AMBER 9

Users' Manual

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Recommended Citations:

When citing Amber Version 9 in the literature, the following citation should be used:

> D.A. Case, T.A. Darden, T.E. Cheatham, III, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, K.M. Merz, D.A. Pearlman, M. Crowley, R.C. Walker, B. Wang, S. Hayik, A. Roitberg, G. Seabra, X. Wu, S. Brozell, V. Tsui, H. Gohlke, L. Yang, C. Tan, J. Mongan, V. Hornak, G. Cui, P. Beroza, D.H. Mathews, C. Schafmeister, W.S. Ross, and P.A. Kollman (2006), AMBER 9, University of California, San Francisco.

The history of the codes and a basic description of the methods can be found in two papers:

> D.A. Pearlman, D.A. Case, J.W. Caldwell, W.S. Ross, T.E. Cheatham, III, S. DeBolt, D. Ferguson, G. Seibel, and P. Kollman. AMBER, a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules. *Comp. Phys. Commun.* **91,** 1-41 (1995).
>
> D.A. Case, T. Cheatham, T. Darden, H. Gohlke, R. Luo, K.M. Merz, Jr., A. Onufriev, C. Simmerling, B. Wang and R. Woods. The Amber biomolecular simulation programs. *J. Computat. Chem.* **26,** 1668-1688 (2005).

Peter Kollman died unexpectedly in May, 2001. We dedicate Amber to his memory.

Cover Illustration

The cover shows three snapshots from an Amber all-atom MD simulation of HIV- 1 protease along with a cyclic urea inhibitor. The inhibitor was manually placed into the binding site of an open structure of the protease. The structure was obtained from Amber MD of the unbound protease. The top structure shows the initial complex, the middle shows a later snapshot in which the active site flaps are seen to be closing over the inhibitor and the lowest image shows the final structure, with the flaps fully closed. The crystal structure of the complex is shown in transparent gray in all three images for reference. All images were generated by Carlos Simmerling using VMD. For further details, see Refs. [1,2].

Table of Contents

1. Introduction

Amber is the collective name for a suite of programs that allow users to carry out molecular dynamics simulations, particularly on biomolecules. None of the individual programs carries this name, but the various parts work reasonably well together, and provide a powerful framework for many common calculations [3,4]. The term *amber* is also sometimes used to refer to the empirical force fields that are implemented here [5]. It should be recognized however, that the code and force field are separate: several other computer packages have implemented the *amber* force fields, and other force fields can be implemented with the *amber* programs. Further, the force fields are in the public domain, whereas the codes are distributed under a license agreement.

Amber 9 (2006) represents a significant change from the most recent previous version, *Amber 8*, which was released in March, 2004. Briefly, the major differences include:

(1) *Force fields:* Many new force field types are available:

- *(a)* updates to existing non-polarizable (*ff99*) and polarizable (*ff02*) protein force fields, with improved torsional parameters for peptides and proteins;
- *(b)* a new united-atom (no non-polar hydrogens) force field, derived with a philosophy similar to the *ff03* all-atom force field [6];
- *(c)* an extension of the General Amber Force Field (*gaff*), that expands the range of applicable molecules, particularly for conjugated systems [7];
- *(d)* support for the AMOEBA polarizable potentials of Ren and Ponder [8,9];
- *(e)* an empirical valence bond model that can be used to construct approximate potentials for chemical reactions;

(2) *QM/MM Simulations:* Amber 9 features new and significantly improved QM/MM support, whereby part of the system can be treated via quantum mechanics (QM). The QM/MM facility supports gas phase, implicit solvent (GB) and periodic boundary (PME) simulations where the energies and forces for the QM part of the system can be derived from a semi-empirical method, such as MNDO, AM1, PM3, or PM3/PDDG. Compared to earlier versions, the QM/MM implementation offers improved accuracy, energy conservation, and performance.

In addition, gas-phase or solvent-cap QM/MM simulations can be carried out with the MNDO/d or SCC-DFTB [10] Hamiltonians. Large quantum regions (with hundreds of atoms) can be modeled with a divide-and-conquer linear-scaling approach, and chemical shifts can be computed from semiempirical wavefunctions.

(3) *Generalized Born models:* Amber 9 features a new model with a pairwise molecular volume correction that shows substantially better agreement with molecular surface Poisson-Boltzmann and explicit solvent results than previous Amber GB models [11].

(4) *Updates to the Poisson-Boltzmann applications:* these include new nonbonded routines, newly optimized atomic cavity radii based on explicit-solvent free energy simulations, improved visualization option for electrostatic potential, and a new nonpolar solvation model with an explicit treatment of dispersion interaction that greatly improves the correlation with nonpolar solvation free energies in explicit solvent.

(5) *Nudged elastic band* simulations can be used to search for approximate transition states in complex transformations. The *self-guided Langevin dynamics* method can be used to accelerate conformational searches.

(6) *Path integral molecular dynamics* simulations can be used to sample equilibrium canonical distributions using quantum dynamics rather than Newton's equations for nuclear motion.

(7) *Free energies* can also be estimated from from non-equilibrium "targeted" or "pulling" simulations, using the Jarzynski identity.

(8) *Updates to the replica exchange methods*, including improvements to the standard replica exchange code and support for a new replica exchange method in which a hybrid solvent model is used to reduce the number of replicas required for large systems in explicit solvent.

(9) *General improvements in speed and parallel scaling* are available in an expanded *pmemd* program, which now includes generalized Born capability.

(10) *NetCDF binary trajectory files* are now supported by *sander*, *pmemd* and *ptraj*. Compared to formatted trajectory files, the binary trajectory files are smaller, higher precision and significantly faster to read and write. NetCDF provides for file portability across architectures, allows for backwards compatible extensibility of the format and enables the files to be self-describing. Support for this format will also be available in VMD 1.8.4.

1.1. What to read next

If you are installing this package see Section 1.3. New users should continue with this Chapter, and should consult the tutorial information in Section 1.4. Everyone should read Chapter 2, which contains information about force fields, and which is extensively revised from earlier versions of Amber. There are also tips and examples on the Amber Web pages at `http://amber.scripps.edu`. Although Amber may appear dauntingly complex at first, it has become easier to use over the past few years, and overall is reasonably straightforward once you understand the basic architecture and option choices. In particular, we have worked hard on the tutorials to make them accessible to new users. Hundreds of people have learned to use Amber; don't be easily discouraged.

If you want to learn more about basic biochemical simulation techniques, there are a variety of good books to consult, ranging from introductory descriptions [12,13], to standard works on liquid state simulation methods [14,15], to multi-author compilations that cover many important aspects of biomolecular modelling [16-18]. Looking for "paradigm" papers that report simulations similar to ones you may want to undertake is also generally a good idea.

1.2. Information flow in Amber

Understanding where to begin in Amber is primarily a problem of managing the flow of information in this package--see Fig. 1. You first need to understand what information is needed by the simulation programs (*sander, pmemd* and *nmode*). You need to know where it comes from, and how it gets into the form that the energy programs require. This section is meant to orient the new user and is not a substitute for the individual program documentation.

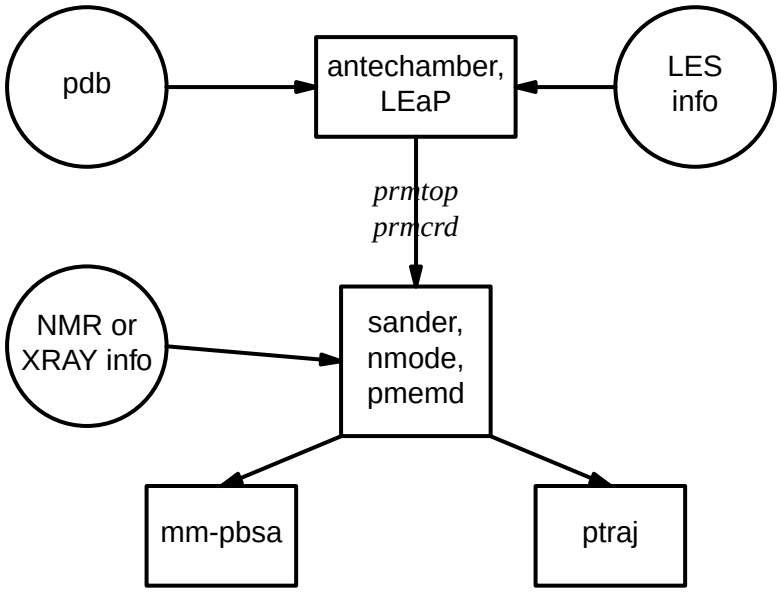


Fig. 1. Basic information flow in Amber

Information that all the simulation programs need:

(1) Cartesian coordinates for each atom in the system. These usually come from Xray crystallography, NMR spectroscopy, or model-building. They should be in Protein Databank (PDB) format. The program *LEaP* provides a platform for carrying out many of these modeling tasks, but users may wish to consider other programs as well.

(2) "Topology": connectivity, atom names, atom types, residue names, and charges. This information comes from the database, which is found in the *amber9/dat/leap/prep* directory, and is described in Chapter 2. It contains topology for the standard amino acids as well as N- and C-terminal charged amino acids, DNA, RNA, and common sugars. The database contains default internal coordinates for these monomer units, but coordinate information is usually obtained from PDB files. Topology information for other molecules (not found in the standard database) is kept in user-generated "residue files", which are generally created using *antechamber*.

(3) Force field: Parameters for all of the bonds, angles, dihedrals, and atom types in the system. The standard parameters for several force fields are found in the *amber9/dat/leap/parm* directory; consult Chapter 2 for more information. These files may be used "as is" for proteins and nucleic acids, or users may prepare their own files that contain modifications to the standard force fields.

(4) Commands: The user specifies the procedural options and state parameters desired. These are specified in the input files (usually called *mdin*) to the sander, pmemd, or nmode programs.

1.2.1. Preparatory programs

LEaP is the primary program to create a new system in Amber, or to modify old systems. It combines the functionality of `prep, link, edit,` and `parm` from earlier versions.

ANTECHAMBER is the main program from the Antechamber suite. If your system contains more than just standard nucleic acids or proteins, this may help you prepare the input for LEaP.

1.2.2. Simulation programs

SANDER is the basic energy minimizer and molecular dynamics program. This program relaxes the structure by iteratively moving the atoms down the energy gradient until a sufficiently low average gradient is obtained. The molecular dynamics portion generates configurations of the system by integrating Newtonian equations of motion. MD will sample more configurational space than minimization, and will allow the structure to cross over small potential energy barriers. Configurations may be saved at regular intervals during the simulation for later analysis, and basic free energy calculations using thermodynamic integration may be performed.

More elaborate conformational searching and modeling MD studies can also be carried out using the SANDER module. This allows a variety of constraints to be added to the basic force field, and has been designed especially for the types of calculations involved in NMR structure refinement.

PMEMD is a version of *sander* that is optimized for speed and for parallel scaling. The name stands for "Particle Mesh Ewald Molecular Dynamics," but this code can now also carry out generalized Born simulations. The input and output have only a few changes from *sander*.

NMODE is both a quasi-Newton Raphson second derivative energy minimizer and vibrational analysis program. NMODE can calculate the normal modes of the system as well as numerous thermochemical properties. Other features include the ability to compute "Langevin modes" (normal modes including viscous coupling to a continuum solvent) and techniques to find transitions states as well as minima.

1.2.3. Analysis programs

PTRAJ is a general purpose utility for analyzing and processing trajectory or coordinate files created from MD simulations (or from various other sources), carrying out superpositions, extractions of coordinates, calculation of bond/angle/dihedral values, atomic positional fluctuations, correlation functions, analysis of hydrogen bonds, etc. The same executable, when named rdparm (from which the program evolved), can examine and modify prmtop files.

MM-PBSA is a script that automates energy analysis of snapshots from a molecular dynamics simulation using ideas generated from continuum solvent models.

1.3. Installation of Amber 9

To compile the basic AMBER distribution, do the following:

(1) Set up the AMBERHOME environment variable to point to where the Amber tree resides on your machine. For example

```
Using csh, tcsh, etc:          setenv AMBERHOME  /usr/local/amber9

Using bash, sh, zsh, etc:      set AMBERHOME=/usr/local/amber9
                               export AMBERHOME
```

NOTE: Be sure to replace the "/usr/local" part above with whatever path is appropriate for your machine. You should then add $AMBERHOME/exe to your PATH.

(2) Go to the $AMBERHOME/src directory, and create a configuration file for a serial version:

```
./configure -help
```

will show you the options available. Choose a machine/compiler name, for example:

```
./configure -p4 g95
```

This will create a config.h file for a single-processor Pentium IV machine using the *g95* compiler (see http://www.g95.org). You can examine and edit this file to match your local environment, if necessary. Do not choose any parallel options (-mpich, -lam,...) at this point. (Note: if you choose one of the "ifort" options, be sure to execute the *ifortvars.sh* or *ifortvars.csh* script, in order to set up the proper environment variables.)

(3) Now compile everything:

```
make serial
```

Loader warnings (especially on SGI) can generally be ignored; compiler warnings should be considered, but most are innocuous. If a program that you don't need initially fails to compile, you should consider invoking "make" with the ignore errors option (make -i) or commenting out that line in the Makefile, and seeing if the rest of the suite can be compiled correctly.

(4) To test the basic AMBER distribution, do this:

```
cd $AMBERHOME/test
make test.serial
```

You can also try "make test.<program-name>", to test individual programs. See $AMBERHOME/test/Makefile for the available options.

Where "possible FAILURE" messages are found, go to the indicated directory under $AMBERHOME/test, and look at the "*.dif" files. Differences should involve round-off in the final digit printed, or occasional messages that differ from machine to machine (see

below for details). As with compilation, if you have trouble with individual tests, you may wish to invoke "make" with the ignore errors option (`make -i`) or comment out certain lines in the Makefile, and/or go directly to the $AMBERHOME/test subdirectories to examine the inputs and outputs in detail. For convenience, all of the failure messages are collected in the file $AMBERHOME/test/TEST_FAILURES.DAT; you can quickly see from these if there is anything more than round-off errors.)

(5) Once you have some experience with the serial version of Amber, you may wish to build a parallel version as well. Because of the vagaries of MPI libraries, this has more pitfalls than installing the serial version; hence you should not do this just "because it is there". Build a parallel version when you know you have a basic understanding of Amber, and you need extra features.

Also note this: *you may want to build a parallel version even for a machine with a single cpu.* The free energy and empirical valence bond (EVB) facilities require a parallel installation, but these will generally run fine using two threads on a single-cpu machine. It is also the case (especially if you have an Intel CPU with hyper-threading enabled) that you will get a modest speedup by running an MPI job with two threads, even on a machine with just one physical CPU.

To build a parallel version, do the following: First, you need to install an MPI library, if one is not already present on your machine. As an example, here are general instructions for installing Open MPI; for more specific information, see its documentation.

```
cd /usr/local                (or wherever you want)
tar xvf openmpi-1.0.tar
cd /usr/local/openmpi-1.0
setenv FC=/opt/intel_fc_80/bin/ifort  (or your compiler)
setenv CC=/opt/intel_cc_80/bin/icc
setenv CXX=/opt/intel_cc_80/bin/icpc
make
make install
setenv MPI_HOME=/usr/local
```

Look out for errors, etc., but this should install Open MPI on your system. You should add $MPI_HOME/bin to your PATH and $MPI_HOME/lib to your LD_LIBRARY_PATH. You are now ready to compile a parallel version of the Amber programs:

```
cd $AMBERHOME/src
make clean                   (important! don't neglect this step)
./configure -mpi ifort       (as an example)
make parallel
```

This creates three new executables: *sander.MPI, sander.LES.MPI* and *sander.PIMD.MPI*. The serial versions will still be available in $AMBERHOME/exe, just without the "MPI" extension. [Note that this is a change from previous releases, where both serial and parallel versions were just called "sander".]

To test parallel programs, you need first to set the DO_PARALLEL environment variable

as follows:

```
cd $AMBERHOME/test
setenv DO_PARALLEL 'mpirun -np 4'
make test.parallel
```

The integer is the number of processors; if your command to run MPI jobs is something different than `mpirun` (e.g. it is `dmpirun` on Tru64 Unix systems), use the command appropriate for your machine.

(6) At this point, you should also compile the PMEMD (particle-mesh Ewald molecular dynamics) program. (Note that, in spite of its name, this code now can do implicit solvent GB calculations as well.) See Chapter 8 of the Users' Manual, and $AMBERHOME/src/pmemd/README for instructions.

1.3.1. More information on parallel machines or clusters

This section contains notes about the various parallel implementations supplied in the current release. Only *sander* and *pmemd* are parallel programs; all others are single threaded. NOTE: Parallel machines and networks fail in unexpected ways. PLEASE check short parallel runs against a single-processor version of Amber before embarking on long parallel simulations!

The MPI (message passing) version was initially developed by James Vincent and Ken Merz, based on 4.0 and later an early prerelease 4.1 version [19]. This version was optimized, integrated and extended by James Vincent, Dave Case, Tom Cheatham, Scott Brozell, and Mike Crowley, with input from Thomas Huber, Asiri Nanyakkar, and Nathalie Godbout.

The bonds, angles, dihedrals, SHAKE (only on bonds involving hydrogen), nonbonded energies and forces, pairlist creation, and integration steps are parallelized. The code is pure SPMD (single program multiple data) using a master/slave, replicated data model. Basically, the master node does all of the initial set-up and performs all the I/O. Depending on the version and/or what particular input options are chosen, either all the non-master nodes execute *force()* in parallel, or all nodes do both the forces and the dynamics in parallel. Communication is done to accumulate partial forces, update coordinates, etc.

For reasons we don't understand, some MPI implementations require a null file for stdin, even though *sander* doesn't take any input from there. This is true for some SGI and HP machines. If you receive a message like "stopped, tty input", try the following:

```
mpirun -np <num-proc> sander  [ options ]   < /dev/null
```

1.3.2. Installing Non-Standard Features

The source files of some Amber programs contain multiple code paths. These code paths are guarded by directives to the C preprocessor. All Amber programs regardless of source language use the C preprocessor. The activation of non-standard features in guarded code paths can be controlled at build time via the -D preprocessor option. For example, to enable the use of a Lennard-Jones 10-12 potential with the *sander* program the HAS_10_12 preprocessor guard must be activated with -DHAS_10_12.

To ease the installers burden we provide a hook into the build process. The hook is the environment variable AMBERBUILDFLAGS. For example, to build *sander* with -DHAS_10_12, assuming that a correct configuration file has already been created, do the following:

```
cd $AMBERHOME/src/sander
make clean
make AMBERBUILDFLAGS='-DHAS_10_12' sander
```

Note that AMBERBUILDFLAGS is accessed by all stages of the build process: preprocessing, compiling, and linking. In rare cases a stage may emit warnings for unknown options in AMBERBUILDFLAGS; these may usually be ignored.

1.3.3. Installing on Microsoft Windows

All of Amber (including the X-windows parts) will compile and run on Windows using the Cygwin development tools: see *http://sources.redhat.com/cygwin*. We recommend (certainly as a first step) using the g95 compiler (see *http://www.g95.org*) along with the gcc compiler that comes with cygwin.

Note that Cygwin provides a POSIX-compatible environment for Windows. Effective use of this environment requires a basic familiarity with the principles of Linux or Unix operating systems. Building the Windows version is thus somewhat more complex (not simpler) than building under other operating systems. You should only attempt this *after* you have a basic familiarity with the cygwin environment. The Windows version has only been tested in a single-cpu environment.

1.3.4. Testing

We have installed and tested Amber 9 on a number of platforms, using UNIX, Linux, Microsoft Windows or Macintosh OSX operating systems. However, owing to time and access limitations, not all combinations of code, compilers, and operating systems have been tested. Therefore we recommend running the test suites.

The distribution contains a validation suite that can be used to help verify correctness. The nature of molecular dynamics, is such that the course of the calculation is very dependent on the order of arithmetical operations and the machine arithmetic implementation, *i.e.* the method used for roundoff. Because each step of the calculation depends on the results of the previous step, the slightest difference will eventually lead to a divergence in trajectories. As an initially identical dynamics run progresses on two different machines, the trajectories will eventually become completely uncorrelated. Neither of them are "wrong;" they are just exploring different regions of phase space. Hence, states at the end of long simulations are not very useful for verifying correctness. Averages are meaningful, provided that normal statistical fluctuations are taken into account. "Different machines" in this context means any difference in floating point hardware, word size, or rounding modes, as well as any differences in compilers or libraries. Differences in the order of arithmetic operations will affect roundoff behavior; (a + b) + c is not necessarily the same as a + (b + c). Different optimization levels will affect operation order, and may therefore affect the course of the calculations.

All initial values reported as integers should be identical. The energies and temperatures on the first cycle should be identical. The RMS and MAX gradients reported in sander are often more precision sensitive than the energies, and may vary by 1 in the last figure on some machines.

In minimization and dynamics calculations, it is not unusual to see small divergences in behavior after as little as 100-200 cycles.

1.3.5. Memory Requirements

The Amber 9 programs mainly use dynamic memory allocation, and do not generally need to be compiled for any specific size of problem. Some sizes related to NMR refinements are defined in `nmr.h` If you receive error messages directing you to look at these files, you may need to edit them, then recompile.

If you get a "Segmentation fault" immediately upon starting a program (particularly if this happens with no arguments), you may not have enough memory to run the program. The "size" command will show you the size of the executable. Also check the limits of your shell; you may need to increase these (especially stacksize, which is sometimes set to quite small values).

1.3.6. Notes for users of previous versions of Amber

The comments here point out some features and compatibility changes that may be affect those who have used previous versions of this package.

(1) The way two system Hamiltonians are prepared for free energy calculations is different from earlier versions. In particular, there is no longer a "perturbed prmtop" file; the beginning and ending states are created in the usual way as individual prmtop files. See Section 6.5 for more information.

(2) In spite of its name, the *pmemd* program can now carry out generalized Born simulations. This means that many more simulations can make use of this program, which is considerably faster than *sander*, especially in parallel environments.

(3) There is a new binary format for trajectory files, using the netCDF libraries and file formats. See the *sander* and *ptraj* chapters for more information.

(4) The *nmode* program is no longer being developed, and will probably be phased out in the next release. Better functionality is available from NAB (Nucleic Acid Builder): see `http://www.scripps.edu/case/nab.html`.

(5) There are some new executable names in this release. In the past, *sander* could refer to either a serial or a parallel program. Now, the command *make serial* will create *sander*, and *make parallel* will create *sander.MPI*. If you have existing scripts that run parallel jobs, you may have to substitute *sander.MPI* for *sander* inside these.

(6) There are two sets of features that require separate compilation because they would slow down the basic code too much if they were present in the base program. For locally enhanced sampling, you will need to use the *sander.LES* (serial) or *sander.LES.MPI* (parallel) programs. Similarly, for path-integral MD or for nudged elastic band, you will need to use the *sander.PIMD* or *sander.PIMD.MPI* programs.

(7) In Amber 8, running replica exchange programs required a special compilation, with *-DREM* specified at compile-time. This is no longer necessary: the basic *sander.MPI* code includes a replica exchange capability; (note that replica exchange implies a parallel mode of execution, so that the serial *sander* does not support replica exchange.)

1.4. Basic tutorials

AMBER is a suite of programs for use in molecular modeling and molecular simulations. It consists of a substructure database, a force field parameter file, and a variety of useful programs. Here we give some commented sample runs to provide an overview of how things are carried out. The examples only cover a fraction of the things that it is possible to do with AMBER. The formats of the example files shown are described in detail later in the manual, in the chapters pertaining to the programs. Tom Cheatham, Bernie Brooks and Peter Kollman have prepared some detailed information on simulation protocols that should be also be consulted [20].

Additional tutorial examples are available at *http://amber.scripps.edu*. Because the web can provide a richer interface than one can get on the printed page (with screen shots, links to the actual input and output files, etc.), most of our recent efforts have been devoted to updating the tutorials on the web site. In particular, new users are advised to look at the following, which can be found at both the web site listed above, and on the distribution CD, under *amber9/tutorial*:

DNA	Basic introduction to *LEaP*, *sander*, and *ptraj*, to build, solvate, run MD and analyze trajectories.
Plastocyanin/ion/water	Basic tutorial for a protein, introducing non-standard residues, NMR restraints, and more complex modeling tasks.
Loop dynamics in HIV integrase	Show how a study of protein dynamical behavior was carried out, illustrating some more complex setups and analyses.
NMR refinement of DNA	Basic introduction to NMR refinement using LEaP and sander.
GB simulation	Carrying out a protein simulation using the generalized Born continuum solvent model.

We are in the process of creating additional tutorials; because of the lead time needed to print this manual, there may be new tutorials available, either in *amber9/tutorial* or at the web site listed above. You should also look at the sample inputs in the chapters devoted to each program, especially for LEaP and sander.

As a basic example, we consider here the minimization of a protein in a simple solvent model. The procedure consists of three steps:

Step 1. Generate some starting coordinates.

The first step is to obtain starting coordinates. We begin with the bovine pancreatic trypsin inhibitor, and consider the file *6pti.pdb*, exactly as distributed by the Protein Data Bank. This file (as with most PDB files) needs some editing before it can be used by Amber. First, alternate conformations are provided for residues 39 and 50, so we need to figure out which one we want. For this example, we choose the "A" conformation, and manually edit the file to remove the alternate conformers. Second, coordinates are provided for a phosphate group and a variety of water molecules. These are not needed for the calculation we are pursuing here, so we also edit the file to remove these. Third, the cysteine residues are involved in disulfide bonds, and need to have

their residue names changed in an editor from CYS to CYX to reflect this. Finally, since we removed the phosphate groups, some of the CONECT records now refer to non-existent atoms; if you are not sure that the CONECT records are all correct then it may be safest to remove all of them, as we do for this example. Let's call this modified file *6pti.mod.pdb.*

Although Amber tries hard to understand pdb-format files, it is typical to have to do some manual editing before proceeding. A general prescription is: "keep running the *loadPdb* step in LEaP (see step 2, below), and editing the pdb file, until there are no error messages."

Step 2. Run LEaP to generate the parameter and topology file.

This is a fairly straightforward exercise in loading in the pdb file, adding the disulfide cross links, and saving the resulting files. Typing the following commands should work in either *tleap* or *xleap*:

```
source leaprc.ff94
bpti = loadPdb 6pti.mod.pdb
bond bpti.5.SG bpti.55.SG
bond bpti.14.SG bpti.38.SG
bond bpti.30.SG bpti.51.SG
saveAmberParm bpti prmtop prmcrd
quit
```

Step 3. Perform some minimization.

Use this script:

Running minimization for BPTI

```
cat << eof > min.in
#  200 steps of minimization, generalized Born solvent model
 &cntrl
    maxcyc=200, imin=1, cut=12.0, igb=1, ntb=0, ntpr=10,
 /
eof
sander -i min.in -o 6pti.min1.out -c prmcrd -r 6pti.min1.xyz
/bin/rm min.in
```

This will perform minimization (`imin=1`) for 200 steps (`maxcyc`), using a nonbonded cut-off of 12 Å (`cut`), a generalized Born solvent model (`igb=1`), and no periodic boundary (`ntb=0`); intermediate results will be printed every 10 steps (`ntpr`). Text output will go to file *6pti.min1.out*, and the final coordinates to file *6pti.min1.xyz*. The "out" file is intended to be read by humans, and gives a summary of the input parameters and a history of the progress of the minimization.

Of course, Amber can do much more than the above minimization. This example illustrates the basic information flow in Amber: Cartesian coordinate preparation (*Step 1.*), topology and force field selection (*Step 2.*), and simulation program command specification (*Step 3.*). Typically the subsequent steps are several stages of equilibration, production molecular dynamics runs, and analyses of trajectories. The tutorials in *amber9/tutorial* should be consulted for examples of these latter steps.

2. Specifying a force field

Amber is designed to work with several simple types of force field, although it is most commonly used with parameterizations developed by Peter Kollman and his co-workers. There are now a variety of such parameterizations, with no obvious "default" value. The "traditional" parameterization uses fixed partial charges, centered on atoms. Examples of this are *ff94*, *ff99* and *ff03* (described below). The default in versions 5 and 6 of Amber was *ff94*; a comparable default now would probably be *ff03* or *ff99SB*, but users should consult the papers listed below to see a detailed discussion of the changes made.

Less extensively used, but very promising, recent modifications add polarizable dipoles to atoms, so that the charge description depends upon the environment; such potentials are called "polarizable" or "non-additive". Examples are *ff02* and *ff02EP*: the former has atom-based charges (as in the traditional parameterization), and the latter adds in off-center charges (or "extra points"), primarily to help describe better the angular dependence of hydrogen bonds. Again, users should consult the papers cited below to see details of how these new force fields have been developed.

In order to tell LEaP which force field is being used, the four types of information described below need to be provided. This is generally accomplished by selecting an appropriate *leaprc* file, which loads the information needed for a specific force field. (See section 2.2, below).

(1) A listing of the atom types, what elements they correspond to, and their hybridizations. This information is encoded as a set of LEaP commands, and is normally read from a *leaprc* file.

(2) Residue descriptions (or "topologies") that describe the chemical nature of amino acids, nucleotides, and so on. These files specify the connectivities, atom types, charges, and other information. These files have a "prep" format (a now-obsolete part of Amber) and have a ".in" extension. Standard libraries of residue descriptions are in the *amber9/dat/leap/prep* directory. The *antechamber* program may be used to generate prep files for other organic molecules.

(3) Parameter files give force constants, equilibrium bond lengths and angles, Lennard-Jones parameters, and the like. Standard files have a ".dat" extension, and are found in *amber9/dat/leap/parm*.

(4) Extensions or changes to the parameters can be included in *frcmod* files. The expectation is that the user will load a large, "standard" parameter file, and (if needed) a smaller frcmod file that keeps track of any changes to the default parameters that are needed. The *frcmod* files for changing the default water model (which is TIP3P) into other water models are in files like *amber9/dat/leap/parm/frcmod.tip4p*. The *parmchk* program (part of *antechamber*) can also generate *frcmod* files.

2.1. Description of the database files

The following files are in the *amber9/dat/leap* directory. Files with a ".in" extension are in the *prep* subdirectory; those with a ".dat" extension are in the *parm* subdirectory, as are the "frcmod" files; files ending with ".off" or ."lib" are in the *lib* subdirectory.

Glycam 2004 (Woods et al.) force field

```
glycam04.dat        Parameters for oligosaccharides
glycam04EP.dat      Parameters for oligosaccharides, using extra points

glycam04.in         Topologies for glycosyl residues
glycam04EP.in       Topologies for glycosyl residues, using extra points
```

Amber 2003 (Duan et al.) force field

```
frcmod.ff03         For proteins: changes to parm99.dat, primarily in the
                    phi and psi torsions.
all_amino03.in      Charges and atom types for proteins.
```

Amber 2003 (Yang et al.) united-atom force field

```
frcmod.ff03ua       For proteins: changes to parm99.dat, primarily in the
                    introduction of new united-atom carbon types and new
                    side chain torsions.
uni_amino03.in      Amino acid input for building database
uni_aminont03.in    NH3+ amino acid input for building database.
uni_aminoct03.in    COO- amino acid input for building database.
```

Amber 2002 polarizable force field, and recent updates

```
parm99.dat          Force field, for amino acids and some organic molecules;
                       can be used with either additive or
                       non-additive treatment of electrostatics.
parm99EP.dat        Like parm99.dat, but with "extra-points": off-center
                       atomic charges, somewhat like lone-pairs.
frcmod.ff02pol.r1   Updated torsion parameters for ff02.

all_nuc02.in        Nucleic acid input for building database, for a non-
                    additive (polarizable) force field without extra points.
all_amino02.in      Amino acid input ...
all_aminoct02.in    COO- amino acid input ...
all_aminont02.in    NH3+ amino acid input ....

all_nuc02EP.in      Nucleic acid input for building database, for a non-
                    additive (polarizable) force field with extra points.
all_amino02EP.in      Amino acid input ...
all_aminoct02EP.in    COO- amino acid input ...
all_aminont02EP.in    NH3+ amino acid input ....
```

Amber 1999 (Wang et al.) force field, and recent updates

parm99.dat	Basic force field parameters
gaff.dat	Force field for general organic molecules.
frcmod.ff99SB	"Stony Brook" modification to ff99 backbone torsions
frcmod.ff99SP	"Sorin/Pande" modification to ff99 backbone torsions

Amber 1994 (Cornell et al.) force field

all_nuc94.in	Nucleic acid input for building database.
all_amino94.in	Amino acid input for building database.
all_aminoct94.in	COO- amino acid input for database.
all_aminont94.in	NH3+ amino acid input for database.
nacl.in	Ion file.
parm94.dat	1994 force field file.
parm96.dat	Modified version of 1994 force field, for proteins.
parm98.dat	Modified version of 1994 force field, for nucleic acids.

Amber 1984, 1986 (Weiner et al.) force fields

all.in	All atom database input.
allct.in	All atom database input, COO- Amino acids.
allnt.in	All atom database input, NH3+ Amino acids.
uni.in	United atom database input.
unict.in	United atom database input, COO- Amino acids.
unint.in	United atom database input, NH3+ Amino acids.
parm91X.dat	Parameters for 1984, 1986 force fields.

Solvent models:

water.in	Topology definition for several water models.
meoh.in	Topology file for methanol.
chcl3.in	Topology file for chloroform.
nma.in	Topology file for N-methylacetamide.
tip3pbox.off	Solvation box for TIP3P water.
tip4pbox.off	Solvation box for TIP4P water.
pol3box.off	Solvation box for POL3 water.
spcebox.off	Solvation box for SPC/E water.
meohbox.off	Solvation box for methanol.
nmabox.off	Solvation box for N-methylacetamide.
chcl3box.off	Solvation box for chloroform.
8Mureabox.off	Solvation box for 8M urea/water mixture (see 8Murea.readme for more information).
frcmod.tip4p	Parameter changes from TIP3P -> TIP4P.
frcmod.tip5p	Parameter changes from TIP3P -> TIP5P.
frcmod.spce	Parameter changes from TIP3P -> SPC/E.
frcmod.pol3	Parameter changes from TIP3P -> POL3.
frcmod.meoh	Parameters for methanol.
frcmod.chcl3	Parameters for chloroform.
frcmod.nma	Parameters for N-methyacetamide.

```
  frcmod.urea        Parameters for urea (or urea-water mixtures).
```

Miscellaneous:

```
  nucgen.dat         Nucgen nucleic acid conformations.
  PROTON_INFO*       Files needed for protonate
  map.DG-AMBER       Needed for NMR input generation.
```

2.2. Specifying which force field you want in LEaP

Various combinations of the above files make sense, and we have moved to an "ff" (force field) nomenclature to identify these; examples would then be *ff94* (which was the default in Amber 5 and 6), *ff99*, etc. The most straightforward way to specify which force field you want is to use one of the *leaprc* files in *$AMBERHOME/dat/leap/cmd*. The sytax is:

```
xleap -s -f <filename>
```

Here, the *-s* flag tells LEaP to ignore any *leaprc* file it might find, and the *-f* flag tells it to start with commands for some other file. Here are the combinations we support and recommend:

How to specify force fields in LEaP

filename	*topology*	*parameters*
leaprc.ff86	Weiner *et al.* 1986	parm91X.dat
leaprc.ff94	Cornell *et al.* 1994	parm94.dat
leaprc.ff96	"	parm96.dat
leaprc.ff98	"	parm98.dat
leaprc.ff99	"	parm99.dat
leaprc.ff99SB	"	parm99.dat+frcmod.ff99SB
leaprc.ff03	Duan *et al.* 2003	parm99.dat+frcmod.ff03
leaprc.ff03ua	Yang *et al.* 2003	parm99.dat+frcmod.ff03+frcmod.ff03ua
leaprc.ff02	reduced (polarizable) charges	parm99.dat+frcmod.ff02pol.r1
leaprc.ff02EP	" + extra points	parm99EP.dat
leaprc.gaff	none	gaff.dat
leaprc.glycam04	Woods *et al.*	glycam04.dat
leaprc.glycam04EP	"	glycam04EP.dat

Notes:

(1) There is no default leaprc file. If you make a link from one of the files above to a file named `leaprc`, then that will become the default. For example:

```
cd $AMBERHOME/dat/leap/cmd
ln -s leaprc.ff03 leaprc
```

or

```
cd $AMBERHOME/dat/leap/cmd
ln -s leaprc.ff99SB leaprc
```

will provide a good default for many users; after this you could just invoke `tleap` or `xleap` without any arguments, and it would automatically load the *ff03* or *ff99SB* force field. A *leaprc* file in the current directory overrides any other such files that might be present in the search path.

(2) The first eight choices in the above table are for additive (non-polarizable) simulations; you should use saveAmberParm (or saveAmberParmPert) to save the *prmtop* file, and keep the default *ipol=0* in sander or gibbs.

(3) The *ff02* entries in the above table are for non-additive (polarizable) force fields. Use saveAmberParmPol to save the *prmtop* file, and set *ipol=1* in the sander input file. Note that POL3 is a polarizable water model, so you need to use saveAmberParmPol for it as well.

(4) The files above assume that nucleic acids are DNA, if not explicitly specified. Use the files *leaprc.rna.ff98, leaprc.rna.ff99, leaprc.rna.ff02* or *leaprc.rna.ff02EP* to make the default RNA. If you have a mixture of DNA and RNA, you will need to edit your PDB file, or use the `loadPdbUsingSequence` command in LEaP (see that chapter) in order to specify which nucelotide is which.

(5) There is also a *leaprc.gaff* file, which sets you up for the "general" Amber force field. This is primarily for use with Antechamber (see that chapter), and does not load any topology files.

(6) The *leaprc.ff86* file gives the 1986 all-atom parameters; Amber no longer directly supports the 1984 united atom parameter set. Instead users interested in simulations in united atom should use the 2003 united-atom parameter set, which can be invoked by *leaprc.ff03ua*.

(7) Our experience with generalized Born simulations is mainly with *ff99* or *ff03*; the current GB models are not compatible with polarizable force fields. Replacing explicit water with a GB model is equivalent to specifying a different force field, and users should be aware that none of the GB options (in Amber or elsewhere) is as "mature" as simulations with explicit solvent; user discretion is advised! For example, it was shown that salt bridges are too strong in some of these models [21] and some of them provide secondary structure distributions that differ significantly from those obtained using the same protein parameters in explicit solvent, with GB having too much α-helix present [22].

2.3. The AMOEBA potentials

The **amoeba** force field for proteins, ions, organic solvents and water, developed by Ponder and Ren [8,9] are available in *sander*. This force field is specified by setting *do_amoeba* to 1 in the sander input file. Setting up the system is done in a special way, described in Chapter 6. Users will need to obtain Tinker, version 4.3 in order to obtain needed parameter files and utilities. See Chapter 6 for more information.

2.4. The Duan et al. (2003) force field

The **ff03** force field [23,24] is a modified version of *ff99* (described below). The main changes are that charges are now derived from quantum calculations that use a continuum

dielectric to mimic solvent polarization, and that the ϕ and ψ backbone torsions for proteins are modified, with the effect of decreasing the preference for helical configurations. The changes are just for proteins; nucleic acid parameters are the same as in *ff99*.

2.5. The Yang et al. (2003) united-atom force field

The **ff03ua** force field [6] is the united-atom counterpart of *ff03*. This force field uses the same charging scheme as *ff03*. In this force field, the aliphatic hydrogen atoms on all amino acid sidechains are united to their corresponding carbon atoms. The aliphatic hydrogen atoms on all alpha carbon atoms are still represented explicitly to minimize the impact of the united-atom approximation on protein backbone conformations. In addition, aromatic hydrogens are also explicitedly represented. Van der Waals parameters of the united carbon atoms are refitted based on solvation free energy calculations. Due to the use of all-atom protein backbone, the ϕ and ψ backbone torsions from *ff03* are left unchanged. The sidechain torsions involving united carbon atoms are all refitted. In this parameter set, nucleic acid parameters are still in all atom and kept the same as in *ff99*.

2.6. 1999 and 2002 force fields and recent updates to these parameters

The **ff99** force field [25] points toward a common force field for proteins for "general" organic and bioorganic systems. The atom types are mostly those of Cornell *et al.* (see below), but changes have been made in many torsional parameters, and this parameterization supports both additive and non-additive (polarizable) force fields. The topology and coordinate files for the small molecule test cases used in the development of this force field are in the *parm99_lib* subdirectory. The *ff99* force field uses these parameters, along with the topologies and charges from the Cornell *et al.* force field, to create an all-atom nonpolarizable force field for proteins and nucleic acids.

Several groups have noticed that *ff99* (and *ff94* as well) do not provide a good energy balance between helical and extended regions of peptide and protein backbones. Another problem is that many of the ff94 variants had incorrect treatment of glycine backbone parameters. *ff99SB* is the recent attempt to improve this behavior, and was developed in the Simmerling group [26]. It presents a careful reparametrization of the backbone torsion terms in ff99 and achieves much better balance of four basic secondary structure elements (PP_{II}, β, α_L, and α_R). A detailed explanation of the parametrization as well as an extensive comparison with many other variants of fixed charge Amber forcefields is given in the reference above. Briefly, dihedral term parameters were obtained through fitting the energies of multiple conformations of glycine and alanine tetrapeptides to high-level ab initio QM calculations. We have shown that this force field provides much improved proportions of helical versus extended structures. In addition, it corrected the glycine sampling and should also perform well for β-turn structures, two things which were especially problematic with most previous Amber force field variants. In order to use *ff99SB*, issue "source leaprc.ff99SB" at the start of your LEaP session.

An alternative is to simply zero out the torsional terms for the ϕ and ψ backbone angles [27]. Another alteration along the same lines has been developed by Sorin and Pande [28], and is implemented in the *frcmod.ff99SP* file. Research in this area is ongoing, and users interested in peptide and protein folding are urged to keep abreast of the current literature.

The **ff02** force field is a polarizable variant of *ff99*. Here, the charges were determined at the B3LYP/cc-pVTZ//HF/6-31G* level, and hence are more like "gas-phase" charges. During charge fitting the correction for intramolecular self polarization has been included [29]. Bond

polarization arising from interactions with a condensed phase environment are achieved through polarizable dipoles attached to the atoms. These are determined from isotropic atomic polarizabilities assigned to each atom, taken from experimental work of Applequist. The dipoles can either be determined at each step through an iterative scheme, or can be treated as additional dynamical variables, and propagated through dynamics along with the atomic positions, in a manner analogous Car-Parinello dynamics. Derivation of the polarizable force field required only minor changes in dihedral terms and a few modification of the van der Waals parameters.

Recently, a set up updated torsion parameters has been developed for the *ff02* polarizable force field [30]. These are available in the *frcmod.ff02pol.r1* file.

The user also has a choice to use the polarizable force field with extra points on which additional point charges are located; this is called **ff02EP**. The additional points are located on electron donating atoms (e.g. O,N,S), which mimic the presence of electron lone pairs [31]. For nucleic acids we chose to use extra interacting points only on nucleic acid bases and not on sugars or phosphate groups.

There is not (yet) a full published description of this, but a good deal of preliminary work on small molecules is available [29,32]. Beyond small molecules, our initial tests have focussed on small proteins and double helical oligonucleotides, in additive TIP3P water solution. Such a simulation model, (using a polarizable solute in a non-polarizable solvent) gains some of the advantages of polarization at only a small extra cost, compared to a standard force field model. In particular, the polarizable force field appears better suited to reproduce intermolecular interactions and directionality of H-bonding in biological systems than the additive force field. Initial tests show *ff02EP* behaves slightly better than *ff02*, but it is not yet clear how significant or widespread these differences will be.

The **gaff.dat** ("general Amber force field") is yet a further step towards general purpose organic molecules [7]. It is primarily used in conjunction with the *antechamber* program, and users should consult that chapter for more information.

2.7. The Cornell et al. (1994) force field

Contained in **ff94** are parameters from the so-called "second generation" force field developed in the Kollman group in the early 1990's [33]. These parameters are especially derived for solvated systems, and when used with an appropriate 1-4 electrostatic scale factor, have been shown to perform well at modeling many organic molecules. The parameters in *parm94.dat* omit the hydrogen bonding terms of earlier force fields. This is an all-atom force field; no united-atom counterpart is provided. 1-4 electrostatic interactions are scaled by 1.2 instead of the value of 2.0 that had been used in earlier force fields.

Charges were derived using Hartree-Fock theory with the 6-31G* basis set, because this exaggerates the dipole moment of most residues by 10-20%. It thus "builds in" the amount of polarization which would be expected in aqueous solution. This is necessary for carrying out condensed phase simulations with an effective two-body force field which does not include explicit polarization. The charge-fitting procedure is described in Chapter 12.2.

The **ff96** force field [34] differs from *parm94.dat* in that the torsions for ϕ and ψ have been modified in response to *ab initio* calculations [35] which showed that the energy difference between conformations were quite different than calculated by Cornell *et al.* (using *parm94.dat*). To create parm96.dat, common V_1 and V_2 parameters were used for ϕ and ψ, which were empirically adjusted to reproduce the energy difference between extended and constrained alpha helical energies for the alanine tetrapeptide. This led to a significant improvement between molecular

mechanical and quantum mechanical relative energies for the remaining members of the set of tetrapeptides studied by Beachy *et al.* Users should be aware that *parm96.dat* has not been as extensively used as *parm94.dat*, and that it almost certainly has its own biases and idiosyncrasies, including strong bias favoring extended β conformations [26,36,37].

The **ff98** force field [38] differs from *parm94.dat* in torsion angle parameters involving the glycosidic torsion in nucleic acids. These serve to improve the predicted helical repeat and sugar pucker profiles.

2.8. The Weiner et al. (1984,1986) force fields

The **ff86** parameters are described in early papers from the Kollman and Case groups [39,40]. [The "parm91" designation is somewhat unfortunate: this file is really only a corrected version of the parameters described in the 1984 and 1986 papers listed above.] These parameters are not generally recommended any more, but may still be useful for vacuum simulations of nucleic acids and proteins using a distance-dependent dielectric, or for comparisons to earlier work. The material in *parm91X.dat* is the parameter set distributed with Amber 4.0. The *STUB* nonbonded set has been copied from *parmuni.dat*; these sets of parameters are appropriate for united atom calculations using the "larger" carbon radii referred to in the "note added in proof" of the 1984 JACS paper. If these values are used for a united atom calculation, the parameter *scnb* should be set to 8.0; for all-atom calculations use 2.0. The *scee* parameter should be set to 2.0 for both united atom and all-atom variants. *Note that the default value for scee is sander is now 1.2 (the value for 1994 and later force fields; users must explicitly change this in their inputs for the earlier force fields.*

parm91X.dat is not recommended. However, for historical completeness a number of terms in the non-bonded list of parm91X.dat should be noted. The non-bonded terms for I(iodine), CU(copper), and MG(magnesium) have not been carefully calibrated, but are given as approximate values. In the STUB set of non-bonded parameters, we have included parameters for a large hydrated monovalent cation (IP) that represent work by Singh *et al.* [41] on large hydrated counterions for DNA. Similar values are included for a hydrated anion (IM).

The non-bonded potentials for hydrogen-bond pairs in *ff86* use a Lennard-Jones 10-12 potential. If you want to run *sander* with *ff86* then you will need to recompile, adding -DHAS_10_12 to the Fortran preprocessor flags; see Chapter 1.3.2.

2.9. Glycam-04 force field for carbohydrates

As in previous versions of Glycam, the parameters are intended for explicitly solvated MD simulations of carbohydrates. Also, as in previous versions, the van der Waals parameters were borrowed from the Parm94 force field. However, in several other areas the development of the Glycam-04 parameters differs significantly from earlier versions of Glycam. This parameter set is unique from the other standard AMBER parameters in that is has been derived for use without the need for scaling 1-4 non-bonded interactions. Other major differences from earlier Glycam versions relate to the partial charge placements. Throughout the development of the parameters the 1-4 electrostatic (SCEE) and non-bonded (SCNB) scaling factors were set to unity. We have shown that this is essential in order to properly treat internal hydrogen bonds, particularly those associated with the hydroxymethyl group. With 1-4 scaling, it was not possible to correctly reproduce the rotamer populations for the C5-C6 bond. For studying carbohydrate-protein interactions, we suggest that the SCEE and SCNB scaling factors be set to the appropriate value according to the protein force field that is chosen. While this may degrade the accuracy of the rotational populations obtained with Glycam, it should not interfere with the stability or structure

of protein-bound carbohydrates. Details of how the new parameters were developed are described elsewhere [42-44].

As in previous versions of Glycam, the atomic partial charges were determined using the RESP formalism, with a weighting factor of 0.01 from a wavefunction computed at the HF/6-31G(d) level. However, to reduce artifactual fluctuations in the charges on saturated carbon atoms, charges on aliphatic hydrogens (types HC, H1, H2, & H3) were set to zero while the partial charges were fit to the remaining atoms. Due to the rotational freedom of hydroxyl groups, partial atomic charges for each sugar were determined by averaging charges obtained from 100 conformations selected evenly from 50 ns solvated MD simulations of the methyl glycoside of each monosaccharide, thus yielding an ensemble averaged charge set.

In order to extend glycam04 to simulations employing the TIP-5P water model, an additional set of carbohydrate prep files has been derived, in which lone pairs (or extra points, EPs) have been incorporated on the oxygen atoms. The optimal O-EP distance was located by obtaining the best fit to the HF/6-31g(d) electrostatic potential. In general, the best fit to the quantum potential coincided with a negligible charge on the oxygen nuclear position. The optimal O-EP distance for an sp3 oxygen atom was found to be 0.70Å; for an sp2 oxygen atom a shorter length of 0.45Å was optimal. When applied to water, this approach to locating the lone pair positions and assigning the partial charges yielded a model that was essentially indistinguishable from TIP-5P. Therefore, we believe this model is well suited for use with TIP-5P.

All valence parameters were determined by fitting to data computed at the B3LYP/6-31++G(2d,2p)//HF/6-31G(d) level of theory. This level was selected due to its inherently low level of basis set superposition error, which manifests itself in a reduction of rotational barrier heights. A major difference from earlier versions of Glycam is that here each parameter term was explicitly parameterized, using a molecular development suite of more than 75 molecules. The parameter test suite included carbohydrates and numerous smaller molecular fragments. Further, in this version of Glycam we have eliminated the atom types AC, EC, and OG. However, to avoid potential conflicts with the CT atom type in the protein parameters, we have employed a new atom type for tetrahedral carbons called CG (C-Glycam) in order to keep the Glycam parameters specific for carbohydrates.

The latest release of the Glycam parameters prep files can always be obtained from the Woods group at `http://glycam.ccrc.uga.edu`.

2.9.1. Notes on the naming of Prep files

Due to the structural diversity of carbohydrates, the prep file nomenclature requires some explanation. The naming of prep files is relatively straightforward. However, to be limited to a three-letter residue name requires some compromises in clarity. Nevertheless, an orthogonal set is presented that encodes the following details: core monosaccharide name (glucose, mannose, etc.), anomericity (α or β), configuration (D or L), sugar linkage position (2, 3, 4, etc.) and ring size (pyranose or furanose).

Here are some examples of pyranoses (linked at the 2-position):

-2)-α-D-mannose	prep file name: 2madp.prep	Residue name: 2MA (A = α)
-2)-β-D-mannose	prep file name: 2mbdp.prep	Residue name: 2MB (B = β)
-2)-α-L-mannose	prep file name: 2malp.prep	Residue name: M2A (mirror image of 2MA)
-2)-β-L-mannose	prep file name: 2mblp.prep	Residue name: M2B (mirror image of 2MA)

Here are some examples of furanoses (linked at the 2-position):

-2)-α-D-xylose	prep file name: 2xadf.prep	Residue name 2XD (D = α, as in Down)
-2)-β-D-xylose	prep file name: 2xbdf.prep	Residue name 2XU (U = β, as in Up)
-2)-α-L-xylose	prep file name: 2xalf.prep	Residue name X2D (mirror image of 2XD)
-2)-β-L-xylose	prep file name: 2xblf.prep	Residue name X2U (mirror image of 2XU)

Finally, here are some examples of terminal residues (thought of as linked at the 1-position):

α-D-mannopyranose-(1-	prep file name: 1madp.prep	Residue name: 1MA
α-D-xylofuranose-(1-	prep file name: 1xadf.prep	Residue name 1XD

2.9.2. Carbohydrate Naming Convention in Glycam-04

Here are the one-letter codes that form the core of the Glycam residue names for carbohydrates. (In the future we may employ lowercase letters to define L-sugars. There are also numerous complex residues that will not fit the rule for simple monosaccharides.)

One-letter codes for carbohydrates			
Carbohydrate	*One-letter*	*Common Abbr.*	*Classification*
Arabinose	A	Ara	Pentose
Lyxose	D	Lyx	Pentose
Ribose	R	Rib	Pentose
Xylose	X	Xyl	Pentose
Allose	N	All	Hexose
Altrose	E	Alt	Hexose
Galactose	L	Gal	Hexose
Glucose	G	Glc	Hexose
Gulose	K	Gul	Hexose
Idose	I	Gul	Hexose
Mannose	M	Man	Hexose
Talose	T	Tal	Hexose
Fructose	C	Fru	Hexulose
Psicose	P	Psi	Hexulose
Sorbose	B	Sor	Hexulose
Tagatose	J	Tag	Hexulose
Fucose	F	Fuc	6-DeoxyHexose
Quinovose	Q	Qui	6-DeoxyHexose
Rhamnose	H	Rha	6-DeoxyHexose
Galacturonic Acid	O	GalA	Hexuronic Acid
Glucuronic Acid	Z	GlcA	Hexuronic Acid
Iduronic Acida	U	IdoA	Hexuronic Acid
N-Acetylgalactosamine	V	GalNac	HexNAc
N-Acetylglucosamine	Y	GlcNAc	HexNAc
N-Acetylmannosamine	W	ManNAc	HexNAc
N-Acetyl-neuraminic acid	S	NeuNAc, Neu5Ac	9-Carbon Acid
KDN	KN	KDN	8-Carbon Acid
KDO	KO	KDO	8-Carbon Acid
N-Glycolyl-neuraminic acid	SG	NeuNGc, Neu5Gc	9-Carbon Acid

The following tables give details of choosing residue names.

Linkage position and anomeric configuration in D-hexopyranoses				
	α-D-Glucose		*β-D-Mannose*	
Linkage Position	*Prep File Prefix*	*Residue Name*	*Prep File Prefix*	*Residue Name*
Terminal	1GADP	1GA	1MBDP	1MB
2-	2GADP	2GA	2MBDP	2MB
3-	3GADP	3GA	3MBDP	3MB
4-	4GADP	4GA	4MBDP	4MB
6-	6GADP	6GA	6MBDP	6MB
2,3-	23GADP	ZGA	23MBDP	ZMB
2,4-	24GADP	YGA	24MBDP	YMB
2,6-	26GADP	XGA	26MBDP	XMB
3,4-	34GADP	WGA	34MBDP	WMB
3,6-	36GADP	VGA	36MBDP	VMB
4,6-	46GADP	UGA	46MBDP	UMB
2,3,4-	234GADP	TGA	234MBDP	TMB
2,3,6-	236GADP	SGA	236MBDP	SMB
2,4,6-	246GADP	RGA	246MBDP	RMB
3,4,6-	346GADP	QGA	346MBDP	QMB
2,3,4,6-	2346GADP	PGA	2346MBDP	PMB

Linkage position and anomeric configuration in L-hexopyranoses				
	α-L-Glucose		*β-L-Mannose*	
Linkage Position	*Prep File Prefix*	*Residue Name*	*Prep File Prefix*	*Residue Name*
Terminal	1GALP	G1A	1MBLP	M1B
2-	2GALP	G2A	2MBLP	M2B
3-	3GALP	G3A	3MBLP	M3B
4-	4GALP	G4A	4MBLP	M4B
6-	6GALP	G6A	6MBLP	M6B
2,3-	23GALP	GZA	23MBLP	MZB
2,4-	24GALP	GYA	24MBLP	MYB
2,6-	26GALP	GXA	26MBLP	MXB
3,4-	34GALP	GWA	34MBLP	MWB
3,6-	36GALP	GVA	36MBLP	MVB
4,6-	46GALP	GUA	46MBLP	MUB
2,3,4-	234GALP	GTA	234MBLP	MTB
2,3,6-	236GALP	GSA	236MBLP	MSB
2,4,6-	246GALP	GRA	246MBLP	MRB
3,4,6-	346GALP	GQA	346MBLP	MQB
2,3,4,6-	2346GALP	GPA	2346MBLP	MPB

A = α, B = β; The D-configuration (mirror image of the L-) is indicated in the three letter residue name by reversing the first two letters.

Linkage position and anomeric configuration in D-pentofuranoses				
	α-D-Arabinose		*β-D-Xylose*	
Linkage Position	*Prep File Prefix*	*Residue Name*	*Prep File Prefix*	*Residue Name*
Terminal	1AADF	1AD	1XBDF	1XU
2-	2AADF	2AD	2XBDF	2XU
3-	3AADF	3AD	3XBDF	3XU
4-	4AADF	4AD	4XBDF	4XU
2,3-	23AADF	ZAD	23XBDF	ZXU
2,4-	24AADF	YAD	24XBDF	YXU
3,4-	34AADF	WAD	34XBDF	WXU
2,3,4-	234AADF	TAD	234XBDF	TXU

Linkage position and anomeric configuration in L-pentofuranoses				
	α-L-Arabinose		*β-L-Xylose*	
Linkage Position	*Prep File Prefix*	*Residue Name*	*Prep File Prefix*	*Residue Name*
Terminal	1AALF	A1D	1XBLF	X1U
2-	2AALF	A2D	2XBLF	X2U
3-	3AALF	A3D	3XBLF	X3U
4-	4AALF	A4D	4XBLF	X4U
2,3-	23AALF	AZD	23XBLF	XZU
2,4-	24AALF	AYD	24XBLF	XYU
3,4-	34AALF	AWD	34XBLF	XWU
2,3,4-	234AALF	ATD	234XBLF	XTU

Linkage position in exceptional cases			
Carbohydrate	*Linkage Position*	*Prep File Prefix*	*Residue Name*
α-D-Sialic Acid	Terminal	1SA	1SA
	7-	7SA	7SA
	8-	8SA	8SA
	9-	9SA	9SA
α-D-N-Acetylglucosamine	Terminal	YA	YA
	3-	3YA	3YA
	4-	4YA	4YA
	6-	6YA	6YA
	3,4-	34YA	WYAa
	3,6-	36YA	VYA
	4,6-	46YA	UYA
	3,4,6-	346YA	RYA

(The single letter specification of linkage position follows the pattern of the earlier tables.)

2.9.3. Building a polysaccharide in LEaP

Here is an example of how to construct a somewhat complex polysaccharide, using the *glycam04* parameters:

```
logFile leap.log
#
#
#          Making Branched Carbohydrates
#
# In general, the head atom is set to C1 in each sugar, where the tail atom
# varies depending on the sugar linkage.  For all sugars, the tail atom is
# specified as the glycosidic oxygen with the largest atomid, i.e. for the
# 2,3,4, linked sugars the tail atoms would be O4.  Therefore, it it possible
# to build the longest chain according to this convention and then connect
# the branched portions.  Simply changing the tail atom enables a different
# connection point for the unit, which can then be utilized to sequence to
# any branched residue/s.
#
#
# Example: Man9
#
# Mana1 - 2Mana1 \
#                   3
#                    Mana1
#                   6      \
# Mana1 - 2Mana1 /           6
#                             ManB-OMe
#                            3
# Mana1 - 2Mana1 - 2Mana1 /
#
#
# First, we must load the necessary parameters and prep files
source leaprc.glycam04

# Find the longest chain and use the sequence command to build it
# (See the manual for naming conventions)
part1 = sequence { OME VMB VMA 2MA 1MA }

# Now set the tail atom of part1 to O3 of VMB
set part1 tail part1.2.9

# Join the first branch to the long chain
part2 = sequence { part1 VMA 2MA 1MA }

# Set the tail atom of part2 to O3 of VMA
set part2 tail part2.6.19

# Add the last branch
man9 = sequence { part2 2MA 1MA }
```

```
# The basic structure has been built, but it clearly does not have the optimal
# glycosidic torsion angles.
#
# Set the psi(1-6) torsion to 180 (C1-O6-C6-C5)
impose man9 { 2 3 4 } { { C5 C6 O6 C1 180.0 } }

# Set the omega angle of 6-linked sugars to 60.0 (O5-C5-C6-O6)
impose man9 { 2 3 }  {{ O6 C6 C5 O5 60.0 } }

# Set the phi angle of all a-linked (not 1-6 linked) sugars to -60.0
impose man9 { 4 5 6 7 8 9 10 } { { H1 C1 O2 C2 -60.0 } }
impose man9 { 6 2 } { { H1 C1 O3 C3 -60.0 } }
impose man9 { 9 3 } { { H1 C1 O3 C3 -60.0 } }

# Set the psi angle of all sugars to 0.0
impose man9 { 4 5 6 7 8 9 10 } { { C1 O2 C2 H2 0.0 } }
impose man9 { 6 2 } { { C1 O3 C3 H3 0.0 } }
impose man9 { 9 3 } { { C1 O3 C3 H3 0.0 } }

# Now we have a reasonable starting structure for AMBER min/md, but we need
# to save a topology and coordinate file as well as a pdb for later use.
saveamberparm man9 man9.top man9.crd
savepdb man9 man9.pdb
quit

# For more info visit the Glycam website www.glycam.ccrc.uga.edu
```

2.10. Ions

For alkali ions with TIP3P waters, we have provided the values of Åqvist [45], which are adjusted for Amber's nonbonded atom pair combining rules to give the same ion-OW potentials as in the original (which were designed for SPC water); these values reproduce the first peak of the radial distribution for ion-OW and the relative free energies of solvation in water of the various ions. Note that these values would have to be changed if a water model other than TIP3P were to be used. These potentials may also need modification if absolute free energies of solvation are important [46].

2.11. Solvent models

Amber now provides direct support for several water models. The default water model is TIP3P [47]. This model will be used for residues with names HOH or WAT. If you want to use other water models, execute the following leap commands after loading your leaprc file:

```
WAT = PL3                       (residues named WAT in pdb file will be POL3)
loadAmberParams frcmod.pol3     (sets the HW,OW parameters to POL3)
```

(The above is obviously for the POL3 model.) The *solvents.lib* file contains TIP3P [47], TIP4P [47,48], TIP5P [49], POL3 [50] and SPC/E [51] models for water; these are called TP3, T4P, T5P, PL3 and SPC, respectively. By default, the residue name in the *prmtop* file will be WAT, regardless of which water model is used. If you want to change this (for example, to keep track of

which water model you are using), you can change the residue name to whatever you like. For example,

```
WAT = TP4
set WAT.1 name "TP4"
```

would make a special label in PDB and *prtmop* files for TIP4P water. Note that Brookhaven format files allow at most three characters for the residue label.

In addition, non-polarizable models for the organic solvents methanol, chloroform and N-methylacetamide are provided, along with a box for an 8M urea-water mixture. The input files for a single molecule are in *amber9/dat/leap/prep*, and the corresponding frcmod files are in *amber9/dat/leap/parm*. Pre-equilibrated boxes are in *amber9/dat/leap/lib*. For example, to solvate a simple peptide in methanol, you could do the following:

```
source leaprc.ff99                      (get a standard force field)
loadAmberParams frcmod.meoh             (get methanol parameters)
peptide = sequence { ACE VAL NME }      (construct a simple peptide)
solvateBox peptide MEOHBOX 12.0 0.8     (solvate the peptide with meoh)
saveAmberParm peptide prmtop prmcrd
quit
```

Similar commands will work for other solvent models.

3. LEaP

3.1. Introduction

LEaP is the generic name given to the programs teLeap and xaLeap, which are generally run *via* the *tleap* and *xleap* shell scripts. These two programs share a common command language but the xleap program has been enhanced through the addition of an X-windows graphical user interface. The name LEaP is an acronym constructed from the names of the older AMBER software modules it replaces: link, edit, and parm. Thus, LEaP can be used to prepare input for the AMBER molecular mechanics programs.

Both *tleap* and *xleap* are written in ANSI C; the former does not support graphics and therefore, it will run in a text window or from a script. The *xleap* script is meant to run on any machine that supports X-windows (Version 11 Revision 4 and latter versions); it does all of its graphics manipulations in generic X-windows. It does not depend on any system-dependent graphics to do 3D transformations or page-flipping. All of the user interface was written using David E. Smyth's Widget Creation Library (Wcl-1.05). This library is included in the LEaP distribution, as is the Xraw 3D widget set by Vladimir Romanovski (modeled on the ATHENA 3D widget set by Kaleb Keithley).

Using *tleap*, the user can:

```
Read AMBER PREP input files
Read AMBER PARM format parameter sets
Read and write Object File Format files (OFF)
Read and write PDB files
Read single residue Mol2 files
Construct new residues and molecules using simple commands
Link together residues and create nonbonded complexes of molecules
Place counterions around a molecule
Solvate molecules in arbitrary solvents
Modify internal coordinates within a molecule
Generate files that contain topology and parameters for AMBER.
```

In addition, with *xleap* the user can:

```
Access commands using a simple point and click interface
Draw new residues and molecules in a graphical environment
View structures graphically
Graphically dock molecules
Modify the properties of atoms, residues, and molecules using a
     spreadsheet editor
Input or alter molecular mechanics parameters using a spreadsheet editor.
```

3.2. Concepts

In order to effectively use LEaP it is necessary to understand the philosophy behind the program, especially the concepts of LEaP *commands*, *variables*, and *objects*. In addition to exploring

these concepts, this section also addresses the use of external files and libraries with the program.

3.2.1. Commands

The heart of LEaP is a command-line interface that accepts text commands which direct the program to perform operations on objects. All LEaP commands have one of the following two forms:

```
command argument1 argument2 argument3 ...
variable = command argument1 argument2 ...
```

For example:

```
edit ALA
trypsin = loadPdb trypsin.pdb
```

Each command is followed by zero or more arguments that are separated by whitespace. Some commands return objects which are then associated with a variable using an assignment (=) statement. Each command acts upon its arguments, and some of the commands modify their arguments' contents. The commands themselves are case- insensitive.

The arguments in the command text may be *objects* such as NUMBERs, STRINGs, or LISTs or they may be *variables*. These two subjects are discussed next.

3.2.2. Variables

A *variable* is a handle for accessing an object. A variable name can be any alphanumeric string whose first character is an alphabetic character. (Alphanumeric means that the characters of the name may be letters, numbers, or special symbols such as "*". LEaP commands should not be used as variable names. Variable names are case-sensitive: "ARG" and "arg" are different variables. Variables are associated with objects using an assignment statement not unlike regular computer languages such as Fortran or C.

```
mole = 6.02E23
MOLE = 6.02E23
myName = "Joe Smith"
listOf7Numbers = { 1.2 2.3 3.4 4.5 6 7 8 }
```

LEaP maintains a list of variables that are currently defined and this list can be displayed using the `list` command. The contents of a variable can be printed using the `desc` command.

3.2.3. Objects

The *object* is the fundamental entity in LEaP. Objects range from the simple objects NUMBERs and STRINGs to the complex objects UNITs, RESIDUEs, and ATOMs. Complex objects have properties that can be altered using the `set` command and some complex objects can contain other objects. For example, RESIDUEs are complex objects that can contain ATOMs and have the properties: residue name, connect atoms, and residue type.

NUMBERs are simple objects and they are identical to double precision variables in Fortran and double variables in C.

STRINGs are simple objects that are identical to character arrays in C and similar to character strings in Fortran. STRINGs are represented by sequences of characters which may be delimited by double quote characters. Example strings are:

```
"Hello there"
"String with a "" (quote) character"
"Strings contain letters and numbers:1231232"
```

LISTs are composed of sequences of other objects delimited by LIST open and close characters. The LIST open character is an open curly bracket ({) and the LIST close character is a close curly bracket (}). LISTs can contain other LISTs and be nested arbitrarily deep. Example LISTs are:

```
{ 1 2 3 4 }
{ 1.2 "string" }
{ 1 2 3 { 1 2 } { 3 4 } }
```

LISTs are used by many commands to provide a more flexible way of passing data to the commands. The `zMatrix` command has two arguments, one of which is a LIST of LISTs where each subLIST contains between three and eight objects.

PARMSETs are objects that contain bond, angle, torsion, and nonbond parameters for AMBER force field calculations. They are normally loaded from *e.g.* `parm94.dat` and `frcmod` files.

ATOMs are complex objects that do not contain any other objects. The ATOM object is similar to the chemical concept of atoms. Thus, it is a single entity that may be bonded to other ATOMs and it may be used as a building block for creating molecules. ATOMs have many properties that can be changed using the `set` command. These properties are defined below.

`name`

This is a case-sensitive STRING property and it is the ATOM's name. The `name`s for all ATOMs in a RESIDUE should be unique. The `name` has no relevance to molecular mechanics force field parameters; it is chosen arbitrarily as a means to identify ATOMs. Ideally, the `name` should correspond to the PDB standard, being 3 characters long except for hydrogens, which can have an extra digit as a 4th character.

`type`

This is a STRING property. It defines the AMBER force field atom type. It is important that the character case match the canonical type definition used in the appropriate "parm.dat" or "frcmod" file. For smooth operation, all atom types need to have element and hybridization defined by the `addAtomTypes` command. The standard AMBER force field atom types are added by the default "leaprc" file.

`charge`

The `charge` property is a NUMBER that represents the ATOM's electrostatic point charge to be used in a molecular mechanics force field.

`element`

The atomic element provides a simpler description of the atom than the `type`, and is used only for LEaP's internal purposes (typically when force field information is not available). The element names correspond to standard nomenclature; the character "?" is used for special cases.

`position`

This property is a LIST of NUMBERs. The LIST must contain three values: the (X, Y, Z) Cartesian coordinates of the ATOM.

RESIDUEs are complex objects that contain ATOMs. RESIDUEs are collections of ATOMs, and are either molecules (e.g. formaldehyde) or are linked together to form molecules (e.g. amino acid monomers). RESIDUEs have several properties that can be changed using the `set` command. (Note that database RESIDUEs are each contained within a UNIT having the same name; the residue GLY is referred to as GLY.1 when setting properties. When two of these single-UNIT residues are joined, the result is a single UNIT containing the two RESIDUEs.)

One property of RESIDUEs is connection ATOMs. Connection ATOMs are ATOMs that are used to make linkages between RESIDUEs. For example, in order to create a protein, the N-terminus of one amino acid residue must be linked to the C-terminus of the next residue. This linkage can be made within LEaP by setting the N ATOM to be a connection ATOM at the N-terminus and the C ATOM to be a connection ATOM at the C-terminus. As another example, two CYX amino acid residues may form a disulfide bridge by crosslinking a connection atom on each residue.

There are several properties of RESIDUEs that can be modified using the `set` command. The properties are described below:

`connect0`

This defines an ATOM that is used in making links to other RESIDUEs. In UNITs containing single RESIDUEs, the RESIDUE's `connect0` ATOM is usually defined as the UNIT's `head` ATOM. (This is how the standard library UNITs are defined.) For amino acids, the convention is to make the N-terminal nitrogen the `connect0` ATOM.

`connect1` This defines an ATOM that is used in making links to other RESIDUEs. In UNITs containing single RESIDUEs, the RESIDUE's `connect1` ATOM is usually defined as the UNIT's `tail` ATOM. (This is done in the standard library UNITs.) For amino acids, the convention is to make the C-terminal oxygen the `connect1` ATOM.

`connect2` This is an ATOM property which defines an ATOM that can be used in making links to other RESIDUEs. In amino acids, the convention is that this is the ATOM to which disulfide bridges are made.

`restype` This property is a STRING that represents the type of the RESIDUE. Currently, it can have one of the following values: `"undefined"`, `"solvent"`, `"protein"`, `"nucleic"`, or `"saccharide"`. Some of the LEaP commands behave in different ways depending on the type of a residue. For example, the solvate commands require that the solvent residues be of type `"solvent"`. It is important that the proper character case be used when defining this property.

`name` The RESIDUE name is a STRING property. It is important that the proper character case be used when defining this property.

UNITs are the most complex objects within LEaP, and the most important. UNITs, when paired with one or more PARMSETs, contain all of the information required to perform a calculation using AMBER. UNITs have the following properties which can be changed using the `set` command:

`head`

`tail` These define the ATOMs within the UNIT that are connected when UNITs are joined together using the `sequence` command or when UNITs are joined together with the PDB or PREP file reading commands. The `tail` ATOM of one UNIT is connected to the `head` ATOM of the next UNIT in any sequence. (Note: a "TER card" in a PDB file causes a new UNIT to be started.)

`box` This property can either be `null`, a NUMBER, or a LIST. The property defines the bounding box of the UNIT. If it is defined as `null` then no bounding box is defined. If the value is a single NUMBER then the bounding box will be defined to be a cube with each side being NUMBER of angstroms across. If the value is a LIST then it must be a LIST containing three numbers, the lengths of the three sides of the bounding box.

`cap` This property can either be `null` or a LIST. The property defines the solvent cap of the UNIT. If it is defined as `null` then no solvent cap is defined. If the value is a LIST then it must contain four numbers, the first three define the Cartesian coordinates (X, Y, Z) of the origin of the solvent cap in angstroms, the fourth NUMBER defines the radius of the solvent cap in angstroms.

Examples of setting the above properties are:

```
set dipeptide head dipeptide.1.N
set dipeptide box { 5.0 10.0 15.0 }
set dipeptide cap { 15.0 10.0 5.0 8.0 }
```

The first example makes the amide nitrogen in the first RESIDUE within "dipeptide" the `head` ATOM. The second example places a rectangular bounding box around the origin with the (X, Y, Z) dimensions of (5.0, 10.0, 15.0) in angstroms. The third example defines a solvent cap centered at (15.0, 10.0, 5.0) angstroms with a radius of 8.0 Å. **Note:** the "set cap" command does not actually solvate, it just sets an attribute. See the `solvateCap` command for a more practical case.

UNITs are complex objects that can contain RESIDUEs and ATOMs. UNITs can be created using the `createUnit` command and modified using the `set` commands. The contents of a UNIT can be modified using the `add` and `remove` commands. There is a loose hierarchy of complex objects and what they are allowed to contain. The hierarchy is as follows:

- UNITs can contain RESIDUEs and ATOMs.
- RESIDUEs can contain ATOMs.

The hierarchy is loose because it does not forbid UNITs from containing ATOMs directly. However, the convention that has evolved within LEaP is to have UNITs directly contain RESIDUEs which directly contain ATOMs.

Objects that are contained within other objects can be accessed using dot "." notation. An example would be a UNIT which describes a dipeptide ALA-PHE. The UNIT contains two RESIDUEs each of which contain several ATOMs. If the UNIT is referenced (named) by the variable `dipeptide`, then the RESIDUE named ALA can be accessed in two ways. The user may type one of the following commands to display the contents of the RESIDUE:

```
desc dipeptide.ALA
desc dipeptide.1
```

The first translates to "some RESIDUE named ALA within the UNIT named dipeptide". The second form translates as "the RESIDUE with sequence number 1 within the UNIT named dipeptide". The second form is more useful because every subobject within an object is guaranteed to have a unique sequence number. If the first form is used and there is more than one RESIDUE with the name ALA, then an arbitrary residue with the name ALA is returned. To access ATOMs within RESIDUEs, the notation to use is as follows:

```
desc dipeptide.1.CA
desc dipeptide.1.3
```

Assuming that the ATOM with the name CA has a sequence number 3, then both of the above commands will print a description of the $alpha$−carbon of RESIDUE dipeptide.ALA or dipeptide.1. The reader should keep in mind that dipeptide.1.CA is the ATOM, an object, contained within the RESIDUE named ALA within the variable dipeptide. This means that dipeptide.1.CA can be used as an argument to any command that requires an ATOM as an argument. However dipeptide.1.CA is not a variable and cannot be used on the left hand side of an assignment statement.

In order to further illustrate the concepts of UNITs, RESIDUEs, and ATOMs, we can examine the log file from a LEaP session. Part of this log file is printed below.

```
> loadOff all_amino94.lib
> desc GLY
UNIT name: GLY
Head atom: .R<GLY 1>.A<N 1>
Tail atom: .R<GLY 1>.A<C 6>
Contents:
R<GLY 1>
> desc GLY.1
RESIDUE name: GLY
RESIDUE sequence number: 1
RESIDUE PDB sequence number: 0
Type: protein
Connection atoms:
 Connect atom 0: A<N 1>
 Connect atom 1: A<C 6>
Contents:
A<N 1>
A<HN 2>
A<CA 3>
A<HA2 4>
A<HA3 5>
A<C 6>
A<O 7>
> desc GLY.1.3
ATOM
Normal                  Perturbed
Name:    CA             CA
Type:    CT             CT
```

```
Charge:  -0.025        0.000
Element: C             (not affected by pert)
Atom position: 3.970048, 2.845795, 0.000000
Atom velocity: 0.000000, 0.000000, 0.000000
  Bonded to .R<GLY 1>.A<N 1> by a single bond.
  Bonded to .R<GLY 1>.A<HA2 4> by a single bond.
  Bonded to .R<GLY 1>.A<HA3 5> by a single bond.
  Bonded to .R<GLY 1>.A<C 6> by a single bond.
```

In this example, command lines are prefaced by ">" and the LEaP program output has no such character preface. The first command,

```
> loadOff all_amino94.lib
```

loads an OFF library containing amino acids. The second command,

```
> desc GLY
```

allows us to examine the contents of the amino acid UNIT, GLY. The UNIT contains one RESIDUE which is named GLY and this RESIDUE is the first residue in the UNIT (`R<GLY 1>`). In fact, it is also the only RESIDUE in the UNIT. The `head` and `tail` ATOMs of the UNIT are defined as the N- and C-termini, respectively. The `box` and `cap` UNIT properties are defined as `null`. If these latter two properties had values other than `null`, the information would have been included in the output of the `desc` command.

The next command line in the session,

```
> desc GLY.1
```

enables us to examine the first residue in the GLY UNIT. This RESIDUE is named GLY and its residue type is that of a `protein`. The `connect0` ATOM (N) is the same as the UNITs' `head` ATOM and the `connect1` ATOM (C) is the same as the UNITs' `tail` ATOM. There are seven ATOM objects contained within the RESIDUE GLY in the UNIT GLY.

Finally, let us look at one of the ATOMs in the GLY RESIDUE.

```
> desc GLY.1.3
```

The ATOM has a name (CA) that is unique among the atoms of the residue. The AMBER force field atom type for CA is CT. The type of element, atomic point charge, and Cartesian coordinates for this ATOM have been defined along with its bonding attributes. Other force field parameters, such as the van der Waals well depth, are obtained from PARMSETs.

3.3. Starting LEaP

```
% xleap [-h] [-I dir] [-f file] [-s]
```

```
% tleap [-h] [-I dir] [-f file] [-s]
```

The user may enter several options when starting the LEaP program. If the option "`-h`" is used (e.g., `xleap -h`), then the program will print a list of start-up options and then exit. A directory may be added to the program's search path by using the option: "`-I dir`". This will cause the program to search `dir` whenever a file is requested. If the user would like to execute LEaP commands at start-up, they should use the option: "`-f file`". Finally, if the user enters the command option "`-s`", the "leaprc" file will not be executed at start-up.

A file called "leaprc" is executed as a script file at the start of the LEaP session unless the user suppresses it with a command line option. Sample files are in *$AMBERHOME/dat/leap/cmd*, and you may wish to copy one of these to become "your" default file. LEaP will look first for a *learpc* file in the user's current directory, then in any directories included with `-I` flags.

3.3.1. Verbosity

The `verbosity` command is used to control how much output LEaP displays to the user. A verbosity level of `0` tells LEaP to print the minimum amount of information. A verbosity level of `1` tells LEaP to print all information it can, and a verbosity level of `2` tells LEaP to print all information and to display each line read from source files executed using the `source` command.

3.3.2. Log File

The command line interface allows the user to specify a log file that is used to log all input and output within the command line environment. The log file is named using the `logFile` command. The file has two purposes: to allow the user to see a complete record of operations performed by LEaP, and to help recover from (and recreate) program crashes. Output from LEaP commands is written to the log file at a verbosity level of `2` regardless of the verbosity level set by the user using the `verbosity` command. Each line in the log file that was typed in by the user begins with the two characters "`> `" (a greater-than sign followed by a space). This allows the user to extract the commands typed into LEaP from the log file to create a script file that can be executed using the `source` command. This provides a type of insurance against program crashes by allowing the user to regenerate their interactive sessions. An example of a command that works on UNIX systems and that will create a script to reenact a LEaP session is:

```
% cat LOGFILE | grep "^> " | sed "s/^> //" > SOURCEFILE.x
```

Note that changes via graphical and table interfaces (xleap) are not captured by command-line traces.

3.4. Using LEaP

In this section, we describe how to use the tleap and xleap user interfaces. Strategies for using LEaP in research are discussed in the subsequent section on using LEaP with AMBER.

tleap (terminal LEaP) is the non-graphical, command-line-only interface to LEaP. It has the same functionality as the *xleap* main window (Universe Editor Command Window, described below), and uses standard text control keys.

xleap is a windowing interface to LEaP. In addition to the command-line interface contained in the Universe Editor window, it has a Unit Editor (graphical molecule editor), an Atom

Properties Editor, and a Parmset Editor. These editors are discussed in subsequent subsections.

3.4.1. Universe Editor

The window that first appears when the user starts xleap is called the Universe Editor. The Universe Editor is the most basic way in which users can interact with xleap. It has two parts, the "command window," which corresponds to the tleap command interface, and the "pulldown" items above the window, which provide mouse-driven methods to generate specific commands for the command window, either directly or via popped-up dialog boxes.

The items in the pulldowns allow the user to generate commands using dialog boxes. To display the "File" pulldown, for example, press the left mouse button on "File;" to select an item in the pulldown, keep the button down, move the mouse to highlight the item, then release the mouse button. A dialog box will then pop up containing fields which the user can fill in, and lists from which values can be chosen; these will be used to generate commands for the command window interface.

3.4.2. Unit Editor

When the user enters the `edit` command from the Universe Editor Command Window, the Unit Editor will be displayed if the argument to the `edit` command is an existing UNIT or a nonexistent (i.e. new) object. The Parmset Editor will be activated if the argument is a PARMSET. The Parmset Editor is discussed later in this subsection.

