BERKELEY LAB U.S. DEPARTMENT OF ENERGY UNIVERSITY OF CALIFORNIA

Phenix workshop at the University of Nebraska Lincoln,
April 30, 2026

Using Predicted models in Phenix (cryo-EM)

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Lawrence Berkeley Laboratory

Predicting models with AlphaFold

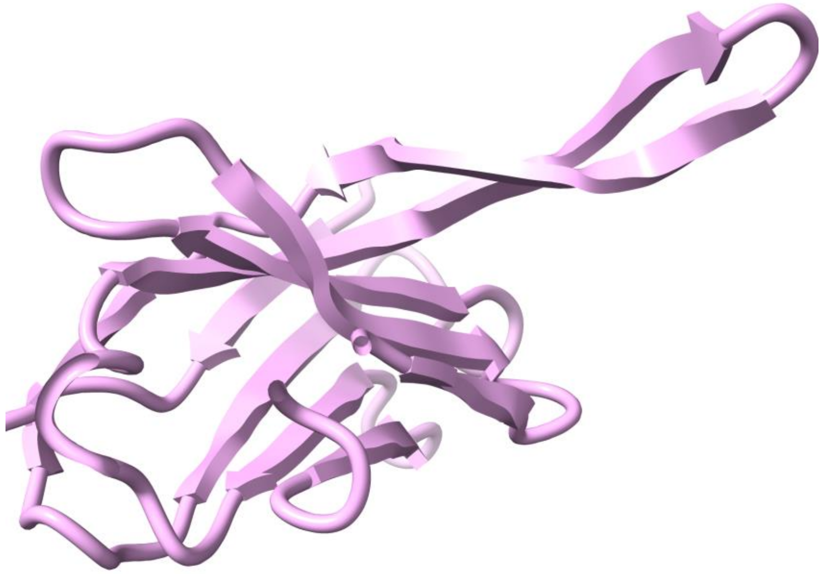
BLOG POST
RESEARCH

30 NOV 2020

AlphaFold: a solution to a 50-year-old grand challenge in biology

```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
............................F....M........Q........
..................K.........Y....L.......A.........
..............A.............................V......
....................A..............................
............................L....V....E............
.........A...................................Q.....
```

Sequence
Multiple sequence alignment



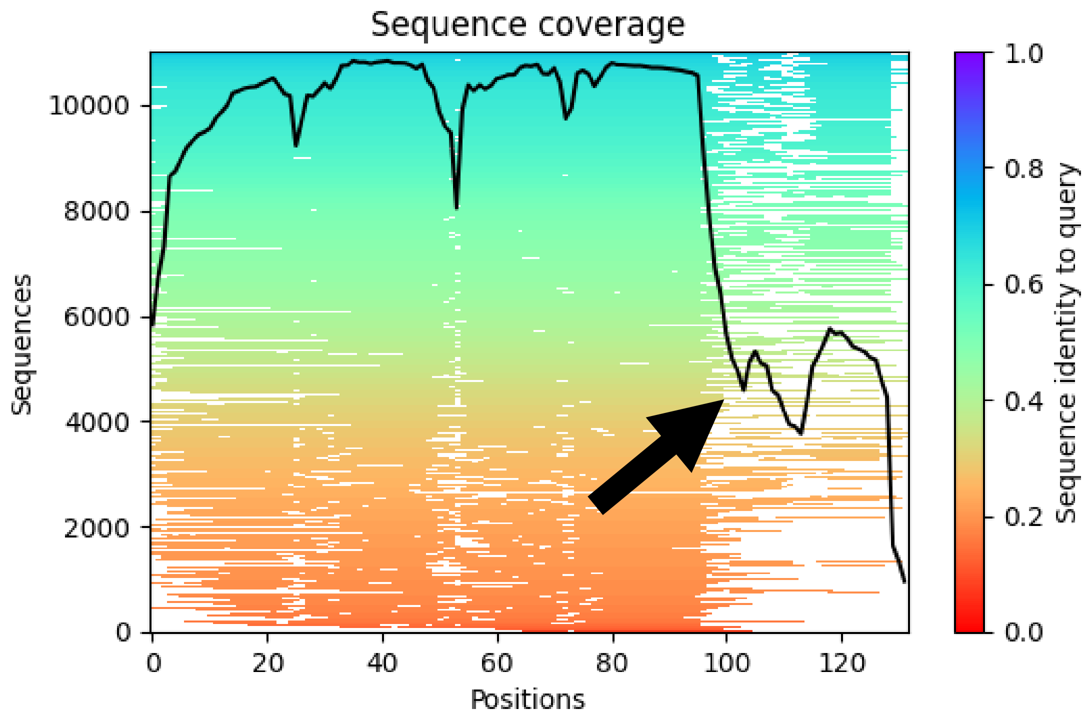
3D prediction

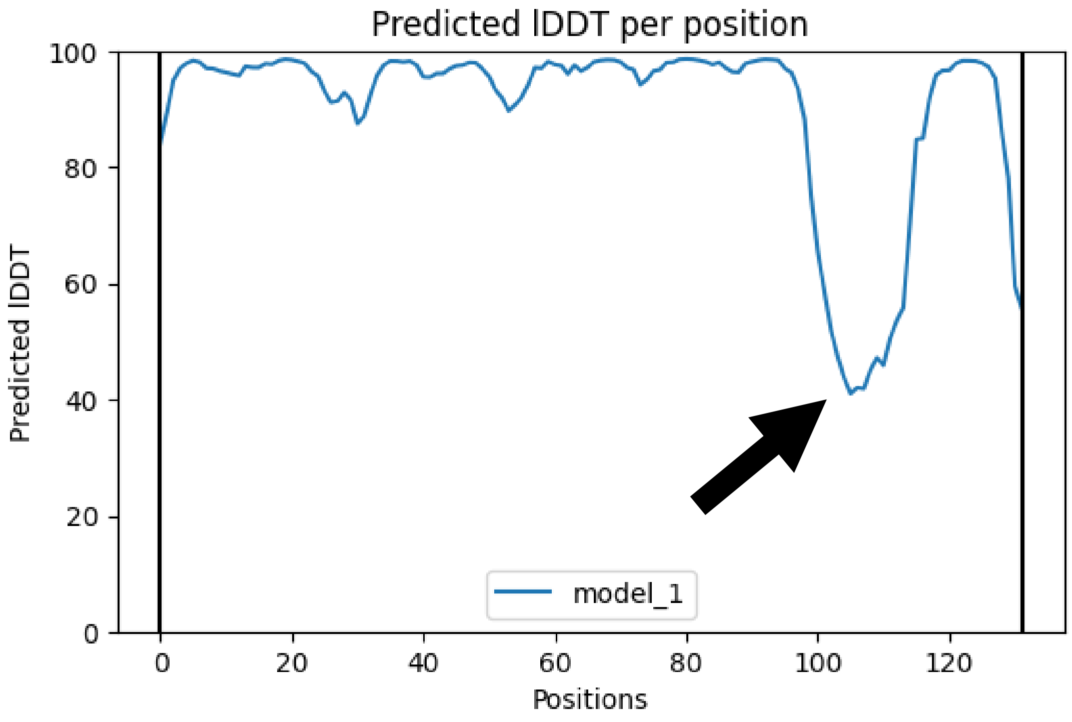
Predictions come with confidence measures

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).

Sequence coverage → **Confidence**

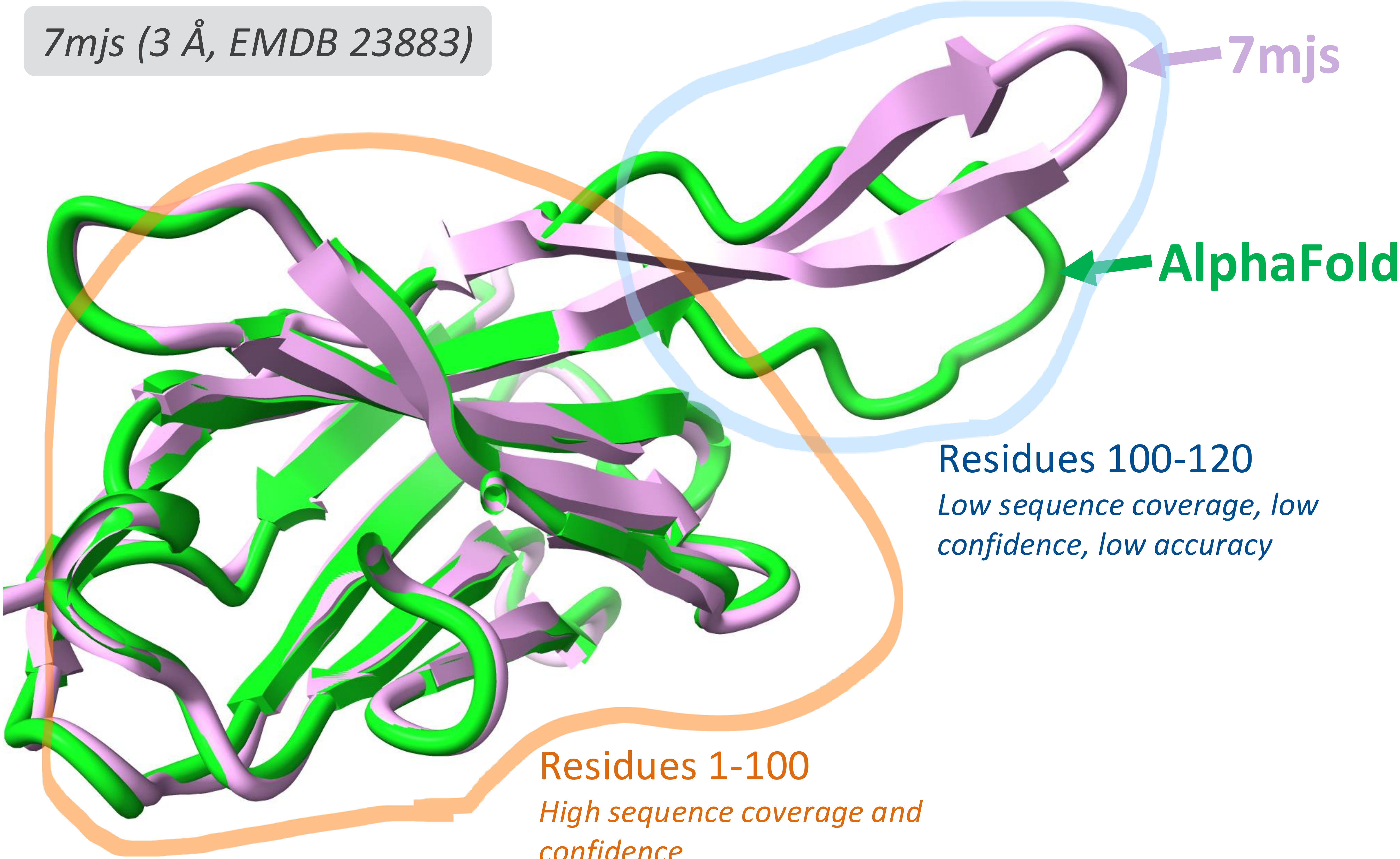




Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Predictions come with confidence measures

7mjs (3 Å, EMDB 23883)



Residues 100-120

Low sequence coverage, low confidence, low accuracy

Residues 1-100

High sequence coverage and confidence

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

Predictions come with confidence measures

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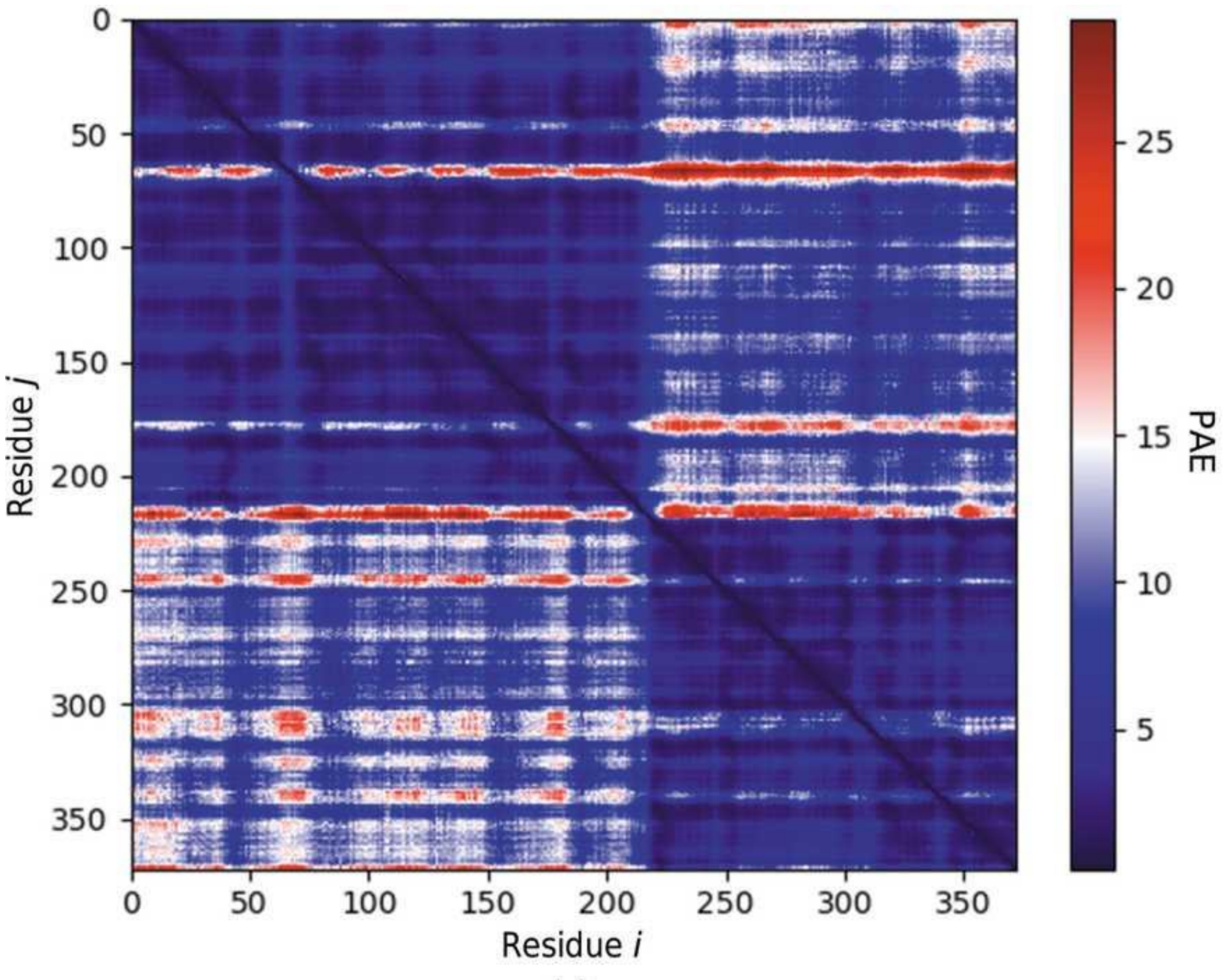
AlphaFold confidence (pLDDT)	Median prediction error (Å)	Percentage with error over 2 Å
>90	**0.6**	**10**
80 - 90	**1.1**	**22**
70 - 80	**1.5**	**33**
<70	**3.5**	**77**

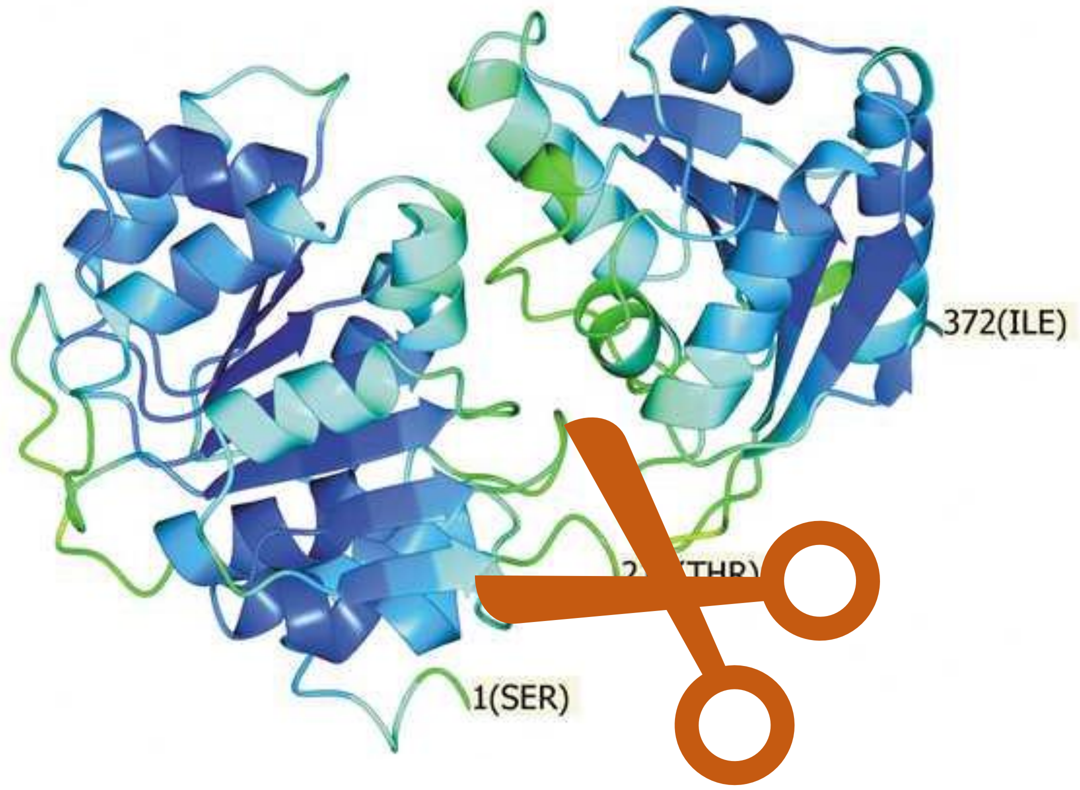
Terwilliger et al. (2023), AlphaFold predictions are valuable hypotheses, and accelerate but do not replace experimental structure determination. Nature Methods 2023: https://doi.org/10.1038/s41592-023-02087-4

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 Å.





- 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)

Oeffner RD, Croll TI, Millán C , Poon BK, Schlicksup CJ, Read RJ, Terwilliger TC. Acta Cryst. D, 2022 (78):1303-1314; https://doi.org/10.1107/S2059798322010026

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.

