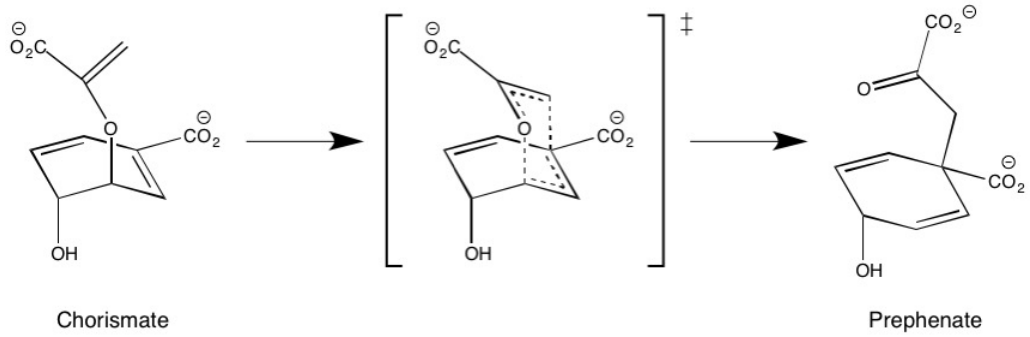
Empirical Valence bond solution reaction

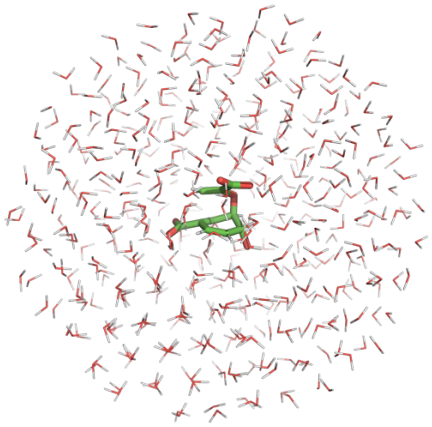
Introduction

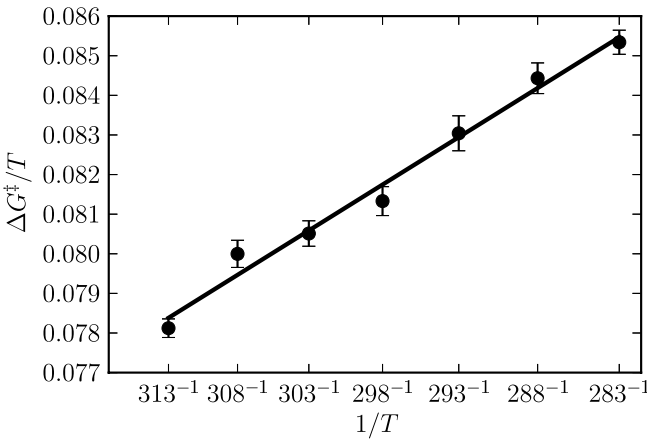
In this practical we are going to calibrate the EVB Hamiltonian against density functional theory (DFT) calculated the activation and reaction free energies ($\Delta G^{\ddagger}$ and ΔG^{0}, respectively) for the uncatalyzed transformation of chorismate to prephenate:



Figur 1: The unimolecular transformation of chorismate to prephenate.

We will then use this to calculate the activation free energies as a function of the temperature to obtain the thermodynamic activation parameters ($\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$) of the uncatalyzed reaction (Arrhenius plot) in water:





Recommended literature

- Åqvist & Warshel (1993) "Simulation of enzyme reactions using valence bond force fields and other hybrid quantum/classical approaches"[1]
- Marelius *et al.* (1999) "Q: A molecular dynamics program for free energy calculations and empirical valence bond simulations in biomolecular systems".[2]
- Isaksen *et al.* (2015) "Qgui: A high-throughput interface for automated setup and analysis of free energy calculations and empirical valence bond simulations in biological systems"[3]

