Experimental Phasing

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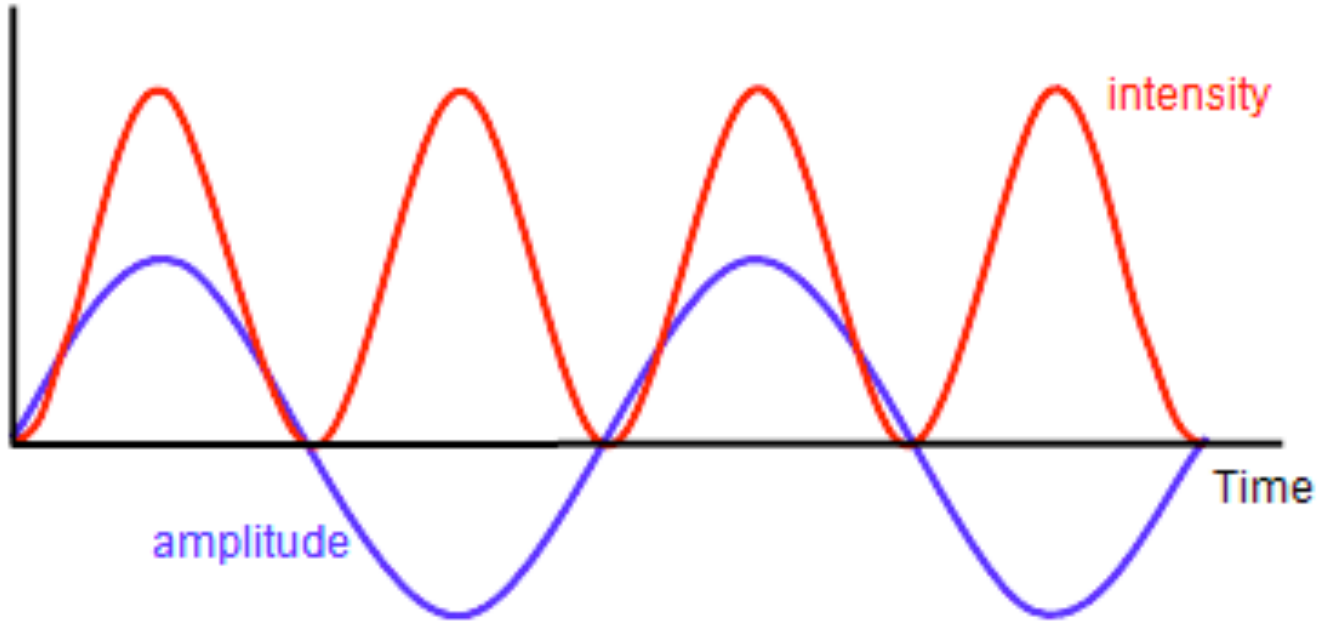
Interference

- X-ray detectors only detect X-ray intensities

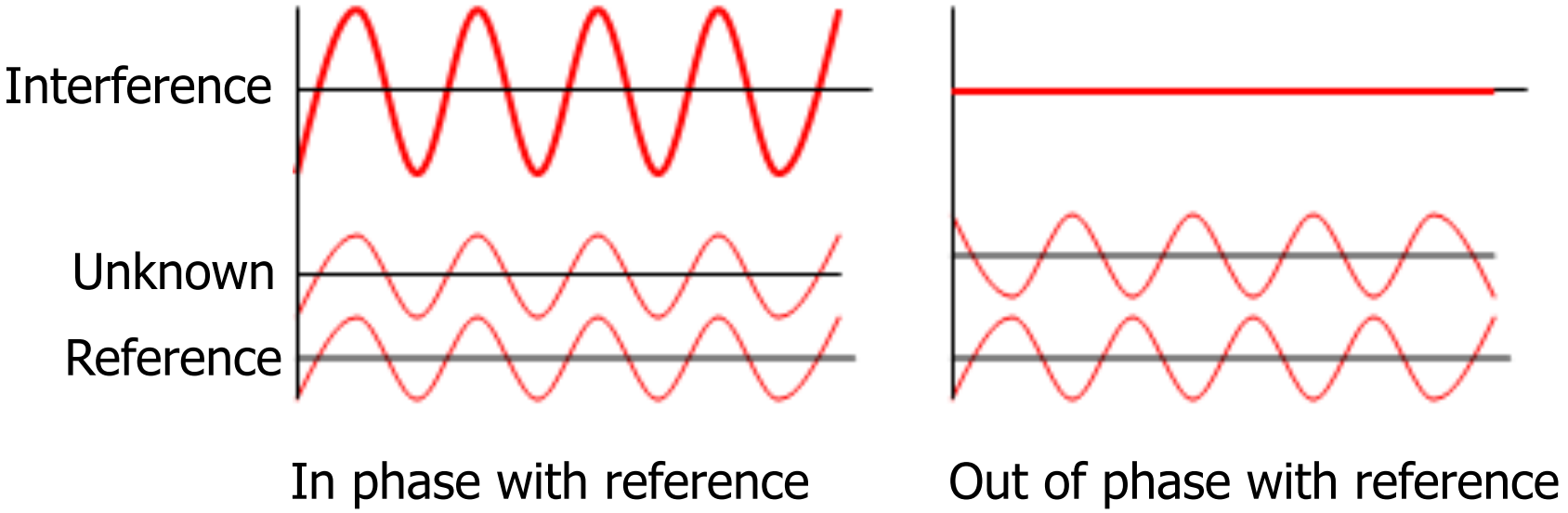


$$E \propto A^2$$

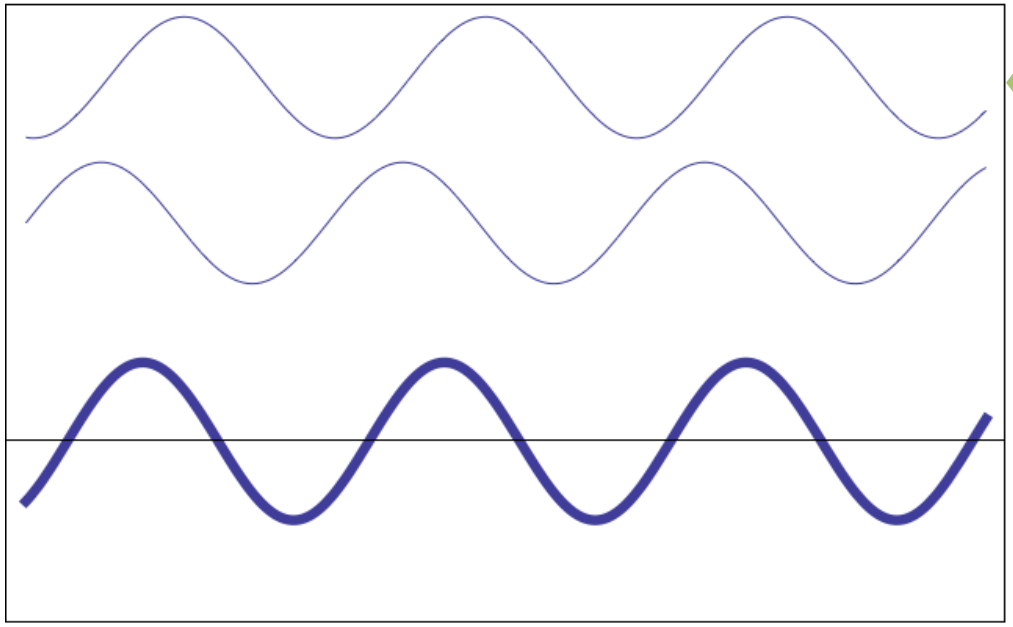
because they respond to the energy of the wave

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

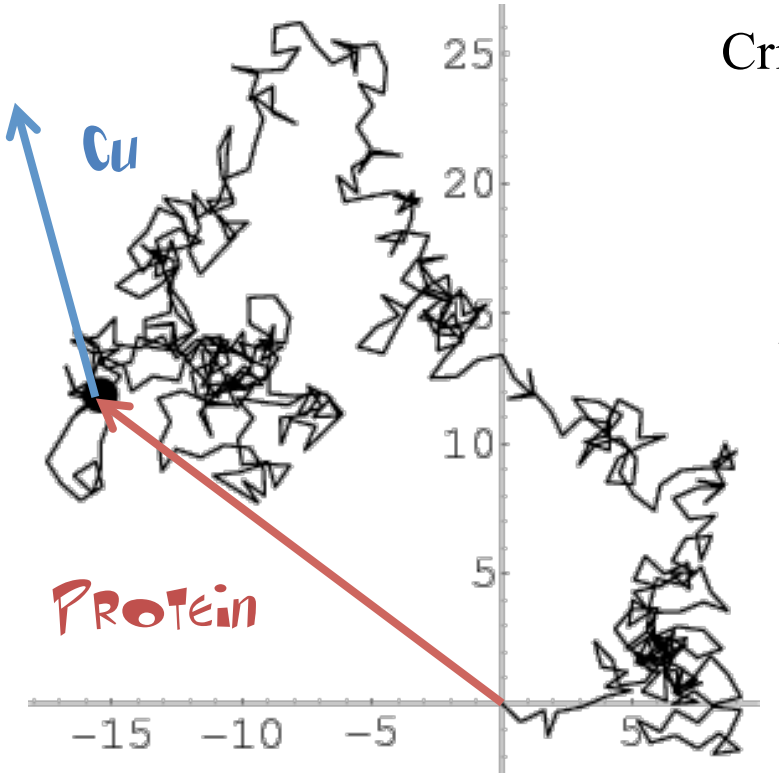
- Use approximations (e.g. Blundell & Johnson 1976)

Types of Interference Experiments

- Isomorphous replacement
- Anomalous scattering

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"

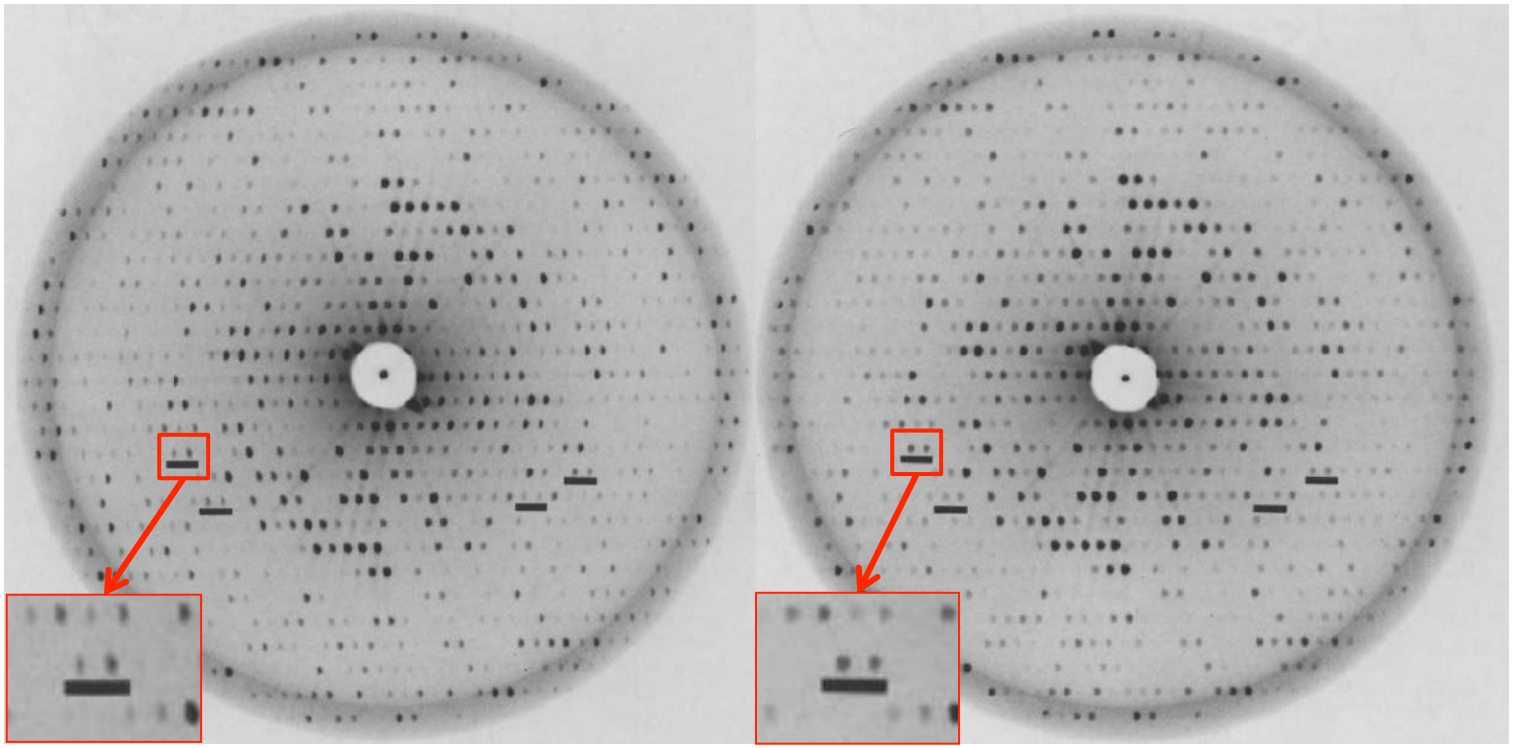


Crick and Magdoff (1956)

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

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

The clue is in the name…

- 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

Anomalous Scattering

A·nom·a·lous

adj.

