MD simulation of a protein-ligand complex

1. Introduction

In this tutorial, you'll launch a molecular dynamics (MD) simulation of 52.5 ns for a protein-ligand complex downloaded from the Protein Data Bank. Then you'll analyze the results of the MD simulation. The MD simulations will be carried out by using AMBER. "AMBER" refers to two things: a set of molecular mechanical force fields for the simulation of biomolecules (which are in the public domain, and are used in a variety of simulation programs); and a package of molecular simulation programs which includes source code and demos (that will be used in this tutorial).
Amber is distributed in two parts: AmberTools (an ensemble of software for preparing and analyze MD simulations) and Amber (the actual MD engine). You can use AmberTools without Amber, but not vice versa.

2. Input files

Like any other software AMBER needs several data:

1. Starting geometry and connectivity
2. Force field parameters
3. Procedures to be executed

Although all MD programs use the same kinds of data, the format of the files in which all data are reported is (very) different, and the programs that generate these files are different too.

In the case of AMBER, the main program for the generation of the MD input files is **tleap,** also available with a graphic interface (**xleap)** that however does not simplify the work. Therefore, also for a teaching purpose, we will not use it.

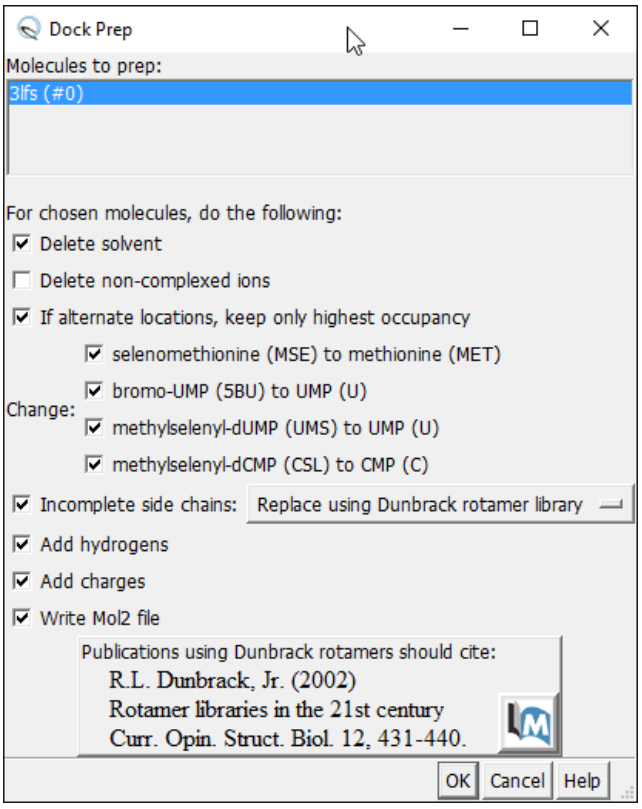
We will use the coordinates of CDK2 with SAR37, an aminoindazole type inhibitor (PDB code 3LFS). We need to generate a pdb file of the protein without hydrogen atoms and a mol2 file of the ligand with hydrogen atoms.

3. Input files generation

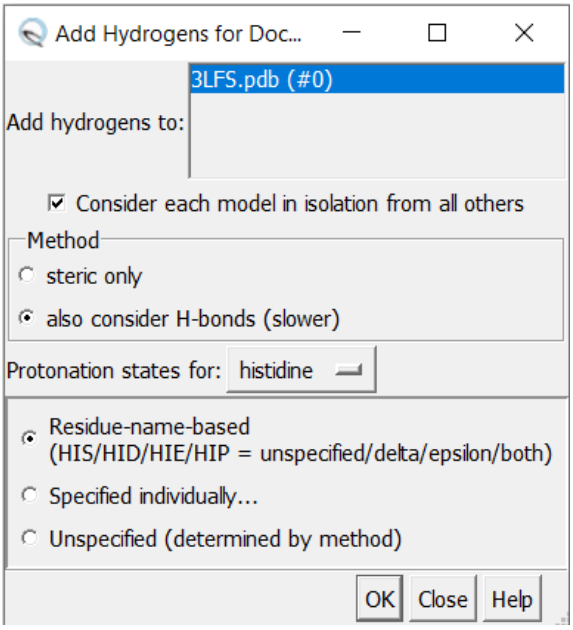
- Open one shell
- Create a directory called 3LFS.MD (type "*mkdir 3LFS.MD*" within the shell and press Enter)
- Open Google Chrome and go to the PDB homepage (www.rcsb.org/)
- In the search panel, digit the code 3LFS and press the Search button: the page related to the desired X-ray structure will be loaded
- Click on "Download Files" button and select "Legacy PDB format" from the drop-down menu
- The file should be automatically saved in the "Downloads" folder of your home directory. Otherwise, select "Save File" in the popup window and click "OK"
- Open Chimera program
- Click on File/Open
- Select the file 3lfs.pdb from the "Open File in Chimera" window
- Click on Open

Use the Dock Prep tool to complete the complex structure preparation.

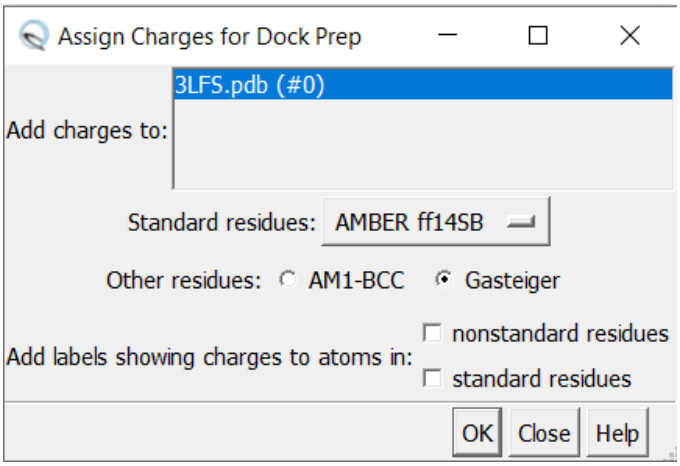
- Click on Tools/Structure editing/Dock Prep. Leave default options and press OK (*first time*)



