Chimera Tutorials Index

Superpositions and Alignments Tutorial

In this tutorial, **MatchMaker** is used to align protein structures (create a superposition), **Match -> Align** is used to generate a multiple sequence alignment from the structural superposition, and **Morph Conformations** is used to morph between related structures.

Sequence alignments are displayed in **Multalign Viewer**, which is covered in more detail in the Sequences and Structures tutorial, and the morphing trajectory is displayed in **MD Movie**, which is covered in more detail in the Trajectory and Ensemble Analysis tutorial.

Internet connectivity is required to fetch the structures used in this tutorial: **1tad**, **121p**, **1r2q**, **1j2j**, **1puj**, **1tnd**, **1tag**

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← Background and Setup

Protein structures are classified within databases such as SCOP, CATH, and HOMSTRAD. Classifications range from groups of highly similar and closely related proteins to larger, more diverse sets. For analysis and comparison, it is often useful to superimpose related structures. Although it is not always clear whether proteins with the same fold are evolutionarily related (homologous), they should still be superimposable. In general, more closely related proteins are easier to superimpose.

G proteins (guanine nucleotide-binding proteins) are used as examples. G proteins are important in signal transduction. They act as molecular switches, changing conformation and interaction partners depending on whether GTP or GDP is bound. Many diverse structures are known. The two main subsets are the small monomeric G proteins, such as Ras, and the larger heterotrimeric G proteins, which act immediately downstream of G-protein-coupled receptors. The α subunits of heterotrimeric G proteins are homologous to the small G proteins.

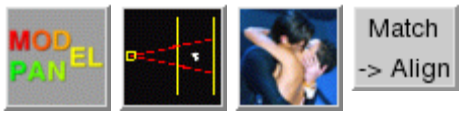
On **Windows**/**Mac**, click the **chimera** icon; on **UNIX**, start Chimera from the system prompt:

```
unix: chimera
```

A splash screen will appear, to be replaced in a few seconds by the main Chimera graphics window or **Rapid Access** interface (it does not matter which, the following instructions will work with either). If you like, resize the Chimera window by dragging its lower right corner.

Show the **Command Line** (**Tools... General Controls... Command Line**). Choose **Favorites... Add to Favorites/Toolbar** to place some icons on the toolbar. This opens the **Tools** section of the preferences, which lists the same tools as Chimera's **Tools** menu. In the **On Toolbar** column, check the boxes for:

- **Model Panel** (under General Controls)
- **Side View** (Viewing Controls)
- **MatchMaker** (Structure Comparison)
- **Match -> Align** (Structure Comparison)



You can also specify tools such as the **Command Line** to **Auto Start** (start when Chimera is started). If you want these settings to apply to subsequent uses of Chimera, click **Save** before closing the preferences.

Fetch a structure from the Protein Data Bank:

Command: **open 1tad**

The structure contains three copies of the α subunit of transducin, a heterotrimeric G protein. Delete solvent and two of the copies, chains B and C:

Command: **del solvent**
Command: **del :.b-c**

Move and scale the structures using the mouse and **Side View** as desired throughout the tutorial.

← Different but Related Proteins

We will superimpose structures of a sample of G proteins, then create a sequence alignment from the superposition.

The α subunit of the heterotrimeric G protein transducin was already opened in the setup. Fetch structures for the monomeric G proteins H-Ras, Rab5a, and ADP-ribosylation factor 1, respectively:

Command: **open 121p**
Command: **open 1r2q**
Command: **open 1j2j**

Use the ribbons preset (which may or may not change the appearance, depending on your preference settings):

Menu: **Presets... Interactive 1 (ribbons)**

This preset displays ribbons plus ions, ligands, and nearby sidechains.

