

Phase Determination using Anomalous Scattering

What is anomalous scattering

- what happens when a single X-ray photon is scattered
- resonance phenomena
- overall absorption spectrum
- fluorescence spectrum
- each element has a characteristic set of edges

Use of anomalous scattering for phasing

- perturbation of scattering power
- conceptual equivalence to SIR/MIR methods
- fundamental differences to SIR/MIR

Introduction of anomalous scattering atoms into the crystal

- metalloproteins
- conventional heavy-metal derivatives
- SeMet
- Kr/Xe derivitization
- Br⁻/I⁻/Rb⁺ quick dip

One wavelength techniques

- breaking Laue symmetry
- enantiomorph determination
- Friedel pairs and Bijvoet differences
- Bijvoet difference Patterson maps
- Bijvoet difference Fourier maps
- identification of heavy atom sites (and which type of atom)
- phase likelihood distributions, Hendrickson-Lattman coefficients
- phase combination
- SIRAS (Single Isomorphous Resolved Anomalous Scattering)
- resolved anomalous phasing
- SAS or SAD (Single-wavelength Anomalous Dispersion)

Multiple wavelength techniques

- Estimating $|F_A|$, $|F_T|$, and $\Delta\phi$
- MAD Patterson types
- Karle/Hendrickson equations
- Pahler equations
- geometric interpretation
- treatment as pseudo-MIR

Combining diffraction with spectroscopy

- EXAFS (Extended X-ray Absorption Fine Structure)
- DAFS (Diffraction Anomalous Fine Structure)

Breaking Friedel's Law

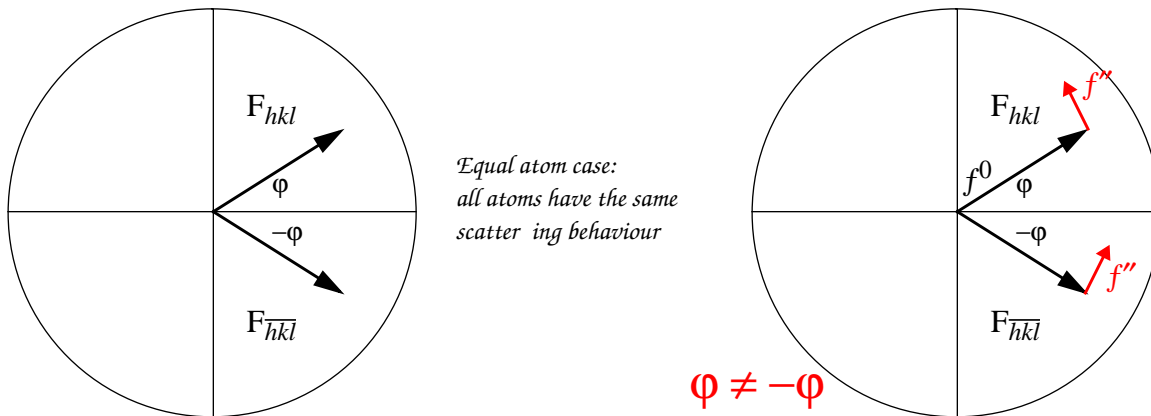
Friedel pairs are Bragg reflections related by inversion through the origin

$$F_{hkl}^+ \equiv F_{hkl} \qquad F_{\bar{h}\bar{k}\bar{l}}^- \equiv F_{\bar{h}\bar{k}\bar{l}}$$

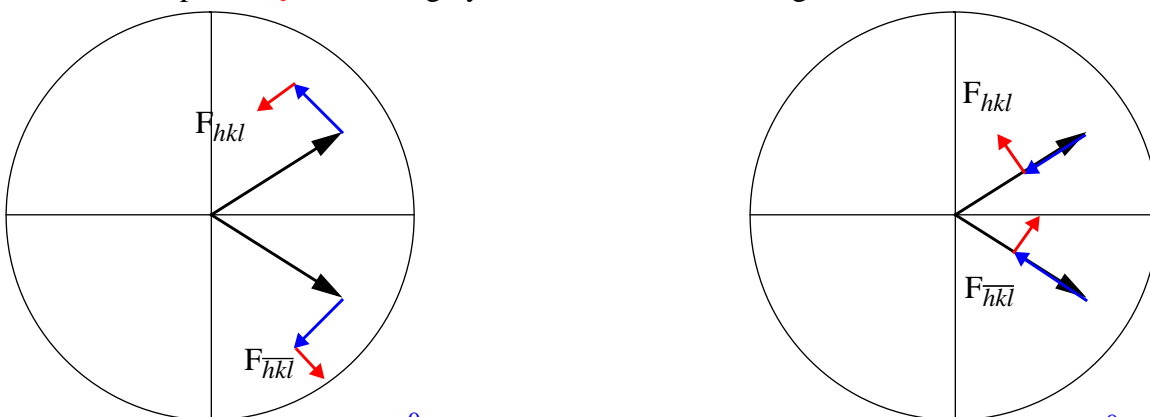
Friedel's Law states that members of a Friedel pair have equal amplitude and opposite phase