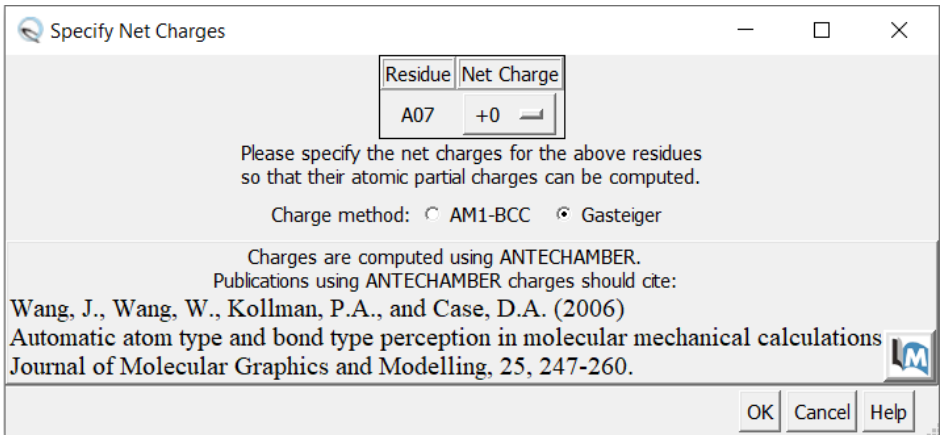
- The Add Hydrogens pane will appear; the default is OK, therefore Press OK (*second time*)



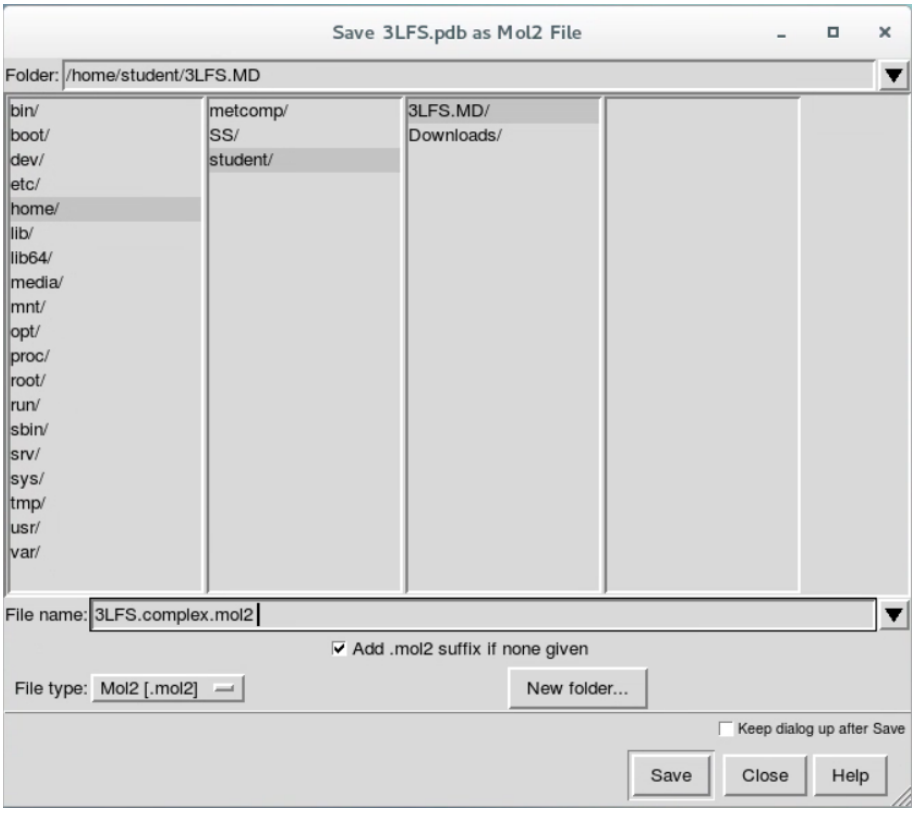
- The Assign Charges pane will appear; select Gasteiger and press OK (*third time*)



- The Specify Net Charges panel (for non-standard residues) will appear; the net charge of the ligand (A07) is zero, therefore press OK (*fourth time*)



- The Save as Mol2 panel will appear, in the File name write 3LFS.complex.mol2 and save the file in the 3LFS.MD directory (clicking on the Save button)



Dock Prep performs several tasks to prepare structures for MD and/or other calculations. Some of the Dock Prep tasks include:

deleting water molecules

repairing truncated sidechains

adding hydrogens

assigning partial charges

writing files in Mol2 format

Creation of the receptor files.

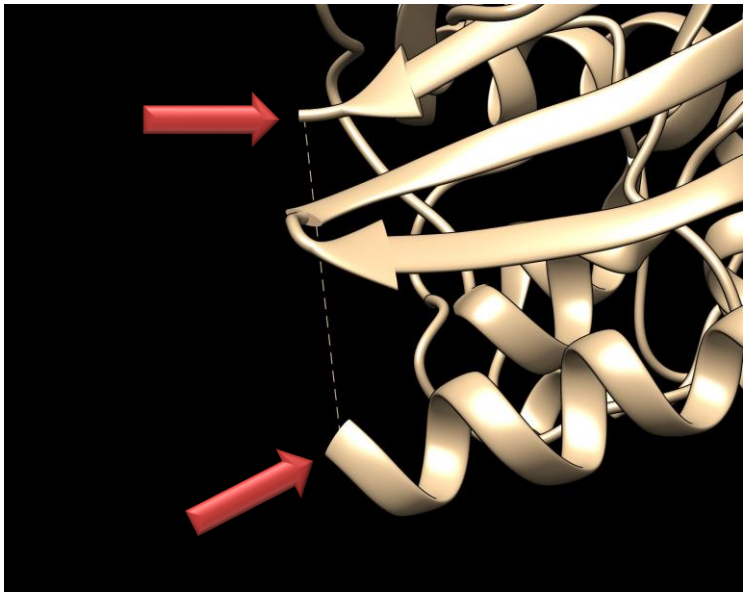
- Click on Select/Residue/A07
- Click on Actions/Atoms/Bonds/delete
- Click on Select/Chemistry/element/H
- Click on Actions/Atoms/Bonds/delete
- Click on File/Save PDB… and save the file in the 3LFS.MD directory as 3LFS.rec.noH.pdb

Creation of the ligand file.

