

Phenix user workshop (IUCr 2023)

Time	Agenda	Lecturers
8:00	Welcome/Introduction to Phenix	D. Liebschner
8:30	Using AlphaFold predictions for structure determination	T. Terwilliger
9:00	MR/Docking	R. Read/A. McCoy
9:30	Density modification in Crystallography and cryo-EM	T. Terwilliger
9:55	Tutorial 1: Density modification of cryo-EM maps	T. Terwilliger
10:10	10 min break	
10:20	Tutorial 2: AlphaFold structure prediction, Cryo-EM docking with Emplace Local, Cryo-EM model refinement with Real space refine	R. Read, A. McCoy, T. Croll, T. Terwilliger
11:10	Tutorial 3: MR/MR-SAD with AlphaFold	R. Read/A. McCoy
11:50	FAQ	
12:00	45 min lunch break and on-on-one discussions (lunch provided)	
12:45	Refinement	O. Sobolev
13:30	Tutorial 4: Automated Cryo-EM and X-ray structure determination with AlphaFold	T. Terwilliger
14:00	5 min break	
14:05	Validation	C. Williams
14.50	Tutorial 5: Refinement with phenix.refine, Validation	D. Liebschner, O. Sobolev, C. Williams
15:35	Discussion and questions	
15:50	Wrap up, Feed-back survey	
16:00	End	

Phenix User Workshop, August 22nd 2023



Introduction

Dorothee Liebschner
Lawrence Berkeley Laboratory

What is Phenix?

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



Los Alamos National Laboratory New Mexico Consortium

Tom Terwilliger, Li-Wei Hung



UTHealth

Matt Baker



University of Cambridge

Randy Read, Airlie McCoy,
Alisia Fadini



Duke University

Jane & David Richardson,
Christopher Williams,
Vincent Chen

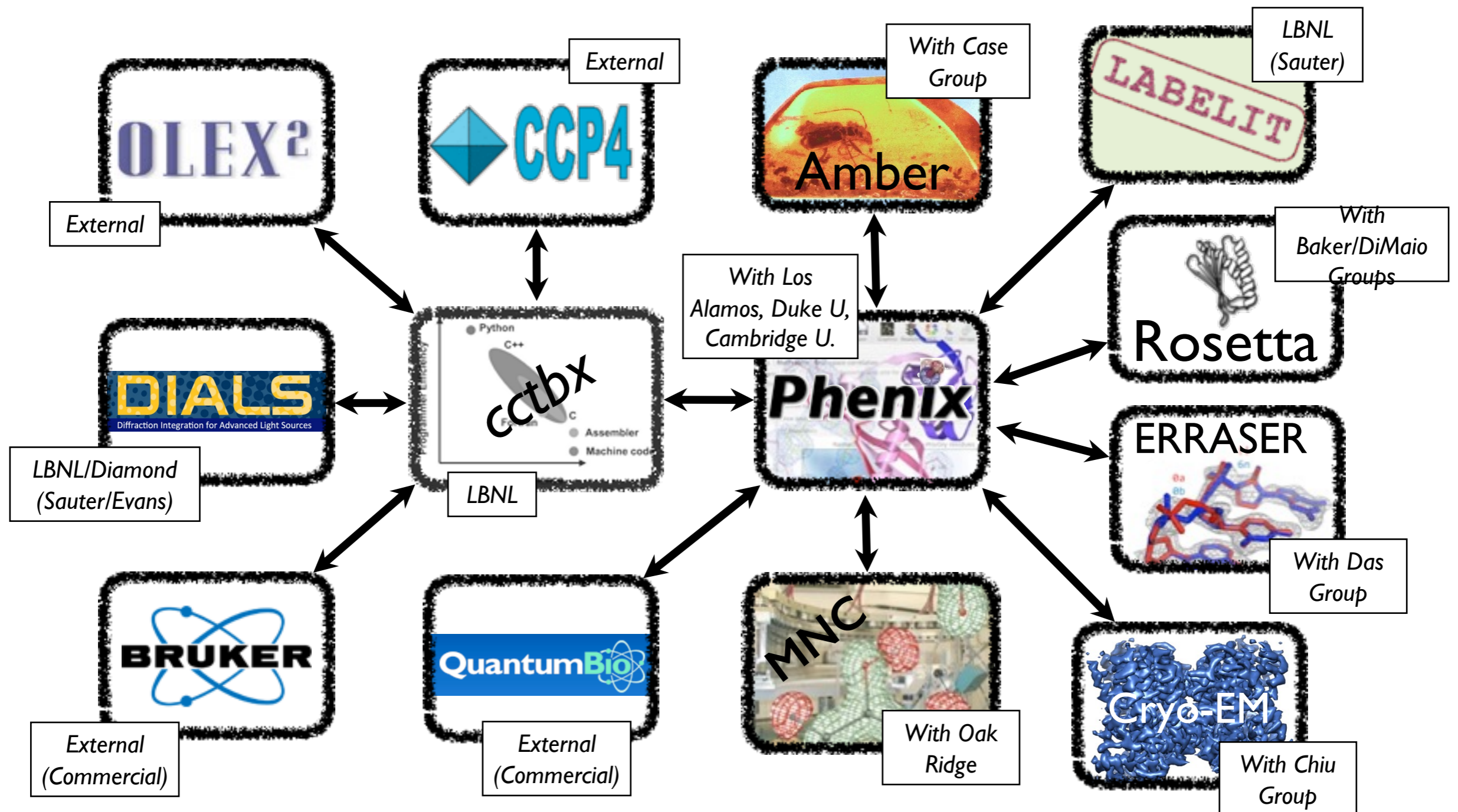


An NIH/NIGMS funded
Program Project

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

Phenix - a Structural Biology Hub

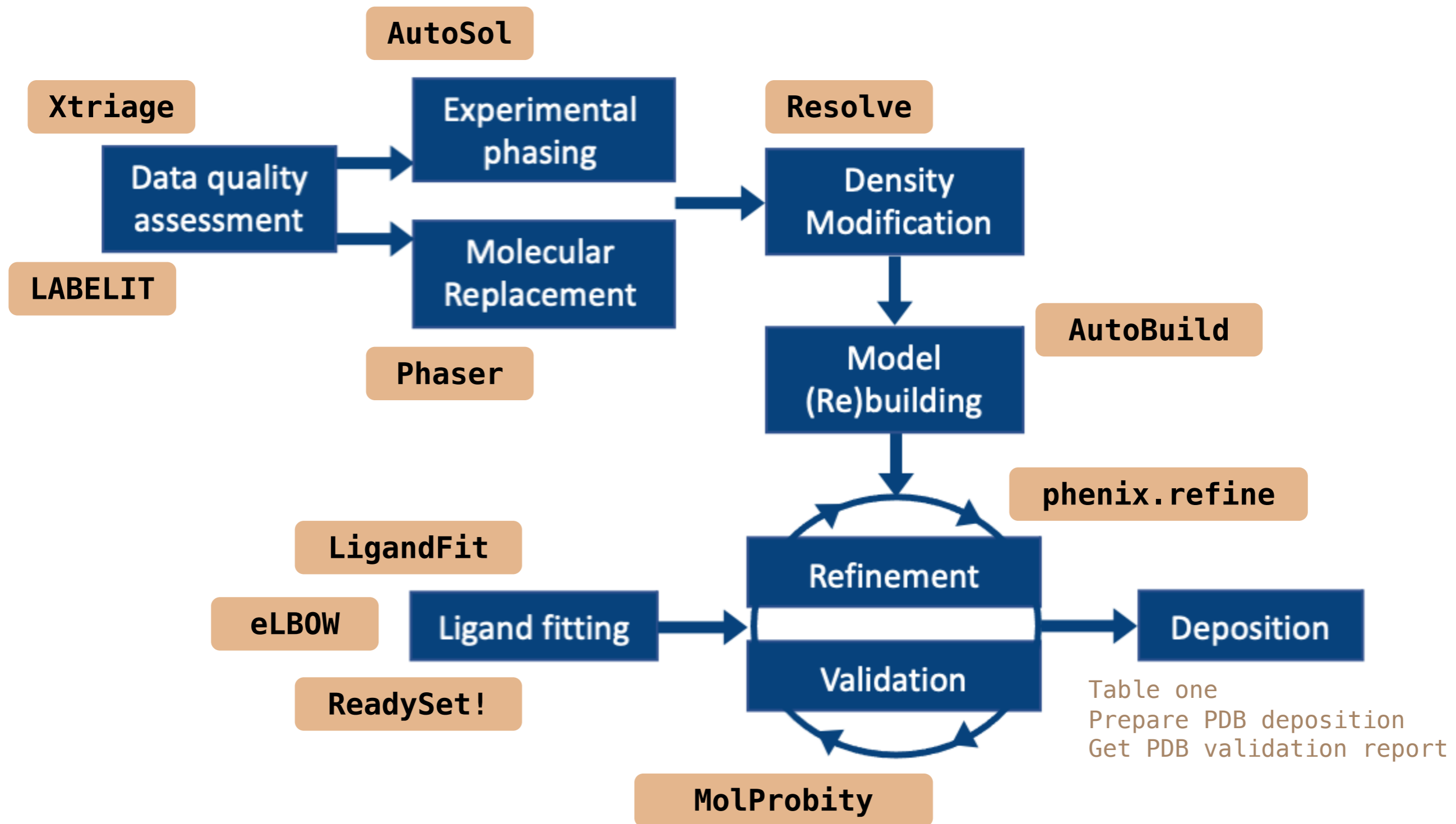
We have nucleated the development of new computational methods for structural biology



