PROTEIN STRUCTURE PREDICTION WITH ALPHAFOLD



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Protein Structure Prediction with AlphaFold

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Protein Structure Prediction Activity

About this activity

Overview

In this activity, students use AlphaFold to predict a protein structure and evaluate confidence in the prediction using pLDDT, PAE, and pTM scores. Students then use UCSF ChimeraX to compare the predicted structure with an experimentally determined structure from the Protein Data Bank and evaluate structural similarity using RMSD values.

Recommended audience: Undergraduate Biochemistry / Structural Biology

Estimated time: one class period (65 min)

Required resources: AlphaFold Server, UCSF ChimeraX
Prerequisites: Basic understanding of protein primary, secondary, and tertiary structure

Learning objectives:

By the end of this activity, students will be able to:

1. Generate a protein structure prediction from an amino-acid sequence using AlphaFold.

2. Interpret pLDDT, PAE, and pTM as measures of confidence in a predicted structure.

3. Use ChimeraX to visualize and align predicted and experimentally determined protein structures.

4. Interpret RMSD as a measure of structural similarity.

5. Evaluate strengths and limitations of computational protein-structure prediction.

1. Predict protein structure from sequence using AlphaFold:

· Go to **AlphaFold Server**.

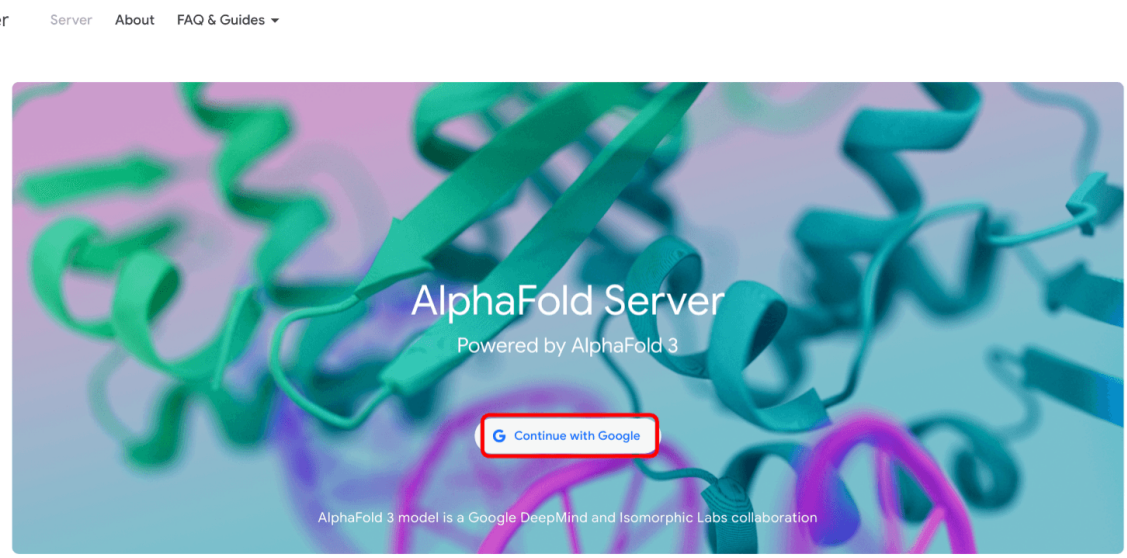
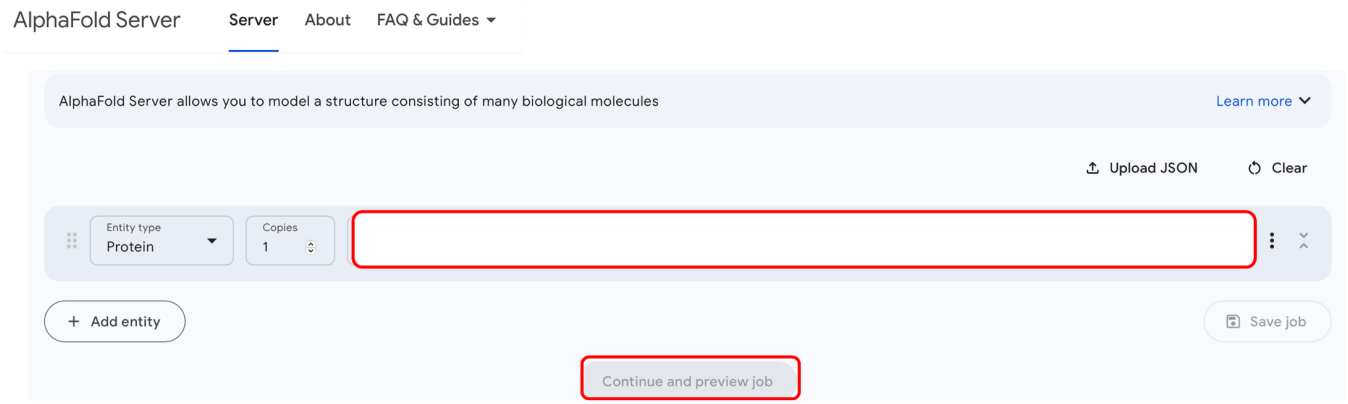


