

# ***Phenix* Tools for Cryo-EM**

# The *Phenix* project

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Duncan Stockwell

Cambridge University



Duke University

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



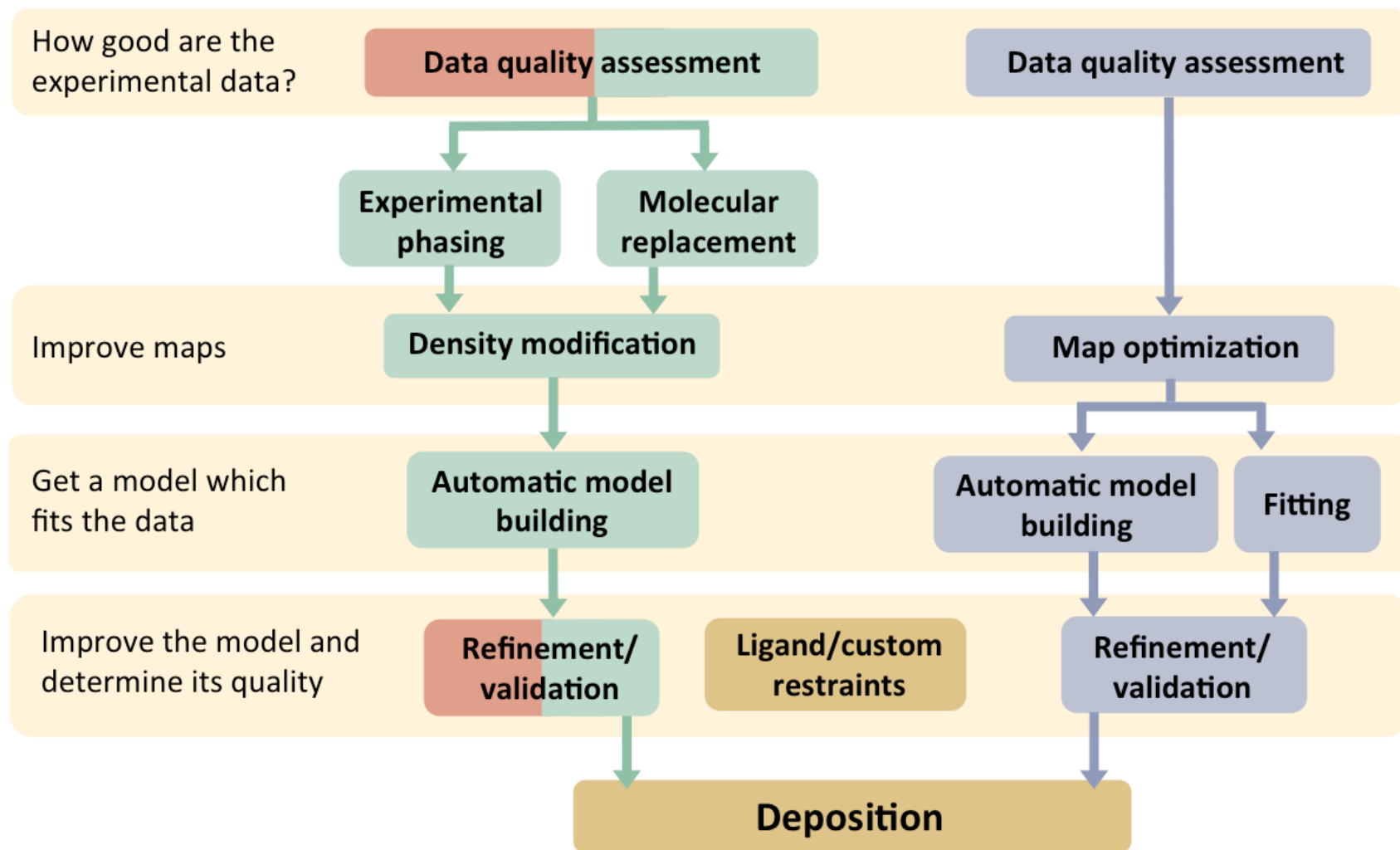
An NIH/NIGMS funded  
Program Project

Liebschner D, *et al.*, Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*. Acta Cryst. 2019 **D75**:861–877

