Structure Analysis and Comparison Tutorial

This tutorial includes binding site analysis and comparison of related structures by superposition and morphing. Internet connectivity is required to fetch the structures **2zcp** and **2zco**.

← Background and Setup

The pathogenic organism *Staphylococcus aureus* makes a pigment called staphyloxanthin. The pigment imparts a golden color (hence *aureus*), but more importantly, contributes to virulence by protecting the bacteria from killing by the host immune system. The *S. aureus* enzyme CrtM may be a good drug target because it catalyzes a key step in staphyloxanthin synthesis:

> C. Liu, G.Y. Liu, Y. Song, F. Yin, M.E. Hensler, W. Jeng, V. Nizet, A. Wang, and E. Oldfield, "A Cholesterol Biosynthesis Inhibitor Blocks *Staphylococcus aureus* Virulence" *Science* **319**:1391 (2008).

We will view and compare different structures of this enzyme.

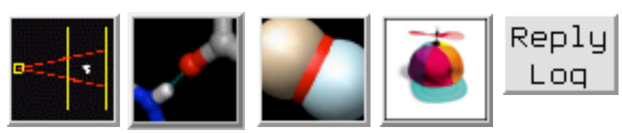
On **Windows**/**Mac**, click the **chimera** icon; on **UNIX**, start Chimera from the system prompt:

> *unix*: **chimera**

A basic Chimera window should appear after a few seconds; resize it as desired. Open the **Command Line** (**Tools... General Controls... Command Line**).

Choose **Favorites... Add to Favorites/Toolbar** to place some icons on the toolbar. This opens the **Preferences**, set to **Category**: **Tools**. In the **On Toolbar** column, check the boxes for:

- **Side View** (under Viewing Controls)
- **FindHBond** (Structure Analysis)
- **Find Clashes/Contacts** (Structure Analysis)
- **Rotamers** (Structure Editing)
- **Reply Log** (Utilities, the last section)



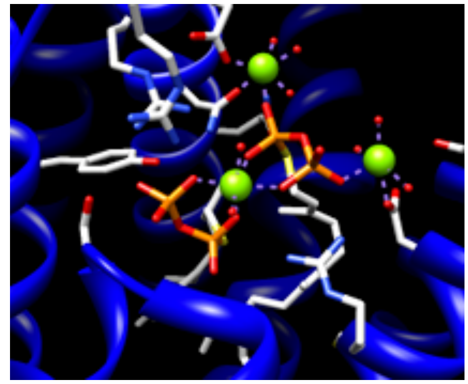
If you want these icons to appear in later uses of Chimera, click **Save** before closing the preferences.

Fetch a structure from the Protein Data Bank and simplify the display:

> *Command*: **open** **2zcp**
> *Menu*: **Presets... Interactive 1 (ribbons)**

This preset shows proteins as ribbons, plus sidechains close to any ligands. The structure includes two copies of the enzyme, chains A

2zcp active site



and B. Delete chain B:

> *Command*: **delete :.b**
> *Command*: **focus**

Move and scale structures using the mouse and **Side View** as desired throughout the tutorial. The front and back clipping planes can be adjusted in the **Side View**.

The enzyme combines two 15-carbon molecules of farnesyl pyrophosphate to form a 30-carbon lipid. This structure contains farnesyl ***thio***pyrophosphate, which differs from the substrate by having a sulfur in the place of one oxygen. Sulfur atoms are yellow, phosphorus orange, oxygen red, and nitrogen blue. Label the ligand residues:

> *Command*: **rlabel ligand**

In this structure, the farnesyl thiopyrophosphate molecules are named FPS and are residues 657 and 658 of chain A. Three metal ions help to offset the negative charges on the phosphates. These are shown as greenish spheres; hovering the mouse cursor over each reveals (assuming default balloon preferences) that they are Mg ions. Metal coordination bonds from FPS, water, and the protein are shown with dashed purple lines. Lines drawn to indicate interactions other than covalent bonds are called ***pseudobonds***. Hovering the cursor over a pseudobond or bond shows balloon information about its end atoms and length.

Delete the water and label residues with displayed atoms:

> *Command*: **del solvent**
> *Command*: **rlab @/display**

Water could be included in the various analyses, but is removed here to simplify the tutorial.

