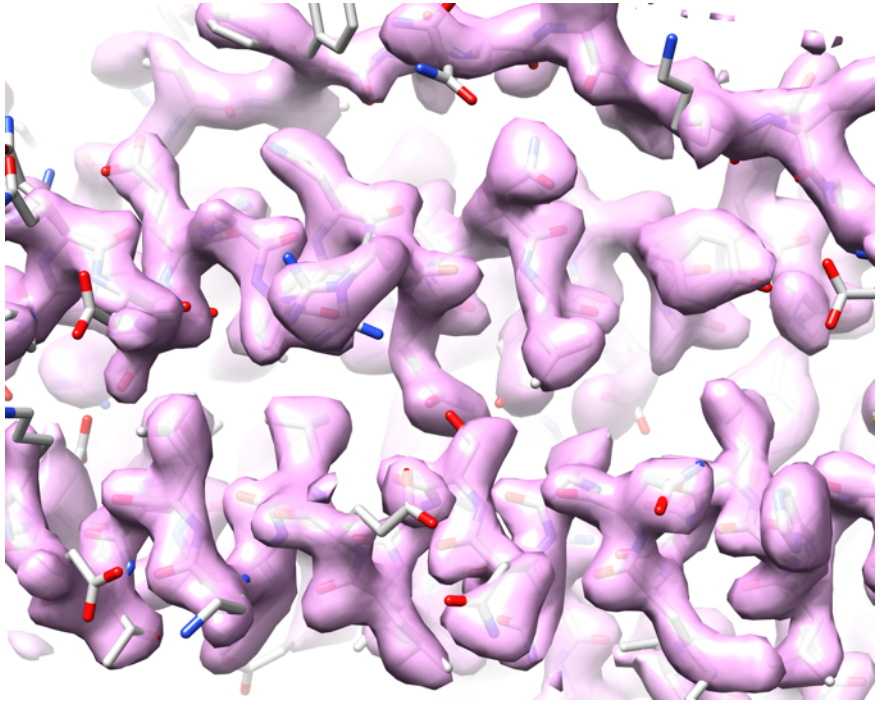
Cryo-EM map improvement



Phenix Workshop

Sept. 15, 2020

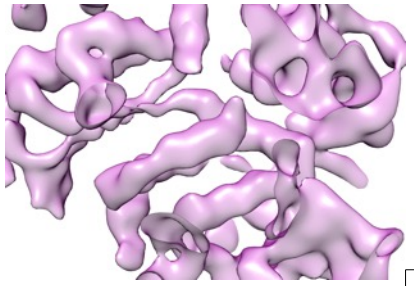
Tom Terwilliger, New Mexico Consortium
Randy Read, Cambridge University
Steve Ludke, Baylor College of Medicine
Pavel Afonine, Paul Adams, Dorothee Liebschner, Lawrence Berkeley National Laboratory

