CU Anschutz Medical School, January 2020

Map tools (cryo-EM)

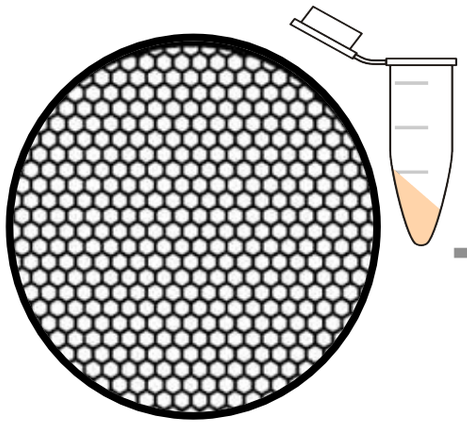
Dorothee Liebschner

Lawrence Berkeley Laboratory

Structure determination workflow: Cryo-EM

Prepare sample

Sample on grid

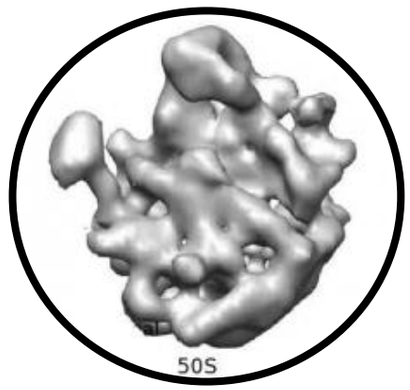


Collect data

Electron microscope

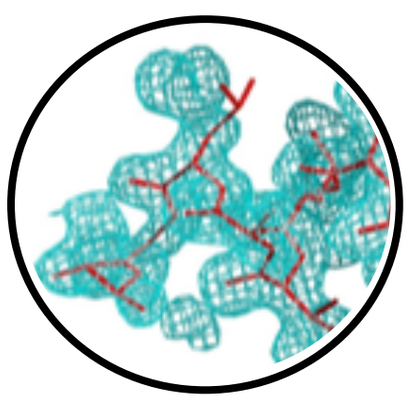
Process data

3d map



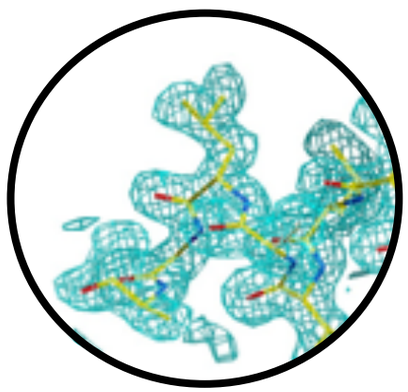
Map manipulations

Build first model

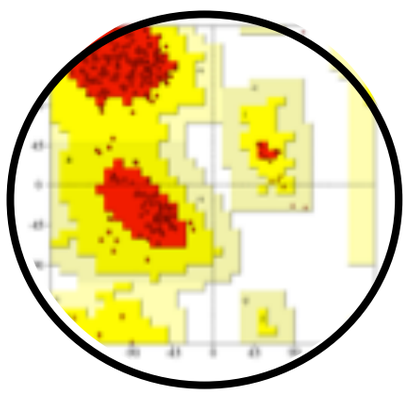


Refinement

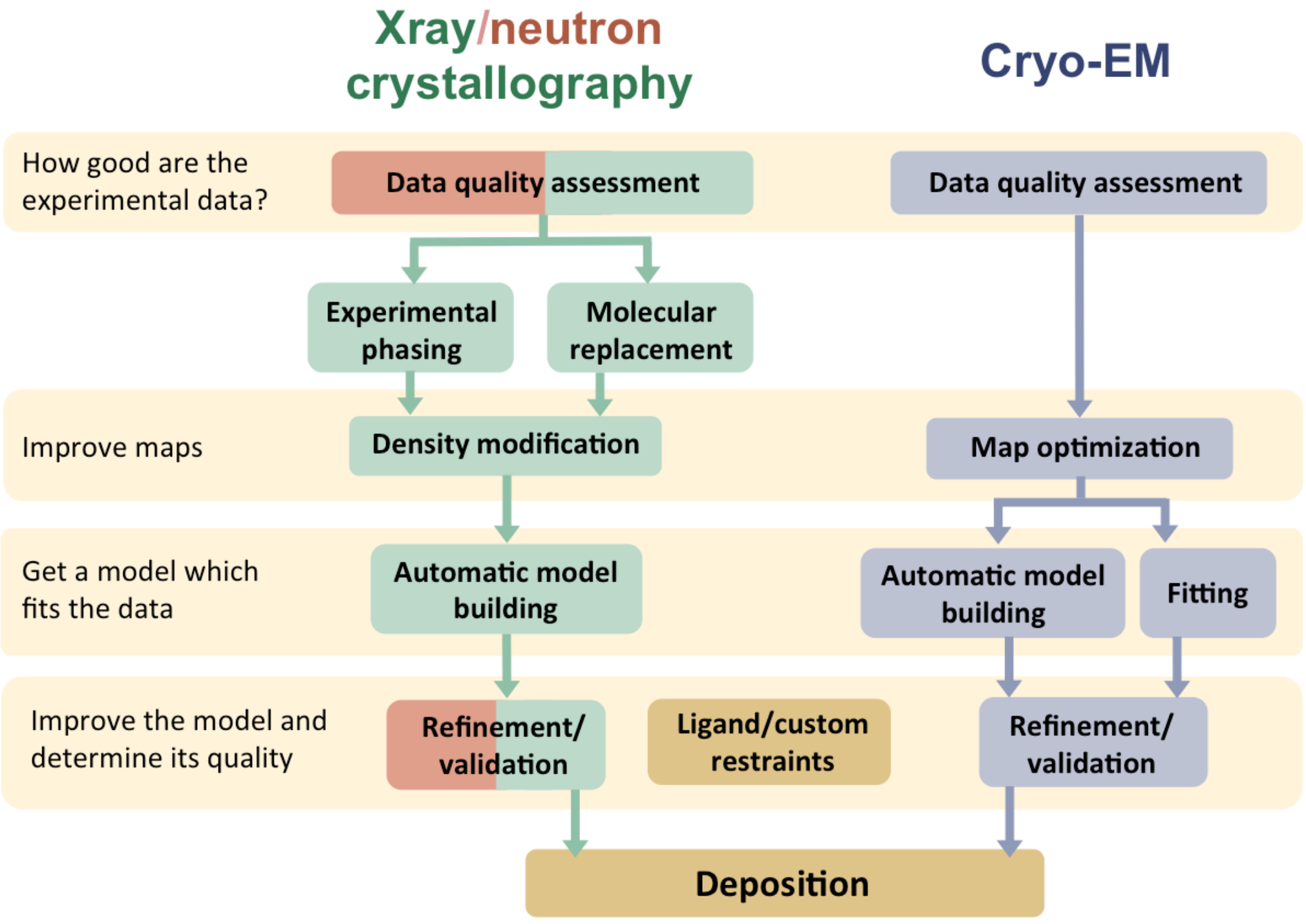
Real Space



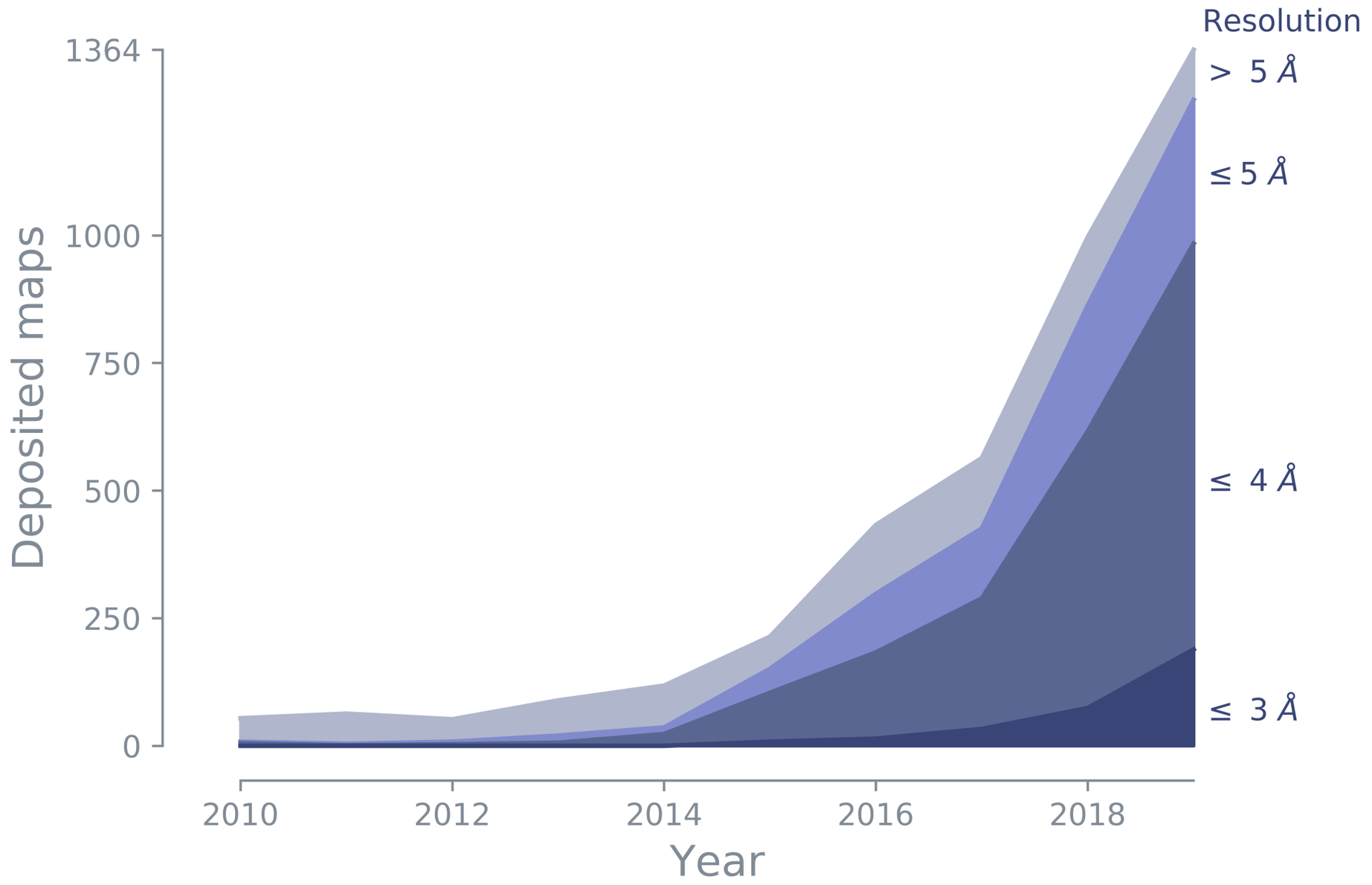
Validation



Structure determination workflow



