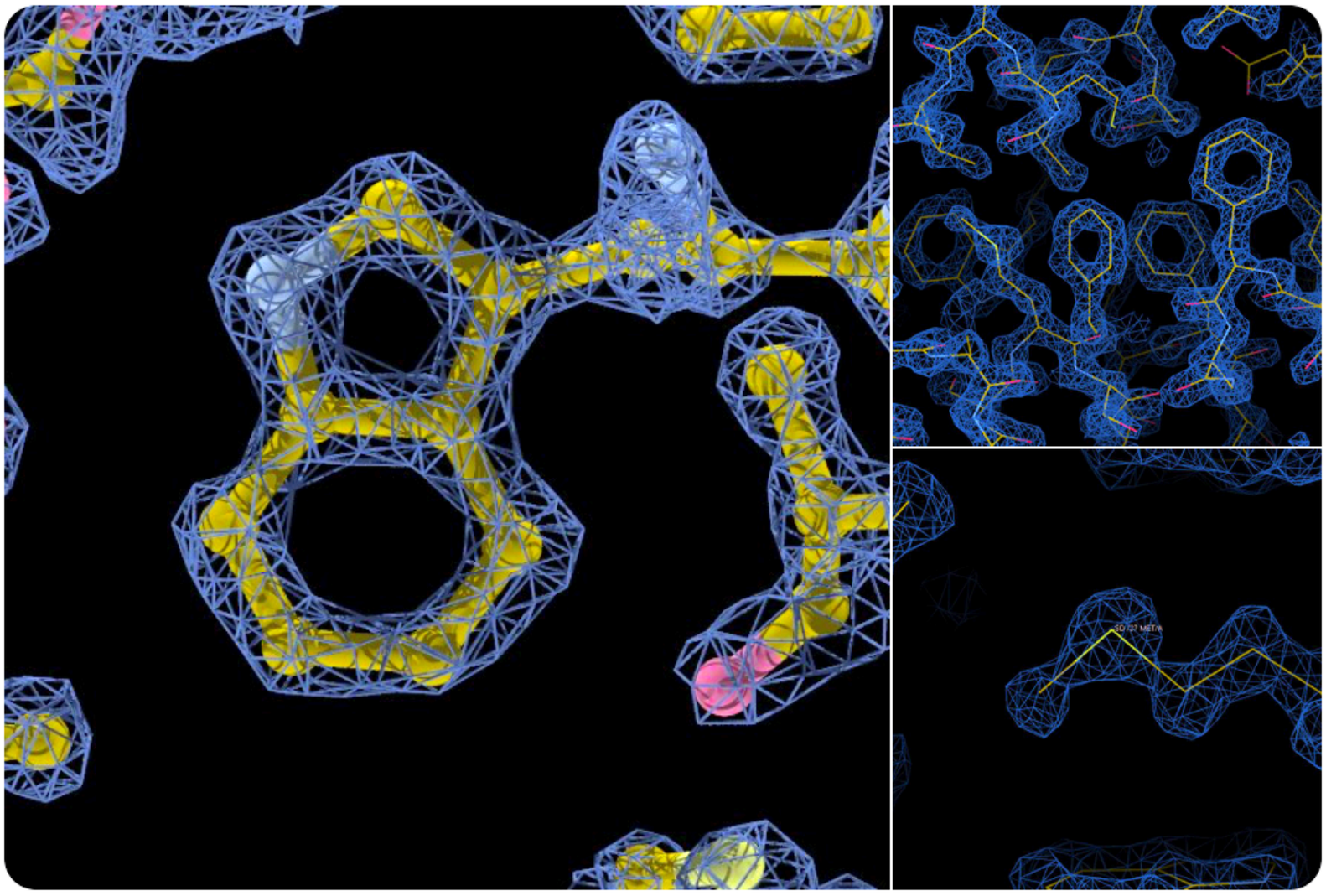
Atomic Models from Cryo-EM Data

ACA Meeting Cryo-EM Workshop
July 2019

Paul Adams
Lawrence Berkeley Laboratory and
Department of Bioengineering UC Berkeley

Impressive Cryo-EM Achievements



Namba Lab, Osaka

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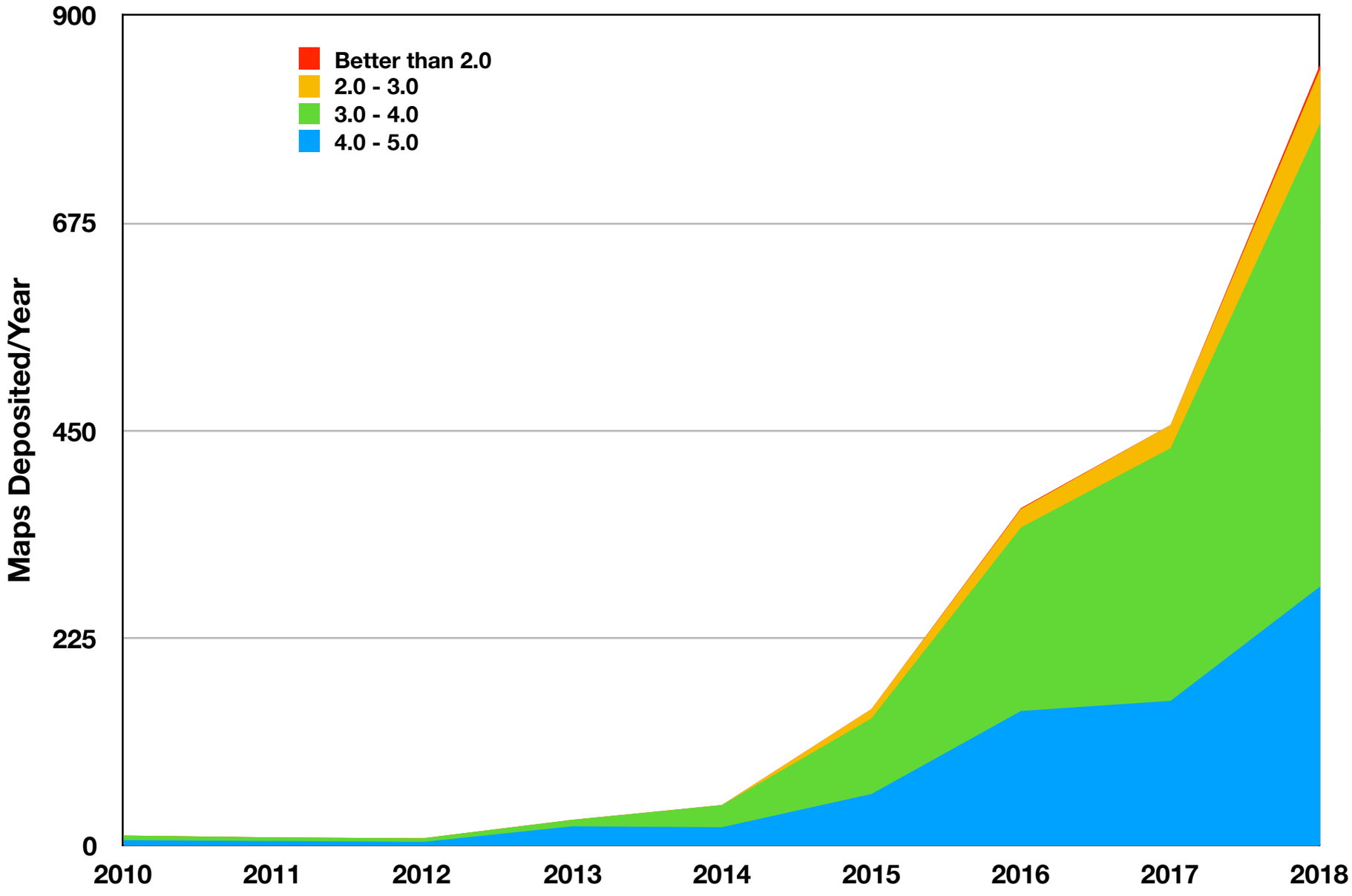
Map Resolution

- Biggest growth is in the 3-4Å range
- Substantial number of maps in 4-5Å range



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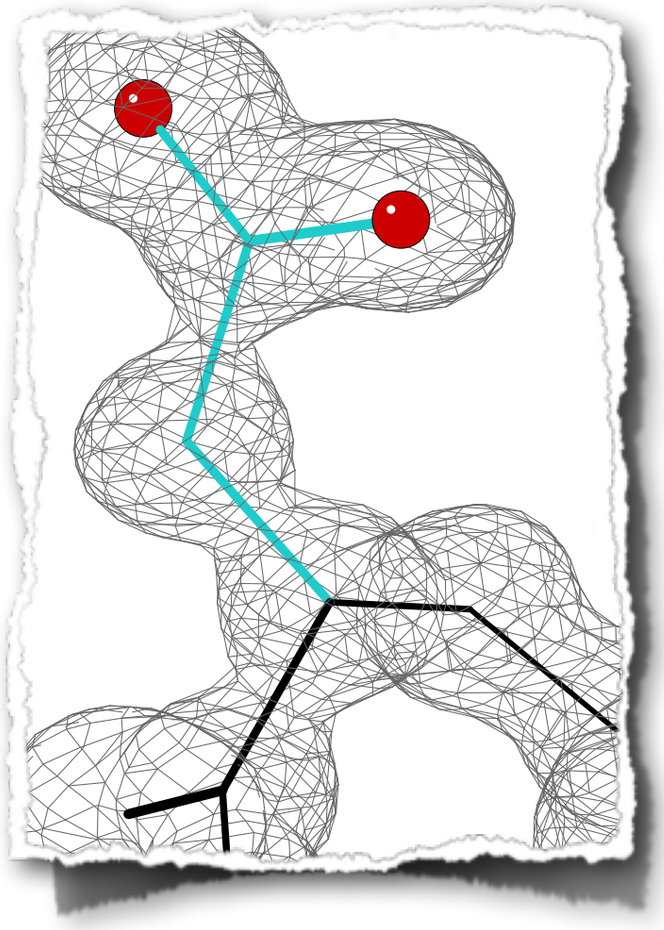
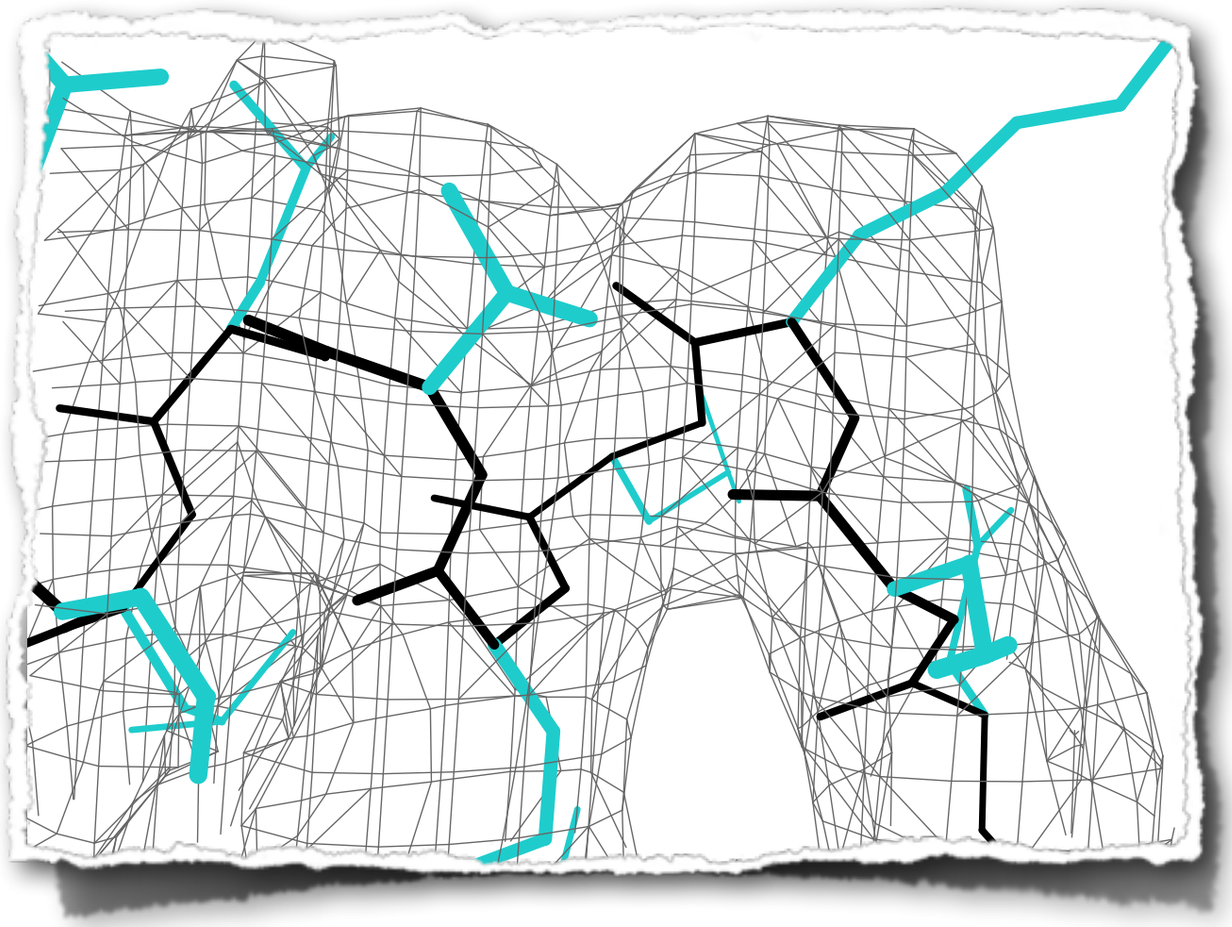
** Not all maps have an associated model*

Low Resolution

PDBID: 2gkg
Resolution: 1.00Å



PDB ID: 3k7a
Resolution: 3.80Å



- Many challenges:
  - How to interpret "featureless" maps (pattern matching, chemical constraints)
  - How to optimize models with sparse data (prior information)

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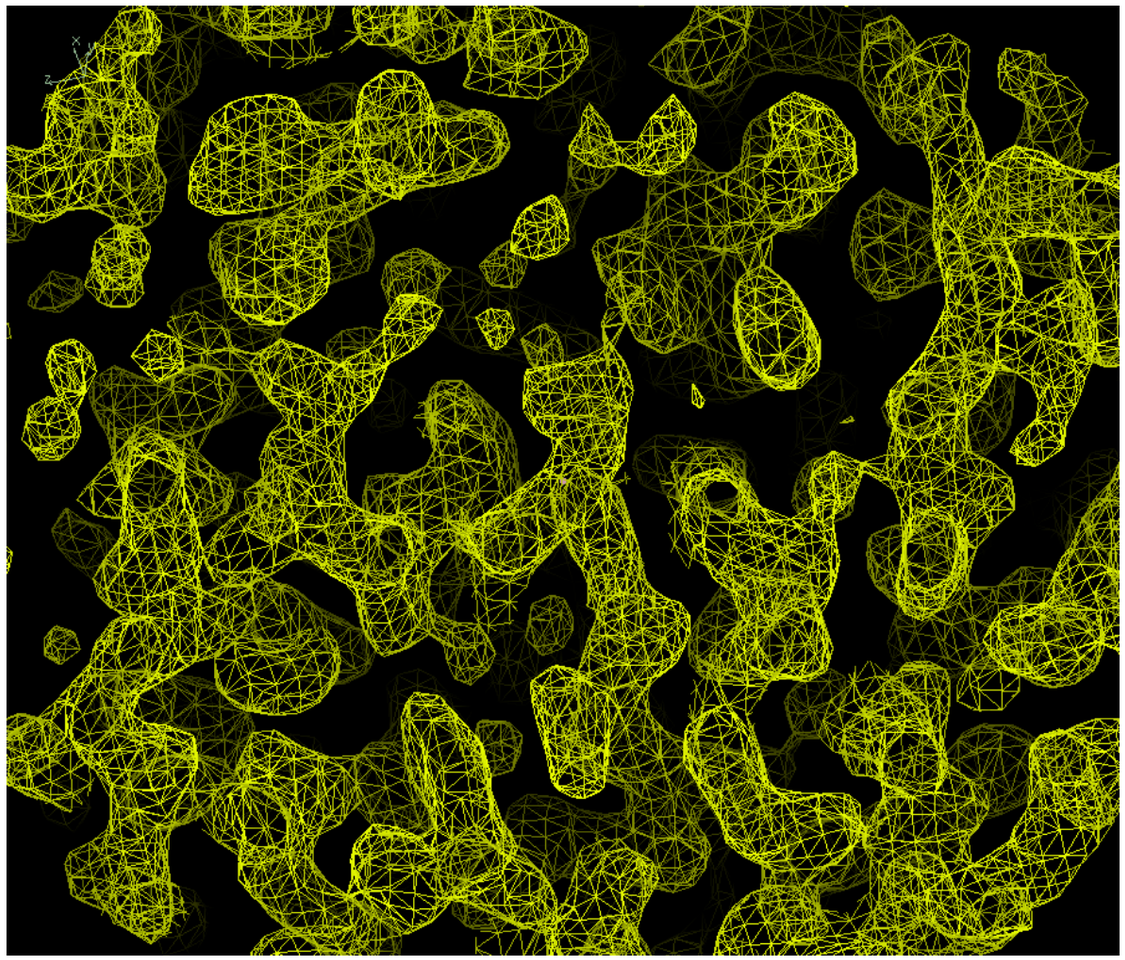
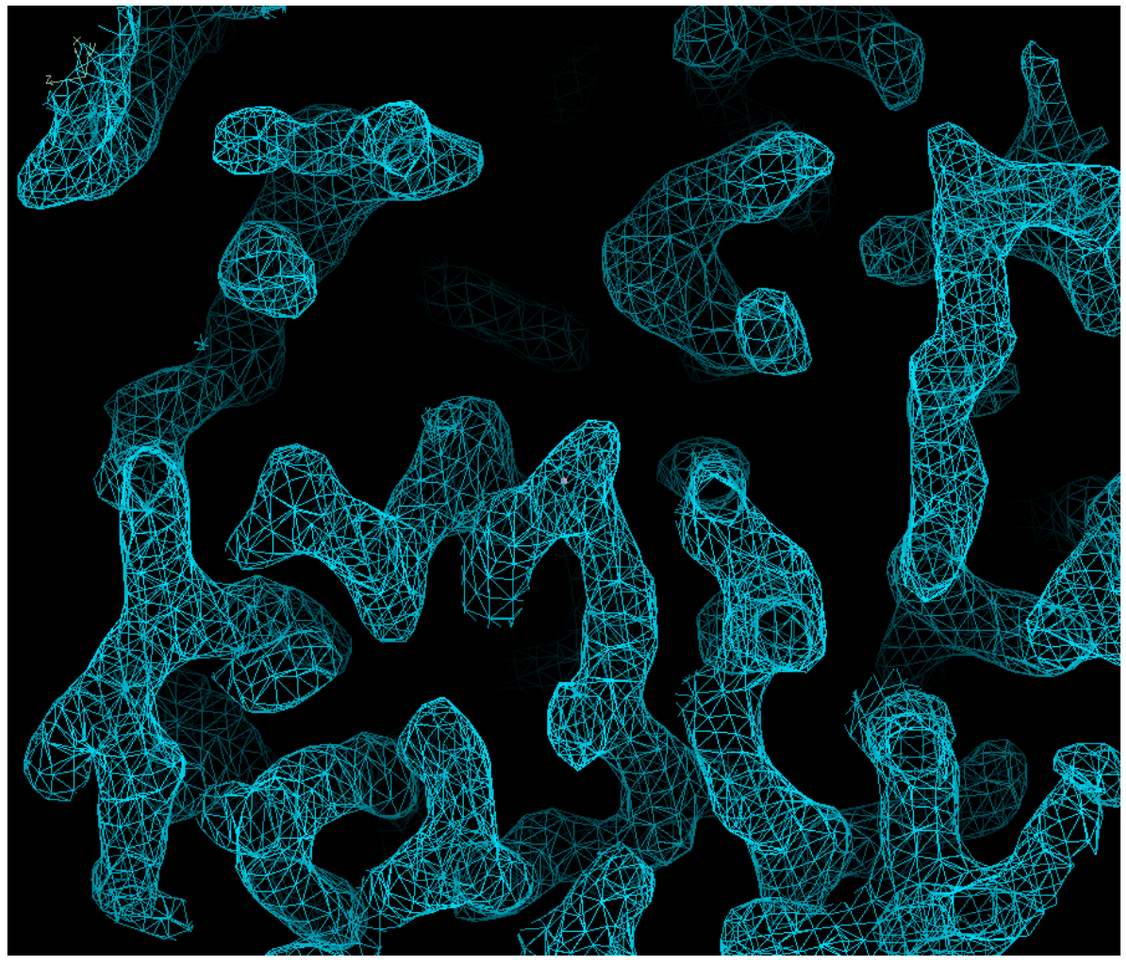
Crystallographic vs. Cryo-EM Maps

