Phenix Tools for Cryo-EM

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March 9, 2022
OpenEye CUP Conference

The Phenix Project

Lawrence Berkeley Laboratory

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

New Mexico Consortium
Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung

University of Cambridge

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

Baylor College of Medicine

Matt Baker, Corey Hryc

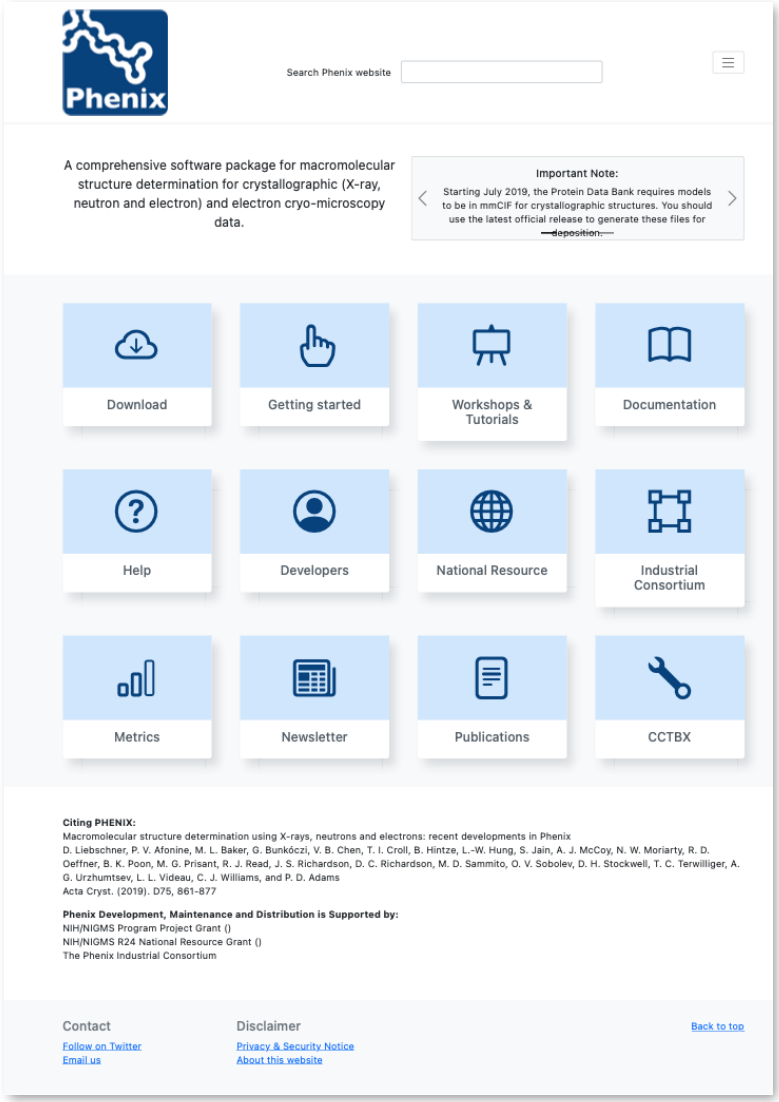
Duke University

Jane & David Richardson, Chris Williams, Vincent Chen

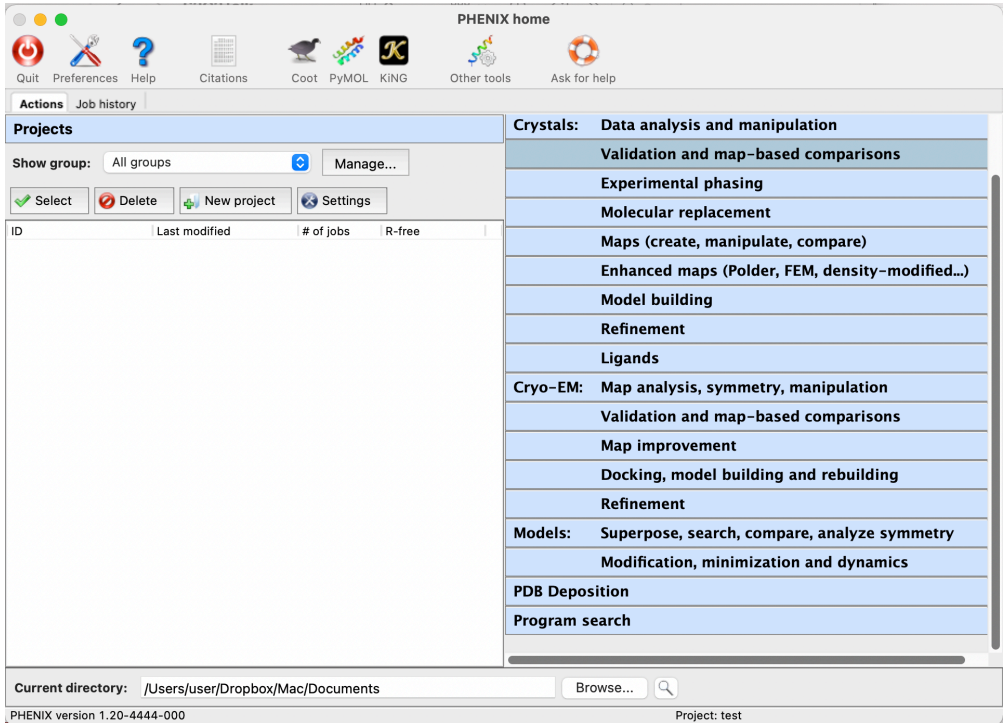
An NIH/NIGMS funded Program Project

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

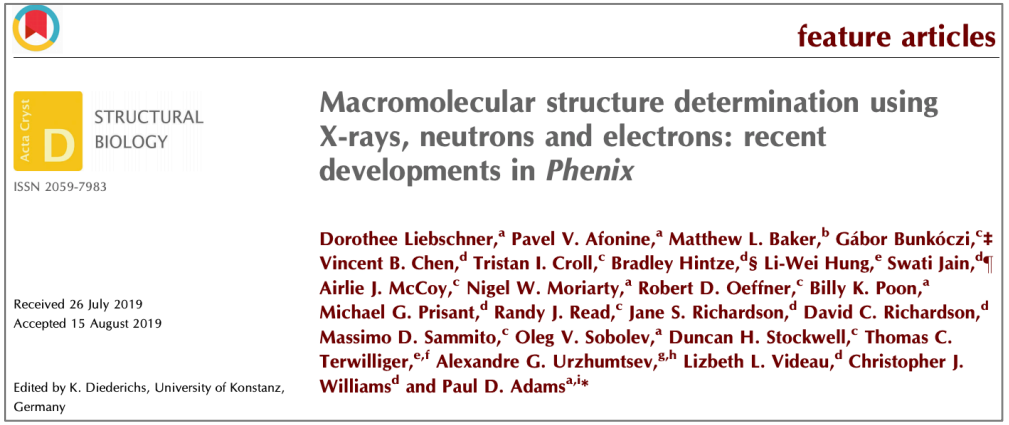
