

Introduction to Molecular Mechanics with MOE:

Why GFP needs a beta-barrel shell?

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This tutorial is designed to introduce molecular dynamics calculations using MOE. This tutorial covers basics of building molecules, visualizing atoms, measuring geometric characteristics, preparing and performing minimization in gas phase and with a solvent, in the protein environment, and performing dihedral energy calculations.

The system of interest is a model of the chromophore of the green fluorescent protein.

Green fluorescent protein (GFP), found in the jellyfish *Aequorea Victoria*, is a highly versatile photoactive protein widely used in molecular and cell biology and in medicine. The Nobel Prize in Chemistry 2008 was awarded to Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien "for the discovery and development of the green fluorescent protein, GFP". (http://nobelprize.org/nobel_prizes/chemistry/laureates/2008/)

Estimated time to complete this tutorial is 1.5 hr.

Outline: I) Prepare, solvate, and minimize the GFP chromophore; measure the bridge dihedral angles; II) Download crystal structure of GFP and minimize it; measure the bridge dihedral angles.

1. Software

VizLab: MOE, text editor, graphing software of your choice.

This tutorial is written to be completed with iMac computers of the VizLab. Small modifications might be needed when used on other computers.

2. Starting MOE

Open Terminal window from the Dock.

Make new directory for current tutorial:

```
>mkdir moe-tutorial-md-1
```

Go to the newly made directory:

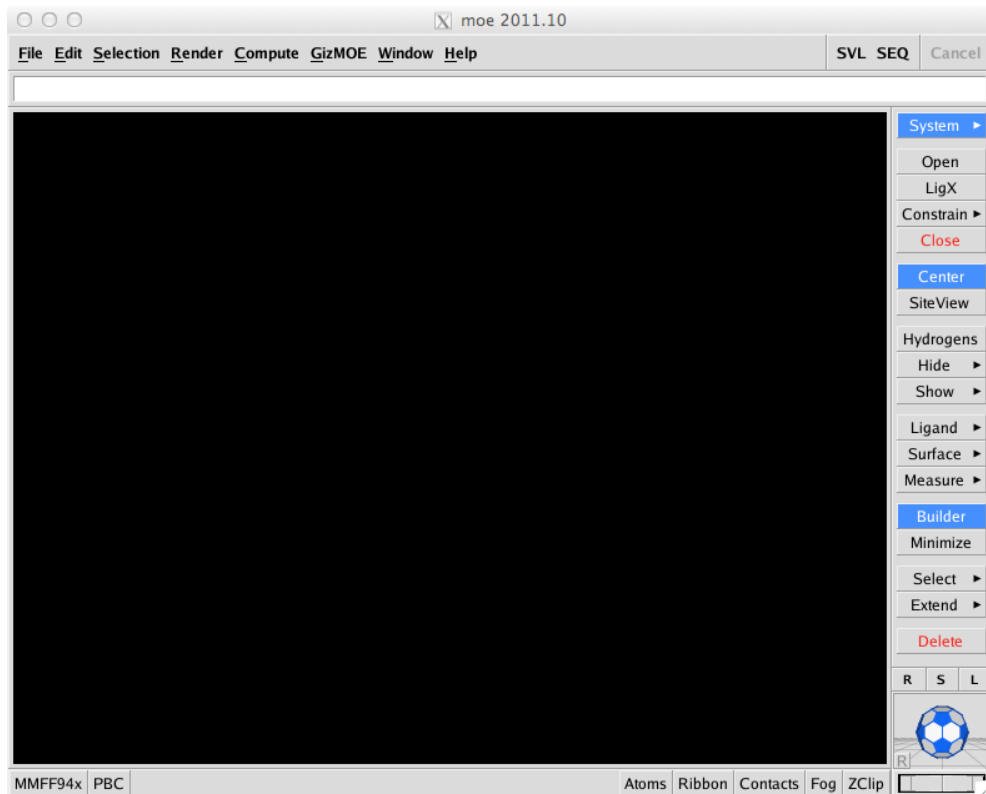
```
>cd moe-tutorial-md-1
```

Start MOE:

>moe

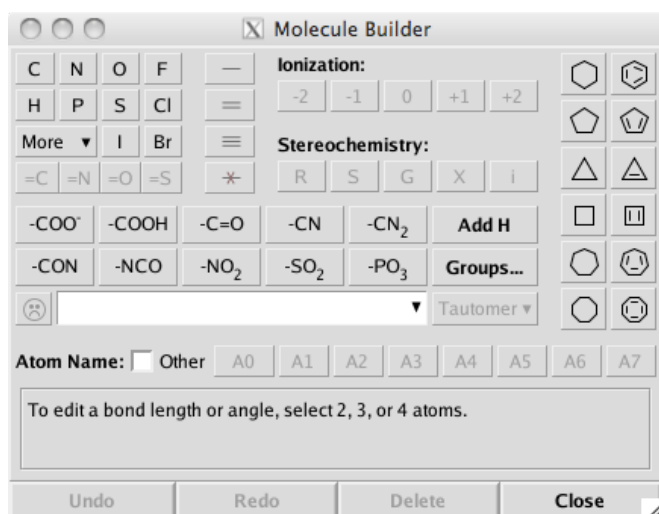
(This command is alias for /Applications/moe2011/bin/moe or a newer version)

The main window of MOE will appear (may look a bit different for new version):



3. Build GFP chromophore using MOE

Open Builder: click on Builder button in the middle of the panel on the right. Builder's window will open:



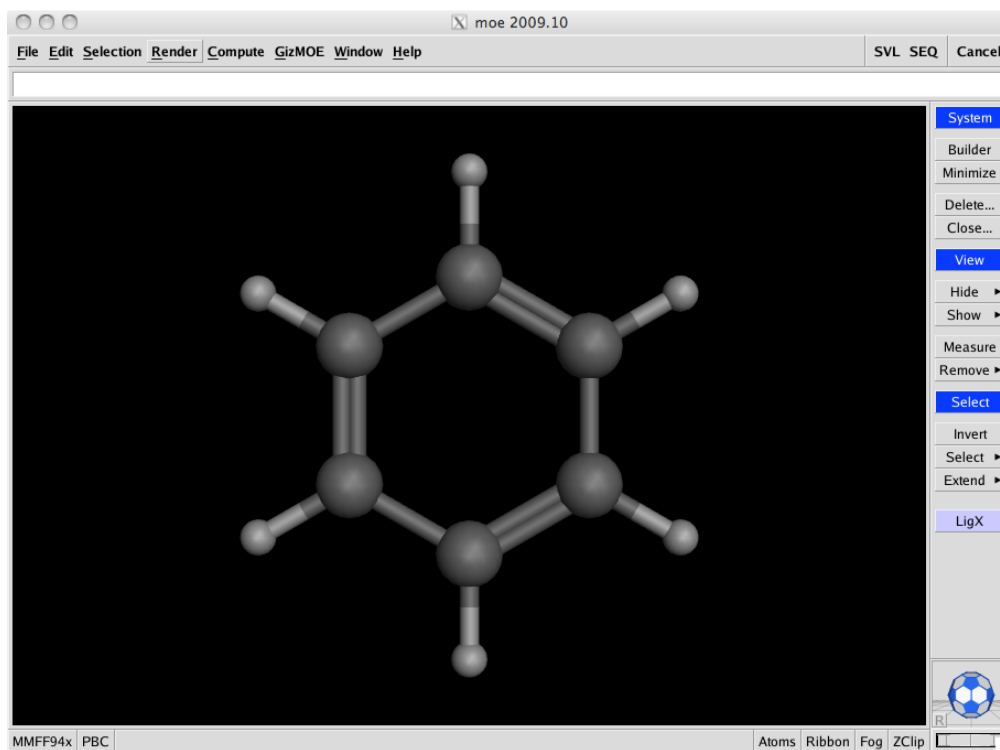
Build a model GFP chromophore - neutral *p*-hydroxybenzylidene-imidazolinone (HBI).

