Phaser

Experimental phasing and MR-SAD

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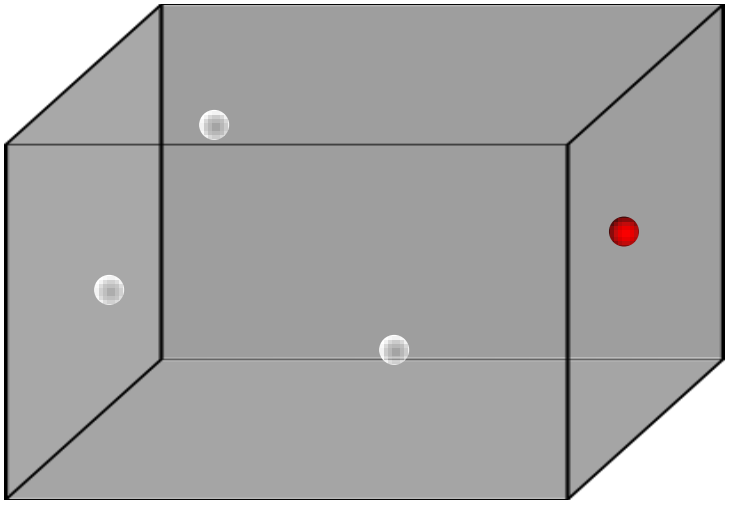
CCP4 Fukuoka School,
31 October 2012

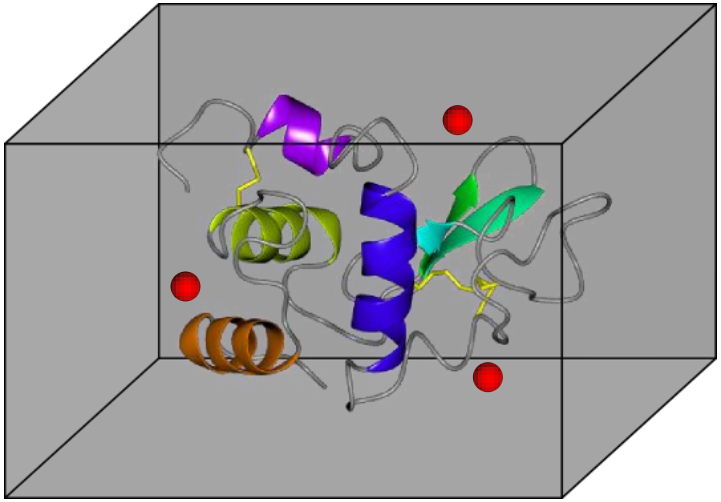
Phasing scenarios



Known anomalous substructure

— missing atoms

— model errors (*xyz*, *occ*, *B*)



Known partial structure

— missing (anomalous) atoms

— (partial) model errors

SAD function – normal scattering

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

F_o^- – measured amplitude

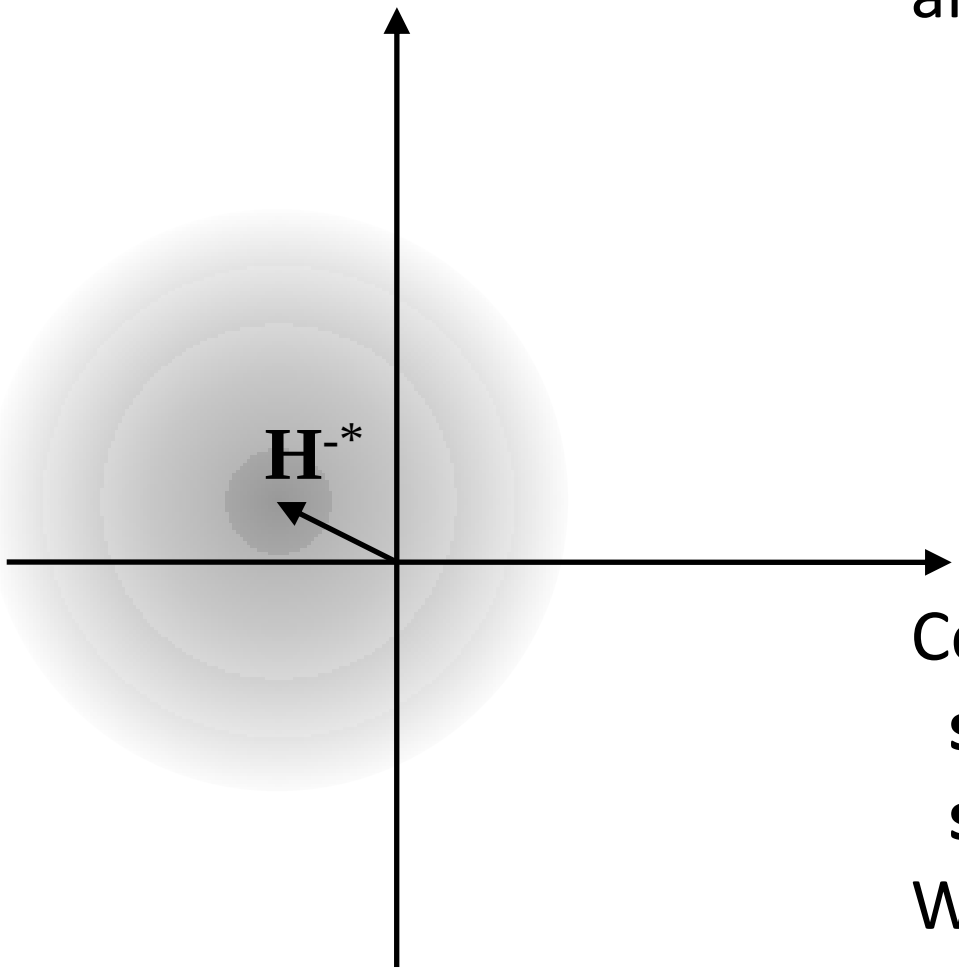
α^- – phase angle

$\mathbf{H}^-$ – structure factor from known substructure

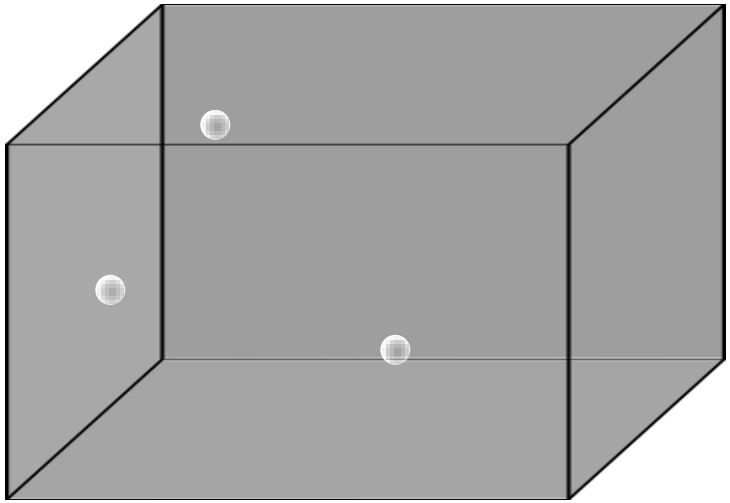
D – substructure error

Σ^- – uncertainty

SAD function – normal scattering



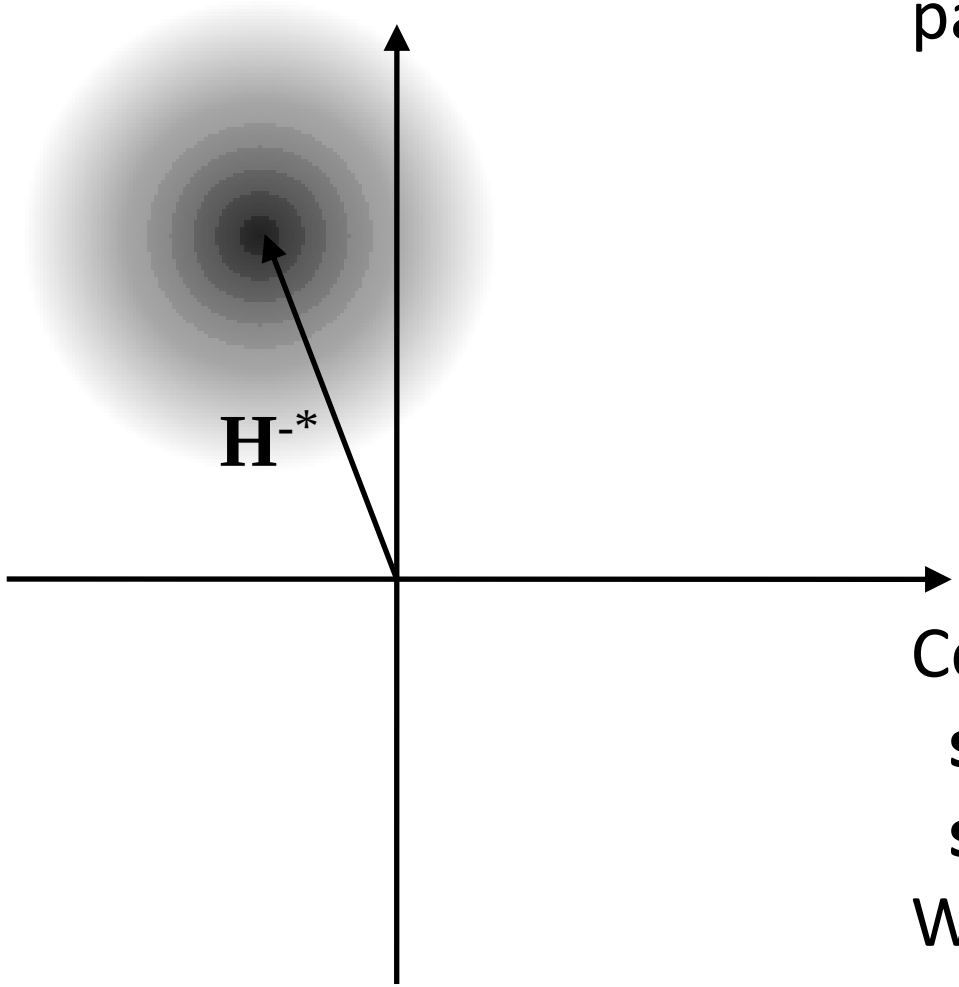
Scenario:
anomalous substructure



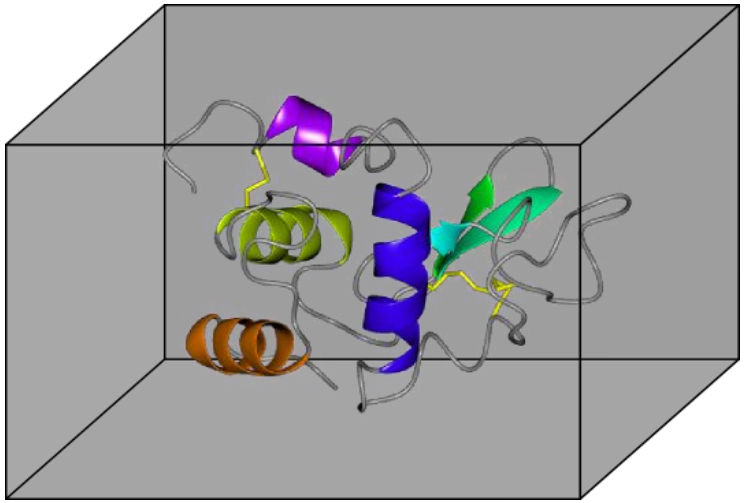
Centered on:
structure factor of known substructure

Width determined by:
model incompleteness

SAD function – normal scattering



Scenario:
partial MR model



Centered on:
structure factor of known substructure
Width determined by:
model errors

SAD function – anomalous scattering

$$P\left(F_o^+;\mathbf{F}^-,\mathbf{H}^+,\mathbf{H}^{-*}\right)=\frac{2F_o^+}{\Sigma^+}\exp\left(-\frac{\left(F_o^+-F_c^+\right)^2}{\Sigma^+}\right)\mathrm{eI}_0\left(\frac{2F_o^+F_c^+}{\Sigma^+}\right)$$

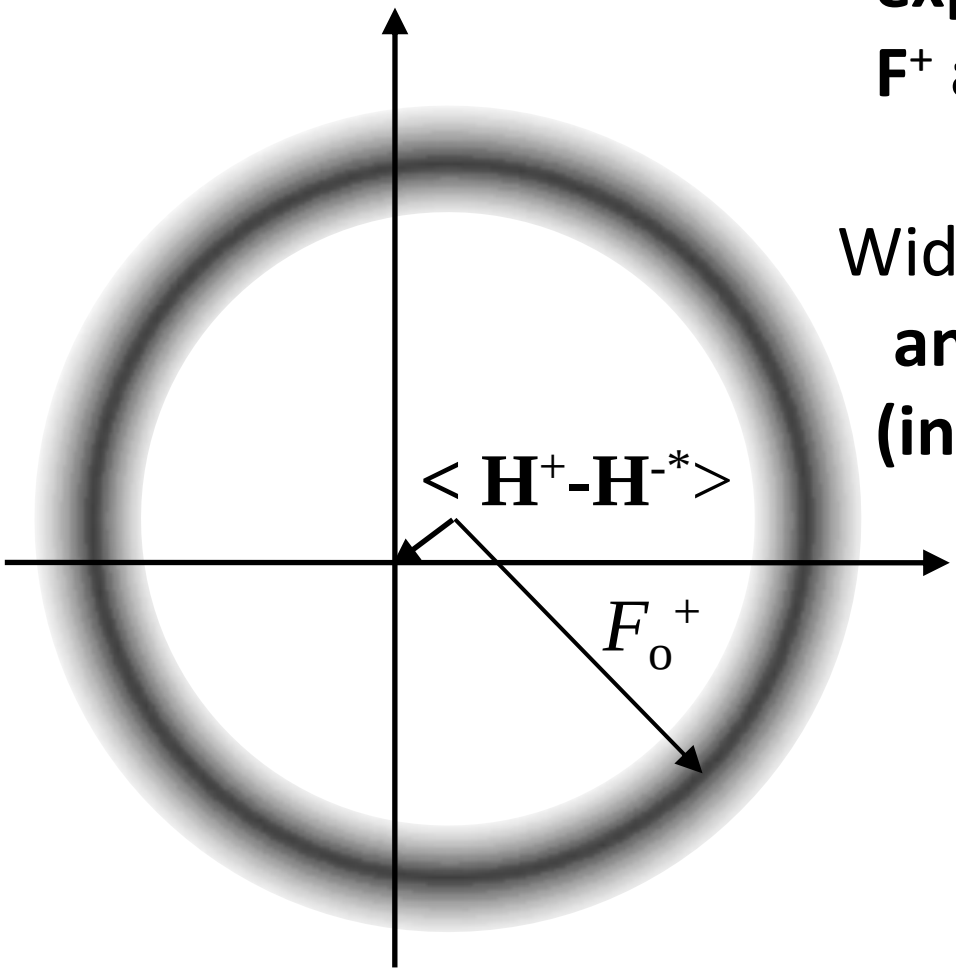
$$F_c^+=\left|D\mathbf{H}^+ + D_\Phi e^{i\alpha_\Phi}\left(\mathbf{F}^- - D\mathbf{H}^{-*}\right)\right|$$

$\mathbf{H}^{\pm}$ – structure factor of known substructure

F_c^+ – estimate of F_o^+ from F_o^-, $\mathbf{H}^+$ and $\mathbf{H}^-$

Σ^+ – error in F_c^+

SAD function – anomalous scattering



Centered on:

expected difference between F^+ and F^-

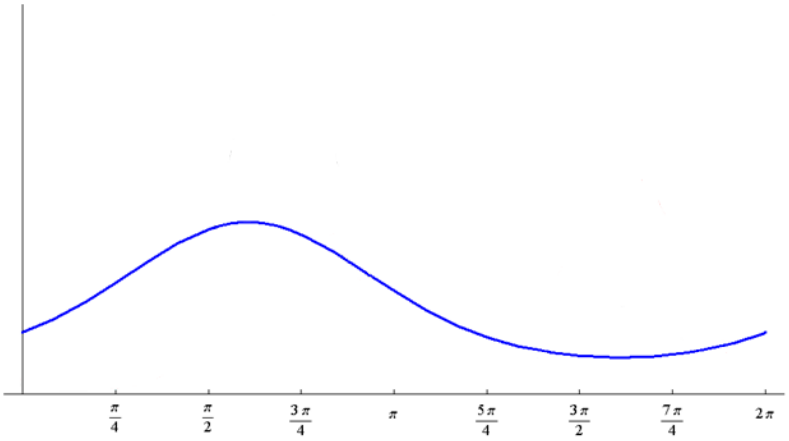
Width determined by:

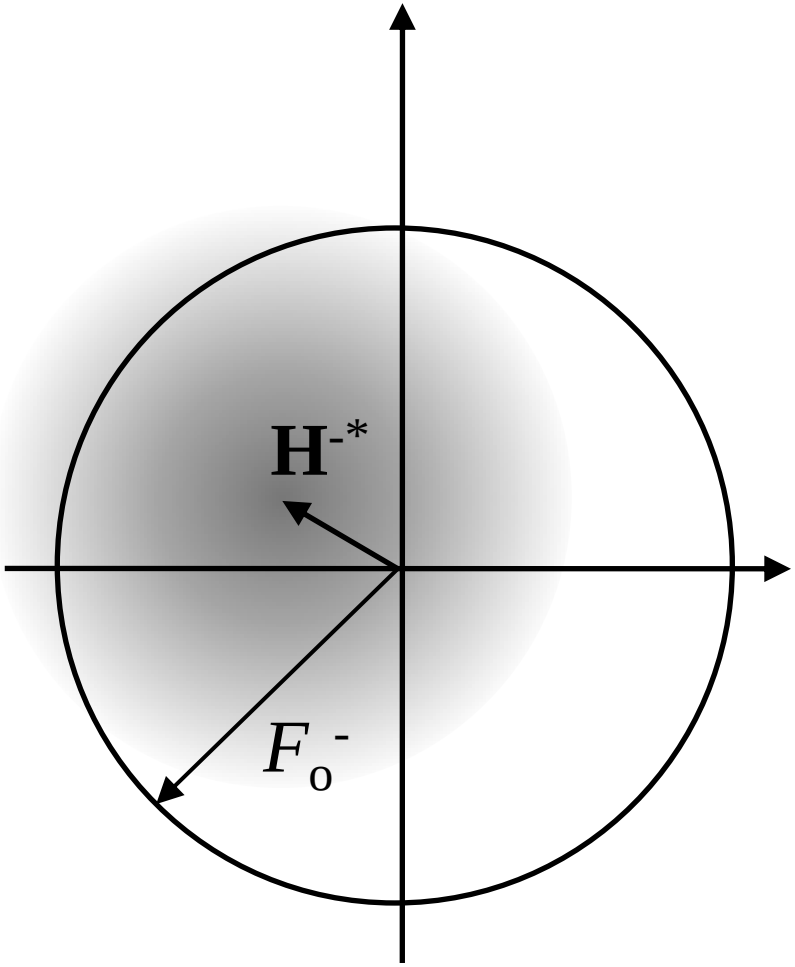
anomalous substructure error (including measurement error)

Phase probability

Normal scattering

Phase probability

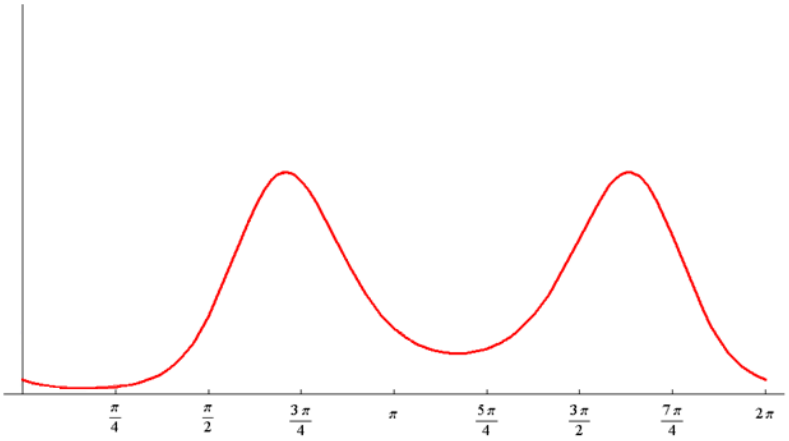


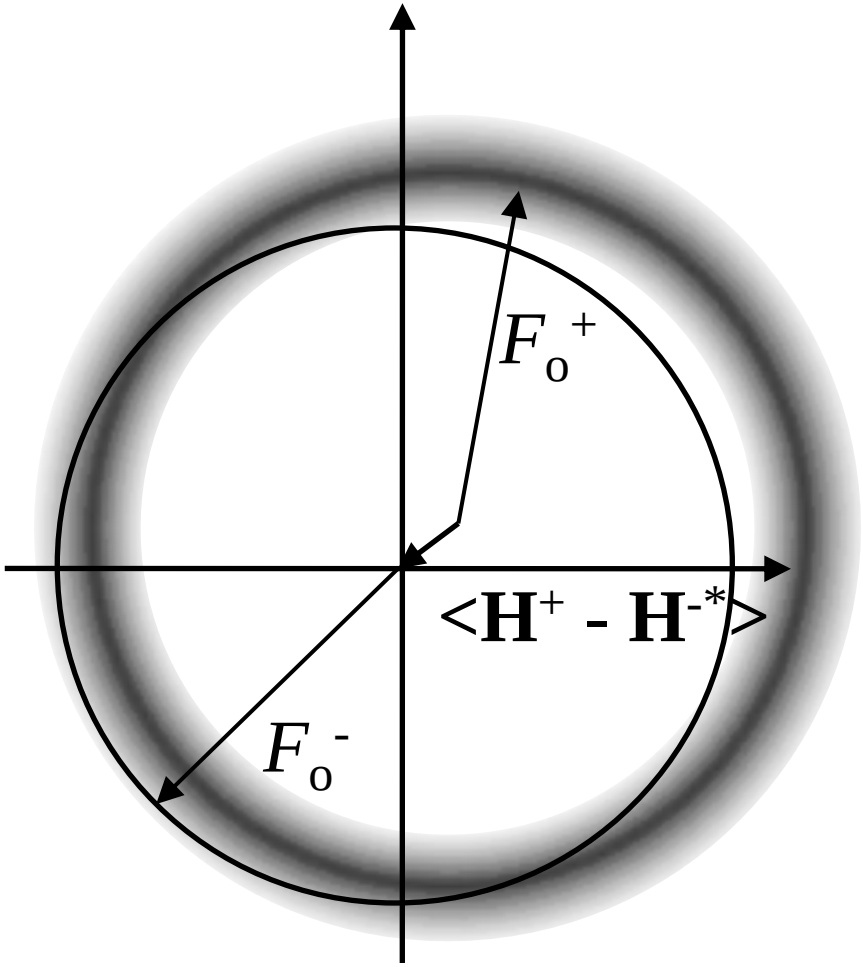


Phase probability

Anomalous scattering

Phase probability



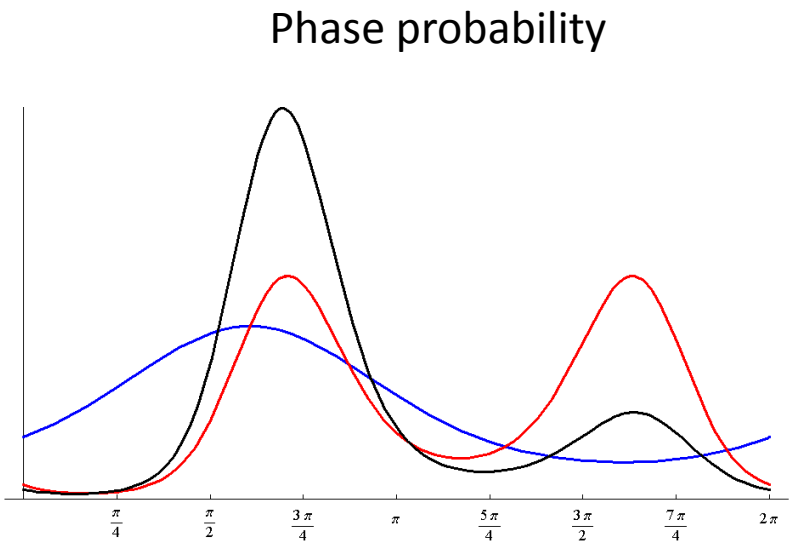


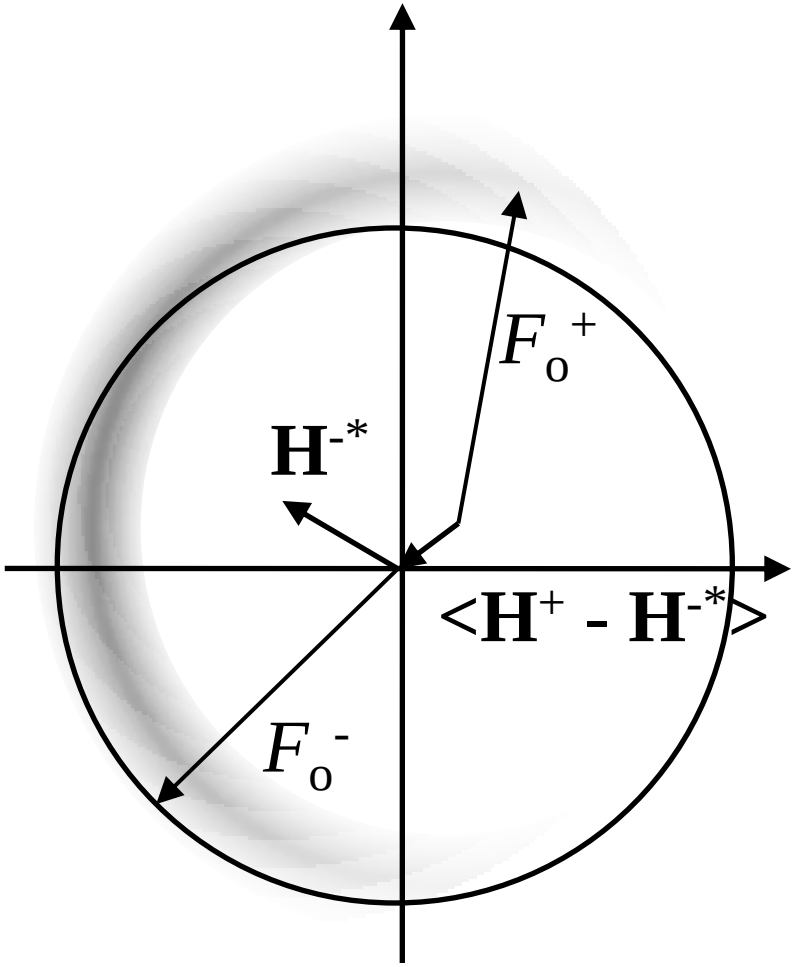
Phase probability

Total

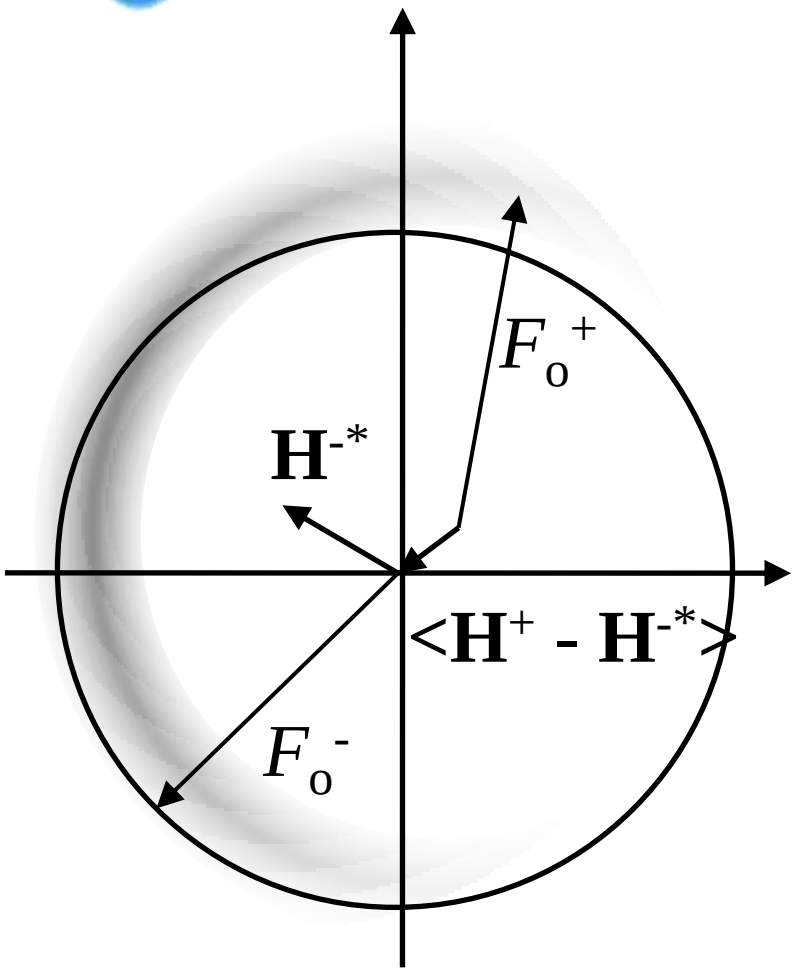
Normal scattering

Anomalous scattering

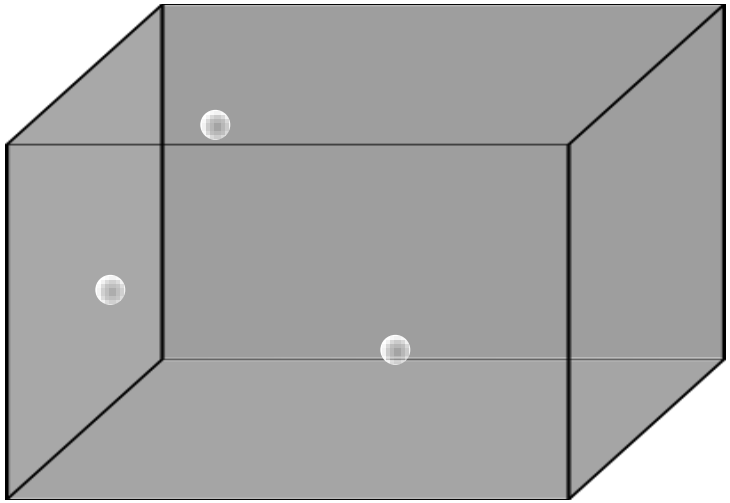




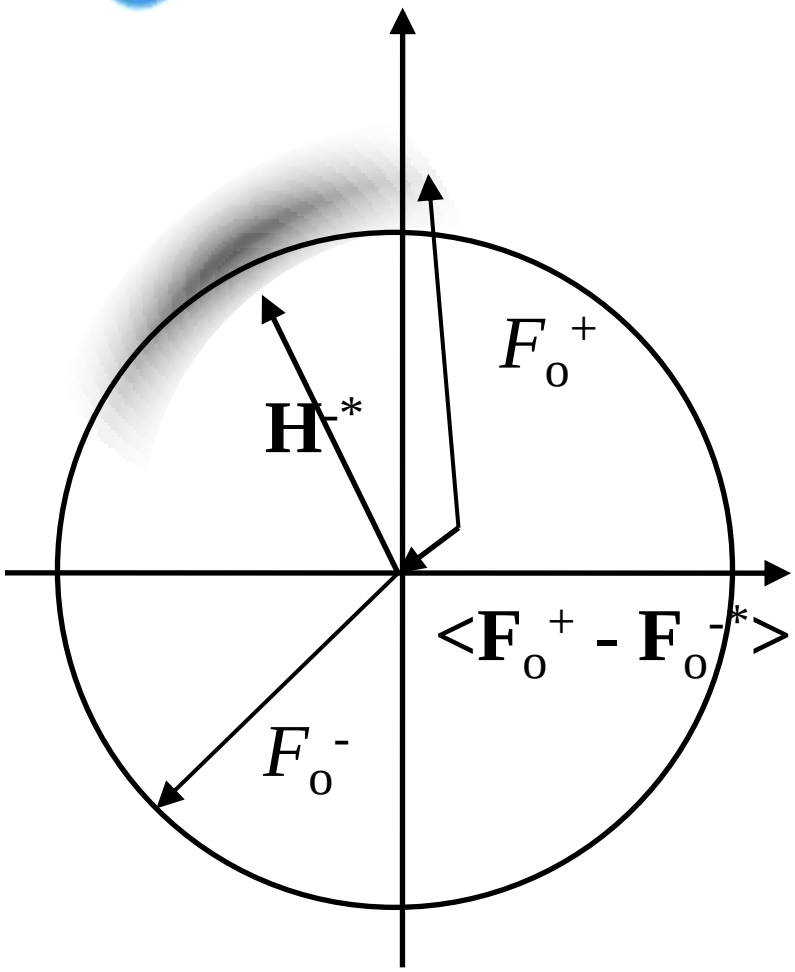
Phase probability – SAD phasing



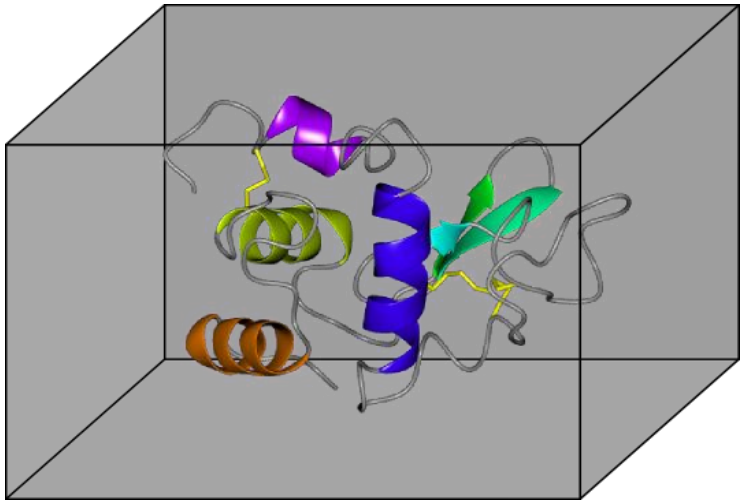
Scenario:
anomalous substructure



Phase probability – MR-SAD phasing

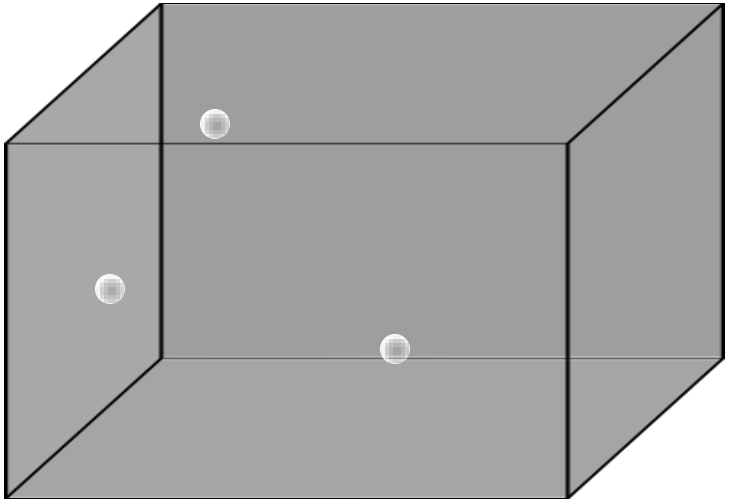


Scenario:
partial MR model



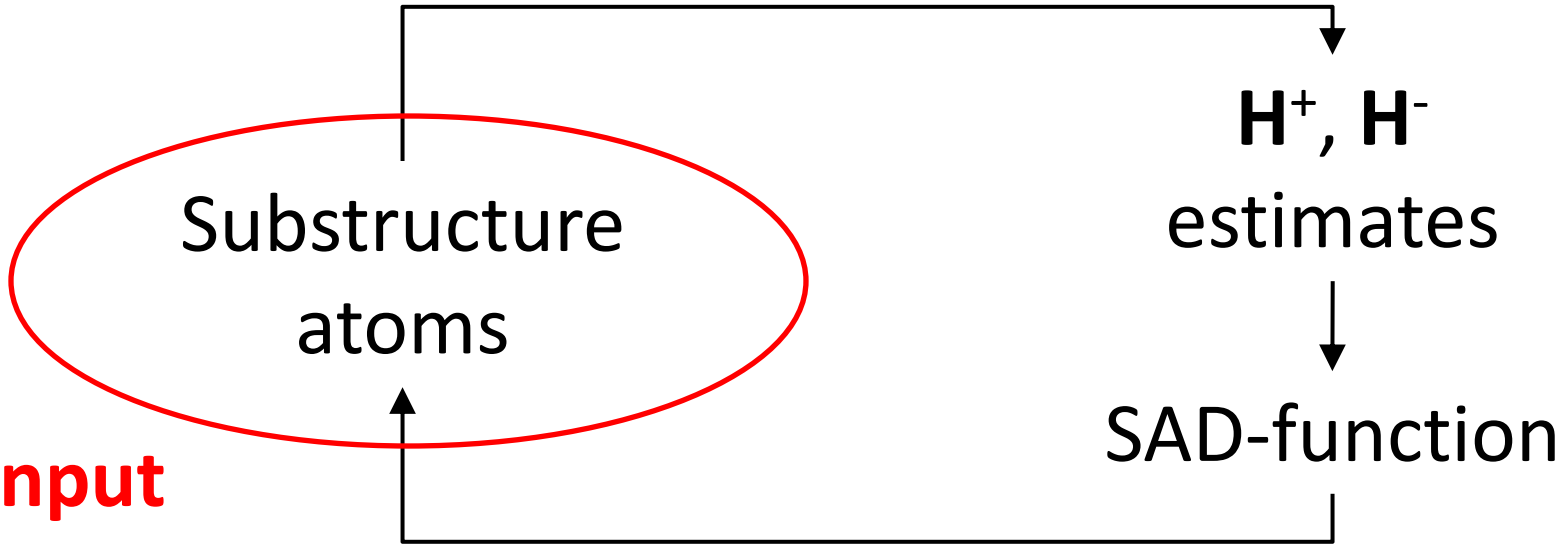
Phases contain **contribution from** known partial (MR) **model** that are weighted with **expected model error**

SAD phasing workflow

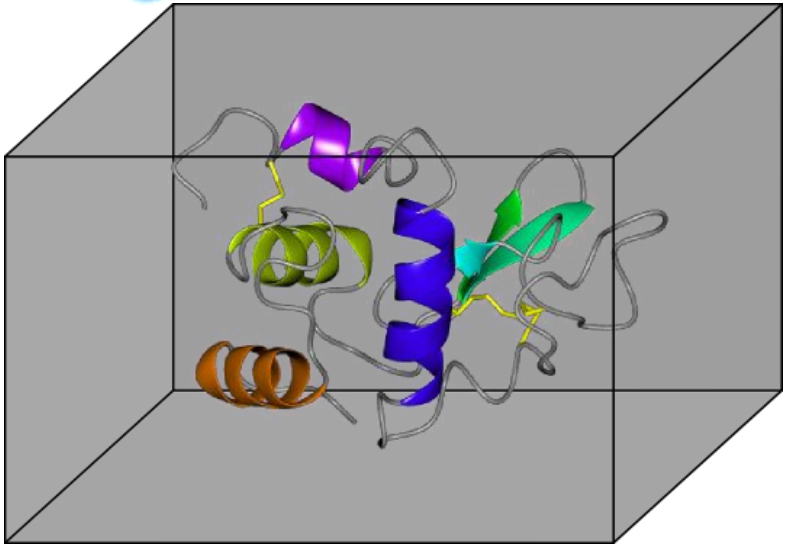


Parameters:

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

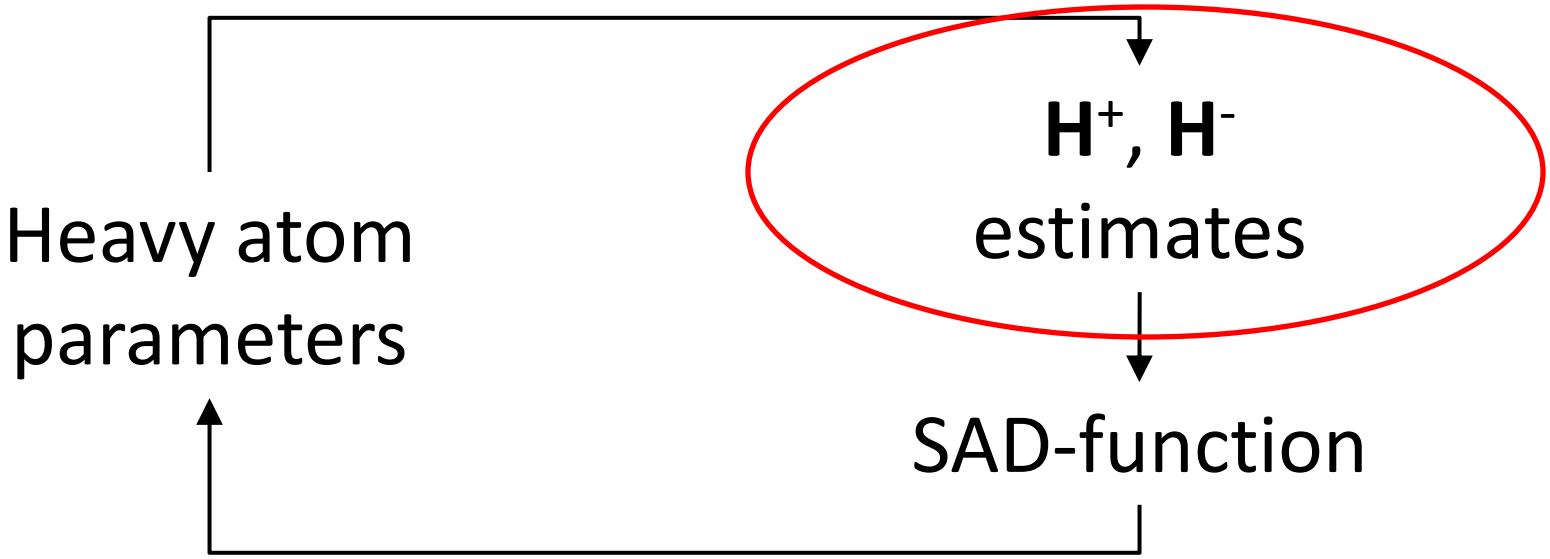


MR-SAD phasing workflow

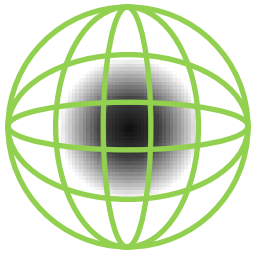


Input:

- partial structure
- possible atom types

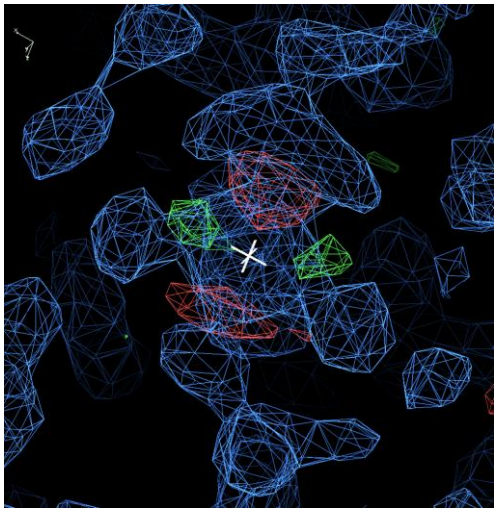


Log-likelihood gradient maps



Peak height dependent on f'/f" ratio

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



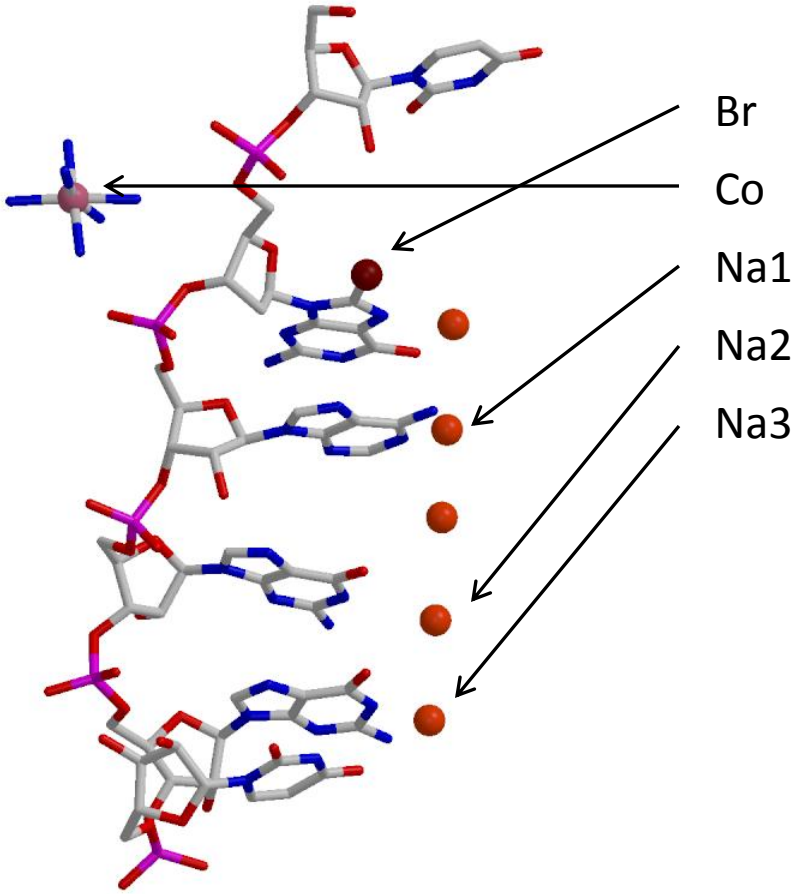
Very sensitive to detail

- e.g. anisotropic temperature motion

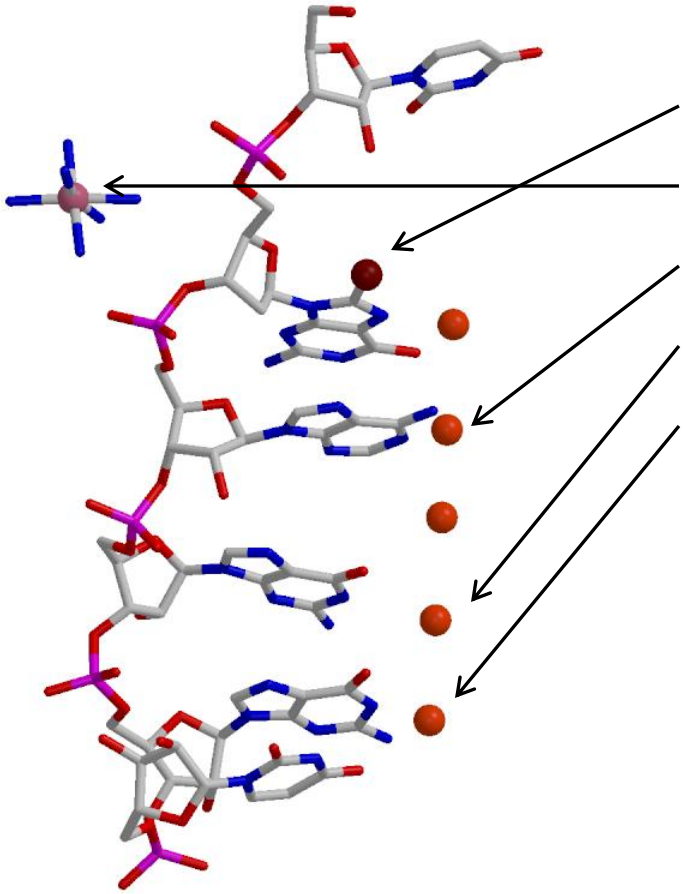
Iterative

- improvements in substructure description increases sensitivity

Log-likelihood gradient maps



Log-likelihood gradient maps



	Δ_{ano}	LLG1	LLG2	LLG3
Br	26.5	28.5	-	-
Co	5.0	5.0	18.5	-
Na1	-	-	7.6	-
Na2	-	-	-	6.6
Na3	-	-	-	6.0

Element	f’	f”
Na	0.05	0.04
P	0.14	0.16
K	0.25	0.41
Ca	0.28	0.50

Common uses of MR-SAD

- Model refinement
  identify/validate anomalous scatterers
- Model building
  Marker atoms to help sequence docking
- Escape model bias
  add weak but independent phase information
- Improve anomalous substructure/phasing

Iterative substructure improvement

- Nitrate reductase (Natalie Strynadka)
  - integral membrane protein, 1976 residues
  - solved by combination of Fe-MAD and MIRAS
  - 21 Fe, 1 Mo and 113 S atoms
- Solve using Fe peak SAD data

Iterative substructure improvement

- Find heavy atoms sites
  - 11 “Fe” sites from phenix.hyss
  - several of super-sites of Fe_4S_4 clusters
- Complete and phase using Phaser
  - 38 Fe-sites, some of which are S atoms
  - still ghosts of super-sites

Iterative substructure improvement

- Improve phases with density modification
- Build structure with ARP/wARP
  798 residues, 18 docked in the sequence
- Completion in Phaser
  - using partial poly-Ala model from ARP/wARP
  - complete using Fe, Mo and S atoms
  - 21 Fe, 1 Mo and 84 of 113 S found
- Rebuild with ARP/wARP
  1392 of 1976 build, 736 docked in the sequence

F1-ATPase testcase

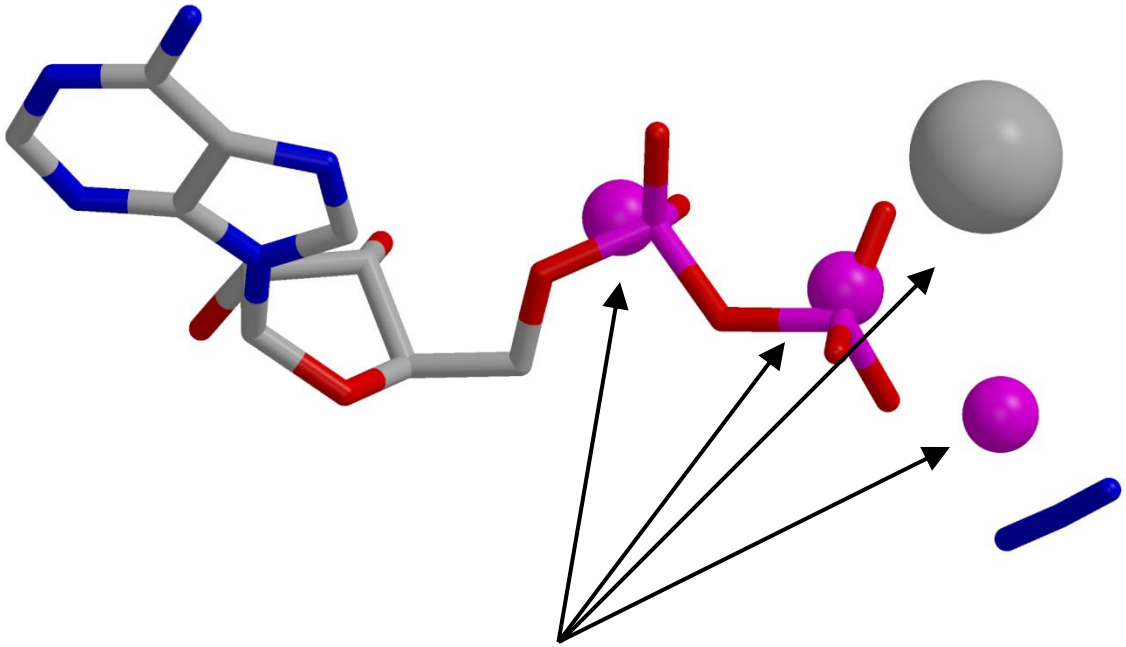
3500 residue protein complex
81 S atoms, 3.50 Å resolution
Wavelength: 1.77 Å (f_S"=0.72 e^-)

phenix.xtriage:

```
The anomalous signal seems to extend to about 21.5 A
(or to 12.7 A, from a more optimistic point of view).
The quoted resolution limits can be used as a guideline
to decide where to cut the resolution for phenix.hyss.
As the anomalous signal is not very strong in this
dataset substructure solution via SAD might prove to be
a challenge. Especially if only low resolution
reflections are used, the resulting substructures could
contain a significant amount of false positives.
```

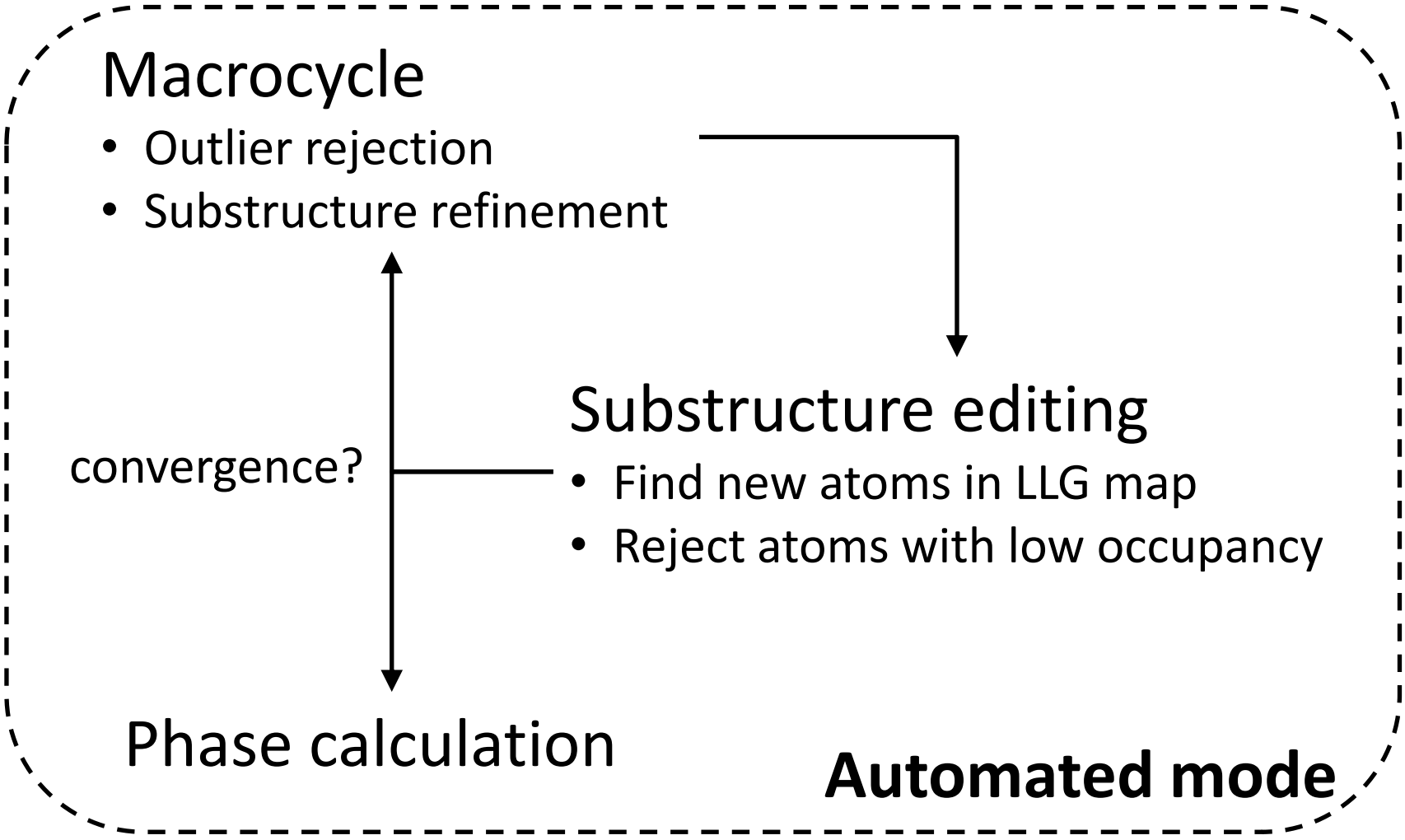
F1-ATPase testcase

ADP in active site
(not included in model)



Located sites

Phaser


Macrocycle
• Outlier rejection
• Substructure refinement
Substructure editing
• Find new atoms in LLG map
• Reject atoms with low occupancy
convergence?
Phase calculation
Automated mode

Results

Phase information

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

PDB file

anomalous substructure

Logfile

Best map or initial map for density modification

Phase probability for density modification or refinement

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

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

Phaser

Mode

- SAD (start from heavy atoms)

Reflection data

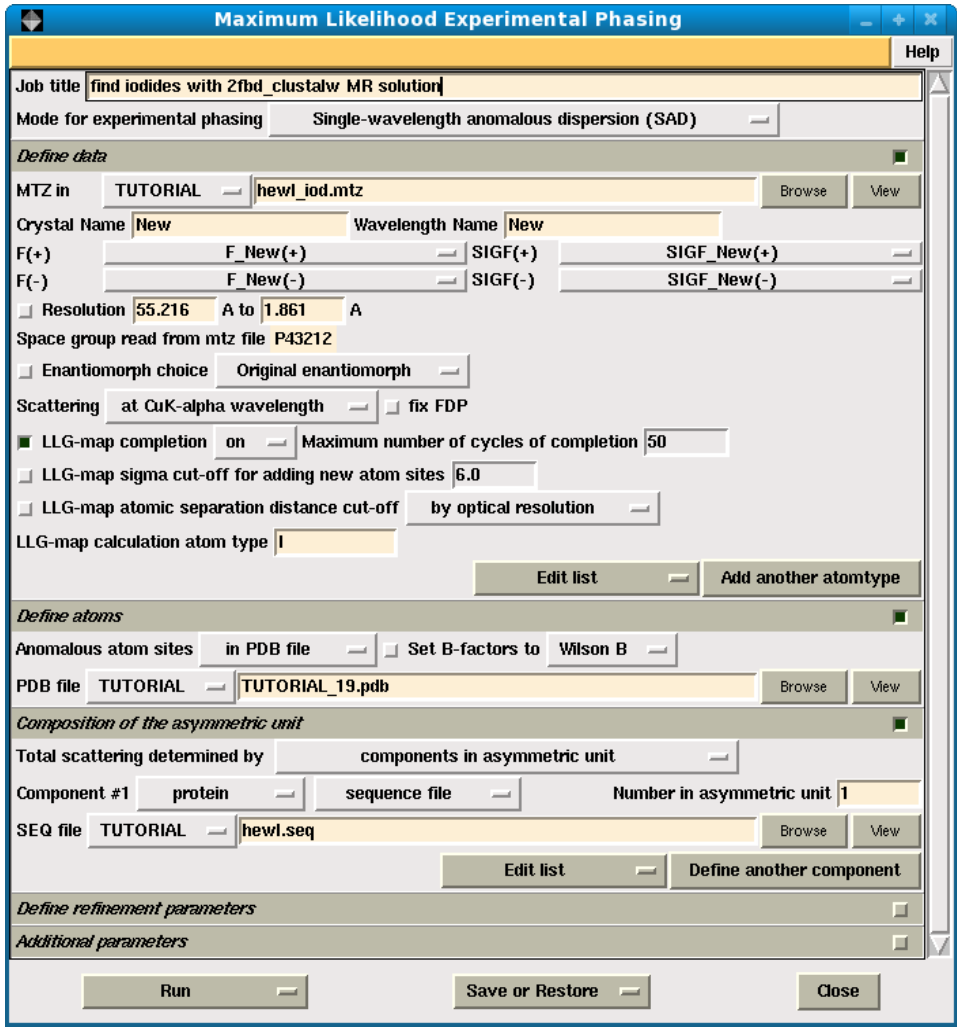
- F^+/F^-
- possible space groups

Scatterer information

- anomalous substructure
- atom type and wavelength
- measured f'/f" values

Absolute scale

macromolecule weight



Phaser

Mode

- MRSAD (partial MR solution)

Reflection data

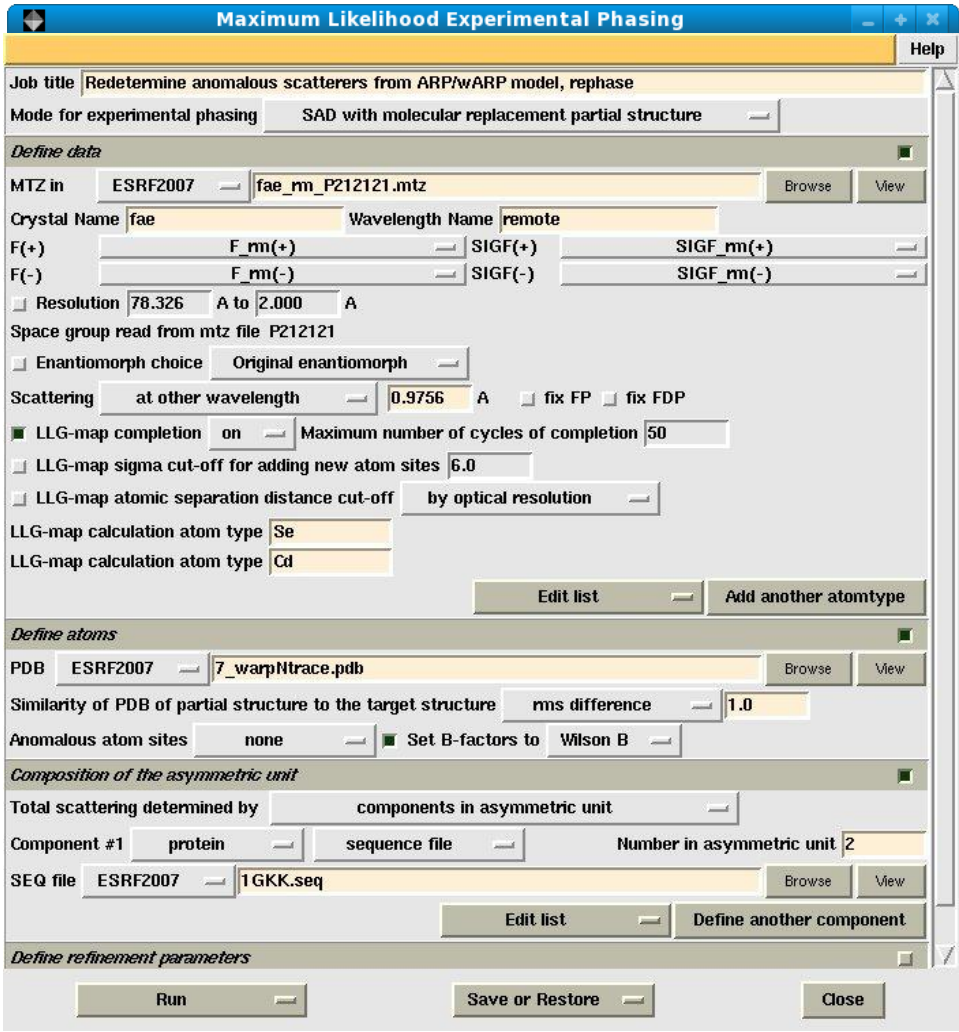
- F^+/F^-
- possible space groups

Scatterer information

- partial MR solution
- (anomalous substructure)
- atom type and wavelength
- measured f'/f" values

Absolute scale

macromolecule weight



Acknowledgements

Python-based Hierarchical Environment for Integrated Xtallography

Computational Crystallography Initiative / LBNL
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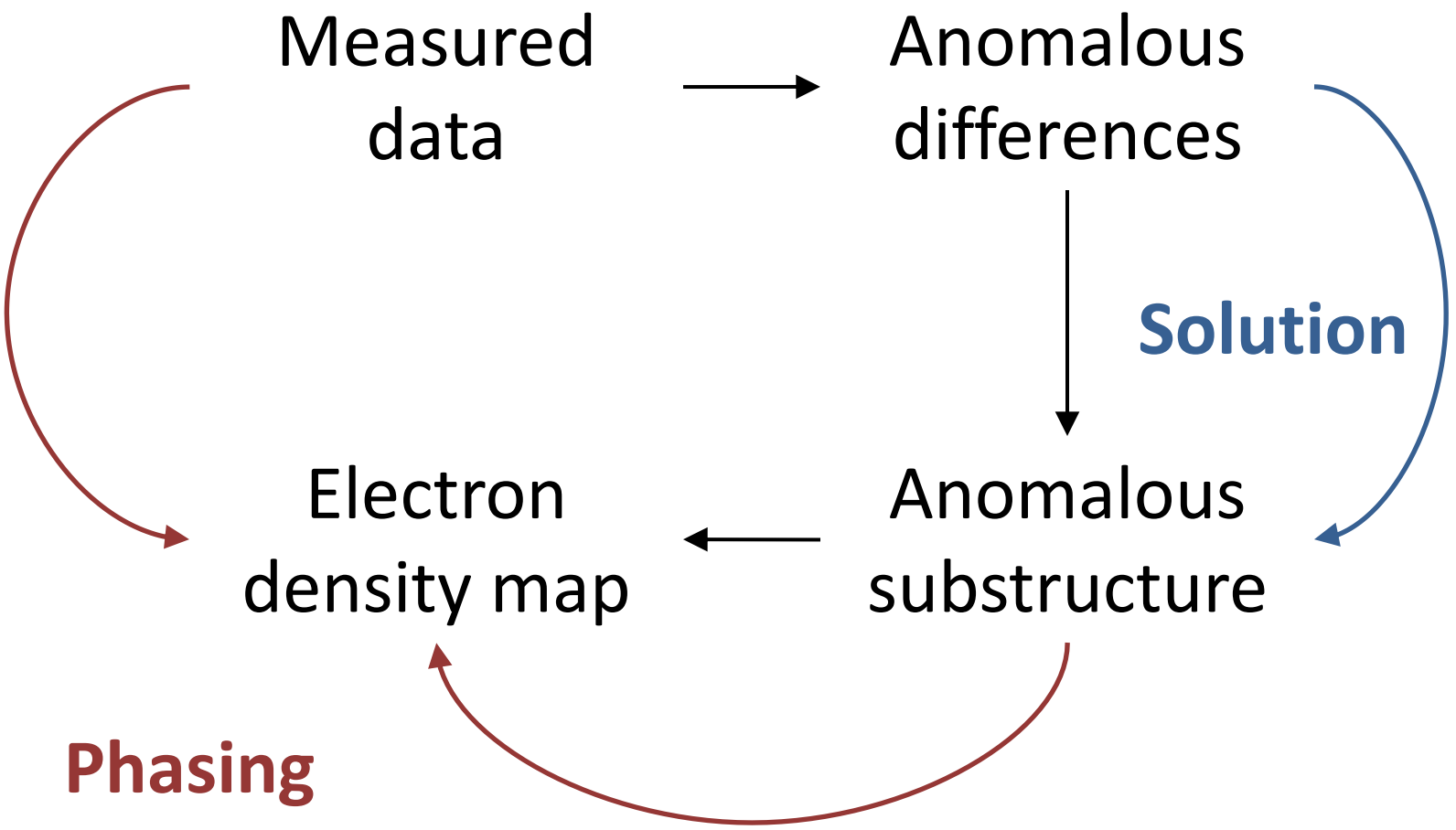
UNIVERSITY OF CAMBRIDGE

