



What's new in: Phaser

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CCP4 Study Weekend

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Phaser – new developments

Next version: 2.2.4 (2.3.0?)

General

- faster minimiser
- changes in keyword input
- map sharpening

Experimental phasing

- reassign anomalous atom type in experimental phasing
- map coefficients without real scattering contribution for density modification
- better treatment of anomalous scatterers inside partial MR models
- wavelength-dependent f' and f'' restraints

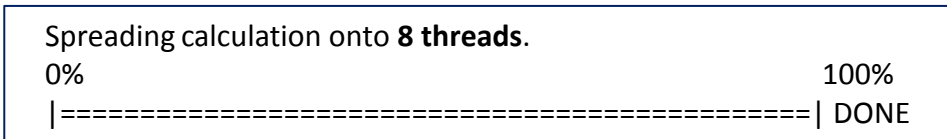
Molecular replacement

- OpenMP – fast rotation function
- OpenMP – brute rotation and translation function
- OpenMP – packing and refinement
- B-factor refinement
- model error refinement
- automatic search order determination
- model symmetry
- fast mode
- analytic second derivatives
- template matching
- faster, more intelligent packing test

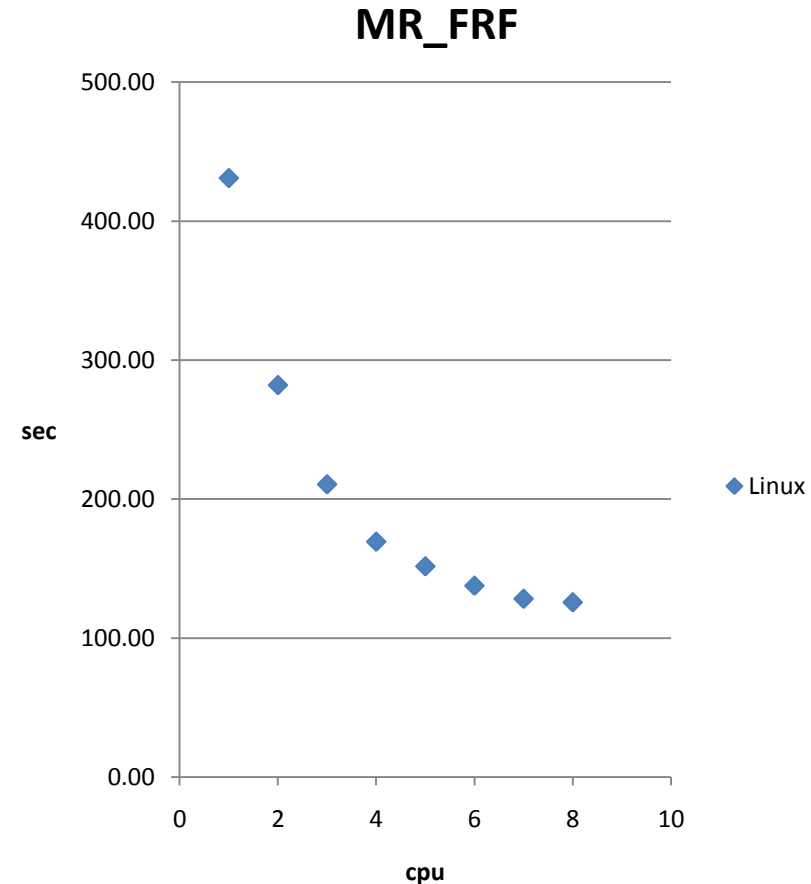


OpenMP support

- everything with a progress bar can be run in parallel



- controlled by JOBS keyword
default: 1
max ≤ number of CPUs
- **uses more memory!**

