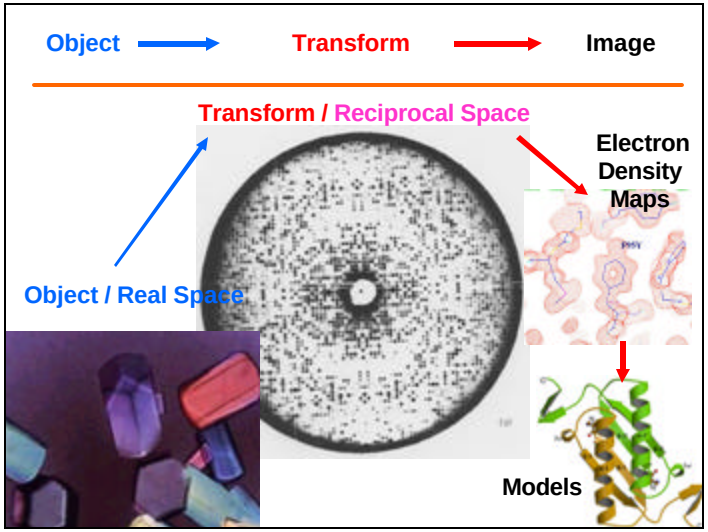


# X-Ray Crystallography

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**Topics:**

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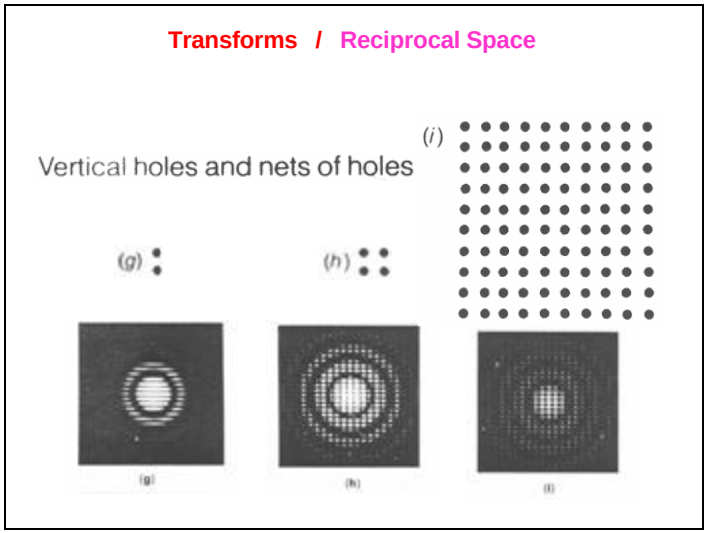
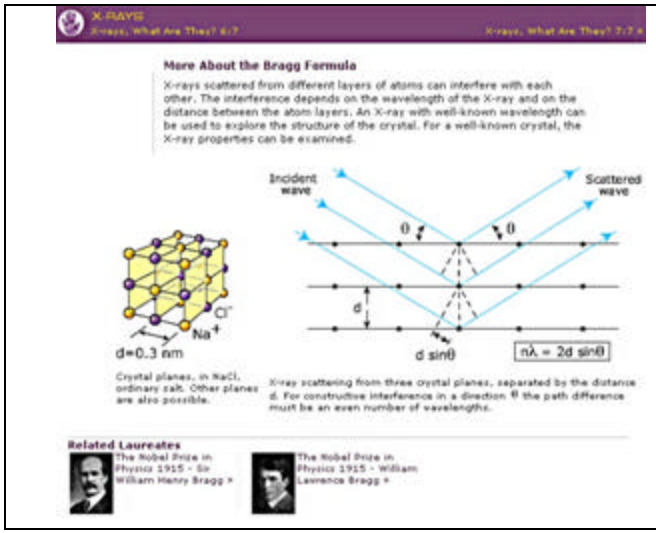


