

## X-Ray Crystallography

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

Topics:

1. Protein Data Bank (PDB)

Data mining and Protein Structure Analysis Tools

2. Image Formation

Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

3. X-Ray Crystallography (after NMR)

a) Crystal Growth – Materials / Methods

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 (part 2) / 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 Fourier Maps ( $2F_o - F_c$ )

## X-ray tubes: the "sealed" tube

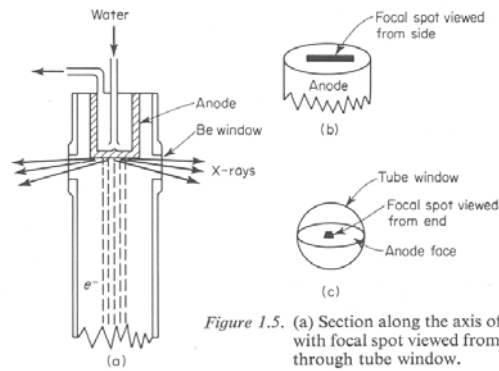


Figure 1.5. (a) Section along the axis of an X-ray tube. (b) Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

## Origin of characteristic X-rays

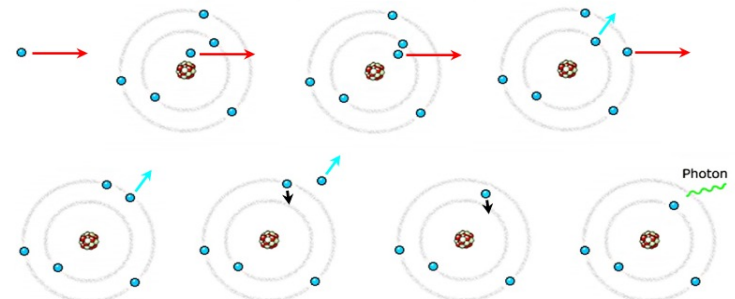
### Characteristic X-ray Lines

The high energy electron can also cause an electron close to the nucleus in a metal atom to be knocked out from its place. This vacancy is filled by an electron further out from the nucleus. The well defined difference in binding energy, characteristic of the material, is emitted as a monoenergetic photon. When detected this X-ray photon gives rise to a characteristic X-ray line in the energy spectrum. C. Barkla observed these lines in 1908-09 and was given the 1917 Nobel Prize for this discovery. He also made the first experiments suggesting that the X-rays are electromagnetic waves.

### Related Laureate



The Nobel Prize in Physics 1917 - Charles Glover Barkla >



## Characteristic X-rays arise from electronic transitions

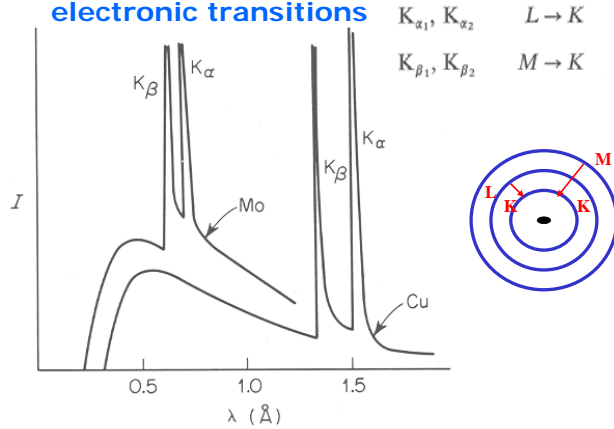


Figure 1.2. X-ray spectra with characteristic peaks: MoK $\alpha$ , 50 Kv; CuK $\alpha$ , 35 Kv.

## Characteristic X-rays have defined $\lambda$

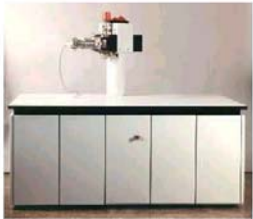
Table 1.1. Target Materials and Associated Constants

	Cr	Fe	Cu	Mo
Z	24	26	29	42
$\alpha_1, \text{\AA}$	2.2896	1.9360	1.5405	0.70926
$\alpha_2, \text{\AA}$	2.2935	1.9399	1.5443	0.71354
$\bar{\alpha}, \text{\AA}$	2.2909	1.9373	1.5418	0.71069
$\beta_1, \text{\AA}$	2.0848	1.7565	1.3922	0.63225
$\beta$ , filt.	V, 0.4 mil†	Mn, 0.4 mil	Ni, 0.6 mil	Nb, 3 mils
$\alpha$ , filt.	Ti	Cr	Co	Y
Resolution, $\text{\AA}$	1.15	0.95	0.75	0.35
Critical potential, kV	5.99	7.11	8.98	20.0
Operating conditions, kV:	30-40	35-45	35-45	50-55
half- or full-wave-rectified, mA	10	10	20	20
constant potential, mA	7	7	14	14

\*  $\bar{\alpha}$  is the intensity-weighted average of  $\alpha_1$  and  $\alpha_2$  and is the figure usually used for the wavelength when the two lines are not resolved.

† 1 mil = 0.001 inch = 0.025 mm.

## X-ray Generators

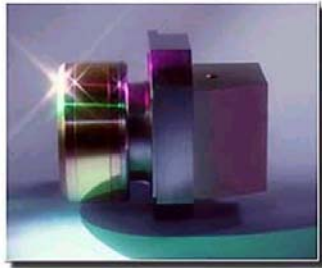


FR591

### FR591 Rotating Anode X-ray Generator

The Nomad® FR591 rotating anode X-ray generator now has dramatically improved the performance of the anode, by a complete redesign. We now have a static shaft and a rotating anode, instead of rotating both. The cooling water flow has also been redesigned to give much higher throughput, higher flow and higher turbulence, which results in better heat transfer and hence better cooling capacity.

Now with the new ULTRA anode you can get 6 kW on a 0.3mm focus!



