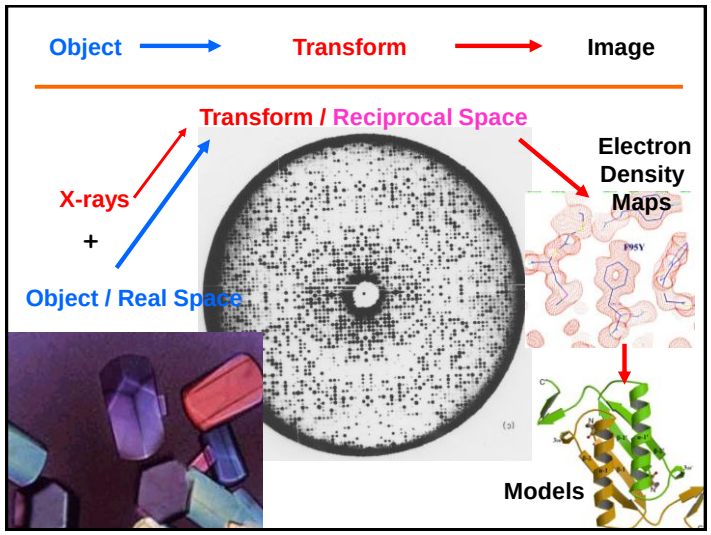


X-Ray Crystallography

"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 ([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

X-ray tubes: the "sealed" tube

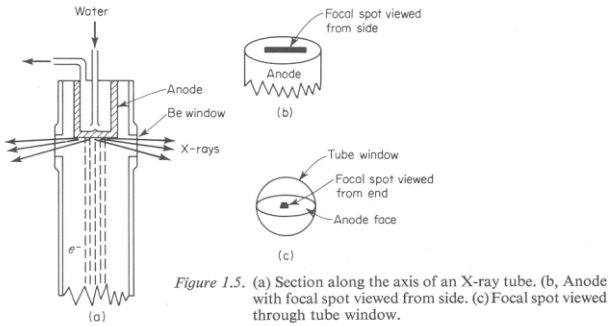
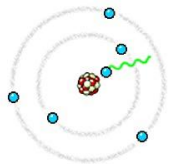


Figure 1.5. (a) Section along the axis of an X-ray tube. (b, Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

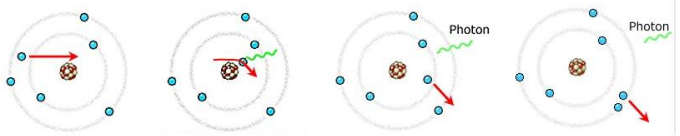
Origin of Non-characteristic X-rays



The electron (much lighter than the nucleus) comes very close to the nucleus and the electromagnetic interaction causes a deviation of the trajectory where the electron looses energy and an X-ray photon is emitted.

Bremsstrahlung X-rays

In an X-ray tube the electrons emitted from the anode are accelerated towards the metal target cathode by an accelerating voltage of typically 50 kV. The high energy electrons interact with the atoms in the metal target. Sometimes the electron comes very close to a nucleus in the target and is deviated by the electromagnetic interaction. In this process, which is called bremsstrahlung (braking radiation), the electron looses much energy and a photon (X-ray) is emitted. The energy of the emitted photon can take any value up to a maximum corresponding to the energy of the incident electron.