# Phenix: tools for crystallography and cryo-EM

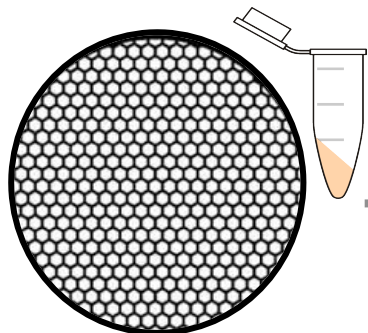
## Xray/neutron crystallography

## Cryo-EM



# Phenix tools for cryo-EM

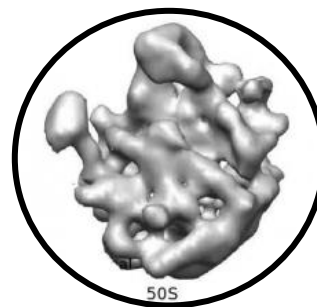
Sample



Data collection

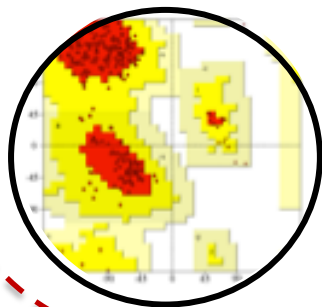


Data processing

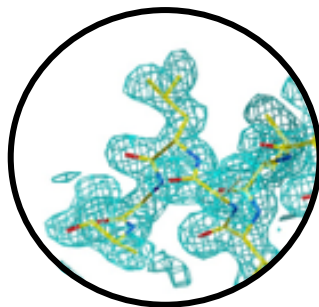


Map manipulations

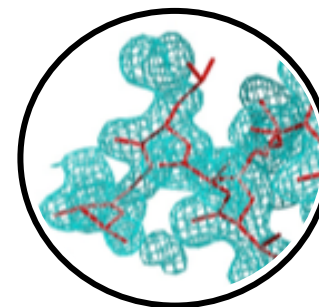
Validation



Model refinement

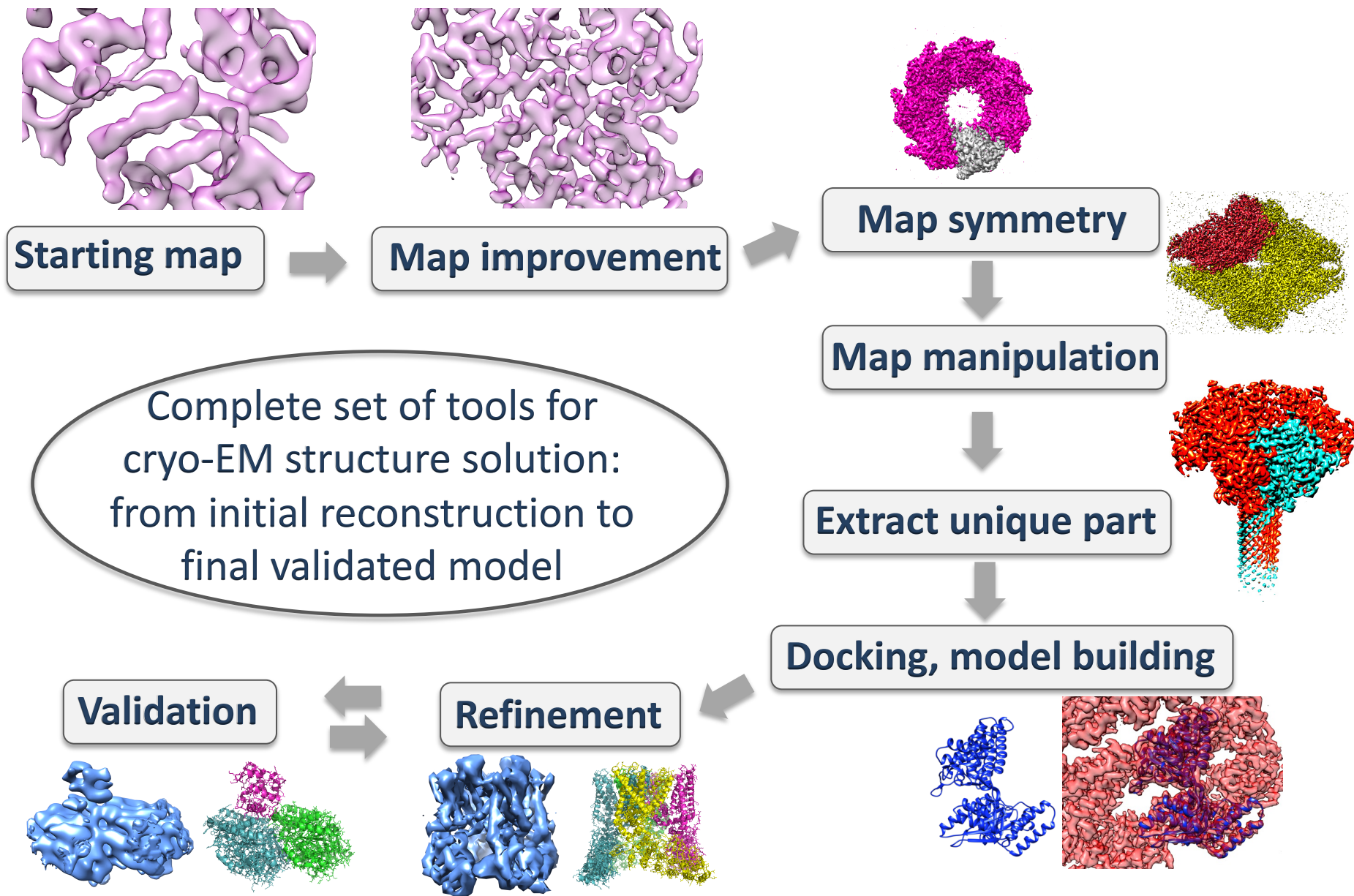


Model building

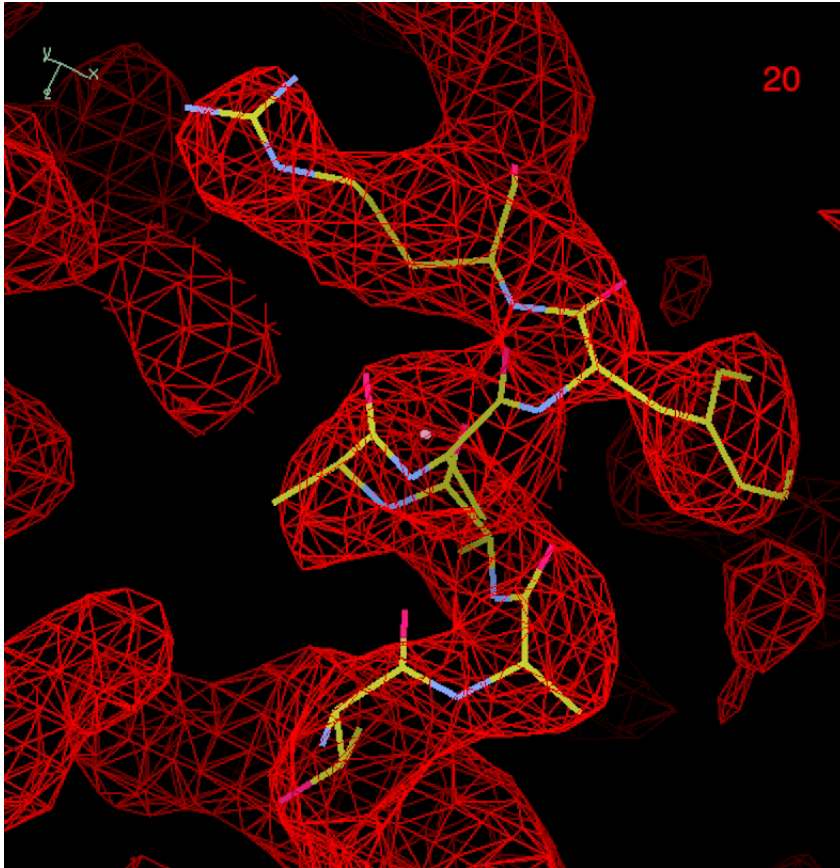


*Phenix*

# Phenix tools for cryo-EM



# Automated map sharpening: *phenix.auto\_sharpen*



Maximize detail in the map

... and connectivity of map

Adjusted surface area



Optimally sharpened map

Fully automatic:

- No manual trial-and-error
- No parameters to adjust
- Only inputs: map and resolution



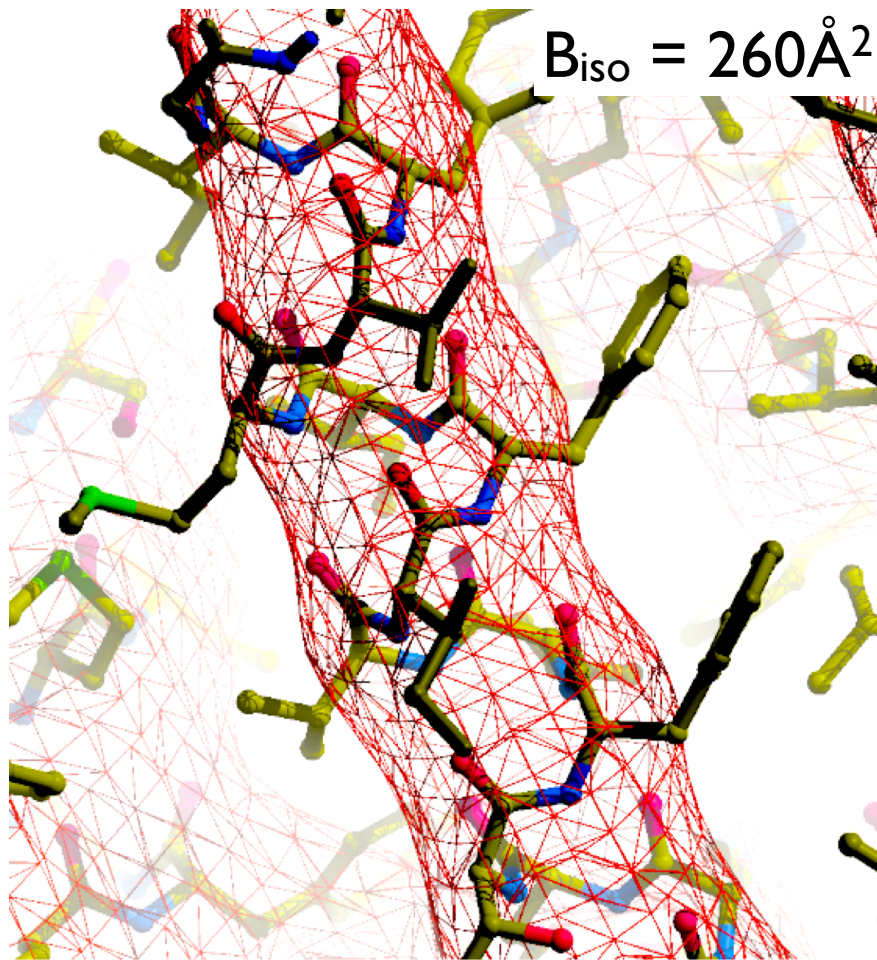
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BIOLOGY

ISSN 2059-7983

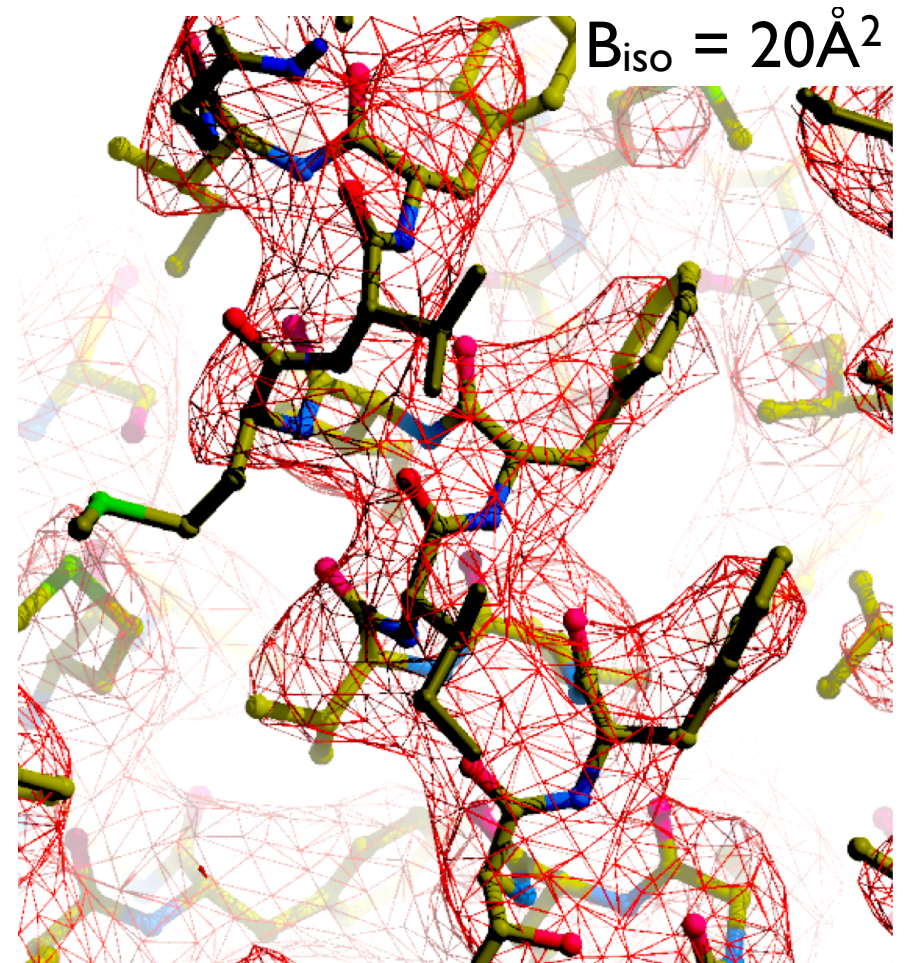
Automated map sharpening by maximization of  
detail and connectivity

Thomas C. Terwilliger,<sup>a,b\*</sup> Oleg V. Sobolev,<sup>c</sup> Pavel V. Afonine<sup>c,d</sup> and  
Paul D. Adams<sup>d,e</sup>

# Automated map sharpening: *phenix.auto\_sharpen*



*Deposited Map*



*Autosharpened Map*

High-conductance  $\text{Ca}(2+)$ -activated  $\text{K}(+)$  channel  
(emd\_8414 and PDB entry 5tji; Hite et al., 2017)

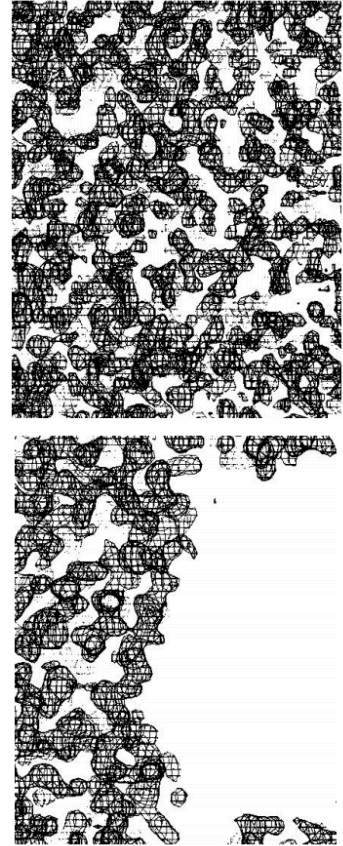
# Density modification: *phenix.density\_modify\_cryo\_em*