Practical step by step

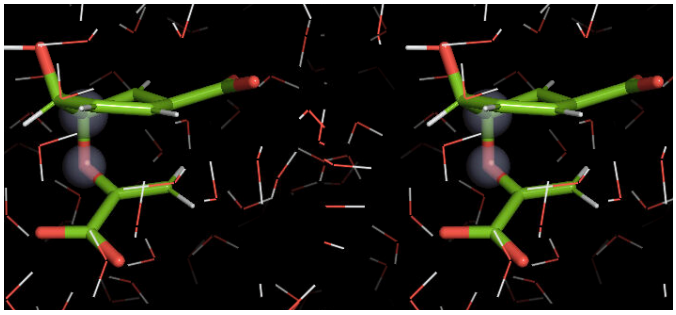
Preparing Qgui and FF parameters on locally

1. Go to /$HOME/biomolmod/practicals/EVB and open Qgui:
    a. Qgui -p

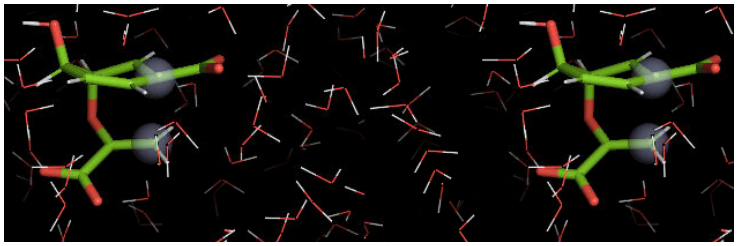
2. Open chorismate.pdb
3. First, go to File→ Settings and change the force field to Qopls_2015.prm and Qopls_2015.lib (They are located in your home in $HOME/QGUI/FF/OPLS). Also, set the *schrodinger* directory to $HOME/biomolmod/software/schrodinger. Press Save to close.
4. We need to generate force field parameters for chorismate. Do this by pressing Prepare→Parameters in Qgui.
    a. Press select, and select all atoms
    b. Call the new residue COA
    c. Press "Run"
        i. This will create the parameter file (.prm) and the library file (.lib), in addition to a new PDB file (COA.pdb) with updated atom names corresponding to the lib.
    d. Close the window and go to "File → Settings" and add the new **prm and lib** file. Press "Save" and close.
5. We now need to create a topology for the EVB simulation.
    a. Load COA.pdb (created from the previous step)
    b. Go to "Prepare → Topology"
    c. Set the simulation sphere size to **15 Å.**
    d. Press "Run" to genereate the topolology.
    e. Close Topology prepare.

Setting up the EVB simulation

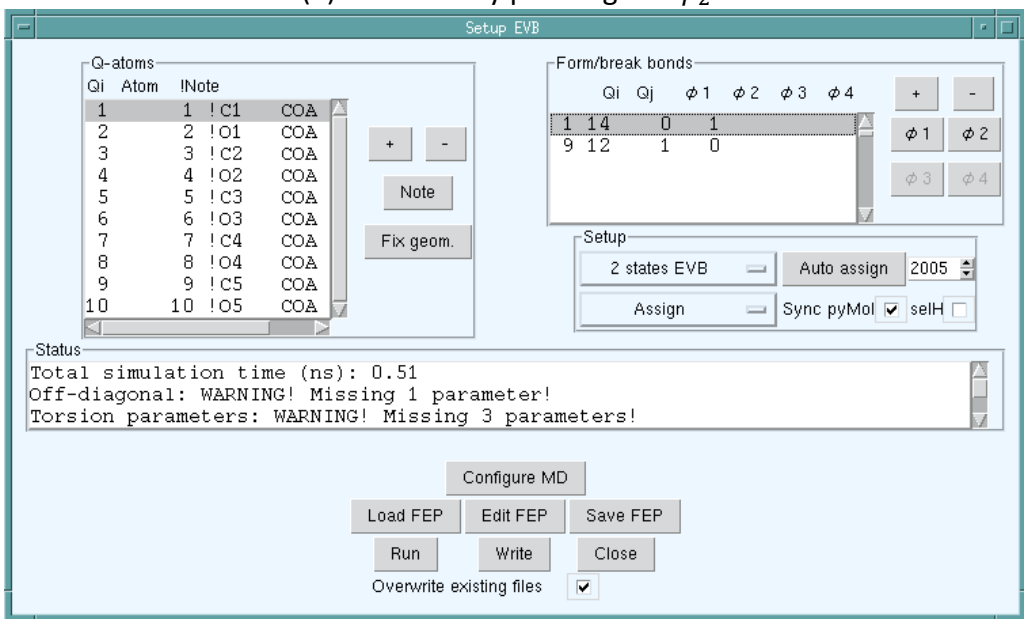
6. Set up EVB simulation:
    a. Load COAQ_top.pdb and COAQ.top in the Qgui main window
    b. Open "Setup → EVB"
    c. In the "Q-atoms" window, press "+" to add Q-atoms.
        i. Select all the atoms in COA (not waters).
    d. Tick the "Sync PyMol" to open the visual helper for setting up the reaction.
    e. Click O7 and C5 (ether bond to be broken) in the pymol window and press "+" in the "Form/break bonds" window (Qgui):



