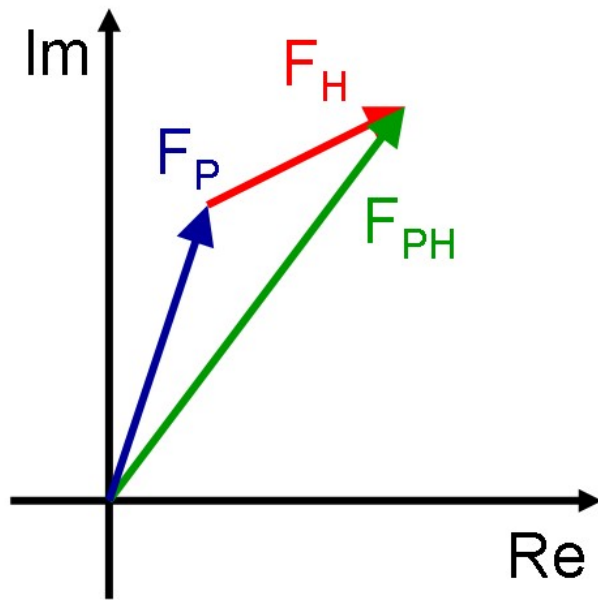
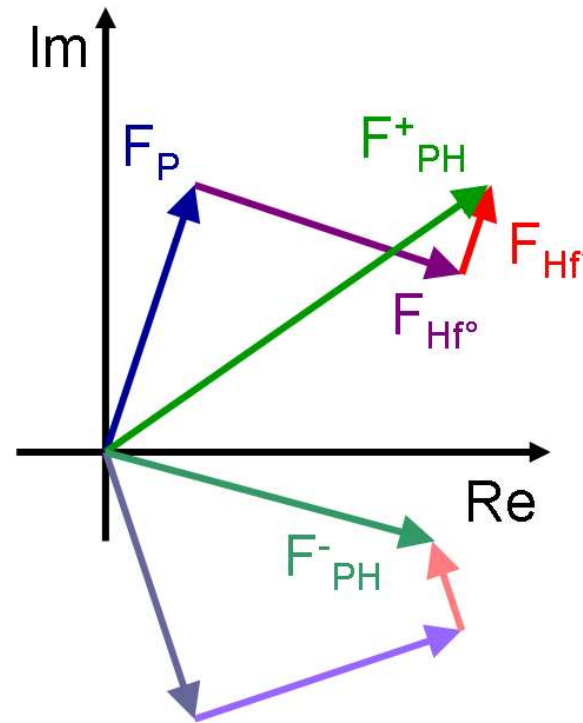


Phasing/2:

Experimental phasing



SIR and MIR

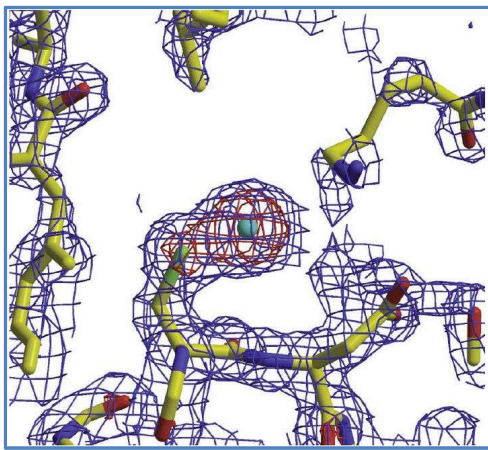


SAD and MAD

Experimental phasing

First, reduce the phase problem to a smaller task: identify the position of a single element, a marker atom.
Then, extend the solution to the whole structure.

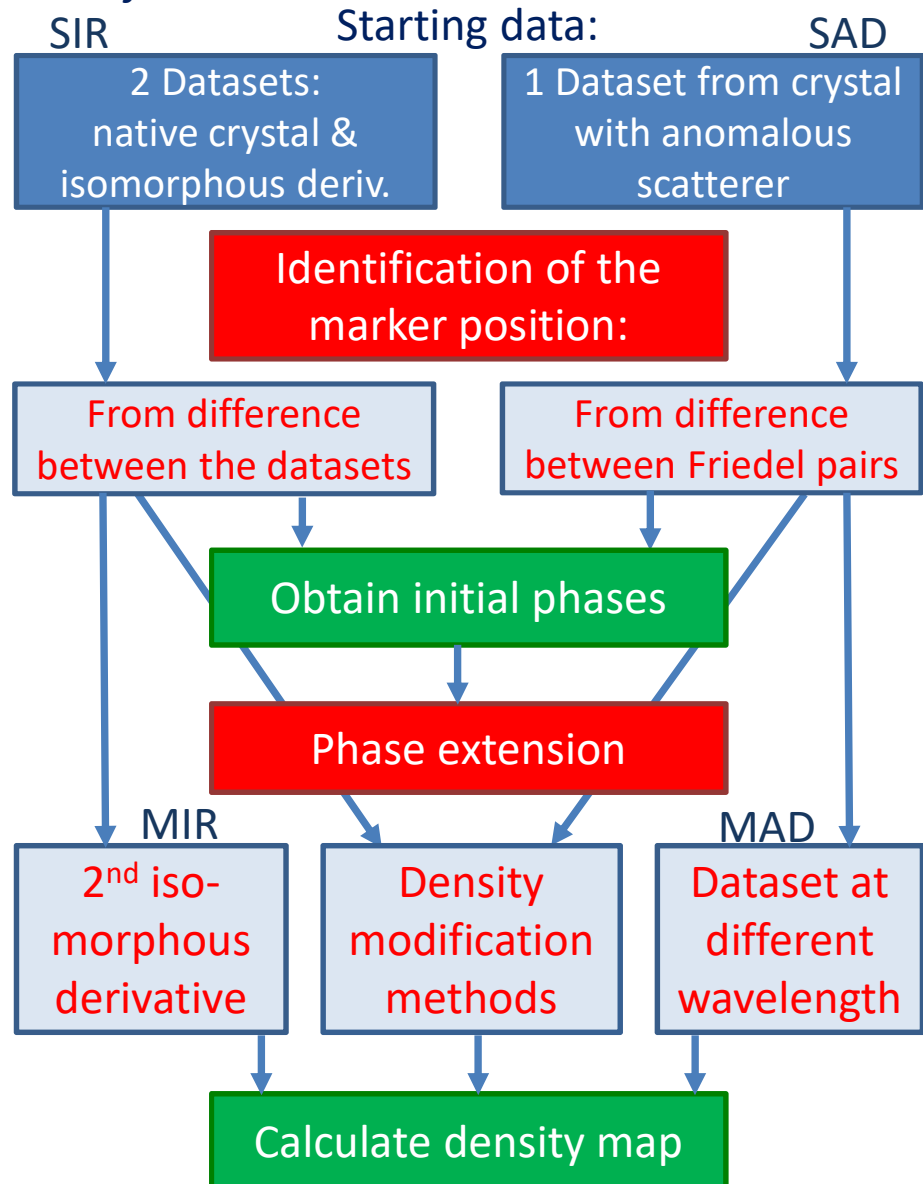
Marker atom substructure



Marker atom = an atom that yields a significant difference in structure factors:

- high number of electrons (heavy atom)
- anomalous scattering

Workflow:



Data preparation

Single/Multiple Isomorphous Replacement (SIR/MIR)

Data from a native crystal and one or more **isomorphous** crystals containing heavy atoms.

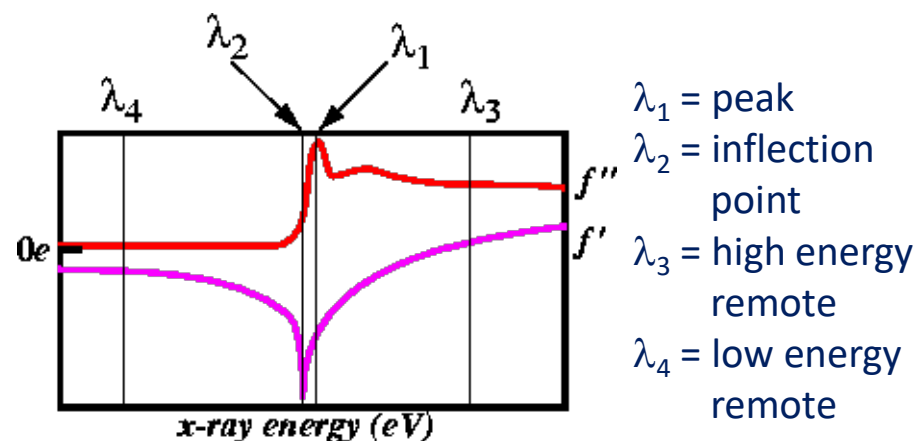
- **Scale together** the data of native ($I_P \propto F_P^2$) and heavy atom-derivative(s) ($I_{PH} \propto F_{PH}^2$)
- Outlayer removal: remove (or downweight) reflections significantly affected by heavy atom presence (only for initial scaling!)
- Remove high resolution reflections (only for substructure solution!)

$$R_{\Delta} = 2 \frac{\sum_h |I_{P,h} - I_{PH,h}|}{\sum_h |I_{P,h} + I_{PH,h}|}$$

For good data: $10\% < R_{\Delta} < 30\%$

Single-/Multiple-wavelength Anomalous Diffraction (SAD/MAD)

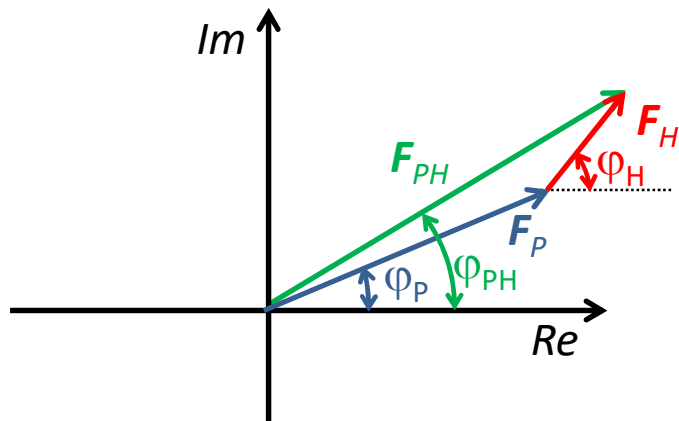
Data from the same crystal at one or more wavelengths around the absorption edge:



- Scale together, but **keep Friedel pairs separate** ($I_+ \propto F_+^2$ and $I_- \propto F_-^2$), merge together Bijvoet mates

$$R_{\Delta} = 2 \frac{\sum_h |I_{+,h} - I_{-,h}|}{\sum_h |I_{+,h} + I_{-,h}|}$$

Marker atom



When **isomorphous replacement** data are available:

Structure factors for native data:

$$\mathbf{F}_P = \sum_{j=1}^{N_P} f_j^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j)$$

Structure factors for heavy atom derivative (with N_H heavy atoms per unit cell):