Los Alamos NATIONAL LABORATORY

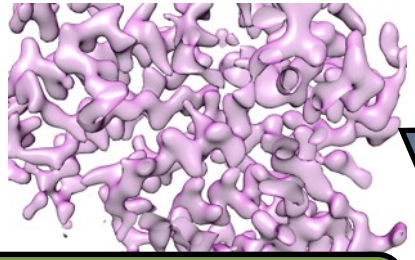
BERKELEY LAB

UNIVERSITY OF CAMBRIDGE

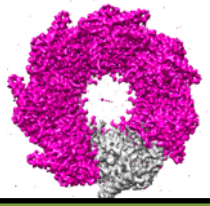
Cryo-EM tools in Phenix



Starting map



Map improvement

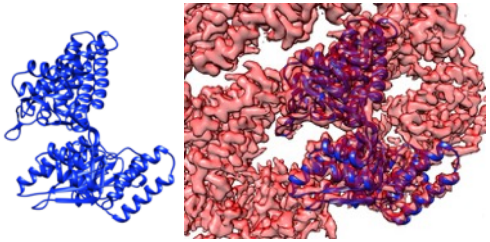


Map symmetry

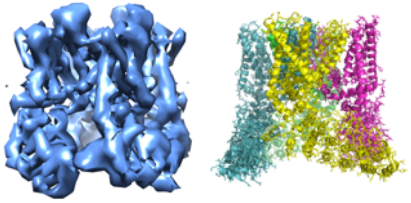
Map manipulation

Extract unique part

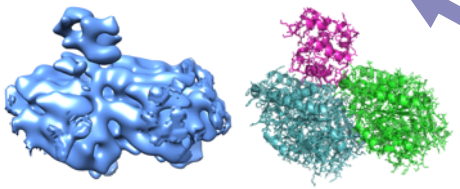
Docking, model-building



Refinement