The Unit Editor has five parts. At the top of the window is a pulldown menu bar; below it is a set of buttons titled "Manipulation" that define the mode of mouse activity in the graphics window, and below that, a list of elements to select for the manipulation "Draw" mode (selecting one automatically selects "Draw" mode). Then comes the graphical molecule-editing ("viewing") window itself, and at the very bottom a text window where status and errors are reported.

3.4.2.1. Unit Editor Menu Bar

The menu bar has three pulldowns: "Unit," "Edit," and "Display."

`Unit` The Unit pulldown contains commands affecting the whole UNIT.

"Check unit" − checks the UNIT in the viewing window for improbable bond lengths, missing force field atom types, close nonbonded contacts, and a non-integral and non-zero total charge. Information is printed in the text window at the bottom of the Unit Editor.

"Calculate charge" − the total electrostatic charge for the UNIT is displayed in the text window at the bottom of the Unit Editor.

"Build," "Add H & Build" − the coordinates of new atoms are adjusted according to hybridization (inferred from bonds) and standard geometries. (See also the `Edit` pulldown's "Relax selection.) Newly-drawn ATOMs are marked as "unbuilt" until they are marked otherwise by one of the Build commands or by the `Edit` pulldown's "Mark selection (un)built." The builder *only* builds coordinates for unbuilt ATOMs. This allows users to draw molecules piecemeal and make adjustments as they draw, without worrying that the builder is going to undo their work. "Add H & Build" adds hydrogens to the ATOMs that do not have a full valence and builds coordinates for the hydrogens and any other ATOMs that are marked "unbuilt." The number of hydrogens added to each ATOM is determined by the hybridization and element type of each ATOM.

"Import unit" − a selection window pops up for the user to incorporate a copy of another unit in the current one. The imported unit will generally superimpose on the existing one. (Hint: select all atoms in the current unit before doing this to simplify dragging them apart using the Manipulation `Move` mode.)

"Close" − Exit the Editor.

`Edit` The Edit pulldown contains commands relating to the currently- selected ATOMs in the viewer window. Selection is described below in the "Manipulation buttons" section.

"Relax selection" − performs a limited energy minimization of all selected ATOMs, leaving unselected ATOMs fixed in place, by relaxing strained bonds, angles, and torsions. If atom types have been assigned and can be found in the currently-loaded force field, force field parameters are used. If no types are available then default parameters are used that are based on ATOM hybridization. This command invokes an iterative algorithm that can take some time to converge for large systems. As the algorithm proceeds, the modified UNIT will be continuously updated within the viewing window. The user can stop the process at any time by placing the mouse pointer within the viewing window and typing `control-C`. Since only internal coordinates are energy minimized, steric overlap can result.

"Edit selected atoms" − pops up an Atom Properties Editor, a tool for examining/setting the properties of the selected ATOMs. The Atom Properties Editor allows the user to edit the ATOM names, types and charges in a convenient table format. It is described in a separate subsection below.

"Flip chirality" This command inverts the chirality of all selected ATOMs. In order for the chirality to be inverted, the ATOM cannot be in more than one ring. The operation causes the lightest chains leaving the ATOM to be moved so as to invert the chirality. If the ATOM has only three chains attached to it, then only one of the chains will be moved. **Note:** this command is rather apt to crash LEaP.

"Select Rings/Residues/Molecules" − expands the currently selected group of atoms to include all partially-contained rings, residues, or molecules.

"Show everything" − causes all ATOMs to become visible.

"Hide selection" − makes all selected ATOMs invisible.

"Show selection only" − makes only selected ATOMs visible.

"Mark selection unbuilt/built" − see "Unit/Build," above.

`Display` The Display pulldown contains commands that determine what information is displayed within the viewing window.

"Names" − toggles display of ATOM names at each ATOM position.

"Types" − toggles display of molecular mechanics atom types. The ATOM types are displayed within parentheses "()".

"Charges" − toggles display of the atomic charges.

"Residue names" − toggles display of residue names. These are displayed at the position of the first ATOM, before any of that ATOM's information that may be displayed. The residue names are displayed within angled brackets "<>".

"Axes" − toggles display of the Cartesian coordinate axes. The origin of the axes coincides with the origin of Cartesian space.

"Periodic box" − toggles display of the periodic box, if the UNIT has one.

3.4.2.2. Unit Editor Manipulation Buttons

The Manipulation buttons are Select, Twist, Move, Erase, and Draw. They determine the behavior of the mouse left-button when the mouse pointer is in the Viewing Window.

`Select` This button allows one to select part or all of a UNIT in anticipation of a subsequent operation or action. In the `Select` mode, the user can highlight ATOMs within the viewing window for special operations. The mouse pointer becomes a pointing hand in the viewing window in this mode. Selected ATOMs are displayed in a different color (or different line styles on monochrome systems) from all other ATOMs. Atoms can be selected with the left-button in several ways: first, clicking on an atom and releasing selects that atom. Clicking twice in a row on an atom (at any speed) selects all atoms (this is a bug - only the residue should be selected). Keeping the button down and moving to release on another atom selects all ATOMs in the shortest chain between the two ATOMs, if such a chain exists. Finally, by first pressing the button in empty space, and holding it down as the mouse is moved, one can "drag a box" enclosing atoms of interest. Note that a current selection can be expanded by using the "Edit" menubar pulldown select option to complete any partial selection of rings, residues or molecules.

If the user holds down the SHIFT key while performing any of the above actions, the same effect will be seen, except ATOMs will be unselected.

`Twist` `Twist` mode operates on previously-`Select`ed atoms. The intention is to allow rotation about dihedrals; if too many atoms are selected, odd transformations can occur. While in the `Twist` mode, the mouse pointer looks like a curved arrow. Twisting is driven by holding down the left-button anywhere in the viewing window and moving the mouse up and down. It is important to select a complete torsion (all four atoms) before trying to "twist" it.

`Move` Like `Twist`, `Move` mode operates on previously-`Select`ed atoms. While in the `Move` mode, the mouse pointer looks like four arrows coming out of one central point. Holding down the left-button anywhere allows movement of these atoms by dragging in any direction in the viewing plane. (The view can be rotated by holding down the middle-button to allow any movement desired.) This option allows the user to move the selected ATOMs relative to the unselected ATOMs.

To *rotate* the selected ATOMs relative to the unselected ones, press and drag the mode (left) button while holding down the SHIFT key. The selected ATOMs will rotate around a central ATOM on a "virtual sphere" (see the subsubsection below on the rotate (middle) button for more information on the "virtual sphere"). The user can change which ATOM is used as the center of rotation by clicking the mode (left) button on any of the ATOMs in the window.

`Erase` `Erase` mode causes the mouse pointer to resemble a chalkboard eraser when it is in the viewing window. Clicking the left-button will delete any atoms or bonds under this mouse pointer, one atom or bond per click.

`Draw` Choosing `Draw` is equivalent to choosing the default "Elements" atom in the next array of buttons; the initial default is carbon. While in the `Draw` mode, the

mouse pointer is a pencil when in the viewing window. Clicking the left-button deposits an atom of the current element, while dragging the mouse pointer with the left-button held down draws a bond: if no atom is found where the button is released, one is created.

When the mouse pointer approaches an ATOM, the end of the line connected to the pointer will "snap" to the nearest ATOM. This is to facilitate drawing of bonds between ATOMs. Any bonds that are drawn will by default be single bonds. To change the order of a bond, the user would move the mouse to any point along the bond and click the mode (left) button. This will cause the order of the bond to increase until it is reset back to a single bond. The user can cycle through the following bond order choices: single, double, triple, and aromatic.

If the user rotates a structure as it is being drawn, she will notice that all of the ATOMs that have been drawn lie in the same plane. New ATOMs are automatically placed in the plane of the screen. The fact that LEaP places the new ATOMs in the same plane is not a handicap because once a rough sketch of part of the structure is compete, the user can invoke one of LEaP's two model building facilities ("Unit/Build" and "Edit/Relax Selection" in the Unit Editor Menu bar) to build full three dimensional coordinates.

3.4.2.3. Unit Editor Elements Buttons

`C, H, O, ...`

These buttons put the viewing window in `Draw` mode if it is not in that mode already, and select the drawing element. The more common elements have their own buttons, and all elements are also found by pulling down the `other elements` button.

3.4.2.4. Unit Editor Viewing Window

The viewing window displays a projection of the UNIT currently being edited. The user can manipulate the structure within the viewing window with the mouse. By moving the mouse and holding down the mouse buttons, the user can rotate, scale, and translate the UNIT within the window. The functions attached to the mouse buttons are:

`Rotate (Middle button)`

By pressing the rotate (middle) button within the viewing window and dragging the mouse, the user can rotate the UNIT around the center of the viewing window. While the rotate (middle) button is down, a circle appears within the viewing window, representing a "virtual sphere trackball." As the user drags the mouse around the outside of the circle, the UNIT will spin around the axis normal to the screen. As the user drags the mouse within the circle, the UNIT will spin around the axis in the screen, perpendicular to the movement of the mouse. The structures that are being viewed can be considered to be embedded within a sphere of glass. The circle is the projection of the edge of the sphere onto the screen. Rotating a UNIT while the mouse is within the circle is akin to placing a hand on a glass sphere and turning the sphere by pulling the hand. The rotate operation does not modify the coordinates of the ATOMs; rather, it simply changes the user's point of view.

`Translate (Right button)`

By pressing the translate (right) button within the viewing window and dragging

the mouse around the viewing window, the user can translate the UNIT within the plane of the screen. The structures will follow the mouse as it moves around the window. This operation does not modify the coordinates of the UNIT.

`Scale (middle plus right button)`
: If the scale "button" (holding the middle and right buttons down at the same time) is depressed, the user will change the size of the structures within the viewing window. Pressing the scale (middle plus right) button and dragging the mouse up and down the screen will increase and decrease the scale of the structures. This operation does not modify the coordinates of the UNIT.

`Mode button (left button) and the viewing window mode`
: The function of the left button is determined by the current mode of the viewing window as described in the "Manipulation" section, above. When the mouse enters the viewing window it changes shape to reflect the current mode of the viewing window.

`Spacebar`
: Another always-available operation when the mouse pointer is in the viewing window is the keyboard spacebar. It centers and normalizes the size of the molecule in the viewing window. This is especially useful if the UNIT becomes "lost" due to some operation.

The functions of the middle and right buttons are fixed and always available to the user. This allows the user to change the viewpoint of the UNIT within the viewing window regardless of its current mode. The user might ask why there are controls to translate in the plane of the screen, but not out of the plane of the screen. This is because LEaP does not have depth-cueing or stereo projection and this makes it difficult for users to perceive changes in the depth of a structure. However, the user can rotate the entire UNIT by 90 degrees which will orient everything so that the direction that was coming out of the screen becomes a direction lying in the plane of the screen. Once the UNIT has been rotated using the rotate (middle) button, the user can translate the structure anywhere in space. While it does take some getting used to, users can become very adept at the combination of rotations and translations.

3.4.3. Atom Properties Editor

The Atom Properties Editor is popped up by the Unit Editor when the user selects the `Edit selected atoms` command from the `Edit` pulldown. The Atom Properties Editor allows the user to edit the properties of ATOMs using a convenient table format. ATOM properties are: name, type, charge, and element.

3.4.4. Parmset Editor

If the user enters the command `edit Foo` in the Universe Editor and `Foo` is a PARMSET, then a Parmset Editor is popped up. First, a window appears which contains a number of buttons. The buttons list the parameters that can be edited − Atom, Bond, Angle, Proper Torsion, Improper Torsion, and Hydrogen Bond − and an option to close the editor. Choosing one of the parameter buttons will pop up a Table Editor. This editor resembles that of the Atom Properties Editor, having three parts: the Menu Bar, Status Window, and Table Window.

3.5. Basic instructions for using LEaP with AMBER

This section gives an overview of how LEaP is most commonly used. Detailed descriptions of all the commands are given in the following section.

3.5.1. Building a Molecule For Molecular Mechanics

In order to prepare a molecule within LEaP for AMBER, three basic tasks need to be completed.

(1) Any needed UNIT or PARMSET objects must be loaded;

(2) The molecule must be constructed within LEaP;

(3) The user must output topology and coordinate files from LEaP to use in AMBER.

The most typical command sequence is the following:

```
source leaprc.ff94              load a force field
x = loadPdb trypsin.pdb         load in a structure
   ....                         add in cross-links, solvate, etc.
saveAmberParm x prmtop prmcrd   save files for sander and other programs
```

There are a number of variants of this:

(1) Although `loadPdb` is by far the most common way to enter a structure, one might use `loadOff`, `loadMol2`, or `loadAmberPrep`, or use the `zMatrix` command to build a molecule from a z-matrix. See the Commands section below for descriptions of these options. For situations where you do not have a starting structure (in the form of a pdb file) LEaP can be used to build the molecule; you may find, however, that this is not always as easy as it might be. Many experienced Amber users turn to other (commercial and non-commercial) programs to create their initial structures.

Be very attentive to any errors produced in the `loadPdb` step; these generally mean that LEaP has mis-read the file. A general rule of thumb is to keep editing your input pdb file until LEaP stops complaining. It is often convenient to use the `addPdbAtomMap` or `addPdbResMap` commands to make systematic changes from the names in your pdb files to those in the Amber topology files; see the *leaprc* files for examples of this. An unknown residue error message indicates that LEaP needs additional information. The antechamber chapter describes procedures for building LEaP input files for small organic molecules. The tutorials contain examples of creating and modifying residues.

3.5.2. Amino Acid Residues

The accompanying table shows the amino acid UNITs and their aliases as defined in the LEaP libraries.

For each of the amino acids found in the LEaP libraries, there has been created an N-terminal and a C-terminal analog. The N-terminal amino acid UNIT/RESIDUE names and aliases are prefaced by the letter N (e.g. NALA) and the C-terminal amino acids by the letter C (e.g. CALA}. If the user models a peptide or protein within LEaP, they may choose one of three ways to represent the terminal amino acids. The user may use 1) standard amino acids, 2) protecting groups (ACE/NME), or 3) the charged C- and N-terminal amino acid UNITs/RESIDUEs. If the standard amino acids are used for the terminal residues, then these residues will have incomplete valences. These three options are illustrated below:

Group or residue	Residue Name, Alias
Acetyl beginning group	ACE
Amine ending group	NHE
N-methylamine ending group	NME
Alanine	ALA
Arginine	ARG
Asparagine	ASN
Aspartic acid	ASP
Aspartic acid--protonated	ASH
Cysteine	CYS
Cysteine--deprotonated	CYM
Cystine, S--S crosslink	CYX
Glutamic acid	GLU
Glutamic acid--protonated	GLH
Glutamine	GLN
Glycine	GLY
Histidine, delta H	HID
Histidine, epsilon H	HIE
Histidine, protonated	HIP
Isoleucine	ILE
Leucine	LEU
Lysine	LYS
Methionine	MET
Phenylalanine	PHE
Proline	PRO
Serine	SER
Threonine	THR
Tryptophan	TRP
Tyrosine	TYR
Valine	VAL

```
{ ALA VAL SER PHE }
{ ACE ALA VAL SER PHE NME }
{ NALA VAL SER CPHE }
```

The default for loading from PDB files is to use N- and C-terminal residues; this is established by the `addPdbResMap` command in the default `leaprc` files. To force incomplete valences with the standard residues, one would have to define a sequence ("`x = { ALA VAL SER PHE }`") and use `loadPdbUsingSeq`, or use `clearPdbResMap` to completely remove the mapping feature.

Histidine can exist either as the protonated species or as a neutral species with a hydrogen at the delta or epsilon position. For this reason, the histidine UNIT/RESIDUE name is either HIP, HID, or HIE (but not HIS). The default "leaprc" files assign the name HIS to HIE. Thus, if a PDB file is read that contains the residue HIS, the residue will be assigned to the HIE UNIT object. This feature can be changed within one's own "leaprc" file.

The AMBER force fields also differentiate between the residue cysteine (CYS) and the similar residue that participates in disulfide bridges, cystine (CYX). The user will need to load the

PDB file using the `loadPdbUsingSeq` command, substituting CYX for CYS in the sequence wherever a disulfide bond will be created. (Or alternatively, the PDB file can be manually edited to change CYS to CYX.) Then the user will have to explicitly define, using the `bond` command, the disulfide bond for a pair of cystines, as this information is not read from the PDB file.

3.5.3. Nucleic Acid Residues

The following are defined for the 1994 force field.

Group or residue	Residue Name, Alias
Adenine	DA,RA
Thymine	DT
Uracil	RU
Cytosine	DC,RC
Guanine	DG,RG

The "D" or "R" prefix can be used to distinguish between deoxyribose and ribose units; with the default `leaprc` file, ambiguous residues are assumed to be deoxy. Residue names like "DA" can be followed by a "5" or "3" ("DA5", "DA3") for residues at the ends of chains; this is also the default established by `addPdbResMap`, even if the "5" or "3" are not added in the PDB file. The "5" and "3" residues are "capped" by a hydrogen; the plain and "3" residues include a "leading" phosphate group. Neutral residues capped by hydrogens end in "N," such as "DAN."

3.5.4. Miscellaneous Residues

Miscellaneous Residue	unit/residue name
TIP3P water molecule	TP3
TIP4P water model	TP4
TIP5P water model	TP5
SPC/E water model	SPC
Cesium cation	Cs+
Potassium cation	K+
Rubidium cation	Rb+
Lithium cation	Li+
Sodium cation	Na+ or IP
Chlorine	Cl- or IM
Large cation	IB

"IB" represents a solvated monovalent cation (say, sodium) for use in vacuum simulations. The cation UNITs are found in the files "ions91.lib" and "ions94.lib", while the water UNITs are in the file "solvents.lib". The `leaprc` files assign the variables WAT and HOH to the TP3 UNIT found in the OFF library file. Thus, if a PDB file is read and that file contains either the residue name HOH or WAT, the TP3 UNIT will be substituted. See the `solvate` commands and Chapter 2.9 for a discussion of how to use other water models.

A periodic box of 216 TIP3P waters (TIP3PBOX) is provided in the file "solvents.lib". The box measures 18.774 angstroms on a side. This box of waters has been equilibrated by a Monte Carlo simulation. It is the UNIT that should be used to solvate systems with TIP3P water molecules within LEaP. It has been provided by W. L. Jorgensen. Boxes are also available for other water models (TIP4P, TIP5P, POL3, SPC/E), and for chloroform, methanol, and N-methylacetamide; these are described in Chapter 2.

3.6. Commands

The following is a description of the commands that can be accessed using the command line interface in *tleap*, or through the command line editor in *xleap*. Whenever an argument in a command line definition is enclosed in brackets ([arg]), then that argument is optional. When examples are shown, the command line is prefaced by "> ", and the program output is shown without this character preface.

Some commands that are almost never used have been removed from this description to save space. You can use the "help" facility to obtain information about these commands; most only make sense if you understand what the program is doing behind the scenes.

3.6.1. add

`add a b`

`UNIT/RESIDUE/ATOM` *a,b*

Add the object *b* to the object *a*. This command is used to place ATOMs within RESIDUEs, and RESIDUEs within UNITs. This command will work only if *b* is not contained by any other object.

The following example illustrates both the `add` command and the way the tip3p water molecule is created for the LEaP distribution tape.

```
> h1 = createAtom H1 HW  0.417
> h2 = createAtom H2 HW  0.417
> o  = createAtom O  OW -0.834
>
> set h1 element H
> set h2 element H
> set o  element O
>
> r = createResidue TIP3
> add r h1
> add r h2
> add r o
>
> bond h1 o
> bond h2 o
> bond h1 h2
>
> TIP3 = createUnit TIP3
>
> add TIP3 r
> set TIP3.1 restype solvent
> set TIP3.1 imagingAtom TIP3.1.O
>
> zMatrix TIP3 {
>       { H1 O 0.9572 }
>       { H2 O H1 0.9572 104.52 }
> }
```

```
>
> saveOff TIP3 water.lib
Saving TIP3.
Building topology.
Building atom parameters.
```

3.6.2. addAtomTypes

`addAtomTypes { { type element hybrid } { ... } ... }`

```
STRING  type
STRING  element
STRING  hybrid
```

Define element and hybridization for force field atom types. This command for the standard force fields can be seen in the default `leaprc` files. The STRINGs are most safely rendered using quotation marks. If atom types are not defined, confusing messages about hybridization can result when loading PDB files.

3.6.3. addIons

`addIons unit ion1 numIon1 [ion2 numIon2]`

```
UNIT    unit
UNIT    ion1
NUMBER  numIon1
UNIT    ion2
NUMBER  numIon2
```

Adds counterions in a shell around *unit* using a Coulombic potential on a grid. If *numIon1* is 0, then the *unit* is neutralized. In this case, *numIon1* must be opposite in charge to *unit* and *numIon2* cannot be specified. If solvent is present, it is ignored in the charge and steric calculations, and if an ion has a steric conflict with a solvent molecule, the ion is moved to the center of said molecule, and the latter is deleted. (To avoid this behavior, solvate after addIons.) Ions must be monoatomic. This procedure is not guaranteed to globally minimize the electrostatic energy. When neutralizing regular-backbone nucleic acids, the first cations will generally be placed between phosphates, leaving the final two ions to be placed somewhere around the middle of the molecule.The default grid resolution is 1 Å, extending from an inner radius of (maxIonVdwRadius + maxSoluteAtomVdwRadius) to an outer radius 4 Å beyond. A distance-dependent dielectric is used for speed.

3.6.4. addPdbAtomMap

`addPdbAtomMap list`

```
LIST    list
```

The atom Name Map is used to try to map atom names read from PDB files to atoms within residue UNITs when the atom name in the PDB file does not match an atom in the

residue. This enables PDB files to be read in without extensive editing of atom names. Typically, this command is placed in the LEaP start-up file, "leaprc", so that assignments are made at the beginning of the session. The LIST is a LIST of LISTs. Each sublist contains two entries to add to the Name Map. Each entry has the form:

```
{ string string }
```

where the first *string* is the name within the PDB file, and the second *string is the name in the residue UNIT.*

3.6.5. addPdbResMap

`addPdbResMap list`

```
LIST    list
```

The Name Map is used to map RESIDUE names read from PDB files to variable names within LEaP. Typically, this command is placed in the LEaP start-up file, "leaprc", so that assignments are made at the beginning of the session. The LIST is a LIST of LISTs. Each sublist contains two or three entries to add to the Name Map. Each entry has the form:

```
{ double string string }
```

where *double* can be 0 or 1, the first string is the name within the PDB file, and the second string is the variable name to which the first string will be mapped. To illustrate, the following is part of the Name Map that exists when LEaP is started from the "leaprc" file included in the distribution tape:

```
  ADE   -->  DADE
  :   :
 0 ALA   -->  NALA
 0 ARG   -->  NARG
    :   :
 1 ALA   -->  CALA
 1 ARG   -->  CARG
    :   :
 1 VAL   -->  CVAL
```

Thus, the residue `ALA` will be mapped to `NALA` if it is the N-terminal residue and `CALA` if it is found at the C-terminus. The above Name Map was produced using the following (edited) command line:

```
> addPdbResMap {
> { 0 ALA NALA }  { 1 ALA CALA }
> { 0 ARG NARG } { 1 ARG CARG }
           :    :
> { 0 VAL NVAL }  { 1 VAL CVAL }
>
           :   :
```

```
> { ADE DADE }
            :  :
> }
```

3.6.6. alias

`alias [ string1 [ string2 ] ]`

```
STRING  string1
STRING  string2
```

This command will add or remove an entry to the Alias Table or list entries in the Alias Table. If both strings are present, then string1 becomes the alias to string2, the original command. If only one string is used as an argument, then this string is removed from the Alias Table. If no arguments are given with the command, the current aliases stored in the Alias Table will be listed.

The proposed alias is first checked for conflict with the LEaP commands and it is rejected if a conflict is found. A proposed alias will replace an existing alias with a warning being issued. The alias can stand for more than a single word, but also as an entire string so the user can quickly repeat entire lines of input.

3.6.7. bond

`bond atom1 atom2 [ order ]`

```
ATOM    atom1
ATOM    atom2
STRING  order
```

Create a bond between atom1 and atom2. Both of these ATOMs must be contained by the same UNIT. By default, the bond will be a single bond. By specifying "S", "D", "T", or "A" as the optional argument, *order*, the user can specify a single, double, triple, or aromatic bond, respectively. Example:

```
bond trx.32.SG trx.35.SG
```

3.6.8. bondByDistance

`bondByDistance container [ maxBond ]`

```
CONT    container
NUMBER  maxBond
```

Create single bonds between all ATOMs in container that are within maxBond angstroms of each other. If maxBond is not specified then a default distance will be used. This command is especially useful in building molecules. Example:

```
bondByDistance alkylChain
```

3.6.9. center

```
center container
```

```
UNIT/RESIDUE/ATOM   container
```

Display the coordinates of the geometric center of the ATOMs within container. In the following example, the alanine UNIT found in the amino acid library has been examined by the center command:

```
> center ALA
The center is at: 4.04, 2.80, 0.49
```

3.6.10. charge

```
charge container
```

```
UNIT/RESIDUE/ATOM   container
```

This command calculates the total charge of the ATOMs within container.

3.6.11. check

```
check unit [ parms ]
```

```
UNIT    unit
PARMSET parms
```

This command can be used to check the UNIT for internal inconsistencies that could cause problems when performing calculations. This is a very useful command that should be used before a UNIT is saved with *saveAmberParm or its variants. Currently it checks for the following possible problems:*

- long bonds
- short bonds
- non-integral total charge of the UNIT.
- missing force field atom types
- close contacts (< 1.5 Å) between nonbonded ATOMs.

The user may collect any missing molecular mechanics parameters in a PARMSET for subsequent editing. In the following example, the alanine UNIT found in the amino acid library has been examined by the *check command:*

```
> check ALA
Checking 'ALA'....
Checking parameters for unit 'ALA'.
Checking for bond parameters.
Checking for angle parameters.
Unit is OK.
```

3.6.12. combine

```
variable = combine  list
```

```
object  variable
LIST    list
```

Combine the contents of the UNITs within list into a single UNIT. The new UNIT is placed in variable. This command is similar to the *sequence* command except it does not link the ATOMs of the UNITs together. In the following example, the input and output should be compared with the example given for the *sequence* command.

```
> tripeptide = combine { ALA GLY PRO }
Sequence: ALA
Sequence: GLY
Sequence: PRO
> desc tripeptide
UNIT name: ALA     !! bug: this should be tripeptide!
Head atom: .R<ALA 1>.A<N 1>
Tail atom: .R<PRO 3>.A<C 13>
Contents:
R<ALA 1>
R<GLY 2>
R<PRO 3>
```

3.6.13. copy

```
newvariable = copy  variable
```

```
object  newvariable
object  variable
```

Creates an exact duplicate of the object variable. Since newvariable is not pointing to the same object as variable, changing the contents of one object will not alter the other object. Example:

```
> tripeptide = sequence { ALA GLY PRO }
> tripeptideSol = copy tripeptide
> solvateBox tripeptideSol TIP3PBOX 8 2
```

In the above example, tripeptide is a separate object from tripeptideSol and is not solvated. Had the user instead entered

```
> tripeptide = sequence { ALA GLY PRO }
> tripeptideSol = tripeptide
> solvateBox tripeptideSol TIP3PBOX 8 2
```

then both tripeptide and tripeptideSol would be solvated since they would both point to the same object.

3.6.14. createAtom

```
variable = createAtom  name type charge
```

```
ATOM    variable
STRING  name
STRING  type
NUMBER  charge
```

Return a new and empty ATOM with name, type, and charge as its atom name, atom type, and electrostatic point charge. (See the *add* command for an example of the *createAtom* command.)

3.6.15. createParmset

```
variable = createParmset  name
```

```
PARMSET variable
STRING  name
```

Return a new and empty PARMSET with the name "name".

```
> newparms = createParmset newParms
```

3.6.16. createResidue

```
variable = createResidue  name
```

```
RESIDUE variable
STRING  name
```

Return a new and empty RESIDUE with the name "name". (See the *add* command for an example of the *createResidue* command.)

3.6.17. createUnit

```
variable = createUnit  name
```

```
UNIT    variable
STRING  name
```

Return a new and empty UNIT with the name "name". (See the *add* command for an example of the *createUnit* command.)

3.6.18. deleteBond

```
deleteBond atom1 atom2
```

```
ATOM    atom1
ATOM    atom2
```

Delete the bond between the ATOMs atom1 and atom2. If no bond exists, an error will be

displayed.

3.6.19. desc

```
desc variable
```

```
object  variable
```

Print a description of the object. In the following example, the alanine UNIT found in the amino acid library has been examined by the *desc* command:

```
> desc ALA
UNIT name: ALA
Head atom: .R<ALA 1>.A<N 1>
Tail atom: .R<ALA 1>.A<C 9>
Contents:
R<ALA 1>
```

Now, the *desc* command is used to examine the first residue (1) of the alanine UNIT:

```
> desc ALA.1
RESIDUE name: ALA
RESIDUE sequence number: 1
Type: protein
Connection atoms:
Connect atom 0: A<N 1>
Connect atom 1: A<C 9>
Contents:
A<N 1>
A<HN 2>
A<CA 3>
A<HA 4>
A<CB 5>
A<HB1 6>
A<HB2 7>
A<HB3 8>
A<C 9>
A<O 10>
```

Next, we illustrate the desc command by examining the ATOM *N* of the first residue (1) of the alanine UNIT:

```
> desc ALA.1.N
ATOM
Name:    N
Type:    N
Charge:  -0.463
Element: N
Atom flags: 20000|posfxd- posblt- posdrn- sel- pert-
notdisp- tchd- posknwn+ int - nmin- nbld-
```

```
Atom position: 3.325770, 1.547909, -0.000002
Atom velocity: 0.000000, 0.000000, 0.000000
Bonded to .R<ALA 1>.A<HN 2> by a single bond.
Bonded to .R<ALA 1>.A<CA 3> by a single bond.
```

Since the N ATOM is also the first atom of the ALA residue, the following command will give the same output as the previous example:

```
> desc ALA.1.1
```

3.6.20. edit

`edit unit`

```
UNIT    unit
```

In xleap this command creates a Unit Editor that contains the UNIT unit. The user can view and edit the contents of the UNIT using the mouse. The command causes a copy of the object to be edited. If the object that the user wants to edit is `null`, then the edit command assumes that the user wants to edit a new UNIT with a single RESIDUE within it. PARMSETs can also be edited. In tleap this command prints an error message.

3.6.21. groupSelectedAtoms

`groupSelectedAtoms unit name`

```
UNIT    unit
STRING  name
```

Create a group within unit with the name, "name", using all of the ATOMs within the UNIT that are selected. If the group has already been defined then overwrite the old group. The *desc* command can be used to list groups. Example:

```
groupSelectedAtoms TRP sideChain
```

An expression like "TRP@sideChain" returns a LIST, so any commands that require LIST ’s can take advantage of this notation. After assignment, one can access groups using the "@" notation. Examples:

```
select TRP@sideChain


center TRP@sideChain
```

The latter example will calculate the center of the atoms in the "sideChain" group. (see the *select* command for a more detailed example.)

3.6.22. help

```
help [string]

      STRING  string
```

This command prints a description of the command in string. If the STRING is not given then a list of help topics is provided.

3.6.23. impose

```
impose unit seqlist internals

      UNIT    unit
      LIST    seqlist
      LIST    internals
```

The impose command allows the user to impose internal coordinates on the UNIT. The list of RESIDUEs to impose the internal coordinates upon is in seqlist. The internal coordinates to impose are in the LIST internals.

The command works by looking into each RESIDUE within the UNIT that is listed in the seqlist argument and attempts to apply each of the internal coordinates within internals. The seqlist argument is a LIST of NUMBERs that represent sequence numbers or ranges of sequence numbers. Ranges of sequence numbers are represented by two element LISTs that contain the first and last sequence number in the range. The user can specify sequence number ranges that are larger than what is found in the UNIT. For example, the range { 1 999 } represents all RESIDUEs in a 200 RESIDUE UNIT.

The internals argument is a LIST of LISTs. Each sublist contains a sequence of ATOM names which are of type STRING followed by the value of the internal coordinate. An example of the impose command would be:

```
impose peptide { 1 2 3 } {
{ N CA C N -40.0 }
{ C N CA C -60.0 }
}
```

This would cause the RESIDUE with sequence numbers 1, 2, and 3 within the UNIT peptide to assume an alpha helical conformation. The command

```
impose peptide { 1 2 { 5 10 } 12 } {
 { CA CB 5.0 } }
```

will impose on the residues with sequence numbers 1, 2, 5, 6, 7, 8, 9, 10, and 12 within the UNIT peptide a bond length of 5.0 angstroms between the alpha and beta carbons. RESIDUEs without an ATOM named CB (like glycine) will be unaffected.

Three types of conformational change are supported: bond length changes, bond angle changes, and torsion angle changes. If the conformational change involves a torsion angle, then all dihedrals around the central pair of atoms are rotated. The entire list of internals are applied to each RESIDUE.

3.6.24. list

List all of the variables currently defined. To illustrate, the following (edited) output shows the variables defined when LEaP is started from the leaprc file included in the distribution tape:

```
> list
A
ACE       ALA
ARG       ASN
 :    :
VAL       W
WAT       Y
```

3.6.25. loadAmberParams

`variable = loadAmberParams  filename`

```
PARMSET variable
STRING  filename
```

Load an AMBER format parameter set file and place it in variable. All interactions defined in the parameter set will be contained within variable. This command causes the loaded parameter set to be included in LEaP 's list of parameter sets that are searched when parameters are required. General proper and improper torsion parameters are modified during the command execution with the LEaP general type "?" replacing the AMBER general type "X".

```
> parm91 = loadAmberParams parm91X.dat
> saveOff parm91 parm91.lib
Saving parm91.
```

3.6.26. loadAmberPrep

`loadAmberPrep filename [ prefix ]`

```
STRING  filename
STRING  prefix
```

This command loads an AMBER PREP input file. For each residue that is loaded, a new UNIT is constructed that contains a single RESIDUE and a variable is created with the same name as the name of the residue within the PREP file. If the optional argument prefix is provided it will be prefixed to each variable name; this feature is used to prefix UATOM residues, which have the same names as AATOM residues with the string "U" to distinguish them. Let us imagine that the following AMBER PREP input file exists:

```
0  0  2

  Crown Fragment A
```

```
cra.res
CRA  INT 0
CORRECT  NOMIT DU  BEG
0.0
1  DUMM  DU M  0  0  0     0.      0.      0.
2  DUMM  DU M  0  0  0     1.000   0.      0.
3  DUMM  DU M  0  0  0     1.000  90.      0.
4  C1    CT M  0  0  0     1.540 112.    169.
5  H1A   HC E  0  0  0     1.098 109.47  -110.0
6  H1B   HC E  0  0  0     1.098 109.47   110.0
7  O2    OS M  0  0  0     1.430 112.    -72.
8  C3    CT M  0  0  0     1.430 112.    169.
9  H3A   HC E  0  0  0     1.098 109.47  -49.0
10 H3B   HC E  0  0  0     1.098 109.47   49.0

CHARGE
 0.2442  -0.0207  -0.0207  -0.4057  0.2442
 -0.0207  -0.0207

DONE
STOP
```

This fragment can be loaded into LEaP using the following command:

```
> loadAmberPrep cra.in
Loaded UNIT: CRA
```

3.6.27. loadOff

`loadOff filename`

```
STRING  filename
```

This command loads the OFF library within the file named filename. All UNITs and PARMSETs within the library will be loaded. The objects are loaded into LEaP under the variable names the objects had when they were saved. Variables already in existence that have the same names as the objects being loaded will be overwritten. Any PARMSETs loaded using this command are included in LEaP 's library of PARMSETs that is searched whenever parameters are required (The old AMBER format is used for PARMSETs rather than the OFF format in the default configuration). Example command line:

```
> loadOff parm91.lib
Loading library: parm91.lib
Loading: PARAMETERS
```

3.6.28. loadMol2

```
variable = loadMol2 filename

      STRING  filename
      object  variable
```

Load a SYBYL Mol2 format file in a UNIT. This command is very much like *loadOff*, except that it only creates a single UNIT. Currently, only Mol2 files that contain a single residue will be read correctly.

3.6.29. loadPdb

```
variable = loadPdb filename

      STRING  filename
      object  variable
```

Load a Protein Databank format file with the file name filename. The sequence numbers of the RESIDUEs will be determined from the order of residues within the PDB file ATOM records. This function will search the variables currently defined within LEaP for variable names that map to residue names within the ATOM records of the PDB file. If a matching variable name is found then the contents of the variable are added to the UNIT that will contain the structure being loaded from the PDB file. Adding the contents of the matching UNIT into the UNIT being constructed means that the contents of the matching UNIT are copied into the UNIT being built and that a bond is created between the connect0 ATOM of the matching UNIT and the connect1 ATOM of the UNIT being built. The UNITs are combined in the same way UNITs are combined using the sequence command. As atoms are read from the ATOM records their coordinates are written into the correspondingly named ATOMs within the UNIT being built. If the entire residue is read and it is found that ATOM coordinates are missing, then external coordinates are built from the internal coordinates that were defined in the matching UNIT. This allows LEaP to build coordinates for hydrogens and lone-pairs which are not specified in PDB files.

```
> crambin = loadPdb 1crn
Loading PDB file
Matching PDB residue names to LEaP variables.
Mapped residue THR, term: 0, seq. number: 0 to: NTHR.
Residue THR, term: M, seq. number: 1 was not
found in name map.
Residue CYS, term: M, seq. number: 2 was not
found in name map.
Residue CYS, term: M, seq. number: 3 was not
found in name map.
Residue PRO, term: M, seq. number: 4 was not
found in name map.
:               :               :
Residue TYR, term: M, seq. number: 43 was not
found in name map.
Residue ALA, term: M, seq. number: 44 was not
found in name map.
```

```
Mapped residue ASN, term: 1, seq. number: 45 to: CASN.
Joining NTHR - THR
Joining THR - CYS
Joining CYS - CYS
Joining CYS - PRO
:               :               :
Joining ASP - TYR
Joining TYR - ALA
Joining ALA - CASN
```

The above edited listing shows the use of this command to load a PDB file for the protein crambin. Several disulfide bonds are present in the protein and these bonds are indicated in the PDB file. The loadPdb command, however, cannot read this information from the PDB file. It is necessary for the user to explicitly define disulfide bonds using the *bond* command.

3.6.30. loadPdbUsingSeq

`loadPdbUsingSeq filename unitlist`

```
STRING  filename
LIST    unitlist
```

This command reads a Protein Data Bank format file from the file named filename. This command is identical to *loadPdb* except it does not use the residue names within the PDB file. Instead the sequence is defined by the user in unitlist. For more details see *loadPdb.*

```
> peptSeq = { UALA UASN UILE UVAL UGLY }
> pept = loadPdbUsingSeq pept.pdb peptSeq
```

In the above example, a variable is first defined as a LIST of united atom RESIDUEs. A PDB file is then loaded, in this sequence order, from the file "pept.pdb".

3.6.31. logFile

`logFile filename`

```
STRING  filename
```

This command opens the file with the file name filename as a log file. User input and all output is written to the log file. Output is written to the log file as if the verbosity level were set to 2. An example of this command is:

```
> logfile /disk/howard/leapTrpSolvate.log
```

3.6.32. measureGeom

`measureGeom atom1 atom2 [ atom3 [ atom4 ] ]`

```
ATOM    atom1
```

```
ATOM    atom2
ATOM    atom3
ATOM    atom4
```

Measure the distance, angle, or torsion between two, three, or four ATOMs, respectively.

In the following example, we first describe the RESIDUE ALA of the ALA UNIT in order to find the identity of the ATOMs. Next, the measureGeom command is used to determine a distance, simple angle, and a dihedral angle. As shown in the example, the ATOMs may be identified using atom names or numbers.

```
> desc ALA.ALA
RESIDUE name: ALA
RESIDUE sequence number: 1
Type: protein
Connection atoms:
Connect atom 0: A<N 1>
Connect atom 1: A<C 9>
Contents:
A<N 1>
A<HN 2>
A<CA 3>
A<HA 4>
A<CB 5>
A<HB1 6>
A<HB2 7>
A<HB3 8>
A<C 9>
A<O 10>
> measureGeom ALA.ALA.1 ALA.ALA.3
Distance: 1.45 angstroms
> measureGeom ALA.ALA.1 ALA.ALA.3 ALA.ALA.5
Angle: 111.10 degrees
> measureGeom ALA.ALA.N ALA.ALA.CA ALA.ALA.C ALA.ALA.O
Torsion angle: 0.00 degrees
```

3.6.33. quit

Quit the LEaP program.

3.6.34. remove

`remove a b`

```
CONT    a
CONT    b
```

Remove the object b from the object a. If b is not contained by a then an error message will be displayed. This command is used to remove ATOMs from RESIDUEs, and

RESIDUEs from UNITs. If the object represented by b is not referenced by some variable name then it will be destroyed.

```
> dipeptide = combine { ALA GLY }
Sequence: ALA
Sequence: GLY
> desc dipeptide
UNIT name: ALA     !! bug: this should be dipeptide!
Head atom: .R<ALA 1>.A<N 1>
Tail atom: .R<GLY 2>.A<C 6>
Contents:
R<ALA 1>
R<GLY 2>
> remove dipeptide dipeptide.2
> desc dipeptide
UNIT name: ALA     !! bug: this should be dipeptide!
Head atom: .R<ALA 1>.A<N 1>
Tail atom: null
Contents:
R<ALA 1>
```

3.6.35. saveAmberParm

`saveAmberParm unit topologyfilename coordinatefilename`

```
UNIT    unit
STRING  topologyfilename
STRING  coordinatefilename
```

Save the AMBER topology and coordinate files for the UNIT into the files named topologyfilename and coordinatefilename respectively. This command will cause LEaP to search its list of PARMSETs for parameters defining all of the interactions between the ATOMs within the UNIT. This command produces topology files and coordinate files that are identical in format to those produced by AMBER PARM and can be read into AMBER for calculations. The output of this operation can be used for minimizations, dynamics, and thermodynamic integration calculations.

In the following example, the topology and coordinates from the all_amino94.lib UNIT ALA are generated:

```
> saveamberparm ALA ala.top ala.crd
Building topology.
Building atom parameters.
Building bond parameters.
Building angle parameters.
Building proper torsion parameters.
Building improper torsion parameters.
Building H-Bond parameters.
```

3.6.36. saveAmberParmPol

```
saveAmberParmPol unit topologyfilename coordinatefilename
```

```
UNIT    unit
STRING  topologyfilename
STRING  coordinatefilename
```

Like saveAmberParm, but includes atomic polarizabilities in the topology file for use with IPOL=1 in Sander. The polarizabilities are according to atom type, and are defined in the 'mass' section of the *parm.dat* or *frcmod* file.

3.6.37. saveOff

```
saveOff object filename
```

```
object  object
STRING  filename
```

The saveOff command allows the user to save UNITs and PARMSETs to a file named *filename*. The file is written using the Object File Format (off) and can accommodate an unlimited number of uniquely named objects. The names by which the objects are stored are the variable names specified in the argument of this command. If the file *filename* already exists then the new objects will be added to the file. If there are objects within the file with the same names as objects being saved then the old objects will be overwritten. The argument object can be a single UNIT, a single PARMSET, or a LIST of mixed UNITs and PARMSETs. (See the *add* command for an example of the *saveOff* command.)

3.6.38. savePdb

```
savePdb unit filename
```

```
UNIT    unit
STRING  filename
```

Write UNIT to the file *filename* as a PDB format file. In the following example, the PDB file from the "all_amino94.lib" UNIT ALA is generated:

```
> savepdb ALA ala.pdb
```

3.6.39. sequence

```
variable = sequence  list
```

```
UNIT    variable
LIST    list
```

The sequence command is used to create a new UNIT by combining the contents of a LIST of UNITs. The first argument is a LIST of UNITs. A new UNIT is constructed by taking each UNIT in the sequence in turn and copying its contents into the UNIT being constructed. As each new UNIT is copied, a bond is created between the tail ATOM of

the UNIT being constructed and the head ATOM of the UNIT being copied, if both connect ATOMs are defined. If only one is defined, a warning is generated and no bond is created. If neither connection ATOM is defined then no bond is created. As each RESIDUE is copied into the UNIT being constructed it is assigned a sequence number which represents the order the RESIDUEs are added. Sequence numbers are assigned to the RESIDUEs so as to maintain the same order as was in the UNIT before it was copied into the UNIT being constructed. This command builds reasonable starting coordinates for all ATOMs within the UNIT; it does this by assigning internal coordinates to the linkages between the RESIDUEs and building the external coordinates from the internal coordinates from the linkages and the internal coordinates that were defined for the individual UNITs in the sequence.

```
> tripeptide = sequence { ALA GLY PRO }
Sequence: ALA
Sequence: GLY
Joining ALA - GLY
Sequence: PRO
Joining GLY - PRO
> desc tripeptide
UNIT name: ALA     !! bug: this should be tripeptide!
Head atom: .R<ALA 1>.A<N 1>
Tail atom: .R<PRO 3>.A<C 13>
Contents:
R<ALA 1>
R<GLY 2>
R<PRO 3>
```

3.6.40. set

```
set default variable value

      STRING  variable
      STRING  value
```

or

```
set container parameter object

      CONT    container
      STRING  parameter
      object  object
```

This command sets the values of some global parameters (when the first argument is "default") or sets various parameters associated with container. The following parameters can be set within LEaP:

For "default" parameters

PBradii — Set to "mbondi" to use modified Bondi radii (where the hydrogens are modified from the Bondi values). Here the radius of hydrogen bonded to oxygen or sulfur is set to 0.8; hydrogen bonded to carbon is 1.3; hydrogen

bonded to nitrogen is 1.3. These parameters are the default, and are those used by Tsui & Case [52], and are the recommended ones when *igb* = 1 in the *sander* input. The code in Amber (version 6) used values like the "mbondi" values, except that the radius for hydrogen bonded to nitrogen was 1.2; you can use the "amber6" keyword for *PBradii* to use these earlier values [53], but this is only recommended if you want to check results against those from Amber6, or if you need to extend a simulation started with the earlier parameters.

Set to "mbondi2" to use another set of modified Bondi radii and Tinker screening parameters for generalized Born calculations. These values are recommended when *igb* = 2 and *igb* = 5 in the *sander* input. Here only the radius of hydrogen bonded to nitrogen is increased to 1.3. Original bondi radii, set by "bondi" also perform reasonably well with this GB model; see [54] for details.

Set to "bondi" when using *igb*=7.

The values specified above are put into the RADII and SCREENING sections of the *prmtop* file, and could be edited by hand from there if further changes were desired.

OldPrmtopFormat

If set to "on", the saveAmberParm command will write a prmtop file in the format used in Amber6 and before; if set to "off" (the default), it will use the new format.

Dielectric If set to "distance" (the default), electrostatic calculations in LEaP will use a distance-dependent dielectric; if set to "constant", and constant dielectric will be used.

PdbWriteCharges

If set to "on", atomic charges will be placed in the "B-factor" field of pdb files saved with the savePdb command; if set to "off" (the default), no such charges will be written.

PdbWriteRadii If set to "on", atomic radii will be placed in the " " field pdb files saved with the savePdb command; if set to "off" (the default), no such radii will be written.

FlexibleWater If set to "on", LEaP will *not* remove the HW-OW-HW angle from water residues; if set to "off" (the default), LEaP will remove this angle. In all cases, the HW-HW-OW angle in water residues is removed if it is present. As the name suggests, you should turn this variable "on" if you are using a flexible water model.

For ATOMs:

name A unique STRING descriptor used to identify ATOMs.

type This is a STRING property that defines the AMBER force field atom type.

charge The charge property is a NUMBER that represents the ATOM’s electrostatic point charge to be used in a molecular mechanics force field.

position This property is a LIST of NUMBERs containing three values: the (X, Y, Z) Cartesian coordinates of the ATOM.

For RESIDUEs:

connect0 This defines an ATOM that is used in making links to other RESIDUEs. In UNITs containing single RESIDUEs, the RESIDUEsS connect0 ATOM is usually defined as the UNIT's head ATOM.

connect1 This is an ATOM property which defines an ATOM that is used in making links to other RESIDUEs. In UNITs containing single RESIDUEs, the RESIDUEsS connect1 ATOM is usually defined as the UNIT's tail ATOM.

connect2 This is an ATOM property which defines an ATOM that can be used in making links to other RESIDUEs. In amino acids, the convention is that this is the ATOM to which disulfide bridges are made.

restype This property is a STRING that represents the type of the RESIDUE. Currently, it can have one of the following values: "undefined", "solvent", "protein", "nucleic", or "saccharide".

name This STRING property is the RESIDUE name.

For UNITs:

head Defines the ATOM within the UNIT that is connected when UNITs are joined together: the tail ATOM of one UNIT is connected to the head ATOM of the subsequent UNIT in any sequence.

tail Defines the ATOM within the UNIT that is connected when UNITs are joined together: the tail ATOM of one UNIT is connected to the head ATOM of the subsequent UNIT in any sequence.

box The property defines the bounding box of the UNIT. If it is defined as `null` then no bounding box is defined. If the value is a single NUMBER then the bounding box will be defined to be a cube with each side being NUMBER of angstroms across. If the value is a LIST then it must be a LIST containing three numbers, the lengths of the three sides of the bounding box.

cap The property defines the solvent cap of the UNIT. If it is defined as `null` then no solvent cap is defined. If the value is a LIST then it must contain four numbers, the first three define the Cartesian coordinates (X, Y, Z) of the origin of the solvent cap in angstroms, the fourth NUMBER defines the radius of the solvent cap in angstroms.

3.6.41. setBox

```
setBox unit   vdw OR centers  [ buffer OR buffer_xyz_list ]

      UNIT    unit
```

The setBox command adds a periodic box to the UNIT, turning it into a periodic system for the simulation programs. It does not add any solvent to the system. The choice of "vdw" or "centers" determines whether the box encloses the entire atoms or just the atom centers - use "centers" if the system has been previously equilibrated as a periodic box. See the solvateBox command for a description of the buffer variable, which extends either type of box by an arbitrary amount.

3.6.42. solvateBox

```
solvateBox solute solvent buffer [ iso ] [ closeness ]

      UNIT    solute
      UNIT    solvent
      object  buffer
      NUMBER  closeness
```

The *solvateBox* command creates a rectangular parallelepiped solvent box around the solute UNIT. The solute UNIT is modified by the addition of solvent RESIDUEs. (For most liquid state simulations, the *solvateOct* command discussed below is probably a better choice.)

The normal choice for a TIP3 _solvent_ UNIT is TIP3PBOX, which is a snapshot from a room-temperature equilibration for this model. If you want to solvate with other water models, look for a "BOX" with that model (*e.g.* TIP4PBOX, POLBOX, SPCBOX, TIP5PBOX). If you need to use a water model other than these, try the following: *(a)* solvate the system with TIP3PBOX, using the default TIP3 model; *(b)* use *ambpdb* to convert your *prmtop* file to Brookhaven format; *(c)* restart LEaP, choose the water model you want (instructions are in the Database chapter), then use loadPdb to bring back in the system you have created. The issue a setBox command to provide a box. It is also best to manually edit the resulting *prmcrd* file so that its last line (which contains the box information) matches what you had from the original run with TIP3PBOX; this corrects a glitch which prevents setBox from making as good a box as does solvateBox or solvateOct.

Note that equilibration will always be required to bring the artificial box to reasonable density, since Van der Waals voids remain due to the impossibility of natural packing of solvent around the solute and at the edges of the box. First, equilibrate the system at constant volume to the temperature you want, then turn on constant pressure to adjust the system density to the desired value.

The solvent UNIT is copied and repeated in all three spatial directions to create a box containing the entire solute and a buffer zone defined by the buffer argument. The buffer argument defines the distance, in angstroms, between the wall of the box and the closest ATOM in the solute. If the buffer argument is a single NUMBER, then the buffer distance is the same for the x, y, and z directions, unless the 'iso' option is used to make the box cubic, with the shortest box clearance = buffer. If the buffer argument is a LIST of three NUMBERs, then the NUMBERs are applied to the x, y, and z axes respectively. As the larger box is created and superimposed on the solute, solvent molecules overlapping the solute are removed.

The optional closeness parameter can be used to control how close, in angstroms, solvent ATOMs can come to solute ATOMs. The default value of the closeness argument is 1.0. Smaller values allow solvent ATOMs to come closer to solute ATOMs. The criterion for rejection of overlapping solvent RESIDUEs is if the distance between any solvent ATOM to the closest solute ATOM is less than the sum of the ATOMs VANDERWAAL distances multiplied by the closeness argument.

This command modifies the _solute_ UNIT in several ways. First, the coordinates of the ATOMs are modified to move the center of a box enclosing the Van der Waals radii of the atoms to the origin. Secondly, the UNIT is modified by the addition of _solvent_

RESIDUEs copied from the _solvent_ UNIT. Finally, the box parameter of the new system (still named for the _solute_) is modified to reflect the fact that a periodic, rectilinear solvent box has been created around it.

In this example, it is assumed that the file solvents.lib, containing TIP3PBOX, has been loaded already (as is done by the default leaprc):

```
>> mol = loadpdb my.pdb
>> solvateBox sol TIP3PBOX 10
  Solute vdw bounding box:              7.512 12.339 12.066
  Total bounding box for atom centers:  27.512 32.339 32.066
  Solvent unit box:                     18.774 18.774 18.774
  Total vdw box size:                   30.995 35.538 35.416 angstroms.
  Total mass 14470.768 amu,  Density 0.616 g/cc
  Added 785 residues.
```

Again, note that the density of 0.601 g/cc points to the need for constant pressure equilibration. (See the discussion of equilibration in the Q&A section of the amber web.)

3.6.43. solvateCap

```
solvateCap solute solvent position radius [ closeness ]
```

```
UNIT    solute
UNIT    solvent
object  position
NUMBER  radius
NUMBER  closeness
```

The solvateCap command creates a solvent cap around the solute UNIT. The solute UNIT is modified by the addition of solvent RESIDUEs. The solvent box will be repeated in all three spatial directions to create a large solvent sphere with a radius of radius angstroms.

The position argument defines where the center of the solvent cap is to be placed. If position is a RESIDUE, ATOM, or a LIST of UNITs, RESIDUEs, or ATOMs, then the geometric center of the ATOMs within the object will be used as the center of the solvent cap sphere. If position is a LIST containing three NUMBERs, then the position argument will be treated as a vector that defines the position of the solvent cap sphere center.

The optional closeness parameter can be used to control how close, in angstroms, solvent ATOMs can come to solute ATOMs. The default value of the closeness argument is 1.0. Smaller values allow solvent ATOMs to come closer to solute ATOMs. The criterion for rejection of overlapping solvent RESIDUEs is if the distance between any solvent ATOM to the closest solute ATOM is less than the sum of the ATOMs VANDERWAAL's distances multiplied by the closeness argument.

This command modifies the solute UNIT in several ways. First, the UNIT is modified by the addition of solvent RESIDUEs copied from the solvent UNIT. Secondly, the cap parameter of the UNIT solute is modified to reflect the fact that a solvent cap has been created around the solute.

```
>> mol = loadpdb my.pdb
```

```
>> solvateCap mol TIP3PBOX mol.2.CA 8.0 2.0
Added 3 residues.
```

3.6.44. solvateDontClip

`solvateDontClip solute solvent buffer [ closeness ]`

```
UNIT    solute
UNIT    solvent
object  buffer
NUMBER  closeness
```

This command is identical to the *solvateBox* command except that the solvent box that is created is not clipped to the boundary of the buffer region. This command forms larger solvent boxes than does *solvateBox* because it does not cause solvent that is outside the buffer region to be discarded. This helps to preserve the periodic structure of properly constructed solvent boxes, preventing hot-spots from forming.

```
>> mol = loadpdb my.pdb
>> solvateDontClip mol TIP3PBOX 10
  Solute vdw bounding box:              7.512 12.339 12.066
  Total bounding box for atom centers:  27.512 32.339 32.066
  Solvent unit box:                     18.774 18.774 18.774
  Total vdw box size:                   41.120 40.899 41.075 angstroms.
  Total mass 30595.088 amu,  Density 0.735 g/cc
  Added 1680 residues.
```

Note the larger number of waters added, compared to solvateBox; in the case of this solute and choice of buffer, the overall box size is increased by about 10 angstroms in each direction.

3.6.45. solvateOct

`solvateOct solute solvent buffer [aniso] [ closeness ]`

```
UNIT                    _solute_
UNIT                    _solvent_
object                  _buffer_
NUMBER                  _closeness_
```

The solvateOct command is the same as solvateBox, except the corners of the box are sliced off, resulting in a truncated octahedron, which typically gives a more uniform distribution of solvent around the solute. In solvateOct, when a LIST is given for the buffer argument, four numbers are given instead of three, where the fourth is the diagonal clearance. If 0.0 is given as the fourth number, the diagonal clearance resulting from the application of the x,y,z clearances is reported. If a non-0 value is given, this may require scaling up the other clearances, which is also reported.

Unless the 'aniso' option is used, an isometric truncated octahedron is produced and rotated to an orientation used by the *sander* PME code. (Note: don't use the 'aniso'

option unless you are sure you know what you are doing; it is only there for expert backward compatibility, and probably has no real use anymore.)

3.6.46. solvateShell

```
solvateShell solute solvent thickness [ closeness ]
```

```
UNIT    solute
UNIT    solvent
NUMBER  thickness
NUMBER  closeness
```

The *solvateShell* command adds a solvent shell to the solute UNIT. The resulting solute/solvent UNIT will be irregular in shape since it will reflect the contours of the solute. The solute UNIT is modified by the addition of solvent RESIDUEs. The solvent box will be repeated in three directions to create a large solvent box that can contain the entire solute and a shell thickness angstroms thick. The solvent RESIDUEs are then added to the solute UNIT if they lie within the shell defined by thickness and do not overlap with the solute ATOMs. The optional closeness parameter can be used to control how close solvent ATOMs can come to solute ATOMs. The default value of the closeness argument is 1.0. Please see the *solvateBox* command for more details on the closeness parameter.

```
>> mol = loadpdb my.pdb
>> solvateShell mol TIP3PBOX 8.0
  Solute vdw bounding box:              7.512 12.339 12.066
  Total bounding box for atom centers:  23.512 28.339 28.066
  Solvent unit box:                     18.774 18.774 18.774
  Added 147 residues.
```

3.6.47. source

```
source filename
```

```
STRING  filename
```

This command executes commands within a text file. To display the commands as they are read, see the *verbosity* command.

3.6.48. transform

```
transform atoms, matrix
```

```
CONT    atoms
LIST    matrix
```

Transform all of the ATOMs within atoms by the (3×3) or (4×4) matrix represented by the nine or sixteen NUMBERs in the LIST of LISTs *matrix*. The general matrix looks like:

```
r11 r12 r13 -tx
r21 r22 r23 -ty
r31 r32 r33 -tz
0   0   0   1
```

The matrix elements represent the intended symmetry operation. For example, a reflection in the (x, y) plane would be produced by the matrix:

```
1   0    0
0   1    0
0   0   -1
```

This reflection could be combined with a six angstrom translation along the x-axis by using the following matrix.

```
1   0    0  6
0   1    0  0
0   0   -1  0
0   0    0  1
```

In the following example, wrB is transformed by an inversion operation:

```
transform wrpB {
 { -1  0  0  }
 {  0 -1  0  }
 {  0  0 -1  }
 }
```

3.6.49. translate

`translate atoms direction`

```
CONT    atoms
LIST    direction
```

Translate all of the ATOMs within atoms by the vector defined by the three NUMBERs in the LIST *direction*.

Example:

```
translate wrpB { 0  0 -24.53333 }
```

3.6.50. verbosity

`verbosity level`

```
NUMBER  level
```

This command sets the level of output that LEaP provides the user. A value of 0 is the

default, providing the minimum of messages. A value of 1 will produce more output, and a value of 2 will produce all of the output of level 1 and display the text of the script lines executed with the *source* command. The following line is an example of this command:

```
> verbosity 2
Verbosity level: 2
```

3.6.51. zMatrix

```
zMatrix object zmatrix
```

```
CONT    object
LIST    matrix
```

The *zMatrix* command is quite complicated. It is used to define the external coordinates of ATOMs within object using internal coordinates. The second parameter of the *zMatrix* command is a LIST of LISTs; each sub-list has several arguments:

```
{ a1 a2 bond12 }
```

This entry defines the coordinate of a1 by placing it bond12 angstroms along the x-axis from ATOM a2. If ATOM a2 does not have coordinates defined then ATOM a2 is placed at the origin.

```
{ a1 a2 a3 bond12 angle123 }
```

This entry defines the coordinate of a1 by placing it bond12 angstroms away from ATOM a2 making an angle of angle123 degrees between a1, a2 and a3. The angle is measured in a right hand sense and in the x-y plane. ATOMs a2 and a3 must have coordinates defined.

```
{ a1 a2 a3 a4 bond12 angle123 torsion1234 }
```

This entry defines the coordinate of a1 by placing it bond12 angstroms away from ATOM a2, creating an angle of angle123 degrees between a1, a2, and a3, and making a torsion angle of torsion1234 between a1, a2, a3, and a4.

```
{ a1 a2 a3 a4 bond12 angle123 angle124 orientation }
```

This entry defines the coordinate of a1 by placing it bond12 angstroms away from ATOM a2, making angles angle123 between ATOMs a1, a2, and a3, and angle124 between ATOMs a1, a2, and a4. The argument orientation defines whether the ATOM a1 is above or below a plane defined by the ATOMs a2, a3, and a4. If orientation is positive then a1 will be placed in such a way so that the inner product of (a3-a2) cross (a4-a2) with (a1-a2) is positive. Otherwise a1 will be placed on the other side of the plane. This allows the coordinates of a molecule like fluoro-chloro-bromo-methane to be defined without having to resort to dummy atoms.

The first arguments within the *zMatrix* entries (a1, a2, a3, a4) are either ATOMs or STRINGs containing names of ATOMs within object. The subsequent arguments are all

NUMBERs. Any ATOM can be placed at the a1 position, even those that have coordinates defined. This feature can be used to provide an endless supply of dummy atoms, if they are required. A predefined dummy atom with the name "*" (a single asterisk, no quotes) can also be used.

There is no order imposed in the sub-lists. The user can place sub-lists in arbitrary order, as long as they maintain the requirement that all atoms a2, a3, and a4 must have external coordinates defined, except for entries that define the coordinate of an ATOM using only a bond length. (See the *add* command for an example of the *zMatrix* command.)

4. Antechamber

This is a set of tools to generate files for organic molecules, which can then be read into LEaP. The Antechamber suite was written by Junmei Wang, and is designed to be used in conjunction with the "general AMBER force field (GAFF)" (*gaff.dat*) [7]. See Ref. [55] for an explanation of the algorithms used to classify atom and bond types, to assign charges, and to estimate force field parameters that may be missing in *gaff.dat.*

Like the traditional AMBER force fields, GAFF uses a simple harmonic function form for bonds and angles. Unlike the traditional AMBER force fields, atom types in GAFF are more general and cover most of the organic chemical space. In total there are 33 basic atom types and 22 special atom types. The charge methods used in GAFF can be HF/6-31G* RESP or AM1-BCC [56,57]. All of the force field parameterizations were carried out with HF/6-31G* RESP charges. However, in most cases, AM1-BCC, which was parameterized to reproduce HF/6-31G* RESP charges, is recommended in large-scale calculations because of its efficiency.

The van der Waals parameters are the same as those used by the traditional AMBER force fields. The equilibrium bond lengths and bond angles came from statistics derived from the Cambridge Structural Database, and *ab initio* calculations at the MP2/6-31G* level. The force constants for bonds and angles were estimated using empirical models, and the parameters in these models were trained using the force field parameters in the traditional AMBER force fields. General torsional angle parameters were extensively applied in order to reduce the huge number of torsional angle parameters to be derived. The force constants and phase angles in the torsional angle parameters were optimized using our PARMSCAN package [58], with an aim to reproduce the rotational profiles depicted by high-level *ab initio* calculations [geometry optimizations at the MP2/6-31G* level, followed by single point calculations at MP4/6-311G(d,p)].

By design, GAFF is a complete force field (so that missing parameters rarely occur), it covers almost all the organic chemical space that is made up of C, N, O, S, P, H, F, Cl, Br and I. Moreover, GAFF is totally compatible to the AMBER macromolecular force fields. We believe that the combination of GAFF with AMBER macromolecular force fields will provide an useful molecular mechanical tool for rational drug design, especially in binding free energy calculations and molecular docking studies. are introduced.

4.1. Principal programs

The *antechamber* program itself is the main program of Antechamber: if your molecule falls in fairly broad categories, this should be all you need to convert an input pdb file into files ready for LEaP.

If there are missing parameters after *antechamber* is finished, you may want to run *parmchk* to generate a *frcmod* template that will assist you in generating the needed parameters.

4.1.1. antechamber

This is the most important program in the package. It can perform many file conversions, and can also assign atomic charges and atom types. As required by the input, *antechamber* executes the following programs: *divcon, atomtype, am1bcc, bondtype, espgen, respgen* and *prepgen*. It may also generate a lot of intermediate files (all in capital letters). If there is a problem with *antechamber*, you may want to run the individual programs that are described below. Antechamber options are given here:

```
-help  print these instructions
  -i   input file name
  -fi  input file format
  -o   output file name
  -fo  output file format
  -c   charge method
  -cf  charge file name
  -nc  net molecular charge (int)
  -a   additional file name
  -fa  additional file format
  -ao  additional file operation
       crd : only read in coordinate
       crg: only read in charge
       name  : only read in atom name
       type  : only read in atom type
       bond  : only read in bond type
  -m   mulitiplicity (2S+1), default is 1
  -rn  residue name, if not available in the input file,
       default is MOL
  -rf  residue toplogy file name in prep input file, default
       is molecule.res
  -ch  check file name in gaussian input file, default is
       molecule
  -mk  divcon or mopac keyword in a pair of quotation marks
  -gk  gaussian keyword in a pair of quotation marks
  -df  use divcon flag, 1 - always use divcon if $AMBERHOME
       is set (the default); 0 - not use divcon if $ACHOME is set
  -at  atom type, can be gaff, amber, bcc and sybyl, default
       is gaff
  -du  check atom name duplications, can be yes(y) or no(n),
       default is yes
  -j   atom type and bond type prediction index, default is 4
       0    : no assignment
       1    : atom type
       2    : full  bond types
       3    : part  bond types
       4    : atom and full bond type
       5    : atom and part bond type
  -s   status information, can be 0 (brief), 1 (the default)
       and 2 (verbose)
  -pf  remove the intermediate files: can be yes (y) and no
       (n), default is no

  -i -o -fi and -fo must appear in command lines and the others are optional

            List of the File Formats
```

```
file format type  abbre. index | file format type abbre. index
-----------------------------------------------------------------
Antechamber        ac       1  | Sybyl Mol2          mol2    2
PDB                pdb      3  | Modifiled PDB       mpdb    4
AMBER PREP (int)   prepi    5  | AMBER PREP (car)    prepc   6
Gaussian Z-Matrix  gzmat    7  | Gaussian Cartesian  gcrt    8
Mopac Internal     mopint   9  | Mopac Cartesian     mopcrt 10
Gaussian Output    gout    11  | Mopac Output        mopout 12
Alchemy            alc     13  | CSD                 csd    14
MDL                mdl     15  | Hyper               hin    16
AMBER Restart      rst     17  | Jaguar Cartesian    jcrt   18
Jaguar Z-Matrix    jzmat   19  | Jaguar Output       jout   20
Divcon Input       divcrt  21  | Divcon Output       divout 22
-----------------------------------------------------------------

AMBER restart file can only be read in as additional file

             List of the Charge Methods

charge method     abbre.  index | charge method      abbre.

-------------------------------------------------------------------
RESP               resp     1  |  AM1-BCC            bcc     2
CM1                cm1      3  |  CM2                cm2     4
ESP (Kollman)      esp      5  |  Mulliken           mul     6
Gasteiger          gas      7  |  Read in charge     rc      8
Write out charge   wc       9
-------------------------------------------------------------------
```

Examples:

```
antechamber -i g98.out -fi gout -o sustiva_resp.mol2 -fo mol2 -c resp
antechamber -i g98.out -fi gout -o sustiva_bcc.mol2 -fo mol2 -c bcc -j 5
antechamber -i g98.out -fi gout -o sustiva_gas.mol2 -fo mol2 -c gas
antechamber -i g98.out -fi gout -o sustiva_cm2.mol2 -fo mol2 -c cm2
antechamber -i g98.out -fi gout -o sustiva.ac -fo ac
antechamber -i sustiva.ac -fi ac -o sustiva.mpdb -fo mpdb
antechamber -i sustiva.ac -fi ac -o sustiva.mol2 -fo mol2
antechamber -i sustiva.mol2 -fi mol2 -o sustiva.gzmat -fo gzmat
antechamber -i sustiva.ac -fi ac -o sustiva_gas.ac -fo ac -c gas
antechamber -i mtx.pdb -fi pdb -o mtx.mol2 -fo mol2 -c rc -cf mtx.charge
```

The *-rn* line specifies the residue name to be used; thus, it must be one to three characters long. The *-at* flag is used to specify whether atom types are to be created for the general AMBER force field (gaff) or for atom types consistent with parm94.dat and parm99.dat (amber). Atom types for gaff are all in lower case, and the AMBER atom types are always in upper case. If you are using *antechamber* to create a modified residue for use with the standard AMBER parm94/parm99 force fields, you should set this flag to `amber`; if you are looking at a more

arbitrary molecule, set this to `gaff`, even if you plan to use this as a ligand bound to a macromolecule described by the AMBER force fields.

4.1.2. parmchk

Parmchk reads in an ac file as well as a force field file (*gaff.dat* in $AMBERHOME/dat/leap/parm). It writes out a frcmod file for the missing parameters. For each atom type, an atom type corresponding file (ATCOR.DAT) lists its replaceable general atom type. Be careful to those problematic parameters indicated with "ATTN, need revision".

```
Usage: parmchk -i input file name
               -o frcmod file name
               -f input file format (prepi, ac ,mol2)
               -p ff parmfile
               -c atom type corresponding file, default is ATCOR.DAT
```

Example:

```
parmchk -i sustiva.prep -f prepi -o frcmod
```

This command reads in sustiva.prep and finds the missing force field parameters listed in frcmod.

4.2. A simple example for antechamber

The most common use of the *antechamber* program suite is to prepare input files for LEaP, starting from a three-dimensional structure, as found in a pdb file. The *antechamber* suite automates the process of developing a charge model and assigning atom types, and partially automates the process of developing parameters for the various combinations of atom types found in the molecule.

As with any automated procedure, caution should be taken to examine the output. Furthermore, the procedure, although carefully tested, has not been widely used by lots of people, so users should certainly be on the lookout for unusual or incorrect behavior.

Suppose you have a PDB-format file for your ligand, say thiophenol, which looks like this:

```
ATOM      1  CG  TP      1      -1.959   0.102   0.795
ATOM      2  CD1 TP      1      -1.249   0.602  -0.303
ATOM      3  CD2 TP      1      -2.071   0.865   1.963
ATOM      4  CE1 TP      1      -0.646   1.863  -0.234
ATOM      5  C6  TP      1      -1.472   2.129   2.031
ATOM      6  CZ  TP      1      -0.759   2.627   0.934
ATOM      7  HE2 TP      1      -1.558   2.719   2.931
ATOM      8  S15 TP      1      -2.782   0.365   3.060
ATOM      9  H19 TP      1      -3.541   0.979   3.274
ATOM     10  H29 TP      1      -0.787  -0.043  -0.938
ATOM     11  H30 TP      1       0.373   2.045  -0.784
ATOM     12  H31 TP      1      -0.092   3.578   0.781
ATOM     13  H32 TP      1      -2.379  -0.916   0.901
```

(This file may be found at *$AMBERHOME/test/antechamber/tp/tp.pdb*). The basic command to create a *mol2* file for LEaP is just:

```
antechamber -i tp.pdb -fi pdb -o tp.mol2 -fo mol2 -c bcc
```

This command says that the input format is pdb, output format is Sybyl mol2, and the BCC charge model is to be used. The output file is shown in the box titled *tp.mol2*. The format of this file is a common one understood by many programs.

tp.mol2

```
@<TRIPOS>MOLECULE
TP
   13    13     1     0     0
SMALL
bcc


@<TRIPOS>ATOM
      1 CG          -1.9590    0.1020    0.7950 ca        1 TP       -0.1186
      2 CD1         -1.2490    0.6020   -0.3030 ca        1 TP       -0.1138
      3 CD2         -2.0710    0.8650    1.9630 ca        1 TP        0.0162
      4 CE1         -0.6460    1.8630   -0.2340 ca        1 TP       -0.1370
      5 C6          -1.4720    2.1290    2.0310 ca        1 TP       -0.1452
      6 CZ          -0.7590    2.6270    0.9340 ca        1 TP       -0.1122
      7 HE2         -1.5580    2.7190    2.9310 ha        1 TP        0.1295
      8 S15         -2.7820    0.3650    3.0600 sh        1 TP       -0.2540
      9 H19         -3.5410    0.9790    3.2740 hs        1 TP        0.1908
     10 H29         -0.7870   -0.0430   -0.9380 ha        1 TP        0.1345
     11 H30          0.3730    2.0450   -0.7840 ha        1 TP        0.1336
     12 H31         -0.0920    3.5780    0.7810 ha        1 TP        0.1332
     13 H32         -2.3790   -0.9160    0.9010 ha        1 TP        0.1432
@<TRIPOS>BOND
     1    1    2 ar
     2    1    3 ar
     3    1   13 1
     4    2    4 ar
     5    2   10 1
     6    3    5 ar
     7    3    8 1
     8    4    6 ar
     9    4   11 1
    10    5    6 ar
    11    5    7 1
    12    6   12 1
    13    8    9 1
@<TRIPOS>SUBSTRUCTURE
     1 TP          1 TEMP              0 ****  ****    0 ROOT
```

You can now run *parmchk* to see if all of the needed force field parameters are available:

```
parmchk -i tp.mol2 -f mol2 -o frcmod
```

This yields the *frcmod* file:

```
remark goes here
MASS

BOND

ANGLE

DIHE

IMPROPER
ca-ca-ca-ha         1.1          180.0         2.0          General improper
ca-ca-ca-sh         1.1          180.0         2.0          Using default value

NONBON
```

In this case, there were two missing dihedral parameters from the *gaff.dat* file, which were assigned a default value. (As *gaff.dat* continues to be developed, there should be fewer and fewer missing parameters to be estimated by *parmchk*.) In rare cases, *parmchk* may be unable to make a good estimate; it will then insert a placeholder (with zeros everywhere) into the *frcmod* file, with the comment "ATTN: needs revision". After manually editing this to take care of the elements that "need revision", you are ready to read this residue into LEaP, either as a residue on its own, or as part of a larger system. The following LEaP input file (*leap.in*) will just create a system with thiophenol in it:

```
source leaprc.gaff
mods = loadAmberParams frcmod
TP = loadMol2 tp.mol2
saveAmberParm TP prmtop inpcrd
quit
```

You can read this into LEaP as follows:

```
tleap -s -f leap.in
```

This will yield a *prmtop* and *inpcrd* file. If you want to use this residue in the context of a larger system, you can insert commands after the loadAmberPrep step to construct the system you want, using standard LEaP commands.

In this respect, it is worth noting that the atom types in *gaff.dat* are all lower-case, whereas the atom types in the standard AMBER force fields are all upper-case. This means that you can load both *gaff.dat* and (say) *parm99.dat* into LEaP at the same time, and there won't be any conflicts. Hence, it is generally expected that you will use one of the AMBER force fields to describe your protein or nucleic acid, and the *gaff.dat* parameters to describe your ligand; as mentioned above, *gaff.dat* has been designed with this in mind, *i.e.* to produce molecular mechanics

descriptions that are generally compatible with the AMBER macromolecular force fields.

The procedure above only works as it stands for neutral molecules. If your molecule is charged, you need to set the *-nc* flag in the initial *antechamber* run. Also note that this procedure depends heavily upon the initial 3D structure: it must have all hydrogens present, and the charges computed are those for the conformation you provide, after minimization in the AM1 Hamiltonian. In fact, this means that you must have an reasonable all-atom initial model of your molecule (so that it can be minimized with the AM1 Hamiltonian), and you must specify what its net charge is. The system should really be a closed-shell molecule, since all of the atom-typing rules assume this implicitly.

Further examples of using *antechamber* to create force field parameters can be found in the *$AMBERHOME/test/antechamber* directory. Here are some practical tips from Junmei Wang:

(1) For the input molecules, make sure there are no open valences and the structures are reasonable.

(2) Failures are most likely produced when *antechamber* infers an incorrect connectivity. In such cases, you can revise by hand the connectivity information in "ac" or "mol2" files. Systematic errors could be corrected by revising the parameters in CONNECT.TPL in $AMBERHOME/dat/antechamber.

(3) It is a good idea to check the intermediate files in case of a program failure, and you can run separate programs one by one. Use the "-s 2" flag to *antechamber* to see details of what it is doing.

(4) Please visit `amber.scripps.edu/antechamber/antechamber.html` to obtain the latest information about *antechamber* development and to download the latest GAFF parameters. Please report program failures to Junmei Wang at <jwang@encysive.com>.

4.3. Programs called by antechamber

The following programs are automatically called by *antechamber* when needed. Generally, you should not need to run them yourself, unless problems arise and/or you want to fine-tune what *antechamber* does.

4.3.1. atomtype

Atomtype reads in an ac file and assigns the atom types. You may find the default definition files in $AMBERHOME/dat/antechamber: ATOMTYPE_AMBER.DEF (AMBER), ATOMTYPE_GFF.DEF (general AMBER force field). ATOMTYPE_GFF.DEF is the default definition file.

```
Usage: atomtype -i input file name
                -o output file name (ac)
                -f input file format(ac (the default) or mol2)
                -p amber or gaff or bcc or gas, it is suppressed by "-d" option
                -d atom type definition file, optional
```

Example:

```
atomtype -i sustiva_resp.ac -o sustiva_resp_at.ac -f ac -p amber
```

This command assigns atom types for *sustiva_resp.ac* with amber atom type definitions. The output file name is *sustiva_resp_at.ac*

4.3.2. am1bcc

Am1bcc first reads in an ac or mol2 file with or without assigned AM1-BCC atom types and bond types. Then the bcc parameter file (the default, BCCPARM.DAT is in $AMBERHOME/dat/antechamber) is read in. An ac file with AM1-BCC charges [56,57] is written out. Be sure the charges in the input ac file are AM1-Mulliken charges.

```
Usage: am1bcc -i input file name in ac format
              -o output file name
              -f output file format(pdb or ac, optional, default is ac)
              -p bcc parm file name (optional))
              -j atom and bond type judge option, default is 0)
                 0: No judgement
                 1: Atom type
                 2: Full bond type
                 3: Partial bond type
                 4: Atom and full bond type
                 5: Atom and partial bond type
```

Example:

```
am1bcc -i comp1.ac -o comp1_bcc.ac -f ac -j 4
```

This command reads in *comp1.ac*, assigns both atom types and bond types and finally performs bond charge correction to get AM1-BCC charges. The '-j' option of 4, which is the default, means that both the atom and bond type information in the input file is ignored and a full atom and bond type assignments are performed. The '-j' option of 3 and 5 implies that bond type information (single bond, double bond, triple bond and aromatic bond) is read in and only a bond type adjustment is performed. If the input file is in mol2 format that contains the basic bond type information, option of 5 is highly recommended. *comp1_bcc.ac* is an ac file with the final AM1-BCC charges.

4.3.3. bondtype

bondtype is a program to assign the atom types and bond types according to the AM1-BCC definitions (BCCTYPE.DEF in $AMBERHOME/dat/antechamber). This program can read an ac file or mol2 file; the output file is an ac file with predicted atom types and bond types. You can choose to determine to assign atom types or bond types or both. If there is some problem with the assignment of bond types, you will get some warnings and for each problematic bond, a "!!!" is appended at the end of the line. In initial tests, the current version works for most organic molecules (>95% overall and >90% for charged molecules).

```
Usage: bondtype -i input file name
                -o output file name
                -f input file format (ac or mol2)
                -j judge bond type level option, default is part
                   full  full judgement
                   part  partial judgement, only do reassignment according
```

```
                              to known bond type information in the input file
```

Example:

```
#! /bin/csh -fv
set mols = `/bin/ls *.ac`
foreach mol ($mols)
   set mol_dir = $mol:r
   antechamber -i $mol_dir.ac -fi ac -fo ac -o $mol_dir.ac -c mul
   bondtype -i $mol_dir.ac -f ac -o $mol_dir.dat -j full
   am1bcc -i $mol_dir.dat -o $mol_dir_bcc.ac -f ac -j 0
end
exit(0)
```

The above script finds all the files with the extension of "ac", calculates the Mulliken charges using *antechamber*, and predicts the atom and bond types with bondtype. Finally, AM1-BCC charges are generated by running am1bcc to do the bond charge correction.

4.3.4. prepgen

Prepgen generates the prep input file from an ac file. By default, the program generates a mainchain itself. However, you may also specify the mainchain atom in the mainchain file. From this file, you can also specify which atoms will be deleted, and whether to do charge correction or not. In order to generate the amino-acid-like residue (this kind of residue has one head atom and one tail atom to be connected to other residues), you need a mainchain file. Sample mainchain files are in $AMBERHOME/dat/antechamber.

```
Usage: prepgen -i  input file name(ac)
               -o  output file name
               -f  output file format (car or int, default: int)
               -m  mainchain file name
               -rn residue name (default: MOL)
               -rf residue file name (default: molecule.res)
                   -f -m -rn -rf are optional
```

Examples:

```
prepgen -i sustiva_resp_at.ac -o sustiva_int.prep -f int -rn SUS -rf SUS.res
prepgen -i sustiva_resp_at.ac -o sustiva_car.prep -f car -rn SUS -rf SUS.res
prepgen -i sustiva_resp_at.ac -o sustiva_int_main.prep -f int -rn SUS
        -rf SUS.res -m mainchain_sus.dat
prepgen -i ala_cm2_at.ac -o ala_cm2_int_main.prep -f int -rn ALA -rf ala.res
        -m mainchain_ala.dat
```

The above commands generate different kinds of prep input files with and without specifying a mainchain file.