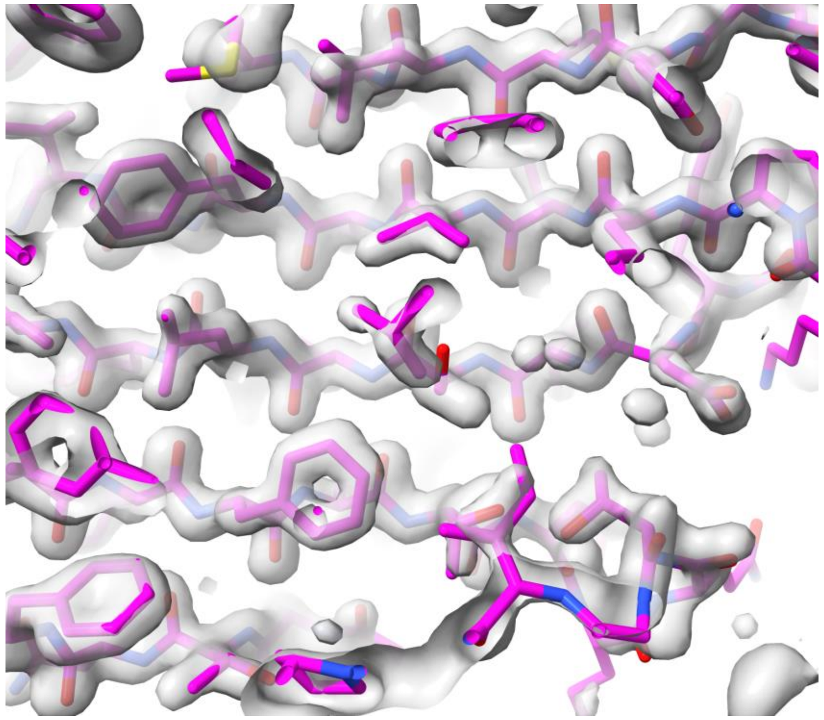
→ Convert pLDDT to pseudo B-factors.

$$\Delta = 1.5\exp[4(0.7 - \mathrm{pLDDT})] \qquad B = \frac{8\pi^2\Delta^2}{3}$$

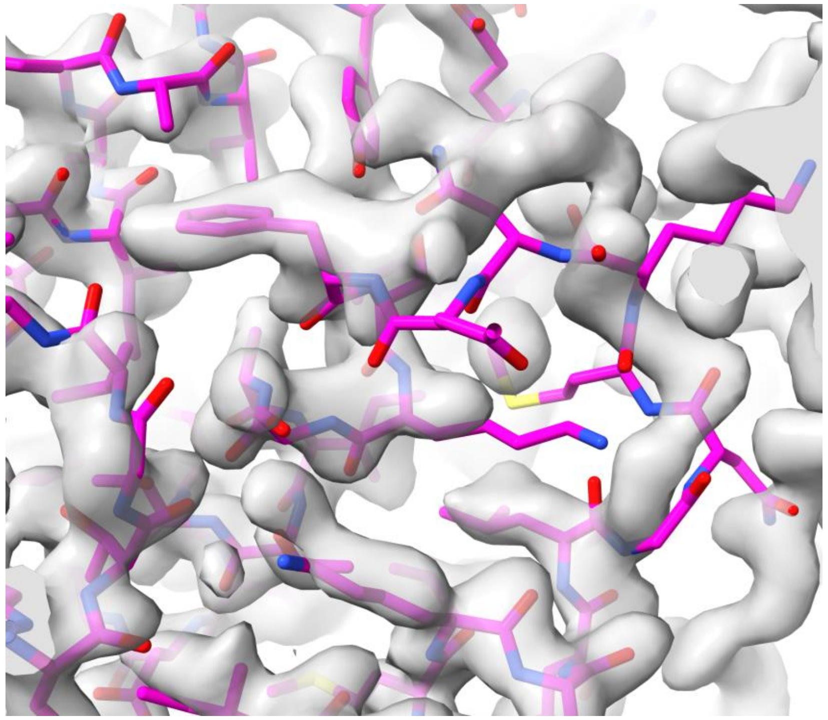
Oeffner RD, Croll TI, Millán C , Poon BK, Schlicksup CJ, Read RJ, Terwilliger TC. Acta Cryst. D, 2022 (78):1303-1314; https://doi.org/10.1107/S2059798322010026

AlphaFold predictions are great hypotheses

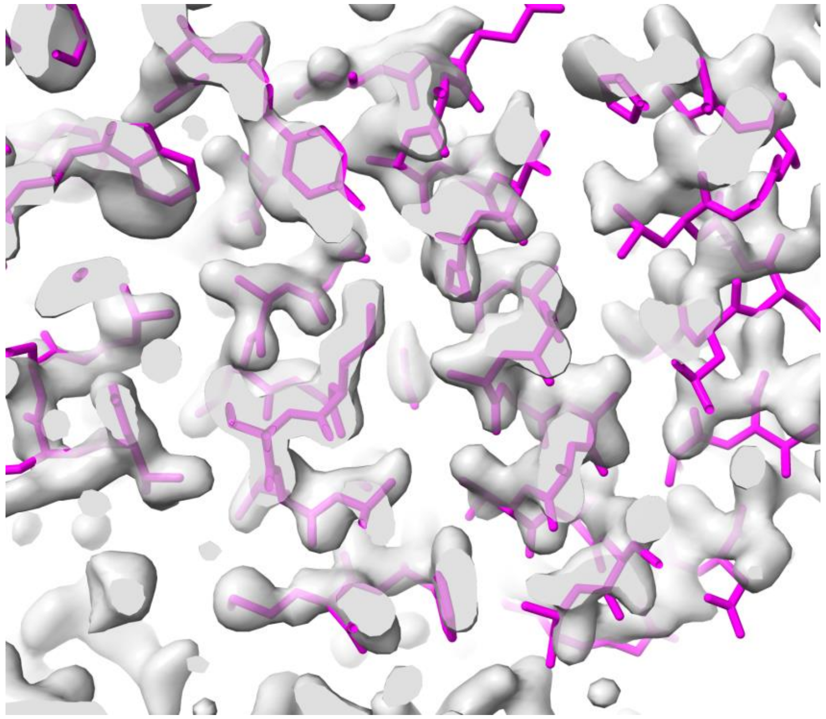
AlphaFold models
can be….



Awesome



Wrong

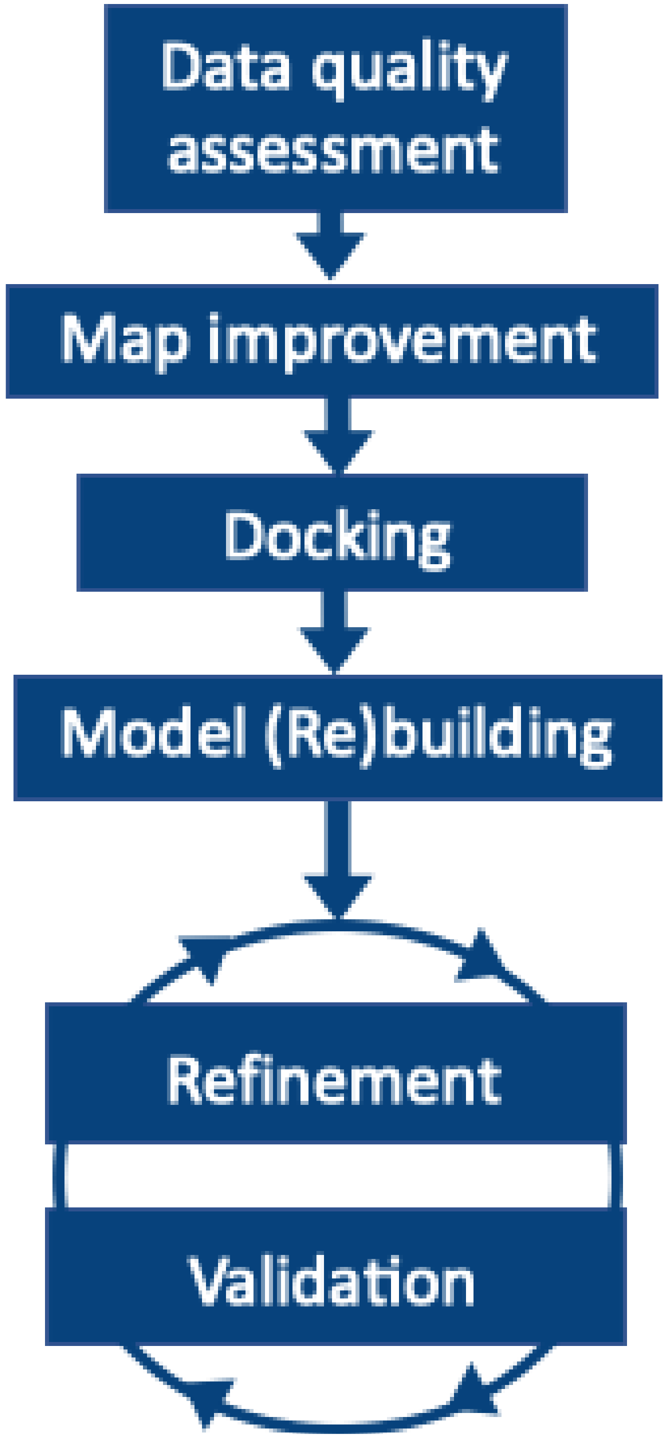


Distorted

Terwilliger et al. (2023), AlphaFold predictions are valuable hypotheses, and accelerate but do not replace experimental structure determination. Nature Methods 2023: https://doi.org/10.1038/s41592-023-02087-4

What can predicted models be used for?

Incorporate predictions into the typical cryo-EM workflow.



Data quality assessment

Map improvement

Docking

Model (Re)building

Refinement

Validation

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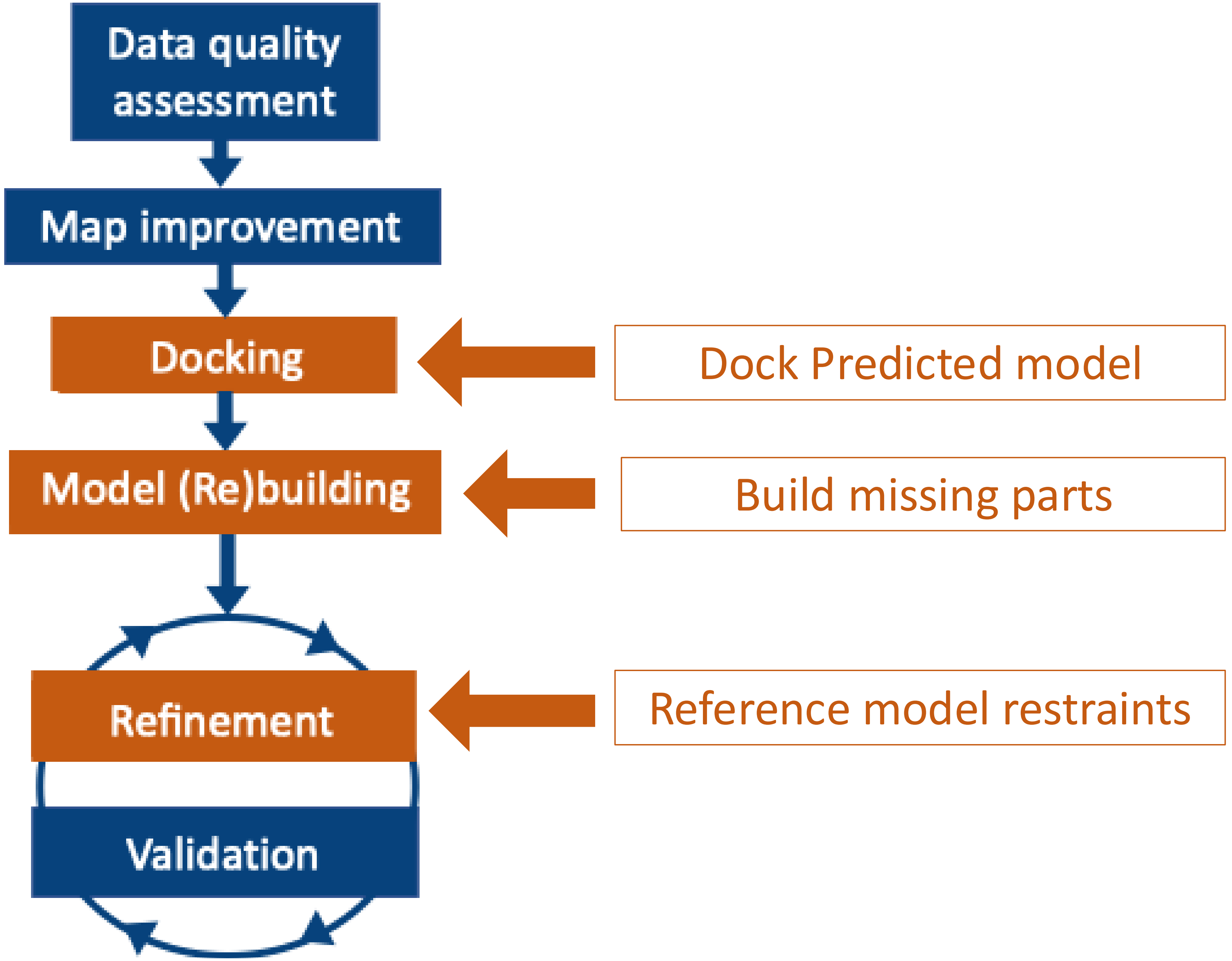
Incorporate predictions into the typical cryo-EM workflow.



Data quality assessment

Map improvement

Docking

Model (Re)building

Refinement

Validation

Dock Predicted model

Build missing parts

Reference model restraints

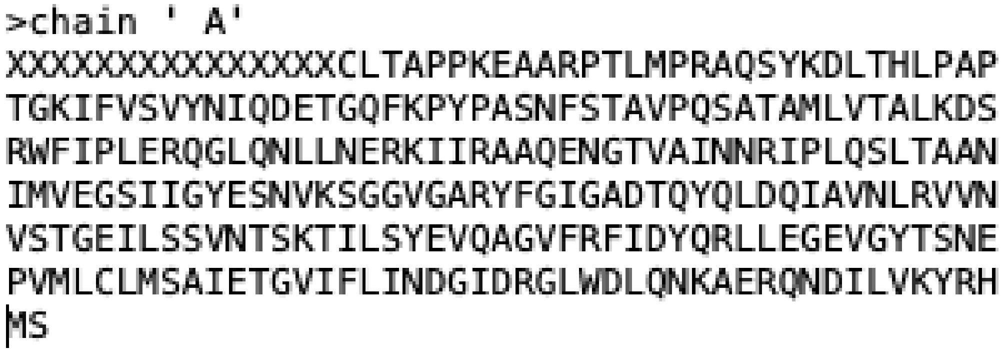
1. Use a prediction for cryo-EM docking

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

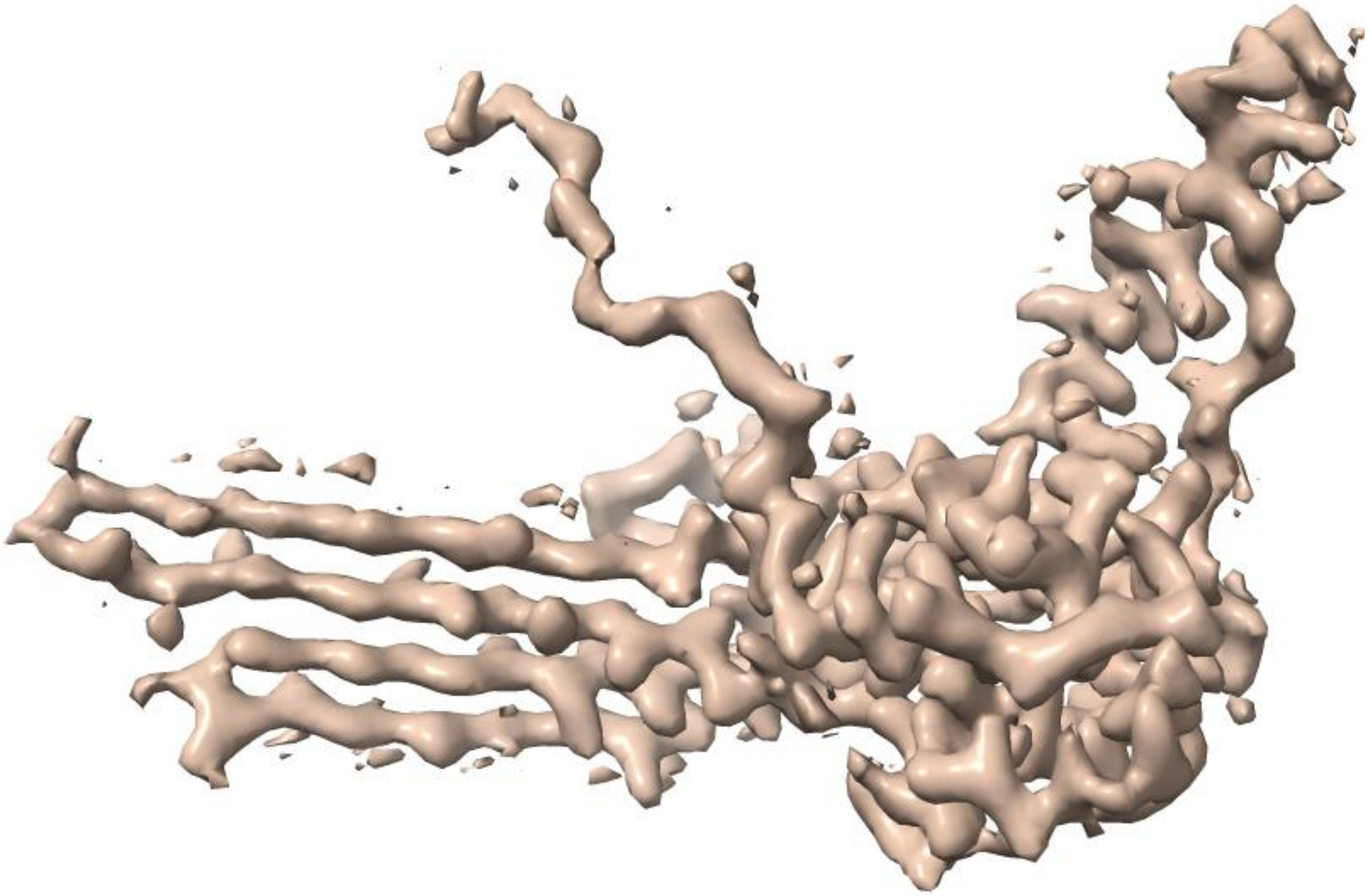
Example:

Cryo-EM map (30160 – 7brm)
3.6 Å



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

sequence



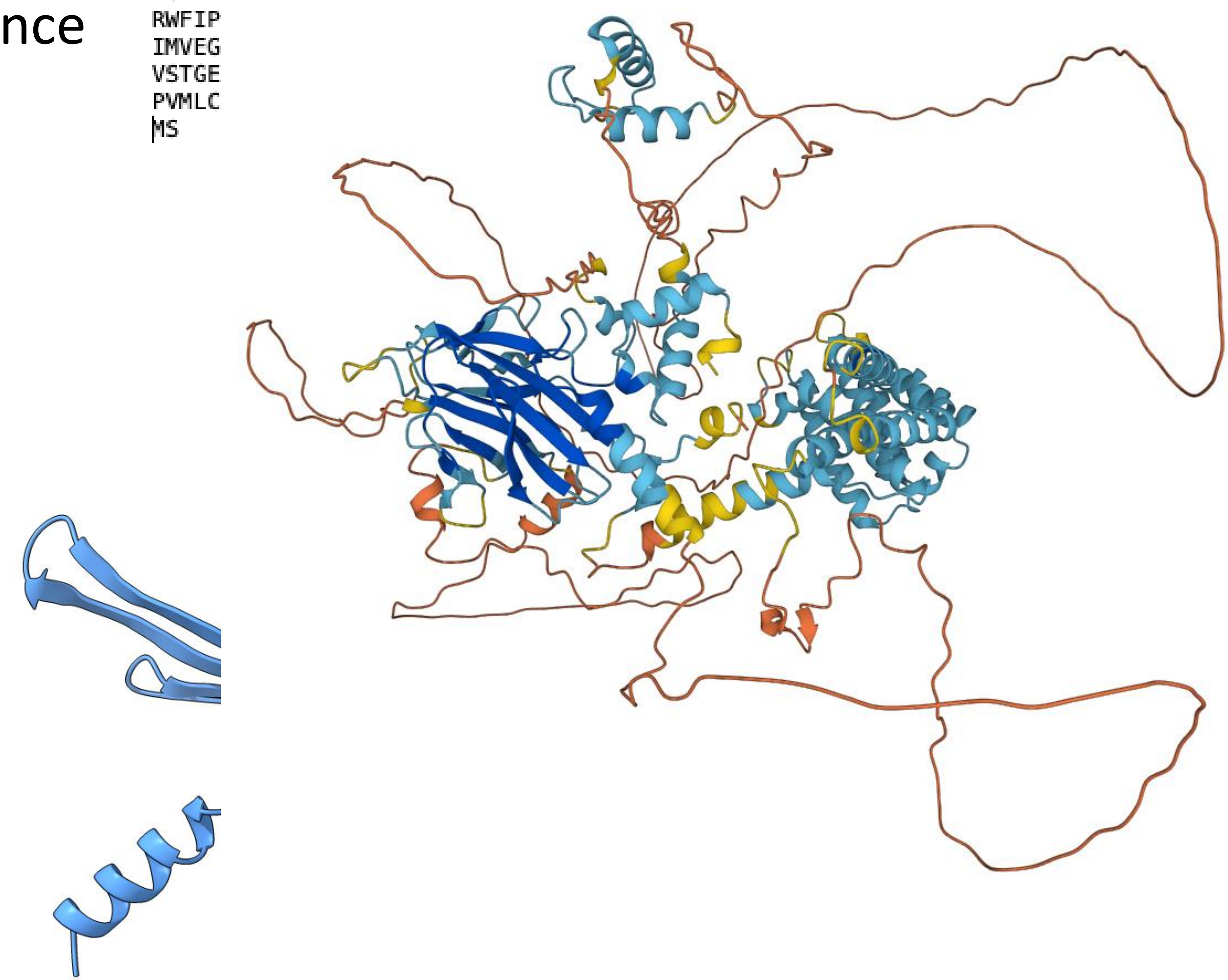
cryo-EM map

Get a prediction

sequence

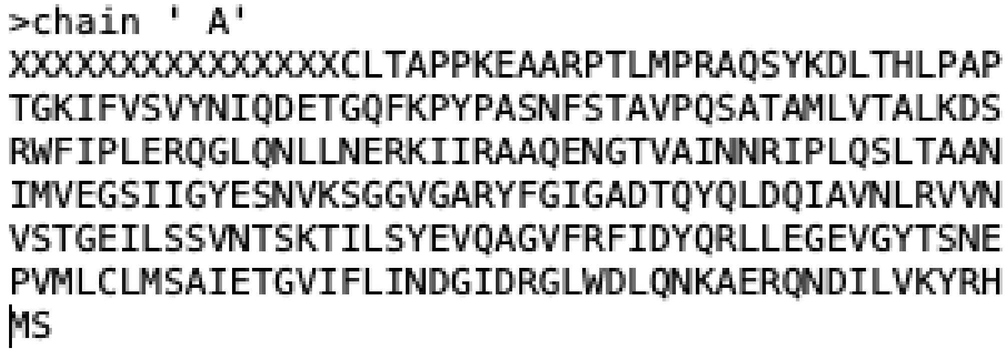
```
>chain ' A'
XXXXXXXXXXXXXXXCLTAPPKEAARPTLMPRAQSYKDLTHLPAP
TGKIF
RWFIP
IMVEG
VSTGE
PVMLC
MS
```