Beta galactosidase at 2.2 Å





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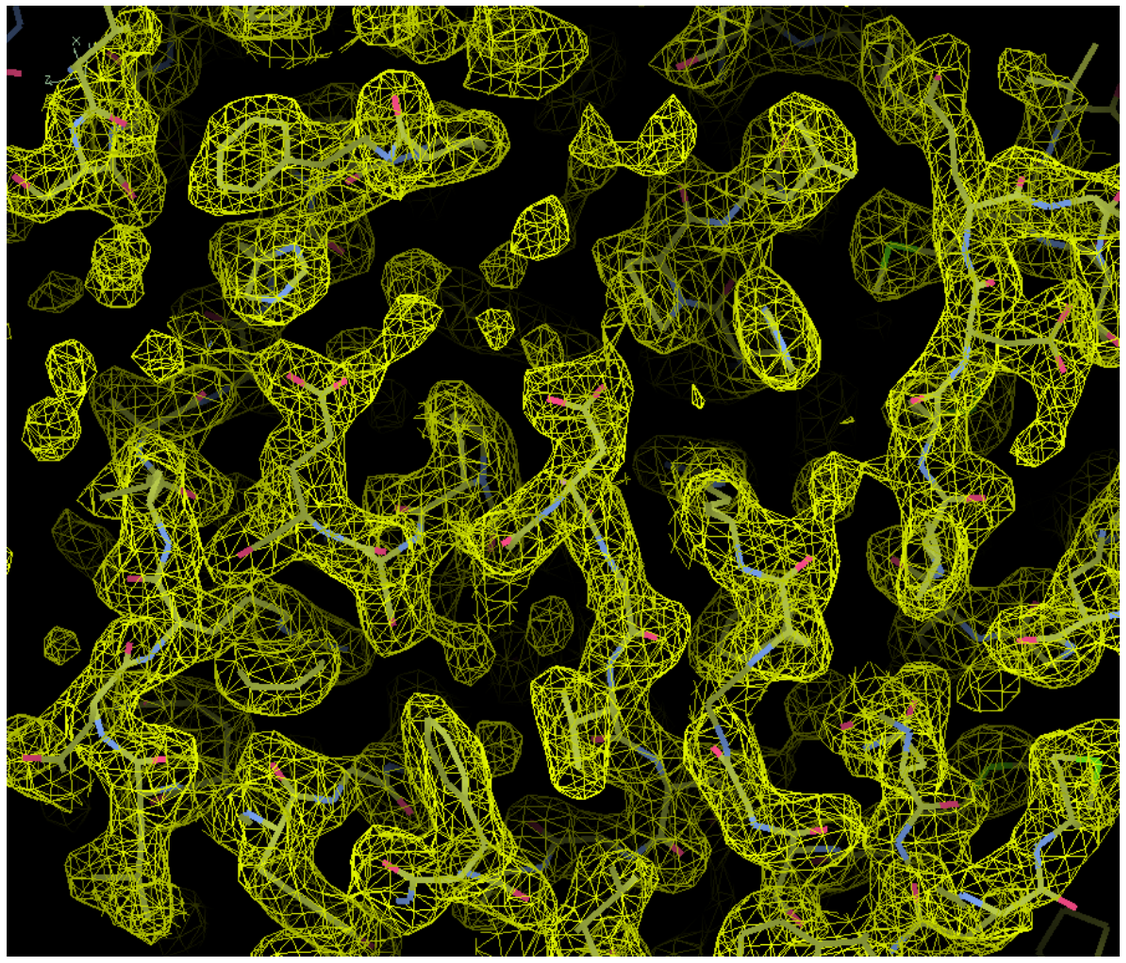
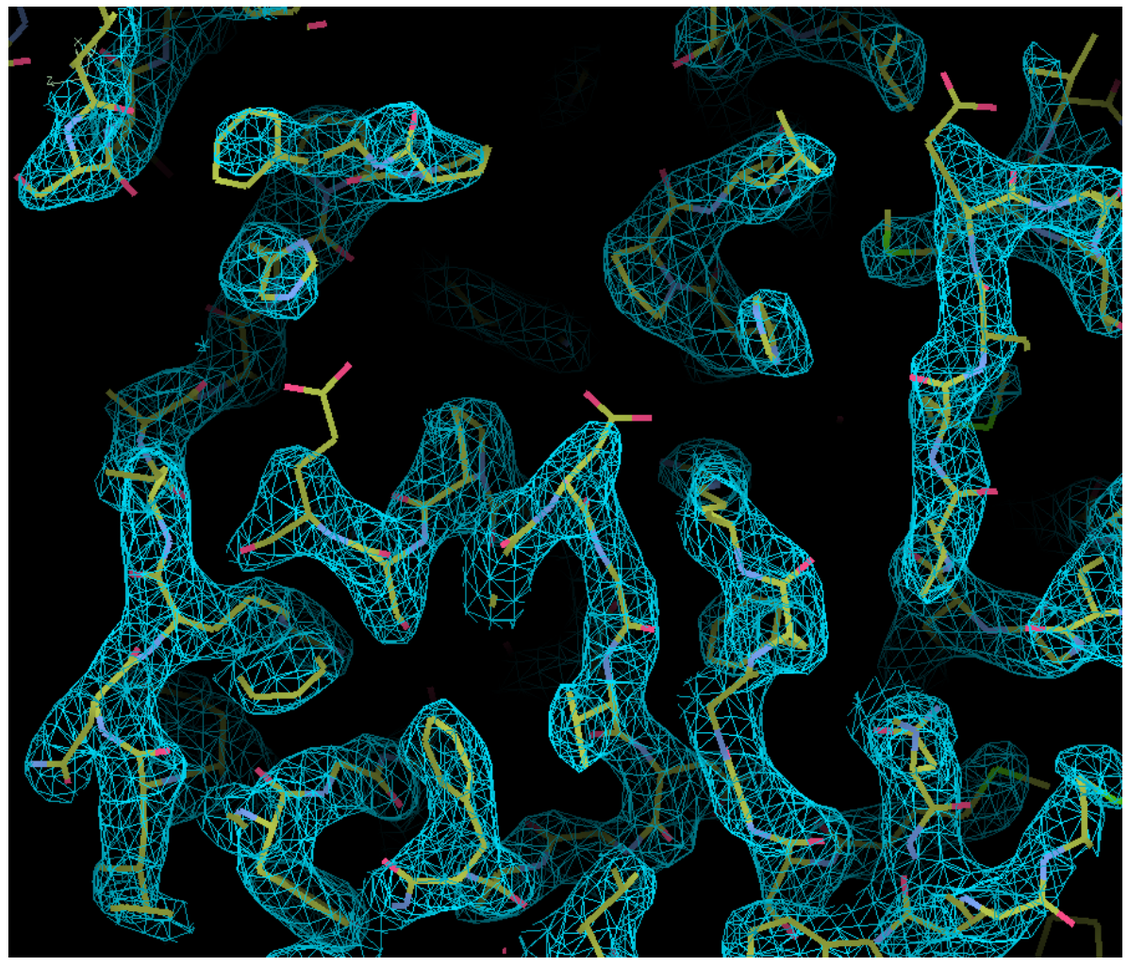
Tom Terwilliger, Los Alamos National Lab

Crystallographic vs. Cryo-EM Maps

Beta galactosidase at 2.2 Å



X-ray (PDB 3i3b)



Cryo-EM (PDB 5a1a)

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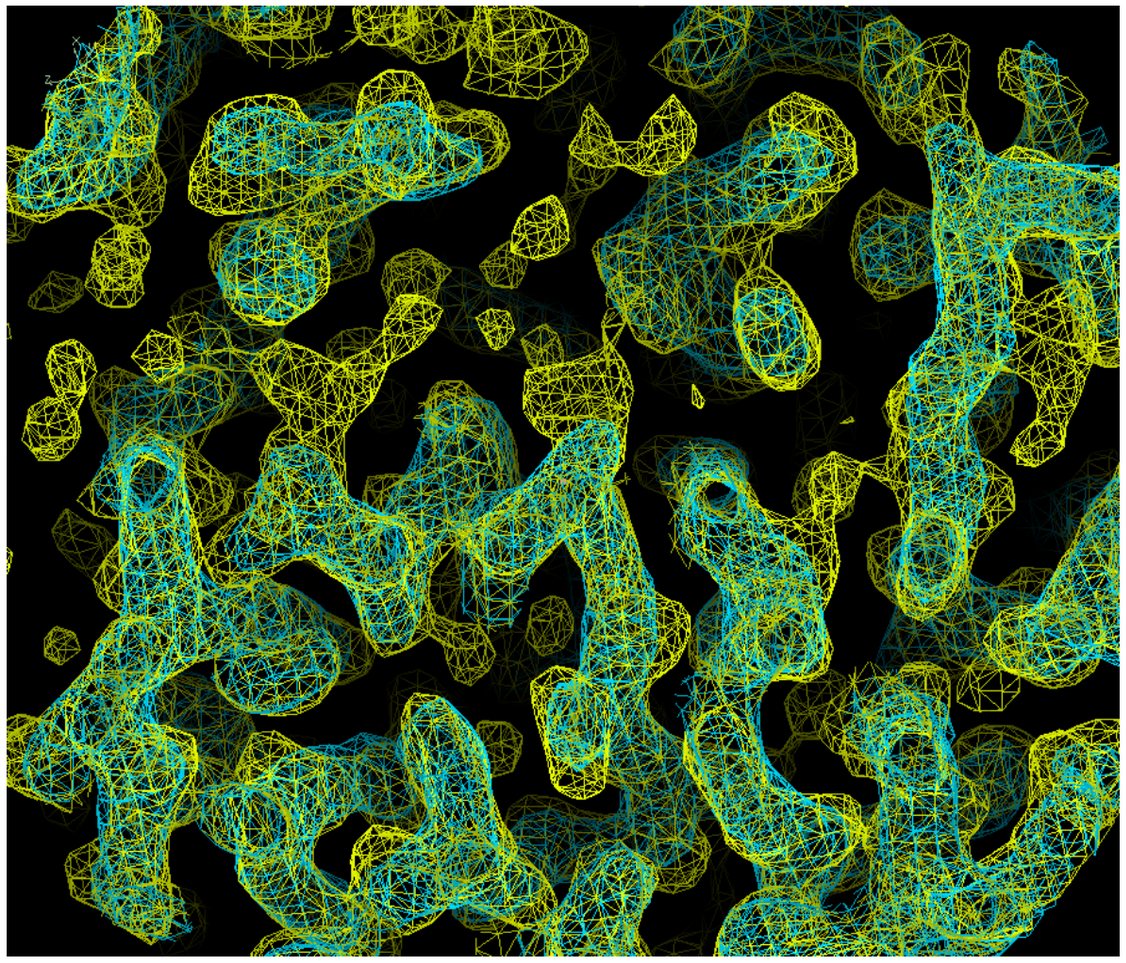
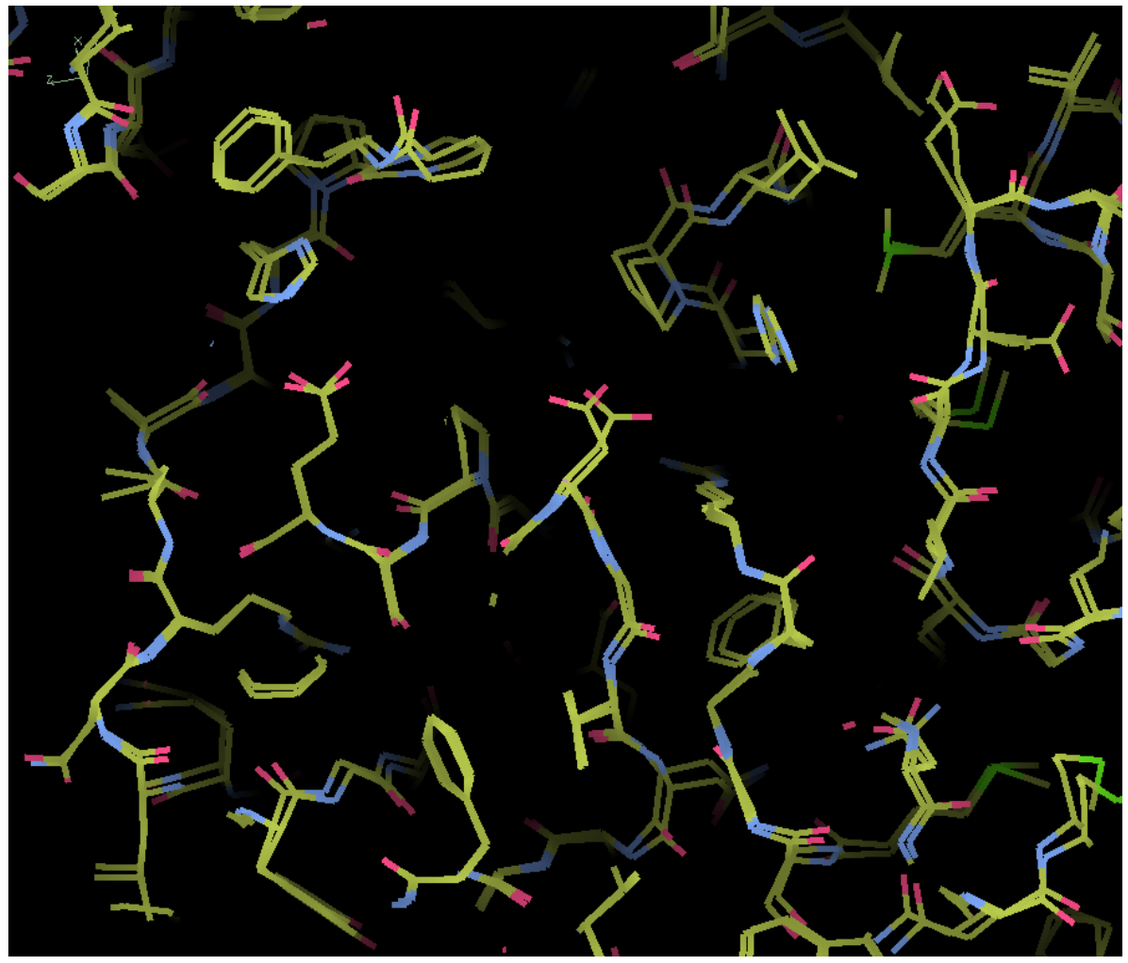
Crystallographic vs. Cryo-EM Maps

- The maps are very similar





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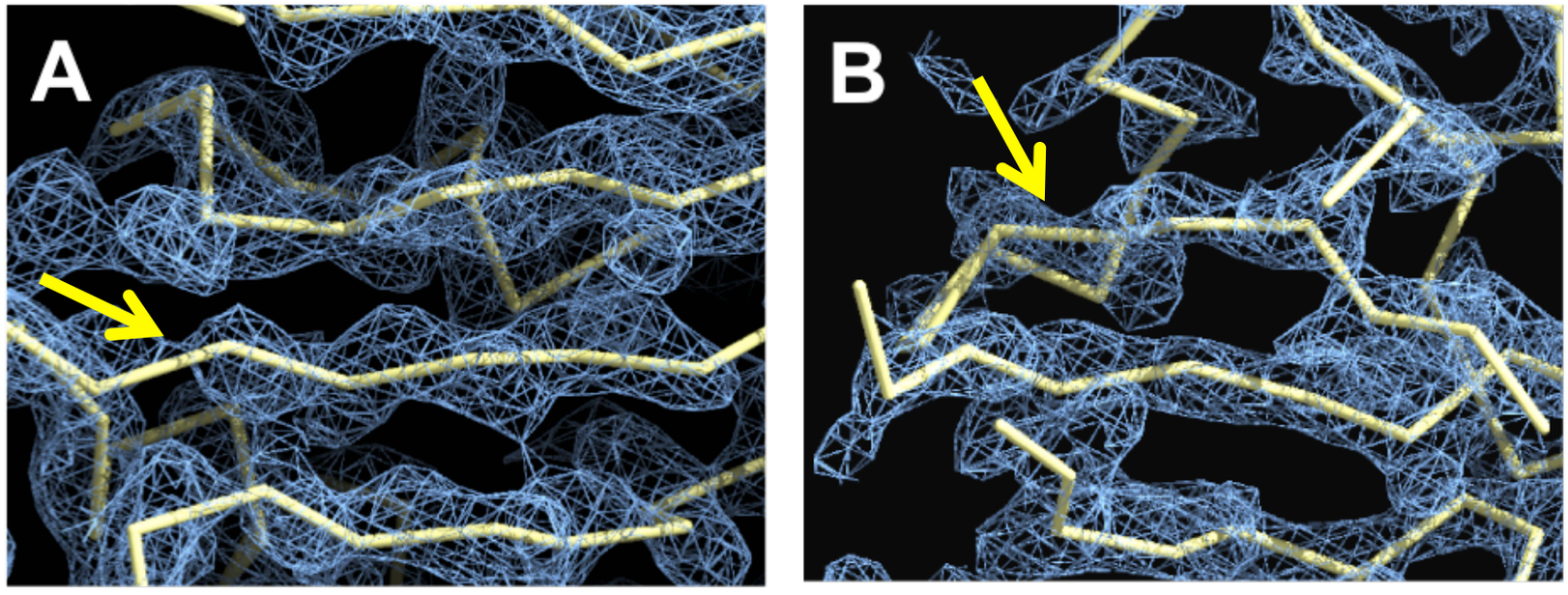
More Accurate Low Resolution Information in Cryo-EM Maps



Original

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Tom Terwilliger, Los Alamos National Lab

The Phenix Project

Lawrence Berkeley Laboratory

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

Los Alamos National Laboratory
New Mexico Consortium
Baylor College of Medicine

Tom Terwilliger, Li-Wei Hung, Matt Baker

Cambridge University

Randy Read, Airlie McCoy, Tristan Croll, Rob Oeffner, Kaushik Hatti, Massimo Sammito, Duncan Stockwell

Duke University

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

An NIH/NIGMS funded Program Project

Adams PD et al., PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Cryst.* 2010, **D66**:213-221.

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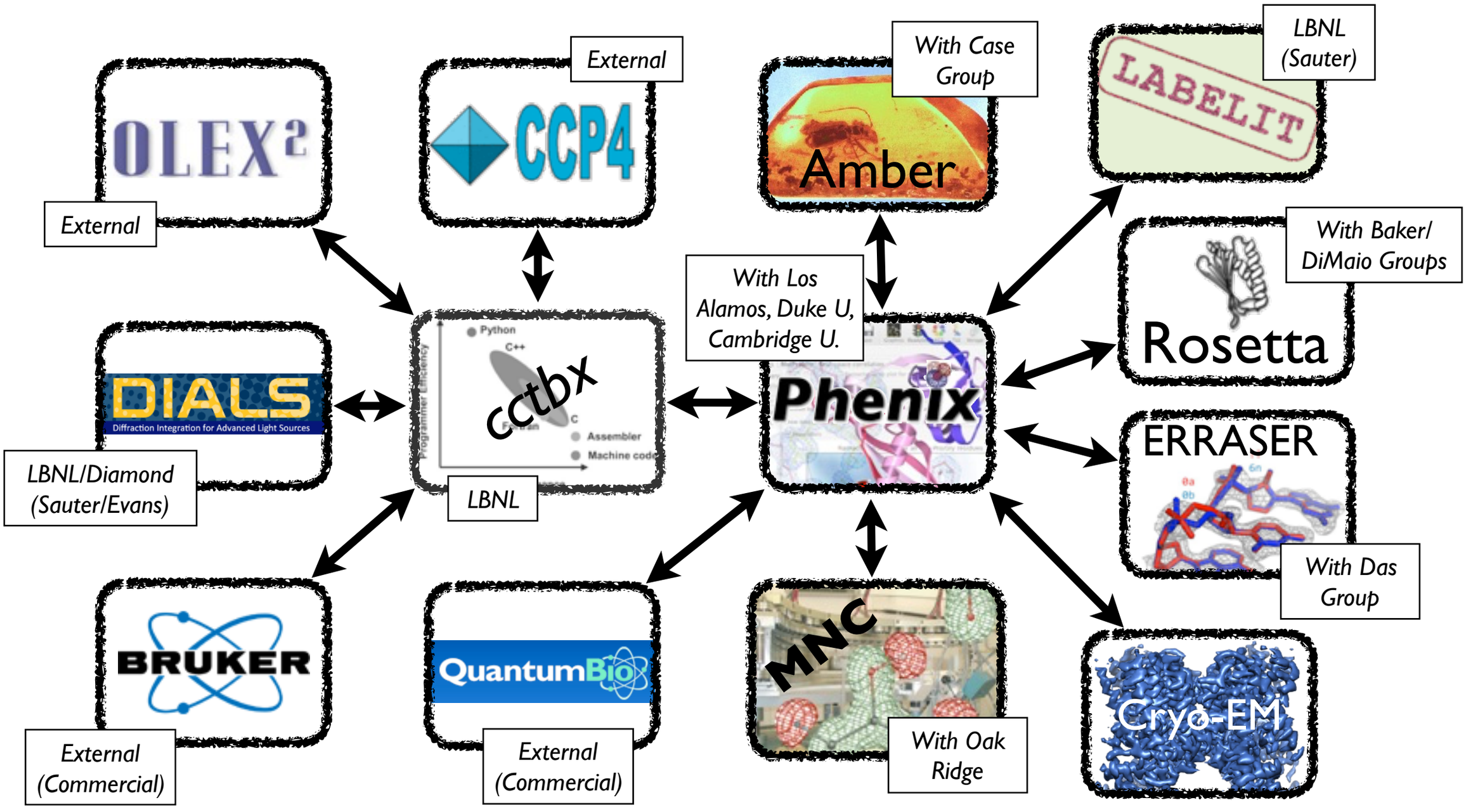
Phenix - a Structural Biology Hub