## Another Source of "X-rays"

### Synchrotron Radiation

X-ray photons can also be created under different conditions. When physicists were operating the first particle accelerators, they discovered that electrons can produce photons without colliding at all. This was possible because the magnetic field in the accelerators was causing the electrons to move in large spirals around magnetic field lines of force. This process is called synchrotron radiation.

In the cosmos particles such as electrons can be accelerated to high energies— near the speed of light— by electric and magnetic fields. These high-energy particles can produce synchrotron photons with wavelengths ranging from radio up through X-ray and gamma-ray energies.



Synchrotron Radiation: Electrons moving in magnetic field radiate photons.

# "X-ray" Sources: X-ray tubes

The **brilliance** of a light source is defined as the number of photons emitted per second, per unit source size, per unit space angle and for a bandwidth of 1/1000 of the photon energy

The Comparison between various sources of X-rays shows large differences in their brilliance.

### X-ray tubes:

Wilhelm Conrad Röntgen discovered X-rays in 1895 whilst working with cathode-ray tubes. Using the principle of fast electrons hitting a metallic target, a first substantial gain in brilliance was not obtained until the introduction of rotating anode sources (~1960).

### Synchrotron Radiation Facilities:

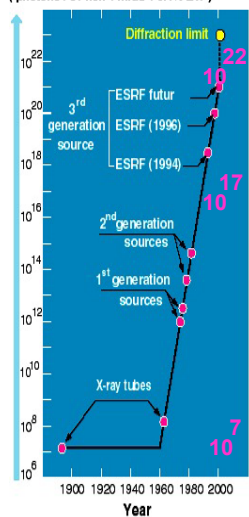
The progress of high energy physics, with the construction of powerful particle accelerators gave birth to what we now call *First* generation synchrotron sources (~1970). Using the deflection of high energy electrons by a magnetic field for the production of X-rays proved so promising that a number of dedicated *Second* generation sources were built (~1980). Relying on the combination of needle than electron beams and Insertion Devices, *Third* generation synchrotron sources (~1995) are now emitting synchrotron X-ray beams that are a trillion ( $10^{12}$ ) times more brilliant than those produced by X-ray tubes.

### Free Electron X-ray Lasers:

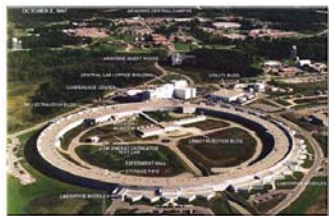
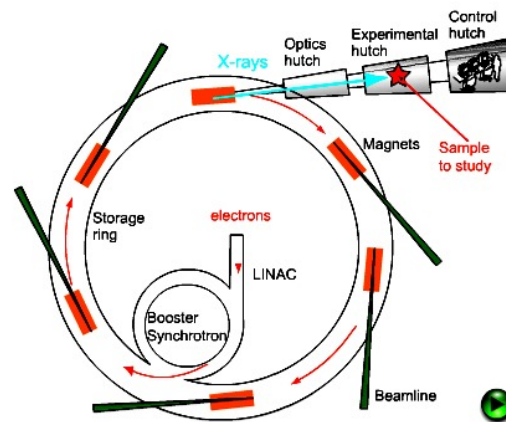
Coupling electron and X-ray beams together, the Free Electron X-ray Lasers currently on the drawing boards could be the next generation of X-ray sources. While they promise to achieve an increase in peak brilliance by another factor of a trillion, the first prototypes may be operational around the year 2010.

Brilliance of the X-ray beams

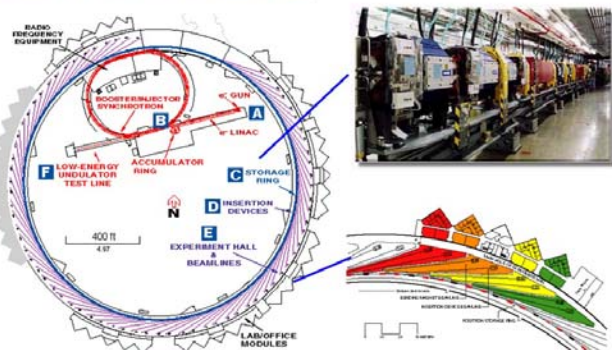
(photons / s / mm<sup>2</sup> / mrad<sup>2</sup> / 0.1% BW)



# How synchrotron light is produced?



APS - Advanced Photon Source  
Argonne National Laboratory

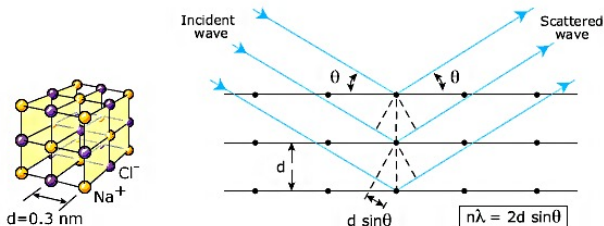


X-RAYS  
X-rays, What Are They? 6:7

X-rays, What Are They? 7:7

### More About the Bragg Formula

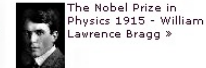
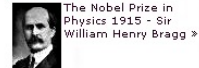
X-rays scattered from different layers of atoms can interfere with each other. The interference depends on the wavelength of the X-ray and on the distance between the atom layers. An X-ray with well-known wavelength can be used to explore the structure of the crystal. For a well-known crystal, the X-ray 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  $\theta$  the path difference must be an even number of wavelengths.

