

Agenda

Start	Title	Presenter
9:00 AM	PHENIX Overview	Paul Adams
9:15 AM	Cryo-EM Automated map improvement & model building	Paul Adams
10:15 AM	<i>Break and set-up of PHENIX on computers</i>	
10:45 AM	Cryo-EM structure refinement in PHENIX	Pavel Afonine
11:45 PM	Model Validation	Jane Richardson
12:30 PM	<i>Lunch</i>	
1:30 PM	Tutorial – Cryo-EM map improvement and model building	Paul Adams
2:30 PM	<i>Break</i>	
3:00 PM	Tutorial – Cryo-EM Refinement	Pavel Afonine
4:00 PM	Tutorial – Cryo-EM Validation	Jane Richardson/ Pavel Afonine
4:45 PM	<i>Workshop Survey and wrap-up</i>	

Phenix Introduction

*ACA Meeting Cryo-EM Workshop
July 2019*

Paul Adams

Lawrence Berkeley Laboratory and
Department of Bioengineering UC Berkeley



The Phenix Project

Lawrence Berkeley Laboratory

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



Los Alamos National Laboratory New Mexico Consortium Baylor College of Medicine

Tom Terwilliger, Li-Wei Hung,
Matt Baker



Randy Read, Airlie McCoy,
Tristan Croll, Rob Oeffner,
Kaushik Hatti, Massimo
Sammito, Duncan Stockwell

Cambridge University



Duke University

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

Adams PD et al., PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Cryst.* 2010, **D66**:213-221.