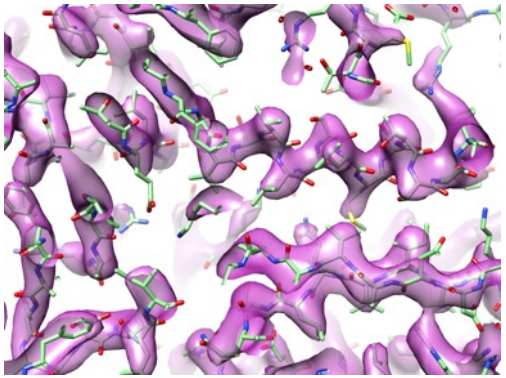


Validation



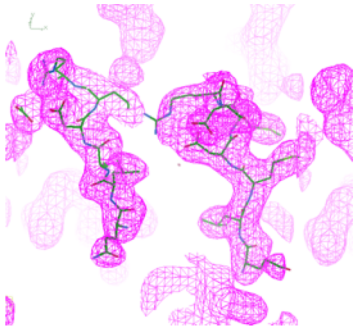
Cryo-EM map improvement tools

Automatic map sharpening

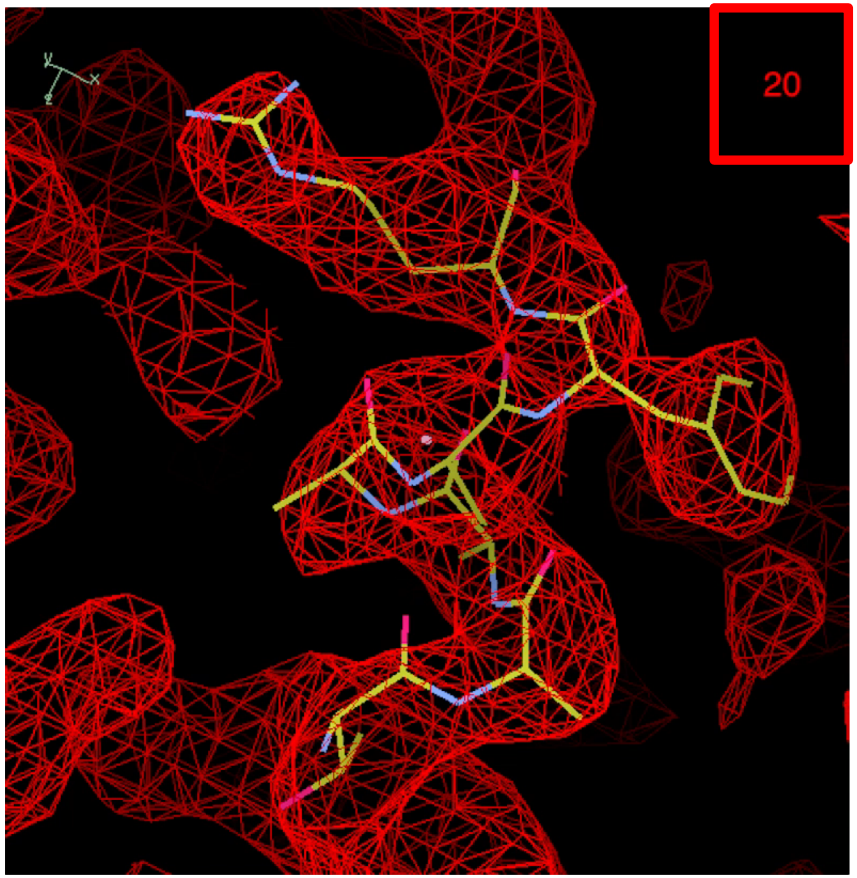


Density modification

Combining focused maps



Automatic map sharpening



Maximize detail in map

… and connectivity of map

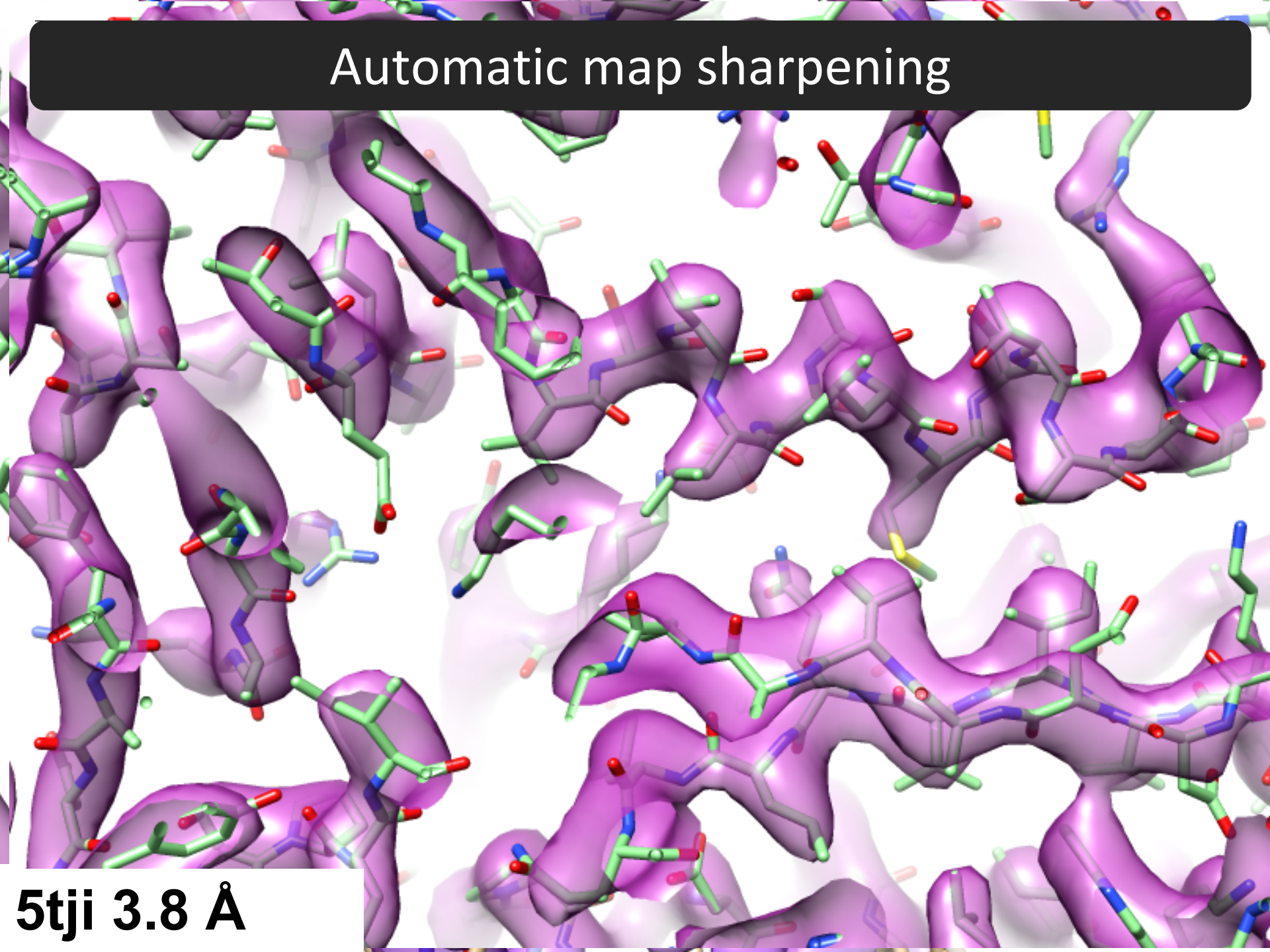
Adjusted surface area

↓

Optimally sharpened map

Terwilliger, et al. (2018) Acta Cryst. D 74, 545-559.

