Likelihood and SAD phasing in *Phaser*

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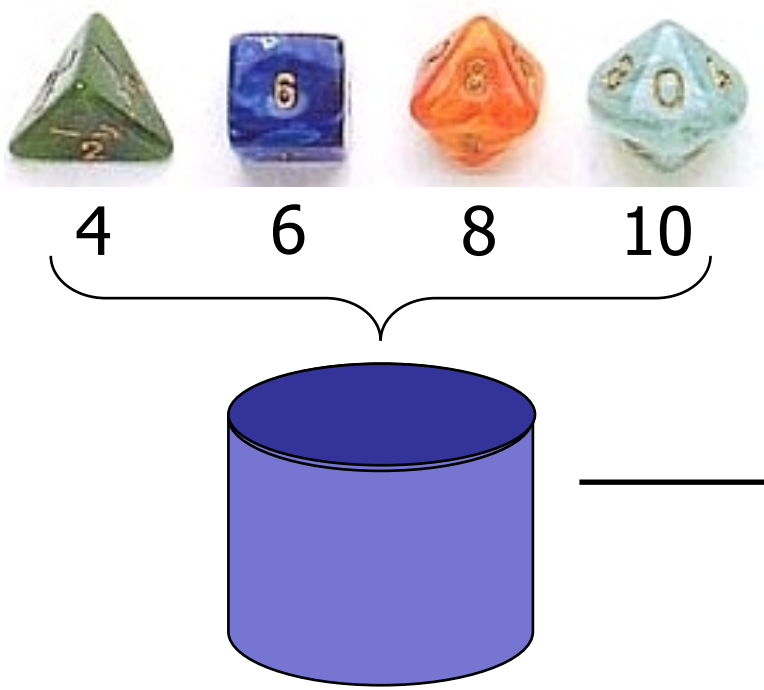
Concept of likelihood

- Likelihood with dice



Roll a seven.

Which die?

p(4)=p(6)=0

p(8)=1/8

p(10)=1/10

Principle of maximum likelihood

- Best model is most consistent with data
- Measure consistency by probabilities
- Optimise model by adjusting parameters in probability distribution

Least squares and likelihood

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

Why not least squares in crystallography?

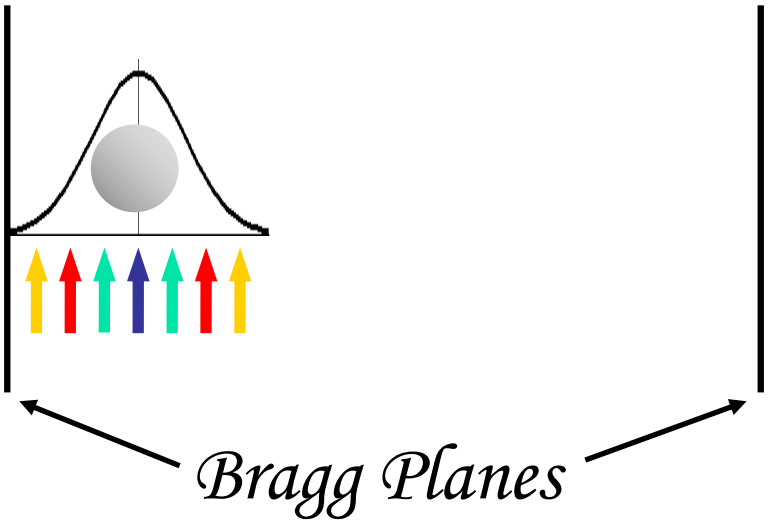
- Gaussian error for observations
- Error in predicting observation generally includes difference between structure factors
  - this is Gaussian in *phased* difference
  - *e.g.* $\mathbf{F}$ *vs.* $\mathbf{F}_C$ from model, $\mathbf{F}_P$ *vs.* $\mathbf{F}_{PH}$
- Phased error usually dominates
  -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

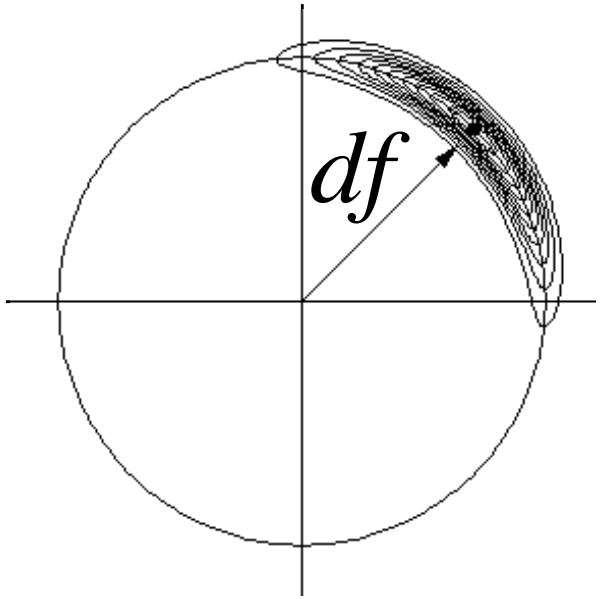
Applying likelihood to crystallography

- 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

Effect of atomic errors (or differences)

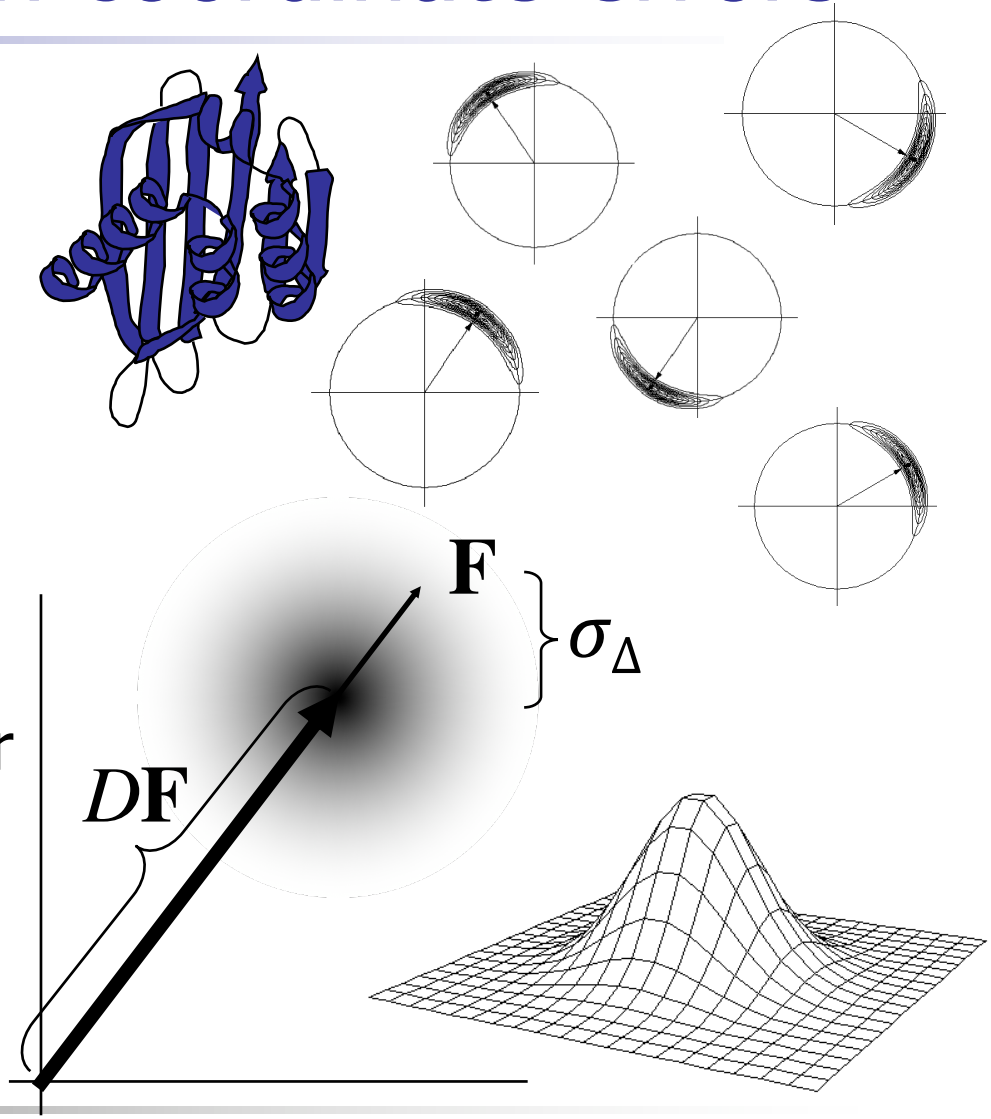
- Atomic errors give “boomerang” distribution of possible atomic contributions
- Portion of atomic contribution is correct





