

Phenix user workshop

Time	Agenda	
8:30	Welcome/Introduction to Phenix (20 min)	D. Liebschner
8:50	Xtrriage (20 min)	D. Liebschner
9:10	Refinement (60 min)	P. Afonine
10:10	10 min break	
10:20	Tutorial 1: Model refinement (30 min)	P. Afonine, Phenix Team
10:50	Molecular replacement (30 min)	D. Liebschner
11:20	Tutorial 2: AlphaFold structure prediction, MR with an AlphaFold search model	D. Liebschner
11:50 am	FAQ	Phenix Team
12:00 pm	1h lunch break	
1:00	Using AlphaFold predictions for structure determination (40 min)	T. Terwilliger
1:40 pm	Tutorial 3: Automated X-ray structure determination with AlphaFold	T. Terwilliger, Phenix Team
2:10 pm	Ligands (30 min)	D. Liebschner
2:40 pm	Experimental phasing	T. Terwilliger
3:00	Tutorial 2: Xtrriage, Experimental phasing	T. Terwilliger, Phenix Team
3:20	FAQ	
3:30 pm	15 min break	
3:45 pm	Model validation	C. Williams
4:30 pm	Tutorial 5: Model validation	C. Williams, Phenix Team
5:00 pm	End	



U.S. DEPARTMENT OF
ENERGY



**UNIVERSITY OF
CALIFORNIA**

*Phenix User Workshop, Pittsburgh Diffraction Conference,
October 14th 2023*



Introduction

Dorothee Liebschner
Lawrence Berkeley Laboratory



**UNIVERSITY OF
CAMBRIDGE**

What is *Phenix*?

- Package for **automated structure solution** (crystallography, cryo-EM)
- Modern programming concepts and new algorithm development
- Designed to be used by **both novices and experienced users**
- Long-term development and **support**
- Why is it called *Phenix*?

Python **H**ierarchical **E**Nvironment for **I**ntegrated **X**tallography

The Project



Lawrence Berkeley Laboratory

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



University of Cambridge

Randy Read, Airlie McCoy,
Alisia Fadini



Los Alamos National Laboratory New Mexico Consortium

Tom Terwilliger, Li-Wei Hung



UTHealth

Matt Baker



Duke University

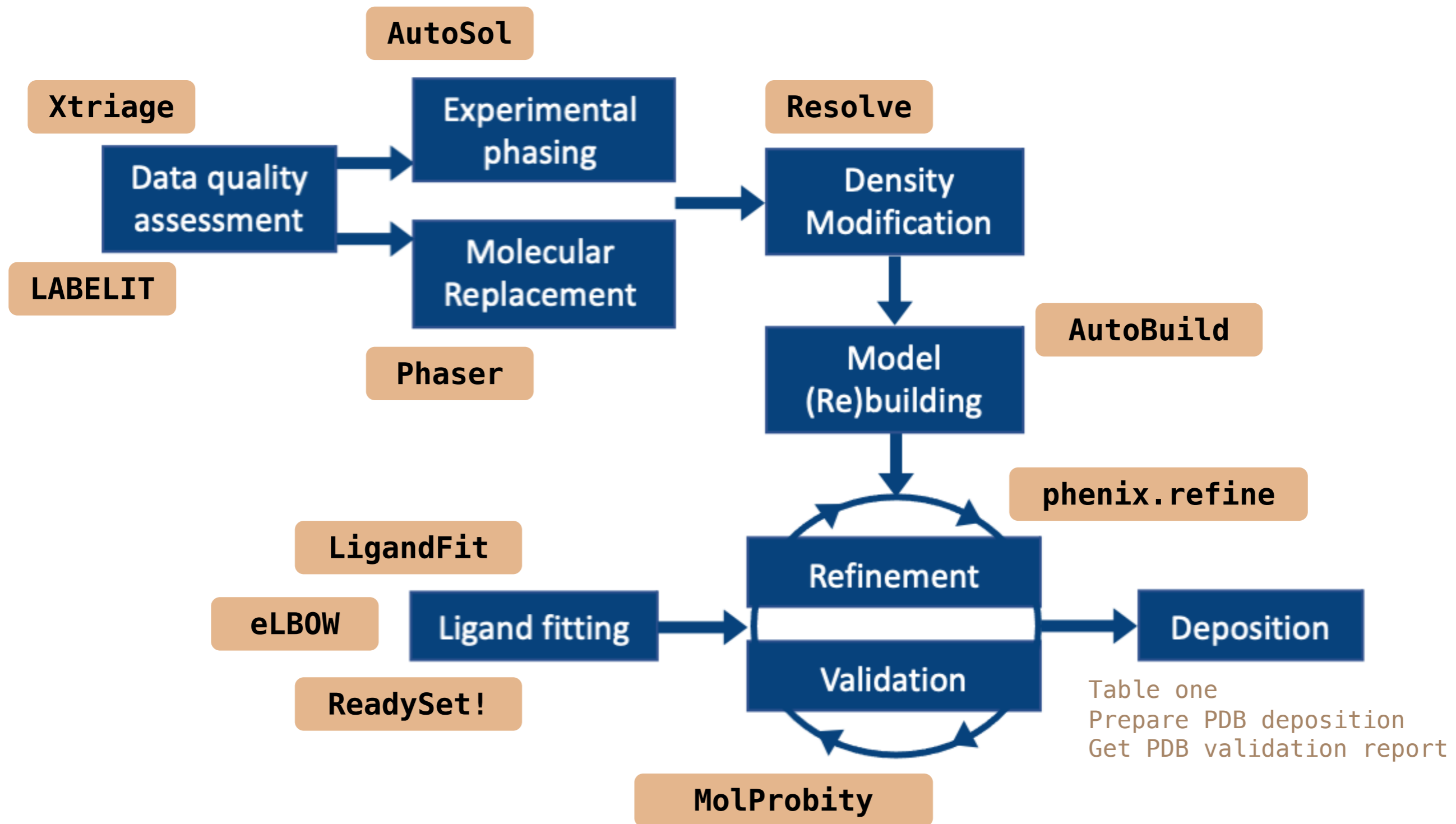
Jane & David Richardson,
Christopher Williams,
Vincent Chen