← Superposition

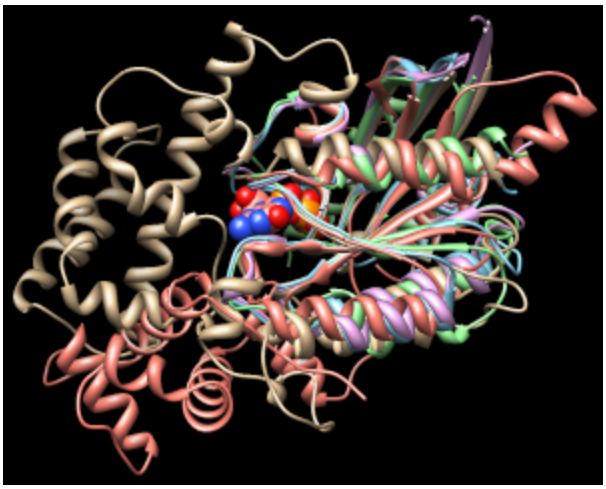
superimposed G proteins

The structures need to be superimposed so that they can be compared. Start **MatchMaker** by clicking its icon: 

MatchMaker superimposes structures pairwise by first aligning their sequences and then fitting the α-carbons of residues in the same columns of the sequence alignment. Usually the fit is iterated so that residue pairs aligned in sequence but far apart in space are not used in the final 3D match.

Several parameters control the sequence alignment step:

- algorithm - Needleman-Wunsch (global; default) or Smith-Waterman (local)
- scoring function -
  - residue similarity matrix (default BLOSUM-62)
  - whether secondary structure information should be used (default yes)
  - weighting of the secondary structure and residue similarity terms (default 30% and 70%, respectively)
  - gap penalties



Click **Reset to defaults** (near the bottom of the dialog) to ensure that the default settings will be used. All of the structures should already be chosen as the **Structure(s) to match**; keep that the same, but choose **1tad** as the **Reference** and click **Apply** to match all the others to it.

The number of α-carbon pairs and RMSD in the final iteration of each pairwise fit are reported in the **Reply Log** (in the menu under **Favorites**). However, simple visual inspection of the overall structures is often the most useful indicator of success.

Another visual indicator is how well similar ligands superimpose. Show only residues classified as ligand, and label them:

Command: **show ligand**
Command: **rlab ligand**

Each of these structures includes GTP or an analog of GTP in the binding site. However, some other ligands were simply present in the crystallization solution and are not biologically relevant. GOL is glycerol and can be removed:

Command: **del :gol**
Command: **~rlab**

Try using different reference structures in **MatchMaker** (click a line in the **Reference structure** list, click **Apply**). With the default alignment parameters, the superposition is similar and basically correct no matter which structure is used as the reference. Detailed examination of the match statistics and guanine nucleotide positions suggests results may be slightly better with **1r2q** as the reference.

Next, try a structure that is harder to superimpose, and display its ligand in the sphere representation:

Command: **open 1puj**
Menu: **Presets... Interactive 1 (ribbons)**
Command: **show ligand**
Command: **repr sphere ligand & #4**

Besides lacking sequence similarity, this protein is ***circularly permuted*** compared to the others: its N-terminal part structurally matches the C-terminal part of other G proteins and vice versa.

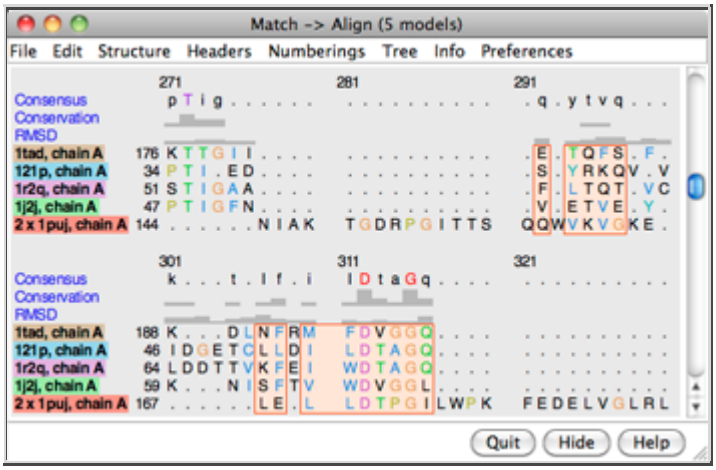
In the **MatchMaker** dialog, change the **Structure to match** to only **1puj** and try the others in turn as the reference. Again, ligand positions can be used to help gauge the match.

