

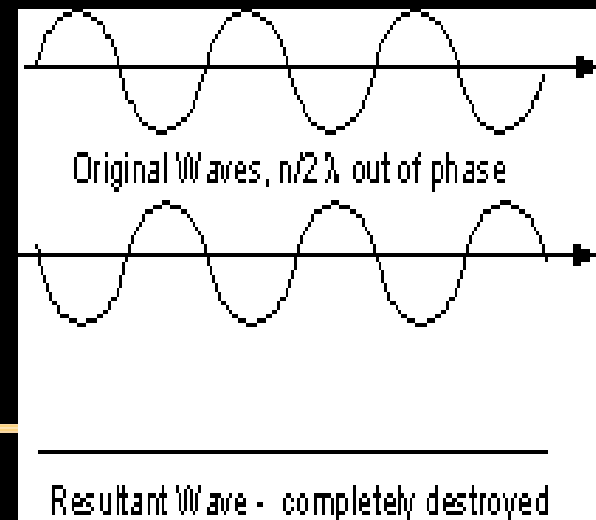
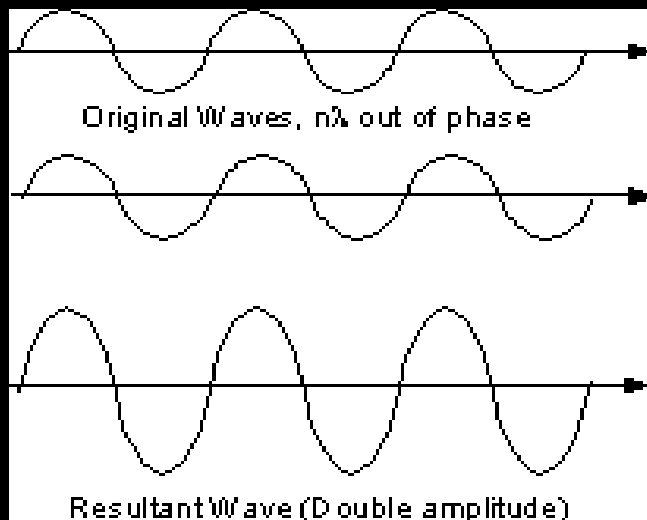
X RAY CRYSTALLOGRAPHY



FUNDAMENTALS OF DIFFRACTION

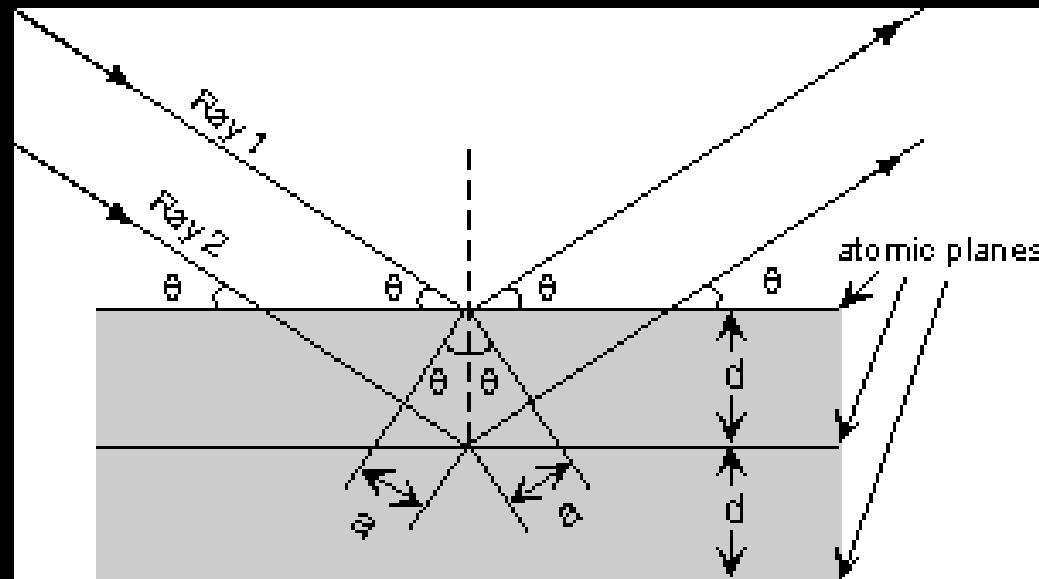
- A beam of X-rays consists of a bundle of separate waves, the waves can interact with one another. Such interaction is termed *interference*.
 - If all the waves in the bundle are in phase, that is their crests and troughs occur at exactly the same position (the same as being an integer number of wavelengths out of phase, $n\lambda$, $n = 1, 2, 3, 4$, etc.), the waves will interfere with one another and their amplitudes will add together to produce a resultant wave that is has a higher amplitude (the sum of all the waves that are in phase.)
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- If the waves are out of phase, being off by a non-integer number of wavelengths, then destructive interference will occur and the amplitude of the waves will be reduced.
- In an extreme case, if the waves are out of phase by an odd multiple of $\frac{1}{2} \lambda$ $[(2n+1)/2 \lambda]$, the resultant wave will have no amplitude and thus be completely destroyed.



