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COMPUTATIONAL CRYSTALLOGRAPHY INITIATIVE

# Automated Crystallographic Structure Solution in PHENIX



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PHYSICAL BIOSCIENCES DIVISION

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# The PHENIX project

- **PHENIX is a (relatively) new package for automated structure solution** that incorporates handling of both: X-ray and neutron data
- PHENIX is not a pipe-line made of existing programs, but a highly integrated software
- Library based development (Python, C++) and new or re-designed algorithms
- Designed to be used by both novices and experienced users
- Long-term development and support
- Large experience of crystallographic software and methods development (previous experience of CNS development – Paul Adams and Ralf Grosse-Kunsteve)

# PHENIX

## Lawrence Berkeley National Laboratory

**Paul Adams**, Pavel Afonine, Nat Echols,  
Jeff Headd, Ralf Grosse-Kunstleve, Nigel  
Moriarty, Nicholas Sauter, Peter Zwart

## Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung

## Cambridge University

Randy Read, Airlie McCoy, Laurent  
Storoni, Gabor Bunkoczi, Robert Oeffner

## Duke University

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

## Funding

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

***Paul Adams – project director***

# PHENIX and Neutron crystallography

## Macromolecular Neutron Crystallography Consortium (MNC)



Los Alamos National Lab

*Paul Langan, Marat Mustyakimov, Benno Schoenborn*



Lawrence Berkeley National Lab (LBNL)

*Paul Adams, Pavel Afonine*

<http://mnc.lanl.gov/>

## Some milestones (publications)

- 2002:
  - Grosse-Kunstleve RW et al. **The Computational Crystallography Toolbox: crystallographic algorithms in a reusable software framework.**
  - Grosse-Kunstleve RW, Adams PD. **On the handling of atomic anisotropic displacement parameters.**
  - Adams PD et al. **PHENIX: building new software for automated crystallographic structure determination.**
- 2003:
  - Grosse-Kunstleve RW et al. **Substructure search procedures for macromolecular structures.**
  - Abrahams D et al. **Building Hybrid Systems with Boost.Python.**
- 2004:
  - Adams PD et al. **Recent developments in the PHENIX software for automated crystallographic structure determination.**
  - Grosse-Kunstleve RW et al. **cctbx news: Geometry restraints and other new features.**
- 2005:
  - McCoy AJ et al. **Likelihood-enhanced fast translation functions**
  - Afonine PV et al. **A robust bulk-solvent correction and anisotropic scaling procedure**
  - Afonine PV et al. **The Phenix refinement framework.**
  - Zwart PH et al. **Xtrriage and Fest: automatic assessment of X-ray data and substructure structure factor estimation.**

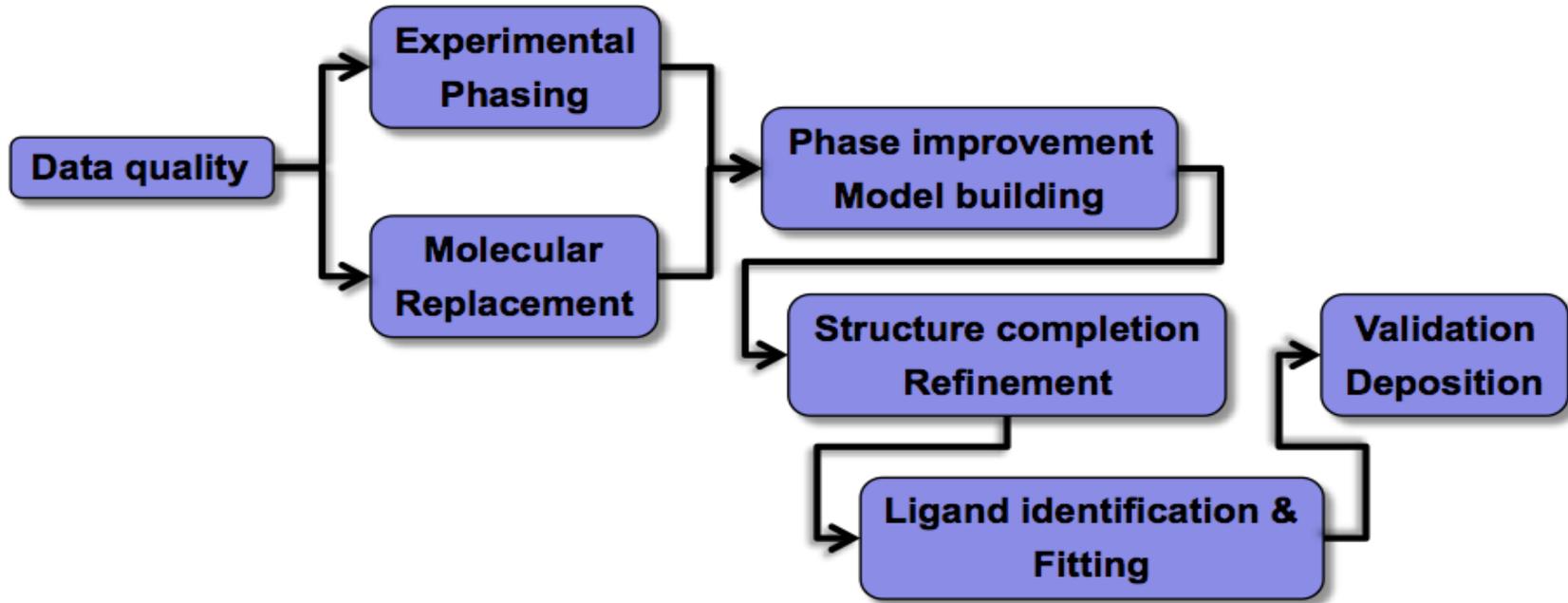
## Some milestones (publications)

- 2007:
  - Terwilliger TC et al. **Interpretation of ensembles created by multiple iterative rebuilding of macromolecular models.**
  - McCoy AJ et al. **Phaser crystallographic software.**
  - Afonine PV et al. **On macromolecular refinement at subatomic resolution with interatomic scatterers.**
- 2008
  - Terwilliger TC et al. **Iterative model building, structure refinement and density modification with the PHENIX AutoBuild wizard**
  - Terwilliger TC et al. **Iterative-build OMIT maps: map improvement by iterative model building and refinement without model bias.**
- 2009:
  - Grosse-Kunstleve RW et al. **Experience converting a large Fortran-77 program to C++**
  - Grosse-Kunstleve RW et al. **Torsion Angle Refinement and Dynamics as a Tool to Aid Crystallographic Structure Determination**
  - Moriarty NW et al. **electronic Ligand Builder and Optimization Workbench (eLBOW): a tool for ligand coordinate and restraint generation**
  - Afonine PV et al. **Automatic multiple-zone rigid-body refinement with a large convergence radius.**
  - Terwilliger TC et al. **Decision-making in structure solution using Bayesian estimates of map quality: the PHENIX AutoSol wizard.**
  - Urzhumtseva L. et al. **Crystallographic model quality at a glance.**

## Some milestone publications

- 2010
  - Afonine PV et al. **Joint X-ray and neutron refinement with phenix.refine**
  - Grosse-Kunstleve RW et al. **cctbx PDB handling tools**
  - Afonine PV et al. **phenix.model\_vs\_data: a high-level tool for the calculation of crystallographic model and data statistics**
  - Adams PD et al. **PHENIX: a comprehensive Python-based system for macromolecular structure solution**
  - Afonine PV et al. **Atomic Displacement Parameters (ADPs), their parameterization and refinement in PHENIX**. Computational Crystallography Newsletter. 1
- 2011
  - Urzhumtsev A, et al. **TLS for dummies**. Computational Crystallography Newsletter. 2.

# Why Automation ?

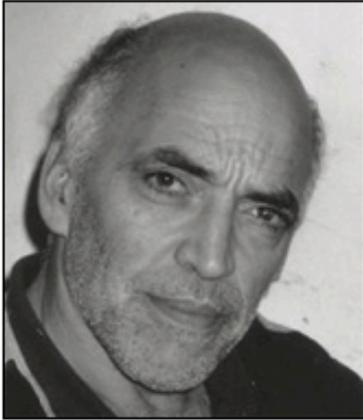


- ✓ Automation can increase efficiency, and reduce human error (especially for non-expert crystallographers)

## Why Automation ?

- Makes difficult cases more feasible for experts
- Routine structure solution cases are accessible to a wider group of structural biologists
- Software can try more possibilities than we are typically willing to bother with
- Multiple trials or use of different parameters can be used to estimate uncertainties
- If a task is modular and automated, you can run it many times...
  - ... checking different space groups, datasets to use
  - ... checking if your model is biasing the map
  - ... checking if you always get the same model
- What is required:
  - Software carrying out individual steps
  - Seamless connection between steps
  - A way to decide what is good
  - Strategies for structure determination and decision-making

## Idea of automation is not new



*“In the field of macromolecular structure determination, AMoRe was the first programme aiming at solving a crystal structure in an automated way.”*

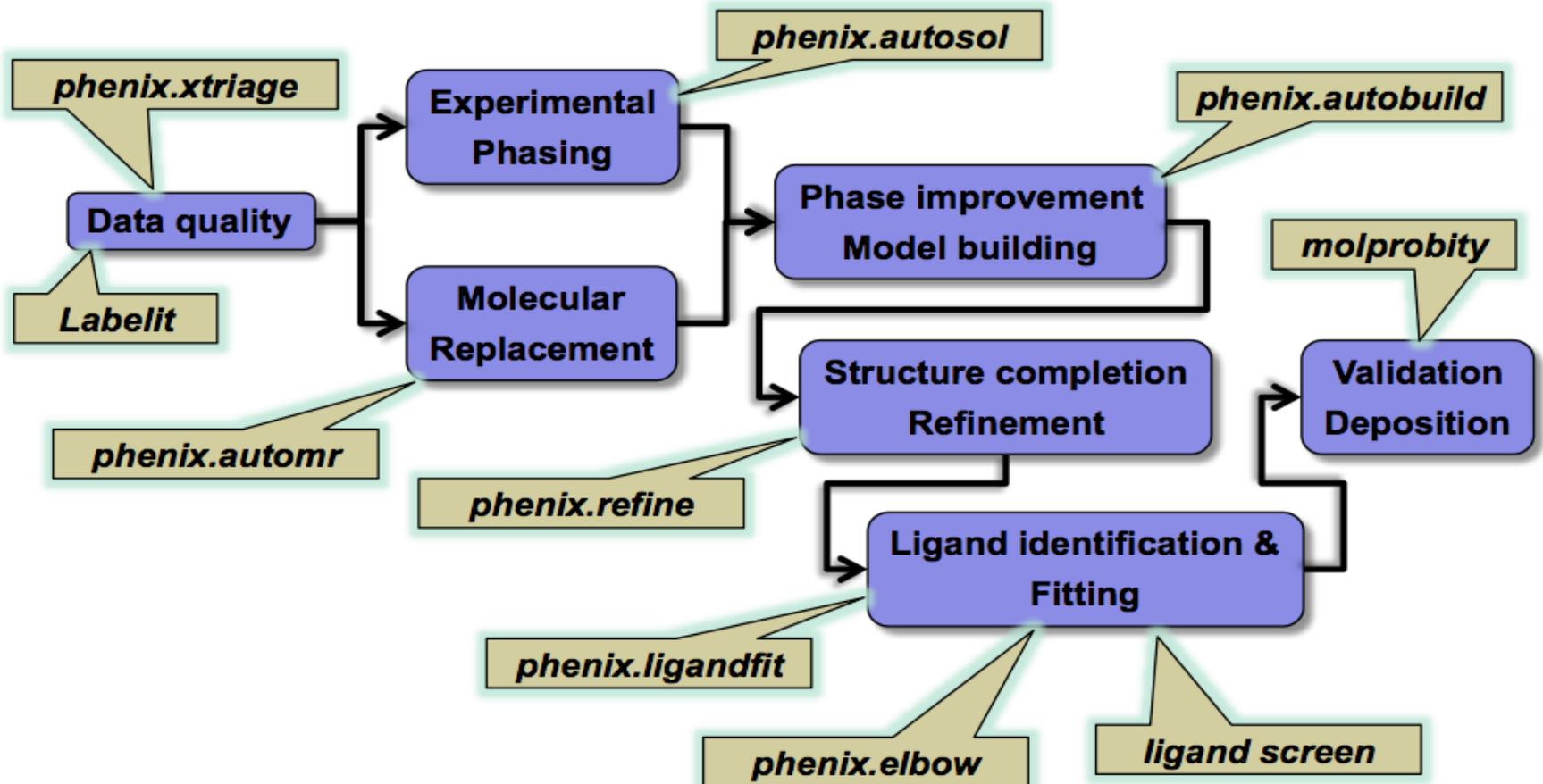
J.Navaza, Acta Cryst. A50, 157-163 (1994)

Source:

<http://www.in-cites.com/papers/JorgeNavaza.html>

# PHENIX: principal tools

Complete set of tools for crystallographic structure determination: from experimental data to PDB deposited structure



## PHENIX: principal tools

- PHENIX programs can be run using:
  - **GUI**: easy for beginners, guided process - less chance of errors
  - **Command line**: convenient for scripting of multiple and large scale tasks
  - Expert developers can run certain tools from their own programs

# GUI examples

PHENIX home

Quit Preferences Help New project Project settings Job history Citations Coot PyMOL

Click or drag-and-drop files onto a program to launch it. To switch to a project, click the "Choose this project" button.