## 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: *phenix.density\_modify\_cryo\_em*

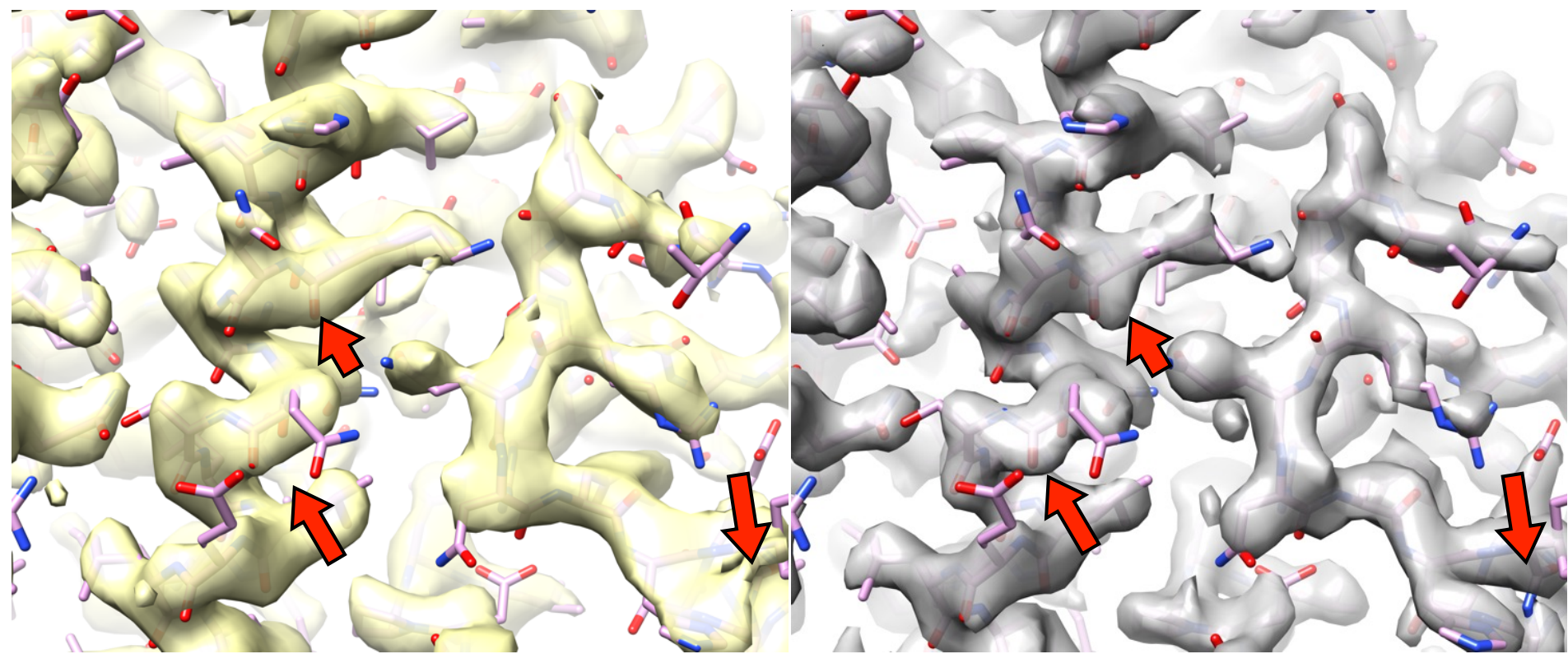
### Cryo-EM:

- 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)

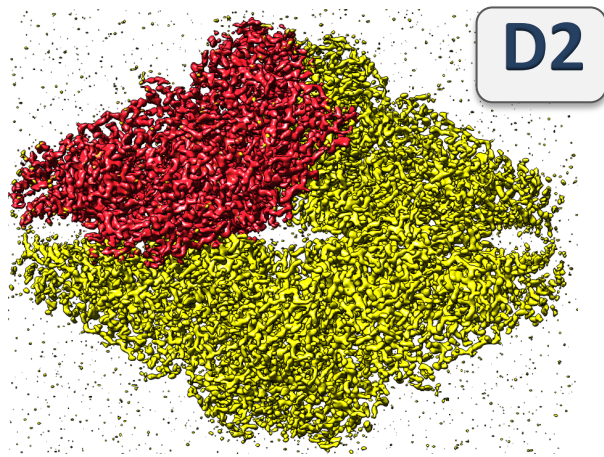
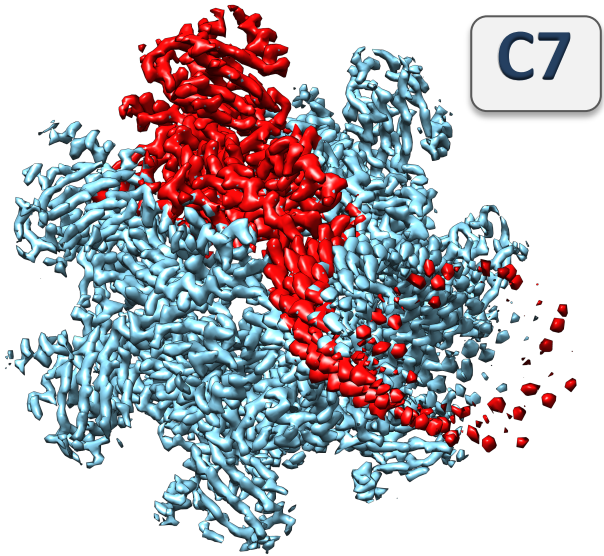
# Density modification: *phenix.density\_modify\_cryo\_em*



## Density modification for cryo-EM maps, facts:

- Can increase map resolution
- Can improve map clarity for interpretation
- Improvements are no huge (compared to crystallography)

# Finding map symmetry: *phenix.symmetry\_from\_map*



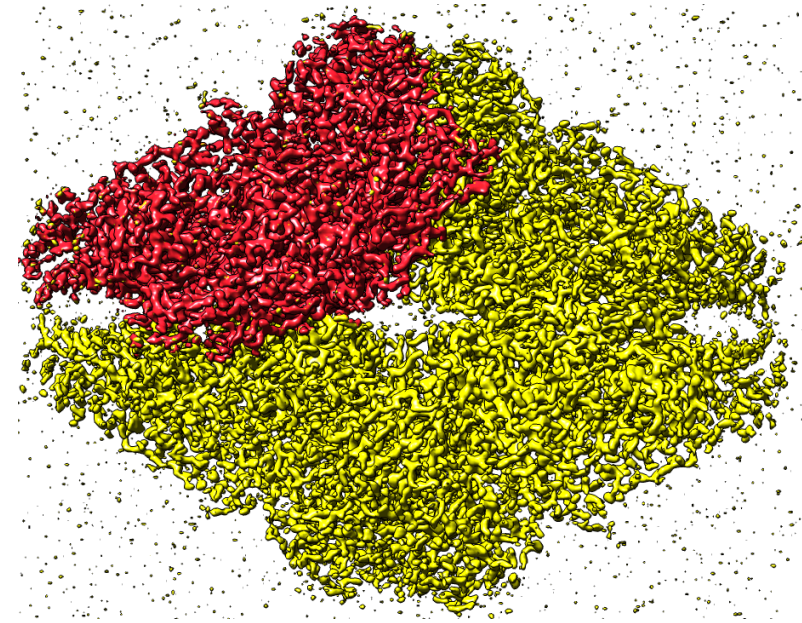
## Procedure for finding symmetry:

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

<http://phenix-online.org/newsletter/>

Tools for interpreting cryo-EM maps using models from the PDB

# Extracting unique part of map: *phenix.map\_box*



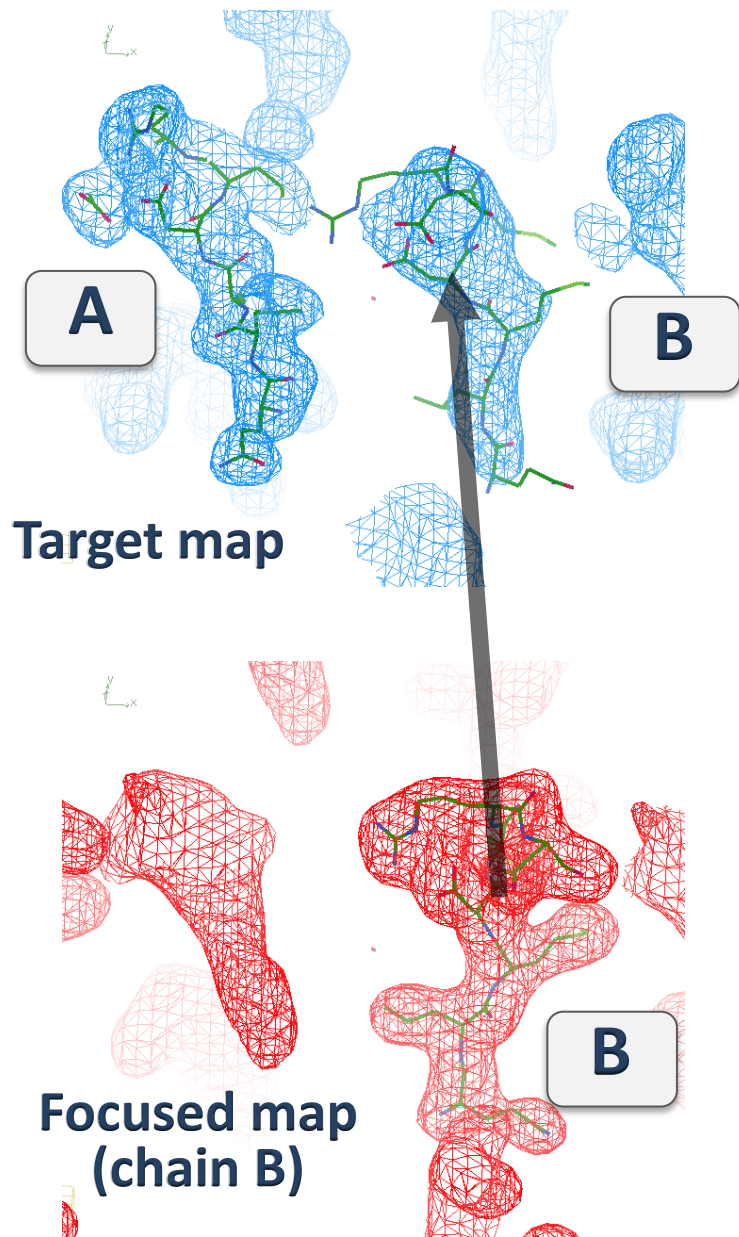
## Procedure:

- 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

<http://phenix-online.org/newsletter/>

Tools for interpreting cryo-EM maps using models from the PDB

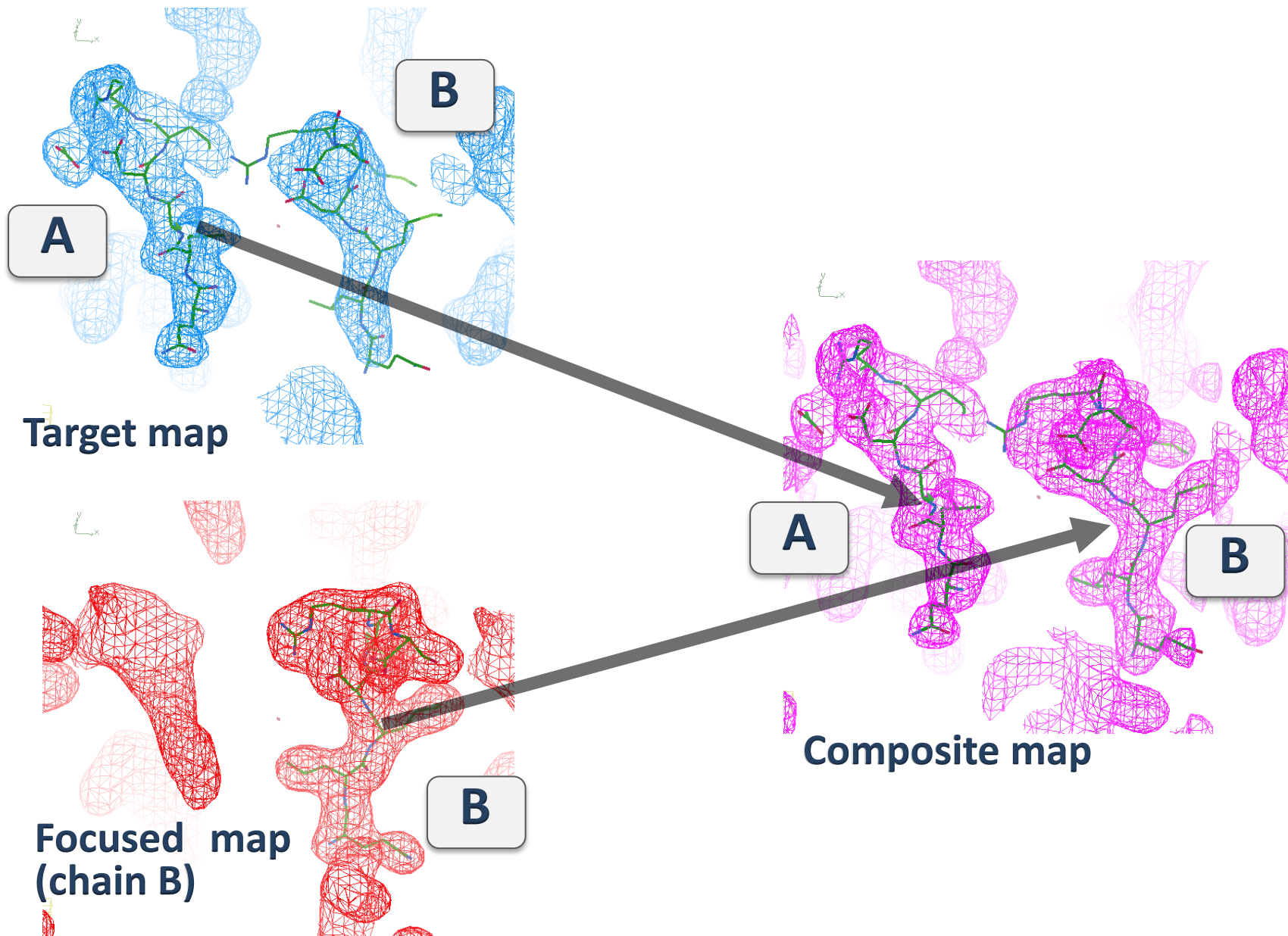
# Combining maps with *phenix.combine\_focused\_maps*



## Procedure for combining maps:

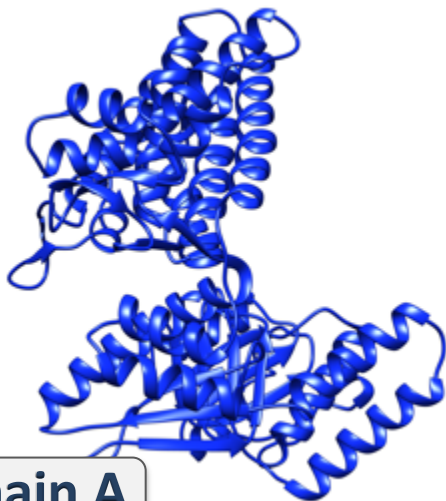
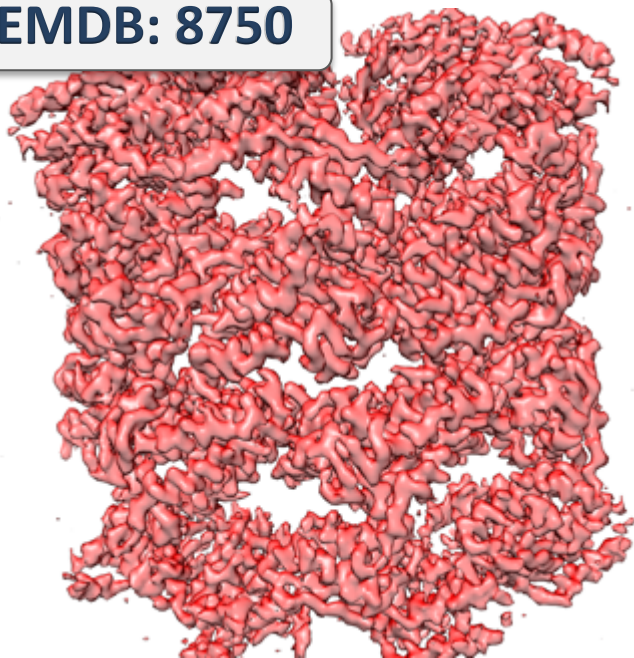
- Superpose density
- Rotation/translation from refined models
- Average target and focused map density
- Weight using map-model correlations

# Combining maps with *phenix.combine\_focused\_maps*



# Docking models with *phenix.dock\_in\_map*

EMDB: 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*

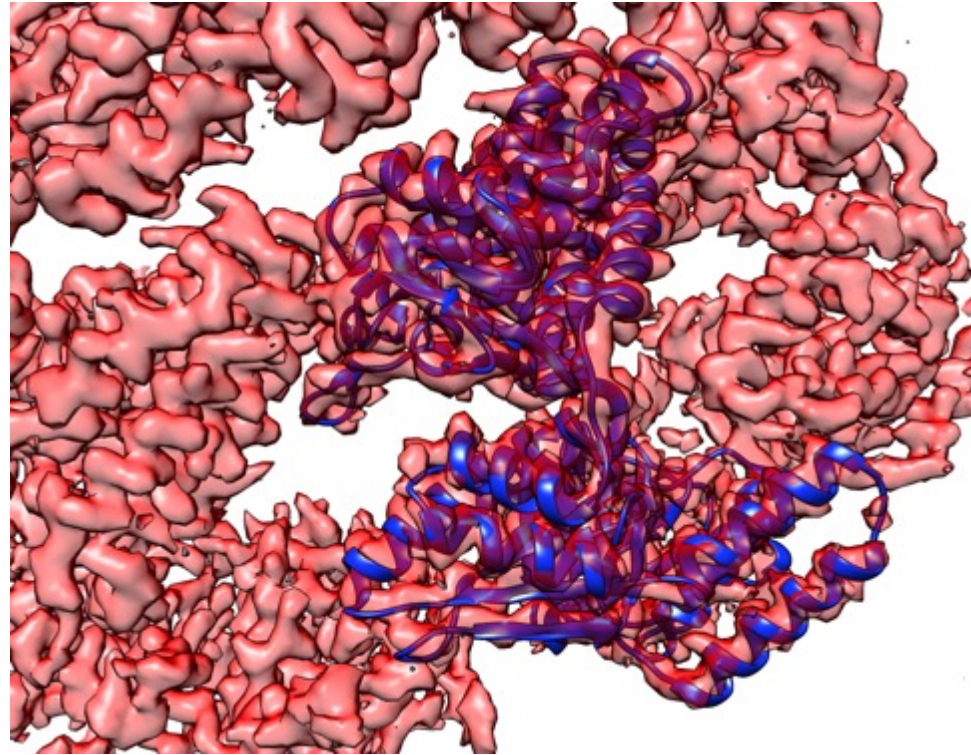
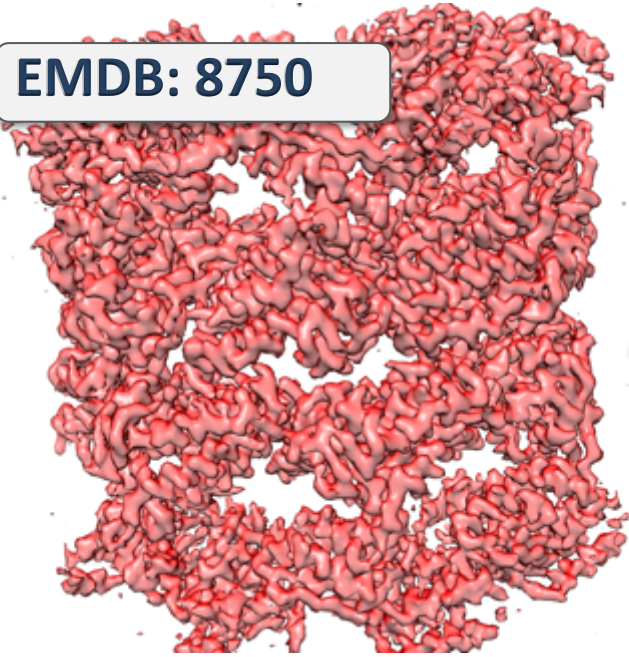
## Features:

- Multiple chains
- Density search
- Symmetry
- Multiprocessing

<http://phenix-online.org/newsletter/>  
Tools for interpreting cryo-EM maps using models from the PDB

# Docking models with *phenix.dock\_in\_map*

EMDB: 8750



Chain A docked in map

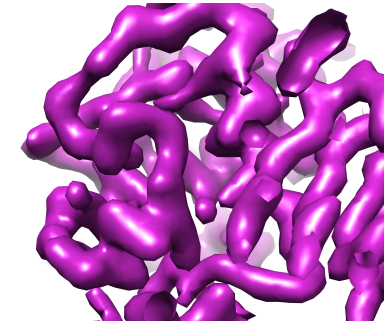
1ss8 chain A





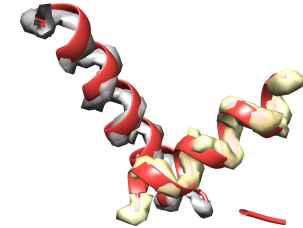
# Automated model building: *phenix.map\_to\_model*

Trace chain the way a person does

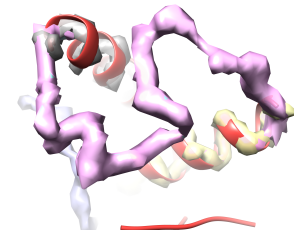


Find secondary structure

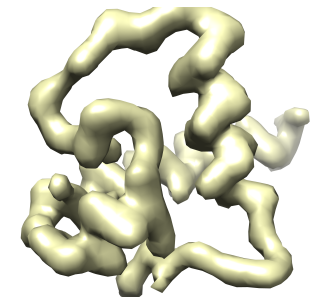
Find clear regions of density



Adjust contour level until a region just connects to one other



Iterate to build up chain



nature **methods**

BRIEF COMMUNICATION

<https://doi.org/10.1038/s41592-018-0173-1>

A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps

Thomas C. Terwilliger<sup>1,2\*</sup>, Paul D. Adams<sup>3,4</sup>, Pavel V. Afonine<sup>3,5</sup> and Oleg V. Sobolev<sup>3</sup>

