X-RAY CRYSTALLOGRAPHY

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Introduction

What is crystallography?

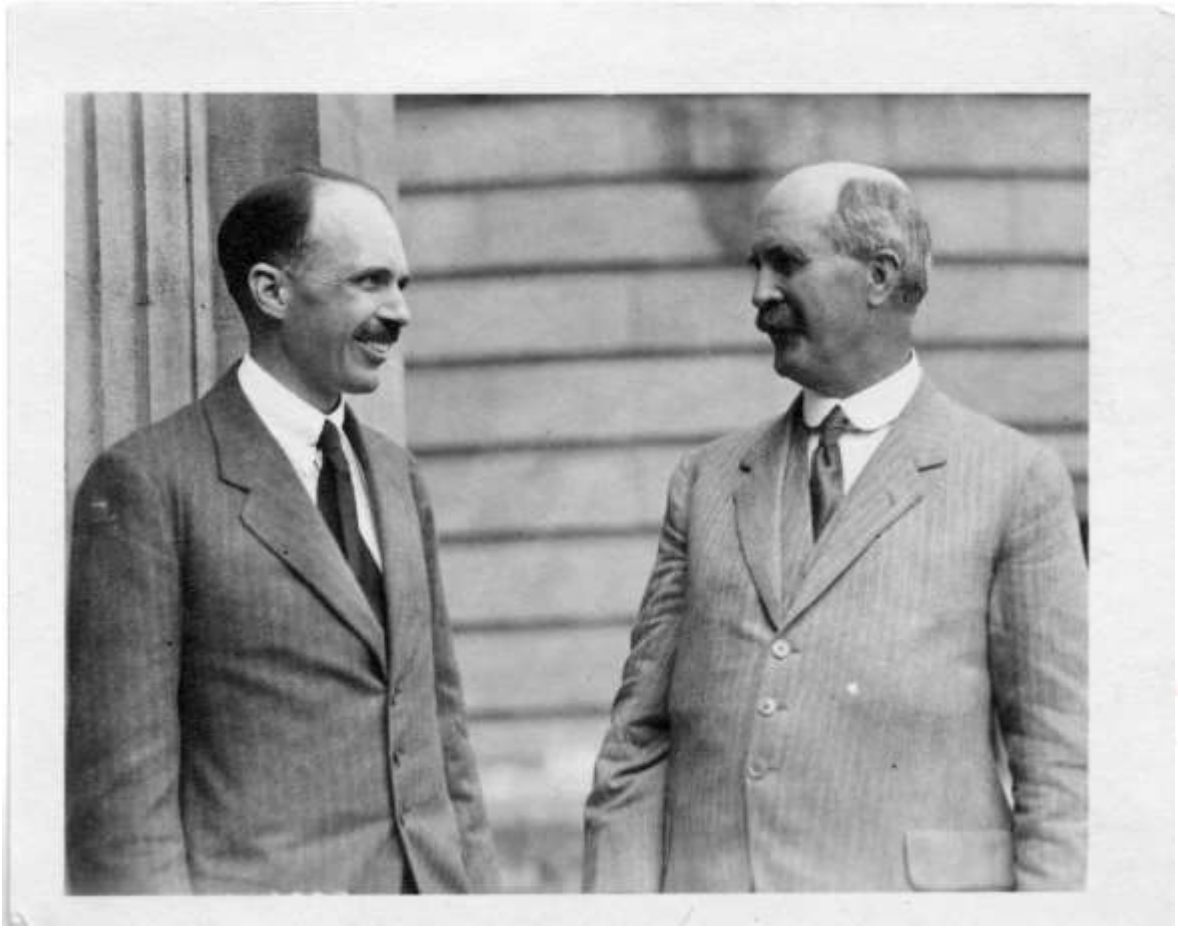
It is a tool used for determining the atomic and molecular structure of a crystal with the help of x-ray.

or more precisely

It is a method of determining the arrangement of atoms within a crystal, in which a beam of x-rays strikes a crystal and causes the x-ray beam to spread into many specific directions. From the angles and intensities of the diffracted beams, a 3 - dimensional picture of the density of electrons within the crystal are produced.

It was first used by William Lawrence Bragg in 1912 and is considered as Father of X-ray crystallography. Both father and son William Henry Bragg and William Lawrence Bragg were awarded with Novel prize in 1915 for this discovery.