    f. Click C1 and C9 (the C-C bond to be formed) in the pymol window and press "+" in the "Form/break bonds" in Qgui:



g. In the "Form/break bonds" the ether bond is defined as bonded in both states 1 and 2 (1 and 1), whereas the carbon bond is defined as unbonded in both states. Change this so that the ether bond is off (unbonded / 0) in state 2 by first selecting the ether bond in the "Form/break bonds" window and pressing the ϕ_2 button. Now select the carbon bond in the "Form/break bonds" window and set this to bonded (1) in state 2 by pressing the ϕ_2 button.



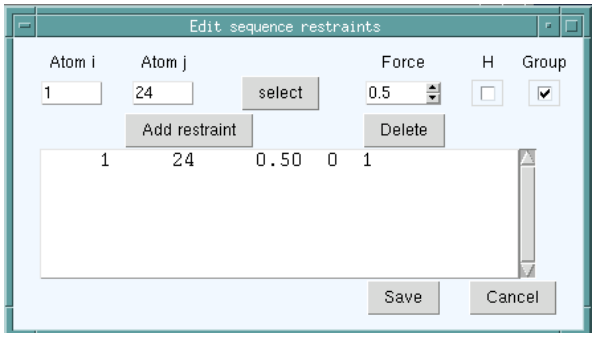
h. You can now automatically assign force field parameters to the two EVB states defined by pressing the "Auto assign" button. This will ask you to check and verify the generated template structure in the PyMol window. In some cases it needs some adjustments, but in this case it is fine, so go ahead and press "Auto assign" once more to complete the assignment of parameters.

i. You can now look through the dropdown list under "Setup" where it says "Assign" and checkout all parameters (atom types, charges, bonds etc). As you will see from the "Status", there is 1 parameter missing – the off-diagonal.

j. Define the off-diagonal – you find this from the drop down list. Select fist the off diagonal by clicking in the window and then select the ether bond in the "Form/break bonds" list. Press + in the off-diagonal to assign this atom pair.

k. We are going to apply a weak force to the chorismate as a group to make it stay in the simulations sphere. Open "Configure md" and press Setup Restraints "Sequence". Press "Select" and select the 24 atoms in COA. Set the force to 0.5

and untick “H”. Press “Add restraint” and “Save” to close the window. Press “Write” in the “Configure MD Settings for EVB” window to apply changes and close the window.



l. The setup is now completed. Press “**Write**” (not Run!) in the “Setup EVB” window and close it. Quit Qgui.
m. If you have not done so already, log in to saga and clone the github repo
    i. git clone https://github.com/isaksengeir/biomolmod
n. Copy the newly created inputfiles directory to Saga:
    i. scp -r inputfiles userXY@saga.sigma2.no:biomolmod/practicals/EVB

o. log on to Saga.
p. We are going to sample downhill instead of forward (better sampling). cd into the /inputfiles and type the following command:
    i. downhill_fep.py
    ii. cd out of inputfiles when done (cd ../)

q. Submit the job from command line. Make sure that you are in the correct directory (.. practicals/EVB). If you type “ls -ltr” you should see the /inputfiles directory. Do not cd into the inputfiles to submit the job! Submit The job (**10 replicas at 298 K**) will be automatically submitted with following command:
    i. mirror_evb.sh
        1. The jobs will be launched at “/cluster/work/users/$USER/nodelete/biomolmod/practicals/EVB/”, which is where you will collect the files for analyzing when they are done.