Figure 1. AlphaFold Server. *AlphaFold Server is a web-based service for predicting biomolecular structures containing proteins, DNA, RNA, ligands, and ions, and can model chemical modifications of proteins and nucleic acids. The server is powered by the AlphaFold 3 model. Screenshot of AlphaFold Server; annotations added by the author.*

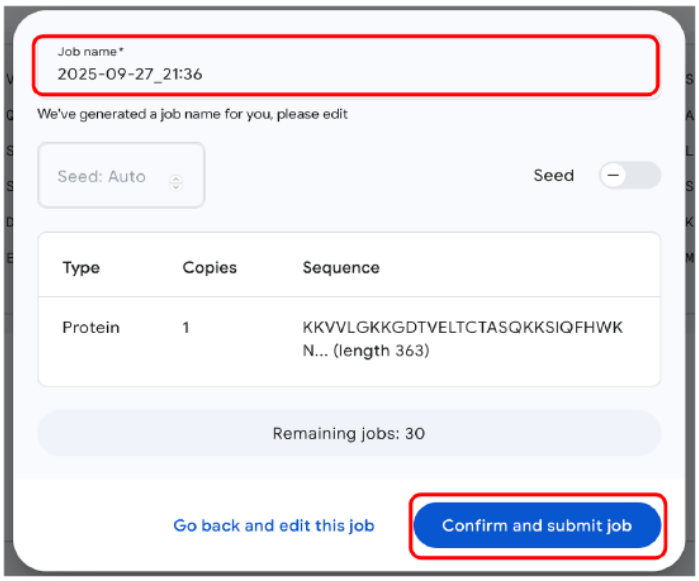
- Click on **Continue with Google** button and sign in with your Google account.
- You will use the following sequence to predict protein 3D structure using AlphaFold:

KKVVLGKKGDTVELTCTASQKKSIQFHWKNSNQIKILGNQGSFLTKGPSKLNDRADSRRSLWDQGNFPLIIKNLKIEDSDTYICEVEDQKEEV

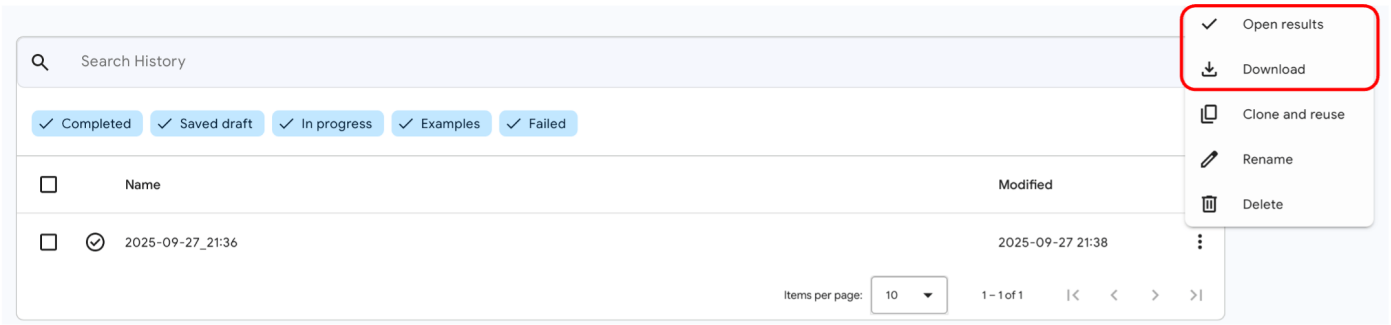
- Go to **Server** tab and input the sequence in the box and click on **Continue and preview job**:



- Update the job name and click on **Confirm and submit job**:



- After the prediction is completed (as indicated by a checkmark next to the job name) you can **Open** and **Download** results. Make sure to also Save the downloaded results for later use.



- Insert the obtained results below, including the predicted three-dimensional structure of your protein and the PAE plot.
- Explain and analyze the following confidence metrics for the predicted protein structure:

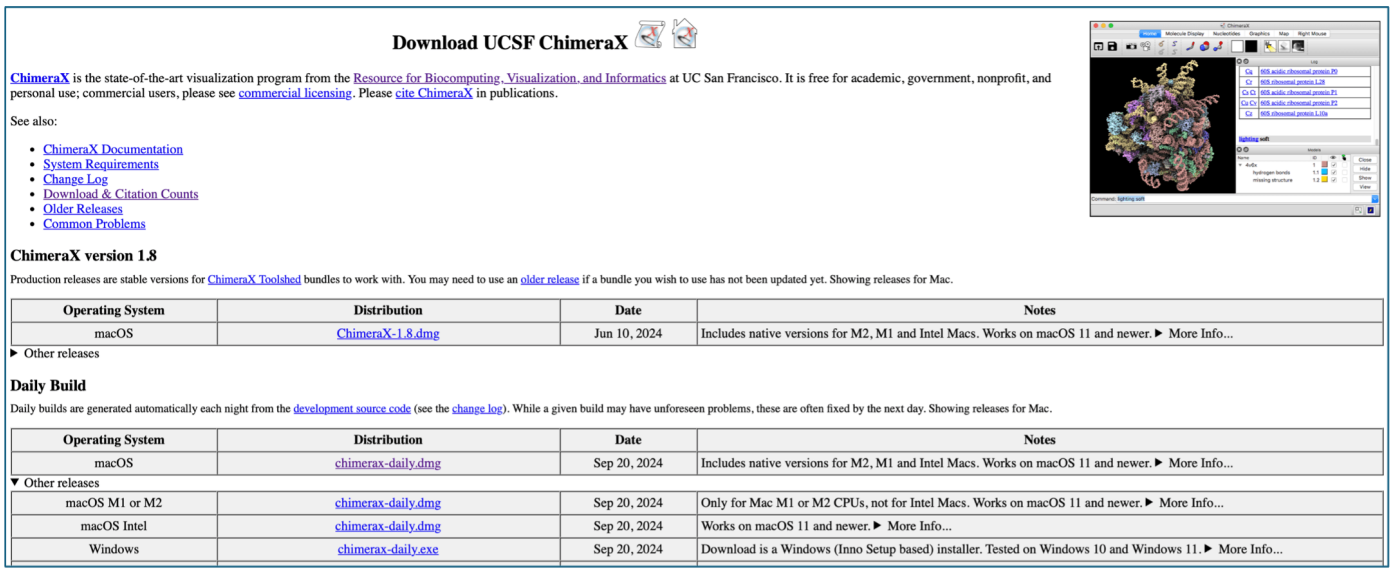
pLDDT scores:

PAE plot:

pTM score:

2. Compare predicted and experimentally determined protein structures

- You will now compare the protein structure predicted by AlphaFold with the experimentally determined structure from the Protein Data Bank (PDB ID: **1WIO**)
- We will use ChimeraX for visualization of your protein structures. UCSF ChimeraX (or simply ChimeraX) is the next-generation molecular visualization program from the Resource for Biocomputing, Visualization, and Informatics (RBVI). ChimeraX can be downloaded free of charge for academic, government, nonprofit, and personal use.
- Download and install UCSF ChimeraX



Download UCSF ChimeraX

ChimeraX is the state-of-the-art visualization program from the Resource for Biocomputing, Visualization, and Informatics at UC San Francisco. It is free for academic, government, nonprofit, and personal use; commercial users, please see commercial licensing. Please cite ChimeraX in publications.

See also:

- ChimeraX Documentation
- System Requirements
- Change Log
- Download & Citation Counts
- Older Releases
- Common Problems

ChimeraX version 1.8

Production releases are stable versions for ChimeraX Toolshed bundles to work with. You may need to use an older release if a bundle you wish to use has not been updated yet. Showing releases for Mac.

Operating System	Distribution	Date	Notes
macOS	ChimeraX-1.8.dmg	Jun 10, 2024	Includes native versions for M2, M1 and Intel Macs. Works on macOS 11 and newer. ▶ More Info...

▶ Other releases

Daily Build

Daily builds are generated automatically each night from the development source code (see the change log). While a given build may have unforeseen problems, these are often fixed by the next day. Showing releases for Mac.

Operating System	Distribution	Date	Notes
macOS	chimerax-daily.dmg	Sep 20, 2024	Includes native versions for M2, M1 and Intel Macs. Works on macOS 11 and newer. ▶ More Info...

▼ Other releases

macOS M1 or M2	chimerax-daily.dmg	Sep 20, 2024	Only for Mac M1 or M2 CPUs, not for Intel Macs. Works on macOS 11 and newer. ▶ More Info...
macOS Intel	chimerax-daily.dmg	Sep 20, 2024	Works on macOS 11 and newer. ▶ More Info...
Windows	chimerax-daily.exe	Sep 20, 2024	Download is a Windows (Inno Setup based) installer. Tested on Windows 10 and Windows 11. ▶ More Info...

Figure 2. UCSF ChimeraX. *UCSF ChimeraX is a molecular visualization program that can be downloaded free of charge for academic and personal use. Screenshot from the UCSF ChimeraX website.*

- Open ChimeraX and click on **Open** button to open your predicted structure (.cif file).