### Projects

ID	Last modified	# of jobs	R-free
rnase	Jul 12 2010 12:14 PM	0	None
✓ industry_MTP	Jul 12 2010 12:17 PM	0	None

### Reflection tools

### Model tools

### Experimental phasing

### Molecular replacement

### Building and refinement

### Maps

### Ligands

### Validation

### Utilities

Switch project Delete project

Output directory : /Users/afonine/Desktop/zz/zz1 Browse...

PHENIX version 1.6.2-432 Project: industry\_MTP

# GUI examples

PHENIX home

Quit Preferences Help New project Project settings Job history Citations Reload last job Coot PyMOL KiNG Fetch PDB

Click or drag-and-drop files onto a program to launch it. To switch to a project, click the "Choose this project" button.

### Projects

ID	Last modified	# of jobs	R-free
rnase-s	Oct 04 2010 03:18 PM	90	0.2201
PknB_L33D_1	Oct 04 2010 03:16 PM	21	0.3575
PknB_L33D_ANP_2	Oct 01 2010 05:41 PM	13	0.2315
FrzS	Sep 30 2010 07:59 ...	21	0.5264
debug	Sep 28 2010 12:16 ...	65	0.1881
beta-blip	Sep 23 2010 05:10 ...	18	None
Rv0577	Sep 21 2010 04:57 ...	9	0.2116
pka-compare_nat	Sep 17 2010 03:18 ...	6	None
✓ p9-sad_nat	Sep 13 2010 03:39 ...	27	0.2418
PknB_D76A	Sep 09 2010 05:02 ...	32	0.2807
nsf-d2-ligand	Sep 03 2010 03:17 ...	20	None
xntest	Jul 15 2010 04:16 PM	3	0.1896

Switch project Delete project

### Reflection tools

- Xtrriage**  
*Analysis of data quality and crystal defects*
- Reflection file editor**  
*Utility for merging and converting reflections*
- Calculate F(model)**  
*Utility for generating structure factors from a PDB file*
- Import CIF structure factors**  
*Convert data deposited in PDB to MTZ format*

### Model tools

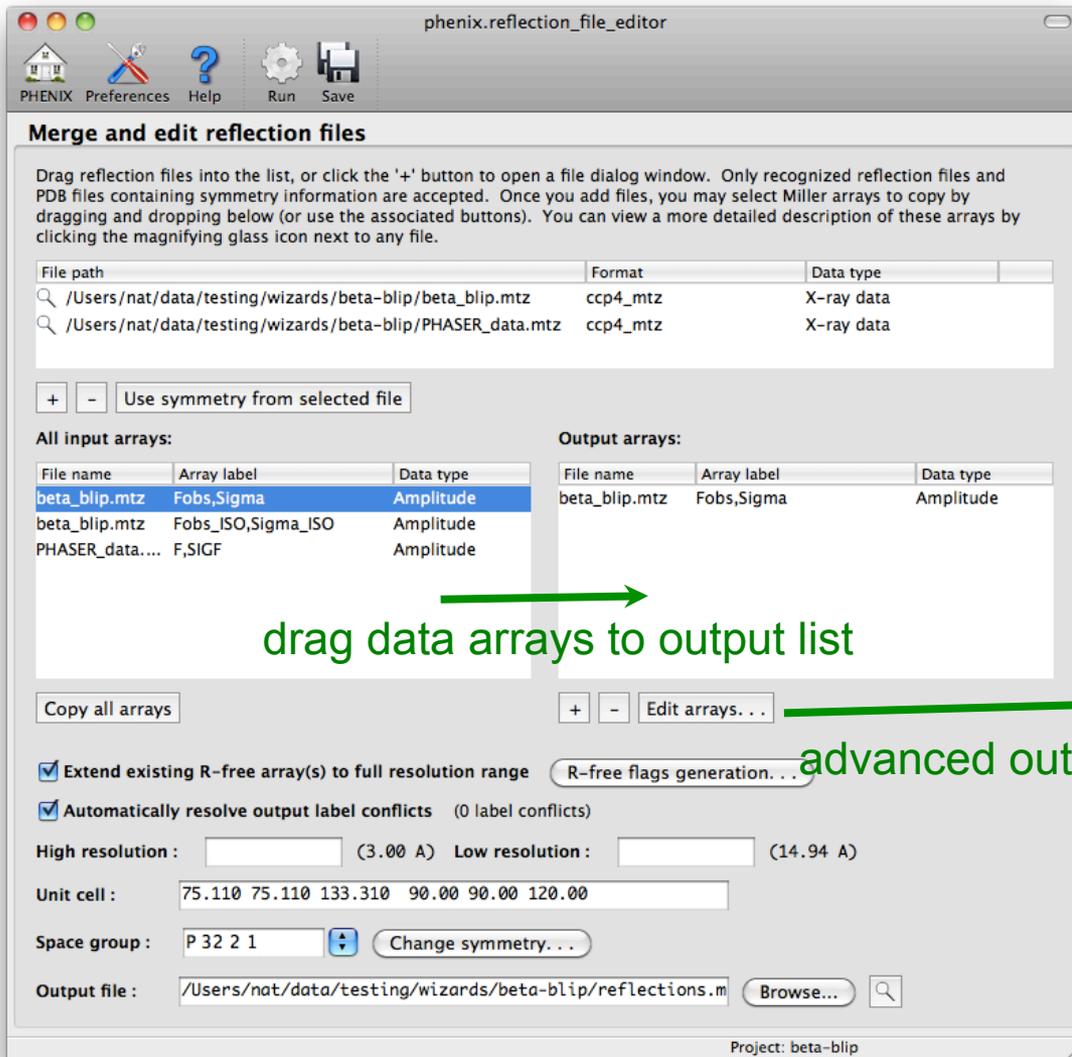
- PDB Tools**  
*Utility for simple modifications of PDB files, including geometry regularization*
- Combine PDB files**  
*Merge a model split across multiple files, with automatic chain renumbering and clash check*
- Superpose PDB files**  
*Simple structure alignment program*
- Find NCS operators**  
*Identify non-crystallographic symmetry in model, heavy-atom sites, or electron density map*
- Apply NCS operators**  
*Transform a molecule by NCS matrices to generate complete structure*

### Experimental phasing

Output directory :

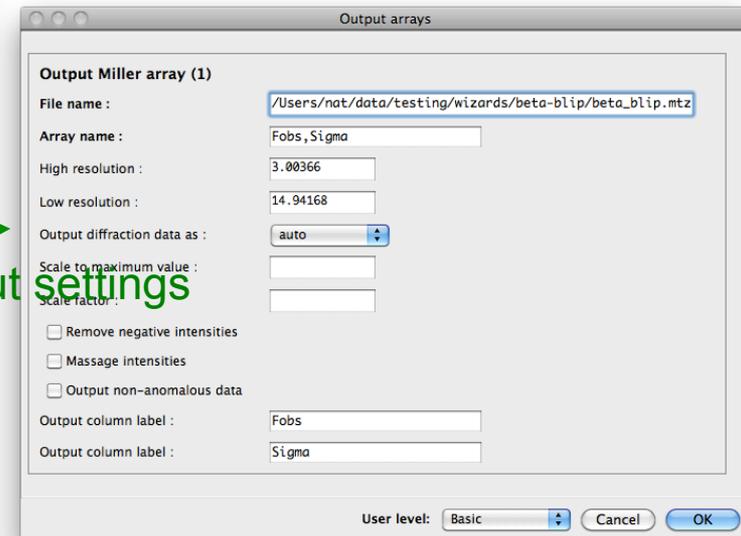
PHENIX version dev-434 Project: p9-sad\_nat

# GUI examples: Reflection file editor



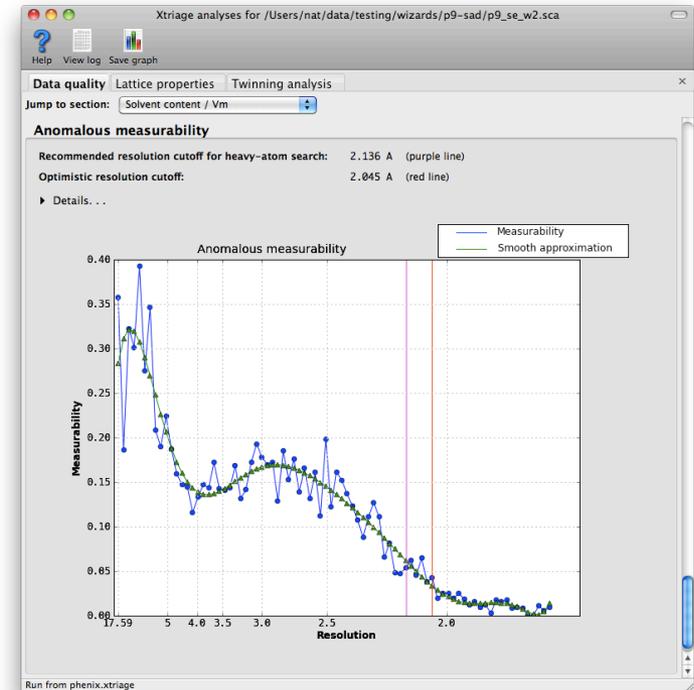
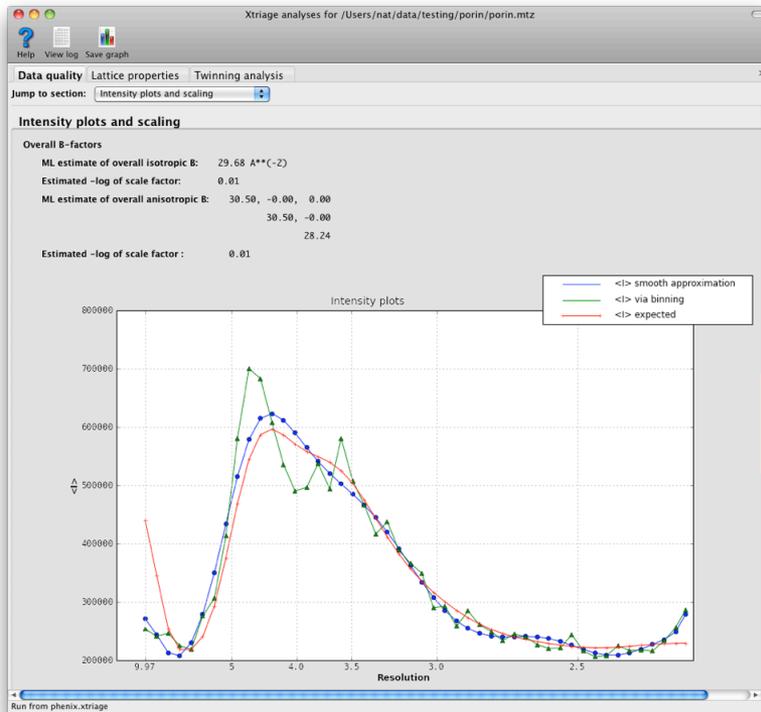
Combine and manipulate reflection files in any format, output as MTZ.

Extend old R-free sets, and generates new sets as thin shells (for refinement in presence of NCS).

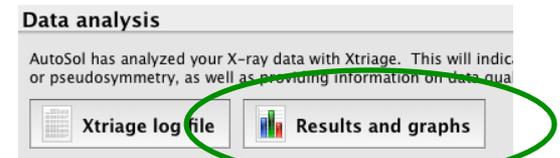


# Data analysis with *phenix.xtriage*

- Analysis of signal-to-noise, data quality, Wilson plot, translational NCS, twinning, symmetry issues, and more



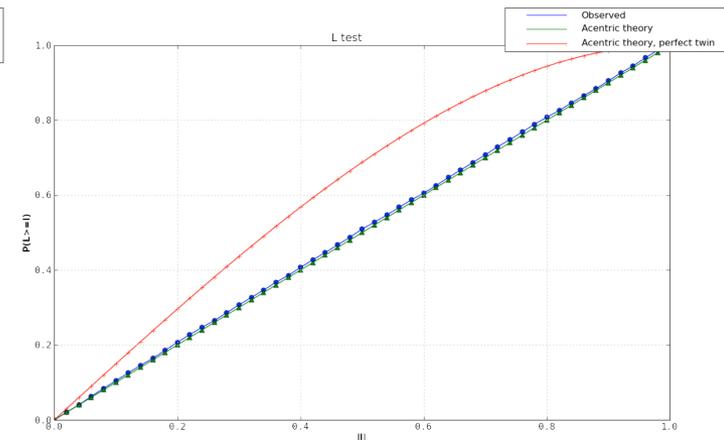
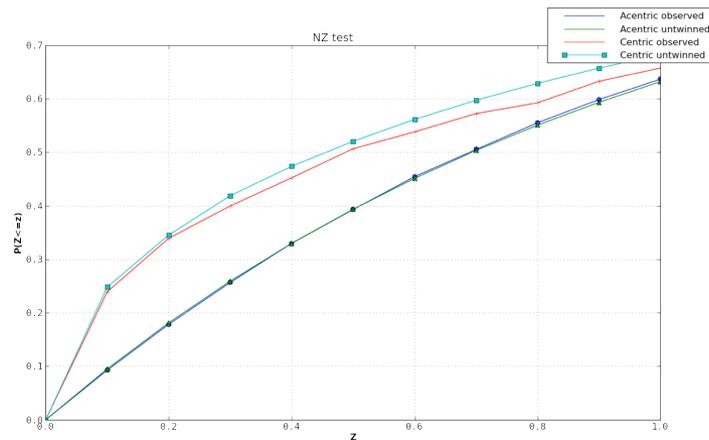
*AutoSol and AutoBuild run Xtriage almost immediately, and results can be viewed from those GUIs. However, it may save time and effort to run Xtriage yourself first.*



