

*Phenix workshop at UT Southwestern Medical Center,
May 21, 2026*

Using Predicted models in Phenix (cryo-EM)

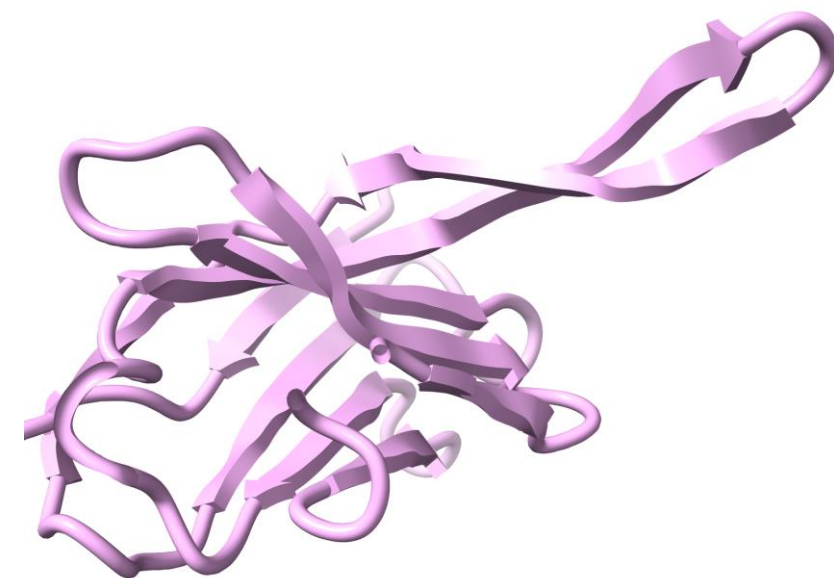


Dorothee Liebschner
Lawrence Berkeley Laboratory

Predicting models with AlphaFold



EVQLVESGGGLVQPGGSLRLSCAASGFTIYSSSIHWVRQAPGKGLEWVAYI
.....F.....M.....Q.....
.....K.....Y.....L.....A.....
.....A.....V.....
.....A.....
.....L.....V.....E.....
.....A.....Q.....



Sequence

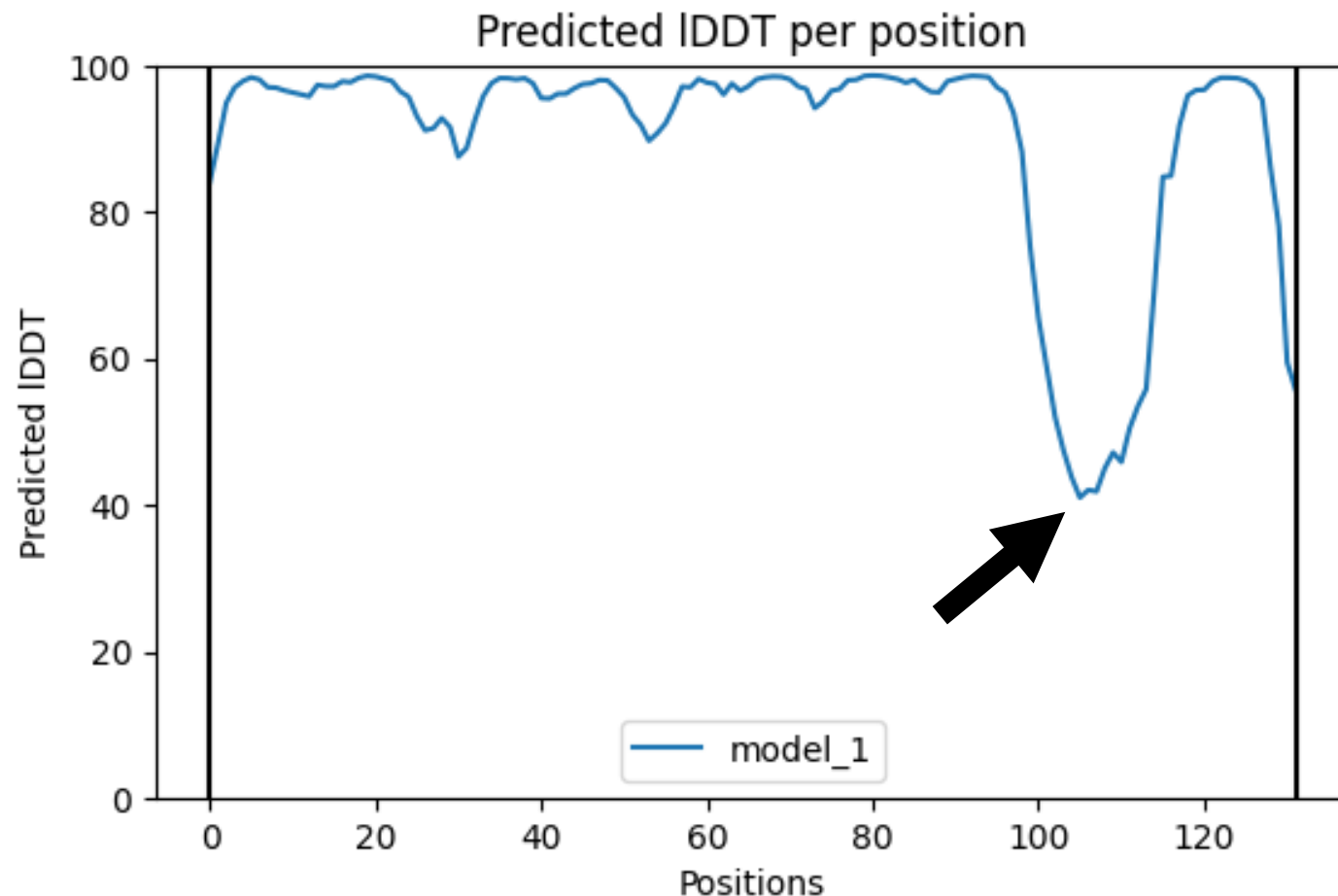
Multiple sequence alignment

3D prediction

Predictions come with confidence measures

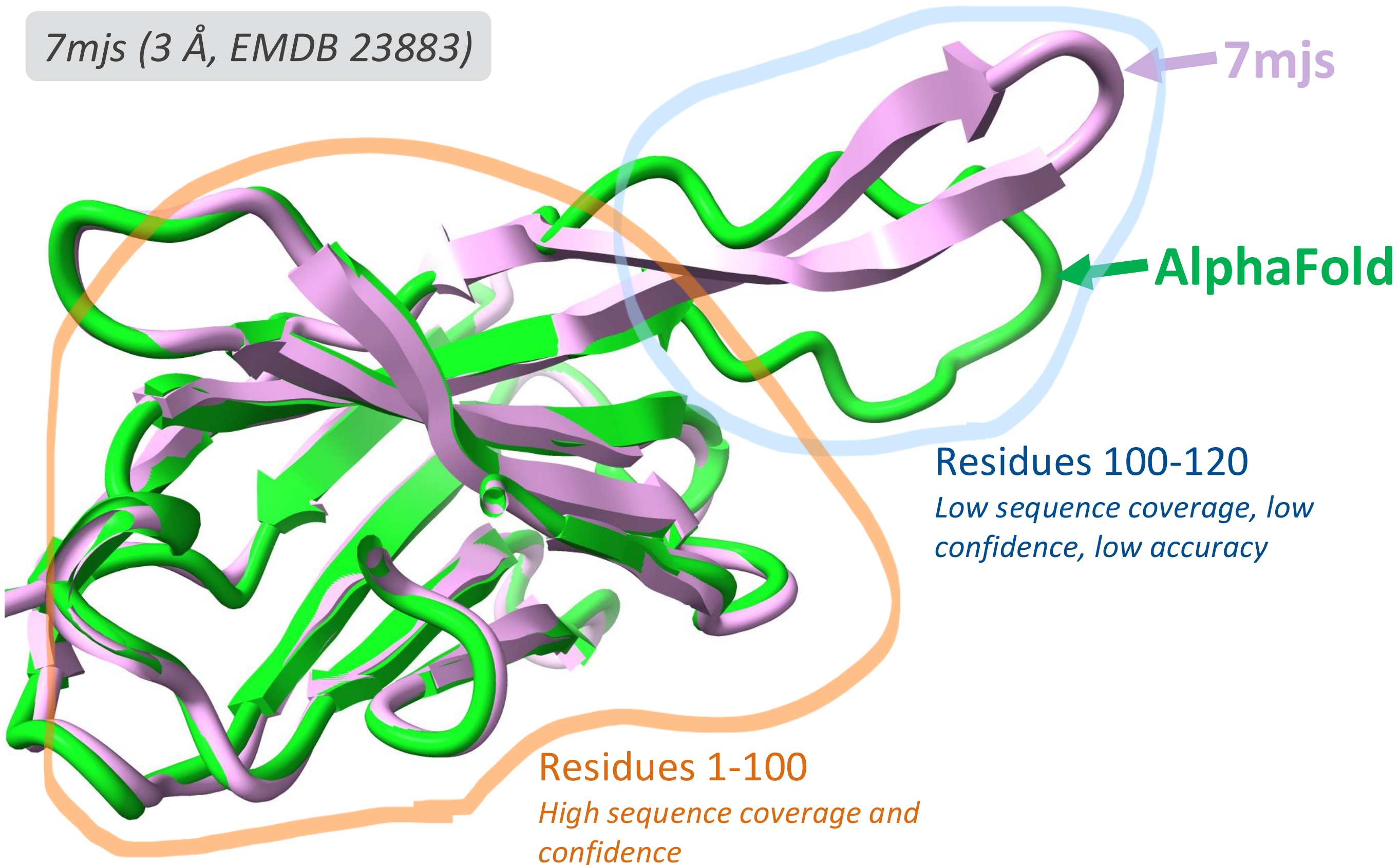
1. pLDDt (predicted Local Distance Difference Test)

- pLDDt identifies where errors are more likely.
- Per-residue confidence measure.
- Scales from 0 – 100 (pLDDt > 90: predicted with high accuracy).



Predictions come with confidence measures

7mjs (3 Å, EMDB 23883)



Predictions come with confidence measures

1. pLDDt (predicted Local Distance Difference Test)

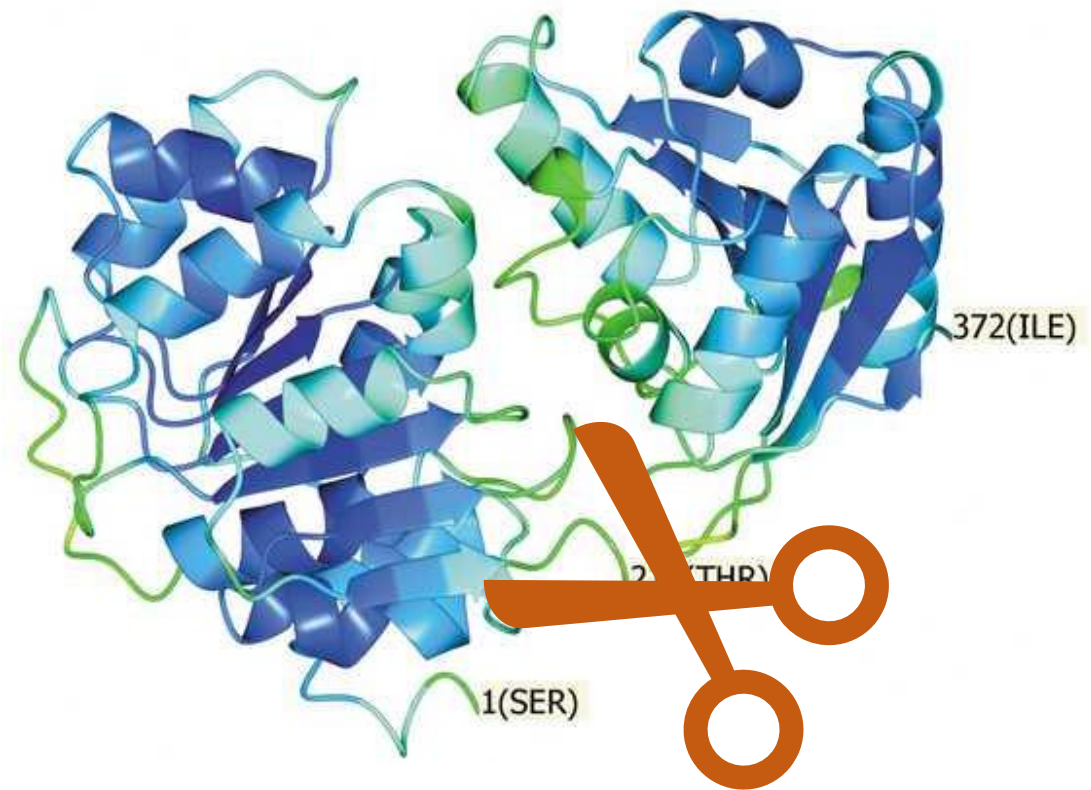
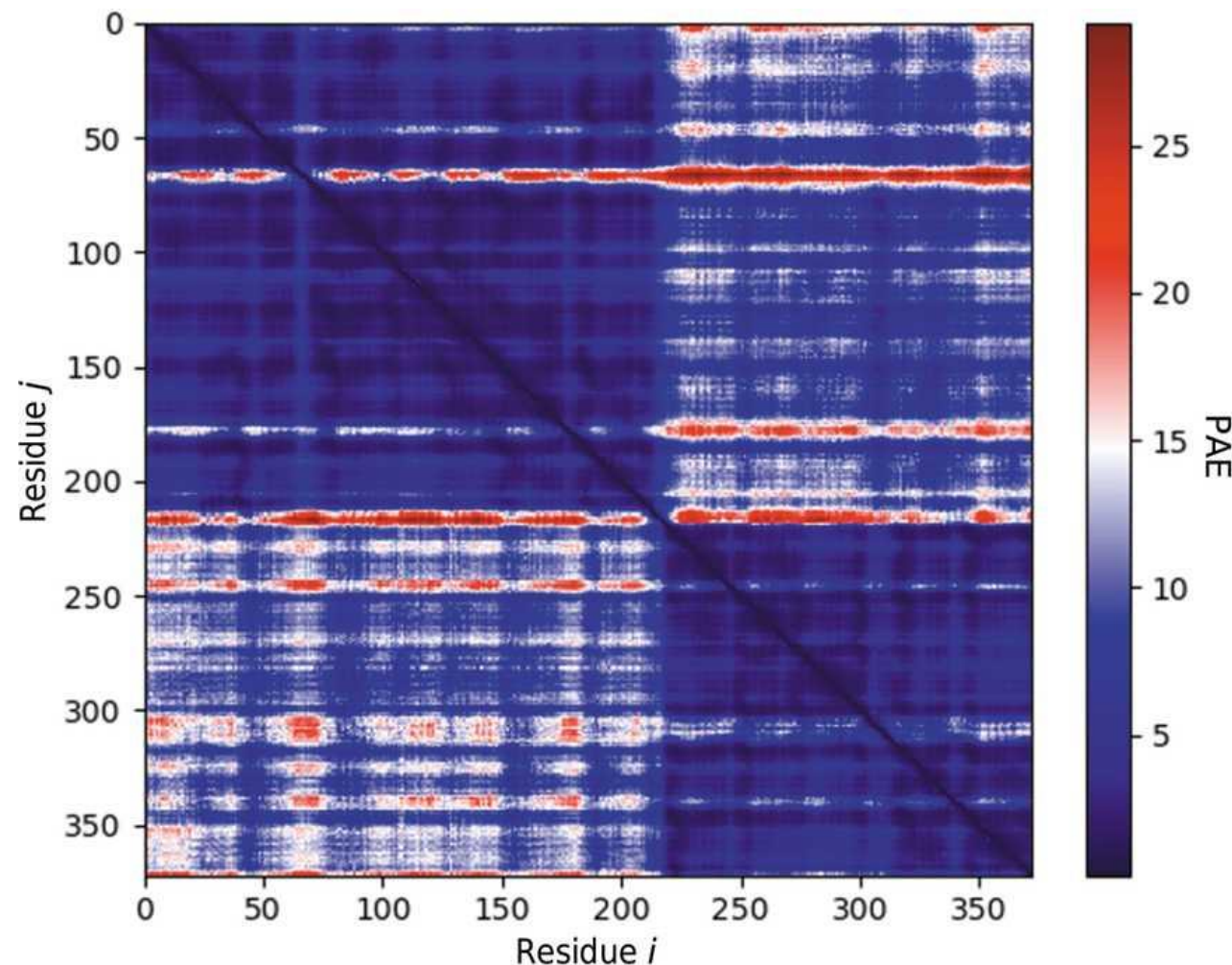
- pLDDt identifies where errors are more likely.
- Per-residue confidence measure.
- Scales from 0 – 100 (pLDDt > 90: predicted with high accuracy).