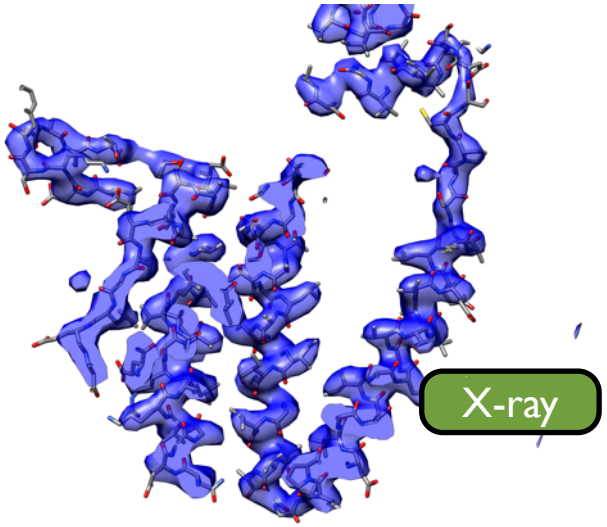
Automatic map sharpening



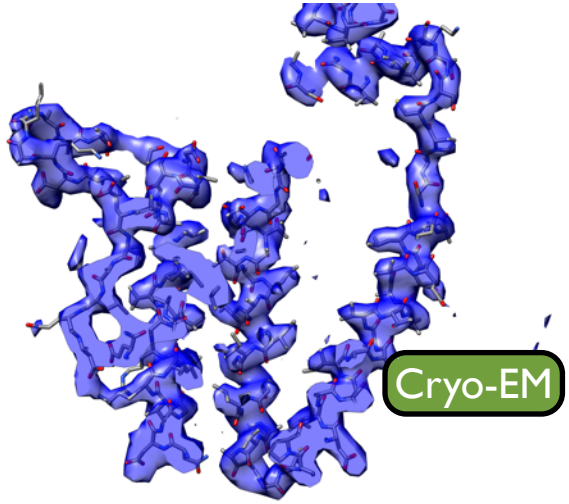
5tji 3.8 Å

Improvement of Cryo-EM maps by density modification

A standard method for improvement of X-ray maps

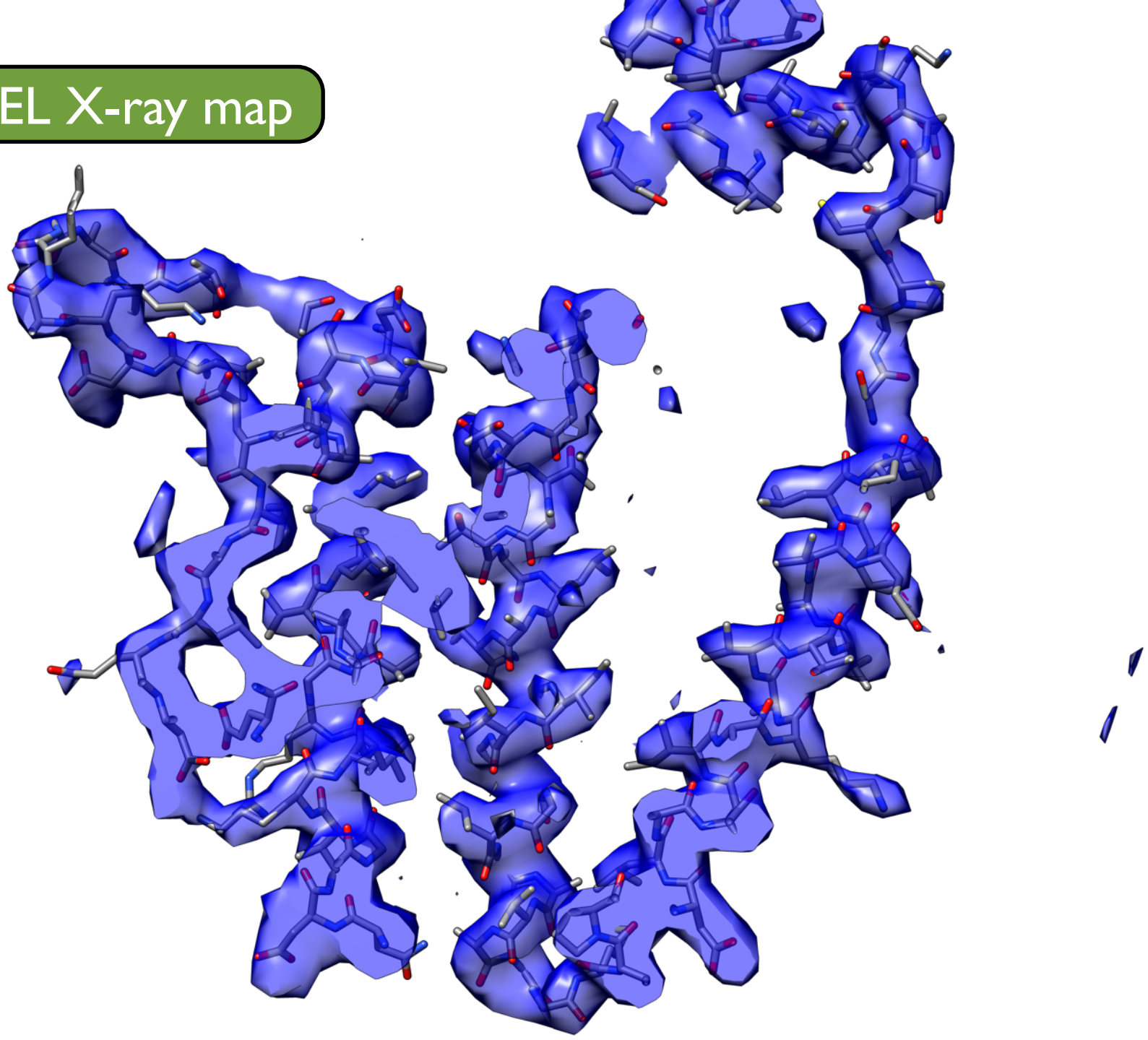


Can we density modify a cryo-EM map?

