X-Ray Crystallography

"If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist." – van Holde

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1. **Protein Data Bank (PDB)**

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2. **Image Formation**

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3. **X-Ray Crystallography (after NMR)**

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   b) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit

   c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

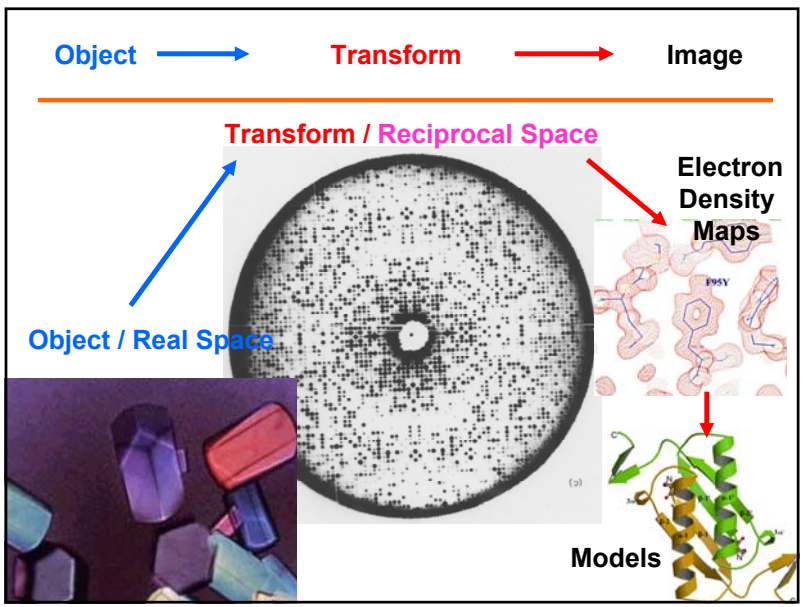
   d)Theory of Diffraction – Bragg's Law / Reciprocal Space

   e) Data Collection – Methods / Detectors / Structure Factors

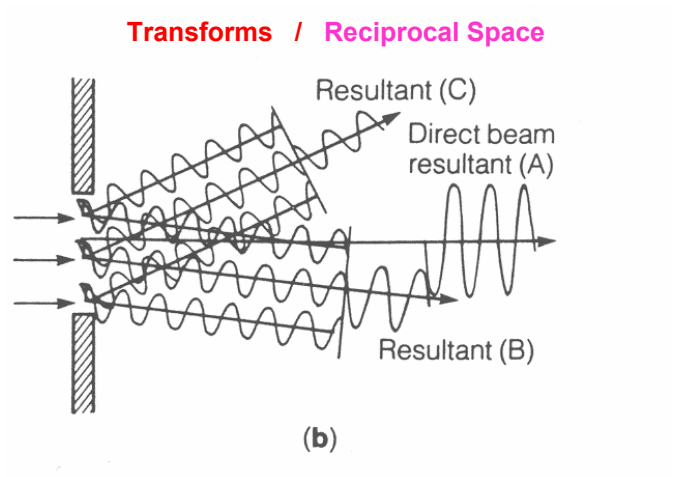
   f) Structure Solution – Phase Problem: MIR / MR / MAD

   h) Refinement, Analysis and Presentation of Results

   i) Use of Difference Fouriers



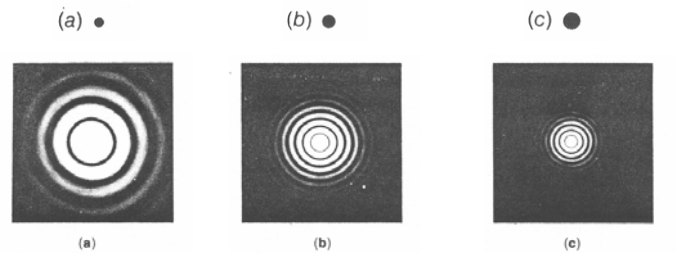
Single Hole Scattering Experiment



Single Hole Scattering Experiment

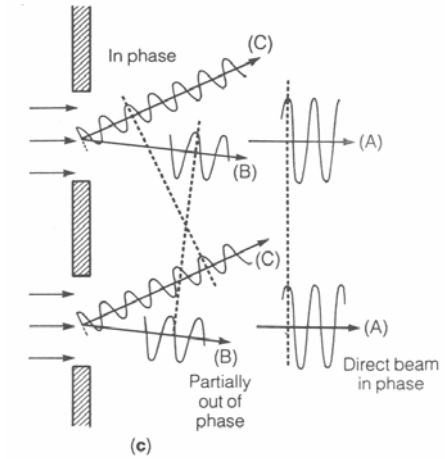
Transforms / Reciprocal Space

Different size holes



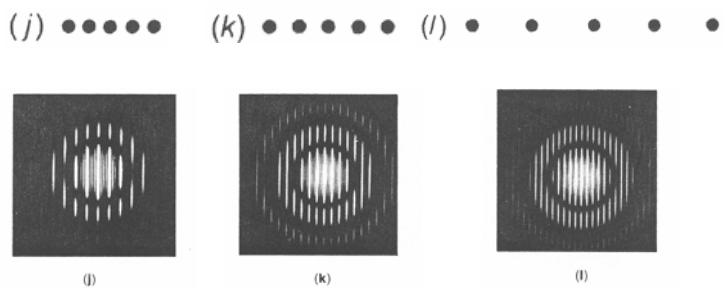
Effect of Multiple "Scatterers"

