Guide to setting up a new system and doing equilibration

From Simmerling Lab

SIMMERLING LAB EQUILIBRATION PROCEDURES AND RECOMMENDATIONS

This is probably the most important of all tutorials- read it carefully! If you do not follow these procedures, you may have to redo all of your work.

You should take detailed notes on the topics below and what you did for your system- this will be needed later for writing your methods section.

If you choose not to follow the steps below (for example, you decide to build mutations with Leap) then you need to write a justification and have it approved by Carlos. NO EXCEPTIONS.

NOTE THAT THIS IS A GUIDE- DON'T PLAN TO USE THE INPUT FILES EXACTLY AS GIVEN! OUR IDEAS OF THE BEST CHOICES CAN CHANGE OVER TIME, AND THESE FILES MAY NOT ALWAYS BE UPDATED. FOR EXAMPLE, THERE MIGHT BE BETTER CHOICES IN WHICH GB MODEL TO USE, AND YOU WILL NEED TO BE AWARE OF THAT AND CHANGE THESE SAMPLE FILES ACCORDINGLY.

>>> **Required:** fill out the **new system checklist** before you do any simulations of a new molecule.

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things to add to this tutorial

- proper wiki formatting
- discussion of periodicity effects in PBC

Contents

IMPORTANT NOTES

- Eventually you will need to write the Methods section of a paper describing this work. Approach it that way. **Can you justify every single choice** (either by citation or good physical rationale)?

If you do not use our lab-default settings in the input file, make sure you can explain why it is ok (defaults meaning what you find in the setup guide, not what someone happened to use in their work here years ago). It will never be good enough to say "I did it that way because XXX did it that way. The same goes with saying that you did it that way to make it run faster. It's not ok.

- If you ever plan to compare two data sets (WT and mutant, 2 methods, 2 water models, or whatever) MAKE SURE THAT IS THE ONLY DIFFERENCE IN EVERY STEP YOU TAKE.
  - **REPEAT: If you ever plan to compare two data sets (WT and mutant, 2 methods, 2 water models, or whatever) MAKE SURE THAT IS THE ONLY DIFFERENCE IN EVERY STEP YOU TAKE.**
  - it must always be crystal clear that the only explanation for different results is the difference you wanted to study
  - Don't change the equilibration protocol.don't change the force field. don't change the cutoff. don't change anything at all, anywhere, except the spot where you want people to focus.
  - if you changed 2 things (sequence and the method) then you MUST have a control that isolates the differences to only 1 change.

- **do not ever set a value in an input file if you don't know what it is doing and why you set that value**
  - examples:
  - don't set a force constant if you don't know the units and the impact on the simulations/dynamics
  - don't set a thermostat or barostat unless you know how they work and what the coupling constant does
  - and so on

Choosing your input structure and checking the details

1. choose and download the pdb file. Check things like resolution, sequence, presence of inhibitors/cofactors/substrates/etc. when deciding on a file if there are several available for your system. If the sequence you want is not in the PDB, remember that mutating to a smaller residue is easier than changing to something larger (which might not fit).
2. evaluate your pdb file data
    1. is it from NMR or xray?
    2. NMR
        1. **Look at the entire family of NMR structures** and understand where the structures match and where they do not.
        2. check/fix names for hydrogens (Amber's protonate command can fix this (protonate –k)
        3. check for multiple models (only keep 1- make sure to read the NMR paper and the PDB file to know which one is most reliable)
        4. read about how the model was generated- force field used, number of restraints, etc. Know the limitations.
        5. compare the multiple models visually to see which parts are well defined and which are not. Parts that vary should probably not be restrained in equlibration, and also not be used in RMSD calculations.
    3. xray structures
        1. **Look at the electron density maps** (if available- look carefully!) in a program like Coot. Make sure to understand the Fo-Fc maps. What parts of the model are not reliable?
        2. **look at crystal packing contacts** (pymol symexp command). What impact might they have on the structure?
        3. make sure you read the info in the paper and file about structure quality- structure quality,

resolution, etc.
4. check to make sure you use the biological unit (is it a monomer/dimer/etc?) Notes about building multimer from "Biological Assembly" pdb file. Make sure you simulate what is biologically relevant? Also make sure to check the experimental evidence for formation of dimer/trimer etc.
5. don't simulate the entire unit cell unless that is what you really want. It usually isn't.
6. check for alternate conformations in high resolution structures. Keep only 1. (leap will usually take the first and ignore the others, but read the leap log to make sure that it is doing what you want). Read the experimental paper and the file to understand the conformations.
7. check for missing density (read the paper). Build missing atoms if needed (see below). Don't restrain these during equilibration.
8. you will usually need to add hydrogens using leap or molprobity (http://molprobity.biochem.duke.edu/) or Amber protonate
    1. molprobity is best, protonate can adapt between different H atom naming conventions. Use leap as a last resort only.

