Protein structure key for function and safety

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 101007939. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA companies.

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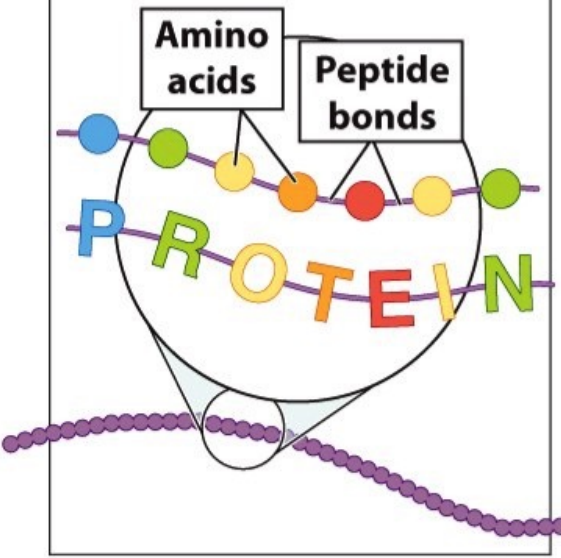
STRUCTURE OF PROTEINS

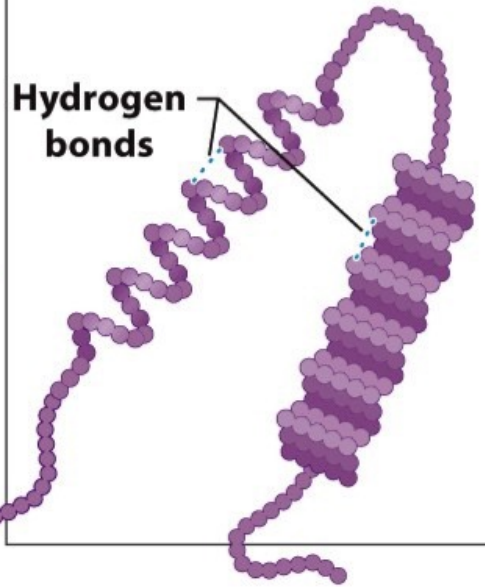
PRIMARY STRUCTURE

The sequence of amino acids in a polypeptide chain, similar to the sequence of letters that spell out a specific word



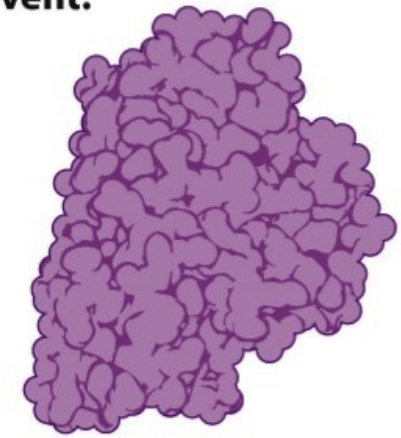
SECONDARY STRUCTURE

The corkscrew-like twists or pleated folds formed by hydrogen bonds between amino acids in the polypeptide chain



TERTIARY STRUCTURE

The complex three-dimensional shape formed by multiple twists and bends in the polypeptide chain, based on the side chains' interactions with each other and with the aqueous solvent.



QUATERNARY STRUCTURE

Two or more polypeptide chains bonded together

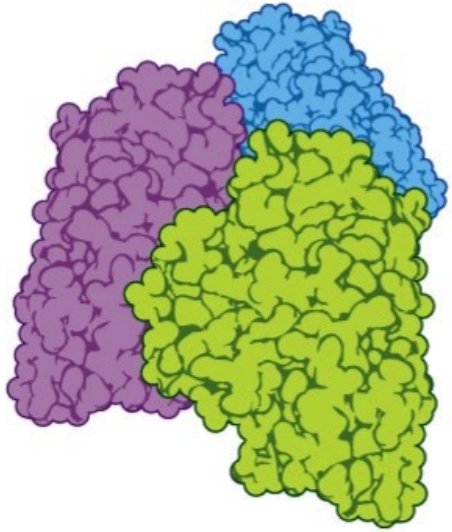
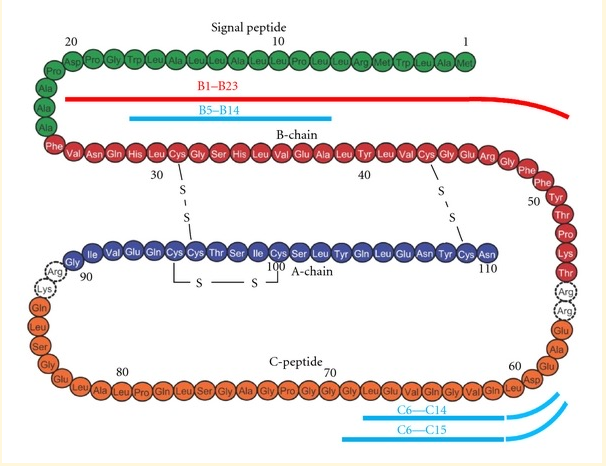


Figure 2-39

***What Is Life? A Guide To Biology*, Second Edition**

Primary structure



The sequence o amino acids

The amino acid sequence of the desired product should be determined to the extent possible and compared to the gene-sequence

- Amino acid composition
- Terminal amino acid sequence
- Peptide map
- Sulfhydryl group(s) and disulphide bridges

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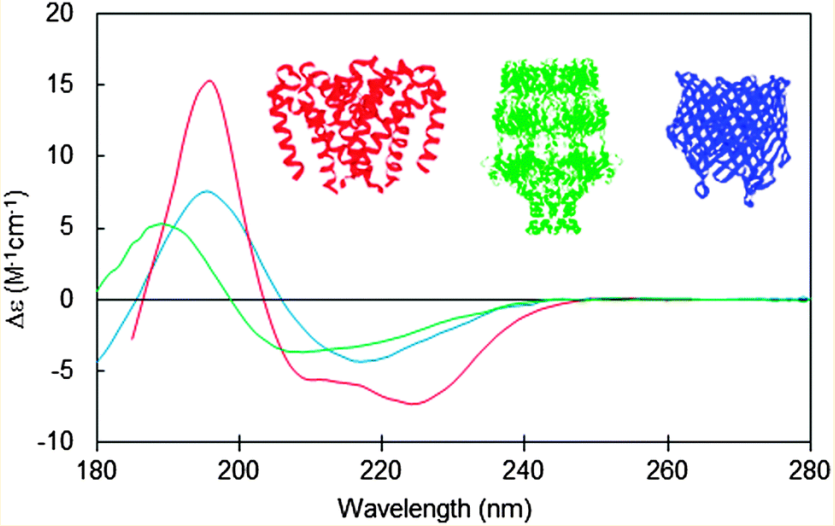
Secondary structure



The secondary structure of a proteins is well defined structural elements such as

- α-helix
- β-sheet
- β-barrel

The secondary structure can be probed by methods such as Circular Dichroism

Misfolded proteins can still express secondary structure

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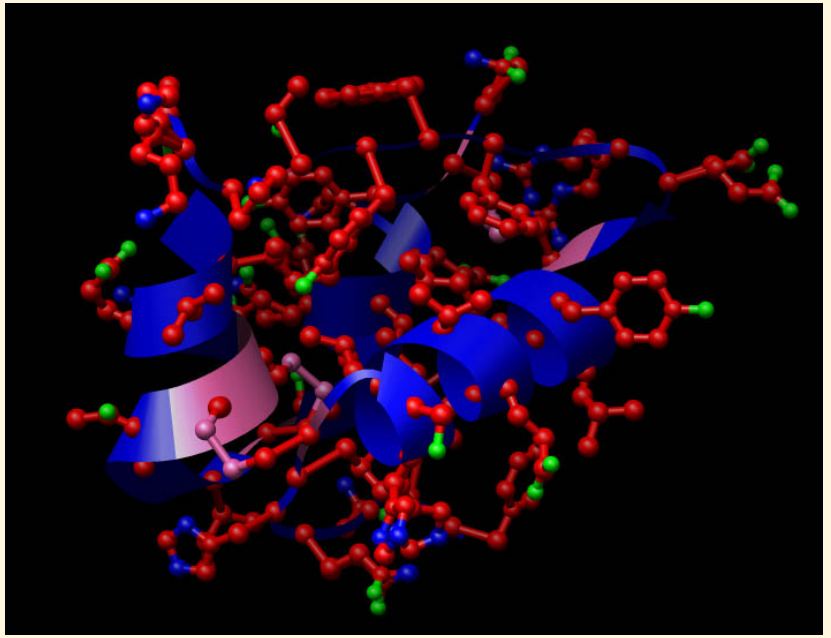
Tertiary structure



- This is the key for the function of the protein
  - Ligand binding
  - Enzymatic activity
- It is affected by solution conditions such as;
  - pH
  - Ionic strength and specific ion effects
  - Interaction with excipients
- It can be disrupted by
  - Heat
  - Denaturant such as Urea and Guanidinium chloride and Surfactants such as SDS
  - Interfaces

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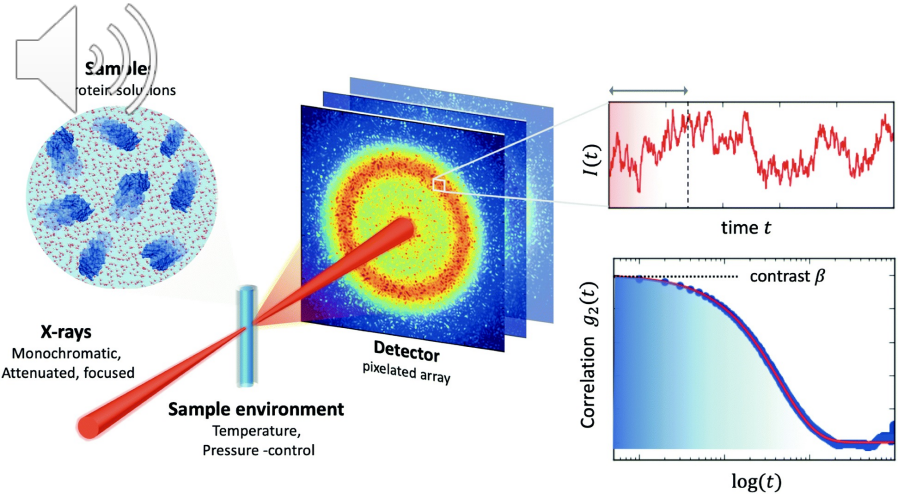

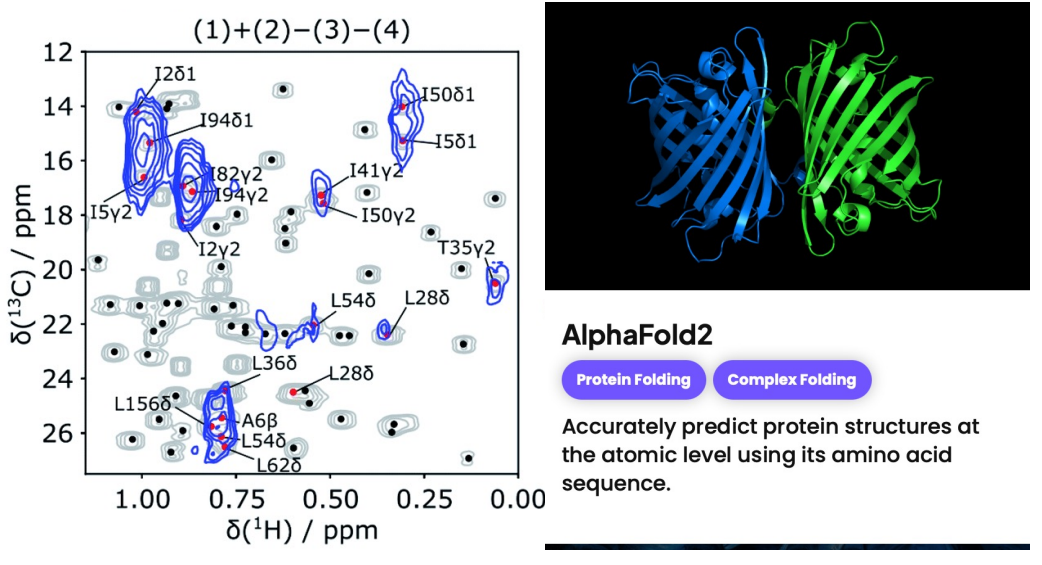

AlphaFold2

Protein Folding | Complex Folding

Accurately predict protein structures at the atomic level using its amino acid sequence.

Determination of protein 3 -D structure

- X-ray crystallography
- NMR
- Computer generated structures - Alphafold

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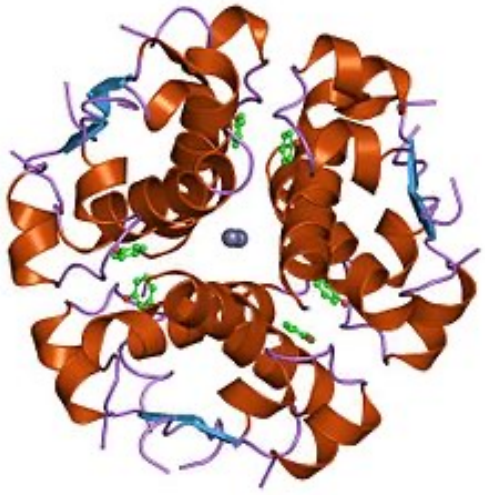
Quaternary structure



- The quaternary structure of a protein refers to the arrangement and interaction of multiple protein subunits to form a functional, multi-subunit protein complex.
- It involves the assembly of two or more protein subunits, each contributing to the overall functionality of the protein and its beahvior in the formulation.
- It is a equlibrium structure and can be affected by solution conditions

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Influencing clearance and uptake– Quaternary structure

The example of Insulin

Insulin has three forms

- Monomer – fast uptake
- Dimer- slow uptake
- Hexamer- No uptake

The forms are affected by concentration Zn^{3+} and site specific mutations

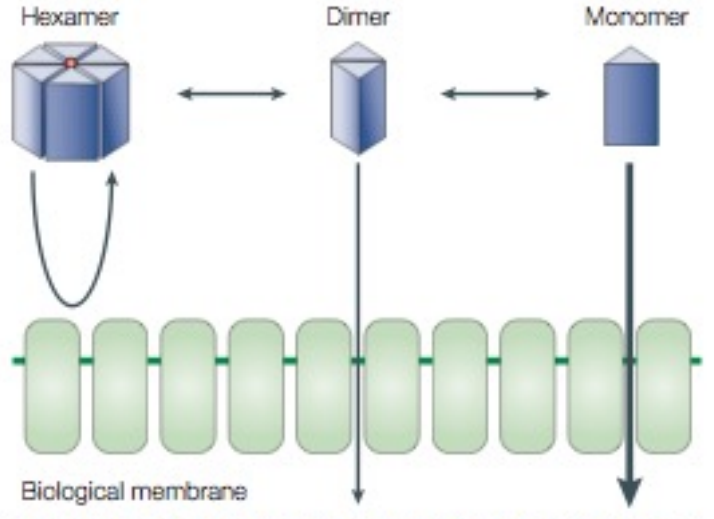

Figure 2 | **Transport of insulin across biological membranes.** Insulin monomer has the highest flux.

Nature Reviews Drug Discovery 4, 298-306 (April 2005) | doi:10.1038/nrd1695
Protein drug stability: a formulation challenge
Sven Frokjaer1 & Daniel E. Otzen2

This presentation was partially developed using material from ReaHope

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Real World Handling of Protein Drugs – Exploration, Evaluation & Education

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