

Lecture 2

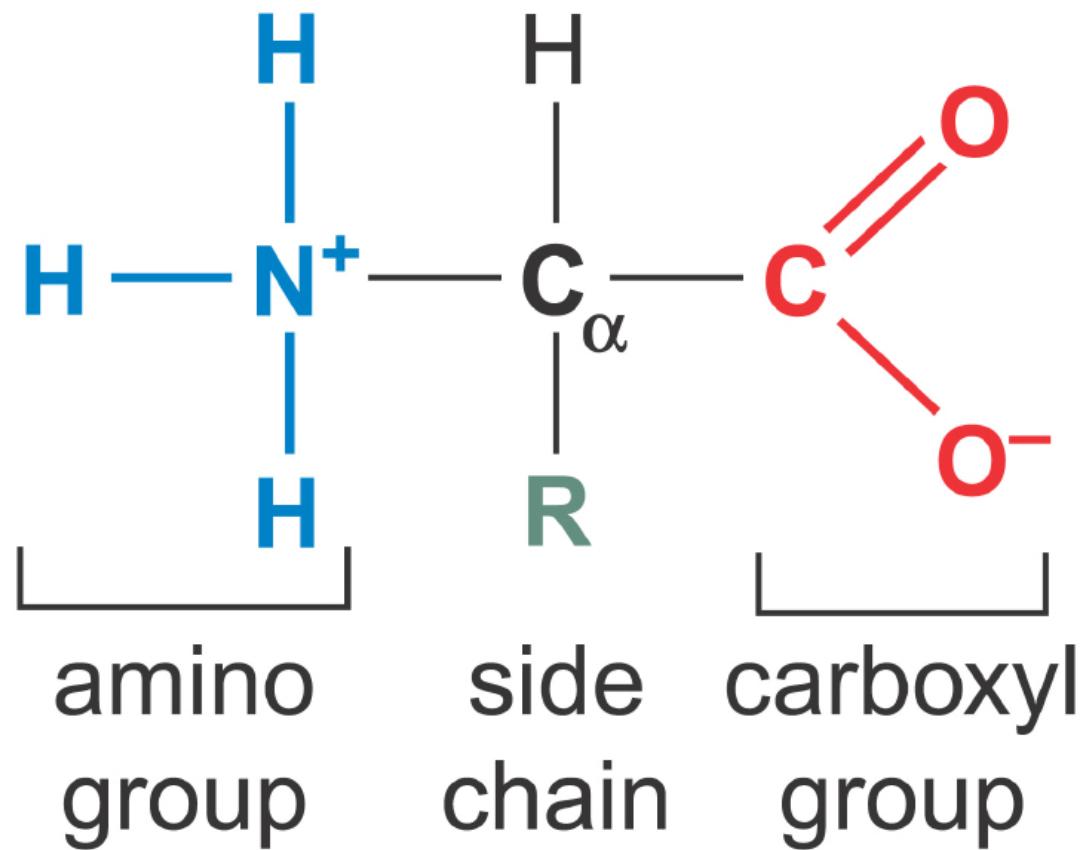
Protein Structure

Mar 31, 2016
Pyle

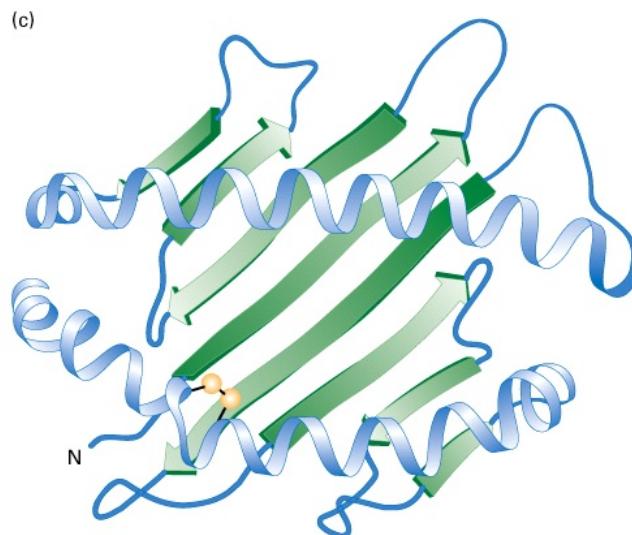
Proteins

- Proteins are polypeptides made up of amino acids
- There are 20 different amino acids, each with different chemical properties
- The 3D shape a protein adopts is determined by its sequence of amino acids
- The large number of 3D shapes that proteins can assume allows them to perform a wide range of functions

Basic Structure of Amino Acids



Amino Acids, Shape and Function of Proteins



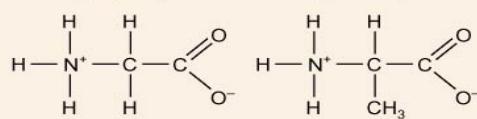
Proteins play countless roles throughout the biological world, from catalyzing chemical reactions to building the structures of all living things. Despite this wide range of functions all proteins are made out of the same twenty amino acids, but combined in different ways. The way these twenty amino acids are arranged dictates the folding of the protein into its unique final shape.

<http://ed.ted.com/on/2vooZ7ac>

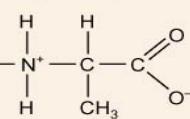
20 Amino Acids in Proteins

neutral-nonpolar amino acids

glycine
(Gly, G)

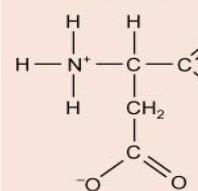


alanine
(Ala, A)

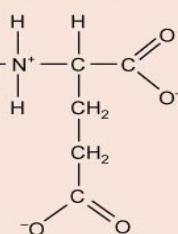


acidic amino acids

aspartic acid
(Asp, D)

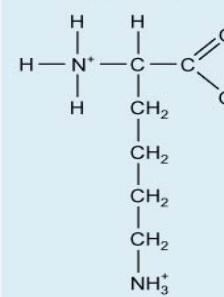


glutamic acid
(Glu, E)

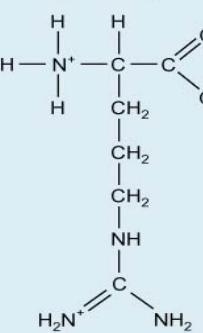


basic amino acids

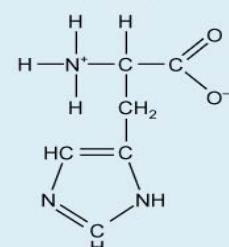
lysine
(Lys, K)



arginine
(Arg, R)

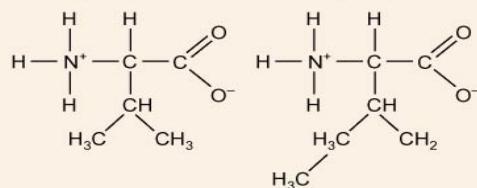


histidine
(His, H)

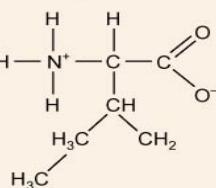


neutral-polar amino acids

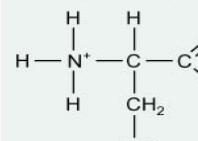
valine
(Val, V)



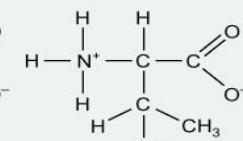
isoleucine
(Ile, I)



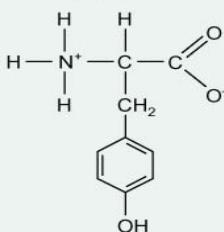
serine
(Ser, S)



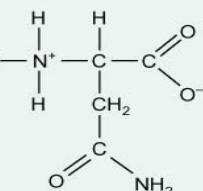
threonine
(Thr, T)



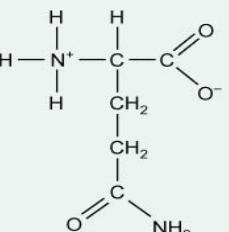
tyrosine
(Tyr, Y)



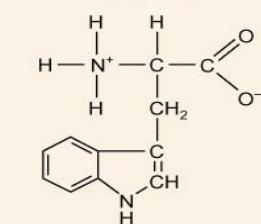
asparagine
(Asn, N)



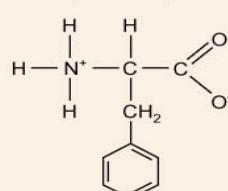
glutamine
(Gln, Q)



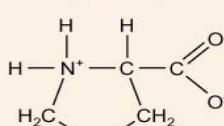
tryptophan
(Trp, W)



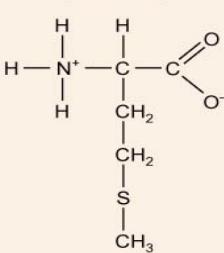
phenylalanine
(Phe, F)



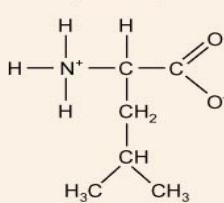
proline
(Pro, P)



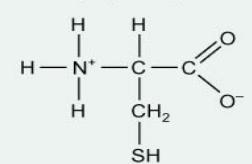
methionine
(Met, M)



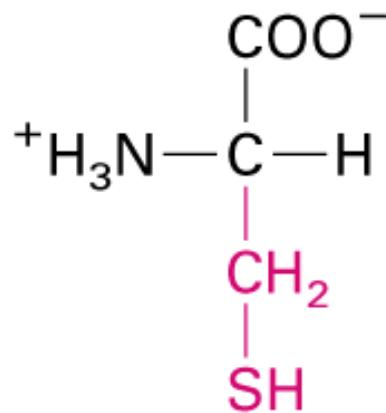
leucine
(Leu, L)



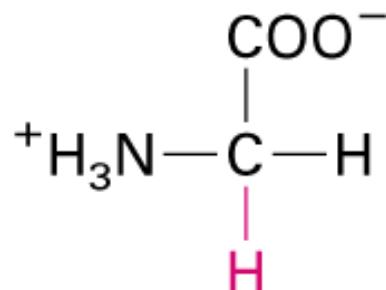
cysteine
(Cys, C)



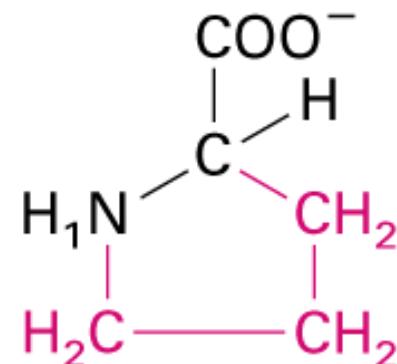
SPECIAL AMINO ACIDS



Cysteine
(Cys or C)



Glycine
(Gly or G)



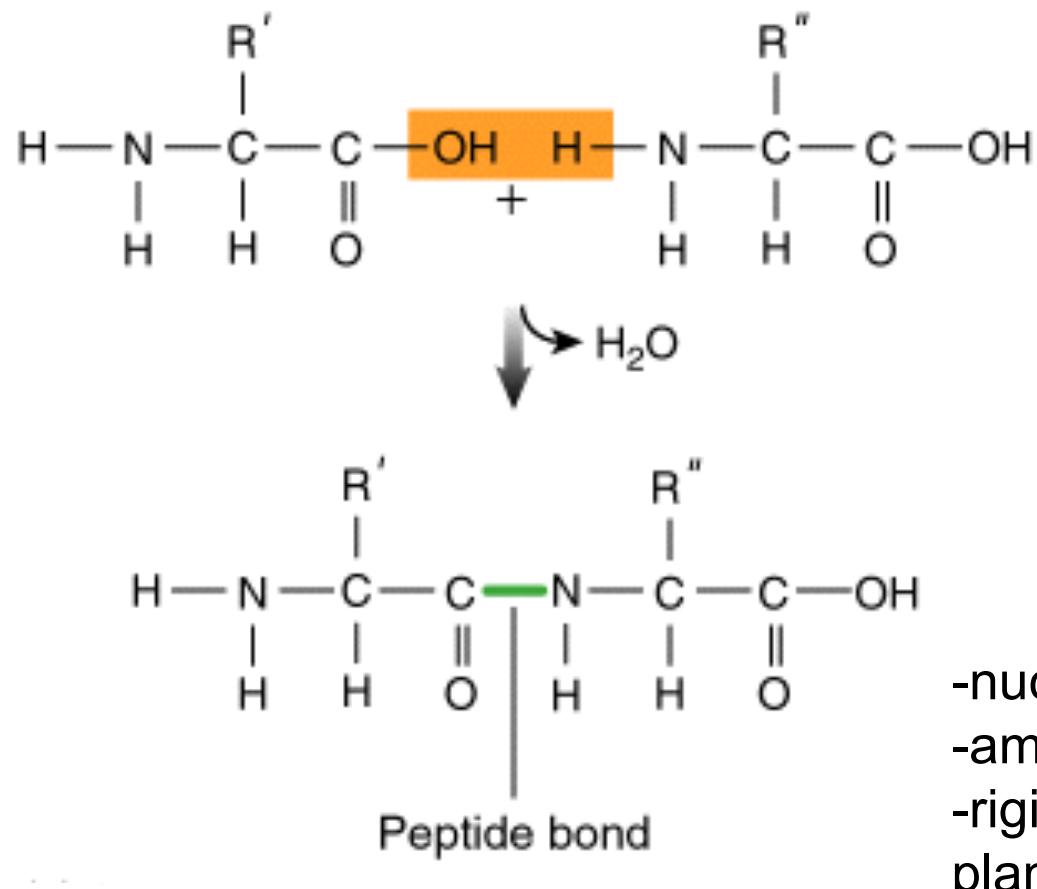
Proline
(Pro or P)

Glycine - smallest side group

Cysteine - forms disulfide bonds (covalent bond)

Proline - the only cyclic amino acid. Often involved in bends in protein chains.

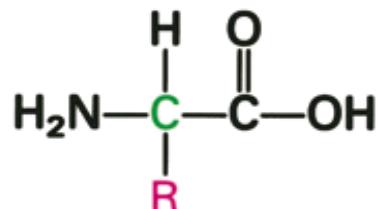
The peptide bond is formed by joining the amino end of one amino acid to the carboxyl end of another amino acid.



- nucleophilic add-elim rxn
- amide bond
- rigid bond/DB character/planar

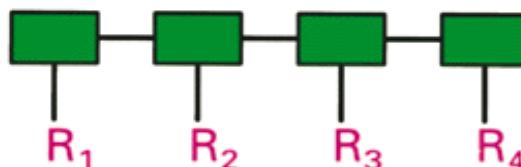
Peptide bonds

MONOMER



Amino acid

POLYMER



Polypeptide

The covalent bond between two adjacent amino acids in a peptide is called peptide bond.

6N HCl breaks peptide bonds and hydrolyzes polypeptides. Specific cleavages by proteases.

Ex. Trypsin cleaves at C terminus of Arg and Lys

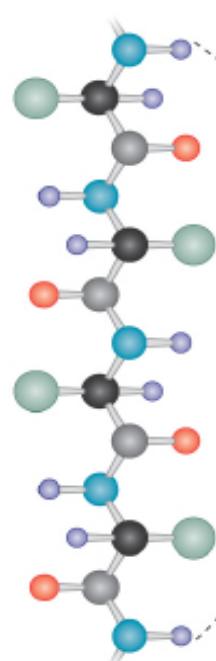
Protein Sequence Comparison and Homology

D.M. Dolphin myoglobin, H.M. human myoglobin, H.H. human hemoglobin

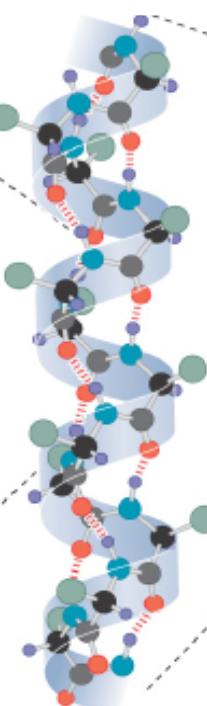
Protein homology : means either identical amino acid or similar amino acid. This is because change of an amino acid in a protein to a different amino acid with similar chemical property usually causes no harm to the protein with respect to its function.

Protein structure: The structure of a protein can be divided into several levels:

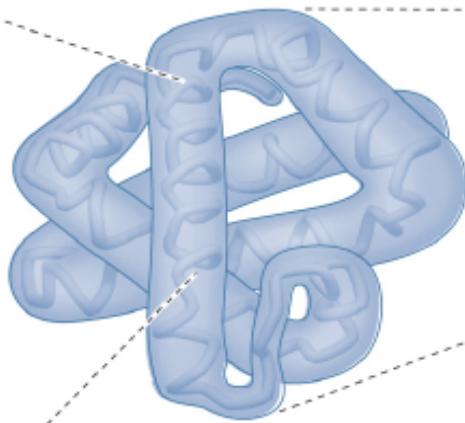
Primary



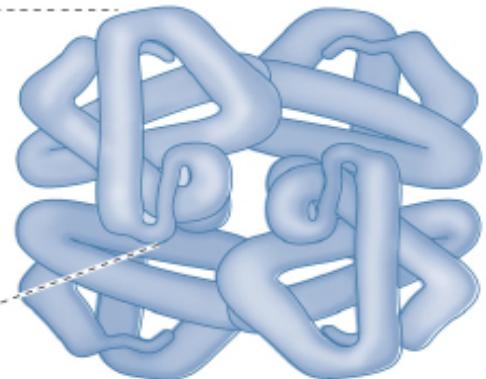
Secondary



Tertiary



Quaternary



Primary (1°) structure: linear amino acids sequences (also called protein sequences)

H₂N-MDEKRHSTQNYAVLIFWGCP-COOH

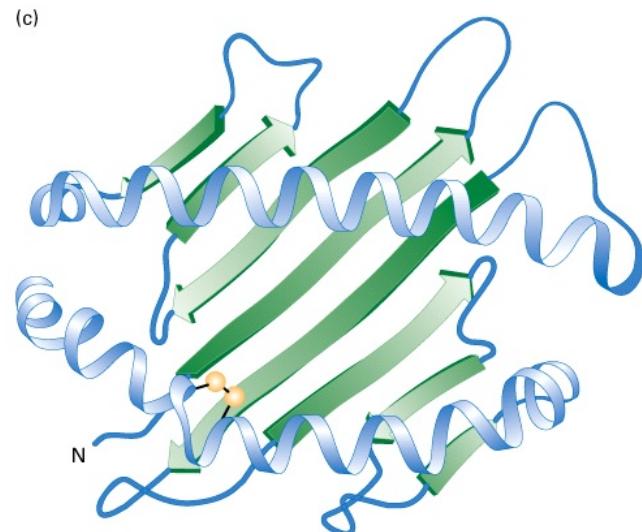
↑
N-terminus

↗
C-terminus

The primary structure of a protein is linked by covalent peptide bonds.

Secondary (2°) structure

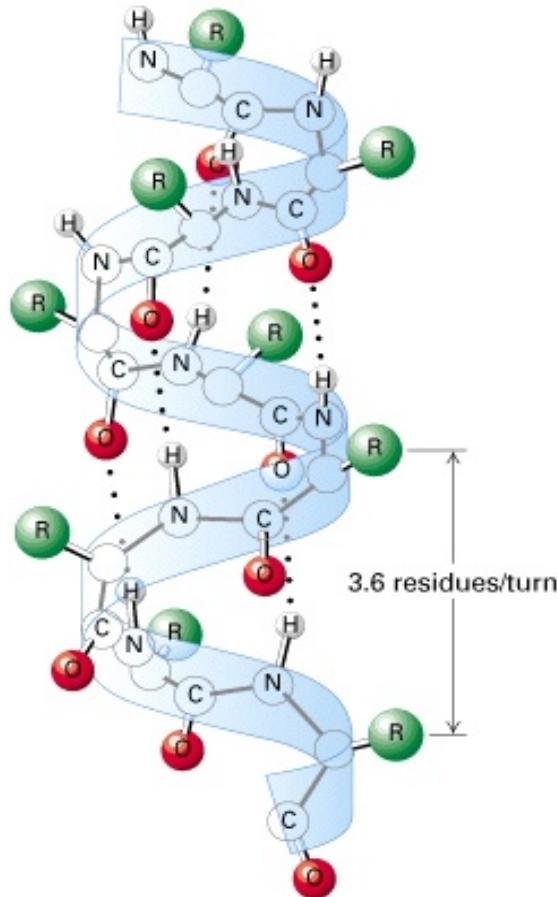
- (1) α helix
- (2) β strand/ β sheet
- (3) random coil



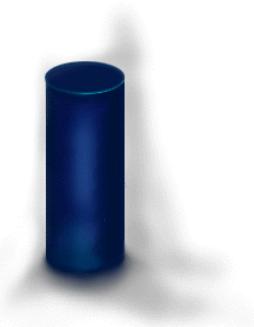
The regularly (or periodically) repeating conformation of the peptide backbone, often refers to the localized organization of parts of polypeptide chain. Stabilized mainly by hydrogen-bonding between N-H and C=O groups of the peptide backbone

A polypeptide usually has different secondary structures at different regions, and this depends on the amino acid sequence of regions.

