AMBER Tutorial

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DNA:DNA, polyA–polyT

This is a summary of the amber tutorial in the AMBER home page (http://amber.scripps.edu/).
In this tutorial, we learn how to set up a standard decamer polyA–polyT duplex DNA model and perform a molecular dynamics simulation for it with the AMBER suite of programs.

The flow of this tutorial is as follows.

1. Making the initial model structure.

Generating the initial models of the DNA duplex 10-mer.

2. Creating the input files for Amber calculation.

This is a description of how to set up the molecular topology/parameter and coordinate files necessary for performing minimization or dynamics with AMBER.

3. Equilibration

Setting up and running equilibration and production minimization and molecular dynamics simulations for this DNA model, in explicit water.

4. Analyzing results.

Performing basic analysis such as calculating root-mean-squared deviations (RMSD) and plotting various energy terms as a function of time.

1. Making the coordinates of the initial model structure

If you use the experimentally determined structures, these can be found by searching through databases of crystal or NMR structures.

Proteins	Protein Data Bank	http://www.rcsb.org/pdb/
Nucleic acids	Nucleic Acid Database	http://ndbserver.rutgers.edu/

If you can not find a good experimental structure for DNA, there are several programs which help us make the initial model nucleic acids structures. (For example, NAB molecular manipulation language has been developed by David Case and Tom Macke of Scripps.)

AMBER comes with a simple program to generate standard canonical α- and β-duplex geometries of nucleic acids.

The program nucgen will build cartesian coordinate canonical α- and β-models of standard nucleic acid duplexes and provide the coordinates in the pdb format.

Reproduced below is the input file for nucgen that will build a 10-mer polyA–polyT duplex into the β -DNA canonical structure. Basically, this is building two strands of DNA.

File nuc.in

```
NUC 1
D
A5   A    A    A    A    A    A    A    A    A3

NUC 2
D
T5   T    T    T    T    T    T    T    T    T3

END
$ABDNA
```

To run nucgen, you need to specify the input file, an output file, the nucgen database and the name of the pdb you wish to write.

The command is as follow.

```
nucgen -O -i nuc.in -o nuc.out -d $AMBERHOME/dat/leap/parm/nucgen.dat -p nuc.pdb
```

The input file is `nuc.in`, and the generated output files are `nuc.out` and `nuc.pdb`.

Now you have got a pdb file of the initial structure of DNA. You should always look at your models before trying to use them. LEaP works fine for displaying models assuming that the appropriate residues were preloaded into LEaP and that they are named appropriately and consistently to what LEaP expects within the pdb file. In addition to names of the residues, LEaP also requires information about the chain connectivity. LEaP is fairly simple in that it expects that all residues are connected in a pdb file unless they are separated by a "TER" card and that the first residue read in is "tail only" and the last residue is "head only". The "TER" separator (a single line that begins with "TER") ends the chain and begins a new one.

File `nuc.pdb`

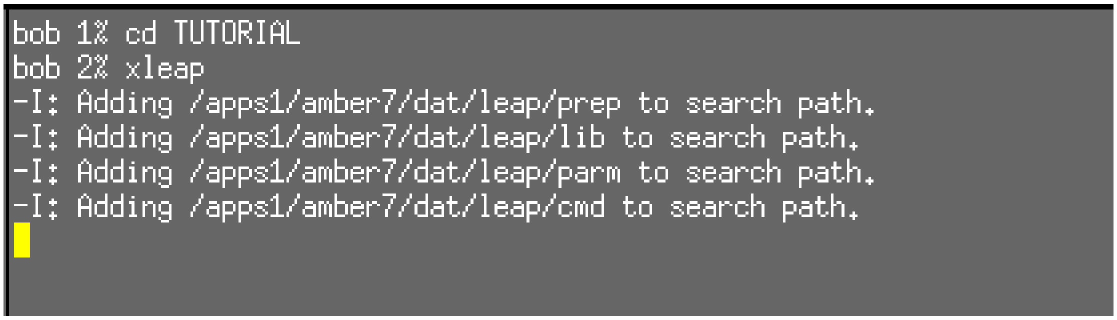
```
....
ATOM    228  N1  A3     10       0.207  -0.835  30.390
ATOM    229  H3T A3     10      -0.808  -8.873  31.720
ATOM    230  H5T T5     11       4.562   7.653  32.500
ATOM    231  O3' T5     11       7.904   3.753  30.670
....
```

File `nuc_ter.pdb`

```
....
ATOM    228  N1  A3     10       0.207  -0.835  30.390
ATOM    229  H3T A3     10      -0.808  -8.873  31.720
TER
ATOM    230  H5T T5     11       4.562   7.653  32.500
ATOM    231  O3' T5     11       7.904   3.753  30.670
....
```