AlphaFold model



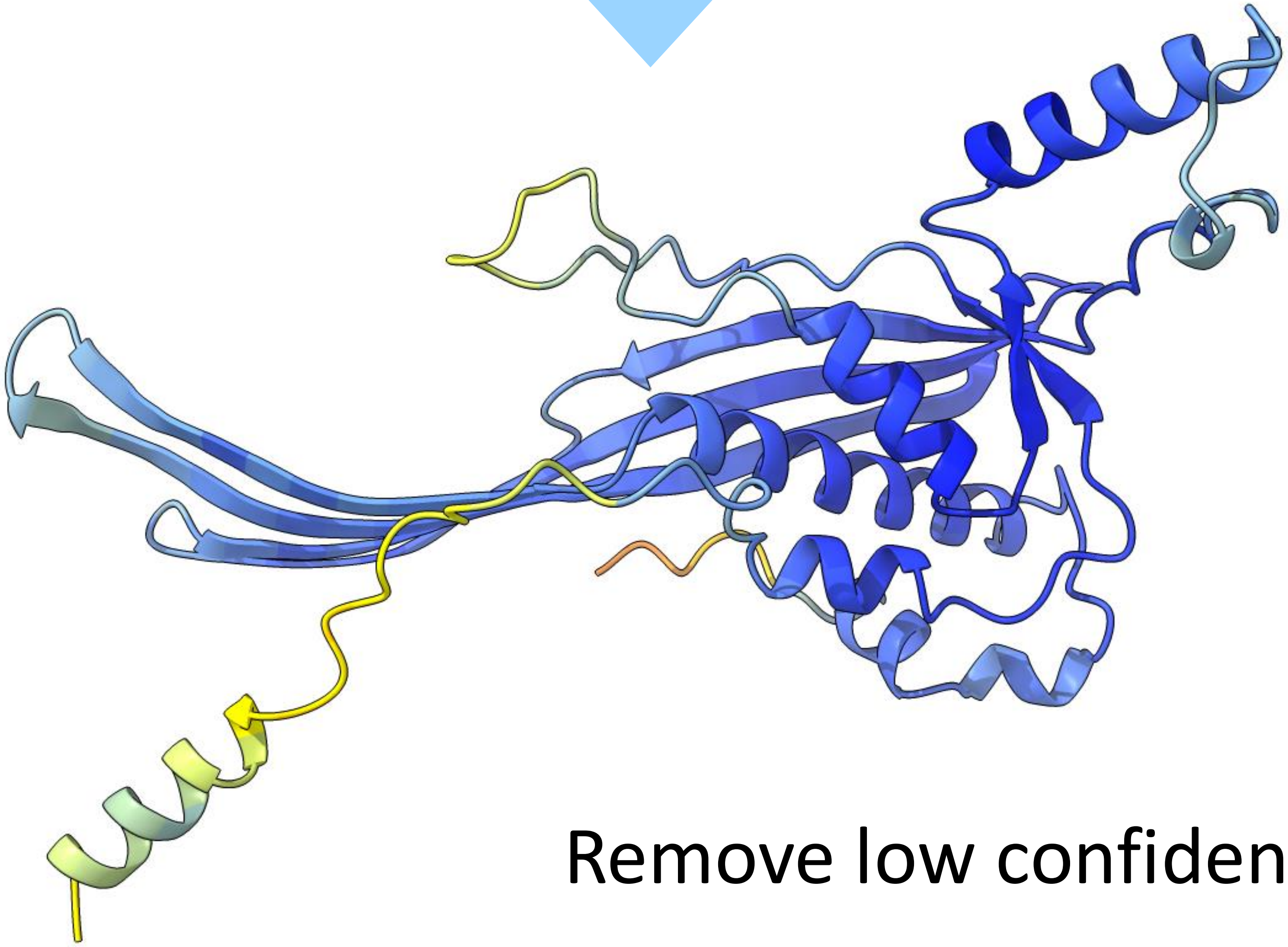
Process prediction

sequence



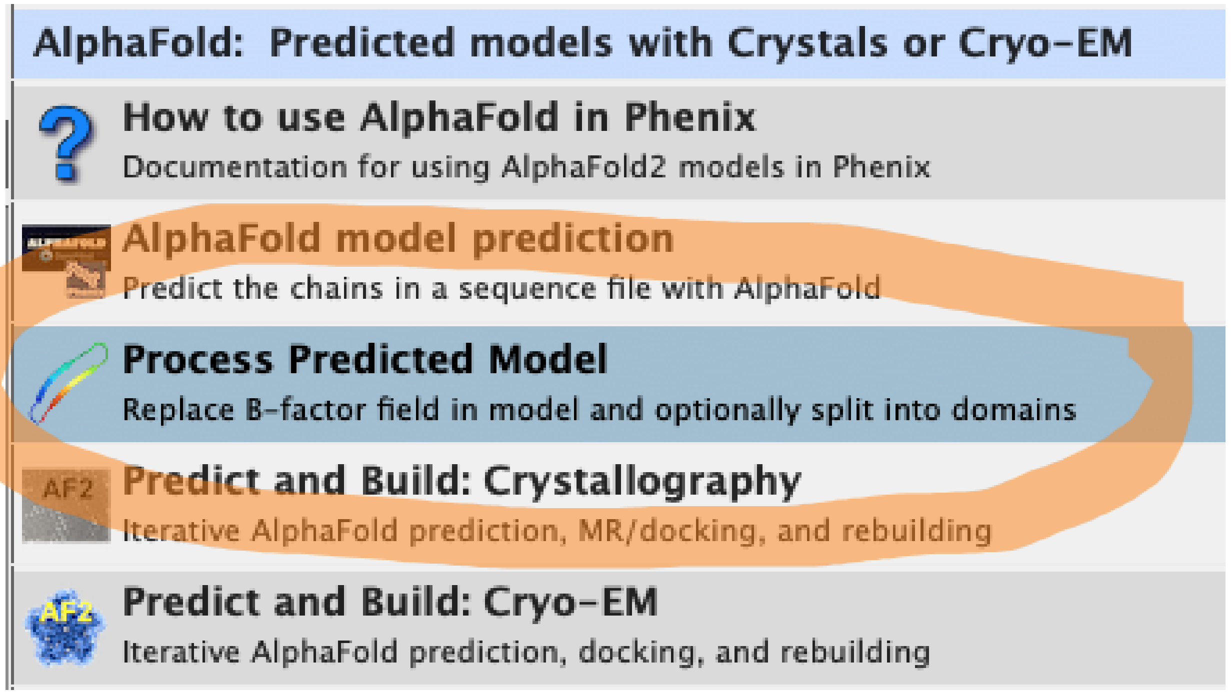
```
>chain ' A'
XXXXXXXXXXXXXXXCLTAPPKEAARPTLMPRAQSYKDLTHLPAP
TGKIFVSVYNIQDETGQFKPYPASNFSTAVPQSATAMLVTALKDS
RWFIPLERQGLQNLLNERKIIRAAQENGTVAINNRIPLQSLTAAN
IMVEGSIIGYESNVKSGGVGARYFGIGADTQYQLDQIAVNLRVVN
VSTGEILSSVNTSKTILSYEVQAGVFRFIDYQRLLEGEVGYTSNE
PVMLCLMSAIETGVIFLINDGIDRGLWDLQNKAERQNDILVKYRH
MS
```

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

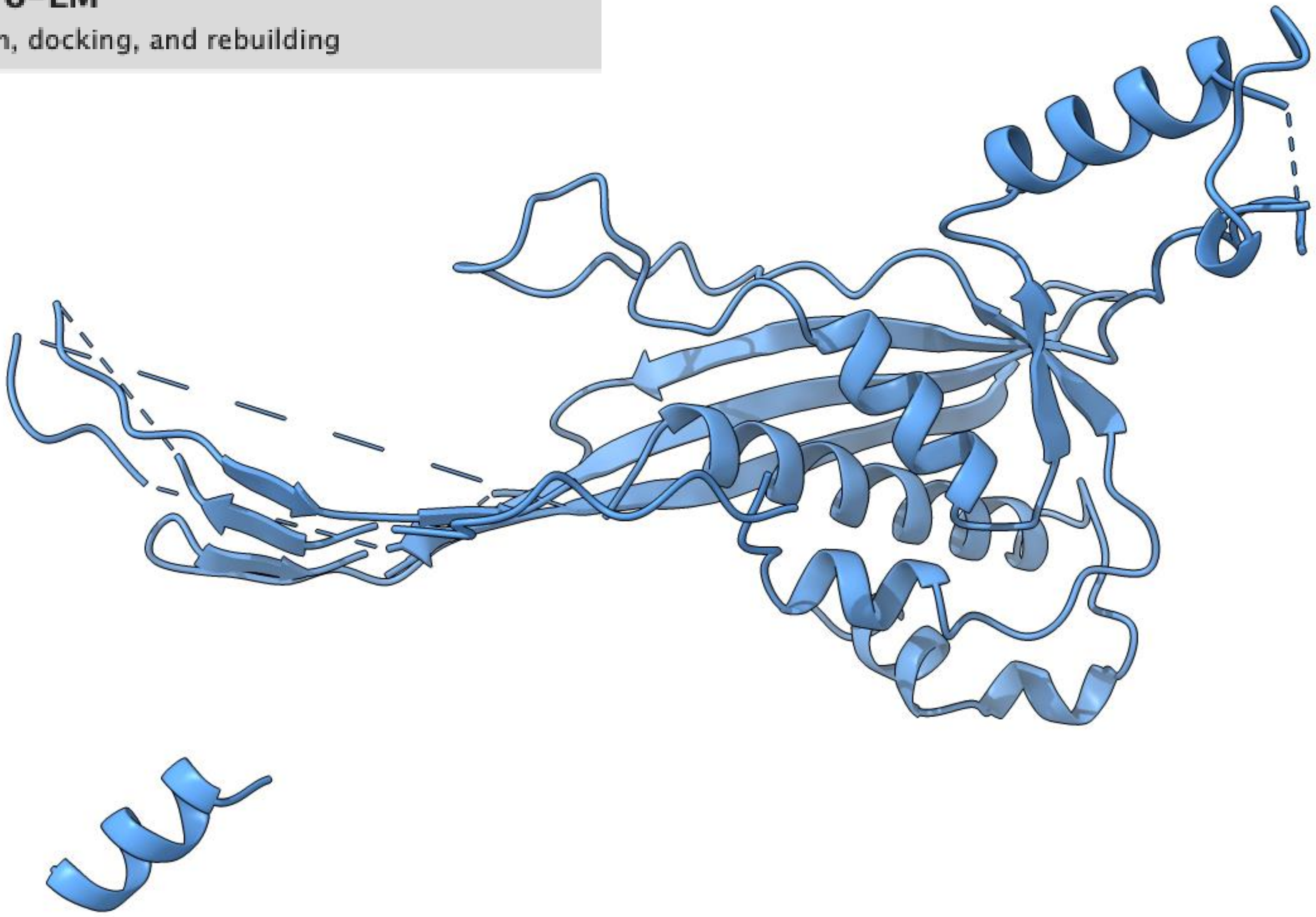
Process Predicted Model
Replace B-factor field in model and optionally split into domains

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

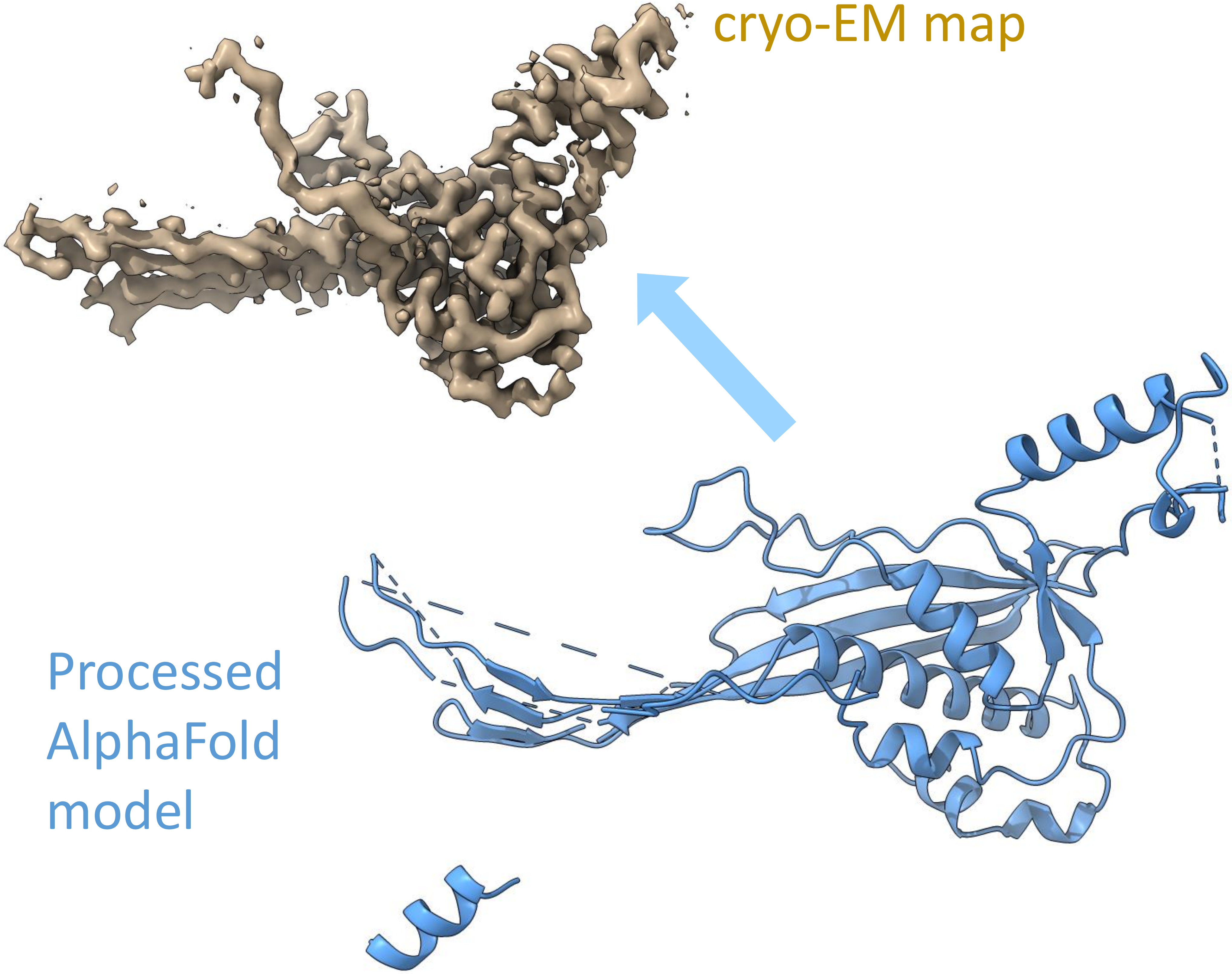
Predict and Build: Cryo-EM
Iterative AlphaFold prediction, docking, and rebuilding

```
phenix.process_predicted_model
```

Processed AlphaFold model



Dock processed model

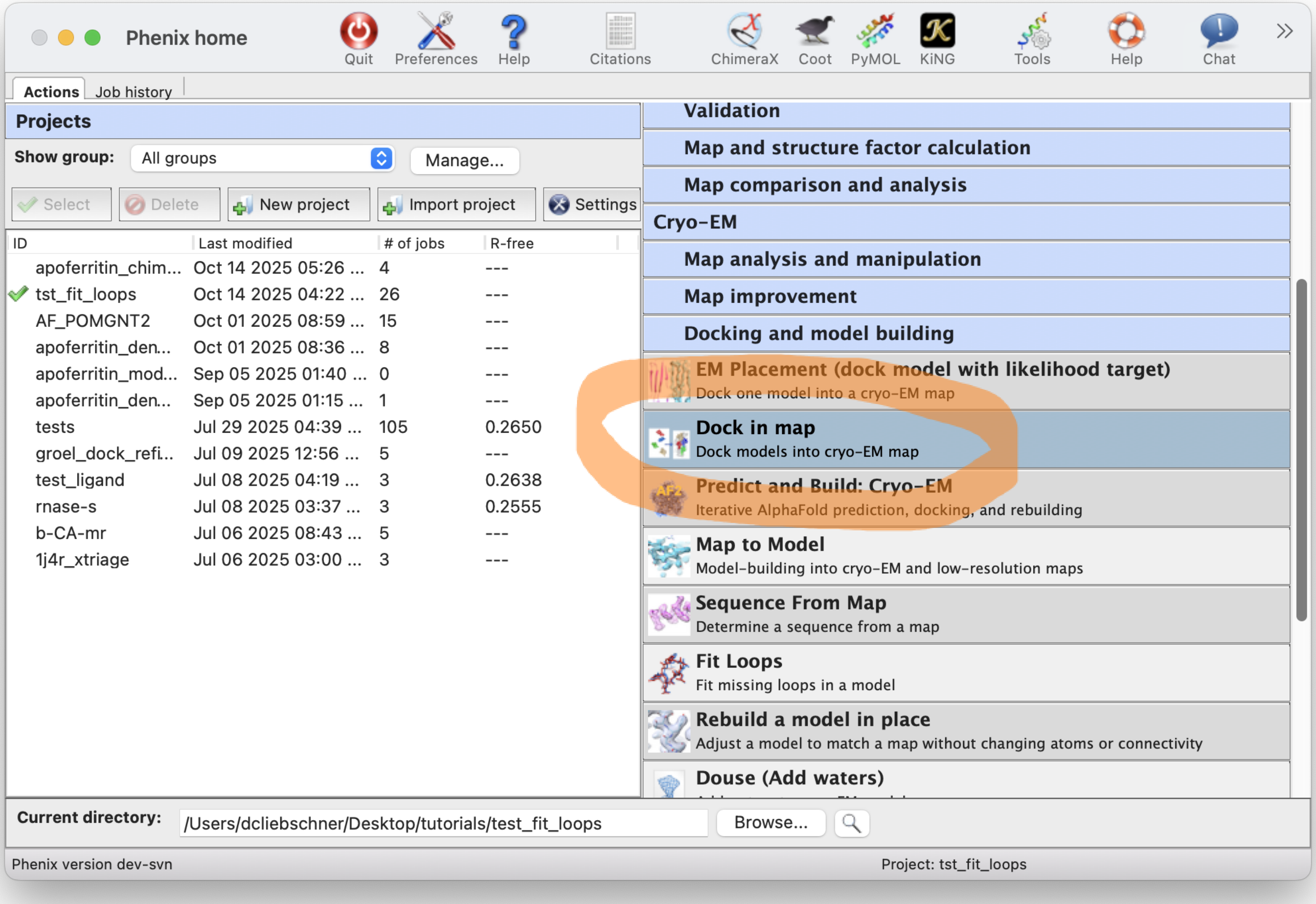


cryo-EM map

Processed AlphaFold model

Docking in Phenix

Dock in map (T. Terwilliger)