Phenix



Website

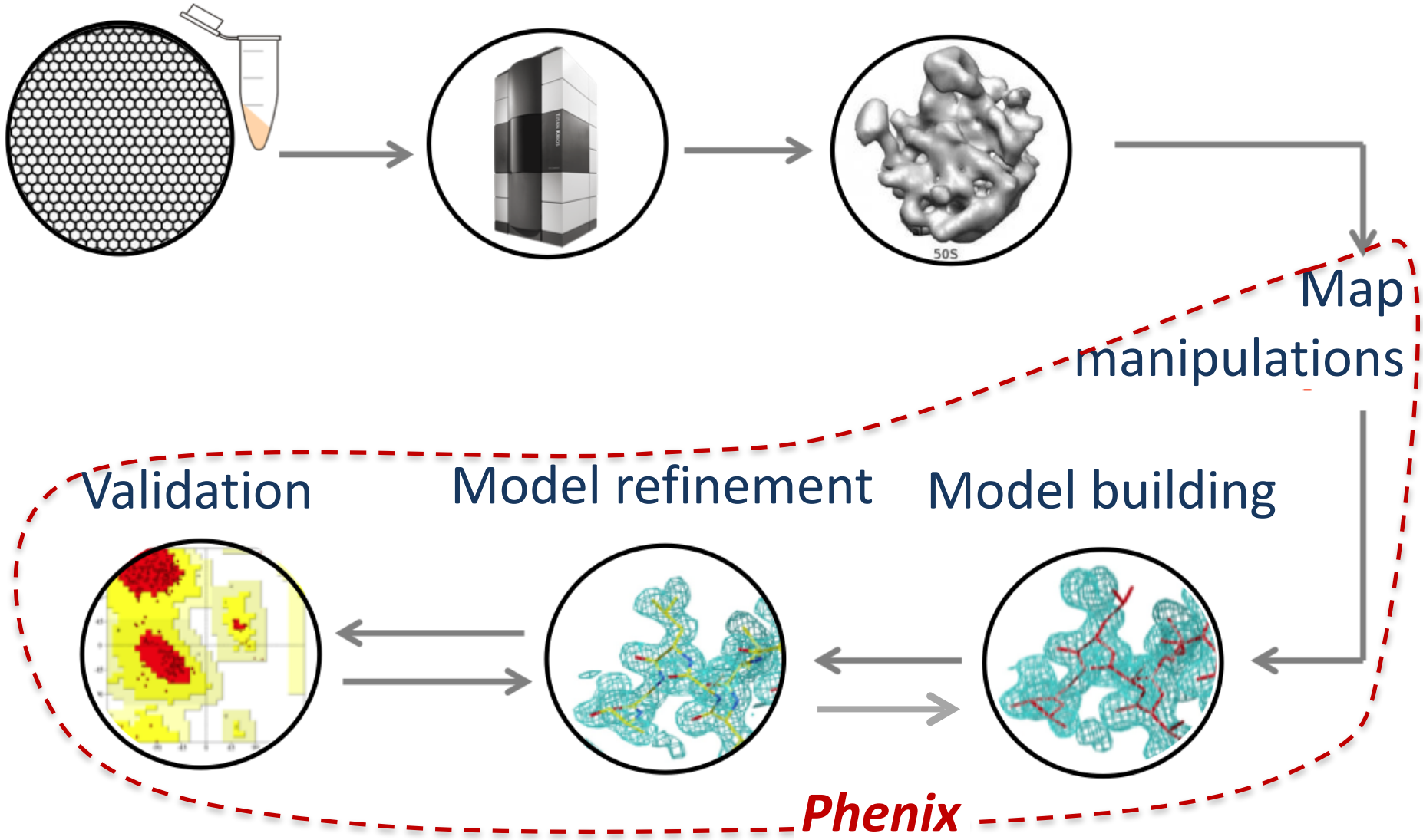


GUI



Most recent publication

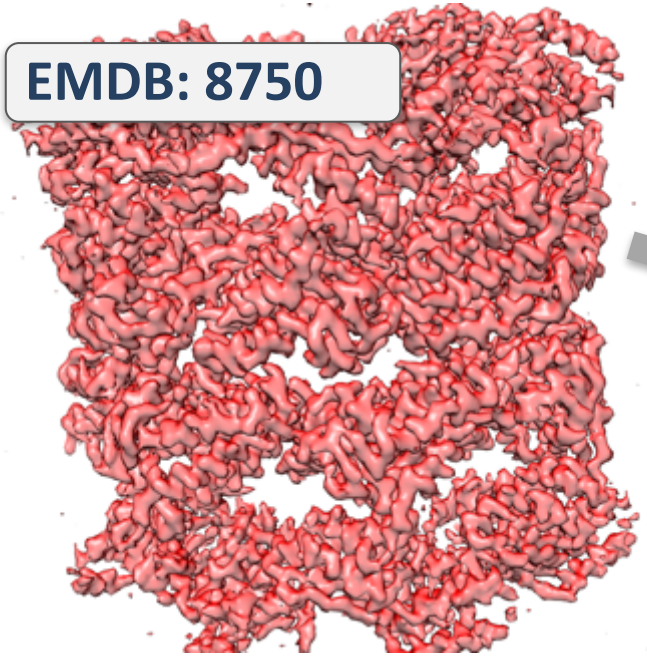
Typical cryo-EM pipeline

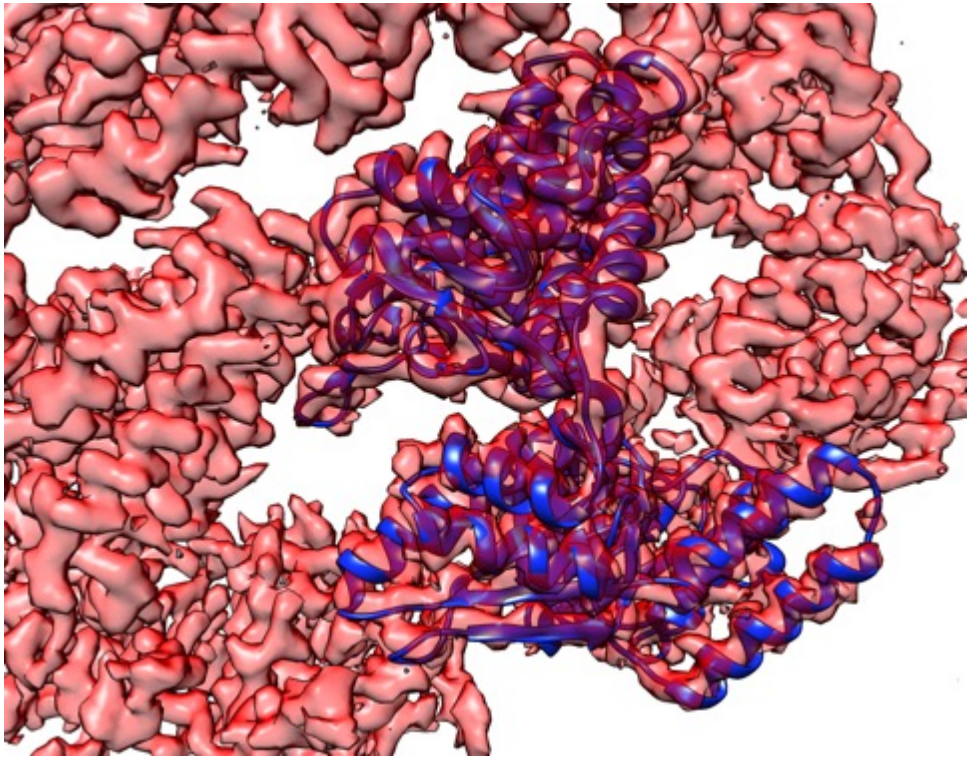


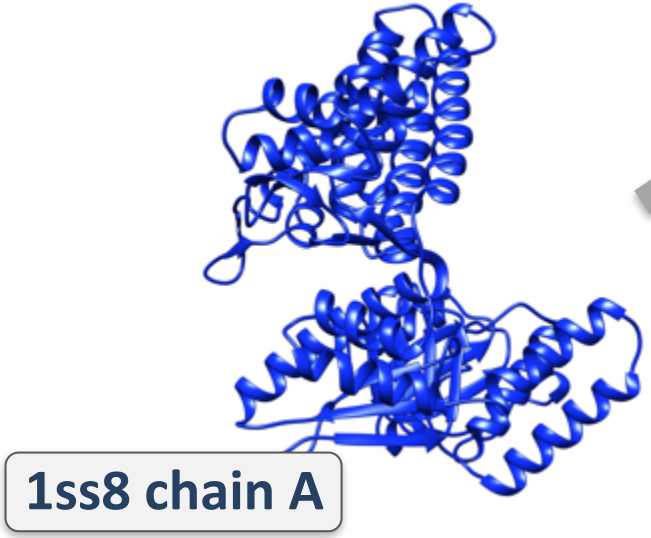
Agenda: A long list of cryo-EM tools within Phenix

If a little is good, more must be better - Mae West (adapted)

Docking models with *phenix.dock_in_map*





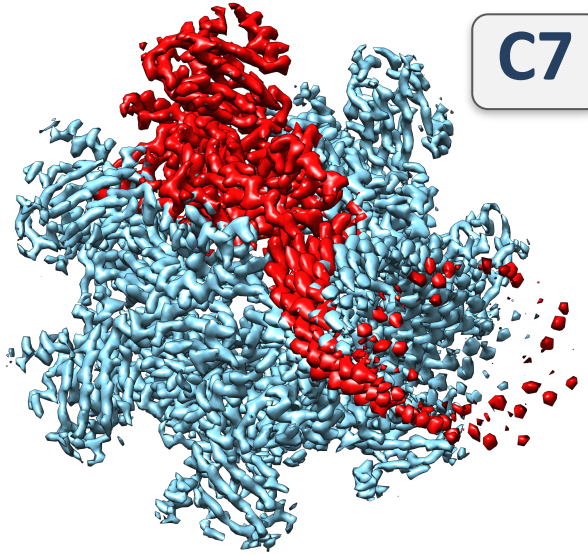


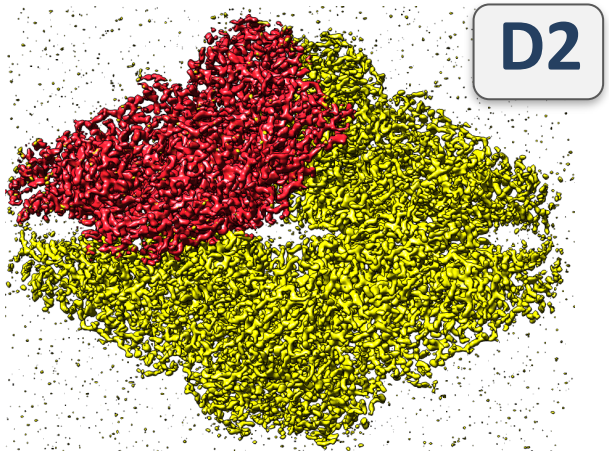
Chain A docked

Features:

- Multiple chains
- Density search
- Symmetry
- Multiprocessing

Finding map symmetry: *phenix.symmetry_from_map*





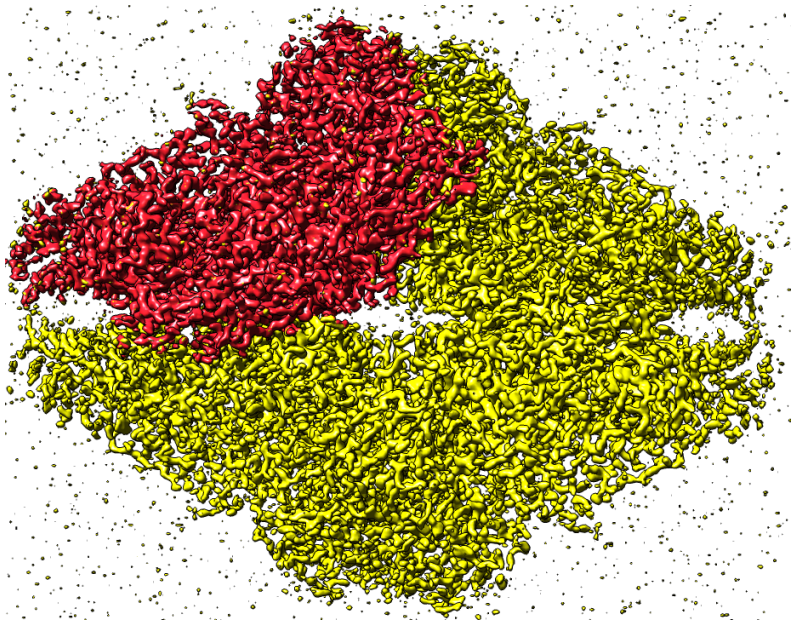
Procedure for finding symmetry:

- 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*



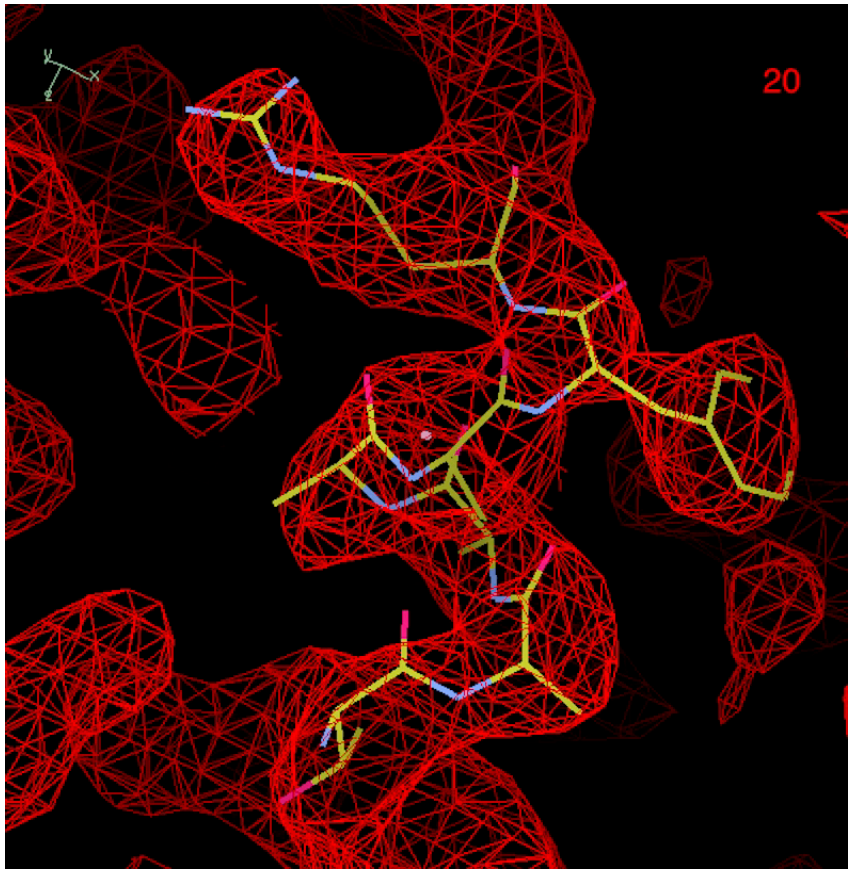
Procedure:

- 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

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

Acta Cryst D STRUCTURAL BIOLOGY
ISSN 2059-7983

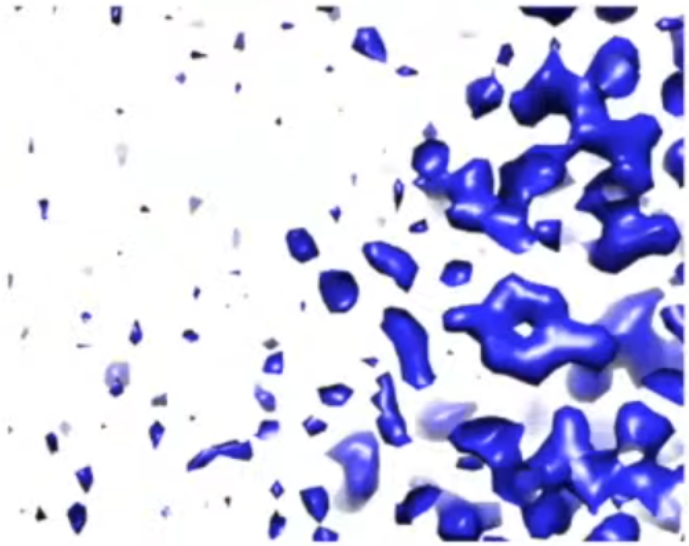
Automated map sharpening by maximization of detail and connectivity

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

Density modification: *phenix.density_modify_cryo_em*

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

Original map



Density modified map

Solvent should be flat

Distribution of density (histograms) should match typical protein

nature methods ARTICLES
https://doi.org/10.1038/s41592-020-0914-9
Check for updates

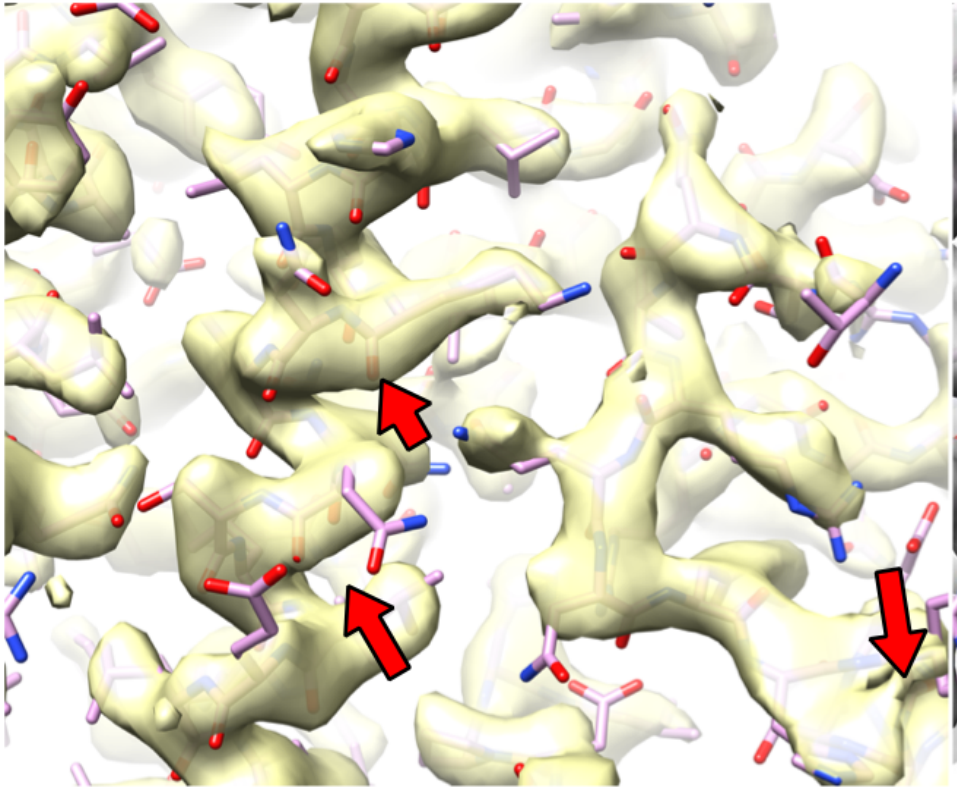
Improvement of cryo-EM maps by density modification

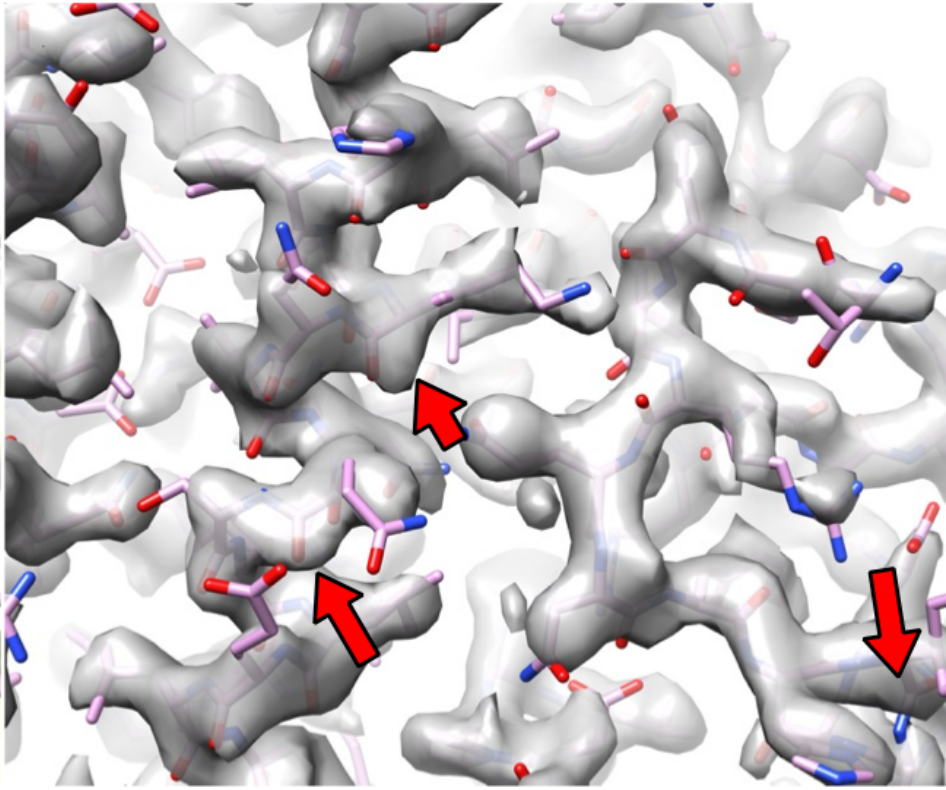
Thomas C. Terwilliger[1,2], Steven J. Ludtke[3], Randy J. Read[4], Paul D. Adams[5,6] and Pavel V. Afonine[5]

