X-ray Crystallography

X-ray Crystallography is a scientific method used to determine the arrangement of atoms of a crystalline solid in three-dimensional space. This technique takes advantage of the interatomic spacing of most crystalline solids by employing them as a diffraction gradient for x-ray light, which has wavelengths on the order of 1 angstrom (10-8 cm). x Ray crystallography is currently the most favoured technique for structure determination of proteins and biological macromolecules.

The aim of x ray crystallography is to obtain a three-dimensional molecular structure from a crystal. A purified sample at high concentration is crystallised and the crystals are exposed to an x ray beam. The resulting diffraction patterns can then be processed, initially to yield information about the crystal packing symmetry and the size of the repeating unit that forms the crystal. This is obtained from the pattern of the diffraction spots. The intensities of the spots can be used to determine the "structure factors" from which a map of the electron density can be calculated.

Introduction:

X-ray crystallography is a technique used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X-rays to diffract into many specific directions.

Then they use an X-ray beam to "hit" the crystallized molecule. The electrons surrounding the molecule diffract as the X-rays hit them. This forms a pattern; this type of pattern is called the X-ray diffraction pattern.

History:

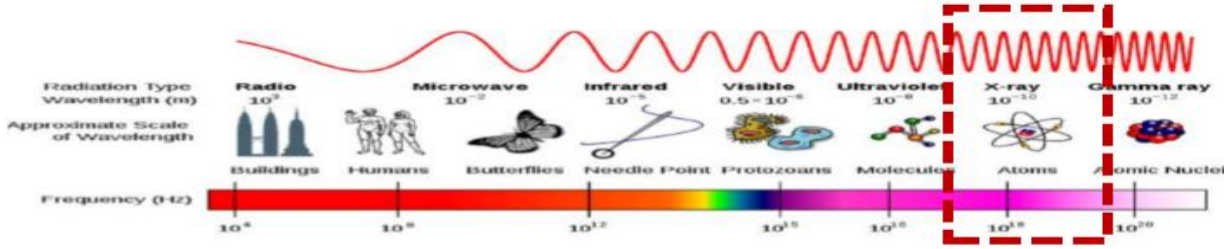
The English physicist Sir William Henry Bragg pioneered the determination of crustal structure by X-ray diffraction methods

X-ray crystallography is a complex field that has been associated with several of science's major breakthroughs in the 20th century

Using X-ray crystal data, Dr. James Watson and Dr. Francis Crick were able to determine the helix structure of DNA in 1953.

Why X rays?

An electromagnetic wave of high energy and very short wavelength, which can pass through many materials opaque to light. (wavelength 1 angstrom)

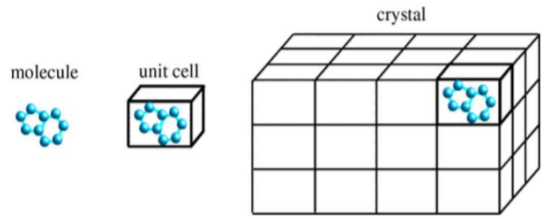


The wavelength of X-ray photons is on the order of the distance between atomic nuclei in solids (bonds are roughly 1.5-2.5 Å). You can think of it like the waves fit nice between the atoms and "fill" the crystal.

Why Crystal?

Researchers crystallize an atom or molecule, because the precise position of each atom in a molecule can only be determined if the molecule is crystallized.

If the molecule or atom is not in a crystallized form, the X-rays will diffract unpredictably, and the data retrieved will be too difficult if not impossible to understand.

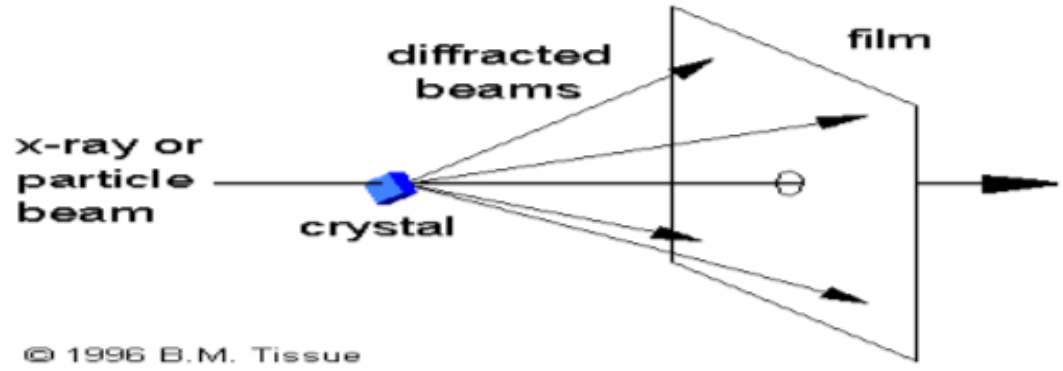


X-ray diffraction

Diffraction is the slight bending of light as it passes around the edge of an object.

X-ray crystallography uses the uniformity of light diffraction of crystals to determine the structure of molecule or atom. Then X-ray beam is used to hit the crystallized molecule.

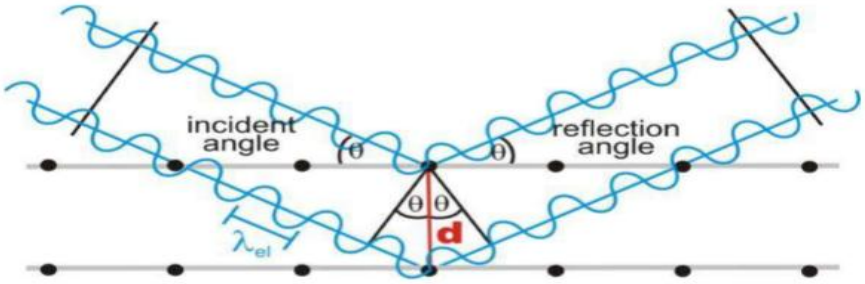
The electron surrounding the molecule diffract as the X-rays hit them. This forms a pattern. This type of pattern is known as X-ray diffraction pattern.



Bragg's Law:

There is a definite relationship between the angle at which a beam of X rays must fall on the parallel planes of atoms in a crystal in order that there be strong reflection, the wavelength of the X rays, and the distance between the crystal planes $2d \sin\Theta = n\lambda$

Here d is the spacing between diffracting planes, Θ is the incident angle, n is any integer, and λ is the wavelength of the beam.

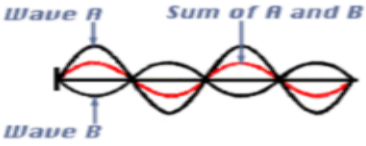


Interference

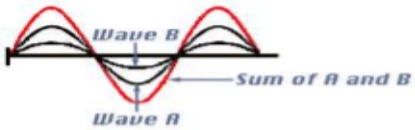
- When an incident x-ray beam **hits a scatterer**, scattered x-rays are emitted in all directions. Most of the scattering wave fronts are **out of phase interfere destructively**. Some sets of wave fronts are in phase and interfere constructively.

- A crystal is composed of many repeating unit cells in 3-dimensions, and therefore, acts like a 3-dimensional diffraction grating. **The constructive interference** from a diffracting crystal is observed as a pattern of points on the detector. The relative positions of these points are related mathematically to the crystal's unit cell dimensions.

Destructive Interference



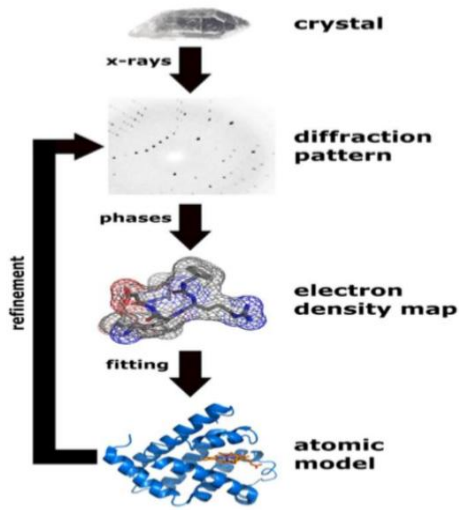
Constructive Interference



Procedure

- The first-and often most difficult-step is to obtain an adequate crystal of the material under study.
- The crystal should be sufficiently large (typically larger than 0.1 mm in all dimensions), pure in composition and regular in structure, with no significant internal imperfections such as cracks.
- Researchers crystallize an atom or molecule, because the precise position of each atom in a molecule can only be determined if the molecule is crystallized.
- The crystal is placed in an intense beam of X-rays, usually of a single wavelength (monochromatic X-rays), producing the regular pattern of reflections. As the crystal is gradually rotated, previous reflections disappear, and new ones appear; the intensity of every spot is recorded at every orientation of the crystal.
- Multiple data sets may have to be collected, with each set covering slightly more than half a full rotation of the crystal and typically containing tens of thousands of reflections.

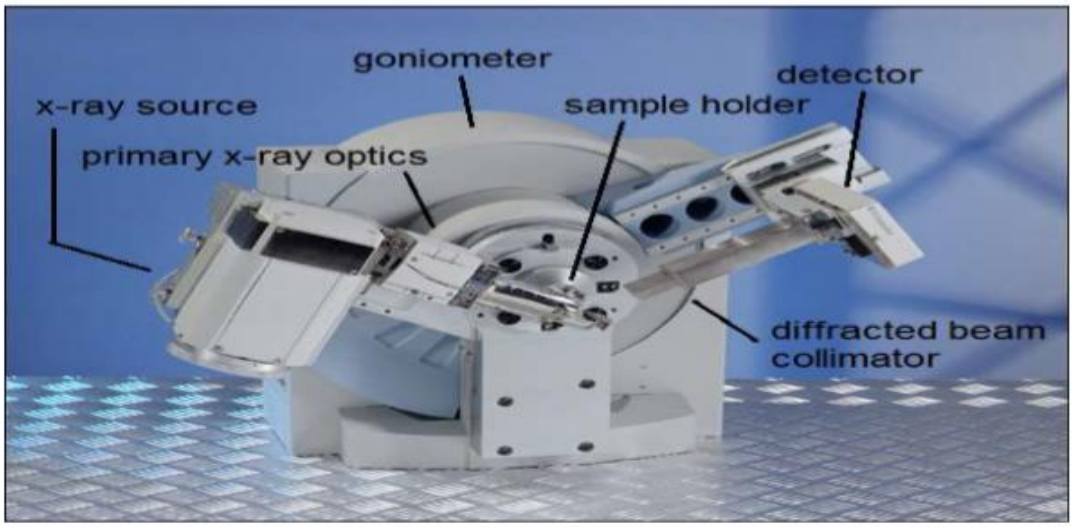
- In the third step, these data are combined computationally with complementary chemical information to produce and refine a model of the arrangement of atoms within the crystal. The final, refined model of the atomic arrangement-now called a crystal structure is usually stored in a public database.
- After the diffraction pattern is obtained, the data is then processed by a computer and the structure of the atom or molecule is deduced and visualized.



Instrumentation:

Generally a typical x-ray diffraction contain below parts:

- X-ray source
- Detector
- Crystal on the end of mounting needle
- Liquid nitrogen steam to keep crystal cold
- Movable mount to rotate crystal
- Collimator



X-ray Tube: the source of X-rays

Incident-beam optics: condition the X-ray beam before it hits the sample

The goniometer: the platform that holds and moves the sample, optics, detector, and/or tube

The sample &sample holder

Receiving-side optics: condition the X-ray beam after it has encountered the sample

Detector: count the number of X Rays scattered by the sample.