- Click on File/Close Session
- Open the 3LFS.complex.mol2 (click on File/Open… select the file and press Open)
- Click on Select/Residue/standard amino acids
- Click on Actions/Atoms/Bonds/delete
- Click on File/Save Mol2… and save the file in the 3LFS.MD directory as 3LFS.lig.mol2

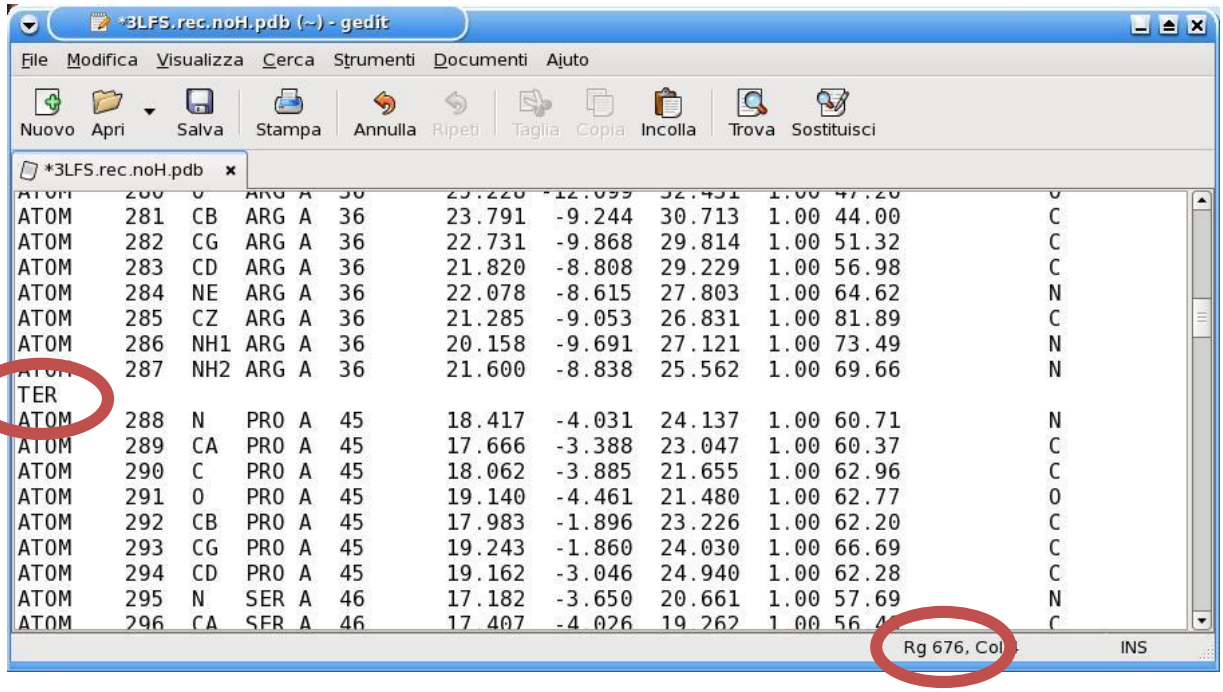
Editing of the 3LFS.rec.noH.pdb.

- Click on File/Close Session
- Open the 3LFS.rec.noH.pdb (click on File/Open… select the file and press Open)
- In the receptor structure there is a missing fragment of eight residues (from residue 37 to 44). You can verify it by moving the mouse on the two cut ends (see Figure)



It is important to edit the structure in order to avoid the possibility that AMBER software connects these two ends with a covalent bond.

- Close Chimera, open a shell, go into the 3LFS.MD directory (type "*cd 3LFS.MD*", press Enter)
- Digit "*gedit 3LFS.rec.noH.pdb*" and press Enter
- Scroll the file looking for the last line of residue ARG36 (line 675)
- Add a line containing the TER command (see Figure)



With the TER line, the two ends will not be connected with a covalent bond

- Save the file overwriting the 3LFS.rec.noH.pdb file and close the gedit software.

Charge and atom type definition for the ligand

AMBER package uses for the protein the Amber force field and for the ligand the GAFF force field (General AMBER Force Field). In order to add the charge (AM1-BCC) and the correct atom type to the ligand, we will use the antechamber program of the AmberTools suite.

- Open one shell and go inside the 3LFS.MD directory
- Digit "`antechamber -i 3LFS.lig.mol2 -fi mol2 -o 3LFS.lig.bcc.mol2 -fo mol2 -pf y -c bcc -nc 0`" and press Enter
- After about 2 minutes, the calculation will finish

```
Usage: antechamber -i   input file name
                   -fi  input file format
                   -o   output file name
                   -fo  output file format
                   -c   charge method
                   -nc  net molecular charge (int)
                   -pf  remove intermediate files: yes(y) or no(n)[default]
List of the Charge Methods
                charge method     abbre.  index | charge method    abbre. index
                ------------------------------------------------------------------
                RESP               resp     1   |  AM1-BCC            bcc     2
                CM1                cm1      3   |  CM2                cm2     4
                ESP (Kollman)      esp      5   |  Mulliken           mul     6
                Gasteiger          gas      7   |  Read in charge     rc      8
                Write out charge   wc       9   |  Delete Charge      dc     10
```

At this point, you should have the following file in your 3LFS.MD directory: 3LFS.complex.mol2, 3LFS.lig.bcc.mol2, 3LFS.lig.mol2, 3LFS.rec.noH.pdb, sqm.in, sqm.out, sqm.pdb (the last three files are produced by antechamber calculations).

Type "*ls*" in the shell and press Enter to check its content and verify to have all files.

4. Preparation of the MD input files

Starting from the 3LFS.rec.noH.pdb and 3LFS.lig.bcc.mol2 we will prepare the two files that will be used for launching the MD simulation: the 3LFS.complex.inpcrd (containing the initial coordinates of the system) and the 3LFS.complex.prmtop file (containing the parameter and topology of the molecules in the system). In order to build and solvate this complex, you will need to start tLEaP. tLEaP has another command line interface for building the system topology and define parameters for the molecules.

- Open one shell and go inside the 3LFS.MD directory
- We use the utility parmchk2 to test if all the parameters required for the ligand are available. Digit "`parmchk2 -i 3LFS.lig.bcc.mol2 -f mol2 -o frcmod_lig`" and press Enter.

