Introduction to biomolecular visualization: What are the characteristics of a folded protein?[*]

Biochemistry textbooks use the term *protein* in the first chapters as if we all know exactly what that means. For example, Chapter 1 of Moran, et al.[1] states that "a functional protein can be a single polypeptide or it can consist of several distinct polypeptide chains that are tightly bound to form a more complex structure." Going further into this definition, a *polypeptide* is a sequence of *amino acids* joined by *peptide bonds*.

In this experiment, we explore further what those terms signify through a visualization study of the bovine serum albumin (BSA) protein. Our main resource is the RCSB Protein Data Bank (https://www.rcsb.org), which is the primary repository for experimentally determined structures of "biological macromolecular structures". We will refer to the Protein Data Bank by its initials, PDB.

Almost every structure deposited in the PDB is connected to a research paper in the peer-reviewed literature. We use a combination of the structural information available in the PDB with the experimental information and analysis in the research paper to enhance our understanding of the structure and function of the biological macromolecule.

We will investigate two of the BSA structures available in the PDB from the Bujacz group, one that is just the protein[2] and a second one that is the protein complexed with the anti-inflammatory drug naproxen[3]. Here are our specific goals.

1. Explore the information about a protein structure available in the PDB.

2. Develop a structural analysis of BSA using the molecular visualization software VMD (Visual Molecular Dynamics).

3. Search the PDB to find related structures of BSA or other serum albumin proteins and use those for structural analysis.

Pre-experiment work

These questions and guidelines should help shape what you include in your lab notebook for your pre-lab work. In addition, be sure to address any other items in the general statement about pre-lab guidelines for any biochemistry experiment.

1. Read what the first chapter or two of your biochemistry textbook says about proteins. Summarize what the textbook implies are the key characteristics of proteins.

2. Read, in the following order, these parts of Reference **Error! Bookmark not defined.**: the abstract, Introduction, Discussion, Figures 2b, 4, 5, and 6, Table 2, and sections 3.1-3.6 of the Results and discussion. Summarize the authors' work being sure to address the following questions with citation to appropriate figures or sections of the paper.

   a. What is the function of serum albumin in mammals?

   b. Why might it be important to compare the structures of serum albumin proteins from several mammals?

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  c. What kind of secondary structure is most prevalent in BSA?

  d. Of the other serum albumin proteins studied in this experiment, which one is BSA most similar to?

  e. What holds the protein together in its tertiary structure? What regions are the most flexible and why is that flexibility important?

3. Use the RCSB Protein Data Bank (RCSB PDB) at https://www.rcsb.org and search for bovine serum albumin. Find the entry that corresponds to the crystal structure submitted to the Protein Data Bank by Bujacz. Use the information in that entry to obtain the following information.

  a. The four-character PDB ID of the BSA crystal structure.

  b. The resolution of the crystal structure (with correct units).

  c. The classification of this macromolecule. Include a definition of the term used.

4. Find the PDB ID for the structure of BSA complexed with naproxen from the Bujacz group.

Visualization and analysis

We will first work on reproducing the BSA structural analysis of Bujacz. We will use the molecular visualization software VMD,[4] available at http://www.ks.uiuc.edu/Research/vmd. Any current version of VMD should have all the key components for this experiment. You should download and install VMD now if you're using your own computer.

The protocols in this section are based on Hsin, et al., "Using VMD – An Introductory Tutorial."[5] You may wish to refer to that article for additional details and additional protocols for other kinds of biomolecular analysis that you can perform with VMD.

Your research team should divide up into pairs for this work on the protocols. Each pair should complete the same protocols on two different computers, including both sets of results in the team notebook. The final report will require the team to choose which set(s) they wish to include. Each team member must become familiar (in the hands-on sense) with the tools and protocols.

All work in Protocols 1-9 and Visualization Tasks 1-2, including answers to any questions, will be in your team's lab notebook. The team's report will focus on the final visualization and analysis task.

Loading, displaying, and manipulating a molecule

To display a molecule, VMD (and any visualization program) needs at least three sets of information:

- The identity of each atom in the molecule.
- The coordinates in Cartesian space (x, y, z) of each atom.
- The other atoms each atom is bonded to.

For files from the Protein Data Bank, this information is stored in a standard format known PDB. Many other formats exist, but you won't have to worry about those for this experiment.

We will therefore load this information into VMD and then let it do its magic in order to display the structure.