Key Features

- **Python**
 - Easy scripting of repetitive tasks
 - Enables rapid prototyping and development
- **Advanced algorithms**
 - Experimental phasing
 - Molecular replacement
 - Automated model building and rebuilding
 - Structure refinement and validation
 - Ligand coordinate and restraint generation
- **Rapid development and bug fixing**

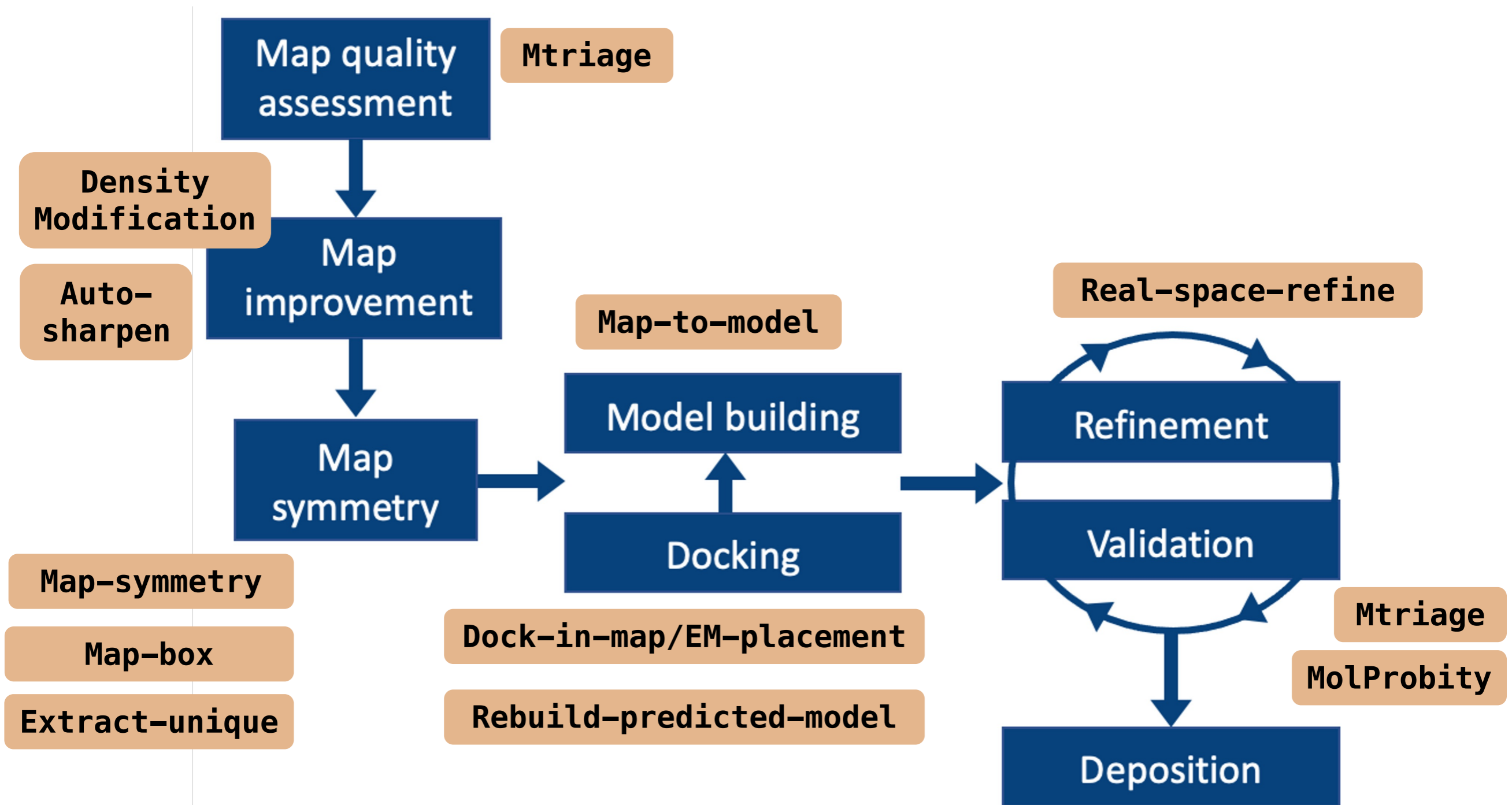
Tools for Crystallography



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

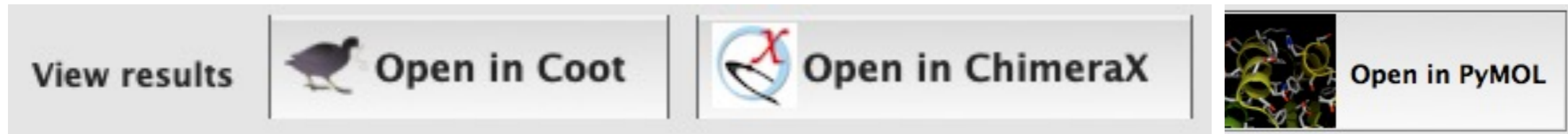
The right panel, titled 'Favorites', lists various tool categories:

- 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

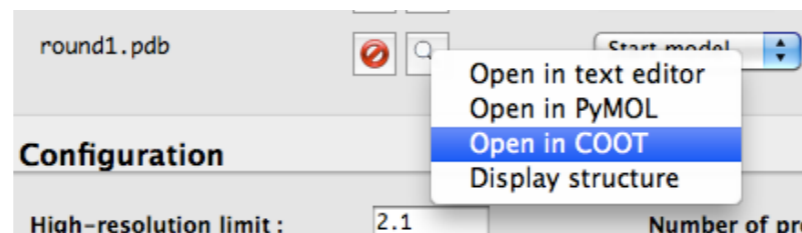
At the bottom, there is a 'Current directory:' field showing '/Users/dcliebschner/Desktop/Projects/test' and a 'Browse...' button. The status bar at the very bottom indicates 'PHENIX version dev-svn-000' and 'Project: test'.

Coot/PyMOL/ChimeraX integration

- Most results can be opened directly in graphics apps



- Any PDB file listed in GUI can also be opened

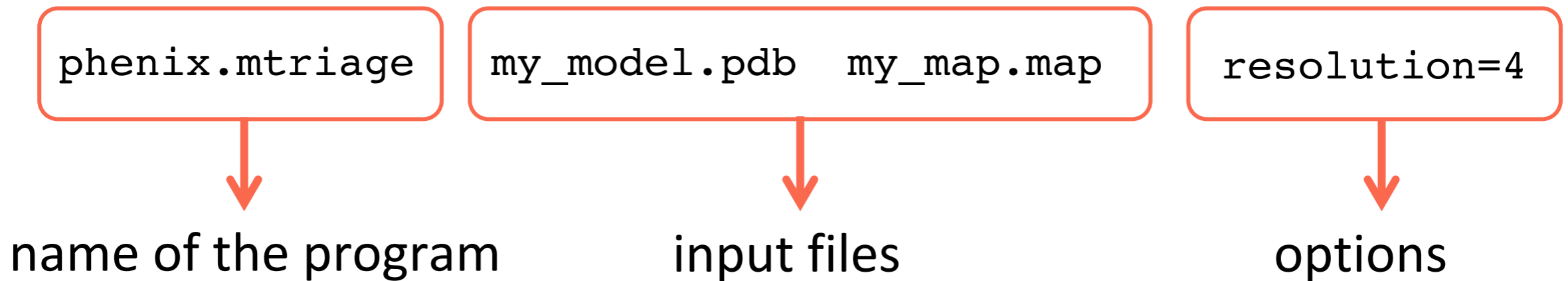


- AutoSol, AutoBuild, and phenix.refine will update Coot continuously while running
- Coot must have Python support (default on Mac)
- Specific paths to executables usually required on Linux