This command will create the frcmod_lig file. This is a parameter file that can be loaded into tLeap in order to add missing parameters. Here it will contain all of the missing parameters. If it can, antechamber will fill in these missing parameters by analogy to a similar parameter. You should check these parameters carefully before running a simulation. If antechamber can't empirically calculate a value or has no analogy, it will either add a default value that it thinks is reasonable or alternatively insert a place holder (with zeros everywhere) and the comment "ATTN: needs revision". In this case you will have to manually parameterize this yourself. It is hoped that as GAFF is developed so the number of missing parameters will decrease. Let's look at our frcmod file:

- Digit "`more frcmod_lig`" and press Enter

```
Remark line goes here
MASS
BOND
ANGLE
DIHE
ca-ca-cd-nc   4   2.800   180.000           2.000      same as X -c2-ca-X , penalty score=232.0
ca-ca-cd-n    4   2.800   180.000           2.000      same as X -c2-ca-X , penalty score=232.0
IMPROPER
ca-ca-cp-cp         1.1     180.0       2.0          Using the default value
ca-cp-ca-ha         1.1     180.0       2.0          Using general improper torsional angle  X- X-ca-ha
ca-ca-ca-ha         1.1     180.0       2.0          Using general improper torsional angle  X- X-ca-ha
```

```
ca-cl-ca-cp         1.1     180.0       2.0          Using the default value

ca-ca-ca-na         1.1     180.0       2.0          Using the default value

ca-ca-ca-cd         1.1     180.0       2.0          Using the default value

ca-hn-na-nc         1.1     180.0       2.0          Same as X -X -na-hn penalty score= 31.9

ca-n -cd-nc         1.1     180.0       2.0          Using the default value

c -cd-n -hn         1.1     180.0       2.0          Using general improper torsional angle  X- X- n-hn

c3-n -c -o         10.5     180.0       2.0          Using general improper torsional angle  X- X- c- o

NONBON
```

We can see that there were a total of 2 missing dihedral and 10 missing improper dihedrals for which Antechamber has suggested similar parameters.

- Digit "`tleap -f leaprc.ff14SB`"

This command will open the tleap interface loading the amber14 force field

- Digit "`source leaprc.gaff`" (once tleap is up and running we also need to ensure that it knows about the GAFF force field. This command will do this for us.
- Digit "`source leaprc.tip3p`" (it will load the TIP3P water parameters).
- Digit "`fmod1 = loadamberparams frcmod_lig`" (it will load the ligand missing parameters)
- Digit "`fmod2 = loadamberparams frcmod.ionsjc_tip3p`" (it will load the information about the solvation of the system)
- Digit "`prot = loadpdb 3LFS.rec.noH.pdb`" (it will load the protein, add the hydrogens and cap the ending residues)
- Digit "`lig = loadmol2 3LFS.lig.bcc.mol2`" (it will load the ligand)
- Digit "`complex = combine {prot lig}`" (it will combine together protein and ligand)
- Digit "`solvatebox complex TIP3PBOX 20`" (it will create a rectangular box of water 20 Å beyond the complex)
- Digit "`charge complex`" (it will give the charge value of the system, if it is positive we have to use Cl^- for neutralizing the system; if it is negative Na^+ ions)
- Digit "`addions complex Cl- 0`" (it will neutralize the system adding chlorine ions)
- Digit "`saveamberparm complex 3LFS.complex.prmtop  3LFS.complex.inpcrd`" (it will create the two input files for launching the MD simulation)
- Digit "`quit`" (it will close the tleap command interface)

- Digit in the shell "`ambpdb -p 3LFS.complex.prmtop -c 3LFS.complex.inpcrd > 3LFS.complex.pdb`" (it will create a pdb file of the AMBER input file)

You can open the 3LFS.complex.pdb with Chimera and analyze the complex.

5. Minimization of the complex

Before the MD simulation, two steps of minimization will be performed: in the first one we will block the protein leaving free only the water molecules, whereas in the second step we will minimize the whole system blocking only the Cα of the protein.

- Open one shell and go inside the 3LFS.MD directory
- Digit "`cp /usr/local/SS/MD/*in .`". (in this way you will copy in your directory all the input files for launching the minimizations and dynamics)
- Digit "`cp /usr/local/SS/MD/script* .`". (in this way you will copy in your directory the file containing the commands for launching the minimizations and dynamics)
- In order to launch the two minimizations digit: "`tcsh script_MD &`". The & command will launch the script in the background mode (the calculation will be independent from the shell)
- In order to analyze the script open it with the linux command "`more`". All the lines start with "mpirun -np 2". It means that the calculation will be clustered using two CPU of your PC.

The first minimization step (min1.in) will minimize the water molecules blocking the protein-ligand complex (it should take about 8 minutes).

min1.in:

```
WATER minimization
&cntrl
  imin   = 1,
  maxcyc = 1000,
  ncyc   = 500,
  ntb    = 1,
  ntr    = 1,
  cut    = 10
  drms   = 0.05
 /
Hold the prot fixed
100.0
RES 1 290
END
END
```

imin = 1 ::: Flag to run minimization, = 0 No minimization (only do molecular dynamics), = 1 Perform minimization (and no molecular dynamics) = 5 Read in a trajectory for analysis.

maxcyc = 1000 ::: The maximum number of cycles of minimization.

ncyc = 500 ::: If NTMIN is 1 then the method of minimization will be switched from steepest descent to conjugate gradient after NCYC cycles.

ntb = 1 ::: Periodic boundary, = 0 no periodicity is applied and PME is off, = 1 constant volume (default), = 2 constant pressure

ntr = 1 ::: Flag for restraining specified atoms in Cartesian space using a harmonic potential, = 0 No position restraints, = 1 restraint of specified atoms. In this simulation residues 1-290 will be fixed with a force constant of 100 kcal/mol•$Å^2$

cut = 10 ::: nonbonded cutoff, in Angstroms.

dmrs = 0.05 gradient convergence criterion (0.05 kcal/mol•Å, the default is 0.0001

When the calculation ends you will obtain as output the 3LFS.complex_1.rst that can be converted in the pdb format with the command: "`ambpdb -p 3LFS.complex.prmtop -c 3LFS.complex_1.rst > 3LFS.complex_1.pdb`"

The second minimization step (min2.in) will minimize the system blocking the Cα of the protein (it will take about 8 minutes).

min2.in:

```
System minimization
 &cntrl
  imin   = 1,
  maxcyc = 1000,
  ncyc   = 500,
  ntb    = 1,
  ntr    = 1,
  cut    = 10,
  drms   = 0.05
 /
Hold the CA fixed
10.0
FIND
CA * * * *
SEARCH
RES 1 290
END
END
```

When the calculation ends you will obtain as output the 3LFS.complex_2.rst that can be converted in the pdb format with the command: "`ambpdb -p 3LFS.complex.prmtop -c 3LFS.complex_2.rst > 3LFS.complex_2.pdb`"

6. MD simulation of the complex

The next stage in our equilibration protocol, after the minimization, is heating our system up from 0 K to 300 K and then maintaining the system at 300 K.