### Related Laureates





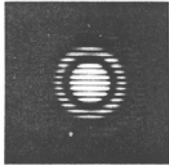
**Related Laureates**

 The Nobel Prize in Physics 1901 - Wilhelm Conrad Röntgen >	 The Nobel Prize in Physics 1914 - Max von Laue >	 The Nobel Prize in Physics 1915 - Sir William Henry Bragg >
 The Nobel Prize in Physics 1915 - William Lawrence Bragg >	 The Nobel Prize in Physics 1917 - Charles Glover Barkla >	 The Nobel Prize in Physics 1924 - Karl Manne Georg Siegbahn >
 The Nobel Prize in Physics 1927 - Arthur Holly Compton >	 The Nobel Prize in Chemistry 1936 - Petrus (Peter) Josephus Wilhelmus Debye >	 The Nobel Prize in Chemistry 1962 - Max Ferdinand Perutz >
 The Nobel Prize in Chemistry 1962 - John Cowdery Kendrew >	 The Nobel Prize in Physiology or Medicine 1962 - Francis Harry Compton Crick >	 The Nobel Prize in Physiology or Medicine 1962 - James Dewey Watson >
 The Nobel Prize in Physiology or Medicine 1962 - Maurice Hugh Frederick Wilkins >	 The Nobel Prize in Chemistry 1964 - Dorothy Crowfoot Hodgkin >	 The Nobel Prize in Chemistry 1976 - William N. Lipscomb >
 The Nobel Prize in Physiology or Medicine 1979 - Allan M. Cormack >	 The Nobel Prize in Physiology or Medicine 1979 - Godfrey N. Hounsfield >	 The Nobel Prize in Physics 1981 - Kai M. Siegbahn >
 The Nobel Prize in Chemistry 1985 - Herbert A. Hauptman >	 The Nobel Prize in Chemistry 1985 - Jerome Karle >	 The Nobel Prize in Chemistry 1988 - Johann Deisenhofer >
 The Nobel Prize in Chemistry 1988 - Robert Huber >	 The Nobel Prize in Chemistry 1988 - Hartmut Michel >	 Chemistry 2003 - Roderick MacKinnon
		 Chemistry 2006 - Roger Kornberg

## Transforms / Reciprocal Space

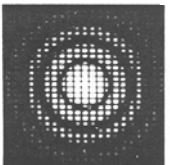
Vertical holes and nets of holes

(g)



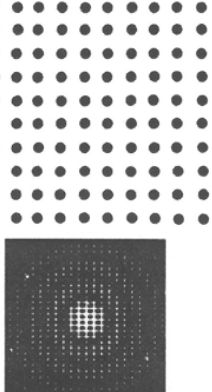
(g)

(h)




(h)

(i)



(i)

## Joseph Fourier / Fourier Series ~1808



Fourier series are named in honor of Joseph Fourier (1768-1830), who made important contributions to the study of trigonometric series, after preliminary investigations by Euler, d'Alembert, and Bernoulli. He applied this technique to find the solution of the heat equation, publishing his initial results in 1807, and publishing his *Théorie analytique de la chaleur* in 1822

$$f(t) = \frac{a_0}{2} + \sum_{n=1}^{\infty} a_n \cos \frac{n\pi t}{L} + \sum_{n=1}^{\infty} b_n \sin \frac{n\pi t}{L}$$

$$a_0 = \frac{1}{L} \int_{-L}^L f(t) dt$$

$$a_n = \frac{1}{L} \int_{-L}^L f(t) \cos \frac{n\pi t}{L} dt \quad b_n = \frac{1}{L} \int_{-L}^L f(t) \sin \frac{n\pi t}{L} dt$$

where  $n = 1, 2, 3 \dots$

## Sines / Cosines / and Exponentials

$$\exp(x) \equiv e^x \equiv \sum_{n=0}^{\infty} \frac{x^n}{n!}$$

$$= 1 + x + \frac{x^2}{2} + \frac{x^3}{6} + \frac{x^4}{24} + \frac{x^5}{120} + \dots$$

If we let  $x$  be imaginary,  $x = i\theta$  (where  $\theta$  is real), then this can be written

$$e^{i\theta} = 1 + i\theta - \frac{\theta^2}{2} - i\frac{\theta^3}{6} + \frac{\theta^4}{24} + i\frac{\theta^5}{120} - \dots$$

recall

$$\cos \theta = 1 - \frac{\theta^2}{2} + \frac{\theta^4}{24} - \dots$$

$$\sin \theta = \theta - \frac{\theta^3}{6} + \frac{\theta^5}{120} - \dots$$

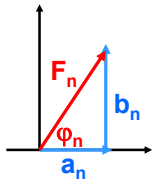
thus

$e^{i\theta} = \cos \theta + i \sin \theta$

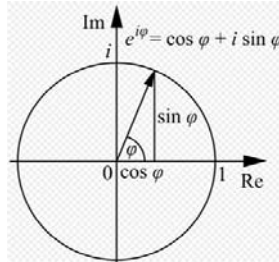
**Euler's Equation**

**Euler's formula** (Leonhard Euler, 1707-1783) gives the relationship between the complex exponential function and common trig terms. For any real number "φ"

→ Replace list of  $a_n / b_n$  with  $F_n$  and  $\varphi_n$



n	a	b	F	φ
1	7	0	7	0
2	0	8	8	90
1	5	5	7.1	??
1	8	6	??	36.9



## Fourier Series / Fourier Transforms

$$f(t) = \frac{a_0}{2} + \sum_{n=1}^{\infty} a_n \cos \frac{n\pi t}{L} + \sum_{n=1}^{\infty} b_n \sin \frac{n\pi t}{L}$$

or →

$$a_0 = \frac{1}{L} \int_{-L}^L f(t) dt$$

$$a_n = \frac{1}{L} \int_{-L}^L f(t) \cos \frac{n\pi t}{L} dt \quad b_n = \frac{1}{L} \int_{-L}^L f(t) \sin \frac{n\pi t}{L} dt$$

Now consider **electron density as a function**

$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}} \mathbf{F}(\mathbf{h}) \exp(-2\pi i \mathbf{h} \cdot \mathbf{x}) \quad \text{or} \quad \mathbf{F}(\mathbf{h}) = \int_{\text{cell}} \rho(\mathbf{x}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x}) dV$$

**AND** –  $F_{hkl}$  can also be **calculated** as the resultant scattering or the **sum of the individual scattering atoms!!**