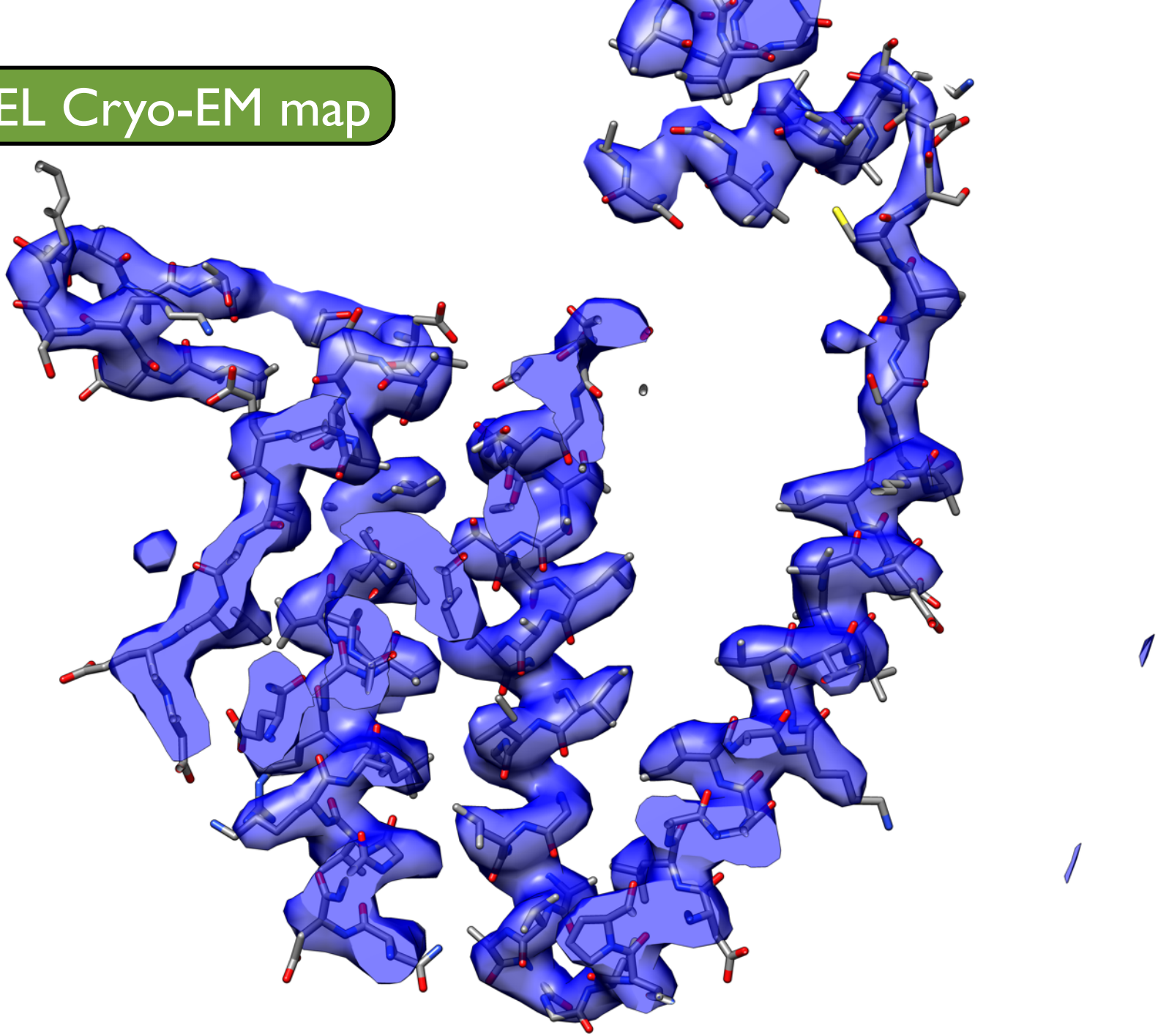


Terwilliger, et al. (2020) Nature Methods 17, 923–927

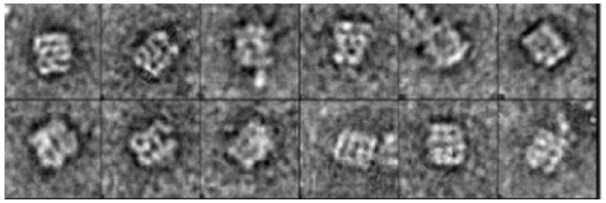
GroEL X-ray map



GroEL Cryo-EM map



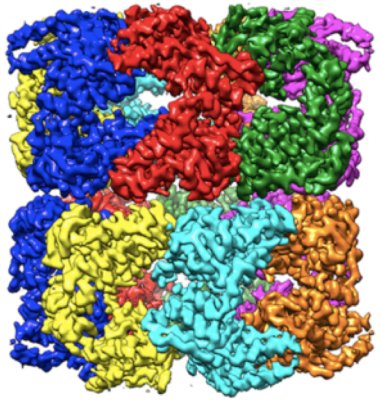
Cryo-EM and X-ray maps are created as Fourier transforms



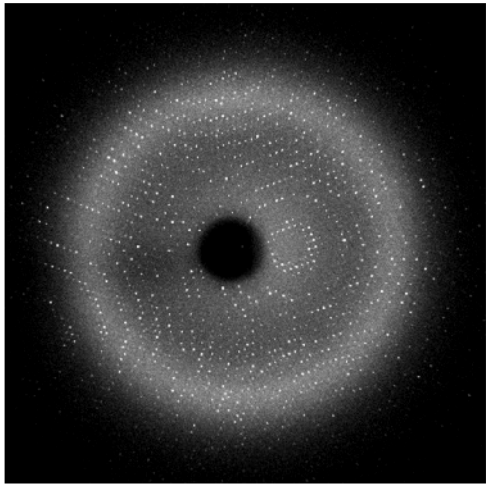
FT of one image is a section through FT of structure

Combine FT sections

Inverse FT



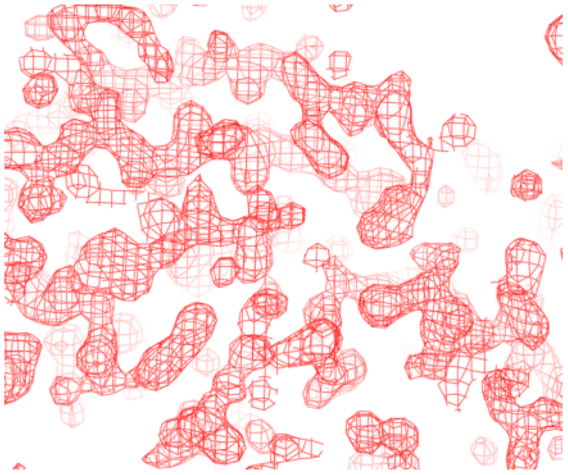
CryoEM Map



Diffraction pattern is (more or less) section through FT of structure (amplitudes only)

