Experimental Phasing

Airlie McCoy

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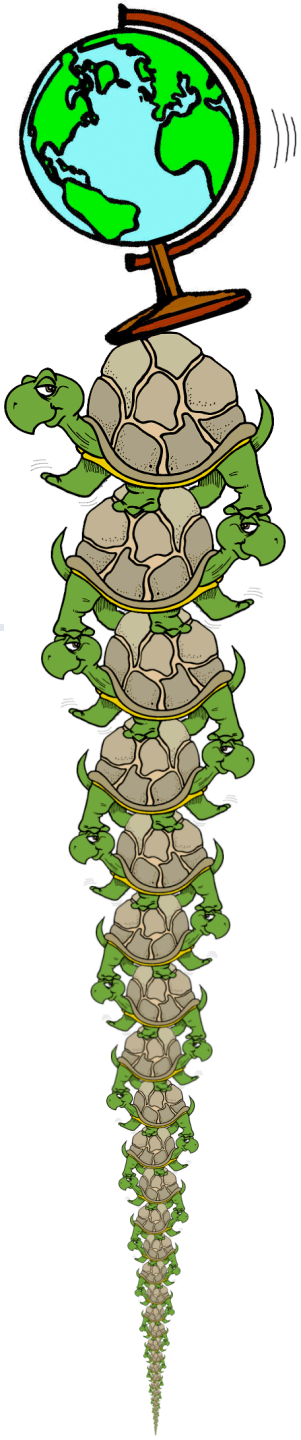
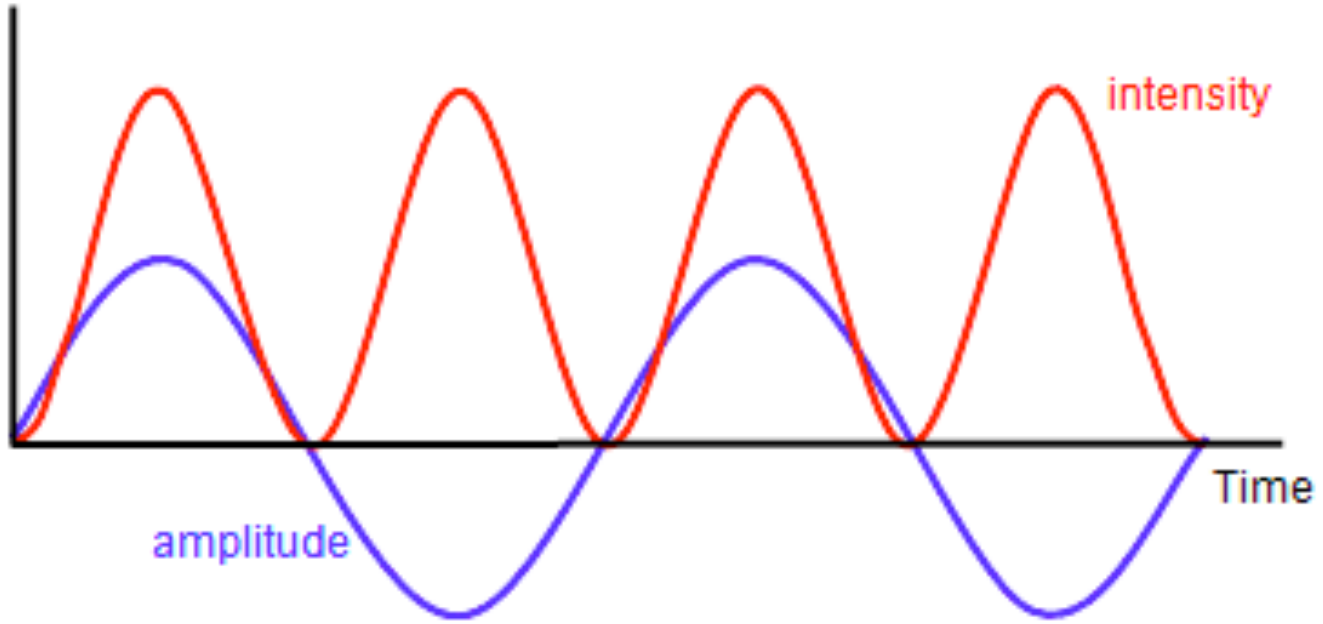


Interference

- X-ray detectors only detect X-ray intensities

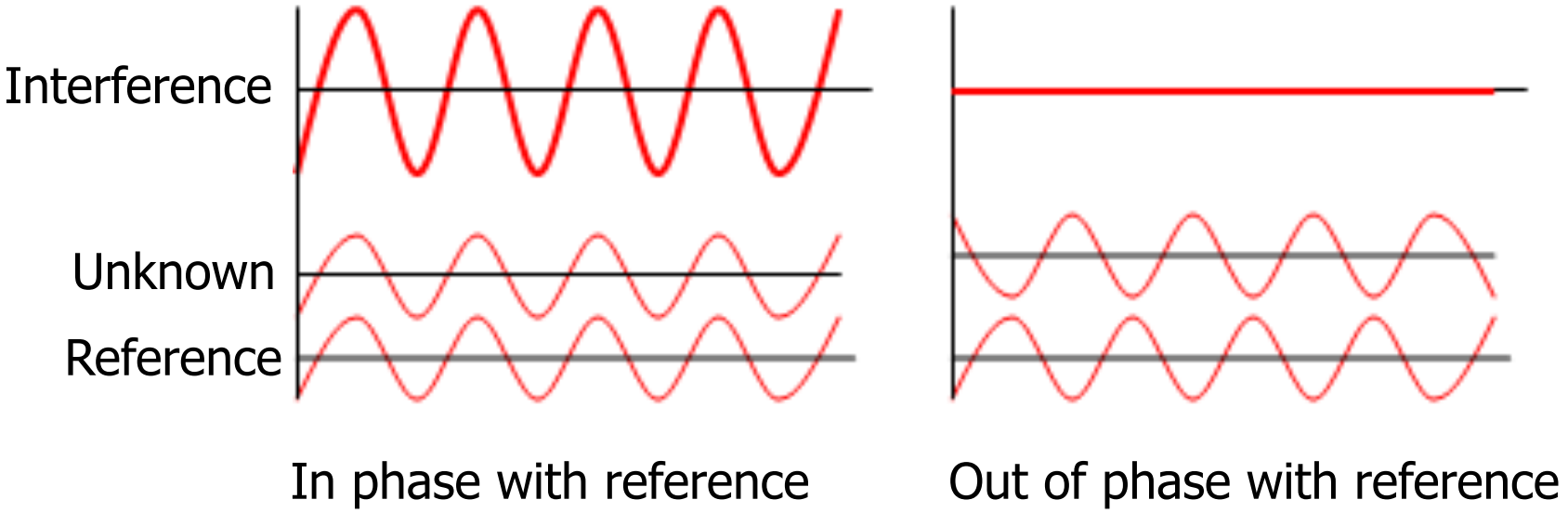


$$E \propto A^2$$

because they respond to the energy of the wave

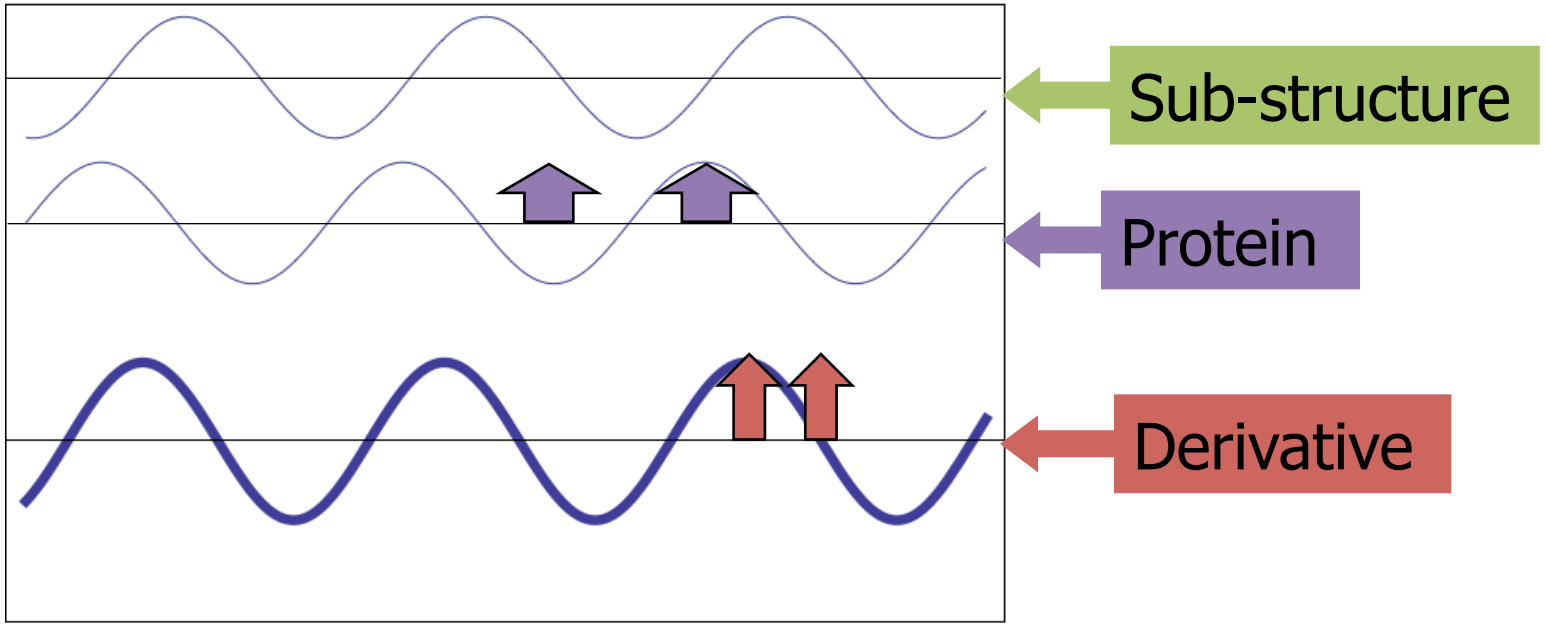
Reference Wave

- To phase a wave of known amplitude by interference, look at the amplitude of the wave after interference with a reference wave of known phase and amplitude



Interference and Phase

- The phase and amplitude of the reference wave are known from calculating them from a substructure of atoms

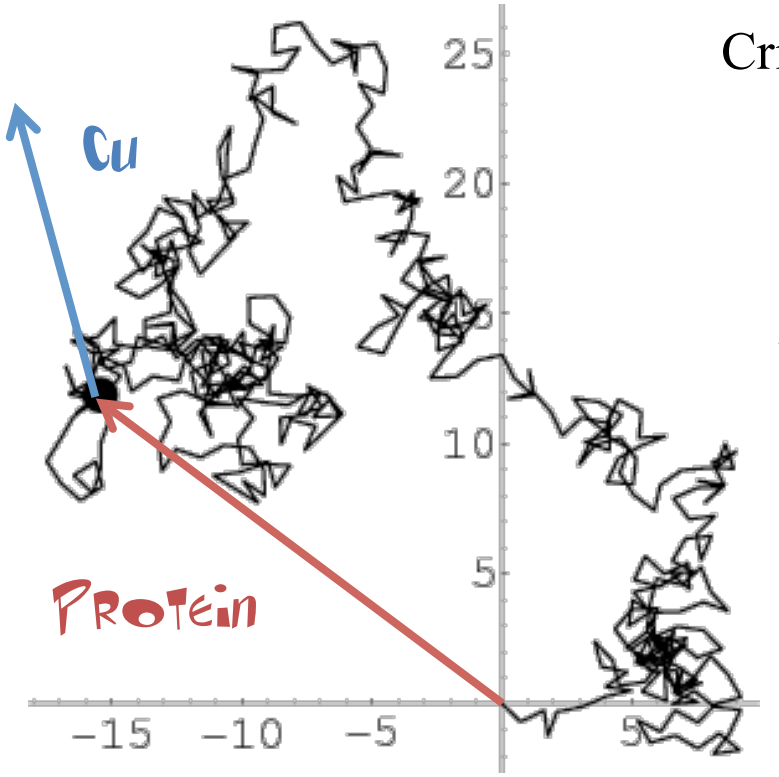


Substructure determination

- How do you determine the substructure?
- If you had the intensities of the substructure atoms floating in space you could solve the substructure
  - Patterson methods
  - Direct methods
  - Dual space methods
- PROBLEM
  - You don’t have these intensities!
- SOLUTION
  - Use approximations (see e.g. Blundell & Johnson 1976)

Heavy Atom Scattering is Significant

- How can e.g. a single Cu atom in a 100 kDa protein make any difference to the intensities?
- Structure factors add up as a "random walk"

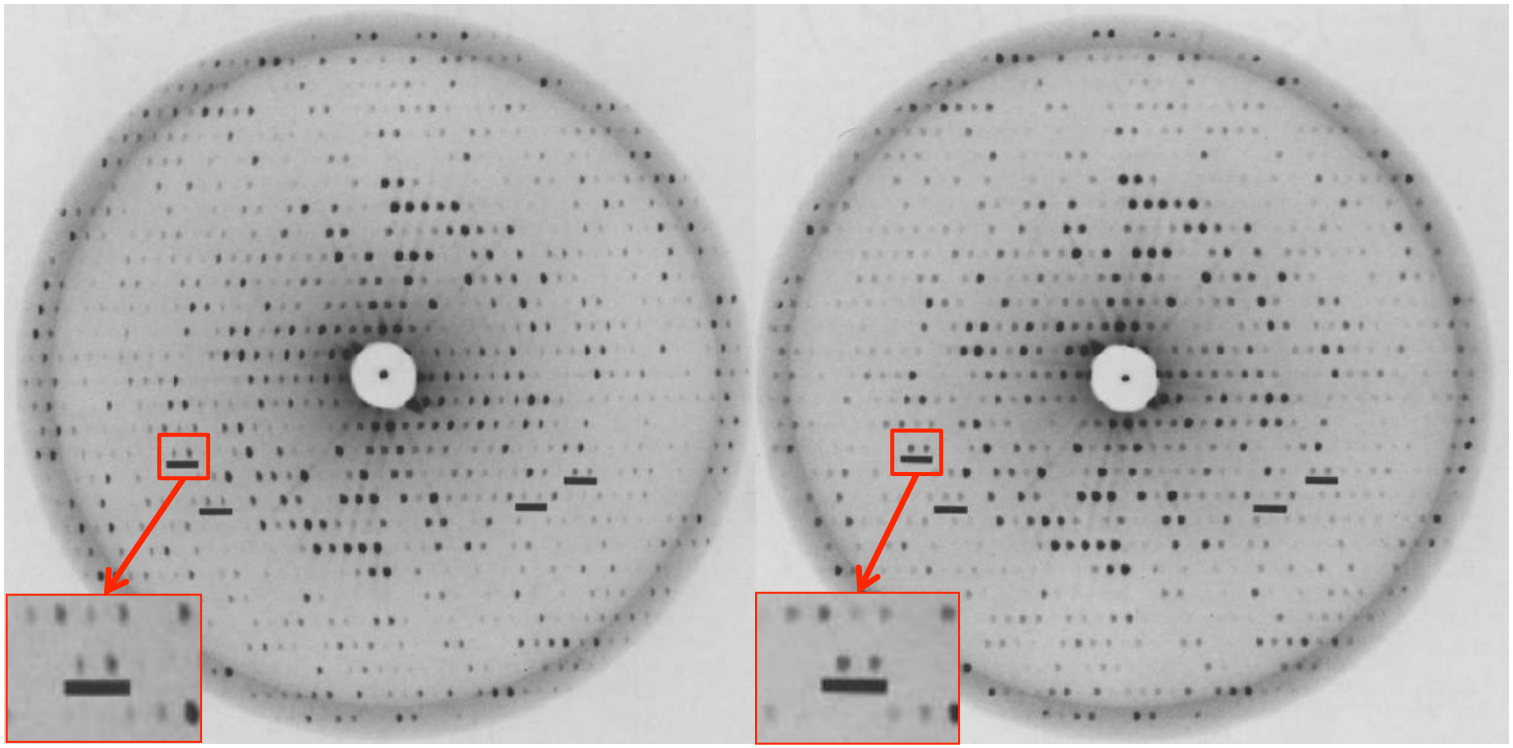


Crick and Magdoff (1956)

$$\frac{\langle \Delta F \rangle}{\langle |F| \rangle} = \frac{Z_H}{Z_{eff}} \sqrt{\frac{2N_H}{N_p}}$$