Our final aim is to run dynamics at constant temperature and pressure, since this procedure more closely resembles laboratory conditions. However, for the first few ps, at low temperatures, the calculation of our system pressure is very inaccurate, and thus using constant pressure periodic boundaries in this situation can lead to problems. Using constant pressure with restraints can also cause problems, so initially we will run 500 ps (0.5 ns) of MD at constant volume. Once our system has equilibrated over approximately 500 ps we will change to constant pressure before running a further 52000 ps (52 ns) of equilibration at 300 K.

dyn1.in

```
MD1
 &cntrl
  imin   = 0,
  irest  = 0,
  ntx    = 1,
  ntb    = 1,
  cut    = 10,
  ntr    = 1,
  ntc    = 2,
  ntf    = 2,
  tempi  = 0.0,
  temp0  = 300.0,
  ntt    = 3,
  gamma_ln = 5.0,
  nstlim = 250000, dt = 0.002,
  ntpr = 5000, ntwx = 50000, ntwr = 10000
  /
 Hold the CA fixed
 10.0
 FIND
 CA * * * *
 SEARCH
 RES 1 290
 END
 END
```

imin = 0 ::: Flag to run minimization, = 0 No minimization (only do molecular dynamics), = 1 Perform minimization (and no molecular dynamics) = 5 Read in a trajectory for analysis.

irest = 0 ::: Flag to restart the run, = 0 No effect (default), = 1 restart calculation. Requires velocities in coordinate input file, so you may also need to reset NTX if restarting MD

ntx = 1 ::: Option to read the initial coordinates, velocities and box size, = 1 X is read formatted with no initial velocity information, = 2 X is read unformatted with no initial velocity information, = 4 X and V are read unformatted, = 5 X and V are read formatted; box information will be read if ntb>0, the velocity

information will only be used if *irest*=1, = 6 X, V and BOX(1..3) are read unformatted; in other respects, this is the same as option "5".

ntb = 1 ::: Periodic boundary, = 0 no periodicity is applied and PME is off, = 1 constant volume (default), = 2 constant pressure

cut = 10 ::: nonbonded cutoff, in Angstroms.

ntr = 1 ::: Flag for restraining specified atoms in Cartesian space using a harmonic potential, = 0 No position restraints, = 1 restraint of specified atoms

ntc = 2 ::: Flag for SHAKE to perform bond length constraints, = 1 SHAKE is not performed (default), = 2 bonds involving hydrogen are constrained, = 3 all bonds are constrained (not available for parallel or qmmm runs in *sander*)

ntf = 2 ::: Force evaluation. Note: If SHAKE is used (see NTC), it is not necessary to calculate forces for the constrained bonds., = 1 complete interaction is calculated (default), = 2 bond interactions involving H-atoms omitted (use with NTC=2), = 3 all the bond interactions are omitted (use with NTC=3), = 4 angle involving H-atoms and all bonds are omitted, = 5 all bond and angle interactions are omitted, = 6 dihedrals involving H-atoms and all bonds and all angle interactions, are omitted, = 7 all bond, angle and dihedral interactions are omitted, = 8 all bond, angle, dihedral and non-bonded interactions are omitted

tempi = 0 and temp0 = 300 ::: Initial temperature and reference temperature at which the system is to be kept.

ntt = 3 ::: Switch for temperature scaling, = 0 Constant total energy classical dynamics (assuming that *ntb*<2, as should probably always be the case when *ntt=0*), = 1 Constant temperature, using the weak-coupling algorithm, = 2 Andersen temperature coupling scheme, = 3 Use Langevin dynamics with the collision frequency γ given by *gamma_ln*

gamma_ln = 5.0 ::: The collision frequency γ, in ps-1, when *ntt* = 3.

nstlim = 250000 ::: Number of MD-steps to be performed.

dt = 0.002 ::: The time step (psec).

ntpr = 5000 ::: Every NTPR steps energy information will be printed in human-readable form to files "mdout" and "mdinfo

ntwx = 50000 ::: Every NTWX steps the coordinates will be written to file "mdcrd".

ntwr = 10000::: Every NTWR steps during dynamics, the "restrt" file will be written.

---

dyn2.in

```
MD2
 &cntrl
  imin = 0, irest = 1, ntx = 5,
  ntb = 2, pres0 = 1.0, ntp = 1,
  taup = 2.0,
  cut = 10, ntr = 1,
```

```
 ntc = 2, ntf = 2,
 tempi = 300.0, temp0 = 300.0,
 ntt = 3, gamma_ln = 5.0,
 nstlim = 1000000, dt = 0.002,
 ntpr = 5000, ntwx = 50000, ntwr = 10000
 /
Hold the CA fixed
10.0
FIND
CA * * * *
SEARCH
RES 1 290
END
END
```

imin = 1 ::: Flag to run minimization, = 0 No minimization (only do molecular dynamics), = 1 Perform minimization (and no molecular dynamics) = 5 Read in a trajectory for analysis.

irest = 1 ::: Flag to restart the run, = 0 No effect (default), = 1 restart calculation. Requires velocities in coordinate input file, so you also may need to reset NTX if restarting MD

ntx = 5 ::: Option to read the initial coordinates, velocities and box size, = 1 X is read formatted with no initial velocity information, = 2 X is read unformatted with no initial velocity information, = 4 X and V are read unformatted, = 5 X and V are read formatted; box information will be read if ntb>0, the velocity information will only be used if *irest*=1, = 6 X, V and BOX(1..3) are read unformatted; in other respects, this is the same as option "5".

ntb = 2 ::: Periodic boundary, = 0 no periodicity is applied and PME is off, = 1 constant volume (default), = 2 constant pressure

pres0 = 1.0 ::: Reference pressure (atm)

ntp = 1 ::: Flag for constant pressure dynamics, = 0 Used with NTB not = 2 no pressure scaling, =1 md with isotropic position scaling, = 2 md with anisotropic (x-,y-,z-) pressure scaling.

taup = 2.0 ::: Pressure relaxation time (in ps)

cut = 10 ::: nonbonded cutoff, in Angstroms

ntr = 1 ::: Flag for restraining specified atoms in Cartesian space using a harmonic potential, = 0 No position restraints, = 1 restraint of specified atoms

ntc = 2 ::: Flag for SHAKE to perform bond length constraints, = 1 SHAKE is not performed (default), = 2 bonds involving hydrogen are constrained, = 3 all bonds are constrained (not available for parallel or qmmm runs in *sander*)

ntf = 2 ::: Force evaluation. Note: If SHAKE is used (see NTC), it is not necessary to calculate forces for the constrained bonds., = 1 complete interaction is calculated (default), = 2 bond interactions involving H-atoms omitted (use with NTC=2), = 3 all the bond interactions are omitted (use with NTC=3), = 4 angle involving H-atoms and all bonds are omitted, = 5 all bond and angle interactions are omitted, = 6 dihedrals involving H-atoms and all bonds and all angle interactions, are omitted, = 7 all bond, angle and dihedral interactions are omitted, = 8 all bond, angle, dihedral and non-bonded interactions are omitted

tempi = 300 and temp0 = 300 ::: Initial temperature and reference temperature at which the system is to be kept.