Transforms / Reciprocal Space



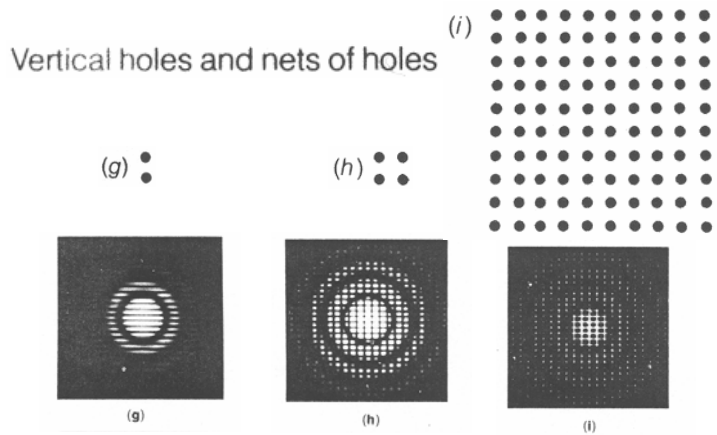
Transforms / Reciprocal Space

Five horizontal holes with various spacings



Transforms / Reciprocal Space

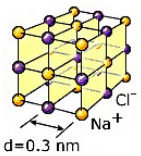
Vertical holes and nets of holes



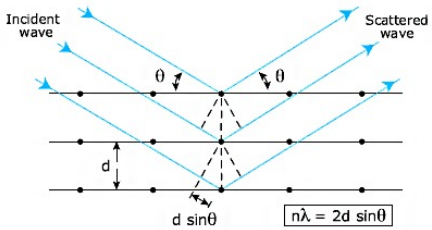
X-RAYS

More About the Bragg Formula

X-rays scattered from different layers of atoms can interfere with each other. The interference depends on the wavelength of the X-ray and on the distance between the atom layers. An X-ray with well-known wavelength can be used to explore the structure of the crystal. For a well-known crystal, the X-ray properties can be examined.



Crystal planes, in NaCl, ordinary salt. Other planes are also possible.



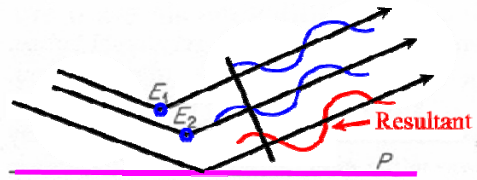
X-ray scattering from three crystal planes, separated by the distance d. For constructive interference in a direction θ the path difference must be an even number of wavelengths.

Related Laureates

The Nobel Prize in Physics 1915 - Sir William Henry Bragg »

The Nobel Prize in Physics 1915 - William Lawrence Bragg »

Diffraction: Scattering from (two) "atoms"



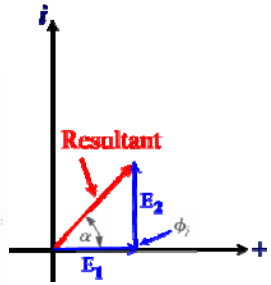


Figure 2.10. Diffraction from E_1 and E_2 as if reflected from plane P.

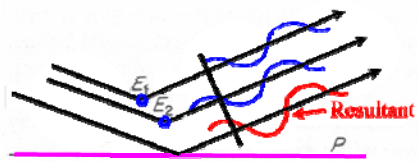
Scattering from "many atoms"

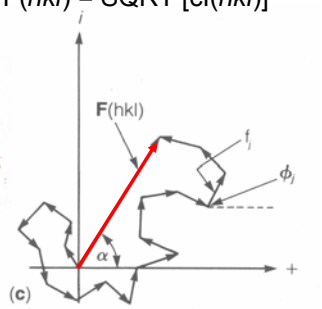
$$\mathbf{F}(hkl) = F(hkl)e^{i\alpha(hkl)} = \sum_{j=1}^{N'} \mathbf{f}_j(hkl) = \sum_{j=1}^{N'} f_j(hkl)e^{i\phi_j(hkl)}$$

Calculated

Experimental

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

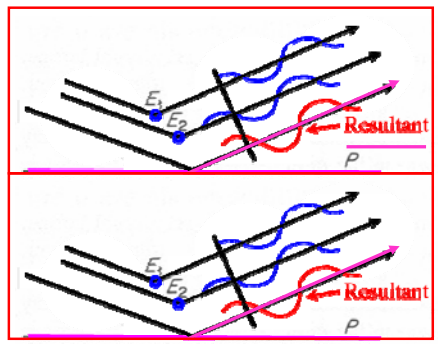




The structure factor for a reflection may be thought of as the vector sum of the x-ray scattering contributions from many atoms.

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"

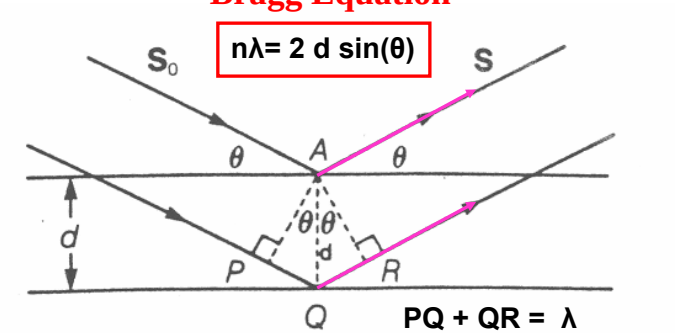


Crystals: Scattering from "planes"

Resultant scattering of resultant scattering!

