



Experimental phasing

Charles Ballard
(original Gábor Bunkóczi)

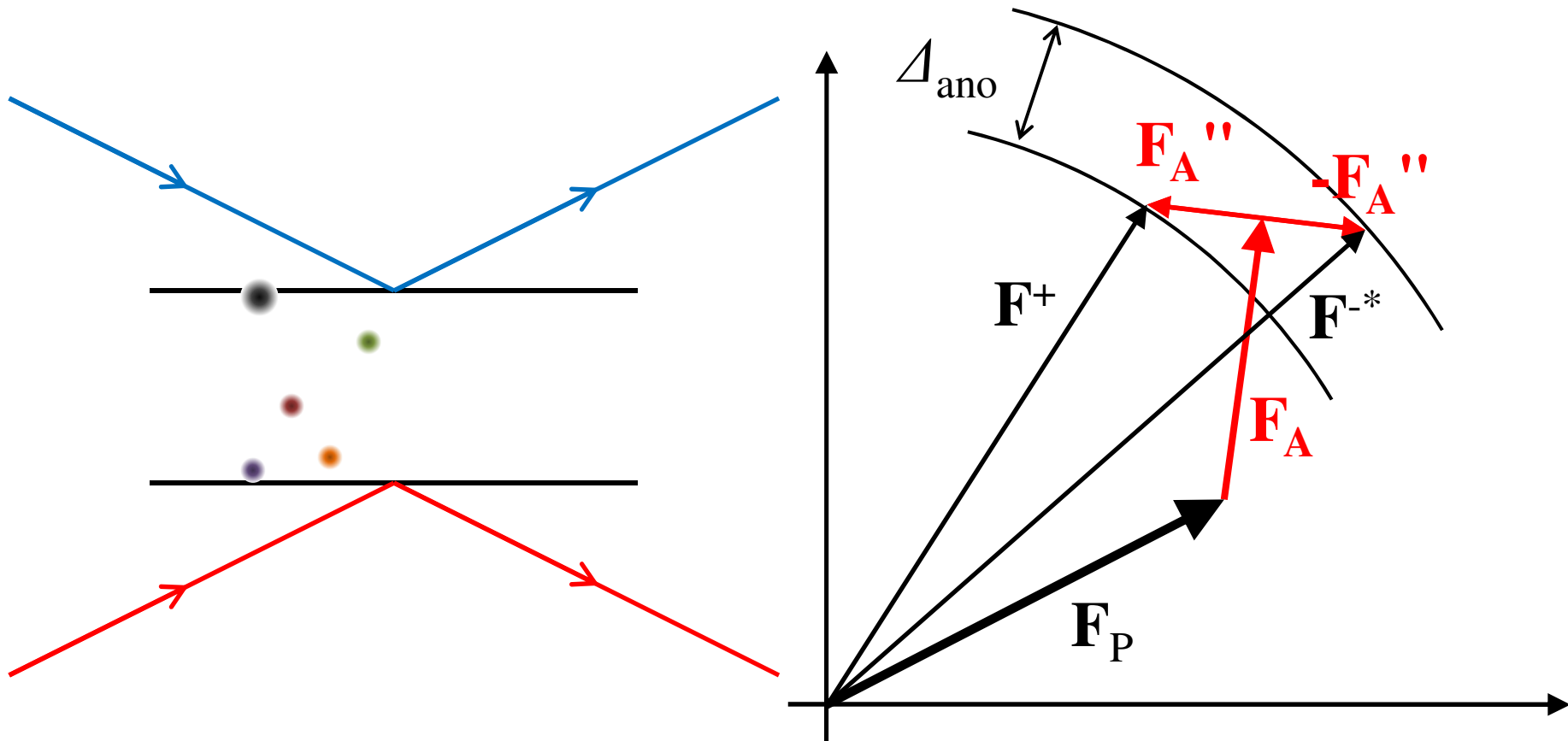
CCP4 Workshop
7 December 2011



Anomalous diffraction

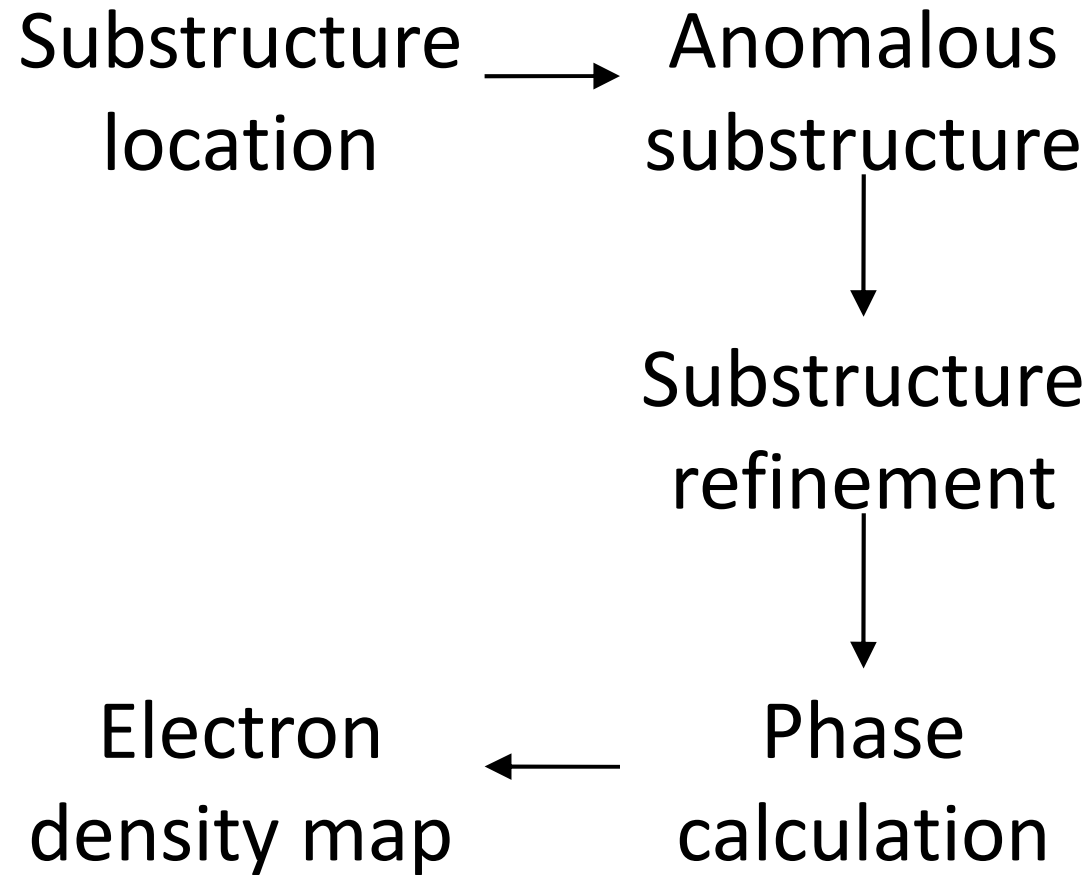
$\mathbf{F}_P$ – protein

$\mathbf{F}_A$ – anomalous substructure





Phasing





Phase calculation in SAD

F_o^+, F_o^- – measurements

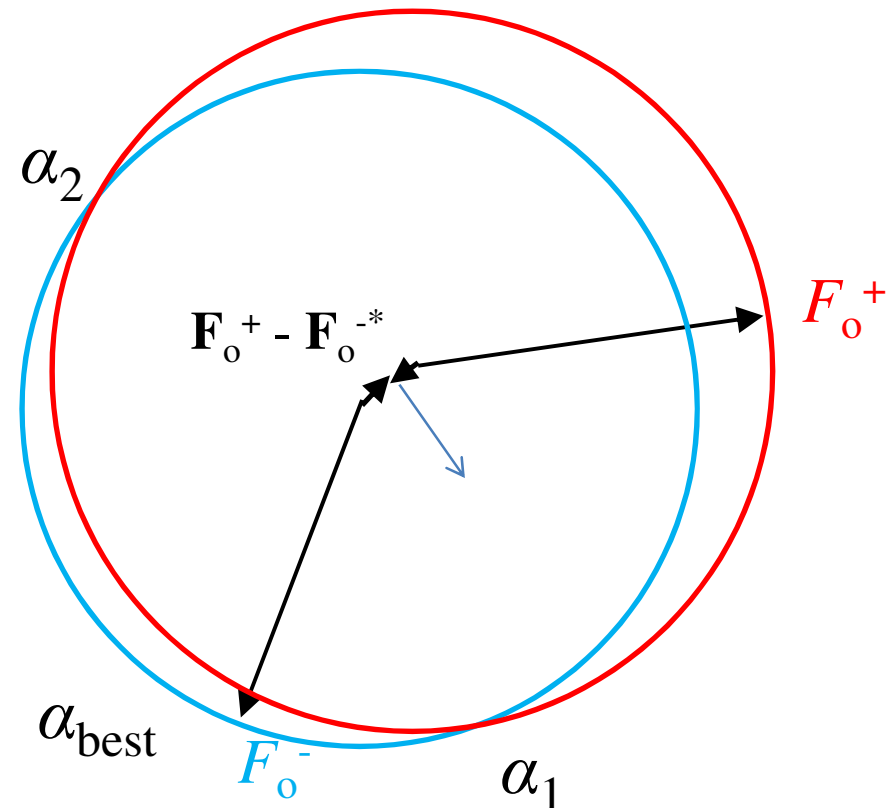
$F_o^+ - F_o^{-*}$ – calculated from substructure ($-2F_A''$)