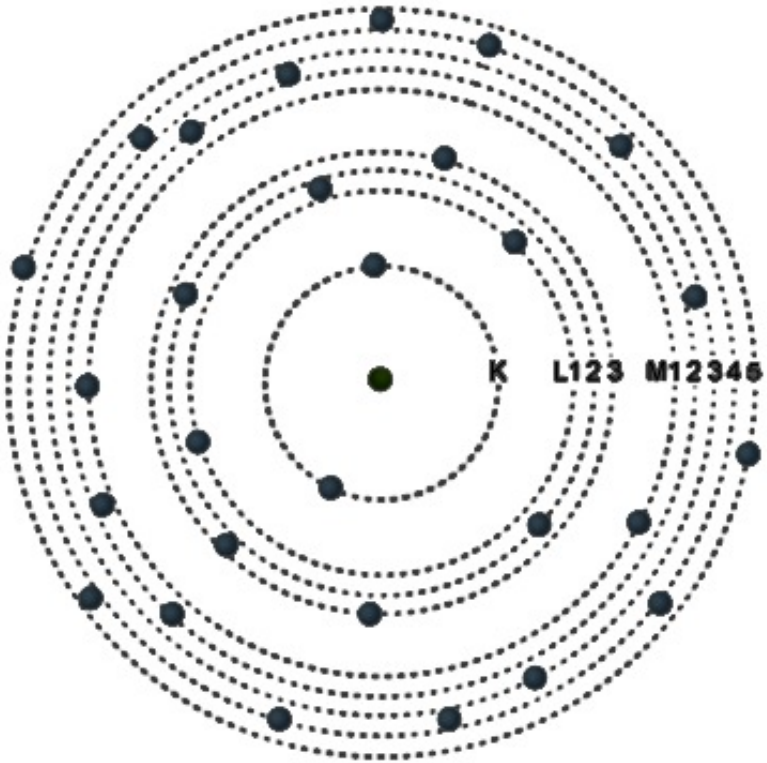
Deviating from the normal or common order, form or rule

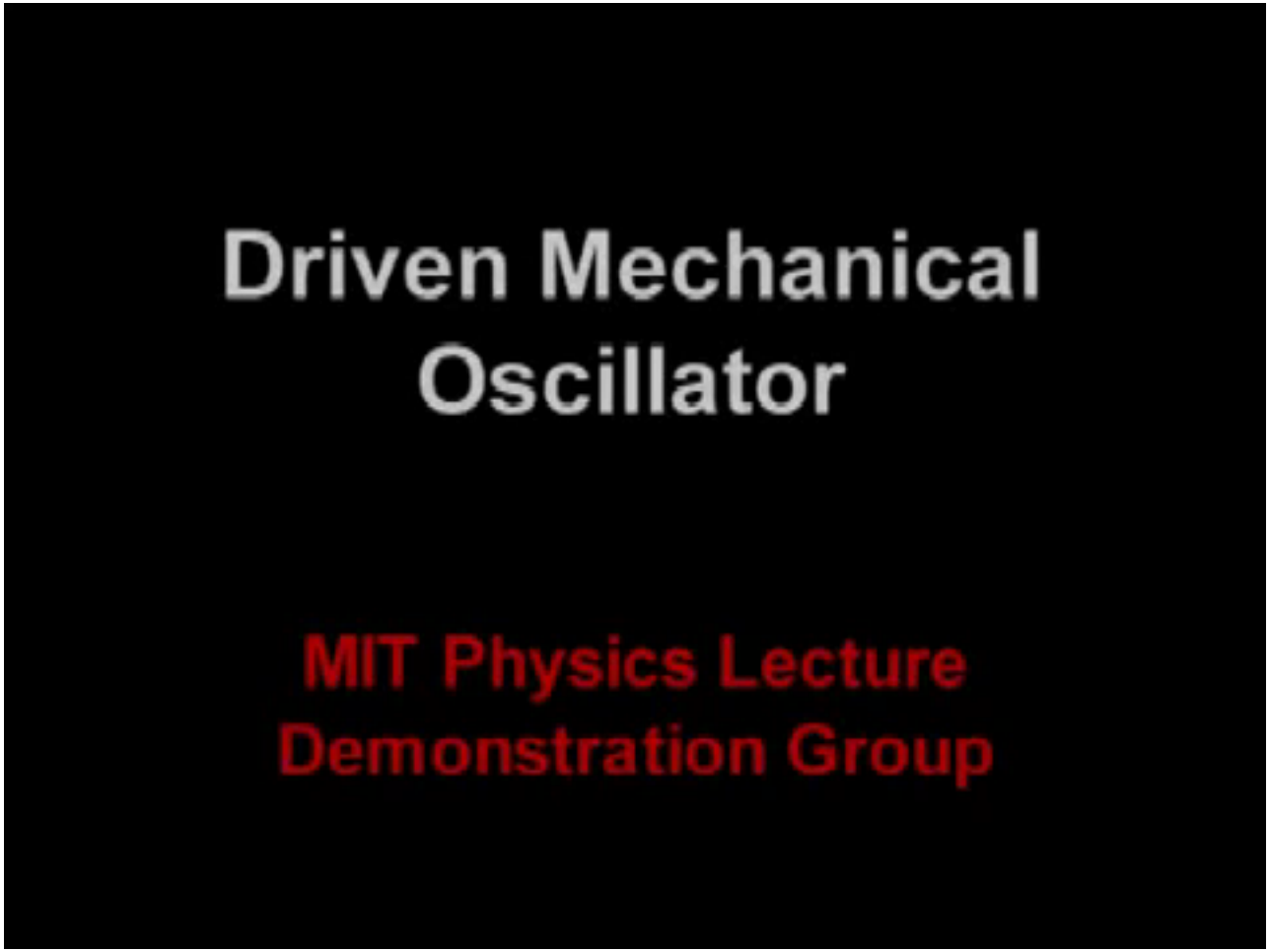
"**Anomalous** scattering" is **absolutely normal** while "**norma**l scattering" occurs only as an ideal, over simplified model, which can be used as a first approximation when studying scattering problems"

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

$$f = \frac{\omega^2}{\omega^2 - \omega_s^2 - ik\omega} = f^0 + \Delta f' + i\Delta f''$$

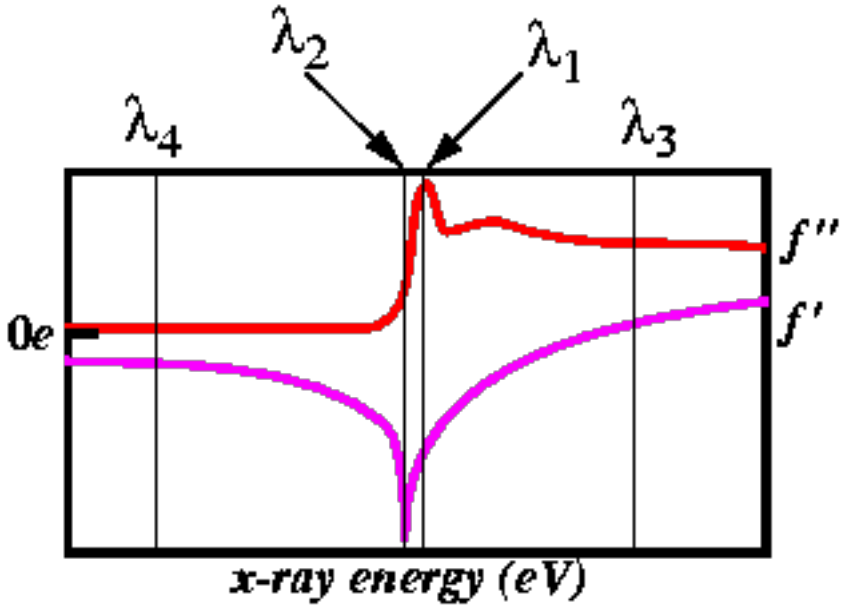
Scattering factor

Frequency of incident wave

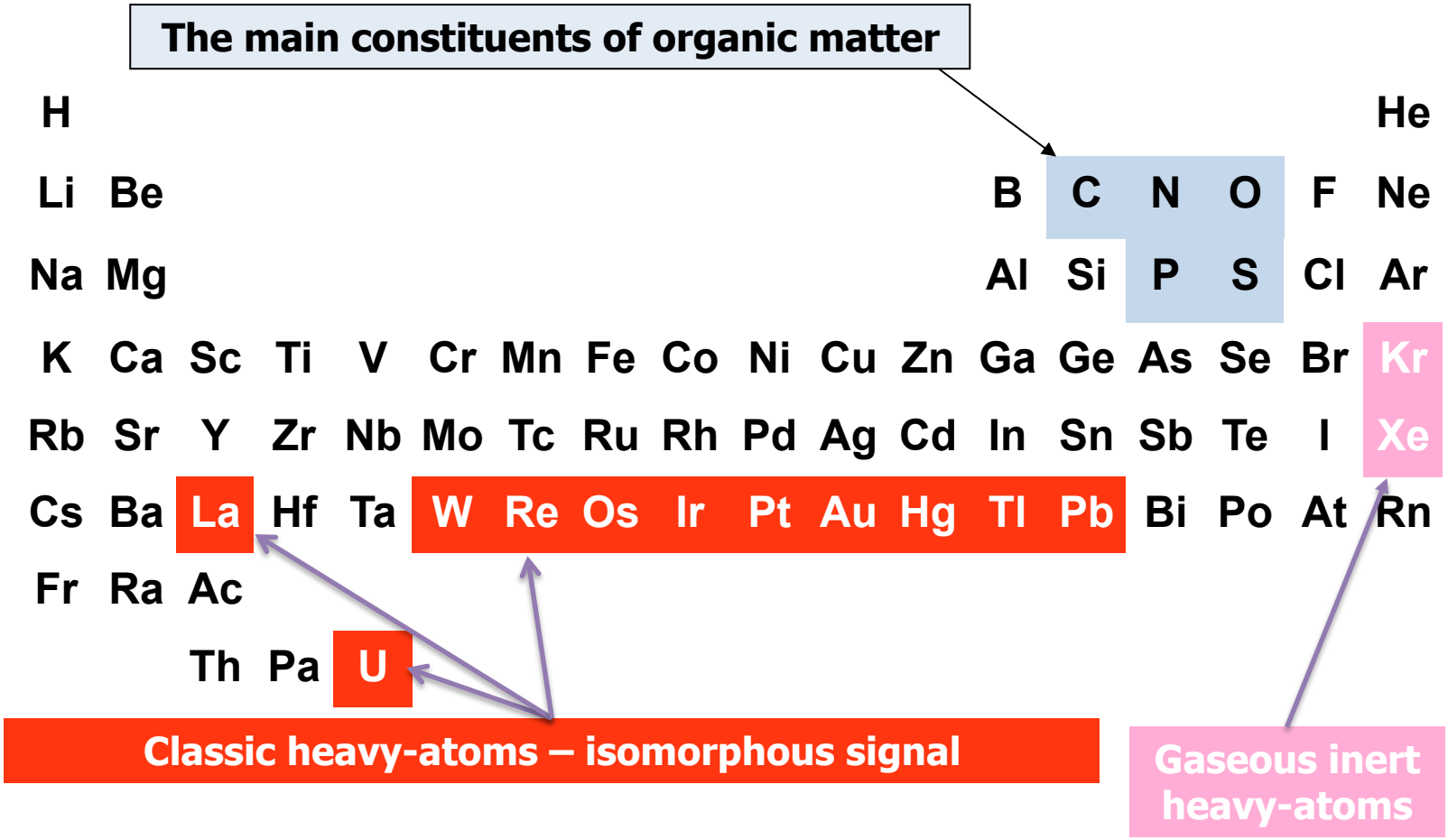
Resonant frequency

Damping factor

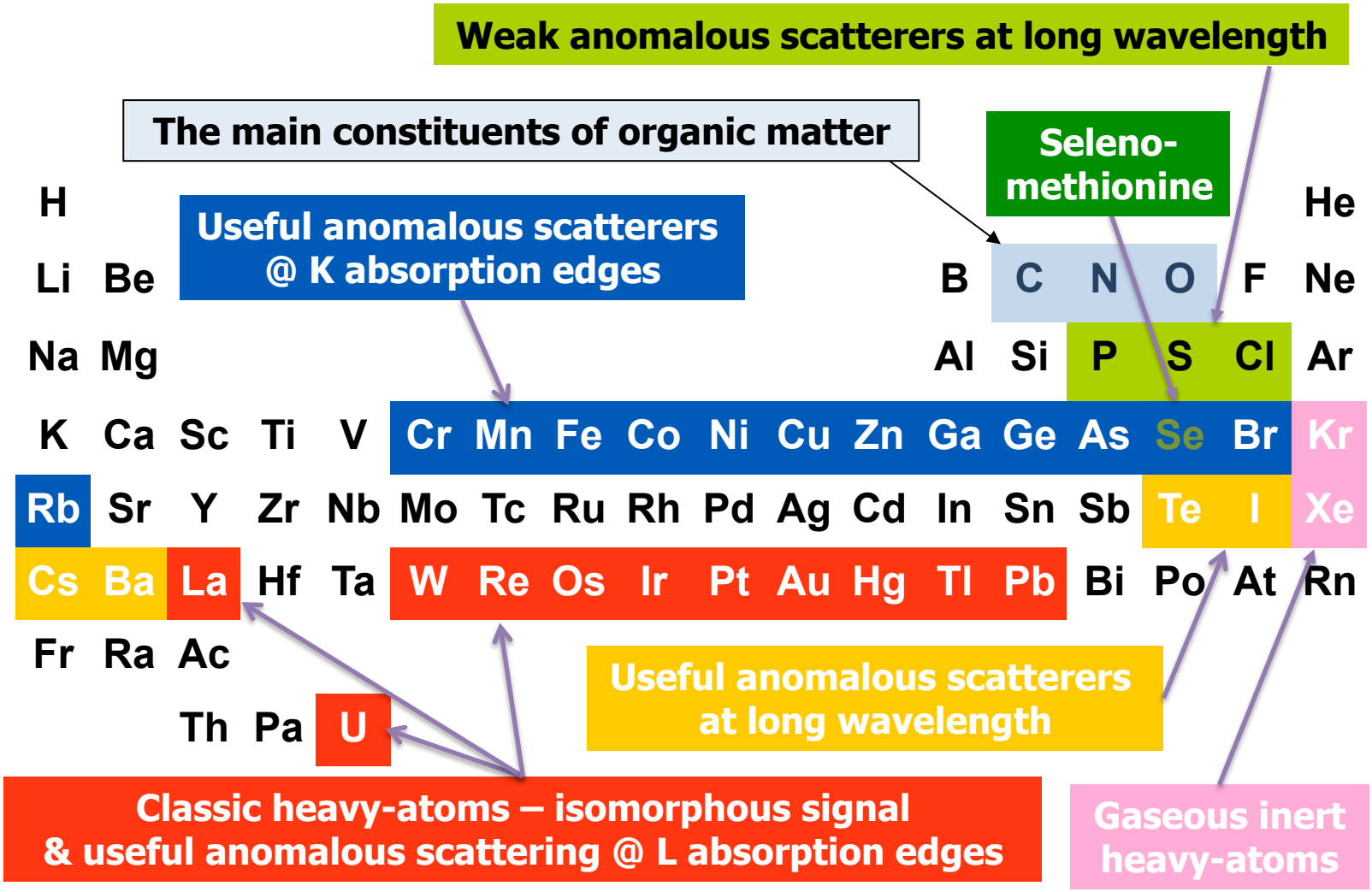
- Both $\Delta f'$ and $\Delta f''$ change with the frequency of the incident radiation
  - Large when the incident frequency is near resonant frequency
- Dispersive component
  $\Delta f'$ is in phase with f^0
- Anomalous component
  $\Delta f''$ is advanced by 90° by with respect to f^0



