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 **EN**vironment for **I**ntegrated **X**tallography

The Phenix 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

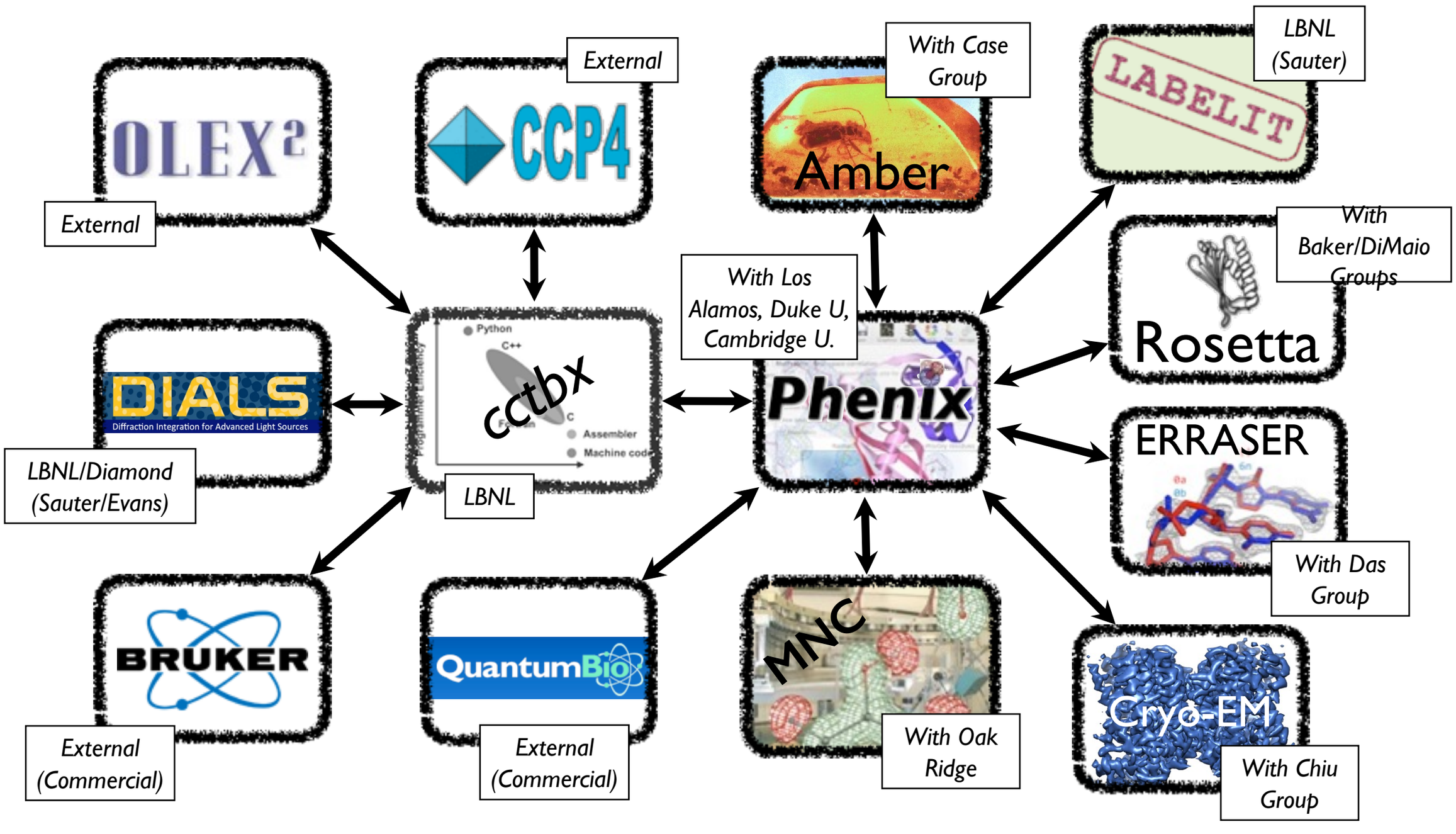
Jane & David Richardson, Christopher Williams, Vincent Chen

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

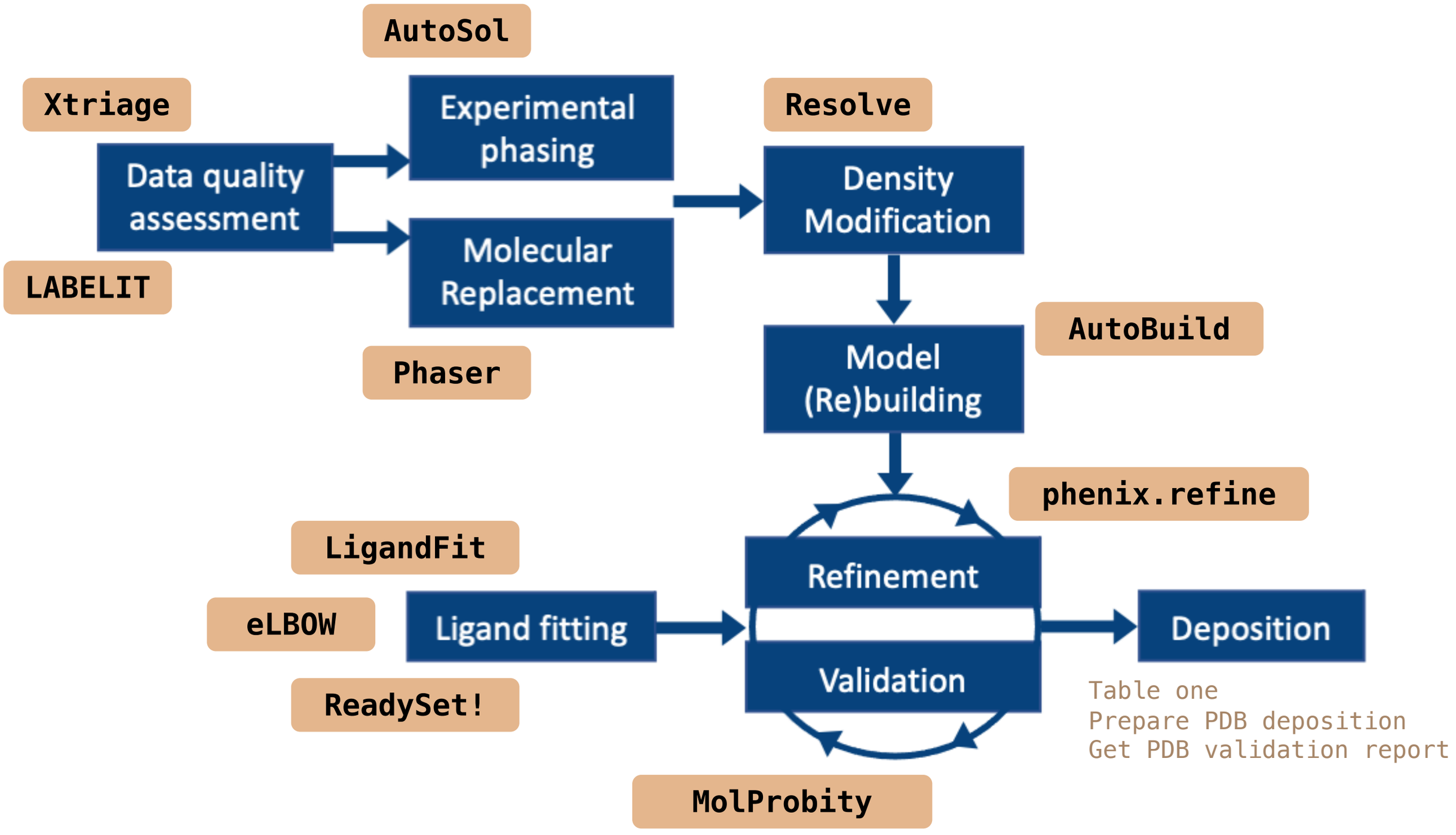
We have nucleated the development of new computational methods for structural biology