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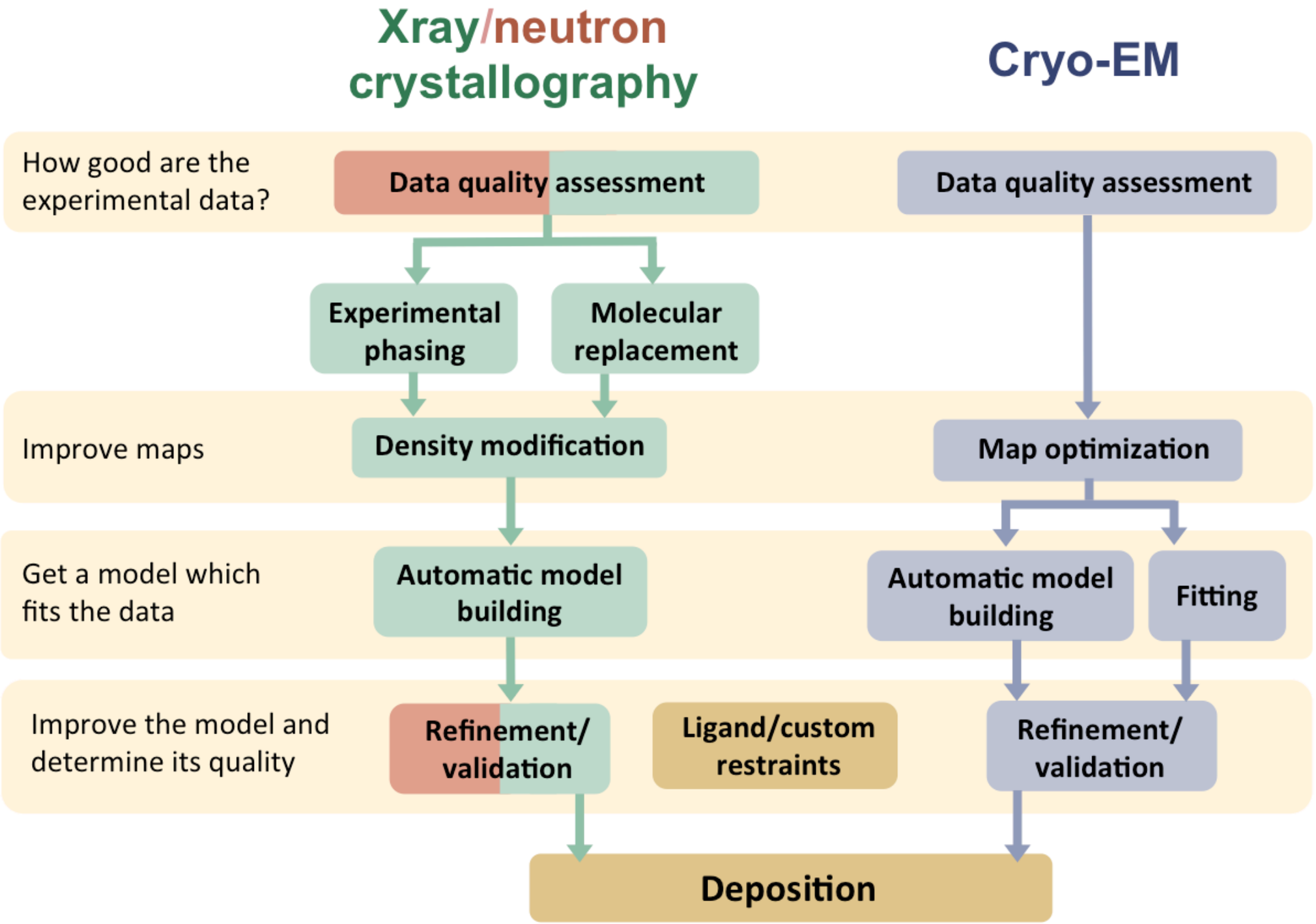
Structural Biology Workflows



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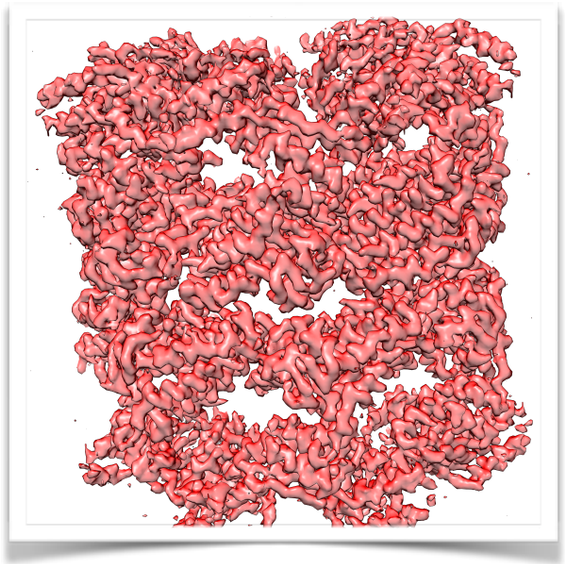
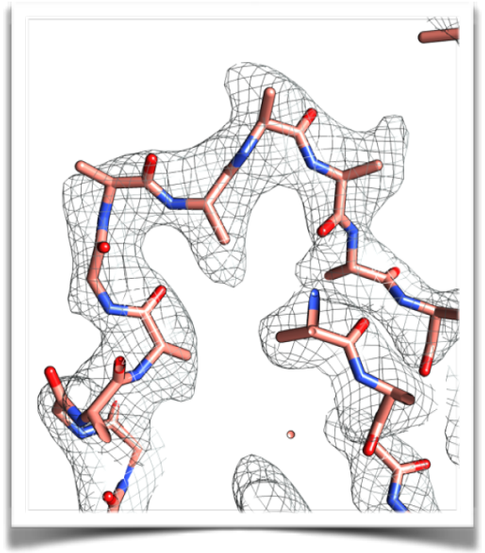
Dorothee Liebschner,
Lawrence Berkeley Lab

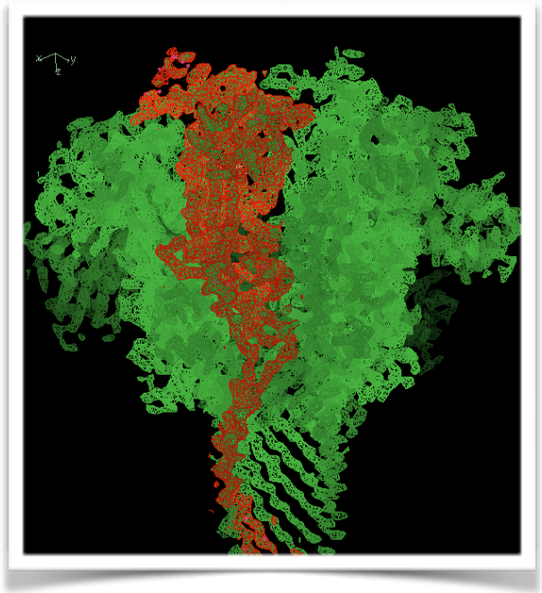
New Tools for Cryo-EM in Phenix



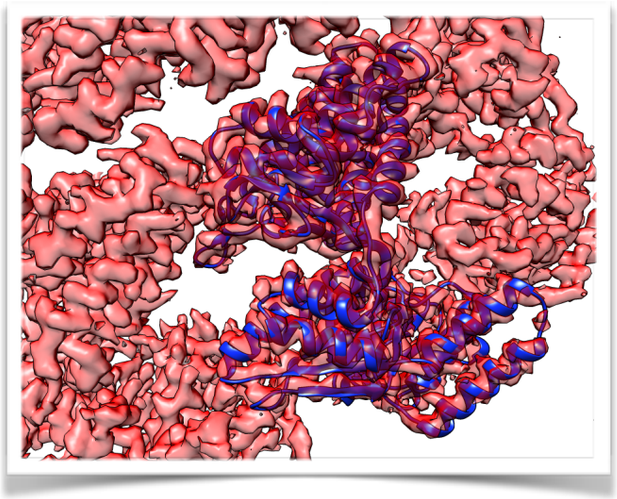
Symmetry from a map



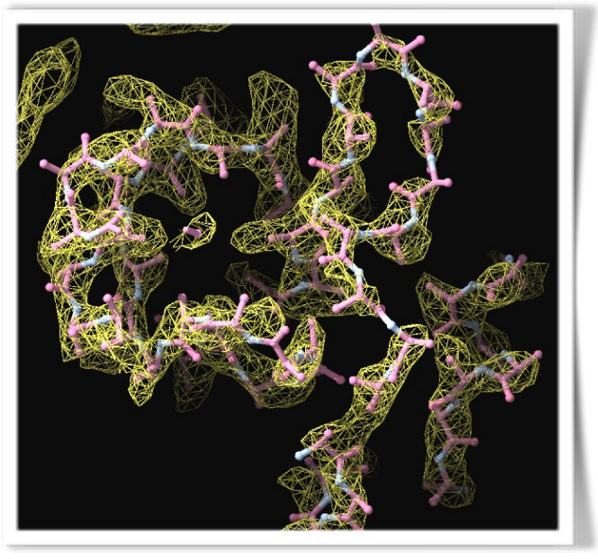
Automated map sharpening



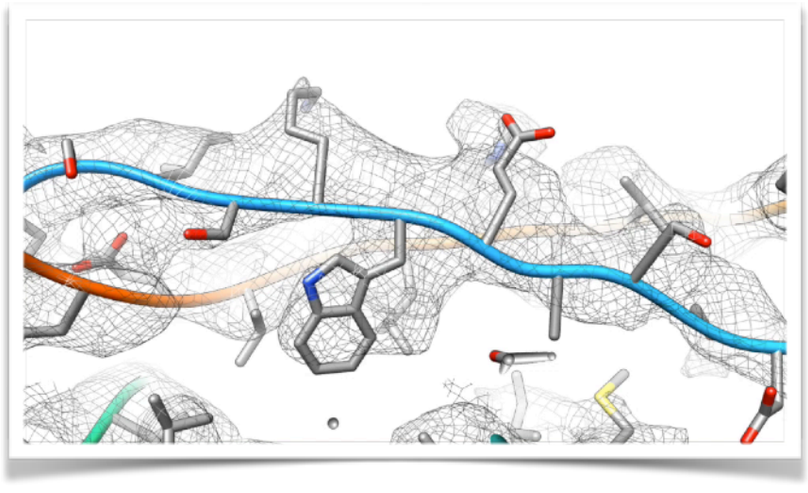
Map segmentation



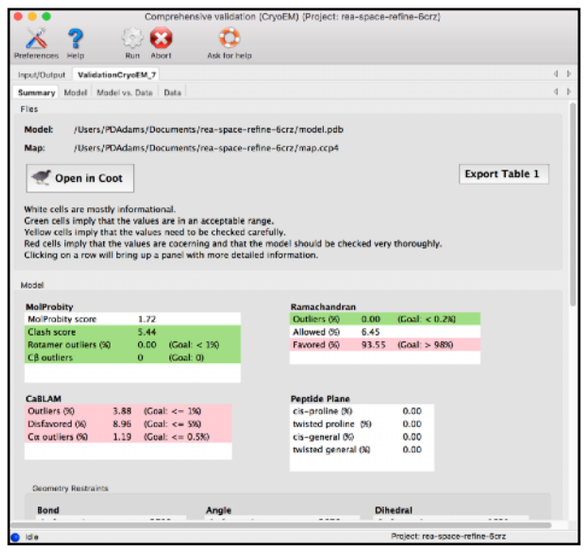
Rigid model docking



Automated model building



Real space refinement



Model and map validation

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Tutorials

- Model placement and building
  - Symmetry determination
  - Rigid body model fitting
  - Map sharpening
  - Map segmentation
  - Automated model building
  - [Focused map/model combination]
- Atomic model optimization and validation
  - Structure refinement
  - Validation

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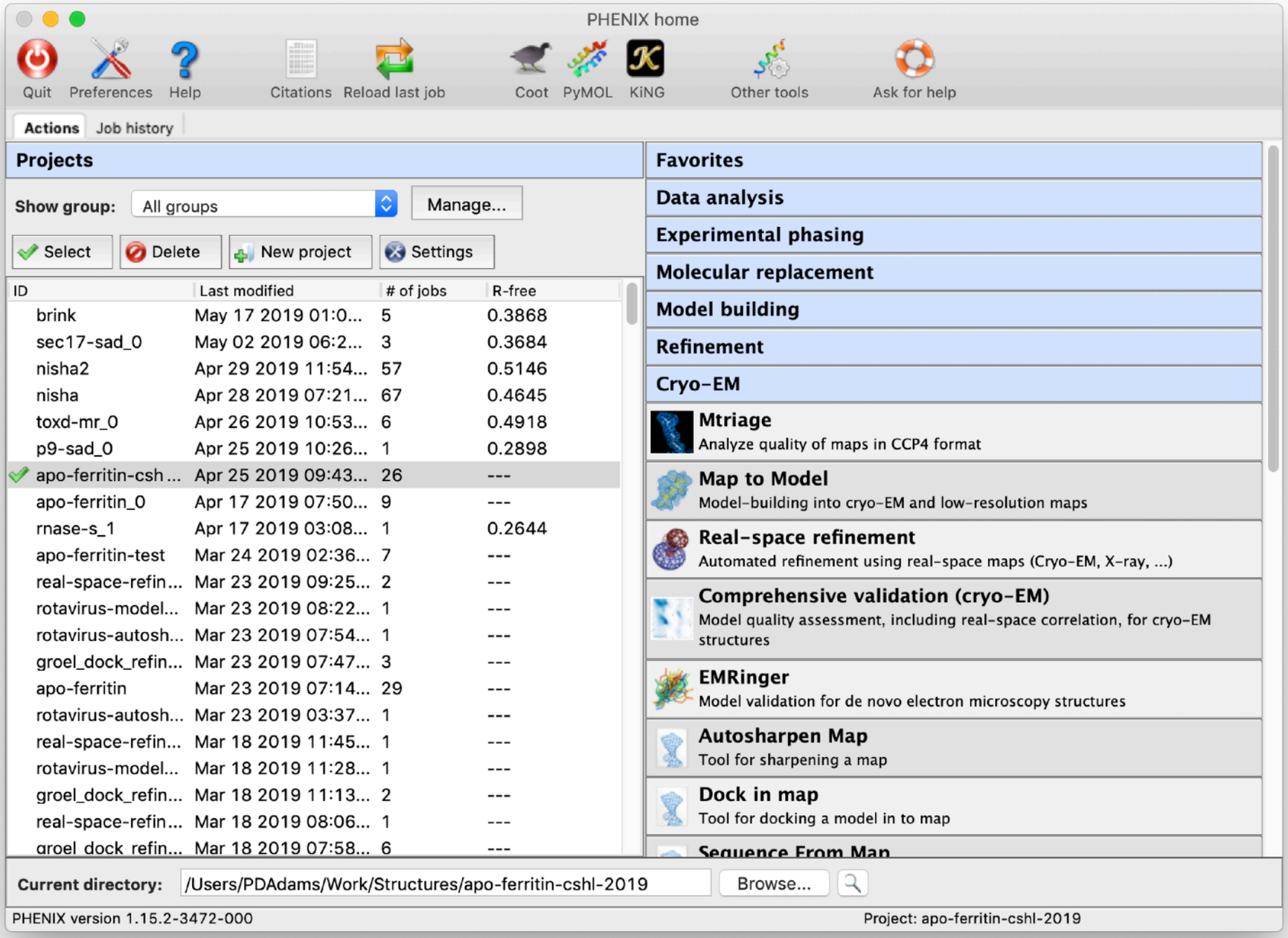
Tutorial Format

- Use graphical user interface



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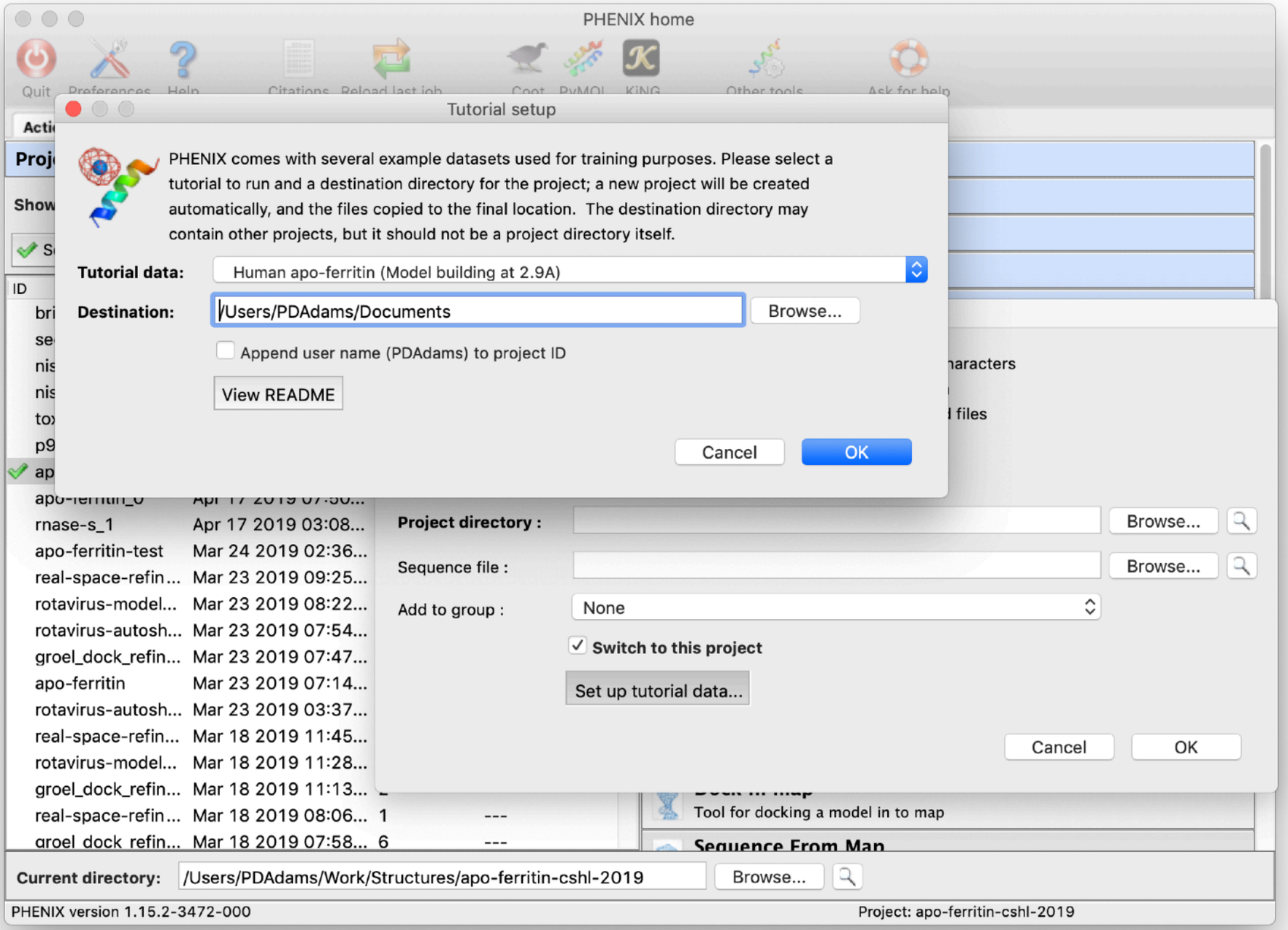
Tutorial Format

- Use tutorial datasets distributed with Phenix
- Should run on most laptops (2GB RAM, multiple CPUs better)



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Challenges

- Automated model building
  - What is the magnification of the map? (can be 5% uncertainty)
  - What is the optimal sharpening of the map?
  - What is the region containing the molecule?
  - Low and variable resolution across maps
- Structure optimization
  - Variable resolution across maps
  - Large molecules
  - Poor initial models
- Validation
  - How to validate a model against moderate resolution maps

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Automated Model Docking

Tom Terwilliger
Los Alamos National Laboratory
Pavel Afonine, Oleg Sobolev
Lawrence Berkeley National Laboratory

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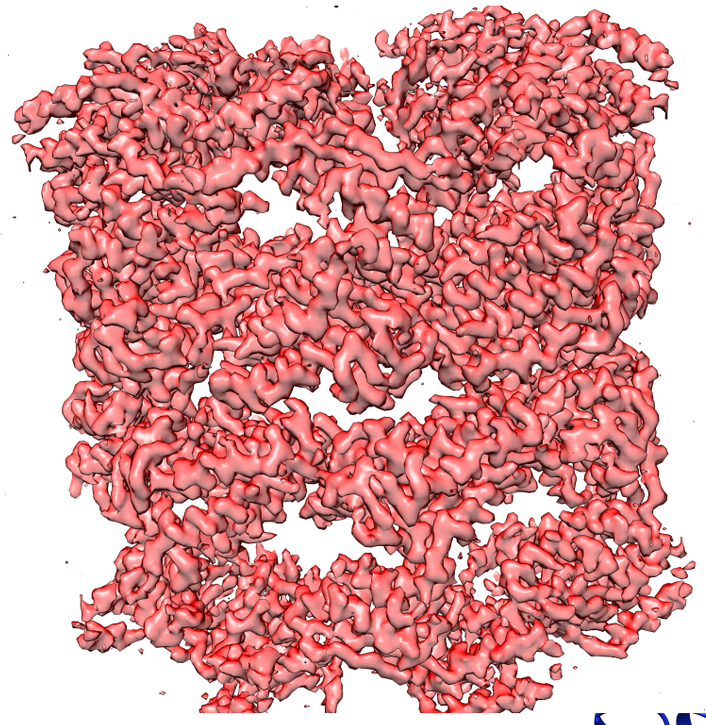
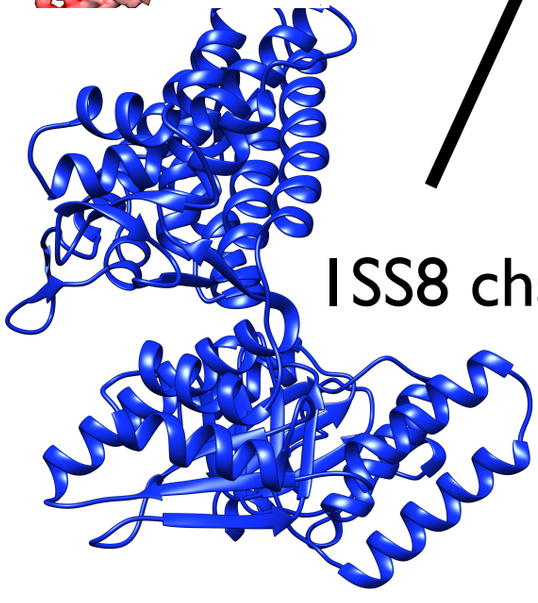
Automated Model Docking