You have a couple of choices to prepare the chromophore: 1) *The fastest*. Copy from the authors directory two files (chromophore and the protein):

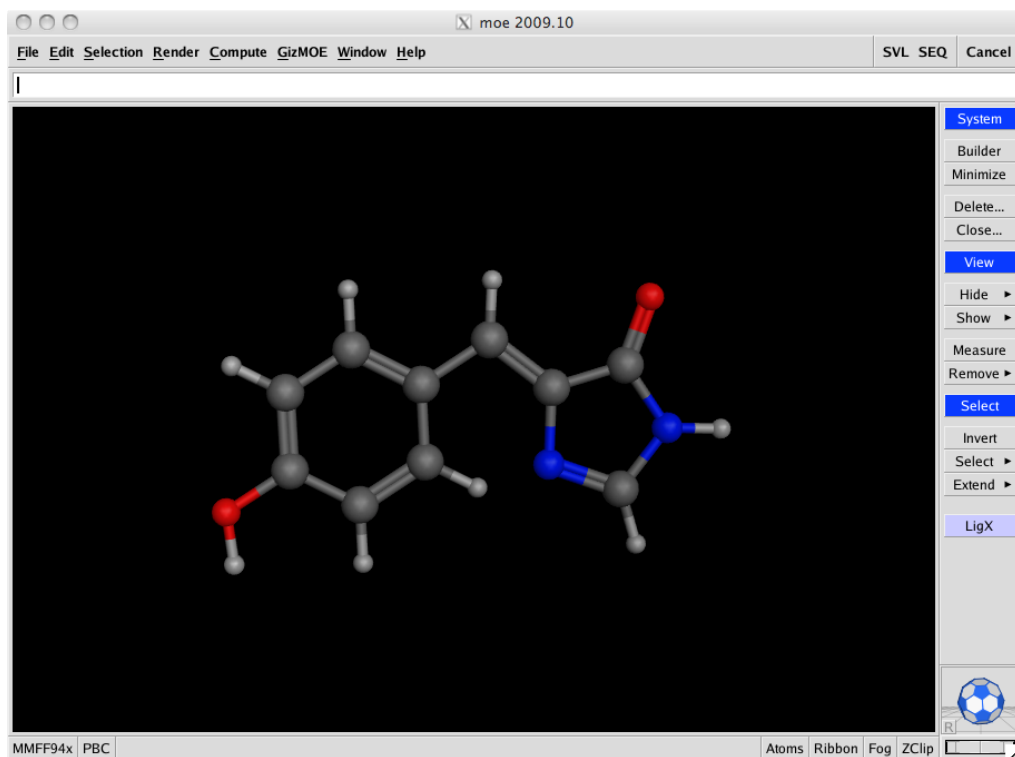
```
> cp /mnt/people/pogorelo/teaching/biophyscamp/moe_gfp/*pdb .
```

2) to download GFP structure from the PDB database (PDB ID: 1ema) into, say VMD and save the chromophore to be loaded to MOE. This step was done for you and you can use the pdb provided on the class website. Save the file gfp-cro-1ema-protonated.pdb to your tutorial directory. Also save the 1EMA.pdb file of the complete GFP.

3) Build yourself the chromophore structure using MOW Builder. Start by clicking the benzene ring button in the Builder. A ring, capped with hydrogen atoms will appear. Note: one atom is selected (colored pink). To choose representation you like: click anywhere on the black background (notice the pink sphere was removed) and draw a rectangle about all atoms (they turn pink) then go to the top Menu: Render-> Atoms-> select icon of choice. TIP: Rotate molecule using the soccer ball in the lower right corner. TIP: translate click on the little "R" button next to the soccer ball to turn it into a "T".



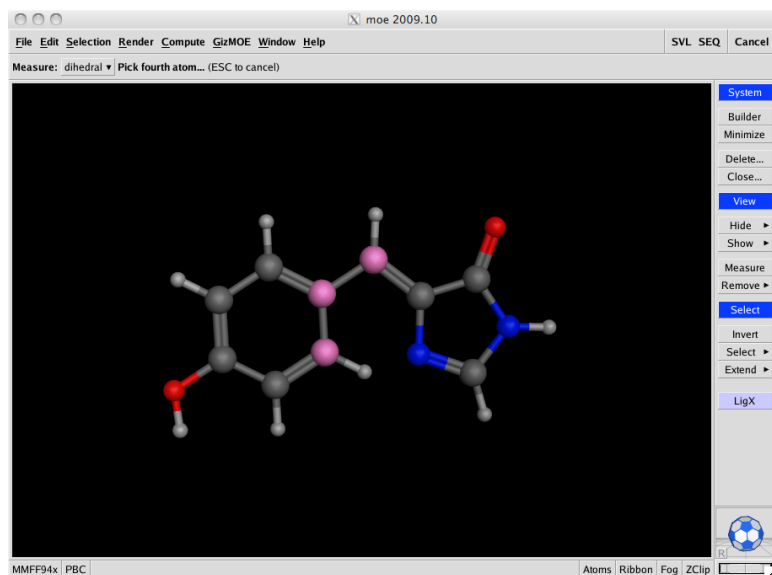
Now modify this starting structure: Click on hydrogen (it turns pink)-> Click on needed button on the Builder window. Proceed in the order of your choosing until you received the final HBI structure (note: one can use the single and double bonds buttons in the Builder):



Save the structure: File-> Save. Select .moe format: hbi.moe

Inspect the newly build structure: is it planar? Are hydrogen atoms in the plane?