Key Features

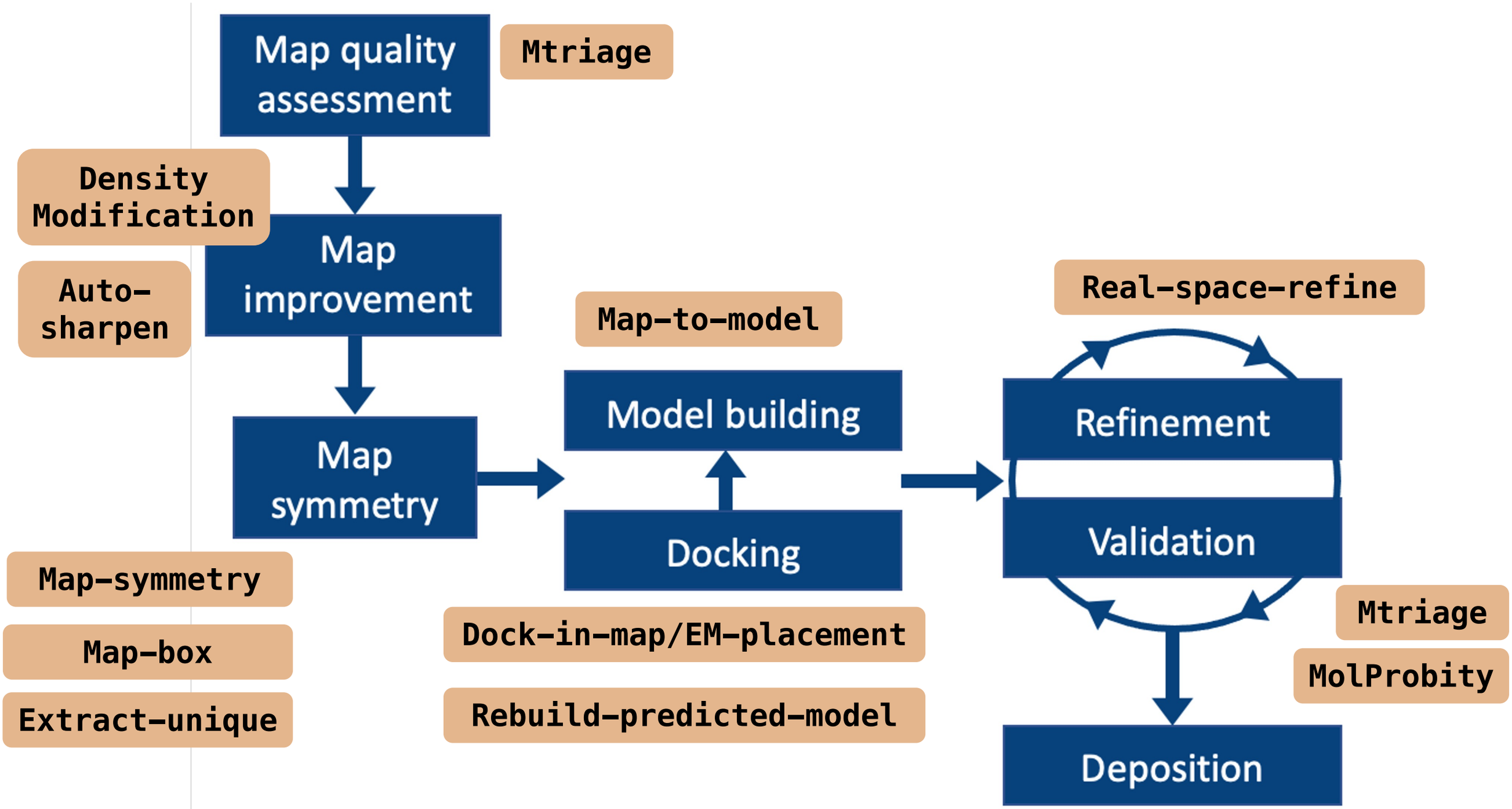
- **Python**
  - Easy scripting of repetitive tasks
  - Enables rapid prototyping and development
- **Advanced algorithms**
  - Experimental phasing
  - Molecular replacement
  - Automated model building and rebuilding
  - Structure refinement and validation
  - Ligand coordinate and restraint generation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

Tools for Crystallography



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

Tools for Cryo-EM



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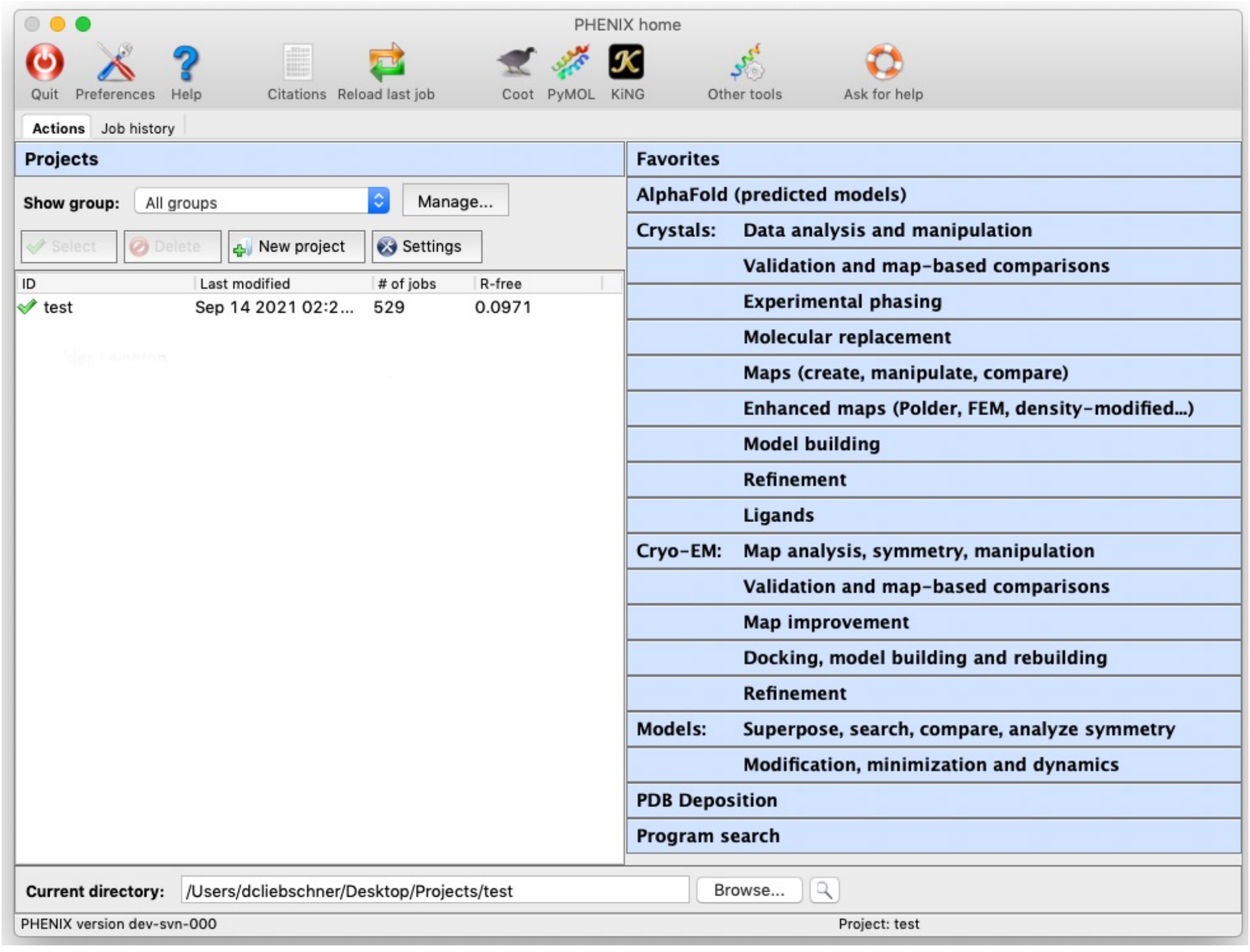
J. Appl. Cryst. 2002, 35:126-136 (cctbx)
Acta Cryst. 2010, D66: 213-221 (Phenix)
Acta Cryst. 2019 D75:861–877 (Phenix)