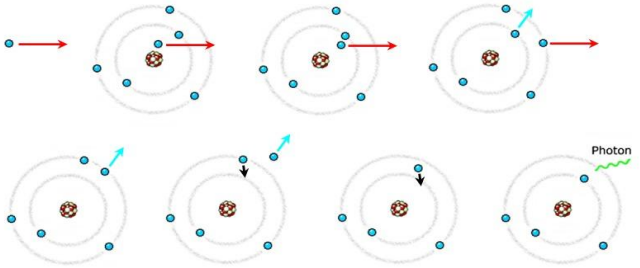
Origin of characteristic X-rays

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

$K_{\alpha_1}, K_{\alpha_2}$ $L \rightarrow K$

K_{β_1}, K_{β_2} $M \rightarrow K$

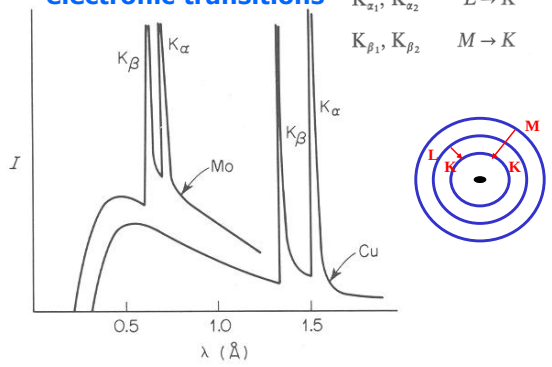


Figure 1.2. X-ray spectra with characteristic peaks: MoKα, 50 Kv; CuKα, 35 Kv.

Characteristic X-rays have defined λ

Table 1.1. Target Materials and Associated Constants

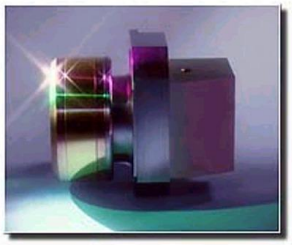
	Cr	Fe	Cu	Mo
Z	24	26	29	42
α_1, Å	2.2896	1.9360	1.5405	0.70926
α_2, Å	2.2935	1.9399	1.5443	0.71354
$\bar{\alpha}$,* Å	2.2909	1.9373	1.5418	0.71069
β_1, Å	2.0848	1.7565	1.3922	0.63225
β, filt.	V, 0.4 mil†	Mn, 0.4 mil	Ni, 0.6 mil	Nb, 3 mils
α, filt.	Ti	Cr	Co	Y
Resolution, Å	1.15	0.95	0.75	0.35
Critical 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 α_1 and α_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 Nonius' 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.

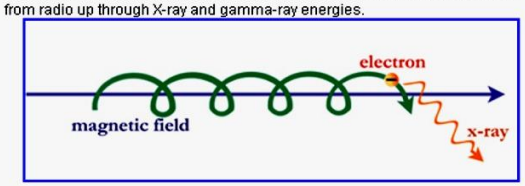
Now with the new ULTRA anode you can get 6 kW on a 0.3mm focus!

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

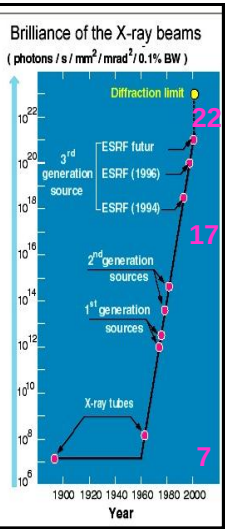
The **brilliance** of a light source is defined as the number of photons emitted per second, per unit source size, per unit space angle and for a bandwidth of 1/1000 of the photon energy

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

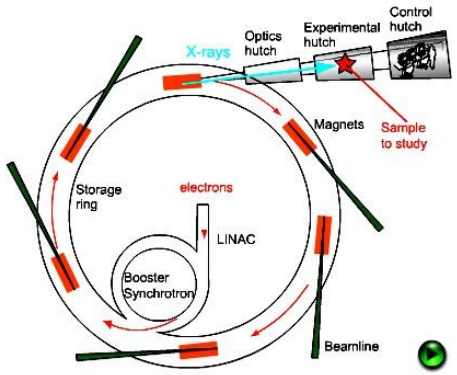
X-ray tubes:
Wilhelm Conrad Röntgen discovered X-rays in 1895 whilst working with cathode-ray tubes. Using the principle of fast electrons hitting a metallic target, a first substantial gain in brilliance was not obtained until the introduction of rotating anod sources (~1960).