4.3.5. espgen

Espgen reads in a gaussian (92,94,98,03) output file and extracts the ESP information. An esp file for the resp program is generated.

```
Usage: espgen -i   input file name
              -o   output file name
```

Example:

```
espgen -i sustiva_g98.out -o sustiva.esp
```

The above command reads in sustiva_g98.out and writes out sustiva.esp, which can be used by the resp program. Note that this program replaces shell scripts formerly found on the AMBER web site that perform equivalent tasks.

4.3.6. respgen

Respgen generates the input files for two-stage resp fitting. The current version only supports single molecule fitting. Atom equivalence is recognized automatically.

```
Usage: respgen -i input file name(ac)
               -o output file name
               -f output file format (resp1 or resp2)
                  resp1 - first stage resp fitting
                  resp2 - second stage resp fitting
```

Example:

```
respgen -i sustiva.ac -o sustiva.respin1 -f resp1
respgen -i sustiva.ac -o sustiva.respin2 -f resp2
resp -O -i sustiva.respin1 -o sustiva.respout1 -e sustiva.esp -t qout_stage1
resp -O -i sustiva.respin2 -o sustiva.respout2 -e sustiva.esp -q qout_stage1
        -t qout_stage2
antechamber -i sustiva.ac -fi ac -o sustiva_resp.ac -fo ac -c rc
            -cf qout_stage2
```

The above commands first generate the input files (sustiva.respin1 and sustiva.respin2) for resp fitting, then do two-stage resp fitting and finally use *antechamber* to read in the resp charges and write out an ac file − sustiva_resp.ac.

4.4. Miscellaneous programs

The Antechamber suite also contains some utility programs that perform various tasks in molecular mechanical calculations. They are listed in alphabetical order.

4.4.1. crdgrow

Crdgrow reads an incomplete pdb file (at least three atoms in this file) and a prep input file, and then generates a complete pdb file. It can be used to do residue mutation. For example, if you want to change one protein residue to another one, you can just keep the mainchain atoms in a pdb file and read in the prep input file of the residue to be changed, and crdgrow will generate the

coordinates of the missing atoms.

```
Usage: crdgrow -i input file name
               -o output file name
               -p prepin file name
               -f prepin file format: prepi (the default)
```

Example:

```
crdgrow -i ref.pdb -o new.pdb -p sustiva_int.prep
```

This command reads in ref.pdb (only four atoms) and prep input file sustiva_int.prep, then generates the coordinates of the missing atoms and writes out a pdb file (new.pdb).

4.4.2. parmcal

Parmcal is an interactive program to calculate the bond length and bond angle parameters, according to the rules outlined in [7].

```
Please select:
1. calculate the bond length parameter: A-B
2. calculate the bond angle parameter: A-B-C
3. exit
```

4.4.3. database

Database reads in a multiple sdf or mol2 file and a description file to run a set of commands for each record sequentially. The commands are defined in the description file.

```
Usage: database -i database file name
                -d definition file name
```

Example:

```
database -i sample_database.mol2 -d mol2.def
```

This command reads in a multiple mol2 database - sample_database.mol2 and a description file mol2.def to run a set of commands (defined in mol2.def) to generate prep input files and merge them to a single file called total.prepi. Both files are located in the following directory: $AMBERHOME/test/antechamber/database/mol2.

5. Sander basics

5.1. Introduction.

This is a guide to *sander*, the Amber module which carries out energy minimization, molecular dynamics, and NMR refinements. The acronym stands for **S**imulated **A**nnealing with **N**MR-**D**erived **E**nergy **R**estraints, but this module is used for a variety of simulations that have nothing to do with NMR refinement. Some general features are outlined in the following paragraphs:

(1) *Sander* provides direct support for several force fields for proteins and nucleic acids, and for several water models and other organic solvents. The basic force field implemented here has the following form, which is about the simplest functional form that preserves the essential nature of molecules in condensed phases:

$$
\begin{aligned}
U(\mathbf{R}) = & \sum_{bonds} K_r\,(r - r_{eq})^2 && \textit{bond} \\
& + \sum_{angles} K_\theta\,(\theta - \theta_{eq})^2 && \textit{angle} \\
& + \sum_{dihedrals} \frac{V_n}{2}\,(1 + \cos[n\phi - \gamma]) && \textit{dihedral} \\
& + \sum_{i<j}^{atoms} \frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} && \textit{van der Waals} \\
& + \sum_{i<j}^{atoms} \frac{q_i q_j}{\varepsilon R_{ij}} && \textit{electrostatic}
\end{aligned}
\qquad (5.1)
$$

"Non-additive" force fields based on atom-centered dipole polarizabilities can also be used. These add a "polarization" term to what was given above:

$$
E_{pol} = -\frac{1}{2} \sum_{i}^{atom} \mu_i \bullet \mathbf{E}_i^{(o)} \qquad \textit{polarization} \qquad (5.2)
$$

where μ_i is an induced atomic dipole. In addition, charges that are not centered on atoms, but are off-center (as for lone-pairs or "extra points") can be included in the force field.

(2) The particle-mesh Ewald (PME) procedure (or, optionally, a "true" Ewald sum) is used to handle long-range electrostatic interactions. Long-range van der Waals interactions are estimated by a continuum model. Biomolecular simulations in the NVE ensemble (*i.e.* with Newtonian dynamics) conserve energy well over multi-nanosecond runs without modification of the equations of motion.

(3) Two periodic imaging geometries are included: rectangular parallelepiped and truncated octahedron (box with corners chopped off). (*Sander* itself can handle many other periodically-replicating boxes, but input and output support in *LEaP* and *ptraj* is only available right now for these two.) The size of the repeating unit can be coupled to a given external pressure, and velocities can be coupled to a given external temperature by several schemes. The external conditions and coupling constants can be varied over time, so various simulated annealing protocols can be specified in a simple and flexible manner.

(4) It is also possible to carry out non-periodic simulations in which aqueous solvation effects are represented *implicitly* by a generalized Born/ surface area model by adding the following two terms to the "vacuum" potential function:

$$+\sum_{ij}^{atoms}\frac{q_i q_j}{f^{gb}(R_{ij})} \qquad +\sigma\mathbf{A} \qquad implicit\ \ solvation \tag{5.3}$$

The first term accounts for the polar part of solvation (free) energy via the f^{gb} function [59] designed to provide an approximation for the reaction field potential, and the second represents the non-polar contribution which is taken to be proportional to the surface area of the molecule *A*.

(5) Users can define internal restraints on bonds, valence angles, and torsions, and the force constants and target values for the restraints can vary during the simulation. The relative weights of various terms in the force field can be varied over time, allowing one to implement a variety of simulated annealing protocols in a single run.

(6) Internal restraints can be defined to be "time-averaged", that is, restraint forces are applied based on the averaged value of an internal coordinate over the course of the dynamics trajectory, not only on its current value. Alternatively, restraints can be "ensemble-averaged" using the locally-enhanced-sampling (LES) option.

(7) Restraints can be directly defined in terms of NOESY intensities (calculated with a relaxation matrix technique), residual dipolar couplings, scalar coupling constants and proton chemical shifts. There are provisions for handling overlapping peaks or ambiguous assignments. In conjunction with distance and angle constraints, this provides a powerful and flexible approach to NMR structural refinements.

(8) Restraints can also be defined in terms of the root-mean-square coordinate distance from some reference structure. This allows one to bias trajectories either towards or away from some target. Free energies can be estimated from non-equilibrium simulations based on targetting restraints.

(9) Free energy calculations, using thermodynamic integration (TI) with a linear or non-linear mixing of the "unperturbed" and "perturbed" Hamiltonian, can be carried out. Alternatively, potentials of mean force can be computed using umbrella sampling.

(10) The empirical valence bond (EVB) scheme can be used to mix "diabatic" states into a potential that can represent many types of chemical reactions that take place in enzymes.

(11) QMMM Calculations where part of the system can be treated quantum mechanically allowing bond breaking and formation during a simulation. Semi-empirical and DFTB Hamiltonians are provided.

5.2. Credits.

The annealing, "weight change," "restraints" and NMR-specific portions of *sander* were primarily written by David Pearlman and David Case. All of the Amber crew listed on the title page contributed to the general portions; the polarization implementation is that of Jim Caldwell, Liem Dang, and Tom Darden, and the "targeted MD" code is from Carlos Simmerling. The pseudocontact shift code was provided by Ivano Bertini of the University of Florence. A brief overview and history of parallel implementations is given in the Installation section, as well as in Ref [3].

Particle Mesh Ewald. The Particle Mesh Ewald (PME) method was implemented originally in Amber 3a by Tom Darden, and has been developed in subsequent versions of Amber by several people, in particular by Tom Darden, Tom Cheatham, Mike Crowley and David Case. The PME method not only provides a better treatment of long range electrostatics (at a modest computational cost), but can be applied in both rectangular and non-rectangular periodic boundary simulations [60-63]. The generalization of this method to systems with polarizable dipoles was carried out by Toukmaji *et al.* [64].

Generalized Born. When *igb*=1, we use the "pairwise" generalized Born model introduced by Hawkins, Cramer and Truhlar [65,66], which is based on earlier ideas by Still and others [59,67-69]. Radii are the Bondi radii [70], optionally with slight modifications of the different types of hydrogen atoms [52] ; the overlap parameters are taken from the TINKER molecular modeling package (`http://tinker.wustl.edu`). The effects of added monovalent salt are included at a level that approximates the solutions of the linearized Poisson-Boltzmann equation [71]. The implementation is by David Case, who thanks Charlie Brooks for inspiration.

When *igb*=2 or *igb*=5, modifications outlined by Onufriev, Bashford and Case are used [54].

When *igb*=7, a pairwise molecular volume correction described by Mongan *et al.* is applied. A custom set of overlap parameters are used with this model [11].

Solvent-accessible surface areas It is also possible to carry out GB/SA simulations, using the surface areas (SA) to approximate the cavity and van der Waals contributions to solvation. The surface area is calculated using the LCPO (Linear Combinations of Pairwise Overlaps) model [72].

Hybrid QM/MM. The built-in semi-empirical QM/MM support was written by Ross Walker, Mike Crowley and David Case [73] This implementation provides support for the MNDO [74] , AM1 [75] , PM3 [76] , MNDO-PDDG [77] , PM3-PDDG [77] and PM3-CARB1 [78] semi-empirical Hamiltonians. The QM/MM Generalised Born implementation uses the model described by Pellegrini and Field [79] while regular QM/MM Ewald support is based on the work of Nam et al [80]. Support for QM/MM PME simulations was developed by Ross Walker, Mike Crowley and David Case [73] The initial parallel implementation of this code was written by Ross Walker. PIMD support is maintained by Wei Zhang. SCC-DFTB support was written by Gustavo Seabra, Ross Walker and Adrian Roitberg.

5.3. File usage.

```
sander [-help] [-O] [-A] -i mdin -o mdout -p prmtop -c inpcrd -r restrt
        -ref refc -x mdcrd -y inptraj -v mdvel -e mden -inf mdinfo -radii radii
        -cpin cpin -cpout cpout -cprestrt cprestrt -mmtsb mmtsb_setup.job
```

`-O` Overwrite output files if they exist.

`-A` Append output files if they exist, (used mainly for replica exchange).

Here is a brief description of the files referred to above; the first five files are used for every run, whereas the remainder are only used when certain options are chosen.

file	*in/out*	*purpose*
mdin	input	control data for the min/md run
mdout	output	user readable state info and diagnostics -o stdout will send output to stdout (to the terminal) instead of to a file.
mdinfo	output	latest mdout-format energy info
prmtop	input	molecular topology, force field, periodic box type, atom and residue names
inpcrd	input	initial coordinates and (optionally) velocities and periodic box size
refc	input	(optional) reference coords for position restraints; also used for targeted MD
mdcrd	output	coordinate sets saved over trajectory
inptraj	input	input coordinate sets in trajectory format, when imin=5
mdvel	output	velocity sets saved over trajectory
mden	output	extensive energy data over trajectory
restrt	output	final coordinates, velocity, and box dimensions if any - for restarting run
inpdip	input	polarizable dipole file, when indmeth=3
rstdip	output	polarizable dipole file, when indmeth=3
cpin	input	protonation state definitions
cprestrt		protonation state definitions, final protonation states for restart (same format as cpin)
cpout	output	protonation state data saved over trajectory

5.4. Example input files.

Here are a couple of sample files, just to establish a basic syntax and appearance. There are more examples of NMR-related files later in this chapter.

1. Simple restrained minimization

```
Minimization with Cartesian restraints
 &cntrl
   imin=1, maxcyc=200,          (invoke minimization)
   ntpr=5,                      (print frequency)
   ntr=1,                       (turn on Cartesian restraints)
   restraint_wt=1.0,            (force constant for restraint)
   restraintmask=':1-58',       (atoms in residues 1-58 restrained)
 /
```

2. "Plain" molecular dynamics run

```
molecular dynamics run
&cntrl
   imin=0, irest=1, ntx=5,                 (restart MD)
   ntt=3, temp0=300.0, gamma_ln=5.0,       (temperature control)
   ntp=1, taup=2.0,                        (pressure control)
   ntb=2, ntc=2, ntf=2,                    (SHAKE, periodic bc.)
   nstlim=500000,                          (run for 0.5 nsec)
   ntwe=100, ntwx=100, ntpr=200,           (output frequency)
/
```

3. Self-guided Langevin dynamics run

```
Self-guided Langevin dynamics run
&cntrl
   imin=0, irest=0, ntx=1,                 (start LD)
   ntt=3, temp0=300.0,gamma_ln=1.0         (temperature control)
   ntc=3, ntf=3,                           (SHAKE)
   nstlim=500000,                          (run for 0.5 nsec)
   ntwe=100, ntwx=100, ntpr=200,           (output frequency)
   isgld=1, tsgavg=0.2,tempsg=1.0          (SGLD)
/
```

5.5. Overview of the information in the input file.

Section	*Format*
Standard minimization and dynamics input	One or more title lines, followed by the (required) `&cntrl` and (optional) `&pb`, `&ewald`, `&qmmm`, `&amoeba` or `&debugf` namelist blocks. The basic input is described in **Sections 5.6 and 5.7.**
Varying conditions	Parameters for changing temperature, restraint weights, etc. during the MD run. Each parameter is specified by a separate `&wt` namelist block, ending with `&wt type='END', /.` This input is described in **Section 5.8.**
File redirection	TYPE=*filename* lines. Section ends with the first non-blank line which does not correspond to a recognized redirection. This input is described in **Section 5.9.**
Group information	Read if *ntr*, *ibelly* or *idecomp* are set to non-zero values, and if some other conditions are satisfied; see sections on these variables, below. This input format is described in **Appendix B.**

5.6. General minimization and dynamics parameters.

Each of the variables listed below is input in a namelist statement with the namelist identifier `&cntrl`. You can enter the parameters in any order, using keyword identifiers. Variables that are not given in the namelist input retain their default values. Support for namelist input is included in almost all current Fortran compilers, and is a standard feature of Fortran 90. A detailed description of the namelist convention is given in Appendix A.

In general, namelist input consists of an arbitrary number of comment cards, followed by a record whose first 7 characters after a " `&`" (e.g. " `&cntrl` ") name a group of variables that can be set by name. This is followed by statements of the form " `maxcyc=500, diel=2.0, ...` ", and is concluded by an " `/` " token. The first line of input contains a title, which is then followed by the `&cntrl` namelist. Note that the first character on each line of a namelist block must be a blank.

Some of the options and variables are much more important, and commonly modified, than are others. We have denoted the "common" options by printing them in **boldface** below. In general, you can skip reading about the non-bold options on a first pass, and you should change these from their defaults only if you think you know what you are doing.

5.6.1. General flags describing the calculation.

IMIN Flag to run minimization

= 0 No minimization (only do molecular dynamics; default)

= 1 Perform minimization (and no molecular dynamics)

= 5 Read in a trajectory for analysis.

Although *sander* will write energy information in the output files (using *ntpr*), it is often desirable to calculate the energies of a set of structures at a later point. In particular, one may wish to post-process a set of structures using a different energy function than was used to generate the structures. A example of this is MM-PBSA analysis, where the explicit water is removed and replaced with a continuum model.

When imin is set to 5 *sander* will expect to read a trajectory file from the inptraj file (specified using `-y` on the command line), and will perform the functions described in the *mdin* file for each of the structures in the trajectory file. The final structures from each minimization will be written to the normal *mdcrd* file.

For example, when *imin=5* and *maxcyc=1000*, sander will minimize each structure in the trajectory for 1000 steps and write a minimized coordinate set for each frame to the *mdcrd* file. If *maxcyc=1*, then the output file can be used to extract the energies of each of the coordinate sets in the *inptraj* file.

NMROPT

= 0 no nmr-type analysis will be done; default (Note: this variable replaces `nmrmax` from previous versions, and has a slightly different meaning.)

> 0 NMR restraints/weight changes will be read

= 2 NOESY volume restraints or chemical shift restraints will be read as well

---

5.6.2. Nature and format of the input.

NTX Option to read the initial coordinates, velocities and box size from the "inpcrd" file. The options 1-2 must be used when one is starting from minimized or model-built coordinates. If an MD restrt file is used as inpcrd, then options 4-7 may be used.

= 1 X is read formatted with no initial velocity information (default)

= 2 X is read unformatted with no initial velocity information

= 4 X and V are read unformatted.

= 5 X and V are read formatted; box information will be read if ntb>0. The velocity information will only be used if *irest*=1.

= 6 X, V and BOX(1..3) are read unformatted; in other respects, this is the same as option "5".

IREST Flag to restart the run.

= 0 No effect (default)

= 1 restart calculation. Requires velocities in coordinate input file, so you also may need to reset NTX if restarting MD

NTRX Format of the Cartesian coordinates for restraint from file "refc". Note: the program expects file "refc" to contain coordinates for all the atoms in the system. A subset for the actual restraints is selected by *restraintmask* in the control namelist.

= 0 Unformatted (binary) form

= 1 Formatted (ascii, default) form

5.6.3. Nature and format of the output.

NTXO Format of the final coordinates, velocities, and box size (if constant volume or pressure run) written to file "restrt".

= 0 Unformatted

= 1 Formatted (default).

NTPR Every NTPR steps energy information will be printed in human-readable form to files "mdout" and "mdinfo". "mdinfo" is closed and reopened each time, so it always contains the most recent energy and temperature. Default 50.

NTAVE Every NTAVE steps of dynamics, running averages of average energies and fluctuations over the last NTAVE steps will be printed out. Default value of 0 disables this printout.

NTWR Every NTWR steps during dynamics, the "restrt" file will be written, ensuring that recovery from a crash will not be so painful. In any case, restrt is written every NSTLIM steps for both dynamics and minimization calculations. If NTWR<0, a unique copy of the file, restrt_nstep, is written every abs(NTWR) steps. This option is useful if for example one wants to run free energy perturbations from multiple starting points or save a series of restrt files for minimization. Default 500.

IWRAP If set to 1, the coordinates written to the restart and trajectory files will be "wrapped" into a primary box. This means that for each molecule, the image closest to the middle of the "primary box" [with x coordinates between 0 and a, y coordinates between 0 and b, and z coordinates between 0 and c] will be the one written to the output file. This often makes the resulting structures look better visually, but has no effect on the energy or forces. Performing such wrapping, however, can mess up diffusion and other calculations. The

default (when *iwrap=0*) is to not perform any such manipulations; in this case it is typical to use *ptraj* as a post-processing program to translate molecules back to the primary box. You may also want to use *iwrap=1* if you are preparing a system for further runs in *gibbs*, since that program requires the coordinates to be wrapped. For very long runs, setting *iwrap*=1 may be required to keep the coordinate output from overflowing the trajectory file format.

NTWX Every NTWX steps the coordinates will be written to file "mdcrd". NTWX=0 inhibits all output. Default 0.

NTWV Every NTWV steps the velocities will be written to file "mdvel". NTWV=0 inhibits all output. Default 0. NTWV=-1 will write velocities into a combined coordinate and velocity file "mdcrd" at the interval defined by NTWX. This option is available only for binary NetCDF output (IOUTFM=1).

NTWE Every NTWE steps the energies and temperatures will be written to file "mden" in compact form. NTWE=0 inhibits all output. Default 0.

IOUTFM Format of velocity and coordinate sets. As of Amber 9, the binary format used in previous versions is no longer supported; binary output is now in NetCDF trajectory format. Binary trajectory files are smaller, higher precision and much faster to read and write than formatted trajectories. You must configure and compile Amber with the -bintraj flag to use the NetCDF format. Note: these values are "backwards" compared to NTRX and NTXO; this is an ancient mistake that we are reluctant to change, since it would break existing scripts.

= 0 Formatted (default)

= 1 Binary NetCDF trajectory

NTWPRT Coordinate/velocity archive limit flag. This flag can be used to decrease the size of the coordinate / velocity archive files, by only including that portion of the system of greatest interest. (E.g. one can print only the solute and not the solvent, if so desired). The Coord/velocity archives will include:

= 0 all atoms of the system (default).

> 0 only atoms 1->NTWPRT.

IDECOMP This option is only really useful in conjunction with *mm_pbsa*, where it is turned on automatically if required. The options are:

= 0 Do nothing (default).

= 1 Decompose energies on a per-residue basis; 1-4 EEL + 1-4 VDW are added to internal (bond, angle, dihedral) energies.

= 2 Decompose energies on a per-residue basis; 1-4 EEL + 1-4 VDW are added to EEL and VDW.

= 3 Decompose energies on a pairwise per-residue basis; the rest is equal to "1".

= 4 Decompose energies on a pairwise per-residue basis; the rest is equal to "2".

If decomp is switched on, residues may be chosen by the RRES and/or LRES card. The RES card determines about which residues information is finally

output. See the *mm_pbsa* chapter for more information. Use of *idecomp* > 0 is incompatible with *ntr* > 0 or *ibelly* > 0.

5.6.4. Frozen or restrained atoms.

IBELLY Flag for belly type dynamics.

= 0 No belly run (default).

= 1 Belly run. A subset of the atoms in the system will be allowed to move, and the coordinates of the rest will be frozen. The *moving* atoms are specified *bellymask*. This option is not available when *igb>0*. Note also that this option does *not* provide any significant speed advantage, and is maintained primarily for backwards compatibility with older version of Amber. Most applications should use the *ntr* variable instead to restrain parts of the system to stay close to some initial configuration.

NTR Flag for restraining specified atoms in Cartesian space using a harmonic potential. The restrained atoms are determined by the *restraintmask* string. The force constant is given by *restraint_wt*. The coordinates are read in "restrt" format from the "refc" file (see NTRX, above).

= 0 No position restraints (default)

= 1 MD with restraint of specified atoms

RESTRAINT_WT

The weight (in $kcal/mol - Å^2$) for the positional restraints. The restraint is of the form $k(\Delta x)^2$, where k is the value given by this variable, and Δx is the difference between one of the Cartesian coordinates of a restrained atom and its reference position. There is a term like this for each Cartesian coordinate of each restrainted atom.

RESTRAINTMASK

String that specifies the *restrained* atoms when *ntr=1*.

BELLYMASK String that specifies the *moving* atoms when *ibelly=1*. The syntax for both *restraintmask* and *bellymask* is given in Chapter 13.5. Note that these mask strings are limited to a maximum of 256 characters.

5.6.5. Energy minimization.

MAXCYC The maximum number of cycles of minimization. Default 1.

NCYC If NTMIN is 1 then the method of minimization will be switched from steepest descent to conjugate gradient after NCYC cycles. Default 10.

NTMIN Flag for the method of minimization.

= 0 Full conjugate gradient minimization. The first 4 cycles are steepest descent at the start of the run and after every nonbonded pairlist update. Note that the Amber7 documentation incorrectly indicated 10 cycles instead of 4. The first Amber version in which the

documentation became incorrect is unknown.

= 1 For NCYC cycles the steepest descent method is used then conjugate gradient is switched on (default).

= 2 Only the steepest descent method is used.

= 3 The XMIN method is used, see *amber8/doc/lmod.pdf*.

= 4 The LMOD method is used, see *amber8/doc/lmod.pdf*.

DX0 The initial step length. If the initial step length is big then the minimizer will try to leap the energy surface and sometimes the first few cycles will give a huge energy, however the minimizer is smart enough to adjust itself. Default 0.01.

DRMS The convergence criterion for the energy gradient: minimization will halt when the root-mean-square of the Cartesian elements of the gradient is less than DRMS. Default 1.0E-4 kcal/mole Å.

5.6.6. Molecular dynamics.

NSTLIM Number of MD-steps to be performed. Default 1.

NSCM Flag for the removal of translational and rotational center-of-mass (COM) motion at regular intervals. For non-periodic simulations, after every NSCM steps, translational and rotational motion will be removed. For periodic systems, just the translational center-of-mass motion will be removed. This flag is ignored for belly simulations. Default 1000.

T The time at the start (psec) this is for your own reference and is not critical. Start time is taken from the coordinate input file if IREST=1. Default 0.0.

DT The time step (psec). Recommended MAXIMUM is .002 if SHAKE is used, or .001 if it isn't. Note that for temperatures above 300K, the step size should be reduced since greater temperatures mean increased velocities and longer distance traveled between each force evaluation, which can lead to anomalously high energies and system blowup. Default 0.001.

NRESPA This variable allows the user to evaluate slowly-varying terms in the force field less frequently. For PME, "slowly-varying" (now) means the reciprocal sum. For generalized Born runs, the "slowly-varying" forces are those involving derivatives with respect to the effective radii, and pair interactions whose distances are greater than the "inner" cutoff, currently hard-wired at 8 Å.
If NRESPA>1 these slowly-varying forces are evaluated every *nrespa* steps. The forces are adjusted appropriately, leading to an impulse at that step. If *nrespa*dt* is less than or equal to 4 fs the energy conservation is not seriously compromised. However if *nrespa*dt* > 4 fs the simulation becomes less stable. Note that energies and related quantities are only accessible every *nrespa* steps, since the values at other times are meaningless.

5.6.7. Self-Guided Langevin dynamics.

Self-guided Langevin dynamics (SGLD) can be used to enhance conformational search efficiency in either a molecular dynamics (MD) simulation (when *gamma_ln=0*) or Langevin dynamics (LD) simulation (when *gamma_ln>0*). This method applies a guiding force calculated during a simulation to accelerate the systematic motion for more efficient conformational sampling [81]. The guiding force can be applied to a part of a simulation system starting from atom *isgsta* to atom *isgend*. The strength of the guiding force is defined by either *tempsg* or *sgft*. A smaller *tempsg* or *sgft* will produce results closer to a normal MD or LD simulation. Normally, *tempsg* or *sgft* is set to the limit that accelerates slow events to an affortable time scale.

ISGLD — The default value of zero disables self-guiding; a positive value enables this feature.

TSGAVG — Local averaging time (*psec*) for the guiding force calculation. Default 0.2 *psec*. A larger value defines a slower motion to be enhanced.

TEMPSG — Guiding temprature (*K*). Defines the strength of the guiding force in temperature unit. Default 1.0 K. The default value is recommended for a noticeable enhancement in conformational search. Once *tempsg* is set, *sgft* will fluctuate and be printed out in the output file.

SGFT — Guiding factor. Defines the strength of the guiding force when *tempsg*=0. Default 0.0. *tempsg*>0 will overide *sgft*. Because *sgft* varies with systems and simulation conditions, it is recommended to read *sgft* values from the output file of a SGLD simulation with *tempsg=1 K*. Setting *tempsg=0 K* and *sgft=0.0* will reduce the simulation to a normal MD or LD. Only experienced users should use the *sgft* variable; for most purposes, setting *tempsg* should be sufficient.

ISGSTA — The first atom index of SGLD region. Default 1.

ISGEND — The last atom index of SGLD region. Default is *natom*.

5.6.8. Temperature regulation.

NTT — Switch for temperature scaling. Note that setting *ntt*=0 corresponds to the microcanonical (NVE) ensemble (which should approach the canonical one for large numbers of degrees of freedom). Some aspects of the "weak-coupling ensemble" (*ntt*=1) have been examined, and roughly interpolate between the microcanonical and canonical ensembles [82,83]. The *ntt*=2 and 3 options correspond to the canonical (constant T) ensemble.

= 0 — Constant total energy classical dynamics (assuming that *ntb*<2, as should probably always be the case when *ntt=0*).

= 1 — Constant temperature, using the weak-coupling algorithm [84]. A single scaling factor is used for all atoms. Note that this algorithm just ensures that the total kinetic energy is appropriate for the desired temperature; it does nothing to ensure that the temperature is even over all parts of the molecule. Atomic collisions will tend to ensure an even temperature distribution, but this is not guaranteed, and there are many subtle problems that can arise with weak temperature

coupling [85]. *Unless you are sure you know what you are doing, please don't use ntt=1!* (This warning is especially relevant for generalized Born simulations, where there are no collisions with solvent to aid in thermalization.) Other temperature coupling options (especially *ntt*=3) should be used instead.

= 2 Andersen temperature coupling scheme [86], in which imaginary "collisions" randomize the velocities to a distribution corresponding to *temp0* every *vrand* steps. Note that in between these "massive collisions", the dynamics is Newtonian. Hence, time correlation functions (etc.) can be computed in these sections, and the results averaged over an initial canonical distribution. Note also that too high a collision rate (too small a value of *vrand*) will slow down the speed at which the molecules explore configuration space, whereas too low a rate means that the canonical distribution of energies will be sampled slowly. A discussion of this rate is given by Andersen [87].

= 3 Use Langevin dynamics with the collision frequency γ given by *gamma_ln*, discussed below. Note that when γ has its default value of zero, this is the same as setting *ntt* = 0.

TEMP0 Reference temperature at which the system is to be kept, if *ntt* > 0. Note that for temperatures above 300K, the step size should be reduced since increased distance traveled between evaluations can lead to SHAKE and other problems. Default 300.

TEMP0LES This is the target temperature for all LES particles (see Chapter 6). If *temp0les*<0, a single temperature bath is used for all atoms, otherwise separate thermostats are used for LES and non-LES particles. Default is -1, corresponding to a single (weak-coupling) temperature bath.

TEMPI Initial temperature. For the initial dynamics run, (NTX .lt. 3) the velocities are assigned from a Maxwellian distribution at TEMPI K. If TEMPI = 0.0, the velocities will be calculated from the forces instead. TEMPI has no effect if NTX .gt. 3. Default 0.0.

IG The seed for the random number generator. The MD starting velocity is dependent on the random number generator seed if NTX .lt. 3 .and. TEMPI .ne. 0.0. Default 71277.

TAUTP Time constant, in ps, for heat bath coupling for the system, if *ntt* = 1. Default is 1.0. Generally, values for TAUTP should be in the range of 0.5-5.0 ps, with a smaller value providing tighter coupling to the heat bath and, thus, faster heating and a less natural trajectory. Smaller values of TAUTP result in smaller fluctuations in kinetic energy, but larger fluctuations in the total energy. Values much larger than the length of the simulation result in a return to constant energy conditions.

GAMMA_LN The collision frequency γ, in ps^{-1}, when *ntt* = 3. A simple Leapfrog integrator is used to propagate the dynamics, with the kinetic energy adjusted to be correct for the harmonic oscillator case [88,89]. Note that it is not necessary that γ approximate the physical collision frequency, which is about 50 ps^{-1} for liquid water. In fact, it is often advantageous, in terms of sampling or stability of integration, to use much smaller values, around 2 to 5 ps^{-1}. Default is 0 [89,90].

VRAND If *vrand>0* and *ntt=2*, the velocities will be randomized to temperature TEMP0 every *vrand* steps.

VLIMIT If not equal to 0.0, then any component of the velocity that is greater than abs(VLIMIT) will be reduced to VLIMIT (preserving the sign). This can be used to avoid occasional instabilities in molecular dynamics runs. VLIMIT should generally be set to a value like 20 (the default), which is well above the most probable velocity in a Maxwell-Boltzmann distribution at room temperature. A warning message will be printed whenever the velocities are modified. Runs that have more than a few such warnings should be carefully examined.

5.6.9. Pressure regulation.

In "constant pressure" dynamics, the volume of the unit cell is adjusted (by small amounts on each step) to make the computed pressure approach the target pressure, *pres0*. Equilibration with *ntp* > 0 is generally necessary to adjust the density of the system to appropriate values. Note that fluctuations in the instantaneous pressure on each step will appear to be large (several hundred bar), but the average value over many steps should be close to the target pressure. Pressure regulation only applies when Constant Pressure periodic boundary conditions are used (*ntb* = 2). Pressure coupling algorithms used in Amber are of the "weak-coupling" variety, analogous to temperature coupling [84]. Please note: in general you will need to equilibrate the temperature to something like the final temperature using constant volume (*ntp*=0) *before* switching on constant pressure simulations to adjust the system to the correct density. If you fail to do this, the program will try to adjust the density too quickly, and bad things (such as SHAKE failures) are likely to happen.

NTP Flag for constant pressure dynamics. This option should be set to 1 or 2 when Constant Pressure periodic boundary conditions are used (NTB = 2).

- = 0 Used with NTB not = 2 (default); no pressure scaling
- = 1 md with isotropic position scaling
- = 2 md with anisotropic (x-,y-,z-) pressure scaling: this should only be used with orthogonal boxes (i.e. with all angles set to 90^o). Anisotropic scaling is primarily intended for non-isotropic systems, such as membrane simulations, where the surface tensions are different in different directions; it is generally not appropriate for solutes dissolved in water.

PRES0 Reference pressure (in units of bars, where 1 bar ˜ 1 atm) at which the system is maintained (when NTP > 0). Default 1.0.

COMP compressibility of the system when NTP > 0. The units are in 1.0E-06/bar; a value of 44.6 (default) is appropriate for water.

TAUP Pressure relaxation time (in ps), when NTP > 0. The recommended value is between 1.0 and 5.0 psec. Default value is 1.0, but larger values may sometimes be necessary (if your trajectories seem unstable).

5.6.10. SHAKE bond length constraints.

NTC Flag for SHAKE to perform bond length constraints [91]. (See also NTF in the **Potential function** section. In particular, typically NTF = NTC.) The SHAKE option should be used for most MD calculations. The size of the MD timestep is determined by the fastest motions in the system. SHAKE removes the bond stretching freedom, which is the fastest motion, and consequently allows a larger timestep to be used. For water models, a special "three-point" algorithm is used [92]. Consequently, to employ TIP3P set NTF = NTC = 2.

Since SHAKE is an algorithm based on dynamics, the minimizer is not aware of what SHAKE is doing; for this reason, minimizations generally should be carried out without SHAKE. One exception is short minimizations whose purpose is to remove bad contacts before dynamics can begin.

For parallel versions of *sander* only intramolecular atoms can be constrained. Thus, such atoms must be in the same chain of the originating PDB file.

= 1 SHAKE is not performed (default)

= 2 bonds involving hydrogen are constrained

= 3 all bonds are constrained (not available for parallel or qmmm runs in *sander*)

TOL Relative geometrical tolerance for coordinate resetting in shake. Recommended maximum: <0.00005 Angstrom Default 0.00001.

JFASTW Fast water definition flag. By default, the system is searched for water residues, and special routines are used to SHAKE these systems [92].

= 0 Normal operation. Waters are identified by the default names (given below), unless they are redefined, as described below.

= 4 Do not use the fast SHAKE routines for waters.

The following variables allow redefinition of the default residue and atom names used by the program to determine which residues are waters.

WATNAM The residue name the program expects for water. Default `'WAT '`.

OWTNM The atom name the program expects for the oxygen of water. Default `'O '`.

HWTNM1 The atom name the program expects for the 1st H of water. Default `'H1 '`.

HWTNM2 The atom name the program expects for the 2nd H of water. Default `'H2 '`.

NOSHAKEMASK String that specifies atoms that are not to be shaken (assuming that ntc>1). Any bond that would otherwise be shaken by virtue of the *ntc* flag, but which involves an atom flagged here, will *not* be shaken. The syntax for this string is given in Chap. 13.5. Default is an empty string, which matches nothing. A typical use would be to remove SHAKE constraints from all or part of a solute, while still shaking rigid water models like TIPnP or SPC/E. Another use would be to turn off SHAKE constraints for the parts of the system that are being changed with thermodynamic integration, or which are the EVB or quantum regions of the system.

If this option is invoked, then all parts of the potential must be evaluated, that

is, *ntf* must be one. The code enforces this by setting *ntf* to 1 when a *noshakemask* string is present in the input.

If you want the *noshakemask* to apply to all or part of the water molecules, you must also set *jfastw=4*, to turn off the special code for water SHAKE. (If you are not shaking waters, you presumably also want to issue the "set default FlexibleWater on" command in LEaP; see that chapter for more information.)

5.6.11. Water cap.

IVCAP Flag to control cap option. The "cap" refers to a spherical portion of water centered on a point in the solute and restrained by a soft half-harmonic potential. For the best physical realism, this option should be combined with *igb=10*, in order to include the reaction field of waters that are beyond the cap radius.

= 0 Cap will be in effect if it is in the *prmtop* file (default).

= 2 Cap will be inactivated, even if parameters are present in the *prmtop* file.

FCAP The force constant for the cap restraint potential.

5.6.12. NMR refinement options.

ISCALE Number of additional variables to optimize beyond the 3N structural parameters. (Default = 0). At present, this is only used with residual dipolar coupling restraints.

NOESKP The NOESY volumes will only be evaluated if mod(nstep, noeskp) = 0; otherwise the last computed values for intensities and derivatives will be used. (default = 1, i.e. evaluate volumes at every step)

IPNLTY

= 1 the program will minimize the sum of the absolute values of the errors; this is akin to minimizing the crystallographic R-factor (default).

= 2 the program will optimize the sum of the squares of the errors.

= 3 For NOESY intensities, the penalty will be of the form

$awt\ [I_c^{(1/6)} - I_o^{(1/6)}]^2$.

Chemical shift penalties will be as for *ipnlty=1*.

MXSUB Maximum number of submolecules that will be used. This is used to determine how much space to allocate for the NOESY calculations. Default 1.

SCALM "Mass" for the additional scaling parameters. Right now they are restricted to all have the same value. The larger this value, the slower these extra variables will respond to their environment. Default 100 amu.

PENCUT In the summaries of the constraint deviations, entries will only be made if the penalty for that term is greater than PENCUT. Default 0.1.

TAUSW For noesy volume calculations (*NMROPT = 2*), intensities with mixing times less that TAUSW (in seconds) will be computed using perturbation theory, whereas those greater than TAUSW will use a more exact theory. See the theory section (below) for details. To always use the "exact" intensities and derivatives, set TAUSW = 0.0; to always use perturbation theory, set TAUSW to a value larger than the largest mixing time in the input. Default is TAUSW of 0.1 second, which should work pretty well for most systems.

5.7. Potential function parameters

The parameters in this section generally control what sort of force field (or potential function) is used for the simulation.

5.7.1. Generic parameters

NTF Force evaluation. Note: If SHAKE is used (see NTC), it is not necessary to calculate forces for the constrained bonds.

= 1 complete interaction is calculated (default)

= 2 bond interactions involving H-atoms omitted (use with NTC=2)

= 3 all the bond interactions are omitted (use with NTC=3)

= 4 angle involving H-atoms and all bonds are omitted

= 5 all bond and angle interactions are omitted

= 6 dihedrals involving H-atoms and all bonds and all angle interactions are omitted

= 7 all bond, angle and dihedral interactions are omitted

= 8 all bond, angle, dihedral and non-bonded interactions are omitted

NTB Periodic boundary. If NTB .EQ. 0 then a boundary is NOT applied regardless of any boundary condition information in the topology file. The value of NTB specifies whether constant volume or constant pressure dynamics will be used. Options for constant pressure are described in a separate section below.

= 0 no periodicity is applied and PME is off

= 1 constant volume (default)

= 2 constant pressure

If NTB .NE. 0, there must be a periodic boundary in the topology file. Constant pressure is not used in minimization (IMIN=1, above).

For a periodic system, constant pressure is the only way to equilibrate density if the starting state is not correct. For example, the solvent packing scheme used in LEaP can result in a net void when solvent molecules are subtracted which can aggregate into "vacuum bubbles" in a constant volume run. Another potential problem are small gaps at the edges of the box. The upshot

is that almost every system needs to be equilibrated at constant pressure (*ntb=2, ntp>0*) to get to a proper density. But be sure to equilibrate first (at constant volume) to something close to the final temperature, before turning on constant pressure.

DIELC — Dielectric multiplicative constant for the electrostatic interactions. Default is 1.0. Please note this is NOT related to dielectric constants for generalized Born simulations.

CUT — This is used to specify the nonbonded cutoff, in Angstroms. For PME, the cutoff is used to limit direct space sum, and the default value of 8.0 is usually a good value. When *igb>0*, the cutoff is used to truncate nonbonded pairs (on an atom-by-atom basis); here a larger value than the default is generally required. A separate parameter (**RGBMAX**) controls the maximum distance between atom pairs that will be considered in carrying out the pairwise summation involved in calculating the effective Born radii, see the generalized Born section below.

SCNB — 1-4 vdw interactions are divided by SCNB. Default 2.0.

SCEE — 1-4 electrostatic interactions are divided by SCEE; the 1991 and previous force fields used 2.0, while the 1994 force field uses 1.2. Default is 1.2.

NSNB — Determines the frequency of nonbonded list updates when *igb=0* and *nbflag=0*; see the description of *nbflag* for more information. Default is 25.

IPOL — When set to 1, use a polarizable force field. See Section 5.7.5 for more information. Default is 0.

IFQNT — Flag for QM/MM run; if set to 1, you must also include a `&qmmm` namelist. See Section 6.4 for details on this option. Default is 0.

IGB — Flag for using the generalized Born or Poisson-Boltzmann implicit solvent models. See Sections 6.1 and 6.2 for information about using this option. Default is 0.

IEVB — If set to 1, use the empirical valence bond method to compute energies and forces. See Section 6.3 for information about this option. Default is 0.

IAMOEBA — Flag for using the *amoeba* polarizable potentials of Ren and Ponder [8,9]. When this option is set to 1, you need to prepare an `amoeba` namelist with additional parameters. Also, the *prmtop* file is built in a special way. See Section 6.14 for more information about this option. Default is 0.

5.7.2. Particle Mesh Ewald.

The Particle Mesh Ewald (PME) method is always "on", unless *ntb* = 0. PME is a fast implementation of the Ewald summation method for calculating the full electrostatic energy of a unit cell (periodic box) in a macroscopic lattice of repeating images. As implemented, the PME in Amber bypasses the "old" pairlist creation and nonbonded energy and force evaluation, calling special PME functions to calculate the Lennard-Jones and electrostatic interactions. The PME method is fast since the reciprocal space Ewald sums are B-spline interpolated on a grid and since the convolutions necessary to evaluate the sums are calculated via fast Fourier transforms. Note that the accuracy of the PME is related to the density of the charge grid (NFFT1, NFFT2, and NFFT3), the spline interpolation order (ORDER), and the direct sum tolerance (DSUM_TOL);

see the descriptions below for more information.

The `&ewald` namelist is read immediately after the `&cntrl` namelist. We have tried hard to make the defaults for these parameters appropriate for solvated simulations. *Please take care in changing any values from their defaults.* The `&ewald` namelist has the following variables:

NFFT1, NFFT2, NFFT3
: These give the size of the charge grid (upon which the reciprocal sums are interpolated) in each dimension. Higher values lead to higher accuracy (when the DSUM_TOL is also lowered) but considerably slow the calculation. Generally it has been found that reasonable results are obtained when NFFT1, NFFT2 and NFFT3 are approximately equal to A, B and C, respectively, leading to a grid spacing (A/NFFT1, etc) of 1.0 Å. Significant performance enhancement in the calculation of the fast Fourier transform is obtained by having each of the integer NFFT1, NFFT2 and NFFT3 values be a *product of powers* of 2, 3, and 5. If the values are not given, the program will chose values to meet these criteria.

ORDER
: The order of the B-spline interpolation. The higher the order, the better the accuracy (unless the charge grid is too coarse). The minimum order is 3. An order of 4 (the default) implies a cubic spline approximation which is a good standard value. Note that the cost of the PME goes as roughly the order to the third power.

VERBOSE
: Standard use is to have VERBOSE = 0. Setting VERBOSE to higher values (up to a maximum of 3) leads to voluminous output of information about the PME run.

EW_TYPE
: Standard use is to have EW_TYPE = 0 which turns on the particle mesh ewald (PME) method. When EW_TYPE = 1, instead of the approximate, interpolated PME, a *regular* Ewald calculation is run. The number of reciprocal vectors used depends upon RSUM_TOL, or can be set by the user. The exact Ewald summation is present mainly to serve as an accuracy check allowing users to determine if the PME grid spacing, order and direct sum tolerance lead to acceptable results. Although the cost of the exact Ewald method formally increases with system size at a much higher rate than the PME, it may be faster for small numbers of atoms (< 500). For larger, macromolecular systems, with > 500 atoms, the PME method is significantly faster.

DSUM_TOL
: This relates to the width of the direct sum part of the Ewald sum, requiring that the value of the direct sum at the Lennard-Jones cutoff value (specified in CUT as during standard dynamics) be less than DSUM_TOL. In practice it has been found that the relative error in the Ewald forces (RMS) due to cutting off the direct sum at CUT is between 10.0 and 50.0 times DSUM_TOL. Standard values for DSUM_TOL are in the range of 10^{-6} to 10^{-5}, leading to estimated RMS deviation force errors of 0.00001 to 0.0005. Default is 10^{-5}.

RSUM_TOL
: This serves as a way to generate the number of reciprocal vectors used in an Ewald sum. Typically the relative RMS reciprocal sum error is about 5-10 times RSUM_TOL. Default is 5 x 10^{-5}.

MLIMIT(1,2,3)
: This allows the user to explicitly set the number of reciprocal vectors used in a regular Ewald run. Note that the sum goes from -MLIMIT(2) to MLIMIT(2) and -MLIMIT(3) to MLIMIT(3) with symmetry being used in first dimension.

Note also the sum is truncated outside an automatically chosen sphere.

EW_COEFF Ewald coefficient, in $Å^{-1}$. Default is determined by *dsum_tol* and *cutoff*. If it is explicitly inputed then that value is used, and *dsum_tol* is computed from *ew_coeff* and *cutoff*.

NBFLAG If *nbflag = 0*, construct the direct sum nonbonded list in the "old" way, *i.e.* update the list every *nsnb* steps. If *nbflag = 1* (the default when *imin = 0* or *ntb > 0*), *nsnb* is ignored, and the list is updated whenever any atom has moved more than 1/2 *skinnb* since the last list update.

SKINNB Width of the nonbonded "skin". The direct sum nonbonded list is extended to *cut + skinnb*, and the van der Waals and direct electrostatic interactions are truncated at *cut*. Default is 2.0 Å. Use of this parameter is required for energy conservation, and recommended for all PME runs.

NBTELL If *nbtell = 1*, a message is printed when any atom has moved far enough to trigger a list update. Use only for debugging or analysis. Default of 0 inhibits the message.

NETFRC The basic "smooth" PME implementation used here does not necessarily conserve momentum. If *netfrc = 1*, (the default) the total force on the system is artificially removed at every step. This parameter is set to 0 if minimization is requested, which implies that the gradient is an accurate derivative of the energy. You should only change this parameter if you really know what you are doing.

VDWMETH Determines the method used for van der Waals interactions beyond those included in the direct sum. A value of 0 includes no correction; the default value of 1 uses a continuum model correction for energy and pressure.

EEDMETH Determines how the switch function for the direct sum Coulomb interaction is evaluated. The default value of 1 uses a cubic spline. A value of 2 implies a linear table lookup. A value of three implies use of an "exact" subroutine call. When *eedmeth=4*, no switch is used (*i.e.* the bare Coulomb potential is evaluated in the direct sum, cut off sharply at CUT). When *eedmeth=5*, there is no switch, and a distance-dependent dielectric is used (*i.e.* the distance dependence is $1/r^2$ rather than 1/r). The last two options are intended for non-periodic calculations, where no reciprocal term is computed.

EEDTBDNS Density of spline or linear lookup table, if *eedmeth* is 1 or 2. Default is 500 points per unit.

COLUMN_FFT 1 or 0 flag to turn on or off, respectively, column-mode fft for parallel runs. The default mode is slab mode which is efficient for low processor counts. The column method can be faster for larger processor counts since there can be more columns than slabs and the communications pattern is less congested. This flag has no effect on non-parallel runs. Users should test the efficiency of the method in comparison to the default method before commiting a long run to the method. Default is 0 (off).

5.7.3. Using IPS for the calculation of nonbonded interactions

Isotropic Periodic Sum (IPS) is a method for long-range interaction calculation [93]. Unlike Ewald method, which uses periodic boundary images to calculate long range interactions, IPS uses isotropic periodic images of a local region to calculate the long-range contribution beyond the cutoff distance *cut*.

IPS — Flag to control nonbonded interaction calculation method. The *cut* value will be used to define the IPS radius. When IPS is used for electrostatic interaction, PME will be turned off.

- = 0 IPS will not be used (default).
- = 1 IPS will be used for both electrostatic and VDW interactions.
- = 2 IPS will be used only for electrostatic interactions.
- = 3 IPS will be used only for VDW interactions.

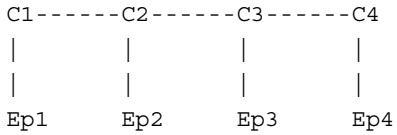
5.7.4. Extra point options

Several parameters deal with "extra-points" (sometimes called lone-pairs), which are force centers that are not at atomic positions. These are currently defined as atoms with "EP" in their names. These input variables are really only for the convenience of force-field developers; *do not change the defaults unless you know what you are doing, and have read the code.* These variables are set in the `&ewald` namelist.

FRAMEON — If *frameon* is set to 1, (default) the bonds, angles and dihedral interactions involving the lone pairs/extra points are removed except for constraints added during parm. The lone pairs are kept in ideal geometry relative to local atoms, and resulting torques are transferred to these atoms. To treat extra points as regular atoms, set frameon=0.

CHNGMASK — If *chngmask*=1 (default), new 1-1, 1-2, 1-3 and 1-4 interactions are calculated. An extra point belonging to an atom has a 1-1 interaction with it, and participates in any 1-2, 1-3 or 1-4 interaction that atom has.

For example, suppose (excusing the geometry) C1,C2,C3,C4 form a dihedral and each has 1 extra point attached as below



The 1-4 interactions include C1−C4, Ep1−C4, C1−Ep4, and Ep1−Ep4. (To see a printout of all 1-1, 1-2, 1-3 and 1-4 interactions set verbose=1.) These interactions are masked out of nonbonds. Thus the amber mask list is rebuilt from these 1-1, 1-2, 1-3 and 1-4 pairs.

A separate list of 1-4 nonbonds is then compiled. This list does not agree in general with the above 1-4, since a 1-4 could also be a 1-3 if its in a ring. See the *ephi()* routine for the precise algorithm involved here. The list of 1-4

nonbonds is printed if *verbose=1*.

5.7.5. Polarizable potentials.

The following parameters are relevant for *polarizable potentials*, that is, when *ipol* is set to 1 in the `&cntrl` namelist. These variables are set in the `&ewald` namelist.

INDMETH If indmeth is 0, 1, or 2 then the nonbond force is called iteratively until successive estimates of the induced dipoles agree to within DIPTOL (default 0.0001 debye) in the root mean square sense. The difference between indmeth = 0, 1, or 2 have to do with the level of extrapolation (1st, 2nd or 3rd-order) used from previous time steps for the initial guess for dipoles to begin the iterative loop. So far 2nd order (indmeth=1) seems to work best.

If indmeth = 3, use a Car-Parinello scheme wherein dipoles are assigned a fictitious mass and integrated each time step. This is much more efficient and is the current default. Note that this method is unstable for dt > 1 fs.

DIPTOL Convergence criterion for dipoles in the iterative methods. Default is 0.0001 Debye.

MAXITER For iterative methods (indmeth<3), this is the maximum number of iterations allowed per time step. Default is 20.

DIPMASS The fictitious mass assigned to dipoles. Default value is 0.33, which works well for 1fs time steps. If dipmass is set much below this, the dynamics are rapidly unstable. If set much above this the dynamics of the system are affected.

DIPTAU This is used for temperature control of the dipoles (for indmeth=3). If *diptau* is greater than 10 (ps units) temperature control of dipoles is turned off. Experiments so far indicate that running the system in NVE with no temperature control on induced dipoles leads to a slow heating, barely noticeable on the 100ps time scale. For runs of length 10ps, the energy conservation with this method rivals that of SPME for standard fixed charge systems. For long runs, we recommend setting a weak temperature control (e.g. 9.99 ps) on dipoles as well as on the atoms. Note that to achieve good energy conservation with iterative method, the diptol must be below 10^{-7} debye, which is much more expensive. Default is 11 ps (*i.e.* default is turned off).

IRSTDIP If indmeth=3, a restart file for dipole positions and velocities is written along with the restart for atomic coordinates and velocities. If irstdip=1, the dipolar positions and velocities from the inpdip file are read in. If irstdip=0, an iterative method is used for step 1, after which Car-Parrinello is used.

SCALDIP To scale 1-4 charge-dipole and dipole-dipole interactions the same as 1-4 charge-charge (i.e. divided by scee) set scaldip=1 (default). If scaldip=0 the 1-4 charge-dipole and dipole-dipole interactions are treated the same as other dipolar interactions (i.e. divided by 1).

5.7.6. Dipole Printing

By including a `&dipoles` namelist containing a series of groups, at the end of the input file, the printing of permanent, induced and total dipoles is enabled.

The X, Y and Z components of the dipole (in debye) for each group will be written to mdout every NTPR steps. In order to avoid ambiguity with charged groups all of the dipoles for a given group are calculated with respect to the centre of mass of that group.

It should be noted that the permanent, inducible and total dipoles will be printed regardless of whether a *polarizable potential* is in use. However, only the permanent dipole will have any physical meaning when *non-polarizable potentials* are in use.

It should also be noted that the groups used in the dipole printing routines are not exclusive to these routines and so the dipole printing procedure can only be used when group input is *not* in use for something else (*i.e.* restraints).

5.8. Weight change information.

This section of information is read (*if NMROPT > 0*) as a series of namelist specifications, with name "`&wt`". This namelist is read repeatedly until a namelist `&wt` statement is found with TYPE=END.

Overview of weight change variables	
variable	*description*
TYPE	Defines quantity being varied; valid options are listed below.
ISTEP1,ISTEP2	This change is applied over steps/iterations ISTEP1 through ISTEP2. If ISTEP2 = 0, this change will remain in effect from step ISTEP1 to the end of the run at a value of VALUE1 (VALUE2 is ignored in this case). *(default= both 0)*
VALUE1,VALUE2	Values of the change corresponding to ISTEP1 and ISTEP2, respectively. If ISTEP2=0, the change is fixed at VALUE1 for the remainder of the run, once step ISTEP1 is reached.
IINC IMULT	If IINC > 0, then the change is applied as a step function, with IINC steps/iterations between each change in the target VALUE (ignored if ISTEP2=0). If IINC =0, the change is done continuously. *(default=0)* If IMULT=0, then the change will be linearly interpolated from VALUE1 to VALUE2 as the step number increases from ISTEP1 to ISTEP2. *(default)* If IMULT=1, then the change will be effected by a series of multiplicative scalings, using a single factor, R, for all scalings. i.e. VALUE2 = (R**INCREMENTS) * VALUE1. INCREMENTS is the number of times the target value changes, which is determined by ISTEP1, ISTEP2, and IINC.

The remainder of this section describes the options for the TYPE parameter. For a few types of cards, the meanings of the other variables differ from that described above; such differences are noted below. Valid Options for TYPE (you must use uppercase) are:

BOND Varies the relative weighting of bond energy terms.

ANGLE Varies the relative weighting of valence angle energy terms.

TORSION Varies the relative weighting of torsion (and J-coupling) energy terms. Note that any restraints defined in the input to the PARM program are included in

	the above. Improper torsions are handled separately (IMPROP).
IMPROP	Varies the relative weighting of the "improper" torsional terms. These are not included in TORSION.
VDW	Varies the relative weighting of van der Waals energy terms. This is equivalent to changing the well depth (epsilon) by the given factor.
HB	Varies the relative weighting of hydrogen-bonding energy terms.
ELEC	Varies the relative weighting of electrostatic energy terms.
NB	Varies the relative weights of the non-bonded (VDW, HB, and ELEC) terms.
ATTRACT	Varies the relative weights of the attractive parts of the van der waals and h-bond terms.
REPULSE	Varies the relative weights of the repulsive parts of the van der waals and h-bond terms.
RSTAR	Varies the effective van der Waals radii for the van der Waals (VDW) interactions by the given factor. Note that this is done by changing the relative attractive and repulsive coefficients, so ATTRACT/REPULSE should not be used over the same step range as RSTAR.
INTERN	Varies the relative weights of the BOND, ANGLE and TORSION terms. "Improper" torsions (IMPROP) must be varied separately.
ALL	Varies the relative weights of all the energy terms above (BOND, ANGLE, TORSION, VDW, HB, and ELEC; does not affect RSTAR or IMPROP).
REST	Varies the relative weights of *all* the NMR restraint energy terms.
RESTS	Varies the weights of the "short-range" NMR restraints. Short- range restraints are defined by the SHORT instruction (see below).
RESTL	Varies the weights of any NMR restraints which are not defined as "short range" by the SHORT instruction (see below). When no SHORT instruction is given, RESTL is equivalent to REST.
NOESY	Varies the overall weight for NOESY volume restraints. Note that this value multiplies the individual weights read into the "awt" array. (Only if NMROPT=2; see Section 4 below).
SHIFTS	Varies the overall weight for chemical shift restraints. Note that this value multiplies the individual weights read into the "wt" array. (Only if NMROPT=2; see section 4 below).
SHORT	Defines the short-range restraints. For this instruction, ISTEP1, ISTEP2, VALUE1, and VALUE2 have different meanings. A short-range restraint can be defined in two ways. *(1)* If the residues containing each pair of bonded atoms comprising the restraint are close enough in the primary sequence: ISTEP1 ≤ ABS(delta_residue) ≤ ISTEP2, where delta_residue is the difference in the numbers of the residues containing the pair of bonded atoms. *(2)* If the distances between each pair of bonded atoms in the restraint fall within a prescribed range: VALUE1 ≤ distance ≤ VALUE2.

Only one SHORT command can be issued, and the values of ISTEP1, ISTEP2, VALUE1, and VALUE2 remain fixed throughout the run. However, if IINC>0, then the short-range interaction list will be re-evaluated every IINC steps.

TGTRMSD Varies the RMSD target value for targeted MD.

TEMP0 Varies the target temperature TEMP0.

TEMP0LES Varies the LES target temperature TEMP0LES.

TAUTP Varies the coupling parameter, TAUTP, used in temperature scaling when temperature coupling options NTT=1 is used.

CUT Varies the non-bonded cutoff distance.

NSTEP0 If present, this instruction will reset the initial value of the step counter (against which ISTEP1/ISTEP2 and NSTEP1/NSTEP2 are compared) to the value ISTEP1. An NSTEP0 instruction only has an effect at the beginning of a run. For this card (only) ISTEP2, VALUE1, VALUE2 and IINC are ignored. If this card is omitted, NSTEP0 = 0. This card can be useful for simulation restarts, where NSTEP0 is set to the final step on the previous run.

STPMLT If present, the NMR step counter will be changed in increments of STPMLT for each actual dynamics step. For this card, only VALUE1 is read. ISTEP1, ISTEP2, VALUE2, IINC, and IMULT are ignored. Default = 1.0.

DISAVE

ANGAVE

TORAVE If present, then by default time-averaged values (rather than instantaneous values) for the appropriate set of restraints will be used. DISAVE controls distance data, ANGAVE controls angle data, TORAVE controls torsion data.

See below for the functional form used in generating time-averaged data.

For these cards: VALUE1 = τ (characteristic time for exponential decay)
VALUE2 = POWER (power used in averaging; the nearest integer of value2 is used)

Note that the range (ISTEP1→ISTEP2) applies only to TAU; The value of POWER is not changed by subsequent cards with the same ITYPE field, and time-averaging will always be turned on for the entire run if one of these cards appears.

Note also that, due to the way that the time averaged internals are calculated, changing τ at any time after the start of the run will only affect the relative weighting of steps occurring after the change in τ.

Separate values for τ and POWER are used for bond, angle, and torsion averaging.

The default value of τ (if it is 0.0 here) is 1.0D+6, which results in no exponential decay weighting. Any value of $\tau \geq$ 1.D+6 will result in no exponential decay.
If DISAVE,ANGAVE, or TORAVE is chosen, one can still force use of an instantaneous value for specific restraints of the particular type (bond, angle, or torsion) by setting the IFNTYP field to "1" when the restraint is defined (IFNTYP is defined in the DISANG file).

If time-averaging for a particular class of restraints is being performed, all restraints of that class that are being averaged (that is, all restraints of that class except those for which IFNTYP=1) *must* have the same values of NSTEP1 and NSTEP2 (NSTEP1 and NSTEP2 are defined below).

(For these cards, IINC and IMULT are ignored)

See the discussion of time-averaged restraints following the input descriptions.

DISAVI

ANGAVI

TORAVI

ISTEP1: Ignored.

ISTEP2: Sets IDMPAV. If IDMPAV > 0, *and* a dump file has been specified (DUMPAVE is set in the file redirection section below), then the time-averaged values of the restraints will be written every IDMPAV steps. Only one value of IDMPAV can be set (corresponding to the first DISAVI/ANGAVI/TORAVI card with ISTEP2 > 0), and *all* restraints (even those with IFNTYP=1) will be "dumped" to this file every IDMPAV steps. The values reported reflect the current value of τ.

VALUE1: The integral which gives the time-averaged values is undefined for the first step. By default, for each time-averaged internal, the integral is assigned the current value of the internal on the first step. If VALUE1≠0, this initial value of internal r is reset as follows:

```
-1000. <  VALUE1 <    1000.:  Initial value = r_initial + VALUE
          VALUE1 <= -1000.:  Initial value = r_target + 1000.
 1000. <= VALUE1           :  Initial value = r_target - 1000.
```

r_target is the target value of the internal, given by R2+R3 (or just R3, if R2 is 0). VALUE1 is in angstroms for bonds, in degrees for angles.

VALUE2: This field can be used to set the value of τ used in calculating the time-averaged values of the internal restraints reported at the end of a simulation (if LISTOUT is specified in the redirection section below). By default, no exponential decay weighting is used in calculating the final reported values, regardless of what value of τ was used during the simulation. If VALUE2>0, then τ = VALUE2 will be used in calculating these final reported averages. Note that the value of VALUE2 = τ specified here only affects the reported averaged values in at the end of a simulation. It does not affect the time-averaged values used during the simulation (those are changed by the VALUE1 field of DISAVE, ANGAVE and TORAVE instructions).

IINC: If IINC = 0, then forces for the class of time-averaged restraints will be calculated exactly as (dE/dr_ave) (dr_ave/dx). If IINC = 1, then then forces for the class of time-averaged restraints will be calculated as (dE/dr_ave) (dr(t)/dx). Note that this latter method results in a non-conservative force, and does not integrate to a standard form. But this latter formulation helps avoid the large forces due to the (1+*i*) term in the exact derivative calculation--and may avert instabilities in the molecular dynamics trajectory for some systems. See the discussion of time-averaged restraints following the input description.

	Note that the DISAVI, ANGAVI, and TORAVI instructions will have no affect unless the corresponding time average request card (DISAVE, ANGAVE or TORAVE, respectively) is also present.
DUMPFREQ	Istep1 is the only parameter read, and it sets the frequency at which the coordinates in the distance or angle restraints are dumped to the file specified by the DUMPAVE command in the I/O redirection section.
	(For these cards, ISTEP1 and IMULT are ignored).
END	END of this section.

NOTES:

(1) All weights are relative to a default of 1.0 in the standard force field.

(2) Weights are not cumulative.

(3) For any range where the weight of a term is not modified by the above, the weight reverts to 1.0. For any range where TEMP0, SOFTR or CUTOFF is not specified, the value of the relevant constant is set to that specified in the input file.

(4) If a weight is set to 0.0, it is set internally to 1.0D-7. This can be overridden by setting the weight to a negative number. In this case, a weight of exactly 0.0 will be used. *However,* if any weight is set to exactly 0.0, it cannot be changed again during this run of the program.

(5) If two (or more) cards change a particular weight over the same range, the weight given on the last applicable card will be the one used.

(6) Once any weight change for which NSTEP2=0 becomes active (i.e. one which will be effective for the remainder of the run), the weight of this term cannot be further modified by other instructions.

(7) Changes to RSTAR result in exponential weighting changes to the attractive and repulsive terms (proportional to the scale factor**6 and **12, respectively). For this reason, scaling RSTAR to a very small value (e.g. ≤0.1) may result in a zeroing-out of the vdw term.

5.9. File redirection commands.

Input/output redirection information can be read as described here. Redirection cards must follow the end of the weight change information. Redirection card input is terminated by the first non-blank line which does not start with a recognized redirection TYPE (e.g. LISTIN, LISTOUT, etc.).

The format of the redirection cards is

TYPE = filename

where TYPE is any valid redirection keyword (see below), and filename is any character string. The equals sign ("=") is required, and TYPE must be given in *uppercase* letters.

Valid redirection keywords are:

LISTIN	An output listing of the restraints which have been read, and their deviations from the target distances *before* the simulation has been run. By default, this listing is not printed. If POUT is used for the filename, these deviations will be printed in the normal output file.
LISTOUT	An output listing of the restraints which have been read, and their deviations from the target distances *_after* the simulation has finished. By default, this listing is not printed. If POUT is used for the filename, these deviations will be printed in the normal output file.
DISANG	The file from which the distance and angle restraint information described below (Section 4) will be read.
NOESY	File from which NOESY volume information (Section 5), if any, will be read.
SHIFTS	File from which chemical shift information (Section 6), if any, will be read.
PCSHIFT	File from which paramagnetic shift information (Section 6), if any, will be read.
DIPOLE	File from which residual dipolar couplings (Section 7), if any, will be read.
DUMPAVE	File to which the time-averaged values of all restraints will be written. If DISAVI / ANGAVI / TORAVI has been used to set IDMPAV≠0, then averaged values will be output. If the DUMPFREQ command has been used, the instantaneous values will be output.

6. Using Sander

This chapter provides a number of sections describing how to use *sander* for particular types of problems. It should be read in conjunction with the previous chapter.

6.1. The Generalized Born/Surface Area Model

The generalized Born solvation model can be used instead of explicit water for non-polarizable force fields such as ff94 or ff99. To estimate the total solvation free energy of a molecule, ΔG_{solv}, one typically assumes that it can be decomposed into the "electrostatic" and "non-electrostatic" parts:

$$\Delta G_{solv} = \Delta G_{el} + \Delta G_{nonel}, \tag{6.1}$$

where ΔG_{nonel} is the free energy of solvating a molecule from which all charges have been removed (i.e. partial charges of every atom are set to zero), and ΔG_{el} is the free energy of first removing all charges in the vacuum, and then adding them back in the presence of a continuum solvent environment. Generally speaking, ΔG_{nonel} comes from the combined effect of two types of interaction: the favorable van der Waals attraction between the solute and solvent molecules, and the unfavorable cost of breaking the structure of the solvent (water) around the solute. In the current Amber codes, ΔG_{nonel} is taken to be proportional to the total solvent accessible surface area (SA) of the molecule, with a proportionality constant derived from experimental solvation energies of small non-polar molecules, and uses a fast LCPO algorithm [72] to compute an analytical approximation to the surface accessible area of the molecule.

The Poisson-Boltzmann approach described in the next section has traditionally been used in calculating ΔG_{el}. However, in molecular dynamics applications, the associated computational costs are often very high, as the Poisson-Boltzmann equation needs to be solved every time the conformation of the molecule changes. Amber developers have pursued an alternative approach, the analytic generalized Born (GB) method, to obtain a a reasonable, computationally efficient estimate of ΔG_{el} to be used in molecular dynamics simulations. The methodology has become popular [59,69,94-99], especially in molecular dynamics applications [53,54,100,101], due to its relative simplicity and computational efficiency, compared to the more standard numerical solution of the Poisson-Boltzmann equation. Within Amber GB models, each atom in a molecule is represented as a sphere of radius ρ_i with a charge q_i at its center; the interior of the atom is assumed to be filled uniformly with a material of dielectric constant of 1. The molecule is surrounded by a solvent of a high dielectric ε_w (80 for water at 300 K). The GB model approximates ΔG_{el} by an analytical formula [59,71],

$$\Delta G_{el} \approx \Delta G_{gb} = -\tfrac{1}{2} \sum_{ij} \frac{q_i q_j}{f_{GB}(r_{ij}, R_i, R_j)} \left(1 - \frac{e^{-\kappa f_{gb_{ij}}}}{\varepsilon_w}\right) \tag{6.2}$$

where r_{ij} is the distance between atoms i and j, the R_i are the so-called *effective Born radii* of atoms i and j, and f_{GB} is a certain smooth function of its arguments. The electrostatic screening effects of (monovalent) salt are incorporated [71] via the Debye-Huckel screening parameter $\kappa \text{Å}^{-1} \approx 0.316\sqrt{[salt][mol/L]}$.

A common choice [59] of f_{GB} is

$$f_{GB} = \left[r_{ij}^2 + R_i R_j \exp(-r_{ij}^2/4R_i R_j) \right]^{\frac{1}{2}} \tag{6.3}$$

although other other expressions have been tried [96,102]. The effective Born radius of an atom reflects the degree of its burial inside the molecule: for an isolated ion, R_i is equal to its van der Waals (VDW) radius ρ_i. Then one obtains the particularly simple form:

$$\Delta G_{el} \approx -\tfrac{1}{2}(1 - \frac{1}{\varepsilon_w})\frac{q^2}{\rho} \tag{6.4}$$

where we assumed $\kappa = 0$ (pure water). This is the famous expression due to Born for the solvation energy of a single ion. The function f_{GB} is designed to interpolate, in a clever manner, between the limit $r_{ij} \to 0$ when atomic spheres merge into one, and the opposite extreme $r_{ij} \to \infty$ when the ions can be treated as point charges obeying the Coulomb's law [98]. For deeply buried atoms, the effective radii are large, $R_i \gg \rho_i$, and for such atoms one can use a rough estimate $R_i \approx L$, where L is the distance from the atom to the molecular surface. Closer to the surface, the effective radii become smaller, and for a completely solvent exposed side-chain one can expect R_i to approach ρ_i.

The effective radii depend on the molecule's conformation, and so have to be re-computed every time the conformation changes. This makes the computational efficiency a critical issue, and various approximations are normally made that facilitate an effective estimate of ΔG_{el}. In particular, the so-called *Coulomb field approximation*, or *CFA*, is often used, which replaces the true electric displacement, $\mathbf{D}^{true}$, around the atom by the Coulomb field $\mathbf{D}_i^0(\mathbf{r}) \equiv (q_i/r^3)\mathbf{r}$. Within this assumption, the following expression for R_i can be derived [98]:

$$R_i^{-1} = \rho_i^{-1} - \frac{1}{4\pi} \int\limits_{solute} \theta(|\mathbf{r}| - \rho_i) \frac{1}{r^4}\, d^3\mathbf{r}. \tag{6.5}$$

where the integral is over the solute volume surrounding atom i. For a realistic molecule, the solute boundary (molecular surface) is anything but trivial, and so further approximations are made to obtain a closed-form analytical expression for the above equation, *e.g.* the so-called pair-wise de-screening approach of Hawkins, Cramer and Truhlar [66], which leads to a GB model termed GB^{HCT}, implement in Amber with *igb=1*. In the GB^{HCT}, the 3D integral used in the estimation of the effective radii, is performed over the van der Waals (VDW) spheres of solute atoms, which implies a definition of the solute volume in terms of a set of spheres, rather than the complex molecular surface [103], commonly used in the PB calculations. For macromolecules, this approach tends to underestimate the effective radii for buried atoms [98], arguably because the standard integration procedure treats the small vacuum--filled crevices between the van der Waals (VDW) spheres of protein atoms as being filled with water, even for structures with large interior [102]. This error is expected to be greatest for deeply buried atoms characterized by large effective radii, while for the surface atoms it is largely canceled by the opposing error arising from the Coulomb approximation, which tends [67,69,94] to overestimate R_i.

The deficiency of the GB^{HCT} model described above can, to some extent, be corrected by noticing that even the optimal packing of hard spheres, which is a reasonable assumption for biomolecules, still occupies only about 3/4 of the space, and so "scaling-up" of the integral by a factor of $\approx 4/3$ should effectively increase the underestimated radii by about the right amount, without any loss of computational efficiency. This idea was developed and applied in the context of pH titration [98], where it was shown to improve the performance of the GB approximation in calculating pK_a values of protein sidechains. However, the one-parameter correction introduced in Ref. [98] was not optimal in keeping the GB^{HCT} model's established performance on small molecules. It was therefore proposed [54] to re-scale the effective radii with the re-scaling

parameters being proportional to the degree of the atom's burial, as quantified by the value I of the 3D integral. The latter is large for the deeply buried atoms and small for exposed ones. Consequently, one seeks a well-behaved re-scaling function, such that $R_i \approx (\tilde{\rho}_i^{-1} - I)^{-1}$ for small I, and $R_i > (\tilde{\rho}_i^{-1} - I)^{-1}$ when I becomes large. The following simple, infinitely differentiable re-scaling function was chosen to replace the GB^{HCT} original expression for the effective radii:

$$R_i^{-1} = \tilde{\rho}_i^{-1} - \rho_i^{-1} \tanh\left(\alpha\Psi - \beta\Psi^2 + \gamma\Psi^3\right) \tag{6.6}$$

where $\Psi = I\tilde{\rho}_i$, and α, β, γ are treated as adjustable dimensionless parameters which were optimized using the guidelines mentioned earlier (primarily agreement with the PB). Currently, Amber supports two GB models (termed GB^{OBC}) based on this idea. These differ by the values of α, β, γ, and are invoked by setting igb to either igb=2 or igb=5. The details of the optimization procedure and the performance of the GB^{OBC} model relative to the PB treatment and in MD simulations on proteins is described in Ref. [54]; an independent comparison to the PB in calculating the electrostatic part of solvation free energy on a large data set of proteins can be found in [104].

6.1.1. GB/SA input parameters

As outlined above, there are several "flavors" of GB available, depending upon the value of *igb*. The version that has been most extensively tested corresponds to *igb*=1; the "OBC" models (*igb*=2 and 5) are newer, but appear to give significant improvements and are recommended for most projects (certainly for peptides or proteins). The newest, most advanced, and least extensively tested model, *GBn* (*igb*=7), yields results in considerably better agreement with molecular surface Poisson-Boltzmann and explicit solvent results than the "OBC" models under many circumstances. The *GBn* model was parameterized for peptide and protein systems and is not recommended for use with nucleic acids. Users should understand that all (current) GB models have limitations and should proceed with caution. Generalized Born simulations can only be run for non-periodic systems, *i.e.* where *ntb*=0. The nonbonded cutoff for GB calculations should be greater than that for PME calculations, perhaps *cut*=16. The slowly-varying forces generally do not have to be evaluated at every step for GB, either *nrespa*=2 or 4.

IGB

= 0 No generalized Born term is used. (Default)

= 1 The Hawkins, Cramer, Truhlar [65,66] pairwise generalized Born model (GB^{HCT}) is used, with parameters described by Tsui and Case [52]. This model uses the default radii set up by LEaP. It is slightly different from the GB model that was included in Amber6. If you want to compare to Amber 6, or need to continue an ongoing simulation, you should use the command "set default PBradii amber6" in LEaP, and set *igb*=1 in *sander*. For reference, the Amber6 values are those used by an earlier Tsui and Case paper [53].

= 2 Use a modified GB model developed by A. Onufriev, D. Bashford and D.A. Case (GB^{OBC}); the main idea was published earlier [98], but the actual implementation here [54] is an elaboration of this initial idea. Within this model, the effective Born radii are re-scaled to account for the interstitial spaces between atom spheres missed by the GB^{HCT} approximation. In that sense, GB^{OBC} is intended to be a closer approximation to true molecular volume, albiet in an average

sense. With *igb*=2, the inverse of the effective Born radius is given by:

$$R_i^{-1} = \bar{\rho}_i^{-1} - \tanh(\alpha\Psi - \beta\Psi^2 + \gamma\Psi^3)/\rho_i \quad (6.7)$$

where $\bar{\rho}_i = \rho_i - offset$, and $\Psi = I\bar{\rho}_i$, with I given in our earlier paper. The parameters α, β, and γ were determined by empirical fits, and have the values 0.8, 0.0, and 2.909125. This corresponds to model I in Ref [54]. With this option, you should use the LEaP command "set default PBradii mbondi2" or "set default PBradii bondi" to prepare the *prmtop* file.

= 3 or 4 These values are unused; they were used in Amber 7 for parameter sets that are no longer supported.

=5 Same as *igb=2*, except that now α, β, γ are 1.0, 0.8, and 4.85. This corresponds to model II in Ref [54]. With this option, you should use the command "set default PBradii mbondi2" in setting up the *prmtop* file, although "set default PBradii bondi" is also OK. When tested in MD simulations of several proteins [54], both of the above parameterizations of the "OBC" model showed equal performance, although further tests [104] on an extensive set of protein structures revealed that the *igb=5* variant agrees better with the Poisson-Boltzmann treatment in calculating the electrostatic part of the solvation free energy.

=6 With this option, there is no continuum solvent model used at all; this corresonds to a non-periodic, "vacuum", model where the non-bonded interactions are just Lennard-Jones and Coulomb interactions. This option is logically equivalent to setting *igb=0* and *eedmeth=4*, although the implementation (and computational efficiency) is not the same.

=7 The *GBn* model described by Mongan, Simmerling, McCammon, Case and Onufriev [11] is employed. This model uses a pairwise correction term to GB^{HCT} to approximate a molecular surface dielectric boundary; that is to eliminate interstitial regions of high dielectric smaller than a solvent molecule. This correction affects all atoms and is geometry-specific, going beyond the geometry-free, "average" rescaling approach of GB^{OBC}, which mostly affects buried atoms. With this method, you should use the bondi radii set. The overlap or screening parameters in the *prmtop* file are ignored, and the model-specific *GBn* optimized values are substituted. The model carries little additional computational overhead relative to the other GB models described above. [11] This method is not recommended for systems involving nucleic acids.

=10 Calculate the reaction field for a non-periodic solute in a spherical "cap" of water, using a numerical Poisson-Boltzmann solver. This option is described in Section 5.15, below. Note that this is *not* a generalized Born simulation, in spite of its use of *igb*; it is rather an alternative continuum solvent model.

INTDIEL Sets the interior dielectric constant of the molecule of interest. Default is 1.0. Other values have not been extensively tested.

EXTDIEL Sets the exterior or solvent dielectric constant. Default is 78.5.

SALTCON Sets the concentration (M) of 1-1 mobile counterions in solution, using a modified generalized Born theory based on the Debye-Hückel limiting law for ion screening of interactions [71]. Default is 0.0 M (*i.e.* no Debye-Hückel screening.) Setting *saltcon* to a non-zero value does result in some increase in computation time.

RGBMAX This parameter controls the maximum distance between atom pairs that will be considered in carrying out the pairwise summation involved in calculating the effective Born radii. Atoms whose associated spheres are farther way than *rgbmax* from given atom will not contribute to that atom's effective Born radius. This is implemented in a "smooth" fashion (thanks mainly to W.A. Svrcek-Seiler), so that when part of an atom's atomic sphere lies inside *rgbmax* cutoff, that part contributes to the low-dielectric region that determines the effective Born radius. The default is 25 Å, which is usually plenty for single-domain proteins of a few hundred residues. Even smaller values (of 10-15 Å) are reasonable, changing the functional form of the generalized Born theory a little bit, in exchange for a considerable speed-up in efficiency, and without introducing the usual cut-off artifacts such as drifts in the total energy.
The *rgbmax* parameter affects only the effective Born radii (and the derivatives of these values with respect to atomic coordinates). The *cut* parameter, on the other hand, determines the maximum distance for the electrostatic, van der Waals and "off-diagonal" terms of the generalized Born interaction. The value of *rgbmax* might be either greater or smaller than that of *cut*: these two parameters are independent of each other. However, values of *cut* that are too small are more likely to lead to artifacts than are small values of *rgbmax*; therefore one typically sets *rgbmax* <= *cut*.

RBORNSTAT If *rbornstat = 1*, the statistics of the effective Born radii for each atom of the molecule throughout the molecular dynamics simulation are reported in the output file. Default is 0.

OFFSET The dielectric radii for generalized Born calculations are decreased by a uniform value "offset" to give the "intrinsic radii" used to obtain effective Born radii. Default 0.09 Å.

GBSA Option to carry out GB/SA (generalized Born/surface area) simulations. For the default value of 0, surface area will not be computed and included in the solvation term. If *gbsa = 1*, surface area will be computed using the LCPO model. [72] If *gbsa = 2*, surface area will be computed by recursively approximating a sphere around an atom, starting from an icosahedra. Note that no forces are generated in this case, hence, *gbsa = 2* only works for a single point energy calculation and is mainly intended for energy decomposition in the realm of MM_GBSA.

SURFTEN Surface tension used to calculate the nonpolar contribution to the free energy of solvation (when *gbsa = 1*), as Enp = surften*SA. The default is 0.005 kcal/mol-Å2 [105].

RDT This parameter is only used for GB simulations with LES (Locally Enhanced Sampling). In GB+LES simulations, non-LES atoms require multiple

effective Born radii due to alternate descreening effects of different LES copies. When the multiple radii for a non-LES atom differ by less than RDT, only a single radius will be used for that atom. See the LES portion of the manual for more details. Default 0.01 Å.