Protocol 1: Loading and initial display

A. Start a VMD session.* Two windows will appear. The **VMD Main** window has many of the commands that we will us in menus and will display the list of all molecules that we have loaded. The **OpenGL Display** window (which we'll call the Display window) is where all the graphics appear.

B. In the Main window, choose File→New Molecule…. The **Molecule File Browser** window will appear. You will choose one of the following two ways for

   a. Version 1.94a of VMD can load files from the RCSB Protein Data Bank directly by using the PDB ID. For the Filename, enter the PDB ID for the BSA crystal structure (4F5S) that you found in the pre-experiment activities, then click Load.

   b. If you're using Version 1.93 of VMD, do the following more complicated set of steps.

      i. Click on the Download Files button on the PDB page for 4F5S and select PDB format. That will create a file 4F5S.pdb on your computer.

      ii. In the Molecule File Browser in VMD, click the Browse button and open the 4F5S.pdb file. Then click on Load.

C. You should now see a line structure of BSA in the Display window. Close the Molecule File Browser window.

Protocol 2: Manipulating the display

A. Click (on Windows, that's click with the left mouse button) and drag on the line structure to see that you can easily rotate the molecule in the window.

B. Now click and drag with the right mouse button (on the Mac, hold down the Command key and click) to see that you can perform a different kind of molecule rotation.

C. Click on the Mouse menu in the VMD Main window. Note that the mouse is currently in Rotate mode. Change the mode to Translate and click and drag using both the left and right mouse buttons to see how you can move the molecule in the window.

D. Go back to the Mouse menu and choose the Scale mode. Click and drag with the left mouse button to see how you can change the size of the molecule.

   a. You can use keyboard shortcuts—*r*, *t*, *s*—to switch between these modes without going to the Mouse menu.

E. When a molecule is first loaded, VMD will rotate it about a point in the center of the molecule. From the Mouse menu, choose Center, or press *c*, and then click on an atom in the molecule. Return to Rotate mode, and you should now see that you are rotating the molecule about the atom you clicked on.

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* If you're running VMD for the first time on a Mac, hold down the control key and click on the VMD icon, then choose Open. In the dialog box that appears, choose Open again. You'll only have to do this step this one time.

F. If you ever want to return to a default view, press = on the keyboard (or select Display→Reset View in the VMD Main window).

G. Let's do three other quick steps to make the display look a little better for what we'll be doing. First, choose Display→Orthographic in the VMD Main window. When you're first looking at structures, this viewing choice is a little easier to interpret. Second, we don't often care about the axes being displayed, so turn those off by choosing Display→Axes→Off. Finally, uncheck Display→Depth Cuing.

H. For the final task in this section, choose Mouse→Query in the VMD Main window (or press 0). Then click on any atom. It may not seem like anything is happening. But go look for the third window that VMD opened. It will contain all kinds of text information about what VMD is doing. When you click on an atom while in Query mode, you get lots of information about that atom. Find examples of each of the following kinds of atoms in this structure and for each, record the following information that VMD reports: the name of the atom, the index (how VMD numbers the atom in the molecule), the residue name, and the values of residue and resid[*].

    a. An α-carbon (the name will be CA)

    b. An oxygen on a tyrosine

    c. A sidechain nitrogen on a tryptophan

    d. An oxygen in a water molecule (look carefully for these; if you have trouble finding these, return to this step after you do Protocol 3)

Protocol 3: Changing the molecular representation

When you first load a molecule, you will see the line structure. VMD allows you to modify the display of those lines as well as choose many other ways to display the molecule, each of which is called a representation. Those methods are not just to make the molecule look good; they are helpful in our analysis. We'll explore several key methods in this section.

A. Choose Graphics→Representation from the VMD Main window. You'll get a new window with the title **Graphical Representations**. The pop-up menu will contain the name of your molecule. You only have one representation right now; when you have more, the one you're editing is highlighted.

B. In the current Lines representation, you can change one parameter, the thickness of the lines. Play with that value until you find a thickness you like.

    a. *Optional* From the Drawing Method window in the Graphical Representations window, select Licorice. This representation is a little nicer, but it can also be a little slower to manipulate. Any place where the Lines representation is mentioned, you can use the Licorice representation instead. (And the water molecules are a little easier to see in this representation.)

C. Let's add a second representation. First, click on Create Rep in this window. You will see that a second, identical representation appears. In the Drawing Method pop-up menu,

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[*] "residue" is the number that VMD assigns to the amino acid, solvent molecule, or ligand, starting with 0 for the first residue in the structure. "resid" is the number assigned to the same species in the original PDB file. Those numbers are not always identical.

choose VDW. What do you now see? Choose a Sphere Resolution you like (but don't go above 30).

