Crystallography

2014 international year of crystallography

Topics:

1. **Image Formation** *(what we see is not always accurate)*
   - **Resolution / Wavelength (Amplitude, Phase) / Diffraction & Interference**
   - **Light Microscopy / EM / X-ray / (NMR)**
2. **Protein Data Bank (PDB)**
   - **Data mining and Protein Structure Analysis Tools**
3. **IYCr 2014 - Celebrating 100 years of X-ray Crystallography**
4. **X-Ray Crystallography – practical aspects**
   - **a) Crystal Growth – Materials / Methods**
   - **c) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit**
   - **d) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron**
   - **e) Data Collection – Methods / Detectors / Structure Factors / Computers**
   - **f) Theory of Diffraction – Bragg's Law / Reciprocal Space**
   - **g) Structure Solution – Phase Problem: MIR / MR / MAD**
   - **h) Refinements and Models / Analysis and presentation of results**

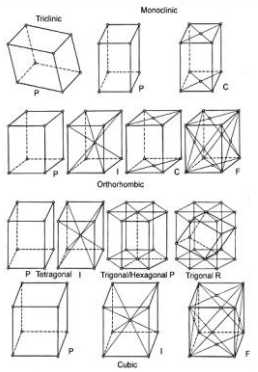
Crystal Systems

Crystal System	Bravais Type(s)	External Minimum Symmetry	Unit Cell Properties
Triclinc	P	None	a, b, c, al, be, ga,
Monoclinic	P, C	One 2-fold axis, parallel b (b unique)	a, b, c, 90, be, 90
Orthorhombic	P, I, F	Three perpendicular 2-folds	a, b, c, 90, 90, 90
Tetragonal	P, I	One 4-fold axis, parallel c	a, a, c, 90, 90, 90
Trigonal	P, R	One 3-fold axis	a, a, c, 90, 90, 120
Hexagonal	P	One 6-fold axis	a, a, c, 90, 90, 120
Cubic	P, F, I	Four 3-folds along space diagonal	a, a, ,a, 90, 90, 90

Symmetry operations : 1,2,3,4,6, -1,-2,-3,-4,-6, m

Crystal System	Point groups	Laue Class	Patterson Symmetry
Triclinc	1, -1	-1	P-1
Monoclinic	2, m, 2/m	2/m	P2/m, C2/m
Orthorhombic	222, mm2 , mmm	mmm	Pmmm, Cmmm, Fmmm, Immm
Tetragonal	4, -4, 4/m, 422, 4mm, -42m, 4/mmm	4/m, 4/mmm	P4/m, I4/m, P4/mmm, I4/mmm
Trigonal	3, -3, 32, 3m, -3 m	-3, -3m	P-3, R-3, P-3m1, P-31m, R-3m
Hexagonal	6, -6, 6/m, 622, 6mm, -62m, 6/mmm	6/m, 6/mmm	P6/m, P6/mmm
Cubic	23, m-3, 432, -43m, m3m	m-3, m-3m	Pm-3, Im-3, F-3m, Pm-3m, Fm-3m, Im-3m

The 14 Bravais Lattices



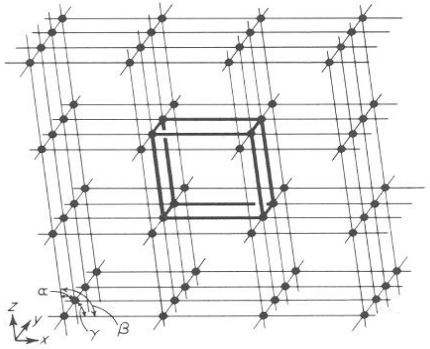
Notes

Note: Only 2-, 3-, 4-, and 6-fold rotations allowed

- Laue class corresponds to symmetry of reciprocal space (diffraction pattern)
- Patterson symmetry is Laue class plus allowed Bravais centering, i.e. centrosymmetric and symorphic

A unit cell is defined by its lattice constants:

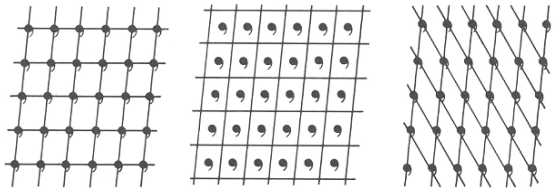
a, b, c and α, β, γ



Three-dimensional lattice, showing unit cell (heavy lines).

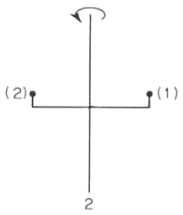
How to identify "the" unit cell ?

Regular two-dimensional array.

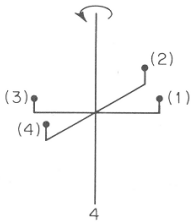


Three different grid systems referred to the array same array.

How to identify "the" unit cell ? Role of Symmetry



A 2-fold rotation axis.



A 4-fold rotation axis.

	2-fold	3-fold	4-fold	6-fold
Rotation axes:	180°	120°	90°	60°
Translations:	1/2	1/3	1/4	1/6

Rot + Trans (Screw Axes) / Mirror / Inversion

How to identify "the" unit cell ? Role of Symmetry

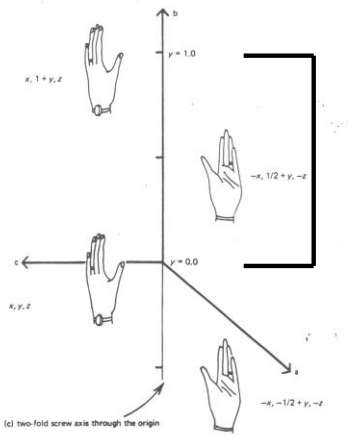
Screw Axes
(rotation + translation)

2-fold screw
180° + 1/2

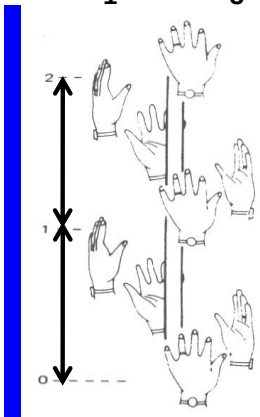
3-fold screw
120° + 1/3

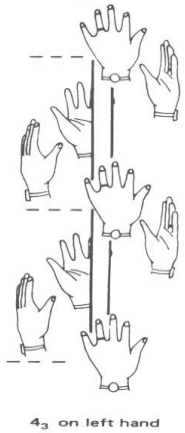
4-fold screw
90° + 1/4

6-fold screw
60° + 1/6



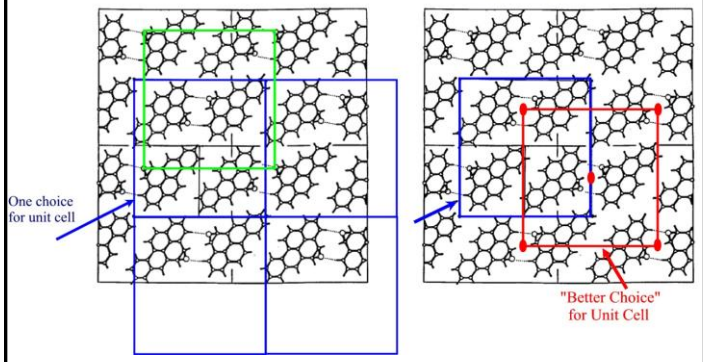
4_1 and 4_3 Screw Axis





We will only look at examples having only 2-fold (2) or 2-fold screw (2_1) axes.