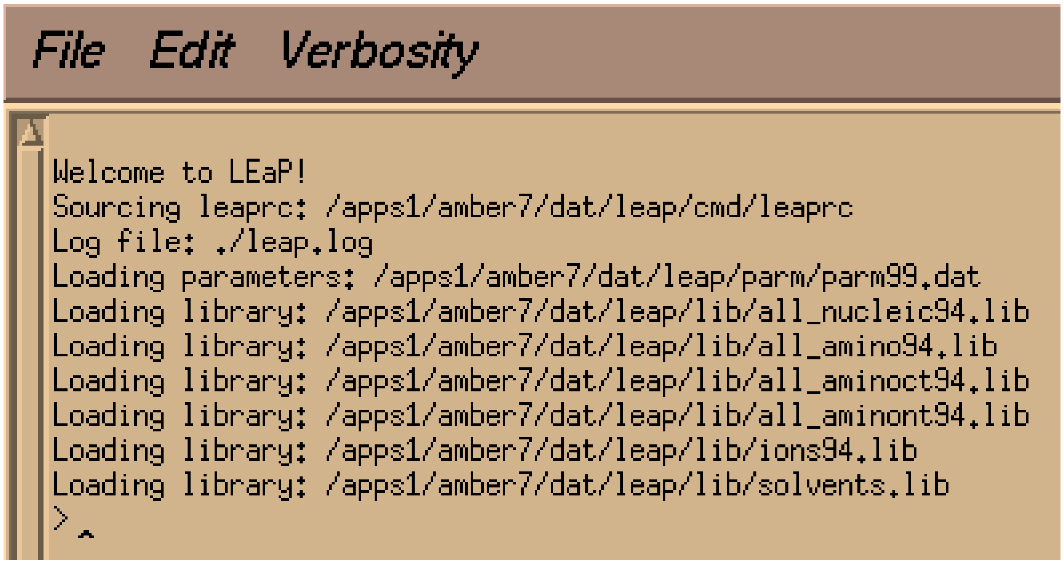
2. Creating the input files for Amber calculation.

To obtain the input and parameter files for a molecular dynamics simulation, start up the graphical version of LEaP, `xleap`.



```
bob 1% cd TUTORIAL
bob 2% xleap
-I: Adding /apps1/amber7/dat/leap/prep to search path.
-I: Adding /apps1/amber7/dat/leap/lib to search path.
-I: Adding /apps1/amber7/dat/leap/parm to search path.
-I: Adding /apps1/amber7/dat/leap/cmd to search path.
```

You will see something like this appear



```
Welcome to LEaP!
Sourcing leaprc: /apps1/amber7/dat/leap/cmd/leaprc
Log file: ./leap.log
Loading parameters: /apps1/amber7/dat/leap/parm/parm99.dat
Loading library: /apps1/amber7/dat/leap/lib/all_nucleic94.lib
Loading library: /apps1/amber7/dat/leap/lib/all_amino94.lib
Loading library: /apps1/amber7/dat/leap/lib/all_aminoct94.lib
Loading library: /apps1/amber7/dat/leap/lib/all_aminont94.lib
Loading library: /apps1/amber7/dat/leap/lib/ions94.lib
Loading library: /apps1/amber7/dat/leap/lib/solvents.lib
> 
```

To load up a pdb and view it, we can use the `loadpdb` and `edit` commands. To do this, we will create a new *unit* in LEaP. A *unit* is a residue or collection of residues.

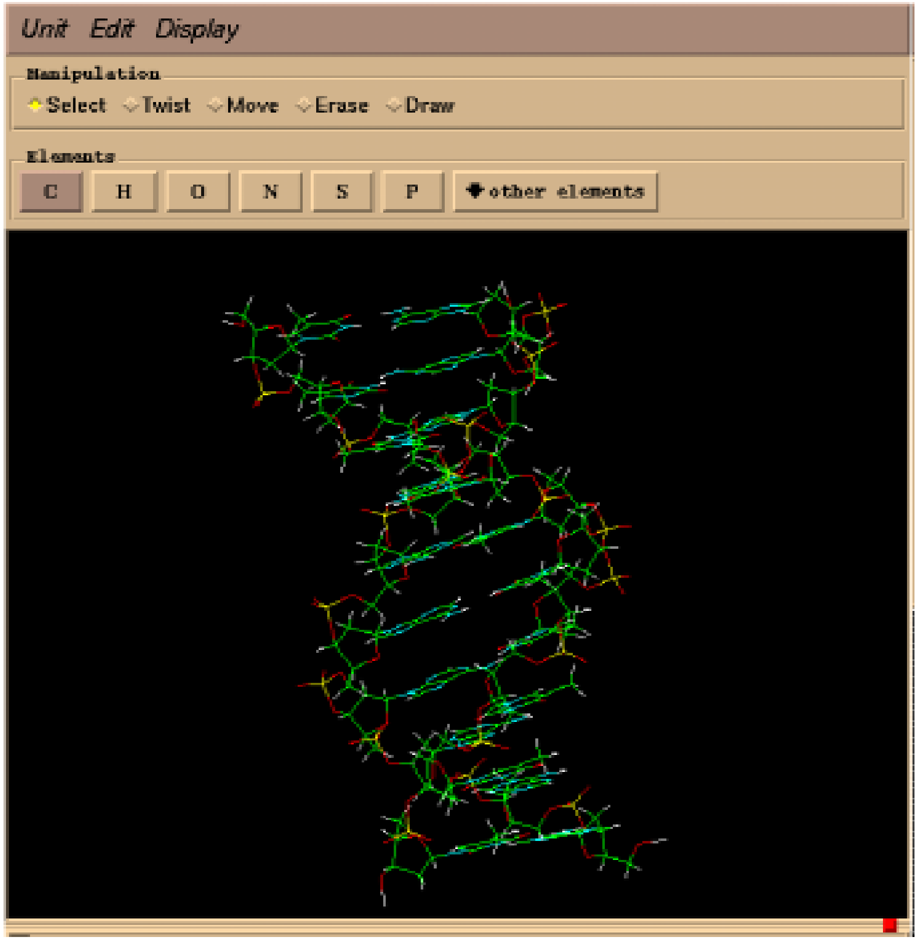
Let us load up the pdb into a *unit* called "dna":

```
dna=loadpdb nuc_ter.pdb
```

When LEaP reads a pdb file, LEaP automatically adds the missing hydrogen atoms (which nucgen does not supply).