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

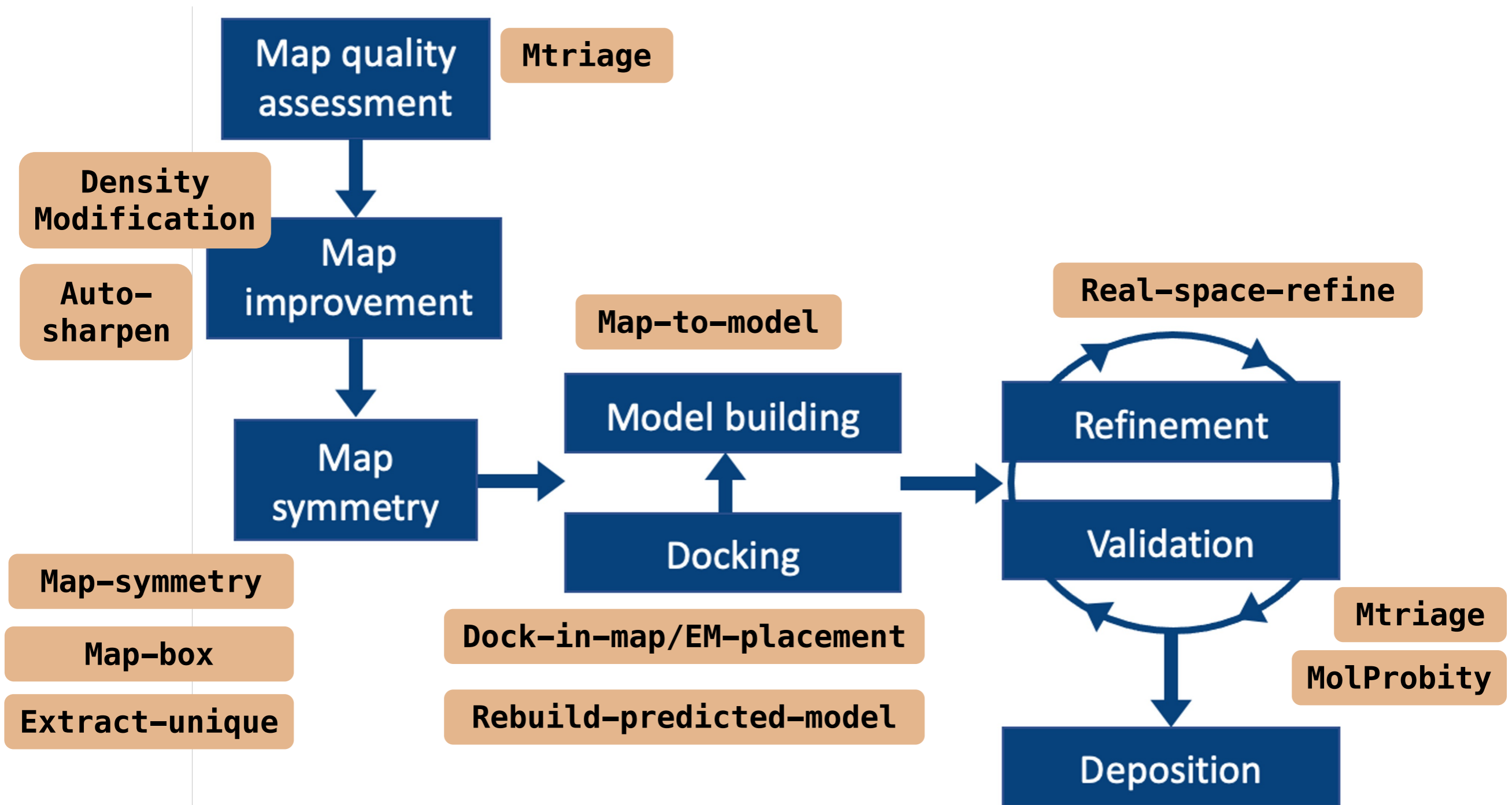
Tools for Crystallography



Acta Cryst. 2002, D58:1948-1954
J. Appl. Cryst. 2002, 35:126-136
Acta Cryst. 2010, D66: 213-221
Acta Cryst. 2019 D75:861-877

(Phenix)
(cctbx)
(Phenix)
(Phenix)

Tools for Cryo-EM



Acta Cryst. 2002, D58:1948-1954
J. Appl. Cryst. 2002, 35:126-136
Acta Cryst. 2010, D66: 213-221
Acta Cryst. 2019 D75:861-877

(Phenix)
 (cctbx)
 (Phenix)
 (Phenix)

Features

Phenix GUI

Central GUI for job control and to launch new jobs

The screenshot displays the Phenix GUI interface. At the top, there is a menu bar with icons for Quit, Preferences, Help, Citations, Reload last job, Coot, PyMOL, KiNG, Other tools, and Ask for help. Below the menu bar, there are two tabs: 'Actions' and 'Job history'. The main area is divided into two panels. The left panel, titled 'Projects', contains a 'Show group:' dropdown menu set to 'All groups' and a 'Manage...' button. Below this are buttons for 'Select', 'Delete', 'New project', and 'Settings'. A table lists the projects:

ID	Last modified	# of jobs	R-free
✓ test	Sep 14 2021 02:2...	529	0.0971

The right panel, titled 'Favorites', lists various tool categories:

- AlphaFold (predicted models)
- Crystals: Data analysis and manipulation, Validation and map-based comparisons, Experimental phasing, Molecular replacement, Maps (create, manipulate, compare), Enhanced maps (Polder, FEM, density-modified...), Model building, Refinement, Ligands
- Cryo-EM: Map analysis, symmetry, manipulation, Validation and map-based comparisons, Map improvement, Docking, model building and rebuilding, Refinement
- Models: Superpose, search, compare, analyze symmetry, Modification, minimization and dynamics
- PDB Deposition
- Program search

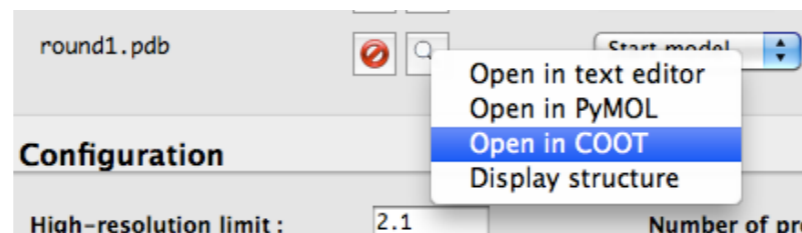
At the bottom, there is a 'Current directory:' field showing '/Users/dcliebschner/Desktop/Projects/test' and a 'Browse...' button. The status bar at the very bottom indicates 'PHENIX version dev-svn-000' and 'Project: test'.