## Diffraction: Scattering from (two) "atoms"

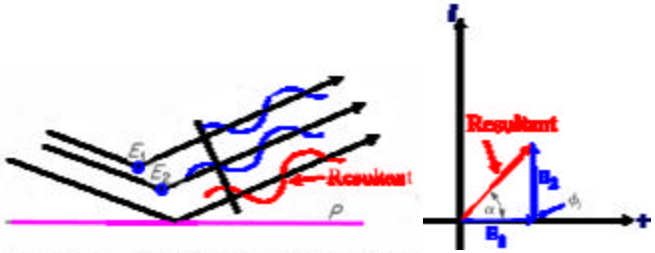



**Figure 2.10.** Diffraction from $E_1$ and $E_2$ as if reflected from plane $P$.

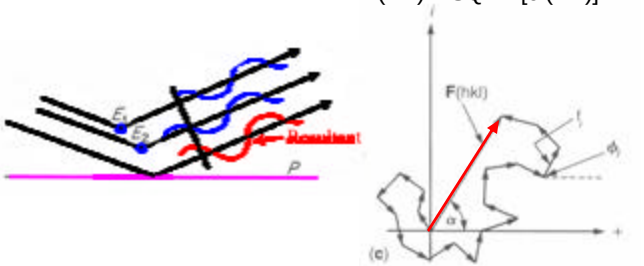
## Scattering from "many atoms"

$$\mathbf{F}(hkl) = F(hkl)e^{i\alpha(hkl)} = \sum_{j=1}^{N} \mathbf{f}_j(hkl) = \sum_{j=1}^{N} f_j(hkl)e^{i\phi_j(hkl)}$$

Calculated

Experimental

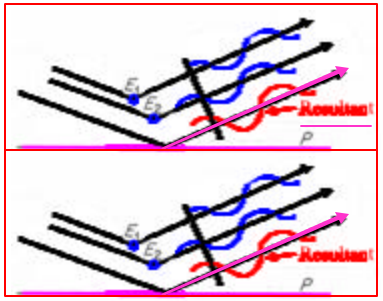
F(*hkl*) = SQRT [cI(*hkl*)]




The structure factor for a reflection may be thought of as the vector sum of the x-ray scattering contributions from many atoms.

Each of the j contributions may be represented as a vector in the complex plane, with amplitude $f_j$ and phase $phi_j$.

## Scattering from "atoms in two unit cells"

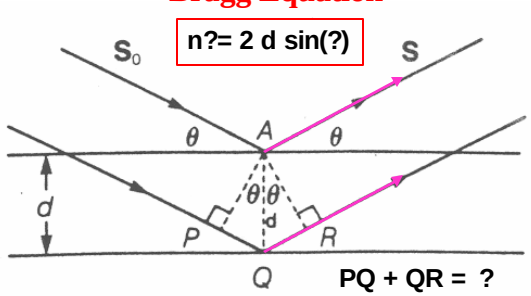



## Crystals: Scattering from "planes"

Resultant scattering of resultant scattering!

### Bragg Equation

$n?= 2\ d\ \sin(?)$




PQ + QR = ?

→ **Scattering** will only be **"observed"** at discrete **Bragg angles** (q )

**The spacings of the Bragg reflections → Lattice Constants**

## Bragg Planes

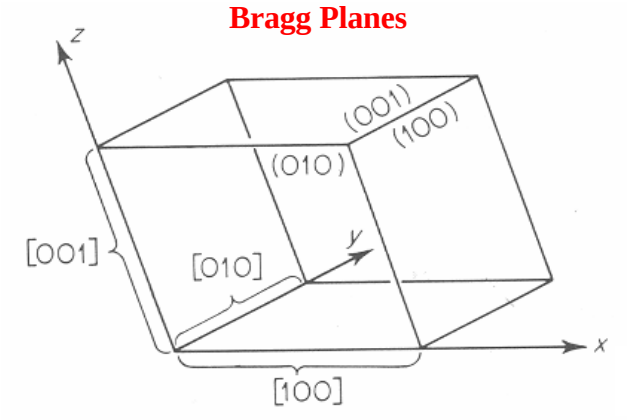



Figure 2.7. Unit cell showing bounding planes and edges.