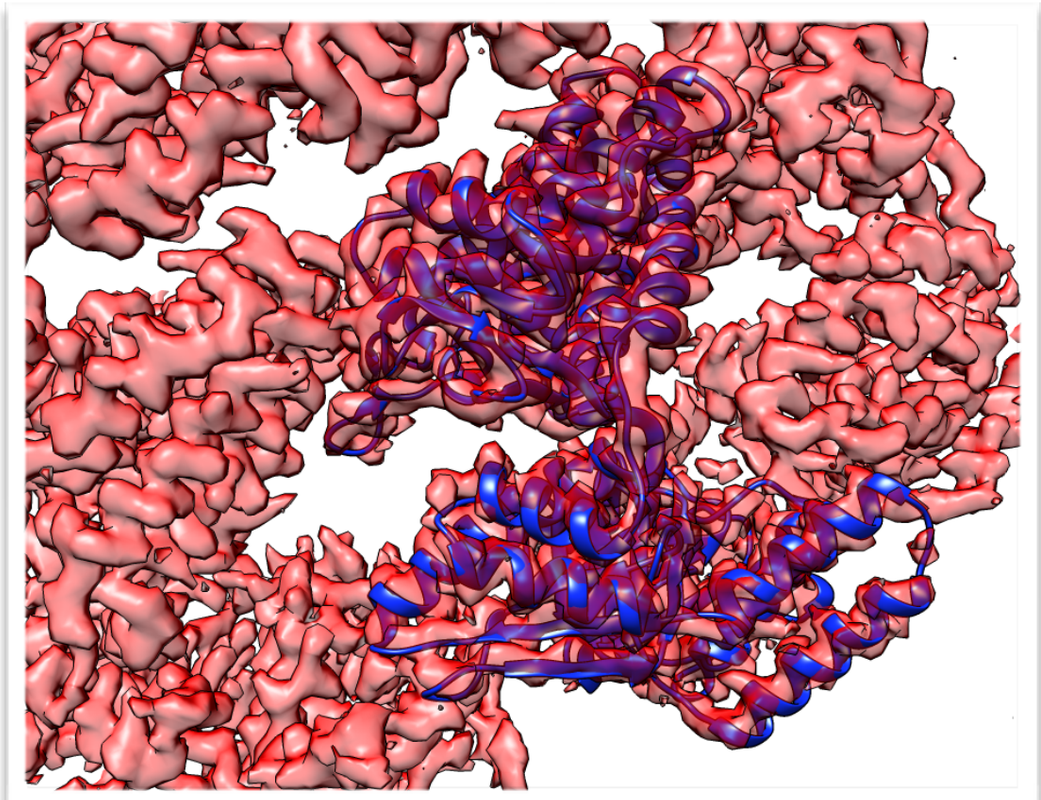
- Systematic cross correlation search of rotations and translations
- Performed in reciprocal space using FFT (very fast)
- Rigid body optimization of position



EMD8750



1SS8 chain A



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Automated Model Sharpening, Segmentation and Model Building

Tom Terwilliger
Los Alamos National Laboratory
Pavel Afonine, Oleg Sobolev
Lawrence Berkeley National Laboratory

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Automated Model Building Procedure

Determine optimal sharpening of the map

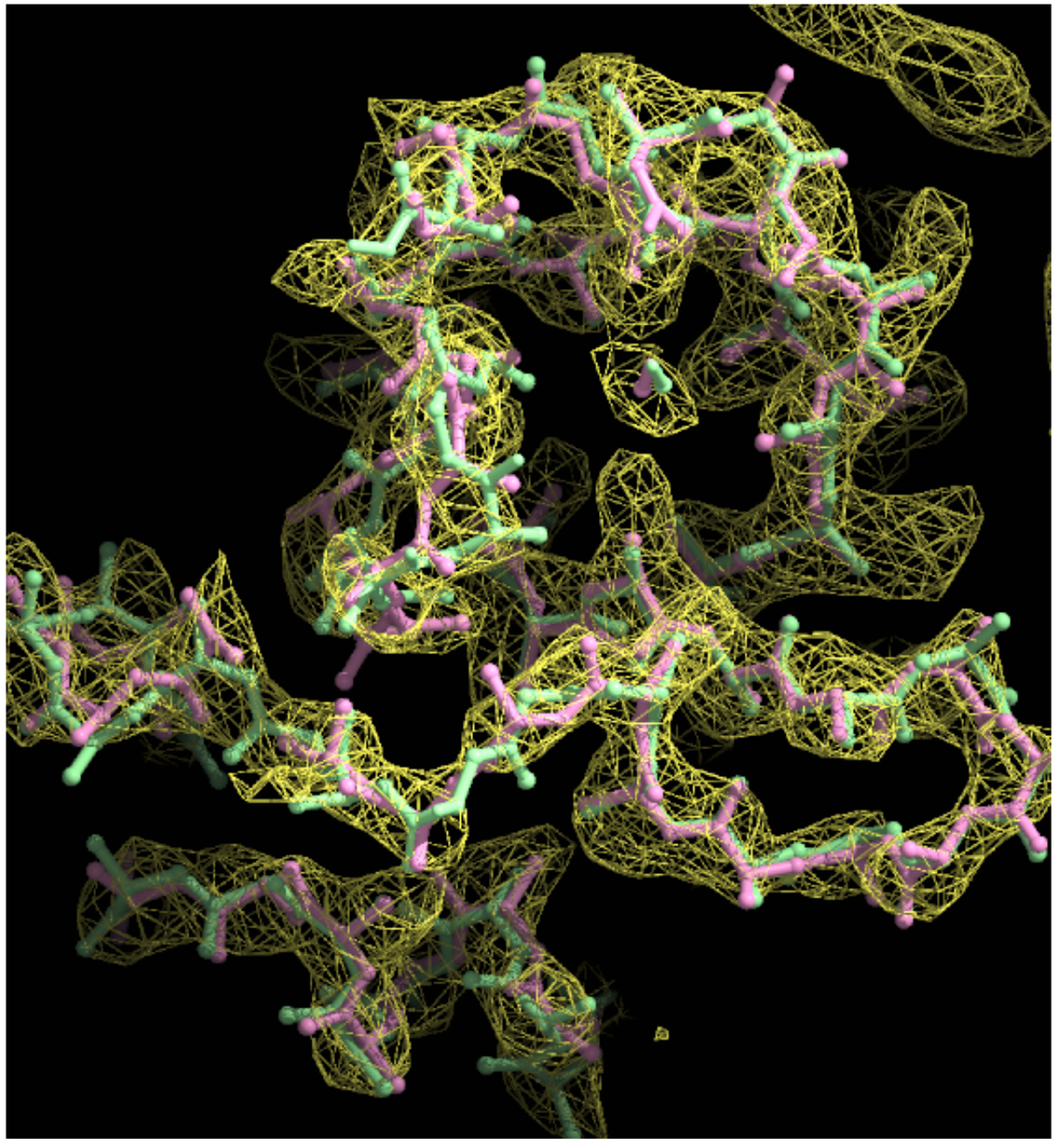
Cut out asymmetric unit of the map

Trace chain and build model

Idealize secondary structure and refine

Assemble and refine (protein/RNA/DNA)

Apply molecular symmetry and re-refine



Cryo-EM map from the yeast mitochondrial ribosome (chain I of large subunit, 3.2Å, Amunts *et al.*, 2014)

Autobuilt model (pink)
Deposited model (green)

Terwilliger et al. A fully automatic method yielding initial models from high-resolution electron cryo-microscopy maps. *Nature Methods*, in press

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Automated Map Sharpening

Create series of maps with variable overall B-values

Analyze maps for detail and connectivity

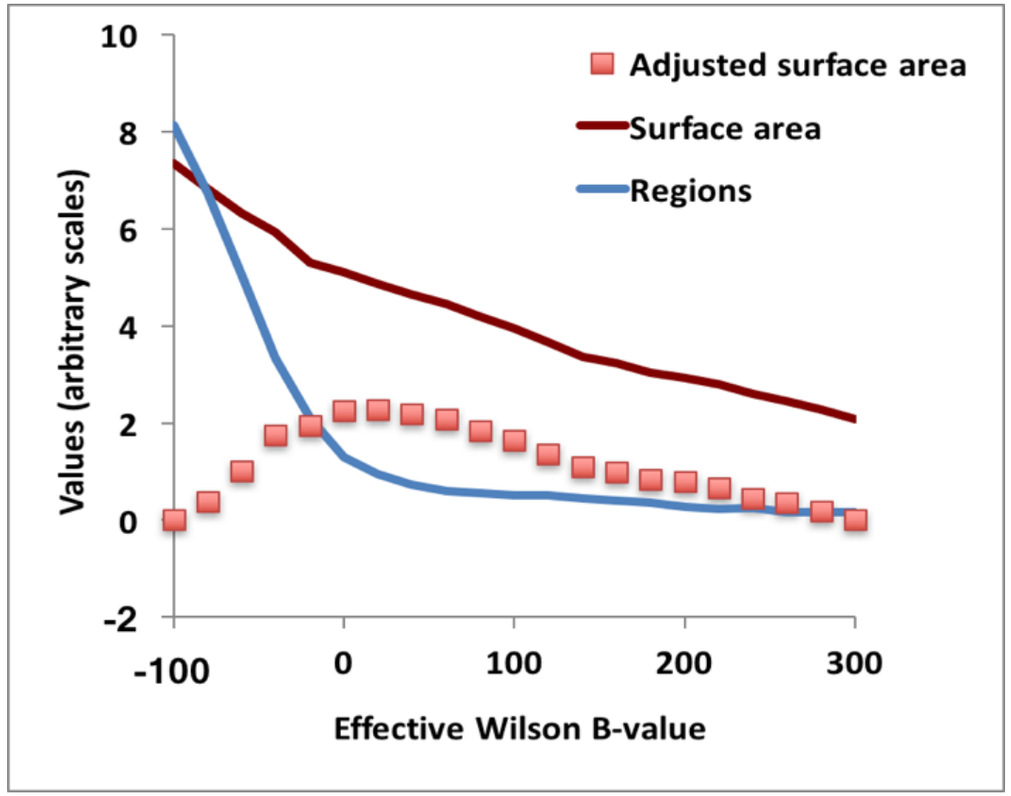
Set contour level enclosing 20% of molecular volume

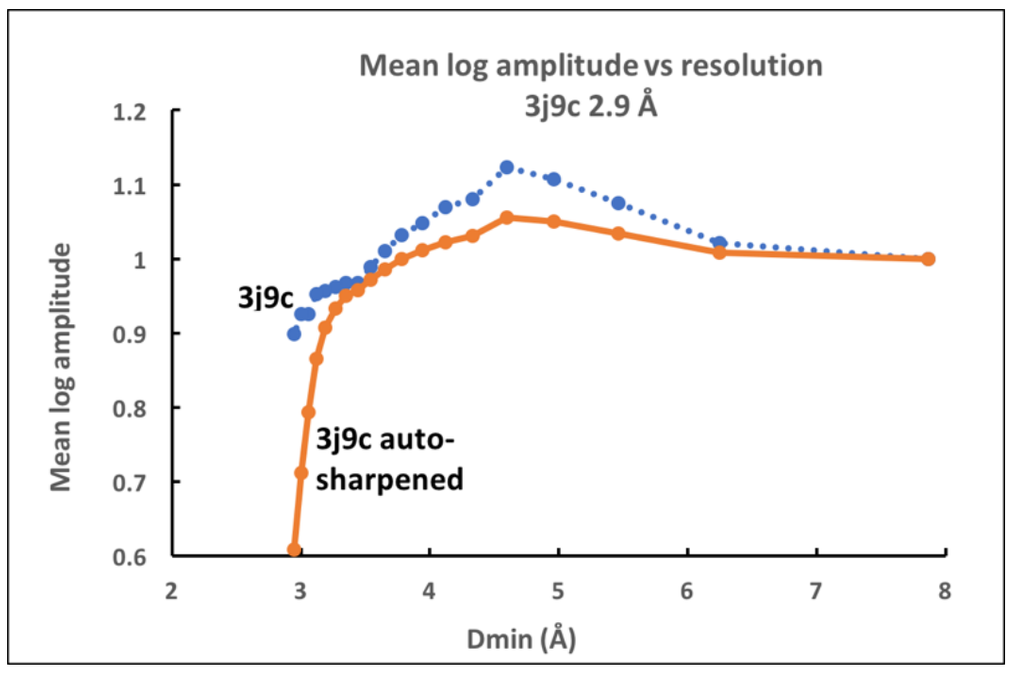
Calculate surface area of contours

Count number of distinct regions enclosed by contours

Choose map with maximum of adjusted surface area

adjusted area = surface area – weight * number of regions





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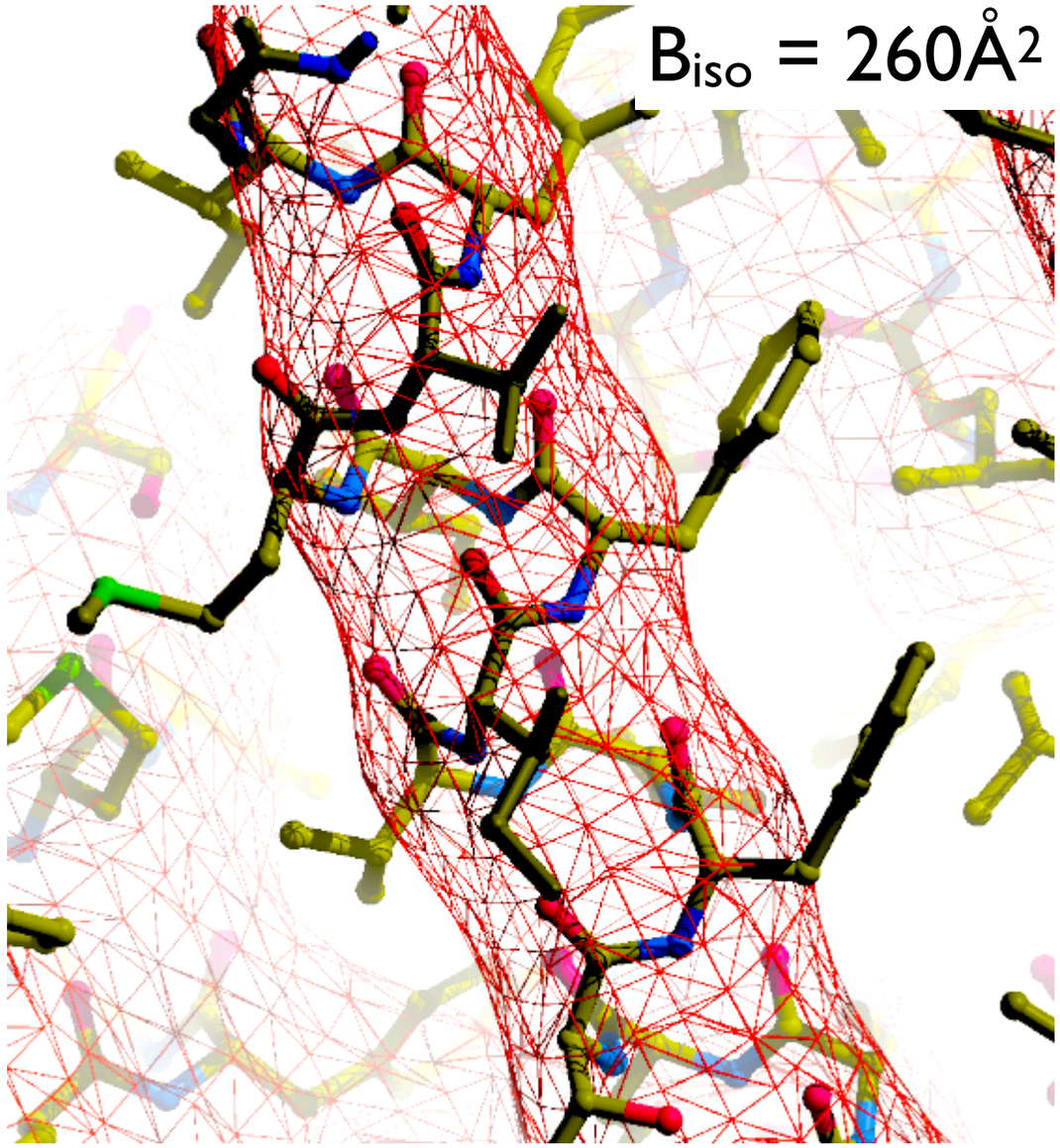
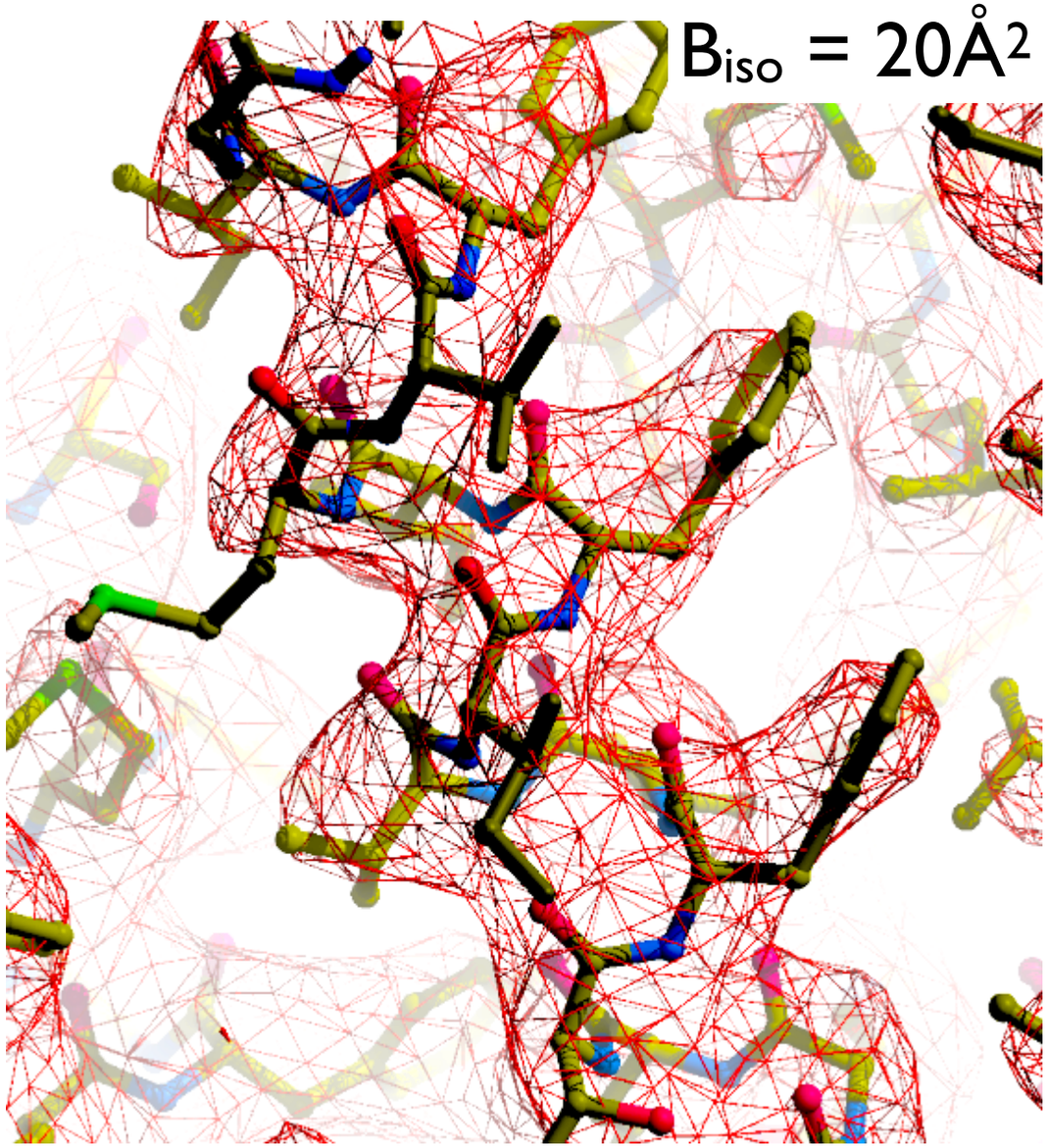
phenix.auto_sharpen

Automated Map Sharpening





Deposited Map *Autosharpened Map*

High-conductance Ca(2+)-activated K(+) channel (emd_8414 and PDB entry 5tji; Hite et al., 2017)