← Distances, H-Bonds, Contacts

It looks like several sidechains could be donating hydrogen bonds to ligand phosphate oxygens. (Although the structure does not include hydrogens, we know they are there!)

One of the displayed residues is Ser 21. To measure a distance:

1. Ctrl-click to pick the sidechain oxygen of Ser 21
2. Shift-Ctrl-doubleclick on the nearest phosphate oxygen
3. click **Show Distance** in the resulting menu

Similarly, measure the distance between the sidechain oxygen of Tyr 248 and the same phosphate oxygen.

The distances seem consistent with hydrogen bonds. However, rather than measuring many distances and trying to remember the appropriate hydrogen-bonding distances for different types of atoms, just use **FindHBond**. We will limit the search to H-bonds involving the FPS residues:

1. Select the FPS residues (for example, with **Select... Residue... FPS** in the menu).

2. Start **FindHBond** by clicking its icon.
3. In that dialog, turn on the options:
    - **Only find H-bonds with at least one end selected**
    - **Write information to reply log**
4. Set **Line width** to 3.
5. Click **OK**.

The H-bonds are shown as pseudobonds of the specified color and line width. Both measured distances were found to be consistent with hydrogen bonding. Details can be viewed in the **Reply Log**, which can be opened by clicking its icon. (In your own work, you may prefer to write the information to a file instead.) Remove the "hydrogen bond" pseudobonds:

> *Command*: **~hbond**

Find Clashes/Contacts has some similarities to **FindHBond**, but it can also identify nonpolar interactions:

- ***clashes*** - unfavorable interactions where atoms are too close together; close contacts
- ***contacts*** - all kinds of direct interactions: polar and nonpolar, favorable and unfavorable (including clashes)

We will identify contacts of the FPS residues:

1. Start **Find Clashes/Contacts** by clicking its icon.
2. With the FPS residues still selected, click **Designate**. 48 atoms should be designated for checking against **all other atoms**.
3. Set the **Clash/Contact Parameters** to the default **contact** criteria (by clicking the button marked **contact**).
4. Set the **Treatment of Clash/Contact Atoms** to:
    - **Select**
    - **Write information to reply log**
    - (turn off any other treatment options)
5. Click **Apply**.

In the **Reply Log**, the atom-atom contacts are listed in order of decreasing VDW overlap.

Even though all of the contacting atoms have been selected, not all of them are displayed. Click into the graphics window, press the keyboard up arrow key to promote the selection from atoms to whole residues, and then display the selection (**Actions... Atoms/Bonds... show**).

One might simply want a list of the interacting residues rather than the details of each atomic contact. A list of the selected residues can be saved. First, deselect the ligand residues and ions, leaving the protein residues selected:

> *Command*: **~sel ligand | ions**

The **Selection Inspector** (**Actions... Inspect**) reports that 26 residues are selected. Click its **Write List...** button or choose **Actions... Write List** from the menu. In the resulting dialog, indicate that **selected residues** should be written. Click **Log** to write the list to the **Reply Log** instead of to a file. Clear the selection by Ctrl-clicking in an empty area of the graphics window.

← Angles, Rotamers, Clashes

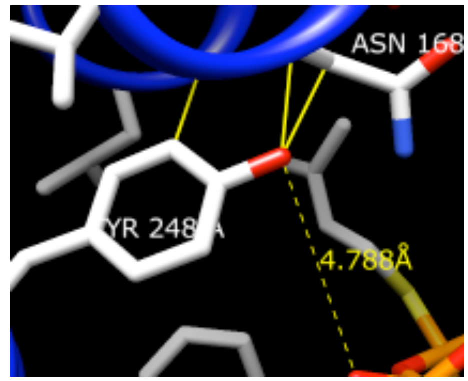
Focus on Tyr 248 and measure its chi angles:

> *Command*: **focus :248**
> *Command*: **angle :248@n@ca@cb@cg**
> *Command*: **angle :248@ca@cb@cg@cd1**

As reported in the status line (transiently) and **Reply Log**, chi1 and chi2 are approximately –107° and –142°, respectively.