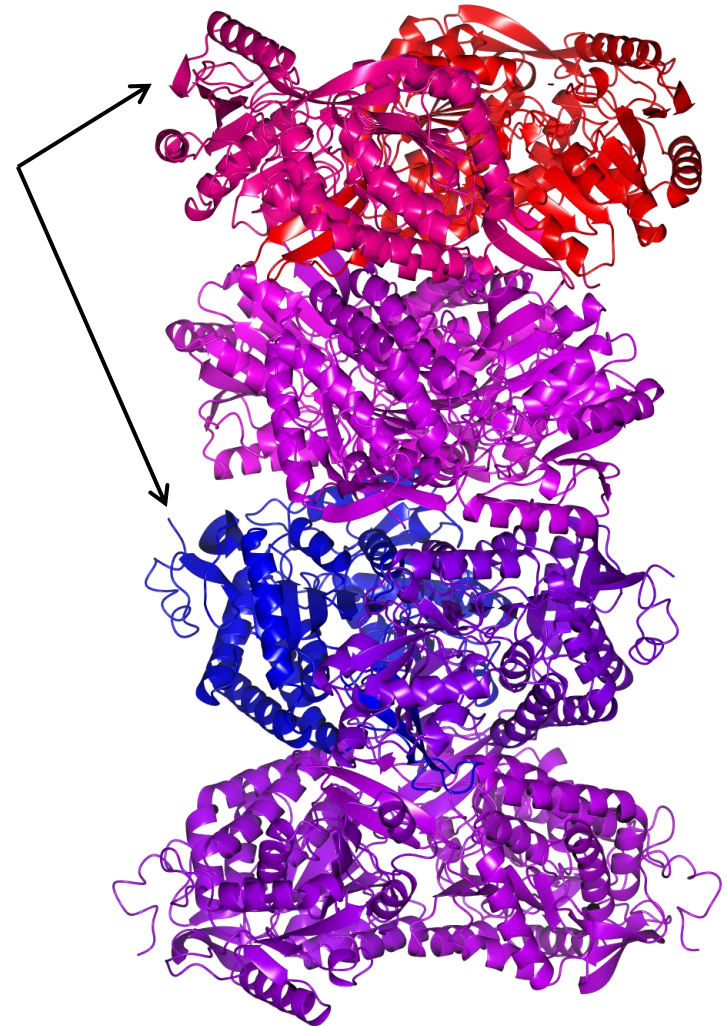




B-factor refinement

For multimer models, B-factors typically differ between chains

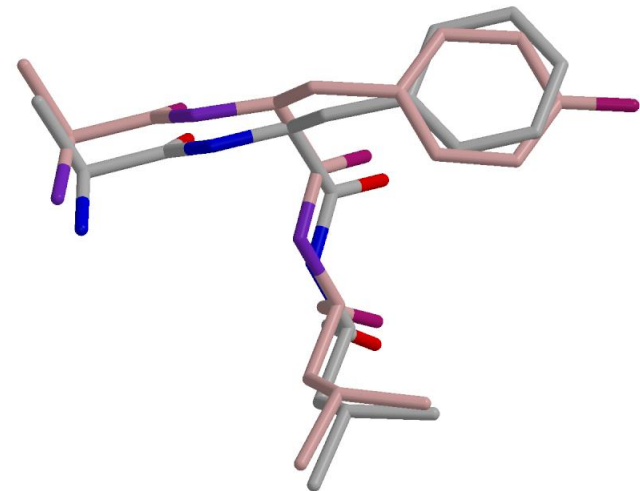
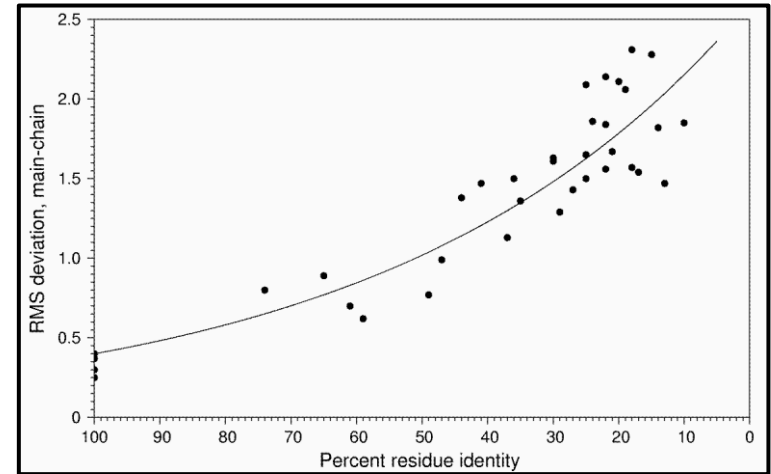
- additional chains are found by taking the present structure into account and trying to explain the difference
- refining the B-factor for a chain makes the estimate for the “missing” structure factor more accurate





