Using SAD data in *Phaser*

Phasing and extended model completion

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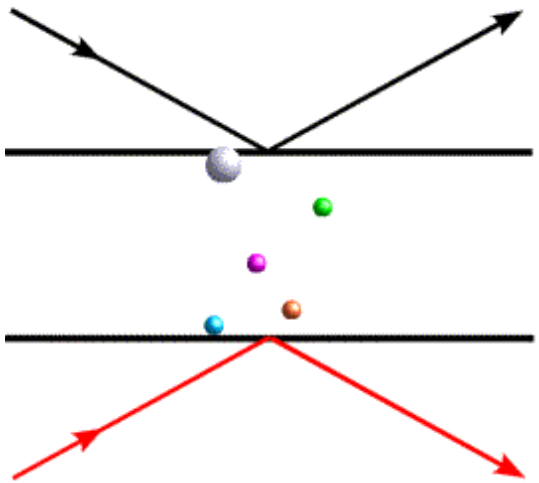
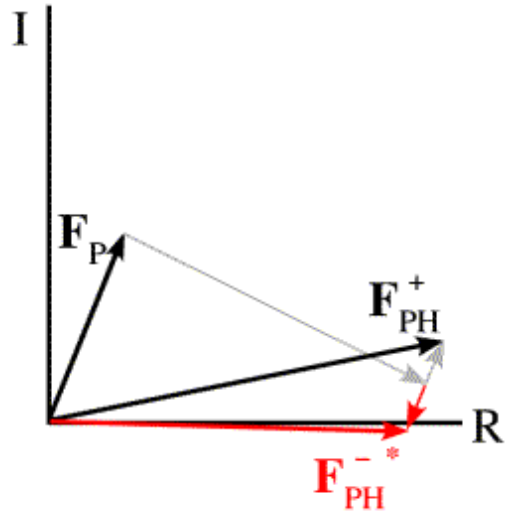
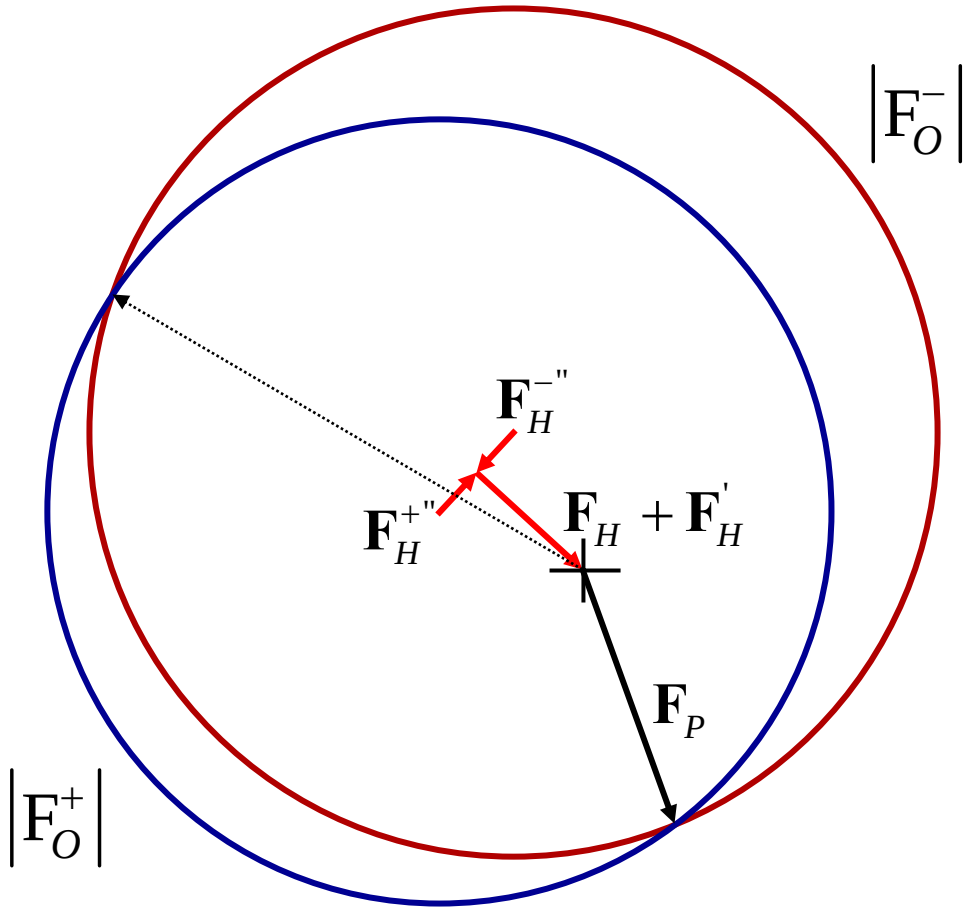
Diffraction with anomalous scatterers

- SAD: single-wavelength anomalous diffraction





Harker construction for SAD phasing



Principle of maximum likelihood

- How consistent is the model with the data?
- *What is the probability that the data would be measured if the model were correct?*

