Phenix Tools for Cryo-EM

The *Phenix* project

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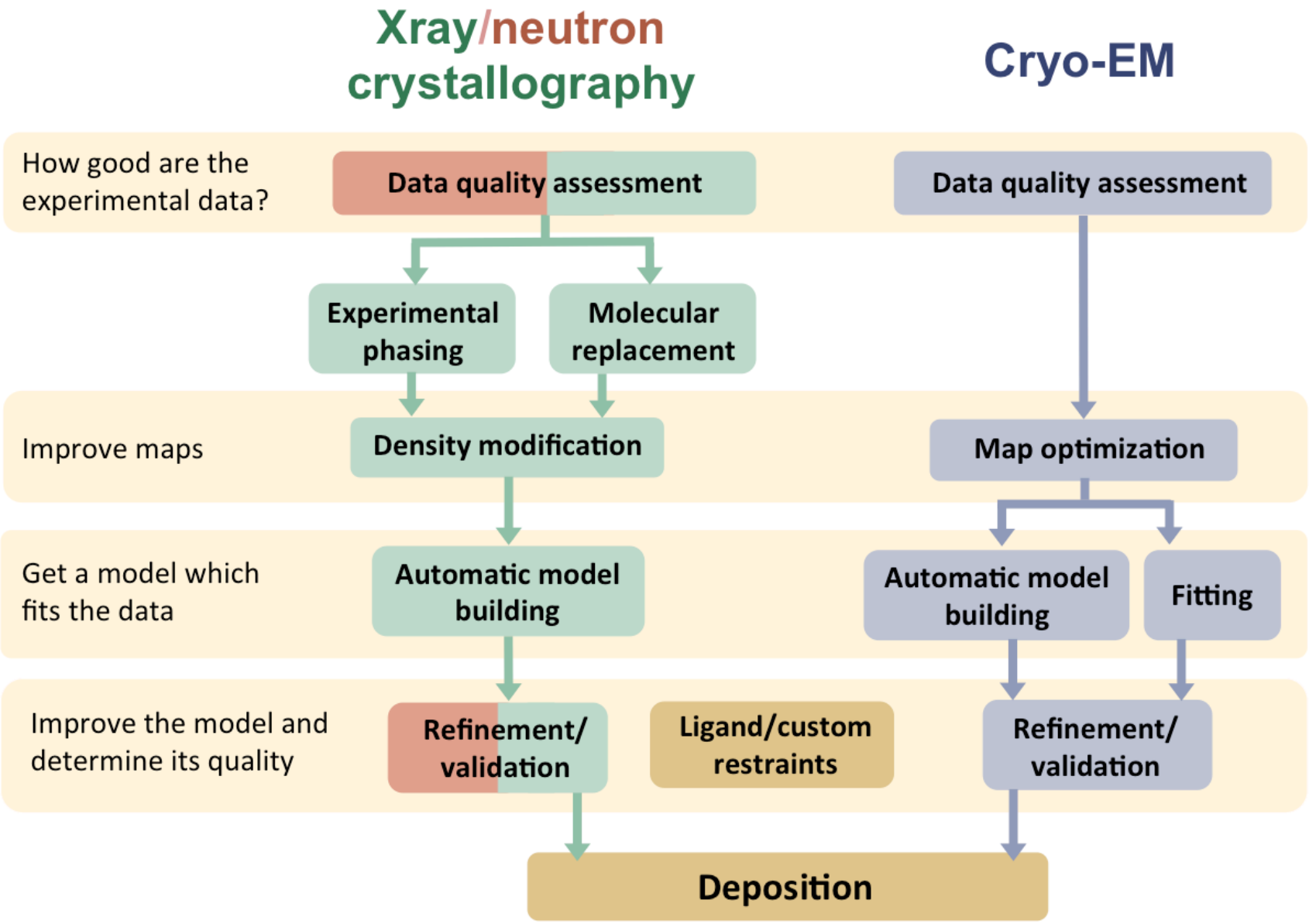
Duke University

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

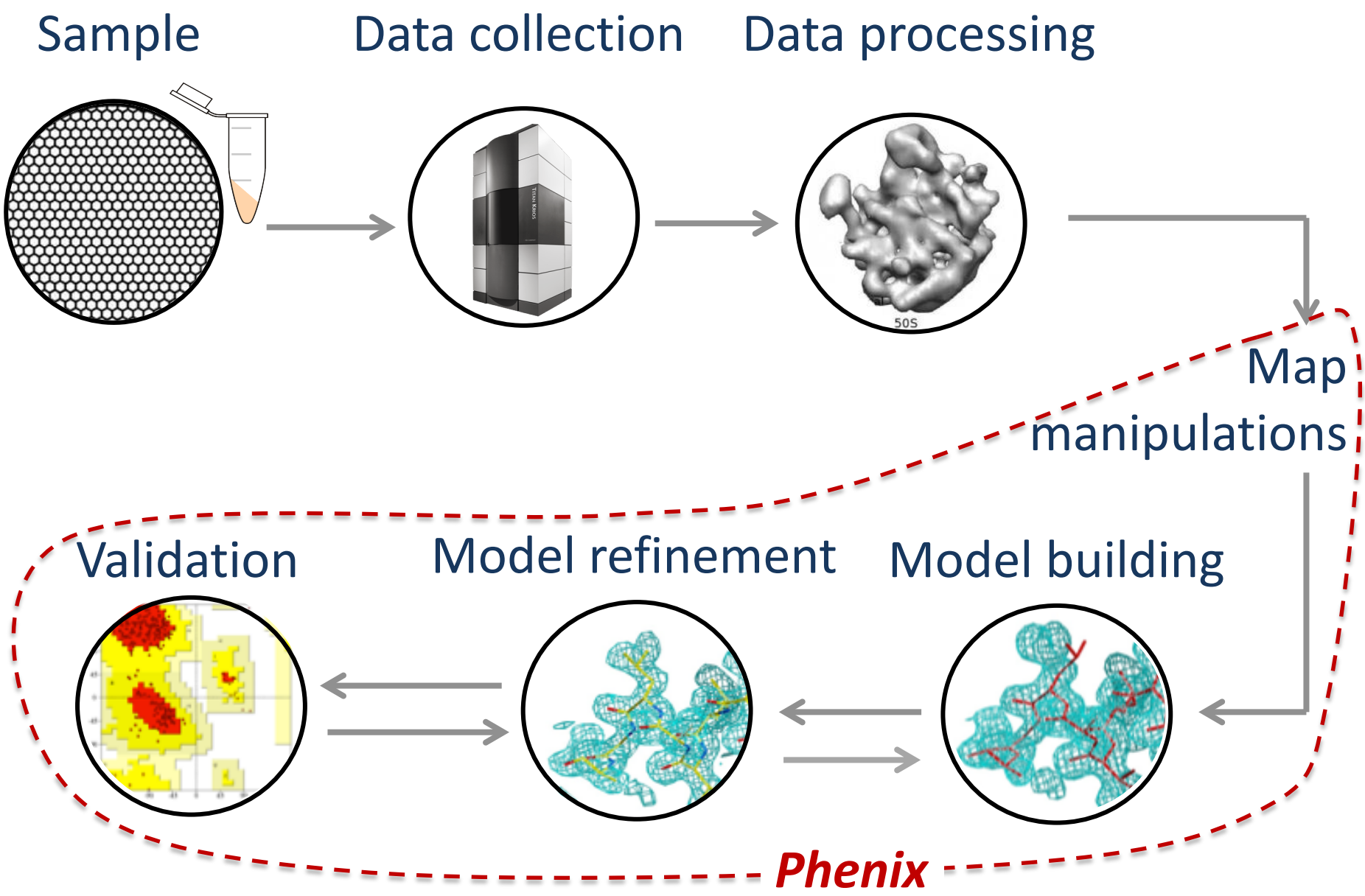
An NIH/NIGMS funded Program Project

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

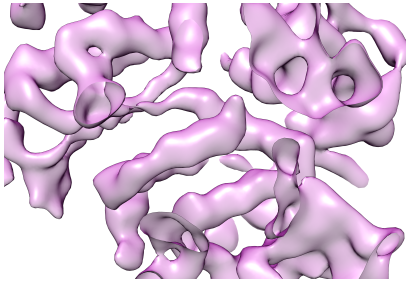
Phenix: tools for crystallography and cryo-EM