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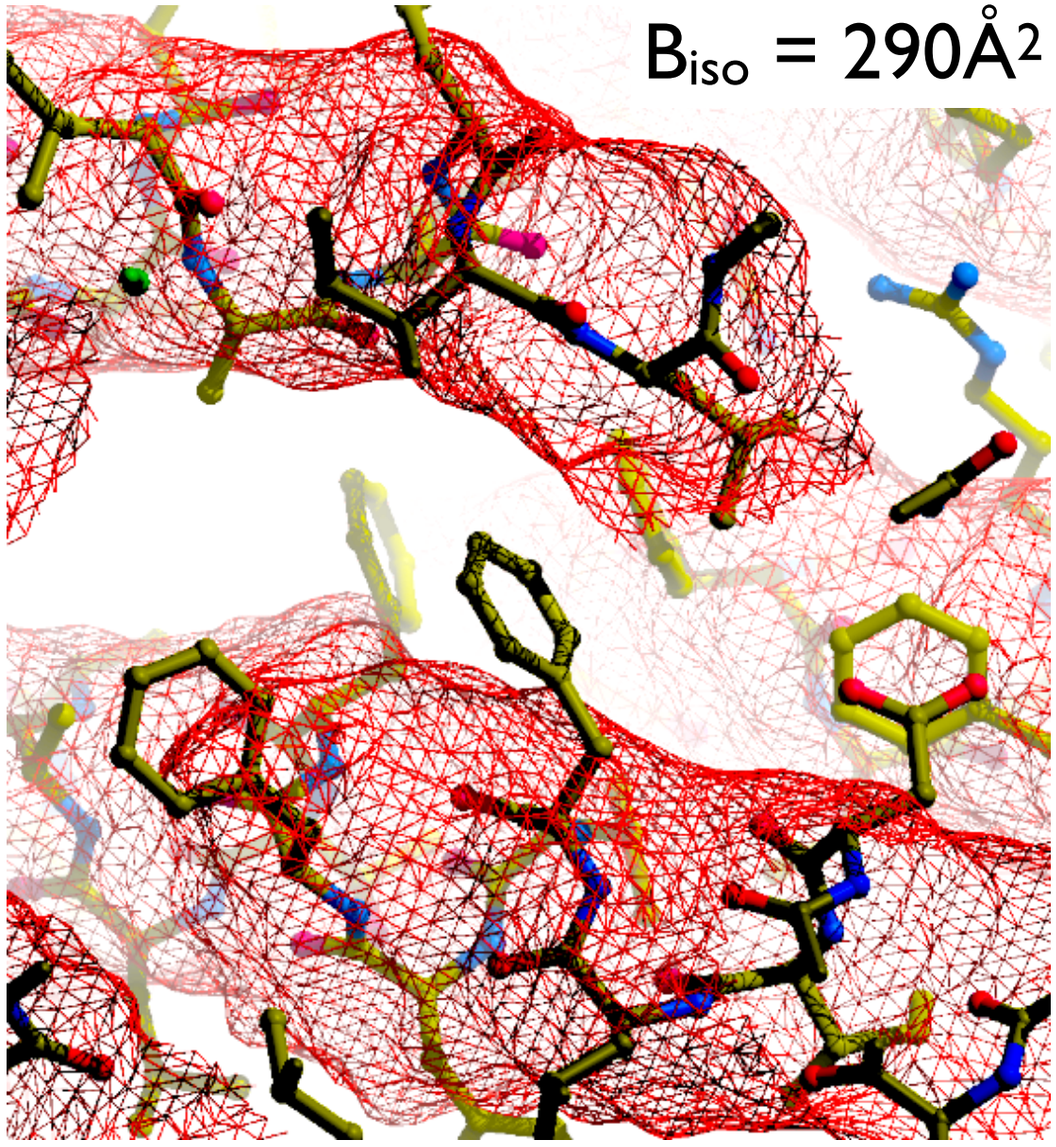
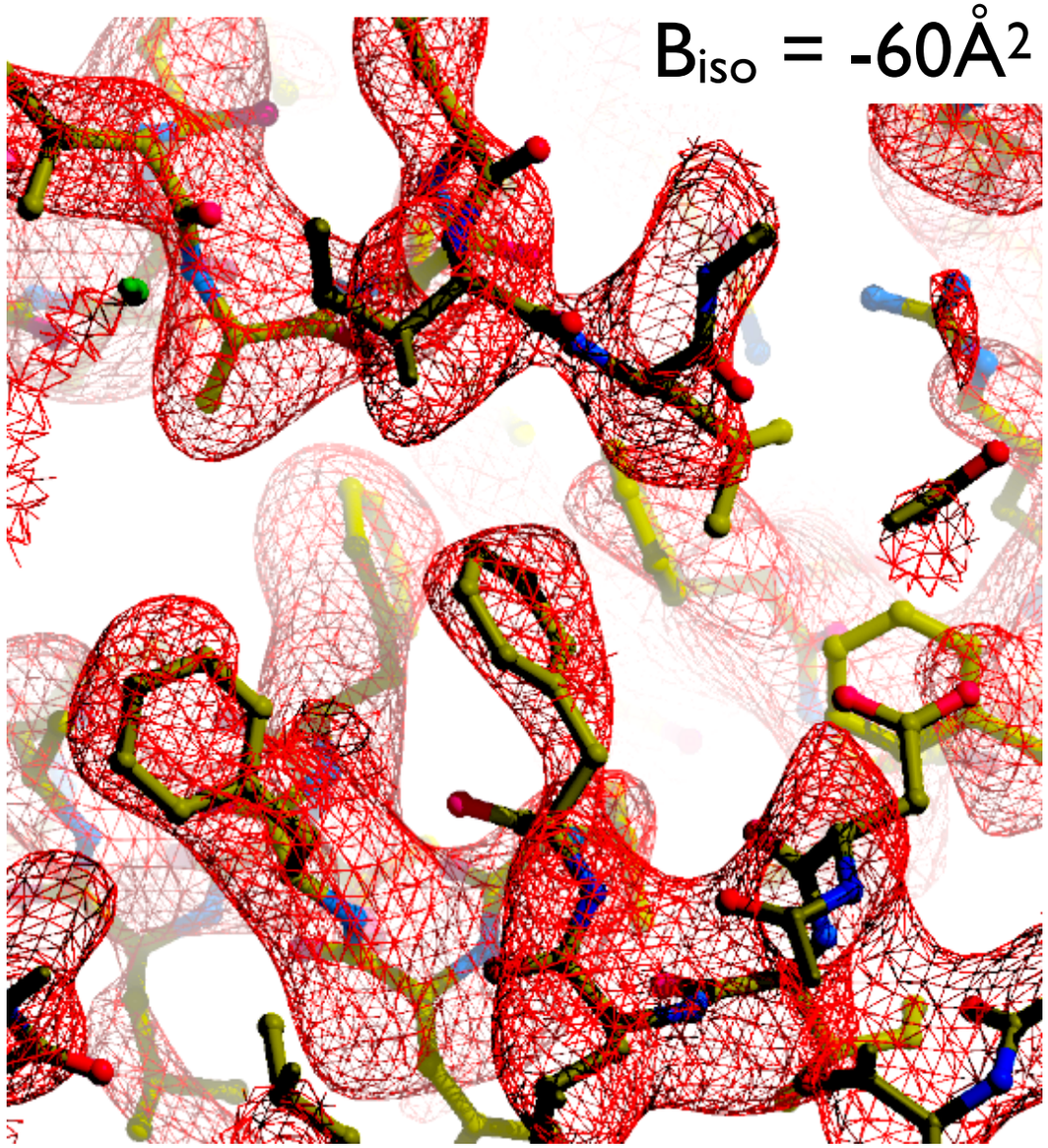
Automated Map Sharpening





Deposited Map *Autosharpened Map*

Cystic fibrosis transmembrane conductance regulator
(emd_8461 and PDB entry 5uar; Zhang and Chen, 2016)

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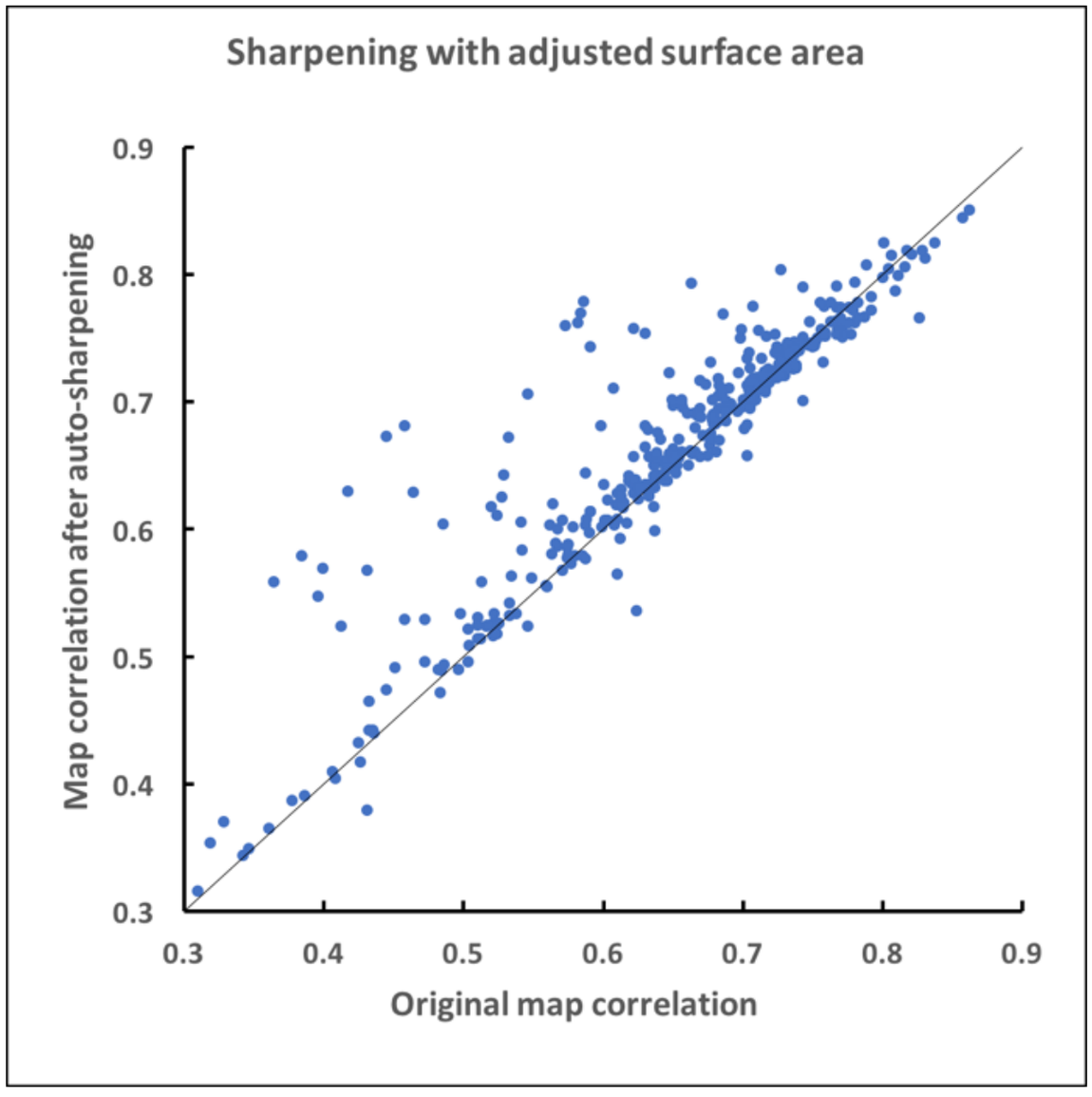
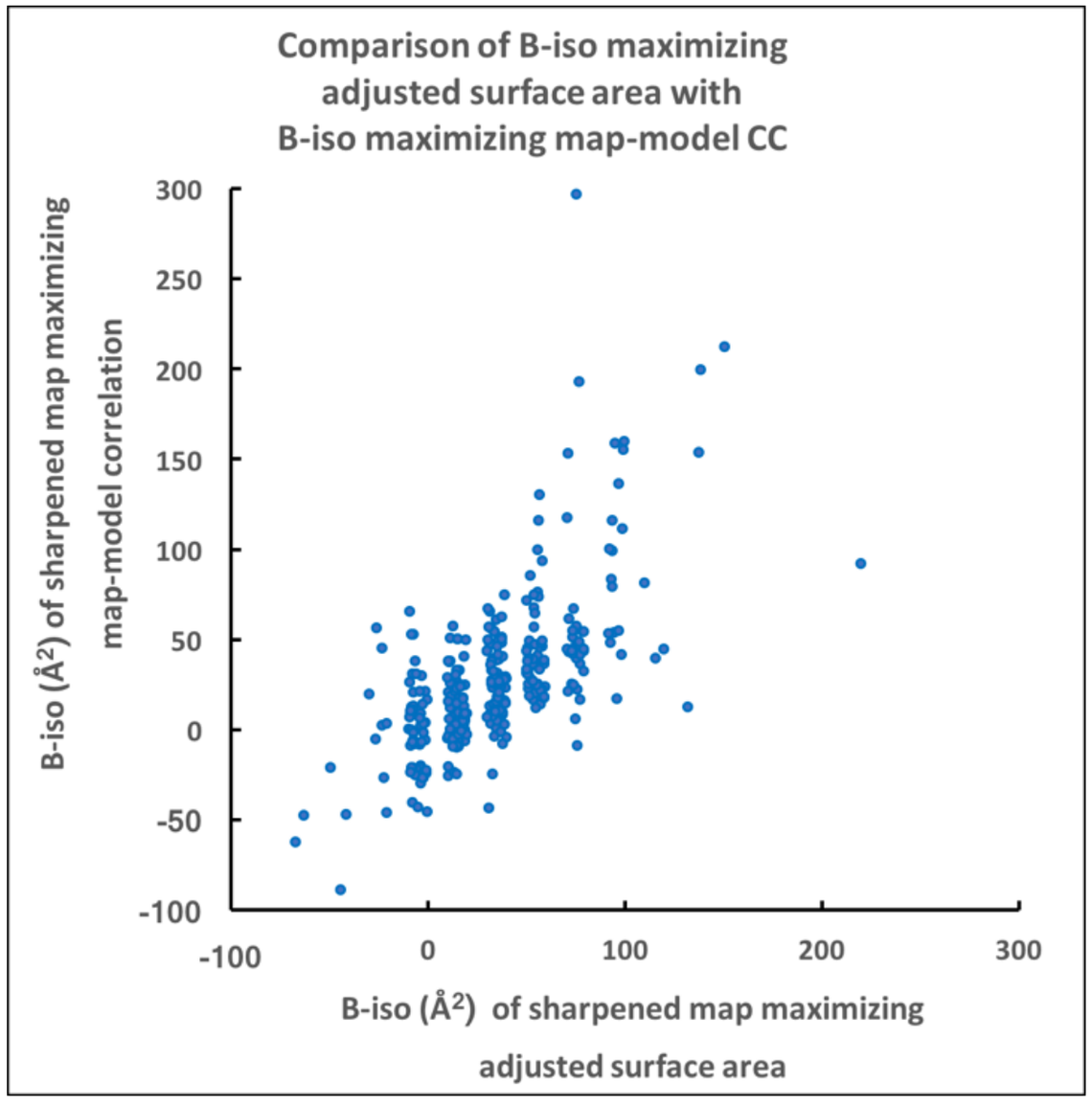
Automated Map Sharpening





Terwilliger et al. Automated map sharpening by maximization of detail and connectivity. *Acta Cryst* 2018, **D74**:545-559

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Automated Segmentation

Determine optimal sharpening of the map

Cut out asymmetric unit of the map

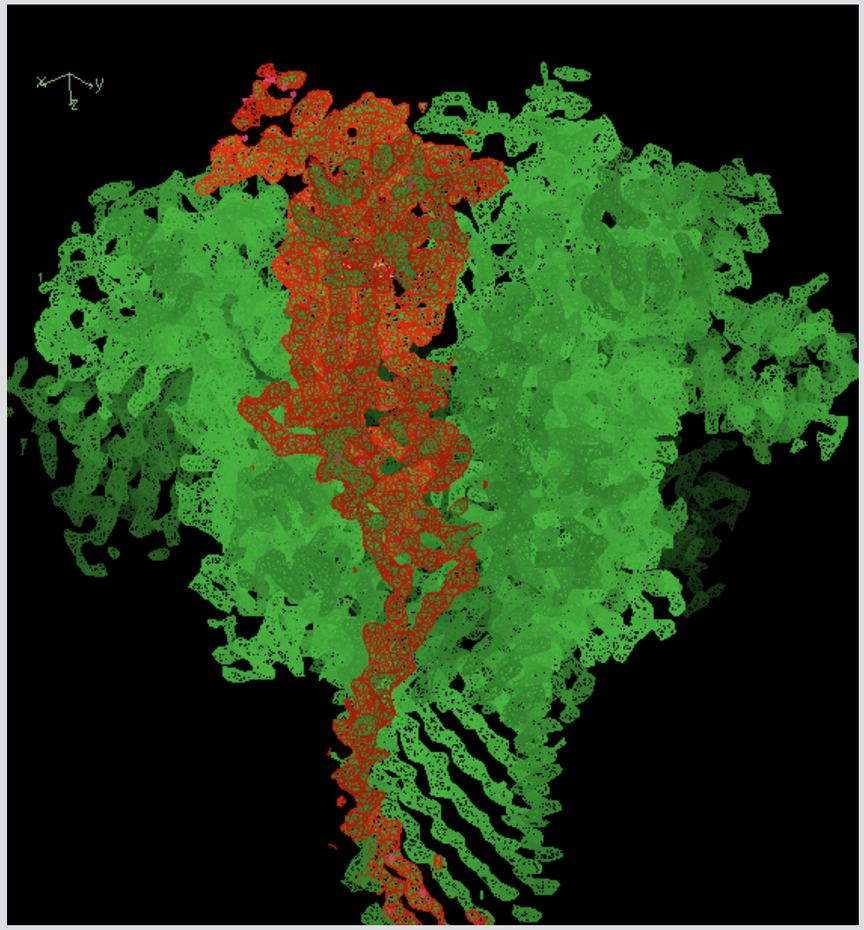
Trace chain and build model

Idealize secondary structure and refine

Assemble and refine (protein/RNA/DNA)

Apply molecular symmetry and re-refine

- Use the symmetry of the map
- Identify contiguous regions representing asymmetric unit of the map
- Choose symmetry-copies that make compact molecule



emd_6224 (anthrax toxin protective antigen pore at 2.9 Å; Jiang *et al.* 2015)

Terwilliger et al. Map segmentation, automated model-building and their application to the Cryo-EM Model Challenge. *J. Struct. Biol.* 2018, in press

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Chain Tracing

Determine optimal sharpening of the map

Cut out asymmetric unit of the map

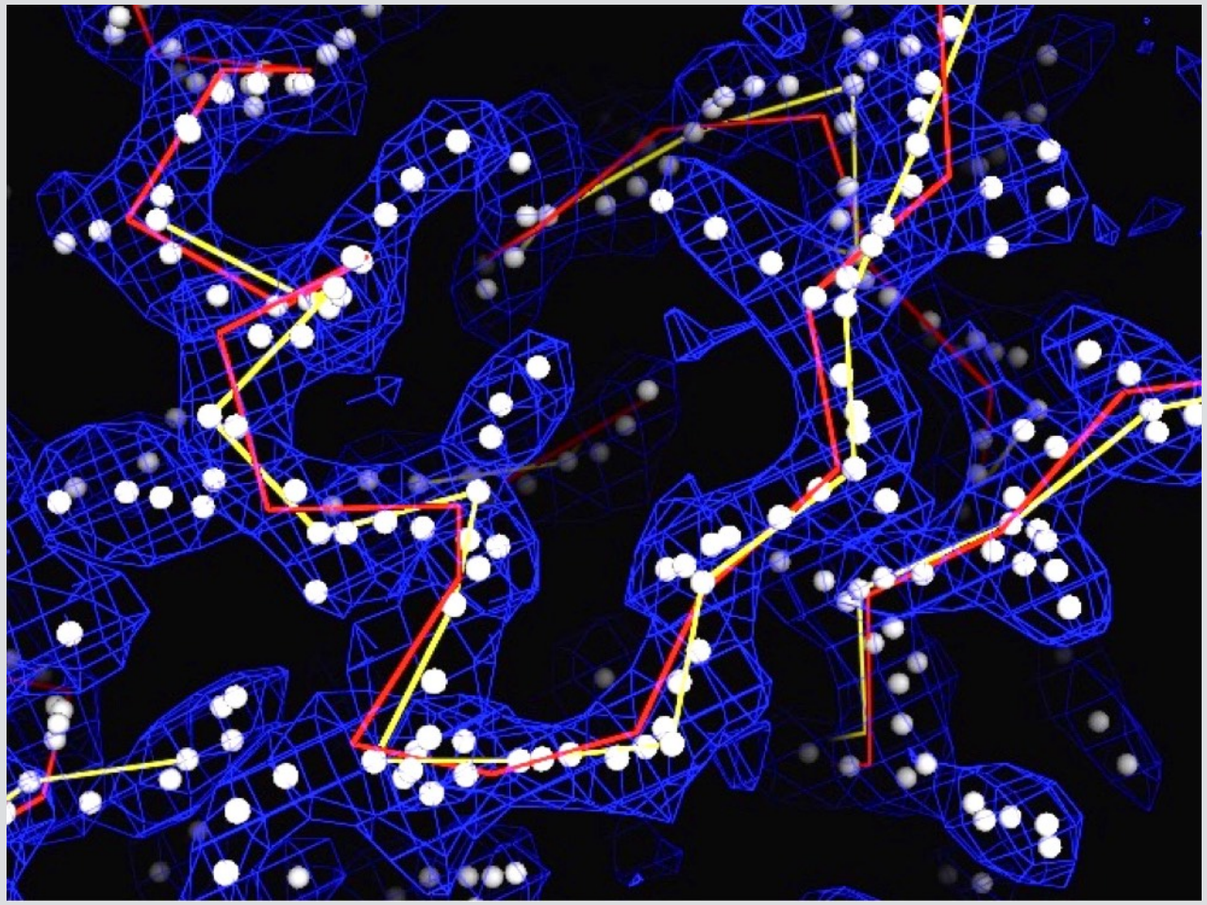
Trace chain and build model

Idealize secondary structure and refine

Assemble and refine (protein/RNA/DNA)

