Project SEED 2007

Research Report:
Phospholipid Docking

Written By
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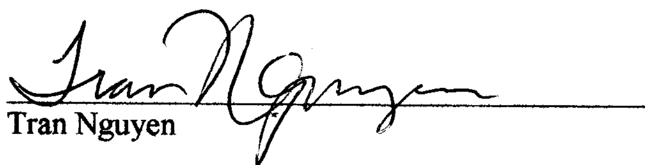
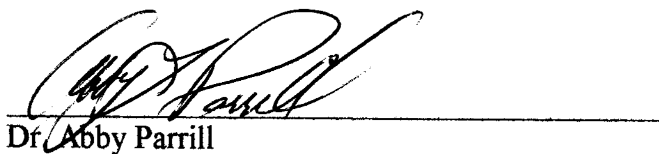


Tran Nguyen

07/27/07
Date



Dr. Abby Parrill

07/27/07
Date

INTRODUCTION

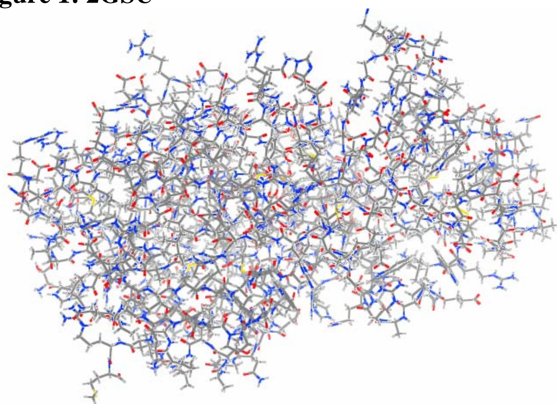
Autotaxin (ATX), or nucleotide pyrophosphatase/phosphodiesterase 2 (NPP2) is different from the rest of the NPP members because it functions as a lysphospholipase D (LPLD) enzyme.[1] In a chain of events, ATX converts lysophosphatidylcholine to lysophosphatidic acid (LPA), a lipid mediator, and LPA is the key to ATX becoming the "tumor cell autocrine motility factor."[1] LPA can promote wound healing,[1] but it can also harm us by promoting the survival and spread of cancer. One of the functions of LPA is cell proliferation.[1] Cell proliferation is an increase in the number of cells. Uncontrolled proliferation is one characteristic of cancer and tumors. Tumors are separated into two categories: benign or malignant. Malignant tumors can spread throughout our body, a process called metastasis, and destroy our tissues. Benign tumors, on the other hand, are no harm to us because it does not spread around our body. In addition to cell proliferation, LPA plays a role in movement of cells and metastasis.[1] Our group at the University of Memphis is studying the structure of autotaxin and its interaction with known substrates to guide the development of autotaxin inhibitors as potential cancer chemotherapeutics. My project focused on identifying the molecular interactions between ATX and known substrates to identify the structural characteristics necessary for ATX inhibition.

METHODS/DETAILS

My project mostly utilizes docking. Docking is finding the right ligand shape and position that fits into the protein structure. In other words, docking is simply like a jigsaw puzzle. You have to find the right place in the picture for each piece.

The 2GSU crystal structure (Figure 1) serves as a template for our model of ATX due to its similar NPP function and high sequence identity. The 2GSU crystal structure includes adenosine 5-phosphate (AMP), and therefore plays a vital role in finding the optimal placement and scoring method because it acts as the control. In order to find the best placement and scoring method, AMP was removed and docked back into the 2GSU crystal structure using all 9 combinations of the placement and scoring methods. It wasn't necessary that the docked AMP have to be on top of the original AMP from the crystal structure, but that the shape and position have to be close to each other and not far apart. In addition, the docked AMP must have at least 3-4 hydrogen bonds like the original structure. (Refer to the Results section)