Unit Cell Selection is Based on Symmetry



M.C. Escher

[Escher Sketch]

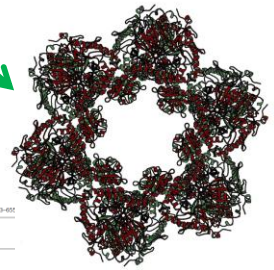
The crystal structure of silver chromate

Marvin L. Hackert[1,2], Robert A. Jacobson[1,2]
[a] Institute for Atomic Research, Iowa State University, Ames, Iowa 50010 USA
[b] Department of Chemistry, Iowa State University, Ames, Iowa 50010 USA

Ag_2CrO_4

Pnma a = 10.06, b = 7.03, c = 5.54; **V = 392 A^3**
*"**325** observed reflections used in the analysis"*

~ 10,000 X



J. Mol. Biol. (1995) 252, 643–655

JMB

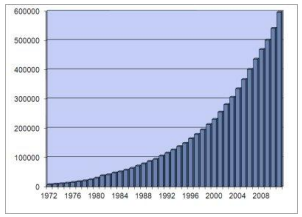
Crystallographic Structure of a PLP-Dependent Ornithine Decarboxylase from *Lactobacillus* 30a to 3.0 Å Resolution

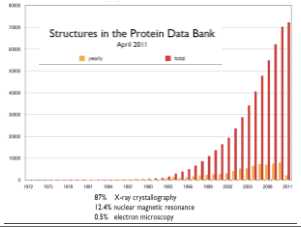
Cory Momany, Stephen Ernst, Ratna Ghosh, Ning-Leh Chang and Marvin L. Hackert*

OrnDC from *L30a* (dodecamer 8760 aa)
P6 a = 195.6, c = 97.6; **V = 3,230,000 A^3**

A Half Century of Advances in Small Molecule Crystallography

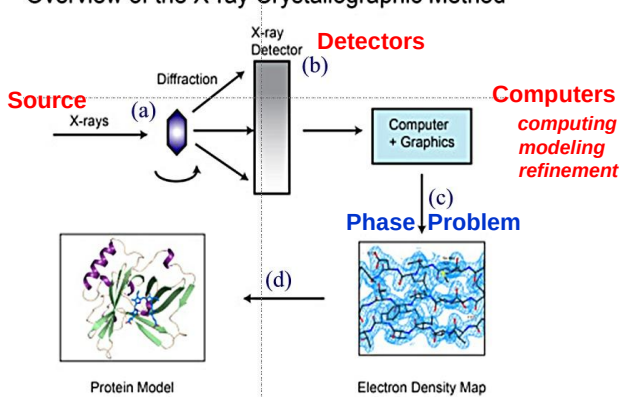
Decade	1950's	1960's	1970's	1980's	1990's	2000's
Size	30 atoms	<100 non-H atoms	<200 non-H atoms	<400 non-H atoms	<600 non-H atoms	<1000 non-H atoms
Time / Structure	~ 1year	3-6 months	1 month	1 week	Few days	Few hours
# Structures	<500	~8300	32,000	95,000	229,000	528,000





Advances in Crystallography - What happened?

Overview of the X-ray Crystallographic Method



X-ray Sources:

X-ray tubes: the "sealed" tube

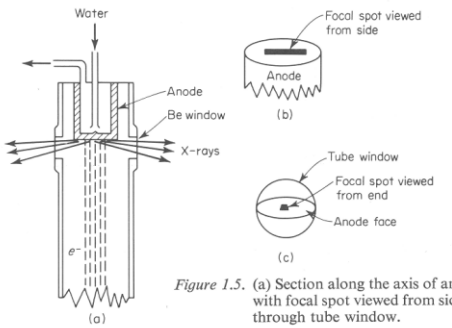


Figure 1.5. (a) Section along the axis of an X-ray tube. (b, Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

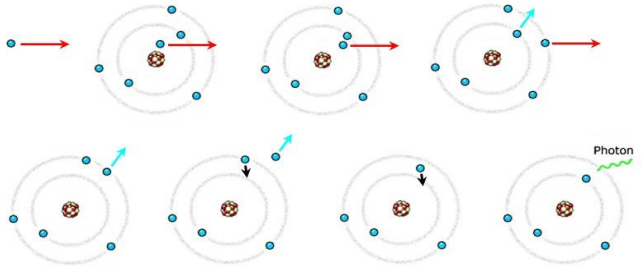
Origin of characteristic X-rays

Related Laureate

The Nobel Prize in Physics 1917 - Charles Glover Barkla »

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.



Characteristic X-rays arise from electronic transitions

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

K_{β_1}, K_{β_2} $M \rightarrow K$

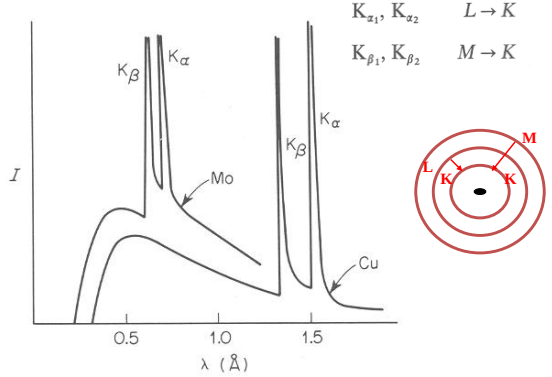


Figure 1.2. X-ray spectra with characteristic peaks: MoKα, 50 Kv; CuKα, 35 Kv.