Features

Phenix GUI

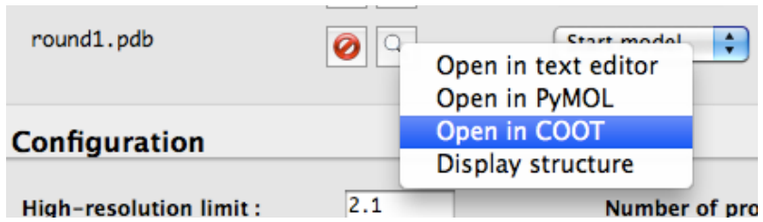
Central GUI for job control and to launch new jobs



Coot/PyMOL/ChimeraX integration

- Most results can be opened directly in graphics apps

- Any PDB file listed in GUI can also be opened

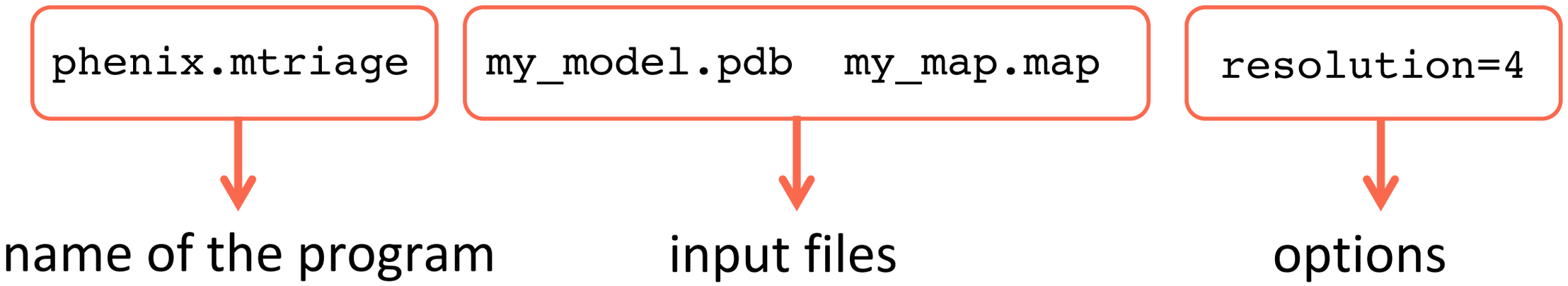


- AutoSol, AutoBuild, and phenix.refine will update Coot continuously while running
- Coot must have Python support (default on Mac)
- Specific paths to executables usually required on Linux

