Introduction

Wednesday, February 14, 2018 1:03 PM

Case Study: All Atom Molecular Dynamics Simulation of LFA-I Complex with Lovastatin

This tutorial is designed as a case study that demonstrates a drug complexing with its protein target:

This tutorial consists of this introduction page and six subsections as follows:

- 1) Building the Structure.
- 2) Creating the topology and coordination files.
- 3) Minimizing the structure.
- 4) Heating the system up.
- 5) Production MD.
- 6) Analyzing the Results

The procedure will also follow those sections step-by-step.

WARNING 1: some of the calculations in this tutorial can take a very long time to run. While I urge you to run these simulations in order to familiarize yourself with SANDER I provide the relevant output files so that you can still follow the tutorial even if you don't have sufficient computing power at your disposal.

WARNING 2: There is no guarantee that if you run these simulations yourself that you will get the exact same answers as I. Difference in machine architecture leads to rounding errors in the calculation which result in different simulations on different machines exploring different regions of phase space. The average properties, however, should be comparable. As you will see though as you progress through this tutorial there is no guarantee that you can reproduce the results described here.

Section-1: Building the structure

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Prepare your working folder:

mkdir (make directory)

You'll need a new directory for the files and folders created in this tutorial. \$ mkdir LFA-1

cd (change directory)

At this point, you'll want to move into your Tutorial directory so that you can save all of your working files there.

\$ cd LFA-1

There is a special directory named "..". This means the parent of the current directory. So to return to the parent of the Tutorial directory use cd ..\$ cd ..

If you ever need to return to your home directory use just the **cd** command by itself. Tilde "~" is a shortcut to your home directory. The following commands both change directory to your home directory. \$ cd

\$ cd ~

ls (list)

Now when you do ${\rm ls}$ you should see your new directory has been created. \$ ls

pwd (print working directory)

Pathnames describe what directory you are in relative to the entire computer's filesystem. You home directory has location within the entire filesystem.

Print the working directory pathname of your home directory with pwd. \$ cd

\$ pwd

/home/username This is the current working directory that you're located in. In this case the directory **username** is in the directory **home** which is in the **/** (root) directory.

Prepare PDB

\$ cd LFA-1 \$ mkdir PDB The directory will be created as ~/LFA-1/PDB/

Download PDB

Download PDB file from protein data bank, PDB ID is 1CQP, and save the pdb file under ~/LFA-1/PDB/ directory.



1cqp

Check your PDB with Chimera:



There are two chains, chain-A and chain-B.

The ligand is name as "803" with residue number 311. In this system, there is a metal ion, MG. Today we will use chain-A for our simulation instead of taking both chains.

Small Malagulas				
Small Wolecules				
ID	Chains	Name / Formula / InChi Key	2D Diagram & Interaction	s 3D Interactions
MG Query on MG	А, В	MAGNESIUM ION Mg	M2+	CLigand Interaction
Download SDF File 🖲			MG	
Download CCD File 🕑				
803 Query on 803	А, В	LOVASTATIN MK-803; LOVALIP; MEVACOR	, Á 🔄	Cigand Interaction
Download SDF File 🛞		PCZOHLXUXFIOCF-BXMDZJJMSA-N	The second se	
Download CCD File 🖲			¥ • •	La
External Ligand Annotat	ions			
ID	Binding Affi	inity (Sequence Identity %)		
803	Kd: 12900 nf	M (99) BINDINGOB		
803	IC50: 2400 n	MPDEBIND		

\$cp 1cqp.pdb 1cqp.A.pdb

\$vi 1cqp.A.pdb

- Check if this molecule has disulfide bonds. If yes, write down the bond list. SSbond will need to specially bond in LEaP in the following step.
- Since we are going to simulate only one chain, delete lines which belong to chain-B. Keep the molecules in chain-A.

The file supposes to contains the protein, LFA-I, and the metal ion, MG, and the compound, 803.

Prepare a pdb file only contains the receptor.

\$ cp 1cqp.A.pdb 1cqp_A_receptor.pdb

\$ vi 1cqp_A_receptor.pdb

Delete ions and ligands. The receptor file is ready for PDB2PQR.

Prepare a file which only has ions and crystal water inside.

\$ cp 1cqp.A.pdb ion.pdb

\$ vi ion.pdb

Keep only Mg ion and water in the pdb. It will be used for building the complex system. The crystal water will be kept in the future calculation.

