Physical Methods used in bioinorganic chemistry

- X-ray crystallography
- X-ray absorption (XAS)
  - **Extended X-ray Absorption Fine Structure (EXAFS)**
  - **X-ray Absorption Near Edge Structure**
- Magnetic Resonance
  - **Electron paramagnetic resonance (EPR)**
  - **Nuclear magnetic resonance (NMR)**
- Mössbauer (MB)
- Optical Spectroscopy:
  - **Electronic absorption (UV-vis)**
  - **Circular dichroism (CD)**
  - **Magnetic circular dichroism (MCD)**
  - **Luminescence (fluorescence & phosphorescence)**
  - **Infrared (IR)**
  - **Resonance Raman (RR)**
- Electrochemistry

Lecture 5: X-ray Crystallography

Guest Lecturer: Dr. Joe Reibenspies

The slides are a compilation from Dr. Reibenspies, Dr. Zhao, Dr. Lu, and MYD

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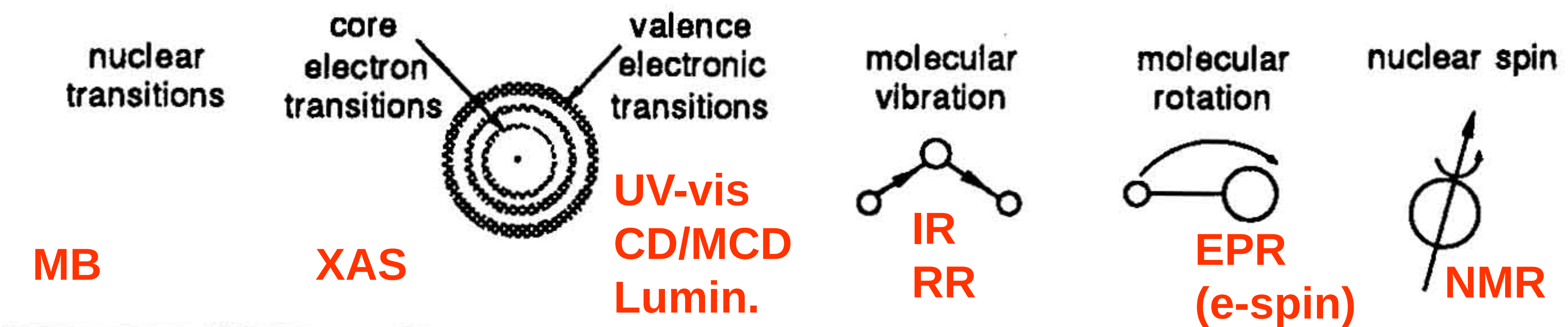
- Electrochemistry
    - **Direct (cyclic voltammetry and differential pulse polarography)**
    - **Indirect (spectro-electrochemistry)**

Spectrum of Electromagnetic Radiation



	γ-ray	x-ray	uv ⋮ visible	IR	microwave	radio
λ(m)	10^{-12}—10^{-10}	10^{-10}—10^{-8}	10^{-8}—10^{-6}	10^{-6}—10^{-4}	10^{-4}—10^{-2}	10^{-2}—10^{2}
ν(Hz)	10^{18}—10^{20}	10^{16}—10^{18}	10^{14}—10^{16}	10^{12}—10^{14}	10^{10}—10^{12}	10^{6}—10^{10}
E(J)	10^{9}—10^{11}	10^{7}—10^{8}	10^{5}—10^{6}	10^{4}—10^{5}	10^{2}—10^{4}	10^{-3}—10^{2}

Figure 2.2 Spectrum of electromagnetic radiation. The boundaries between domains are not sharp, and each region is not shown to scale. The visible domain actually forms an extremely small part (~350–750 nm) of the electromagnetic spectrum.

Interaction between X-ray and Matter

wavelength λ_o

intensity I_o

- incoherent scattering

  λ_c (Compton-Scattering)
- coherent scattering (XRD)

  λ_o (Bragg´s-scattering)
- Absorption (XANSE etc)

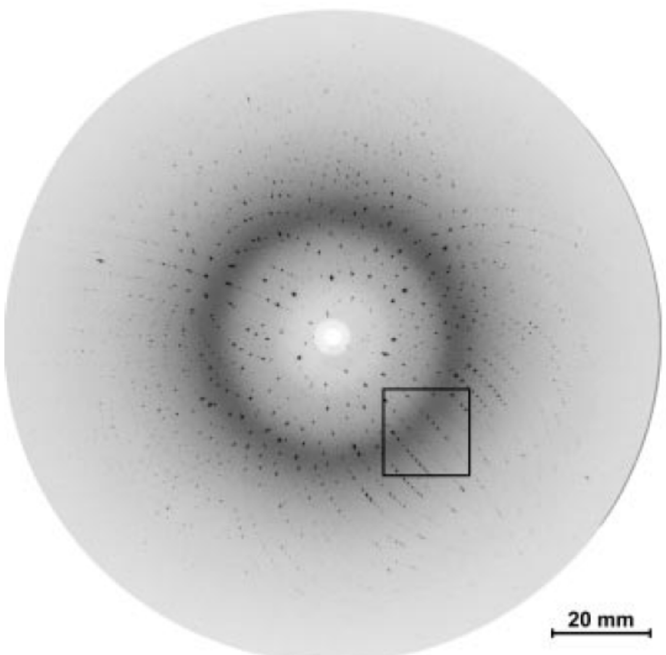
  Beer´s law $I = I_o * e^{-\mu d}$
- Fluorescence (XRF)

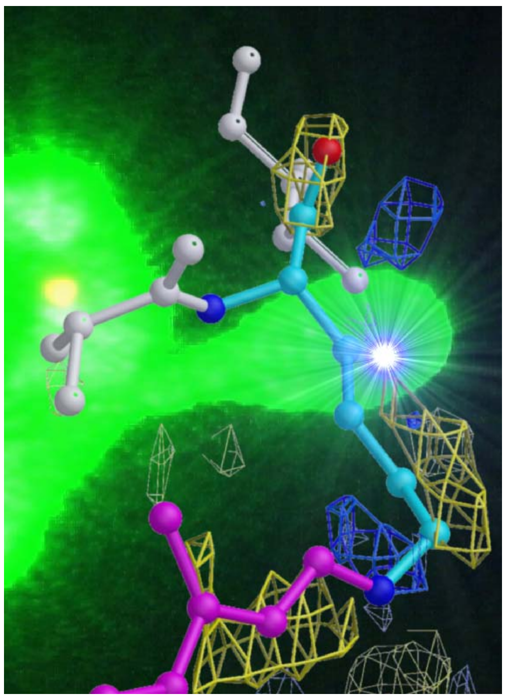
  $\lambda > \lambda_o$
- Photoelectrons (XPS)

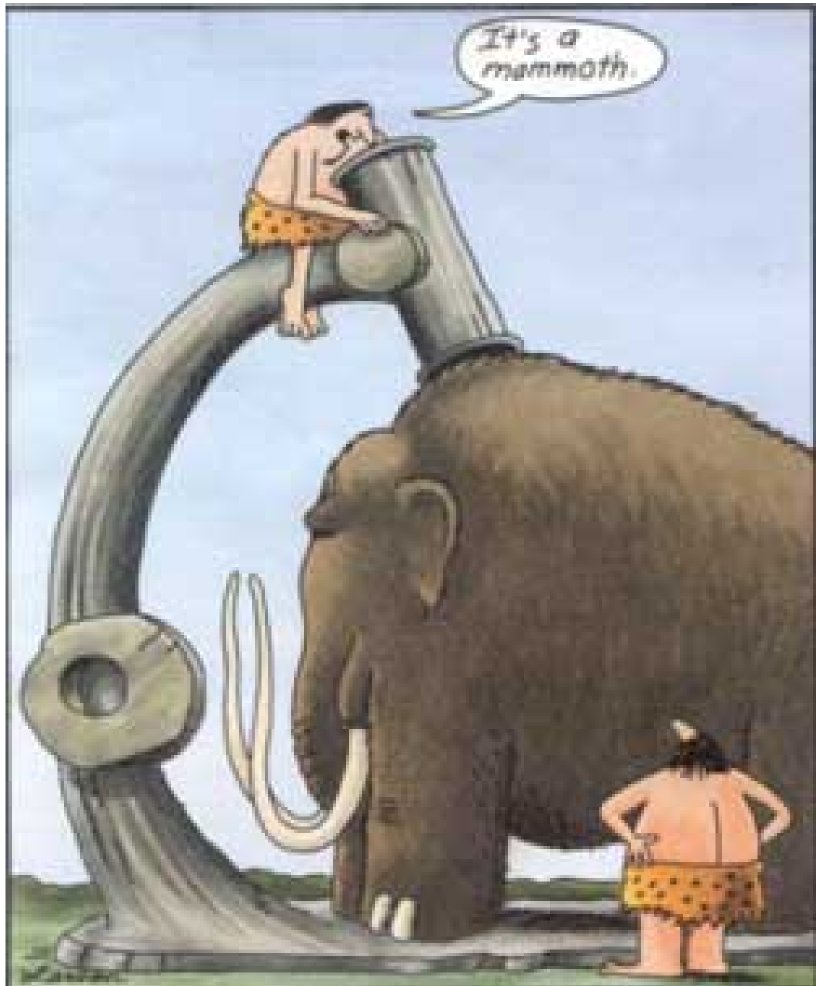
X-ray Diffraction

X-ray Crystallography

from crystals to spots to displays of atom-atom connections



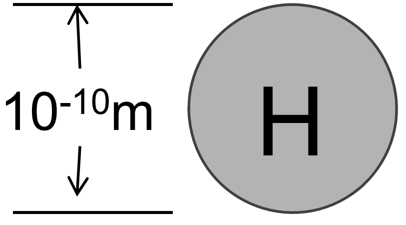




Primitive Crystallographers

Structure

Molecular, Extended, Quasi, etc



Angstrom (Å)

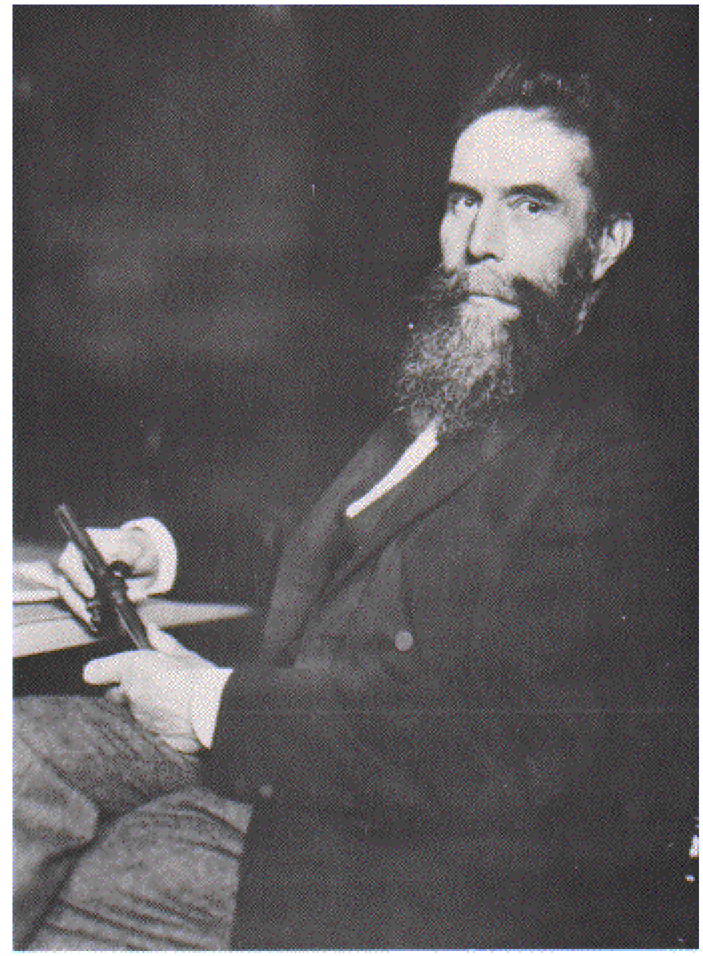
Visible Light $4x10^{-7}$-$9x10^{-7}$m
4000-9000 Å

Leptons
X-rays 0.1 to 14 Å
Fermions
Neutrons
Electrons

X-rays

- Discovered by Röntgen (rent-gen) 1895
- Cause of tremdous scientific excitement.
- 1,500 scientific communications within first twelve months.
- US scientists repeated experiments within four weeks.

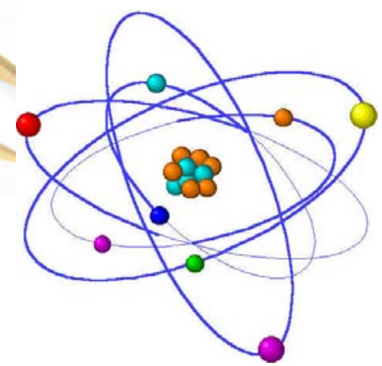
Mrs Röntgen
(Anna Bertha)



William Röntgen

The crystal.

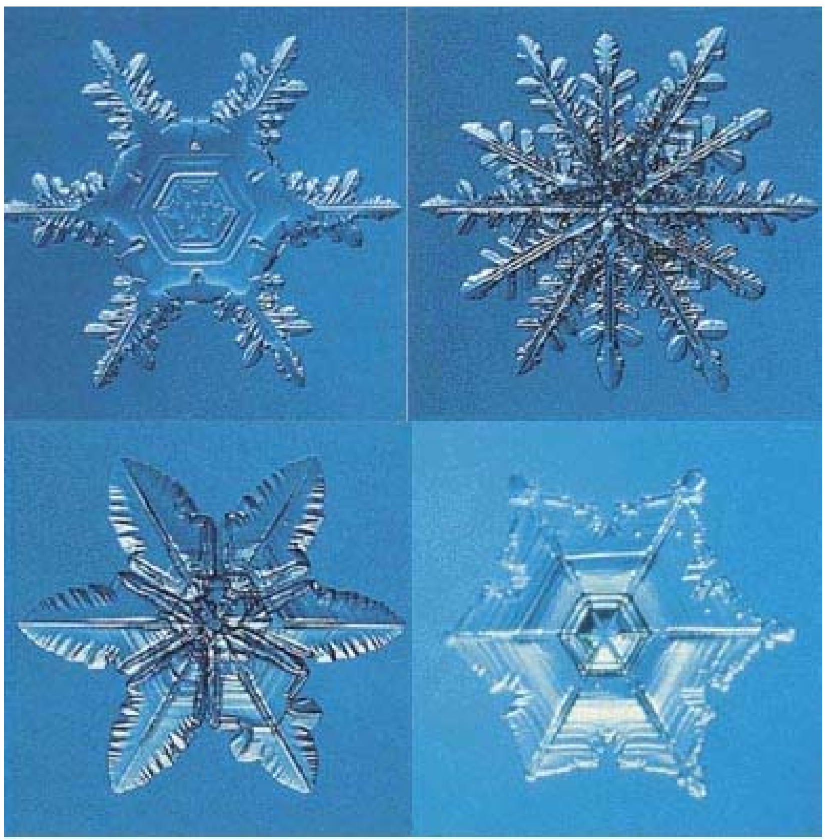
To view an atom (structure)with X-rays
you must find a way to hold it in place!