Structure factor with coordinate errors

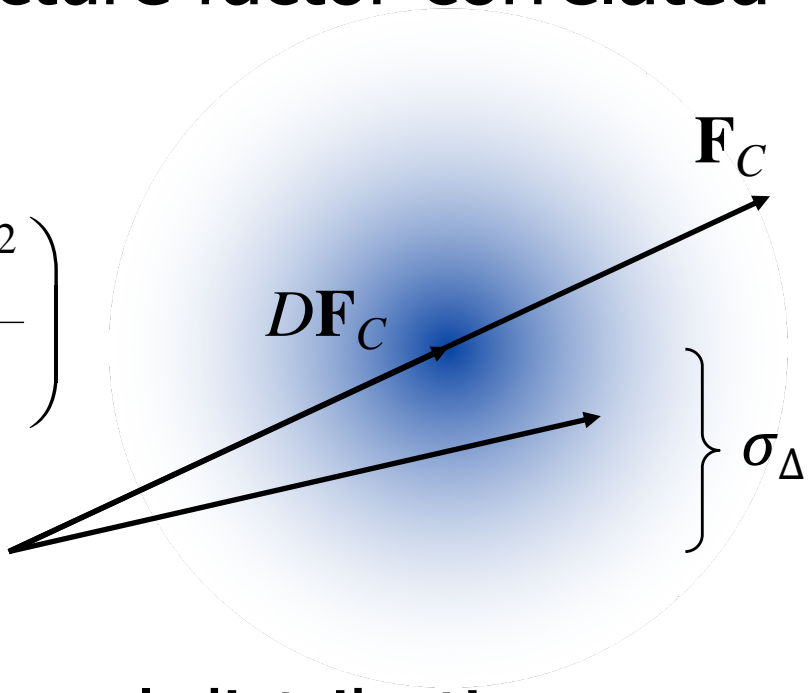
- Same direction as the sum of the atomic f
  - but shorter by $0 < D < 1$
  - D=f(resolution)
- Central Limit Theorem
  - Many small atoms
  - Gaussian distribution for the total summed **F**
  - σ_Δ=f(resolution)



Probability distribution for related structure factors

- Fraction of calculated structure factor correlated to true structure factor: D

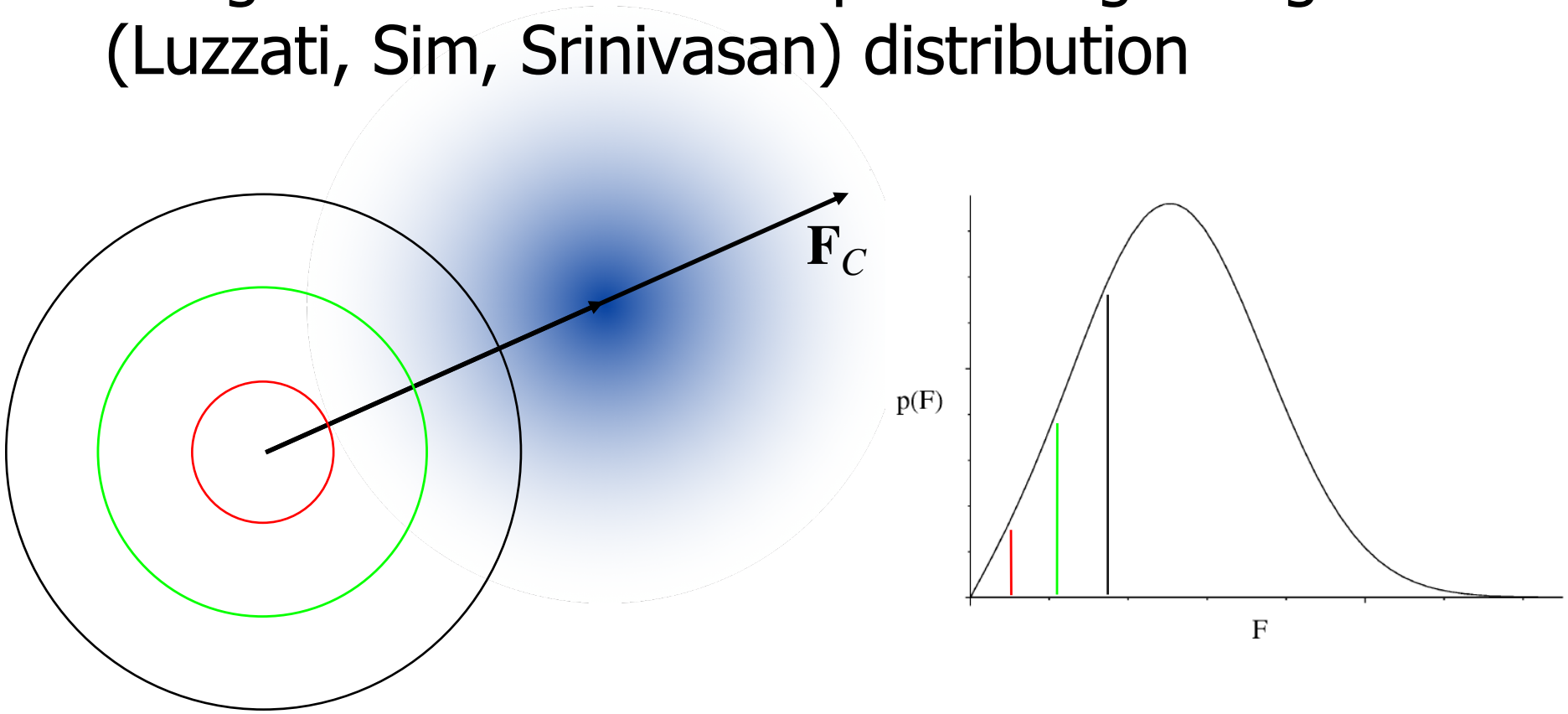
$$p(\mathbf{F};\mathbf{F}_C) = \frac{1}{\pi\varepsilon\sigma_\Delta^2}\exp\left(-\frac{|\mathbf{F}-D\mathbf{F}_C|^2}{\varepsilon\sigma_\Delta^2}\right)$$



  - (Sim, Luzzati, Srinivasan…)
- Takes form of complex normal distribution

Amplitude probability distribution

- Integrate over unknown phase angle to get Rice (Luzzati, Sim, Srinivasan) distribution