N_h, N_p are number of heavy, protein atoms
Z_h, Z_{eff} are atomic numbers of heavy atom and average of protein atoms (~6.7)

100 kDa protein with Copper (Z=28)
5.6%



Native

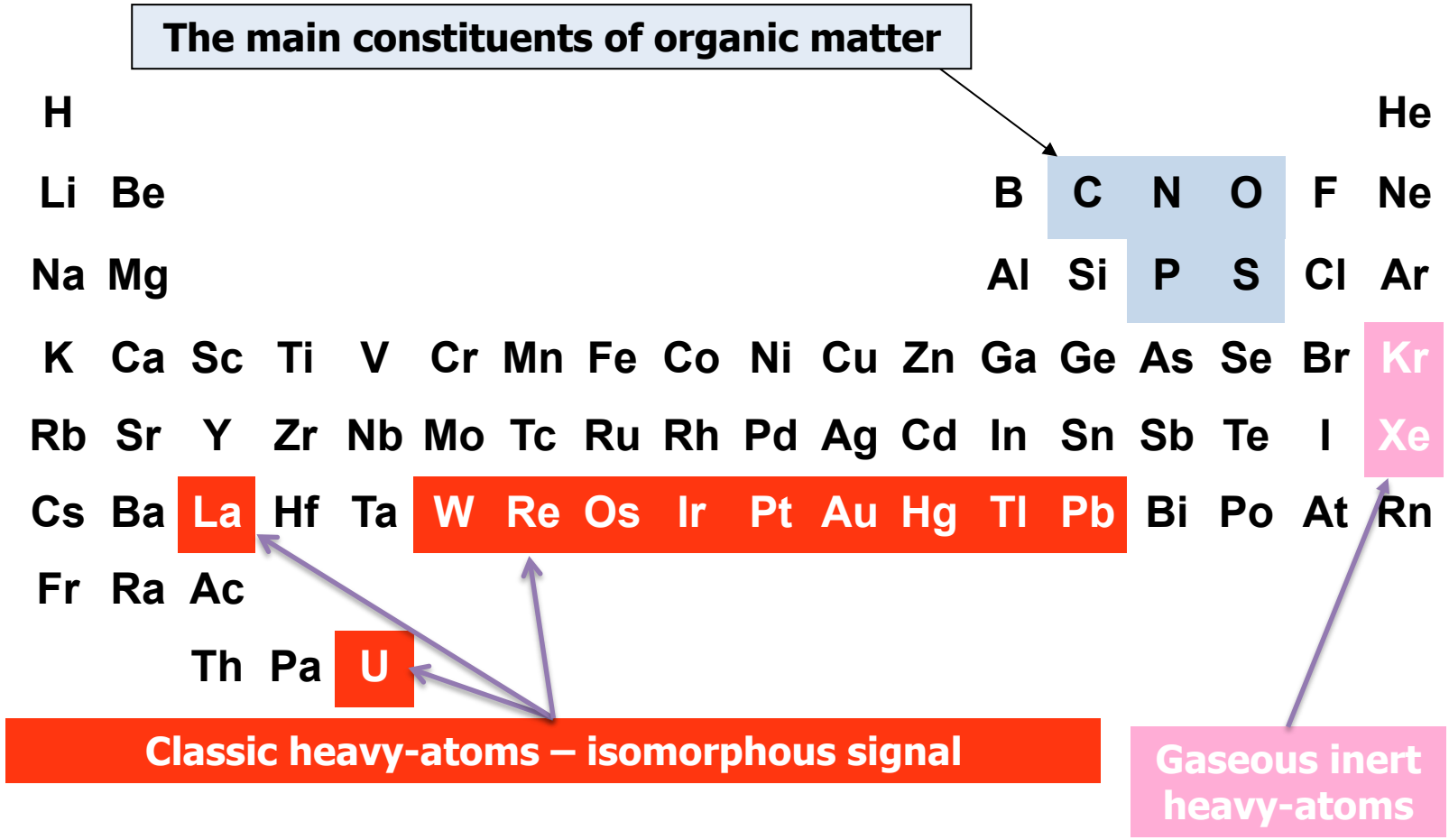
Heavy atom derivative

Intensity reversals (underlined)
indicate heavy atom has bound

Isomorphous Replacement

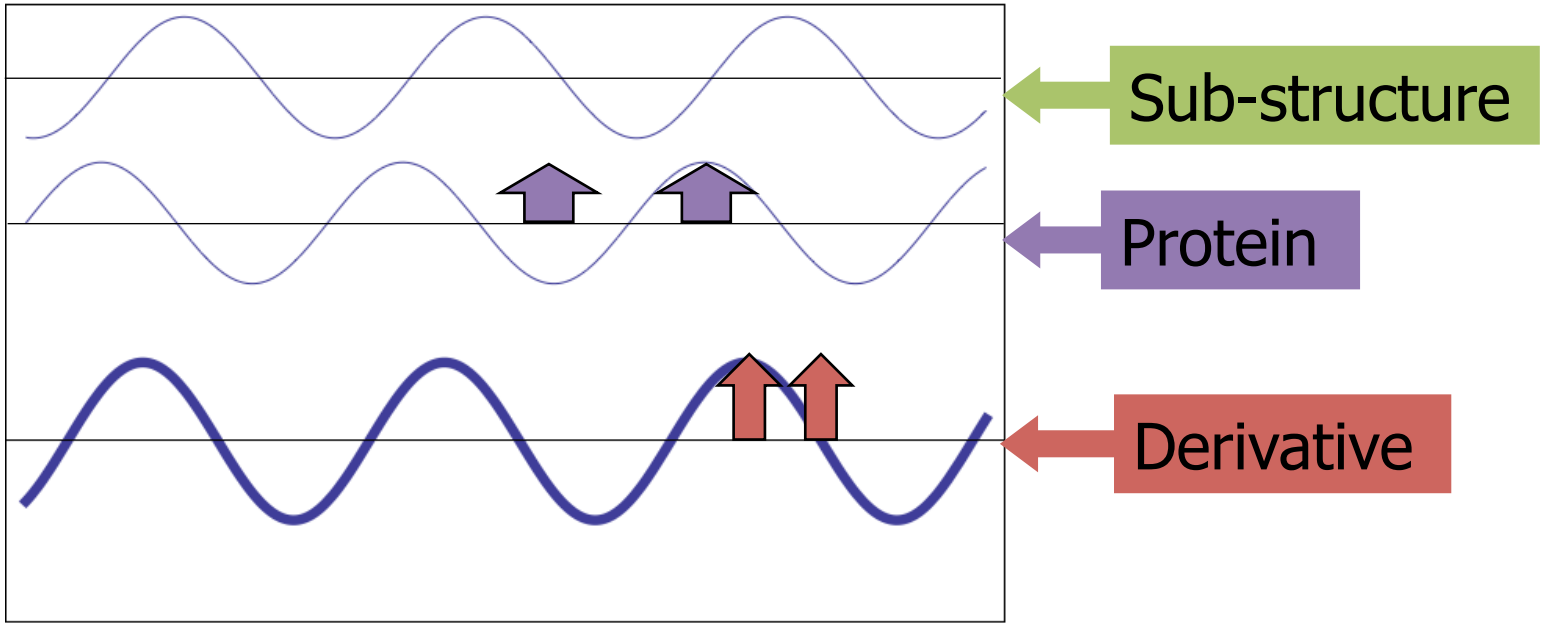
- Native and derivative must be isomorphous
  - Same unit cell and space group
  - Same position and orientation of protein in unit cell
- Can require searching many different compounds to find one or two isomorphous ones
  - Merge datasets from different crystals
  - e.g. BLEND to find isomorphous (partial) datasets

Isomorphous replacement

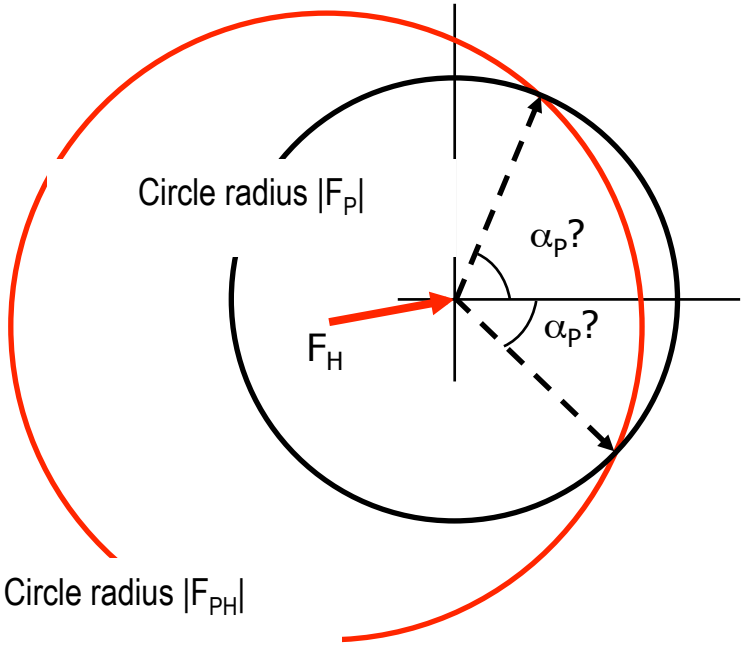


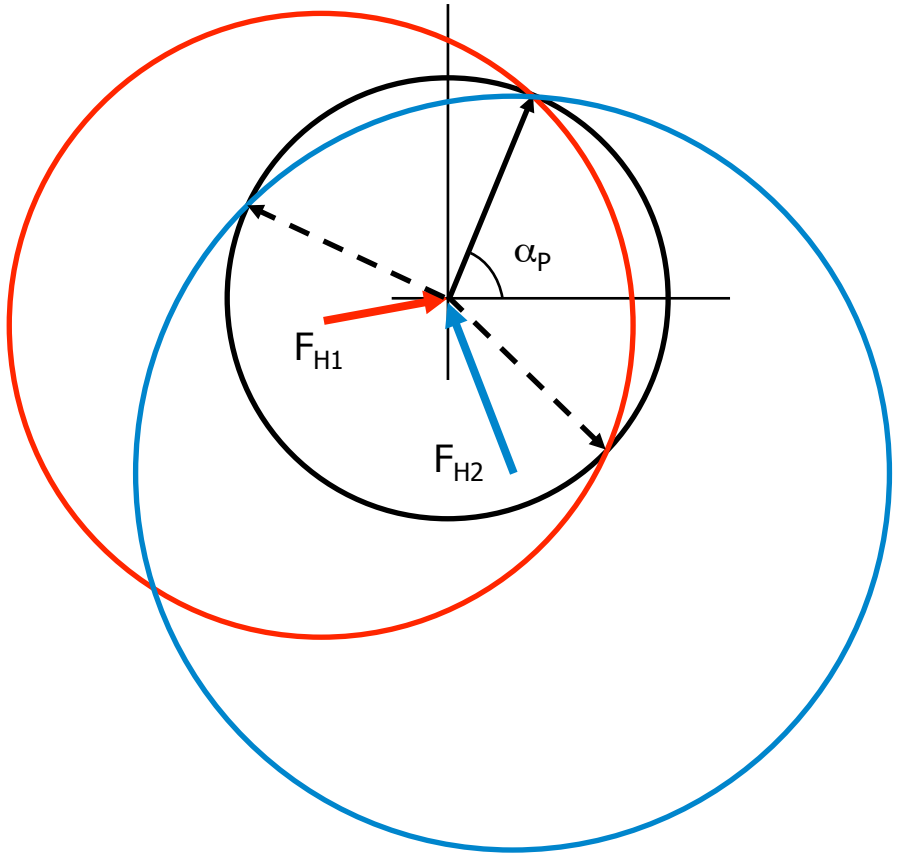
Harker Diagrams

- Two solutions for phase of unknown wave
- Also two solutions for phase of interference wave



