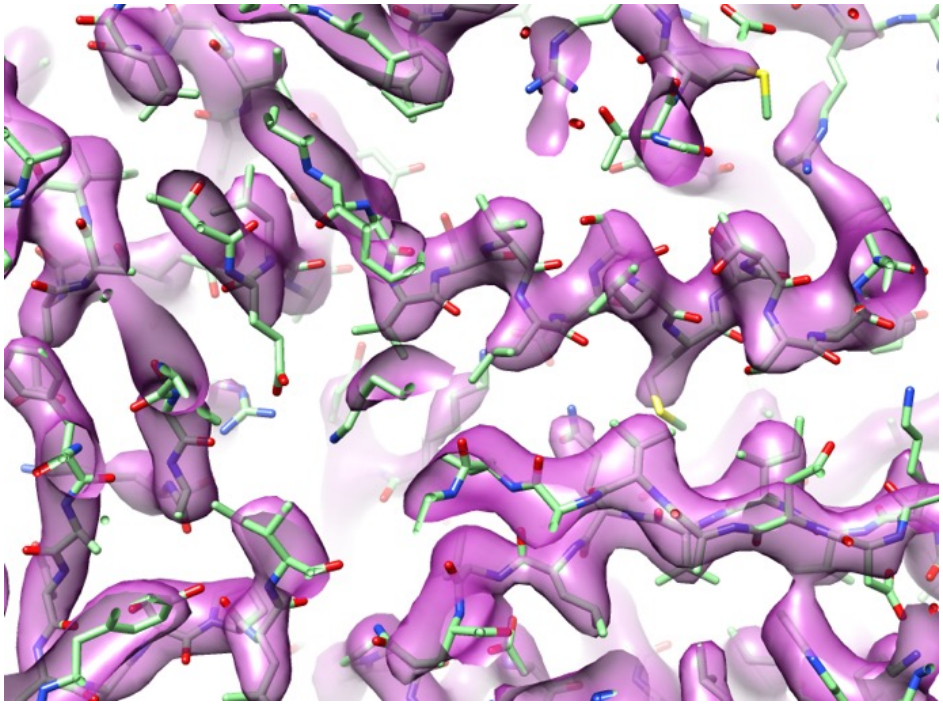
Cryo-EM Model-building

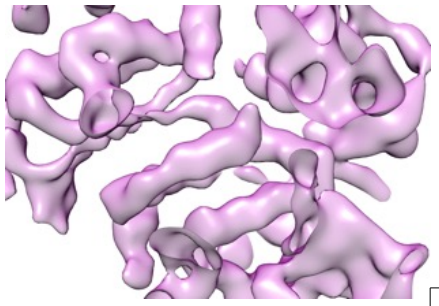


Phenix workshop

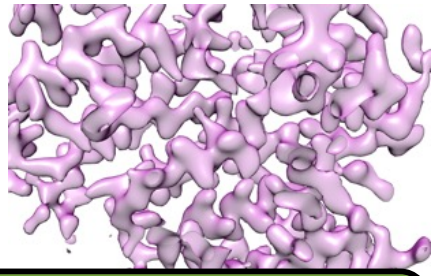
Sept. 15, 2020

Tom Terwilliger, New Mexico Consortium
Li-Wei Hung, Los Alamos National Laboratory
Pavel Afonine, Paul Adams, Dorothee Liebschner, Lawrence Berkeley National Laboratory

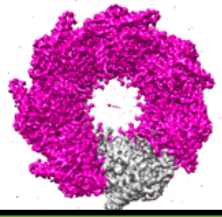
Cryo-EM tools in Phenix



Starting map



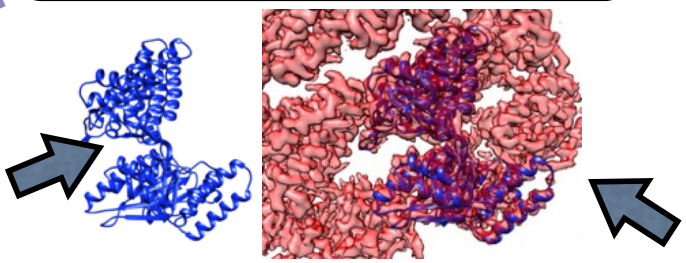
Map improvement



Map symmetry

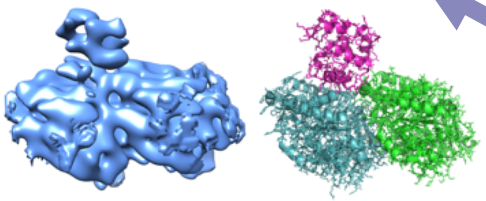
Map manipulation

Extract unique part



Docking, model-building

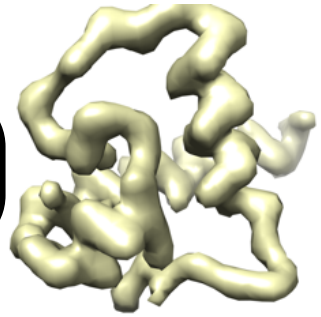
Refinement



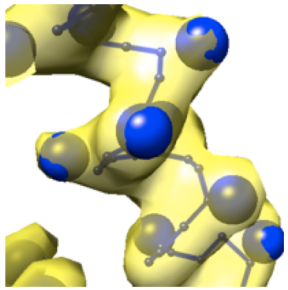
Validation

Map-to-model: Cryo-EM model building

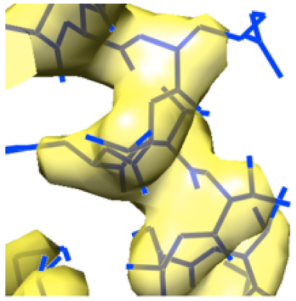
Isolate density for a chain



Identify C_α and C_β positions from side-chain density



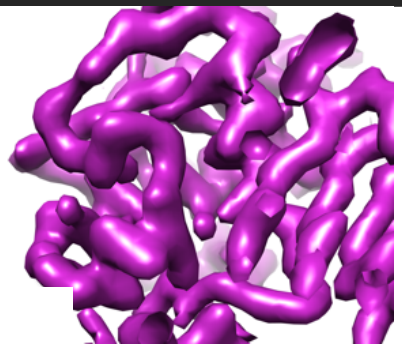
Construct and refine all-atom model



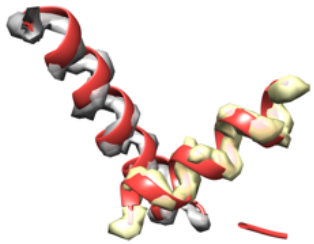
Terwilliger et al., (2018) Nature Methods 15, 905–908
Terwilliger et al., (2020) Protein Science 29, 87-99

Isolating density for a chain

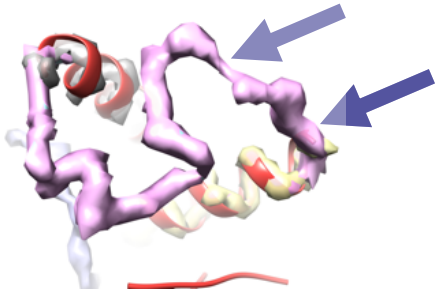
Trace chain the way a person does



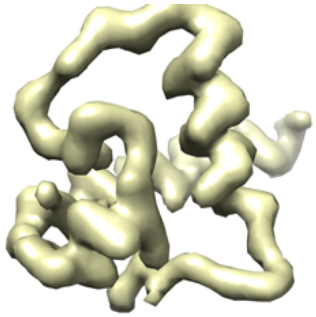
Find secondary structure (helices, strands)