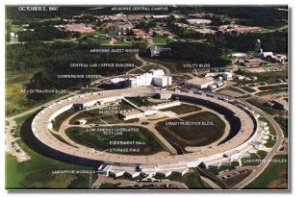
Characteristic X-rays have defined λ

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

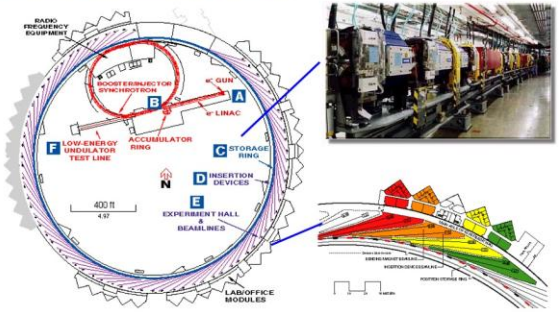
* $\bar{\alpha}$ is the intensity-weighted average of α_1 and α_2 and is the figure usually used for the wavelength when the two lines are not resolved.

† 1 mil = 0.001 inch = 0.025 mm.



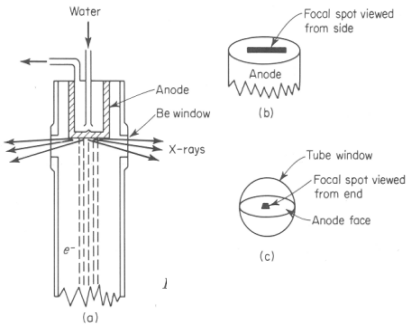
APS - Advanced Photon Source

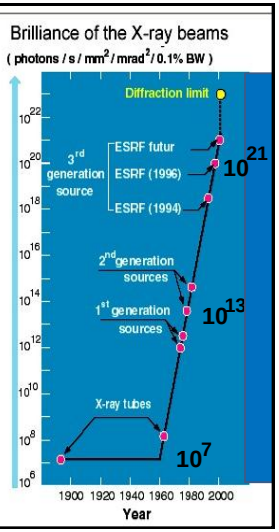
Argonne National Laboratory



X-ray Sources:

the "sealed" tube

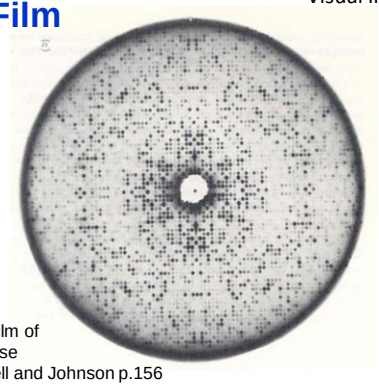




Detectors

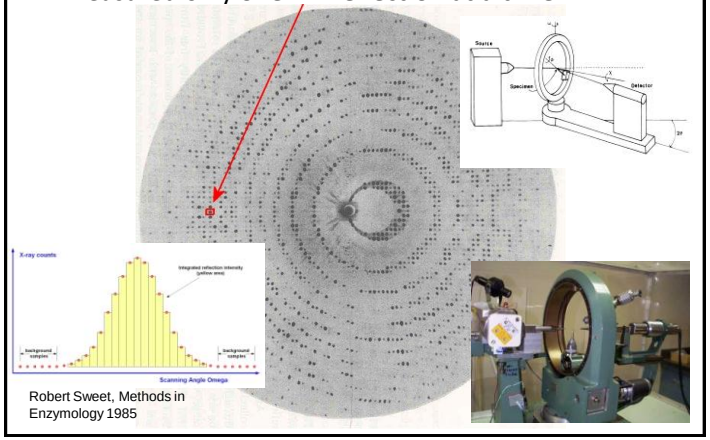
Film

Visual intensity estimation



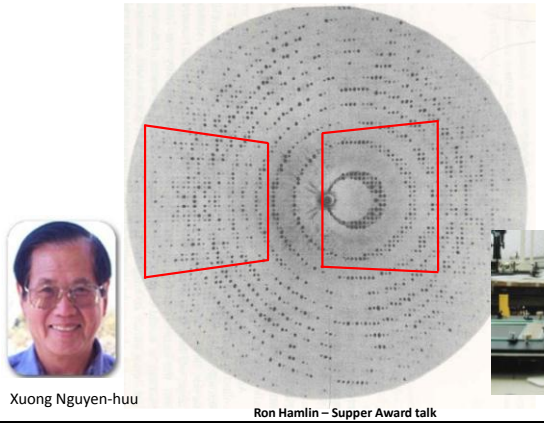
Precession film of Phosphorylase
From Blundell and Johnson p.156

Diffractometer: automatic but measured only **one hkl reflection at a time**



Robert Sweet, Methods in Enzymology 1985

Area Detectors: Typical coverage of diffraction pattern by a pair of ADSC multiwire detectors