$$L = \mathrm{p}(data; model)$$

- Optimise model by adjusting parameters in probability distribution

Illustration of likelihood

- Generate data randomly from Gaussian distribution with $\mu=5$, $\sigma=1$

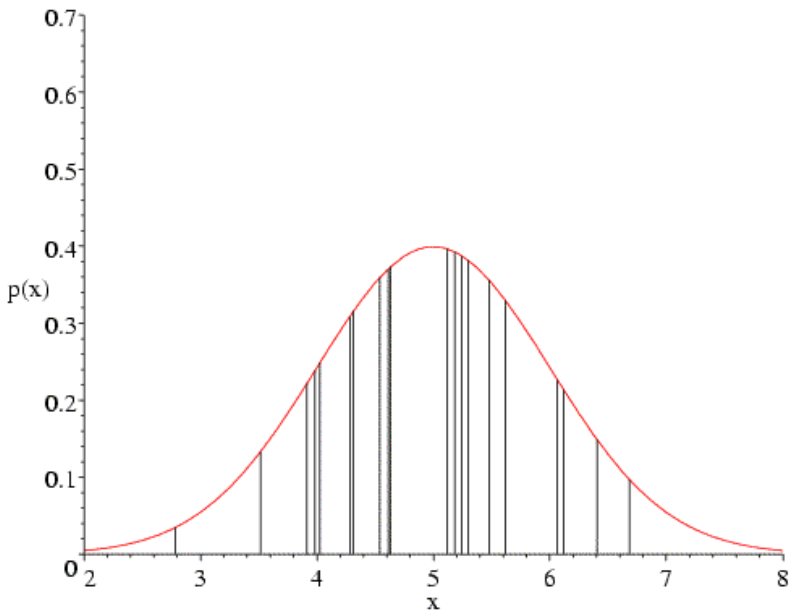


Illustration of likelihood

- With incorrect mean, some points become improbable

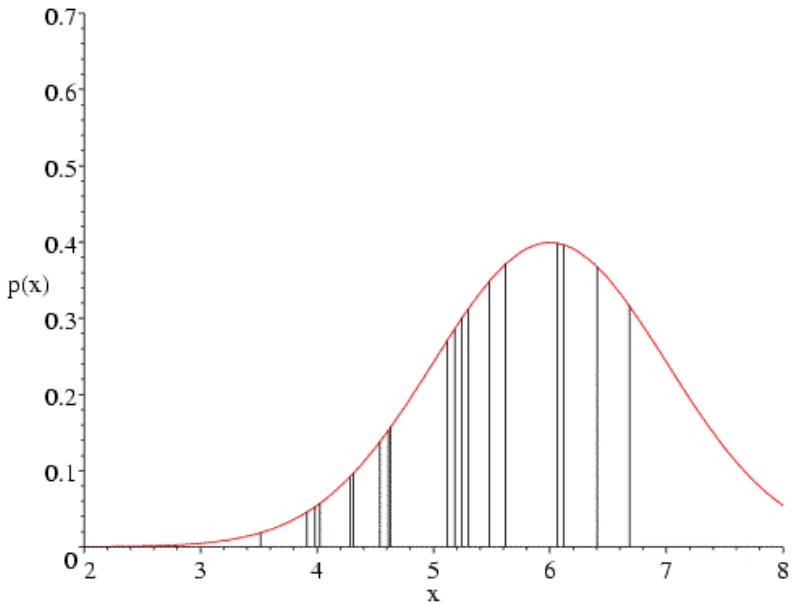


Illustration of likelihood

- With incorrect standard deviation, some points become improbable

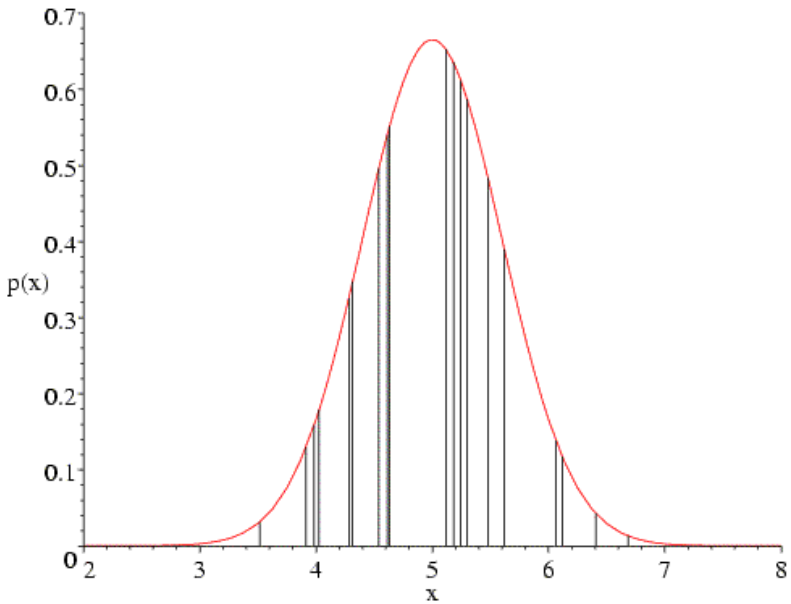
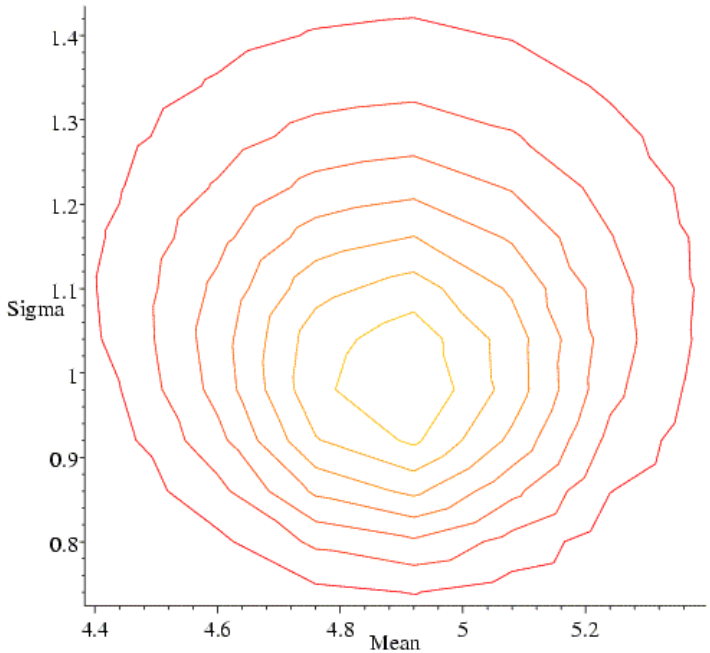


