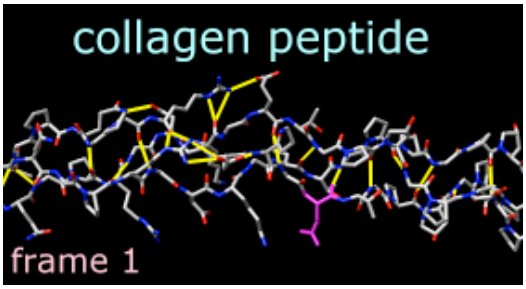
Trajectory and Ensemble Analysis Tutorial

This tutorial focuses on visualization and analysis of molecular dynamics (MD) trajectories and other structural ensembles with the **MD Movie** tool. Part 1 uses an MD trajectory of a collagen peptide, and Part 2 uses an NMR ensemble of Met-enkephalin.

Part 1 - Collagen Peptide

We will view an MD trajectory of the nonmutant collagen peptide described in:

> R. J. Radmer and T. E. Klein, "Severity of Osteogenesis Imperfecta and Structure of a Collagen-like Peptide Modeling a Lethal Mutation Site" *Biochemistry* **43**:5314 (2004).



(Thanks to the authors for providing the data!) To follow along, download the data files:

- leap.top - **Amber** parameter/topology file
- md01.crd - **Amber** trajectory file
- collagen.meta - metafile specifying these input files for **MD Movie**

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. Show the **Command Line** (**Tools... General Controls... Command Line**) and start **MD Movie** (**Tools... MD/Ensemble Analysis... MD Movie**). In the resulting dialog, the inputs can be specified in two different ways:

- by setting the **Trajectory format** to **Amber** and browsing to the **Prmtop** file leap.top and the **Trajectory** file md01.crd
- by setting the **Trajectory format** to **metafile** and browsing to the file collagen.meta (it must be in the same directory or folder as the other two files). It contains the following lines, which simply specify the options and filenames that would otherwise be entered into the dialog:

```
amber
leap.top
md01.crd
```

Once the inputs have been specified, click **OK**. The first set of coordinates will be displayed and the **MD Movie** controller will appear. Move and scale the structure as desired throughout the tutorial.

Show the structure with sticks and ribbons, color by element, and undisplay the hydrogens:

```
Command: repr stick
Command: ribbon
Command: ribrepr smooth
Command: col byhet
Command: ~disp H
```

The structure contains three chains. Each chain is in a left-handed polyproline II helix conformation, and together the chains form the right-handed triple helix characteristic of fibrillar collagen. The

ribbons are narrow because the peptides are not in a standard alpha-helical conformation.

MD Movie controller buttons

Use the **MD Movie** controller to play the trajectory. From left to right, the buttons mean: play backward continuously; go back one step; stop; go forward one step; and play forward continuously. The rate of continuous play can be adjusted with the **Playback speed** slider. The **Frame** number is reported and can also be entered directly to view a specific frame. **Frame** number and **Step size** changes take effect when return (**Enter**) is pressed. If the controller becomes obscured by other windows, it can be raised using its instance in the **Tools** menu (near the bottom of the menu, below the horizontal line).

Show the amino acid sequence with **Tools... Structure Analysis... Sequence** (pick any one of the three chains). Fibrillar collagen typically contains many -Gly-X-Y- repeats, where X is often Pro (proline) and Y is often Hyp (hydroxyproline). Both Pro and Hyp are shown as P in the sequence panel. Selecting residues highlights both the sequences and the structures:

Command: **sel :gly**
Command: **sel :pro**
Command: **sel :hyp**

Quit from the sequence panel.

It may be useful to hold certain atoms steady during trajectory playback. For example, hold Glu-86 steady to view its interactions:

Command: **sel :86**
(from the controller menu) **Actions... Hold selection steady**
Command: **color magenta sel**
Command: **~sel**

Even though it is no longer selected, residue 86 will be held steady during playback (as possible; there will still be internal motions) until **Hold selection steady** with a different selection or **Stop holding steady** is used. The structure can still be moved with the mouse, however. Try playing the trajectory with residue 86 held steady and then without holding any atoms steady (from the controller menu, choose **Actions... Stop holding steady**).