Find connections: adjust contour level until a region just connects to one other



Iterate to build up chain



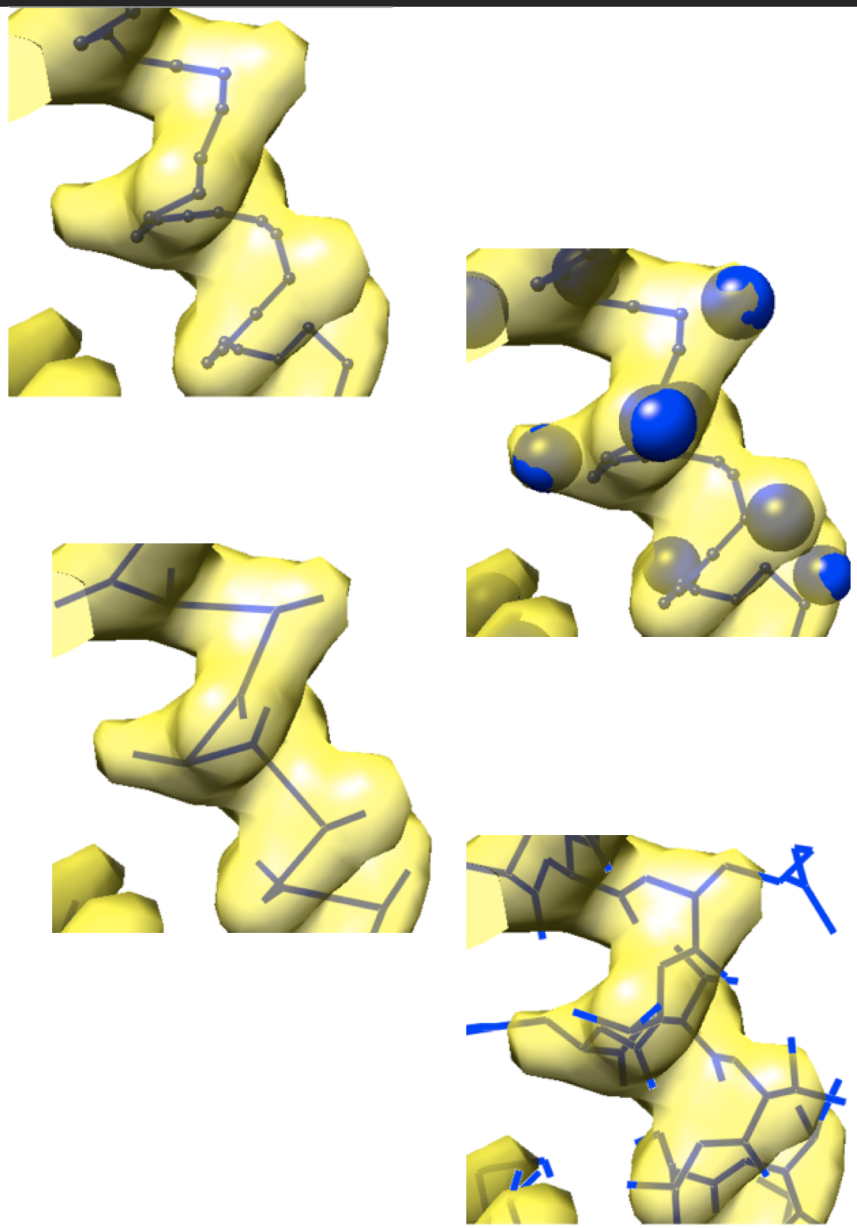
Finding C_α and C_β positions

Trace chain path through high density

Find C_β positions from side-chain density

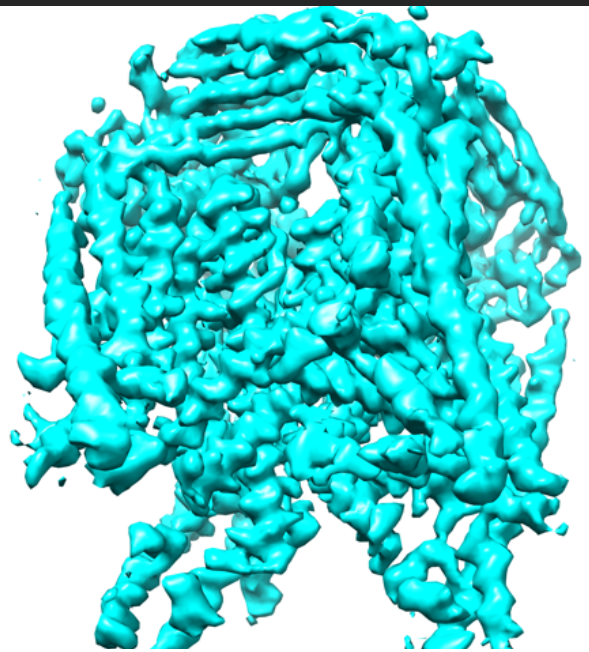
Choose C_α positions 3.8 Å apart and next to C_β positions

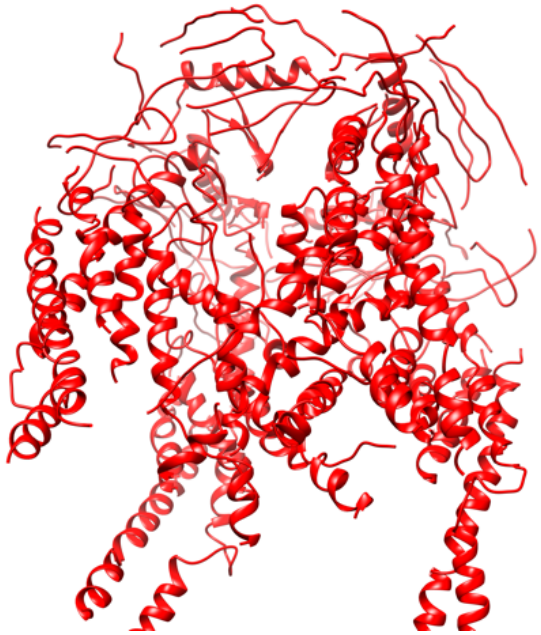
Construct all-atom model with Pulchra* and refine

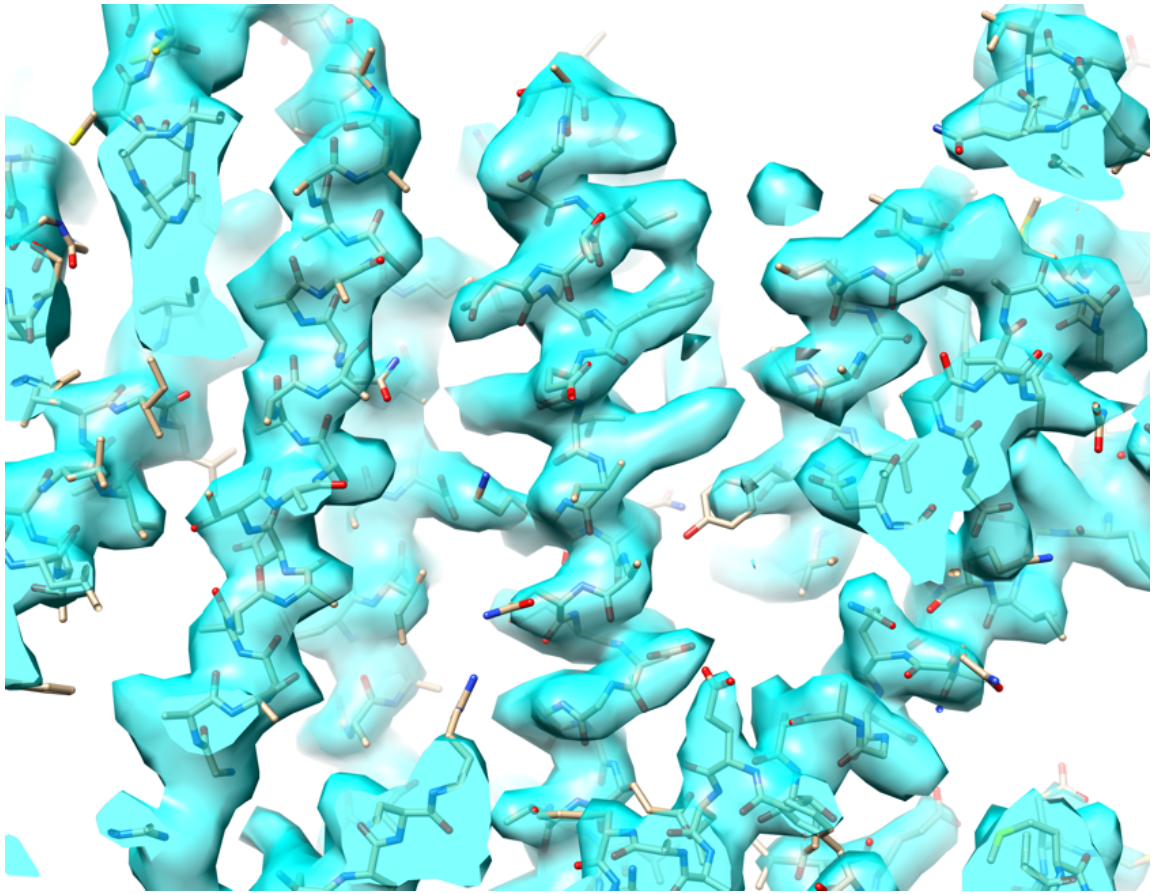


*Rotkiewicz & Skolnick (2008). J. Comp. Chem. 29, 1460.

