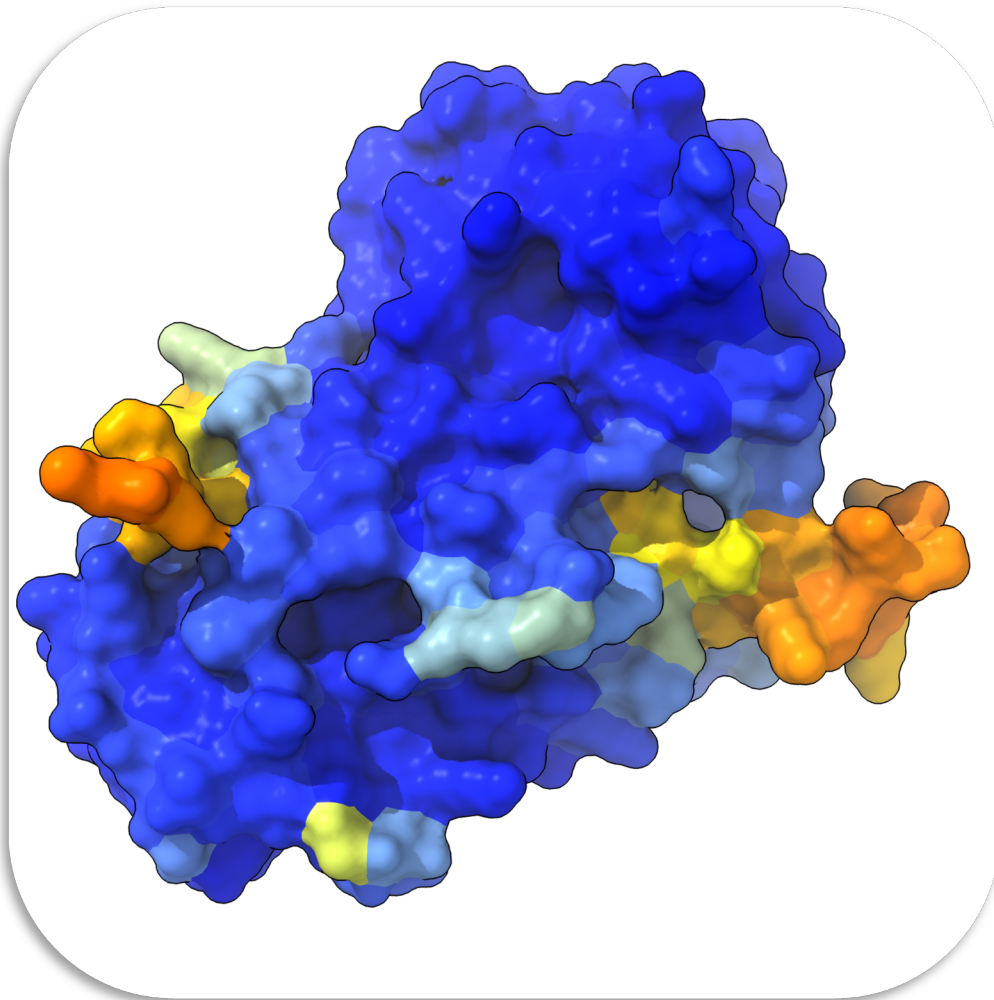


Practice Session 7: Analysis of Predicted Structures



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Green boxes contain commands to type in the command line interface.

Pink boxes contain important notes and troubleshooting hints.

Yellow boxes contain notes that allow users to explore possibilities beyond those exposed in the main practice or to provide additional information not strictly necessary to complete the exercises.

In this exercise, we will work with the heme enzyme indoleamine 2,3-dioxygenase 1 (IDO1), which plays an essential role in immunity, neuronal function, and aging through catalysis of the rate-limiting step in the kynurenine pathway of tryptophan metabolism. Many IDO1 inhibitors with different chemotypes have been developed, mainly for use in anti-cancer immunotherapy.

Exercise 9: Visualizing a Predicted Protein Structure

ChimeraX provides support for different structure prediction methods such as Modeller, AlphaFold, Boltz, ESMFold, and OpenFold. Concerning AlphaFold, ChimeraX can *fetch* models from the AlphaFold database, find models with a similar sequence as an opened structure in the AlphaFold database using a BLAST *search*, and *predict* new structures using AlphaFold on Google servers. At present however, these functionalities use AlphaFold version 2, and not version 3, which also provides modeling of complexes of proteins, nucleic acids, and ligands.

In this exercise, we will compare an experimentally resolved structure of IDO1 bound to heme and an inhibitor with its apo-protein model from the AlphaFold database.