To see the structure, "edit" the *unit* dna:

```
edit dna
```



We are ready to build the input files necessary for sander. Since we want to perform a molecular dynamics simulation for a 10-mer polyA–polyT duplex into the β-DNA canonical structure in explicit net neutralizing counterions and water. First, the counterions are placed at the points of lowest/highest electrostatic potential to neutralize the net charges. The command is as follows.

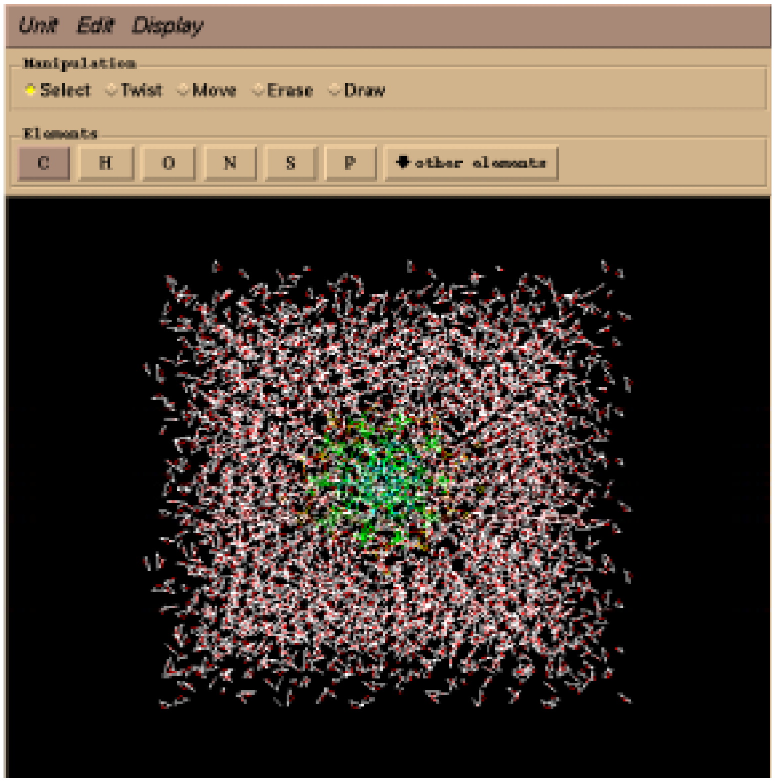
```
addions dna Na+ 0
```

This command places Na^+ ions (18 Na^+ ions) around the system ("dna") to make the net charge zero.

The system ("dna") with explicit counterions (Na^+) is solvated. This is done with the command "solvatebox". For this system, we will put 10 angstroms of water around DNA in each direction.

```
solvatebox dna WATBOX216 10.0
```

This is as shown.



To create prmtop and inpcrd files, we simply use the command:

```
saveamberparm dna polyAT.prmtop polyAT.inpcrd
```

prmtop: The parameter/topology file. This defines the connectivity and parameters for our current model. This information does not change during the simulation. The prmtop we created above is called polyAT.prmtop.

inpcrd: The coordinates (and box coordinates, if present and optionally the velocities). This changes during simulation. Above we created an initial set of coordinates called `polyAT.inpcrd`.

Now you can go on to the next section which introduces us to running molecular dynamics and minimization.

3. Equilibration.

After you have built up the structure, you are ready to begin minimization and molecular dynamics simulations to equilibrate the system.

The procedure of the minimization and molecular dynamics simulations are shown as follows.

A. Minimization holding the solute (DNA) fixed

A short minimization run to remove any potentially bad contacts between solute and solvent molecules.

B. Dynamics holding the solute (DNA) fixed

The bulk of the water relaxation takes place. Minimization does not really sample the accessible conformational space of the water.

C. Moving the DNA

We gradually reduce the restraints on the DNA in a series of minimizations.

D. Equilibration of the DNA

The DNA model is equilibrated by a 20 ps molecular dynamics simulation.

A. Minimization holding the solute (DNA) fixed

Given our `prmtop` (polyAT.prmtop) and `inpcrd` (polyAT.inpcrd) we created, we will now use sander in a short minimization run, with position restraints, to remove bad interactions between solute and solvent.

The basic usage for `sander` is as follows:

```
sander [-O] -i mdin -o mdout -p prmtop -c inpcrd -r restrt
           [-ref refc -x mdcrd -v mdvel -e mden -inf mdinfo]
```

Arguments in []'s are optional.

If an argument is not specified, the default name will be used.