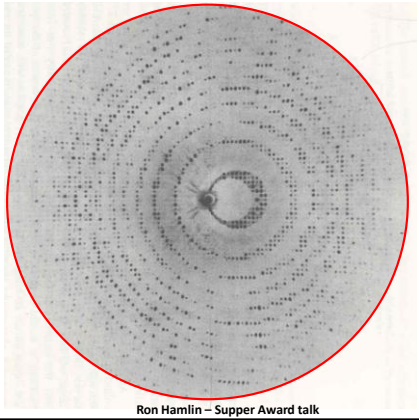
Xuong Nguyen-huu

Ron Hamlin

Ron Hamlin – Supper Award talk

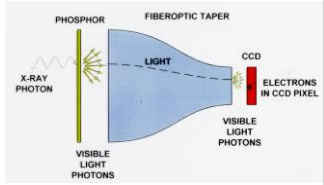
Image Plate Detectors

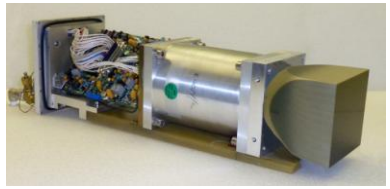
brute force solid angle coverage



Ron Hamlin – Supper Award talk

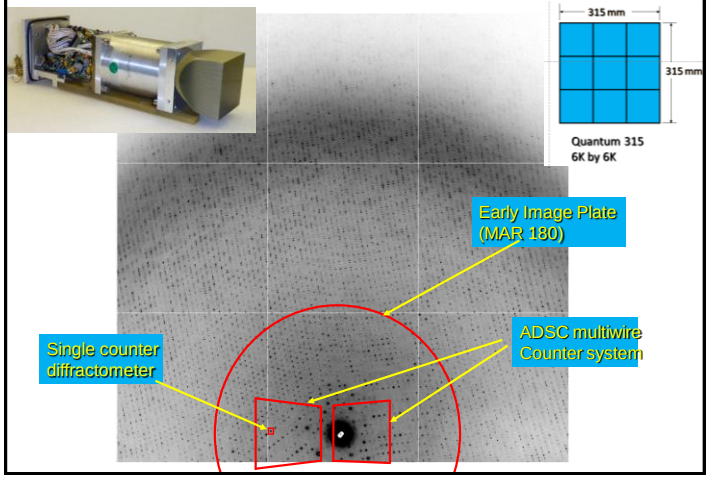
Fiberoptic Tapers / CCD Dectors



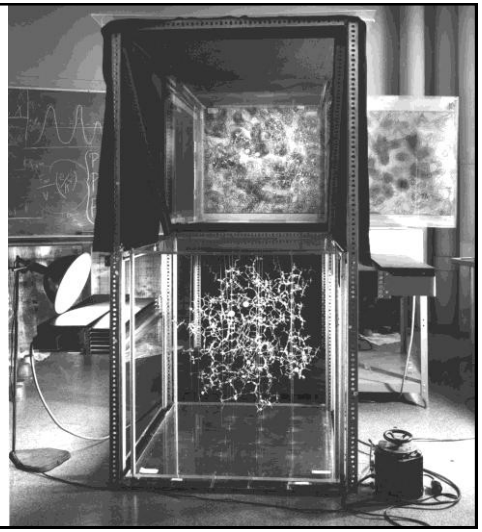


Ron Hamlin – Supper Award talk

CCD Detectors: Diffraction pattern from a Quantum 315



Computers
~ 1960s



Computers
~late 1970s

Computer - $100,000

Display - $100,000

Computers

Crystallography

2014 international year of crystallography

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   - **Light Microscopy / EM / X-ray / (NMR)**
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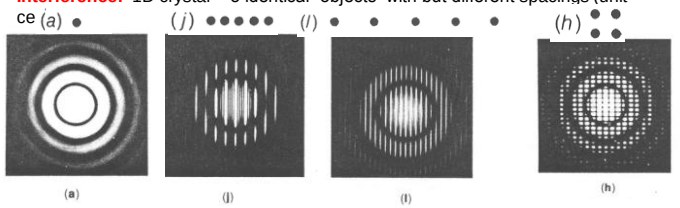
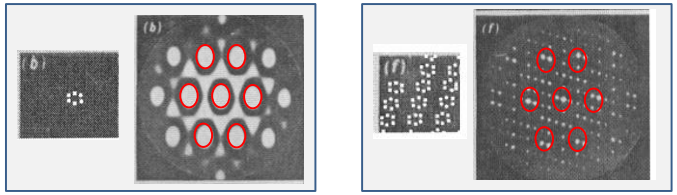
Wave Properties: Diffraction / Interference

Interference: 1D crystal - 5 identical "objects" with but different spacings (unit ce

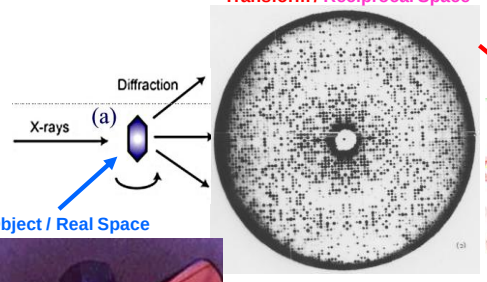


Diffraction + Interference: 2D Crystal

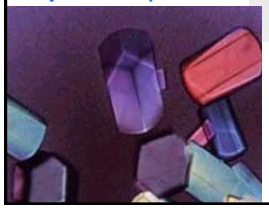


Object → **Transform** → **Image**

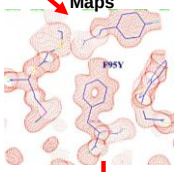
Transform / Reciprocal Space

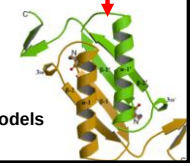


Object / Real Space



Electron Density Maps

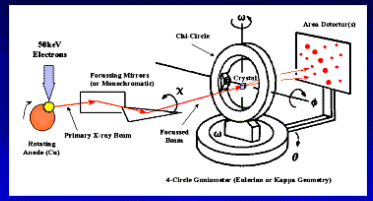


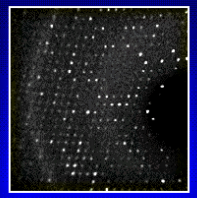


Models

Advanced Methods in Modern Biomolecular Crystallography

The information we get from a single diffraction experiment……

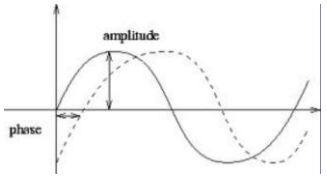


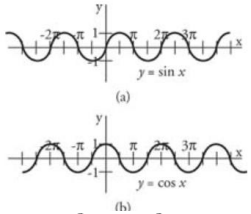


The reflections are indexed (consistent assignment of reciprocal cell indices h,k,l) and all we get for the money is a long list of intensities from several ten thousand reflections

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

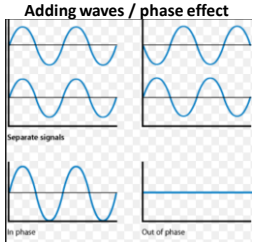
Waves (Amplitudes & Phases)

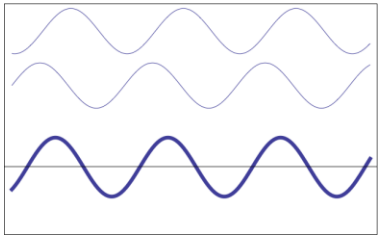


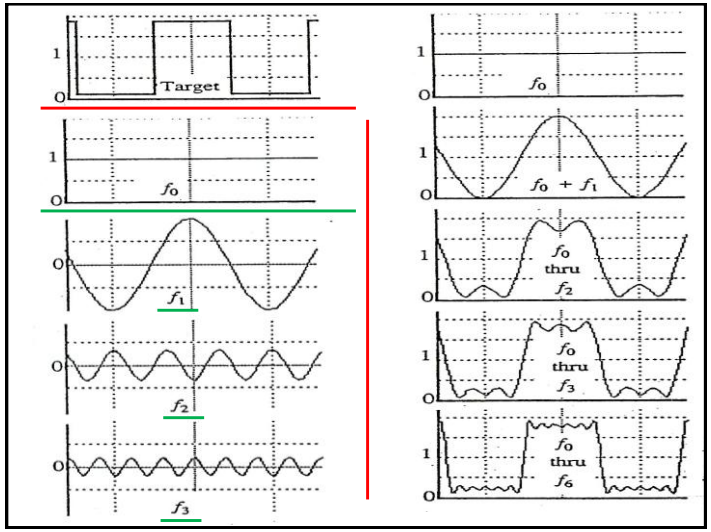


$$\sin^2\theta + \cos^2\theta = 1$$

Adding waves / phase effect







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

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

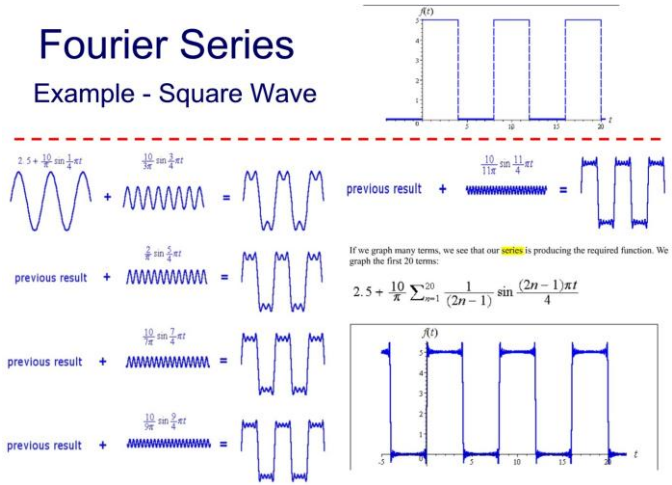
$$a_0 = \frac{1}{L}\int_{-L}^{L} f(t)\,dt$$