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

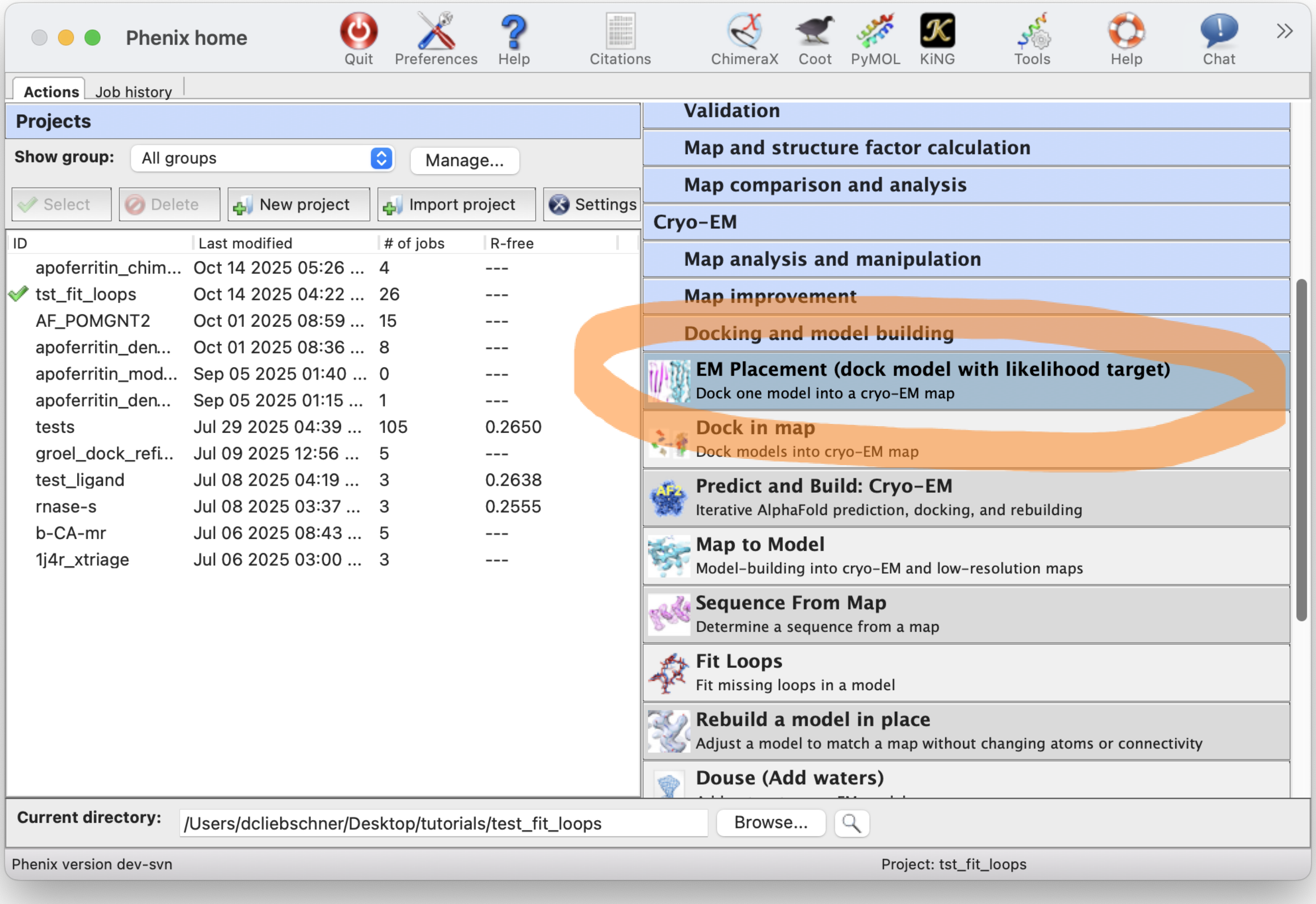
Phenix version dev-svn

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)



Show group: All groups

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	---
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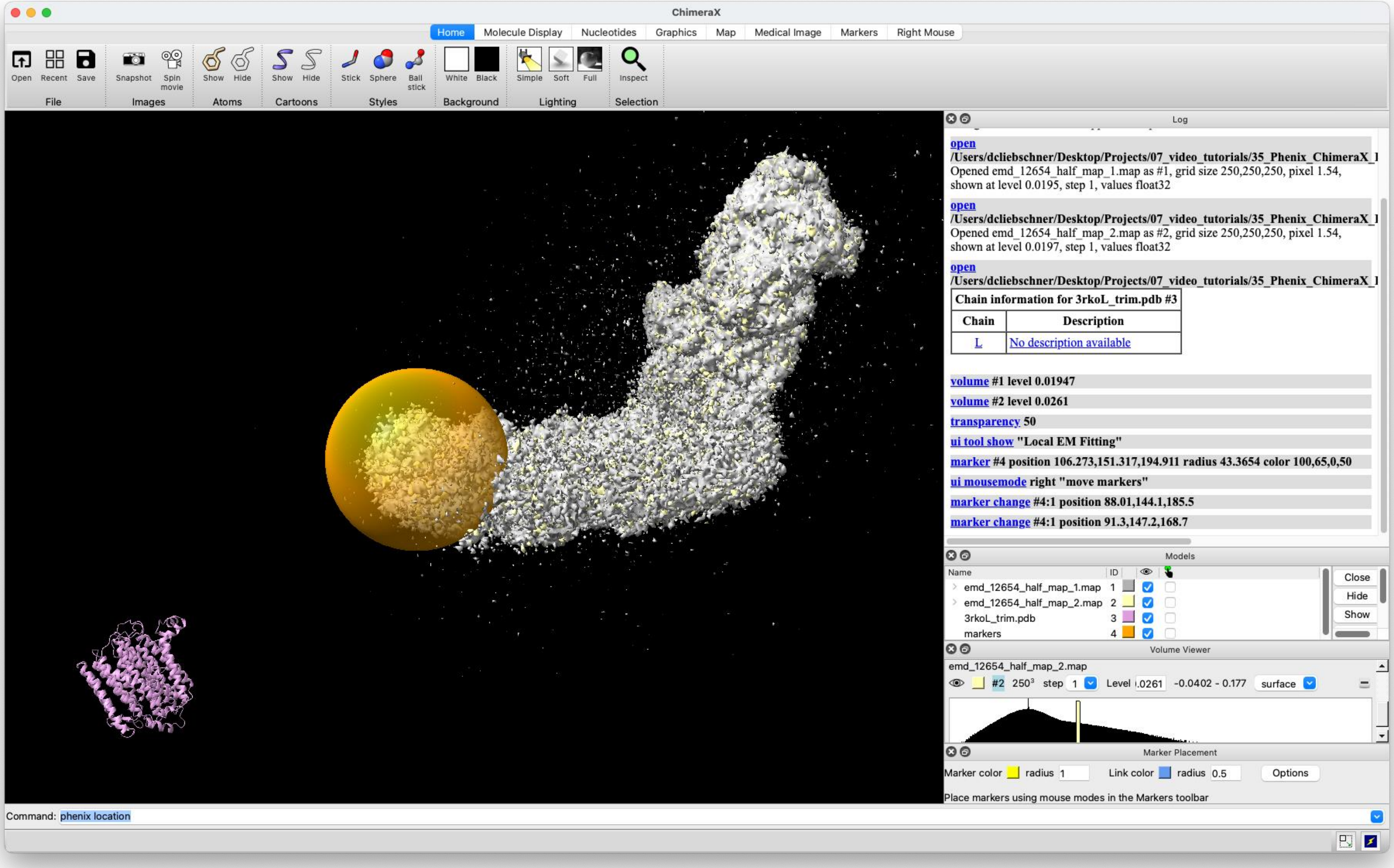
Current directory: /Users/dcliebschner/Desktop/tutorials/test_fit_loops

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.



```
open
/Users/dcliebschner/Desktop/Projects/07_video_tutorials/35_Phenix_ChimeraX_
Opened emd_12654_half_map_1.map as #1, grid size 250,250,250, pixel 1.54,
shown at level 0.0195, step 1, values float32
open
/Users/dcliebschner/Desktop/Projects/07_video_tutorials/35_Phenix_ChimeraX_
Opened emd_12654_half_map_2.map as #2, grid size 250,250,250, pixel 1.54,
shown at level 0.0197, step 1, values float32
open
/Users/dcliebschner/Desktop/Projects/07_video_tutorials/35_Phenix_ChimeraX_
```

Chain information for 3rkoL_trim.pdb #3

Chain	Description
L	No description available

```
volume #1 level 0.01947
volume #2 level 0.0261
transparency 50
ui tool show "Local EM Fitting"
marker #4 position 106.273,151.317,194.911 radius 43.3654 color 100,65,0,50
ui mousemode right "move markers"
marker change #4:1 position 88.01,144.1,185.5
marker change #4:1 position 91.3,147.2,168.7
```

Name	ID		👁	
emd_12654_half_map_1.map	1	⬜	☑	☐
emd_12654_half_map_2.map	2	⬜	☑	☐
3rkoL_trim.pdb	3	🟪	☑	☐
markers	4	🟧	☑	☐

emd_12654_half_map_2.map

#2 250³ step 1 Level 0.0261 -0.0402 - 0.177 surface

Marker color 🟨 radius 1 Link color 🟦 radius 0.5

Place markers using mouse modes in the Markers toolbar

Command: phenix location

2. Use a prediction as reference model

Concept

- Use a related model to generate a set of **torsion restraints**.
- Restrain **each torsion angle** in the working model to the corresponding torsion angle in the reference model.
- Allows for structural differences.

When to use

Low resolution (worse than 3Å).

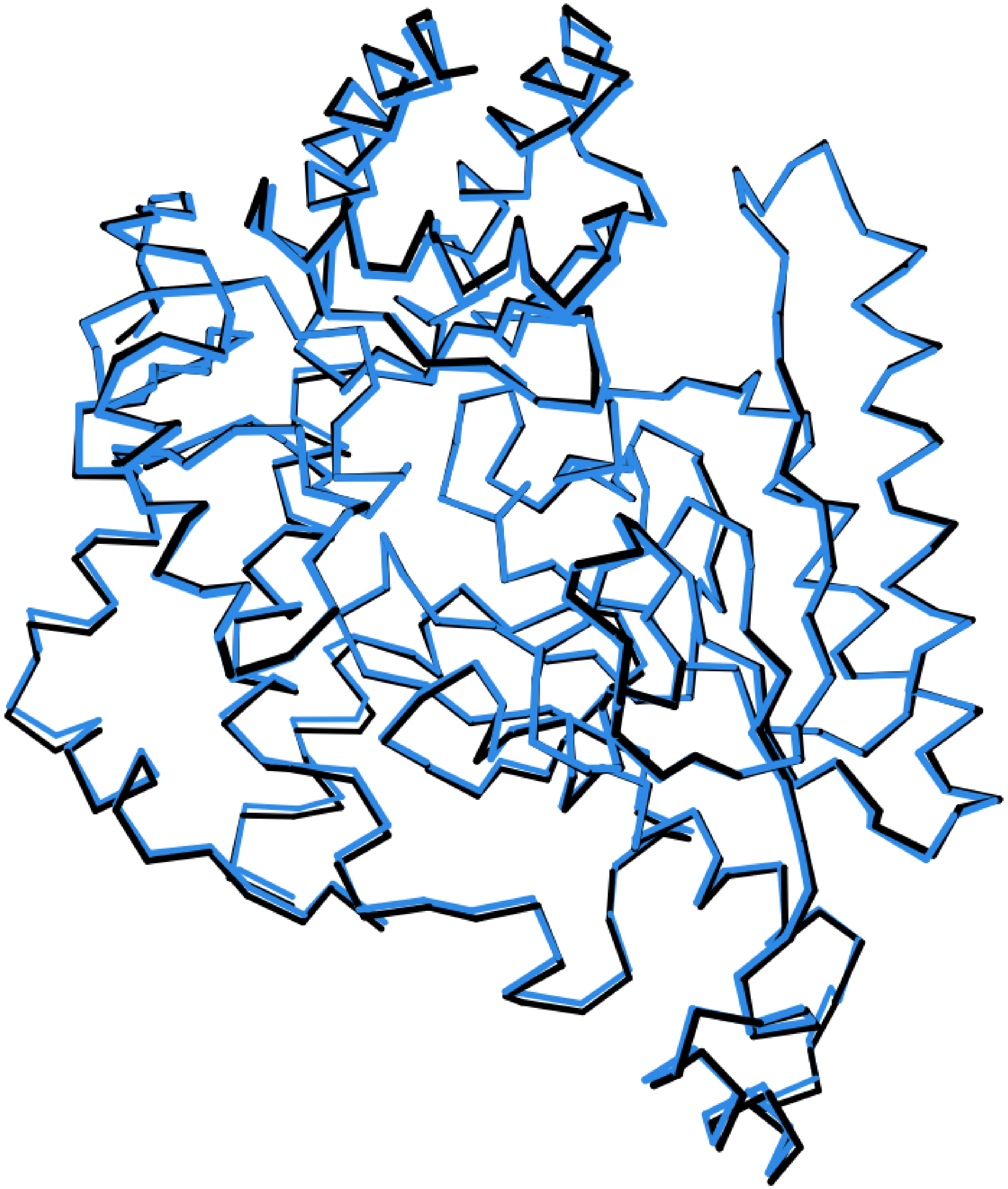
If no high resolution homologue available, can use AF model for reference model (AF models have good geometry).

Headd JJ et al., 2012, *Acta Cryst.* **D68**:381-390

Reference model Restraints: example

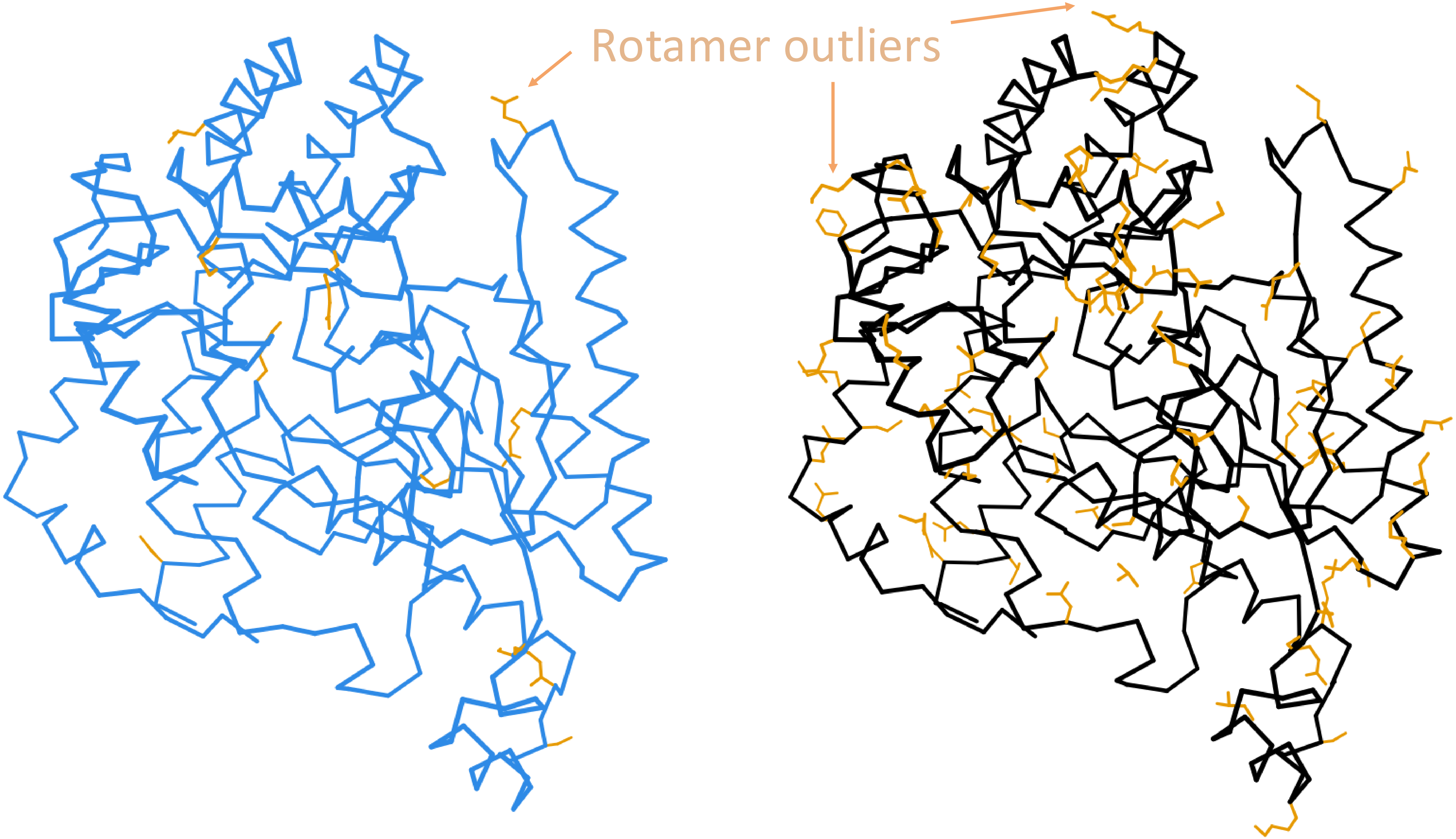
1GTX: 3.0 Å

1OHV: 2.3 Å



4-aminobutyrate-aminotransferase

Reference model Restraints: example



Rotamer outliers

1OHV: 2.3 Å

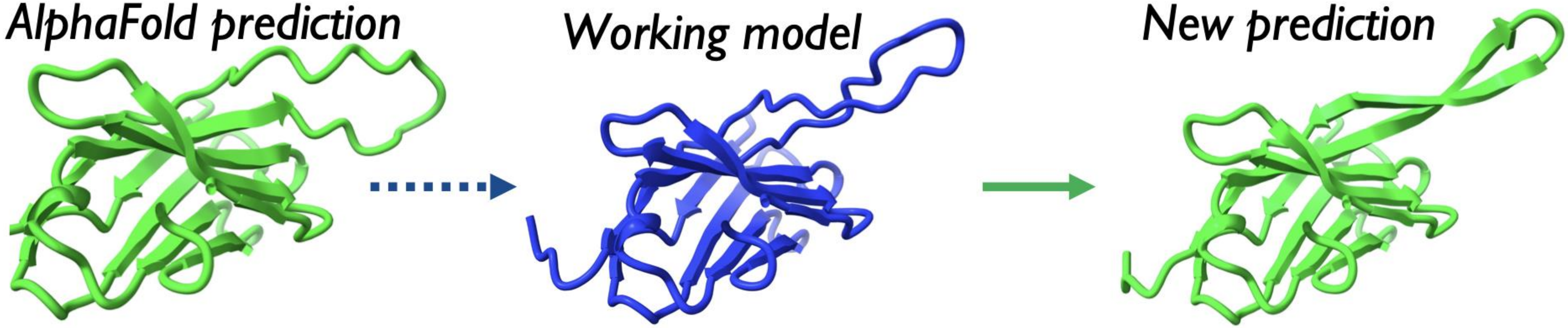
1GTX: 3.0 Å

4-aminobutyrate-aminotransferase

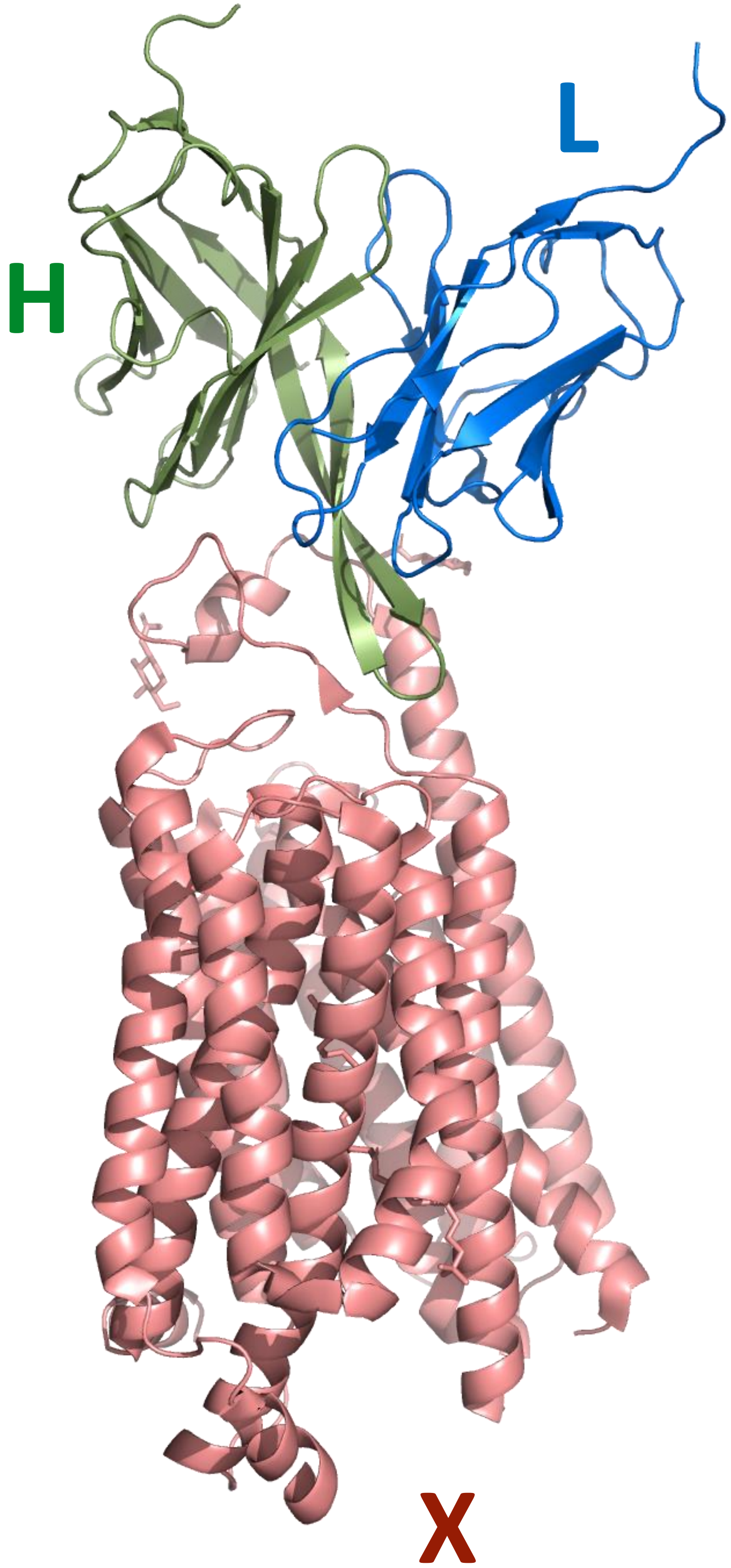
Use your working model to get a new AlphaFold prediction

Why?