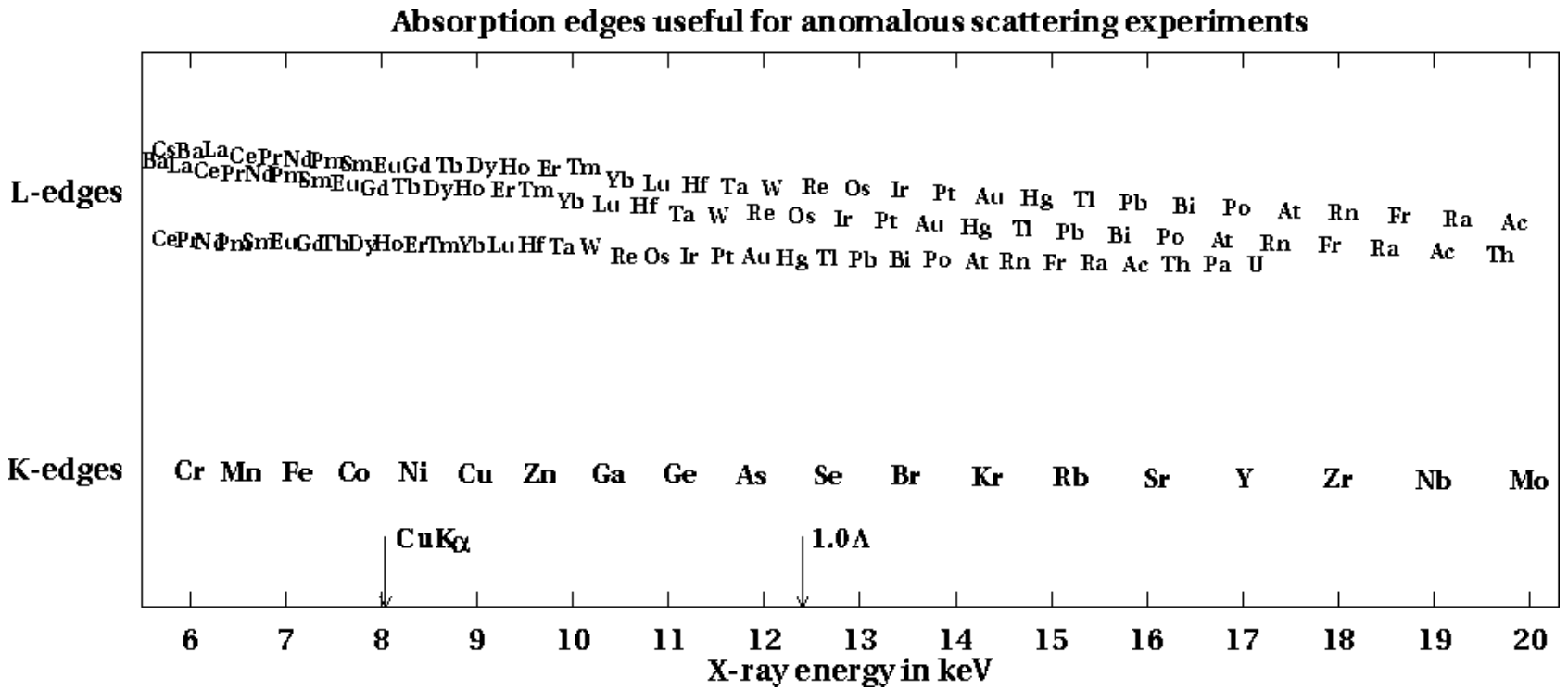
Isomorphous replacement



Anomalous Scatterering



Absorption edges




MIR
SAD
SIR
SIRAS
MIRAS
MAD
RIP

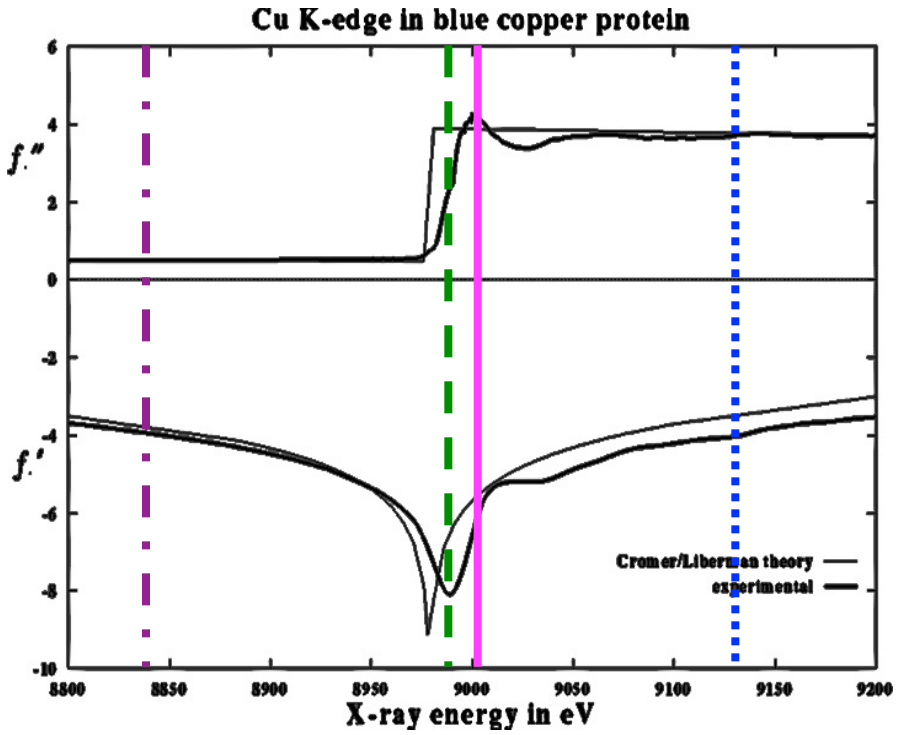
SAD

- The most popular way of solving structures by experimental phasing (over 70% and rising)
- 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

MAD

- Usually performed using seleno-methionines
- Can use intrinsically bound metal ions
  - e.g. Fe, Ni, Cu, Zn
- Or introduced heavy atoms
  - e.g. U, Pt, Au, Hg, Pb
- Requires a synchrotron
  - Tune wavelength to optimise f’ and f” values
- Theoretical problems in the phasing algorithms make MAD data not as useful as they could be

Wavelength Choice



PEAK: |f”| is large

INFLECTION: |f’| is large

REMOTE: low energy

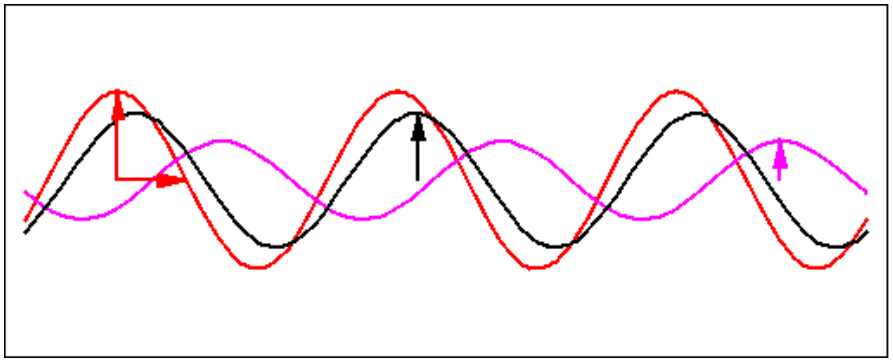
REMOTE: high energy

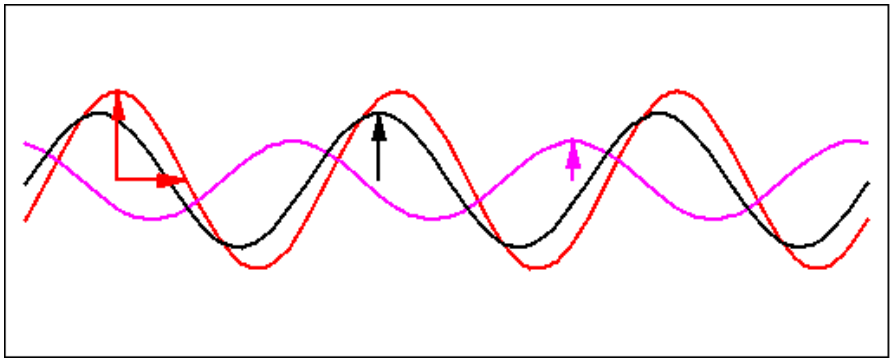
Warning
Radiation
Damage
INCREASES
at peak

Harker diagrams

Reference Wave

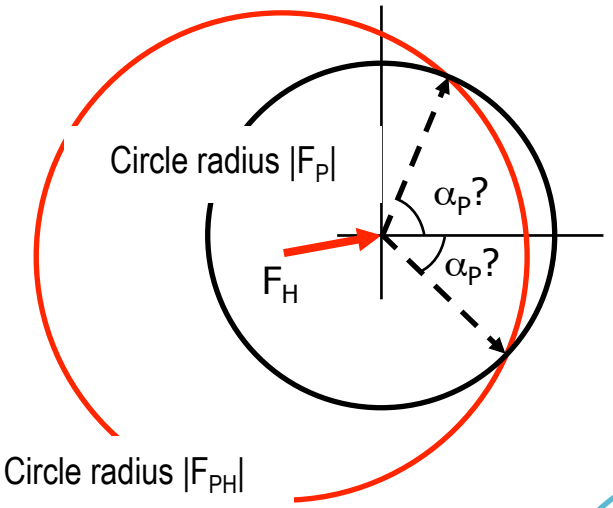
- Reference wave (red)
  - Amplitude and phase known
- Interference wave (black)
  - Amplitude known
- Unknown wave (magenta)
  - Amplitude known

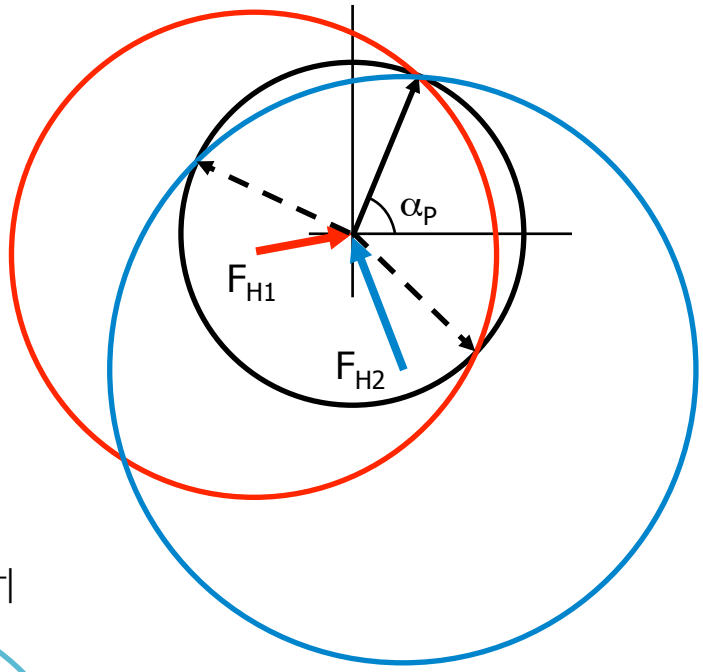


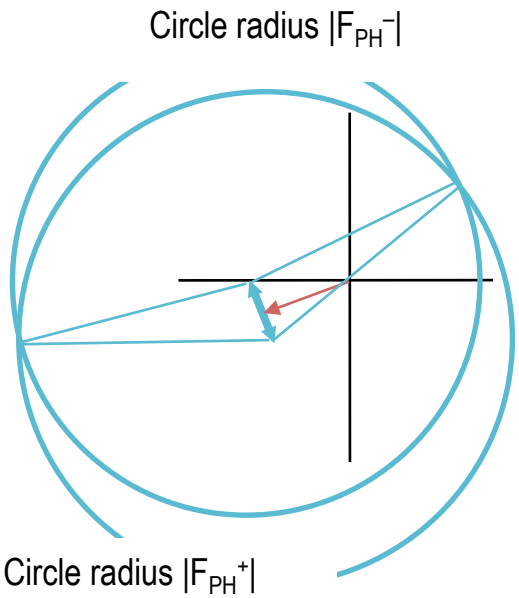


- Two solutions for phase of unknown wave
- *Also two solutions for phase of interference wave*