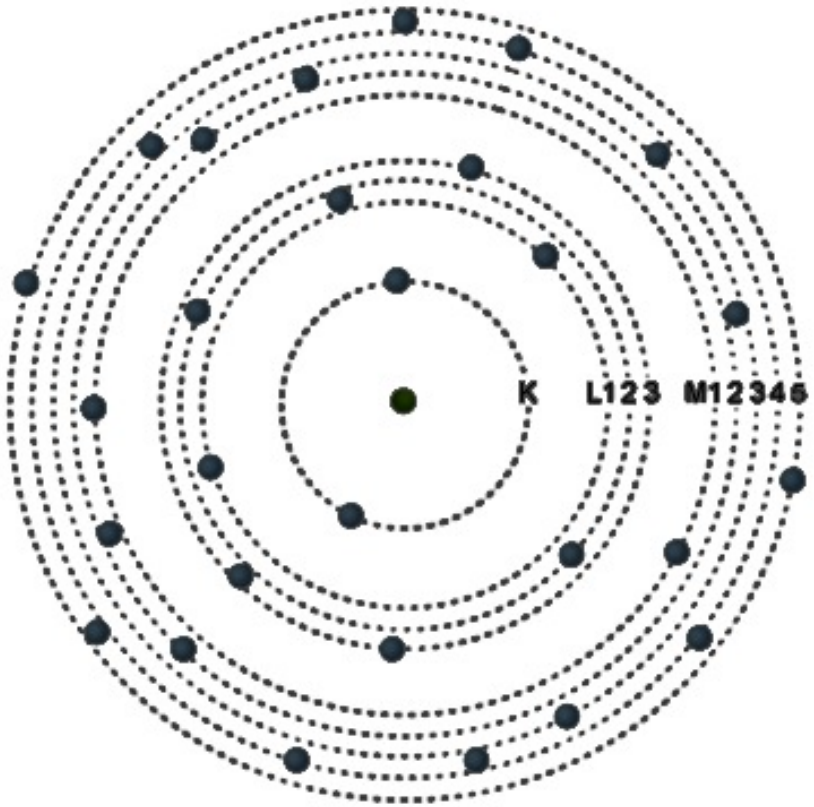


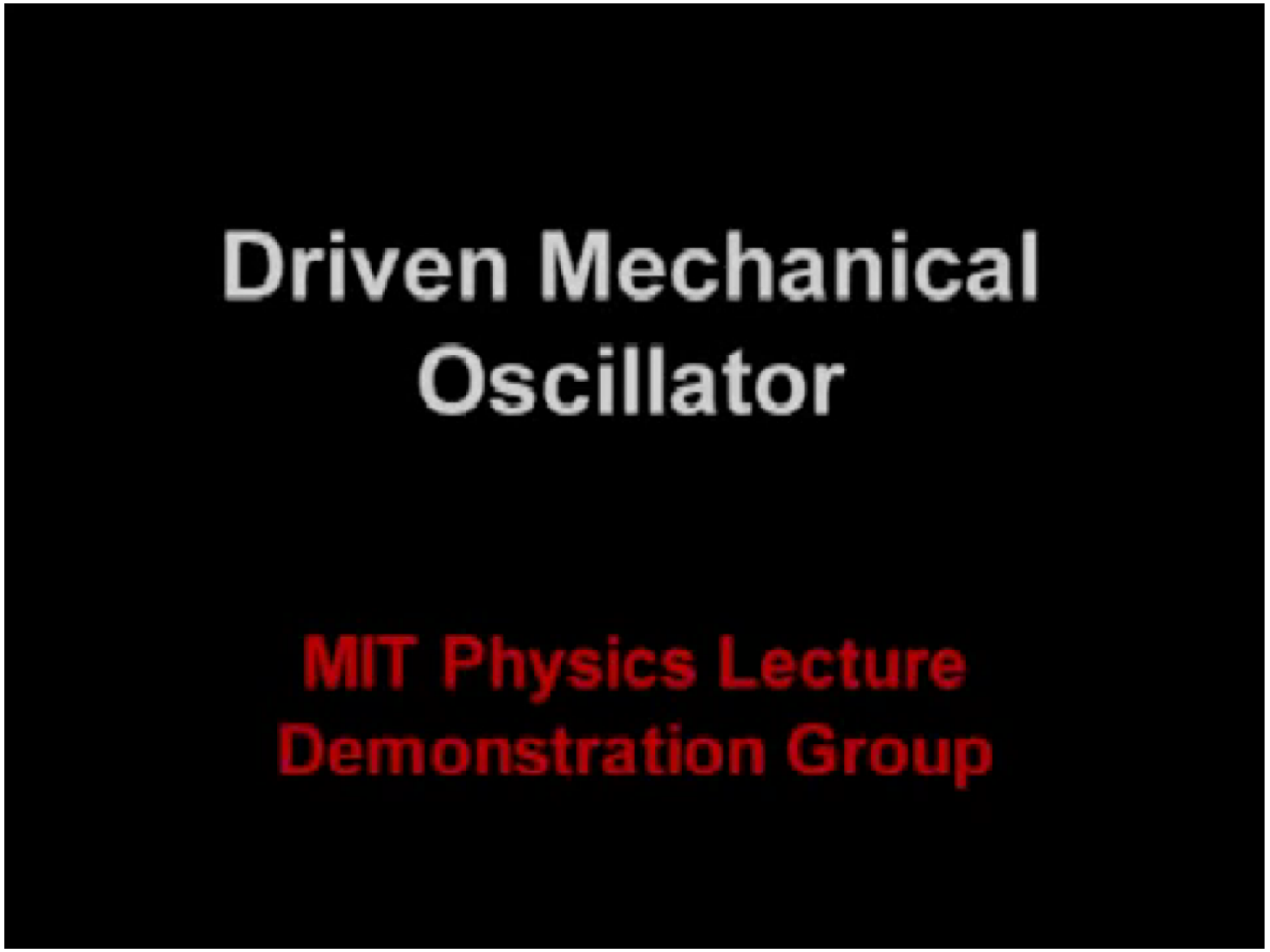
SAD: single-wavelength anomalous diffraction

- Most popular way to solve 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

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

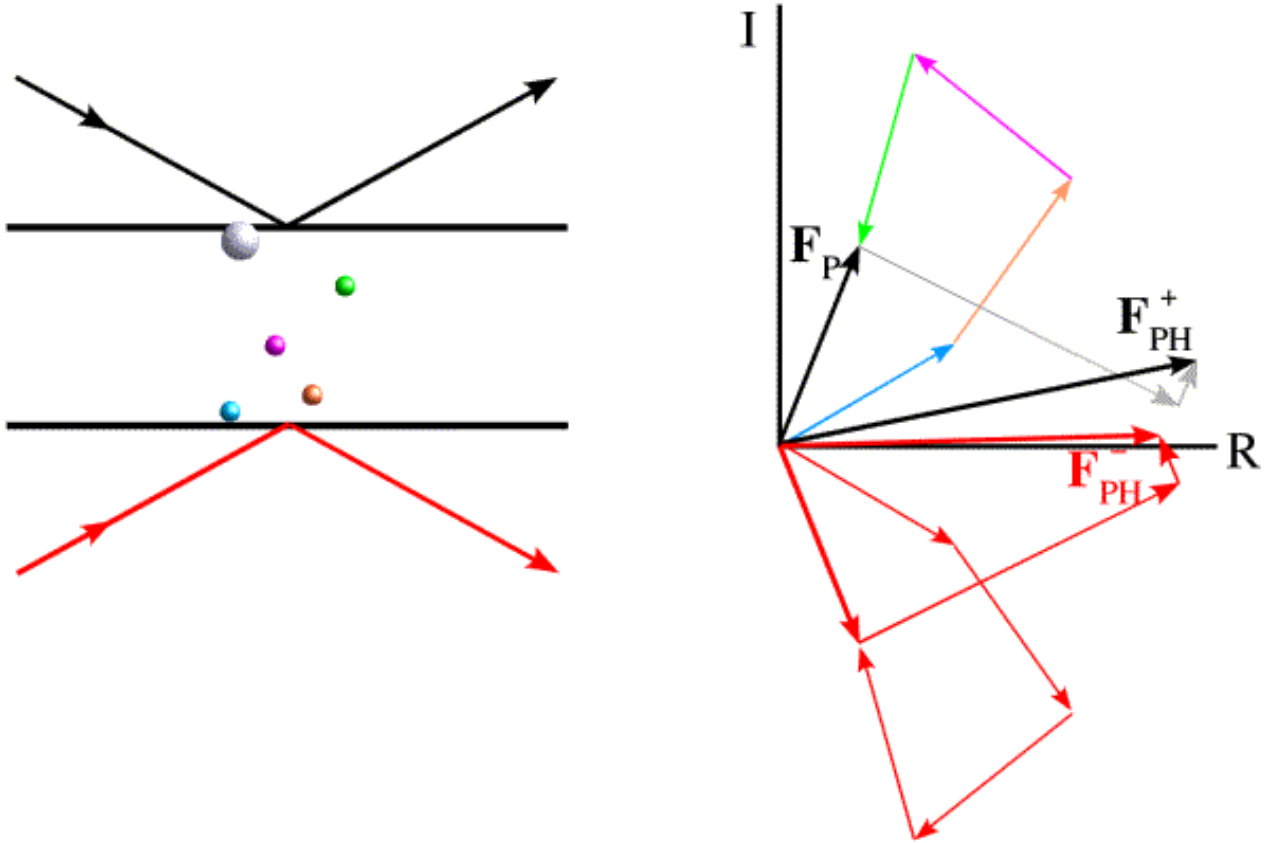




https://www.youtube.com/watch?v=aZNnwQ8HJHU

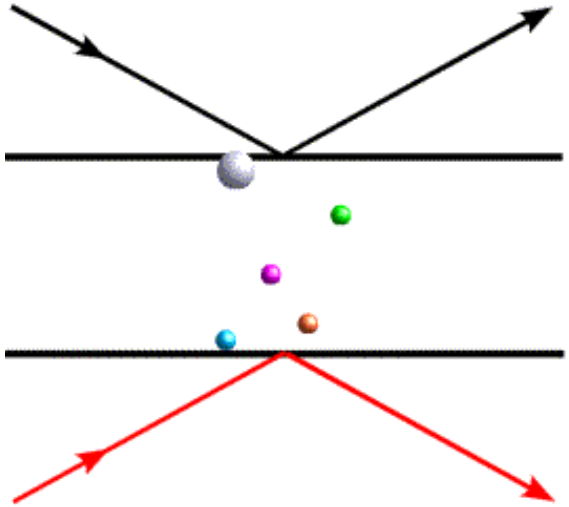
Diffraction with anomalous scatterers

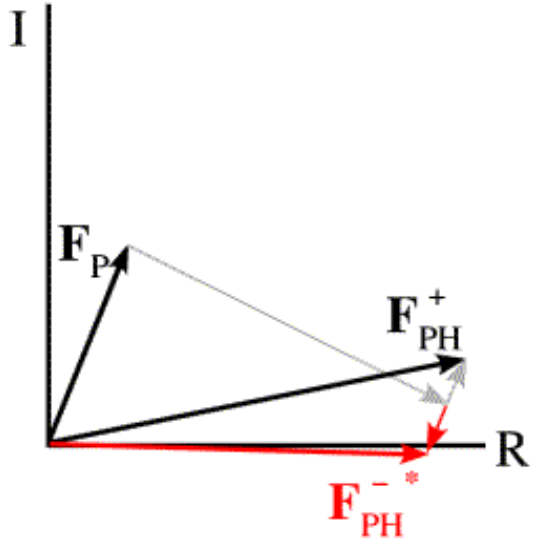
- SAD: single-wavelength anomalous diffraction