Prepare a file which contains only lovastatin from 1cqp

\$ cp 1cqp.A.pdb lova.pdb

\$ vi lova.pdb

Keep only the 803 in the file. And, add "TER" at the first line of the file and last line of the file.

```
_____
TER
                        52.497 19.025 23.052 1.00 43.13
HETATM 1472 C1 803 A 311
                                                        .129 C
HETATM 1473 C2 803 A 311 52.393 17.482 22.971 1.00 36.38
                                                        .035 C
HETATM 1474 C3 803 A 311
                         52.359 16.834 24.377 1.00 33.15
                                                        .024 C
                          53.777 16.458 24.857 1.00 27.12
HETATM 1475 C21 803 A 311
                                                        .005 C
HETATM 1476 C4 803 A 311
                         51.698 17.697 25.428 1.00 31.06
                                                        .031 C
                                                        .025 C
HETATM 1477 C24 803 A 311 51.206 18.918 25.189 1.00 35.91
```

:

:						
:						
HETATM 1494	O4 803 A 311	53.678	19.407	23.772	1.00 53.81	.304 O
HETATM 1495	C23 803 A 311	54.765	19.395	22.995	1.00 49.98	.271 C
HETATM 1496	O5 803 A 311	54.734	19.102	21.809	1.00 43.97	.264 O
HETATM 1497	C16 803 A 311	56.030	19.778	23.712	1.00 43.96	.086 C
HETATM 1498	C19 803 A 311	57.059	18.637	23.609	1.00 37.46	.010 C
HETATM 1499	C17 803 A 311	56.607	21.070	23.116	1.00 40.94	.010 C
HETATM 1500	C18 803 A 311	55.592	22.202	23.360	1.00 49.33	.001 C
TER						

Therefore, we will have pdb files of

- receptor
- ligand
- and its ions, crystal waters.

Section-2: Creating parameters files

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Prepare topology and coordinate files

For this tutorial, you will build the following molecule in the preparatory program called xLEaP for simulation in AMBER.

In order to build and solvate this molecule, you will need to start xLEaP. xLEaP has another command line interface and simple molecular graphics for building the system topology and define parameters for the molecules.

Add protons on the protein structure

The structure was solved by X-ray so the protons are missing in the PDB file. We need to add protons carefully by using the program "PDB2PQR".

\$ cd .. \$ mkdir 0_PDB2PQR \$ cd 0_PDB2PQR \$ /opt/PDB2PQR/pdb2pqr/pdb2pqr --with-ph=7.4 --ph-calc-method=propka --apbs-input -ff=amber --ffout=amber --verbose --summary ../PDB/1cqp_A_receptor.pdb 1cqp.pqr >pdb2pqr.log

The output file 1cqp.pqr is the pdb after adding protons.

Prepare topology and coordinate files for the drug-target complex

Go back to the working folder ~/LFA-1

\$ cd ..

Create a folder for drug-target complex input file

\$ mkdir 1_LEaP

\$ cd 1_LEaP/

Link the parameter files for the compound to the current directory

\$ In ../0_Gaussian/LVA.lib .

\$ In ../0_Gaussian/frcmod.LVA .

Prepare a full PDB which contains the protonated receptor with the compound pdb and ion pdb

Take those files prepare in the section-1.

\$ cp ../0_PDB2PQR/1cqp.pqr 1cqp_H.pdb

\$ cp ../PDB/1cqp_803.pdb .

\$ cat 1cqp_803.pdb | sed "s/ 803 / LVA /" > LVA.pdb

#change residue name from 803 to LVA

\$ cp ../PDB/1cqp.pdb MG.pdb \$ vi MG.pdb #1,1908d #2,\$d #:wq \$ vi 1cqp_all.pdb

#put LVA back. go to the last line of protein

#:r LVA.pdb

#put Mg back. go to the last line of LVA

#:r MG.pdb

#change WAT Atom HW to H1

#:%s/HW/H1/

#change WAT Atom OW to O

#:%s/OW/O /

#:wq

Start xLEaP now with the **xleap** command.

\$ xleap

-I: Adding /usr/local/amber_14/amber/dat/leap/prep to search path.

-I: Adding /usr/local/amber_14/amber/dat/leap/lib to search path.

-I: Adding /usr/local/amber_14/amber/dat/leap/parm to search path.

-I: Adding /usr/local/amber_14/amber/dat/leap/cmd to search path. You should see a window like this:



Warning:

Do NOT click the "X" on any LEaP window. It will quit LEaP entirely.

Note:

At this point it's a good idea to turn Num Lock off for the menus to work.

Load a protein and nucleic acid force field

A MD force field is the Hamiltonian (potential energy function) and the related parameters that describe the intra- and intermolecular interactions between the molecules in the system. In MD, the Hamiltonian is integrated to describe the forces and velocities of the molecules.