Alpha (α)- helix

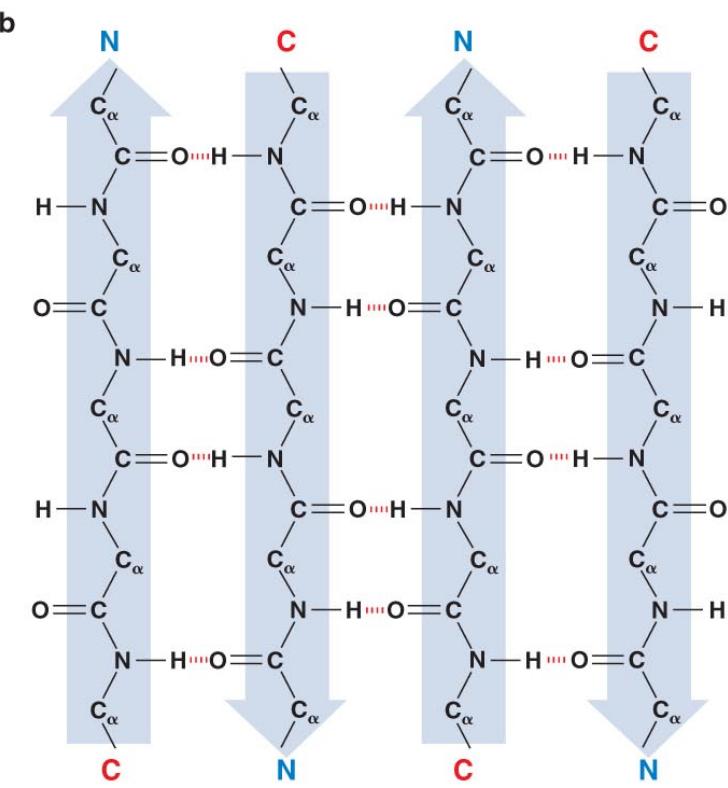
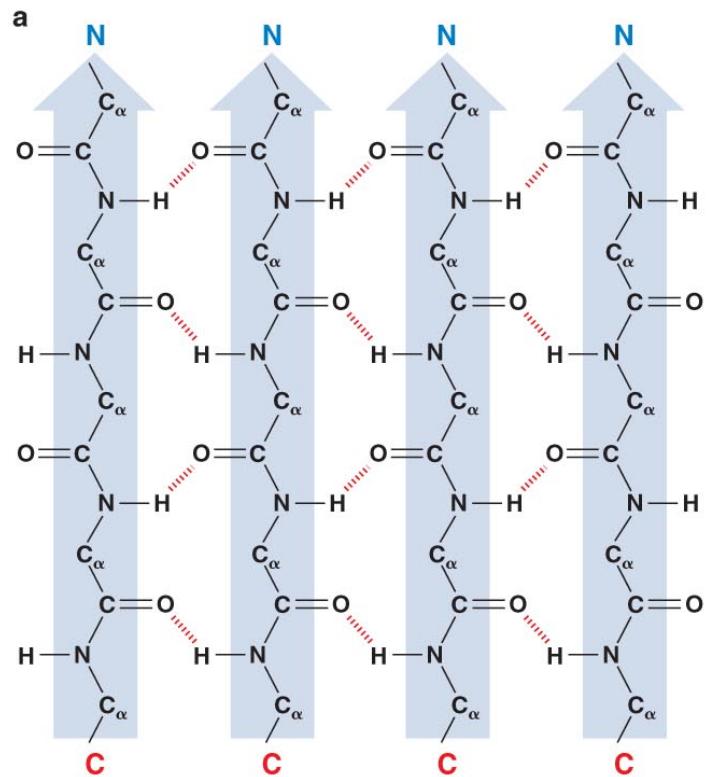


Frequently
represented
by a cylinder



- Right handed helix
- 3.6 residues/turn
- H-bond between every 4th aa
- Forms a rigid structure
- R groups project out from helix
- H-bonds are parallel to axis of the helix

Two Orientations of Beta-strands:

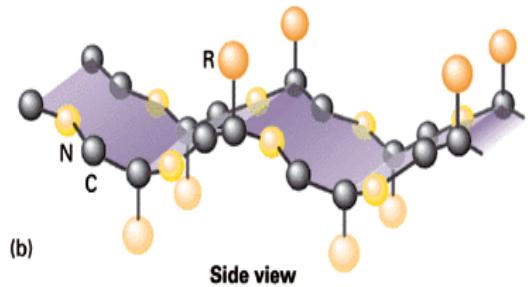
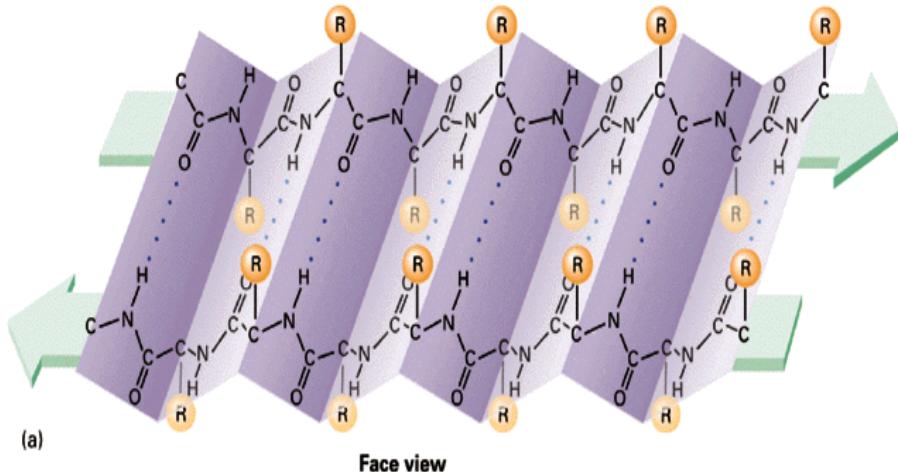


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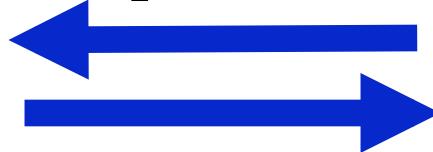
Parallel

Antiparallel

Beta (β)-pleated sheet

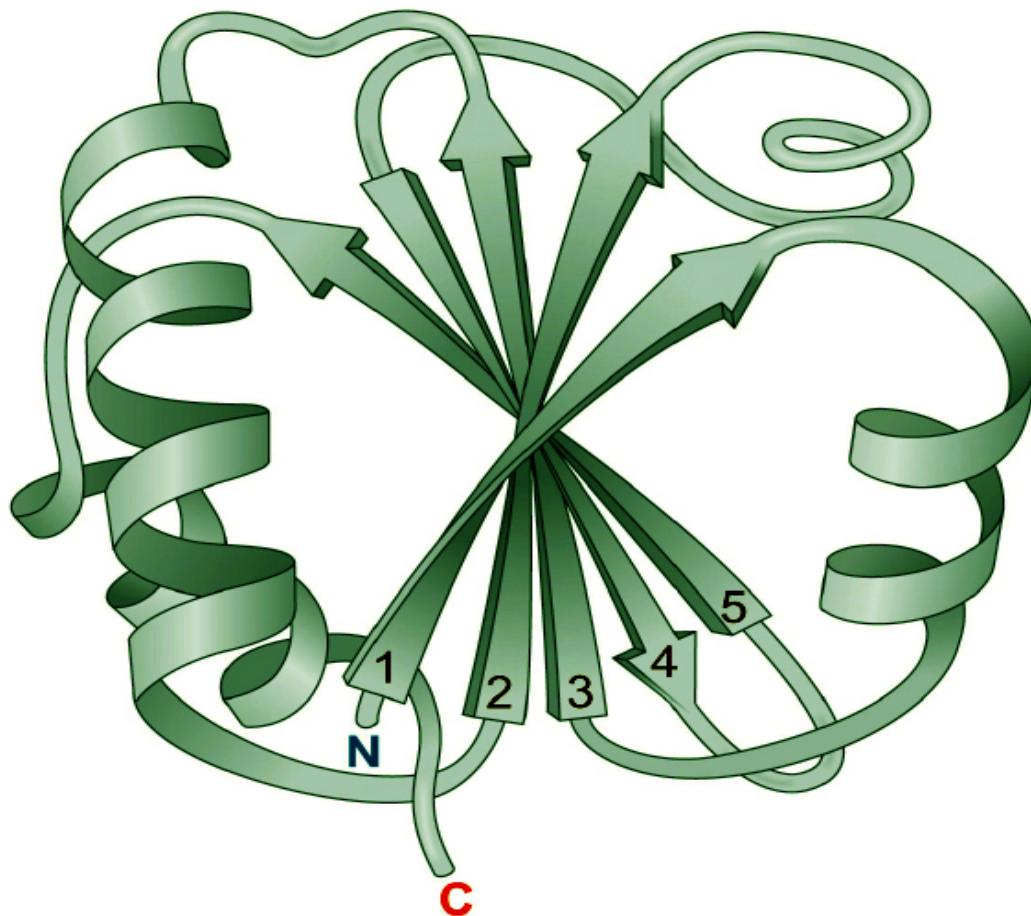


Frequently represented by arrows (point to carboxyl)



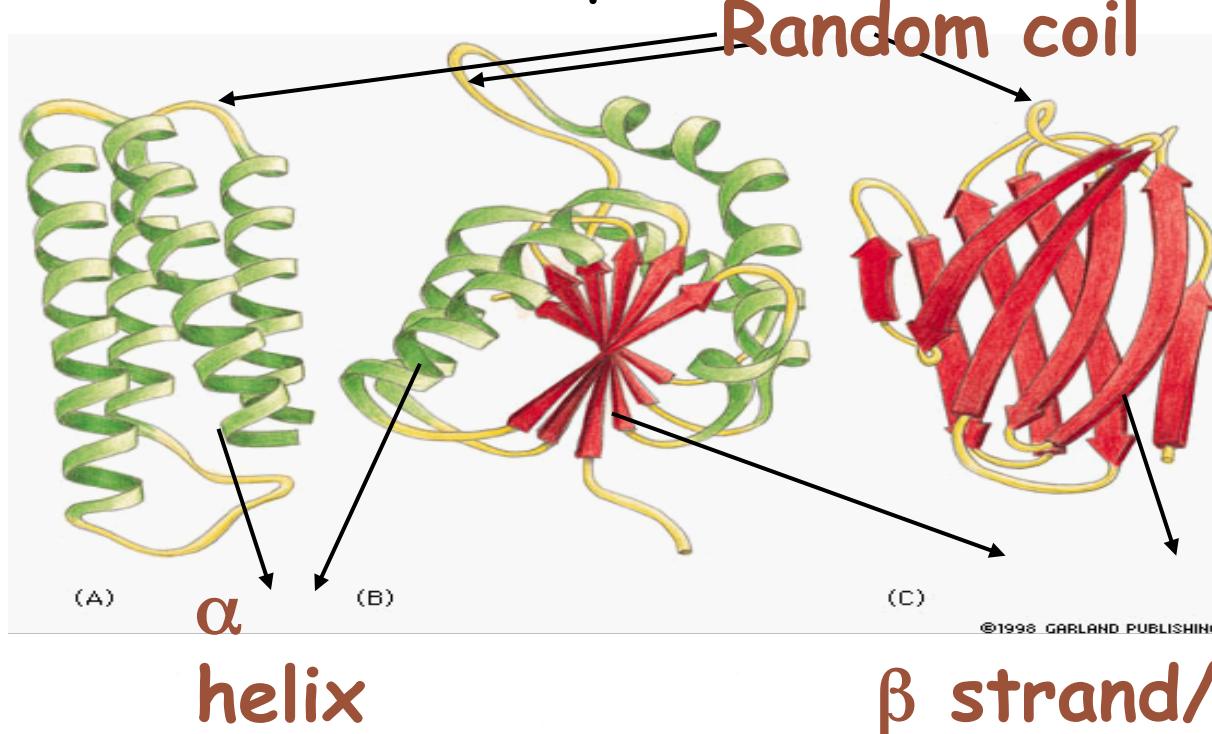
- Planar peptide bonds with bend at $C\alpha$
- H-bonding between one beta strand and another
- R groups alternate above and below sheet