We will create a short movie of several frames in the trajectory. The following procedure is just one example; there are many possibilities of what to show, how to show it, whether to use a script, and so on.

Adjust the Chimera window to the dimensions desired for the movie. If needed, use the **Side View** (**Tools... Viewing Controls... Side View**) to adjust the clipping planes. Turn off the ribbon to reveal the backbone atoms:

Command: **~ribbon**

Use **2D Labels** to add a title (**Tools... Utilities... 2D Labels**). When the **Mouse** setting in the **2D Labels** dialog is **labeling**, the left mouse button (button 1) is reassigned to labeling: clicking starts a new 2D label and previously created 2D labels can be repositioned by dragging. Click in the Chimera window where you would like to start a title and type in the title text; drag the text if you want to reposition it. Adjust the **Font size** and **Color** (click the color well, use the **Color Editor**) to your liking. Changing the **Mouse** setting to **normal** returns the left mouse button to its previous function (by default, rotation of models).

Create another 2D label, this time using the **2dlabels** command so that the label will have a name:

Command: **2dlabels create mylabel text temp**

This label will be used to display the frame number. Make sure that the **Mouse** setting in the **2D Labels** dialog is **labeling**, then drag the temporary text to near the lower left corner of the Chimera window. Adjust its **Font size** and **Color** to your liking, then **Close** the **2D Labels** dialog.

Next, define a script to execute at each frame. Halt any playback. From the **MD Movie** controller menu, choose **Per-Frame... Define script**. Enter a script to be interpreted as **Chimera commands**:

```
findhbond linewidth 2 color yellow
2dlabels change mylabel text "frame <FRAME>"
```

Uncheck the option to **Use leading zeroes...** This script will calculate the hydrogen bonds in each frame, show them as yellow lines, and display the current frame number in the label named **mylabel**. Click **OK** to dismiss the dialog with the script. Play a few steps by clicking the button to go forward or backward one step at a time. The number and arrangement of H-bonds vary somewhat from step to step. (Although the number of H-bonds cannot be accessed in **Chimera commands**, a **Python** script could be used to display this information. For example, hbcount.py would show the H-bond count instead of the frame number in **mylabel**.)

Halt any playback, but move the **Playback speed** slider all the way to the right. From the controller menu, choose **File... Record movie**. If a dialog with an MPEG license agreement appears, click **Accept** since the movie will not be used for commercial purposes. In the dialog for recording,

- choose a **File type** you will be able to play back on your computer (the choices are MPEG-1, MPEG-2, MPEG-4, AVI MSMPEG-4v2, and Quicktime)
- specify a convenient name and location for the output file
- change the **Ending frame** to 25
- click **Record**

Frames 1-25 will then be played, saved as images, and automatically assembled into a movie file. (***Do not obscure any parts of the Chimera window while this is occurring***.) View the resulting 1-second movie with the appropriate application on your computer.

Click **Quit** on the controller to close the trajectory and exit from **MD Movie**. An easy way to delete all of the 2D labels is with **File... Close Session**. Go on to **Part 2** below, **OR** terminate the Chimera session:

```
Command: stop
```

Part 2 - Met-Enkephalin

We will view an NMR ensemble of Met-enkephalin in negatively charged bicelles, as described in:

> I. Marcotte, F. Separovic, M. Auger, and S. M. Gagne, "A Multidimensional ^{1}H NMR Investigation of the Conformation of Methionine-Enkephalin in Fast-Tumbling Bicelles" *Biophys J* **86**:1587 (2004).

To follow along, download the data file 1plx.pdb.

With Chimera started and the **Command Line** shown (as in Part 1), choose **Tools... MD/Ensemble Analysis... MD Movie**. In the resulting dialog, choose **PDB** as the **Trajectory format** and indicate that the frames are contained in a **single file**. Browse to the file 1plx.pdb, set the input location, and then click **OK**.