Introduction



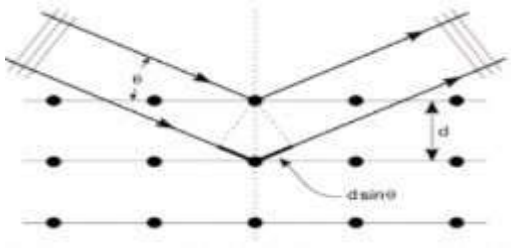
Lawrence Bragg (left) and William Henry Bragg.
Smithsonian Institution

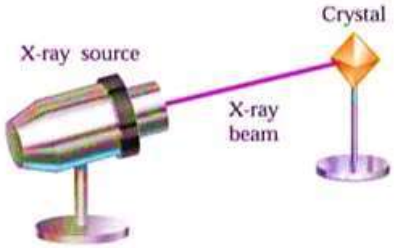
Original Bragg x-ray spectrometer, developed by William Bragg at Leeds University, 1910–1926

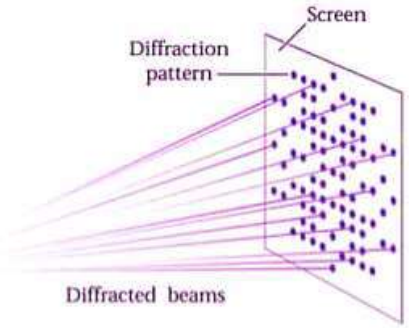
Introduction

- X-rays have an appropriate wavelength (~10^{-10}m) to be scattered by the electron cloud of an atom of the crystal.
- A diffraction pattern is obtained from X-rays scattered from the periodic assembly of molecules or atoms in the crystal.
- The electron density, and the positions of atoms, can be reconstructed from the diffraction pattern.
- The data from diffraction pattern show the intensities arising from constructive and destructive interference of the scattered wave fronts, but do not show the phases of wave fronts – a phenomenon known as phase problem.
- *[The phase problem can be resolved by getting additional information either from the diffraction data itself or by supplementing diffraction experiments with additional information from multiple isomorphous replacement (MIR), multiwavelength anomalous diffraction (MAD), and multiple X-ray wavelengths or Laue diffractometry.]*

Introduction







Diffraction of X-ray from crystal lattice

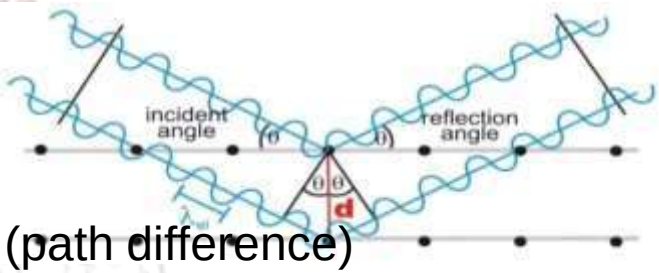
Why X-Rays? Not Others?

Microscopy	Wavelength	Visualization
Light	300 nm	Individual cells and sub-cellular organelles
Electron	10 nm	Cellular architecture Shapes of large protein molecules
X-Rays	0.1 nm or 1 Å	Atomic detail of protein

Principle

- It uses principle of diffraction from crystal of the substance.
- Diffraction of light as well as X-ray obeyed by Bragg's law, which states that when the x-ray is incident onto a crystal surface, its angle of incidence, θθ, will reflect back with a same angle of scattering, θθ. And, when the path difference, dd is equal to a whole number, nn, of wavelength, a constructive interference will occur. Thus according to Bragg's law:

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



where:

λ is the wavelength of the x-ray

d is the spacing of the crystal layers (path difference)

θ is the incident angle (the angle between incident ray and the scatter plane)

n is an integer

These specific direction appear as spots on diffraction pattern called reflections.