ntt = 3 ::: Switch for temperature scaling, = 0 Constant total energy classical dynamics (assuming that *ntb*<2, as should probably always be the case when *ntt=0*), = 1 Constant temperature, using the weak-coupling algorithm, = 2 Andersen temperature coupling scheme, = 3 Use Langevin dynamics with the collision frequency γ given by *gamma_ln*

gamma_ln = 5.0 ::: The collision frequency γ, in ps-1, when *ntt* = 3.

nstlim = 1000000 ::: Number of MD-steps to be performed.

dt = 0.002 ::: The time step (psec).

ntpr = 5000 ::: Every NTPR steps energy information will be printed in human-readable form to files "mdout" and "mdinfo

ntwx = 50000 ::: Every NTWX steps the coordinates will be written to file "mdcrd".

ntwr = 10000 ::: Every NTWR steps during dynamics, the "restrt" file will be written.

The third MD simulation (dyn3.in) differs only for the length (50 ns).

For time reasons you will not launch the dyn1.in, dyn2.in and dyn3.in calculations. However, you can copy the results from the /usr/local/SS/MD directory.

- Open one shell and go inside the 3LFS.MD directory
- Digit "`cp /usr/local/SS/MD/1/mdcrd* .`" and press Enter.

In this way you will copy in your directory all the resulting trajectory files that will be analyzed with chimera.

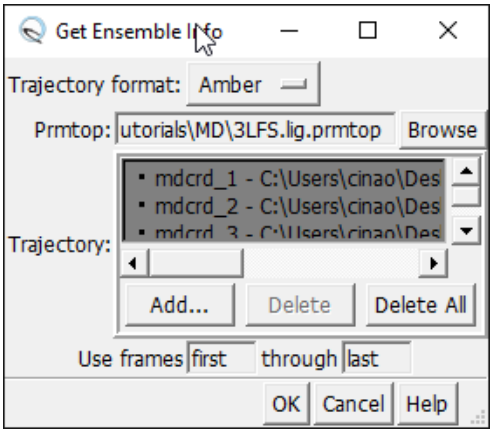
7. Analysis of the MD results

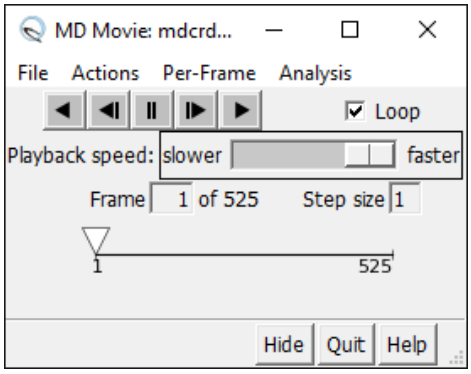
The MD results will be evaluated by means of Chimera.

- Open one shell and go inside the 3LFS.MD directory
- Open Chimera
- Click on Tools/MD/Ensemble Analysis/MD Movie
- In the Prmtop line of the "Get Ensemble info" panel (left image below) select the 3LFS.complex.prmtop file (clicking on Browse)

- If the Trajectory task is not empty click on "Delete All"; then click on "Add…", select mdcrd_1 and press Add
- Click on "Add…" again and load the mdrcd_2
- Click on "Add…" again and load the mdrcd_3
- Click on "OK"

The MD simulation will be opened, together with the **MD Movie** (right image below) that is a tool for viewing and analyzing trajectories and other ensembles.