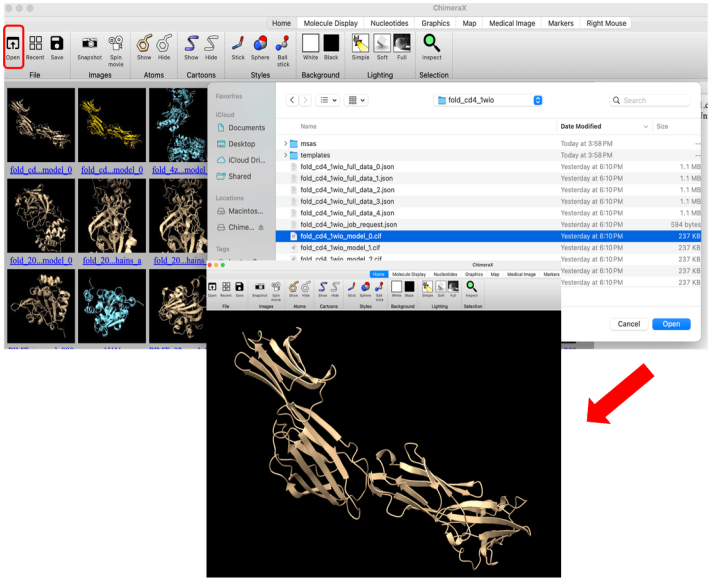
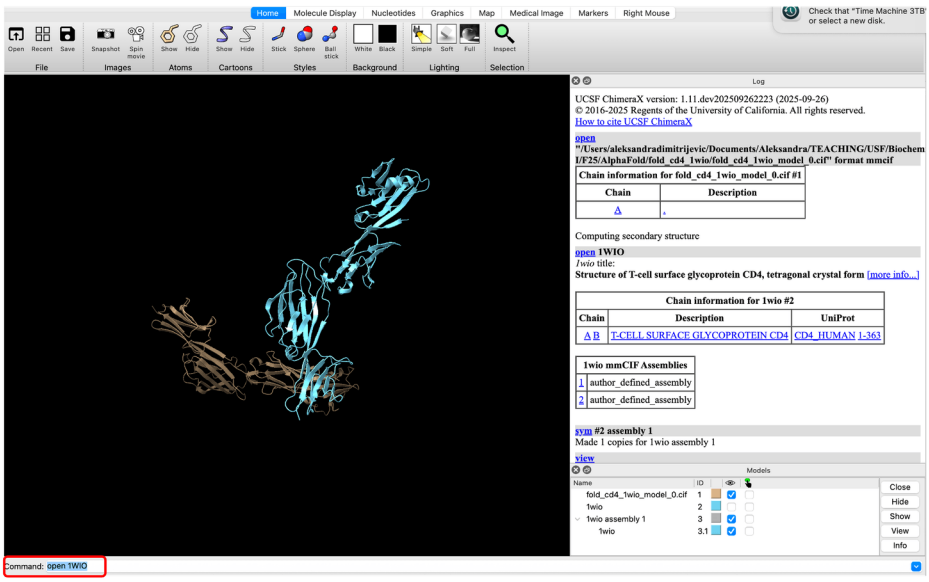


Figure 3. *UCSF ChimeraX can be used to visualize and analyze protein structures. Screenshot generated by the author using UCSF ChimeraX.*

- Open experimentally derived structure of the same protein (PDB ID 1WIO). In the Command box, type **“open 1WIO”** and you will see a second, experimentally derived structure (blue) appear in the vicinity of your predicted structure (beige).



- Align the two structures by typing **“matchmaker #1 to #2”** in the Command dialog box. Insert the obtained image below and clearly label predicted and experimentally determined structures:
- Explain and analyze the obtained RMSD values:
- Based on your AlphaFold confidence metrics and your comparison with the experimentally determined structure, what conclusions can you draw about the strengths and limitations of the AlphaFold prediction for this protein?

References and Resources:

AlphaFold 3:

Abramson, J., Adler, J., Dunger, J., et al. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature, 630*, 493–500. https://doi.org/10.1038/s41586-024-07487-w

ChimeraX:

Meng, E. C., Goddard, T. D., Pettersen, E. F., Couch, G. S., Pearson, Z. J., Morris, J. H., & Ferrin, T. E. (2023). UCSF ChimeraX: Tools for structure building and analysis. *Protein Science, 32*(11), e4792.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., Morris, J. H., & Ferrin, T. E. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Science, 30*(1), 70–82.

Experimental Structure:

Wu, H., Kwong, P. D., & Hendrickson, W. A. (1997). Dimeric association and segmental variability in the structure of human CD4. *Nature, 387*, 527–530. https://doi.org/10.1038/387527a0

Protein Data Bank:

RCSB Protein Data Bank. PDB ID **1WIO**: *Structure of T-cell surface glycoprotein CD4, tetragonal crystal form*. https://doi.org/10.2210/pdb1WIO/pdb

Software Acknowledgment

This activity uses AlphaFold Server for protein structure prediction and UCSF ChimeraX for molecular visualization and structural comparison. AlphaFold Server is developed by Google DeepMind, and UCSF ChimeraX is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from the National

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S

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