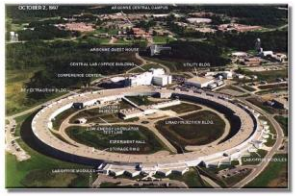
Synchrotron Radiation Facilities:
The progress of high energy physics, with the construction of powerful particle accelerators gave birth to what we now call *First* generation synchrotron sources (~1970). Using the deflection of high energy electrons by a magnetic field for the production of X-rays proved so promising that a number of dedicated *Second* generation sources were built (~1980). Relying on the combination of needle thin 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.



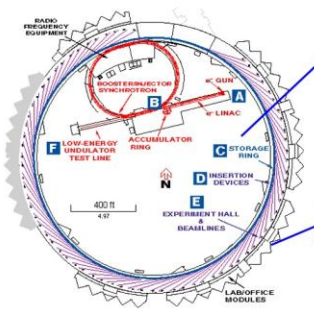
How synchrotron light is produced?

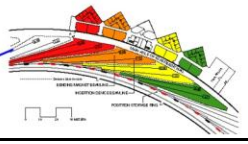




APS - Advanced Photon Source

Argonne National Laboratory





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   d)Theory of Diffraction – Bragg's Equation (take 1) / Reciprocal Space

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   h) Refinements and Models

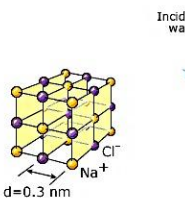
   i) Analysis and presentation of results

X-RAYS

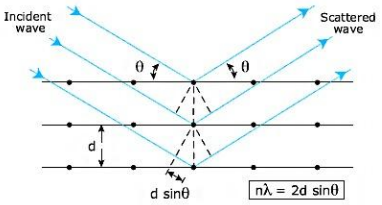
X-rays, What Are They? 6:7 — X-rays, What Are They? 7:7 »

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 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 »

Related Laureates

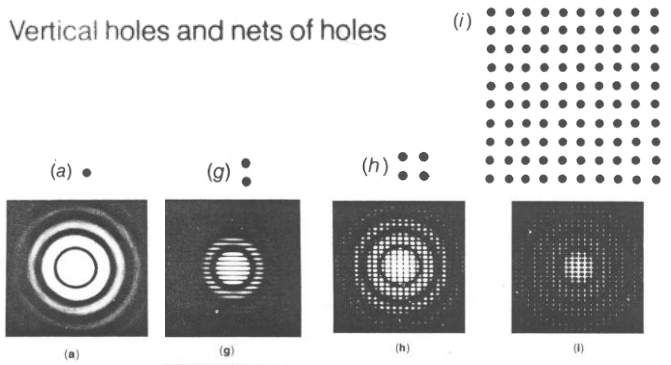
The Nobel Prize in Physics 1901 - Wilhelm Conrad Röntgen »
The Nobel Prize in Physics 1914 - Max von Laue »
The Nobel Prize in Physics 1915 - Sir William Henry Bragg »
The Nobel Prize in Physics 1915 - William Lawrence Bragg »
The Nobel Prize in Physics 1917 - Charles Glover Barkla »
The Nobel Prize 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



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

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

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

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

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

Sines / Cosines / and Expoentials

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

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

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

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

recall

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

&

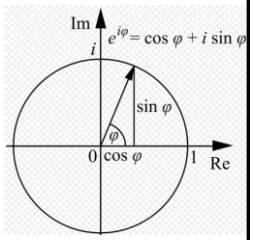
$$\sin\theta = \theta - \frac{\theta^3}{6} + \frac{\theta^5}{120} - \ldots$$

thus

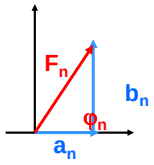
$$e^{i\theta} = \cos\theta + i\sin\theta$$

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 φ_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

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

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

Now consider **electron density** (as a function or a set of coefficients)