## Name that Bragg "plane"

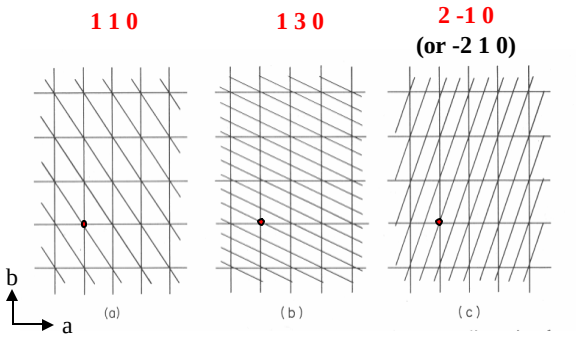
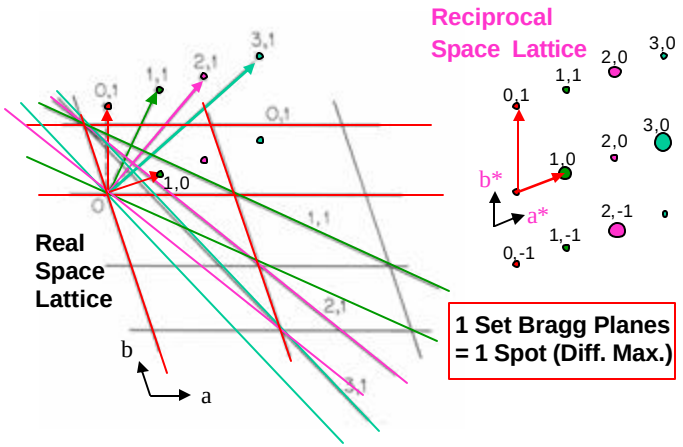



Figure 2.5. Three families of lattice "planes" in a two-dimensional lattice.

## Reciprocal Space Lattice

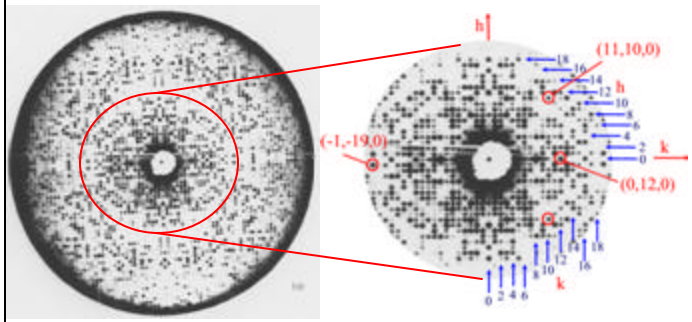



**1 Set Bragg Planes = 1 Spot (Diff. Max.)**

Figure 2.11. Planes in direct space represented by points in reciprocal space.

## Electron Density Function

$$\rho(X,Y,Z) = \frac{1}{V}\sum_h\sum_k\sum_l F(hkl)\exp[i\alpha(hkl)]\exp[-2\pi i(hX + kY + lZ)]$$




Measure thousands of **Amplitudes** - [$F_{hkl}$]'s - ?? How do we obtain **Phases** $\alpha_{hkl}$ ??

