Determination of protein three-dimensional structure

Protein three-dimensional structures are obtained using two popular experimental techniques, x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

X-ray Crystallography

- In X-ray protein crystallography, proteins need to be grown into large crystals in which their positions are fixed in a repeated, ordered fashion.
- The protein crystals are then illuminated with an intense x-ray beam.
- The x-rays are deflected by the electron clouds surrounding the atoms in the crystal producing a regular pattern of diffraction.
- The diffraction pattern is composed of thousands of tiny spots recorded on an x-ray film.
- The diffraction pattern can be converted into an electron density map using a mathematical procedure known as Fourier transform.
- To interpret a three-dimensional structure from two-dimensional electron density maps requires solving the phases in the diffraction data.
- The phases refer to the relative timing of different diffraction waves hitting the detector.
- Knowing the phases can help to determine the relative positions of atoms in a crystal. Phase solving can be carried out by two methods, molecular replacement, and multiple isomorphous replacement.

✓ Molecular replacement uses a homologous protein structure as template to derive an initial estimate of the phases.

✓ Multiple isomorphous replacement derives phases by comparing electron intensity changes in protein crystals containing heavy metal atoms and the ones without heavy metal atoms.

- The heavy atoms diffract x-rays with unusual intensities, which can serve as a marker for relative positions of atoms.
- Once the phases are available, protein structures can be solved by modeling with amino acid residues that best fit the electron density map.
- The quality of the final model is measured by an R factor, which indicates how well the model reproduces the experimental electron intensity data.
- The R factor is expressed as a percentage of difference between theoretically reproduced diffraction data and experimentally determined diffraction data. R values can range from 0.0, which is complete agreement, to 0.59, which is complete disagreement.
- A major limitation of x-ray crystallography is whether suitable crystals of proteins of interest can be obtained.

Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy detects spinning patterns of atomic nuclei in a magnetic field.

- Protein samples are labelled with radioisotopes such as ^{13}C and ^{15}N.
- A radiofrequency radiation is used to induce transitions between nuclear spin states in a magnetic field.
- Interactions between spinning isotope pairs produce radio signal peaks that correlate with the distances between them.
- By interpreting the signals observed using NMR, proximity between atoms can be determined.
- Knowledge of distances between all labelled atoms in a protein allows a protein model to be built that satisfies all the constraints.

Advantages: NMR determines protein structures in solution, which has the advantage of not requiring the crystallization process. However, the proteins in solution are mobile and vibrating, reflecting the dynamic behaviour of proteins. For that reason, usually a number of slightly different models (twenty to forty) have to be constructed that satisfy all the NMR distance measurements. The NMR technique obviates the need of growing protein crystals and can solve structures relatively more quickly than x-ray crystallography.

Disadvantages: The major problem associated with using NMR is the current limit of protein size (<200 residues) that can be determined. Another problem is the requirement of heavy instrumentation.