

Molecular Graphics for Ligand Binding Experiment

The purpose of this exercise is to utilize the power of molecular graphics to help understand the nature of the binding of ligands to avidin and to correlate those structures to the values for the K_d 's that you will obtain for HABA and desthiobiotin binding to egg white avidin. The relevant PDB files that you can download from the RCSB web site are:

1ave: apo-avidin

1avd: avidin with biotin (a model for the desthiobiotin) bound

1rst: streptavidin with the strept-tag bound

1sre: streptavidin with HABA bound

Unfortunately, there is no structure determined for avidin with desthiobiotin (DTB). A brief explanation of avidin and these ligands will hopefully shed some light on their usage. In vivo, the function of avidin is to bind and transport biotin. HABA is a commonly used ligand for avidin because the spectrum of HABA changes dramatically when it is bound to the protein, giving one a convenient spectrophotometric handle to study ligand binding as you will see next week. The strept-tag is a small peptide that is incorporated into an expression vector, resulting in a tag at the end of the protein you wish to purify. Typically a column using strept-avidin (avidin expressed by the bacterium *Streptococcus*) that is covalently linked to the resin material. There is great commercial interest in optimization of the binding of the strept-tag to strept-avidin. In fact both species have been engineered to minimize K_d . Desthiobiotin is a biotin analog in which the sulfur from the 5 membered ring of biotin has been removed, leaving a methyl group and the lysine side chain. The K_d for DTB is a couple orders magnitude higher than that for biotin. Experimentally DTB is usually used in the following manner. When purifying a protein containing the strept tag, after the cell lysate is passed through the column and the junk proteins rinsed off, DTB is used to elute the tagged protein. In order to regenerate the column, DTB must be removed and this accomplished using HABA. Finally, HABA has to be removed by flushing a large volume of buffer through the column, the HABA being released by mass action dilution. Thus, over time, there has been a great deal of interest in aviding and its ligands.

The following exercise will be performed in two parts. In Part A you will use Jmol to examine general features of the avidin structure with and without ligands bound to the protein. This portion of the assignment is to be completed before coming to class on Tuesday. In Part B you will be introduced to a new molecular graphics program (CN3D) that has a very handy superimposing function built into the software. The answers to both Parts need to be incorporated into the lab report for the ligand binding experiment (see below).

Part A: Jmol examination of avidin ± ligands.

1. What is the overall folding motif of avidin? Considering the chemical structures of HABA and biotin, what do you think the chemical nature of

- the residues lining this structure is with respect to hydrophobicity? Verify your answer by displaying the hydrophobic vs. hydrophilic side chains in the interior of this structure.
2. When any ligand binds to a protein, there may be significant changes in the protein structure necessary for the ligand to gain access to its binding site. Examining the apo-avidin structure, can you envision how HABA, biotin, or desthiobiotin can gain access to the binding site without a significant change in protein structure? The best way to answer this question is examine carefully the ligands, their steric bulk via CPK representation, and the CPK structure of the protein. Now carefully examine the protein structure with the strept-tag bound. You will notice that a loop at the top of the binding site undergoes a significant conformational change allowing the strep-tag peptide to bind. Locate this loop and note the residue numbers for the amino acids comprising the loop. It might be helpful to look at the two structures side by side opening either two Jmol windows from the RCSB website or two stand-alone Jmol.jar windows. 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 (i.e., the structure in the presence of the strept-tag ligand) might be thermodynamically unfavorable for the apo-protein (no ligand bound)?
 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 hard to decipher unless one does a very detailed analysis of **ALL** the interactions between the protein and ligand. However, you certainly should be able to explain why the strept-tag is less tightly bound to avidin than HABA and how that is related to the K_d values for the different ligands.

Part B: CN3D examination of avidin \pm ligands.

In a previous exercise you examined the structures of apo-avidin (1ave) and avidin with the strept-avidin tag bound (1rst), concentrating on the fact that a loop at that top of the beta-barrel was swung out when the tag was bound to the protein, looking at the molecules displayed side by side. Another molecular graphics program, CN3D, and a related program, VAST, will be used to more closely look at the avidin structures.

VAST (Vector Alignment Search Tool) is an application located on the NCBI (www.ncbi.org) website. It is part of the Structure functions. In a nutshell, VAST is a program that allows you to directly superposition protein structures regardless of the proteins amino acid sequences, something that most molecular graphics programs cannot do. The way in which we are going to use VAST **is not** the intended purpose of the program (the details of which go way beyond this short description), but VAST will allow us to visualize two structures

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superimposed on each other, a function many molecular graphics programs can not perform. In order to carry out this assignment, go to the NCBI website and download CN3D onto your computer. CN3D will now be automatically executed by VAST when you want to display the structures. For those who are really interested in how VAST works, NCBI has placed excellent tutorials and notes on their website under Education. You can follow lecture notes from workshops they periodically hold as well as follow along their hands on exercises.