An NIH/NIGMS funded
Program Project

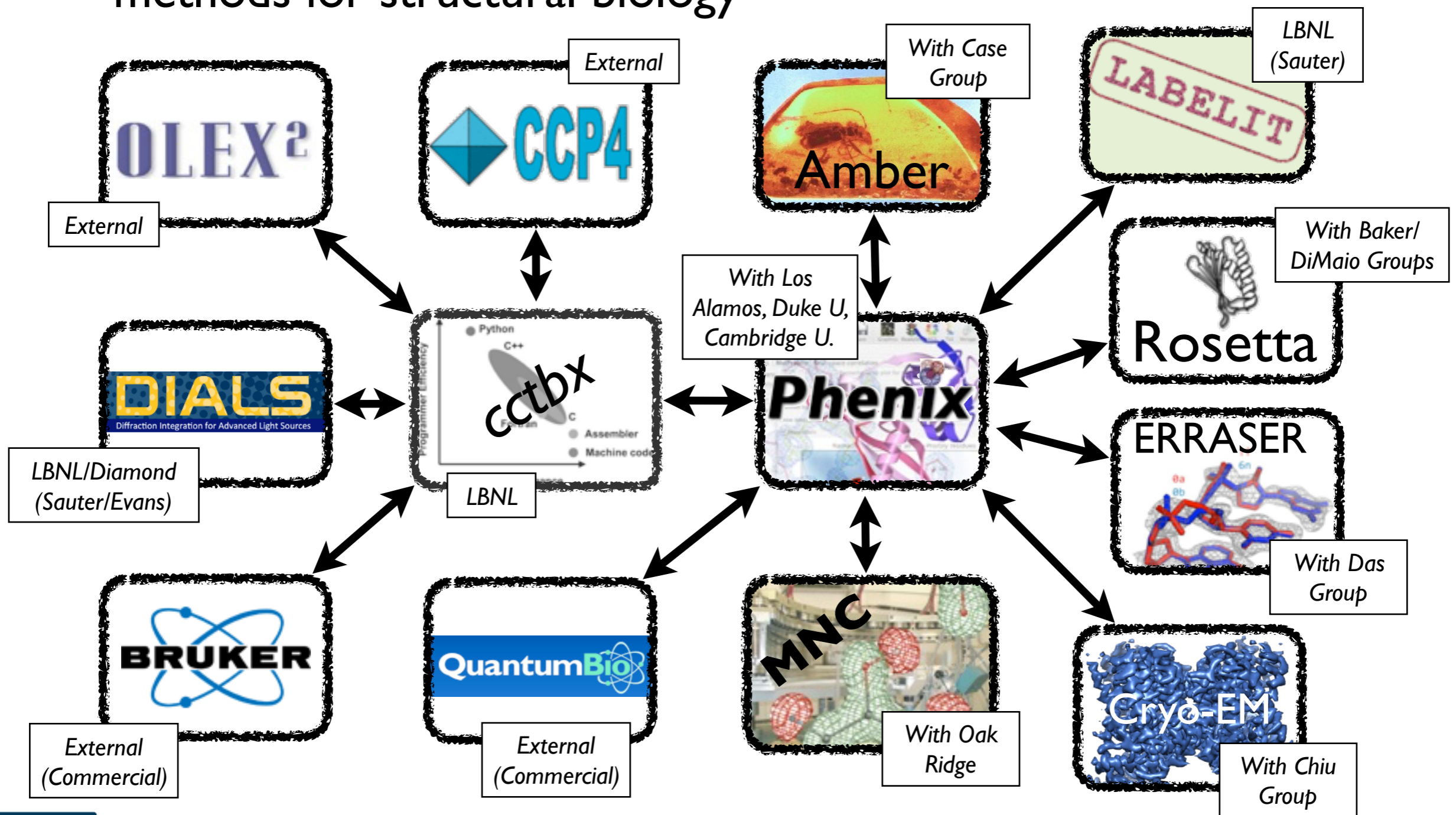


Phenix



Phenix - a Structural Biology Hub

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



Phenix

What is PHENIX?

- Package for automated structure solution
- 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 Hierarchical Environment for Integrated Xtallography