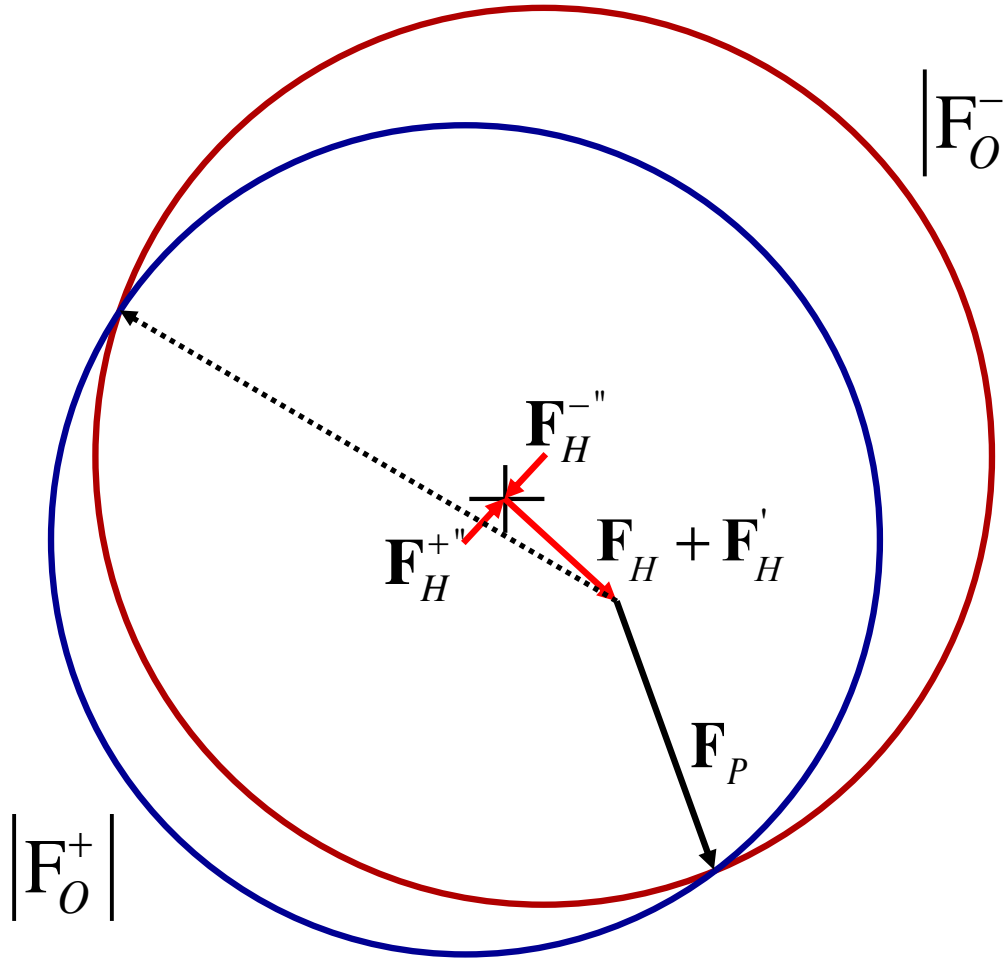
Diffraction with anomalous scatterers

- SAD: single-wavelength anomalous diffraction



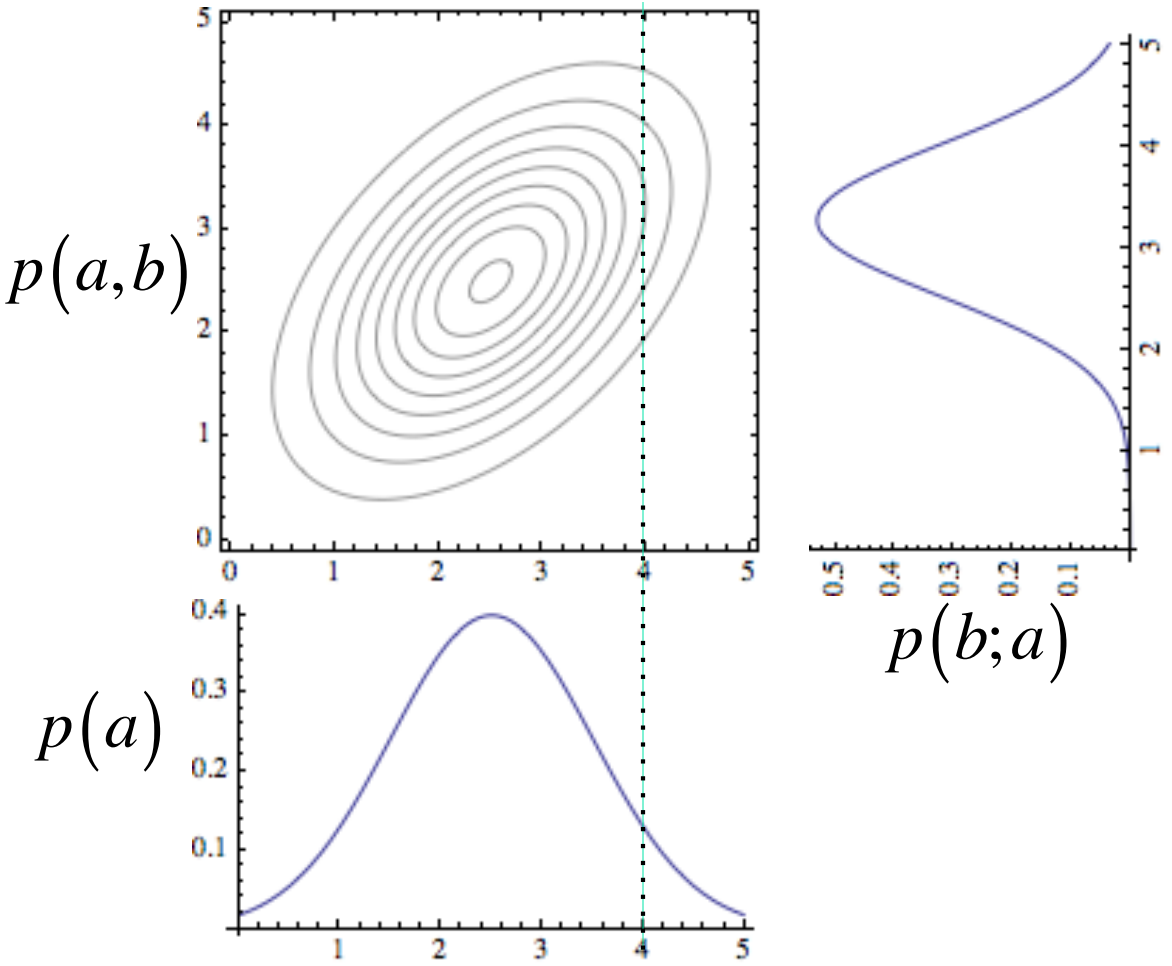


Harker construction for SAD phasing



Multiplication law of probabilities

$$p(a,b) = p(a;b)\,p(b) = p(b;a)\,p(a)$$

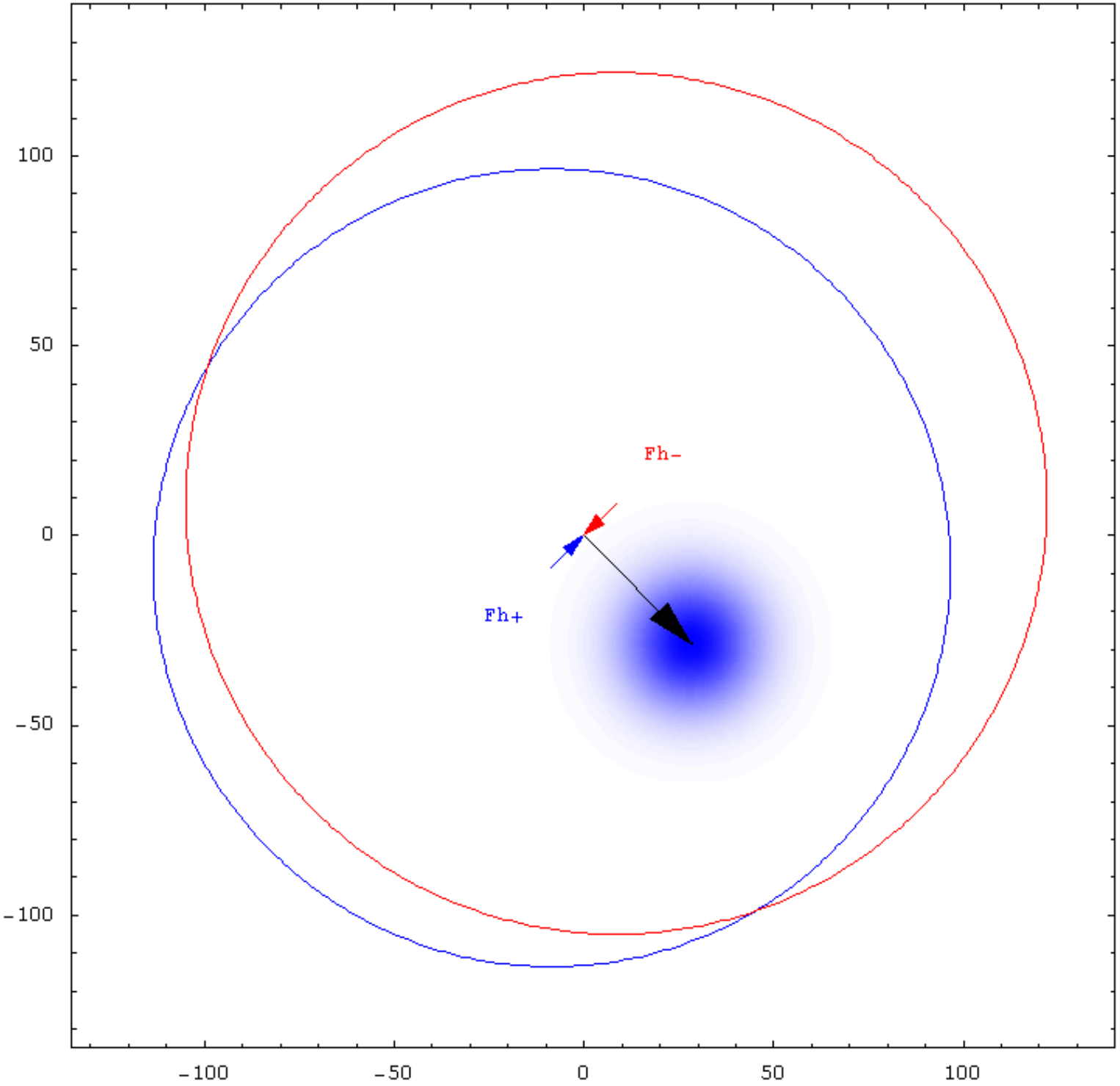


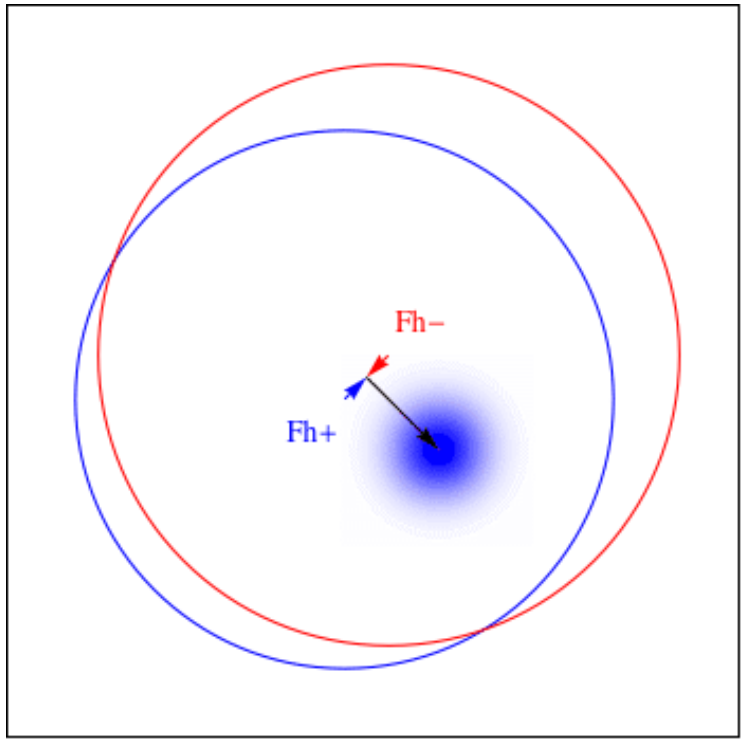
