IIT Guwahati

Lecture 14

Course BT 631

Protein Structure function and Crystallography

Prof. Arun Goyal

Dept. of Biosciences and Bioengineering

Tertiary structure

The tertiary structure represents the folded polypeptide chain.

Asking the question "What is the tertiary structure of a protein?"

is the same as asking "What is the protein fold?"

Tertiary structure is defined as the spatial arrangement of amino acid residues that are widely separated in the primary sequence or as the overall topology formed by the polypeptide.

TOPOLOGY- संस्थिति (Sansthiti) · स्थान-विज्ञान (Sthan-vigyan) · किसी स्थान की प्राकृतिक दशा का वर्णन

Tertiary structure is formed by the packing of protein secondary structure elements into compact globular units.

Therefore the dividing line between the Secondary and Tertiary structure is thin.

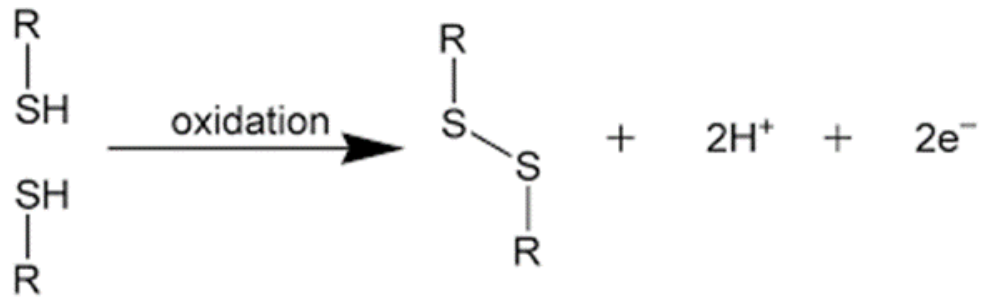
The fold arises from linking together secondary structures forming a compact globular structure. These globular structures are called domains.

Tertiary structure

- In tertiary structure cavities are formed due to folding of polypeptide chain which acts as enzyme active site.
- Tertiary structure is important for the biological function.