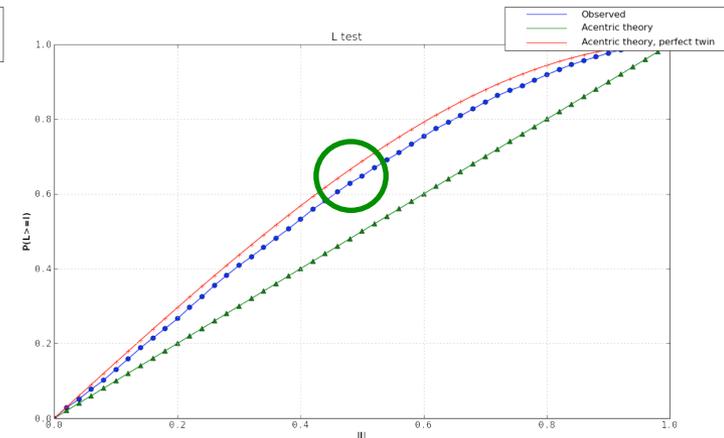
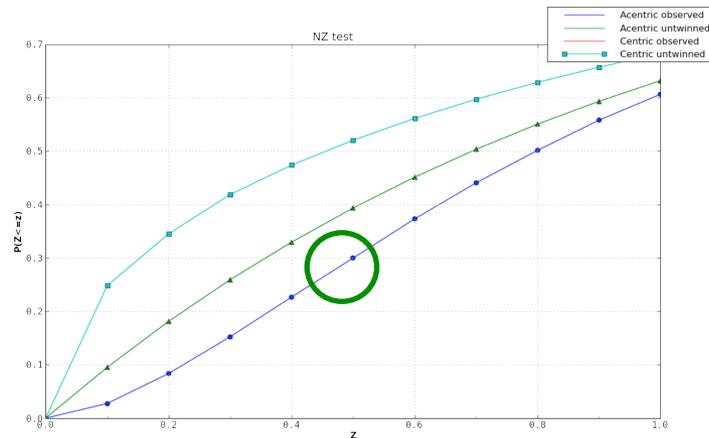
# Identifying twinned structures in Xtriage

- Twinning changes the distribution of intensity values in predictable ways

Good data (p9-sad example): observed intensity distributions are close to expected values



Twinned data (porin-twin example): NZ test curve is sigmoidal, L test curve is shifted upwards



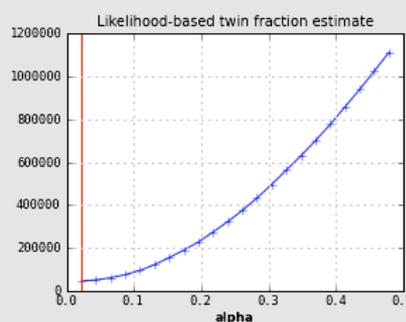
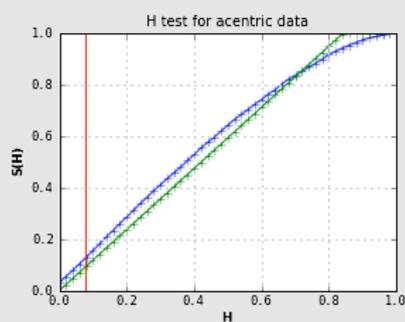
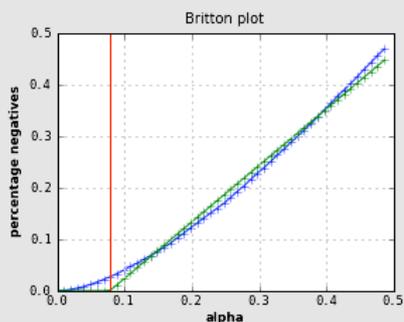
*Intensity distributions can also be affected by pseudotranslation (especially NZ test); make sure you look at all of the evidence for twinning!*

# Identifying twinned structures in Xtrriage

- The twin fraction for all possible twin laws will be estimated; usually one of these is obviously different

$-1/3*h-2/3*k-2/3*l, -2/3*h-1/3*k+2/3*l, -2/3*h+2/3*k-1/3*l$ :

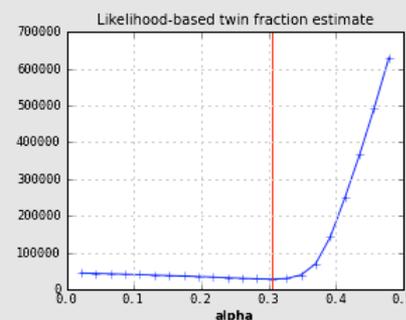
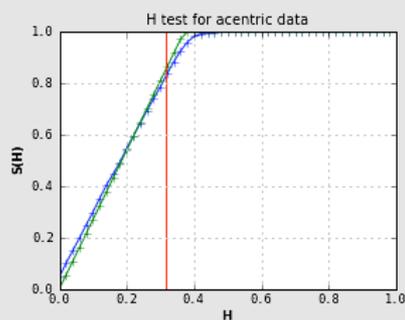
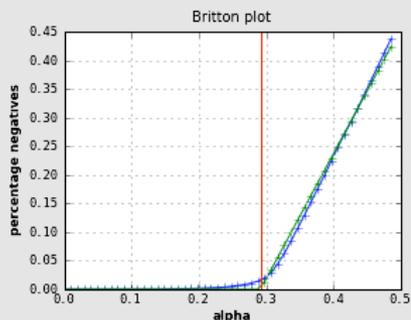
Estimated twin fraction: 0.078 (Britton analyses)  
0.079 (H-test)  
0.022 (Maximum likelihood method)



Show tables

$h,-h-k,-l$ :

Estimated twin fraction: 0.292 (Britton analyses)  
0.315 (H-test)  
0.304 (Maximum likelihood method)



Show tables

Two twin laws from the porin-twin example are shown; in this case  $h,-h-k,-l$  is the actual twin law for this crystal. This can be used in phenix.refine, which will determine the true twin fraction based on the refined model.



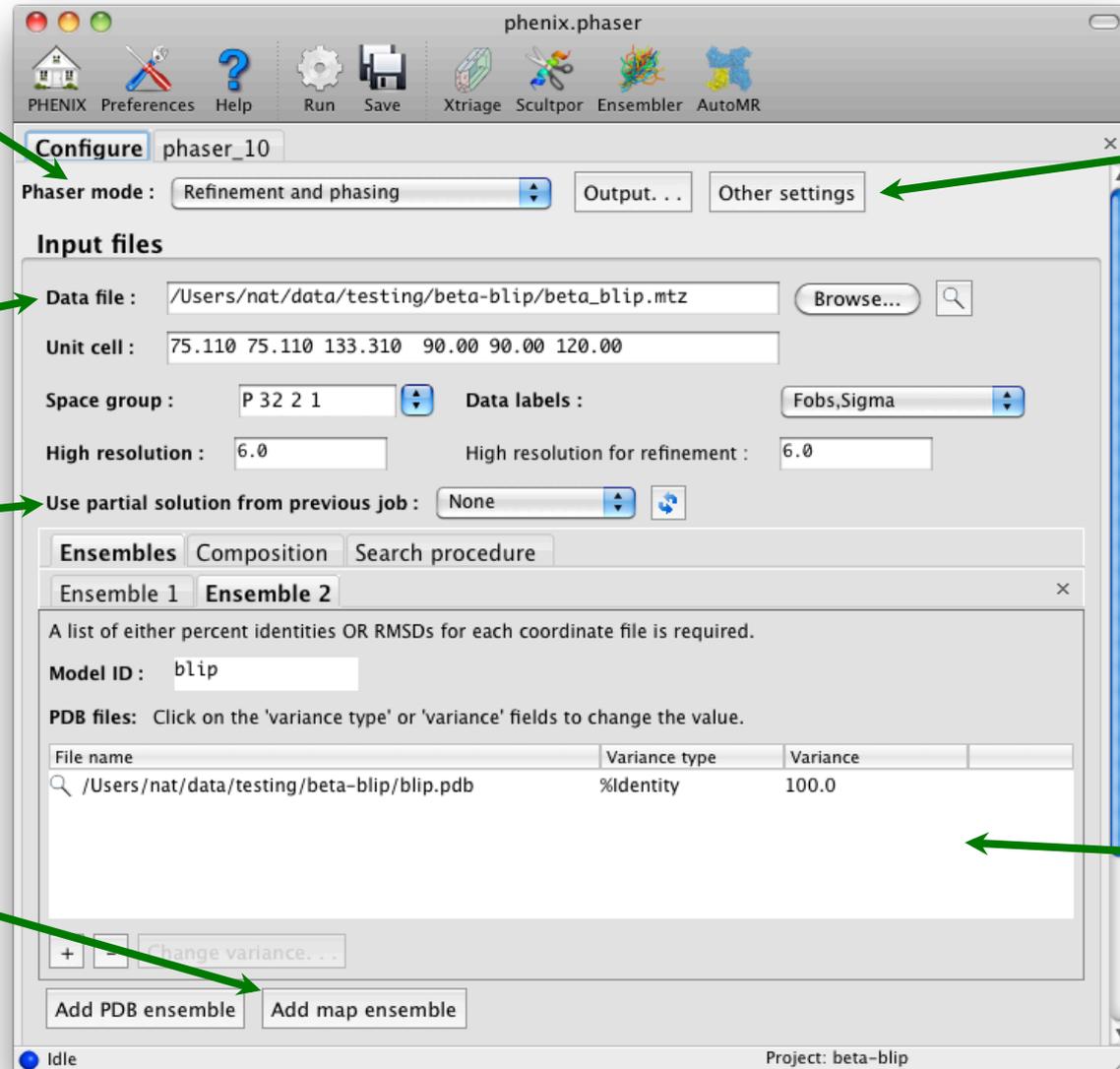
# GUI examples: PHASER

Supports modes  
MR\_AUTO,  
MR\_FRF, MR\_BRF,  
MR\_FTF, MR\_BTF,  
MR\_PAK, MR\_RNP

Any reflection  
file  
format permitted

One-click re-use  
of partial solutions  
from past runs

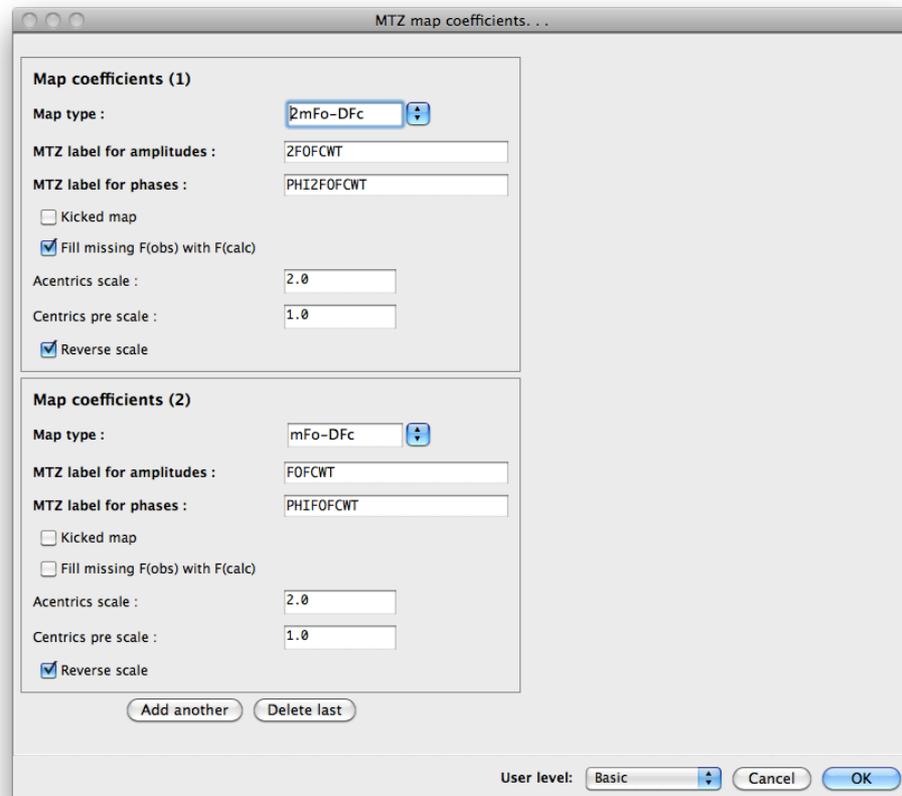
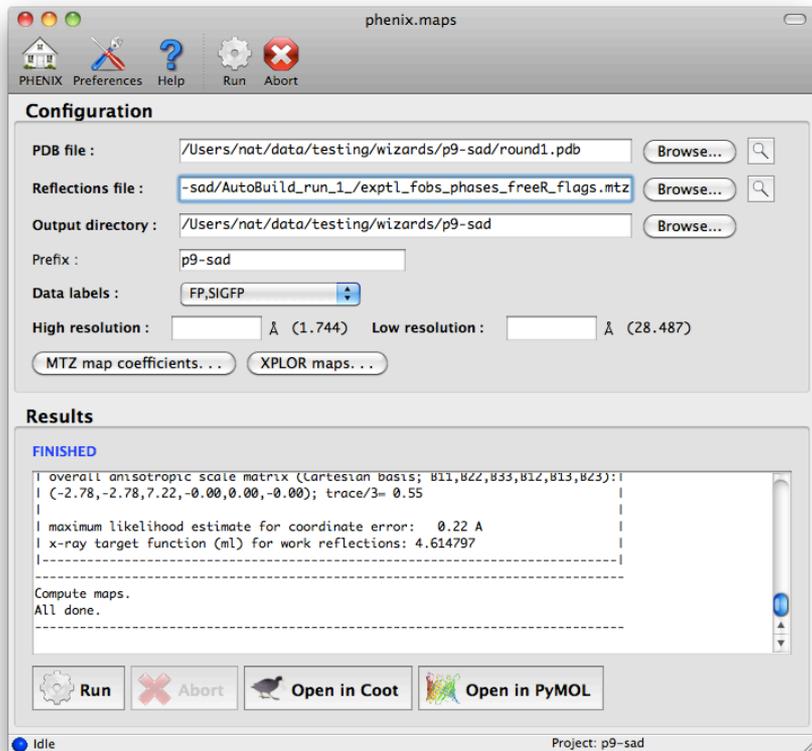
Can use a low-  
resolution map  
as a search  
model



Most  
keywords  
found here

Drag-and-  
drop  
supported

# GUI examples: phenix.maps



- Any kind of map defined as  $[p][m]Fo + [q][D]Fc$  (Example:  $3.2Fo - 1.7Fc$ )
- “kicked” map: removes bias by averaging maps calculated with shaken coordinates
- Fill missing F(obs) with F(calc): often improves  $2mFo - DFc$  maps, but watch out for bias! (phenix.refine and Refmac both do this)
- B-factor sharpened maps (with automatic Bsharp determination)
- Anomalous difference maps

# GUI examples: Parallel Structure Validation/Comparison

- Identifies points of difference between structures of the same protein, with optional map superpositioning

The screenshot displays the Coot software interface for parallel structure comparison. The main window is titled 'Parallel structure comparison' and shows a table of comparison results for various protein chains. The table has columns for different residue types and their corresponding comparison results, with some cells highlighted in red to indicate outliers.

Rotamers	Ramachandran	Missing atoms	Secondary structure	19 LEU	21 LYS	23 LYS	24 GLU	25 ASP	27 LEU	28 LYS	29 LYS	31 GLU
3fhi.pdb:A	---	OUTLIER	tt0	m-20	mt	---	mttm	---	---	---	---	m
3dnd.pdb:A	TLIER	OUTLIER	tttt	tt0	m-20	OUTLIER	mtmm	mttt	mm-40	OL	---	OL
113r.pdb:E	---	---	tttt	m-20	mt	---	mttt	mttt	mm-40	---	---	m
3fjq.pdb:E	---	tmtm?	tttt	mt-10	m-20	mt	mttt	mttt	mm-40	---	---	m
1syk.pdb:A	n?	mttt	tptm	tp10	t70	OUTLIER	mppt	mttt	tp10	---	---	m
1syk.pdb:B	n?	mttt	tptm	tp10	t70	OUTLIER	mppt	mttt	mt-10	---	---	m
3dne.pdb:A	TLIER	mppt	ttpt	mt-10	m-20	OUTLIER	mtmm	mttt	mm-40	---	---	OL

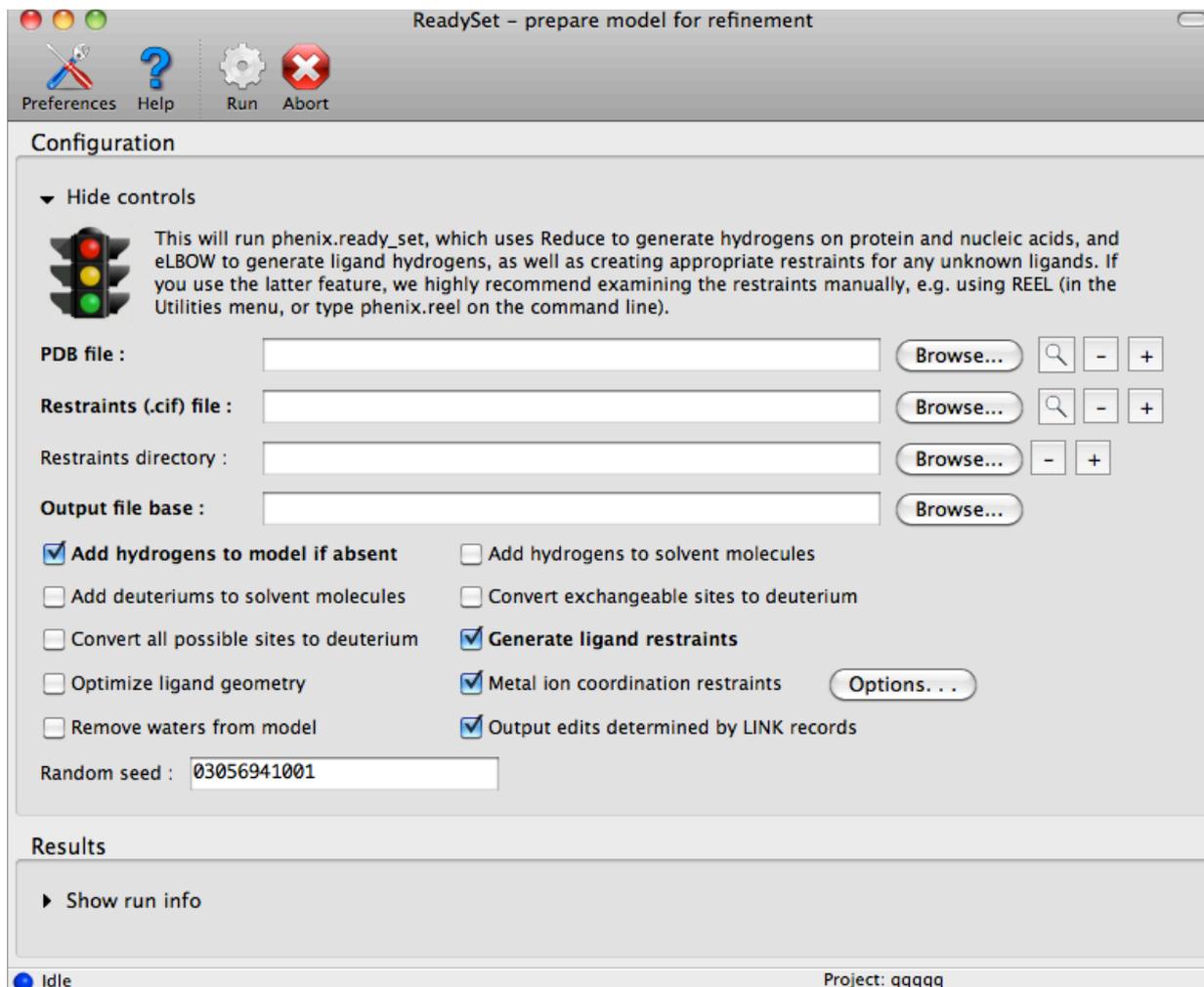
The 3D molecular model on the right shows the protein structure with various chains highlighted in different colors (blue, red, green, yellow) to represent the different structures being compared. The interface includes a 'Coot controls' dialog box with a list of models to be compared:

- 3fhi.pdb:chain A
- 3dnd.pdb:chain A
- 113r.pdb:chain E
- 3fjq.pdb:chain E
- 1syk.pdb:chain A
- 1syk.pdb:chain B
- 3dne.pdb:chain A

The 'Coot controls' dialog also includes buttons for 'Load models and maps' and 'Fetch modified structures', and a description of the comparison process. The main window also shows a 'Structure comparison' panel with a 'Browse...' button.

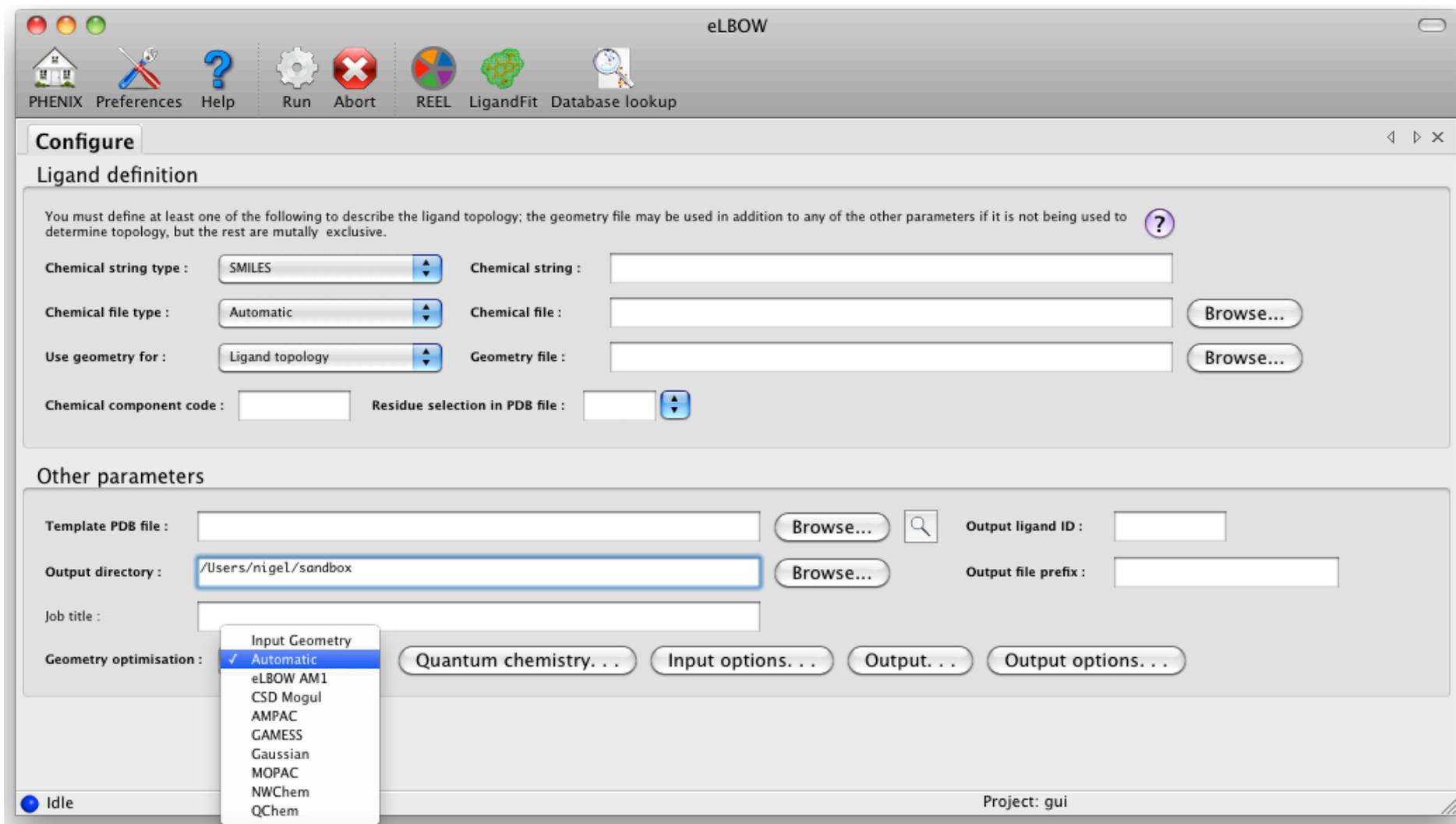
# Ligands: ReadySet! - One-stop preparation for your refinement needs

- Generates files for refinement
  - Adds hydrogens, deuteriums, metal-coordination, CIF file
  - Uses Reduce for protein hydrogens and eLBOW for ligands
  - Command line **phenix.ready\_set model.pdb** will do this all!



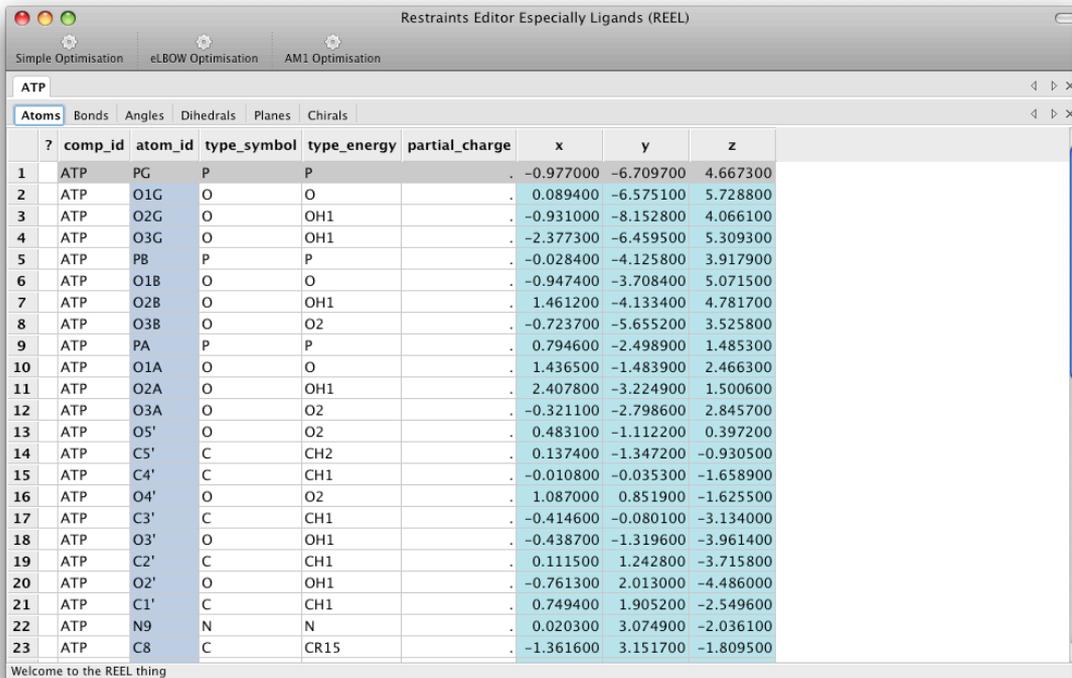
# Ligands: eLBOW

- eLBOW - electronic Ligand Builder & Optimisation Workbench: uses a semi-empirical method to generate atomic coordinates from a chemical topology, then calculates restraint values



# Ligands: REEL (Restrains Editor, Especially Ligands)

- phenix.reel provides a graphical interface to manipulate restraints
  - Editing of restraints using a molecule & table view
  - Two-way and set-intersection highlighting
  - Comparison of ligand geometries
  - eLBOW interface
  - Generate files required to link a ligand to a protein



Restraints Editor Especially Ligands (REEL)

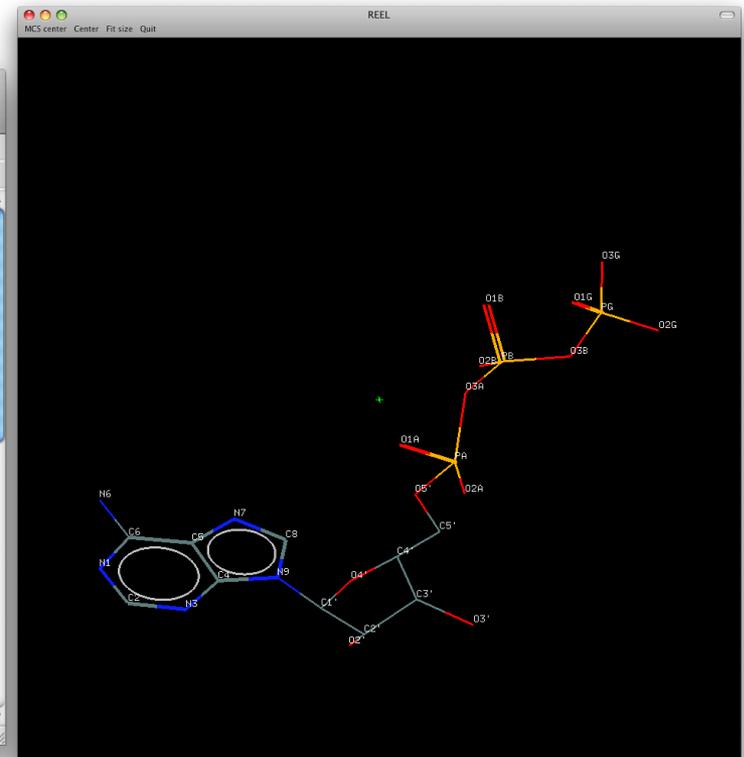
Simple Optimisation eLBOW Optimisation AM1 Optimisation

ATP

Atoms Bonds Angles Dihedrals Planes Chirals

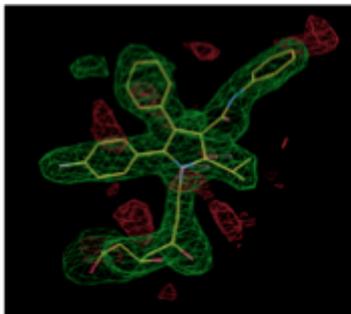
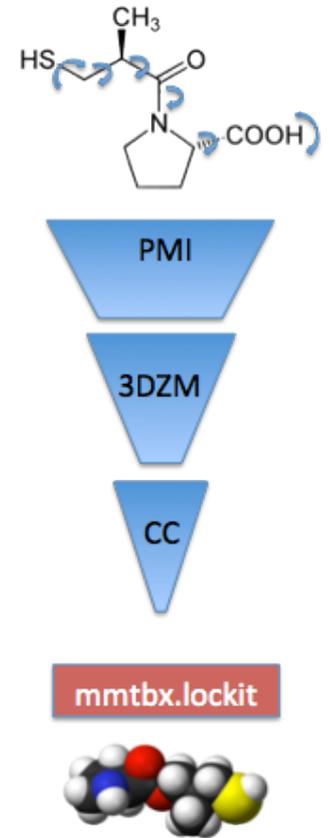
?	comp_id	atom_id	type_symbol	type_energy	partial_charge	x	y	z
1	ATP	PG	P	P		-0.977000	-6.709700	4.667300
2	ATP	O1G	O	O		0.089400	-6.575100	5.728800
3	ATP	O2G	O	OH1		-0.931000	-8.152800	4.066100
4	ATP	O3G	O	OH1		-2.377300	-6.459500	5.309300
5	ATP	PB	P	P		-0.028400	-4.125800	3.917900
6	ATP	O1B	O	O		-0.947400	-3.708400	5.071500
7	ATP	O2B	O	OH1		1.461200	-4.133400	4.781700
8	ATP	O3B	O	O2		-0.723700	-5.655200	3.525800
9	ATP	PA	P	P		0.794600	-2.498900	1.485300
10	ATP	O1A	O	O		1.436500	-1.483900	2.466300
11	ATP	O2A	O	OH1		2.407800	-3.224900	1.500600
12	ATP	O3A	O	O2		-0.321100	-2.798600	2.845700
13	ATP	O5'	O	O2		0.483100	-1.112200	0.397200
14	ATP	C5'	C	CH2		0.137400	-1.347200	-0.930500
15	ATP	C4'	C	CH1		-0.010800	-0.035300	-1.658900
16	ATP	O4'	O	O2		1.087000	0.851900	-1.625500
17	ATP	C3'	C	CH1		-0.414600	-0.080100	-3.134000
18	ATP	O3'	O	OH1		-0.438700	-1.319600	-3.961400
19	ATP	C2'	C	CH1		0.111500	1.242800	-3.715800
20	ATP	O2'	O	OH1		-0.761300	2.013000	-4.486000
21	ATP	C1'	C	CH1		0.749400	1.905200	-2.549600
22	ATP	N9	N	N		0.020300	3.074900	-2.036100
23	ATP	C8	C	CR15		-1.361600	3.151700	-1.809500

Welcome to the REEL thing

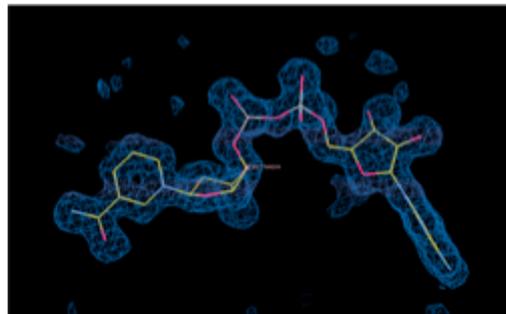


# Ligands: ez-Ligand – fast ligand building into density map (in progress)

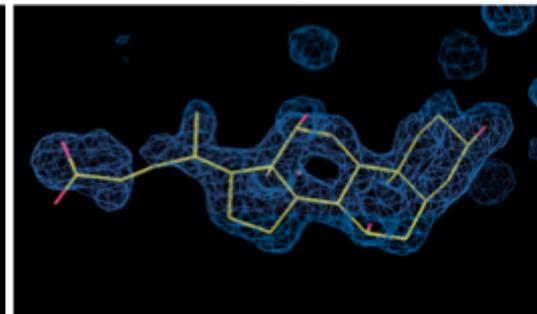
- General procedure
  - Find blob in difference density
  - Generate lots (>1e5) random conformations of a ligand via ultra-fast torsion angle sampling
  - For each conformation apply a series of ‘shape filters’ of increasing complexity
    - Principal moment of Inertia match (PMI)
    - Orientation independent shape features (3DZM)
    - Low resolution Real Space Correlation (CC)
    - FFT based fast rotational matching
    - Real Space Refinement



Lipitor: 50 seconds

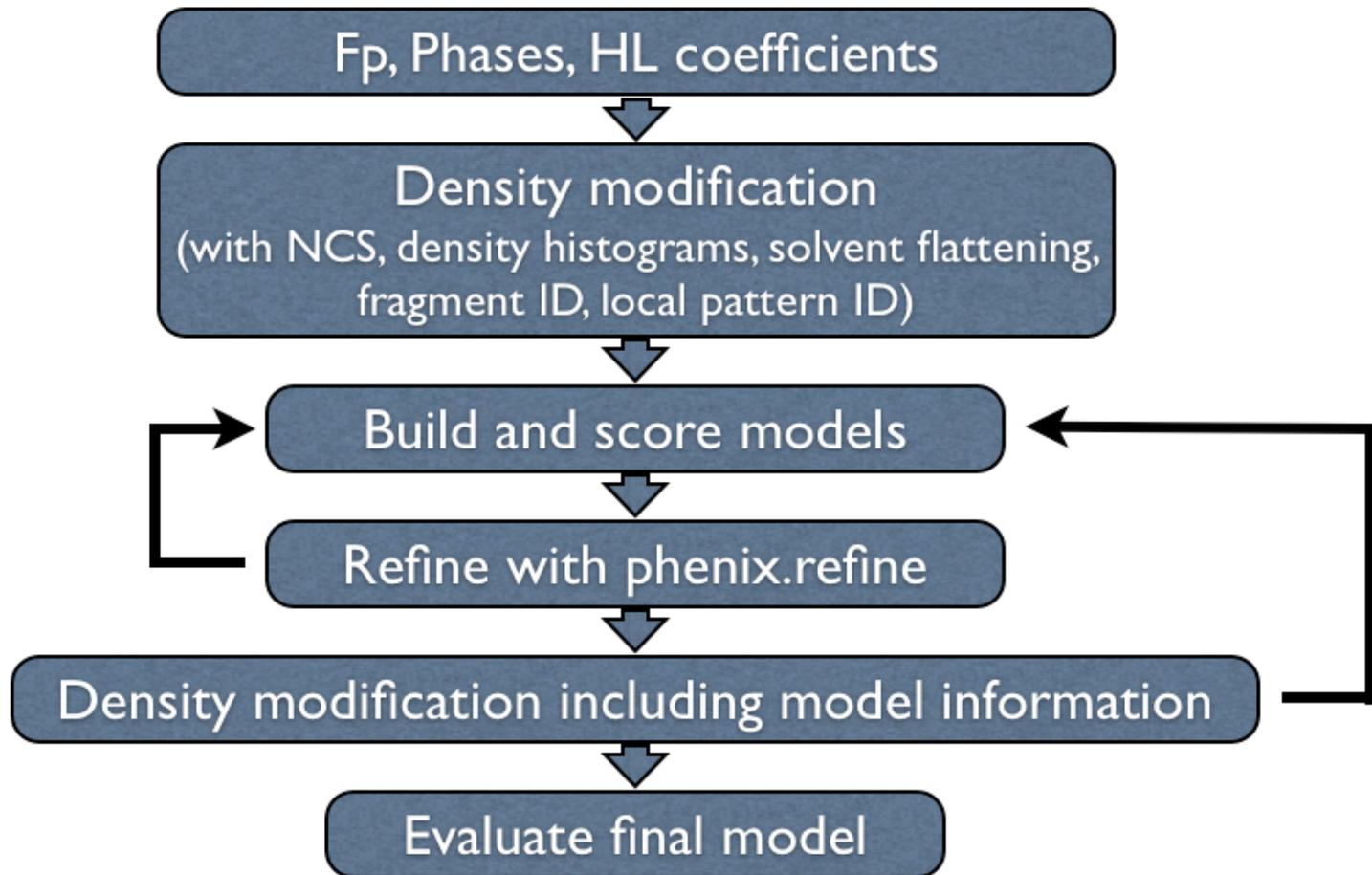


NAD: 2 minutes



Cholic Acid: 50 seconds

# Automated model building and rebuilding with AutoBuild



- Proteins and nucleic acids
- General model building: low to high resolution (3.5Å or better)
- Location of secondary structure elements (few seconds, tested at ~4Å)
- Loop building and extension and side chain docking

# Automated model building and rebuilding with AutoBuild

The screenshot displays the AutoBuild application window. At the top, a toolbar contains icons for PHENIX, Preferences, Help, Run, Abort, Save, Xtrriage, Coot, and PyMOL. The main window is titled 'Configure' and contains a 'Configuration' section with a text box explaining file input methods. Below this is a table with columns for 'File path', 'Format', and 'Data type'. Further down are controls for '+', '-', 'Modify file data type...', and 'Input file options...'. The configuration parameters include 'Space group', 'Unit cell', 'High-resolution limit' (0.0), 'Rebuild in place' (Auto), 'Max. iterative build cycles' (6), 'Max. iterative rebuild cycles' (15), 'NCS copies', and 'Number of processors' (1). Checkboxes for 'Include input model', 'Quick mode', and 'Build SeMet residues' are also present. An 'Output' section at the bottom has a 'Run title' field. The status bar at the bottom shows 'Idle' and 'Project: qqqqq'.

**AutoBuild**

PHENIX Preferences Help Run Abort Save Xtrriage Coot PyMOL

### Configure

#### Configuration

You can add input files by either dragging them from the desktop into this window, or clicking the '+' button and selecting a file from the browser. All reflection file formats, PDB files (starting model, heavy atoms, or ligands), CIF (restraint) files, and phenix.refine parameter files are supported. Please see the documentation ("Help" button on the toolbar) for instructions on what files are required to run the program.

File path	Format	Data type
-----------	--------	-----------

+ - Modify file data type... Input file options...

Space group : [ ] [v] Unit cell : [ ]

High-resolution limit : [ 0.0 ] Rebuild in place : [ Auto ] [v]  Include input model

Max. iterative build cycles : [ 6 ] [v] Max. iterative rebuild cycles : [ 15 ] [v]  Quick mode

NCS copies : [ ] Number of processors : [ 1 ] [v]  Build SeMet residues

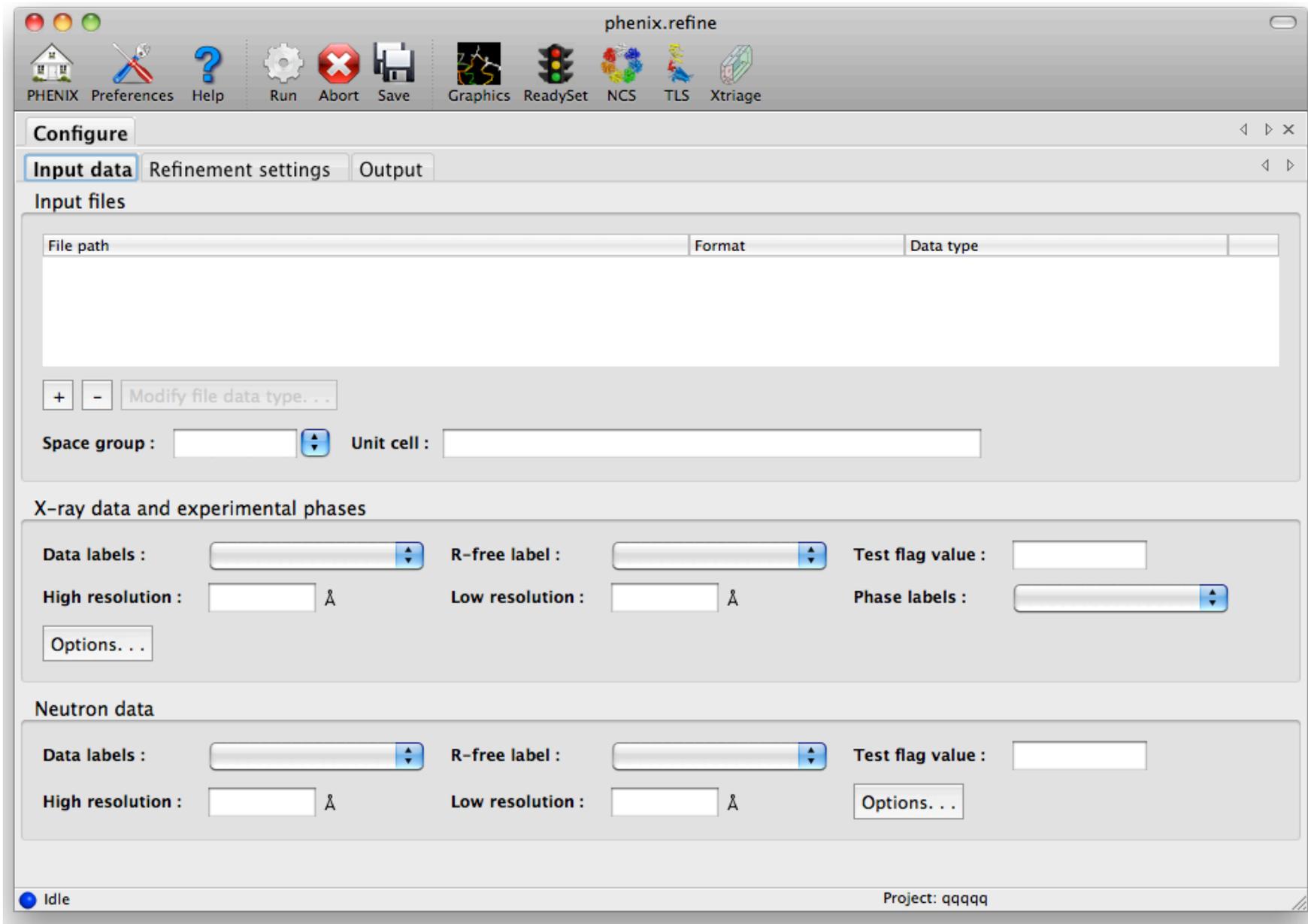
All parameters...

#### Output

Run title : [ ]

Idle Project: qqqqq

# phenix.refine GUI

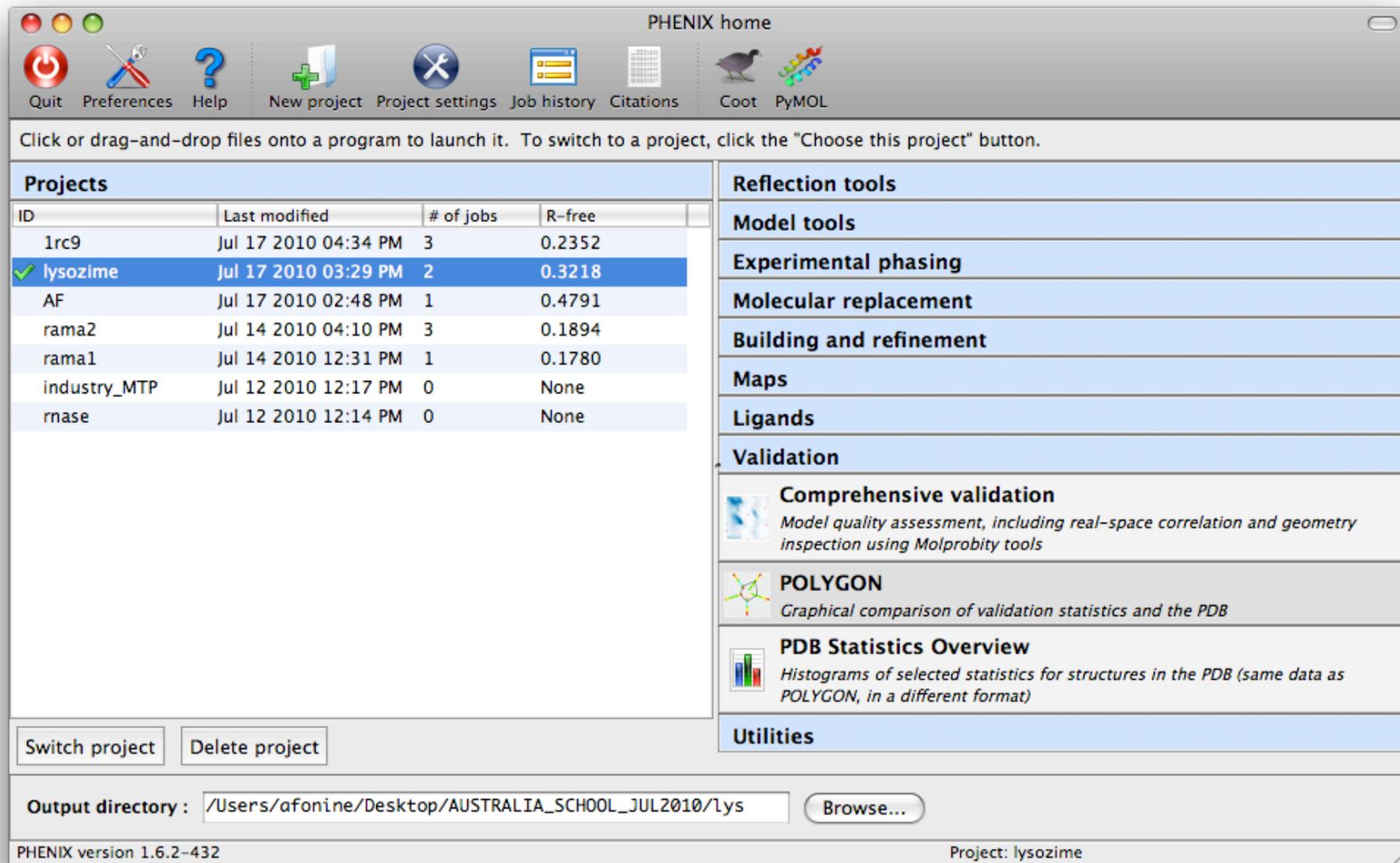


# PHENIX tools for model validation

- **Comprehensive validation** option available from PHENIX GUI:
  - MolProbity scores;
  - Real-space correlation (map CC), 2mFo-DFc and mFo-DFc listed for each atom or residue;
  - Basic geometry statistics (rmsd and max deviation for bonds, angles, ...)
  - phenix.model\_vs\_data report;
  - POLYGON.
- phenix.refine .log file contains lots of information.
- Tools to create various maps (iterative build omit maps, SA omit maps, Average kick maps,  $i^*mFo-j^*DFc$  maps)...
- Getting uncertainties by building multiple models.

# PHENIX tools for model validation

- Comprehensive validation option available from PHENIX GUI:



PHENIX home

Click or drag-and-drop files onto a program to launch it. To switch to a project, click the "Choose this project" button.

Projects			
ID	Last modified	# of jobs	R-free
1rc9	Jul 17 2010 04:34 PM	3	0.2352
✓ lysozyme	Jul 17 2010 03:29 PM	2	0.3218
AF	Jul 17 2010 02:48 PM	1	0.4791
rama2	Jul 14 2010 04:10 PM	3	0.1894
rama1	Jul 14 2010 12:31 PM	1	0.1780
industry_MTP	Jul 12 2010 12:17 PM	0	None
rnase	Jul 12 2010 12:14 PM	0	None

Reflection tools

Model tools

Experimental phasing

Molecular replacement

Building and refinement

Maps

Ligands

Validation

-  **Comprehensive validation**  
*Model quality assessment, including real-space correlation and geometry inspection using Molprobit tools*
-  **POLYGON**  
*Graphical comparison of validation statistics and the PDB*
-  **PDB Statistics Overview**  
*Histograms of selected statistics for structures in the PDB (same data as POLYGON, in a different format)*

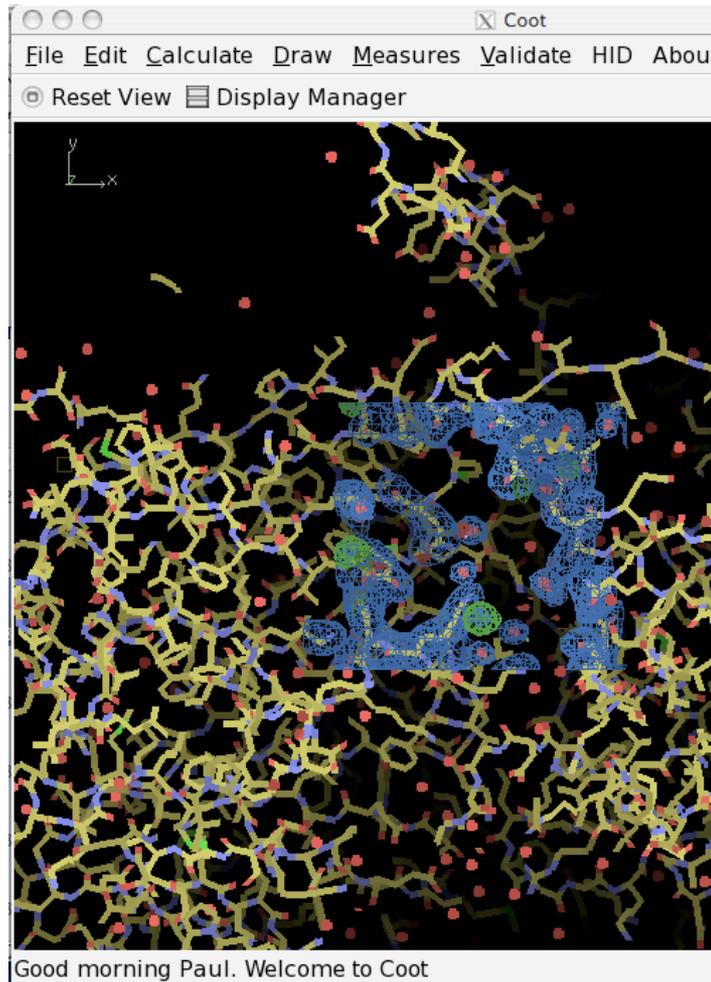
Utilities

Switch project Delete project

Output directory : /Users/afonine/Desktop/AUSTRALIA\_SCHOOL\_JUL2010/Lys Browse...

PHENIX version 1.6.2-432 Project: lysozyme

# PHENIX tools for model validation



Validation results for /Users/pdadams/Work/Scratch/phenix-test/refine/bad/Refine\_0/refine\_001.pdb

The validation performed by PHENIX is currently a subset of the full MolprobitY analysis available on the web server. We recommend that academic groups use the server version to obtain more detailed information on structure quality, as well as files necessary to show validation results in Coot. You can start this process by clicking the MolprobitY button on the left.

**Ramachandran outliers:**

Chain	Residue	Residue type	Score	Phi	Psi		
A	LEU 45	General	0.02	68.0	106.1		
A	SER 49	General	0.02	-79.0	-110.8		
A	SER 76	General	0.02	-70.5	-97.4		
A	LYS 78	General	0.04	-61.4	56.4		
A	GLY 79	Glycine	0.04	-46.3	3.8		
A	ASN 81	General	0.02	62.4	122.9		
A	LYS 203	General	0.00	42.7	-77.8		
A	GLU 204	General	0.00	134.3	152.6		
A	LEU 207	Prepro	0.00	76.2	118.6		
A	PHE 210	General	0.00	43.6	166.6		
A	LEU 292	General	0.00	4.2	174.2		
A	ARG 293	General	0.04	72.9	137.8		
A	TYR 424	General	0.00	-68.5	-122.0		
A	SER 425	General	0.02	-171.3	-47.3		
A	THR 426	General	0.02	48.0	96.9		

**Rotamer outliers:**

Chain	Residue	Score	Chi1	Chi2	Chi3	Chi4	
A	ILE 6	0.39	163.3	97.6	-	-	
A	THR 8	0.00	154.8	-	-	-	

Refinement complete Project: None

# PHENIX tools for model validation

Structure validation

Summary | Geometry outliers | **Model validation** | Real-space correlation

### Validation summary

The validation performed by PHENIX is currently a subset of the full Molprobtity analysis available on the web server. We recommend that academic groups use the server version to obtain more detailed information on structure quality, as well as files necessary to show validation results in Coot. You can start this process by clicking the icons.

**Basic statistics for overall\_best.pdb:**

- Ramachandran outliers: 1.7% (Good)
- Ramachandran favored: 96.6% (Good)
- Rotamer outliers: 2.9% (Good)
- C-beta outliers: 1 (Good)
- Clashscore: 13.40

### Ramachandran analysis

View Ramachandran plots

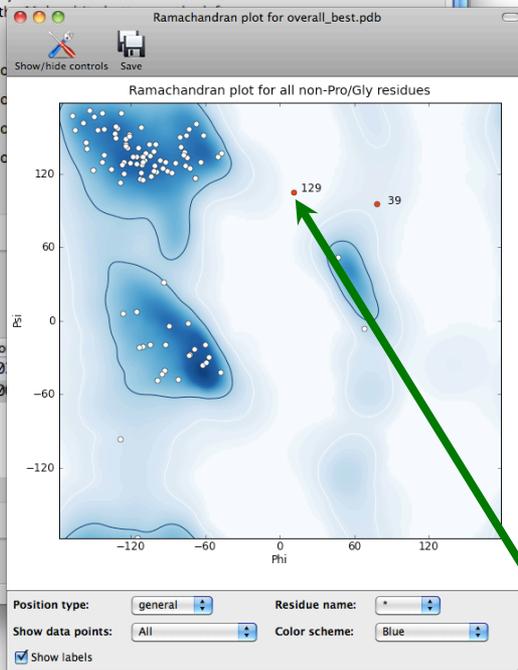
Ramachandran outliers:

Chain	Residue	Residue type	Score
	LYS 39	General	0.00
	LEU 129	General	0.00

### Rotamer analysis

View Chi1-Chi2 plots

Idle



Structure validation

Summary | Geometry outliers | Model validation | **Real-space correlation**

### Real-space correlation

If you have a graphics window open, clicking an atom or residue in the list will recenter the display (you may disable this in the "Graphics" section of the preferences). Click on a column label to re-sort the list by values in that column.

By residue: Show only residues with CC less than 0.80 | Update list

Chain	Residue	CC	B-factor	Occ.	Fc	2mFo-DFc
	GLY 115	0.795	37.40	1.00	3.07	2.49

Show residues: 11 - 110

Idle | Project: p9-sad

*outliers in graphs also recenter Coot*

# New developments: integration of PHENIX with Rosetta

Frank DiMaio et al. Nature (2011)

Improved molecular replacement by density- and energy-guided protein structure optimization.

- Molecular replacement or the subsequent rebuilding usually fail with divergent starting models based on remote homologues with less than 30% sequence identity.
- It is demonstrated that this limitation can be substantially reduced by combining algorithms for protein structure modelling with those developed for crystallographic structure determination.
- Integrating *Rosetta structure modelling* with *PHENIX Autobuild chain tracing* yielded for 8 of 13 X-ray diffraction data sets that could not be solved in the laboratories of expert crystallographers and that remained unsolved after application of an extensive array of alternative approaches.
- An estimate is that the new method should allow rapid structure determination without experimental phase information for over half the cases where current methods fail, given diffraction data sets of better than 3.2 Å resolution, four or fewer copies in the asymmetric unit, and the availability of structures of homologous proteins with >20% sequence identity.

## PHENIX command line tools

- Most of PHENIX command-line tools are invoked as  
***phenix.command\_name***  
Example: phenix.refine, phenix.maps, etc.
- To see all available commands and quick hint about what it is:  
***phenix.commands***
- Typically, running a command without arguments will give a quick help message
- Currently there are 276 commands

# PHENIX: principal tools

- Command line tools are still easy to run:

- **Autobuild** (*from starting phases to complete and refined model*):

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

- **Ligandfit** (automatically find and build ligands into density):

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

- **AutoMR** (molecular replacement with Phaser + Autobuild = refined model):

```
phenix.automr native.sca search.pdb RMS=0.8 mass=23000 copies=1
```

- **phenix.refine** (highly automated structure refinement, X-ray, Neutron):

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

- **phenix.xtriage** (complete data analysis):

```
phenix.xtriage porin_fp.mtz
```

## Some commands

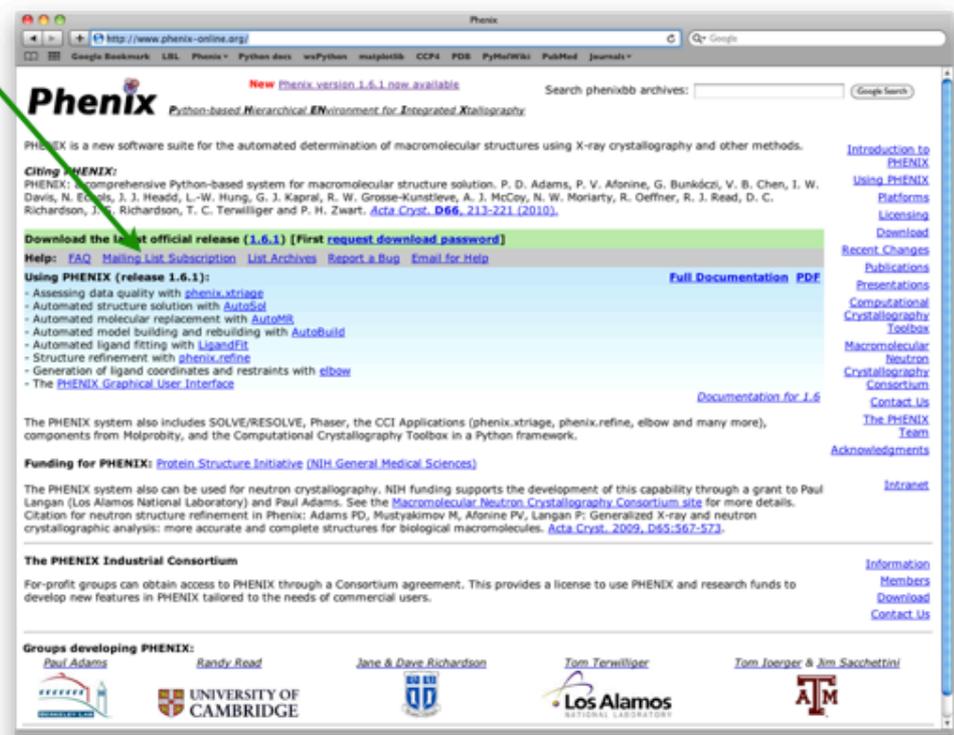
phenix:	Run Phenix graphical user interface
phenix.about:	Summarize contributors, packages, and info for phenix
phenix.acknowledgments:	Summarize third-party components of Phenix
phenix.autobuild:	Iterative model-building density modification and refinement
phenix.automr:	Automated MR and model-building
phenix.autosol:	Automated structure solution by MR/MAD/SAD
phenix.cif_as_mtz:	Convert CIF to MTZ
phenix.clashscore:	Evaluate model based on all-atom contacts
phenix.cns_as_mtz:	Convert CNS to MTZ
phenix.commands:	List command line Phenix methods
phenix.elbow:	Ligand builder (CIF from PDB, SMILES etc)
phenix.enssembler:	Superpose PDB files to create ensemble for MR
phenix.fetch_pdb:	Download a model from PDB (specify PDB code)
phenix.find_all_ligands:	Find ligands from a list in a map
phenix.find_helices_strands:	Build helices and strands into a map
phenix.find_tls_groups:	Automatic identification of appropriate TLS groups in model
phenix.fit_loops:	Fit missing loops in a model
phenix.fmodel:	Calculate structure factors from model
phenix.form_factor_query:	f' and f'' table lookup given element and wavelength
phenix.ksdssp:	Identify secondary structure in a model
phenix.map_value_at_point:	Get map value at given coordinates
phenix.maps:	Create maps from PDB and MTZ files
phenix.metal_coordination:	Generate restraints for metal coordination sites
phenix.model_vs_data:	Evaluate model using experimental data
phenix.mtz.dump:	Dump MTZ file contents
phenix.mtz2map:	Convert MTZ file to map (superseded by phenix.maps)

## Some commands

phenix.multi_crystal_average:	Multi-crystal averaging
phenix.pdb.hierarchy:	Quick summary of PDB file content
phenix.pdb_atom_selection:	Extract selected atoms from PDB file
phenix.pdb_interpretation:	Read PDB file and build restraints for refinement
phenix.pdbtools:	Manipulate PDB files
phenix.phaser:	Run PHASER
phenix.polygon:	Compare model statistics to expected distributions
phenix.print_sequence:	Print sequence from PDB file
phenix.r_factor_statistics:	R-factor statistics at given resolution
phenix.ramalyze:	Validate protein backbone Ramachandran dihedral angles
phenix.ready_set:	Prepare for refinement
phenix.reduce:	Run REDUCE, software for addition or trimming of hydrogens
phenix.refine:	Carry out refinement of a model
phenix.resolve:	Run resolve
phenix.rotalyze:	Validate protein sidechain rotamers
phenix.solve:	Run SOLVE
phenix.superpose_ligands:	Superimpose two ligands
phenix.superpose_maps:	Superimpose PDB files and transform map to match
phenix.superpose_pdbs:	Superimpose PDB files using aligned sequences
phenix.table_one:	Prepare Table 1 for publication
phenix.tls:	Extract/Combine TLS from PDB file
phenix.version:	Print version of Phenix
phenix.xtriage:	Analyze data files for quality and unusual conditions

# PHENIX resources online

- [help@phenix-online.org](mailto:help@phenix-online.org): user support
- [bugs@phenix-online.org](mailto:bugs@phenix-online.org): bug reports
- [phenixbb@phenix-online.org](mailto:phenixbb@phenix-online.org): message board (subscribers only)



The screenshot shows the Phenix website homepage. At the top, there is a navigation bar with the Phenix logo and the tagline "Python-based Hierarchical Environment for Integrated Xtallography". Below the navigation bar, there is a search bar and a "New Phenix version 1.6.1 now available" notification. The main content area includes a description of Phenix as a software suite for macromolecular structure determination, a list of authors, and a section for downloading the latest official release (1.6.1). A green arrow points to the "Download the latest official release (1.6.1) [First request download password]" link. The page also features a sidebar with various links such as "Introduction to PHENIX", "Using PHENIX", "Platforms", "Licensing", "Download", "Recent Changes", "Publications", "Presentations", "Computational Crystallography", "Toolbox", "Macromolecular Neutron Crystallography Consortium", "Contact Us", "The PHENIX Team", and "Acknowledgments". At the bottom, there is a section for "Groups developing PHENIX" with logos for Paul Adams, Randy Read, Jane & Dave Richardson, Tom Terwilliger, Tom Joerper & Jim Sacchettini, and the University of Cambridge.

# Obtaining PHENIX

- Free to academic users; simple online registration required (*please use your academic email address!*)
- Regular official releases (typically 2-8 months)
- Nightly builds

**Phenix** Python-based Hierarchical Environment for Integrated Xtallography

### PHENIX nightly build distribution

These installers are built automatically using the current source code. Although some automated testing occurs as part of the build system, and we do not release installers that have known major problems, they have not been checked as thoroughly as the official distributions, and individual programs may be broken. **Use at your own risk!** Each installer directory should contain reports about any errors that occurred during building/testing. In most cases, if you do not see any errors, the installer can be considered functional enough for daily use. Official releases and builds deemed to be "stable" are noted as such and highlighted in bold type.

If you do choose to use the nightly builds, we recommend updating frequently; once a build disappears from the list here, it can be assumed to be obsolete.

If you encounter errors when using any of these builds, please update to the most recent version before sending a bug report. (It is generally a good idea to update frequently anyway, since the code changes rapidly.) We recommend keeping the most recent official distribution (version 1.6) installed as well, since it is more stable.