SAD likelihood function

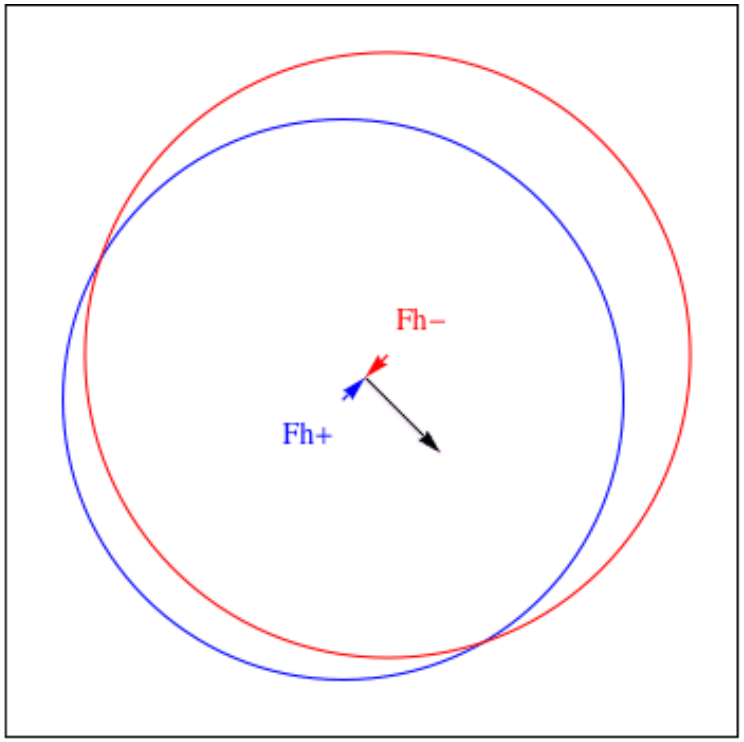
- Factor joint probability into two parts

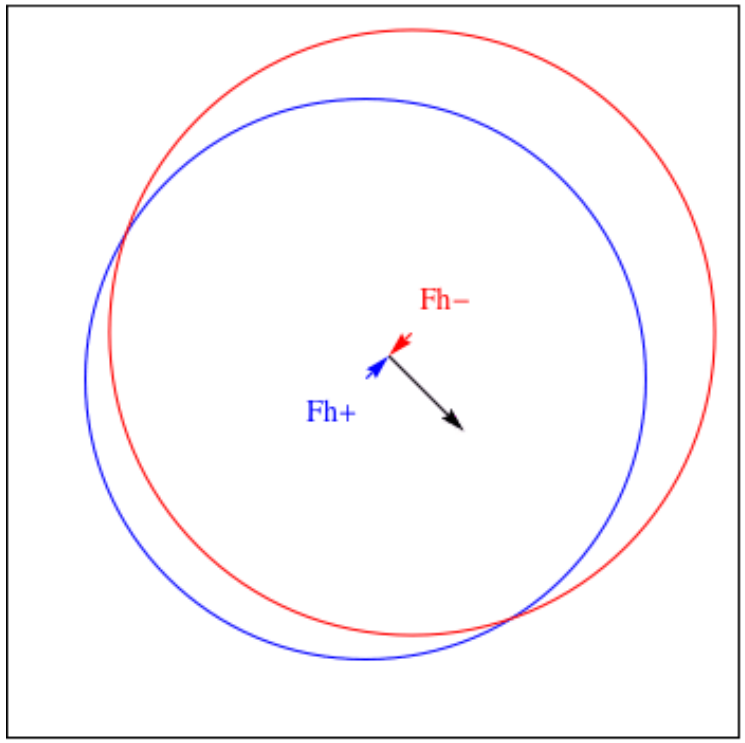
$$p\left(\mathbf{F}_O^+, \mathbf{F}_O^-; \mathbf{H}^+, \mathbf{H}^-\right) = p\left(\mathbf{F}_O^+; \mathbf{F}_O^-, \mathbf{H}^+, \mathbf{H}^-\right) p\left(\mathbf{F}_O^-; \mathbf{H}^-\right)$$