AlphaFold confidence (pLDDT)	Median prediction error (Å)	Percentage with error over 2 Å
>90	0.6	10
80 - 90	1.1	22
70 - 80	1.5	33
<70	3.5	77

Predictions come with confidence measures

2. Predicted aligned error (PAE)

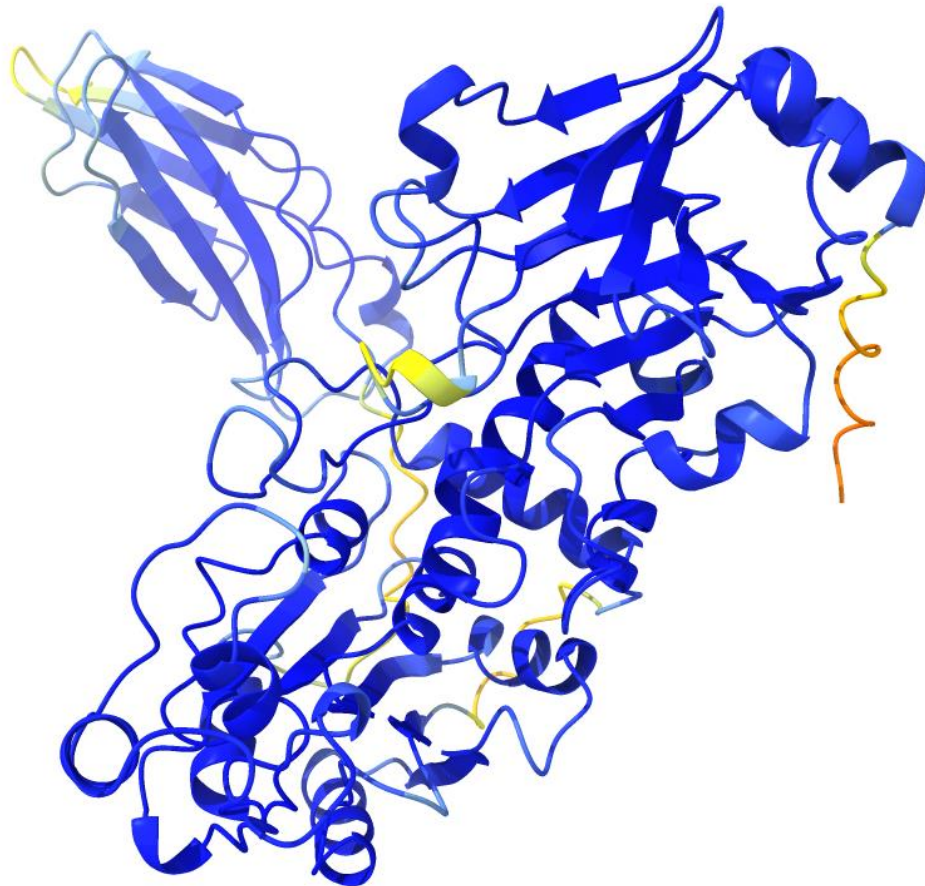
- Certainty of relative positions between two residues.
- Identifies accurately-predicted domains.
- Dark blue: uncertainty in relative positions $< 5 \text{ \AA}$.



- Suggests 2 domains

Using predicted models: B-factors

Color by pLDDT



Color by B-factor



high pLDDT (high confidence)

low pLDDT (low confidence, uncertain)



high B-factor (disordered, uncertain)

low B-factor (ordered)

Using predicted models: B-factors

high pLDDT (high confidence)

low pLDDT (low confidence, uncertain)



high B-factor (disordered, uncertain)

low B-factor (ordered)

B-factor may be used in downstream calculations, e.g. to calculate weights for docking. Residues with high B-factors are downweighed.

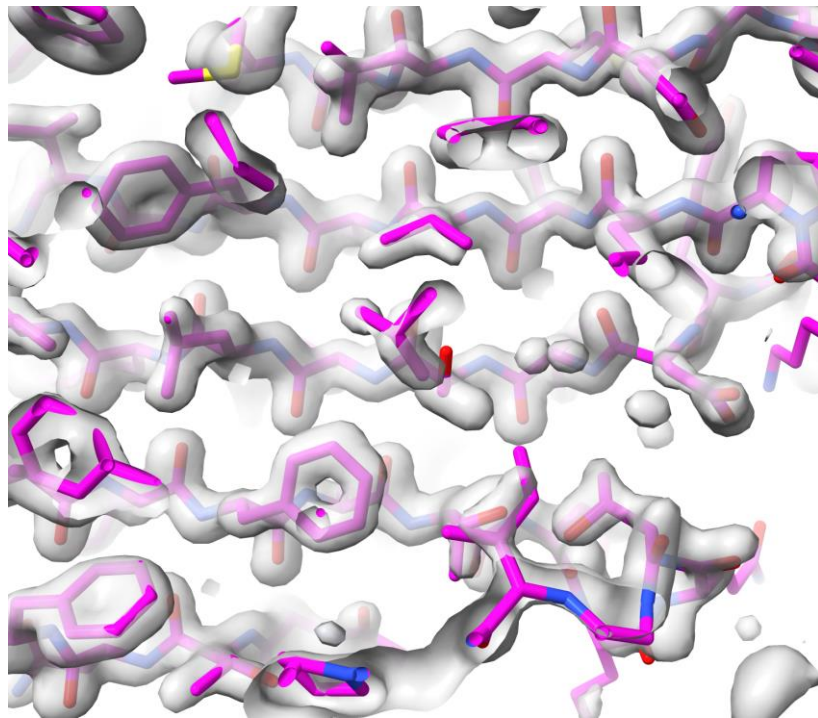
→ Convert pLDDT to pseudo B-factors.

$$\Delta = 1.5 \exp[4(0.7 - \text{pLDDT})]$$

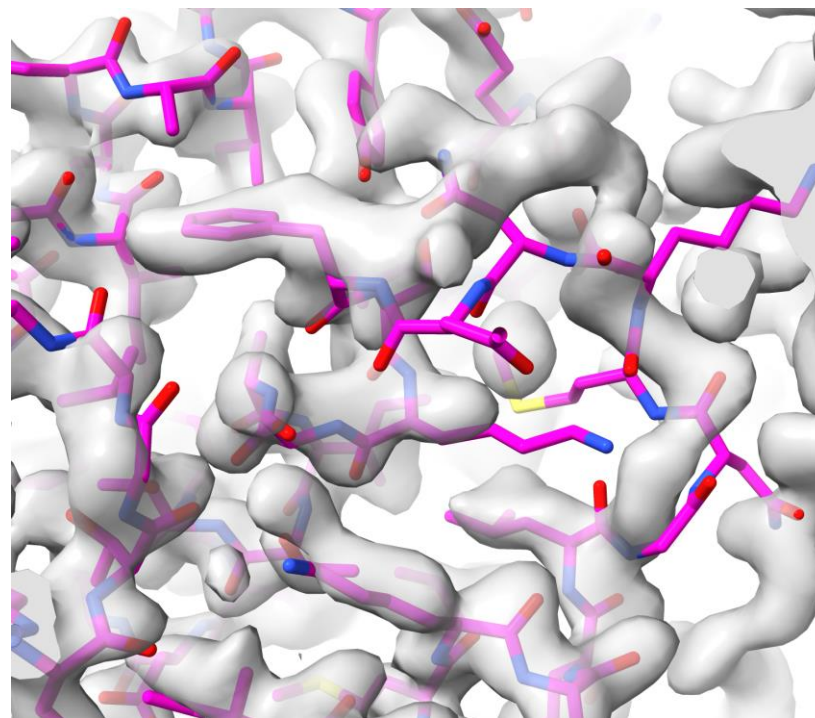
$$B = \frac{8\pi^2 \Delta^2}{3}$$

AlphaFold predictions are great hypotheses

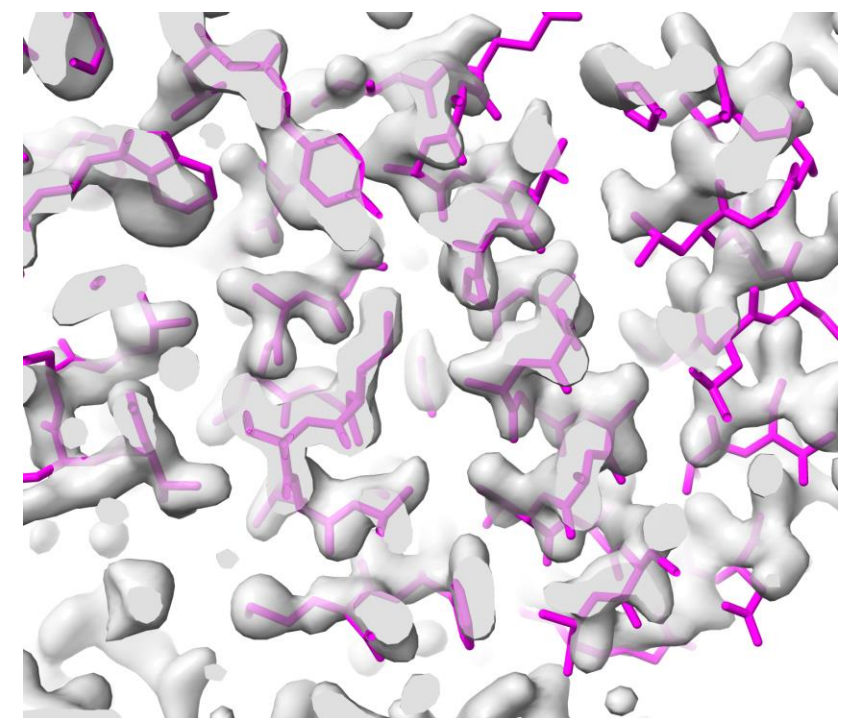
AlphaFold models
can be....



Awesome



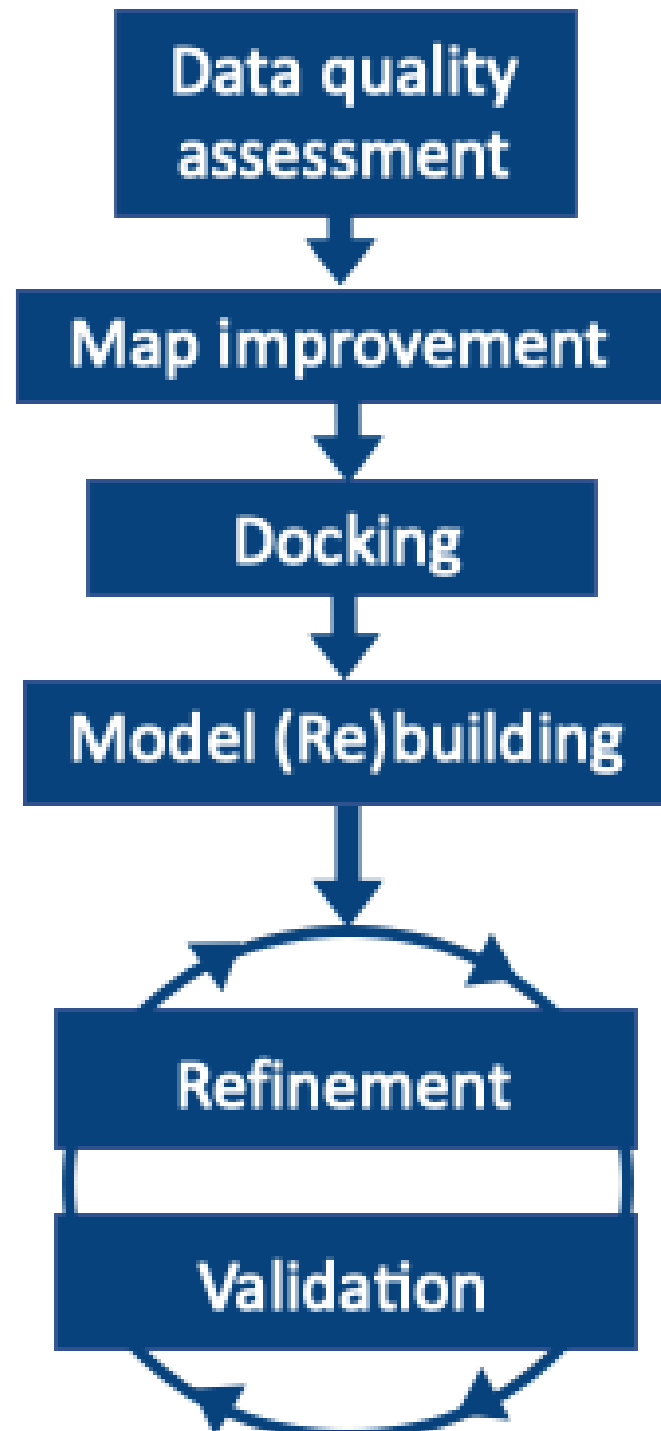
Wrong



Distorted

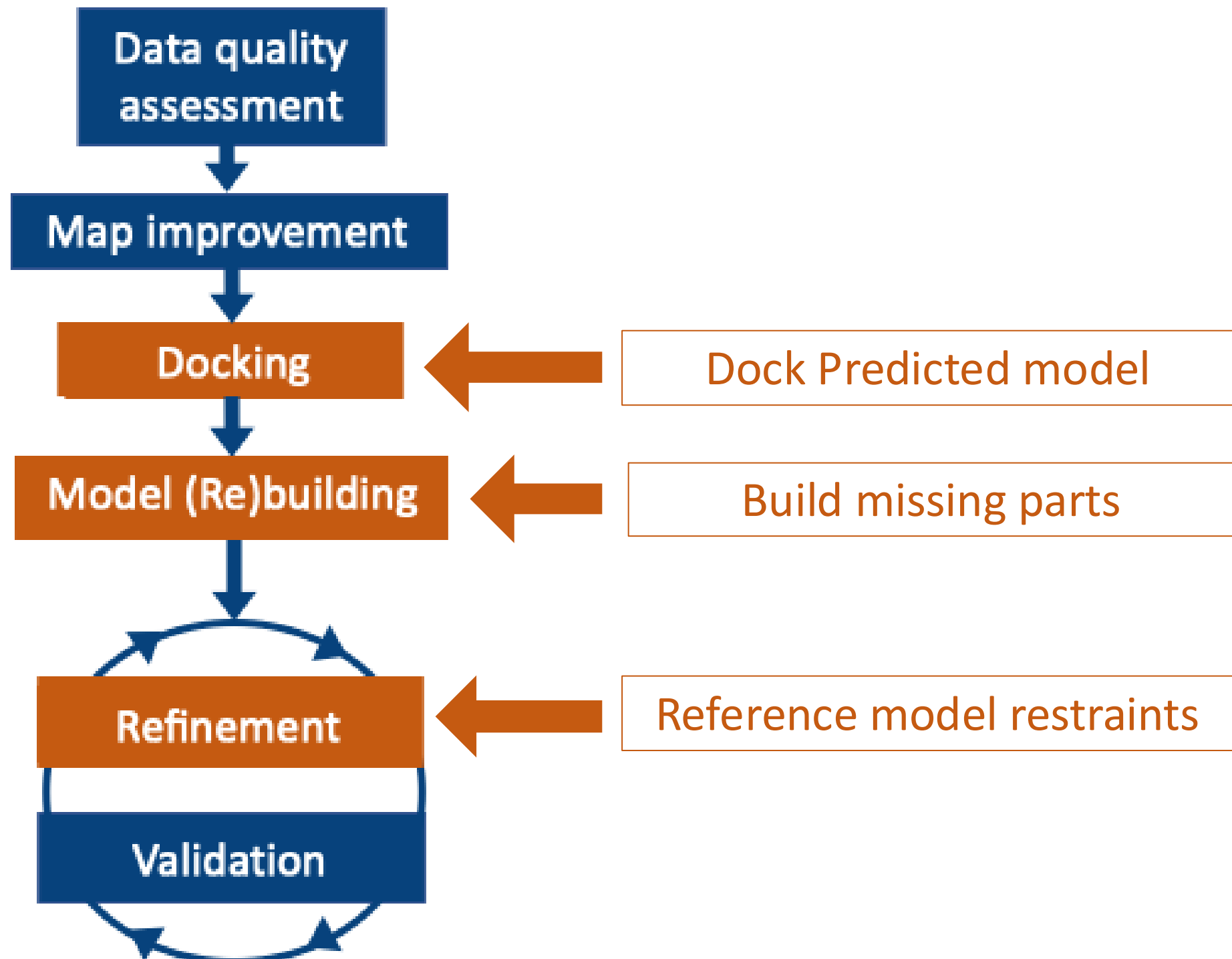
What can predicted models be used for?

Incorporate predictions into the typical cryo-EM workflow.



What can predicted models be used for?

Incorporate predictions into the typical cryo-EM workflow.



1. Use a prediction for cryo-EM docking

Cryo-EM maps are often insufficiently resolved for reliable ab initio model building.

→ Dock a pre-existing model into the map.