Measure and record the two dihedral angles of the bridge (P- closer to the benzene ring and I – closer to the imidazole ring): Click Measure button in the middle of the right pane.



Note a new button appeared under File in the left top corner. Select Measure: dihedral and start selecting atoms. Note that there more than one way to select the same dihedral angle. As one need 4 atoms where the middle two atoms form the rotation axis, atoms 1 and 4 can be any other connected atoms. Here is how MOE main window look after selecting first three atoms (figure on the left).

Record the values. Now perform initial in vacuum minimization

by pressing the Minimize button. Measure the two dihedral angles and record the values. Did they change? Why?

We will plot dihedral energy as a function of angle for P- and I-angles.

Compute->Conformations->Dihedral Energy Plot.

Note: a number of minima exist.

Once the recorded values are displayed on the structure it can be removed: click Measure -> Remove All.

4. MOE minimization of HBI in gas phase

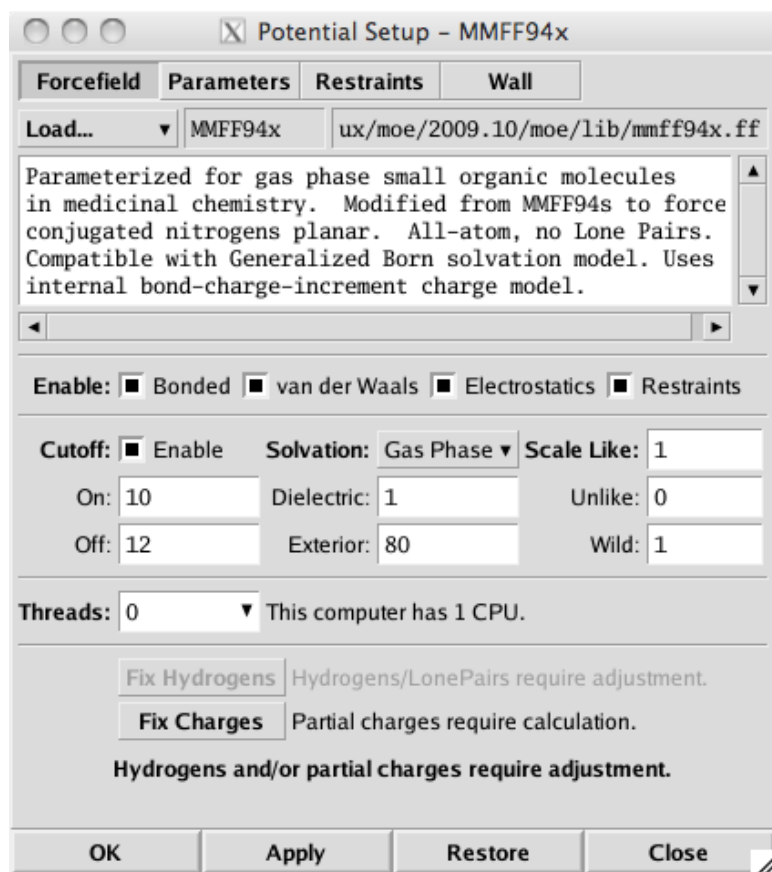
Note: the following list will be used almost every time when you need to set up a simulation

Step 1: Select the force field: Click the MMFF94x button in the lower left corner.