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)
```

Phenix Availability

phenix-online.org

Supported on:

- Linux
- macOS
- Windows

Extensive documentation
(online and via GUI)

Nightly builds

Phenix

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

NEW: Phenix with AlphaFold models

- Trim, weight, create domains and use for molecular replacement
- Trim, dock into cryo-EM maps and fill in gaps
- Reference models for refinement

[Learn more](#)

Cryo-EM map and superposed, refined AlphaFold model

Download | Getting Started | Workshops & Tutorials | Documentation | Help | Developers | National Resource | Industrial Consortium

Version	Date	Status	Logs	Info
dev-3758	2020-01-22	successful	ci , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64 , intel-windows-x86_64	docs ; changelog
dev-3753	2020-01-17	successful	ci , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64 , intel-windows-x86_64	docs ; changelog
dev-3751	2020-01-15	successful	ci , intel-windows-x86_64 , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64	docs ; changelog
1.17.1-3660	2019-10-16	successful	ci , intel-windows-x86_64 , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64	Official 1.17.1 release; docs ; changelog

Video Tutorials

www.youtube.com/channel/UCcdl0hfHngWAZLJWynxPQWg/videos

YouTube IT Search

Phenix Tutorials

560 subscribers

SUBSCRIBE 560

HOME VIDEOS PLAYLISTS CHANNELS ABOUT

Uploads PLAY ALL SORT BY

Video Title	Duration	Views	Upload Date
real_space_refine Tutorial	5:27	763 views	7 months ago
Secondary Structure Restraints Tutorial	6:23	399 views	8 months ago
Multiple refinement strategies Tutorial	5:46	281 views	8 months ago
Planning a SAD experiment Tutorial	6:00	483 views	1 year ago
Map-to-model Tutorial	5:33	1.3K views	1 year ago
Scale-and-merge Tutorial	7:31	387 views	1 year ago
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

What's new?

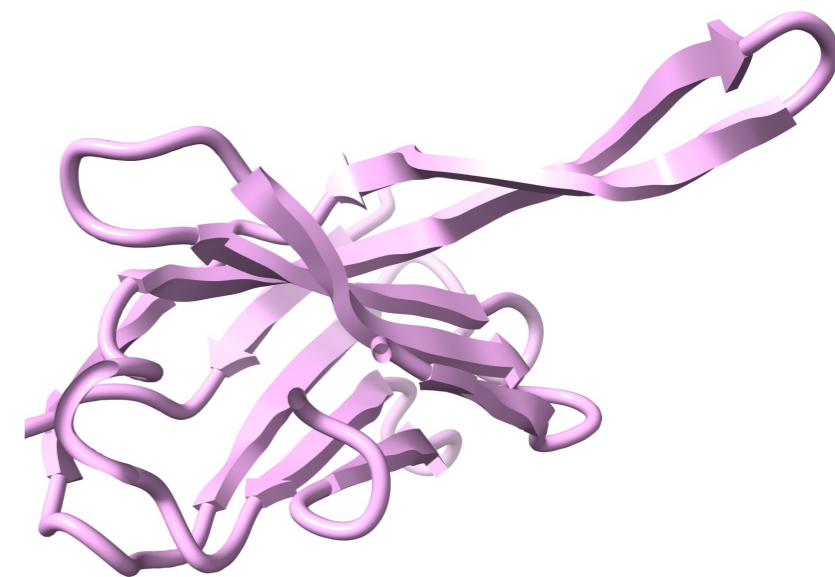
Accurate predicted models