Bragg Equation

$n\lambda = 2\,d\sin(\theta)$



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

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

Bragg Planes

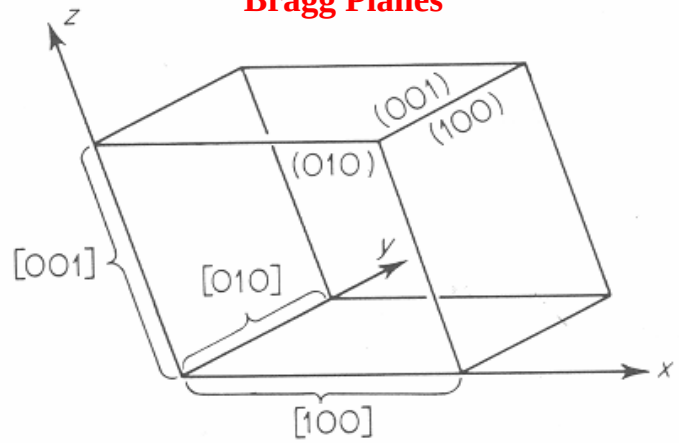


Figure 2.7. Unit cell showing bounding planes and edges.

Name that Bragg "plane"

1 1 0 | 1 3 0 | 2 -1 0 (or -2 1 0)

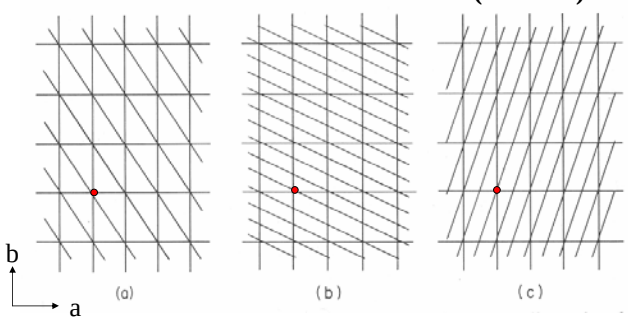


Figure 2.5. Three families of lattice "planes" in a two-dimensional lattice.

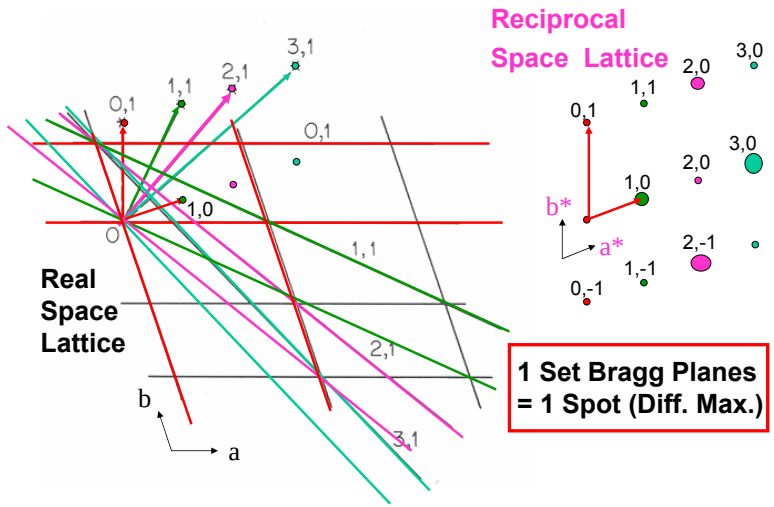
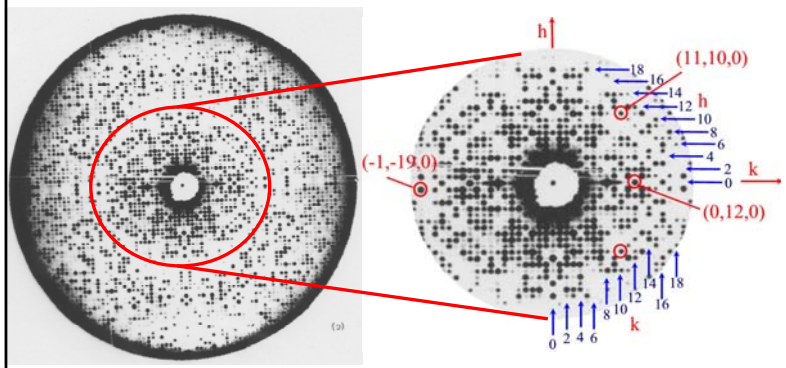


Figure 2.11. Planes in direct space represented by points in reciprocal space.

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** - [Fhkl]'s - ?? How do we obtain **Phases** αhkl ??