Illustration of likelihood

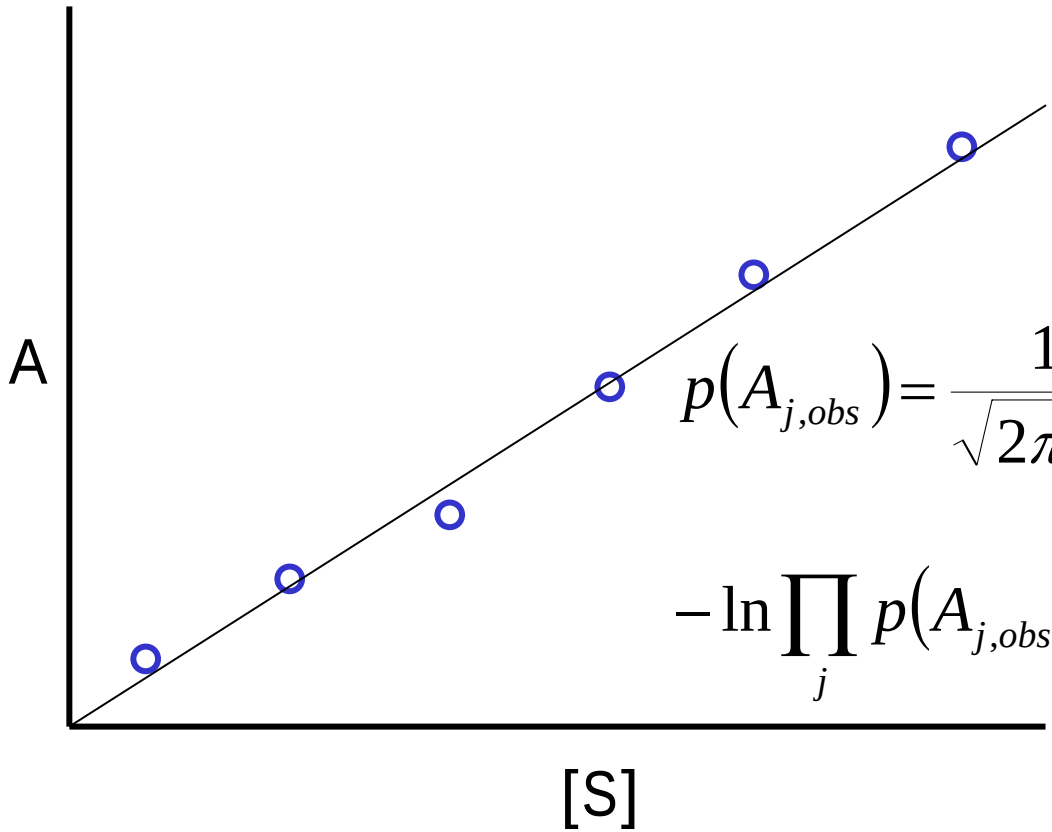
- Plot likelihood as function of mean and sigma



Least squares and likelihood

- Most experiments have multiple sources of error: Gaussian error in observations
  - Central Limit Theorem
- Likelihood for Gaussians = least squares

Least-squares line fitting



$$p\left(A_{j,obs}\right) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[-\frac{\left(A_{j,obs} - A_{j,calc}\right)^2}{2\sigma^2}\right]$$

$$-\ln \prod_j p\left(A_{j,obs}\right) \propto c + \sum_j \frac{\left(A_{j,obs} - A_{j,calc}\right)^2}{\sigma^2}$$

Why not least squares in crystallography?

- Gaussian error for observations
- Error in predicting observation generally includes difference between structure factors
  - this is Gaussian in *phased* difference
  - *e.g.* $\mathbf{F}$ *vs.* $\mathbf{F}_C$ from model, $\mathbf{F}_P$ *vs.* $\mathbf{F}_{PH}$
- Phased error usually dominates
  - elimination of unknown phase changes probabilities

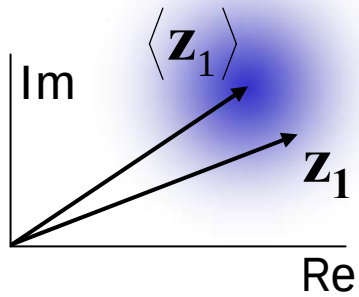
Applying likelihood to crystallography

- Find probability distribution for observations
  - start from structure factor probabilities
  - eliminate unknown phase angles
- Adjust parameters to optimise likelihood
- Applications:
  - calculating model phase probabilities
  - structure refinement
  - experimental phasing (isomorphous/anomalous)
  - likelihood-based molecular replacement

Multivariate complex normal distribution

- Complex normal

$$p(\mathbf{z}_1) = \frac{1}{\pi\Sigma}\exp\left[-\frac{|\mathbf{z}_1 - \langle \mathbf{z}_1 \rangle|^2}{\Sigma}\right]$$



$$= \frac{1}{\pi\Sigma}\exp\left[-(\mathbf{z}_1 - \langle \mathbf{z}_1 \rangle)^* \Sigma^{-1}(\mathbf{z}_1 - \langle \mathbf{z}_1 \rangle)\right]$$

- Multivariate complex normal distribution

$$p(\mathbf{z}) = \frac{1}{|\pi\boldsymbol{\Sigma}|}\exp\left[-(\mathbf{z} - \langle \mathbf{z} \rangle)^H \boldsymbol{\Sigma}^{-1}(\mathbf{z} - \langle \mathbf{z} \rangle)\right], \text{ where}$$

$$\text{elements of } \boldsymbol{\Sigma} \text{ given by } \boldsymbol{\sigma}_{ij} = \left\langle (\mathbf{z}_i - \langle \mathbf{z}_i \rangle)(\mathbf{z}_j - \langle \mathbf{z}_j \rangle)^* \right\rangle$$