The first set of coordinates will be displayed and the **MD Movie** controller will appear. Show the structure with sticks colored by element:

Command: **repr stick**
Command: **col byelement**

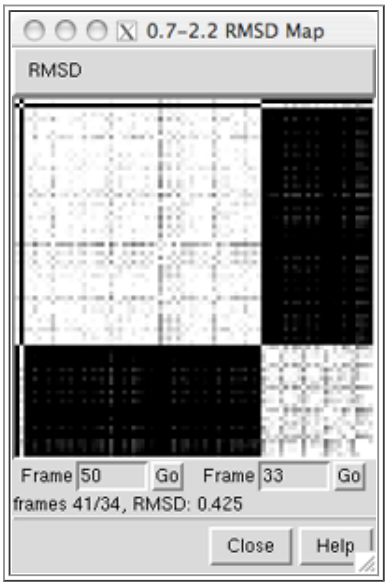
Move and scale the structure as desired throughout the tutorial. This structure is Met-enkephalin, with the sequence Tyr-Gly-Gly-Phe-Met. Enkephalins are neuropeptides that activate opioid receptors. Different subtypes of opioid receptors mediate different but overlapping responses in the body. For example, molecules that selectively activate μ-opioid receptors are more effective for treating severe pain than molecules that selectively activate δ-opioid receptors, but are also more likely to cause constipation. The conformations of molecules that bind opioid receptors (enkephalins, morphine, *etc.*) are of interest because they influence the selectivity of receptor binding and thus the physiological response.

Use the **MD Movie** controller to flip through the different conformations, as described above. The frames do not reflect time ordering, as this is an NMR ensemble rather than a trajectory. If desired, simplify the view by undisplaying hydrogens:

Command: **~disp H**

It is thought (see the reference and papers cited therein) that a conformation of enkephalin in which the Tyr and Phe rings point in different directions (like frames 1 and 25) binds to μ-opioid receptors and a conformation in which they point toward each other (like frames 2 and 80) binds to δ-opioid receptors.

One way to analyze the ensemble is to calculate root-mean-square deviations (RMSDs) between pairs of frames. From the controller menu, choose **Analysis... RMSD map**. Click **Apply** on the RMSD parameters dialog to perform the calculation without closing the dialog. This will compute all pairwise RMSDs between frames and show the result as a map in grayscale. After the initial calculation, the map will be recolored to enhance contrast.



In the RMSD map, the axes are frame numbers; lighter squares reflect pairs of frames with lower RMSDs (more similar structures) and darker squares reflect pairs of frames with higher RMSDs (less similar structures). Mousing over the map shows the RMSD values and the numbers of the frames being compared. Clicking on the map enters the corresponding pair of **Frame** numbers below the map. Clicking **Go** then displays that frame in the main Chimera window.

Roughly, the upper left block of white in the map represents conformations more similar to to a μ-binding conformation, and the lower right block of white represents conformations more similar to a δ-binding conformation. Similar conformations are mostly grouped together in this ensemble, but will not necessarily be grouped together in ensembles in general. Also, ensembles usually contain many more groups of conformations, especially for larger structures with more degrees of freedom.

All atoms were used in the RMSD calculation, because although the parameter **Restrict map to current selection, if any** was set to **true**, nothing was selected. Select the nonhydrogen backbone atoms only,

Command: **sel @n,ca,c,o**

and this time click **OK** to dismiss the RMSD parameters dialog and perform the calculation. Although the two maps span different ranges in RMSD, they reveal essentially the same groups of conformations. **Close** both maps.

Another way to analyze the ensemble is to calculate spatial occupancy maps for atoms of interest. From the controller menu, choose **Analysis... Calculate occupancy**. The resulting dialog will show the warning message "No atoms being held steady." This means you have not previously selected a set of atoms and chosen **Actions... Hold selection steady** from the controller menu for this ensemble.

Why might it be useful to hold atoms steady while calculating occupancy? The contents of different frames may move around enough to obscure certain spatial patterns. Even if the structure as a whole is held fairly steady, one may want to hold a particular set of atoms (such as a sidechain) steady to examine local interactions. However, if the structure or region of interest is already sufficiently steady, the "hold steady" step can be omitted.