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

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"

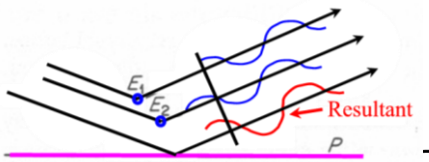
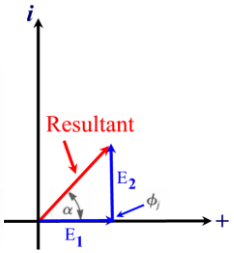


Figure 2.10. Diffraction from E_1 and E_2 as if reflected from plane P.



Represent a wave as an amplitude + phase.

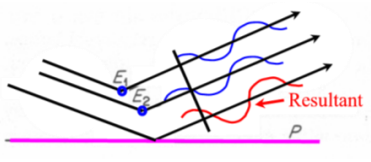
Scattering from "many atoms"

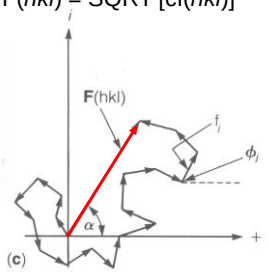
$$\mathbf{F}(hkl) = F(hkl)e^{i\alpha(hkl)} = \sum_{j=1}^{N'} \mathbf{f}_j(hkl) = \sum_{j=1}^{N'} f_j(hkl)e^{i\phi_j(hkl)}$$

Calculated

Experimental

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

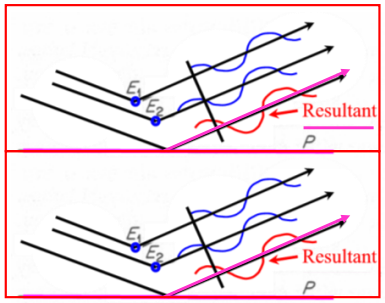




The structure factor for a reflection may be thought of as the vector sum of the x-ray scattering contributions from many atoms.

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"

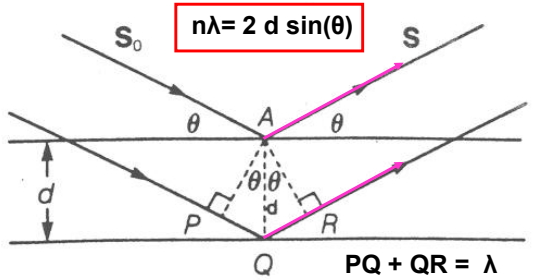


Crystals: Scattering from "planes"

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

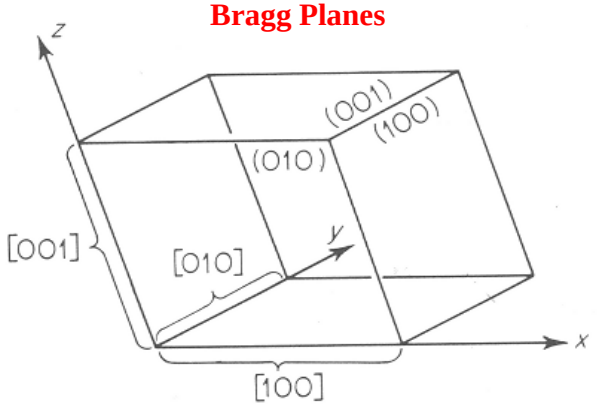


Figure 2.7. Unit cell showing bounding planes and edges.