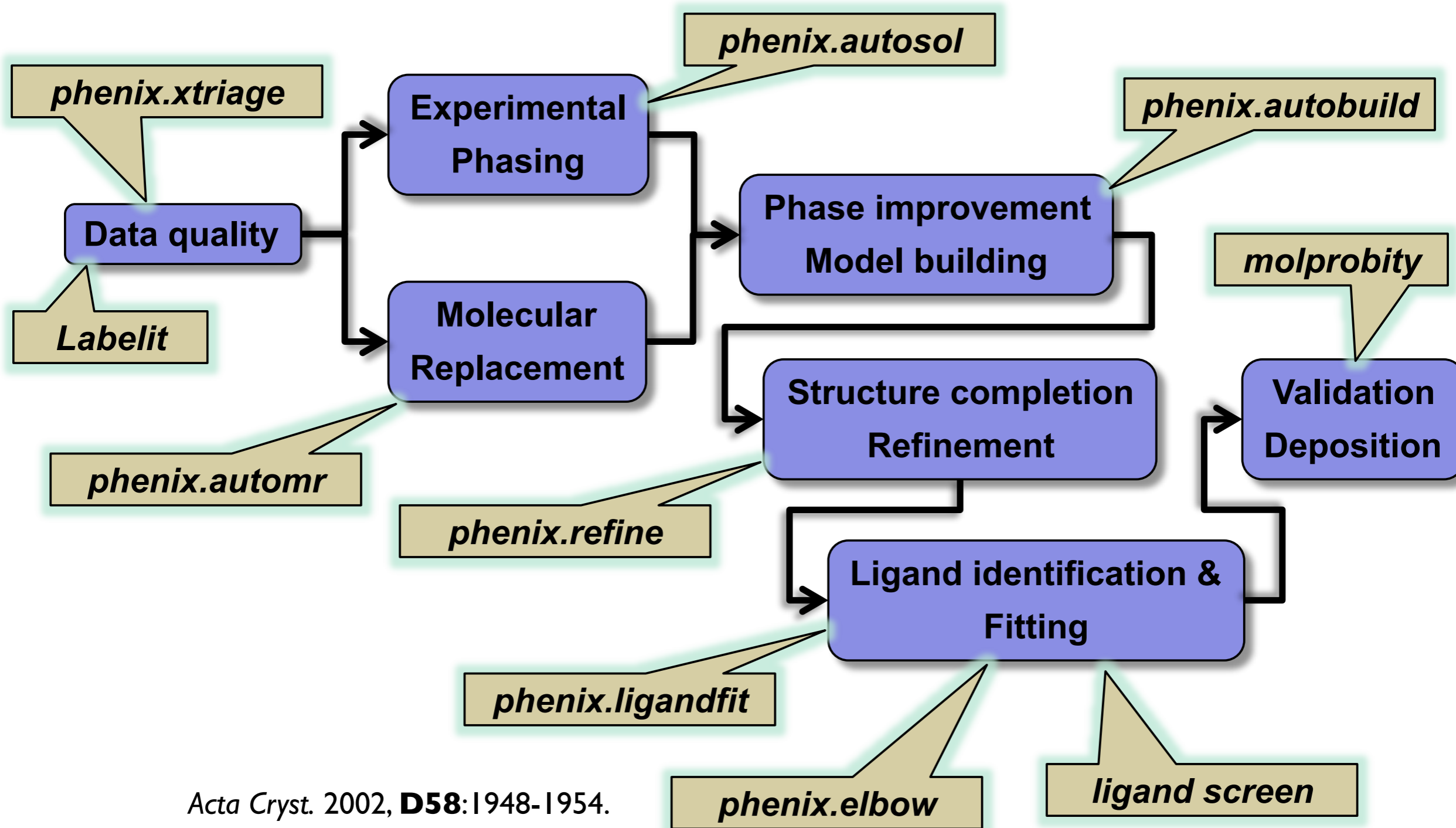


Key Features

- 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



Automation of Structure Solution



Acta Cryst. 2002, **D58**:1948-1954.

J. Appl. Cryst. 2002, **35**:126-136.

Acta Cryst. 2010, **D66**: 213-221

Phenix



Command Line Tools

- **Data validation**

- `phenix.xtriage porin_fp.mtz`

- **Automated structure solution**

- `phenix.autosol data=peak.sca seq_file=nsf-d2.seq`

- **Automated model building**

- `phenix.autobuild data=scale.mtz model=mr.pdb
seq_file=correct.seq`

- **Automated ligand fitting**

- `phenix.ligandfit data=nsf-d2.mtz model=noligand.pdb
ligand=atp.pdb`

- **Structure refinement**

- `phenix.refine nsf-d2.mtz nsf.pdb`

- **Building ligand coordinates and restraints**

- `phenix.elbow --smiles="C12CC3CC(C2)CC(C1)C3"`

Phenix GUI

- Central GUI to view job control and launch new jobs

The screenshot displays the Phenix GUI interface. At the top, there is a menu bar with icons for Quit, Preferences, Help, New project, Project settings, Job history, Citations, Reload last job, Coot, PyMOL, and KING. Below the menu bar is a text prompt: "Click or drag-and-drop files onto a program to launch it. To switch to a project, click the 'Choose this project' button."

The main interface is divided into two main sections. On the left is a table titled "Projects" with columns for ID, Last modified, # of jobs, and R-free. On the right is a vertical list of tool categories and their descriptions.