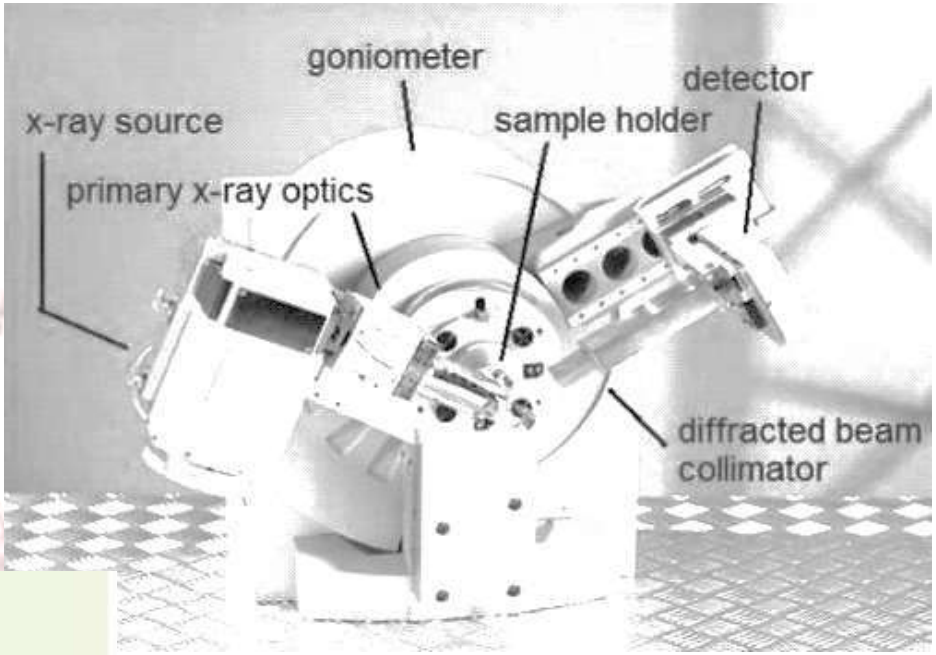
X-ray diffractometer (XRD)

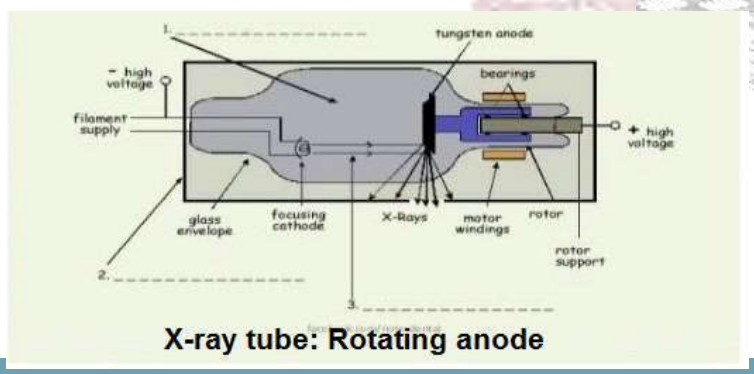
- X-ray diffraction is performed by a machine known as X-ray diffractometer (XRD).

X-ray diffractometer (XRD)

Components of diffractometer

- X-ray detector
- X-ray source
- Crystal or sample holder
- Liquid nitrogen steam to keep crystal cold
- Movable mount to rotate crystal
- Goniometer