4. what are the experimental conditions? pH, etc. Are any of the amino acids or other groups in a non-standard protonation state? Especially consider His, but others as well.
5. TER lines should be present between any molecules that should not be connected together with a bond.

3. deal with things that need to be added, removed or changed
    1. check to see what was included in the experiment, as opposed to what coordinates are present. The resulting structure is not always what was used for the experiment! Missing atoms, residues called ALA that were not (missing side chain density). Carefully read the paper and all of the comments in the PDB file.
    2. What are the termini? Are they blocked, acetylated, amidated?
    3. Mutations (you want a different sequence than the PDB has)can e done with the following. Visually inspect every mutated residue! DO NOT USE LEAP FOR THIS PURPOSE.
        1. use swissPDBviewer
        2. use the SCWRL program available at http://dunbrack.fccc.edu/scwrl4/SCWRL4.php
        3. VMD may also be an option but we have not tested it.
    4. Mutations other than protein residues can be done with leap, but be careful since leap always uses the Z matrix that you have in the library file to add missing atoms. Check the results visually before doing next steps – leap does not consider energies or anything else when it adds missing atoms.
    5. Run molprobity to check these things:
        1. Asn/Gln flips
        2. Histidine flips (note that it will not change name to HID/HIE/HIP, see below)
        3. Bad geometries
        4. It can add hydrogens (probably more accurate than leap is)
    6. Should salt be included? How, which ions and what concentration? Vtot*(10**-24)*0.001*(Conc.Salt in Molar)*6.022*10**23
        1. Are there ions in the actual crystal structure? Should they be kept? Do you know for sure which ions they are? (not always the same as the PDB file names them)
        2. Tom Cheatham's advice on adding ions
    7. Are there water molecules in the crystal structure?
        1. For explicit water simulations, these will usually be kept.
        2. For implicit water simulations, these are usually removed, but they might need to be kept if they are not going to diffuse away (if they are buried, or in the interface between molecules).
        3. In either case this requires some thought and visual analysis. You need to be able to justify your choices.
    8. Are there any metals? these can be important, but are often challenging. It will require discussion at lab meeting.
    9. Are there any other molecules, such as crystallization buffer? These normally are not important to function and can usually be removed.
    10. check for any unusual non-standard residues – ligands, cofactors, modified amino acids, etc). carefully

consider if and how they should be modeled.