ID	Last modified	# of jobs	R-free
✓ 1016B	Aug 17 2010 11:51 ...	19	0.3665
1BOV	Jul 05 2010 02:33 PM	2	None
2hr0	Sep 19 2009 01:16 ...	2	None
3g5u	Nov 14 2009 03:14 ...	1	None
3lna	May 18 2010 06:45 ...	5	0.3901
actin-2010	May 31 2010 06:18 ...	0	None
calmodulin	None	0	None
chennai-a2u-gl ...	Jan 19 2010 01:37 AM	6	0.2547
chennai_p9_sad	Mar 03 2010 07:18 ...	4	0.3157
ggr-2010	Mar 16 2010 03:50 ...	0	None
gui	None	0	None
haemoglobin	Mar 27 2010 09:34 ...	6	0.2977
horseHB	Oct 26 2009 08:57 ...	2	0.5026
insulin	Nov 15 2009 11:33 ...	1	0.2039
jason-kim	Jul 05 2010 05:12 PM	25	0.4706
lysozyme	Oct 24 2009 09:25 ...	3	0.3336
lysozyme-mono ...	Mar 08 2010 12:33 ...	6	0.5638
lysozyme_test	Nov 15 2009 11:11 ...	1	0.2329
mmcpn-compari ...	Aug 03 2010 04:15 ...	3	None
mmcpn_dec_2009	Dec 12 2009 09:27 ...	9	0.2624
nsf-ligand	Mar 06 2010 08:56 ...	1	0.3002
p9	Nov 26 2009 11:16 ...	11	0.2612
p9-sad_pdadams	Jun 15 2010 09:58 AM	3	0.2473
porin-twin	Apr 30 2010 11:32 ...	1	None
porin-twin_pdad	Jun 09 2010 03:45 PM	3	0.1481

Below the table are buttons for "Switch project" and "Delete project".

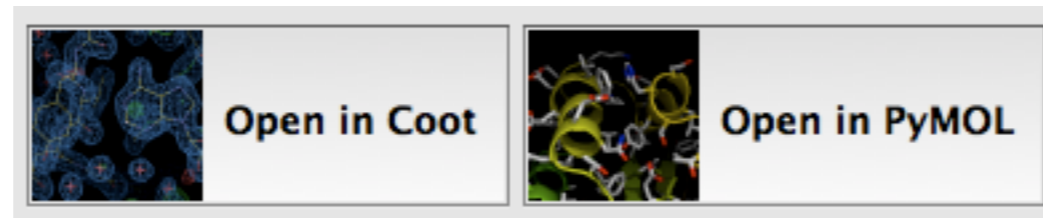
The right-hand side of the interface lists various tools under different categories:

- Reflection tools**
- Model tools**
- Experimental phasing**
 - AutoSol**: Automated experimental phasing with model-building
 - Phaser-EP**: Maximum-likelihood SAD experimental phasing
 - Density modification**: Simple interface for running density modification only using AutoBuild and RESOLVE
- Molecular replacement**
- Model building**
 - AutoBuild**: Automated model-building and refinement
 - Find Helices and Strands**: Fast chain tracing
 - Fit Loops**: Fast placement of missing loops in electron density
- Refinement**
 - phenix.refine**: Automated X-ray and neutron refinement
 - ReadySet**: Utility for preparing PDB files for refinement – automatically generate restraints and add hydrogens
- Maps**

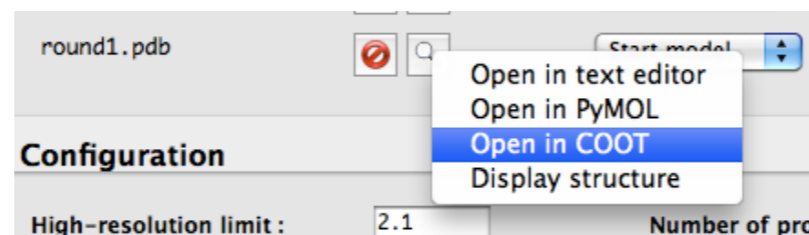
At the bottom, there is an "Output directory:" field with the path "/Users/pdadams/Work/Structures/1016B" and a "Browse..." button. The status bar at the bottom shows "PHENIX version 1.6.4-486" and "Project: 1016B".