Density modification: *phenix.density_modify_cryo_em*

Original map

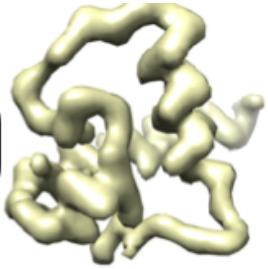
Density modified map



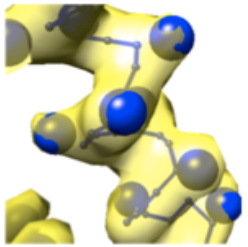


Automated model building: *phenix.map_to_model*

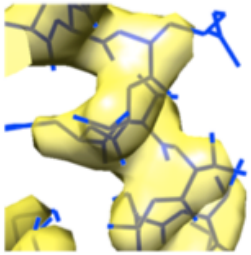
Isolate density for a chain



Identify C_α and C_β positions from side-chain density



Construct and refine all-atom model



nature methods

BRIEF COMMUNICATION

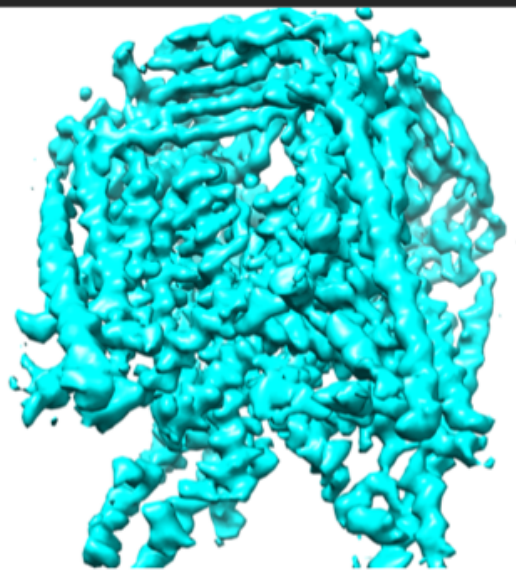
https://doi.org/10.1038/s41592-018-0173-1

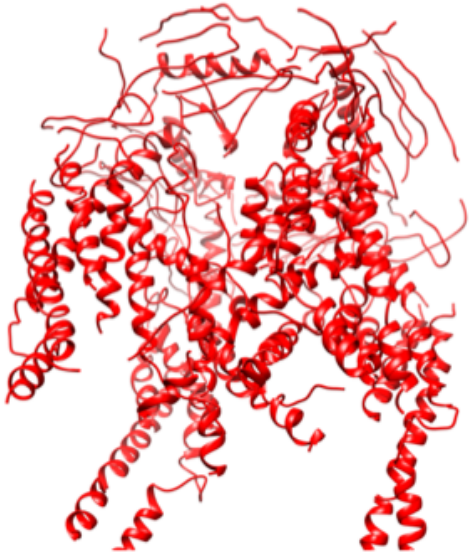
A fully automatic method yielding initial models from high-resolution cryo-electron microscopy maps

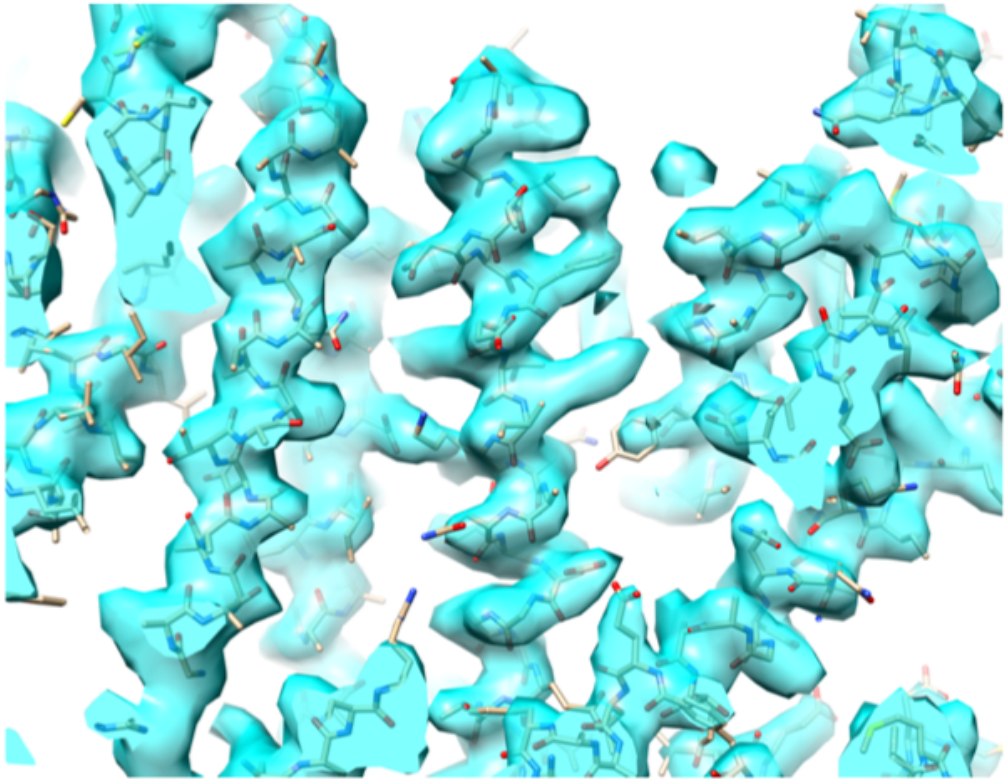
Thomas C. Terwilliger[1,2*], Paul D. Adams[3,4], Pavel V. Afonine[3,5] and Oleg V. Sobolev[3]

Automated model building: *phenix.map_to_model*

TRPML3 channel (4.1 Å, 78% built, 1.3 Å rmsd)