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

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

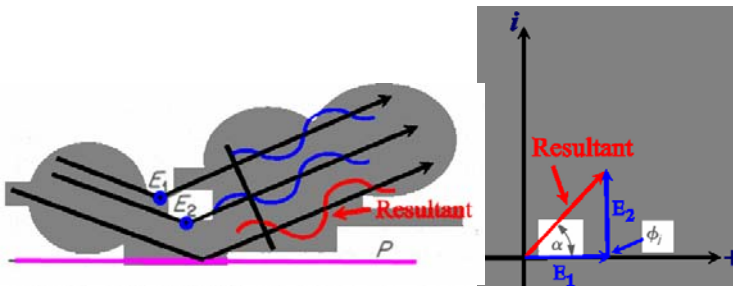


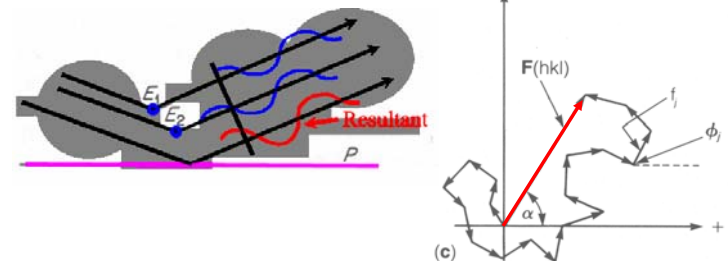
Figure 2.10. Diffraction from  $E_1$  and  $E_2$  as if reflected from plane  $P$ .

Represent a wave as an **amplitude** + **phase**.

## Scattering from "many atoms"

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

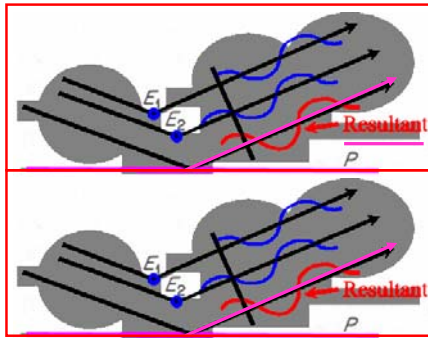
$$\mathbf{F}(hkl) = \text{SQRT} [c_l(hkl)]$$



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”

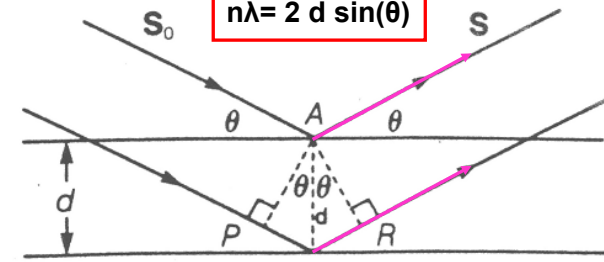


## Crystals: Scattering from “planes”

Resultant scattering of resultant scattering!

### Bragg Equation

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



→ Scattering will only be “observed” at discrete **Bragg angles** ( $\theta$ )  
 The spacings of the Bragg reflections → **Lattice Constants**

## Bragg Planes

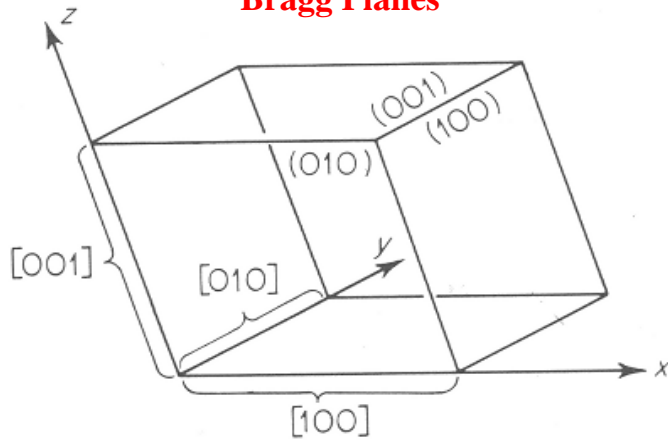


Figure 2.7. Unit cell showing bounding planes and edges.