Model error refinement

- model error is typically an estimate from sequence identity
- if a solution is found, the error can be determined more accurately
- better model error improves phases and helps locating subsequent molecules

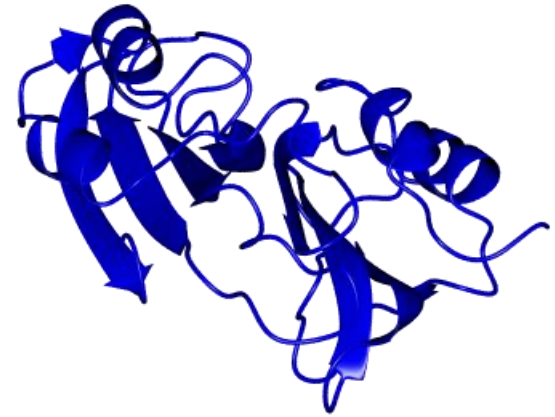




Search order determination



65% of structure
35% identical model



35% of structure
50% identical model

Which model is easier to find?



Search order determination

A better model explains more of the data



65% of structure
35% identical model

**Better model if data
resolution is low**



35% of structure
50% identical model

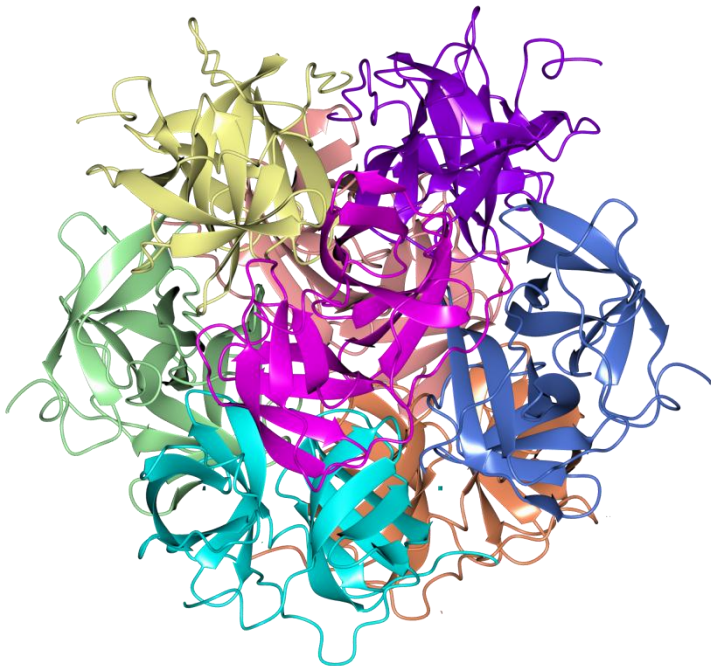
**Better model if data
resolution is high**



Model symmetry

Model with point group symmetry

- several identical solutions
- difficulty in identifying solutions



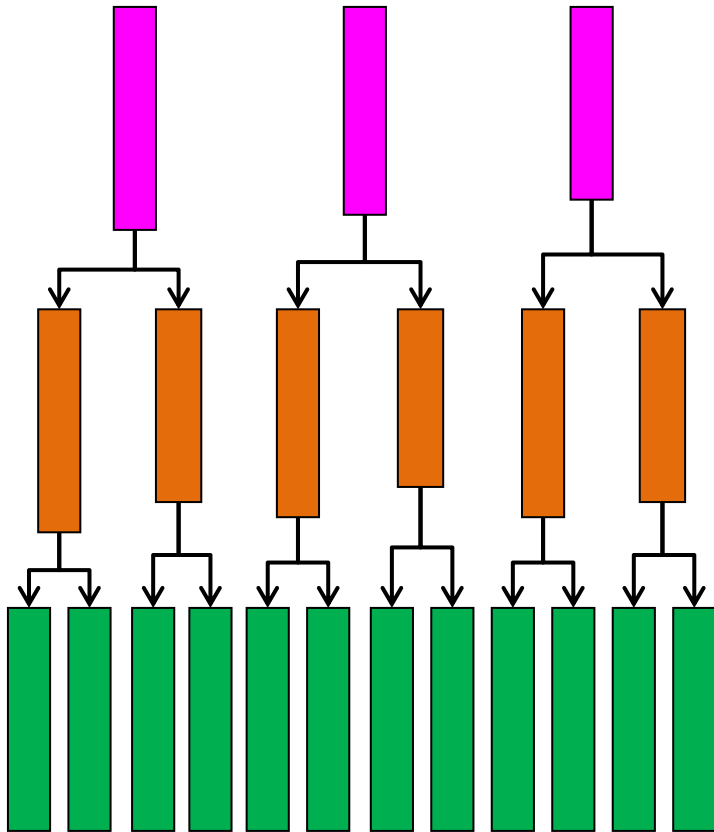
Phaser

- extended solution identity determination taking internal symmetry into account
- automatic point group symmetry detection tolerating small differences between chains



Fast mode

MR_AUTO mode

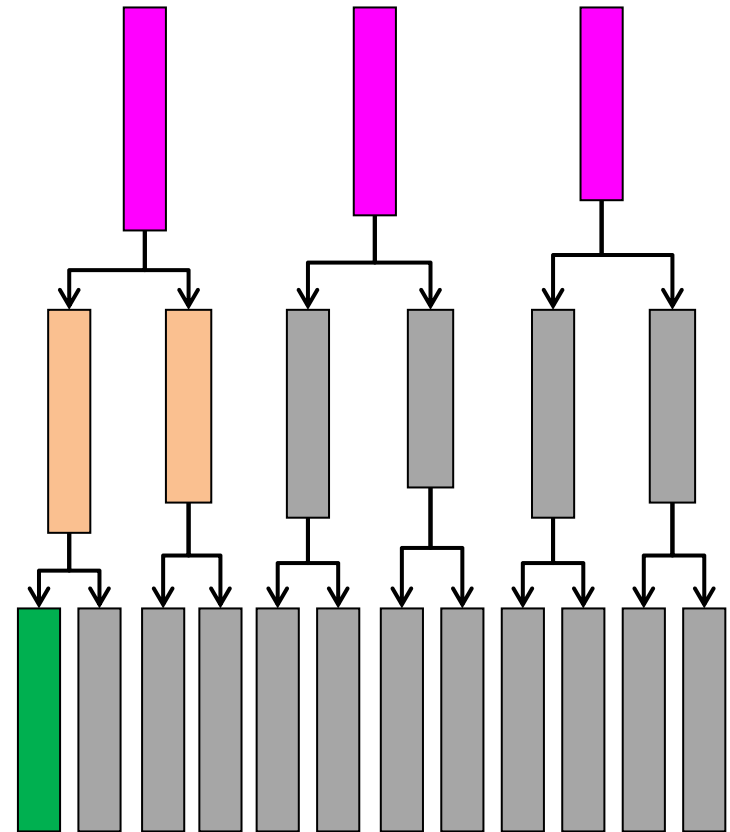


MR_FAST mode

1st

2nd

3rd





Fast mode

identify solutions ($8.0 \leq \text{TFZ}$)



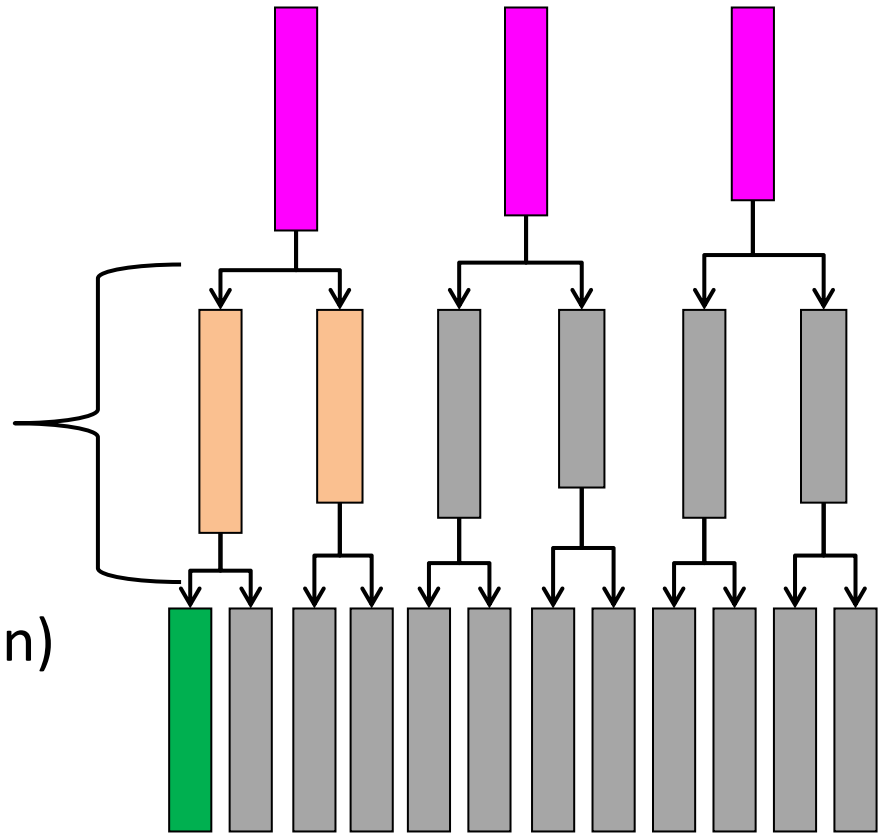
“amalgamate” independent molecules

1. discard if clashing
2. discard if LLG lower

(repeat if more than one solution)



final solution





Packing test

- **scale allowed clashes with structure:**
specify allowed clashes as percentage
- **fast packing test:** do not count all clashes,
only up to maximum allowed



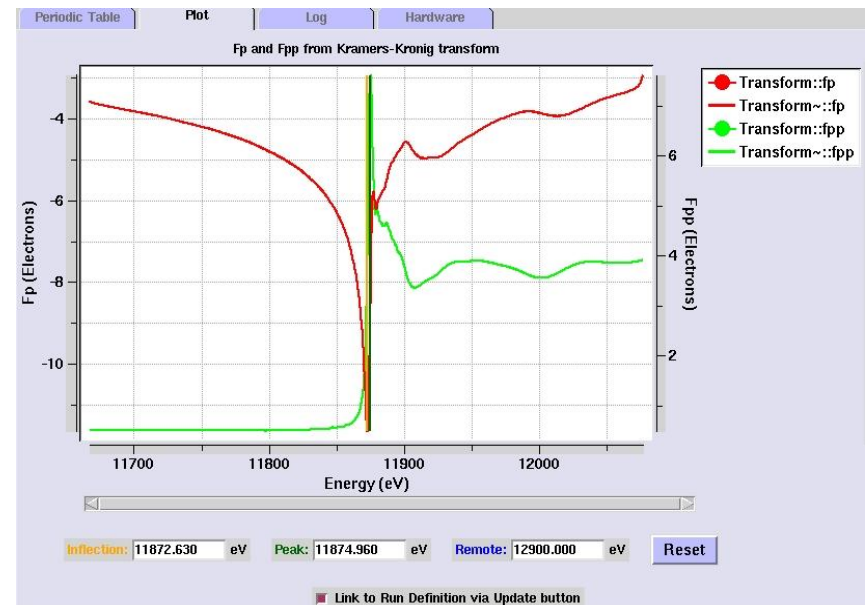
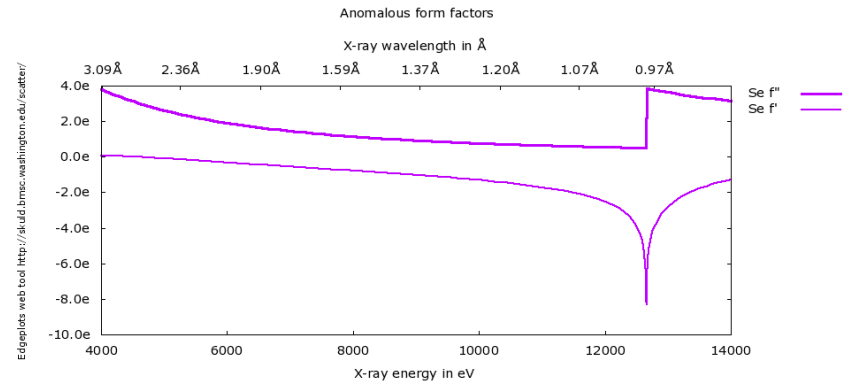
Other improvements in MR

- faster refinement (analytic second derivatives)
- ability to match solution with previously established solutions (“templates”)



f' and f'' restraints

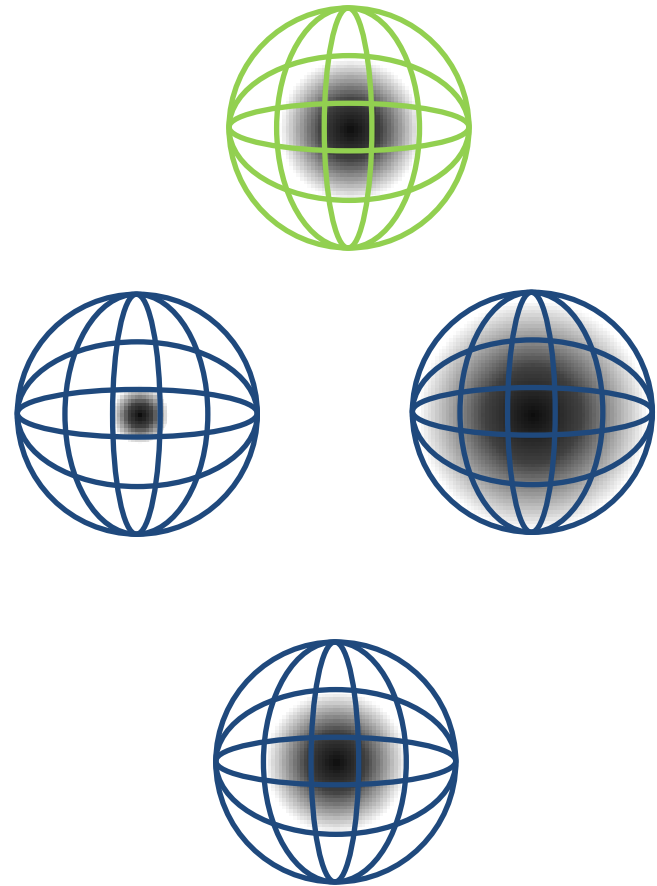
- f' and f'' are restrained to stabilize refinement (correlation with occupancy)
- tight restraints are used if not near an absorption edge
- loose restraints are used when near an edge
- **atom type** and **wavelength** now compulsory inputs





Atom type reassignment

- atom identity is guessed when site is located
- this may turn out to be incorrect when the model improves
- checks for site identity in every cycle using site occupancy calculated assuming every possible heavy atom type





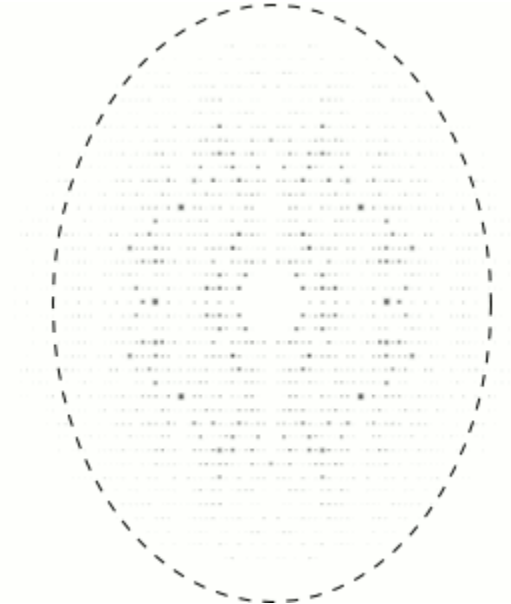
Other improvements in EP

- delete only nearest atom from partial model if an anomalous atom is found inside the model
- output map coefficients without real scattering contribution (use in density modification)



Map sharpening

- scale map coefficients according to steepest anisotropic fall-off
- visual increase in map quality





New CCP4i

Maximum Likelihood Molecular Replacement

Job title

Mode for molecular replacement: automated search

Number of processors: 1

Define data

MTZ in: GENERIC

F: SIGF

Resolution range: A to A

Space group read from mtz file; Run Phaser with

Define ensembles (models)

Ensemble # 1

Ensemble Name: ensemble1

Define ensemble via: pdb file(s)

PDB #1: GENERIC

Similarity of PDB #1 to the target structure: sequence identity

Define composition of the asymmetric unit

Total scattering determined by: solvent content of protein crystal

Search parameters

Determine ensemble search order automatically: on

Allow search with alternative ensembles (models) for a single component of the ASU: off

Perform search using: Number of copies to search for: 1

Additional parameters

Output control

Expert parameters

Run Save or Restore Close

Maximum Likelihood Experimental Phasing

Job title

Mode for experimental phasing: Single-wavelength anomalous dispersion (SAD)

Define data

MTZ in: GENERIC

Crystal Name: GENERIC

Wavelength Name:

F(+): SIGF(+)

F(-): SIGF(-)

Resolution: A to A

Space group read from mtz file

Enantiomorph choice: Original enantiomorph

Scattering: at CuK-alpha wavelength; fix FDP

LLG-map completion: on; Maximum number of cycles of completion: 50

LLG-map sigma cut-off for adding new atom sites: 6.0

LLG-map atomic separation distance cut-off: by optical resolution

LLG-map calculation atom type: Se

Define atoms

Anomalous atom sites: in PDB file; Set B-factors to: Wilson B

PDB file: GENERIC

Composition of the asymmetric unit

Total scattering determined by: components in asymmetric unit

Component #1: protein; sequence file; Number in asymmetric unit: 1

SEQ file: GENERIC

Define refinement parameters

Additional parameters

Run Save or Restore Close

(keyword input changed, old parameter files cannot be used!)



www.phaser.cimr.cam.ac.uk

- wiki format documentation (more up-to-date)
- portal to share recipes and scripts for the community
- (nightly builds distribution – coming!)

Phaserwiki - Mozilla Firefox

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http://www.phaser.cimr.cam.ac.uk/index.php/Phaser_Crystall... W Wikipedia (en)

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Phaser Crystallographic Software

Phaser is a program for phasing macromolecular crystal structures with maximum likelihood methods. It has been developed by Randy Read's group at the University of Cambridge and is available through the [Phenix](#) and [CCP4](#) software suites, and directly from this site.

This website supersedes <http://www-structmed.cimr.cam.ac.uk/phaser/> which is now **obsolete** (and redirects to this page). A copy of the obsolete website can be found at http://www-structmed.cimr.cam.ac.uk/phaser_obsolete/

Documentation

Use the sidebar to navigate through the extensive documentation for Phaser.

- Current Phenix release is **Phaser-2.3** → [Manual](#)
- Current CCP4 release is **Phaser-2.1** → [Manual](#)

User Stories

Users are invited to contribute to the [User Stories](#) section of Phaserwiki. If you have managed to solve a difficult crystallographic problem with Phaser, please write a summary of your structure solution here.

Referencing Phaser

Citing crystallographic software in your paper is important for funding new software development. We rely on your citations to convince funding bodies that our software is being used.

If you solve a structure with Phaser, please cite

Phaser crystallographic software pdf

McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, Read RJ. *J. Appl. Cryst.* (2007). 40, 658-674.

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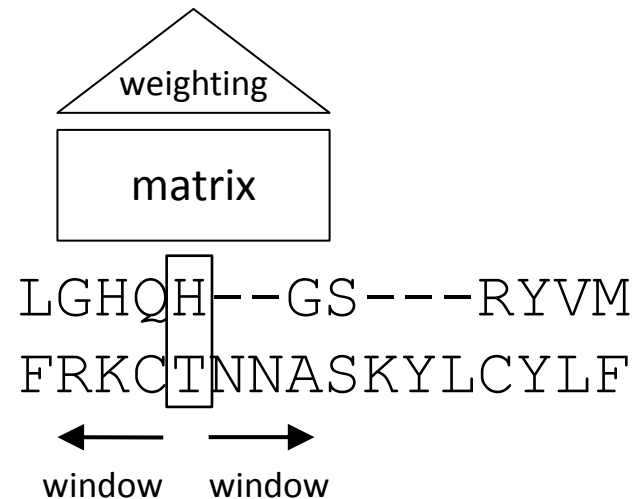
This page was last modified on 10 August 2010, at 06:40. This page has been accessed 3,998 times. [About Phaserwiki](#)

Done



Sculptor: model improvement

- improve models using information from sequence alignment (sequence similarity)
- modifies main chain, side chain and B-factors
- pre-defined protocols to generate good model variability



sequence similarity
calculation



Sculptor: model improvement

Model
CHAINSAW

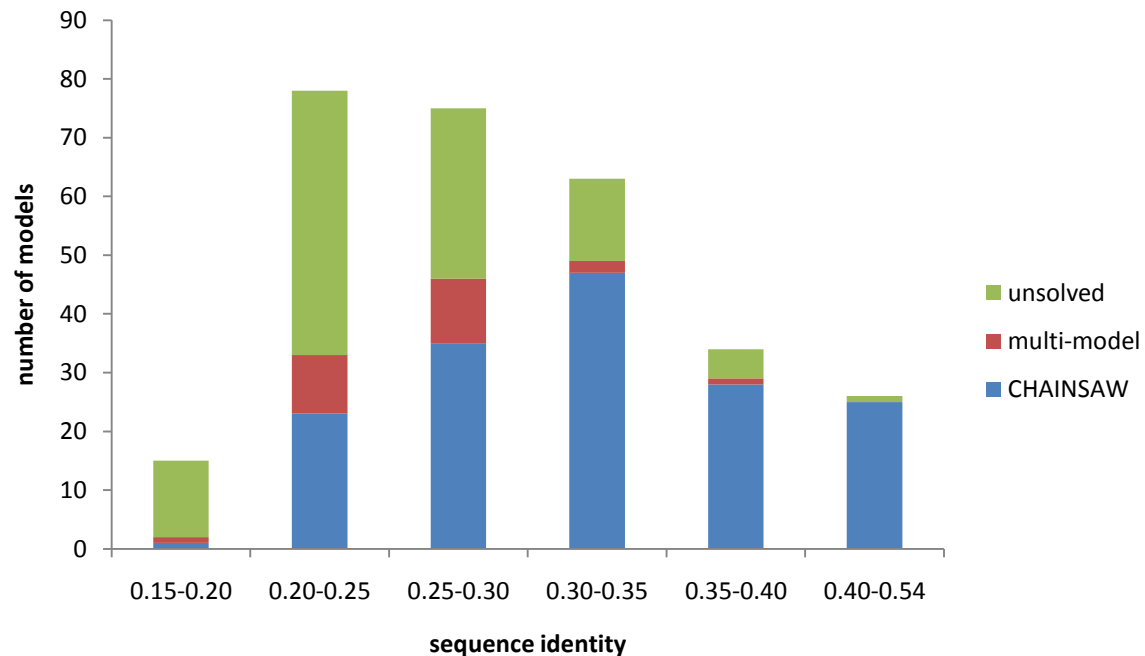
Model
Sculptor

Model
Sculptor
+ASA B-factors

Model
Sculptor
+sequence similarity B-factors

Model
Sculptor
+sequence similarity B-factors
+ ASA B-factors

Calculations with CLUSTALW alignments





Sculptor: interactive editing

The screenshot shows the Coot software interface with the Sculptor GUI open. The main window displays a protein backbone in blue and red. The Sculptor GUI is titled "Sculptor GUI" and contains the following fields and options:

- Alignment:
- Model:
- Chain:
- General | Mainchain | Sidechain (selected)
- Renumbering:
 - Target
 - Model
 - None
- Rename residues
- Generate missing Cbeta
-

At the bottom of the window, a status bar reads: "Successfully read coordinates file /tmp/tmp8g9EMg/tmpgRcopP_baseline.pdb. Molecule number 2 created."

The system tray at the bottom shows: Applications Places System [icons] GBr [icons] 9 °C Fri Mar 5, 2:50 PM Gabor Bunkoczi



Acknowledgements

Python-based Hierarchical Environment for Integrated Xtallography



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