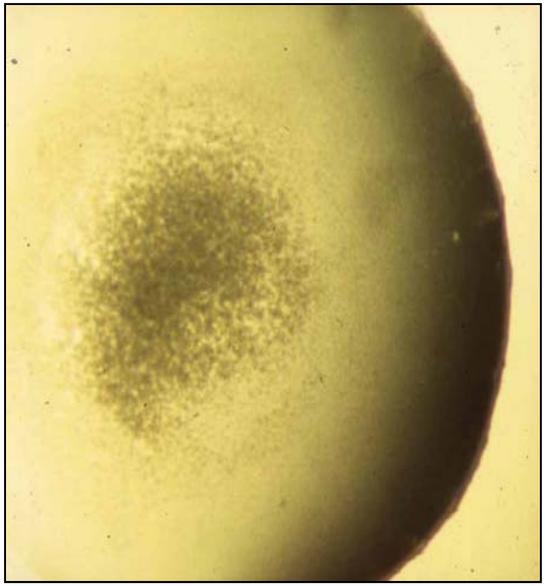
Crystal Concept

• In 1611 **Kepler** suggested that snowflakes derived from a regular arrangement of minute brick-like units:

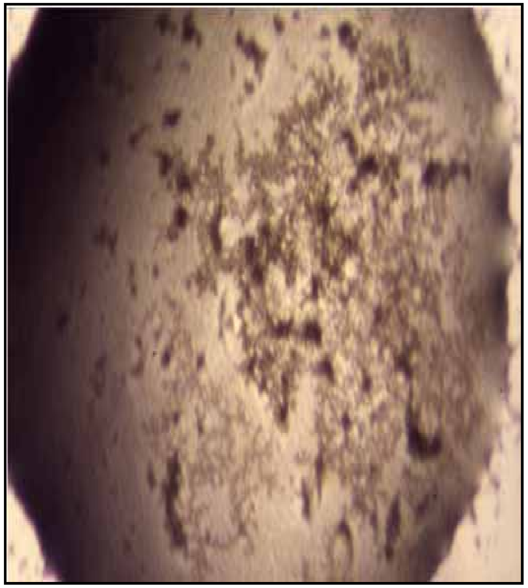
the essential idea of a **crystal**



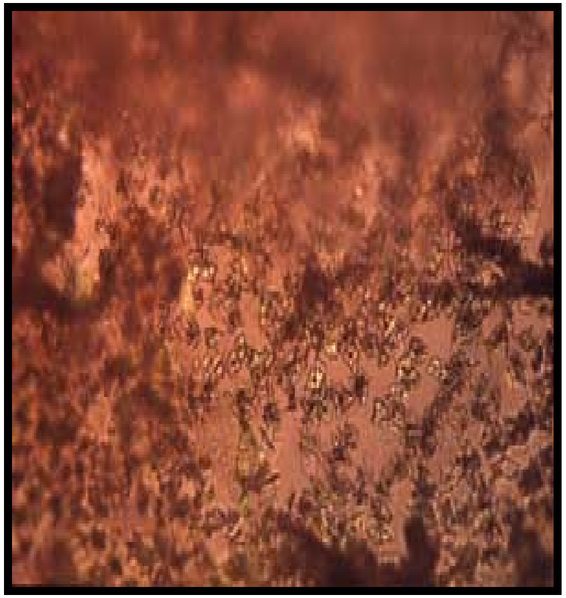
Types of preciptiation



amorphous



non-amorphous



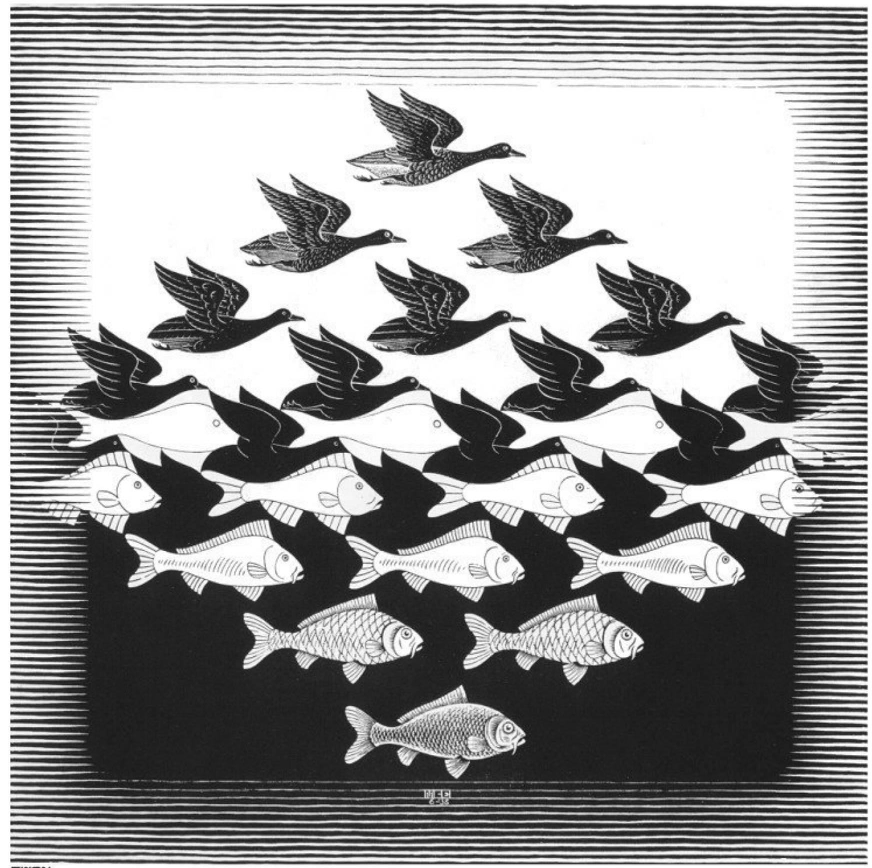
microcrystals

A skilled person can ”read” the drops & knows what to try next.

Crystal definition

A crystal is an object with translational symmetry:

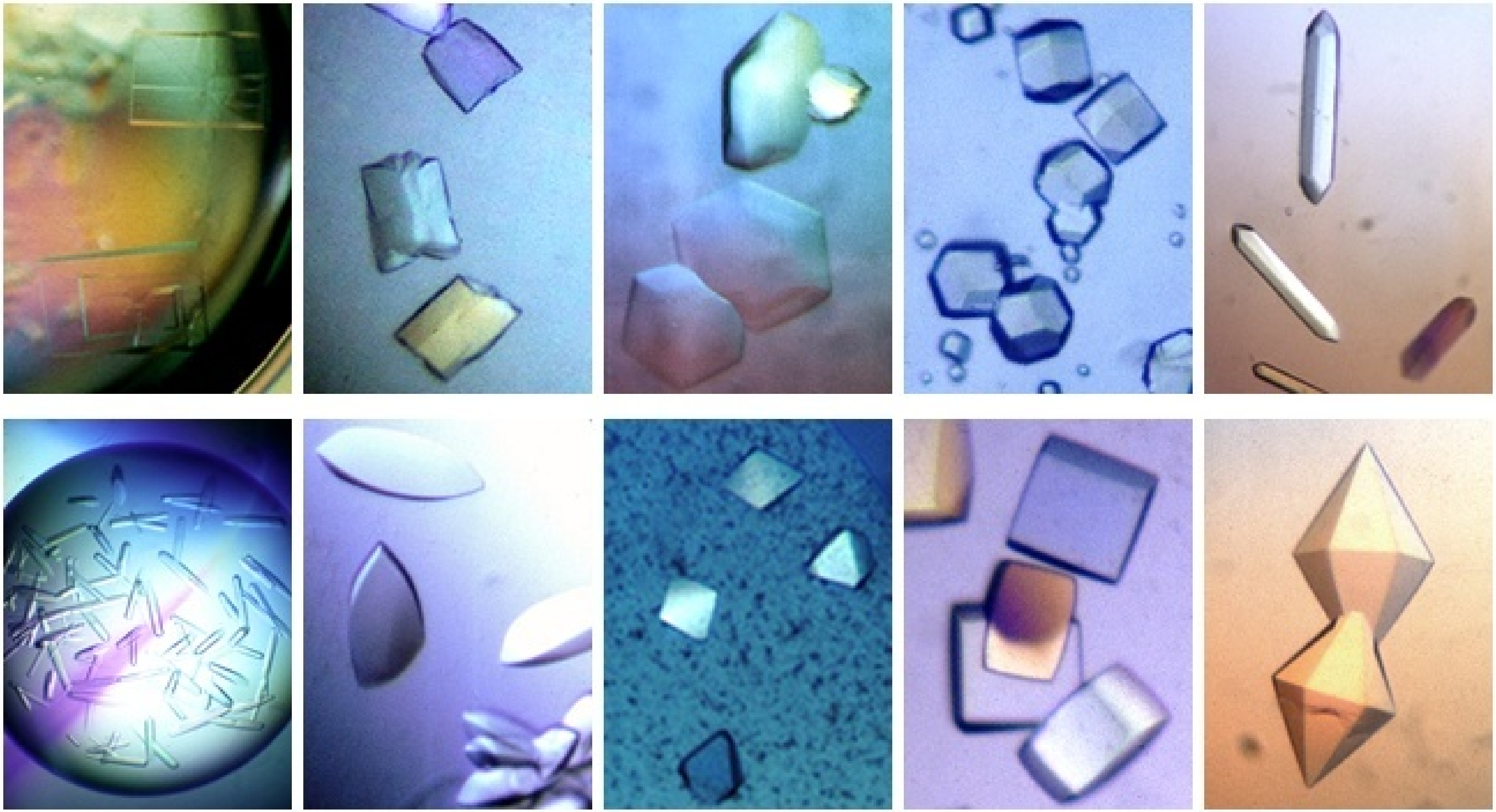
$$\rho(\underline{r}) = \rho(\underline{r}) + a\cdot\underline{x} + b\cdot\underline{y} + c\cdot\underline{z}$$



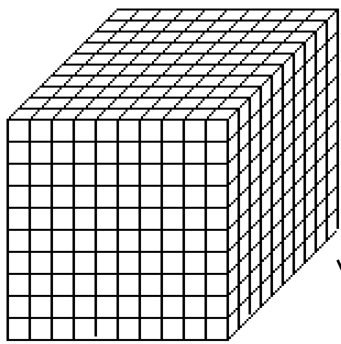
Has crystal symmetry

Doesn't have crystal symmetry

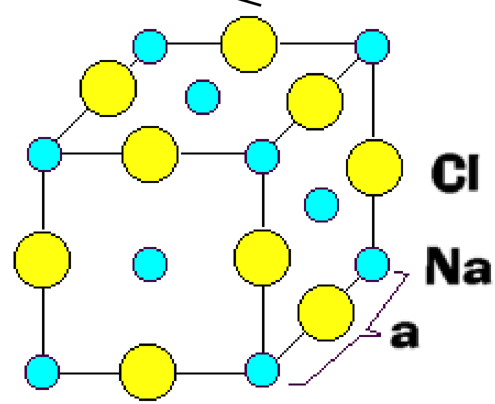
Crystals



The Crystal



A crystal is a solid material whose constituent atoms (molecules and ions) are arranged in an orderly repeating pattern extending in all three spatial dimensions.



Unit Cell
Sides : a,b,c
Angles : α,β,γ
e.g. Cubic : a=b=c $\alpha=\beta=\gamma=90$

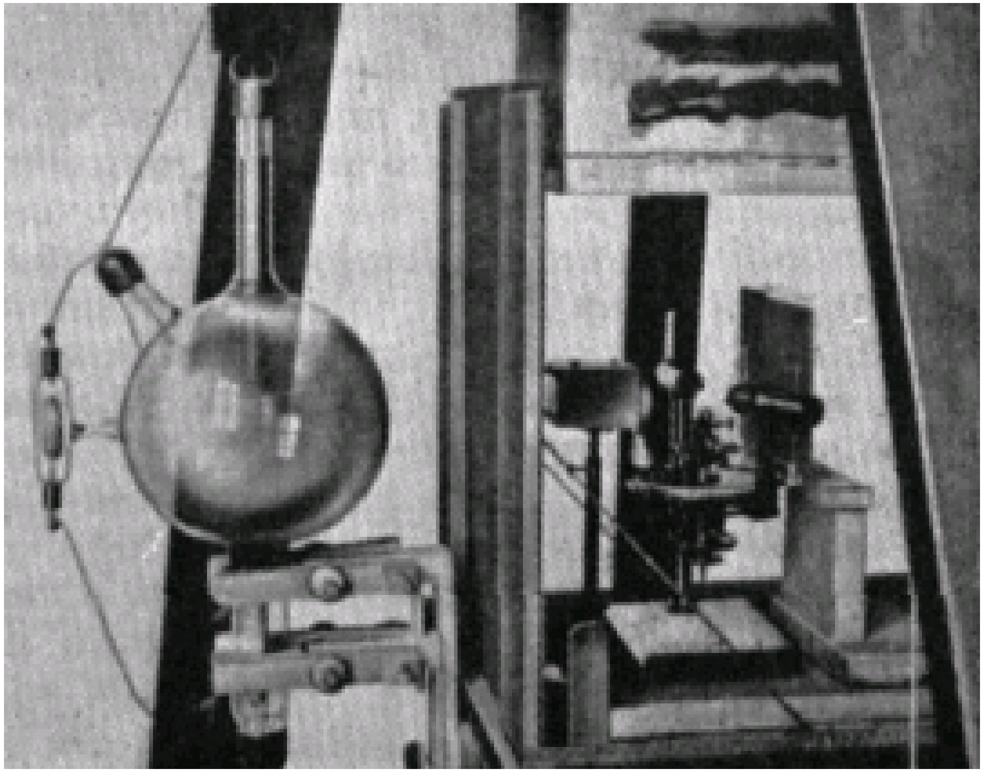
Problems

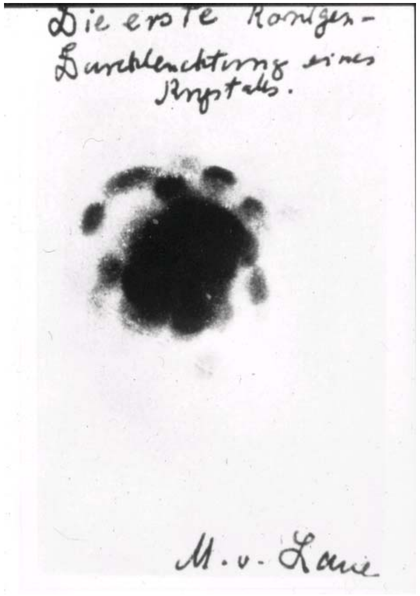
- Just get percipitate:
  - Protein denatured?
  - Micro-hetrogeniety? (seriously disrupts crystal packing).
- Too many micro-crystals.
  - Micro-seeding (adding crushed up & diluted crystal seeds).
  - Streak seeding (touching a crystal with a cat-whisker & streaking it through a new drop).