Trials with the default alignment parameters are not successful. When proteins are very distantly related, it may be useful to switch to a lower-number BLOSUM matrix and/or increase the proportion of secondary structure scoring. Usually a range of parameters will give similar results. For example, with **121p** as the reference structure, **1puj** can be superimposed as shown in the figure with any of BLOSUM 30-75 if secondary structure weighting is raised to 90% and the Smith-Waterman algorithm is used (leaving other settings as defaults). Keep in mind that when proteins are very distantly related, their backbones may diverge even in the best possible superposition.

← Structure-Based Sequence Alignment

When all five proteins are superimposed to your satisfaction, **Cancel** the **MatchMaker** dialog. We will generate a structure-based alignment of the five sequences using **Match -> Align**; start that tool by clicking its icon: 

Match -> Align uses only the distances between α-carbons to create an alignment. Residue types and how the structures were superimposed are not important. All of the A chains should already be chosen in the dialog; the B chain of **1j2j** is an unrelated peptide and should not be chosen. Use a cutoff of **5.0** Å, specify **Residue aligned in column if within cutoff of [at least one other]**, and turn on **Allow for circular permutation**. Click **OK** to start the calculation.



It may take a minute or two to create the alignment; progress is reported in the status line. When the calculation is finished, the new alignment will be displayed in **Multalign Viewer** and can be saved to a file from that tool.

The output multiple sequence alignment (example: 5gees.afa) shows that **1puj** was correctly recognized as a circular permutation relative to the others. **Match -> Align** doubled its sequence to allow C-terminal residues (in the first copy of the sequence) to appear before more N-terminal residues (in the second copy) within the alignment. The columns with residues from all five structures are highlighted as a region in light orange with dark orange outline. Clicking the region will select the corresponding parts of the structures, in effect their common cores. The alignment header named **RMSD** shows the spatial variation per column (α-carbon root-mean-square distance).

Keep the sequence alignment, but close most of the structures:

1. start the **Model Panel** by clicking its icon: 

2. in the **Model Panel**, choose all of the models except **1tad** on the left side and click the **close** button on the right (not at the bottom of the dialog!)
3. **Close** the **Model Panel**

If **Multalign Viewer** (the sequence alignment window) is hidden behind other windows, it can be

resurrected by choosing **MAV - *alignment-name*... Raise** from near the bottom of the **Tools** menu. In **Multalign Viewer**:

- choose **Preferences... Appearance** and adjust settings for **Multiple alignments** as desired
- use **Info... Percent Identity** to compare **all sequences** with **all sequences**, confirming that the pairwise identities are <30% for these examples
- use **Edit... Delete Sequences/Gaps** to delete the sequence named **2 x 1puj, chain A** and any resulting all-gap columns

Now the alignment clearly shows the large insertion in α-transducin (**1tad**) relative to the small monomeric G proteins. Select and display residues that are completely conserved in the sequence alignment:

Command: **sel :/mavPercentConserved=100**
Command: **disp sel**

Some of the conserved residues are Gly (no sidechain). Clear the selection by Ctrl-clicking in an empty area of the graphics window.

← Different Conformations of the Same Protein

(To jump to this section right after performing the setup, open the sequence alignment file 4gees.afa included with this tutorial.)