6.1.2. ALPB (Analytical Linearized Poisson-Boltzmann)

Like the GB model, the ALPB approximation [106,107] can be used to replace the need for explicit solvent, with similar benefits (such as enhanced conformational sampling) and caveats. The basic ALPB equation that approximates the electrostatic part of the solvation free energy is:

$$\Delta G_{el} \approx \Delta G_{alpb} = -\tfrac{1}{2}\left(\frac{1}{\varepsilon_{in}} - \frac{1}{\varepsilon_{ex}}\right)\frac{1}{1+\alpha\beta}\sum_{ij} q_i q_j \left(\frac{1}{f_{GB}} + \frac{\alpha\beta}{A}\right) \tag{6.8}$$

where $\beta = \varepsilon_{in}/\varepsilon_{ex}$ is the ratio of the internal and external dielectrics, $\alpha = 0.571412$, and A is the so-called *effective electrostatic size* of the molecule, see the definition of *Arad* below. Here f_{GB} is the same smooth function as in the GB model. The GB approximation is then just the special case of ALPB when the solvent dielectric is infinite; however, for finite values of solvent dielectric the ALPB tends to be more accurate. For aqueous solvation, the accuracy advantage offered by the ALPB is still noticeable, and becomes more pronounced for less polar solvents. Statistically significant tests on macromolecular structures [107] have shown that the ALPB is more likely to be a better approximation to PB than the GB. At the same time, the ALPB has virtually no additional computational overhead relative to the GB. However, users should realize that at this point the new model has not yet been tested nearly as extensively as the GB model, and is therefore in its experimental stage. The model can potentially replace GB in the energy analysis of snapshots via the MMGB/SA scheme. The electrostatic screening effects of monovalent salt are currently introduced into the ALPB in the same manner as in the GB, and are determined by the parameter *saltcon* .

ALPB Flag for using ALPB to handle electrostatic interactions within the implicit solvent model.

- =0 No ALPB (default)
- =1 ALPB is turned on. Requires that one of the GB models is also used to compute the effective Born radii, that is one must set *igb*=1,2,5, or 7. The ALPB uses the same sets of radii as required by the particular GB model.

ARAD Effective electrostatic size (radius) of the molecule. Characterizes its over-all dimensions and global shape, and is not to be confused with the effective Born radius of an atom. An appropriate value of *Arad* must be set if *alpb=1*: this can be conveniently estimated for your input structure with the utility *elsize* that comes with the main distribution. The default is 15 Å. While *Arad* may change during the course of a simulation, these changes are usually not very large; the accuracy of the ALPB is found to be rather insensitive to these variations. In the current version of Amber *Arad* is treated as constant throughout the simulation, the validity of this assumption is discussed in [107] Currently, the effective electrostatic size is only defined for "single-connected" molecules. However, the ALPB model can still be used to treat the important case of complex formation. In the docked state, the compound is

considered as one, with its electrostatic size well defined. When the ligand and receptor become infinitely separated, each can be assigned its own value of *Arad*.

6.2. Poisson-Boltzmann calculations.

An efficient finite-difference numerical solver [108,109] is implemented in *sander* for various applications of the Poisson-Boltzmann (PB) method. In the following, a brief introduction to the PB method and the numerical solver is given first. This is followed by a brief discussion of the supported PB applications and a detailed description of the keywords. Finally example input files are explained for typical PB applications. For more background information and how to use the PB method, please consult cited references and the online AMBER PB tuitorial pages.

6.2.1. Introduction.

Solvation interactions, especially solvent-mediated dielectric screening and Debye-Huckel screening, are thought to be one of the essential determinants of the structure and function of proteins and nucleic acids [110]. Ideally, one would like to provide a detailed description of solvent through explicit simulation of a large number of solvent molecules. This approach is frequently used in molecular dynamics simulations of solution systems. In many applications, however, the solute is the focus of interest, and the detailed properties of the solvent are not of central importance. In such cases, a simplified representation of the solvent, based on an approximation of the mean-force potential for the solvation interactions, can be employed to accelerate the computation. The mean-force potential averages out the degrees of freedom of solvent molecules, so that they are often called implicit or continuum solvents.

The Poisson-Boltzmann (PB) solvents are a class of widely used implicit solvents [111,112]. In these models, a solute is represented by an atomic-detail model as in a molecular mechanics force field, while the solvent molecules and any dissolved electrolyte are treated as a structureless continuum. The solute intramolecular interactions are computed by the usual molecular mechanics force field terms, while the solute-solvent and solvent-solvent interactions are computed by a mean-field approximation through the use of the PB electrostatic theory. The electrostatic model represents the solute as a dielectric body whose shape is defined by atomic coordinates and atomic cavity radii [113]. The solute contains a set of point charges at atomic centers that produce an electrostatic field in the solute region and the solvent region. The electrostatic fields in such a system, including the solvent reaction field and the Coulombic field, may be computed by solving the PB equation [114,115]. The nonelectrostatic or nonpolar solvation interactions are typically modeled with a term proportional to the solvent accessible surface area. An alternative method to model the nonpolar solvation interactions is also implemented in this release (Tan and Luo, In preparation). The new method separates the nonpolar solvation interactions into two terms: the attractive (dispersion) and repulsive (cavity) interactions. Doing so significantly improves the correlation between the cavity free energies and solvent accessible surface areas for branched and cyclic organic molecules [116]. This is in contrast to the commonly used strategy that correlates total nonpolar solvation energies with solvent accessible surface areas, which only correlats well for linear aliphatic molecules [105]. In the new method, the attractive free energy is computed by a numerical integration over the solvent accessible surface area that accounts for solvation attractive interactions with an infinite cutoff [117]. The PB solvent models has been demonstrated to be reliable in reproducing the energetics and conformations as

compared with explicit solvent simulations and experimental measurements for a wide range of systems.

The formalism with which a PB solvent can be applied in molecular mechanics simulations is based on rigorous foundation in statistical mechanics, at least for additive molecular mechanics force fields. PB solvents approximate the solvent-induced electrostatic mean-force potential by computing the reversible work done in the process of charging the atomic charges in a solute molecule as $1/2\sum_j Q_j\phi_j$ with j running over all charged atoms. The electrostatic potential ϕ_j at atomic charge site is computed by solving the PB equation:

$$\nabla\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r}) = -4\pi\rho(\mathbf{r}) - 4\pi\sum_i z_i \exp(-z_i\phi(\mathbf{r})/k_BT)$$

where $\varepsilon(\mathbf{r})$ is the dielectric constant, $\phi(\mathbf{r})$ is the electrostatic potential, $\rho(\mathbf{r})$ is the solute charge, z_i is the charge of ion type i, c_i is the number density of ion type i far from the solute, k_B is the Boltzmann constant, and T is temperature; the summation is over all different ion types. In this release, only the linearized form of the PB equation is used.

Many numerical methods may be used to solve the linearized PB equation. The finite-difference (FD) method is one of the most popular methods in computational studies of biomolecules [118-120]. It involves the following steps: mapping atomic charges to the FD grid points (termed grid charges below); assigning non-periodic/periodic boundary conditions, i.e. electrostatic potentials on the boundary surfaces of the finite difference grid; and applying a dielectric model to define the boundary between high-dielectric (i.e. water) and low-dielectric (i.e. solute interior) regions [121].

These steps allow the partial differential equation to be converted into a linear system A x = b with the electrostatic potential on grid points, x as unknowns, the charge distribution on the grid points as the source b, and the dielectric constant on the grid edges and salt-related terms wrapped into the coefficient matrix A, which is a seven-banded symmetric matrix. Once the linear system is solved, the solution is used to compute the electrostatic energies and forces.

It has been shown that the total electrostatic energy of a solute molecule can be approximated through the FD approach by subtracting the self FD Coulombic energy ($G_{FD,coul,self}$) and the short-range FD Coulombic energy ($G_{FD,coul,short}$) from the total FD electrostatic energy, and adding back the analytical short-range Coulombic energy ($G_{ana,coul,short}$) (see for example [109]). The self FD Coulombic energy is due to interactions of grid charges within one single atom. The self energy exists even when the atomic charge is exactly positioned on one grid point. It also exists in the absence of solvent and any other charges. It apparently is a pure artifact of the FD approach and must be removed. The short-range FD Coulombic energy is due to interactions between grid charges in two different atoms that are separated by a short distance, usually less than 14 grid units. The short-range Coulombic energy is inaccurate because the atomic charges are mapped onto their eight nearest FD grids, thus causing deviation from the analytical Coulomb energy. The correction of $G_{FD,coul,self}$ and $G_{FD,coul,short}$ is made possible by the work of Luty and McCammon's analytical approach to compute FD Coulombic interactions [122]. Therefore, the PB electrostatic interactions include both Coulombic interactions and reaction field interactions for all atoms of the solute. The total electrostatic energy is given in the energy component EEL(EC) in the output file. The term that is reserved for reaction field energy, EPB, is zero if this method is used. If you want to know how much of EEL(EC) is reaction field energy, you can run FDPB twice, once with EPSOUT = 80, and once with EPSOUT = 1.

An alternative method of computing the total electrostatic interactions is also implemented in this release. In this method, reaction field energy is be computed directly after the induced

surface charges are first computed at the dielectric boundary (i.e. the surface that separate solute and solvent). These surface charges are then used to compute reaction field energy [110], and is given as the EPB term. It has been shown that doing so improves the convergence of reaction field energy with respect to the FD grid spacing. However, a drawback of this method is that the Coulombic energy has to be recomputed analytically with a pairwise summation procedure. The EEL(EC) term only gives the Coulombic energy with a specified cutoff distance.

If requested, the ECAVITY term returns the solvent-accessible-surface-area dependent nonpolar solvation free energy (either the total nonpolar solvation free energy or the cavity solvation free energy), the EDISPER term returns the dispersion solvation free energy.

Note that the accuracy of PB is related to the FD grid spacing, the convergence criterion for the PB solver, and the method used for computing total electrostatic interactions. In Lu and Luo [109], the accuracy of the first method for total electrostatic interactions is discussed in detail. In the second method presented above, the total electrostatic interactions strongly depend on the cutoff distance used in the pairwise summations of charge-charge interactions. The accuracy of nonpolar solvation energy depends on the quality of solvent accessbile surface area which is computed numerically by representing each atomic surface by sphereically distributed dots. Thus a higher dot density gives more accurate atomic surface area and molecular surface area. However, it is found by the developers that the default setting for the dot density is quite sufficient for typical applications (Tan and Luo, In preparation).

PB calculations are memory intensive for macromolecules. Thus, the efficiency of PB depends on how much memory is allocated for it and the performance of the memory subsystem. The option directly related its memory allocation is the finite-difference grid spacing. To make PB run faster, it is possible to change the PB code to single precision as in many widely available numerical PB solvers. Make sure you have successfully installed *sander* by running all related test cases before you do this.

6.2.2. Usage and keywords.

The PB procedure can be turned on by setting *igb* = 10. The procedure can be used for both static (single point) and dynamic applications. The default setting of keywords is for static calulculations, so please carefully follow the keyword descriptions and examples to change the input files for dynamic applications.

The current PB implementation can be used both as a pure implicit solvent just as GB (see section 6.1) and as a limited hybrid explicit-implicit solvent for water CAP simulations (see section 5.6.14). The water CAP should be set up in Leap using the solvateCap option (see section 3.6 and example inputs in the next section).

6.2.2.1. Static calculations.

The PB procedure can be invoked by using IMIN = 1 or 5 for static calculations. It is recommended that the second method (DBFOPT = 1) for total electrostatic interactions be used for static calculations. As discussed above, the cutoff distance for charge-charge interactions strongly influences the accuracy of electrostatic interactions. The default setting is infinity, i.e. no cutoff is used (CUTNB = 0). In this method, the convergence of reaction field energy with respect to the grid spacing (SPACE) is much better than that of the first method. Our experience shows that reaction field energy converges within 1% for over 800 tested proteins, protein domains, and nucleic acids at a grid spacing of 0.5 Å. The reaction field energy computed with this method is

also in excellent agreement with the *Delphi* program for the tested systems.

For static calculations, NPOPT can be set to nonzero to choose one of the two treatments of nonpolar solvation interactions (Tan and Luo, In preparation). You can use the solvent-accessible-surface area (SASA) to correlate total nonpolar solvation free energy. I.e. G_{np} = NP_TENSION * SASA + NP_OFFSET as in PARSE [105]. You can also use SASA to correlate the repulsive (cavity) term only and use a surface-integration approach to compute the attractive (dispersion) term. I.e. $G_{np} = G_{disp} + G_{cavity}$, with G_{cavity}= CAVITY_TENSION * SASA + CAVITY_OFFSET. When this option is used, RADIOPT has to be set to 1, i.e. the radii set optimized by Tan and Luo (In preparation) to mimic G_{np} in the TIP3P explicit solvent. Otherwise, there is no guarantee of consistence between the implemented PB solvent and the TIP3P explicit solvent.

6.2.2.2. Dynamic calculations.

The PB procedure can also be invoked by setting IMIN = 0 for dynamics calculations. Since the nonpolar solvation energy has not been implimented for dynamics, please set NPOPT to 0 to turn it off. It is recommended that the first method (DBFOPT = 0) for total electrostatic interactions be used for *hybrid explicit-implicit solvent* for water CAP simulations. This is a special case of the procedure described by Lu and Luo [109]. Specifically, the electrostatic energies and forces are determined with the first method described in Introduction, but the dielectric surface is fixed at the boundary of the CAP waters. That is, in regions of space that are less than CAP radius from the CAP center (both of these are set with the "solvateCap" command in LEaP), the dielectric is taken to be EPSIN (typically 1.0); otherwise, the dielectric is EPSOUT (typically 80). This means that all electrostatic interactions are computed, and that the electrostatic cutoffs (CUTRES and CUTFD, below) are just used to partition the electrostatic interactions into "short-range" and "long-range" contributions. (This is analogous to the way the CUT variable is used in PME.) Covalent interactions are computed in the usual way, and the Lennard-Jones interactions are computed out to a distance CUTNB, with no long-range correction for the missing dispersion terms.

It should be pointed out that "solvateCap" can be used to solvate either a small portion of a solute or all of a solute, depending on the center and radius of the water CAP. The two scenarios require very different implementations for efficiency even if the fundamental algorithm is the same. The implementation in this release is for the situation where a solute is solvated completely by the water CAP. If the water CAP option is detected in the prmtop file, i.e. IFCAP > 0, the PB procedure will ignore whatever atoms outside the water CAP for its dielectric setup.

Because PB treats regions outside the water CAP (augmented by a buffer) as continuum, the explicit water molecules should stay inside the water CAP throughout a simulation. Thus a strong restraining harmonic potential should be used, the recommended value for FCAP is 10 kcal/mol-Å2. Note that the restraining force is only turned on when a water molecule moves outside the water CAP, so that its interference to the solute dynamics is small. Incidentally, this is also why the water CAP is augmented by a buffer in the definition of low dielectric region.

Users interested in dynamics simulations with *pure implicit solvent* are encouraged to test out the second method (DBFOPT = 1) for total electrostatic interactions with an infinite cutoff distance (CUTNB = 0). Doing so would be slow for most systems, but this is a safe way to perform PB dynamics due to the second method's very good convergence behavior. The first method (DBFOPT = 0) for total electrostatic interactions is not implemented for pure implicit solvent dynamics simulations in this release. Also keep in mind that NPOPT should be set to zero to turn off nonpolar solvation treatments just as dynamics simulations in hybrid solvent mentioned above.

All PB options described below can be defined in the `&pb` namelist, which is read immediately after the `&cntrl` namelist. We have tried hard to make the defaults for these parameters appropriate for solvated simulations. Please take care in changing any values. Note that it is not necessary to use the `&pb` namelist at all to turn on PB as long as *igb* = 10. Of course, this means that you only want to use default options for default applications of PB. The `&pb` namelist has the following variables:

EPSIN
: Sets the dielectric constant of the solute region, default to 1.0. The solute region is defined to be the solvent excluded volume which in turn is computed numerically based on a numerical solvent accessible surface area represented as surface dots.

EPSOUT
: Sets the implicit solvent dielectric constant, default to 80. The solvent region is defined to be the space not occupied the solute region. I.e. only two dielectric regions are allowed in the current release.

ISTRNG
: Sets the ionic strength (in mM) for the Poisson-Boltzmann solvent; default is 0 mM.

PBTEMP
: Temperature used for the PB equation, needed to compute the Boltzmann factor for salt effects; default is 300 K.

RADIOPT
: The option to set up atomic radii.

  = 0 Use radii from the prmtop file for both the PB calculation and for the nonpolar solvation energy calculation (see below on NPOPT).

  = 1 Use atom-type/charge-based radii by Tan and Luo (In preparation) for the PB calculation. Note that the radii are optimized for AMBER atom types as in standard residues from the AMBER database. If a residue is build by *antechamber*, i.e. if GAFF atom types are used, radii from the prmtop file will be used. Please see AMBER PB tutorials on how these radii are optimized. The procedure in the tutorial can also be used to optimize radii for non-standard residues. These optimized radii can be read in if they are incorporated into the prmtop file. This option also instructs *sander* to use van der Waals radii from the prmtop file for nonpolar solvation energy calculations (see below on NPOPT). Default.

SPROB
: Solvent probe radius, default to 1.6 Å, the sigma value of TIP3P water. The radii set up by RADIOPT = 1 are optimized with respect to the reaction field energies computed by the thermodynamic intergration method for all AMBER database residues in explicit TIP3P solvents and PME.

NSAS
: The PB procedure uses a numerical method to compute solvent accessible surface area. NSAS variable gives the approximate number of dots to represent the maximum atomic solvent accessible surface, default to 400. These dots are first checked against covalently bonded atoms to see whether any of the dots are buried. The exposed dots from the first step are then checked against a nonbonded pair list for van der Waals interactions (see below) to see whether any of the exposed dots from the first step are buried. The exposed dots of each atom after the second step then represent the solvent accessible portion of the atom and are used to compute the SASA of the atom. The molecular SASA is simply a summation of the atomic SASA's. A molecular SASA is

used for both PB dielectric map assignment and for nonpolar solvation energy calculations.

SMOOTHOPT SMOOTHOPT instructs PB how to set up dielectric values for finite-difference edges that are located on the dielectric boundary.

= 0 The dielectric constant of the boundary edges is always set to the harmonic average of EPSIN and EPSOUT. Default.

= 1 A weighted harmonic average of EPSIN and EPSOUT is used. The weights for EPSIN and EPSOUT are fractions of the boundary edges that are inside or outside the solute [112].

FILLRATIO The ratio between the longest dimension of the rectangular finite-difference grid and that of the solute. Default to 2.0. It is suggested that a larger FILLRATIO, for example 4.0, be used for a small solute. Otherwise, part of the small solute may lie outside of the finite-difference grid, causing the finite-difference solver to fail.

SPACE Sets the grid spacing for the finite difference solver; default is 0.5 Å.

NBUFFER Sets how far away (in grid units) the boundary of the finite difference grid is away from the solute surface; default is 0 grids, i.e. automatically set to be at least a solvent probe (diameter) away.

ACCEPT Sets the convergence criterion (relative) for the finite difference solver; default is 0.001.

MAXITN Sets the maximum number of iterations for the finite difference solver, default to 100. If MAXITN is reached during a simulation (with an accept value of 0.001), it usually indicates there is something wrong in the installation of the program.

DBFOPT Option to compute total electrostatic energy and forces.

= 0 Compute total electrostatic energy and forces with an infinite cutoff distance with the particle-particle particle-mesh procedure outlined in Lu and Luo [109]. In doing so, energy term EPB in the output file is set to zero, while EEL(EC) includes both reaction field energy and Coulombic energy.

= 1 Use dielectric boundary surface charges to compute reaction field energy and forces with an infinite cutoff distance. Default. Energy term EPB in the output file is reaction field energy. EEL(EC) is Coulombic energy computed according to the cutoff distance as speficied by CUTNB below.

SCALEC Option to compute reaction field energy and forces.

= 0 Do not scale dielectric boundary surface charges before computing reaction field energy and forces. Default.

= 1 Scale dielectric boundary surface charges using Gauss's Law before computing reaction field energy and forces.

NPBGRID Sets how often the finite difference grid is regenerated; default is 1 steps. For molecular dynamics simulations, it is recommended to be set to at least 100. If IFCAP is nonzero, a value as high as 1000 can be used because the water CAP dimension does not change during simulation. Note that the PB solver

effectively takes advantage of the fact that the electrostatic potential distribution varies very slowly during dynamics simulations. This requires that the finite-difference grid be fixed in space for the code to be efficient. However, molecules do move freely in simulations. Thus, it is necessary to set up the finite-difference grid once in a while to make sure a molecule is well within the grid.

NSNBR Sets how often residue-based pairlist is generated; default is 1 steps. For molecular dynamics simulations, a value of 25 is recommended.

NSNBA Sets how often atom-based pairlist is generated; default is 1 steps. For molecular dynamics simulations, a value of 5 is recommended.

CUTRES Residue-based cutoff distance; default is 12 Å. The residue-based nonbonded list is used to make the generation of the atom-based cutoff list efficient.

CUTFD Atom-based cutoff distance to remove short-range finite-difference Coulombic interactions, and to add pairwise Coulombic interactions, default is 5 Å. See Eqn (20) in Lu and Luo [109].

CUTNB Atom-based cutoff distance for van der Waals interactions, and pairwise Coulombic interactions when DBFOPT = 1. Default to 0 Å. When CUTNB is set to the default value of 0, no cutoff will be used for van der Waals and Coulombic interactions, i.e. all pairwise interactions will be included. When DBFOPT = 0, this is the cutoff distance used for van der Waals interactions only. Coulombic interactions are computed without cutoff.

NPOPT Option to select different methods to compute nonpolar solvation free energy.

= 0 No nonpolar solvation free energy is computed.

= 1 The total nonpolar solvation free energy is modeled as a single term linearly proportional to the solvent accessible surface area, as in the PARSE parameter set. See Usage and keywords above.

= 2 The total nonpolar solvation free energy is modeled as two terms: the repulsive (cavity) term and the dispersion term. Default. The dispersion term is computed with a surface-based integration method (Tan and Luo, In preparation) closely related to the PCM solvent for quantum chemical programs [117] Under this framework, the repulsive term is still computed as a term linearly proportional to the solvent accessible surface area. With this option, please do not use RADIOPT = 0, i.e. the radii in the prmtop file. Otherwise, a warning will be issued in the output file.

CAVITY_SURFTEN
The surface tension for the linear relation between the total nonpolar solvation free energy (NPOPT = 1) or the cavity free energy (NPOPT = 2) and the solvent accessible surface area. The default value is for NPOPT = 2, but not for NPOPT = 1.

CAVITY_OFFSET The offset for the linear relation between the total nonpolar solvation free energy (NPOPT = 1) or the cavity free energy (NPOPT = 2) and the solvent accessible surface area. The default value is for NPOPT = 2, but not for NPOPT = 1.

PHIOUT The PB procedure can be used to output spacial distribution of electrostatic potential (kcal/mol-e) for visualization.

= 0 No potential file is printed out. Default.

= 1 Electrostatic potential will be printed out in a file named *pbsa.phi*. Please see AMBER PB tutorials on how to display electrostatic potential on molecular surface.

PHIFORM

Controls the format of the electrostatic potential file.

= 0 The electrostatic potential is printed in the *Delphi* binary format. Default.

= 1 The electrostatic potential is printed in the AMBER ascii format.

NPBVERB

When set to 1, turns on verbose mode for PB calculations; default is 0.

6.2.3. Example inputs.

6.2.3.1. Static calculations.

Here is a sample input file that might be used to perform single structure calculations.

```
Sample single point PB calculation
&cntrl
   ntx=1, irest=0,
   imin=1, ntmin=2, maxcyc=0,
   ntpr=1, igb=10, ntb=0,
   ntc=1, ntf=1
 /
&pb
   npbverb=1, istrng=0, epsout=80.0, epsin=1.0,
   sprob=1.6, radiopt=1,
   space=0.5, nbuffer=0, accept=0.001,
   cutnb=0, dbfopt=1, npopt=2
/
```

Note that NPBVERB = 1 above. This generates many detailed information in the output file for the PB and NP calculations. A useful printout is atomic SASA data for both PB and NP calculations which may or may not use the same atomic radius definition. Since the FD solver for PB is called twice to perform electrostatic focus calculations, two PB printouts are shown for each single point calculation. NPOPT is set to default value of 2, which calls for nonpolar solvation calculation with the new method that separates cavity and dispersion interactions. The EDISPER term gives the dispersion solvation free energy, and the ECAVITY term gives the cavity solvation free energy. If NPOPT is set to 1, the ECAVITY term would give the total nonpolar solvation free energy. If IMIN is set to be 5, the above input file can also be used to post-process a *sander* trajectory. See section 5.6.1 and related AMBER test cases for details of the IMIN = 5 option. Also keep in mind that such calculations are usually for structures without explicit water molecules. You can use *ptraj* to generate water-free *inpcrd* and trajectory files for these calculations.

You can also use *sander* to produce an electrostatic potential map for visualization in *pymol* when setting PHIOUT = 1. By default, *sander* outputs a file *pbsa.phi* in the *Delphi* bindary format. The sample input file is listed below:

```
Sample PB visualization input
&cntrl
   ntx=1, irest=0,
   imin=1, ntmin=2, maxcyc=0,
   ntpr=1, igb=10, ntb=0,
   ntc=1, ntf=1
/
&pb
   npbverb=1, istrng=0, epsout=80.0, epsin=1.0,
   space=1., accept=0.001,
   sprob=1.4, cutnb=9,
   phiout=1, phiform=0
/
```

To be consistent with the surface routine of *pymol*, the option PHIOUT = 1 instructs *sander* to use the radii as defined in *pymol*. The finite-difference grid is also set to be cubic as in *Delphi*. The SPROB value should be set to that used in *pymol*, 1.4 Å. A large grid spacing, e.g. 1 Å or higher, is recommended for visualization purposes. Otherwise, the potential file would be very large. In principle, it is possible to visualize the potential file in *VMD*, but we have not validated this program. More detailed information on static single-point PB calculations can be found on the AMBER PB tutorial pages.

6.2.3.2. Dynamic calculations.

Since the PB procedure is called for static calculations by default, several default options must be changed if you want to test the procedure for dynamics simulations. For hybrid explicit-implicit solvent dynamics simulations, the following sample input file can be used:

```
Sample water CAP simulation with PB reaction field correction
&cntrl
   ntx=1, irest=0, imin=0,
   ntpr=500, ntwx=500, ntwr=5000,
   nstlim=1000, dt=0.001,
   ntt=1, temp0=300, tempi=0, tautp=0.1,
   igb=10, ntb=0, cut=0, fcap=10.0, ivcap=0,
   ntc=2, ntf=2, tol=0.000001
/
 &pb
   npbverb=0, npbgrid=1000, nsnbr=25, nsnba=5,
   epsin=1.0, epsout=80.0,
   space=0.7, accept=0.001,
   smoothopt=1, dbfopt=0,
   npopt=0,
   cutres=11, cutnb=9, cutfd=9
/
```

Here NPOPT should be set to zero to turn off nonpolar solvation because the nonpolar implementations are not ready for dynamics simulations and also because the nonpolar interactions are supposed to be taken care of by explicit solvent molecules in the systems. The second point is that DBFOPT should be set to zero to use the procedure in Lu and Luo [109]. A related keyword is SMOOTHOPT that has to be set to 1 to turn on the weighted harmonic averaging of dielectric constants for boundary dielectric edges when using DBFOPT = 0 for dynamics. Finally the cutoff distances and their update frequencies should be set as in the input file.

The PB procedure implemented in *sander* can be invoked for pure implicit solvent dynamics simulations as well. Here is a sample input:

```
Sample PB implicit solvent dynamics
&cntrl
  ntx=1, irest=0, imin=0,
  ntpr=500, ntwx=500, nscm=100, ntwr=5000,
  dt=0.001, nstlim=1000,
  temp0=300, tempi=0, ntt=1, tautp=0.1,
  igb=10, cut=0, ntb=0,
  ntc=2, ntf=2, tol=0.000001
/
&pb
  npbverb=0, nsnbr=25, nsnba=5, npbgrid=100,
  npopt=0, istrng=0, epsout=80.0, epsin=1.0,
  space=1., fillratio=2,
  sprob=1.6, radiopt=1,
  accept=0.001
/
```

Note here NPOPT is also set to turn off nonpolar solvation interactions. DBFOPT is the default value of 1, i.e. induced surface charges are first computed for reaction field energy and forces. For dynamics simulation of small molecules, it might be necessary to set FILLRATIO to 4. Since CUTNB is set to the default value of zero, an inifite cutoff distance is used for both Coulombic and van der Waals interactions. Note that the surface routine also needs the nonbonded list, though with a much shorter cutoff distance (9 Å), so the nonbonded list needs regular updates as specified in the input file.

6.3. Empirical Valence Bond

6.3.1. Introduction.

Chemical reactivity can be formulated within the empirical valence bond (EVB) model, whereby the reactive surface is defined traditionally as the lowest adiabatic surface obtained by diagonalization of the Hamiltonian matrix in the representation of non-reactive diabatic states. These diabatic states can be described by a force field approach, such as Amber. The coupling elements in the Hamiltonian matrix embody all the physics needed for describing transitions between the diabatic states.

For example, the intramolecular proton transfer reaction involved in the intercoversion between hydroxypyridine and pyridone (Figure 1) is described by a 2-state EVB Hamiltonian

matrix

$$\mathbf{H} = \begin{bmatrix} H_{11} & H_{12} \\ H_{21} & H_{22} \end{bmatrix} \tag{6.10}$$

where valence bond state 1 represents the reactant state (RS) with the proton bonded to the oxygen and valence bond state 2 represents the product state (PS) with the proton bonded to the nitrogen. The matrix elements H_{11} and H_{22} are simply the energies of the reactant and product systems, respectively, as calculated from Amber. The off-diagonal elements of the symmetric Hamiltonian matrix, *i.e.* $H_{12} = H_{21}$, couple these two well states.

Now, how do we go about determining the coupling element H_{12} for describing the proton tranfer reaction (involving bond breakage and bond formation)? The standard prescription is to fit the coupling elements such that the results from EVB molecular dynamics reproduce experimental observables or results from high-level electronic structure methods. Amber offers several functional forms for describing the couplings. Additionally, the EVB facility can handle an arbitrary N-state system as well as perform MD or energy minimization on the EVB ground-state surface and biased sampling along an energy gap reaction coordinate.

6.3.2. General usage description.

The EVB facility is built on top of the multisander infrastructure in Amber. As such, the user will need to build the parallel version of sander in order to utilize the EVB feature. Information for each EVB diabatic state are obtained from separate (simultaneous) instances of sander. The energies and forces of all the states are communicated via MPI to the master node, which is responsible for computing the EVB energy and forces and broadcasting these to the other nodes for the next MD cycle.

The required input files are (1) an EVB multisander groupfile that contains (per line) all the commandline options for each sander job, (2) the mdin, coordinate, and parmtop files that are specified in the groupfile, and (3) the EVB input files. The contents of the EVB groupfile is

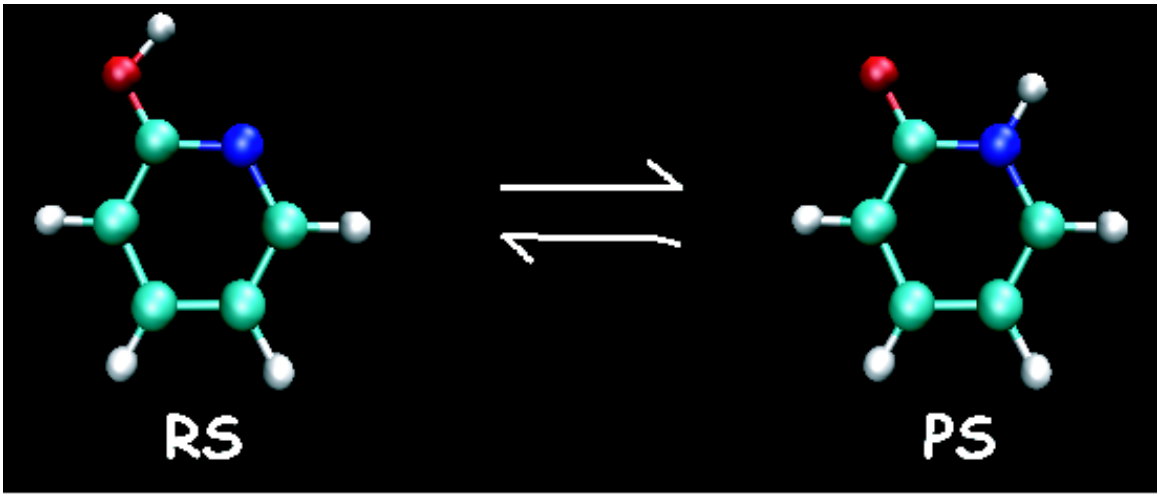


Figure 1. Hydroxypyridine to pyridone conversion via intramolecular proton transfer.

similar to that for a typical multisander execution, with the exception of an additional commandline flag `-evbin` for specifying the name of the EVB input file. Below is an example groupfile:

```
# 2-state EVB example; start the system in the po configuration space
-O -i mdin -p poh.top -c poh.crd -o poh.out -r poh.rst -evbin input.poh
-O -i mdin -p  po.top -c poh.crd -o  po.out -r  po.rst -evbin input.po
```

Each line corresponds to a diabatic state; comments are preceeded by a # symbol in the first column of a line. Now, it is critical to notice in the above example that the starting configurations for both sander jobs are the same, although the topology files are different. This is absolutely necessary, and the code does check to ensure that the file names for the initial configurations are identical. This constraint is to guarantee that the system starts in a physically meaningful part of configuration space. All other alternatives are nonsense in our part of the physical universe. The only additional flag in the `&cntrl` namelist of the `mdin` file is `ievb`, which has the following values

IEVB Flag to run EVB

- = 0 No effect (default)
- = 1 Enable EVB. The value of **IMIN** specifies if the sander calculation is a molecular dynamics (`imin=0`) or an energy minimization (`imin=1`). The variable **EVB_DYN** in the `&evb` namelist of the EVB input file refines this choice to specify if the calculation type is on the EVB ground-state surface, on a mapping potential, or on an umbrella potential (see below for details).

The argument of the commandline flag `-evbin` provides the name of the EVB inputfile. Corresponding to the above groupfile example, the inputs for EVB state 1 are provided in the file input.poh and those for EVB state 2 are provided in input.po. For the case of constant coupling between the EVB states, the input.poh file may look something like the following

```
# Hydroxypyridine: proton = atom no. 2 and Oxygen = atom no. 12
&evb nevb=2, nmorse=1,
     dia_type    = 'force_field',
     xch_type    = 'constant',
     evb_dyn     = 'groundstate',
     dia_shift(1)% st = 1, dia_shift(1)% nrg_offset = -42.0,
     dia_shift(2)% st = 2, dia_shift(2)% nrg_offset =   0.0,
     xch_cnst(1)% ist = 1, xch_cnst(1)% jst = 2, xch_cnst(1)% xcnst = 20.0,
     morsify(1)% iatom=2, morsify(1)% jatom=12, morsify(1)% D=25.0,
     morsify(1)% a=1.5, morsify(1)% r0=1.0,
&end
```

and the file input.po may appear as follows:

```
# Pyridone: proton = atom no. 2 and Nitrogen = atom no. 1
&evb nevb=2, nmorse=1,
```

```
     dia_type    = 'force_field',
     xch_type    = 'constant',
     evb_dyn     = 'groundstate',
     dia_shift(1)% st = 1, dia_shift(1)% nrg_offset = -42.0,
     dia_shift(2)% st = 2, dia_shift(2)% nrg_offset =   0.0,
     xch_cnst(1)% ist = 1, xch_cnst(1)% jst = 2, xch_cnst(1)% xcnst = 20.0,
     morsify(1)% iatom=1, morsify(1)% jatom=2, morsify(1)% D=20.0,
     morsify(1)% a=3.2, morsify(1)% r0=1.2,
&end
```

Note that the only difference between the two input files is contained within the derived datatype variable `morsify(:)`, which requests the replacement of specified Amber harmonic bond interactions with Morse type interactions. The above EVB input files specify that the system is described by a 2-state model where the diabatic states are computed from a force field approach, the coupling between the 2-states is a constant, and the dynamics is on the EVB ground-state surface. The energies of the diabatic states were also adjusted by 42.0 kcal/mol so that the relative energies agree with *ab initio* calculation. The constant value coupling between the 2-states was parameterized to give an EVB energy barrier that agrees with the *ab initio* barrier of ....

This parameterization of the EVB surface to provide observables that match either results from high-level quantum chemistry calculations or experimental measurements is the most tedious, boring, and time-consuming aspect of the EVB model. However, after the EVB surface has been calibrated, the user has access to reactive chemical dynamics simulation timescales and lengthscales which would be otherwise unachievable using standard *ab initio* MD approaches, for example.

Now, let us suppose that the constant coupling prescription does not provide the detailed features needed to described the transition state region in going from diabatic state 1 to diabatic state 2. Furthermore, we find that the couplings, as a function of nuclear coordinates, can be described quite well (from comparison to *ab initio* data) using a Gaussian functional form. How should we modify the above EVB input files to obtain a more accurate reactive surface?

We would need to change the `xch_type` variable from a `'constant'` to `'gauss'` as well as replace the variable `xch_cnst` by the variable `xch_gauss(:)`, which contains the parameters for the Gaussian functional form. Of course, these parameters need to be optimized to provide the more accurate surface; otherwise, the surface may turn out to be worse than that provided by the constant coupling approximation. The two modified EVB files may look something like the following:

```
# Hydroxypyridine: proton = atom no. 2 and Oxygen = atom no. 12
&evb nevb=2, nmorse=1,
     dia_type    = 'force_field',
     xch_type    = 'gauss',
     evb_dyn     = 'groundstate',
     dia_shift(1)% st = 1, dia_shift(1)% nrg_offset = -42.0,
     dia_shift(2)% st = 2, dia_shift(2)% nrg_offset =   0.0,
     xch_gauss(1)% ist=1, xch_gauss(1)% jst=2,
     xch_gauss(1)% iatom=12, xch_gauss(1)% iatom=1,
     xch_gauss(1)% a=20.0, xch_gauss(1)% sigma=1.0, xch_gauss(1)% r0=2.11,
     morsify(1)% iatom=2, morsify(1)% jatom=12, morsify(1)% D=25.0,
```

```
     morsify(1)% a=1.5, morsify(1)% r0=1.0,
&end

--------------------------------------------------------------------

# Pyridone: proton = atom no. 2 and Nitrogen = atom no. 1
&evb nevb=2, nmorse=1,
     dia_type    = 'force_field',
     xch_type    = 'gauss',
     evb_dyn     = 'groundstate',
     dia_shift(1)% st = 1, dia_shift(1)% nrg_offset = -42.0,
     dia_shift(2)% st = 2, dia_shift(2)% nrg_offset =   0.0,
     xch_gauss(1)% ist=1, xch_gauss(1)% jst=2,
     xch_gauss(1)% iatom=12, xch_gauss(1)% iatom=1,
     xch_gauss(1)% a=20.0, xch_gauss(1)% sigma=1.0, xch_gauss(1)% r0=2.11,
     morsify(1)% iatom=1, morsify(1)% jatom=2, morsify(1)% D=20.0,
     morsify(1)% a=3.2, morsify(1)% r0=1.2,
&end
```

6.3.3. EVB input variables and interdependencies.

The variables in the `&evb` namelist of the EVB input file are described below. The style of the input file is the same as that for the standard `mdin` used in a sander run. Assignment to character type variables need to be assigned values encapsulated within quotation marks (for example, `evb_dyn='groundstate'`). Array variables are denote below by a colon enclosed within parenthesis (for example, DIA_SHIFT(:)). Conforming to the Fortran 90 standard, derived datatype variables can be assigned element-wise, *i.e.*, `dia_shift(1)%st=1, dia_shift(1)%nrg_offset=0.0, dia_shift(2)%st=2, dia_shift(2)%nrg_offset=42.0.`

NEVB [integer] Number of EVB states. For example, NEVB = 3 specifies that the system is described by a 3×3 Hamiltonian matrix in the representation of 3 diabatic states. The EVB groupfile will contain 3 lines of sander command-line options specifying the mdin, coordinate, parmtop, and EVB input files.

NMORSE [integer] Number of Amber harmonic bond interactions that will be changed to a Morse type interaction. User will need to provide parameters for the variable MORSIFY(:).

NBIAS [integer] Number of biasing potentials to include in the system Hamiltonian. The supported biased sampling approaches include (1) mapping potential and (2) energy gap umbrella potential. See EVB_DYN and associated dependencies.

DIA_TYPE [character] Diabatic state type

= 'force_field' Diabatic states energies are computed using Amber.

= 'ab_initio' Diabatic states energies are computed based on energy, gradient, and hessian information from an external ab initio calculation. User will need to provide parameters for the variable DIA_XFILE(:).

XCH_TYPE [character] Coupling element type

= 'constant' H_{ij} is a constant

= 'exp' H_{ij} is described by an exponential function of the form $A_{ij}\exp\left[-u_{ij}\left(r_{kl}-r_{kl}^{(0,ij)}\right)\right]$. The user will need to provide parameters for this function in the variable XCH_EXP(:).

= 'gauss' H_{ij} is described by a gaussian function of the form $A_{ij}\exp\left[-\frac{1}{\sigma_{ij}^2}\left(r_{kl}-r_{kl}^{(0,ij)}\right)^2\right]$. The user will need to provide parameters for this function in the variabe XCH_GAUSS(:).

= 'chang_miller' H_{ij} is described by the Chang-Miller framework

EVB_DYN [character] EVB dynamics type

= 'groundstate' Dynamics on EVB ground-state potential surface

= 'evb_map' Biased sampling based on Ariel Warshel's mapping potential approach. User will need to provide parameters for the variable EMAP(:).

= 'egap_umb' Harmonic umbrella sampling on an energy gap reaction coordinate. User will need to provide parameters for the variable EGAP_UMB(:).

DIA_SHIFT(:) [derived type] Diabatic state energy shift. The size of this derived datatype array is NEVB. The components of the derived type are

%st [integer] Diabatic state index

%nrg_offset [real] Energy offset for EVB state `st`

XCH_CNST(:) [derived type] Constant coupling. The size of this derived datatype array is NXCH, which is calculated internally by NEVB (NEVB - 1) / 2. The components of the derived type are

%ist [integer] Diabatic state index involved in the coupling

%jst [integer] Diabatic state index involved in the coupling

%xcnst [real] Constant exchange parameter

DIA_XFILE(:) [derived type] External file containing the energy, gradient, and hessian for a particular diabatic state. The size of this derived datatype array is NEVB. The components of this derived type are

%st [integer] Diabatic state index

%fname [character] External file name

XCH_XFILE(:) [derived type] External file containing the energy, gradient, and hessian used for computing an exchange term. The size of this derived datatype array is

NXCH, which is calculated internally by NEVB (NEVB - 1) / 2. The components of this derived type are

%ist	[integer] Diabatic state index involved in the coupling
%jst	[integer] Diabatic state index involved in the coupling
%fname	[character] External file name

XCH_EXP(:) [derived type] Parameters for the exponential functional form of the coupling terms. The size of this derived datatype array is NXCH, which is calculated internally by NEVB (NEVB - 1) / 2. The components of this derived type are

%ist	[integer] Diabatic state index involved in the coupling
%jst	[integer] Diabatic state index involved in the coupling
%iatom	[integer] Index of atom involved in the bond
%jatom	[integer] Index of atom involved in the bond
%a	[real] Prefactor parameter outside of exponential
%u	[real] Prefactor parameter inside of exponential
%r0	[real] Equilibrium distance parameter

XCH_GAUSS(:) [derived type] Parameters for the Gaussian functional form of the coupling terms. The size of this derived datatype array is NXCH, which is calculated internally by NEVB (NEVB - 1) / 2. The components of this derived type are

%ist	[integer] Diabatic state index involved in the coupling
%jst	[integer] Diabatic state index involved in the coupling
%iatom	[integer] Index of atom involved in the bond
%jatom	[integer] Index of atom involved in the bond
%a	[real] Prefactor parameter outside of exponential
%sigma	[real] Related to the variance
%r0	[real] Equilibrium distance parameter

MORSIFY(:) [derived type] Morse potential parameters used form converting the NMORSE Amber harmonic bond interactions to the Morse type. The size of this derived datatype array is NMORSE. The components in the derived type are

%iatom	[integer] Index of atom involved in the bond
%jatom	[integer] Index of atom involved in the bond
%d	[real] Well depth in Morse potential
%a	[real] Prefactor inside exponential
%r0	[real] Equilibrium distance parameter

EMAP(:) [derived type] Mapping potential parameters required for the function $V_\lambda = (1-\lambda)H_{ii} + \lambda H_{ff}$. The size of this derived datatype array is NBIAS. The components of the derived type are

	%ist	[integer] Diabatic state index for the initial state
	%jst	[integer] Diabatic state index for the final state
	%lambda	[real] Mapping potential parameter
EGAP_UMB(:)	[derived type] Harmonic umbrella potential parameters required for the function $V_{\text{umb}} = \frac{1}{2}\, k[(H_{ii} - H_{ff}) - \text{RC}_0]^2$. The size of this derived datatype array is NBIAS. The components of the derived type are	
	%ist	[integer] Diabatic state index for the initial state
	%jst	[integer] Diabatic state index for the final state
	%k	[real] Harmonic force constant
	%ezero	[real] Equilibrium RC position in harmonic potential

6.3.4. Biased sampling.

When a reactive event is described by an intrinsic high free energy barrier, standard molecular dynamics on the EVB ground-state surface will not adequately sample the important transition state region. Under these conditions, chemical reactions are rare events and sampling on the EVB surface effectively reduces to sampling on a diabatic surface. One framework for enhancing the sampling of rare events is through the modification of the system Hamiltonian with the addition of biasing potentials. The EVB facility in Amber offers two options for biased sampling: (1) Arieh Warshel's mapping potential approach (2) Dave Case's harmonic umbrella potential approach. Currently, only the energy gap reaction coordinate (RC) is supported; support for other types of RC will be included in future releases.

In the mapping potential framework, the system Hamiltonian (and hence, the molecular dynamics) is described by the modified potential

$$V_\lambda = (1 - \lambda)H_{ii} + \lambda H_{ff} \tag{6.11}$$

where H_{ii} is the EVB matrix element for the *initial* state and H_{ff} is the EVB matrix element for the *final* state. As the value of mapping potential parameter λ changes from 0 to 1, the system *evolves* from the initial state to the final state. As an example, for $\lambda = 0.50$, the system Hamiltonian is an equal linear combination of the initial and final states and molecular dynamics sample the region in the vicinity of the transition state. Each mapping potential only samples a portion of the reaction coordinate. In practice, a series of mapping potentials are used to biase the sampling across the whole range of the RC. The average distribution of the RC for each mapping potential are then *unbiased* and the set of unbiased distributions are combined to give the whole free energy profile or potential of mean force (PMF). Figure 2 shows a PMF for the hydroxypyridine to pyridone intramolecular proton transfer reaction obtained from 19 mapping potential simulations with λ ranging from 0.05 to 0.95 at 0.05 interval.

In the harmonic umbrella sampling framework, the system Hamiltonian is described by the modified potential

$$V^{(n)}_{\text{biased}}(\mathbf{q}) = V_{\text{el0}}(\mathbf{q}) + V^{(n)}_{\text{umb}}(\mathbf{q})$$

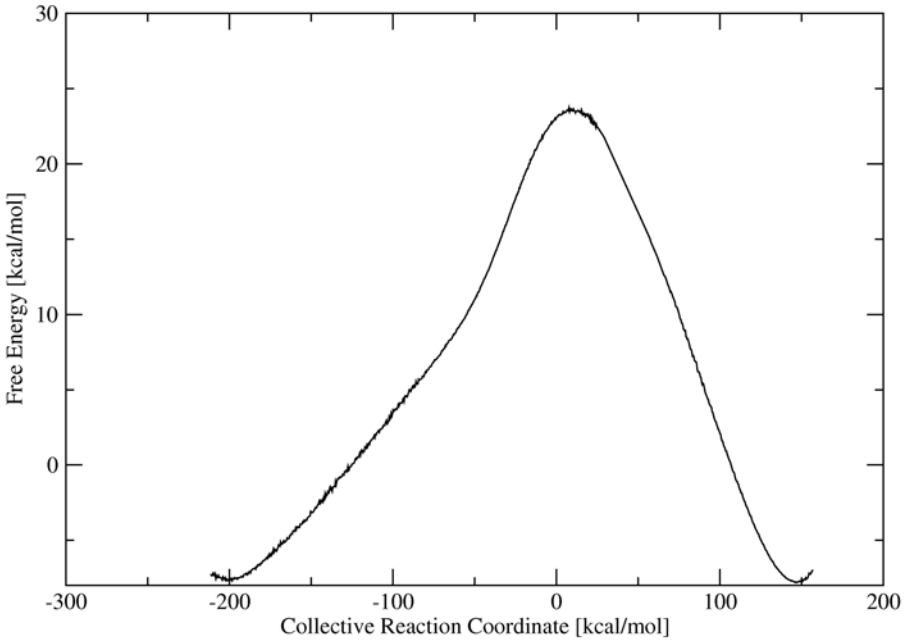


Figure 2. Potential of mean force for the hydroxypyridine to pyridone conversion via intramolecular proton transfer as obtained from a series of mapping potential simulations.

$$= V_{\mathrm{el0}}(\mathbf{q}) + \tfrac{1}{2}\, k^{(n)}\left[\mathrm{RC}(\mathbf{q}) - \mathrm{RC}_0^{(n)}\right]^2 \qquad (6.12)$$

where **q** is the set of system coordinates, k is the *harmonic force constant* parameter, and V_{umb} is an umbrella potential that is added to the original system potential V_{el0} (obtained from diagonalization of the EVB matrix) to biase the sampling towards a particular value of the reaction coordinate RC_0. The superscript (n) denotes that a series of biased sampling simulations, each enhancing the sampling of a particular window of the RC, is required to map out the entire PMF. In the current implementation, the reaction coordinate is chosen to be the difference between the energies of the initial and final diabatic states

$$\mathrm{RC}(\mathbf{q}) = H_{ii}(\mathbf{q}) - H_{ff}(\mathbf{q}) \qquad (6.13)$$

The results from each biased sampling are then unbiased and combined to generate the PMF for the chemical reaction to occur on the original (physically relevant) EVB ground-state potential energy surface, V_{el0}. Figure 3 depicts the PMF for the hydroxypyridine to pyridone conversion that is obtained from 21 umbrella sampling simulations. The probability for sampling the whole range of the RC on V_{el0} was obtained from the biased sampling trajectories using the weighted histogram analysis method. The supporting program to generate the PMF from a set of mapping potential simulations or from a set of umbrella sampling MD can be obtained from the Amber website, http://amber.scripps.edu/.

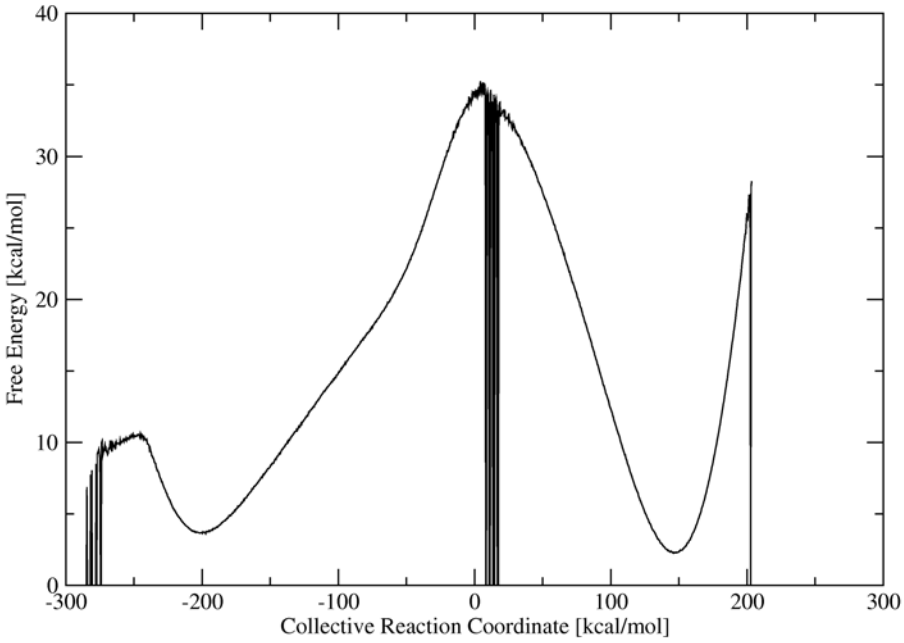


Figure 3. Potential of mean force for the hydroxypyridine to pyridone conversion via intramolecular proton transfer as obtained from a series of umbrella sampling simulations.

6.3.5. Biased sampling usage.

The biased sampling function is accessed through the `evb_dyn` and `nbias` variables in the EVB input file. We have already discussed in the previous section the ground-state dynamics option, *i.e.*, `evb_dyn='groundstate'`. Mapping potential dynamics is invoked using the assignment `evb_dyn='evb_map'`; while, biased sampling via umbrella potentials is invoked with the assignment `evb_dyn='egap_umb'`. The variable `nbias` specifies the number of biasing potentials to include in the system Hamiltonian. Associated with each choice of biased sampling approach is an additional dependent derived-type variable

EMAP(:) [derived type] Mapping potential parameters required for the function $V_\lambda = (1-\lambda)H_{ii} + \lambda H_{ff}$. The size of this derived datatype array is NBIAS. The components of the derived type are

- %ist [integer] Diabatic state index for the initial state
- %jst [integer] Diabatic state index for the final state
- %lambda [real] Mapping potential parameter

EGAP_UMB(:) [derived type] Harmonic umbrella potential parameters required for the function $V_{\text{umb}} = \frac{1}{2}\,k[(H_{ii} - H_{ff}) - \text{RC}_0]^2$. The size of this derived datatype array is NBIAS. The components of the derived type are

- %ist [integer] Diabatic state index for the initial state
- %jst [integer] Diabatic state index for the final state
- %k [real] Harmonic force constant

%ezero [real] Equilibrium RC position in harmonic potential

Shown below is an example mapping potential EVB inputfile for the hydroxypyridine diabatic state; one will also have a corresponding file for the pyridone diabatic state. Now, these pairs of inputfiles will comprise a single mapping potential simulation, where diabatic state 1 has been assigned arbitrarily as the initial state and diabatic state 2 has been assigned as the final state and the coupling parameter between these two states is $\lambda = 0.50$. Molecular dynamics on this effective potential will sample the transition state region of the reaction coordinate. To sample the other parts of the RC, one will need to perform mapping potential trajectories for other values of λ in the interval between 0.0 and 1.0. The PMF shown in Figure 2 was obtain from a set of 19 mapping potential trajectories, with $\lambda = \{0.05, 0.10, \ldots, 0.90, 0.95\}$. The hydroxypyridine diabatic state inputfiles are identical for all 19 simulations, except for the assignment of the λ value.

```
# Hydroxypyridine: proton = atom no. 2 and Oxygen = atom no. 12
&evb nevb=2, nmorse=1, nbias=1, ntw_evb=50
     dia_type    = 'force_field',
     xch_type    = 'constant',
     evb_dyn     = 'evb_map',
     dia_shift(1)% st = 1, dia_shift(1)% nrg_offset = -42.0,
     dia_shift(2)% st = 2, dia_shift(2)% nrg_offset =   0.0,
     xch_cnst(1)% ist = 1, xch_cnst(1)% jst = 2, xch_cnst(1)% xcnst = 20.0,
     morsify(1)% iatom=2, morsify(1)% jatom=12, morsify(1)% D=25.0,
     morsify(1)% a=1.5, morsify(1)% r0=1.0,
     emap(1)% ist = 1, emap(1)% jst = 2, emap(1)% lambda = .50,
&end
```

Shown below is an example umbrella sampling EVB inputfile for the hydroxypyridine diabatic state; the pyridone diabatic state will have a corresponding file. Here, the EVB ground-state potential V_{el0} is augmented with a single (`nbias=1`) harmonic umbrella potential with force constant of `k=0.010` and a RC equilibrium position of `ezero=-75.00`. The PMF shown in Figure 3 was generated from a set of 21 umbrella sampling trajectories where the RC equilibrium position was varied from -250.00 kcal/mol to 250.00 kcal/mol at 25.0 kcal/mol increments. The number of umbrella sampling windows as well as the choice of values for the force constant parameter and RC equilibrium position will ultimately depend on the nature of the free energy landscape of the system in question.

```
# Hydroxypyridine: proton = atom no. 2 and Oxygen = atom no. 12
&evb nevb=2, nmorse=1, nbias=1, ntw_evb=50
     dia_type    = 'force_field',
     xch_type    = 'constant',
     evb_dyn     = 'egap_umb',
     dia_shift(1)% st = 1, dia_shift(1)% nrg_offset = -42.0,
     dia_shift(2)% st = 2, dia_shift(2)% nrg_offset =   0.0,
     xch_cnst(1)% ist = 1, xch_cnst(1)% jst = 2, xch_cnst(1)% xcnst = 20.0,
     morsify(1)% iatom=2, morsify(1)% jatom=12, morsify(1)% D=25.0,
     morsify(1)% a=1.5, morsify(1)% r0=1.0,
     egap_umb(1)% ist = 1, egap_umb(1)% jst = 2, egap_umb(1)% k = 0.010,
```

```
     egap_umb(1)% ezero = -75.00,
&end
```

6.4. QM/MM calculations

Sander now supports the option of allowing part of the system to be described quantum mechanically [73] in an approach known as a hybid (or coupled potential) QM/MM simulation. The QM/MM support has been completely re-written in Amber 9 and the way in which QM/MM calculations are initiated has been greatly simplified. As such Amber 8 QM/MM input files are no longer compatible with Amber 9. You should read this section of the manual carefully to familiarise yourself with how to run QM/MM simulations in Amber 9. The most important change is that QM/MM support is now provided in the regular sander executable and so the only change required to your execution protocol used for a simulation is some minor modification of the input file (`mdin`). Other important changes include the way in which calculations are set up, the way the non-bond cut off works for QM atoms, the way link atoms are treated and the way in which the SHAKE algorithm deals with QM atoms.

QM/MM calculations are implemented via two interfaces. The first interface provides seemless semi-empirical QM/MM integration via a `&qmmm` namelist supplied in the regular *mdin* file. This interface is accessed by setting *ifqnt=1* and *idc=0*. The second interface provides support for QM/MM simulations via the DivCon library. This interface is accessed by setting *idc>0*, and specifying additional parameters in a *divcon.in* file. Chapter 7 discusses DivCon, and the rest of this section assumes that *idc=0*.

Support currently exists for gas phase, generalized Born and PME periodic simulations. Available semi- empirical Hamiltonians are PM3 [76], AM1 [75], MNDO [74], PDDG/PM3 [77], PDDG/MNDO [77], and PM3CARB1 [78]. Support is also available, on a functionally limited basis at present (see below) for the Density Functional Theory-based-tight- binding (DFTB) Hamiltonian [123,124] as well as the Self-Consistent-Charge version, SCC-DFTB [125]. DFTB/SCC-DFTB also supports approximate inclusion of dispersion effects [126].

The elements supported by each QM method are:

```
MNDO: H, Li, Be, B, C, N, O, F, Al, Si, P, S, Cl, Zn, Ge, Br, Sn, I, Hg, Pb

AM1:  H, C, N, O, F, Al, Si, P, S, Cl, Zn, Ge, Br, I, Hg

PM3:  H, Be, C, N, O, F, Mg, Al, Si, P, S, Cl, Zn, Ga, Ge, As, Se, Br, Cd,
      In, Sn, Sb, Te, I, Hg, Tl, Pb, Bi

PDDG/PM3: H, C, N, O, F, Si, P, S, Cl, Br, I

PDDG/MNDO: H, C, N, O, F, Cl, Br, I

PM3CARB1: H, C, O

DFTB/SCC-DFTB: H, C, N, O, S, Zn
```

The DFTB/SCC-DFTB implementation does not currently support generalized Born, PME or Ewald calculations, and the performance has not been as aggressively optimised as the other semi-empirical methods. The DFTB/SCC- DFTB code is an adaptation of the DFT/DYLAX code by Marcus Elstner *et al.*. In order to use DFTB (*qmtheory=7*) a set of integral parameter files are required. These are not distributed with Amber, and must be obtained directly from Marcus Elstner, by completing the license agreement *DFTB_license.pdf* found in the $AMBEROME/doc directory, and sending it to Marcus Elstner as indicated in the file.

6.4.1. Changes from earlier versions of Amber

A primary aim in making recent changes has been to improve the accuracy of the code as well as the simplicity with which QM/MM calculations can be setup and run. Energy conservation with even small systems proves a problem with a number of commonly used QM/MM MD packages, since energies and forces may not be completely consistent with each other. QM/MM simulations with default parameers in Amber 9 should generally conserve energy about as well as one would find for a corresponding pure MM simulation.

A second aim has been to make the code as efficient as possible, so that the hybrid potential can be used for simulations on biological macromolecules in condensed phase on the nanosecond time scale. A further goal has been to make selection of a QM region orthogonal to other choices, by implementing the same generalized Born and periodic PME options that one already has for MM potentials. We have attempted to make the QM/MM interface as compatible with the regular Sander options as possible so that a user does not need to worry about incompatibilities between classical only simulations and QM/MM simulations. A QM/MM MD simulation is enabled simply by adding the keyword *ifqnt=1* to the regular `i&cntrl` namelist and then adding a second namelist called `&qmmm` with QM-specific settings such as which atoms to apply the QM potential to and what Hamiltonian to use.

6.4.2. The hybrid QM/MM potential

When running a QM/MM simulation in Sander the system is partitioned into two regions, a QM region consisting of the atoms defined by either the *qmmask* or *iqmatoms* keyword, and a MM region consisting of all the atoms that are not part of the QM region. For a typical protein simulation in explicit solvent the number of MM atoms will be much greater than the number of QM atoms. Either region can contain zero atoms, giving either a pure QM simulation or a standard classical simulation. For periodic simulations, the quantum region must be *compact*, so that the extent (or diameter) of the QM region (in any direction) plus twice the QM/MM cutoff must be less that the box size. Hence, you can define an "active site" to be the QM region, but in most cases could not ask that all cysteine residues (for example) be quantum objects. The restrictions are looser for non-periodic (gas-phase or generalized Born) simulations, but the codes are written and tested for the case of a single, compact quantum region.

The partitioned system is characterized by an effective Hamiltonian, H_{eff}, which operates on the system's wavefunction Ψ, which is dependent on the position of the MM nuclei, x_{MM}, the QM nuclei, x_{QM}, and the position of the QM electrons, x_e, to yield the system energy E_{eff}:

$$H_{eff}\Psi(x_e, x_{QM}, x_{MM}) = E(x_{QM}, x_{MM})\Psi(x_e, x_{QM}, x_{MM}) \quad (6.14)$$

The effective Hamiltonian consists of three components - one for the QM region, one for the MM region and a term that describes the interaction of the QM and MM regions, implying that likewise the energy of the system can be divided into three components. If the total energy of the

system is re-written as the expectation value of H_{eff} then the MM term can be removed from the integral since it is independent of the position of the electrons:

$$E_{eff} = \langle \Psi | H_{QM} + H_{QM/MM} | \Psi \rangle + E_{MM} \tag{6.15}$$

In the QM/MM implementation in *sander*, E_{mm} is calculated classically from the MM atom positions using the Amber force field equation and parameters, whereas H_{QM} is evaluated using the chosen QM method.

The interaction term $H_{QM/MM}$ is more complicated, representing the interaction of the MM point charges with the electron cloud of the QM atoms as well as the interaction between MM point charges and the QM atomic cores. For the case where there are no covalent bonds between the atoms of the QM and MM regions this term is the sum of an electrostatic term and a Lennard-Jones (VDW) term and can be written as:

$$H_{QM/MM} = -\sum_e \sum_m \left[q_m h_{electron}(x_e, x_{MM}) + z_q q_m h_{core}(x_{QM}, x_{MM}) + \left(\frac{A}{r^{12}} - \frac{C}{r^6} \right) \right] \tag{6.16}$$

where the subscripts e, m and q refer to the electrons, the MM nuclei and the QM nuclei repectively. Here q_m is the charge on MM atom m, z_q is the core charge (nucleus minus core electrons) on QM atom q, r_{qm} is the distance between atoms q and m, and A and C are Lennard-Jones interaction parameters. For systems that have covalent bonds between the QM and MM regions, the situation is more complicated, as dicsussed later.

If one evalutes the expectation values in Eq. (6.15) over a single determinant built from molecular orbitals

$$\phi_i = \sum_j c_{ij} \chi_j \tag{6.17}$$

where the c_{ij} are molecular orbital coefficients and the χ_j are atomic basis functions, the total energy depends upon the c_{ij} and on the postions x_{QM} and x_{MM} of the atoms. Once the energy is known, the forces on the atoms can be obtained by using the chain rule and setting $\partial E_{eff}/\partial c_{ij}$ to zero. This leads to a self-consistent (SCF) procedure to determine the c_{ij}, (with a modified Fock matrix that contains the electric field arising from the MM charges).

The main subtelty that arises is that, for a periodic system, there are formally in infinite number of QM/MM interactions; even for a non-periodic system, the (finite) number of such interactions may be prohibitively large. These problems are addressed in a manner analogous to that used for pure MM systems: a PME approach is used for periodic systems, and a (large) cutoff may be invoked for non-periodic systems. Some details are discussed below.

6.4.3. The QM/MM interface and link atoms

The sections above dealt with situations where there are no covalent bonds between the QM and MM regions. In many protein simulations, however, it is necessary to have the QM/MM boundary cut covalent bonds, and a number of additional approximations have to be made. There are a variety of approaches to this problem, including hybrid orbitals, capping potentials, and explicit link atoms. The last option is the method available in *sander*.

There are a number of ways to implement a link atom approach that deal with the way the link atom is positioned, the way the forces on the link atom are propagated, and the way non-bonding interactions around the link atom are treated. In Amber 9 we have implemented an approach that treats link atoms in a different way than earlier versions of the codes. Each time an energy or gradient calculation is to be done, the link atom coordinates are re-generated from the

current coordinates of the QM and MM atoms making up the QM-MM covalent pair. The link atom is placed along the bond vector joining the QM and MM atom, at a distance d_{L-QM} from the QM atom. By default d_{L-QM} is set to the equilibrium distance of a methyl C-H atom pair (1.09 Å) but this can be set in the input file. The default link atom type is hydrogen, but this can also be specified as an input.

Since the link atom position is a function of the coordinates of the "real" atoms, it does not introduce any new degrees of freedom into the system. The chain rule is used to re-write forces on the link atom itself in terms of forces on the two real atoms that define its position. This is analogous to the way in which "extra points" or "lone-pairs" are handled in MM force fields.

The remaining details of how the QM-MM boundary is treated are as follows: for the interactions surrounding the link atom, the MM bond term between the QM and MM atoms is calculated classically using the Amber force field parameters, as are any angle or dihedral terms that include at least one MM atom. The Lennard-Jones interactions between QM-MM atom pairs are calculated in the same way as described in the section above with exclusion of 1-2 and 1-3 interactions and scaling of 1-4 interactions. What remains is to specify the electrostatic interactions between QM and MM atoms around the region of the link atom.

A number of different schemes have been proposed for handling link-atom electrostatics. Many of these have been tested or calibrated on (small) gas-phase systems, but such testing can neglect some considerations that are very important for more extended, condensed-phase simulations. In choosing our scheme, we wanted to ensure that the total charge of the system is rigorously conserved (at the correct value) during an MD simulation. Further, we strove to have the Mulliken charge on the link atom (and the polarity of its bond to the nearest QM atom) adopt reasonable values and to exhibit only small fluctuations during MD simulations. In Amber 9, *place a real description of the electrostatic model here!*

Since the MM atoms that make up the QM region (including the MM link pair atom) have their charges from the prmtop file essentially replaced with Mulliken charges it is important to consider the issue of charge conservation. The QM region (including the link atoms) by definition must have an integer charge. This is defined by the `&qmmm` namelist variable *qmcharge*. If the MM atoms (including the MM link pair atoms) that make up the QM region have prmtop charges that sum to the value of *qmcharge* then there is no problem. If not, there are two options for dealing with this charge, defined by the namelist variable *adjust_q*. A value of 1 will distribute the difference in charge equally between the nearest *nlink* MM atoms to the MM link pair atoms. A value of 2 will distribute this charge equally over all of the MM atoms in the simulation (excluding MM link pair atoms).

6.4.4. Generalized Born implicit solvent

The implementation of Generalized Born (GB) for QM/MM calculations is based on the method described by Pellegrini and Field [79]. Here, the total energy is taken to be E_{eff} from Eq. (6.15) plus E_{gb} from Eq. (6.2). In E_{gb}, charges on the QM atoms are taken to be the Mulliken charges determined from the quantum calculation; hence these charges depend upon the moleucular orbital coefficients c_{ij} as well as the positions of the atoms.

As with conventional QM/MM simulations, one then solves for the c_{ij} by setting $\partial E_{tot}/\partial c_{ij} = 0$. This leads to a set of SCF equations with a Fock matrix modified not only by the presence of MM atoms (as in "ordinary" QM/MM simulations), but also modified by the presence of the GB polarization terms. Once self-consistency is achieved, the resulting Mulliken charges can be used in the ordinary way to compute the GB contribution to the total energy and forces on

the atoms.

6.4.5. Ewald and PME

The support for long range electrostatics in QM/MM calculations is based on a modification of the Nam, Gao and York Ewald method for QM/MM calculations [80]. This approach works in a similar fashion to GB in that Mulliken charges are used to represent long range interactions. Within the cut-off, interactions between QM and MM atoms are calculated using a full multipole treatment. Outside of the cut off the interaction is based on pairwise point charge interactions. This leads to a slight discontinuity at the QM/MM cut off boundary but this does not so far seem to be a significant limitation.

The implementation in Ref [80]. uses an Ewald sum for both QM/QM and QM/MM electrostatic interactions. This can be expensive for large MM regions, and *sander* uses a PME model (rather than an Ewald sum) for QM/MM interactions. This is controlled by the *qm_pme* variable discussed below.

6.4.6. Hints for running successful QM/MM calculations

Required Parameters and Prmtop Creation

QM/MM calculations without link atoms require only mass, van der Waals and GB radii in the *prmtop* file. All charges and bonds, angle and dihedral parameters involving QM atoms are neglected. (Note that when SHAKE is applied, the bonds are constrained to the ideal MM values, even when these are part of a QM region; hence, for this case, it is important to have correct bond parameters in the QM region.) The simplest general prescription for setting things up is to use *antehcamber* and *LeaP* to create a reference force field, since "placeholders" are required in the *prmtop* file even for things that will be neglected. This also allows you to run comparison simulations between pure MM and QM/MM simulations, which can be helpful if problems are encountered in the QM/MM calculations.

The use of *antechamber* to construct a pure MM reference system is even more useful when there are link atoms, since here MM parameters for bonds, angles and dihedrals that cross the QM/MM boundary are also needed.

Choosing the QM region

There are no good universal rules here. Generally, one might want to have as large a QM region as possible, but having more than 80-100 atoms in the QM region will lead to simulations that are very expensive. One should also remember that for many features of conformational analysis, a good MM force field may be better than a semiempirical or DFTB quantum description. In choosing the QM/MM boundary, it is better to cut non-polar bonds (such as C−C single bonds) than to cut unsaturated or polar bonds. Link atoms are not placed between bonds to hydrogen. Thus cutting across a C-H bond will NOT give you a link atom across that bond. (This is not currently tested for in the code and so it is up to the user to avoid such a situation.) Furthermore, link atoms are restricted to one per MM link pair atom. This is tested for during the detection of link atoms and an error is generated if this requirement is violated. This would seem to be a sensible policy otherwise you could have two link atoms too close together. See the comments in *qm_link_atoms.f* for a more in-depth discussion of this limitation.

Choice of electrostatic cutoff

The implementation of the non-bonded cut off in QM/MM simulations is slightly different to regular MM simulations. The cut off between MM-MM atoms is still handled in a pairwise fashion. However, for QM atoms any MM atom that is within *qmcut* of ANY QM atom is included in the interaction list for all QM atoms. This means that the value of *qmcut* essentially specifies a shell around the QM region rather than a spherical shell around each individual QM atom. Ideally the cut off should be large enough that the energy as a function of the cutoff has converged. For non-periodic, generalized Born simulations, a cutoff of 15 to 20 Å seems sufficient in some tests. (Remember that long-range electrostatic interactions are reduced by a factor of 80 from their gas-phase counterparts, and by more if a non-zero salt concentration is used.) For periodic simulations, the cutoff only serves to divide the interactions between "direct" and "reciprocal" parts; as with pure MM calculations, a cutoff of 8 or 9 Å is sufficient here.

Parallel simulations

The built-in QM/MM implementation currently supports execution in parallel, however, the implementation is not fully parallel. At present all parts of the QM simulation (for *qmtheory<=6* and *idc=0*) are parallel except the density matrix build and the matrix diagonalisation. For small QM systems these two operations do not take a large percentage of time and so acceptable scaling can be seen to around 8 cpus (depending on interconnect speed). However, for large QM systems the matrix diagonalisation time will dominate and so the scaling will not be as good.

6.4.7. General QM/MM &qmmm Namelist Variables

An example input file for running a simple QM/MM MD simulation is given in the box. The `&qmmm` namelist contains variables that allow you to control the options used for a QM/MM

Example QMMM MD Script for Sander 9

```
Example QMMM MD Script for Sander 9
 &cntrl
  imin=0, nstlim=10000,          (perform MD for 10,000 steps)
  dt=0.0002,                     (2 fs time step)
  ntt=1, tempi=0.1, temp0=300.0 (Berendsen temperature control)
  ntb=1,                         (Constant volume periodic boundaries)
  ntf=2, ntc=2,                  (Shake hydrogen atoms)
  cut=8.0,                       (8 angstrom classical non-bond cut off)
  ifqnt=1                        (Switch on QM/MM coupled potential)
 /
 &qmmm
  qmmask=':753'                  (Residue 753 should be treated using QM)
  qmcharge=-2,                   (Charge on QM region is -2)
  qmtheory=1,                    (Use the PM3 semi-empirical Hamiltonian)
  qmcut=8.0                      (Use 8 angstrom cut off for QM region)
 /
```

simulation. This namelist must be present when running QM/MM simulations and at the very least must contain either the `iqmatoms` or `qmmask` variable which define the region to be treated quantum mechanically. If `ifqnt` is set to zero then the contents of this namelist are ignored.

QM region definition. Specify one of either `iqmatoms` or `qmmask`. Link atoms will be added automatically along bonds (as defined in the prmtop file) that cross the QM/MM boundary.

IQMATOMS comma-separated integer list containg the atom numbers (from the prmtop file) of the atoms to be treated quantum mechanically.

QMMASK Mask specifying the quantum atoms. E.g. :1-2, = residues 1 and 2. See mask documentation for more info.

IDC Specifies use of the Amber built-in or DivCon for QM/MM calculations.

= 0 (default) Use the built-in Amber QM/MM code.

= 1 Use standard DivCon for QM/MM calculations. You must prepare a separate *divcon.in* file to specify DivCon keywords; see Chapter 7 for a full discussion of these options.

= 2 Use divide and conquer DivCon QM/MM calculations. This option may be the preferred option for larger systems. It also requires a *divcon.in* file for DivCon keywords; see Chapter 7.

QMCUT Specifies the size of the electrostatic cutoff in Angstroms for QM/MM electrostatic interactions. By default this is the same as the value of cut chosen for the classical region, and the default generally does not need to be changed. Any classical atom that is within qmcut of *any* QM atom is included in the pair list. For PME calculations, this parameter just affects the division of forces between direct and reciprocal space. *Note:* this option only effects the electrostatic interactions between the QM and MM regions. Within the QM region all QM atoms see all other QM atoms regardless of their separation. QM-MM van der Waals interactions are handled classically, using the cutoff value specified by *cut*.

QM_EWALD This option specifies how long range electrostatics for the QM region should be treated.

= 0 Use a real-space cutoff for QM-QM and QM-MM long range interactions. In this situation QM atoms do not see their images and QM-MM interactions are truncated at the cutoff. This is the default for non-periodic simulations, and is the only option available for DFTB calculations.

= 1 (default) Use PME or an Ewald sum to calculate long range QM-QM and QM-MM electrostatic interactions. This is the default when running QMMM with periodic boundaries and PME.

= 2 This option is similar to option 1 but instead of varying the charges on the QM images as the central QM region changes the QM image charges are fixed at the Mulliken charges obtained from the previous MD step. This approach offers a speed improvement over *qm_ewald=1*, since the SCF typically converges in fewer steps, with only a minor loss of accuracy in the long range electrostatics. This option has not been extensively tested, although it becomes

increasingly accurate as the box size gets larger.

KMAXQX,Y,Z Specifies the maximum number of kspace vectors to use in the x, y and z dimensions respectively when doing an Ewald sum for QM-MM and QM-QM interactions. Higher values give greater accuracy in the long range electrostatics but at the expensive of calculation speed. The default value of 5 should be optimal for most systems.

KSQMAXQ Specifies the maximum number of K squared values for the spherical cut off in reciprocal space when doing a QM-MM Ewald sum. The default value of 27 should be optimal for most systems.

QM_PME Specifies whether a PME approach or regular Ewald approach should be used for calculating the long range QM-QM and QM-MM electrostatic interactions.

= 0 Use a regular Ewald approach for calculating QM-MM and QM-QM long range electrostatics. Note this option is often much slower than a pme approach and typically requires very large amounts of memory. It is recommended only for testing purposes.

= 1 (default) Use a QM compatible PME approach to calculate the long range QM-MM electrostatic energies and forces and the long range QM-QM forces. The long range QM-QM energies are calculated using a regular Ewald approach.

QMGB Specifies how the QM region should treated with generalised Born. (Note that DFTB/SCC-DFTB calculations do not currently work with generalized Born.)

= 2 (default) As described above, the electrostatic and "polarization" fields from the MM charges and the exterior dielectric (respectively) are included in the Fock matrix for the QM Hamiltonian.

= 3 This is intended as a debugging option and should only be used for single point calculations. With this option the GB energy is calculated using the Mulliken charges as with option 2 above but the fock matrix is NOT modified by the GB field. This allows one to calculate what the GB energy would be for a given structure using the gas phase quantm charges. When combined with a simulation using *qmgb=2*, this allows the strain energy from solvation to be calculated.

QMTHEORY Level of theory to use for the QM region of the simulation. (Hamiltonian). Default is to use the semi-empirical hamiltonian PM3.

= 1 PM3 (default)

= 2 AM1

= 3 MNDO

= 4 PDDG/PM3

=5 PDDG/MNDO

= 6 PM3CARB1

= 7 DFTB/SCC-DFTB

DFTB_DOSCC Flag turning on (1) or off (0) the self-consistent-charge version of DFTB, SCC-DFTB. Requires *qm_method=7*. (Default = 1)

DFTB_DISPER Flag turning on (1) or off (0) the use of a dispersion correction to the DFTB/SCC-DFTB energy. Requires *qm_method=7*. It is assumed that you have the file DISPERSION.INP_ONCHSP in your $AMBERHOME/dat/slko/ directory. This file must be obtained directly form Marcus Elstner, as described in the beggining of this chapter. Not available for the Zn atom. (Default = 0)

QMCHARGE Charge on the QM system in electron units (must be an integer). (Default = 0)

SPIN Spin state of the QM system. Valid values are 1 to 6. Default is 1 = singlet. Note for a doublet(2), quartet(4) or sextet(6) you require an odd number of electrons. Note that this option is ignored by DFTB/SCC-DFTB, which allows only ground state calculations. In this case, the spin state will be calculated from the number of electrons and orbital occupancy.

VERBOSITY Controls the verbosity of QM/MM related output. *Warning:* Values of 2 or higher will produce a *lot* of output.

= 0 (default) - only minimial information is printed - Initial QM geometry and link atom positions as well as the SCF energy at every ntpr steps.

= 1 Print SCF energy at every step to many more significant figures than usual. Also print the number of SCF cycles needed on each step.

= 2 As 1 but also print info about memory reallocations, number of pairs per QM atom. Also prints QM core - QM core energy, QM core - MM charge energy and total energy.

= 3 As 2 but also print SCF convergence information at every step.

= 4 As 3 but also print forces on QM atoms due to the SCF calculation and the coordinates of the link atoms at every step.

= 5 As 4 but also print all of the info in KJ/mol as well as KCal/mol.

TIGHT_P_CONV Controls the tightness of the convergence criteria on the density matrix in the SCF.

= 0 (default) - loose convergence on the density matrix (or Mulliken charges, in case of a SCC-DFTB calculation). SCF will converge if the energy is converged to within scfconv and the largest change in the density matrix is within 0.05*sqrt(scfconv).

= 1 Tight convergence on density(or Mulliken charges, in case of a SCC-DFTB calculation). Use same convergence (scfconv) for both energy and density (charges) in SCF. Note: in the SCC-DFTB case, this option can lead to instabilities.

SCFCONV Controls the convergence criteria for the SCF calculation, in kcal/mol. In order to conserve energy in a dynamics simulation with no thermostat it is often necessary to use a convergence criterion of 1.0d-9 or tighter. Note, the tighter the convergence the longer the calculation will take. Values tighter than 1.0d-11 are not recommended as these can lead to oscillations in the SCF, due to limitations in machine precision, that can lead to convergence failures. Default is 1.0d-8 kcal/mol. Mininum usable value is 1.0d-14.

PSEUDO_DIAG Controls the use of 'fast' pseudo diagonalisations in the SCF routine. By default the code will attempt to do pseudo diagonalisations whenever

possible. However, if you experience convergence problems then turning this option off may help. Not available for DFTB/SCC-DFTB.

= 0 Always do full diagonalisation.

= 1 Do pseudo diagonalisations when possible (default).

PSEUDO_DIAG_CRITERIA

Float controlling criteria used to determine if a pseudo diagonalisation can be done. If the difference in the largest density matrix element between two SCF iterations is less than this criteria then a pseudo diagonalisation can be done. This is really a tuning parameter designed for expert use only. Most users should have no cause to adjust this parameter. (Not applicable to DFTB/SCC-DFTB calculations.) Default = 0.05

PRINTCHARGES

= 0 Don't print any info about QM atom charges to the output file (default)

= 1 Print Mulliken QM atom charges to output file on every step.