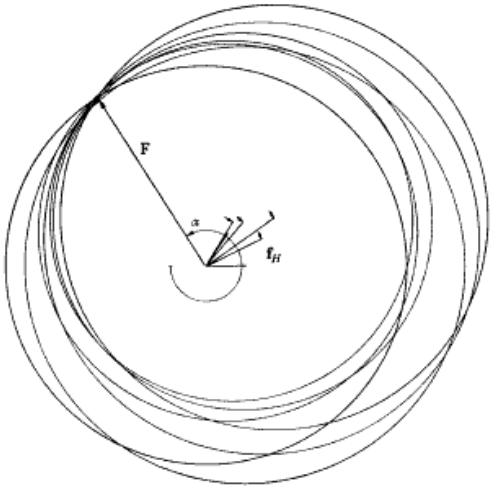
Harker Diagrams

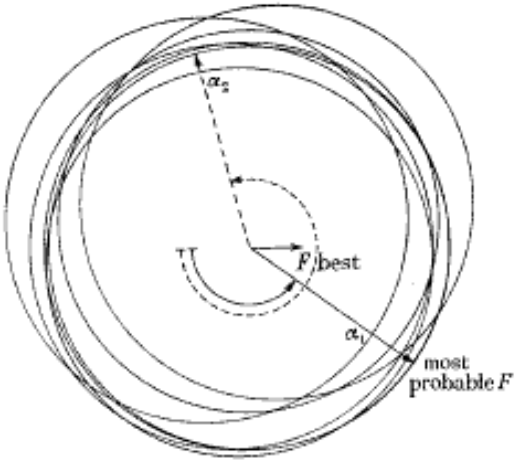


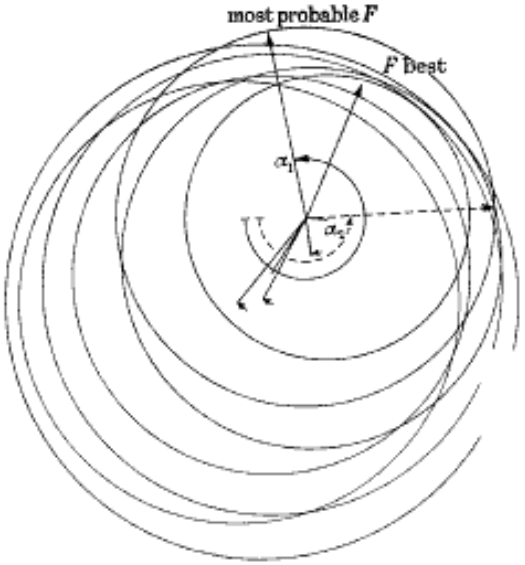


Reality…

- Some real Harker diagrams from the phasing of haemoglobin with 6 derivatives



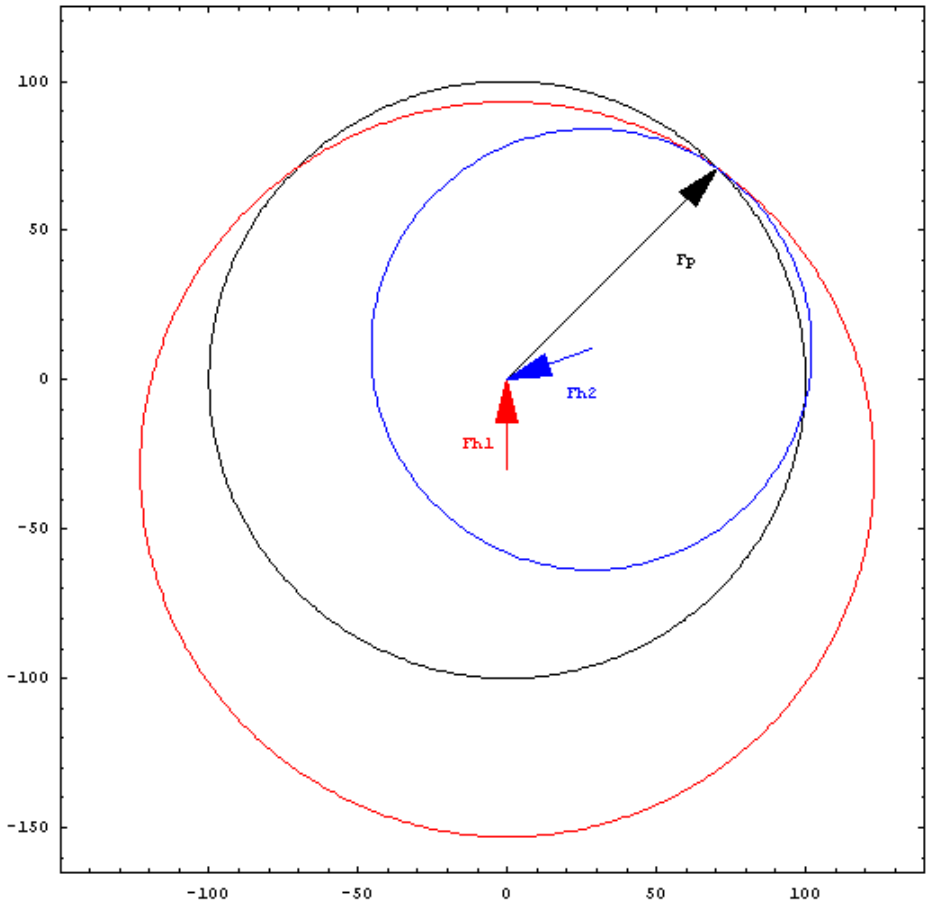




- Phase circles rarely cross exactly
- Need a **probabilistic** approach to determining the phase

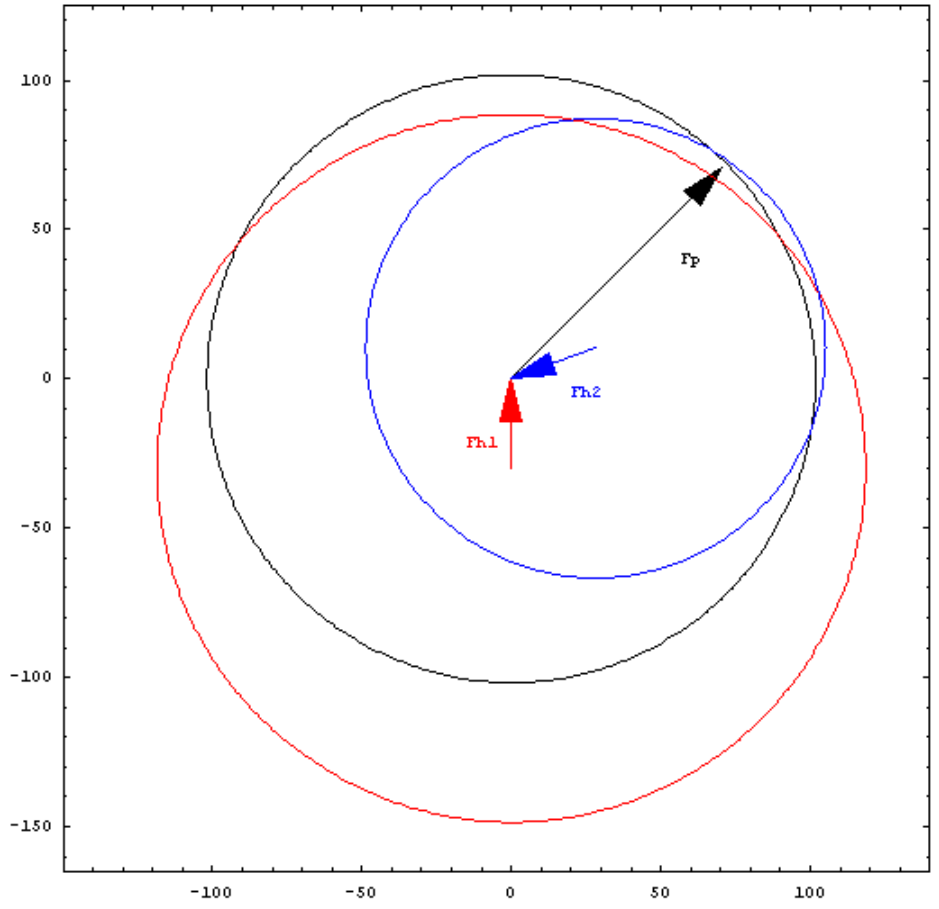
Harker construction

- Phasing of one refection using two derivatives with no errors
- Phase determined with very high probability



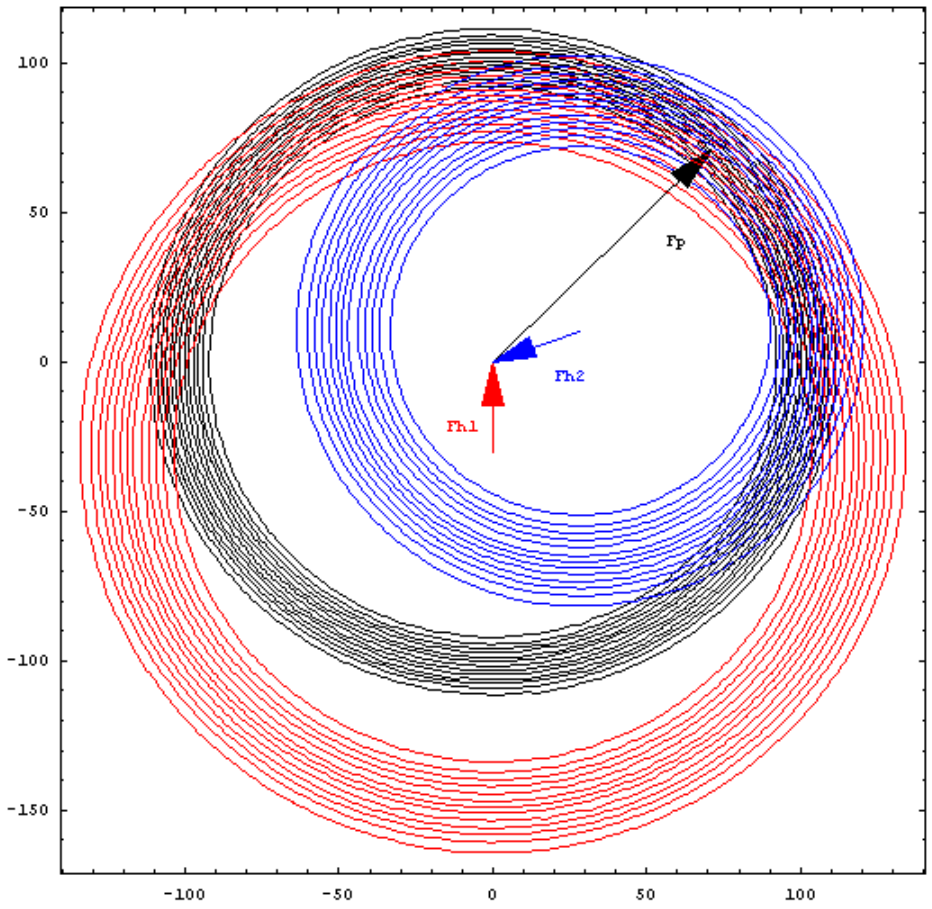
Harker construction

- There are many sources of error in the experiments
  - Mainly model errors
  - Also data errors
- The errors are large
- We are looking for the best phase
- We therefore need a probability function



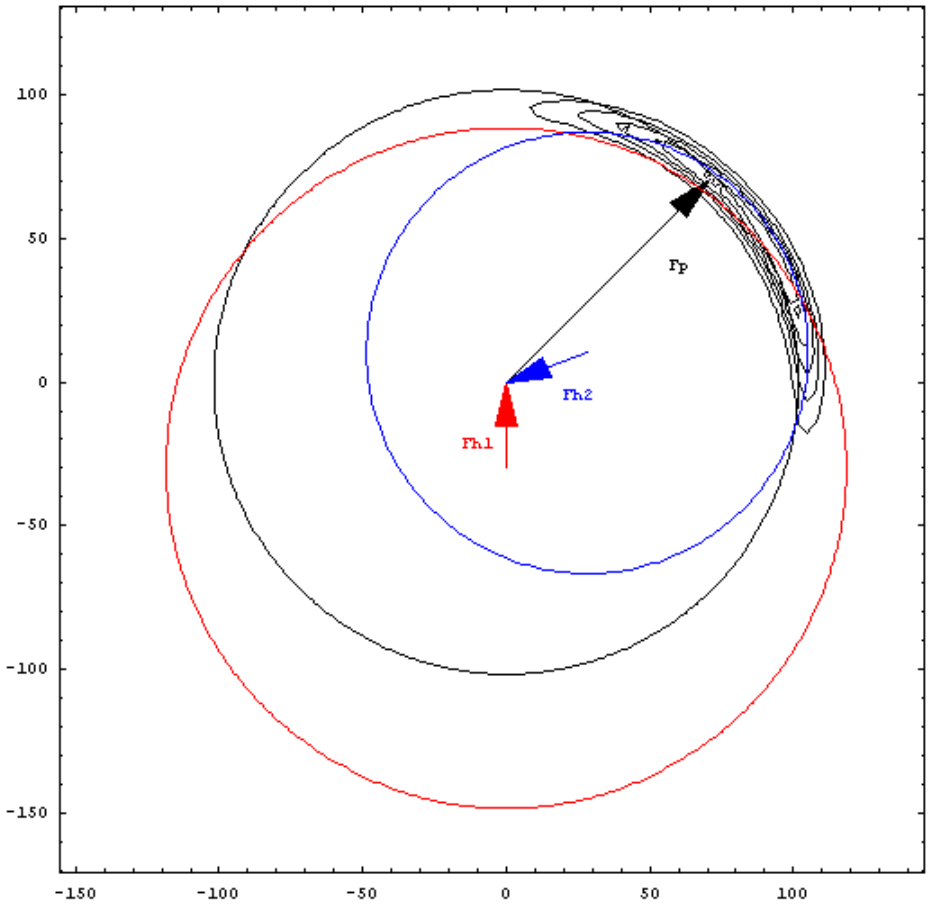
Probabilistic Harker Diagram

- Each circle has an error associated with it to give a distribution
- The total likelihood is the volume under the curve of the product of the distributions



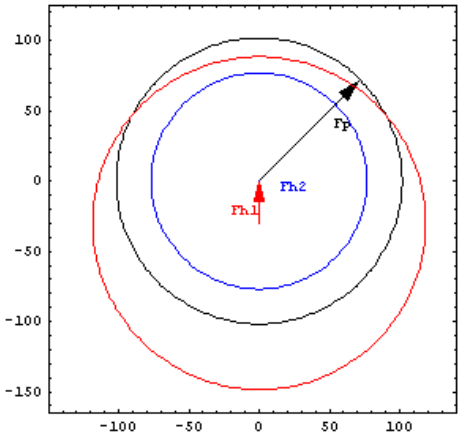
Probabilistic Harker Diagram

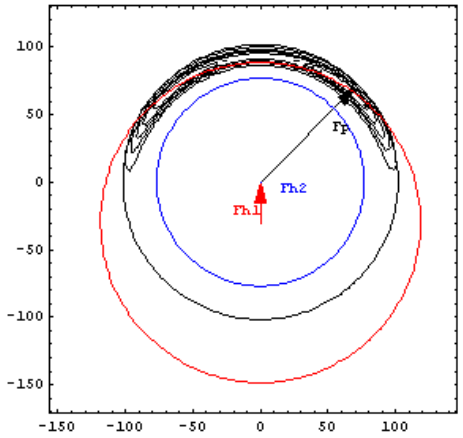
- The final distribution is high only where all three circles overlap



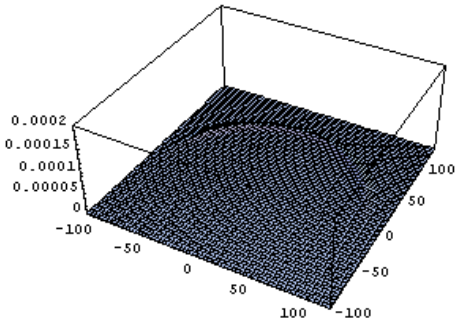
Refining Occupancy

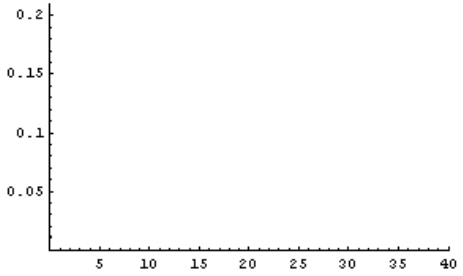
To refine the occupancy of a heavy atom, maximise the likelihood (area under the curve)