Combine FT sections

Inverse FT with phases

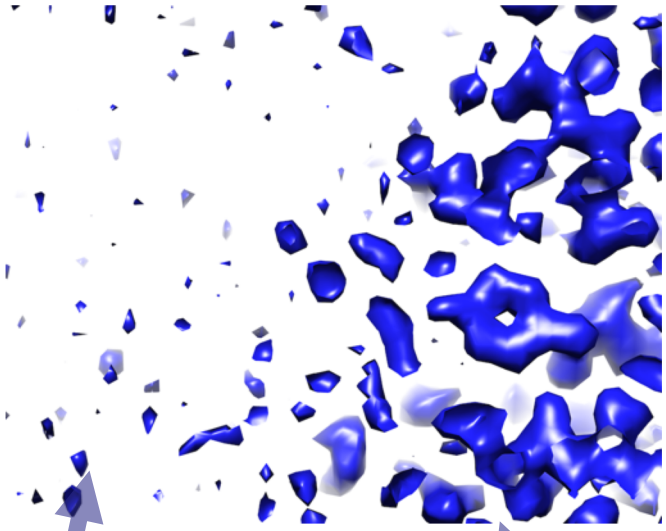


X-ray Map

Density modification with cryo-EM maps

Using expectations about one part of a map to improve another part of the map

Original map



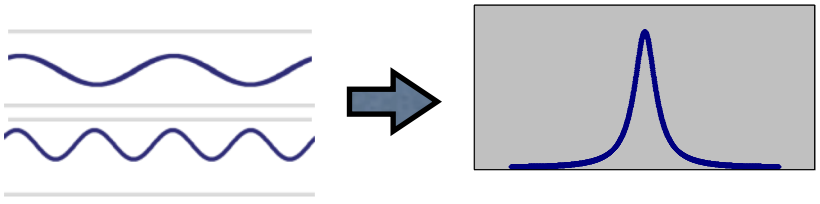
Density-modified

Solvent should be flat

Distribution of density values (histograms) should match typical protein

How density modification works

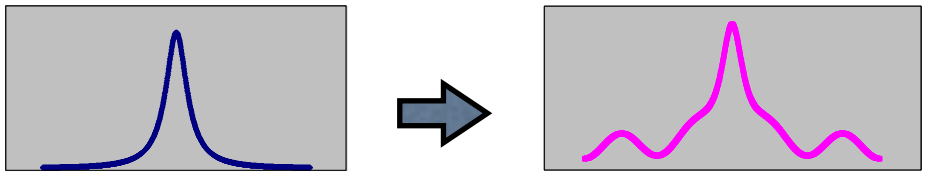
Maps are calculated from Fourier terms…



Fourier terms

Map

A change in one Fourier term affects all points in the map



Change one Fourier term

An error in one Fourier term leads to correlated errors throughout the map

Fixing a Fourier term based on one part of the map can improve another part of the map

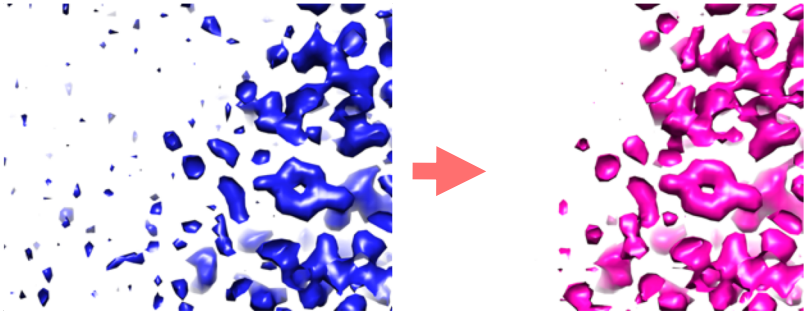
Map phasing step in density modification

Fourier terms that improve the map in one place improve it everywhere

One Fourier term at a time, find value that yields most plausible map (all other terms fixed)

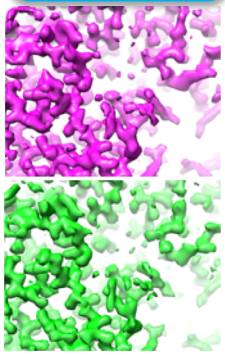
Flatter solvent
Better histograms

New Fourier terms improve entire map
("map-phasing")



Density modification with cryo-EM maps

Two half-maps



Sharpen/average to create full map

Identify macromolecule and solvent regions

Target histograms for macromolecule and solvent

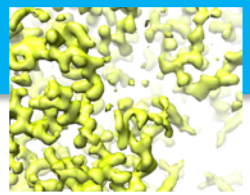
Fourier terms for two original half-maps

Map-phasing estimates of Fourier terms

(one set for each half-map)

Calculate weighted average of two original and two map-phasing sets of Fourier terms

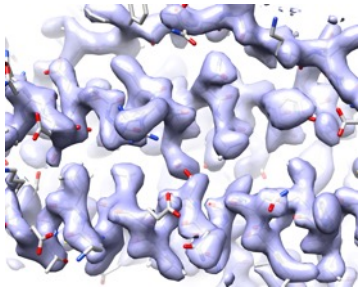
6. Density-modified map



Density modification

What to try and what to keep in mind

Density modification on your half-maps (*resolve_cryo_em*)