→ **Phase Problem**

# X-Ray Crystallography

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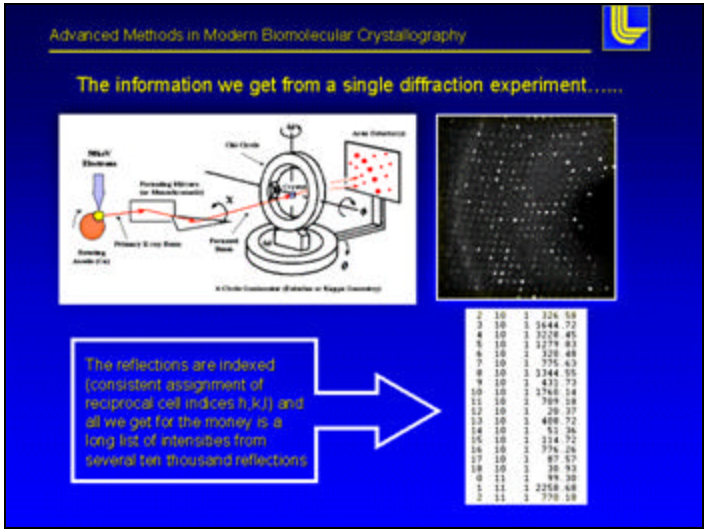
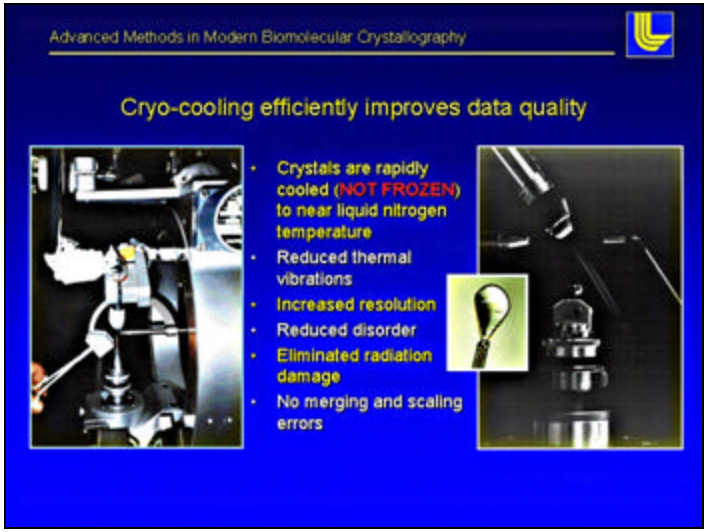
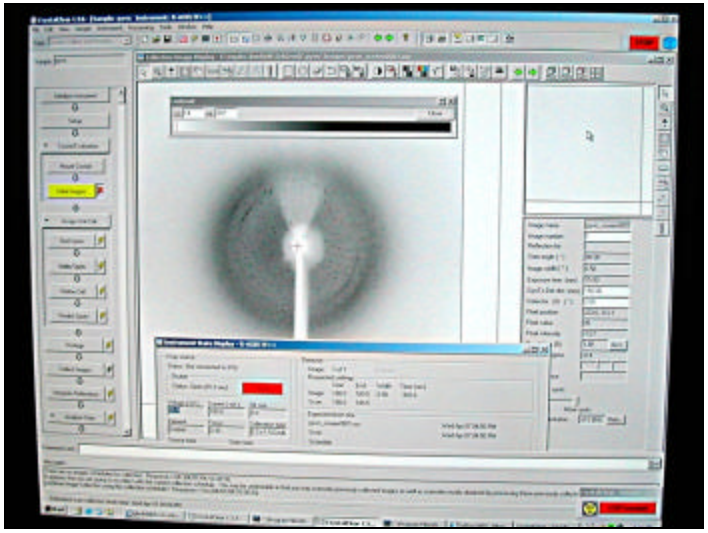
   **f) Structure Solution – Phase Problem: MIR / MR / MAD**