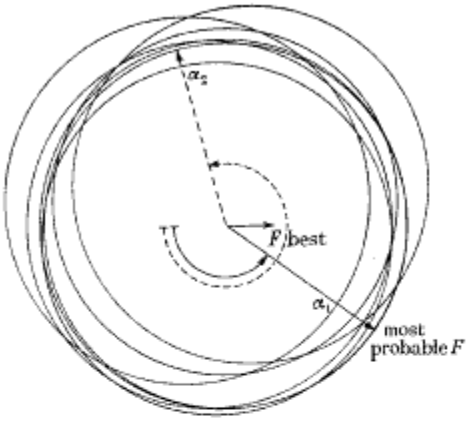
Final refined value is the optimum for ALL reflections (movie shows ONE reflection)



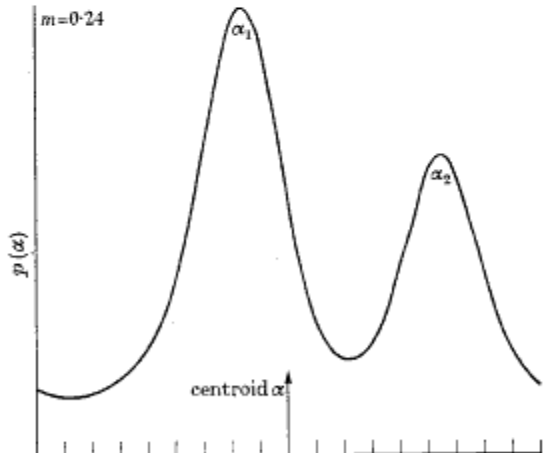


Phase probability

- Each reflection really has a phase probability density function (PDF) rather than a single phase
- This is a complicated mathematical function
  - Requires lots of memory
- Four Hendrickson-Lattman coefficients (A,B,C,D) are used to store this PDF in a compact form



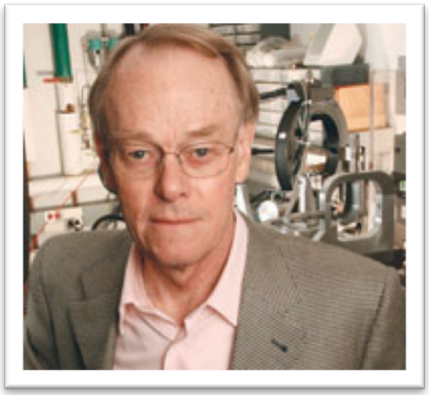
(b)



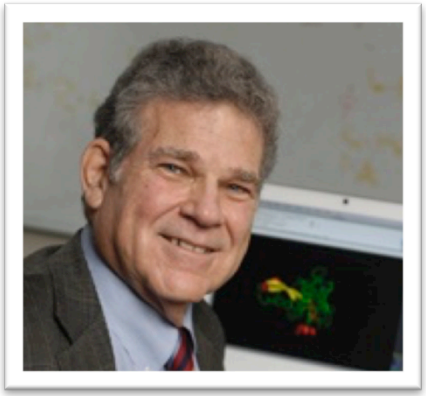
Hendrickson-Lattman Coefficients

$$P(\alpha) \propto \exp\left[A\cos(\alpha) + B\sin(\alpha) + C\cos(2\alpha) + D\sin(2\alpha)\right]$$

- HL coefficients allow for easy combination of phase information from multiple sources
  - the combined PDF is formed simply by adding the A,B,C, and D from the two distributions



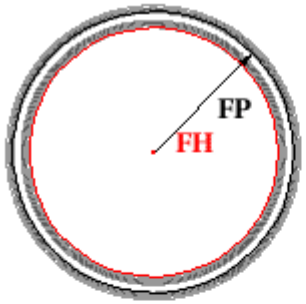
Wayne Hendrickson

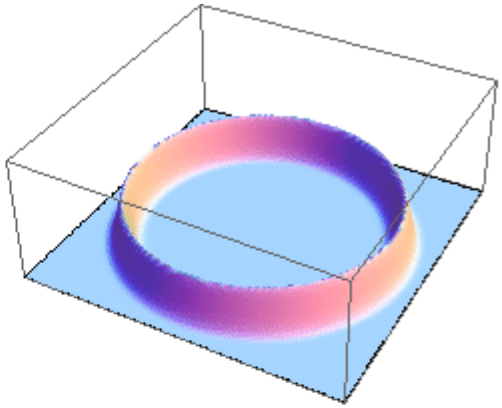


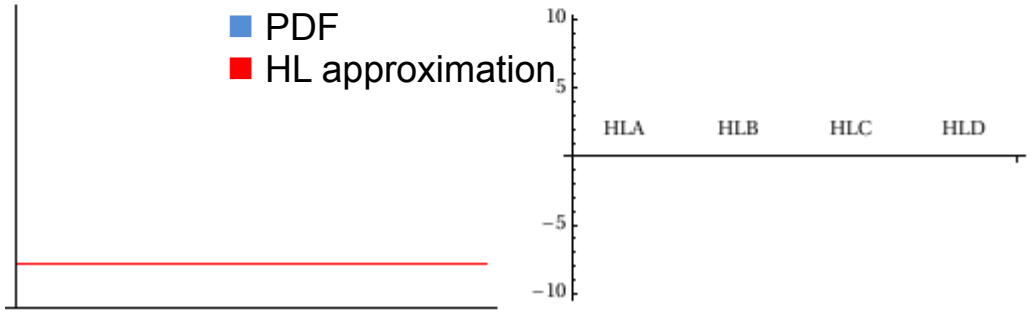
Eaton Lattman

Hendrickson-Lattman Coefficients

HL coefficients as a function of F_H occupancy








MIR
SAD
SIR
SIRAS
MIRAS
MAD
RIP

SAD

- The most popular way of solving structures by experimental phasing (over 70%)
- Can be done with intrinsic S and CuKα X-rays
- SAD phasing theory is very good
- Easy to automate
- Can be very fast
  - Can be done from single dataset
- May need multiple crystals
  - And careful data processing

Anomalous Scattering

A·nom·a·lous

adj.

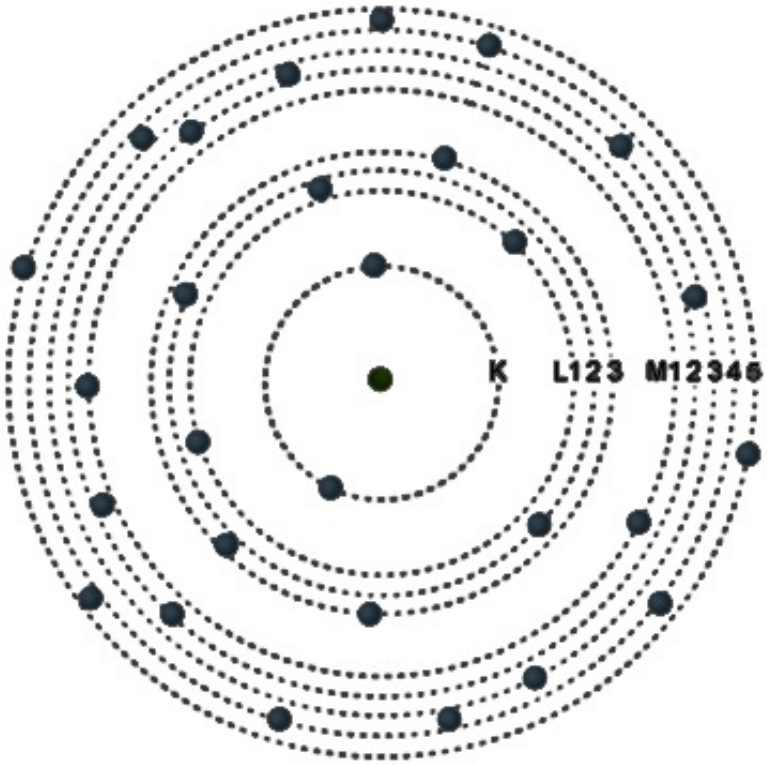
Deviating from the normal or common order, form or rule

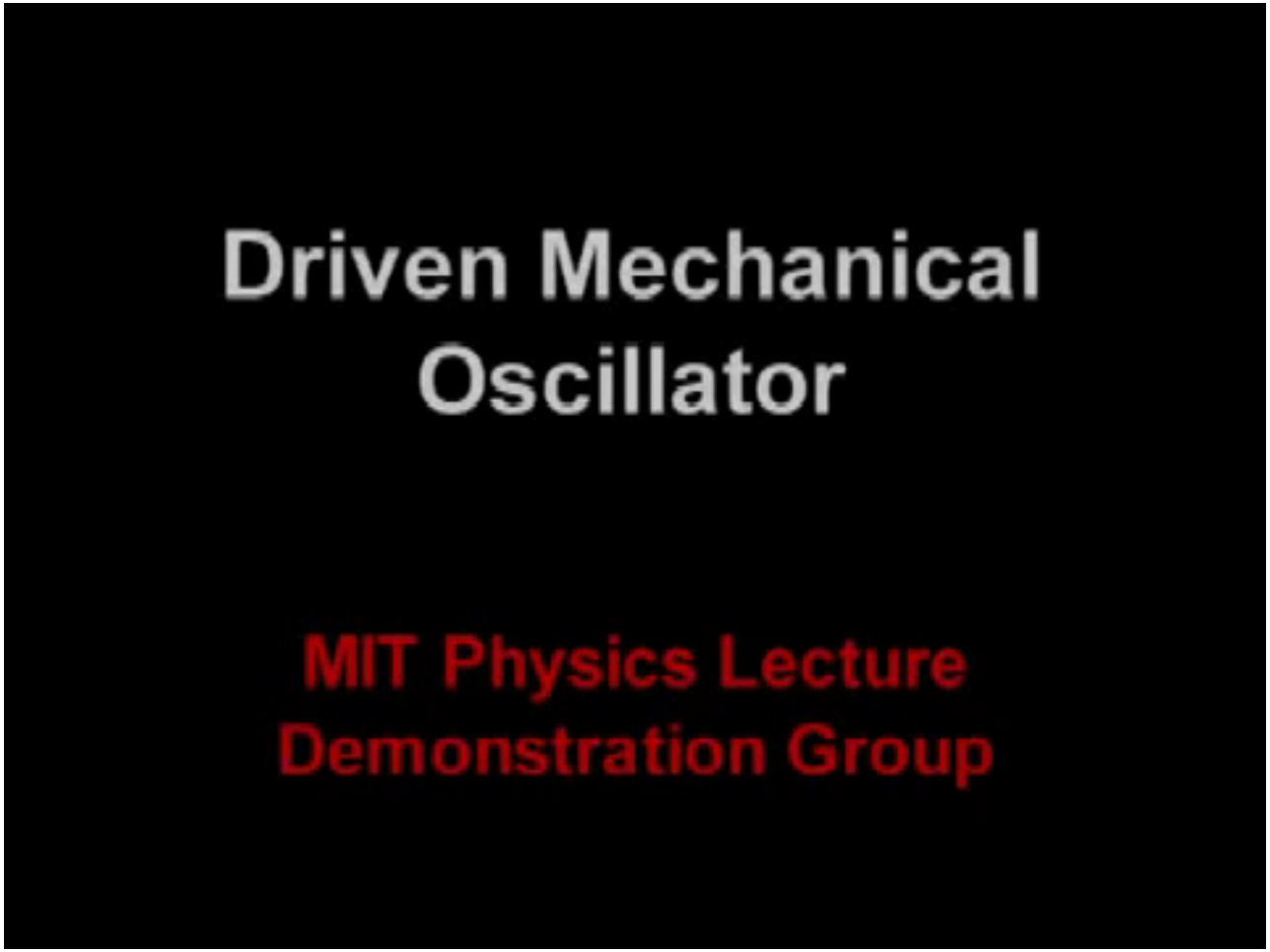
"**Anomalous** scattering" is **absolutely normal** while "**norma**l scattering" occurs only as an ideal, over simplified model, which can be used as a first approximation when studying scattering problems"

IUCR Pamphlet "Anomalous Dispersion of X-rays in Crystallography"
S. Caticha-Ellis (1998)

Anomalous Scattering

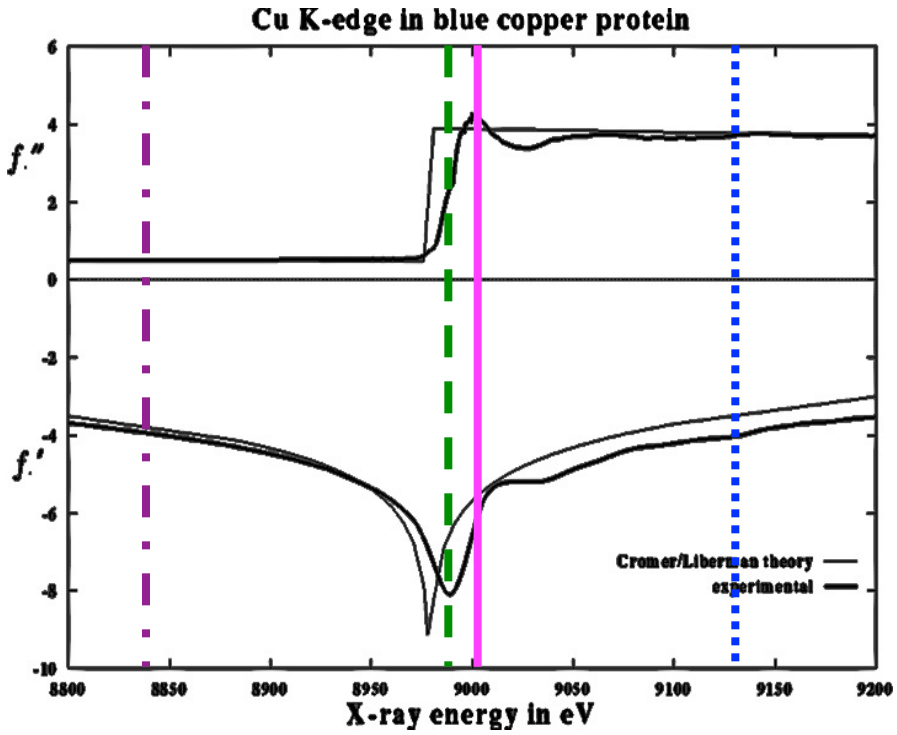
- Anomalous scattering is due to the electrons being tightly bound (particularly in K & L shells)
- In classical terms, the electrons scatter as though they have resonant frequencies