The basic form of the Amber Hamiltonian is:

$$V_{\text{AMBER}} = \sum_{i}^{n_{\text{bounds}}} b_i (r_i - r_{i,\text{eq}})^2 + \sum_{j}^{n_{\text{amples}}} a_i (\theta_i - \theta_{i,\text{eq}})^2 + \sum_{i}^{n_{\text{dimerals}}} \sum_{n}^{n_{\text{dimerals}}} (V_{i,n}/2) [1 + \cos(n\phi_i - \gamma_{i,n})] + \sum_{i < j}^{n_{\text{atoms}}} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^6} \right) + \sum_{i < j}^{n_{\text{atoms}}} \left(\frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \right)$$

In order to run a molecular dynamics simulation, we need to load a force field to describe the potential energy of alanine dipeptide. We will use the AMBER force field FF14SB for proteins and nucleic acids. FF14SB is based off FF12SB, an updated version of FF99SB, which in turn was based on the original Amber Cornell *et al* (1995) [ff94] force field. The most significant changes to the FF14SB force field included updated torsion terms for the protein Phi-Psi angles and refits of the torsion terms for side chains. Together these improved the estimation of alpha helices in these molecules.

source (load) the FF14SB force field now.

> source leaprc.protein.ff14SB

	XLEaP: Universe Editor	- • ×
File Edit	Verbosity	
Please to Welcome (no leap > source So Log file Loading J Reading Reading Reading ff14SB p Loading Loading Loading	<pre>arn Num Lock off for the menus to function! co LEaP! cc in search path) leaprc.protein.ff14SB arce: /opt/amber16/dat/leap/cmd/leaprc.protein.ff14SB done correction of /opt/amber16/dat/leap/cmd/leaprc.protein.ff14SB done c./leap.log parameters: /opt/amber16/dat/leap/parm/parm10.dat title: fromod.ff99SB + fromod.parmbsc0 + OL3 for RNA parameters: /opt/amber16/dat/leap/parm/fromod.ff14SB force field modification type file (fromod) title: rotein backbone and sidechain parameters library: /opt/amber16/dat/leap/lib/amino12.1ib library: /opt/amber16/dat/leap/lib/aminot12.1ib library: /opt/amber16/dat/leap/lib/aminont12.1ib</pre>	

Source (load) force field for water model, TIP3P. And, load the force field parameters for metal ions.

>source leaprc.water.tip3p

>source leaprc.gaff2

<pre>File Edit Verbosity Loading parameters: /opt/amber16/dat/leap/parm/parm10.dat Reading title: PARM99 + fromod.ff99SB + fromod.parmbsc0 + OL3 for RNA Loading parameters: /opt/amber16/dat/leap/parm/fromod.ff14SB Reading force field modification type file (fromod) Reading title: ff14SB protein backbone and sidechain parameters Loading library: /opt/amber16/dat/leap/lib/aminot12.lib Loading library: /opt/amber16/dat/leap/lib/aminot12.lib Loading library: /opt/amber16/dat/leap/lib/aminot12.lib > source leaprc.water.tip3p Source: /opt/amber16/dat/leap/cmd/leaprc.water.tip3p done Loading library: /opt/amber16/dat/leap/lib/solvents.lib Loading library: /opt/amber16/dat/leap/lib/solvents.lib Loading library: /opt/amber16/dat/leap/lib/solvents.lib Loading force field modification type file (fromod) Reading fiele: Li/Merz ion parameters of divalent to tetravalent ions for TIP3P water model</pre>			XLEaP: Universe Editor _ D X
Loading parameters: /opt/amber16/dat/leap/parm/parm10.dat Reading title: PARM99 + fromod.ff99SB + fromod.parmbsc0 + OL3 for RNA Loading parameters: /opt/amber16/dat/leap/parm/fromod.ff14SB Reading force field modification type file (fromod) Reading title: ff14SB protein backbone and sidechain parameters Loading library: /opt/amber16/dat/leap/lib/amino12.lib Loading library: /opt/amber16/dat/leap/lib/aminot12.lib Loading library: /opt/amber16/dat/leap/lib/aminot12.lib Loading library: /opt/amber16/dat/leap/cmd/leaprc.water.tip3p Source: /opt/amber16/dat/leap/cmd/leaprc.water.tip3p done Loading library: /opt/amber16/dat/leap/lib/solvents.lib Loading library: /opt/amber16/dat/leap/lib/solvents.lib Loading parameters: /opt/amber16/dat/leap/lib/solvents.lib Loading parameters: /opt/amber16/dat/leap/parm/fromod.ionsjc_tip3p Reading force field modification type file (fromod) Reading title: Monovalent ion parameters for Ewald and TIP3P water from Joung & Cheatham JPCB (2008) Loading parameters: /opt/amber16/dat/leap/parm/fromod.ions2341m_126_tip3p Reading force field modification type file (fromod) Reading title: Li/Merz ion parameters of divalent to tetravalent ions for TIP3P water model (12-6 nor	File	Edit	Verbosity
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Load the parameters for the compound