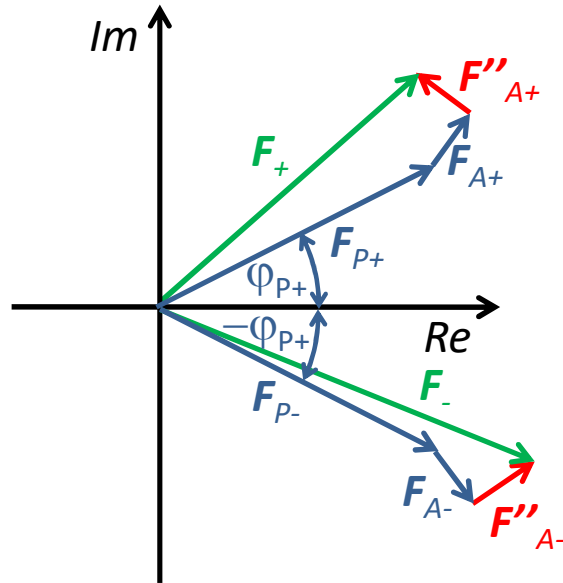
$$\begin{aligned} \mathbf{F}_{PH} &= \sum_{j=1}^{N_P+N_H} f_j^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j) = \\ &= \sum_{j=1}^{N_P} f_j^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j) + \sum_{j=1}^{N_H} f_j^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j) \end{aligned}$$

If the crystals are isomorphous:

$$\mathbf{F}_{PH} = \mathbf{F}_P + \mathbf{F}_H$$

Unfortunately, neither moduli F_P , F_H and F_{PH} ,
nor phases φ_P , φ_H and φ_{PH} of the structure factors
are additive.

Marker atom



For an anomalous scatterer H :

$$\mathbf{F}_+ = \sum_{j=1}^{N_P} f_j^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j) + \sum_{j=1}^{N_H} f_H^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j)$$

$$\text{with } f_H^B = f(\vartheta) + f'(\lambda) + if''(\lambda)$$

$$\mathbf{F}_+ = \mathbf{F}_{P+} + \mathbf{F}_{A+} + \mathbf{F}''_{A+}$$

$$\text{with } \varphi''_{A+} = \varphi_{A+} + \frac{\pi}{2}$$

$$\mathbf{F}_- = \mathbf{F}_{P-} + \mathbf{F}_{A-} + \mathbf{F}''_{A-}$$

$$\varphi_{P-} = -\varphi_{P+}, \quad \varphi_{A-} = -\varphi_{A+},$$

$$\text{but } \varphi'_{A-} = \varphi_{A-} + \frac{\pi}{2} = -\varphi_{A+} + \frac{\pi}{2}$$

Patterson method for substructure solution

Difference Patterson map:

$$FT[I_{PH} - I_P] = FT[F_{PH}^2 - F_P^2] = FT[F_H^2] + noise$$

Isomorphous difference Patterson map:

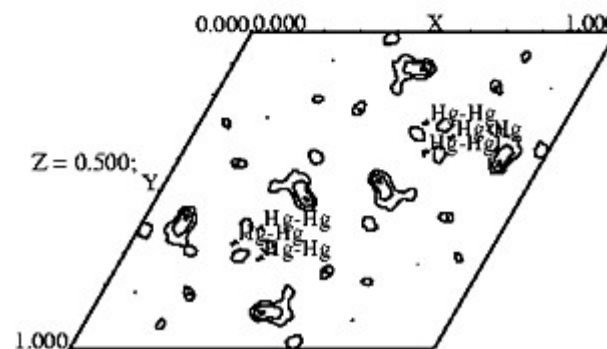
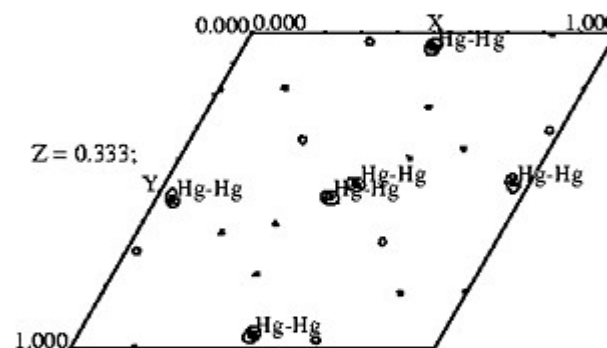
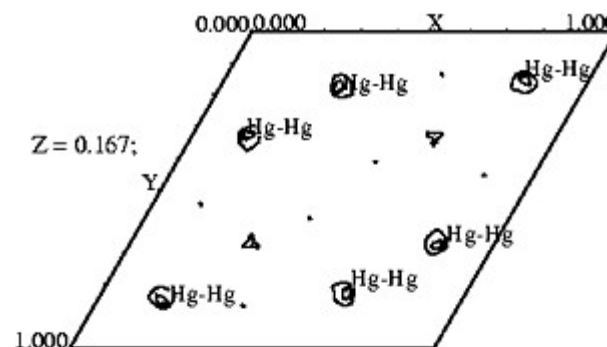
$$FT[(F_{PH} - F_P)^2] = FT[F_H^2]$$

Anomalous difference Patterson map:

$$FT[(F_+ - F_-)^2] = FT[F_A^2]$$

The main contribution to the difference Patterson maps will be the contribution of the heavy atoms (or anomalous scatterers).

If the crystal contains N_H heavy atoms (or N_A anomalous scatterers), the difference maps are expected to contain $N_H(N_H - 1)/2$ independent peaks corresponding to heavy atom-heavy atom interatomic vectors (Patterson maps are centrosymmetric).



Considering the position of the peaks of the Patterson map and the symmetry operators of the space group, the position of the heavy atoms/anomalous scatterers can be obtained. Vectors between symmetry related heavy atoms have specific coordinates in the Patterson map.