Name that Bragg "plane"

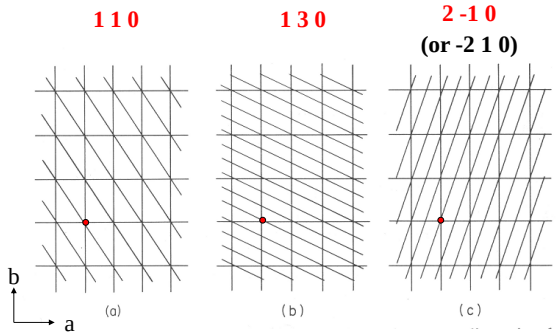
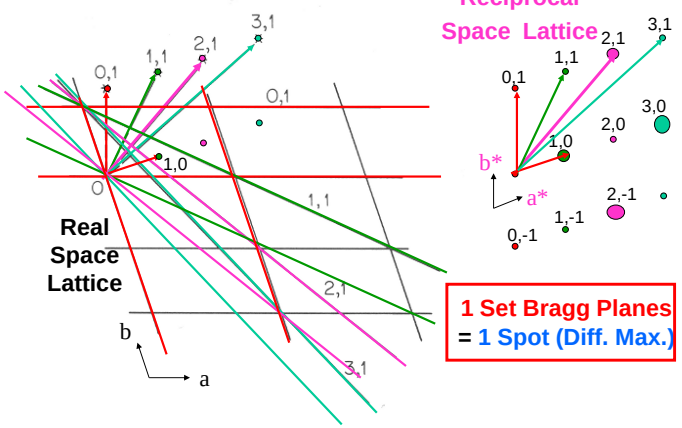
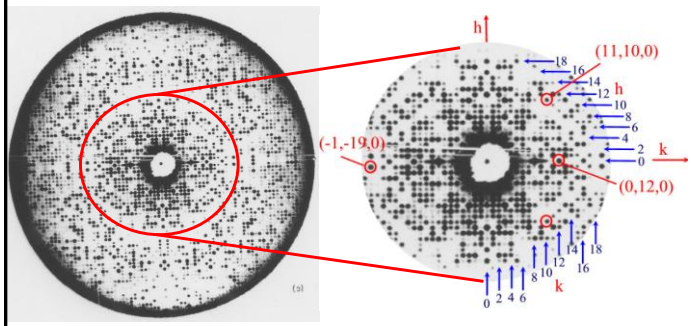


Figure 2.5. Three families of lattice "planes" in a two-dimensional lattice.



Electron Density Function

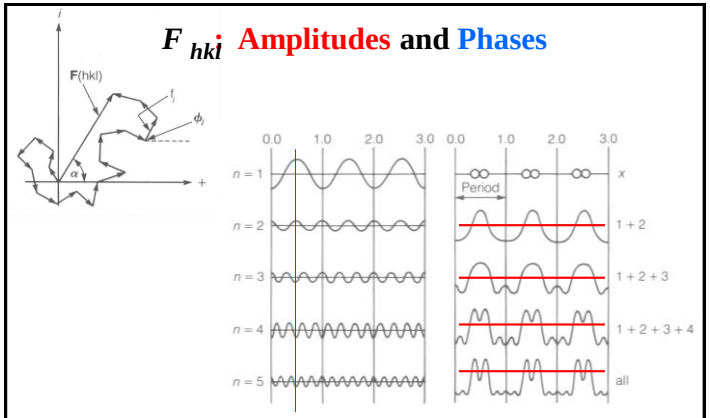
$$\rho(X,Y,Z) = \frac{1}{V}\sum_h\sum_k\sum_l F(hkl)\ \exp[i\alpha(hkl)]\exp[-2\pi i(hX + kY + lZ)]$$



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

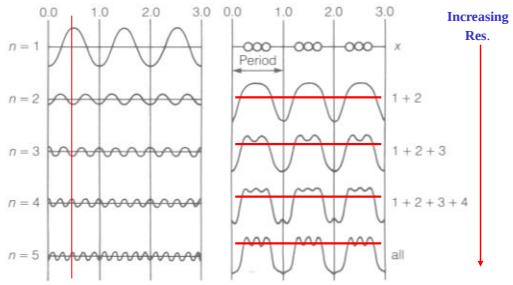
→ **Phase Problem**

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.)