Select MMFF94x: ForceField->Load-> MMFF94x

Select gas phase simulation: Solvation->Gas Phase

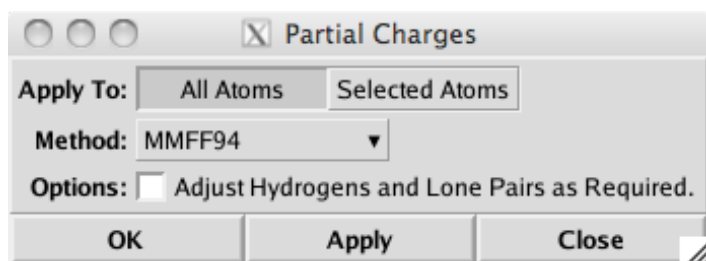
Increase cutoffs: from 8/10 Å to 10/12 Å. The resulting Potential Setup window:



Note: which force field components are enabled? What is the role of dielectric constant in the setup?

Note: Hydrogen atoms are missing and need to be added. Click the Fix Hydrogens button and check that the atoms were added by clicking Show-> All Atoms. Partial charges need to be calculated. Click Apply if the Fix Charges button highlighted.

Step 2: calculate charges. Compute-> Partial Charges. Select MMFF94 force field:



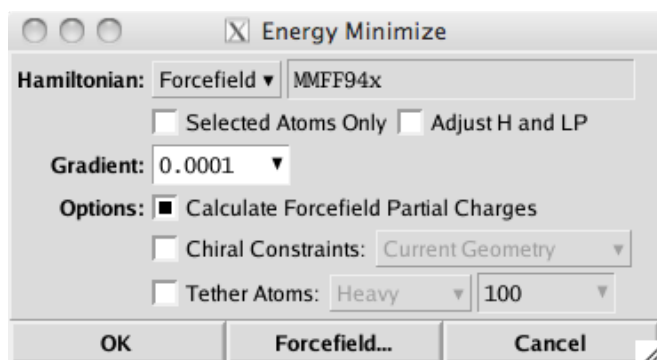
Click OK.

Display calculated charges: Render->Atoms->Charges. Inspect the calculated charges (symmetry, hydrogen atoms vs. heavy atoms).

Step 3: Minimization.

First observe current energy: GizMOE->Energy. Reflect on the value: is conformation stable? Why? Record the value.

Set up minimization: Compute-> Energy Minimize. Select MMFF94x force field. Change gradient to 0.0001 (What this value reflects?):



Click OK.

Record the total energy and contributing terms: Compute-> Potential Energy.

Save your results as hbi-min-gas.moe.

Reflect: is the new conformation more stable with respect to the initial one? How each term contributes to the stability?

Repeat minimization with 0.000001 gradient. Did the energy change?

Measure P- and I- dihedral angles now. How they changed with respect to the initial structure? Why?

Calculated all components that contribute to the potential energy: Compute->Potential Energy. Which two components contribute the largest positive values? The values will be shown briefly in the main window. Feel free to repeat the calculation if needed.

Review and summarize the steps we followed for minimization: we will need them often.

5. MOE minimization of HBI in water solution

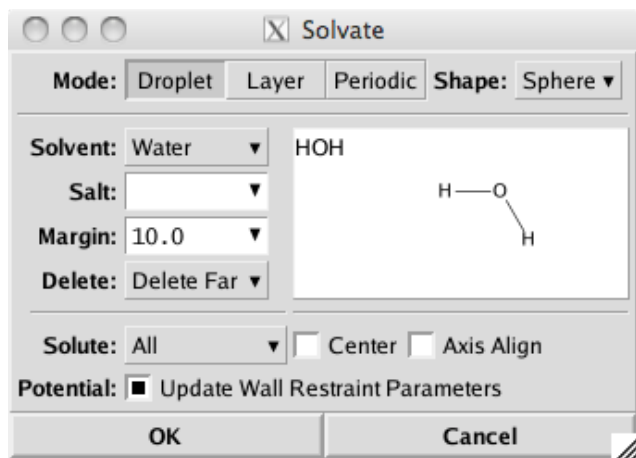
Now we will study how presence of explicit water molecules changes the minimized conformation.