Figure 1: 2GSU



After finding the best scoring method, I started building molecules in several structural classes such as the lysophosphotidyl choline (LPC), lysophosphatidic acid (LPA), alkyl phosphocholine (a.phos), alkyl phosphonocholine (APPNC), and lysoPAF using the program MOE. These structures were then docked into the homology model of the ATX catalytic domain (model generated by Abby Parrill, Refer to Figure 2). After the docking results were finished, it was time to observe the ligand interactions. The ligand interactions show where the ligand exposures occur, the residues around the structure,

and the interactions. A ligand exposure means that the ligand is not tightly bound against the enzyme at that location (shown in figure 3). Instead, it will be exposed to the surrounding solution (water in the case of a soluble enzyme such as the autotaxin). This is most unfavorable for non-polar segments of a ligand such as the hydrocarbon segments, but it doesn't have any affect on polar segments such as electronegative like oxygen, nitrogen, and bromide.

Figure 2: ATX catalytic domain model based on 2GSU crystal structure.

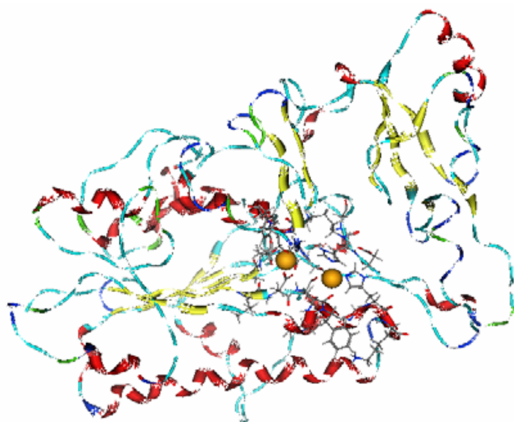
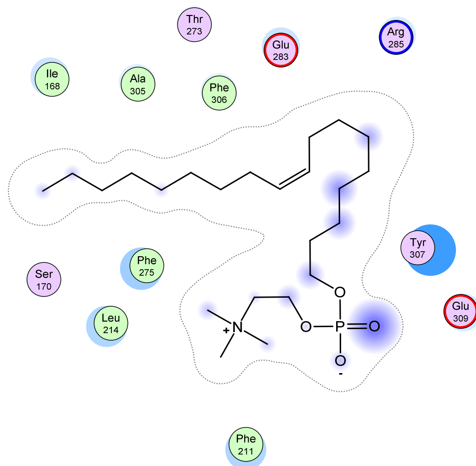


Figure 3: To the left is an example of a ligand interaction picture. Blue spots represent exposed atoms. The surrounding circles are the residues, which are amino acids.



RESULTS

When the docking results for AMP into 2GSU were finished, it was time to compare which scoring and placement method gave results most like the experimental AMP position. In the crystal structure, there were 3 main interactions between the histidine residues and the phosphate head group of AMP. In addition, the zinc atoms were really close to the phosphate, and other key interactions of AMP were tyrosine, phenylalanine, and lysine. The best result was obtained with the combination of alpha-pmi placement and affinity dG scoring out of the nine scoring and placement combinations. Alpha-pmi/affinity dG gave 4 out of 5 structures that displayed the positions most similar to those seen in the crystal structure. As you can see in figure 5a, the phosphate group from the docked AMP has a close distance to the original AMP phosphate, and the furan, which is the pentagon figure, as well as the other hexagon and pentagon at the end are almost on top of each other; however, it did not have enough hydrogen bonds, so I changed the energetic contribution of hydrogen bonds and the samples per conformation from 10 and –0.66 to 100 and –0.05, respectively. (Refer to Figure 5)

Figure 4. To the right is Jeff North's (graduate student in Parrill group) experimental data that shows that inhibition increases with chain length and unsaturation.