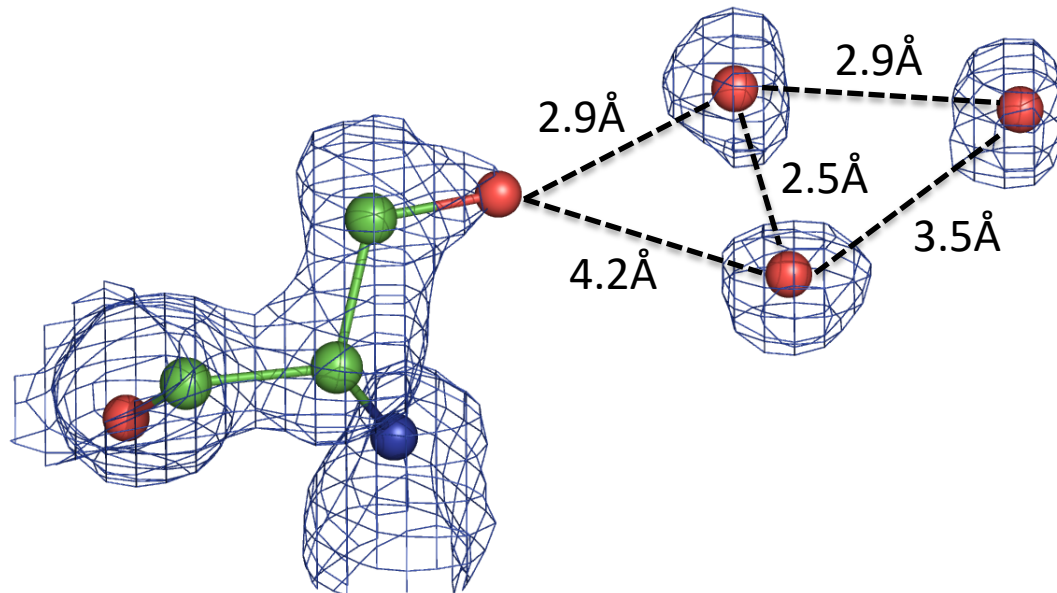
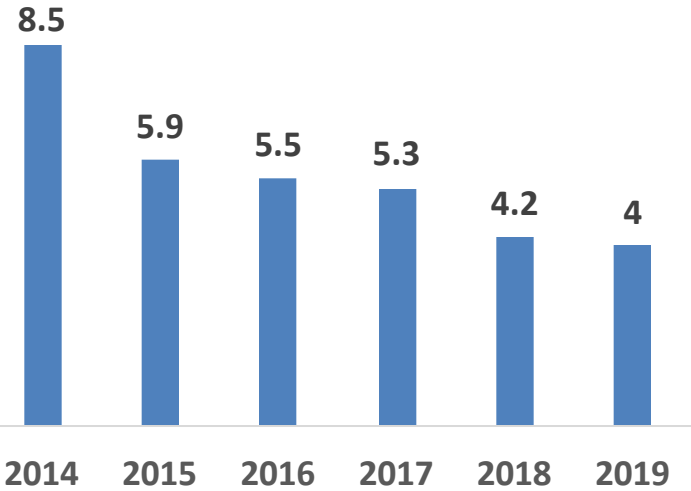
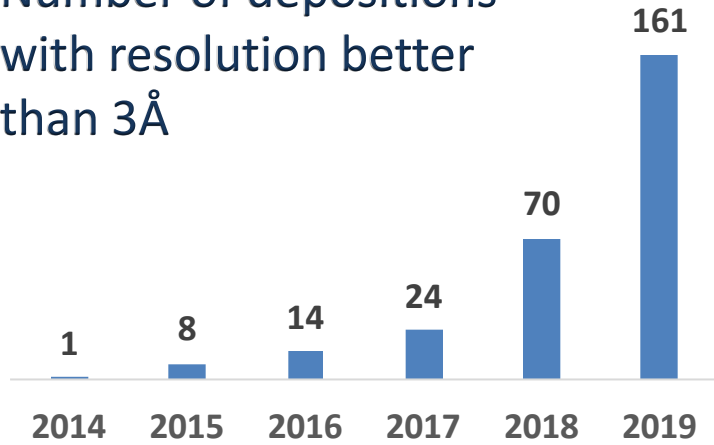
# Automated model building: *phenix.map\_to\_model*

## Automated model building, facts:

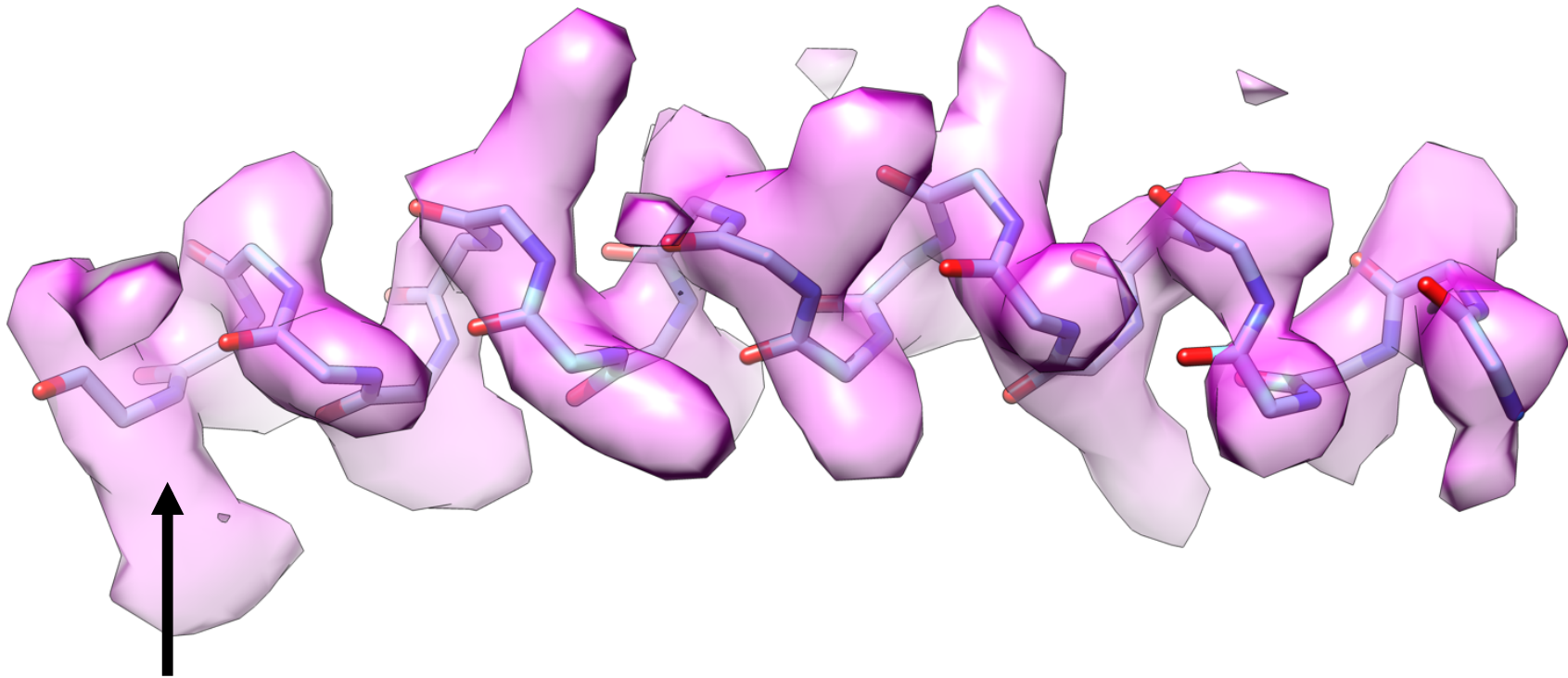
- No automated model building produces 100% complete and accurate model
- Produces initial model for further manual building
- The lower the resolution, the less complete and accurate the auto built model

# Automated water building: *phenix.douse*

Number of depositions with resolution better than 3Å



# Sequence from map: *phenix.sequence\_from\_map*

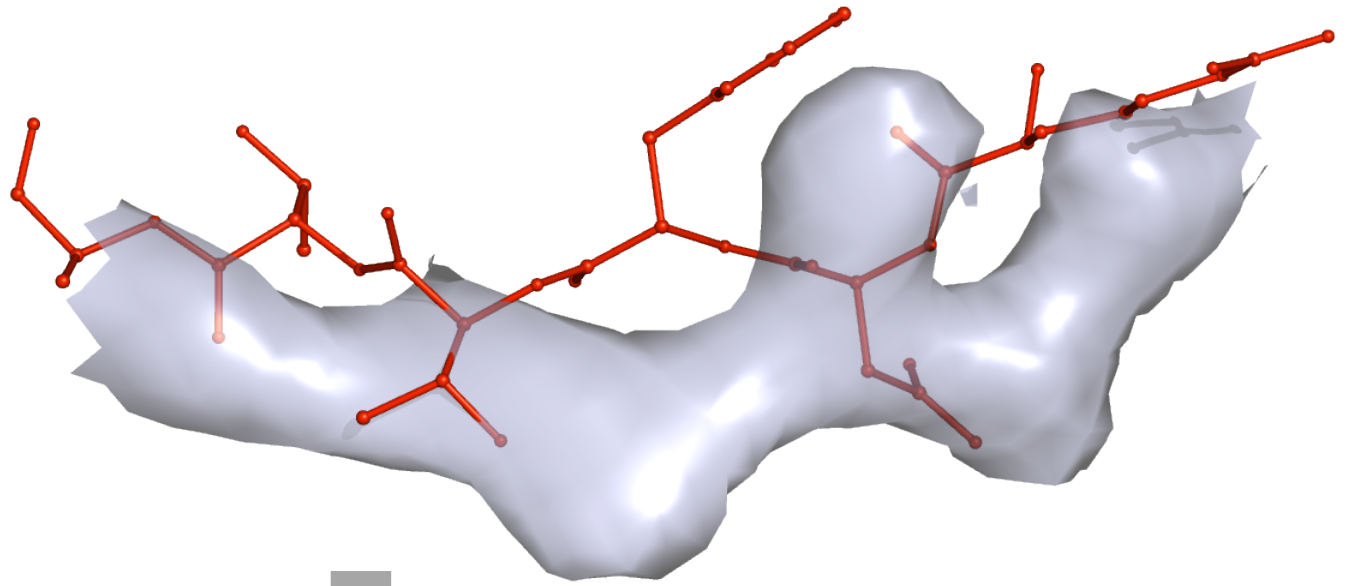


Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

- Determine probability of side chain at each C $\alpha$
- Align sequence to maximize total probability for the chain