TRPML3 channel (4.1 Å, 78% built, 1.3 Å rmsd)

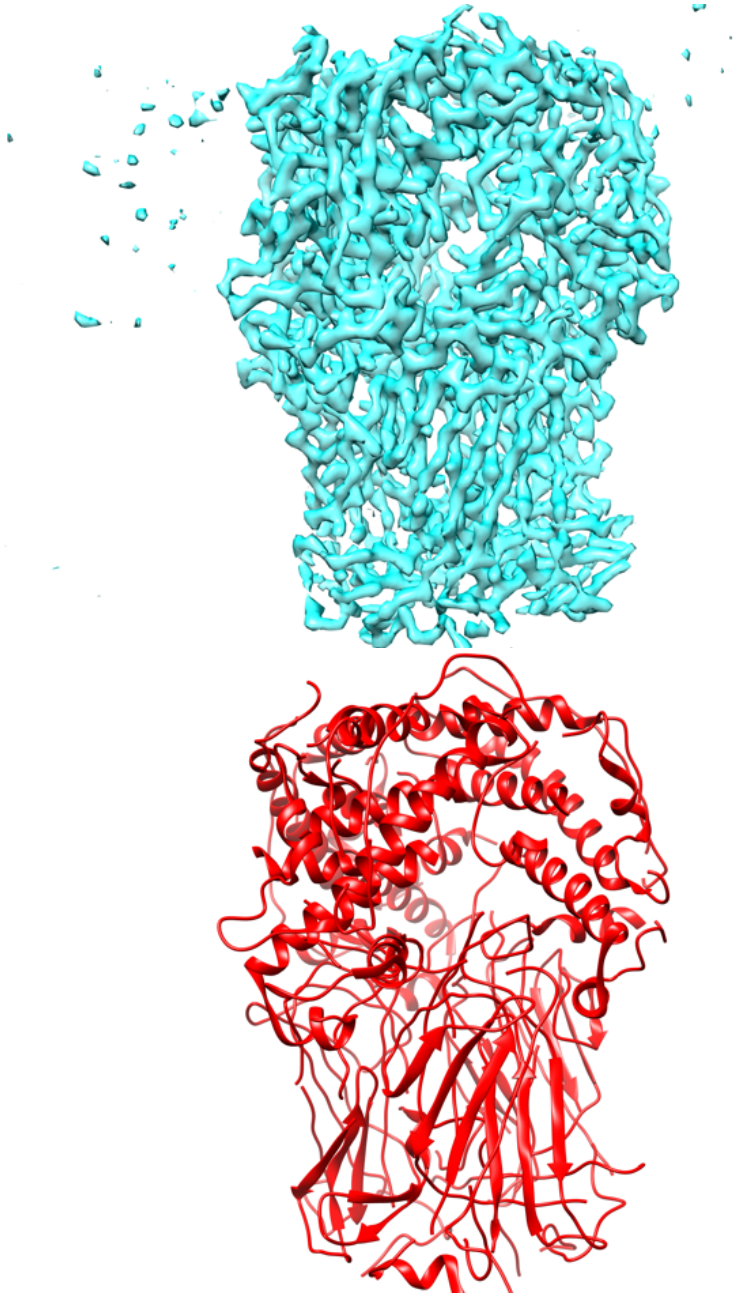


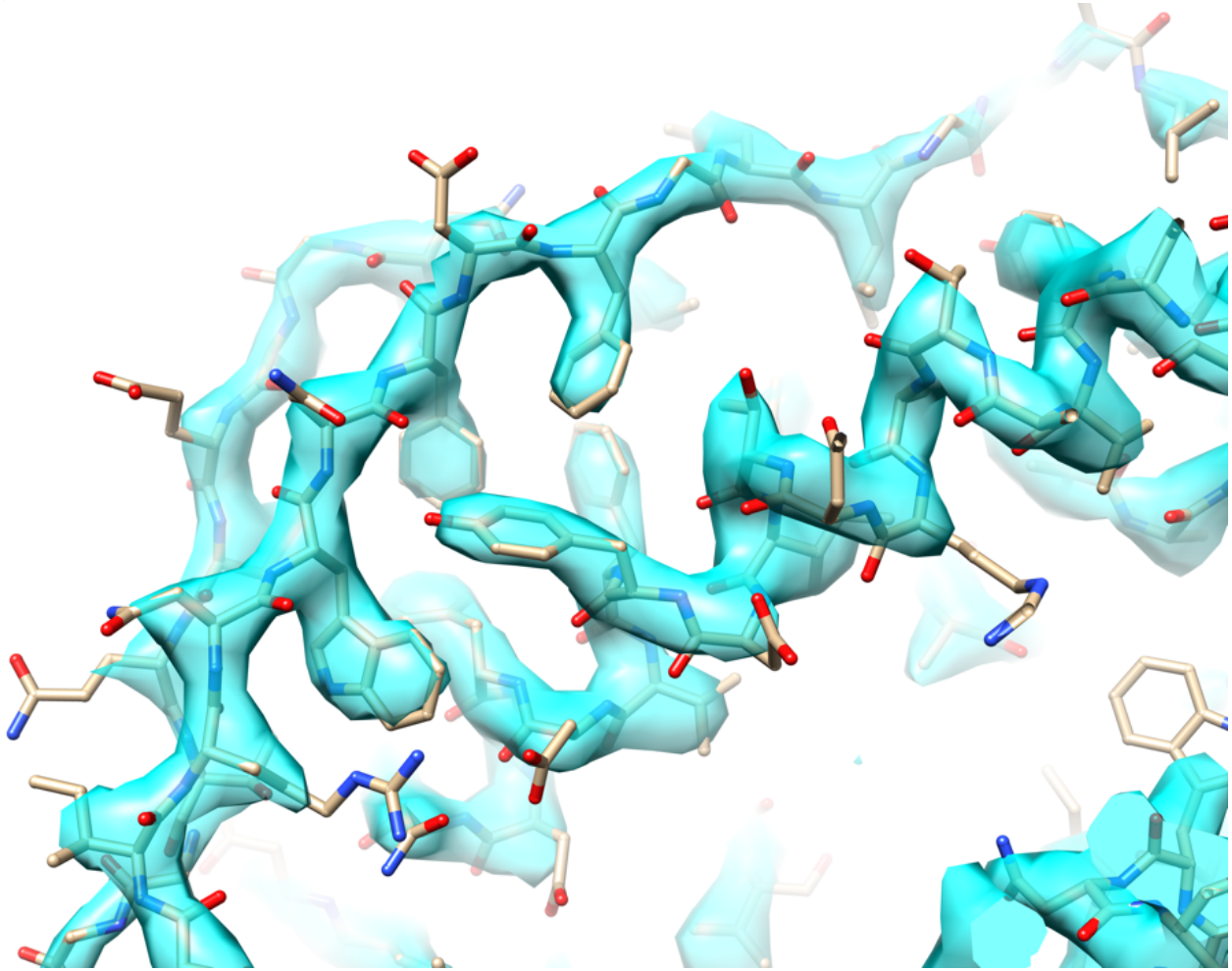




Data from Zhou, X. et al. (2017) Nat. Struct. Mol. Biol. 24: 1146

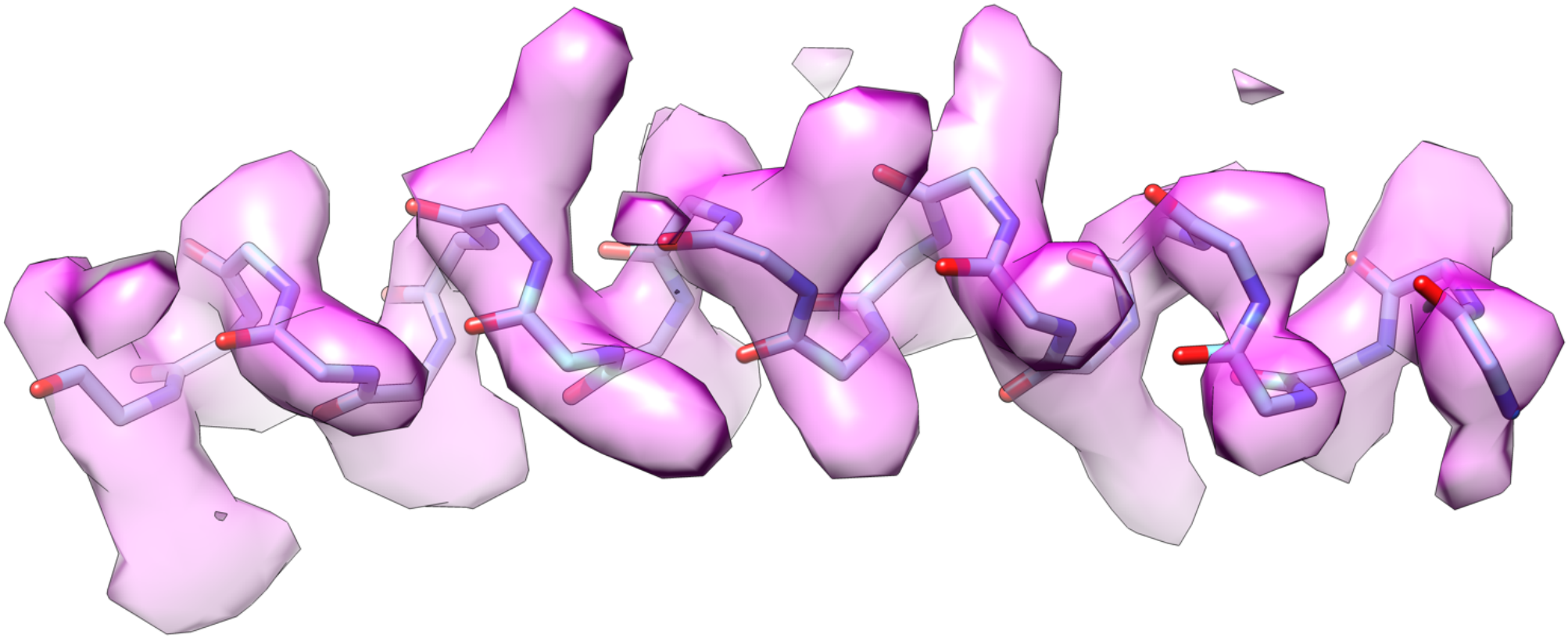
Rotavirus VP6 (2.6 Å, 100% built, 0.9 Å rmsd)