$\alpha_1, \alpha_2, \alpha_{\text{best}}$ – phase angles

Approximate map given by

$$mF_{\text{best}} e^{i\alpha_{\text{best}}}$$

With $F_{\text{best}} = \frac{|F^+| + |F^-|}{2.0}$, $m = \cos \frac{1}{2}(\alpha_1 - \alpha_2)$





SAD likelihood function

Probability of experimental observations. Observations correlated, multivariate distribution.

$$\begin{aligned} P(F_o^+, F_o^{-*}, H^+, H^{-*}) &\rightarrow P(F_o^+, F_o^{-*}; H^+, H^{-*}) \\ &\rightarrow P(F_o^+, \alpha^+, F_o^-, \alpha^-; H^+, H^{-*}) \end{aligned}$$

Factor joint probability into two parts

$$P(F_o^+, F_o^-; H^+, H^{-*}) = P(F_o^+; F_o^-, H^+, H^{-*}) P(F_o^-, H^{-*})$$

Marginalise phases α^+ and α^-



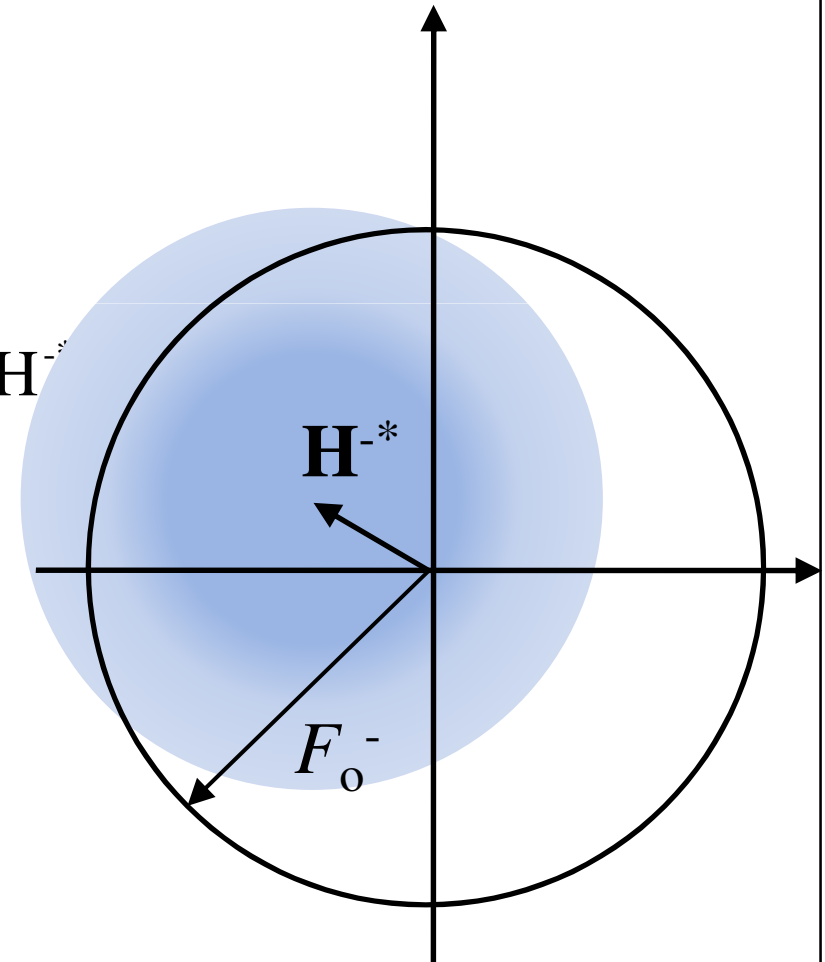
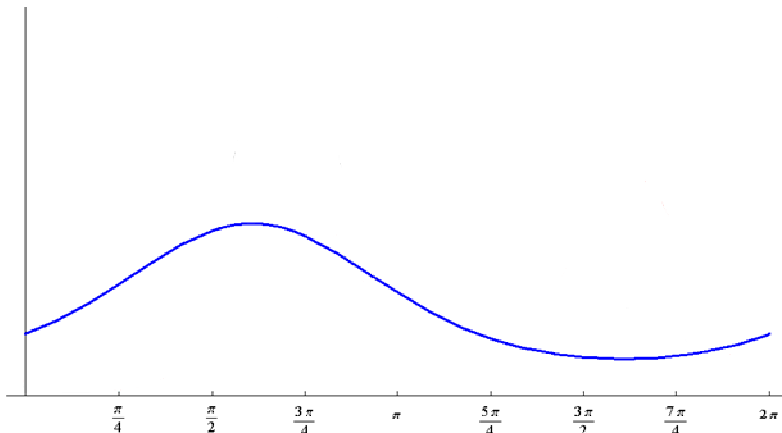
SAD function – normal scattering

$$P(F_o^-, \alpha^-; \mathbf{H}^-) =$$

$$\frac{F_o^-}{\pi \Sigma^-} \exp \left(- \frac{|F_o^- e^{i\alpha^-} - D\mathbf{H}^{-*}|^2}{\Sigma^-} \right)$$

Σ^- accounts for errors between F_o^- and $\mathbf{H}^{-*}$

Primarily normal scattering.





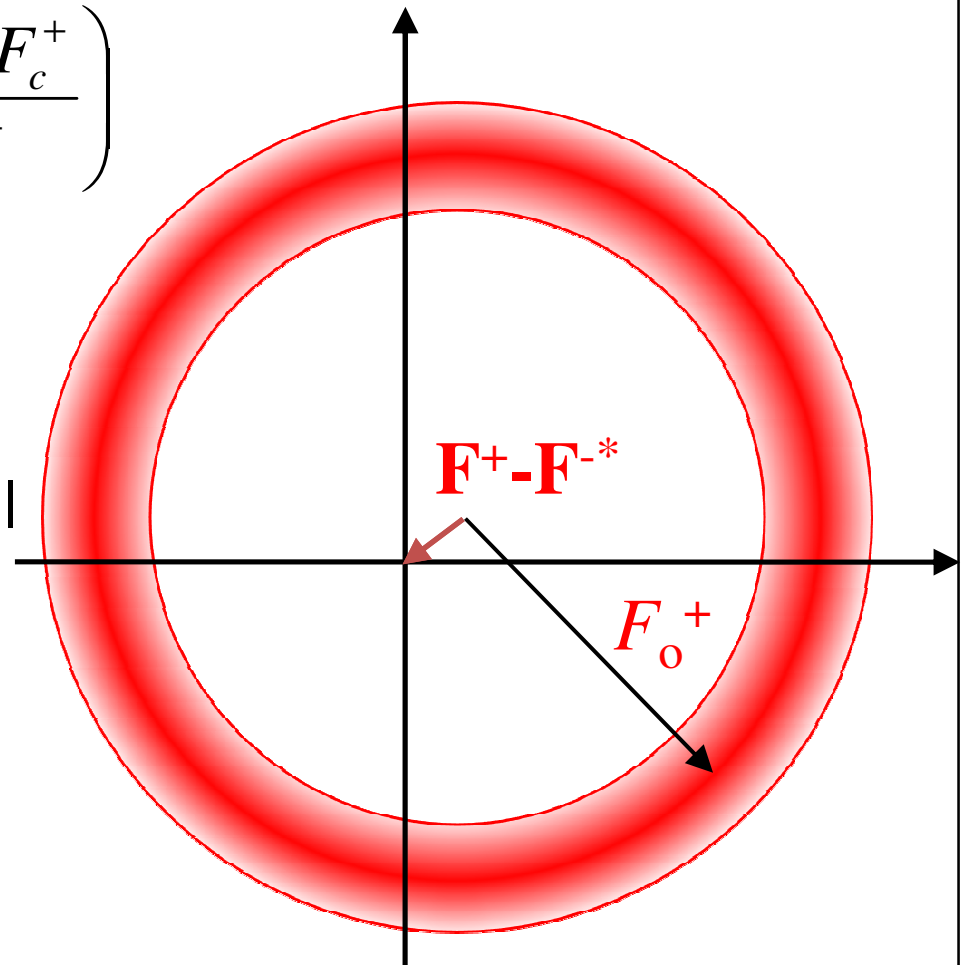
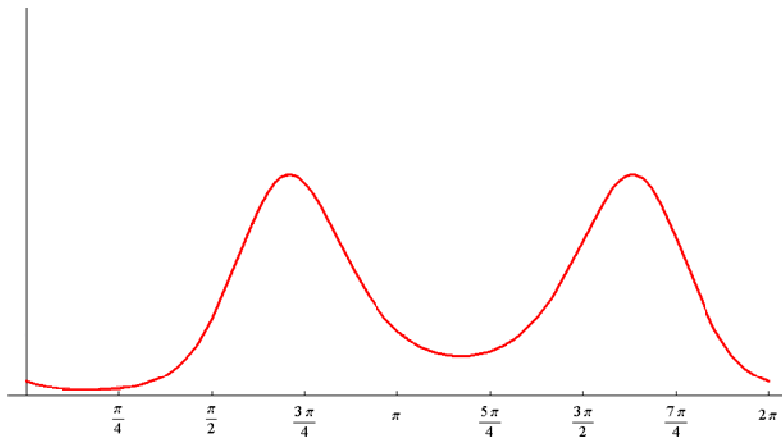
SAD function - anomalous

$$P(F_o^+; \mathbf{F}_o^-, \mathbf{H}^+, \mathbf{H}^{-*}) = \frac{2F_o^+}{\Sigma^+} \exp\left(-\frac{(F_o^+ - F_c^+)^2}{\Sigma^+}\right) eI_0\left(\frac{2F_o^+ F_c^+}{\Sigma^+}\right)$$