Both parallel and anti-parallel strands can be found in single protein



Random coils are linkers between 2° structures

- U shaped or unshaped structure, 3-4 residues per unit
- usually formed by Glycine or Proline
- needed to connect different helices/strands
- often located on the surface of a protein
- longer turns are called loops and random coils

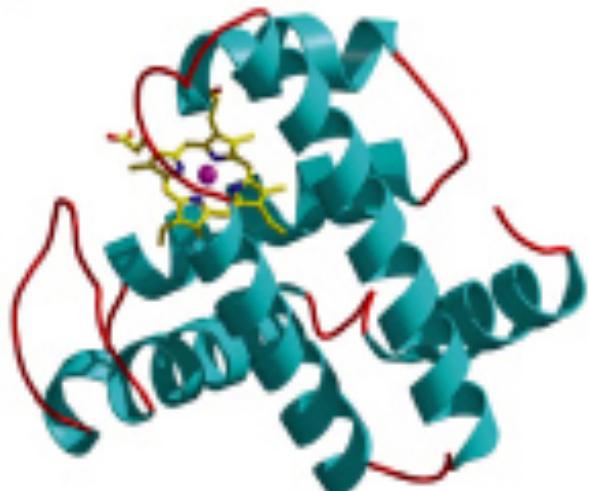


Tertiary (3°) structures

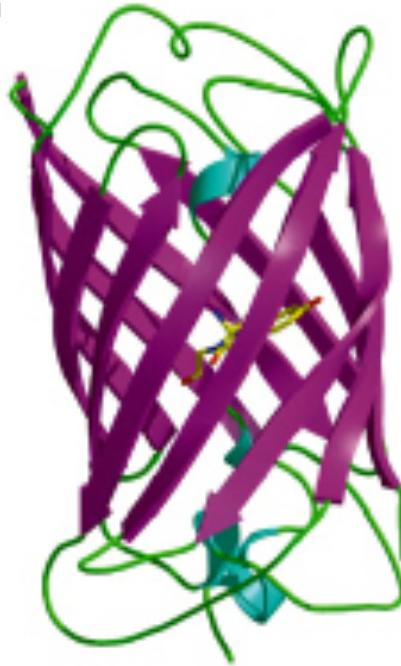
Tertiary structure is the particular 3-D folding pattern (or shape, or conformation) of the entire polypeptide chain.

The tertiary structure is stabilized by hydrophobic interactions between the nonpolar side chains, hydrogen bonds between polar side chains and in some cases also by disulfide bonds between cysteine residues.

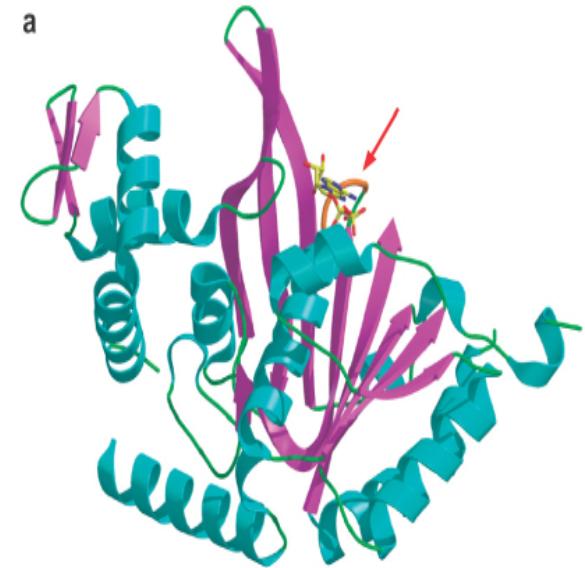
Tertiary structures often reflect important protein functions



Myoglobin-carrys
oxygen



**Green fluorescent
Protein**-exhibits bright
green fluorescence when
exposed to ultraviolet blue light
Carries chromophore
responsible for fluorescence



Rec A-
carries ATP and
Binds DNA

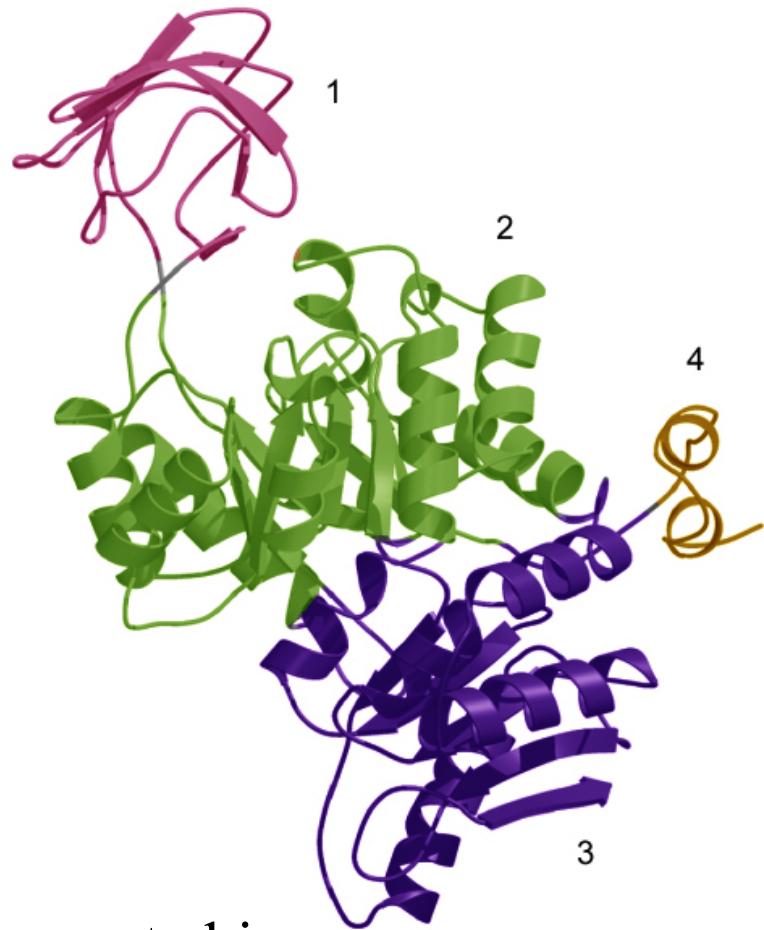
Quaternary (4°) structures

Quaternary structure is the structure organization of multiple polypeptide subunits e.g. structure of antibody IgG, or hemoglobin

**For a single subunit protein, Tertiary structure =
Quaternary structure**

The quaternary structure is held together by noncovalent bonds (in some cases also by disulfide bonds) between protein subunits.