Harker Diagrams

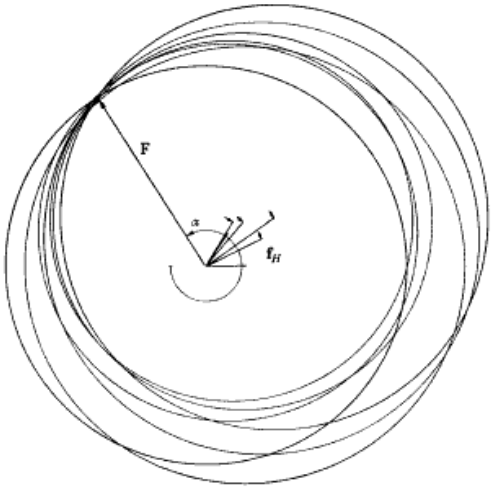


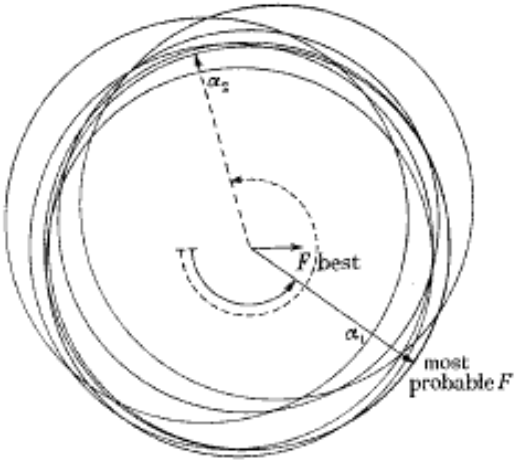


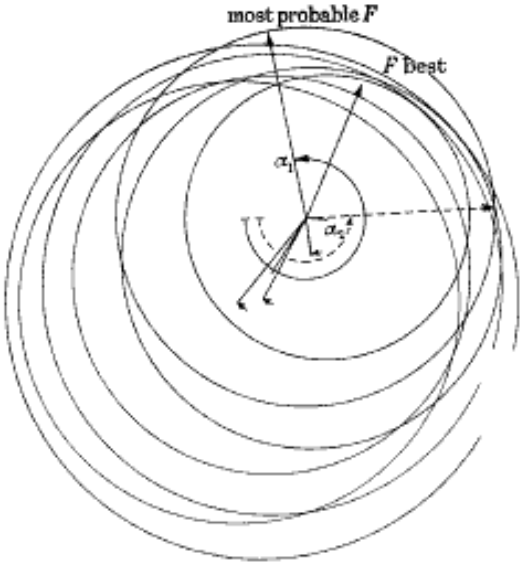


Reality…

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



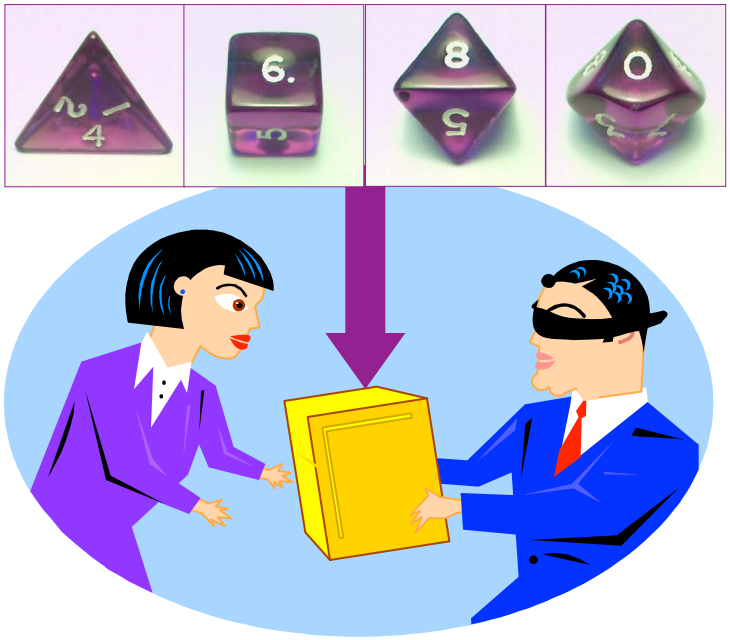


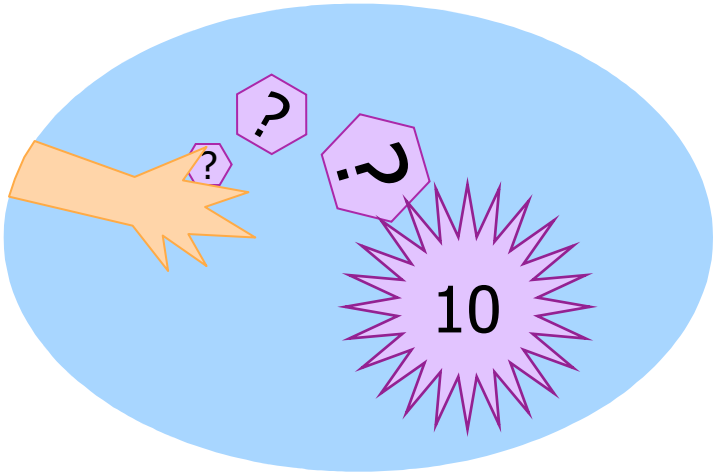


- Phase circles rarely cross exactly
- Need a **probabilistic** approach to determining the phase

Maximum Likelihood

A game of dice

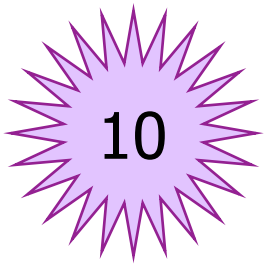




- Put four unbiased dice in a box
- I select a die at random
- I roll the die and tell you the result of the roll
- Which die did I most likely select?

Roll a 10

- The die obviously must have been the 10 sided die
- What does "must" mean in probabilities?



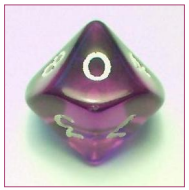
$$P(10;\boxed{10}) = \tfrac{1}{10}$$

$$P(10;\boxed{8}) = 0$$

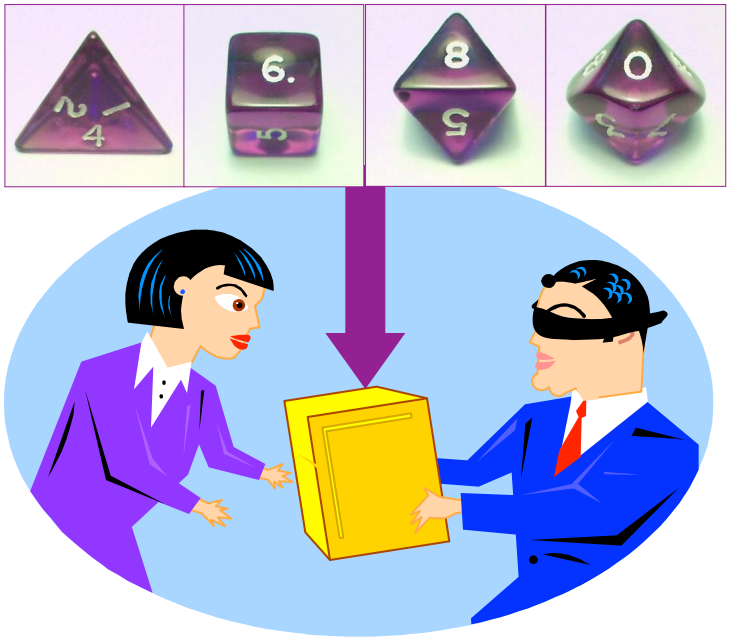
$$P(10;\boxed{6}) = 0$$

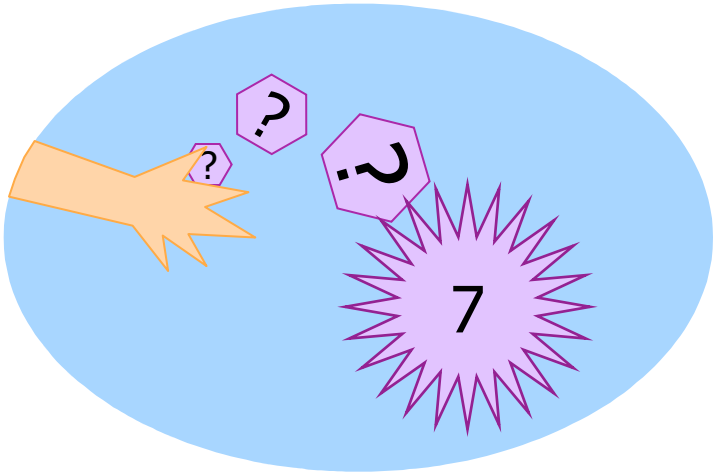
$$P(10;\boxed{4}) = 0$$

most likely



A game of dice with data

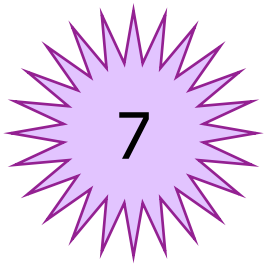




- Put four unbiased dice in a box
- I select a die at random
- I roll the die and tell you the result of the roll
- Which die did I most likely select?

Roll a 7

- The die could have been the 10 sided or the 8 sided die
- Which die is most likely?



$$P(7;\boxed{10}) = \frac{1}{10}$$

$$P(7;\boxed{8}) = \frac{1}{8}$$

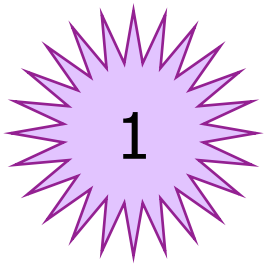
$$P(7;\boxed{6}) = 0$$

$$P(7;\boxed{4}) = 0$$

most likely

Roll a 1

- Could have been rolled by any of the dice
- The most likely die is the one with the highest probability of generating the data



$$P(1;\boxed{10}) = \frac{1}{10}$$

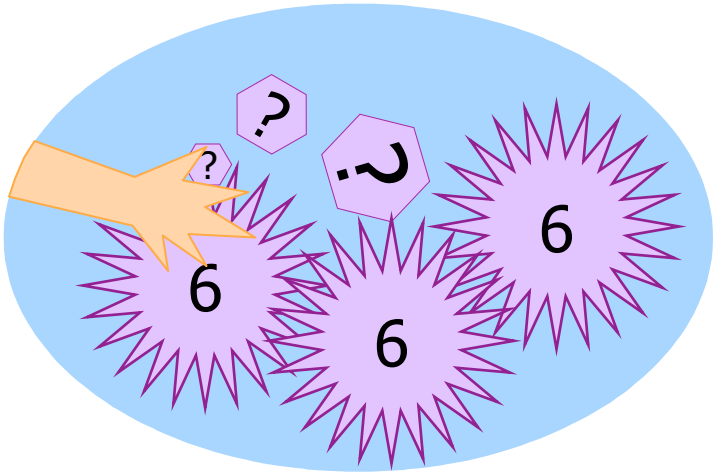
$$P(1;\boxed{8}) = \frac{1}{8}$$

$$P(1;\boxed{6}) = \frac{1}{6}$$

$$P(1;\boxed{4}) = \frac{1}{4}$$

most likely

A game of dice with more data

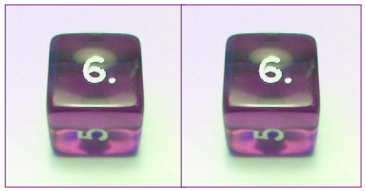


- Put four unbiased dice in a box
- I select a die at random
- I roll that die three times and tell you the results
- Which die did I most likely select?

Multiplying probabilities

- When probabilities are independent they multiply

$$P(6;\boxed{6}) = \tfrac{1}{6} = 0.16666667$$



$$P(6,6;\boxed{6}) = \tfrac{1}{6} \times \tfrac{1}{6} = \tfrac{1}{36} = 0.0277778$$

$$P(6,6,6;\boxed{6}) = \tfrac{1}{6} \times \tfrac{1}{6} \times \tfrac{1}{6} = \tfrac{1}{216} = 0.0046296$$

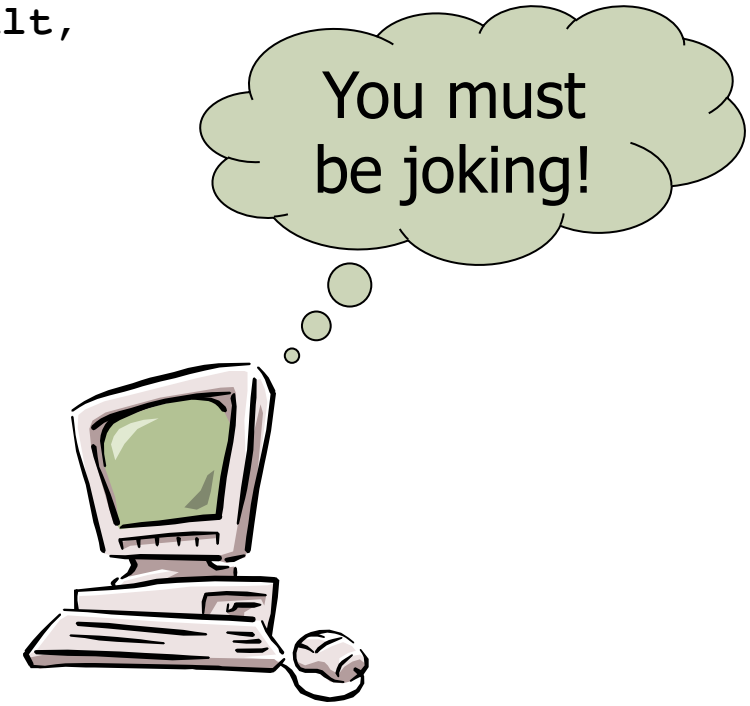
100 times

$$P(6\text{K} \times 100;\boxed{6}) = 6^{-100} = 1.53064 \times 10^{-78}$$

Computers and small numbers

"Oh great one, what is the probability of throwing a 6 from a six sided die one billion times?"

```
> SYSTEM-F-FLTOVF_F, arithmetic fault,
floating overflow at PC=00006244,
PSL=03C0 0020 %TRACE-F-TRACEBACK,
symbolic stack dump follows
module name     routine name    line
OVERF           OVERF           104
DPARA$MAIN      DPARA$MAIN      276
```



Computers can not store numbers very close to zero

Computers and log(small numbers)

"Oh great one, what is the logarithm of the probability of throwing a 6 from a six sided die one billion times?"