Interactions that stabilize the tertiary structure

1. Disulfide bridges

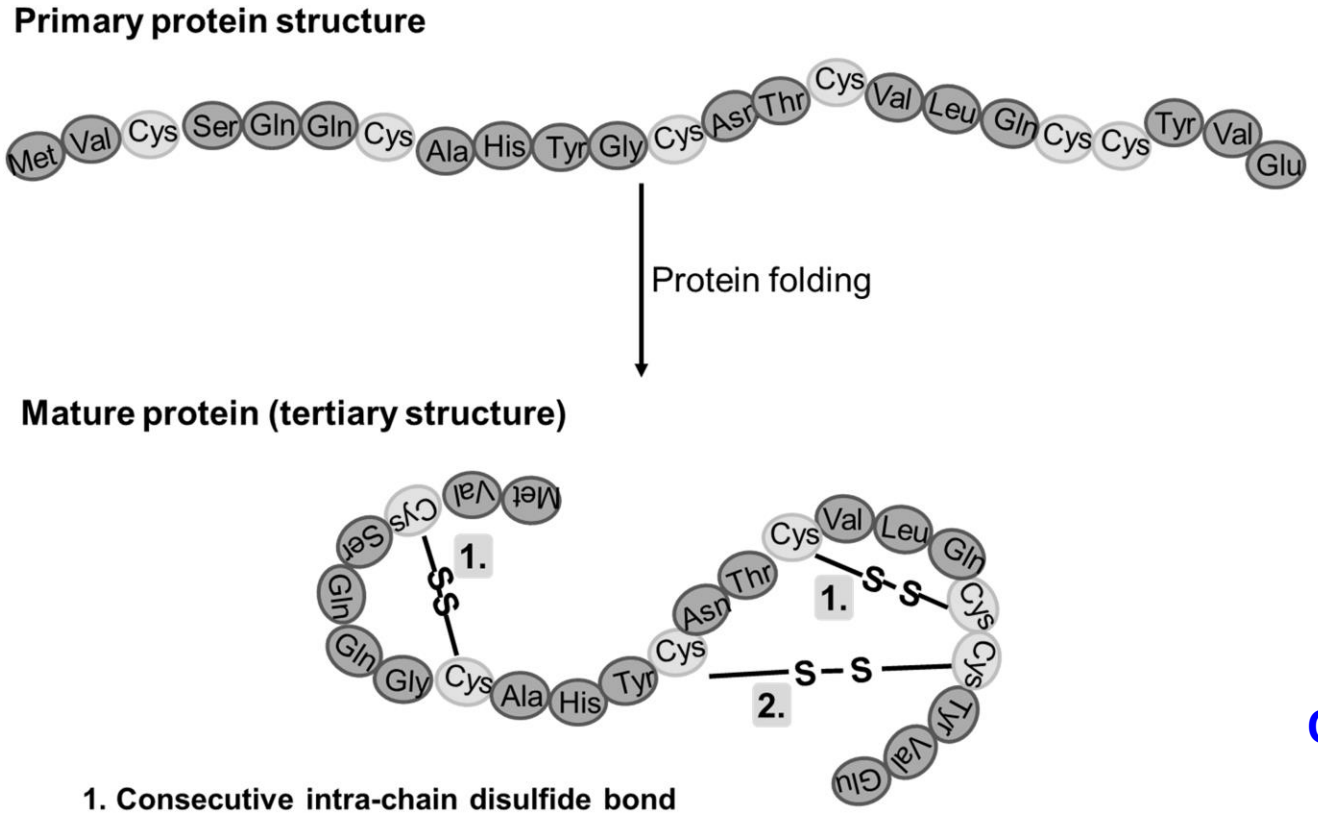


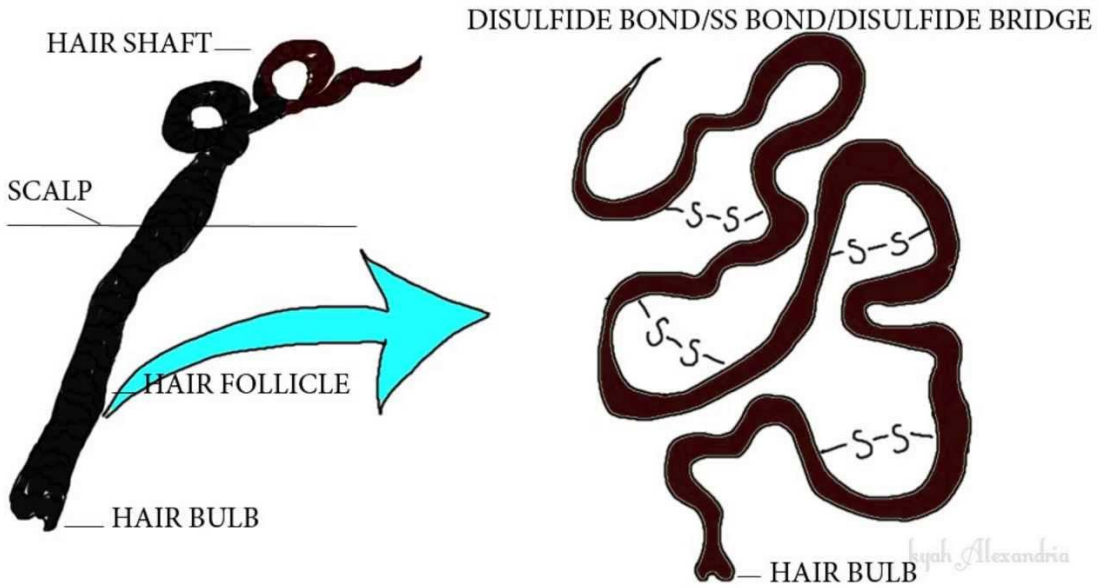
Two cysteines can form a S-S bond upon oxidation

- Disulfide bridges dictate a protein fold by forming strong covalent bonds between cysteine side chains that are often widely separated in the primary sequence.
- A disulfide bond cannot form between two consecutive Cys residues.
- Generally the two cysteines that form S-S bond are 5 amino acid residues apart.
- A disulfide bridge restrains the overall conformation of the polypeptide.
- Bovine pancreatic trypsin inhibitor is a small 58 amino acid residues protein and contains three S-S bridges.

Interactions that stabilize tertiary structure

Disulfide bridges





Curley hair are due to formation of S-S bonds

Disulfide bonds are only broken at high temperatures, acidic pH or by reducing agents (e.g. β-mercapto-ethanol).