Apply molecular symmetry and re-refine

- Variable map thresholding
- Trace protein main chain
- Identify direction of main chain by fit to density



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Idealization and Refinement

Determine optimal sharpening of the map

Cut out asymmetric unit of the map

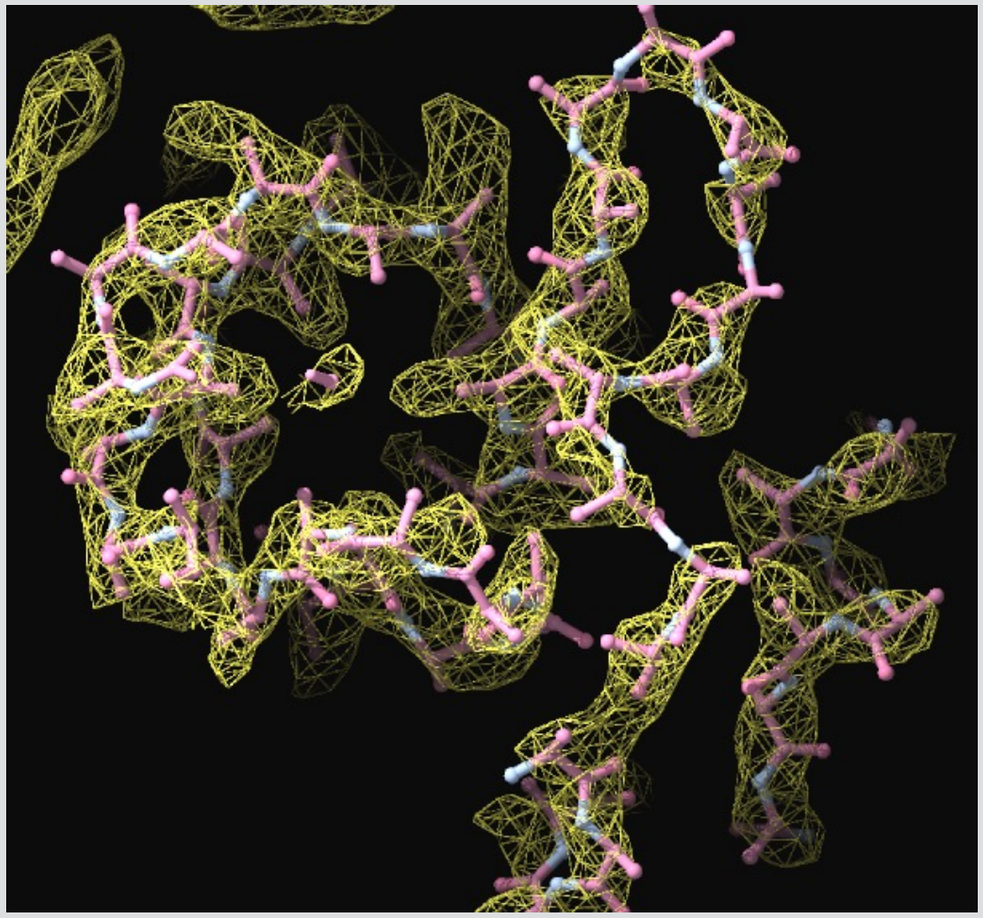
Trace chain and build model

Idealize secondary structure and refine

Assemble and refine (protein/RNA/DNA)

Apply molecular symmetry and re-refine

- Refine and rebuild model (simulated annealing, rebuilding and combination of best parts of each model)
- Replace segments with idealized structure
- Identify hydrogen-bonding (β-sheets, α-helices) and use them as restraints in real-space refinement



Chain I, yeast mitochondrial ribosome large subunit, 3.2 Å, 3j6b

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Assembly and Polymer Recognition

Determine optimal sharpening of the map

Cut out asymmetric unit of the map

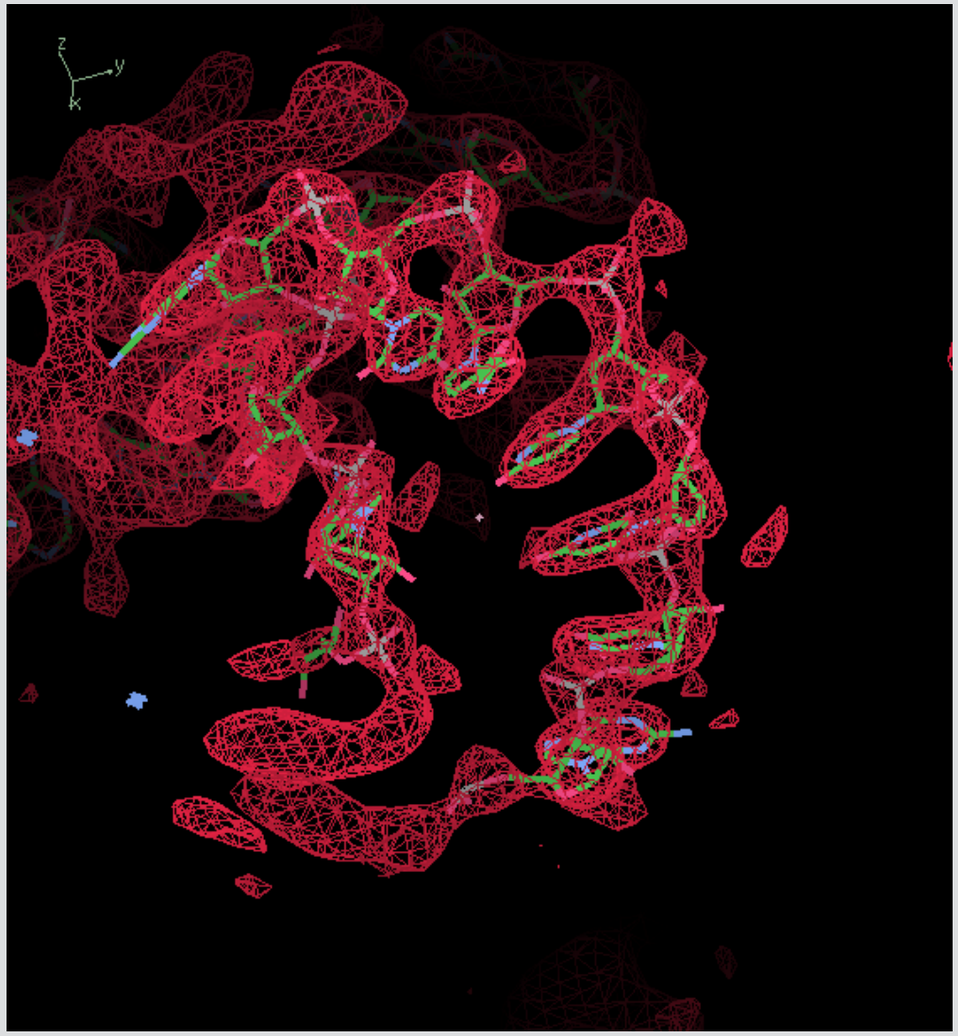
Trace chain and build model

Idealize secondary structure and refine

Assemble and refine (protein/RNA/DNA)

Apply molecular symmetry and re-refine

- Try building protein/RNA/DNA (whatever may be there)
- Choose segment type by map correlation



70S ribosome at 2.9 Å

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The Final Model

Determine optimal sharpening of the map

Cut out asymmetric unit of the map

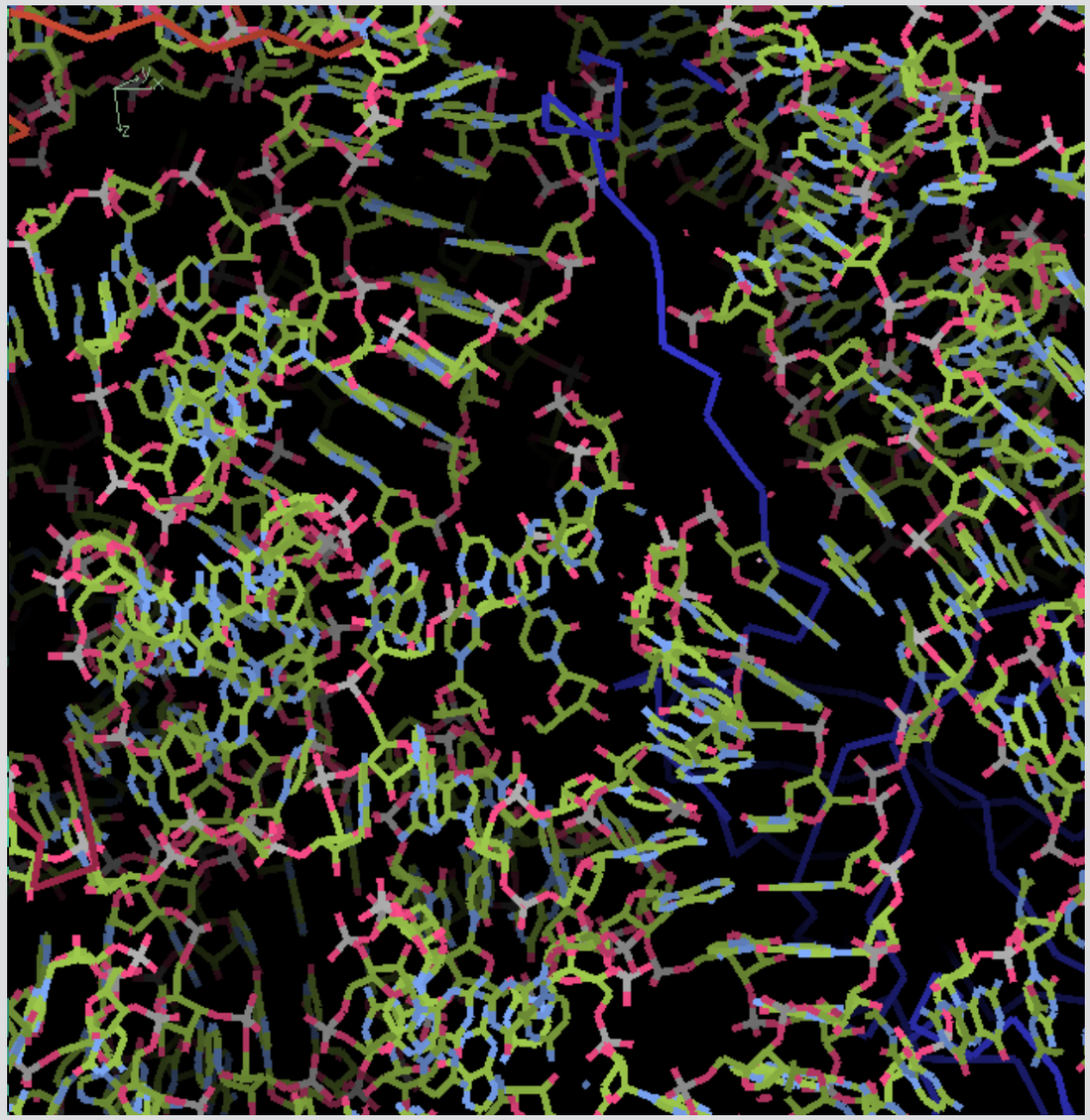
Trace chain and build model

Idealize secondary structure and refine

Assemble and refine (protein/RNA/DNA)

Apply molecular symmetry and re-refine

- `phenix.map_to_model`



30S Ribosome (1j5e, 2.9 Å)

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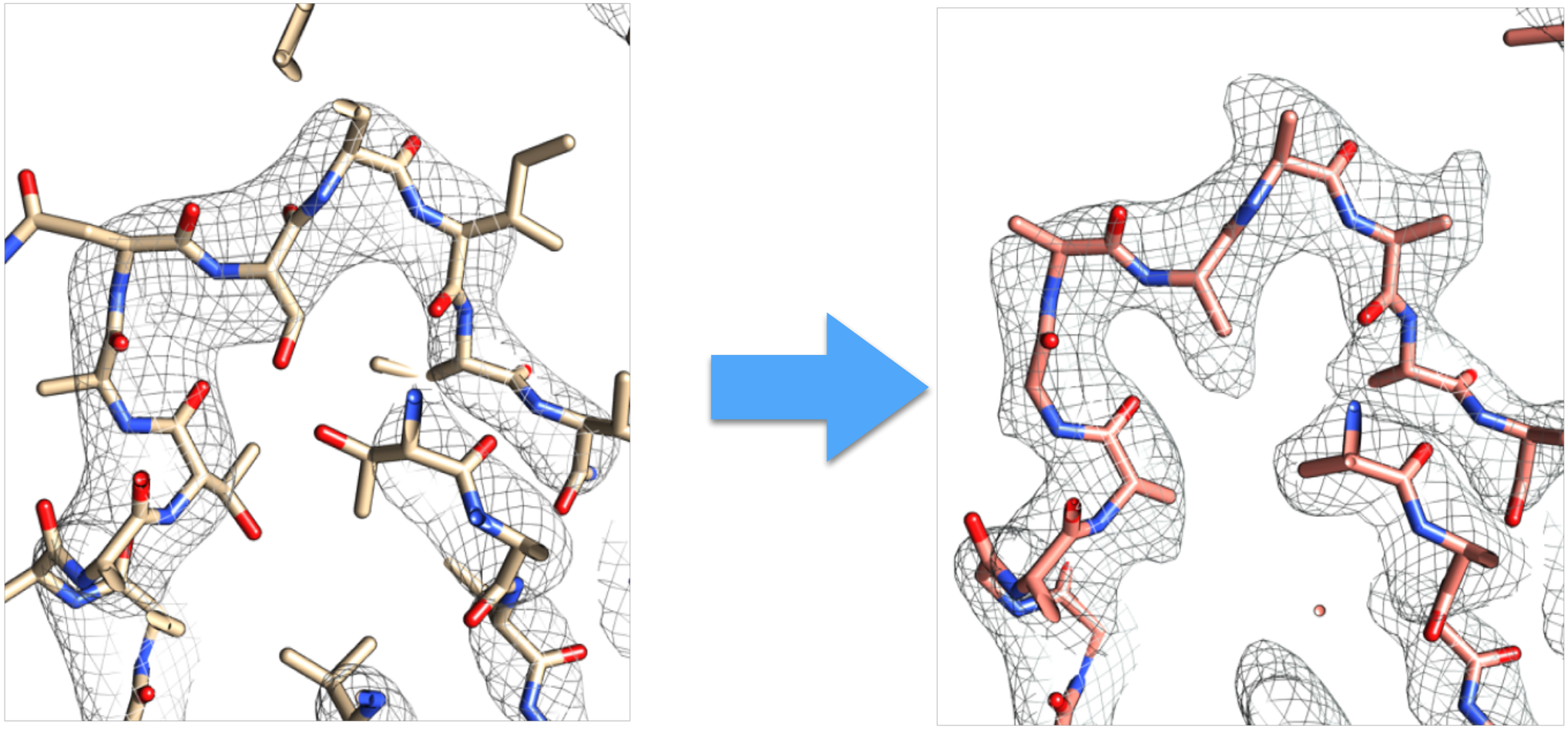
Automated Building - Sharpening



Original

Automatically Sharpened

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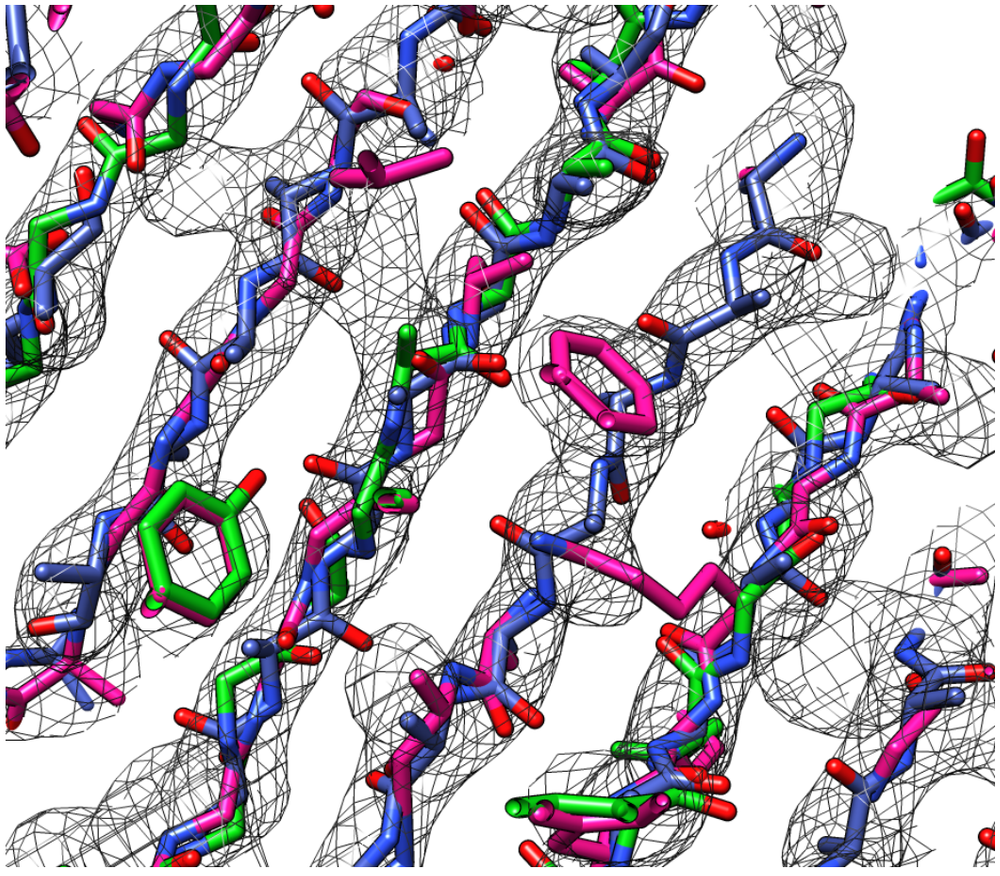
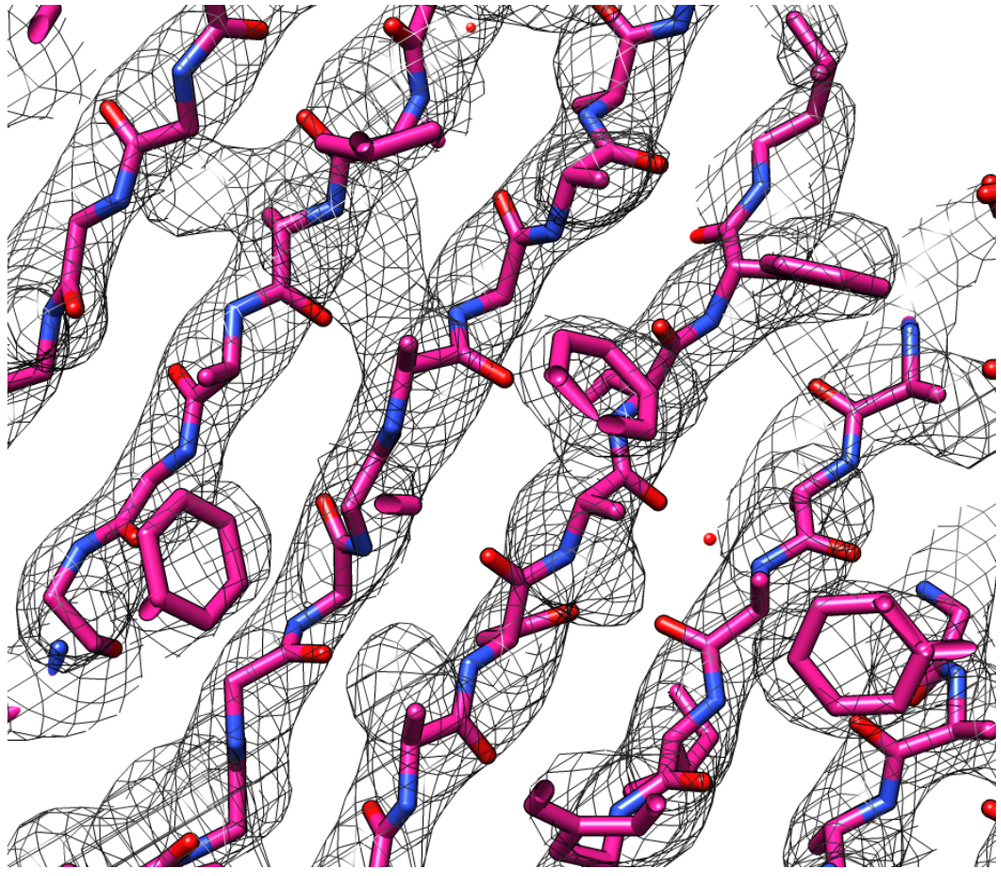
Automated Building - Combining Multiple Models