-O overwrite all the output files

-i the name of the input file, `mdin` by default

-o the name of the output file, `mdout` by default

-p the parameter/topology file, `prmtop` by default

-c the set of initial coordinates for this run, `inpcrd` by default

-r the final set of coordinates from this MD or minimization run, `restrt` by default

-ref reference coordinates for positional restraints, if this option is specified in the input file, refc by default
-x the molecular dynamics trajectory (if running MD), mdcrd by default
-v the molecular dynamics velocities (if running MD), mdvel by default
-e a summary file of the energies (if running MD), mden by default
-inf a summary file written every time energy information is printed in the output file for the current step of the minimization or MD, mdinfo by default

Now that we have the prmtop and inpcrd from LEaP, all we need to run sander is the mdin file which specifies the myriad of possible options for this run.

File min_ntr.in

```
Initial minimization w/ position restraints on DNA, 9.0 cut
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 10,

  ntr    = 1,

  imin   = 1, maxcyc = 1000, ncyc   = 5000, ntmin  = 1,
  dx0    = 0.1, drms   = 0.0001,

  ntf    = 1,  ntb    = 1, cut    = 9.0, nsnb   = 10,

  ntc    = 1,
 &end
Hold the DNA fixed
500.0
RES  1  20
END
END
```

In the absence of a variable specification in the input file, default values are chosen; every specified variable needs to be followed by a comma. The sander manual describes all of these inputs, for each of the possible namelists. Which namelist is used depends on the specification above, such as `&cntrl`. At minimum, the `&cntrl` namelist must be specified. Also notice the space or empty first column before specification of the namelist control variable; this is necessary. It is also necessary to end each namelist with `&end`. After the namelist, some other information may be specified, such as "GROUP" input, which allows atom selections for restraints.

Now, let us build up a minimal input file for performing minimization with position restraints on the DNA atoms. Here are things to notice in the input file:

The first part shows the format of output.

> **ntx = 1:**
>
> The initial coordinates are read formatted with no initial velocity information (default)
>
> **irest = 0:**
>
> This is not a continuation of a previous dynamics run.
>
> **ntpr = 10:**
>
> Every 10 steps energy information will be printed in human-readable form to files "`mdout`". Default is 50.

The second part shows the restraint of this calculation.

> **ntr = 1**:
>
> This means that we have turned on position restraints and therefore have to specify via GROUP input the atoms which are restrained as well as the force constant. In this example, after specification of the namelists, a title is given, followed by the force constant for the restraint (in kcal mol^{-1} angstrom^{-2}) and then a specification of residues or atoms to restrain. Residues can be specified using the "RES" keyword. We have chosen a force constant of 500 kcal mol^{-1} angstrom^{-2} and restrained residues 1 through 20.

The third part shows the minimization regulation.

> **imin = 1:**
>
> Minimization is turned on.
>
> **maxcyc = 1000**, **ncyc > maxcyc**; for example **ncyc = 5000:**
>
> This means that we will be running 1000 steps of steepest descent minimization. Conjugate gradient minimization typically fails with rigid water (TIP3P). So by keeping `ncyc` > `maxcyc` we never changes the method of minimization from steepest descent to

conjugate gradient method.

dx0 = 0.1:

The initial step length. Default is 0.01.

drms = 0.0001:

Convergence criterion for the energy gradient. Default 0.0001 kcal mol^{-1} Å^{-1}.

The fourth part controls a molecular dynamics.

ntf =1:

Complete interaction is calculated. (default)

ntb = 1:

Periodic boundary is applied under constant temperature.

cut = 9.0:

We are running with a 9 angstrom cutoff

nsnb = 10:

Updating the pairlist every 10 steps.

The last part indicates SHAKE regulation.

ntc = 1,

SHAKE is not performed. (default)

SHAKE

When the simulation involves conformationally flexible molecules such as proteins then the high frequency motions (e.g. bond vibrations) are usually of less interest than the lower frequency modes, which often correspond to major conformational changes. Unfortunately, the time step of a molecular dynamics simulation is dictated by the highest frequency motion present in the system. It would therefore be of considerable benefit to be able to increase the time step without prejudicing the accuracy of the simulation. Constraint dynamics enables individual internal coordinates or combinations of specified coordinates to be constrained, or 'fixed' during the simulation without affecting the other internal degrees of freedom. The most commonly used method for applying constraints is the **SHAKE** procedure.

To run the minimization, we should make `cmd` file (`min_ntr.cmd`) and submit this file to the queue system using `qsub`. The `min_ntr.cmd` file is shown as follows:

file min_ntr.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N min_ntr
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i min_ntr.in -o min_ntr.out -c polyAT.inpcrd  ¥
-p polyAT.prmtop -r min_ntr.restrt -ref polyAT.inpcrd
wait
```

[Note: the "¥" in the lines above are just continuations for the shell to allow you to type over multiple lines. When you actually type this in, just type it in on one command line.]

The command to run the minimization is as follows:

```
qsub min_ntr.cmd
```

Input files: min_ntr.in, polyAT.inpcrd, polyAT.prmtop
Output files: min_ntr.out, min_ntr.restrt

B. Dynamics holding the solute (DNA) fixed

Using the restrt file generated from the previous step (min_ntr.restrt), we now run dynamics, holding the DNA fixed with positional restraints. This is where the bulk of the water relaxation takes place. Minimization does not really sample the accessible conformational space of the water; it simply serves to move the energy down hill. Large "moves" or changes in the coordinates are not possible. During the dynamics on the other hand, the water is free to move around (subject to the kinetic energy or temperature supplied to the system initially) and also the box size is changing in an attempt to maintain constant pressure.

file `md_ntr.in`