Step 1: add solvent

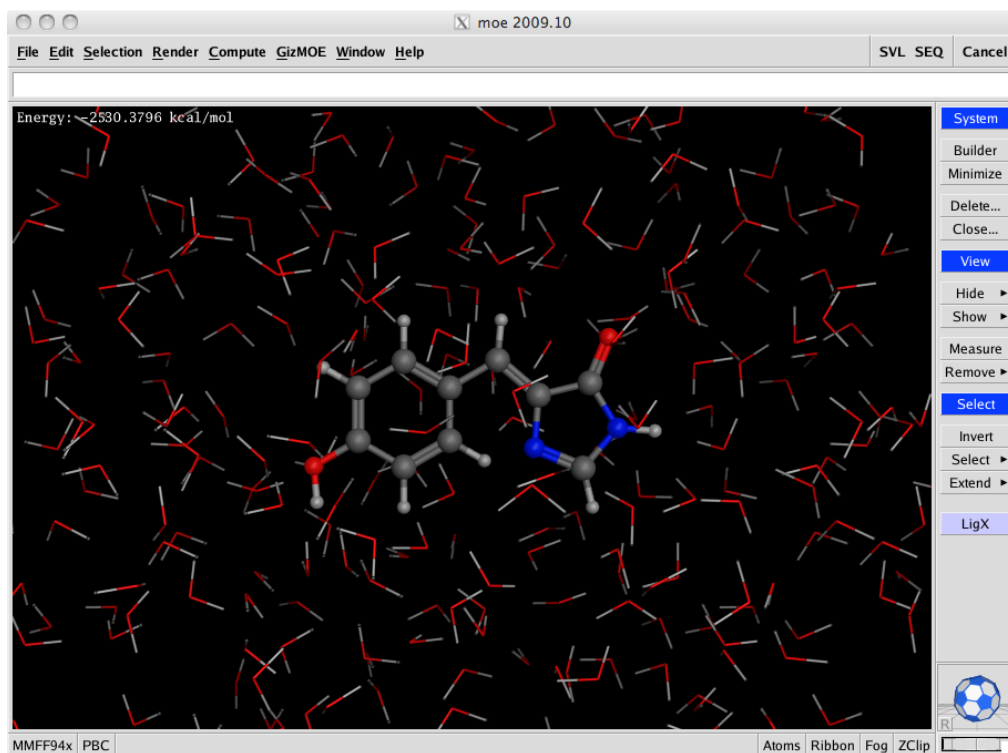
Close any molecules you might have open in the main window.

Load the initial HBI structure we made (prior to minimization in gas phase).

Add water: Edit->Build->Solvate. Mode: Droplet. Increase solvent margin to 10Å:



Click OK. Inspect the system. Hint: use the wheel of the mouse to zoom out.



Step 2: minimize the solvated system using the protocol: potential setup as before, select “Gas Phase” (since water is explicit), calculate partial charges, and minimize with set gradient to 0.01.

Hint: you can hide water molecules during minimization to simplify view:
Render->Hide->Solvent

Note: to view how dihedral angles change during minimization select the two angles (please make sure the Measure: dihedral button is visible below the File button on top of the main window).

Save as hbi-min-water.moe

Step 3: analysis

Record the total energy and contributing components

Measure P- and I- dihedral angles. Are the values different from the initial and from the values after minimization in gas phase? Did both angles changed? Why?

6. Dihedral angles in solution.

We will plot dihedral energy as a function of angle for P- and I-angles.

Compute->Conformations->Dihedral Energy Plot.

Hint: if an error is made during selection press Esc and start again.

HBI in water: plot P-/I- angles and observe changes in energy as the angles change.

Compare the number of minima to the Dihedral Energy Plot for the initial structure.

Reflect on role of water and stable conformation of the GFP chromophore.

What role, do you think, the GFP *protein backbone* plays? Hint: in order to be active GFP chromophore has to be planar.

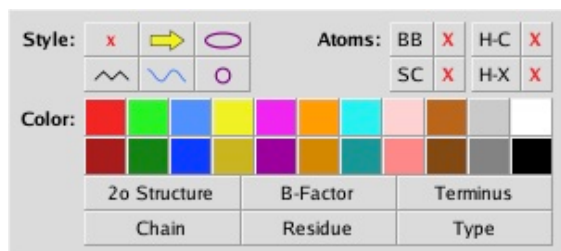
7. Minimization of HBI in the native environment for the GFP

Close all existing molecules in MOE. (File -> Close)