`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)
```

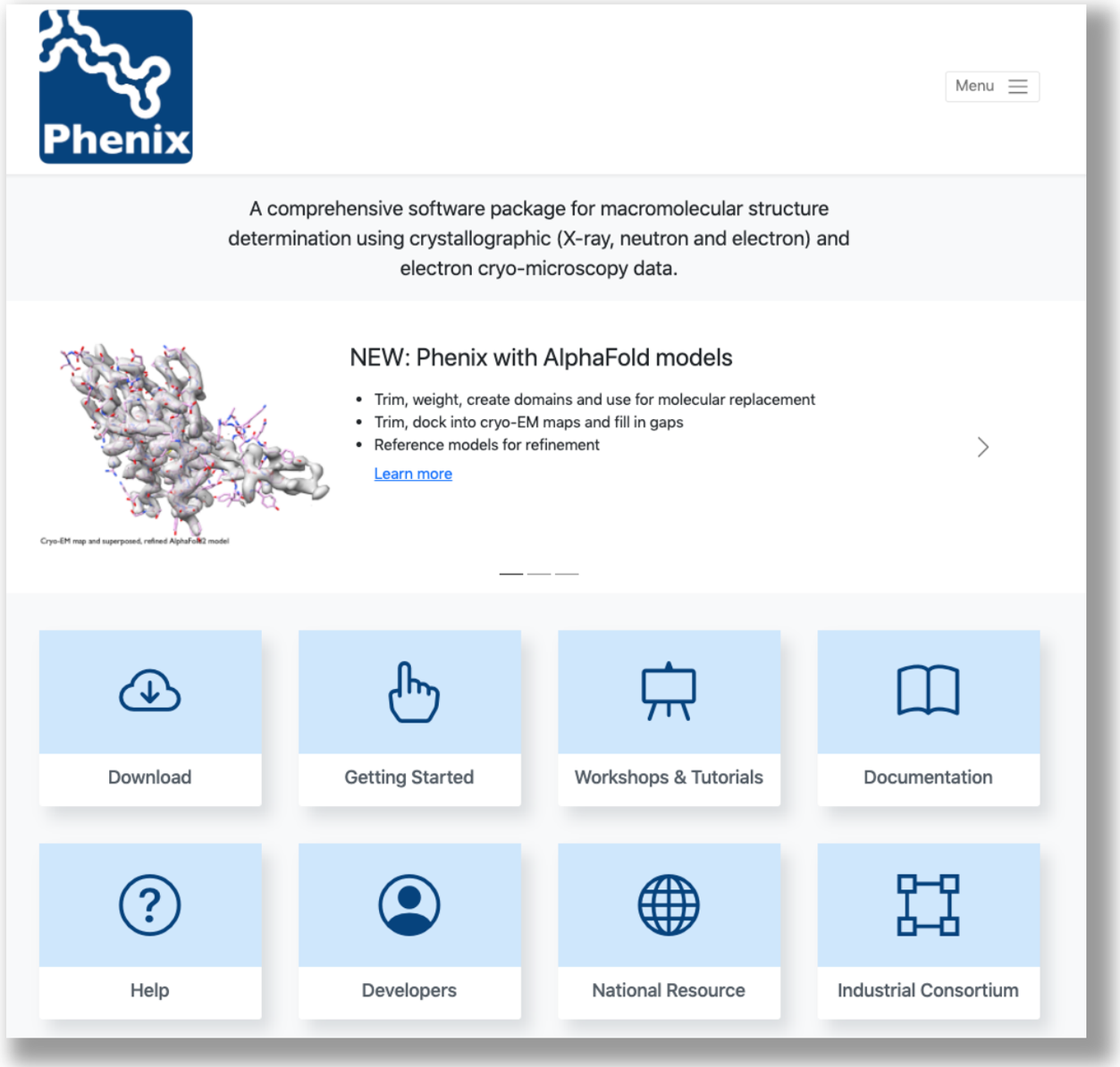
Phenix Availability

phenix-online.org

Supported on:

- Linux
- macOS
- Windows

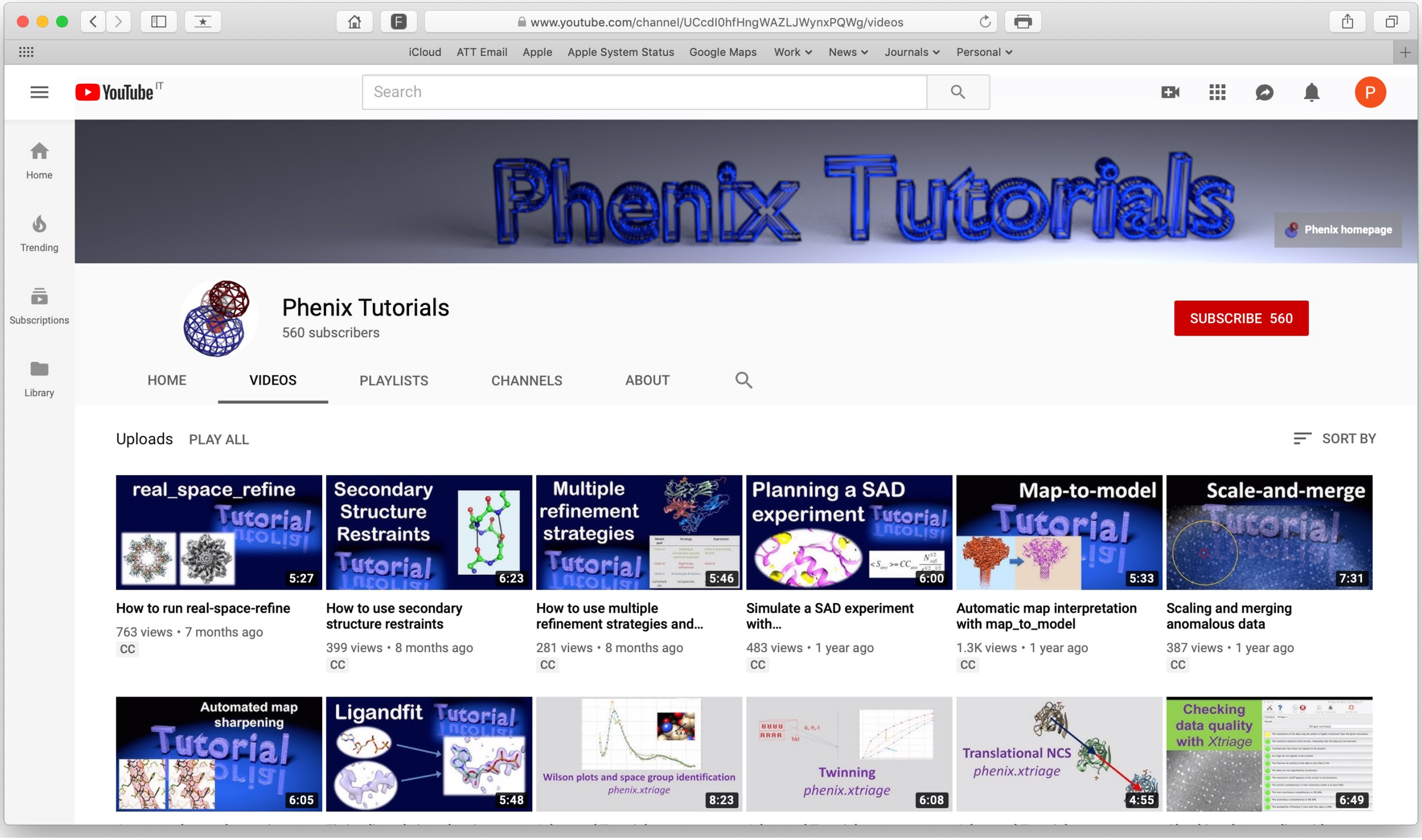
Extensive documentation (online and via GUI)



Nightly builds

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

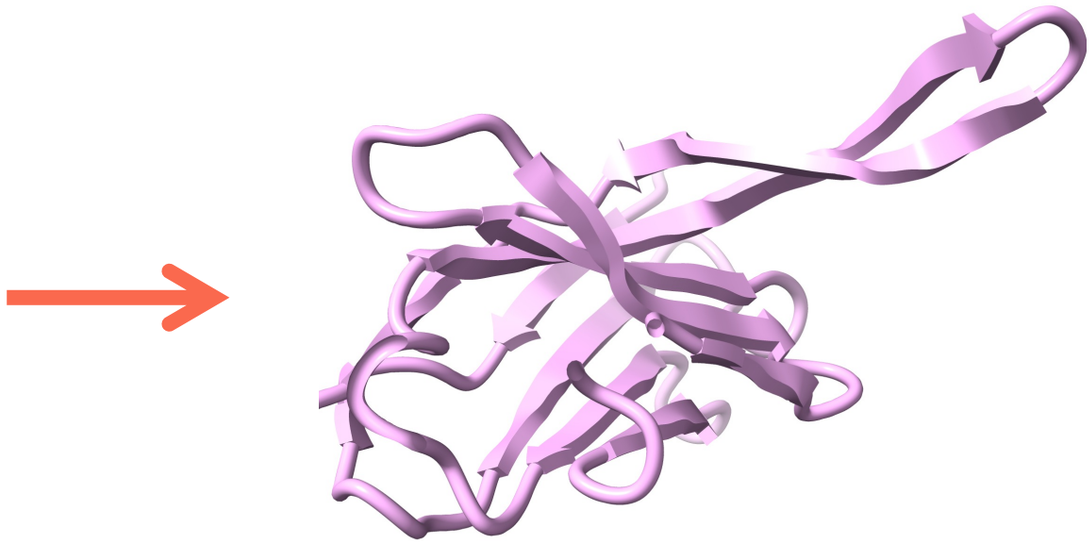


Dorothee Liebschner, Nigel Moriarty,
Tom Terwilliger, Christopher Schlicksup, Vincent Chen

What’s new?

Accurate predicted models


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

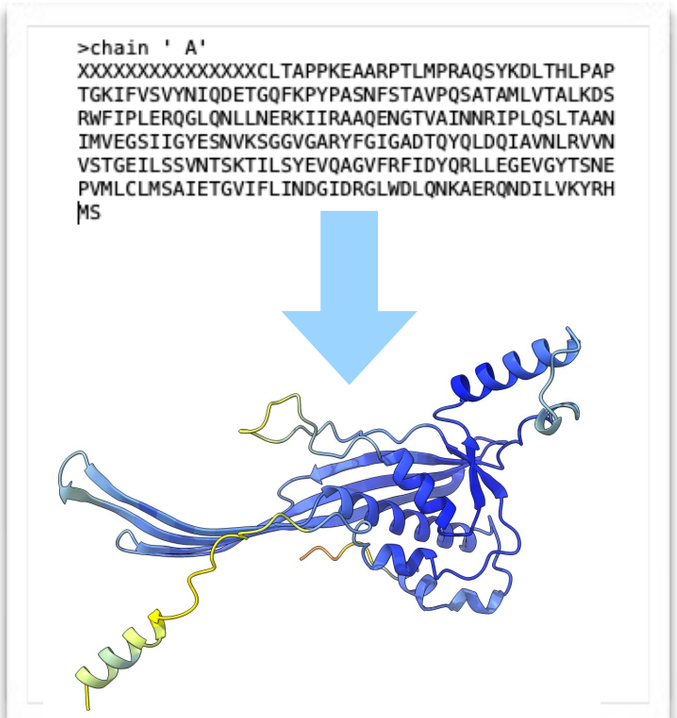


Sequence

Multiple sequence alignment

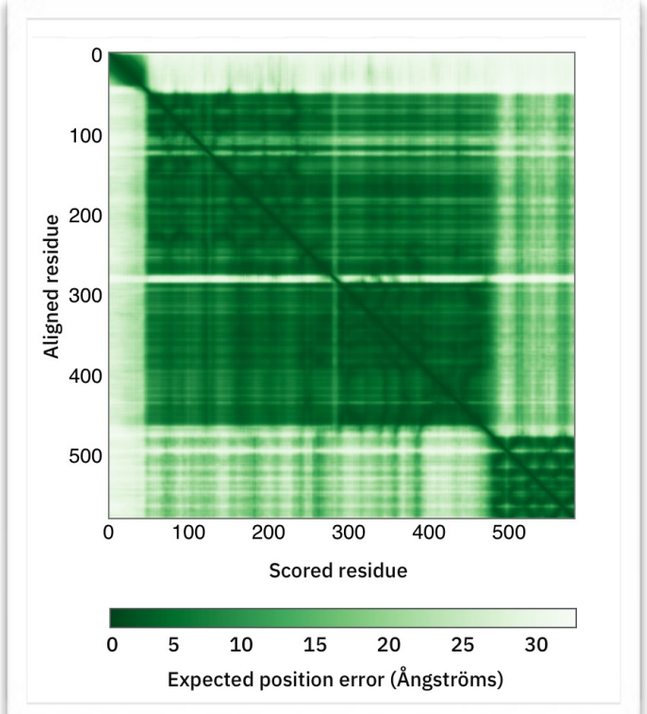