Running leap to generate coordinates and prmtop

1. use leaprc.ff14SB for all simulations unless you are absolutely sure you should use something different.
2. if using DNA, make sure to modify force field for parm.bsc0
3. set your PBradii. Usually you want mbondi2, but this depends on the GB model. **unless you are sure what you are doing, ALWAYS SET PBRADII TO MBONDI2 EVEN FOR EXPLICIT WATER PRMTOPS**
4. you must explicitly add disulfide bonds in leap, it is not automatic.
5. Leap will use HIE for histidine unless you change the name from HIS to HID or HIP. Think about whether your histidine should be charged, and if it should be HID or HIE. If these issues aren't clear, ask for help from a senior lab member. You may need to edit the name in your PDB to get HID instead of HIE (or to get HIP). **Don't assume HIS is done correctly** unless you check carefully!
6. If you are trying to model something far from pH=7, to probably should run pKa calculations or use another method to check protonation states. Check the termini too.
7. Add solvent- your buffer size will depend on how large the box needs to be during MD. Will the system significantly change size (unfold)? If so, then you need to estimate how large your box might be (maybe run GB md and plot end to end distance). Usually a buffer of about 8Å should be ok.
8. When you are building multiple coordinate files of the same system (for independent runs, or for NEB endpoints, etc) make SURE to get the same # water molecules in every coordinate set This will make the analysis much easier in the end. Multiple versions of the same system should have the same atoms. Modify the buffer and rerun leap. If needed, convert the prmtop and prmcrd to a pdb using ambpdb, and then delete some water molecules and load this pdb into leap instead of the one with no water.
9. Think about whether you want a truncated octahedron box or not- usually yes, but sometimes no. make sure you understand the issues.
10. Think about inclusion of ions – neutralizing ions and bulk salt.
11. READ THE LEAP.LOG FILE. You can usually ignore “warnings” about missing improper dihedrals. Never ignore errors. Make sure that every “added missing heavy atom” is something that you wanted leap to do.
12. **If you have a dimer or another system with more than 1 solute molecule, it is very important to modify your solvent pointers in the prmtop so that there is only 1 solute “molecule”.**
    1. This keeps sander/pmemd from splitting apart the complex when it wraps molecules at the boundary of the periodic box. You can fix it later, but it is easiest to avoid it altogether.
    2. It also will keep your multimer from compressing during the pressure equilibration as the box changes size
    3. Make sure to put **bound** ions (like zinc) in the solute too
    4. This is very important!
13. You will almost always need a copy of your system without the solvent and ions, so go ahead and build that now using the same procedure.

Check the results in VMD

1. Load your prmtop and inpcrd into VMD or another program that uses the prmtop file. If you had any non-standard residues or small molecules, check them carefully for correct bonding.

Equilibration

1. general philosophy
    1. **the most important thing to do is to keep in mind that you want to relax any portion that was not in the experimental (PDB) file before you let the “known” part move.** If something was built in a non-ideal position, you want it to move, not the rest of the system. Restrain everything from the experiment until the parts you added (water, ions, hydrogens, missing atoms) have been relaxed through minimization and MD. The high energies from the parts you added can be enough to melt the

parts that the experiment determined- so you need to relax them before removing restraints! Note that ions can take a LONG time to equilibrate (tens of ns or more). Make sure you understand what you are doing when adding ions.

2. crystallographic water molecules are part of the "known" structure (at least the O atoms). You probably should restrain the crystal water O atoms, but not their H atoms. (see note above about not keeping these for implicit solvent).
3. If you added large sections, such as loops, you may want to equilibrate the solute using GB before adding explicit water. You will want strong restraints on the rest so it doesn't move during implicit solvent simulation.
    1. Don't do this unless you really added something more than a sidechain or hydrogens- it isn't needed. GB has issues and unless you must use it during your equilibration, you probably should not. It's only for adding large sections of solute that might need to move a lot.
    2. This will make it easier for the added atoms to relax and not bump into water.
    3. Restrain the coordinates from experiment, and then minimize/MD the added parts following a procedure similar to what is below.
    4. Keep everything known from experiment restrained while you use GB to relax the part added.
    5. Don't turn off restraints until you do it in the normal explicit water equilibration!
    6. Take the coordinates from the GB equilibration and convert to pdb using ambpdb, then read them into leap and add solvent and ions if needed. Then continue below.
    7. Be very careful when using GB on a system with ions (metals, salt) or other unusual atoms – the radii for these may not be correct. Look into it before running the simulation and be prepared to justify your choices.
4. It is a good idea to always use the same coordinates for the reference for restraints (the coordinates from the experiment). **The exception** is that minimization and MD appear to not have the same coordinate origin (as of amber10), so after minimization you should use the minimization restart file as both the inpcrd and refc for the next MD step. If you don’t, the energies will get very high and the system will be unstable.For all following MD steps, do not change the reference coordinates unless you do minimization again.
5. Usually it's a good idea to turn off restraints on side chains prior to the backbone- often side chains tend to have more crystal contacts and may need more adjustment. It's good to let these "fix" themselves prior to letting a strained conformation in a side chain force the backbone to change.

restraintmask syntax

- **note that '&' is logical AND and '|' is logical OR.** Make sure you understand logical expressions before writing a mask. a AND b means it must be in both groups a and B. OR means it can be in either group to match. Saying "water and backbone" will match nothing. In most cases where people think "and" they really mean logical OR. NOT a and NOT b usually gives what you want- stuff that isn't in a and it also isn't in b. a AND b will give the intersection- stuff that is in group a and also in group b. a OR b gives things that are either in a or in b- what you often want.
- keep in mind that AND is intersection and B is union.
- use parentheses if needed
- read the section on restraint masks (ambmask) in the Amber manual. It works the same way as in ptraj.
- make sure you carefully check your sander output to make sure it restrained what you wanted. THIS IS IMPORTANT.