1. Use a prediction for cryo-EM docking

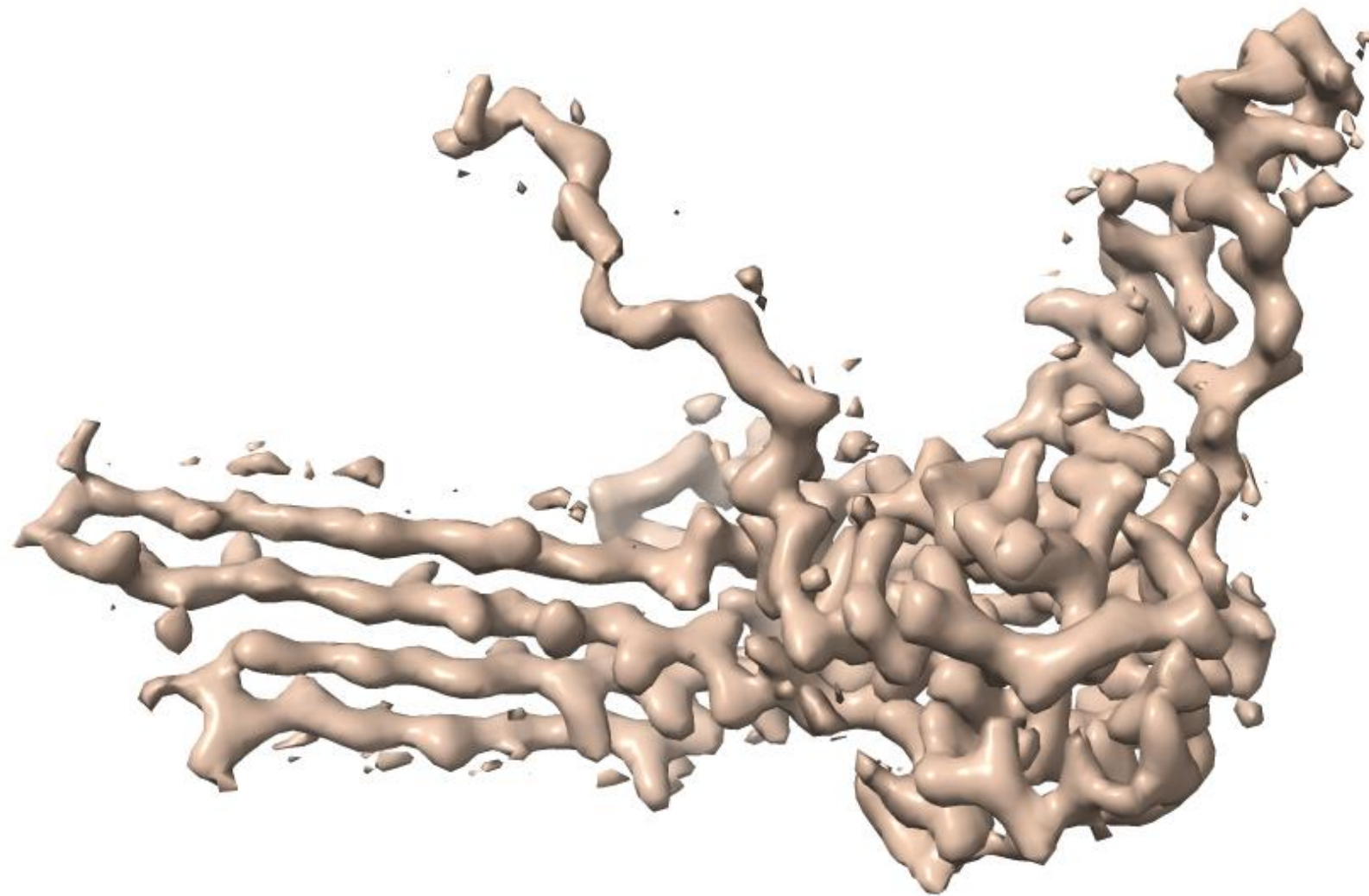
Example:

Cryo-EM map (30160 – 7brm)

3.6 Å

```
>chain ' A'  
XXXXXXXXXXXXXXXXCLTAPPKEAARPTLMPRAQSYKDLTHLPAP  
TGKIFVSVYNIQDETGQFKPYPASNFSTAVPQSATAMLVTALKDS  
RWFIPLERQGLQNLLNERKIIRAAQENGTVAINNRIPLQSLTAAN  
IMVEGSIIGYESNVKSGGVGARYFGIGADTQYQLDQIAVNLRVVN  
VSTGEILSSVNTSKTILSYEVQAGVFRFIDYQRLLEGEVGYTSNE  
PVMLCLMSAIETGVIFLINDGIDRGLWDLQNKAERQNDILVKYRH  
MS
```

sequence

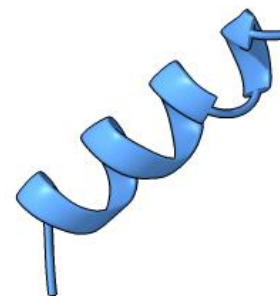
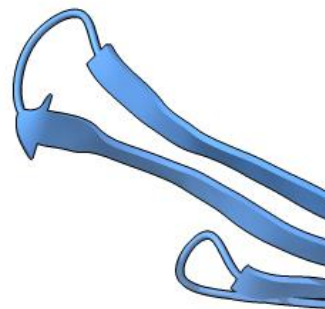
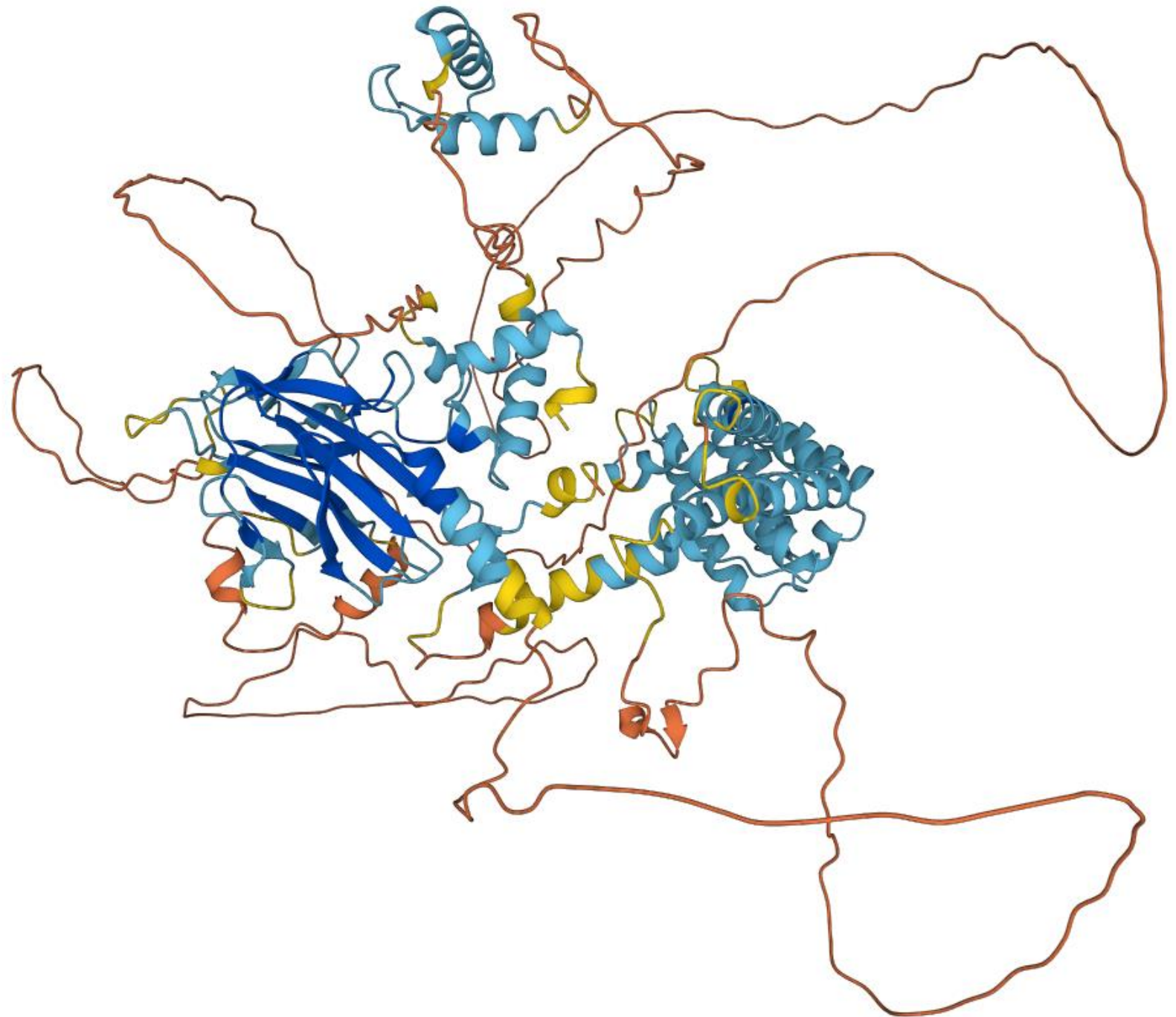


cryo-EM map

Get a prediction

sequence

```
>chain ' A'  
XXXXXXXXXXXXXXXXXCLTAPPKEAARPTLMPRAQSYKDLTHLPAP  
TGKIFVSLVNTAGETGAEKDYBAGNEETAUDAGATAMILITAIKDE  
RWFIP  
IMVEG  
VSTGE  
PVMLC  
MS
```

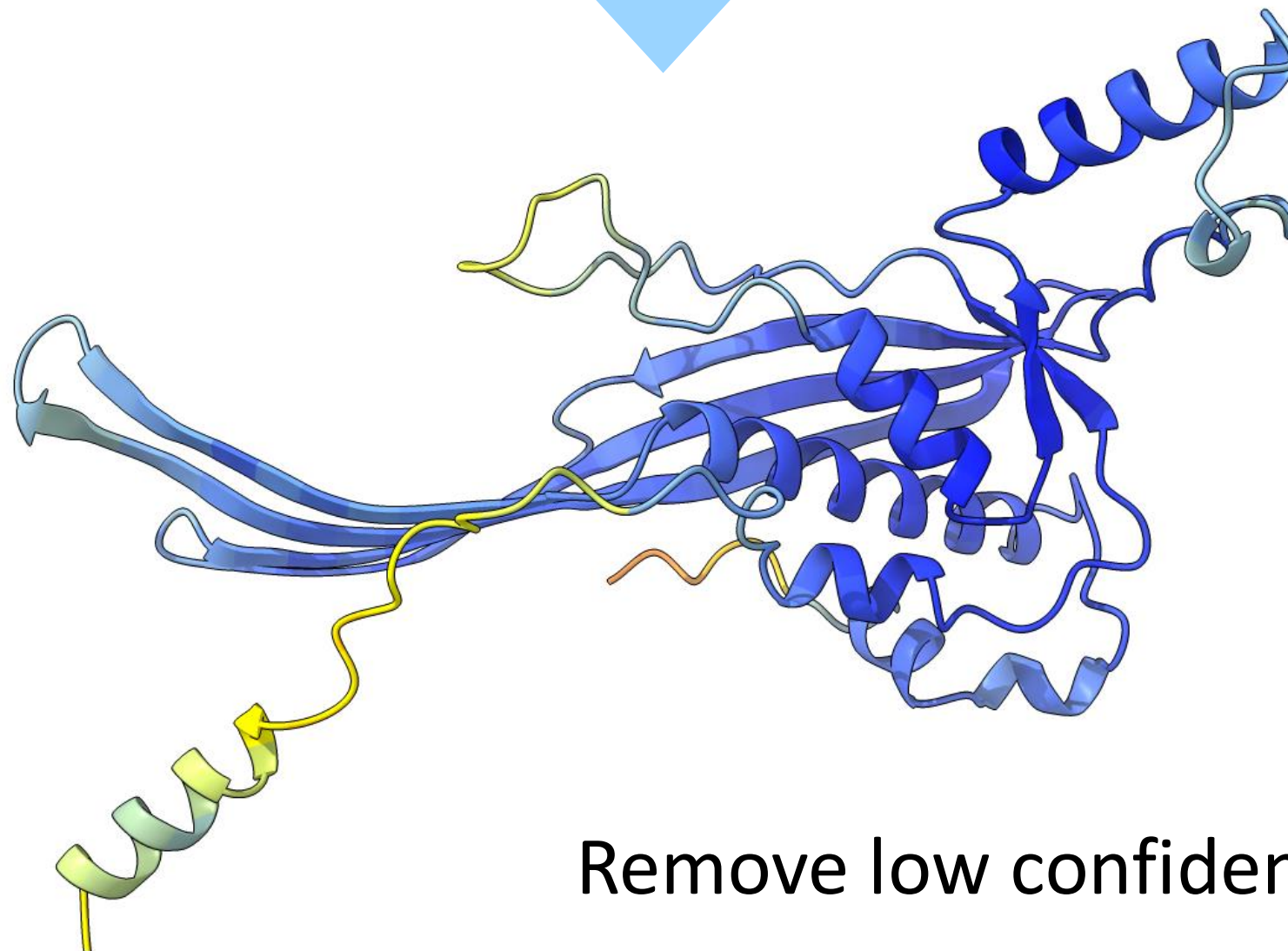
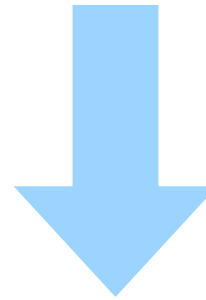


AlphaFold
model

Process prediction

sequence

```
>chain ' A'  
XXXXXXXXXXXXXXXXXCLTAPPKEAARPTLMPRAQSYKDLTHLPAP  
TGKIFVSVYNIQDETGQFKPYPA SNFSTAVPQSATAMLVTALKDS  
RWFIPLERQGLQNLLNERKIIRAAQENGTVAINNRIP LQSLTAAN  
IMVEGSIIGYESNVKSGGVGARYFGIGADTQYQLDQIAVNLRVVN  
VSTGEILSSVNTSKTILSYEVQAGVFRFIDYQRLLEGEVGYTSNE  
PVMLCLMSAIETGVIFLINDGIDRGLWDLQNK AERQNDILVKYRH  
MS
```



AlphaFold
model

Remove low confidence parts

Process prediction

AlphaFold: Predicted models with Crystals or Cryo-EM

? How to use AlphaFold in Phenix
Documentation for using AlphaFold2 models in Phenix

AlphaFold model prediction
Predict the chains in a sequence file with AlphaFold

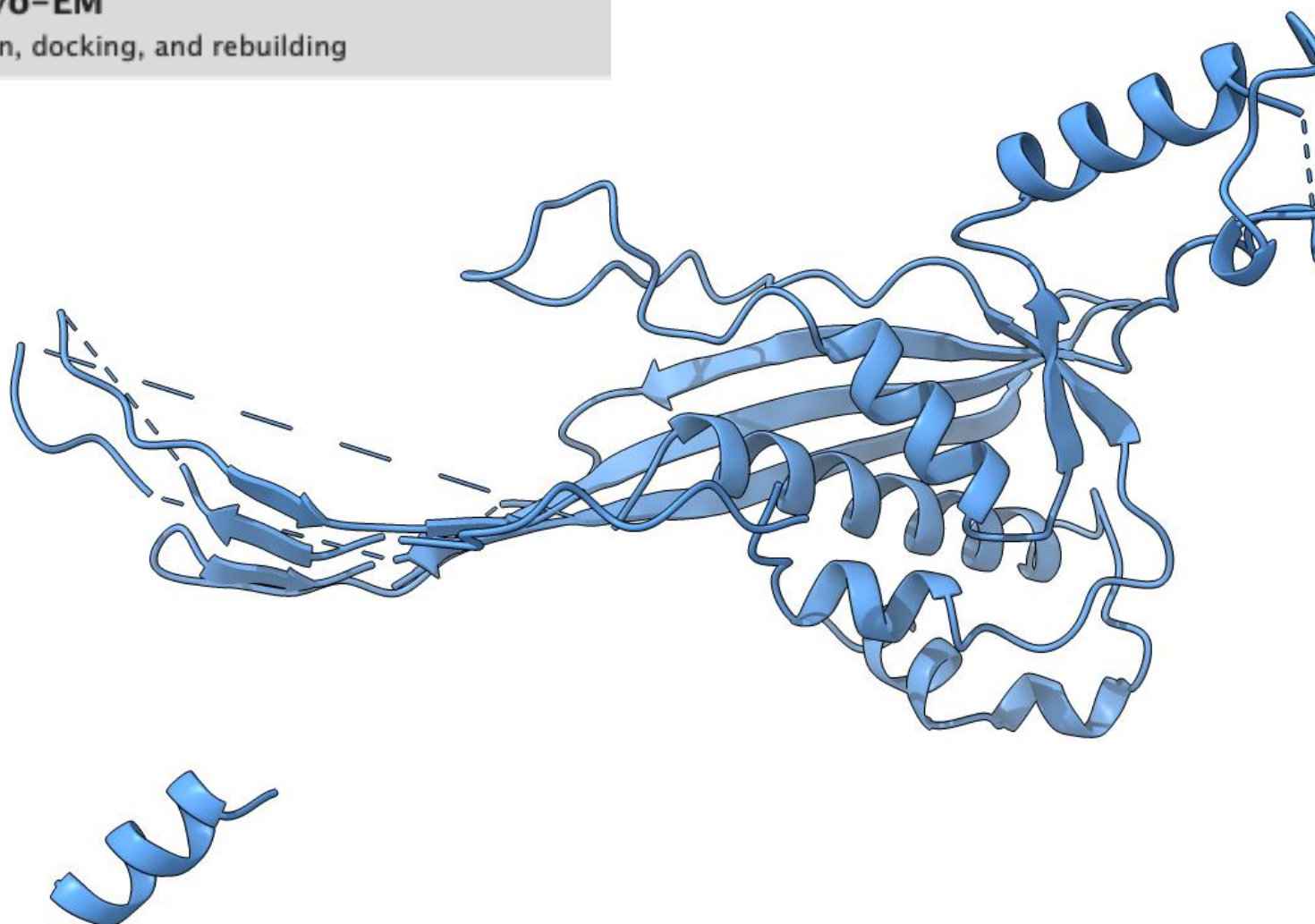
Process Predicted Model
Replace B-factor field in model and optionally split into domains

AF2 Predict and Build: Crystallography
Iterative AlphaFold prediction, MR/docking, and rebuilding