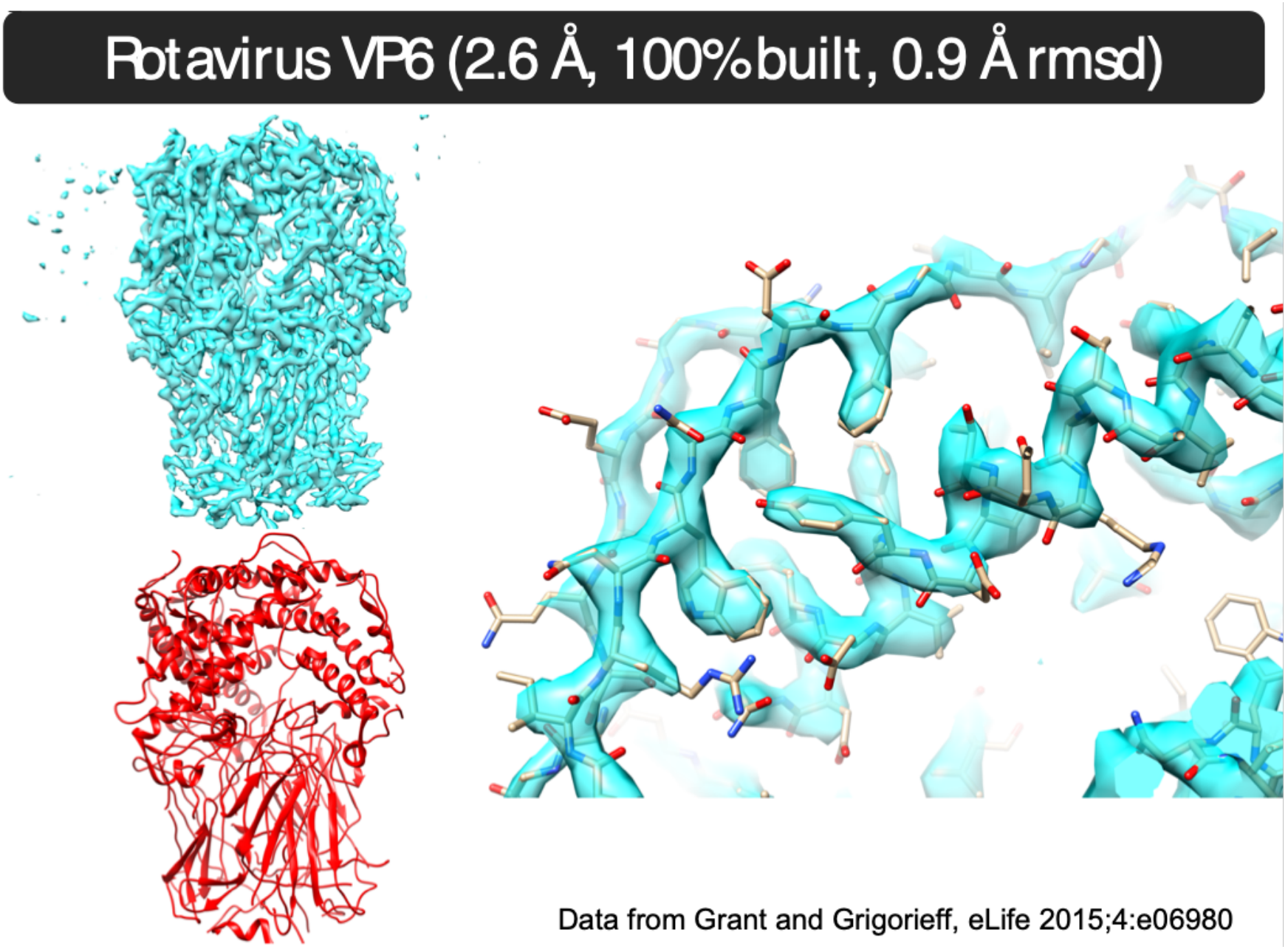






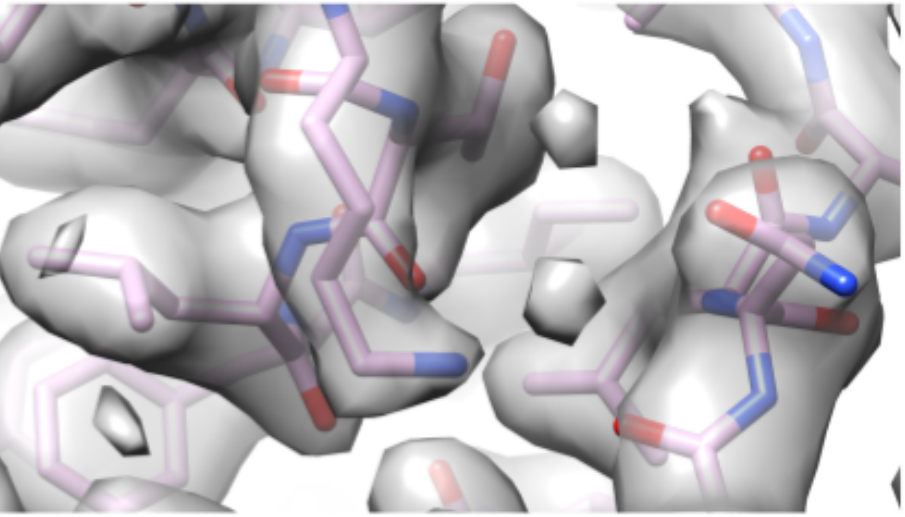
Data from Zhou, X. et al. (2017) Nat. Struct. Mol. Biol. 24: 1146

Automated model building: *phenix.map_to_model*

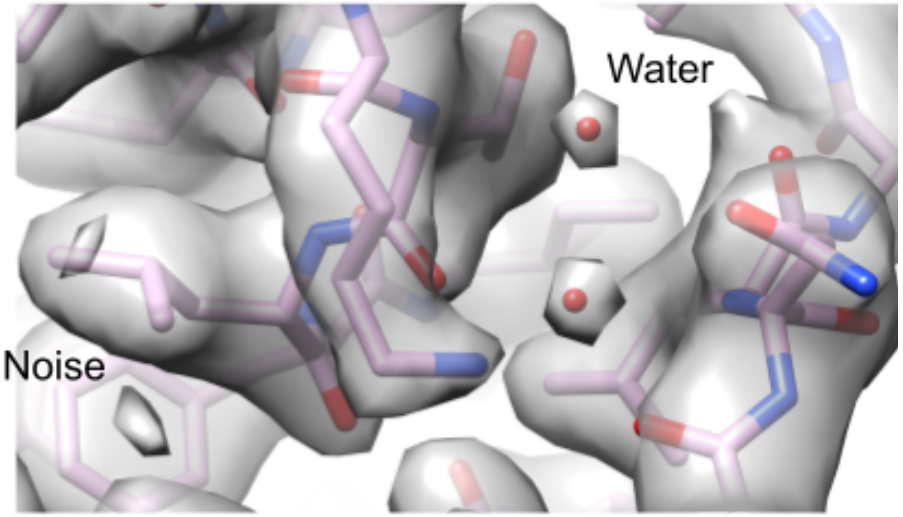


Automated water building: *phenix.douse*

Beta-gal, isolated density peaks
near LYS 897

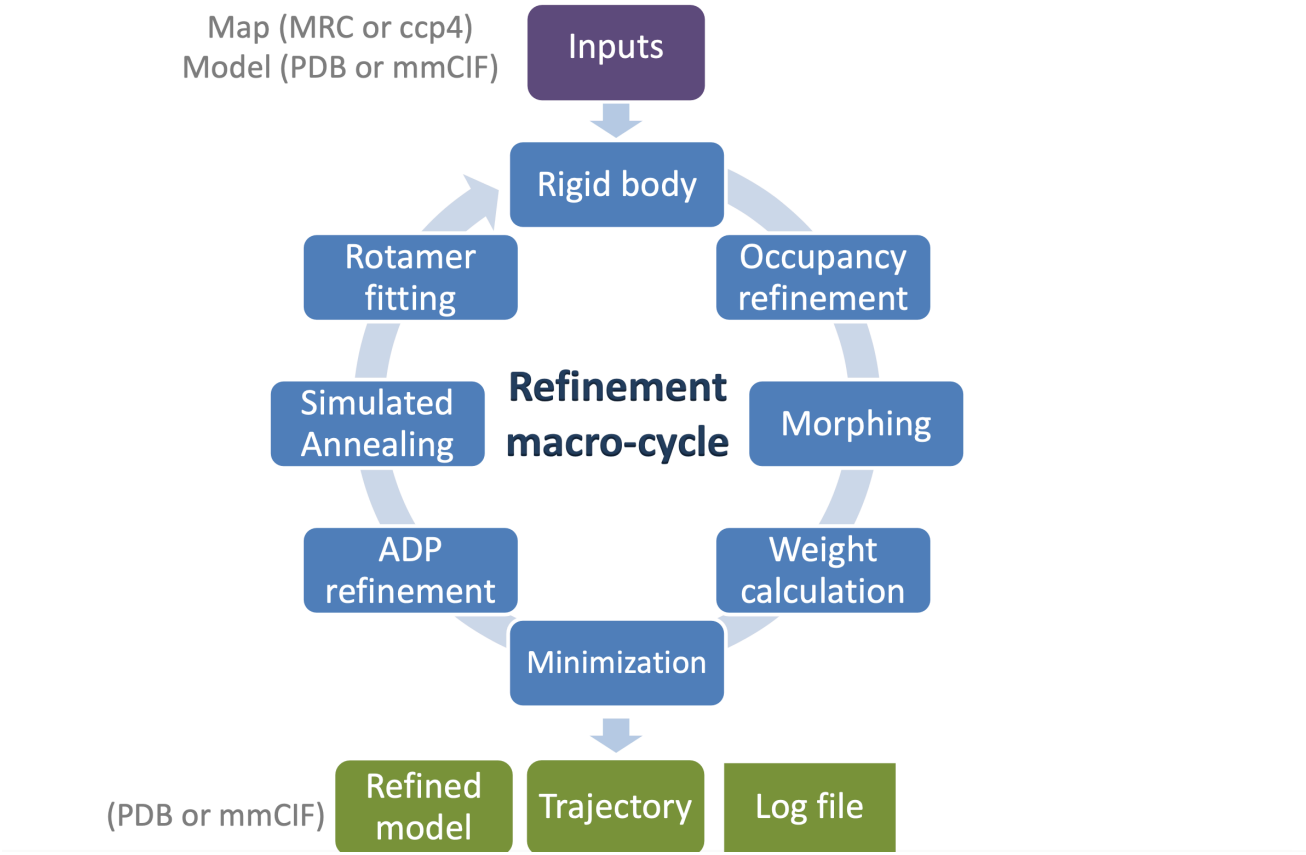


Automated water placement



https://phenix-online.org/presentations/water.pdf

Atomic model refinement: *phenix.real_space_refine*



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ISSN 2059-7983

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]

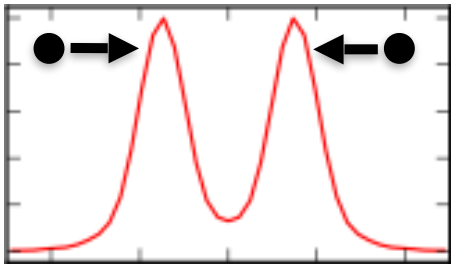
Refinement target

- **Atom-centered:**

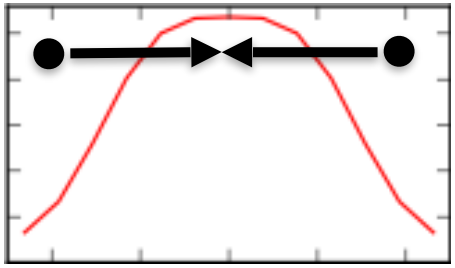
$$T = -\sum_{atoms} \rho_{obs}\left(x_{atom}, y_{atom}, z_{atom}\right)$$