D. Demonstrate that you can turn the VDW representation off and on by double-clicking it in the Graphical Representation window. Being able to turn representations off and on is a very valuable analysis tool.

E. Turn the VDW representation off, create another representation, and choose NewCartoon for the drawing method. Then, in the Coloring Method pop-up menu, choose Secondary Structure. You may also want to turn off the Lines representation.

    a. To determine the kinds of secondary structure present in BSA, we need to interpret the colors. In the VMD Main window, choose Graphics→Colors. In the **Color Controls** window that appears, select Structure from the Categories list. In the Names list, you'll see each kind of structure that VMD knows about and its corresponding color.

    b. List the different kinds of secondary structure present in BSA. Is the secondary structure information VMD displays consistent with what you found in the pre-experiment questions?

F. Now just display the Lines (or Licorice, if you chose to do that) representation. When a protein crystallizes, it often incorporates solvent molecules and other solutes into its structure. To see that here, select that representation, replace the word *all* in the Selected Atoms field with *not protein* and press enter. You should now see one small molecule and a bunch of red dots. (And you may need to turn off all the other representations to see those.)

    a. Go back to the 4F5S page in the PDB and identify the small molecule. (Hint: The Structure Summary tab has a Small Molecules section.)

    b. The red dots are the oxygen atoms of all the water molecules in the structure. So it's now a good time to research the answer to this question: Why aren't hydrogen atoms typically present in protein structures determined by x-ray crystallography?

G. We won't be interested in anything that's not the protein for the moment, so let's turn off all the other molecules. Do the following for each representation. Select the representation and change the Selected Atoms field to "protein". Then press Enter. Be sure to turn each representation on as you make the change so that you can see the effect. For the Lines and VDW representations, it should be clear that the water and small molecules are no longer displayed. We'll work more with atom selections later.

H. One other valuable representation shows the volume the molecule occupies in a way that is more accurate than the VDW space-filling mode. Leave only the Lines (or Licorice) representation on. Then, create a new representation and choose Surf (short for solvent-accessible surface) as the Drawing Method and ResType as the Coloring Method. Verify that "protein" is still the selection. As you did with the secondary structure coloring method, determine which residue type each color corresponds to.

    a. For each of the residue types in BSA, find one residue in the structure and verify that it has the correct color. Record the residue number, the 1 or 3 letter code for

the residue, the color assigned to it by VMD in the ResType coloring method, and the type that color corresponds to.[*]

I. The last task of this section is to use the tools introduced so far to create an image of BSA that your team likes. That image should use more than one representation, but otherwise you have your choice of drawing methods, coloring methods, and materials. To save the image in the Display window, select File→Render in the VMD Main window. You can just click on Start Rendering to get a snapshot of the Display window, but you might also try selecting "Tachyon (internal, in-memory rendering)" in the first pop-up menu and see what you get.

You should save an image of any significant visualization you've done. For example, you will be asked later to highlight key sets of residues in the BSA structure. Each time you do that, you should save an image and put it in your lab notebook.

J. Before you go on, turn off all the representations except the Lines (or Licorice) representation.

Interlude: Information available in the PDB

So far, we've only looked at the Structure Summary tab on the 4F5S PDB page. Several other tabs on that page will be useful to us as well. Return to that page and complete the following steps.

A. Review the following elements from the Structure Summary tab that you have seen previously in this activity.

    a. Experimental data snapshot: Method and resolution

    b. Literature reference

    c. Small molecules

B. From the Macromolecule Content and Macromolecules section in the Structure Summary tab, record the following information.[†]

    a. Total structure weight

    b. Number of chains and the letters corresponding to those chains

    c. Sequence length (and define this quantity)

C. Let's investigate the Macromoleules section a little more.

    a. Verify that chains A and B have the same Entity ID and sequence length. Why does this information indicate that chains A and B are identical?

    b. Protein crystal structures often appear as dimers, trimers, tetramers, and even higher mers. Why does the information in the Macromolecule section imply that 4F5S has crystallized as a *dimer*?

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[*] You may find it useful to change the Material of the surface representation to Transparent and turn on the Lines or Licorice representation.

[†] There is also a "Protein Feature View" subsection, which is way cool, but beyond the scope of what we're doing in this experiment.

c. There is one other chain in this protein. What is it?

d. Bujacz states that serum albumin is a 67 kDa transport protein. But the molecular weight you found for the structure in the PDB is larger. Neither value is wrong, so what is the explanation for the different values? (Hint: How much larger is the value given in the PDB?)

