Brief Description of Selected Theoretical Techniques in the Molecular Modeling of Inhibitors and Their Receptor Enzymes

Molecular Mechanics and Force Fields

Molecular modeling of inhibitors and their host receptor enzymes has relied heavily upon molecular mechanics, which is a computational technique that utilizes empirical equations to calculate conformational energies. Molecular mechanics uses potential energy functions to describe atom-atom van der Waals and electrostatic interactions, bond stretching, bond bending, torsional rotations, and so forth. These potential energy functions, along with their appropriate parameters (determined from empirical data or from quantum-mechanical calculations), are called a force field.

Energy Minimization

In energy minimization, the computer uses the previously-mentioned force field to obtain a potential energy of a starting conformation, then varies its structural features (bond lengths, bond angles, dihedral rotations) in a systematic way to obtain a local potential energy minimum, that is, the minimum-energy conformation closest to the starting conformation, but not necessarily the global minimum (the conformation with overall lowest energy).

Determination of Relative Interaction Energies of Inhibitors to a Receptor Enzyme

The interaction energy $\Delta E_i$ between a host receptor enzyme and an inhibitor can be defined with the following relation: $\Delta E_i = E[\text{Enz}:\text{I}] - E[\text{I}] - E[\text{Enz}]$ where $E[\text{Enz}:\text{I}]$, $E[\text{I}]$, and $E[\text{Enz}]$ are the potential energies of the enzyme-inhibitor complex, inhibitor, and enzyme, respectively.

Based on the above definition for the interaction energy, a negative value of $\Delta E_i$ means that the interaction is energetically favorable, whereas a positive value means that the interaction is unfavorable.

The relative interaction energy $\Delta \Delta E_i$ can be used to compare the interaction energies of different inhibitors (reference and analogue) to the same receptor enzyme. For example, the $\Delta \Delta E_i$ calculated when we subtract the interaction energy of the reference inhibitor:enzyme complex from the interaction energy of the analogue:enzyme complex. In other words, $\Delta \Delta E_i = \Delta E_i,\text{analogue} - \Delta E_i,\text{reference}$, where a positive value means that the interaction of the analogue inhibitor to the enzyme is less energetically favorable than the
interaction of the reference inhibitor. As an added advantage, the calculation of $\Delta \Delta E_i$ may lead to the cancellation of errors due to the approximation involved in force field simulations$^2$.

**Determination of Relative Interaction Energies of various carbocyclic inhibitor analogues to Neuraminidase of Influenza Virus**

Influenza neuraminidase (NA) is a critical enzyme necessary for the cleavage of terminal sialic acid from glycoconjugates and subsequently releases newly produced virion particles. NA from all types of influenza possess similar active site amino acid residues necessary for interaction with the sialic acid. This conservative residue nature makes NA a desirable pharmaceutical target.

A crystal structure$^3$ has been submitted to the Protein Data Bank of the potent antiviral oseltamivir (Tamiflu) in complex with NA. This is the same structure (2QWK) that we looked at during the previous week. Gilead Sciences, the pharmaceutical company that discovered and developed Tamiflu in conjunction with Hoffman-LaRoche, has also published$^4$ structure activity relationships of various carbocyclic analogues that inhibit NA with a wide range of effectiveness. This is the same article that included figure 1 from last week’s experiment.

This experiment will give you the opportunity of confirming that the magnitude of calculated $\Delta E_i$ correlates well with published IC$_{50}$ values. In addition, this enzyme system provides an ideal setting for the design of new carbocyclic inhibitors containing derivatives that produce $\Delta \Delta E_i$ values indicative of enhanced binding properties. Obviously, this type of information would be of value to medicinal chemists that synthesize and screen new drug candidates.

**Lab Notebook Preparation**

As with the other experiments that will be performed throughout the semester, all procedures, observations, results, conclusions, references etc. are to be placed in your laboratory notebook.

**Protocol**

**Mimimization introduction**

1. We will begin by having you perform some simple minimizations. Open a ViewerPro window and build 6 separate water molecules that are fairly close to each other. We will now minimize this system of water molecules by setting up the following minimization – click on the Dreiding icon located near the top right corner of the listed icons of ViewerPro and enter the following minimization parameters: number of iterations – 1000, convergence criterion – 0.01, all options selected with checks, and 1 for
the value of every iteration shown. Now select OK to begin the minimization. Notice that the water molecules have been reoriented during the minimization to optimize the intermolecular (or hydrogen bonding) interactions.

2. It was previously mentioned above that the minimization will proceed to find the nearest local minimum but may not provide the lowest energy minimum\(^1\). We will demonstrate this by performing two different minimizations with a cyclohexane molecule. Open a new ViewerPro window and create cyclohexane by using the ring draw tool located in your tool bar. Add the hydrogens to your cyclohexane molecule by clicking on the add hydrogens (H) button. Notice that the cyclohexane ring is planar. Now perform an energy minimization using the same parameters used above in step 1. Now go to the Window/New Data Table command. Once you click on the molecule tab of the Data Table, you can locate the molecule’s Final Dreiding Energy value. Record this energy value in your lab notebook. Now change the planar cyclohexane conformation into the more familiar “chair” conformation by selecting and translating two of the cyclohexane carbon atoms. Your TA can assist you in helping to produce the chair conformation. Now perform a second minimization using the same parameters as before. Using the Window/New Data Table command again, determine the new Final Dreiding Energy value. Note that this minimized energy is lower than the planar structure energy.