3D prediction

New tools for predicted models in Phenix

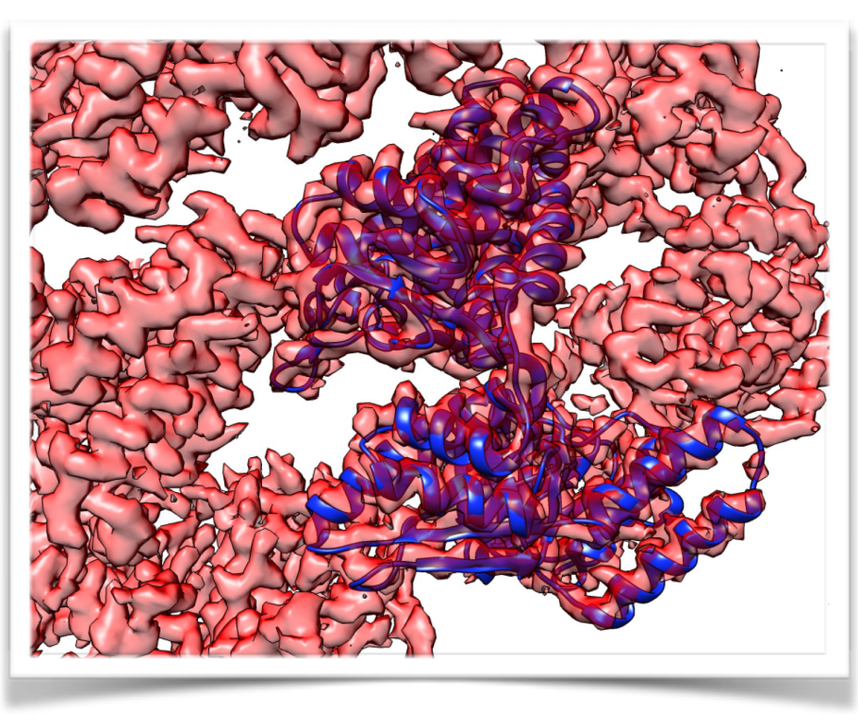


AlphaFold model prediction

(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

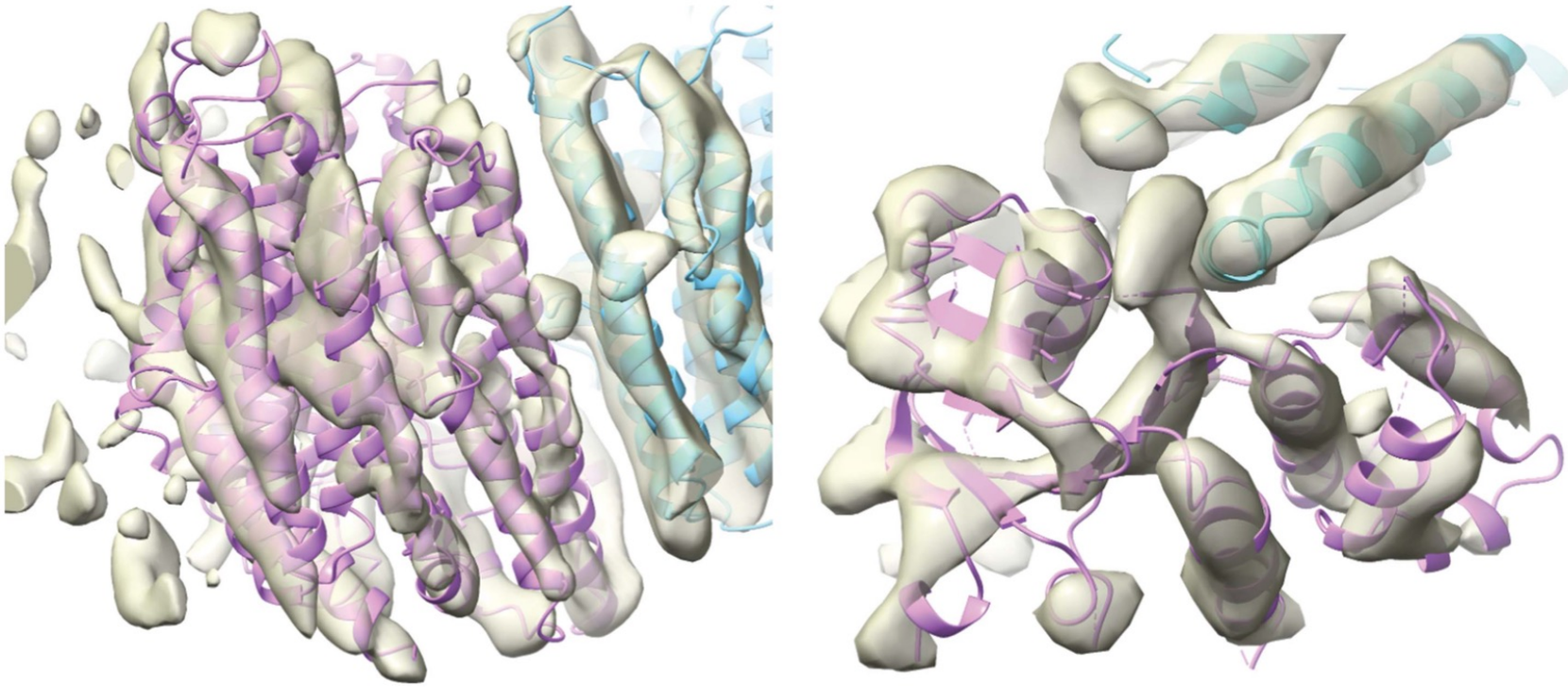
Tom Terwilliger

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)

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



Wednesday, August 23rd

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

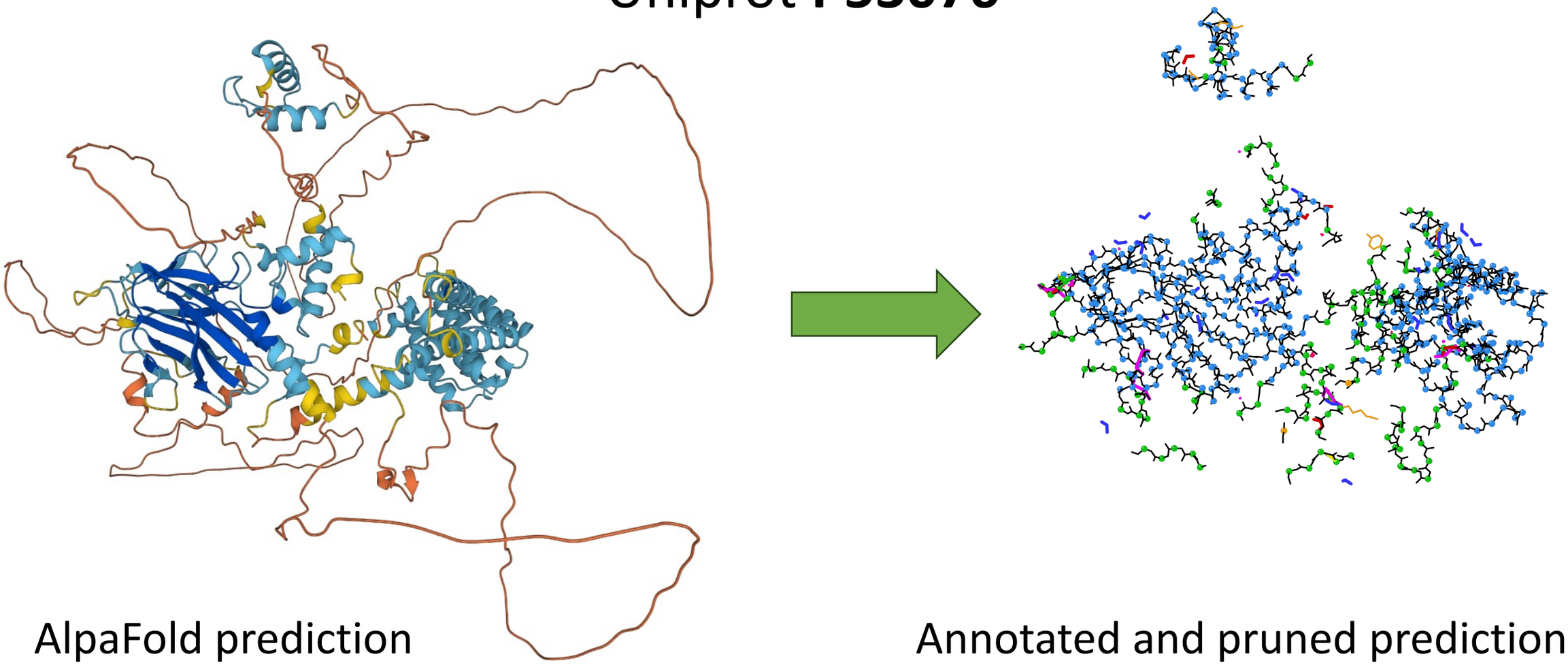
Read RJ, Millán C, McCoy AJ, Terwilliger TC. Likelihood-based signal and noise analysis for docking of models into cryo-EM maps. Acta Crystallogr D Struct Biol. 2023 Apr 1;79(Pt 4):271–80.