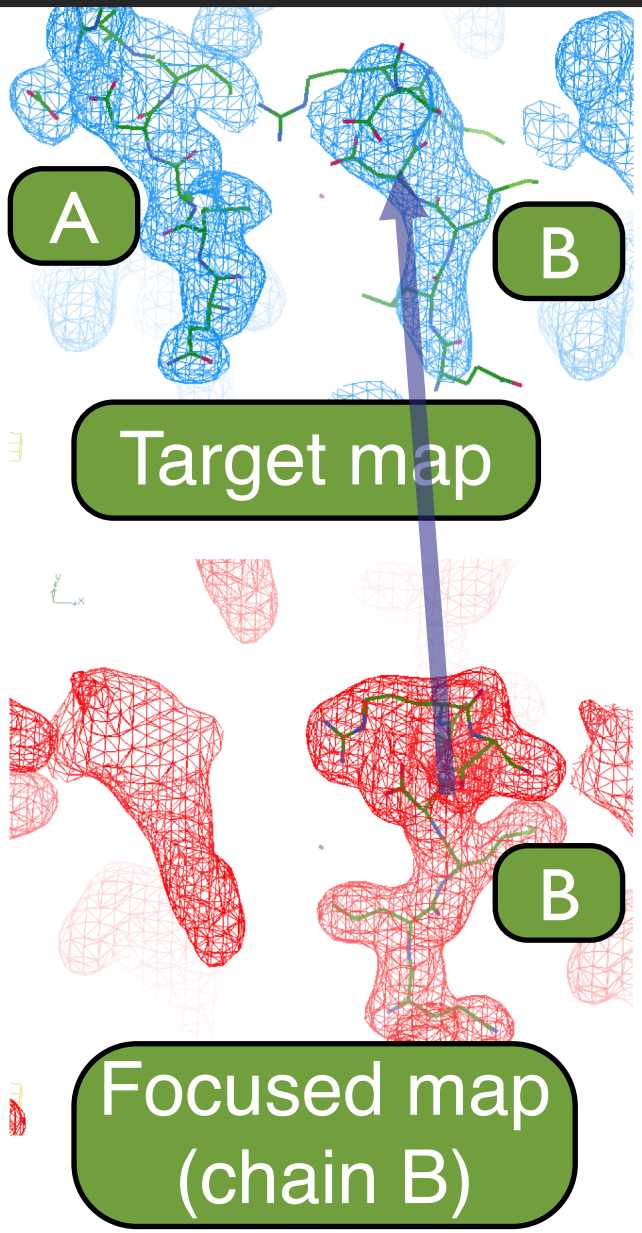


Look for 0.05-0.3 Å estimated improvement in resolution

Works best with half-maps that have uniform noise

Tutorial data, walk-through and scripts supplied with latest versions of *Phenix*

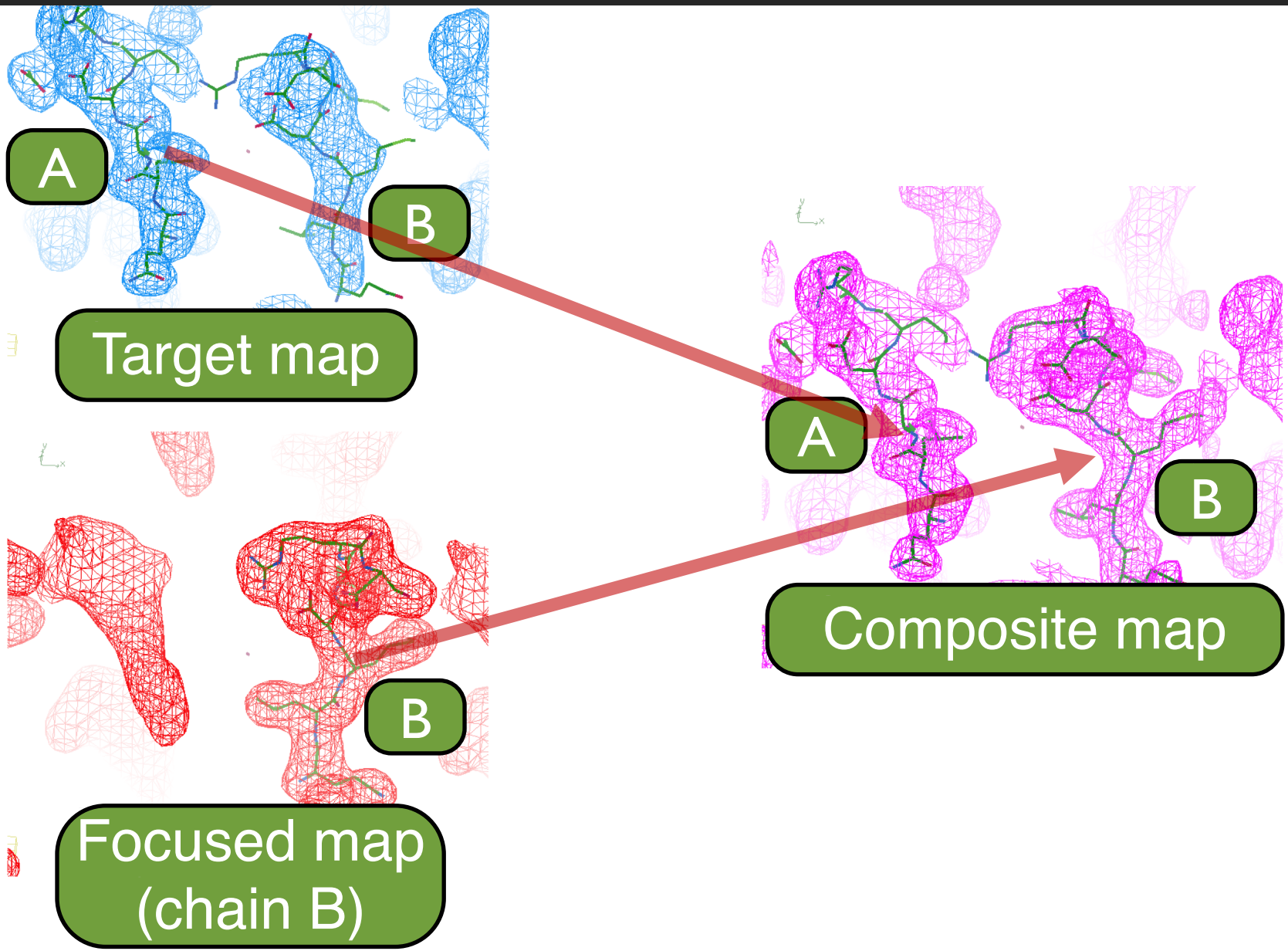
Combining maps with combine_focused_maps