> frcmods = loadAmberParams frcmod.MOL

>loadOff MOL.lib

		XLEaP: Universe Editor – \Box ×
File	Edit	Verbosity
Los Res Res Res Los Res Los Los Res Res Res Res Res Res Res Res Res Re	ading ading ading ading ading ading ading ading source source source ading ading ading ading ading ading ading ading ading	<pre>library: /opt/amber16/dat/leap/lib/solvents.lib parameters: /opt/amber16/dat/leap/parm/frcmod.ionsjc_tip3p force field modification type file (frcmod) title: nt ion parameters for Ewald and TIP3P water from Joung & Cheatham JPCB (2008) parameters: /opt/amber16/dat/leap/parm/frcmod.ions2341m_126_tip3p force field modification type file (frcmod) title: ion parameters of divalent to tetravalent ions for TIP3P water model (12-6 nor leaprc.gaff2 urce /opt/amber16/dat/leap/cmd/leaprc.gaff2 urce of /opt/amber16/dat/leap/cmd/leaprc.gaff2 done : ./leap.log parameters: /opt/amber16/dat/leap/parm/gaff2.dat title: neral Force Field for organic molecules (Version 2.1, April 2016) s = loadAmberParams frcmod.803 parameters: ./frcmod.803 force field modification type file (frcmod) title: oes here f 803.lib library: ./803.lib</pre>
11-		

Load amber parameters for metal ions which are charge +2,+3, +4 in the force field parameter of Lenard-Jons 12-6 in the tip3p water model.

> loadamberparams frcmod.ions234lm_126_tip3p

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```
File Edit Verbosity
Loading parameters: /opt/amber16/dat/leap/parm/frcmod.ions2341m_126_tip3p
 Reading force field modification type file (frcmod)
 Reading title:
 Li/Merz ion parameters of divalent to tetravalent ions for TIP3P water model (12-6 nor
 > source leaprc.gaff2
 ----- Source: /opt/amber16/dat/leap/cmd/leaprc.gaff2
----- Source of /opt/amber16/dat/leap/cmd/leaprc.gaff2 done
 Log file: ./leap.log
 Loading parameters: /opt/amber16/dat/leap/parm/gaff2.dat
Reading title:
 AMBER General Force Field for organic molecules (Version 2.1, April 2016)
 > frcmods = loadAmberParams frcmod.803
 Loading parameters: ./frcmod.803
Reading force field modification type file (frcmod)
 Reading title:
 remark goes here
> loadOff 803.1ib
 Loading library: ./803.lib
> loadamberparams frcmod.ions2341m_126_tip3p
 Loading parameters: /opt/amber16/dat/leap/parm/frcmod.ions2341m_126_tip3p
Reading force field modification type file (frcmod)
 Reading title:
Li/Merz ion parameters of divalent to tetravalent ions for TIP3P water model (12-6 nor
```

Load PDB

> _ M_

> 1cqp = loadpdb all.pdb

> 1cqptip3pbox = copy 1cqp

Solvate the system with the **solvatebox** command.

> solvatebox 1cqptip3pbox TIP3PBOX 10.0 1 #defualt radii is 1.5

Add ions to neutralize the system

Assuming the targeting salt concentration is 150mM. The water concentration is 55M in the solution. Therefore,

Number of water/Number of salt = 55 000/150

In this system, the total amount of water is about 8518. Thus, the amount of salt ion is about 20. The drug-target system is charged –2. In order to neutralize the system, two sodium ions are addionally added.

> addions 1cqptip3pbox Na+ 22 Cl- 20

×

I	XLEaP: Universe Editor _ O X
	File Edit Verbosity
/	A residue 362: duplicate [H1] atoms (total 2) residue 363: duplicate [H1] atoms (total 2) residue 364: duplicate [H1] atoms (total 2)
1	Warning: Atom names in each residue should be unique. (Same-name atoms are handled by using the first occurrence and by ignoring the rest. Frequently duplicate atom names stem from alternate conformations in the PDB file.)
	total atoms in file: 3142 Leap added 91 missing atoms according to residue templates: 91 H / lone pairs
,	Solute vdw bounding box: 45.023 50.825 46.725 Total bounding box for atom centers: 65.023 70.825 66.725 Solvent unit box: 18.774 18.774 18.774
1	Total vdw box size: 68.524 73.396 69.110 angstroms. Volume: 347578.515 A^3 Total mass 168319.397 amu, Density 0.804 g/cc Added 8110 residues.
11.1.1	Adding 42 counter ions to "1cqptip3pbox" using 1A grid Grid extends from solute vdw + 8.80 to 14.80 Resolution: 1.00 Angstrom. grid build: 0 sec
1	Solvent present: replacing closest with ion

To save the prmtop and inpcrd file use the **saveamberparm** command.

> savepdb 1cqptip3pbox 1cqptip3pbox.pdb

> saveamberparm 1cqptip3pbox 1cqptip3pbox.top 1cqptip3pbox.crd

		XLEaP: Universe Editor	- • ×
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B B B B B B B B B B B B B B B B B B B	igno uilding uilding uilding uilding total 5 uilding ncorpor ot Mark arking (Resid these	ring the warning. topology. atom parameters. bond parameters. angle parameters. proper torsion parameters. improper torsions applied H-Bond parameters. ating Non-Bonded adjustments. ing per-residue atom chain types. per-residue atom chain types. ues lacking connect0/connect1 - don't have chain types marked:	
]>) (no res ^	res total affected CILE 1 NGLY 1 WAT 8158 traints)	

Two input files are ready:

1cqptip3pbox.top

1cqptip3pbox.crd

And, a pdb of the whole system, 1cqptip3pbox.pdb

Quit XLEaP > quit Run tleap in batch

To execute in command mode of LEaP alternatively, you can also prepare a in-file then execute the

source leaprc.protein.ff14SB source leaprc.water.tip3p source leaprc.gaff2 frcmods = loadAmberParams frcmod.LVA loadOff LVA.lib loadamberparams frcmod.ions1lm_126_tip3p loadamberparams frcmod.ions234lm_126_tip3p loadoff atomic_ions.lib 1cqp = loadpdb 1cqp_H.pdb 1cqptip3pbox = copy 1cqp solvatebox 1cqptip3pbox TIP3PBOX 10.0 1 addions 1cqptip3pbox Na+ 24 Cl- 22 savepdb 1cqptip3pbox 1cqptip3pbox.pdb saveamberparm 1cqptip3pbox 1cqptip3pbox.top 1cqptip3pbox.crd quit _____

tleap

Prepare restraint atom lis

\$./SelectRestrainedAtoms

Back to the ~/LFA-1 > cd ..

Section-3: Minimizing the system

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The last components needed are the input files that define the program settings for each MD run. For this system, we will perform an energy minimization on the system, then slowly heat the system, and then do production MD at the desired temperature and pressure.

- 1. Minimization
- 2. Heating with constant volume and temperature (NVT) for 20ps from 0K to 300K

3. Production MD with constant pressure and temperature (NPT) at 300K and 1atm for 1ns We will save the trajectory and write to the output file every 1ps. The Langevin thermostat will be used to control the temperature. The random number generator will be initialized with a random seed.

Prepare workspace \$ mkdir 2_MIN \$ cd 2_MIN

Minimization input

Create the file min.in that includes the following settings for minimization: File: min.in