→ **Phase Problem**

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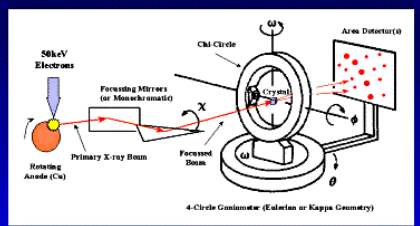
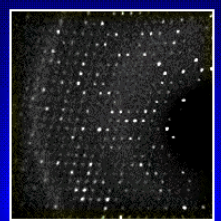
Advanced Methods in Modern Biomolecular Crystallography

The information we get from a single diffraction experiment……





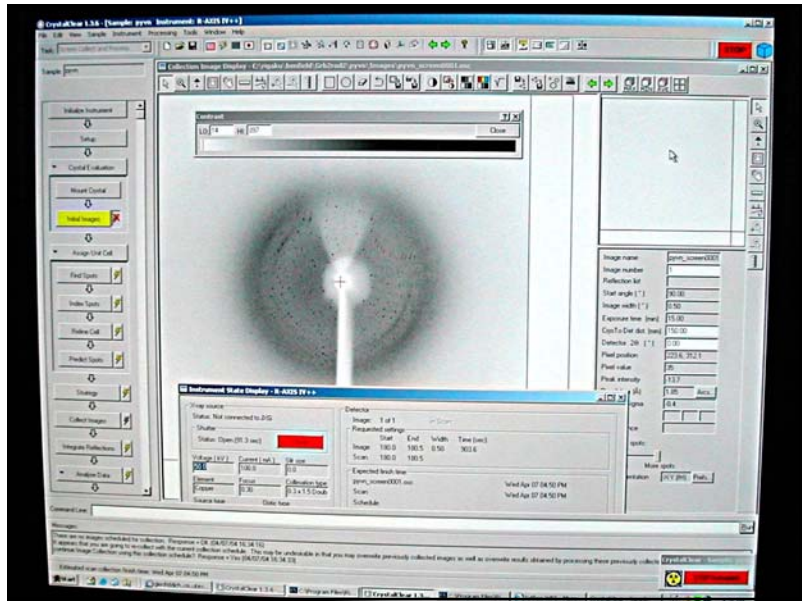
2	10	1	326.58
3	10	1	1644.72
4	10	1	3228.45
5	10	1	1279.83
6	10	1	320.48
7	10	1	775.63
8	10	1	1344.55
9	10	1	431.73
10	10	1	1760.14
11	10	1	709.18
12	10	1	20.37
13	10	1	408.72
14	10	1	51.36
15	10	1	114.72
16	10	1	776.26
17	10	1	87.57
18	10	1	30.93
0	11	1	99.30
1	11	1	2258.68
2	11	1	770.18

The reflections are indexed (consistent assignment of reciprocal cell indices h,k,l) and all we get for the money is a long list of intensities from several ten thousand reflections

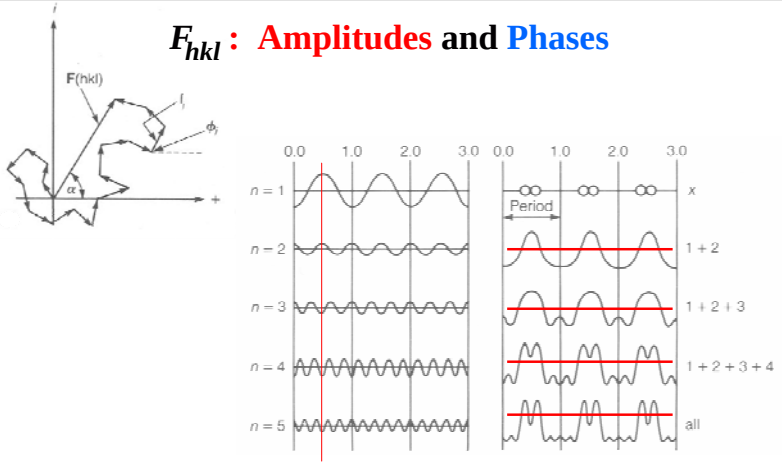
Advanced Methods in Modern Biomolecular Crystallography

Cryo-cooling efficiently improves data quality

- Crystals are rapidly cooled (NOT FROZEN) to near liquid nitrogen temperature
- Reduced thermal vibrations
- Increased resolution
- Reduced disorder
- Eliminated radiation damage
- No merging and scaling errors

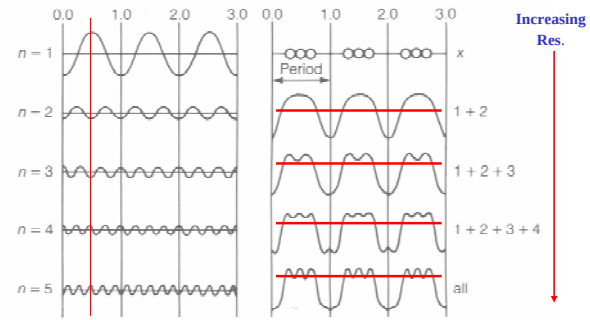


F_{hkl} : Amplitudes and Phases



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.)

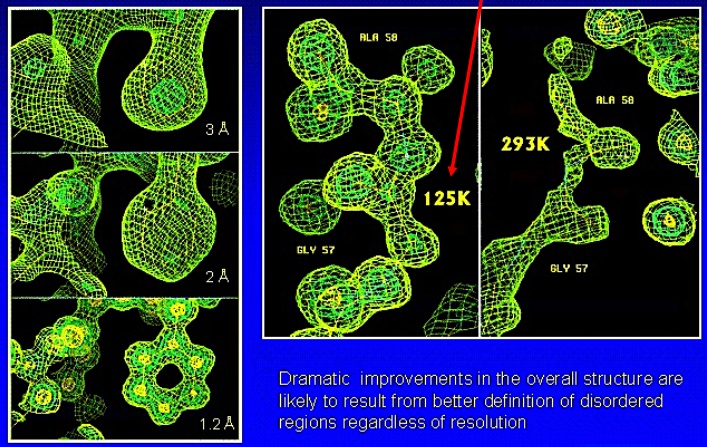
F_{hkl} : Amplitudes and Phases



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.)