SAD likelihood function

- Based on probability of F^+ and F^- given model

$$p\left(\mathbf{F}_O^+, \mathbf{F}_O^-, \mathbf{H}^+, \mathbf{H}^-\right) \rightarrow p\left(\mathbf{F}_O^+, \mathbf{F}_O^-; \mathbf{H}^+, \mathbf{H}^-\right)$$

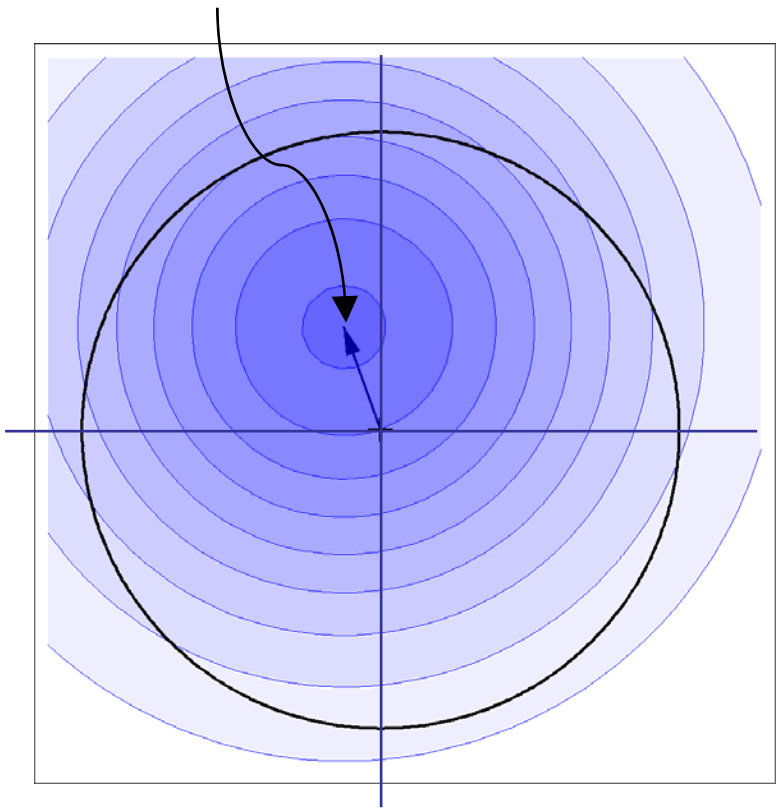
- Factor joint probability into two parts

$$p\left(\mathbf{F}_O^+, \mathbf{F}_O^-; \mathbf{H}^+, \mathbf{H}^-\right) = p\left(\mathbf{F}_O^+; \mathbf{F}_O^-, \mathbf{H}^+, \mathbf{H}^-\right) p\left(\mathbf{F}_O^-; \mathbf{H}^-\right)$$

- Integrate out unknown phases, α^+ and α^-

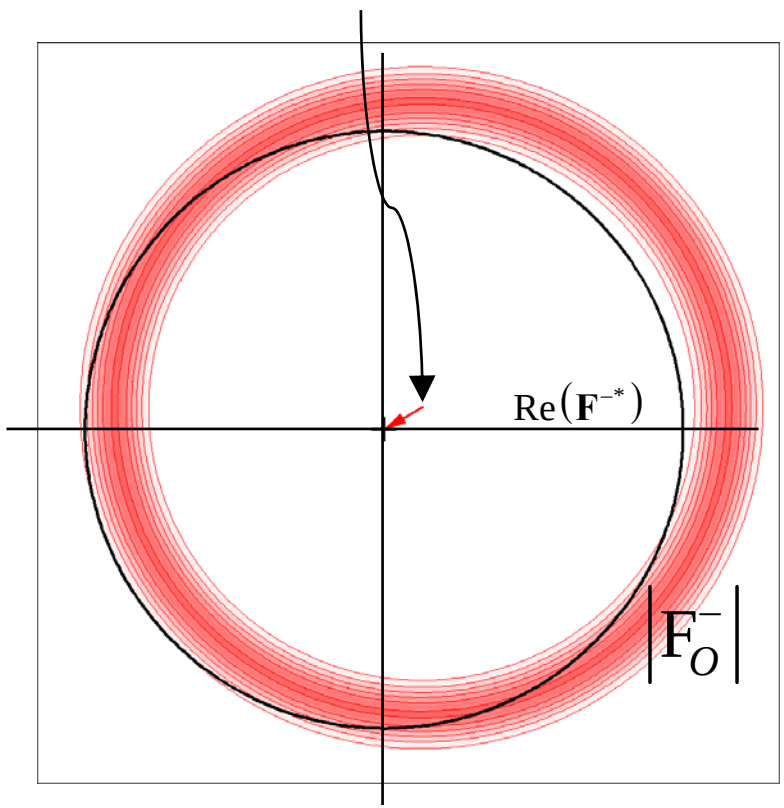
Intuitive understanding of SAD phasing

Expected value of $\mathbf{F}^{-*}$ $(\mathbf{H}^{-*})$



$P\left(\mathrm{F}_O^-, \alpha_O^-; \mathbf{H}^{-*}\right)$

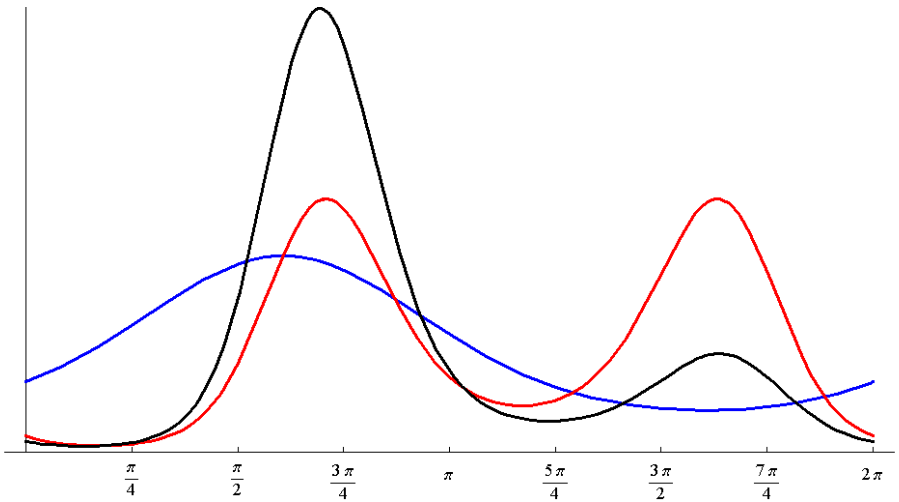
Expected difference between $\mathbf{F}^{+}$ and $\mathbf{F}^{-*}$



$P\left(\mathrm{F}_O^+; \mathrm{F}_O^-, \mathbf{H}^+, \mathbf{H}^{-*}\right)$

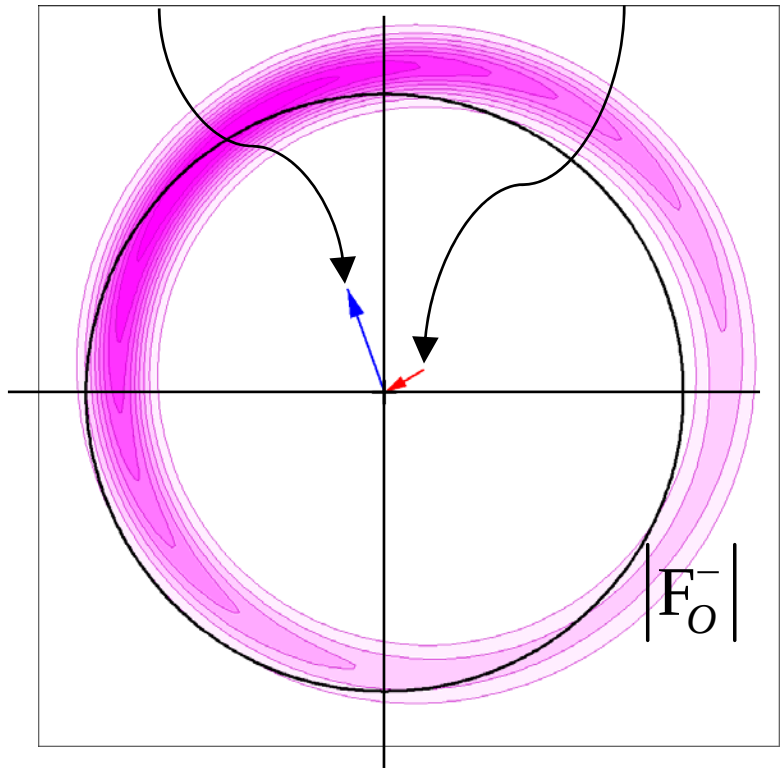
Intuitive understanding of SAD phasing

Total likelihood is integral of the product of the two distributions under the black circle



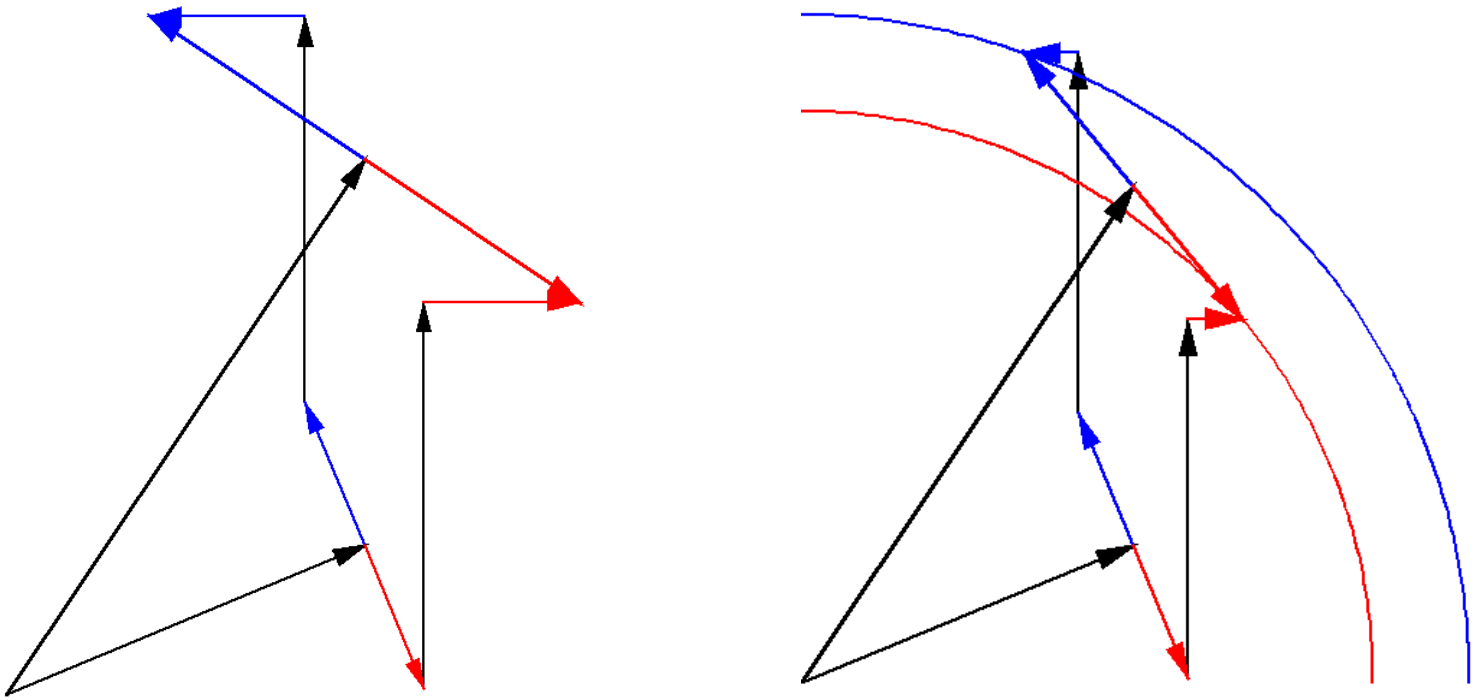
Expected value of $\mathbf{F}^{-*}$ $(\mathbf{H}^{-*})$