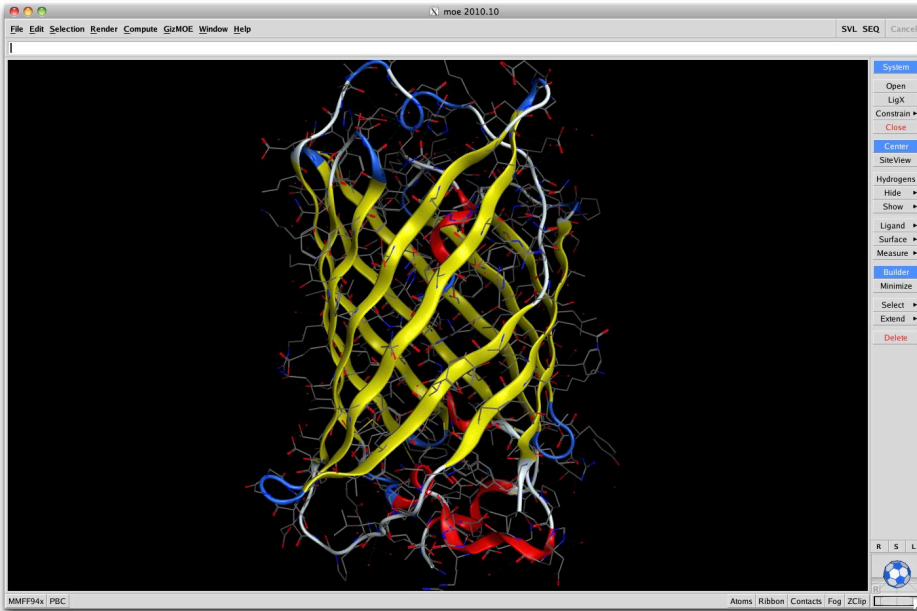
Load GFP coordinate file (1EMA.pdb) from the class website or (gfp.pdb) that you have saved in the tutorial directory.

Step 1: Explore the structure of the GFP.

Hit Ribbon button in the lower right corner and select the Oval button. Now the GFP secondary structure is shown in the ribbon representation. To simplify the view remove representation of backbone and side chains by selecting "X" in Ribbon -> Atoms for BB/SC/H-C/H-X.



Observe: central location of the chromophore, type of the secondary structure of the GFP backbone.



Questions:

Is the chromophore planar?

What do the small red spheres represent?

(Hint: this is a crystal structure of fairly high resolution)?

Why no hydrogens are present?

Step 2: Quick look on sequence.

Press the SEQ button in the upper right corner. This opens the sequence viewer. Locate the chromophore as residue 65 (CRO) and observe crystallographic water molecules.

Note: there is another way to display the chromophore: select CRO in the Sequence Editor and Selection->Atoms->Isolate Atoms of Selected Residue.

When done close the Sequence Editor.

Step 3: Minimization

Add hydrogen atoms: Edit-> Hydrogens->Add Hydrogens

Minimize the solvated system using the protocol: potential setup as before, select "Gas Phase", calculate partial charges, and minimize with set gradient to 0.05.

Note: after clicking OK, warning regarding unparametrized atom types will be displayed. Select NO for now. Investigate this further. In the Energy Minimize window click the Forcefield button. In the Potential Setup window select Parameters and click Display Missing Parameters Only.

Questions: which parameters are missing? Where these atoms are located? (Hint: use the Sequence Editor). Select MSE (residue 78) and the chromophore (residue 65). Is MSE close to the chromophore? Check also at www.pdb.org: find 1ema and see what is MSE.

Since the missing parameters correspond to few atoms that are far away from the chromophore we will proceed as is. Note: for a proper simulation one would need either to develop missing parameters (this is beyond the scope of this tutorial) or mutate the modified amino acid to its natural form.

Run the minimization. It will take few minutes.

Step 3: Analysis

Measure P- and I- dihedral angles. Are the values different from the initial and from the values after minimization in gas phase and in the water? Did both angles changed? Why?

Plot dihedral energy as a function of angle for P- and I-angles and compare the graphs received for minimizations in the gas phase and in water. Reflect on the differences and similarities.

7. Summary

This tutorial covered basics of building molecules, visualizing atoms, measuring geometric characteristics, preparing and performing minimization in gas phase and with solvent, in the protein environment, and performing dihedral energy calculations. We also able to demonstrate that the GFP chromophore remains planar in the protein environment while twists in water.

8. Contact

If you found errors/typos or have suggestions or comments on material in this tutorial please contact us.

9. Acknowledgment

We are very grateful to the Chemical Computing Group, especially Ms. Audrey Bonin and Ms. Petrina Kamyra, for generous support in providing us with MOE teaching licenses.