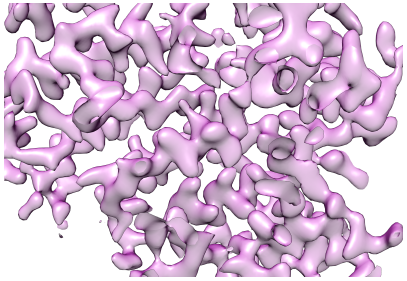
Phenix tools for cryo-EM



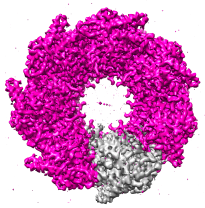
Phenix tools for cryo-EM



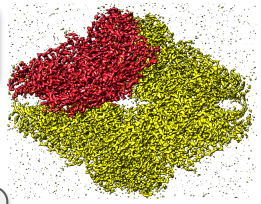
Starting map



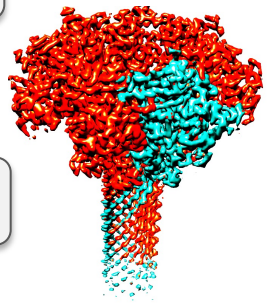
Map improvement



Map symmetry

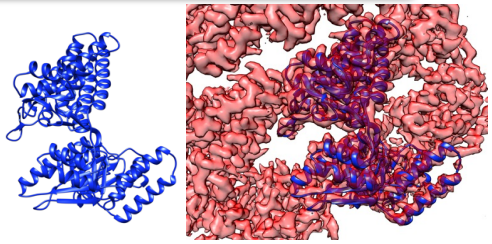


Map manipulation

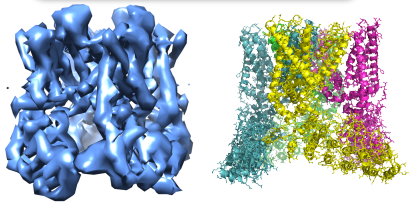


Extract unique part

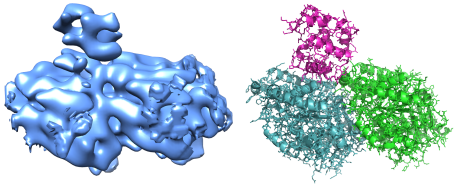
Docking, model building



Refinement

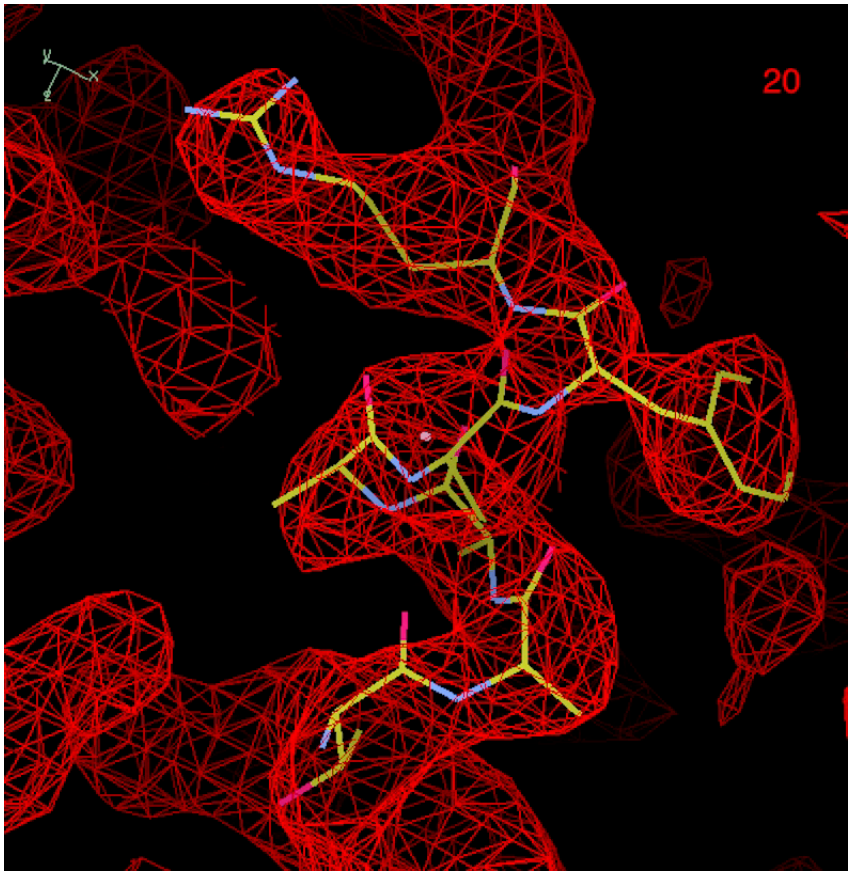


Validation



Complete set of tools for cryo-EM structure solution: from initial reconstruction to final validated model

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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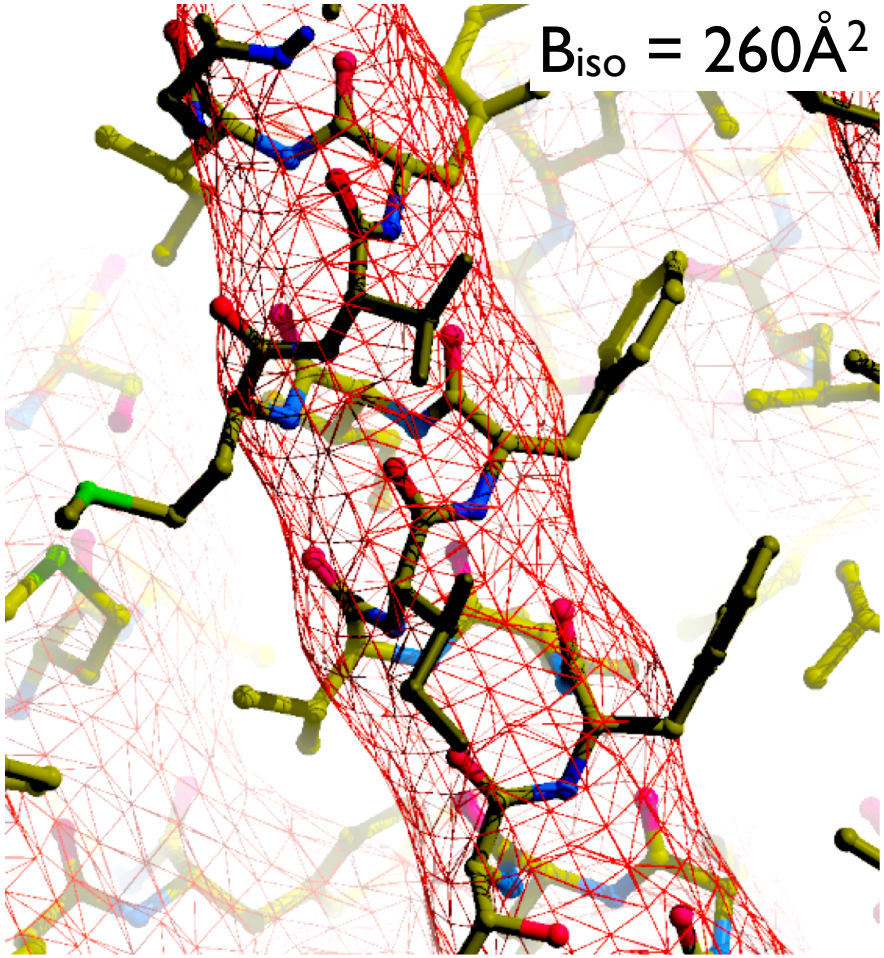
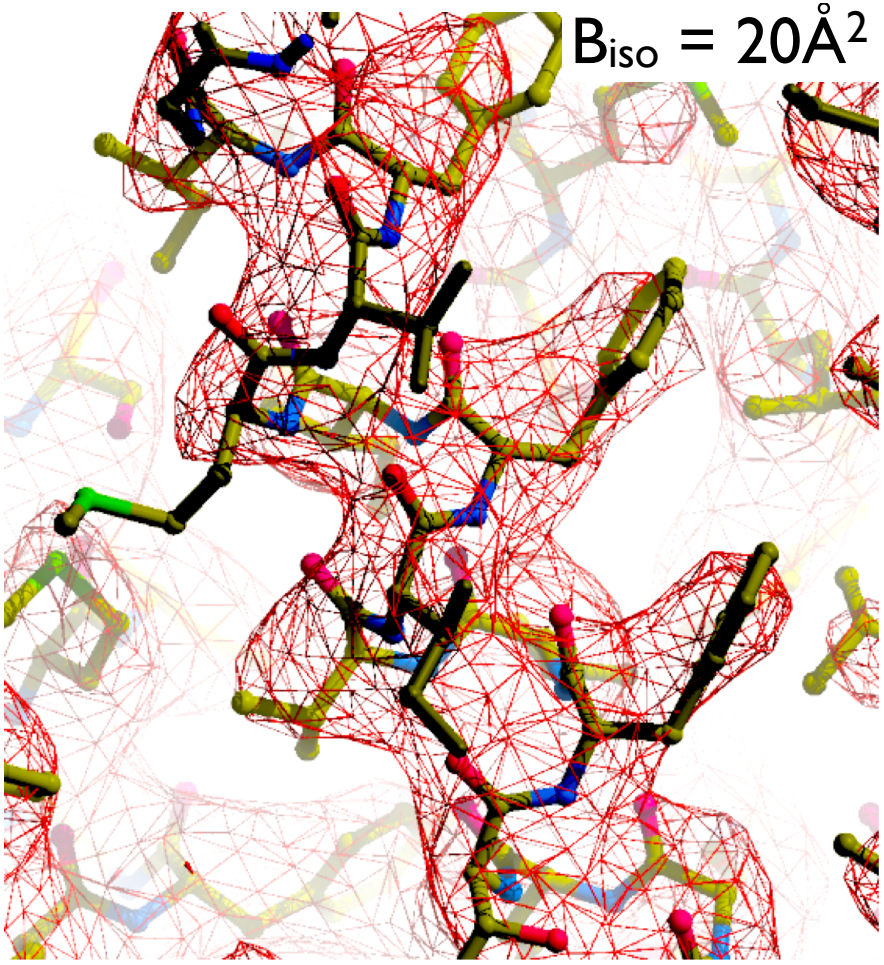
Automated map sharpening by maximization of detail and connectivity

Thomas C. Terwilliger,[a,b]* Oleg V. Sobolev,[c] Pavel V. Afonine[c,d] and Paul D. Adams[d,e]

Automated map sharpening: *phenix.auto_sharpen*



Deposited Map



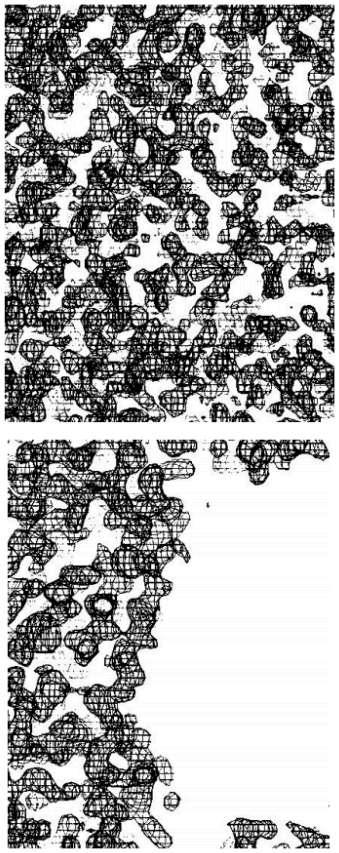
Autosharpened Map

High-conductance Ca(2+)-activated 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:

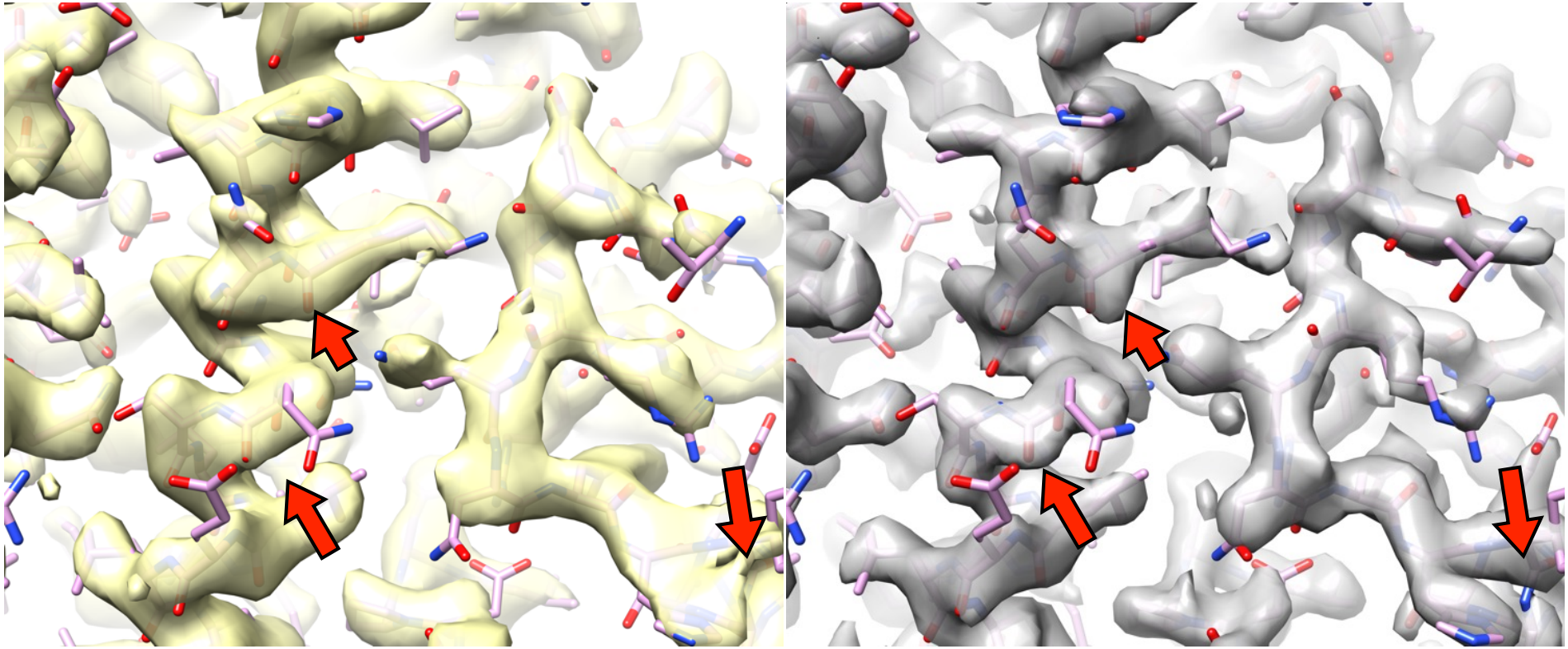
- Both amplitudes and phases have errors
- Half-maps are available

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

- Macromolecular density distributions (histogram matching)

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

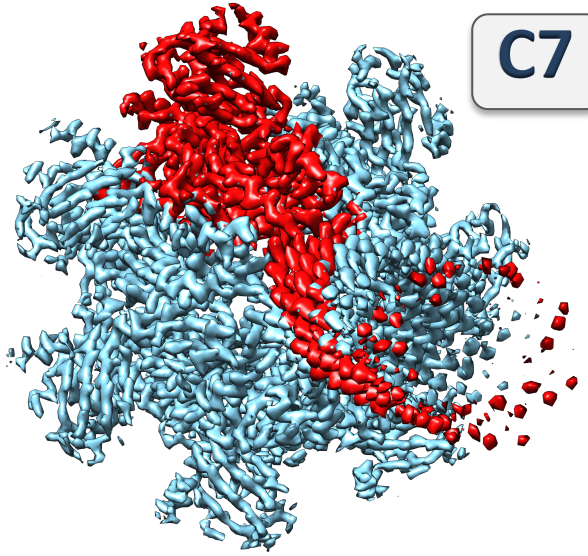
Density modification: *phenix.density_modify_cryo_em*

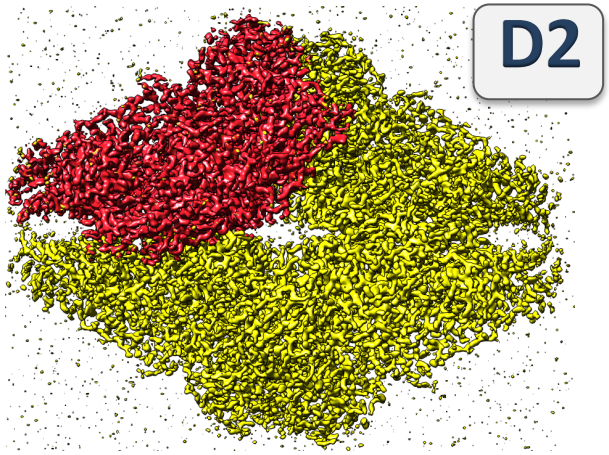


Density modification for cryo-EM maps, facts:

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

Finding map symmetry: *phenix.symmetry_from_map*





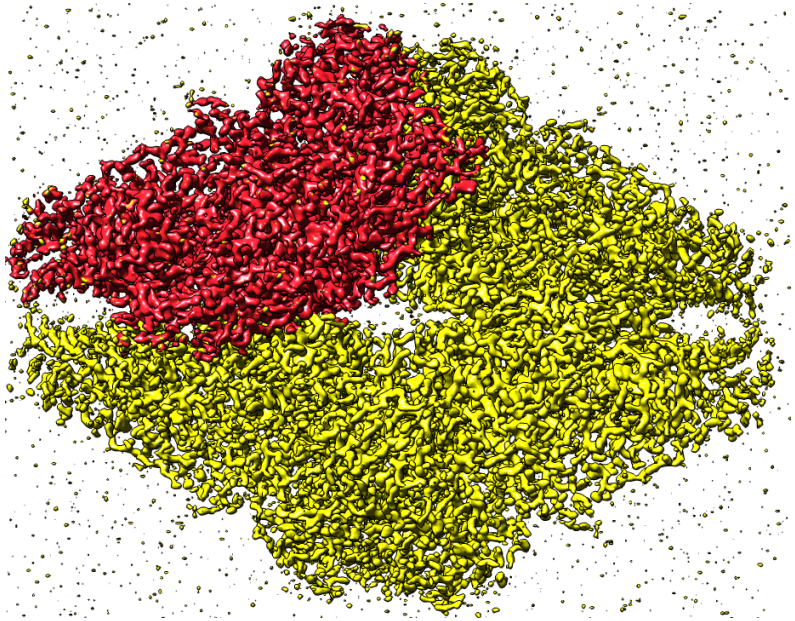
Procedure for finding symmetry:

- 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*



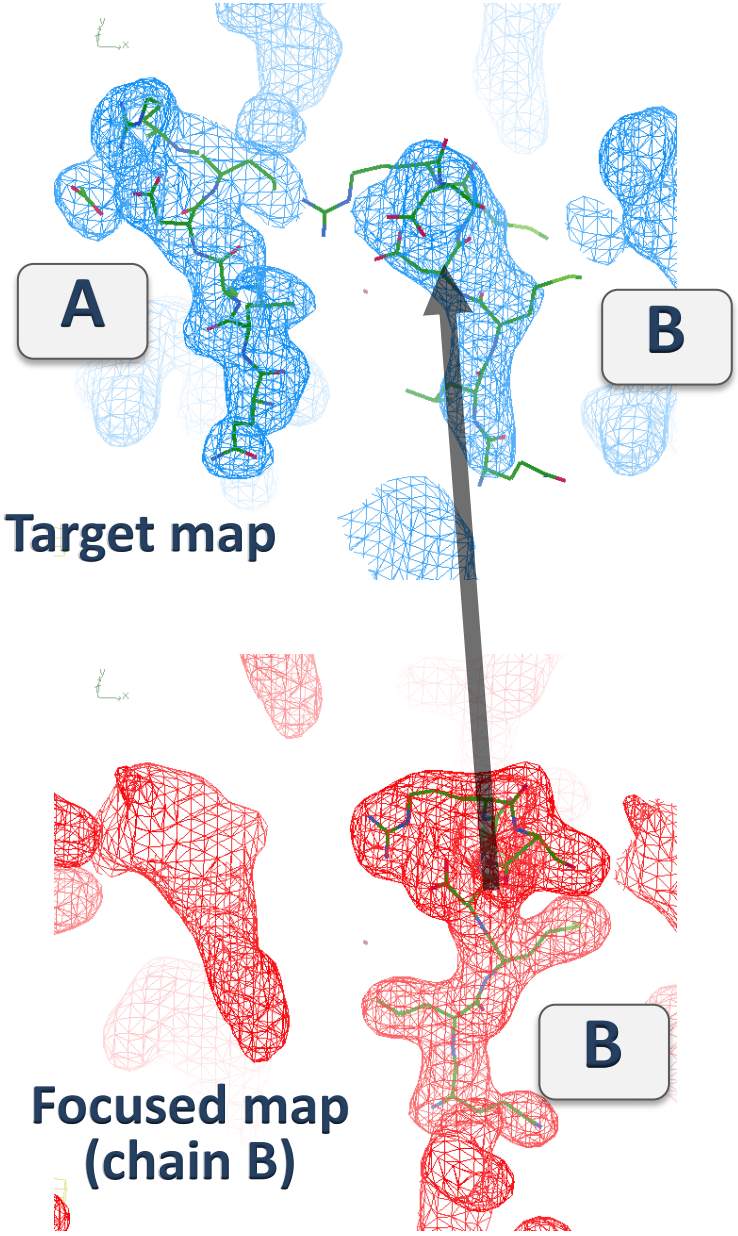
Procedure:

- 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

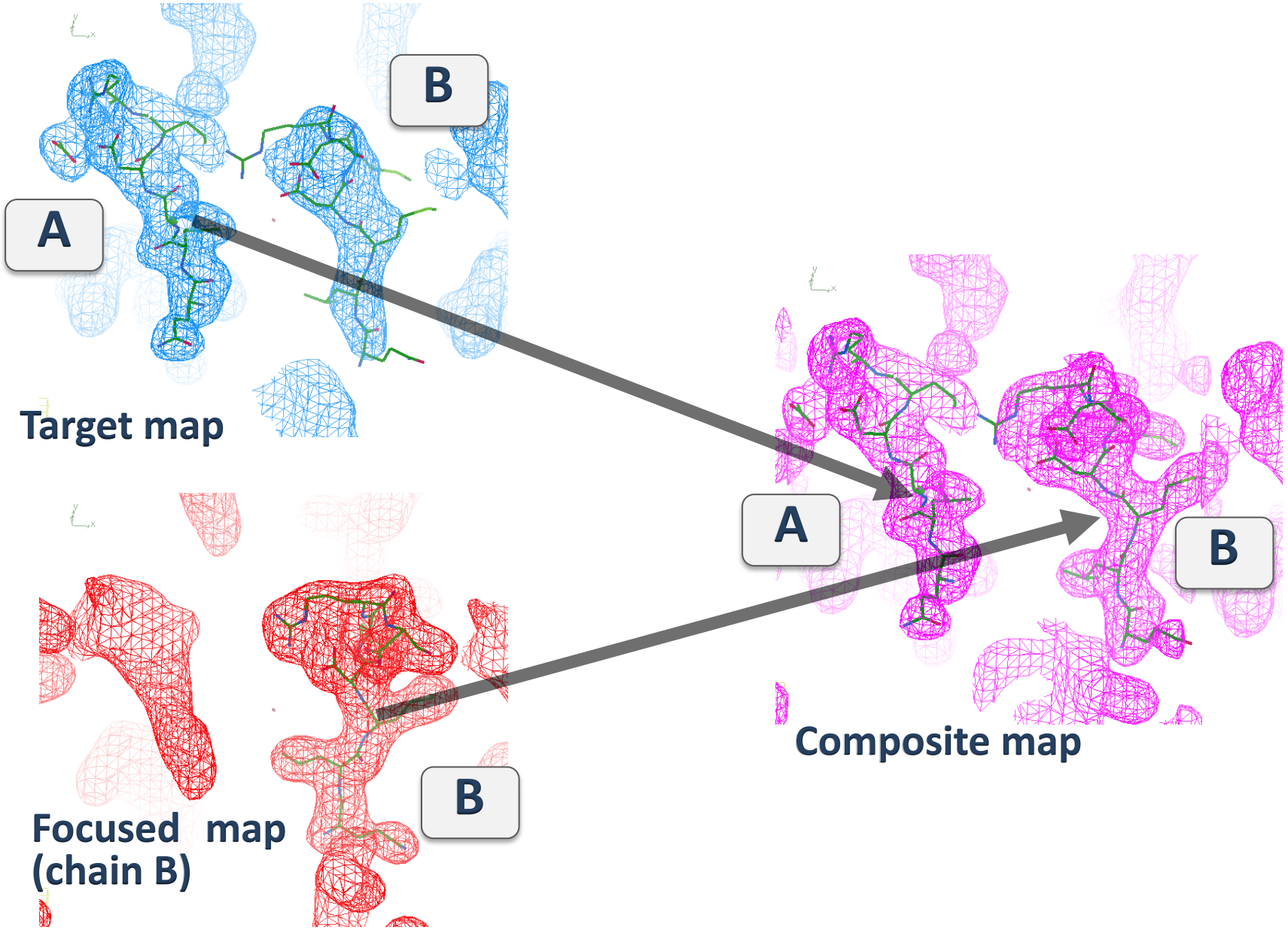
Combining maps with *phenix.combine_focused_maps*



Procedure for combining maps:

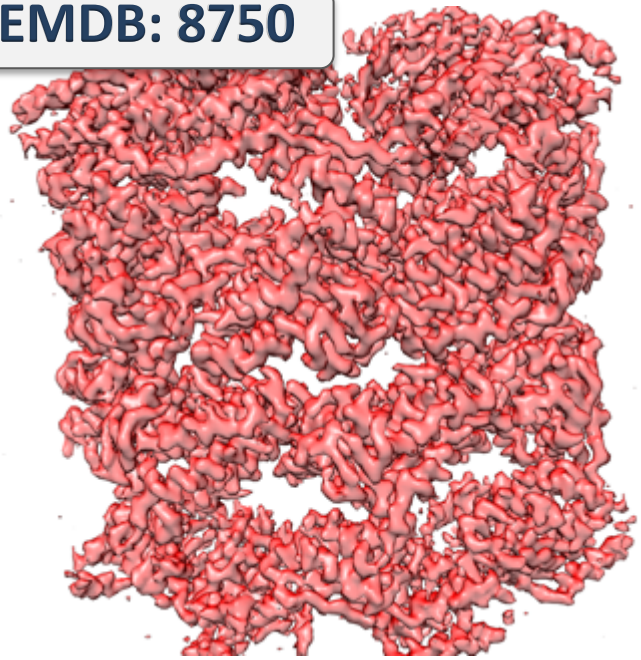
- Superpose density
- Rotation/translation from refined models
- Average target and focused map density
- Weight using map-model correlations

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

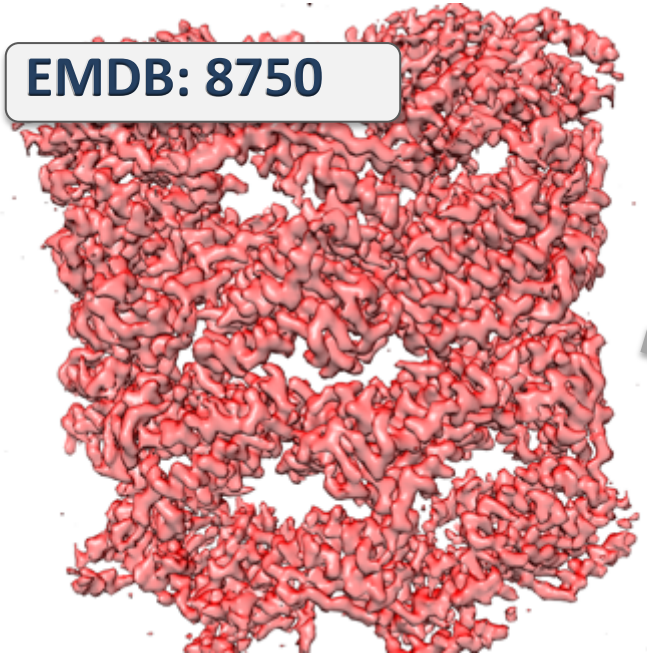
Score based on rigid-body refinement map-model correlation

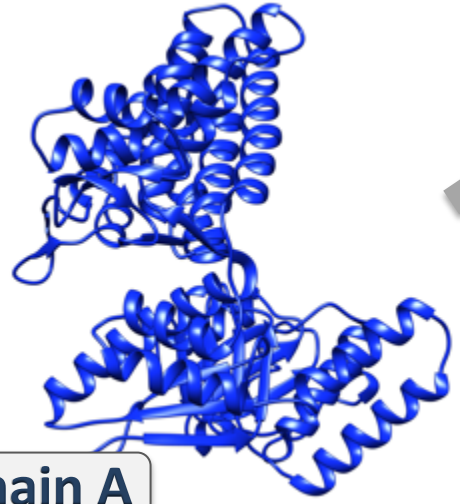
Features:

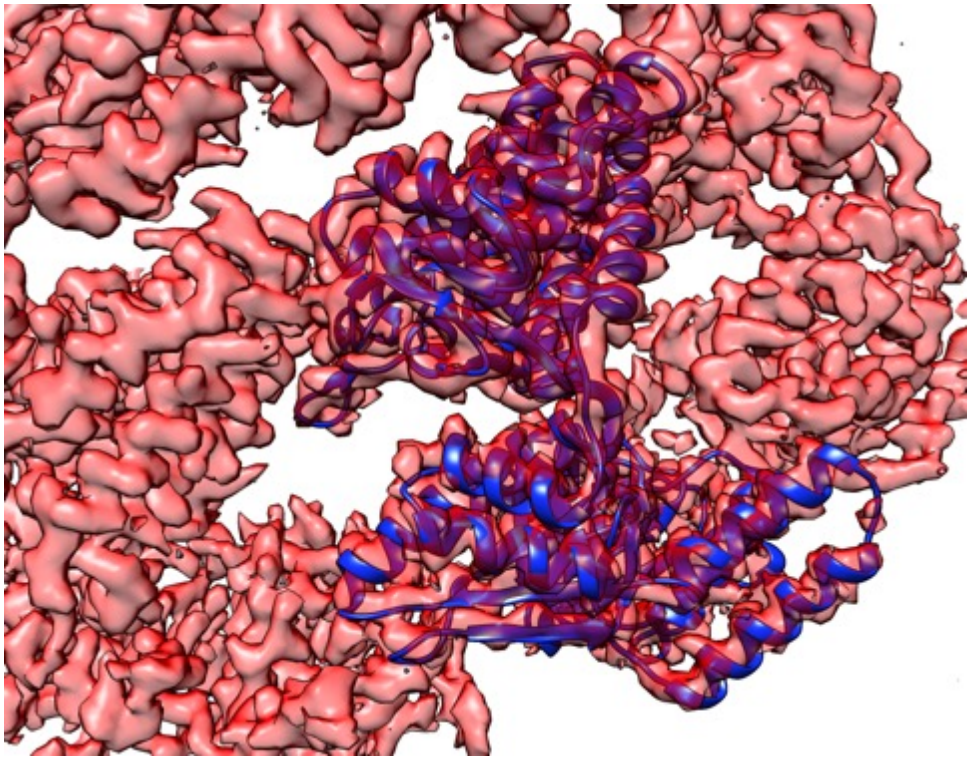
- Multiple chains
- Density search
- Symmetry
- Multiprocessing