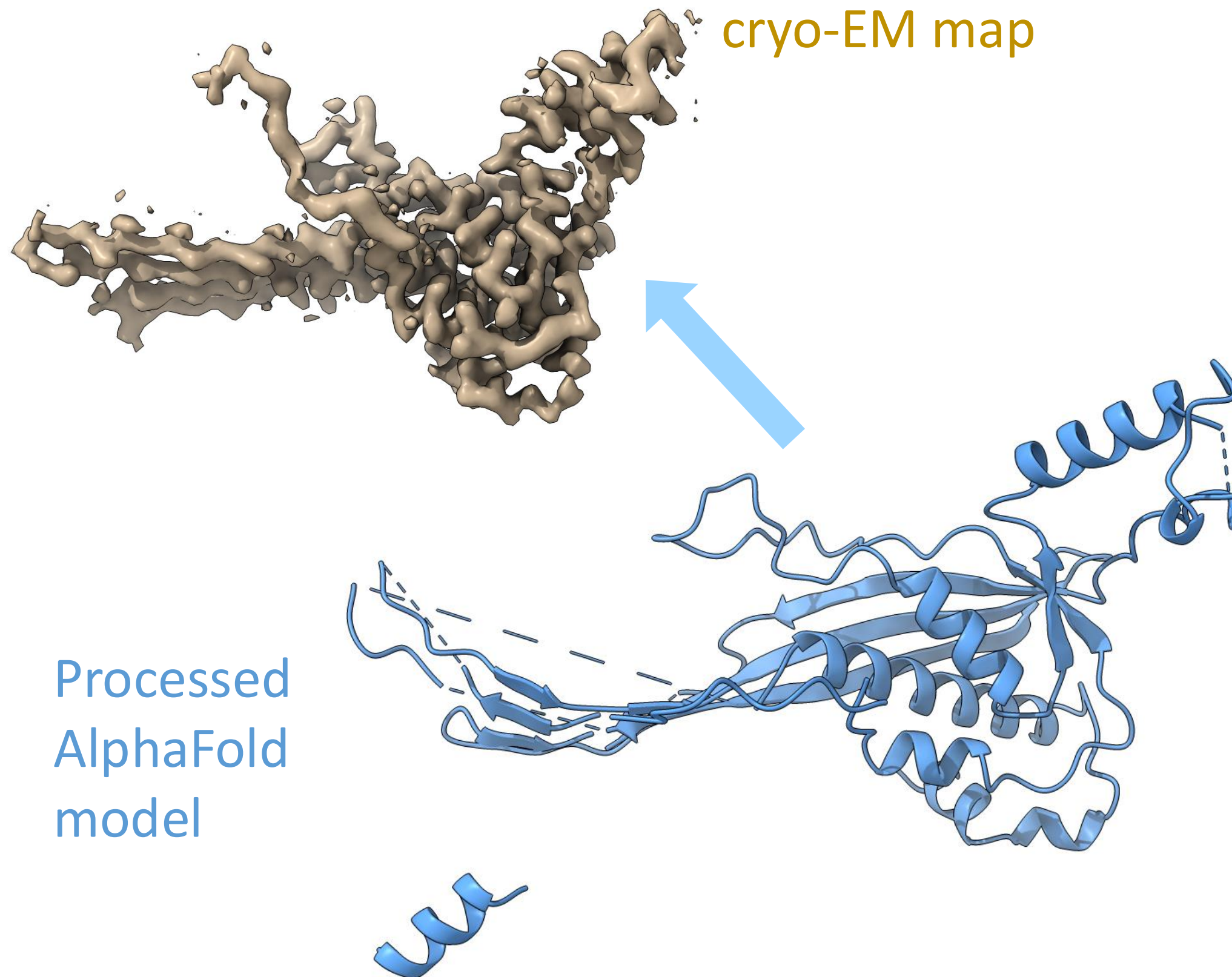
AF2 Predict and Build: Cryo-EM
Iterative AlphaFold prediction, docking, and rebuilding

`phenix.process_predicted_model`

Processed
AlphaFold
model



Dock processed model



Docking in Phenix

Dock in map (T. Terwilliger)

The screenshot displays the Phenix software interface. The top toolbar includes icons for Quit, Preferences, Help, Citations, ChimeraX, Coot, PyMOL, KING, Tools, Help, and Chat. The main window is divided into two panes. The left pane, titled 'Projects', shows a table of project entries with columns for ID, Last modified, # of jobs, and R-free. The right pane, titled 'Validation', lists various actions. The 'Cryo-EM' section is expanded, and the 'Dock in map' option is highlighted with an orange circle. Below this, other options like 'EM Placement', 'Predict and Build: Cryo-EM', 'Map to Model', 'Sequence From Map', 'Fit Loops', 'Rebuild a model in place', and 'Douse (Add waters)' are visible. The bottom status bar shows the current directory and project name.

Projects

Show group: All groups Manage...

Select Delete New project Import project Settings

ID	Last modified	# of jobs	R-free
apoferritin_chim...	Oct 14 2025 05:26 ...	4	---
✓ tst_fit_loops	Oct 14 2025 04:22 ...	26	---
AF_POMGNT2	Oct 01 2025 08:59 ...	15	---
apoferritin_den...	Oct 01 2025 08:36 ...	8	---
apoferritin_mod...	Sep 05 2025 01:40 ...	0	---
apoferritin_den...	Sep 05 2025 01:15 ...	1	---
tests	Jul 29 2025 04:39 ...	105	0.2650
groel_dock_refi..	Jul 09 2025 12:56 ...	5	---
test_ligand	Jul 08 2025 04:19 ...	3	0.2638
rnase-s	Jul 08 2025 03:37 ...	3	0.2555
b-CA-mr	Jul 06 2025 08:43 ...	5	---
1j4r_xtriage	Jul 06 2025 03:00 ...	3	---

Validation

- Map and structure factor calculation
- Map comparison and analysis
- Cryo-EM**
 - Map analysis and manipulation
 - Map improvement
 - Docking and model building
 - EM Placement (dock model with likelihood target)
Dock one model into a cryo-EM map
 - Dock in map**
Dock models into cryo-EM map
 - Predict and Build: Cryo-EM
Iterative AlphaFold prediction, docking, and rebuilding
 - Map to Model
Model-building into cryo-EM and low-resolution maps
 - Sequence From Map
Determine a sequence from a map
 - Fit Loops
Fit missing loops in a model
 - Rebuild a model in place
Adjust a model to match a map without changing atoms or connectivity
 - Douse (Add waters)

Current directory: /Users/dcliebschner/Desktop/tutorials/test_fit_loops Browse...

Phenix version dev-svn Project: tst_fit_loops

Docking in Phenix

Likelihood-based EM docking (R. Read)

- Use likelihood scores to dock a model into a map
- Works at low resolution (8.5 Å)

The screenshot displays the Phenix software interface. The top menu bar includes 'Phenix home', 'Quit', 'Preferences', 'Help', 'Citations', 'ChimeraX', 'Coot', 'PyMOL', 'KING', 'Tools', 'Help', and 'Chat'. Below the menu bar, there are tabs for 'Actions' and 'Job history'. The main area is divided into two panels. The left panel, titled 'Projects', shows a table of projects with columns for ID, Last modified, # of jobs, and R-free. The right panel, titled 'Validation', lists various actions: 'Map and structure factor calculation', 'Map comparison and analysis', 'Cryo-EM', 'Map analysis and manipulation', 'Map improvement', 'Docking and model building' (highlighted in orange), 'EM Placement (dock model with likelihood target)' (highlighted in blue), 'Dock in map', 'Predict and Build: Cryo-EM', 'Map to Model', 'Sequence From Map', 'Fit Loops', 'Rebuild a model in place', and 'Douse (Add waters)'. The 'Current directory' is shown as '/Users/dcliebschner/Desktop/tutorials/test_fit_loops'.

ID	Last modified	# of jobs	R-free
apoferritin_chim...	Oct 14 2025 05:26 ...	4	---
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AF_POMGNT2	Oct 01 2025 08:59 ...	15	---
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apoferritin_mod...	Sep 05 2025 01:40 ...	0	---
apoferritin_den...	Sep 05 2025 01:15 ...	1	---
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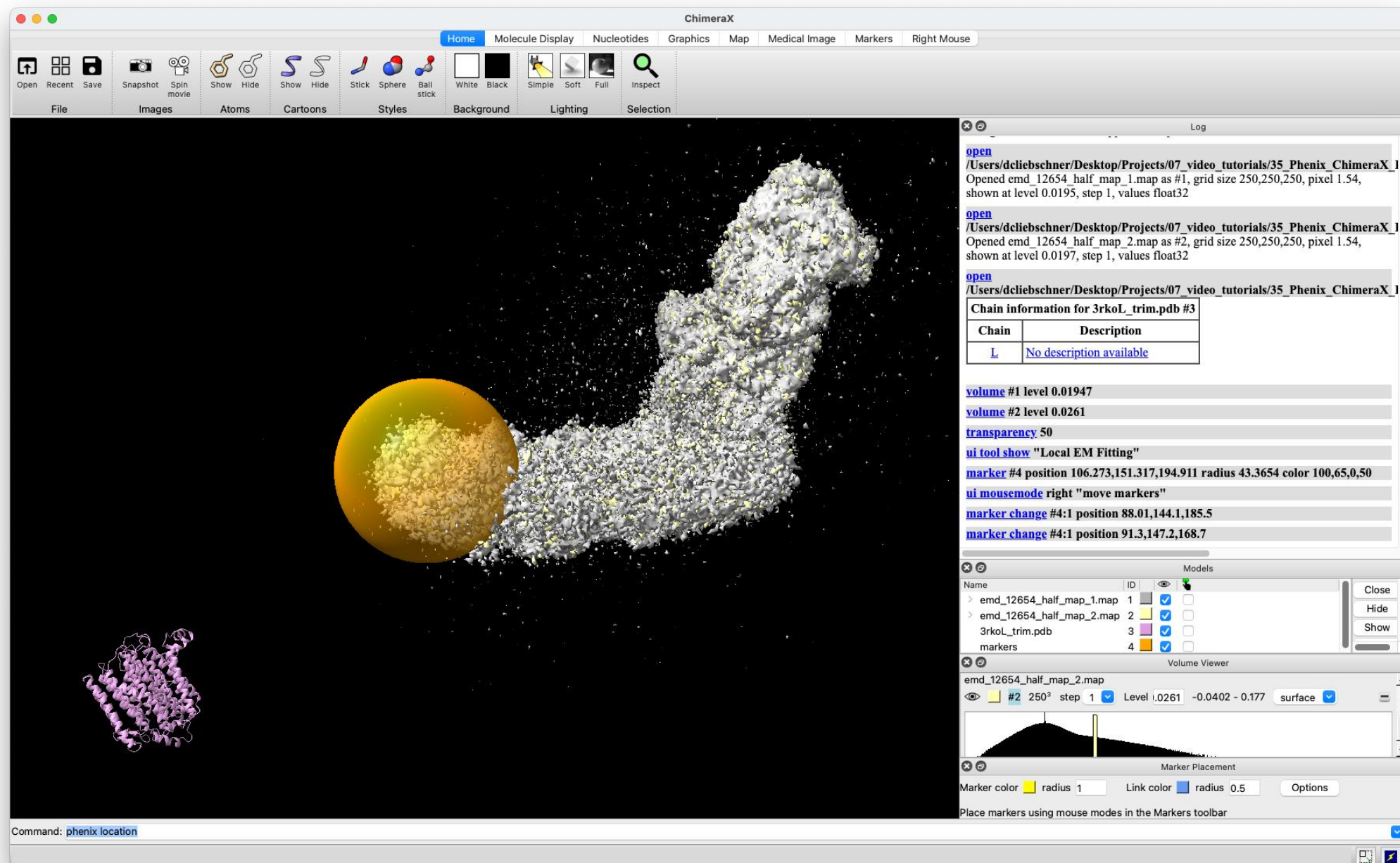
Current directory: /Users/dcliebschner/Desktop/tutorials/test_fit_loops

Phenix version dev-svn

Project: tst_fit_loops

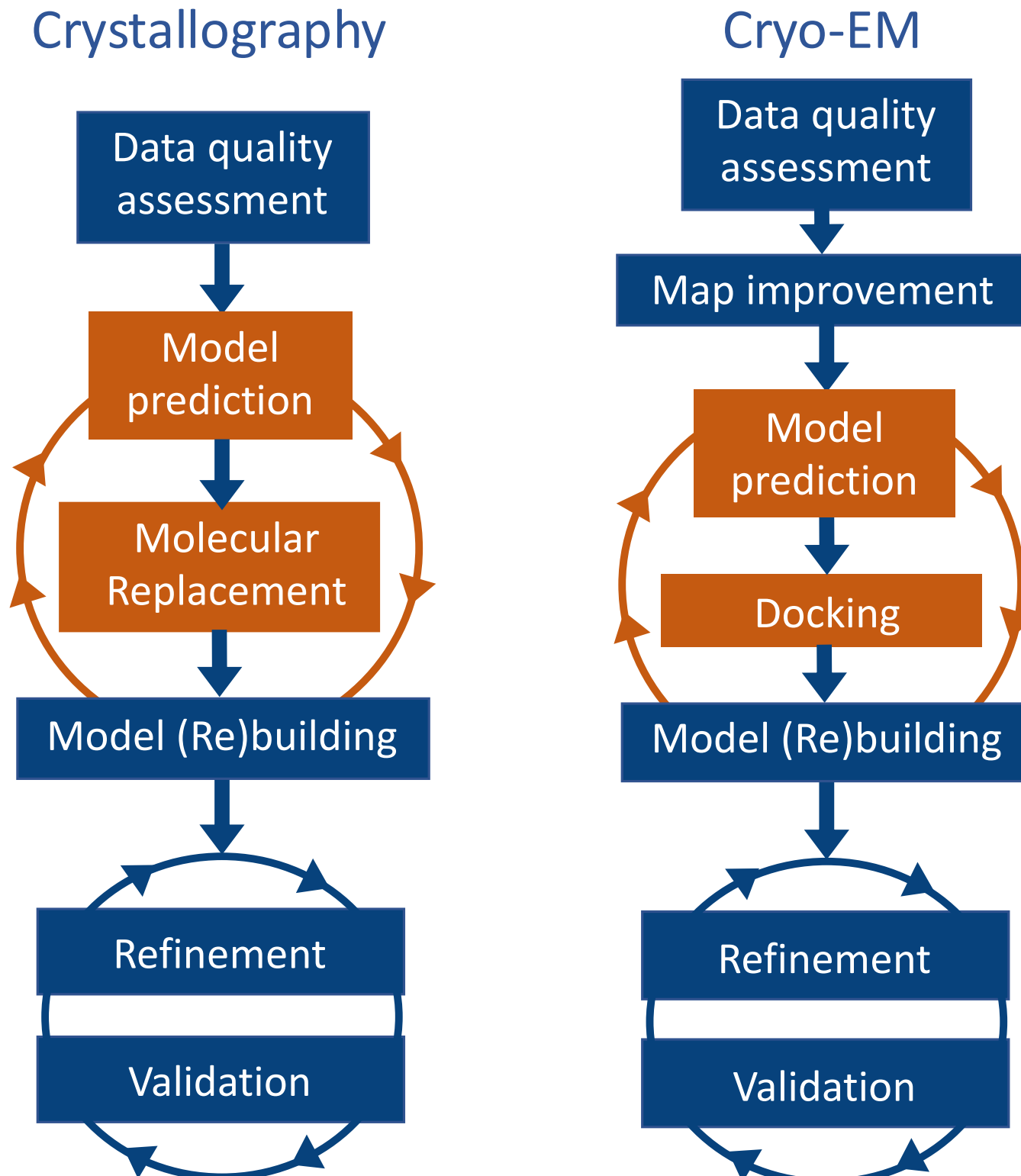
Docking in Phenix/ChimeraX

- Likelihood-based docking can be done via ChimeraX.
- Can select the region into which the model should be docked.



Using predicted models

Updated approach: Iterate prediction and model building

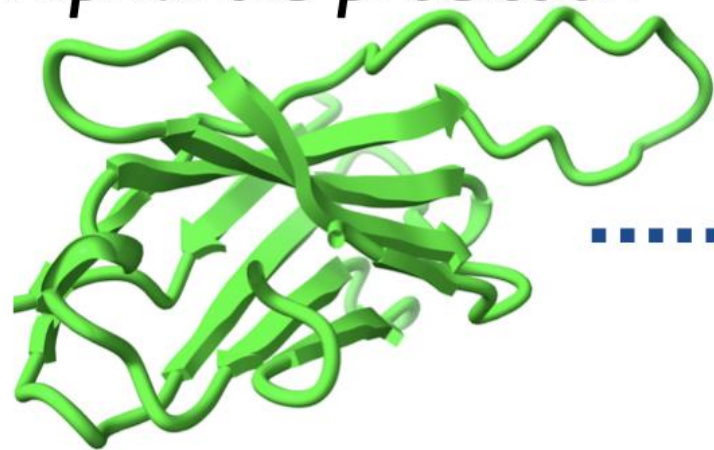


Use your working model to get a new AlphaFold prediction

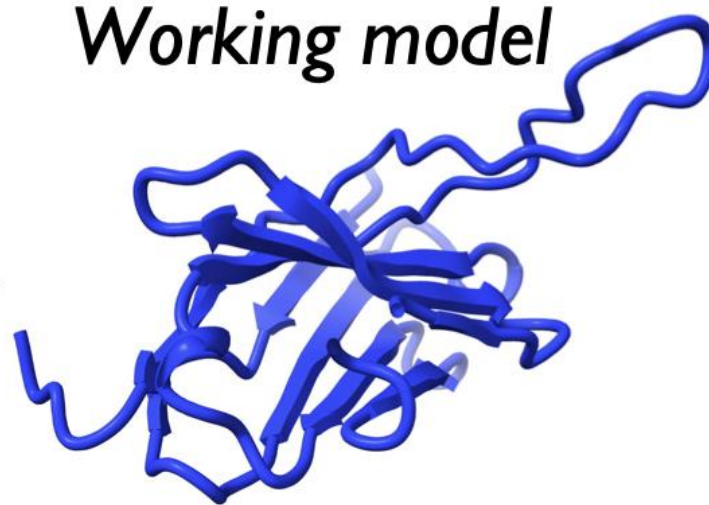
Why?

