Proteins are chains of ~100-500 amino acids connected by "peptide bonds".

Driven by Hydrogen Bonding, these chains fold into regular secondary structures.

Spacings in molecules can be interrogated with x-rays.

E.g. C-C bond in a protein 0.15 nm
No X-Ray Lenses!

X-Ray Diffraction from crystalline proteins.

Figures from Glusker, "Crystal Structure Analysis"

Diffraction grating reminder; more slits, sharper images.

Closer spacings on grating \rightarrow wider spacings on diffraction pattern.

Interference leads to discrete diffraction “spots”.

Diffraction from atoms ---
Electrons within atoms act as new x-ray point sources just as edges of slits do in diffraction grating.

Diffraction from a 6-atom molecule.
Transform of molecule convoluted with diffraction from lattice spacings is diffraction pattern from crystalline array of identical molecules.

Diffraction from a protein crystal (lysozyme).

The Concept of Electron Density

Recall: ALL the positions in the diffraction pattern are contributed to by scattering from each slit in the grating (or each atom in a protein).

How to get from “spots” to electron density - the phase problem.

\[ \rho \leftrightarrow \sum F \]

We have only measured the Amplitudes of the Structure Factors (F). To calculate the Electron Density (\(\rho\)) we also need the Phases.

The Phase Problem

Structure determination requires calculation of the phase shifts to “recombine” the diffraction amplitudes into an image in the absence of an x-ray lens.

Guess.
Patterson methods.
Heavy atom substitution to perturb intensities.

Given protein --- Growing crystals

![Graph showing the relationship between protein concentration and growth stages.](image)
Precipitate of all kinds and sometimes crystals

Data collection --
Cryopreservation
Radiation source

Useful crystals with expected spectra

Early Electron Density for D.r. Phytochrome
How to get from electron density to a structure?

Overall Fold

Protein chromophore interactions

GGBP Crystallization

High Quality Diffraction

Strong Data to Detector Edge at 0.92 Å Resolution

Space group C2: a=119.9 Å, b=36.2 Å, c=80.1 Å, β=124.5°
Glucose Binding

Domain Swings

Beta sheet electron density