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

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

Fourier Series

Example - Square Wave

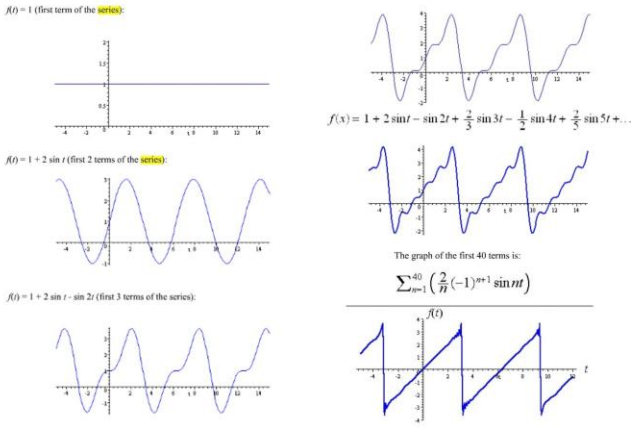


If we graph many terms, we see that our series is producing the required function. We graph the first 20 terms:

$$2.5 + \frac{10}{\pi}\sum_{n=1}^{20}\frac{1}{(2n-1)}\sin\frac{(2n-1)\pi t}{4}$$

Example - Saw Tooth Function

$f(t) = 1 + 2\sin t - \sin 2t + \frac{2}{3}\sin 3t$



Fourier Series Applet http://www.falstad.com/fourier/

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

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

&

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

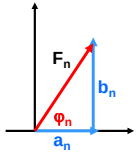
thus

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

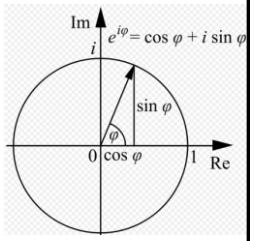
Euler's Equation

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
3	5	5	7.1	??
4	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)**

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

Real Space — Reciprocal Space

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"

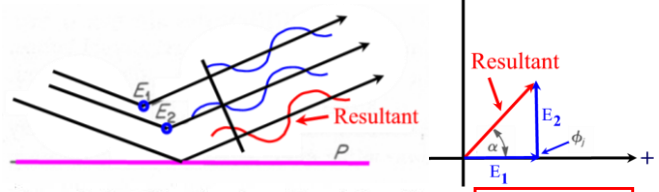


Figure 2.10. Diffraction from E_1 and E_2 as if reflected from plane P.

Represent a wave as an amplitude + phase.

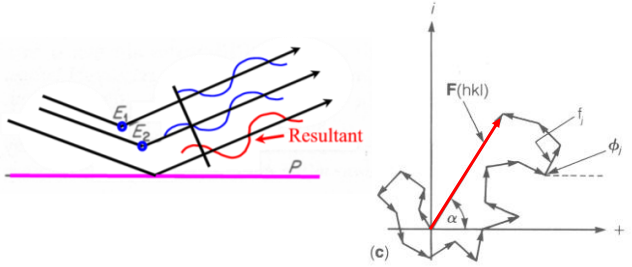
Scattering from "many atoms"

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

Calculated

Experimental

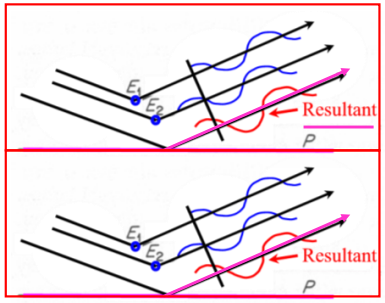
F(*hkl*) = SQRT [cI(*hkl*)]



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.

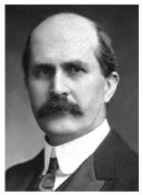
Scattering from "atoms in two unit cells"



The Nobel Prize in Physics 1915
William Bragg, Lawrence Bragg

2014 international year of crystallography

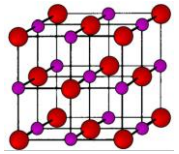
The Nobel Prize in Physics 1915



Sir William Henry Bragg

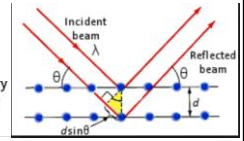
William Lawrence Bragg

X-ray apparatus



Sodium Chloride (NaCl)

The Nobel Prize in Physics 1915 was awarded jointly to Sir William Henry Bragg and William Lawrence Bragg *"for their services in the analysis of crystal structure by means of X-rays"*

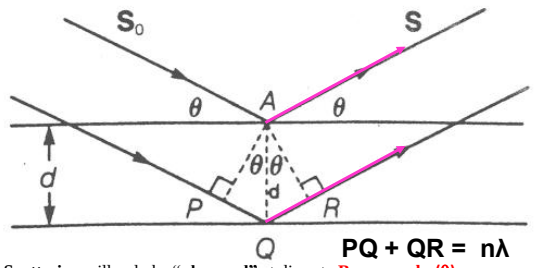


Bragg's Law ($n\lambda = 2d \sin\theta$)

Crystals: Scattering from "planes"

Resultant scattering of resultant scattering!