PEPTIDE_CORR

= 0 Don't apply MM correction to peptide linkages. (default)

= 1 Apply a MM correction to peptide linkages. This correction is of the form $escf = escf + htype(itype)\sin(\phi)^2$, where ϕ is the dihedral angle of the H-N-C-O linkage and *htype* is a constant dependent on the Hamiltonian used. (Recommended, except for DFTB/SCC-DFTB.)

ITRMAX Integer specifying the maximum number of SCF iterations to perform before assuming that convergence has failed. Default is 1000. Typically higher values will not do much good since if the SCF hasn't converged after 1000 steps it is unlikely to. If the convergence criteria have not been met after itrmax steps the SCF will stop and the MD or minimisation will proceed with the gradient at itrmax. Hence if you have a system which does not converge well you can set itrmax smaller so less time is wasted before assuming the system won't converge. In this way you may be able to get out of a bad geometry quite quickly. Once in a better geometry SCF convergence should improve.

QMSHAKE Controls whether shake is applied to QM atoms. Using shake on the QM region will allow you to use larger time steps such as 2 fs with NTC=2. If, however, you expect bonds involving hydrogen to be broken during a simulation you should not SHAKE the QM region. WARNING: the shake routine uses the equilibrium bond lengths as specified in the prmtop file to reset the atom positions. Thus while bond force constants and equilibrium distances are not used in the energy calculation for QM atoms the equilibrium bond length is still required if QM shake is on.

= 0 Do not shake QM H atoms.

= 1 Shake QM H atoms if shake is turned on (NTC>1) (default).

WRITEPDB

= 0 Do not write a pdb file of the selected QM region. (default).

= 1 Write a pdb file of the QM region. This option is designed to act as an aid to the user to allow easy checking of what atoms were

included in the QM region. When this option is set a crude pdb file of the atoms in the QM region will be written on the very first step to the file *qmmm_region.pdb*.

6.4.8. Link Atom Specific QM/MM &qmmm Namelist Variables

The following options go in the qmmm namelist and control the link atom behaviour.

LNK_DIS Distance in Å from the QM atom to its link atom. Currently all link atoms must be placed at the same distance. Default = 1.09 Å.

LNK_ATOMIC_NO
The atomic number of the link atoms. This selects what element the link atoms are to be. Default = 1 (Hydrogen). Note this must be an integer and an atomic number supported by the chosen qm theory.

ADJUST_Q This controls how charge is conserved during a QMMM calculation involving link atoms. When the QM region is defined the QM atoms and any MM atoms involved in link bonds have their RESP charges zeroed. If the sum of these RESP charges does not exactly match the value of *qmcharge* then the total charge of the system will not be correct.

= 0 (default) - No adjustment of the charge is done.

= 1 The charge correction is applied to the nearest *nlink* MM atoms to MM atoms that form link pairs. Typically this will be any MM atom that is bonded to a MM link pair atom (a MM atom that is part of a QM-MM bond). This results in the total charge of QM+QMlink+MM equalling the original total system charge from the prmtop file.

= 2 This option is similar to option 1 but instead the correction is divided among all MM atoms (except for those adjacent to link atoms). As with option 1 this ensures that the total charge of the QM/MM system is the same as that in the *prmtop* file.

6.5. Free energies using thermodynamic integration.

Sander has the capability of doing simple thermodynamic free energy calculations, using either PME or generalized Born potentials. When *icfe* is set to 1 or 2, information useful for doing thermodynamic integration estimates of free energy changes will be computed. You must use the "multisander" capability to create two groups, one corresponding to the starting state, and a second corresponding to the ending state; you will need a *prmtop* file for each of these two end points. Then a mixing parameter λ is used (see Eqs. 4 and 5, below) to interpolate between the "unperturbed" and "perturbed" potential functions.

The two prmtop files that you create must have the same number of atoms, and the atoms must appear *in the same order* in the two files. This is because there is only one set of coordinates that are propagated in the molecular dynamics algorithm. If there are more atoms in the intial state than in the final, "dummy" atoms must be introduced into the final state to make up the difference. Although there is quite a bit of flexibility in choosing the initial and final states, it is important in general that the system be able to morph "smoothly" from the intial to the final

states.

In a free energy calculation, the system evolves according to a mixed potential (such as in Eqs. 4 or 5, below). The essence of free energy calculations is to record and analyze the fluctuations in the values of V_0 and V_1 (that is, what the energies *would have been* with the endpoint potentials) as the simulation progresses. For thermodynamic integration (which is a very straightforward form of analysis) the required averages can be computed "on-the-fly" (as the simulation progresses), and printed out at the end of a run. For more complex analyses (such as the Bennett acceptance ratio scheme), one needs to write out the history of the values of V_0 and V_1 to a file, and later post-process this file to obtain the final free energy estimates.

There is not room here to discuss the theory of free energy simulations, and there are many excellent discussions elsewhere [15,127,128]. Such calculations are demanding, both in terms of computer time, and in a level of sophistication to avoid pitfalls that can lead to poor convergence. Since there is no one "best way" to estimate free energies, *sander* primarily provides the tools to collect the statistics that are needed. Assembling these into a final answer, and assessing the accuracy and significance of the results, general requires some calculations outside of what Amber provides, *per se*. The discussion here will assume a certain level of familiarity with the basis of free energy calculations.

The basics of the multisander functionality are given below, but the mechanics are really quite simple. You start a free energy calculation as follows:

```
mpirun -np 4 sander -ng 2 -groupfile <filename>
```

Since there are 4 total cpu's in this example, each of the two groups will run in parallel with 2 cpu's each. The number of processors must be a multiple of two. The *groups* file might look like this:

```
-O -i mdin -p prmtop.0 -c eq1.x  -o md1.o  -r md1.x  -inf mdinfo
-O -i mdin -p prmtop.1 -c eq1.x  -o md1b.o -r md1b.x -inf mdinfob
```

The input (*mdin*) and starting coordinate files must be the same for the two groups. Furthermore, the two *prmtop* files must have the same number number of atoms, in the same order (since one common set of coordinates will be used for both.) The simulation will use the masses found in the first *prmtop* file; in classical statistical mechanics, the Boltzmann distribution in coordinates is independent of the masses so this should not represent any real restriction.

On output, the two restart files should be identical, and the two output files should differ only in trivial ways such as timings; there should be no differences in any energy-related quantities. For our example, this means that one could delete the *md1b.o* and *md1b.x* files, since the information they contain is also in *md1.o* and *md1.x*. (It is a good practice, however, to check these file identities, to make sure that nothing has gone wrong.)

ICFE — The basic flag for free energy calculations. The default value of 0 skips such calcuations. Setting this flag to 1 turns them on, using the mixing rules in Eq. (5), below. Setting the flag to 2 uses the mixing rules of Eqs. (6) and (8), below.

CLAMBDA — The value of λ for this run, as in Eqs. (4) and (5), below. Zero corresponds to the unperturbed Hamiltonian (or the first of the two multisander groups) λ=1 corresponds to the perturbed Hamiltonian, or the second of the two multisander groups.

KLAMBDA The exponent in Eq. (5), below.

Unlike *gibbs*, the program itself does not compute free energies; it is up to the user to combine the output of several runs (at different values of λ) and to numerically estimate the integral:

$$\Delta A \equiv A(\lambda = 1) - A(\lambda = 0) = \int_0^1 \langle \partial V/\partial \lambda \rangle_\lambda \, d\lambda \tag{6.18}$$

If you understand how free energies work, this should not be at all difficult. However, since the actual values of λ that are needed, and the exact method of numerical integration, depend upon the problem and upon the precision desired, we have not tried to pre-code these into the program.

The simplest numerical integration is to evaluate the integrand at the midpoint:

$$\Delta A \approx \langle \partial V/\partial \lambda \rangle_{\frac{1}{2}} \tag{6.19}$$

This might be a good first thing to do to get some picture of what is going on, but is only expected to be accurate for very smooth or small changes, such as changing just the charges on some atoms. Gaussian quadrature formulas of higher order are generally more useful:

$$\Delta A \approx \sum_{i=1}^{n} w_i \langle \partial V/\partial \lambda \rangle_{\lambda_i} \tag{6.20}$$

Some weights and quadrature points are given in the accompanying table; other formulas are possible [129], but the Gaussian ones listed there are probably the most useful. The formulas are always symmetrical about λ = 0.5, so that λ_i^a and λ_i^b both have the same weight. For example, if you wanted to use 5-point quadrature, you would need to run five *sander* jobs, setting λ to 0.04691, 0.23076, 0.5, 0.76923, and 0.95308 in turn. (Each value of λ should have an equilibration period as well as a sampling period; this can be achieved by setting the *ntave* parameter.) You would then multiply the values of $< \partial V/\partial \lambda >$ by the weights listed in the Table, and compute the sum.

When *icfe=1* and *klambda* has its default value of 1, the simulation uses the mixed potential function:

$$V(\lambda) = (1 - \lambda)V_0 + \lambda V_1 \tag{6.21}$$

where V_0 is the potential with the original Hamiltonian, and V_1 is the potential with the perturbed Hamiltonian. The program also computes and prints $\partial V/\partial \lambda$ and its averages; note that in this case, $\partial V/\partial \lambda = V_1 - V_0$. This is referred to as linear mixing, and is often what you want unless you are making atoms appear or disappear. If some of the perturbed atoms are "dummy" atoms (with no van der Waals terms, so that you are making these atoms "disappear" in the perturbed state), the integrand in Eq. (6.18) diverges at λ = 1; this is a mild enough divergence that the overall integral remains finite, but it still requires special numerical integration techniques to obtain a good estimate of the integral [128]. *Sander* implements one simple way of handling this problem: if you set *klambda* > 1, the mixing rules are:

$$V(\lambda) = (1 - \lambda)^k V_0 + [1 - (1 - \lambda)^k] V_1 \tag{6.22}$$

where *k* is given by *klambda*. Note that this reduces to Eq. (6.21) when *k* = 1, which is the default. If *klambda* $\geq$ 4, the integrand remains finite as $\lambda \rightarrow 1$ [128]. We have found that setting *klambda* = 6 with disappearing groups as large as tryptophan works well. Note that the behavior of $\partial V/\partial \lambda$ as a function of λ is not monotonic when *klambda* > 1. You may need a fairly fine quadrature to get converged results for the integral, and you may want to sample more carefully in

Abcissas and weights for Gaussian integration			
n	λ_i^a	λ_i^b	w_i
1	0.50000		1.00000
2	0.21132	0.78867	0.50000
3	0.11270	0.88729	0.27777
	0.50000		0.44444
5	0.04691	0.95308	0.11846
	0.23076	0.76923	0.23931
	0.50000		0.28444
7	0.02544	0.97455	0.06474
	0.12923	0.87076	0.13985
	0.29707	0.70292	0.19091
	0.50000		0.20897
9	0.01592	0.98408	0.04064
	0.08198	0.91802	0.09032
	0.19331	0.80669	0.13031
	0.33787	0.66213	0.15617
	0.50000		0.16512
12	0.00922	0.99078	0.02359
	0.04794	0.95206	0.05347
	0.11505	0.88495	0.08004
	0.20634	0.79366	0.10158
	0.31608	0.68392	0.11675
	0.43738	0.56262	0.12457

regions where $\partial V/\partial\lambda$ is changing rapidly.

When *icfe* is set to 2, a different set of mixing rules is used. This was implemented by Ilyas Yildirm and Harry Stern and the University of Rochester. Let the "mixing" function be $f(\lambda)$ so that the effective potential becomes

$$V(\lambda) = f(\lambda)V_0 + [1 - f(\lambda)]V_1 \tag{6.23}$$

Then we want to ensure the following conditions on the function f:

$$f(\lambda = 0) = 1; \quad f(\lambda = 1) = 0; \quad f^{(n)}(\lambda = 0) = f^{(n)}(\lambda = 1) = 0 \tag{6.24}$$

where $f^{(n)}(\lambda)$ is the nth derivative of the mixing function, and Eq. (6.24) should hold for all values of n up to at least 4. One function that satisfies this for $n < k$ is

$$f(\lambda, k) = (1 - \lambda)^k \sum_{i=0}^{k-1} \mathrm{comb}(k - 1 + i, i)\lambda^i \tag{6.25}$$

where the combination funciton is

$$\text{comb}(x, y) = x!/[(x-y)!y!] \quad (6.26)$$

[Note that when $k = 1$, Eq. (6.25) reduces to Eq. (6.21).] This modification allows dummy atoms to be a either (or both) of the end points of the modification for sufficiently large values of k. Values of k (variable *klambda*) of 6 or 7 have given good results; see the test cases or tutorials for more information.

Notes:

(1) This capability in *sander* is implemented by calling the force() routine twice on each step, once for λ=0 and once for λ=1. This increases the cost of the simulation, but involves extremely simple coding.

(2) Eq. (6.22) is designed for having dummy atoms in the perturbed Hamiltonian, and "real" atoms in the regular Hamiltonian. You must ensure that this is the case when you set up the system in LEaP. If you need dummy atoms in V_0, or in both end states, use *icfe*=2.

(3) One common application of this model is to pK_a calculations, where the charges are mutated from the protonated to the deprotonated form. Since H atoms bonded to oxygen already have zero van der Waals radii (in the Amber force fields and in TIP3P water), once their charge is removed (in the deprotonated form) they are really then like dummy atoms. For this special situation, there is no need to use *klambda* > 1: since the van der Waals terms are missing from both the perturbed and unperturbed states, the proton's position can never lead to the large contributions to $< V_1 - V_0 >$ that can occur when one is changing from a zero van der Waals term to a finite one.

(4) The implementation requires that the masses of all atoms be the same on all threads. To enforce this, the masses found in the first *prmtop* file (for V_0) are used for V_1 as well. In classical statistical mechanics, the canonical distribution of configurations (and hence of potential energies) is unaffected by changes in the masses, so this should not pose a limitation. Since the masses in the second *prmtop* file are ignored, they do not have to match those in the first *prmtop* file.

(5) Special care needs to be taken when using SHAKE for atoms whose force field parameters differ in the two end points. The same bonds must be SHAKEN in both cases, and the equilibrium bond lengths must also be the same. The easiest way to ensure this is to use the *noshakemask* input to remove SHAKE from the regions that are being perturbed. You must do this manually, as the current code does not have any internal idea of "perturbed" and "unperturbed" atoms. (This is a change from earlier versions of Amber, which used a *pertprmtop* file, and which automatically removed SHAKE from the perturbed parts of the system.)

6.6. Targeted MD

The targeted MD option adds an additional term to the energy function based on the mass-weighted root mean square deviation of a set of atoms in the current structure compared to a reference structure. The reference structure is specified using the *-ref* flag in the same manner as is used for Cartesian coordinate restraints (NTR=1). Targeted MD can be used with or without positional restraints. If positional restraints are not applied (ntr=0), *sander* performs a best-fit of the reference structure to the simulation structure based on selection in *tgtfitmask* and calculates the RMSD for the atoms selected by *tgtrmsmask*. The two masks can be identical or different. This way, fitting to one part of the structure but calculating the RMSD (and thus restraint force) for another part of the structure is possible. If targeted MD is used in conjunction with positional

restraints (ntr=1), only *tgtrmsmask* should be given in the control input because the molecule is 'fitted' implicitly by applying positional restraints to atoms specified in *restraintmask*.
The energy term has the form:

```
E = 0.5 * TGTMDFRC * NATTGTRMS * (RMSD-TGTRMSD)**2
```

The energy will be added to the RESTRAINT term. Note that the energy is weighted by the number of atoms that were specified in the *tgtrmsmask* (NATTGTRMS). The RMSD is the root mean square deviation and is mass weighted. The force constant is defined using the *tgtmdfrc* variable (see below). This option can be used with molecular dynamics or minimization. When targeted MD is used, *sander* will print the current values for the actual and target RMSD to the energy summary in the output file.

ITGTMD

= 0 no targeted MD (default)

= 1 use targeted MD

TGTRMSD Value of the target RMSD. The default value is 0. This value can be changed during the simulation by using the weight change option.

TGTMDFRC This is the force constant for targeted MD. The default value is 0, which will result in no penalty for structure deviations regardless of the RMSD value. Note that this value can be negative, which would force the coordinates AWAY from the reference structure.

TGTRMSMASK Define the atoms that will be used for the rms superposition between the current structure and the reference structure. Syntax is in Chapter 11.5.

TGTFITMASK Define the atoms that will be used for the rms difference calculation (and hence the restraint force), as outlined above. Syntax is in Chapter 11.5.

One can imagine many uses for this option, but a few things should be kept in mind. Since there is currently only one reference coordinate set, there is no way to force the coordinates to any specific structure other than the reference. To move a structure toward a reference coordinate set, one might use an initial *tgtrmsd* value corresponding to the actual RMSD between the input and reference (*inpcrd* and *refc*). Then the weight change option could be used to decrease this value to 0 during the simulation. To move a structure away from the reference, one can increase *tgtrmsd* to values larger than zero. The minimum for this energy term will then be at structures with an RMSD value that matches *tgtrmsd*. Keep in mind that many different structures may have similar RMSD values to the reference, and therefore one cannot be sure that increasing *tgtrmsd* to a given value will result in a particular structure that has that RMSD value. In this case it is probably wiser to use the final structure, rather than the initial structure, as the reference coordinate set, and decrease *tgtrmsd* during the simulation. A negative force constant *tgtmdfrc* can be used, but this can cause problems since the energy will continue to decrease as the RMSD to the reference increases.

Also keep in mind that phase space for molecular systems can be quite complex, and this method does not guarantee that a low energy path between initial and target structures will be followed. It is possible for the simulation to become unstable if the restraint energies become too large if a low-energy path between a simulated structure and the reference is not accessible.

Note also that the input and reference coordinates are expected to match the *prmtop* file and have atoms in the same sequence. No provision is made for symmetry; rotation of a methyl group by 120° would result in a non-zero RMSD value.

6.7. Potentials of mean force using umbrella sampling.

Another free energy quantity that is accessible within *sander* is the ability to compute potentials of mean force (at least for simple distance, angle, or torsion variables) using umbrella sampling. The basic idea is as follows. You add an artificial restraint to the system to bias it to sample some coordinate in a certain range of values, and you keep track of the distribution of values of this coordinate during the simulation. Then, you repeatedly move the minimum of the biassing potential to different ranges of the coordinate of interest, and carry out more simulations. These different simulations (often called "windows") must have some overlap; that is, any particular value of the coordinate must be sampled to a significant extent in more than one window. After the fact, you can remove the effect of the biassing potential, and construct a potential of mean force, which is the free energy profile along the chosen coordinate.

The basic ideas have been presented in many places [130-134], and will not be repeated here. The implementation in *sander* follows two main steps. First, restraints are set up (using the distance and angle restraint files) and the DUMPFREQ parameter is used to create "history" files that contain sampled values of the restraint coordinate. Second, a collection of these history files is analyzed (using the so-called "weighted histogram" or WHAM method [132-134]) to generate the potentials of mean force. As with thermodynamic integration, the *sander* program itself does not compute these free energies; it is up to the user to combine the output of several windows into a final result. For many problems, the programs prepared by Alan Grossfield (`http://dasher.wustl.edu/alan/`) are very convenient, and the *sander* output files are compatible with these codes.

A simple example. The input below shows how one window of a potential of mean force might be carried out. The coordinate of interest here is the chi angle of a base in an RNA duplex. Here is the *mdin* file:

```
test of umbrella sampling of a chi torsion angle
 &cntrl
   nstlim=50000, cut=20.0, igb=1, saltcon=0.1,
   ntpr=1, ntwr=100000, ntt=3, gamma_ln=0.2,
   ntx=5, irest=1,
   ntc=2, ntf=2, tol=0.000001,
   dt=0.001, ntb=0,
   nmropt=1,
 /
 &wt type='DUMPFREQ', istep1=10  /
 &wt type='END'   /
DISANG=chi.RST
DUMPAVE=chi_vs_t.170
```

The items in the *&cntrl* namelist are pretty standard, and not important here, except for specifying *nmropt=1*, which allows restraints to be defined. (The name of this variable is an historical artifact: distance and angle restraints were originally introduced to allow NMR-related structure calculations to be carried out. But they are also very useful for cases, like this one, that have nothing to do with NMR.) The DUMPFREQ command is used to request a separate file be created to hold values of the torsion angle; this will have the name *chi_vs_t.170* given in the DUMPAVE file redirection command.

The torsion angle restraint itself is given in the *chi.RST* file:

```
#  torsion restraint for chi of residue 2
 &rst  iat=39,40,42,43, r1=0., r2=170., r3=170., r4=360., rk2 = 30.,
       rk3 = 30.,   /
```

The *iat* variable gives the atom numbers of the four atoms that define the torsion of interest. We set *r2 = r3* and *rk2 = rk3* to obtain a harmonic biassing potential, with a minimum at 170^{o}. The values *r1* and *r4* should be far away from 170, so that the potential is essentially harmonic everywhere. (It is not required that biassing potentials be harmonic, but Dr. Grossfield's programs assume that they are, so we enforce that here.) Subsequent runs would change the minimum in the potential to values other than 170, creating other *chi_vs_t* files. These files would then be used to create potentials of mean force. Note that the conventionally defined "force constant" is twice the value *rk2*, and that the Grossfield program uses force constants measured in degrees, rather than radians. So you must perform a unit conversion in using those programs, multiplying *rk2* by 0.0006092 (= $2(\pi/180)^2$) to get a equivalent force constant for a torsional restraint.

6.8. Steered Molecular Dynamics (SMD) and the Jarzynski Relationship

Background

SMD applies an external force onto a physical system, and drives a change in coordinates within a certain time. Many leading applications have come from Klaus Schulten's group [135]. An implementation where the coordinate in question changes in time at constant velocity is coded in this version of Amber. The present implementation has been done by the group of Prof. Dario Estrin in Buenos Aires <dario@q1.fcen.uba.ar> by Marcelo Marti <marcelomarti@yahoo.com> and Alejandro Crespo <alec@qi.fcen.uba.ar>, and in the group of Prof. Adrian Roitberg at the University of Florida <roitberg@ufl.edu> [136].

The method should be thought of as an umbrella sampling where the center of the restraint is time-dependent as in:

$$V_{rest}(t) = (1/2)k[x - x_0(t)]2$$

where x could be a distance, and angle or a torsion between atoms or groups of atoms.

This methodology can be used then to drive a physical process such as ion diffusion, conformational changes and many other applications. By integrating the force over time (or distance), a generalized work can be computed. This work can be used to compute free energy differences using the so- called Jarzynski relationship [137-139]. This method states that the free energy difference between two states A and B (differing in their values of the generalized coordinate x) can be calculated as

$$\exp(-\Delta G/k_B T) = < \exp(-W/k_B T) >_A$$

This means that by computing the work between the two states in question, and averaging over the initial state, equilibrium free energies can be extracted from non-equilibrium calculations. In order to make use of this feature, SMD calculations should be done, with different starting coordinates taken from equilibrium simulations. This can be done by running sander multiple times, or by running multisander. There are examples of the various modes of action under the *test/jar* directories in the amber distribution.

Implementation and usage

To set up a SMD run, set the *jar* variable in the `&cntrl` namelist to 1. The change in coordinates is performed from a starting to an end value in *nstlim* steps.

To specify the type and conditions of the restraint an additional ".RST" file is used as in *nmropt=1*. (Note that *jar=1* internally sets *nmropt=1*.) The restraint file is similar to that of NMR restraints (see Section 6.12.1), but fewer parameters are required. For instance, the following RST file could be used:

```
#  Change distance between atoms 485 and 134 from 15 A to 20 A
&rst  iat=485,134,  r2=15., rk2 = 5000., r2a=20. /
```

Note that only *r2, r2a* and *rk2* are required; *rk3* and *r3* are set equal to these so that the harmonic restraint is always symmetric, and *r1* and *r4* are internally set so that the restrain is always operative. An SMD run changing an angle, would use three *iat* entries, and one changing a torsion needs four. As in the case of NMR restraints, group inputs can also be used, using *iat<0* and defining the corresponding groups using the *igr* flag.

The output file differs substantially from that used in the case of nmr restraints. It contains 4 columns:

$x_{0(t)}$, x, force, work

Here work is computed as the integrated force over distances (or angle, or torsion). These files can be used for later processing in order to obtain the free energy along the selected reaction coordinate using Jarzynski's equality.

Example

The following example changes the distance between two atoms along 1000 steps:

```
 Sample pulling input
&cntrl
  nstlim=1000, cut=99.0, igb=1, saltcon=0.1,
  ntpr=100, ntwr=100000, ntt=3, gamma_ln=5.0,
  ntx=5, irest=1, ntwx=100000000, ig = 256251,
  ntc=2, ntf=2, tol=0.000001,
  dt=0.002, ntb=0, tempi=300., temp0=300.,
  jar=1,
/
 &wt type='DUMPFREQ', istep1=1, /
 &wt type='END',   /
DISANG=dist.RST
DUMPAVE=dist_vs_t
LISTIN=POUT
LISTOUT=POUT
```

Note that the flag *jar* is set to 1, and redirections to the *dist.RST* file are given. In this example the values in the output file *dist_vs_t* are written every *istep=1* steps.

The restraint file *dist.RST* in this example is:

```
# Change distance between atoms 485 and 134 from 15 A to 15.3 A
 &rst  iat=485,134,  r2=15., rk2 = 5000., r2a=15.3, /
```

and the output *dist_vs_t* file might contain:

```
   15.00000      15.12396     -12.39555       0.00000
   15.01500      15.09446      -7.93769      -0.15250
   15.03000      15.06527      -3.51962      -0.23843
   15.04500      15.03889       0.60920      -0.26026
   15.06000      15.02062       3.92188      -0.22627
   15.07500      15.01051       6.41707      -0.14873
   15.09000      15.00853       8.09781      -0.03987
   15.10500      15.01917       8.52326       0.08479
   15.12000      15.03271       8.65886       0.21365
   15.13500      15.05121       8.30316       0.34087
   15.15000      15.07348       7.57521       0.45996
   15.16500      15.09981       6.44775       0.56513
   15.18000      15.12038       5.89042       0.65766
   15.19500      15.14401       5.03225       0.73958
   15.21000      15.16543       4.39421       0.81028
   15.22500      15.17906       4.52505       0.87718
   15.24000      15.18605       5.30866       0.95093
   15.25500      15.18570       6.81170       1.04183
   15.27000      15.18451       8.39536       1.15589
   15.28500      15.17963      10.33724       1.29638
   15.30000      15.17747      12.00823       1.46397
```

explain how to interpret the results of the above file; how to run this many times, etc.

6.9. Replica Exchange Molecular Dynamics (REMD)

In the one dimensional replica-exchange method, noninteracting copies of the system (replicas) are simulated concurrently at different values of some independent variable, such as temperature. Replicas are subjected to Monte Carlo move evaluation periodically, thus effecting exchange between values of the independent variable. The replica-exchange method enables simulation in a generalized ensemble --- one in which states may be weighted by non-Boltzmann probabilities. (However, one advantage of replica-exchange is the simplicity inherent in its use of Boltzmann factors.) Consequently, local potential energy wells may not dominate traversal through phase space because a replica trapped in a local minimum can escape via exchange to a different value of the independent variable [140]. The *multisander* approach runs multiple *sander* jobs concurrently under a single MPI program. This can be used to just run unconnected parallel jobs, but it is more useful to use this as a platform for the *replica exchange method.*

The replica exchange method in temperature space for molecular dynamics (REMD) [140-142] has been implemented on top of the framework that multisander provides. N non-interacting replicas are simultaneously simulated in N separate MPI groups, each of which has its own set of input and output files. One process from each MPI group is chosen to form another MPI group (called the master group), in which exchanges are attempted.

In this REMD implementation, sander is called as a subroutine from the multisander program. In particular, at the start of the replica exchange run, sander is called once to obtain the current potential energies of each of the input coordinates. The current temp0 value for each replica is also updated if it is present in the restart files (as would be the case if this is a restart of a replica run). Note that no actual MD steps are taken during this initial sander call. Multisander then enters a loop over the number of exchange attempts. In each loop, the first step is to calculate the exchange probabilities between neighboring pairs of temperatures.

The N replicas are first automatically sorted in an array by their target temperatures. Half of the N replicas (replicas with even array indices) are chosen to be exchange initiators. These initiators pair with their right and left neighbors alternatively after each sander call. Topologically, the N temperature-sorted replicas form a loop, in which the first and the last replicas are neighbors. Therefore, N/2 exchanges are attempted in each iteration. The current potential energies and target (temp0) temperatures are used in a Metropolis-type calculation to determine the probability of making the exchange. If the exchange is allowed between the pair, the target temperatures for the two replicas are swapped before the next sander call. The velocities of each replica involved in successful exchange are then adjusted by a scaling factor related to the previous and new target temperatures.

After the exchange calculation, sander is called to perform MD following the mdin file. After the sander run, the exchange probability is calculated again, and so on.

Before starting a replica exchange simulation, an optimal set of target temperatures should be determined so that the exchange ratio is roughly a constant. These target temperatures determine the probability of exchange among the replicas, and the user is referred to the literature for a more complete description of the influence of various factors on the exchange probability.

Each replica requires (for input files) or generates (for output files) its own *mdin, inpcrd, mdout, mdcrd, restrt, mdinfo,* and associated files. The names are provided through the specification of a *groupfile* on the command line with the *-groupfile groupfile* option. The *groupfile* file contains a separate command line for each of the replicas or multisander instances, one per line (with no extra lines except for comments, which must have a '#' in the first column). To choose the number of replicas or multisander instances, the *-ng N* command line option is used (in this case to specify *N* separate instances.) If the number of processors (for the MPI run) is larger than *N* (and also a multiple of *N*), each replica or multisander instance will run on a number of processors equal to the total specified on the command line divided by *N*. Note that in the *groupfile*, the *-np* option is currently ignored, *i.e.* each replica or multisander instance is currently hardcoded to run on an equivalent number of processors.

For example, an 8-replica REMD job will need 8 mdin and 8 inpcrd files. Then, the groupfile might look like this:

```
#
# multisander or replica exchange group file
#
# replica 1
-O -i mdin.rep1 -o mdout.rep1 -c inpcrd.rep1 -r restrt.rep1 -x mdcrd.rep1
# replica 2
-O -i mdin.rep2 -o mdout.rep2 -c inpcrd.rep2 -r restrt.rep2 -x mdcrd.rep2
# replica 3
-O -i mdin.rep3 -o mdout.rep3 -c inpcrd.rep3 -r restrt.rep3 -x mdcrd.rep3
```

```
# replica 4
-O -i mdin.rep4 -o mdout.rep4 -c inpcrd.rep4 -r restrt.rep4 -x mdcrd.rep4
# replica 5
-O -i mdin.rep5 -o mdout.rep5 -c inpcrd.rep5 -r restrt.rep5 -x mdcrd.rep5
# replica 6
-O -i mdin.rep6 -o mdout.rep6 -c inpcrd.rep6 -r restrt.rep6 -x mdcrd.rep6
# replica 7
-O -i mdin.rep7 -o mdout.rep7 -c inpcrd.rep7 -r restrt.rep7 -x mdcrd.rep7
# replica 8
-O -i mdin.rep8 -o mdout.rep8 -c inpcrd.rep8 -r restrt.rep8 -x mdcrd.rep8
```

Note that the *mdin* and *inpcrd* files are *not* required to be ordered by their target temperatures since the temperatures of the replicas will not remain sorted during the simulation. Sorting is performed automatically at each REMD iteration as described above. Thus one can restart REMD simulations without modifying the restart files from the previous REMD run (see below for more information about restarting REMD).

It is important to ensure that the target temperature (specified using *temp0*) is the only difference among the mdin files for the replicas, otherwise the outcome of an REMD simulation may be unpredictable since each replica may be performing a different type of simulation. However, in order to accommodate advanced users, the input files are not explicitly compared.

Using replica excange leads to some changes in the default behavior and output files since REMD calls the sander routine multiple times during a single REMD simulation, with output files combined from *each* REMD iteration. Thus, the multiple iterations of a single REMD MD simulation will generate a single set of output files. The trajectory outputs (mdcrd) of each sander call (each REMD iteration) are combined by enabling file appending.

6.9.1. Restarting REMD simulations

It is recommended that each REMD run generate a new set of output files (such as mdcrd), but for convenience one may use -A in the command line in order to append output to existing output files. This can be a useful option when restarting REMD simulations. As noted above, file appending is always used after the first iteration (first exchange attempt) in REMD. The use of -A on the command line only affects how sander treats any existing files during the first iteration of REMD. If -A is used, files that were present before starting the REMD simulation are appended to throughout the new simulation. Note that this can seriously affect performance on systems where the file writing becomes rate limiting. If -O is used, any files present are overwritten during the first iteration, and then subsequent iterations append to these new files.

At the end of a REMD simulation, the target temperature of each replica is most likely not the same as it was at the start of the simulation (due to exchanges). If one wishes to continue this simulation, sander will need to know that the target temperatures have changed. Since the target temperature is normally specified in the mdin file (using temp0), the previous mdin files would all need to be modified to reflect changes in target temperature of each replica. In order to simplify this process, the program will write the current target temperature as additional information in the restart files during an REMD simulation. When an REMD simulation is started, the program will check to see if the target temperature is present in the restart file. If it is present, this value will override the target temperature specified using temp0 in the mdin file. In this manner, one can restart the simulation from the set of restart files and the program will automatically update the

target temperature of each replica to correspond to the final target temperature from the previous run. If the target temperature is not present (as would be the case for the first REMD run), the correct values should be present in the mdin files.

6.9.2. Content of the output files

Standard (non-REMD) sander simulations produce a significant amount of data at the start and end of the simulation. Since sander is called after each REMD exchange attempt (possibly each 100-1000 MD steps), the output files will become quite large. In order to maintain an energy archive while saving disk space, the roles of mdout and mdinfo are therefore switched during REMD simulation. The mdout file is created and overwritten for each sander call, and only contains information pertinent to this call. The energy archive for the entire set of REMD iterations is placed into the mdinfo file. In non-REMD simulations, mdinfo only contains the most recent energy information.

An important user-controllable option exists for writing output files (repcrd `&cntrl` namelist variable, see below). For mdinfo and mdcrd, two methods of file writing are permitted. An individual mdcrd or mdinfo file (for example, mdcrd.001) can contain the history of a single replica (which samples multiple temperatures), or it can contain all of the information for a single target temperature (regardless of the index of the replica that employed that target temperature. In the former case (files containing data for a single replica index), the files contain a continuous trajectory in phase space but discontinuous in target temperature. Thermodynamic analysis will most likely require postprocessing of these files such that data for each temperature can be analyzed separately. The information required for this postprocessing (the target temperature of each replica during the simulation) can be found in the replica log file. One should note, however, that the current sander trajectory format does not store any information in the trajectory files other than coordinates (such as time index or target temperature). Thus it can be difficult (or impossible) to postprocess the data if any file corruption has occurred.

For this reason, the default behavior is to write mdcrd and mdinfo files that represent a particular target temperature rather than replica index. Thus no postprocessing is required for thermodynamic analysis, but the trajectories do not represent a path that was physically sampled. If desired, a continuous trajectory can be obtained by the postprocessing described above.

As mentioned earlier, mdin and inpcrd files need not be ordered by target temperature. If the user requests that mdcrd, mdinfo and mdout files correspond to a single temperature, these files will be ordered by target temperature. For example, mdcrd.000 would correspond to the coordinate archive for the lowest temperature, mdcrd.001 would be the next highest temperature, and so on. Note that this reordering only occurs if temperature-based output files are requested. If replica-based output files are requested, each output file will have the same index value as the corresponding input files for that replica.

6.9.3. Major changes from sander when using replica exchange

Within an MPI job, as discussed above, it is now possible to run multiple sander jobs at once, such that each job gets a subset of the total processors. To run multisander (or to specify the number of replicas to use in a REMD run), a new command line argument:

-ng specifies the number of sander runs (replicas) to perform concurrently. Note that at present, the number of replicas must be a divisor of the total number of processors (specified by the MPI run command). The input and output file

information must be provided in a groupfile (as described earlier in this section).

In the -DREM compiled code enabling replica-exchange, the following additional options are available and changes in behavior from standard sander are present. First, there are two new command line arguments:

-rem specifies the type of replica exchange simulation. Only two options are currently available. 0, no replica exchange (standard MD) (default behavior if -rem is not specified on command line); 1, regular replica exchange (requires -ng).

-remlog specifies the filename of a log file. This file records from left to right, for every replica and every exchange attempt, the velocity scaling factor (negative if the exchange attempt failed), current actual temperature, current potential energy, current target temperature, and the new target temperature. The default value is *rem.log*.

Next, there are new variables in the `&cntrl` namelist:

REPCRD REMD output file (mdinfo and mdcrd) specifications. If 0, output files contain data for a particular temperature (default); if 1, output files contain data for a particular replica.

NUMEXCHG The number of exchange attempts, default 0.

NSTLIM the number of MD steps *between exchange attempts*. Note that NSTLIM is not a new variable for REMD, but the meaning is somewhat different. The total length of the REMD simulation will be nstlim*numexchg steps long.

6.9.4. Cautions when using replica exchange

While many variations of replica exchange have been tested with sander, all possible variations have not been tested and the option is intended for use by advanced researchers that already have a comprehensive understanding of standard molecular dynamics simulations.

Caution should be used when creating REMD input files. Amber will check for the most obvious errors but due to the nature of the multiple output files the reason for the error may not be readily apparent.

The following is only a subset of things that users should keep in mind:

The number of replicas must be an even number (so that all replicas have a partner for exchange).

Temp0 values for each replica must be unique.

Other than temp0, mdin files should normally be identical.

Temp0 values should not be changed in the nmropt=1 weight change section.

The value of irest must be 1. This means that inpcrd files must have velocities.

A groupfile is required (this was not the case in Amber8).

If high temperatures are used, it may be necessary to use a smaller time step and possibly restraints to prevent cis/trans isomerization or chirality inversion.

Due to increased diffusion rates at high temperature, it may be good to use iwrap=1 to prevent coordinates from becoming too large to fit in the restart format.

Note that the optimal temperature range and spacing will depend on the system. The user is strongly recommended to read the literature in this area.

6.9.5. Replica exchange example

Below is an example of an 8-replica REMD run on 16 processors, assuming that relevant environment variables have been properly set.

```
$MPIRUN -np 16 sander -ng 8 -groupfile groupfile
```

This input specifies that REMD should be used (-rem 1), with 8 replicas (-ng 8) and 2 processors per replica (-np 16). Note that the total number of processors should always be a multiple of the number of replicas.

Here is a section of a sample rem.log file produced by Amber:

```
# replica exchange log file
 # Replica #, Velocity Scaling, T, Eptot, Temp0, NewTemp0, Success rate (i,i+1)
 # exchange            1
 1      1.46      0.00   -541.20    269.50    570.90      0.00
 2      1.06      0.00   -541.20    300.00    334.00      2.00
 3      0.95      0.00   -541.20    334.00    300.00      0.00
 4      1.06      0.00   -541.20    371.80    413.90      2.00
 5      0.95      0.00   -541.20    413.90    371.80      0.00
 6      1.06      0.00   -541.20    460.70    512.90      2.00
 7      0.95      0.00   -541.20    512.90    460.70      0.00
 8      0.69      0.00   -541.20    570.90    269.50      2.00
 # exchange            2
 1     -1.00      0.00   -491.39    570.90    570.90      1.00
 2     -1.00      0.00   -547.98    334.00    334.00      0.00
 3     -1.00      0.00   -553.87    300.00    300.00      1.00
 4     -1.00      0.00   -518.92    413.90    413.90      0.00
 5     -1.00      0.00   -538.17    371.80    371.80      1.00
 6     -1.00      0.00   -494.00    512.90    512.90      0.00
 7     -1.00      0.00   -498.12    460.70    460.70      1.00
 8     -1.00      0.00   -567.18    269.50    269.50      0.00
 # exchange            3
 1     -1.00      0.00   -462.14    570.90    570.90      0.67
 2      0.95      0.00   -539.83    334.00    300.00      0.00
 3      1.06      0.00   -537.76    300.00    334.00      1.33
 4     -1.00      0.00   -510.33    413.90    413.90      0.00
 5     -1.00      0.00   -540.74    371.80    371.80      0.67
 6     -1.00      0.00   -491.99    512.90    512.90      0.00
 7     -1.00      0.00   -522.01    460.70    460.70      0.67
 8     -1.00      0.00   -568.87    269.50    269.50      0.00
```

Note that a section of the log file is written for each exchange attempt. For each exchange, the log contains a line for each replica. This line lists the replica number, the velocity scaling factor, the actual instantaneous temperature, the potential energy, the old and new target

temperatures, and the current overall success rate for exchange between this temperature and the next higher temperature. Note that the velocity scaling factor will be -1.0 if the exchange was not successful. In that case, the old and new target temperatures will be identical.

In this particular example, all of the inpcrd files were identical, and thus the potential energies listed for exchange 1 are identical. For this reason, all of the exchanges are successful. After this exchange, MD is performed for nstlim steps, and so the potential energies are no longer identical at exchange #2.

Note that the exchange success rate may be larger than 1.0 during the first few attempts, since each particular pair is considered only every other attempt. The success rate is the number of accepted exchanges for the pair divided by the total number of exchange attempts, multiplied by 2 to account for the alternating neighbors.

6.9.6. Replica exchange using a hybrid solvent model

This section describes an advanced feature of Amber that is currently under development [22]. Users that are not already comfortable with standard replica exchange simulations should likely get more experience with them before attempting hybrid solvent REMD calculations.

For large systems, REMD becomes intractable since the number of replicas needed to span a given temperature range increases roughly with the square root of the number of degrees of freedom in the system. Recognizing that the main difficulty in applying REMD with explicit solvent lies in the number of simulations required, rather than just the complexity of each simulation, we recently developed (OKUR JCTC 2006) a new approach in which each replica is simulated in explicit solvent using standard methods such as periodic boundary conditions and inclusion of long-range electrostatic interactions using PME. However, the calculation of exchange probabilities (which determines the temperature spacing and thus the number of replicas) is handled differently. Only a subset of closest water molecules is retained, with the remainder temporarily replaced by a continuum representation. The energy is calculated using the hybrid model, and the exchange probability is determined. The original solvent coordinates are then restored and the simulation proceeds as a continuous trajectory with fully explicit solvation. This way the perceived system size for evaluation of exchange probability is dramatically reduced and fewer replicas are needed.

An important difference from existing hybrid solvent models is that the system is fully solvated throughout the entire MD simulation, and thus the distribution functions and solvent properties should not be affected by the use of the hybrid model in the exchange calculation. In addition, no restraints of any type are needed for the solvent, and the solute shape and volume may change since the solvation shells are generated for each replica on the fly at every exchange calculation. Nearly no computational overhead is involved since the calculation is performed infrequently as compared to the normal force evaluations. Thus the hybrid REMD approach can employ more accurate continuum models that are too computationally demanding for use in each time step of a standard molecular dynamics simulation. However, since the Hamiltonian used for the exchange differs from that employed during dynamics, these simulations are approximate and are not guaranteed to provide correct canonical ensembles.

To use hybrid solvent REMD in Amber, 2 sets of *prmtop* files are needed. One is the fully solvated *prmtop* used for the MD simulations. The second is the hybrid *prmtop*, containing only the solute and the desired number of water molecules. Two sets of *mdin* files are also required, the first set for the dynamics between exchanges and the second set for the exchange calculation. The first set of *mdin* files describe the fully solvated system and are essentially the same as standard

REMD with explicit solvent with the addition of the *numwatkeep* namelist variable described below. The second *mdin* set is used for the energy evaluation of the reduced system using a continuum model.

To summarize, this type of calculation requires 2 sets of mdin files, 2 prmtop files and generates 2 sets of output files. The grouplist will specify the names for the large system, and the hybrid calculation file names will be identical to what was specified in the groupfile with the exception that ".strip" will be appended to each input/output file name. For example, if the input file for replica 1 is called mdin.001, a file called mdin.001.strip must also be present for the hybrid part of the calculation.

Two sets of *inpcrd* files are not needed. At each exchange calculation (including the first) sander will create the hybrid inpcrd based on the current coordinates for the fully solvated system. This is done by calculating the distance of each water oxygen to the nearest solute atom, and sorting the water by increasing shortest distance. The closest *numwatkeep* are retained and sander will write a coordinate file containing the solute and the *numwatkeep* closest waters.

Note that the user must create a *prmtop* file for the hybrid run that matches the full system but has only *numwatkeep* waters. Both *prmtop*s must be present during the calculation. If the hybrid *prmtop* has a different number of atoms than the smaller system file written by *sander*, then the calculation will fail.

For a more complete example, users are directed to the hybridREMD test case in the Amber test directory.

NUMWATKEEP The number of explicit waters that should be retained for the calculation of potential energy to be used for the exchange calculation. Before each exchange attempt, the closest numwatkeep waters will be retained (closest to the solute) and the rest will be temporarily removed and then replaced after the exchange probability has been calculated. The default value is -1, indicating that all waters should be retained (standard REMD). A value of 0 would direct Amber to remove all of the explicit water (as in MM-PBSA) while a non-zero value will result in some water close to the solute being retained while the rest is removed. Currently it is not possible to select a subset of solute atoms for determing which waters are "close". Determining the optimal numwatkeep value is a topic of current research.

6.9.7. Cautions for hybrid solvent replica exchange

This option has not been extensively tested. The following would not be expected to work without further modification of the code:

(1) Only the water is imaged for the creation of the stripped system. Care should be taken with dimers (such as DNA duplexes) to ensure that the imaging is correct.

(2) Explicit counterions should probably not be used.

(3) The choice of implicit solvent model will likely have a large effect on the resulting ensemble.

6.10. Nudged elastic band calculations

6.10.1. Background

In nudged elastic band method [143,144], the path for a conformational change is approximated with a series of images of the molecule describing the path. Minimization, with the images at the endpoints fixed in space, of the total system energy provides a minimum energy path. Each image between is connected to the previous and next image by "springs" along the path that keep each image from sliding down the energy landscape onto adjacent images. NEB derives from the plain elastic band method, pioneered by Elber and Karplus [145], which added the spring forces to the potential of energy surface and minimized the energy of the system. The plain elastic band method found low energy paths, but tended to cut corners in the energy landscape. NEB prevents corner cutting by truncating the spring forces in directions perpendicular to the tangent of the path. Furthermore, the forces from the molecular potential are truncated along the path, so that images remain evenly spaced along the path. This leads to:

$$\mathbf{F} = \mathbf{F}^{\perp} + \mathbf{F}^{\parallel}$$

$$\mathbf{F}^{\perp} = -\nabla V(\mathbf{P}) + ((\nabla V(\mathbf{P}) \cdot \tau)\tau \tag{6.27}$$

$$\mathbf{F}^{\parallel} = [(k_{i+1}|\mathbf{P}_{i+1} - \mathbf{P}_i| - k_i|\mathbf{P}_i - \mathbf{P}_{i-1}|) \cdot \tau]\tau$$

where, if N is the number of atoms per image, $\mathbf{F}$ is the force on image i, $\mathbf{P}_i$ is the 3N dimensional position vector of image i, k_i is the spring constant between image $i-1$ and image i, V is the potential described by the force field, and τ is the 3N dimensional tangent unit vector that describes the path.

The simplest definition of τ is:

$$\tau = (\mathbf{P}_i - \mathbf{P}_{i-1})/|\mathbf{P}_i - \mathbf{P}_{i-1}| \tag{6.28}$$

This definition leads to instability in the path caused by kinks that occur where the magnitude of $\mathbf{F}^{\parallel}$ is much larger than the magnitude of $\mathbf{F}^{\perp}$. A more stable tangent definition was derived to prevent kinks in the path [146] that depends upon the energies, E, of adjacent images:

$$\textit{If } (E_{i+1} > E_i > E_{i-1}) \textit{ then } \tau = (\mathbf{P}_{i+1} - \mathbf{P}_i)/|\mathbf{P}_{i+1} - \mathbf{P}_i|$$

$$\textit{If } (E_{i+1} < E_i < E_{i-1}) \textit{ then } \tau = (\mathbf{P}_i - \mathbf{P}_{i-1})/|\mathbf{P}_i - \mathbf{P}_{i-1}| \tag{6.29}$$

Or, if E_i is a local minima or maxima, then:

$$\textit{If } (E_{i+1} > E_{i-1}) \textit{ then } \tau = [(\mathbf{P}sub+1 - \mathbf{P}_i)E^{+} + (\mathbf{P}_i - \mathbf{P}_{i-1})E^{-}]/|(\mathbf{P}sub+1 - \mathbf{P}_i)E^{+} + (\mathbf{P}_i - \mathbf{P}_{i-1})E^{-}|$$

$$\textit{If } (E_{i+1} < E_{i-1}) \textit{ then } \tau = [(\mathbf{P}_{i+1} - \mathbf{P}_i)E^{-} + (\mathbf{P}_i - \mathbf{P}_{i-1})E^{+}]/|(\mathbf{P}_{i+1} - \mathbf{P}_i)E^{-} + (\mathbf{P}_i - \mathbf{P}_{i-1})E^{+}| \tag{6.30}$$

where:

$$E^{+} = \max[\,|E_{i+1} - E_i|, |E_{i-1} - E_i|\,]$$

$$E^{-} = \min[\,|E_{i+1} - E_i|, |E_{i-1} - E_i|\,] \tag{6.31}$$

The spring constants can be the same between all images or they can be scaled to move the images closer together in the regions of transition states [147]:

$$\textit{If } (E_i > E_{ref}) \textit{ then } k_i = k_{\max} - \Delta k(E_{\max} - E_i)/(E_{\max} - E_{ref})$$

$$If\ (E_i \le E_{ref})\ then\ k_i = k_{\max} - \Delta k \tag{6.32}$$

Here $E_{\max}$ is the highest energy for an image along the path, E_{ref} is the energy of the higher energy endpoint, and $k_{\max}$ and Δk are parameters with units of force per length. Because the spring force applies only in directions along the path and because the potential of the energy surface is zeroed along the path, the calculation is relatively insensitive to the magnitude of the spring constants. Care must be taken, however, to select a spring constant that does not result in higher frequency motions than those found in the system of interest [148]. At each step, before calculating the spring forces that compose $\mathbf{F}^{||}$, the images, starting with the second image, are rotated and translated onto the previous image to find the RMSD minimum.

Energy minimization of the path is complicated by the fact that the forces are truncated according to the tangent direction, making it impossible to define a Lagrangian [148]. Conjugate gradient minimization, therefore, cannot be used to find the minimum energy path. An algorithm for quenched molecular dynamics has been used to find the minimum [144]. With this method, the component of the velocity || to the force is kept, but perpendicular components are scaled:

$$If\ (\mathbf{v}\cdot\mathbf{f} \ge 0)\ then\ \mathbf{v} = (\mathbf{v}\cdot\mathbf{f})\mathbf{f}$$

$$If\ (\mathbf{v}\cdot\mathbf{f} < 0)\ then\ \mathbf{v} = x(\mathbf{v}\cdot\mathbf{f})\mathbf{f} \tag{6.33}$$

where **f** is the 3N-dimensional unit force vector, **v** is the 3N-dimensional velocity vector, and x is a scaling factor less than one. Recently, a super-linear minimization method was described using an adopted basis Newton-Raphson minimizer [148].

The implementation of NEB in *sander.PIMD* [149] allows minimization by simulated annealing. This requires no hypothesis for a starting path, but does require careful judgment of the temperature and length of time required to populate the minimum energy path. The initial coordinates can have multiple copies of the structure superimposed on the start and endpoints. When adjacent structures are superimposed, the tangent, τ is 0 in every direction. This case is explicitly handled so that the calculation is stable.

6.10.2. Preparing input file for NEB

The nudged elastic band capability is implemented inside *sander.PIMD* because of the similarity between PIMD and NEB, and *addles* should be used to prepare the input file for NEB. The input *prmtop* and *inpcrd* files for NEB are generated using *addles*. To use *addles*, generally you need prmtop and inpcrd files of single molecule, which can be generated by using LEaP, then a control parameter for addles to explictly define how the NEB prmtop and inpcrd file should be generated. An example control parameter file (addles.in) for addles is illustrated as follows:

```
file rprm name=(input.prmtop) read
file rcrd name=(input.inpcrd) pack=2 read
file wprm name=(neb.prmtop) wovr
file wcrd name=(neb.inpcrd) wovr

action
~ use original mass
omas
pimd
~ make 20 copies of atom 1 to 22 (the whole system)
```

```
space numc=20 pick #prt 1 22 done

*EOD
```

For a full description of addles please refer to Section 9.2, the following is something tricks in preparing NEB input files:

(1) Always turn on the pimd tag, otherwise you may get an unexpectabely big prmtop file because of a huge nonbond exclusion list contain all atoms in different copy.

(2) Make sure your are making copies of the whole system, since now the PIMD implementation of sander is an all-or-nothing thing, means you can't run partial NEB simulation currently.

(3) Make use of the pack option of rcrd(rcvd,rcbd,rcvb) to assign different coordinates for different copies. It is necessary for NEB that different images be assigned different configurations, that is why the option "pack" is added to "rcrd". To use this option, the user need first concatenate desired coordinates together, than specify the number of coordinate sets via "pack=n", addles then will assign coordinates to images averagely. For example, if the inpcrd file has 4 sets of coordinates and you have 20 images. Then image 1-5 will have coordinate set 1, image 6-10 have set 2, and so on.

6.10.3. Input Variables

INEB	Flag for nudged elastic band. A value of 0 (default) means that no nudged elastic band will be used. A value of 1 means that a NEB simulation is being performed.
SKMAX	Spring constant or kmax from above (100 by default).
SKMIN	If skmin = skmax, a constant spring constant is used. Otherwise, skmin is taken from above for scaled spring constants (50 by default).
TMODE	If 1 (default), use the revised tangent definition that prevents kinks. For any other value, use the simple (original) tangent definition.
VV	If this is 1, use the quenched velocity Verlet minimization; otherwise, do not.
VFAC	Scaling factor for quenched velocity Verlet algorithm. (0.0 by default)

6.11. Constant pH calculations

The constant pH molecular dynamics method has been implemented in *sander* by John Mongan [150]. Constant pH is limited to implicit solvent simulations. Using the constant pH method requires minor modifications to the process of generating the prmtop file, as well as generation of a second input file from the prmtop file, describing the titrating residues.

6.11.1. Background

Traditionally, molecular dynamics simulations have employed constant protonation states for titratable residues. This approach has many drawbacks. First, assigning protonation states requires knowledge of pK_a values for the protein's titratable groups. Second, if any of these pK_a values are near the solvent pH there may be no single protonation state that adequately represents the ensemble of protonation states appropriate at that pH. Finally, since protonation states are

constant, this approach decouples the dynamic dependence of pK_a and protonation state on conformation.

The constant pH method implemented in *sander* addresses these issues through Monte Carlo sampling of the Boltzmann distribution of protonation states concurrent with the molecular dynamics simulation. The nature of the distribution is affected by solvent pH, which is set as an external parameter. Residue protonation states are changed by changing the partial charges on the atoms.

6.11.2. Preparing a system for constant pH

Amber provides definitions for titrating side chains of ASP, GLU, HIS, LYS and TYR. See below if you need other titrating groups.

Begin by preparing your PDB file as you normally would for use with LEaP. Edit the PDB file, replacing all histidine residue names (HIS, HID, or HIE) with HIP. Change all ASP and ASH to AS4 and all GLU and GLH to GL4. This ensures that the prmtop file will have a hydrogen defined at every possible point of protonation.

Run leap, and enter the following commands:

```
source leaprc.ff99
loadAmberParams frcmod.mod_phipsi.1
set default PBRadii mbondi2
loadoff constph.lib
loadamberparams frcmod.constph
```

This loads constph.lib, which contains residue definitions for AS4 and GL4 (aspartate and glutamate residues with syn and anti hydrogens on each carboxyl oxygen), and frcmod.constph which defines improper torsions to keep the syn and anti protons on AS4 and GL4 from rotating into the same position. Now load your edited PDB file and proceed as usual to create the prmtop and prmcrd files.

Once you have the prmtop file, you need to generate a cpin file. The cpin file describes which residues should titrate, and defines the possible protonation states and their relative energies. A perl script, *cpinutil.pl*, is provided to generate this file. It takes a PDB file as input, either on the command line or on STDIN, and writes the cpin file to STDOUT. Note that you *must* generate this PDB file from the prmtop file; do not use your original PDB file. Since LEaP has inserted extra hydrogens, the atom numbering in your original PDB file will not correspond to the prmtop file. Here is an example of generating the PDB file and using it to create the cpin file in a single step:

```
ambpdb -p prmtop < prmcrd | cpinutil.pl > cpin
```

The *cpinutil.pl* program accepts a number of flags that modify its behavior. By default, all residues start in protonation state 0: deprotonated for ASP and GLU, protonated for LYS and TYR, doubly protonated for HIS (i.e. HIP). Initial protonation states can be specified using the -states flag followed by a comma delimited list of initial protonation states (see below for more about protonation state definitions) as follows:

```
ambpdb -p prmtop < prmcrd | cpinutil.pl -states 1,3,0,0,0,1 > cpin
```

The -system flag can be used to provide a name for the titrating system. If experimental pKa values have been defined for the system (see below), they will be written into the cpin file. Note that experimental pKa values are used only by the analysis scripts to calculate pKa prediction error; they are not used in any way by *sander* and do not need to be included.

```
ambpdb -p prmtop < prmcrd | cpinutil.pl -system HEWL > cpin
```

A number of flags are available for filtering which residues are included in the cpin file. All residues in the cpin file, and only the residues in the cpin file, will be titrated. In general it is safe to exclude TYR and LYS for acidic simulations and GL4 and AS4 for basic simulations. HIP should be included in all except very acidic simulations. Note that there is currently no support for titrating N or C terminal residues. If you have an N or C terminal residue with a titratable sidechain, you should explicitly exclude it from the cpin file. The -resnum flag may be used to specify which residue numbers should be retained; all others are deleted. Conversely, the -notresnum flag can be used to specify which residue numbers are deleted; all others are retained. Residue number refers to the numbering in the PDB file, not the index number among titrating residues. Similarly, -resname and -notresname can be used to filter by residue type. For instance, -notresname TYR,LYS would eliminate basic residues from the cpin file. If experimental pKa values are known through use of the -system flag, the -minpka and -maxpka flags can be used to filter residues by experimental pK_a values.

cpinutil.pl can also take an existing cpin file as input, allowing modification or further filtering of existing cpin files. See *cpinutil.pl -h* for a summary of options and flags.

6.11.3. Running at constant pH

Running constant pH under *sander* has few differences from normal operation. In the mdin file, you must set *icnstph*=1 to turn on constant pH. *solvph* is used to set the solvent pH value. You must also specify the period for Monte Carlo steps, *ntcnstph* (for period *n*, a Monte Carlo step is performed every *n* steps). Note that only one residue is examined on each step, so you should decrease the step period as the number of titrating residues increases to maintain a constant effective step period for each residue. We have seen good results with fairly short periods, in the neighborhood of 100 fs effective period for each residue (e.g. *ntcnstph*=5, *dt*=0.002 with about 10 residues titrating).

In order to avoid having to calculate non-electrostatic contributions to protonation state transition energies, this method uses correction factors based on the relative energy differences of the different protonation states in the Amber force field. These relative energies were calculated under the following parameters:

```
cut=30.0, scee=1.2, igb=2, saltcon=0.1,
ntb=0, dt=0.002, nrespa=1,
ntt=1, tempi=300.0, temp0 = 300., tautp=2.0,
ntc=2, ntf=2, tol=0.000001,
```

Deviations from these parameters, or from the force field or GB radii specified above may affect the relative energies of the protonation states, which will cause erroneous results. If you must

deviate from these settings, you can test whether your changes will cause problems by running long (multiple ns) titrations of the model compounds, with solvent pH equal to the model compound pKa value. The model compounds are ACE-X-NME, where X is AS4, GL4, HIP, LYS or TYR. If these titrations predict the model pKa value (4.0, 4.4, 6.5, 10.4 and 9.6, respectively), then the parameter set is probably OK. If not, you must either change the parameter set or recalculate the relative energies (see section below).

Some additional command line flags have been added to *sander* to support constant pH operation. The cpin file must be specified using the -cpin option. Additionally, a history of the protonation states sampled is written to the filename specified by -cpout. Finally, a constant pH restart file is written to the filename specified by -cprestrt. This is used to ensure that titrating residues retain the same protonation state across restarts. The constant pH restart file is a cpin-format file, and should be used as the cpin file when restarting the run. It will generally be longer than the original cpin file, as it contains some amount of zeroed data, due to limitations in the Fortran namelist implementation. The excess zero data can be removed by filtering it through *cpinutil.pl*, e.g.

```
cpinutil.pl cprestrt > cpin2
```

6.11.4. Analyzing constant pH simulations

As the simulation progresses, the protonation states that are sampled are written to the cpout file. A section of a cpout file is included here:

```
Solvent pH:  2.00000
Monte Carlo step size:        2
Time step:        0
Time:      0.000
Residue    0 State:  1
Residue    1 State:  0
Residue    2 State:  1
Residue    3 State:  0
Residue    4 State:  1
Residue    5 State:  0

Residue    2 State:  0

Residue    4 State:  0

Residue    0 State:  3

Residue    1 State:  0

Residue    0 State:  0
```

One record is written on each Monte Carlo step. Each record is terminated by a blank line. There are two types of records, full records (at the top of the file) and delta records (single lines, remainder of file). Full records are written before the run begins, on timesteps where restart files are

written, and on the final time step (assuming these are Monte Carlo steps); delta records are written in all other cases. The full record specifies the protonation state of each residue, along with some additional information, while the delta records give only the protonation state for the residue selected on the corresponding Monte Carlo step. Note that in some cases, the protonation state for a delta record may be the same as that in an earlier record: this indicates that the Monte Carlo protonation move was rejected. The residue numbers in cpout are indices over the titrating residues included in the cpin file; cpout must be analyzed in conjunction with cpin to map these indices back to the original system.

The Perl script *calcpka.pl* is provided as an example parser for the cpout format, and as a utility for calculating predicted pK_a values from cpout files. It takes a cpin file as its first argument and any number of cpout files for its remaining arguments. For instance:

```
calcpka.pl cpin cpout1 cpout2 cpout3
```

Output contains one line for each titrating residue in the system. *Offset* is the difference between the predited pK_a and the system pH. *Pred* is the predicted pK_a. Note that predictions are calculated assuming Henderson-Hasselbalch titration curves. Predictions are most accurate when the absolute value of the offset is less than 2.0. If experimental pK_a values have been defined for the system (see following), then experimental and error values are also printed. *Frac Prot* is the fraction of time the residue spends protonated and *Transitions* gives the number of accepted protonation state transitions. Note that transitions between states with the same total protonation (e.g. *syn* and *anti* protonated states of a carboxylic acid) are not included in this total. *Average total molecular protonation* is the sum of the fractional protonations. It ranges between zero and the number of titrating residues, and gives the average protonation of the molecule as a whole.

6.11.5. Extending constant pH to additional titratable groups

There are two major components to defining a new titrating group for constant pH. First you must define the partial charges for each atom in the residue for each protonation state. Then you must set the relative energies of each state.

6.11.5.1. Defining charge sets

Partial charges are most easily calculated using Antechamber and Gaussian. You must set up a model to calculate charges for each protonation state. If the titrating group you are defining is a polymer subunit (e.g. amino acid residue), you must adjust the charges on atoms that have bonded interactions (including 1-4) with atoms in neighboring residues. The charges on these atoms must be changed so they are constant across all protonation states − otherwise relative energies of protonation states become sequence dependent. For an amino acid, this means that all backbone atoms must have constant charges. For the residues defined here, we arbitrarily selected the backbone charges of the protonated state to be used across all protonation states. The total charge difference between states should remain 1; we achieved this by adjusting the charge on the beta carbon.

6.11.5.2. Calculating relative energies

Relative energies are used to calibrate the method such that when a model compound is titrated at pH equal to its pK_a, the energies (and thus populations) of the protonated and deprotonated states are equal. Relative energies of the different protonation states are calculated using thermodynamic integration of a model compound between the charge sets defined for the different protonation states. The model compound should be a small molecule that mimics the bonded environment of the titratable group of interest, and for which experimental pK_a data are available. For instance, the model compound for an amino acid X is generally ACE-X-NME; the model compound for a ligand might be the free ligand. The thermodynamic integration calculations must be performed using exactly the same parameters and force field as you plan to use in your constant pH simulations. Once the relative energies of the states are calculated by thermodynamic integration, the energy difference must be adjusted to account for the pK_a: the energy of the more protonated states should be increased by $pK_a RT \ln(10)$.

For example suppose one were developing a model for an artificial amino acid, ART, with pK_a 3.5 and two protonation states: ARP, having one proton and ARD having zero protons. After calculating partial charges as above, you would construct a model compound having the sequence ACE-ARP-NME and generate a prtprmtop file where the ARP charges were perturbed to the ARD values. You would then use sander to perform thermodynamic integration between ARP and ARD. Suppose that this showed that the energy of ARD relative to ARP was -6.3 kcal/mol. You would assign a relative energy of -6.3 to ARD and a relative energy of 3.5RTln(10) to ARP.

6.11.5.3. Testing the titratable group definitions

Prior to large scale use of your new titratable group definition, it's a good idea to test it by performing a constant pH simulation on your model compound, with pH set to the model pK_a. Doing this requires generation of a cpin file, so this is a good point to modify the table of titratable group definitions used by *cpinutil.pl*. These tables are found near the end of CPin.pm. The table is a perl hash of 2D arrays. Each hash entry is an array of states that define a titratable group. Each state array consists of the relative energy, the relative protonation, and the partial charges for the state, in that order. An entry for the example given above might look like (charge list shortened for brevity):

```
"ARP" => [
          # State 0, ARP
          [3.5 * 1.3818, # Relative energy (300K)
           1, # Relative protonation
           -0.4157, 0.2719, -0.0014, 0.0876, -0.0152, 0.0295, ],
          # State 1, ARD
          [-6.3, # Energy
           0, # Protonation
           -0.4157, 0.2719, -0.0014, 0.0876, -0.0858, 0.019, ]
         ]
```

Below this table is another table of experimental pK_a values. Entries for new systems can be created following the example already present for HEWL (the keys are residue numbers, the values are their pK_a values). As discussed above, this is optional and does not affect the constant pH simulations − these data are used only by *calcpka.pl* and *cpinutil.pl*.

Having added your titratable group definition to the table, you should be able to prepare a cpin file as described above, run your simulation and calculate the predicted pK_a using *calcpka.pl*. Since the model compound is usually very small, runs of tens of nanoseconds are easily accessible for these tests. In general, the run to run variation of predicted pK_a values is a few hundredths of a pK_a unit for long runs with pH near pK_a. In most cases, the thermodynamic integration procedure described above yields acceptable results, but if your predicted pK_a differs significantly from the model pK_a, you may want to adjust your relative energies, regenerate your cpin file and rerun the test until you achieve good predictions.

6.12. NMR refinement using SANDER.

We find the *sander* module to be a flexible way of incorporating a variety of restraints into a optimization procedure that includes energy minimization and dynamical simulated annealing. The "standard" sorts of NMR restraints, derived from NOE and J-coupling data, can be entered in a way very similar to that of programs like DISGEO, DIANA or X-PLOR; an aliasing syntax allows for definitions of pseudo-atoms, connections with peak numbers in spectra, and the use of "ambiguous" constraints from incompletely-assigned spectra. More "advanced" features include the use of time-averaged constraints, use of multiple copies (LES) in conjunction with NMR refinement, and direct refinement against NOESY intensities, paramagnetic and diamagnetic chemical shifts, or residual dipolar couplings. In addition, a key strength of the program is its ability to carry out the refinements (usually near the final stages) using an explicit-solvent representation that incorporates force fields and simulation protocols that are known to give pretty accurate results in many cases for unconstrained simulations; this ability should improve predictions in regions of low constraint density and should help reduce the number of places where the force field and the NMR constraints are in "competition" with one another.

Since there is no generally-accepted "recipe" for obtaining solution structures from NMR data, the comments below are intended to provide a guide to some commonly-used procedures. Generally speaking, the programs that need to be run to obtain NMR structures can be divided into three parts:

(1) *front-end* modules, which interact with NMR databases that provide information about assignments, chemical shifts, coupling constants, NOESY intensities, and so on. We have tried to make the general format of the input straightforward enough so that it could be interfaced to a variety of programs. At TSRI, we generally use the FELIX and NMRView codes, but the principles should be similar for other ways of keeping track of a database of NMR spectral information. As the flow-chart on the next page indicates, there are only a few files that need to be created for NMR restraints; these are indicated by the solid rectangles. The primary distance and torsion angle files have a fairly simple format that is largely compatible with the DIANA programs; if one wishes to use information from ambiguous or overlapped peaks, there is an additional "MAP" file that makes a translation from peak identifiers to ambiguous (or partial) assignments. Finally, there are some specialized (but still pretty straightforward) file formats for chemical shift or residual dipolar coupling restraints.

There are a variety of tools, besides the ones described below, that can assist in preparing input for structure refinement in Amber. The SANE (Structure Assisted NOE Evaluation) package,

`http://garbanzo.scripps.edu/nmrgrp/wisdom/sane/sane.html`

is widely used at The Scripps Research Institute [151]. If you use Bruce Johnson's NmrView package, you might also want to look at the TSRI additions to that:

`http://garbanzo.scripps.edu/nmrgrp/wisdom/pipe/tips_scripts.html`

In particular, the *xpkTOupl* and *starTOupl* scripts there convert NmrView peak lists into the "7-column" needed for input to makeDIST_RST.

Users of the MARDIGRAS programs from UCSF can use the *mardi2amber* program to do conversion to Amber format:

`http://picasso.ucsf.edu/mardihome.html`

(2) *restrained molecular dynamics*, which is at the heart of the conformational searching procedures. This is the part that *sander* itself handles.

(3) *back-end* routines that do things like compare families of structures, generate statistics, simulate spectra, and the like. For many purposes, such as visualization, or the running of procheck-NMR, the "interface" to such programs is just the set of pdb-format files that contain the family of structures to be analyzed. These general-purpose structure analysis programs are available in many locations and are not discussed here. The principal *sander*-specific tool is *sviol*, which prepares tables and statistics of energies, restraint violations, and the like.

6.12.1. Distance, angle and torsional restraints.

Distance and angle restraints are read from the DISANG file if *nmropt* > 0. Namelist rst ("`&rst`") contains the following variables; it is read repeatedly until a namelist `&rst` statement is found with IAT(1)=0, or until reaching the end of the DISANG file.

If you wish to include weight changes but have no internal constraints, set *nmropt*=1, but do not include a DISANG line in the file redirection section. (Note that, unlike earlier versions of Amber, the `&rst` namelists must be in the DISANG file, and not in the *mdin* file.)

In many cases, the user will not prepare this section of the input by hand, but will use the auxiliary programs *makeDIST_RST, makeANG_RST* and *makeCHIR_RST* to prepare input from simpler files.

Variables in the `&rst` namelist:

IAT(1)→IAT(4) *If IRESID = 0 (normal operation):*

The atoms defining the restraint. If IAT(3) ≤0, this is a distance restraint. If IAT(4) ≤0, this is an angle restraint. Otherwise, this is a torsional (or J-coupling, if desired) restraint.

If this is a distance restraint, and IAT1 <0, then a group of atoms is defined below, and the coordinate-averaged position of this group will be used in place of the coordinates of atom 1 [IAT(1)].

Similarly, if IAT(2) < 0, a group of atoms will be defined below whose coordinate-averaged position will be used in place of the coordinates for atom 2 [IAT(2)].

If IRESID=1:

IAT(1) → IAT(4) point to the numbers of the *residues* containing the atoms comprising the internal. Residue numbers are the absolute numbers in the entire system. In this case, the variables ATNAM(1) → ATNAM(4) must be specified, and give the character names of the desired atoms within the respective residues.

If IAT(1) < 0 or IAT(2) < 0, then group input will still be read in place of the corresponding atom, as described below.

Defaults for IAT(1)→IAT(4) are 0.

ATNAM If IRESID = 1, then the character names of the atoms defining the internal are contained in ATNAM(1)→ATNAM(4). Residue IAT(1) is searched for atom name ATNAM(1); residue IAT(2) is searched for atom name ATNAM(2); etc. On machines using the portable namelist code, the form is atnam(1)='AT1',atnam(2)='AT2' etc, otherwise the form atnam='AT1','AT2' etc can be used.

Defaults for ATNAM(1)→ATNAM(4) are ' '.

IRESID Indicates whether IAT(I) points to an atom # or a residue #. See descriptions of IAT() and ATNAM() above.

Default = 0.

NSTEP1

NSTEP2 This restraint is applied for steps/iterations NSTEP1 through NSTEP2. If NSTEP2 = 0, the restraint will be applied from NSTEP1 through the end of the run. Note that the first step/iteration is considered step zero (0).

Defaults for NSTEP1, NSTEP2 are both 0.

IRSTYP Normally, the restraint target values defined below (R1→R4) are used directly. If IRSTYP = 1, the values given for R1→R4 define relative displacements from the current value (value determined from the starting coordinates) of the restrained internal. For example, if IRSTYP=1, the current value of a restrained distance is 1.25, and R1 (below) is -0.20, then a value of R1=1.05 will be used.

Default is IRSTYP=0.

IALTD Determines what happens when a distance restraint gets very large. If IALTD=1, then the potential "flattens out", and there is no force for large violations; this allows for errors in constraint lists, but might tend to ignore constraints that *should* be included to pull a bad initial structure towards a more correct one. When IALTD=0 the penalty energy continues to rise for large violations. See below for the detailed functional forms that are used for distance restraints. Set IALTD=0 to recover the behavior of earlier versions of *sander*. Default value is 0, or the last value that was explicitly set in a previous restraint. This value is set to 1 if *makeDIST_RST* is called with the *-altdis* flag.

IFVARI If IFVARI > 0, then the force constants/positions of the restraint will vary with step number. Otherwise, they are constant throughout the run. If IFVARI >0, then the values R1A→R4A, RK2A, and RK3A must be specified (see below).

Default is IFVARI=0.

NINC If IFVARI > and NINC > 0, then the change in the target values of of R1→R4 and K2,K3 is applied as a step function, with NINC steps/ iterations between each change in the target values. If NINC = 0, the change is effected continuously (at every step).

Default for NINC is the value assigned to NINC in the most recent namelist where NINC was specified. If NINC has not been specified in any namelist, it defaults to 0.

IMULT If IMULT=0, and the values of force constants RK2 and RK3 are changing with step number, then the changes in the force constants will be linearly interpolated from rk2→rk2a and rk3→rk3a as the step number changes.

If IMULT=1 and the force constants are changing with step number, then the changes in the force constants will be effected by a series of multiplicative scalings, using a single factor, R, for all scalings. *i.e.*

rk2a = R**INCREMENTS * rk2
rk3a = R**INCREMENTS * rk3.

INCREMENTS is the number of times the target value changes, which is determined by NSTEP1, NSTEP2, and NINC.

Default for IMULT is the value assigned to IMULT in the most recent namelist where IMULT was specified. If IMULT has not been specified in any namelist, it defaults to 0.

R1→R4

RK2,RK3

R1A→R4A

RK2A,RK3A If IALTD=0, the restraint is a well with a square bottom with parabolic sides out to a defined distance, and then linear sides beyond that. Force constants are in units of kcal/mol. If R is the value of the restraint in question:

R < r1	Linear, with the slope of the "left-hand" parabola at the point R=r1.
r1 <= R < r2	Parabolic, with restraint energy $k_2(R - r_2)^2$.
r2 <= R < r3	E = 0.
r3 <= R < r4	Parabolic, with restraint energy $k_3(R - r_3)^2$.
r4 <= R	Linear, with the slope of the "right-hand" parabola at the point R=r4.

For torsional restraints, the value of the torsion is translated by +-n*360, if necessary, so that it falls closest to the mean of r2 and r3.

Specified distances are in Angstroms. Specified angles are in degrees. Force constants for distances are in kcal/mol-Å^2 Force constants for angles are in kcal/mol-rad^2. (Note that angle positions are specified in degrees, but force constants are in radians, consistent with typical reporting procedures in the

literature).

If IALTD=1, distance restraints are interpreted in a slightly different fashion. Again, If R is the value of the restraint in question:

R < r2 Parabolic, with restraint energy $k2(R-r2)^2$.

r2 <= R < r3 E = 0.

r3 <= R < r4 Parabolic, with restraint energy $k3(R-r3)^2$.

r4 <= R Hyperbolic, with energy $k_3[b/(R-r_3)+a]$, where $a=3(r_4-r_3)^2$ and $b=-2(r_4-r_3)^3$. This function matches smoothly to the parabola at $R=r_3$, and tends to an asymptote of ak_3 are large R. The functional form is adapted from that suggested by Michael Nilges, *Prot. Eng.* **2,** 27-38 (1988). Note that if *ialtd=1*, the value of r1 is ignored.

IFVARI = 0 The values of r1→r4, rk2, and rk3 will remain constant throughout the run.

IFVARI > 0 The values r1a, r2a, r3a, r4a, r2ka and r3ka are also used. These variables are defined as for r1→r4 and rk2, rk3, but correspond to the values appropriate for NSTEP = NSTEP2: e.g., if IVARI >0, then the value of r1 will vary between NSTEP1 and NSTEP2, so that, e.g. r1(NSTEP1) = r1 and r1(NSTEP2) = r1a. Note that you *must* specify an explicit value for *nstep1* and *nstep2* if you use this option.

Defaults for r1→r4,rk2,rk3,r1a→r4a,rk2a and rk3a are the values assigned to them in the most recent namelist where they were specified. They should always be specified in the first `&rst` *namelist.*

(IGR1(i),i=1→200)

If IAT(1) < 0 and IAT(3)=IAT(4)=0, then IGR1() gives the atoms defining the group whose coordinate averaged position is used to define "atom 1" in a distance restraint. If IRESID = 0, absolute atom numbers are specified by the elements of IGR1(). If IRESID = 1, then IGR1(I) specifies the number of the residue containing atom I, and the name of atom I must be specified using GRNAM1(I). A maximum of 200 atoms are allowed in any group. Only specify those atoms which are needed.

RJCOEF(1)→RJCOEF(3)

By default, 4-atom sequences specify torsional restraints. It is also possible to impose restraints on the vicinal ^{3}J-coupling value related to the underlying torsion. J is related to the torsion τ by the approximate Karplus relationship: $J=A\cos^2(\tau)+B\cos(\tau)+C$. If you specify a non-zero value for either RJCOEF(1) or RJCOEF(2), then a J-coupling restraint, rather than a torsional restraint, will be imposed. At every MD step, J will be calculated from the Karplus relationship with A = RJCOEF(1), B = RJCOEF(2) and C = RJCOEF(3). In this case, the target values (R1->R4, R1A->R4A) and force constants (RK2, RK3, RK2A, RK3A) refer to J-values for this restraint. RJCOEF(1)->RJCOEF(3) must be set individually for each torsion for which you wish to apply a J-coupling restraint, and RJCOEF(1)->RJCOEF(3) may be different for each J-coupling restraint.

With respect to other options and reporting, J-coupling restraints are treated identically to torsional restraints. This means that if time-averaging is requested for torsional restraints, it will apply to J-coupling restraints as well. The J-coupling restraint contribution to the energy is included in the "torsional" total. And changes in the relative weights of the torsional force constants also change the relative weights of the J-coupling restraint terms.

Setting RJCOEF has no effect for distance and angle restraints.

Defaults for RJCOEF(1)->RJCOEF(3) are 0.0.

(IGR2(i),i=1→200)

If IAT(2) < 0 and IAT(3)=IAT(4)=0, then IGR1 gives the atoms defining the group whose coordinate averaged position is used to define "atom 2" in a distance restraint. If IRESID = 0, absolute atom numbers are specified by the elements of IGR2(). If IRESID = 1, then IGR2(I) specifies the number of the residue containing atom I, and the name of atom I must be specified using GRNAM1(I). A maximum of 200 atoms are allowed in any group. Only specify those atoms which are needed.

Default value for any unspecified element of IGR1 or IGR2 is 0.

(GRNAM1(i),i=1→200)

(GRNAM2(i),i=1→200)

If group input is being specified (IAT(1) or IAT(2) < 0 and IAT(3)=IAT(4)=0), *and* IRESID = 1, then the character names of the atoms defining the group are contained in GRNAM1(i) or GRNAM2(i)), as described above. In the case IAT(1) < 0, each residue IGR1(i) is searched for an atom name GRNAM1(i) and added to the first group list. In the case IAT(2) < 0, each residue IGR2(i) is searched for an atom name GRNAM2(i) and added to the second group list.

Defaults for GRNAM1(i) and GRNAM2(i) are ' '.

IR6 If a group coordinate-averaged position is being used (see IGR1 and IGR2 above), the average position can be calculated in either of two manners: If IR6 = 0, center-of-mass averaging will be used. If IR6=1, the $< r^{-6} >^{-1/6}$ average of all interaction distances to atoms of the group will be used.

Default for IR6 is the value assigned to IR6 in the most recent namelist where IR6 was specified. If IR6 has not been specified in any namelist, it defaults to 0.

IFNTYP If time-averaged restraints have been requested (see DISAVE/ANGAVE/TORAVE above), they are, by default, applied to all restraints of the class specified. Time-averaging can be overridden for specific internals of that class by setting IFNTYP for that internal to 1. IFNTYP has no effect if time-averaged restraint are not being used.

Default value is IFNTYP=0.

IXPK

NXPK These are user-defined integers than can be set for each constraint. They are typically the "peak number" and "spectrum number" associated with the cross-peak that led to this particular distance restraint. Nothing is ever done with them except to print them out in the "violation summaries", so that NMR people can more easily go from a constraint violation to the corresponding

peak in their spectral database. Default values are zero.