Structural domains often represent key protein functions

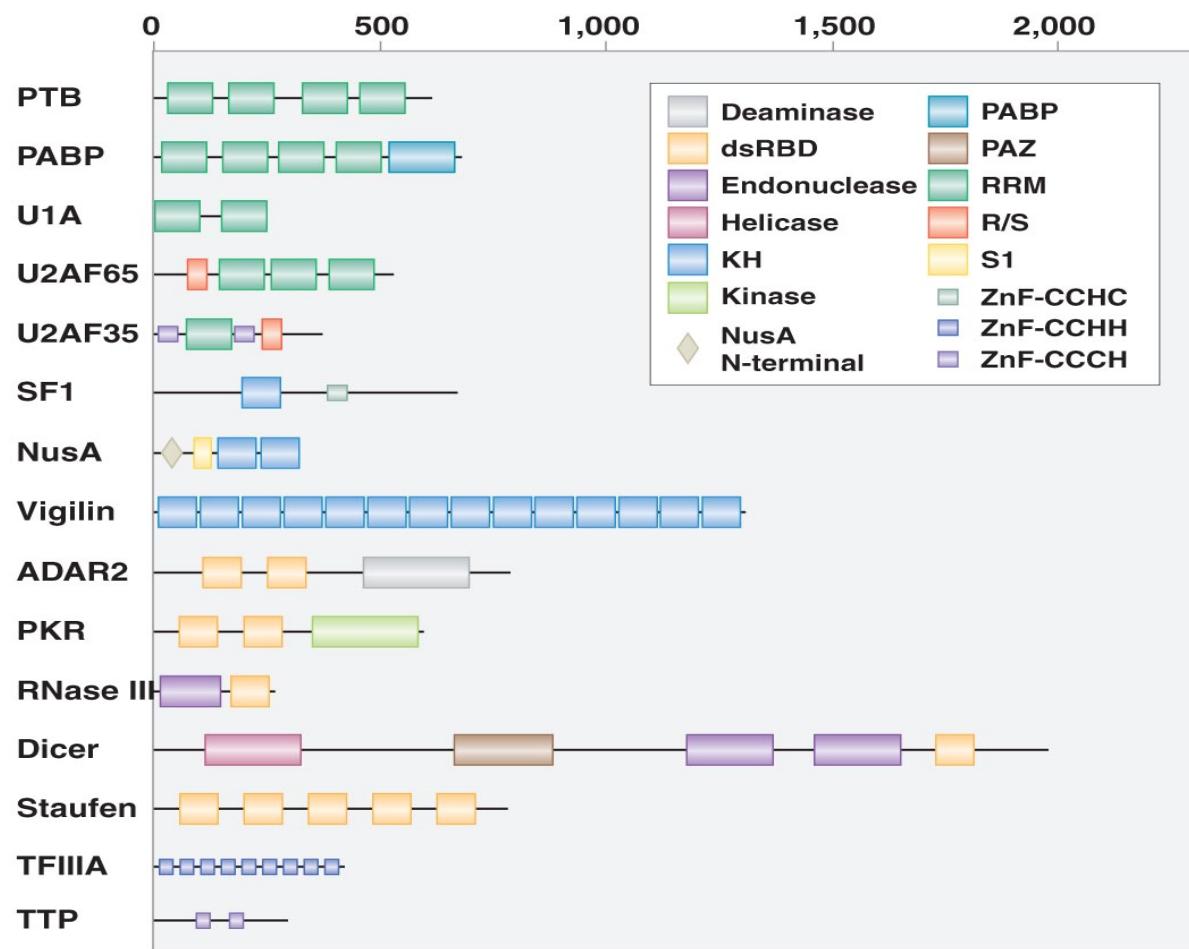


Pyruvate kinase

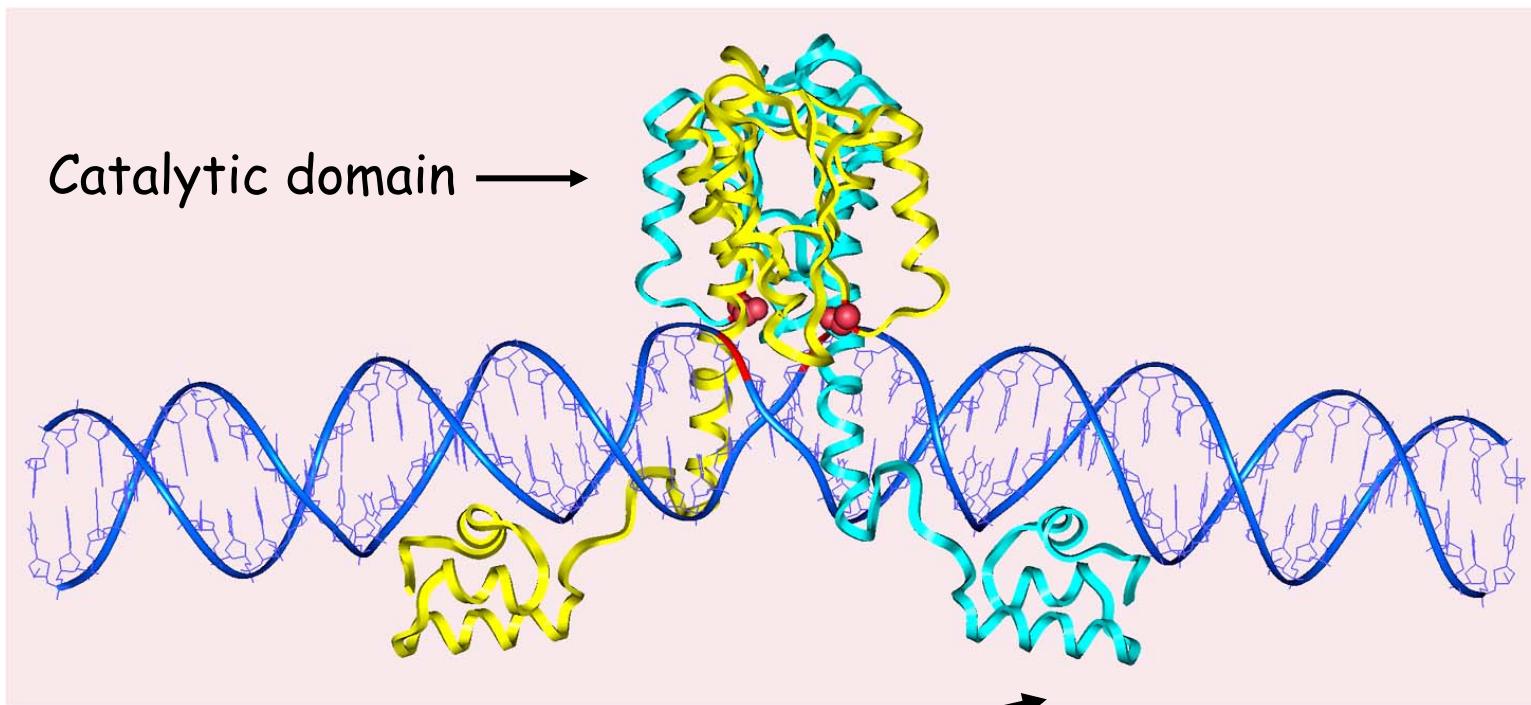
- Part of the polypeptide chain that can fold independently into a compact stable structure.
- Large proteins may have many domains
- Domains are usually composed of a continuous sequence of amino acids

Book Box 6-2 Glossary of terms for proteins

Proteins often contain Combinations of Multiple Domains Important for Protein Function

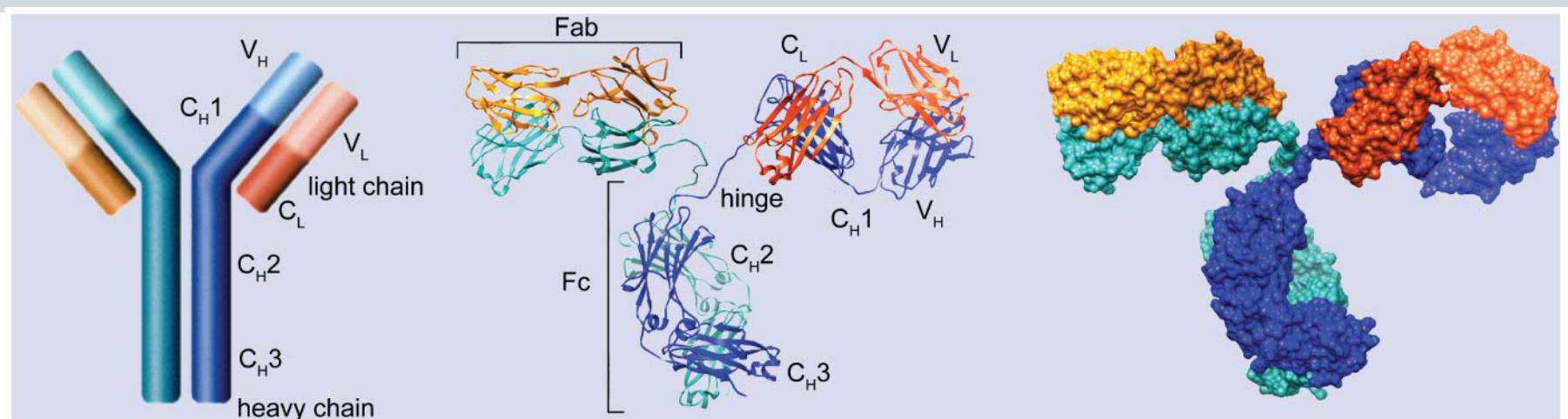


Individual Domains of proteins are often associated with different functions



DNA binding domains

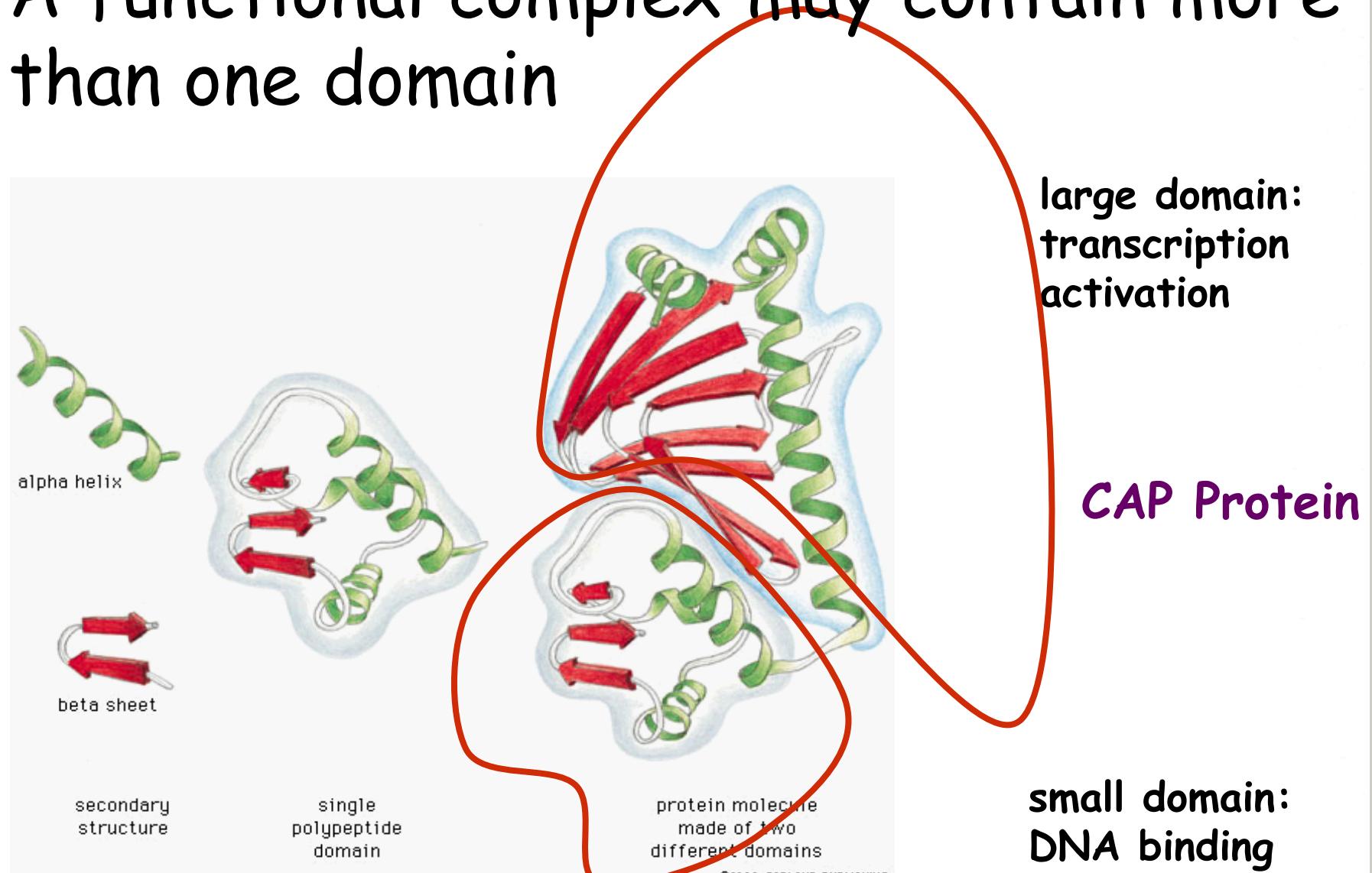
Antibodies (ABs) Illustrate Protein Domains



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- The immune system uses specialized cells to recognize invading organisms
- B cells produce antibodies that circulate in bloodstream to attack "foreigners"
- ABs have two copies of a light chain and a heavy chain and interface with antigens at V (variable) sites
 - Your very own Randy Wall was one of the first to study B cell development and the importance of AB/antigen interactions and diversity!!

A functional complex may contain more than one domain



Regions in a protein can be defined based on their properties

- **Binding site**

region that interacts with another molecule (ligand) through non-covalent interactions

- **Dimerization region**

region where two different polypeptides interact

- **Active site**

region where catalysis takes place on enzyme

- **Regulatory site**

binding site for molecule which affects activity of protein (frequently through conformational changes)

Protein Folding

Proteins fold up to form specific 3-D conformations. The center of a protein is usually hydrophobic and the exterior of a protein is typically hydrophilic.