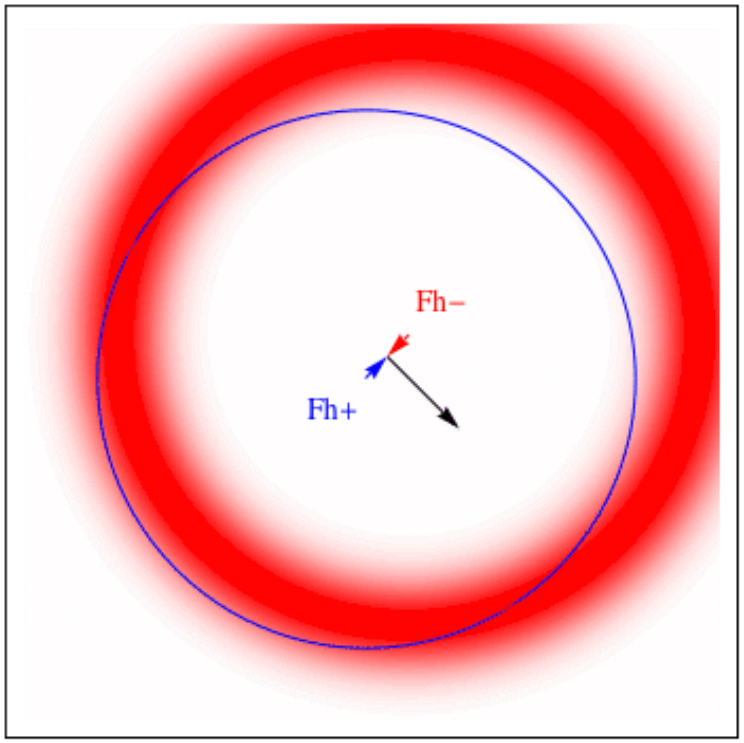
- Integrate out unknown phases, α^+ and α^-


100
50
0
-50
-100
-100
-50
0
50
100
Fh-
Fh+


Fh−
Fh+


Fh−
Fh+


Fh−
Fh+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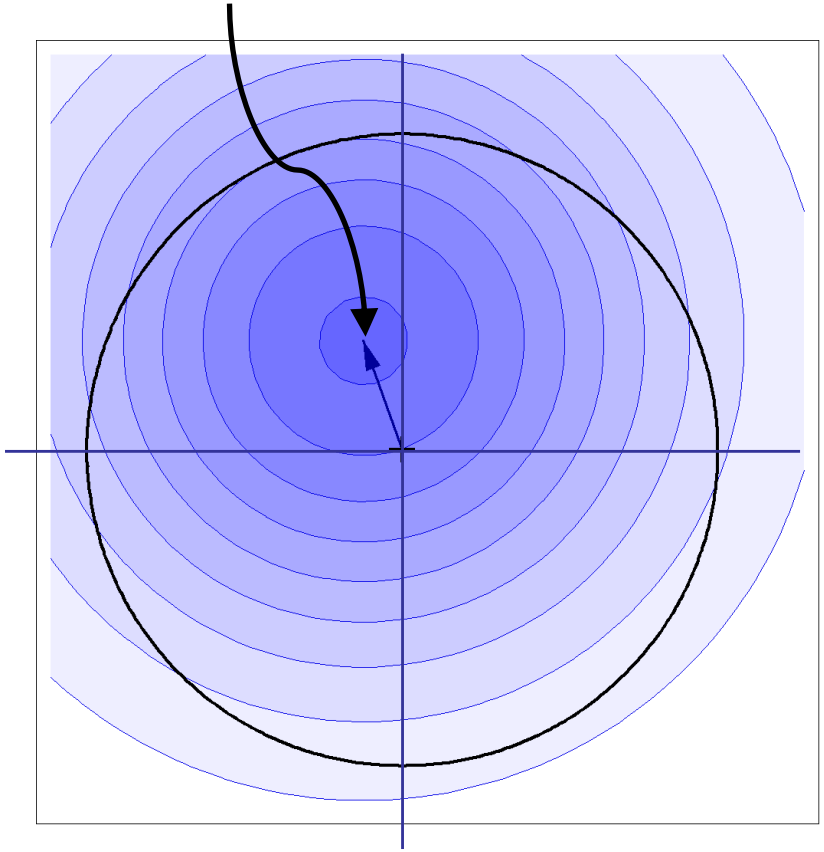
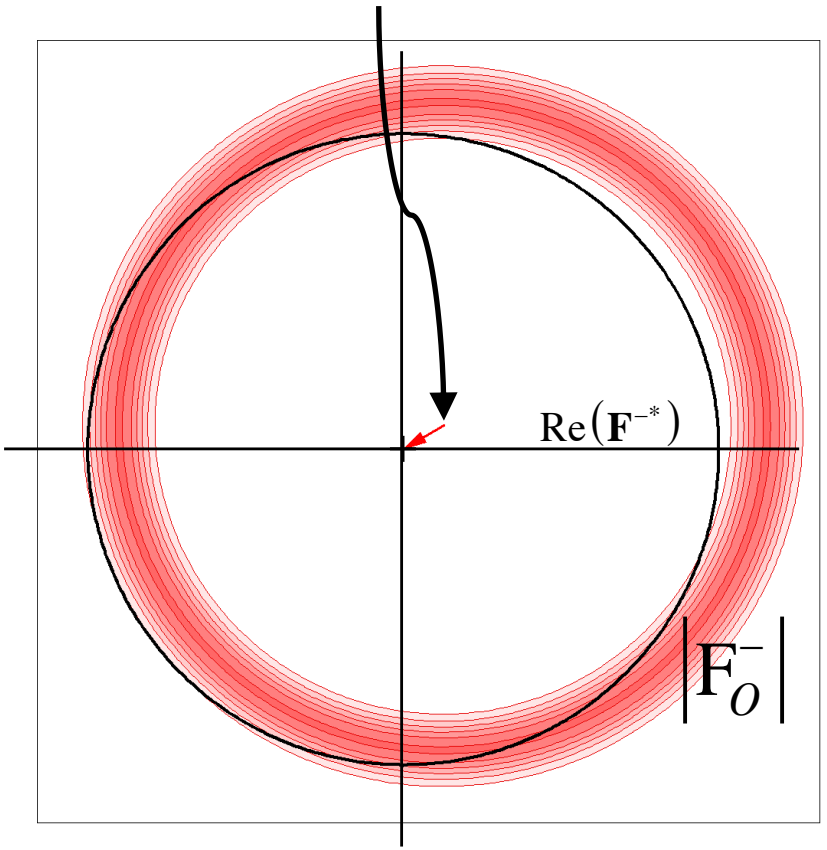
Intuitive understanding of SAD phasing

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



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

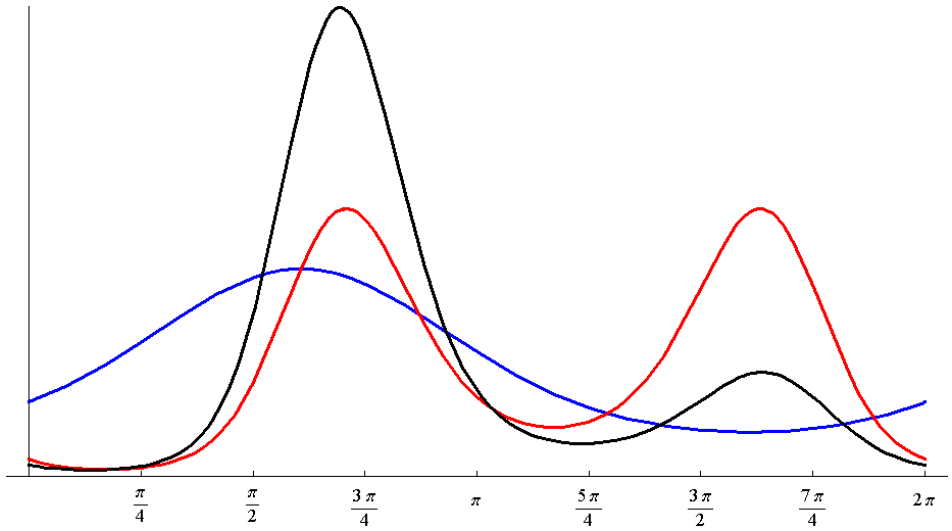
Expected difference between $\mathbf{F}^{+}$ and $\mathbf{F}^{-*}$