## Name that Bragg “plane”

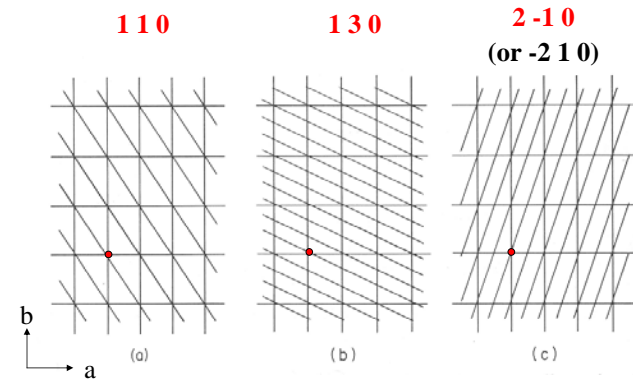
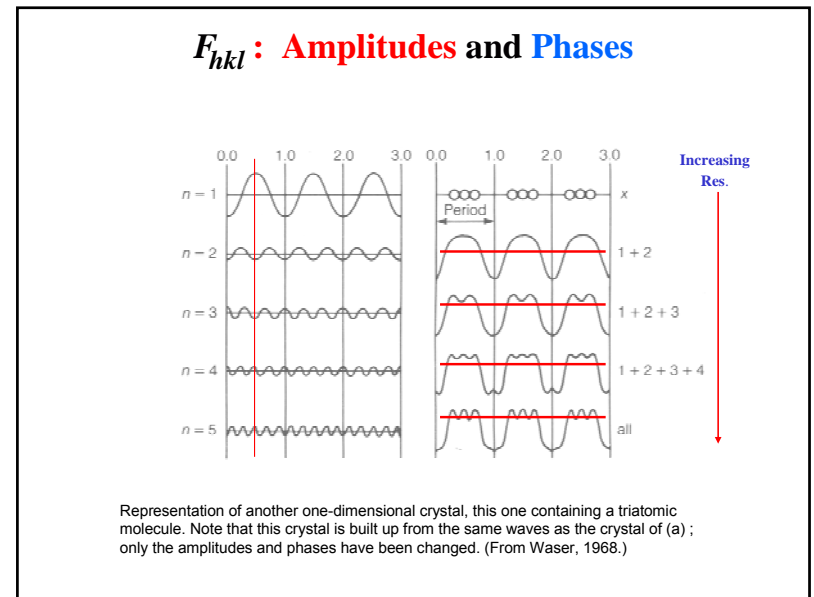
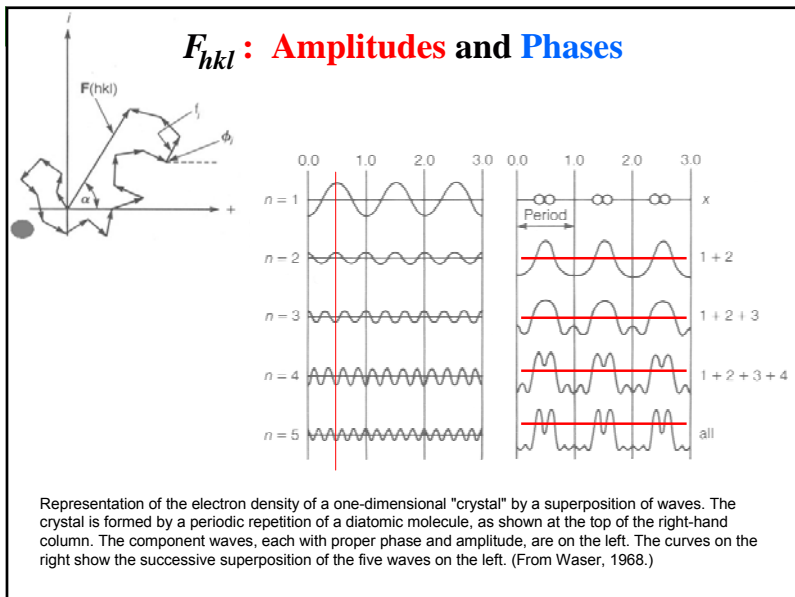
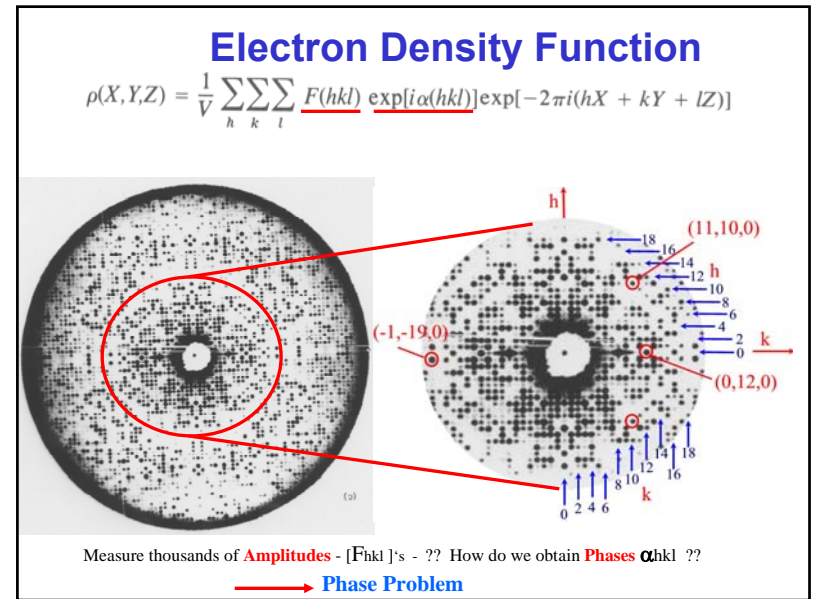
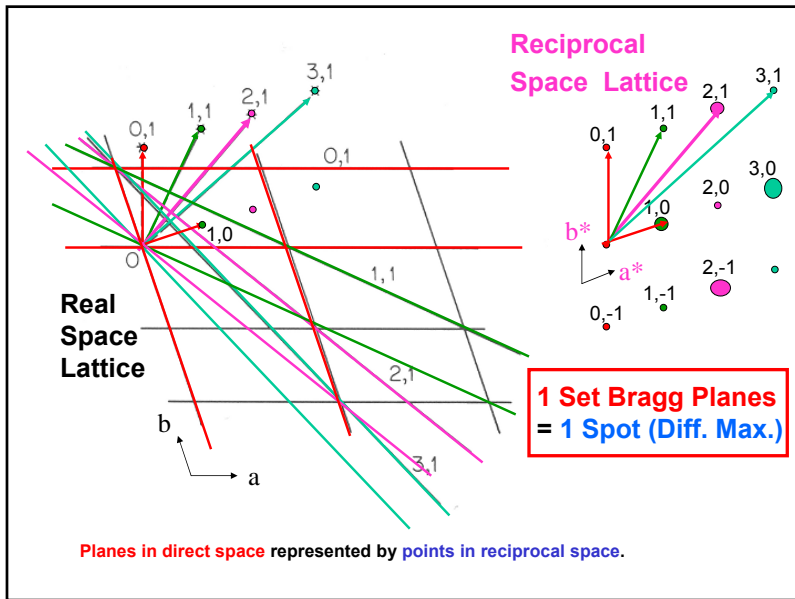


Figure 2.5. Three families of lattice “planes” in a two-dimensional lattice.