University of Cambridge
Randy Read, Airlie McCoy, Robert Oeffner

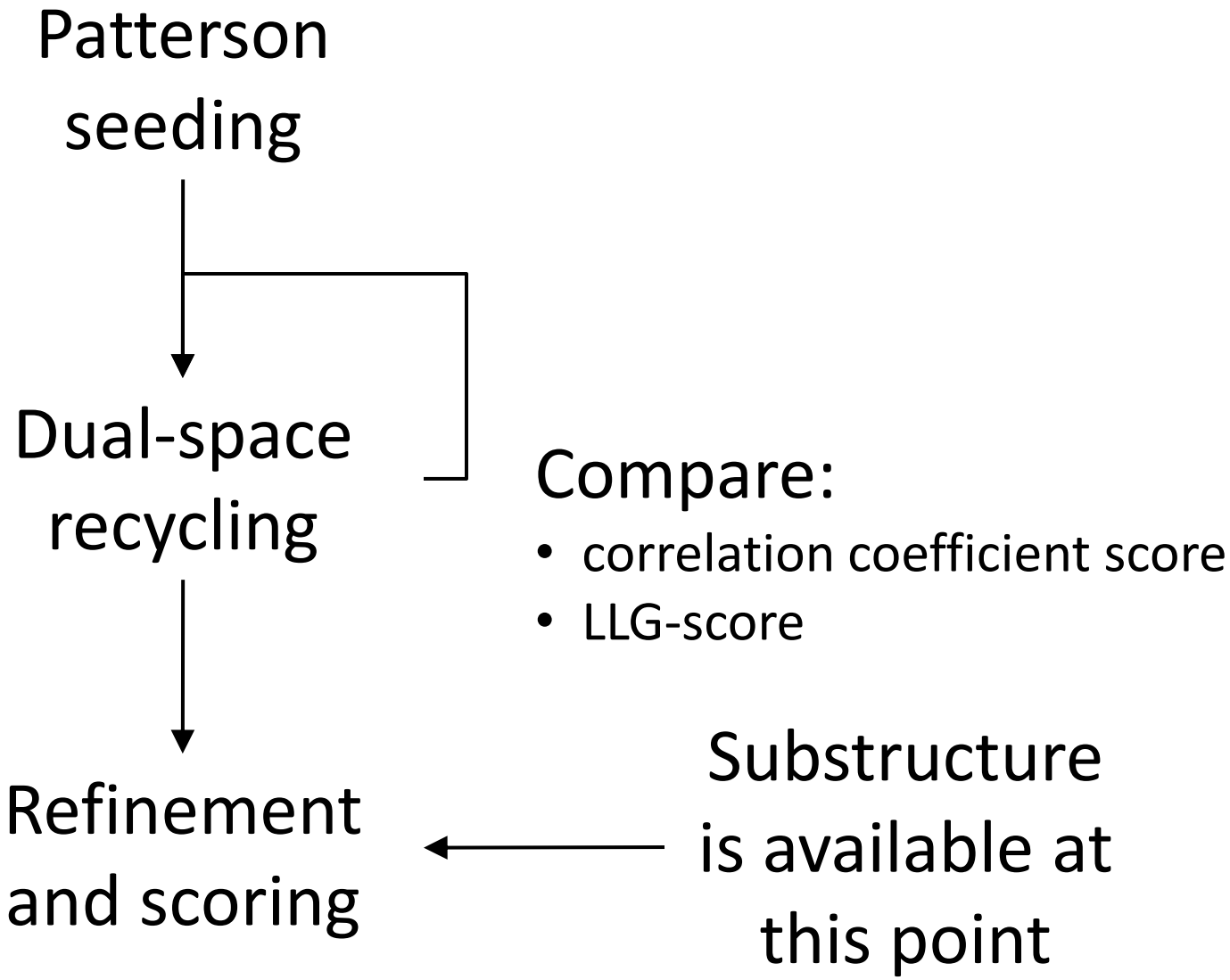
DUKE University

Duke University
Jane and Dave Richardson, Vincent Chen

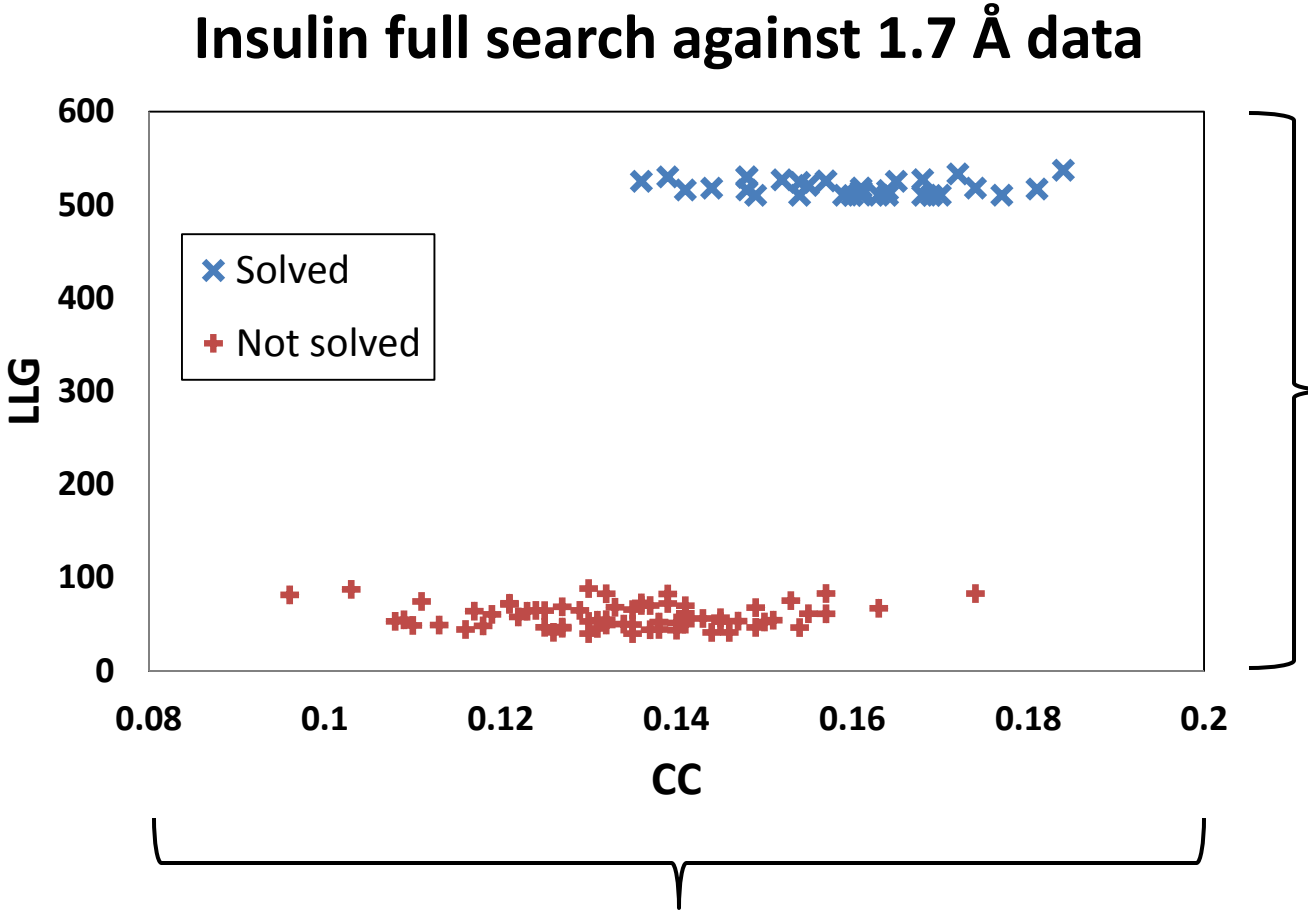
Substructure solution



Substructure solution workflow



Rescore trials with phaser

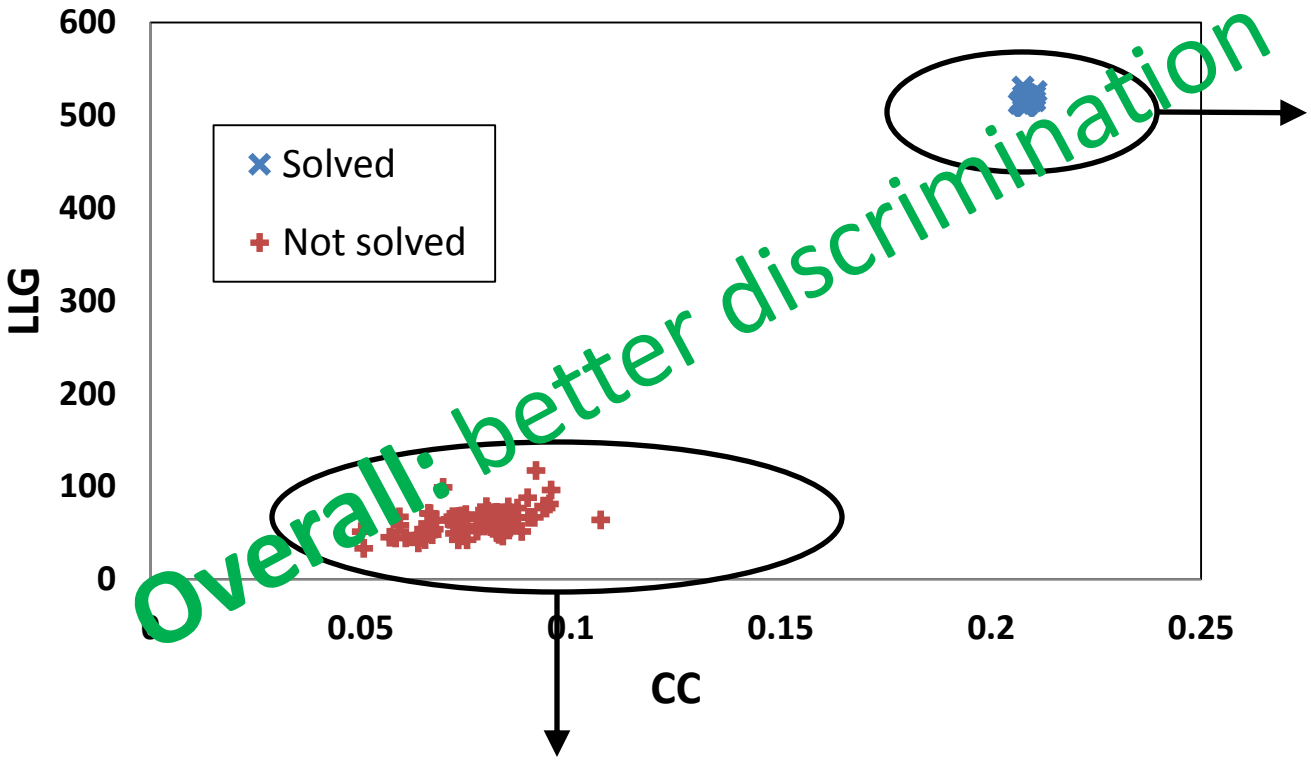


Clearly separated solution and non-solution groups

Impossible to identify solutions

CC for phaser-refined substructures

Insulin full search against 1.7 Å data



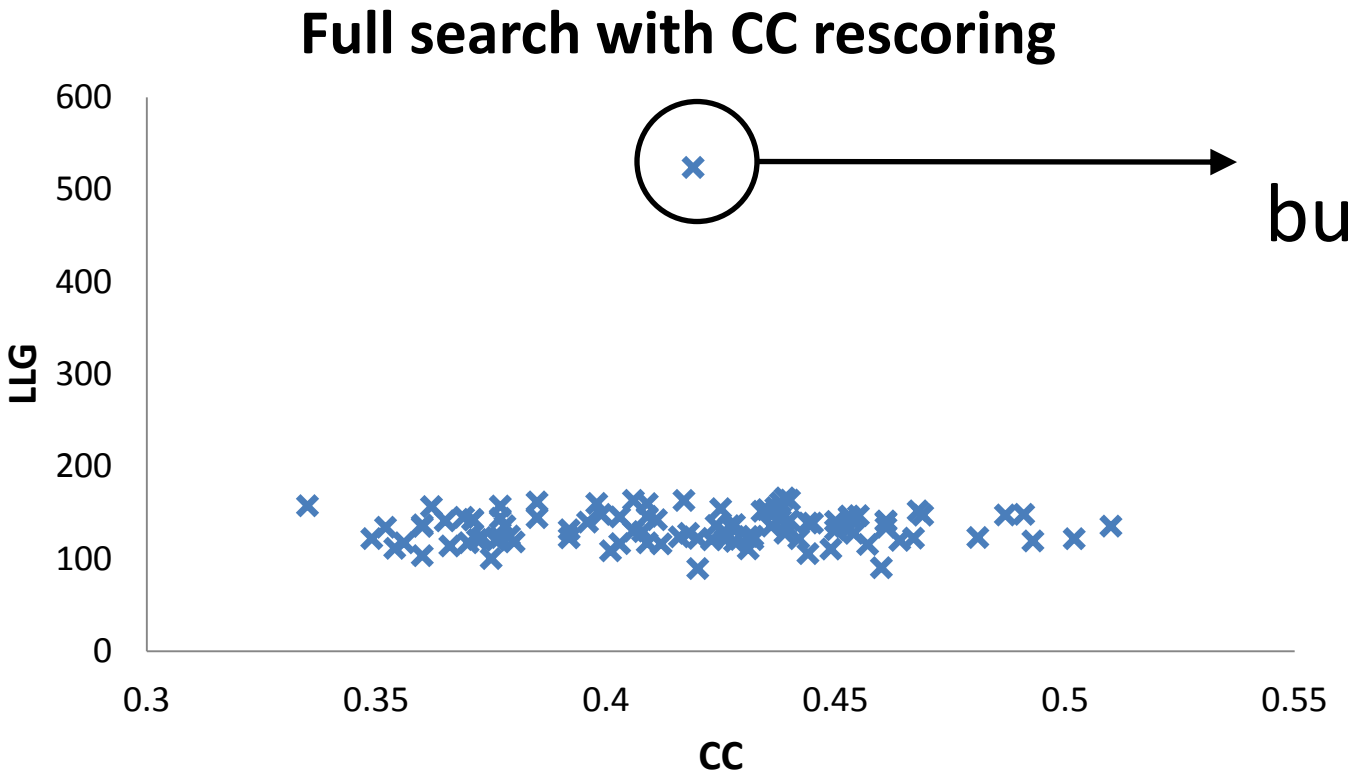
Increased CC for solutions

Completion: improved substructure

Lower CC for non-solutions

Refinement: reduced overfitting

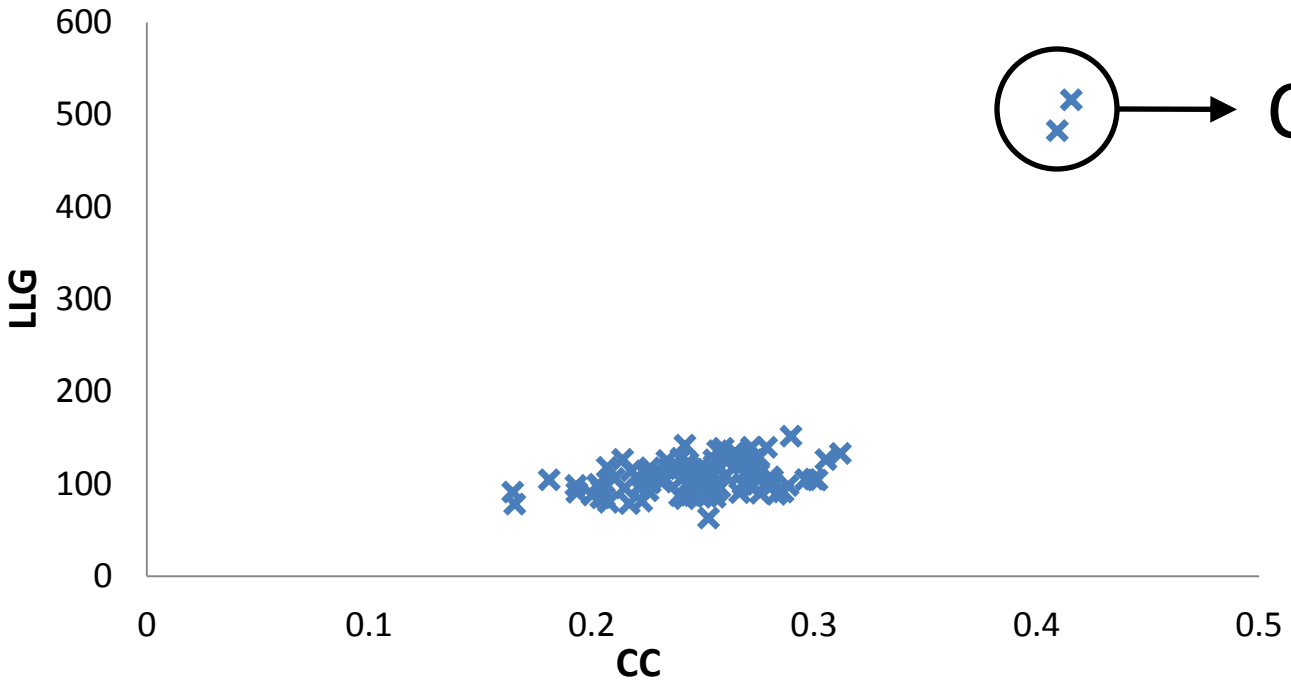
Fibronectin test case



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 test case

Full search with phaser rescoring



Clear solutions

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