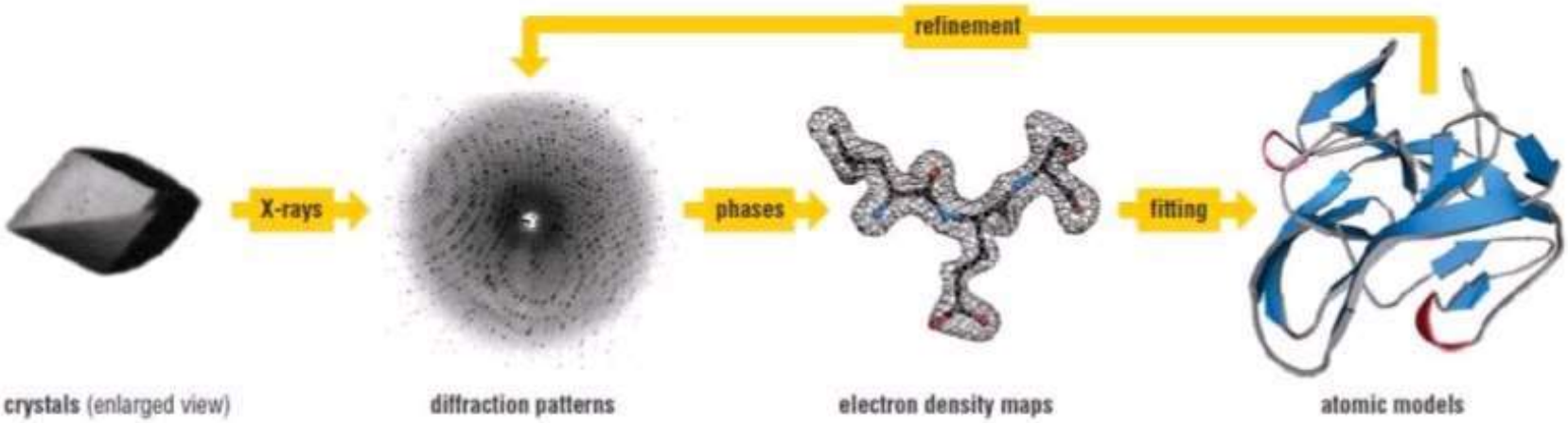
X-ray tube: Rotating anode

X-ray crystallography

Procedure

It is multistep process which can be outlined as below:

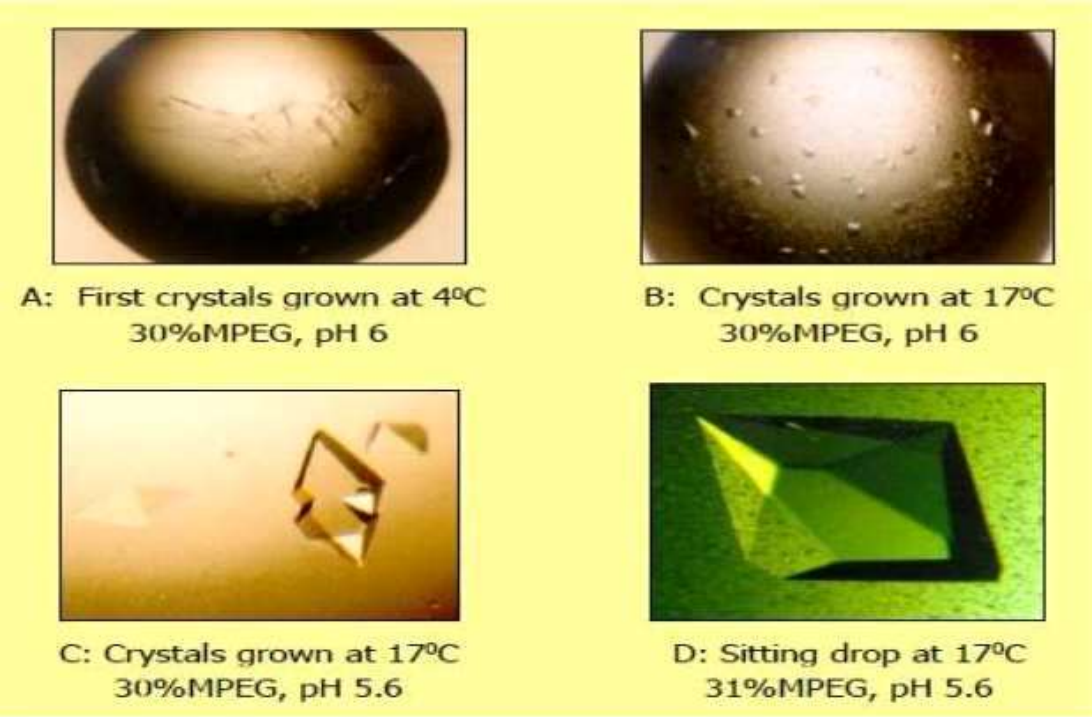
1. Protein purification
2. Protein crystallization
3. Data collection
4. Structure determination (Model building and refinement) - Identification