We will set up clash checking and rotate the sidechain interactively.

clash checking



1. Display additional residues within 4 Å of Tyr 248:

   Command: **disp :248 z<4**

2. Ctrl-click to select any atom or bond in Tyr 248 and press the up arrow key on the keyboard to promote the selection to the whole residue.
3. Start **Find Clashes/Contacts** by clicking its icon.
4. In that dialog, click **Designate**. The 12 atoms of Tyr 248 should be designated for checking against **all other atoms**.
5. Set the **Clash/Contact Parameters** to the default **clash** criteria (by clicking the button marked **clash**).
6. Set the **Treatment of Clash/Contact Atoms** to **Draw pseudobonds**; turn off any other treatment options.
7. Set the **Frequency of Checking** to **after relative motions**.

No clashes are found in the current position. To rotate the sidechain of Tyr 248 interactively:

1. Ctrl-doubleclick its CA-CB bond (the stick segment right next to the ribbon)
2. click **Rotate Bond** in the resulting menu

The rotatable bond will be listed in the **Adjust Torsions** dialog. In that dialog, if you change the **Near** atom to **N**, the value reported is the chi1 angle. There are several ways to rotate a bond, including dragging the pointer on the dial, clicking the black arrowheads, and editing the angle value directly.

Use whichever method you prefer to rotate the bond. As the sidechain moves, yellow pseudobonds show any clashes and the distance monitor updates automatically. The sidechain is fairly constrained; only chi1 angles of approximately –(100-120)° avoid clashes if only that bond is rotated. The sidechain can be frozen in a new position, but for tutorial purposes, simply restore it to its original position: in the **Adjust Torsions** dialog, click the entry under **Bond** to show a menu. In that menu, choose **Revert** (move back to original position) and then **Deactivate** (make the bond no longer rotatable). **Close** the torsion dialog.

Click the **Find Clashes/Contacts** icon to bring the dialog to the front and **Close** it to halt clash checking.

Next, we will compare the conformation of Tyr 248 in the structure to tyrosine rotamers from a library. This will indicate whether the sidechain is in a frequently observed conformation and whether other conformations might also fit into the structure.

With part of Tyr 248 still selected (if not, Ctrl-click to to select any of its atoms or bonds), start **Rotamers** by clicking its icon. Click **OK** to show **TYR** rotamers from the **Dunbrack** backbone-dependent library. The rotamers are shown in the wire representation and listed in another dialog. Clicking a line in the rotamer dialog displays just that rotamer. Clicking the lines one by one shows that none of the rotamers match the conformation in the structure.

The rotamer dialog reports chi1 and chi2 angles and probabilities from the library based on the residue's backbone conformation. The residue's backbone angles (phi and psi) are reported in the **Reply Log**. The probabilities do not take into account any interactions specific to this structure. However, the rotamer dialog is integrated with clash and H-bond detection. Choose **Columns... Add... Clashes** and click **OK** to use the default settings; repeat with **Columns... Add... H-bonds**.

The new columns show that each rotamer forms several clashes but no hydrogen bonds. Formation of an H-bond with the ligand and avoidance of clashes may compensate for the nonrotameric (presumably strained) chi angles of Tyr 248 in this structure.

When a single rotamer is displayed, it can be substituted for the current sidechain. Since rotamers of different residue types can be shown at a given position, "mutations" of one type to another can be performed. Either click **OK** to replace the sidechain with the currently shown rotamer and remove the others, or simply **Close** the dialog to remove the rotamers without replacing the sidechain.

Unlike Tyr 248, Tyr 41 closely resembles the highest-probability tyrosine rotamer given its backbone angles. If you like, focus on Tyr 41, select one of its atoms or bonds, and use **Rotamers** to show and evaluate rotamers of tyrosine or some other amino acid at that position.