Expected difference between $\mathbf{F}^{+}$ and $\mathbf{F}^{-*}$



Breakdown of Friedel's law

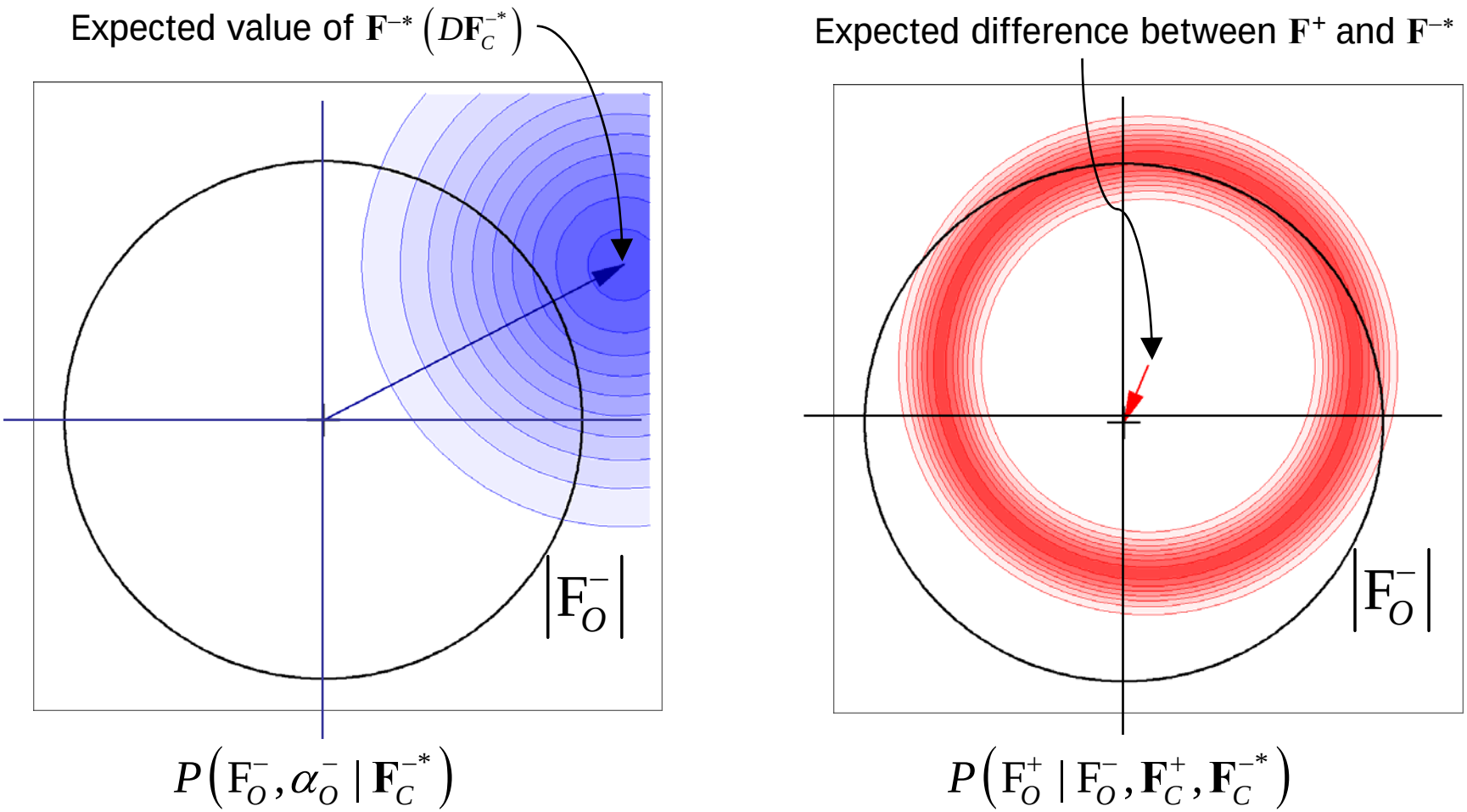
- Friedel's law breaks down for mixture of scatterers differing in real:anomalous ratio