Because your new prediction might be better than your model



Example: Fab heavy chain



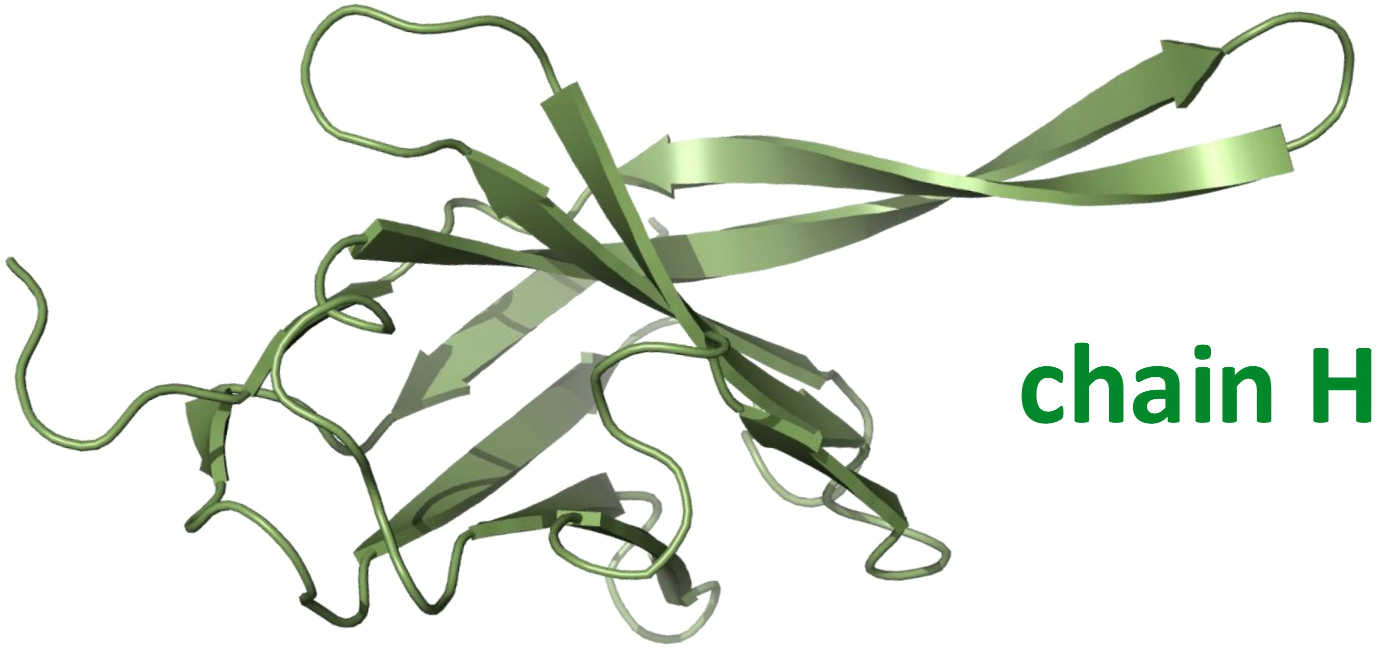

7MJS

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 EMDataResource

3.03 Å resolution

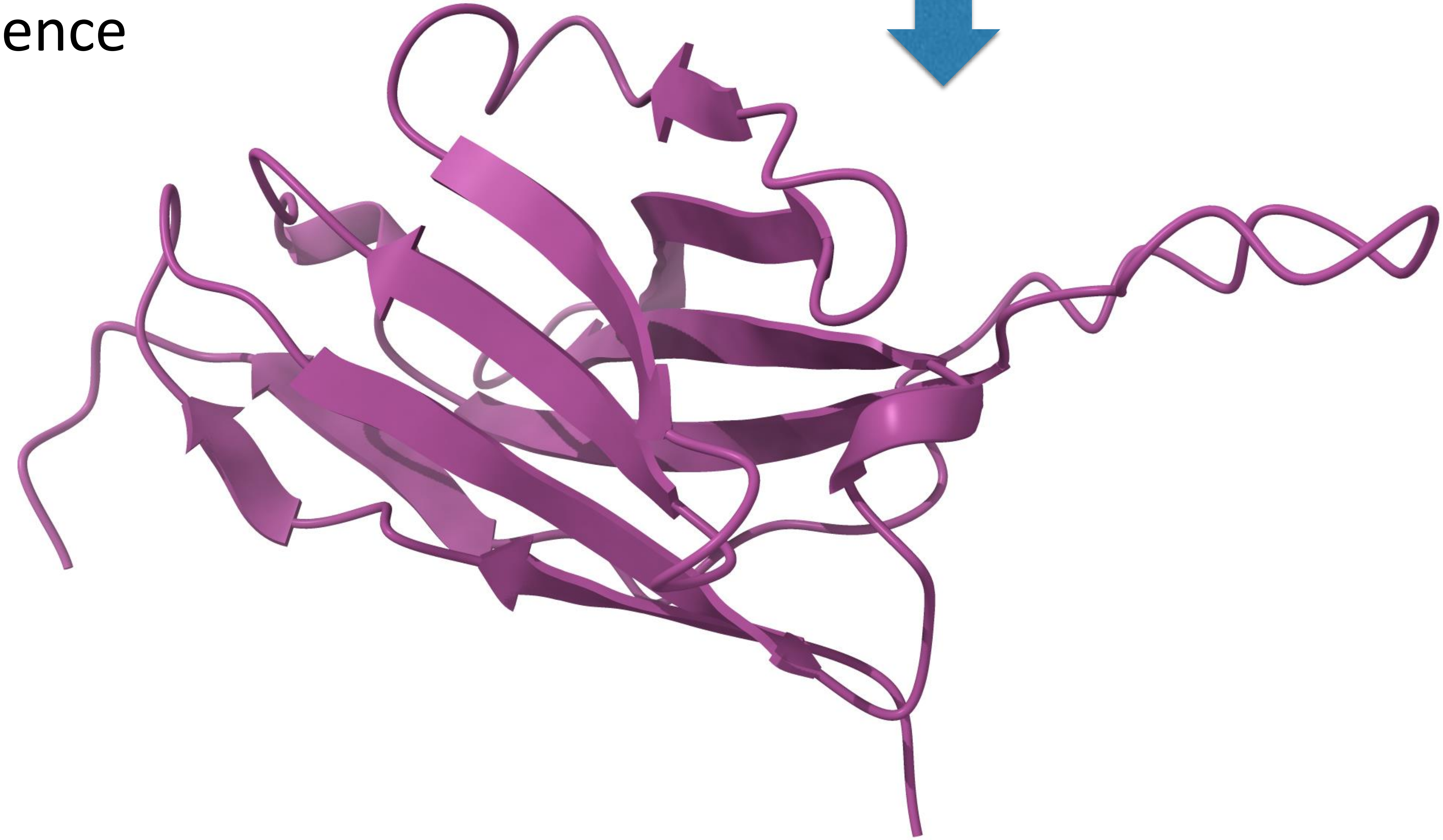


A loop that interacts with other chains is not correctly predicted.

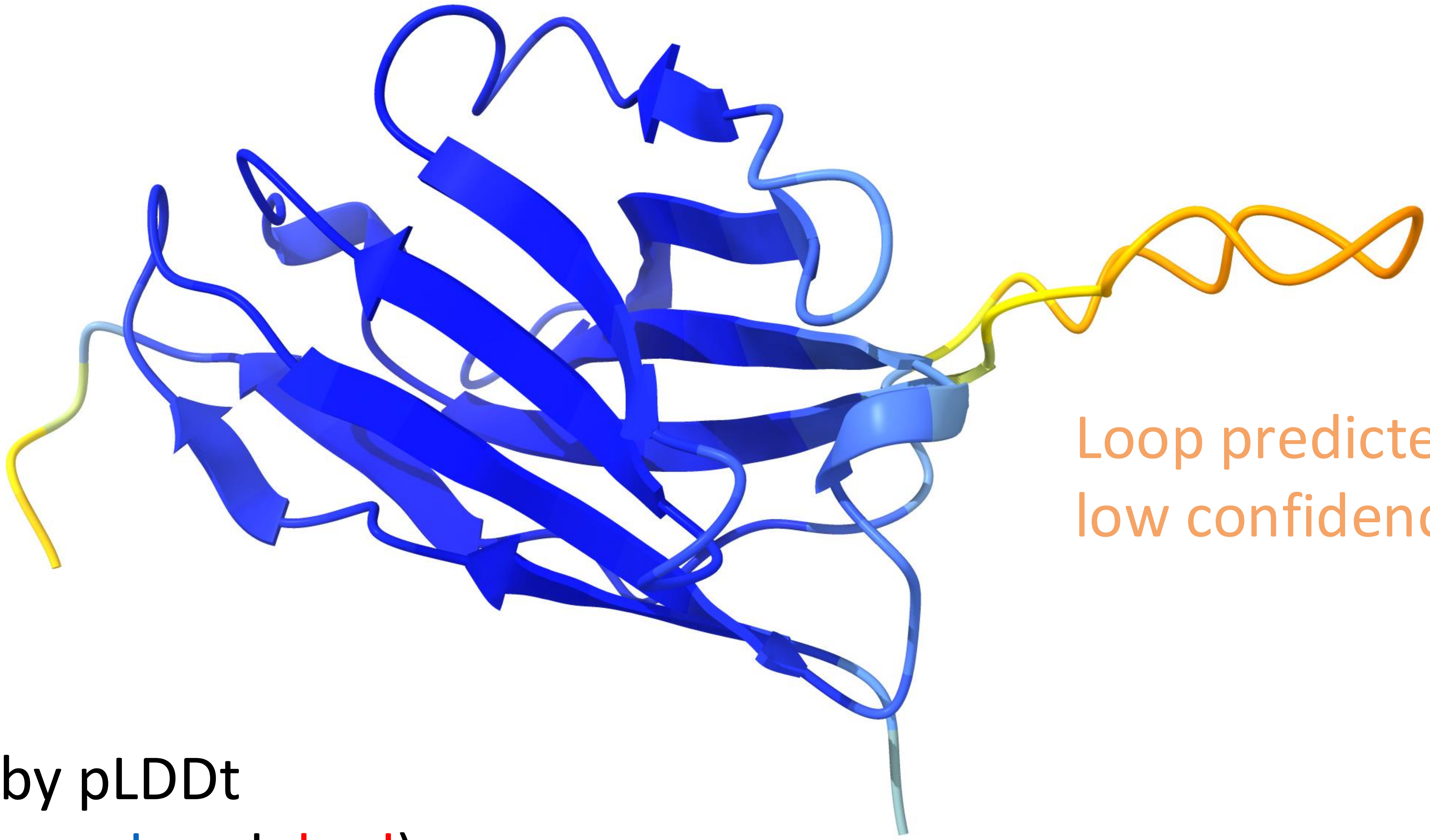
AF2 prediction of chain H

EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQ
APGKGLEWVAYIYSYSGYTSYADSVKGRFTISADTSKNTA
YLQMNSLRAEDTAVYYCARSLEYLYSSGYQYKWATGLDY
WGQGTLVTVSSAST

