

Bioscience & Bioengineering 101: BB101

Lecture – 10

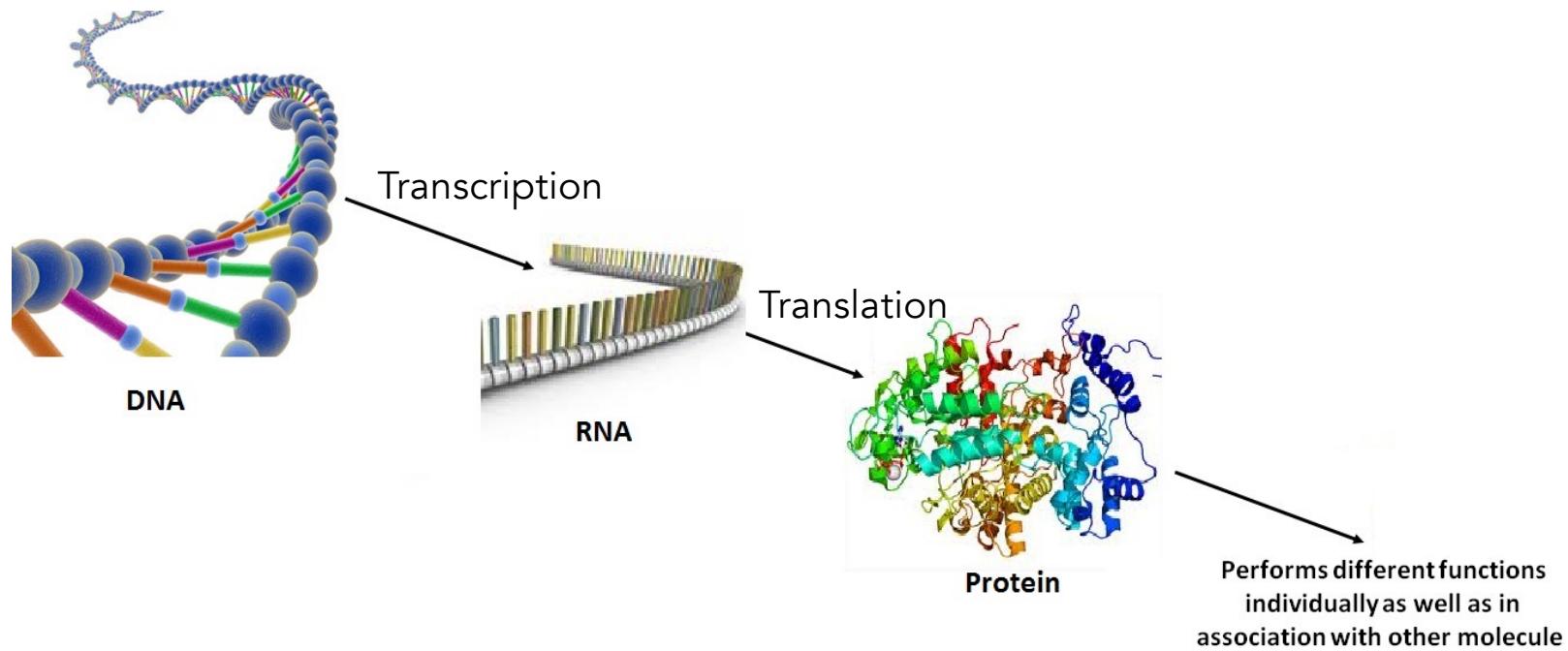
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BSBE, IIT Bombay

Proteins: Basic concepts & Techniques

Outline

- Central dogma & why to study proteins
- Amino Acids: Building Blocks of Proteins
- Different Levels of Protein Structure
- Sequence alignment
- Techniques to Study Proteins: Chromatography & Electrophoresis
- Contributions of Genomics & Proteomics to Biology

