Chemistry 430 — Simulation in Chemistry & Biochemistry

Laboratory #10 — Docking of Fisetin to Protein Kinase B

In this lab exercise you will use the AutoDock Vina from within the Chimera program to dock the fisetin ligand to protein kinase B (PKB, also known as Akt). PKB is a Ser/Thr kinase that phosphorylates serine and threonine residues in a variety of proteins that regulate metabolism, apoptosis and proliferation. Fisetin is bioactive flavonol antioxidant found in fruits and vegetables such as strawberry, apple, onion and cucumber.

The protocol described here is a simplified version of the tutorial article published in *JMIR Bioinformatics and Biotechnology*, ***1***, e14232 (2020), which is provided on the web site for this exercise.

Protocol

(1) Open Force Field Explorer (Tinker-FFE) and use the **`Download from PubChem…`** option to get the fisetin molecule. Once the molecule appears, you can close FFE.

(2) The Tinker xyz file for fisetin is saved under the Tinker-FFE installation, so we need to move it to the directory where you want to perform the lab. In a terminal window, change to your lab directory, and issue the following command:

```
mv ~/Tinker-FFE/NIHdownloads/fisetin.xyz .
```

(3) Convert the Tinker xyz file to the MOL2 format that is readable by the Chimera program. This can be done using command **`xyzmol2 fisetin.xyz`**. Look at the MOL2 file that is produced, and see if you can understand what it contains.

(4) Open the **`fisetin.mol2`** file in Chimera. We need to modify the MOL2 file to assign partial charges to each of the atoms. Invoke the following option via the Chimera menu: **`Tools -> Surface/Binding Analysis -> Dock Prep`**. In the popup box, select all options, and then click on **`OK`**. In the following **`Add Hydrogens`** popup box, keep the default options, and then click on **`OK`**. In the next **`Assign Charges`** popup box, choose Gasteiger charges, and then click on **`OK`**. In the **`Specify Net Charges`** popup, click on the **`OK`** button. Finally in the **`Save as Mol2 File`** popup box, save the modified MOL2 file under a new name, for example by entering **`fisetin-prep.mol2`** as the **`File Name`**, then clicking **`Save`**.

(5) Next, prepare the Protein Kinase B receptor protein. Using Chimera, get the PDB file of the protein using the **`File -> Fetch by ID…`** menu option. The PDB ID of the structure we will use is 3QKK.

(6) Remove the bound ligand and water molecules from the 3QKK structure using the menu option **`Select -> Residue -> all non standard`**, followed by the option **`Actions -> Atoms/Bonds -> Delete`**.

(7) Repeat the same docking preparation procedure for the receptor that was used for the ligand in step (4) above. In the end you should save a file called **`3QKK-prep.mol2`**.

(8) Close and reopen the Chimera program. Then open in the same session the two dock prepped MOL2 files prepared above, one for the receptor protein and one for the ligand. These should both appear in the viewing window, but the ligand will not be at random coordinates and not docked with the receptor.

(9) Initiate the AutoDock Vina protocol via **`Tools -> Surface/Binding Analysis -> AutoDock Vina`**. In the popup window that appears, enter in Angstroms under **`Receptor search volume options`** the center and size of the region that contains the binding site:

Center:	28.60	0.65	10.94
Size:	10.20	17.20	8.80

Under **`Receptor options`** and **`Ligand options`**, make sure everything is set to **`True`**. And under **`Executable location`** enter **`/Applications/vina/bin/vina`**, which is the location of the AutoDock Vina binary application on the lab computers. At the top of the popup windows, next to **`Output file`**, click on **`Browse`**, find your directory for this lab, and in the Save File box enter a name to use for the output files, for example **`results`**, then click on **`Set Output Location`**. Finally, back in the AutoDock Vina window, set the **`Receptor`** to be **`3QKK`** and the **`Ligand`** to be **`fisetin`**, then click on **`OK`** at the bottom of the window to start the docking calculation. The calculation requires several seconds to a minute to complete.

(10) Once AutoDock Vina has finished, a results window will appear with energies and other information on several docked poses. Following the JMIR article found on the lab web site, you can step through the poses, look for hydrogen bonds, *etc*. The best pose found, based on AutoDock's scoring function, is the one with the highest value.

Questions

(1) Save screen snapshots showing the best and second-best poses found for fisetin, and include them in your lab report. How do the poses compare? Is the ligand in the same location? As you inspect all of the docked poses, does the conformation of fisetin change? According to the AutoDock Vina output, how many ligand torsions are "active"? Does this number make sense, and can you identify the torsions?

(2) Have Chimera show the side chains of the receptor. Which side chains interact directly with the ligand in the best pose? Do any of the receptor side chains within the active site change their conformation in the different pose structures?

(3) Go back to the original 3QKK structure as downloaded from the PDB. That structure contains a ligand referred to as SMH, which is bound to the same general site within Protein Kinase B. Is the structure of SMH similar to fisetin? Does the PDB crystal structure show SMH bound in a location similar to any of the AutoDock poses for fisetin?

(4) Look online or into the published literature, and briefly describe the components that go into the scoring function used by AutoDock Vina. The AutoDock Vina package is provided in the Software Resources section of the course web site, and the manual is found in under this lab's web site. The developers of the software maintain a web site at the Scripps Research Institute in San Diego, and the source code is available from a GitHub repository.