Remove the distance pseudobonds, unlabel the residues, and zoom back out:

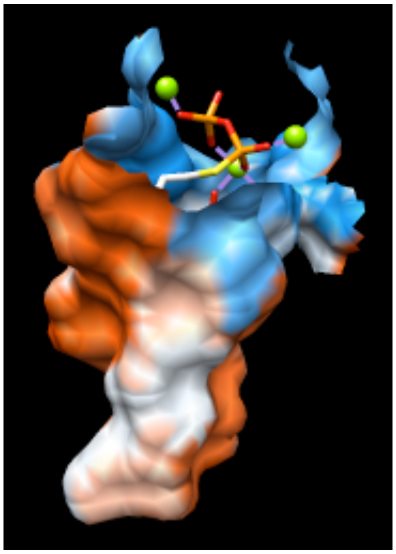
> *Command*: **~dist**
> *Command*: **~rlab**
> *Command*: **focus**

← Surfaces and Attributes

FPS and the natural substrate both have a highly polar/charged "head" and a long hydrocarbon tail. The enzyme pocket is very large and deep, as needed to accommodate two of these molecules.

A surface representation is best for showing the shape of the pocket. First, choose **Favorites... Preferences** from the menu, change to **Category**: **New Surfaces**, and set **show disjoint surfaces** to **false**. This will make the surface simpler by ignoring small enclosed pockets inside the protein. **Close** the preferences.

2zcp pocket surface	Choose **Presets... Interactive 3 (hydrophobicity surface)** from the menu to display a molecular surface color-coded by amino acid hydrophobicity.



This preset colors the surface ranging from dodger blue for the most polar residues to white to orange red for the most hydrophobic residues.

As one might expect, the mouth of the pocket near the phosphates and ions is mostly polar, while the rest of the pocket is largely hydrophobic. However, the pocket is so deep that it is hard to see when the whole surface is shown. Restricting the surface display to the pocket region allows it to be viewed from the outside, as shown in the figure.

Hide the surface, the ribbon, and the protein atoms:

Command: **~surf**
Command: **~ribbon**
Command: **~disp protein**

Surface display can be limited to a zone in a couple of different ways. Command-line zone specifications use atom-atom distances. For example, the following displays the surface patches of protein atoms within 6.5 Å of ligand atoms:

Command: **surf protein & ligand za<6.5**

A fairly large cutoff is needed to avoid holes in the pocket surface. Apparently, this pocket includes some "wiggle room."

The second way entails selecting the atoms of interest (in this case, the ligand residues) and using **Surface Zone** (under **Tools... Surface/Binding Analysis**). This tool measures distances to surface vertices, and it allows the cutoff to be adjusted interactively with a slider. The figure shows results for a cutoff of 4.7 Å. If you use this method, clear the selection afterward by Ctrl-clicking in an empty area of the graphics window.

Amino acid hydrophobicity is one example of an ***attribute***. Another attribute commonly used in structure analysis is atomic B-factor. B-factor values indicate which parts of the structure are more or less flexible or disordered. Display all atoms by choosing **Presets... Interactive 2 (all atoms)** from the menu.

The **Render by Attribute** tool shows attribute values with colors and other visual cues. Choose **Tools... Depiction... Render by Attribute** from the menu to start this tool. The dialog is set to **atoms** when it appears. Choose **bfactor** from the attribute menu. The B-factor values are shown in a histogram, and the vertical bars on the histogram define a color mapping. These bars or ***thresholds*** can be added, deleted, moved, and/or their colors changed. Clicking the square color well allows changing the color of the threshold that was most recently clicked or moved. If any sidechain was replaced with a rotamer, the new atoms will not have B-factor values, but they can be assigned a **No-value color**. Adjust the color mapping as desired, then **Apply** it to the structure. The lowest B-factors are in the protein core, the highest in a loop over the active site and the C-terminus on the opposite side.

In the **Render** dialog, switch from **atoms** to **residues** and show the histogram for the attribute **kdHydrophobicity**, the Kyte-Doolittle hydrophobicity. This is the same attribute used by the surface preset, but this tool allows different color schemes to be applied. Higher numbers correspond to more hydrophobic (less polar) amino acids. The **No-value color** pertains to residues without Kyte-Doolittle hydrophobicity values, namely any that are not amino acids. **Apply** coloring by hydrophobicity if you like, then **Close** the dialog.

← Superposition and Morphing

Comparing different structures of a protein is another way to evaluate flexibility. We have been viewing a structure bound to substrate analogs, **2zcp**. A structure of the same enzyme without ligands is also available.