Spontaneous folding

Most proteins can fold to their native conformation spontaneously

Chaperone assisted folding

Some proteins won't appropriately fold by themselves, they need help of chaperones, many "heat shock" (hsp) proteins are chaperones

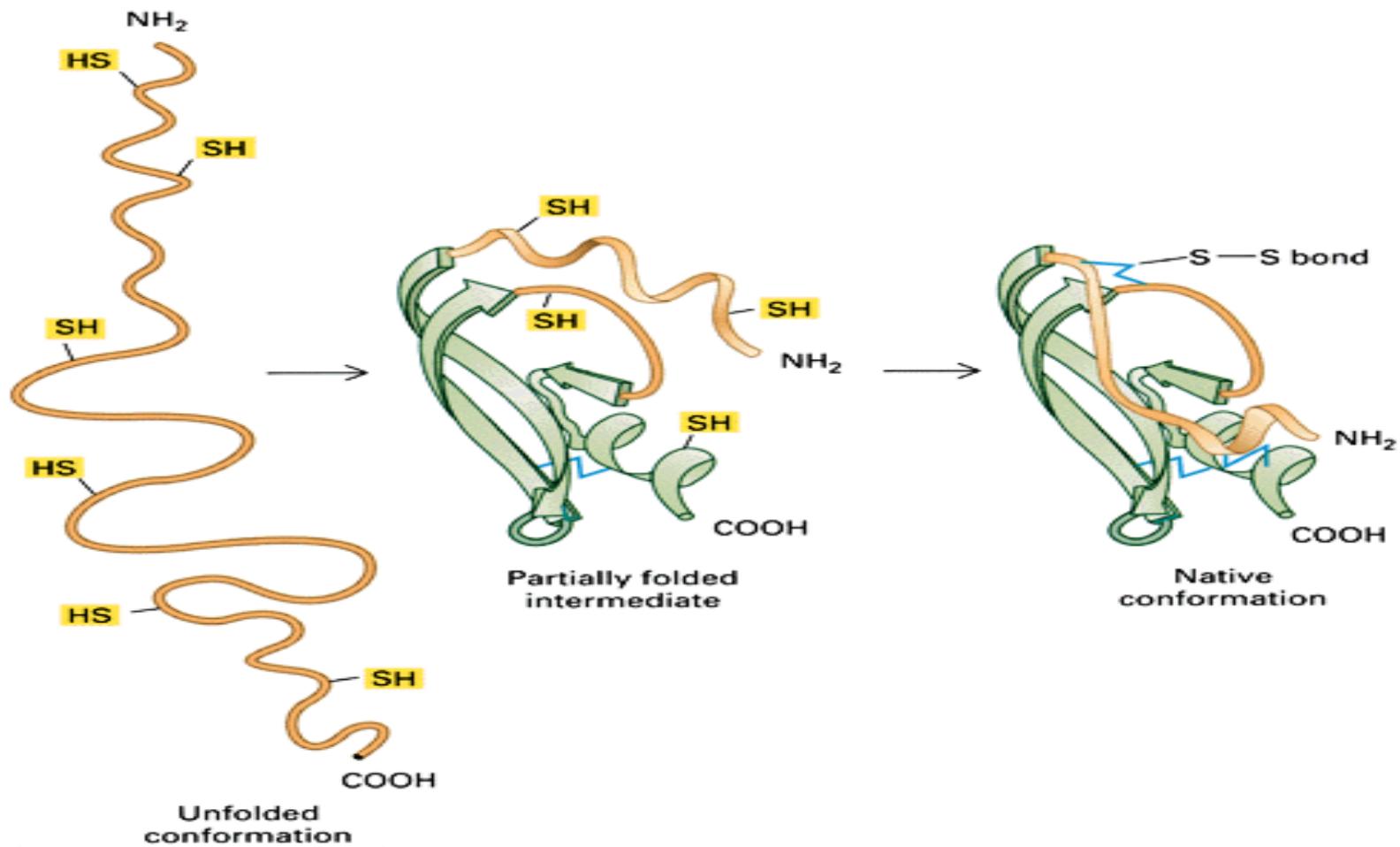
Denature

Loss of native folding due to the break of weak (H, ionic etc) or strong (S-S) bonds, by heat, freezing/thaw, extreme pH or ionic conditions, detergents, reducing agents, etc. If a protein become insoluble, it is likely denatured.

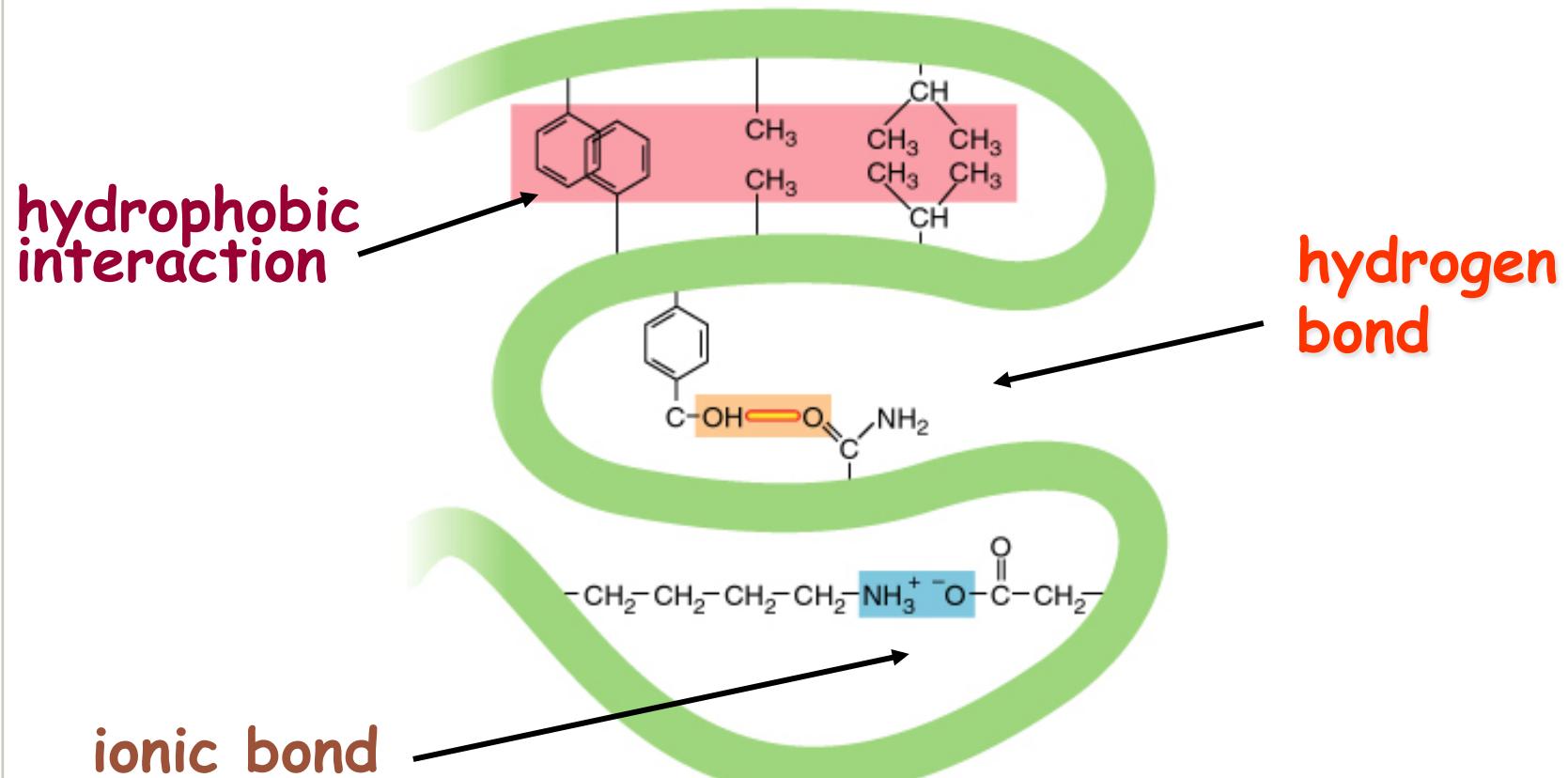
Renature

Regain of the native conformation (and solubility in aqueous solution)

Protein folding can proceed through intermediate states



Noncovalent bonds/Protein composition are important in maintaining the conformation of a protein



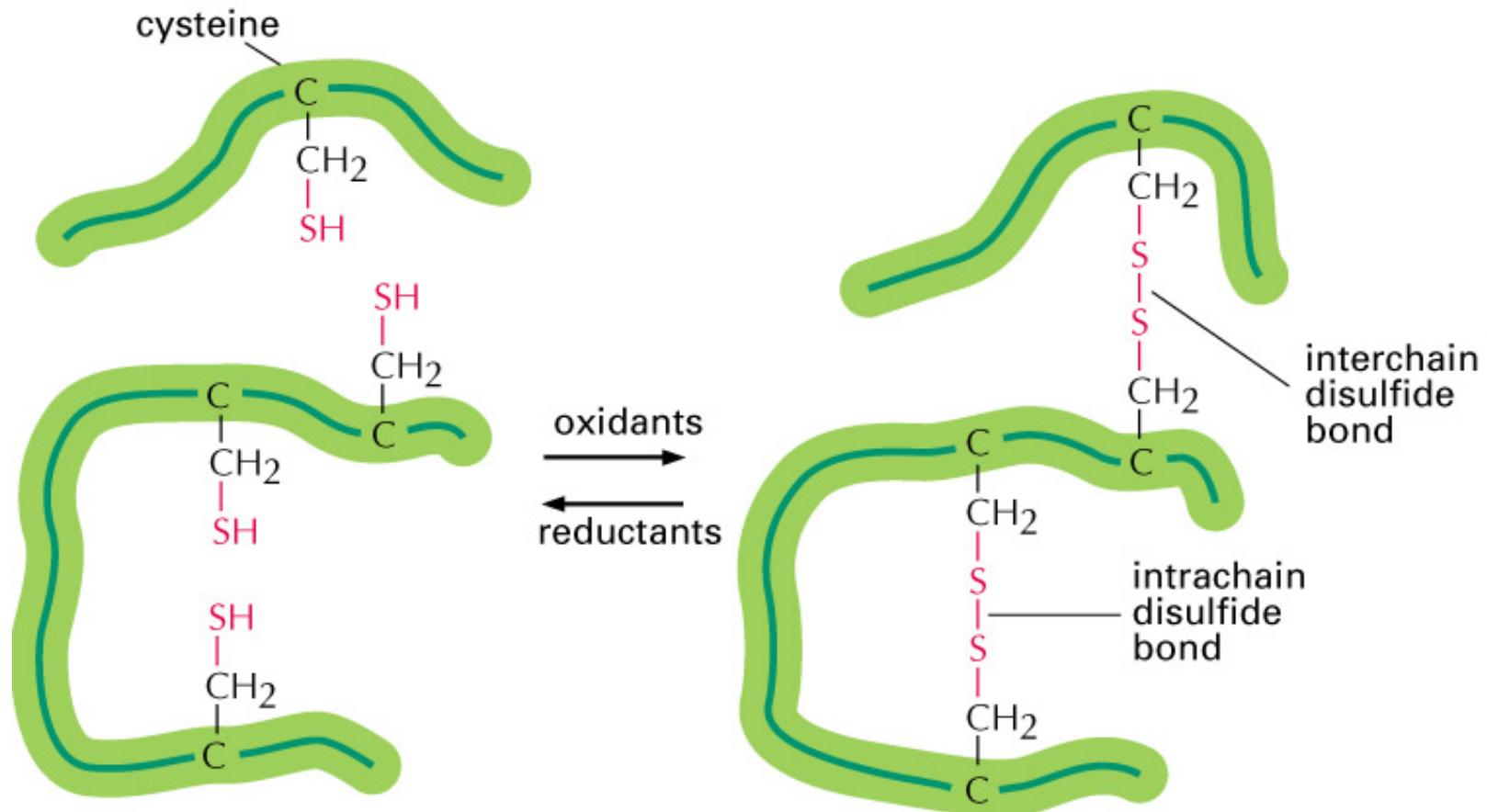
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Protein Modifications Regulate Protein Activity

Post-translational modifications are often essential for the regulation of the structure and function of a protein

1. Disulfide-bond formation
2. Group addition
 - protein phosphorylation
 - protein glycosylation
 - protein Ubiquitination
3. Proteolytic modification (cleavage)