X-Ray Crystallography

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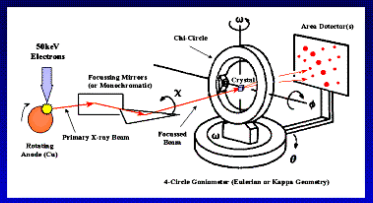
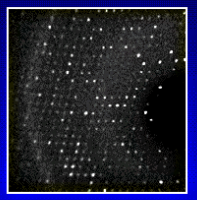
The information we get from a single diffraction experiment……



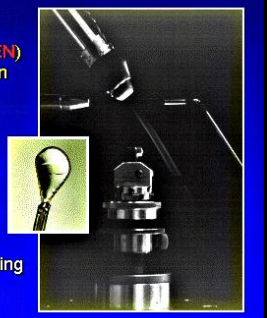


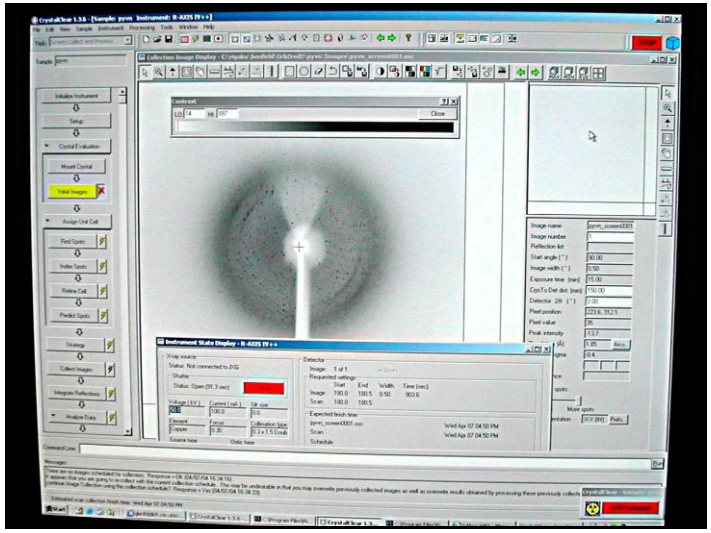
The reflections are indexed (consistent assignment of reciprocal cell indices h,k,l) and all we get for the money is a long list of intensities from several ten thousand reflections

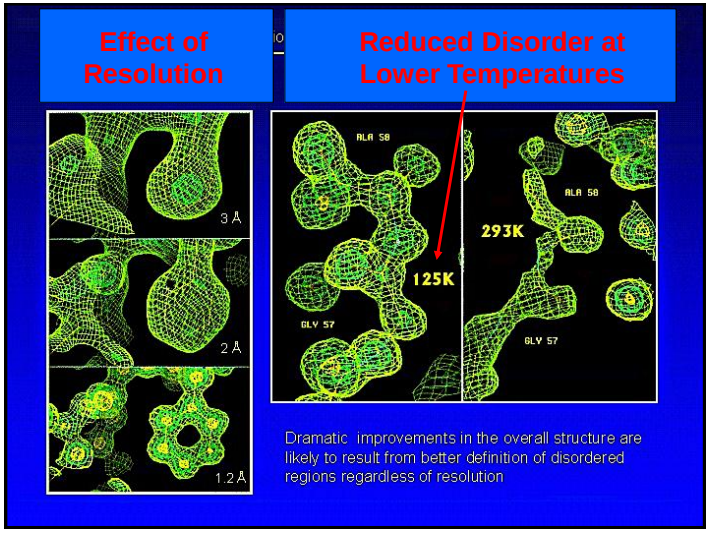
2	10	1	326.58
3	10	1	1644.72
4	10	1	3228.45
5	10	1	1279.83
6	10	1	320.48
7	10	1	775.63
8	10	1	1344.55
9	10	1	431.73
10	10	1	1760.14
11	10	1	709.18
12	10	1	20.37
13	10	1	408.72
14	10	1	51.36
15	10	1	114.72
16	10	1	776.26
17	10	1	87.57
18	10	1	30.93
0	11	1	99.30
1	11	1	2258.68
2	11	1	770.18