Interactions that stabilize tertiary structure

2.The hydrophobic effect

The main driving force for folding water-soluble globular protein molecules is to pack hydrophobic side chains into the interior of the molecule thus creating a hydrophobic core and a hydrophilic surface.

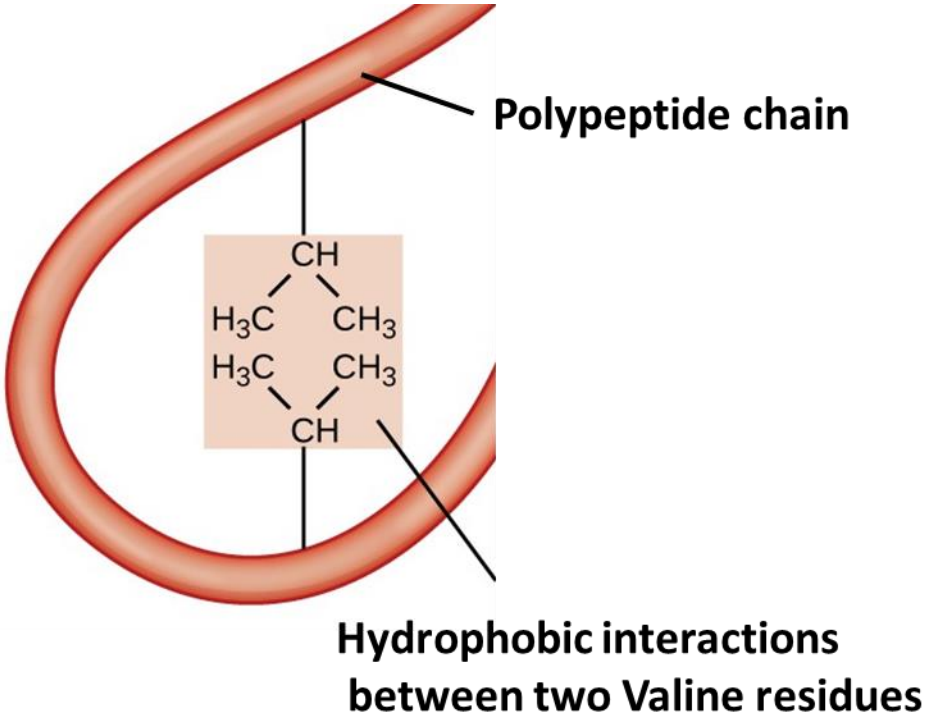
Non-polar (Hydrophobic) molecules cannot form H-bonds with water and do not dissolve in water so the interactions between them is non-existent.

The result is an enhancement in the interactions between the non-polar (Hydrophobic) molecules and the formation of hydrophobic clusters with in water.

The interactions between non-polar (Hydrophobic) molecules in the presence of water are the basis for the hydrophobic effect.

Tertiary structure

The side chains of many amino acid residues are hydrophobic, so the hydrophobic effect contributes to the intra-molecular interactions.



Interactions that stabilize tertiary structure

2.The hydrophobic effect

- The magnitude of the hydrophobic effect is difficult to estimate but is possible by measuring the Free Energy associated with transfer of a non polar molecule into aqueous state through the relationship

$$\Delta G = \Delta H - T\Delta S$$

- A large number of correlations have been made between accessible area, solubility of non polar solutes in aqueous solutions and the energetics associated with the hydrophobic interaction.
- The **hydropathy index** established for amino acids is one manifestation of hydrophobic interactions.

Hydropathy index

The hydropathy index of an amino acid is a number representing the hydrophobic or hydrophilic properties of its side chain.

It was proposed in 1982 by Jack Kyte and Russell F. Doolittle.

The larger the number is, the more hydrophobic is the amino acid.