Coot/PyMOL 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]*

Recent Developments

- Automated structure solution with weak anomalous data
- Translational NCS corrections in SAD phasing

- MR_Rosetta and morphing for rescuing poor MR solutions
- Translational NCS corrections in MR

- New Rosetta methods for RNA rebuilding (ERRASER)

- Automated ion placement in refinement
- Automated ligand pipeline

- DIALS included in Phenix distribution

- Support for mmCIF format (for structure deposition)
- Video Tutorials

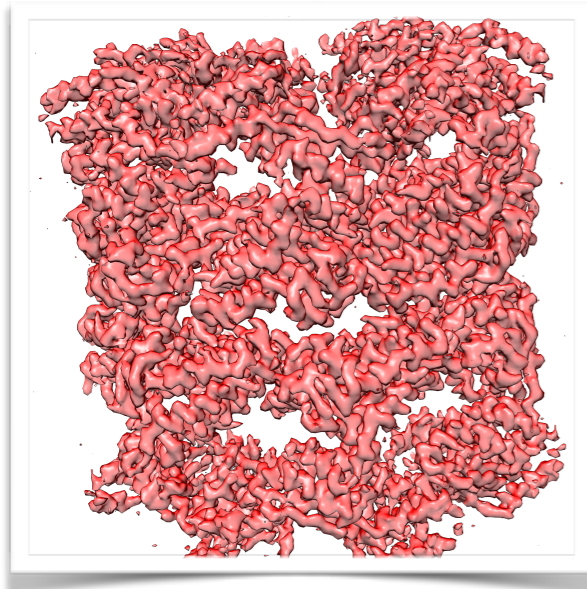
- Low resolution refinement algorithms:
 - Rosetta refinement
 - Reference model
 - Torsion NCS
 - Structure restraints

- New model validation metrics

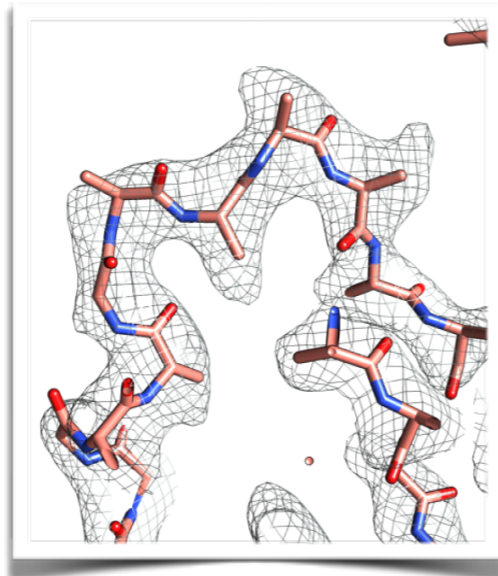
- New hydrogen validation tools
- New riding hydrogen model

- Feature Enhanced Maps to improve interpretability
- Polder maps for better ligand density
- Ensemble refinement to understand dynamics and structural variability