```
&cntrl

imin=1, irest=0,

ntc=2, ntf=2,

ntb=1, cut=10.0, iwrap=1,

ntmin=2, maxcyc=500, ntpr=50, ntr=1,

&end

restraining 1CQP

5.0

[ATOM list]

:
```

A list of heavy atoms are restraining to avoid the whole system crash during the minimization step. A perl script is ready for extracting the heavy atom automatically.

\$ cp 1_LEaP/1captip3pbox.pdb .

\$./SelectRestrainedAtoms

\$ extractHeavyAtom.pl 1cqptip3pbox_restrained_atoms.pdb > atomlist

Merge the input file and atom list: \$cat min.in atomlist > min_r.in

/ The settings can be summarized as follows:

imin=1	Choose a minimization run
irest=0	Do not restart simulation. (not applicable to minimization)
maxcyc=500	Maximum minimization cycles
ncyc=1000	The steepest descent algorithm for the first 0-ncyc cycles, then switches the conjugate

	gradient algorithm for ncyc-maxcyc cycles
ntpr=50	Print to the Amber mdout output file every ntpr cycles

Link top and crd files in this working folder

\$ In 1_LEaP/1cqptip3pbox.top 2_MIN/.

\$ In 1_LEaP/1cqptip3pbox.crd 2_MIN/.

Execute minimization

Source Amber setup

\$ source /opt/setup_amber.sh

Execute sander

\$ mpirun -np 4 \$AMBERHOME/bin/sander.MPI -O -i min_r.in -c 1cqptip3pbox.crd -p 1cqptip3pbox.top - ref 1cqptip3pbox.crd -o min.out -r min.rst

The files coming from this step of calculation are:

min.out min.rst <== this one will be used for the subsequent step.

Run in Batch Mode

\$./RUN

#!/bin/sh

source /opt/setup_amber16gnu.sh

In ../1_LEaP/1cqptip3pbox.crd .

In ../1_LEaP/1cqptip3pbox.top .

mpirun -np 16 \$AMBERHOME/bin/sander.MPI -O \

-i min_r.in \

-c 1cqptip3pbox.crd \

- -p 1cqptip3pbox.top \
- -ref 1cqptip3pbox.crd \

-o min.out \

-r min.rst

RUN

Back to ~/LFA-1 \$ cd ..

Section-4: Heating-up the system

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Prepare working space

\$ mkdir 3_MD0 \$ cd 3_MD0

Heating input

Create the file md0.in that includes the following settings for heating:

md, npt, v	warm to 300K quickly, constant pressure
&cntrl	
imin = 0,	irest = 0, ntx = 1,
nstlim =	100000, dt=0.001, nsnb = 20,
tempi=0	.0, temp0 = 300.0, iwrap=1,
tautp=0.	1, taup=1,
ntt = 1, r	ntb = 2, ntp = 1,
ntc = 2, r	ntf = 2, cut = 10.0,
ntwe = 5	00, ntwx=500, ntr=1,
&end	
restrainin	g all
5.0	
[ATOM LI	ST]
:	
ntx=5	Read coordinates and velocities from unformatted inpcrd coordinate file
irest=1	Restart previous MD run [This means velocities are expected in the inpcrd file and will used to provide initial atom velocities]
temp0= 300.0	Thermostat temperature. Run at 300K
ntb=2	Use periodic boundary conditions with constant pressure
ntp=1	Use the Berendsen barostat for constant pressure simulation
Convithe	atom list generated from last step in 2 MIN
> cn /2	MIN/atomlist
· · · · · /	

be

Merge input file together with atom list

> cat md0.in atomlist > md0_r.in

Link top and crd files in this working folder > ln 1_LEaP/1cqptip3pbox.top 3_MD0/. > ln 1_LEaP/1cqptip3pbox.crd 3_MD0/.

Execute heating-up

Source Amber setup \$ source /opt/setup_amber.sh Execute pmemd \$ mpirun -np 4 \$AMBERHOME/bin/pmemd.MPI -O \ -i md0_r.in \ -c ../2_MIN/min.rst \ -p 1cqptip3pbox.top \ -o md0.out \ -e md0.en \ -ref 1cqptip3pbox.crd \ -x md0.trj \ -r md0.rst Back to ~/LFA-1

\$ cd ..

Section-5: Production MD

2018年2月14日 下午 01:11

Prepare working space

\$ mkdir 4_MDN \$ cd 4_MDN

Production MD input

Create the file mdN.in that includes the following settings for heating:

```
md, npt, warm to 300K quickly, constant volume
&cntrl
imin = 0, irest = 1, ntx = 5,
nstlim = 100000, dt=0.001,
tempi=300.0, temp0 = 300.0, nscm=500,
ntt = 1, npscal = 1, ntb = 2, ntp = 1, tautp=0.01, taup = 0.01,
ntc = 2, ntf = 2, cut = 9.0,
ntwx= 100, ntwe = 100, ntwv=100,
iwrap=0,
/
```

Create the file RUN_MD that includes the following commands:

#!/bin/sh

```
source /opt/setup_amber.sh
```

```
In ../2_MIN/1cqptip3pbox.top .
In ../2_MIN/1cuqtip3pbox.crd .
In ../3_MD0/md0.rst .
```

```
mkdir RST OUT EN FRC VEL TRJ
cp md0.rst RST/
```

pseq=0

```
seq=1
```

```
while [ $seq -le 10 ]
do
mpirun -np 4 $AMBERHOME/bin/pmemd.MPI -O \
-i mdN.in \
```