Disulfide-bond formation



Regulation and Importance of disulfide-bond formation

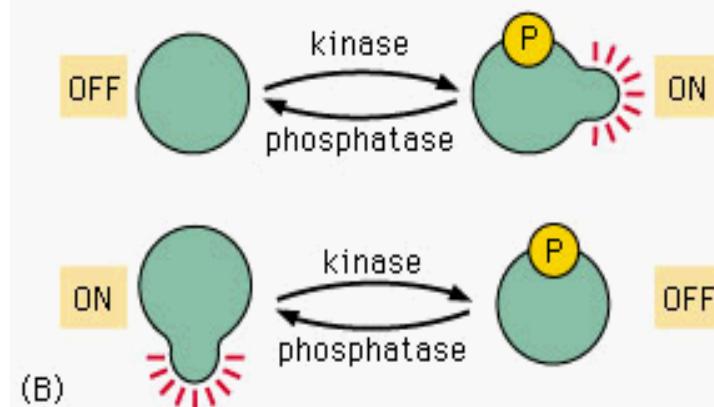
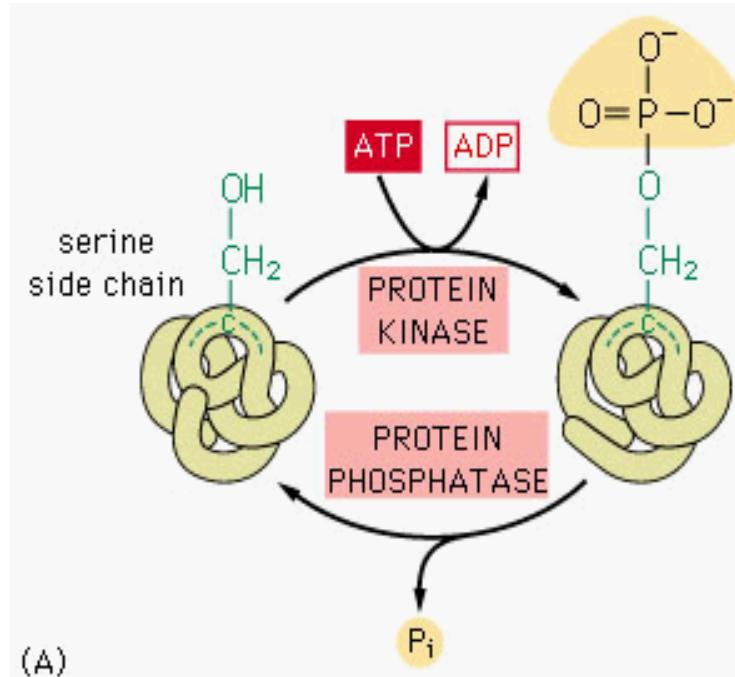
- Disulfide bonds are covalent sulfur-sulfur bonds formed between two cysteines (-cys-S-S-cys-) under oxidative conditions
- Disulfide bonds do not form in the cytosol because of the high levels of reducing agents
- Disulfide bonds are formed in the lumen of the RER (rough endoplasmic reticulum) but not in the cytosol.
- Disulfide bonds help to stabilize protein structure from pH changes or degradative enzymes
- Reducing agents such as β -mercaptoethanol and dithiothreitol (DTT) break S-S bonds (turn it to -SH + SH-), and they are often used in SDS-PAGE

Phosphorylation/ Dephosphorylation are key protein regulatory mechanisms

Kinases add phosphates to
the hydroxyl group of **serine**,
threonine, or **tyrosine** residues
of a protein

Phosphatases remove
phosphates from the protein

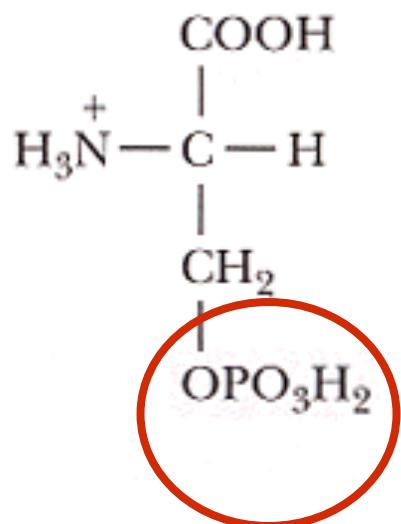
**Phosphorylation or
dephosphorylation often
affects protein activity**



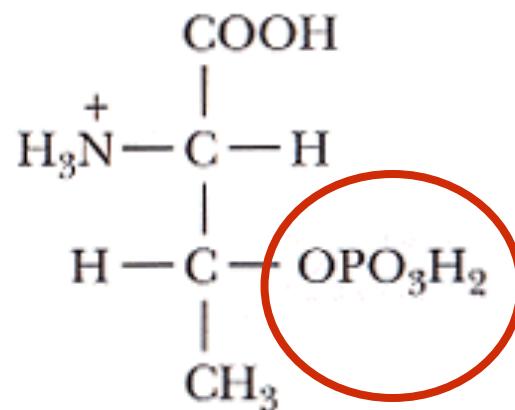
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Phosphorylation $[\text{PO}_4^{2-}]$: Phosphates can replace the OH in serines, threonines and tyrosines

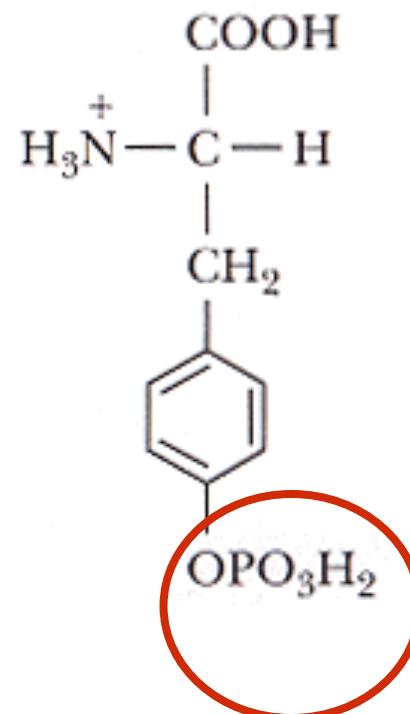
Phosphoserine



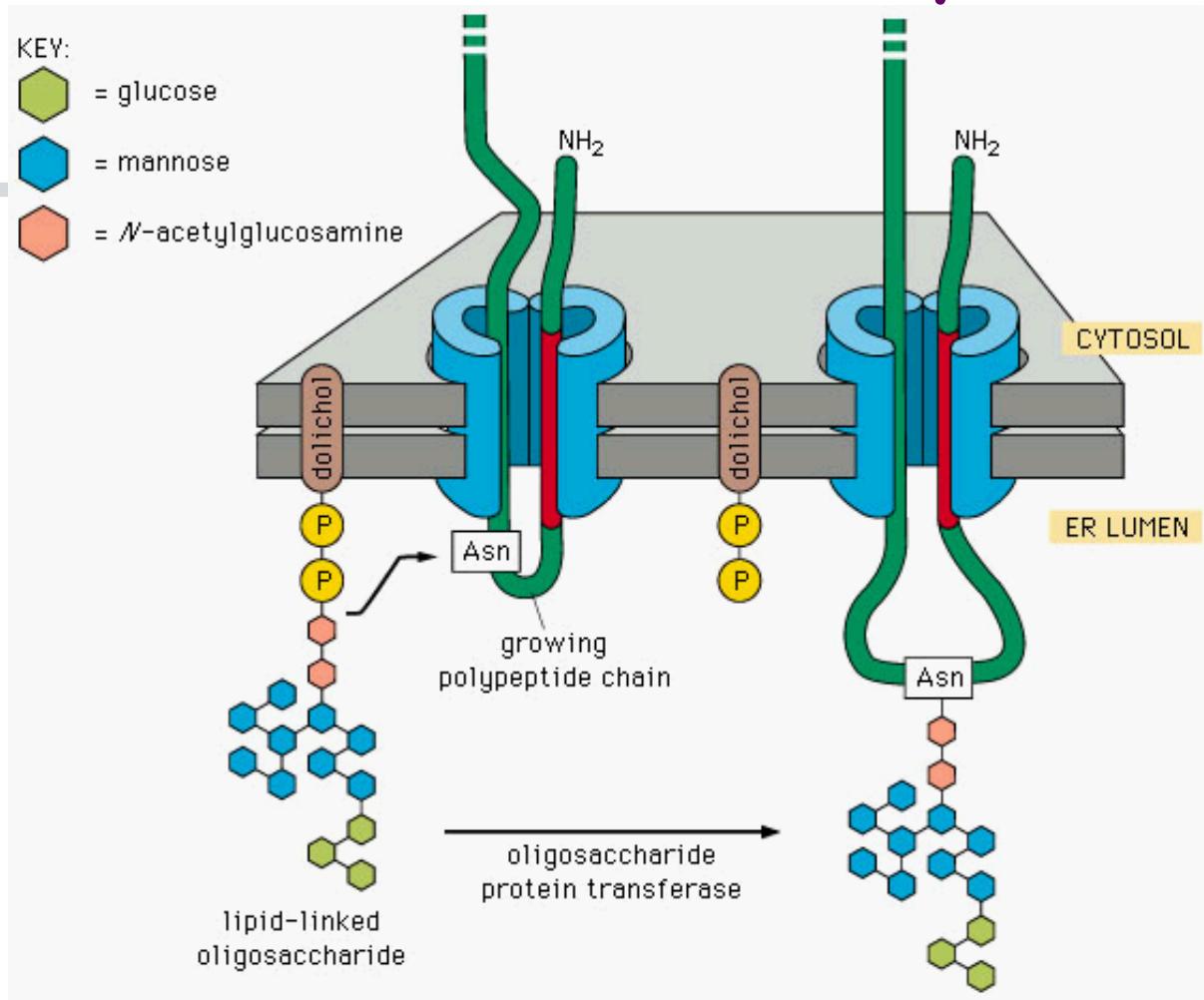
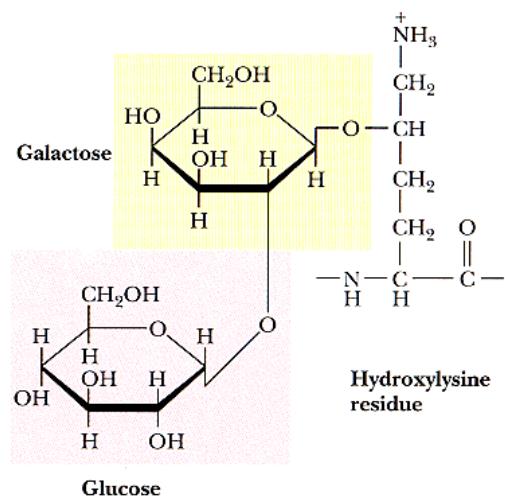
Phosphothreonine



Phosphotyrosine



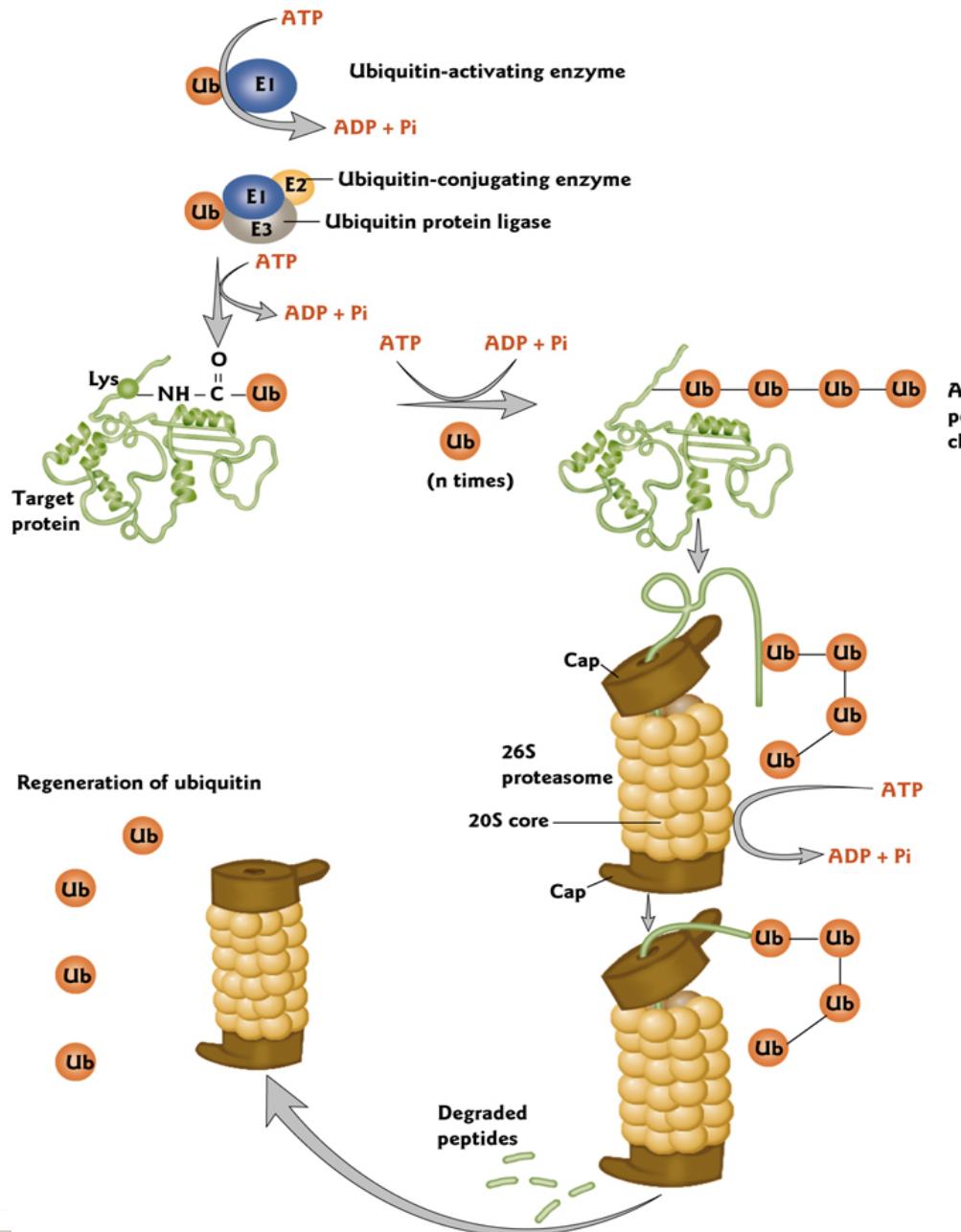
Protein Glycosylation: addition of carbohydrates



Carbohydrate groups can be added to the side chains of asparagine, serine, threonine or hydroxylysine

Glycosylation is often found for proteins secreted to the outside of the cell

Protein Ubiquitination



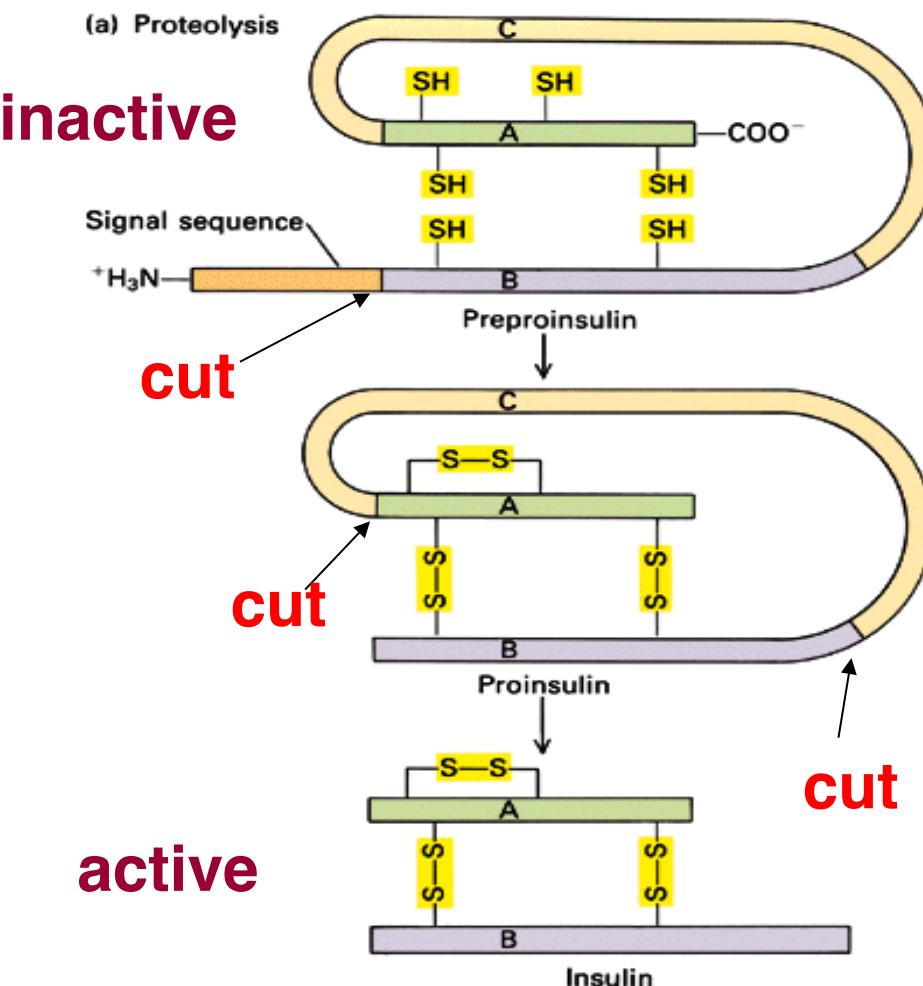
Ubiquitin is a 76 amino acid polypeptide that can be covalently linked to the lysine residue of its targeting proteins. Ubiquitin generally acts as a tag for protein degradation in a cell.

Proteolytic processing:

Removal of a fraction of a protein by a specific protease is often important for protein activation and transportation

-Pre-proinsulin is activated by proteolytic cleavage and formation of disulfide bonds

-A mutation in the insulin gene can prevent proinsulin from being cleaved, and is a cause of diabetes



Networks of Protein Interactions often controlled by Protein Modification Regulate Biological Processes: Not just one Protein!

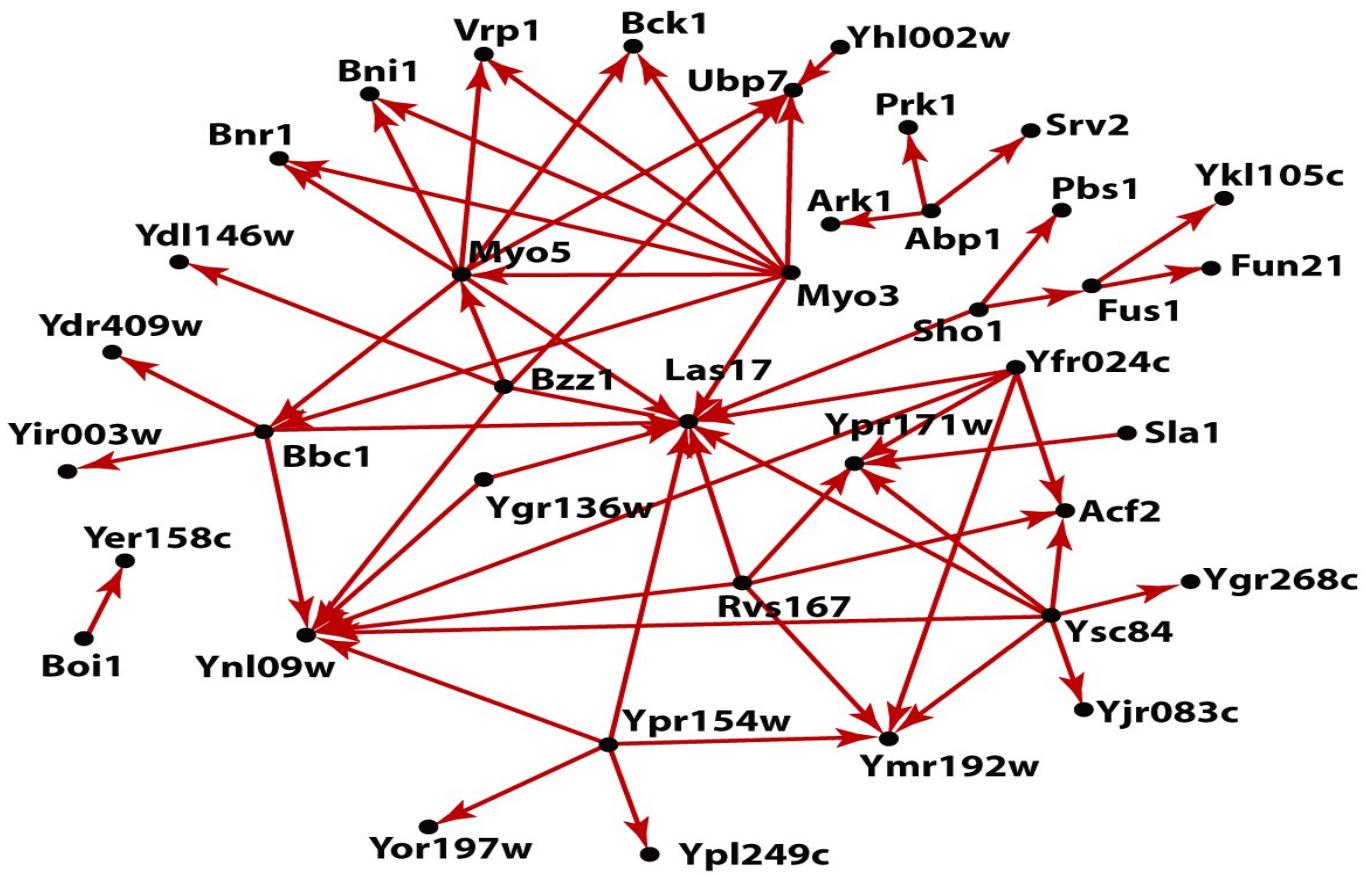


Figure 2-41 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Critical Thinking and Implications-2

- To function properly, polypeptides must fold into exactly the right three-dimensional structure
- In certain cases, aberrant protein folding has been suggested to play a role in disease progression in a number of disorders.
- What are examples of how protein mis-folding could lead to disease?