NOTES ON RECOMMENDED DEFAULT SETTINGS

1. **Always use the input files below unless you are SURE of what you are doing.** Modify them as needed (restraints, etc.)
2. make sure the amber version you are using is fully up to date with all patches from the amber web site.
3. **Use pmemd as a simulation program unless you need a feature that only sander supports.** pmemd

benchmark tests should be done separately from sander- it will behave differently (faster and better parallel scaling)

4. keep iwrap=1 at all times unless you really know what you are doing.
5. time steps should usually be 0.002, but use 0.001 for (GB or explicit solvent) MD where you want to compare to experimental dynamics such as nmr order parameters, or during free energy calculations (TI).
6. **for GB runs using RESPA** (multiple time stepping), do a short (10ps) run with time step 0.5fs and no respa with constant energy (ntt=0) and then repeat for your respa setting to make sure you can still conserve energy. this may vary from 1 system to another- so test it!
7. the value of the nonbonded cutoff "cut" can strongly affect simulation time. For PME calculations, the cutoff does not really affect energies (make sure you understand why!), but does affect timings and scaling. Try values of cut=8 or 10 and benchmark them. Longer than 8 is probably not a good idea, it will be too slow.
8. repeat! GB can use respa. **You should run benchmarks of both timing and energy conservation** (ntt=0), but a good starting point is nrespa=4, nrespai=2, cut=9999, and cut_inner=15.
9. when you are using restraints (ntr=1), make sure to set nscm=0. during free MD (production), you MUST set ncsm>0.
10. GB simulations should always be run with Langevin dynamics (ntt=3, gamma_ln>0). gamma_ln should be small (1) unless you specifically want to slow down motions. Make sure you know what you're doing. Don't just follow an input someone showed you. That won't help when the reviewers reject your manuscript.
11. **explicit solvent simulations should probably also use Langevin dynamics- but be aware that explicit water already introduces friction/viscosity, and using a high gamma_ln value will only make the dynamics slower. It is probably best to use Langevin dynamics with a small gamma_ln value for explicit solvent, such as 0.1 to 1, but no higher than 1.**
12. The Berendsen thermostat has been shown in many articles to not produce correct ensembles- although it isn't really clear how bad it is.
13. In many simulations, you may not want any temperature control and just want "normal" Newtonian dynamics. In this case do not use a thermostat. Make sure to equilibrate with a thermostat first and then turn it off after equilibration is complete. This is needed when you want unperturbed dynamics and energy conservation, such as with order parameter calculations.
14. use fairly strong pressure coupling during equilibration, but weak during MD. Strong is 0.1 for tau and weak is 10. Weaker coupling is always closer to "normal" dynamics. Make sure to check the energy in your output- it may drift too much in long runs without a thermostat.
15. make sure to set the random number seed (ig) to different numbers for independent simulations. **The ig value sholud be different for every restart run. Otherwise the simulation will be periodic and nothing new will be observed on restart. It's recommended to set ig = -1**
    1. Setting ig to different values does not make a difference for anything except Langevin dynamics or a simulation with tempi=0 and irest=0.
    2. **currently there is a bug** in setting initial velocities for simulations with ntb>0 (periodic systems) and ntt=1, where irest=0 and tempi>0 does not actually use the seed to assign velocities. For independent simulations in these cases, you need different input coordinates or different temp0.
    3. Note that **Langevin dynamics in general does not scale well to large numbers of processors**. Benchmark carefully- the parallel scaling using different thermostats is quite different. You might want to use ntt=1 for large parallel runs. This is fixed in Amber11 but requires special compile-time flags and special flags when running MD. Check back here for more details. **This needs updating!**
16. Note that **scee** is **not supported in AMBER11**. Delete the **scee** altogether from the input files below, if you're using AMBER11.
17. **use netcdf formatting for all output files - this results in smaller files, increased precision, faster processing in ptraj, and higher compressibility.**

```
ioutfm=1 (invoke netcdf formatting, =0 Default)
```