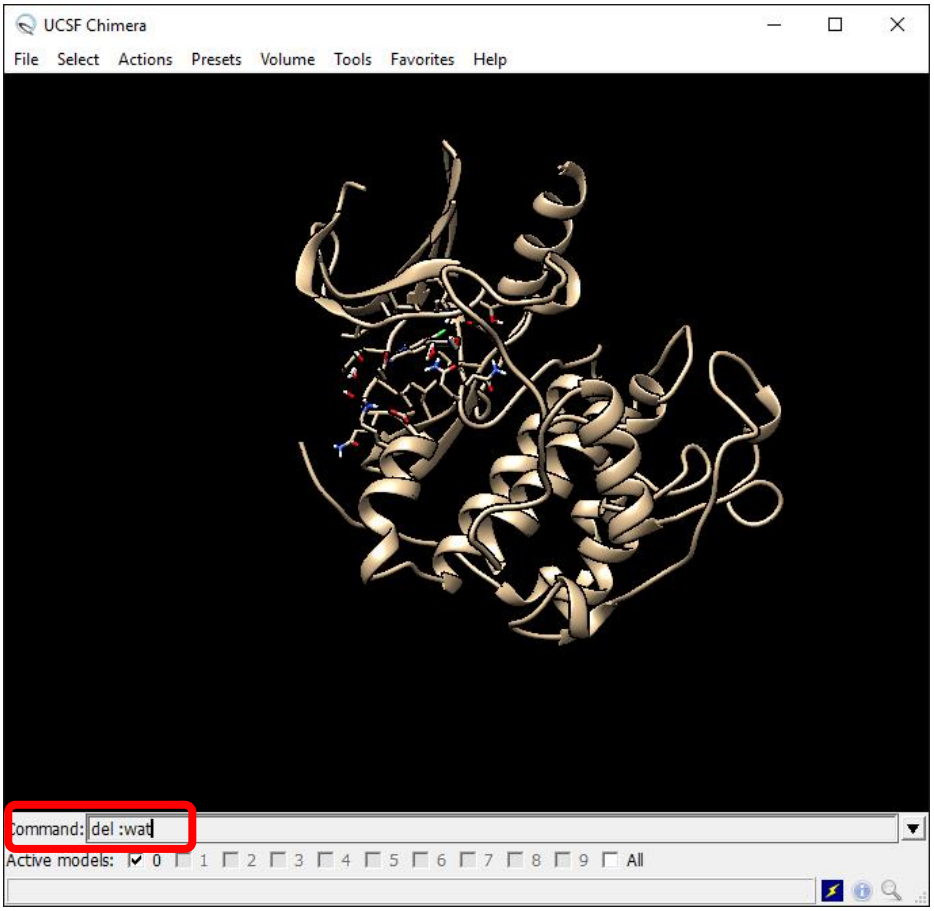




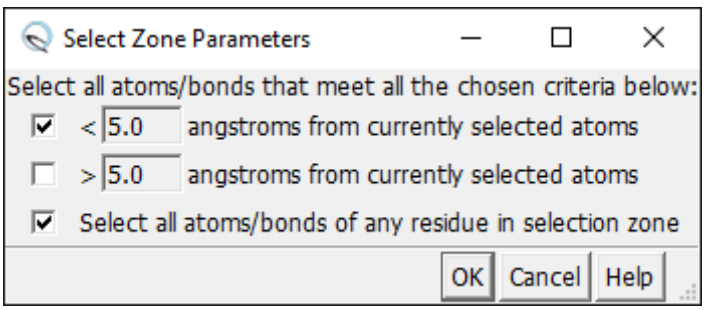
From left to right, the buttons mean: play backward continuously; go back one step; stop; go forward one step; and play forward continuously. When the Loop option is on, forward play can wrap from the end to the beginning of the loaded trajectory and reverse play can wrap from the beginning to the end. The rate of continuous play can be adjusted with the Playback speed slider; up to a 1-second delay can be added between frame advances. When continuous play is not in use, the display can also be controlled by moving the pointer (inverted triangle) on the timeline or by entering a new Frame number. The Step size controls the level of sampling for continuous playback. For example, a step size of 3 indicates that only every third frame will be shown; however, when forward play loops from end to beginning, the movie will start at the first frame loaded, and when reverse play loops from beginning to end, the movie will start at the last frame loaded. Frame number and step size changes take effect when return (Enter) is pressed.

It will be useful to delete water, ions and hold alpha carbon steady during trajectory playback.

- Click on Favorites/Command Line
- In the Command Line digit "`del :wat`" and press the Enter keyboard button. This command will delete the water molecules.



- In the Command Line erase "`del :wat`", type "`del :Cl-`" and press the Enter keyboard button. This command will delete the Chlorine ions.
- In the Command Line erase "`del :Cl-`", type "`sel @CA`" and press the Enter keyboard button. This command will select the alpha Carbon of the protein.
- In the **MD Movie** tool click on Actions/Hold selection steady. This command will hold alpha carbon steady during trajectory playback.
- In the Chimera menu click on Select/Residue/A07
- Click on Actions/Color/green
- Click on Actions/Color/by heteroatom
- Click on Select/Zone… adjust the parameters as shown in the Figure and click on OK.



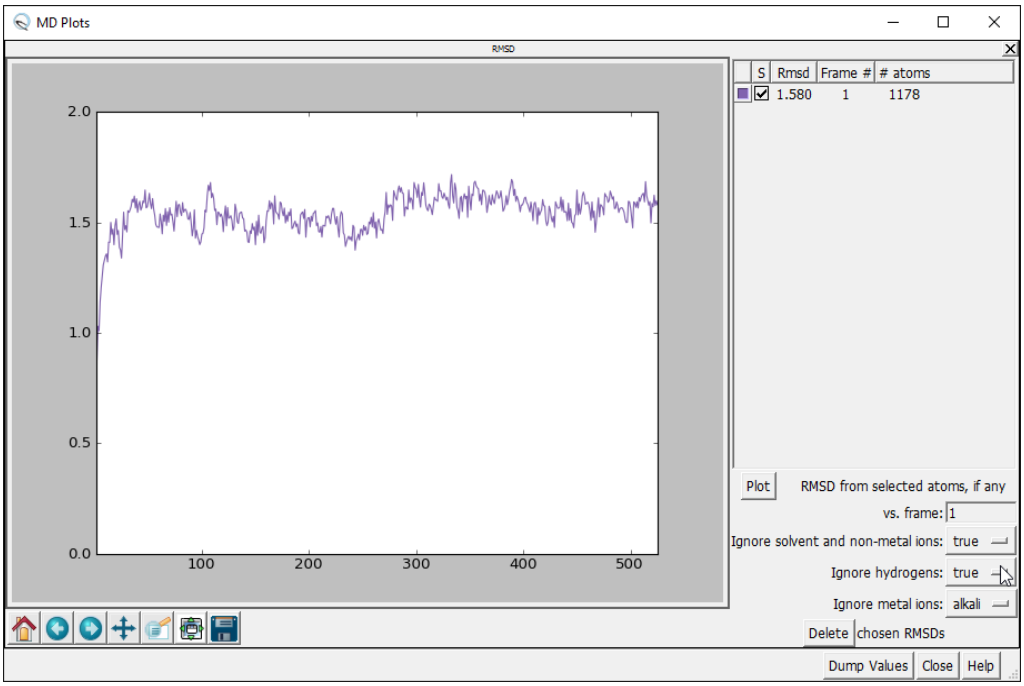
- Click on Actions/Atoms/Bonds/show
- Click on Select/Invert (all models)
- Click on Actions/Atoms/Bonds/hide
- Click on Select/Chemistry/IDATM type/HC
- Click on Actions/Atoms/Bonds/hide
- Click on Select/Clear Selection

At this point we can visualize the MD simulation:

- In the **MD Movie** tool deactivate the loop button and click on the Play button  .

Analysis of the protein side-chain movements:

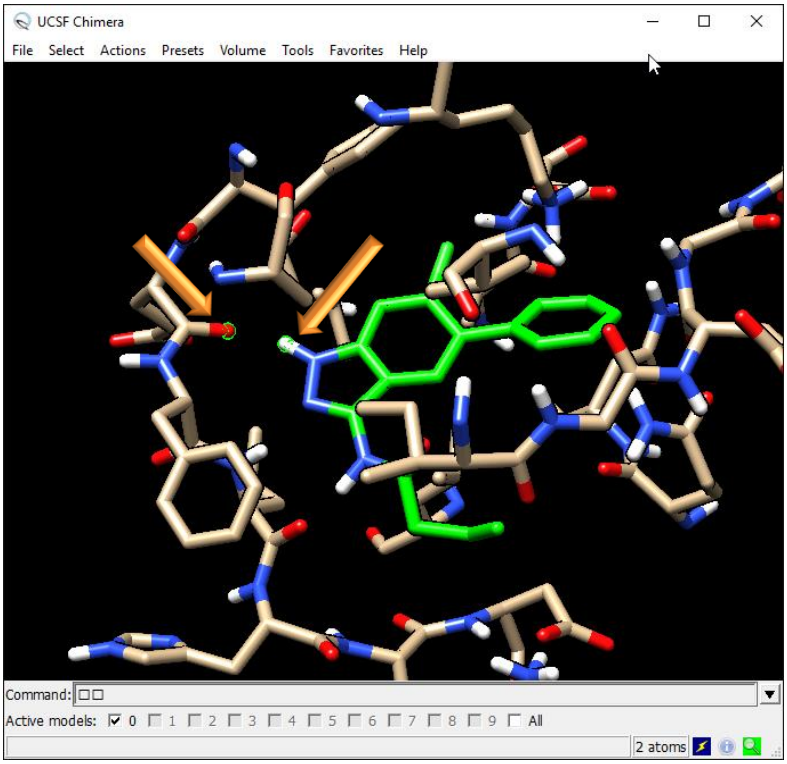
- In the Chimera menu click on Select/Structure/side chain/base/without CA/C1'
- In the **MD Movie** tool click on Analysis/Plot/RMSD
- In the MD Plots window press the Plot button. The movement of the side chains will be reported in the plot (X = MD steps, Y= Angstrom). As you can see the heavy atoms are stable as they show deviations under 2.0 Å.