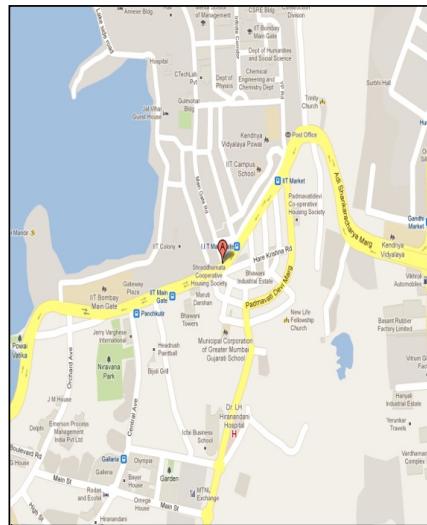
Central Dogma: Why Proteins?



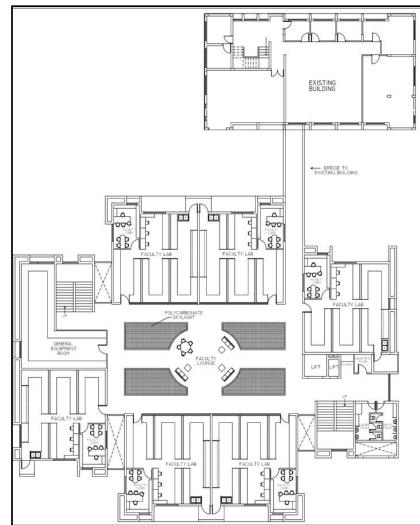
Proteins are the functional entities that encode and perform the coded messages in DNA to carry out all biological activities