```
-c RST/md$pseq.rst \
    -p 5cuqtip3pbox_1264.top \
    -o OUT/md$seq.out \
    -e EN/md$seq.en \
    -v VEL/md$seq.vel \
    -ref 5cuqtip3pbox_1264.crd \
    -frc FRX/md$seq.frc \
    -x TRJ/md$seq.trj \
    -r RST/md$seq.rst
pseq=`expr $pseq + 1`
seq=`expr $seq + 1`
```

done

Launch production MD

Change command mode by adding executable option \$ chmod +x RUN_MD Execute the command \$./RUN_MD &

*Add "&" at the end of command is to execute the command in the background

The total duration of production MD here is 10ns. Every loop is 1ns. The RUN_MD contains 10loops.

Section-6: Analyzing the results

2018年2月14日 下午 01:11

Visualize the result

You've now run an MD simulation. In order to visualize the results, we will now use <u>Chimera</u> to check the outcoming structures and trajectory.

Open the software chimera \$ chimera

							UCSF Chimera	- 0
0	Select	Actions	Presets	Tools	Fayorites	Help		
								MOL.pdb
								803_model.pdb
								all.pdb
								5cuq.pdb
								5dto.pdb
								Cmp8_MTase.pdbqt
								Cmp10_MTase.pdbqt
								Cmp11_MTase.pdbqt
								receptor.pdbqt
								Cmp10_MTase_HB.pdb
							ocenes	alitip3pbox.pdb
								3acl.pdb
	ol Ico	ns						receptor.pqr
								sqm.pdb
								3acl.pdb
								LBD.pdb
								p2.pdb
								p1.pdb
								LBD_A5.pdb
								ali.pdb
							Scenes are under development and may not	Cmp10_RdRp.pdbqt
							be fully functional	receptor.pdbqt
							Save scene	all.pdb
								receptor.pdbqt
								ligand.pdbqt
		_					Named Selections	all.pdb
	d Tool Ico	on						alitip3pbox.pdb
	Dialoga None							5hmx.pdb
	Sheers I Led					17		Province Extern
Î	snow Hel	P					Name current selection	Browse Fetch

Load the crystal structure and make the superimpose of these two structures. "File -> open " open the crystal structure which has chain A only.



Go to "Tools -> MD/Ensemble Analysis -> MD Movie" A small window will pop out for selection of Prmtop and trajectory. In our case, we need to choose "1cqptip3pbox.top" as our Prmtop. And, the trajectory can be selected according to the run sequence.

1		Get En	semble Ir	nfo –	• ×				
	Trajectory format: Amber								
	Prmtop: hingyu/LFA-1/4_MDN/1cqptip3pbox.top Browse								
	• md1.trj - /backup/chingyu/LFA-1/4_MDN/TRJ • md2.trj - /backup/chingyu/LFA-1/4_MDN/TRJ • md3.trj - /backup/chingyu/LFA-1/4_MDN/TRJ • md3.trj - /backup/chingyu/LFA-1/4_MDN/TRJ • Md3.trj - /backup/chingyu/LFA-1/4_MDN/TRJ								
		Use frames fi	rst throug	ghlast					
			ок	Cancel	Help				

Go to the small movie panel and select "Per-Frame -> Define script"

				1000			
	Per-Fr	ame Co	mmands		-		×
Interpret script as	Chimera commands 🦳						
	Substitute text <	FRAME:	with frame	number			
	✓ Use leading zeroes so	all fram	e numbers	are equal le	ngth		
	Commands prefixed wit	th #N: wi	I be execut	ted at frame	N		
Script							_
							1
Insert text file	Save to file						i
					-		1
		OK	Apply	Clear	Close	Help	1

Input "mm #1 #0" and click "Apply"



And centering the molecule in the display. Find a proper view to see the compound.

After selecting the proper top file and trajectory, simply click "OK" The molecule will show together with salt and some contacting waters. To have a clear view of the compound and the receptor, we can unselect the water and salt.

Select salt and hide bonds and atoms: "Select -> Residue -> Cl-" "Actions -> Atoms/Bonds/ -> hide" "Select -> Residue -> Na+" "Actions -> Atoms/Bonds/ -> hide"

"Select -> chain -> water" [Ctrl + Shift] mouse select the target water which is closed to MG. "Actions -> Atoms/Bonds -> hide"