```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
.....F.....M.....Q.....
.....K.....Y.....L.....A.....
.....A.....V.....
.....A.....
.....L.....V.....E.....
.....A.....Q.....
```

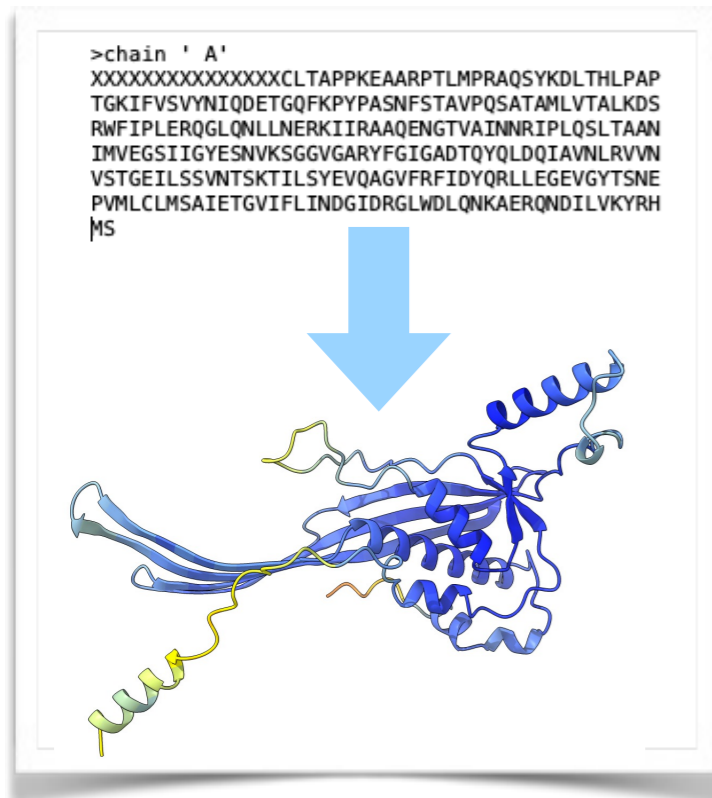
Sequence

Multiple sequence alignment



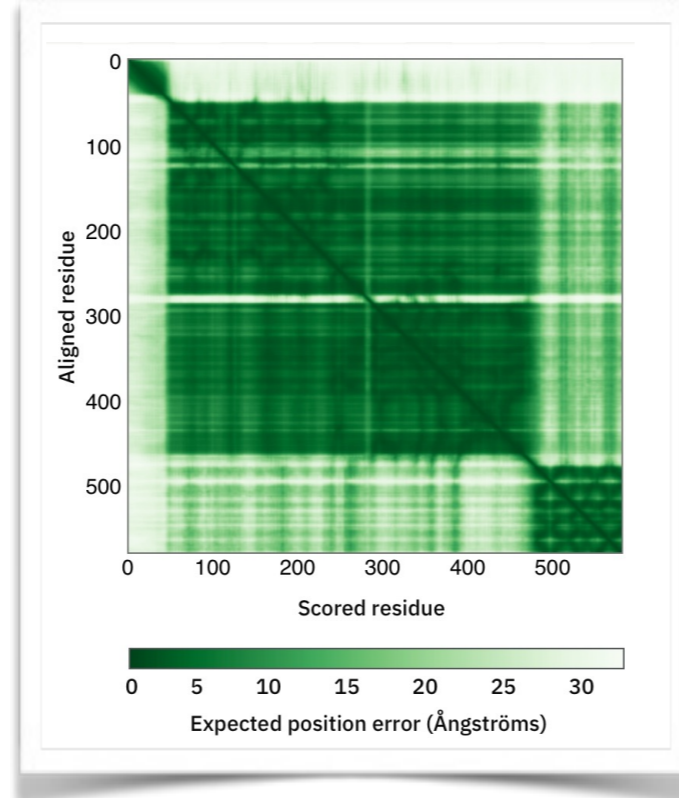
3D prediction

New tools for predicted models in Phenix

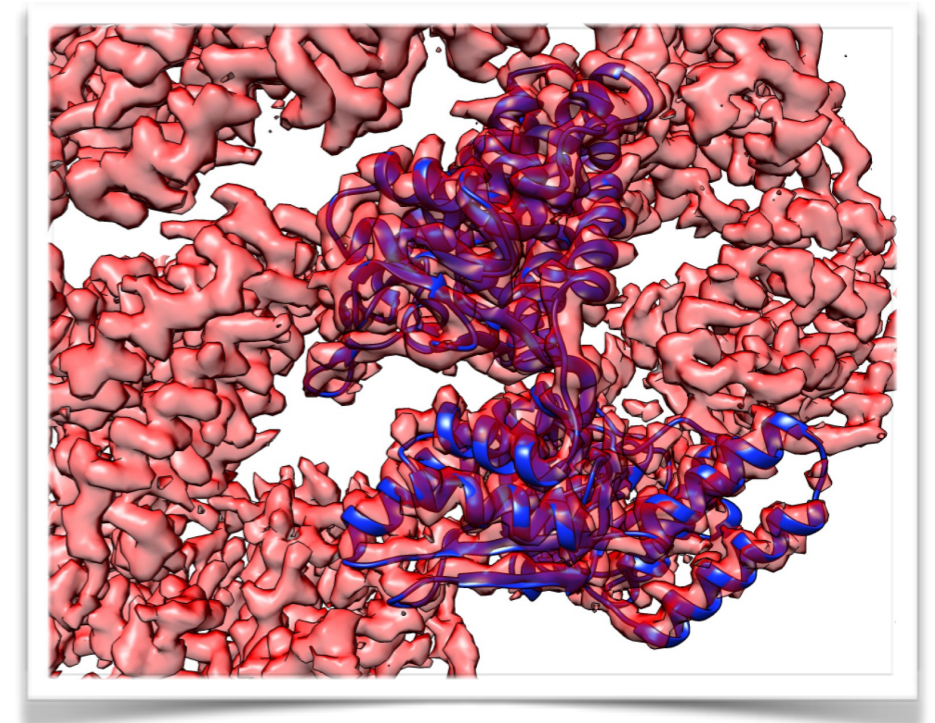


AlphaFold model prediction

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



Process predicted model



Predict and Build

(Iterative AlphaFold prediction, docking, and rebuilding)

Fully automatic!

Wednesday, August 23rd

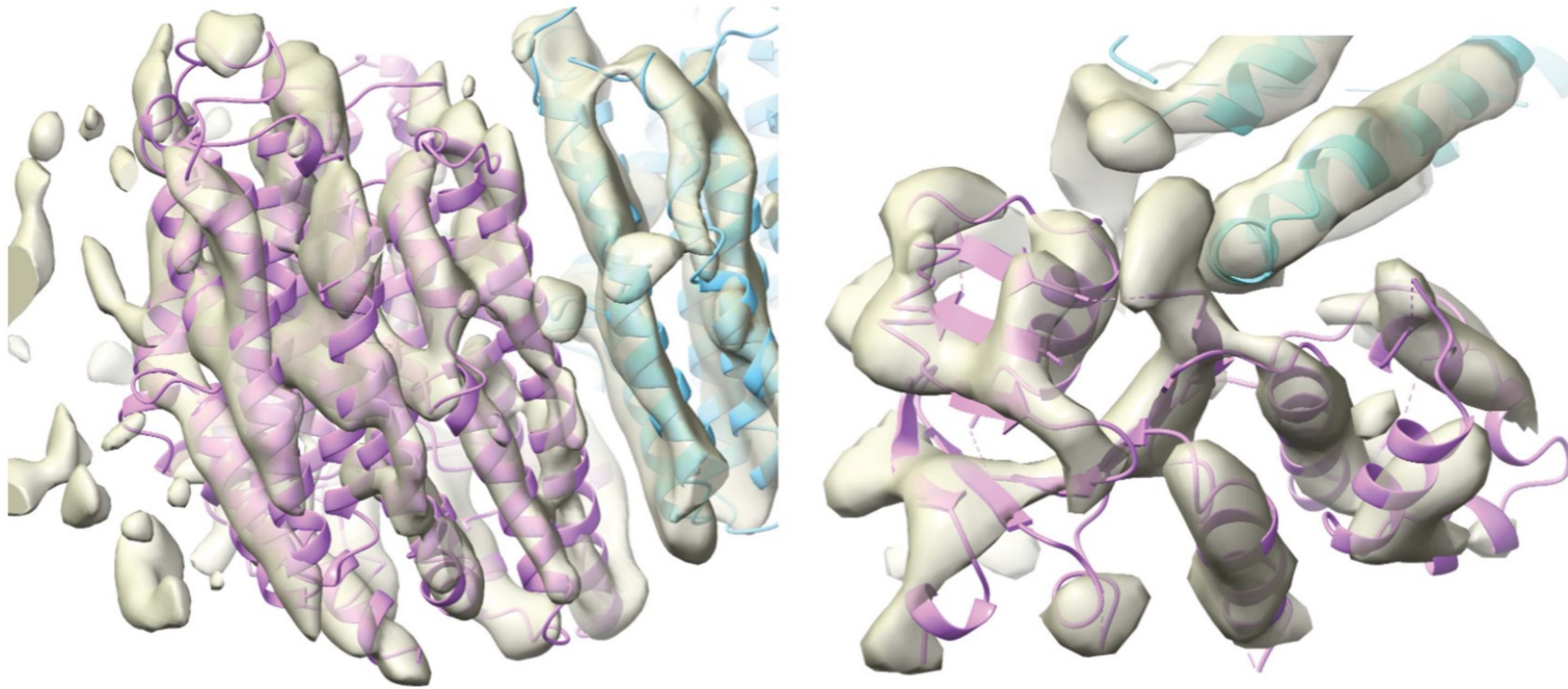
Tom Terwilliger

AlphaFold changes everything (and nothing)

Likelihood-based EM docking

- Use likelihood scores to dock a model into a map
- Works at low resolution (8.5 Å)

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



Wednesday, August 23rd

2:40 pm, Randy Read, Likelihood-based docking (A020)

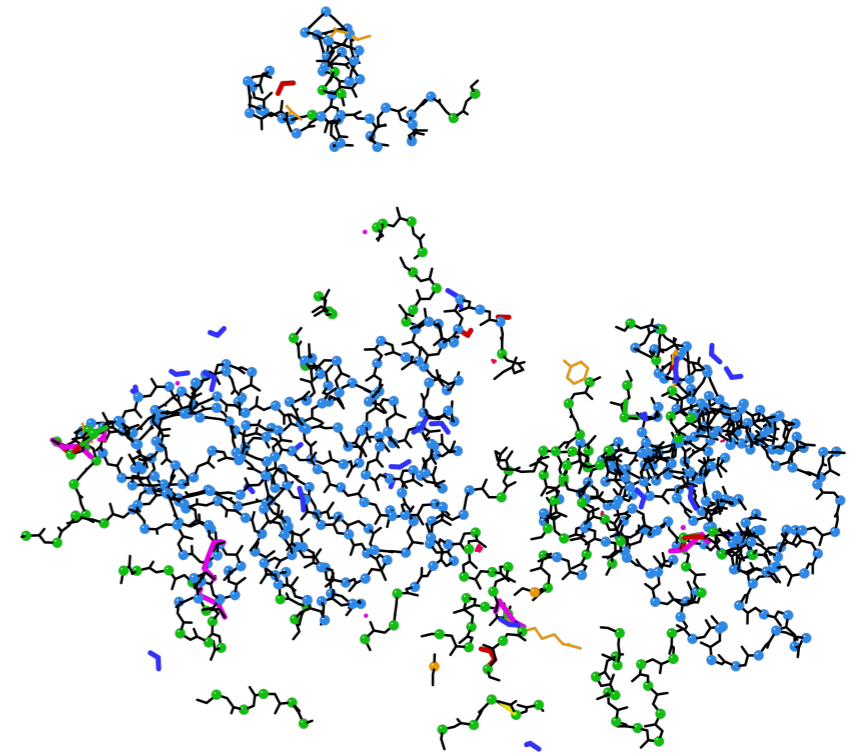
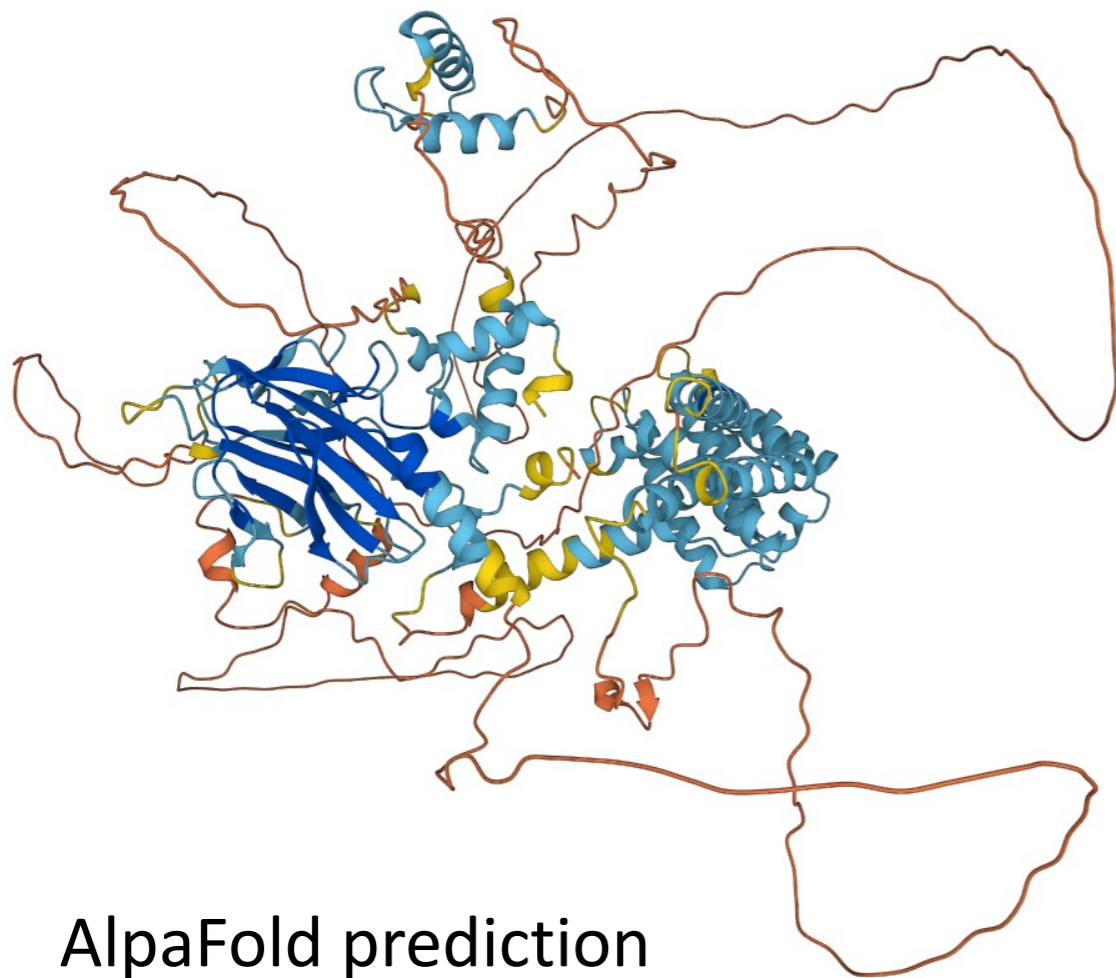
Read RJ, Millán C, McCoy AJ, Terwilliger TC. Likelihood-based signal and noise analysis for docking of models into cryo-EM maps. *Acta Crystallogr D Struct Biol.* 2023 Apr 1;79(Pt 4):271–80.

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.

Barbed wire analysis

- Automatically select the most promising regions of an AlphaFold prediction
- Annotate a prediction to help you make informed decisions about it.

Uniprot **P53076**



Sunday, August 27th 5:55 pm, Christopher Williams, *Identification and Validation of low-pLDDT regions in AF2 predictions (A011)*

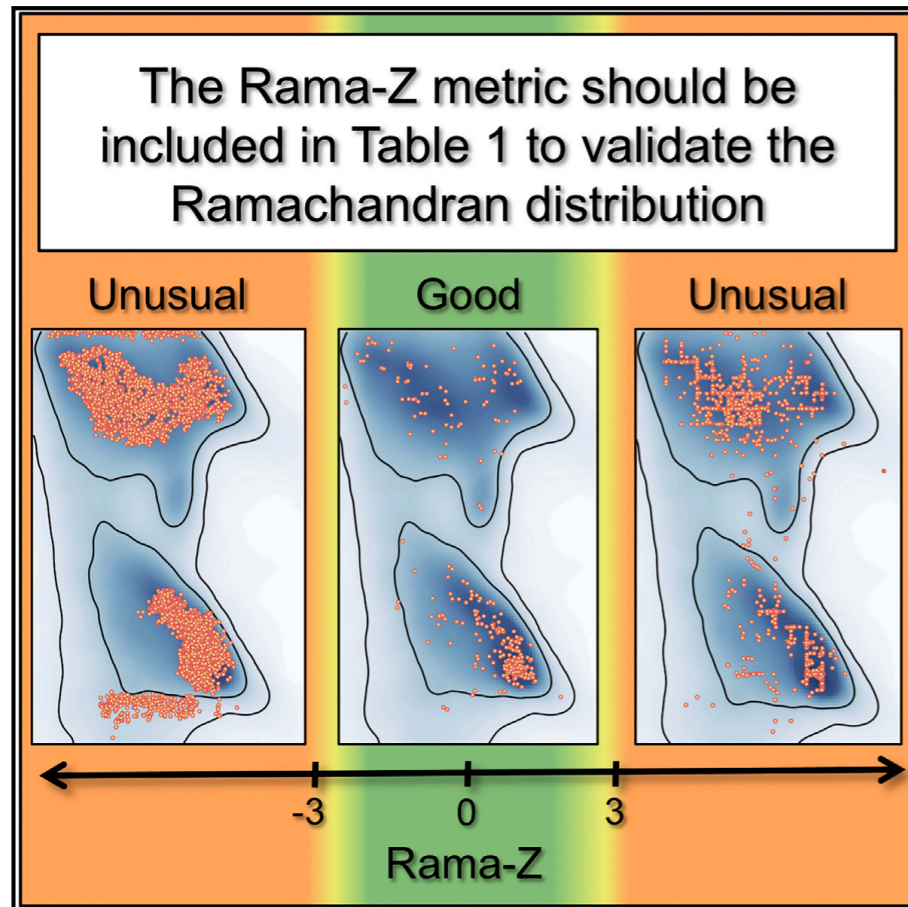
Global Ramachandran Score

Resource

Structure

A Global Ramachandran Score Identifies Protein Structures with Unlikely Stereochemistry