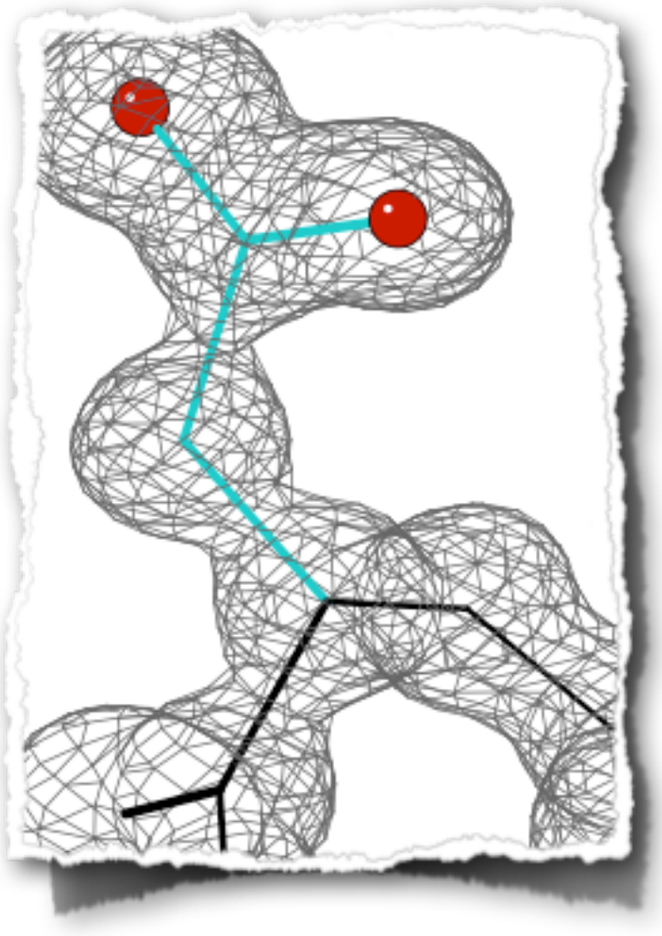
EM map resolutions



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

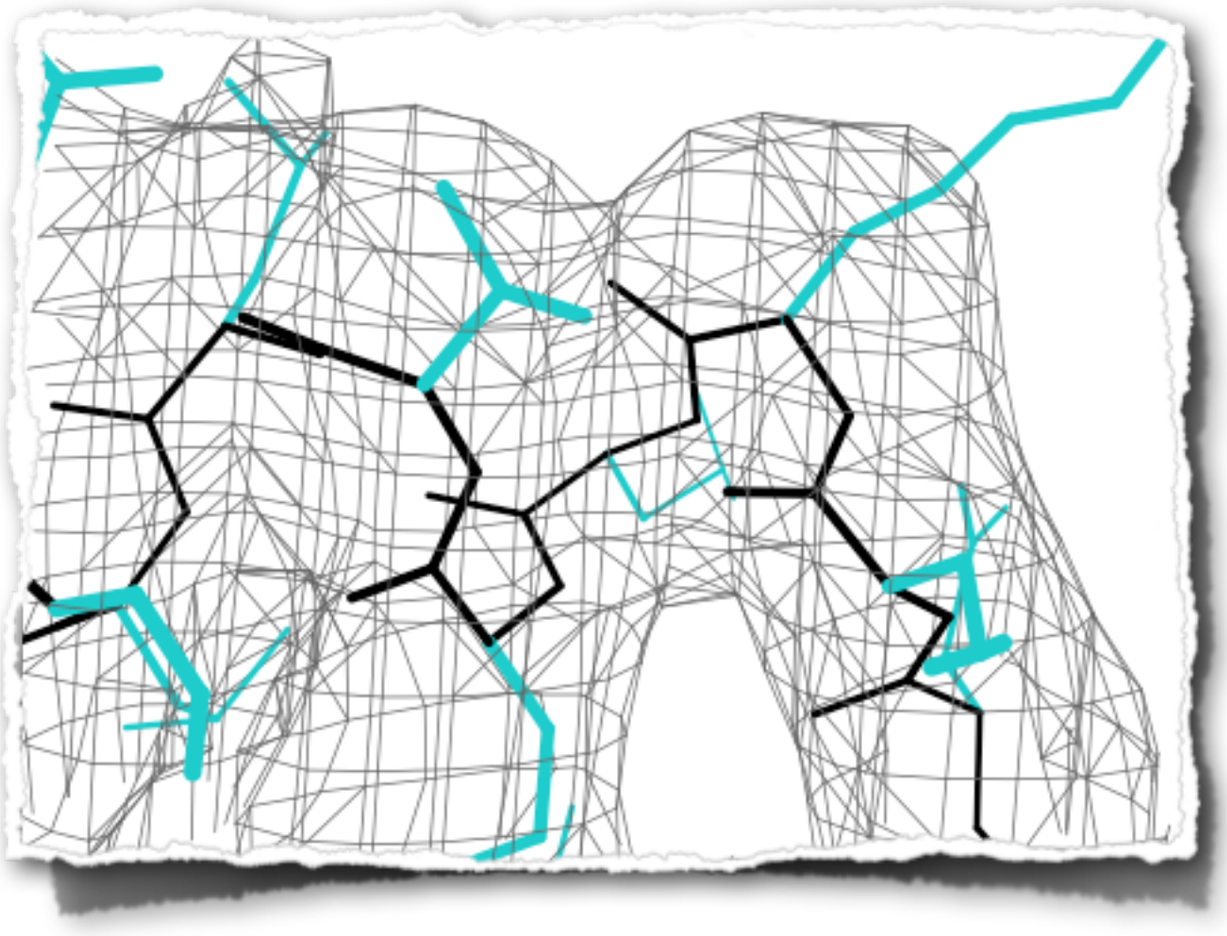
EM maps have low resolution

1.00Å resolution



2gkg

3.80Å resolution



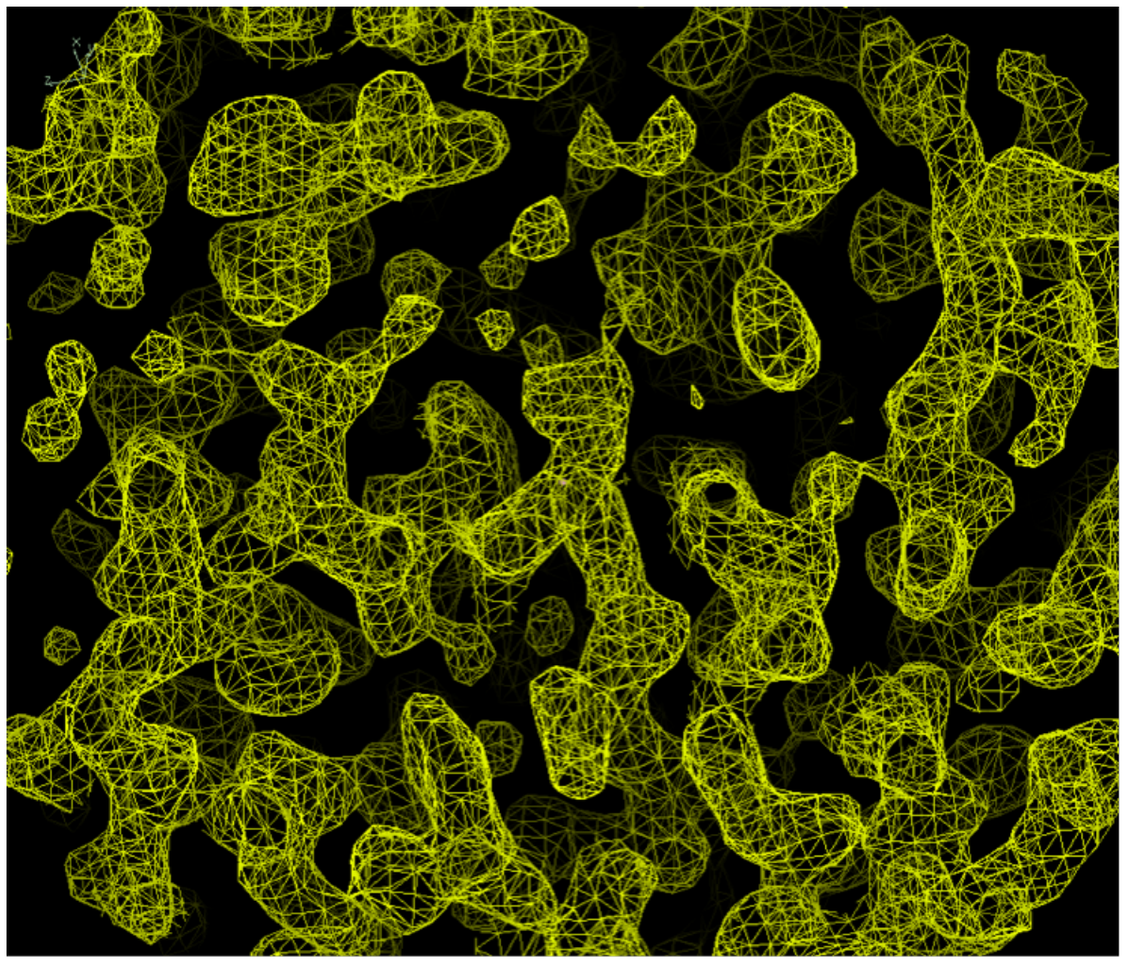
3k7a

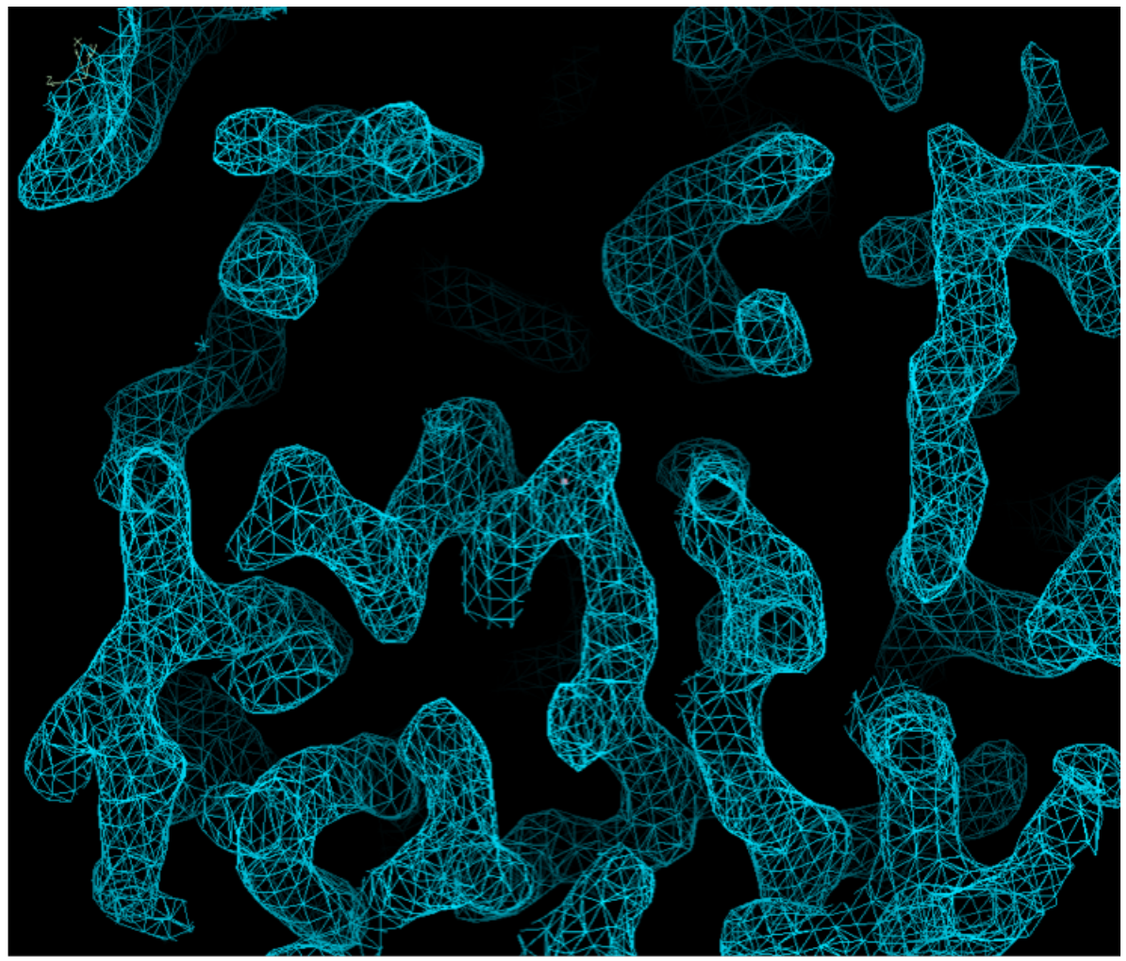
Challenges:

- How to interpret “featureless” maps?
- How to optimize models with sparse data?

Crystallographic vs. cryo-EM maps

Beta galactosidase at 2.2 Å

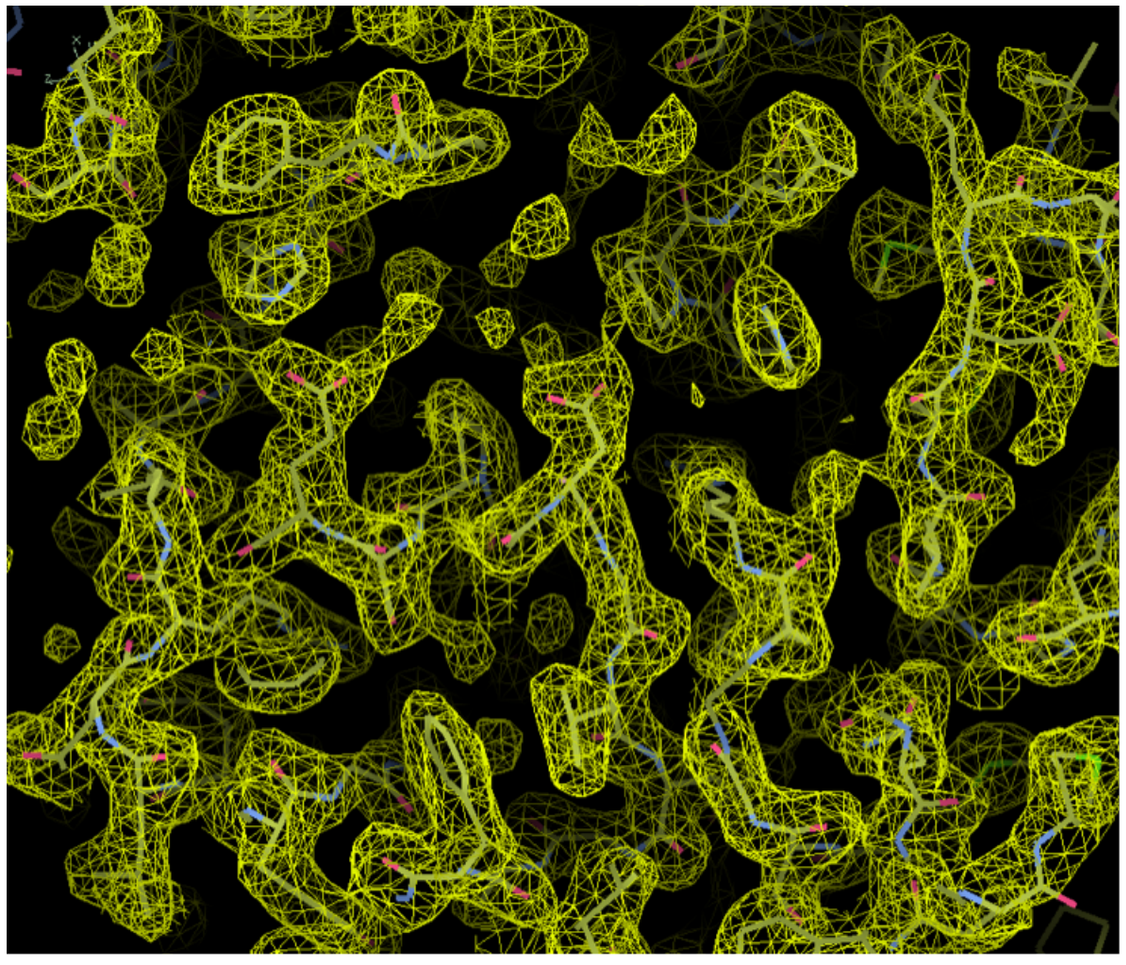




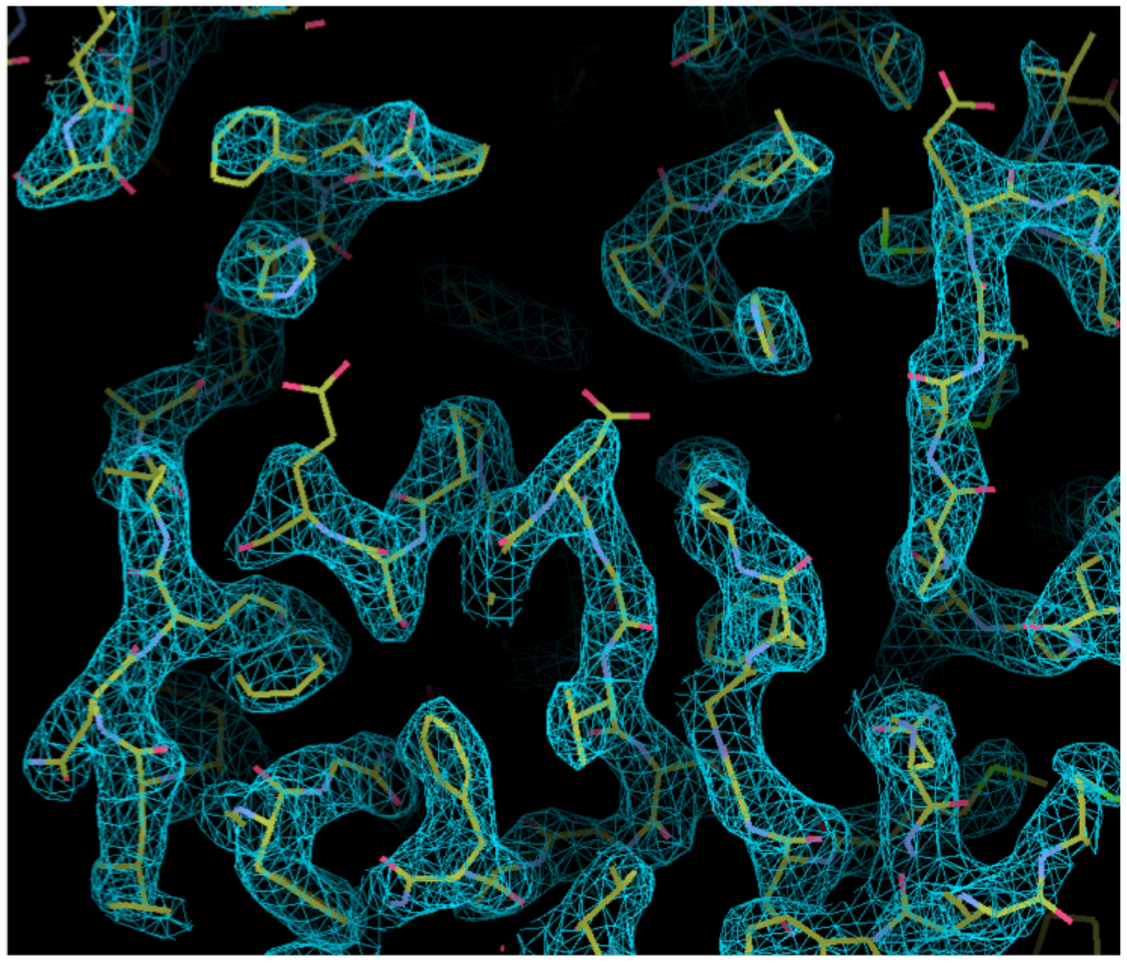
Tom Terwilliger, Los Alamos National Lab

Crystallographic vs. cryo-EM maps

Beta galactosidase at 2.2 Å



X-ray (PDB 3i3b)