**You will need proper authentication to download the installer; see the [main download page](#) for details.**

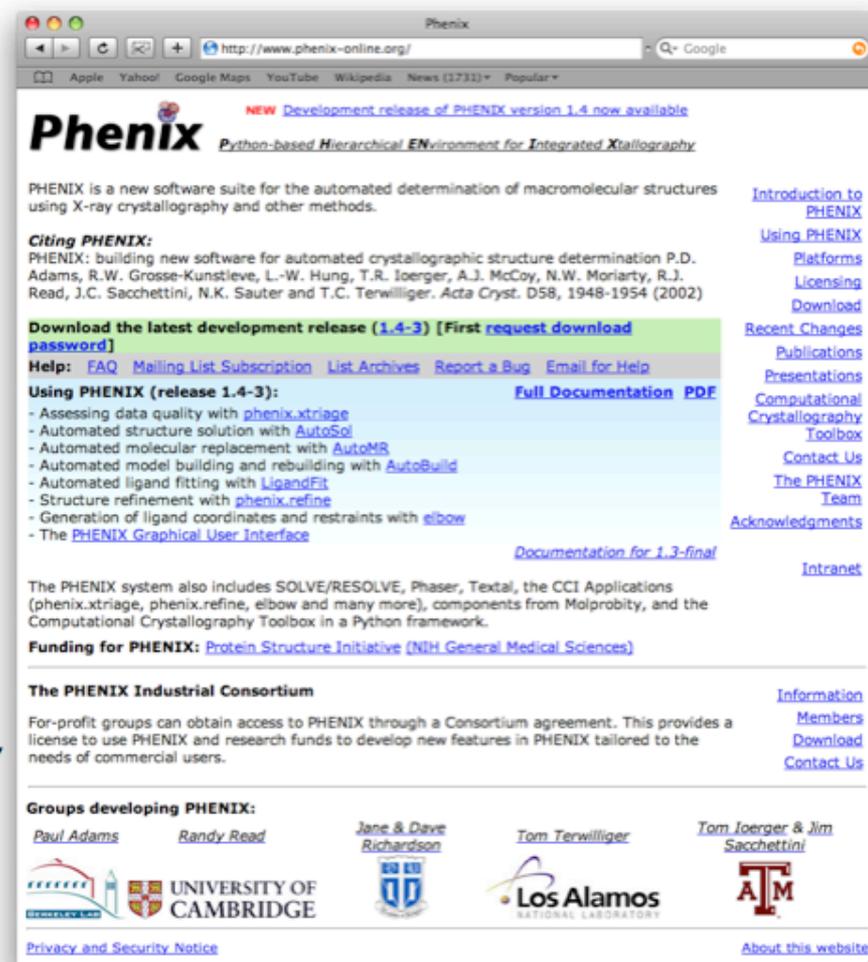
Version	Date	Status	Logs	Info
<b>dev-387</b>	2010-04-27	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	Reverted to PC3 for intel-linux-2.6+68_04 build: <a href="#">0000</a>
<b>dev-386</b>	2010-04-26	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-385</b>	2010-04-25	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-382</b>	2010-04-23	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-378</b>	2010-04-18	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-377</b>	2010-04-17	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-374</b>	2010-04-14	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-373</b>	2010-04-13	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-371</b>	2010-04-11	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-370</b>	2010-04-11	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>dev-368</b>	2010-04-08	successful	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<a href="#">0000</a>
<b>1.6.1-357</b>	<b>2010-03-29</b>	<b>successful</b>	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<b>Official 1.6.1 release: <a href="#">0000</a></b>
<b>1.6-289</b>	<b>2010-01-20</b>	<b>successful</b>	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<b>Official 1.6 release: <a href="#">0000</a></b>
<b>1.5-2</b>	<b>2009-09-28</b>	<b>successful</b>	<a href="#">x0b6_01_0000_0b000_0b000_0b000</a>	<b>Official release for version 1.5: <a href="#">0000</a></b>

Questions/comments to [help@phenix-online.org](mailto:help@phenix-online.org)  
[Administrative interface](#)

[http://www.phenix-online.org/download/nightly\\_builds.cgi](http://www.phenix-online.org/download/nightly_builds.cgi)

# PHENIX Distribution

- Regular releases
- Supported on:
  - Linux (RedHat, Fedora)
  - Mac OSX
- Regular development releases:
  - Nightly builds
  - <http://www.phenix-online.org/>
- Extensive documentation



The screenshot shows the Phenix website homepage in a browser window. The URL is <http://www.phenix-online.org/>. The page features a navigation menu on the right with links such as "Introduction to PHENIX", "Using PHENIX", "Platforms", "Licensing", "Download", "Recent Changes", "Publications", "Presentations", "Computational Crystallography", "Toolbox", "Contact Us", "The PHENIX Team", "Acknowledgments", and "Intranet". The main content area includes a "NEW Development release of PHENIX version 1.4 now available" banner, a description of PHENIX as a software suite for macromolecular structure determination, a "Citing PHENIX" section with a list of authors and a 2002 publication, and a "Download the latest development release (1.4-3)" section with a "Full Documentation PDF" link. Below this is a "Using PHENIX (release 1.4-3)" section listing various tools like phenix.xtriage, AutoSol, AutoMR, AutoBuild, LigandFit, phenix.refine, and elbow. The page also mentions funding from the Protein Structure Initiative (NIH) and the Phenix Industrial Consortium, and lists groups developing PHENIX, including Paul Adams, Randy Read, Jane & Dave Richardson, Tom Terwilliger, and Tom Ioerger & Jim Sacchettini, with their respective institutional logos (Berkeley Lab, University of Cambridge, Los Alamos National Laboratory, and ATM).



**Phenix**



# PHENIX: installation

- **You can do it:** you don't have to have administrative privileges to install PHENIX: you can always install it under your own account in the place of your choice.
- **Use** the latest version from nightly builds: it has latest bug fixes, newest features, ...
- **Keep** the latest official release in case the nightly build is broken (you can have several versions of PHENIX installed on your computer).



## **Phenix** *Python-based Hierarchical ENvironment for Integrated Xtallography*

### PHENIX nightly build distribution

These installers are built automatically using the current source code. Although some automated testing occurs as part of the build system, and we do not release installers that have known major problems, they have not been checked as thoroughly as the official distributions, and individual programs may be broken. **Use at your own risk!** Each installer directory should contain reports about any errors that occurred during building/testing. In most cases, if you do not see any errors, the installer can be considered functional enough for daily use. Official releases and builds deemed to be "stable" are noted as such and highlighted in bold type.

If you do choose to use the nightly builds, we recommend updating frequently; once a build disappears from the list here, it can be assumed to be obsolete.

If you encounter errors when using any of these builds, please update to the most recent version before sending a bug report. (It is generally a good idea to update frequently anyway, since the code changes rapidly.) We recommend keeping the most recent official distribution (version 1.7.1) installed as well, since it is more stable.

**You will need proper authentication to download the installer; see the [main download page](#) for details.**

Version	Date	Status	Logs	Info
<a href="#">dev-759</a>	2011-05-13	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-757</a>	2011-05-11	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-756</a>	2011-05-10	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-755</a>	2011-05-09	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-753</a>	2011-05-07	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-752</a>	2011-05-06	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-751</a>	2011-05-05	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	new program (+GUI): <a href="#">phenix.cut_out_density</a> ; <a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-750</a>	2011-05-04	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<a href="#">dev-749</a>	2011-05-03	successful	<a href="#">cctbx</a> , <a href="#">cci_apps</a> , <a href="#">phaser</a> , <a href="#">phenix</a> , <a href="#">misc</a>	<a href="#">docs</a> ; <a href="#">changelog</a>
<b><a href="#">1.7.1-743</a></b>	<b>2011-04-27</b>	<b>successful</b>	<b><a href="#">cctbx</a>, <a href="#">cci_apps</a>, <a href="#">phaser</a>, <a href="#">phenix</a>, <a href="#">misc</a></b>	<b>1.7.1 official release; <a href="#">docs</a>; <a href="#">changelog</a></b>

## Reporting bugs, problems, asking questions

- **Something didn't work as expected?... program crashed?... missing feature?...**

**Not Good:** silently give up and run away looking for alternative software (or write your own program).

**Good:** report us a problem, ask a question, request a feature (explain why it's good to have), ask for help.

- **Reporting a bug:**

**Not good:** “Hi! PHENIX crashed, I don't know what to do.”

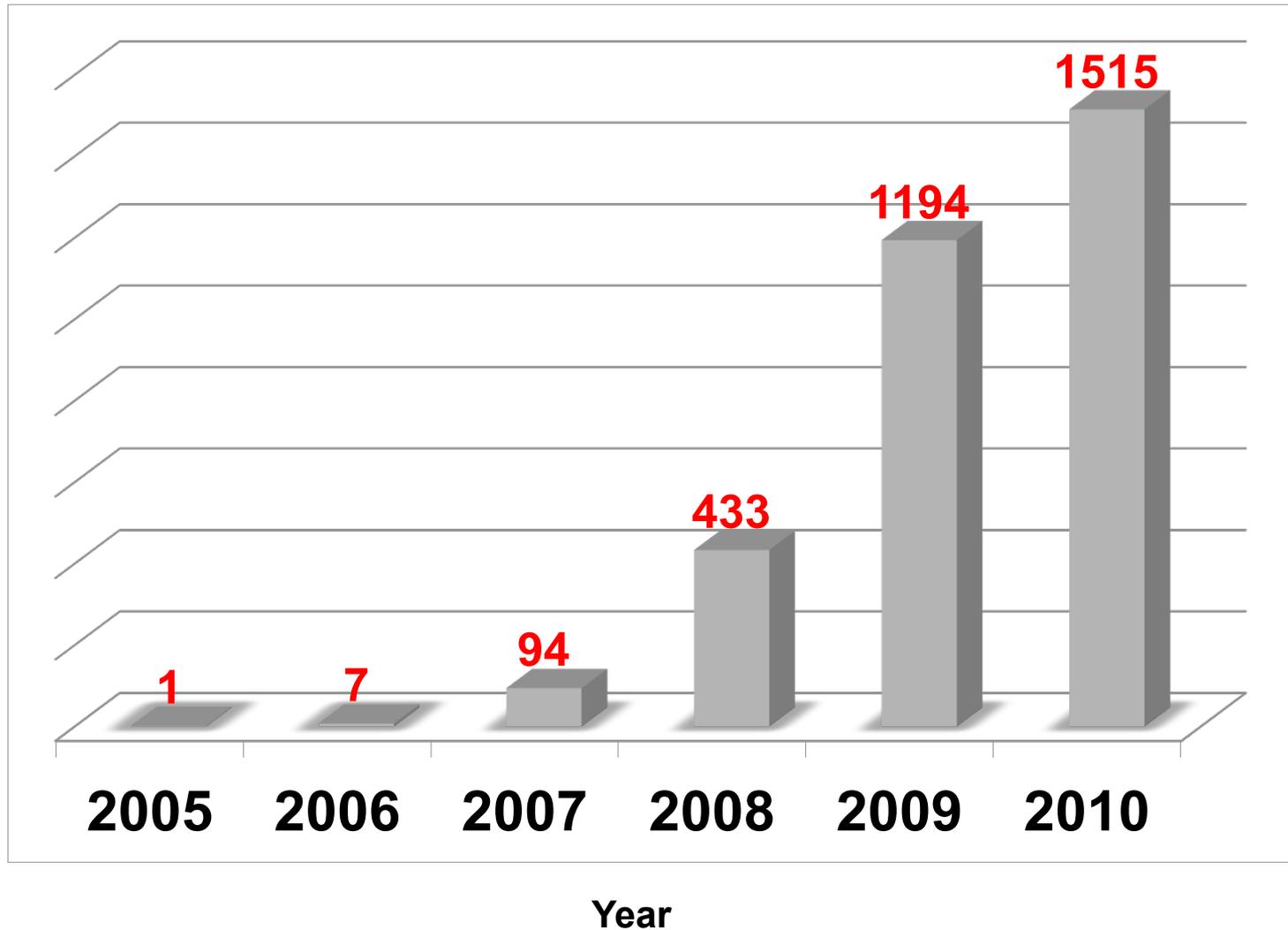
**Good:** “Hi! PHENIX crashed. Here are:

- 1) PHENIX version;
- 2) Command and parameters I used;
- 3) Input and output files (at least logs).”

**Subscribe to PHENIX bulletin board: [www.phenix-online.org](http://www.phenix-online.org)**

# PHENIX use (May 18, 2011)

Number of structures in PDB with “REMARK 3 PROGRAM PHENIX”



Phenix  
http://www.phenix-online.org/

**Phenix** NEW [Development release of PHENIX version 1.4 now available](#)  
*Python-based Hierarchical ENvironment for Integrated Xtallography*

PHENIX is a new software suite for the automated determination of macromolecular structures using X-ray crystallography and other methods.

**Citing PHENIX:**  
PHENIX: building new software for automated crystallographic structure determination P.D. Adams, R.W. Grosse-Kunstleve, L.-W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter and T.C. Terwilliger. *Acta Cryst.* D58, 1948-1954 (2002)

**Download the latest development release (1.4-3) [First request download password]**

**Help:** [FAQ](#) [Mailing List Subscription](#) [List Archives](#) [Report a Bug](#) [Email for Help](#)

**Using PHENIX (release 1.4-3):** [Full Documentation](#) [PDF](#)

- Assessing data quality with [phenix.xtriage](#)
- Automated structure solution with [AutoSol](#)
- Automated molecular replacement with [AutoMR](#)
- 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 Graphical User Interface](#)

[Documentation for 1.3-final](#)

The PHENIX system also includes SOLVE/RESOLVE, Phaser, Textal, the CCI Applications (phenix.xtriage, phenix.refine, elbow and many more), components from Molprobit, and the Computational Crystallography Toolbox in a Python framework.

**Funding for PHENIX:** [Protein Structure Initiative \(NIH General Medical Sciences\)](#)

**The PHENIX Industrial Consortium** [Information](#)

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