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

Docking models with *phenix.dock_in_map*

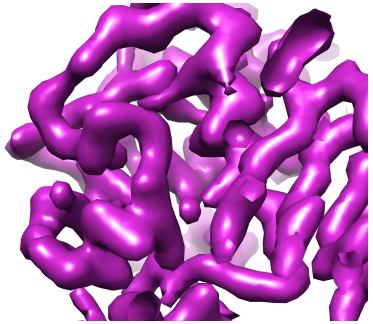






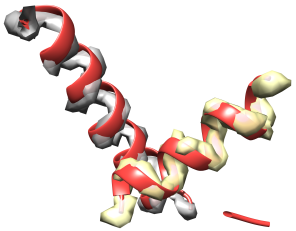
Automated model building: *phenix.map_to_model*

Trace chain the way a person does

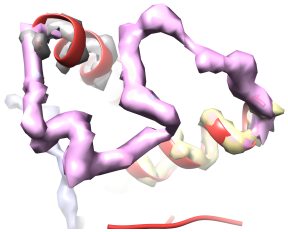


Find secondary structure

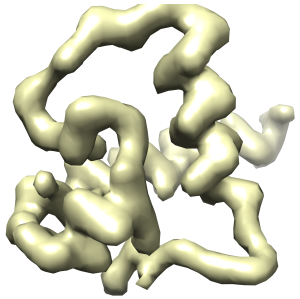
Find clear regions of density



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

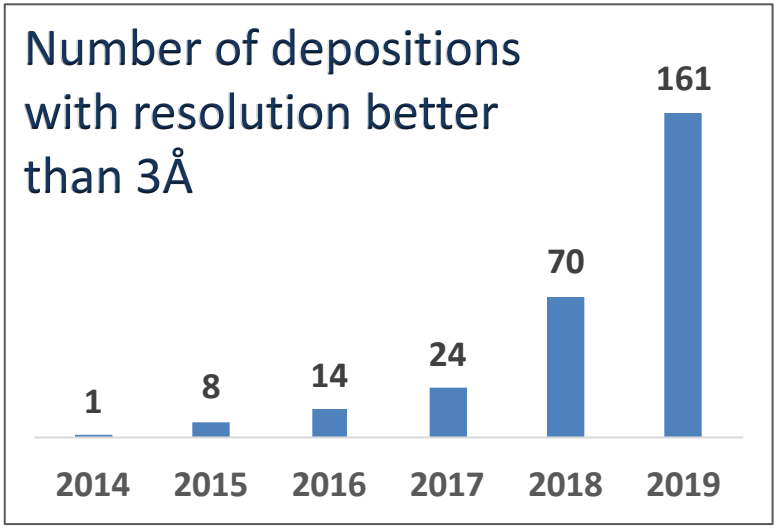
Thomas C. Terwilliger[1,2*], Paul D. Adams[3,4], Pavel V. Afonine[3,5] and Oleg V. Sobolev[3]

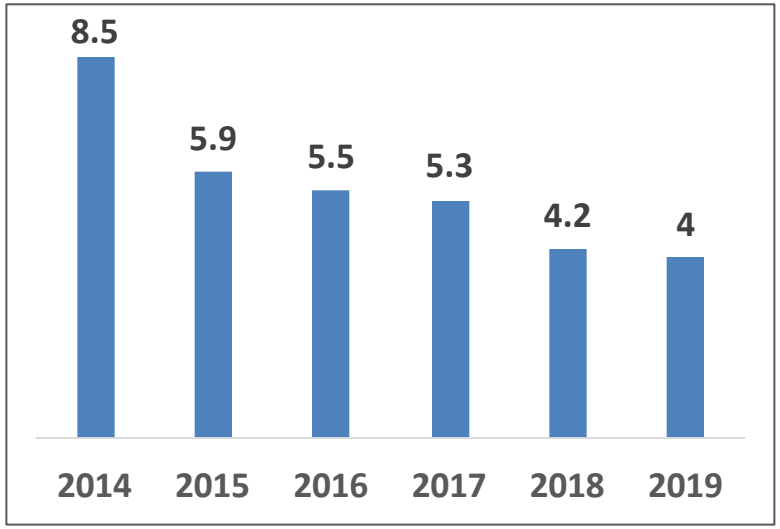
Automated model building: *phenix.map_to_model*

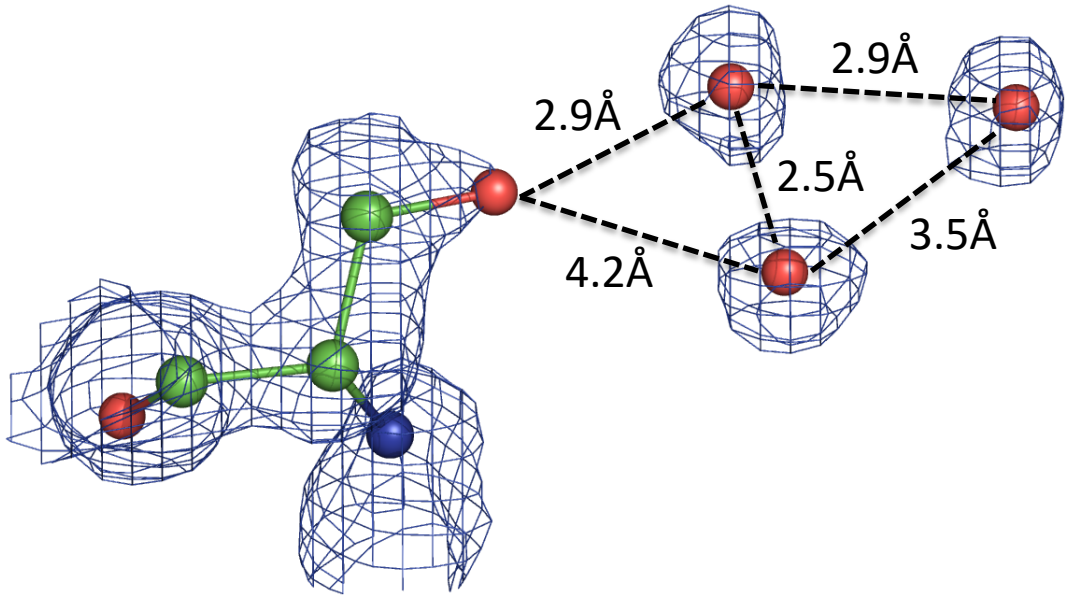
Automated model building, facts:

- 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

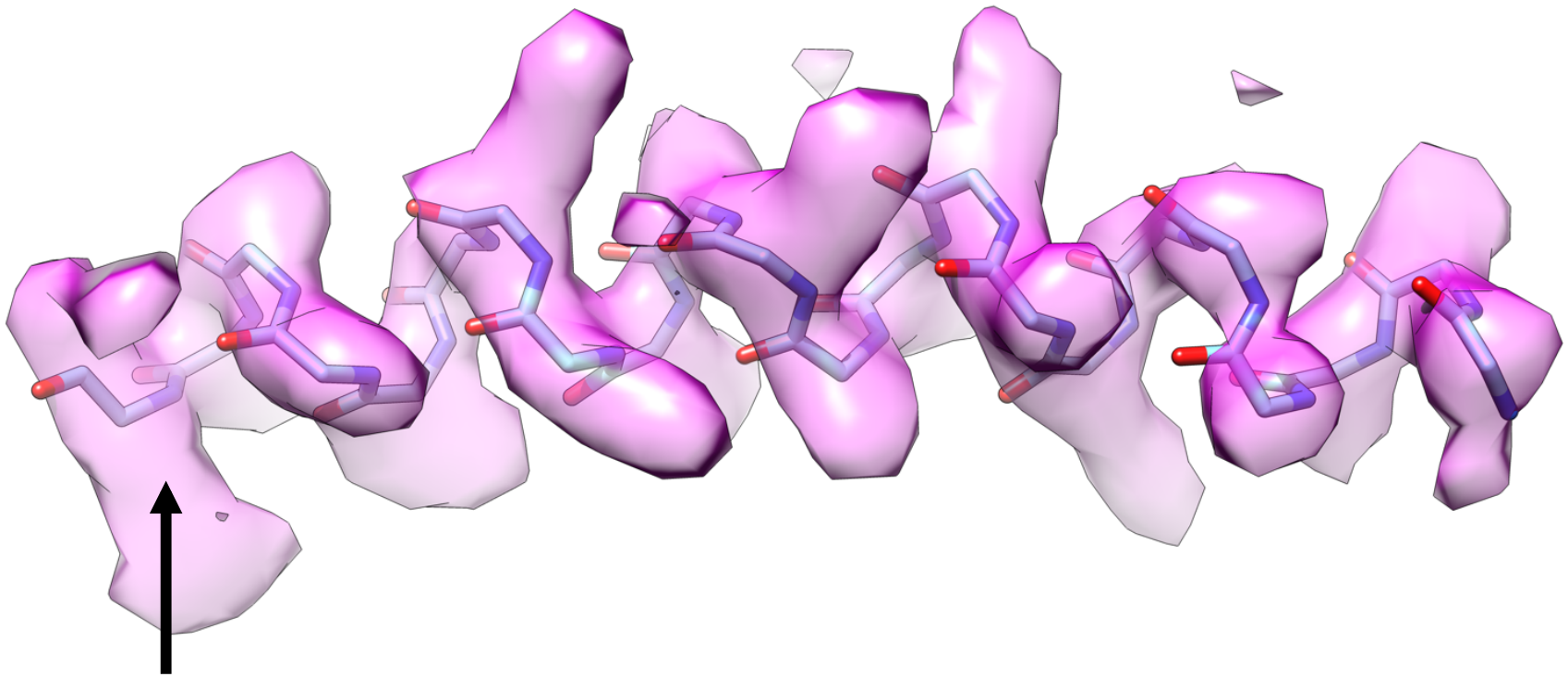
Automated water building: *phenix.douse*