Now, we will use VAST and CN3D to view apo-avidin (1ave) and the strept-tag bound avidin (1rst) structures.

1. Go to NCBI website, go to the **VAST** page and enter **1ave** and then GET.
2. A **Structure Summary** page will now be displayed. You can display the molecule in CN3D or examine other features of the page. Scroll down and click on the **Sequence A** bar. You will now see a page **Related Structures** (VAST) page. The structures listed on this page are proteins with similar secondary and tertiary structures to avidin, however they have amino acid sequences that are distinctly different than either avidin or streptavidin. In other words, VAST compares structures of proteins without taking amino acid sequences into account. This is distinctly different than the amino acid sequence homology program BLAST, another NCBI data base mining tool. You will notice that none of the structures given above are shown because they have a high degree of amino acid sequence homology or exact identity. Therefore, we must force VAST to call up those structures.
3. On the **Related Structures** page, locate the “**Advanced related structure search**” line and click on the + symbol. This allows you to specify and find the structures you want to compare directly.
4. Enter **1rst** in the **PDB id** box then **FIND**. A new **Related Structures** page will be opened containing a line for **1rst**. , Click on the box next to **1rst** and then **VIEW 3D**. The CN3D window will open with the two structures superimposed. The first task you must accomplish is to determine the identities of the two structures.
5. You can determine the structure identities by the **SHOW** pull down menu and then choose the **Pick Structures** option. Point and click your way around this option. You have to hit **Apply** every time you change the display and **DONE** to exit this window. Notice that the strept-tag is not initially shown in the **1rst** structure. Using the above instructions, display the ligand. Since the strept-tag is a peptide itself it is unfortunately not considered a “hetero” group.
6. A second very important pull down menu is the **STYLE** option. Open **Style** and choose **EDIT GLOBAL STYLE**. This will open a window that will allow you customize the display. There are also display changing options in the list below **EDIT GLOBAL STYLE** that you can explore. Again, **APPLY** and **DONE** must be clicked every time you want to change the display. Since you are now familiar with molecular graphics, explore the different options for displaying the two structures.

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Comparing the two structures suggests that this loop at the top of the beta-barrel is flexible. If you now envision the mechanism of how a ligand binds to the apo-protein, what does the strept-tag structure suggest to you about this process? In other words, does the strept-tag structure represent a transient avidin species that has been “frozen” in an open form? In order to answer this question, compare the apo-avidin and either the HABA bound or biotin bound proteins. Taking all the factors into consideration, you should now be able to rationally propose a rational mechanism for ligand binding.

The next question is related to changes in the protein structure upon ligand binding. Recall, that when hemoglobin binds O_2 , the structure of each subunit and the tetramer is altered. Is a similar effect observed when either HABA or biotin are bound to avidin? Superimpose either of the ligand bound structures with the apo-avidin structure. Are there any significant changes in the backbone structures of the two proteins?

Finally, we will compare the structures of HABA bound (1sre) and biotin bound (1avd) avidin. Taking into consideration the K_d you determined for HABA, the K_d you determined for desthiobiotin, and literature value for biotin, are there any gross structural changes in the protein structure that are related to the different K_d values? The magnitude of the K_d values suggests significantly different interactions of the ligands with the protein. Closely examine the two structures, 1avd and 1sre, and try to rationalize the experimental results. Take into consideration the types of bonding that might exist: hydrophobic interactions, van der Waals, ionic, hydrogen bonding. For the former, determine which groups on the ligands are involved and to what groups on the protein as well as the distance involved. Jmol is a better program to use for this study since you can measure distance with it. One student suggested that the interactions might be related to the observed thermal motions in the side chains when the ligands were bound. What do you think?

Now that you have done quite a bit of structural examinations, what will you do with all this information? Quite simply, you will incorporate the knowledge you have gained from these exercises in the lab report for the ligand binding experiment. The most logical place to discuss this material is in the discussion section of the report. As in previous lab reports, use the questions posed above as a framework around which you will compose your thoughts. You might even want to export images from either Jmol or CN3D (be sure to use a white background to save ink) to facilitate your discussion!

Have fun with this and ENJOY!

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