Effect of Resolution

Reduced Disorder at Lower Temperatures



Dramatic improvements in the overall structure are likely to result from better definition of disordered regions regardless of resolution

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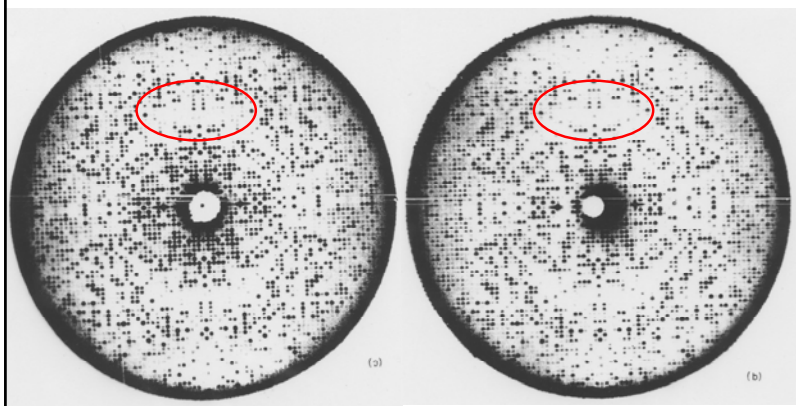
Solving the Phase Problem

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

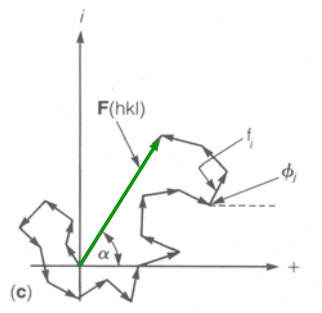
Use of Heavy Metal Ions for Phasing by MIR Methods



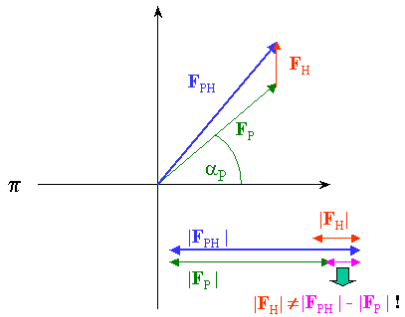
Native Phosphorylase

Phosphorylase + Ethyl Hg thiosalicylate

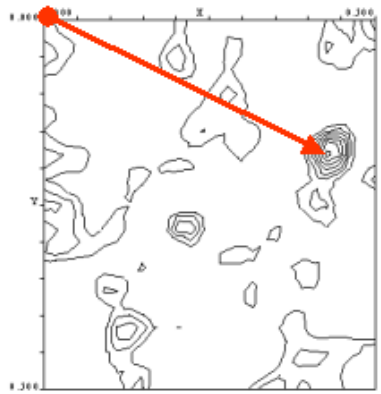
Effect of adding 1 "heavy" atom with lots of electrons!



$F_{PH} = F_P + F_H$



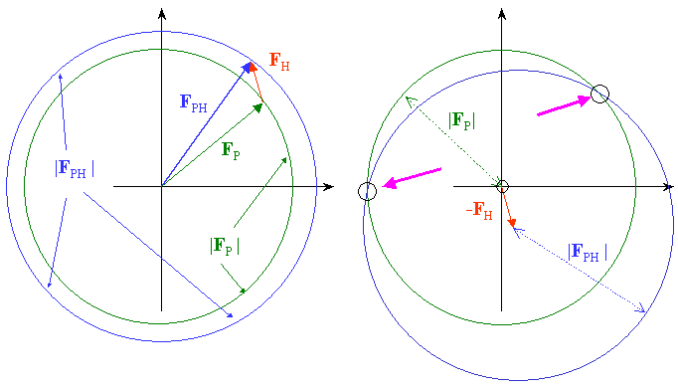
Difference Patterson Map $(F_{hkl})^2$



Multiple Isomorphous Replacement (MIR) method

$F_{PH} = F_P + F_H$

$F_P = F_{PH} - F_H$



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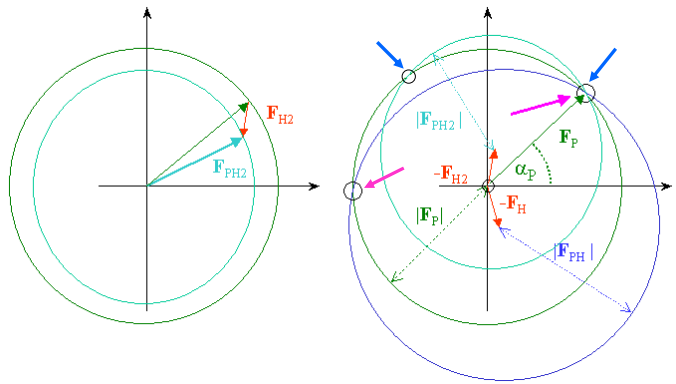


Solving the phase problem by "**Molecular Replacement**".