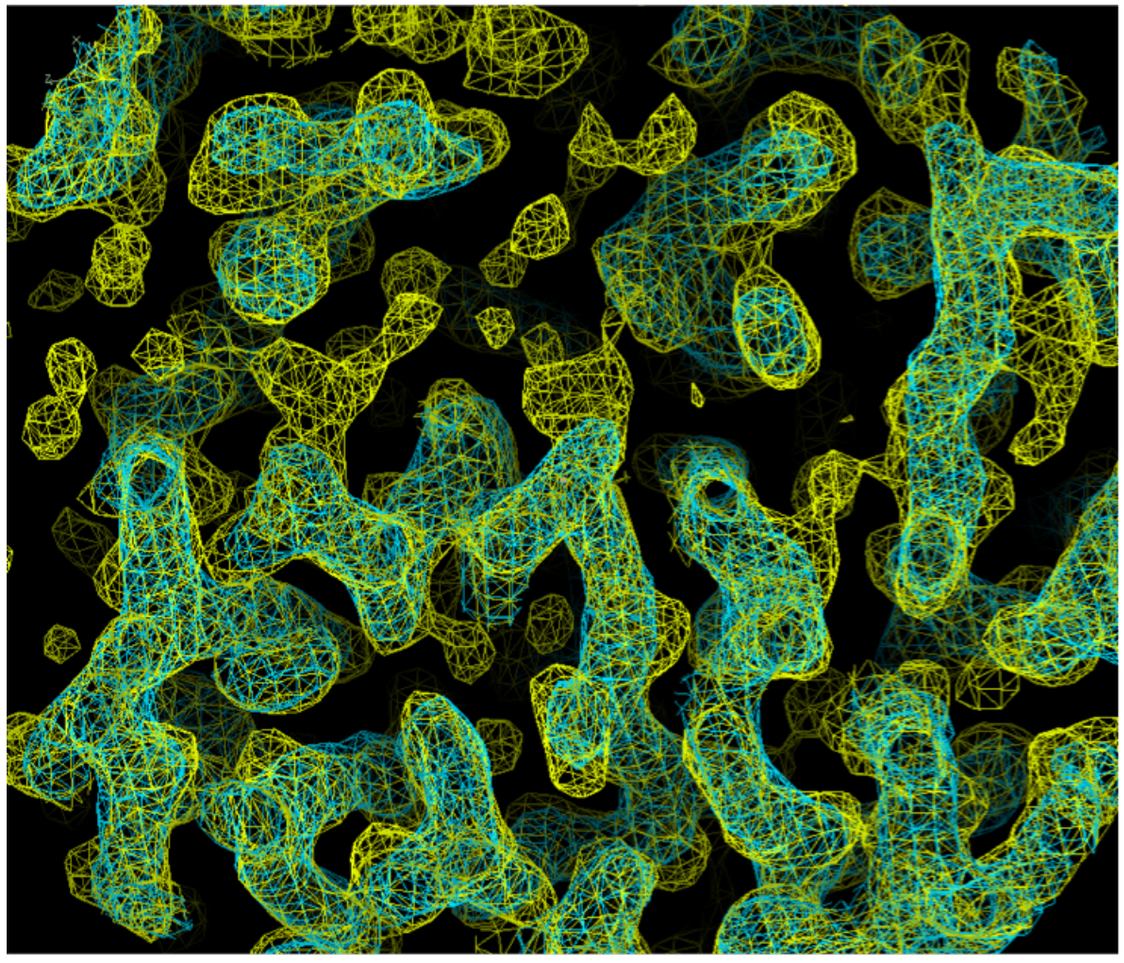


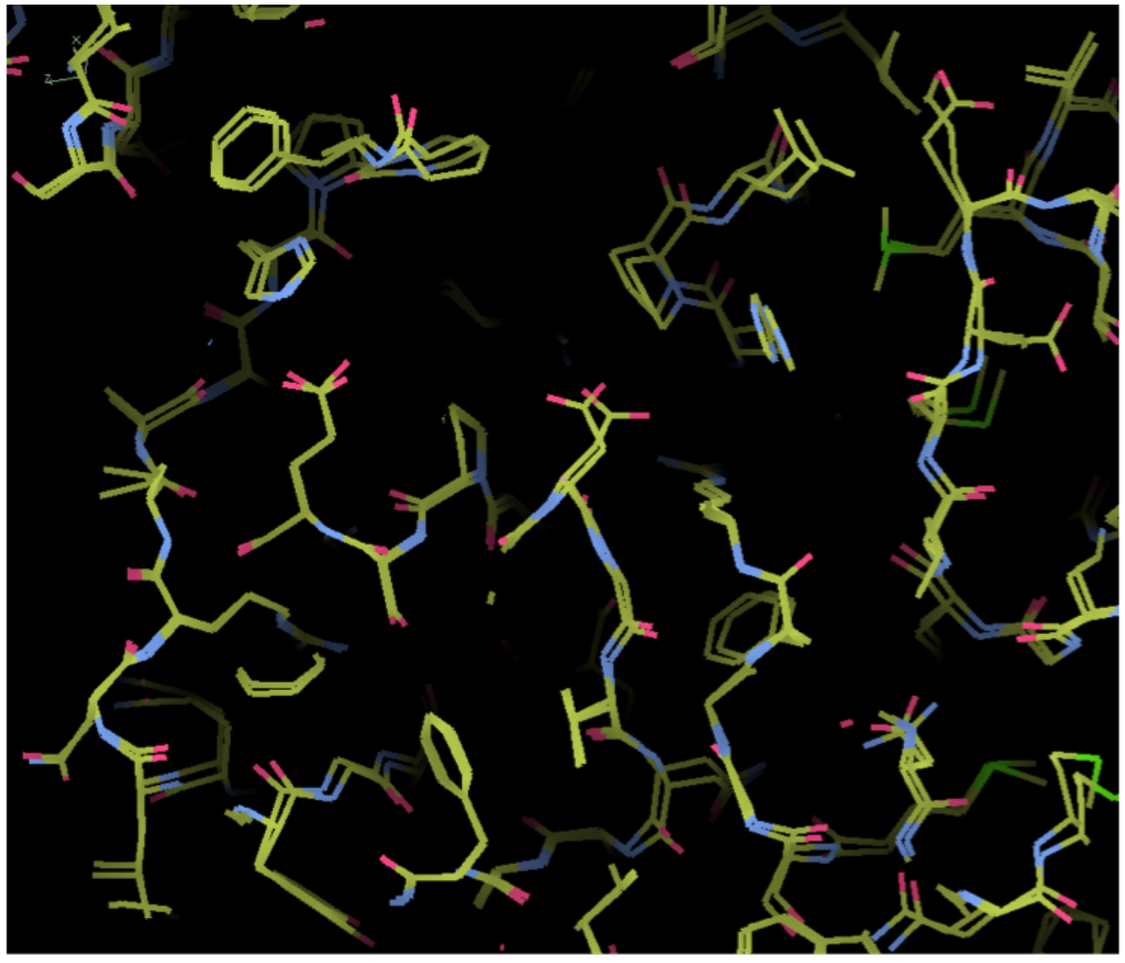
Cryo-EM (PDB 5a1a)

Tom Terwilliger, Los Alamos National Lab

Crystallographic vs. cryo-EM maps

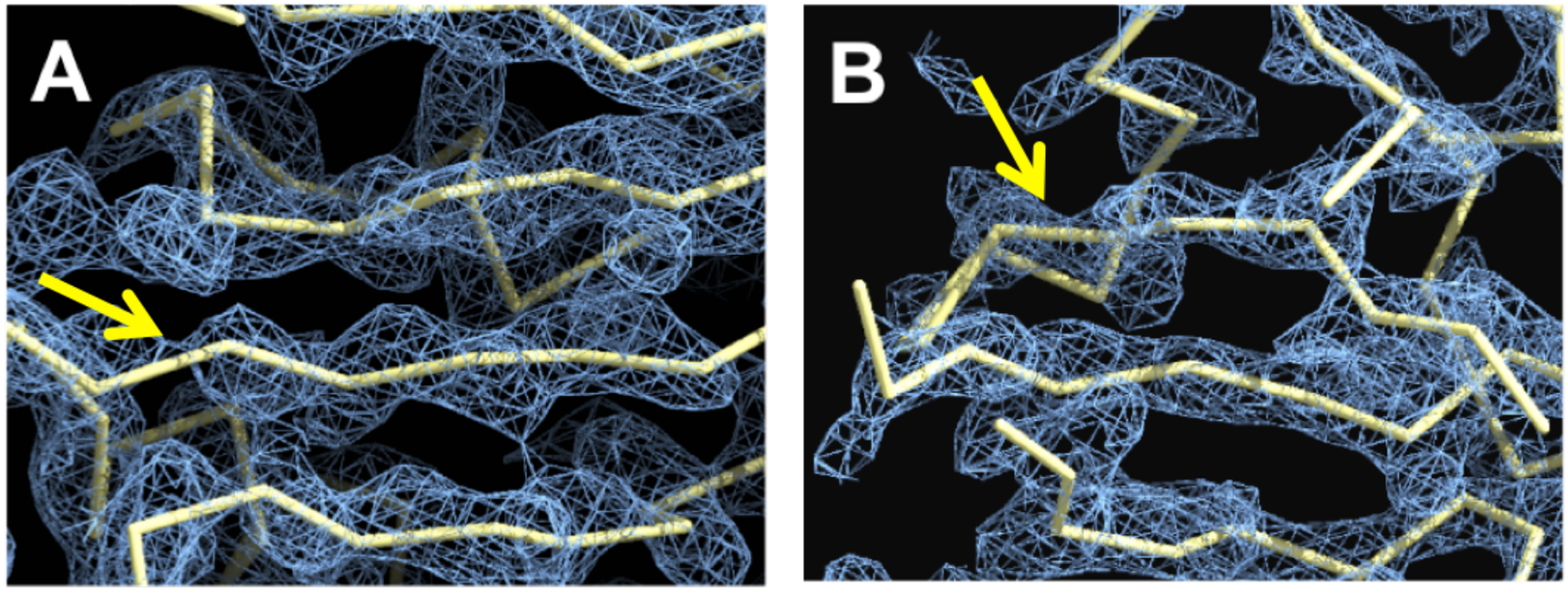
The maps are very similar





Tom Terwilliger, Los Alamos National Lab

More accurate low-resolution info in cryo-EM maps



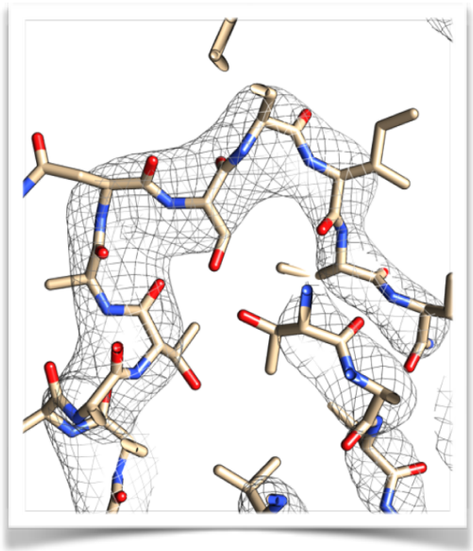
Original

Tom Terwilliger, Los Alamos National Lab

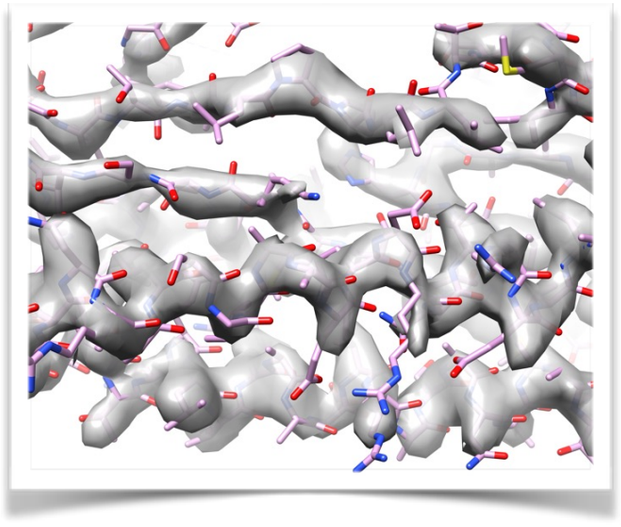
Map tools in *Phenix*

Goal:

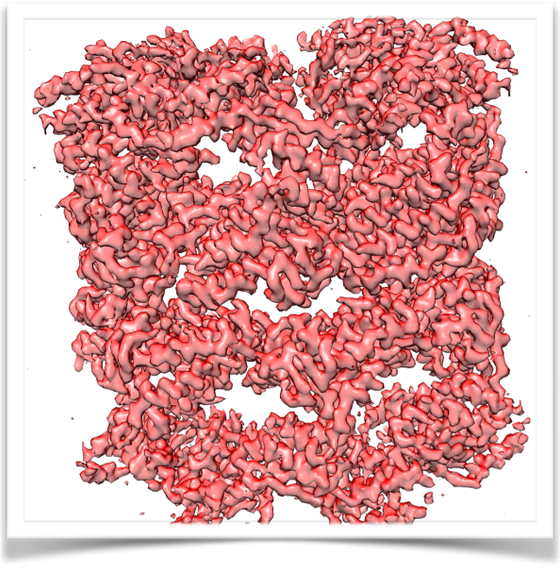
Get the best possible map to facilitate subsequent steps (model building, refinement)



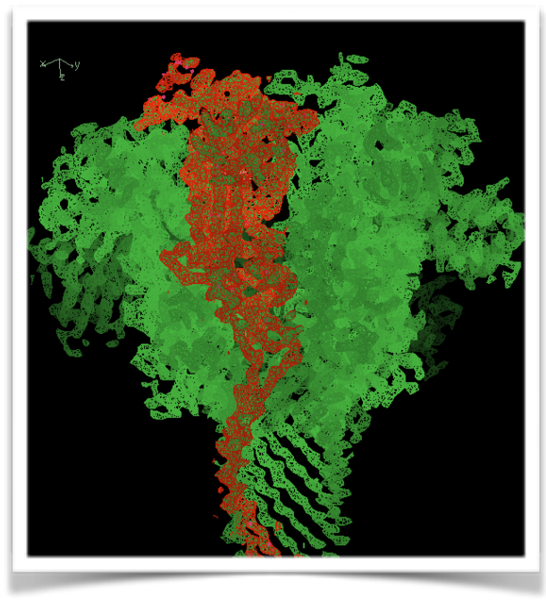
Automated map sharpening



EM density modification



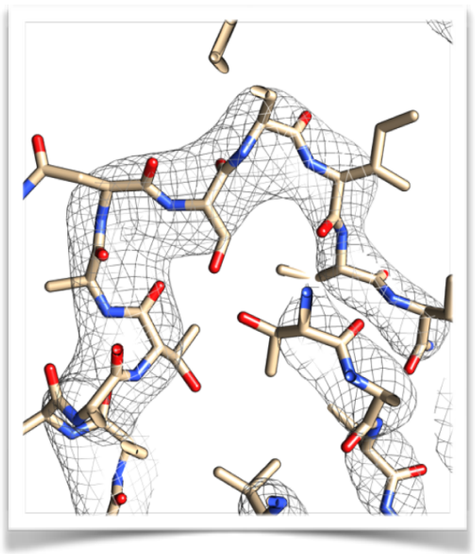
Symmetry from a map



Map segmentation

Map tools in *Phenix*

1. **Automated map sharpening**
   (improve interpretability of the map)



Automated map sharpening

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

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

Map sharpening

Principle:
Adjust the resolution dependence of the map to maximize its clarity.

Apply a resolution-dependent scale factor to Fourier coefficients:

$$A(d) = w_{\text{sharpen}}(d)e^{\frac{B_{\text{sharpen}}}{4 \cdot d^2}} + w_{\text{blurr}}(d)e^{-\frac{B_{\text{blurr}}}{4 \cdot d^2}}$$

d: resolution
A: scale factor
$B_{sharpen}$, B_{blurr}: sharpening/blurring B-factor
w: resolution-dependent weights

Map sharpening

Principle:
Adjust the resolution dependence of the map to maximize its clarity by reducing the contribution of high resolution noise.

Apply a resolution-dependent scale factor to Fourier coefficients:

$$A(d) = w_{\text{sharpen}}(d)e^{\frac{B_{\text{sharpen}}}{4 \cdot d^2}} + w_{\text{blurr}}(d)e^{-\frac{B_{\text{blurr}}}{4 \cdot d^2}}$$

Stronger weight at low resolution

$B_{sharpen} > 0$: amplitudes increase at high resolution (sharpen)

$B_{sharpen} < 0$: amplitudes decrease at high resolution (blurr)

Map sharpening

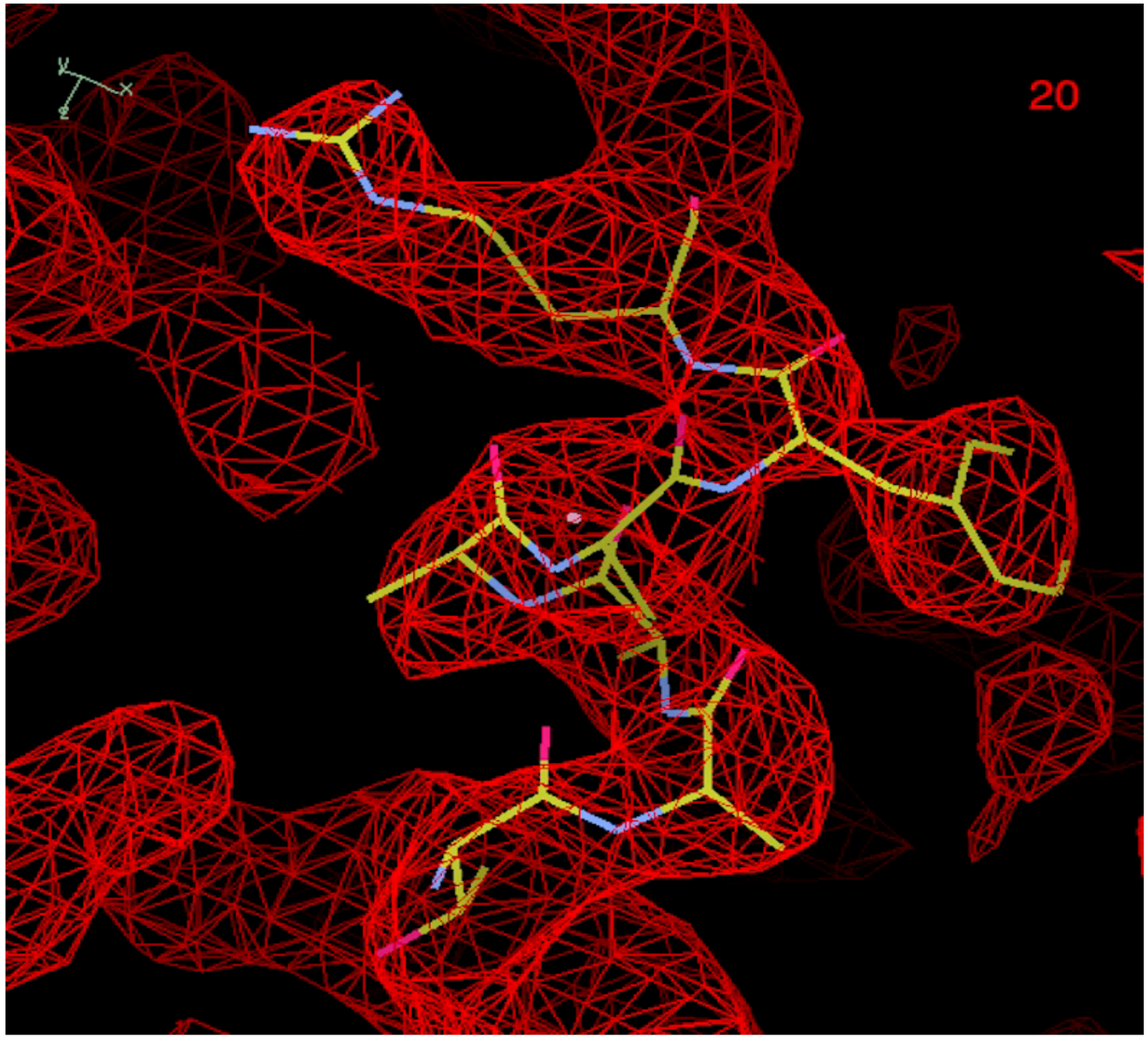
Principle:
Adjust the resolution dependence of the map to maximize its clarity by reducing the contribution of high resolution noise.