$x_{atom}, y_{atom}, z_{atom}$ = coordinates of atom center

High resolution

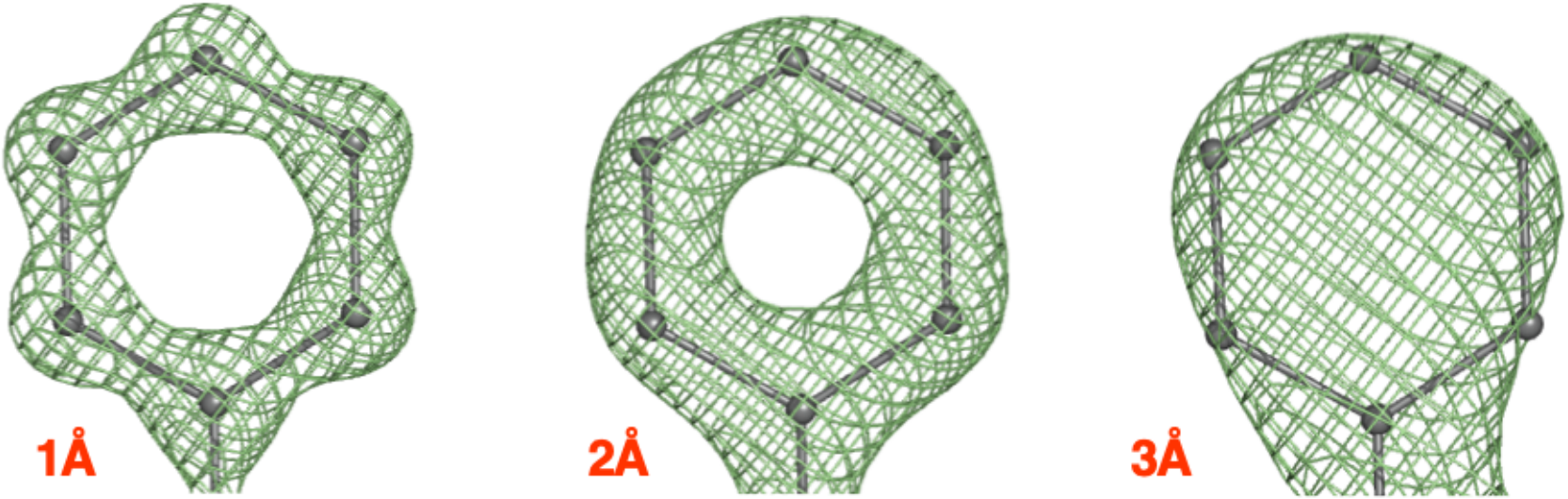
Atom 1  Atom 2

Low resolution



Moving atoms to nearest peaks $\neq$ making correct model

Restraints



- Lower the resolution, less detailed the map
  - Need extra information to keep correct geometry during refinement

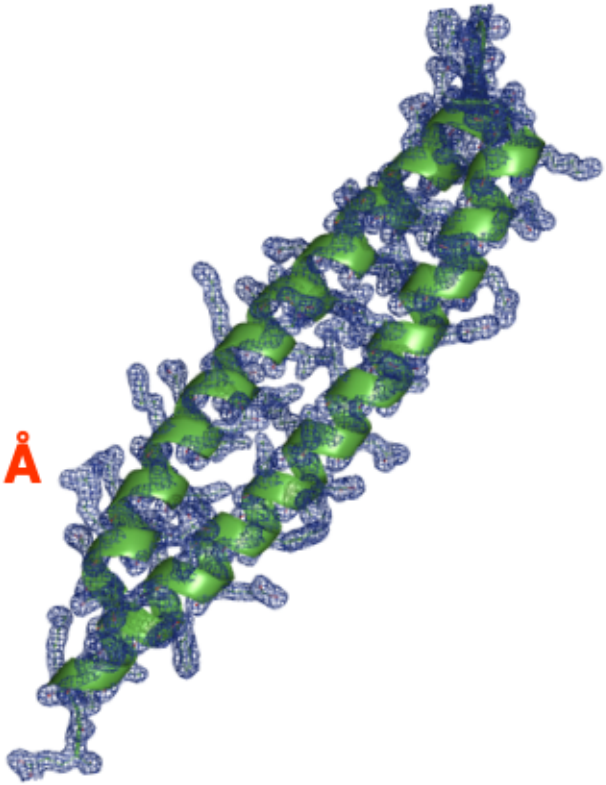
$$T = T_{\mathrm{DATA}} + wT_{\mathrm{RESTRAINTS}}$$

$$T_{RESTRAINTS} = T_{BOND} + T_{ANGLE} + T_{DIHEDRAL} + T_{PLANARITY} + T_{NONBONDED} + T_{CHIRALITY}$$

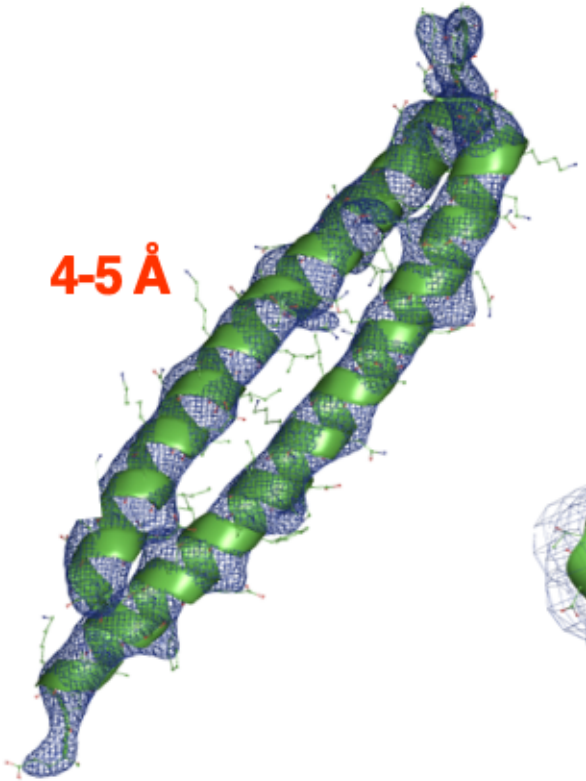
Restraints

- **Low resolution map is not sufficient to maintain secondary**

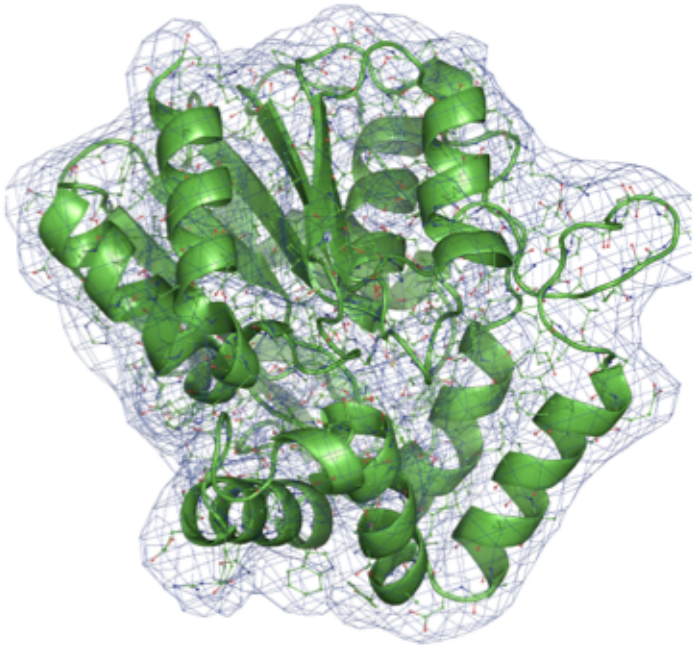
2 Å



4-5 Å



6Å-lower



- Secondary structure
- Ramachandran plot
- RNA/DNA parallelity
- Reference model
- Rotamer, starting position, etc

Ligand parameterization: *phenix.elbow*

What if you have a small molecule ligand?

1. We may ship restraints for that ligand:
   - Restraints are pre-calculated, archived in our GeoStd library
   - This is a subset of the CCD, molecules that have been previously appeared in macromolecular structures

2. Your ligand is new:
   - Need to use phenix.elbow to generate a restraints file
   - Restraints written to a cif file (eq. values for bonds, angles, torsions, etc)
   - Can use internal simplified force field, or external plugins (QM packages, Mogul, etc)

***electronic Ligand Builder and Optimization Workbench* (*eLBOW*): a tool for ligand coordinate and restraint generation**

Nigel W. Moriarty,[a]* Ralf W. Grosse-Kunstleve[a] and Paul D. Adams[a,b]

[a]Lawrence Berkeley National Laboratory, One Cyclotron Road, Mailstop 64R0246, Berkeley, CA 94720, USA, and [b]Department of Bioengineering, UC Berkeley, CA 94720, USA
*Correspondence e-mail: nwmoriarty@lbl.gov

(Received 27 April 2009; accepted 23 July 2009)

The *electronic Ligand Builder and Optimization Workbench* (*eLBOW*) is a program module of the *PHENIX* suite of computational crystallographic software. It is designed to be a flexible procedure that uses simple and fast quantum-chemical techniques to provide chemically accurate information for novel and known ligands alike. A variety of input formats and options allow the attainment of a number of diverse goals including geometry optimization and generation of restraints.