Limitations

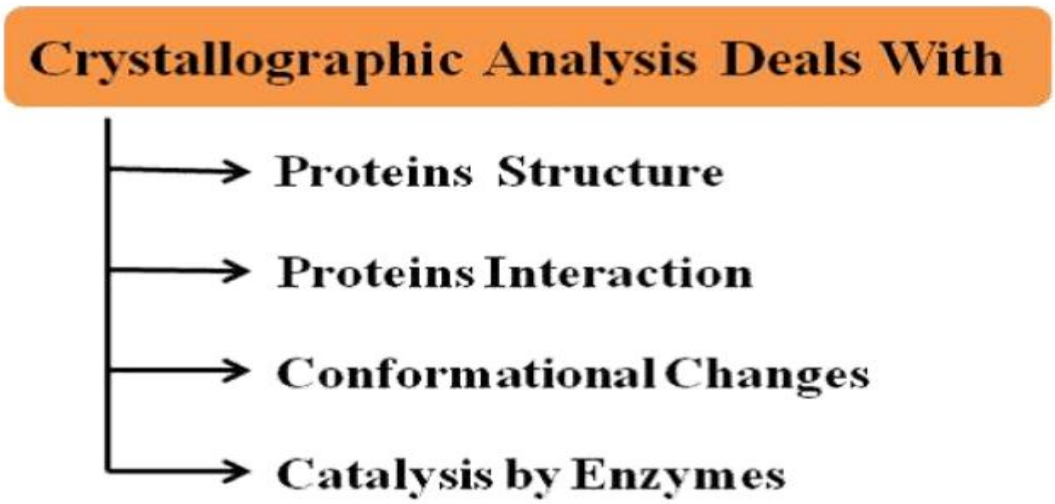
- Small-molecule crystallography typically involves crystals with fewer than 100 atoms in their asymmetric unit; such crystal structures are usually so well resolved that the atoms can be discerned as isolated "blobs." of electron density.
- By contrast, macromolecular crystallography often involves tens of thousands of atoms in the unit cell. Such crystal structures are generally less well-resolved (more "smeared out"); the atoms and chemical bonds appear as tubes of electron density, rather than as isolated atoms.
- In general, small molecules are also easier to crystallize than macromolecules; however, X-ray crystallography has proven possible even for viruses with hundreds of thousands of atoms.

Application of X ray Crystallography

- XRD is a non-destructive technique
- To identify crystalline phases and orientation
- To determine structural properties.
- To measure thickness of thin films and multi-layers
- To determine atomic arrangement
- X-ray diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.
- identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically.
- determination of unit cell dimensions measurement of sample purity

Applications of X ray Crystallography in proteomics

Proteomics: A branch of biotechnology concerned with applying the techniques of molecular biology, biochemistry, and genetics to analysing the structure, function, and interactions of the proteins produced by the genes of a particular cell, tissue, or organism, with organizing the information in databases, and with applications of the data.



Protein Structure Determination:

Protein structure determination refers to finding the exact orientations and arrangements of different amino acids present in the protein.

X ray crystallography helps us to determine the structure of proteins which further helps us to even determine its function.

Protein Interaction Studies:

Protein interaction refers to the way in which two or more proteins interact with each other. Studies include the orientation, site of action and the major amino acids (of protein) taking part in a reaction ray crystallography helps to determine their Interactions.

Conformational Studies:

Conformational studies refer to spatial arrangements of atoms in a molecule that can come about through free rotation of atoms about a chemical bond.

It is necessary to determine the arrangements as it determines the structure and function of Protein X ray crystallography is an efficient technique to determine it.

Enzyme catalysis Determination:

Enzymes are protein. Determination of structure (especially active site's) and type of amino acids present in active sites determines catalytic activities, Interaction level of enzymes.

X ray crystallography helps us to determine and predict the catalytic efficiency of enzymes.

Conclusion:

X-ray crystallography is essentially a form of very high-resolution microscopy.

It enables us to visualize protein structures at the atomic level and enhances our understanding of protein function.

Specifically, we can study how proteins interact with other molecules, how they undergo conformational changes, and how they perform catalysis in the case of enzymes.

Armed with this information we can design novel drugs that target a protein, or rationally engineer an enzyme for a specific industrial process.