- Crystals diffract to only very low resolution.
  - Check protein preparation & try seeding.
  - Try to slow down growth
- Number of experiments?
  - May need to do thousands of crystal screens.
  - May need tens-of-thousands of optimisation experiments.
  - May be lucky with first experiments.

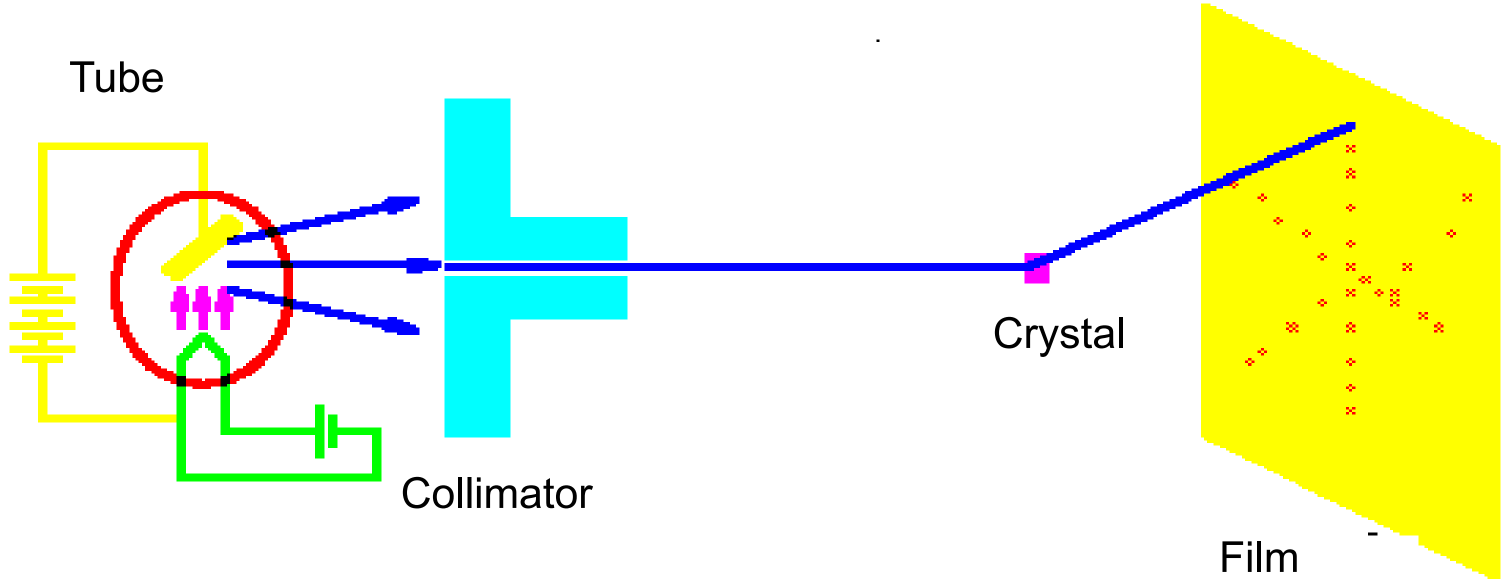
Theory of Diffraction

- 1910 Max von Laue derived theory of diffraction from a lattice.





Laue's Experiment in 1912
Single Crystal X-ray Diffraction



Bragg's Law of diffraction 1912

- Diffraction observed if X-rays scattering from a plane add in phase.
- Path difference $\Delta P = 2d \sin \theta$.

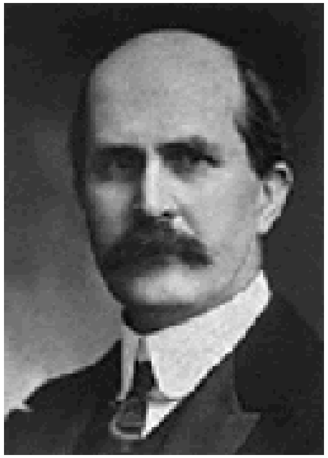
    - d is the spacing between planes & θ is the angle of incidence.
- Scatter in phase if path difference is $n\lambda$

    - n is an integer & λ is the X-ray wavelength.

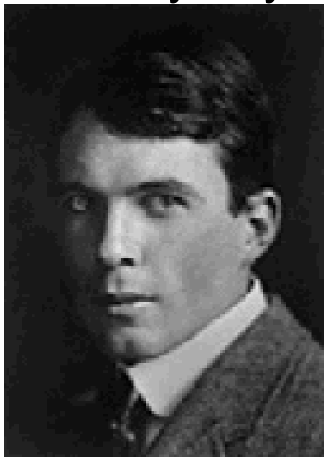
$$2d \sin \theta = n \lambda$$

- First structure (NaCl) in 1912.

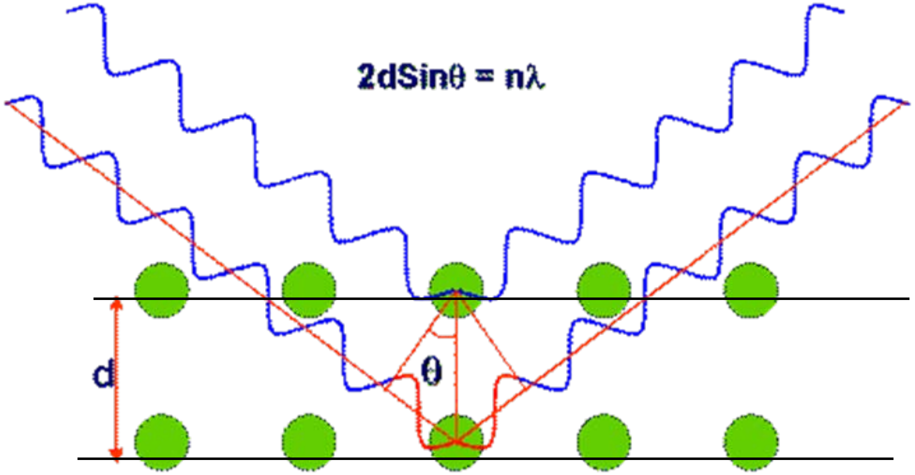
PaPa



Sonny Boy



W. H. Bragg & W. L. Bragg

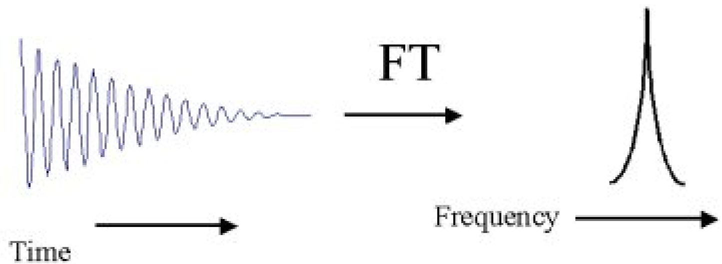


$$\frac{\lambda}{d} = 2\sin\theta$$

Fourier Transform
Real Space to Reciprocal Space

$$\lambda d^{*} = 2\sin\theta$$

Reciprocal   Real



2D : time   1/time
Chart

3D :   Map

$$\frac{1}{d} = \left(h^2a^{*2} + k^2b^{*2} + l^2c^{*2} + 2hka^{*}b^{*}\cos\gamma^{*} + 2hla^{*}c^{*}\cos\beta^{*} + 2klb^{*}c^{*}\cos\alpha^{*}\right)^{1/2} = d^{*}$$

The atoms in a crystal are a periodic array of coherent scatterers and thus can diffract X-rays.

- Diffraction occurs when each object in a periodic array scatters radiation coherently, producing concerted constructive interference at specific angles.

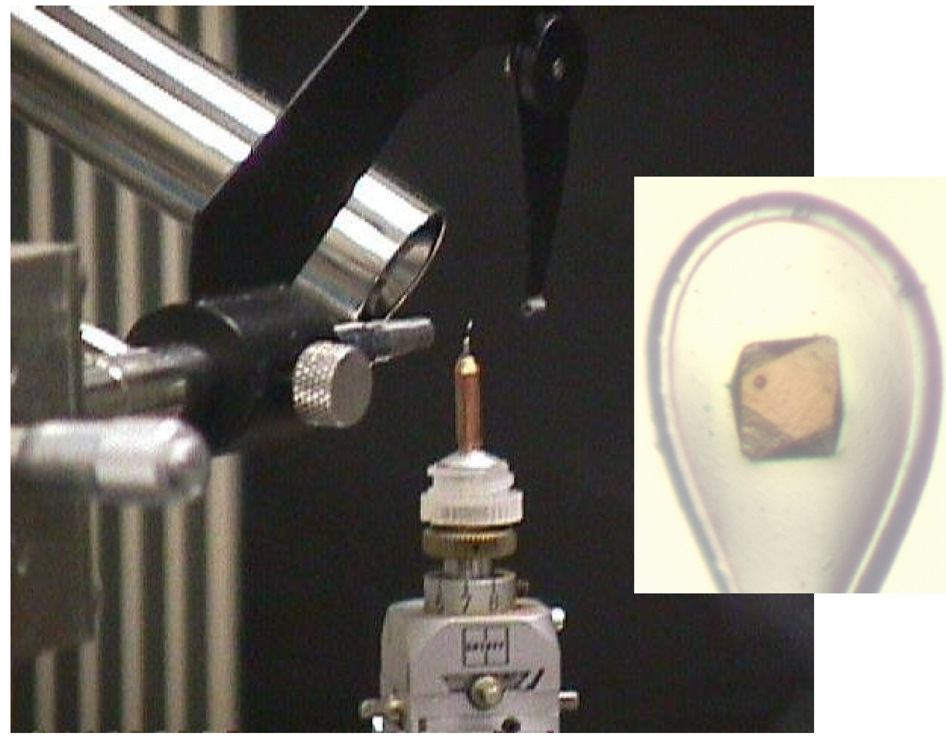
- The electrons in an atom coherently scatter X-rays.
    - The electrons interact with the oscillating electric field of the X-ray.

- Atoms in a crystal form a periodic array of coherent scatterers.
    - The wavelength of X rays are similar to the distance between atoms.
    - Diffraction from different planes of atoms produces a diffraction pattern, which contains information about the atomic arrangement within the crystal

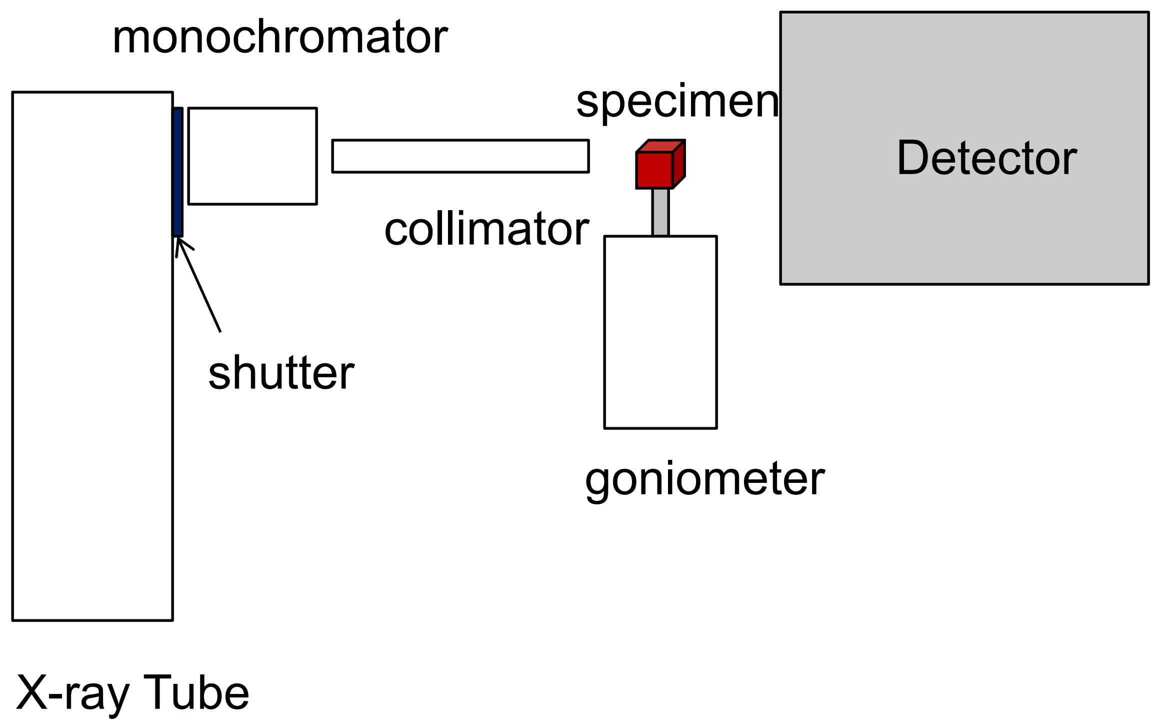
- X-Rays are also reflected, scattered incoherently, absorbed, refracted, and transmitted when they interact with matter.

