FAQ

1. **Describe properties of X-Rays?**

X-rays are produced when any electrically charged particle of sufficient kinetic energy rapidly decelerates. Electrons are usually used for this purpose, the radiation being produced in an *x-ray tube* which contains a source of electrons and two metal electrodes. The high voltage maintained across these electrodes, some tens of thousands of volts, rapidly draws the electrons to the anode, or *target*, which they strike with very high velocity. X-rays are produced at the point of impact and radiate in all directions. If *e* is the charge on the electron (1.60 x 10^{-19} coulomb) and *V* the voltage across the electrodes, then the kinetic energy (in joules) of the electrons on impact is given by the equation

$$KE = eV = \frac{1}{2}mv^2$$

where *m* is the mass of the electron (9.11 x 10^{-31} kg) and *v* its velocity in m/sec just before impact. At a tube voltage of 30,000 volts, this velocity is about one-third that of light. Most of the kinetic energy of the electrons striking the target is converted into heat, less than 1 percent being transformed into x-rays

2. **Why use X-rays?**

In all forms of microscopy, the amount of detail 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 may be below 10 nm, one can see detailed cellular architecture and the shapes of large protein molecules. In order to see proteins in atomic detail, we need 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.

In light microscopy, the subject is irradiated with light and causes the incident radiation to be diffracted in all directions. The diffracted beams are then collected, focused and magnified by the lenses in the microscope to give an enlarged image of the object. The situation with electron microscopy is similar only in this case the diffracted beams are focused using magnets. Unfortunately it is not possible to physically focus an X-ray diffraction pattern, so it has to be done mathematically and this is where the computers come in. The diffraction pattern is recorded using some sort of detector which used to be X-ray sensitive film, but nowadays is usually an image plate or a charge-coupled device (CCD).

3. **Why do we need a crystal?**

The diffraction from a single molecule would be too weak to be measurable. So we use an ordered three-dimensional array of molecules, in other words a crystal, to magnify the signal. Even a small protein crystal might contain a billion molecules. If the internal order of the crystal is poor, then the X-rays will not be diffracted to high angles or high resolution and the data will not yield a detailed structure. If the crystal is well ordered, then diffraction will be measurable at high angles or high resolution and a detailed structure should result. The X-rays are diffracted by the electrons in the structure and consequently the result of an X-ray experiment is a 3-dimensional map showing the distribution of electrons in the structure.

A crystal behaves like a three-dimensional diffraction grating, which gives rise to both constructive and destructive interference effects in the diffraction pattern, such that it appears on the detector as a series of discrete spots which are known as reflections. Each reflection contains information on all atoms in the structure and conversely each atom contributes to the intensity of each reflection. As with all forms of electro-magnetic radiation, X-rays have wave properties, in other words they have both an amplitude and a phase. In order to recombine a diffraction pattern, both of these parameters are required for each reflection. Unfortunately, only the amplitudes can be recorded experimentally. All phase information is lost. This is known

as "the phase problem". When crystallographers say they have solved a structure, it means that they have solved "the phase problem".

4. **How X-rays are generated?**

X-rays are produced when electrons strike a metal target. The electrons are liberated from the heated filament and accelerated by a high voltage towards the metal target. The X-rays are produced when the electrons collide with the atoms and nuclei of the metal target.

5. **Define terms crystals and lattice?**

A crystal may be defined as *a solid composed of atoms, ions or molecules arranged in a pattern periodic in three dimensions*. As such, crystals differ in a fundamentalway from gases and liquids because the atomic, ionic or molecular arrangements inthe latter do not possess the essential requirement of periodicity. Many solids arecrystalline; if they are not single-crystals they consist of many contiguous crystals,i.e., they are polycrystalline. Not all solids are crystalline, however; some are *amorphous*,like glass, and do not have any regular interior arrangement of atoms, ionsor molecules.

The crystal is then represented as a *lattice*, that is, a three-dimensional array of points (*lattice points*), each of which has identical surroundings. As mathematical constructs, lattices are infinite in extent whereas crystals are not.

6. **Why do I need to learn crystallography?**

The simple answer is that crystallography is the most widely used experimental method to

visualize structures at the atomic level. For example, Watson and Crick used Rosalind Franklin's

X-ray diffraction data to generate a model of the DNA double helix. Molecular machines such as

myosin, the ribosome, RNA polymerase, topoisomerases, ion channels, Gprotein signal

transduction, RISC and many more are known because of crystallography. Structures have

proven essential to develop drugs (e.g. HIV-1 protease). The basis for antigen recognition by

and antibody, and MHC complex displays of self and foreign peptides are known from

crystallography. The structure of histones are known from crystallography.