Sequence



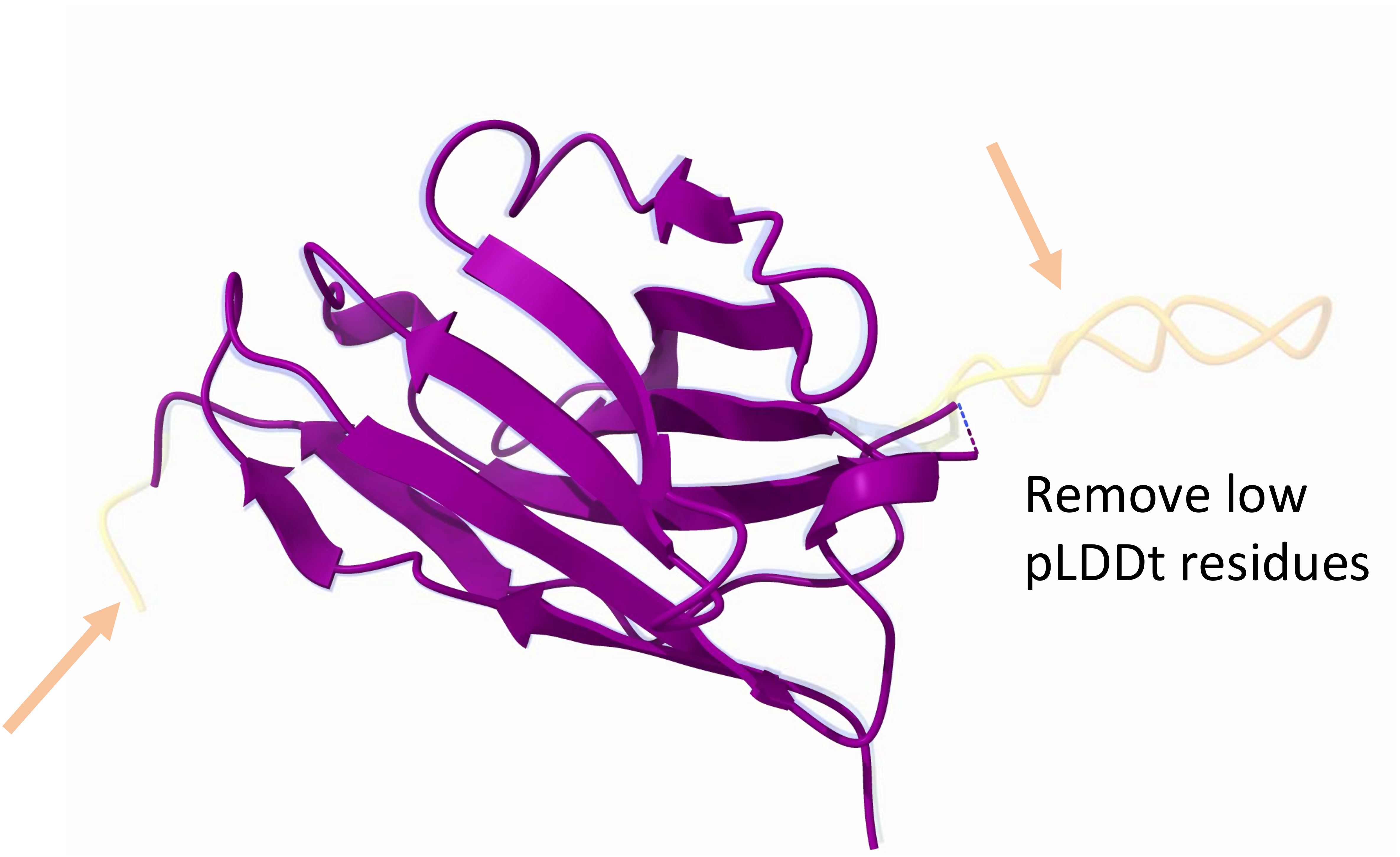
AF2 prediction of chain H



Loop predicted with low confidence

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

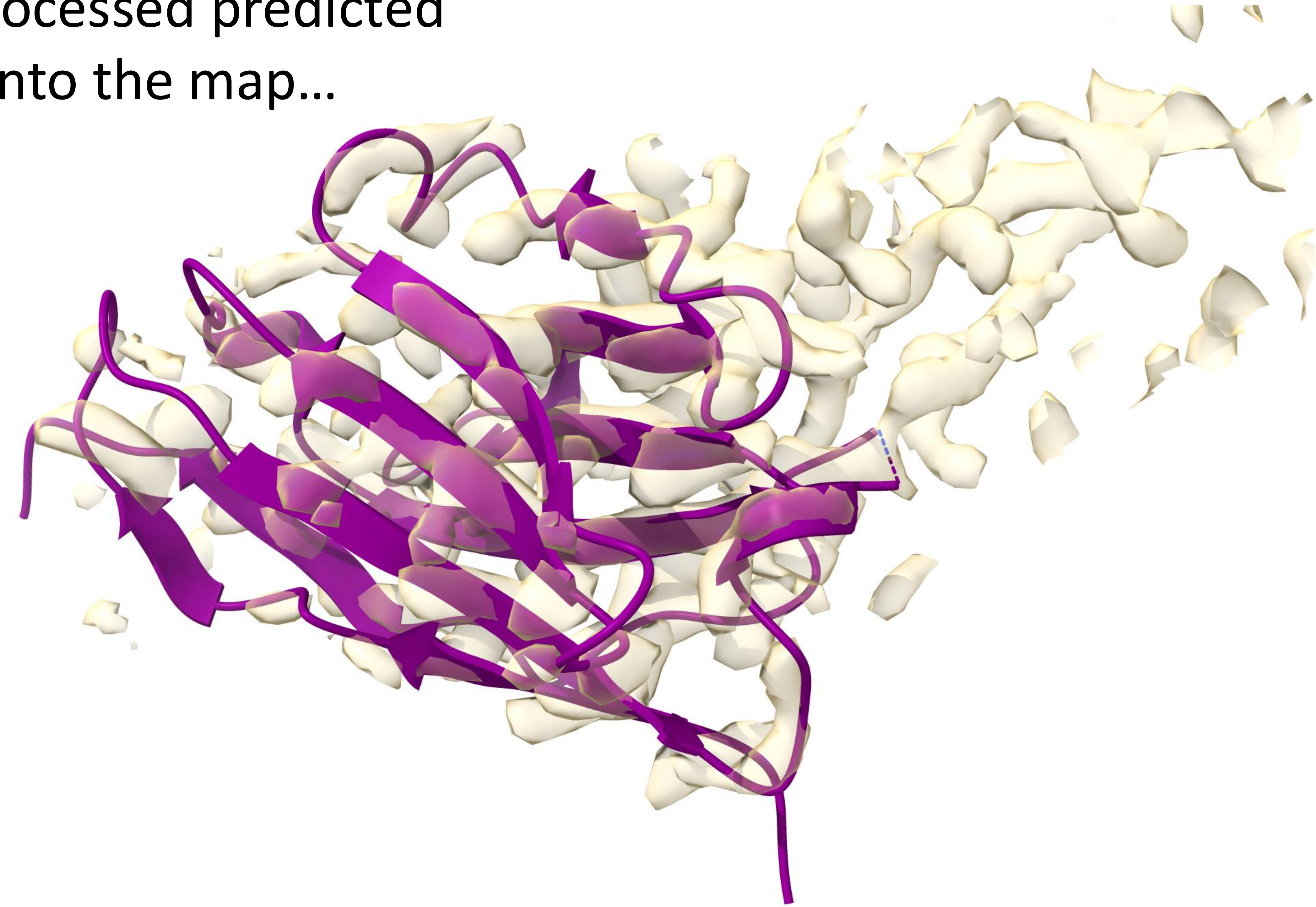
Process AF2 prediction



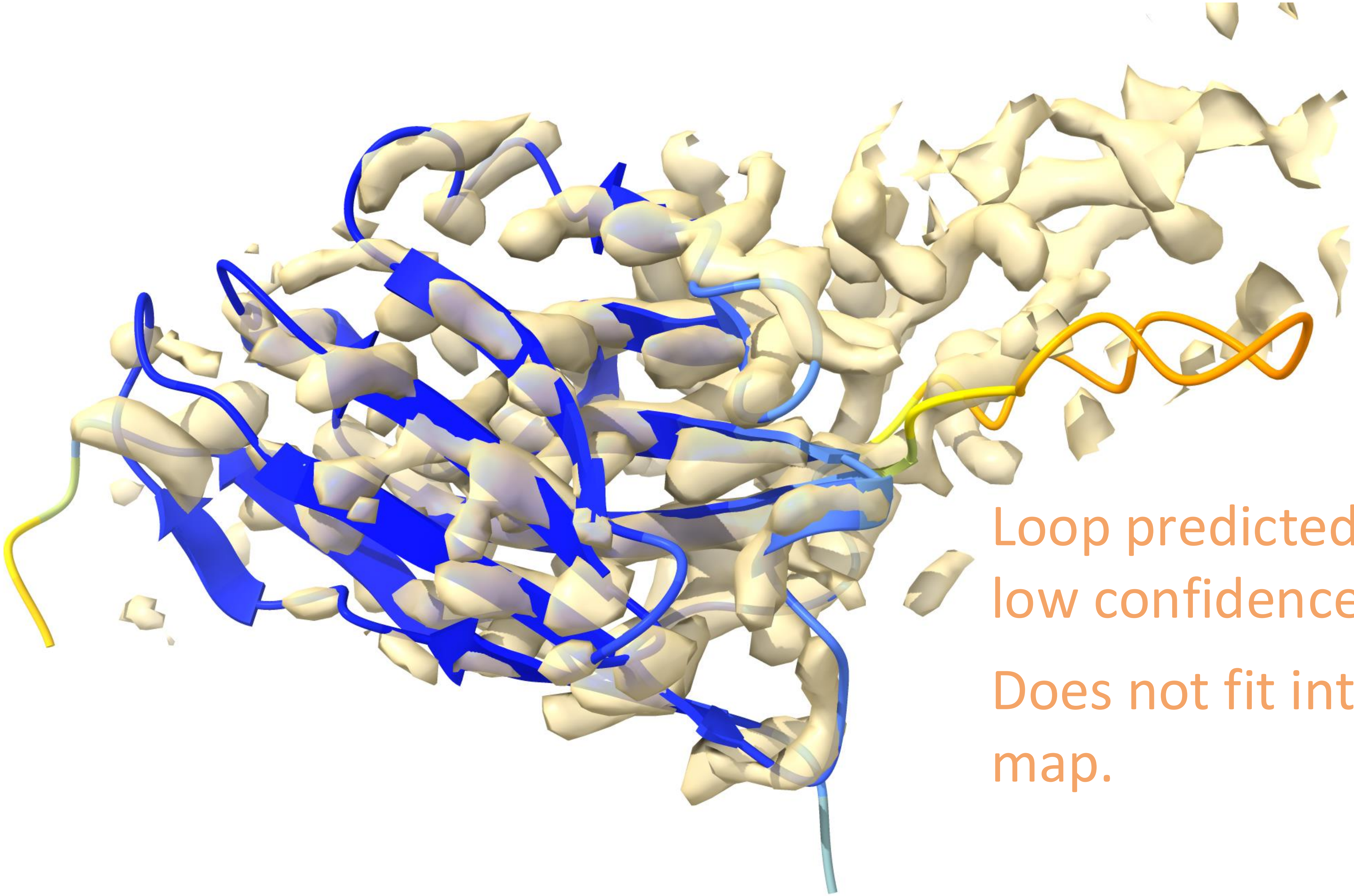
Remove low pLDDt residues

Dock processed model into the map

Dock processed predicted model into the map…



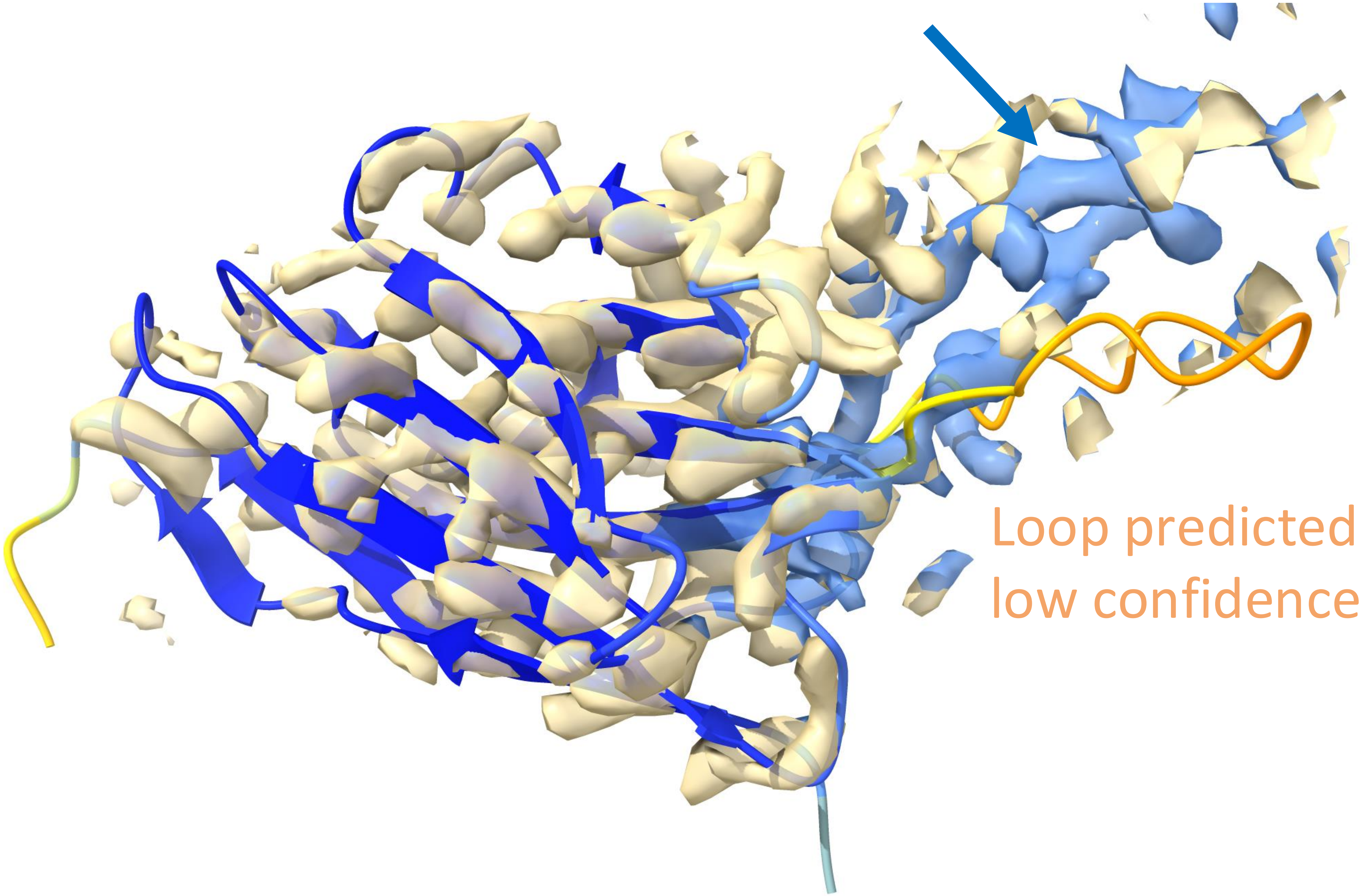
AF2 prediction of chain H



Loop predicted with low confidence

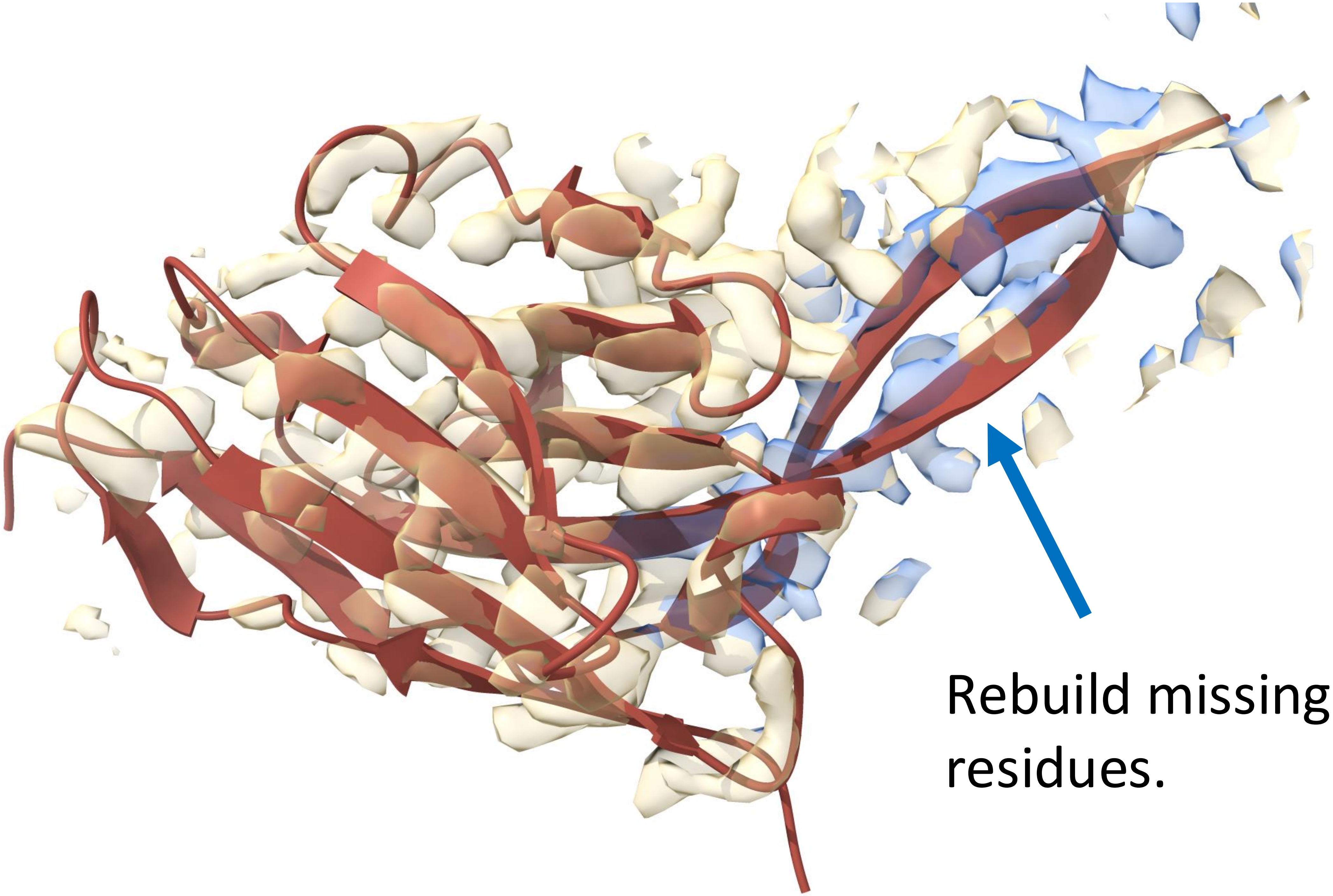
Does not fit into map.

AF2 prediction of chain H



Loop predicted with low confidence

Dock and rebuild model



Rebuild missing residues.

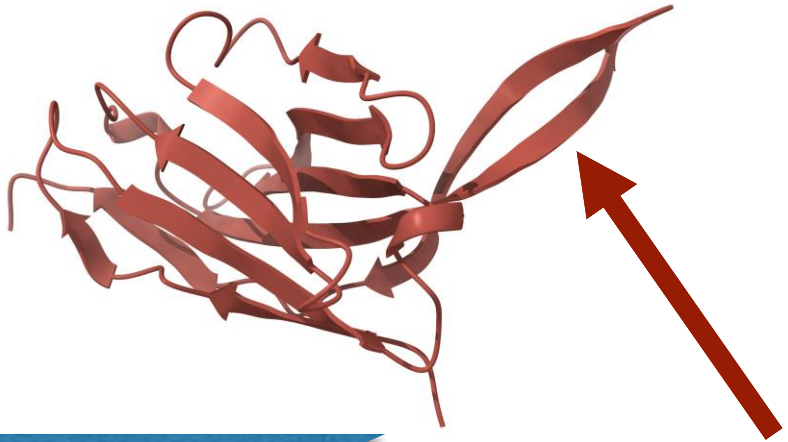
“predicted-processed-docked-rebuilt” model

Make a new prediction

Sequence

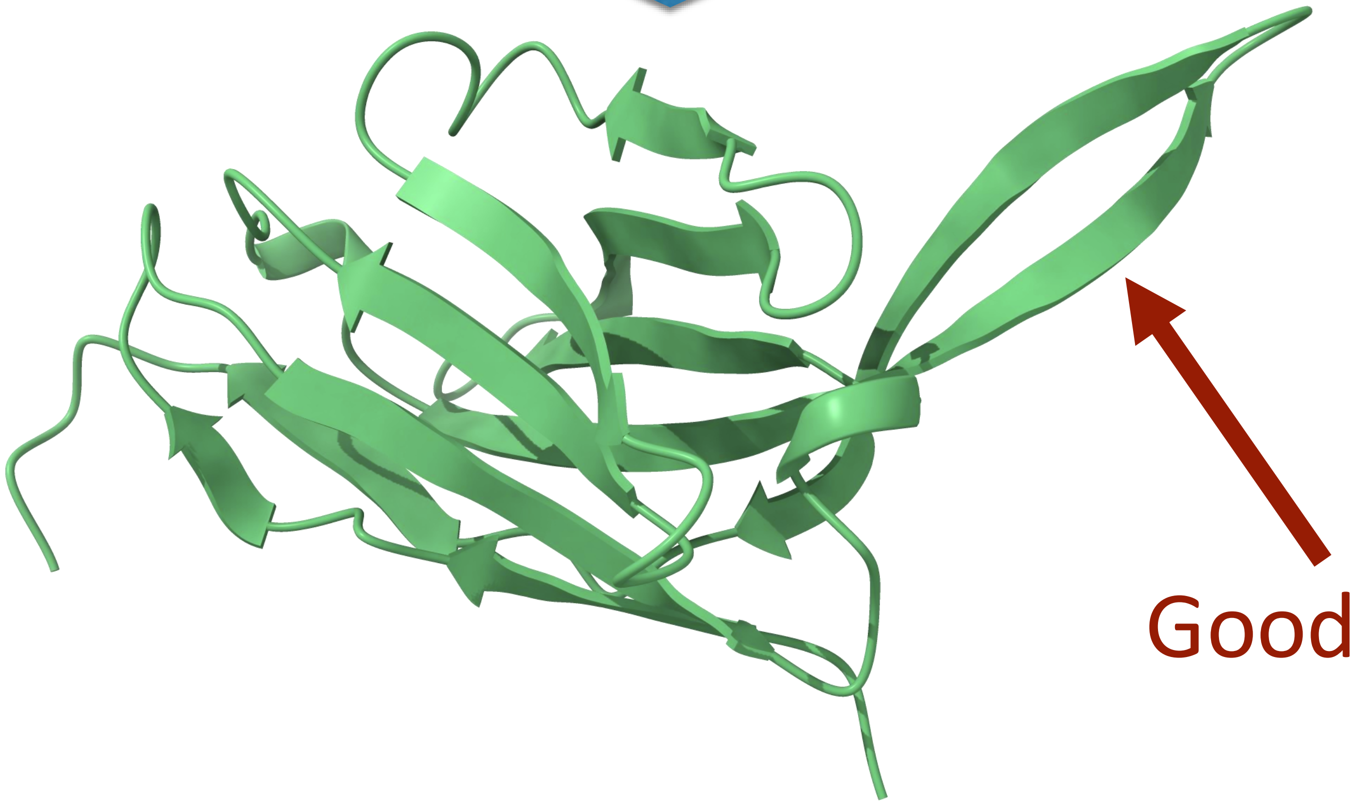
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQ
APGKGLEWVAYIYSYSGYTSYADSVKGRFTISADTSKNTA
YLQMNSLRAEDTAVYYCARSLEYLYSSGYQYKWATGLDY
WGQGTLVTVSSAST