For example:

Space group

$P3_121$

General position (x_H, y_H, z_H) [1]

Equivalent positions generated by 3_1 : $(-y_H, x_H - y_H, z_H + 1/3)$ [2]

$(y_H - x_H, -x_H, z_H + 2/3)$ [3]

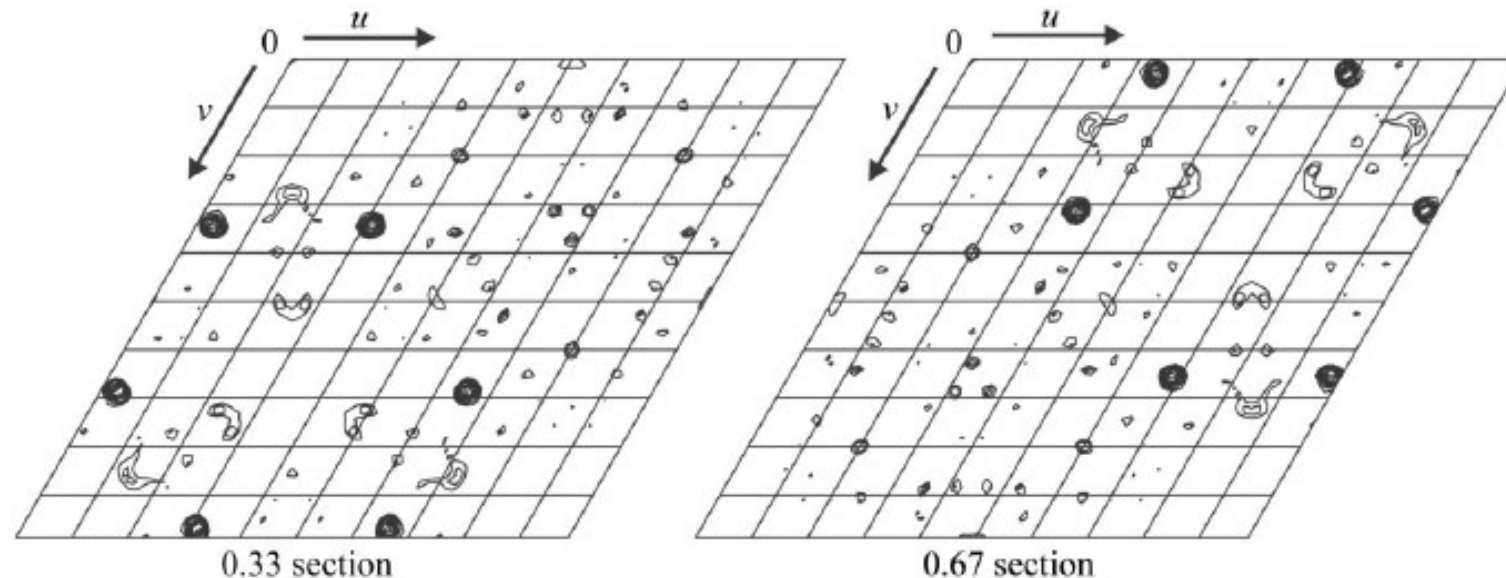
Difference vectors:

[2-1] $[-x_H - y_H, x_H - 2y_H, 1/3]$

[3-1] $[y_H - 2x_H, -x_H - y_H, 2/3]$

[3-2] $[2y_H - x_H, y_H - 2x_H, 1/3]$

Difference
Patterson
sections at
 $z=1/3$ and
 $z=2/3$,
respectively
(Harker
sections)



Single Isomorphous Replacement (SIR)