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**



AlpaFold prediction

Annotated and pruned prediction

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

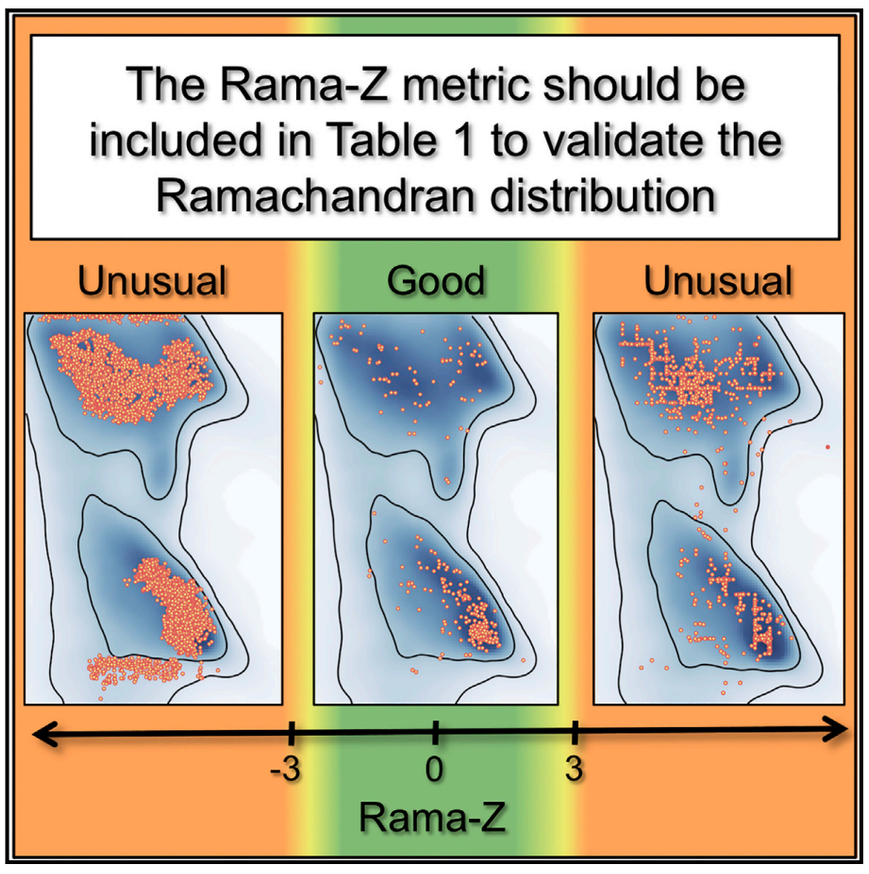
Global Ramachandran Score

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 reimplementation in Phenix and PDB-REDO and showcase its utility. They advocate including it in the validation reports provided by the Protein Data Bank.

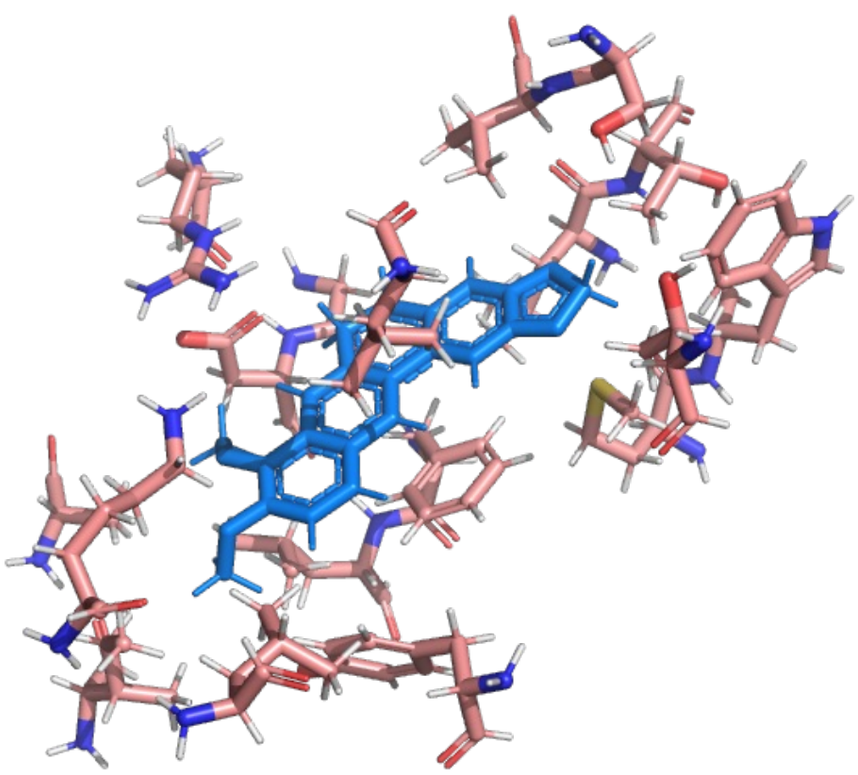
Structure, **28**. 1249-1258.

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.



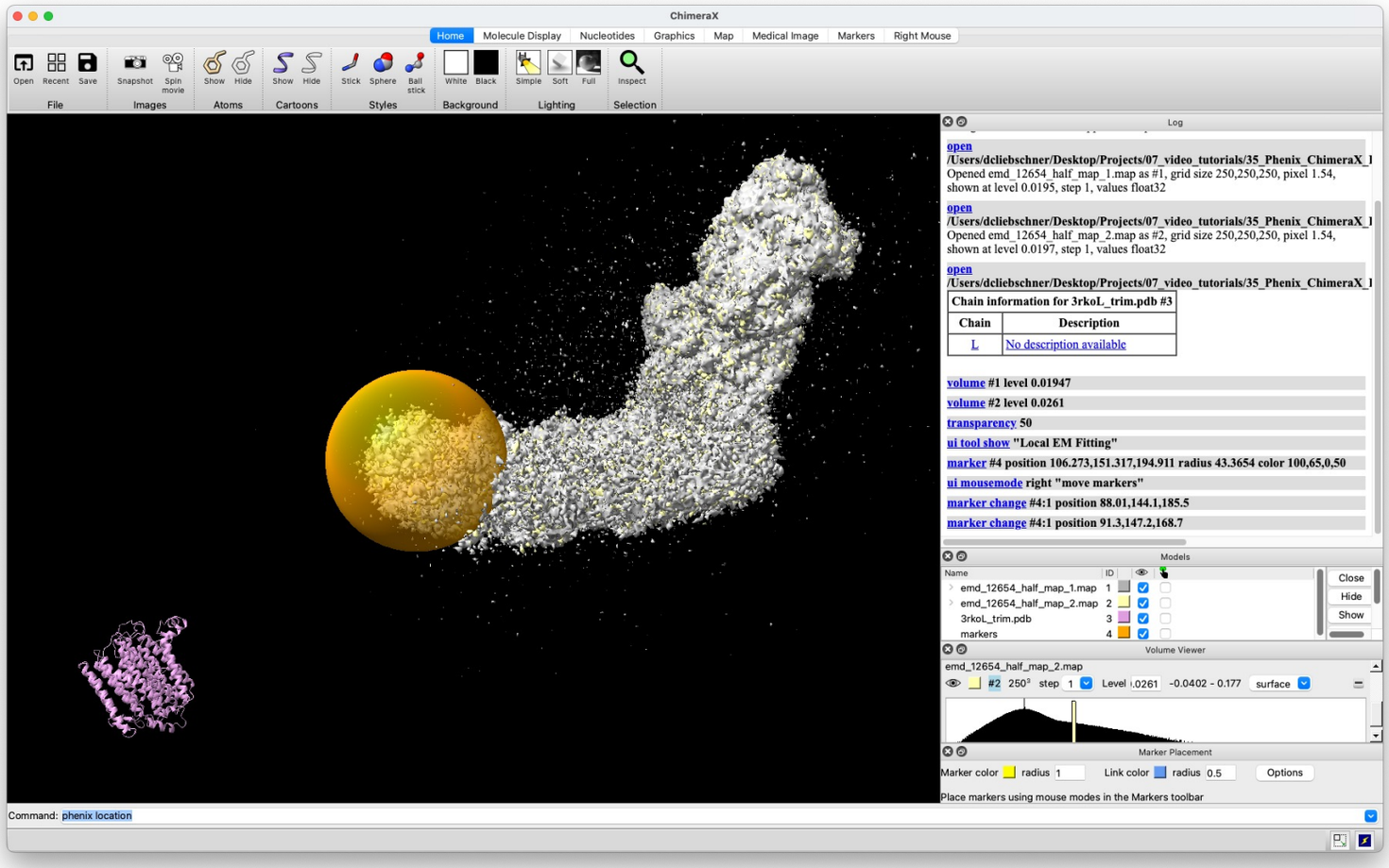
BER in 3vw2

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)