Apply a resolution-dependent scale factor to Fourier coefficients:

$B_{sharpen} > 0$: amplitudes increase at high resolution (sharpen)

$B_{sharpen} < 0$: amplitudes decrease at high resolution (blurr)

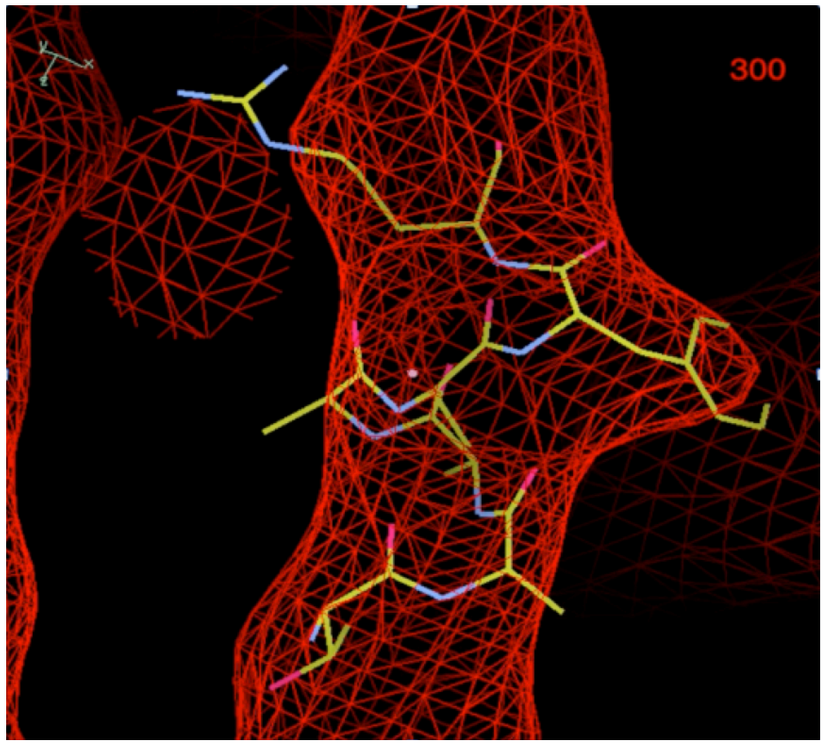
Map sharpening



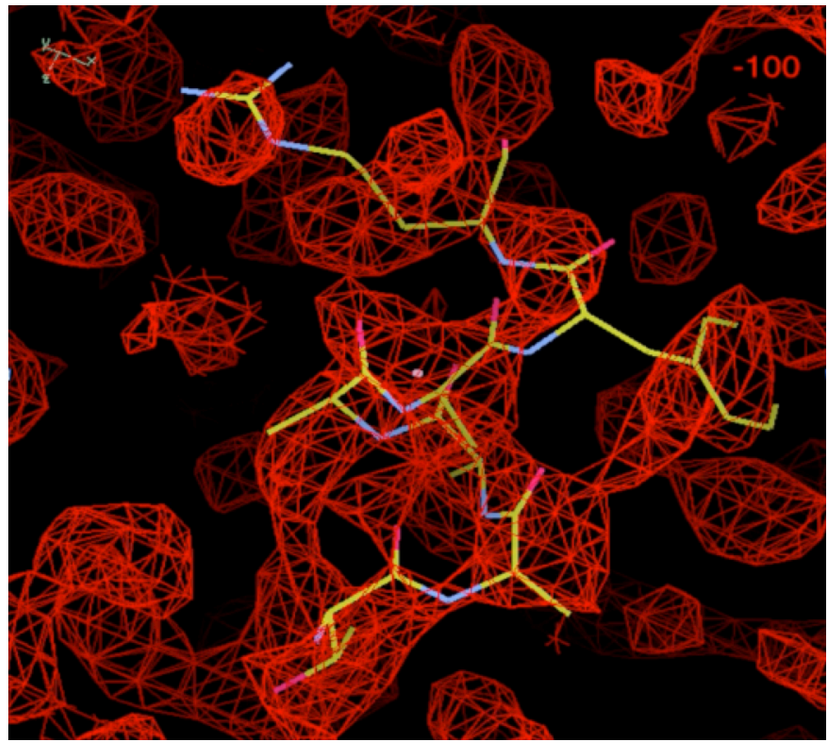
Map sharpening

High connectivity

Low connectivity



Find best compromise between connectivity and detail



Few details

Lots of detail

Map sharpening approach

Create series of maps for different overall B-values

Analyze maps for detail and connectivity

Set contour level enclosing 20% of molecular volume

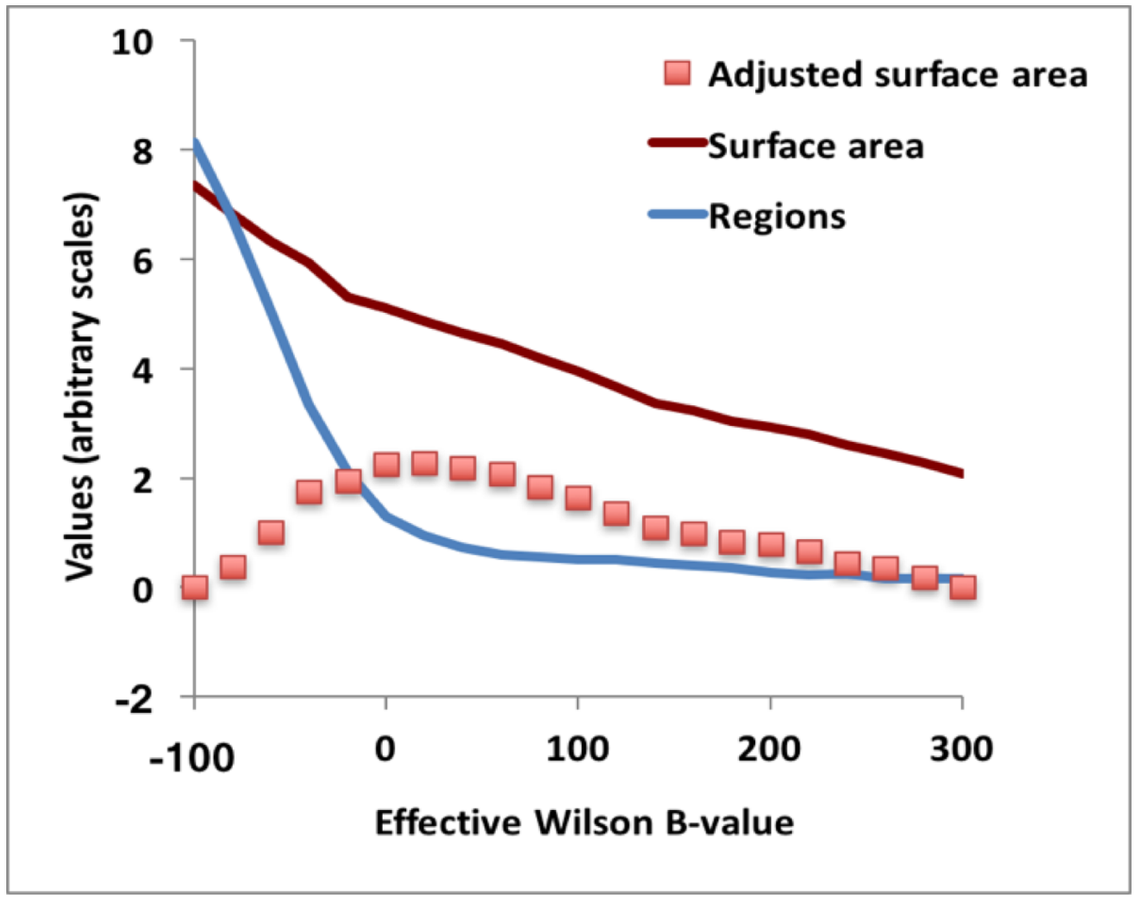
Calculate surface area of contours

Count number of distinct regions enclosed by contours

Choose map with maximum of adjusted surface area

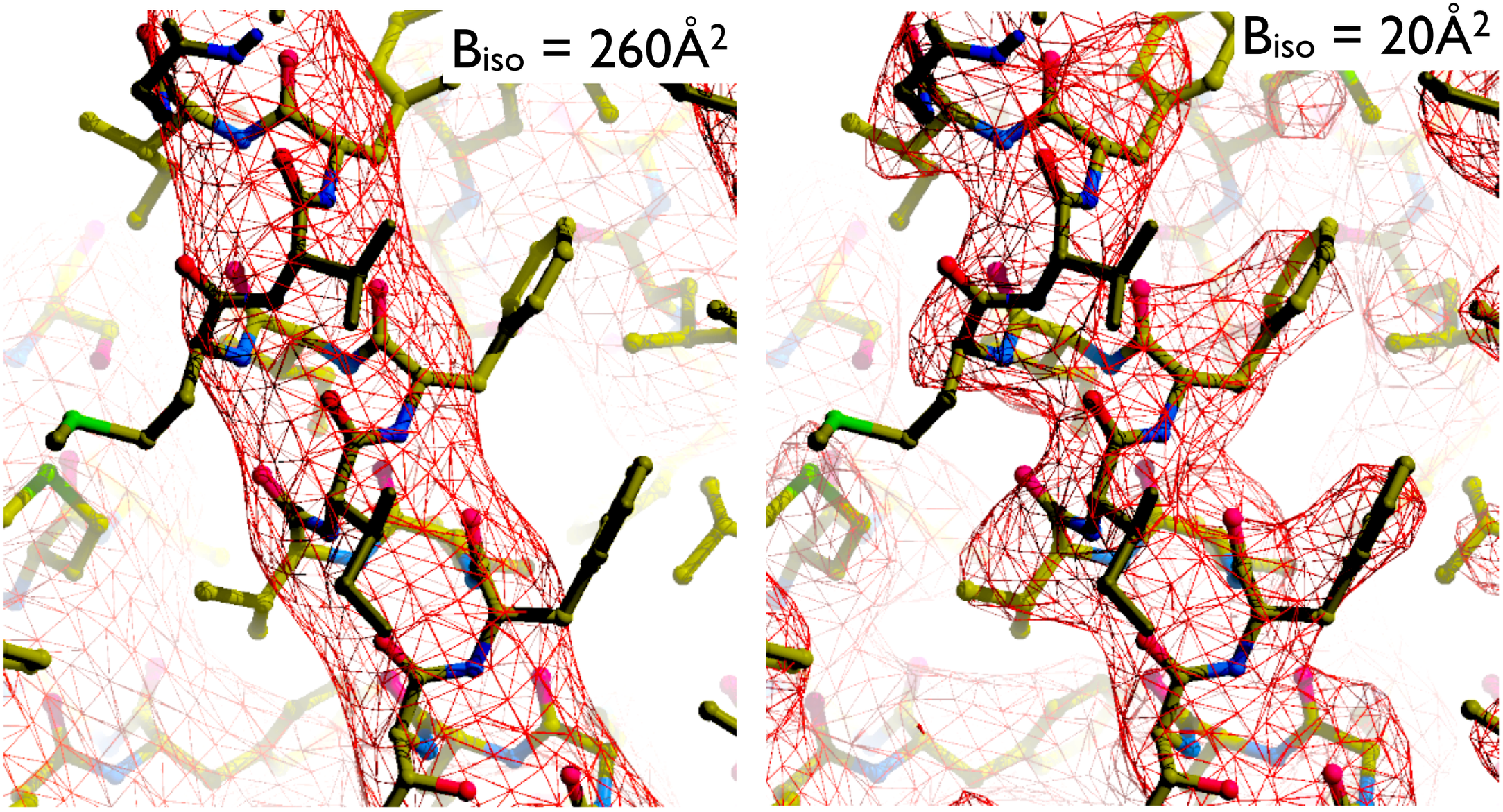
adjusted area = surface area – weight * number of regions