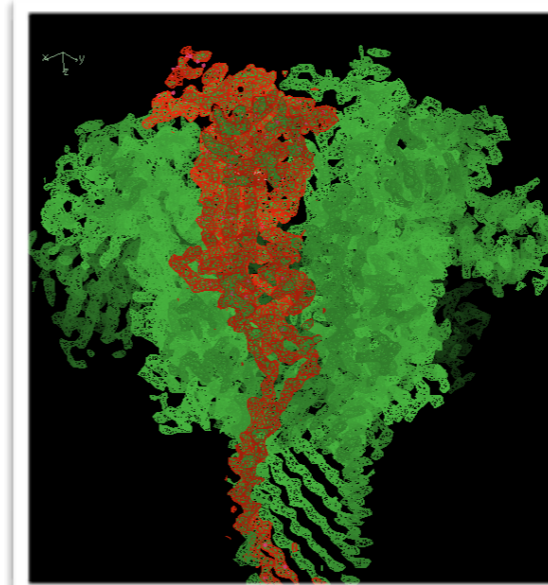
New Tools for Cryo-EM in Phenix



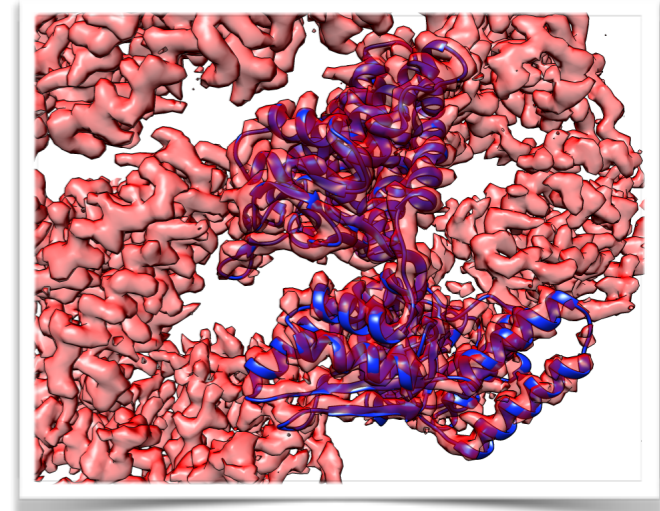
Symmetry from a map



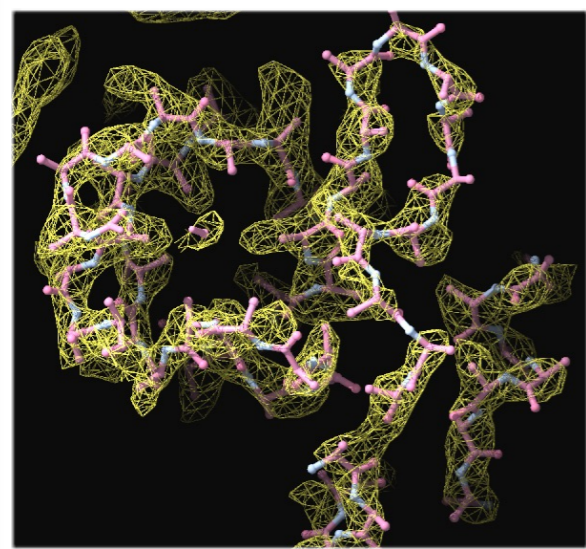
Automated map sharpening



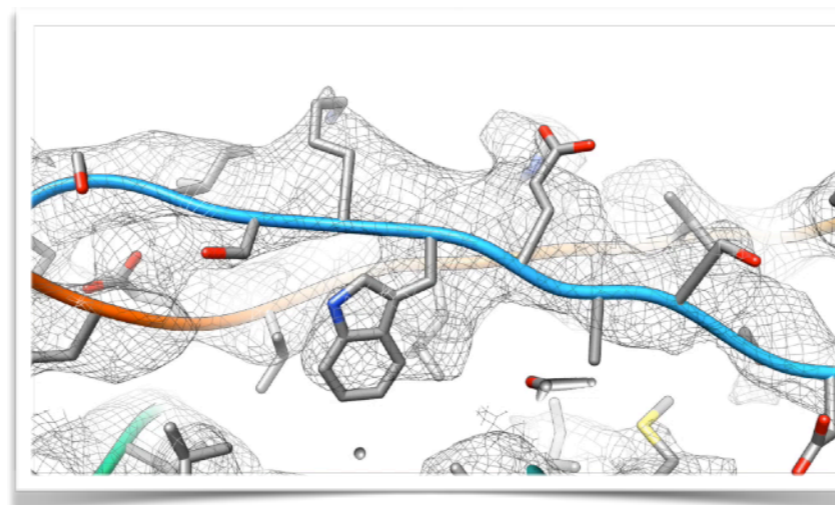
Map segmentation



Rigid model docking



Automated model building



Real space refinement

Model		Ramachandran	
MolProbity		Outliers (%)	0.00 (Goal: < 0.2%)
MolProbity score	1.72	Allowed (%)	6.45
Clash score	5.44	Favored (%)	93.55 (Goal: > 98%)
Rotamer outliers (%)	0.00 (Goal: < 1%)		
CB outliers	0 (Goal: 0)		

CaBLAM		Peptide Plane	
Outliers (%)	3.88 (Goal: <= 1%)	cis-proline (%)	0.00
Disfavored (%)	8.96 (Goal: <= 5%)	twisted proline (%)	0.00
Cis outliers (%)	1.13 (Goal: <= 0.5%)	cis-general (%)	0.00
		twisted general (%)	0.00