7. **How are X-rays obtained from an accelerator?**

Electromagnetic waves are emitted when a charged particle is submitted to an acceleration. In a circular accelerator such as a synchrotron or a storage ring, electrons are deviated by magnetic fields. This deviation is due to the radial force (i.e. a radial acceleration) which attracts the electrons towards the centre or the ring. The light emitted by electrons in a synchrotron or a storage ring is called "synchrotron radiation". Its wide spectrum reaches the X-ray range only when the energy of the electrons is high enough (of the order of several billion electronvolts - GeV).

8. **Why is the X-ray diffraction pattern a reciprocal picture instead of a real one, like in electron microscopy for example?**

In an electron microscope, the scattered electrons are refocussed to form an image by the lenses of the microscope's optics. The electron is a charged particle so it can be focussed via electromagnets. The focussing is the equivalent of converting the reciprocal to the real. In general, X-rays cannot be focussed, so stay as the reciprocal. (Note that lenses do now exist for X-rays, but with very small apertures and very small focussing power so this is not yet practicable for X-rays scattered by diffraction from a crystal).

9. **What are the applications of X-ray crystallography?**

   Currently, X-ray crystallography is the most accurate and precise method available for chemical structure determination (although some

NMR technologies are coming close). However, due to the nature of diffraction and need for uniform samples, only crystalline compounds can be used. "Growing" or designing these samples is relatively easy for simple and/or small compounds, but as the complexity increases (proteins, viruses, DNA), creating crystal lattices to analyze becomes more difficult. The crystal structure ensures uniform distribution and orientation of one molecule relative to the molecules surrounding it, which is crucial to ensure the diffraction pattern is only determined by the bond orientation *within* the molecules. Non-crystallized samples on the other hand have molecules in random relative orientations, which would produce jumbled, and essentially useless diffraction patterns.

10. **How XRD is useful in pharmaceutical industry?**

X-ray diffraction (XRD) can be used to unambiguously characterize the composition of pharmaceuticals. An XRD-pattern is a direct result of the crystal structures, which are present in the pharmaceutical under study. As such, the parameters typically associated with crystal structure can be simply accessed. For example, once an active drug has been isolated, an indexed X-ray powder diffraction pattern is required to analyze the crystal structure, secure a patent and protect the company's investment.

For multi-component formulations, the actual percentages of the active ingredients in the final dosage form can be accurately analyzed in situ, along with the percentage of any amorphous packing ingredients used.

11. **What is involved in a crystal structure determination?**

Protein preparation

Firstly we need to obtain a pure sample of our target protein. We can do this by either isolating it from its source, or by cloning its gene into a high expression system. The sample then needs be assessed for

suitability according to the following criteria:

Is it pure and homogeneous?

we can test this by various electrophoretic methods and mass spectrometry .

Is the protein soluble and folded?

If protein estimations suggest that a lot of protein is being lost, then it may be due to precipitation.

The degree of ordered secondary structure can be tested with circular dichroism. If this is very low then the protein may be misfolded. This may occur if the protein is being produced faster than it can fold and may result in the formation of insoluble inclusion bodies. Attenuating the induction can alleviate this problem e.g. using a lower temperature.

Is the sample stable?

Occasionally good protein crystals will form overnight at room temperature, but usually it may take several days to one or two weeks before suitable crystals can grow. Therefore, ideally the sample needs to remain stable over that period

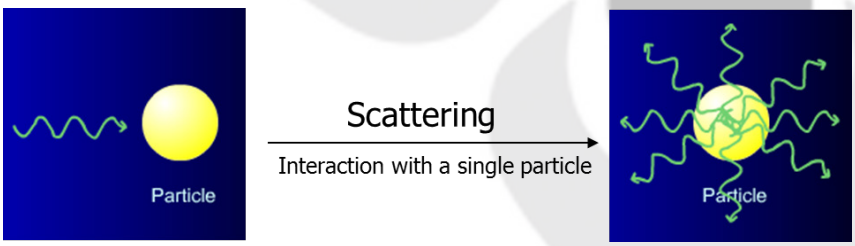
If the sample fails one or more of the above criteria, it may be worthwhile returning to the expression and purification protocols and trying something different, such as the addition of ligands known to interact with the protein, or adding extra purification steps. In extreme cases it may be worthwhile switching to a different expression system altogether or working with a mutated or truncated construct. It may be possible to refold protein successfully using chaotropic reagents such as urea. Aggregated or polydisperse samples may be made monodisperse by simply changing pH or adding some salt. However, without DLS, this is very difficult to assess.

