IIT Guwahati Lecture 29

Course BT 631

Protein Structure, Function and Crystallography

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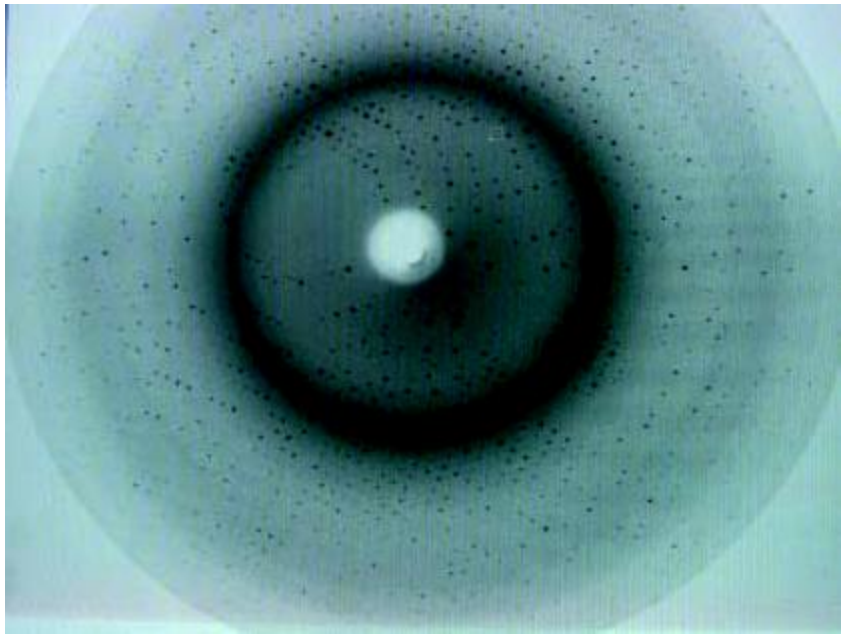
Diffraction Pattern from Protein Crystal

In a typical diffraction pattern the irradiation of a protein crystal with monochromatic X-rays results in the detection of thousands of spots or reflections.

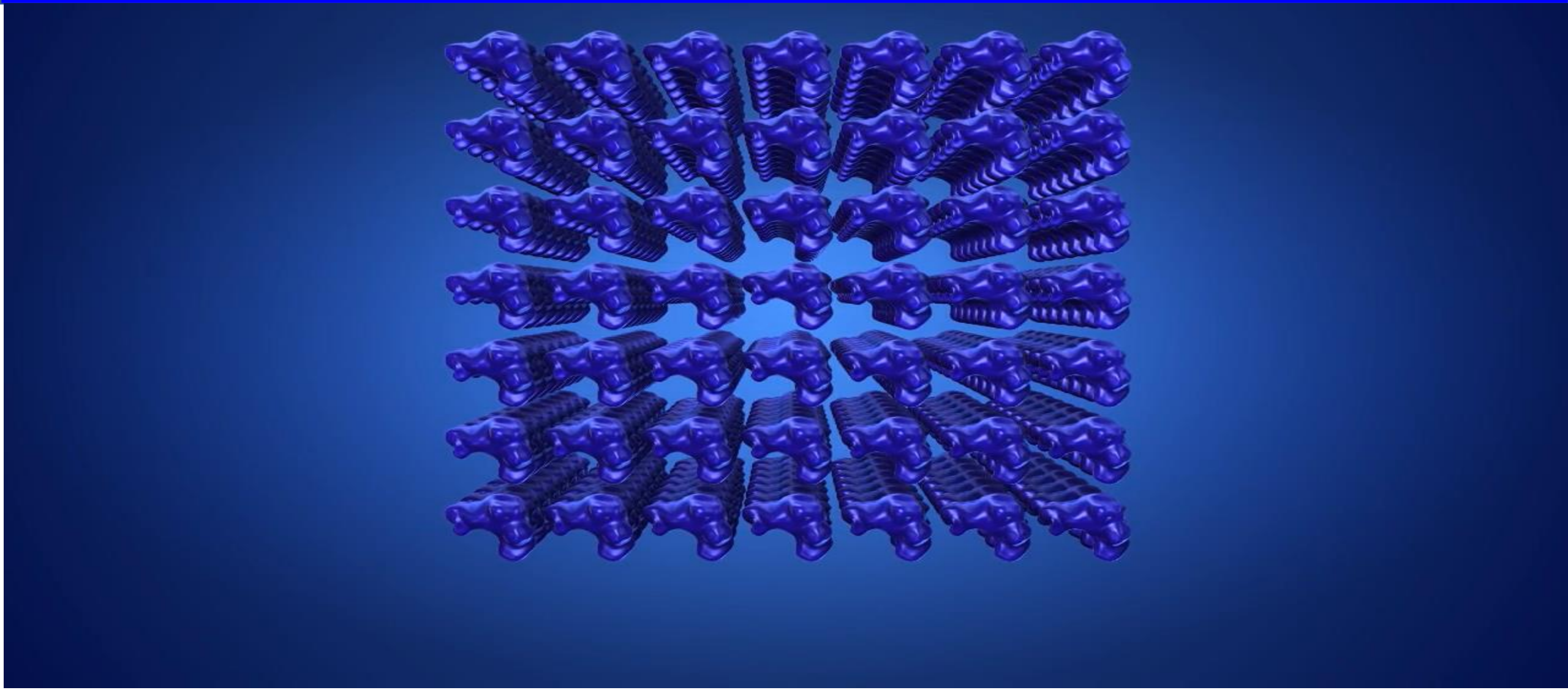
These ‘spots’ are the raw data of crystallography and arise from all atoms within the unit cell.