BRAGG'S LAW

- Two such X-rays are shown here, where the spacing between the atomic planes occurs over the distance, d . Ray 1 reflects off of the upper atomic plane at an angle θ equal to its angle of incidence. Similarly, Ray 2 reflects off the lower atomic plane at the same angle θ . While Ray 2 is in the crystal, however, it travels a distance of $2a$ farther than Ray 1. If this distance $2a$ is equal to an integral number of wavelengths ($n\lambda$), then Rays 1 and 2 will be in phase on their exit from the crystal and constructive interference will occur. If not destructive interference will occur and the waves will not be as strong as when they entered the crystal.



Condition for constructive interference to occur is

$$n\lambda = 2a$$

but, from trigonometry, we can figure out what the distance $2a$ is in terms of the spacing, d , between the atomic planes.

$$a = d \sin\theta$$

$$\text{or } 2a = 2d \sin\theta$$

$$\text{thus, } n\lambda = 2d \sin\theta$$

This is known as **Bragg's Law** for X-ray diffraction.

X RAY CRYSTALLOGRAPHY

INTRODUCTION

- **X-ray crystallography** 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 beam of light to spread into many specific directions. From the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal.
 - Because X-rays have wavelengths similar to the size of atoms, they are useful to explore within crystals.
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USES

- Used to study many materials which form crystals like salts, metals, minerals, semiconductors, as well as various inorganic, organic and biological molecules.
 - Determine electron density, the mean positions of the atoms in the crystal their chemical bonds, their disorder and various other information.
 - Size of atoms, the lengths and types of chemical bonds, and the atomic-scale differences among various materials, especially minerals and alloys. The method also revealed the structure and function of many biological molecules, including vitamins, drugs, proteins and nucleic acids such as DNA.
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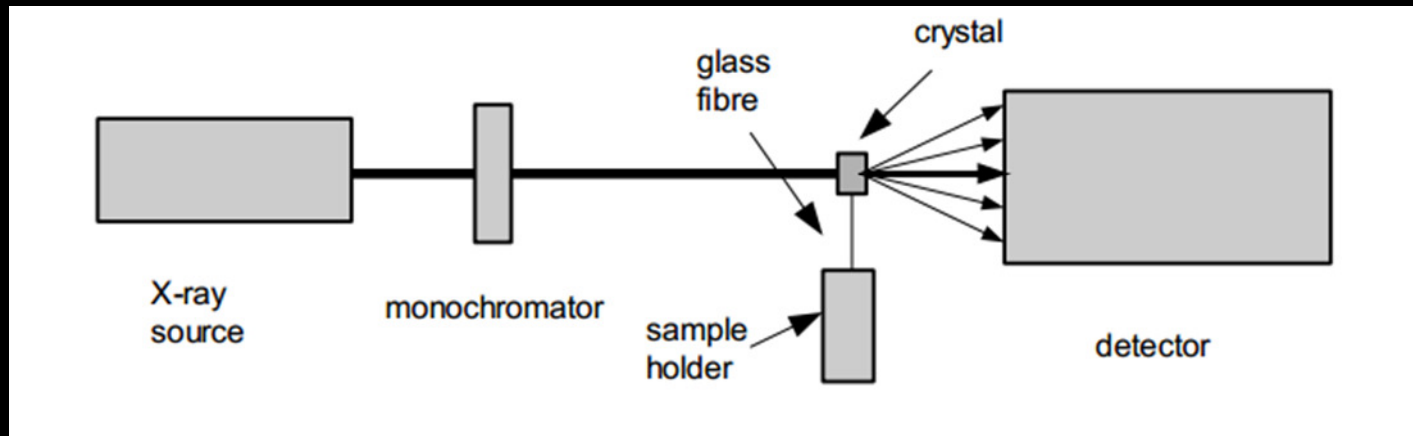
- Characterizing the atomic structure of new materials and in discerning materials that appear similar by other experiments
- X-ray crystal structures can also account for unusual electronic or elastic properties of a material, shed light on chemical interactions and processes, or serve as the basis for designing pharmaceuticals against diseases.