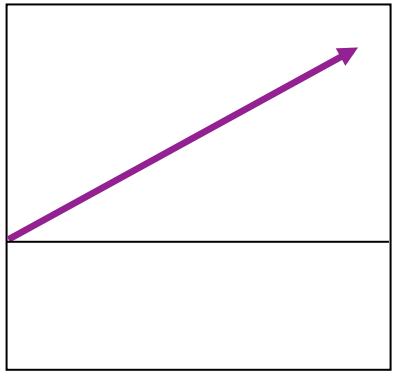
```
> -778151250.4
```

log(likelihood) is not close to zero

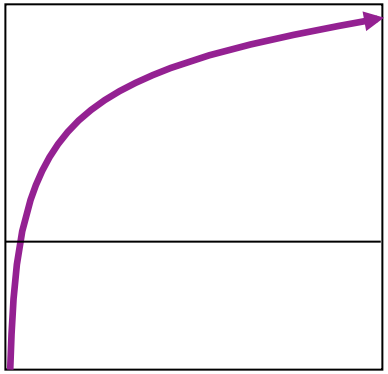
- So the log(likelihood) solves the small number problem
- But can we just switch to using the log(likelihood)?

Optimisation and logarithms

- Logarithmic functions are "monotonic" functions
  - *i.e.* they "preserve the given order"
  - If $y_1 < y_2$ for all $x_1 < x_2$ then $\log(x_1) < \log(x_2)$
- The parameter values obtained optimising log(likelihood) are the same as those obtained optimising likelihood
  - **Optimising log(likelihood) ≡ Optimising likelihood**



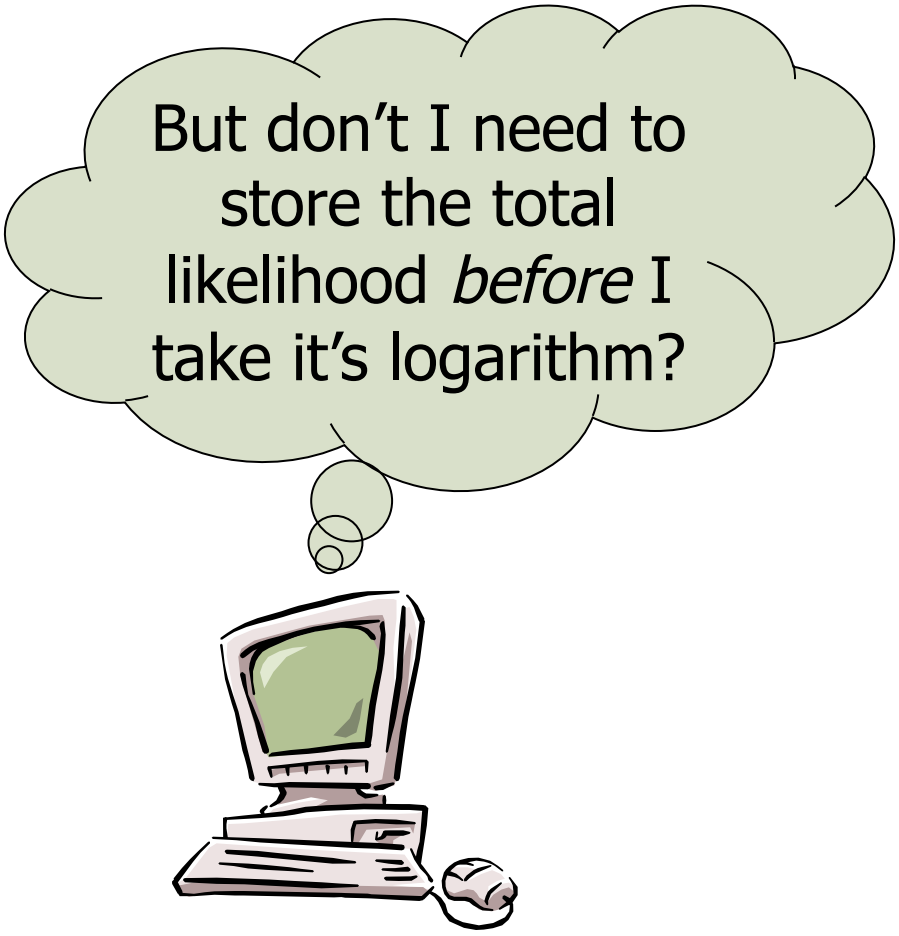
$y = x$



$y = \log(x)$

Logarithms, products and sums

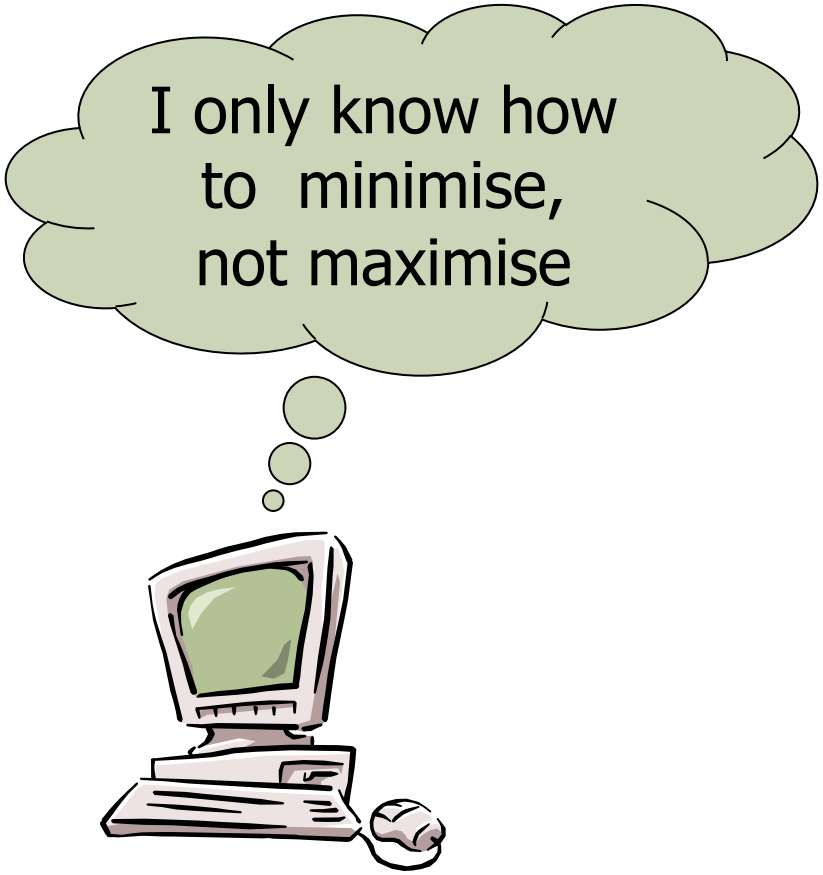
- No, there is a shortcut to the log(total likelihood) when total likelihood is a product of likelihoods
- If log(total likelihood) equals log(Π likelihoods)
  - product
- Then log(total likelihood) also equals Σlog(likelihoods)
  - sum



Minimising

- Computer algorithms are designed to minimise
- Therefore we optimise our parameters by minimising the

  -log(likelihood)



Logarithms and independence

$$\log(\prod \text{likelihoods}) = \sum \log(\text{likelihoods})$$

$$\log\left(P\left(3,3;\boxed{6}\right)\right)$$

$$= \log\left(P\left(3;\boxed{6}\right) \times P\left(3;\boxed{6}\right)\right)$$

$$= \log\left(\tfrac{1}{6} \times \tfrac{1}{6}\right)$$

$$= \log(0.0277)$$

$$= -1.556$$

$$\log\left(P\left(3,3;\boxed{6}\right)\right)$$

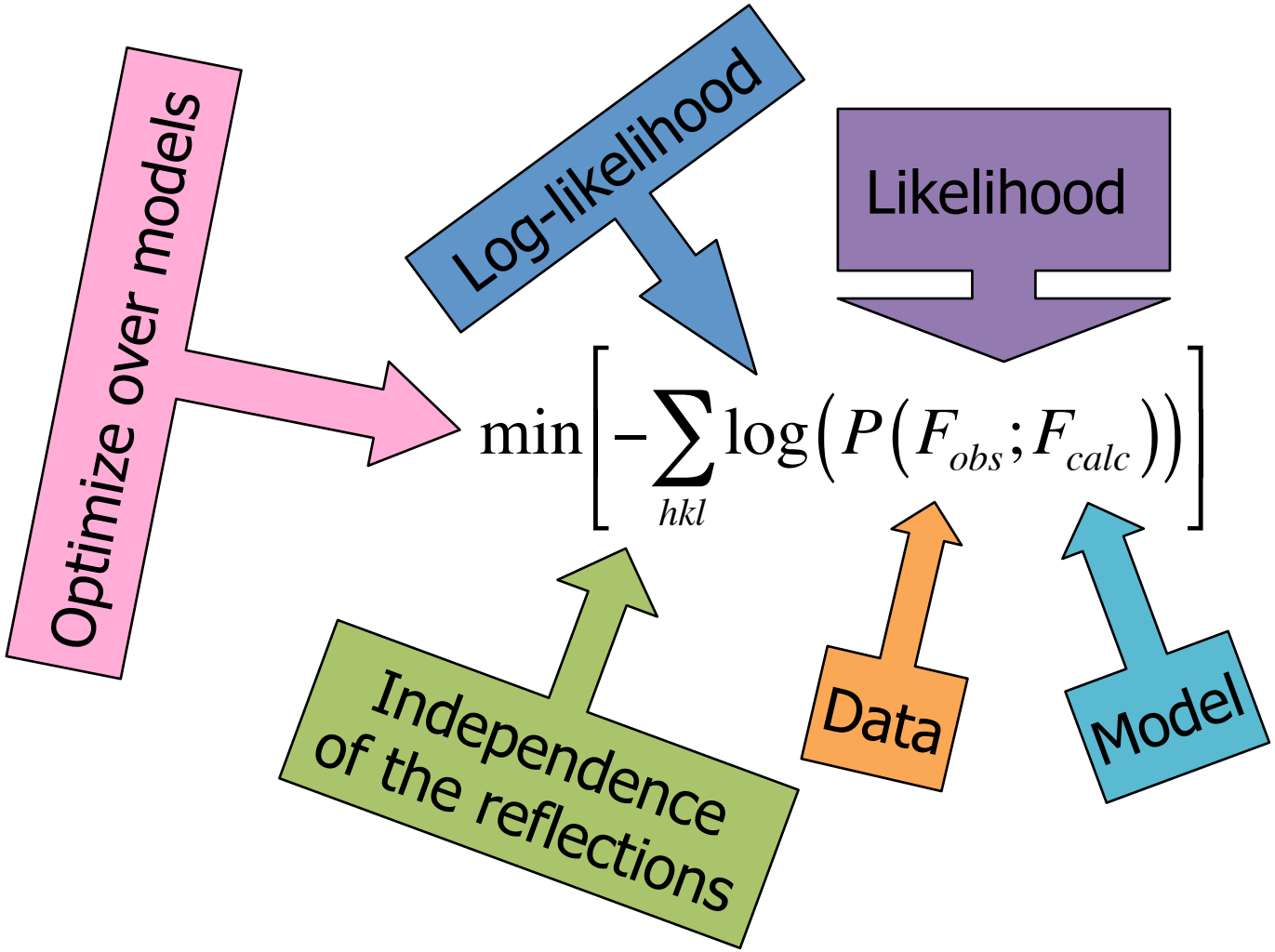
$$= \log\left(P\left(3;\boxed{6}\right)\right) + \log\left(P\left(3;\boxed{6}\right)\right)$$

