7. Exploring

(If you are resuming the tutorial after a break, start your desktop copy of RasMol, arrange the windows as in section 1, and open **3b5c.pdb**. Restrict the view to residues 64-72, mainchain only. Turn on hydrogen bonds.)

Display: Spacefill
Rotate this model to view it end-on. Notice that stick models make protein structures appear very open and empty, but even an isolated helix is quite densely packed.

RasMol > **restrict 64-72** < return >
RasMol > **hbonds off** < return >
Display: Spacefill
Now you see a space filling model of the entire helix. The side chains reappear because this **restrict** command includes them.

RasMol > **select** < return >
Now all atoms are selected.

Display: Backbone
Colours: Structure
RasMol > **reset** < return >
This returns the full backbone to the screen, centers rotation on the center of the whole model, and returns it to the original orientation. The last four commands are useful when you get lost and need to redisplay everything and get your bearings again.

RasMol > **restrict sheet** < return >
This command removes all residues except those in pleated sheet (*sheet* is another *set*). Center rotation on one of the alpha carbons in the middle of the sheet (look back at previous commands if you don't remember how, and remember to set picking back to **ident** after you change the center of rotation). Display the model as sticks and color it CPK. Display hydrogen bonds. To help you see the sheet structure more clearly, remove the sidechains as follows:

RasMol > **restrict sheet and mainchain** < return >
Decide whether the three central strands in the sheet are parallel or antiparallel. What about the edge strands? Are they parallel or antiparallel to their neighbors? Here's how to check your answers:

Display: Cartoons
Cartoon displays show sheet strands as arrows pointing toward the C-terminal end of the chain. A pair of chains with arrows at the same end are parallel. If arrows on neighboring strands are at opposite ends, the strands are antiparallel.

RasMol > **select** < return >
RasMol > **hbonds off** < return >
Display: Cartoons
Colours: Structure
Now you see the whole protein as a cartoon. This is a vivid display that is easy to interpret, but it

has one disadvantage for further exploration: picking doesn't identify atoms. Remember that you must us a display function that shows the exact location of atoms in order to identify atoms by picking.

RasMol > **restrict turn** < return >
Display: Backbone
Now you see the beta turns in the model.

RasMol > **restrict 17-21** < return >
This shows just one turn. Display it as sticks in CPK colors, and turn on hydrogen bonds. Notice that residue 19, the middle residue, is lysine.

RasMol > **center lys19.ca** < return >
Confirm that the hydrogen bond in a beta turn connects the carbonyl oxygen of residue *n* with the N-H of residue *n+3*. From information in a standard biochemistry textbook, decide whether this is a type I or type II turn. One way to look at it: if the three carbonyls that make the turn (residues 17, 18, and 19) all point in the same general direction (up or down when you look at the turn edgewise), then it the turn is type I. If the middle carbonyl points in the opposite direction from the other two, it's type II.

RasMol > **reset** < return >
RasMol > **select** < return >
RasMol > **hbonds off** < return >
Display: Cartoons
Colours: Structure
RasMol > **select hetero and not hoh**
Display: Spacefill
Colours: CPK
Now you see the heme prosthetic group in its cartoon-protein binding site. You will now use RasMol to explore the binding of the heme to the protein.

Rotate the molecule so that you see the heme edge-on, protruding from the right- hand side of the protein. Notice that the binding pocket is composed of four alpha helices above and below the heme, and a four-strand pleated sheet on its inside edge. The molecule looks somewhat like a pair of jaws holding a heme. The teeth are four alpha helices, and the throat is pleated sheet. However, this cartoon view does not display any of the chemical details of heme binding.

RasMol > **restrict within (7.0, hem)** < return >
Display: Wireframe
Colours: CPK
RasMol > **center hem.fe** < return >
RasMol > **select hem** < return >
Display: Ball & Stick
Now you see the heme as a ball and stick model surrounded only by atoms that lie within 7.0 angstroms of heme atoms. The **restrict within** and **select within** commands are powerful tools for directing your attention to specific interactions within macromolecules.

Look for possible electrostatic interactions and hydrogen bonds between the heme (ball & stick) and the protein (wireframe). First, look at the yellow iron (III) ion in the center of the heme. The ion is part of an octahedral transition metal complex. Four of its six ligands are the light blue nitrogens of the heme porphyrin ring. What are the other two ligands? Use picking to identify them. When you have learned the residue numbers of the side chains that contain the ligands, display them in stick form as follows:

RasMol > **select (#1 or #2) and sidechain** < return >
(substitute the residue numbers of the ligands for "#1" and "#2").
Display: Sticks
Now you should see two histidine side chains providing nitrogens as the fifth and sixth ligands of iron(III). The stick display does not include the alpha carbon in the main chain, because the alpha carbon is not included in the selection **sidechain**, which selects CB but not CA. You can, however, include CA in the display, and complete the stick model of the histidine side chains without changing the selection, as follows:

RasMol > **set bondmode or** < return >
Display: Sticks
Notice that one bond, the CA-CB bond, is added to the display for each histidine. RasMol has two *bond modes*, called *and* and *or*. In bond mode *and*, RasMol draws bond CA-CB if both CA *and* CB are selected. In bond mode *or*, RasMol draws bond CA-CB if either CA *or* CB is selected. When you start RasMol, the bond mode is *and* until you change it. Consider your last two **sticks** commands, with CB selected and CA unselected. On the first try, in the *and* bond mode, RasMol did not draw the the CA-CB bond as a stick because CB was selected and CA was not. Then on the second try, in the *or* mode, RasMol drew the CA-CB bond as a stick because CB was selected. Changing the bond mode does not change the current display, but it does change the behavior of subsequent display commands.

RasMol > **set picking monitors** < return >
Working without stereo, click first on the iron (III) ion, and then on one of its histidine nitrogen ligands. A dotted line appears between the atoms, along with a label showing the distance between the atoms in angstroms. The label is colored that same as the first atom picked. If you accidentally pick the wrong atoms, you can remove the dotted line and label by picking the same two atoms again. Now measure the distance between iron (III) and the other histidine ligand. This distance, about 2.0 anstroms, is the length of the bond between iron (III) and nitrogen in the transition-metal complex. Such bonds were once called coordinate-covalent bonds, because one of the two bonded atoms, in this case the ligand nitrogen, donates both of the electrons to form the bond.

The heme has two chains that extend from the edge that sticks out of the binding pocket. Both chains end with carboxyl groups. Can you find any heme-protein interactions that involve the heme carboxyls? There are two hydrogen bonds involving serine 64. Find and measure them. To make all your measurements easier to see, use the cursor-up key to retrieve and execute your command **restrict within (7.0, hem)**. Then display all the atoms of the heme and its binding pocket in wireframe. Another quirk of RasMol is that the heme iron is not displayed in wireframe. Select only the iron (hem.fe) it and display it as a ball using **Ball & Stick**.

Now you will study the remaining interactions that hold the heme in place, which are primarily hydrophobic.

RasMol > **set picking ident** < return >
RasMol > **monitors off** < return >
This sequence resets picking to the default and removes all measurement lines and labels.

RasMol > **select hydrophobic and sidechain** < return >
RasMol > **color yellow** < return >
This sequence selects only the sidechains of hydrophobic residues, and colors them yellow. The **hydrophobic** set is another set of atoms you can use in **select** commands. The **color** command does not add atoms to, or remove atoms from, the display. To see the names of other colors you can use in commands, look at **help** for **color** and **colors**. (In the **colors** help information, **RGB** stands for "red-green-blue".)

RasMol > **select hem.c??** < return >
RasMol > **color cyan** < return >
This sequence selects all carbons in the hem and colors them cyan. The question marks stand for unspecified characters. The selection **hem.c??** means, "atoms of heme designated **c** followed by up to two additional unspecified characters." Now you will display the heme and its binding pocket as a space filling model.

RasMol > **restrict within (7.0, hem) and not hoh** < return >
Arrange the model so that you are looking into the opening of the hem pocket, with the two heme carboxyls pointing at you. It is much easier to do it stereo. If you can't tell front from back, try displaying in ball and stick, which gives better depth cues.

Display: Spacefill
Now you see the heme peeking out of its pocket. One carboxyl points out into space, and the other is in contact with two atoms of serine 64. The cyan carbons of the heme are hydrophobic, as are the yellow carbons of the hydrophobic sidechains. In this view, you see hydrophobic interactions, therefore, as contact between cyan and yellow. But much of the contact area is buried by the space-filling models. Let's look inside.

RasMol > **slab 100** < return >
Now hold down the control key at the bottom left of the keyboard (not the command key). While holding the key down, move the mouse pointer up the screen. As you move the mouse, an invisible plane slides back through the model, cutting away everything that lies in front of it. Thus you can slice into the model to any depth, removing all foreground as you go. Slide the pointer up and down the screen to change the position of this cutting plane. (You may find that the action is choppy, because the computer is doing many calculations to produce each successive view.) As you cut into the model, notice the contacts between heme carbons (cyan) and atoms of hydrophobic side chains (yellow). By releasing the control key, you can rotate the model to cut into it from other directions.

RasMol > **slab off** < return >
Again orient the model so you are looking at the heme peeking out of its pocket.

RasMol > **select hem** < return >
Display: Wireframe
RasMol > **select hem.fe** < return >
Display: Ball&Stick
Now you can clearly see the interior of the pocket, and observe its strongly hydrophobic nature. You can also see the two histidine side chains that protrude into the pocket to interact with the iron (III).

RasMol > **select hem** < return >
RasMol > **dots 200** < return >
This display colors the surface of the heme with about 200 dots per atom. Dot displays give a feeling of solidity, but you can see through them to neighboring atoms. Zoom in and try to see contacts between heme carbons and other atoms in the pocket. These contacts are much easier to see in stereo. For a dramatic view of the pocket, try turning the model around and slabbing in through the back. You will see what the world looks like from the perspective of the iron (III) ion.

File: Close

Cytochrome b5 is a small protein consisting of a heme and only one polypeptide chain. In your biochemistry class, you will also study oligomeric proteins, which consist of more than one polypeptide subunit, as well as protein-protein and protein- nucleic acid complexes. RasMol has some features that are very useful with models that contain more than one chain. Now you will briefly examine such a model -- part of an antigen-antibody complex.

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8. Oligomeric Proteins