Template



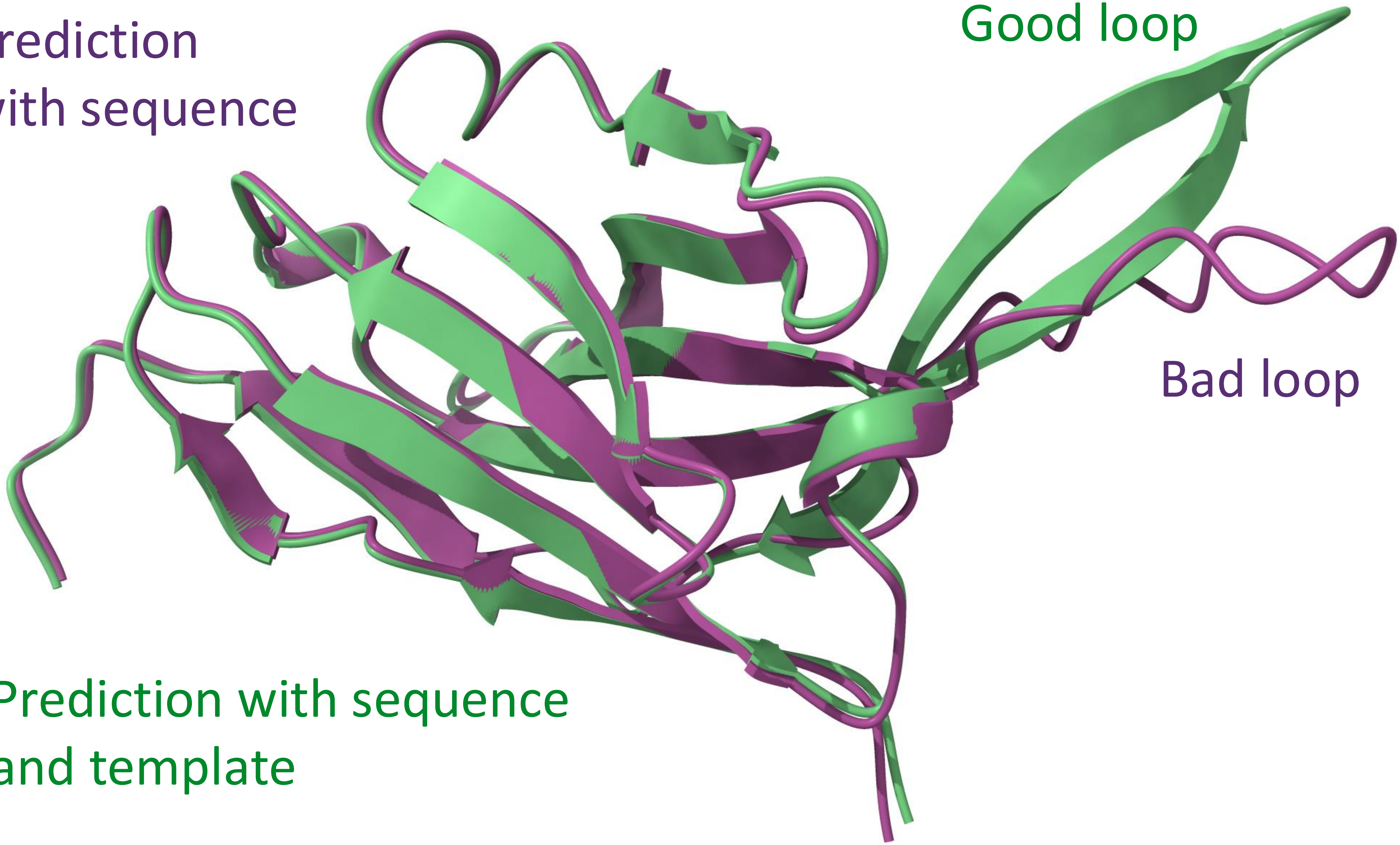
Good loop

AlphaFold



Good loop

Using a template improves prediction



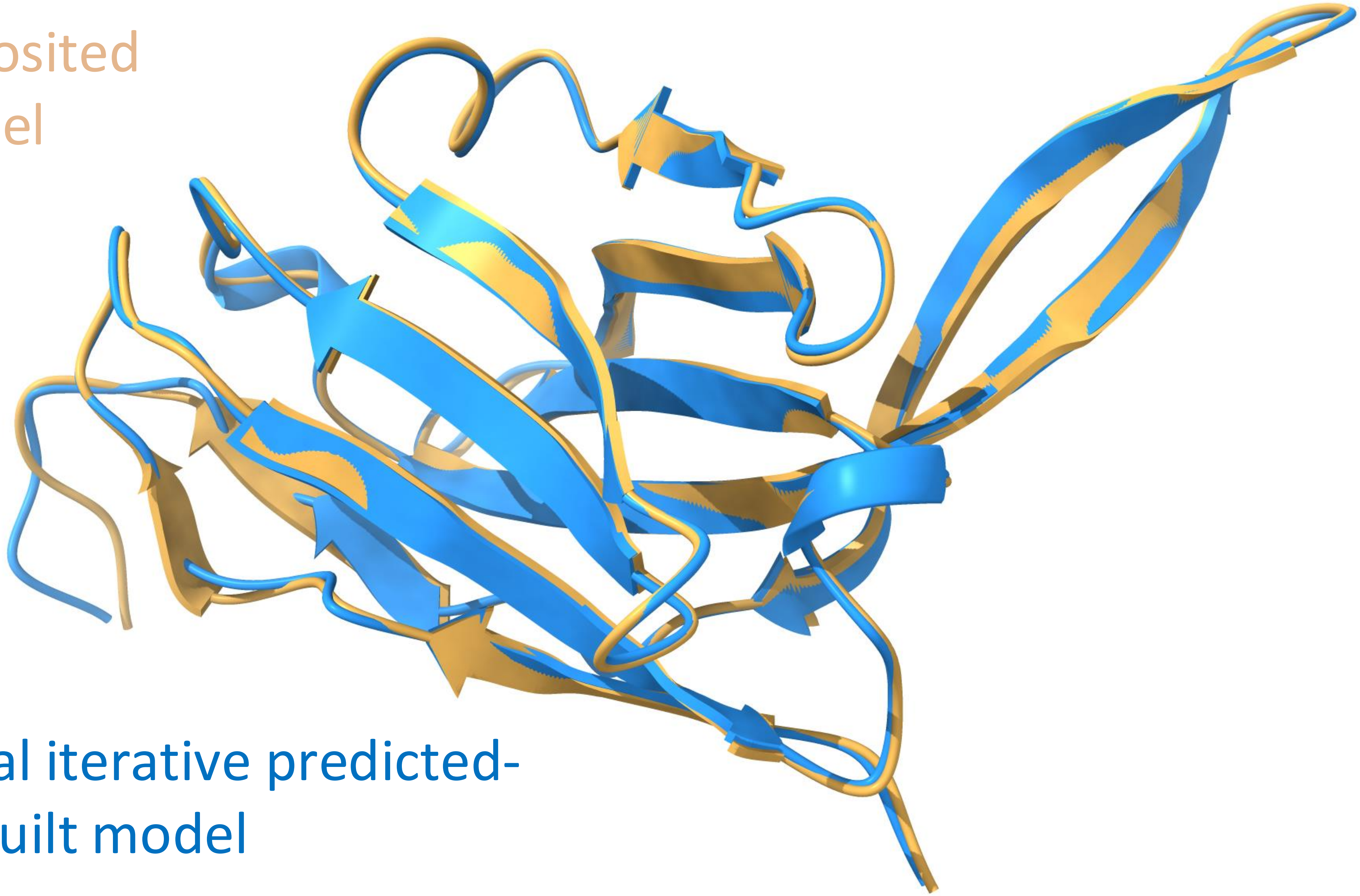
Prediction with sequence

Good loop

Bad loop

Prediction with sequence and template

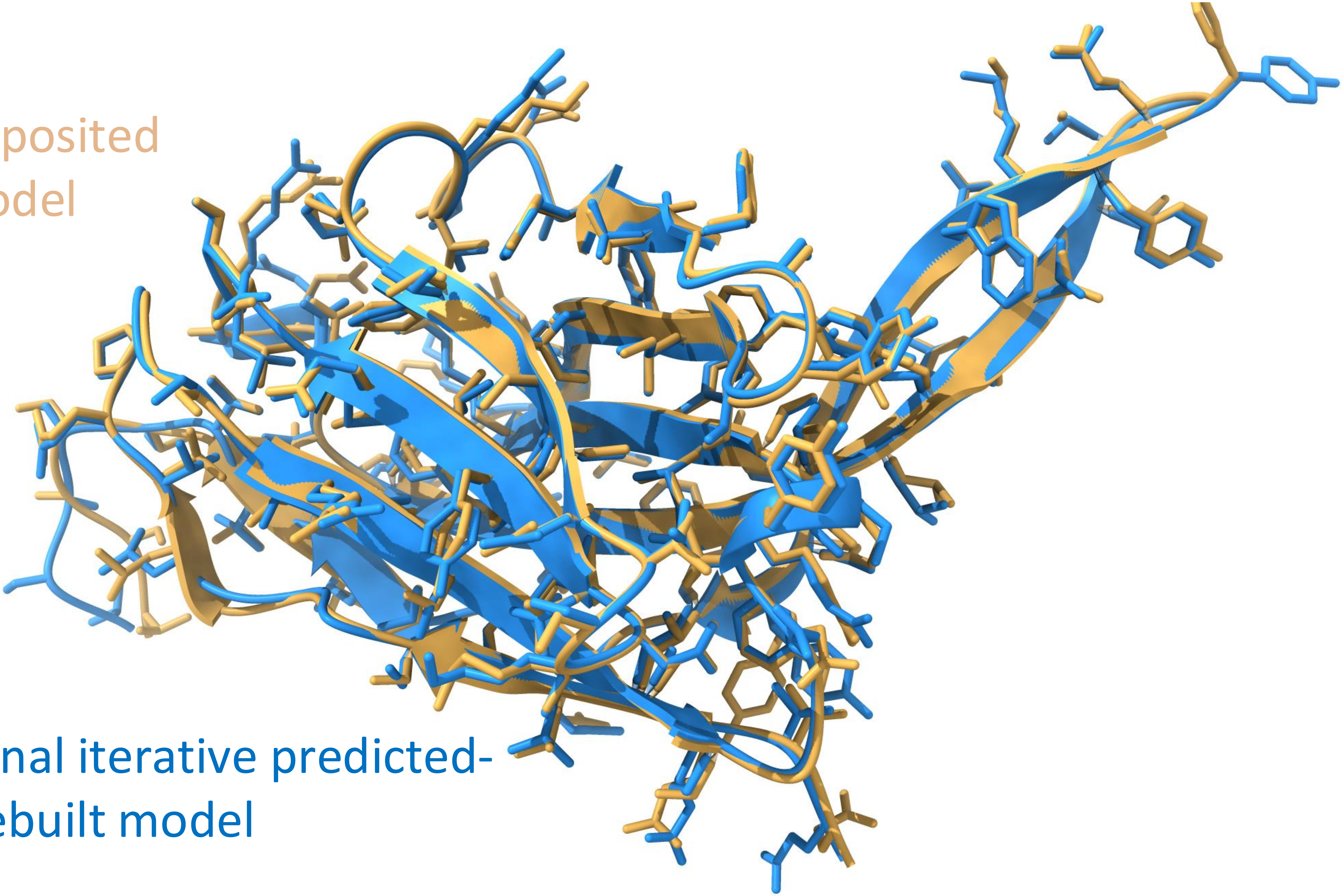
Iterate prediction and rebuilding



Deposited model

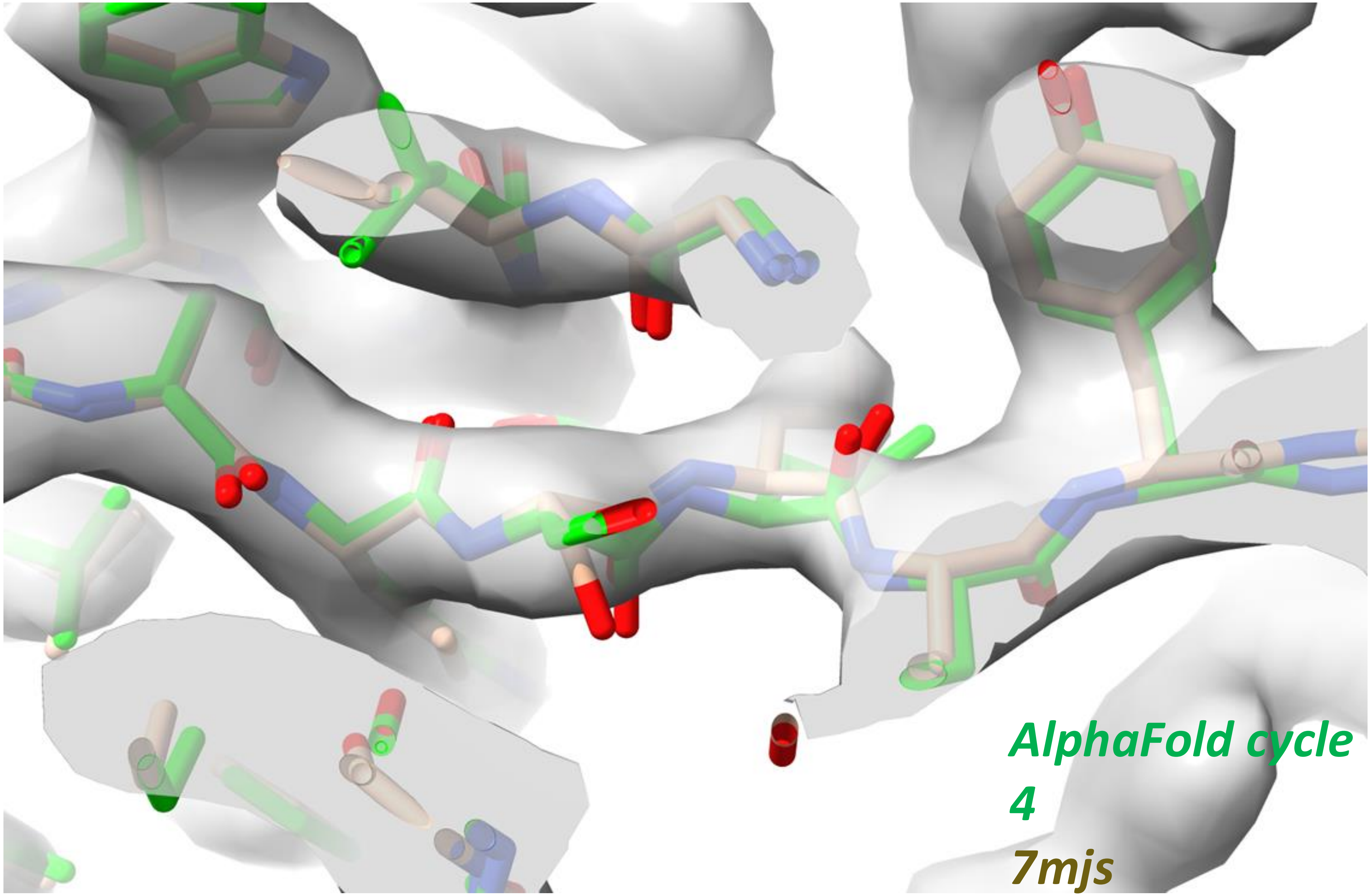
Final iterative predicted-rebuilt model

Iterate prediction and rebuilding



Deposited model

Final iterative predicted-rebuilt model



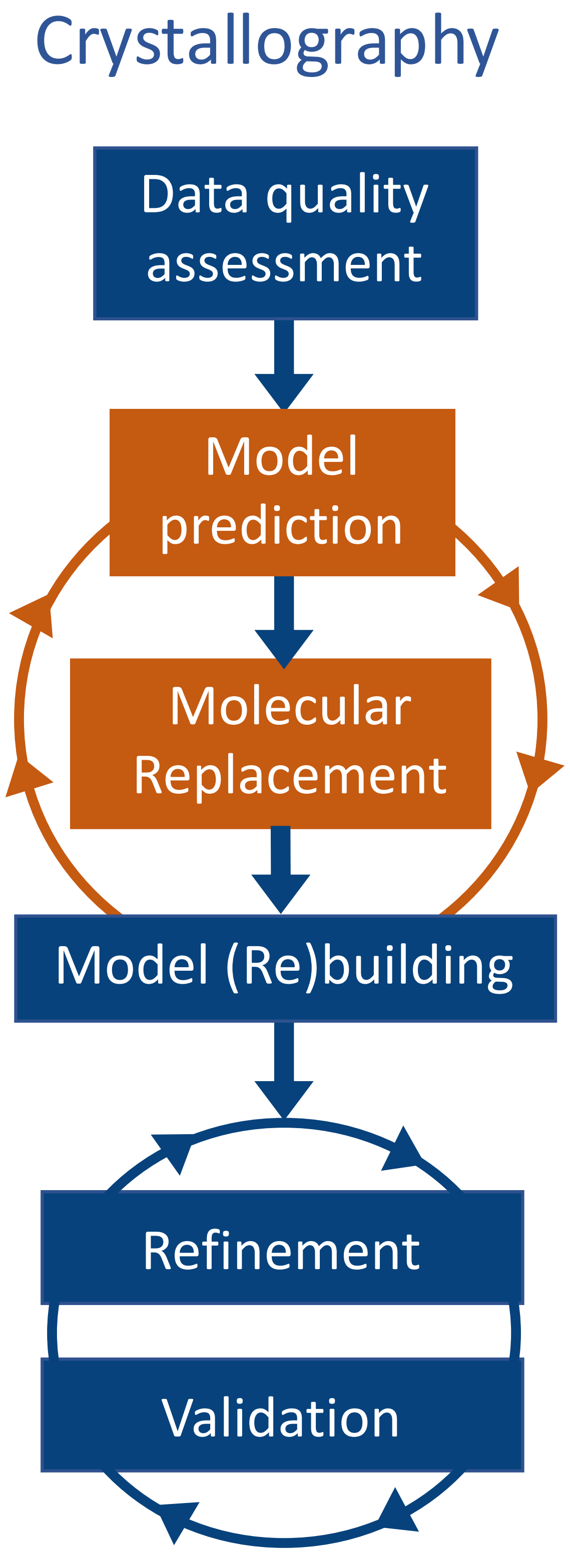
AlphaFold cycle 4

7mjs

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

Using predicted models

Updated approach: Iterate prediction and model building



Crystallography

Data quality assessment

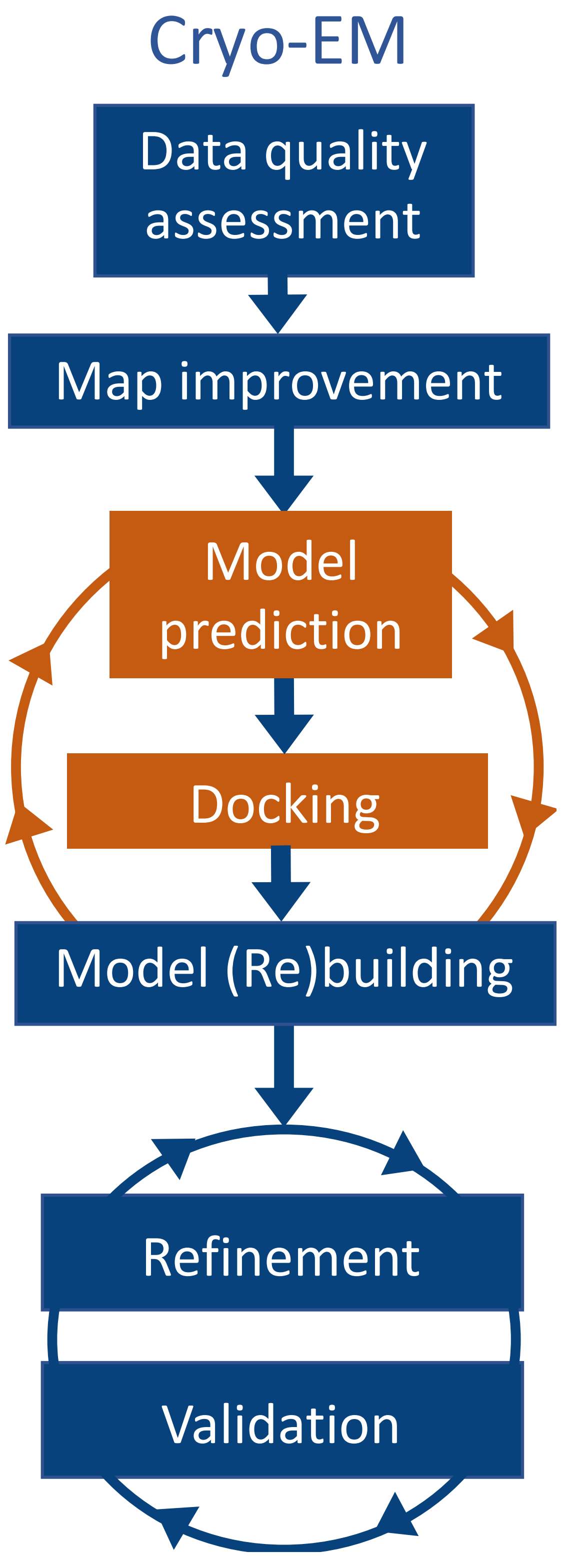
Model prediction

Molecular Replacement

Model (Re)building

Refinement

Validation



Cryo-EM

Data quality assessment

Map improvement

Model prediction

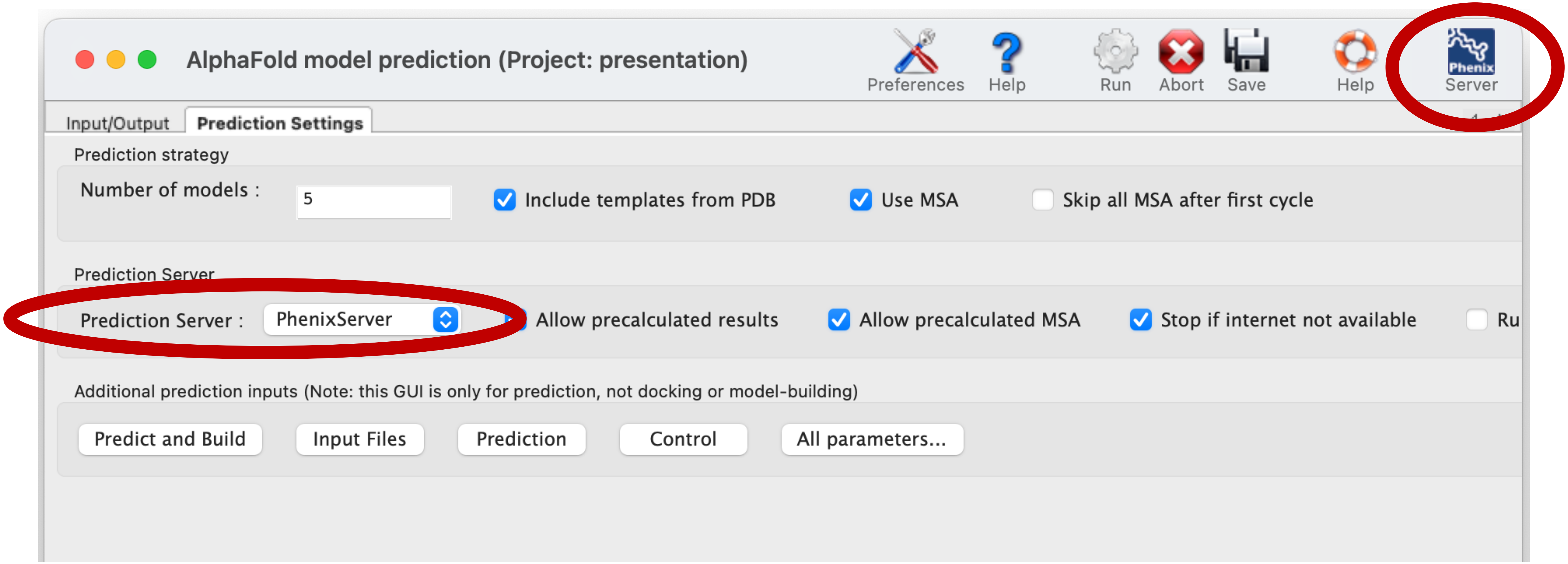
Docking

Model (Re)building

Refinement

Validation

Phenix Server for running AlphaFold



AlphaFold model prediction (Project: presentation)