Sequence from map: *phenix.sequence_from_map*

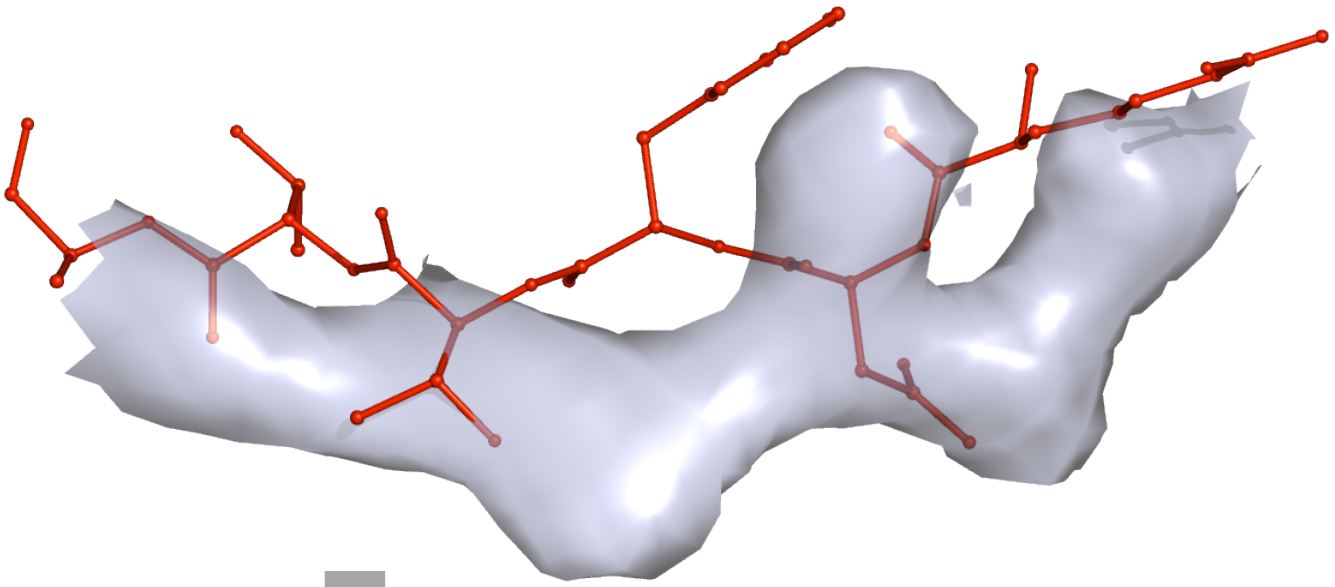


Residue	G	A	S	V	I	L	M	C	F	Y	K	R	W	H	E	D	Q	N	P	T
CC	0.30	0.50	0.53	0.47	0.58	0.62	0.68	0.59	0.83	0.77	0.71	0.69	0.70	0.82	0.65	0.64	0.60	0.60	0.35	0.47
Prob	3	0	0	0	0	0	1	0	40	23	5	5	4	9	2	2	1	0	2	0

- Determine probability of side chain at each C**α**
- Align sequence to maximize total probability for the chain

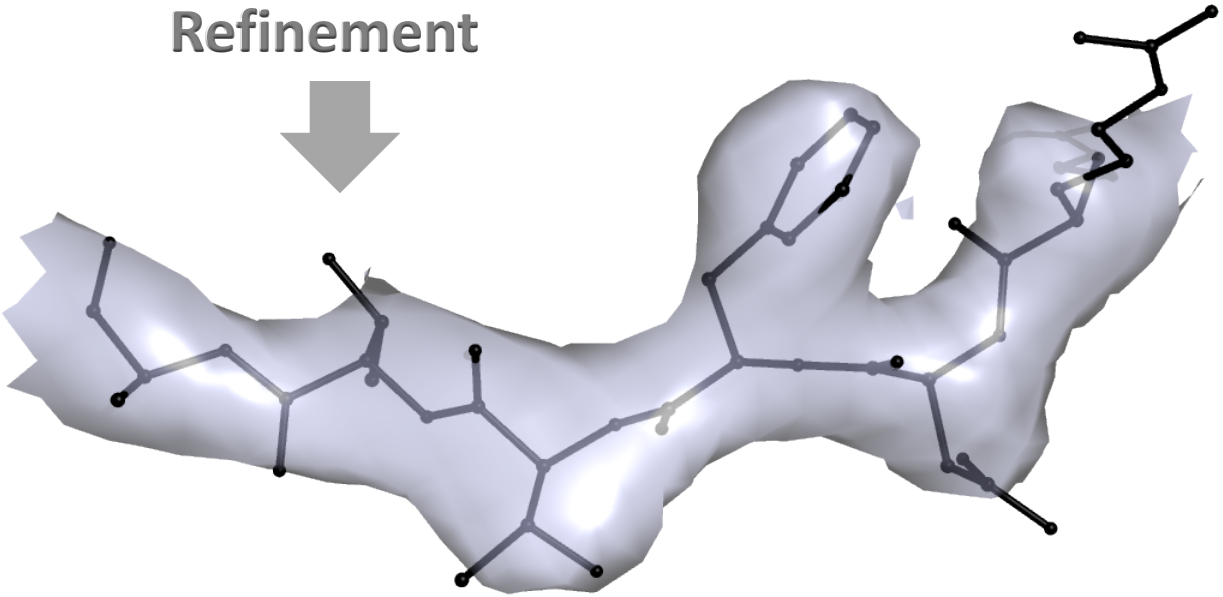
Atomic model refinement: *phenix.real_space_refine*

Initial (poor) model



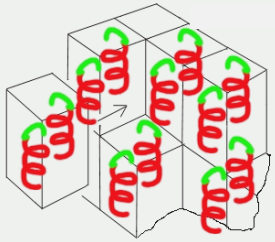
Refinement

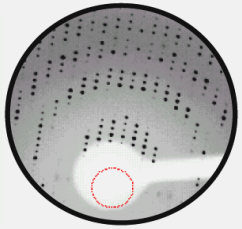
Improved (refined) model



Atomic model refinement: *phenix.real_space_refine*

Crystallography





Initial model

Experimental data

A priori knowledge

Score

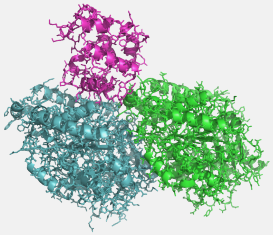
Modify model parameters

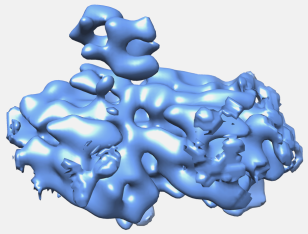
Improved model

phenix.refine

Available since 2005

Cryo-EM





Initial model

Experimental data

A priori knowledge

Score

Modify model parameters

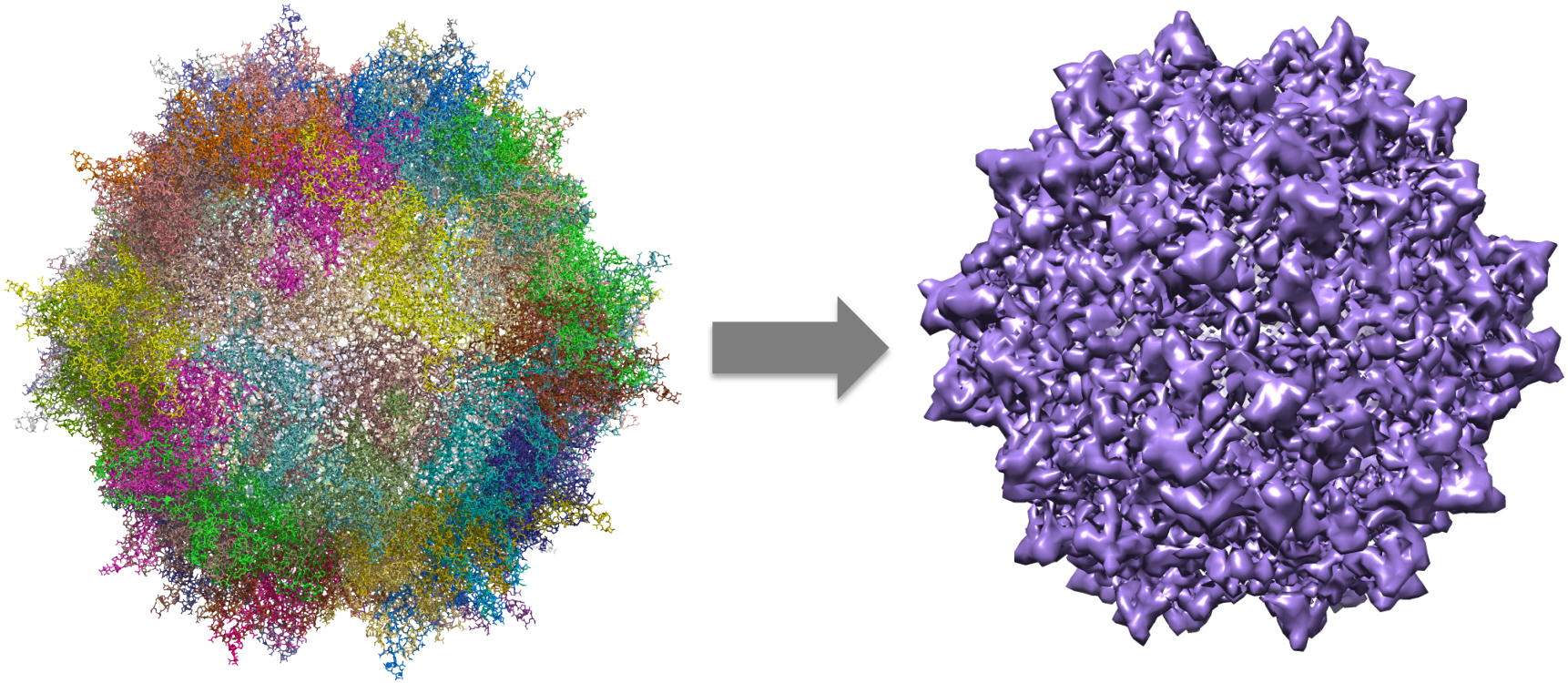
Improved model

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

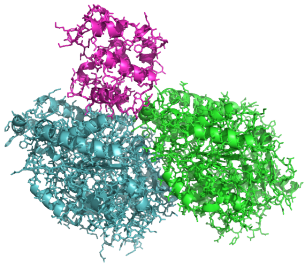
Pavel V. Afonine,[a,b]* Billy K. Poon,[a] Randy J. Read,[c] Oleg V. Sobolev,[a] Thomas C. Terwilliger,[d,e] Alexandre Urzhumtsev[f,g] and Paul D. Adams[a,h]