Fetch the "empty" structure and apply the ribbons preset:

> *Command*: open **2zco**
> *Command*: preset **apply int 1**

Now the first structure is white and the new structure is magenta, as shown in the **Model Panel** (**Tools... General Controls... Model Panel**).

The structures are in completely different positions, so the next step is to superimpose them. This is easily accomplished with **MatchMaker** or its command equivalent:

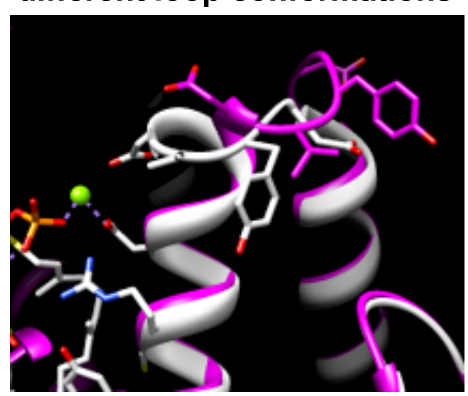
> *Command*: mm **#0 #1**

MatchMaker generates a sequence alignment using residue types and secondary structure (tries to align helix with helix and strand with strand) and then fits CA-CA pairs in the same columns of the sequence alignment. By default, it iterates the fit so that far-apart pairs are not included in the final match. Match statistics are reported in the **Reply Log**. The sequence alignment is not shown by default, but can be displayed by adding **show true** to the above command.

Alternatively, the following produces exactly the same superposition:

> *Command*: match **iterate 2.0 #1@ca #0@ca**

different loop conformations



This command works because the structures have equal numbers of CA atoms, and the first CA atom in one structure correctly pairs with the first in the other, the second with the second, and so on. The **match** command does not generate a sequence alignment, so it is faster than **MatchMaker**. Another potential advantage is that it can use any atoms, not just CA. Its main disadvantage is that in most cases, one must figure out and specify which residues in one structure should pair with which residues in the other. While not used by default, iteration can be turned on, as shown above.

Either way, iteration is recommended so that similar parts will superimpose well and dissimilar parts will stand out better. These two structures are very similar except for the loop at approximately residues 51-56 (you can see residue numbers by hovering the cursor over the ribbon):

> *Command*: disp **:51-56**

Morphing involves calculating a series of intermediate, interpolated structures between the original input structures. In Chimera, the series of structures is treated as a trajectory that can be replayed, saved to a coordinate file, or saved as a movie using **MD Movie**.

Start the morphing tool:

Command: **start** **Morph Conformations**

Click **Add...** and in the resulting list of models, doubleclick to choose #0, #1, and #0 again, corresponding to a morph trajectory from the ligand-bound structure to the empty structure and back. **Close** the model list. In the main **Morph Conformations** dialog, set the **Action on Create** to **hide Conformations**, and then click **Create**.

The progress of the calculation is reported in the status line. When all the intermediate structures have been calculated, the input structures are hidden, the trajectory is opened as a third model (#2), and the **MD Movie** tool appears. The trajectory can be played continuously or one step at a time by clicking buttons on the tool, or a frame number can be entered directly.

The initial display style of the trajectory and which atoms are shown/hidden mimic the input structures, but in fact all atoms that are present in both input structures are also present in the trajectory. The coloring and display can be controlled just as in any other structure. For example, to show only aromatic amino acids along with the ribbon:

Command: **show** **#2 & aromatic**

Ligand residues were only present in one of the structures, so they are not included in the morph trajectory. However, part or all of the original structures can be displayed along with the trajectory. Display of the other models can be re-enabled by checking the **Shown** boxes in the **Model Panel** (**Tools... General Controls... Model Panel**) or with a command, for example:

Command: **modeldisp** **#0**

Then, to show only the ligand residues in that model:

Command: **~rib** **#0**
Command: **~disp** **#0**
Command: **disp** **:fps**

In this case, the secondary structure does not change much between the input structures. When conformational differences are larger and ribbons will be displayed, one may want to re-evaluate the secondary structure at each step of the trajectory. That can be done by putting the command **ksdssp** in a per-frame Chimera script in **MD Movie**.

The **Morph Conformations** page lists a few additional example systems.

When finished enjoying the morph trajectory, choose **File... Quit** from the menu to exit from Chimera.

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