Steps for a good equilibration protocol

1. Keep the “known” part restrained with ntr=1 and restraint_wt=100.
    1. Do not restrain solvent, ions, hydrogens, or anything else added in. For example, if the crystal structure resolves the "O" atom of water(s), restraint just that atom of the crystal water(s) and not the hydrogens added by leap; If NMR resolves the "H"'s and "O" of water, restrain the whole water molecule(s).
    2. Minimize everything
        1. Note that the minimization output will tell you the atom with "GMAX", meaning the highest forces. It can be useful to help you visualize the structure and see where the problem area might be.
    3. Heat to the desired temperature with ntb=1 and ntp=0 (constant V) (**starting at tempi=100. Do NOT start at 0K. NEVER.** This can be a disaster for some thermostats- hot spots will get huge temperatures if you scale by this much.).
        1. Do this using nmropt=1 and changing temp0 in the weight change section
        2. Use about 100ps or so to go from 100 to 300K
    4. Run MD on everything and ntb=2, ntp=1 (unless you plan to do all of the MD at constant V) for long enough to reach a plateau in the energies and density/pressure. Even if you want to run constant V later, you should equilibrate the volume and pressure here. Use strong (0.1) tautp and taup to equilibrate T and P faster.
2. Slowly reduce the restraints on the coordinates determined from experiment. For each step below, longer is better but you should probably run for at least 1ns.
    1. Minimize the results of the first equilibration stage, but reduce restraints to 10
    2. Run MD, heating from 100K to 300K over 100ps
    3. Run md with restraints at 10
    4. Run md with restraints at 1
        1. For crystal structure that resolved waters
    5. For proteins, change restraints to be only on the protein backbone (so that surface side chains can change in case they were influenced by crystal contacts).
    6. Run md with restraints at 0.1
    7. Turn off restraints completely. Use weak (1.0) T and P coupling (if any).
3. For GB, perform the same steps, but obviously there is no water in the unrestrained region and no constant V/P changes.

Checking the results

1. check these things during the equilibration
    1. energies at every step of your equilibration- does it every get very high? Why?
    2. Check densities, pressures, potential/kinetic energies – extract them using scripts and plot them. Check any unusual peaks. Make sure they reach plateau values.
    3. Pay special attention to bond and angle energies! Should end up low and never spike. Increasing values usually indicated some structural strain that the equilibration can’t fix, such as steric overlap. Visually inspect these areas.
        1. Ross Walker's Perl Script (http://simmerlinglab.org/wiki/images/4/46/E_asis.txt) to extract above quantities from mdout files. Usage: "./E_asis mdout". Creates many files.
    4. Look at atoms that have GMAX in minimization output. Why is the force high?
    5. Visually inspect your structure, especially the things that were added or changed in the steps above, or anything that needed a separate force field library (like a ligand).
2. check these things in your final equilibrated structure
    1. peptide bonds cis/trans
    2. chiralities of protein backbone (mdhelper)
    3. chiralities of these side chains: Thr, Ile
    4. nucleic acids: check sugar puckers if needed. Check chiralities if possible.
    5. Geometries of anything that is not standard amino acids or nucleic acids or water. Check bonds, angles, planarity, etc. Does it make chemical sense?

Production runs

1. collect production MD data by extending the unrestrained simulation described in the last step above
2. some calculations are better off without using a thermostat/barostat. For example, if you want to calculate NMR order parameters, you probably want to run in the NVE ensemble with a small time step.
    1. Note: equilibrate the volume/density with a barostat before doing constant volume (or else the volume will be incorrect)
    2. Note: equilibrate the temperature with a thermostat before doing constant energy- so that your system has close to the right temperature
        1. Note: tighten SHAKE and PME direct sum tolerances to 1e-6; you probably won't conserve energy without this
    3. REMD simulations require constant volume

Final thoughts

1. make sure you have a backup of the data. The data storage disks WILL fail. It's just a questino of when. It will happen. Don't let it hurt your career.