X-ray source Diffractometer

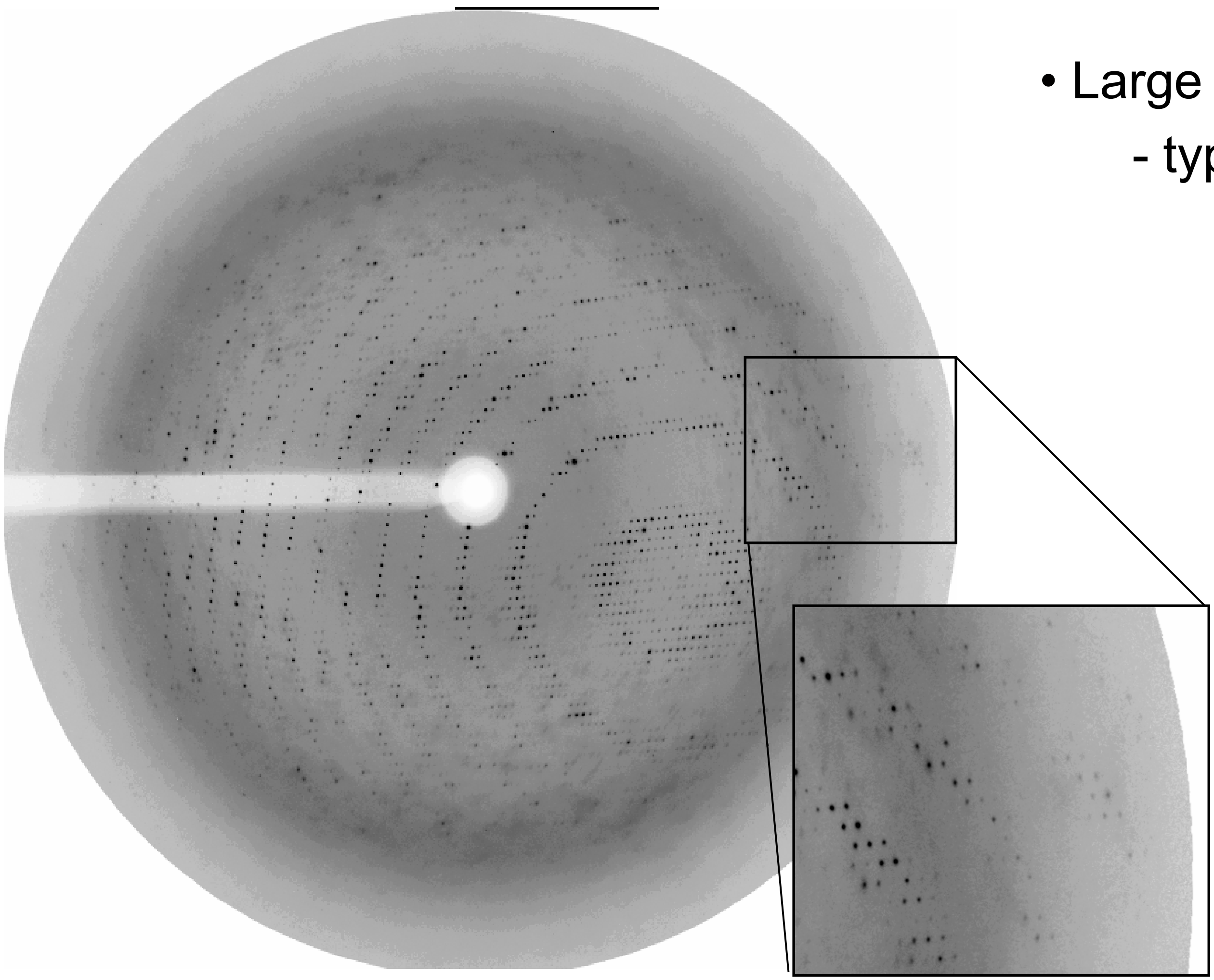
• Freeze a crystal on a loop & mount in an X-ray beam.



The X-ray Diffractometer

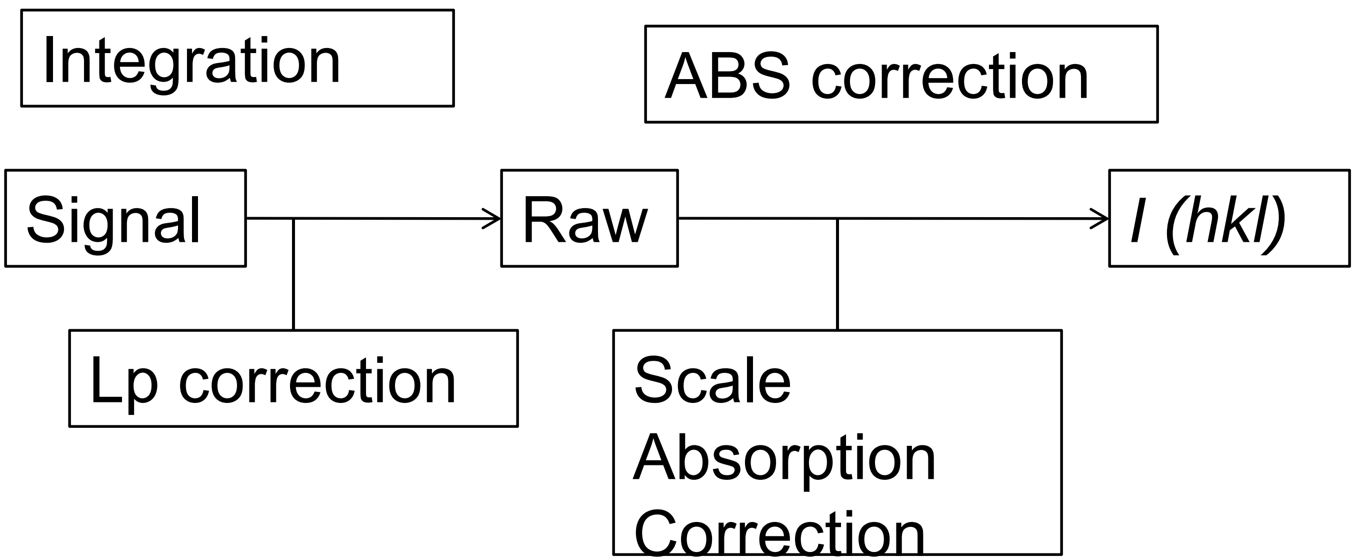


X-ray diffraction



- Large number of spots
  - typically 10 to 300 Å.

From Signal to Intensity



The MATH

$$I(hkl) = K\left|F(hkl)_{obs}\right|^2$$

Structure Factor : *I* and Phase

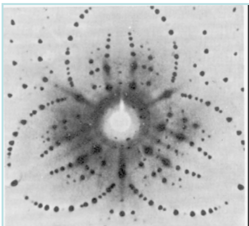
Phase information : Focusing X-Rays cannot be focused

$$\rho(xyz) = \frac{1}{V}\sum_{h}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l}^{\infty}\left|F_{hkl}\right|\cos 2\pi\left(hx + ky + lz - \alpha'_{hkl}\right)$$

Real Space
Electron Density

Reciprocal Space

Model building employing known Intensities



Structure Solution
Phase Refinement
Fourier

Guess?

$$\rho(xyz) = \frac{1}{V}\sum_{h}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l}^{\infty}|F_{hkl}|\cos 2\pi\left(hx+ky+lz-\alpha'_{hkl}\right)$$

atom (i)

Calc. structure factor for atom (i)

$$I(hkl) = K\left|F(hkl)_{obs}\right|^2$$

$$F(hkl)_{cal} = \sum_i f_i \exp\left[2\pi\left(hx_i + ky_i + lz_i\right)\right]$$

$$\rho(xyz) = \frac{1}{V}\sum_{h}^{\infty}\sum_{k=-\infty}^{\infty}\sum_{l}^{\infty}|F_{hkl}|\cos 2\pi\left(hx+ky+lz-\alpha'_{hkl}\right)$$

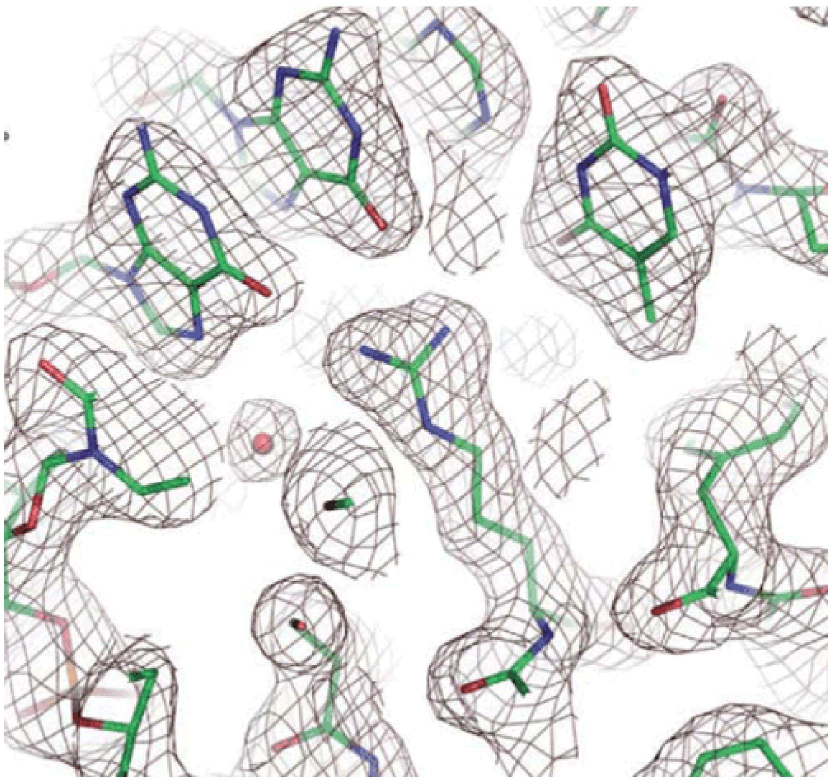
$$D = \sum_{hkl} w_{hkl}\left(\left|F_{obs}\right|^2 - \left|kF_{calc}\right|^2\right)^2$$

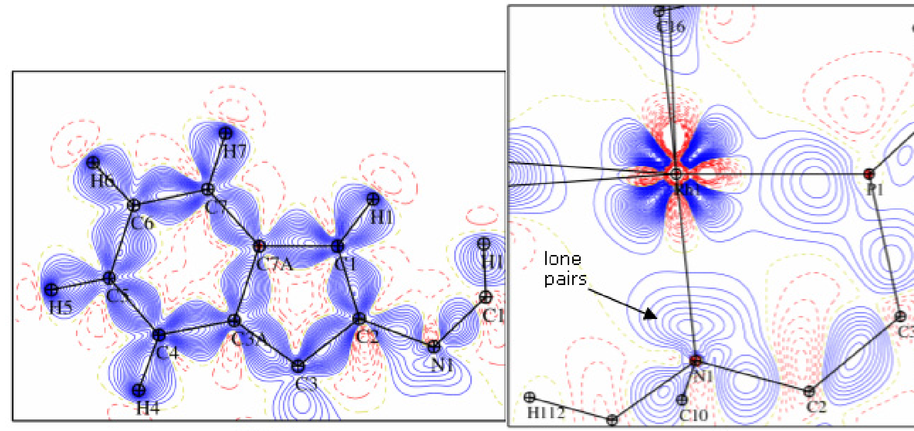
L.S./Fourier

Minimize D
(modify x,y,z +
Thermal p's)

$$wR2 = \left(\frac{\sum_{hkl}\left(w\left|F_{obs}\right|^2 - \left|kF_{calc}\right|^2\right)^2}{\sum_{hkl}\left(w\left|F_{obs}\right|^2\right)^2}\right)^{1/2}$$

$$\frac{\partial D}{\partial p} \sim \frac{\Delta D}{\Delta p}$$





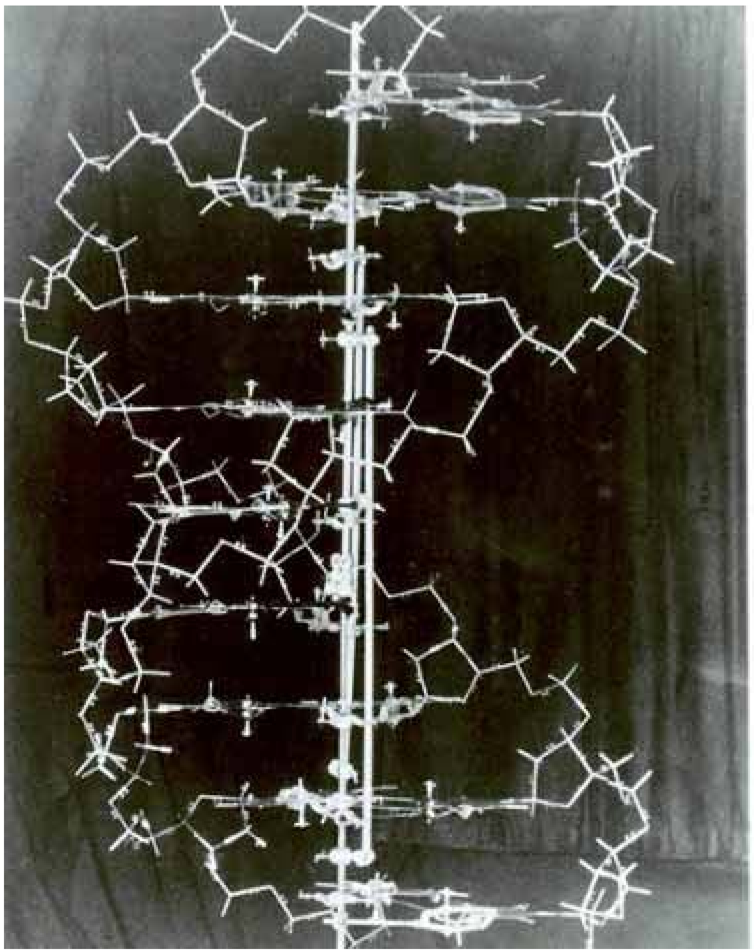
(a) (b)

rhodium complex $C_{25}H_{37}NPRh$

A view of the 2fo-fc electron density map of the I-Dmo/ DNA complex structure contoured at 1.25σ.

1953: Double helix structure of DNA

- Crick & Watson used X-ray diffraction to work out the way genes are encoded.