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

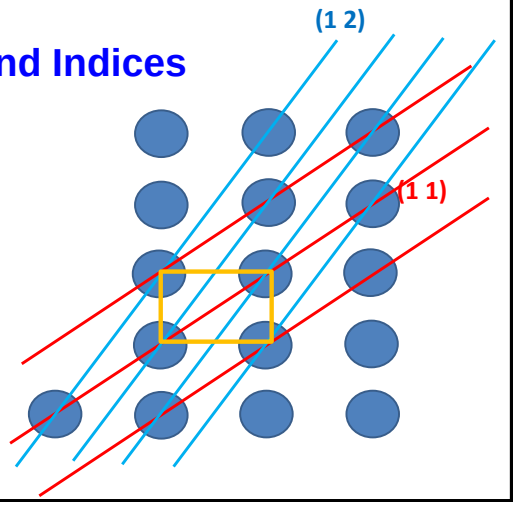


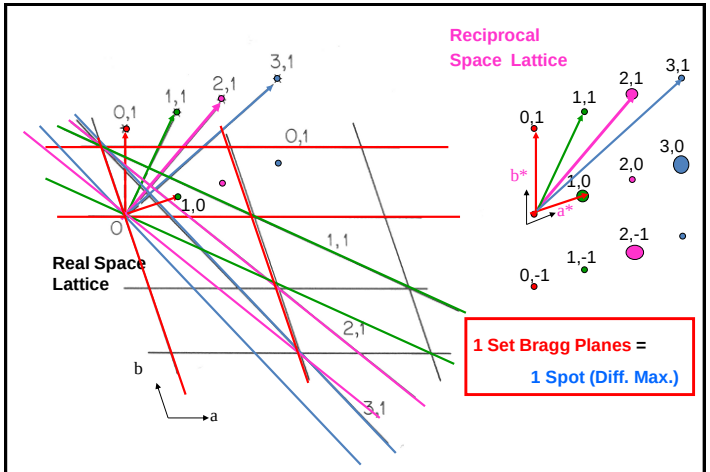
$PQ + QR = n\lambda$

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

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

Planes and Indices

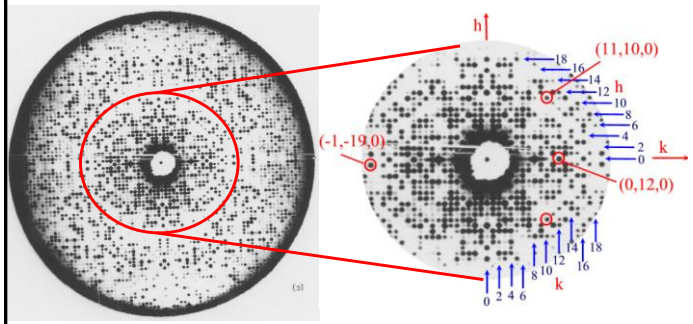




Planes in direct space represented by **points in reciprocal space**.

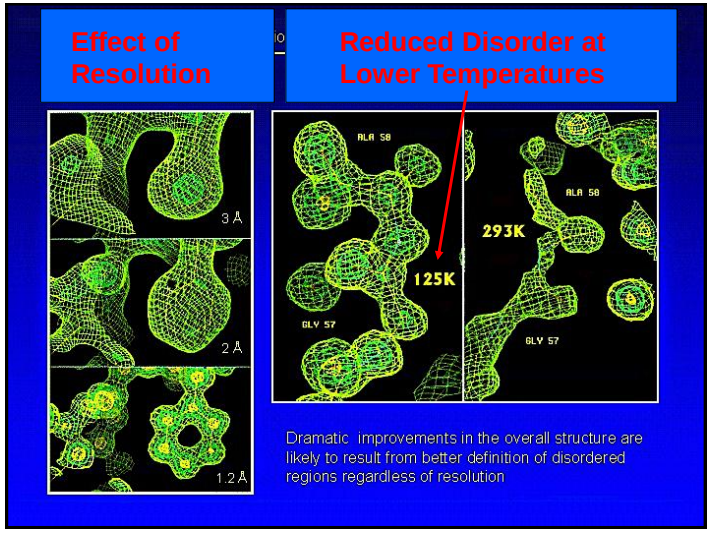
Electron Density Function

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



Measure thousands of **Amplitudes** - [Fhkl]'s - ?? How do we obtain **Phases** αhkl ??

→ **Phase Problem**



Crystallography

2014 international year of crystallography

Topics:

1. **Image Formation** *(what we see is not always accurate)*
   - Resolution / Wavelength (Amplitude, Phase) / Diffraction & Interference
   - Light Microscopy / EM / X-ray / (NMR)
2. **Protein Data Bank (PDB)**
   - Data mining and Protein Structure Analysis Tools
3. **IYCr 2014 - Celebrating 100 years of X-ray Crystallography**
4. **X-Ray Crystallography – practical aspects**
   - a) Crystal Growth – Materials / Methods
   - c) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit
   - d) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron
   - e) Data Collection – Methods / Detectors / Structure Factors / Computers
   - f) Theory of Diffraction – Bragg's Law / Reciprocal Space
   - g) Structure Solution – Phase Problem: MIR / MR / MAD
   - h) Refinements and Models / Analysis and presentation of results

Solving the Phase Problem

Early Days:

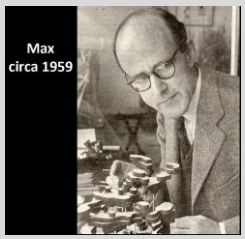
Centric structures (all phases 0º or 180º) Heavy atom / Patterson method

Macromolecular Crystallography

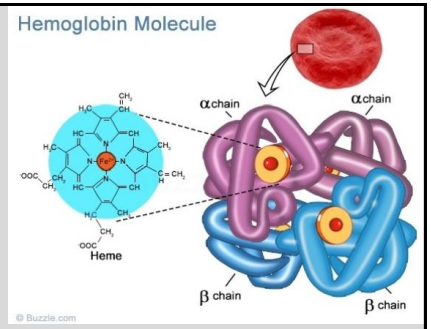
1. **MIR:** Multiple Isomorphous Replacement (Heavy Atom)
2. **MR:** Molecular Replacement
3. **MAD:** Multiwavelength anomolous dispersion

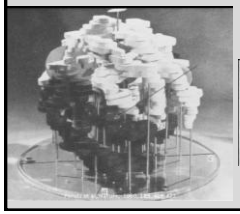
****

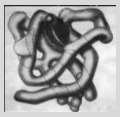
- **Molecular Modeling** (predicting starting structure from sequence alone)

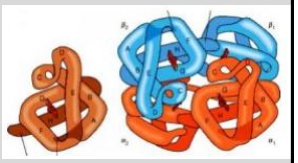


The first protein structures were of myoglobin and hemoglobin ~ 1960.