Σ^+ accounts for errors between

F_o^+ and F_c^+ , where

$$F_c^+ = |H^+ + D_\Phi e^{i\alpha_\Phi} (F^- e^{i\alpha_-} - H^{-*})|$$





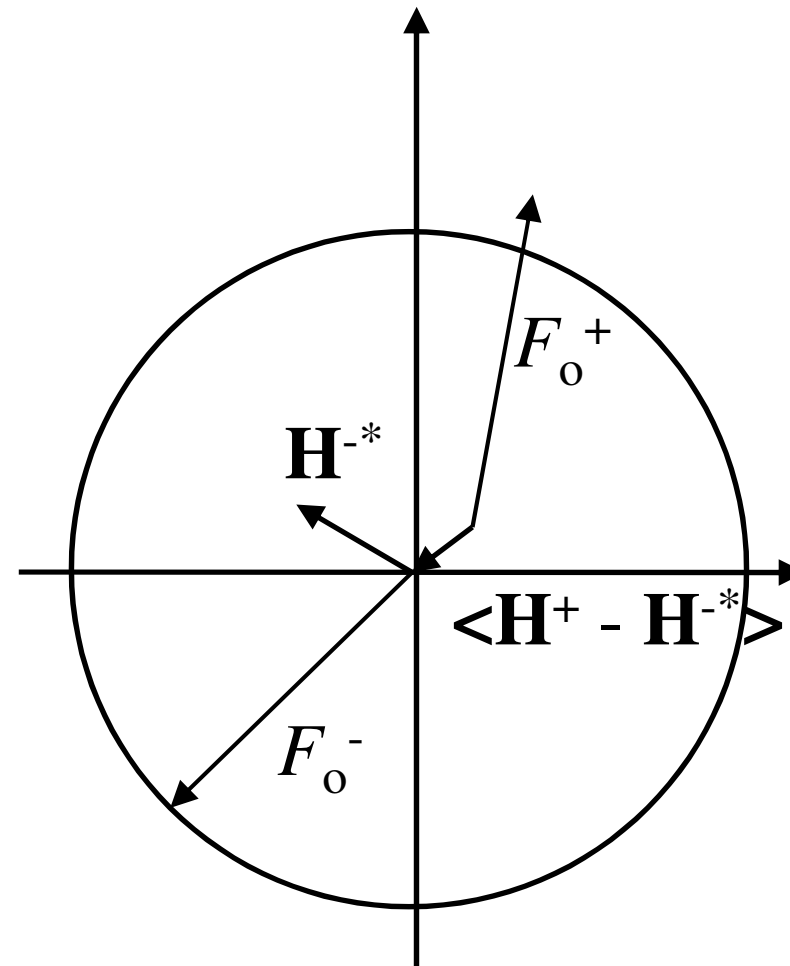
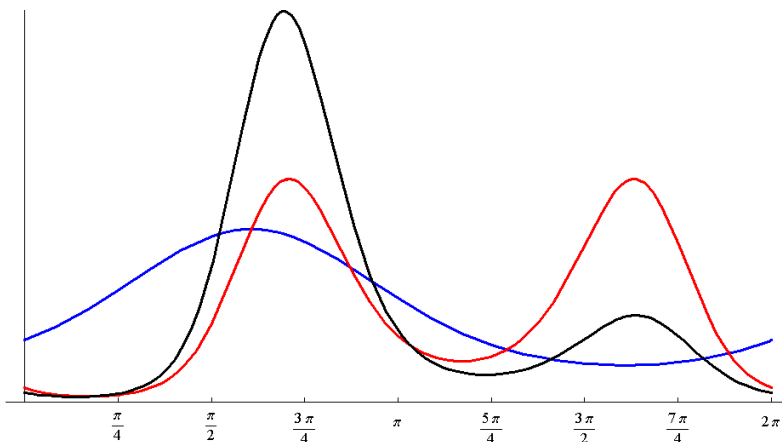
SAD function

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

Normal scattering

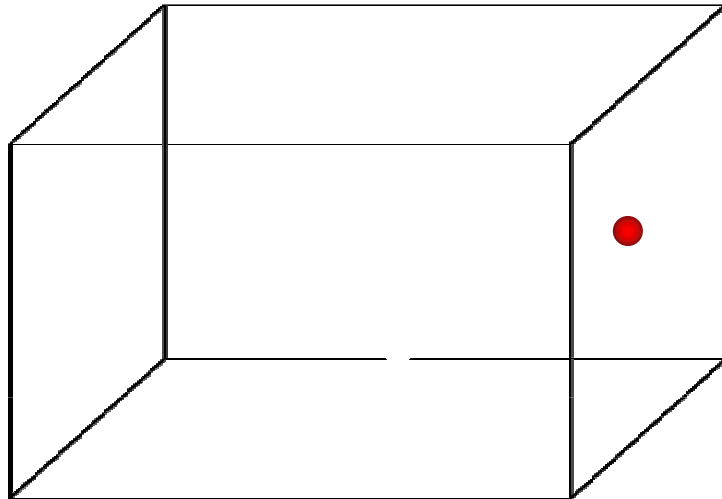
Anomalous scattering

Phase probability



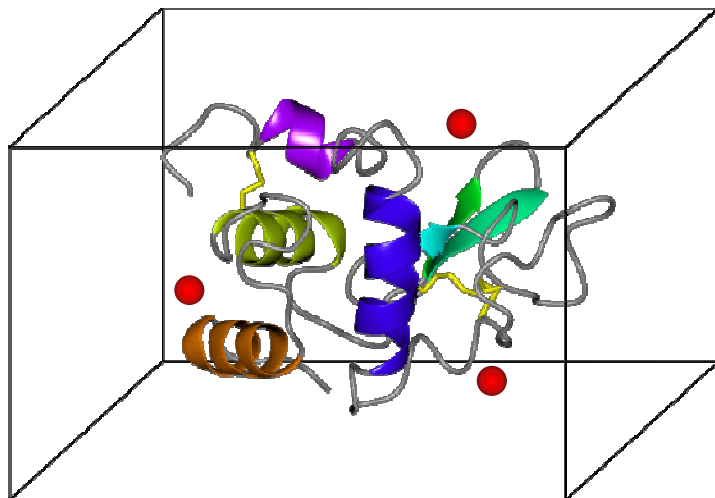


Phaser phasing scenarios



Known anomalous substructure

- missing atoms
- model errors (xyz , occ , B)
- weak normal component

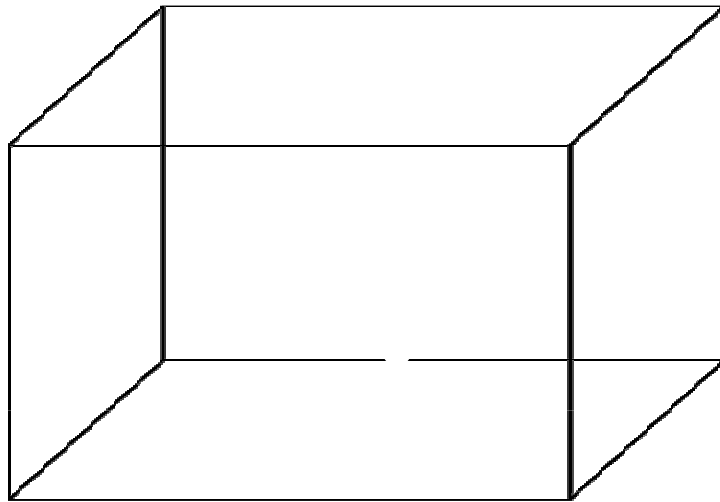


Known partial structure

- missing (anomalous) atoms
- (partial) model errors
- weak anomalous component

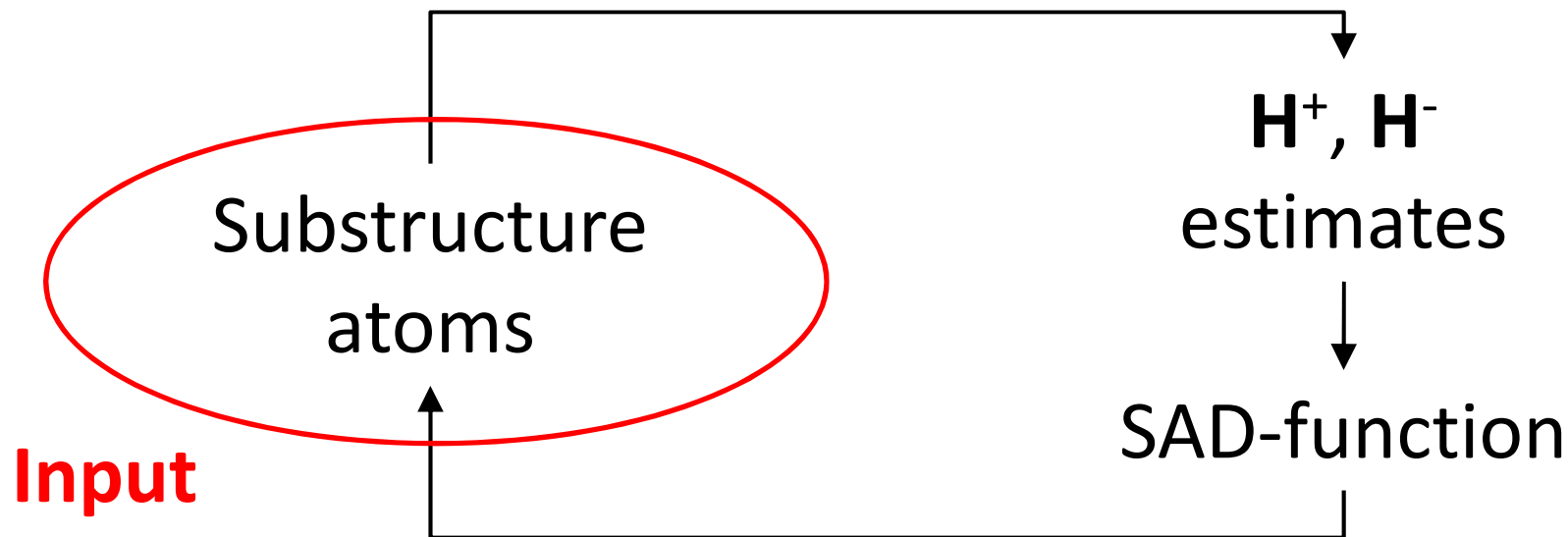


SAD phasing



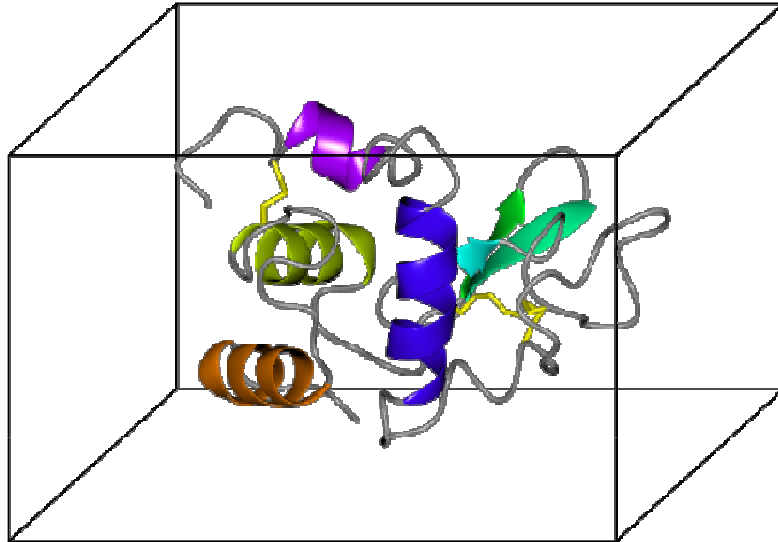
Parameters:

- atom type
- position
- occupancy
- B-factor
- f''





MR-SAD phasing



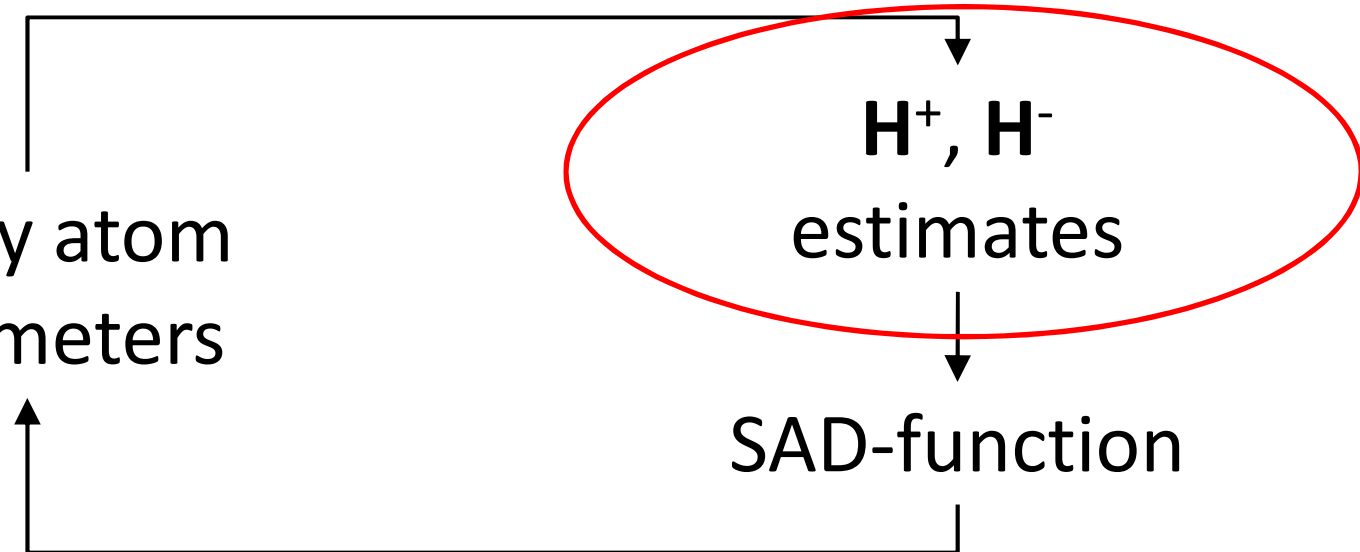
Input:

- partial structure
- possible atom types

Heavy atom
parameters

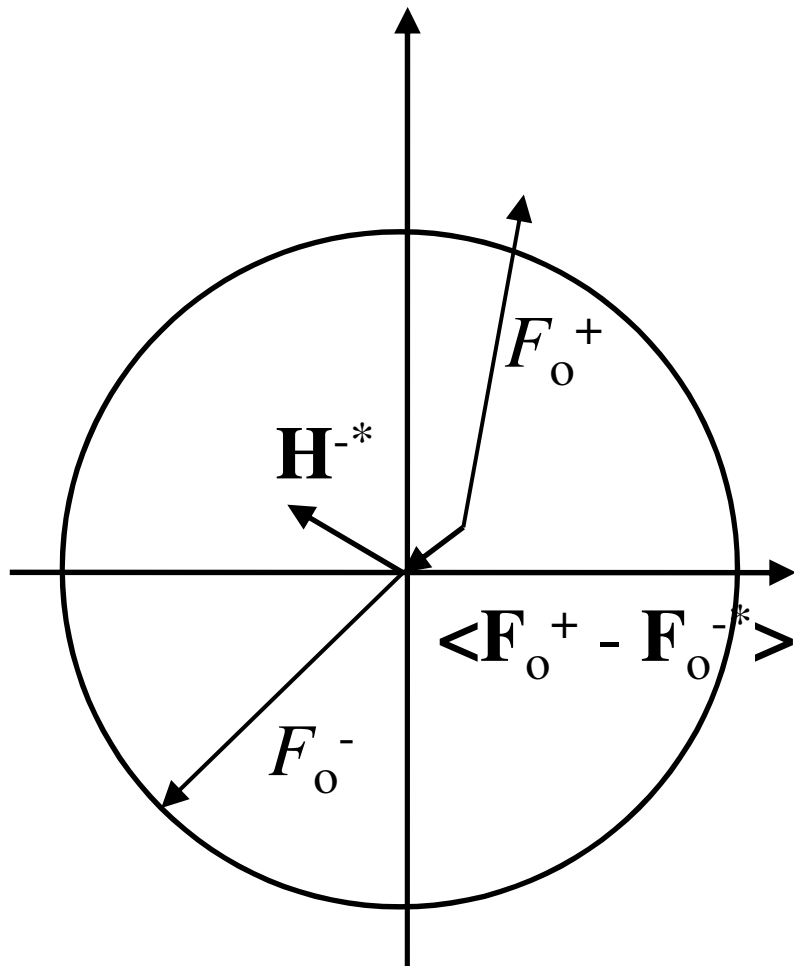
H^+ , H^-
estimates

SAD-function

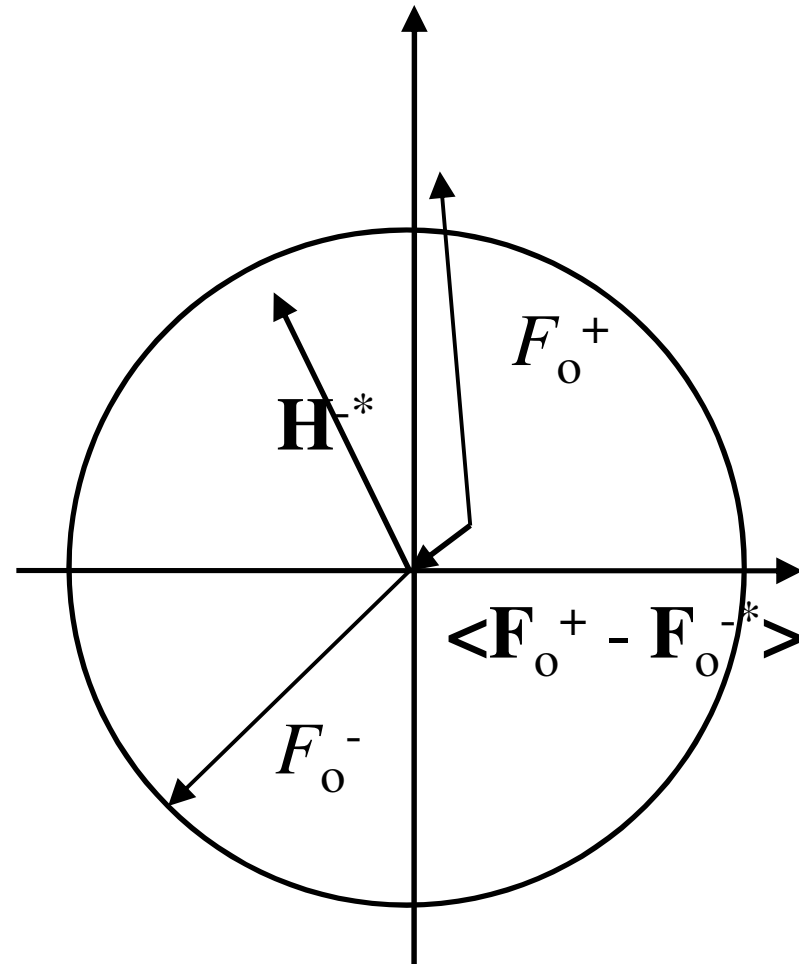




SAD function



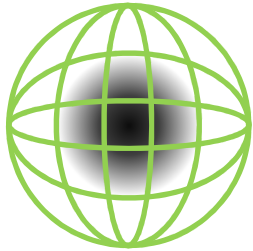
Scenario:
anomalous substructure



Scenario:
partial MR model

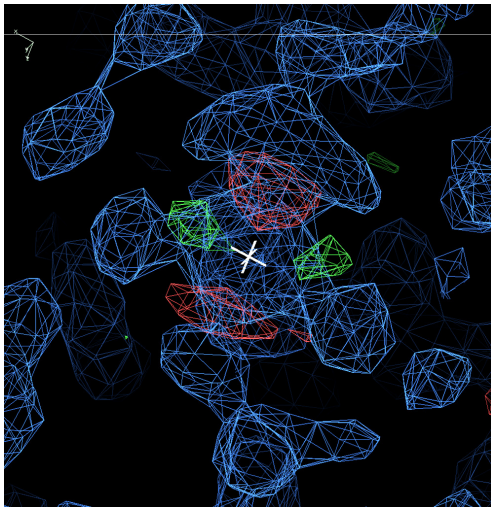


Log-likelihood gradient maps



Peak height dependent on f'/f'' ratio

- distinguish atom type
- (fooled if normal scattering is present at site)



Very sensitive to detail

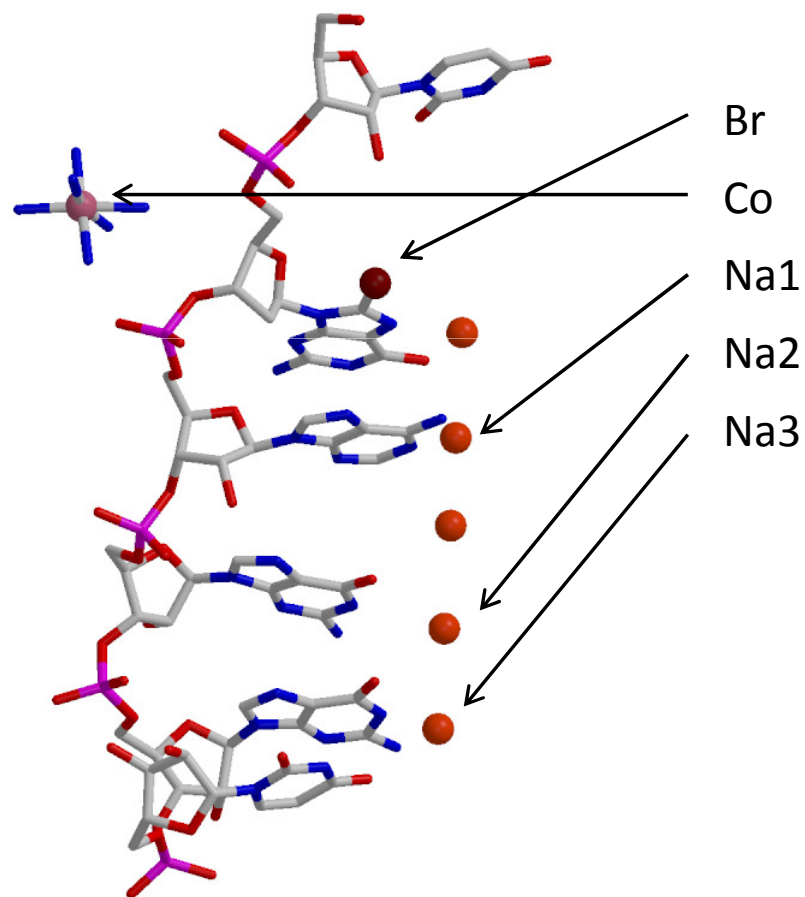
- e.g. anisotropic temperature motion

Iterative

- improvements in substructure description increases sensitivity



Log-likelihood gradient maps



Δ_{ano}	LLG1	LLG2
-----------------------	------	------

Br	26.5	28.5	-
----	------	------	---

Co	5.0	5.0	18.5
----	-----	-----	------

Na1	-	-	7.6
-----	---	---	-----

Na2	-	-	-
-----	---	---	---

Na3	-	-	-
-----	---	---	---

Element	f'	f''
---------	------	-------

Na	0.05	0.04
----	------	------

P	0.14	0.16
---	------	------

K	0.25	0.41
---	------	------

Ca	0.28	0.50
----	------	------



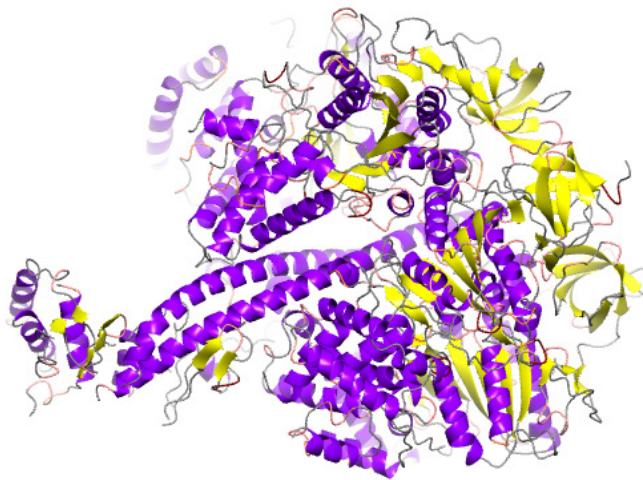
F1-ATPase testcase

3500 residue protein complex

81 S atoms, 3.50 Å resolution

Wavelength: 1.77 Å ($f_s''=0.72$ e⁻)

Anomalous signal extends to 21.5 Å, or to 12.7 Å very optimistically.



1bmf -

Abrahams J.P., Leslie A.G.W., Lutter R.,
Walker J.E.

Nature (1994) **370**:621

2ck3 - high resolution (1.95 Å) equivalent
Bowler M.D., Montgomery M.G. Leslie, A.G
Walker J.E.

Proc. Natl. Acad. Sci. USA (2006) **103**:8646



F1 ATPase testcase

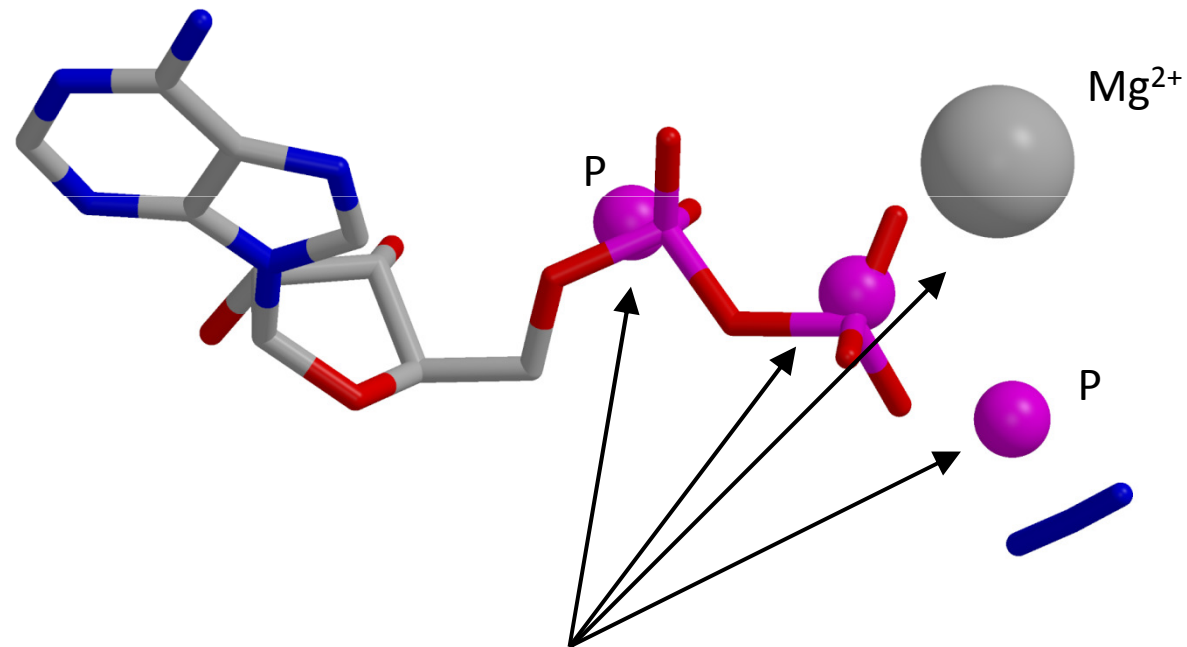
	Present sites			
	Met	Cys	PO ₄	Ion
pdb 2CK3	73	8	15	?

Model	Found sites			
	Met	Cys	PO ₄	Ion
F1-ATPase	53	8	12	8
F1 ATPase 3 α chains	10	3	7	5
Yeast ATPase	24	5	8	3



F1 ATPase testcase

ADP in active site
(not included in model)



Located sites



Phaser

Macrocycle

- Outlier rejection
- Substructure refinement

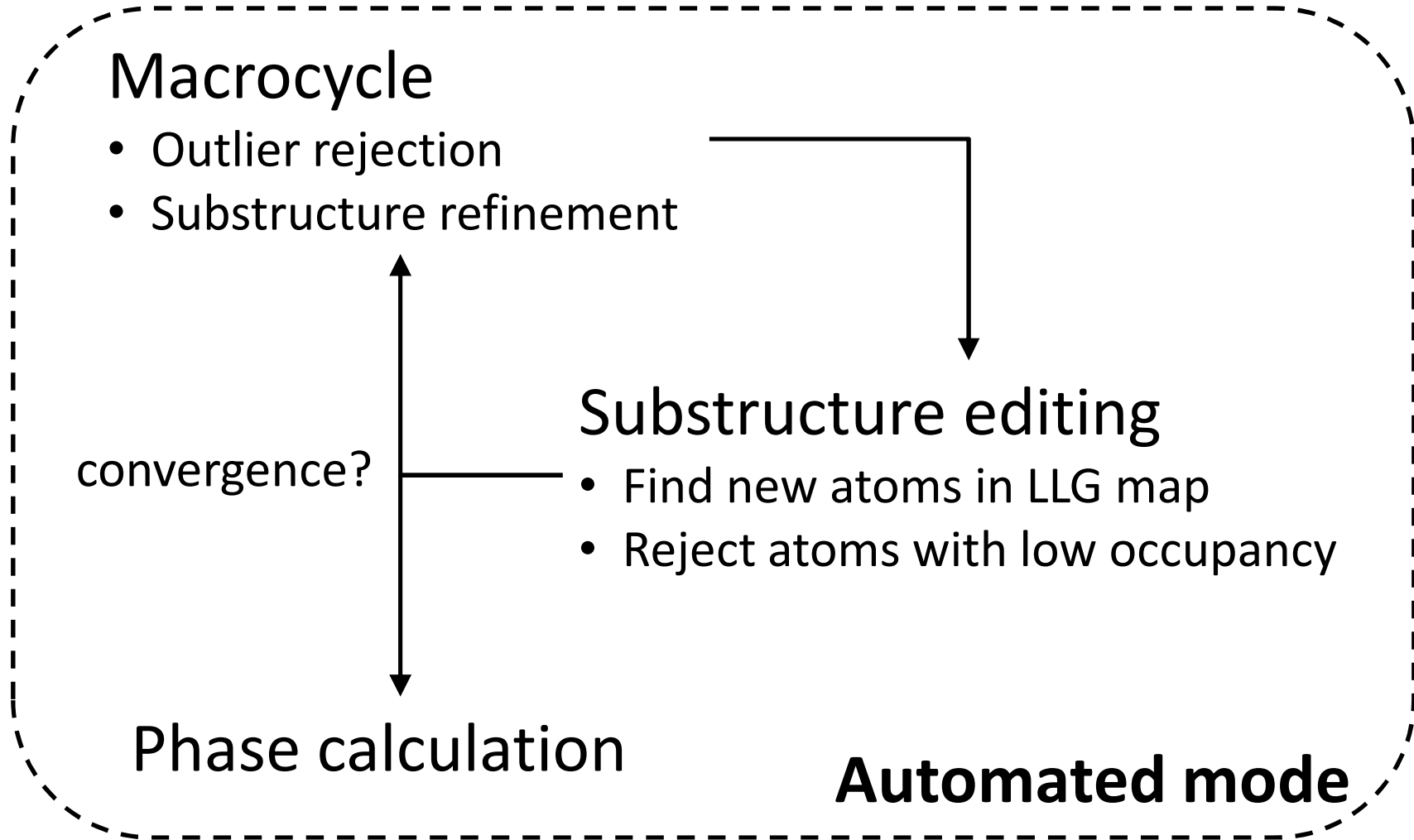
convergence?