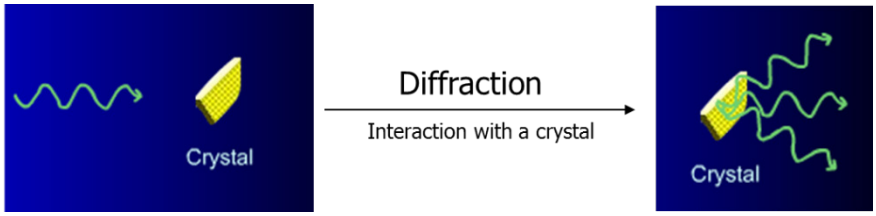
12. Explain x-ray Diffraction

German physicist von Laue in 1912 reasoned that *if* crystals were composed of regularly spaced atoms which might act as scattering centers for x-rays, and *if* x-rays were electromagnetic waves of wavelength about equal to the interatomic distance in crystals, then it should be possible to diffract x-rays by means of crystals.

The rays scattered by all the atoms in all the planes are completely in phase and reinforce one another (constructive interference) to form a diffracted beam in the direction shown. In all other directions of space the scattered beams are out of phase and annul one another (destructive interference). The diffracted beam is rather strong compared to the sum of all the rays scattered in the same direction, simply because of the reinforcement which occurs, but is extremely weak compared to the incident beam since the atoms of a crystal scatter only a small fraction of the energy incident on them.

A diffracted beam may be defined as a beam composed of a large number of scattered rays mutually reinforcing each other





Random arrangement of atoms in space gives rise to scattering in all directions: weak effect and intensities add

By atoms arranged periodically in space

In a few specific directions satisfying Bragg's law: strong intensities of the scattered beam: Diffraction

No scattering along directions not satisfying Bragg's law

13.How X-ray Diffraction is a very useful to characterize materials?

X-ray Diffraction is a very useful to characterize materials for following information

- Phase analysis
- Lattice parameter determination
- Strain determination
- Texture and orientation analysis
- Order-disorder transformation
- Semi-conductor industry
- and many more things

14.What are the applications of X-Ray diffraction?

In Pharmaceutical industry

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indexed X-ray powder diffraction pattern is required to analyze the crystal structure, secure a patent and protect the company's investment.

For multi-component formulations, the actual percentages of the active ingredients in the final dosage form can be accurately analyzed in situ, along with the percentage of any amorphous packing ingredients used.

XRD is the key technique for solid-state drug analysis, benefiting all stages of drug development, testing and production.

Forensic science

XRD is used mainly in contact trace analysis. Examples of contact traces are paint flakes, hair, glass fragments, stains of any description and loose powdered materials. Identification and comparison of trace quantities of material can help in the conviction or exoneration of a person suspected of involvement in a crime.

Geological applications

XRD is the key tool in mineral exploration. Mineralogists have been amongst the foremost to develop and promote the new field of X-ray crystallography after its discovery. Thus, the advent of XRD has literally revolutionized the geological sciences to such a degree that they have become unthinkable without this tool. Nowadays, any geological group actively involved in mineralogical studies would be lost without XRD to

unambiguously characterize the individual crystal structures. Each mineral type is defined by a characteristic crystal structure, which will give a unique x-ray diffraction pattern, allowing rapid identification of minerals present within a rock or soil sample. The XRD data can be analyzed to determine the proportion of the different minerals present.

In Microelectronics industry

As the microelectronics industry uses silicon and gallium arsenide single crystal substrates in integrated circuit production, there is a need to fully characterize these materials using the XRD. XRD topography can easily detect and image the presence of defects within a crystal, making it a powerful non-destructive evaluation tool for characterizing industrially important single crystal specimens.

In Glass industry

While glasses are X-ray amorphous and do not themselves give X-ray diffraction patterns, there are still manifold uses of XRD in the glass industry. They include identification of crystalline particles which cause tiny faults in bulk glass, and measurements of crystalline coatings for texture, crystallite size and crystallinity.

15.Write a short note on X-rays?

The wavelength of X-rays is given by $\lambda = C/V$. X-rays of 1Å are used because of more penetration. Longer wavelength X-rays are used in medical applications. In X-ray tube cathode is used as source of electrons. The X-rays have smaller wavelength than white radiation