X-ray crystallography

Procedure

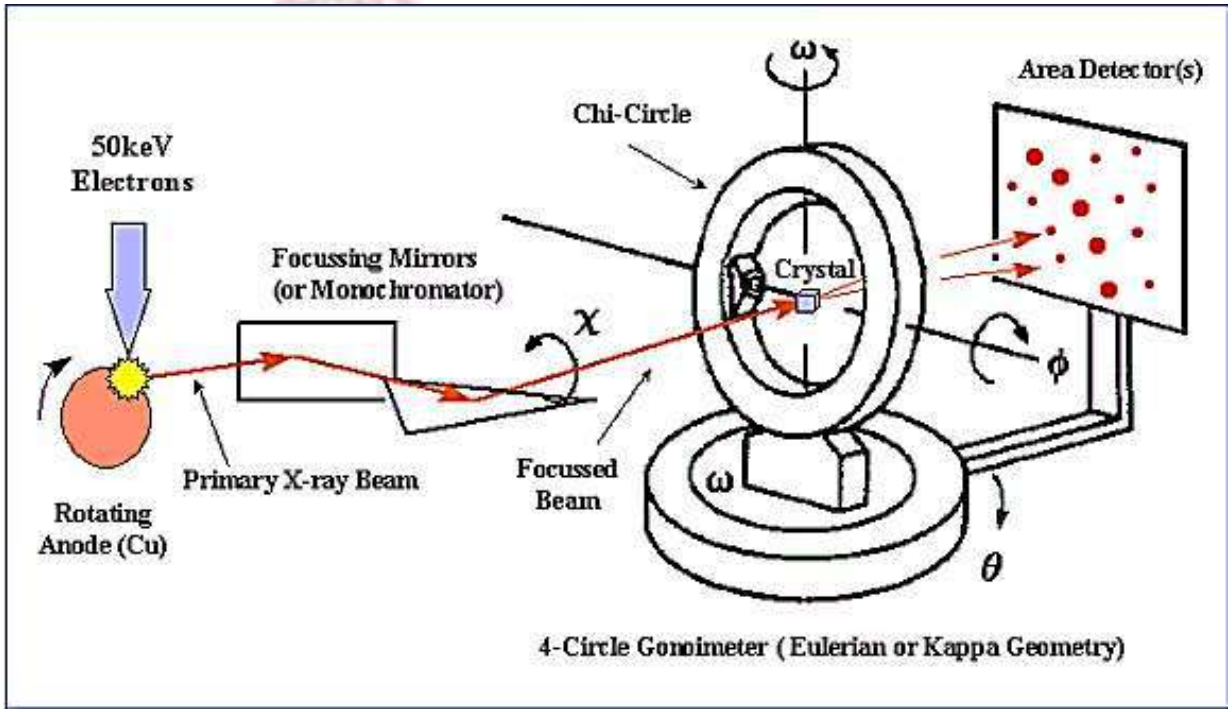
1. **Protein purification:** It involves isolation of one or few proteins from a mixture of compounds from cells, tissues or whole organisms. Purity of protein is taken to about 99% (usually >99%) by dissolving the first elute in an appropriate solvent (water-buffer solution with organic salt such as 2-methyl-2,4-pentanediol).
2. **Protein crystallization:** The pure protein solution is brought to saturation by adding a salt to the concentrated solution of the protein. The solution is then left for crystal of protein to grow.



X-ray crystallography

Procedure

3. **Data collection:** X-ray is generated and directed toward the crystallized protein and X-ray is shot at the protein crystal (crystal size typically is 0.3 x 0.3 x 0.1 mm) that hit the crystal from all sides and angles resulting in scattering of the X-ray beam; some of the x-ray pass throug the crystal. The scattered X-ray is detected by the phosphor detector as diffraction pattern which is analyzed according to the intensities of the spots and their positions.



X-ray crystallography

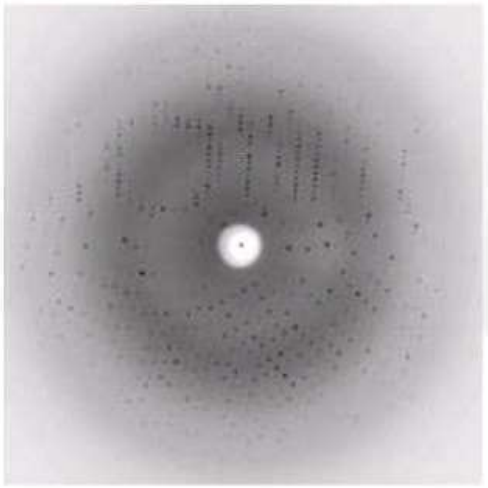
Procedure

4. **Structure determination (Model building and refinement) and identification:** Diffraction pattern is converted into electron density maps by Fourier transforms. The obtained shows contouir lines of electron density. Since electrons more or less surround atoms uniformly, it is possible to determine where atoms are located.
   - For getting 2-D picture, the crystal is rotated (Goniometer) while a computerized detector produces 2-dimensional electron density maps.
   - For getting 3-D picture, the image is produced by comparing the electron density maps obtained from rotation of the crystal with the series of images.
   - *Since hydrogen has only one electron, the electron density map of hydrogen is found difficult to obtain.*
   - *Atoms with higher atomic numbers have more electrons and therefore diffract x-rays more effectively. Diffractive power is Fe > C > H.*
   - *Electron density map provides location of atoms relative to each other .*
   - *Bond angles, bond lengths may be determined which gives position of atoms in space.*
   - *Molecular structure is obtained by connecting the dots.*