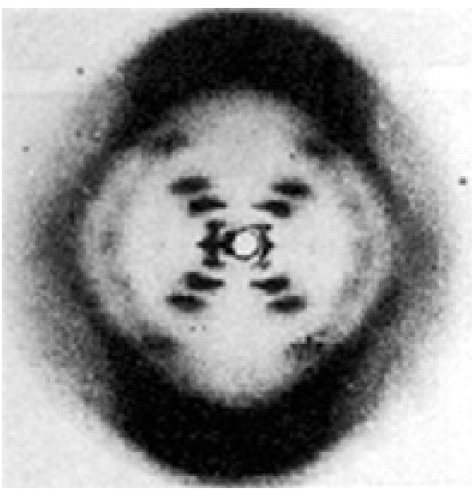
Francis Crick (L) and James Watson (R)

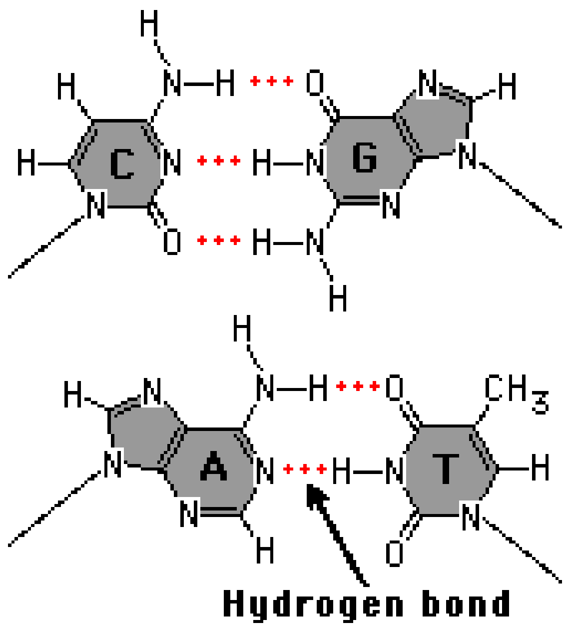
Credit: DNA From the Beginning; http://vector.cshl.org/dnaftb/asp/

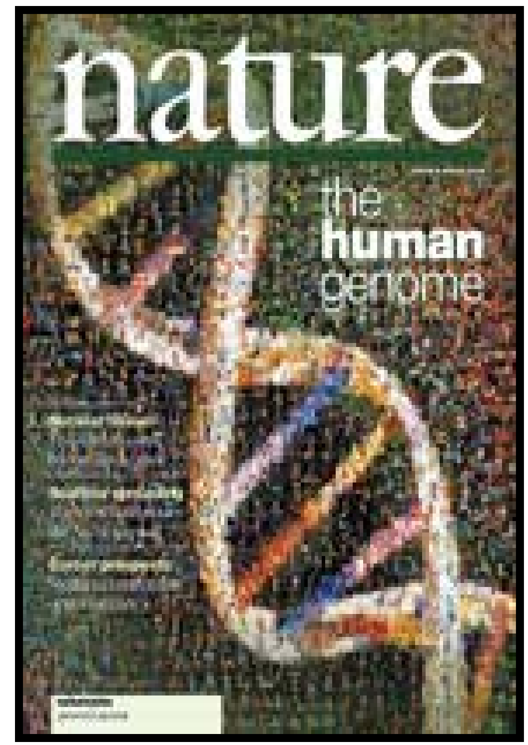
Diffraction pattern

• Crystals & diffraction pattern recorded by Rosalind Franklin and Maurice Wilkins .

•Revealed the symmetry of the helix & pitch of helix.

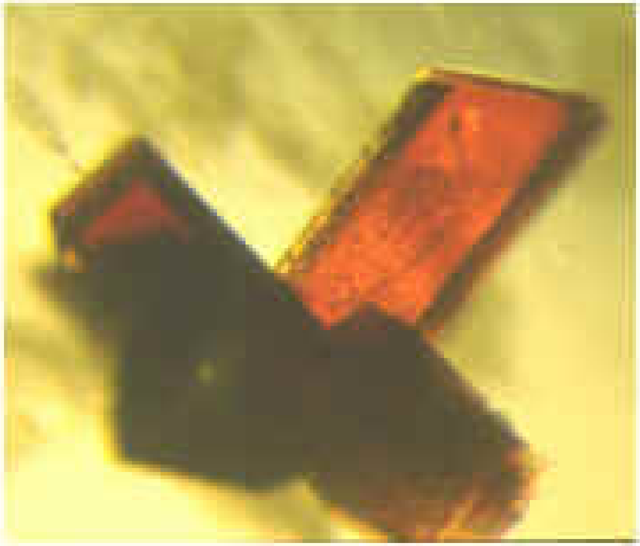






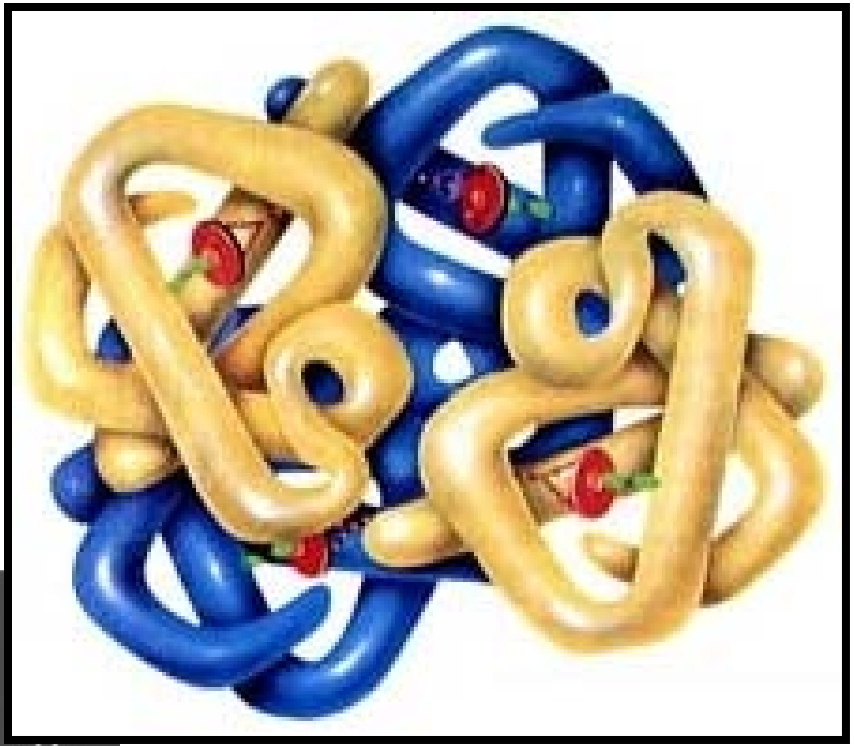
First Protein Structure

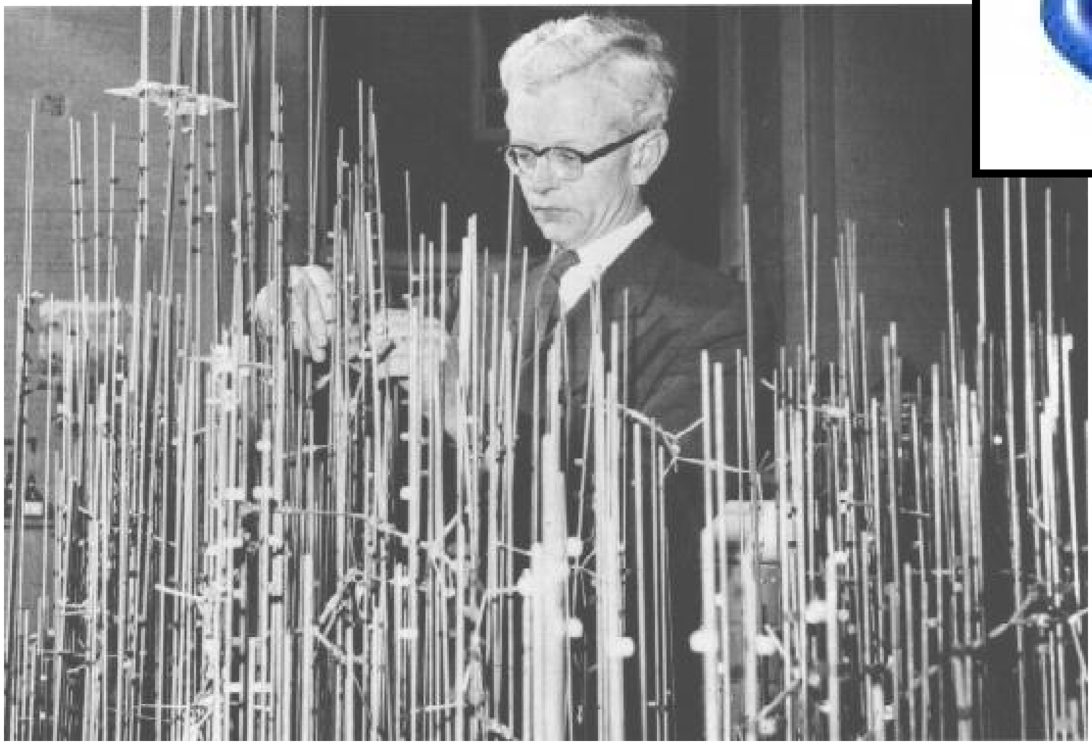
- Myoglobin.
- Protein purified from whale blood.
- Max Perutz 1958.
- Showed a 75% α-helical fold.
- 155 amino acids, ~ 17 kDa.



First Protein Complex

- Hemoglobin.
- Two copies each of α & β chains of myoglobin in a complex.
- Solved by John Kendrew.



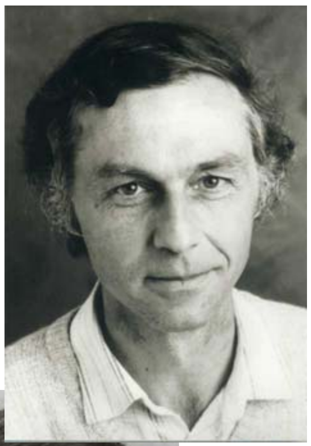


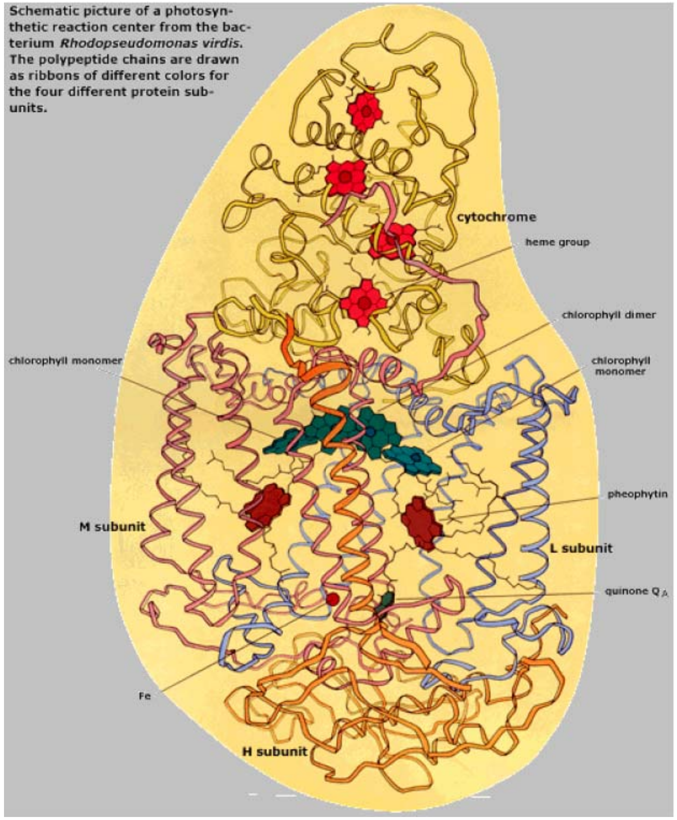
Photosynthetic Reaction Centre Structure

• First membrane protein structure in 1985.

• Nobel prize to Michel, Deisenhofer & Huber 1988.

• Showed the technique of detergent solubilized membrane protein crystallisation.

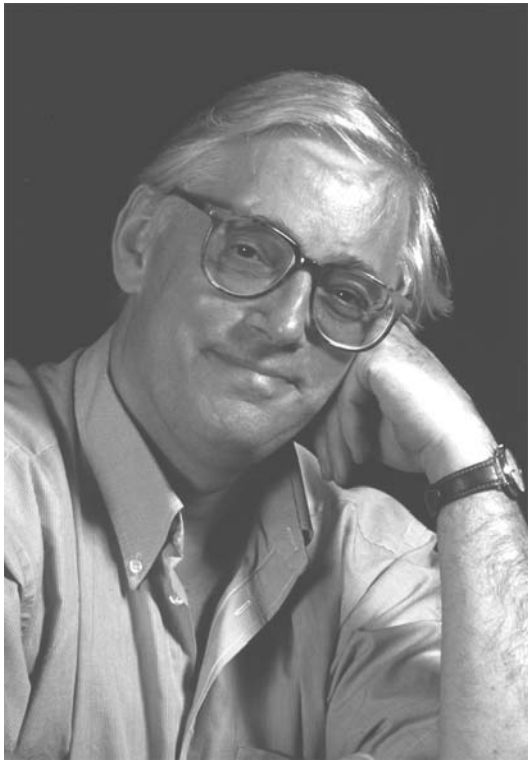




Structure of F1-ATPase

• Revealed the details of the rotational mechanism of ATP-synthase.

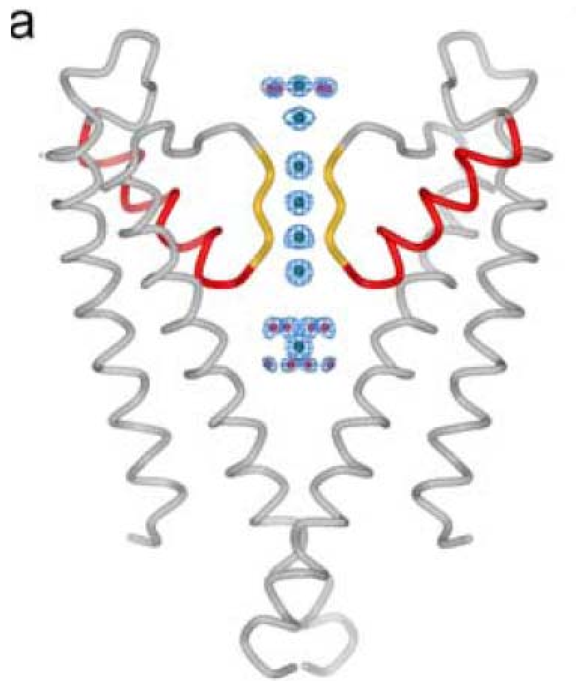
• Nobel prize in 1997 for John E. Walker .



Structure of the K^+ channels

• Revealed the structural basis for ion transport across a membrane.

• Deep physiological relevance.



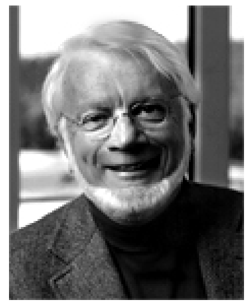
Prof. Roderic MacKinnon
Rockefeller University

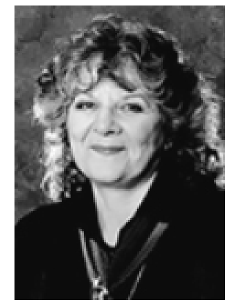
1996: moved from Harvard Medical School to Rockefeller Univ to study protein crystallography

1998: determined the first high-resolution structure of an ion channel, called KcsA, from the bacterium *Streptomyces lividans*
SCIENCE 1998 APR;280(5360):69-77

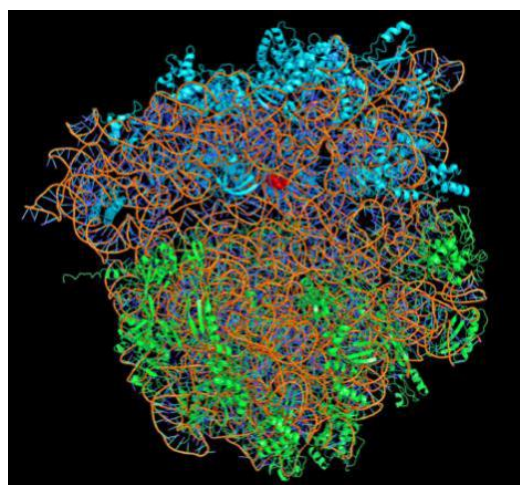
2003: Nobel Prize in Chemistry

Venkatraman Ramakrishnan, Thomas Steitz and Ada Yonath
2009 Nobel Prize in Chemistry.