Liebschner D., Moriarty, N.W., Poon, B. K. & Adams, P. D., In situ ligand restraints from quantum-mechanical methods. *Acta Cryst. D*. **79**, 100–110, (2023)

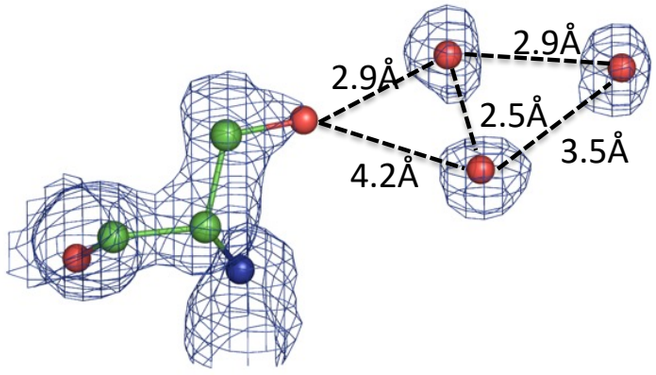
Run Phenix tools through ChimeraX

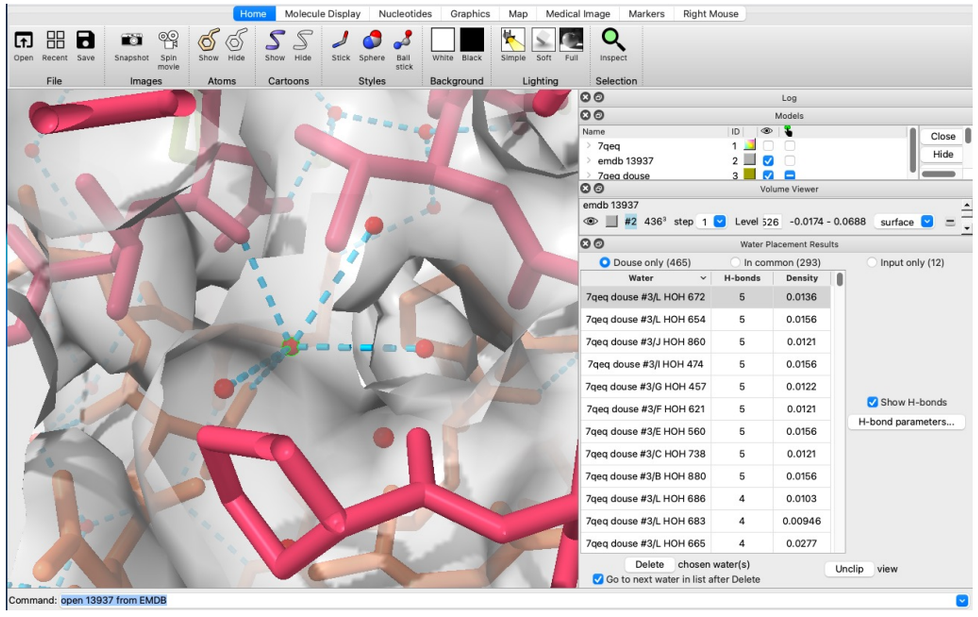


Local EM fitting
(EM placement)

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 Valdiation of low-pLDDT regions in AF2 predictions* (A011)

Monday, August 28th

- 9:00 am, Airlie McCoy, Trekkin' 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

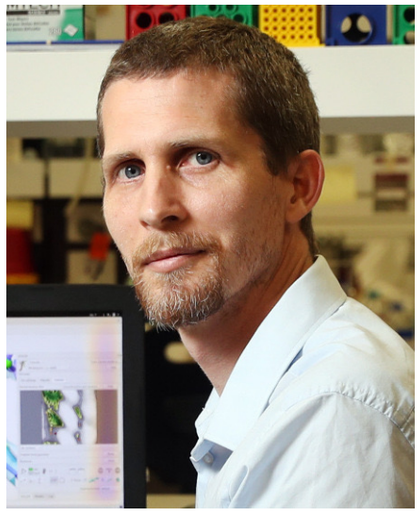
Funding
- NIH/NIGMS: P01GM063210, P50GM062412, P01GM064692, R01GM071939
- PHENIX Industrial Consortium