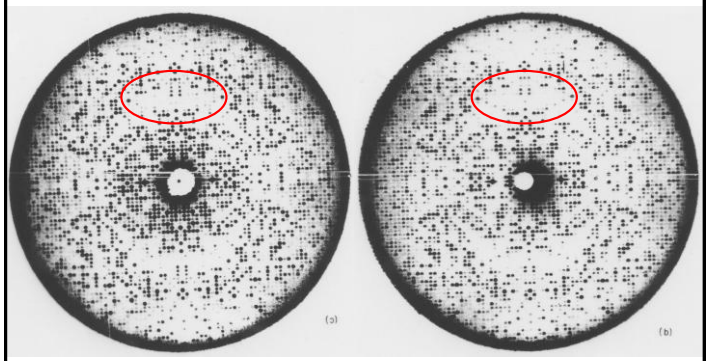








Use of Heavy Metal Ions for Phasing by MIR Methods



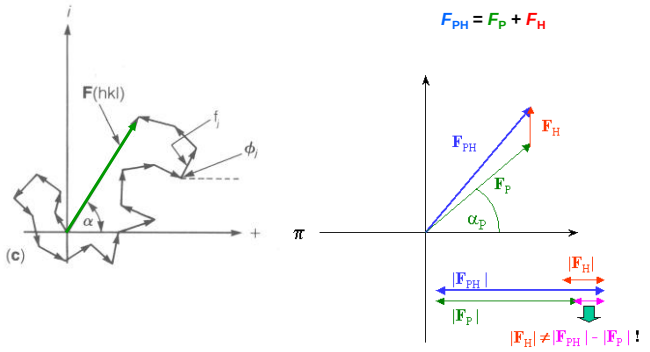
Native Phosphorylase

Phosphorylase + Ethyl Hg thiosalicylate

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

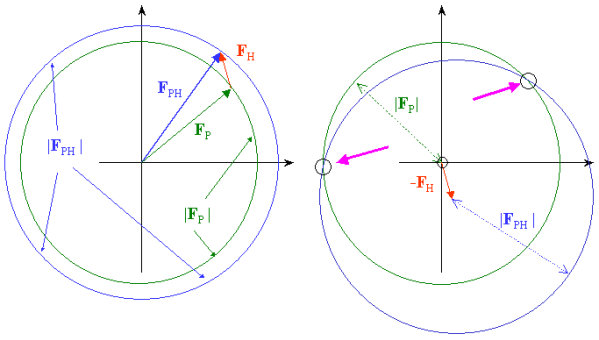
(e.g. Hg, Pt)

$F_{PH} = F_P + F_H$



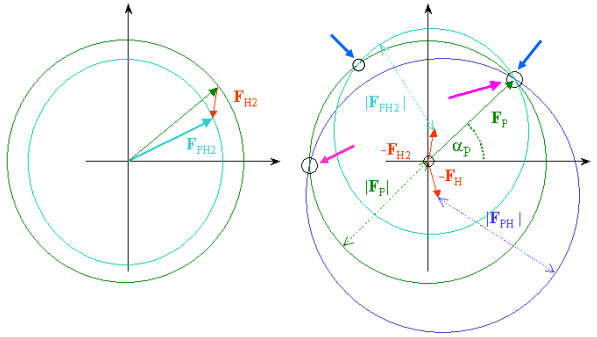
Multiple Isomorphous Replacement (MIR) method

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



Multiple Isomorphous Replacement (MIR) method

$F_P = F_{PH} - F_H$



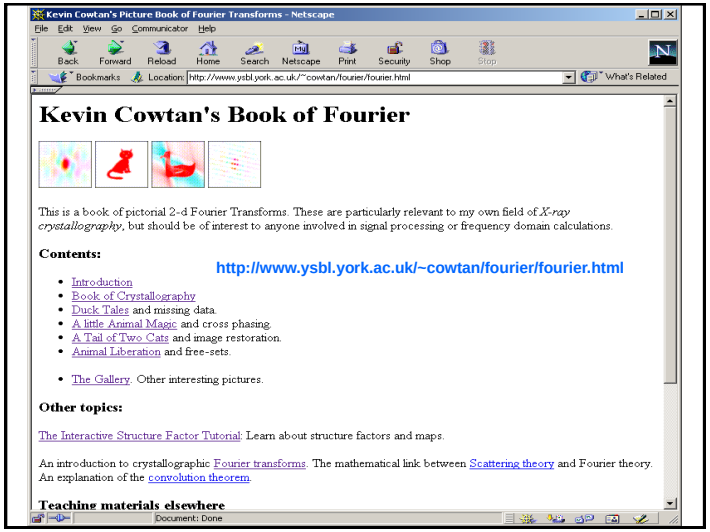
Solving the phase problem by "**Molecular Replacement**".

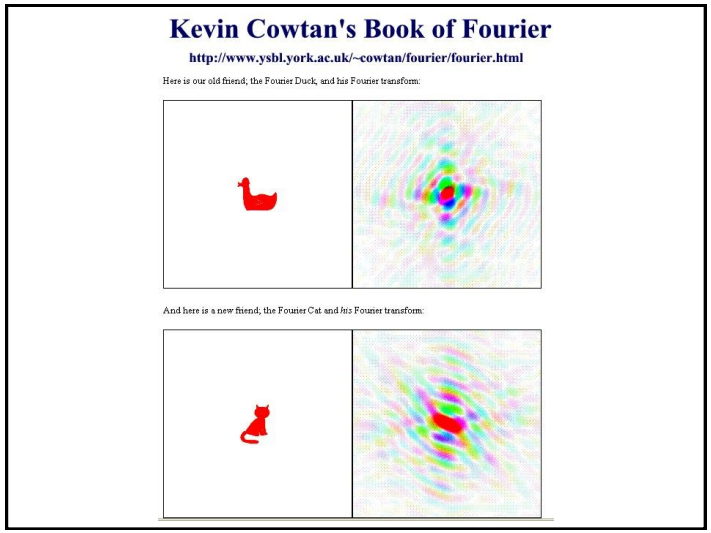
If an approximate model of the protein structure is known in advance, approximate phases can be guessed, and the unknown parts of the structure can be calculated in an iterative procedure.

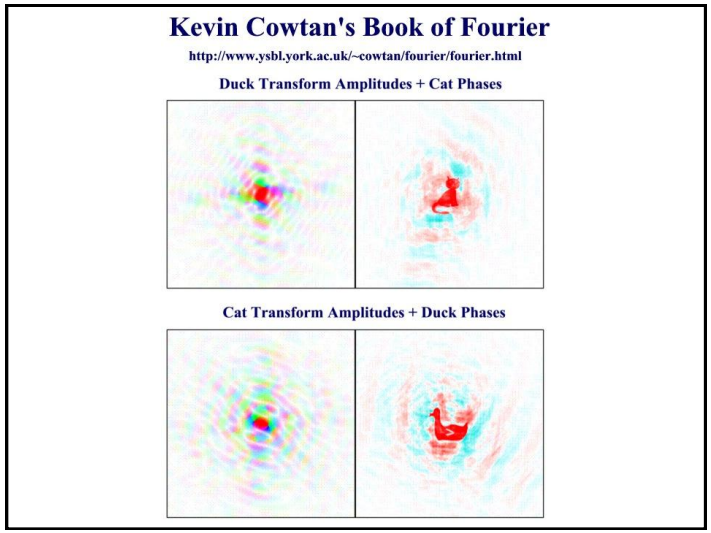
No heavy atom derivative required.