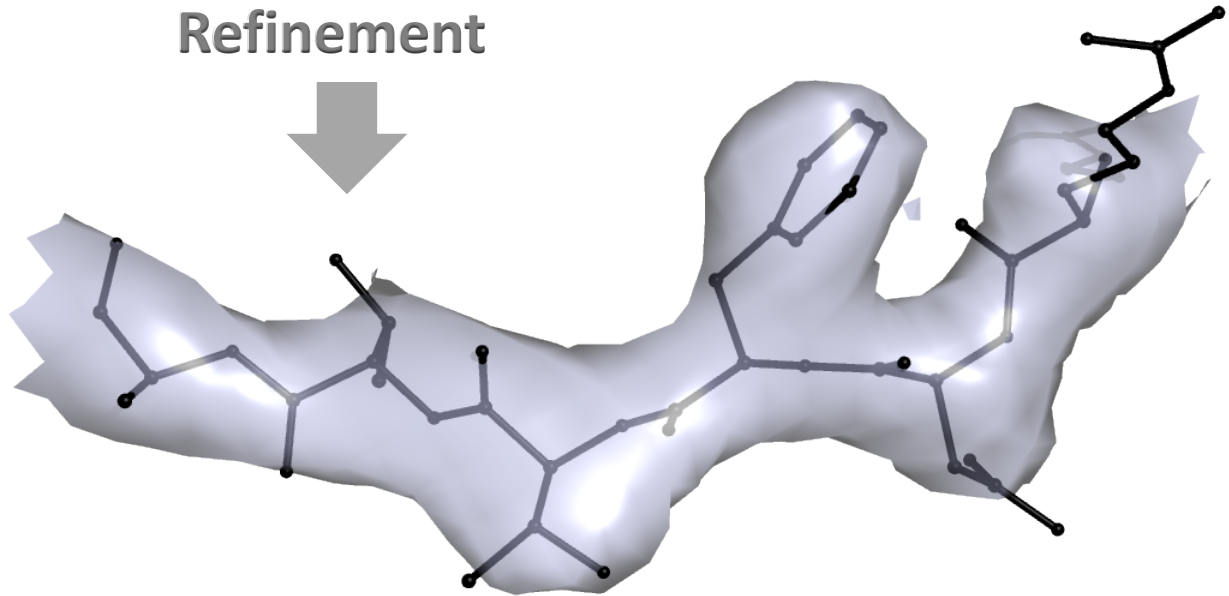
# Atomic model refinement: *phenix.real\_space\_refine*