https://github.com/phenix-project/geostd

Using AlphaFold via Phenix GUI

Sequence

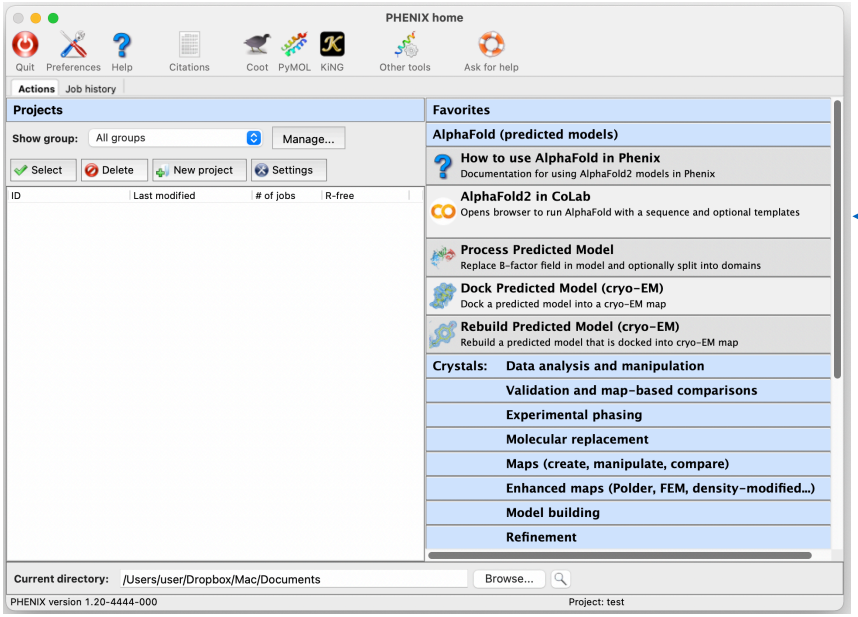
Templates from PDB

Templates you have created

AlphaFold Colab

AlphaFold

Prediction (hypothesis)



Highly accurate protein structure prediction with AlphaFold

John Jumper, Richard Evans, ... Demis Hassabis + Show authors

Nature **596**, 583–589 (2021) | Cite this article

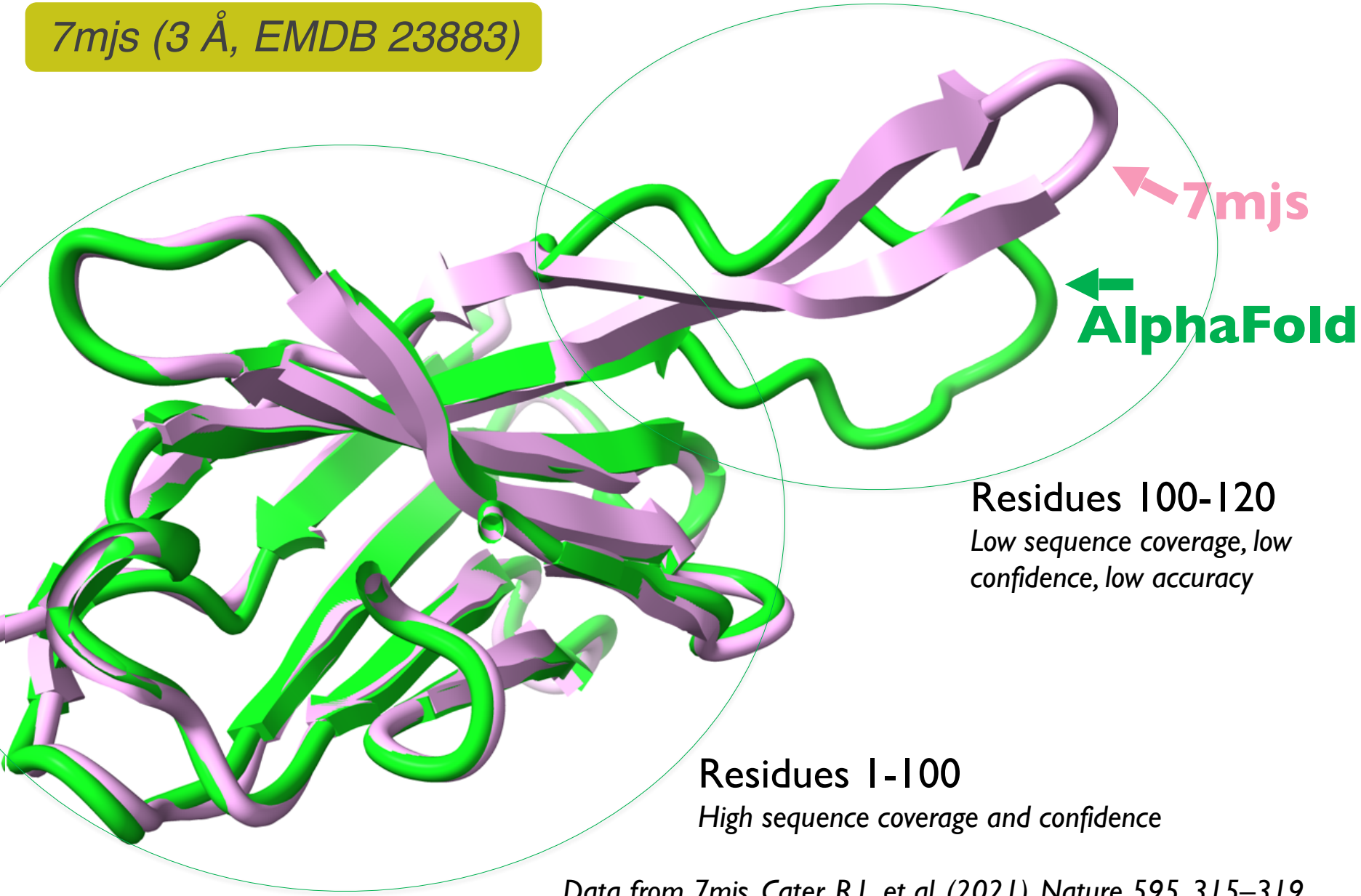
ColabFold - Making protein folding accessible to all

Milot Mirdita, Konstantin Schütze, Yoshitaka Moriwaki, Lim Heo, Sergey Ovchinnikov, Martin Steinegger

doi: https://doi.org/10.1101/2021.08.15.456425

Models are accurate where sequence coverage is high

7mjs (3 Å, EMDB 23883)



Residues 100-120

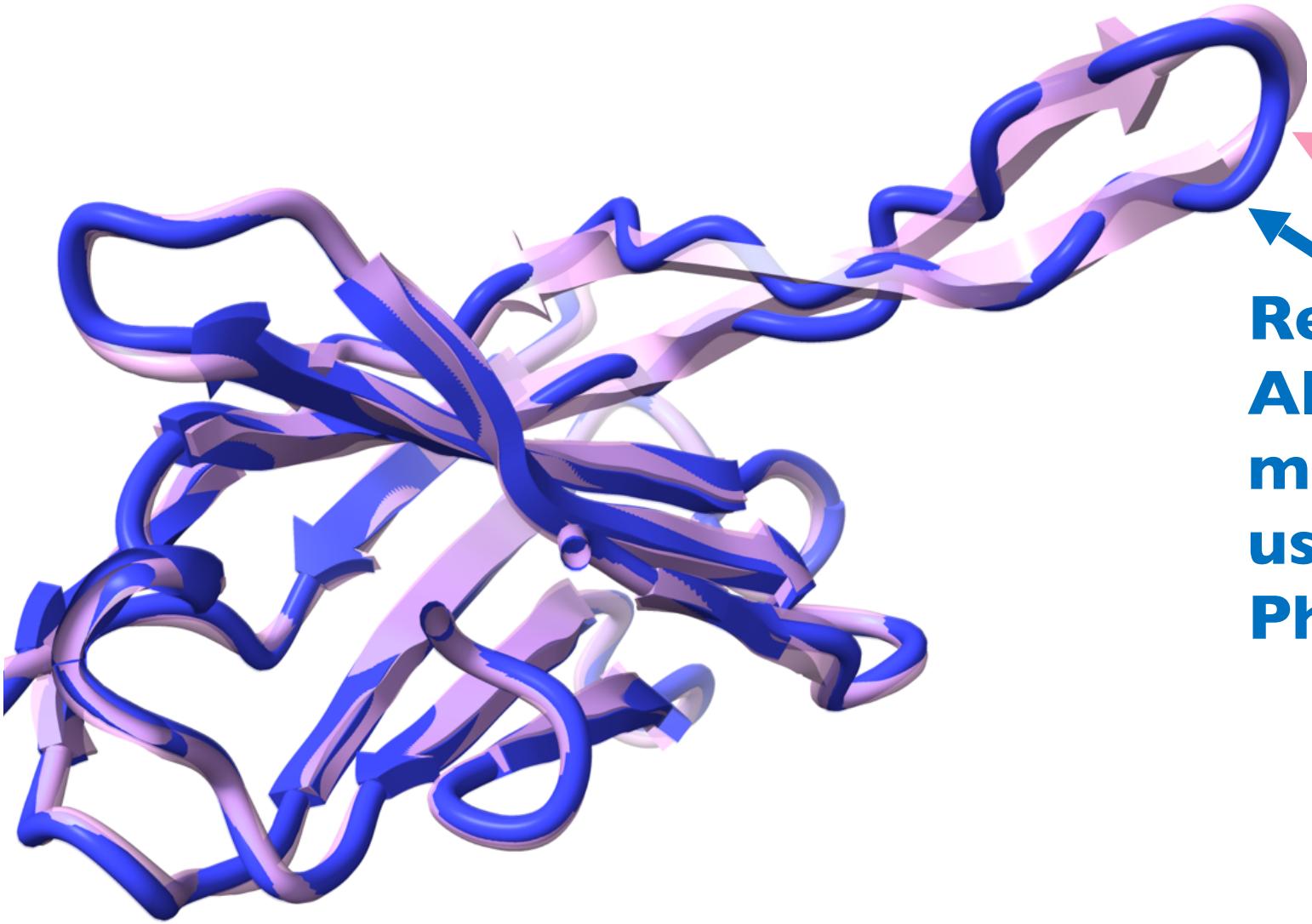
Low sequence coverage, low confidence, low accuracy