$$= \log\left(\tfrac{1}{6}\right) + \log\left(\tfrac{1}{6}\right)$$

$$= -0.778 - 0.778$$

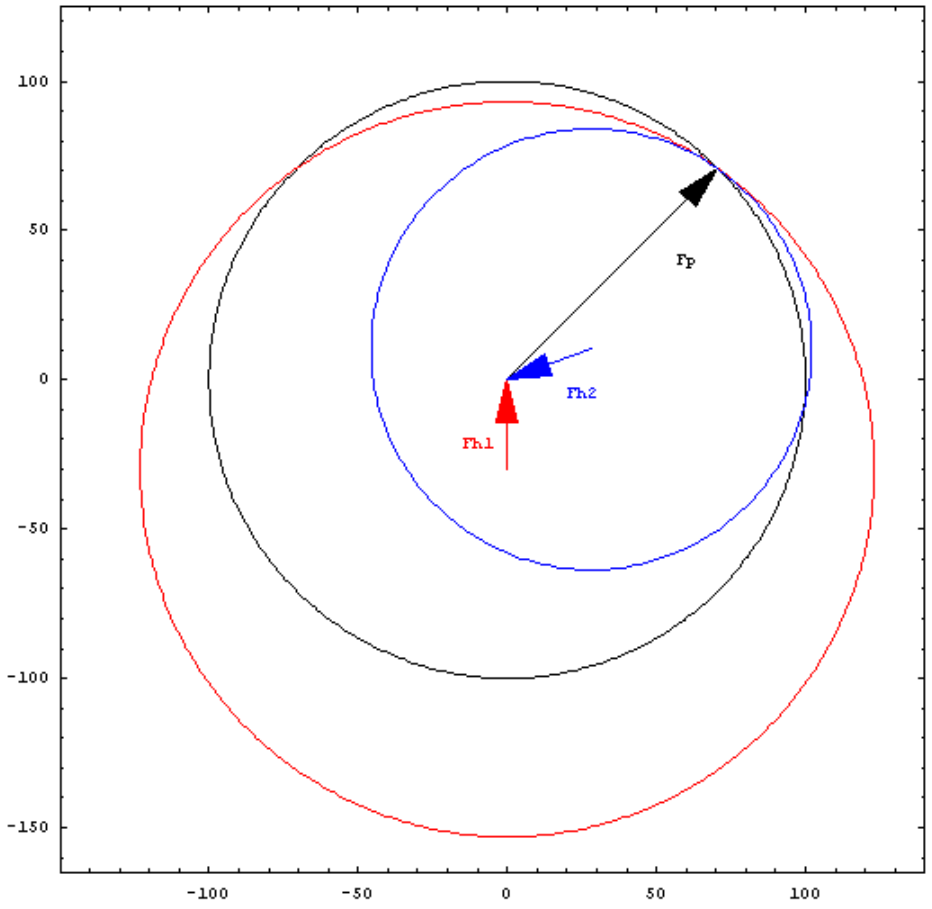
$$= -1.556$$

Likelihood Functions



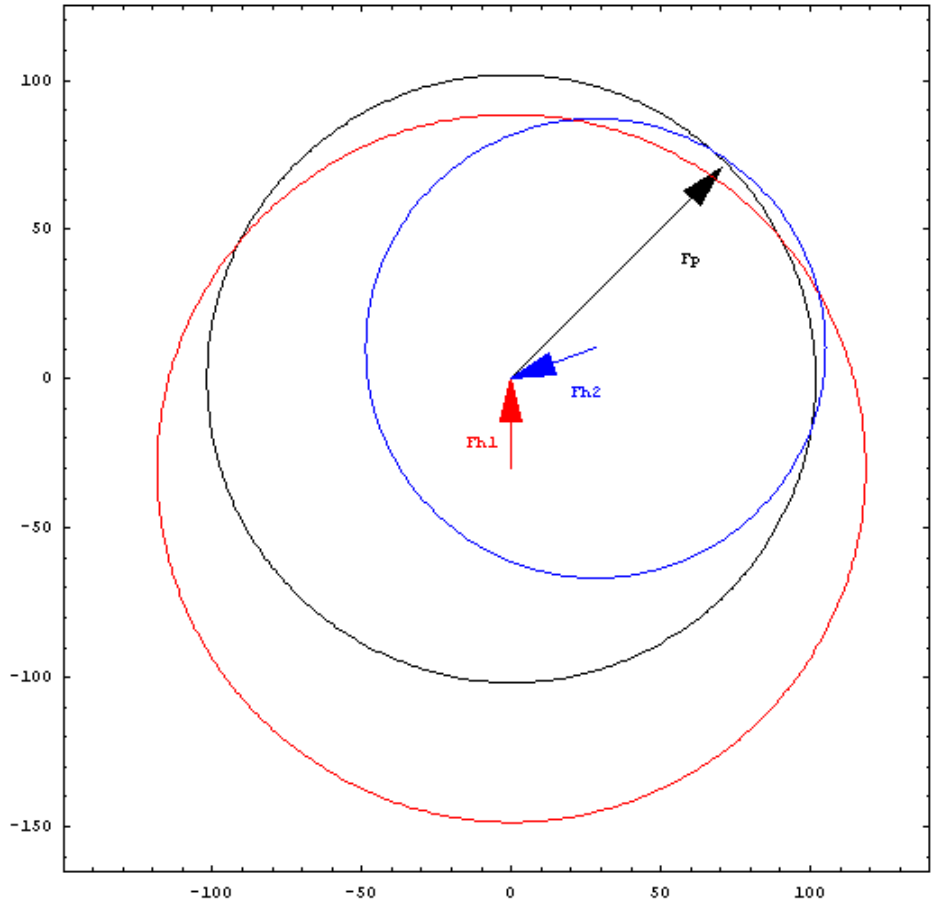
Harker construction

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



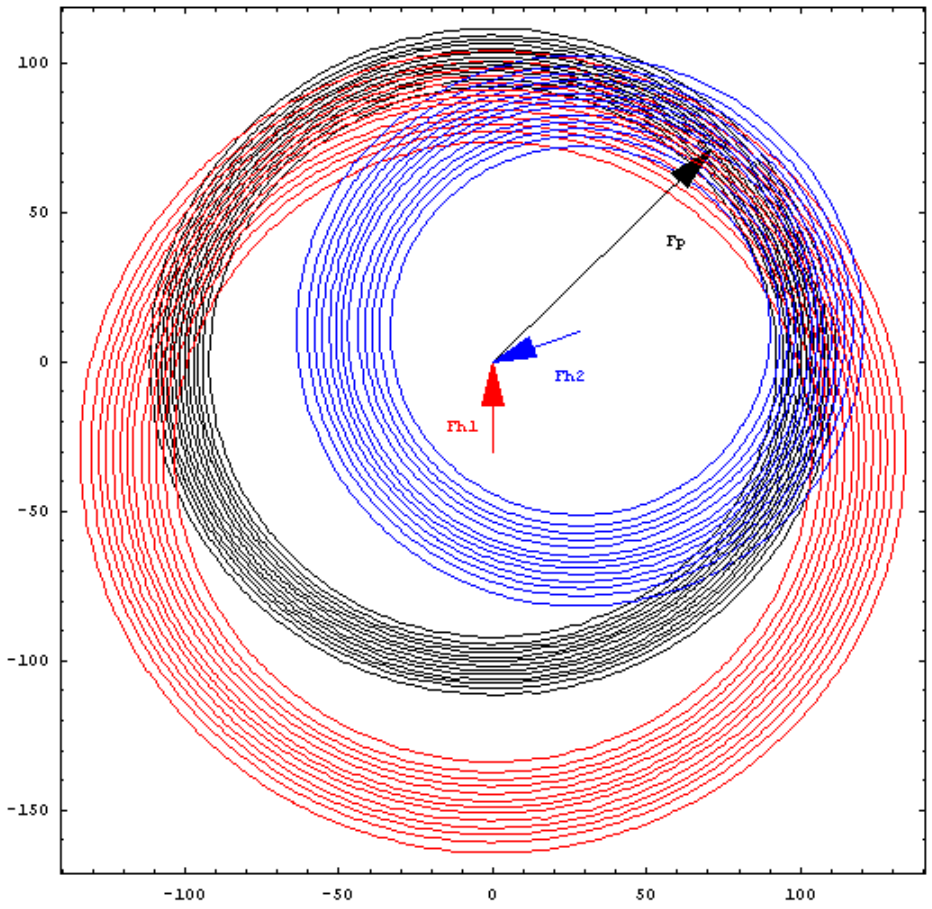
Harker construction

- 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



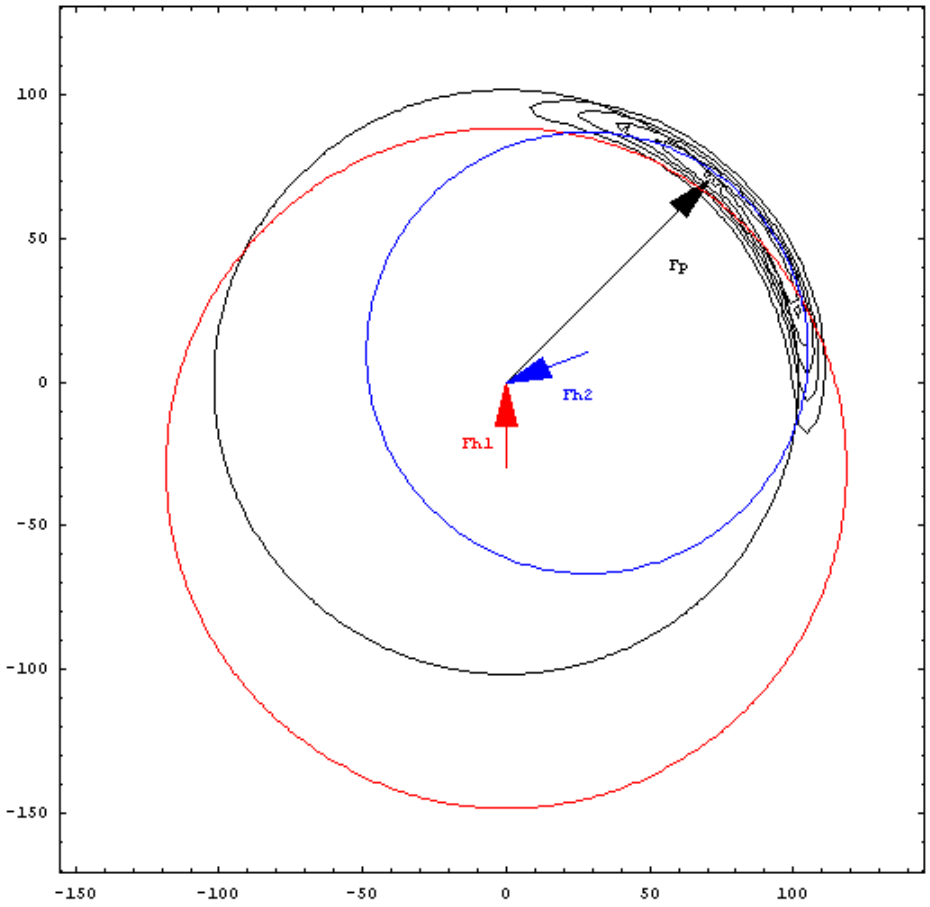
Probabilistic Harker Diagram

- 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



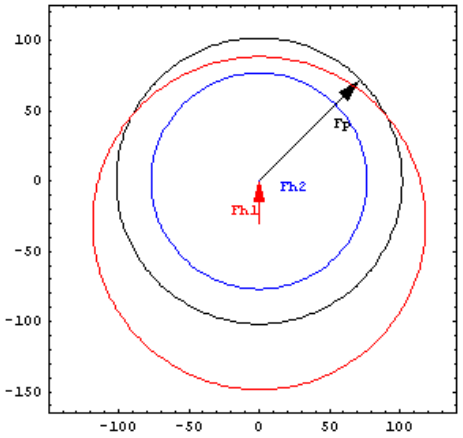
Probabilistic Harker Diagram

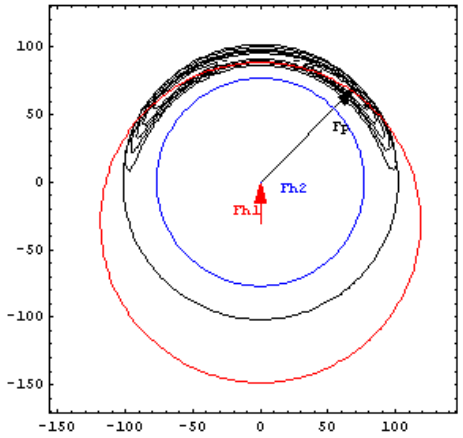
- The final distribution is high only where all three circles overlap



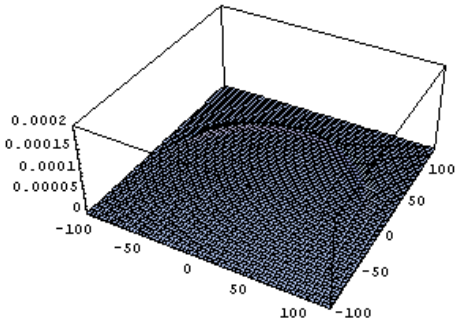
Refining Occupancy

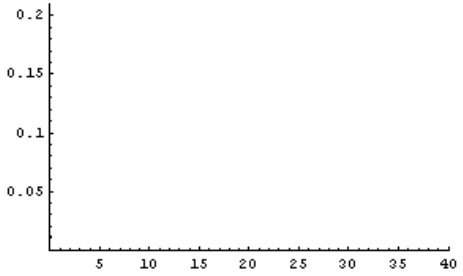
To refine the occupancy of a heavy atom, maximise the likelihood (area under the curve)





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

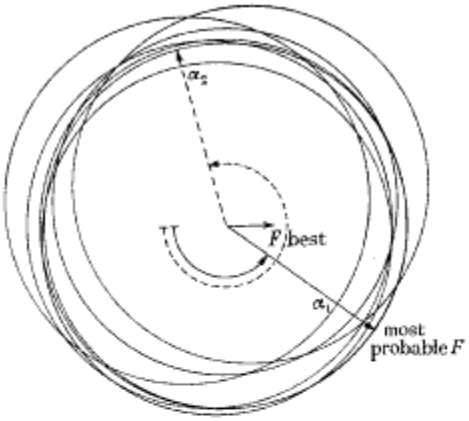




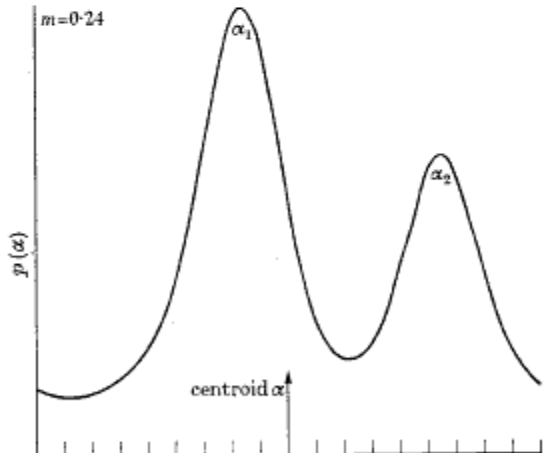
Density modification

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



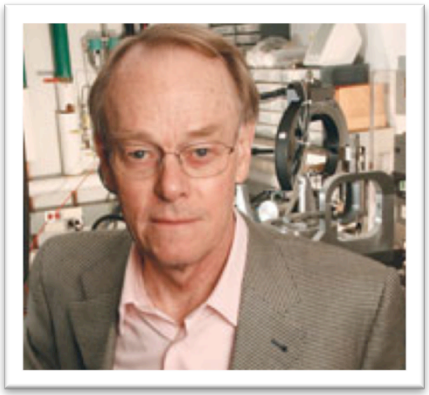
(b)