Diffraction experiments on native crystal and heavy atom derivative

Patterson solution or direct methods solution: position of the heavy atom

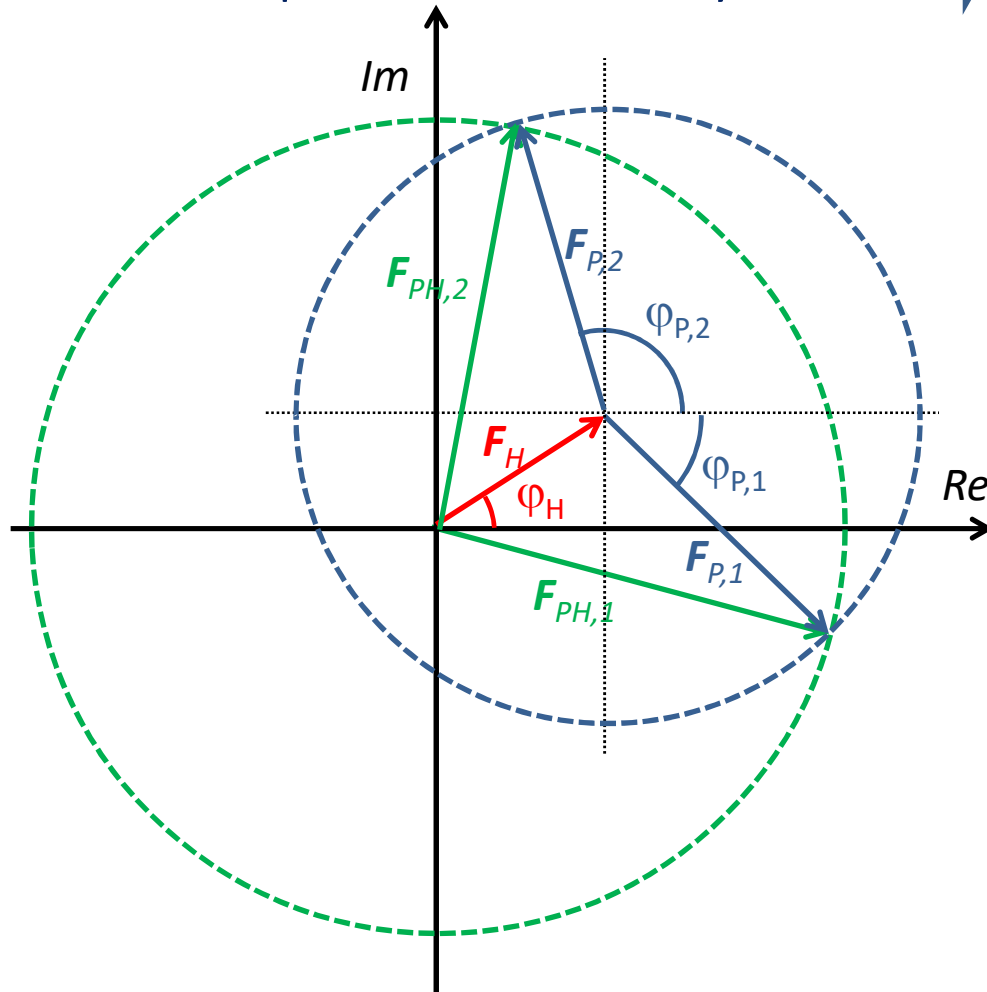


F_P, F_{PH}

$$F_{PH} = F_P + F_H$$



F_H, φ_H



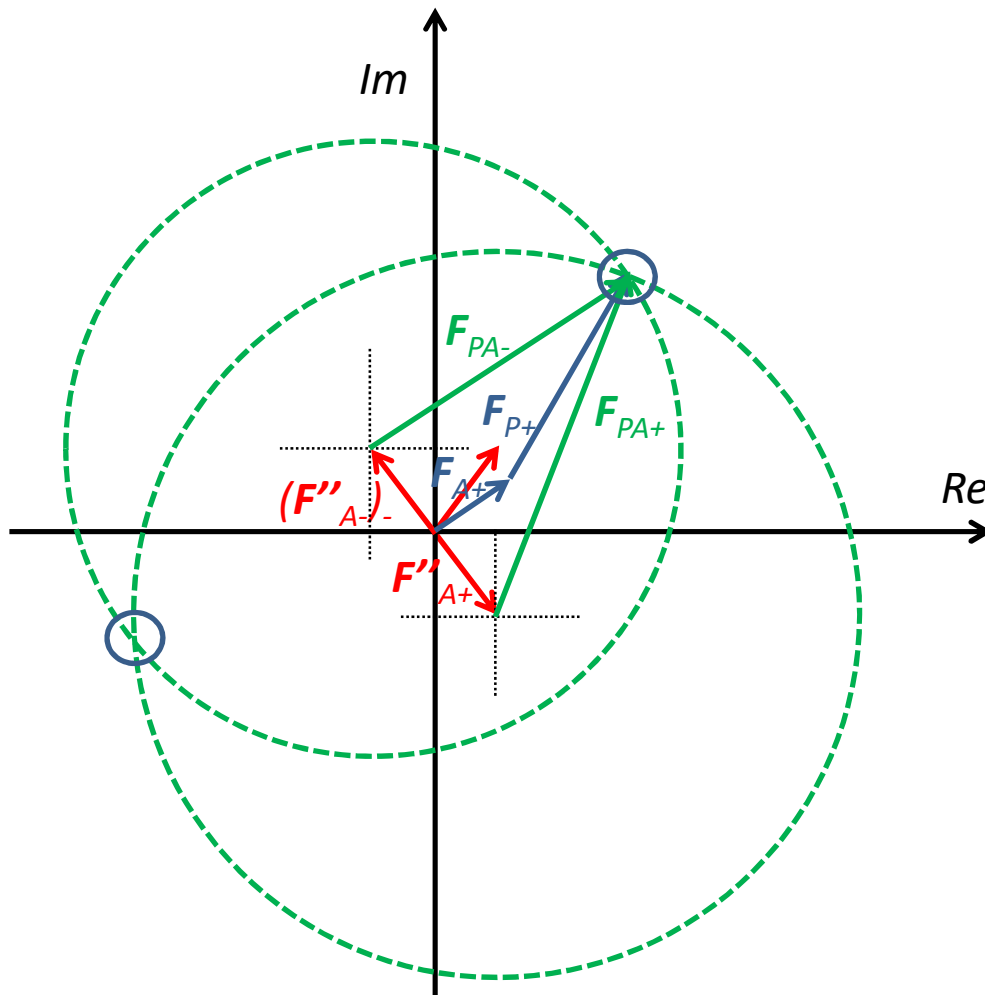
Graphical solution of SIR:

1. Draw vector F_H
2. Phase of vector F_{PH} is unknown, but it must lie on a circle of radius $|F_{PH}|$
3. Phase of vector F_P is unknown, but it must lie on a circle of radius $|F_P|$; the center of the circle is positioned at end of F_H
4. Two intersections allow to obtain two possible solutions (only one is correct!)
5. More information is required to select correct solution

Single-wavelength Anomalous Diffraction (SAD)

Diffraction experiment $\Rightarrow F_{PA+}, F_{PA}$

Patterson solution or direct methods solution:
position of the anomalous scatterer $\Rightarrow F_A, \varphi_A, F''_A, \varphi''_A$



Graphical solution of SAD:

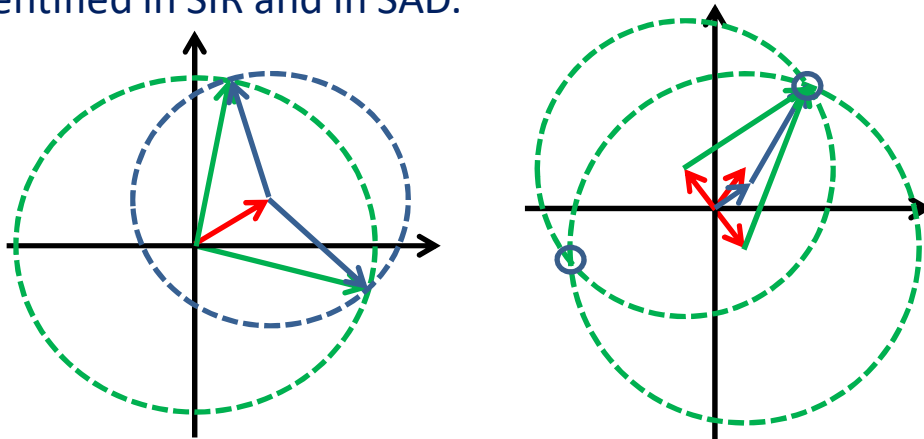
1. Draw vector F''_{A+} , ending on the origin
2. Phase of vector F_{PA+} is unknown, but it must lie on a circle of radius $|F_{PA}|$, centered on the start of F''_{A+}
3. Considering that F_{P-} has an inverted phase compared to F_{P+} , the phase of F''_{A-} is inverted and moved similarly to F''_{A+}
4. Phase of vector F_{PA-} is unknown, but it must lie on a circle of radius $|F_{PA}|$, centered on the start of $(F''_{A-})_{-}$
5. Vector F_{A+} is known
6. Two possible solutions (only one is correct!) for vector F_{P+}

Multiple possible solutions, only one correct!

Space group ambiguity: for space groups of enantiomorphic pairs.

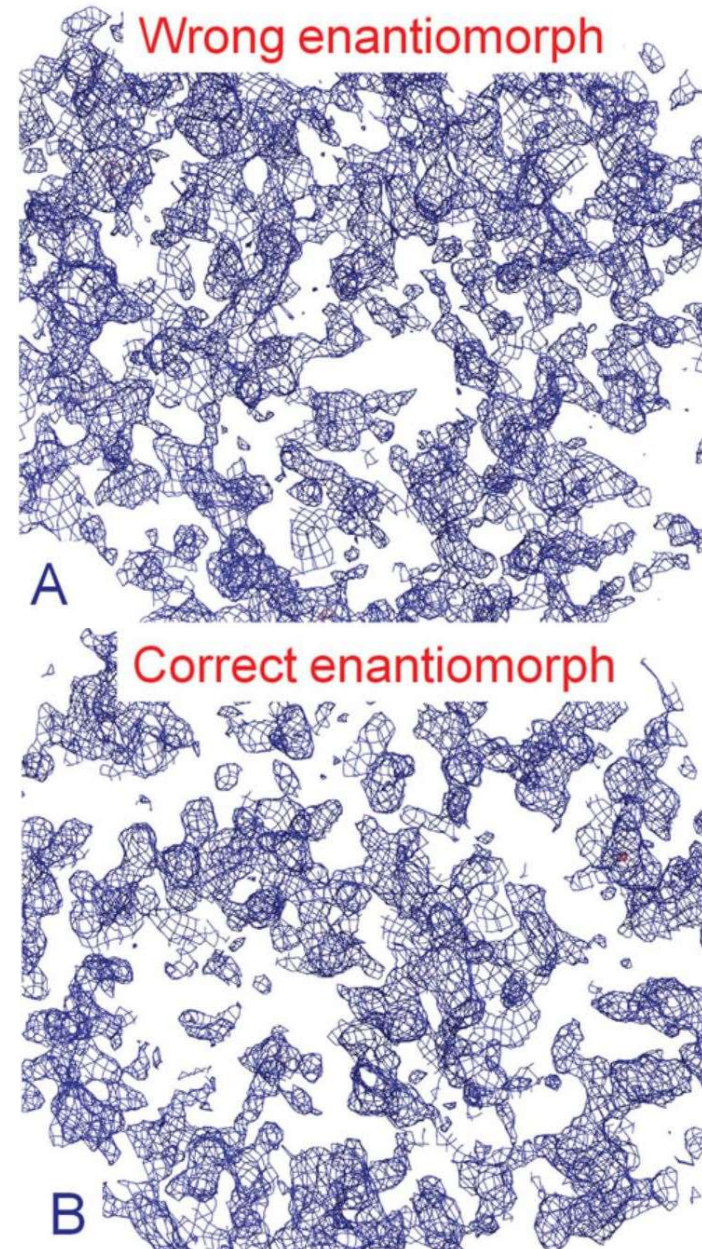
Enantiomorph ambiguity: since the reciprocal space is centrosymmetric, 2 centrosymmetric positions of the marker atom are possible. Only testing both solutions can clarify the ambiguity.

Phase ambiguity: two possible phase solutions are identified in SIR and in SAD.