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Topics:

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## 2. Image Formation

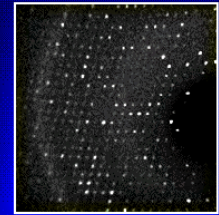
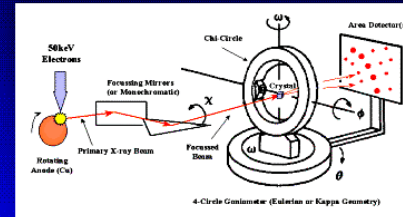
Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

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

- Crystal Growth – Materials / Methods
- Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit
- X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron
- Theory of Diffraction – Bragg's Law / Reciprocal Space
- Data Collection – Methods / Detectors / Structure Factors
- Structure Solution – Phase Problem: MIR / MR / MAD
- Refinement, Analysis and Presentation of Results
- Use of Difference Fourier



The information we get from a single diffraction experiment.....

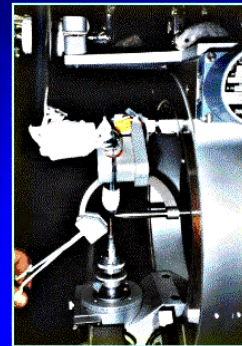


The reflections are indexed (consistent assignment of reciprocal cell indices  $h, k, l$ ) and all we get for the money is a long list of intensities from several ten thousand reflections

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	775	26
17	10	1	87	57
18	10	1	30	93
0	11	1	93	30
1	11	1	2258	68
2	11	1	770	18



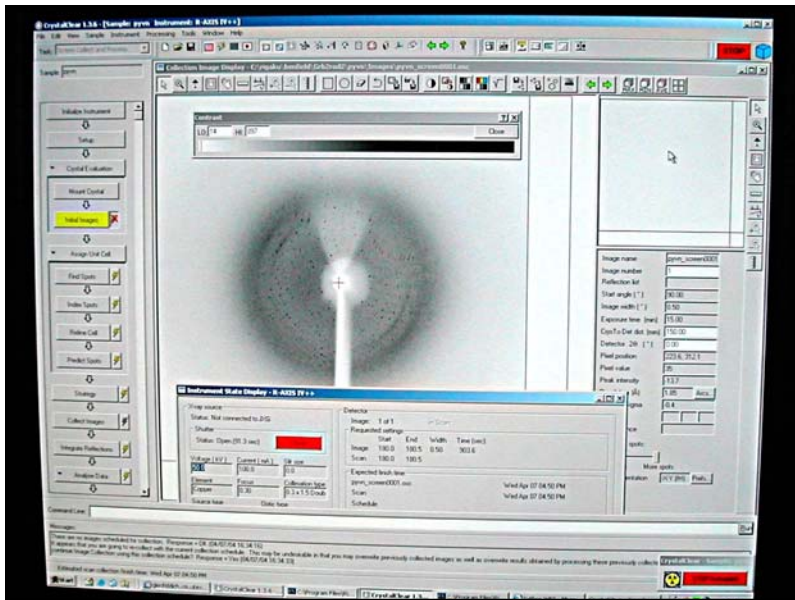
Cryo-cooling efficiently improves data quality



- 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







### Effect of Resolution

The three panels show a molecular model at different resolutions: 3 Å (top), 2 Å (middle), and 1.2 Å (bottom). As resolution improves, the model becomes more detailed and defined.

### Reduced Disorder at Lower Temperatures

The two panels show a molecular model at different temperatures: 293K (left) and 125K (right). A red arrow points from the 293K model to the 125K model, indicating a transition to a more ordered state. Labels 'ALA 58' and 'GLV 57' are visible on the models.

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

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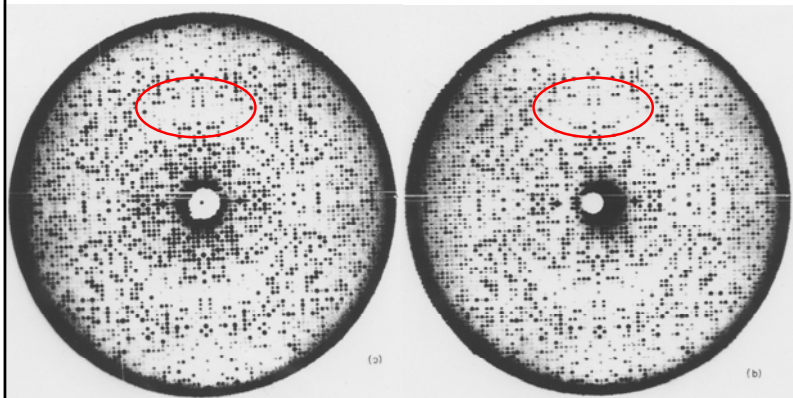
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## Solving the Phase Problem

1. **MIR:** Multiple Isomorphous Replacement (Heavy Atom)
2. **MR:** Molecular Replacement
3. **MAD:** multiwavelength anomolous dispersion
  - \*\*\*\*\*
  - **Molecular Modeling** (predicting starting structure from sequence alone)

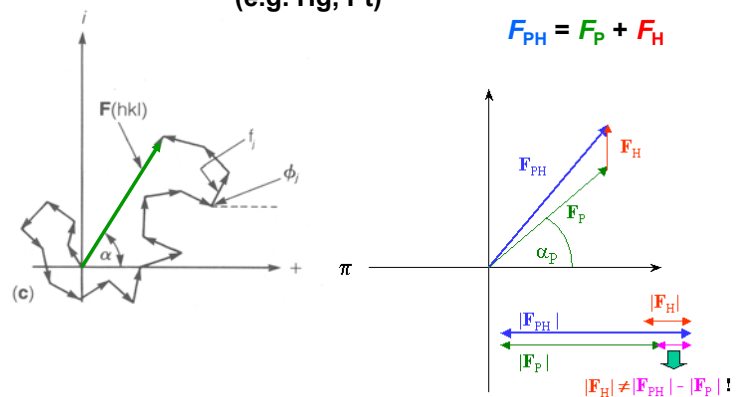
### Use of Heavy Metal Ions for Phasing by MIR Methods



