Amber Tutorial for Heidelberg-ND Summer School

This tutorial covers the basics of the program AMBER. We will use a protein-ligand complex as an example. The PDB code is 3HTB. It is a lysozyme complexed with 2-propylphenol.

1. Visualize the protein in VMD (or your favorite visualization program)

Download 3HTB from Protein Data Bank (http://www.rcsb.org). Visualize the protein in VMD.

2. Prepare the ligand for Amber

```
grep JZ4 3htb.pdb > lig-noH.pdb
module load amber
reduce lig-noH.pdb > lig.pdb
```

These commands save the coordinates of the ligand as a pdb file and add hydrogens to the ligand.

```
awk '{print $12, $7, $8, $9}' lig.pdb
```

This command generates the coordinates in a format which can used in Gaussian input file. We are now ready to perform geometry optimization using Gaussian 09. The Gaussian input file 'lig-opt.com' is provided to you. Copy and paste the coordinates into the input file.

On CRC, we use scripts to submit jobs. The submission script 'gaussian-opt.sh' is provided to you. Remember to change '#$ -M YOUR_EMAIL_ADDRESS'. To submit the job, use

```
qsub gaussian-opt.sh
```

This job takes ~30 minutes. We do not have to wait till it is done. The output file 'lig-opt.log' is provided to you. In the 'lig-opt.log' file, get the optimized coordinates.

```
C,0,24.2860518605,-23.9483933108,0.045568526
C,0,21.4649915601,-27.2619570916,-4.2550806875
C,0,21.9140039067,-26.7422806942,-5.4709703284
…AND MORE…
```

The input file for charge calculation 'lig-opt-charge.com' is provided to you. Paste the optimized coordinates into 'lig-opt-charge.com' file. Use the script 'gaussian-charge.sh' to submit this job. When the job is done, the charge of the ligand is saved in 'lig-opt-charge.gesp' file. We will then run `antechamber` to generate the mol2 file of the ligand.

```
antechamber -eq 2 -fo mol2 -fi gesp -i lig-opt-charge.gesp -o
lig.mol2 -a lig.pdb -fa pdb -ao crd -nc 0 -rn LIG -c resp
```

Note: `-nc 0` specifies the charge of the ligand. If your ligand is not neutral, change it appropriately.

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

This command generates the 'lig.frcmod' file with any parameter not included in the GAFF force field that will be needed for the simulation.

```
tleap -f lig-tleap-prep
```

The 'lig-tleap-prep' file is provided to you. The `tleap` command generates a library file 'lig.off' for ligand and the 'lig-tleaped.pdb' file.

```
cp 3htb.pdb protein-lig.pdb
```

Copy the coordinates in 'lig-tleaped.pdb' file and paste into 'protein-lig.pdb' file.

3. Prepare the protein-ligand complex for Amber

```
tleap –f protein-lig-ions-solv-tleap-prep
```

This `tleap` command generates `protein-lig-ions-solv.prmtop` and `protein-lig-ions-solv.inpcrd` files, which are Amber input files.

4. Time to Run Amber!

We will run the simulation by several steps: minimization, equilibration and production run. The commands (.in file) for all these steps are provided in the eqscripts folder.

The submission subscript for Amber is 'md-run.sh'. To submit the job, use

```
qsub md-run.sh
```

5. Analysis

When the MD job is done, the trajectories are saved in the mdcrd file. You can get RMSD, snapshots and many other cool stuff from the trajectory file. You first need to prepare a script file. As an example, a script '1ns-snapshot.ptraj' to get a trajectory snapshot is provided.

```
cpptraj -p protein-lig-ions-solv.prmtop -i 1ns-snapshot.ptraj
```

This generates '1ns-snapshot.pdb' which is the 1 ns snapshot of the simulation.

`cpptraj` can be used to perform many other useful analysis. Consult to the Amber manual for details.

This tutorial covers the basics of Amber. Of course, we cannot teach you everything in this short tutorial. If you get into troubles (I am sure you will!) when running Amber, consult to the Amber manual or even just google your question.

Happy Computing!