Coot/PyMOL/ChimeraX integration

- Most results can be opened directly in graphics apps



- Any PDB file listed in GUI can also be opened

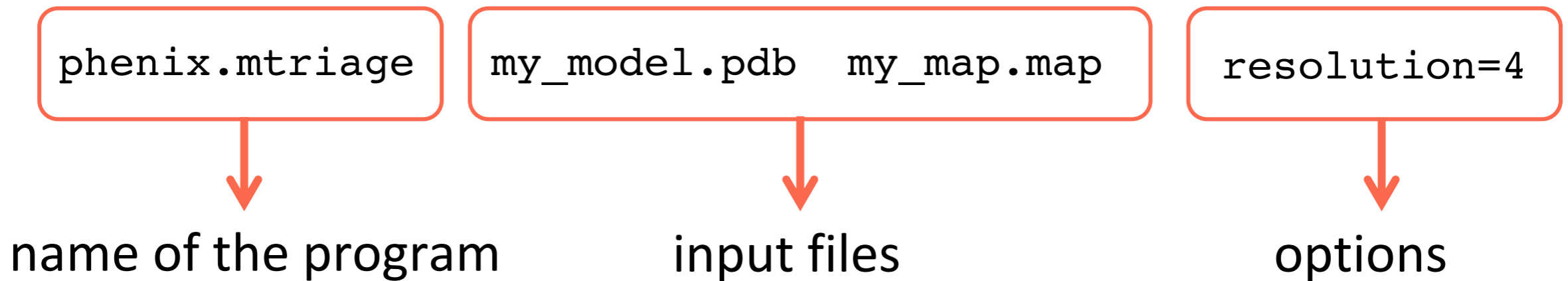


- AutoSol, AutoBuild, and phenix.refine will update Coot continuously while running
- Coot must have Python support (default on Mac)
- Specific paths to executables usually required on Linux

Preferences → Graphics → Full path to Coot [...PyMOL]

Command Line Tools

Run on the terminal



Run in a python script

```
try:  
    easy_run.call("phenix.mtriage my_model.pdb my_map.map")  
except Exception as e:  
    msg = traceback.format_exc()  
    print(msg)
```

Video Tutorials

www.youtube.com/channel/UCcdl0hfHngWAZLJWynxPQWg/videos

YouTube IT Search

Phenix Tutorials

Phenix homepage

Phenix Tutorials
560 subscribers

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Uploads PLAY ALL SORT BY

- real_space_refine Tutorial** (5:27)
How to run real-space-refine
763 views • 7 months ago
CC
- Secondary Structure Restraints Tutorial** (6:23)
How to use secondary structure restraints
399 views • 8 months ago
CC
- Multiple refinement strategies Tutorial** (5:46)
How to use multiple refinement strategies and...
281 views • 8 months ago
CC
- Planning a SAD experiment Tutorial** (6:00)
Simulate a SAD experiment with...
483 views • 1 year ago
CC
- Map-to-model Tutorial** (5:33)
Automatic map interpretation with map_to_model
1.3K views • 1 year ago
CC
- Scale-and-merge Tutorial** (7:31)
Scaling and merging anomalous data
387 views • 1 year ago
CC
- Automated map sharpening Tutorial** (6:05)
- Ligandfit Tutorial** (5:48)
- Wilson plots and space group identification phenix.xtriage** (8:23)
- Twinning phenix.xtriage** (6:08)
- Translational NCS phenix.xtriage** (4:55)
- Checking data quality with Xtriage** (6:49)

Dorothee Liebschner, Nigel Moriarty,
Tom Terwilliger, Christopher Schlicksup, Vincent Chen

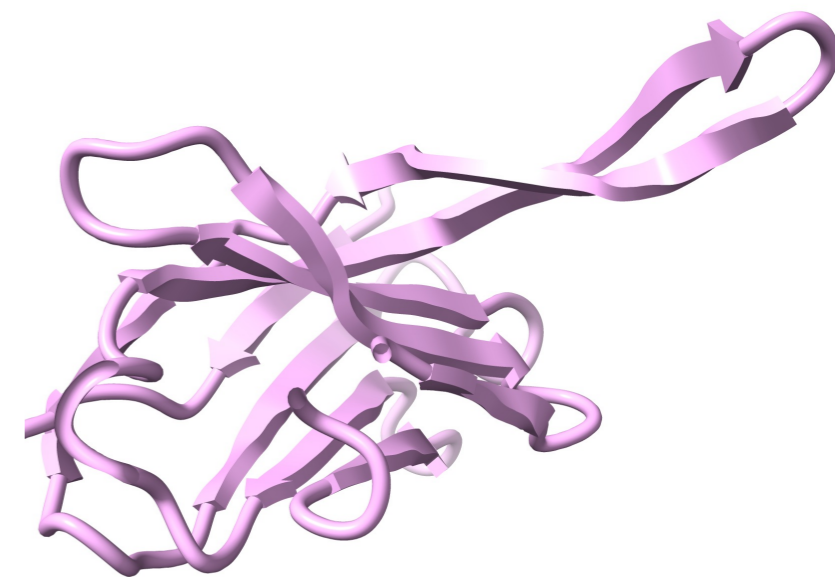
Accurate predicted models



EVQLVESGGGLVQPGGSLRLSCAASGFN**I**YSS**S**IHWVRQAPGKGLEWVAYI
.....**F**.....**M**.....Q.....
.....K.....**Y**.....**L**.....A.....
.....A.....V.....
.....A.....
.....**L**.....**V**.....E.....
.....A.....Q.....