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

Intuitive understanding of SAD phasing

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



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

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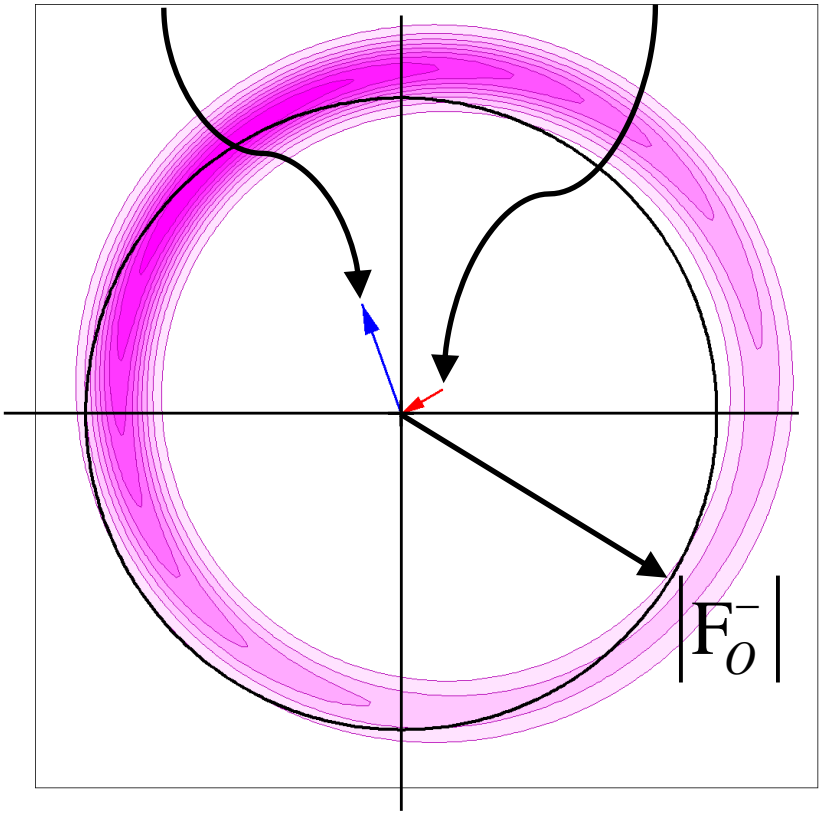
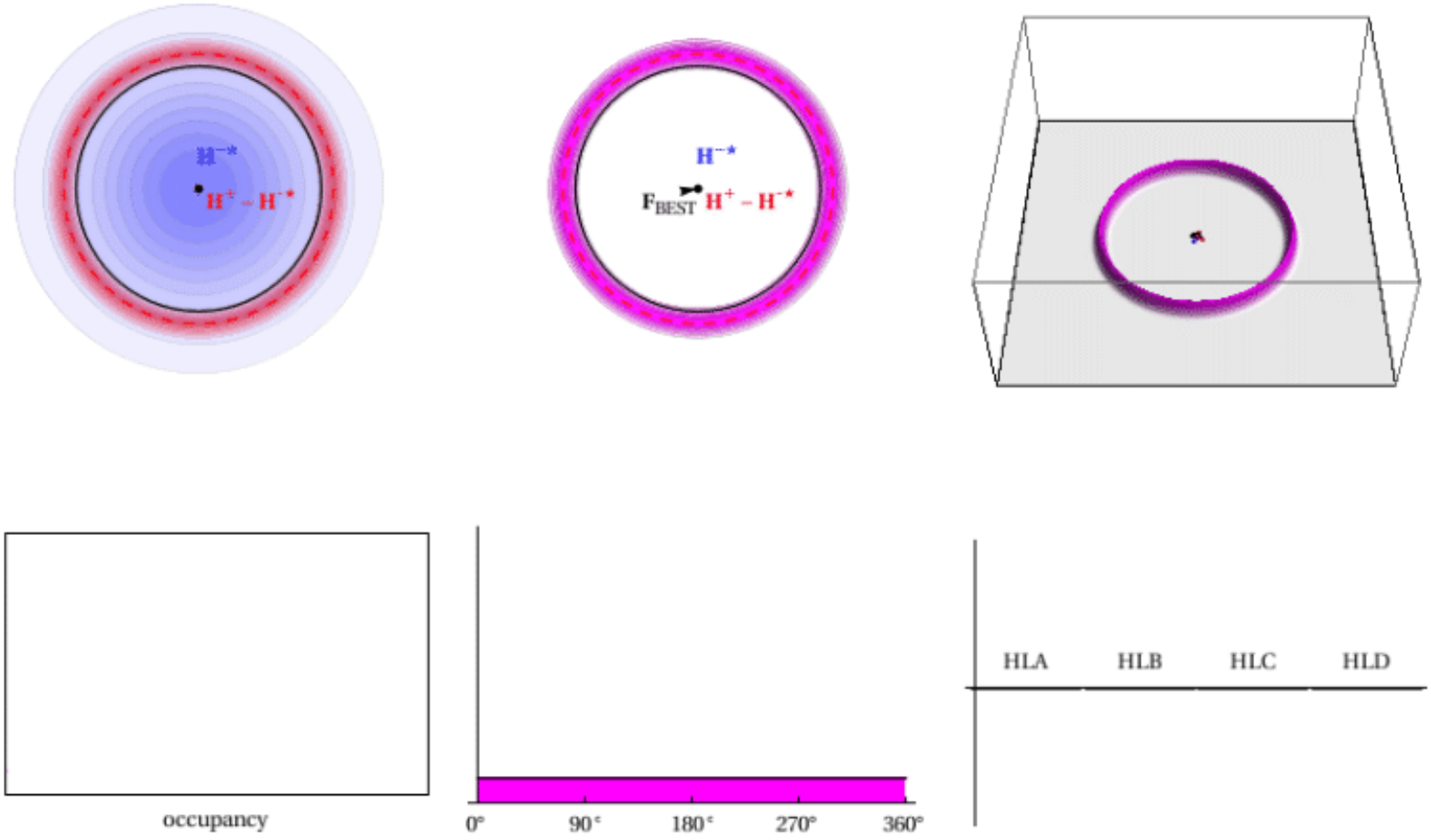


Best (centroid) map for SAD phasing



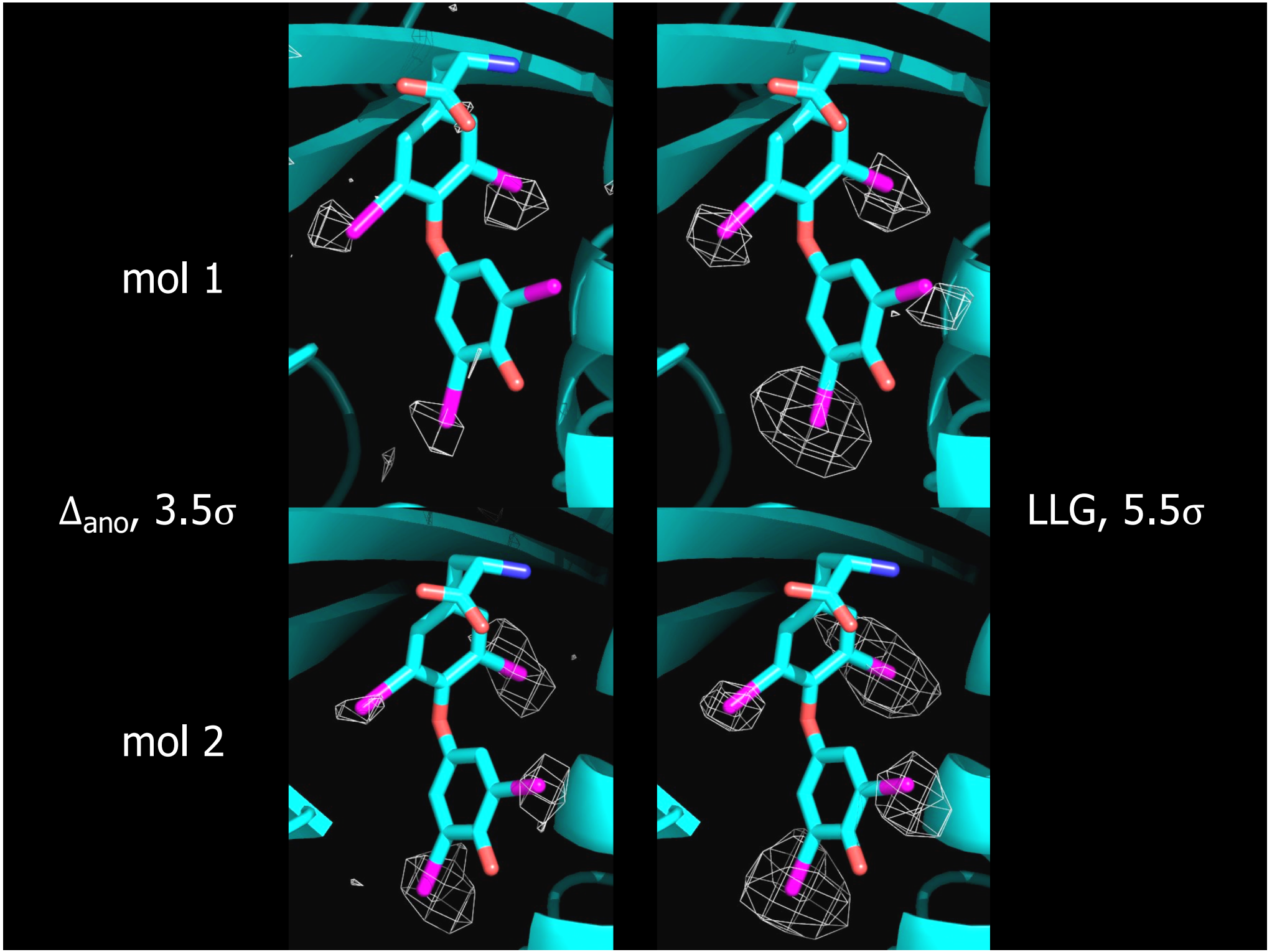
animation produced by Airlie McCoy

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
- Used to improve substructure determination in phenix.hyss (Tom Terwilliger, Gabór Bunkóczi)
- http://www.phaser.cimr.cam.ac.uk/index.php/Tutorials
  - tutorial with data for lysozyme iodide soak