- Lawrence Berkeley Laboratory

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

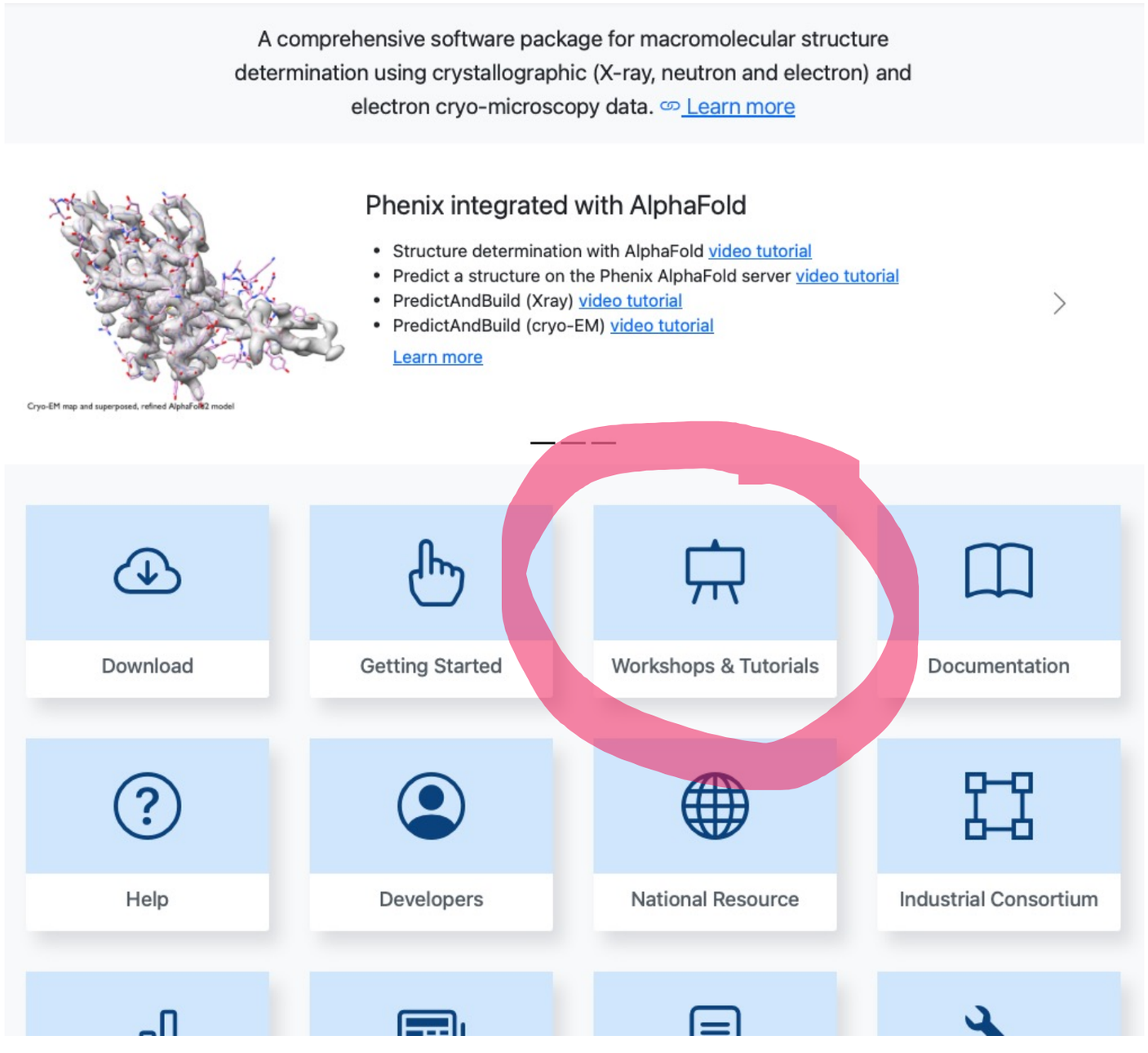
Christopher Williams

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



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 IUCr 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 Xtriage (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)

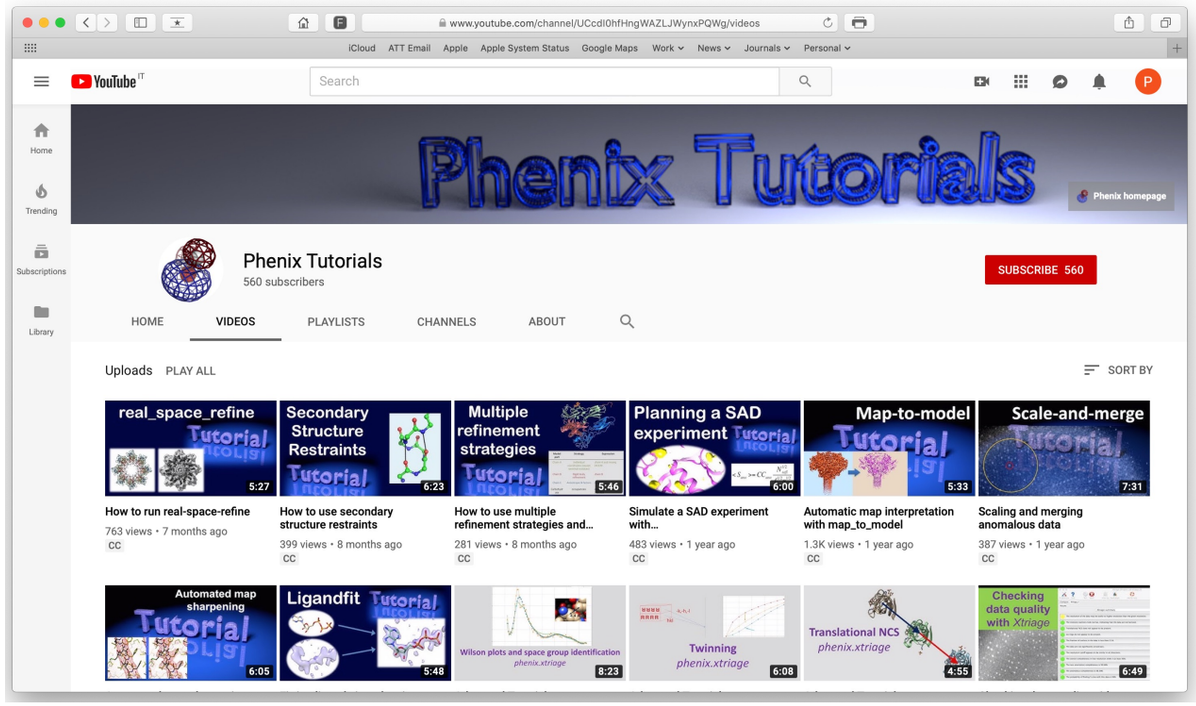
Phenix workshop 04/2021 - cryo-EM (Virtual)

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



Wrap up

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

https://forms.gle/1my2D3fssCye4sVr8

Program

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