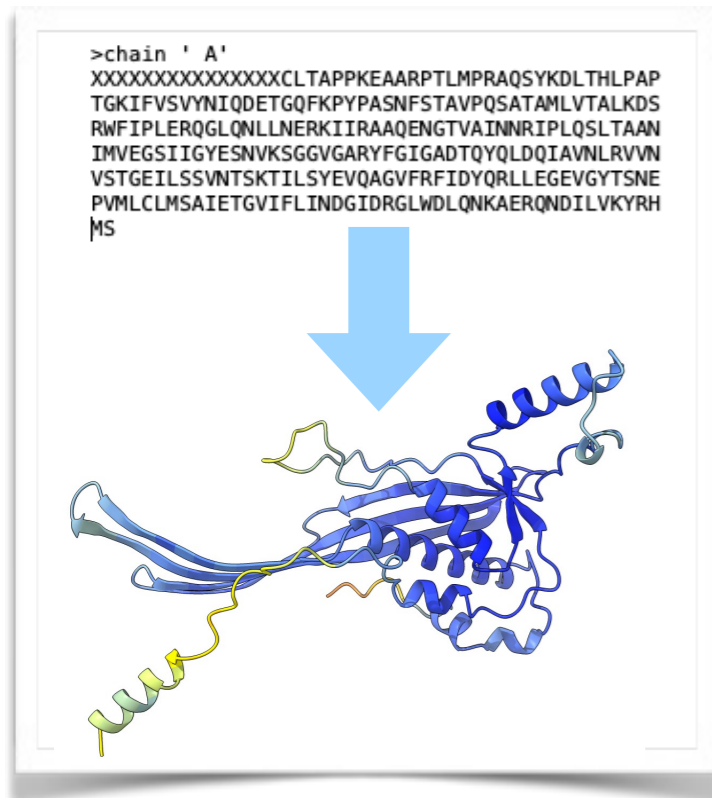
Sequence

Multiple sequence alignment



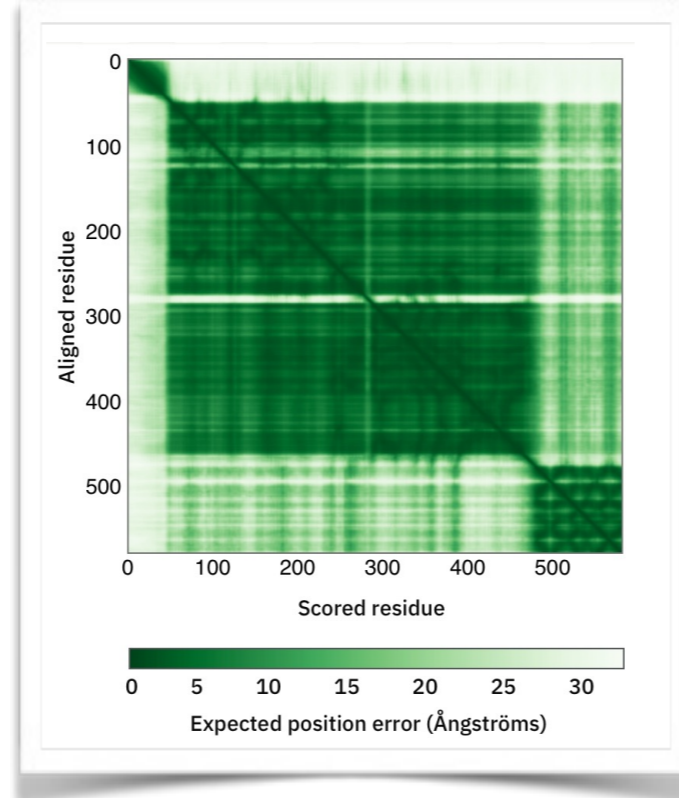
3D prediction

New tools for predicted models in Phenix

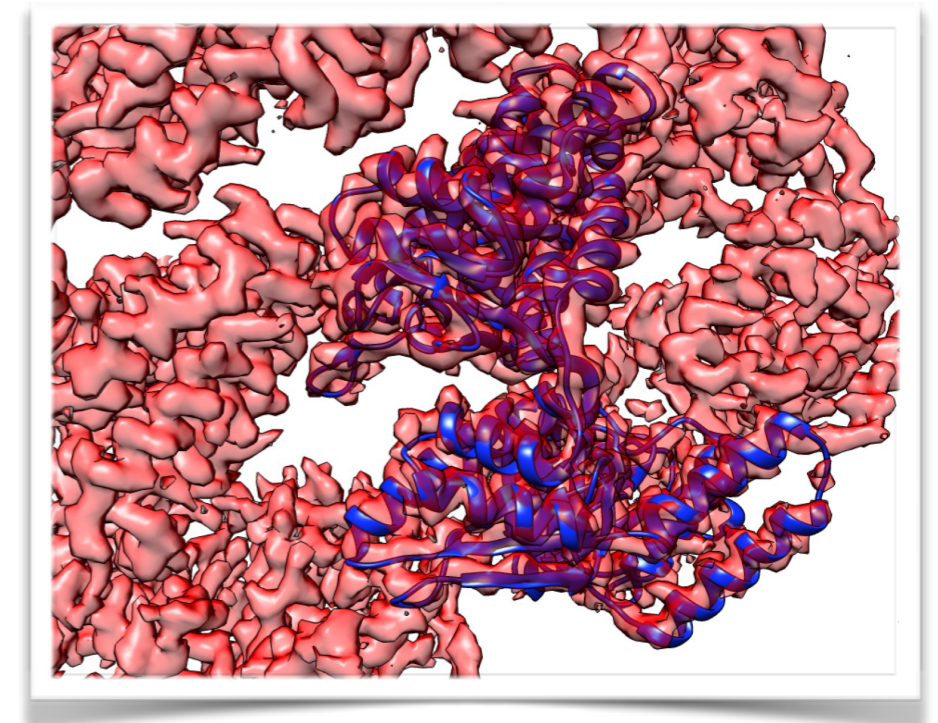


AlphaFold model prediction

(Phenix server, no need to have AF installed locally)