A complete analysis of the diffraction pattern (Fig.) will allow the electron density map, and by implication, the position of atoms can be deciphered.



Protein crystal diffraction pattern

Protein Crystal Diffraction



Phasing problem

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 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*".

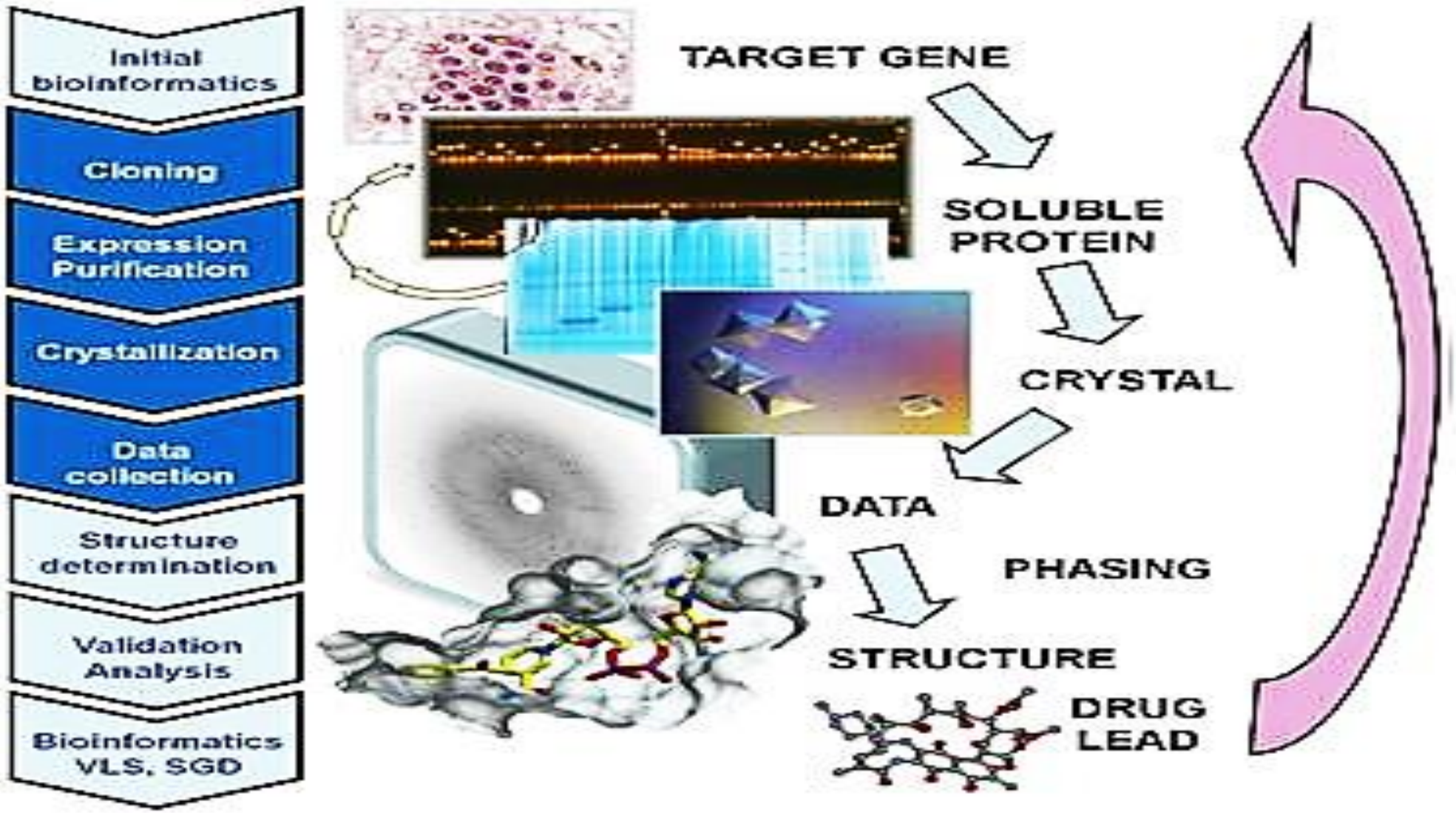
When crystallographers say they have solved a structure, it means that they have solved "the phase problem".

In other words they have obtained phase information sufficient to enable an interpretable electron density map to be calculated.