https://www.youtube.com/watch?v=aZNnwQ8HJHU

Wavelength Choice



PEAK: |f”| is large

INFLECTION: |f’| is large

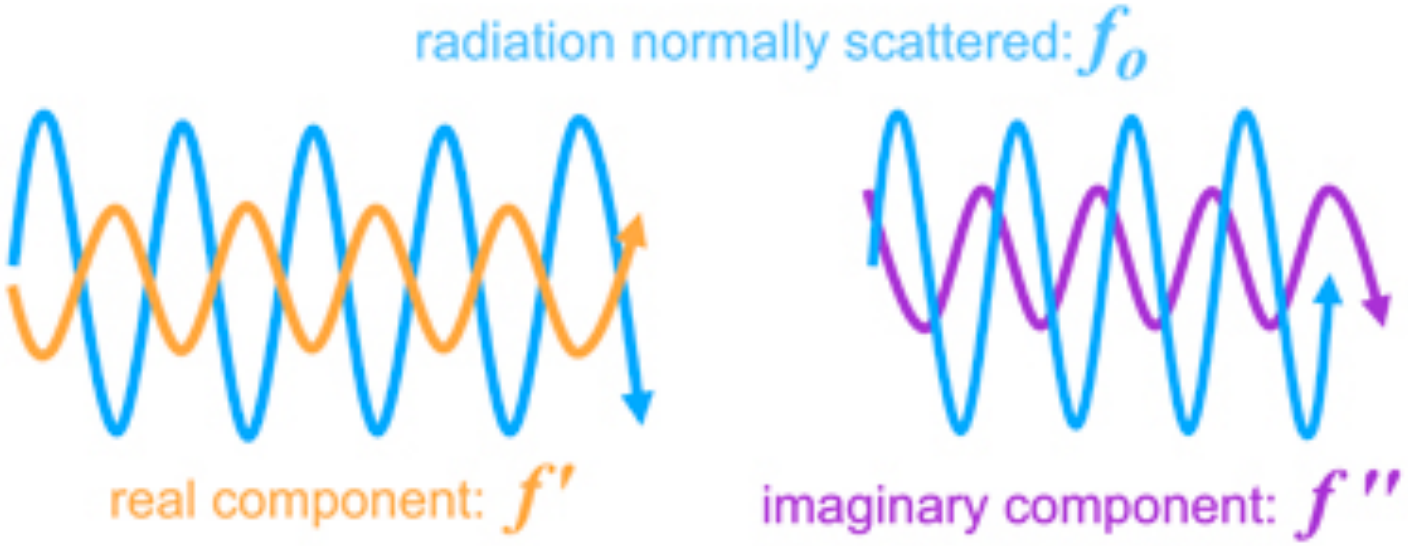
REMOTE: low energy

REMOTE: high energy

Warning
Radiation
Damage
INCREASES
at peak

Anomalous Scattering

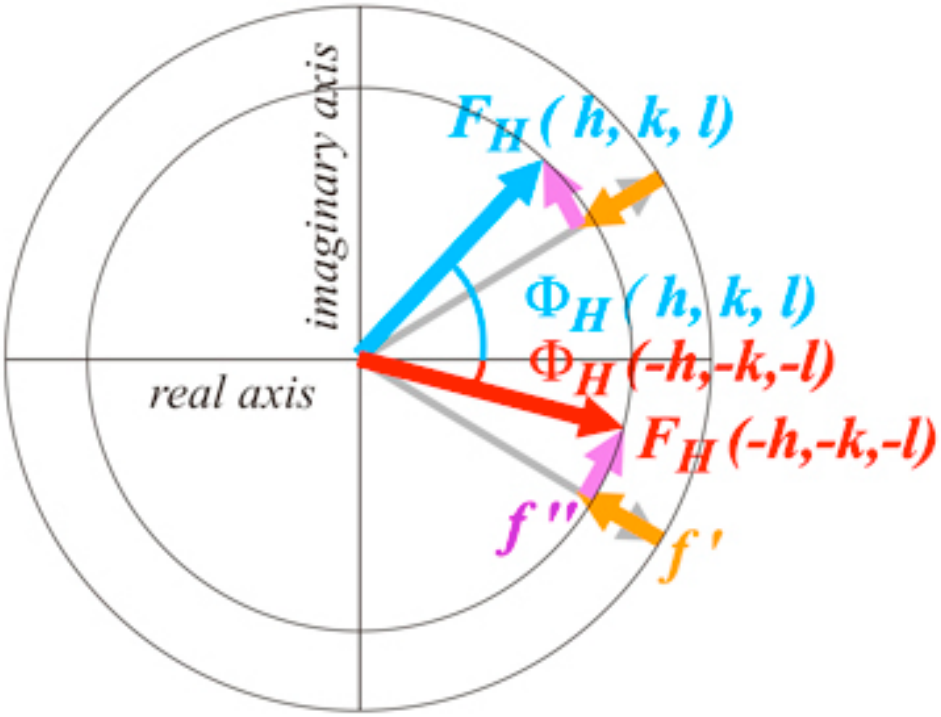
$$f = f_o + f' + if''$$



The real component (f ') is 180 degrees out of phase with the normally scattered radiation.

The imaginary component (f ") is 90 degrees out of phase.

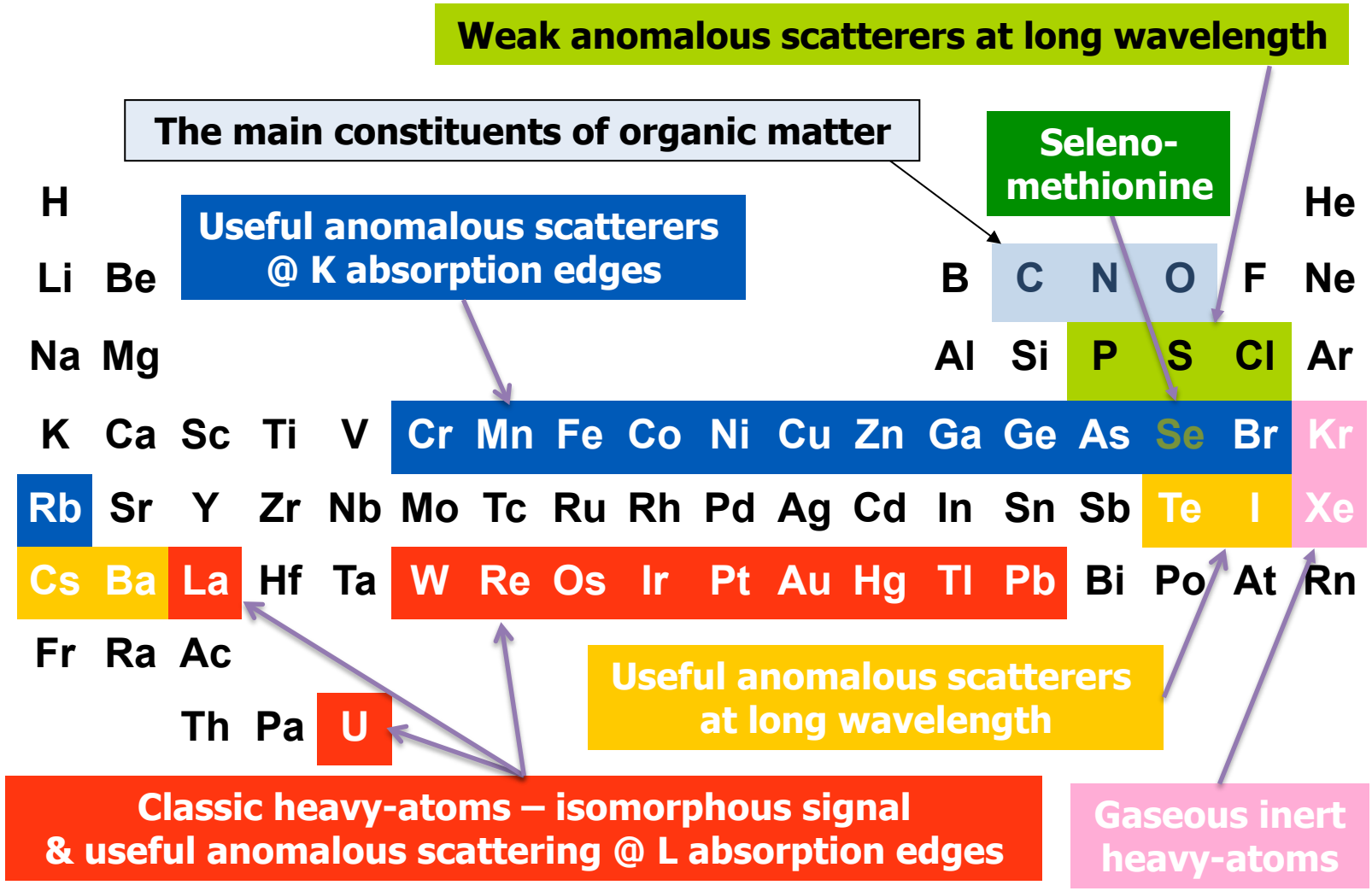
Anomalous Scattering



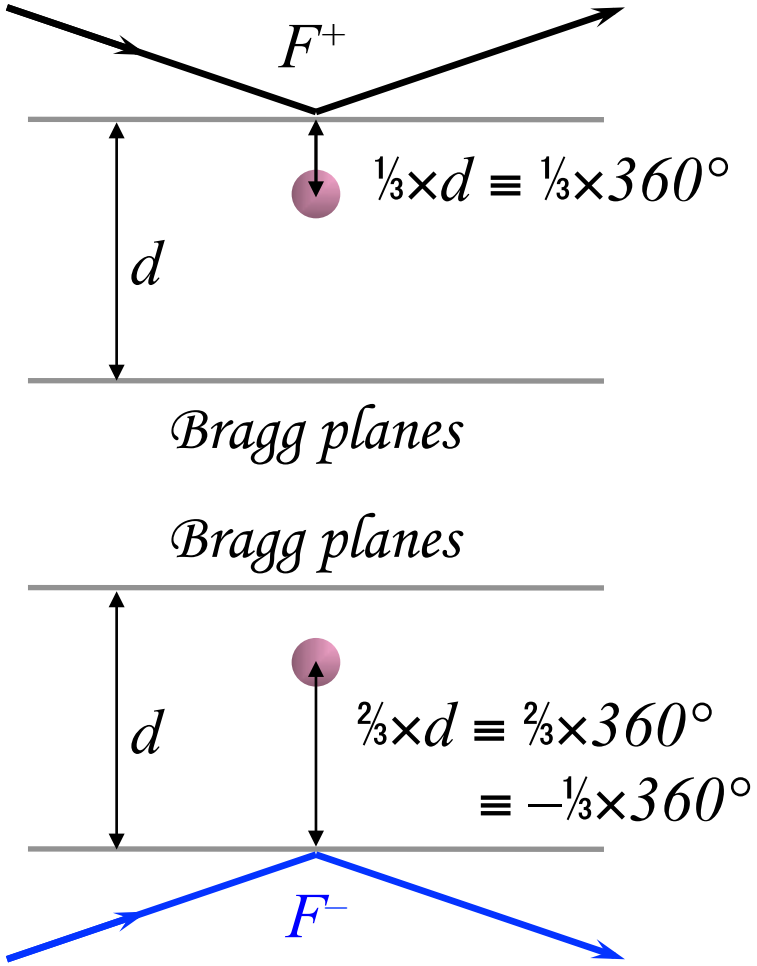
$$I_H(h, k, l) = I_H(-h,-k,-l)$$

$$\Phi_H(h, k, l) \neq -\Phi_H(-h,-k,-l)$$

Anomalous Scatterering

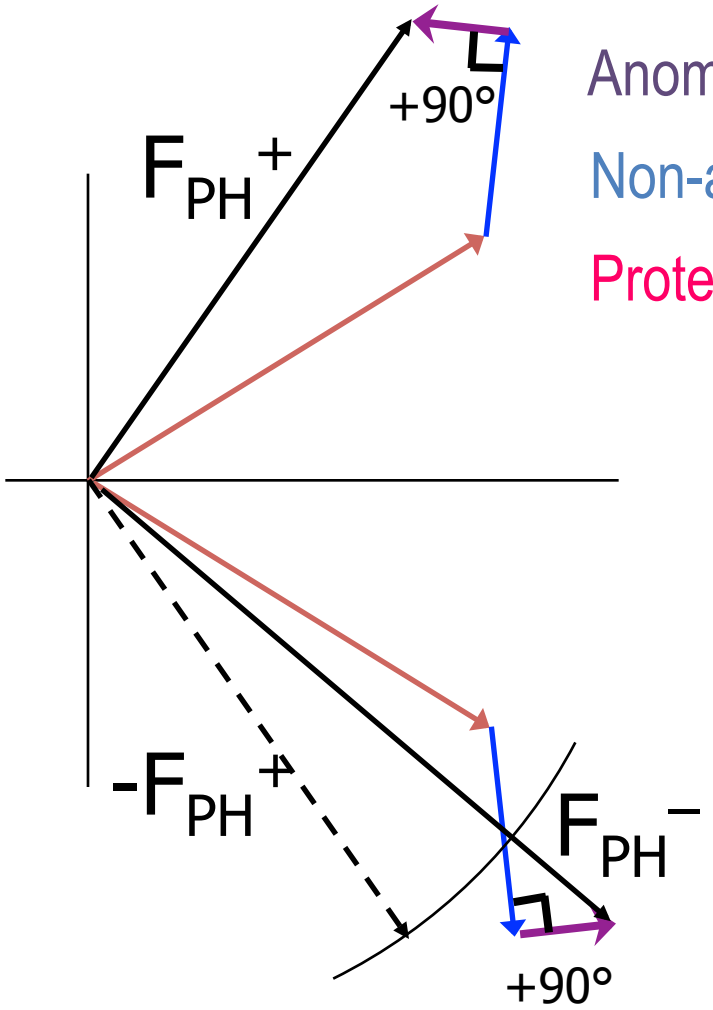


SAD Harker Diagram



- Phase of non-anomalous component depends on the atom's location between Bragg planes
- Anomalous scattering component is always advanced by 90°
  - irrespective of position of atom between Bragg planes

SAD Harker Diagram



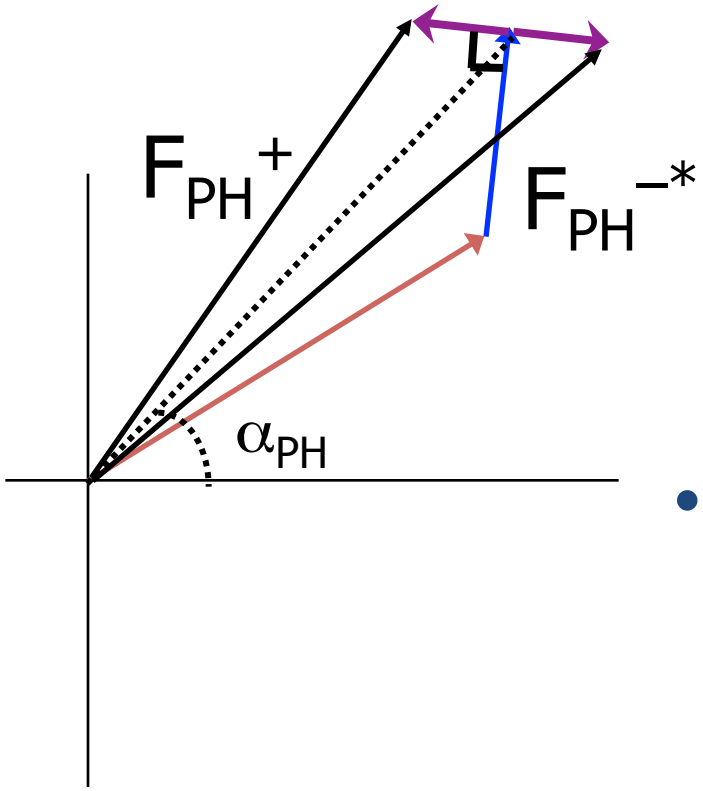
Anomalous part of heavy atom

Non-anomalous part of heavy atom

Protein (non-anomalous scattering)