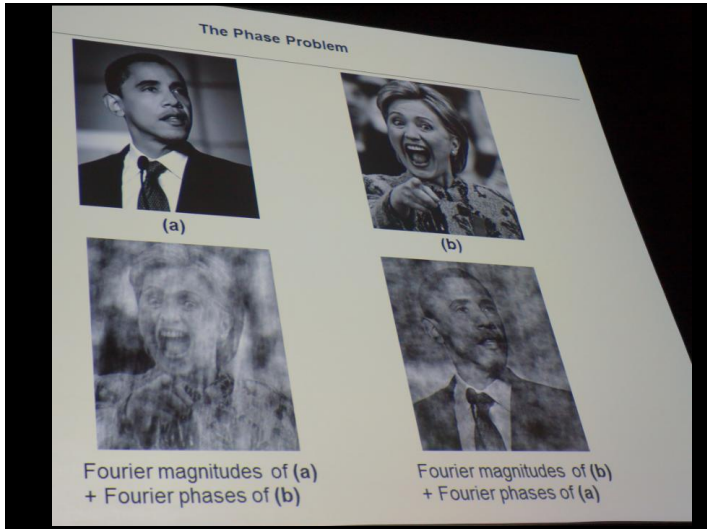
BUT – need starting model and orientation (rotation and translation)

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









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. **Image Formation** *(optical illusions)*

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

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

   Data mining and Protein Structure Analysis Tools

3. **X-Ray Crystallography**

   a) 100 years of X-ray Crystallography

   b) Crystal Growth – Materials / Methods

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

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

   e) Data Collection – Methods / Detectors / Structure Factors

   f) Theory of Diffraction – Waves, Fourier / Bragg's Law / Reciprocal Space

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

   → **h) Refinements and Models / Analysis and presentation of results**

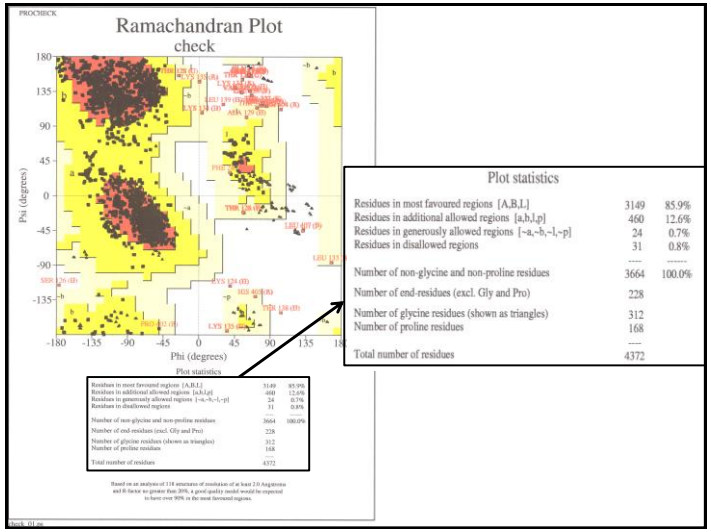
Energy Refinement

(Simulated Annealing)

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

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

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

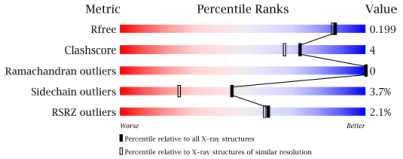


wwPDB Structure Validation Report

1 Overall quality at a glance

The reported resolution of this entry is 1.85 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	66092	1269 (1.86-1.86)
Clashscore	79885	1470 (1.86-1.86)
Ramachandran outliers	78287	1451 (1.86-1.86)
Sidechain outliers	78261	1451 (1.86-1.86)
RSRZ outliers	66119	1269 (1.86-1.86)

Crystal Structure of *M. tuberculosis* Alanine Racemase

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

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

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

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 = $\sum|F_{obs} - F_{calc}|/\sum|F_{obs}|$. [b] All isotropic model.

Difference Fourier

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

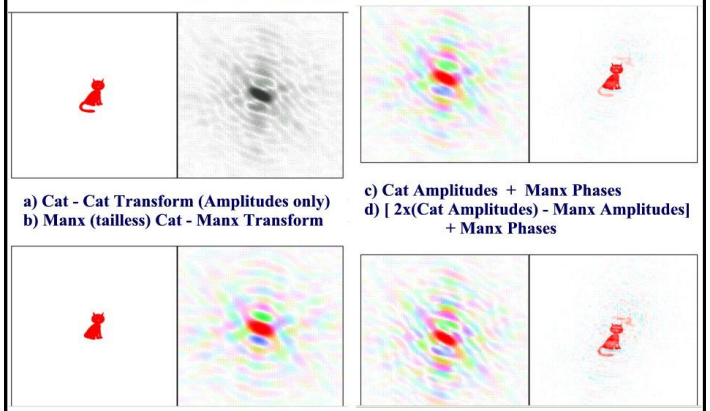
Calc. $$\rho_c(x, y, z) = \frac{1}{V}\sum_h\sum_k\sum_l F_{c,hkl}\, e^{-2\pi i(hx+ky+lz)} + R'$$

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

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

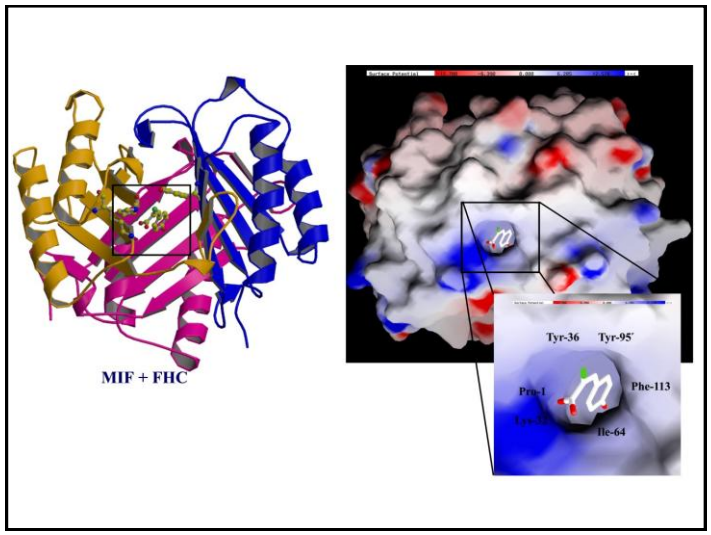
Kevin Cowtan's Book of Fourier

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



Examples of Difference Fouriers