Challenges of Crystallography

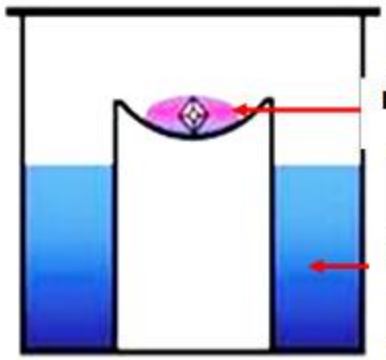
1. Proteins are usually large, flexible molecules which rarely self-assemble readily into regular and periodically repeating arrangement.
2. Obtaining the protein in sufficient amount proves to be difficult and may need to be engineered or modified.
3. Biological materials are susceptible to radiation damage. To prevent this decay, crystals need to be cooled near -196°C.
4. Some crystals are difficult to flash cool and it is difficult to predict which cryo-protection conditions would work.
5. The refractive index of X-rays is close to 1 in different materials and refractive lens can't be used like it is done in case of light microscopes. Thus electron density must be reconstructed by Fourier Transform techniques.
6. The Structure factor amplitude (F_{hkl}) which is proportional to the square root of intensity of diffraction spot) and relative phase angle (α_{hkl}) of each reflection is required.
7. The F_{hkl} is derived from diffraction spot intensities of reflected X-rays, but α_{hkl} is not accessible and must be determined by phasing experiments.

Overview of protein structure determination

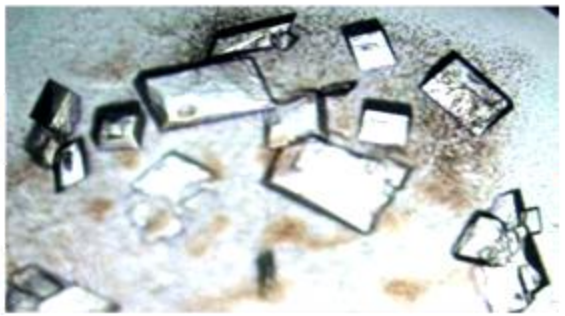


Overview of protein structure determination

Crystallization and X-ray crystallographic analysis

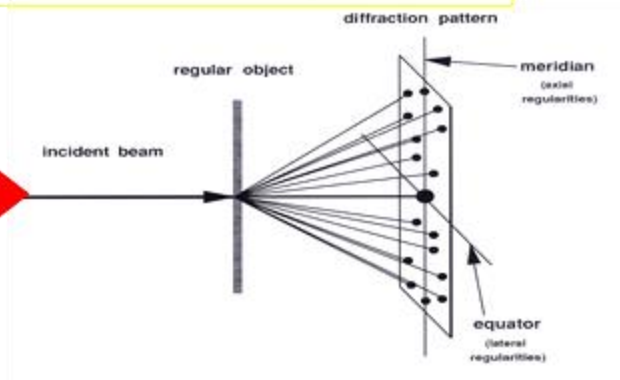


A



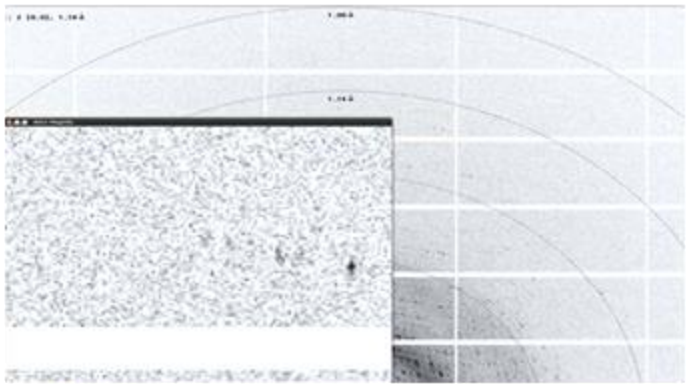
B

Crystals of Xyn30A



C

Diffraction of Xyn30A crystal

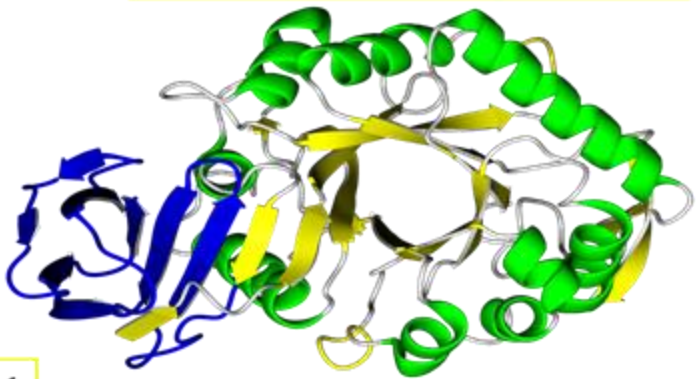


D

Diffraction pattern of Xyn30A crystal with resolution rings

E

Molecular replacement using Phaser



F

3-D structure of Xyn30A