- Non-anomalous and anomalous scattering components sum differently in the Friedel mates F_{PH}^{+} and F_{PH}^{-}
  - $F_{PH}^{+}$and F_{PH}^{-} have different intensities

SAD Harker Diagram



Anomalous part of heavy atom

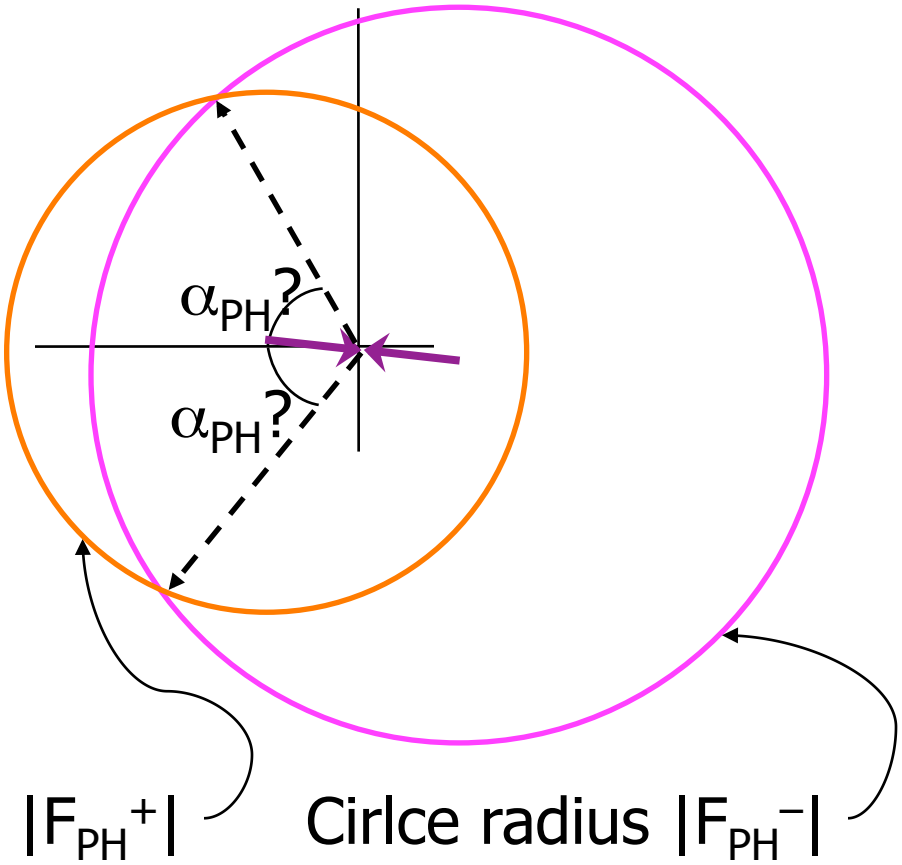
Non-anomalous part of heavy atom

Protein (non-anomalous scattering)

- It is easier/usual to use the complex conjugate of all Friedel F^{-}
  - i.e. all structure factors for (-h,-k,-l)
  - Phase F_{PH} (α_{PH})

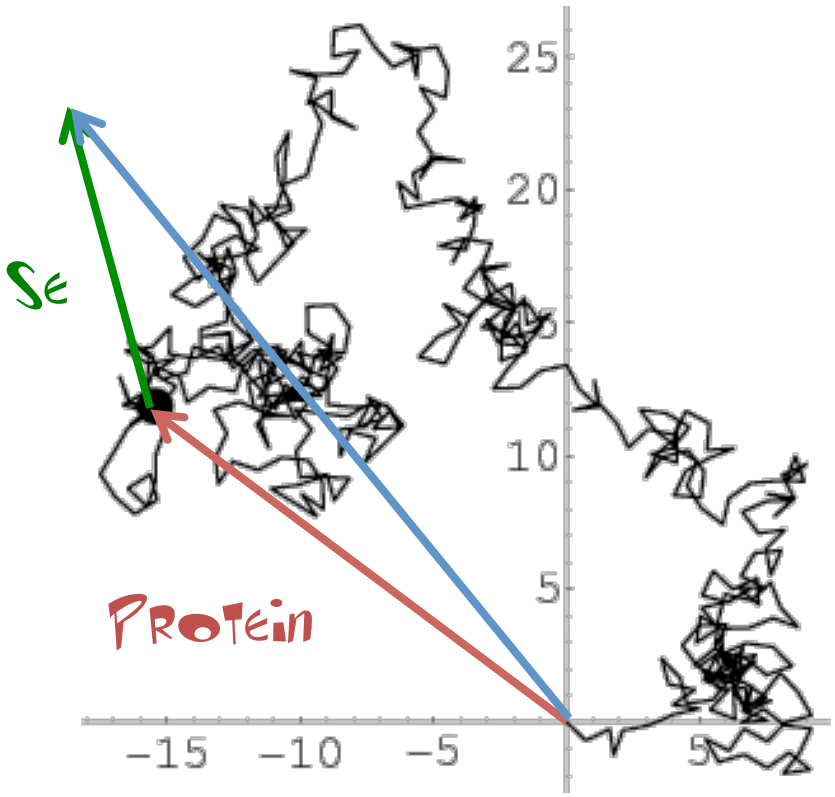
SAD Harker Diagram

- There is a phase ambiguity for FPH given by the two places where the two circles with centres displaced by the anomalous scattering vectors overlap



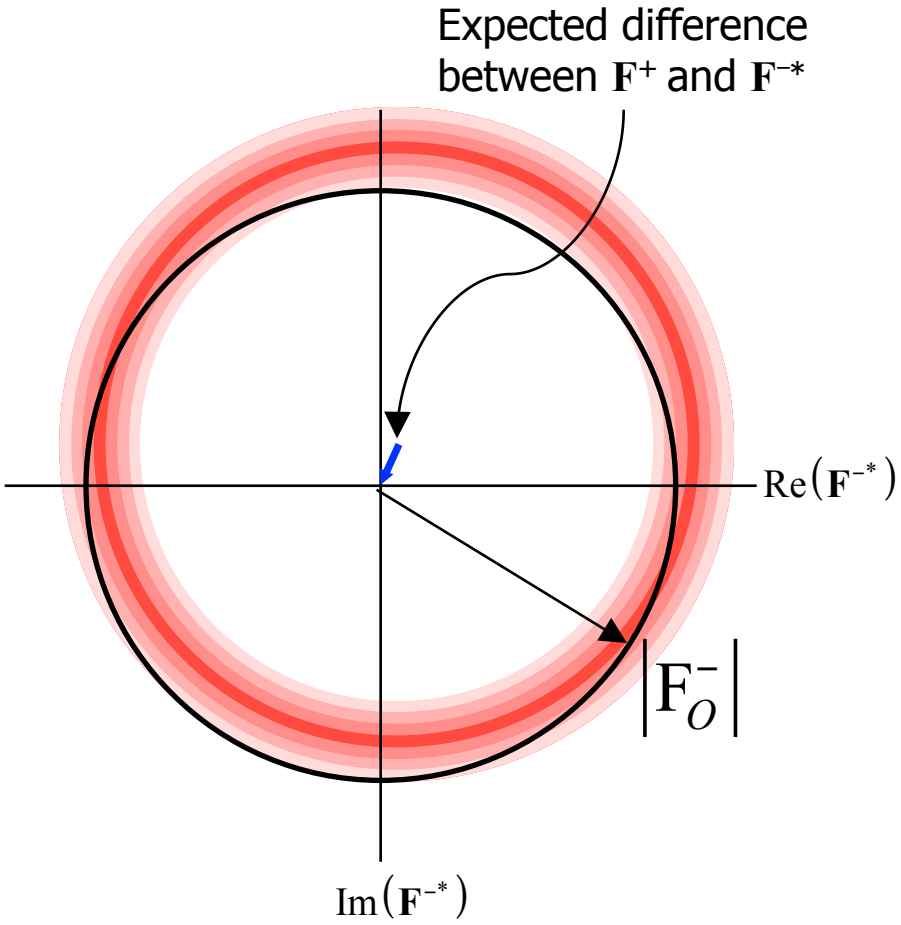
Partial Structure

- But there is more phase information!
- The anomalous scatterer is also a part of the structure
  - Gives additional phase information
  - Begins to break phase ambiguity



SAD Phasing

- Primarily anomalous scattering
- Gives a narrow probability distribution



SAD Phasing

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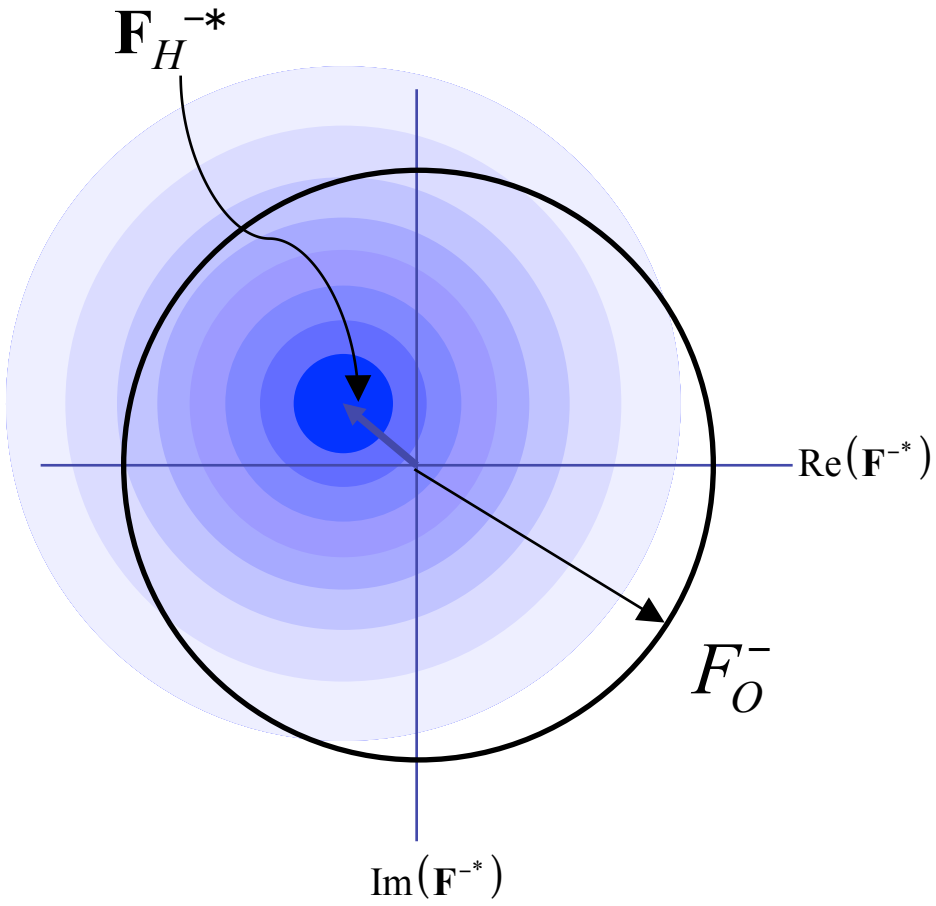
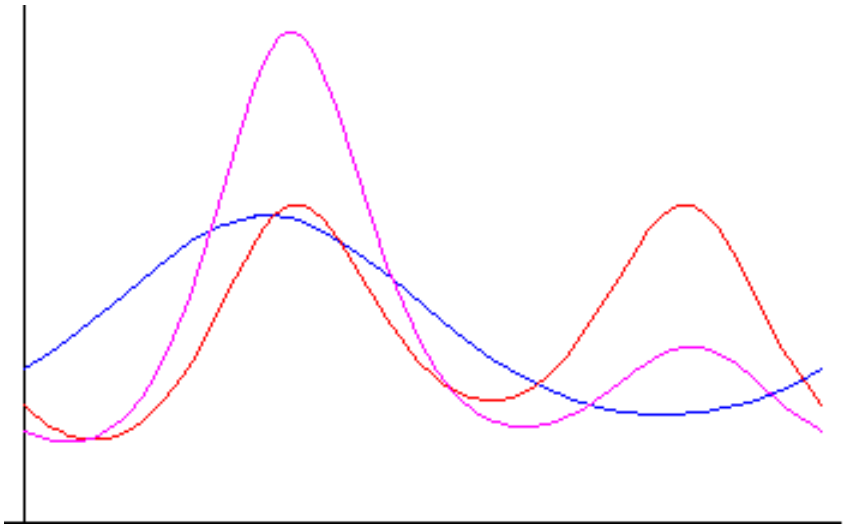
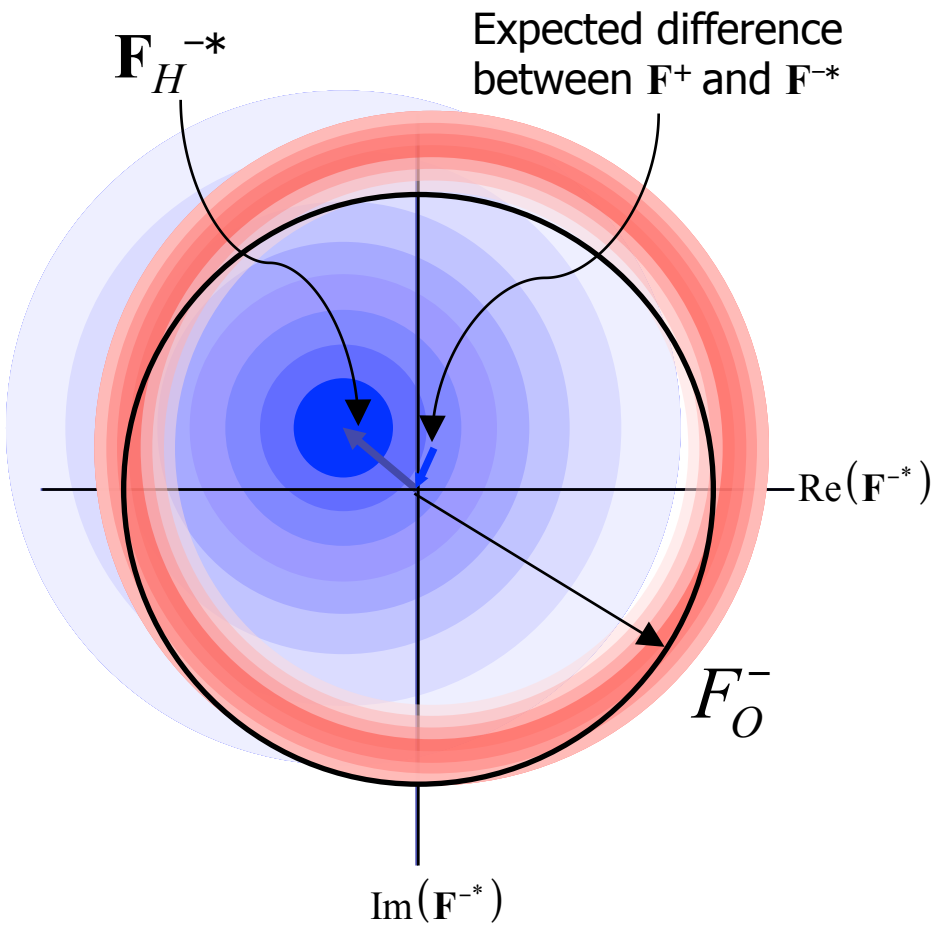