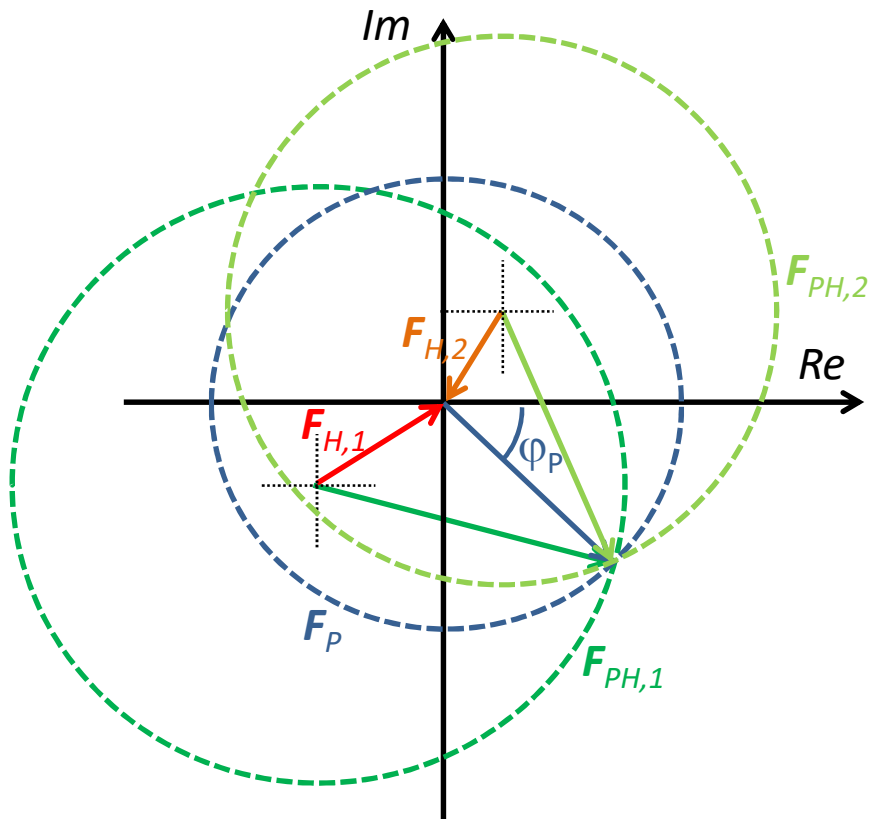


Phase ambiguity can be solved with multiple isomorphous crystals (MIR), with data collections at different λ (MAD), by combining information from SAD and SIR (SIRAS).

Also: use of density modification techniques to solve phase ambiguity.



Multiple Isomorphous Replacement (MIR)



Data from the second derivative, containing a different heavy atom, can solve the phase ambiguity.

But crystals must be ISOMORPHOUS!!

Multiple-wavelength Anomalous Diffraction (MAD)

$$\mathbf{F}_+ = \mathbf{F}_{P+} + \sum_{j=1}^{N_H} f_H^B \exp(2\pi i \mathbf{h} \cdot \mathbf{x}_j)$$

$$\text{with } f_H^B = f(\vartheta) + f'(\lambda) + if''(\lambda)$$

For the data collection at wavelength λ_1 :

$$\mathbf{F}_+(\lambda_1) = \mathbf{F}_{P+} + \mathbf{F}_{H+} + \mathbf{F}'_{H+}(\lambda_1) + \mathbf{F}''_{H+}(\lambda_1)$$

$$\mathbf{F}_-(\lambda_1) = \mathbf{F}_{P-} + \mathbf{F}_{H-} + \mathbf{F}'_{H-}(\lambda_1) + \mathbf{F}''_{H-}(\lambda_1)$$

For the data collection at wavelength λ_2 :

$$\mathbf{F}_+(\lambda_2) = \mathbf{F}_{P+} + \mathbf{F}_{H+} + \mathbf{F}'_{H+}(\lambda_2) + \mathbf{F}''_{H+}(\lambda_2)$$

$$\mathbf{F}_-(\lambda_2) = \mathbf{F}_{P-} + \mathbf{F}_{H-} + \mathbf{F}'_{H-}(\lambda_2) + \mathbf{F}''_{H-}(\lambda_2)$$

The terms \mathbf{F}_{P+} , \mathbf{F}_{H+} , \mathbf{F}_{P-} and \mathbf{F}_{H-} are independent from the wavelength and, therefore, have the same value of both phase and module in the 2 datasets.

The same relations seen for SAD phasing apply to wavelength dependent terms

\mathbf{F}'_{H+} , \mathbf{F}''_{H+} , \mathbf{F}'_{H-} , and \mathbf{F}''_{H-} .

Electron density maps

To calculate electron density map: real space discretized in voxels (the smallest portion of volume in the real space).

A density value is calculated for each voxel and the map is represented as a contour level.

Electron density map: observed moduli ($F_{h,obs}$) and calculated phases ($\varphi_{h,calc}$).

$$\rho(\mathbf{r}) = \frac{1}{V} \sum_h F_{h,obs} \exp(i\varphi_{h,calc})$$

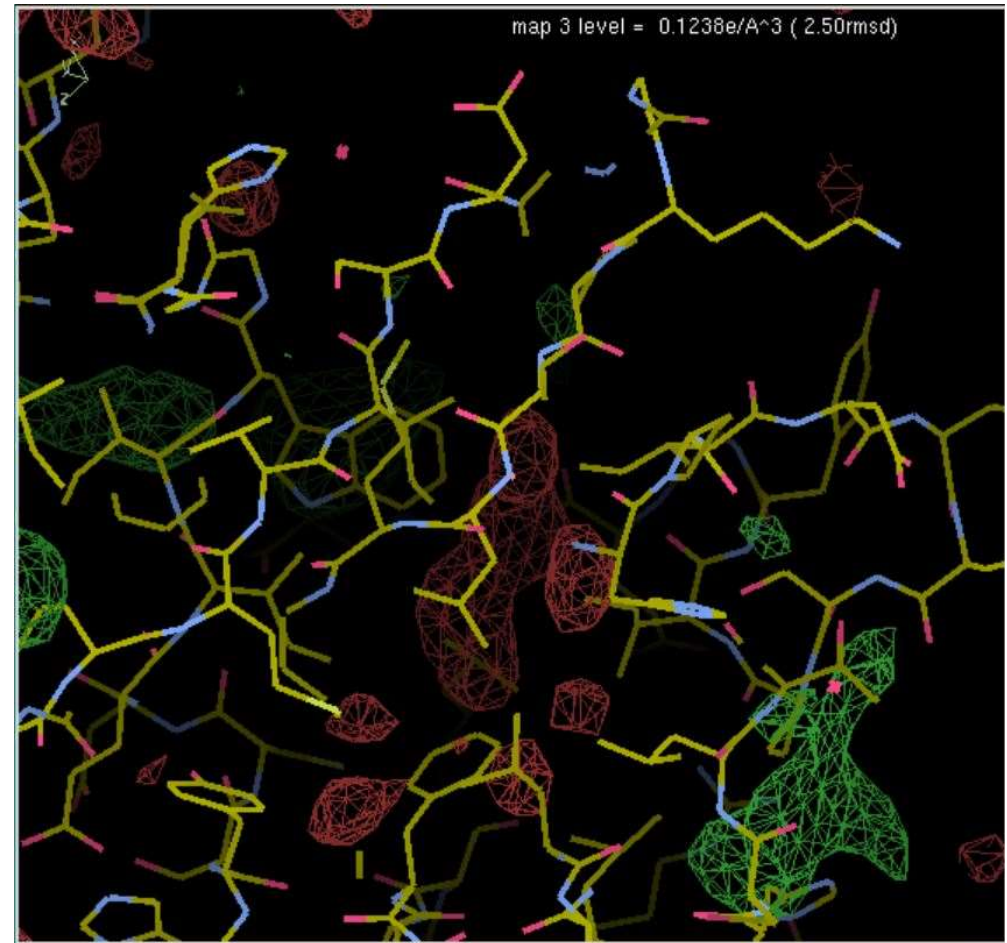
2Fo-Fc map: to reduce bias of the model, a different calculation of the electron density is usually performed.