connectivity



detail

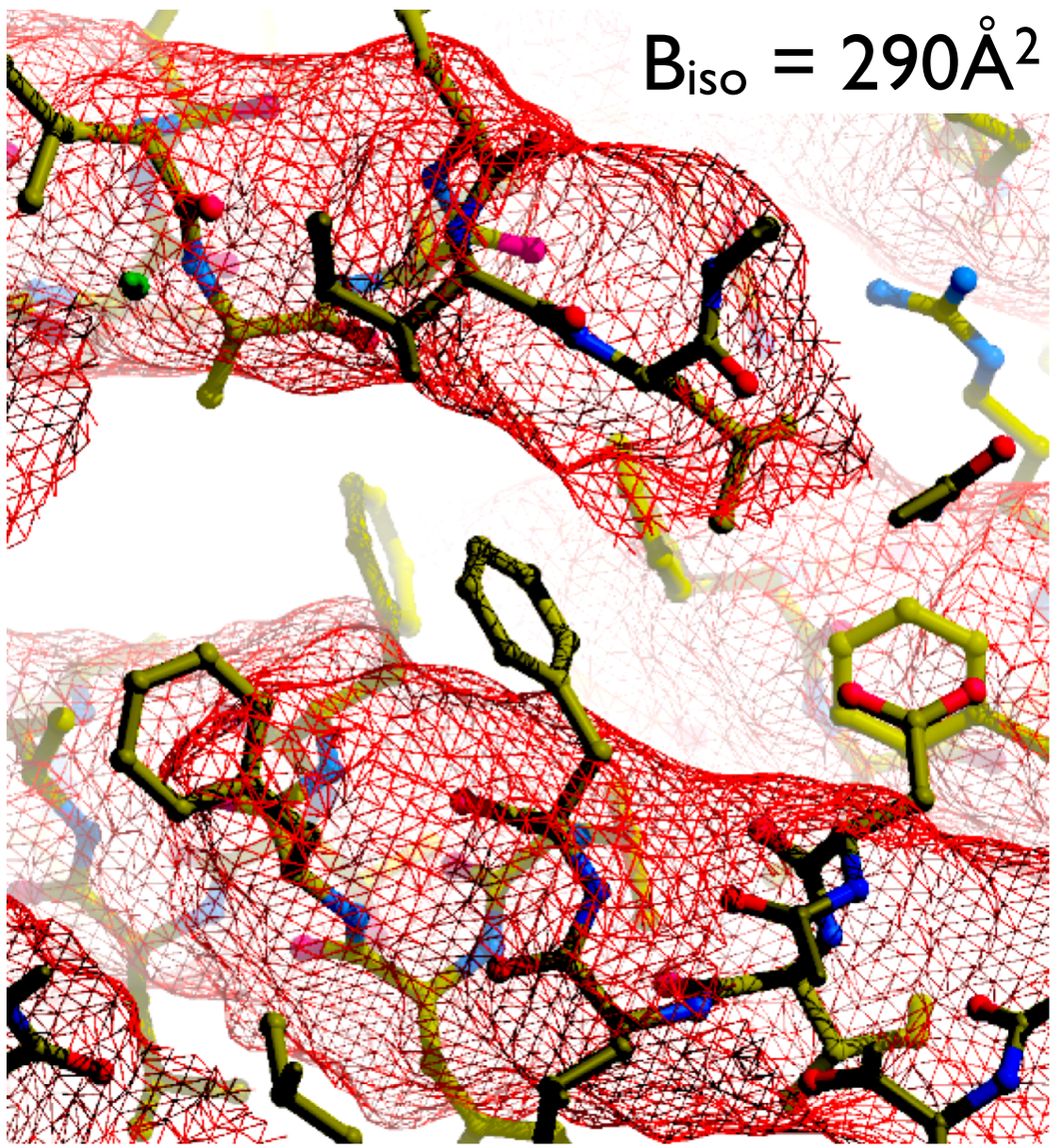
Map sharpening: Examples

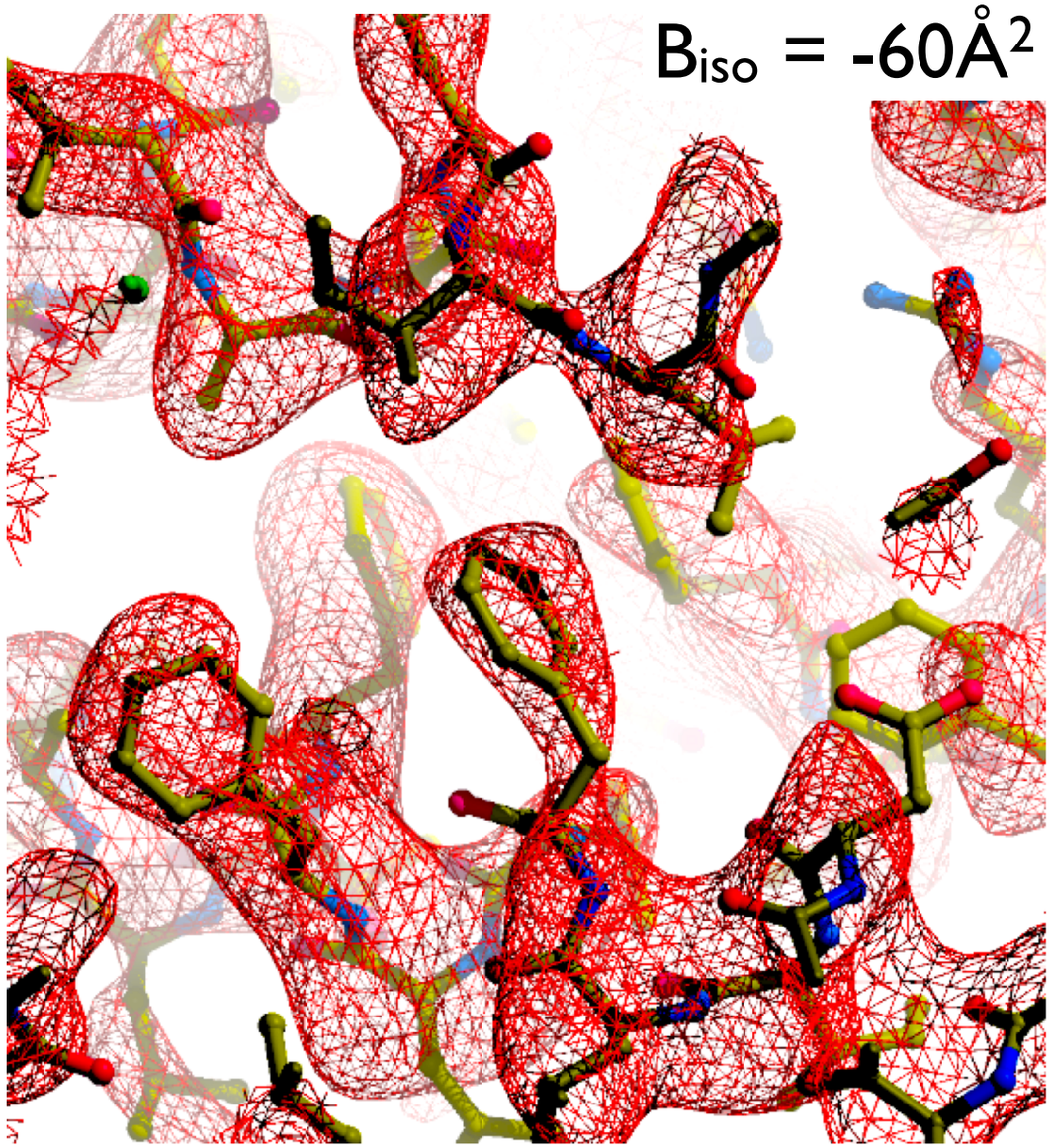


Deposited Map *Autosharpened Map*

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

Map sharpening: Examples





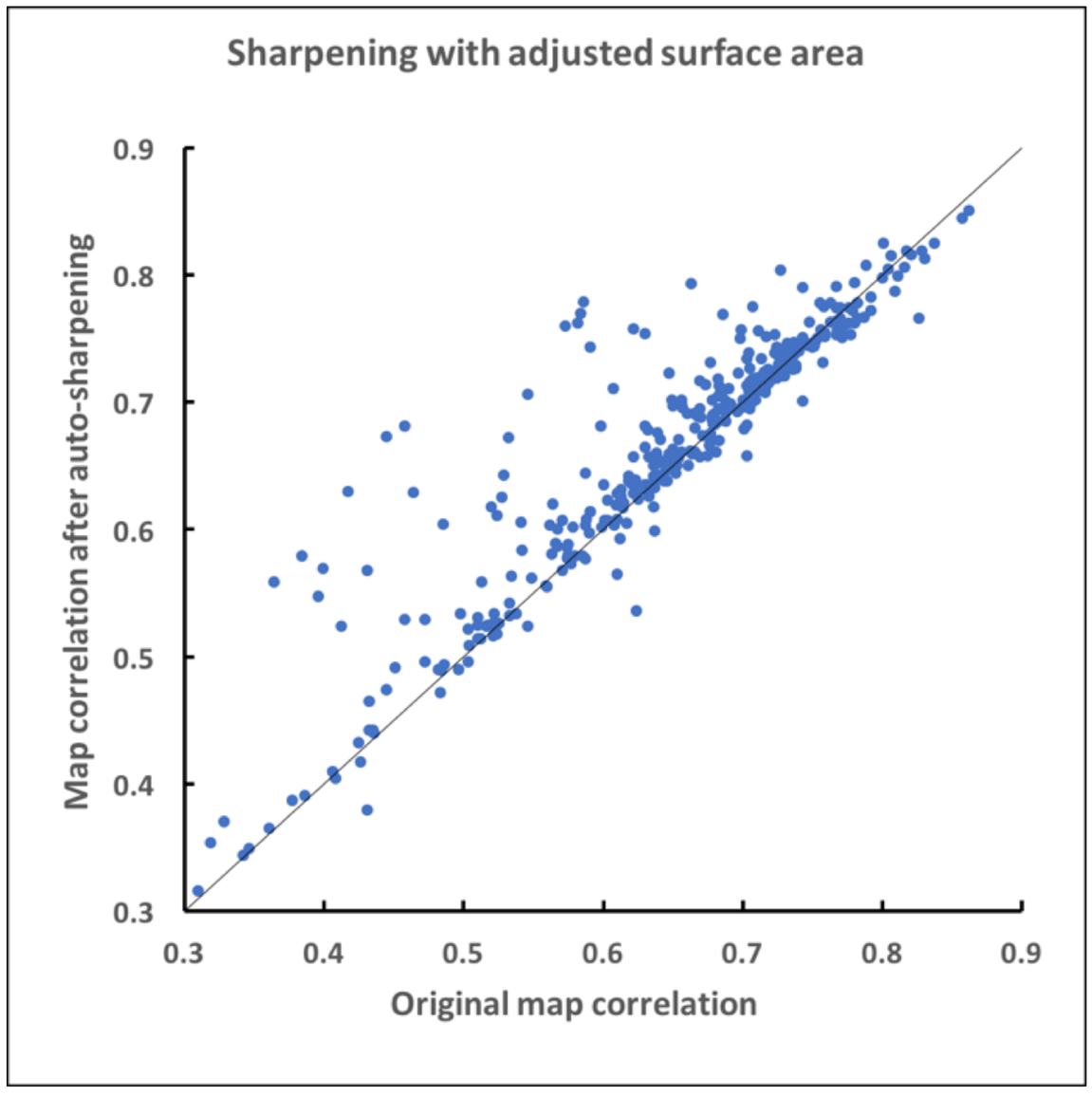
Deposited Map

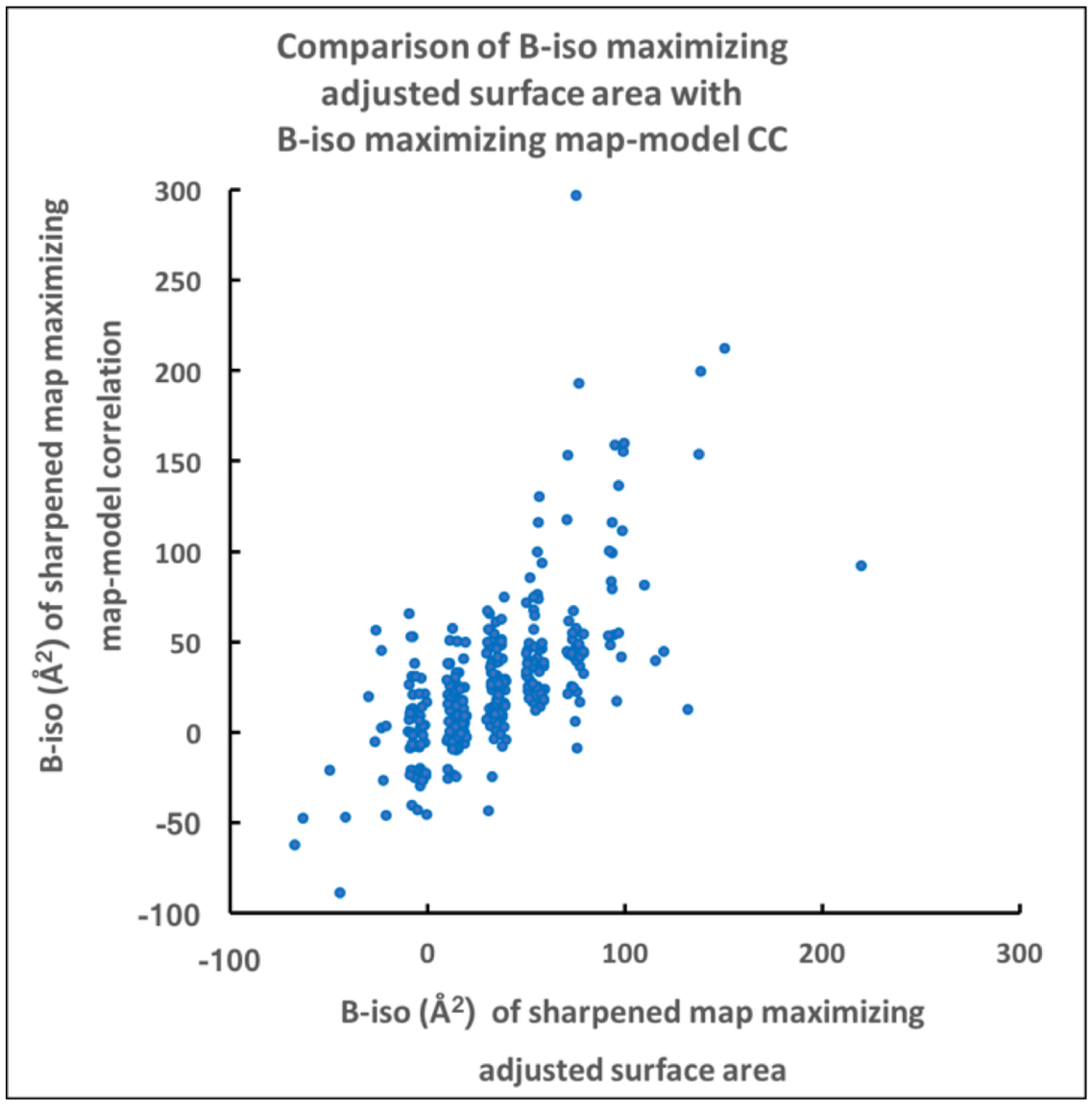
Autosharpened Map

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

Impact of sharpening

Sharpening aims to get an interpretable map, but what about map-model correlation?

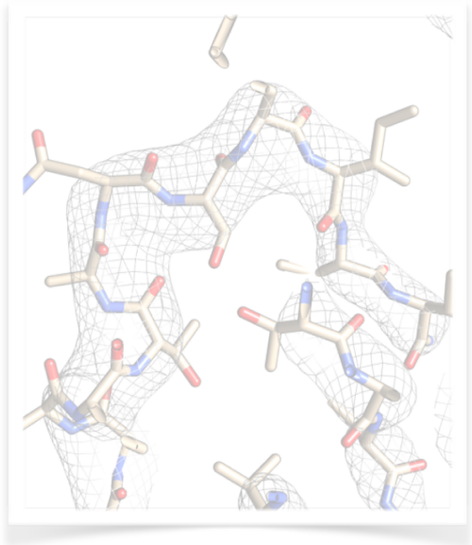




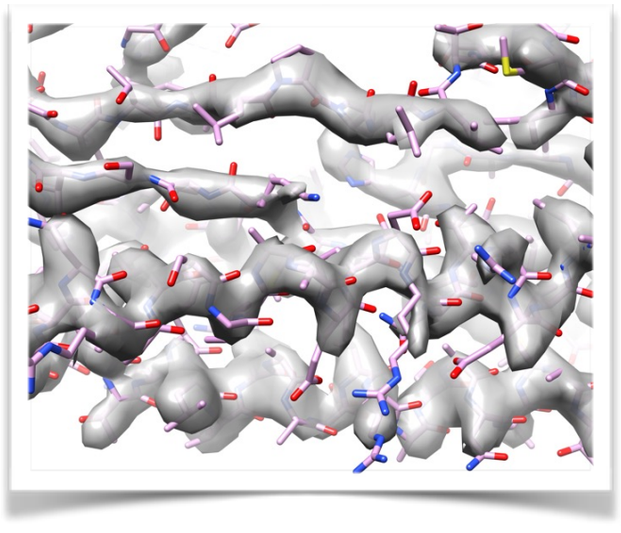
Terwilliger et al. Automated map sharpening by maximization of detail and connectivity. *Acta Cryst* 2018, **D74**:545-559

Map tools in *Phenix*

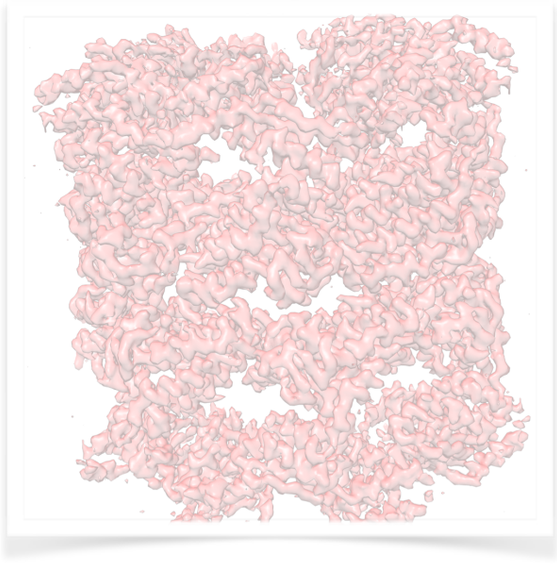
2. **Cryo-EM density modification**
(improve interpretability of the map)