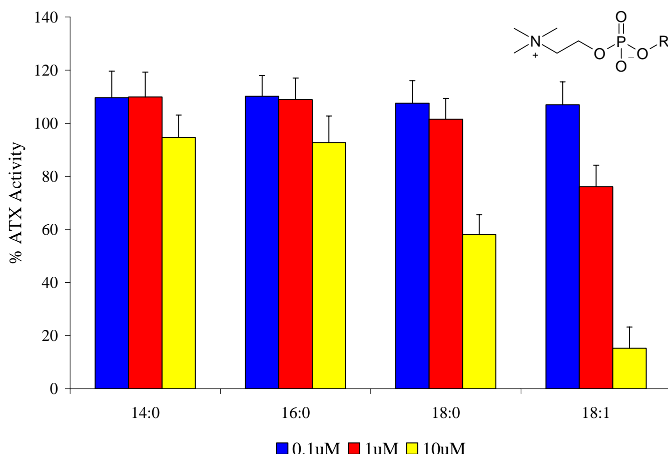
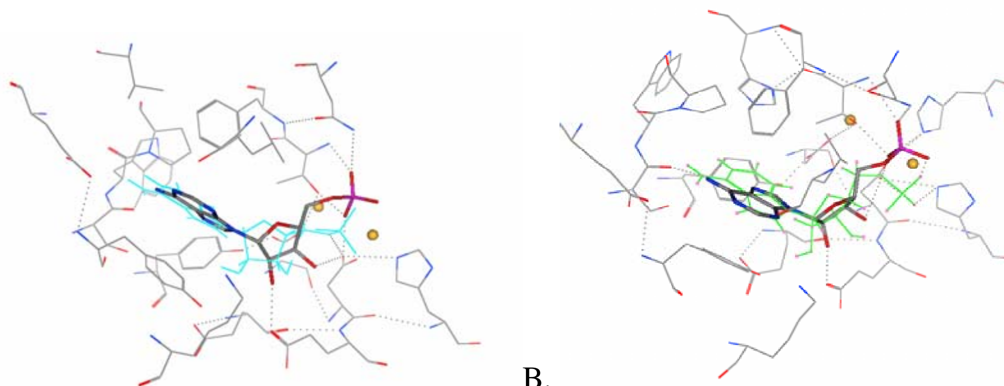


Figure 5 (A): This AMP has samples per conformations of 10 and a hydrogen bond of -0.66. The blue AMP structure was the one that I docked, and the other one is the original crystal structure. The surrounding structures are residues. As you can see, there are not that many hydrogen bonds compared to the AMP in figure5 (B).



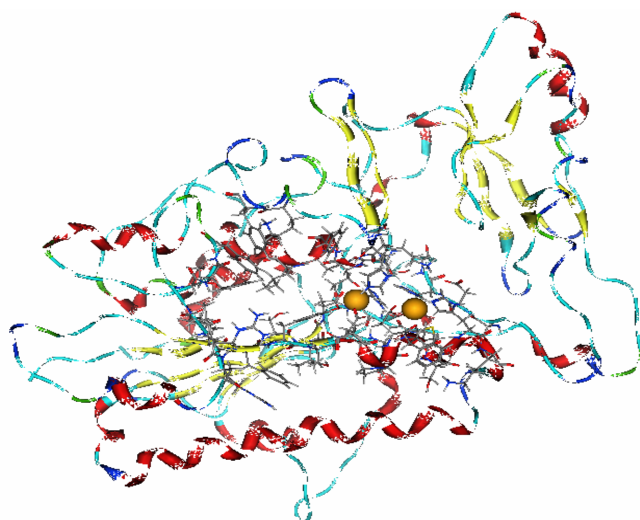
A. B.

Figure 5 (B): This AMP has samples per conformations of 100 and a hydrogen bond of -0.05. The green structure in this picture is the docked AMP while the other structure with the pink phosphate is the original structure from the crystal. As you can see, there are a lot more hydrogen bonds if you compare this to figure 5 (A).

