IIT Guwahati Lecture 27

Course BT 631

Protein Structure, Function and Crystallography

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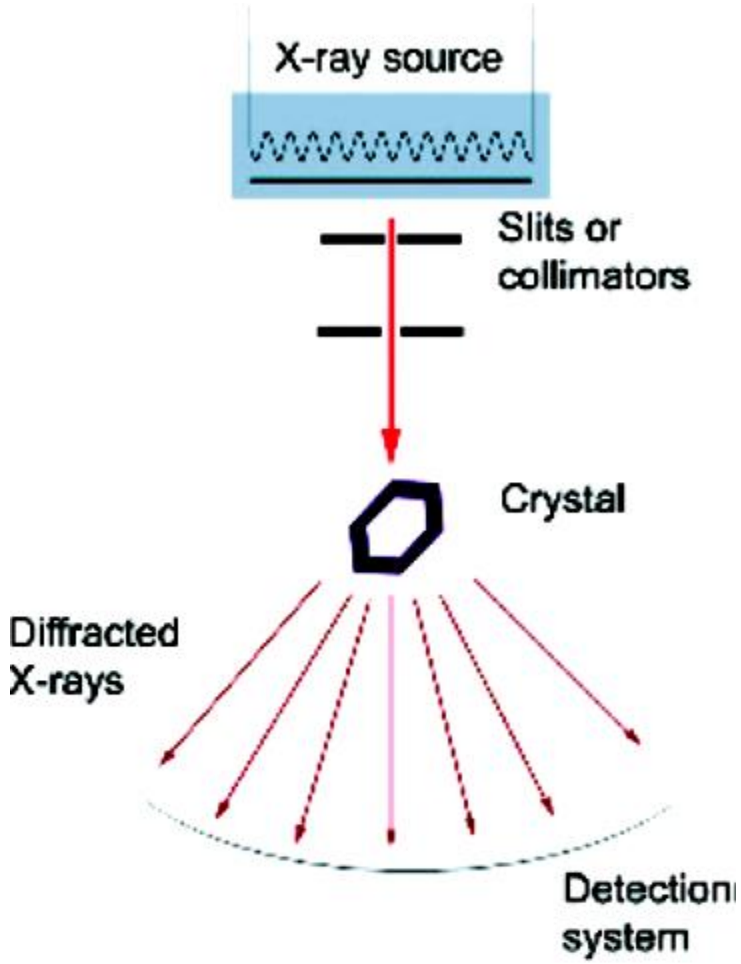
X-ray Crystallography

- Properties and functions of all proteins are influenced by their molecular structures.
- Atomic details of protein structure can provide the basis for enzymatic mechanisms.
- The HIV protease inhibitors amprenavir and nelfinavir were developed by using the knowledge of crystal structure of HIV protease.

- X-ray crystallography is a technique to determine the arrangement of atoms in a crystal.
- When X-rays are incident on a crystal they are diffracted into several directions.
- This diffraction pattern is used to reconstruct the electron density within the crystal.
- This electron density is then used to determine the mean positions of the atoms in the crystal.

X-ray crystallography

- X-ray beams (from source) are shot through a crystal of the atom.
- The crystal causes the beam of X-ray to diffract in a pattern based on their crystal lattice structure.
- This results in a diffraction pattern. (Fig.)
- After observing the reflections of the photons on the detector, the angle at which the X-ray beam hits the crystal is changed.
- All the reflections combined together to find the three-dimensional diffraction pattern and derive the electron density map by using the Fourier transform technique. Diffraction is caused by electron clouds.
- The higher the atomic number of an element, the larger is its electron cloud.



Basic crystallography 'set-up' used in X-ray diffraction.

Mono-chromatic X-rays after passing through filters strike the crystal.

X-ray crystallography

X-rays were discovered by W. C. Röntgen in 1895.

Single-slit experiments suggested the wavelength of X-rays was about 1.0 Å.

However, X-rays are composed of photons and thus are not only waves of electromagnetic radiation but also exhibit particle-like properties.

X-rays are used to produce the diffraction pattern because their wavelength, λ is typically the same order of magnitude (1–100 Å) as the spacing, d between planes in the crystal.

W. C. Röntgen

X-ray crystallography

To produce significant diffraction, the spacing between the scattering particles and the wavelength of the impinging wave should be similar.

In all forms of microscopy, the fineness or the resolution is limited by the wavelength of the electro-magnetic radiation used.

With Light microscopy, where the shortest wavelength is about 300 nm, one can see individual cells and sub-cellular organelles.

With Electron microscopy, where the wavelength is below 10 nm, one can see detailed cellular architecture and the shapes of large protein molecules.

In order to see proteins in atomic detail, one needs to work with electro-magnetic radiation with a wavelength of around 0.1 nm (or 1 Å), in other words we need to use X-rays.

X-ray crystallography

Typical wavelength used for crystallography is 1.0 Å (0.1 nm), which is on the scale of covalent chemical bonds and the radius of a single atom.

Longer-wavelength photons (such as Ultraviolet radiation) will not have sufficient resolution to determine the atomic positions.

At the other extreme, the shorter-wavelength photons such as Gamma rays are difficult to produce in large numbers, difficult to focus and interact too strongly with matter, producing particle-antiparticle pairs.

Bragg's Law

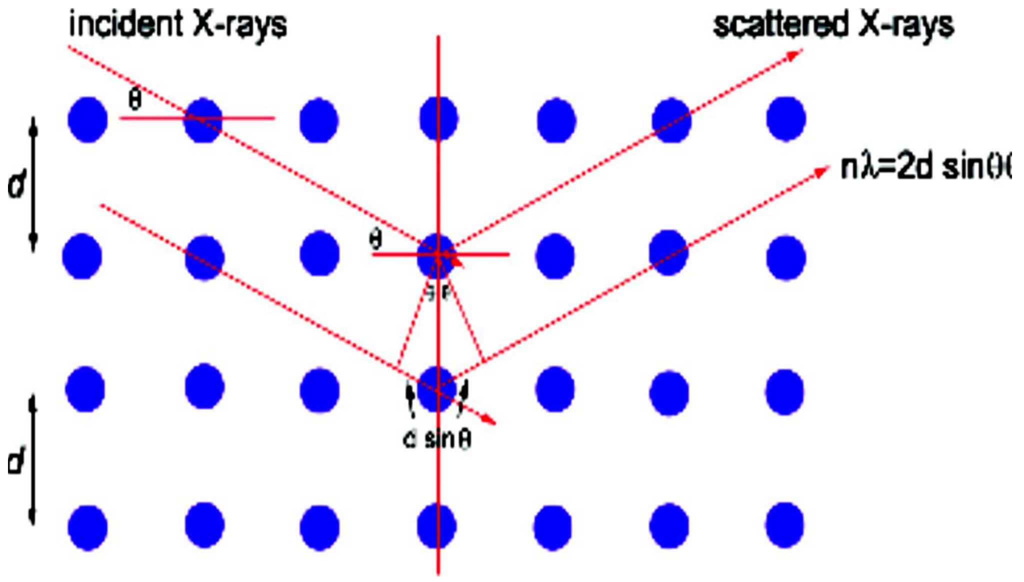
Diffraction of an X-ray beam occurs when the light interacts with the electron cloud surrounding the atoms of the crystalline solid.

Due to the periodic crystalline structure of a solid, it is possible to describe it as a series of planes with an equal interplanar distance.

As an X-ray beam hits the surface of the crystal at an angle θ, some of the light will be diffracted at that same angle away from the solid (Fig.).

The remainder of the light will travel into the crystal and some of that light will interact with the second plane of atoms.

This process will repeat for the many planes in the crystal.



Bragg's Law

The X-ray beams travel different path lengths before hitting the various planes of the crystal, so after diffraction, the beams will interact constructively only if the path length difference is equal to an integer number (n) of wavelengths. In the figure, the difference in path lengths of the beam striking the first plane and the beam striking the second plane is equal to AB + BC.

So, the two diffracted beams will constructively interfere (be in phase) only if AB+BC = nλ. Eq. 1

Basic trigonometry will tell that the two segments are equal to one another with the interplanar distance times the sine of the angle θ.

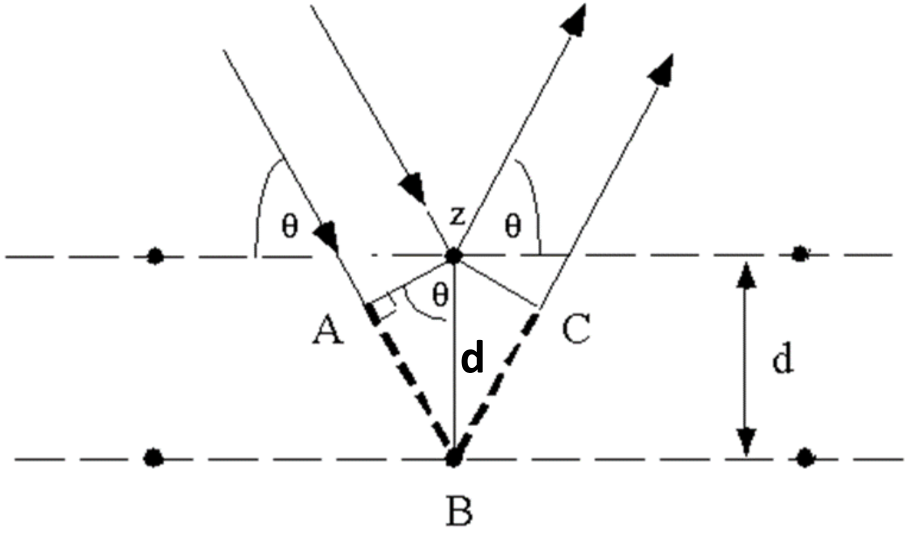
Recognizing d as the hypotenuse of the right triangle ABz, we can use trigonometry to relate d and θ to the distance (AB+BC).

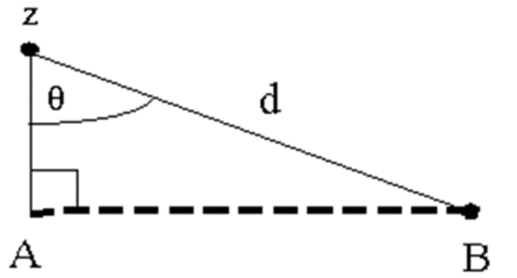
$$AB = d\sin\theta \quad \text{(Eq. 2)}$$

Because AB = BC, (AB= BC= d$\sin\theta$) the Eq. 1 becomes

$$2AB = n\lambda \quad \text{(Eq. 3)}$$

Thus, from Eq. 2 and 3, $2d\sin\theta = n\lambda$





The lower beam must travel the extra distance (AB + BC) to continue traveling parallel and adjacent to the top beam.

$\sin\theta$ = Perpendicular/hypotenuse = AB/d

Thus AB = d$\sin\theta$

Bragg's Law

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

This equation is known as Bragg's Law, named after W. H. Bragg and his son, W. L. Bragg, who discovered this geometric relationship in 1912.

Bragg's Law relates the distance between two planes in a crystal and the angle of reflection to the x-ray wavelength.

The X-rays that are diffracted off the crystal have to be in-phase in order to get signal.