Calculation of Enzyme/Inhibitor Interaction Energies

3. We will be using a smaller section of the 2QWK crystal structure for our subsequent \(\Delta E\) calculations. This is done to reduce the amount of time necessary for the energy minimization calculations. Other computational studies have also focused on part of the crystal structure (the active site) that will be freely minimized while the rest of the structure (amino acid residues 3 to 8 angstroms away from the active site) are held fixed during the minimization\(^4,5,6\).

4. Go to the /My Documents/3710 folder. Create and designate a new folder with your partnership initials to save any desired minimized structures.

5. Open the 2QWK 10000 min complex file from the 3710 directory. The resulting structure contains Tamiflu and any amino acid/water atoms within 15 angstroms. Highlight the active site by going to the Edit/Select option and selecting “Active Site 8 A” from the group option. The atoms that are highlighted in yellow represent a special subset of atoms that are within 8 angstroms from Tamiflu. This subset of atoms will be used for the calculation of the relative interaction energies.

Calculation of the Enzyme/Inhibitor Complex Energy

6. Set up the first energy minimization \((E[Enz:I])\) by copying the active site to a new file. You now have a much simpler system to work with that will require much shorter minimization times. In the process of copying/pasting
the active site and Tamiflu to the new file, the inhibitor and all active site atoms have been grouped together as one molecule. To separate Tamiflu from the rest of the atoms, simply select Tamiflu by double clicking on it and perform a cut/paste. In so doing, Tamiflu will be now identified as its own molecule.

We will now minimize the inhibitor within the active site. Double click on Tamiflu so that it is the only molecule highlighted. Now click on the Dreiding icon located near the top right corner of the listed icons of ViewerPro. Enter the following minimization parameters: number of iterations – 1000, convergence criterion – 0.01, all options selected with checks, and 1 for the value of every iteration shown. Now select OK to begin the minimization. Once the minimization has completed, make sure that no atoms are selected by removing the selection from Tamiflu.

Perform a second zero-step Dreiding minimization. This is done to obtain the enzyme/inhibitor complex energy.

Now go to the Window/New Data Table command. Once you click on the molecule tab of the Data Table, you can copy and paste the two molecules' Final Dreiding Energy values to an Excel spread sheet. $E_{[\text{Enz:I}]}$ is obtained by adding these two energy values.

Calculation of the Enzyme and Inhibitor Energies
7. Now you need to obtain the energy of the enzyme ($E_{[\text{Enz}]}$) and the energy of the inhibitor ($E_{[I]}$). This is done by performing another zero-step Dreiding minimization but this time unchecking the “Include Intermolecular Forces” option. Again go to the Window/New Data Table command to obtain the two molecule Final Dreiding energy values. The larger energy value represents $E_{[\text{Enz}]}$ and the smaller energy value represents $E_{[I]}$.

Calculation of the Interaction Energy
8. From your Excel window, you can now calculate $\Delta E_i$ using the relation:

$$\Delta E_i = E_{[\text{Enz:I}]} - E_{[I]} - E_{[\text{Enz}]}.$$ Is your calculated $\Delta E_i$ negative indicating a favorable enzyme/inhibitor interaction?

Structure-Based Drug Design (the “fun” part)
9. By using the same method described above, go back to your original 2QWK 10000 min file and modify the structure of Tamiflu to represent one of the analogues depicted on the white board. Once this modification is done, copy/paste this new active site selection to a new file and repeat steps 6 and 7 to calculate a new $\Delta E_i$.

Once you have calculated $\Delta E_i$ for the modified analogue, calculate the relative interaction energy by using the following relation:

$$\Delta \Delta E_i = \Delta E_{i, \text{your newly designed analogue}} - \Delta E_{i, \text{reference (original Tamiflu)}}.$$ Is your calculated $\Delta \Delta E_i$ value positive indicating that the interaction of the analogue inhibitor to the enzyme is less energetically favorable than the
interaction of the reference Tamiflu inhibitor? Does this agree with the experimentally reported IC$_{50}$ values?

Calculate $\Delta\Delta E_i$ values for two more reported analogues using the same methods as before. Do these calculated values agree or disagree with the trends established by the reported IC$_{50}$ values?

Now try making your own modification to the reference Tamiflu structure and determine if your calculated $\Delta\Delta E_i$ is negative indicating a more favorable interaction energy of your newly designed analogue within the NA active site. Your TA will be able to show you some interesting ViewerPro tools that you may find helpful in the design of your new analogue.

Report your findings and have fun experimenting.

References: