X-ray crystallography

- ➔ Proteins are grown into large crystals
- ➔ The crystals are illuminated with intense X-ray beam
- ➔ X-rays are deflected by electron clouds surrounding that atoms in the crystal
- ➔ The diffraction pattern can be converted into and electron density map
- ➔ Turns this 2D diffraction pattern into 3D molecule requires phase solving
- ➔ Phase solving has predominantly two methods:
  - ➔ Molecular replacement
    - ➔ Uses homologous protein structure as template
  - ➔ Multiple isomorphous replacement
    - ➔ Derives phases by comparing electron intensity changers in protein crystals
- ➔ Once phases are available structures can be solved by modeling
- ➔ Quality of final model is measured by R factor
- ➔ R factor
  - ➔ Expressed as percentage of difference between theoretucally reproduced diffraction data
  - ➔ Range from 0.0 [complete agreement] to 0.59 [complete disagreement]
- ➔ Major limitation is that suitable crystals of proteins cant be obtained

NMR Spectroscopy

- ➔ NMR spectroscopy detects spinning patterns of atomic nuclei
- ➔ Samples are labeled with radioisotopes 13C and 15N
- ➔ Radio frequency is used to transition between nuclear spin states in magnetic field
- ➔ Interactions for spinning isotope pairs produce signal peaks that correlates to the distance between the pair
- ➔ Proximity between atoms can be determined by interpreting signals
- ➔ NMR determine protein structure in solution which has advantage over crystallization
- ➔ Proteins keeps moving in the solution hence 20 to 40 slightly different models are constrcuted to account for the protein movement
- ➔ NMR protein size limit is <200 residues

Cryo electron microscopy

- ➔ Simila to TEM in principle
- ➔ Uses beam of electrons to examine structures of molecules
- ➔ But protein materials are not compatible with high vacuum conditions and intense lasers
- ➔ Cryo EM uses frozen samples and gentler electron beams with sophisticated image processing to produce models
- ➔ The sample is first frozen and the the technique of xray crystallography is used to produce a set of 2D images
- ➔ These images are then synthesized into a 3D structure
- ➔