Initial (poor)  
model



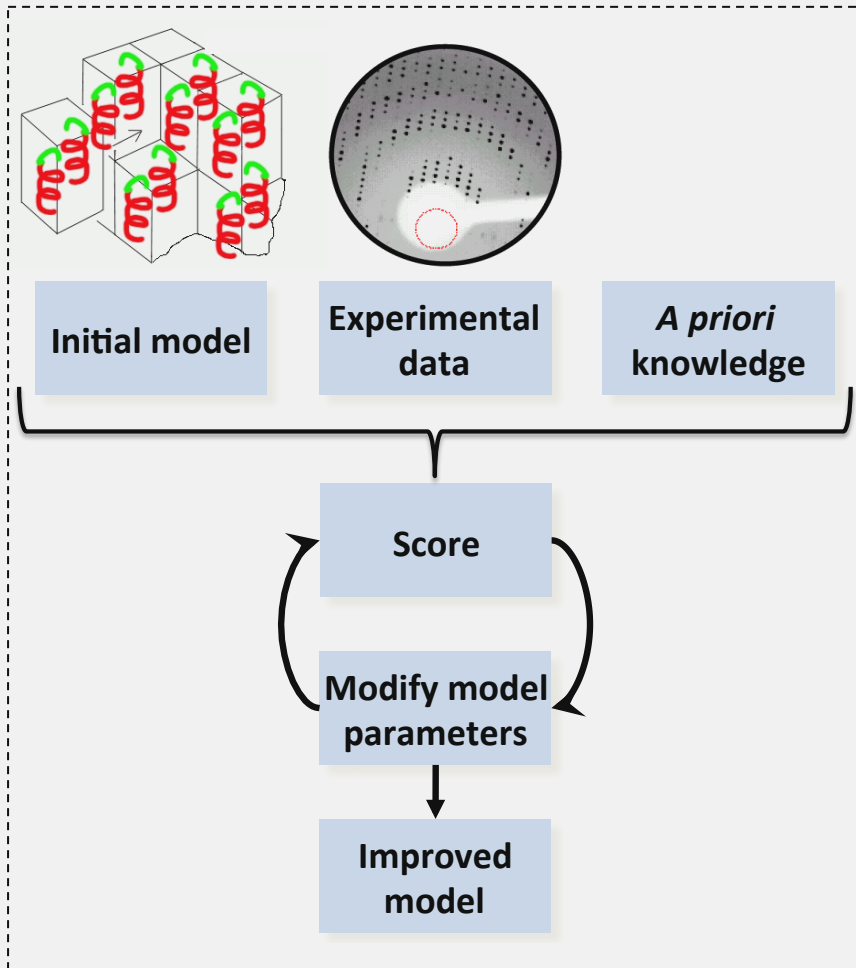
Refinement

Improved  
(refined)  
model



# Atomic model refinement: *phenix.real\_space\_refine*

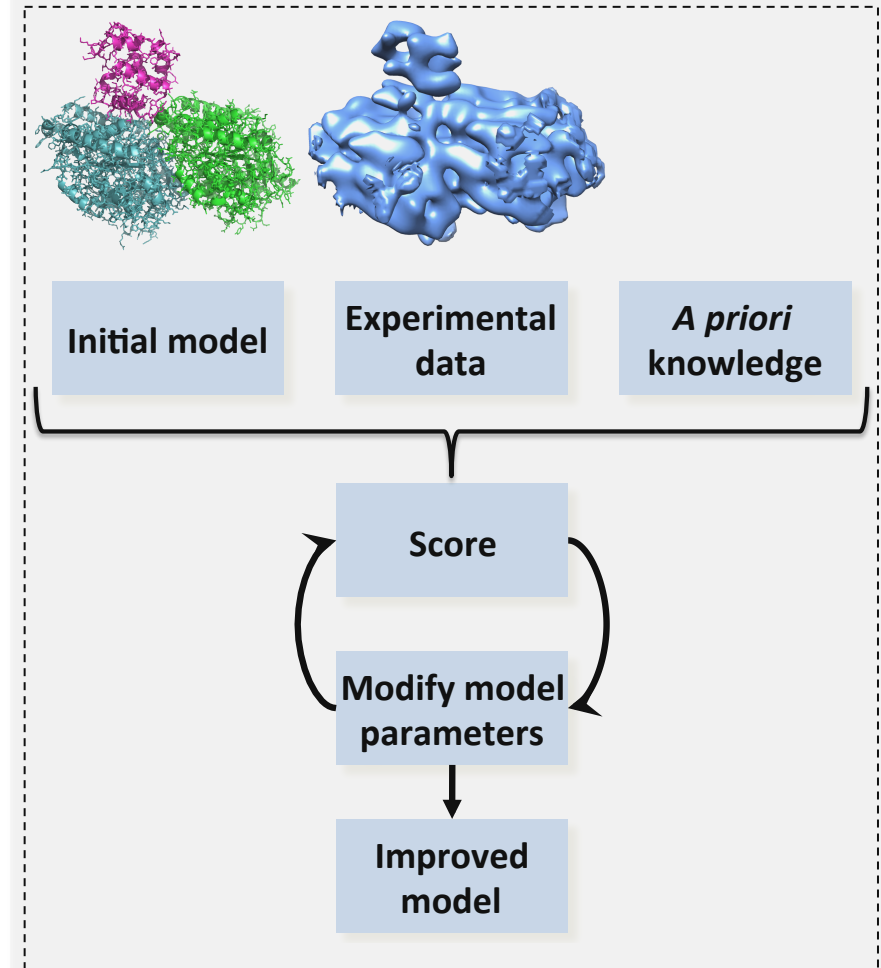
## Crystallography



phenix.refine

Available since 2005

## Cryo-EM

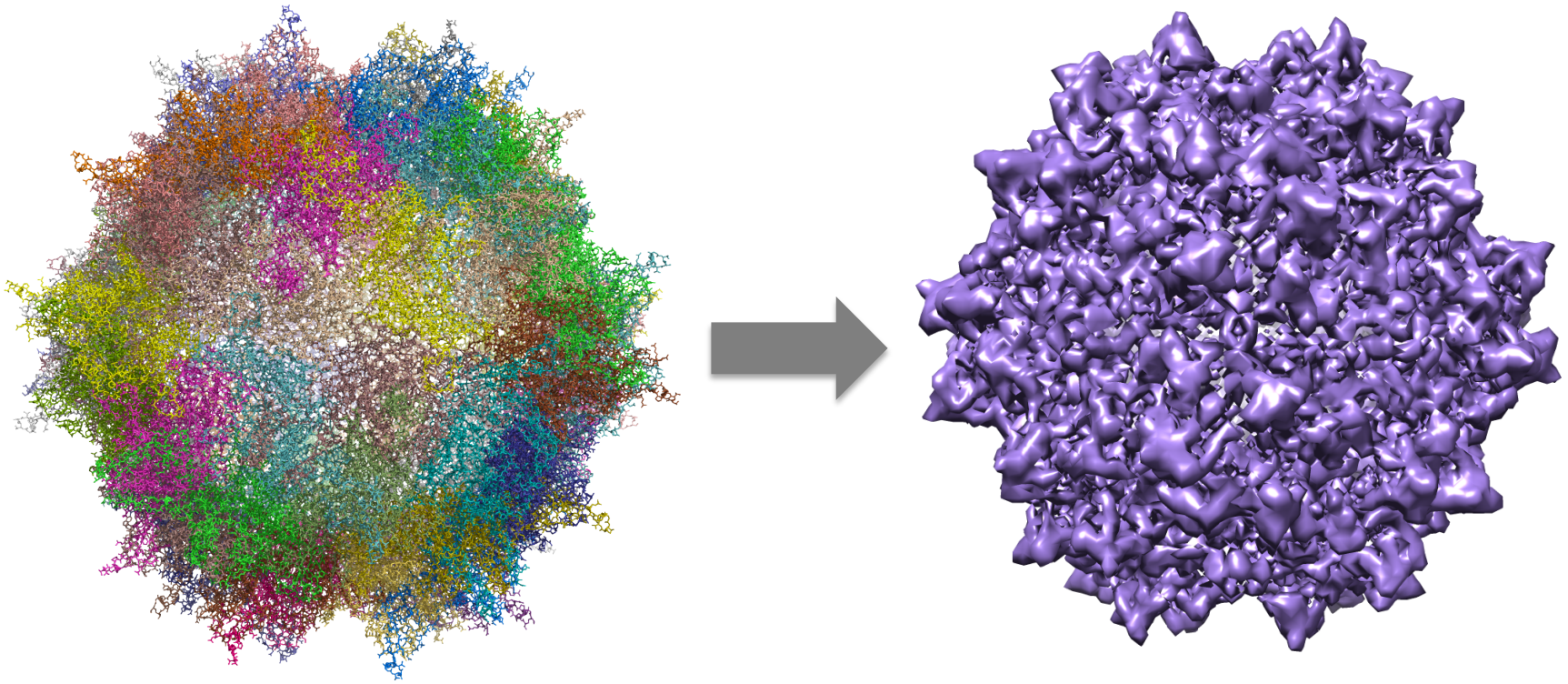


phenix.real\_space\_refine

Available since 2013

# Atomic model refinement: *phenix.real\_space\_refine*

- Direct refinement against the map
  - No Fourier space involved



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ISSN 2059-7983

Real-space refinement in *PHENIX* for cryo-EM and crystallography

Pavel V. Afonine,<sup>a,b\*</sup> Billy K. Poon,<sup>a</sup> Randy J. Read,<sup>c</sup> Oleg V. Sobolev,<sup>a</sup> Thomas C. Terwilliger,<sup>d,e</sup> Alexandre Urzhumtsev<sup>f,g</sup> and Paul D. Adams<sup>a,h</sup>

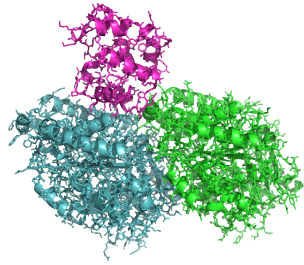
# Atomic model refinement: *phenix.real\_space\_refine*

- Best model-map fit. Any map: X-ray, neutron, EM. Any resolution
- Refined models: no poor validation metrics
- Fast (minutes – a few hours, not days or many hours)
  - **Make use of multiple CPUs: as many as available**
- Large convergence radius
- Easy to use: map and model in, refined model out
- Accessible: no special hardware requirements

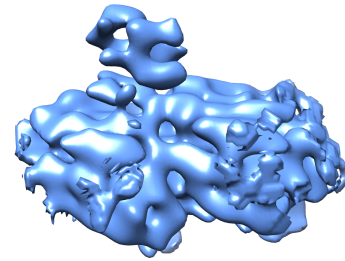


# Validation

**Model**

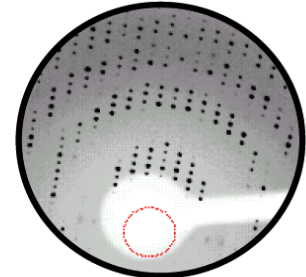


**Data**



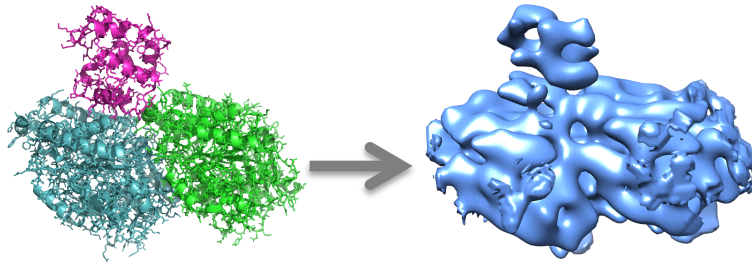
**Cryo-EM**

or



**Diffraction**

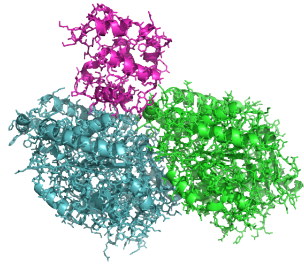
**Model to data fit**



# Validation: crystallography vs cryo-EM

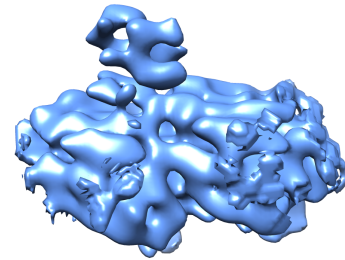
Exactly same

Model



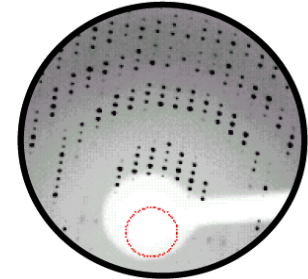
Different

Data



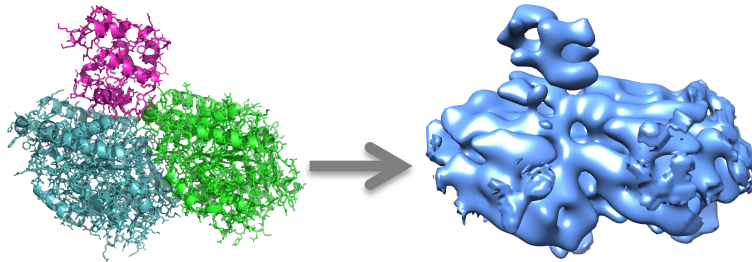
Cryo-EM

or



Diffraction

Model to data fit



Similar

# Validation: **why to do?**

- **Helps to save time later**
- **Helps to produce better models**
- **Helps to set correct expectations**
- **Minimize fraud or true mistakes**



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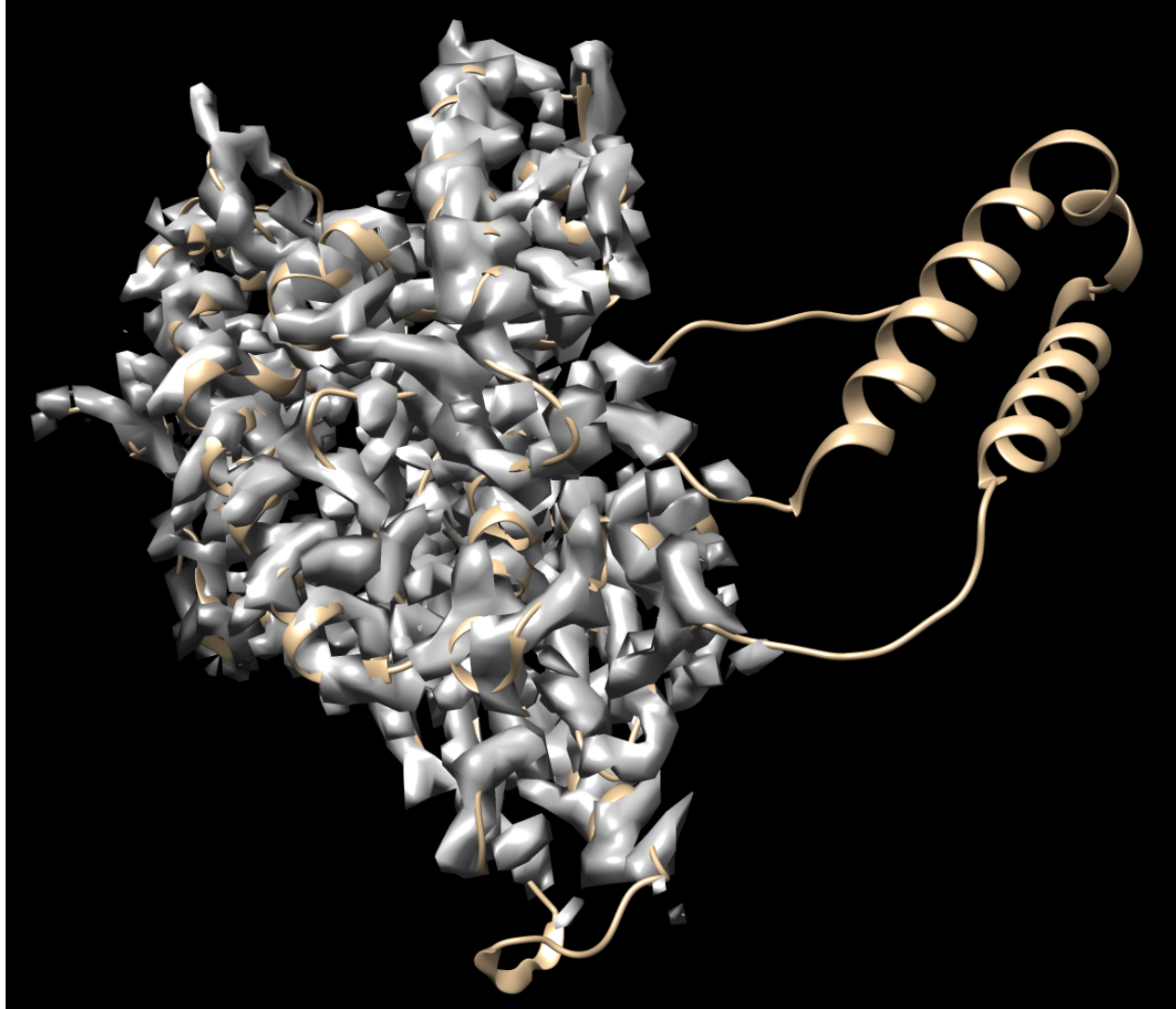
ISSN 2059-7983

**New tools for the analysis and validation of cryo-EM maps and atomic models**

**Pavel V. Afonine,<sup>a,b\*</sup> Bruno P. Klaholz,<sup>c</sup> Nigel W. Moriarty,<sup>a</sup> Billy K. Poon,<sup>a</sup> Oleg V. Sobolev,<sup>a</sup> Thomas C. Terwilliger,<sup>d,e</sup> Paul D. Adams<sup>a,f</sup> and Alexandre Urzhumtsev<sup>c,g</sup>**