For studies of the structure and function of the ribosome



Nobel Prize winners associated with crystallography

2009 Chemistry
V. Ramakrishnan, T.A. Steitz, A.E. Yonath
Studies of the structure and function of the ribosome

2006 Chemistry
R.D. Kornberg
Studies of the molecular basis of eukaryotic transcription

2003 Chemistry
R. MacKinnon
Potassium channels

1997 Chemistry
P.D. Boyer, J.E. Walker, J.C. Skou
Elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP) and discovery of an ion-transporting enzyme

1996 Chemistry
R.Curl, H. Kroto, R. Smalley
Discovery of the fullerene form of carbon

1994 Physics
C. Shull and N. Brockhouse
Neutron diffraction

1992 Physics
G. Charpak
Discovery of the multi wire proportional chamber

1991 Physics
P.-G. de Gennes
Methods of discovering order in simple systems can be applied to polymers and liquid crystals

1988 Chemistry
J. Deisenhofer, R. Huber, H. Michel
For the determination of the three-dimensional structure of a photosynthetic reaction centre

1985 Chemistry
H. Hauptman and J. Karle
Development of direct methods for the determination of crystal structures

1982 Chemistry
A. Klug
Development of crystallographic electron microscopy and discovery of the structure of biologically important nucleic acid–protein complexes

1982 Physics
K.G. Wilson
Theory of critical phenomena in connection with phase transitions

1976 Chemistry
W.N. Lipscomb
Structure of boranes

1972 Chemistry
C.B. Anfinsen
Folding of protein chains

1964 Chemistry
D. Hodgkin
Structure of many biochemical substances including Vitamin B12

1962 Physiology or Medicine
F. Crick, J. Watson, M. Wilkins
The helical structure of DNA

1962 Chemistry
J.C. Kendrew, M. Perutz
For their studies of the structures of globular proteins

1954 Chemistry
L.C. Pauling
For his research into the nature of the chemical bond and its application to the elucidation of the structure of complex substances

1946 Chemistry
J.B. Sumner
For his discovery that enzymes can be crystallised

1937 Physics
C.J. Davisson and G. Thompson
Diffraction of electrons by crystals

1929 Physics
L.-V. de Broglie
The wave nature of the electron

1915 Physics
W.H. Bragg and W.L. Bragg
Use of X-rays to determine crystal structure

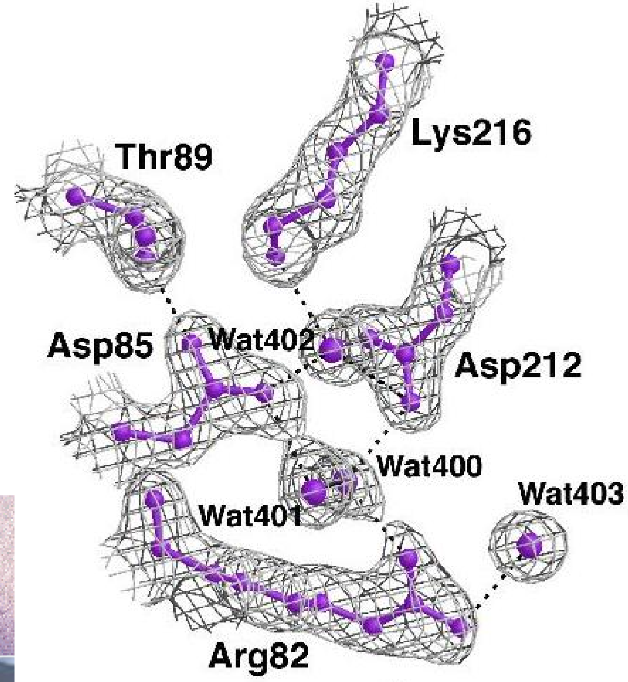
1914 Physics
M. Von Laue
Diffraction of X-rays by crystals

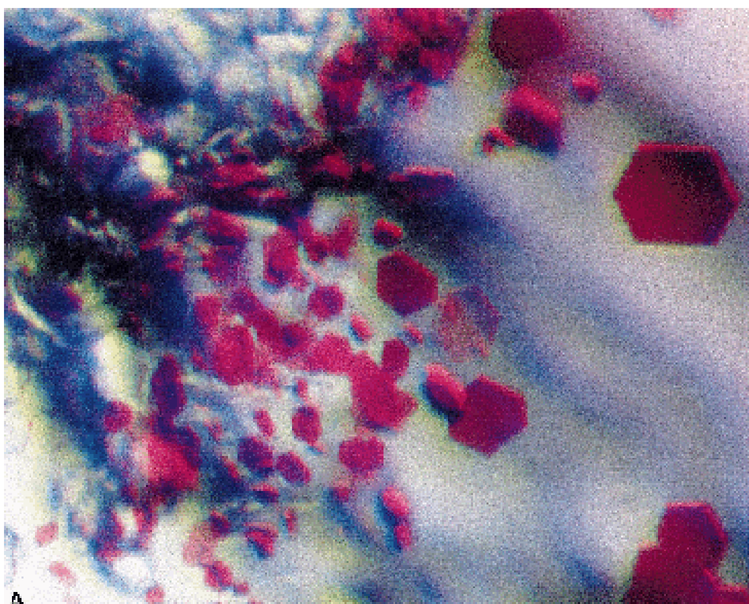
1901 Physics
W.C. Röntgen
Discovery of X-rays

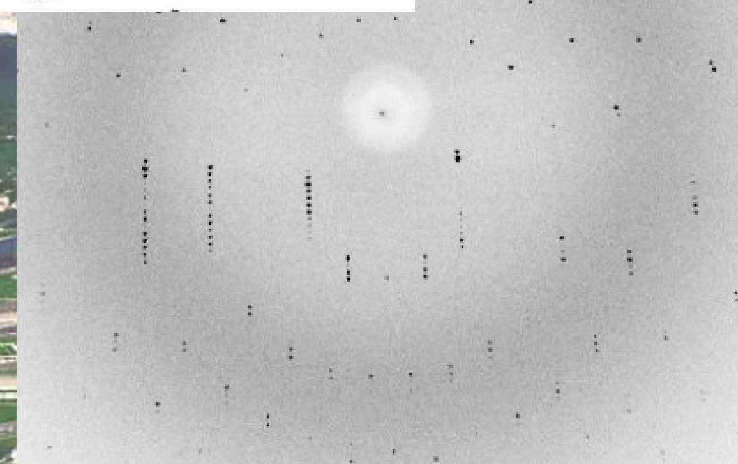
24 Nobel Prizes

X-ray Crystallography Summary

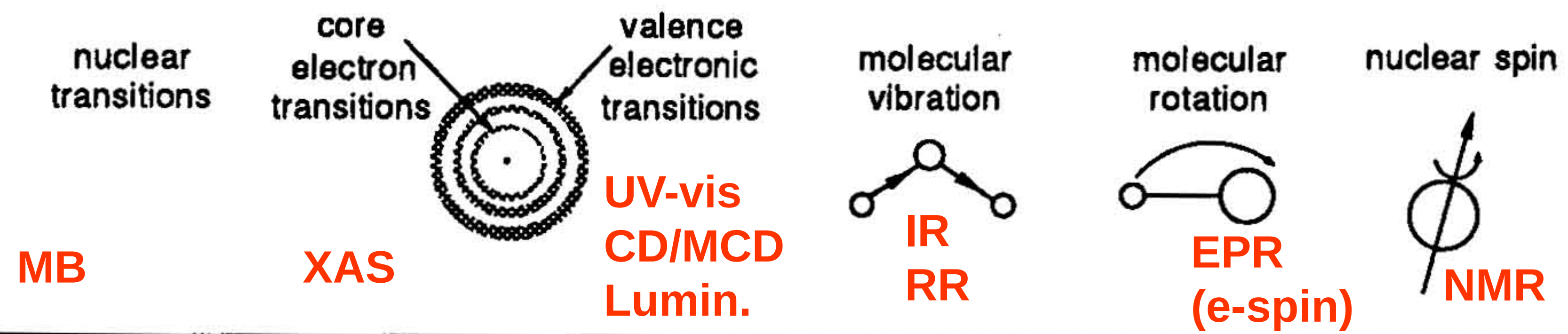
- Grow protein crystals.
- Collect diffraction data.
- Interpret electron density.
- More than 55000 Structures in the protein data bank (www.pdb.org).







Spectrum of Electromagnetic Radiation



	γ-ray	x-ray	uv ⁞ visible	IR	microwave	radio
λ(m)	10^{-12}—10^{-10}	10^{-10}—10^{-8}	10^{-8}—10^{-6}	10^{-6}—10^{-4}	10^{-4}—10^{-2}	10^{-2}—10^{2}
ν(Hz)	10^{18}—10^{20}	10^{16}—10^{18}	10^{14}—10^{16}	10^{12}—10^{14}	10^{10}—10^{12}	10^{6}—10^{10}
E(J)	10^{9}—10^{11}	10^{7}—10^{8}	10^{5}—10^{6}	10^{4}—10^{5}	10^{2}—10^{4}	10^{-3}—10^{2}

Figure 2.2 Spectrum of electromagnetic radiation. The boundaries between domains are not sharp, and each region is not shown to scale. The visible domain actually forms an extremely small part (~350–750 nm) of the electromagnetic spectrum.

Robert A. Scott
Center for Metalloenzyme Studies

X-Ray Absorption Spectroscopy of Metallobiomolecules

The Outskirts of Structural Biology

rscott.myweb.uga.edu/XAS/XAS2007.pdf

Glossary of Acronyms

XAS **X-Ray Absorption Spectroscopy** Generic term for various techniques involving absorption of x-rays

XAFS **X-Ray Absorption Fine Structure** Alternate generic term for following two techniques

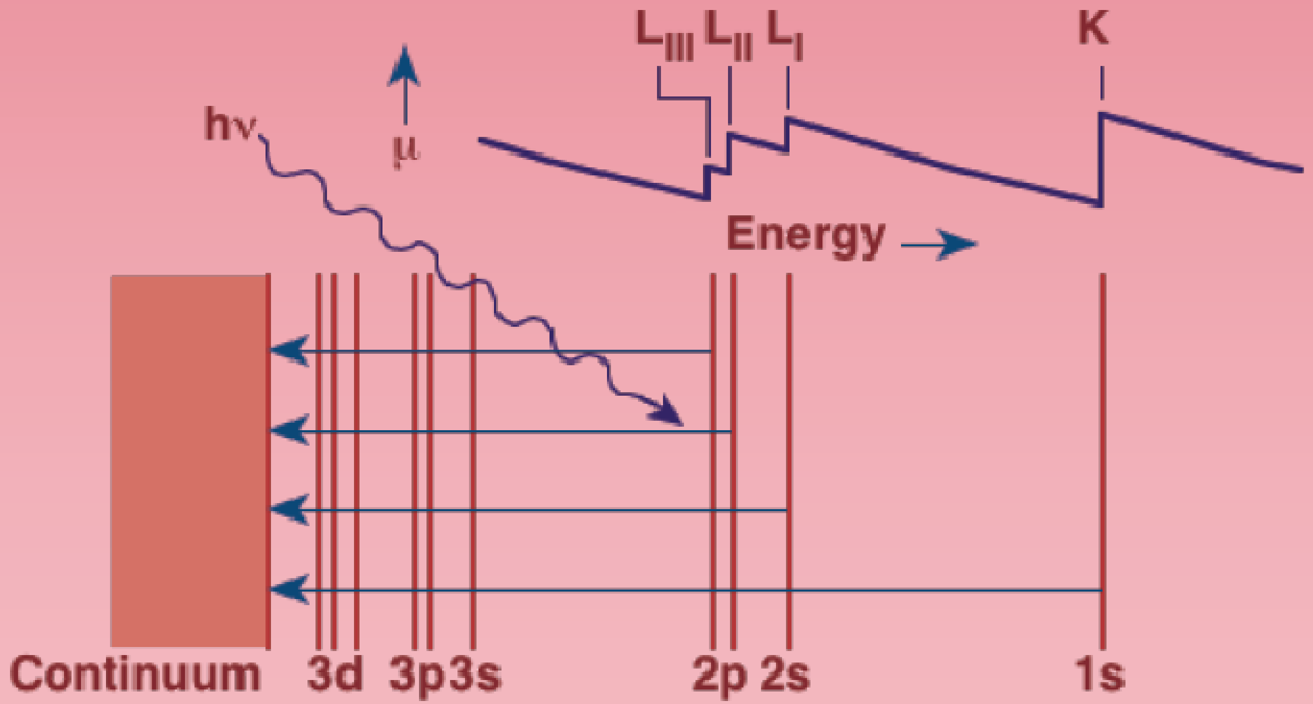
XANES **X-Ray Absorption Near Edge Structure** Structure in x-ray absorption spectrum just before and after edge and its analysis – a spectroscopic technique

EXAFS **Extended X-Ray Absorption Fine Structure** Structure in x-ray absorption spectrum after edge and its analysis – a structural technique

Questions XAS Can Address

- What types of atoms are in the first coordination sphcrc of a mctal sitc ?
- What is the molecular symmetry of this metal site ?
- How covalent are the metal ligand bonds ?
- Does a particular treatment ...
  - generate a redox change at this metal site?
  - result in a structural change at this metal site?
- Is this metal part of a metal cluster ?