Loading a Structure and Splitting it into Submodels

Open the structure of the complex of IDO1 bound to heme and the inhibitor MMG-0358 from the Protein Data Bank (PDB ID 6r63) using your favorite method, for example by typing the following in the command line.

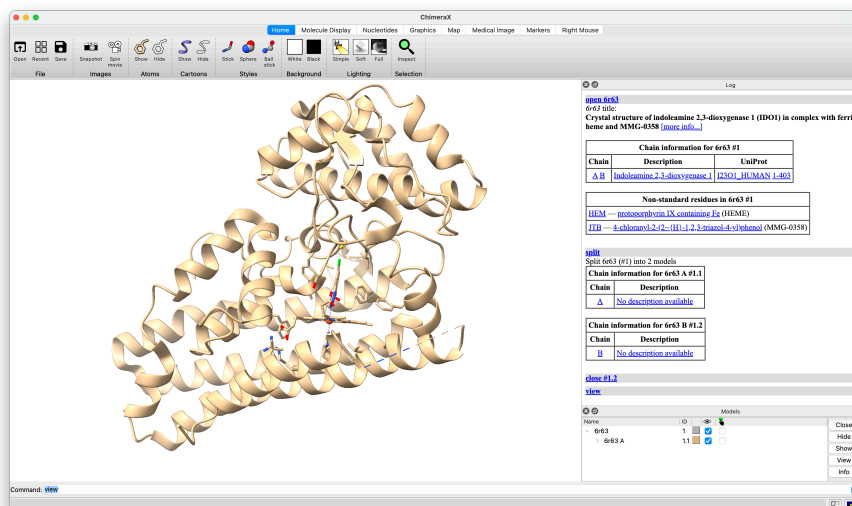
```
open 6r63
```

Then type the following command in the command line:

```
split
```

This is a useful command that splits a model into separate sub-models by protein chain. In the “Models” panel, it can be seen that the structure 6r63 (model #1), which contains two chains of IDO1, is now separated into two sub models #1.1 and #1.2.

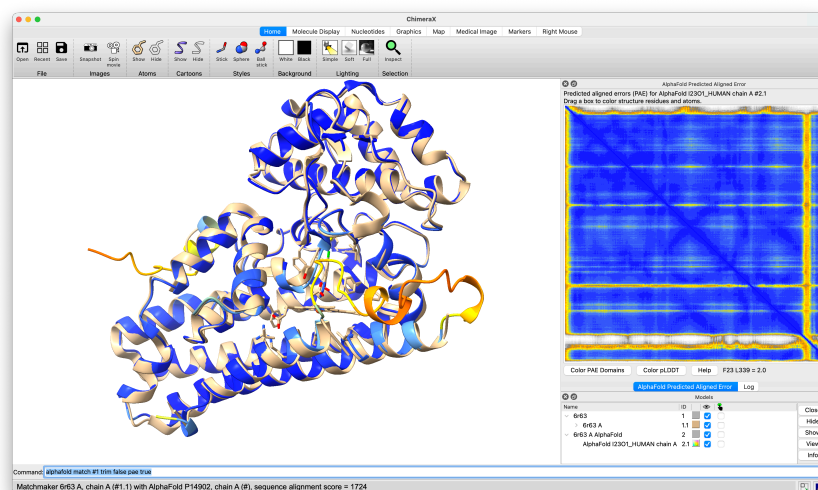
Now close model #1.2 (chain B) by selecting its line in the **Models** window and choosing “**close**”.



Opening a Predicted Protein Structure

In the command line, type `alphafold match #1 trim false pae true`. This will search the AlphaFold database for a structure corresponding to the sequence of the structure in model #1, open the structure and superimpose it to model #1. It will keep also residues that are not resolved in model #1 ("trim false") and open the predicted aligned error ("pae true") plot.

Attention, this command may not work in older ChimeraX versions (<1.11.). Please update ChimeraX in that case.



First hide model #1 by unselecting its box under the eye in the Model panel, so that only the predicted structure (model #2) is displayed.

If the AlphaFold sequence search web service from UCSF is not reachable, please download the [files](#) AF-P14902-F1-model_v6.cif and AF-P14902-F1-predicted_aligned_error_v6.json from the course website and save them on your computer. The first one (.cif) contains the model and can be opened as seen before. The second (.json) contains the PAE information and has to be associated to the model. This can be done with the following command: `alphafold pae #2 file ~/Downloads/AF-P14902-F1-predicted_aligned_error_v6.json` where the path to your downloaded file must be given.