Process predicted model



Predict and Build

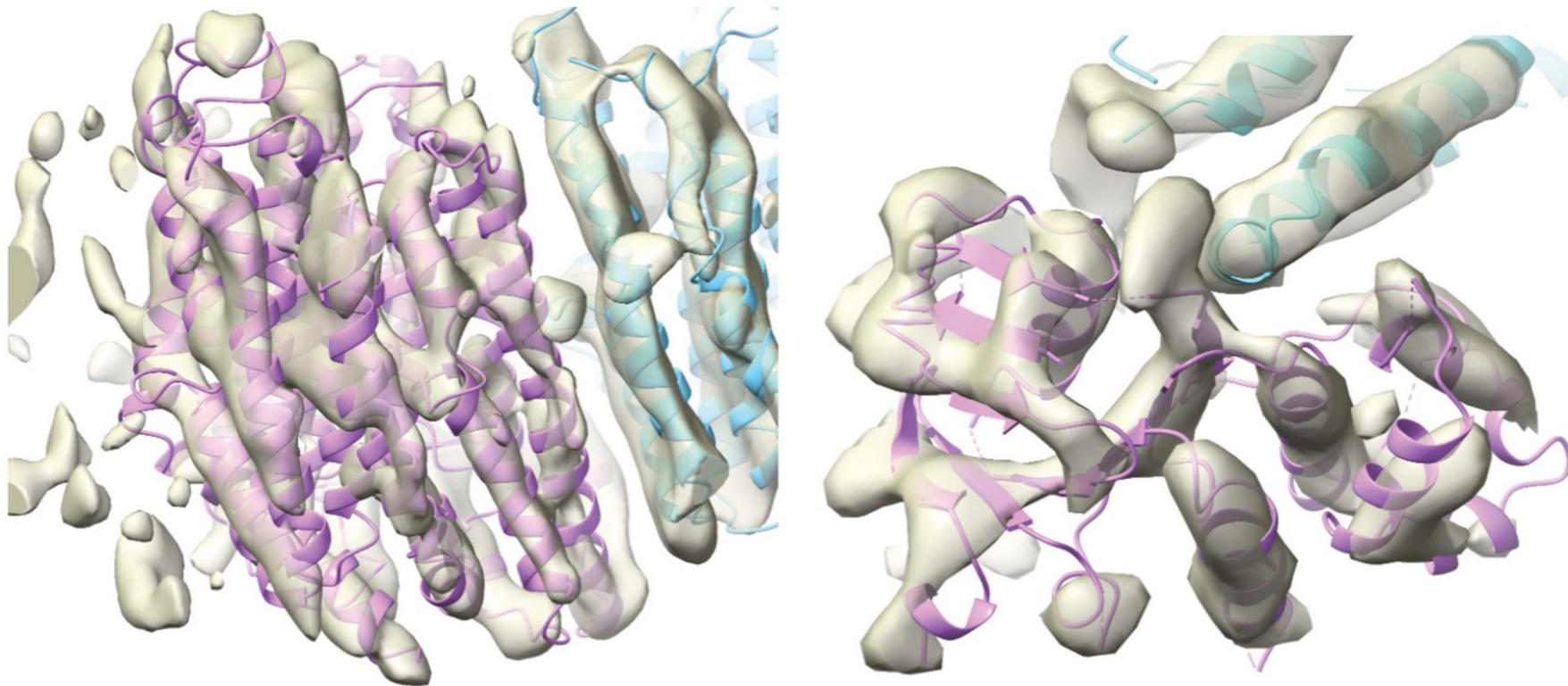
(Iterative AlphaFold prediction, docking, and rebuilding)

Fully automatic!

Likelihood-based EM docking

- Use likelihood scores to dock a model into a map
- Works at low resolution (30 Å!)

$$\text{LLG}(\mathbf{E}_{\text{mean}}; \mathbf{E}_C) = \frac{2}{1 - D_{\text{obs}}^2 \sigma_A^2} D_{\text{obs}} \sigma_A E_{\text{mean}} E_C \cos(\Delta\varphi) - \frac{D_{\text{obs}}^2 \sigma_A^2 (E_{\text{mean}}^2 + E_C^2)}{1 - D_{\text{obs}}^2 \sigma_A^2} - \ln(1 - D_{\text{obs}}^2 \sigma_A^2).$$