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"

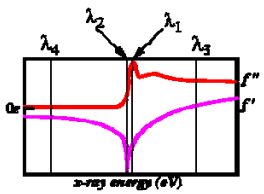
(MAD) methods

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" (λ_1 in the figure above).
- The second wavelength is usually chosen to have maximal |f`| (λ_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 λ_3 and λ_4 are between 100eV and 1000eV from the absorption edge.

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Least-Squares Refinement

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

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

$$\vdots$$

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

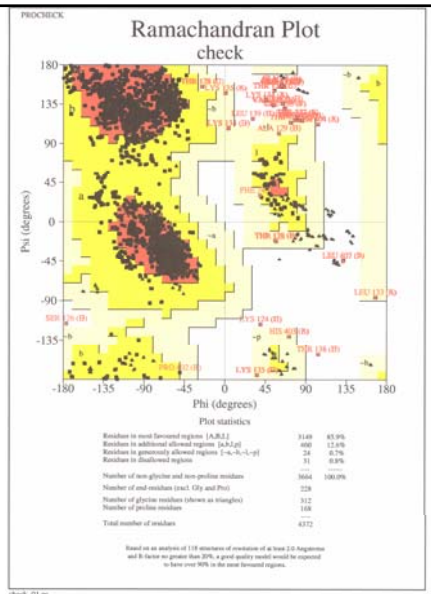
Energy Refinement

(Simulated Annealing)

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

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

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



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)
$\langle I/\sigma \rangle$	30.3	34.3	41.6	50.9	34.5 (2.6)

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

Biochemistry **2005**, *44*, 1471−1481 1471

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

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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)[b]	
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] R-factor = $\Sigma|F_{obs} - F_{calc}|/\Sigma|F_{obs}|$. [b] All isotropic model.

Analyze – structure (Ramachandran Plot) and biochemistry

Publish in leading biochemical or structural biology journal

Contribute results (coordinates, etc.) to PDB

****

Data Mining

- **Visualization programs (Cn3D / RasMol / SwissPDBV / etc)**
- **SCOP – Structural Classification of Proteins**
- **CATH – Classification / Arch / Topology**

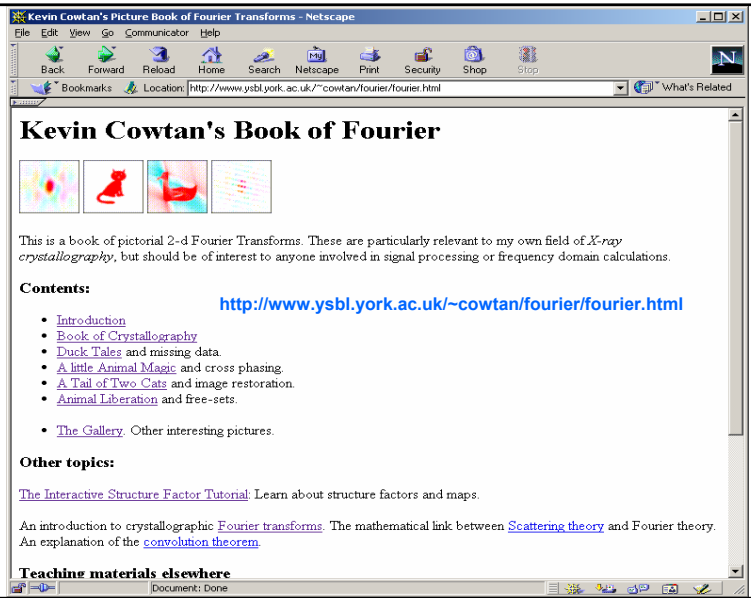
Difference Fourier

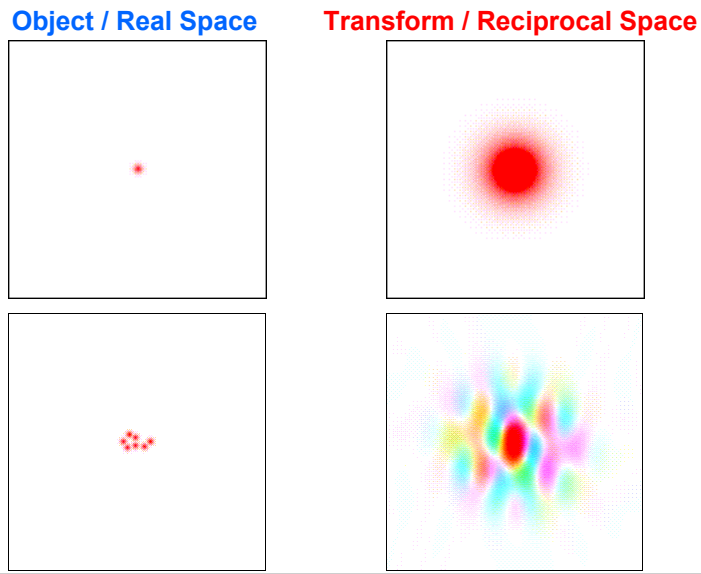
Obs. $$\rho_o(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l F_{o,hkl}\, e^{-2\pi i(hx+ky+lz)} + R$$

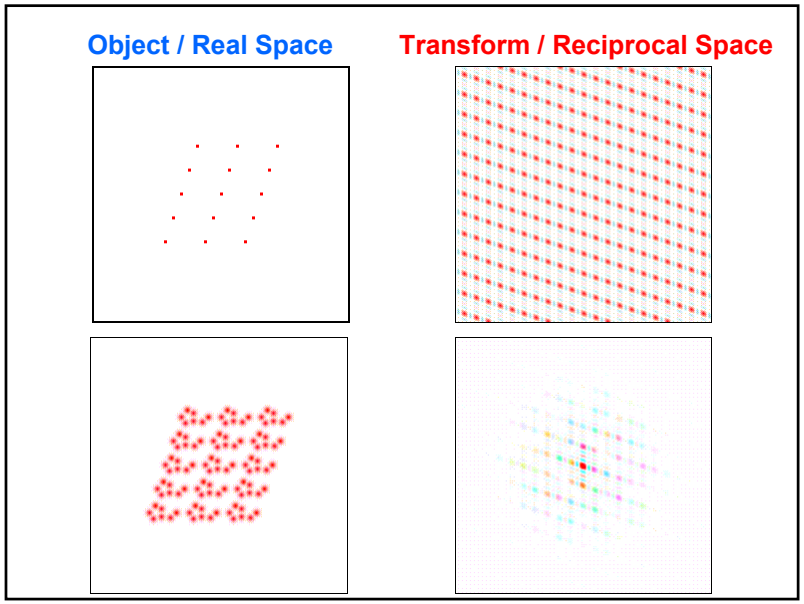
Calc. $$\rho_c(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l F_{c,hkl}\, e^{-2\pi i(hx+ky+lz)} + R'$$

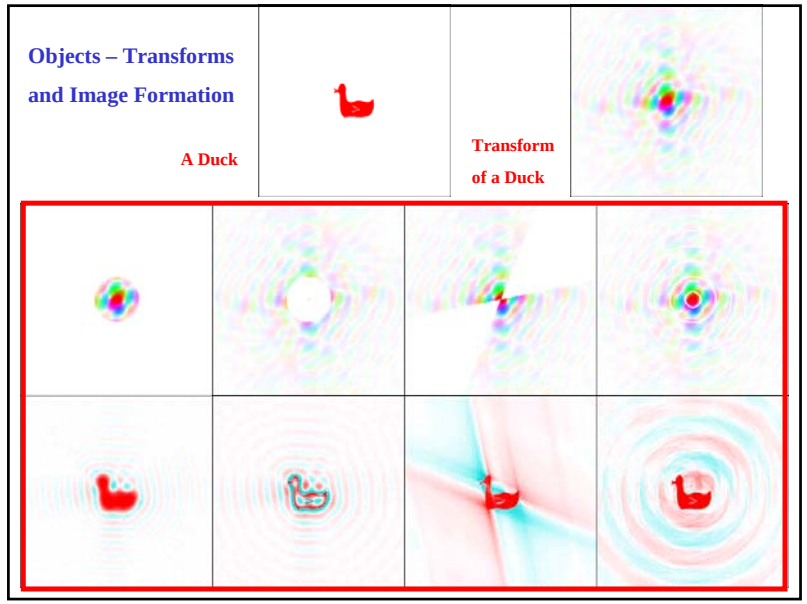
$$\rho_o(x, y, z) - \rho_c(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l (F_o - F_c)_{hkl} e^{-2\pi i(hx+ky+lz)} + R - R'$$

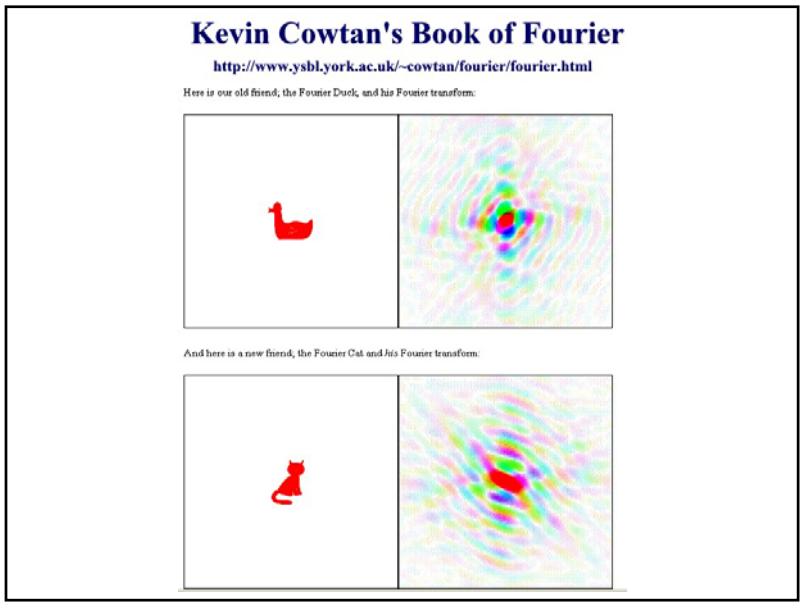
$$\rho_o - \rho_c = \frac{1}{V}\sum_h\sum_k\sum_l \Delta F_{hkl} e^{-2\pi i(hx+ky+lz)}$$