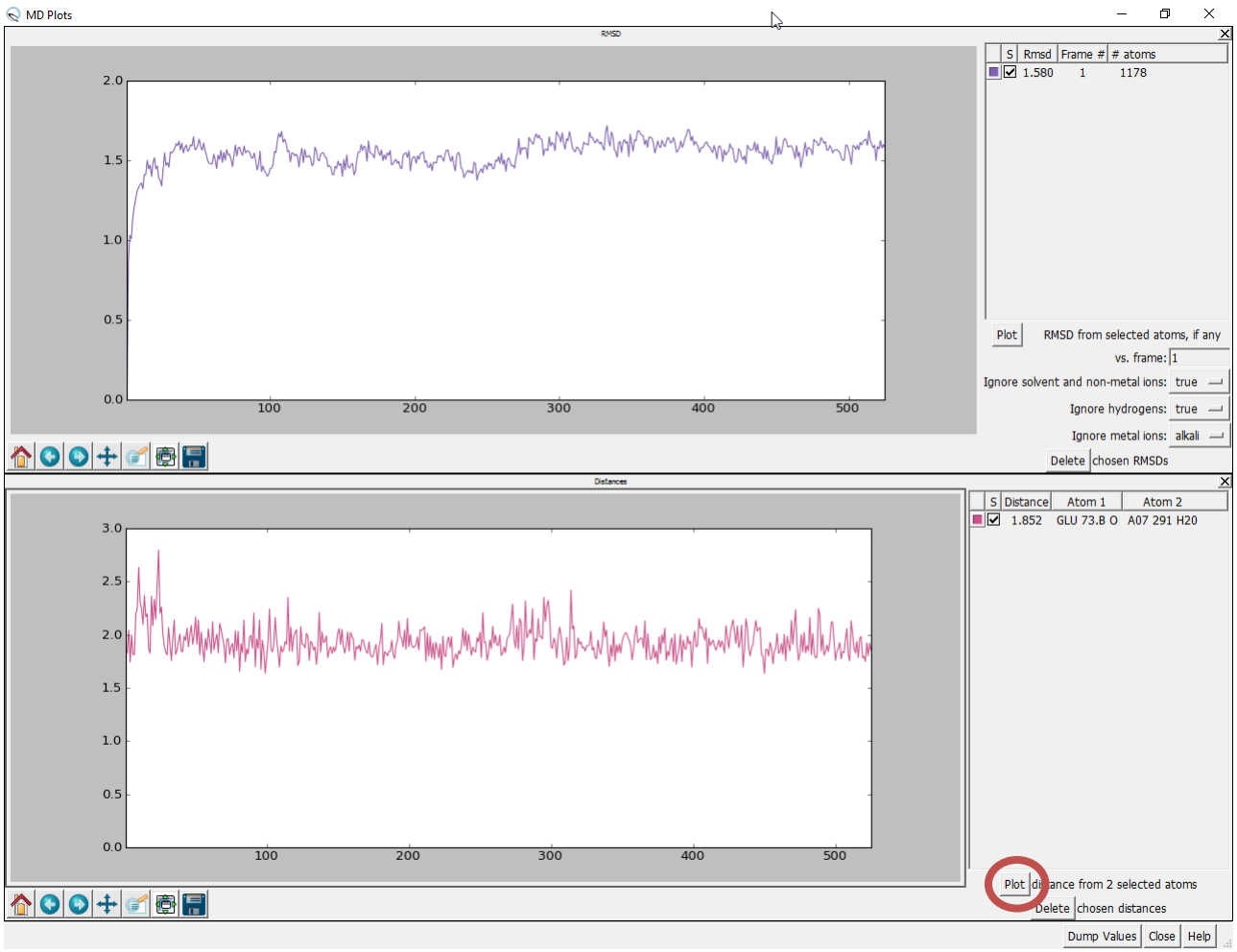
Analysis of the ligand-protein H-bonds

- Click on Select/Clear Selection
- Click on Actions/Ribbon/Hide
- Click on the O atom of GLU73.B with the CTRL-left mouse and click on the H20 atom of the ligand with the CTRL-Shift-left mouse combination (remember: for selecting one atom

press “Ctrl” and then click with the left button on one atom. For adding a selection hold down the “Ctrl” and “Shift” key, and click with the left mouse button on the atom).



- In the **MD Movie** tool click on Analysis/Plot/Distance
- Press the Plot button in the MD plots task (see Figure). The distance between the oxygen of GLU73 and the hydrogen of the ligand will be plotted. As you can see, except in the first frames of the MD, the distance is very stable around the value of 2.0 Å.



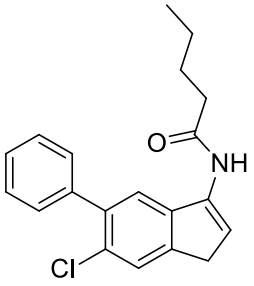
Do the same analysis for the other two H-bonds. You will find out that all the three H-bonds are very stable during the whole MD simulation.

Clicking on the two plots you will visualize on chimera the corresponding MD snapshot.

Exercise.

- Open one shell and go inside the 3LFS.MD directory
- Create the LIG2 directory and go inside this directory
- Digit "`cp /usr/local/SS/MD/2/lig.mol2 .`" to copy inside LIG2 directory the structure of a new compound (lig.mol2)

This compound is a modified version of the active compound that you analyzed previously.



Lig2

- Digit "`cp ../3LFS.rec.noH.pdb .` " to copy the processed reptor file into LIG2 directory

Prepare the input file for the MD simulation using the new ligand file and launch the first two minimization steps. Then, in order to analyze the MD simulation of the new complex:

- Digit "`cp /usr/local/SS/MD/2/mdcrd* .` ".

Visualize the new trajectory and check the difference with respect to the previous one.