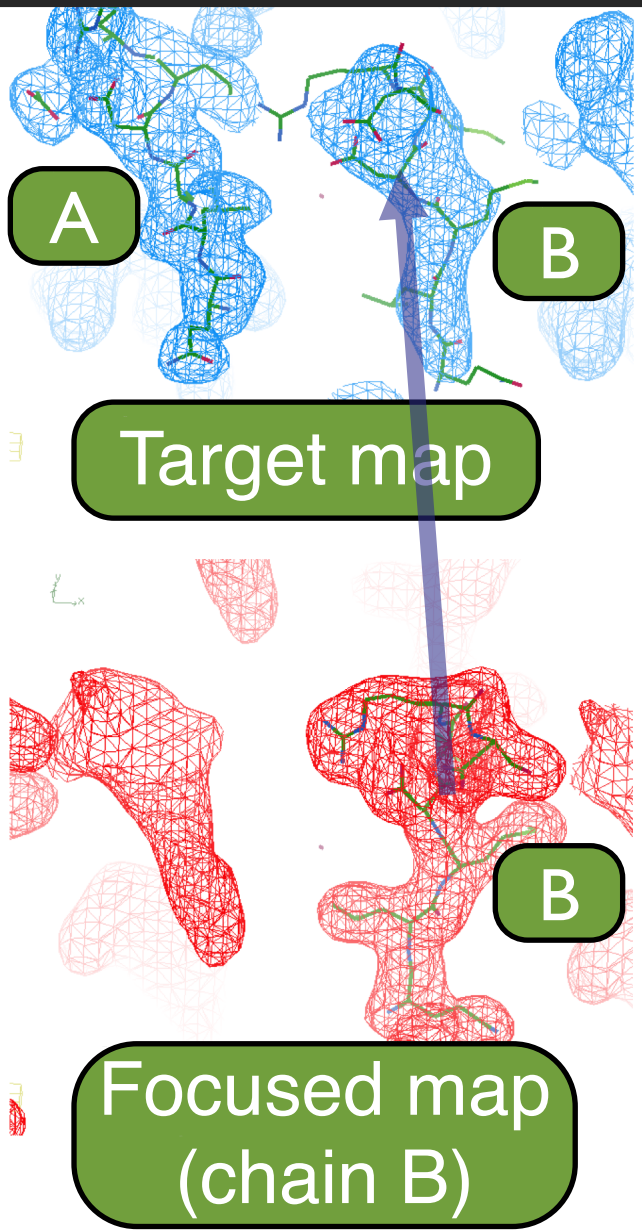
Procedure for combining maps:

- Superimpose density
- Rotation/translation from refined models
- Average target and focused map density
- Weight using map-model correlations

Combining maps with combine_focused_maps



Combining maps with combine_focused_maps



Features

- Averaging of entire chains or local regions
- Application of symmetry to focused map (e.g., superimpose chain B of focused map on chains EFGH of target map)

The Phenix Project

Lawrence Berkeley Laboratory

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

Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung

Duke University

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

Cambridge University

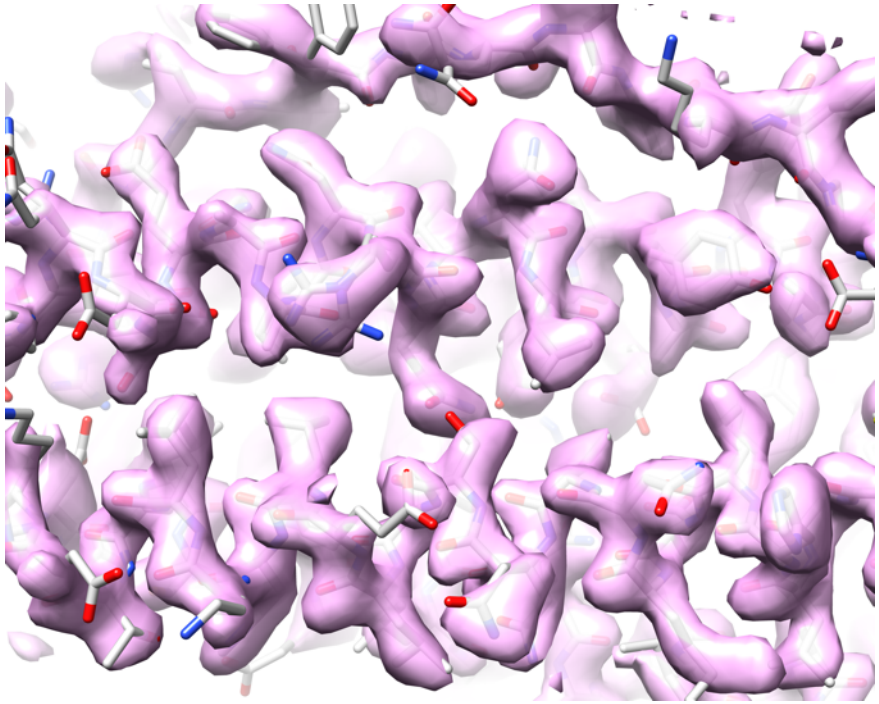
Randy Read, Airlie McCoy, Tristan Croll, Rob Oeffner, Massimo Sammito, Claudia Millán Nebot

University of Texas

Matthew Baker, Cory Hryc

An NIH/NIGMS funded Program Project

Cryo-EM map improvement demos



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