One approach to analyzing the relative positions of the aromatic rings is to hold the Phe ring atoms steady and map the occupancy of the Tyr ring atoms:

1. Select the six Phe ring atoms. One way is to pick them from the screen (Ctrl-click on one, Shift-Ctrl-click on each of the other five). Another way is with a command:

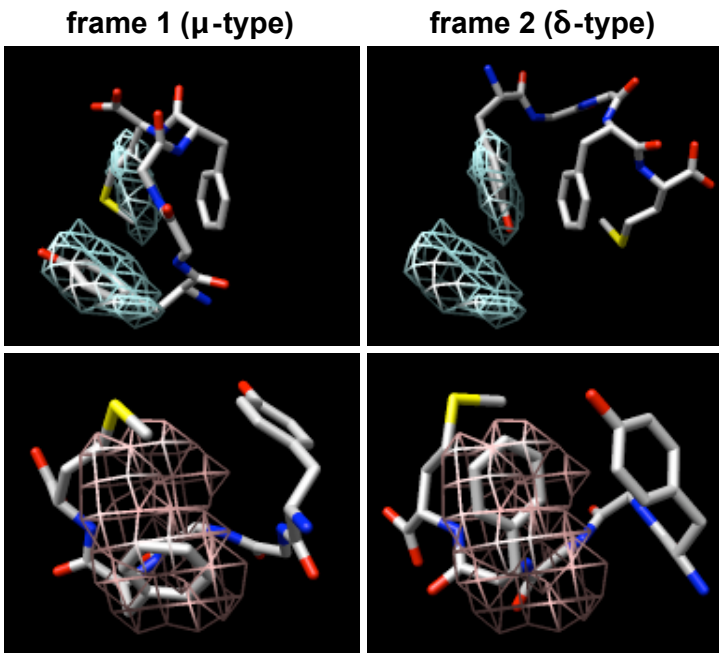
   Command: **sel :phe & aromatic ring**

2. Choose **Actions... Hold selection steady** from the controller menu.
3. Select the six Tyr ring atoms, by picking or with a command:

   Command: **sel :tyr & aromatic ring**

4. If the occupancy dialog is not already up, choose **Analysis... Calculate occupancy** from the controller menu.
5. Click **OK** on the occupancy dialog.

When the map has been computed, the **Volume Viewer** tool will appear. This tool shows a histogram of the values in the map; the slider controls what contour level is displayed.



Clear the selection in Chimera (**Select... Clear Selection**). In the **Volume Viewer** tool, change from **surface** to **mesh** and move the slider to a value of approximately 2. If necessary, use the **Side View** (**Tools... Viewing Controls... Side View**) to move the clipping planes so that all of the mesh is visible. Optionally, change the color of the mesh surface by clicking the color well below the histogram and using the **Color Editor**. (**Volume Viewer** is complex tool with many features. For more on this tool, see the **Volume Viewer** manual page and the Chimera tutorials on the web.)

You will see two main blobs or volumes representing probable positions of the Tyr ring relative to

the Phe ring (similar to the upper images). The larger volume represents μ-type conformations and the smaller represents δ-type conformations.

If desired, flip through the ensemble (still holding the Phe ring steady) to verify that the volumes show areas occupied by the Tyr ring. To delete the volume display, choose **File... Close map** from the **Volume Viewer** menu.

Finally, calculate a map without holding any atoms steady. Choose **Actions... Stop holding steady** from the controller menu. Since this ensemble maintains the Tyr ring in roughly the same place, simply map the occupancy of the Phe ring atoms. Select the Phe ring atoms (as above), choose **Analysis... Calculate occupancy**, and click **OK**. This time, the conformations are not as well separated; two lobes of occupancy are apparent at a contour level of approximately 2 (similar to the lower images). One lobe represents μ-type conformations and the other represents δ-type conformations.

When finished with the Met-enkephalin ensemble, quit from Chimera (**File... Quit**).

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