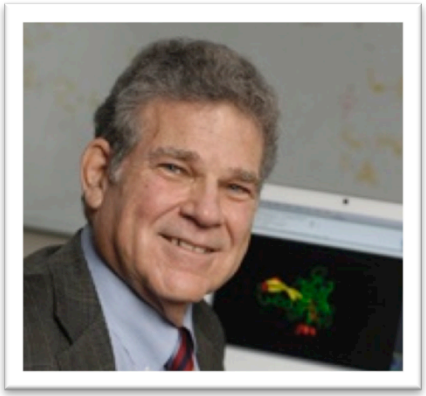
Hendrickson-Lattman Coefficients

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

- 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



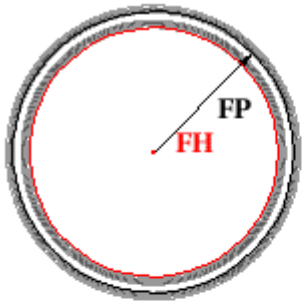
Wayne Hendrickson

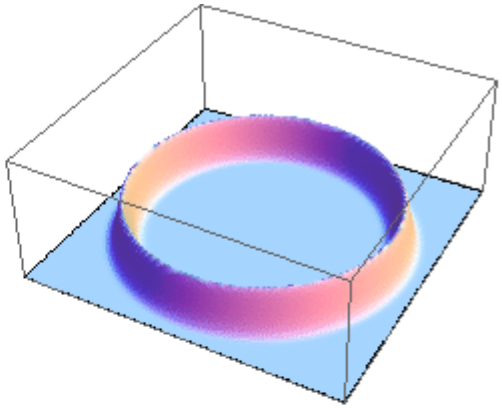


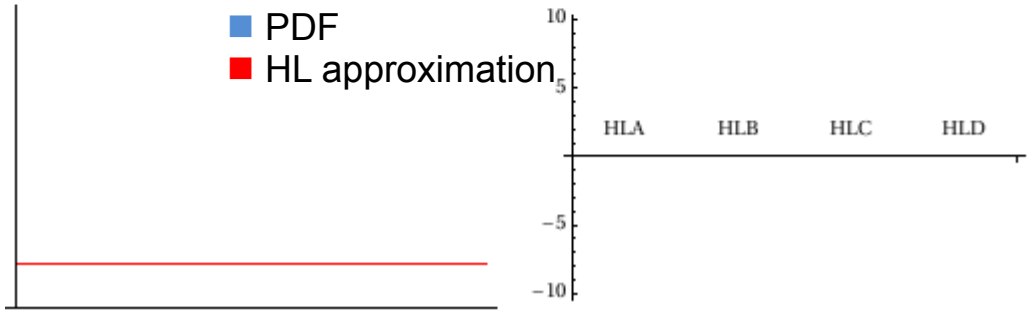
Eaton Lattman

Hendrickson-Lattman Coefficients

HL coefficients as a function of F_H occupancy



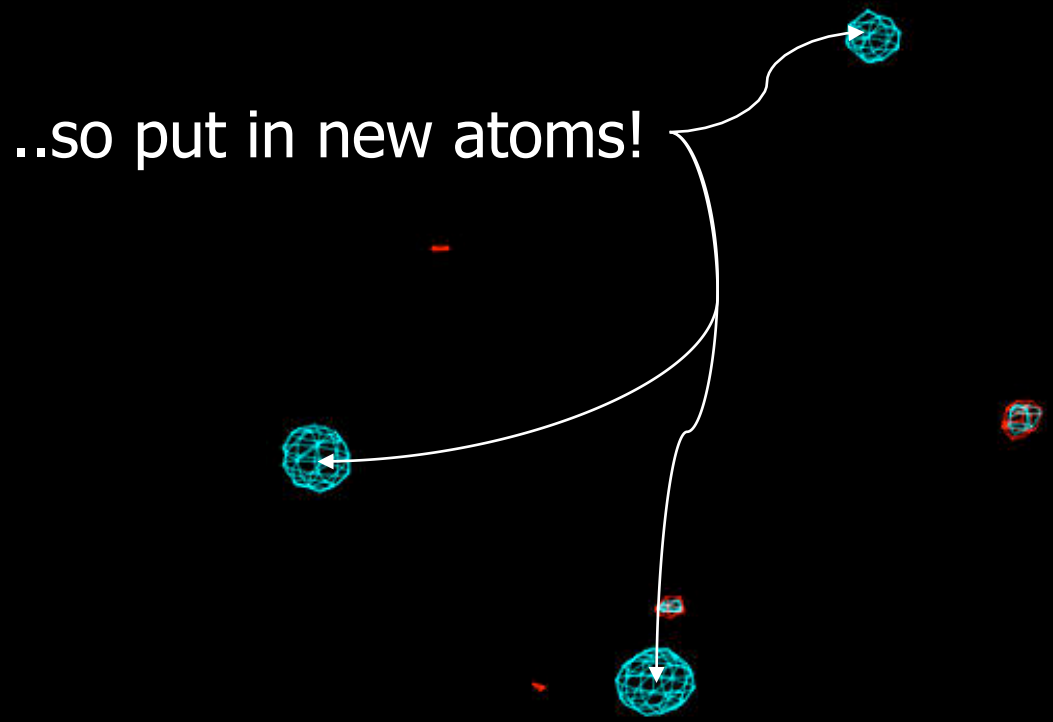




Log-likelihood gradient maps

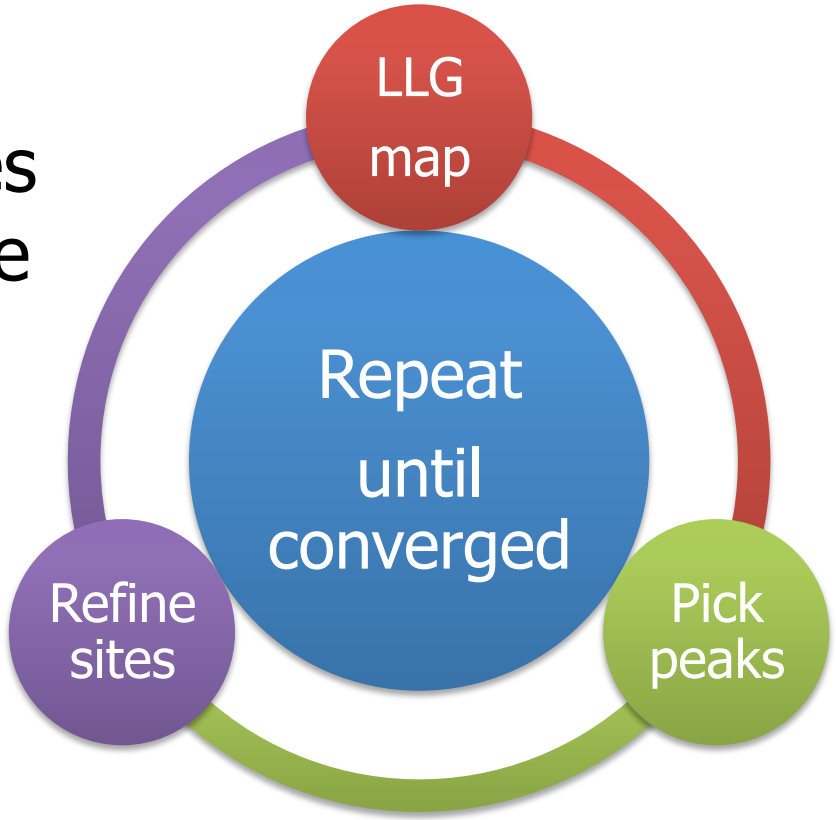
Log-likelihood gradient maps

Gradient of likelihood plotted with respect to coordinates shows where the likelihood would increase if there were atoms present…



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



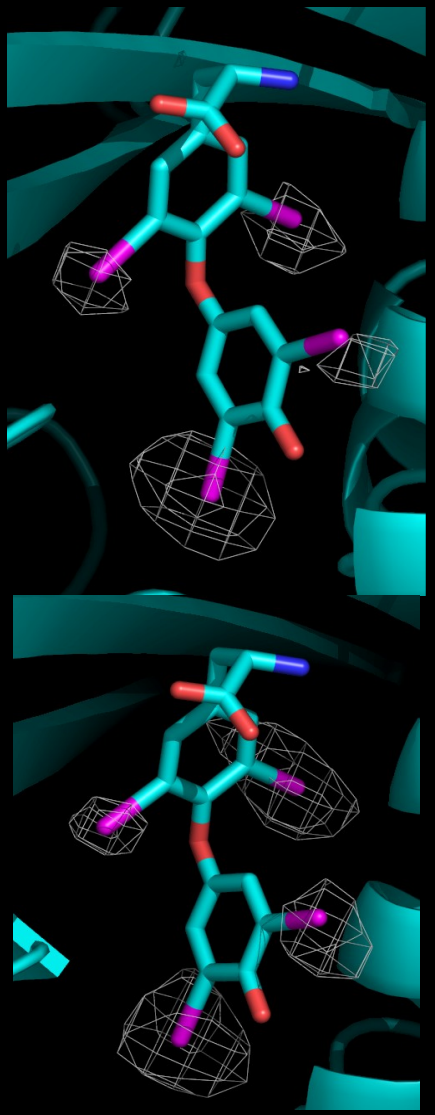
Thyroxine binding globulin

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



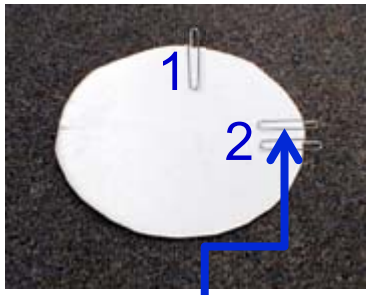
Zhou *et al*. (2006). *PNAS* **103**: 13321