$$|F_{hkl}| = |F_{\bar{h}\bar{k}\bar{l}}| \qquad \phi_{hkl} = -\phi_{\bar{h}\bar{k}\bar{l}}$$

Friedel's Law is broken whenever there is anomalous scattering. If all atoms scatter equally then only the phase half of the law is broken.



If some atoms scatter anomalously and some don't, then both the phase and the amplitude relationships of Friedel's law are broken. In this figure the black arrows represent the scattering by non-anomalous atoms, the blue arrows represent the f^0 scattering by anomalous scattering atoms, and red arrows represent f'' scattering by the anomalous scattering atoms.



ΔF is largest when the contribution f^0 from the anomalous scattering atoms is $\pi/2$ out of phase with that of the non-anomalous atoms

ΔF is smallest (zero) when the f^0 contribution is in phase with that of the non-anomalous atoms

Breaking Friedel's Law

Bijvoet pairs are Bragg reflections which are true symmetry equivalents to a Friedel pair. These true symmetry equivalents have equal amplitudes, even in the presence of anomalous scattering. For example, in Space Group $P2_1$

$$|F^+| \equiv |F_{hkl}| = |F_{\bar{h}\bar{k}\bar{l}}| \qquad |F^-| \equiv |F_{\bar{h}k\bar{l}}| = |F_{h\bar{k}l}|$$

A *Bijvoet difference* refers to the difference in measured amplitude for a Bijvoet pair

$$\Delta F = |F^+| - |F^-|$$

A *Bijvoet difference Patterson* map, calculated with coefficients ΔF^2 , should contain peaks corresponding to interatomic vectors between anomalous scattering atoms only.

A *Bijvoet difference Fourier* map is calculated with coefficients

$$(\Delta F; \varphi + 90^\circ)$$

(Important note: the phases are coming from somewhere else!) This should give a beautiful map with a peak at each point in the unit cell where there is an anomalous scattering atom.

Kramers-Kronig Equation

$$f'(\omega) = \frac{2}{\pi} \int_0^\infty \frac{\omega' f''(\omega') \partial \omega'}{\omega^2 - \omega'^2}$$

MAD Phasing

Data collection

- Measure fluorescence spectrum
- Choose wavelengths (edge, peak, high, low); need at least 2 for MAD
- Measure data and process keeping Bijvoet pairs separate

Locate anomalous scattering atoms

- Bijvoet difference Patterson needs only F^+ , F^- at single wavelength above edge
- Better to generate estimates for $|F_A|$ (contribution of anomalous scattering atoms only)
 - Karle equations [J Karle, 1980] for $|F_A|$, $|F_T|$ and $\Delta\phi$.
 - Bayesian correction to $|F_A|$ [T Terwilliger, 1994]
 - 'quick and dirty' estimate [J Smith, 19??] = $\sqrt{(\Delta f')^2 + (\Delta f'')^2}$
- Direct methods
 - 'Dual space' approach of SHAKE'N'BAKE, SHELXD

Assign phases for protein

- simplest estimate is $\phi = \phi_A + \Delta\phi$ (from Karle/Hendrickson equations)
- geometric minimization of lack-of-closure (equivalent to MIR method of Blow & Crick)
- phase likelihood combinations
 - Hendrickson/Lattman coefficients ABCD express phase likelihood distribution as
$$P_i(\alpha) = e^{k + A\cos\alpha + B\sin\alpha + C\cos 2\alpha + D\sin 2\alpha}$$
 - Always gives bimodal distribution
 - ABCD coefficients can be calculated directly from the MAD data (Pahler, Smith & Hendrickson, 1990)
- implementation as pseudo-MIR (Terwilliger, 1994) is convenient but not optimal
 - MLPHARE (CCP4, E Dodson)
 - SOLVE (Terwilliger)
- most powerful is maximum likelihood refinement of all parameters jointly with fit to data
 - SHARP (de la Fortelle & Bricogne)