# Validation: **why to do?**

3j9e (emd\_6240) | 3.3Å | CC= 0.85 | Year: 2015



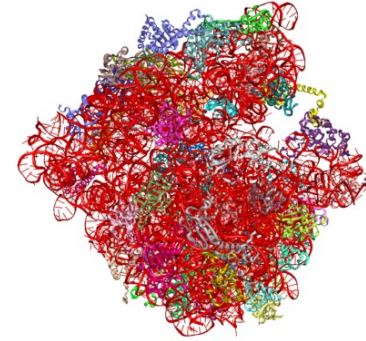
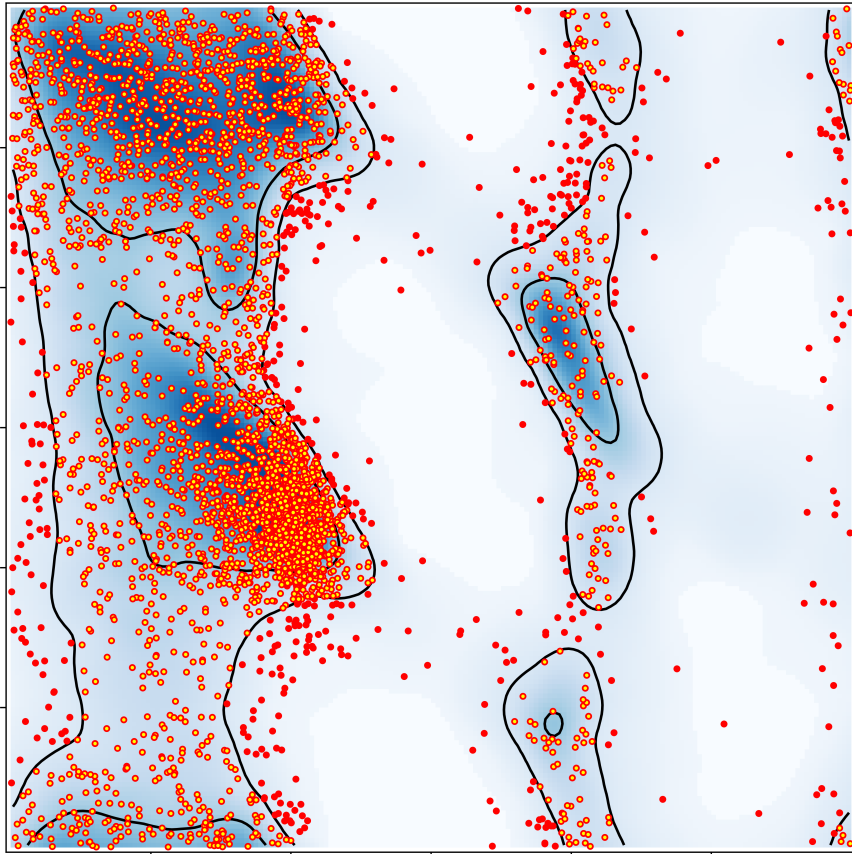
# Validation: **why to do?**

3a5x (emd\_1641) | 4.0Å | CC < 0



# Validation: **why to do?**

(2019) Nature 570: 400-404 | PDB: 6o9j | EMDB: 0661 | 3.9Å



Metric	6o9j	Expected
Clashcore	70	Less than 10
Ramachandran favored, %	59	More than 98
Ramachandran outliers, %	15	0
Rotamer outliers, %	23	0
C <sub>β</sub> deviations, %	0.5	0

# Phenix tools for cryo-EM

PHENIX home

Quit Preferences Help Citations Coot PyMOL KiNG Other tools Ask for help

Actions Job history

### Projects

Show group: All groups Manage...

Select Delete New project Settings

ID	Last modified	# of jobs	R-free
✓ sach	Sep 28 2018 01:2...	1	---
real-space-refin...	Sep 14 2018 09:07...	7	---

### Refinement

#### Cryo-EM

- Mtriage**  
Analyze quality of maps in CCP4 format
- Map to Model**  
Model-building into cryo-EM and low-resolution maps
- CryoFit**  
Flexibly fit a model to a cryo-EM map
- Real-space refinement**  
Automated refinement using real-space maps (Cryo-EM)
- Comprehensive validation (cryo-EM)**  
Model quality assessment, including real-space correlat structures
- EMRinger**  
Model validation for de novo electron microscopy struct
- Autosharpen Map**  
Tool for sharpening a map
- Dock in map**  
Tool for docking a model in to map
- Sequence From Map**  
Determines a sequence from a map
- Map Symmetry**  
Tool for determining the symmetry in a map

Current directory: /Users/pafonine/Desktop/all/projects/real\_space/paper\_01\_magni Browse...

PHENIX version dev-svn-000 Project: sach

# Resources

**Phenix** *Python-based Hierarchical Environment for Integrated Xtallography*  
Documentation: ([HTML](#)) [FAQ](#) Search Documentation [What's New](#)

**PHENIX is a software suite for the automated determination of molecular structures using X-ray crystallography and other methods.**

**Important Note:**  
Starting July 2019, the Protein Data Bank requires models to be in mmCIF for crystallographic structures. You should use the latest official release to generate these files for deposition.

Download the latest official release ([1.17.1](#)) [[First request download password](#)]  
Download the latest [nightly build or prerelease](#)

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**Using PHENIX (release 1.17.1):**

- The [PHENIX Graphical User Interface](#)
- Assessing data quality with [phenix.xtriage](#)
- Automated structure solution with [AutoSol](#)
- Automated molecular replacement with [Phaser-MR](#)
- Automated model building and rebuilding with [AutoBuild](#)
- Automated ligand fitting with [LigandFit](#)
- Structure refinement with [phenix.refine](#)
- Generation of ligand coordinates and restraints with [eLBOW](#)

The PHENIX system also includes SOLVE/RESOLVE, Phaser, the CCI Applications (phenix.xtriage, phenix.refine, elbow and many more), components from MolProbity, and the Computational Crystallography Toolbox in a Python framework.

**Citing PHENIX:**  
Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix D. Liebschner, P. V. Afonine, M. L. Baker, G. Bunkóczi, V. B. Chen, T. I. Croll, B. Hintze, L.-W. Hung, S. Jain, A. J. McCoy, N. W. Moriarty, R. D. Oeffner, B. K. Poon, M. G. Prisant, R. J. Read, J. S. Richardson, D. C. Richardson, M. D. Sammito, O. V. Sobolev, D. H. Stockwell, T. C. Terwilliger, A. G. Urzhumtsev, L. L. Videau, C. J. Williams, and P. D. Adams *Acta Cryst.* (2019), **D75**, 861-877

**Funding for PHENIX:** [NIH General Medical Sciences](#)

The PHENIX system also can be used for neutron crystallography. NIH funding supports the development of this capability through a grant to Paul Langan (Los Alamos National Laboratory) and Paul Adams. See the [Macromolecular Neutron Crystallography Consortium site](#) for more details. Citation for neutron structure refinement in Phenix: Adams PD, Mustyakimov M, Afonine PV, Langan P: Generalized X-ray and neutron crystallographic analysis: more accurate and complete structures for biological macromolecules. *Acta Cryst.* 2009, **D65**:567-573.

**The PHENIX Industrial Consortium**

For-profit groups can obtain access to PHENIX through a Consortium agreement. This provides a license to use PHENIX and research funds to develop new features in PHENIX tailored to the needs of commercial users.

**Groups developing PHENIX:**

[Paul Adams](#) [Randy Read](#) [Jane & Dave Richardson](#) [Tom Terwilliger](#)

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Documentation  
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# Resources

PHENIX Documentation

phenix-online.org/documentation/

## Phenix Python-based Hierarchical Environment for Integrated Xtallography

**Phenix Documentation - 1.17.1-3660**

- [Phenix programs and their functions](#)
- [The Phenix graphical interface](#)
- [Overview of video tutorials](#)
- [Tutorials and Examples](#)
- [Dictionary of crystallographic and other terms](#)
- [FAQs: Frequently asked questions](#)
- [How to install, setup and run Phenix](#)
- [Complete Phenix reference documentation](#)
- [Bibliography](#)
- [Index](#)

### Crystallographic Structure Solution with Phenix

```
graph TD; DQA[Data Quality Assessment] --> EP[Experimental Phasing]; DQA --> MR[Molecular Replacement]; EP --> DM[Density Modification]; MR --> DM; DM --> MRB[Model (Re)building]; MRB --> RV[Refinement & Validation]; RV --> LF[Ligand Fitting]; LF --> MRB; RV --> SD[Structure Deposition];
```

**Phenix Documentation for X-ray Crystallography**

- [Checking data quality](#) | [Experimental phasing](#) | [Molecular replacement](#) | [Model building](#) | [Structure refinement](#) | [Structure validation](#) | [Ligand fitting](#) | [Making geometry restraints](#) | [Structure deposition](#) | [All](#)

### Phenix Documentation for Neutron Crystallography

- [Structure refinement](#) | [Structure validation](#) | [Making geometry restraints](#) | [Structure deposition](#) | [All](#)

### Cryo-EM Structure Solution with Phenix

```
graph TD; ASM[Auto-sharpen Map] --> DMI[Dock Model into Map]; ASM --> AMI[Autobuild Model into Map]; DMI --> RSR[Real Space Refinement]; AMI --> RSR; RSR --> VMM[Validation of Model and Map]; VMM --> SD[Structure Deposition];
```

**Phenix Documentation for Electron Microscopy (EM)**

- [Structure refinement](#) | [Map statistics \(resolution, etc\)](#) | [Auto-sharpen a map](#) | [Dock a model into a map](#) | [Rapid model-building](#) | [Guess sequences from map](#) | [Flexibly fit a model to a map](#) | [Build a model](#) | [Making geometry restraints](#) | [Compare CA/P in two models](#) | [Identify symmetry in a map](#) | [Combine best parts of focused maps](#) | [Extract box with map and model](#) | [Convert map to structure factors](#) | [Segment a map](#) | [Adjust a CA/CB model](#) | [Fix register errors](#) | [All](#)

# User support

- **Feedback, questions, help**

Mailing list (all, developers and users): [phenixbb@phenix-online.org](mailto:phenixbb@phenix-online.org)

Bug reports (developers only): [bugs@phenix-online.org](mailto:bugs@phenix-online.org)

Ask for help (developers only): [help@phenix-online.org](mailto:help@phenix-online.org)

- **Reporting a bug or asking for help:**

- We can't help you if you don't help us to understand your problem

- Make sure the problem still exist using the latest *Phenix* version

- Send us all inputs (files, non-default parameters) and tell us steps that lead to the problem

- All data sent to us kept confidentially

- **We are local (LBNL, main site): come over and chat with us!**