Locating anomalous scatterers in model solved by MR

- Structure of thyroxine-binding globulin
  - thyroxine doesn't bind where most people expected
- Thyroxine contains 4 iodine atoms
  - f" ≈ 3e for λ=0.979Å
- Compare conventional model-phased anomalous difference map with *Phaser* LLG map

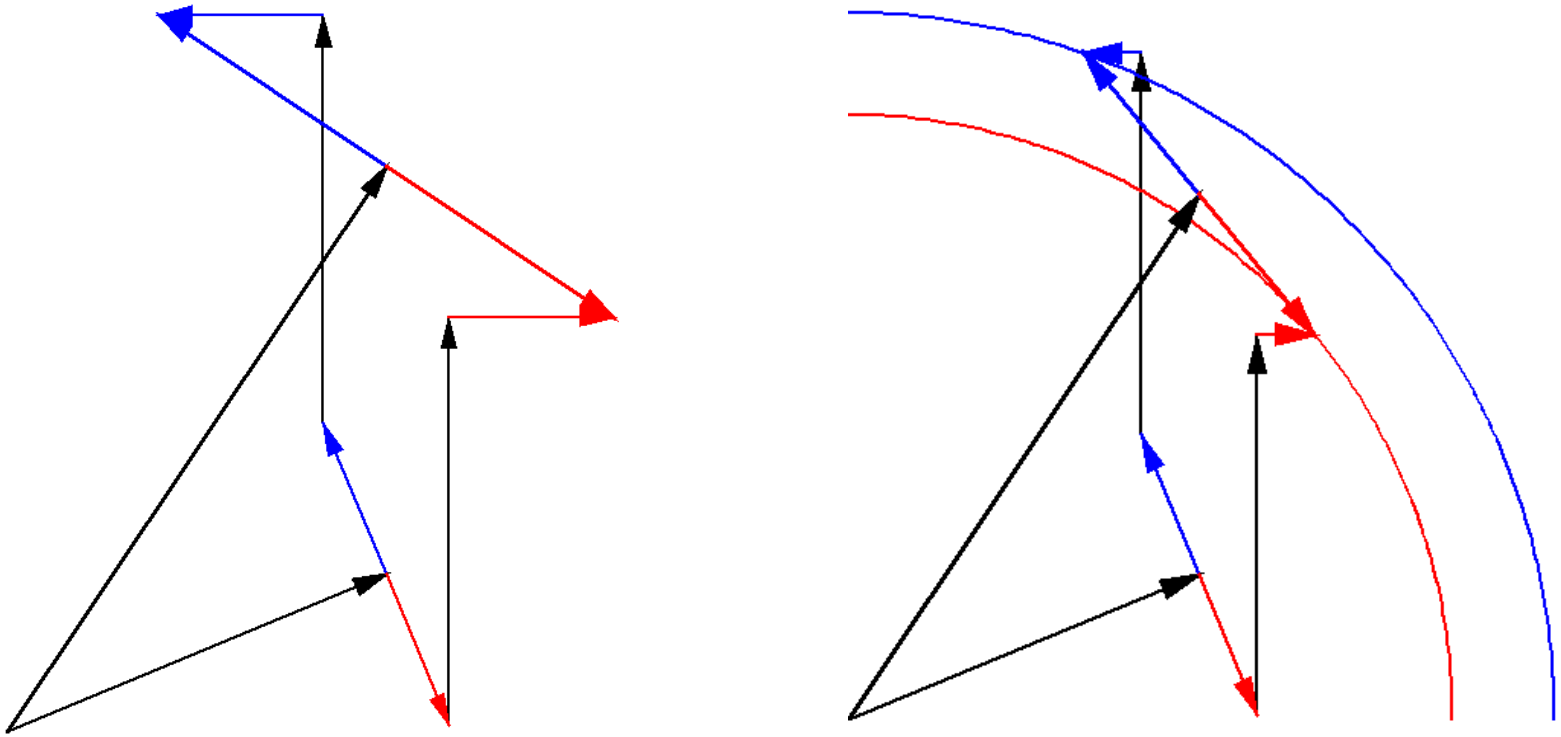

mol 1
Δ_{ano}, 3.5σ
mol 2
LLG, 5.5σ

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
  - tutorial with these data is available

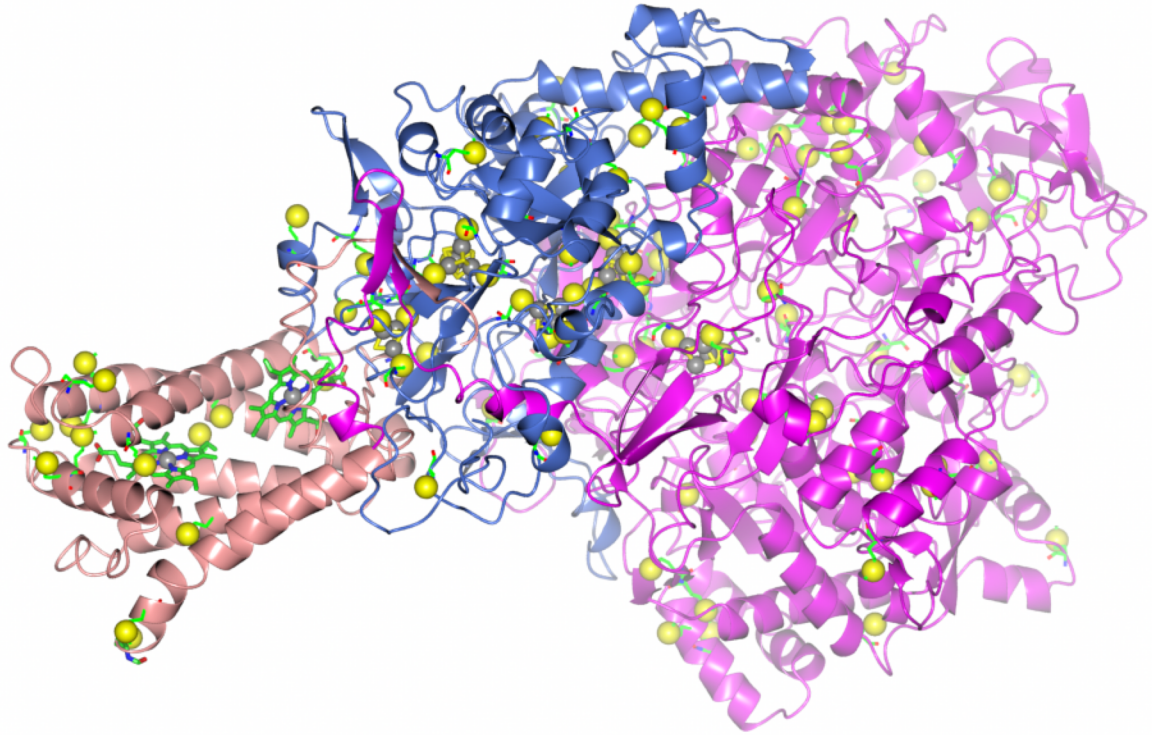
Breakdown of Friedel's law

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



Nitrate reductase (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



SAD phasing of nitrate reductase

- 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 PHENIX AutoSol GUI
  - used as optional substructure completion method in phenix.hyss
- Log-likelihood-gradient completion
  - look for one or several types of scatterer
    - start from MR model (atoms or density) or partial substructure
  - analyse map to add sites, make atoms anisotropic
  - delete atoms that fade away
  - repeat to convergence

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 near edge
- Try both hands if uncertain
  - separate completion if mixture of atom types

SAD phasing in CCP4

- ccp4i GUI
  - modes for SAD phasing or MR+SAD
  - SAD phasing pipeline
    - find substructure with Hyss or SHELXD
    - phase both hands
    - density modification with parrot
    - quick model-building with buccaneer
  - MR+SAD
    - provide MR model
    - only phase in hand of MR solution

SAD phasing in Phenix

- AutoSol GUI
  - finds sites with Hyss
    - new brute-force method uses *Phaser* to complete partial substructures
  - 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.phaser.cimr.cam.ac.uk/index.php
- http://www.phaser.cimr.cam.ac.uk/index.php/Tutorials
- http://www-structmed.cimr.cam.ac.uk/Course

Contributors

- Experimental phasing
  - Airlie McCoy, Laurent Storoni
- “BEST” data
  - Sasha Popov
- PHENIX collaboration
  - Ralf Grosse-Kunstleve, Nigel Moriarty, Paul Adams
  - Tom Terwilliger
- CCP4 SAD pipeline
  - Kevin Cowtan