   **h) Refinement, Analysis and Presentation of Results**

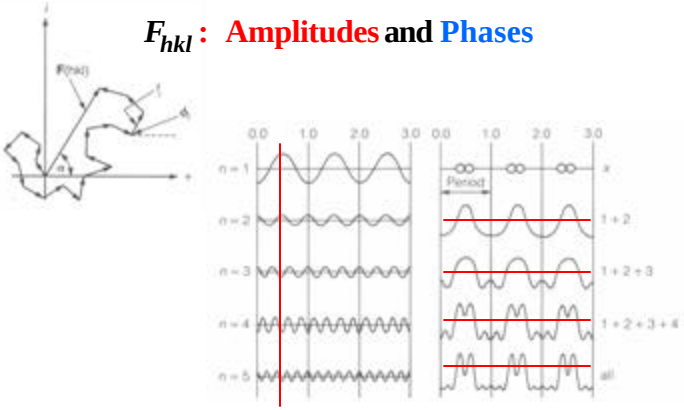
   **i) Use of Difference Fouriers**



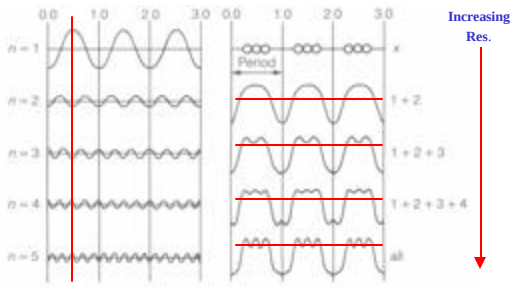


## $F_{hkl}$ : Amplitudes and Phases

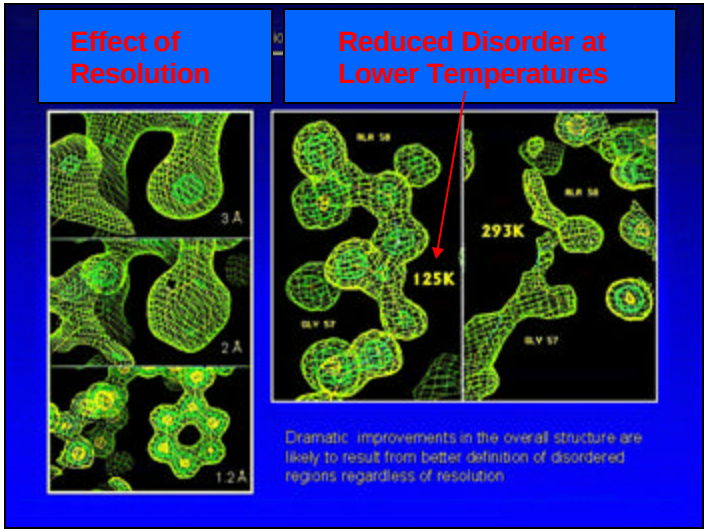



Representation of the electron density of a one-dimensional "crystal" by a superposition of waves. The crystal is formed by a periodic repetition of a diatomic molecule, as shown at the top of the right-hand column. The component waves, each with proper phase and amplitude, are on the left. The curves on the right show the successive superposition of the five waves on the left. (From Waser, 1968.)

## $F_{hkl}$ : Amplitudes and Phases




Representation of another one-dimensional crystal, this one containing a triatomic molecule. Note that this crystal is built up from the same waves as the crystal of (a) ; only the amplitudes and phases have been changed. (From Waser, 1968.)



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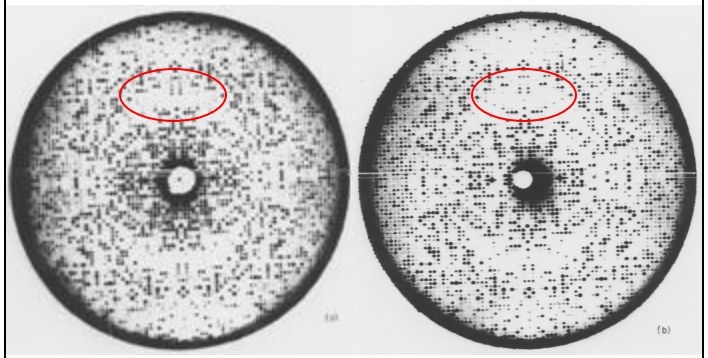
## Solving the Phase Problem

1. MIR: Multiple Isomorphous Replacement (Heavy Atom)
2. MR: Molecular Replacement
3. MAD: multiwavelength anomolous dispersion

**********

- Molecular Modeling (predicting starting structure from sequence alone)

