

This purpose of this exercise is to correlate the data you obtained from the ligand binding experiment with the three dimensional structure of avidin determined by X-ray crystallography. You can either use JMOL or VAST and CN3D (the graphics program from the NCBI website) to do this exercise. The latter program will allow you to directly superimpose structures which might make it easier to answer some of these questions. We will also examine the NCBI website, it contains a wealth of information.

A note about molecular graphics programs: most researchers have learned that there is no single program that will do everything you need to do when it comes to looking at, studying, and trying to relate experimental data with X-ray crystal structures of proteins. Thus, it is often necessary to work with at least two or three different programs and know how to use them well. This is only accomplished by practice and requires patience. RasMol was one of the first robust molecular graphics programs for PCs, however JMOL came along and made a lot of the functions of RasMol point and clickable. However, you will be severely limited if all you do is point and click in JMOL. Learning how to use RasMol command lines will greatly enhance the ability to adjust the display to your needs. We will cover two very useful websites that will enable you to do this. The first is a RasMol tutorial site describing how to use the SELECT and RESTRICT commands and line syntax:

<http://www.umass.edu/microbio/rasmol/rastut.htm>

and

<http://www.umass.edu/microbio/rasmol/rastut.htm>

The second is located on the JMOL website (<http://jmol.sourceforge.net/>)
<http://chemapps.stolaf.edu/jmol/docs/>

this page shows you almost all of the important RasMol or JMOL commands written out and in operation, a very useful resource.

For this exercise, go to the Protein Data Base (RCSB) and exam the following structures of egg white avidin, or the bacterial homolog streptavidin:

1ave : apo-avidin

1sre: streptavidin with bound HABA

1avd: avidin with bound biotin

1rst: streptavidin with bound strept-tag

Please examine the structures and determine:

1. What is the overall folding motif of avidin? Considering the chemical nature of HABA and biotin, what do you think the nature of the residues lining this structure is with respect to hydrophobicity? Verify you answer by displaying the hydrophobic vs. hydrophilic side chains in the interior of this structure.

2. When a ligand binds to avidin, a loop at the top of the binding site undergoes a significant conformational change. Locate this loop and give the residue numbers for the amino acids comprising the loop. You are advised to look at apo-avidin side by side with any avidin structure with the ligand bound. Taking into account the nature of the amino acids on the interior of the ligand binding site (#1 above), what do you think the purpose of this loop is in the apo-avidin structure. You might want to switch to a space-filling representation to verify your answer. Hint: why do you think the “open” conformation (ie. The structure in the presence of the ligand) might be thermodynamically unfavorable for the apo protein that has no ligand bound to it?
3. The order of K_d values for the three ligands show the following relationship: $K_d(\text{biotin}) \ll K_d(\text{HABA}) < K_d(\text{strept-tag})$

The very large difference in K_d for biotin and HABA is very hard to decipher unless one does a very detailed analysis of ALL the interactions between the protein and ligand. However, comparing the HABA bound structure that of the strept-tag avidin, explain why the strept-tag is less tightly bound.

Now compare the biotin and HABA bound structures. Looking at the structure of the ligand (CN3D is good for this since they give you ligand structures), consider the types of interactions that would exist between them and the protein. All of the types of bonding you have learned about in BIOC 462A except electrostatic are involved. Carefully look at the two structures and attempt to reconcile the large difference in K_d for biotin (using the data obtained for des-thio-biotin) and HABA. For those who are particularly adventuresome, try to display the relative B-factors of the amino acids lining the beta-barrel of avidin with the two ligands bound. What does this tell you?