We have just touched on the surface of what you can find in Protein Data Bank. Explore some of the other tabs to see what kind of information is available. But we'll now return to our visualization work.

Protocol 4: SequenceViewer and selecting residues

VMD provides a very nice tool called SequenceViewer that allows us to highlight specific residues.

A. Before we start, let's look at only one of the unique chains in our representation. For each representation in the Graphical Representations window, change the Selected Atoms field to "protein and chain A" and press Enter. That then only shows atoms that are **both** part of the protein **and** in chain A. For this section, only display the Lines (or Licorice) representation.

B. Open SequenceViewer by choosing Extensions→Analysis→SequenceViewer from the VMD Main window. The **VMD Sequence** window will appear.

C. This window contains a list of amino acids in the molecule and a color-coded structure column.[*] Click on any amino acid to see it highlighted in the VMD Display window. The Graphical Representations window now contains a new representation. The atom selection for that representation is controlled by what you select in the VMD Sequence window, but you can change its Drawing Method and Coloring Method. Do so to get something that you like.

D. You can select multiple residues by holding down the shift key while clicking. Do that in order to highlight one of the helix structures you identified in the previous section in the Display window.

Protocol 5: Saving your work

A. You can save a file of commands that VMD will use to reproduce your visualization as precisely as it can—loading molecules, choosing representations, and setting the parameters for those representations. Start by selecting File→Save Visualization State… from the VMD Main window. Enter a filename that ends in the extension .vmd and does not contain any spaces, like "4f5sfirstvis.vmd", and save that file in a place you can find it.

B. Quit and restart VMD. Choose File→Load Visualization State…, navigate to the file you saved in the previous step and click Open. You should see something very close to what you had when you saved your work.

Throughout this experiment, you should save images and visualization states whenever you have done something that has any possibility of ending up in your final report.

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[*] We're not going to use the B value column in this experiment.

Using VMD to analyze molecular structure

VMD (and any similar program) has powerful measurement and analysis tools that provide a great deal of structural insight. We're now going to use the BSA structure that has naproxen bound to it, and we'll be spending time looking at Reference **Error! Bookmark not defined.**. That makes it best to restart VMD so that we have a blank slate.

Protocol 6: Distances, bond angles and dihedral angles

A. Load the BSA/naproxen structure (PDB: 4OR0) into VMD. We'll start with the default Lines representation. And also be sure to display only the protein atoms in Chain A.

B. Choose Mouse→Label→Atoms in the VMD Main window. Click on any atom and a label with the residue name, number, and atom name will appear (although not always be easy to see). Clicking on the same atom turns the label off. Label three atoms in this way.

C. Choose Graphics→Labels in the VMD Main window to bring up the **Labels** window. Select Atoms in the pop-up menu if it's not already selected, and you'll see the list of atoms you've labelled. Black means a label is on; red means it's off. If you click on any label, you'll find all the information about the atom that we saw earlier in this experiment. (You can also show, hide, and delete labels from this window.)

D. Let's go back to the VMD Main menu and choose Mouse→Label→Bonds. Now click successively on two non-bonded atoms that are near each other. You'll see the atoms connected by a dashed line that is labelled by the distance in Å. Sometimes that's hard to read, but no worries! Just go to the Labels window and choose Bonds from the pop-up menus to see all the distance labels you've made. Each label has the distance stored in the Value field.

E. We'll also be analyzing dihedral angles. Choose Mouse→Label→Dihedrals in the VMD Main window. Now click on four bonded atoms in succession—the first atom should be bonded to the second atom, the second atom should be bonded to the third atom, and the third atom should be bonded to the final atom. The value of the dihedral angle will now be displayed.

   a. *Section 4.3 of your text describes the Ramachandran* plot and its relation to the dihedral angles about the N-Cα bond (φ or phi) and the Cα-C bond (ψ or psi). Find three residues in different regions of the BSA protein that are not glycine or proline. Verify that

      i. The φ and ψ angles are consistent with the Ramachandran plot.

      ii. The peptide bond angle is close to +180° or –180°, indicating a trans conformation.

F. Before continuing to the next protocol, do the following.

   a. Delete or hide all the labels you've created in this section.

   b. On a separate piece of paper or in a program like ChemDraw, draw the structure of naproxen. Identify the regions of the molecule that are more polar, that are less polar, and that could participate in hydrogen bonds.

Protocol 7: Selecting a subset of atoms or residues

More detailed analysis of protein structure requires better ways to select subsets of atoms or residues that we can then focus our attention on. VMD has very good tools for doing that and we'll explore those that are useful in the BSA analysis.

In looking at Figure 3a of Reference 3, we see that the authors have identified four BSA residues that interact with naproxen in the binding pocket. Our goal is to reproduce that figure, but we'll get there by seeing a variety of ways we can select and display atoms in the structure. Much of this work will happen in the Graphical Representations window and specifically the Selected Atoms field. Two things to remember about using this field.

- You *must* have the representation you want to affect highlighted in the Graphical Representations window.

- You *must* press Enter each time you change the selected atoms.

Not doing both these steps means your changes won't take effect.

A. Quite often, we are just interested in the backbone of the protein. If we just want to view those atoms, we can change the Selected Atoms from "protein and chain A" to "backbone and chain A" and press Enter. Our representation changes to show only the backbone atoms on chain A. (Throughout this section, we'll be looking just at chain A since BSA crystallizes as a dimer in this structure as well.)

   a. Describe the potential value of examining only the backbone of the protein.

B. Suppose we only want to look at the tyrosine amino acids. Enter "resname TYR and chain A" into the Selected Atoms field and press Enter. You'll see only the TYR residues in the structure.

C. We can also examine a single residue by using its resid value. Enter "resid 127 and chain A" in the Selected Atoms field to see just that residue (which looks like a lysine). If we want to look at a few residues, we can enter each number like "resid 127 131" or a range like "resid 127 to 131". Try those two selections to see what happens (don't forget to include "and chain A").

D. We can also combine these selections. If we want the backbone atoms of the turn in this polypeptide, we would enter "backbone and resid 127 to 131" in the Selected Atoms field.

E. Many, many more selections are possible. Click on the Selections tab in the Graphical Representations window and you'll see all the keywords that VMD understands.

F. Return to the original selected atoms: "protein and chain A". Change the Drawing Method to New Cartoon and the Coloring Method to Secondary Structure

G. To set up for the next task, create another representation and have it display only the residues that interact with naproxen: 409, 410, 413, and 488. Use Licorice for the Drawing Method and Element for the Coloring Method. Orient the protein so that you can see those four residues.

H. So now we just need to display the naproxen. But we need to tell VMD how to identify it. We can figure that out by going to the 4OR0 PDB page and finding the Small Molecules section on the Structure Summary tab. You'll see naproxen listed as one of the ligands.

    a. Verify that the structure shown for naproxen is the same as what you previously drew.

    b. What is the three letter ID for naproxen?

I. Back to VMD. Create a new representation. Remember how we displayed all the tyrosine residues with "resname TYR and chain A". Use the three letter representation for naproxen to display that molecule. You should again use Licorice for the Drawing Method and Element for the Coloring Method. There is more than one naproxen molecule in the structure, so orient your visualization so that you're looking at the naproxen that is binding to the residues identified in the figure.

Visualization Task 1

- Reproduce as completely as you can Figure 3a from Reference 3. Your image does not have to be visually identical, but it should show all four interactions of the naproxen oxygen atoms with the BSA residues, including the distances. Certainly play with Drawing Methods and Coloring Methods to get as clear a visualization as you can.

- For the binding of this naproxen, the authors state that "The leucines present in this [binding] pocket create hydrophobic contacts with naphthalene ring of the [naproxen] stabilizing its position." The leucine residues in the binding pocket are L452, L456, L386, L406, and L429. Also, N390 may play a role as well. Add these residues to your visualization and evaluate the validity of the authors' statement.

Visualization Task 2

Let's return to Reference **Error! Bookmark not defined.**. Figure 2 denotes the binding pockets for fatty acids (FA) in human serum albumin, which we can assume are the same in the other serum albumin proteins.

- Create a list of residues for three of the FA binding pockets in BSA. For example, for FA1, the residues are L115, K116, L122, F133, W134, Y137, L138, I141, A157, Y160, F164, I181, R185.

- Use these lists to recreate Figure 2b as best you can using the 4OR0 structure and for the three FA binding pockets you identified.

- Evaluate whether naproxen molecules occupy the fatty acid binding pockets.

Comparing structures

All major molecular visualization programs provide many tools to compare structures of similar biomolecules. We will learn how to do two tasks: (a) loading multiple molecules; (b) aligning the molecules so that we can see similarities and differences in their structures.

Protocol 8: Loading multiple molecules

We now have two structures that we're working with: BSA and BSA/naproxen. An important question in protein function is how the structure of the protein changes when it binds a molecule.

To investigate that we first want to load both structures into VMD. When we do that, each structure becomes its own molecule with its own set of representations. Let's see how that works.

    A. Launch (or re-launch) VMD so that you have a clean workspace to start. Load the crystal structure of BSA like you did in the first part of this experiment.

    B. With the Molecule File Browser window open, select "New Molecule" in the "Load Files for" pop-up menu. Then, load BSA/naproxen structure. A second molecule appears in the VMD Main window.

    C. When loading multiple molecules, they sometimes are not in the same region of space (although in this case, they are). You can always use the Scale mode to zoom out so that you can see both structures. And note that pressing the = key can always bring the structure back to a default position.

    D. When we have multiple molecules in our visualization, the Graphical Representations window has a drop-down menu that allows us to select each molecule and modify the representations just for that molecule. Set up representations so that you're showing chain A in both molecules (protein only) and the naproxen molecules in the BSA/naproxen structure. For the protein, use the NewCartoon drawing method in order to make the image a little easier to view.

Protocol 9: Aligning different molecules

When we ask VMD to align molecules, the program finds the best possible orientation of the structures that minimizes the distance between equivalent atoms in each structure. We therefore must have a 1:1 correspondence between atoms in each structure. For example, we could align a structure based on two tyrosine residues in different molecules, but we can't align a tyrosine and an alanine. We'll therefore tell VMD a selection of atoms that we want to use for the alignment that we know represents the same kind and number of atoms in each molecule.

The tool we'll use is the RMSD Trajectory Tool.

    A. Launch this tool by choosing Extensions→Analysis→RMSD Trajectory Tool from the VMD Main window.

    B. The text box in the upper left contains the atom selection we want to use for the alignment. At the bottom are the molecules we want to align. By default, that selection is "protein". But if we click "ALIGN" (try it!), we get an error message because the BSA structure doesn't have naproxen molecules in it.

    C. So we're going to choose a subset of residues to perform the alignment on. It turns out that there is a glitch in the BSA PDB file because some residues are duplicated. That doesn't affect what we've done so far, but makes what we want to do here a little problematic. So here is the alignment selection that we're going to use in the RMSD Trajectory Tool: "protein and backbone and resid 100 to 180". Enter that and click "ALIGN". You should now see that the helices in the two structure are almost superimposed.

    D. Investigate the protein structure near each of the naproxen molecules. To what degree (a lot, a little, not at all) does the backbone structure change when a naproxen molecule is

bound? Choosing different drawing methods for the naproxen molecules may assist you in answering this question.

E. Now let's add back in the residues interacting with naproxen that were identified in Protocol 7. Do that for both structures. What changes are evident in the orientation of the sidechains when the naproxen molecule binds?

F. Create an image (or more than one image) that best illustrates the changes in the naproxen binding pocket as the naproxen binds.

There is so much more we can do with VMD, and the tutorial by Hsin, et al. can help you discover some of the additional tools. But what we've done so far is enough to reproduce much of the structural analysis of BSA by the Bujacz group and give you a resource that is useful.

Final visualization and analysis task

There are a variety of serum albumin structures in the Protein Data Bank. While many of those have ligands bound to them, none of them have the 8-anilino-1-naphthalenesulfonate (ANS) molecule that is a fluorescent marker for whether BSA is folded or unfolded.[6]

Each team should find a serum albumin (not necessarily BSA) in the Protein Data Bank complexed with a ligand that seems structurally similar to ANS. No two teams should use the same serum albumin/ligand combination. Develop a visualization that shows how the ligand binds to the serum albumin and develop answers to the following questions for your report.

- What are the structural similarities and differences between ANS and ligand in your team's structure?

- Does the ligand use any of the fatty acid binding pockets identified in Reference **Error! Bookmark not defined.**?

- In what ways is the binding of the ligand similar to and different from the binding of naproxen to BSA?

- How might BSA unfold in a way that ANS (or other similar ligands) would no longer be able to bind to it?

In answering these questions, you should demonstrate appropriate use of the VMD visualization and PDB analysis techniques. Your team's final report will focus on your team's chosen serum albumin, an analysis of how it binds the ligand, and a discussion of how that analysis informs our knowledge of how ANS might bind to folded BSA, but not to unfolded BSA. Your report should include well-annotated images that you've created using VMD.

References

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