Graphical Abstract



Authors

Oleg V. Sobolev, Pavel V. Afonine,
Nigel W. Moriarty,
Maarten L. Hekkelman,
Robbie P. Joosten,
Anastassis Perrakis, Paul D. Adams

Correspondence

osobolev@lbl.gov (O.V.S.),
r.joosten@nki.nl (R.P.J.)

In Brief

Counting the number of Ramachandran outliers is not sufficient for protein backbone validation. Sobolev et al. revisited the underutilized Ramachandran Z score. The authors describe its reimplemention in Phenix and PDB-REDO and showcase its utility. They advocate including it in the validation reports provided by the Protein Data Bank.

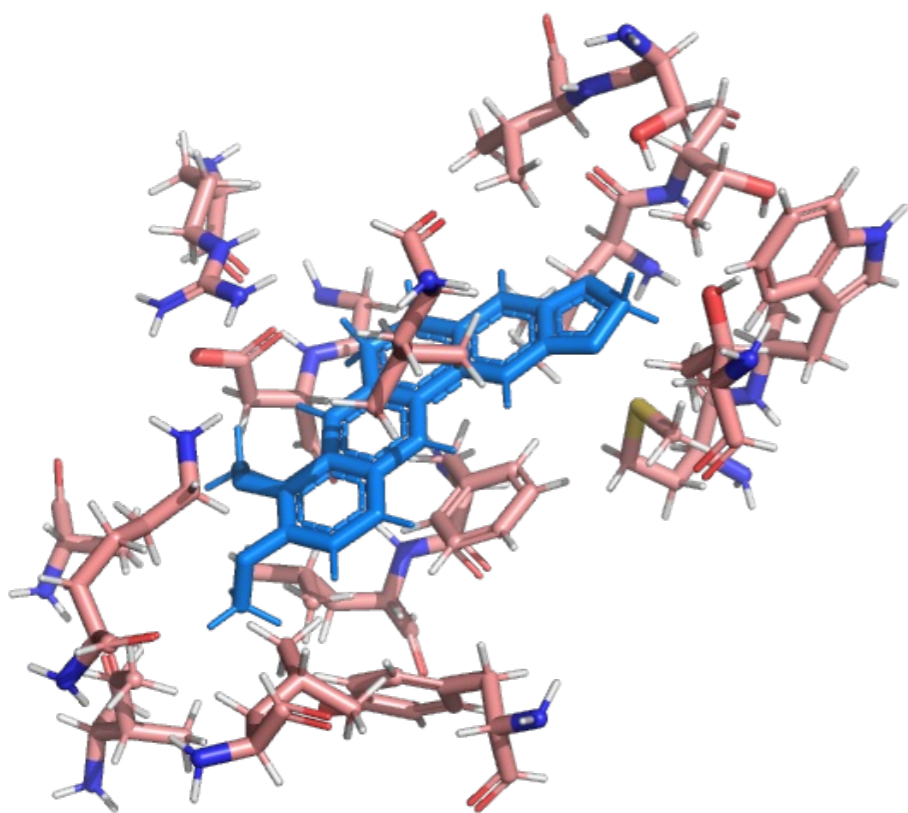
Structure, **28**. 1249-1258.

Wednesday, August 23rd

1:30 pm, Oleg Sobolev (A020, room 209)

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

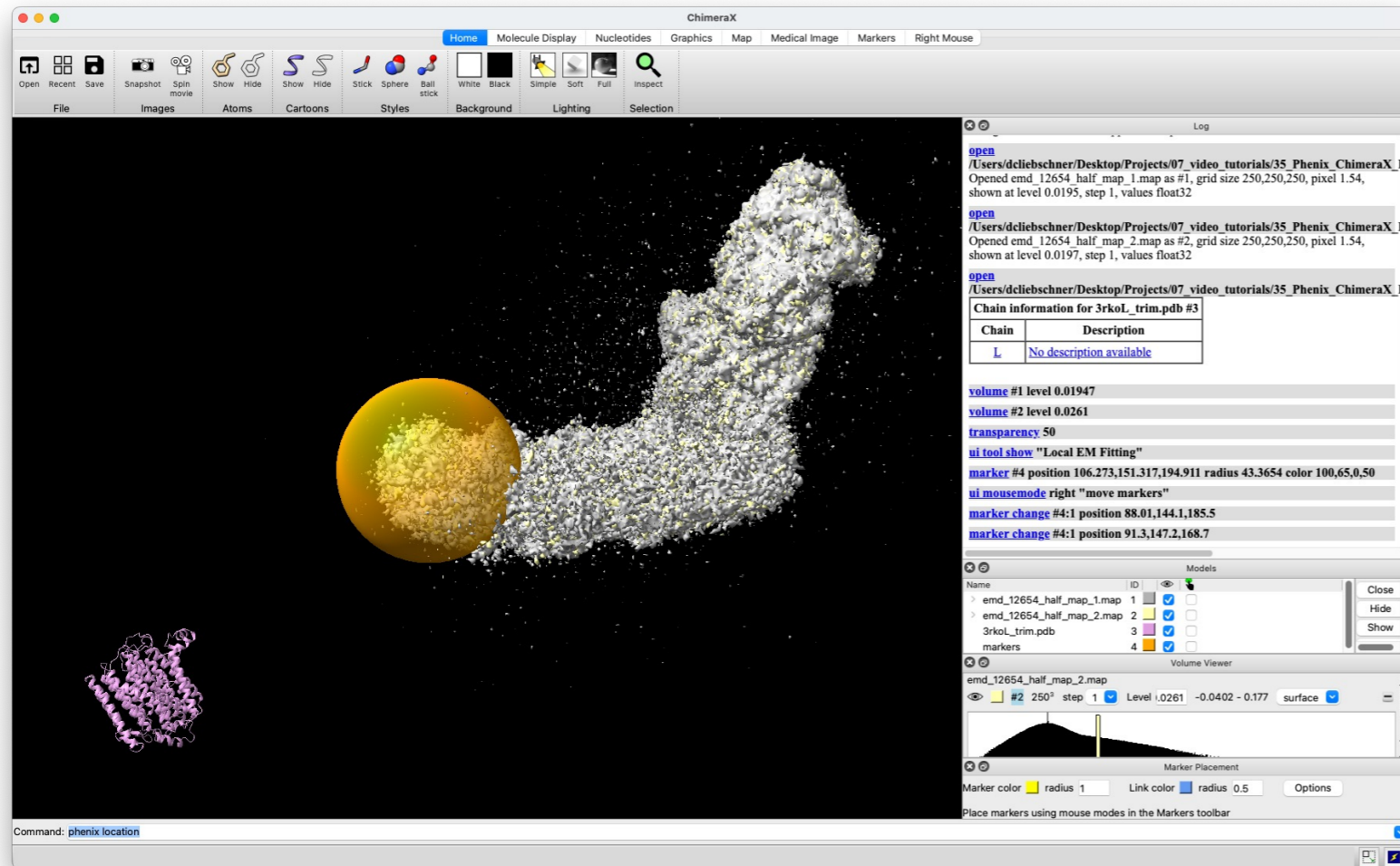
QMR approach:

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

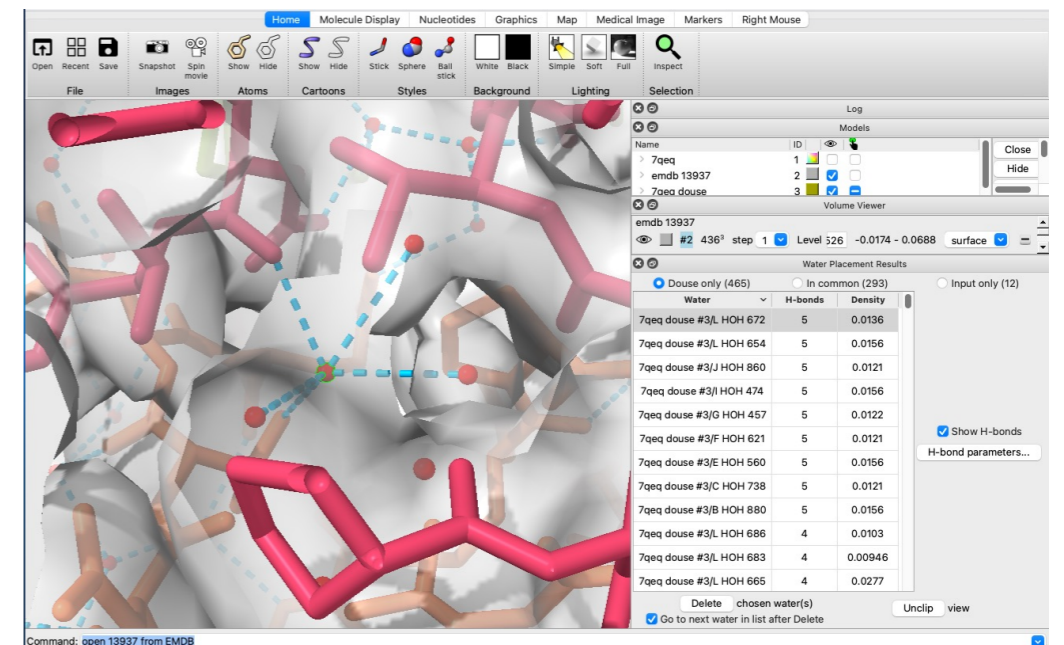
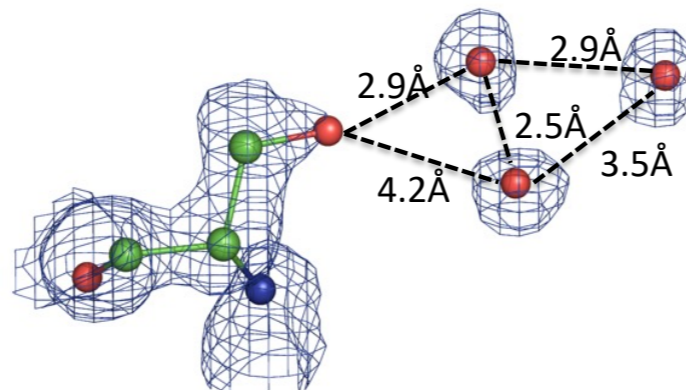
Run Phenix tools through ChimeraX

Local EM fitting
(EM placement)

Tutorial 2



Automated water building
(phenix.douse)



Talks of Phenix team members at the IUCr 2023

Wednesday, August 23rd

- 9:00 am, Tom Terwilliger, *AlphaFold changes everything (and nothing)* (Keynote 1)
- 1:30 pm, Oleg Sobolev, Global Ramachandran Score (A020)
- 2:40 pm, Randy Read, Likelihood-based docking (A020)

Sunday, August 27th

- 5:55 pm, Christopher Williams, *Identification and Validation of low-pLDDT regions in AF2 predictions* (A011)

Monday, August 28th

- 9:00 am, Airlie McCoy, Trekin' through reciprocal space with Phaser (keynote 31)
- 2:20 pm, Dorothee Liebschner, *Using the PDB and EMDB for testing new algorithms* (A011)

Acknowledgements

Berkeley Laboratory