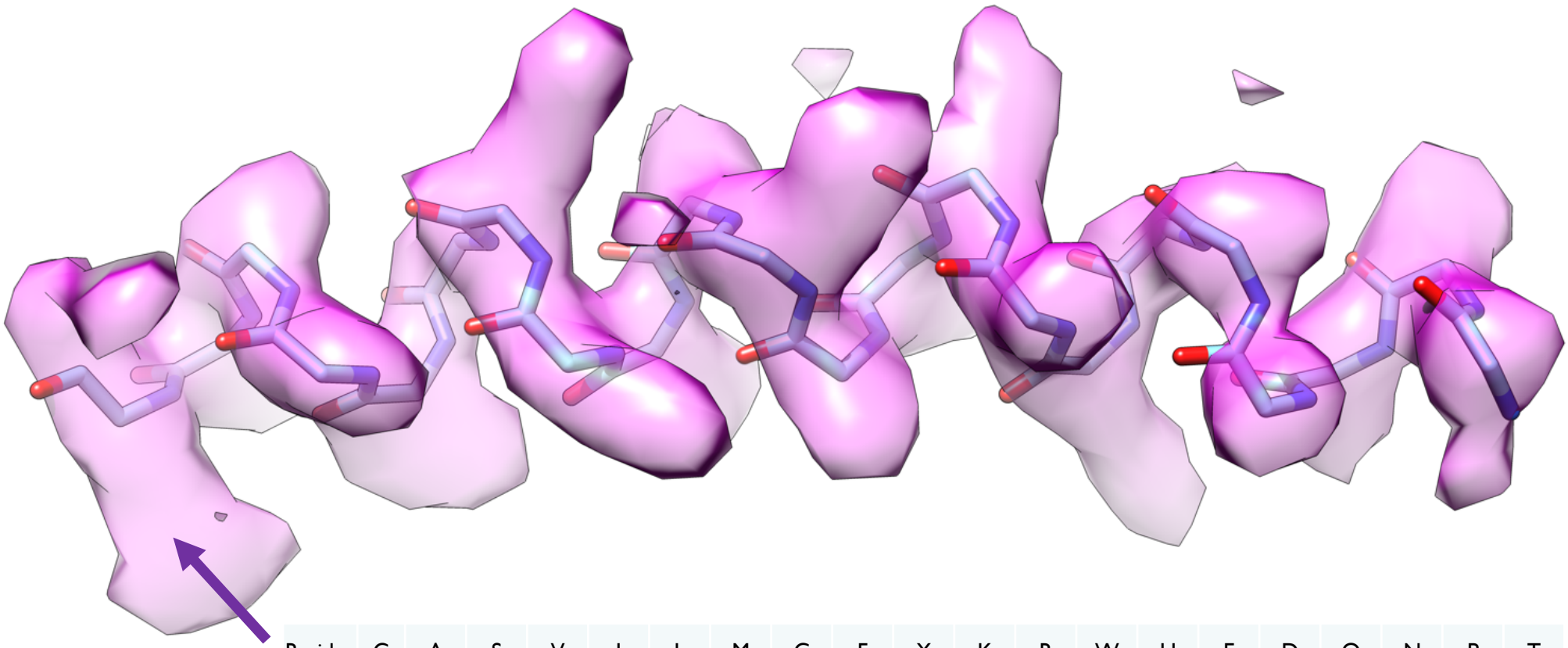
Data from Grant and Grigorieff, eLife 2015;4:e06980

Sequence from density in a map



Can we identify what part of the sequence goes with this helix?