Because your new prediction might be better than your model

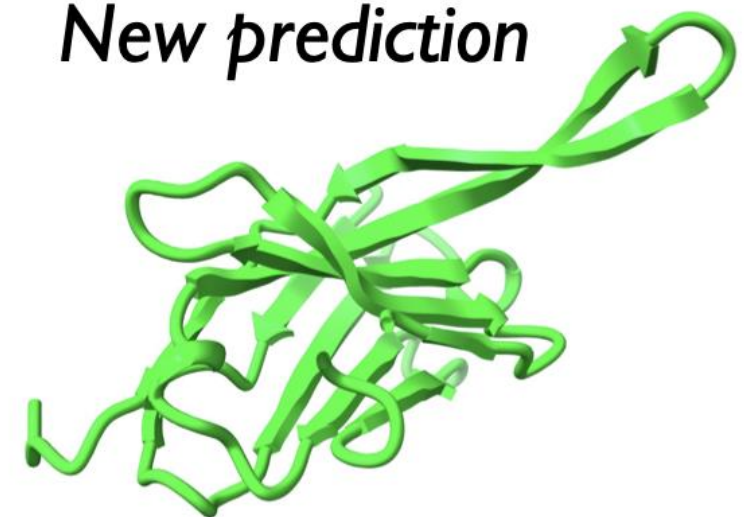
AlphaFold prediction



Working model



New prediction



Strategy for structure determination

1. Predict your structure

Design your experiment accordingly

(choose experimental approach, consider trimming at domain boundaries)

2. Solve your structure

Cryo-EM: docking

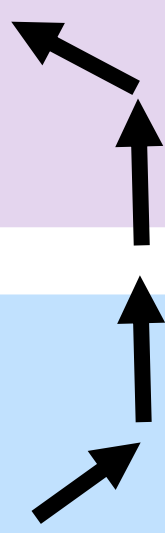
X-ray: MR; SAD

3. Update your prediction

Run AlphaFold again with your best model as a template

4. Improve your structure

Use your new prediction as hypothesis, rebuild parts

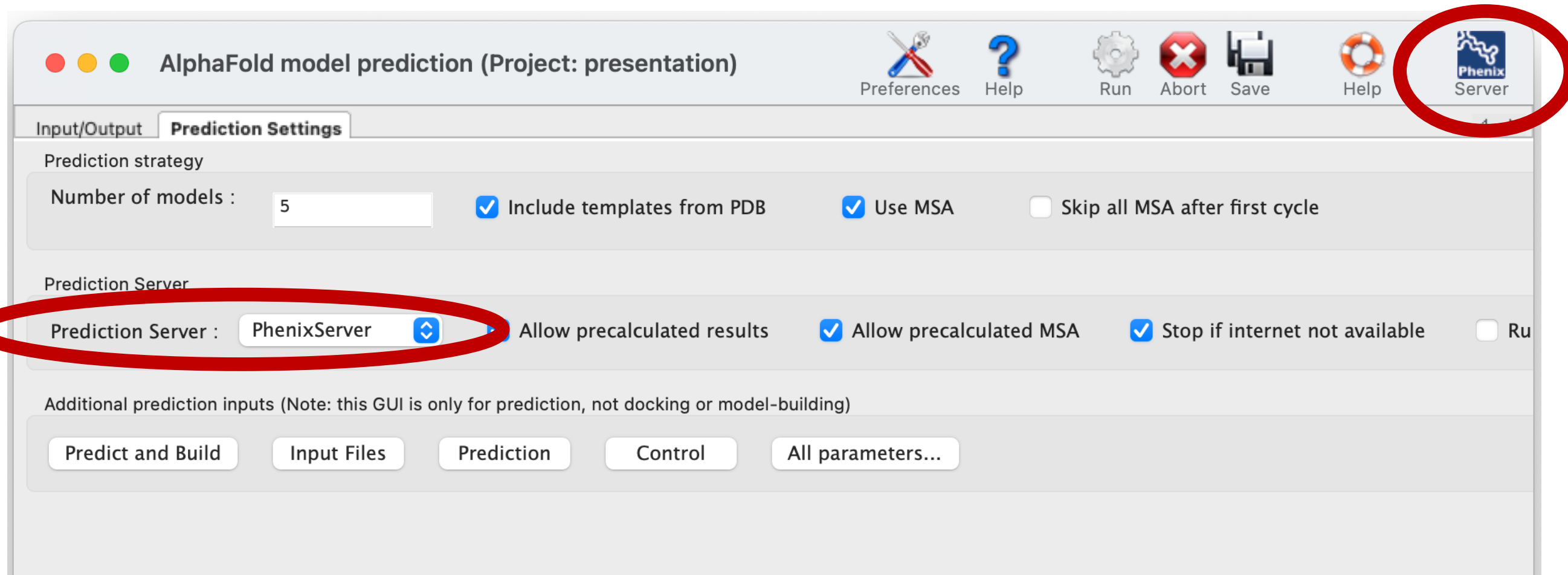


Iterate

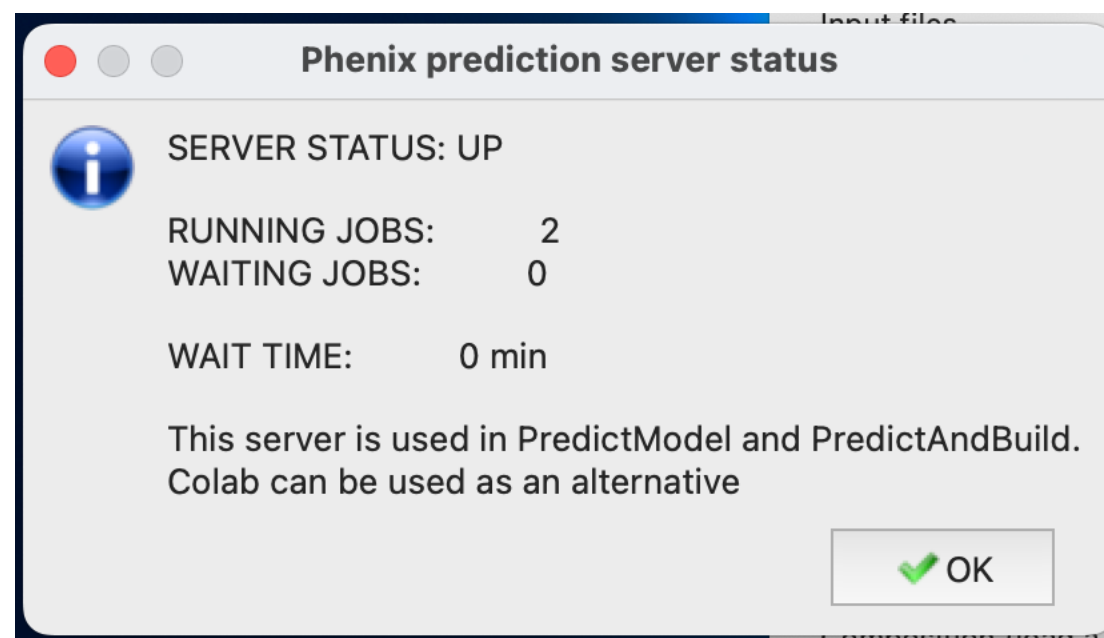
The diagram illustrates an iterative process. A green rounded rectangle labeled 'Iterate' is positioned to the right of the bottom two steps. Three black arrows originate from this box: one points up to step 3, one points up to step 4, and one points down to step 4, indicating a feedback loop between the prediction and improvement stages.



Phenix Server for running AlphaFold



No need for a local
AlphaFold installation



Process predicted model

Process Predicted Model (Project: 7rpq_AF_reference_model)

Preferences Help Run Abort Help

Configure

ProcessPredictedModel: Prepare predicted model for structure determination

Replace values in B-factor field with estimated B values.
Optionally remove low-confidence residues and split into domains.

Inputs: Model file (PDB, mmCIF)

Job title :

Input

Note: The B-value field in your model can be pLDDT, RMSD, or B

Predicted model : Browse...

Contents of B-value field for input models :

Optional input files

Output

Output file prefix (optional) :

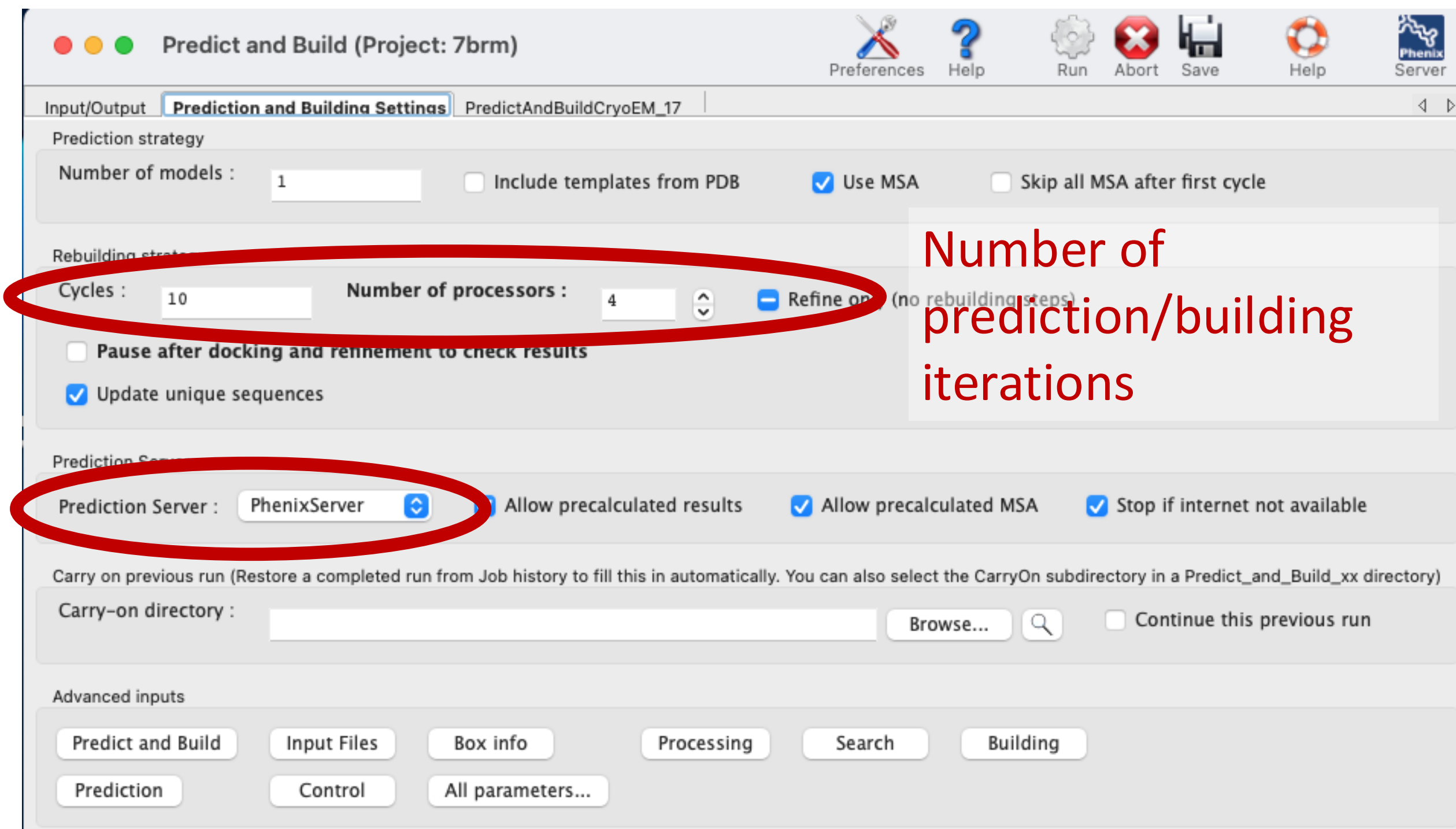
Options

☒ Remove low-confidence residues ☒ Split model into compact regions Maximum output B :

☒ Remove hydrogen ☐ Use only single-letter chain ID ☐ Maintain continuous chain

Processing options All parameters...

Iterate with Predict and Build



The screenshot shows the Phenix Predict and Build window for project 7brm. The interface is divided into several sections: Prediction strategy, Rebuilding strategy, Prediction Server, Carry-on directory, and Advanced inputs. Two red ovals highlight specific settings: the first oval encircles the 'Cycles' field (set to 10) and the 'Number of processors' field (set to 4) in the Rebuilding strategy section; the second oval encircles the 'Prediction Server' dropdown menu (set to PhenixServer) in the Prediction Server section. A red text box with the text 'Number of prediction/building iterations' is positioned to the right of the first oval. The top toolbar contains icons for Preferences, Help, Run, Abort, Save, and Help, along with the Phenix Server icon. The bottom toolbar contains buttons for Predict and Build, Input Files, Box info, Processing, Search, Building, Prediction, Control, and All parameters...

Predict and Build (Project: 7brm)

Input/Output **Prediction and Building Settings** PredictAndBuildCryoEM_17

Prediction strategy

Number of models : 1 ☐ Include templates from PDB ☒ Use MSA ☐ Skip all MSA after first cycle

Rebuilding strategy

Cycles : 10 Number of processors : 4 ☒ Refine on (no rebuilding steps)

☐ Pause after docking and refinement to check results

☒ Update unique sequences

Prediction Server

Prediction Server : PhenixServer ☒ Allow precalculated results ☒ Allow precalculated MSA ☒ Stop if internet not available

Carry on previous run (Restore a completed run from Job history to fill this in automatically. You can also select the CarryOn subdirectory in a Predict_and_Build_xx directory)

Carry-on directory : Browse... ☐ Continue this previous run

Advanced inputs

Predict and Build Input Files Box info Processing Search Building

Prediction Control All parameters...

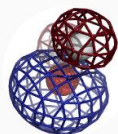
Fully automatic – AF prediction, processing, building, refinement.

Further reading/material

Documentation:

<https://www.phenix-online.org/documentation/>

Phenix Tutorials



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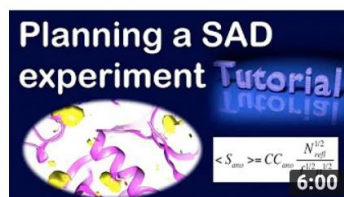
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ABOUT



Uploads PLAY ALL

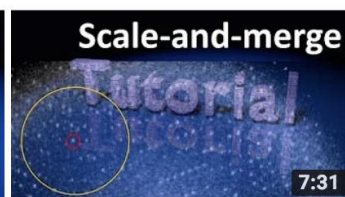
SORT BY



Simulate a SAD experiment with...



Automatic map interpretation with map_to_model



Scaling and merging anomalous data



Automated map sharpening with phenix.autosharpen

Video tutorials

<https://www.youtube.com/c/phenixtutorials>

Working with AlphaFold2, RoseTTAFold and other predicted models

You can use the predicted models from AlphaFold and other prediction software in Phenix. Using these models can be very helpful in structure determination because the models can be very accurate over much of their length and the models come with accuracy estimates that allow removal of poorly-predicted regions.

How to use predicted models in Phenix

Use [Predict and build](#) to incorporate predicted models in the structure solution workflow.

Predict_and_build generates predicted models and uses them to solve an X-ray structure by MR or to interpret a cryo-EM map. The tool then carries out iterative model rebuilding and prediction to improve the models. The iterative procedure allows creating more accurate predicted models than can be obtained with a simple prediction.

Predict and build can automatically generate a fairly accurate model starting from just a sequence file and either cryo-EM half-maps or X-ray data. Additionally, it provides morphed versions of unrefined predicted models that can be useful as reference models for refinement.