Pavel Afonine, Youval Dar, Nat Echols, Jeff Headd, Richard Gildea, Ralf Grosse-Kunstleve, Dorothee Liebschner, Nigel Moriarty, Nader Morshed, Billy Poon, Ian Rees, Nicholas Sauter, Oleg Sobolev, Peter Zwart

Los Alamos Laboratory/New Mexico Consortium

Tom Terwilliger, Li-Wei Hung

Baylor College of Medicine

Matt Baker

Cambridge University

Randy Read, Airlie McCoy, Gabor Bunckozi, Tristan Croll, Rob Oeffner, Kaushik Hatti, Massimo Sammito, Duncan Stockwell, Laurent Storoni

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

Funding

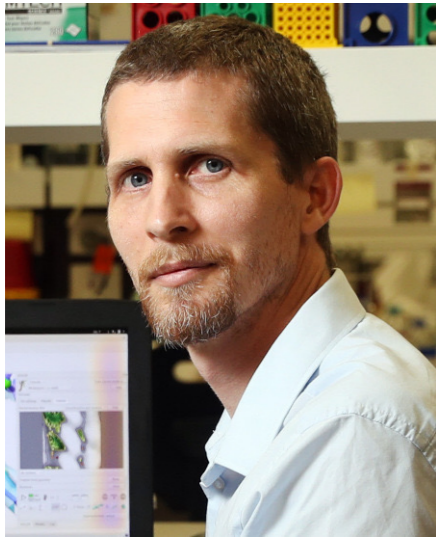
- NIH/NIGMS: P01GM063210, P50GM062412, P01GM064692, R01GM071939
- PHENIX Industrial Consortium
- Lawrence Berkeley Laboratory

Practical information
about this workshop

Today's workshop

- **8am – 4pm**
- **Breaks:**
 - 10:10 am (10 min)
 - 12pm (45 min)
 - 2pm (5 min)
- **Lunch is provided (wrap, meat, vegetarian, dietary restriction)**

Lecturers



Tristan Croll



Dorothee Liebschner



Airlie McCoy



Randy Read



Oleg Sobolev



Tom Terwilliger



Christopher Williams

Today's workshop

- **Lectures**

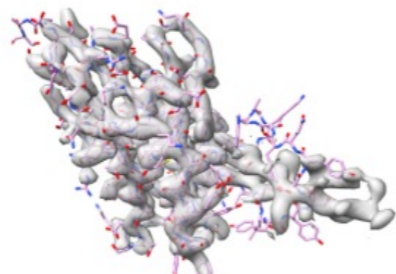
- **Tutorials**

- *If you haven't installed Phenix () prior to the workshop, there is no time to download and install it now (conference WiFi has a limit of 100Mb).*
- If you cannot follow along with your computer
 - Just watch
 - You can do (most) tutorials at home with the tutorial data in the Phenix installation

Presentation slides

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

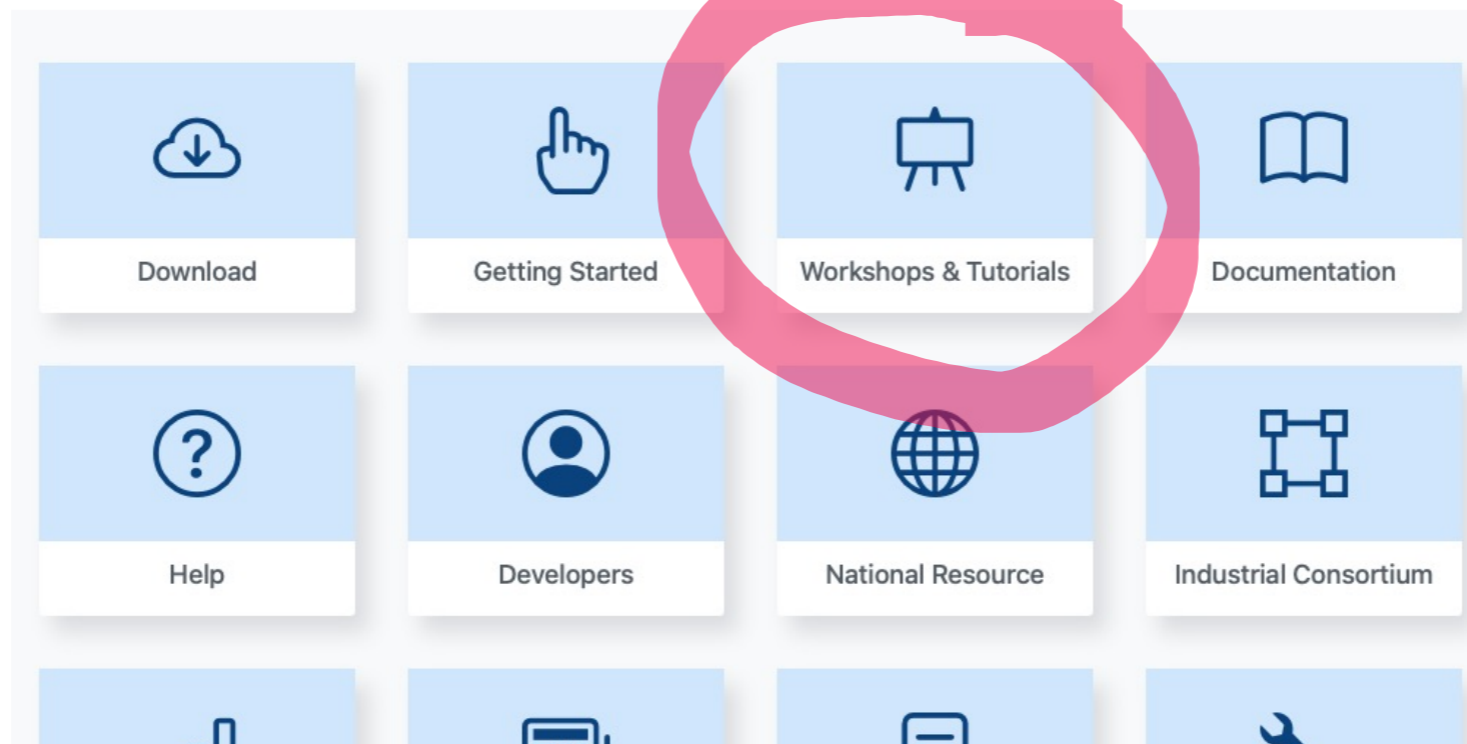
A comprehensive software package for macromolecular structure determination using crystallographic (X-ray, neutron and electron) and electron cryo-microscopy data. [Learn more](#)



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](#)



