IIT Guwahati Lecture 34

Course BT 631

Protein Structure, Function and Crystallography

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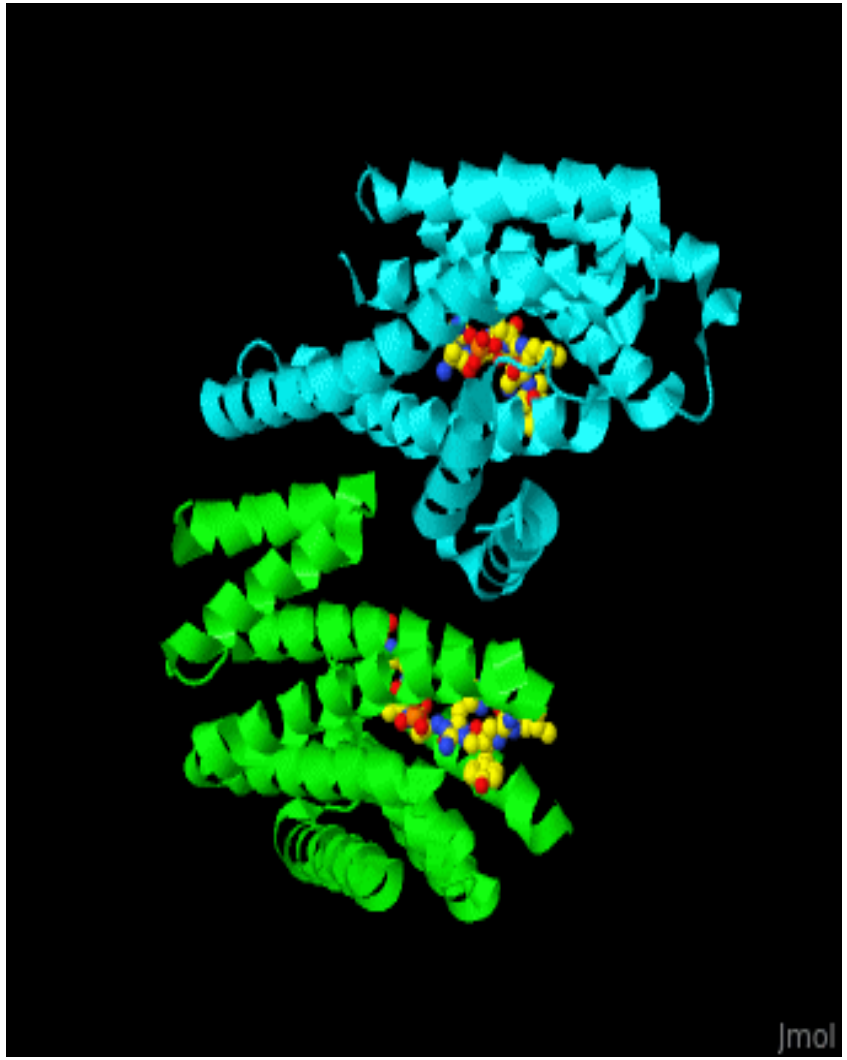


Instrumentation for X-ray diffraction

Instrument used for X-ray data collection is the Diffractometer.

It measures the position (angular distribution) and intensity of the diffracted X-rays.

Its basic components are:

- An intense source for X-rays in the range of 2-25 keV.
- Optical equipment to select monochromatic X-rays and focus them into a strong beam.
- Device that orients the crystal in X-ray beam called Goniostat.
- A detector for the diffracted rays.
- A cryocooler to keep the crystals cool, a microscope to align the crystal.
- A robot for changing the samples.

Instrumentation for X-ray diffraction

X-ray sources

3 types of devices are used for generation of X-rays:

- ***Laboratory X-ray generators*** **that use X-ray tubes.**
- ***Synchrotrons*** **in which electrons moving in an orbit at high energy emit X-rays.**
- ***Compton sources*** **that use lasers to generate X-rays by inducing oscillations in electron beams.**

X-ray detectors

The detectors that are in use these days are CCD detectors (charge-coupled device), that contain semiconductor chips, which record the digital image of diffraction pattern.