ICONSTR If *iconstr* > 0, (default is 0) a Lagrangian multiplier is also applied to the two-center internal coordinate defined by IAT(1) and IAT(2). The effect of this Lagrangian multiplier is to maintain the initial orientation of the internal coordinate. The rotation of the vector IAT(1)->IAT(2) is prohibited, though translation is allowed. For each defined two-center internal coordinate, a separate Lagrangian multiplier is used. Therefore, although one can use as many multipliers as needed, defining centers should NOT appear in more than one multiplier. This option is compatible with mass centers (i.e., negative IAT(1) or IAT(2)). ICONSTR can be used together with harmonic restraints. RK2 and RK3 should be set to 0.0 if the two-center internal coordinate is a simple Lagrangian multiplier. An example has been included in $AMBERHOME/example/lagmul.

Namelist `&rst` is read for each restraint. Restraint input ends when a namelist statement with iat(1) = 0 (or iat(1) not specified) is found. Note that comments can precede or follow any namelist statement, allowing comments and restraint definitions to be freely mixed.

6.12.2. NOESY volume restraints.

After the previous section, NOESY volume restraints may be read. This data described in this section is only read if NMROPT = 2. The molecule may be broken in overlapping submolecules, in order to reduce time and space requirements. Input *for each submolecule* consists of namelist "`&noeexp`", followed *immediately* by standard Amber "group" cards defining the atoms in the submolecule. In addition to the submolecule input ("`&noeexp`"), you may also need to specify some additional variables in the `cntrl` namelist; see the "NMR variables" description in that section.

In many cases, the user will not prepare this section of the input by hand, but will use the auxiliary program *makeDIST_RST* to prepare input from simpler files.

Variables in the `&noeexp` namelist:

For each submolecule, the namelist "`&noeexp`" is read (either from *stdin* or from the NOESY redirection file) which contains the following variables. There are no effective defaults for *npeak, emix, ihp, jhp,* and *aexp*: you must specify these.

NPEAK*(imix)* Number of peaks for each of the "imix" mixing times; if the last mixing time is *mxmix*, set NPEAK(*mxmix+1*) = -1. End the input when NPEAK(1) < 0.

EMIX*(imix)* Mixing times (in seconds) for each mixing time.

IHP*(imix,ipeak)*

JHP*(imix,ipeak)* Atom numbers for the atoms involved in cross-peak "ipeak" at mixing time "imix"

AEXP(imix,ipeak) Experimental target integrated intensity for this cross peak. If AEXP is negative, this cross peak is part of a set of overlapped peaks. The computed intensity is added to the peak that follows; the next time a peak with AEXP > 0 is encountered, the running sum for the calculated peaks will be compared to the value of AEXP for that last peak in the list. In other words, a set of

overlapped peaks is represented by one or more peaks with AEXP < 0 followed by a peak with AEXP > 0. The computed total intensity for these peaks will be compared to the value of AEXP for the final peak.

ARANGE*(imix,ipeak)*
"Uncertainty" range for this peak: if the calculated value is within ±ARANGE of AEXP, then no penalty will be assessed. Default uncertainties are all zero.

AWT*(imix,ipeak)* Relative weight for this cross peak. Note that this will be multiplied by the overall weight given by the NOESY weight change cards in the weight changes section (Section 1). Default values are 1.0, unless INVWT1,INVWT2 are set (see below), in which case the input values of AWT are ignored.

INVWT1,INVWT2
Lower and upper bounds on the weights for the peaks respectively, such that the relative weight for each peak is 1/intensity if 1/intensity lies between the lower and upper bounds. This is the intensity after being scaled by *oscale*. The inverse weighing scheme adopted by this option prevents placing too much influence on the strong peaks at the expense of weaker peaks and was previously invoked using the compilation flag "INVWGT". Default values are INVWT1=INVWT2=1.0, placing equal weights on all peaks.

OMEGA Spectrometer frequency, in Mhz. Default is 500. It is possible for different sub-molecules to have different frequencies, but omega will only change when it is explicitly re-set. Hence, if all of your data is at 600 Mhz, you need only set *omega* to 600. in the first submolecule.

TAUROT Rotational tumbling time of the molecule, in nsec. Default is 1.0 nsec. Like *omega*, this value is "sticky", so that a value set in one submolecule will remain until it is explicitly reset.

TAUMET Correlation time for methyl jump motion, in ns. This is only used in computing the intra-methyl contribution to the rate matrix. The ideas of Woessner are used, specifically as recommended by Kalk & Berendsen [152]. Default is 0.0001 ns, which is effectively the fast motion limit. The default is consistent with the way the rest of the rate matrix elements are determined (also in the fast motion limit,) but probably is not the best value to use, since methyl groups appear to have T1 values that are systematically shorter than other protons, and this is likely to arise from the fact that the methyl correlation time can be near to the inverse of the spectrometer frequency. A value of 0.02 - 0.05 ns is probably better than 0.0001, but this is still an active research area, and you are on your own here, and should consult the literature for further discussion [153]. As with *omega*, *taumet* can be different for different submolecules, but will only change when it is explicitly re-set.

ID2O Flag for determining if exchangeable protons are to be included in the spin-diffusion calculation. If ID2O=0 (default) then all protons are included. If ID2O=1, then all protons bonded to nitrogen or oxygen are assumed to not be present for the purposes of computing the relaxation matrix. No other options exist at present, but they could easily be added to the subroutine *indexn*. Alternatively, you can manually rename hydrogens in the *prmtop* file so that they do not begin with "H": such protons will not be included in the relaxation matrix. (*Note:* for technical reasons, the HOH proton of tyrosine must

always be present, so setting ID2O=1 will not remove it; we hope that this limitation will be of minor importance to most users.) The *id2o* variable retains its value across namelist reads, *i.e.* its value will only change if it is explicitly reset.

OSCALE — overall scaling factor between experimental and computed volume units. The experimental intensities are multiplied by *oscale* before being compared to calculated intensities. This means that the weights WNOESY and AWT always refer to "theoretical" intensity scales rather than to the (arbitrary) experimental units. The *oscale* variable retains its value across namelist reads, *i.e.* its value will only change if it is explicitly reset. The initial (default) value is 1.0.

The atom numbers *ihp* and *jhp* are the absolute atom numbers. For methyl groups, use the number of the last proton of the group; for the delta and epsilon protons of aromatic rings, use the delta-2 or epsilon-2 atom numbers. Since this input requires you to know the absolute atom numbers assigned by Amber to each of the protons, you may wish to use the separate *makeDIST_RST* program which provides a facility for more turning human-readable input into the required file for *sander*.

Following the `&noeexp` namelist, give the Amber "group" cards that identify this submolecule. This combination of "`&noeexp`" and "group" cards can be repeated as often as needed for many submolecules, subject to the limits described in the *nmr.h* file. As mentioned above, this input section ends when NPEAK(1) < 0, or when and end-of-file is reached.

6.12.3. Chemical shift restraints.

After reading NOESY restraints above (if any), read the chemical shift restraints in namelist *&shf*, or the pseudocontact restraints in namelist *&pcshift*. Reading this input is triggered by the presence of a SHIFTS or PCSHIFT line in the I/O redirection section. In many cases, the user will not prepare this section of the input by hand, but will use the auxiliary programs *makeSHF* or *fantasian* to prepare input from simpler files.

Variables in the `&shf` namelist. (Defaults are only available for *shrang*, *wt*, *nter*, and *shcut*; you must specify the rest.)

NRING — Number of rings in the system.

NATR*(i)* — Number of atoms in the *i-th* ring.

IATR*(j,i)* — Absolute atom number for the *j-th* atom of the *i-th* ring.

NAMR*(i)* — Eight-character string that labels the *i-th* ring. The first three characters give the residue name (in caps); the next three characters contain the residue number (right justified); column 7 is blank; column 8 may optionally contain an extra letter to distinguish the two rings of trp, or the 5 or 8 rings of the heme group.

STR*(i)* — Ring current intensity factor for the *i-th* ring. Older values are summarized by Cross and Wright [154]; more recent empirical parametrizations seem to give improved results [155,156].

NPROT — Number of protons for which penalty functions are to be set up.

IPROT*(i)* — Absolute atom number of the *i-th* proton whose shifts are to be evaluated. For equivalent protons, such as methyl groups or rapidly flipping phenylalanine rings, enter all two or three atom numbers in sequence; averaging will be controlled by the *wt* parameter, described below.

OBS*(i)* — Observed secondary shift for the *i-th* proton. This is typically calculated as the observed value minus a random coil reference value.

SHRANG*(i)* — "Uncertainty" range for the observed shift: if the calculated shift is within ±SHRANG of the observed shift, then no penalty will be imposed. The default value is zero for all shifts.

WT*(i)* — Weight to be assigned to this penalty function. Note that this value will be multiplied by the overall weight (if any) given by the SHIFTS command in the assignment of weights (above). Default values are 1.0. For sets of equivalent protons, give a negative weight for all but the last proton in the group; the last proton gets a normal, positive value. The average computed shift of the group will be compared to *obs* entered for the last proton.

SHCUT — Values of calculated shifts will be printed only if the absolute error between calculated and observed shifts is greater than this value. *Default = 0.3 ppm.*

NTER — Residue number of the N-terminus, for protein shift calculations; *default = 1.*

CTER — Residue number of the C-terminus, for protein shift calculations. Believe it or not, the current code cannot figure this out for itself.

The PCSHIFT module allows the inclusion of pseudocontact shifts as constraints in energy minimization and molecular dynamics calculations on paramagnetic molecules. The pseudocontact shift depends on the magnetic susceptibility anisotropy of the metal ion and on the location of the resonating nucleus with respect to the axes of the magnetic susceptibility tensor. For the nucleus i, it is given by:

$$\delta^i_{pc} = \sum_j \frac{1}{12\pi r^3_{ij}} \left[\Delta\chi^j_{ax}(3n^2_{ij} - 1) + (3/2)\Delta\chi^j_{rh}(l^2_{ij} - m^2_{ij}) \right] \qquad (6.34)$$

where l_{ij}, m_{ij}, and n_{ij} are the direction cosines of the position vector of atom i with respect to the j-th magnetic susceptibility tensor coordinate system, r_{ij} is the distance between the j-th paramagnetic center and the proton i, *jax* and *jrh* are the axial and the equatorial anisotropies of the magnetic susceptibility tensor of the j-th paramagnetic center. For a discussion, see Ref. [157].

The PCSHIFT module to be used needs a namelist file which includes information on the magnetic susceptibility tensor and on the paramagnetic center, and a line of information for each nucleus. This module allows to include more than one paramagnetic center in the calculations. To include pseudocontact shifts as constraints in energy minimization and molecular dynamics calculations the NMROPT flag should be set to 2, and a *PCSHIFT=filename* statement entered in the I/O redirection section.

To perform molecular dynamics calculations it is necessary to eliminate the rotational and translational degree of freedom about the center of mass (this because during molecular dynamics

calculations the relative orientation between the external reference coordinate system and the magnetic anisotropy tensor coordinate system has to be fixed).This option can be obtained with the NSCM flag of *sander*.

Variables in the `pcshift` namelist.

NPROT	number of pseudocontact shift constraints.
NME	number of paramagnetic centers.
NMPMC	name of the paramagnetic atom
OPTPHI(n)	
OPTTET(n)	
OPTOMG(n)	
OPTA1(n)	
OPTA2(n)	the five parameters of the magnetic anisotropy tensor for each paramagnetic center.
OPTKON	force constant for the pseudocontact shift constraints

Following this, there is a line for each nucleus for which the pseudocontact shift information is given has to be added. Each line contains :

IPROT(i)	atom number of the i-th proton whose shift is to be used as constraint.
OBS(i)	observed pseudocontact shift value, in ppm
WT(i)	relative weight
TOLPRO(i)	relative tolerance ix mltpro
MLTPRO(i)	multiplicity of the NMR signal (for example the protons of a methyl group have mltprot(i)=3)

Example. Here is a &pcshf namelist example: a molecule with three paramagnetic centers and 205 pseudocontact shift constraints.

```
&pcshf
nprot=205,
nme=3,
nmpcm='FE ',
optphi(1)=-0.315416,
opttet(1)=0.407499,
optomg(1)=0.0251676,
opta1(1)=-71.233,
opta2(1)=1214.511,
optphi(2)=0.567127,
opttet(2)=-0.750526,
optomg(2)=0.355576,
opta1(2)=-60.390,
opta2(2)=377.459,
optphi(3)=0.451203,
opttet(3)=-0.0113097,
optomg(3)=0.334824,
```

```
 opta1(3)=-8.657,
 opta2(3)=704.786,
 optkon=30,
 iprot(1)=26, obs(1)=1.140, wt(1)=1.000, tolpro(1)=1.00, mltpro(1)=1,
 iprot(2)=28, obs(2)=2.740, wt(2)=1.000, tolpro(2)=.500, mltpro(2)=1,
 iprot(3)=30, obs(3)=1.170, wt(3)=1.000, tolpro(3)=.500, mltpro(3)=1,
 iprot(4)=32, obs(4)=1.060, wt(4)=1.000, tolpro(4)=.500, mltpro(4)=3,
 iprot(5)=33, obs(5)=1.060, wt(5)=1.000, tolpro(5)=.500, mltpro(5)=3,
 iprot(6)=34, obs(6)=1.060, wt(6)=1.000, tolpro(6)=.500, mltpro(6)=3,
 ...
 ...
 iprot(205)=1215, obs(205)=.730, wt(205)=1.000, tolpro(205)=.500,
     mltpro(205)=1,
 /
```

An `mdin` file that might go along with this, to perform a maximum of 5000 minimization cycles, starting with 500 cycles of steepest descent. PCSHIFT=./pcs.in redirects the input from the namelist "pcs.in" which contains the pseudocontact shift information.

```
Example of minimization including pseudocontact shift constraints
 &cntrl
 ibelly=0,imin=1,ntpr=100,
 ntwx=100,ntwe=100,ioutfm=0,ntr=0,maxcyc=500,
 ncyc=50,ntmin=1,dx0=0.0001,
 drms=.1,cut=10.,scee=2.0,
 nmropt=2,pencut=0.1, ipnlty=2,
 /
 &wt type='REST', istep1=0,istep2=1,value1=0.,
     value2=1.0,  /
 &wt type='END'  /
DISANG=./noe.in
PCSHIFT=./pcs.in
LISTOUT=POUT
```

6.12.4. Direct dipolar coupling restraints

Energy restraints based on direct dipolar coupling constants are entered in this section. All variables are in the namelist `&align`; reading of this section is triggered by the presence of a DIPOLE line in the I/O redirection section.

When dipolar coupling restraints are turned on, the five unique elements of the alignment tensor are treated as additional variables, and are optimized along with the structural parameters. Their effective masses are determined by the *scalm* parameter entered in the &cntrl namelist. Unlike some other programs, the variables used are the Cartesian components of the alignment tensor in the axis system defined by the molecule itself: *e.g.* $S_{mn} \equiv 10^5 < (3\cos\theta_m \cos\theta_n - \delta_{mn})/2 >$, where θ_x is the angle between the x axis and the spectrometer field [158]. The factor of 10^5 is just to make the values commensurate with atomic coordinates, since both the coordinates and the alignment tensor values will be updated during the

refinement. The calculated dipolar splitting is then

$$D_{calc} = -\left(\frac{10^{-5}\gamma_i\gamma_j h}{2\pi^2 r_{ij}^3}\right)\sum_{m,n=x,y,z}\cos\phi_m \cdot S_{mn} \cdot \cos\phi_n \qquad (6.35)$$

where ϕ_x is the angle between the internuclear vector and the *x* axis. Geometrically, the splitting is proportional to the transformation of the alignment tensor onto the internuclear axis. This is just Eqs. (5) and (13) of the above reference, with any internal motion corrections (which might be a part of S_{system}) set to unity. If there is an internal motion correction which is the same for all observations, this can be assimilated into the alignment tensor. The current code does not allow for variable corrections for internal motion, but this is coming. See ref. [159] for a fuller discussion of these issues.

At the end of the calculation, the alignment tensor is diagonalized to obtain information about its principal components. This allows the alignment tensor to be written in terms of the "axial" and "rhombic" components that are often used to describe alignment.

Variables in the `&align` namelist.

NDIP — Number of observed dipolar coupling restraints to be used as restraints.

ID,JD — Atom numbers of the two atoms involved in the dipolar coupling.

DOBSL, DOBSU — Limiting values for the observed dipolar splitting, in Hz. If the calculated coupling is less than *dobsl*, the energy penalty is proportional to $(D_{calc} - D_{obs,l})^2$; if it is larger than *dobsu*, the penalty is proportional to $(D_{calc} - D_{obs,u})^2$. Calculated values between *dobsl* and *dobsu* are not penalized. Note that *dobsl* must be less than *dobsu*; for example, if the observed coupling is -6 Hz, and a 1 Hz "buffer" is desired, you could set *dobsl* to -7 and *dobsu* to -5.

DWT — The relative weight of each observed value. Default is 1.0. The penalty function is thus:

$$E^i_{align} = D^i_{wt}(D^i_{calc} - D^i_{obs(u,l)})^2 \qquad (6.36)$$

where D_{wt} may vary from one observed value to the next. Note that the default value is arbitrary, and a smaller value may be required to avoid overfitting the dipolar coupling data [159].

DATASET — Each dipolar peak can be associated with a "dataset", and a separate alignment tensor will be computed for each dataset. This is generally used if there are several sets of experiments, each with a different sample or temperature, etc., that would imply a different value for the alignment tensor. By default, there is one dataset to which each observed value is assigned.

NUM_DATASETS — The number of datasets in the constraint list. Default is 1.

S11,S12,S13,S22,S23 — Initial values for the Cartesian components of the alignment tensor. The tensor is traceless, so S33 is calculated as −(S11+S22). In order to have the order of magnitude of the S values be roughly commensurate with coordinates in Angstroms, the alignment tensor values must be multiplied by 10^5.

GIGJ — Product of the nuclear "g" factors for this dipolar coupling restraint. These are related to the nuclear gyromagnetic rations by $\gamma_N = g_N\beta_N/\hbar$. Common values are ^{1}H = 5.5856, ^{13}C = 1.4048, ^{15}N = -0.5663, ^{31}P = 2.2632.

DIJ — The internuclear distance for observed dipolar coupling. If a non-zero value is given, the distance is considered to be fixed at the given value. If a *dij* value is zero, its value is computed from the structure, and it is assumed to be a variable distance. For one-bond couplings, it is usually best to treat the bond distance as "fixed" to an effective zero-point vibration value [160].

DCUT — Controls printing of calculated and observed dipolar couplings. Only values where abs(dobs(u,l) - dexp) is greater than dcut will be printed. Default is 0.1 Hz. Set to a negative value to print all dipolar restraint information.

FREEZEMOL — If this is set to *.true.*, the molecular coordinates are not allowed to vary during dynamics or minimization: only the elements of the alignment tensor will change. This is useful to fit just an alignment tensor to a given structure. Default is *.false.*.

6.12.5. Preparing restraint files for Sander

Figure 1 shows the general information flow for auxiliary programs that help prepare the restraint files. Once the restraint files are made, Figure 2 shows a flow-chart of the general way in which *sander* refinements are carried out.

The basic ideas of this scheme owe a lot to the general experience of the NMR community over the past decade. Several papers outline procedures in the Scripps group, from which a lot of the NMR parts of *sander* are derived [151,161-166]. They are by no means the only way to proceed. We hope that the flexibility incorporated into *sander* will encourage folks to experiment with refinement protocols.

6.12.6. Preparing distance restraints: makeDIST_RST.

The *makeDIST_RST* program converts a simplified description of distance bounds into a detailed input for *sander*. A variety of input and output filenames may be specified on the command line:

```
input:
   -upb <filename>          7-col file of upper distance bounds, OR
   -ual <filename>          8-col file of upper and lower bounds, OR
   -vol <filename>          7-col file of NOESY volumes

   -pdb <filename>          Brookhaven format file
   -map <filename>          MAP file  (default:map.DG-AMBER)
   -les <filename>          LES atom mappings, made by addles

output:
   -dgm <filename>          DGEOM95 restraint format
   -rst <filename>          SANDER restraint format
   -svf <filename>          Sander Volume Format, for NOESY refinement

other options:
   -help       (gives you this explanation, overrides other parameters)
   -report     (gives you short runtime diagnostic output)
   -nocorr     (do not correct upper bound for r**-6 averaging)
```

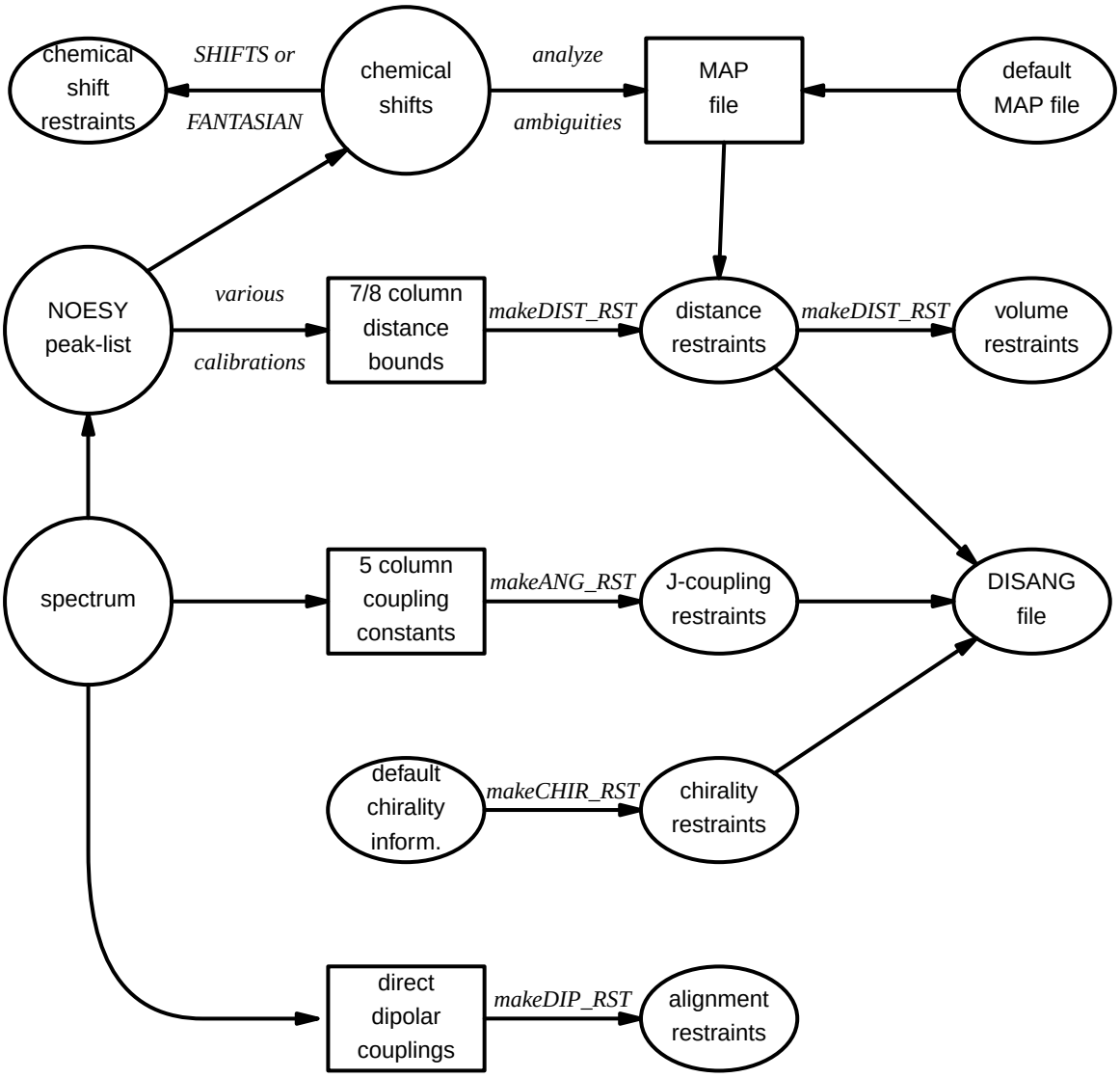


Fig. 1. Notation: *circles* represent logical information, whose format might differ from one project to the next; *solid rectangles* are in a specific format (largely compatible with DIANA and other programs), and are intended to be read and edited by the user; *ellipses* are specific to *sander*, and are generally not intended to be read or edited manually. The conversion of NOESY volumes to distance bounds can be carried out by a variety of programs such as *mardigras* or *xpk2bound* that are not included with Amber. Similarly, the analysis and partial assignment of ambiguous or overlapped peaks is a separate task; at TSRI, these are typically carried out using the programs *xpkasgn* and *filter.pl*.

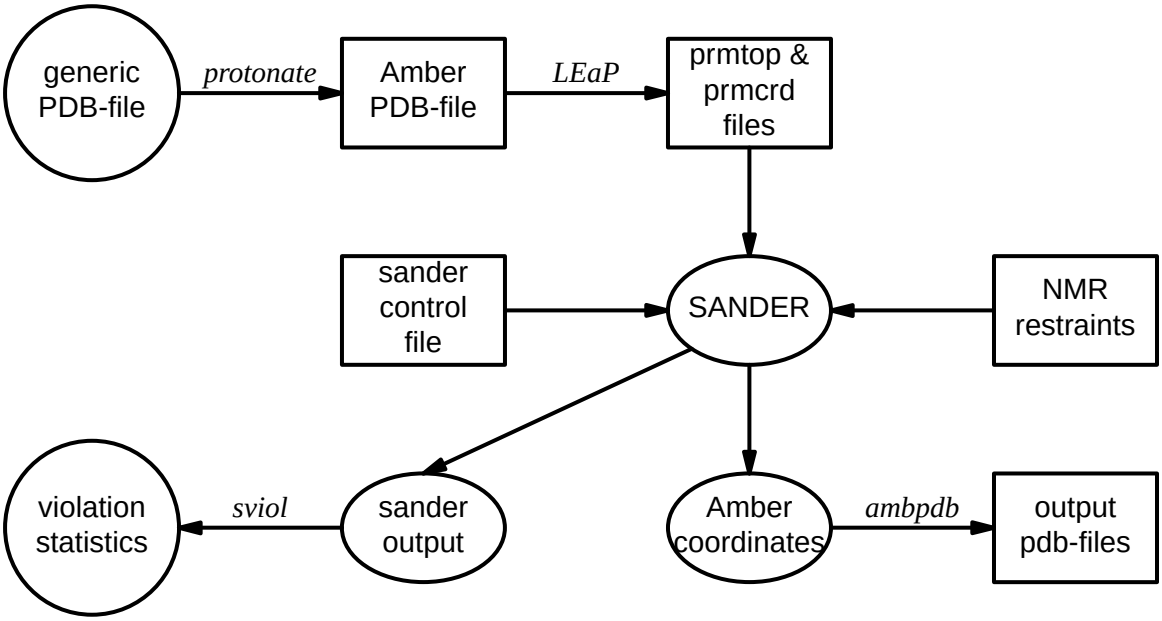


Fig. 2

`-altdis` *(use alternative form for the distance restraints)*

The *7/8 column distance bound* file is essentially that used by the DIANA or DISGEO programs. It consists of one-line per restraint, which would typically look like the following:

```
23   ALA   HA      52  VAL  H    3.8  # comments go here
```

The first three columns identify the first proton, the next three the second proton, and the seventh column gives the upper bound. Only the first three letters of the residue name are used, so that DIANA files that contain residues like "ASP−" will be correctly interpreted. An alternate, 8-column, format has both upper and lower bounds as the seventh and eighth columns, respectively. A typical line might in an "8-col" file might look like this:

```
23   ALA   HA      52  VAL  H   3.2   3.8  # comments go here
```

Here the lower bound is 3.2 Å and the upper bound is 3.8 Å. Comments typically identify the spectrum and peak-number or other identification that allow cross-referencing back to the appropriate spectrum. If the comment contains the pattern "<integer>:<integer>", then the first integer is treated as a peak-identifier, and the second as a spectrum-identifier. These identifiers go into the *ixpk* and *nxpk* variables, and will later be printed out in *sander*, to facilitate going back to the original spectra to track down violations, etc.

The format for the *-vol* option is the same as for the *-upb* option except that the seventh column holds a peak intensity (volume) value, rather than a distance upper bound.

The input pdb file must exactly match the Amber *prmtop* file that will be used; use the `ambpdb -aatm` command to create this.

If all peaks involved just single protons, and were fully assigned, this is all that one would need. In general, though, some peaks (especially methyl groups or fast-rotating aromatic rings) represent contributions from more than one proton, and many other peaks may not be fully assigned. *Sander* handles both of these situations in the same way, through the notion of an "ambiguous" peak, that may correspond to several assignments. These peaks are given two types of special names in the 7/8-column format file:

(1) Commonly-occurring ambiguities, like the lack of stereospecific assignments to two methylene protons, are given names defined in the default MAP file. These names, also more-or-less consistent with DIANA, are like the names of "pseudo-atoms" that have long been used to identify such partially assigned peaks, e.g. "QB" refers to the (HB2,HB3) combination in most residues, and "MG1" in valine refers collectively to the three methyl protons at position CG1, etc.

(2) There are generally also molecule-specific ambiguities, arising from potential overlap in a NOESY spectrum. Here, the user assigns a unique name to each such ambiguity or overlap, and prepares a list of the potential assignments. The names are arbitrary, but might be constructed, for example, from the chemical shifts that identify the peak, e.g. "p_2.52" might identify the set of protons that could contribute to a peak at 2.52 ppm. The chemical shift list can be used to prepare a list of potential assignments, and these lists can often be pruned by comparison to approximate or initial structures.

The default and molecule-specific MAP files are combined into a single file, which is used, along with the 7-column restraint file, the the program *makeDIST_RST* to construct the actual *sander* input files. You should consult the help file for makeDIST_RST for more information. For example, here are some lines added to the MAP file for a recent TSRI refinement:

```
AMBIG n2:68  =  HE 86 HZ 86
AMBIG n2:72  =  HE 24 HD 24 HZ 24
AMBIG n2:73  =  HN 81 HZ 13 HE 13 HD 13 HZ 24
AMBIG n2:78  =  HN 76 HZ 13 HE 13 HZ 24
AMBIG n2:83  =  HN 96 HN 97 HD 97 HD 91
AMBIG n2:86  =  HD1 66 HZ2 66
AMBIG n2:87  =  HN 71 HH2 66 HZ3 66 HD1 66
```

Here the spectrum name and peak number were used to construct a label for each ambiguous peak. Then, an entry in the restraint file might look like this:

```
123 GLY HN  0   AMB n2:68   5.5
```

indicating a 5.5 Å upper bound between the amide proton of Gly 123 and a second proton, which might be either the HE or HZ protons of residue 86. (The "zero" residue number just serves as a placeholder, so that there will be the same number of columns as for non-ambiguous restraints.) If it is possible that the ambiguous list might not be exhaustive (e.g. if some protons have not been assigned), it is safest to set *ialtd*=1, which will allow "mistakes" to be present in the constraint list. On the other hand, if you want to be sure that every violation is "active", set *ialtd*=0.

If the *-les* flag is set, the program will prepare distance restraints for multiple copies (LES) simulations. In this case, the input pdb file is one *without* LES copies, i.e. with just a single copy of the molecule. The "lesfile" specified by this flag is created by the *addles* program, and contains a mapping from original atom numbers into the copy numbers used in the multiple-copies simulation.

The *-rst* and *-svf* flags specify outputs for *sander*, for distance restraints and NOESY restraints, respectively. In each case, you may need to hand-edit the outputs to add additional parameters. You should make it a habit to compare the outputs with the descriptions given earlier in this chapter to make sure that the restraints are what you want them to be.

It is common to run makeDIST_RST several times, with different inputs that correspond to different spectra, different mixing times, etc. It is then expected that you will manually edit the various ouptut files to combine them into the single file required by *sander*.

6.12.7. Preparing torsion angle restraints: makeANG_RST

There are fewer "standards" for representing coupling constant information. We have followed the DIANA convention in the program *makeANG_RST*. This program takes as input a five-column torsion angle constraint file along with an Amber pdb file of the molecule. It creates as output (to standard out) a list of constraints in RST format that is readable by Amber.

```
Usage: makeANG_RST -help
       makeANG_RST -pdb ambpdb_file [-con constraint] [-lib libfile]
                [-les lesfile ]
```

The input torsion angle constraint file can be read from standard in or from a file specified by the -con option on the command line. The input constraint file should look something like this:

```
1   GUA   PPA     111.5   144.0
2   CYT   EPSILN   20.9   100.0
2   CYT   PPA     115.9   134.2
3   THY   ALPHA    20.4   35.6
4   ADE   GAMMA    54.7   78.8
5   GLY   PHI      30.5   60.3
6   ALA   CHI      20.0   50.0
   ....
```

Lines beginning with "#" are ignored. The first column is the residue number; the second is the residue name (three letter code, or as defined in your personal torsion library file). Only the first three letters of the residue name are used, so that DIANA files that contain residues like "ASP-" will be correctly interpreted. Third is the angle name (taken from the torsion library described below).The fourth column contains the lower bound, and the fifth column specifies the upper bound. Additional material on the line is (presently) ignored.

Note: It is assumed that the lower bound and the upper bound define a region of allowed conformation on the unit circle that is swept out in a clockwise direction from $lb \rightarrow ub$. If the number in the *lb* column is greater than the the number in the *ub* column, 360^o will successively be subtracted from the *lb* until $lb < ub$. This preserves the clockwise definition of the allowed conformation space, while also making the number that specifies the lower bound less than the

number that specifies the upper bound, as is required by Amber. If this occurs, a warning message will be printed to *stderr* to notify the user that the data has been modified.

The angles that one can constrain in this manner are defined in the library file that can be optionally specified on the command line with the -lib flag, or the default library "tordef.lib" (written by Garry P. Gippert) will be used. If you wish to specify your own nomenclature, or add angles that are not already defined in the default file, you should make a copy of this file and modify it to suit your needs. The general format for an entry in the library is:

```
LEU   PSI     N    CA   C    N+
```

where the first column is the residue name, the second column is the angle name that will appear in the input file when specifying this angle, and the last four columns are the atom names that define the torsion angle. When a torsion angle contains atom(s) from a preceding or succeeding residue in the structure, a "-" or "+" is appended to those atom names in the library, thereby specifying that this is the case. In the example above, the atoms that define PSI for LEU residues are the N, CA, and C atoms of that same LEU and the N atom of the residue after that LEU in the primary structure. Note that the order of atoms in the definition is important and should reflect that the torsion angle rotates about the two central atoms as well as the fact that the four atoms are bonded in the order that is specified in the definition.

If the first letter of the second field is "J", this torsion is assumed to be a J-coupling constraint. In that case, three additional floats are read at the end of the line, giving the A,B and C coefficients for the Karplus relation for this torsion. For example:

```
ALA JHNA    H    N    CA   HA   9.5  -1.4  0.3
```

will set up a J-coupling restraint for the HN-HA 3-bond coupling, assuming a Karplus relation with A,B, C as 9.5, -1.4 and 0.3. (These particular values are from Brüschweiler and Case, JACS 116: 11199 (1994).)

This program also supports pseudorotation phase angle constraints for prolines and nucleic acid sugars; each of these will generate restraints for the 5 component angles which correspond to the *lb* and *ub* values of the input pseudorotation constraint. In the torsion library, a pseudorotation definition looks like:

```
PSEUDO        CYT     PPA     NU0     NU1     NU2     NU3     NU4
CYT   NU0     C4'     O4'     C1'     C2'
CYT   NU1     O4'     C1'     C2'     C3'
CYT   NU2     C1'     C2'     C3'     C4'
CYT   NU3     C2'     C3'     C4'     O4'
CYT   NU4     C3'     C4'     O4'     C1'
```

The first line describes that a PSEUDOrotation angle is to be defined for CYT that is called PPA and is made up of the five angles NU0-NU4. Then the definition for NU0-NU4 should also appear in the file in the same format as the example given above for LEU PSI.

PPA stands for Pseudorotation Phase Angle and is the angle that should appear in the input constraint file when using pseudorotation constraints. The program then uses the definition of that PPA angle in the library file to look for the 5 other angles (NU0-NU4 in this case) which it then generates restraints for. PPA for proline residues is included in the standard library as well as for the DNA nucleotides.

If the *-les* flag is set, the program will prepare torsion angle restraints for multiple copies (LES) simulations. In this case, the input pdb file is one *without* LES copies, i.e. with just a single copy of the molecule. The "lesfile" specified by this flag is created by the *addles* program, and contains a mapping from original atom numbers into the copy numbers used in the multiple-copies simulation.

Torsion angle constraints defined here cannot span two different copy sets, i.e., there cannot be some atoms of a particular torsion that are in one multiple copy set, and other atoms from the same torsion that are in other copy sets. It *is* OK to have some atoms with single copies, and others with multiple copies in the same torsion. The program will create as many duplicate torsions as there are copies.

A good alternative to interpreting J-coupling constants in terms of torsion angle restraints is to refine directly against the coupling constants themselves, using an appropriate Karplus relation. See the discussion of the variable `RJCOEF`, above.

6.12.8. Chirality restraints: makeCHIR_RST

```
Usage:  makeCHIR_RST <pdb-file> <output-constraint-file>
```

We also find it useful to add chirality constraints and *trans*-peptide ω constraints (where appropriate) to prevent chirality inversions or peptide bond flips during the high-temperature portions of simulated annealing runs. The program *makeCHIR_RST* will create these constraints. Note that you may have to edit the output of this program to change *trans* peptide constraints to *cis*, as appropriate.

6.12.9. Direct dipolar coupling restraints: makeDIP_RST

For simulations with residual dipolar coupling restraints, the *makeDIP_RST.protein*, *makeDIP_RST.dna* and *makeDIP_RST.diana* are simple codes to prepare the input file. Use *-help* to obtain a more detailed description of the usage. For now, this code only handles backbone NH and CαH data. The header specifying values for various parameters needs to be manually added to the output of *makeDIP_RST*.

Use of residual dipolar coupling restraints is new both for Amber and for the general NMR community. Refinement against these data should be carried out with care, and the optimal values for the force constant, penalty function, and initial guesses for the alignment tensor components are still under investigation. Here are some suggestions from the experiences so far:

(1) Beware of overfitting the dipolar coupling data in the expense of Amber force field energy. These dipolar coupling data are very sensitive to tiny changes in the structure. It is often possible to drastically improve the fitting by making small distortions in the backbone angles. We recommend inclusion of explicit angle restraints to enforce ideal backbone geometry, especially for those residues that have corresponding residual dipolar coupling data.

(2) The initial values for the Cartesian components of the alignment tensor can influence the final structure and alignment if the structure is not fixed (ibelly = 0). For a fixed structure (ibelly = 1), these values do not matter. Therefore, the current "best" strategy is to fit the experimental data to the fixed starting structure, and use the alignment tensor[s] obtained from this fitting as the initial guesses for further refinement.

(3) Amber is capable of simultaneously fitting more than one set of alignment data. This allows the use of individually obtained datasets with different alignment tensors. However, if the different sets of data have equal directions of alignment but different magnitudes, using an overall scaling factor for these data with a single alignment tensor could greatly reduce the number of fitting parameters.

(4) Because the dipolar coupling splittings depend on the square root of the order parameters ($0 \leq S2 \leq 1$), these order parameters describing internal motion of individual residues are often neglected (N. Tjandra and A. Bax, *Science* **278**, 1111-1113, 1997). However, the square root of a small number can still be noticeably smaller than 1, so this may introduce undesirable errors in the calculations.

6.12.10. Getting summaries of NMR violations

If you specify `LISTOUT=POUT` when running *sander*, the output file will contain a lot of detailed information about the remaining restraint violations at the end of the run. When running a family of structures, it can be useful to process these ouput files with *sviol*, which takes a list of *sander* output files on the command line, and sends a summary of energies and violations to STDOUT. If you have more than 20 or so structures to analyze, the output from *sviol* becomes unwieldy. In this case you may also wish to use *sviol2*, which prints out somewhat less detailed information, but which can be used on larger families of structures. The *senergy* script gives a more detailed view of force-field energies from a series of structures. (We thank the TSRI NMR community for helping to put these scripts together, and for providing many useful suggestions.)

6.12.11. Time-averaged restraints.

The model of the previous sections involves the "single-average-structure" idea, and tries to fit all constraints to a single model, with minimal deviations. A generalization of this model treats distance constraints arising from from NOE crosspeaks (for example) as being the average distance determined from a trajectory, rather than as the single distance derived from an average structure. Time-averaged bonds and angles are calculated as

$$\bar{r} \;=\; (1/C)\;\left\{\;\int_0^t e^{(t'-t)/\tau} r(t')^{-i}\,dt'\;\right\}^{-1/i} \qquad (6.37)$$

where

$\bar{r}$ = time-averaged value of the internal coordinate (distance or angle)

t = the current time

τ = the exponential decay constant

$r(t')$ = the value of the internal coordinate at time t'

i = average is over internals to the inverse of i. Usually i = 3 or 6 for NOE distances, and −1 (linear averaging) for angles and torsions.

C = a normalization integral.

Time-averaged torsions are calculated as

$$< \phi > \;=\; \tan^{-1}(< \sin(\phi) > / < \cos(\phi) >) \qquad (6.38)$$

where ϕ is the torsion, and $< \sin(\phi) >$ and $< \cos(\phi) >$ are calculated using the equation above with

$\sin(\phi(t'))$ or $\cos(\phi(t'))$ substituted for r(t').

Forces for time-averaged restraints can be calculated either of two ways. This option is chosen with the DISAVI / ANGAVI / TORAVI commands (Section 1). In the first (the default),

$$\partial E/\partial x \;=\; (\partial E/\partial \bar{r})\,(\partial \bar{r}/\partial r(t))\,(\partial r(t)/\partial x) \tag{6.39}$$

(and analogously for y and z). The forces then correspond to the standard flat-bottomed well functional form, with the instantaneous value of the internal replaced by the time-averaged value. For example, when $r_3 < \bar{r} < r_4$,

$$E \;=\; k_3(\bar{r} - r_3)^2 \tag{6.40}$$

and similarly for other ranges of $\bar{r}$.

When the second option for calculating forces is chosen (IINC = 1 on a DISAVI, ANGAVI or TORAVI card), forces are calculated as

$$\partial E/\partial x \;=\; (\partial E/\partial \bar{r})\,(\partial r(t)/\partial x) \tag{6.41}$$

For example, when $r_3 < \bar{r} < r_4$,

$$\partial E/\partial x \;=\; 2\;k_3\;(\bar{r} - r_3)\,(\partial r(t)/\partial x) \tag{6.42}$$

Integration of this equation does not give Equation (6.40), but rather a non-intuitive expression for the energy (although one that still forces the bond to the target range). The reason that it may sometimes be preferable to use this second option is that the term $\partial \bar{r}/\partial r(t)$, which occurs in the exact expression [Eq. (6.39)], varies as $(\bar{r}/r(t))^{1+i}$. When i=3, this means the forces can be varying with the fourth power the distance, which can possibly lead to very large transient forces and instabilities in the molecular dynamics trajectory. [Note that this will not be the case when linear scaling is performed, i.e. when i=−1, as is generally the case for valence and torsion angles. Thus, for linear scaling, the default (exact) force calculation should be used].

It should be noted that forces calculated using Equation (6.41) are not conservative forces, and would cause the system to gradually heat up, if no velocity rescaling were performed. The temperature coupling algorithm should act to maintain the average temperature near the target value. At any rate, this heating tendency should not be a problem in simulations, such as fitting NMR data, where MD is being used to sample conformational space rather than to extract thermodynamic data.

This section has described the methods of time-averaged restraints. For more discussion, the interested user is urged to consult studies where this method has been used [167-171].

6.12.12. Multiple copies refinement using LES

NMR restraints can be made compatible with the multiple copies (LES) facility; see the following chapter for more information about LES. To use NMR constraints with LES, you need to do two things:

(1) Add a line like "`file wnmr name=(lesnmr) wovr`" to your input to *addles*. The filename (`lesnmr` in this example) may be whatever you wish. This will cause *addles* to output an additional file that is needed at the next step.

(2) Add "`-les lesnmr`" to the command line arguments to *makeDIST_RST*. This will read in the file created by *addles* containing information about the copies. All NMR restraints will then be interpreted as "ambiguous" restraints, so that if any of the copies satisfies the restraint, the penalty goes to zero.

Note that although this scheme has worked well on small peptide test cases, we have yet not used it extensively for larger problems. This should be treated as an experimental option, and users should use caution in applying or interpreting the results.

6.12.13. Some sample input files

The next few pages contain excerpts from some sample NMR refinement files used at TSRI. The first example just sets up a simple (but often effective) simulated annealing run. You may have to adjust the length, temperature maximum, etc. somewhat to fit your problem, but these values work well for many "ordinary" NMR problems.

1. Simulated annealing NMR refinement

```
 15ps simulated annealing protocol
 &cntrl
    nstlim=15000, ntt=1,          (time limit, temp. control)
    scee=1.2,                     (scee must be set - 1-4 scale factor)
    ntpr=500, pencut=0.1,         (control of printout)
    ipnlty=1, nmropt=1,           (NMR penalty function options)
    vlimit=10,                    (prevent bad temp. jumps)
    ntb=0,                        (non-periodic simulation)
 /
 &ewald
    eedmeth=5,                    (use r dielectric)
 /
#
# Simple simulated annealing algorithm:
#
#  from steps      0 to  1000: raise target temperature 10->1200K
#  from steps  1000 to  3000: leave at 1200K
#  from steps  3000 to 15000: re-cool to low temperatures
#
 &wt type='TEMP0', istep1=0,istep2=1000,value1=10.,
            value2=1200.,    /
 &wt type='TEMP0', istep1=1001, istep2=3000, value1=1200.,
            value2=1200.0,     /
 &wt type='TEMP0', istep1=3001, istep2=15000, value1=0.,
            value2=0.0,     /
#
# Strength of temperature coupling:
#   steps      0 to  3000: tight coupling for heating and equilibration
#   steps  3000 to 11000: slow cooling phase
#   steps 11000 to 13000: somewhat faster cooling
#   steps 13000 to 15000: fast cooling, like a minimization
#
 &wt type='TAUTP', istep1=0,istep2=3000,value1=0.2,
            value2=0.2,     /
 &wt type='TAUTP', istep1=3001,istep2=11000,value1=4.0,
            value2=2.0,     /
 &wt type='TAUTP', istep1=11001,istep2=13000,value1=1.0,
            value2=1.0,     /
 &wt type='TAUTP', istep1=13001,istep2=14000,value1=0.5,
            value2=0.5,    /
 &wt type='TAUTP', istep1=14001,istep2=15000,value1=0.05,
            value2=0.05,    /
```

(continued on next page)

1. Simulated annealing NMR refinement *(continued)*

```
#
# "Ramp up" the restraints over the first 3000 steps:
#
 &wt type='REST', istep1=0,istep2=3000,value1=0.1,
            value2=1.0,  /
 &wt type='REST', istep1=3001,istep2=15000,value1=1.0,
            value2=1.0,  /

 &wt type='END'  /
LISTOUT=POUT                    (get restraint violation list)
DISANG=RST.f                    (file containing NMR restraints)
```

The next example just shows some parts of the actual `RST` file that *sander* would read. This file would ordinarily *not* be made or edited by hand; rather, run the programs *makeDIST_RST*, *makeANG_RST* and *makeCHIR_RST*, combining the three outputs together to construct the `RST` file.

2. Part of the RST.f file referred to above

```
# first, some distance constraints prepared by makeDIST_RST:
#     (comment line is input to makeRST, &rst namelist is output)
#
#(   proton 1          proton 2          upper bound)
#---------------------------------------------
#
# 2 ILE HA       3 ALA HN         4.00
#
 &rst iat=  23,  40, r3= 4.00, r4= 4.50,
      r1 = 1.3, r2 = 1.8, rk2=0.0, rk3=32.0, ir6=1, /
#
# 3 ALA HA       4 GLU HN         4.00
#
 &rst iat=  42,  50, r3= 4.00, r4= 4.50,  /
#
# 3 ALA HN       3 ALA MB         5.50
#
 &rst iat=  40,  -1, r3= 6.22, r4= 6.72,
      igr1=   0,   0,   0,   0, igr2=  44,  45,  46,   0,   /
#
# .....etc.....
```

2. Part of the RST.f file referred to above *(continued)*

```
#
#  next, some dihedral angle constraints, from makeANG_RST:
#
 &rst iat= 213, 215, 217, 233, r1=-190.0,
      r2=-160.0, r3= -80.0, r4= -50.0, /

 &rst iat= 233, 235, 237, 249, r1=-190.0,
      r2=-160.0, r3= -80.0, r4= -50.0, /

# .......etc.......
#
#  next, chirality and omega constraints prepared by makeCHIR_RST:
#
#
#  chirality for residue  1  atoms:   CA CG HB2 HB3
 &rst iat= 3 , 8 , 6 , 7 ,
   r1=10., r2=60., r3=80., r4=130., rk2 = 10., rk3=10.,  /
#
#  chirality for residue  1  atoms:   CB SD HG2 HG3
 &rst iat= 5 , 11 , 9 , 10 , /
#
#  chirality for residue  1  atoms:   N C HA CB
 &rst iat= 1 , 18 , 4 , 5 , /
#
#  chirality for residue  2  atoms:   CA CG2 CG1 HB
 &rst iat= 22 , 26 , 30 , 25 , /
#
 ......etc.........

# trans-omega constraint for residue  2
 &rst iat= 22 , 20 , 18 , 3 ,
   r1=155., r2=175., r3=185., r4=205., rk2 = 80., rk3=80.,  /
#
# trans-omega constraint for residue  3
 &rst iat= 41 , 39 , 37 , 22 , /
#
# trans-omega constraint for residue  4
 &rst iat= 51 , 49 , 47 , 41 , /
#
# ......etc.........
#
```

The next example is an input file for volume-based NOE refinement. As with the distance/angle `RST` file shown above, the user would generally not construct this file, but create it from a "7-column" file using the makeDIST_RST program. Hand-editing might be used at the top of the file, to change the correlation times, etc.

3. Sample NOESY intensity input file

```
# A part of a NOESY intensity file:

 &noeexp
  id2o=1,                  (exchangeable protons removed)
  oscale=6.21e-4,          (scale between exp. and calc. intensity units)
  taumet=0.04,             (correlation time for methyl rotation, in ns.)
  taurot=4.2,              (protein tumbling time, in ns.)
  NPEAK = 13*3,            (three peaks, each with 13 mixing times)
  EMIX = 2.0E-02,  3.0E-02,  4.0E-02,  5.0E-02,  6.0E-02,
     8.0E-02,  0.1,  0.126,  0.175,  0.2,  0.25,  0.3,  0.35,
                           (mixing times, in sec.)
  IHP(1,1) = 13*423,  IHP(1,2) = 13*1029,  IHP(1,3) = 13*421,
                           (number of the first proton)
  JHP(1,1) = 78*568,  JHP(1,2) = 65*1057,  JHP(1,3) = 13*421,
                           (number of the second proton)
  AEXP(1,1) = 5.7244,  7.6276,  7.7677,  9.3519,
              10.733,  15.348,  18.601,
              21.314,  26.999,  30.579,
              33.57,  37.23,  40.011,
                           (intensities for the first cross-peak)
  AEXP(1,2) =  8.067,  11.095,  13.127,  18.316,
              22.19,   26.514,  30.748,
              39.438,  44.065,  47.336,
              54.467,  56.06,   60.113,
  AEXP(1,3) =  7.708,  13.019,  15.943,  19.374,
              25.322,  28.118,  35.118,
              40.581,  49.054,  53.083,
              56.297,  59.326,  62.174,
 /
SUBMOL1
RES 27 27 29 29 39 41 57 57 70 70 72 72 82 82     (residues in this submol)
END
END
```

Next, we illustrate the form of the file that holds residual dipolar coupling restraints. Again, this would generally be created from a human-readable input using the program *makeDIP_RST*.

5. Residual dipolar restraints, prepared by makeDIP_RST:

```
&align
  ndip=91, dcut=-1.0, gigj = 37*-3.1631, 54*7.8467,
  s11=3.883, s22=53.922, s12=33.855, s13=-4.508, s23=-0.559,
  id(1)=188,   jd(1)=189,  dobsu(1)=  6.24, dobsl(1)= 6.24,
  id(2)=208,   jd(2)=209,  dobsu(2)= -10.39, dobsl(1)= -10.39,
  id(3)=243,   jd(3)=244,  dobsu(3)= -8.12, dobsl(1)= -8.12,

  ....
  id(91)=1393,   jd(91)=1394,  dobsu(91)= -19.64,  dobsl(91) = -19.64,
/
```

Finally, we show how the detailed input to *sander* could be used to generate a more complicated restraint. Here is where the user would have to understand the details of the `RST` file, since there are no "canned" programs to create this sort of restraint. This illustrates, though, the potential power of the program.

5. A more complicated constraint

```
# 1)   Define two centers of mass. COM1 is defined by
#      {C1 in residue 1; C1 in residue 2; N2 in residue 3; C1 in residue 4}.
#      COM2 is defined by {C4 in residue 1; O4 in residue 1;  N* in residue 1}.
#      (These definitions are effected by the igr1/igr2 and grnam1/grnam2
#      variables; You can use up to 200 atoms to define a center-of-mass
#      group)
#
# 2)   Set up a distance restraint between COM1 and COM2 which goes from a
#      target value of 5.0A to 2.5A, with a force constant of 1.0, over steps 1-5000.
#
# 3)   Set up a distance restraint between COM1 and COM2 which remains fixed
#      at the value of 2.5A as the force slowly constant decreases from
#      1.0 to 0.01 over steps 5001-10000.
#
# 4)   Sets up no distance restraint past step 10000, so that free (unrestrained)
#      dynamics takes place past this step.
#

 &rst iat=-1,-1, nstep1=1,nstep2=5000,
    iresid=1,irstyp=0,ifvari=1,ninc=0,imult=0,ir6=0,ifntyp=0,
    r1=0.00000E+00,r2=5.0000,r3=5.0000,
       r4=99.000,rk2=1.0000,rk3=1.0000,
    r1a=0.00000E+00,r2a=2.5000,r3a=2.5000,
       r4a=99.000,rk2a=1.0000,rk3a=1.0000,
    igr1 = 2,3,4,5,0,
    grnam1(1)='C1',grnam1(2)='C1',grnam1(3)='N2',grnam1(4)='C1',
    igr2 = 1,1,1,0,
    grnam2(1)='C4',grnam2(2)='O4',grnam2(3)='N*',
 /
 &rst iat=-1,-1, nstep1=5001,nstep2=10000,
    iresid=1,irstyp=0,ifvari=1,ninc=0,imult=0,ir6=0,ifntyp=0,
    r1=0.00000E+00,r2=2.5000,r3=2.5000,
       r4=99.000,rk2=1.0000,rk3=1.0000,
    r1a=0.00000E+00,r2a=2.5000,r3a=2.5000,
       r4a=99.000,rk2a=1.0000,rk3a=0.0100,
    igr1 = 2,3,4,5,0,
    grnam1(1)='C1',grnam1(2)='C1',grnam1(3)='N2',grnam1(4)='C1',
    igr2 = 1,1,1,0,
    grnam2(1)='C4',grnam2(2)='O4',grnam2(3)='N*',
 /
```

6.13. Path-Integral Molecular Dynamics

6.13.1. General theory.

Based on Feynman's formulation of quantum statistical mechanics in terms of path-integral, Path-Integral Molecular Dynamics (PIMD) is a computationally efficient method for calculating equilibrium properties (both thermodynamic and structural) of a quantum many-body system that is described by the canonical (NVT) ensemble. In the following we briefly illustrate the main principles of the path-integral approach, and we derive the fundamental equations underlying its implementation using standard molecular dynamics methods. Given the broad area of this field, we strongly recommend the user to consult the literature for a more detailed discussion path-integral methods [172-174].

Within the framework of quantum statistical mechanics we consider an ensemble of systems described by the Hamiltonian H. Using Dirac notation, each system defines a state vector $|\Psi^{(k)}>$ in the Hilbert space, with k=1,...,Z, and Z representing the total number of members in the ensemble. If $\{|\phi_i>\}$ is a complete set of vectors in the Hilbert space, then each state vector can be expanded as

$$|\Psi^{(k)}> = \sum_i c_i^{(k)} |\phi_i> \tag{6.43}$$

The expectation value of any observable described by an operator A is thus obtained as an average over the members of the ensemble

$$< A > = \frac{1}{Z}\sum_{k=1}^{Z} < \Psi^{(k)}|A|\Psi^{(k)} > = \sum_{i,j}\left(\frac{1}{Z}\sum_{k=1}^{Z} c_j^{(k)*} c_i^{(k)}\right) < \phi_j|A|\phi_i > = \sum_{i,j} \rho_{i,j} A_{j,i} = Tr(\rho A) \tag{6.44}$$

Here, $\rho_{i,j}$ is the density matrix:

$$\rho_{i,j} = \frac{1}{Z}\sum_{k=1}^{Z} c_j^{(k)*} c_i^{(k)}, \tag{6.45}$$

$Tr(\rho A)$ is the trace of (ρA):

$$Tr(\rho A) = \sum_i < \phi_i|\rho A|\phi_i > \tag{6.46}$$

and $c_j^{(k)*}$ indicates the complex conjugate of $c_j^{(k)}$.

It is possible to show that if $\{|\zeta_i>\}$ is a complete set of vectors which diagonalize ρ, i.e. $\rho\,|\zeta_i> = \zeta_i\,|\zeta_i>$, then the eigenvalues ζ_i satisfy the following relations:

$$0 \le \zeta_i \le 1 \quad \text{and} \quad \sum_i \zeta_i = 1 \tag{6.47}$$

It is thus manifest that the eigenvalues ζ_i of ρ can be interpreted as probabilities.

The equation of motion for the density matrix can be derived from the action of the time-evolution operator $U(t) = e^{-iHt/\hbar}$ on $|\Psi^{(k)}(0)>$:

$$\rho(t) = \frac{1}{Z}\sum_{k=1}^{Z} |\Psi^{(k)}(t)>< \Psi^{(k)}(t)| = \frac{1}{Z}\sum_{k=1}^{Z} e^{-itH/\hbar}|\Psi^{(k)}(0)>< \Psi^{(k)}(0)|e^{itH/\hbar} = e^{-itH/\hbar}\rho(0)e^{itH/\hbar} \tag{6.48}$$

Differentiating both sides with respect to time, one obtains

$$\frac{\partial \rho(t)}{\partial t} = -\frac{iH}{\hbar} e^{-itH/\hbar}\rho(0)e^{itH/\hbar} + e^{-itH/\hbar}\rho(0)\frac{iH}{\hbar}e^{itH/\hbar} = -\frac{i}{\hbar}(H\rho(t) - \rho(t)H) = -\frac{i}{\hbar}[H, \rho(t)]$$

where [H,ρ(t)] is the commutator between H and ρ(t).

Since in equilibrium ρ(t) must be independent of time, i.e. $\partial\rho(t)/\partial t = 0$, it follows that [H,$\rho$(t)]=0. This means that it is possible to diagonalize H and ρ simultaneously, that is:

$$\rho = f(H) = \sum_i f(E_i)|E_i >< E_i| \tag{6.50}$$

where E_i and $|E_i>$ are the eigenvalues and eigenfunctions of H, respectively.

The particular form of $f(E_i)$ depends on the specific ensemble under consideration. In the canonical (NVT) ensemble,

$$f(E_i) = \frac{e^{-\beta E_i}}{Z} \tag{6.51}$$

where $\beta = 1/kT$, and Z is the canonical partition function

$$Z = \sum_i e^{-\beta E_i} \tag{6.52}$$

From the previous equations it follows that the canonical density matrix is defined as

$$\rho = \frac{e^{-\beta H}}{Z} \tag{6.53}$$

and the expectation value of any operator A can thus be computed as

$$< A > = Tr(\rho A) = \frac{1}{Z} Tr(Ae^{-\beta H}) \tag{6.54}$$

In order to describe the path-integral formulation of the canonical density matrix and partition function, we consider here a single quantum particle of mass m, with momentum p and coordinate x, in a one-dimensional potential v(x). Generalization to a multidimensional many-particle system is straightforward. The Hamiltonian for the one-dimensional problem is written as

$$H = \frac{p^2}{2m} + v(x) = T + V \tag{6.55}$$

where T and V are the kinetic and potential operators, respectively. Using the coordinate basis set {|x>}, the canonical partition function can be computed as

$$Z = \int dx < x|e^{-\beta H}|x >= \int dx < x|e^{-\beta(T+V)}|x > \tag{6.56}$$

In general T and V do not commute, i.e. [T,V]$\neq$ 0, and consequently $e^{-\beta(T+V)}$ cannot be calculated directly. However, the Trotter theorem [175] states that for any two operators A and B:

$$e^{\lambda(A+B)} = \lim_{P->\infty}\left[e^{\frac{\lambda B}{2P}} e^{\frac{\lambda A}{P}} e^{\frac{\lambda B}{2P}}\right] \tag{6.57}$$

and, therefore, the canonical partition function can be written as

$$Z = \lim_{P->\infty} \int dx < x|e^{-\frac{\beta V}{2P}} e^{-\frac{\beta T}{P}} e^{-\frac{\beta V}{2P}}|x > \tag{6.58}$$

Defining $\Omega = e^{-\beta V/2P} e^{\beta T/P} e^{-\beta V/2P}$ and using the completeness of the coordinate basis, the quantum partition function then becomes

$$Z = \lim_{P->\infty} \int dx < x|\Omega^P|x > = \lim_{P->\infty} \int dx_1 dx_2 \cdots dx_P < x_1|\Omega|x_2 >< x_2|\Omega|x_{3>} \cdots < x_P|\Omega|x_1 > \quad (6.59)$$

After some algebra, it is possible to show that

$$Z = \lim_{P->\infty} \int dx_1 dx_2 \cdots dx_P \left(\frac{mP}{\hbar^2 \beta}\right)^{P/2} e^{-\sum_{i=1}^{P}\left[\frac{mP}{\beta\hbar^2}(x_{i+1}-x_i)^2+\frac{\beta}{P}v(x_i)\right]_{x_{P+1}=x_1}} \quad (6.60)$$

After introducing a "chain" frequency

$$\omega_P = \frac{\sqrt{P}}{\beta\hbar} \quad (6.61)$$

and defining an effective potential as

$$U_{eff}(x_1, \ldots, x_P) = \sum_{i=1}^{P}\left[\frac{1}{2} m\omega_P^2 (x_{i+1} - x_i)^2 + \frac{\beta}{P} v(x_i)\right]_{x_{P+1}=x_1} \quad (6.62)$$

the canonical partition function is finally expressed as

$$Z = \lim_{P->\infty} \int dx_1 dx_2 \cdots dx_P \left(\frac{mP}{\hbar^2 \beta}\right)^{P/2} e^{-\beta U_{eff}(x_1,\ldots,x_P)} \quad (6.63)$$

In this form, the quantum partition function looks like a classical configurational partition function for a P-particle systems, where the P particles (generally referred to as "beads") are discrete points along a cyclic path [176]. Each bead is coupled to its nearest neighbors by harmonic springs with frequency ω_P, and is subject to the external potential v(x). It is possible to make the connection between the quantum partition function and a fictitious classical P-particle system more manifest by introducing a set of P Gaussian integrals:

$$Z = \lim_{P->\infty} \Lambda \int dp_1 dp_2 \cdots dp_P \int dx_1 dx_2 \cdots dx_P \left(\frac{mP}{\hbar^2 \beta}\right)^{P/2} e^{-\beta\left[\sum_{i=1}^{P}\frac{p_i^2}{2\mu_i}+U_{eff}(x_1,\ldots,x_P)\right]} \quad (6.64)$$

The new Gaussian variables are regarded as fictitious classical "momenta" and, consequently, the constants μ_i have units of mass. Since these Gaussian integrals are uncoupled and can be calculated analytically, the overall constant Λ can be chosen so as to reproduce the correct prefactor. Therefore, one has complete freedom to choose μ_i. The quantum partition function can thus be evaluated using classical molecular dynamics based on equations of motion derived from a fictitious classical Hamiltonian of the form

$$H(p, x) = \sum_{i=1}^{P} \frac{p_i^2}{2\mu_i} + U_{eff}(x_1, \ldots, x_P) \quad (6.65)$$

However, ordinary MD generates a microcanonical distribution of H, i.e. a distribution function of the form δ(H(p,x)-E), where E is the conserved energy. This is clearly not the form appearing in the quantum partition function which requires a canonical distribution of the form $e^{-\beta H}$. In order to satisfy this condition the system is coupled to a thermostat which garantees that the canonical distribution is rigorously obtained. As shown above the exact quantum partition function is obtained in the limit of an infinite number of beads P. In practice this is obviously not possible, and therefore P must be chosen large enough that all thermodynamic properties are converged. Since P is directly related to the quantum nature of the system under consideration, a larger number of beads is necessary for systems containing light atoms (e.g., H and D) and for low temperature.

The current implementation of PIMD in Amber follows the so-called primitive approximation [177] which is directly obtained from the formulation provided above with the fictitious mass of each bead chosen as μ_i= m/P. The equations of motion are propagated using the Leapfrog algorithm and a canonical distribution is obtained from the use of a Langevin thermostat. The energetics of the quantum system (total, kinetic and potential energy) is computed using the so-called "virial estimator" [177,178]. Comparison with the results obtained with a more common implementation of PIMD, which makes use of the velocity-Verlet propagator coupled to Nosé-Hoover chains of thermostats, shows that the two implementations provide identical values for all observables tested.

6.13.2. Preparing PIMD input files

The *mdin* input for a PIMD run is the same as for a regular (classical MD) run. The difference is that your must use *sander.PIMD* as your executable, and you must prepare the *prmtop* file in a special way.

PIMD input files are generated using *addles* (see Chapter 9). Basically, a normal topology file and coordinate file are needed, then a control scrippt (usually named *addles.in*) should be written. PIMD input files can then be generated by running "addles < addles.in". The following is what a typical addles.in will look like(lines start with a '~' are comments):

```
~designate normal topology file
file rprm name=(input.prmtop) read

~designate normal coordinate file
file rcrd name=(input.inpcrd) read

~where to put PIMD topology file
file wprm name=(pimd.prmtop) wovr

~where to put PIMD coordinate file
file wcrd name=(pimd.inpcrd) wovr

action
~use original mass(it is required path integral theory)
omas

~make pimd style topology file(do not put other copy in the exclusion list)
pimd

~make 4 copies of atom 1-648(should be the whole system)
space numc=4 pick #prt 1 648 done

*EOD
```

Several things should be emphasized here about writting *addles.in* for PIMD:

(1) Make sure you are making copies of the whole system, since currently *sander.PIMD* can't run when only part of the system is copied. (Our hope is that future versions of the code will allow different parts of the system to be represented by different numbers of "beads",

but this functionality is not available at present.)

(2) The "omas" tag must be turned on to make every atom use original mass during the simulation; this is required by the path integral theory.

(3) The "pimd" tag should be turned to get a smaller topology file. The normal action of addles is to put atoms from other copies to the exclusion list for the current copy. Since PIMD always make copies of the whole system, this would create a huge exclusion list and a huge topology file. This can be avoided by turning on the "pimd" tag.

(4) How many copies to create is a tradeoff between accuracy and efficiency. To get converged total energies, 15-30 copies may be required; however, other aspects of quantum behavior may be seen with fewer copies. Be prepared to experiment on your system to see what is required.

6.14. Using the AMOEBA force field

The Amoeba force field is a recently developed polarizable force field with parameters for water, univalent ions, small organic molecules and proteins [8,9,179,180]. Differences from the current amber force fields include more complex valence terms including anharmonic bond and angle corrections and bond angle and bond dihedral cross terms, and a two dimensional spline fit for the phi-psi bitorsional energy. The differences in the nonbond treatment include the use of atomic multipoles up to quadrupole order, induced dipoles using a Thole' screening model, and the use of the Halgren buffered 7-14 functional form for van der Waals interactions. The PME implementation used here, as well as a multigrid approach for atomic multipoles, is described in Ref. [181].

Preparation of the necessary coordinate and parameter files for performing simulations using the amoeba forcefield is currently somewhat complex. Please check the Amber web site for updates, as we are working to make the process simpler. Also, detailed examples will also be posted on the web site; what we give here is an overview to help you understand what is needed. Use of the Tinker package is critical, so step one is to obtain that package available from Jay Ponder: `http://dasher.wustl.edu/tinker/`. You will need version 4.3 or later.

In general, to create Amber *inpcrd* and *prmtop* files, one runs the *amoeba_parm* which is built as a standard part of Amber. The *amoeba_parm* program requires five inputs:

(1) a Tinker .xyz file;

(2) the output of the *analyze* code of Tinker, run on that .xyz file, using the PC options;

(3) a Tinker parameter file (needed for the phi-psi bicubic splines);

(4) a .pdb file whose atomic coords agree with the .xyz file. This latter requirement (needed to assign amber atom and residue names) is best met by using the *xyzpdb* executable from Tinker. Check however that the pdb names make sense. Tinker still has trouble with some non-standard residue types, and some hand editing may be needed.

(5) although the above is sufficient for an initial configuration, if a Tinker restart in the form of a *.dyn* file is available, *amoeba_parm* will use the velocities and accelerations from that file to get an equivalent start for sander. This is important for checking equivalence of dynamics in *sander* vs. Tinker.

In addition to the above files, you need to set parameters in the *mdin* file for *sander* that are equivalent to those used by Tinker (in its *.key* file). Note that currently Tinker uses a standard ewald summation for amoeba, but a nearly equivalent PME formulation will be available soon.

With the use of Amoeba, minimization as well as usual *sander* methods of molecular dynamics can be used, including constant temperature and pressure simulations. In addition, with the amoeba implementation it is possible to use the Beeman dynamics integrator, which is helpful in making detailed comparisons to Tinker results. Note that the Amoeba forcefield is parametrized for fully flexible molecules. At this time it is not possible to use SHAKE with this forcefield.

The parameters *ew_coeff, nfft1, nfft2, nfft3,* and *order* from the &ewald section of input all relate to the accuracy of the PME method, which is used in the Amoeba implementation in *sander*. Due to the use of atomic quadrupoles, *order* (i.e. the B-spline polynomial degree plus one) needs to be at least 5 since the B-spline needs 3 continuous derivatives. The *ew_coeff* together with the direct sum cutoff (see below) controls the accuracy in the Ewald direct sum, and *ew_coeff* together with the PME grid dimensions *nfft1,2,3* and *order* controls the accuracy in the reciprocal sum. Since Amoeba atomic multipoles are typically dominated by the charges, experience gained in the usual use of PME is pertinent. Typical values we have used for a good cost *vs.* accuracy balance are *ew_coeff=0.45, order=5,* and *nfft1,2,3* approximately 1.25 times the cell length in that direction.

Some specific amoeba-related input parameters are given here. They should be placed in the `&amoeba` namelist, following the `&cntrl` namelist where *iamoeba* has been set to 1.

BEEMAN_INTEGRATOR

Setting this to be one turns on the Bbeeman integrator. This is the default integrator for Amoeba in Tinker. In sander this integrator can be used for NVE simulations, or for NVT or NTP simulations using the Berendson coupling scheme. (This means that you must set *ntt* to 0 or 1 if you use the Beeman integrator.) By default, *beeman_integrator*=0, and the usual velocity Verlet integration scheme is used instead..

VERBOSE In addition to the usual sander output, by setting the logical variable verbose=.true., energy and virial components can be output. By default, verbose=.false.

EE_DSUM_CUT This is the ewald direct sum cutoff. In the amoeba implementation this is allowed to be different from the nonbond cutoff specifid by *cut*. It should be less than or equal to the latter. (Note, this feature does not apply to the direct sum for standard amber force fields, which use the nonbond cutoff for the Ewald direct sum as well as van der Waals interactions. The default is 7.0 Angstroms, which is conservative for energy conservation with ew_coeff=0.45.

DIPOLE_SCF_TOL

The induced dipoles in the amoeba force field are solutions to a set of linear equations (like the Applequist model but modified by Thole' damping for close dipole-dipole interactions). These equations are solved iteratively by the method of successive over-relaxation. *dipole_scf_tol* is the convergence criterion for the iterative solution to the linear equations. The iterations towards convergence stop when the RMS difference between successive sets of induced dipoles is less than this tolerance in Debye. The default is set to 0.01 Debye, which has been seen to give reasonable energetic and dynamic, but

requires mild temperature restraints. Good energy conservation in NVE simulations requires a tolerance of about 10ˆ-6 Debye tolerance.

SOR_COEFFICIENT

This is the successive over-relaxation parameter. This can be adjusted to optimize the number of iterations needed to achieve convergence. Default value is 0.75. Productive values seem to be in the range 0.6-0.8 .The optimal values seem to depend on the polarizabilities of the system atoms.

DIPOLE_SCF_ITER_MAX

This prevents infinite iterations when the polarization equations are somehow not converging. Possible reasons for this are a bad sor_coefficient, exacerbated by a close contact. Default is 50. For comparison, with typical sor_coefficient values and an equilibrated system it should take 4-7 iterations to achieve 0.01 Debye convergence and 18-25 iterations to achieve 10^{-6} Debye.

EE_DAMPED_CUT

This is used to cutoff the Thole' damping interactions. The default value is 4.5 Angstroms, which should work for the typical sized polarizabilities encountered, and the default Thole' screening parameter (0.39).

DO_VDW_TAPER

Amoeba uses a Halgren buffered 7-14 form for the van der Waals interactions. In the Tinker code these are typically evaluated out to 12 Angstroms, with a taper turned on and no long-range isotropic continuum corrections to the energy and virial. In the sander implementation, the usual nonbond cutoff from the &ctrl namelist is used for van der Waals interactions. The long range correction is available to allow for shorter cutoffs. Setting *do_vdw_taper* to one causes VDW interactions to be tapered to zero beginning at 0.9 times the van der waals cutoff. The taper is a 5th order polynomial switch on the energy term, which gets differentiated for the forces (atom based switching). Its turned on by default.

DO_VDW_LONGRANGE

Setting this to one causes the long-range isotropic continuum correction to be turned on. This adjusts the energy and virial, and in most cases will result in energies and virials that are fairly invariant to van der Waals cutoff, with or without the above taper function. The integrals involved in this correction are done numerically.

7. Divcon

7.1. Introduction.

DivCon is a linear scaling semi-empirical program for calculation of energies, charges and geometries of systems up to ˜20,000 atoms. Available features include:

(1) Linear scaling Divide and Conquer (D&C) calculations [182-184].

(2) Cubic scaling standard calculations [74-76].

(3) Single point AM1 [75], PM3 [76], or MNDO [74] calculations.

(4) Geometry Optimization (steepest decent, conjugate gradient, BFGS, and LBFGS available)

(5) Mulliken, CM1 [185] and CM2 [186] charge analysis

(6) Nuclear Magnetic Resonance prediction and simulation

The program was mainly developed by Steve Dixon. His work includes the development of the semiempirical Divide and Conquer algorithm, implementation of the D&C and standard energy and gradient calculations, geometry optimization routines, Mulliken charge analysis, cluster based subsetting strategy and front end of the program. Arjan van der Vaart added the Monte Carlo routines (single and multi processing), Particle Mesh Ewald routines, grid based subsetting routines, extension of the cluster based subsetting schemes, CM1 and CM2 charge analysis, density matrix build routines, density of state analysis, frozen density matrix routines the interaction energy decomposition routines (serial and parallel), and Talman's algorithm. Valentin Gogonea added the SCRF routines. Jim Vincent parallellized the single point energy and geometry optimization routines, the transition state routines and the sodium parameters. Ed Brothers added dipole and ionization potential routines, the parametrization routines and the sodium parameters. Dimas Sua'rez added the LBFGS optimization routines, the transition state routines and the frequency calculation routines. Ning Liao has added support for a native Poisson-Boltzmannn(PB) implementation, and Andrew Wollocott has added support for restrained minimization. Subsequently, Hwanho Kim and Lance Westerhoff of QuantumBio Inc. fully audited, optimized, and modernized much of the source code in order to impart increased stability and extensibility upon the application. QuantumBio continues to develop DivCon with these same principles in mind.

7.2. Getting Started

DivCon05 packaged with AMBER is capable of performing mixed quantum mechanics/molecular mechanics(QM/MM) linear scaling Semi-Empirical calculations. This allows large patches of a protein to be studied at a quantum mechanical level of theory while still retaining charge effects from the surrouding protein. DivCon contains many options that may aid in the simulation of protein systems with large quantum patches whose keywords can be found within this manual for a more detailed discussion of their applications and uses. This section will provide a brief overview of how to get started using DivCon with AMBER. This section should only be used as a starting point for QM/MM calculations involving DivCon after which the manual may be consulted for more options and uses. These examples should be a good starting point for the divcon.in files needed for these QM/MM jobs. DivCon has several default keywords that can be found in the Keywords section of the manual that are good for general uses, but can easily be

changed if desired.

7.2.1. Standard Jobs

These jobs are run without the use of DivCon's linear scaling feature. Standard should only be used for smaller patches(around 250-300 atoms), afterwhich it will become quite expensive. Below there is a simple divcon.in file for use in standard jobs when running QM/MM calculations. This may not be the best input file for every application, just a place to get started when using DivCon. The manual should be consulted for a more detailed discussion of the keywords used in this divcon.in file.

```
DIRECT CARTESIAN AM1 CHARGE=0.0 &
STANDARD CUTBOND=9.0 SHIFT=3.0
END_COORD
```

7.2.2. Divide and Conquer Jobs

One of DivCon's best features is the ability to scale linearly to system size for Semi-Empirical calculations. This is an excellent feature for larger systems(>˜300 atoms) which maybe not be able to be calculated in other programs. Using divide and conquer requires that the system be broken into smaller subsystems which is done by keywords in the divcon.in file. The most common, and easiest, clustering system for proteins is to make each residue a subsystem. These subsystems are then surrounded by a buffer to be considered in the subsystem calculations, the size of which can be declared in the divcon.in file. For more information on the Divide and Conquer or buffering methods references 1,2, and 3 should be consulted. Again, the example below is a place to get started on using DivCon and more detailed calculations may require different keywords and/or values which can be found in the Keywords section of this manual.

```
DIRECT CARTESIAN AM1 CHARGE=0.0 &
RESIDUE CLUSTER CUTBOND=9.0 SHIFT=3.0
END_COORD
CLUSTER
  NCORE=1
  DBUFF1=4.5 DBUFF2=2.0
END_CLUSTER
```

More detailed information on all these keywords and more can be found within the Keywords section. Also, the keywords that are used by default can be found in the manual along with directions how to change and use them. These simple examples will give a good starting point for doing general QM/MM calculations using DivCon and should be acceptable in many cases, but are not, by any means, a complete input file for DivCon.

7.3. Keywords

7.3.1. Hamiltonians

AM1	AM1 Hamiltonian to be used.
PM3	PM3 Hamiltonian to be used.
MNDO	MNDO Hamiltonian to be used.
MNDO/d	MNDO/d Hamiltonian to be used.
PDDG-PM3	PDDG-PM3 Hamiltonian to be used.

NOTE: One Hamiltonian must be selected. There is no default.

7.3.2. Convergence Criterion

ETEST=FLOAT	user defined geometry optimization energy change criterion. Default : 0.002 kcal/mol.
GTEST=FLOAT	user defined maximum gradient component criterion. Default : 0.500 kcal/(mol ˜A).
XTEST=FLOAT	user defined geometry optimization coordinate change criterion. Default : 0.001 ˜A / 0.001 degrees.

7.3.3. Restrained Atoms

BELLY A subset of the atoms in the system, the belly group, will be allowed to relax their position during optimization while the rest of the atoms will be kept at fixed positions by zeroing the corresponding forces. Currently, the BELLY option requires optimization of both minimum or transition structures using cartesian coordinates (a FREQ calculation can be also subjected to the BELLY option).

The BELLY parameter must be included in the input file in order to specify the BELLY group. Two formats are possible:

```
BELLY
  ATOMS  144-178 310-332
END_BELLY
```

This means that the BELLY group of moving atoms will be constituted from atom 144 to atom 178, and from atom 310 to atom 332. Alternatively, the BELLY group can be selected using residue numbering:

```
BELLY
  RESIDUES 10-13 20
END_BELLY
```

Only residues from 10 to 13 and residue 20 will be allowed to move during minimization.

7.3.4. Output

PRTSUB print subsystem atom lists.

PRTVEC print final eigenvectors. All eigenvectors and eigenvalues will be printed by default. If the input file contains PRTVEC parameters, only some eigenvectors will be printed:

```
PRTVEC
  1-458 all
  558-960 -15.0 -10.0
  45-460 ef 10.0
END_PRTVEC
```

The first line indicates that only the eigenvectors for atoms 1-458 need to be printed. These are all eigenvectors when a standard calculation is performed. For a D&C calculations, these are the eigenstates for subsystems that contain atoms 1-458. The second line indicates that the eigenstates for atoms 558 through 960 will be printed if the associated eigenvalues are between ?15.0 and -10.0 eV. The third line indicates that the eigenvectors of atoms 45-460 will be printed if the associated eigenvalues are within 10 eV of the Fermi energy.

DOS perform a density of state analysis. By default, a DOS analysis will be performed on all eigenvalues for all atoms, with interval of 0.5 eV. Intervals and extend of the DOS analysis can be set by the DOS parameters:

```
DOS
        1-435 0.2
        1015-4452 0.3
END_DOS
```

Here the DOS will be printed for all subsystems that contain atoms 1-435 with interval of 0.2 eV and for all subsystems that contain atoms 1015 through 4452 with interval of 0.3 eV. Note that for a standard calculation the DOS will always extend over all atoms.

DIPOLE calculate the magnitude of themolecular dipole moment using all three charge methods.

IP calculate ionization potential

HOMOLUMO calculate homo-lumo gap. For a D&C run, the homo-lumo gap of all subsystems will be printed.

PRTCOORDS print atomic coordinates .

PRTPAR Print the AM1/PM3/MNDO parameters for all atom types that are found in the input file.

SCREEN output vital information to screen. If not included DivCon will run silently and only reture access to the user once the job is complete.

WRTPDB write final coordinates of an optimization in a "standard" pdb format.

DUMP=INT write restart file (divcon.rst) every INT cycles.

PDUMP=INT write density matrix file (divcon.dmx) every INT SCF iteration

SNAPGEOM Write coordinates during energy optimization (divcon_snapshot.N) at every N-th optimization step. This can be useful when optimizing very large systems.