SAD Phasing

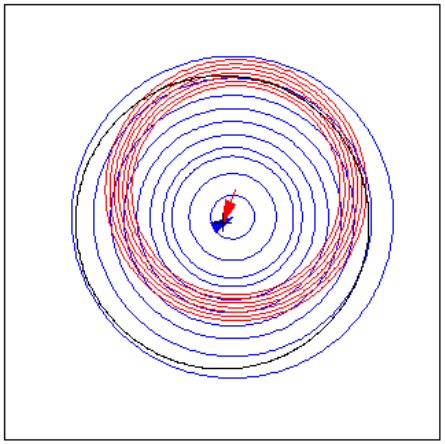
- Likelihood is proportional to the product of the two distributions (magenta) under the black circle

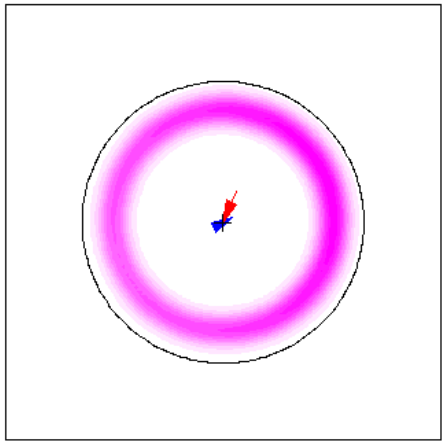




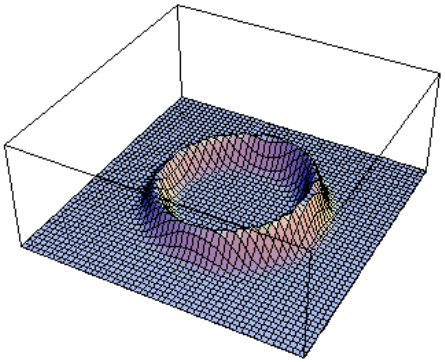
Refining Occupancy

To refine the occupancy of a heavy atom, maximise the SAD likelihood

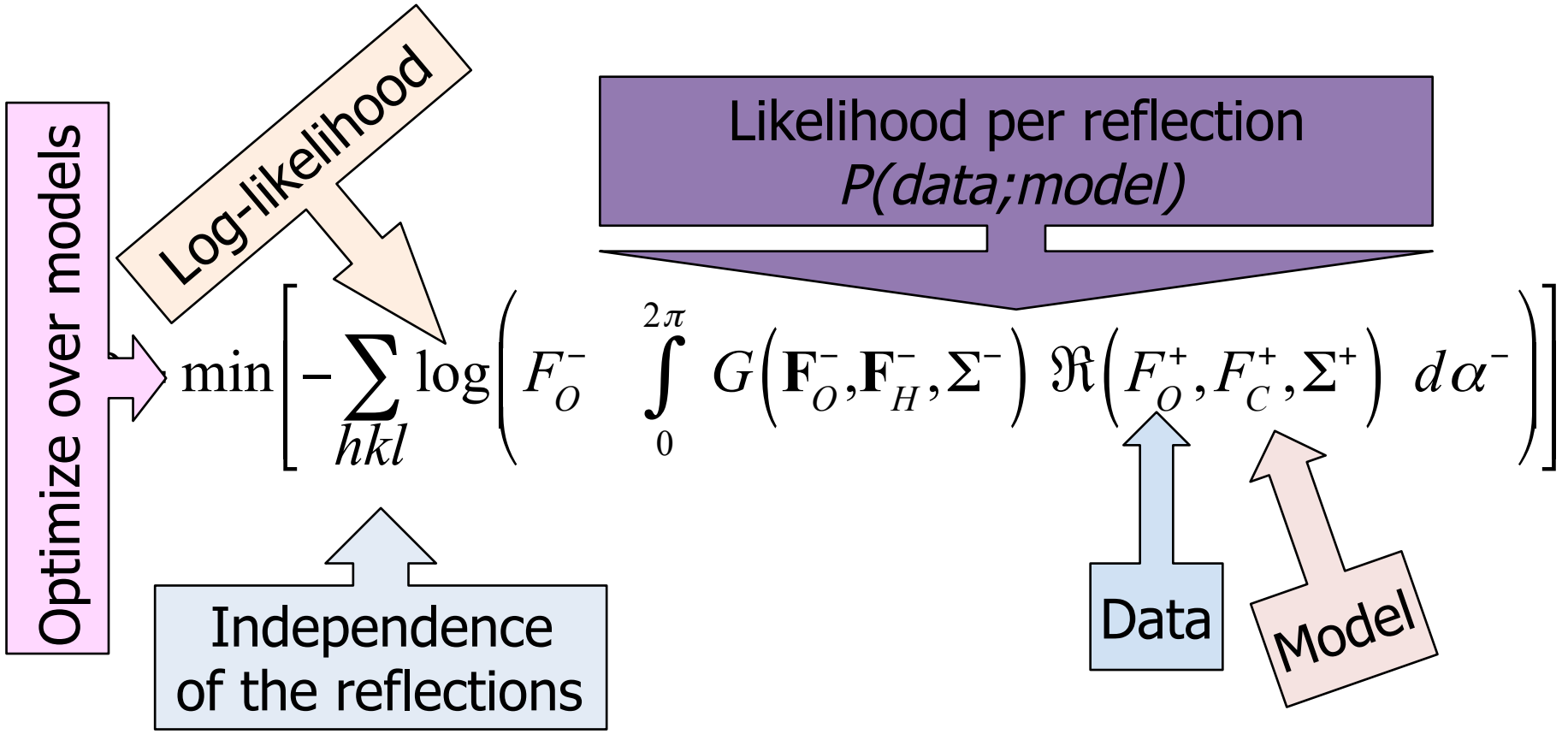




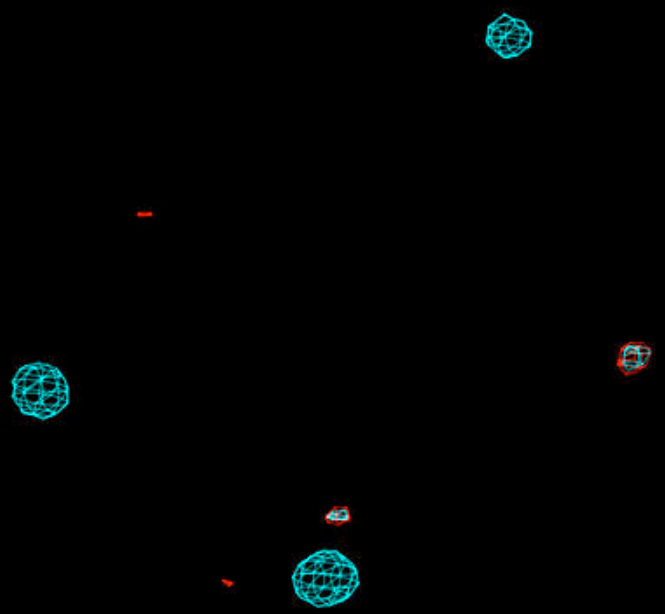
Final refined value is the optimum for ALL reflections (movie shows ONE reflection)



Correlated SAD Likelihood Function

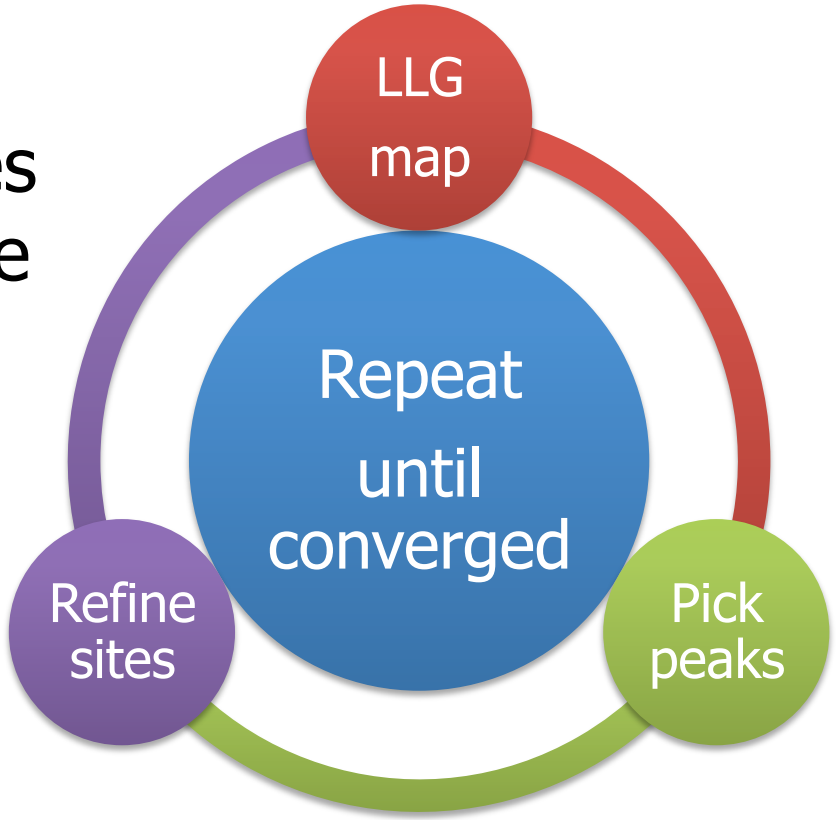


Log-likelihood gradient maps



Completion of sub-structure

- LLG maps are very sensitive
- Inclusion of minor sites **greatly improves** the phases
- Could include all intrinsic sulphurs
- Also finds bound halides



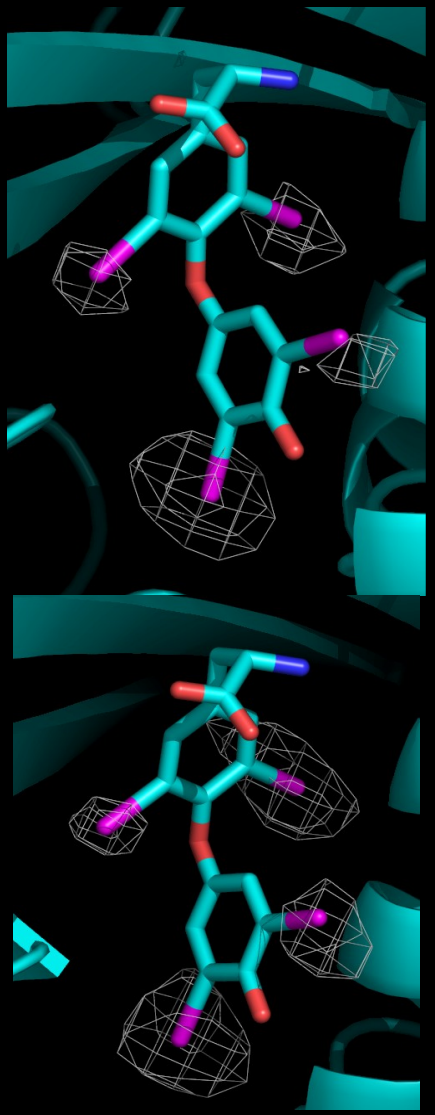
Thyroxine binding globulin

Where does thyroxine bind?

Thyroxine contains 4 iodine atoms

Two molecules in asymmetric unit
d_{min}=2.8Å
λ=0.979Å
f'' ≈ $3e^-$

Phaser LLG map @5.5σ

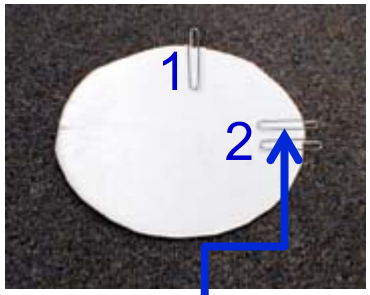


Zhou *et al*. (2006). *PNAS* **103**: 13321

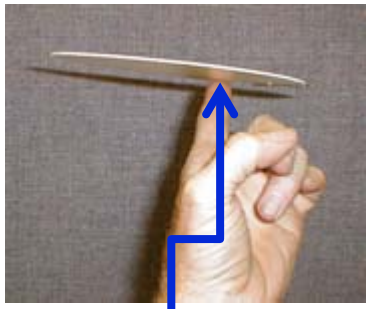
Calculating Electron Density

- ML function is good for refining the parameters, but what phase should be used in the electron density equation?
  - Have to pick one phase
- **We want the phase that gives the electron density with the lowest rms error**
  - Parseval's theorem relates the rms error in real space to the rms error in reciprocal space and vice versa
- This phase (the "**best phase**") is the probability-weighted average of all the phases
  - It is not the "most probable phase"

- Cut the centre out of a polystyrene foam plate
- Balance the disk on your finger
  - The centre of mass is at the centre
- Now put 3 paperclips on the edge of the disc
  - 2 together
  - 1 a distance away
- The balancing point is **between** the 3 paperclips
  - Not on the 2 paperclips