Other tools for using predicted models:

- [Overview](#): AlphaFold and Phenix
- [Processing](#) a predicted model
- [Docking](#) a processed predicted model in a cryo-EM map
- [Rebuilding](#) a docked predicted model in a cryo-EM map
- [Processing, docking and rebuilding](#) a predicted model in a cryo-EM map
- [Trimming overlapping parts](#) of models

AlphaFold changes everything:
Incorporating predicted models in X-ray and Cryo-EM structure determination

Presentation available at:
phenix-online.org/presentations

January 18, 2022
RéNaFoBiS Webinar
Tom Terwilliger
The New Mexico Consortium
Los Alamos National Laboratory

How you can benefit from AlphaFold ...
... by determining structures more easily

Randy Read, Tristan Croll, Claudia Millán, (Cambridge, University), Paul Adams, Billy Poon, Pavel Afonine, Christopher J. Schlicksup (Lawrence Berkeley National Laboratory); Jane Richardson (Duke University)

Phenix
Los Alamos NATIONAL LABORATORY
BERKELEY LAB
UNIVERSITY OF CAMBRIDGE
New Mexico CONSORTIUM

Tom Terwilliger: AlphaFold changes everything

Tom Terwilliger: AlphaFold changes everything

<https://youtu.be/ugMPYdPo8Bc?feature=shared>

The Project



Lawrence Berkeley Laboratory

Paul Adams, Pavel Afonine,
Dorothee Liebschner, Nigel
Moriarty, Billy Poon,
Christopher Schlicksup,
Oleg Sobolev



University of Cambridge

Randy Read, Airlie McCoy,
Alisia Fadini



Los Alamos National Laboratory New Mexico Consortium

Tom Terwilliger, Li-Wei Hung



UTHealth

Matt Baker



Duke University

Jane Richardson, Christopher
Williams, Vincent Chen



An NIH/NIGMS funded
Program Project

Liebschner D, *et al.*, Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*. Acta Cryst. 2019 **D75**:861–877

Example: Fab heavy chain

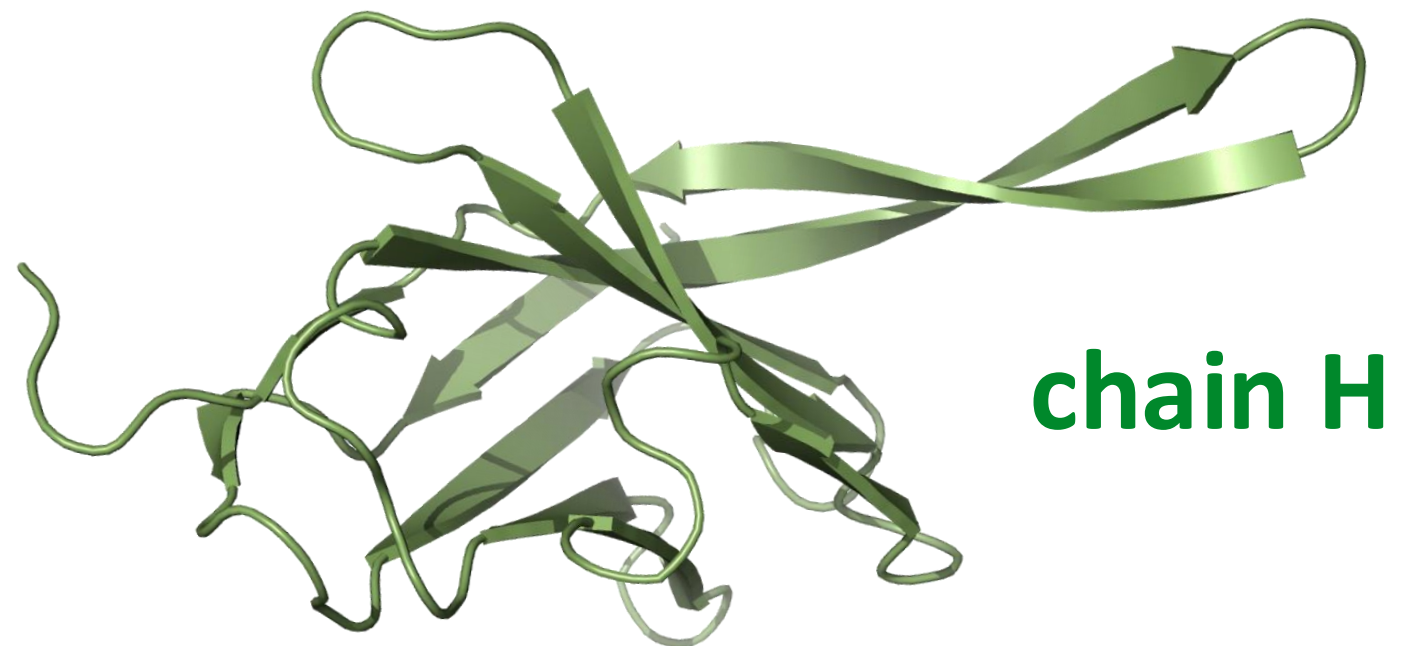
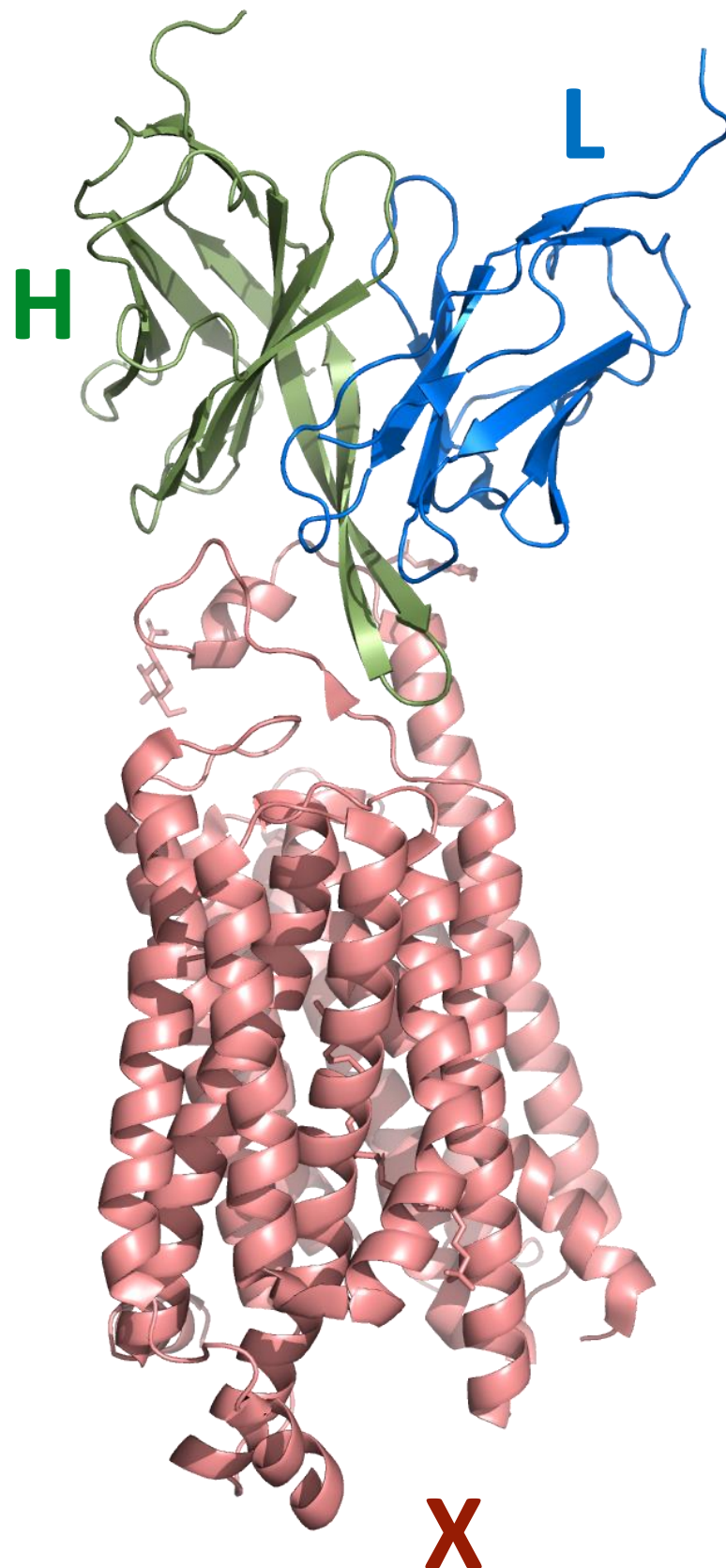
 **7MJS**

Single-Particle Cryo-EM Structure of Major Facilitator Superfamily Domain containing 2A in complex with LPC-18:3

PDB DOI: <https://doi.org/10.2210/pdb7MJS/pdb>

EM Map EMD-23883: [EMDB EMDDataResource](https://www.ebi.ac.uk/emdb/EMD-23883)

3.03 Å resolution

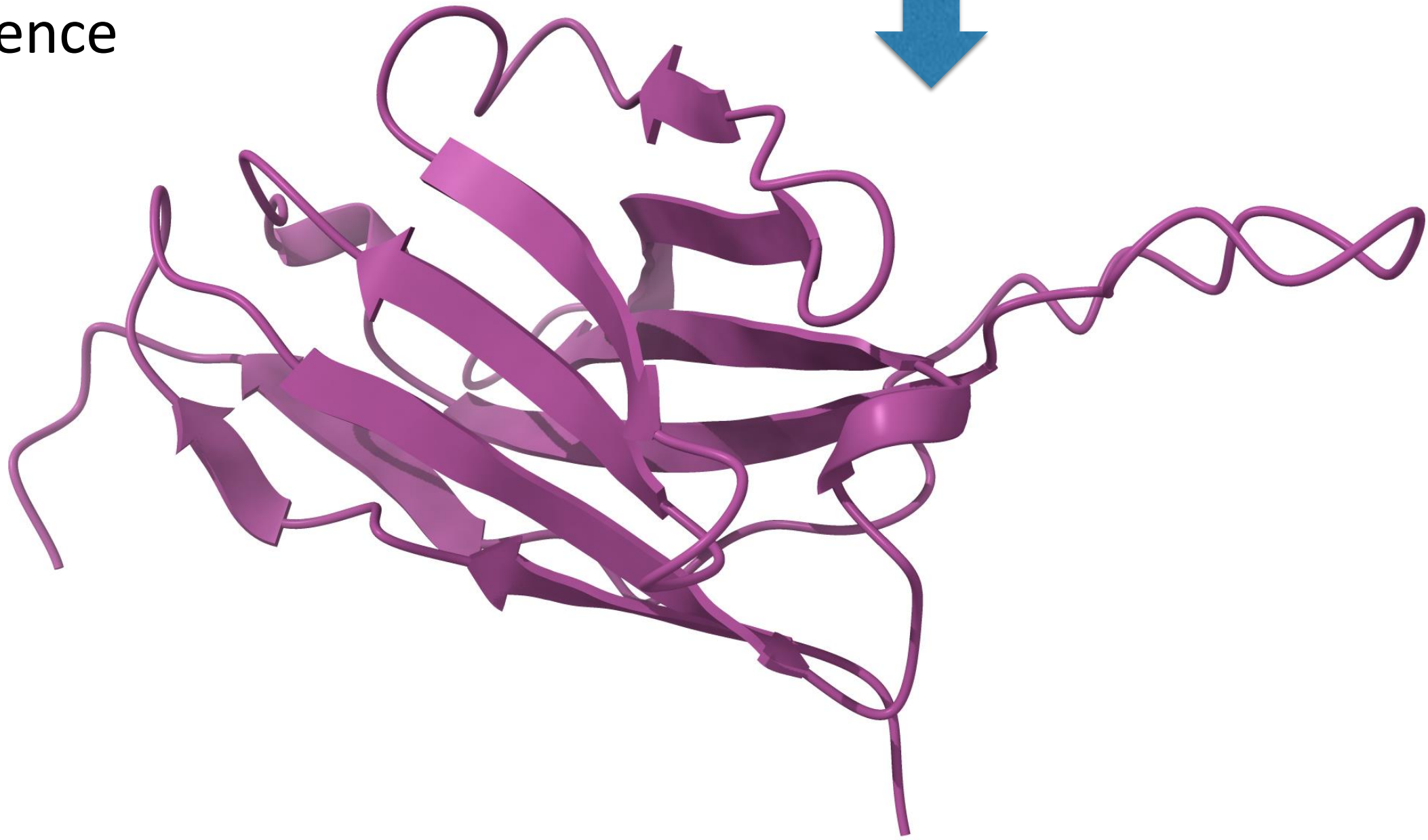


A loop that interacts with other chains is not correctly predicted.

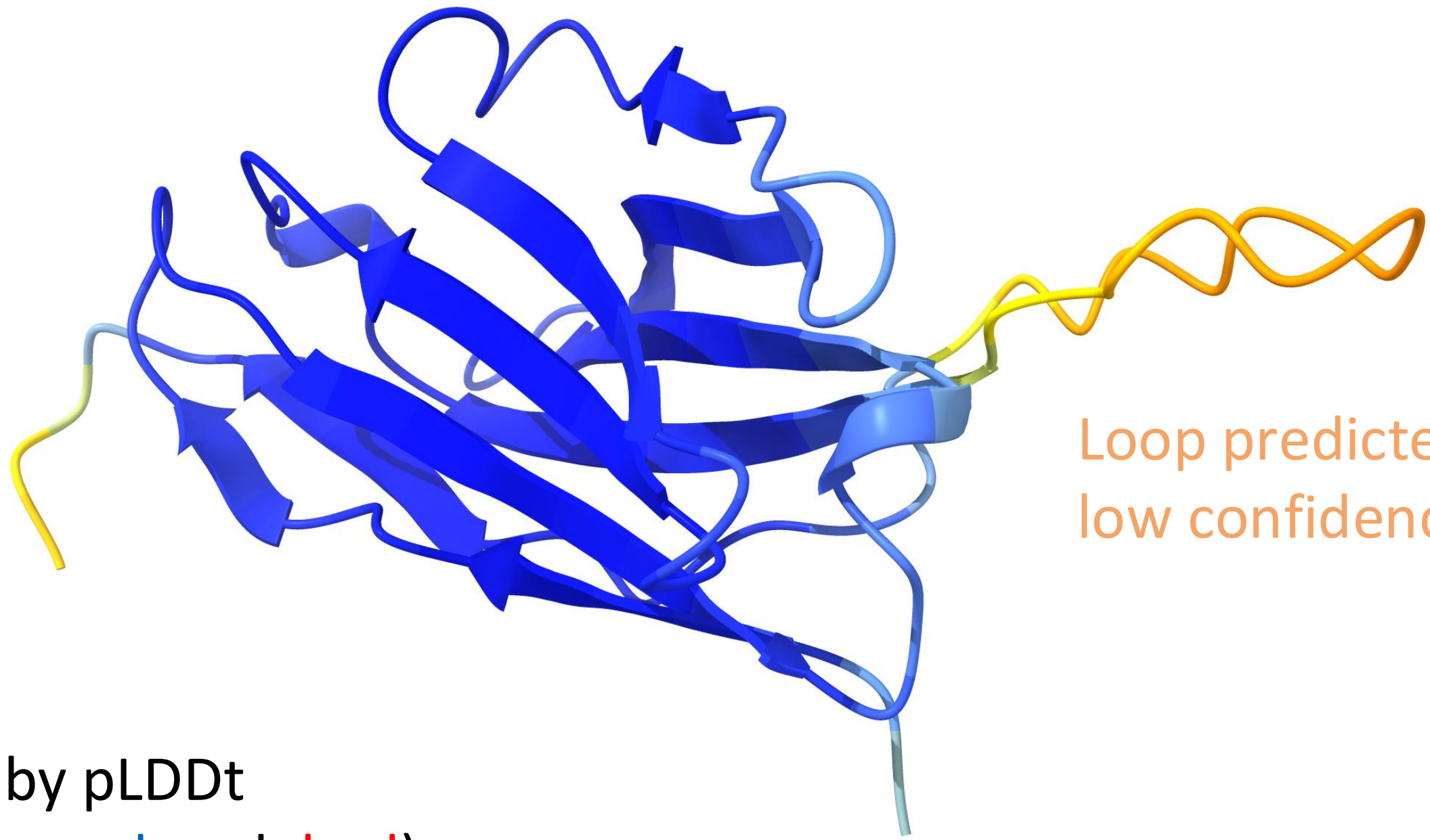
AF2 prediction of chain H

EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQ
APGKGLEWVAYIYSYSGYTSYADSVKGRFTISADTSKNTA
YLQMNSLRAEDTAVYYCARSLEYLYSSGYQYKWATGLDY
WGQGTLVTVSSAST