Model and map validation

Challenges

- Low resolution structure solution and refinement
- Structure completion
 - Automated identification, fitting and refinement of ligands, metals, ions, and water
 - Identification, fitting and refinement of discrete disorder (multiple conformations)
 - Representing other forms of disorder
- Automated parameterization of models in refinement
 - ADPs, TLS groups, NCS, hydrogens
- Handling different kinds of twinning and integrating it into the whole structure solution process
- Automated understanding of chemistry

PHENIX Availability

- phenix-online.org
- Supported on:
 - Linux (RedHat, Fedora)
 - Mac OSX
 - Windows
- Extensive documentation
- Nightly builds:

The screenshot shows the Phenix website with the following content:

- Phenix** Python-based Hierarchical Environment for Integrated Xtallography
- Documentation: (HTML) FAQ
- Search Documentation
- What's New
- PHENIX is a software suite for the automated determination of molecular structures using X-ray crystallography and other methods.
- Download the latest official release (1.15.2) [First request download password]
- Download the latest nightly build or prerelease
- Help: Email Us Report a Bug List Archives Subscribe to Mailing List Search Phenix Mailing List
- Using PHENIX (release 1.15.2):**
 - The PHENIX Graphical User Interface
 - Assessing data quality with phenix.xtriage
 - Automated structure solution with AutoSol
 - Automated molecular replacement with Phaser-MR
 - Automated model building and rebuilding with AutoBuild
 - Automated ligand fitting with LigandFit
 - Structure refinement with phenix.refine
 - Generation of ligand coordinates and restraints with eLBOW
- The PHENIX system also includes SOLVE/RESOLVE, Phaser, the CCI Applications (phenix.xtriage, phenix.refine, elbow and many more), components from MolProbity, and the Computational Crystallography Toolbox in a Python framework.
- Citing PHENIX:** PHENIX: a comprehensive Python-based system for macromolecular structure solution. P. D. Adams, P. V. Afonine, G. Bunkóczy, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L.-W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner, R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart. *Acta Cryst. D66*, 213-221 (2010).
- Funding for PHENIX: NIH General Medical Sciences
- The PHENIX system also can be used for neutron crystallography. NIH funding supports the development of this capability

Version	Date	Status	Logs	Info
dev-3512	2019-05-21	successful	ci , intel-windows-x86_64 , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64	docs ; changelog
dev-3500	2019-05-09	successful	ci , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64 , intel-windows-x86_64	docs ; changelog
dev-3494	2019-05-03	successful	intel-linux-2.6-x86_64-centos6 , ci , mac-intel-osx-x86_64 , intel-windows-x86_64	docs ; changelog
1.15.2-3472	2019-04-11	successful	ci , intel-windows-x86_64 , intel-linux-2.6-x86_64-centos6 , mac-intel-osx-x86_64	Official 1.15.2 release; docs ; changelog



Video Tutorials

www.youtube.com/channel/UCcdl0hfHngWAZLJWynxPQWg/videos

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Phenix Tutorials

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Thumbnail Title	Description	Views	Upload Date
real_space_refine Tutorial	How to run real-space-refine	763 views	7 months ago
Secondary Structure Restraints Tutorial	How to use secondary structure restraints	399 views	8 months ago
Multiple refinement strategies Tutorial	How to use multiple refinement strategies and...	281 views	8 months ago
Planning a SAD experiment Tutorial	Simulate a SAD experiment with...	483 views	1 year ago
Map-to-model Tutorial	Automatic map interpretation with map_to_model	1.3K views	1 year ago
Scale-and-merge Tutorial	Scaling and merging anomalous data	387 views	1 year ago
Automated map sharpening Tutorial			
Ligandfit Tutorial			
Wilson plots and space group identification phenix.xtrriage			
Twinning phenix.xtrriage			
Translational NCS phenix.xtrriage			
Checking data quality with Xtrriage			

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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
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David Abrahams
PHENIX Testers & Users

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