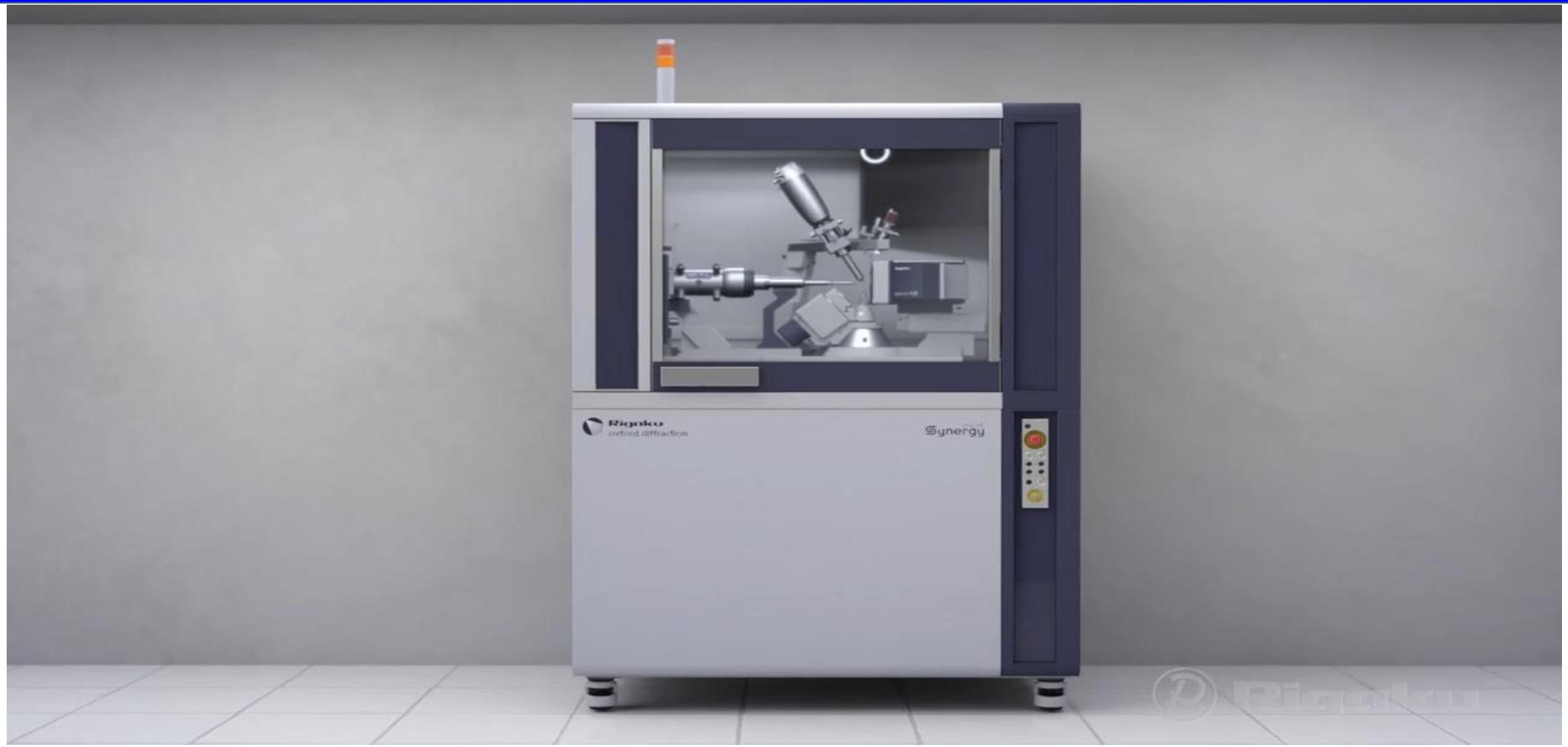
Goniostats

To collect one complete set of diffraction data, the crystal needs to be rotated around at least one axis, this is achieved using a goniostat. During exposure to X-rays the crystal is constantly kept at -196°C using a *cryo-cooler* that blows a stream of liquid N_2 gas.

Mounting using Robots

Almost all structure determinations require X-ray screening of a large number of samples at a fast rate with reliable mounting. This is accurately done by a mounting robot which also saves a lot of beamline time. These robots perform automatic mounting of cryo-pins (or loops) carrying the harvested and cryo-protected crystals.

Instrumentation for X-ray diffraction



Crystal harvesting and mounting

- Any data collecting exercise starts with *harvesting of crystal* from the crystallisation drop followed by a *cryo-protection step* and eventually *mounting of the crystals* on the diffractometer.
- Once crystals have grown to the size of 10-100 µm, they are harvested.
- Crystals can be picked up by capillary mounting procedure, when the data are to be collected at room temperature, using a thin walled quartz or glass capillary.
- While cryo-mounting, the procedure is followed at cryogenic temperatures using a prefabricated nylon loops for data collection.
- After harvesting, during mounting and data collection the crystals must always remain in crystallization buffer and at Liquid nitrogen temperature.

X-Ray Data Collection

- The process of data collection has become highly automated now a days.
- The most common data collection method in macromolecular crystallography is the rotation method. It involves rotation of the crystal around a single axis in small increments ~ 0.1° to 1.5°, while the crystal is exposed to X-rays.
- The diffraction image collected during each of these small rotations is called a frame.
- The electronic signal from the detector is corrected by detector software for specific defects or distortions.
- The corrected image includes experimental parameters such as detector distance, beam centre position and wavelength.
- A diffraction image has single, resolved and strong spots and can be used to identify salt crystals which give few sharp spots at resolution higher than 10Å as their cell dimensions are small.

X-Ray Data Collection

- The first diffraction image tells the extent to which a crystal diffracts and if the resolution of image is sufficient for the required model quality.
- The exposure time can also be adjusted based on the first image, if the exposure is too short, the image obtained will be noisy with poor resolution and too long exposures cause saturation of high intensity spots.
- In the next step of a protein structure determination, the diffraction data are subjected to mathematical treatment using Fourier Transformation to reconstruct an electron density map for the protein. This reconstruction of electron density requires the phases for the diffraction.
- The phasing methods that commonly used are:
  a) Experimental substructure phasing
  b) Molecular replacement
  c) Density modification

Model Building

- Successful model building depends on the quality of the electron density maps.
- Building a protein model into an empty electron density map is generally achievable with reasonable efforts, when the high resolution data are available.
- Very distinct residues like tryptophan, phenylalanine, S-S bridges or heavy metal positions make good anchor points from which the sequence can be assigned.
- The structure validation procedures, that follow the model building and refinement steps, are integral to determining the plausibility of a model.