Residues 1-100

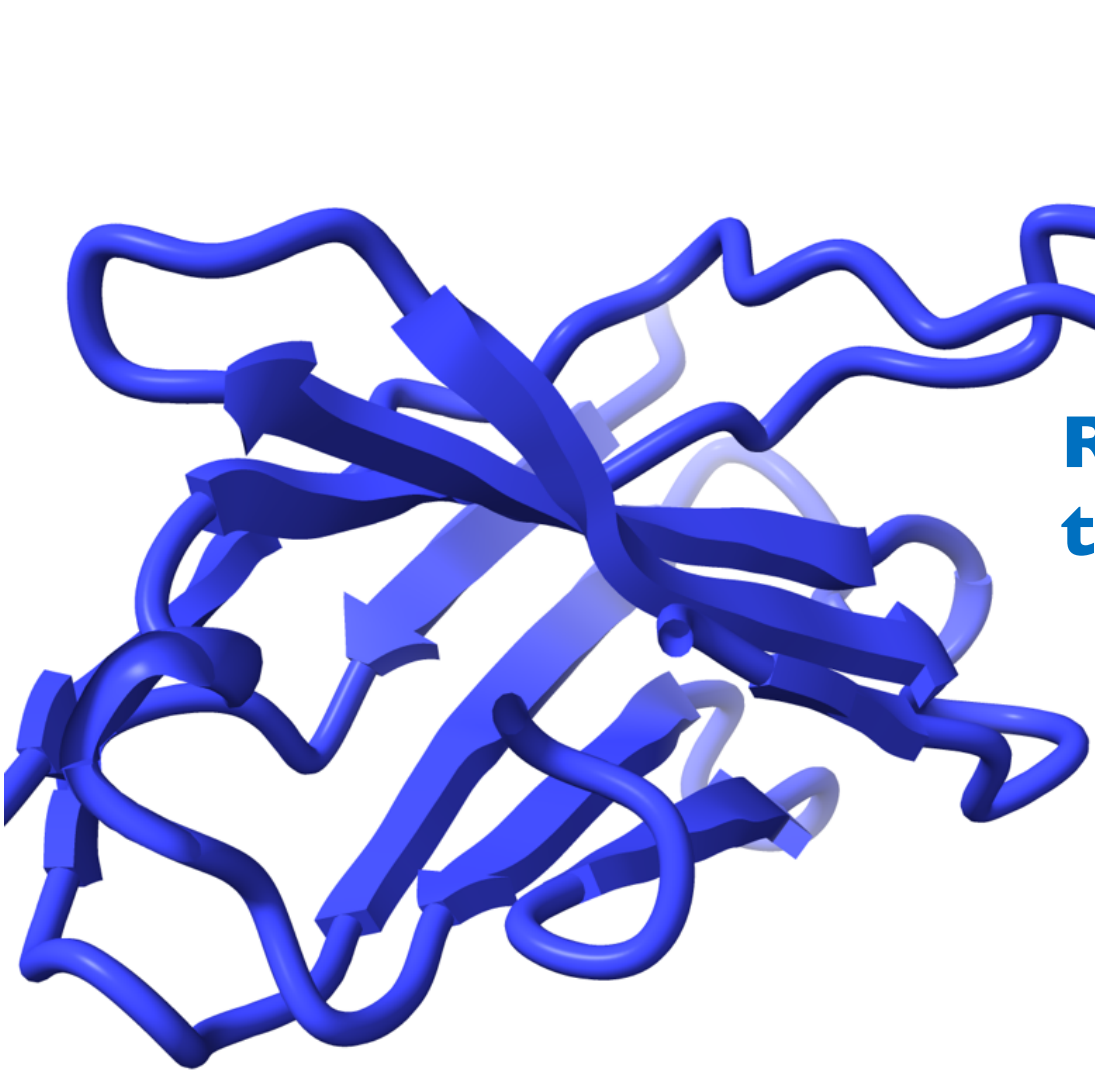
High sequence coverage and confidence

Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

AlphaFold as a starting model (7mjs 3 Å, EMDB 23883)



Iterative AlphaFold prediction and rebuilding



Rebuilt model as a template …

… can we get a better AlphaFold model?

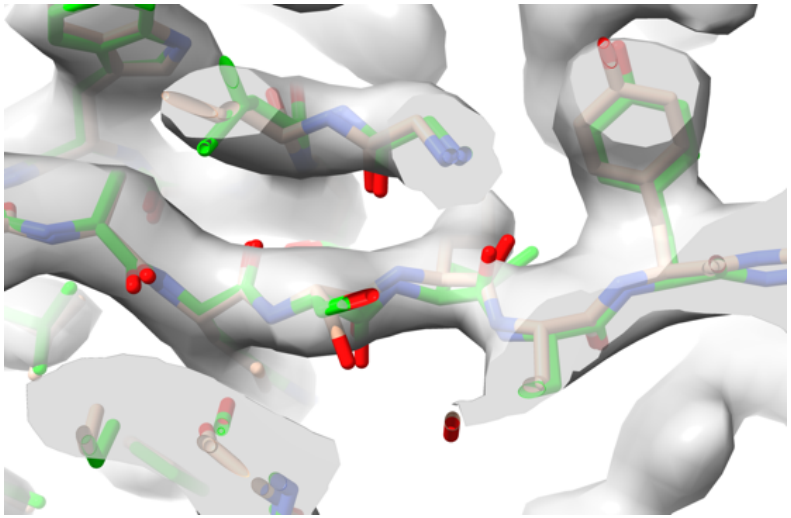
Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Iterative AlphaFold prediction and rebuilding

Rebuild AlphaFold model with density map

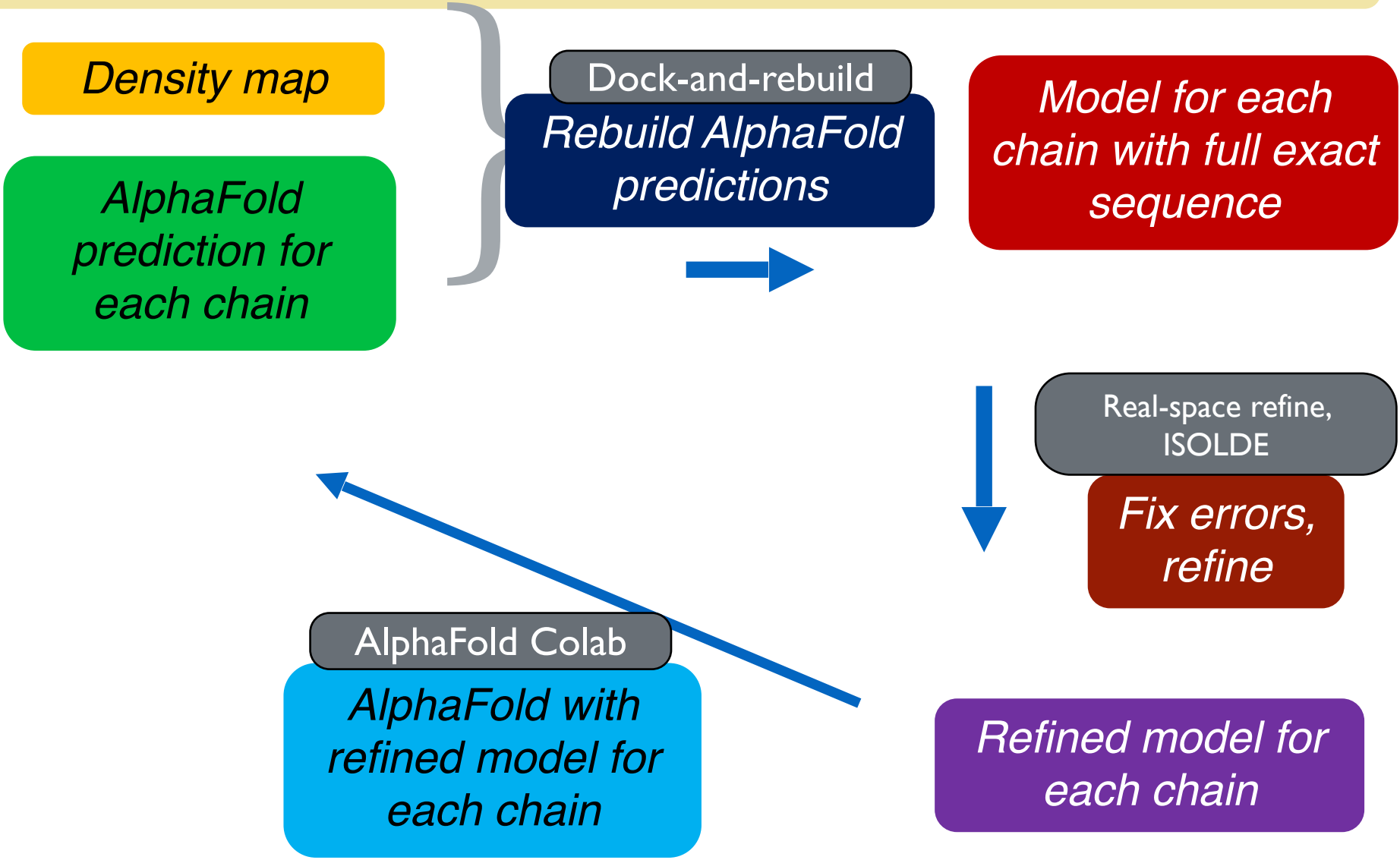
Rebuilt model improves next AlphaFold prediction

Iterate to improve model



Terwilliger et al. (2022). Improving AlphaFold modeling using implicit information from experimental density maps. BioRxiv 2022.01.07.475350

Workflow for Iterative Predict/Rebuild



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:**

  - Please include a detailed description of the 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

Thanks!