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







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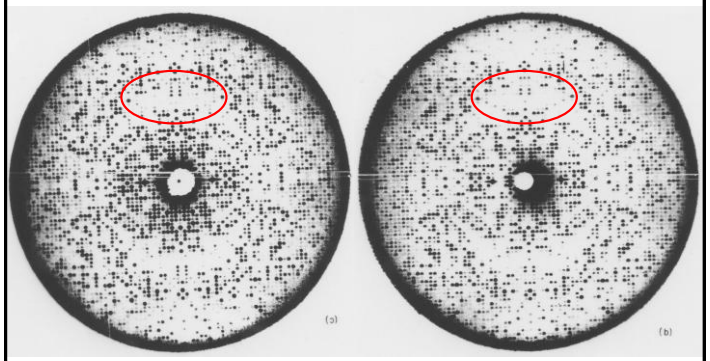
Solving the Phase Problem

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

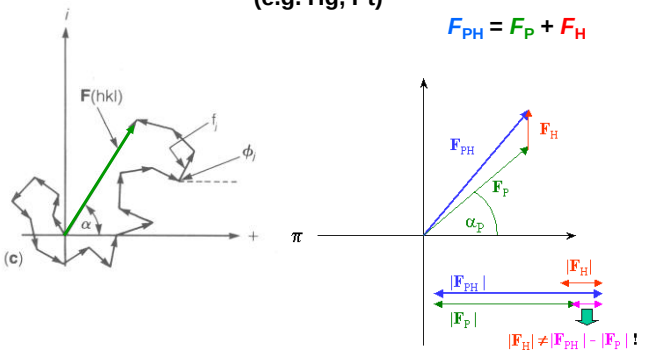


Native Phosphorylase

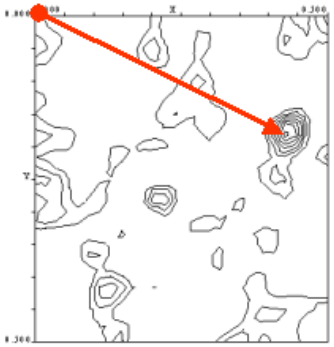
Phosphorylase + Ethyl Hg thiosalicylate

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

$F_{PH} = F_P + F_H$

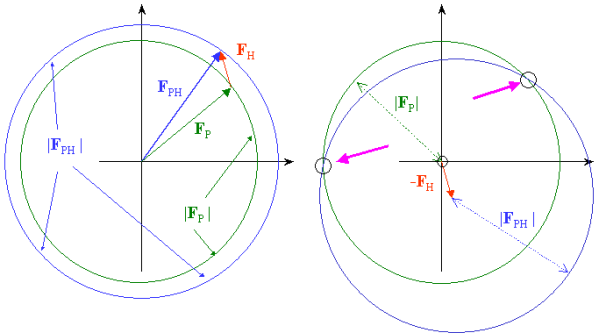


Difference Patterson Map $(F_{hkl})^2$



Multiple Isomorphous Replacement (MIR) method

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



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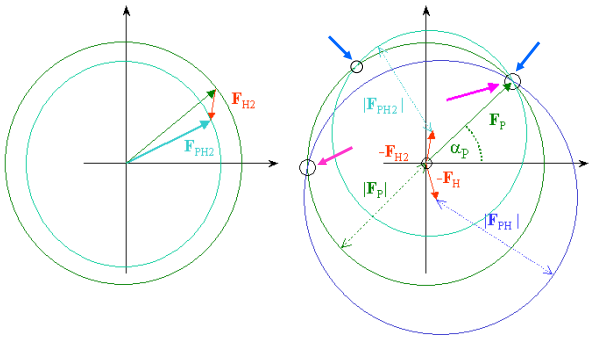


Solving the phase problem by "**Molecular Replacement**".