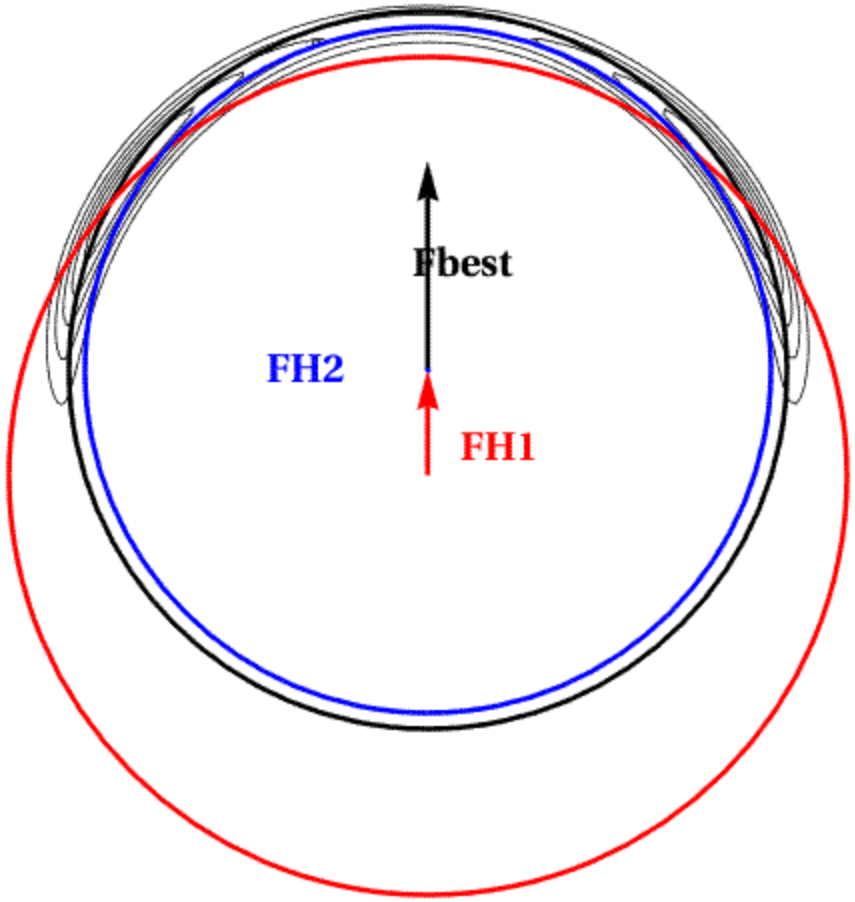


Most Probable Structure Factor



Best Structure Factor

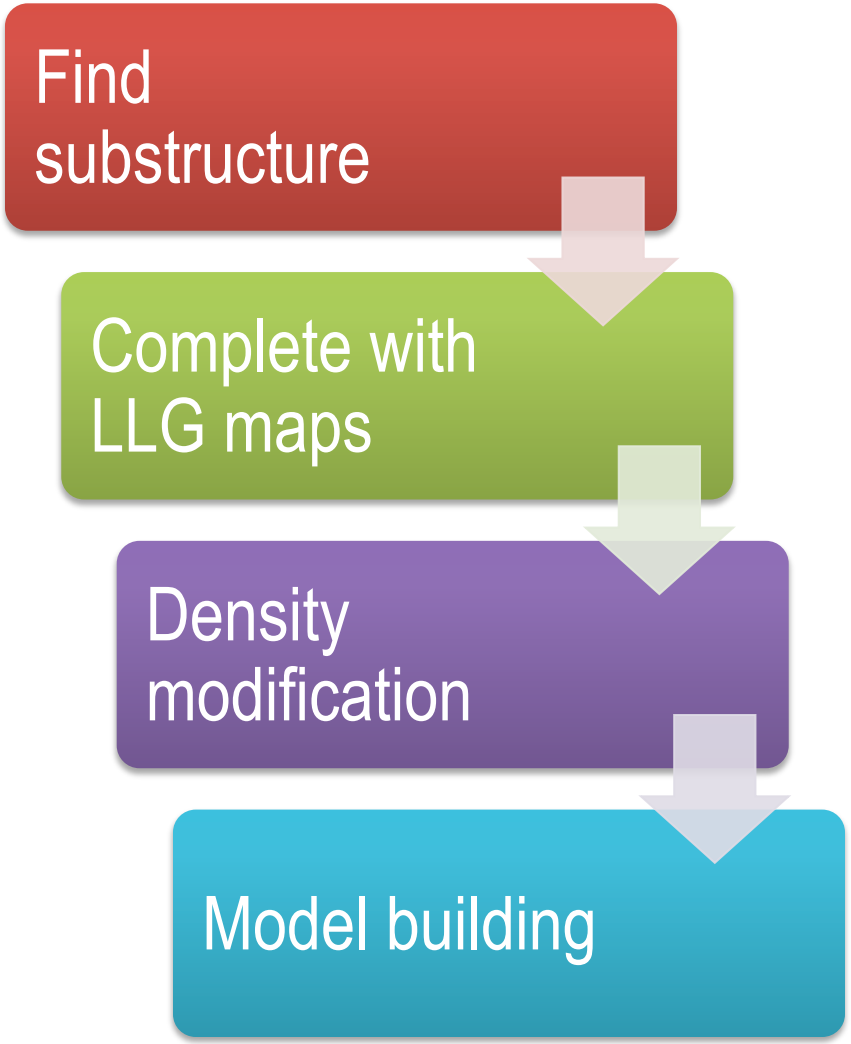
- F_{best} has a lower |F| amplitude than F_{obs}
- The reduction in F_{obs} to give F_{best} is expressed as the "figure of merit" (m)
  - **0<m <1:** F_{best} lies inside the F_{obs} circle
  - **m=1 :** Perfect phase information
  - **m=0:** No phase information
  - The higher the average value of the figure of merit, the better



New approaches

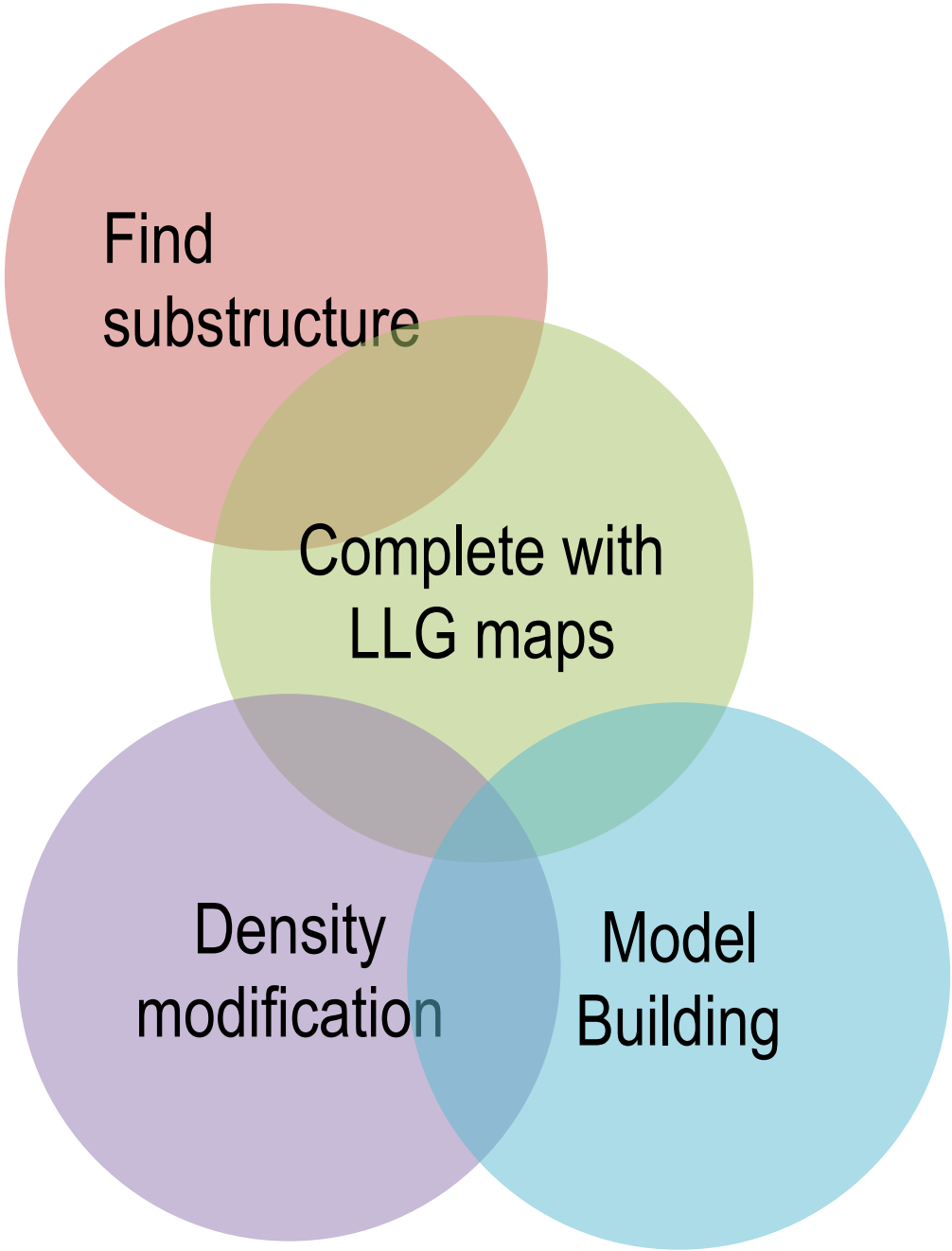
The pathway of structure solution

- Historically, there has been a linear progression through structure solution
- You had to be sure each step is correct before progressing to the next
- When signal is low you cannot be sure (of anything)

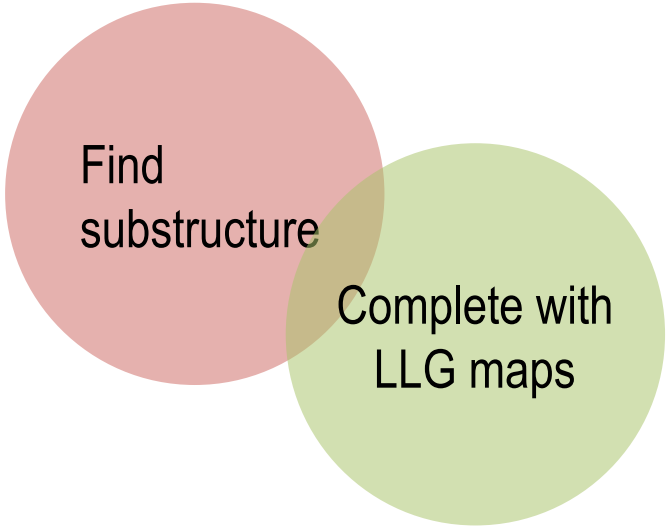


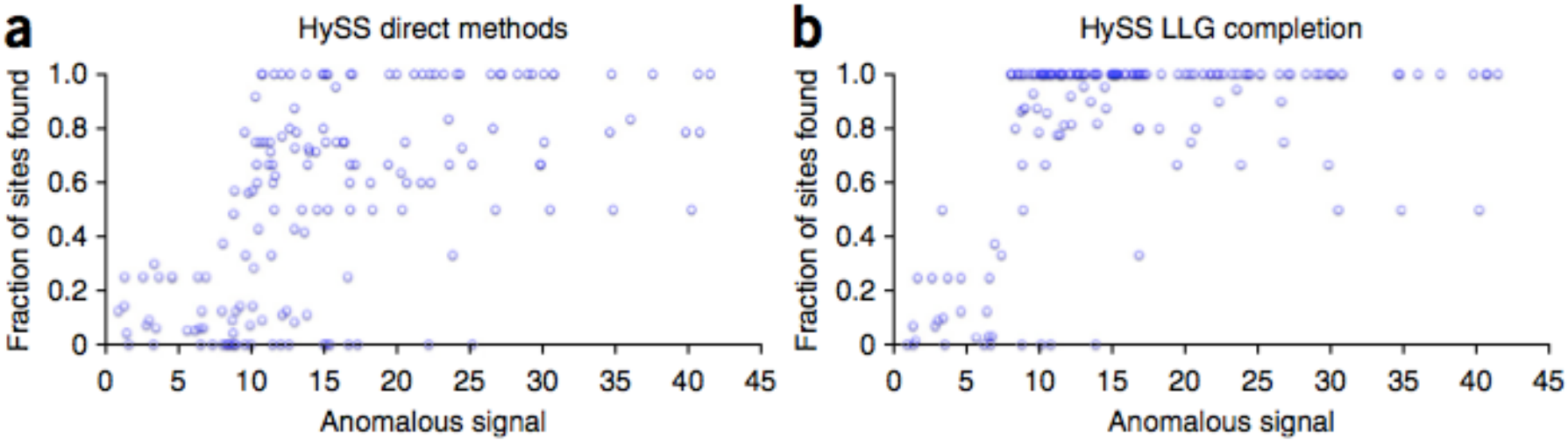
New approaches

- Take multiple possibilities for each step and uses subsequent steps to distinguish correct from incorrect solutions
- Enables structure solution when signal is low

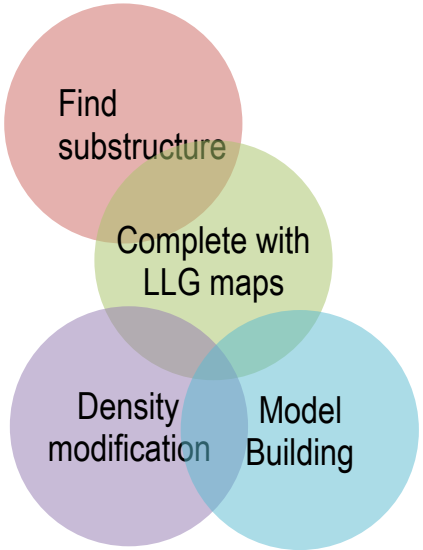


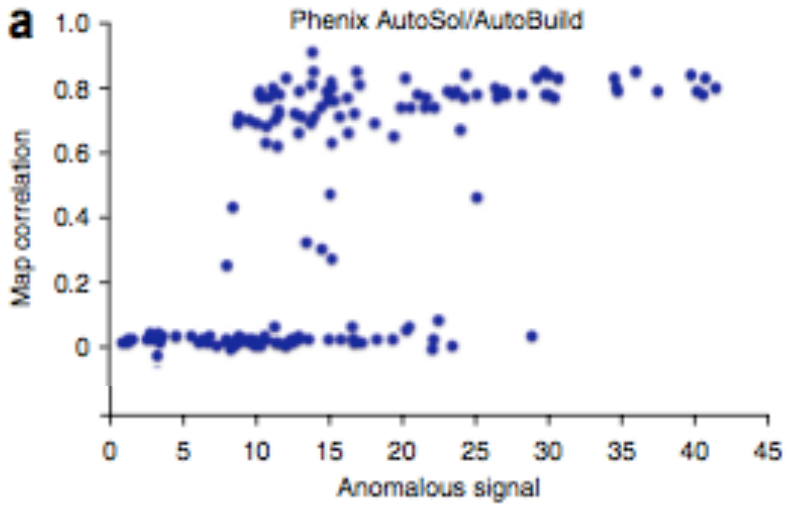
Find phasing substructure

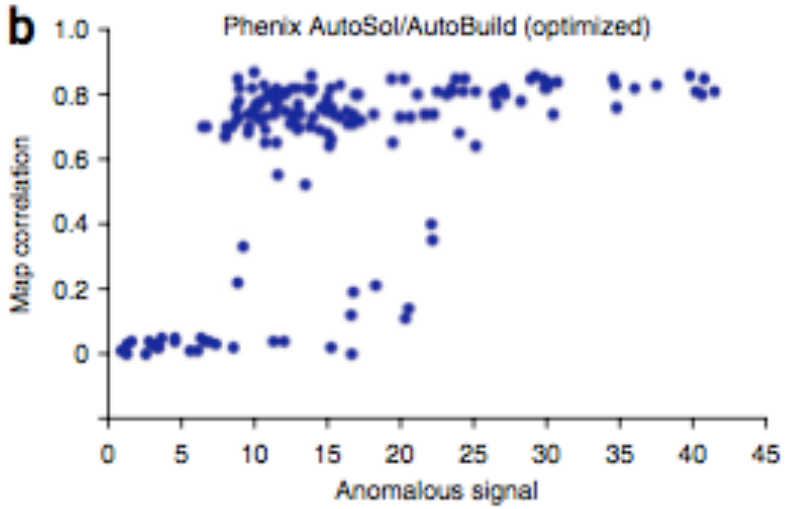





Find substructure
Complete with LLG maps
Density modification
Model building


Find substructure
Complete with LLG maps
Density modification
Model Building


a
Phenix AutoSol/AutoBuild
Map correlation
1.0
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45
Anomalous signal


b
Phenix AutoSol/AutoBuild (optimized)
Map correlation
1.0
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45
Anomalous signal

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