Substructure editing

- Find new atoms in LLG map
- Reject atoms with low occupancy

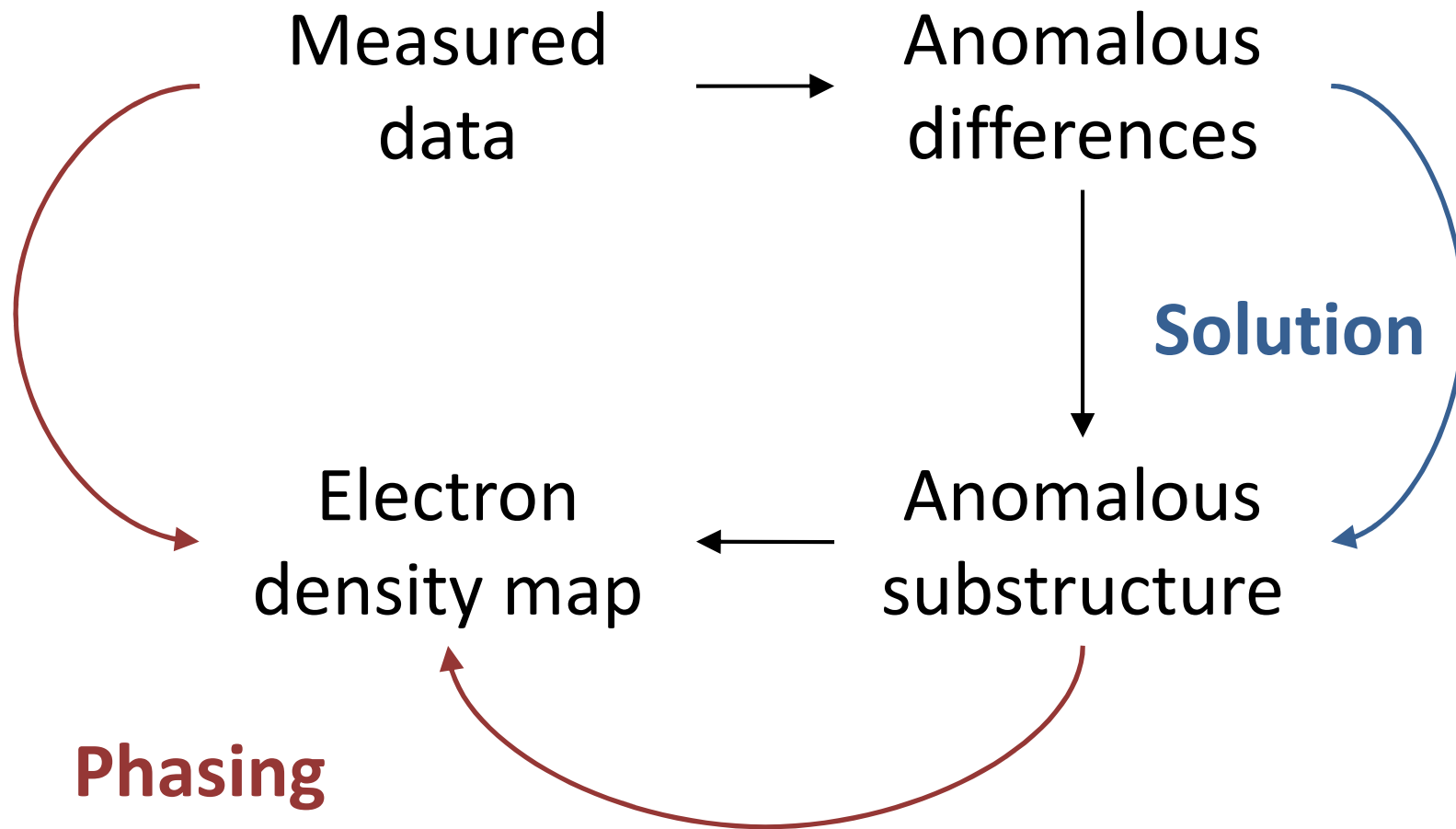
Phase calculation

Automated mode





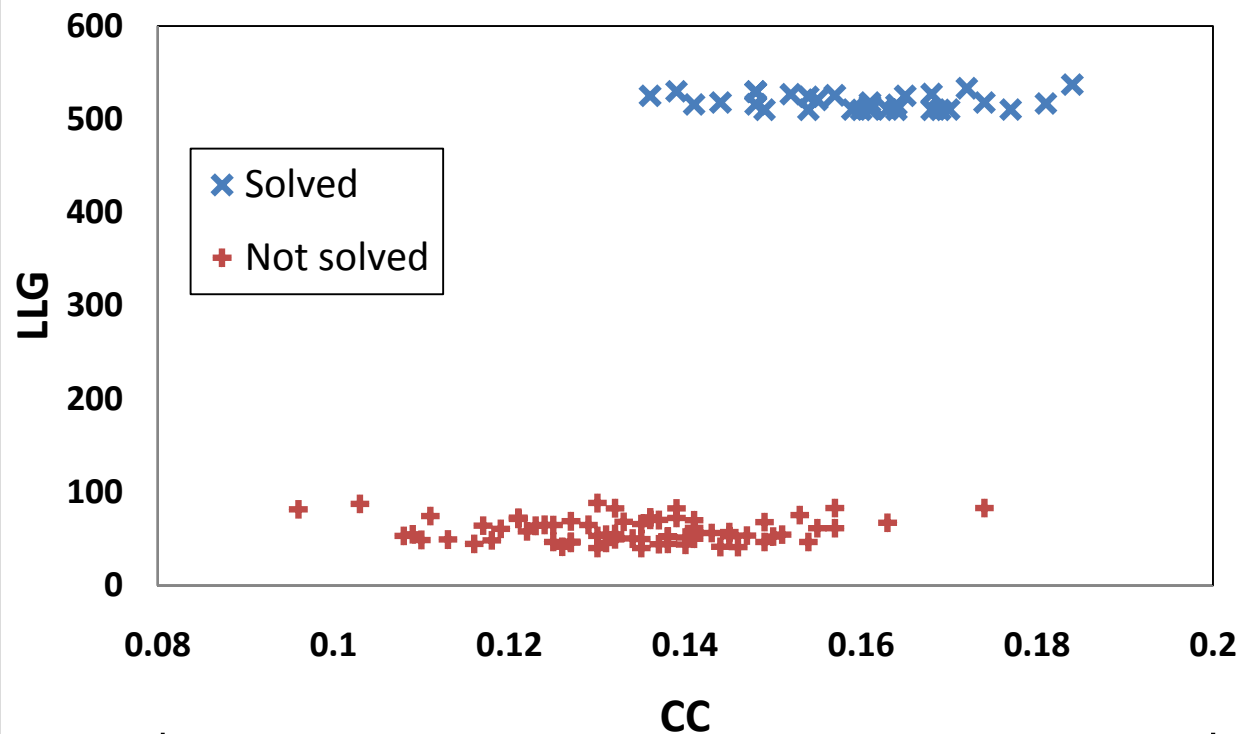
Data flow in substructure solution





Rescore trials with phaser

Insulin full search against 1.7 Å data



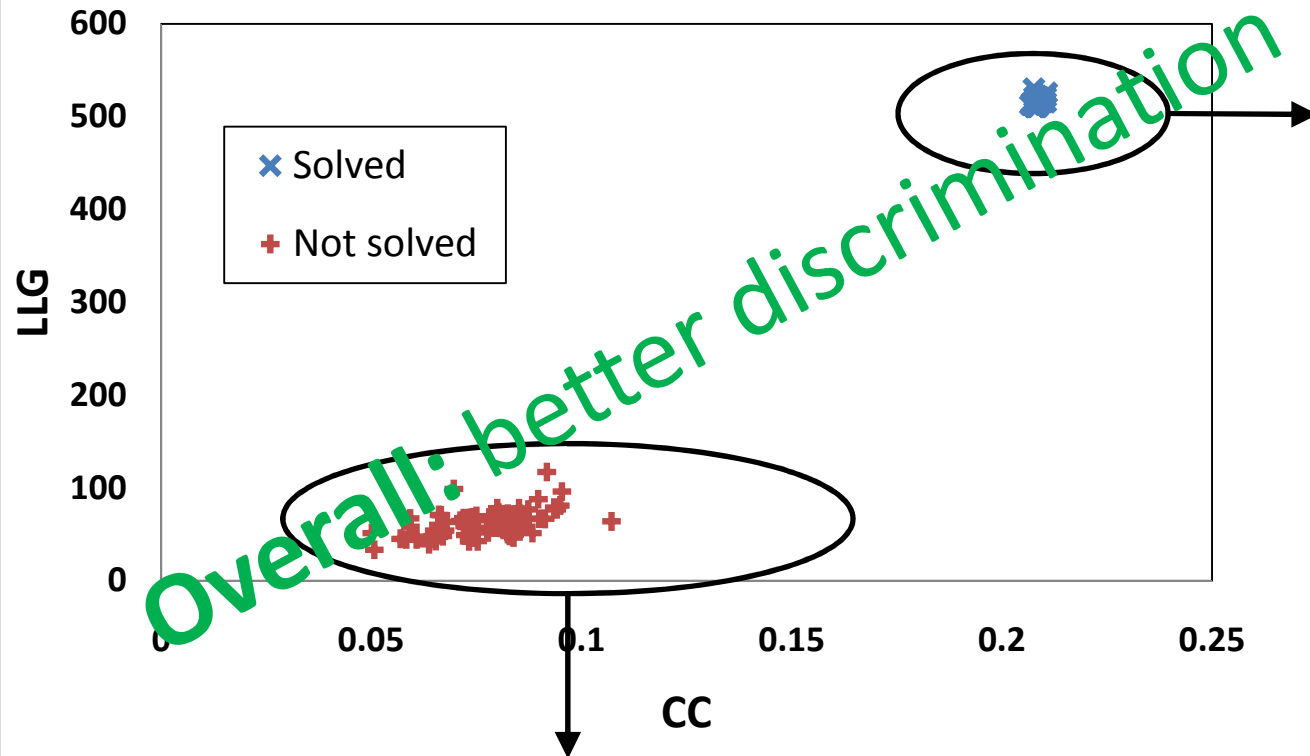
Clearly
separated
solution and
non-solution
groups