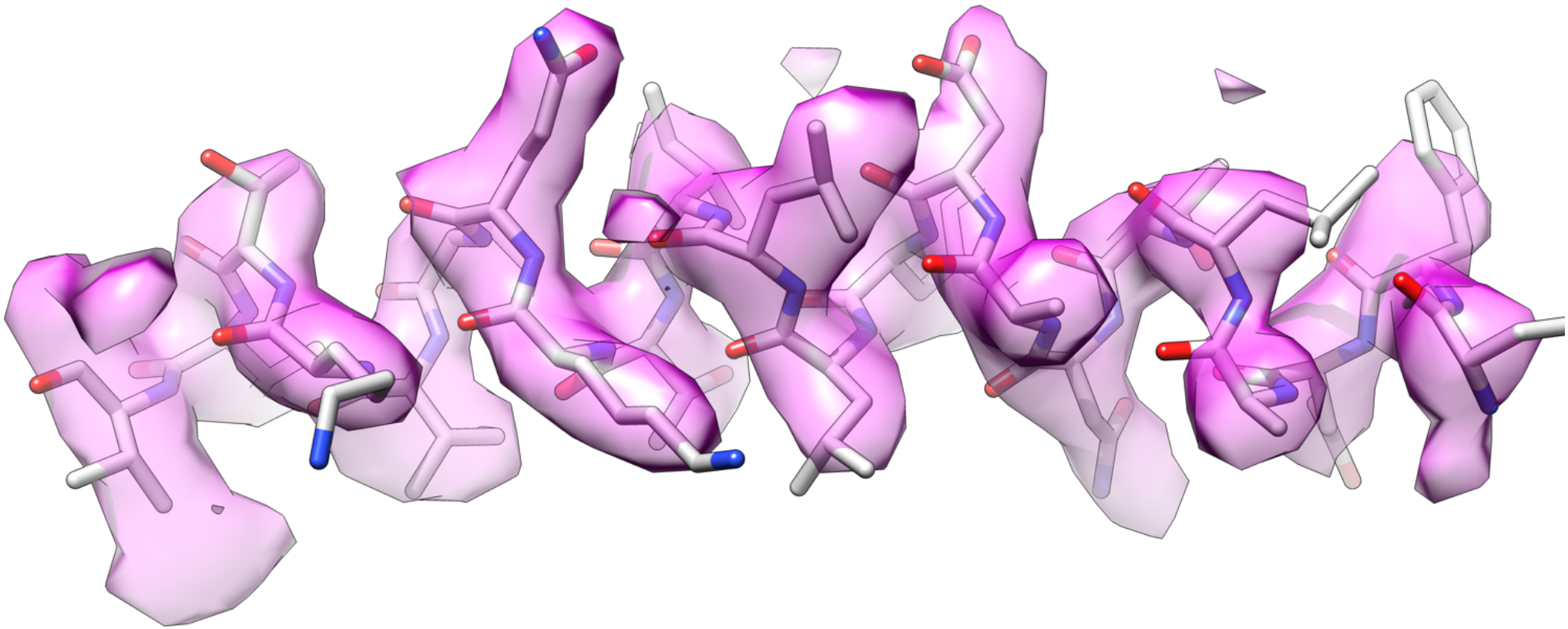
Sequence assignment



Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

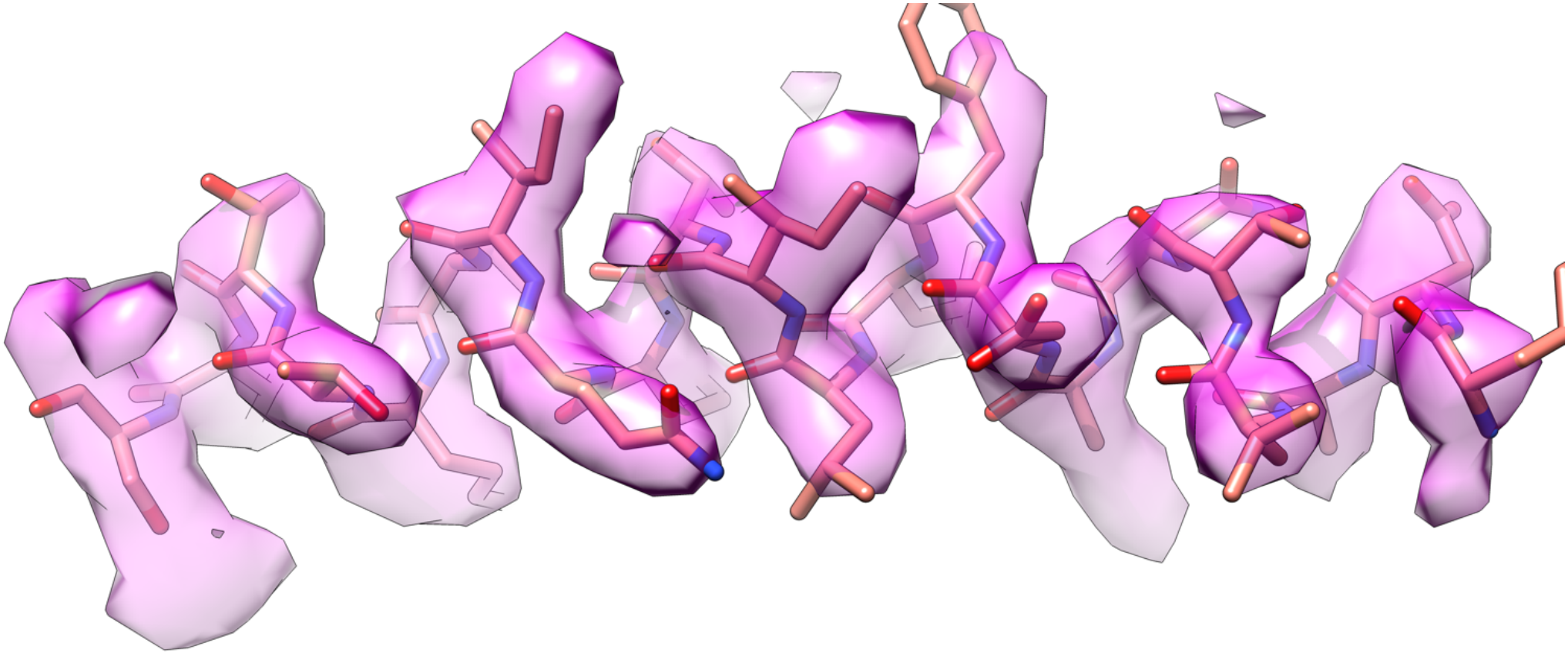
Compatibility of density at position 21 with each amino acid