## Use of Heavy Metal Ions for Phasing by MIR Methods

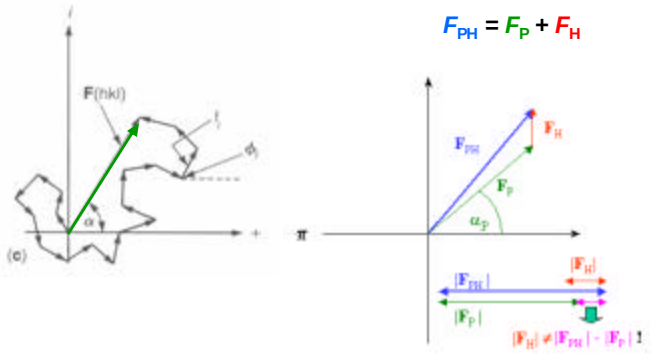


Native Phosphorylase

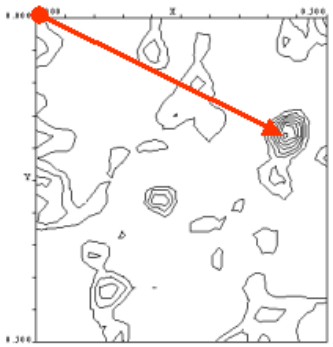
Phosphorylase + Ethyl Hg thiosalicylate

## Effect of adding 1 "heavy" atom with lots of electrons!

$F_{PH} = F_P + F_H$

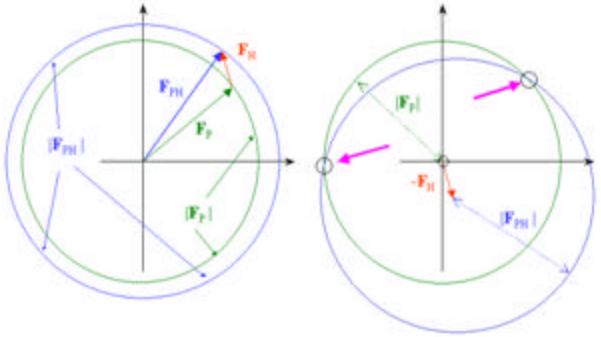


## Difference Patterson Map $(F_{hkl})^2$



## Multiple Isomorphous Replacement (MIR) method

$F_{PH} = F_P + F_H$ $\quad F_P = F_{PH} - F_H$



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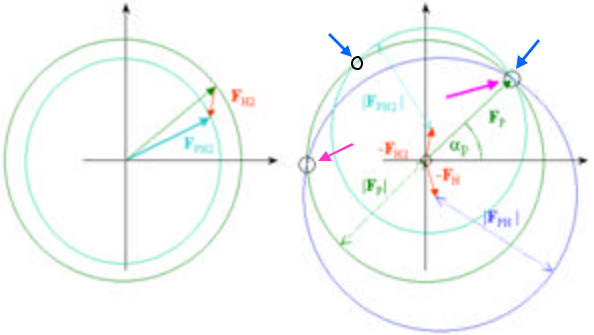
$F_P = F_{PH} - F_H$



Solving the phase problem by "**Molecular Replacement**".

If an approximate model of the protein structure is known in advance, approximate phases can be guessed, and the unknown parts of the structure can be calculated in an iterative procedure.

No heavy atom derivative required.

**BUT – need starting model and orientation (rotation and translation)**

For example, molecular replacement can be used to determine the structure of an complex with inhibitor bound to an enzyme active site, if the structure of the enzyme itself is already known. Also, MR is often used to solve the structures of closely related proteins in a superfamily.

## "Multiwavelength Anomolous Dispersion"

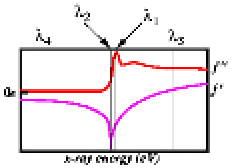
## (MAD) methods

Additional information used in calculating phases can be obtained if x-ray diffraction intensities can be measured at wavelengths near the absorption edge of the heavy atom derivative.

A tunable x-ray source is required (provided by a synchrotron). In a synchrotron, accelerated electrons traveling near the speed of light emit intense x-rays.

a) often only a single heavy atom derivative is required to solve a structure (selenomethionine).

b) it is possible to solve structure of higher molecular weight molecules (such as the ribosome, at MW = 2,500,000).



## What should they be?

- The largest signal will come from choosing the wavelength with maximal f" ($?_1$ in the figure above).
- The second wavelength is usually chosen to have maximal |f`| ($?_2$ in the figure above). Note that (1 and 2) are very close together, requiring great precision in setting up the apparatus which controls wavelength during data collection.
- Additional wavelengths (3 and 4) are chosen at points remote from the absorption edge. The available signal increasing slowly as the distance from the first two wavelengths increases. However the diffraction conditions (crystal absorption and diffracting power, diffraction geometry, etc) become more disparate as the distance increases. The choice usually comes down to the practical limitations imposed by the particular beamline apparatus being used. Typically $?_3$ and $?_4$ are between 100eV and 1000eV from the absorption edge.

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## Least-Squares Refinement

$$\sum_{r=1}^{m} w_r \left(\frac{\partial |kF_{c,r}|}{\partial p_1}\right)^2 \Delta p_1 + \sum_{r=1}^{m} w_r \frac{\partial |kF_{c,r}|}{\partial p_1}\frac{\partial |kF_{c,r}|}{\partial p_2} \Delta p_2 + \cdots + \sum_{r=1}^{m} w_r \frac{\partial |kF_{c,r}|}{\partial p_1}\frac{\partial |kF_{c,r}|}{\partial p_n} \Delta p_n = \sum_{r=1}^{m} w_r \Delta F_r \frac{\partial |kF_{c,r}|}{\partial p_1}$$

$$\sum_{r=1}^{m} w_r \frac{\partial |kF_{c,r}|}{\partial p_2}\frac{\partial |kF_{c,r}|}{\partial p_1} \Delta p_1 + \sum_{r=1}^{m} \left(\frac{\partial |kF_{c,r}|}{\partial p_2}\right)^2 \Delta p_2 + \cdots + \sum_{r=1}^{m} w_r \frac{\partial |kF_{c,r}|}{\partial p_2}\frac{\partial |kF_{c,r}|}{\partial p_n} \Delta p_n = \sum_{r=1}^{m} w_r \Delta F_r \frac{\partial |kF_{c,r}|}{\partial p_2}$$

$$\vdots$$

$$\sum_{r=1}^{m} w_r \frac{\partial |kF_{c,r}|}{\partial p_n}\frac{\partial |kF_{c,r}|}{\partial p_1} \Delta p_1 + \sum_{r=1}^{m} w_r \frac{\partial |kF_{c,r}|}{\partial p_n}\frac{\partial |kF_{c,r}|}{\partial p_2} \Delta p_2 + \cdots + \sum_{r=1}^{m} w_r \left(\frac{\partial |kF_{c,r}|}{\partial p_n}\right)^2 \Delta p_n = \sum_{r=1}^{m} w_r \Delta F_r \frac{\partial |kF_{c,r}|}{\partial p_n}$$