Atomic model refinement: *phenix.real_space_refine*

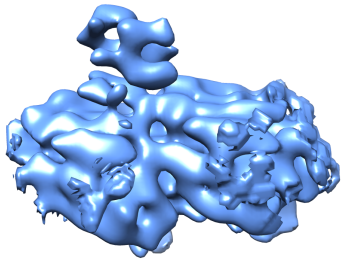
- Best model-map fit. Any map: X-ray, neutron, EM. Any resolution
- Refined models: no poor validation metrics
- Fast (minutes – a few hours, not days or many hours)
  - Make use of multiple CPUs: as many as available
- Large convergence radius
- Easy to use: map and model in, refined model out
- Accessible: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

Validation

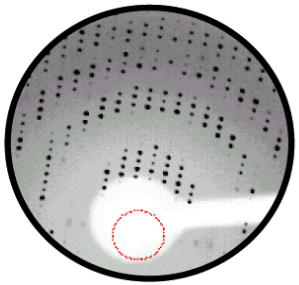
Model



Data



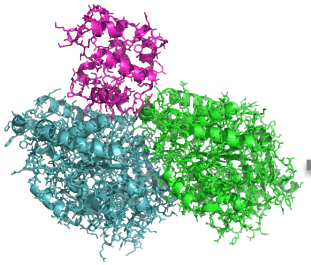
or

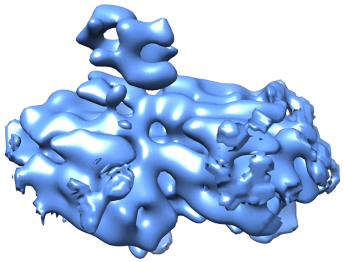


Cryo-EM

Diffraction

Model to data fit

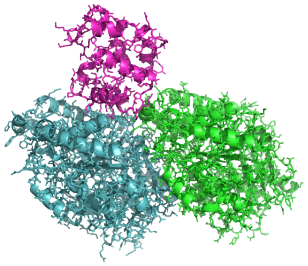




Validation: crystallography vs cryo-EM

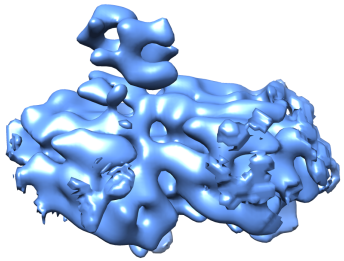
Exactly same

Model

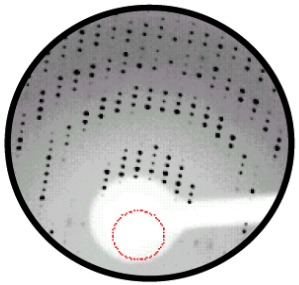


Different

Data



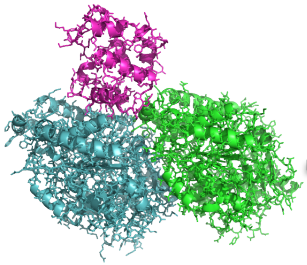
or

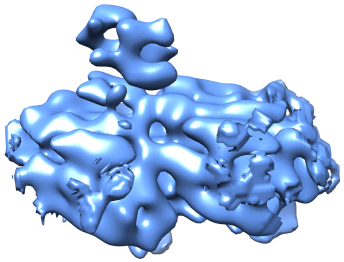


Cryo-EM

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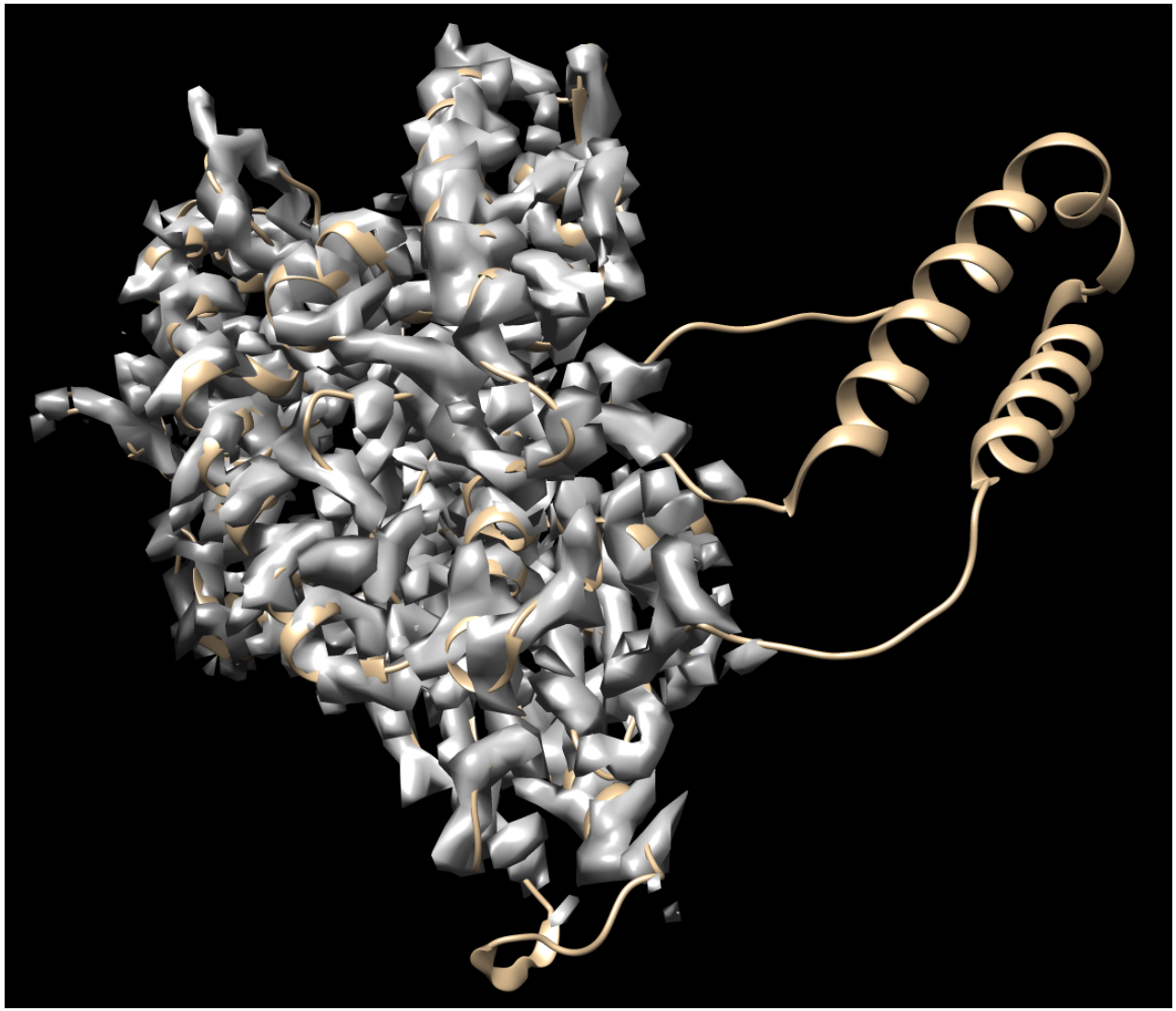
New tools for the analysis and validation of cryo-EM maps and atomic models

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

Validation: why to do?

3j9e (emd_6240) | 3.3Å | CC= 0.85 | Year: 2015

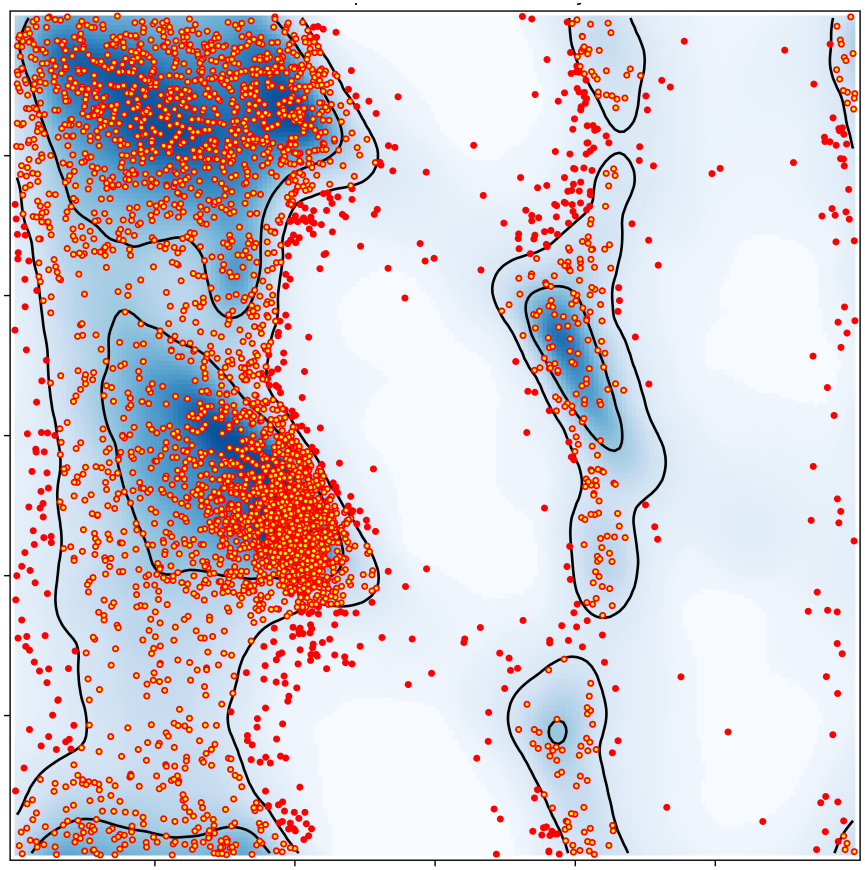


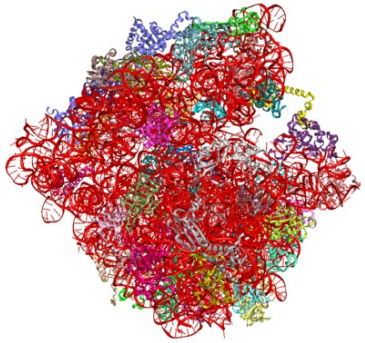
Validation: why to do?