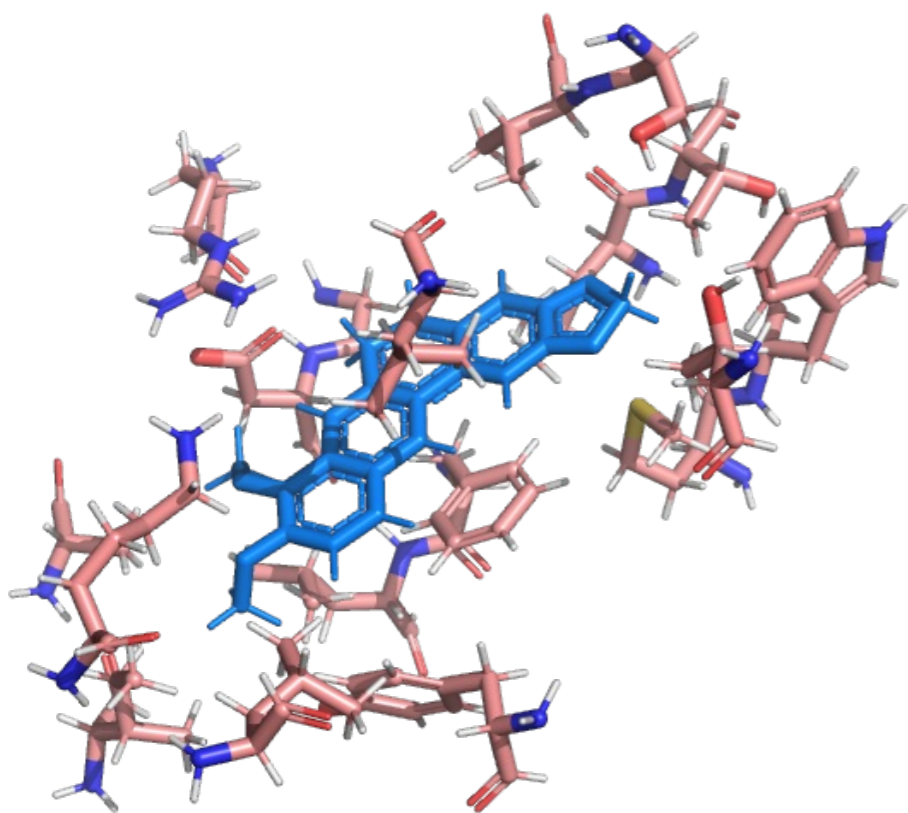


Read RJ, Millán C, McCoy AJ, Terwilliger TC. Likelihood-based signal and noise analysis for docking of models into cryo-EM maps. *Acta Crystallogr D Struct Biol.* 2023 Apr 1;79(Pt 4):271–80.

Millán C, McCoy AJ, Terwilliger TC, Read RJ. Likelihood-based docking of models into cryo-EM maps. *Acta Crystallogr D Struct Biol.* 2023 Apr 1;79(Pt 4):281–9.

QMR – quantum mechanical restraints

- Ligands need restraints (description of chemical structure) for refinement.
- Restraint generators often ignore chemical variability & specific binding interactions.

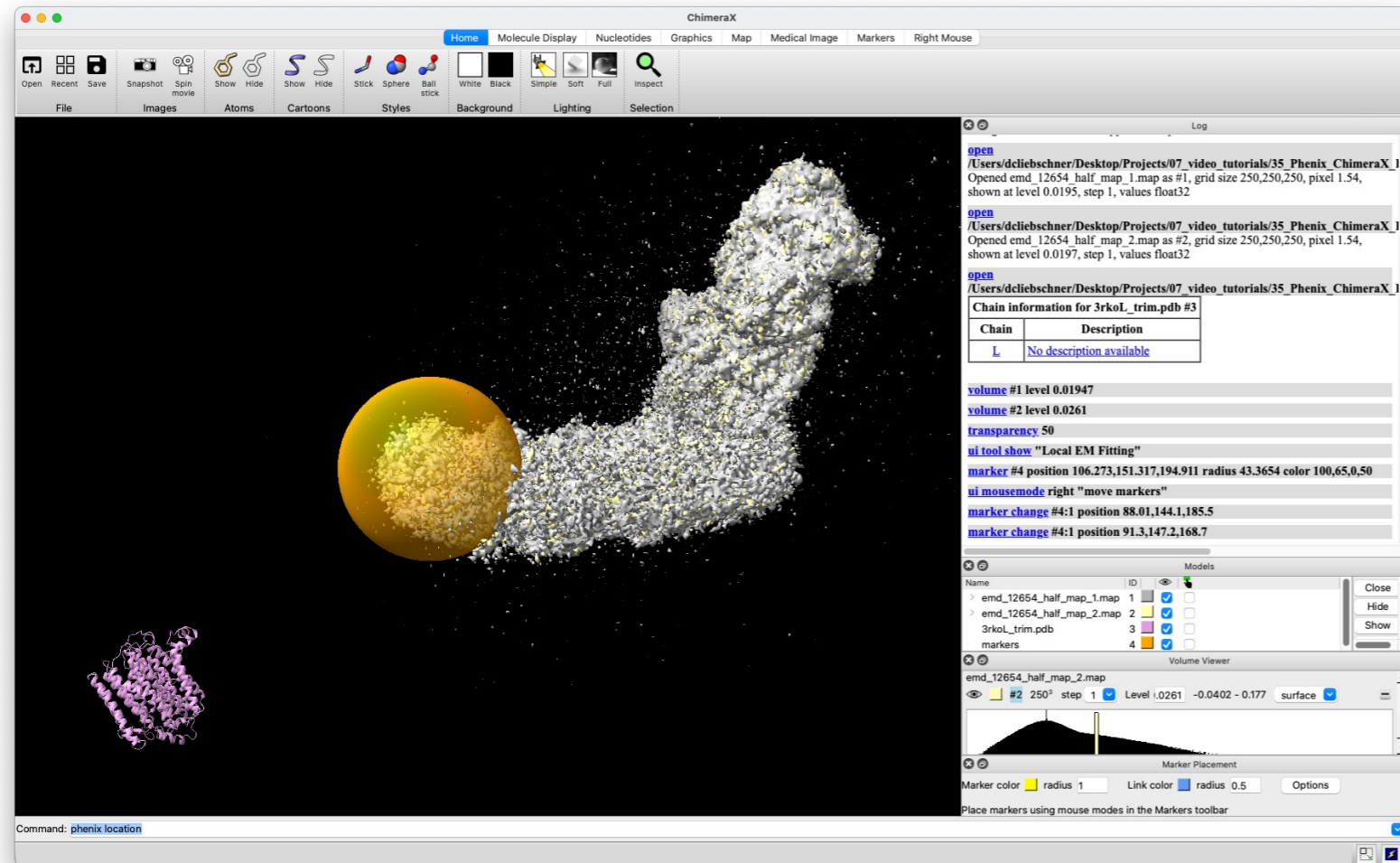


BER in 3vw2

QMR approach:

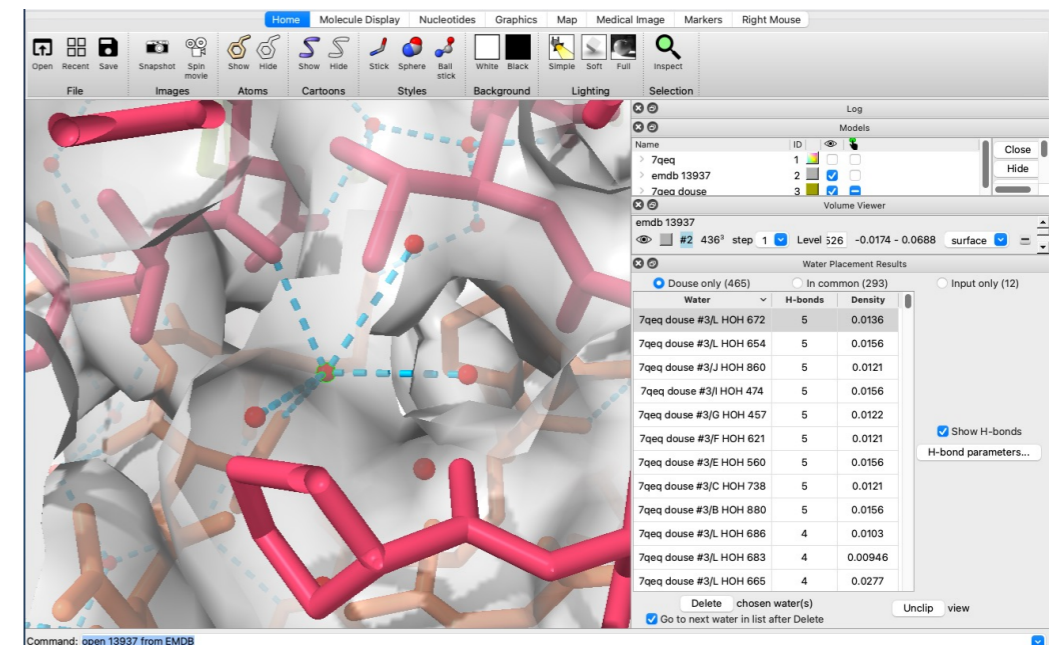
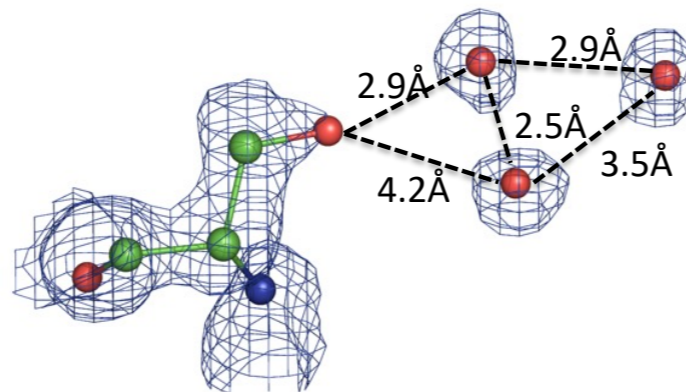
- Minimize the ligand geometry in the binding pocket.
- Use minimized ligand geometry as targets for restraints.
(forget about what happened with the residues)

Run Phenix tools through ChimeraX



Local EM fitting
(EM placement)

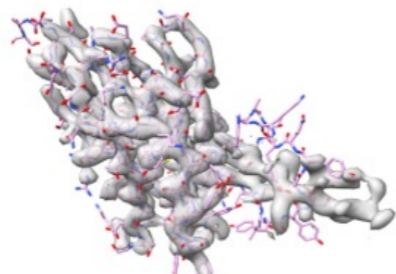
Automated water building
(phenix.douse)



Presentation slides

- **Lectures**
<https://phenix-online.org>

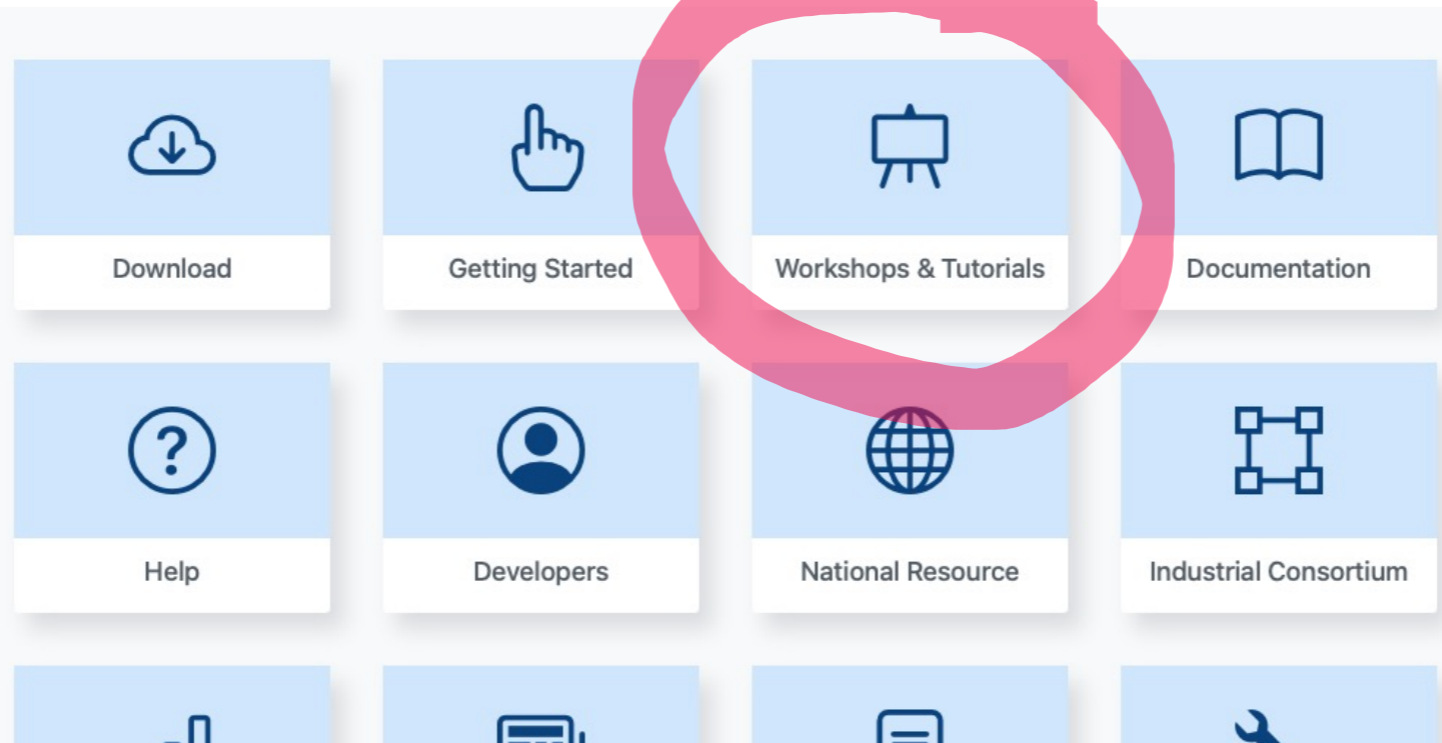
A comprehensive software package for macromolecular structure determination using crystallographic (X-ray, neutron and electron) and electron cryo-microscopy data. [Learn more](#)