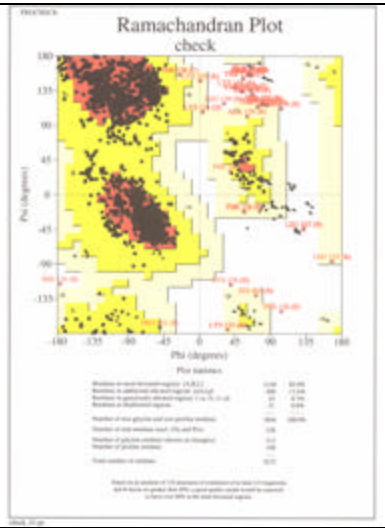
## Energy Refinement

**(Simulated Annealing)**

$$E_{TOTAL} = E_{EMPIRICAL} + E_{EFFECTIVE}$$

$$E_{EFFECTIVE} = E_{XREF} + E_{NOE} + E_{HARM} + E_{CDIH} + E_{NCS} + E_{DG} + E_{RELA} + E_{PLAN}$$

$$E_{EMPIRICAL} = S^{N}_{p=1} [w^{p}_{BOND}E_{BOND} + w^{p}_{ANGL}E_{ANGL} + w^{p}_{DIHE}E_{DIHE} + w^{p}_{IMPR}E_{IMPR} + w^{p}_{VDW}E_{VDW} + w^{p}_{ELEC}E_{ELEC} + w^{p}_{PVDW}E_{PVDW} + w^{p}_{PELE}E_{PELE} + w^{p}_{HBON}E_{HBON}].$$




Crystal Structure of *M. tuberculosis* Alanine Racemase

Table 1: Data Collection and Processing Statistics for the MAD and Native Data Sets of $Alr_{Mtb}$

| | MAD 1 | MAD 2 | MAD 3 | MAD 4 | native |
|---|---|---|---|---|---|
| λ (Å) | 0.9788 | 0.9790 | 0.9562 | 0.9809 | 0.9160 |
| resolution (Å) | | 2.20 | | | 1.80 |
| mosaicity | | 0.50 | | | 0.65 |
| no. of reflections observed > 1σ | 432376 | 446744 | 431524 | 336135 | 779600 |
| no. of unique reflections > 1σ | 35817 | 37506 | 36020 | 36242 | 67592 |
| $R_{merge}$[a] (%) | 6.9 | 6.4 | 5.1 | 3.7 | 6.0 (67.2) |
| completeness (%) | 91.8 | 95.8 | 92.1 | 92.1 | 99.3 (95.6) |
| ⟨I/σ⟩ | 30.3 | 34.3 | 41.6 | 50.9 | 34.5 (2.6) |

[a] $R_{merge} = \Sigma|I_{obs} - I_{avg}|/\Sigma|I_{avg}|$.

The 1.9 Å Crystal Structure of Alanine Racemase from *Mycobacterium tuberculosis* Contains a Conserved Entryway into the Active Site[†]

Table 2: Final Refinement Statistics for $Alr_{Mtb}$ at 1.9 Å Resolution

| | |
|---|---|
| *R* factor[a] (%) | 20.4 |
| $R_{free}$ (%) (for 1747 reflections) | 25.4 |
| average *B* factor (Å$^2$)[a] | |
| main chain | 25.5 |
| side chain | 31.5 |
| PLP | 21.9 |
| waters | 32.4 |
| rms deviations | |
| bond lengths (Å) | 0.006 |
| bond angles (deg) | 1.9 |
| no. of reflections > 2σ | 55001 |
| no. of residues | 722 |
| no. of protein atoms | 5360 |
| no. of PLP atoms | 30 |
| no. of water molecules | 350 |

[a] *B*-factor = $\Sigma|F_{obs} - F_{calc}|/\Sigma|F_{calc}|$. All isotropic model.