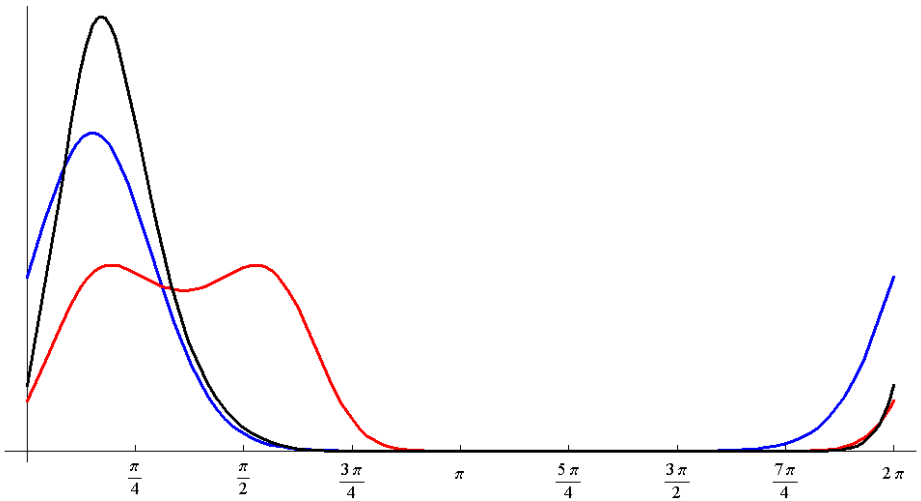
SAD log-likelihood gradient (LLG) map

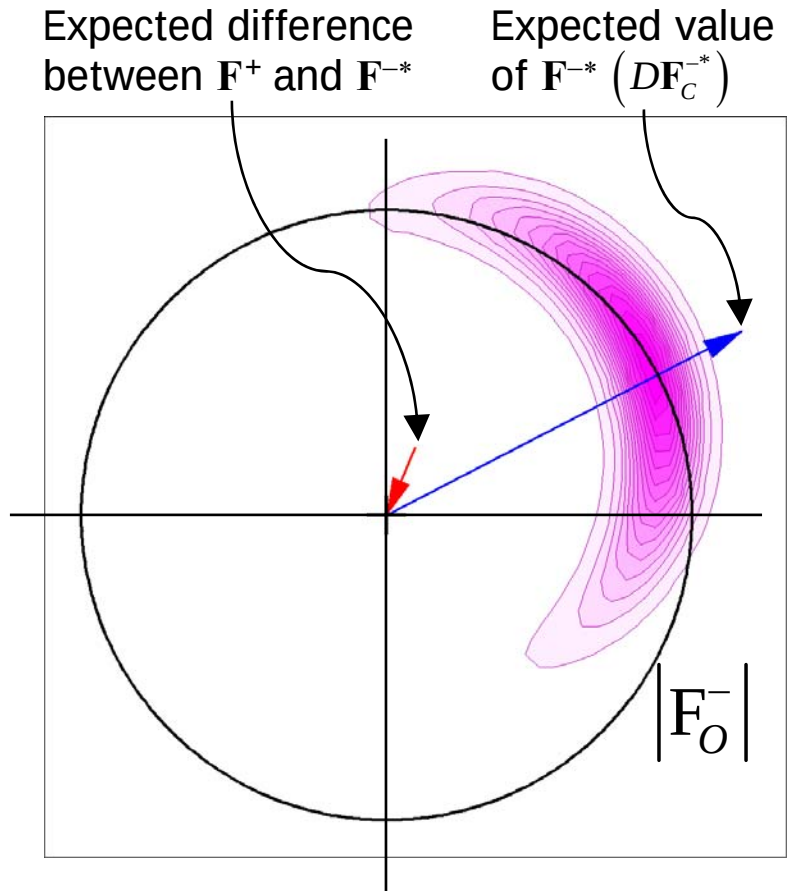
- Compute derivative of log-likelihood with respect to heavy atom structure factor
- Fourier transform gives map of where likelihood target would like to see changes in anomalous scatterer model
- Very sensitive to minor sites
  - picks up sites identified as water molecules in refined structures determined by halide soaks
- http://www-structmed.cimr.cam.ac.uk/phaser/tutorial
  - tutorial with data for lysozyme iodide soak

SAD with partial model



SAD with partial model





Combining MR and SAD

- CuKα data to 1.9Å on hen egg-white lysozyme
  - can't find sulfurs with HySS or SHELXD
- Solve by MR with goat alpha-lactalbumin (40% identical)
- Use MR model as "substructure" for SAD
  - look for S atoms in LLG map (finds all 10 S, 5-9 Cl^-)
  - phases automatically combine MR and SAD
- Automated fitting with density-modified map
- http://www-structmed.cimr.cam.ac.uk/phaser/tutorial
  - tutorial with these data

Iterative model-building with SAD

- Nitrate reductase structure (Natalie Strynadka)
  - integral membrane protein, 1976 residues
  - contains 21 Fe atoms, 1 Mo, 118 S, 5 P (146 total)
  - solved using combination of Fe-MAD, MIRAS
- Fe peak SAD data only
  - find 11 “Fe” sites with phenix.hyss
    - several are super-sites of Fe_4S_4 clusters
  - phase and complete adding Fe, Mo, S with *Phaser*
    - total of 57 sites: 20 Fe, 6 Mo, 31 S
    - superatoms are resolved, 51 of 57 are identified correctly
    - correct hand indicated by number of sites, LLG score

Iterative model-building and phasing

- Improve phases by density modification
- Build with ARP/wARP (or Resolve)
  - 1607 residues, 1368 docked in sequence
- LLG completion from ARP/wARP model
  - 105 sites, 92 correctly identified
- Repeat DM and ARP/wARP
  - 1813 residues, 1775 docked in sequence

Automation of SAD phasing

- Functions are all available from Python
  - used for SAD in AutoSolve wizard
  - can run from HAPPy (CCP4)
- Log-likelihood-gradient completion
  - look for one or several types of scatterer
    - start from MR model or partial substructure
  - analyse map to add sites, make atoms anisotropic
  - delete atoms that fade away
  - change atom type if occupancy far from one
  - repeat to convergence