Cryo-EM map and superposed, refined AlphaFold2 model

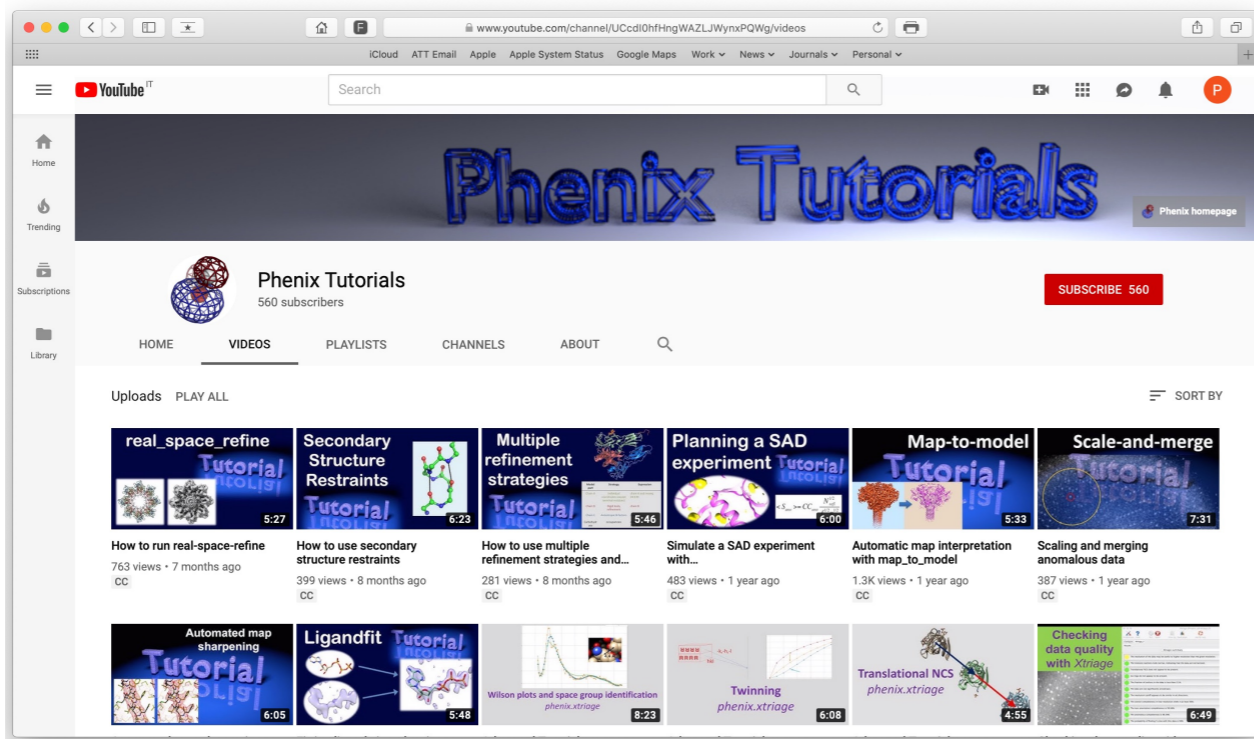
Phenix integrated with AlphaFold

- Structure determination with AlphaFold [video tutorial](#)
 - Predict a structure on the Phenix AlphaFold server [video tutorial](#)
 - PredictAndBuild (Xray) [video tutorial](#)
 - PredictAndBuild (cryo-EM) [video tutorial](#)
- [Learn more](#)



Troubleshooting

- Phenix team members will be in the room
- YouTube video tutorials
<https://www.youtube.com/c/phenixtutorials>



The image shows a screenshot of the Phenix Tutorials YouTube channel page. The channel name is "Phenix Tutorials" with 560 subscribers. The page displays a grid of video thumbnails, each with a title, duration, and view count. The videos cover various topics in X-ray crystallography, including real-space refinement, secondary structure restraints, multiple refinement strategies, SAD experiments, map-to-model, scaling and merging, automated map sharpening, ligandfit, Wilson plots and space group identification, twinning, translational NCS, and checking data quality with Xtriage.

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Acknowledgements

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Los Alamos Laboratory/New Mexico Consortium

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Baylor College of Medicine

Matt Baker

Cambridge University

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Duke University

Jane Richardson & David Richardson, Ian Davis, Vincent Chen, Jeff Headd, Chris Williams, Bryan Arendall, Bradley Hintze, Laura Murray

UC San Francisco

Ben Barad, Yifan Cheng, Jaime Fraser

University of Washington

Frank DiMaio, Ray Wang, David Baker

Oak Ridge National Laboratory

Marat Mustyakimov, Paul Langan

Other Collaborators

Corey Hryc, Zhao Wang, Wah Chiu
Pawel Janowski, David Case
Dale Tronrud, Donnie Berholz, Andy Karplus
Alexandre Urzhumtsev & Vladimir Lunin
Garib Murshudov & Alexi Vagin
Paul Emsley, Bernhard Lohkamp, Kevin Cowtan
David Abrahams
Phenix Testers & Users

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- PHENIX Industrial Consortium
- Lawrence Berkeley Laboratory

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