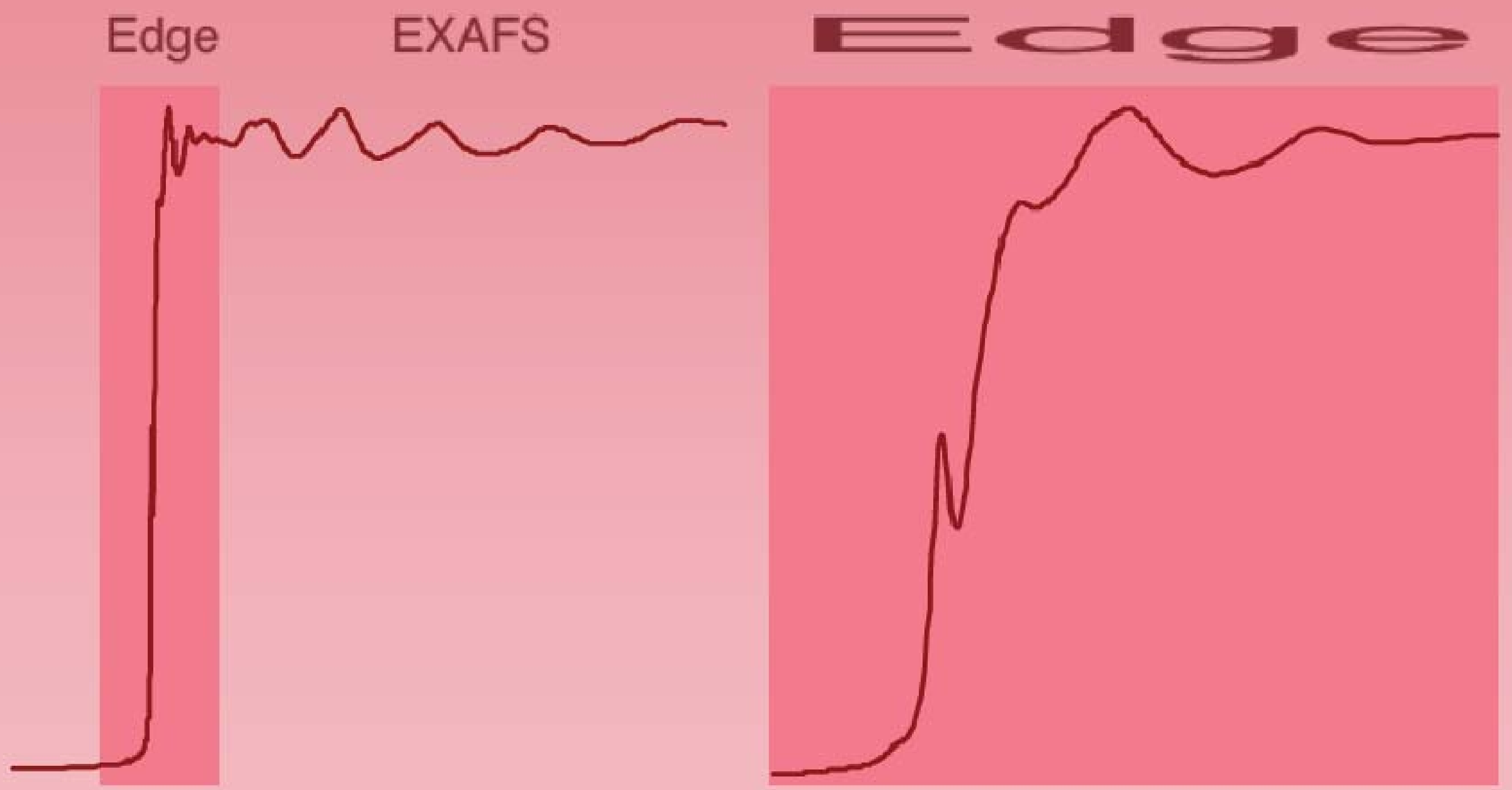
XAS Edge Results from Core Ionization



Energies (keV)

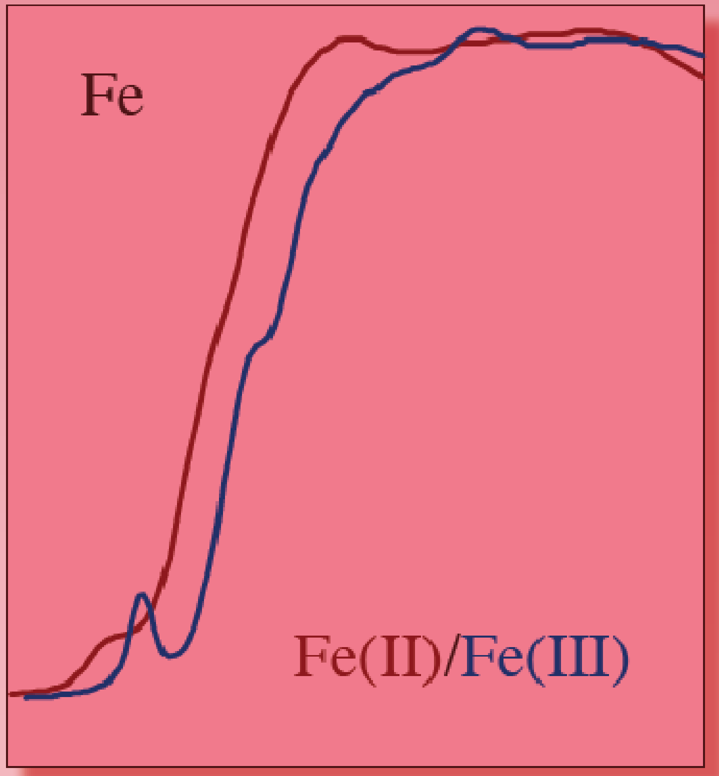
	K	L_{III}
S	2.5	
Mn	6.5	0.6
Fe	7.1	0.7
Ni	8.3	0.8
Cu	9.0	0.9
Mo	20.0	2.5
Tb	52.0	7.5
W	69.5	10.2
Hg	83.1	12.3