```
5DNB, initial dynamics w/ belly on DNA, model1, 9.0 cut
 &cntrl
  nmropt = 1,
  ntx    = 1,  irest  = 0,   ntpr   = 100,  ntwx   = 500,
  ntf    = 2,  ntb    = 2,  cut    = 9.0,  nsnb   = 10,
  ntr    = 1,
  imin   = 0,  nstlim = 25000,  nscm   = 0,  dt   = 0.001,
  temp0  = 300.0,  tempi  = 100.0,  ig     = 71277,
  ntt    = 1,  tautp  = 0.2,  vlimit = 15.0,
  ntp    = 1,  pres0  = 1.0, comp   = 44.6, taup   = 0.2,
  ntc    = 2,  tol    = 0.00001,
 &end
 &wt
   type='TEMP0',   istep1=0,      istep2=1000,
                   value1=100.0,  value2=300.0,
 &end
 &wt
   type='TEMP0',   istep1=1000,   istep2=25000,
                   value1=300.0,  value2=300.0,
 &end
 &wt
   type='END',
 &end
 &rst
   iat=0,
 &end
Hold the DNA fixed
500.0
RES  1  20
END
END
```

In this run, minimization is turned off (`imin = 0`) and dynamics can now proceed (for `nstlim` steps). In addition to this change in the input file, there are a few other major differences:

nmropt = 1:

We are going to use some of the "NMR" options. This is just to control temperature. We want to start the temperature off at 100 K and then ramp it up to 300 K. This is done with the `&wt` to `&end` namelists.

```
&wt
   type='TEMP0',   istep1=0,   istep2=1000,
   value1=100.0,   value2=300.0,
&end
```

Over 1000 steps raise the temperature from 100.0 to 300.0 K.

```
&wt
   type='TEMP0',   istep1=1000,   istep2=25000,
   value1=300.0,   value2=300.0,
&end
```

Over the next 24000 steps, keep the temperature at 300 K.

Do not forget that you need to terminate the `&rst` namelist even if you are not specifying NMR restraints. This is then followed by the same group input as before.

ntwx = 500:

Every 500 steps the coordinates will be written to file "`mdcrd`". Default is 0.

ntf = 2:

Bond interactions involving H-atoms omitted. (use with `ntc = 2`)

ntb = 2:

Periodic boundary is applied under constant pressure.

nstlim = 25000, dt = 0.001:

We are running 25000 steps at a time step of 1 femtosecond. This implies a total simulation of 25 picoseconds.

ntt = 1, tautp = 0.2:

We use standard temperature coupling (`ntt = 1`) to keep the temperature with 0.2ps of time constant for heat bath coupling for the system (`tautp = 0.2`). The default of `tautp` is 1.0.

temp0 = 300.0:

The simulation keeps the temperature at 300.0 K. Default is 300.0 K.

tempi = 100.0:

The initial temperature (`tempi = 100.0`) to make the initial distribution. Default is 300.0 K.

ig = 71277:

The random number generator seed. Note that the initial distribution of temperatures is dependent on `ig`; in other words, every time you run with `ig = 71277` on the same system, you will get the same distribution of initial velocities.

vlimit = 15.0:

Any component of the velocity that is greater than abs(`vlimit`) will be reduced to `vlimit` (preserving the sign).

ntp = 1:

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`. This dynamics is performed with isotropic position scaling.

pres0 = 1.0:

Reference pressure (in units of bars, where 1 bar ~ 1 atm) at which the system maintained. Default is 1.0.

comp = 44.6:

Compressibility of the system. The units are in 0.000001 bar; a value of 44.6 (default) is appropriate for water.

taup = 0.2:

Pressure relaxation time (in ps). Default value of 0.2 could be used for equilibration to more quickly get the desired density.

ntc = 2, tol = 0.00001:

SHAKE on hydrogens is now turned on. This is necessary when using rigid water models such as TIP3P. The SHAKE tolerance is 0.00001.

file `md_ntr.cmd`

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N md_ntr
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i md_ntr.in -o md_ntr.out -c min_ntr.restrt  ¥
-p polyAT.prmtop -r md_ntr.restrt -ref min_ntr.restrt -x md_ntr.mdcrd
wait
```

The command to run the dynamics is as follows:

```
qsub md_ntr.cmd
```

Input files: `md_ntr.in`, `min_ntr.restrt`, `polyAT.prmtop`
Output files: `md_ntr.out`, `md_ntr.restrt`, `md_ntr.mdcrd`

C. Moving the DNA

All throughout the previous steps, the DNA was held relatively rigid. Now we gradually reduce the restraints on the DNA in a series of minimizations.