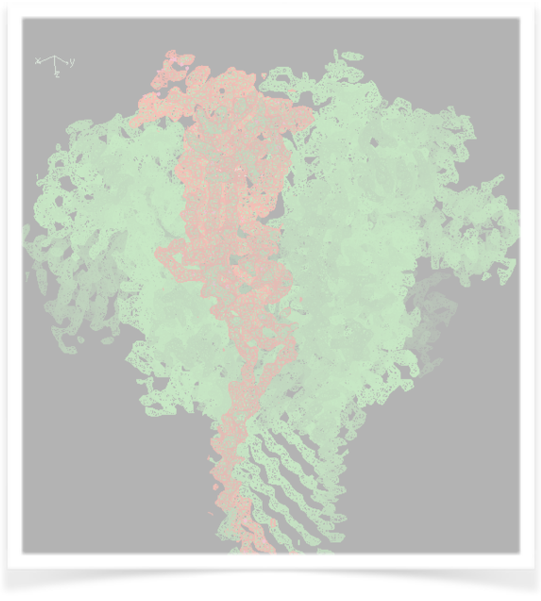
Automated map sharpening



EM density modification



Symmetry from a map



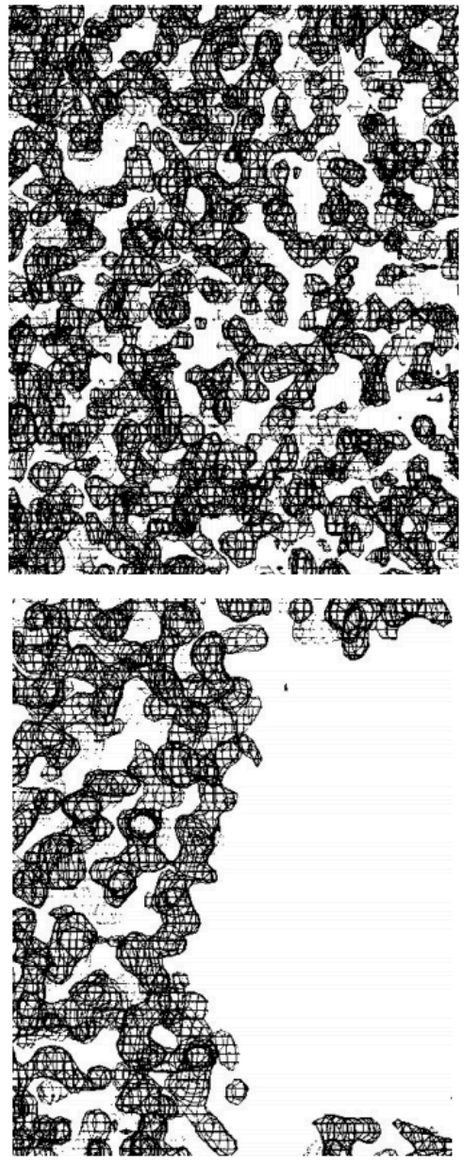
Map segmentation

Terwilliger et al. https://www.biorxiv.org/content/10.1101/845032v1.full.pdf

Density modification: Crystallography

Crystallography:

- Amplitudes are measured quite accurately
- Phases have large errors



Modify phases to produce a map most consistent with what we know about macromolecular structures:

- Solvent density distribution (Solvent flattening)
- Atomicity and positivity
- Macromolecular density distributions (histogram matching)
- Similarity between molecules (symmetry averaging)

Density modification: Cryo-EM

Cryo-EM:

- Both amplitudes and phases have errors
- Half-maps are available

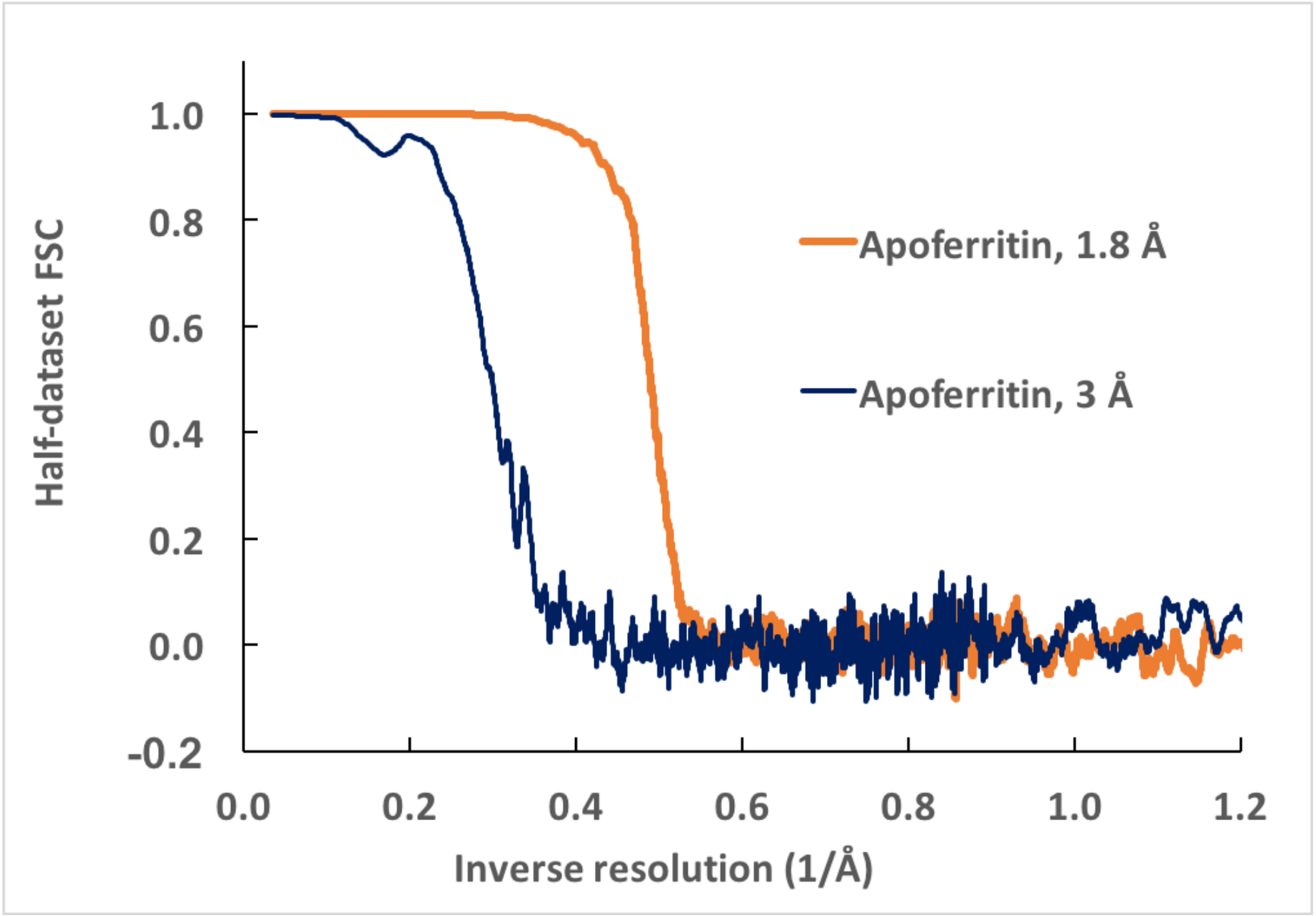
Modify phases to produce a map most consistent with what we know about macromolecular structures:

- Macromolecular density distributions (histogram matching)

Terwilliger et al. https://www.biorxiv.org/content/10.1101/845032v1.full.pdf

Testing density modification

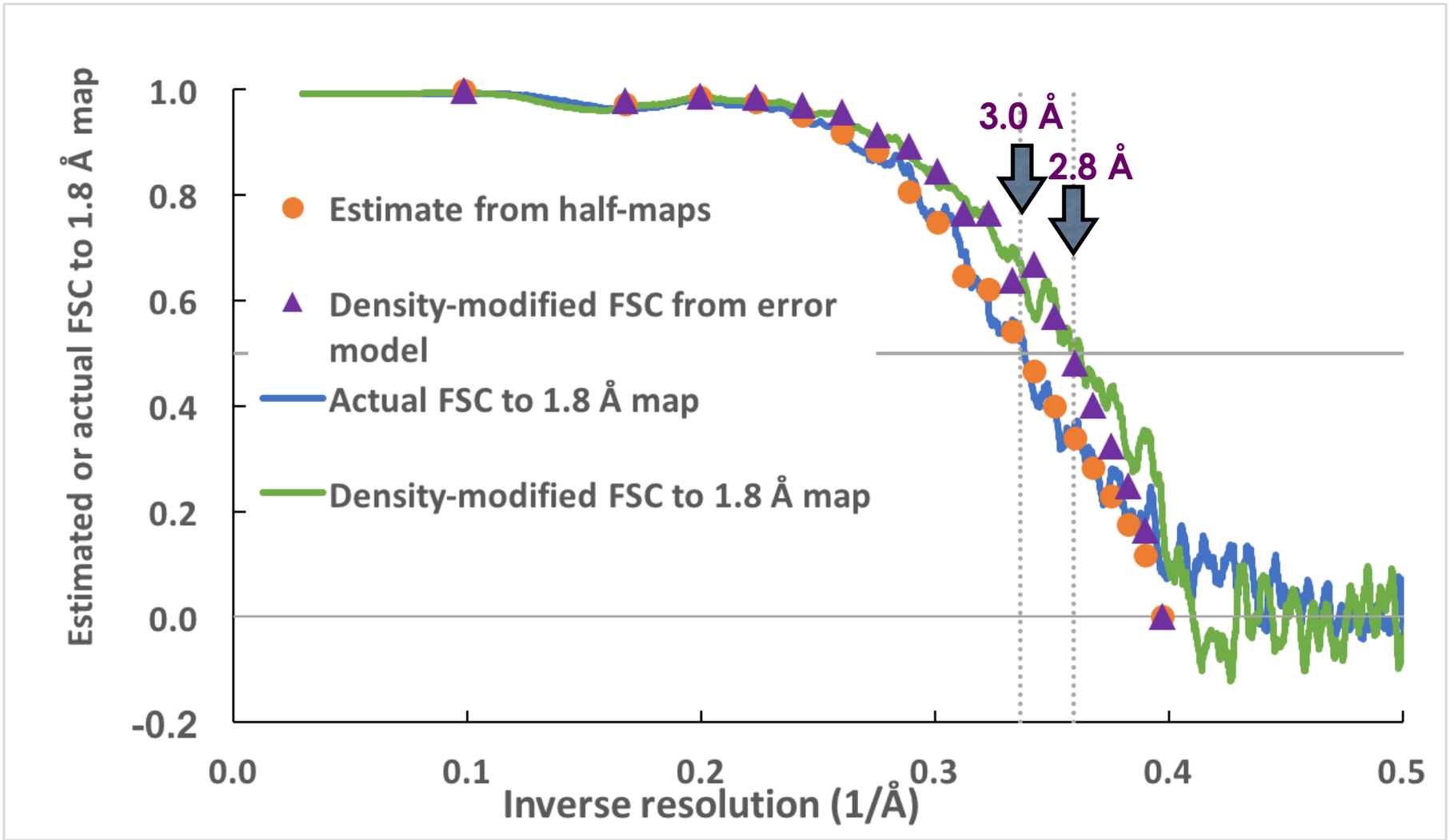
Use 1.8 Å data as ”perfect” for comparison with 3 Å data



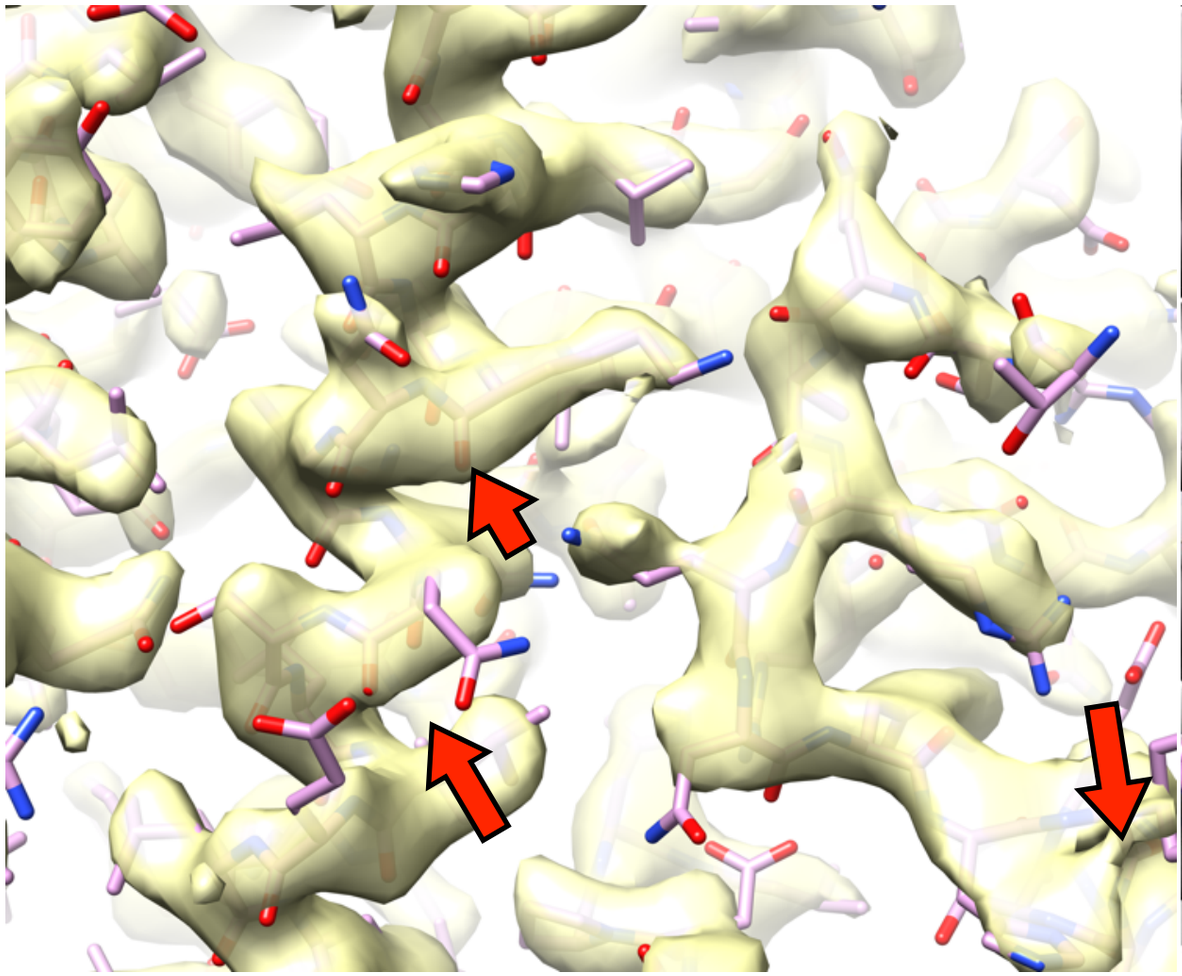
Data from Kaiming Zhang, Greg Pintilie, Shanshan Li, Wah Chiu

Testing density modification

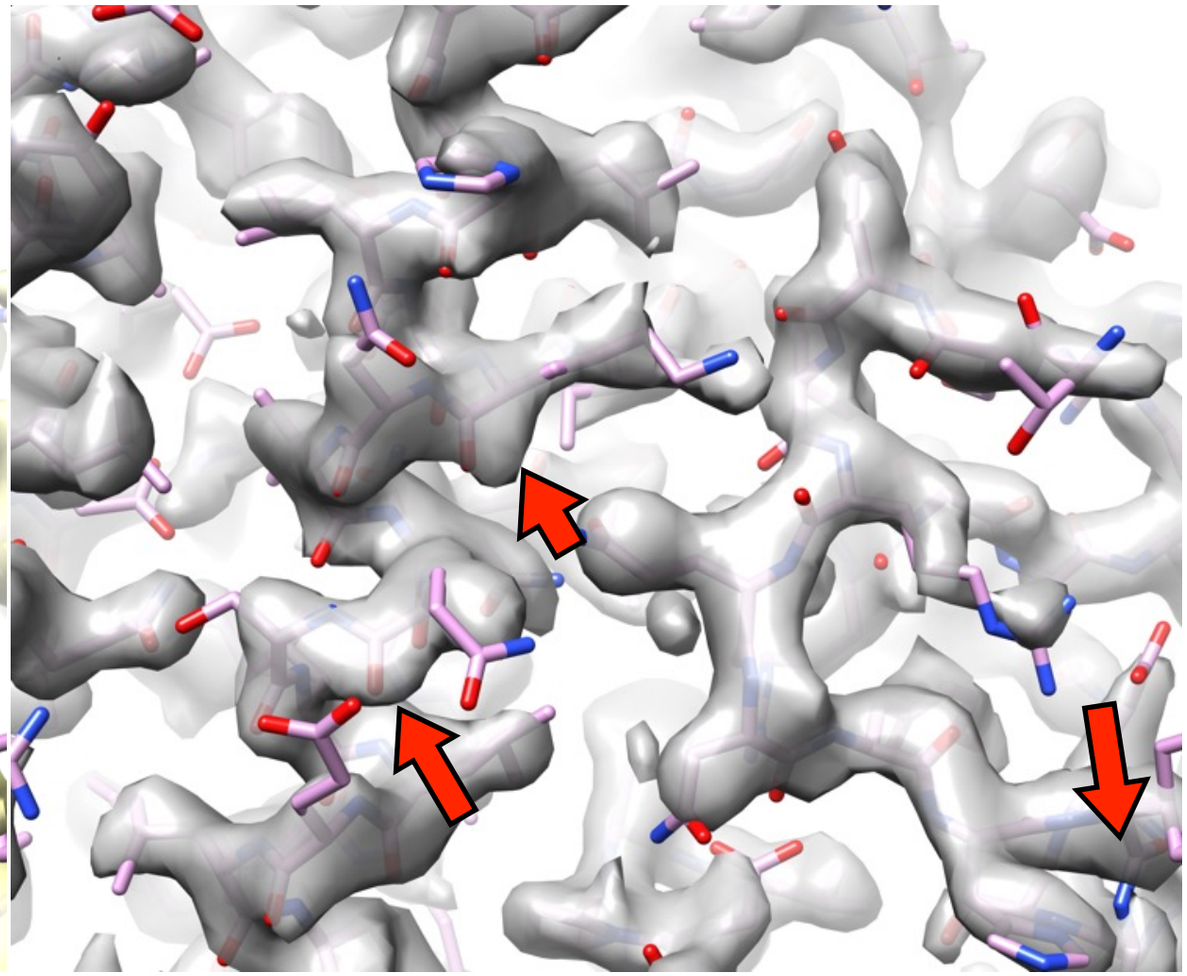
2 half-maps + 2 map-phasing half-maps → *Error model* → Density-modified map