Model building

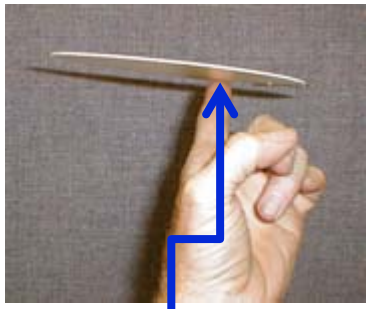
Calculating Electron Density

- 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

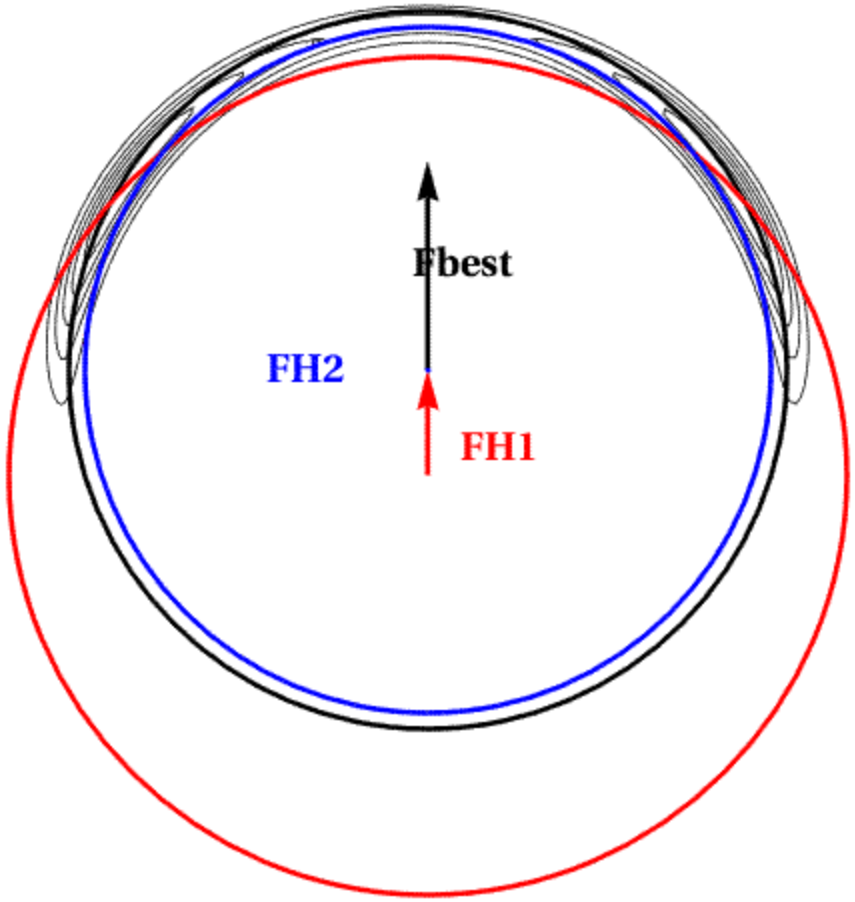


Most Probable Structure Factor



Best Structure Factor

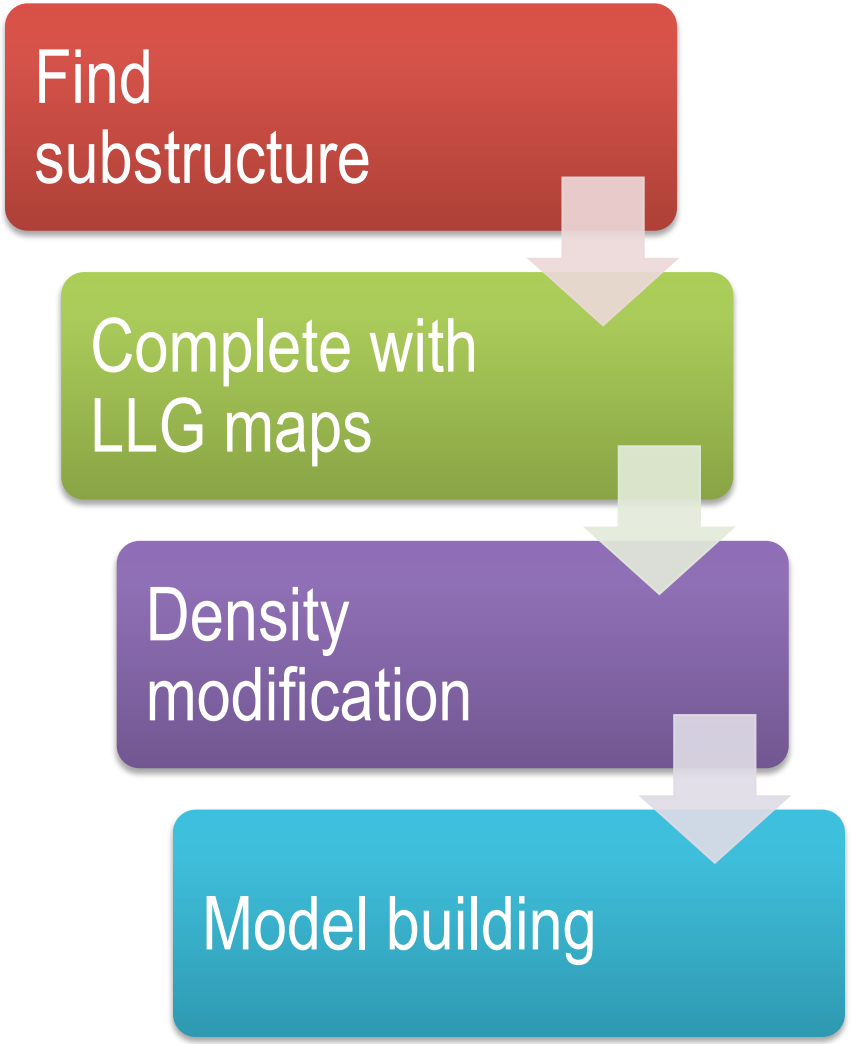
- F_{best} has a lower |F| amplitude than F_{obs}
- The reduction in F_{obs} to give F_{best} is expressed as the "figure of merit" (m)
  - **0<m <1:** F_{best} lies inside the F_{obs} circle
  - **m=1 :** Perfect phase information
  - **m=0:** No phase information
  - The higher the average value of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



New approaches

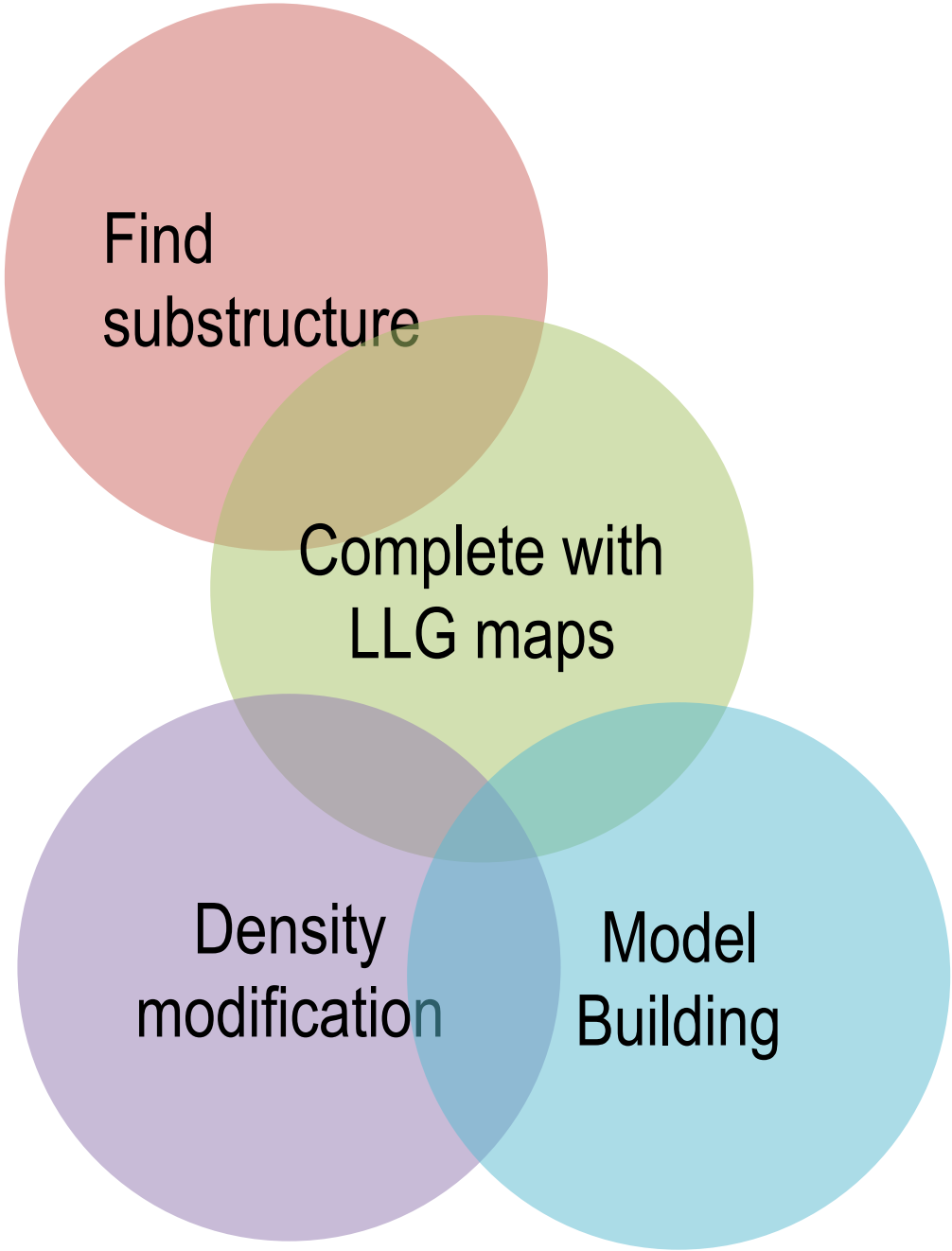
The pathway of structure solution

- 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)

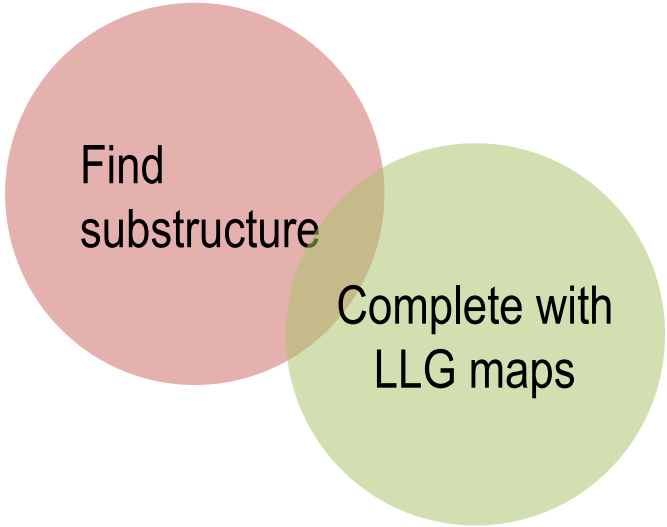


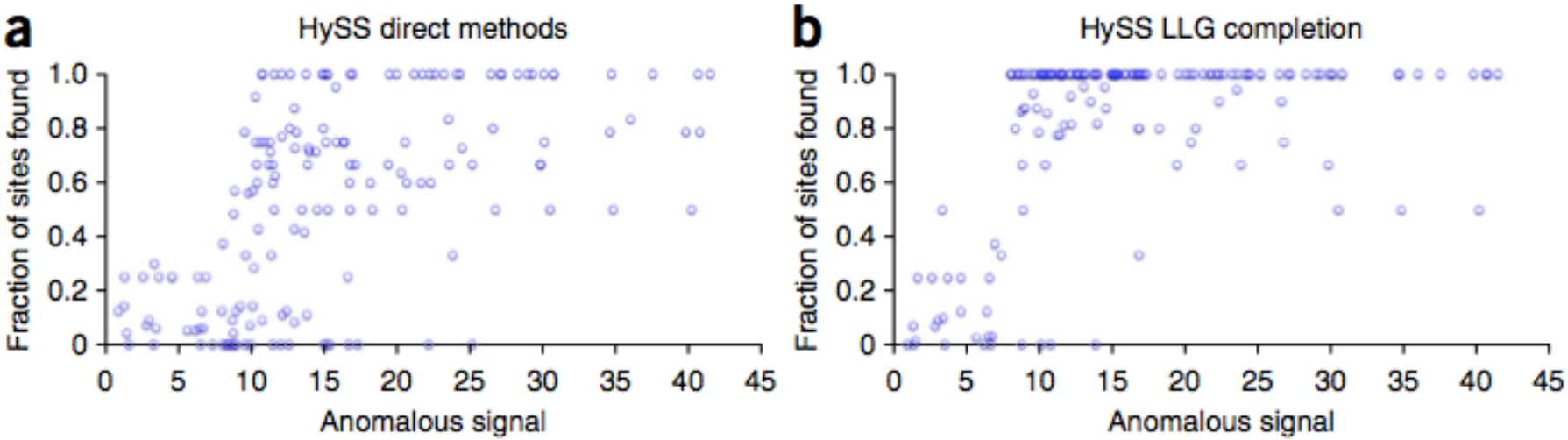
New approaches

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

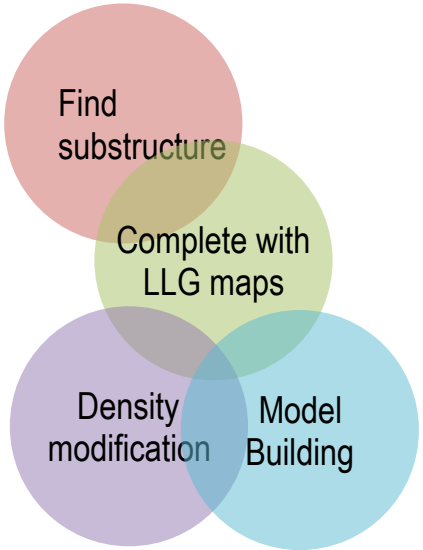


Find phasing substructure






Find substructure
Complete with LLG maps
Density modification
Model building


Find substructure
Complete with LLG maps
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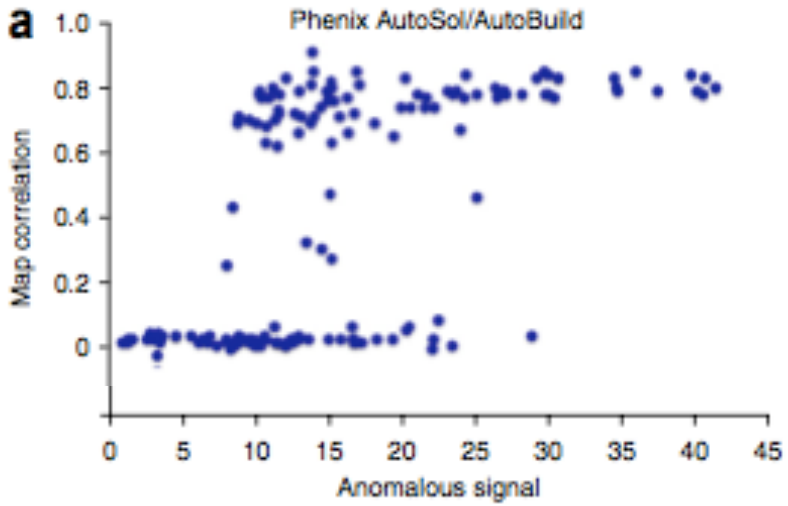
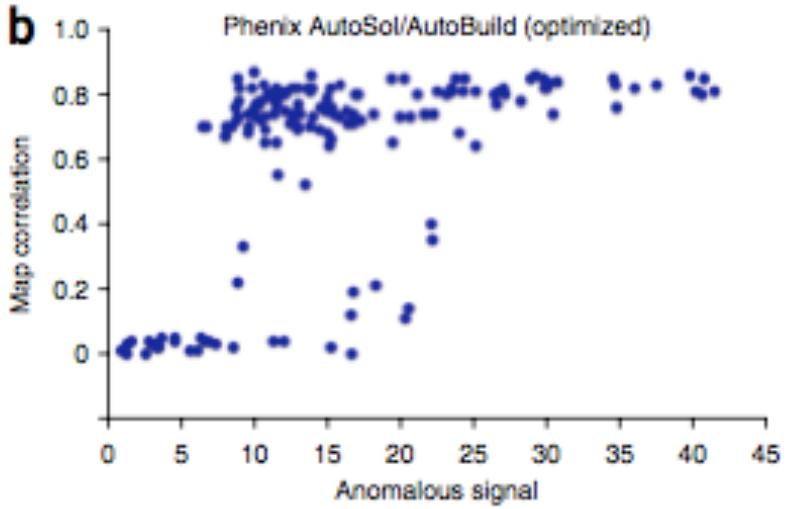
Model Building


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

The Phenix Project

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An NIH/NIGMS funded Program Project