X-ray Crystallography

III
National Synchrotron Light Source
Experimental Floor at NSLS
X29 Beamline NSLS
“Home” Source - Rotating Anode
• Only get constructive reinforcement if $a(\cos \alpha - \cos \alpha_0) = h\lambda$
Diffraction Three-Dimensional Lattice

- Only get constructive reinforcement if
  \[ a(\cos \alpha - \cos \alpha_o) = h\lambda \]
  \[ b(\cos \alpha - \cos \alpha_o) = k\lambda \]
  \[ c(\cos \alpha - \cos \alpha_o) = l\lambda \]
  von Laue’s equations

hkl are called Miller indices

Each reflection is given a unique set of Miller indices

Reciprocal Lattice
Monochromatic vs Polychromatic X-rays

Monochromatic

Polychromatic

Fig. 8.1.4.2. Single-crystal SR diffraction patterns. (a) Rhinovirus monochromatic oscillation photograph recorded at CHESS (Arnold et al. 1987; see also Rossmann & Erickson, 1983). Copyright (1987) International Union of Crystallography. (b) Prediction of a protein crystal Laue diffraction pattern for an illuminating bandpass, without monochromator, \( \lambda < 2.6 \) Å. The colour coding is according to the multiplicity of each spot: turquoise for singlet reflections, yellow for doubles, orange for triplets and blue for quartet or higher-multiplicity Laue spots. Reproduced with permission from Cruickshank et al. (1991). Copyright (1991) International Union of Crystallography.
Scattering Factors

- Scattering factor ($f_0$) ratio of the amplitude scattered by an atom to the amplitude scattered by a point electron
Intensity of Reflections

- For theoretical point scatter (a point that scatters X-rays in all directions) the intensity of the reflection is the same regardless of angle.

- For real atoms the intensity of the reflection is a function of \( \frac{\sin \theta}{\lambda} \).

- Can predict the diffraction pattern if we know the lattice (space group), unit cell dimensions, distance from crystal to detector, orientation of lattice relative to X-ray beam.

- BUT the intensity of the reflection depends on the composition and orientation of molecules in the asymmetric unit.
Two Atoms in a Lattice

- Spacing between one row of A and another row of A is the same as a row of B and another row of B (distance $d$ unit cell)
- Therefore the same angle $\theta$ will be will cause reinforcement
- The distance between A and B as well as the scattering factors for each atom will dictate intensity
Determination of Molecular Structure

- Given the atomic coordinates of molecule in unit cell and the scattering factors for each atom we can calculate the phase difference for the scattered waves.
- Each wave is characterized by an amplitude and a phase.
- We need to add the scattered X-rays from all atoms in unit cell.
- The amplitude depends on the atomic number (number of electrons) and the phase depends on the coordinates and scattering angle.
- \( F(hkl) = \sum f e^{i\alpha} \) for all atoms in the asymmetric unit.
  - \( F(hkl) \) structure factor for the entire molecule.
  - \( f \) = scattering factor of an atom.
  - \( \alpha \) = phase factor for scattering from the atom.
  - \( i = \sqrt{-1} \)
- Intensity (\( I \)) of the reflection can be measured but phases (\( \alpha \)) need to be calculated.
- \( I(hkl) = F(hkl)^2 \)
Calculation of Electron Density

- Use Fourier transformation to calculate the electron density at position X, Y, Z
- electron density represented by $\rho(X,Y,Z)$
- can use this to calculate the density over the entire unit cell
- obtain a distribution of electron density
Dramatic improvements in the overall structure are likely to result from better definition of disordered regions regardless of resolution.