```
Color the compound:
Open command mode
> select #1:184
"Select -> Residue -> LVA"
"Actions -> Color -> forest green"
"Actions -> Color -> by heteroatoms"
"Clear selection"
```

Go to the small movie window and play. If you would like to record a movie, you can go to "File -> Record movie.. "

	Record Animati	ion of Trajectory	- 0	×
Folder: /work/chingyu/LFA-	1/Analysis			•
Chingyu/	Dengue/ DSSP/ EphA2/ FKBP/ LFA-1/ Lovastatin/ MDjeep/ PIRIN/ RadiiOfGyration/	0_LOV_LEaP/ 0_PDB2PQR/ 1_LEaP/ 2_MIN/ 3_HEAT/ 4_MDN/ 5_CheckEq/ 6_UmbrellaSampling/ Analysis/ PDB/		-12
rile name: j	☑ Add .mp4 su	affix if none given		-
File type: MPEG-4 [.mp4	1 [New folder		
	Starting frame: 1			
	Step size: 334			
	Ending frame: 100000	- (
Encode forward then backy	vard ("roundtrip"): false	<u> </u>		
	Rendering: Chimer	a		
	Supersample: 3x3 -	4		
Advanced Options				
Triangle buttons reveal recently-used folders/files	3	Image Tips Record	Close Hel	р

Name the file and click "Record".

Analyze the MD results

Amber includes a suite of tools to examine and analyze MD trajectories. In this tutorial, we will do a simple analysis with several Amber programs and plot the results. The analysis will primarily done from the command line in the terminal.

Open a terminal and change directory to your tutorial files. $\$ cd $\/LFA-1$

Make an **Analysis** directory and change to that directory. \$ mkdir Analysis

\$ cd Analysis

Now we will use an analysis script **process_mdout.perl** to analyze the MD output files. This script will extract the energies, temperature, pressure, density, and volume from the MD output files and save them to individual data files.

Process the MD output files with process_mdout.perl

\$ source /opt/setup_amber.sh \$ process_mdout.perl ../3_HEAT/md0.out It is now quite simple to plot the data saved in the output files.

summary_avg.DENSITY summary_avg.TSOLVENT summary_rms.DENSITY summary_rms.TSOLVENT summary_avg.EKCMT summary_avg.VOLUME summary_rms.EKCMT summary_rms.VOLUME summary_avg.EKTOT summary.DENSITY summary_rms.EKTOT summary.TEMP summary_avg.EPTOT summary.EKCMT summary_rms.EPTOT summary.TSOLUTE summary_avg.ESCF summary.EKTOT summary_rms.ESCF summary.TSOLVENT summary_avg.ETOT summary.EPTOT summary_rms.ETOT summary.VOLUME summary_avg.PRES summary.ESCF summary_rms.PRES summary_avg.TEMP summary.ETOT summary_rms.TEMP summary_avg.TSOLUTE summary.PRES summary_rms.TSOLUTE ____

We will use **gnuplot** to automatically generate plots for the following MD simulation properties throughout the simulation. We use this for our convenience, but you can use any plotting program of your choice.

- 1. MD simulation temperature
- 2. MD simulation density
- 3. MD simulation total, potential, and kinetic energies.

However, for the MD simulation density, the heating portion of the simulation does not include a density output. You will need to edit the **summary.DENSITY** file to remove the empty data points for **gnuplot** to work.

Go into gnuplot: \$ gnuplot G N U P L O T Version 4.6 patchlevel 2 last modified 2013-03-14 Build System: Linux x86_64

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Thomas Williams, Colin Kelley and many others

gnuplot home: <u>http://www.gnuplot.info</u> faq, bugs, etc: type "help FAQ" immediate help: type "help" (plot window: hit 'h')

Terminal type set to 'x11'

gnuplot> set term png gnuplot> set output "DENSITY_time.png" gnuplot> set xlabel "time(ps)" gnuplot> set ylabel "Density" gnuplot> plot "summary.DENSITY" w l title "Density vs. Time" gnuplot> q

