# Lab on host-guest binding free energies: Free energy calculation of organic compounds with BEDAM

Mauro Lapelosa: maurolap@eden.rutgers.edu
Emilio Gallicchio: emilio@biomaps.rutgers.edu

In this lab exercise you will use BEDAM to calculate the standard binding free energies of organic compounds and analyze the data provided by the BEDAM method.
First you need to create a folder with initial data, namely β-Cyclodextrin structure file in the maestro format, a text file with SMILES designation for some guests (smiles.smi), and a sample input_file (benzene.inp), you need to run:
**cp /home/mauro/class/* $your_directory**
and put the files in your directory. Use a different working directory for each ligand. This is an example of input file:

```
#name of receptor .mae file
RECEPTOR_FILE 'bcy_noprop.maegz'
#name of ligand .mae file
LIGAND_FILE 'benzene.maegz'
#list of lambdas for each replica (16 replicas)
LAMBDAS '0.0,0.001,0.002,0.004,0.005,0.006,0.008,0.01,0.02,0.04,0.07,0.1,0.25,0.5,0.75,1.0'
#the atoms of the receptor and ligand that define each centroid.
REST_LIGAND_CMRECASL '( all) AND NOT (( atom.ele H ) )'
REST_LIGAND_CMLIGASL '( all) AND NOT (( atom.ele H ) )'
#parameters of the flat-bottol harmonic restraints between the centroids
#defined above.
# force constant in kcal/mol/A^2
REST_LIGAND_CMKF    3.0
# equilibrium distance in Angstroms
REST_LIGAND_CMDIST0 0.0
# distance tolerance in Angstroms
REST_LIGAND_CMTOL   6.0
#the atoms of the receptor that are harmonically restrained
REST_RECEPTOR_ASL    '(not (atom.num 3, 5, 10, 11, 14, 16, 21, 22, 25, 27, 32, 33, 36, 38, 43, 44, 47, 49, 54, 55, 58, 60,
65, 66, 69, 71, 76, 77 ) ) AND NOT (( atom.ele H ) )'
# force constant of receptor harmonic restraint in kcal/mol/A^2
REST_RECEPTOR_KF    0.6
#Temperature in K
TEMPERATURE          300
# number of equilibration MD steps (each step 1fs)
EQUILIBRATION_STEPS  10000
# number of production MD step (each step 1fs).
PRODUCTION_STEPS    500000
#Frequency of printing information in output file in number of steps. The number
#of binding energy samples collected for each replica is
#PRODUCTION_STEPS/PRNT_FREQUENCY = 500 in this case.
#500 x 16replicas  = 800 samples in total
PRNT_FREQUENCY       1000
# Frequency of saving trajectory frames. 500 frames per replica.
TRJ_FREQUENCY        1000
```

The top of the file specifies the ligand and receptor files, and the parameters of the simulations are at the bottom.
You need to edit this input file according to the assigned ligand, and rename it accordingly, (for example ibuprofen.inp). The basename of the file you choose will be the <jobname> identifier for the BEDAM calculations.

The structures before the simulations need some preparation. Obtain an initial structure for the ligand assigned to you (this may involve manual building or automatic creation with SMILES/LIGPREP). LIGPREP is under the menu applications in Maestro.
The ligand has to be placed properly in the binding pocket. You need initially to place the ligand inside the receptor (β-Cyclodextrin) using Maestro. You will open Maestro and go to build under menu edit, then you click on build and you go to fragment. Then you import the structure of the receptor. You select both items in the project table and use the local transformation on the left side of the screen in Maestro. Export the ligand structure to a maestro file and place it in the ligand working directory.

Each student will be have a different compound plus benzene that is used as an example and it will be used by all the students.

We use BEDAM on hugin, so you need you have a folder on hugin and run a
command to copy your files from your directory to hugin. You may want to login in hugin first to make your folder where you run the BEDAM calculation:
**rsync -azv * hugin:$your_hugin_directory**

Again, make sure to run each ligand in a separate directory.

Then you need to log in hugin:
**ssh hugin**
and go to your working directory:
**/net/huginp/u1/$name/$your_directory**

Once you are there just make sure that you have all the files ready for using BEDAM.
Then you need to run this command to prepare the files to run BEDAM:
**export SCHRODINGER=/net/huginp/u1/emilio/schrod/r2012/b1**
and then
**$SCHRODINGER/run ~emilio/utils/bedam_scripts/bedam_prep.py benzene.inp**

Replace “benzene.inp” with your ligand input file as appropriate

The initial prepared complex need to be thermalized to run a proper calculation, so you need to run this command:
**$SCHRODINGER/impact -i <jobname>_mintherm.inp -HOST hugin -LOCAL**

Then you need to wait till the thermalization is complete (you can monitor using **qstat** in the command line) and you run the production calculation with this command:

**$SCHRODINGER/impact -i <jobname>_remd.inp -HOST hugin -LOCAL**

then you need to run a script to analyze the data using this command:

**$SCHRODINGER/run ~emilio/utils/bedam_scripts/bedam_analyze.py <jobname>.inp**

You exit the hugin with **exit** command.
Finally you need to rsync files to your workstation with this command:

**rsync -azv hugin:$your_hugin_directory $local_directory**

and then do the analysis.

| Name | Solvent | DG[kJ/mol] |
|---|---|---|
| Alkanols | | |
| 1-butanol | H2O (pH 6.90) | -6.9 |
| 1-hexanol | H2O | -13.3 |
| (R)-(-)-2-hexanol | H2O (pH 6.90) | -11.8 |
| cyclohexanol | H2O (pH 6.9) | -16.19 |
| cyclooctanol | H2O (pH 6.9) | -16.4 |
| Aromatic | | |
| naphthalene | H2O | -16.2 |
| naproxenate | H2O (pH 9.0) | -16.1 |
| flurbiprofen | H2O (pH 7.0) | -18.8 |
| ibuprofen | H2O (pH 2) | -33.3 |
| 2-naphthol | H2O | -15.4 |
| Admantyl | | |
| 1-adamantanecarboxylate | H2O (pH 8.50) | -25.74(0.2) |
| 1-adamantylammonium | H2O (pH <2.5) | -9.68(0.04) |
| 2-adamantylammonium | H2O (pH 2.5) | -22.1(0.3) |
| 1-adamantylmethyl-ammonium | H2O (pH 2.5) | -25.5(0.5) |
| 1-adamantyl-trimethylammonium | H2O (pH 8.6) | -20.5(0.1) |

Reference Paper:

Complexation Thermodynamics of Cyclodextrins
Mikhail V. Rekharsky and Yoshihisa Inoue
Chem. Rev. 1998, 98, 1875−1917