Purified Protein → Solve Phase

Build model and refine

Crystal lattice

“Real Space”

Reflections

“Reciprocal Space”

\[ \rho(x, y, z) \quad \text{pronounced rho} \]

\[ |F_{hkl}|^2 \propto I \]

\[ \rho_{(x,y,z)} = \frac{1}{V} \sum_h \sum_k \sum_l |F_{(h,k,l)}| \exp[-2\pi i (hx + ky + lz - \alpha_{(h,k,l)})] \]
Each reflection defines a set of parallel planes that slice through the crystal.

Reflections are the result of constructive interference off a set of parallel planes.

One dimensional waves

\[ f(x) = F \cos 2\pi (hx + \alpha) \]

The frequency term designates one of several Fourier terms.
**Fourier Series**

\[ f(x) = F_0 \cos 2\pi (0x + \alpha_0) + F_1 \cos 2\pi (1x + \alpha_1) + F_2 \cos 2\pi (2x + \alpha_2) + \ldots + F_n \cos 2\pi (nx + \alpha_n), \]

so that:

\[ f(x) = \sum_{n=0}^\infty F_n \cos 2\pi (nx + \alpha_n). \]

**So what does it do for you?**

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**Closer to Crystallography**

\[ f(x) = \sum_{n=0}^n F_n [\cos 2\pi (hx) + i \sin 2\pi (hx)] \]

Setting \( \theta = 2\pi (hx) \)

and using:

\[ \cos \theta + i \sin \theta = e^{i\theta} \]

The really scary equation on top is a little less scary:

\[ f(x) = \sum_{n=0}^n F_n e^{2\pi i (hx)} \]

**Complex number**

\[ a + ib \]

\[ \begin{array}{c}
\text{real} \\
\text{imaginary}
\end{array} \]

\[ i = (-1)^{1/2} \]
Let’s Move to a 3-Dimensional Wave

\[ f(x, y, z) = \sum_{h} \sum_{k} \sum_{l} F_{hkl} e^{2\pi i(hx + ky + lz)} \]

real space \( x = \text{Å} \)

reciprocal space \( h = \text{Å}^{-1} \)

fourier transform or FT

\[ F(h) = \int_{-\infty}^{\infty} f(x) e^{2\pi i(hx)} dx \]

\[ f(x) = \int_{-\infty}^{\infty} F(h) e^{-2\pi i(hx)} dh \]

\[ F_{hkl} \text{ is a Complex Function} \]

Decompose \( F_{hkl} \)

\[ \rho(x, y, z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} |F_{hkl}| e^{-2\pi i(hx + ky + lz)} \]

\[ |F_{hkl}| = (I_{hkl})^{1/2} \]

phase angle \( \alpha_{hkl} \)
$F_{hkl}$ is a complex function - continued

imaginary

\[ i \ b \]

real

\[ a \]

$N = a + ib$

imaginary

\[ F_{hkl} \]

real

angle is the phase \( \alpha_{hkl} \)

Vector length

$|F_{hkl}| = (I_{hkl})^{1/2}$

Fourier Terms Add Up to Give Structure Factor $F_{hkl}$

$$F_{hkl} = \sum_{j=1}^{n} f_j e^{2\pi i(hx_j + ky_j + lz_j)}$$

Assume we have a structure with only 3-atoms in unit cell, so $n=3$
isomorphous replacement gives $F_{hkl}$

$F_P$ - protein

$F_{PH}$ – derivatized protein + heavy atom

$F_H$ – heavy atom alone

$|F_H|$ from simple subtraction

$\alpha$ – for $F_H$ solved by direct methods – computational or

Patterson Function $(|F_{PH}| - |F_P|)^2$

(page 124-128 of CMCC)

Introduce a heavy atom into protein crystal

$[PH] - [P] = [H]$
Now a graphical picture of how MIR works

1) First draw a circle of radius $|F_P|$

2) Then place the $F_{H1}$ vector and draw circle from its tip of radius $|F_{PH1}|$

3) Now place $F_{H2}$ from second derivative

and draw a circle from tip of $F_{H2}$ with a radius of $|F_{PH2}|$
Either deriv. alone does not determine phase.

Only both derivatives together solve phase.

**The Phase Problem is Solved**

**Methods to Obtain Phase**

0. Multiple Isomorphous Replacement (MIR) _i.e._ Hg compound binding Cys residue

0. Molecular Replacement (MR) – use a homologous protein, (>50% identity)

0. Multi-wavelength Anomalous Dispersion (MAD phasing) – the fastest way to solve a structure due to better quality initial phases
$|F_{hkl}| = (I_{hkl})^{1/2}$

Decompose $F_{hkl}$

$$\rho(x,y,z) = \frac{1}{V} \sum_h \sum_k \sum_l |F_{hkl}| \ e^{-2\pi i (hx + ky + lz - \alpha_{hkl})}$$

real space $x = \text{Å}$

reciprocal space $h = \text{Å}^{-1}$

**Molecular Replacement**

Molecular Replacement (MR) – another method to estimate phases
– use a structurally homologous protein
  - >25% sequence identity is sometimes possible
  - >50% sequence identity is a safe bet

1) Make search model
   - find structural model of sequence homolog
   - from sequence alignment and homolog structure, create model
   - mutate or trim down to what the two proteins have in common
   - energy minimize to eliminate bad geometry (intro to refinement)

2) Search model defines electron density – “FT the electron density”
Molecular Replacement - continued

3) Search model defines electron density – “FT the electron density”

\[ F_{hkl} = \iiint \rho(x, y, z) e^{2\pi i (hx + ky + lz)} \, dx \, dy \, dz \]

theoretical reciprocal space

\[ |F_{\text{calc}}| \rightarrow |F_{\text{obs}}| \]
\[ \alpha_{\text{calc}} \]

4) Rotation and Translation MR Search of \(|F_{\text{calc}}|\) into x-ray data set (\(|F_{\text{obs}}|\))

5) Once rotation and translation alignment is found, use this matrix to apply \(\alpha_{\text{calc}}\) to measured x-ray data set (\(|F_{\text{obs}}|\)).