Sequence



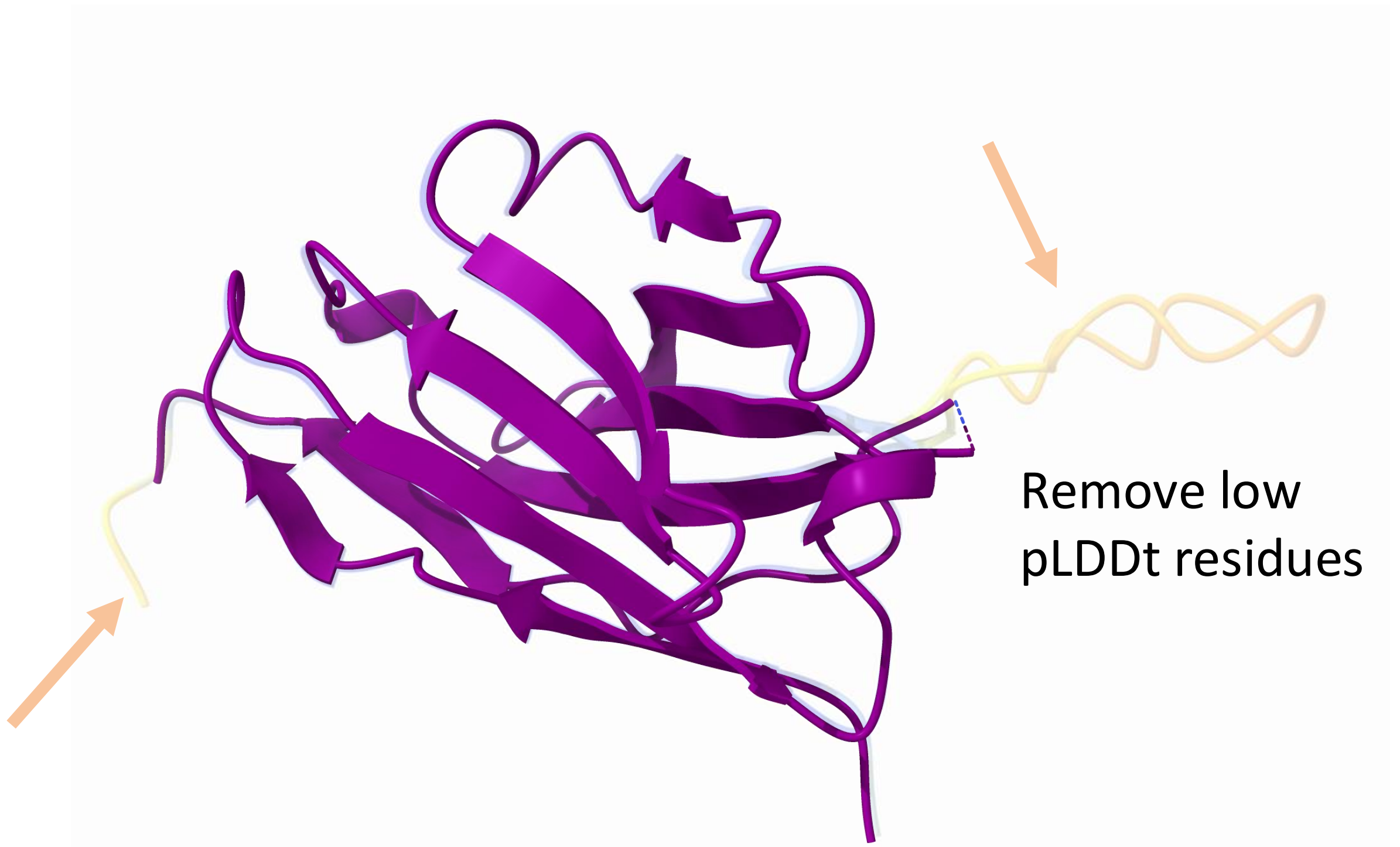
AF2 prediction of chain H



Loop predicted with
low confidence

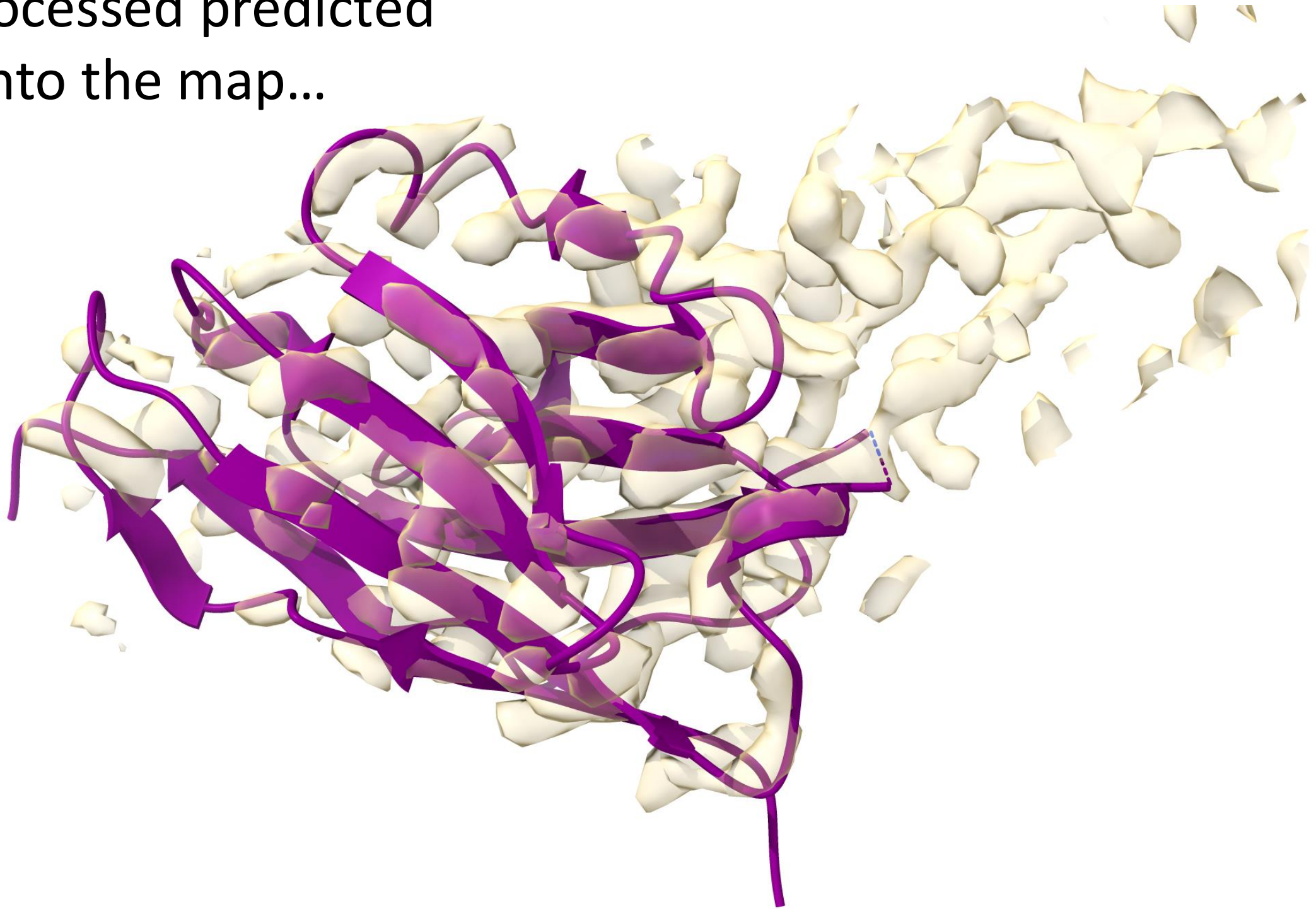
Color by pLDDt
(blue: **good**, red: **bad**)

Process AF2 prediction

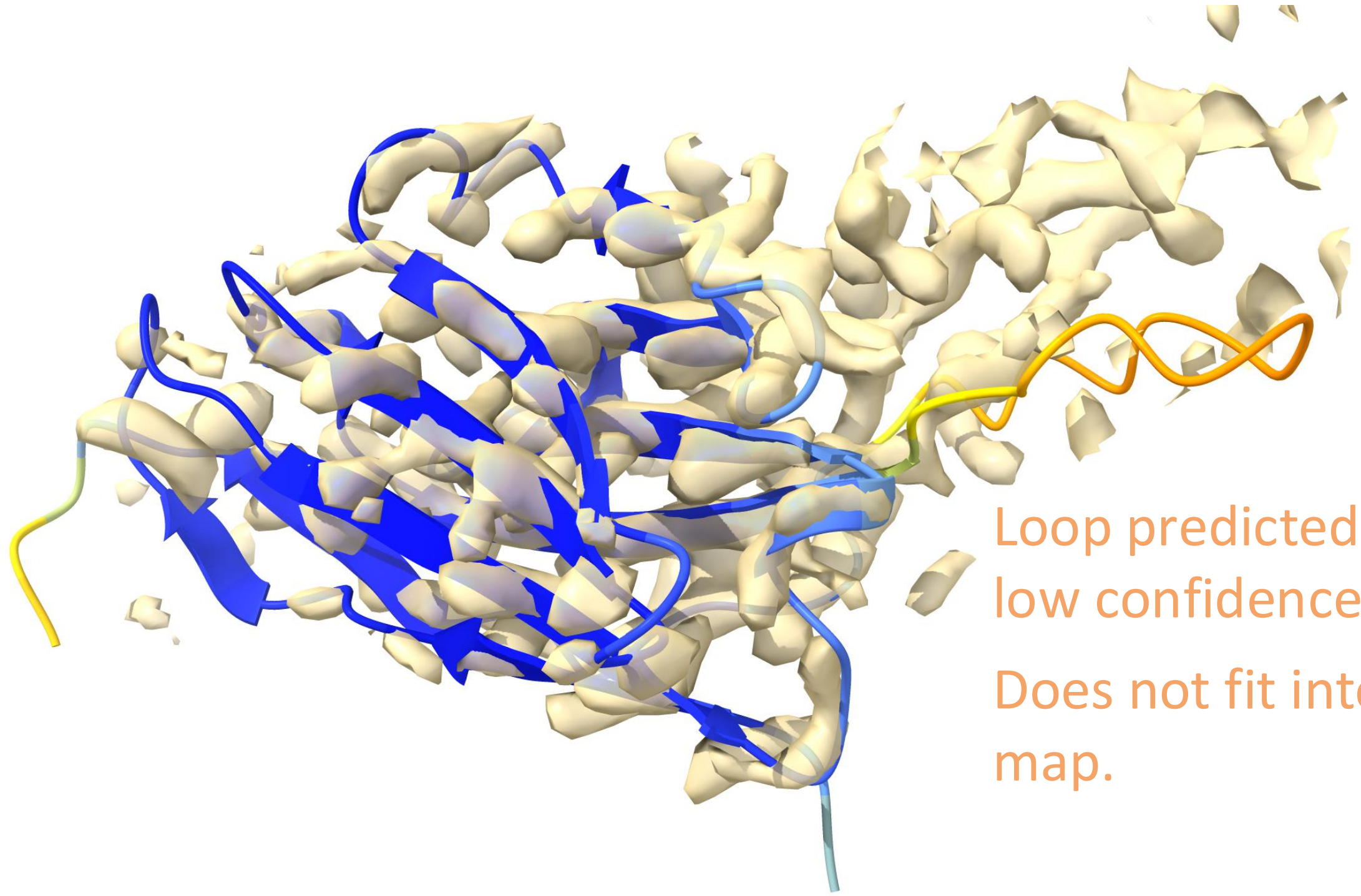


Dock processed model into the map

Dock processed predicted
model into the map...



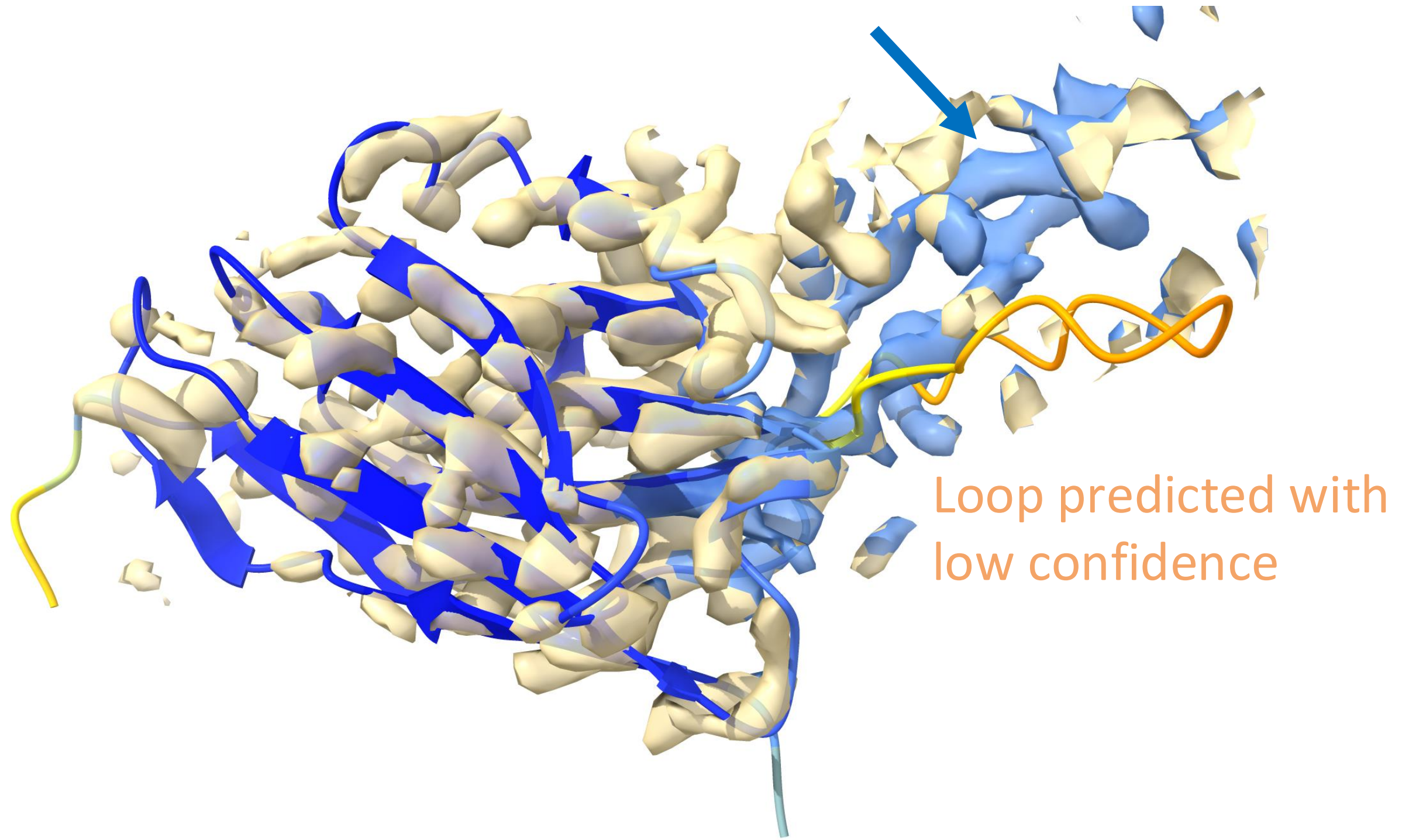
AF2 prediction of chain H



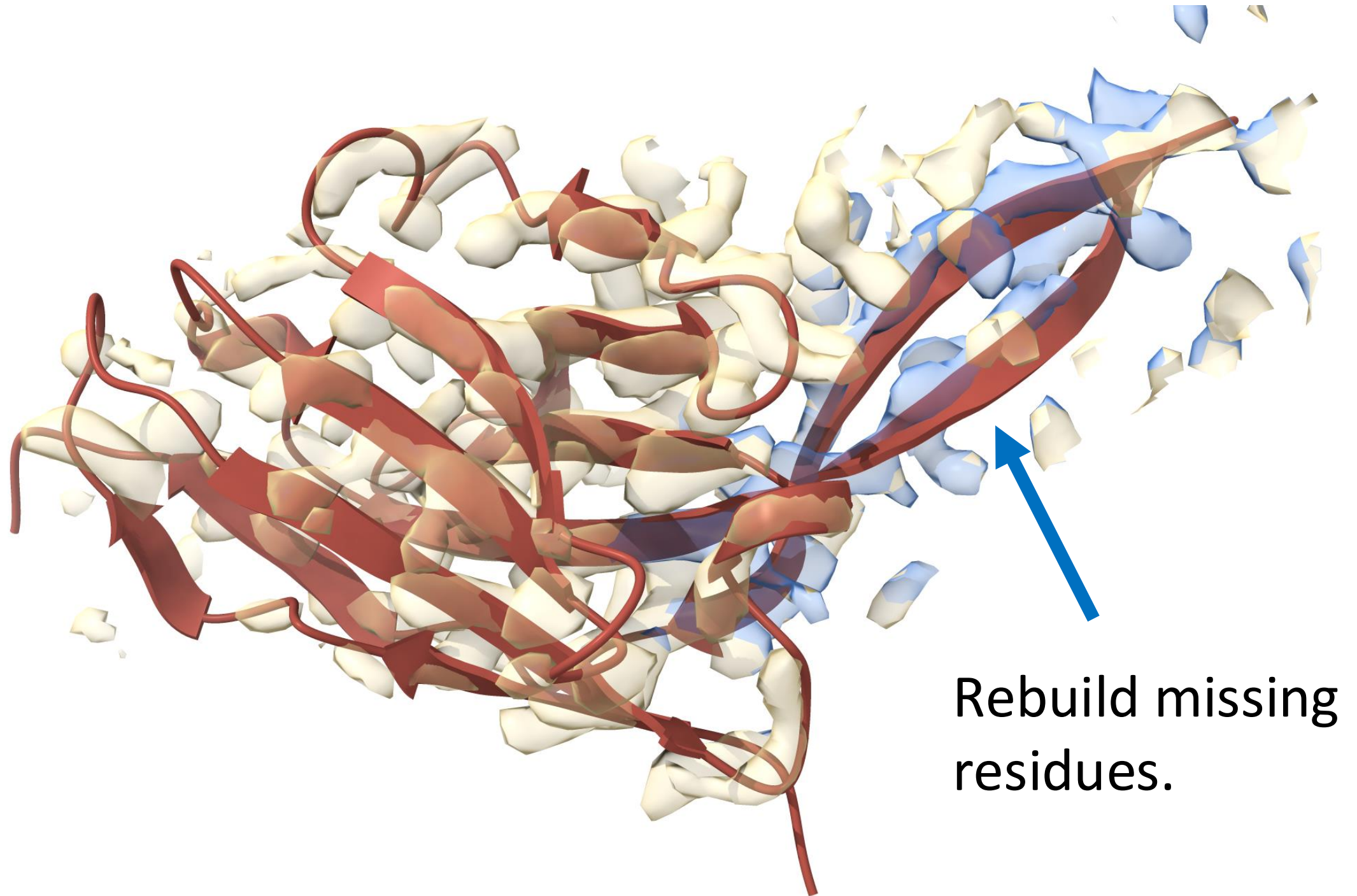
Loop predicted with
low confidence

Does not fit into
map.

AF2 prediction of chain H



Dock and rebuild model



Rebuild missing
residues.