X-ray crystallography

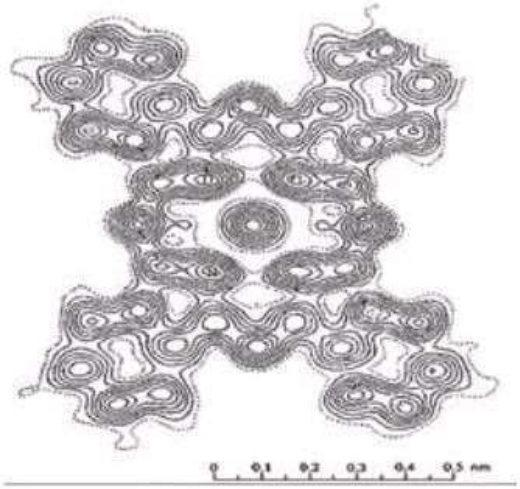
Procedure

4. **Structure determination (Model building and refinement) and identification**



A typical diffraction pattern from a protein crystal

http://www.chem.ucla.edu/harding/IGOC/D/diffraction_pattern01.jpg



The 3D structure obtained above is the electron density map of the crystal.

http://www.chem.ucla.edu/harding/IGOC/E/electron_density_ma

X-ray crystallography

Procedure

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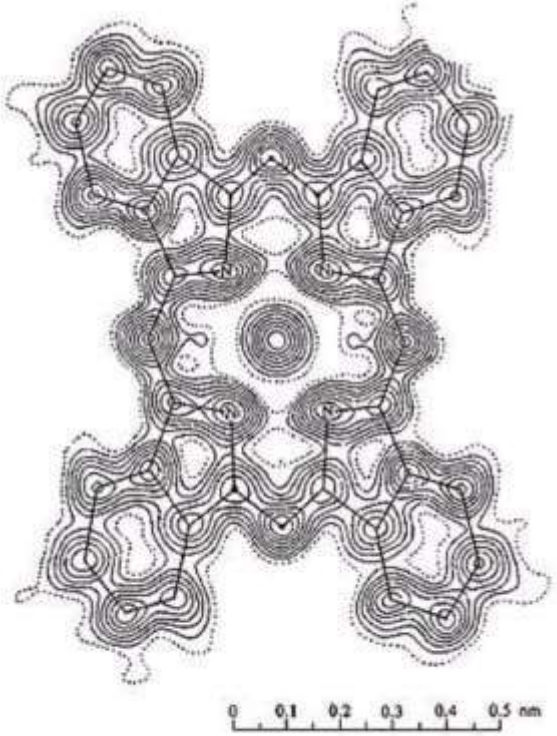
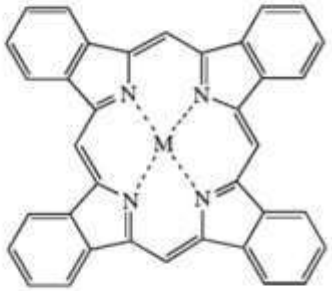


Electron Density Map



Molecular Structure

Uses

- It is used to study many materials which form crystals like salts, metals, minerals, semiconductors, as well as various inorganic, inorganic and biological molecules.
- It is used to determine electron density, the mean positions of the atoms in the crystal, their chemical bonds and various other information.
- It is used to determine size of atoms, the lengths and types of chemical bonds, and the atomic-scale differences among various materials, especially minerals.
- It is used to determine the structure and functions of many biological molecules, including vitamins, drugs, proteins and nucleic acids.

Further reading

- Claycomb J.R., Tran J.Q.P. 2010. Introductory Biophysics – Perspective on the living state 1st Ed. Jones & Bartlett Learning, Burlington, Massachusetts, USA.
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- X-ray crystallography: Revealing our molecular world. https://www.sciencemuseum.org.uk/objects-and-stories/chemistry/x-ray-crystallography-revealing-our-molecular-world
- X-ray crystallography: https://www.slideshare.net/HasanulKarim2/x-ray-crystallograpy
- X-ray Crystallography: https://www.slideshare.net/AashishPatel14/x-ray-crystellography