The X-Ray Absorption Spectrum

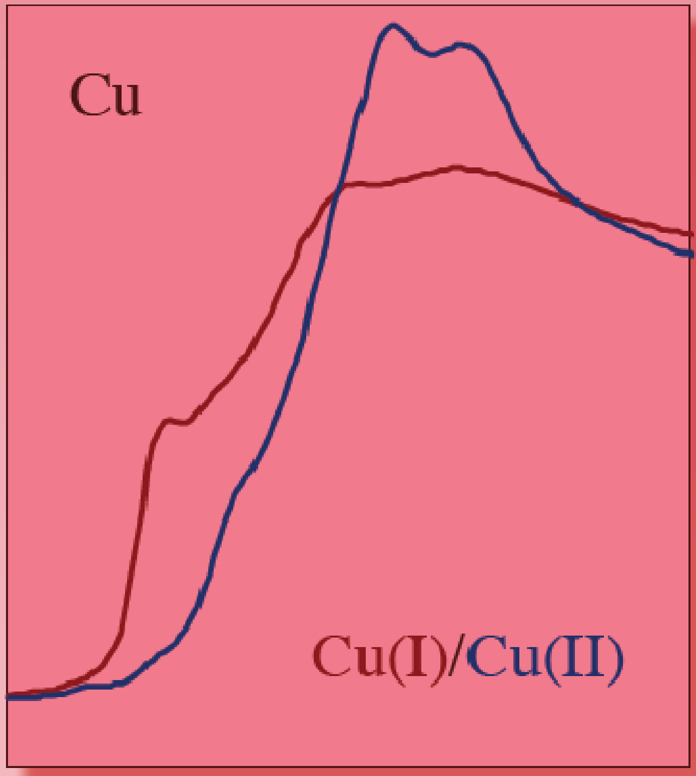


X-Ray Energy

Edge Energy Reflects Redox State

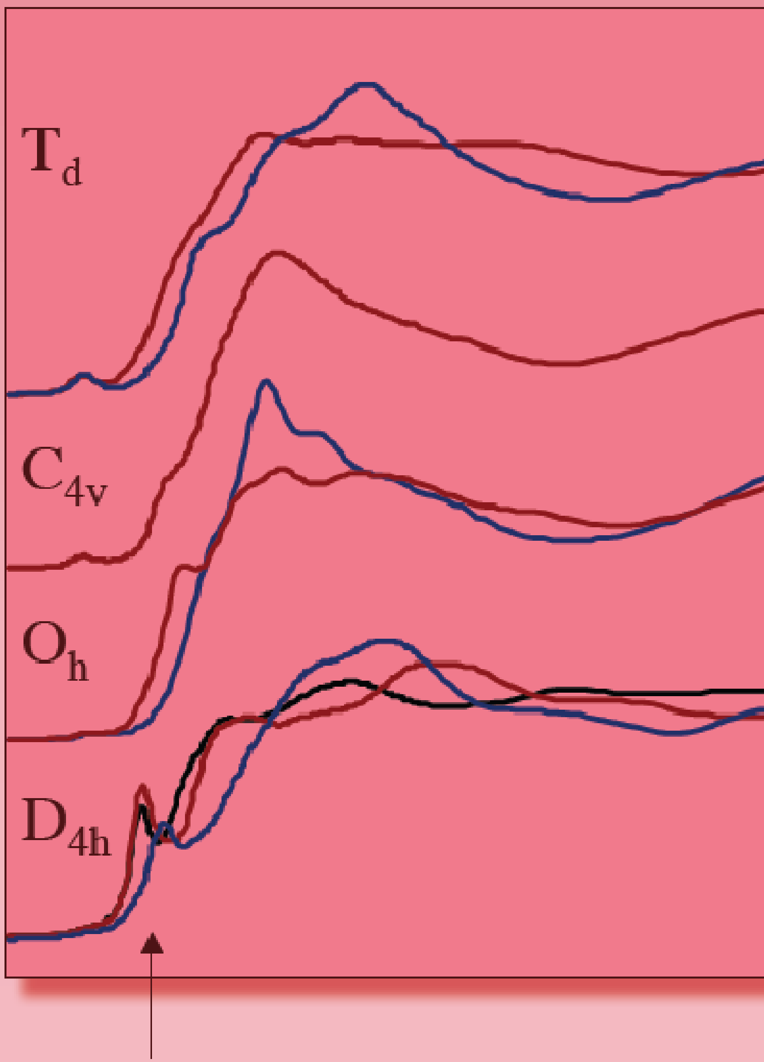


Rubredoxin



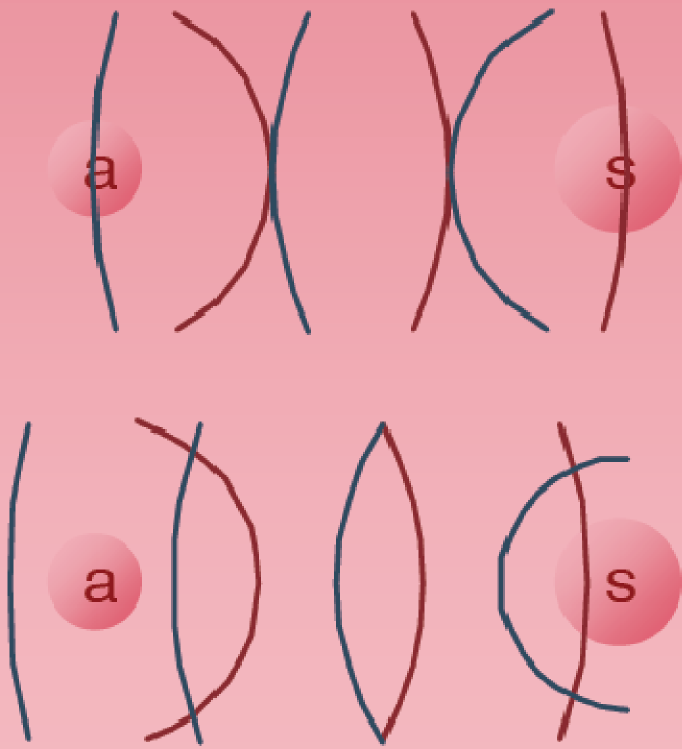
Amine Oxidase

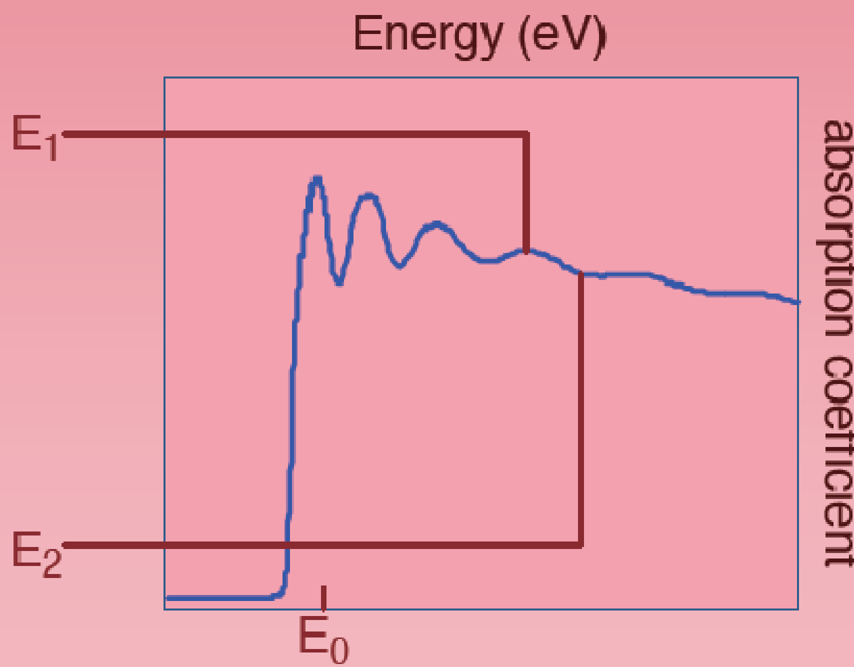
Pre-edge Transitions Reflect Symmetry



T_d	NiN_4
	NiS_4
C_{4v}	NiN_4Br
O_h	NiN_6
	NiS_6
D_{4h}	NiN_4
	NiS_4
	$NiSe_4$

EXAFS – Photoelectron Scattering



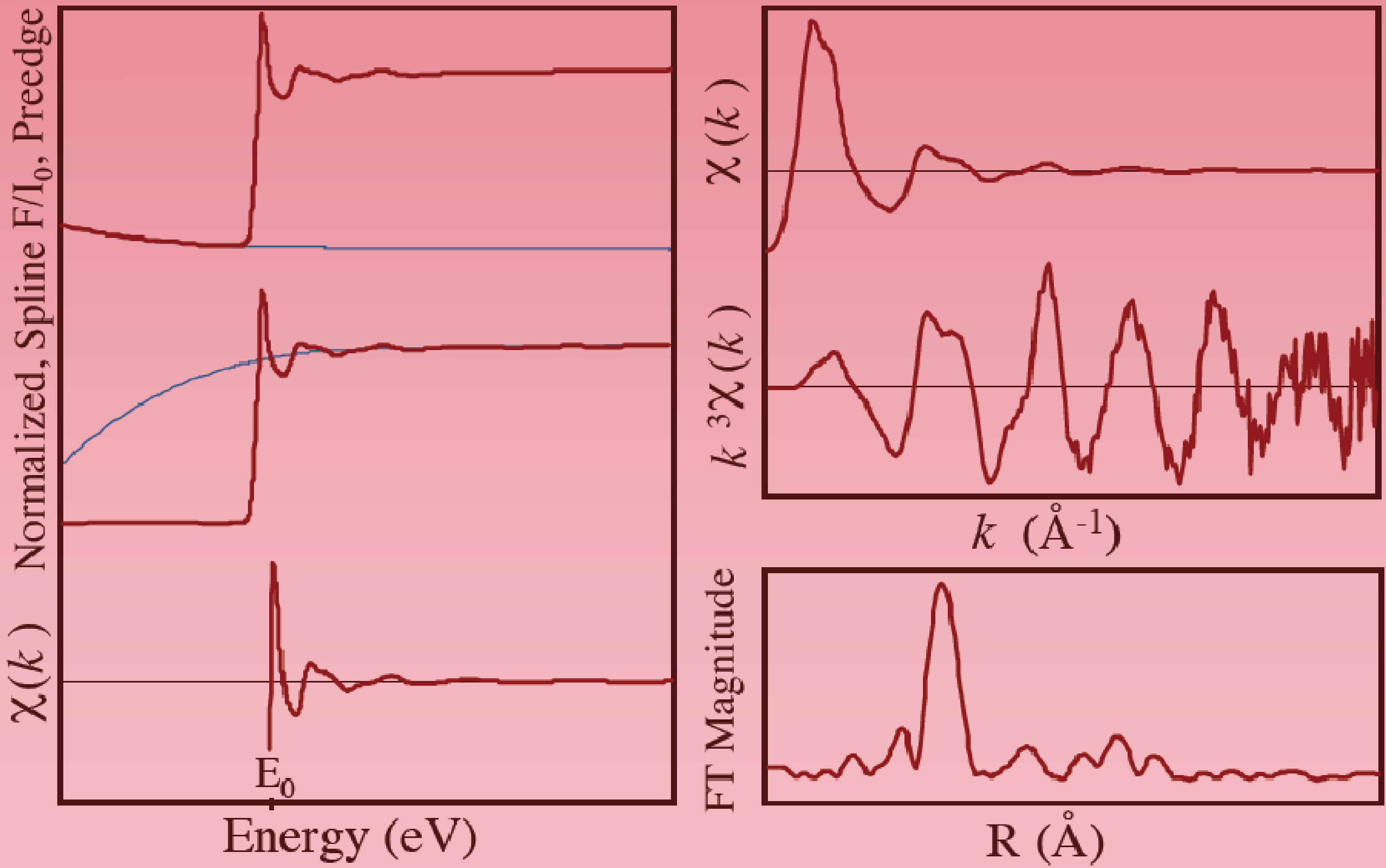


Essential Information from EXAFS

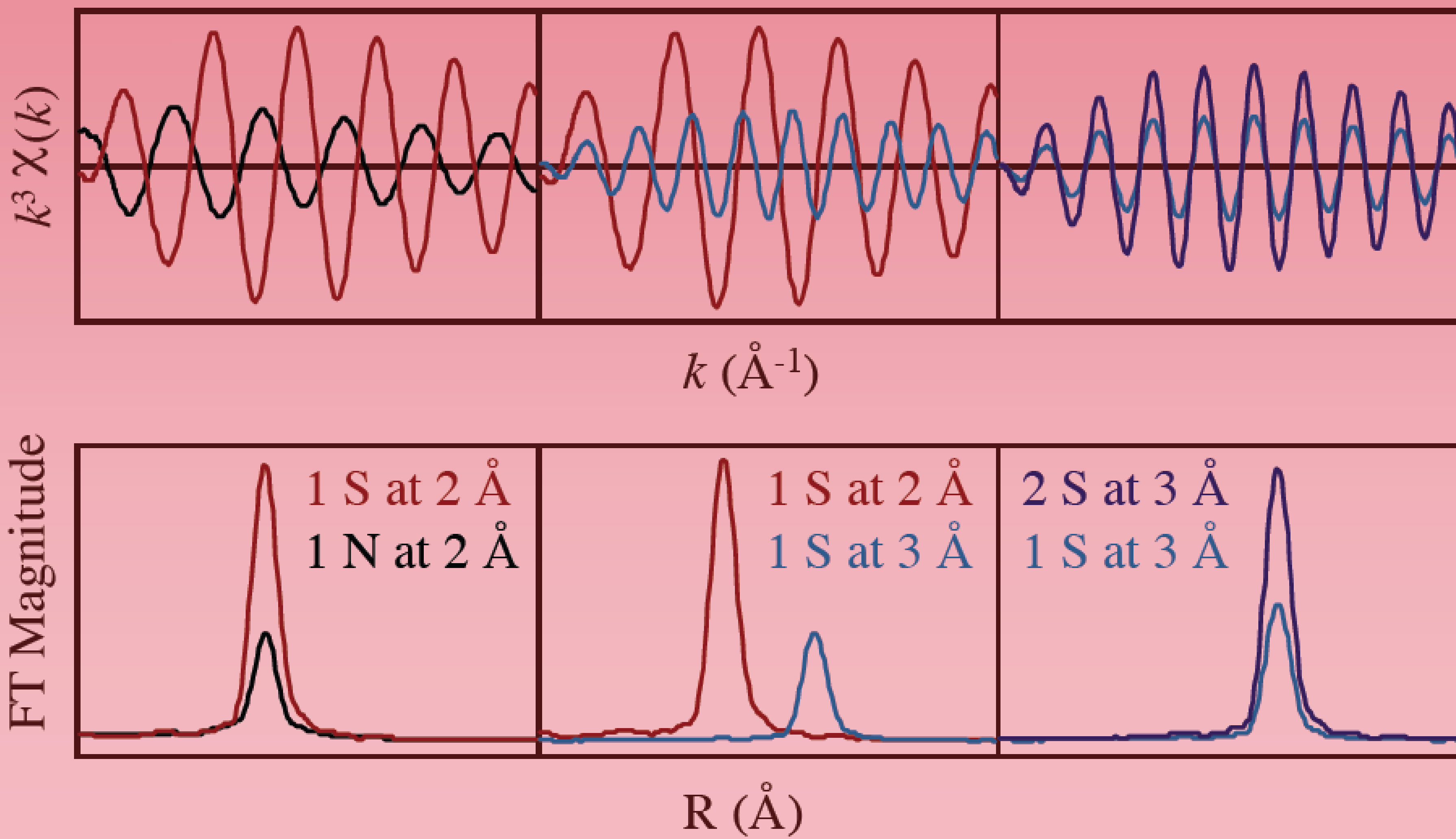
How many of what type of ligands are at what distance from metal?

Observable		Information
Frequency	→	Distance
Phase Shift	→	Type of Atom
Amplitude	→	Type of Atom
Amplitude	→	# of Atoms

EXAFS Data Reduction



EXAFS Simulations

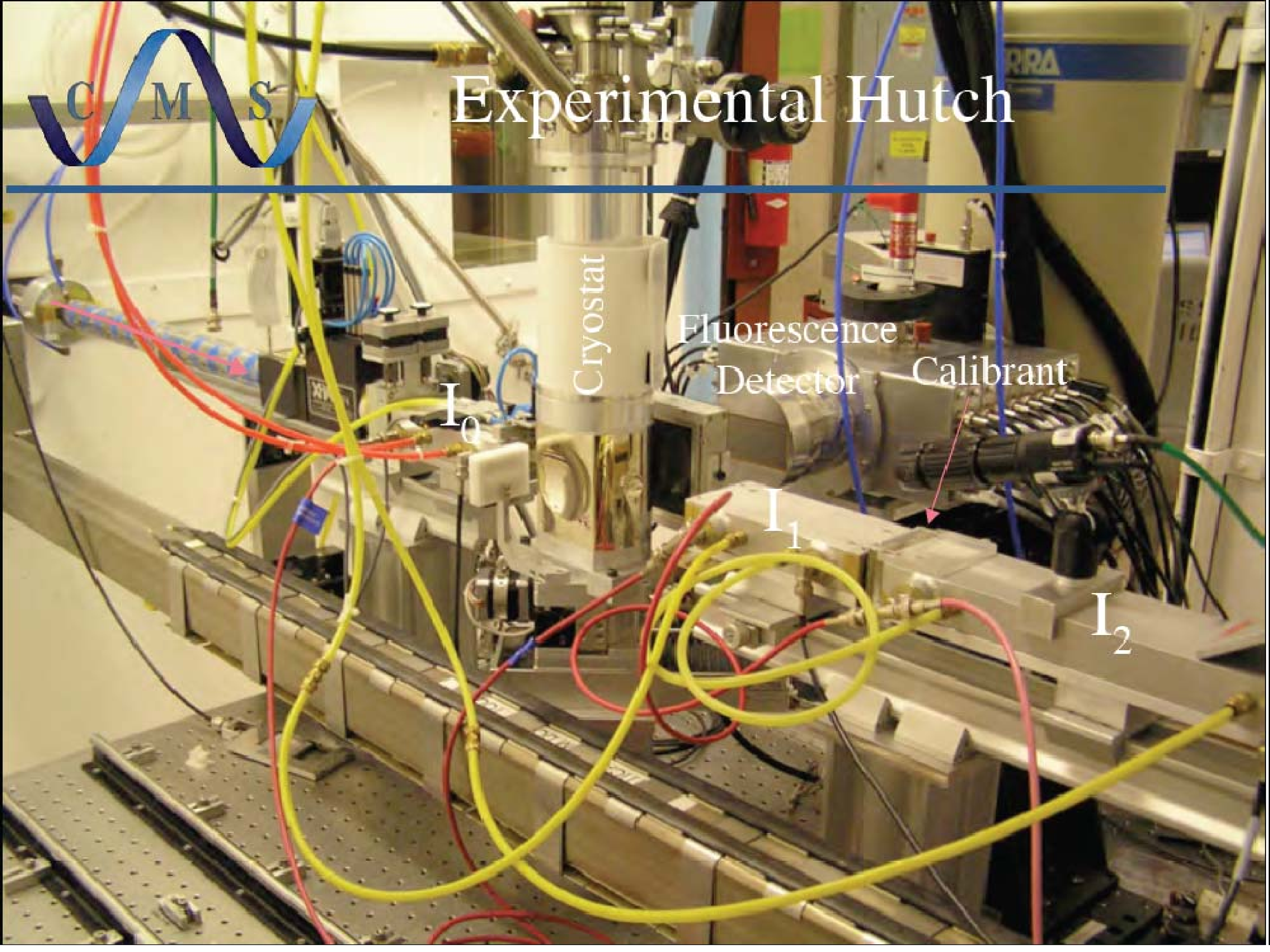


EXAFS Accuracy

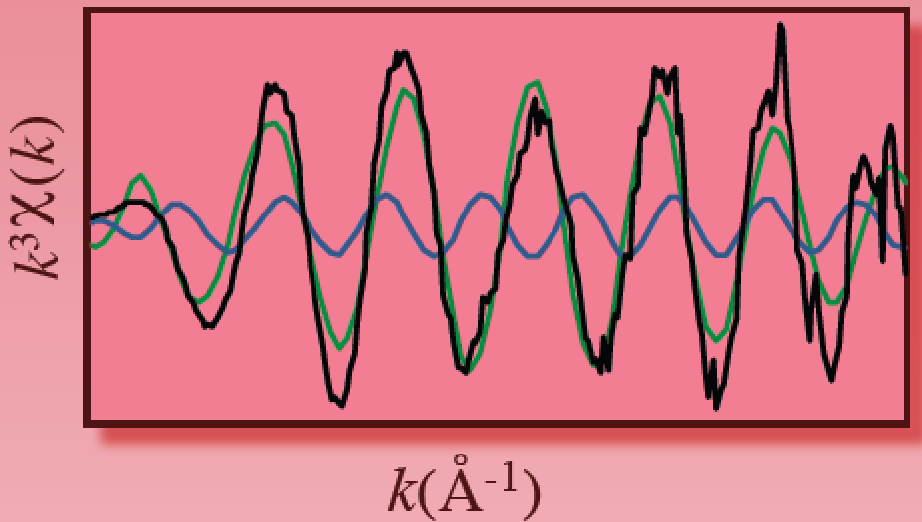
Distances	± 0.02 Å
Coordination Nos.	± 20-25 %
Scattering Atom	ΔZ ± 1 (Z=6-17) ΔZ ± 3 (Z=20-35)

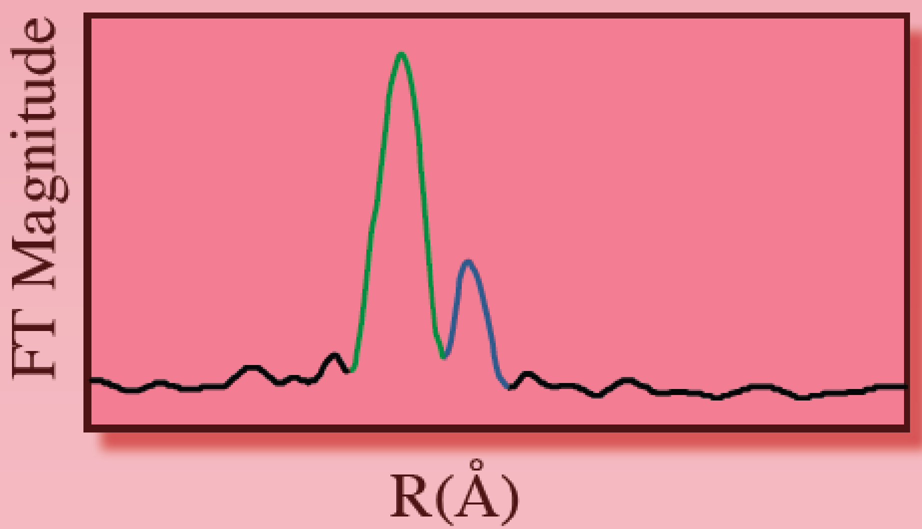
SLAC

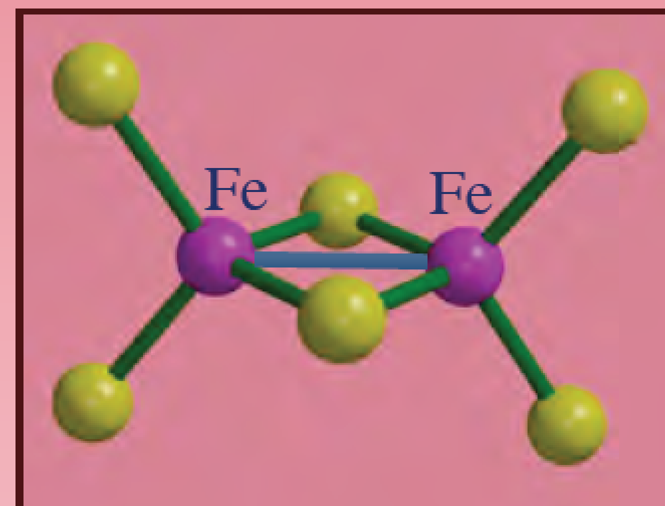
Experimental Hutch



[2Fe] Ferredoxin







Utility of XAS

Spectroscopy (XANES)

- Oxidation State
- Covalency
- Site Symmetry
- Coordination Number

Structure (EXAFS)

- Radial Distances
- Coordination Numbers
- Types of Ligands
- Cluster Nuclearity

How many of what type of ligands are at what distance from metal?