“predicted-processed-docked-rebuilt” model

Make a new prediction

Sequence

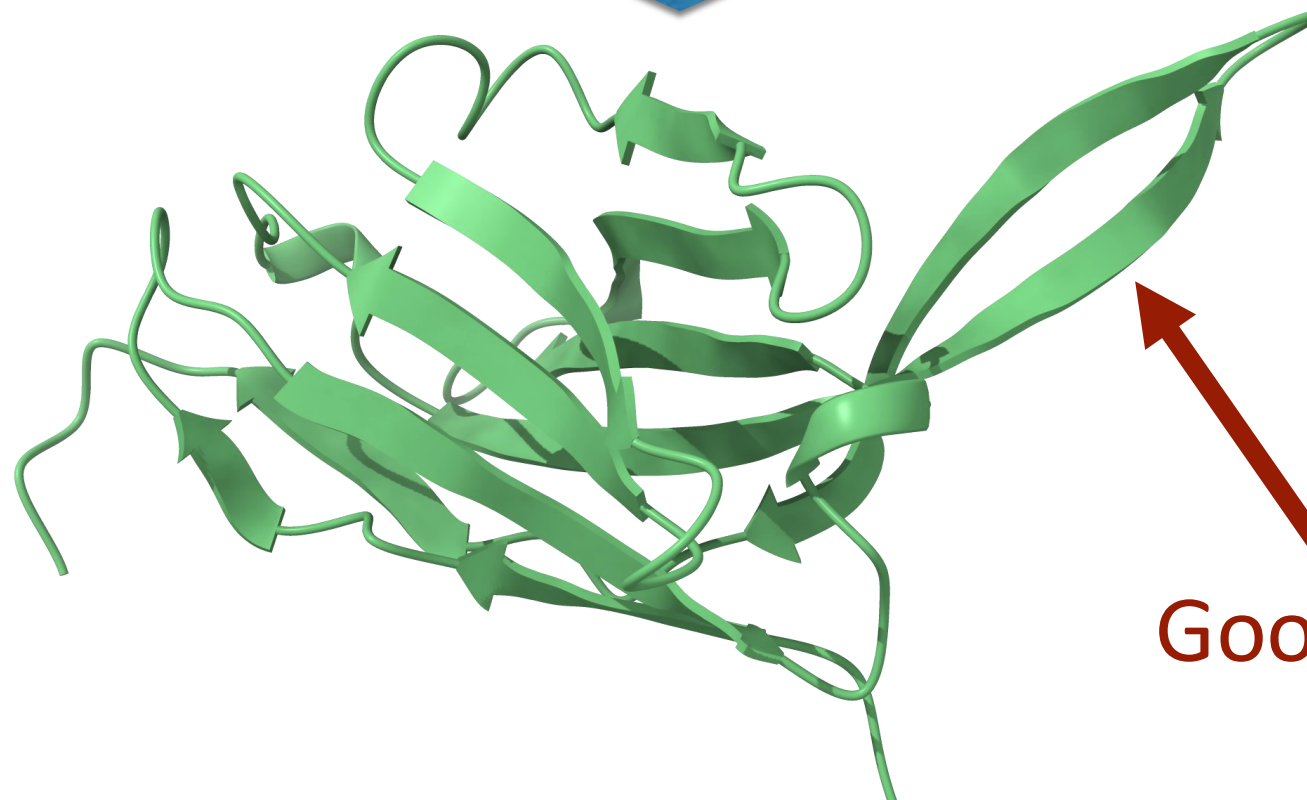
EVQLVESGGGLVQPGGSLRLSCAASGFNISYSSSIHWVRQ
APGKGLEWVAYIYSYSGYTSYADSVKGRFTISADTSKNTA
YLQMNSLR AEDTAVYYCARSLEYLYSSGYQYKWATGLDY
WGQGTLVTVSSAST

Template



Good loop

AlphaFold



Good loop

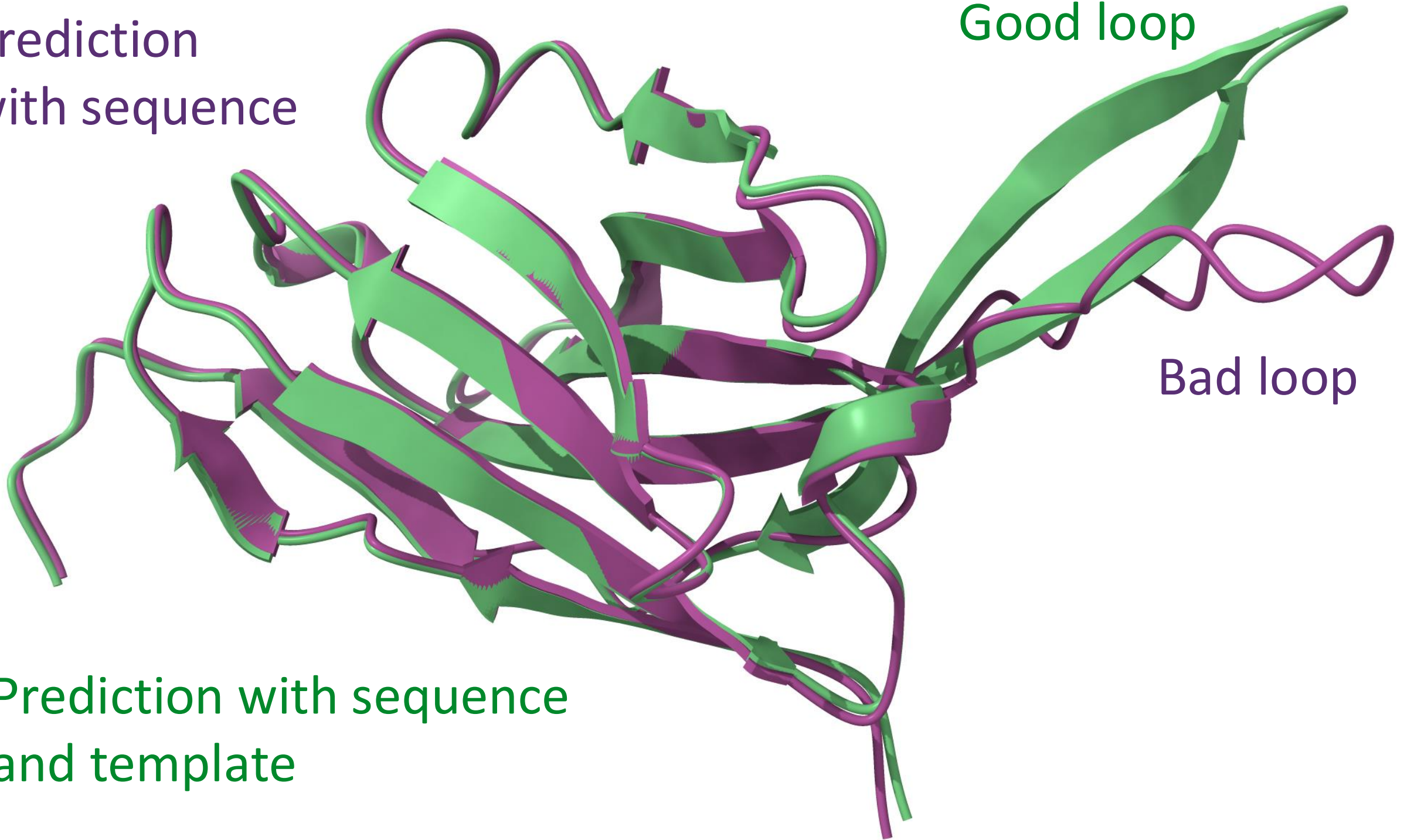
Using a template improves prediction

Prediction
with sequence

Good loop

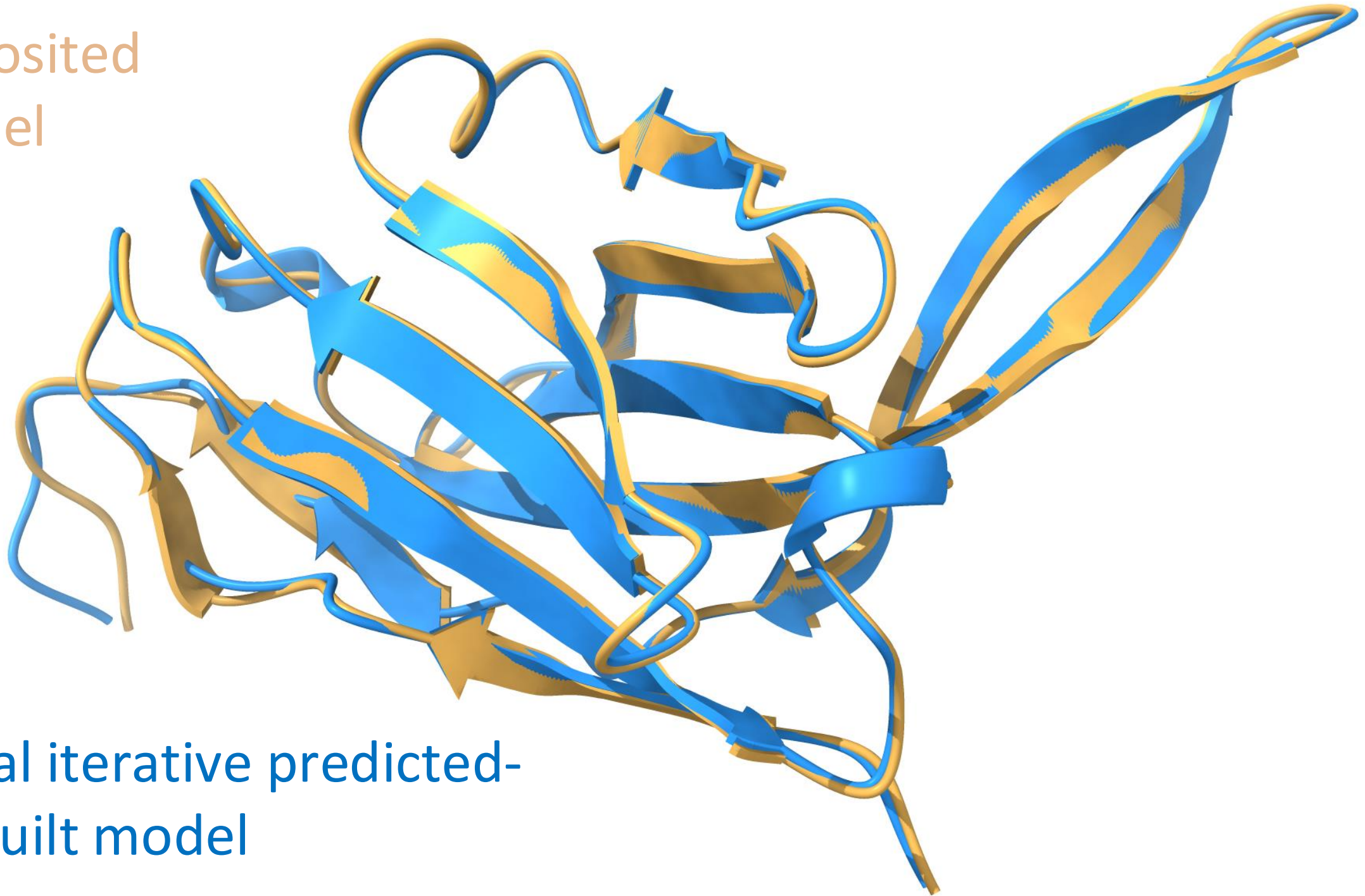
Bad loop

Prediction with sequence
and template



Iterate prediction and rebuilding

Deposited
model

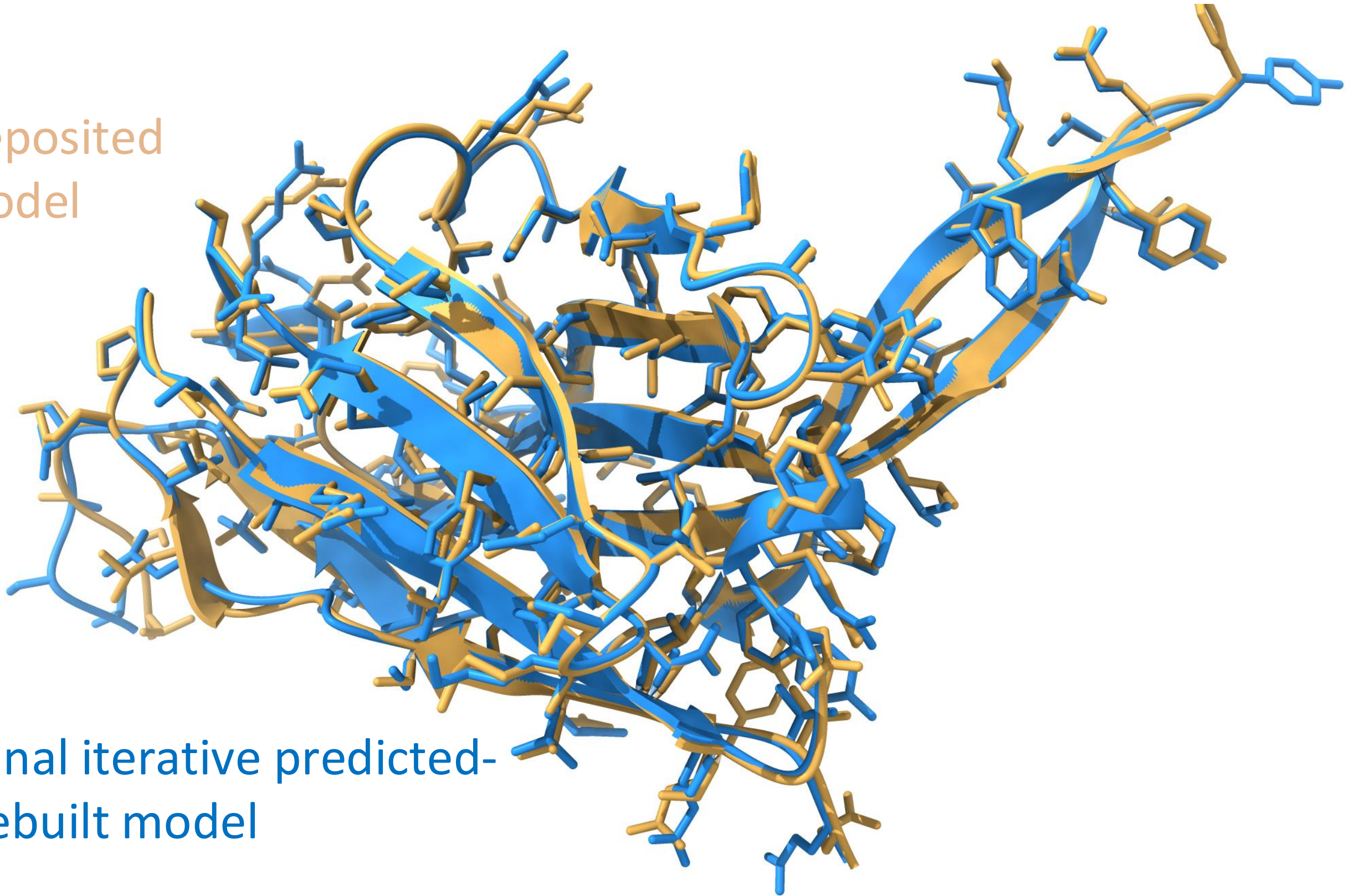


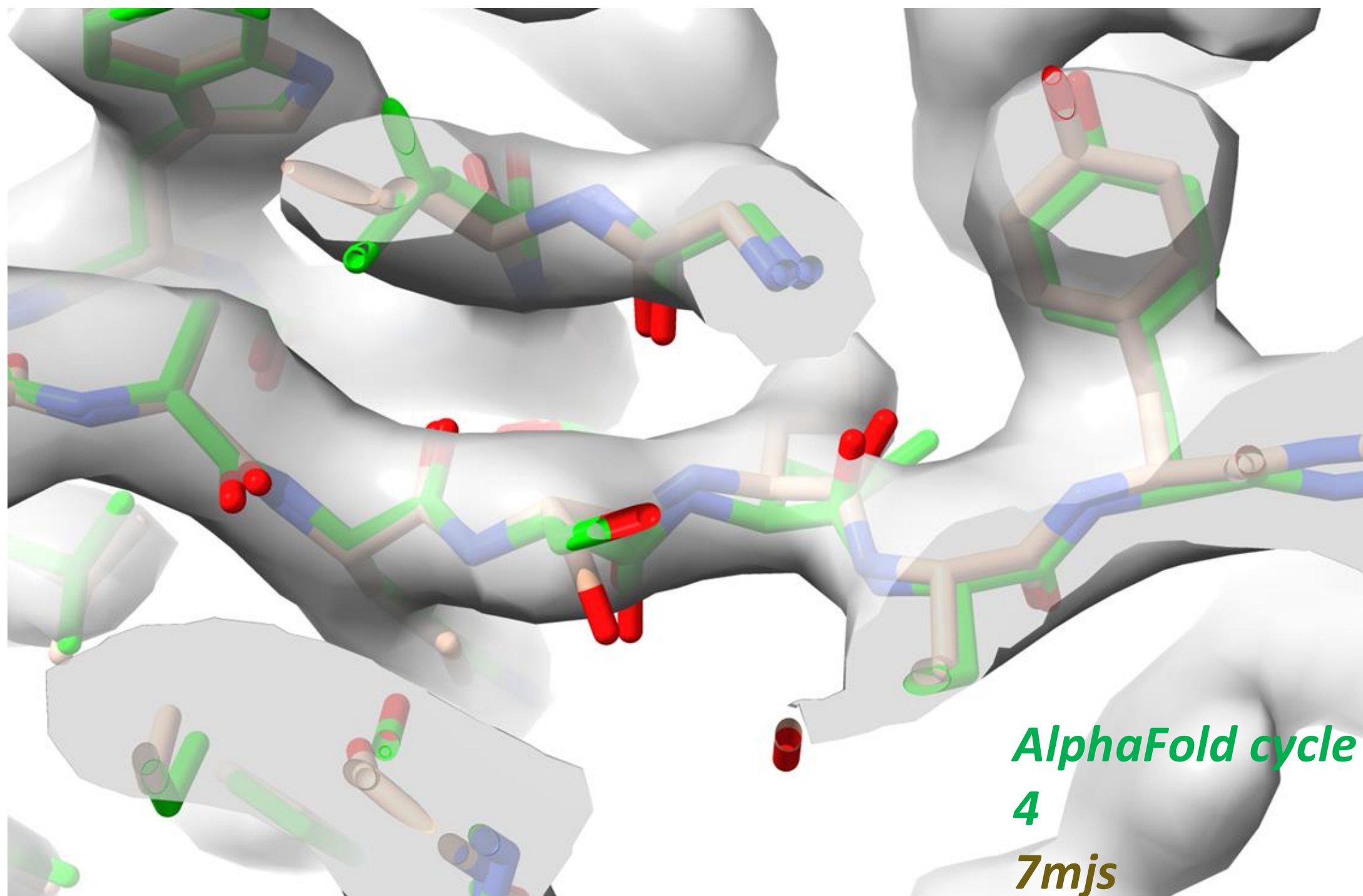
Final iterative predicted-
rebuilt model

Iterate prediction and rebuilding

Deposited
model

Final iterative predicted-
rebuilt model





Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319