The basic plan is

1) 1000 steps minimization with 25.0 kcal mol^{-1} angstrom^{-2} position restraints on the DNA; (equil_min1)

2) 600 steps minimization, reducing the positional restraints by 5.0 kcal mol^{-1} angstrom^{-2} each run; (equil_min2 (20.0 kcal mol^{-1} angstrom^{-2}) — equil_min6 (0.0 kcal mol^{-1} angstrom^{-2}))

Since you already have all the machinery necessary to follow through, details are not shown. Actual cmd and input files are shown as follows. (equil_min1 — equil_min6)

file equil_min1.in

```
5DNB, equil step 1
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 500,
  ntf    = 1,  ntb    = 1,  cut    = 9.0,  nsnb   = 10,
  ntr    = 1,
  imin   = 1,  maxcyc = 1000, ncyc   = 5000,
  ntmin  = 1,  dx0    = 0.1,  drms   = 0.0001,
  ntc    = 1,
 &end
Constraints
25.0
RES 1  20
END
END
```

file equil_min1.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_min1
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_min1.in -o equil_min1.out ¥
                                -c md_ntr.restrt -p polyAT.prmtop ¥
                                -r equil_min1.restrt -ref md_ntr.restrt ¥
wait
```

```
qsub equil_min1.cmd
```

file equil_min2.in

```
5DNB, equil step 2
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 500,
  ntf    = 1,  ntb    = 1,  cut    = 9.0,  nsnb   = 10,
  ntr    = 1,
  imin   = 1,  maxcyc = 1000, ncyc   = 5000,
  ntmin  = 1,  dx0    = 0.1,  drms   = 0.0001,
  ntc    = 1,
 &end
Constraints
20.0
RES 1  20
END
END
```

file equil_min2.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_min2
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_min2.in -o equil_min2.out ¥
                                -c equil_min1.restrt -p polyAT.prmtop ¥
                                -r equil_min2.restrt -ref equil_min1.restrt ¥
wait
```

```
qsub equil_min2.cmd
```

file equil_min3.in

```
5DNB, equil step 3
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 500,
  ntf    = 1,  ntb    = 1,  cut    = 9.0,  nsnb   = 10,
  ntr    = 1,
  imin   = 1,  maxcyc = 1000, ncyc   = 5000,
  ntmin  = 1,  dx0    = 0.1,  drms   = 0.0001,
  ntc    = 1,
 &end
Constraints
15.0
RES 1  20
END
END
```

file equil_min3.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_min3
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_min3.in -o equil_min3.out ¥
                                -c equil_min2.restrt -p polyAT.prmtop ¥
                                -r equil_min3.restrt -ref equil_min2.restrt ¥
wait
```

```
qsub equil_min3.cmd
```

file equil_min4.in

```
5DNB, equil step 4
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 500,
  ntf    = 1,  ntb    = 1,  cut    = 9.0,  nsnb   = 10,
  ntr    = 1,
  imin   = 1,  maxcyc = 1000, ncyc   = 5000,
  ntmin  = 1,  dx0    = 0.1,  drms   = 0.0001,
  ntc    = 1,
 &end
Constraints
10.0
RES 1  20
END
END
```

file equil_min4.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_min4
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_min4.in -o equil_min4.out ¥
                                -c equil_min3.restrt -p polyAT.prmtop ¥
                                -r equil_min4.restrt -ref equil_min3.restrt ¥
wait
```

```
qsub equil_min4.cmd
```

file equil_min5.in

```
5DNB, equil step 5
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 500,
  ntf    = 1,  ntb    = 1,  cut    = 9.0,  nsnb   = 10,
  ntr    = 1,
  imin   = 1,  maxcyc = 1000, ncyc   = 5000,
  ntmin  = 1,  dx0    = 0.1,  drms   = 0.0001,
  ntc    = 1,
 &end
Constraints
5.0
RES 1  20
END
END
```

file equil_min5.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_min5
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_min5.in -o equil_min5.out ¥
                                -c equil_min4.restrt -p polyAT.prmtop ¥
                                -r equil_min5.restrt -ref equil_min4.restrt ¥
wait
```

```
qsub equil_min5.cmd
```

file equil_min6.in

```
5DNB, equil step 6
 &cntrl
  ntx    = 1,  irest  = 0,  ntpr   = 500,
  ntf    = 1,  ntb    = 1,  cut    = 9.0,     nsnb   = 10,
  ntr    = 1,
  imin   = 1,  maxcyc = 1000,  ncyc   = 5000,
  ntmin  = 1,  dx0    = 0.1,   drms   = 0.0001,
  ntc    = 1,
 &end
```

file equil_min6.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_min6
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_min6.in -o equil_min6.out ¥
                                -c equil_min5.restrt -p polyAT.prmtop ¥
                                -r equil_min6.restrt ¥
wait
```

```
qsub equil_min6.cmd
```

D. Equilibration of the DNA

The DNA model is equilibrated by a 20 ps constant temperature and pressure molecular dynamics simulation. The DNA model is first heated from 100 K to 300 K in 2 picoseconds and then equilibrated at 300 K.