By clicking on the AlphaFold Predicted Aligned Error window and drawing a rectangle, the corresponding residues will be colored in green in the structure, while all other residues will be colored in grey. When clicking on **Color pLDDT**, the structure will be colored according to the per residue confidence measure with blue being the most confident and red the least confident.

Model Confidence ^	
	Very high (pLDDT > 90)
	High (90 > pLDDT > 70)
	Low (70 > pLDDT > 50)
	Very low (pLDDT < 50)

You can learn more about the confidence measures by clicking on the **Help** button in the AlphaFold Predicted Aligned Error window or here: <https://alphafold.ebi.ac.uk/faq#faq-12>.

After familiarizing yourself with the AlphaFold model and the PAE window, color by pLDDT value and display also model #1 again by ticking its box under the eye in the Model panel,

-  *What parts of the AlphaFold model have the lowest confidence (low pLDDT values)? Give their approximate residue numbers. How do the structures of these regions compare to the experimental structure? In the environment of the inhibitor, does the AlphaFold model have a low or high confidence? Can you explain your observation?*

Save this Chimera session if you would like to come back to it, then close it.

Exercise 10: Visualizing Predicted Protein-Ligand Complexes

In this exercise, we will analyze structures of IDO1-ligand complexes predicted with OpenFold (<https://openfold.io/>), a free and open-source software, and compare them to experimentally resolved structures.

17 apo-IDO1 inhibitors were selected, which bind to IDO1 in absence of the heme co-factor, and for which experimental structures are available in the protein data bank. For each of these ligands, OpenFold produced a structure associated with an ipTM score, and a file to generate the error plot.

The ligand with the best ipTM score, which was not part of the training set, was the ligand with PDB ID A1JLA (0.92). The worst ipTM score was obtained by the ligand with PDB ID 6IZ (0.79). As the installation of OpenFold in ChimeraX may be problematic depending on your hardware, the calculations were performed separately and the results for these two ligands in complex with IDO1 are provided.

Download the file [ido1-predicted.zip](#) from the course website and uncompress it on your computer.

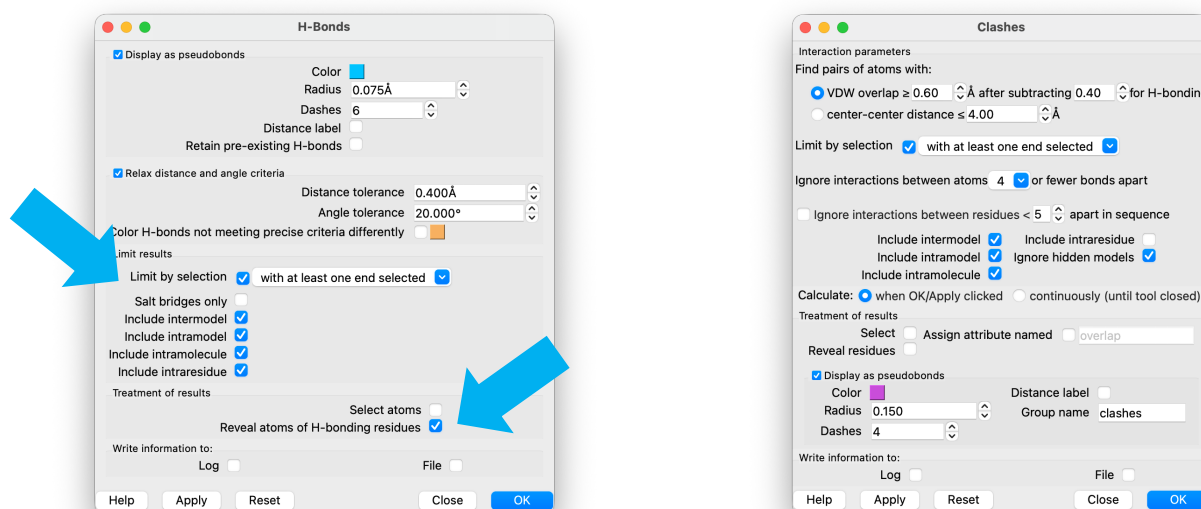
Please note that both ligands carry the name LIG0 in the predicted structures.

Opening the Predicted Complex Structure of IDO1 with A1JLA

Open the model of ligand A1JLA in IDO1 with filename A1JLA_model.cif with ChimeraX. Associate the error file to this structure by typing the following command in the command line, using the correct path for your file:

```
alphafold pae #1 file ~/Downloads/ido1-predicted/A1JLA_confidences.npz
```

Click on “Color pLDDT” in the new window to color all residues by their pLDDT value. Select the ligand and calculate its hydrogen bonds to the protein (> **Tools > Structure Analysis > H-Bonds**). Also calculate its clashes with the protein (> **Tools > Structure Analysis > Clashes**).