Presentation slides

- **Lectures**



Menu ☰

Videos and slides from Phenix user workshops

Video recordings and/or slides from Phenix user workshops.

General information

- All videos are available on the Phenix tutorials YouTube channel: www.youtube.com/c/phenixtutorials.
- Tutorials with example data are described [here](#).

Future user workshops

Phenix user workshop on 8/22/2023 at the [UCr meeting](#) in Melbourne, Australia.

Past user workshops

Phenix workshop at the ACA 2022 in Portland (OR) ⤴

- Introduction to Phenix (Dorothee Liebschner) [\(pdf\)](#)
- Strategy for X-ray or Cryo-EM structure determination using AlphaFold models (Tom Terwilliger) [\(pdf\)](#)
- Evaluating X-ray data with Xtrriage (Dorothee Liebschner) [\(pdf\)](#)
- Xray refinement (Pavel Afonine) [\(pdf\)](#)
- Ligands (Dorothee Liebschner) [\(pdf\)](#)
- Cryo-EM Data evaluation with Mtriage (Pavel Afonine) [\(pdf\)](#)
- Cryo-EM refinement (Pavel Afonine) [\(pdf\)](#)

Phenix workshop 04/2021 - cryo-EM (Virtual) ⤴

Phenix workshop 11/2020 - Xtallography (Virtual) ⤴

Phenix workshop 09/2020 - cryo-EM (Virtual) ⤴

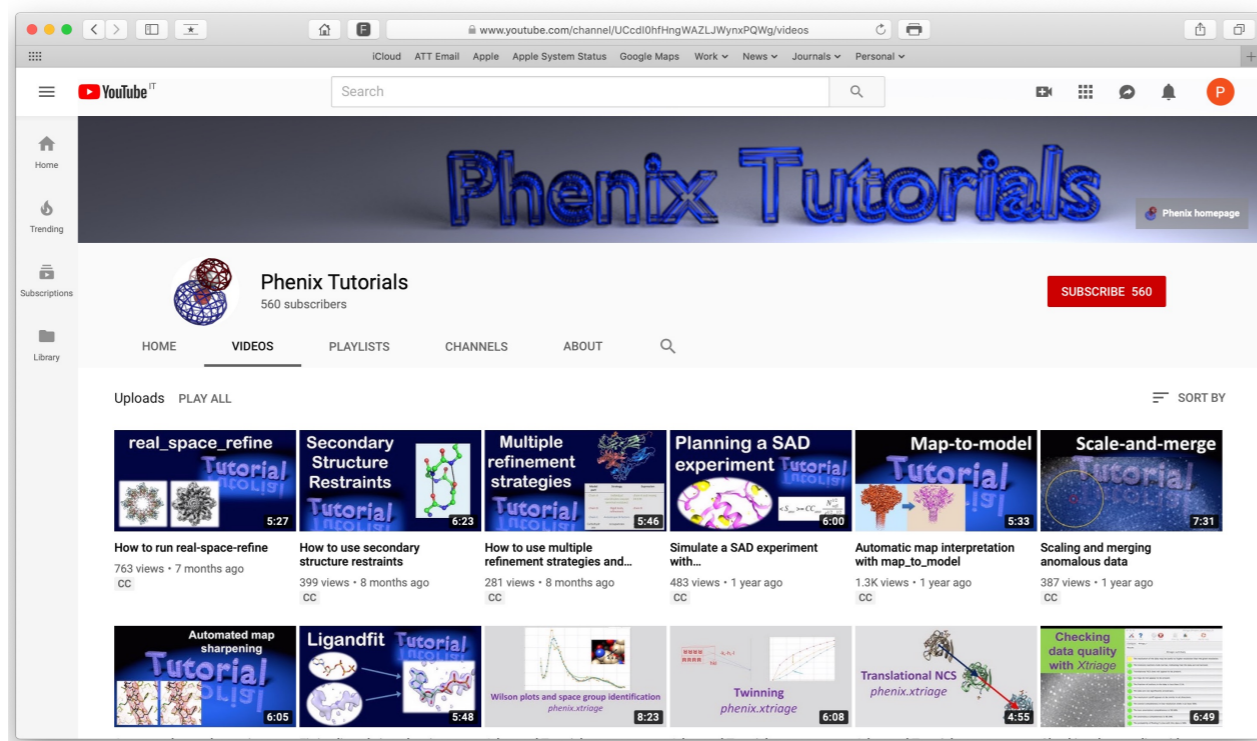
Troubleshooting

- **Tutorials**

- Phenix team members will be in the room

- YouTube video tutorials

<https://www.youtube.com/c/phenixtutorials>



The image shows a screenshot of a web browser displaying the YouTube channel page for "Phenix Tutorials". The channel has 560 subscribers and a "SUBSCRIBE 560" button. The page features a navigation menu with "HOME", "VIDEOS", "PLAYLISTS", "CHANNELS", and "ABOUT". Under the "VIDEOS" tab, there is a grid of video thumbnails. The first row includes:

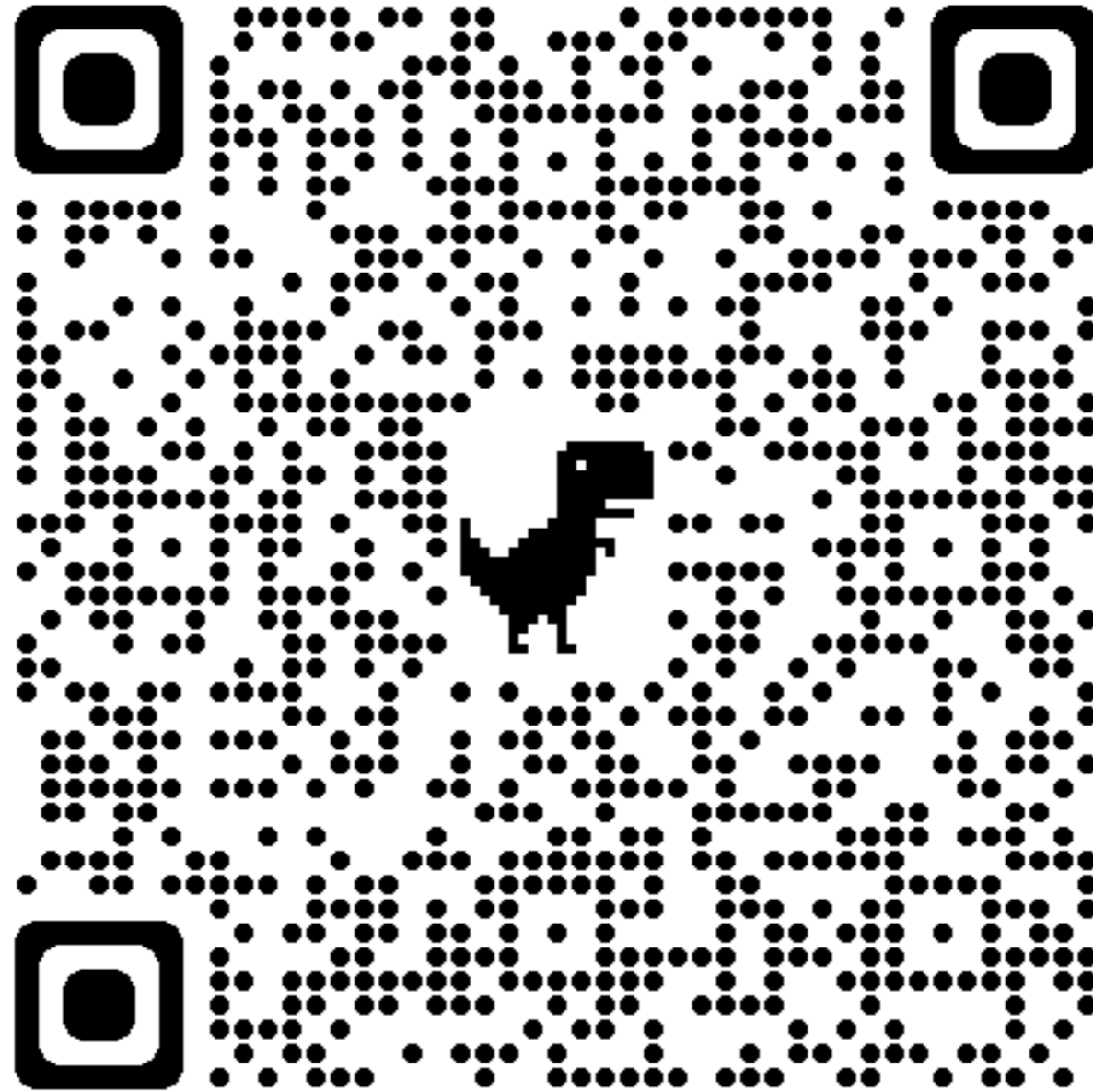
- real_space_refine Tutorial** (5:27): How to run real-space-refine (763 views, 7 months ago)