## Difference Fourier

Obs. $$\rho_o(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l F_{o,hkl}e^{-2\pi i(hx+ky+lz)} + R$$

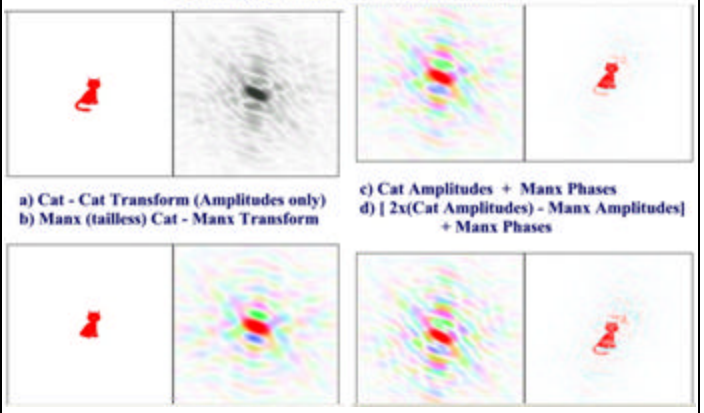
Calc. $$\rho_c(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l F_{c,hkl}e^{-2\pi i(hx+ky+lz)} + R'$$

$$\rho_o(x, y, z) - \rho_c(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l (F_o - F_c)_{hkl}e^{-2\pi i(hx+ky+lz)} + R - R'$$

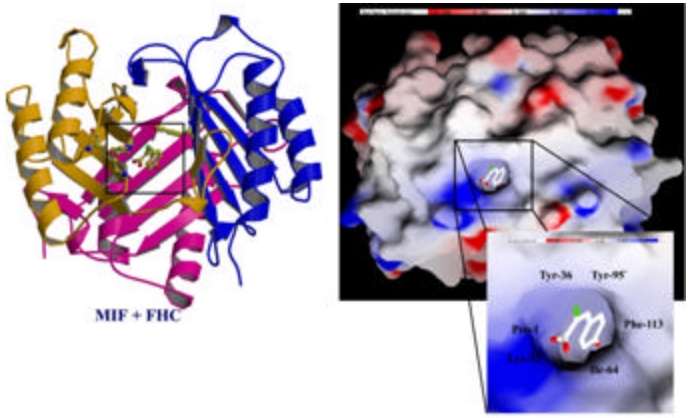
$$\rho_o - \rho_c = \frac{1}{V}\sum_h\sum_k\sum_l \Delta F_{hkl}e^{-2\pi i(hx+ky+lz)}$$

## Kevin Cowtan's Book of Fourier

http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html



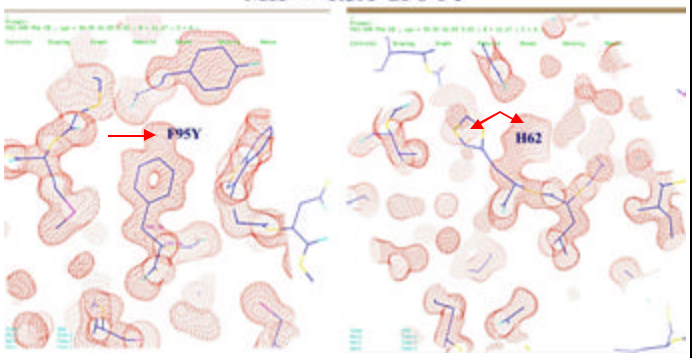
a) Cat - Cat Transform (Amplitudes only)
b) Manx (tailless) Cat - Manx Transform
c) Cat Amplitudes + Manx Phases
d) [ 2x(Cat Amplitudes) - Manx Amplitudes] + Manx Phases




MIF + FHC

## Examples of Difference Fouriers

MIF - 1.5A 2Fo-Fc




## X-Ray Crystallography

**Quiz questions:**

1. Crystal Growth – Materials / Methods
   - What is the single most important factor that determines crystal growth?
   - What are the two most common precipitating agents for growing protein crystals?
2. Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit
   - Identify the unit cell, asymmetric unit and symmetry present in the pattern shown.

3. X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron
   - What is responsible for "characteristic" X-rays?
   - What are the major advantages of using synchrotron radiation?
4. Theory of Diffraction – Bragg's Law / Reciprocal Space
   - When collecting an X-ray data set, what is being measured and how is that data useful?
5. Phasing and Refinement
   - Identify the meaning of the terms: MIR, MR, MAD, Difference Map, Simulated Annealing