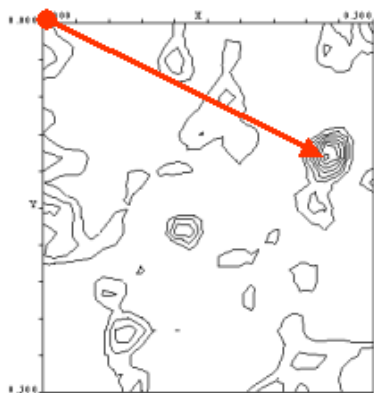
Native Phosphorylase

Phosphorylase + Ethyl Hg thiosalicylate

### Effect of adding 1 "heavy" atom with lots of electrons! (e.g. Hg, Pt)



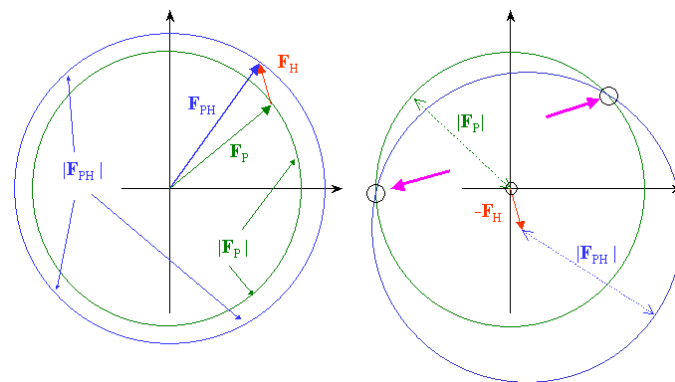
### Difference Patterson Map $(F_{hkl})^2$



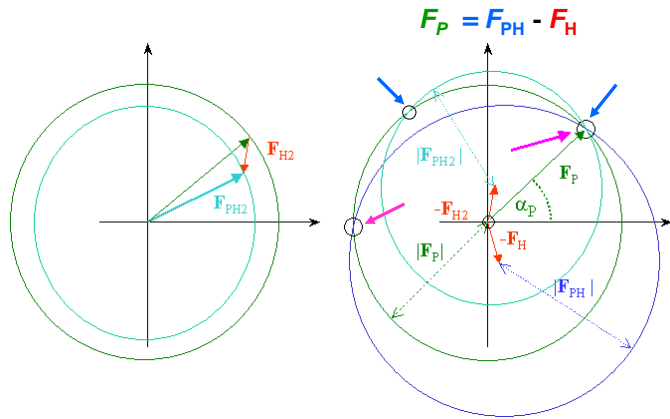
### Multiple Isomorphous Replacement (MIR) method

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

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



## Multiple Isomorphous Replacement (MIR) method



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 a **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.

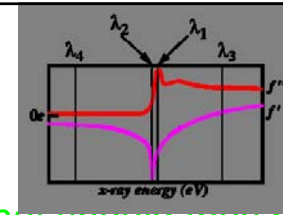
## "Multiwavelength Anomalous Dispersion" (MAD) methods

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  $f''$  ( $\lambda_1$  in the figure above).
- The second wavelength is usually chosen to have maximal  $|f''|$  ( $\lambda_2$  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  $\lambda_3$  and  $\lambda_4$  are between 100eV and 1000eV from the absorption edge.

# X-Ray Crystallography

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

Topics:

## 1. Protein Data Bank (PDB)

Data mining and Protein Structure Analysis Tools

## 2. Image Formation

Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

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

- Crystal Growth – Materials / Methods
- Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit
- X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron
- Theory of Diffraction – Bragg’s Law / Reciprocal Space
- Data Collection – Methods / Detectors / Structure Factors
- Structure Solution – Phase Problem: MIR / MR / MAD
- Refinement, Analysis and Presentation of Results
- Use of Difference Fouriers

# Least-Squares Refinement

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

$$+ \sum_{r=1}^m w_r \frac{\partial |kF_{c,r}|}{\partial p_1} \frac{\partial |kF_{c,r}|}{\partial p_n} \Delta p_n = \sum_{r=1}^m w_r \Delta F_r \frac{\partial |kF_{c,r}|}{\partial p_1}$$

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

$$+ \sum_{r=1}^m w_r \frac{\partial |kF_{c,r}|}{\partial p_2} \frac{\partial |kF_{c,r}|}{\partial p_n} \Delta p_n = \sum_{r=1}^m w_r \Delta F_r \frac{\partial |kF_{c,r}|}{\partial p_2}$$

$$\vdots$$

$$\sum_{r=1}^m w_r \frac{\partial |kF_{c,r}|}{\partial p_n} \frac{\partial |kF_{c,r}|}{\partial p_1} \Delta p_1 + \sum_{r=1}^m w_r \frac{\partial |kF_{c,r}|}{\partial p_n} \frac{\partial |kF_{c,r}|}{\partial p_2} \Delta p_2 + \dots$$

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

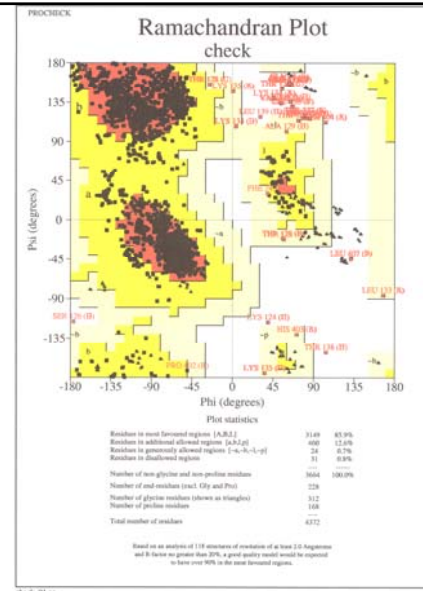
# Energy Refinement

(Simulated Annealing)