Sequence assignment: Test all alignments



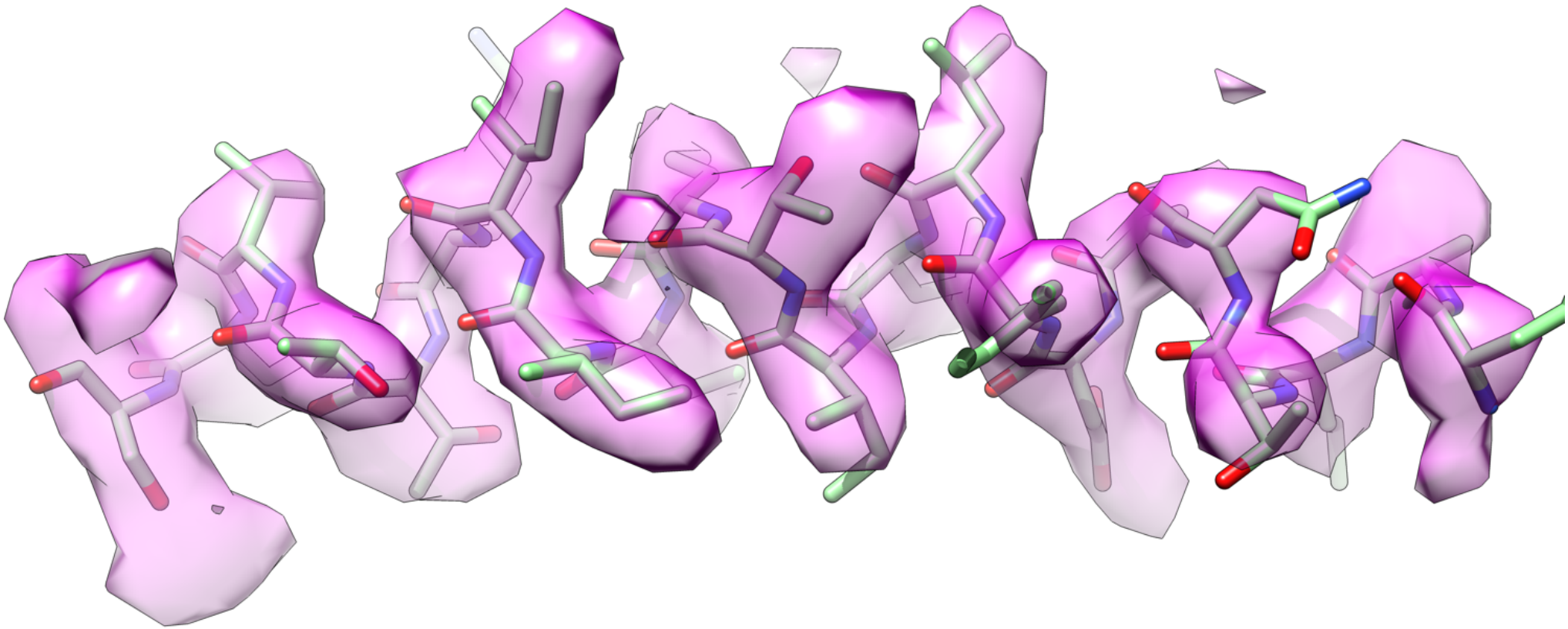
Offset: -3 Score: -4.3

Testing all alignments…



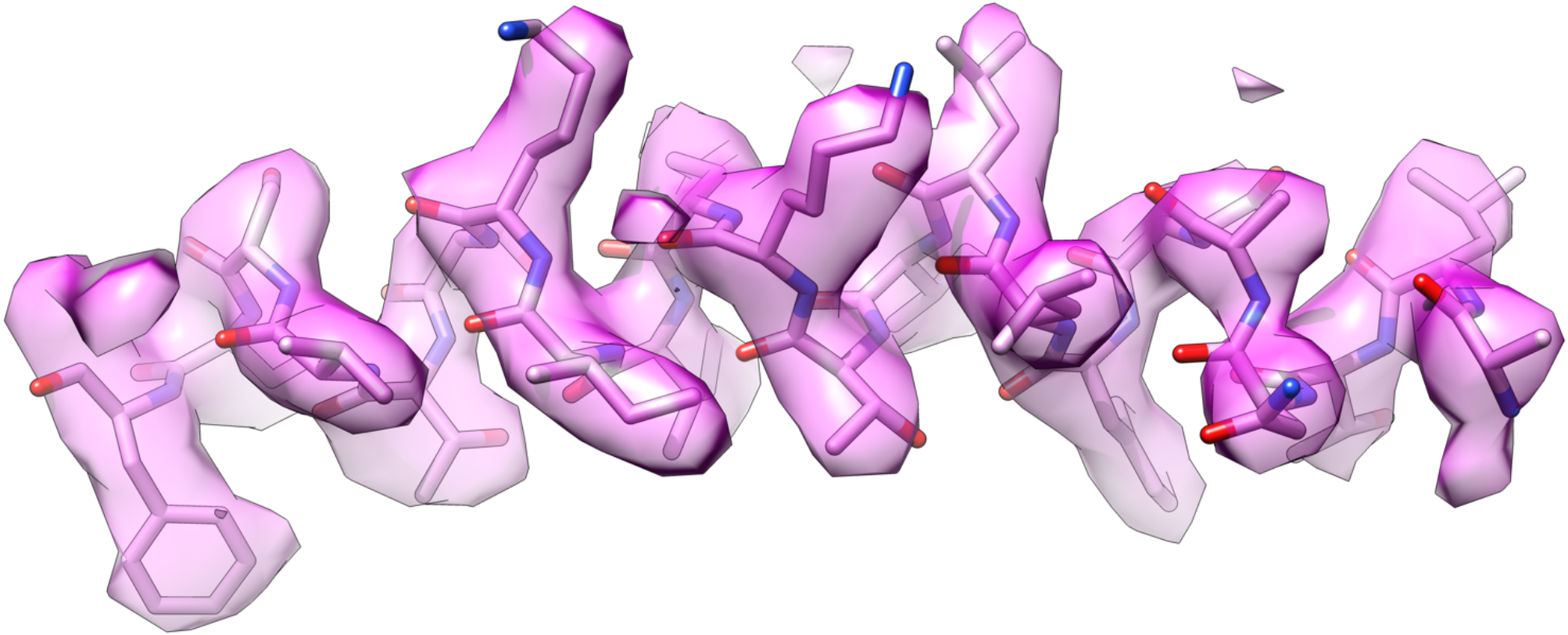
Offset: -2 Score: -0.4

Testing all alignments…



Offset: -1 Score: 4.0

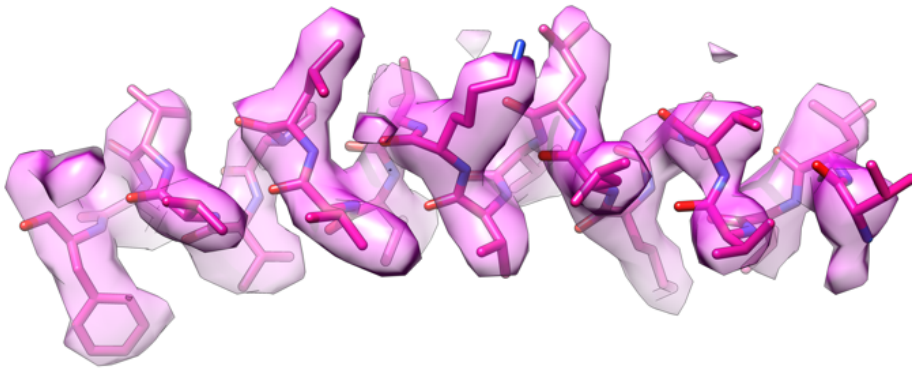
Testing all alignments…

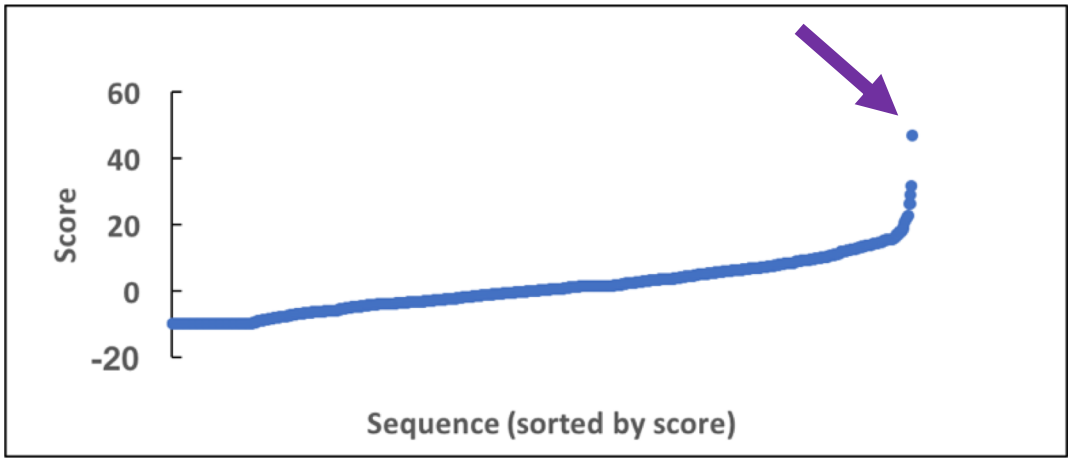


Offset: 0 Score: 25.4

Identifying a protein from a map

With Xiaorun Li, Chi-min Ho & Hong Zhou, UCLA



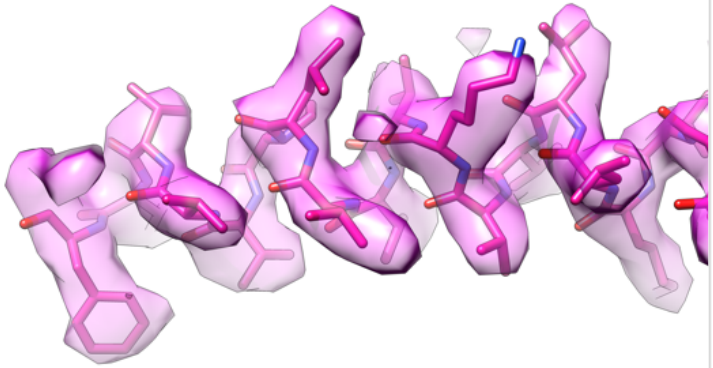


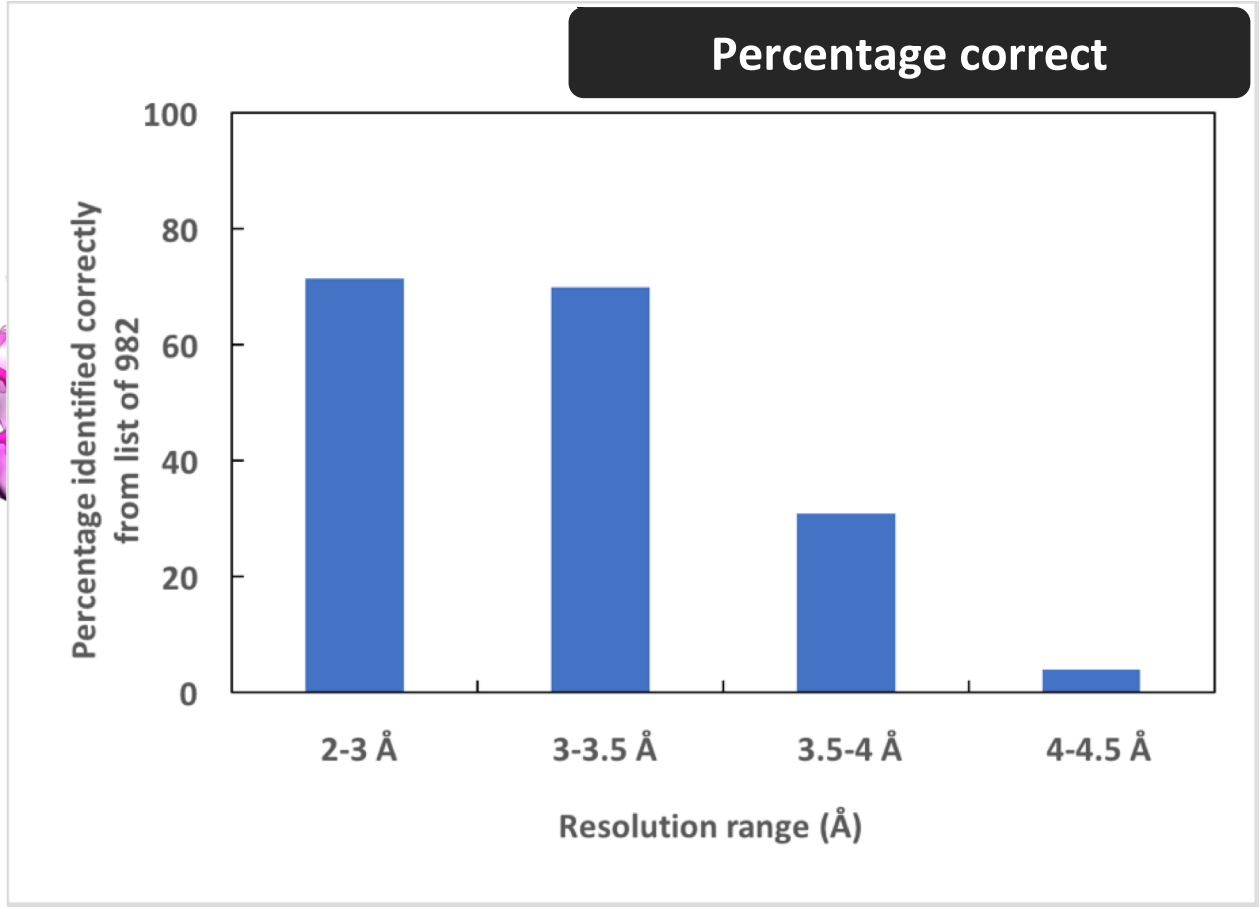
Sequence alignment of full chain against 883 sequences