Sample input files for GB equilibration

(all atoms defined in NMR structure, residues 24,25,30,33,45,49,50 mutated, inputs generated by carlos) Note that for protein:DNA complex, a useful backbone restraint mask might be restraintmask="@N,CA,C,O,O3',O4',O5',C1',C2',C3',C4',C5',P & !:10-13,22-25,253-259", (this restrains backbone of either protein or DNA, except in the residue ranges given) Note that the blue gene and other machines may not like “&end”, try “/” instead.

note that '&' is logical AND and '|' is logical OR. Make sure you understand logical expressions before writing a mask. a AND b means it must be in both groups a and B. OR means it can be in either group to match. Saying "water and backbone" will match nothing. In most cases where people think "and" they really mean logical OR.

1min.in minimize H atoms

```
&cntrl
       imin = 1, maxcyc=1000,
       ntx = 1,
       ntwr = 100, ntpr = 100,
       cut = 99.0,
       ntb=0, igb = 8,  saltcon = 0.0,
       ntr=1, restraint_wt=10, restraintmask="!@H= & !:24,25,30,33,45,49,50",
/
```

2md.in 500ps LD, only H moving, heat during first 250ps

```
&cntrl
       imin = 0, nstlim = 500000, dt = 0.001,
       ntx = 1, irest=0,
       ntt=3, tempi = 100.0, temp0 = 300.0, gamma_ln=1.,
       ntc = 2, ntf = 2,
       ntwx =  500, ntwe = 0, ntwr = 500, ntpr = 500,
       cut = 99.0,
       ntb=0, igb = 8,  saltcon = 0.0,
       ntr=1, restraint_wt=10, restraintmask="!@H= & !:24,25,30,33,45,49,50", nscm=0,
```

```
       ioutfm=1, ntxo=2,
       nmropt=1,
       ig=-1,
/
&wt
   TYPE="TEMP0",istep1=0, istep2=250000,
   value1=100., value2=300.,
/
&wt
   TYPE="TEMP0",istep1=250001, istep2=500000,
   value1=300., value2=300.,
/
&wt
   TYPE="END",
/
```

3min.in minimize side chains, H atoms

```
&cntrl
       imin = 1, maxcyc=1000,
       ntx = 1,
       ntwr = 100, ntpr = 100,
       cut = 99.0,
       ntb=0, igb = 8,  saltcon = 0.0,
       ntr=1, restraint_wt=10, restraintmask="@N,CA,C,O & !:24,25,30,33,45,49,50",
/
```

4md.in 500ps LD, backbone strong restraints, heat during first 250ps

```
&cntrl
       imin = 0, nstlim = 500000, dt = 0.001,
       ntx = 1, irest=0,
       ntt=3, tempi = 100.0, temp0 = 300.0, gamma_ln=1.,
       ntc = 2, ntf = 2,
       ntwx =  500, ntwe = 0, ntwr = 500, ntpr = 500,
       cut = 99.0,
       ntb=0, igb = 8,  saltcon = 0.0,
       ntr=1, restraint_wt=10., restraintmask="@CA,C,N,O & !:24,25,30,33,45,49,50", nscm=0,
       ioutfm=1, ntxo=2,
       nmropt=1,
       ig=-1,
/
&wt
   TYPE="TEMP0",istep1=0, istep2=250000,
   value1=100., value2=300.,
/
&wt
   TYPE="TEMP0",istep1=250001, istep2=500000,
   value1=300., value2=300.,
/
&wt
   TYPE="END",
/
```

5md.in 500ps LD, backbone medium restraints

```
&cntrl
       imin = 0, nstlim = 500000, dt = 0.001,
       ntx = 5, irest=1,
       ntt=3, temp0 = 300.0, gamma_ln=1.,
       ntc = 2, ntf = 2,
       ntwx =  500, ntwe = 0, ntwr = 500, ntpr = 500,
       cut = 99.0,
       ntb=0, igb = 8,  saltcon = 0.0,
```

```
      ntr=1, restraint_wt=1., restraintmask="@CA,C,N,O & !:24,25,30,33,45,49,50", nscm=0,
      ioutfm=1, ntxo=2,
      ig=-1,
/
```