```
Output file will be "DENSITY_time.png".
```



We can use the similar way to produce temperature profile or total energy profiles.





CPPTRAJ

This tutorial will give a brief overview of analyzing simulation data with CPPTRAJ. CPPTRAJ is the successor to PTRAJ, with many additional features. Some basic and common types of analysis will be covered, as well as the basics of data set handling in CPPTRAJ. In addition, <u>gnuplot</u> will be required to view some of the output data.

Running in Batch Mode

Instead of running interactively, CPPTRAJ can also be run using one or more input files. Since the log file 'cpptraj.log' has recorded every command used, you can use the log file as the basis for a cpptraj "script". For example to generate everything we have done so far the following input could be used:

Input file: trj_dist_temp.in

```
parm 1cqptip3pbox.top
trajin TRJ/mdtemp.trj
distance D1 :183@O1 :130@OH out dist_LVA_O1-TYR130_OH_temp.dat
distance D2 :183@C3 :108@CD1 out dist_LVA_C3-ILE108_CD1_temp.dat
distance D3 :183@C20 :106@CG1 out dist_LVA_C20-VAL106_CG1_temp.dat
distance D4 :183@C21 :171@CD1 out dist_LVA_C21-LEU171_CD1_temp.dat
distance D5 :183@C18 :175@CD1 out dist_LVA_C18-LEU175_CD1_temp.dat
distance D6 :184@MG :12@OG out dist_MG-SER12_OG_temp.dat
distance D7 :184@MG :14@OG out dist_MG-SER14_OG_temp.dat
distance D9 :184@MG :233@O out dist_MG-ASP112_OD1_temp.dat
distance D1 :184@MG :238@O out dist_MG-WAT233_O_temp.dat
distance D1 :184@MG :246@O out dist_MG-WAT246_O_temp.dat
distance D1 :184@MG :246@O out dist_MG-WAT246_O_temp.dat
```

We use "distance" command to extract the distance between lovastatin and its binding pocket as

well as the Mg ion to its nearby molecules.

Run script "run_cpp_distance.sh"

\$./run_cpp_distance.sh

Output files will be:

dist_LVA_O1-TYR130_OH.dat dist_LVA_C3-ILE108_CD1.dat dist_LVA_C20-VAL106_CG1.dat dist_LVA_C21-LEU171_CD1.dat dist_LVA_C18-LEU175_CD1.dat dist_MG-SER12_OG.dat dist_MG-ASP112_OD1.dat dist_MG-WAT233_O.dat dist_MG-WAT238_O.dat dist_MG-WAT246_O.dat dist_MG-THR79_OG1.dat

We can use "gnuplot" as well to plot those files. \$ gnuplot gnuplot> set term png gnuplot> set output "[the output filename]" gnuplot> plot "[the file-1 you wish to plot]" w l, "[the file-2 you wish to plot]" w l The plot will present inside the output file.

gnuplot> q