ID of correct sequence (*P. falciparum* glutamine synthetase) with Z-score of 6.0

Reliability of identifying a protein from a map

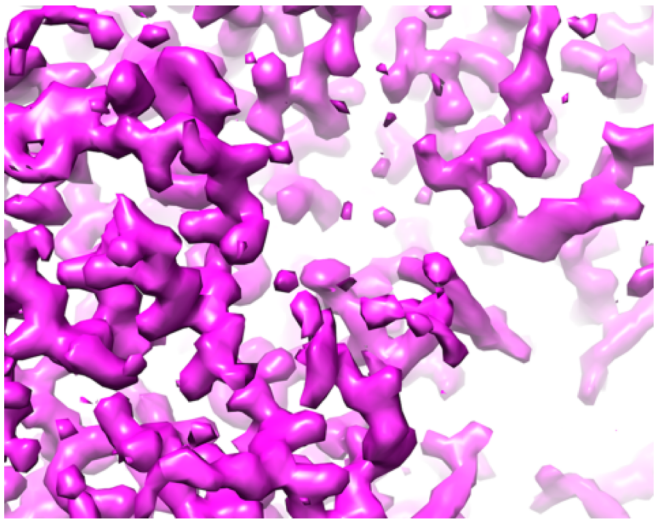
With Xiaorun Li, Chi-min Ho & Hong Zhou, UCLA

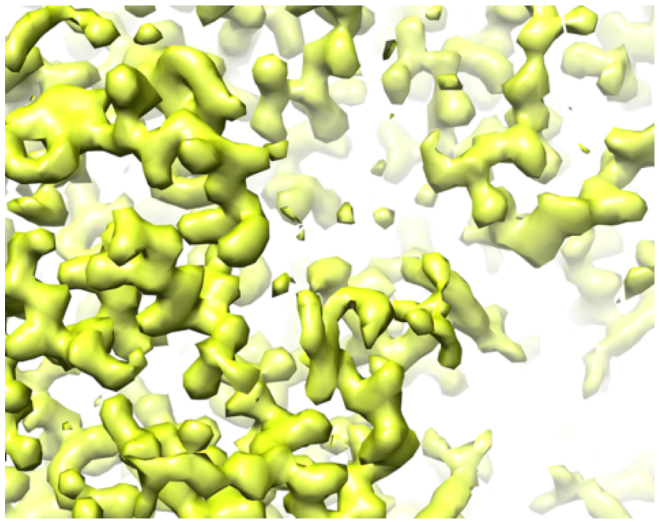


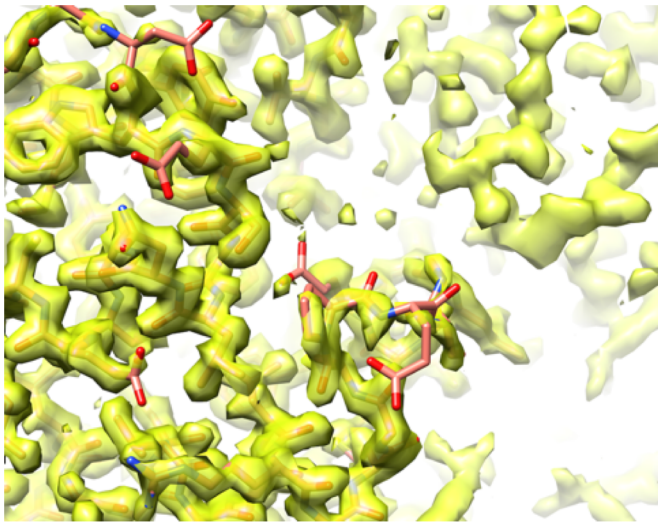


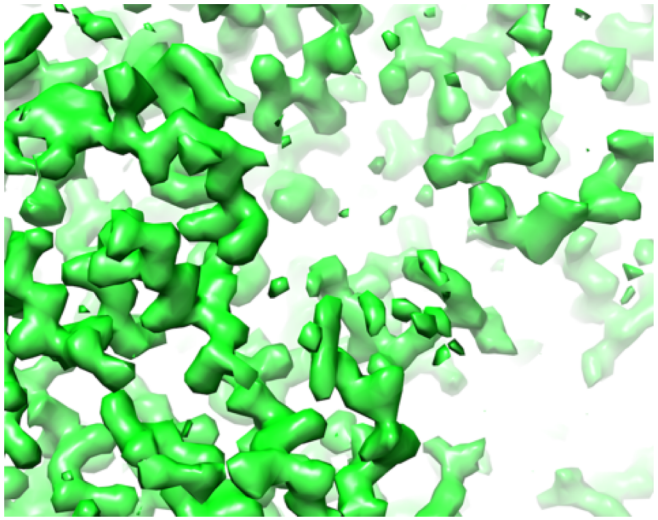
Build models for 92 maps from EMDB
Each model: Rank 982 sequences by Z-score of fit to density

Demo: model-building from a density-modified map



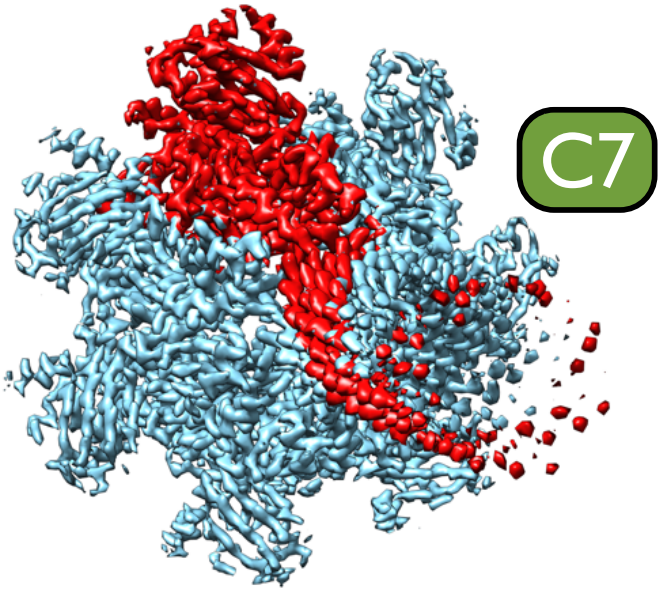


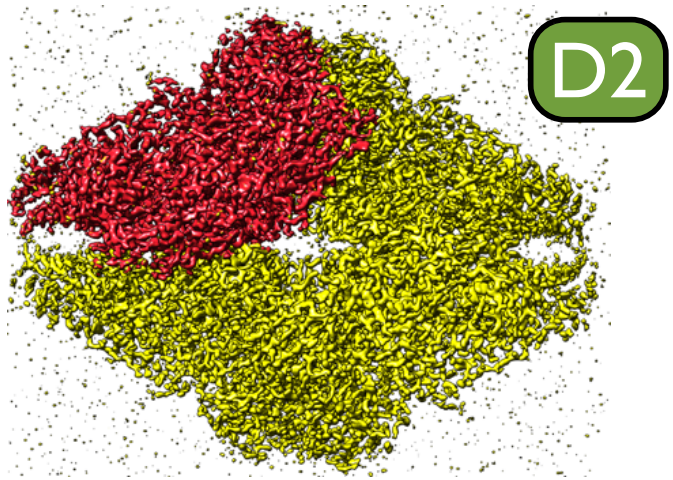




2 half-maps → Density-modified map → Model

Finding map symmetry with symmetry_from_map

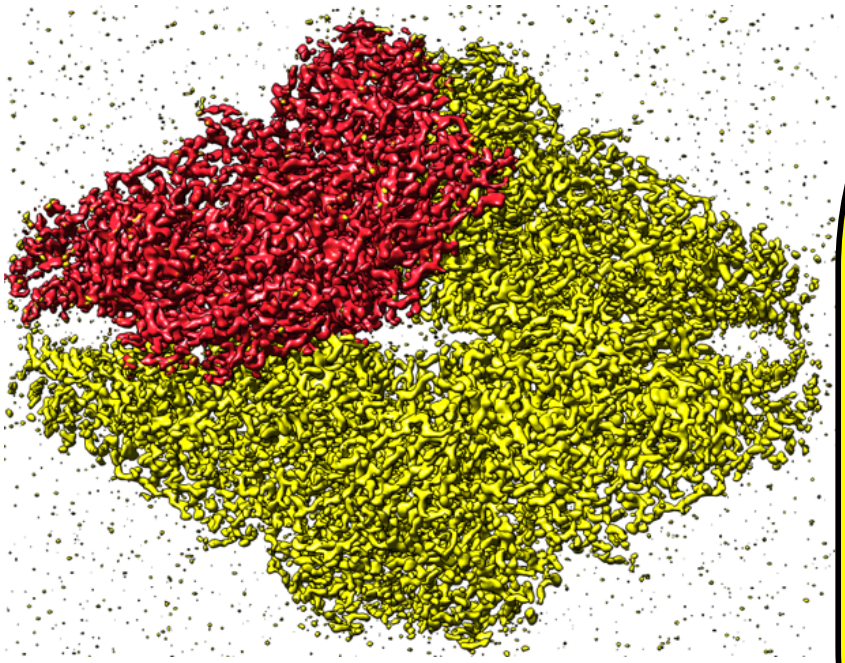




Procedure for finding symmetry:

- Test point group symmetries (e.g., C7, D2, I, O, T)
- Helical symmetry
- Principal rotation axes along z, x, y
- Score based on map correlation for symmetry-related points and number of operators

Extracting unique part of map with map_box (extract_unique option)

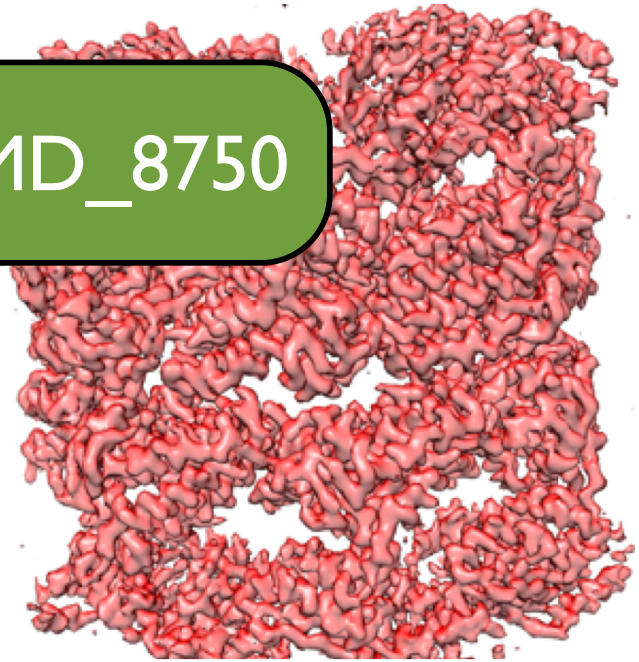


Procedure:

- Use symmetry of map
- Contour map at level that yields regions about 50 residues in size
- Group symmetry-related regions
- Choose one member of each group
- Optimize compactness and connectivity of unique part of map

Docking models with dock-in-map

EMD_8750



1ss8 chain A

Search procedure:

Pure translation

- low-res
- high-res

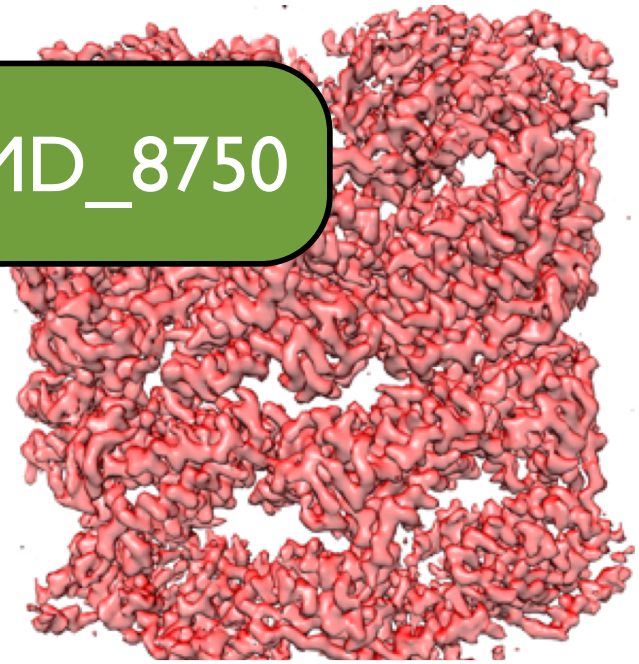
Rotation / translation

- low-res
- high-res

Score based on rigid-body refinement map-model correlation

Docking models with dock-in-map

EMD_8750



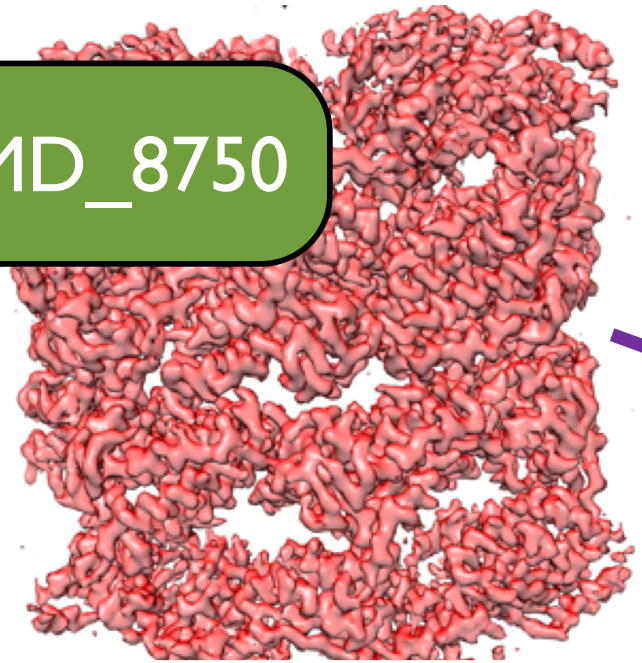
1ss8 chain A

Features

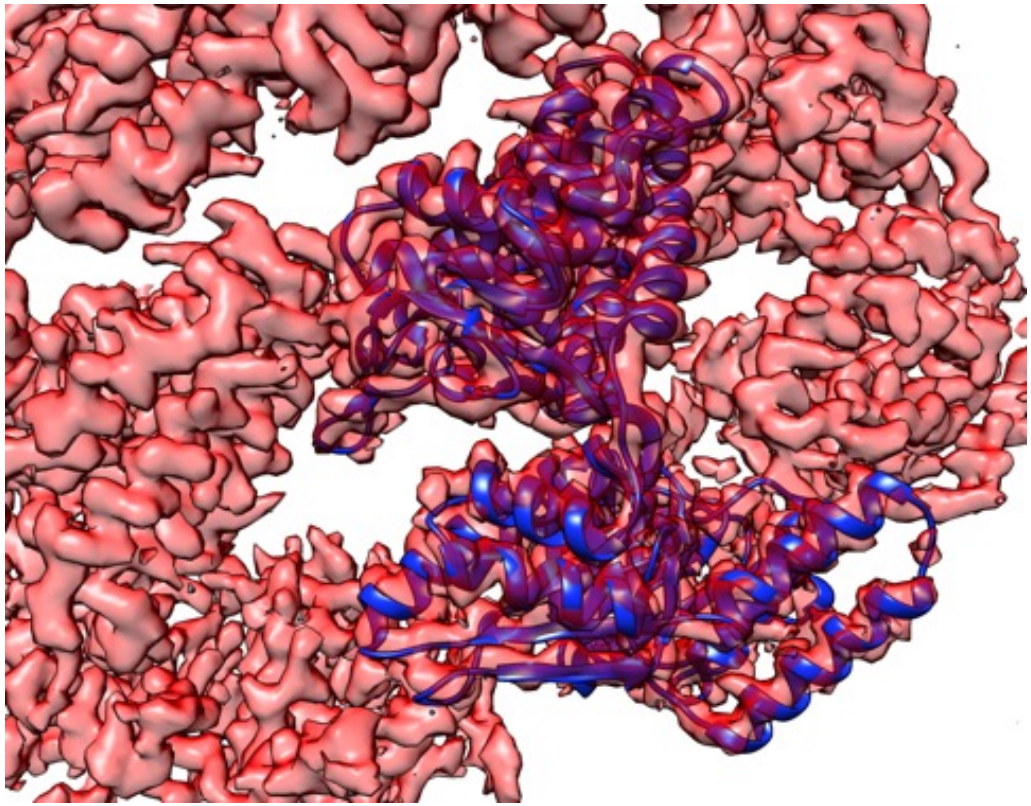
- Multiple chains
- Density search
- Symmetry
- Multiprocessing

Docking models with dock-in-map

EMD_8750



1ss8 chain A



1ss8 chain A docked in map

The *Phenix* Project

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Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung

Duke University

Jane & David Richardson, Chris Williams, Bradley Hintze, Vincent Chen

Cambridge University

Randy Read, Airlie McCoy, Tristan Croll, Rob Oeffner, Massimo Sammito, Claudia Millán Nebot

University of Texas

Matthew Baker, Cory Hryc

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