Testing density modification

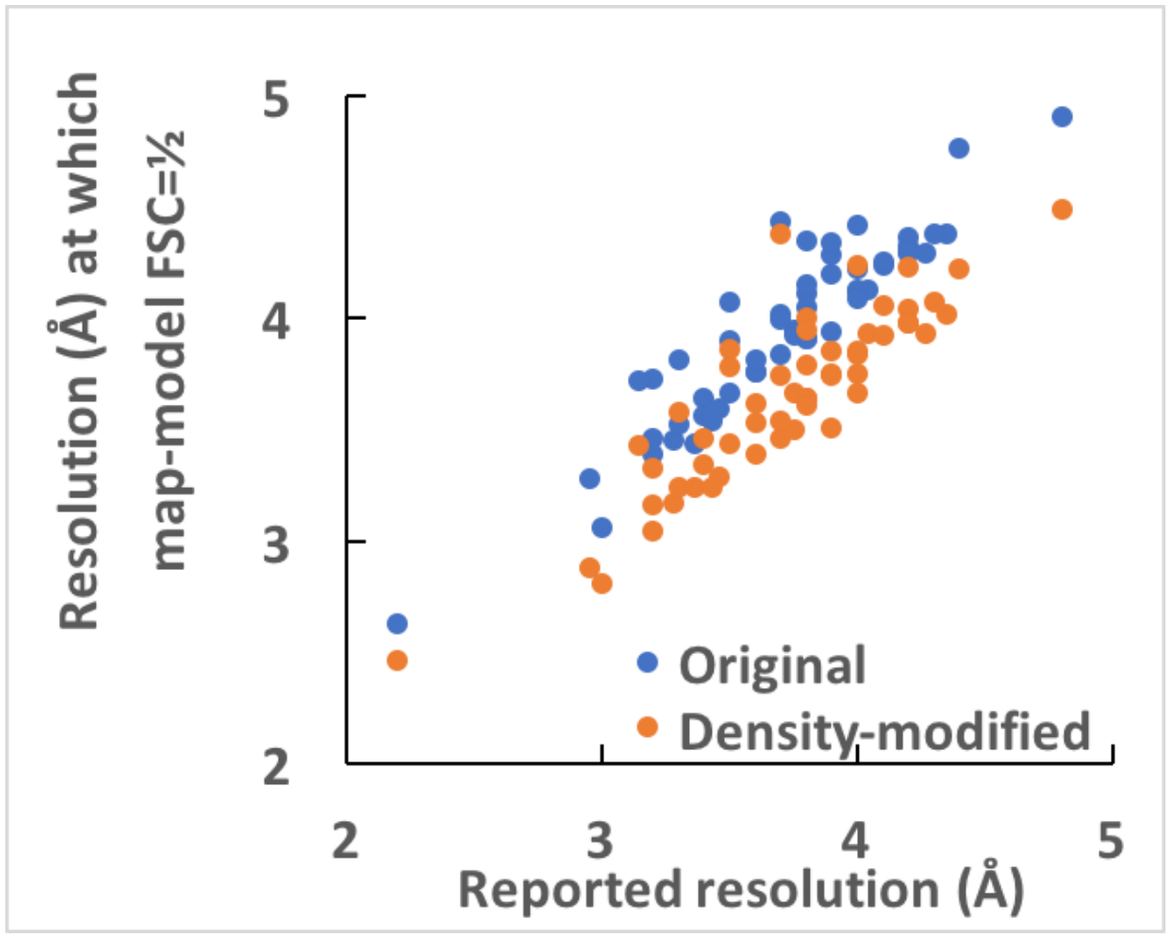


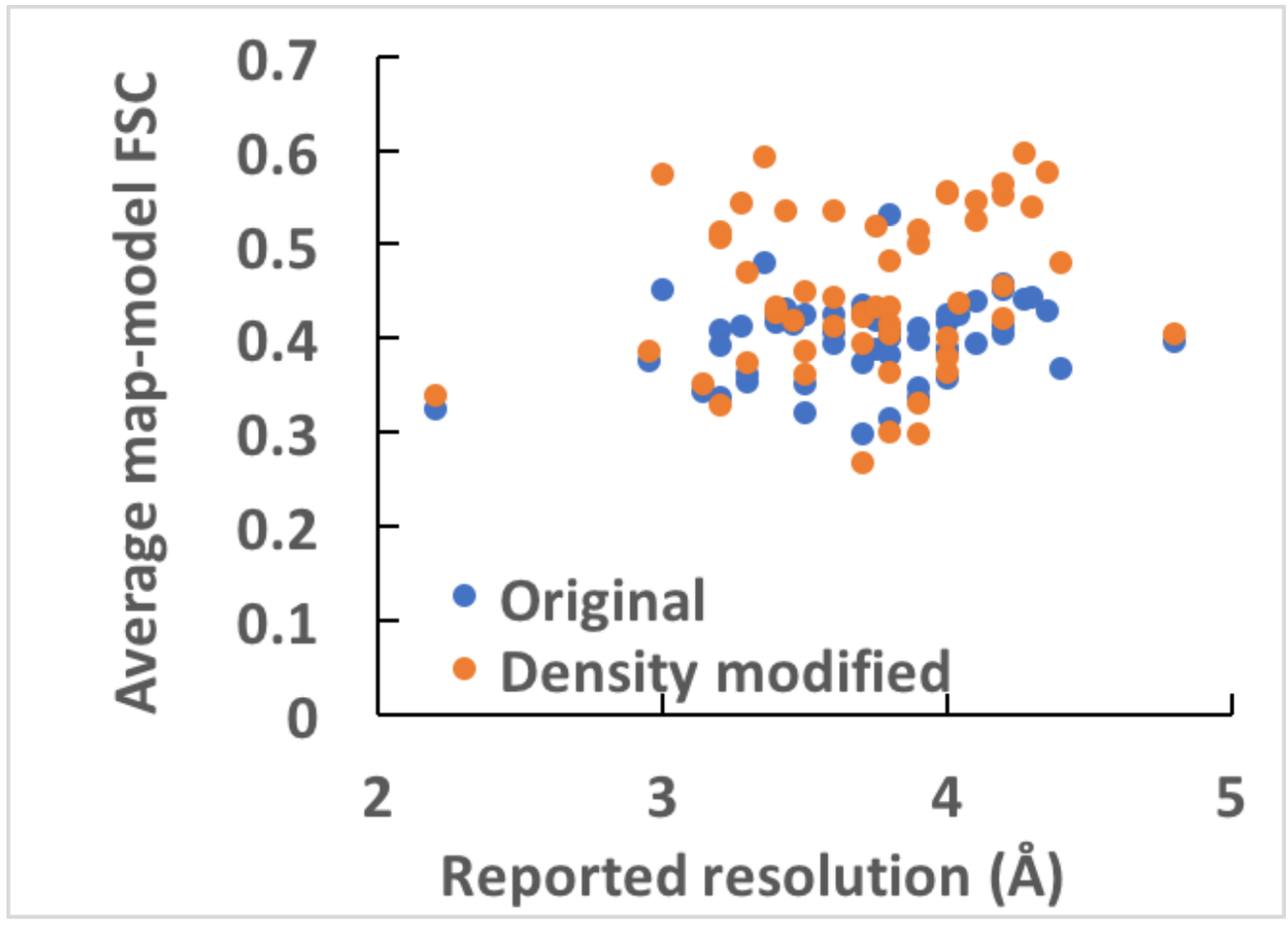
Original map, model-sharpened



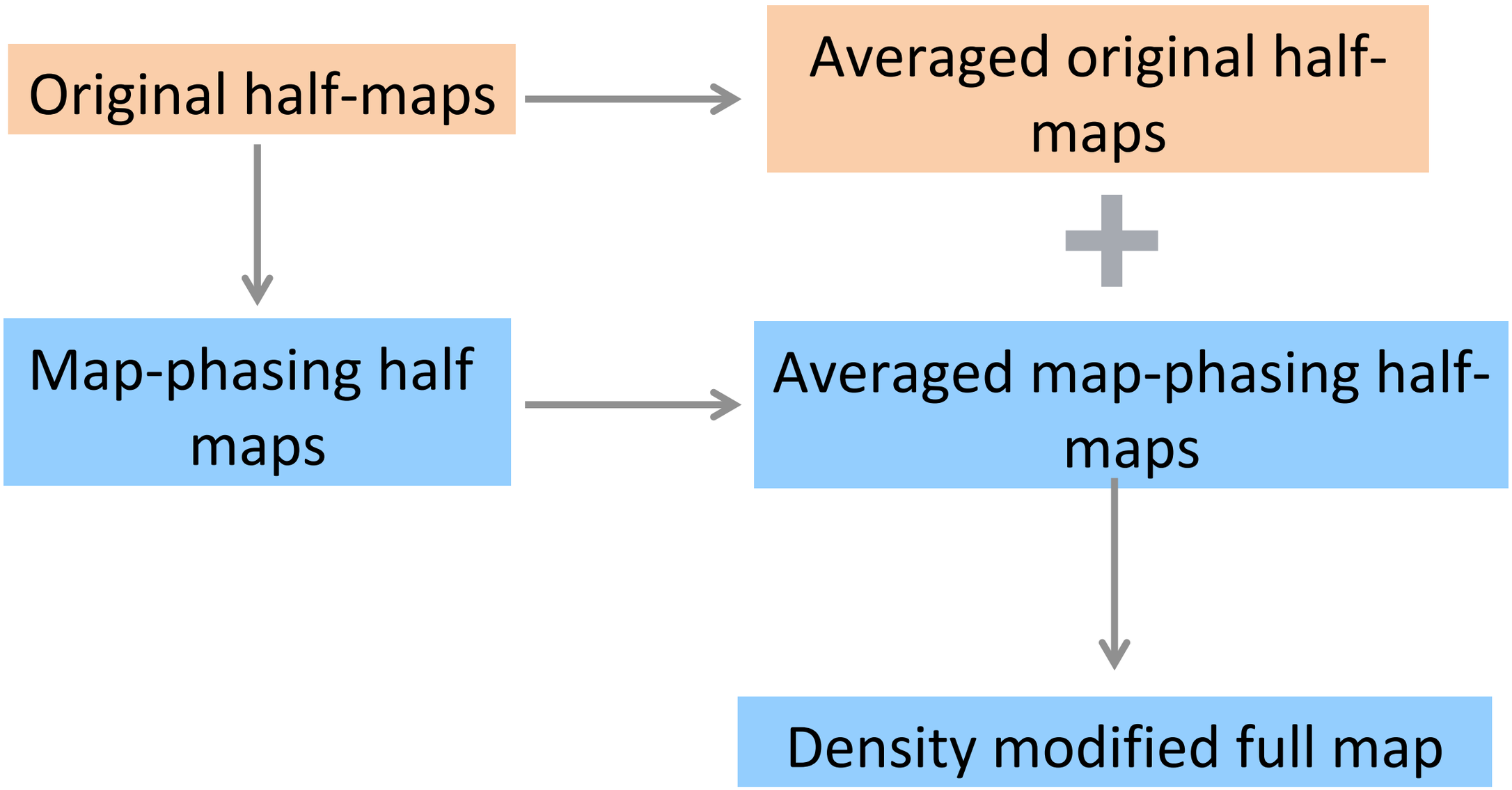
Density-modified map, model-sharpened

Testing density modification





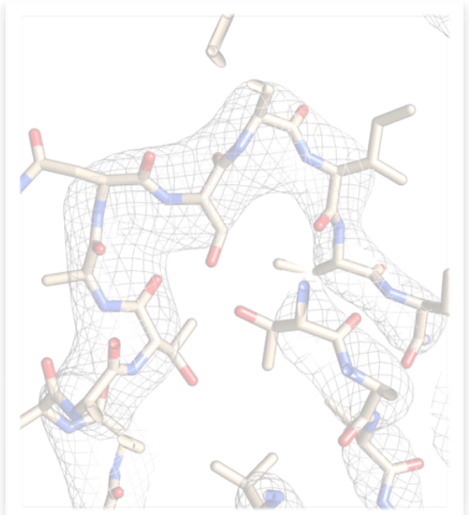
Map sharpening



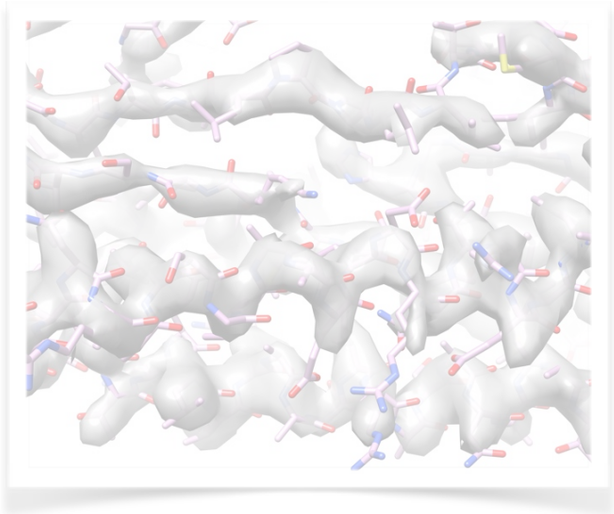
Map tools in *Phenix*

3. Map segmentation

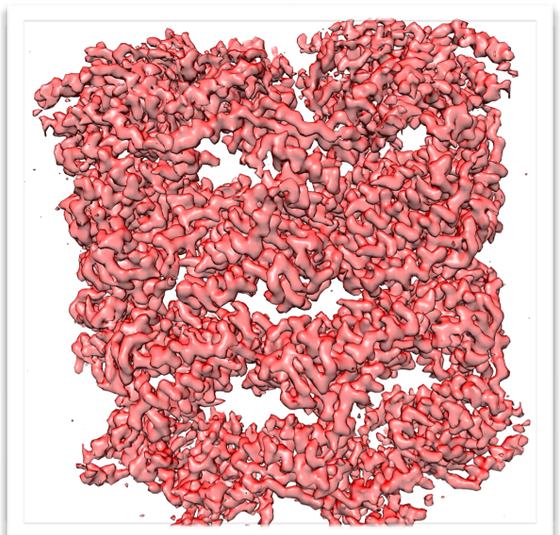
(Find asymmetric unit of the map)