You can monitor your jobs with the command “squeue -u username”

Calibrating EVB Hamiltonian

1. Copy the resulting output files to your local machine to calibrate the EVB Hamiltonian.
    a. cp -r userXY@saga.sigma2.no:cluster/work/users/userXY/nodelete/biomolmod/practicals/EVB/298/ .
2. When download is completed - open Qgui with “Qgui -p”
3. Press “Analyze→EVB → Reference reaction”
4. Set the target activation and reaction free energies to the values you obtained with DFT ($\Delta G^{\ddagger}$ and ΔG^{0}).
5. Tick the “show target lines”

6. Now, you will calibrate (1 at the time) the 10 runs by pressing "Import from dir" and select first "298/1/'. Press "Sort" and then "Scan". When the calibration has converged, take a note of the α and H_{12}.
    a. Repeat this for all 10 runs ("Clear all" → "Import from dir" → "Sort" → "Scan")
7. Calculate the average α and H_{12}. These values will be used for all future calculations (in solution and enzyme) for this reaction.

Submitting Arrhenius simulations

We will now simulate the chorismate→prephenate reaction at 5 different temperatures (288, 293, 298, 303 and 308 K) to construct an Arrhenius plot. We will only do 5 replicas for each temperature.

1. On saga, cd to your /$HOME/biomolmod/practicals/EVB
2. Make a new directory for the Arrhenius calculation:
    a. mkdir arrhenius
3. copy the EVB inputfiles to the Arrhenius directory:
    a. cp -r inputfiles Arrhenius
4. cd to arrhenius and submit the Arrhenius calculation with the following command:
    a. mirror_arrhenius.py
        i. The jobs will be launched at "/cluster/work/users/$USER/nodelete/biomolmod/practicals/EVB/arrhenius", which is where you will analyze them when they are done.

Calculating thermodynamic activation parameters

1. cd to your work directory (cluster/work/users/$USER/nodelete/biomolmod/practicals/EVB/arrhenius") and start Qgui:
    a. module load Qgui/Q6-GCCcore-10.2.0
    b. Start Qgui with **qgui.py -p**
2. Open "Analyze→EVB→Thermodynamic parameters"
3. Enter the values for α and H_{12} that you calculated before when calibrating the EVB Hamiltonian.
4. Press "+" in the Titles, and give the calculation a name, for example "Chorismate".
5. Press "+" in T (for temperatures) to add the temperatures you have simulated over. You do not have to add on and one, just press ok when you are in window where you see the directories "288 293 298 303 308".
    a. Qgui will now automatically go through all EVB simulations at each temperature and calculate the reaction free energy profiles. This will take some minutes or more – be patient.
6. When the calculations are done, you can visualize the Arrhenius plot, check the regression paramters and the Model VS computed.

For the report?

- Calibrated EVB hamiltonian values
- What is the average activation free energies at the different temperatures?
- What values did you get for $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$?
- Suggestions to improve the results?

The experimental values for the uncatalyzed chorismate → prephenate reaction:

$\Delta G^{‡}$ = 24.5 kcal/mol

$\Delta H^{‡}$ = 20.7 kcal/mol

$T\Delta S^{‡}$ = -3.8 kcal/mol (at 298 K)

References

1 Åqvist, J. & Warshel, A. Simulation of enzyme reactions using valence bond force fields and other hybrid quantum/classical approaches. *Chemical Reviews* **93**, 2523-2544, doi:10.1021/cr00023a010 (1993).

2 Marelius, J., Kolmodin, K., Feierberg, I. & Aqvist, J. Q: a molecular dynamics program for free energy calculations and empirical valence bond simulations in biomolecular systems. *J Mol Graph Model* **16**, 213-225, 261, doi:10.1016/s1093-3263(98)80006-5 (1998).

3 Isaksen, G. V., Andberg, T. A., Aqvist, J. & Brandsdal, B. O. Qgui: A high-throughput interface for automated setup and analysis of free energy calculations and empirical valence bond simulations in biological systems. *J Mol Graph Model* **60**, 15-23, doi:10.1016/j.jmgm.2015.05.007 (2015).