Absolute scaling

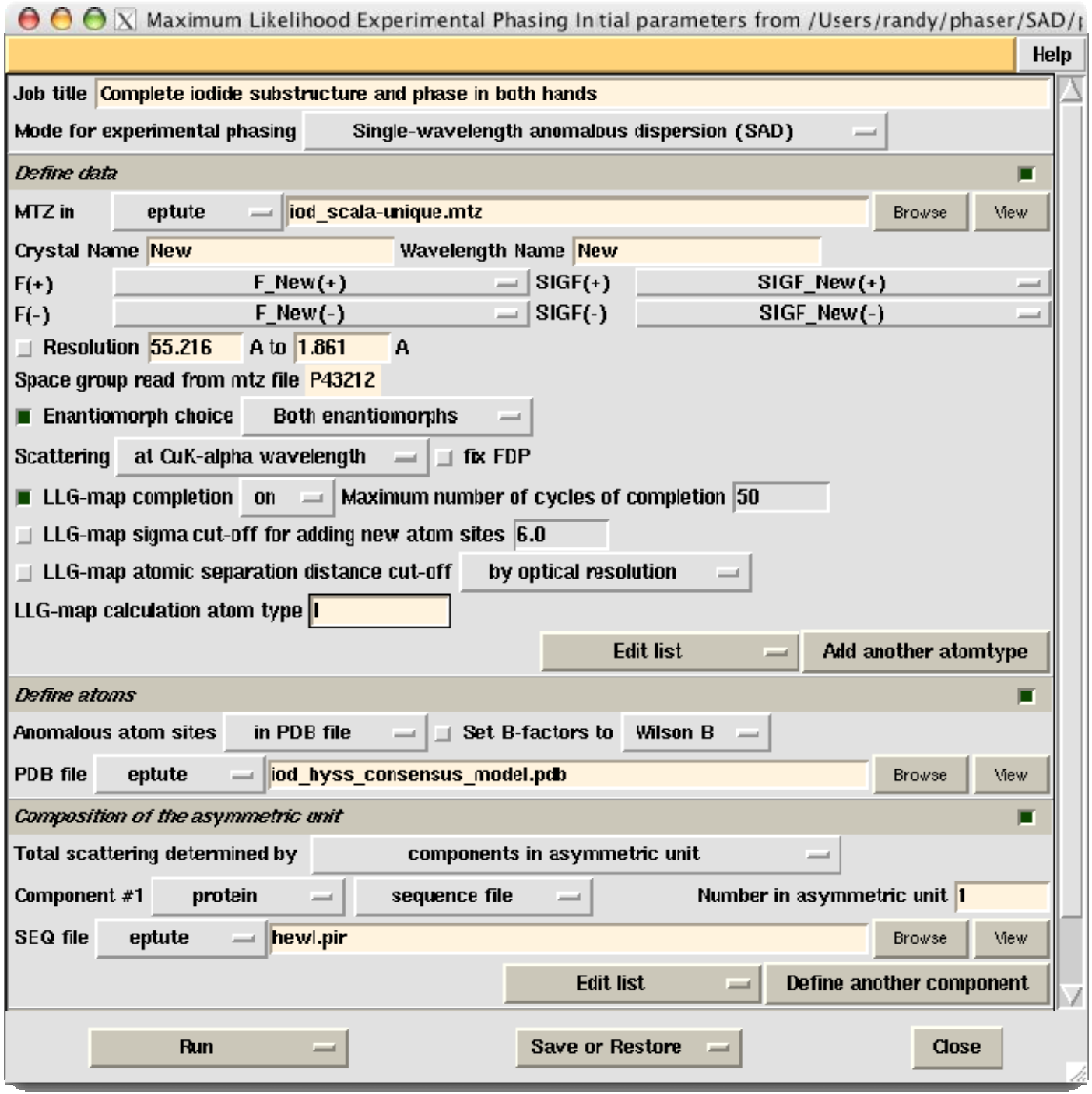
- SAD target uses real (partial structure) scattering and anomalous scattering
  - best results if f'' known precisely
  - helps to have data on absolute scale
- use BEST data from Sasha Popov
  - average intensities as function of resolution
- get Wilson B-factor, absolute scale
  - have to define composition of crystal

Practical aspects of SAD phasing in *Phaser*

- Provide information about cell content
  - sequence, molecular weight, percent solvent...
  - used to put data on absolute scale
    - occupancies are reasonably accurate
- Provide information about f” values
  - wavelength (table lookup) or measured
  - refined by default if only one atom type
- Try both hands if uncertain
  - separate completion if mixture of atom types

SAD phasing in CCP4

- ccp4i interface has *Phaser* SAD phasing module
- Two modes:
  - “Single-wavelength anomalous dispersion (SAD)”
    - start from substructure of anomalous scatterers
    - can test both hands, complete with multiple scatterers
  - “SAD with molecular replacement partial structure”
    - start from substructure of non-anomalous scatterers
    - optionally include known anomalous scatterers


Maximum Likelihood Experimental Phasing Initial parameters from /Users/randy/phaser/SAD/
Help
Job title
Complete iodide substructure and phase in both hands
Mode for experimental phasing
Single-wavelength anomalous dispersion (SAD)
Define data
MTZ in
eptute
iod_scala-unique.mtz
Browse
View
Crystal Name
New
Wavelength Name
New
F(+)
F_New(+)
SIGF(+)
SIGF_New(+)
F(-)
F_New(-)
SIGF(-)
SIGF_New(-)
Resolution
55.216
A to
1.861
A
Space group read from mtz file
P43212
Enantiomorph choice
Both enantiomorphs
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
I
Edit list
Add another atomtype
Define atoms
Anomalous atom sites
in PDB file
Set B-factors to
Wilson B
PDB file
eptute
iod_hyss_consensus_model.pdb
Browse
View
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
eptute
hewl.pir
Browse
View
Edit list
Define another component
Run
Save or Restore
Close

SAD phasing in Phenix

- Use AutoSolve wizard
  - GUI version prompts for necessary information
    - command-line version is faster
  - finds sites with Hyss
  - automatically uses *Phaser* for phasing if SAD data
  - tests both hands, chooses best hand
  - carries out Resolve density modification and model-building

Background information

- "*Phaser* crystallographic software", McCoy, Grosse-Kunstleve, Adams, Winn, Storoni & Read (2007), *J. Appl. Cryst.* **40**, 658-674.
  - plus papers cited here
- "Liking likelihood", Airlie J. McCoy (2004), *Acta Cryst. D***60**, 2169-2183.

- http://www-structmed.cimr.cam.ac.uk/phaser
- http://www-structmed.cimr.cam.ac.uk/Course

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  - Airlie McCoy, Laurent Storoni, Gabor Bunkoczi, Rob Oeffner
- Experimental phasing
  - Raj Pannu, Airlie McCoy, Laurent Storoni
- PHENIX collaboration
  - Ralf Grosse-Kunstleve, Nigel Moriarty, Paul Adams
  - Tom Terwilliger

wellcome trust

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CCP4