3a5x (emd_1641) | 4.0Å | CC <0

Validation: why to do?

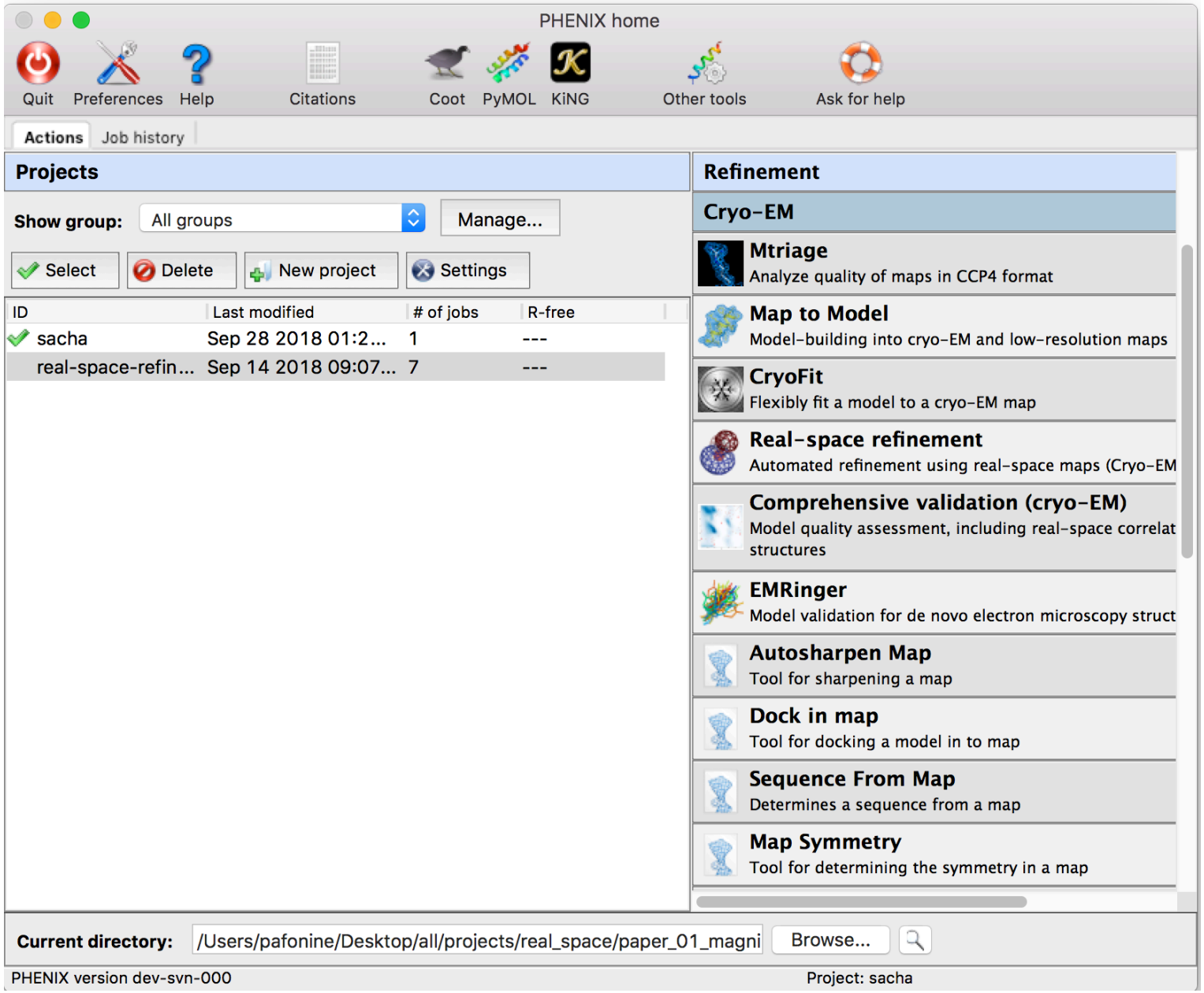
(2019) Nature 570: 400-404 | PDB: 6o9j | EMDB: 0661 | 3.9Å



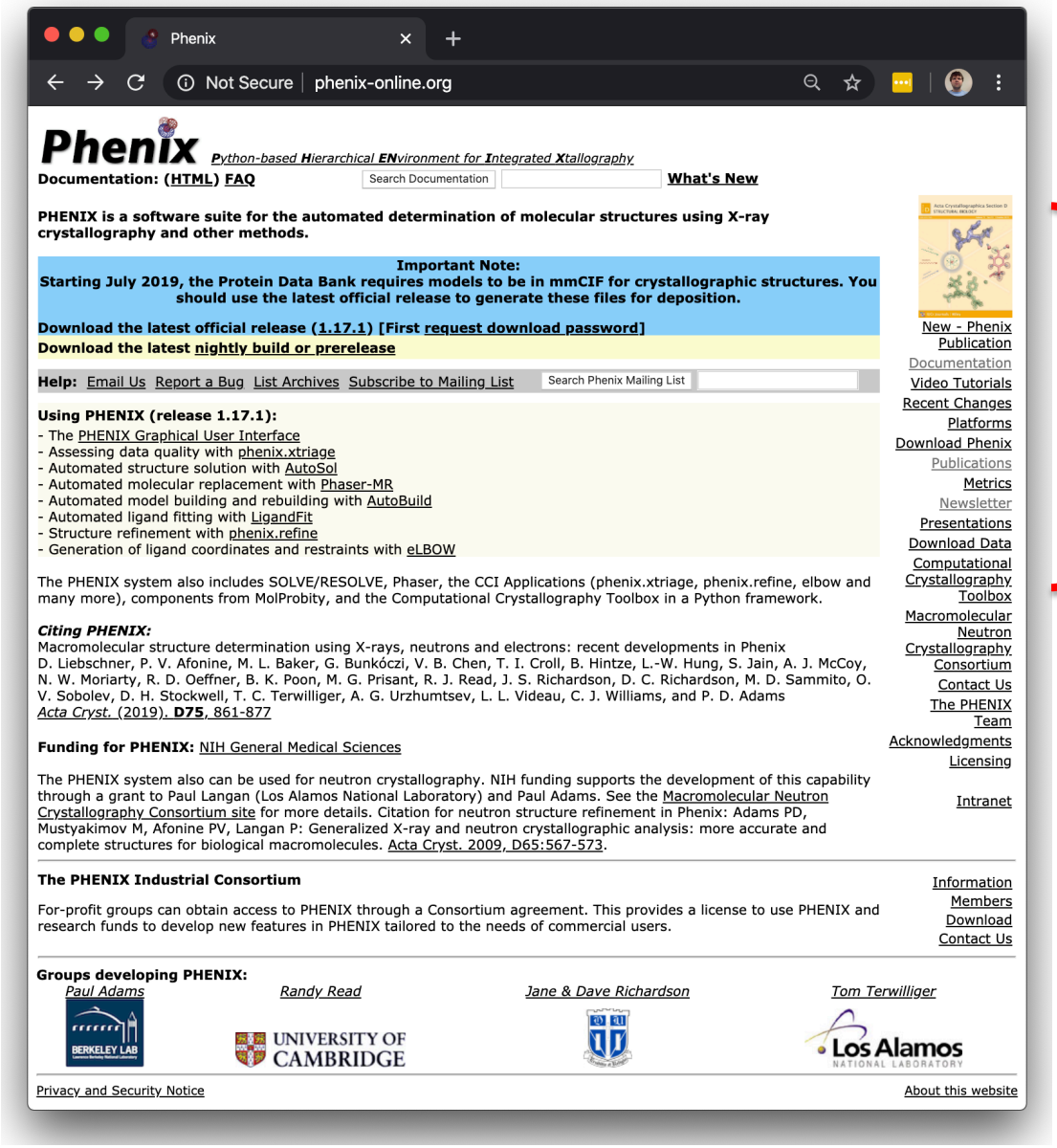


Metric	6o9j	Expected
Clashcore	70	Less than 10
Ramachandran favored, %	59	More than 98
Ramachandran outliers, %	15	0
Rotamer outliers, %	23	0
C_β deviations, %	0.5	0

Phenix tools for cryo-EM



Resources



Phenix paper

Video tutorials

Documentation

Relevant papers

Bi-annual newsletters

Slides from workshops

Resources

PHENIX Documentation

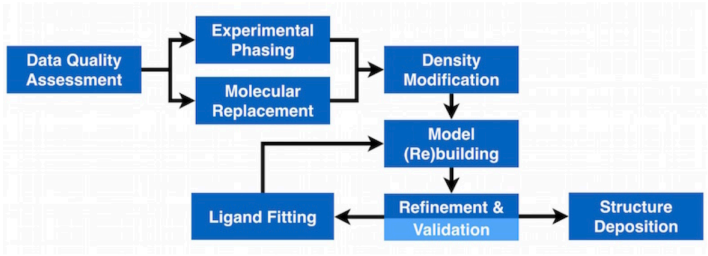
Not Secure | phenix-online.org/documentation/

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



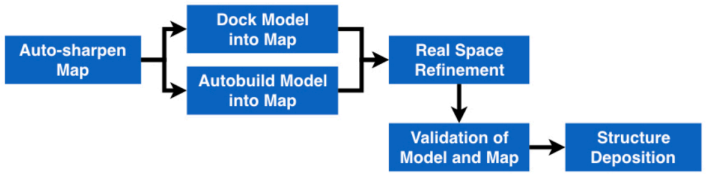
Phenix Documentation for X-ray Crystallography

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



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
  Bug reports (developers only): bugs@phenix-online.org
  Ask for help (developers only): 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!**