TRAJECTORY dump coordinates to trajectory file (divcon.trj) at restart points.

GEOCALC Calculates geometric parameters. Input takes the form (after the END_COORD line):

```
GEO
     DISTANCE
          1-2
     END_DISTANCE
     ANGLE
          1-2-3
      END_ANGLE
      DIHEDRAL
           1-2-4-3
       END_DIHEDRAL
END_GEO
```

Note that if an equals sign is included after the atom numbers (i.e. 1-2=2.0) then a set of differences between the calculated values and these numbers are returned.

ERROR Calculates the difference between accepted and calculated values. An example list is shown below, with each component being explained afterward. Note this is only usable with standard calculations, and this list follows the END_COORDS line.

```
ERROR
     HEAT=FLOAT
     IP=FLOAT
     DIPOLE=FLOAT
     ASSOCIATION=FLOAT
```

```
            FILExINTEGER
            FILExINTEGER
      END_ASSOC
      ETOTDIFF=FLOAT
            FILExINTEGER
            FILExINTEGER
       END_ETOTDIFF
END_ERROR
```

HEAT is the heat of formation in kcal/mol. IP is ionization potential. DIPOLE is the Mulliken dipole. ASSOCIATION is the energy of association, and the lines following it are the files to be used to calculate it. For instance, if the association energy of a methanol-2 water complex was to calculated, and methanol was in divcon001.in and water was in divcon002.in, the values on the two subsequent line would be 1x1 and 2x2. ETOTDIFF is the total energy difference, and it's files are designated the same way. Note also that a geometry list can be placed inside the ERROR/END_ERROR delimiters using the format given above.

ZMAKE output a z-matrix using the DivCon z-matrix format. Note that this uses the first three atoms as the defining atoms, and thus they may not be collinear.

7.3.5. General

ADDMM add MM correction to peptide torsional barrier. (on by default)

NOMM do not use MM correction to peptide torsional barrier.

CARTESIAN Cartesian coordinate format. DivCon reads cartesian coordinates in the format shown in the following example:

```
 1  N   -0.26120  -0.98976   0.00000
 2  C    0.64694   0.01940   0.00000
 3  C   -0.47100   1.06738   0.00000
 4  C   -1.44202  -0.13945   0.00000
 5  O    1.83331   0.04003   0.00000
 6  H   -0.13870  -1.97802   0.00000
 7  H   -0.49385   1.68899  -0.88436
 8  H   -0.49385   1.68899   0.88436
 9  H   -2.05887  -0.23715  -0.88402
10  H   -2.05887  -0.23715   0.88402
```

Coordinates are in ˜A. The specification of symbols and coordinates is format free and the maximum characters per line is 80.

RMIN=FLOAT The minimum allowed distance between atoms (results in an error for single point calculations and geometry optimizations, configuration will be rejected

in an MC-run when a smaller distance is encountered).

ECRIT=FLOAT set the convergence for the energy in units of eV. (default value is 4x10-6 eV). The actual value of ECRIT will be relaxed if the gradient norm is large and the structure is not tiny. This should speed up convergence without any loss of accuracy

DCRIT=FLOAT set the convergence for the density matrix in atomic units (default value 5x10-4 e). The actual value of DCRIT will be relaxed if the gradient norm is large and the strucutre is not tiny. This should speed up convergence without any loss of accuracy.

DESCF=FLOAT related to ECRIT in that it defines the SCF energy convergence criterion. However, unlike ECRIT, this values is considered absolute(in eV). In affect, the SCF calculation will not stop until this criterion is reached.

DPSCF=FLOAT related to DCRIT in that it defines the SCF energy convergence criterion. However, unlike DCRIT, this values is considered absolute(in eV). In affect, the SCF calculation will not stop until this criterion is reached.

CUTREPUL=FLOAT

set the [xy|xy], [xz|xz], [xx|yy], [xx|zz],[zz|xx], [xx|xx] and [zz|zz] integrals to zero when the interatomic distance is larger than FLOAT. The CUTREPUL keyword can be used to speed up a DivCon simulation by limiting the number of calculations performed.

CUTBOND=FLOAT

cutoff bonding for the H, P and F matrixes beyond FLOAT angstroms. The CUTBOND keyword can be used to speed up a DivCon simulation by limiting the number of calculations performed.

DIRECT causes all 2-electron integrals to be kept in memory and recalculated at each step instead of being written out to file. This is the suggested approad as generally with how fast processor are today and how much memory users have at their disposal, accessing disk may be more expensive.

FULLSCF turns off pseudo diagonalizations and turns on full diagonalizations. This is more expensive than pseudo diagonalizations but may be necessary sometimes.

RESIDUE stores residue pointers within DivCon. Also requires that the user denote the beginning of each residue in the input file by using the "RES" delimiter after the "z" coordinate.

CHKRES check inter-atomic distances for each residue.

TEMPK=FLOAT user defined divide and conquer temperature. Units are Kelvin, and default is 1000K.

TESTRUN do setup work and stop before first energy evaluation.

TMAX=FLOAT user defined maximum CPU time in seconds. The default limit is 20^20 seconds.

SHIFT=FLOAT user defined initial dynamic level shift [187] parameter in eV.

XYZSPACE do all operations in xyz space.

MAXIT=INT Set the maximum number of SCF iterations (default: 100). If it takes more than 100 SCF iterations to converge, it is generally thought that the system will probably not converge and is exhibiting problems.

DOUBLE=INT perform a double SCF step during a certain number (int) of SCF iteractions. if INT=0, a double SCF will be done for every SCF iteration (only for non-geometry optimizations). This will aid in convergence as it guarantees that the values calculated at each step are based completely on the current step. By default the first step of an SCF calculation will be a double. Please note, using DOUBLE will significantly increase the CPU time requred to execute a DivCon job.

1SCF Perform only the first SCF iteration, i.e. calculate the energy through E = 0.5(H+F)P. Note that this is not equivalent to MAXIT=1, since no diagonalization is performed.

GUESS Build the initial density matrix from one or more density matrix files. These files are listed by means of the GUESS parameters:

```
GUESS
        A.dmx
        b.dmx
END_GUESS
```

Build the initial density matrix from the files A.dmx and b.dmx. The density matrix elements of atoms 1 through a are read from A.dmx, for atoms a+1 through n from file b.dmx.

```
GUESS
        2-10 A.dmx 33-41 f
        20-30 b.dmx 1-11
END_GUESS
```

Density matrix information for atoms 2-10 is read from the density matrix elements of atoms 33-41 of file A.dmx, density matrix information for atoms

20-30 is read from the density matrix elements of atoms 1- 11 from file b.dmx. Missing density matrix elements are auto-initialized and a correction will be applied to constrain the total number of electrons. The density matrix elements of atoms 2-10 will be kept constant during the SCF iterations by using the Frozen Density Matrix approximation [188]. It is imperative that the number of orbitals on a certain atom in the divcon.in file and density matrix file are the same, i.e. the number of orbitals on atom 2 from divcon.in and atom 33 from A.dmx should be identical.

Note that the maximum length of a density matrix file name is 20 characters, no dashes ("-") are allowed in the density matrix file name.

INTGLS=string The INTGLS keyword can have two values. If the value is "INTGLS=TALMAN" then the Talman method of integrals will be used. If the Value is "INTGLS=STEWART" then the Stewart integrals will be used. The default, and recommended value is Talman integrals as this approach has been found to be the most stable and accurate.

PUSH Only in combination with the CLUSTER keyword (page 22) and when multiple cores (i.e. multiple cluster groups) are defined. Push the different cluster groups apart. Default is 106 ˚A, the user can define this distance by using PUSH=FLOAT. When more than two cluster groups are defined, each group is place on a gridpoint with gridspacing of 106 ˚A or the user defined value.

7.3.6. Gradient

GRADIENT output final gradient. (Note: The gradient for MC calculations contains only intermolecular terms. No intramolecular terms are involved.)

CENTRAL use central difference in gradient calculation

7.3.7. Atomic Charges

CHARGE=INT a net charge is to be placed on system.

MULLIKEN write Mulliken, CM1, and CM2 charges to output file. NOTE: For a single point calculation and geometry optimization both Mulliken, CM1 and CM2 charges are calculated.

7.3.8. Subsetting

This is the basis of divide and conquer methodology [182-184]. Subsetting can be performed by hand through the SUB parameters (page 30), or automatically through the keywords listed below. Subsystems consists of a core surrounded by an inner and outer buffer.

CLUSTER do cluster based subsetting. Specification of the cluster based subsetting is through the cluster parameters:

```
CLUSTER
    NCORE=3 DBUFF1=4.0 DBUFF2=2.0
END_CLUSTER
```

This means that the cores will be build from 3 residues, the first buffer region extends 4.0 ˚A from the core, the second buffer region 2.0 ˚A from the first buffer region. Multiple cores (i.e. multiple cluster groups) can be defined by:

```
CLUSTER
       NCORE=2 (1-6 7 8 12-14)
       NCORE=3 (9 10 15-25 )
       NCORE=1 (26 27)
       DBUFF1=4.3 DBUFF2=2.0
END_CLUSTER
```

Cores will be build from 2 residues for residues 1-6, 7, 8, 12-14, from 3 residues for residues 9, 10, 15-25 and from 1 core for residue 26 and 27. The first buffer region is 4.3 ˚A, the second 2.0 ˚A. Note that all residues should be used (and only once) in this syntax.

```
CLUSTER
        NCORE=1 (1-20) [-1]
        NCORE=1 (21-100) [0]
END_CLUSTER
```

Cores will be build from 1 residue for residues 1-20 and from 1 residue for residues 21-100. Moreover, the charge of the subsystems build from residues 1-20 will be contrained to ?1 electron and the charge of the subsystems build from residues 21-100 to 0 electrons. Only effective when the NO-OVERLAP keyword is used (see page 23). Charges are constrained by use of multiple Fermi energies [189].

NO-OVERLAP Only in combination with the CLUSTER keyword.

```
CLUSTER
        NCORE=1 (1-10)
        NCORE=1 (11-20)
END_CLUSTER
```

When the NO-OVERLAP keyword is used, subsystems made from residues 1-10 only overlap with subsystems made from residues 1-10 and subsystems made from residues 11-20 only overlap with subsystems made from residues 11-20. In other words, density matrix elements between subsystems 1-10 and 11-20 are zero.

ATGRID do grid based, atom-wise subsetting (core and buffers will be build from atoms).

RESGRID do grid based, residue-wise subsetting (core and buffers will be build from residues).

MIXGRID do grid based, residue-wise subsetting for cores, grid based, atom-wise subsetting for buffers.

NOTE: Specify Grid parameters when a grid based subsetting is selected. The syntax for these parameters is:

```
GRID
   XCORE=4.0 YCORE=4.0 ZCORE=4.0 OVERLAP=0.5
   DBUFF1=2.5 DBUFF2=1.0
END_GRID
```

Meaning that the total system will be divided into rectangular boxes of 4.0_4.0_4.0 ˜A that overlap 0.5 ˜A in each dimension. The first buffer region is 2.5 ˜A wide, the second 1.0 ˜A.

NOTE: In Monte Carlo simulations only a residue-wise grid-based subsetting scheme is allowed. Reason for this is rather subtle: Imagine that during the MC-simulation a molecule would penetrate the box, such that the geometric center is still inside the box, but some atoms are outside the box. If an atom based subsetting was performed, the atoms outside the box wouldn’t be included in any subsystem. Making the "grid-subsetting"-box artificially larger than the pbc-box wouldn’t work either: in that case there’s is an artificially larger distance between the molecules and the (virtual, pbc) images of other molecules. This would mean that some atoms will be skipped in making the buffer regions: atoms that, according to their pbc-image should be included. This will lead to non-optimal subsettings and can have a rather drastic effect on energies as was found experimentally.

COMBSUB do a combination subsetting; certain residues will be subsetted grid based, others cluster based. Use the combsub parameters to specify this subsetting:

```
COMBSUB
      CLUSTER
              1-10 13
      RESGRID
              11-12 14-20
END_COMBSUB
```

Here, cluster based subsetting will be done for residues 1-10 and 13, grid-based residue-wise subsetting will be done for residues 11-12 and 14-20. The cores of the subsystems will be selected from the specified residues, buffers from all residues / atoms of the system. COMBSUB can only be defined as a

combination of CLUSTER with one of RESGRID, ATGRID of MIXGRID. Note that you have to specify the CLUSTER and GRID parameters when you use COMBSUB. Note that all residues should be used (and only once) in COMBSUB.

STANDARD standard closed-shell calculation (no divide and conquer). All subsetting parameters are ignored, only one subsystem containing all atoms will be generated.

7.3.9. Nuclear Magnetic Resonance(NMR)

This section details the current NMR facilities found in the DivCon application. This functionality is under active research and development.

NMR used to activate NMR functionality. The keyword will cause DivCon to perform an NMR shielding calculation on the atoms denated in the NMR parameters entry at the end of the divcon.in file. In the first example, the shielding calculation will be performed on a number of atoms. In the second example below the calculation will instead be performed on a residue basis.

```
Example 1:
           NMR
             Atom 1-100 150-255
           END_NMR

Example 2:
           NMR
             Residue 1-10 12-34
             Residue 40-45
           END_NMR
```

CALNUC set what atoms chemical shifts are calculated for. CALNUC=1 for proton chemical shift calculations CLANUC=2 for carbon-13 chemical shift calculations

7.3.10. Default Keywords

The keywords in the following section represent keywords on by default in DivCon and the values that they are given when applicable. The sections above should be consulted for more information on the keywords presented below.

ECRIT Default value=4.0e-6.

DCRIT Default value=5.0e-4.

MAXIT	Default value=100.
TEMPK	Default value=1000.0.
ADDMM	On by default.
RMIN	Default value=0.5.
INTGLS	Default setting is TALMAN.

7.4. Citation Information

Should you publish data generated using DivCon, please include references [183,184] , along with the general citation:

> B. Wang, K. Raha, N. Liao, M.B. Peters, H. Kim, L.M. Westerhoff, A. M. Wollacott, A. van der Vaart, V. Gogonea, D. Suarez, S.L. Dixon, J.J. Vincent, E.N. Brothers and K.M. Merz Jr., DivCon

When executing a pairwise energy decomposition calculation using the PWD module, add references [189,190] ; when using the NMR module, you should add references [191-193].

8. PMEMD

8.1. Introduction.

PMEMD (Particle Mesh Ewald Molecular Dynamics) is a reimplementation of a subset of sander functionality that has been written with the major goal of improving the performance of the most frequently used methods of sander. In release 9, PMEMD supports Particle Mesh Ewald simulations, Generalized Born simulations, and ALPB (Analytical Linearized Poisson-Boltzmann) simulations. The performance and scaling for PME simulations is typically significantly better than that attained by sander. For Generalized Born and ALPB simulations, not much optimization work has been done yet, and performance is slightly better on some platforms. For the supported functionality, the input required and output produced are intended to exactly replicate sander 9 within the limits of roundoff errors. PMEMD just runs more rapidly, scales better on parallel processors using MPI, can be used profitably on significantly higher numbers of processors, and uses less resident memory. Dynamic memory allocation is used so memory configuration is not required. PMEMD is ideal for molecular dynamics simulations of large solvated systems for long periods of time, especially if supercomputer resources are available. At time of release, using pmemd on 320 processors of an IBM sp5 with 8 cpu's/node, we attained max throughputs for the factor ix benchmark (90,906 atoms, PME with 8 angstrom cutoff, 1.5 fsec timestep) of 14.56 nsec/day (NPT ensemble) and 16.63 nsec/day (NVT ensemble).

PMEMD accepts Amber 9 sander input files (mdin, prmtop, inpcrd, refc), and is also backward compatible in regard to input to the same extent as sander 9. All options documented in the sander section of this manual should be properly parsed.

8.2. Functionality

As mentioned above, pmemd is not a complete implementation of sander 9. Instead, it is intended to be a fast implementation of the functionality most likely to be used by someone doing simulations on large solvated systems. We have also slightly improved generalized Born performance, and will continue this effort in the future.

The following functionality is missing entirely:

1	*imin = 5*	In &ctrl. Trajectory analysis is not supported.
2	*nmropt = 2*	In &ctrl. A variety of NMR-specific options such as NOESY restraints, chemical shift restraints, pseudocontact restraints, and direct dipolar coupling restraints are not supported.
3	*idecomp != 0*	In &ctrl. Energy decomposition options, used in conjunction with mm_pbsa, are not supported.
4	*ipol != 0*	In &ctrl. Polarizable force field simulations are not supported.
5	*igb == 10*	In &ctrl. Poisson-Boltzmann simulations are not supported.
6	*gbsa != 0*	In &ctrl. GB/SA (generalized Born/surface area) simulations are not supported.
7		In &ctrl. The new format for specifying frozen or restrained atoms, which uses the restraint_wt, restraintmask, and bellymask options, is not supported. This functionality is still supported through use of the Amber 6/7 GROUP format instead.
8	*ntmin > 2*	In &ctrl. XMIN and LMOD minimization methods are not supported.
9	*isgld != 0*	In &ctrl. Self-guided Langevin dynamics is not supported.
10	*Water Caps*	Water cap simulations are not supported.
11	*ips != 0*	In &ctrl. Isotropic Periodic Sum simulations are not supported.
12	*Extra Points*	PRMTOP files containing extra points data are not supported, and the frameon option (in the &ewald namelist) should not be specified with any value.
13	*icfe != 0*	In &ctrl. Calculation of free energies via thermodynamic integration is not supported.
14	*itgtmd != 0*	In &ctrl. Targeted molecular dynamics is not supported.
15	*ievb != 0*	In &ctrl. Empirical Valence Bond methods are not supported.
16	*ifqnt != 0*	In &ctrl. QM/MM methods are not supported.
17	*icnstph != 0*	In &ctrl. Constant pH calculations are not supported.

18	*&debugf namelist*	Use of the &debugf namelist and options it contains is not supported. This functionality is nice for developers but not very useful for production.
19	*ineb != 0*	In &ctrl. Nudged elastic band (NEB) calculations are not supported. These calculations are done by sander.PIMD.
20	*LES*	The Locally Enhanced Sampling method is not supported.
21	*REM*	The Replica-Exchange method is not supported.
22	*MMTSB*	The Replica Exchange using MMTSB method is not supported.

The following `&ewald` options are supported, but only with the indicated default values:

1	*ew_type = 0*	Only Particle Mesh Ewald calculations are supported. ew_type = 1 (regular Ewald calculations) must be done in sander 9.
2	*nbflag = 1*	The nbflag option is basically ignored, and all nonbonded list updates are scheduled based on "skin" checks. This is more reliable and has little cost. The variable nsnb still can be set and has an influence on minimizations. For PME calculations, list building may also be scheduled based on heuristics to suit load balancing requirements in multiprocessor runs.
3	*nbtell = 0*	The nbtell option is not particularly useful and is ignored.
4	*eedmeth = 1*	Only a cubic spline switch function (eedmeth = 1) for the direct sum Coulomb interaction is supported. This is the default, and most widely used setting for eedmeth. On some machine architectures, we actually spline energies and forces as a function of r**2 to a higher precision than the cubic spline switch. One consequence of only supporting eedmeth 1 is that vacuum simulations cannot be done (though generalized Born nonperiodic simulations are available).
5	*cutoff + skinnb >= 6.d0*	In PMEMD an assumption is made that all nonbonded interaction excluded atoms will be found within a distance of cutoff + skinnb. To insure that this is a safe assumption, a minimum length check on cutoff + skinnb is made.
6	*column_fft = 0*	This is a sander-specific performance optimization option. PMEMD uses different mechanisms to enhance performance, and ignores this option.

I would strongly suggest that new PMEMD users simply take an existing sander 9 mdin file and attempt a short 10-30 step run. The output will tell you whether or not PMEMD will handle the particular problem at hand for all the functionality that is supported by "standard" sander. For

functionality that requires special builds of sander or sander-derived executables (NEB, LES, REM, MMTSB), there will probably be failures in namelist parsing.

8.3. New variables

A minimum of new variables have been introduced into the mdin namelists in PMEMD. The new variables are:

mdout_flush_interval

In &ctrl, this variable can be used to control the minimum time in integer seconds between "flushes" of the mdout file. PMEMD DOES NOT use file flush() calls at all because flush functionality does not work for all fortran compilers used in building pmemd. Thus, pmemd does an open/close cycle on mdout at a default minimum interval of 300 seconds. This interval can be changed with this variable if desired in the range of 0-3600. If mdout_flush_interval is set to 0, then mdout will be reopened and closed for each printed step. This functionality is provided in pmemd because some large systems have such large file i/o buffers that mdout will have 0 length on the disk through 100's of psec of simulated time. The default of 300 seconds provides a good compromise between efficiency and being able to observe the progress of the simulation.

mdinfo_flush_interval

In &ctrl, this variable can be used to control the minimum time in integer seconds between "flushes" of the mdinfo file. PMEMD DOES NOT use file flush() calls at all because flush functionality does not work for all fortran compilers used in building pmemd. Thus, pmemd does an open/close cycle on mdinfo at a default minimum interval of 60 seconds. This interval can be changed with this variable if desired in the range of 0-3600. Note that mdinfo under pmemd simply serves as a heartbeat for the simulation at mdinfo_flush_interval, and mdinfo probably will not be updated with the last step data at the end of a run. If mdinfo_flush_interval is set to 0, then mdinfo will be reopened and closed for each printed step.

es_cutoff, vdw_cutoff

In &ctrl, these variables can be used to control the cutoffs used for vdw and electrostatic direct force interactions in PME calculations separately. If you specify these variables, you should not specify the cut variable, and there is a requirement that vdw_cutoff >= es_cutoff. These were introduced anticipating the need to support force fields where the direct force calculations are more expensive. For the current force fields, one can get slightly improved performance and about the same accuracy as one would get using a single cutoff. A good example would be using vdw_cutoff=9.0, es_cutoff=8.0. For this scenario, one gets about the accuracy in calculations associated with 9.0 angstrom cutoffs, but at a cost intermediate between an 8.0 and a 9.0 angstrom cutoff.

use_axis_opt In &ewald. For parallel runs, the most favorable orientation of an orthogonal unit cell is with the longest side in the Z direction. Starting with pmemd 3.00, we were actually reorienting internal coordinates to take advantage of this, and in high processor count runs on oblong unit cells, using axis optimization can

improve performance on the order of 10%. However, if a system has hotspots, the results produced with axes oriented differently may vary by on the order of 0.05% relatively quickly. This effect has to do with the fact that axis optimization changes the order of LOTS of operations and also the fft slab layout, and under mpi if the system has serious hotspots, shake will come up with slightly different coordinate sets. This is really only a problem in pathological situations, and then it is probably mostly telling you that the situation is pathological, and neither set of results is more correct (typically the ewald error term is also high). In routine regression testing with over a dozen tests, axis reorientation has no effect on results. Nonetheless, we have changed defaults recently to be in favor of higher reproducibility of results. Now, axis optimization is only done for mpi runs in which an orthogonal unit cell has an aspect ratio of at least 3 to 2. It is turned off for all minimization runs and for runs in which velocities are randomized (ntt = 2 or 3). If you want to force axis optimization, you may set use_axis_opt = 1 in the &ewald namelist. If you set it to 0, you will force it off in scenarios where it would otherwise be used.

fft_grids_per_ang

In &ewald. This variable may be used to set the desired reciprocal space fft grid density in terms of fft grids/angstrom. The nearest grid dimensions, given the prime factors supported by the underlying fft implementation, that meet or exceed this density will be used (ie., nfft1,2,3 are set based on this specification). The default value is 1.0 grids/angstrom and gives very reasonable accuracy. PMEMD is actually more stringent now than sander in that it will meet or exceed the desired density instead of just approximating it. Thus, to get identical results with sander, one may have to specify grid dimensions to be used with the nfft1,2,3 variables.

Slightly changed functionality:

An I/O optimization has been introduced into PMEMD. The NTWR default value (frequency of writing the restart file) has been modified such that the default minimum is 500 steps, and this value is increased incrementally for multiprocessor runs. In general, frequent writes of restrt, especially in runs with a high processor count, is wasteful. Also, if the mden file is being written, it is always written as formatted output, regardless of the value of ioutfm. SANDER now conforms to this convention regarding ioutfm and mden.

8.4. New command line options

The following command line options are new in pmemd 9:

-l <logfile name>

A name may now be assigned to the log file on the command line.

-suffix <output files suffix>

A suffix may now be appended, following a ".", to all the default output file names for a pmemd run by simply entering the -suffix option. The suffix will apply to mdout, restrt, mdcrd, mdvel, mden, mdinfo, and logfile names. However, if an output file name is explicitly provided on the command line, the

provided name takes precedence. Entering "pmemd -suffix foo" will write mdout output to mdout.foo, and so on. This provides an easy way to group output files with minimal effort.

8.5. Some Performance Hints

Performance depends not only on proper setup of hardware and software, but also on making good choices in simulation configuration. There are many tradeoffs between accuracy and cost, as one might expect, and understanding all of these comes with experience. However, I would like to suggest a couple of good choices for your simulations, if you have facilities where you can routinely run at high processor count, say 32 processors or more. First of all, there is a new implementation of binary trajectory files in pmemd and sander, based on the netCDF binary file format. This is invoked now using ioutfm == 1, assuming you have built either pmemd or sander with "bintraj" support. Using this output format, i/o from the master process will be more efficient and your filesize will be about half what it would otherwise be. In Amber 9, ptraj can read these new netCDF trajectory files, but if you want to visualize them you may have to wait until viewers support the format. At really high processor count though, using this format can be on the order of 10% more efficient than using the standard formatted trajectory output. Secondly, other simulation packages standardly use respa methods as an efficiency measure. These methods basically sample reciprocal space forces for PME less frequently. This can slightly improve performance for pmemd at low processor count, but at higher processor counts using respa actually makes loadbalancing difficult, and there can be a net loss of performance. If you wish to use respa for pme simulations (done typically by setting nrespa to 2 or 4), then you should check whether you actually get better performance. You may well not, and it will be at a cost of a loss in accuracy. Using respa for generalized Born simulations is fine in all cases, however.

8.6. Installation

The build process for PMEMD is similar to the build process for the rest of Amber 9, but must be invoked separately in the src/pmemd directory. There is a configure script that generates a config.h header that is used in the build process. Generation of config.h files is dependendent on use of information in the src/pmemd/config_data database. This system is similar to the old Amber MACHINE files configuration system, but is a bit more automated in that the configure script will set up config.h for a lot of common machine setups. The PMEMD installation process has remained separate from the Amber 9 installation process because PMEMD does not support all systems that can be automatically configured by Amber 9, and vice versa. Also, there is an emphasis on performance in PMEMD, and there was a desire to be able to fine tune the optimization process to a larger extent than was possible with the Amber 9 configuration process. Finally, user definition of configuration files in the src/config_data database is a fairly simple process, and this allows users to easily target new machines or machines with unusual configuration requirements. For more PMEMD installation details, please read src/pmemd/README.

8.7. Acknowledgements

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When citing PMEMD (Particle Mesh Ewald Molecular Dynamics) in the literature, please use the Amber Version 9 citation given in the Amber 9 manual.

9. LES

The LES functionality for sander and gibbs was written by Carlos Simmerling, based on his thesis work and the experiences of the Elber group. It basically functions by modifying the *prmtop* file using the program `addles`. The modified *prmtop* file is then used with a slightly modified version of sander called `sander.LES`.

Background material for LES can be found in Chapter 12.

9.1. Preparing to use LES with AMBER

The first decision that must be made is whether LES is an appropriate technique for the system that you are studying. For further guidance, you may wish to consult published articles to see where LES has proven useful in the past. Several examples will also be given at the end of this section in order to provide models that you may wish to follow.

There are three main issues to consider before running the ADDLES module of AMBER.

(1) What should be copied?

(2) How many copies should be used?

(3) How many regions should be defined?

A brief summary of my experience with LES follows.

(1) You should make copies of flexible regions of interest. This sounds obvious, and in some cases it is. If you are interested in determining the conformation of a protein loop, copy the loop region. If you need to determine the position of a side chain in a protein after a single point mutation, copy that side chain. If the entire biomolecule needs refinement, then copy the entire molecule. Some other cases may not be obvious- you may need to decide how far away from a particular site structural changes may propagate, and how far to extend the LES region.

(2) You should use as few copies as are necessary. While this doesn't sound useful, it illustrates the general point--too few copies and you won't get the full advantages of LES, and too many will not only increase your system size unnecessarily but will also flatten the energy surface to the point where minima are no longer well defined and a wide variety of structures become populated. In addition, remember that LES is an approximation, and more copies make it more approximate. Luckily, published articles that explore the sensitivity of the results to the number of copies show that 3-10 copies are usually reasonable and provide similar results, with 5 copies being a good place to start.

(3) Placing the divisions between regions can be the most difficult choice when using LES. This is essentially a compromise between surface smoothing and copy independence. The most effective surface-smoothing in LES takes places between LES regions. This is because Na copies in region A interact with all Nb copies in region B, resulting in Na*Nb interactions, with each scaled by 1/(Na*Nb) compared to the original interaction. This is better both from the statistics of how many different versions of this interaction contribute to the LES average, and how much the barriers are reduced. Remember that since the copies of a given region do not interact with different copies of that same region, interactions inside a region are only scaled by 1/N.

The other thing to consider is whether these enhanced statistics are actually helpful. For example, if the copies cannot move apart, you will obtain many copies of the same conformation--obviously not very helpful. This will also result in less effective reduction in barriers, since the average energy barriers will be very similar to the non-average barrier. The independence of the copies is also related to how the copies are attached. For example, different copies of an

amino acid side chain are free to rotate independently (at least within restrictions imposed by the surroundings and intrinsic potential) and therefore each side chain in the sequence could be placed into a separate LES region. If you are interested in backbone motion, however, placing each amino acid into a separate region is not the best choice. Each copy of a given amino acid will be bonded to the neighbor residues on each side. This restriction means that the copies are not very independent, since the endpoints for each copy need to be in nearly the same places. A better choice is to use regions of 2-4 amino acids. As the regions get larger, each copy can start to have more variety in conformation- for example, one segment may have some copies in a helical conformation while others are more strand-like or turn-like. The general rule is that larger regions are more independent, though you need to consider what types of motions you expect to see.

The best way to approach the division of the atoms that you wish to copy into regions is to make sure that you have several LES regions (unless you are copying a very small region such as a short loop or a small ligand). This will ensure plenty of inter-copy averaging. Larger regions permit wider variations in structure, but result in less surface smoothing. A subtle point should be addressed here- the statistical improvement available with LES is not a benefit in all cases and care must be taken in the choice of regions. For example, consider a ligand exiting a protein cavity in which a side chain acts as a *gate* and needs to move before the ligand can escape. If we make multiple copies of the gate, and do not copy the ligand, the ligand will interact in an average way with the *gates*. If the gate was so large that even the softer copies can block the exit, then the ligand would have to wait until ALL of the gate copies opened in order to exit. This may be more statistically difficult than waiting for the original, single gate to open despite the reduced barriers. Another way to envision this is to consider the ligand trying to escape against a true probability distribution of the gate- if it was open 50% of the time and closed 50%, then the exit may still be completely blocked. Continuum representations are therefore not always the best choice.

Specific examples will be given later to illustrate how these decisions can be made for a particular system.

9.2. Using the ADDLES program

The ADDLES module of AMBER is used to prepare input for simulations using LES. A non-LES prmtop and prmcrd file are generated using a program such as LEaP. This prmtop file is then given to ADDLES and replaced by a new prmtop file corresponding to the LES system. All residues are left intact- copies of atoms are placed in the same residue as the original atom, so that analysis based on sequence is preserved. Atom numbering is changed, but atom names are unchanged, meaning that a given residue may have several atoms with the same name. A different program is available for taking this new topology file and splitting the copies apart into separate residues, if desired. All copies are given the same coordinates as in the input coordinate file for the non-LES system.

Using addles:

```
addles < inputfile > outputfile
```

SAMPLE INPUT FILE:

```
~ a line beginning with ~ is a comment line.
~ all commands are 4 letters.
~ the maximum line length is 80 characters;
~ a trailing hyphen, "-", is the line continuation token.
```

```
~
~ use 'file' to specify an input/output file
~ then the type of file
~ 'rprm' means this is the file to read the prmtop
~ the 'read' means it is an input file
~
file rprm name=(solv200.topo) read
~
~ 'rcrd' reads the original coordinates- optional, only if you want
~ a set of coords for the new topology
~ you can also use 'rcvd' for coords+velocities, 'rcvb' for coords,
~ velos and box dimensions, 'rcbd' for coords and box dimensions.
~ use "pack=n" option to read in multiple sets of coordinates and
~ assign different coordinates to different copies.
~
file rcrd name=(501v200.coords) read
~
~ 'wprm' is the new topology file to be written. the 'wovr' means to
~ write over the file if it exists, 'writ' means don't write over.
~
file wprm name=(lesparm) wovr
~
~'wcrd is for writing coords, it will automatically write velo and box
~ if they were read in by 'rcvd' or 'rcvb'
~
file wcrd name=(lescrd) wovr
~
~ now put 'action' before creating the subspaces
~
action
~
~ the default behavior is to scale masses by 1/N.
~ omas leaves all masses at the original values
~
omas
~
~ now we specify LES subspaces using the 'spac' keyword, followed
~ by the number of copies to make and then a pick command to tell which
~ atom to copy for this subspace
~ 3 copies of the fragment consisting of monomers 1 and 2
~
spac numc=3 pick #mon 1 2 done
~
~ 3 copies of the fragment consisting of monomers 3 and 4
~
spac numc=3 pick #mon 3 4 done
~
~ 3 copies of the fragment consisting of residues 5 and 6
~
```

```
spac numc=3 pick #mon 5 6 done
~
~ 2 copies of the side chain on residue 1
~ note that this replaces each of the side chains ON EACH OF THE 3
~ COPIES MADE ABOVE with 2 copies - net 6 copies
~ each of the 3 copies of residue 1-2 has 2 side chain copies.
~ the '#sid' command picks all atoms in the residue except
~ C,O,CA,HA,N,H and HN.
~
spac numc=2 pick #sid 1 1 done
spac numc=2 pick *sid 2 2 done
spac numc=2 pick #sid 3 3 done
spac numc=2 pick #sid 4 4 done
spac numc=2 pick #sid 5 5 done
~
use the *EOD to end the input
*EOD
```

What this does: all of the force constants are scaled in the new prmtop file by 1/N for N copies, so that this scaling does not need to be done for each pair during the nonbond calculation. Charges and VDW epsilon values are also scaled. New bond, angle, torsion and atom types are created. Any of the original types that were not used are discarded. Since each LES copy should not interact with other copies of the SAME subspace, the other copies are placed in the exclusion list. If you define very large LES regions, the exclusion list will get large and you may have trouble with the fixed length for this entry in the prmtop file- currently 8 digits.

The coordinates are simply copied - that means that all of the LES copies initially occupy the same positions in space. In this setup, the potential energy should be identical to the original system- this is a good test to make sure everything is functioning properly. Do a single energy evaluation of the LES system and the original system, using the copied coordinate file. All terms should be nearly identical (to within machine precision and roundoff). With PME on non- neutral systems, all charges are slightly modified to neutralize the system. For LES, there are a different number of atoms than in the original system, and therefore this charge modification to each atom will differ from the non-LES system and electrostatic energies will not match perfectly.

IMPORTANT: After creating the LES system, the copies will all feel the same forces, and since the coordinates are identical, they will move together unless the initial velocities are different. If you are initializing velocities using INIT=3 and TEMPI>0, this is not a problem. In order to circumvent this problem, addles slightly (and randomly) modifies the copy velocities if they were read from the coordinate input file. If the keyword "nomodv" is specified, the program will leave all of the velocities in the same values as the original file. If you do not read velocities, make sure to assign an initial non-zero temperature to the system. You should think about this and change the behavior to suit your needs. In addition, the program scales the velocities by sqrt(N) for N copies to maintain the correct thermal energy (˜mv2), but only when the masses are scaled (not using omas option). Again, this requires some thought and you may want different behavior. Regardless of what options are used for the velocities, further equilibration should be carried out. These options are simple attempts to keep the system close to the original state [194].

Sometimes it is critical that different copies can have different initial coordinates (NEB for example), this is why the option "pack" is added to command rcrd(rcvd,rcvb,rcbd). To use this option, user need first concatenate different coordinates into a single file, and use "pack=n" to

indicate how many sets of coordinates there are in the file, like the following example:

```
file rcrd name=(input.inpcrd) pack=4 read
```

Then addles will assign coordinates averagely. For example, if 4 sets coordinates exists in input file, and 20 copies are generated, then copy 1-5 will have coordinate set 1, copy 6-10 will have coordinate set 2, and so on. Note this option can't work with multiple copy regions now.

It is important to understand that each subsequent pick command acts on the ORIGINAL particle numbers. Making a copy of a given atom number also makes copies of all copies of that atom that were already created. This was the simplest way to be able to have a hierarchical LES setup, but you can't make extra copies of part of one of the copies already made. I'm not sure why you would want to, or if it is even correct to do so, but you should be warned. Copies can be anything -spanning residues, copies of fragments already copied, non-contiguous fragments, etc. Pay attention to the order in which you make the copies, and look carefully at the output to make sure you get what you had in mind. Addles will provide a list at the end of all atoms, the original parent atom, and how many copies were made.

There are array size limits in the file SIZE.h, I apologize in advance for the poor documentation on these. Mail *carlos.simmerling@stonybrook.edu* if you have any questions or problems.

9.3. More information on the ADDLES commands and options

```
file: open a file, also use one of
rcrd:  read coords from this file
rcvd:  read coords + velo from file
rcvb:  read coords, velo and box from file
wcrd:  write coords (and more if rcvd, rcvb) to file
wprm:  write new topology file

action:     start run, all of the following options must come AFTER action

nomodv:     do NOT slightly randomize the velocities of the copies

spac:   add a new subspace definition, using a pick command (see below).
            follow with numc=#  pickcmd where # is the number of copies to make
            and pickcmd is a pick command that selects the group of atoms
            to copy.

omas:   leave all masses at original values (otherwise scale 1/N)

pimd:   write an prmtop file for PIMD simulation, which contains a much smaller non-bond
        exclusion list, atoms from other copy will not be included in this non-bond
        exclusion list.
```

Syntax for 'pick' commands

Currently, the syntax for picking atoms is somewhat limited. Simple Boolean logic is followed, but operations are carried out in order and parentheses are not allowed.

```
#prt A B         picks the atom range from A to B by atom number
#mon A B         picks the residue range from A to B by residue number
#cca A B         picks the residue range from A to B by residue number,
                     but dividing the residue between CA and C; the CO for A
                     is included, and the CO for monomer B is not.  See
                     Simmerling and Elber, 1994 for an example of where this
                     can be useful.
chem prtc A      picks all atoms named A, case sensitive
chem mono A      picks all residues named A, case sensitive
```

Completion wildcards are acceptable for names: H* picks H, HA, etc. Note that H*2 will select all atoms starting with H and ignore the 2.

Boolean logic:

```
|   or              atoms in either group are selected
&   and             atoms must be in both groups to be selected
!=  not             A != B will pick all atoms in A that are NOT in B
```

The user should carefully check the output file to ensure that the proper atoms were selected.

Examples:

```
pick commandatoms selected

pick #mon 4 19 done                       all atoms in residues 4 through 19
pick #mon 1 50 & chem mono GLY done       only GLY in residues 1 to 50
pick chem mono LYS | chem mono GLU done   any GLU or LYS residue
pick #mon 1 5 != #prt 1 3 done            residues 1 to 5 but not atoms 1 to 3
```

so, a full command to add a new subspace (LES region) with 4 copies of atoms 15 to 35 is:

```
spac numc=4 pick #prt 15 35 done
```

9.4. Using the new topology/coordinate files with SANDER

These topology files are ready to use in Sander with one exception: all of the FF parameters have been scaled by 1/N for N copies. This is done to provide the energy of the new system as an average of the energies of the individual copies (note that it is an average energy or force, not the energy or force from an average copy coordinate). However, one additional correction is required for interactions between pairs of atoms in the same LES region. Sander will make these corrections for you, and this information is just to explain what is being done. For example, consider a system where you make 2 copies of a sidechain in a protein. Each charge is scaled by 1/2. For these atoms interacting with the rest of the system, each interaction is scaled by 1/2 and there are 2 such interactions. For a pair of particles inside the sub-space, however, the interaction is scaled by 1/2*1/2=1/4, and since the copies do not interact, there are only 2 such interactions and the sum does not correspond to the correct average. Therefore, the interaction must be scaled up by a

factor of N. When the PME technique is requested, this simple scaling cannot be used since the entire charge set is used in the construction of the PME grid and individual charges are not used in the reciprocal space calculation. Therefore, the intra-copy energies and forces are corrected in a separate step for PME calculations. Sander will print out the number of correction interactions that need to be calculated, and very large amounts of these will make the calculation run more slowly. PME also needs to do a separate correction calculation for excluded atom pairs (atoms that should not have a nonbonded interaction, such as those that are connected by a bond). Large LES regions result in large numbers of excluded atoms, and these will result in a larger computational penalty for LES compared to non-LES simulations. For both of these reasons, it is more efficient computationally to use smaller LES regions- but see the discussion above for how region size affects simulation efficiency. These changes are included in the LES version of Sander (sander.LES). Each particle is assigned a LES 'type' (each new set of copies is a new type), and for each pair of types there is a scaling factor for the nonbond interactions between LES particles of those types. Most of the scaling factors are 1.0, but some are not - such as the diagonal terms which correspond to interactions inside a given subspace, and also off-diagonal terms where only some of the copies are in common. An example of this type is the side chain example given above- each of the 3 backbone copies has 2 sidechains, and while interactions inside the side chains need a factor of 6, interactions between the side chain and backbone need a factor of 3. This matrix of scaling factors is stored in the new topology file, along with the type for each atom, and the number of types. The changes made in sander relate to reading and using these scale factors.

9.5. Using LES with the Generalized Born solvation model

LES simulations can be performed using the GB solvent model, with some limitations. It is strongly recommended that the user read the article describing the derivation of the GB+LES approach [195]. The current code only allows igb=1 when using LES. Surface area calculations are not yet supported with LES. Only a single LES region is permitted for GB+LES simulations. A new namelist variable was introduced (RDT) in sander to control the compromise of speed and accuracy for GB+LES simulations. The article referenced above provides more detail on the function of this variable. RDT is the effective radii deviation threshold. When using GB+LES, non-LES atoms require multiple effective Born radii for an exact calculation. Using these multiple radii can significantly increase calculation time required for GB calculations. When the difference between the multiple radii for a non-LES atom is less than RDT, only a single effective radius will be used. A value of 0.01 has been found to provide a reasonable compromise between speed and accuracy, and is the default value.

9.6. Case studies: Examples of application of LES

9.6.1. Enhanced sampling for individual functional groups: Glucose.

The first example will deal with enhancing sampling for small parts of a molecule, such as individual functional groups or protein side chains. In this case we wanted to carry out separate simulations of α and β (not converting between anomers, only for conversions involving rotations about bonds) glucose, but the 5 hydroxyl groups and the strong hydrogen bonds between neighboring hydroxyls make conversion between different rotamers slow relative to affordable simulation times. The eventual goal was to carry out free energy simulations converting between anomers, but we need to ensure that each window during the Gibbs calculation would be able to

sample all relevant orientations of hydroxyl groups in their proper Boltzmann-weighted populations. We were initially unsure how many different types of structures should be populated and carried out non-LES simulations starting from different conformations. We found that transitions between different conformations were separated by several hundred picoseconds, far too long to expect converged populations during each window of the free energy calculation. We therefore decided to enhance conformational sampling for each hydroxyl group by making 5 copies of each hydroxyl hydrogen and also 5 copies of the entire hydroxymethyl group. Since the hydroxyl rotamer for each copy should be relatively independent, we decided to place each group in a different LES region. This meant that each hydroxyl copy interacted with all copies of the neighboring groups, with a total of 5*5*5*5*5 or 3125 structural combinations contributing to the LES average energy at each point in time. The input file is given below.

```
file rprm name=(parm.solv.top) read
file rcvb name=(glucose.solv.equ.crd) read
file wprm name=(les.prmtop) wovr
file wcrd name=(glucose.les.crd) wovr
action
~
omas
~
~ 5 copies of each hydroxyl hydrogen- copying oxygen will make no difference
~ since they will not be able to move significantly apart anyway
~
spac numc=5 pick chem prtc HO1 done
spac numc=5 pick chem prtc HO2 done
spac numc=5 pick chem prtc HO3 done
spac numc=5 pick chem prtc HO4 done
~
~ take the entire hydroxy methyl group
~
spac numc=5 pick #prt 20 24 done
*EOD
```

This worked quite well, with transitions now occurring every few ps and populations that were essentially independent of initial conformation [196].

9.6.2. Enhanced sampling for a small region: Application of LES to a nucleic acid loop

In this example, we consider a biomolecule (in this case a single RNA strand) for which part of the structure is reliable and another part is potentially less accurate. This can be the case in a number of different modeling situations, such as with homologous proteins or when the experimental data is incomplete. In this case two different structures were available for the same RNA sequence. While both structures were hairpins with a tetraloop, the loop conformations differed, and one was more accurate. We tested whether MD would be able to show that one structure was not stable and would convert to the other on an affordable timescale.

Standard MD simulations of several ns were not able to undergo any conversion between these two structures (the initial structure was always retained). Since the stem portion of the RNA

was considered to be accurate, LES was only applied to the tetraloop region. In this case, both of the ends of the LES region would be attached to the same locations in space, and there was no concern about copies diffusing too far apart to re-converge to the same positions after optimization. The issues that need to be addressed once again are the number of copies to use, and how to place the LES region(s). I usually start with the simplest choices and used 5 LES copies and only a single LES region consisting of the entire loop. If each half of the loop was copied, then it might become too *crowded* with copies near the base-pair hydrogen bonds and conformational changes that required moving a base through this regions could become even more difficult (see the background section for details). Therefore, one region was chosen, and the RNA stem, counterions and solvent were not copied. The ADDLES input file is given below.

```
~
file rprm name=(prm.top) read
file rcvb name=(rna.crd) read
file wprm name=(les.parm) wovr
file wcrd name=(les.crd) wovr
action
~
omas
~
~ copy the UUCG loop region- residues 5 to 8.
~ pick by atom number, though #mon 5 8 would work the same way
~
spac numc=5 pick #prt 131 255  done
*EOD
```

Subsequent LES simulations were able to reproducibly convert from what was known to be the incorrect structure to the correct one, and stay in the correct structure in simulations that started there. Different numbers of LES copies as well as slightly changing the size of the LES region (from 4 residues to 6, extending 1 residue beyond the loop on either side) were not found to affect the results. Fewer copies still converted between structures, but on a slower timescale, consistent with the barrier heights being reduced roughly proportional to the number of copies used. See Simmerling, Miller and Kollman, 1998, for further details.

9.6.3. Improving conformational sampling in a small peptide

In this example, we were interested not just in improving sampling of small functional groups or even individual atoms, but in the entire structure of a peptide. The peptide sequence is AVPA, with ACE and NME terminal groups. Copying just the side chains might be helpful, but would not dramatically reduce the barriers to backbone conformational changes, especially in this case with so little conformational variety inherent in the Ala and Pro residues. We therefore apply LES to all atoms. If we copied the entire peptide in 1 LES regions, the copies could float apart. While this would not be a disaster, it would make it difficult to bring all of the copies back together if we were searching for the global energy minimum, as described above. We therefore use more than one LES region, and need to decide where to place the boundaries between regions. A useful rule of thumb is that regions should be at least two amino acids in size, so we pick our two regions as Ace-Ala-Val and Pro-Ala- Nme. If we make five LES copies of each region and each copy does not interact with other copies of the same regions, each half the

peptide will be represented by five potentially different conformations at each point in time. In addition, since each copy interacts with all copies of the rest of the system, there are 25 different combinations of the two halves of the peptide that contribute at each point in time. This statistical improvement alone is valuable, but the corresponding barriers are also reduced by approximately the same factors. When we place the peptide in a solvent box the solvent interacts in an average way with each of the copies. The input file is given below, and all of the related files can be found in the test directory for LES.

```
~
~ all file names are specified at the beginning, before "action"
~
~ specify input prmtop
~
file rprm name=(prmtop) read
~
~ specify input coordinates, velocities and box (this is a restart file)
~
file rcvb name=(md.solv.crd) read
~
~ specify LES prmtop
~
file wprm name=(LES.prmtop) wovr
~
~ specify LES coordinates (and velocities and box since they
~ were input)
~
file wcrd name=(LES.crd) wovr
~
~ now the action command reads the files and tells addles to
~ process commands
~
action
~
~ do not scale masses of copied particles
~
omas
~
~ divide the peptide into 2 regions.
~ use the CCA option to place the division between carbonyl and
~ alpha carbon
~ use the "or" to make sure all atoms in the terminal residues
~ are included since the CCA option places the region division at C/CA
~ and we want all of the terminal residue included on each end
~
~ make 5 copies of each half
~
~ "spac" defines a LES subspace (or region)
~
```

```
spac numc=5 pick #cca 1 3 | #mon 1 1 done
~
spac numc=5 pick #cca 4 6 | #mon 6 6 done
~
~ the following line is required at the end
*EOD
```

This example brings up several important questions:

(1) should I make LES copies before or after adding solvent? Since LEaP is used to add solvent, and LEaP will not be able to load and understand a LES structure, you must run ADDLES after you have solvated the peptide in LEaP. ADDLES should be the last step before running SANDER.

(2) which structure should be used as input to ADDLES? If you will also be carrying out non-LES simulations, then you can equilibrate the non-LES simulation and carry out any amount of production simulation desired before taking the structure and running ADDLES. At the point you may switch to only LES simulations, or continue both LES and non-LES from the same point (using different versions of SANDER). Typically I equilibrate my system without LES to ensure that it has initial stability and that everything looks OK, then switch to LES afterward. This way I separate any potential problems from incorrect LES setup from those arising from problems with the non-LES setup, such as in initial coordinates, LEaP setup, solvent box dimensions and equilibration protocols.

(3) how can I analyze the resulting LES simulation? This is probably the most difficult part of using LES. With all of the extra atoms, most programs will have difficulty. For example, a given amino acid with LES will have multiple phi and psi backbone dihedral angles. There are basically two options: first, you can process your trajectory such that you obtain a single structure (non-LES). This might be just extracting one of the copies, or it might be one by taking the average of the LES copies. After that, you can proceed to traditional analysis but must keep in mind that the average structure may be non-physical and may not represent any actual structure being sampled by the copies, especially if they move apart significantly. A better way is to use LES-friendly analysis tools, such as those developed in the group of Carlos Simmerling. The visualization program MOIL-View (*http://morita.chem.sunysb.edu/~carlos/moil-view.html*) is one example of these programs, and has many analysis tools that are fully LES compatible. Read the program web page or manual for more details. A version of MOIL-View is included on the Amber 8 CD.

9.7. Unresolved issues with LES in AMBER

(1) Sander can't currently maintain groups of particles at different temperatures (important for dynamics, less so for optimization.) [197,198] Users can set *temp0les* to maintain all LES atoms at a temperature that is different from that for the system as a whole, but all LES atoms are then coupled to the same bath.

(2) Initial velocity issues as mentioned above- works properly, user must be careful.

(3) Analysis programs may not be compatible. See *http://morita.chem.sunysb.edu/~carlos/moil- view.htm*l for an LES-friendly analysis and visualization program.

(4) Visualization can be difficult, especially with programs that use distance-based algorithms to determine bonds. See #3 above.

(5) Water should not be copied- the fast water routines have not been modified. For most users this won't matter.

(6) Copies should not span different 'molecules' for pressure coupling and periodic imaging issues. Copies of an entire 'molecule' should result in the copies being placed in new, separate molecules- currently this is not done. This would include copying things such as counterions and entire protein or nucleic acid chains.

(7) Copies are placed into the same residue as the original atoms- this can make some residues much larger than others, and may result in less efficient parallelization with algorithms that assign nonbond workload based on residue numbers.

10. ptraj

The current version of *ptraj* is really two programs:

rdparm: a program to read, print (and modify) Amber prmtop files
usage: `rdparm` *prmtop*

ptraj: a program to process coordinates/trajectories
usage: `ptraj` *prmtop script*

Which code is used at runtime depends on the name of the executable (note that both *rdparm* and *ptraj* are created by default from the same source code when the programs are compiled with the supplied Makefile). If the executable name contains the string "rdparm", then the *rdparm* functionality is obtained. *rdparm* is semi-interactive (type `?` or `help` for a list of commands) and requires specification of an Amber prmtop file (this *prmtop* is specified as a filename typed on the command line; note that if no filename is specified you will be prompted for a filename).

If the executable name does not contain the string "rdparm", *ptraj* is run instead. *ptraj* also requires specification of parameter/topology information, however it currently supports both the Amber prmtop format and (I know, sacrilege!) CHARMM psf files. Note that the *ptraj* program can also be accessed from *rdparm* by typing *ptraj*.

The commands to *ptraj* can either be piped in through standard input or supplied in a file, where the filename (*script*) is passed in as the second command line argument. Note that if the *prmtop* filename is absent, the user will be prompted for a filename.

The code is written in ANSI compliant C and is fairly extensively documented and meant to be extended by able users!. Along with this code is distributed public domain C code from the Computer Graphics Lab at UCSF for reading and writing PDB files. Note that this program is updated more frequently than the general Amber release and that new versions and documentation may be obtained through links on the Amber WWW page.

Usage: `ptraj` *prmtop script*

ptraj is a program to process and analyze sets of 3-D coordinates read in from a series of input coordinate files (in various formats as discussed below). For each coordinate set read in, a sequence of events or *ACTIONS* is performed (in the order specified) on each of the configurations (set of coordinates) read in. After processing all the configurations, a trajectory file and other supplementary data can be optionally written out.

To use the program it is necessary to (1) read in a parameter/topology file, (2) set up a list of input coordinate files, (3) optionally specify an output file and (4) specify a series of actions to be performed on each coordinate set read in.

(1) **reading in a parameter/topology file**:

This is done at startup and currently either an Amber prmtop or CHARMM psf file can be read in. The type of the file is autodetected. The information in these files is used to setup the global *STATE* (ptrajState *) which gives information about the number of atoms, residues, atom names, residue names, residue boundaries, *etc*. This information is used to guide the reading of input coordinates which MUST match the order specified by the state, otherwise garbage may be obtained (although this may be detected by the program for some file

formats, leading to a warning to the user). In other words, when reading a pdb file, the atom order must correspond exactly to that of the parameter/topology information; in the pdb the names/residues are ignored and only the coordinates are read in based.

(2) **set up a list of input coordinate files**:

This is done with the `trajin` command (described in more detail below) which specifies the name of a coordinate file and optionally the start, stop and offset for reading coordinates. The type of coordinate file is detected automatically and currently the following input coordinate types are supported:

- Amber trajectory
- Amber restart (or inpcrd)
- PDB
- CHARMM (binary) trajectory
- Scripps "binpos" binary trajectory

(3) **optionally specify an output trajectory file**:

This is done with the `trajout` command (discussed in more detail below). Trajectories can currently be written in Amber trajectory (default), Amber restrt, Scripps binpos, PDB or CHARMM trajectory (in little or big endian binary format).

(4) **specify a list of actions**:

There are a variety of coordinate analysis/manipulation **actions** provided and each of these specified-- note that each action can be repeated-- is applied sequentially to the coordinates in the order listed by the user in the input file.

As mentioned above, input to `ptraj` is in the form of commands listed in a script (or if absent, from text on standard input). An example input file to `ptraj` follows:

```
trajin traj1.Z 1 20
trajin traj2.Z 1 100
trajin restrt.Z
trajout fixed.traj
rms first out rms @CA,C,N
center :1-20
image
strip :WAT
go
```

This reads in three files of coordinates (which can be compressed and the type is autodetected), a trajectory file is output (by default to Amber trajectory format), rms fitting is performed to the first coordinate frame using atoms names CA, C and N (storing the RMSd values to a file named "rms"), the center of geometry of residues 1-20 is placed at the origin, the coordinates are imaged (which requires periodic boundary conditions) to move coordinates outside the periodic box back in, and then the coordinates of all the residues named "WAT" are deleted.

10.1. ptraj command prerequisites

Before going into the details of each of the commands, some prerequisites are necessary to describe the command flow and the standard argument types. Effectively, all the commands are

processed from the input file in the order listed, except for the input/output commands. Input is the first step and involves reading in all the coordinates sets from each file specified, in the order specified, a single coordinate set at a time. For each coordinate set read in, all of the actions specified are applied and then the potentially modified coordinates are output. Not all of the actions actually modify the coordinates and some of the commands simply change the state (such as **solvent** which just changes the definition of what the solvent molecules are). Some of the actions just accumulate data (such as distances, angles and sugar puckers). Writing out of any accumulated data is deferred until all of the coordinate sets have been read in. Some of the actions load up contiguous sets of coordinates into main memory; with large coordinate sets this may require large amounts of memory. In these cases, such as with the command **2dRMS**, it may be useful only to "save" the necessary coordinates by performing a **strip** of unnecessary coordinates prior to the **2dRMS** call.

In the discussion that follows commands are listed in **bold** type. Words in *italics* are values that need to be specified by the user, and words in standard text are keywords to specify an option (which may or may not be followed by a value). In the specification of the commands, arguments in square brackets (`[]`'s) are optional and the "`|`" character represents "or". Arguments that are not in square brackets are required. In general, if there is an error in processing a particular action, that action will be ignored and the user warned (rather than terminating the program), so check the printed WARNING's carefully... In what follows is listed a few standard argument types:

mask: this is an atom or residue mask; it represents the list of active atoms. The current parser recognizes a simplified midas style format for picking atoms and residues. The "`@`" character represents an atom selection and the "`:`" character represents a residue selection. Either the atom and residue names or numbers can be specified. The "`-`" character represents a continuation. The "`~`" represents "not" and in this naive implementation, if this character is specified anywhere in the string, the "not" flag will be turned on. The "`*`" character is a wild card and will match all the atoms if specified alone. When specified in atom or residue name specifications, sometimes it will correctly work as a wildcard. The "`?`" character is also a wildcard, however only one character is matched. Note that the current parser is not very sophisticated. Until this is "fixed", check the output very carefully; note that whenever an atom mask is used, a summary of the atoms selected is printed, so check this out...

filename: this refers to the full path to a file and note that no checking is done for existing files, i.e. data will be overwritten if you attempt to write to an existing file.

10.2. ptraj input/output commands

trajin *filename* [*start stop offset*]

Load the trajectory file specified by *filename* using only the frames starting with *start* (default 1) and ending with (and including) *stop* (default, the final configuration) using an offset of *offset* (default 1) if specified. Amber trajectory, restrt/inpcrd, PDB, Scripps BINPOS and CHARMM binary trajectory files are all currently supported and the type of file is auto-detected (including the CHARMM binary file byte ordering). Compressed files (filenames with an appended `.Z` or `.gz` are also recognized and treated appropriately). Note that the coordinates *must* match the names/ordering of the parameter/topology information previously read in.

reference *filename*

Load up a the first coordinate set from the trajectory specified by the file named *filename* and save this for use as a reference structure. Currently only the **rms** command potentially uses this reference structure. Note that as the state is modified (for example by **strip** or **closestwaters**), the reference coordinates are also modified internally.

trajout *filename* [*format*] [nobox] [little | big] [dumpq | parse] [nowrap] [les split|average]

Specify the name of the file of output coordinates to write (*filename*) and the format (*format*). Currently supported formats are "trajectory" (or Amber trajectory, the default), "restart" (Amber restart), "binpos" (Scripps binary format), "pdb" (PDB), or "charmm" (CHARMM binary trajectory). With the CHARMM files, it is possible to specify the byte ordering as "little" or "big" endian, with the default being that which the first CHARMM trajectory file was read in as, or if none was read in, big endian. With the PDB output, it is possible to include charges and radii in higher precision temperature/occupancy columns with the additional keyword "dumpq" (to dump Amber charges and radii, assuming a Amber prmtop has been previously read in) or "parse" (to dump charges and parse radii). By default (and differing from earlier versions of `ptraj`), atom names are wrapped in the PDB file to put the 4th letter of the atom name first. If you want to avoid this behavior, specify "nowrap"; the former is more consistent with standard PDB usage but departs from the format written in previous versions of this program. Note that if more than one coordinate set is to be output, with the pdb and restrt/inpcrd formats, extensions (based on the current configuration number) will be appended to the filenames and therefore only one coordinate set will be written per file. The optional keyword "nobox" will prevent box coordinates from being dumped to Amber trajectory files; this is useful if one is stripping the solvent from a trajectory file and you don’t want that pesky box information cluttering up the trajectory and messing with other programs... Note that if periodic box information is present in the CHARMM trajectory file, when a new CHARMM trajectory file is written (in versions > 22) the symmetric box information will be *very* slightly different due to numerical issues in the diagonalization procedure; this will not effect analysis but shows up if diffing the binary files. The option keyword "les" is used for the analyze of LES trajectory, option "split" will output P non-LES trajectory(P is copy number), and option "average" will output one non- LES trajectory contain the averaged conformation, currently only single LES region is allowed.

10.3. ptraj commands that modify the state

These commands change the state of the system, such as to delete atoms.

box [x *value*] [y *value*] [z *value*] [alpha *value*] [beta *value*] [gamma *value*]
[fixx] [fixy] [fixz] [fixalpha] [fixbeta] +[fixgamma]

This command allows specification and optionally fixing of the periodic box (unit cell) dimensions. This can be useful when reading PDB files that do not contain box

information. In the standard usage, without the "fix*N*" keywords, if the box information is not already present in the input trajectory (such as the case with restart files or trajectory files) this command can be used to set the default values that will be applied. If you want to force a particular box size or shape, the "fixx", "fixy", etc commands can be used to override any box information already present in the input coordinate files.

solvent [byres | bytype | byname] *mask1* [*mask2*] [*mask3*] ...

This command can be used to override the solvent information specified in the Amber prmtop file or that which is set by default (based on residue name) upon reading a CHARMM psf. Applying this command overwrites any previously set solvent definitions. The solvent can be selected by residue with the "byres" modifier using all the residues specified in the one or more atom masks listed. The byname option searches for solvent by residue name (where the mask contains the name of the residue), searching over all residues. The "bytype" option is intended for use in selecting solvents that span multiple residues, however it is not yet implemented since I haven't found a case where it is necessary (and setting the solvent information in the code is a real nightmare).

As an example, say you want to select the solvent to be all residues from 20-100, then you would do

```
solvent byres :20-100
```

Note that if you don't know the final residue number of your system offhand, yet you do know that the solvent spans all residues starting at residue 20 until the end of the system, just chose an upper bound and the program will reset accordingly, *i.e.*

```
solvent byres :20-9999
```

To select all residues named "WAT" and "TIP3" and "ST2":

```
solvent byname WAT TIP3 ST2
```

Note that if you just want to peruse what the current solvent information is (or more generally get some information about the current state), specify **solvent** with no arguments and a summary of the current state will be printed.

Other commands which also modify the state are **strip** and **closestwaters**. These commands are described in the next section since they also modify the coordinates.

10.4. **ptraj** *action* commands

The following are commands that involve an *action* performed on each coordinate set as it is read in. The commands are listed in alphabetical order. Note that in the script the commands are applied in the order specified and some may change the overall state (more on this later). All of the actions can be applied repeatedly. Note that in general (except where otherwise mentioned) imaging in non-orthorhombic systems is now supported, however note that this code has not been

extensively tested.

angle *name mask1 mask2 mask3* [out *filename*] [time *interval*]

Calculate the angle between the three atoms listed, each specified in a mask, *mask1* through *mask3*. If more than one atom is listed in each mask, then the center of mass of the atoms in that mask is used at the position. The results are saved internally with the name *name* (which must be unique) on the `scalarStack` for later processing (with the **analyze** command). Data will be dumped to a file named *filename* if "out" is specified (with a time interval between configurations of *interval* if "time" is listed). All the angles are stored in degrees.

atomicfluct [out *filename*] [*mask*] [start *start*] [stop *stop*] [offset *offset*]
[byres | byatom | bymask] [bfactor]

Compute the atomic positional fluctuations for all the atoms; output is performed only for the atoms in *mask*. If "byatom" is specified, dump the calculated fluctuations by atom (default). If "byres" is specified, dump the average (mass-weighted) for each residue. If "bymask" is specified, dump the average (mass-weighted) over all the atoms in the original *mask*. If "out" is specified, the data will be dumped to *filename* (otherwise the values will be dumped to the standard output). The "start", "stop" and "offset" keywords can be used to specify the range of coordinates processed (as a subset of all of those read in across all input files, not to be confused with the individual specification in each **trajin** command). If the keyword "bfactor" is specified, the data is output as B-factors rather than atomic positional fluctuations (which simply means multiplying the results by (8/3)pi**2).

So, to dump the mass-weighted B-factors for the protein backbone atoms, by residue:

```
atomicfluct out back.apf @C,CA,N byres bfactor
```

average *filename* [*mask*] [start *start*] [stop *stop*] [offset *offset*] [pdb [parse | dumpq]
[nowrap] | binpos | rest] [nobox] [stddev]

Compute the average structure over all the configurations read in (subject to start, stop and offset if set) dumping the results to a file named *filename*. If the keyword "stddev" is present, save the standard deviations (fluctuations) instead of the average coordinates. Output is by default to an Amber trajectory, however can also be to a pdb, binpos or restrt file (depending on the keyword chosen). The "nobox" keyword will suppress box coordinates, and with the PDB format, it is possible to dump charges and radii (with the "dumpq" keyword for Amber radii and charges or the "parse" for parse radii and Amber charges) and prevent atom name wrapping "nowrap". The optional *mask* trims the output coordinates (but does not change the state). This command does not alter the coordinates as they are processed. If you want to alter the coordinates by averaging (for use by actions further on), use the **runningaverage** command.

center [*mask*] [origin] [mass]

If we are in periodic boundary conditions, center all the atoms based on the center of geometry of the atoms in the *mask* to the center of the periodic box or the origin if the optional argument "origin" is specified. If the trajectory is not a periodic boundary trajectory, then the molecule is implicitly centered to the origin. If no *mask* is specified, centering is relative to all the atoms. If "mass" is specified, center with respect to the center of mass instead.

checkoverlap [*mask*] [min *value*] [max *value*] [noimage]

Look for pair distances in the selected atoms (all by default) that are less than the specified minimum value (in angstroms, 0.95 by default) apart or greater than the maximum value (if specified). This command is rather computationally demanding, particularly if imaging is turned on (by default), but it is extremely useful for diagnosing problems in input coordinates related to poor model building.

closest *total mask* [oxygen | first] [noimage]

Retain only *total* solvent molecules (using the solvent information specified, see **solvent** above) in each coordinate set. The solvent molecules saved are those which are closest to the atoms in the *mask*. If "oxygen" or "first" are specified, only the distance to the first atom in the solvent molecule (to each atom in the mask) is measured. This command is rather time consuming since many distances need to be measured. Note that imaging is implicitly performed on the distances and this gets extremely expensive in non-orthorhombic systems due to the need to possibly check all the distances of the nearest images (up to 26!). Imaging can be disabled by specifying the "noimage" keyword.

Note that the behavior of this command is slightly different than in previous `ptraj` versions; now the solvent molecules are ordered at output such that the closest solvent is first and the PDB file residue numbers no longer represent the identity of the water in the original coordinate set. This command should now work with non-sequential solvent molecules and be independent of where the water is located. Like the **strip** command, this modifies the current state (i.e. pars down the size of the trajectory which is useful in cases where subsets of a trajectory may be loaded into memory). A restriction of this command is that each of the solvent molecules must have the same number of atoms; this leads to a fixed size "configuration" in each coordinate set output which is necessary for most of the file formats and to avoid really complicating the code.

Of course, say you have two solvents of differing sizes and you want to perform closest to each of these, this can be done sequentially. Say we have both ethanol ":ETH" and water ":WAT" present, and you want to save the closest 50 of each to residues :1-20

```
solvent byres :WAT
closestwater 50 :1-20 first
```

```
solvent byres :ETH
closestwater 50 :1-20 first
```

contacts [first|reference] [byresidue] [out *filename*] [time *interval*]
[distance *cutoff*] [*mask*]

For each atom given in *mask*, calculate the number of other atoms (contacts) within the distance *cutoff*. The default cutoff is 7.0 A. Only atoms in *mask* are potential interaction partners (e.g., a mask @CA will evaluate only contacts between CA atoms). The results are dumped to *filename* if the keyword "out" is specified. Thereby, the time between snapshots is taken to be *interval*. In addition to the number of overall contacts, the number of native contacts is also determined. Native contacts are those that have been found either in the first snapshot of the trajectory (if the keyword "first" is given) or in a reference structure (if the keyword "reference" is given). Finally, if the keyword "byresidue" is provided, results are output on a per-residue basis for each snapshot, whereby the number of native contacts is written to *filename*.native .

dihedral *name mask1 mask2 mask3 mask4* [out *filename*]

Calculated the dihedral angle for the four atoms listed in *mask1* through *mask4* (representing rotation about the bond from *mask2* to *mask3*). If more than one atom is listed in each mask, treat the position of that atom as the center of mass of the atoms in the mask. The results are saved internally with the name *name* (which must be unique) and the data is stored on the `scalarStack` for later processing. Data will be dumped to a file if "out" is specified (with a *filename* appended). All the angles are listed in degrees.

diffusion *mask time_per_frame* [average] [*filenameroot*]

Compute a mean square displacement plot for the atoms in the *mask*. The time between frames in picoseconds is specified by *time_per_frame*. If "average" is specified, then the average mean square displacement is calculated and dumped (only). If "average" is not specified, then the average and individual mean squared displacements are dumped. They are all dumped to a file in the format appropriate for xmgr (dumped in multicolumn format if necessary, i.e. use xmgr -nxy). The units are displacements (in angstroms**2) vs time (in ps). The *filenameroot* is used as the root of the filename to be dumped. The average mean square displacements are dumped to "filenameroot_r.xmgr", the x, y and z mean square displacements to "filenameroot_x.xmgr", etc and the total distance traveled to "filenameroot_a.xmgr".

This will fail if a coordinate moves more than 1/2 the box in a single step. Also, this command implicitly unfolds the trajectory (in periodic boundary simulations) hence will currently only work with orthorhombic unit cells.

dipole *filename nx x_spacing ny y_spacing nz z_spacing mask1* origin | box [max *max_percent*]

Same as **grid** (see below) except that dipoles of the solvent molecules are binned. Dumping is to a grid in a format for Chris Bayly's discern delegate program that comes with Midas/Plus.

distance *name mask1 mask2* [out *filename*] [noimage]

This command will calculate a distance between the center of mass of the atoms in *mask1* to the center of mass of the atoms in *mask2* and store this information into an array with *name* as the identifier (a name which must be unique and which is placed on the `scalarStack` for later processing) for each frame in the trajectory. If the optional keyword "out" is specified, then the data is dumped to a file named *filename*. The distance is implicitly imaged (for both orthorhombic and non-orthorhombic unit cells) and the shortest imaged distance will be saved (unless the "noimage" keyword is specified which disables imaging).

grid *filename nx x_spacing ny y_spacing nz z_spacing mask1* [origin | box] [negative] [max *fraction*]

Create a grid representing the histogram of atoms in *mask1* on the 3D grid that is "*nx* * *x_spacing* by *ny* * *y_spacing* by *nz* * *z_spacing* angstroms (cubed). Either "origin" or "box" can be specified and this states whether the grid is centered on the origin or half box. Note that to provide any meaningful representation of the density, the solute of interest (about which the atomic densities are binned) should be rms fit, centered and imaged prior to the **grid** call. If the optional keyword "negative" is also specified, then these density will be stored as negative numbers. Output is in the format of a XPLOR formatted contour file (which can be visualized by the density delegate to Midas/Plus or other programs). Upon dumping the file, pseudo-pdb HETATM records are also dumped to standard out which have the most probable grid entries (those that are 80% of the maximum by default which can be changed with the max keyword, i.e. max .5 makes the dumping at 50% of the maximum).

Note that as currently implemented, since the XPLOR grids are integer based, the grid is offset from the origin (towards the negative size) by half the grid spacing.

image [origin] [center] *mask* [bymol | byres | byatom | bymask] *mask*
[triclinic | familiar [com *mask*]]

Under periodic boundary conditions, which particular unit cell a given molecule is in does not matter as long as, as a whole, all the molecules "image" into a single unit cell. In an MD simulation, molecules drift over time and may span multiple periodic cells unless "imaging" is enabled to shift molecules that leave back into the primary unit cell. In sander, the IWRAP variable controls this, with IWRAP=1 implying turning on imaging. This command, **image** allows post-processing of the imaging to force all the molecules into the primary unit cell.

If the optional argument "origin" is specified, then imaging will occur to the coordinate origin (like in SPASMS) rather than the center of the box (as is the Amber standard). By default all atoms are imaged *by molecule* based on the position of the first

atom (or the center of mass of the molecule if "center" is specified; the latter is recommended). If the *mask* is specified, only the atoms in the *mask* will be imaged. It is now possible to image by atom (byatom), by residue (byres), by molecule (bymol, default) or by atom mask (where all the atoms in the mask are treated as belonging to a single molecule). The behavior of the "by molecule" imaging is different in CHARMM and Amber; with Amber the molecules are specified directly by the periodic box information whereas with the CHARMM parameter/topology, each segid is treated as a different molecule. With this newer implementation of the imaging code, it is possible to avoid breaking up double stranded DNA during imaging, *i.e.*:

```
image :1-20 bymask :1-20
image byres :WAT
```

[Of course this assumes that the coordinates of the two strands were not displaced during the dynamics as well!] Imaging only makes sense if there is periodic box information present.

Non-orthorhombic unit cells are now supported! Use of the triclinic imaging can be forced with the "triclinic" keyword. Note that this puts the box into the triclinic shape, not the more familiar, more spherical shapes one might expect for some of the unit cells (i.e. truncated octahedron). To get into the more familiar shape, specify the "familiar" keyword. In this case, to specify atoms that imaged molecules should be closest to, specify a center of the atoms in the mask specified with the "com" keyword. Note that imaging "familiar" is time consuming (but recommended) since each of the possible imaged distances (27) are checked to see which is closest to the center.

principal *mask* [dorotation]

Principal axis transformation to align the atoms specified in *mask*. This is reasonably functional as there are still issues with degenerate eigenvalues and unwanted coordinate swapping. To align whole system along the principal axes specify "dorotation".

pucker *name mask1 mask2 mask3 mask4 mask5* [out *filename*] [amplitude]
[altona | cremer] [offset *offset*]

Calculate the pucker for the five atoms specified in each of the mask's, *mask1* through *mask5*, associating *name* (which must be unique) with the calculated values. If more than one atom is specified in a given mask, the center of mass of all the atoms in that mask is used to define the position. If the "out" keyword is specified the data is dumped to *filename*. If the keyword "amplitude" is present, the amplitudes are saved rather than the pseudorotation values. If the keyword "altona" is listed, use the Altona & Sundarlingam conventions/algorithm (for nucleic acids) (the default) [see Altona & Sundaralingam, *JACS* 94, 8205-8212 (1972) or Harvey & Prabhakaran, *JACS* 108, 6128-6136 (1986).] In this convention, both the pseudorotation phase and amplitude are in degrees.

If "cremer" is specified, use the Cremer & Pople conventions/algorithm [see Cremer & Pople, *JACS* 97:6, 1354-1358 (1975).]

Note that to calculate nucleic acid puckers, specify C1' first, followed by C2', C3', C4' and finally O4'. Also note that the Cremer & Pople convention is offset from the Altona & Sundarlingam convention (with nucleic acids) by 90.0; to add in an extra 90.0 to "cremer" (offset -90.0) or subtract 90.0 from the "Altona" (offset 90.0) specify an *offset* with the offset keyword; this value is subtracted from the calculated pseudorotation value (or amplitude).

radial *root-filename spacing maximum solvent-mask* [*solute-mask*] [closest] [density *value*] [noimage]

Compute radial distribution functions and store the results into files with *root-filename* as the root of the filename. Three files are currently produced, "*root-filename*_carnal.xmgr" (which corresponds to a carnal style RDF), "*root-filename*_standard.xmgr" (which uses the more traditional RDF with a density input by the user) and "*root-filename*_volume.xmgr" (which uses the more traditional RDF and the average volume of the system). The total number of bins for the histogram is determined by the spacing between bins (*spacing*) and the range which runs from zero to *maximum*. If only a *solvent-mask* is listed (i.e. a list of atoms) then the RDF will be calculated for the interaction of every solute-mask atom with ALL the other solute-mask atoms.

If the optional *solute-mask* is specified, then the RDF will represent the interaction of each solute-mask atom with ALL of the solvent-mask atoms. If the optional keyword "closest" is specified, then the histogram will bin, over all the solvent-mask atoms, the distance of the closest atom in the solute mask. If the *solute-mask* and *solvent-mask* atoms are not mutually exclusive, zero distances will be binned (although this should not break the code). If the optional keyword "density", followed by the density *value* is specified, this will be used in the calculations. The default value is 0.033456 molecules/angstrom**3 which corresponds to a density of water equal to 1.0 g/mL. To convert a standard density in g/mL, multiply the density by "6.022 / (10 * weight)" where "weight" is the mass of the molecule in atomic mass units. This will only effect the "*root-filename*_standard.xmgr" file.

Note that although imaging of distances is performed (to find the shortest imaged distance unless the "noimage" keyword is specified), minimum image conventions are applied.

Also note that when LES prmtop and trajectories is processed, the interaction between atoms from different copy is ignored, which allowes users to get the right RDF, but users still need to adjust the density to get the right answer.

radgyr [out *filename*] [time *interval*] [*mask*]

Calculate the radius of gyration considering atoms in *mask*. The results are dumped to *filename* if the keyword "out" is specified. Thereby, the time between snapshots is

taken to be *interval*.

rms *mode* [*mass*] [out *filename*] [time *interval*] *mask* [name *name*] [nofit]

This will RMS fit all the atoms in the *mask* based on the current *mode* which is:

previous: fit to previous frame
first: fit to the "start" frame of the first trajectory specified.
reference: fit to a the reference structure (which must have been previously read in)

If the keyword "mass" is specified, then a mass-weighted RMSd will be performed. If the keyword "out" is specified (followed immediately by a *filename*), the RMSd values will be dumped to a file. If you want to specify an time interval between frames (used only when dumping the RMSd vs time), this can be done with the "time" keyword. To save the calculated values for later processing, associate a name with the "name" keyword (where the chosen *name must be unique and the data will be stored on the* `scalarStack` for later processing. If the keyword "nofit" is specified, then the coordinates are not modified, just the RMSd values are calculated (and stored or output if the name or out keywords were specified).

secstruct [out *filename*] [time *interval*] [*mask*]
Calculate the secondary structure information for residues of atoms contained in *mask*, following the DSSP method by Kabsch & Sander. The *mask* is primarily intended to strip water molecules etc. Not providing contiguous protein sequences may result in erroneous secondary structure assignments (even at residues that are included in the mask!). The results are dumped to *filename* if the keyword "out" is specified. Thereby, the time between snapshots is taken to be *interval*. For every snapshot and every residue, an alpha-helix is indicated by "H", a 3-10-helix by "G", a pi-helix by "I", a parallel beta-sheet by "b", and an antiparallel beta-sheet by "B". A summary providing the percentage for each residue to adopt one of the above secondary structure types over the course of the analyzed snapshots is given in *filename*.sum.

strip *mask* Strip all atoms matching the atoms in *mask*. This changes the state of the system such that all commands (actions) following the strip (including output of the coordinates which is done last) are performed on the stripped coordinates (*i.e.* if you strip all the waters and then on a later action try to do something with the waters, you will have trouble since the waters are gone). A benefit of stripping, beyong paring down trajectories is with the data intensive commands that read entire sections of the trajectory into memory; with the strip to retain only selected atoms, it is much less likely that you will blow memory.

translate *mask* [x *x-value*] [y *y-value*] [z *z-value*]

Move the coordinates for the atoms in the *mask* in each direction by the offset(s) specified.

truncoct *mask distance* [prmtop *filename*]

Create a truncated octahedron box with solvent stripped to a distance *distance* away from the atoms in the *mask*. Coordinates are output to the trajectory specified with the **trajout** command. *Note that this is a special command* and will only really make sense if a single coordinate set is processed (*i.e.* any prmtop written out will only correspond to the first configuration!) and commands after the **truncoct** will have undefined behavior since the state will not be consistent with the modified coordinates. It is intended only as an aid for creating truncated octahedron restrt files for running in Amber.

The "prmtop" keyword can be used to specify the writing of a new prmtop (to a file named *filename*; this prmtop is only consistent with the first set of coordinates written. Moreover, this command will only work with Amber prmtop files and assumes an Amber prmtop file has previously been read in (rather than a CHARMM PSF). This command also assumes that all the solvent is located contiguously at the end of the file and that the solvent information has previously been set (see the **solvent** command).

watershell *mask filename* [lower *lower* upper *upper*] [*solvent-mask*] [noimage]

This option will count the number of waters within a certain distance of the atoms in the *mask* in order to represent the first and second solvation shells. The output is a file into *filename* (appropriate for xmgr) which has, on each line, the frame number, number of waters in the first shell and number of waters in the second shell. If *lower* is specified, this represents the distance from the *mask* which represents the first solvation shell; if this is absent 3.4 angstroms is assumed. Likewise, *upper* represents the range of the second solvation shell and if absent is assumed to be 5.0 angstroms. The optional *solvent-mask* can be used to consider other atoms as the solvent; the default is ":WAT". Imaging on the distances is done implicitly unless the "noimage" keyword has been specified.

10.5. Correlation and fluctuation facility

The *ptraj* program now contains several related sets of commands to analyze correlations and fluctuations, both from trajectories and from normal modes. These items replace the *correlation* command in previous versions of *ptraj*, and also replace what used to be done in the *nmanal* program. Some examples of command sequences are given at the end of this section.

vector *name mask* [principal [x|y|z] | dipole | box | corrplane | ired *mask2* |
corr *mask2* | corrired *mask2*] [out *filename*] [order *order*] [modes *modesfile*] [beg *beg*] [end *end*] [npair *npair*]

This command will keep track of a vector value (and its origin) over the trajectory; the data can be referenced for later use based on the *name* (which must be unique among the vector specifications). "Ired" vectors, however, may only be used in connection with the command "matrix ired". If the optional keyword "out" is specified (not valid for "ired" vectors), the data will be dumped to the file named *filename*.