X RAY DIFFRACTION

- X-Ray Crystallography uses the uniformity of light diffraction of crystals to determine the structure of a molecule or atom.
- 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

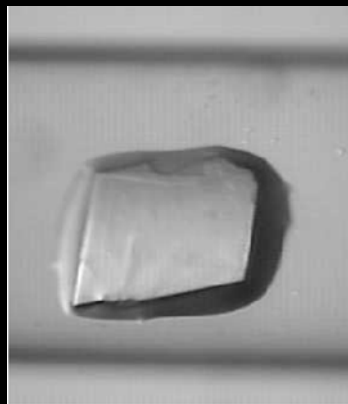




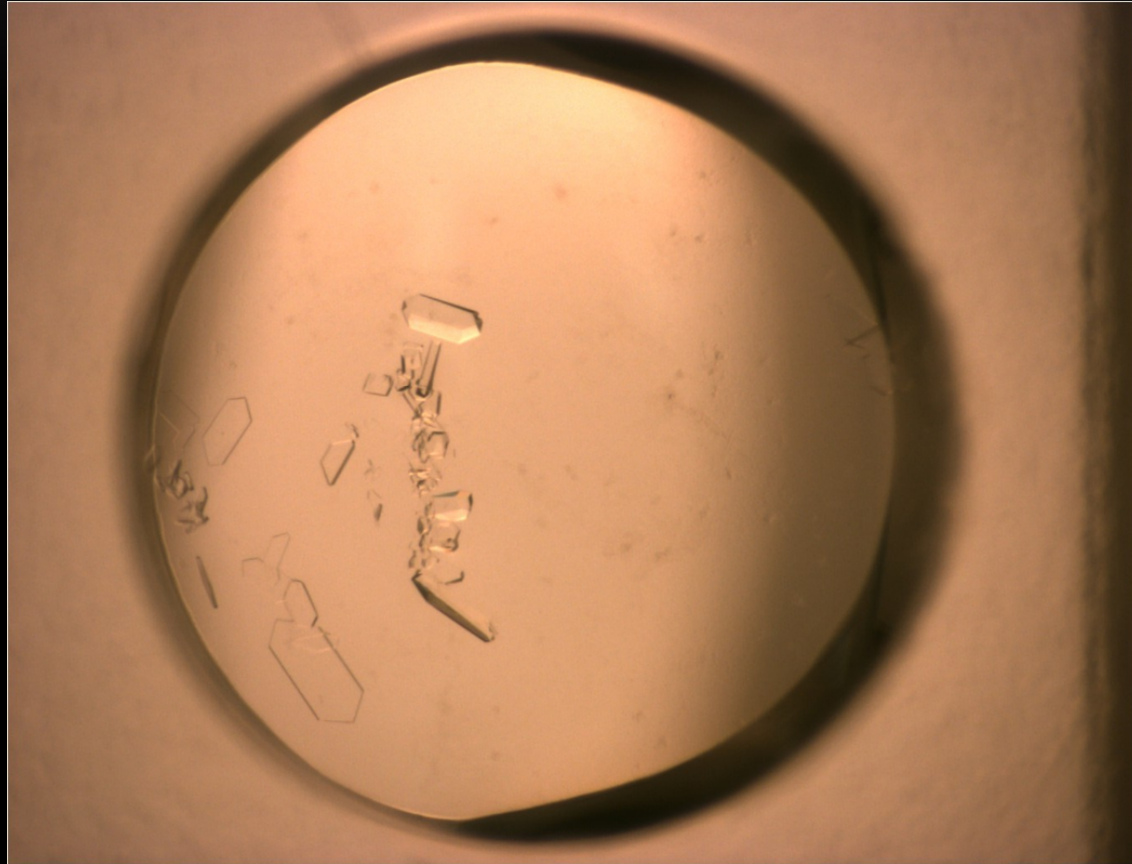
PROCEDURE- THE FIRST STEP

- 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 or twinning.
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- 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.