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"

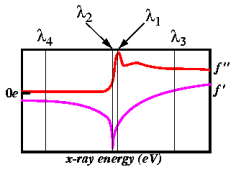
(MAD) methods

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

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

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

$$\vdots$$

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

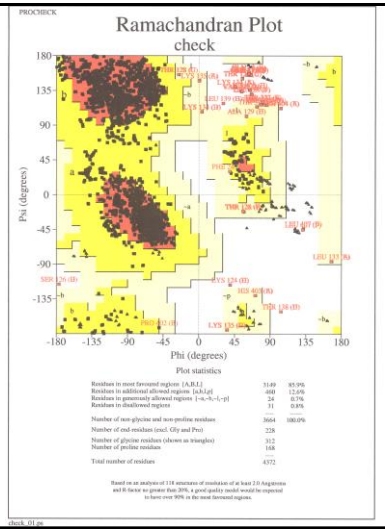
Energy Refinement

(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 $AlrR_{Mtb}$

	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†,‡

Pierre LeMagueres,§ Hookang Im,§ Jerry Ebalunode,§ Ulrich Strych,§ Michael J. Benedik,§ James M. Briggs,§ Harold Kohn,*,§ and Kurt L. Krause*,§,⊥

Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204-5001, Department of Biology, Texas A&M University, College Station, Texas 77843-3258, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360, and Section of Infectious Diseases, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

Received June 27, 2004; Revised Manuscript Received October 22, 2004

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

R factor[a] (%)	20.4
R_{free} (%) (for 1747 reflections)	25.4
average *B* factor (Å²)[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.

Difference Fourier

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

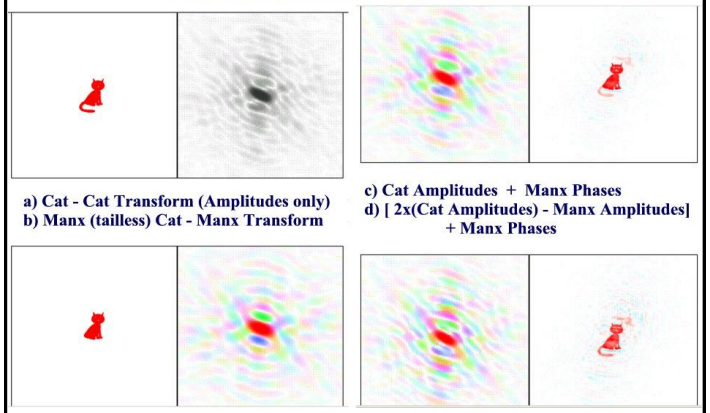
Calc. $$\rho_c(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l F_{c,hkl}\, e^{-2\pi i(hx+ky+lz)} + R'$$

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

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

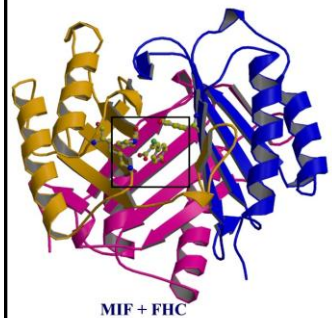
Kevin Cowtan's Book of Fourier

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

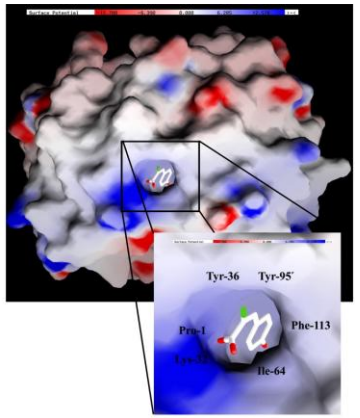


a) Cat - Cat Transform (Amplitudes only)
b) Manx (tailless) Cat - Manx Transform

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



MIF + FHC



Examples of Difference Fouriers