The format is frame number, followed by the value of the vector, the reference point, and the reference point plus the vector. What kind of vector is stored depends on the keyword chosen.

principal [x | y | z]: store one of the principal axis vectors determined by diagonalization of the intertia matrix from the coordinates of the atoms specified by the *mask*. If none of x | y | z are specified, then the principal axis (i.e. the eigenvector associated with the largest eigenvalue) is stored. The eigenvector with the largest eigenvalue is "x" (i.e. the hardest axis to rotate around) and the eigenvector with the smallest eigenvalue is "z" and if one of x | y | z are specified, that eigenvector will be dumped. The reference point for the vector is the center of mass of the *mask* atoms.

dipole: store the dipole and center of mass of the atoms specified in the *mask*. The vector is not converted to appropriate units, nor is the value well-defined if the atoms in the mask are not overall charge neutral.

box: store the box coordinates of the trajectory. The reference point is the origin or (0.0, 0.0, 0.0).

ired *mask2*: This defines ired vectors necessary to compute an ired matrix (see matrix command). The vectors must be defined *prior* to the matrix command.

corrplane: This defines a vector perpendicular to the (least-squares best) plane through a series of atoms given in *mask*, for which a time correlation function can be calculated subsequently with the command "analyze timecorr ...". *order* specifies the order of the Legendre polynomial used (0 <= order <= 2). It defaults to 2.

corr *mask2*: This defines a vector between the center of mass of *mask* and the one of *mask2*, for which a time correlation function can be calculated subsequently with the command "analyze timecorr ...". *order* specifies the order of the Legendre polynomial used (0 <= order <= 2). It defaults to 2.

corrired *mask2*: This defines a vector between the center of mass of *mask* and the one of *mask2*, for which a time correlation function according to the Isotropic Reorientational Eigenmode Dynamics (ired) approach [199] can be calculated. *order* specifies the order of the Legendre polynomial used (0 <= order <= 2). It defaults to 2. To calculate this vector, ired modes need to be provided by *modesfile*. They can be calculated by the commands "matrix ired ...", followed by "analyze matrix ...". Only modes <beg> to <end> are considered. Default is beg = 1, end = 50. To obtain meaningful results, it is important that the vector definition agrees with the one used for calculation of the ired matrix (there is no internal check for this). Along these lines, *npair* needs to be specified, which relates to the position of this definition in the sequence of ired (not corrired!) vectors used to obtain the ired matrix.

matrix dist | covar | mwcovar | distcovar | correl | idea | ired [name *name*] [order *order*] [*mask1*] [*mask2*] [out *filename*]" [start *start*] [stop *stop*] [offset *offset*] [byatom | byres | bymask] [mass]

Compute DISTance, COVARiance, Mass-Weighted COVARiance, CORRELation,

DISTance-COVARiance, Isotropically Distributed Ensemble Analysis [200], or Isotropic Reorientational Eigenmode Dynamics [199] matrices. Results are output to *filename* if given. Be aware, matrix dimension will be of the order of N x M for dist, correl, idea, and ired, 3N x 3M for covar and mwcovar, and N(N-1) x N(N-1) / 4 for distcovar (with N being the number of atoms in *mask1* and M being the number of atoms either in *mask1* or *mask2*).

"byatom" dumps the results by atom (default). This is the sole option for covar, mwcovar, distcovar, idea, and ired. In the case of dist or correl, "byres" calculates an average for each residue and "bymask" dumps the average over all atoms in the mask(s). With "mass", mass-weighted averages will be computed.

In the case of ired, mask information must not be given. Instead, "ired vectors" need to be defined *prior* to the matrix command by using the vector command. Otherwise, if no mask is given, all atoms against all are used. If only *mask1* is given, a symmetric matrix is computed. In the case of distcovar and idea, only *mask1* (or none) may be given. In the case of distcovar, mwcovar, and correl, if *mask1* and *mask2* is given, on output *mask1* atoms are listed column-wise while *mask2* atoms are listed row-wise. The number of atoms covered by *mask1* must be >= the number of atoms covered by *mask2* (this is also checked in the function).

The matrix may be stored internally on the matrixStack with the name *name* for latter processing (with the "analyze matrix" command). Since at the moment this only involves diagonalization, storing is only available for (symmetric) matrices generated with *mask1* (or no mask or ired matrices).

The *start*, *stop*, and *offset* parameters can be used to specify the range of coordinates processed (as a subset of all of those read in across all input files).

The *order* parameter chooses the order of the Legendre polynomial used to calculate the ired matrix.

analyze matrix *matrixname* [out *filename*] [thermo] [vecs *vecs*] [reduce]

Diagonalizes the matrix *matrixname*, which has been generated and stored before by the matrix command. This is followed by Principal Component Analysis (in cartesian coordinate space in the case of a covariance matrix or in distance space in the case of a distance-covariance matrix), or Quasiharmonic Analysis (in the case of a mass-weighted covariance matrix). Diagonalization of distance, correlation, idea, and ired matrices are also possible. Eigenvalues are given in cm^{-1} in the case of a mass-weighted covariance matrix and in the units of the matrix elements in all other cases. In the case of a mass-weighted covariance matrix, the eigenvectors are mass-weighted.

Results [average coordinates (in the case of covar, mwcovar, correl), average distances (in the case of distcovar), main diagonal elements (in the case of idea and ired), eigenvalues, eigenvectors] are output to *filename*. *vecs* determines, how many eigenvectors and eigenvalues are calculated. The value must be >= 1, except if the "thermo" flag is given (see below). In that case, setting vecs = 0 results in

calculating all eigenvalues, but no eigenvectors. This option is mainly intended for saving memory in the case of thermodynamic calculations. "reduce" (only possible for covar, mwcovar, and distcovar) results in reduced eigenvectors [Abseher & Nilges, *J. Mol. Biol.* **279,** 911, (1998)]. They may be used to compare results from PCA in distance space with those from PCA in cartesian-coordinate space.

"thermo" calculates entropy, heat capacity, and internal energy from the structure of a molecule (average coordinates, see above) and its vibrational frequencies using standard statistical mechanical formulas for an ideal gas. This option is only available for mwcovar matrices.

analyze modes fluct|displ|corr stack *stackname* | file *filename*
[beg *beg*] [end *end*] [bose] [factor *factor*] [out *outfile*] [maskp *mask1 mask2* [...]]

Calculates rms fluctuations ("fluct"), displacements of cartesian coordinates along mode directions ("displ"), or dipole-dipole correlation functions ("corr") for modes obtained from principal component analyses (of covariance matrices) or quasiharmonic analyses (of mass-weighted covariance matrices). Thus, a possible series of commands would be "matrix covar|mwcovar ..." to generate the matrix, "analyze matrix ..." to calculate the modes, and, finally, "analyze modes ...". Modes can be taken either from an internal stack, identified by their name on the stack, *stackname*, or can be read from a file *filename*. Only modes *beg* to *end* are considered. Default for *beg* is 7 (which skips the first 6 zero-frequency modes in the case of a normal mode analysis); for *end* it is 50. If "bose" is given, quantum (Bose) statistics is used in populating the modes. By default, classical (Boltzmann) statistics is used. *factor* is used as multiplicative constant on the amplitude of displacement. Default is factor = 1. Results are written to *outfile*, if specified, otherwise to stdout. In the case of "corr", pairs of atom masks (*mask1*, *mask2*; each pair preceded by "maskp" and each mask defining only a single atom) have to be given that specify the atoms for which the correlation functions are desired.

analyze timecorr vec1 *vecname1* vec2 *vecname2* [tstep *tstep*] [tcorr *tcorr*]
[drct] [dplr] [norm] out *filename*

Calculates time correlation functions for vectors *vecname1* (*vecname2*) of type "corr" or "corrired", using a fast Fourier method. If two different vectors are specified for "vec1" and "vec2", a cross-correlation function is calculated; if the two vectors are the same, the result is an autocorrelation function. If the *drct* keyword is given, a direct approach is used instead of the FFT approach. Note that this is less efficient than the FFT route. If *dplr* is given, in addition to the P_l correlation function, also correlation functions $C_l \equiv < P_l/(r(t)^3 r(t+\tau)^3) >$ and $< 1/(r(t)^3 r(t+\tau)^3) >$ are output. If *norm* is given, all correlation functions are normalized, i.e. $P_l(t=0) = C_l(t=0) = 1/(r(t)^3 r(t)^3)(t=0) = 1.0$. Results are written to *filename*. *tstep* specifies the time between snapshots (default: 1.0), and *tcorr* denotes the maximum time for which the correlations functions are to be computed (default: 10000.0).

projection modes *modesfile* out *outfile* [beg *beg*] [end *end*] [*mask*]
[start *start*] [stop *stop*] [offset *offset*]

Projects snapshots onto modes obtained by diagonalizing covariance or mass-weighted covariance matrices. The modes are read from *modesfile*. The results are written to *outfile*. Only modes *beg* to *end* are considered. Default values are beg = 1, end = 2. *mask* specifies the atoms that will be projected. The user has to make sure that these atoms agree with the ones used to calculate the modes (i.e., if mask1 = @CA was used in the "matrix" command, mask = @CA needs to be set here as well). The *start*, *stop*, and *offset* parameters can be used to specify the range of coordinates processed (as a subset of all of those read in across all input files).

10.6. Examples

Please note that in most cases the trajectory needs to be aligned against a reference structure to obtain meaningful results. Use the "rms" command for this.

Calculating and analyzing matrices and modes

As a simple example, a distance matrix of all CA atoms is generated and output to distmat.dat.

```
matrix dist @CA out distmat.dat
```

In the following, a mass-weighted covariance matrix of all atoms is generated and stored internally with the name mwcvmat (as well as output). Subsequently, the matrix is analyzed by performing a quasiharmonic analysis, whereby 5 eigenvectors and eigenvalues are calculated and output to evecs.dat.

```
matrix mwcovar name mwcvmat out mwcvmat.dat
analyze matrix mwcvmat out evecs.dat vecs 5
```

Alternatively, the eigenvectors can be stored internally and used for calculating rms fluctuations or displacements of cartesian coordinates.

```
analyze matrix mwcvmat name evecs vecs 5
analyze modes fluct out rmsfluct.dat stack evecs beg 1 end 3
analyze modes displ out resdispl.dat stack evecs beg 1 end 3
```

Finally, dipole-dipole correlation functions for modes obtained from principle component analysis or quasiharmonic analysis can be computed.

```
analyze modes corr out cffromvec.dat stack evecs beg 1 end 3 ...
                  ... maskp @1 @2 maskp @3 @4 maskp @5 @6
```

Projecting snapshots onto modes

After calculating modes, snapshots can be projected onto these in an additional "sweep" through the trajectory. Here, snapshots are projected onto modes 1 and 2 read from evecs.dat (which have been obtained by the "matrix mwcovar", "analyze

matrix" commands from above).

```
projection modes evecs.dat out project.dat beg 1 end 2
```

Calculating time correlation functions

Vectors between atoms 5 and 6 as well as 7 and 8 are calculated below, for which auto and cross time correlation functions are obtained.

```
vector v0 @5 corr @6 order 2
vector v1 @7 corr @8 order 2
analyze timecorr vec1 v0 tstep 1.0 tcorr 100.0 out v0.out
analyze timecorr vec1 v1 tstep 1.0 tcorr 100.0 out v1.out
analyze timecorr vec1 v0 vec2 v1 tstep 1.0 tcorr 100.0 out v0_v1.out
```

Similarly, a vector perpendicular to the plane through atoms 18, 19, and 20 is obtained and further analyzed.

```
vector v2 @18,@19,@20 corrplane order 2
analyze timecorr vec1 v3 tstep 1.0 tcorr 100.0 out v2.out
```

For obtaining time correlation functions according to the ired approach, two "sweeps" through the trajectory are necessary. First, ired vectors are defined and an ired matrix is calculated and analyzed. Ired eigenvectors are output to ired.vec.

```
vector v0 @5 ired @6
vector v1 @7 ired @8
 ...
vector v5 @15 ired @16
vector v6 @17 ired @18
matrix ired name matired order 2
analyze matrix matired vecs 6 out ired.vec
```

In a subsequent ptraj run, ired time correlation functions are calculated by projecting the snapshots onto the ired eigenvectors (read from ired.vec), which results in corrired vectors. Then, time correlation functions are computed. Please note that it is important that the corrired vector definition agrees with the one used for calculation of the ired matrix.

```
vector v0 @5 corrired @6 order 2 modes ired.vec beg 1 end 6 npair 1
vector v1 @7 corrired @8 order 2 modes ired.vec beg 1 end 6 npair 2
 ...
vector v5 @15 corrired @16 order 2 modes ired.vec beg 1 end 6 npair 6
vector v6 @17 corrired @18 order 2 modes ired.vec beg 1 end 6 npair 7
analyze timecorr vec1 v0 tstep 1.0 tcorr 100.0 out v0.out
 ...
analyze timecorr vec1 v6 tstep 1.0 tcorr 100.0 out v6.out
```

10.7. Hydrogen bonding facility

The *ptraj* program now contains a generic facility for keeping track of lists of pair interactions (subject to a distance and angle cutoff) useful for calculation hydrogen bonding or other interactions. It is designed to be able to track the interactions between a list of hydrogen bond "donors" and hydrogen bond "acceptors" that the user specifies.

donor *resname atomname* | mask *mask* | clear | print

This command sets the list of hydrogen bond donors. It can be specified repeatedly to add to the list of potential donors. The usage is either as a pair of residue and atom names or as a specified atom mask. The former usage,

```
donor ADE N7
```

would set all atoms named N7 in residues named ADE to be potential donors.

```
donor mask :10@N7
```

would set the atom named N7 in residue 10 to be a potential donor.

The keyword "clear" will clear the list of donors specified so far and the keyword "print" will print the list of donors set so far.

The acceptor command is similar except that both the heavy atom and the hydrogen atom are specified. If the same atom is specified twice (as might be the case to probe ion interactions) then no angle is calculated between the donor and acceptor.

acceptor *resname atomname atomnameH* | mask *mask maskH* | clear | print

The **donor** and **acceptor** commands do not actually keep track of distances but instead simply set of the list of potential interactions. To actually keep track of the distances, the **hbond** command needs to be specified:

hbond [distance *value*] [angle *value*] [solventneighbor *value*]
[solventdonor *donor-spec*] [solventacceptor *acceptor-spec*]
[nosort] [time *value*] [print *value*] [series *name*]

The optional "distance" keyword specifies the cutoff distance for the pair interactions and the optional "angle" keyword specifies the angle cutoff for the hydrogen bond. The default is no angle cutoff and a distance of 3.5 angstroms. To keep track of potential hydrogen bond interactions where we don't care *which* molecule of a given type is interaction as long as one is (such as with water), the "solvent" keywords can be specified. An example would be keeping track of water or ions interacting with a particular donor or acceptor. The maximum number of possible interactions per a given donor or acceptor is specified with the "solventneighbor" keyword. The list of potential "solvent" donors/acceptors is specified with the solventdonor and solventacceptor keywords (with a format the same as the donor/acceptor keywords above).

As an example, if we want to keep track of water interactions with our list of donors/acceptors:

```
hbond distance 3.5 angle 120.0 solventneighbor 6 solventdonor WAT O
  solventacceptor WAT O H1 solventacceptor WAT O H2
```

If you wanted to keep track of interactions with Na+ ions (assuming the atom name was Na+ and residue name was also Na+):

```
hbond distance 3.5 angle 0.0 solventneighbor 6 solventdonor Na+ Na+
  solventacceptor Na+ Na+ Na+
```

To print out information related to the time series, such as maximum occupancy and lifetimes, specify the "series" keyword.

10.8. rdparm

`rdparm` requires an Amber *prmtop* file for operation and is menu driven. Rudimentary online help is available with the "?" command. The basic commands are summarized here.

angles <mask>
: Print all the angles in the file. If the <mask> is present, only print angles involving these atoms. For example, atoms :CYT@C? will print all angles involving atoms which have 2-letter names beginning with "C" from "CYT" residues.

atoms <mask>
: Print all the atoms in the file. If the <mask> is present, only print these atoms.

bonds <mask>
: Print all the bonds in the file. If the <mask> is present, only print bonds involving these atoms.

checkcoords <Amber trajectory>
: Perform a rudimentary check of the coordinates from the filename specified. This is to look for obvious problems (such as overflow) and to count the number of frames.

dihedrals <mask>
: Print all the dihedrals in the file. If the <mask> is present, only print dihedrals involving one of these atoms.

ddrive <filename>
: Create an input file for the SPASMS dihedral driver.

delete <bond || angle || dihedral> <number>
: This command will delete a given bond, angle or dihedral angle based on the number specified from the current prmtop. The number specified should match that shown by the corresponding print command. Note that a new prmtop file is not actually saved. To do this, use the writeparm command. For example, "delete bond 5" will delete with 5th bond from the parameter/topology file.

delperturbed <bond || angle || dihedral> <number>
: Same as delete above but to delete perturbed bonds, angles or dihedrals.

restrain <bond || angle || dihedral>
: This is a means to add restraints as is possible with the "parm" program. Its usage is somewhat obsolete because more flexible restraints can be specified with the NMR functionality of sander. To use this command, specify whether the restraint is to a bond, angle or dihedral and the program will prompt for atom numbers (as specified in the "atom" or "printatom" command). As before, the prmtop is not actually saved until a "writeparm" command is issued.

openparm <filename>
: Open up the prmtop file specified.

writeparm <filename>
: Write a new prmtop file to "filename" based on the current (and perhaps modified) parameter/topology file.

system <string>
: Execute the command "string" on the system.

mardi2sander <constraint file>
: A rudimentary conversion of Mardigras style restraints to sander NMR restraint format.

rms <Amber trajectory>
: Create a 2D RMSd plot in postscript or PlotMTV format using the trajectory specified. The user will be prompted for information. This command is rather slow and should be integrated into the "ptraj" code, however it hasn't been yet.

stripwater
: This command will remove or add three point waters to a prmtop file that already has water. The user will be prompted for information. This is useful to take an existing prmtop and create another with a different amount of water. Of course, corresponding coordinates will also have to be built and this is not done by "rdparm". To do this, ideally construct a PDB file and convert to Amber coordinate format using "ptraj".

ptraj <script-file>
: This command reads a file or from standard input a series of commands to perform processing of trajectory files. See the supplemental documentation.

transform <Amber trajectory>
: Perform rudimentary trajectory processing; this command is obsolete.

translateBox <Amber coords>
: Translate the coordinates (only if they contain periodic box information) specified to place either at the origin (SPASMS format) or at half the box (Amber format).

modifyBoxInfo
: This is a command to modify the box information, such as to change the box size. The changes are not saved until a writeparm command is issued.

modifyMolInfo
: This command checks the molecule info (present with periodic box coordinates are specified) and points out problems if they exist. In particular, this is useful to overcome the deficiency in edit which places all the "add" waters into a single molecule.

parmInfo
: Print out information about the current prmtop file.

pertbonds, perturbedBonds
: Print out the perturbed bonds.

pertangles, perturbedAngles
: Print out the perturbed angles.

pertdihedrals, perturbedDihedrals
: Print out the perturbed dihedrals.

printAngles
: Same as "angles".

printAtoms
: Same as "atoms".

printBonds
: Same as "bonds".

printDihedrals
: Same as "dihedrals".

printExcluded
: Print the excluded atom list.

printLennardJones
: Print out the Lennard-Jones parameters.

printTypes
: Print out the atom types.

quit
: Quit the program.

11. MM_PBSA

The MM_PBSA approach represents the postprocessing method to evaluate free energies of binding or to calculate absolute free energies of molecules in solution. The sets of structures are usually collected with molecular dynamics or Monte Carlo methods. However, the collections of structures should be stored in the format of an AMBER trajectory file. The MM_PBSA/GBSA method combines the molecular mechanical energies with the continuum solvent approaches. The molecular mechanical energies are determined with the *sander* program from AMBER and represent the internal energy (bond, angle and dihedral), and van der Waals and electrostatic interactions. An infinite cutoff for all interactions is used. The electrostatic contribution to the solvation free energy is calculated with a numerical solver for the Poisson-Boltzmann (PB) method, for example, as implemented in the *pbsa* program [108] or by generalized Born (GB) methods implemented in *sander*. Previous MM_PBSA applications were mostly performed with a numerical PB solver in the widely used *DelPhi* program [110], which has been shown by AMBER developers to be numerically consistent with the *pbsa* program. The nonpolar contribution to the solvation free energy has been determined with solvent-accessible-surface-area-dependent terms [105]. The surface area is computed with Paul Beroza's *molsurf* program, which is based on analytical ideas primarily developed by Mike Connolly [201]. An alternative method for nonpolar solvation energy is also included here (Tan and Luo, in preparation). The new method separates nonpolar contribution into two terms: the attractive (dispersion) and repulsive (cavity) interactions. Doing so significantly improves the correlation between the cavity free energies and solvent accessible surface areas for branched and cyclic organic molecules [116]. This is in contrast to the commonly used strategy that correlates total nonpolar solvation energies with solvent accessible surface areas, which only correlates well for linear aliphatic molecules [105]. In the new method, the attractive interaction is computed by a numerical integration over the solvent accessible surface area that accounts for solute solvent attractive interactions with an infinite cutoff [117]. Finally, estimates of conformational entropies can be made with the *nmode* module from AMBER.

Although the basic ideas here have many precedents, the first application of this model in its present form was to the A- and B-forms of RNA and DNA, where many details of the basic method are given [202]. You may also wish to refer to a review summarizing many of the initial applications of this model [203], as well as to papers describing more recent applications [204-208].

The initial MM_PBSA scripts were written by Irina Massova. These were later modified and mostly turned into Perl scripts by Holger Gohlke, who also added GB/SA (generalized Born/surface area) options, and techniques to decompose energies into pairwise contributions from groups (where possible).

11.1. General instructions

The general procedure is to edit the *mm_pbsa.in* file (see below), and then to run the code as follows:

```
mm_pbsa.pl  mm_pbsa.in > mm_pbsa.log
```

The *mm_pbsa.in* file refers to "receptor", "ligand" and "complex", but the chemical nature of these is up to the user, and these could equally well be referred to as "A", "B", and "AB". The procedure can also be used to estimate the free energy of a single species, and this is usually considered to be the "receptor".

The user also needs to prepare *prmtop* files for receptor, ligand, and complex using LEaP; if you are just doing "stability" calculations, only one of the *prmtop* files is required.

The output files are labeled ".out", and the most useful summaries are in the "statistics.out" files. These give averages and standard deviations for various quantities, using the following labeling scheme:

```
*** Abbreviations for mm_pbsa output ***

ELE   - non-bonded electrostatic energy + 1,4-electrostatic energy
VDW   - non-bonded van der Waals energy + 1,4-van der Waals energy
INT   - bond, angle, dihedral energies
GAS   - ELE + VDW + INT

PBSUR - hydrophobic contrib. to solv. free energy for PB calculations
PBCAL - reaction field energy calculated by PB
PBSOL - PBSUR + PBCAL
PBELE - PBCAL + ELE
PBTOT - PBSOL + GAS

GBSUR - hydrophobic contrib. to solv. free energy for GB calculations
GB    - reaction field energy calculated by GB
GBSOL - GBSUR + GB
GBELE - GB + ELE
GBTOT - GBSOL + GAS

TSTRA - translational entropy (as calculated by nmode) times temperature
TSROT - rotational entropy (as calculated by nmode) times temperature
TSVIB - vibrational entropy (as calculated by nmode) times temperature


*** Prefixes in front of abbreviations for energy decomposition ***

"T" - energy part due to _T_otal residue
"S" - energy part due to _S_idechain atoms
"B" - energy part due to _B_ackbone atoms
```

The *$AMBERHOME/src/mm_pbsa/Examples* directory shows examples of running a "Stability" calculation (*i.e.*, estimating the free energy of one species), a "Binding" calculation (estimating ΔG for A + B $\rightarrow$ AB), an "Nmode" calculation (to estimate entropies), and two examples of how total energies can be decomposed (either by residue, or pair-wise by residue). You should study the inputs and outputs in these directories to see how the program is typically used.

11.2. Preparing the input file

Below is a prototype *mm_pbsa.in* file; items in boldface would typically vary from run to run.

```
#
# Input parameters for mm_pbsa.pl
#
# Holger Gohlke
# 08.01.2002
#
###############################################################################
@GENERAL
#
# General parameters
#   0: means NO; >0: means YES
#
#   mm_pbsa allows to calculate (absolute) free energies for one molecular
#     species or a free energy difference according to:
#
#     Receptor + Ligand = Complex,
#     DeltaG = G(Complex) - G(Receptor) - G(Ligand).
#
#   PREFIX - To the prefix, "{_com, _rec, _lig}.crd.Number" is added during
#            generation of snapshots as well as during mm_pbsa calculations.
#   PATH - Specifies the location where to store or get snapshots.
#
#   COMPLEX - Set to 1 if free energy difference is calculated.
#   RECEPTOR - Set to 1 if either (absolute) free energy or free energy
#              difference are calculated.
#   LIGAND - Set to 1 if free energy difference is calculated.
#
#   COMPT - parmtop file for the complex (not necessary for option GC).
#   RECPT - parmtop file for the receptor (not necessary for option GC).
#   LIGPT - parmtop file for the ligand (not necessary for option GC).
#
#   GC - Snapshots are generated from trajectories (see below).
#   AS - Residues are mutated during generation of snapshots from trajectories.
#   DC - Decompose the free energies into individual contributions
#        (only works with MM and GB).
#
#   MM - Calculation of gas phase energies using sander.
#   GB - Calculation of desolvation free energies using the GB models in sander
#        (see below).
#   PB - Calculation of polar solvation free energies by using pbsa (see below).
#        Calculation of nonpolar solvation free energies according to
#        the NPOPT option in pbsa (see below).
#   MS - Calculation of nonpolar contributions to desolvation using molsurf
```

```
#        (see below).
#        If MS == 0 and GB == 1, nonpolar contributions are calculated with the
#        LCPO method in sander.
#        If MS == 0 and PB == 1, nonpolar contributions are calculated according
#        the NPOPT option in pbsa (see below).
#   NM - Calculation of entropies with nmode.
#
PREFIX                snapshot
PATH                  ./
#
COMPLEX               1
RECEPTOR              1
LIGAND                1
#
COMPT                 ./parm_com.top
RECPT                 ./parm_rec.top
LIGPT                 ./parm_lig.top
#
GC                    0
AS                    0
DC                    0
#
MM                    1
GB                    0
PB                    1
MS                    0
#
NM                    0
#
###############################################################################
@DECOMP
#
# Energy decomposition parameters (this section is only relevant if DC = 1 above)
#
#   Energy decomposition is performed for gasphase energies, desolvation free
#     energies calculated with GB, and nonpolar contributions to desolvation
#     using the LCPO method.
#   For amino acids, decomposition is also performed with respect to backbone
#     and sidechain atoms.
#
#   DCTYPE - Values of 1 or 2 yield a decomposition on a per-residue basis,
#            values of 3 or 4 yield a decomposition on a pairwise per-residue
#               basis. For the latter, so far the number of pairs must not
#               exceed the number of residues in the molecule considered.
#            Values 1 or 3 add 1-4 interactions to bond contributions.
#            Values 2 or 4 add 1-4 interactions to either electrostatic or vdW
#              contributions.
#
#   COMREC - Residues belonging to the receptor molecule IN THE COMPLEX.
```

```
#   COMLIG - Residues belonging to the ligand molecule IN THE COMPLEX.
#   RECRES - Residues in the receptor molecule.
#   LIGRES - Residues in the ligand molecule.
#   {COM,REC,LIG}PRI - Residues considered for output.
#   {REC,LIG}MAP - Residues in the complex which are equivalent to the residues
#                  in the receptor molecule or the ligand molecule.
#
DCTYPE                2
#
COMREC                1-166 254-255
COMLIG                167-253
COMPRI                1-255
RECRES                1-168
RECPRI                1-168
RECMAP                1-166 254-255
LIGRES                1-87
LIGPRI                1-87
LIGMAP                167-253
################################################################################
@PB
#
# PB parameters (this section is only relevant if PB = 1 above)
#
#   The following parameters are passed to the PB solver.
#   Additional input parameters may also be added here. See the sander PB
#   documention for more options.
#
#   PROC -  Determines which method is used for solving the PB equation:
#           By default, PROC = 2, the pbsa program of the AMBER suite is used.
#   REFE -  Determines which reference state is taken for PB calc:
#           By default, REFE = 0, reaction field energy is calculated with
#           EXDI/INDI. Here, INDI must agree with DIELC from MM part.
#   INDI -  Dielectric constant for the solute.
#   EXDI -  Dielectric constant for the surrounding solvent.
#   ISTRNG - Ionic strength (in mM) for the Poisson-Boltzmann solvent.
#   PRBRAD - Solvent probe radius in Angstrom:
#           1.4: with the radii in the prmtop files. Default.
#           1.6: with the radii optimized by Tan and Luo (In preparation).
#           See RADIOPT on how to choose a cavity radii set.
#   RADIOPT - Option to set up radii for PB calc:
#           0: uses the radii from the prmtop file. Default.
#           1: uses the radii optimized by Tan and Luo (In preparation)
#           with respect to the reaction field energies computed
#           in the TIP3P explicit solvents. Note that optimized radii
#           are based on AMBER atom types (upper case) and charges.
#           Radii from the prmtop files are used if the atom types
#           are defined by antechamber (lower case).
#   SCALE - Lattice spacing in no. of grids per Angstrom.
#   LINIT - No. of iterations with linear PB equation.
```

```
#
# NP Parameters for nonpolar solvation energies if MS = 0
#
#   NPOPT - Option for modeling nonpolar solvation free energy.
#           See pbsa below for more information on the implementations
#           by Tan and Luo (In preparation).
#           1: uses the solvent-accessible-surface area to correlate total
#           nonpolar solvation free energy:
#           Gnp = CAVITY_SURFTEN * SASA + CAVITY_OFFSET. Default.
#           2. uses the solvent-accessible-surface area to correlate the
#           repulsive (cavity) term only, and uses a surface-integration
#           approach to compute the attractive (dispersion) term:
#           Gnp = Gdisp + Gcavity
#               = Gdisp + CAVITY_SURFTEN * SASA + CAVITY_OFFSET.
#           When this option is used, RADIOPT has to be set to 1,
#           i.e. the radii set optimized by Tan and Luo to mimic Gnp
#           in TIP3P explicit solvents. Otherwise, there is no guarantee
#           that Gnp matches that in explicit solvents.
#   CAVITY_SURFTEN/CAVITY_OFFSET - Values used to compute the nonpolar
#           solvation free energy Gnp according NPOPT. The default values
#           are for NPOPT set to 0 and RADIOPT set to 0 (see above).
#           If NPOPT is set to 1 and RADIOPT set to 1, these two lines
#           can be removed, i.e. use the default values set in pbsa
#           for this nonpolar solvation model. Otherwise, please
#           set these to the following:
#           CAVITY_SURFTEN: 0.04356
#           CAVITY_OFFSET: -1.008
#
# NP Parameters for nonpolar solvation energies if MS = 1
#
#   SURFTEN/SURFOFF - Values used to compute the nonpolar contribution Gnp to
#           the desolvation according to Gnp = SURFTEN * SASA + SURFOFF.
#
PROC                 2
REFE                 0
INDI                 1.0
EXDI                 80.0
SCALE                2
LINIT                1000
PRBRAD               1.4
ISTRNG               0.0
RADIOPT              0
NPOPT                1
CAVITY_SURFTEN       0.0072
CAVITY_OFFSET        0.00
#
SURFTEN              0.0072
SURFOFF              0.00
#
```

```
##############################################################################
@MM
#
# MM parameters (this section is only relevant if MM = 1 above)
#
#   The following parameters are passed to sander.
#   For further details see the sander documentation.
#
#   DIELC - Dielectricity constant for electrostatic interactions.
#           Note: This is not related to GB calculations.
#
DIELC                 1.0
#
##############################################################################
@GB
#
# GB parameters (this section is only relevant if GB = 1 above)
#
#   The first group of the following parameters are passed to sander.
#   For further details see the sander documentation.
#
#   IGB - Switches between Tsui's GB (1) and Onufriev's GB (2, 5).
#   GBSA - Switches between LCPO (1) and ICOSA (2) method for SASA calc.
#          Decomposition only works with ICOSA.
#   SALTCON - Concentration (in M) of 1-1 mobile counterions in solution.
#   EXTDIEL - Dielectricity constant for the surrounding solvent.
#   INTDIEL - Dielectricity constant for the solute.
#
#   SURFTEN / SURFOFF - Values used to compute the nonpolar contribution Gnp to
#                    the desolvation according to Gnp = SURFTEN * SASA + SURFOFF.
#
IGB                   2
GBSA                  1
SALTCON               0.00
EXTDIEL               80.0
INTDIEL               1.0
#
SURFTEN               0.0072
SURFOFF               0.00
#
##############################################################################
@MS
#
# Molsurf parameters (this section is only relevant if MS = 1 above)
#
#   PROBE - Radius of the probe sphere used to calculate the SAS.
#           Since Bondi radii are already augmented by 1.4A, PROBE should be 0.0
#
PROBE                 0.0
```

```
#
###############################################################################
@NM
#
# Parameters for sander/nmode calculation (this section is only relevant
#                                          if NM = 1 above)
#
#   The following parameters are passed to sander (for minimization) and nmode
#     (for entropy calculation using gasphase statistical mechanics).
#   For further details see documentation.
#
#   DIELC - (Distance-dependent) dielectric constant
#   MAXCYC - Maximum number of cycles of minimization.
#   DRMS - Convergence criterion for the energy gradient.
#
DIELC                 4
MAXCYC                10000
DRMS                  0.0001
#
###############################################################################
@MAKECRD
#
# The following parameters are passed to make_crd_hg, which extracts snapshots
#   from trajectory files. (this section is only relevant if GC = 1 OR AS = 1 above.)
#
#   BOX - "YES" means that periodic boundary conditions were used during MD
#         simulation and that box information has been printed in the
#         trajectory files; "NO" means opposite.
#   NTOTAL - Total number of atoms per snapshot printed in the trajectory file
#            (including water, ions, ...).
#   NSTART - Start structure extraction from the NSTART-th snapshot.
#   NSTOP - Stop structure extraction at the NSTOP-th snapshot.
#   NFREQ - Every NFREQ structure will be extracted from the trajectory.
#
#   NUMBER_LIG_GROUPS - Number of subsequent LSTART/LSTOP combinations to
#                       extract atoms belonging to the ligand.
#   LSTART - Number of first ligand atom in the trajectory entry.
#   LSTOP - Number of last ligand atom in the trajectory entry.
#   NUMBER_REC_GROUPS - Number of subsequent RSTART/RSTOP combinations to
#                       extract atoms belonging to the receptor.
#   RSTART - Number of first receptor atom in the trajectory entry.
#   RSTOP - Number of last receptor atom in the trajectory entry.
#   Note: If only one molecular species is extracted, use only the receptor
#         parameters (NUMBER_REC_GROUPS, RSTART, RSTOP).
#
BOX                   YES
NTOTAL                25570
NSTART                1
NSTOP                 5000
```

```
NFREQ                 500
#
NUMBER_LIG_GROUPS     0
LSTART                0
LSTOP                 0
NUMBER_REC_GROUPS     1
RSTART                1
RSTOP                 2666
#
###############################################################################
@ALASCAN
#
# The following parameters are additionally passed to make_crd_hg in conjunction
#   with the ones from the @MAKECRD section if "alanine scanning" is requested.
#          (this section is only relevant if AS = 1 above.)
#
# The description of the parameters is taken from Irina Massova.
#
#   NUMBER_MUTANT_GROUPS - Total number of mutated residues. For each mutated
#                          residue, the following four parameters must be given
#                          subsequently.
#   MUTANT_ATOM1 - If residue is mutated to Ala then this is a pointer on CG
#                  atom of the mutated residue for all residues except Thr,
#                  Ile and Val.
#                  A pointer to CG2 if Thr, Ile or Val residue is mutated to Ala
#                  If residue is mutated to Gly then this is a pointer on CB.
#   MUTANT_ATOM2 - If residue is mutated to Ala then this should be zero for
#                  all mutated residues except Thr and VAL.
#                  A pointer on OG1 if Thr residue is mutated to Ala.
#                  A pointer on CG1 if VAL or ILE residue is mutated to Ala.
#                  If residue is mutated to Gly then this should be always zero.
#   MUTANT_KEEP  - A pointer on C atom (carbonyl atom) for the mutated residue.
#   MUTANT_REFERENCE - If residue is mutated to Ala then this is a pointer on
#                      CB atom for the mutated residue.
#                      If residue is mutated to Gly then this is a pointer on
#                      CA atom for the mutated residue.
#   Note: The method will not work for a smaller residue mutation to a bigger
#         for example Gly -> Ala mutation.
#   Note: Maximum number of the simultaneously mutated residues is 40.
#
NUMBER_MUTANT_GROUPS 3
MUTANT_ATOM1         1480
MUTANT_ATOM2         0
MUTANT_KEEP          1486
MUTANT_REFERENCE     1477
MUTANT_ATOM2         1498
MUTANT_ATOM1         1494
MUTANT_KEEP          1500
MUTANT_REFERENCE     1492
```

```
MUTANT_ATOM1          1552
MUTANT_ATOM2          0
MUTANT_KEEP           1562
MUTANT_REFERENCE      1549
#
###############################################################################
@TRAJECTORY
#
# Trajectory names
#
#   The following trajectories are used to extract snapshots with "make_crd_hg":
#   Each trajectory name must be preceded by the TRAJECTORY card.
#   Subsequent trajectories are considered together; trajectories may be
#     in ascii as well as in .gz format.
#   To be able to identify the title line, it must be identical in all files.
#
TRAJECTORY            ../prod_II/md_nvt_prod_pme_01.mdcrd.gz
TRAJECTORY            ../prod_II/md_nvt_prod_pme_02.mdcrd.gz
TRAJECTORY            ../prod_II/md_nvt_prod_pme_03.mdcrd.gz
TRAJECTORY            ../prod_II/md_nvt_prod_pme_04.mdcrd.gz
TRAJECTORY            ../prod_II/md_nvt_prod_pme_05.mdcrd.gz
#
###############################################################################
@PROGRAMS
#
# Additional program executables can be defined here
#
#
###############################################################################
```

11.3. Auxiliary programs used by MM_PBSA

Several programs can be used to compute numerical solutions to the Poisson-Boltzmann equation. The default is *pbsa*, which is described here. Other programs for computing numerical Poisson-Boltzmann results are also available, such as *Delphi*, *MEAD* and *UHBD*. These could be merged into the Perl scripts developed here with a little work. See:

`http://honiglab.cpmc.columbia.edu/`	*(for DELPHI)*
`http://www.scripps.edu/bashford`	*(for MEAD)*
`http://adrik.bchs.uh.edu/uhbd.html`	*(for UHBD)*

The program *pbsa* is a stand-alone program that is much like *sander* with the IGB = 10 option. Please see *sander* PB pages in Section 6.2 for detailed description.

12. Nmode

Usage:

```
nmode [-O] -i nmdin -o nmdout -c inpcrd -p prmtop -r restrt
       -ref refc -v vecs -l lmode -t tstate -e expfile
```

−O: Overwrite output files if they exist.

12.1. Introduction

This program performs molecular mechanics calculations on proteins and nucleic acids, using first and second derivative information to find local minima, transition states, and to perform vibrational analyses. It is designed to read the *prmtop* and *inpcrd* files from the Amber suite of programs. Both Additive and Non-additive Hamiltonians are available in this version. *Nmode* was originally written at the University of California, Davis, by D.T. Nguyen and D.A. Case, based in part on code in the Amber 2.0 package. Major revisions were made at the Research Institute of Scripps Clinic by J. Kottalam and D.A. Case. M. Pique has provided valuable advice and help in porting it to many different machines. J.W.Caldwell implemented the non-additive capabilities.

The second derivative routines are based on expressions used in the Consistent Force Field programs [209]. The code also contains routines to search for transition state, starting (generally) from a minimum. This procedure uses a modification of the procedure of Cerjan and Miller [210], as described by Nguyen and Case [211]. Langevin modes are analogous to normal modes, but in the presence of a viscous coupling to a continuum solvent. The basic ideas are presented by Lamm and Szabo [212], and were implemented in the Amber environment by Kottalam and Case [213].

12.2. General description This program performs five tasks, depending on the value of the input variable ntrun (see below):

(1) Perform a normal mode analysis from starting coordinates. Requires an input structure that has already been minimized, from process (4), below, or by some other method. In addition to the computation of normal mode frequencies, thermodynamic parameters are calculated.

(2) Search for transition state, starting (generally) from a minimum. See the references above for a detailed description of the method.

(3) Perform a conjugate gradient minimization from the starting coordinates. This routine uses an IMSL library routine for this purpose, which is not supplied with this program. Persons who do not have access to the IMSL library should probably use the AMBER "sander" program to carry out conjugate gradient minimizations.

(4) Does a Newton-Raphson minimization from starting coordinates. A constant (tlamba) is added to the diagonal elements of the Hessian matrix to make it positive definite. Tlamba is chosen in a manner such that the step is always downhill in all directions. Whenever the

change in energy is > emx or the rms of step length is > smx, the step length is scaled back repeatedly until the above two conditions are satisfied. Note that this routine will not converge to a transition state.

(5) Perform a langevin mode calculation, starting from a minimized structure. This option is similar to (1), but includes the viscous effects of a solvent in the calculation.

Input files for this program are the same as for the regular AMBER minimization and molecular dynamics programs, with the exception of *nmdin*, whose parameters are given below. The defaults have been carefully selected, so that for most purposes, few of them need to be changed. See the sample runs for more information.

12.3. Files

```
nmdin  : control input for the run
nmdout : standard output file for print and error messages
prmtop : parameter and topology file
inpcrd : starting coordinates
refc   : input coordinates for constraints
restrt : output coordinates at end of minimization
prlist : file for reading or storing the non-bonded pair list
vecs   : file containing output normal mode frequencies and eigenvectors
tstate : output coordinates at a transition state
expfile: file to read exposed surface area for atoms
lmode  : file to write Langevin modes
```

12.4. Input description

Input found on *nmdin:* You can use as many title cards as you want, followed by the namelist `&data`, which contains the following variables.

General flags describing the calculation

---

ntrun — 1: do normal mode analysis *(default)*
2: search for transition states
3: do conjugate gradient minimization (requires IMSL library)
4: do Newton-Raphson minimization
5: do Langevin mode analysis

ibelly — 1: some atoms are to be held fixed (default=0)

icons — 1: do constrained minimization to initial coordinates specified in *refc*. (default=0)

maxcyc — max. number of cycles for minimization (default=100)

drms	rms gradient to stop minimization (default=1.e-5)
nvect	number of vectors for normal mode analysis (default=0)
ismem	set to 1 for "small memory model" for normal modes, which uses about one-third the memory of the default (ismem=0) model. The tradeoff is that no eigenvectors can be computed, so that nvect is set to zero whenever ismem=1
nsave	for every nsave steps the coordinates are saved. (default= 20)
nprint	every nprint-th step the energy will be printed
ilevel	if .ne. 0, then adjust second derivative matrix to put rotation and translation vectors to a high frequency; this can be useful if you want to perform a normal mode analysis from a not-completely-minimized structure, so that rotations and translations don't mix with the low-lying modes (default=0).
ivform	0 if the normal mode eigenvectors are to be written out in unformatted form; 1 to use the Amber standard formatted option (default); 2 to write out the normal modes in MKL format for the *molekel* program (see *http://www.cscs.ch/molekel*).
ntx	0 if the input coordinates are to be read in unformatted form; 1 to use the formatted option (default).
ntxo	0 if the output (restart) coordinates are to be written out in unformatted form; 1 to use the formatted option (default).
t	Temperature to use in calculating thermodynamic properties from the modes; default is 298.15.

Control of certain force field parameters

---

cut	radius for non-bonded cutoff (default=99.)
scnb	1-4 nonbonded scale factor (default=2.0)
scee	1-4 electrostatic scale factor (default=2.0)
dielc	dielectric constant (default=1.0)
idiel	0 for r**2 dielectric dependence (default); 1 for constant dielectric.
ipol	= 0 no polarization (default) = 1 include polarization modules
i3bod	= 0 no three-body interactions(default) = 1 readin and use specified three body interactions (see the sander input area for details)
iprr	1: read in a non-bonded pair list from *prlist*; (default = 0)
iprw	1: write out non-bonded pair list to *prlist*; (default = 0)

control of Newton-Raphson and transition-state searches

smx maximum rms step length (default = 0.08)

emx maximum energy change per step (default =0.3)

alpha scale factor for step length (default = 0.8) (See Nguyen and Case paper for description of smx, emx, and alpha.)

bdwnhl constant to determine tlamba, the value to be subtracted from the diagonal elements of Hessian matrix for a downhill step. tlamba is chosen as min ((lowest eigenvalue - bdwnhl) , 0). (default bdwnhl = 0.1)

ndiag for every ndiag steps, the matrix is diagonalized to calculate tlamba, when ntrun=4

dfpred a rough estimate of the expected reduction in energy for the initial step (only for ntrun = 3). (default = 0.01 kcal/mol)

parameters for running Langevin modes (set ntrun = 5)

eta viscosity in centipoise

ioseen 0: Stokes Law used for hydrodynamic interaction
1: Oseen interaction included
2: Rotne-Prager correction included

hrmax hydrodynamic radius for the atom with largest area exposed to solvent. If a file named 'expfile' is present, then the relative exposed areas are read from that file as a namelist

```
namelist /exposure/ expr(natom)
```

If 'expfile' does not exist, then all atoms are assigned a hydrodynamic radius of hrmax.

parameters for transition state search (when ntrun = 2)

istart	0: new calc. (default) 1: restart calc.
iflag	0: search for transition state then minimum (default) 1: search for minimum from a transition state -1: search for a transition state, then stop
ivect	no. of eigenvectors wanted (default=2) (ivect has to be >=isdir)
isdir	eigenvector along which search for transition state is to be made. Note that translations and rotations are removed from the Hessian, so this number refers to the ordering of the "true" vibrational normal modes. (default=1)
idir	search direction: = 1 along isdir direction (default); = -1 opposite isdir direction
isw	no. of steps before switching to the lowest mode (default=40)
hnot	initial step length (default=0.1 Ang.)
buphl	switch to Newton-Raphson step when lowest eigenvalue is less than this for uphill walk. (default=-0.1)

Cards 3	group cards for the parts of the molecule that move, if ibelly.ne.0. See group documentation for format.
Cards 4	group cards for the part of the molecule to be constrained, along with the constraint weights, if icons.ne.0. See group documentation for format.

Memory usage: Normal mode analysis can take a lot of memory; users should consult the `alloc.f` file to see all of the details. The biggest memory hog is generally for the second derivative (Hessian) matrix. In the "normal" case, for *ntrun=1*, the program requires $9N(3N-1)/2$ 8-byte words of storage. For a 400 atom system, this is about 2.1 million words, or 17 Mbytes, which is generally no problem. For a 4000 atom system, however, this translates to 216 million words, or 1.7 Gbytes, which may not always be available. For larger systems, normal mode calculations that store everything in memory become increasingly impractical.

By setting *ismem* to 1, you can reduce the memory usage to 1/3 of the above estimate, at the expense of not calculating eigenvectors. This can sometimes make calculations feasible that otherwise would not be, but only for a fairly narrow range of problem sizes. More elaborate schemes, involving sparse matrix storage, are certainly possible, but have not yet been implemented in *nmode*.

13. Miscellaneous

13.1. Resp

RESP (Restrained ElectroStatic Potential) fits the quantum mechanically calculated electrostatic potential (esp) at molecular surfaces using an atom-centered point charge model. This method was developed primarily by Christopher Bayly [214-216]. A quantum mechanical program, such as Gaussian, Jaguar, or GAMESS, must be used to generate the ESP input for RESP. See $AMBERHOME/src/resp/0README for tips for interfacing such programs with RESP. Note that *antechamber* automates most of this process: use the *-fo gcrt* option to create a Gaussian input file; then run Gaussian; then use the *-fi gout -c resp* option to automatically create the resp input file and run a two-stage fitting procedure. If you don't use Gaussian, you can still run *respgen* to automatically create the input files needed for resp.

Another alternative is to use the "RED" programs to create RESP input and to manage the calculations. See `http://www.u-picardie.fr/labo/lbpd/RED/` for information about this option.

Because so few users run RESP directly anymore, we have removed the input description from this manual. The instructions can be accessed online at the Amber web site.

13.2. nucgen

Usage: `nucgen [-O] -i ngin -o ngout -d ngdat -p pdbout`

−O Overwrite output files.

This program generates cartesian coordinate models for either double helical DNA or RNA with a number of possible conformations. The conformations are taken from fibre-diffraction studies [217]. The helical topology of the double helix is stored in a file for individual types in terms of cylindrical coordinates. The program loads the required topoplogy and applies two fold symmetry with necessary helical repeat and height values. The cartesian coordinates are output in PDB format. The residue information is read as in the link module either for DNA or RNA. The input is described below.

NUCGEN requires specification of two strands: if only one is given, it will wrap it into two with highly stretched base-phosphate bonds across the end, so for single strands, specify a dummy strand and edit it out of the resulting PDB file. NUCGEN only generates reasonable geometries for complementary base pairs.

NUCGEN can generate PDB files using the 1994 Amber force field convention, which does not have explicit terminal hydrogen or phosphate residues. For the new residue names, only the bases need to be specified, while for the old convention, terminal hydrogen residues (HB and HE) and phosphates (POM) must also be specified. In the 1994 convention, residues are indicated by the first letter (A, G, C, T) and terminal residues have an additional 5 or 3 appended (*e.g.* A5, A3). See the LEaP chapter for a table of these names.

```
file    unit        purpose
```

```
-------------------------------------------------------------
ngin      5   Input:   Control and sequence data for the run
ngout     6   Output:  Diagnostics
ngdat     7   Input:   Monomer geometry file, found in amber41/dat
pdbout   10   Output:  PDB output coordinates
-------------------------------------------------------------
```

Nucleic Acid sequence information is given as described here for each strand. Both strands are entered in the 5' to 3' direction.

```
-------------------------------------------------------------

  - 1A -     A TITLE FOR EACH STRAND (20A4)

  TITLE      A title for the molecule.

-------------------------------------------------------------

  - 1B -     ILBMOL (A4)

  ILBMOL     Label for the type of molecule.

        'D'  DNA
        'R'  RNA

-------------------------------------------------------------

  - 1C -     RESIDUE INFORMATION FOR EACH STRAND
             it is read in the following format until a blank
             card is encounterd (card 1D).

             LBRES(I) , I = 1,NRESM     (16(A4,1X))

  LBRES(I)   Residue name.

-------------------------------------------------------------
  - 1D -     Blank Card to terminate residue input
-------------------------------------------------------------
  NOTE:  Cards 1A-1D are repeated for the second strand.
-------------------------------------------------------------

  - 2 -      KEND    (A4)

  KEND       Control to stop reading the nucleotide strands.

     'END '  end of reading the sequence information

-------------------------------------------------------------
```

```
  - 3 -      CONTROL FOR THE TYPE OF DNA OR RNA CONFORMATION

             TYPM     (A8)

  TYPM       Name of the type of conformation to be generated.

   '$ARNA'     right handed a-rna (arnott)
   '$APRNA'    right handed a-prime rna (arnott)
   '$LBDNA'    right handed bdna (langridge)
   '$ABDNA'    right handed bdna (arnott)
   '$SBDNA'    left handed bdna (sasisekharan)
   '$ADNA'     right handed a-dna (arnott)
   '$SPECIAL'  special type by the user

-----------------------------------------------------------

  - 4 -       special helical parameter

              ***** only if typm .eq. '$SPECIAL' *****

              hxrep , hxht    (2f10.5)

  hxrep       Helical repeat angle in degrees for the special
              type of conformation.

  hxht        Helical height.

              NOTE: If you use '$SPECIAL', you will have to
                    add the appropriate data to file ngdat (found
                    in the database directory).  Consult subroutine
                    gennuc for details.
-----------------------------------------------------------
```

13.3. ambpdb

NAME

ambpdb − convert amber-format coordinate files to pdb format

SYNOPSIS

ambpdb [-p prmtop-file][-tit title] [-pqr|-bnd|-atm] [-aatm] [-bres] [-noter] [-offset #] [-bin] [-first]

DESCRIPTION

ambpdb is a filter to take a coordinate "restart" file from an AMBER dynamics or minimization run (on STDIN) and prepare a pdb-format file (on STDOUT). The

program assumes that a *prmtop* file is available, from which it gets atom and residue names.

OPTIONS

title The title, if given, will be output as a REMARK at the top of the file. It should be protected by quotes or double quotes if it contains spaces or special characters.

-pqr If *-pqr* is set, output will be in the format needed for the MEAD suite of programs created by Don Bashford. The *-atm* option creates files used by Mike Connolly's surface area/volume programs. The *-bnd* option creates a file that lists the bonds in the molecule, one per line.

-aatm This switch controls whether the output atom names follow Amber or Brookhaven (PDB) formats. With the default (when this switch is not set), atom names will be placed into four columns in an approximation to the rules used by the Protein Data Base. This gives files that look very much like PDB files, EXCEPT that PDB uses "1" and "2" for amino-acid beta-protons (for example) whereas the standard Amber database (along with many in the NMR field) use "2" and "3", i.e. we have 2HB and 3HB, whereas Brookhaven files use 1HB and 2HB. The *protonate* program can be used to check and re-name proton names to various conventions.

If *-aatm* is set, Amber atom names will be left-justified in the output file, starting in column 13.

Generally speaking, Amber programs that read PDB files (like *protonate* and *LEaP*, work with either style of atom names. Programs like RASMOL, that expect more strict conformance to Brookhaven standards, require the default behavior; some other programs may work better with *-aatm* set, so that (for example) all hydrogen atoms begin with "H", etc.

-bin If *-bin* is set, an unformatted (binary) "restart" file is read instead of a formatted one (default). Please note that no detection of the byte ordering happens, so binary files should be read on the machine they were created on.

-bres If *-bres* (Brookhaven-residue-names) is not set (the default), Amber-specific atom names (like CYX, HIE, DG5, etc.) will be kept in the pdb file; otherwise, these will be converted to PDB-standard names (CYS, HIS, G, in the above example). Note that setting -bres creates a naming ambiguity between protonated and uprotonated forms of amino acids, and between DNA and RNA.

If you plan to re-read the pdb file back into Amber programs, you should use the default behavior; for programs that demand stricter conformance to Brookhaven standards, set *-bres*.

-first If *-first* is set, a pdb file augmented by additional information about hydrogen bonds, salt bridges, and hydrophobic tethers is generated, which can serve as input to the standalone version of the FIRST software by D. J. Jacobs, L. A. Kuhn, and M. F. Thorpe.

-noter If *-noter* is set, the output PDB file not include TER cards between molecules. Otherwise, TER cards will be added whenever there is not bond between adjacent residues. Note that this means there will be a TER card between each water molecule, for example, unless *-noter is set*. The PDB is idiosyncratic about TER

cards: they are generally present between separate protein chains, but generally not present between cofactors or solvent molecules. This behavior is not mimicked by *ambpdb*.

-offset If a number is given here, it will be added to all residue numbers in the output pdb file. This is useful if you want the first residue (which is always "1" in an Amber prmtop file, to be a larger number, (say to more closely match a file from Brookhaven, where initial residues may be missing). Note that the number you provide is one less than what you want the first residue to have.

Residue numbers greater than 9999 will not "fit" into the Brookhaven format; ambpdb actually prints mod(resno,10000); that is, after 9999, the residue number re-cycles to 0.

FILES

Assumes that a *prmtop* file (with that name, or the one given in the *-p* option) exists in the current directory; reads AMBER coordinates from STDIN, and writes pdb-file to STDOUT.

BUGS

Inevitably, various niceties of the Brookhaven format are not as well supported as they should be. The *protonate* program can be used to fix up hydrogen atom names, but that functionality should really be integrated here. There is no good solution to the PDB problem of using the same residue name for different chemcial species; depending on how the output file is to be used, the two options supported (setting or not setting *-bres*) may or may not suffice. Radii used for the *-pqr* option are hard-wired into the code, requiring a re-compilation if they are to be changed. Atom name output may be incorrect for atoms with two-character atomic symbols, like calcium or iron. The *-offset* flag is a very limited start toward more flexible handling of residue numbers; in the future (we hope!) Amber *prmtop* files will keep track of the "original" residue identifiers from input pdb files, so that this information would be available on output.

13.4. protonate

NAME

protonate − add protons to a heavy-atom protein or DNA PDB file; convert proton names between various conventions; check (pro)-chirality.

SYNOPSIS

```
Usage: protonate [-bcfhkmp] [-d datafile]
 [-i input-pdb-file] [-o output-pdb-file] [-l logfile]
 [-al link-file] [-ae edit-file] [-ap parm-file]
   -b to write Brookhaven-like atom names
   -c to write chains as separate molecules
   -f to force write of atoms found (debugging)
   -h to write ONLY hydrogens to output file
   -k to keep original coordinates of matched protons
```

```
-m to list mismatched protons
-p to print proton substitutions
-d to specify datafile (default is PROTON_INFO)
-i to specify input file (default is stdin)
-o to specify output file (default is stdout)
-l to specify logfile (default is stderr)
```

DESCRIPTION

Protonate combines a program originally written by K. Cross to add protons to a heavy-atom pdb file, with many extensions by J. Holland, G.P. Gippert & D.A. Case. Names and descriptions of the output protons are contained in the info-file (see below.) *Protonate* can be used to add protons that don't exist, to change the names of existing protons to some new convention, and to check pro-chirality of protons in an input pdb file. The source code is in the `src/protonate/` directory. Protonate generally will not do a careful job of orienting polar hydrogens, particularly for hydroxyls of serine, threonine and tyrosine; you can use the *pol_h* program (described below) for this purpose.

OPTIONS

−k The output pdb file will keep the proton coordinates of the input file, to the extent consistent with how well it can identify what names they should really have. Otherwise it will replace input protons with ones it builds.

−b The program will insert a space before the name of each heavy atom in the output file. This is most often used to convert input files whose atom names begin in column 13 to the Brookhaven format where most heavy atom names begin in column 14. NOTE: two-letter heavy atom names (like FE or CA [calcium]) will not be correct; the resulting output file must be hand-edited to check for this.

−d info_file Specifies the file containing information on how to build and name protons. The default name is PROTON_INFO. This information used to determine where on the amino acids the protons should be placed. The file provided handles funny Amber residue names like HIE, HIP and HID and HEM. Other files provided include PROTON_INFO.Brook, which uses Brookhaven proton naming convention (such as 1HB, etc.), and PROTON_INFO.oldnames, which uses old amber names. For example, to take an Amber pdb file and convert to the Brookhaven naming convention, set -d PROTON_INFO.Brook.

Output to LOGFILE includes matches of protons the program builds with any found in the input file, plus a list of any input protons that could not be matched. Questionable matches are flagged and should be checked manually.

BUGS

Format of the PROTON_INFO file is not obvious unless you have read the code.

Methyl protons are built in a staggered conformation; hydroxyl protons in a arbitrary (and generally sub-optimal) conformation. A program like *pol_h* or its equivalent should be used (if desired) to place polar hydrogens on LYS, SER, THR, and SER residues.

HIS in the input file is assumed to be HID. Users should generally explicitly figure out the desired protonation state for histidines.

No attempt is made to identify heavy atoms in the input file that have two-letter element names; this means that Brookhaven-style output may require some hand-editing if atoms like calcium or iron are present.

It is assumed that the alternate conformer flag in column 17 of the PDB file is either blank, or A. The program needs to be recompiled to change this; perhaps this should become an input option.

13.5. ambmask

NAME

ambmask − test group input FIND mask (or mask string given in the &cntrl section) and dump the resulting atom selection in a given format

SYNOPSIS

```
ambmask -p prmtop -c inpcrd -prnlev [0-3] -out [short|
pdb| amber] -find [maskstr]
```

DESCRIPTION

ambmask acts as a filter which takes amber topology and coordinate "restart" file and applies the "maskstr" selection string (similar syntactically to UCSF Chimera/Midas) to select specific atoms or residues. Residues can be selected by their numbers or names. Atoms can be selected by numbers, names, or amber (forcefield) type. Selections are case insensitive. The selected atoms are printed to **stdout** (by default, in amber−style pdb format). Atom and residue names and numbers are taken from amber topology. Beware that selection string works on those names and not the ones from the original pdb file. If you are not sure how atoms or residues are named or numbered in the amber topology, use **ambmask** with a selection string ":*" (which is the default) to dump the whole pdb file with corresponding amber atom/residue names and numbers.

The "maskstr" selection expression is composed of "elementary selections". These start with ":" to select by residues, or "@" to select by atoms. Residues can be selected by numbers (given as numbers separated by commas, or as ranges separated by a dash) or by names (given as a list of residue names separated by commas). The same holds true for atom selections by atom numbers or atom names. In addition, atoms can be selected by amber atom type, in which case "@" must be immediately followed by "%". ":*" means all residues and "@*" means all atoms. The following examples show the usage of this syntax. Square brackets should not be used in actual expressions, they are only used for clarity here:

```
:{residue numlist}          [:1-10] [:1,3,5] [:1-3,5,7-9]
:{residue namelist}         [:LYS] [:ARG,ALA,GLY]
@{atom numlist}             [@12,17] [@54-85] [@12,54-85,90]
@{atom namelist}            [@CA] [@CA,C,O,N,H]
@%{atom typelist}           [@%CT] [@%N*,N3]
```

These "elementary selections" can be combined into more complex selections using binary

operators "&" (and) and "|" (or), unary operator "!" (negation), distance binary operators "<:", ">:", "<@", ">@", and parentheses. Spaces around operators are irrelevant. Parentheses have the highest priority, followed by distance operators ("<:", ">:", "<@", ">@"), "!" (negation), "&" (and) and "|" (or) in order of descending priority. A wildcard "=" in an atom or residue name matches any name starting with a given character (or characters). For example, `[:AS=]` would match all aspartic acid residues (ASP), and asparagines (ASN); `[@H=]` would match all atom names starting with H (which are effectively all hydrogens). It cannot be used to match the end part of names (such as `[:=A]`). Some examples of more complex selections follow:

`[@C= & !@CA,C]`
.. all carbons except backbone alpha and carbonyl carbons
`[(:1-3@CA | :5-7@CB)]`
.. alpha carbons in residues 1-3 and beta carbons in residues 5-7
`[:CYS,ARG & !(:1-10 | @CA,CB)]`
.. all CYS and ARG atoms except those which are in residues 1-10 and which are CA or CB
`[:* & !@H=]` or `[!@H=]`
.. all heavy atoms (i.e. except hydrogens)
`[:5 <@4.5]`
.. all atoms within 4.5A from residue 5
`[(:1-55 <:3.0) & :WAT]`
.. all water molecules within 3A from residues 1-55

Compound expressions of the following type are also allowed:

`:{residue numlist|namelist}@{atom numlist|namelist|typelist}`
`[:1-10@CA]` is equivalent to `[:1-10 & @CA]`
`[:LYS@H=]` is equivalent to `[:LYS & @H=]`

OPTIONS

The program needs an amber topology file and coordinates (restrt format). The filename specified with the *−p* option is amber topology, while the filename given with the *−c* option is a coordinate file. If *−p* or *−c* options are not given, the program expects that files "prmtop" and/or "inpcrd" exist in the current directory, which will be taken as topology and coordinate files correspondingly. If no command line options are given, the program prints the usage statement.

The option *−prnlev* specifies how much (debugging) information is printed to **stdout**. If it is 0, only selected atoms are printed. More verbose output (which might be useful for debugging purposes) is achieved with higher values: 1 prints original "maskstr" in its tokenized (with operands enclosed in square brackets) and postfix (or Reverse Polish Notation) forms; number of atoms and residues in the topology file and number of selected atoms are also printed to **stdout**. 2 prints the resulting mask array, which is an array of integer values, with ’1’ representing a selected atom, and ’0’ an unselected one. Value of 3, in addition, prints mask arrays as they are pushed or popped from the stack (this is really only useful for tracing the problems occurring during stack operations). The *−prnlev* values of 0 or 1 should suffice for most uses.

The option *−out* specifies the format of printed atoms. "short" means a condensed output using residue (:) and atom (@) designators followed by residue ranges and atom names. "pdb" (default) prints atoms in amber-like pdb format with the original "maskstr" printed as a REMARK at the top of the pdb file, and "amber" prints atom/residue ranges in the format suitable for

copying into group input section of amber input file.

The option −*find* is followed by "maskstr" expression. This is a string where some characters have a special meaning and thus express what parts (atoms/residues) of the molecule will get selected. The syntax of this string is explained in the section above (DESCRIPTION). If this option is left out, it defaults to ":*", which selects all atoms in the given topology file. The length of "maskstr" is limited to 80 characters. If the "maskstr" contains spaces or some special characters (which would be expanded by the shell), it should be protected by single or double quotes (depending on the shell).

FILES

Assumes that a *prmtop* and *inpcrd* files exists in the current directory if they are not specified with −*p* and −*c* options. Resulting (i.e. selected) atoms are written to **stdout**.

BUGS

Because all atom names are left justified in amber topology and the selections are case insensitive, there is no way to distinguish some atom names: alpha carbon CA and a calcium ion Ca are a notorious example of that.

13.6. pol_h and gwh

NAME

pol_h − set positions of polar hydrogens in proteins
gwh − set positions of polar hydrogens onto water oxygen positions

SYNOPSIS

pol_h < input-pqr-file > output-pdb-file

gwh [-p <prmtop>] [-w <water.pdb>] [-c] [-e] < input_pdb_file
> output_pdb_file

DESCRIPTION

The program *pol_h* resets positions of polar hydrogens of protein residues (Lys, Ser, Tyr and Thr), by optimizing simple electrostatic interactions. The input *pqr* file can be created by *ambpdb*.

The program *gwh* sets positions of water hydrogens onto water oxygen positions that may be present in PDB files, by optimizing simple electrostatic interactions. If the -w flag is set, the program reads water oxygen positions from the file *water-position-file*, rather than the default name *watpdb*. If *-c* is set, a constant dielectric will be used to construct potentials, otherwise the (default) distant-dependent dielectric will be used. If *-e* is set, the electrostatic potential will be used to determine which hydrogens are placed first; otherwise, a distance criterion will be used.

Accuracy of pol_h & gwh:

```
* In the following the results for BPTI and RSA(ribosuclease A) are
   given together with those of Karplus(1) and Ornstein(2) groups.
   In the case of Ornstein's method, it handles only some of hydrogens
   in question and therfore I normalized(scaled) their results using
   expected values for random generation.  The rms deviation from the
   experimental positions (neutron difraction) and the number of
   hydrogens are shown below.

      BPTI        Lys    Ser    Tyr    Thr       Wat
-------------------------------------------------------------
      # of H     12      1       4       3      112  (4~)
    Pol_H       0.39    0.36    1.08    0.20    0.98(0.38)
    Karplus     0.25    0.71    0.81    0.19     -  (0.35)
    Ornstein    0.22    0.96    0.00    0.07     -
 Ornstn(scaled) 0.51    0.96    1.28    0.07        (1.17)^
-------------------------------------------------------------
      ~internal waters.  ^by random generation


 RSA        Lys    Ser    Tyr    Thr    Wat
-----------------------------------------------------------
    # of H     30     15     6     10    256
GuesWatH       0.61    0.96    1.22    0.96    0.98
Karplus        0.60    0.98    0.60    1.12    1.20
Ornstein       0.20    0.61    0.60    0.30     -
Ornstn(scaled) 0.49    0.89    0.76    0.93   (1.14)^
-----------------------------------------------------------
      ^by random generation

   1) A. T. Brunger and M. Karplus, Proteins, 4, 148 (1988).
   2) M. B. Bass,,, R. L. Ornstein, Proteins, 12, 266 (1992).

* The accuracies seem to be similar among three approaches
  if scaled values of Ornstein's data are considered.
```

FILES Default for <prmtop-file> is "prmtop". The input-pdb-file must have been generated by LEaP or ambpdb, *i.e.* it must have exactly the same atoms (in the same order) as the prmtop file.

13.7. fantasian

```
A program to evaluate magnetic anisotropy tensor parameters
                        Ivano Bertini
    Depart. of Chemistry, Univ. of Florence, Florence, Italy
```

e-mail: bertini@risc1.lrm.fi.cnr.it

INPUT FILES:

Observed shifts file (pcshifts.in):

```
1st    column   -->   residue number
2nd    column   -->   residue name
3rd    column   -->   proton name
4th    column   -->   observed pseudocontact shift value
5th    column   -->   multiplicity of the NMR signal (for example
                      it is 3 for of a methyl group)
6th    column   -->   relative tolerance
7th    column   -->   relative weight
```

Amber pdb file (parm.pdb): coordinates file in PDB format. If you need to use a solution NMR family of structures you have to superimpose the structures before to use them.

OUTPUT FILES:

Observed out file (obs.out): This file is built and read by the program itself, it reports the data read from the input files.

output file (res.out): The main output file. In this file the result of the fitting is reported. Using fantasian it is possible to define an internal reference system to visualize the orientation of the tensor axes. Then in this file you can find PDB format lines (ATOM) which can be included in a PDB file to visualize the internal reference system and the tensor axes. In the main output file all the three equivalent permutations of the tensor parameters with respect to the reference system are reported. The summary of the minimum and maximum errors and that of errors^2 are also reported.

Example files: in the directory example there are all the files necessary to run a fantasian calculation:

```
fantasian.com  --> run file
pcshifts.in    --> observed shifts file
parm.pdb       --> coordinate file in PDB format
obs.out        --> data read from input files
res.out        --> main output file
```

13.8. elsize

NAME

elsize − Given the structure, estimates its effective electrostatic size (parameter *Arad*) need by the ALPB model.

SYNOPSIS

```
Usage: elsize input-pqr-file [-options]
   -det an estimate based on structural invariants. DEFAULT.
   -ell an estimate via elliptic integral (numerical).
   -elf same as above, but via elementary functions.
   -abc prints semi-axes of the effective ellipsoid.
   -tab prints all of the above into a table without header.
   -hea prints same table as -tab but with a header.
   -deb prints same as -tab with some debugging information.
   -xyz uses a file containing only XYZ coordinates.
```

DESCRIPTION

elsize is a program originally written by G. Sigalov to estimate the effective electrostatic size of a structure via a quick, analytical method. The algorithm is presented in detail in Ref. [107] You will need your structure in a pqr format as input, which can be easily obtained from the prmtop and inpcrd files using *ambpdb* utility described above:
ambpdb -p prmtop -pqr < inpcrd > input-file-pqr . After that you can simply do: *elsize input-file-pqr* , the value of electrostatic size in Angstroms will be output on stdout. The source code is in the `src/etc/` directory, its comments contain more extensive decription of the options and give an outline of the algorithm. A somewhat less accurate estimate uses just the XYZ coordinates of the molecule and assumes the default radius size of for all atoms: *elsize input-file-xyz* . This option is not recommended for very small compounds. The code should not be used on structures made up of two or more completely disjoint" compounds -- while the code will still produce a finite value of *Arad* , it is not very meaningful. Instead, one should obtain estimates for each compound separately.

14. Appendices

14.1. Appendix A: Namelist Input Syntax

Namelist provides list-directed input, and convenient specification of default values. It dates back to the early 1960's on the IBM 709, but was regrettably not part of Fortran 77. It is a part of the Fortran 90 standard, and is supported as well by most Fortran 77 compilers (including g77).

Namelist input groups take the form:

```
&name
 var1=value, var2=value, var3(sub)=value,
 var4(sub,sub,sub)=value,value,
 var5=repeat*value,value,
/
```

The variables must be names in the Namelist variable list. The order of the variables in the input list is of no significance, except that if a variable is specified more than once, later assignments may overwrite earlier ones. Blanks may occur anywhere in the input, except embedded in constants (other than string constants, where they count as ordinary characters).

It is common in older inputs for the ending "/" to be replaced by "&end"; this is non-standard-conforming.

Letter case is ignored in all character comparisons, but case is preserved in string constants. String constants must be enclosed by single quotes ('). If the text string itself contains single quotes, indicate them by two consecutive single quotes, e.g. C1' becomes 'C1'' as a character string constant.

Array variables may be subscripted or unsubscripted. An unsubscripted array variable is the same as if the subscript (1) had been specified. If a subscript list is given, it must have either one constant, or exactly as many as the number in the declared dimension of the array. Bounds checking is performed for ALL subscript positions, although if only one is given for a multi-dimension array, the check is against the entire array size, not against the first dimension. If more than one constant appears after an array assignment, the values go into successive locations of the array. It is NOT necessary to input all elements of an array.

Any constant may optionally be preceded by a positive (1,2,3,..) integer repeat factor, so that, for example, 25*3.1415 is equivalent to twenty-five successive values 3.1415. The repeat count separator, *, may be preceded and followed by 0 or more blanks. Valid LOGICAL constants are 0, F, .F., .FALSE., 1, T, .T., and .TRUE.; lower case versions of these also work.

14.2. Appendix B: GROUP Specification

Entering Group Information

This section describes the format used to define groups of atoms in various AMBER programs. In *sander*, a group can be specified as a movable "belly" while the other atoms are fixed absolutely in space (aside from scaling caused by constant pressure simulation), and/or a group of movable atoms can independently restrained (held by a potential) at their positions. In *anal*, groups can be defined for energy analysis.

Except in the analysis module where different groups of atoms are considered with different group numbers for energy decomposition, in all other places the groups of atoms defined are considered as marked atoms to be included for certain types of calculations. In the case of constrained minimization or dynamics, the atoms to be constrained are read as groups with a different weight for each group.

Reading of groups is performed by the routine RGROUP and you are advised to consult it if there is still some ambiguity in the documentation.

Input description:

```
   - 1 -       Title

               format(20a4)

   ITITL       Group title for identification.

        Setting ITITL = 'END' ends group input.

-------------------------------------------------------------------------

   - 1A -      Weight

        This line is only provided/read when using GROUP input to
        define restrained atoms.

               format(f)

   WT   The harmonic force constants in kcal/mol-A**2 for the group
        of atoms for restraining to a reference position.

-------------------------------------------------------------------------

   - 1B -     Control to define the group

        KTYPG , (IGRP(I) , JGRP(I) , I = 1,7)

               format(a,14i)

   KTYPG      Type of atom selection performed.  A molecule can be
```

defined by using only 'ATOM' or 'RES', or part of the molecule can be defined by 'ATOM' and part by 'RES'.

'ATOM' The group is defined in terms of atom numbers. The atom number list is given in igrp and jgrp.

'RES' The group is defined in terms of residue numbers. The residue number list is given in igrp and jgrp.

'FIND' This control is used to make additional conditions (apart from the 'ATOM' and 'RES' controls) which a given atom must satisfy to be included in the current group. The conditions are read in the next section (1C) and are terminated by a SEARCH card.

Note that the conditions defined by FIND filter any set(s) of atoms defined by the following ATOM/RES instructions. For example,

```
-- group input: select main chain atoms --
FIND
* * M *
SEARCH
RES 1 999
END
END
```

'END' End input for the current group. Followed by either another group definition (starting again with line 1 above), or by a second 'END' "card", which terminates all group input.

IGRP(I) , JGRP(I)

The atom or residue pointers. If ktypg .eq. 'ATOM' all atoms numbered from igrp(i) to jgrp(i) will be put into the current group. If ktypg .eq. 'RES' all atoms in the residues numbered from igrp(i) to jgrp(i) will be put into the current group. If igrp(i) = 0 the next control card is read.

It is not necessary to fill groups according to the numerical order of the residues. In other words, Group 1 could contain residues 40-95 of a protein, Group 2 could contain residues 1-40 and Group 3 could contain residues 96-105.

If ktypg .eq. 'RES', then associating a minus sign with igrp(i) will cause all residues igrp(i) through jgrp(i) to be placed in separate groups.

In the analysis modules, all atoms not explicitly defined as members of a group will be combined as a unit in the (n + 1) group, where the (n) group in the last defined group.

- 1C - Section to read atom characteristics

***** Read only if KTYPG = 'FIND' *****

JGRAPH(I) , JSYMBL(I) , JTREE(I) , JRESNM(I)

format(4a)

A series of filter specifications are read. Each filter consists of four fields (JGRAPH,JSYMBL,JTREE,JRESNM), and each filter is placed on a separate line. Filter specification is terminated by a line with JGRAPH = 'SEARCH'. A maximum of 10 filters may be specified for a single 'FIND' command.

The union of the filter specifications is applied to the atoms defined by the following ATOM/RES cards. I.e. if an atom satisfies any of the filters, it will be included in the current group. Otherwise, it is not included. For example, to select all non main chain atoms from residues 1 through 999:

```
-- group input: select non main chain atoms --
FIND
* * S *
* * B *
* * 3 *
* * E *
SEARCH
RES 1 999
END
END
```

'END' End input for the current group. Followed by either another
The four fields for each filter line are:

JGRAPH(I) The atom name of atom to be included. If this and the following three characteristics are satisfied the atom is included in the group. The wild card '*' may be used to to indicate that any atom name will satisfy the search.

JSYMBL(I) Amber atom type of atom to be included. The wild card '*' may be used to indicate that any atom type will

```
          satisfy the search.

     JTREE(I)   The tree name (M, S, B, 3, E) of the atom to be included.
          The wild card '*' may be used to indicate that any tree
          name will satisfy the search.

     JRESNM(I)  The residue name to which the atom has to belong to be
          included in the group.  The wild card '*' may be used to
          indicate that any residue name will satisfy the search.
```

Examples:

The molecule 18-crown-6 will be used to illustrate the group options. This molecule is composed of six repeating (-CH2-O-CH2-) units. Let us suppose that one created three residues in the PREP unit: CRA, CRB, CRC. Each of these is a (-CH2-O-CH2-) moiety and they differ by their dihedral angles. In order to construct 18-crown-6, the residues CRA, CRB, CRC, CRB, CRC, CRB are linked together during the LINK module with the ring closure being between CRA(residue 1) and CRB(residue 6).

Input 1:

```
Title one
RES   1  5
END
Title two
RES   6
END
END
```

Output 1: Group 1 will contain residues 1 through 5 (CRA, CRB, CRC, CRB, CRC) and Group 2 will contain residue 6 (CRB).

Input 2:

```
Title one
RES   1  5
END
Title two
ATOM 36 42
END
END
```

Output 2: Group 1 will contain residues 1 through 5 (CRA, CRB, CRC, CRB, CRC) and Group 2 will contain atoms 36 through 42. Coincidentally, atoms 36 through 42 are also all the atoms in residue 6.

Input 3:

```
Title one
RES  -1  6
END
END
```

Output 3: Six groups will be created; Group 1: CRA, Group 2: CRB,..., Group 6: CRB.

Input 4:

```
Title one
FIND
O2 OS M CRA
SEARCH
RES   1  6
END
END
```

Output 4: Group 1 will contain those atoms with the atom name 'O2', atom type 'OS', tree name 'M' and residue name 'CRA'.

Input 5:

```
Title one
FIND
O2 OS * *
SEARCH
RES   1  6
END
END
```

Output 5: Group 1 will contain those atoms with the atom name 'O2', atom type 'OS', any tree name and any residue name.

14.3. Appendix C: Retired Namelist Variables

Unfortunately, many implementations of Fortran namelist reading do not emit specific errors for invalid variables. To remedy this situation retired input variables are kept in their namelist, and a warning is produced in the output file. Listed below are input variables that have been eliminated since version 7. For each variable the relevant program, namelist, and the version in which the deletion occurred are specified. A terse description of the original meaning is followed by the new behavior.

DOBS — Sander `&align` namelist; retired in version 8. The observed dipolar splitting, in Hz. Now lower and upper bounds are specified with DOBSL and DOBSU.

DTEMP Sander `&cntrl` namelist; retired in version 8. A control of temperature for NTT = 4 via the reassignment of velocities. Now NTT = 4 is included only for historical reasons and with reduced functionality.

DXM Sander `&cntrl` namelist; retired in version 8. The maximum step length in an energy minimization. Now no limit on the step length exists. In fact, this behavior is identical to that in Amber 7 since DXM was unused.

FRC_INT Sander `&ewald` namelist; retired in version 8. A control of the computation of forces in PME. Now forces cannot be obtained by interpolation; the forces are always calculated via differentiation of the energy.

HEAT Sander `&cntrl` namelist; retired in version 8. The initial velocities scaling factor for temperature regulation. Now the initial velocities are controlled solely by TEMPI.

ISCHRGD Sander `&ewald` namelist; retired in version 8. The control of charge neutralization in a unit cell. Now an insignificant nonzero net charge, one less than or equal to 0.01, is assumed to be due to roundoff error and is always neutralized. In fact, this behavior is identical to that in Amber 7 since ISCHRGD was silently ignored.

MATCAP Sander `&cntrl` namelist; retired in version 8. The modified cap atom pointer. Now the cap atom pointer in the *prmtop* cannot be modified.

NTU Sander `&cntrl` namelist; retired in version 8. The behavior is identical to that in Amber 7 since NTU was reset to 1 even if it appeared in the namelist.

PLEVEL Sander `&cntrl` namelist; retired in version 8. The parallelization level for constant pressure simulations. Now all the atoms of a small molecule are assigned to a single processor, and, consequently, the velocities do not need to be distributed to compute the correct virial.

TIMLIM Sander `&cntrl` namelist; pbsa `&cntrl` namelist; retired in version 9. The execution time limit, in seconds, for the job. Now no limit on the execution time exists. In fact, this behavior is identical to that in Amber 7 and 8 since TIMLIM was unused.

NPSCAL Sander `&cntrl` namelist; retired in version 9. This controls the way in which pressure scaling takes place. The only valid value now is "1", which invokes a center-of-mass scaling. This is really no change, since atom-based scaling was never correctly implemented. (Note that scaling in the amoeba_runmd code *does* use atom-based model, since it has a atom-based virial; but this behavior is not controlled by the *npscal* input variable.)

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