Now we will compare **1tad** with different structures of the same protein, transducin-α:

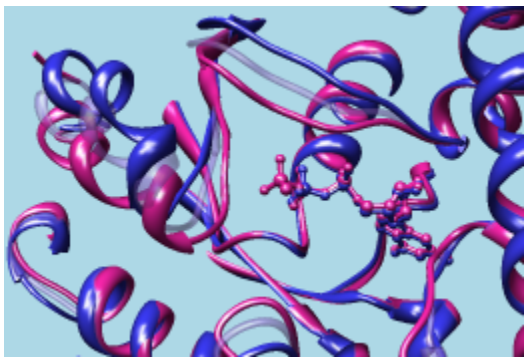
Command: **open 1tnd**
Command: **open 1tag**

Delete solvent and chains B-C (extra copies in **1tag**):

Command: **del solvent**
Command: **del :.b-c**

If **Multalign Viewer** (the sequence alignment window) is hidden, bring it to the front by choosing **MAV - *alignment-name*... Raise** from near the bottom of the **Tools** menu.

GTP-binding switch
(**1tagA**, **1tndA**, morph intermediate)



Multalign Viewer displays lines of information called headers above the sequences in the alignment. Use the **Headers** menu to hide **Consensus** and **Conservation** and to show **RMSD**, if not already shown. The sequence name **1tad, chain A** has a dashed green line around it, indicating that the sequence is associated with multiple structures. The **RMSD** header shows the spatial variability of residues associated with each column (α-carbon root-mean-square distance); currently, it contains high values everywhere because the structures are not all superimposed.

To superimpose the structures using the sequence alignment, choose **Structure... Match** from the **Multalign Viewer** menu. One structure (it does not matter which) should be designated as the reference, and all three can be designated as the structures to match. Check the option to **Iterate by pruning...** using a **2.0**-Å cutoff and click **OK**. The **RMSD** header is automatically recomputed, showing much lower values.

Superposition of proteins with the same or nearly the same sequence is generally trivial. We used

Multalign Viewer since we already had a sequence alignment, but **MatchMaker** (or its command equivalent) or the command **match** could have been used instead. These other methods are used and discussed in the Structure Analysis and Comparison tutorial.

Use the ribbons preset (which may or may not change the appearance, depending on your preference settings) and focus on the ligand residues:

Command: **preset apply int 1**
Command: **focus ligand**
Command: **rlab ligand**

Open the **Model Panel** and use the **S**(hown) checkboxes to view the structures individually.

The **1tad** structure (tan) represents the activated form of a G protein; even though it includes GDP, the GDP and ALF (AlF_4^-) residues together mimic the transition state of GTP hydrolysis. **1tnd** (light blue) contains the GTP analog GSP and also represents the activated form. The third structure, **1tag** (purplish pink), includes GDP and represents the nonactivated form.

Use the **Model Panel** checkboxes to show all three structures together. Remove the labels and focus on the overall structures:

Command: **~rlab**
Command: **focus**

Although the structures are mostly similar, the nonactive conformation (pink) differs from the activated ones (tan and light blue) in specific areas, termed ***switch regions***.

In the sequence alignment window, the three most prominent "humps" in the **RMSD** header correspond to the known G protein switch regions at approximately residues 173-183, 195-215, and 227-238 of transducin-α. The third switch region is unique to heterotrimeric G proteins; it is an insertion relative to the monomeric G proteins. Placing the cursor over a position in the **1tad** sequence lists the associated structure residues near the bottom of the sequence window, and drawing a box around residues in the sequence alignment (click to start, drag to expand) selects the associated parts of the structures.

Close **1tad**:

Command: **close 0**

The RMSD histogram looks much the same; now it simply shows the CA-CA distances between the two remaining structures, **1tnd** representing the activated form and **1tag** representing the nonactivated form.

← Morphing

Finally, morph between the two structures. Morphing involves calculating a series of intermediate 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 #2, #1, and #2 again,

corresponding to a morph trajectory from the nonactivated structure to the activated 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 model #0, and the **MD Movie** tool appears.

The trajectory can be played continuously or one step at a time using the buttons on the tool. If the player dialog becomes obscured by other windows, it can be resurrected by choosing **MD Movie - *trajectory-name*... Raise** from near the bottom of the **Tools** menu. If you want to see the original structures again, use the **S**(hown) checkboxes in the **Model Panel**.

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

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