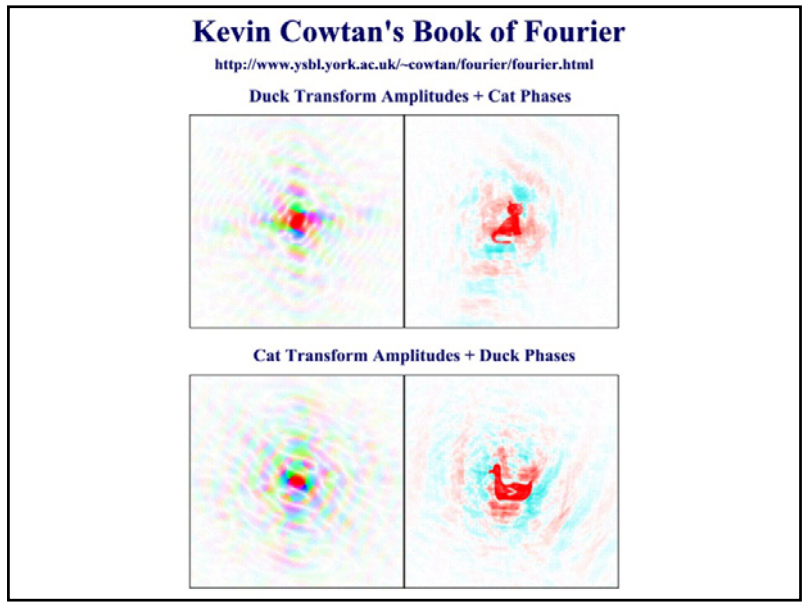





Object / Real Space
Transform / Reciprocal Space


Objects – Transforms
and Image Formation
A Duck
Transform
of a Duck


Kevin Cowtan's Book of Fourier
http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html
Here is our old friend, the Fourier Duck, and his Fourier transform:
And here is a new friend, the Fourier Cat and his Fourier transform: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Duck Transform Amplitudes + Cat Phases
Cat Transform Amplitudes + Duck Phases

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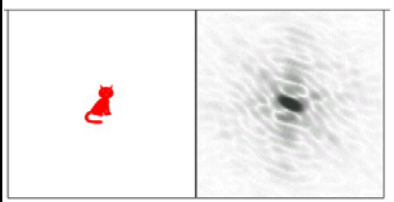
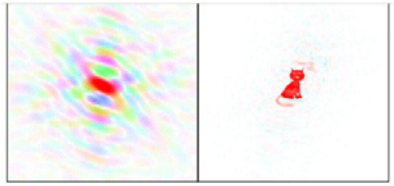
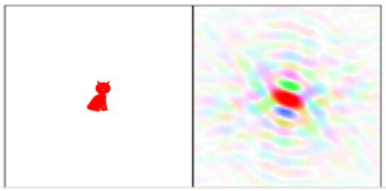
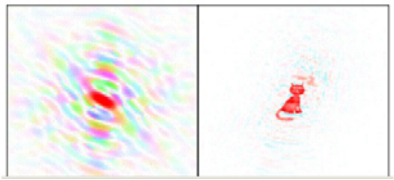


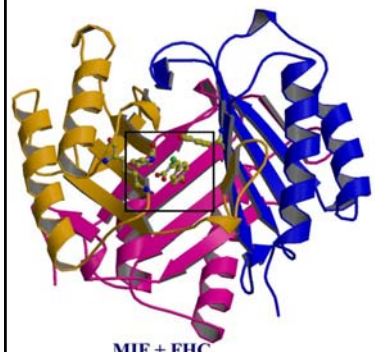
a) Cat - Cat Transform (Amplitudes only)
b) Manx (tailless) Cat - Manx Transform



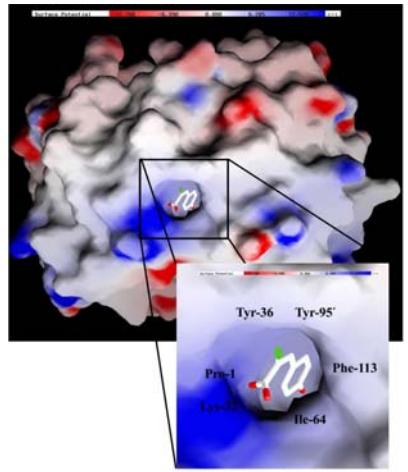
c) Cat Amplitudes + Manx Phases
d) [2x(Cat Amplitudes) - Manx Amplitudes] + Manx Phases





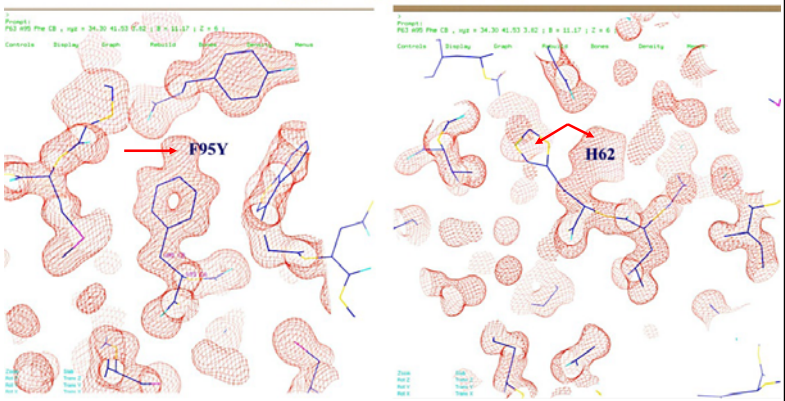


MIF + FHC



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