Three Independently Built Models



Composite Model

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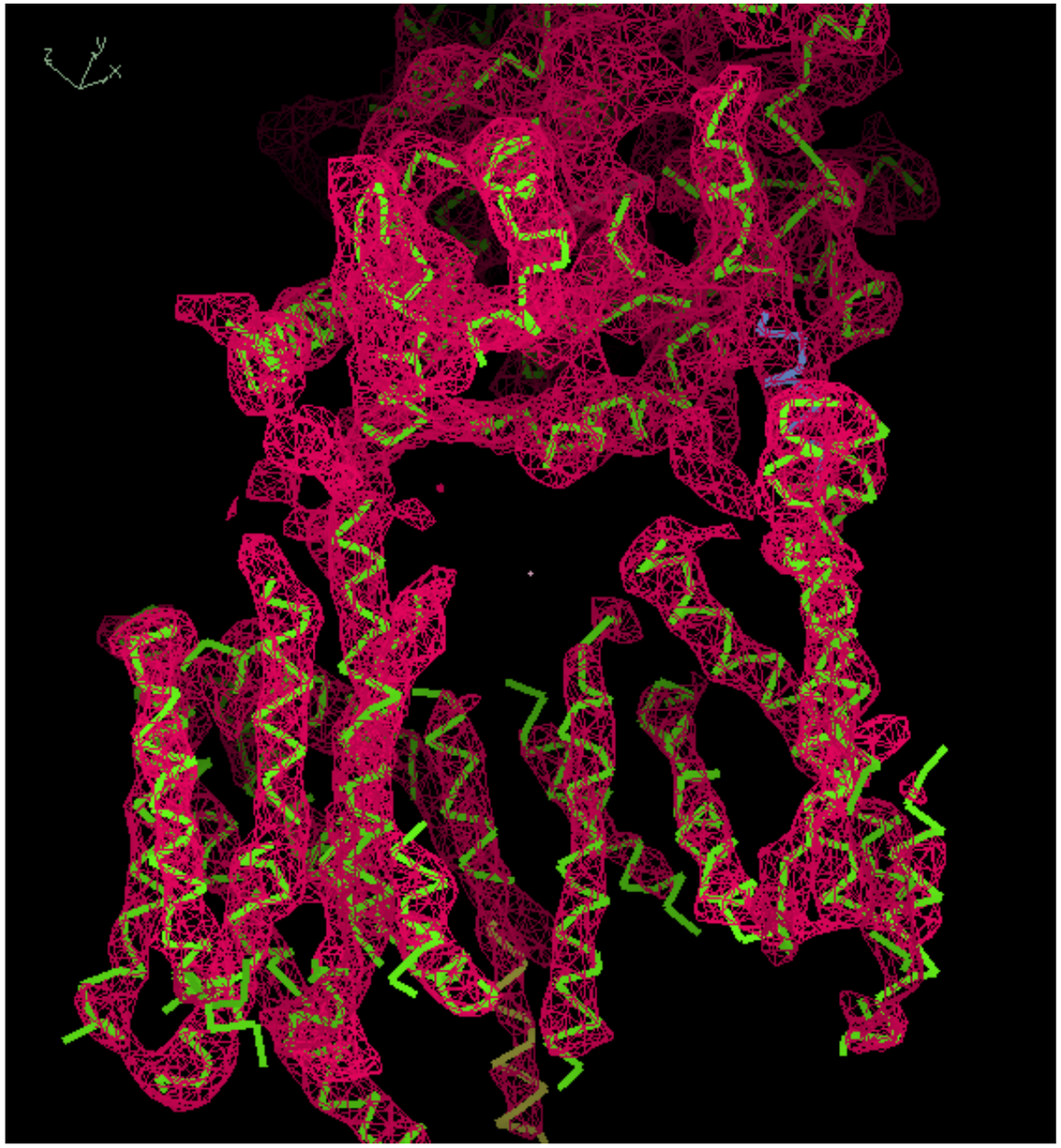
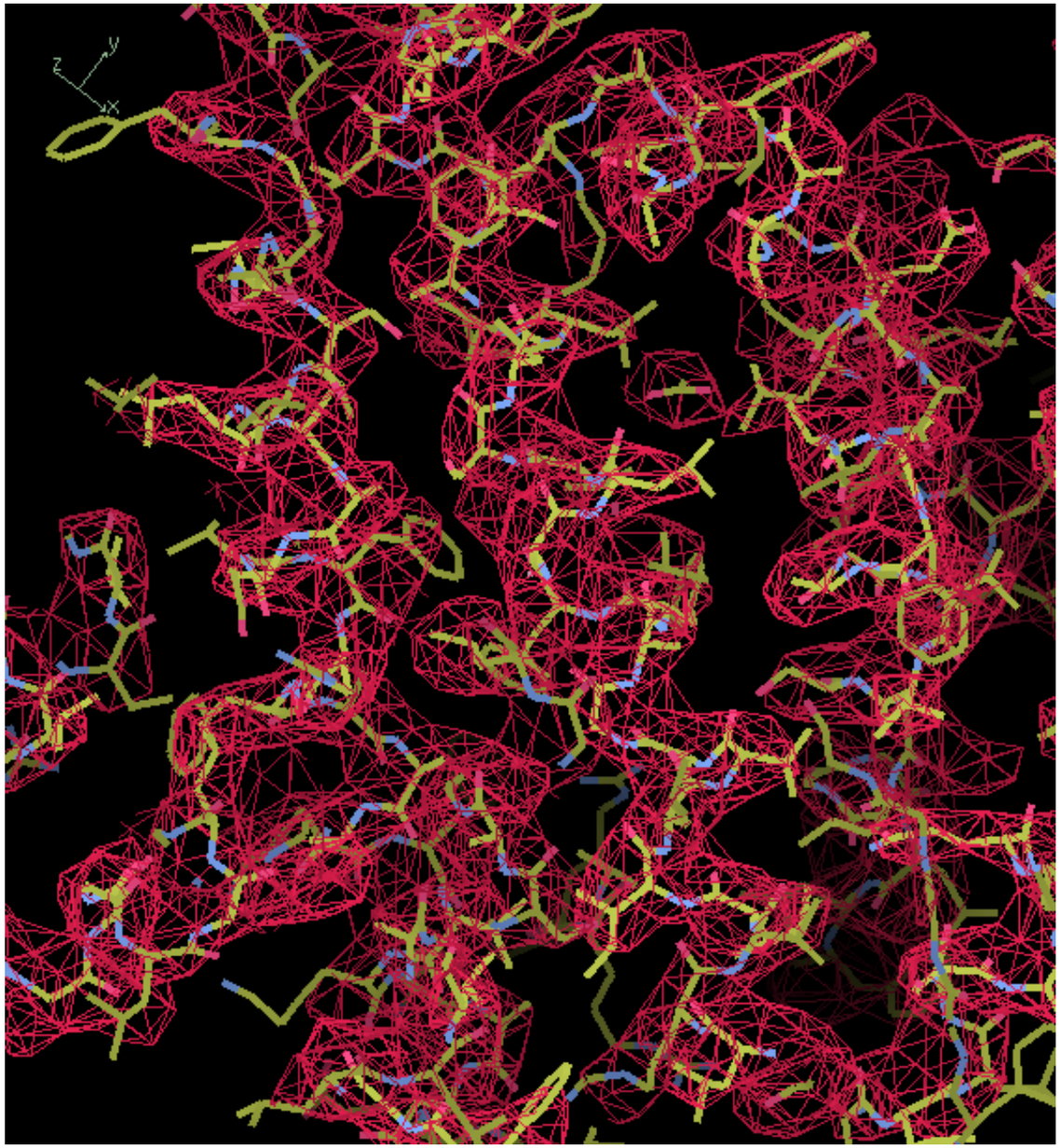
Building at Low Resolution



Gamma-secretase at 4.5 Å
(autobuilt model; emd_2677)



Gamma-secretase structure at 3.4 Å
(autobuilt model; emd_3061)

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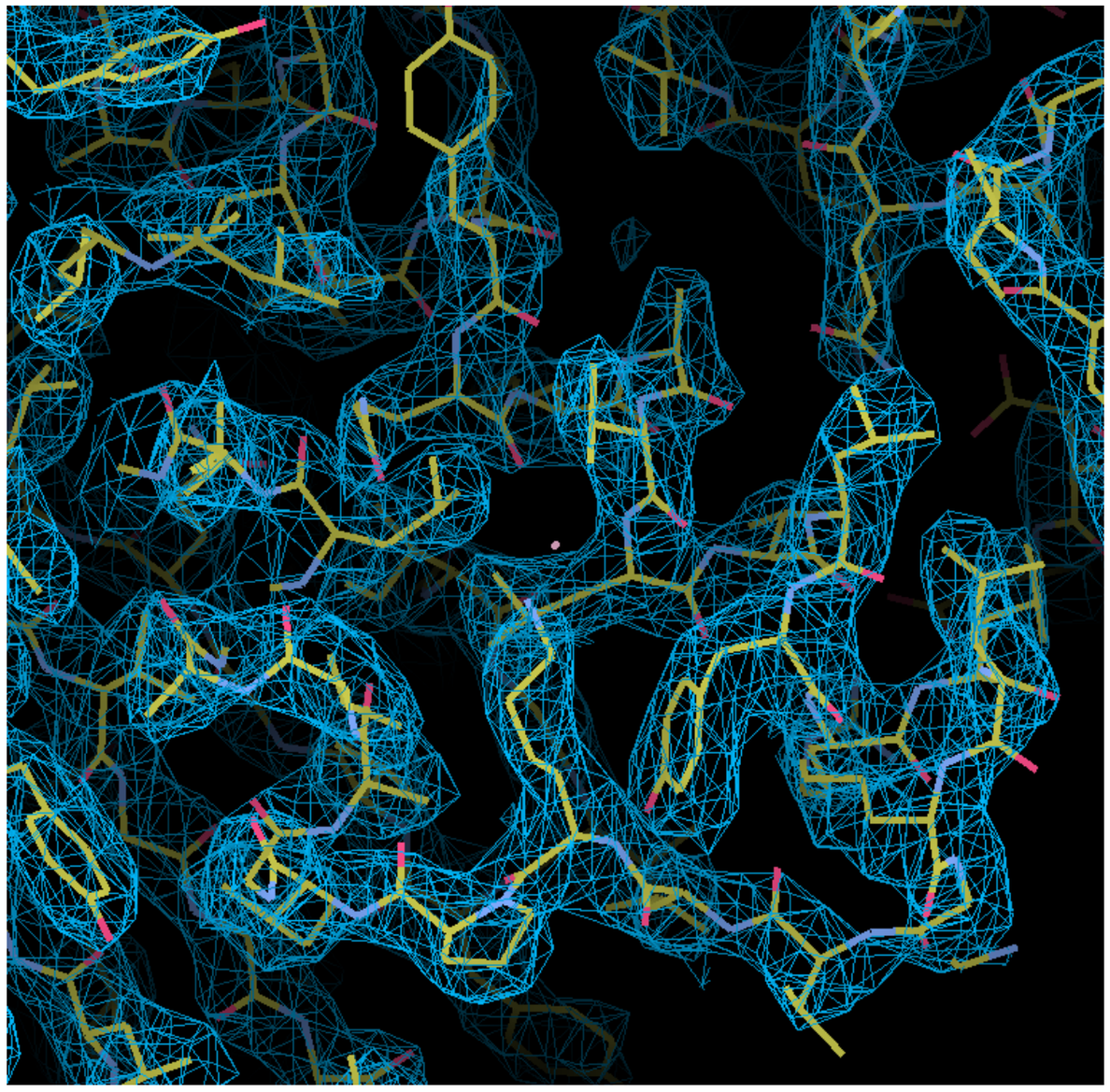
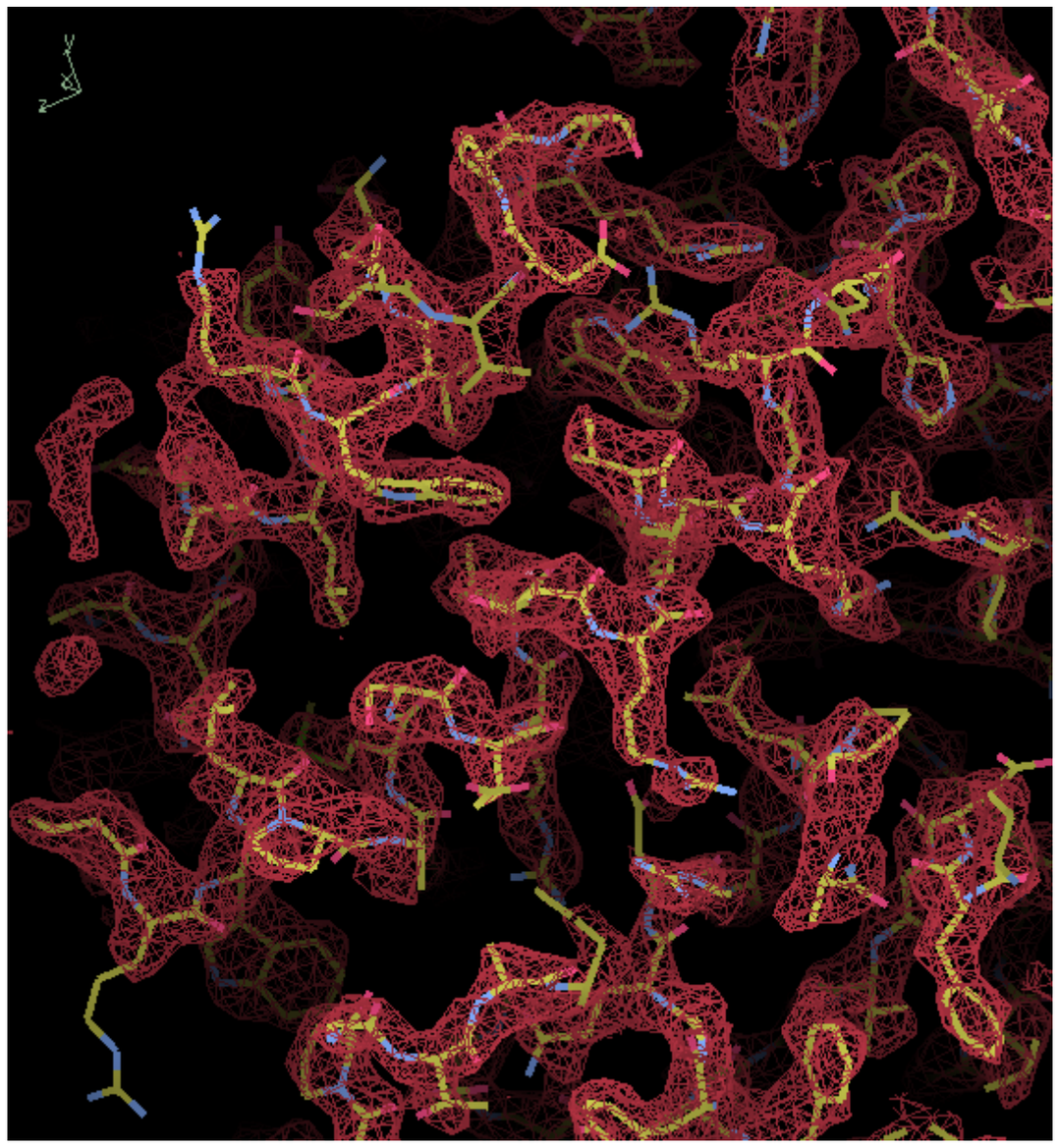
Building at Medium/High Resolution



Proteasome at 2.8 Å
(autobuilt model; emd_6287)



Beta-galactosidase at 2.2 Å
(autobuilt model; emd_2984)

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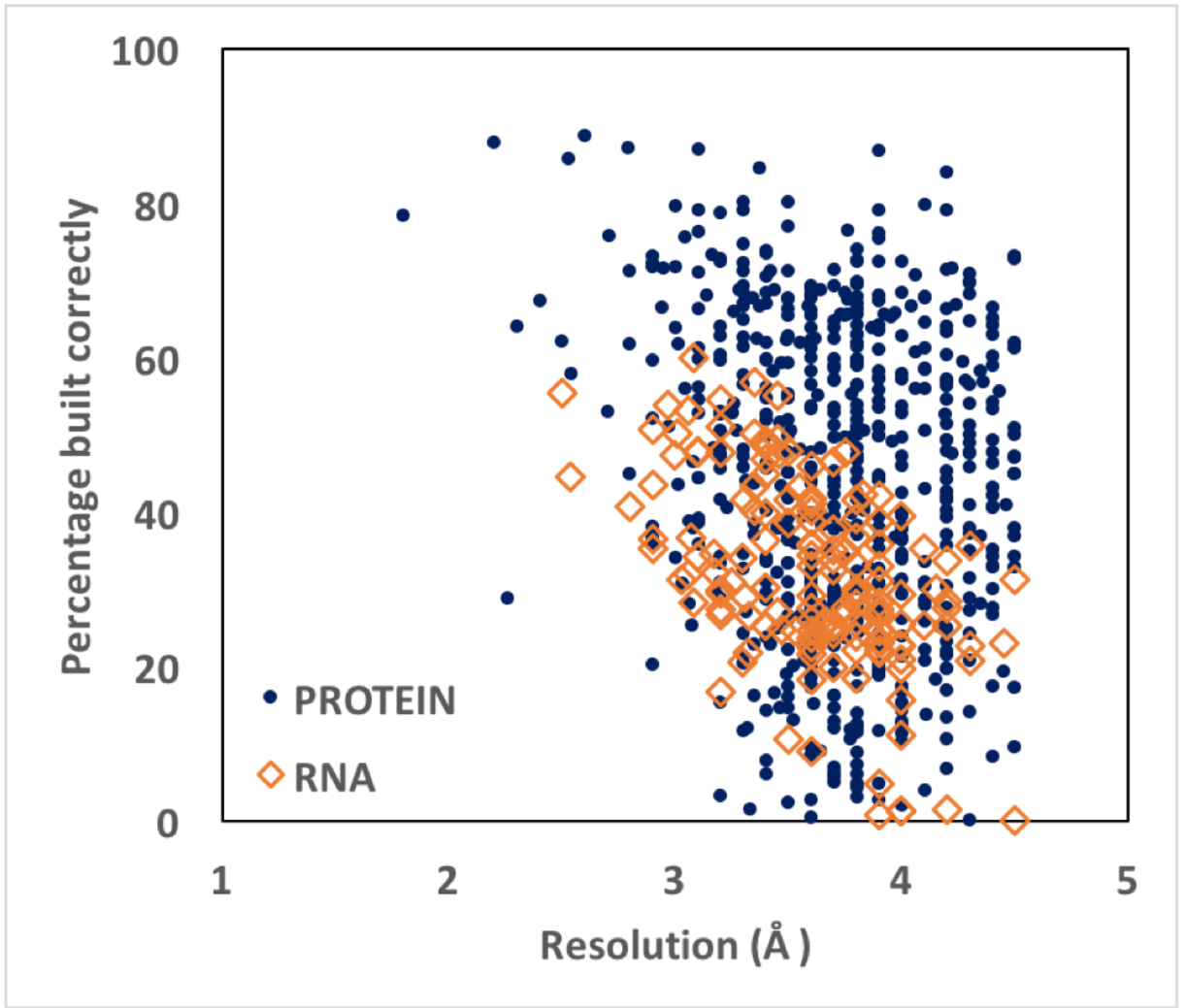
Autobuilding Performance



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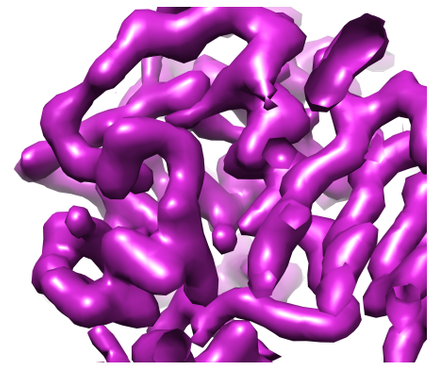
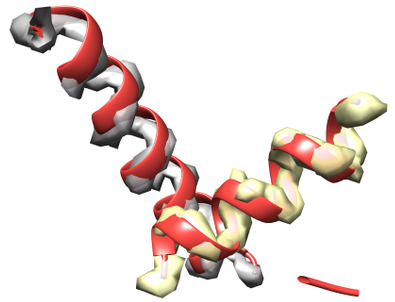
Model Building Version 2