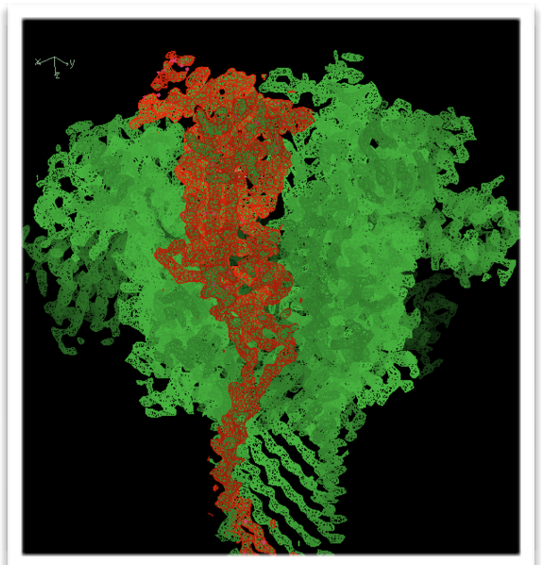
Automated map sharpening



EM density modification



Symmetry from a map

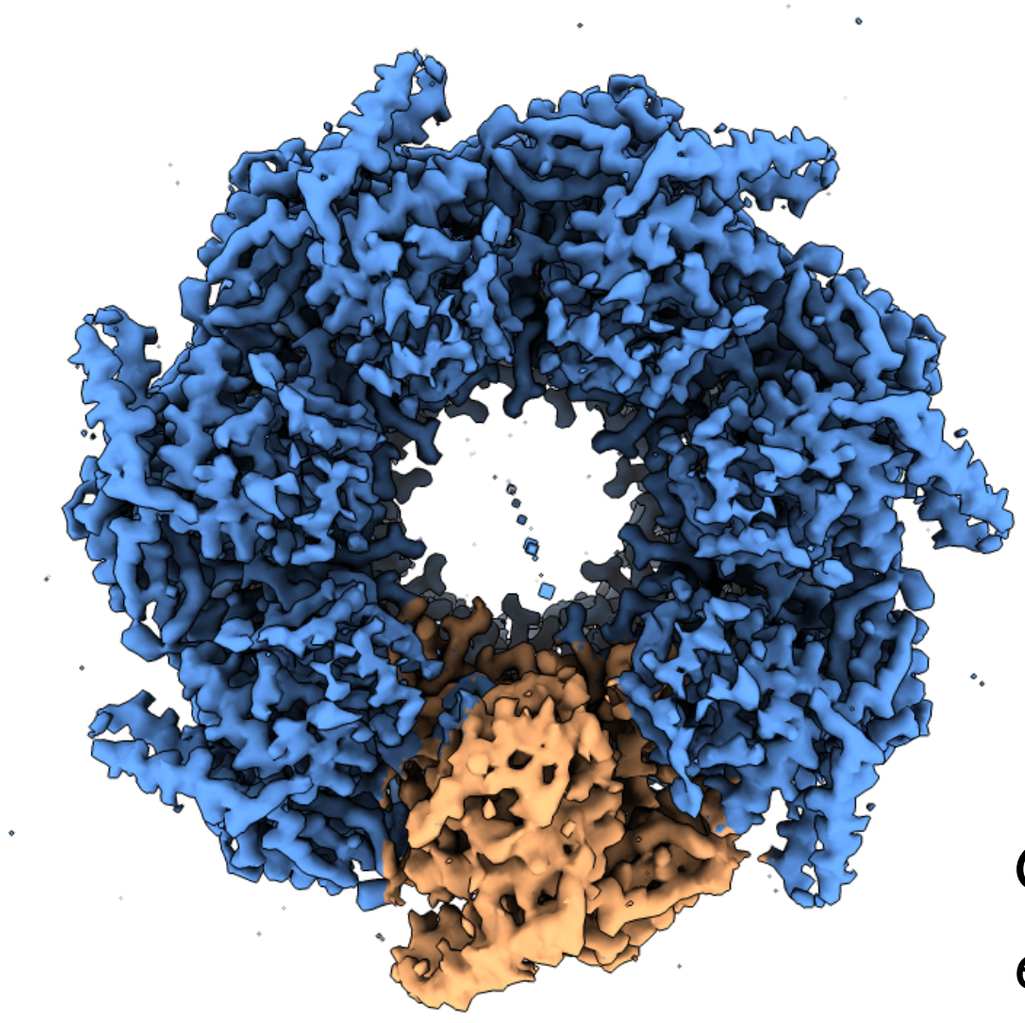


Map segmentation

Map symmetry

Cryo-EM maps may have symmetry

It is more efficient to work with the unique part of a map

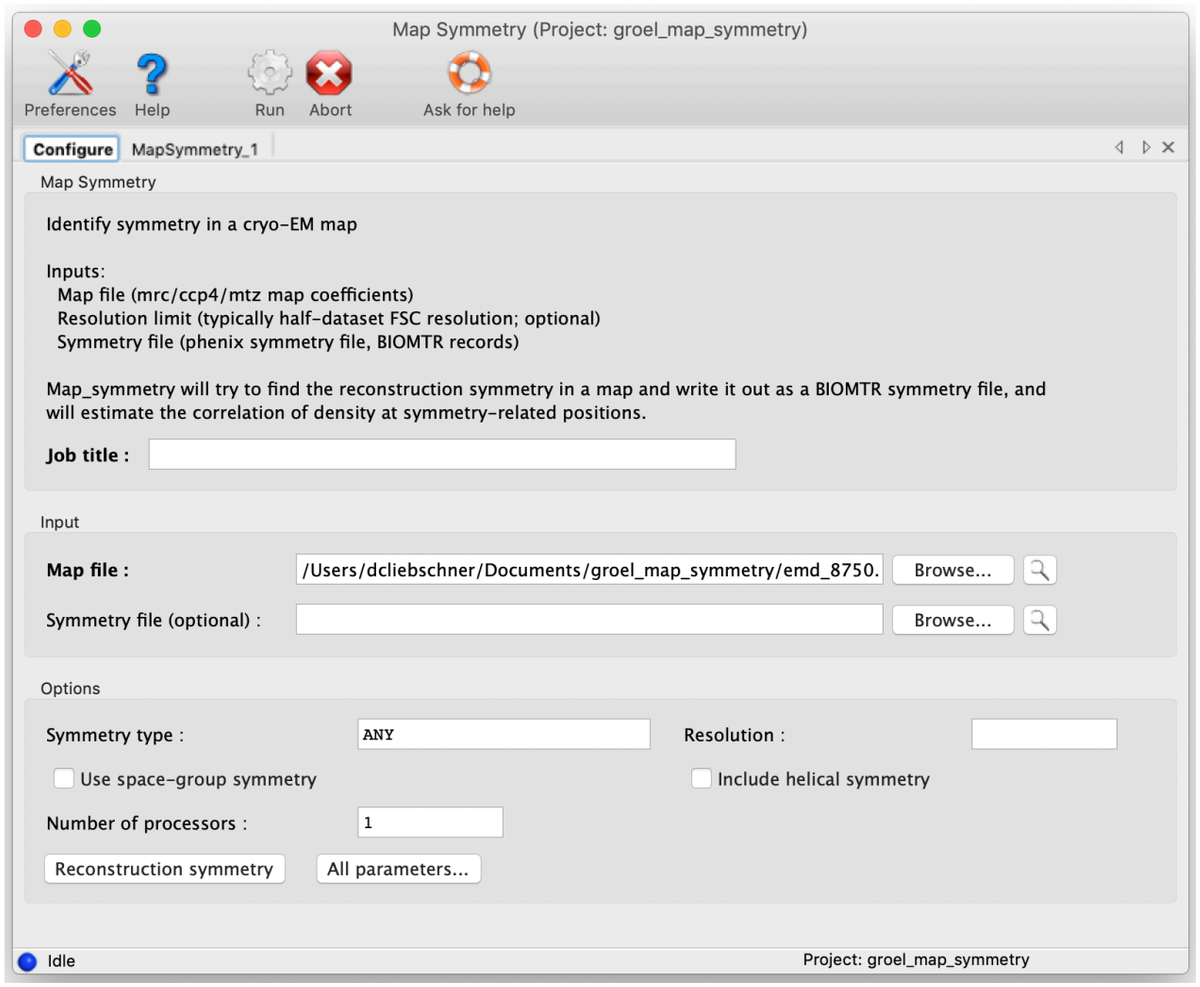


GroEL, 3.5 Å resolution

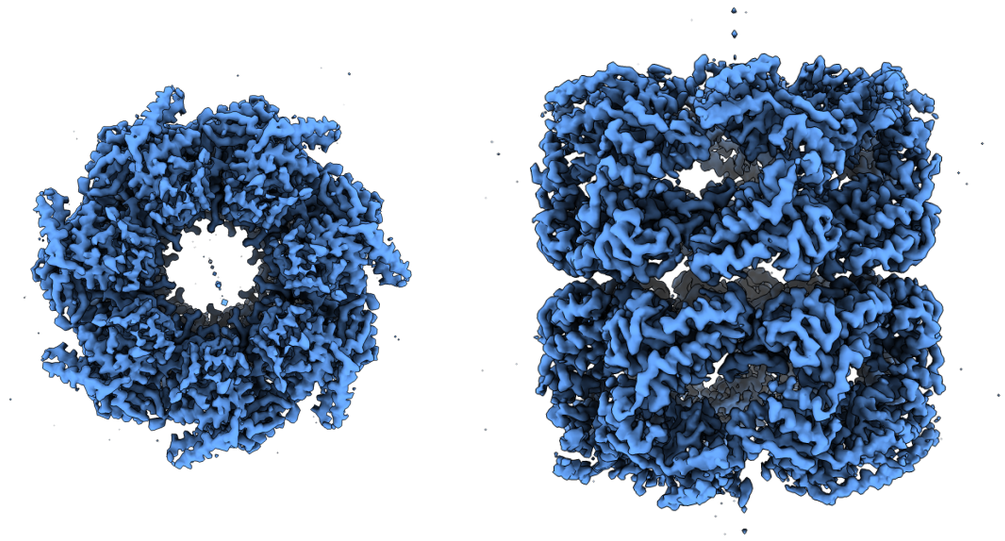
emd_8750

Map symmetry

Map symmetry (GUI)
phenix.map_symmetry



Map
Optional: symmetry



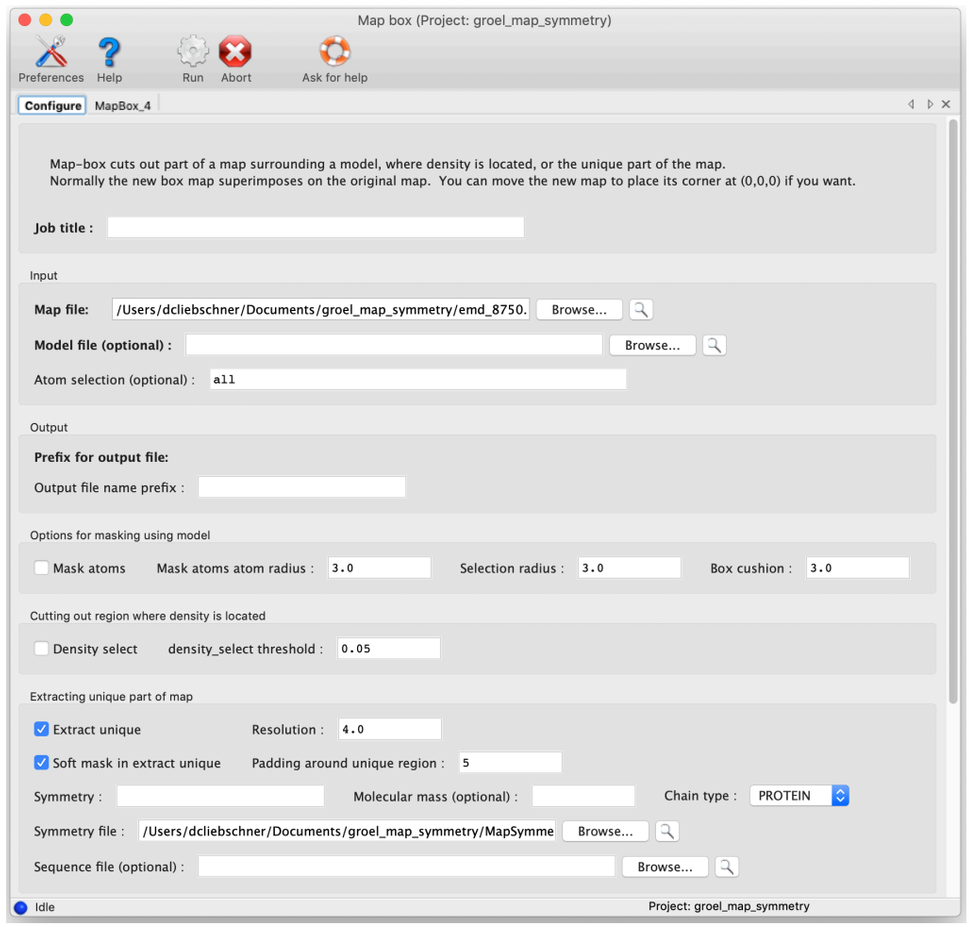
14 symmetry operators

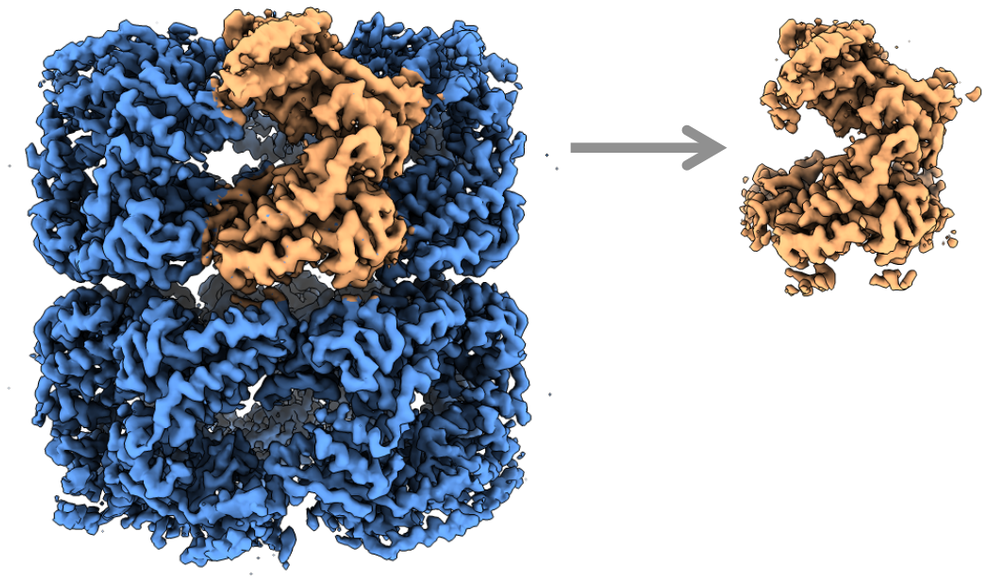
File with symmetry operators

Map box

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

Map box (GUI)
phenix.map_box



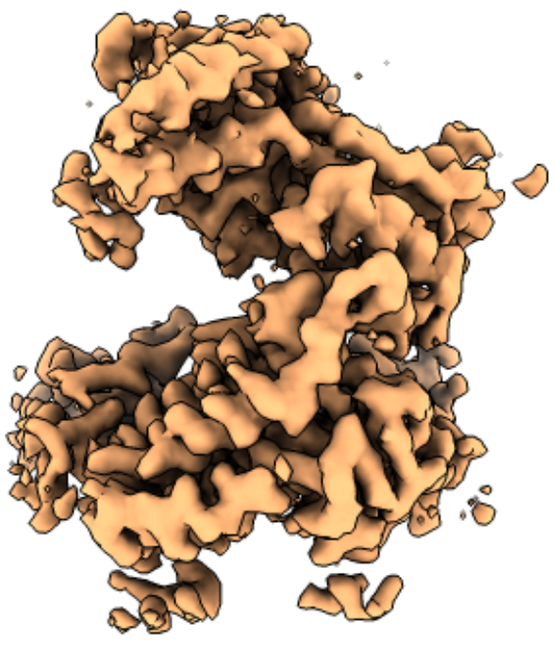


Map, (model)
Symmetry operators

Density of unique part of the map

Map manipulations: next steps

Once the best interpretable map has been obtained,
we try to build/dock a molecular model into it.



Docking

Automated
model building

Refinement

Schedule

8:30 AM: Introduction to Phenix and overview of tools for cryo-EM
8:45 AM: Map tools (density modification, sharpening, map symmetry)
9:30 AM: Break and computer setup
10:00 AM Model building (docking, ab initio building)
10:30 AM: Atomic model refinement
11:30 AM: Validation (map, model, model to map fit)

12:00: Lunch

1:00 PM: Introduction to the GUI and setup
1:15 PM Map improvement and model building
(DM, sharpening, symmetry, segmentation + automated model building)
3:00 PM: Break
3:30 PM: Refinement and validation
4:30 PM: User questions, more select tutorials, discussion, etc
5:30 PM: Finish