6md.in 500ps LD, backbone weak restraints

```
&cntrl
      imin = 0, nstlim = 500000, dt = 0.001,
      ntx = 5, irest=1,
      ntt=3, temp0 = 300.0, gamma_ln=1.,
      ntc = 2, ntf = 2,
      ntwx =  500, ntwe = 0, ntwr = 500, ntpr = 500,
      cut = 99.0,
      ntb=0, igb = 8,  saltcon = 0.0,
      ntr=1, restraint_wt=0.1, restraintmask="@CA,C,N,O & !:24,25,30,33,45,49,50", nscm=0,
      ioutfm=1, ntxo=2,
      ig=-1,
/
```

7md.in 5ns LD unrestrained, using respa

```
&cntrl
      imin = 0, nstlim = 5000000, dt = 0.001,
      ntx = 5, irest=1,
      ntt=3, temp0 = 300.0, gamma_ln=1.,
      ntc = 2, ntf = 2,
      ntwx =  500, ntwe = 0, ntwr = 500, ntpr = 500,
      cut = 99.0,
      ntb=0, igb = 8,  saltcon = 0.0,
      ntr=0, nscm=0,
      nrespa=4,nrespai=2,cut_inner=15.,
      ioutfm=1, ntxo=2,
      ig=-1,
/
```