The cmd and input files are shown as follows. (equil_md)

file `equil_md.in`

```
5DNB, warm it up
 &cntrl
  nmropt = 1,
  ntx    = 1,      irest  = 0,  ntpr   = 100,    ntwx   = 500,
  ntf    = 2,      ntb    = 2,       cut    = 9.0,    nsnb   = 10,
  nstlim = 20000,  dt     = 0.001,
  temp0  = 300.0,  tempi  = 100.0,   ig     = 71277,
  ntt    = 1,  tautp  = 0.2,  vlimit = 20.0,
  ntp    = 1,  pres0  = 1.0, comp   = 44.6, taup   = 0.2,
  ntc    = 2,  tol    = 0.0005,
 &end
 &wt
   type='TEMP0', istep1=0,     istep2=2000,
                 value1=100.0, value2=300.0,
 &end
 &wt
   type='TEMP0', istep1=2000,   istep2=20000,
                 value1=300.0, value2=300.0,
 &end
 &wt
   type='END',
 &end
 &rst
   iat = 0,
 &end
```

file equil_md.cmd

```
#PBS -S/bin/bash
#PBS -l pmem=200mb
#PBS -l nodes=1:m200mb
#PBS -N equil_md
cd /users1/$USER/TUTORIAL
export AMBERHOME='/usr/local/amber7'
export PATH=$PATH:/usr/local/amber7/exe
wait
/usr/local/amber7/exe/sander -O -i equil_md.in -o equil_md.out ¥
                                -c equil_min6.restrt -p polyAT.prmtop ¥
                                -r equil_md.restrt  -x equil_md.mdcrd ¥
wait
```

```
qsub equil_md.cmd
```

Input files: equil_md.in, equil_min6.restrt, polyAT.prmtop

Output files: equil_md.out, equil_md.restrt, equil_md.mdcrd

4. Analyzing results.

After equilibrating the system by a molecular dynamics simulation, we should analyze the simulated results from a molecular dynamics run using some of the tools supplied within AMBER and some available through other sources.

Creating pdb files from the AMBER coordinates files

You may want to generate a new pdb file so you can look at the structure using the minimized coordinates from `restrt`. The pdb can be created from the parm topology and coordinates (`inpcrd` or `restrt`) using the program `ambpdb`.

```
ambpdb -p polyAT.prmtop < equil_md.restrt > equil_md.pdb
```

This will take the prmtop and inpcrd (`polyAT.prmtop` and `equil_md.restrt`) specified and create the file (`equil_md.pdb`).

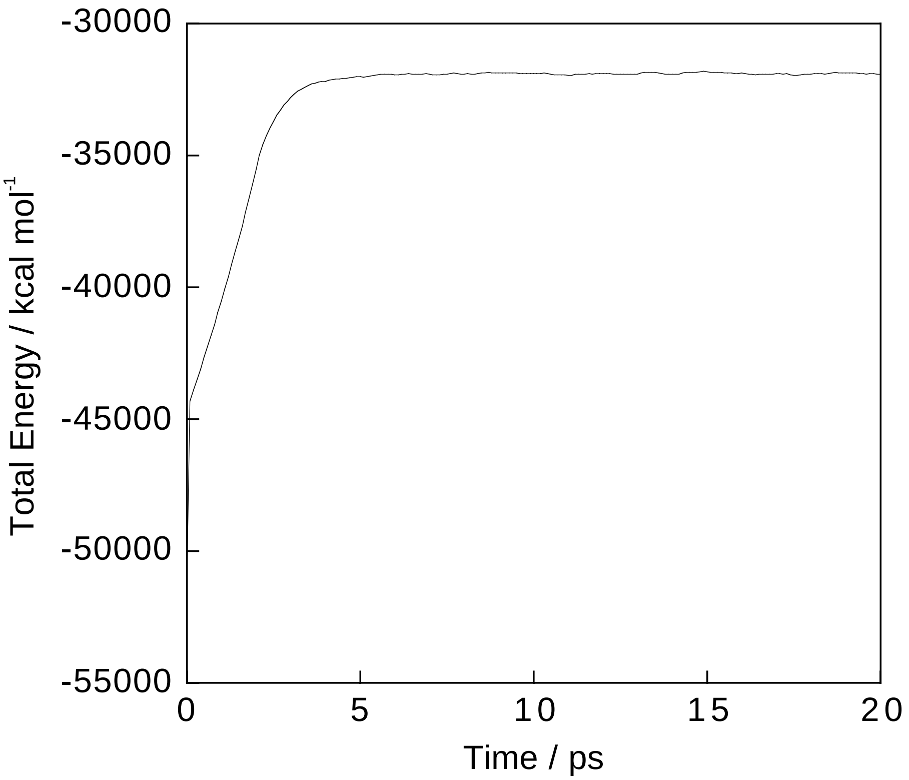
Extracting the energies, etc. from the mdout file

If you want to pull out the energies, etc. directly from `mdout` file, you can write an awk, sed, or perl script or use a perl script prepared in the AMBER web page.

```
process_mdout.perl equil_md.out
```

This script will take a whole series of mdout files and will create a whole series, leading off with the prefix "summary." such as "summary.ETOT". These files are just columns of times vs. the value for each of the energy components. You can plot this with a program.