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

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

$$E_{EMPIRICAL} = \sum_{p=1}^N [W^p_{BOND} E_{BOND} + W^p_{ANGL} E_{ANGL} + W^p_{DIHE} E_{DIHE} + W^p_{IMPR} E_{IMPR} + W^p_{VDW} E_{VDW} + W^p_{ELEC} E_{ELEC} + W^p_{PVDW} E_{PVDW} + W^p_{PELE} E_{PELE} + W^p_{HBON} E_{HBON}]$$





### Crystal Structure of *M. tuberculosis* Alanine Racemase

Table 1: Data Collection and Processing Statistics for the MAD and Native Data Sets of Alr<sub>166</sub>

	MAD 1	MAD 2	MAD 3	MAD 4	native
$\lambda$ (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	432376	446744	431524	336135	779600
observed $> 1\sigma$					
no. of unique reflections $> 1\sigma$	35817	37506	36020	36242	67592
$R_{\text{merge}}^a$ (%)	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)
( $I/\sigma$ )	30.3	34.3	41.6	50.9	34.5 (2.6)

$$^a R_{\text{merge}} = \sum |I_{\text{obs}} - I_{\text{avg}}| / \sum I_{\text{avg}}$$

Table 2: Final Refinement Statistics for Alr<sub>166</sub> at 1.9 Å Resolution

$R$ factor <sup>a</sup> (%)	20.4
$R_{\text{free}}$ (%) (for 1747 reflections)	25.4
average $B$ factor (Å <sup>2</sup> ) <sup>b</sup>	
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 $> 2\sigma$	55001
no. of residues	722
no. of protein atoms	5360
no. of PLP atoms	30
no. of water molecules	350

$$^a R\text{-factor} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum |F_{\text{obs}}|, ^b \text{All isotropic model.}$$

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The 1.9 Å Crystal Structure of Alanine Racemase from *Mycobacterium tuberculosis* Contains a Conserved Entryway into the Active Site<sup>1,2</sup>

Pierre LeMagueres,<sup>3</sup> Hockang Im,<sup>3</sup> Jerry Dhanasek,<sup>3</sup> Ulrich Storch,<sup>3</sup> Michael J. Benedik,<sup>4</sup> James M. Briggs,<sup>3</sup> Harold Kohn,<sup>1</sup> and Kurt L. Wilson<sup>1,4\*</sup>

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## Difference Fourier

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

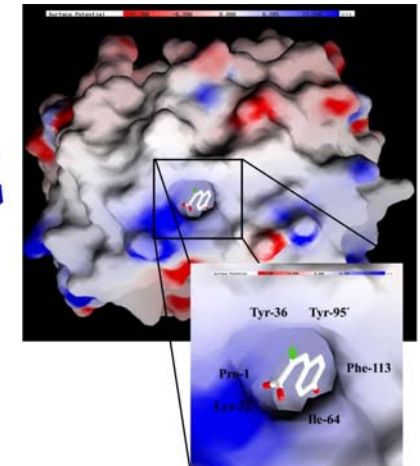
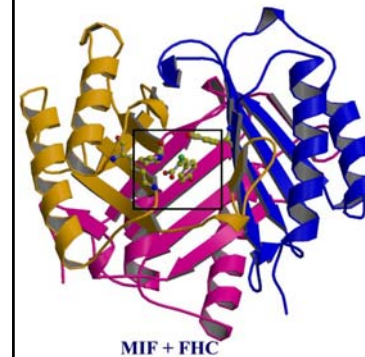
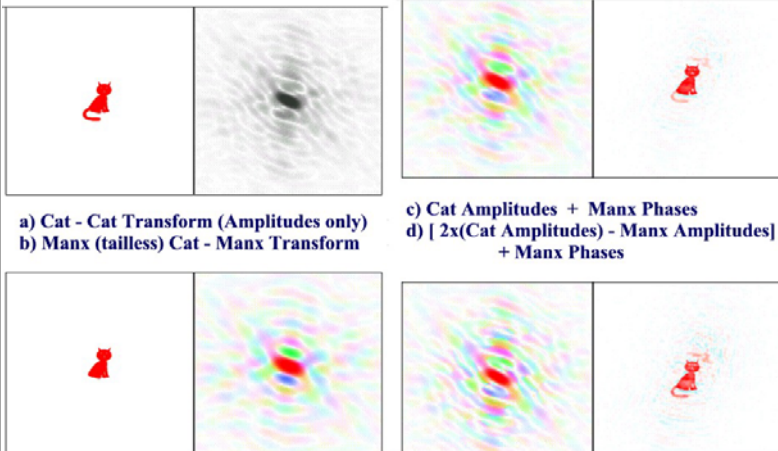
$$\text{Calc. } \rho_c(x, y, z) = \frac{1}{V} \sum_h \sum_k \sum_l F_{c,hkl} e^{-2\pi i(hx+ky+lz)} + R'$$

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

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

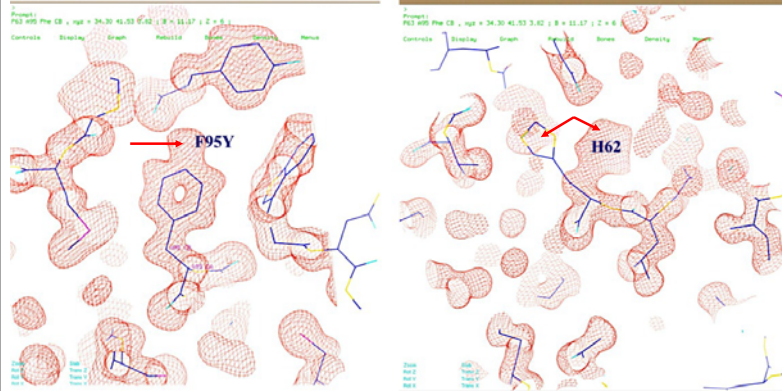
## Kevin Cowtan's Book of Fourier

<http://www.yzbl.york.ac.uk/~cowtan/fourier/fourier.html>



## Examples of Difference Fourier

### 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