Prediction strategy

Number of models : 5

☑ Include templates from PDB

☑ Use MSA

☐ Skip all MSA after first cycle

Prediction Server

Prediction Server : PhenixServer

☑ Allow precalculated results

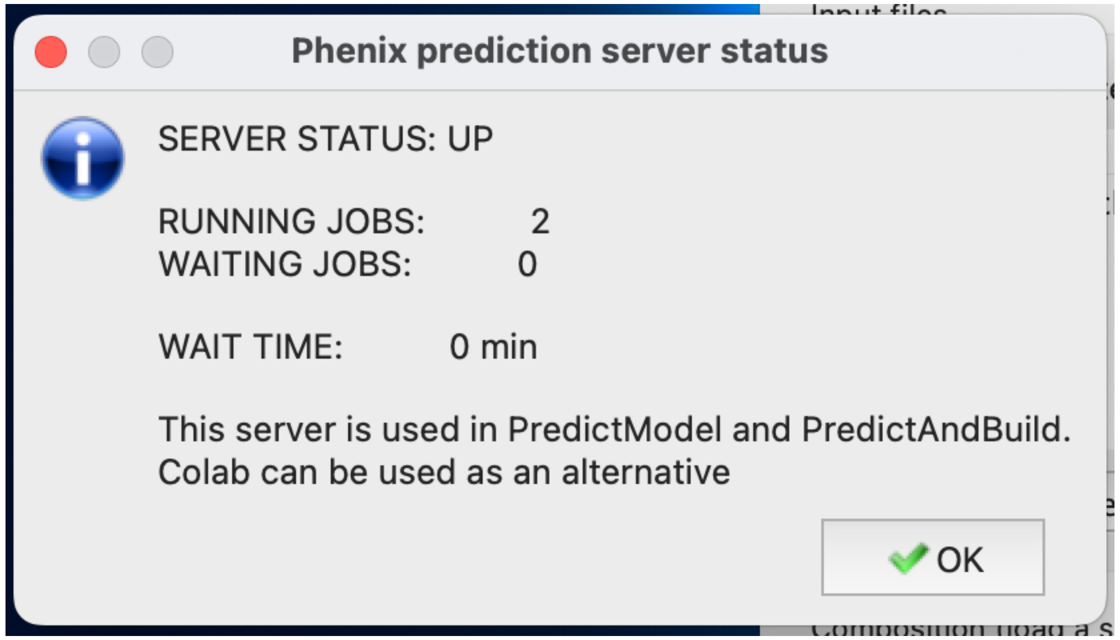
☑ Allow precalculated MSA

☑ Stop if internet not available

☐ Ru

Additional prediction inputs (Note: this GUI is only for prediction, not docking or model-building)

No need for a local AlphaFold installation



Phenix prediction server status

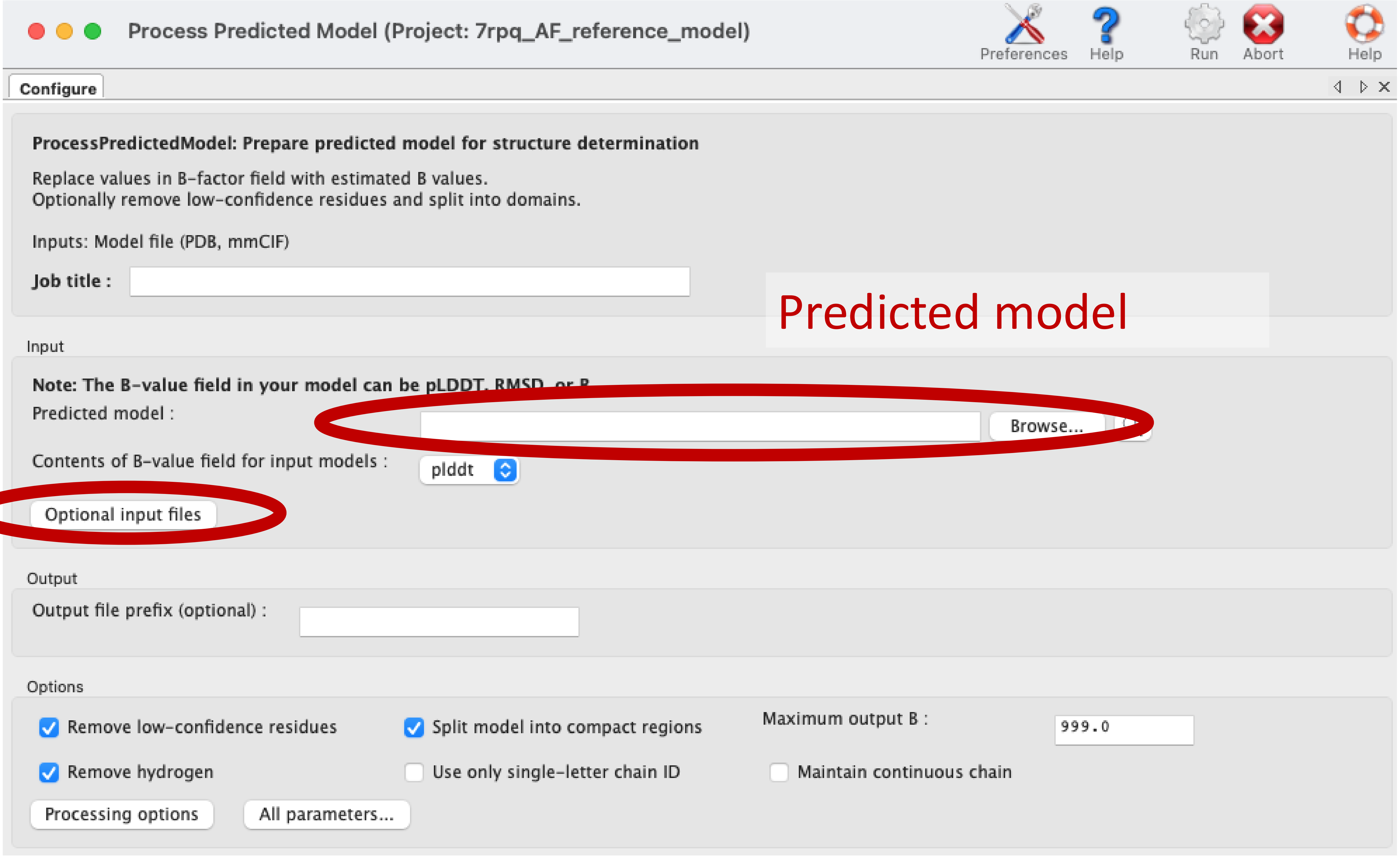
SERVER STATUS: UP

RUNNING JOBS: 2
WAITING JOBS: 0

WAIT TIME: 0 min

This server is used in PredictModel and PredictAndBuild.
Colab can be used as an alternative

Process predicted model



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 :

Contents of B-value field for input models : plddt

Output

Output file prefix (optional) :

Options

☑ Remove low-confidence residues

☑ Split model into compact regions

Maximum output B : 999.0

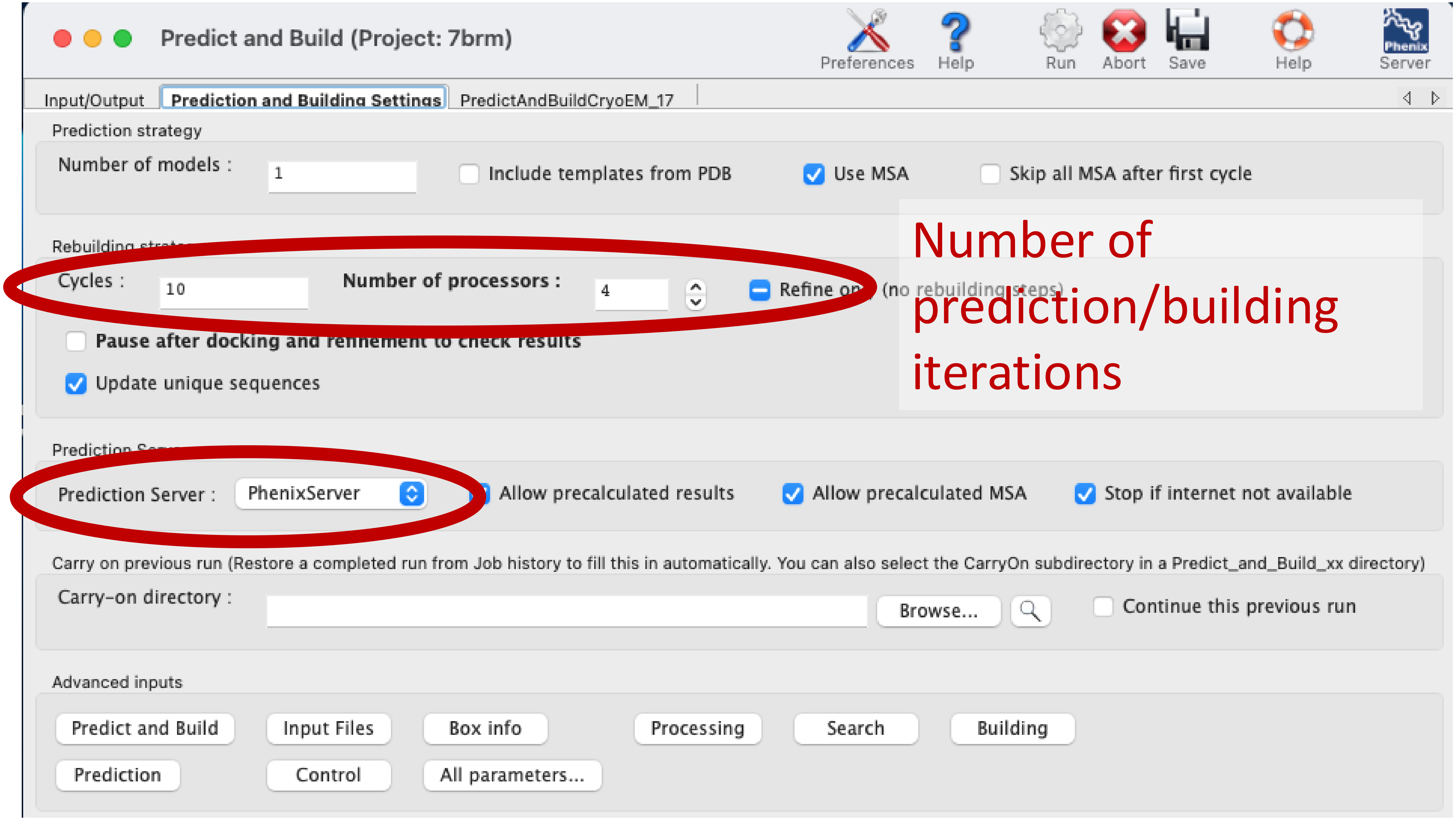
☑ Remove hydrogen

☐ Use only single-letter chain ID

☐ Maintain continuous chain

Predicted model

Iterate with Predict and Build



Prediction strategy

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

Rebuilding strat

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

☐ **Pause after docking and refinement to check results**

☑ Update unique sequences

Prediction Se

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 : ☐ Continue this previous run

Number of prediction/building iterations

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

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

Iterate

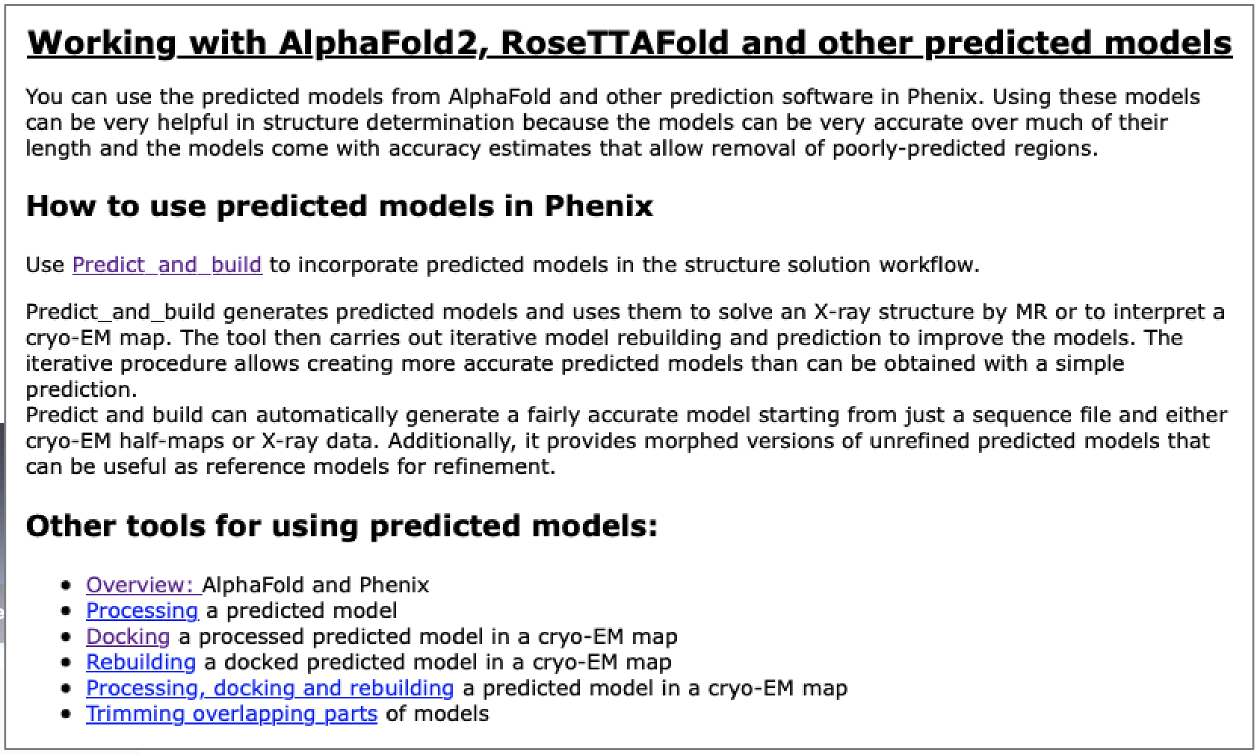
4. Improve your structure

Use your new prediction as hypothesis, rebuild parts

Further reading/material

Documentation:

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



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.

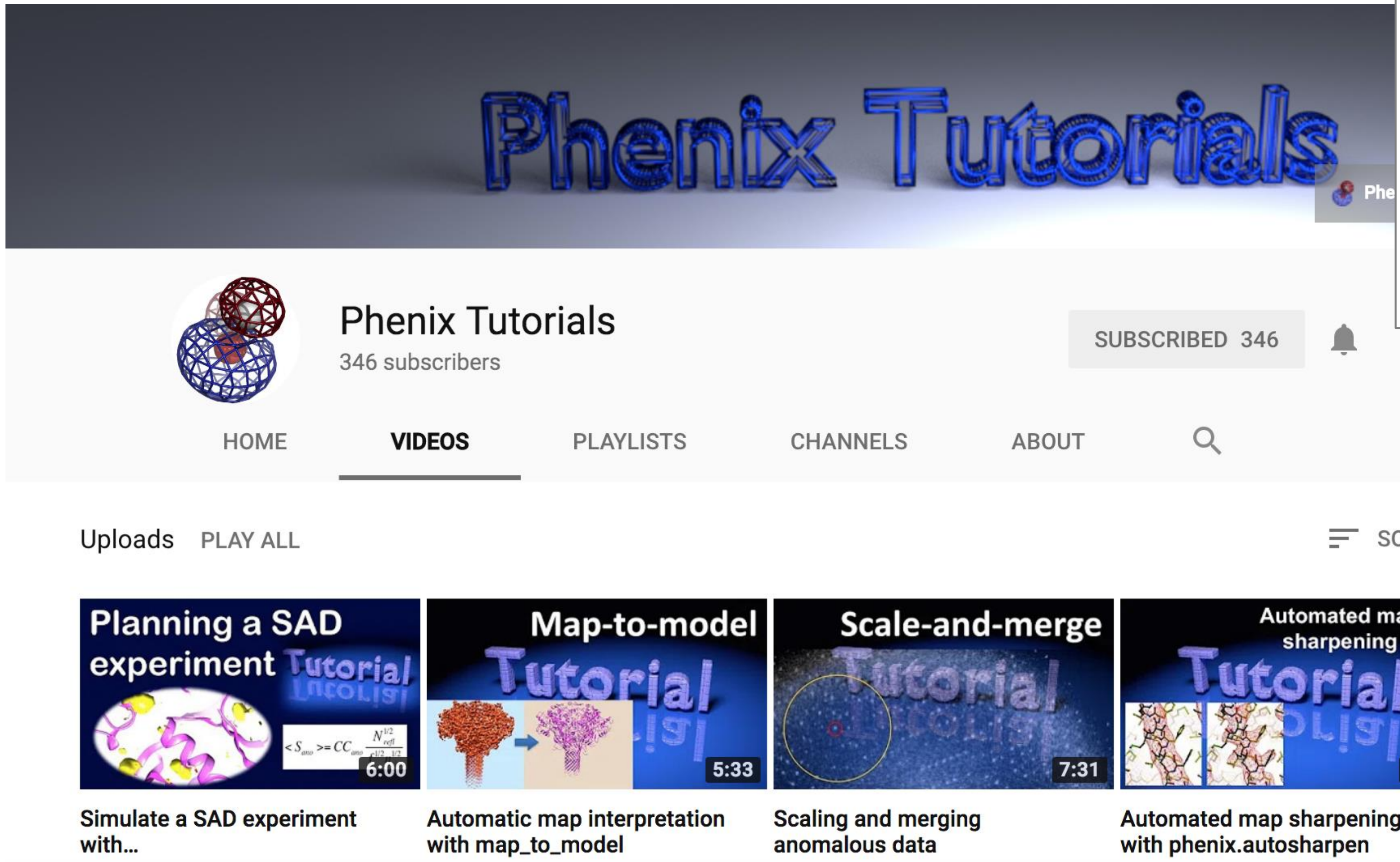
How to use predicted models in Phenix

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.

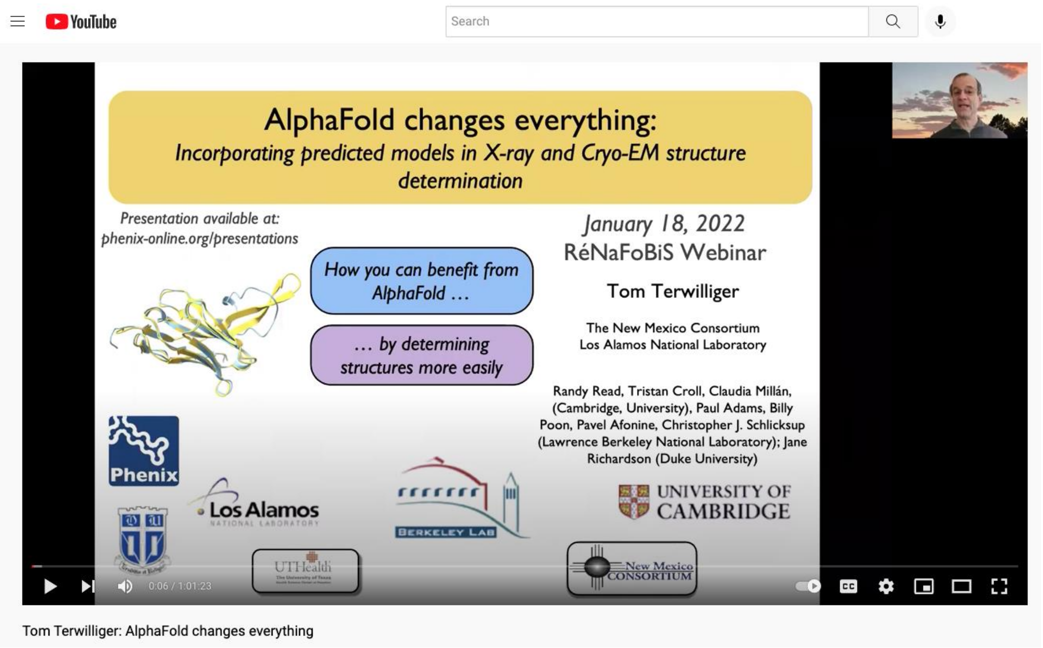
Other tools for using predicted models:

- 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



Video tutorials

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



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

Presentation available at:
phenix-online.org/presentations

How you can benefit from AlphaFold …

… by determining structures more easily

January 18, 2022
RéNaFoBiS Webinar

Tom Terwilliger

The New Mexico Consortium
Los Alamos National Laboratory

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)

Tom Terwilliger: AlphaFold changes everything

Tom Terwilliger: AlphaFold changes everything

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

The Phenix 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