CRYSTAL MOLECULES OF PROTEIN



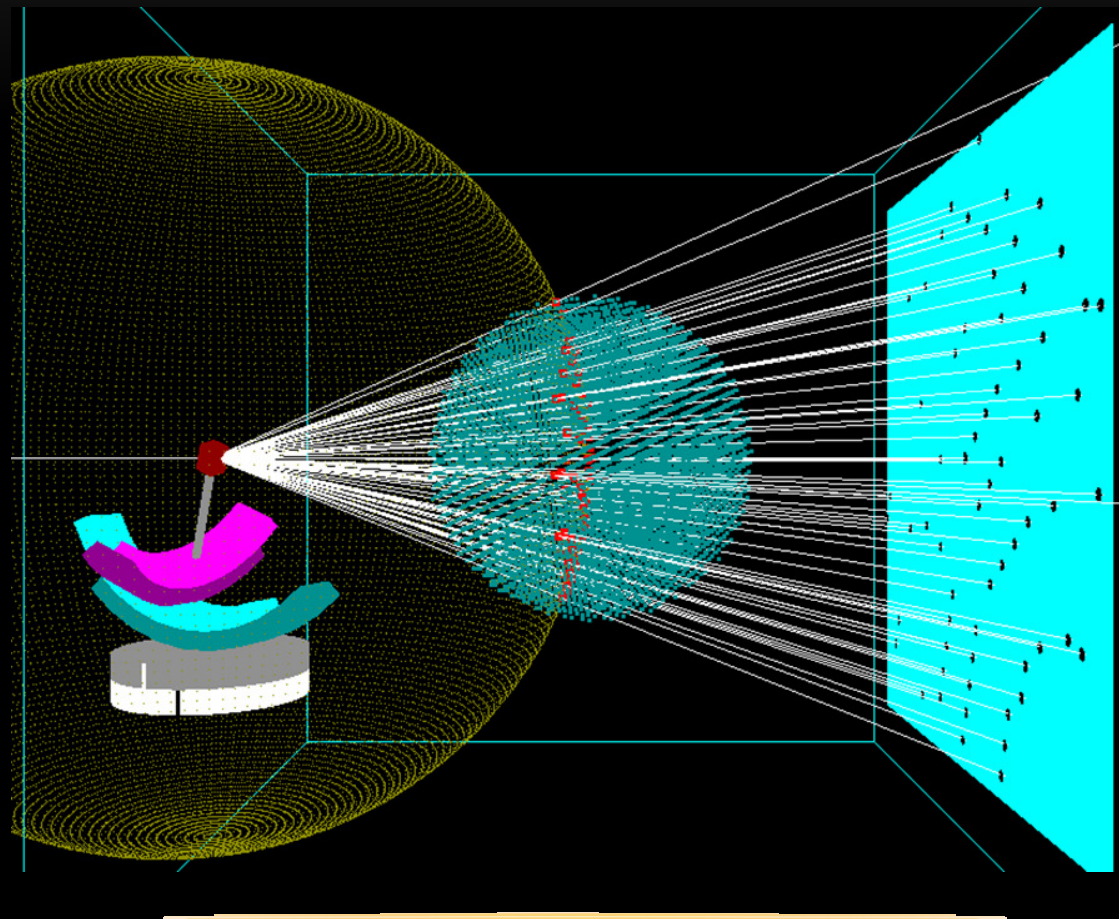
SECOND STEP

- 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.
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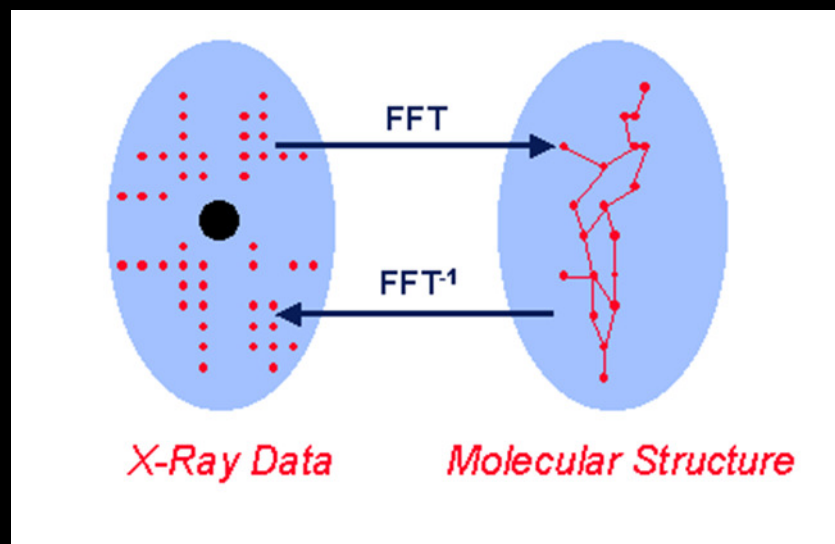
THIRD AND FINAL STEP

- 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.



ELECTRON DENSITY MAP

- An X-ray crystallographic experiment produces an electron density map for the average unit cell of the protein crystal. The amino acid (or nucleotide) sequence of the crystallized polymer(s) is known in advance. The crystallographer fits the atoms of the known molecules into the electron density map, and refines the model and map to the limits of the resolution of the crystal (which is limited by the level of order or disorder in the crystal). The crystallographer then deposits a model of the asymmetric unit of the crystal in the PDB, along with the experimental diffraction data (amplitudes and widths of the X-ray reflection spots, or "structure factors") from which the electron density map can be reconstructed.



- A three-dimensional description of the electron density in a crystal structure, determined from X-ray diffraction experiments. X-rays scatter from the electron clouds of atoms in the crystal lattice; the diffracted waves from scattering planes h,k,l are described by structure factors. The electron density as a function of position x,y,z is the Fourier transform of the structure factors:

$$\rho(xyz) = \frac{1}{V} \sum_{hkl} F(hkl) \exp[-2\pi i(hx + ky + lz)]$$

- The electron density map describes the contents of the unit cells averaged over the whole crystal and not the contents of a single unit cell (a distinction that is important where structural disorder is present).
 - Three-dimensional maps are often evaluated as parallel two-dimensional contoured sections at different heights in the unit cell.
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X RAY INFRASTRUCTURE

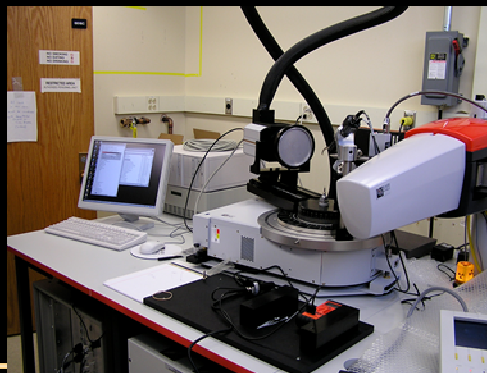
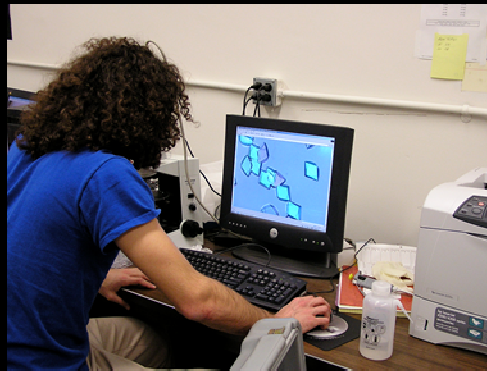
Initial screening



Optimization



Identification



LIMITATIONS

- Two limiting cases of X-ray crystallography.
 - *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
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- 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~~.

CONCLUSION

- X-Ray crystallography allowed for the discovery of the structure of DNA
- Allows researchers today to see how certain factors may effect protein structure
- Allows researchers today to see how secondary protein structures in protein residues can fold depending on different environmental factors



THANK YOU