Once the docking results were done, I observed all of the molecules and compared my structures with Jeff Norths's experimental data. It turned out that in Jeff's experiment, the 18:1 alkyl phosphocholine inhibits the best, but compared to my result, the 18:1 alkyl phosphocholine does not seems that active because there weren't that many interactions; moreover, there were about 7 ligand exposures at the hydrocarbon areas. As you can see (Shown in figure 4), the far right is 18:1. Experimentally, alkyl phosphocholine 18:1 inhibits ATX activity. We looked back at all of my structures, and we found out that the structures of all molecules were folded on top of each other. This suggests that performing a conformational search as part of the docking simulation does not produce realistic ligand geometries. To solve this problem, conformational searches were performed for all of my structures before docking. A conformational search is basically rotating single bonds in the molecules to different angles. After running the conformational search, the structures were docked again using a larger target site. (Shown in Figure 6) After the results were done, I measured distances from zinc atoms and the

threonine 210 oxygen to the phosphate. The best distance should be about 3.5 to 5.7 angstroms because if two atoms are too close the interaction will be repulsive, whereas if it is too far than the interaction would be weak.

Figure 6: This is the new site that I docked the molecule in. The pocket is a lot bigger than shown in Figure 2.



CONCLUSION

Using docking I was able to studied the interactions of ATX and known substrates. This can be used in the near future to find inhibitors of ATX. A key issue in docking the structures was to find the scoring method and placement that best reproduced that of the crystal structure.

ACKNOWLEDGEMENTS

I'm grateful that Dr. Burkey and ACS have given me the opportunity to have this wonderful experience. This experience has given me the opportunity to learn and experience what college class is really like. I had the chance to use software such as MOE before my classmates. In addition, I had the chance of meeting a lot of great people such as Dr. Parrill, James, Jeff, and everyone else in the lab. They all have helped me so

much this summer. They tried their best to answer all the questions I have to where I can understand. Also, I learned that a little thing could make a whole lot of difference. If you have forgotten to push one little button, it can mess up your whole docking result. From this experience, I have learned to be more careful and organized. One last thing I learned is that you will need more than just the time to do research; you will need determination, effort, and patience. Sometimes you will have to fall down several times before you get things right. If you are unfortunate, sometime you will have to fall several times; yet you probably won't have the result that you wished; but either way, you still have to work hard, and maybe one day you will find a research that will make an impact on others lives.

Reference

(1) van Meeteren, L. A.; Moolenaar, W. H. Regulation and biological activities of the autotaxin-LPA axis. *Prog Lipid Res* **2007**, *46*, 145-160.

FIGURE APPENDIX

Figure 7: This figure is an example of MOE. You use the molecule builder to build the molecules.

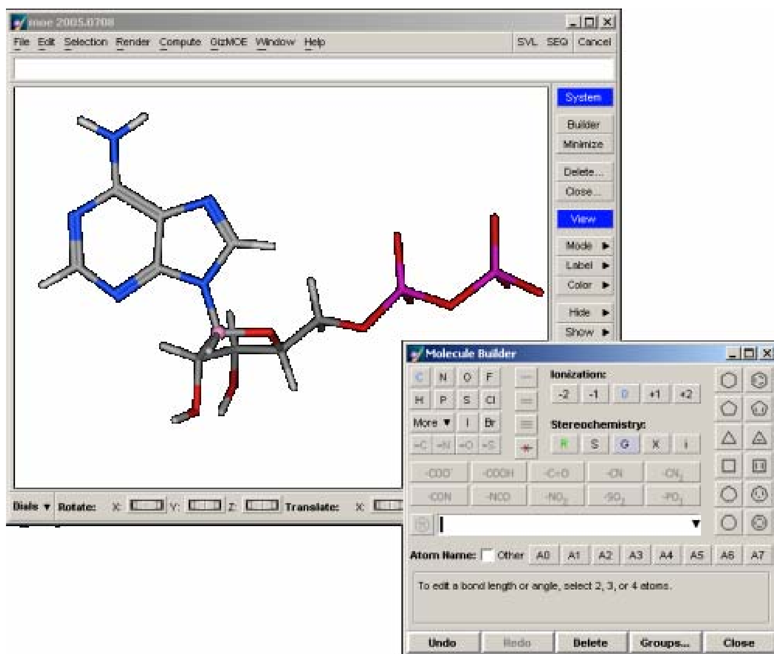


Figure 8: This is an example of a dock box. There are 3 placements options and 3 scoring methods. The second time when I run the conformation search I have to turn off the conformation search.