For better visualization, you can display the ligand in ball-and-stick representation and color its non-carbon atoms by element (> **Actions** > **Color** > **By Heteroatom**).

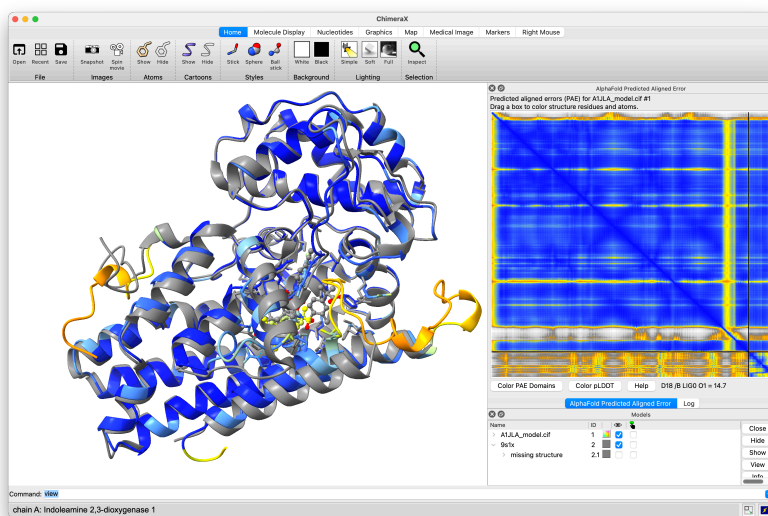
🔗 *How many hydrogen bonds and clashes does the ligand form?*

The experimental co-crystal structure of IDO1 with A1JLA has the PDB ID 9S1X. Open this structure, superimpose it to the predicted model (> **Tools** > **Structure Analysis** > **Matchmaker**) and assess the quality of the predicted model.

Apply some commands learned earlier to facilitate the visualization of the comparison between the structures. As a hint, you can, for example, type the following sequence of commands in the command line:

```
delete H I solvent I :PEG
color #2 grey
hide #2
show :A1JLA :<5
style :A1JLA ball
```

```
#delete hydrogens, solvent, and other ligands
#color model #2 in grey
#hide all atoms of model #2
#show all residues within 5 Å of the ligand
#show the ligand in ball-and-stick
```



Switch off the cartoon representation to obtain a detailed view of the ligand binding (**Home tab** > **Cartoons** > **Hide**).

🔗 *In the predicted model, does the ligand bind to the correct binding pocket? How well does it superimpose with the native ligand structure? How well does the protein structure agree between the model and the experimental structure?*

Save this ChimeraX session, then close it.

Opening the Predicted Complex Structure of IDO1 with 6IZ

Now do the same exercise for ligand 6IZ by visualizing its predicted model with IDO1 with filename 6IZ_model.cif with ChimeraX. Color the model by pLDDT and display the ligand's hydrogen bonds and clashes

The co-crystal structure of IDO1 with 6IZ has the PDB ID 7RRD. Open this structure and keep only its chain A, as seen above. Superimpose the experimental structure to the predicted model and assess the quality of the predicted model.

- 🔗 *In this predicted model, does the ligand bind to the correct binding pocket? How well does it superimpose with the native ligand structure? How well does the protein structure agree between the model and the experimental structure?*

Save this ChimeraX session. You can now open the first session again to compare the results. Unfortunately, the error window will not be saved in the session, but you can open it again with the command shown above.

If you want to come back to the coloring by pLDDT independently of the PAE window, you can use the following command: `color bfactor <spec> palette alphafold`

- 🔗 *When comparing these two structure predictions, does the calculated confidence (pLDDT and ipTM) correlate with their structural correctness? Assess both the protein and the ligand structures and explain your assessment.*

Bonus Exercise 11: Predicting a Protein-Ligand Complex with OpenFold in ChimeraX

The installation of OpenFold from within ChimeraX requires about 3 GB of disk space and ~10 min of installation time. A complete guide to using OpenFold from ChimeraX can be found here: <https://www.rbvi.ucsf.edu/chimerax/data/openfold-feb2026/openfold.html>

You can try a co-folding of your favorite system, or you can try to reproduce one of the co-foldings from this exercise, using IDO1 (UniProt identifier P14902) and one of the ligands (CCD codes A1JLA or 6IZ) as input molecules.

Please note that these calculations will run much faster on a GPU than on a CPU.