- Secondary Structure Restraints Tutorial** (6:23): How to use secondary structure restraints (399 views, 8 months ago)
- Multiple refinement strategies Tutorial** (5:46): How to use multiple refinement strategies and... (281 views, 8 months ago)
- Planning a SAD experiment Tutorial** (6:00): Simulate a SAD experiment with... (483 views, 1 year ago)
- Map-to-model Tutorial** (5:33): Automatic map interpretation with map_to_model (1.3K views, 1 year ago)
- Scale-and-merge Tutorial** (7:31): Scaling and merging anomalous data (387 views, 1 year ago)

The second row includes:

- 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)

Wrap up

- Please fill out the survey at the end (~1min)



<https://forms.gle/1my2D3fssCye4sVr8>

Program

Time	Agenda	Lecturers
8:00	Welcome/Introduction to Phenix	D. Liebschner
8:30	Using AlphaFold predictions for structure determination	T. Terwilliger
9:00	MR/Docking	R. Read/A. McCoy
9:30	Density modification in Crystallography and cryo-EM	T. Terwilliger
9:55	Tutorial 1: Density modification of cryo-EM maps	T. Terwilliger
10:10	10 min break	
10:20	Tutorial 2: AlphaFold structure prediction, Cryo-EM docking with Emplace Local, Cryo-EM model refinement with Real space refine	R. Read, A. McCoy, T. Croll, T. Terwilliger
11:10	Tutorial 3: MR/MR-SAD with AlphaFold	R. Read/A. McCoy
11:50	FAQ	
12:00	45 min lunch break and on-on-one discussions (lunch provided)	
12:45	Refinement	O. Sobolev
13:30	Tutorial 4: Automated Cryo-EM and X-ray structure determination with AlphaFold	T. Terwilliger
14:00	5 min break	
14:05	Validation	C. Williams
14.50	Tutorial 5: Refinement with phenix.refine, Validation	D. Liebschner, O. Sobolev, C. Williams
15:35	Discussion and questions	
15:50	Wrap up, Feed-back survey	
16:00	End	