Impossible to identify solutions



CC for phaser-refined substructures

Insulin full search against 1.7 Å data



Increased CC
for solutions

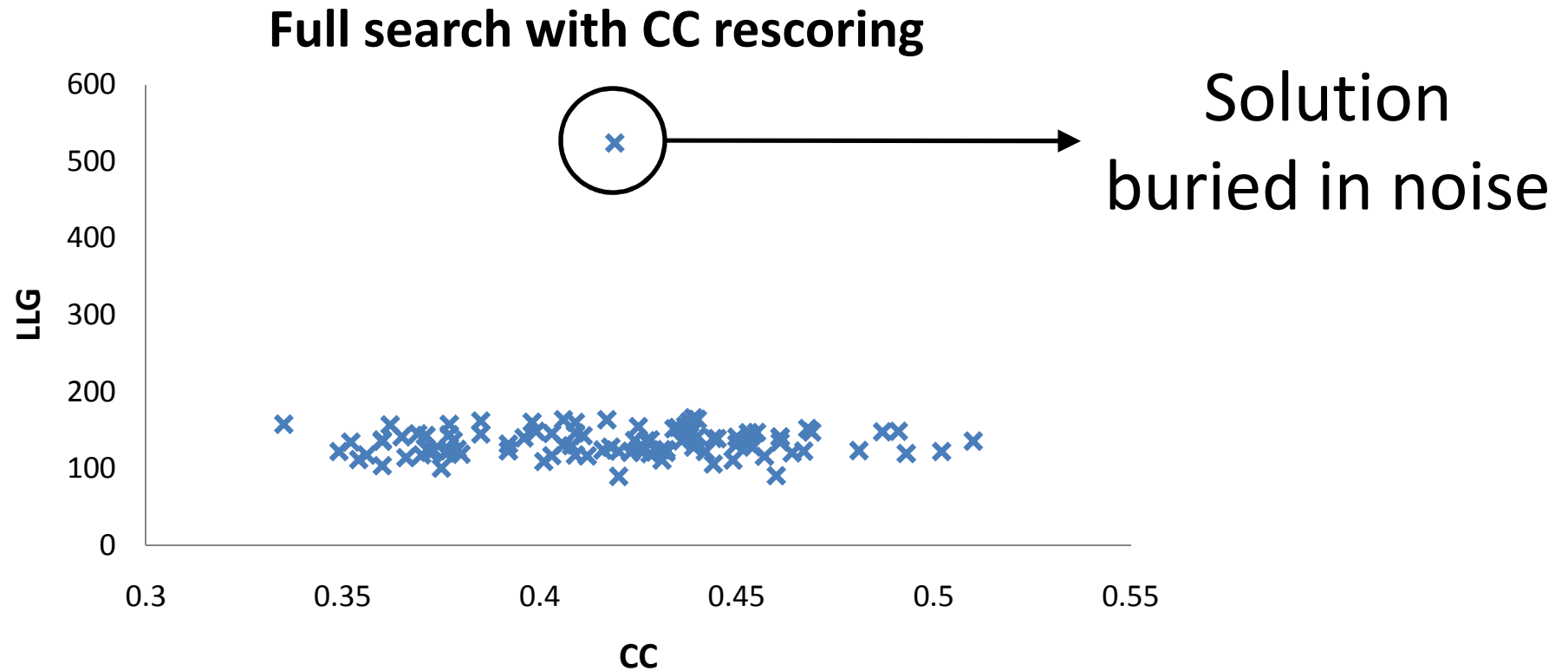
Completion:
improved
substructure

Lower CC for non-solutions

Refinement: reduced overfitting



Fibronectin testcase

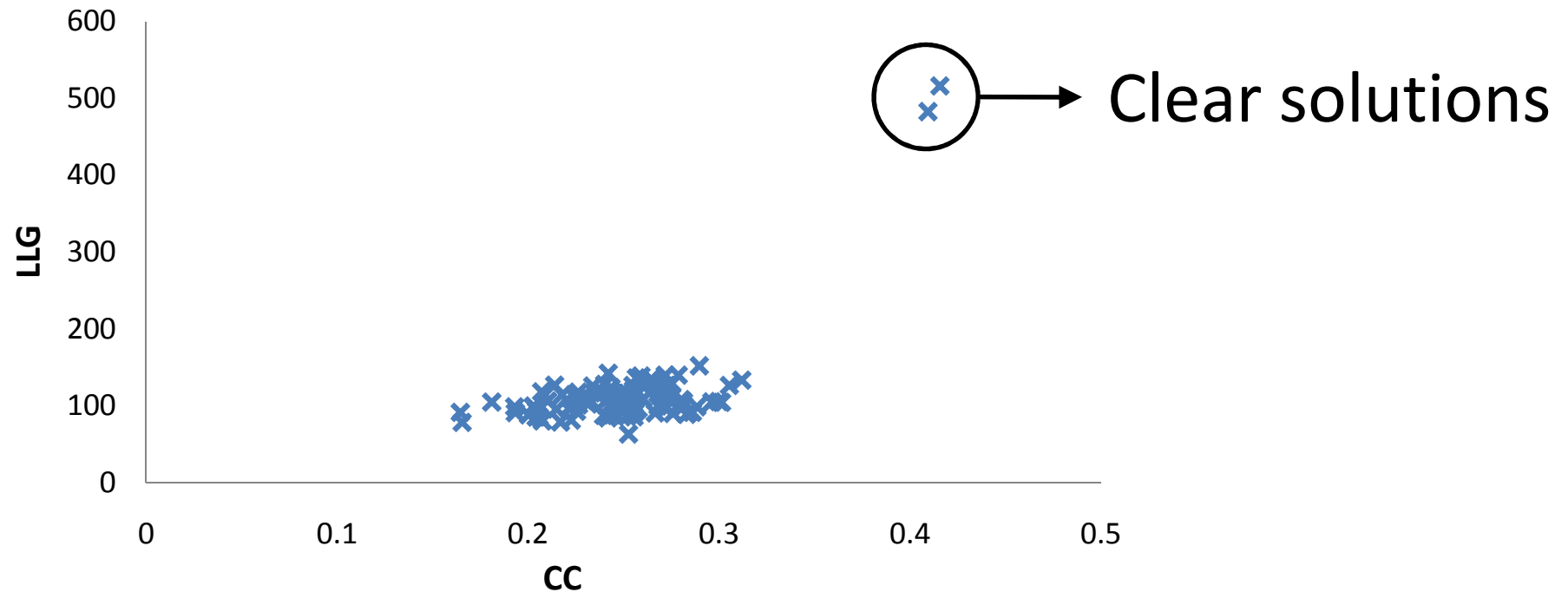


Rudiño-Piñera E, Ravelli RB, Sheldrick GM, Nanao MH, Korostelev VV,
Werner JM, Schwarz-Linek U, Potts JR, Garman EF
J Mol Biol. **368**, 833-44



Fibronectin testcase

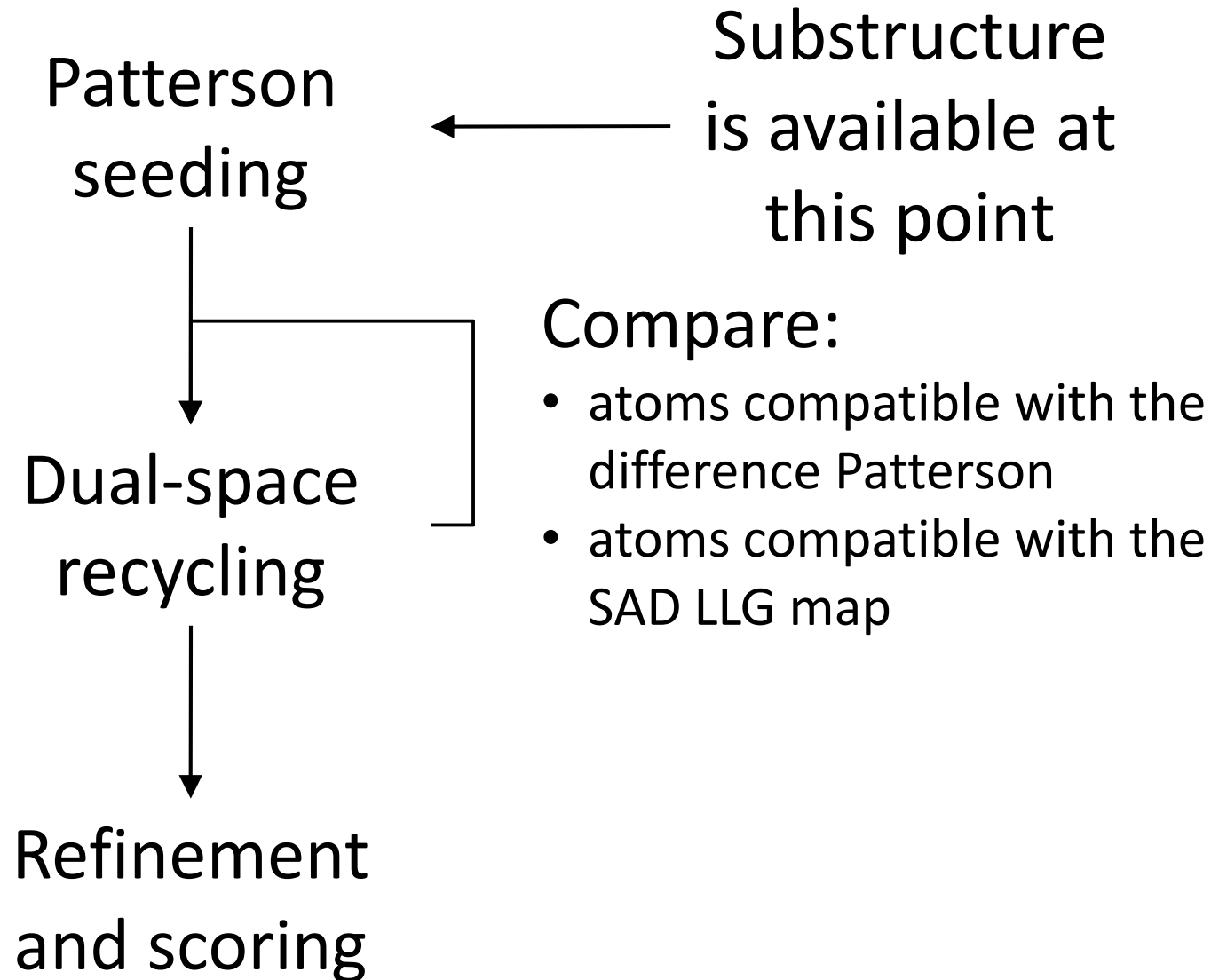
Full search with phaser rescoring



Rudiño-Piñera E, Ravelli RB, Sheldrick GM, Nanao MH, Korostelev VV,
Werner JM, Schwarz-Linek U, Potts JR, Garman EF
J Mol Biol. **368**, 833-44



Substructure seeding





Comparison

Transaminase, 60 Se sites

	Patterson seeding	LLG-map seeding
CC scoring	17	25
Phaser scoring	20	25

KSM42, 160 Se sites

	Patterson seeding	LLG-map seeding
CC scoring	23	28
Phaser scoring	28	37



When to use

- Phaser scoring

- default run
- default give no solution



- LLG-map seeding

- default run
- large substructures
- with phaser scoring





Phaser

Mode

- SAD (start from heavy atoms)
- MRSAD (start with partial MR model)

Reflection data

- F^+/F^-
- possible space groups

Scatterer information

- atom type and wavelength
- measured f'/f'' values

Absolute scale

macromolecule weight

The screenshot shows the 'Maximum Likelihood Experimental Phasing' window. The 'Job title' is 'find iodides with 2fbd_clustalw MR solution'. The 'Mode for experimental phasing' is set to 'Single-wavelength anomalous dispersion (SAD)'. Under 'Define data', 'MTZ in' is 'TUTORIAL' and 'hewl_iod.mtz'. 'Crystal Name' is 'New' and 'Wavelength Name' is 'New'. 'F(+)' is 'F_New(+)' and 'SIGF(+)' is 'SIGF_New(+)'. 'F(-)' is 'F_New(-)' and 'SIGF(-)' is 'SIGF_New(-)'. 'Resolution' is '55.216 A to 1.861 A'. 'Space group read from mtz file' is 'P43212'. 'Enantiomorph choice' is 'Original enantiomorph'. 'Scattering' is 'at CuK-alpha wavelength' and 'fix FDP' is unchecked. 'LLG-map completion' is 'on' and 'Maximum number of cycles of completion' is '50'. 'LLG-map sigma cut-off for adding new atom sites' is '6.0'. 'LLG-map atomic separation distance cut-off' is 'by optical resolution'. 'LLG-map calculation atom type' is 'I'. There are 'Edit list' and 'Add another atom type' buttons. Under 'Define atoms', 'Anomalous atom sites' is 'in PDB file' and 'Set B-factors to' is 'Wilson B'. 'PDB file' is 'TUTORIAL' and 'TUTORIAL_19.pdb'. There are 'Browse' and 'View' buttons. Under 'Composition of the asymmetric unit', 'Total scattering determined by' is 'components in asymmetric unit'. 'Component #1' is 'protein' and 'sequence file' is 'hewl.seq'. 'Number in asymmetric unit' is '1'. There are 'Edit list' and 'Define another component' buttons. At the bottom, there are 'Run', 'Save or Restore', and 'Close' buttons.



Results

Phase information

- FWT, PHWT
- HLA, HLB, HLC, HLD
- PHIB
- FLLG, PHILLG

Best map or initial map for density modification

Phase probability for density modification or refinement

Centroid phase for density modification/refinement
(use HL-coefficients!)

PDB file

anomalous substructure

Log-likelihood gradient map
(flat after automated mode!)

Logfile



www.phaser.cimr.cam.ac.uk

Phaserwiki - Mozilla Firefox

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http://www.phaser.cimr.cam.ac.uk/index.php/Phaser_Crystallo... 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, Storoni LC, Read RJ.
J. Appl. Cryst. (2007). 40, 658-674.

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Done



Acknowledgements

Python-based Hierarchical Environment for Integrated Xtallography



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Los Alamos National Lab

Tom Terwilliger, Li-Wei Hung



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Randy Read, Airlie McCoy, Robert Oeffner, Gabor Bunkoczi



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Jane and Dave Richardson, Vincent Chen