Hydropathy Plot

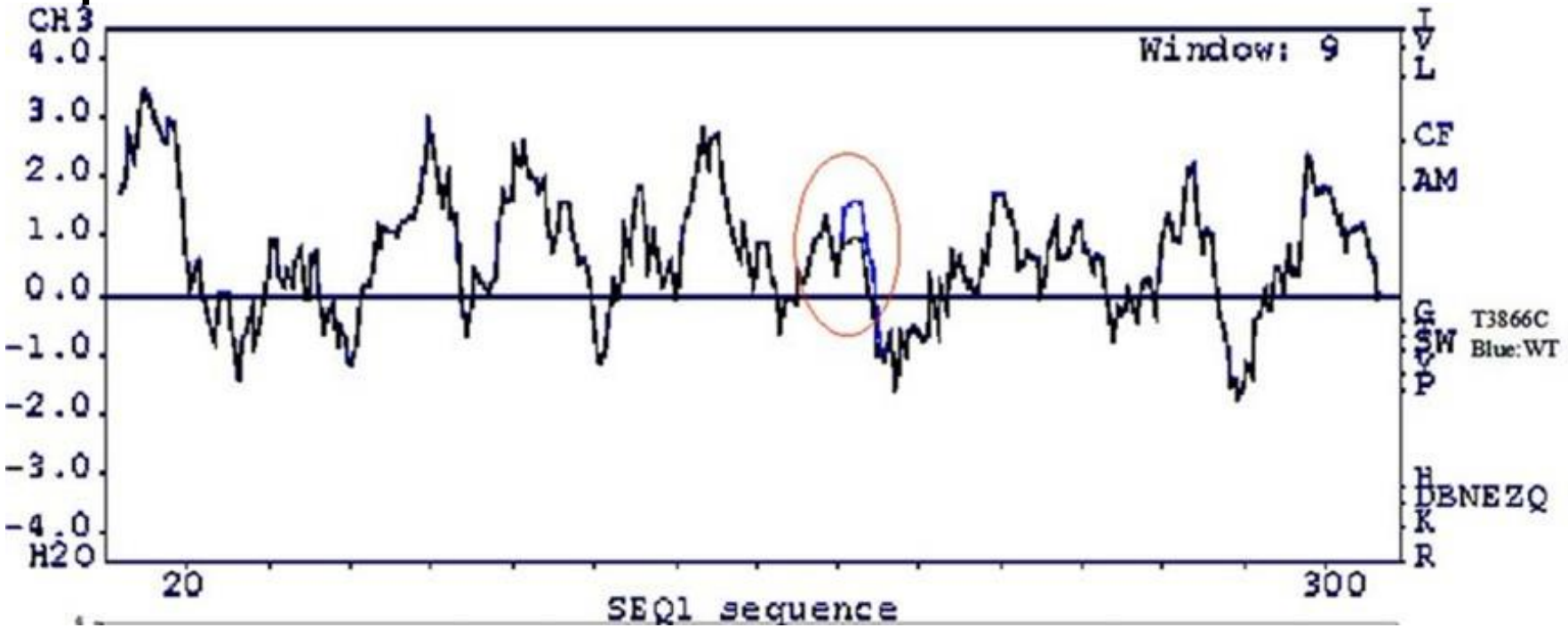
The data in the table is generated by using a computer program that evaluates the average hydrophobicity of segments within a protein.

Amino Acid Hydropathy Scores [1]		
Amino Acid	One Letter Code	Hydropathy Score
Isoleucine	I	4.5
Valine	V	4.2
Leucine	L	3.8
Phenylalanine	F	2.8
Cysteine	C	2.5
Methionine	M	1.9
Alanine	A	1.8
Glycine	G	-0.4
Threonine	T	-0.7
Serine	S	-0.8
Tryptophan	W	-0.9
Tyrosine	Y	-1.3
Proline	P	-1.6
Histidine	H	-3.2
Glutamic acid	E	-3.5
Glutamine	Q	-3.5
Aspartic acid	D	-3.5
Asparagine	N	-3.5
Lysine	K	-3.9
Arginine	R	-4.5

Hydropathy Plot

The plot has amino acid sequence of a protein on its x-axis, and degree of hydrophobicity and hydrophilicity on its y-axis.

There are a number of methods to measure the degree of interaction of polar solvents such as water with specific amino acids.



Kyte-Doolittle Hydropathy Plot of Dehydrogenase

Hydropathy Plot

Hydropathy plots **allow for the visualization of hydrophobicity over the length of a peptide sequence**.

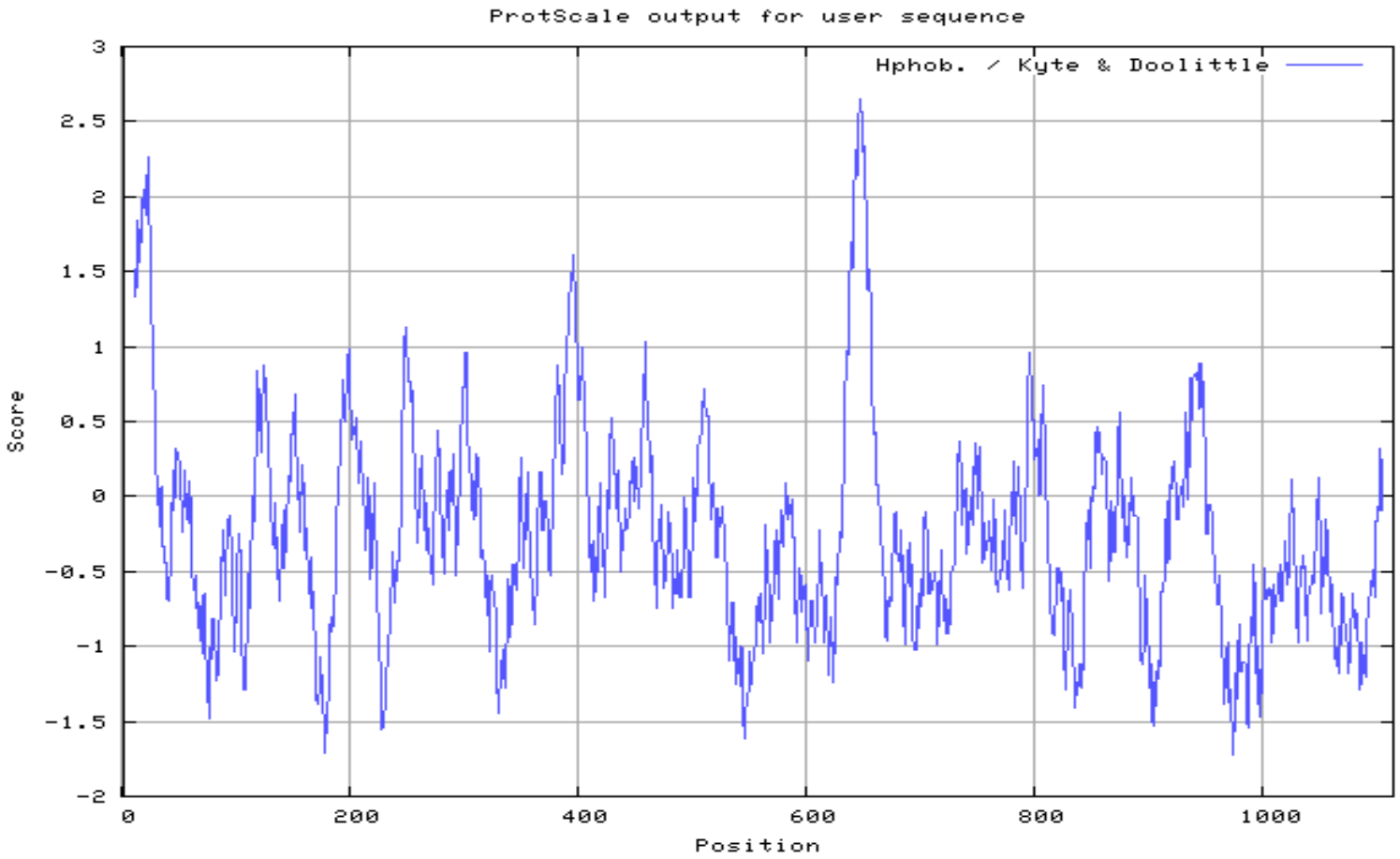
Such plots are useful in determining the hydrophobic interior portions of globular proteins as well as determining membrane spanning regions of membrane bound proteins

A hydropathy plot is a quantitative analysis of the degree of hydrophobicity or hydrophilicity of amino acids of a protein. It is used to characterize or identify possible structure or domains of a protein.

Analyzing the shape of the plot gives information about partial structure of the protein. For instance, if a stretch of about 20 amino acids shows positive for hydrophobicity, these amino acids may be part of alpha-helix spanning across a lipid bilayer, which is composed of hydrophobic fatty acids.

On the converse, amino acids with high hydrophilicity indicate that these residues are in contact with solvent, or water, and that they are therefore likely to reside on the outer surface of the protein.

Hydropathy Plot



Kyte-Doolittle-Hydropathy Plot for Human RET proto-oncogene. Plot was created by using the ExPASy Protscale tool (http://web.expasy.org/protscale/).