$$\rho(\mathbf{r}) = \frac{1}{V} \sum_h (2F_{h,obs} - F_{h,calc}) \exp(i\varphi_{h,calc})$$

Difference Fo-Fc map: highlights difference between data and model (green map

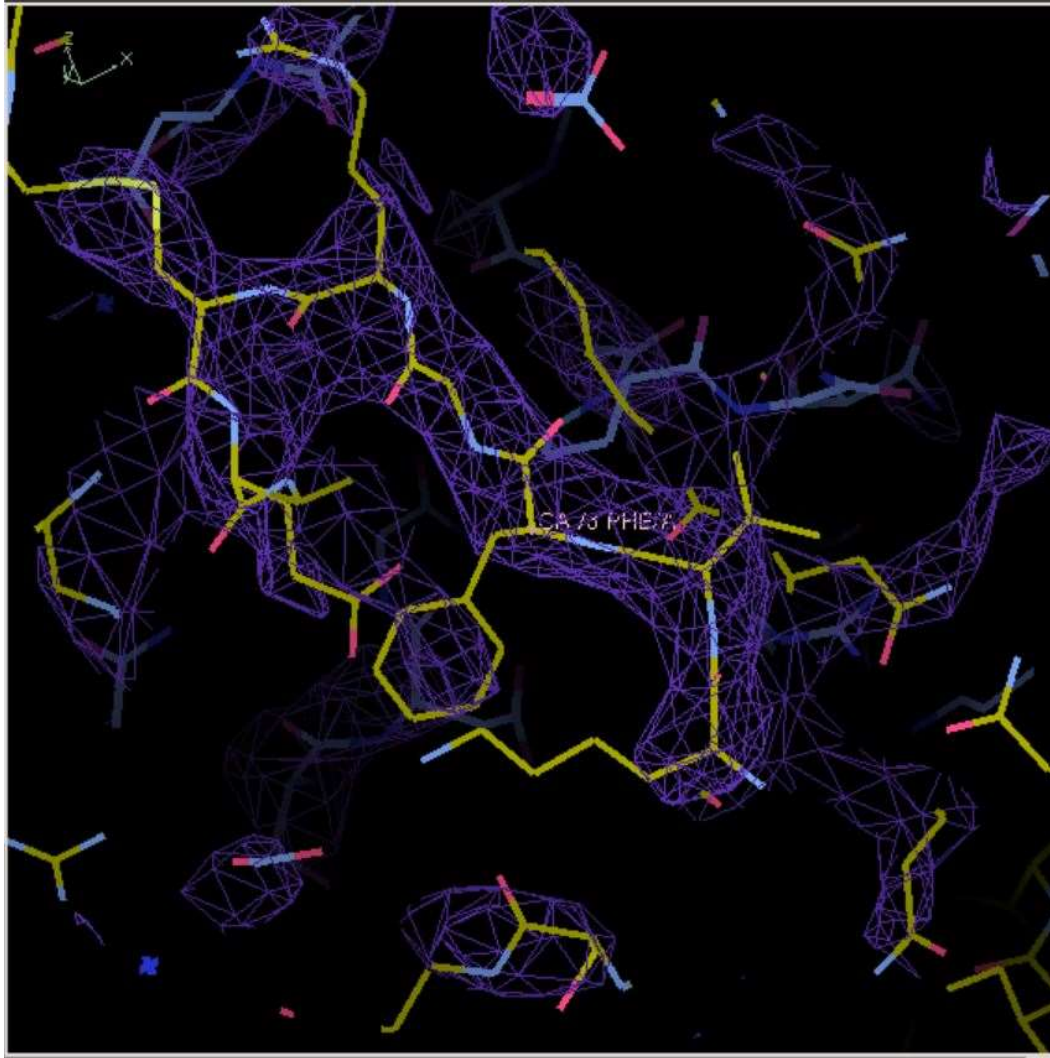
$\rho_{model} > \rho_{data}$; red map $\rho_{model} < \rho_{data}$)

$$\rho(\mathbf{r}) = \frac{1}{V} \sum_h (F_{h,obs} - F_{h,calc}) \exp(i\varphi_{h,calc})$$



Resolution and structural details

What details are visible at a defined resolution?



1 Å resolution → single atoms are visible

1.5 Å resolution → side chain groups clear, but not atoms

2 Å resolution → residue side chains can be distinguished

4 Å resolution → main chain still visible, but few side chains can be traced