Here is the total energy (ETOT) as a function of time (20 ps):



Calculating the RMS deviation vs. time

To calculate the RMS deviation as a function of time, we can use carnal or ptraj. In ptraj, this is done with the list of commands specified in the file calc_rms.1.

```
ptraj polyAT.prmtop < calc_rms.1
```

Input files: polyAT.prmtop, equil_md.mdcrd, calc_rms.1
Output file: rms.1

carnal is also useful for calculating the RMS deviation of each frame in our trajectory to the first frame in the trajectory. This is useful to see how far we have drifted during the dynamics. We create a carnal input carnal_rms_strat.in as follows:

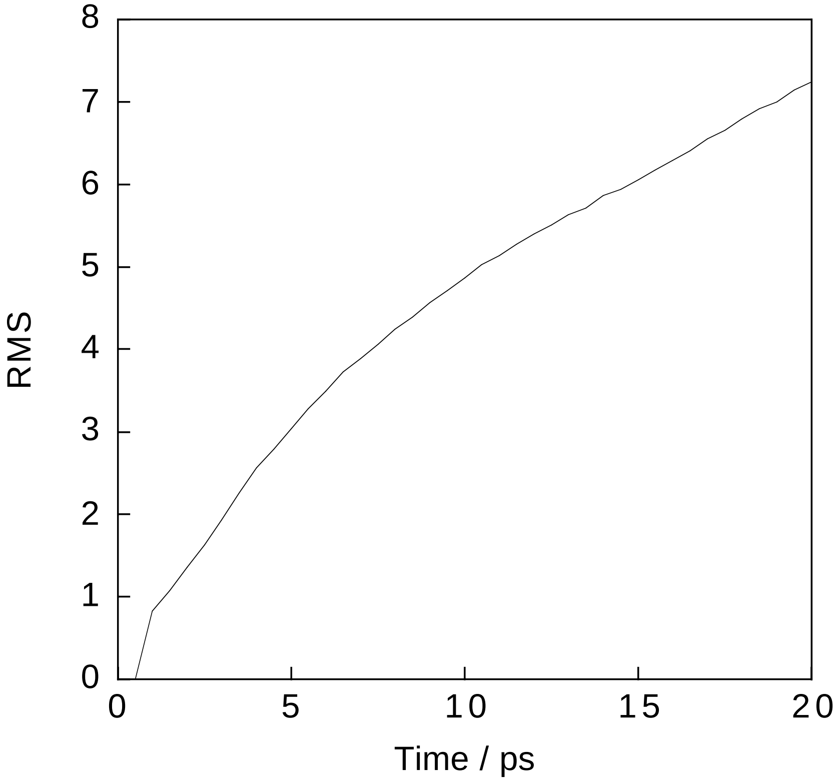
file carnal_rms_strat.in

```
FILES_IN
  PARM p1 polyAT.prmtop;
  STREAM s1 equil_md.mdcrd;
FILES_OUT
  TABLE tab1 rms_to_start;
DECLARE
  GROUP gALL (RES 1 - 3547);
  RMS r1 FIT gALL s1;
OUTPUT
  TABLE tab1 r1;
END
```

In the input file, we specified our parm file and trajectory as input and the file carnal_rms_start as output. After carnal is run, this file will contain the "TABLE" of "RMS" (r1) values. In the "DECLARE" section we specified a "group" representing the atoms we would like to RMS fit. We are going to use all the DNA atoms, or residues 1 through 20. The key to this run of carnal is the RMS command which says to perform the RMS fit.

```
carnal < carnal_rms_strat.in
```

The RMS deviation values are in angstroms.



Measuring atomic positional fluctuations with carnal

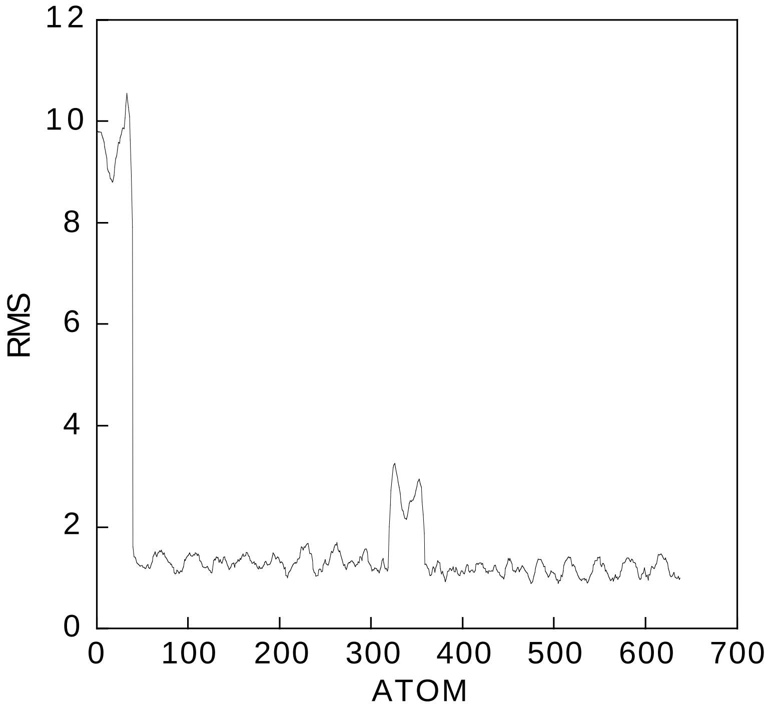
Note that carnal can also calculating atomic positional fluctuations which are directly related to the atomic B-factors.

The calculation is a two-step procedure, running carnal twice. In the first step you calculate the average structure of the trajectory. In the second step the RMS distances between the averaged structure and the trajectory is calculated.

```
carnal < carnal_fluctuation1
```

```
carnal < carnal_fluctuation2 > fluctuation
```

Here is the atomic positional fluctuations of the solute (DNA model).


RMS
ATOM