Trace chain the way a person does

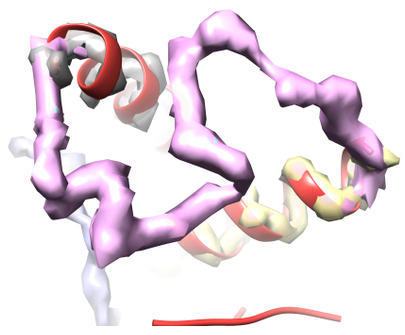


Find secondary structure

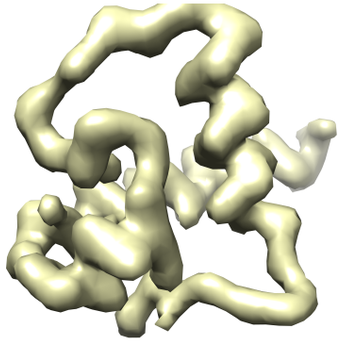


Find clear regions of density

Adjust contour level until a region just connects to another



Iterate to build up a connected chain



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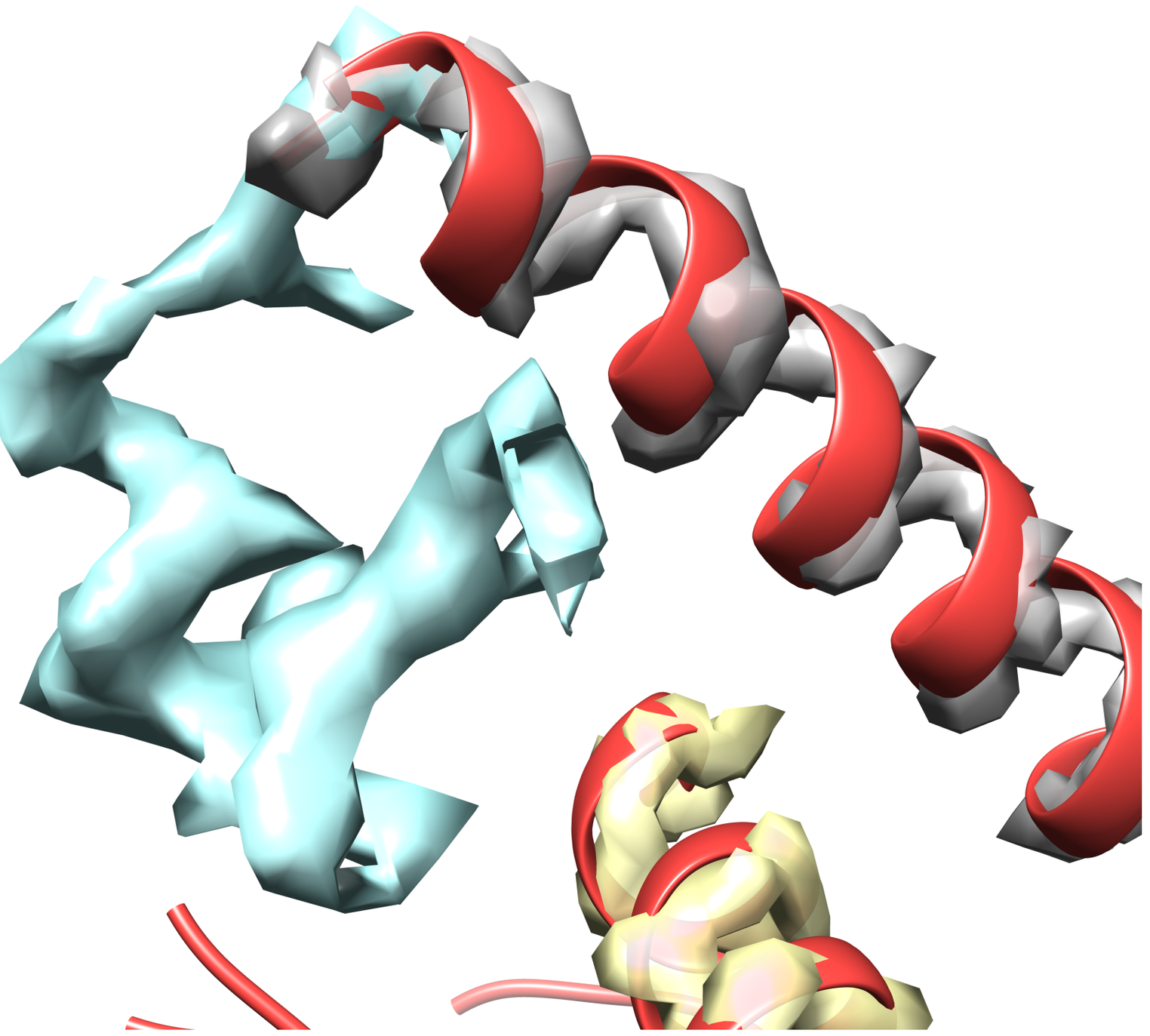
Model Building Version 2



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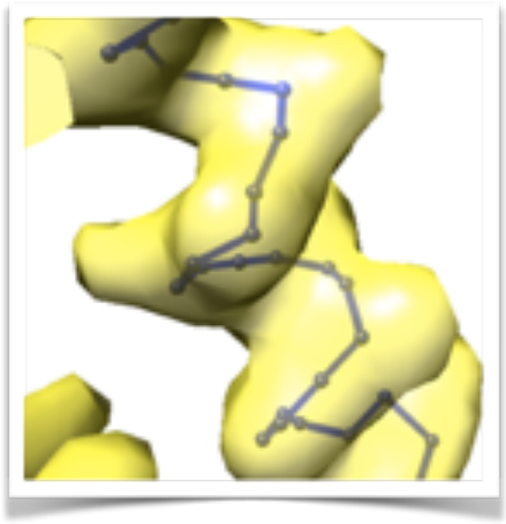
Finding C_α and C_β positions

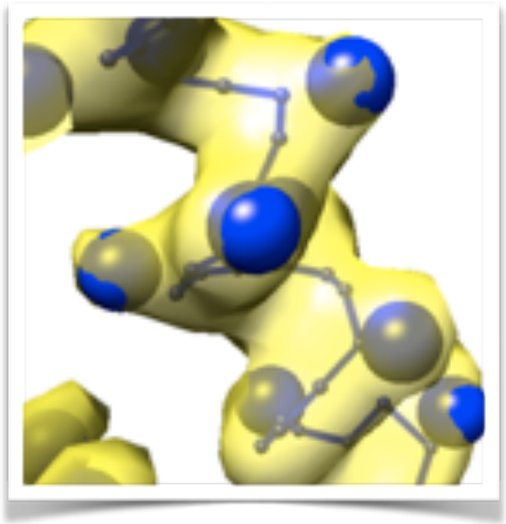
Trace chain path through high density

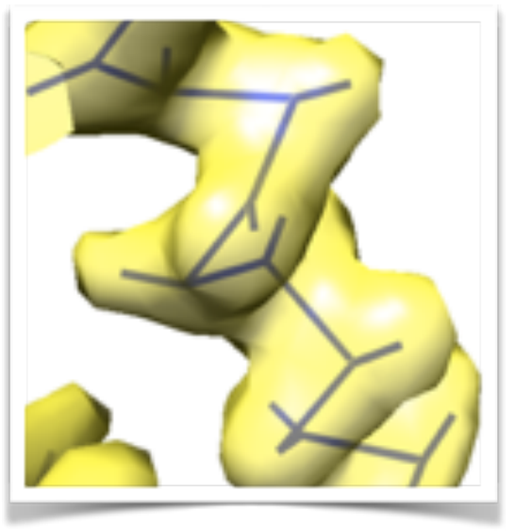
Find C_β positions from side-chain density

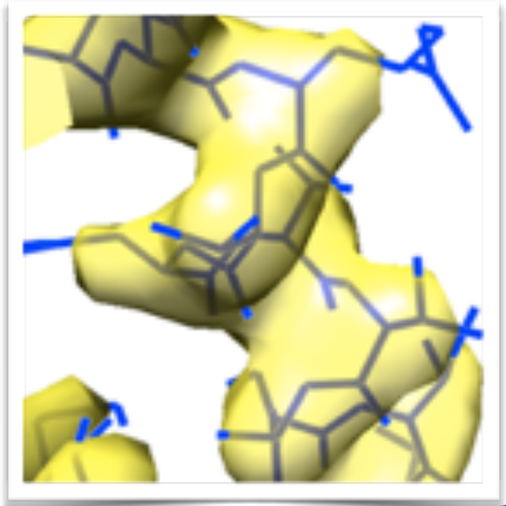
Choose C_α positions 3.8 Å apart and next to C_β positions

Construct all-atom model with Pulchra* and refine









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

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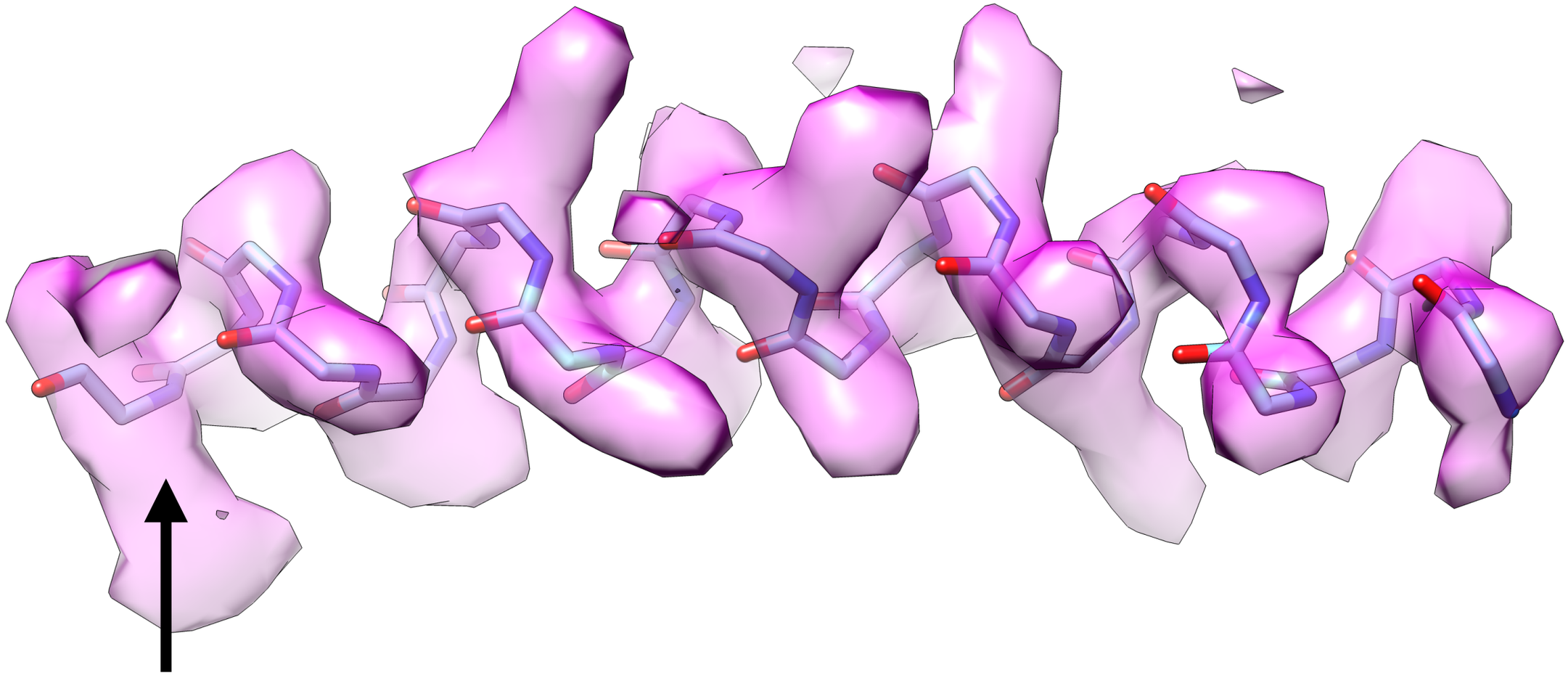
Sequence Assignment



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

- Determine probability of side chain at each C_α
- Align sequence to maximize total probability for the chain

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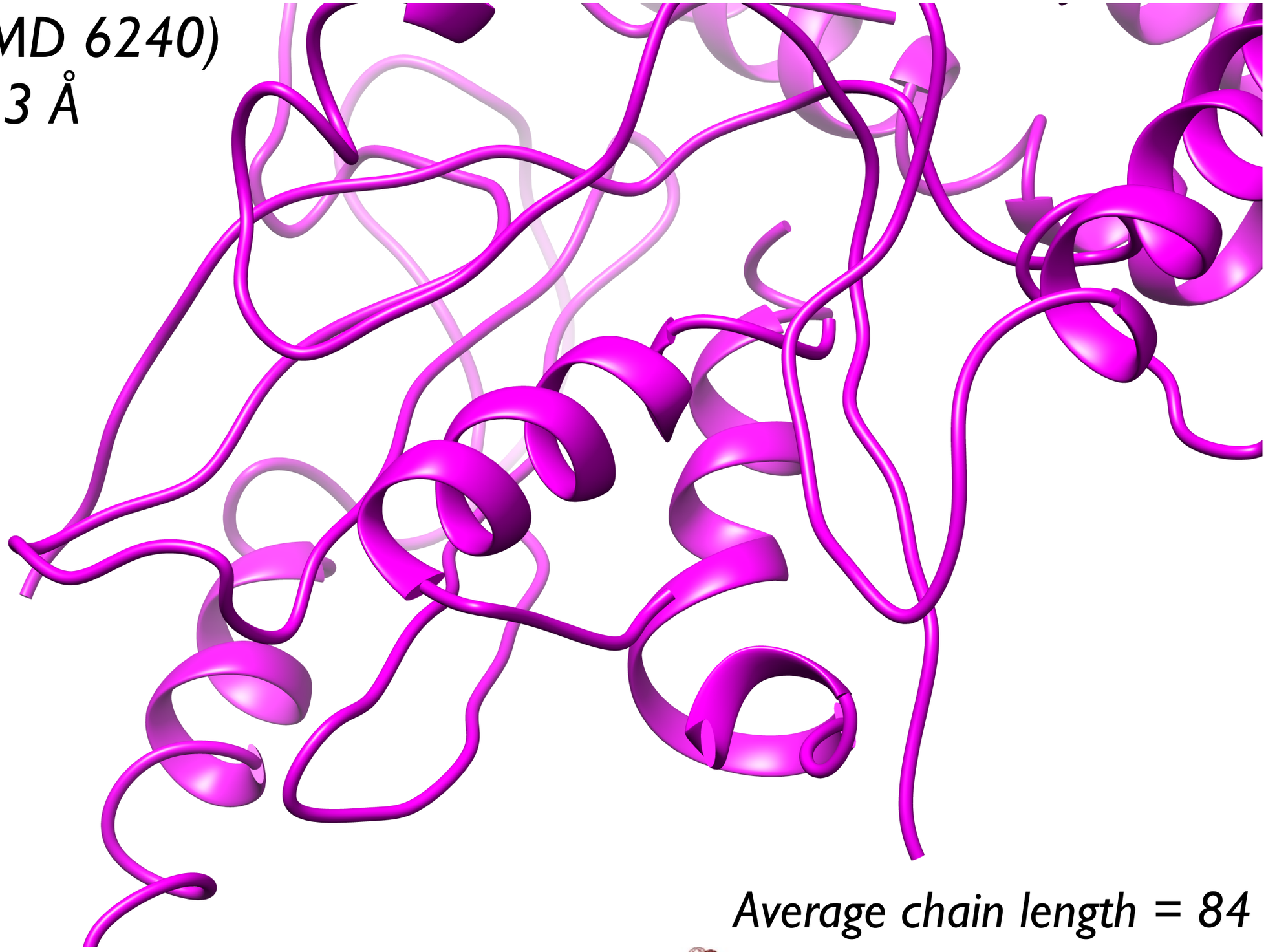
Improved Connectivity

3j9e (EMD 6240)
3.3 Å



Average chain length = 84

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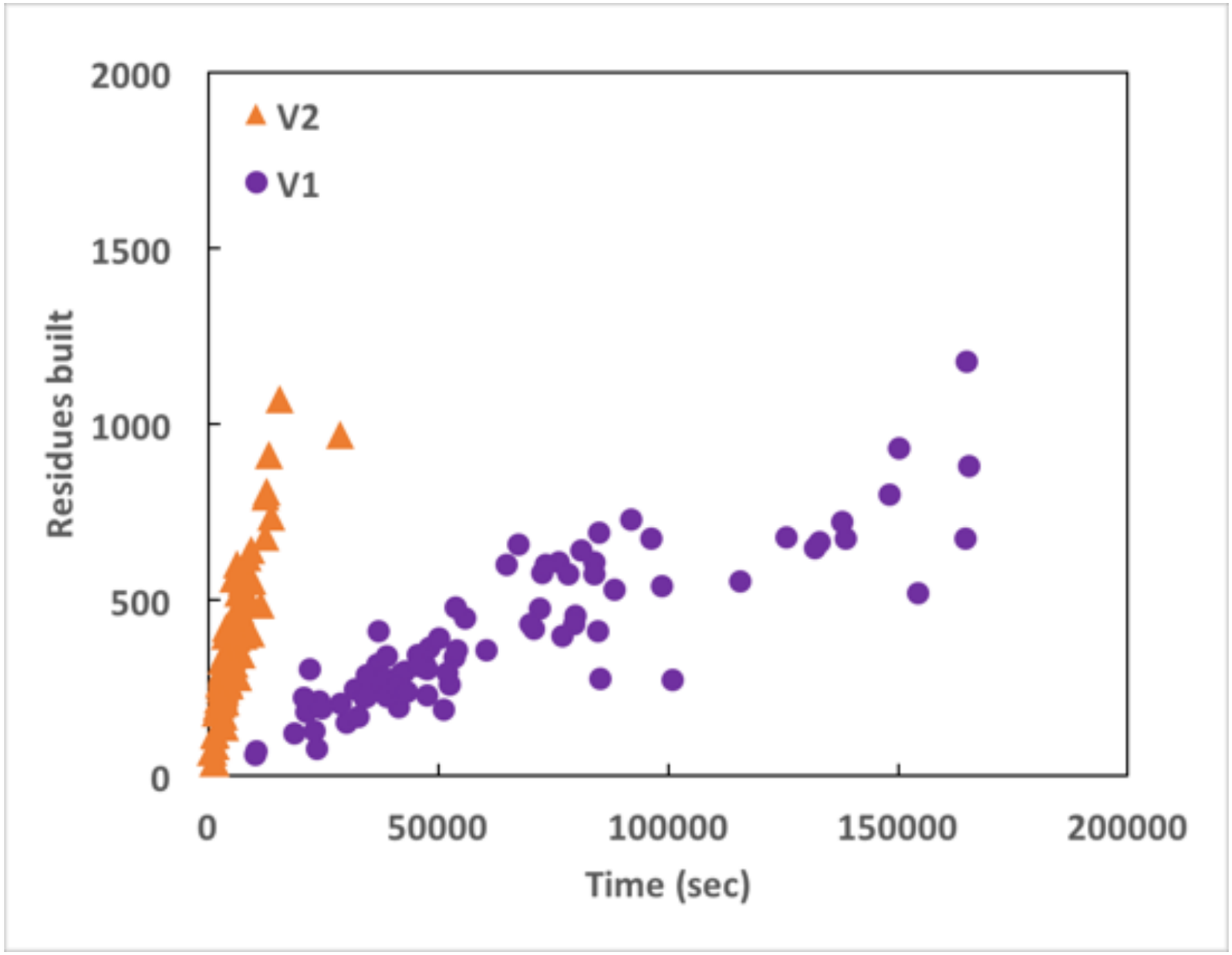
Improved Performance



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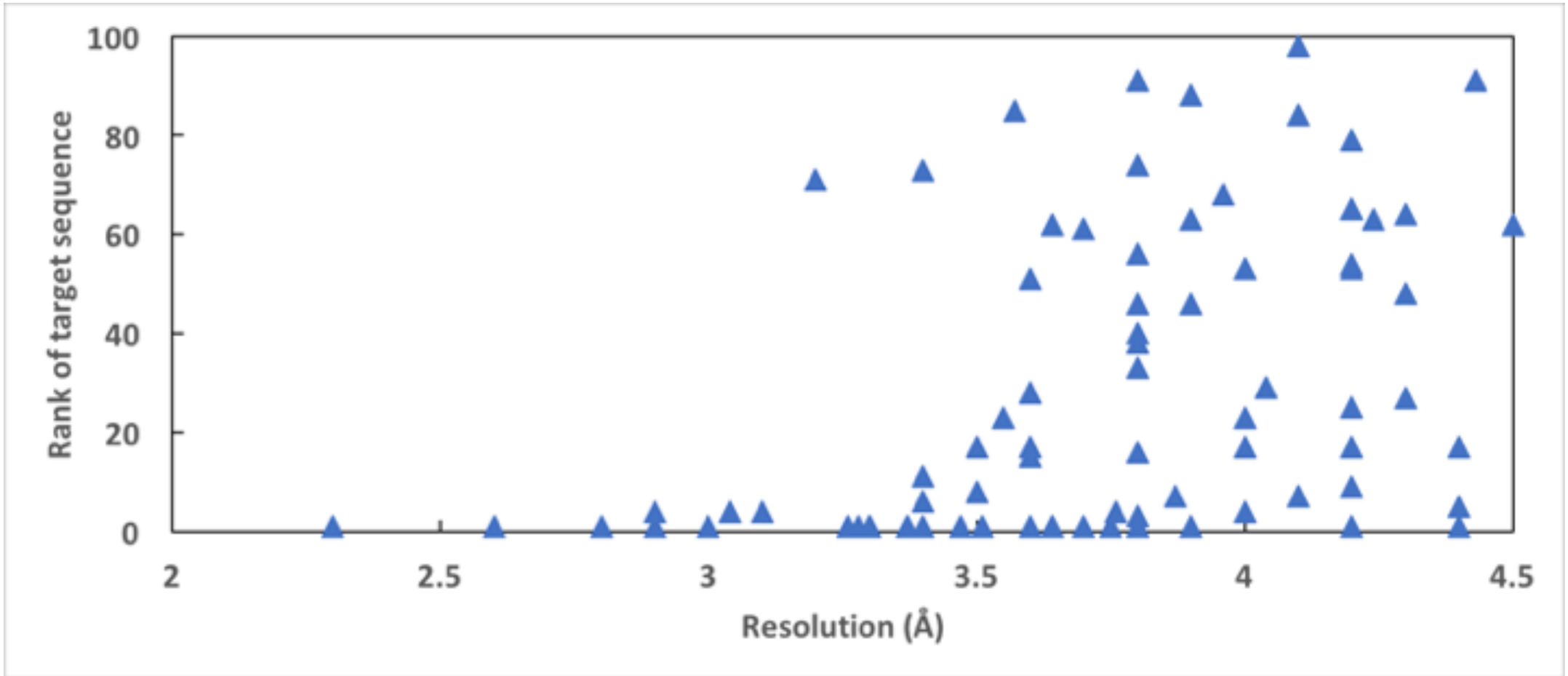
What's The Molecule?

- Use the highest side chain probabilities to determine a sequence (from the map)
- Search the sequence database to identify the molecule



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

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Conclusions

- Automated model building is possible, but can be improved
  - Include information from secondary structure prediction, evolution etc.
  - Combine structure-modeling tools (Rosetta) with Phenix model-building
- Many challenges remain:
  - Reliably accounting for uncertainty in magnification
  - Local variation in resolution leads to uncertainties in interpretation

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