Sample input files for TIP3P equilibration

(All atoms defined in NMR structure, water added by leap, no ions, nothing mutated; inputs generated by Carlos, for Kraken)

```
aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/1min.in \
-p ./prmtop \
-c ./inpcrd \
-ref ./inpcrd \
-o ./1min.out \
-x ./1min.trj \
-inf ./1min.info \
-r ./1min.rst

aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/2mdheat.in \
-p ./prmtop \
-c ./1min.rst \
-ref ./1min.rst \
-o ./2mdheat.out \
-x ./2mdheat.trj \
-inf ./2mdheat.info \
-r ./2mdheat.rst
```

```
aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/3md.in \
-p ./prmtop \
-c ./2mdheat.rst \
-ref ./2mdheat.rst \
-o ./3md.out \
-x ./3md.trj \
-inf ./3md.info \
-r ./3md.rst

aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/4md.in \
-p ./prmtop \
-c ./3md.rst \
-ref ./3md.rst \
-o ./4md.out \
-x ./4md.trj \
-inf ./4md.info \
-r ./4md.rst

aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/5min.in \
-p ./prmtop \
-c ./4md.rst \
-ref ./3md.rst \
-o ./5min.out \
-x ./5min.trj \
-inf ./5min.info \
-r ./5min.rst

aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/6md.in \
-p ./prmtop \
-c ./5min.rst \
-ref ./5min.rst \
-o ./6md.out \
-x ./6md.trj \
-inf ./6md.info \
-r ./6md.rst

aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/7md.in \
-p ./prmtop \
-c ./6md.rst \
-ref ./6md.rst \
-o ./7md.out \
-x ./7md.trj \
-inf ./7md.info \
-r ./7md.rst

aprun -n 128 -N2 /nics/c/home/csimmerl/scratch/amber10/src/sander/sander.MPI -O \
-i ../inputs/8md.in \
-p ./prmtop \
-c ./7md.rst \
-ref ./6md.rst \
-o ./8md.out \
-x ./8md.trj \
-inf ./8md.info \
-r ./8md.rst

aprun -n 128 -N2 /lustre/scratch/csimmerl/amber10/exe/pmemd -O \
-i ../inputs/9md.in \
-p ./prmtop \
-c ./8md.rst \
-o ./9md.out \
-x ./9md.trj \
-inf ./9md.info \
-r ./9md.rst
```

```
1min.in
 &cntrl
```

```
 imin = 1, maxcyc = 10000,
 ntx = 1,
 ntwx =  50, ntwe = 0, ntwr = 500, ntpr = 50,
 ntc = 1, ntf = 1, ntb = 1, ntp = 0,
 cut = 8.0,
 ntr=1, restraintmask = '!:WAT  & !@H=',
 restraint_wt = 100.,
/
```

```
2mdheat.in
 &cntrl
  imin = 0, nstlim = 100000, dt = 0.001,
  irest = 0, ntx = 1, ig = -1,
  tempi = 100.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 0.1, taup = 0.1,
  ntwx = 1000, ntwe = 0, ntwr = 1000, ntpr = 1000,
  cut = 8.0, iwrap = 1,
  ntt =1, ntb = 1, ntp = 0,
  nscm = 0,
  ntr=1, restraintmask="!:WAT  & !@H=", restraint_wt=100.0
  nmropt=1,
  ioutfm=1, ntxo=2,
 /
 &wt
   TYPE="TEMP0", istep1=0, istep2=100000,
   value1=100., value2=298.,
 /
 &wt
   TYPE="END",
 /
/
```

```
3md.in
restrained molecular dynamics, 100ps
 &cntrl
  imin = 0, nstlim = 100000, dt = 0.001,
  irest = 1, ntx = 5, ig = -1,
  tempi = 298.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 0.1, taup = 0.1,
  ntwx = 1000, ntwe = 0, ntwr = 1000, ntpr = 1000,
  cut = 8.0, iwrap = 1,
  ntt =1, ntb = 2, ntp = 1,
  nscm = 0,
  ntr=1, restraintmask="!:WAT & !@H=", restraint_wt=100.
  ioutfm=1, ntxo=2,
/
```

```
4md.in
restrained molecular dynamics, 250ps
 &cntrl
  imin = 0, nstlim = 250000, dt = 0.001,
  irest = 1, ntx = 5, ig = -1,
  tempi = 298.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 0.5, taup = 0.5,
  ntwx = 1000, ntwe = 0, ntwr = 1000, ntpr = 1000,
  cut = 8.0, iwrap = 1,
  ntt =1, ntb = 2, ntp = 1,
  nscm = 0,
  ntr=1, restraintmask="!:WAT  & !@H=", restraint_wt=10.
  ioutfm=1, ntxo=2,
 /
```

```
5min.in
 &cntrl
  imin = 1, maxcyc = 10000,
  ntx = 1,
  ntwx =  50, ntwe = 0, ntwr = 500, ntpr = 50,
  ntc = 1, ntf = 1, ntb = 1, ntp = 0,
  cut = 8.0,
  ntr=1, restraintmask="@CA,N,C", restraint_wt=10.
 /
```

```
6md.in
 &cntrl
  imin = 0, nstlim = 100000, dt = 0.001,
  irest = 0, ntx = 1, ig = -1,
  tempi = 298.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 0.5, taup = 0.5,
  ntwx = 1000, ntwe = 0, ntwr = 1000, ntpr = 1000,
  cut = 8.0, iwrap = 1,
  ntt =1, ntb = 2, ntp = 1,
  nscm = 0,
  ntr=1, restraintmask="@CA,N,C", restraint_wt=10.
  ioutfm=1, ntxo=2,
  /
```

```
7md.in
 &cntrl
  imin = 0, nstlim = 100000, dt = 0.001,
  irest = 1, ntx = 5, ig = -1,
  tempi = 298.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 0.5, taup = 0.5,
  ntwx = 1000, ntwe = 0, ntwr = 1000, ntpr = 1000,
  cut = 8.0, iwrap = 1,
  ntt =1, ntb = 2, ntp = 1,
  nscm = 0,
  ntr=1, restraintmask="@CA,N,C", restraint_wt=1.
  ioutfm=1, ntxo=2,
  /
```

```
8md.in
 &cntrl
  imin = 0, nstlim = 100000, dt = 0.001,
  irest = 1, ntx = 5, ig = -1,
  tempi = 298.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 0.5, taup = 0.5,
  ntwx = 1000, ntwe = 0, ntwr = 1000, ntpr = 1000,
  cut = 8.0, iwrap = 1,
  ntt =1, ntb = 2, ntp = 1,
  nscm = 0,
  ntr=1, restraintmask="@CA,N,C", restraint_wt=0.1
  ioutfm=1, ntxo=2,
  /
```

```
9md.in
 &cntrl
  imin = 0, nstlim = 250000, dt = 0.002,
  irest = 1, ntx = 5, ig = -1,
  tempi = 298.0, temp0 = 298.0,
  ntc = 2, ntf = 2, tol = 0.00001,
  tautp = 1.0, taup = 1.0,
  ntwx = 500, ntwe = 0, ntwr = 500, ntpr = 500,
```

```
 cut = 8.0, iwrap = 1,
 ntt =1, ntb = 2, ntp = 1,
 nscm = 500
 ioutfm=1, ntxo=2,
 /
```