Understanding Protein Function is Key to Biology

DNA
Genetic
blueprint



RNA
Molecular
photocopy

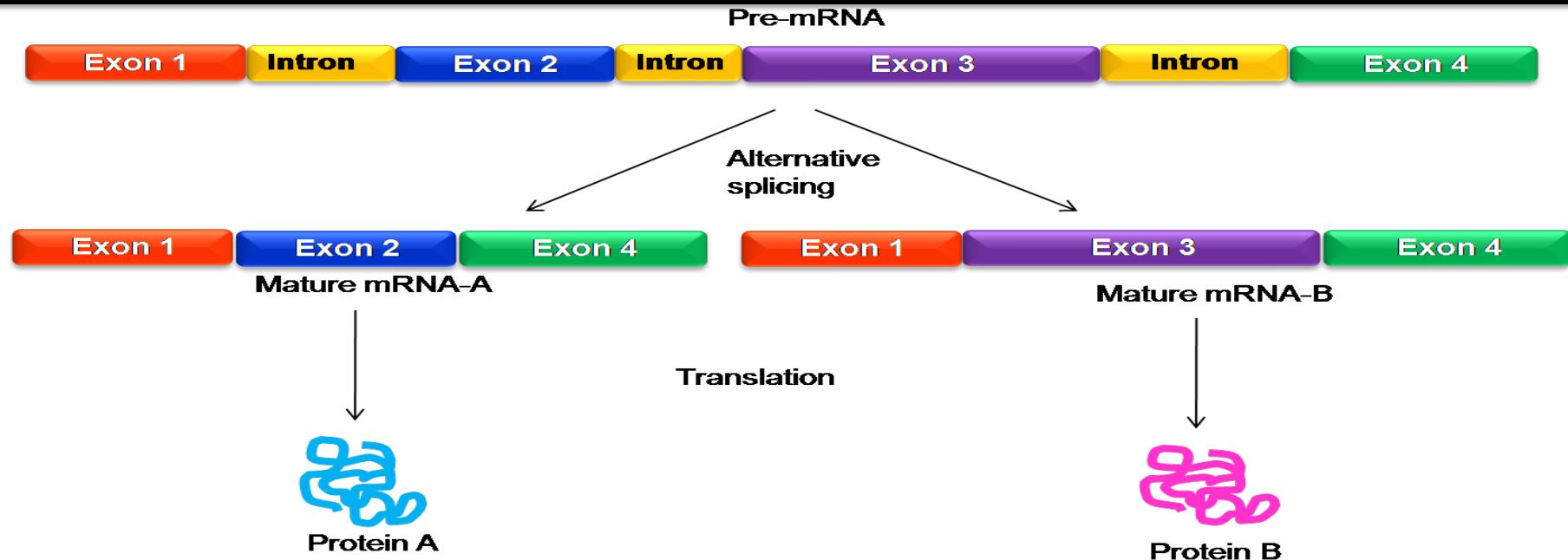


Proteins
Building
blocks



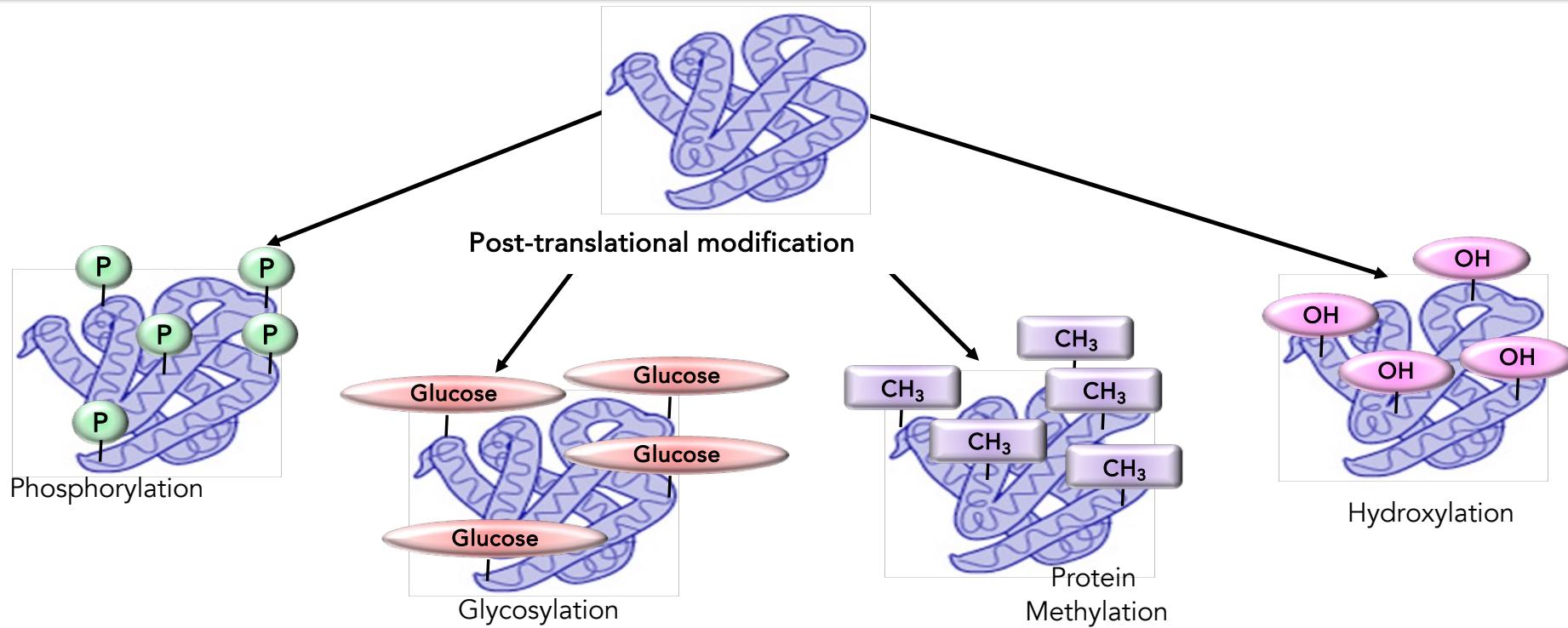
- Proteins perform a range of cellular functions
- Disease is the result of protein malfunction
- Drugs either alter protein function or are proteins themselves

Genes to Proteins

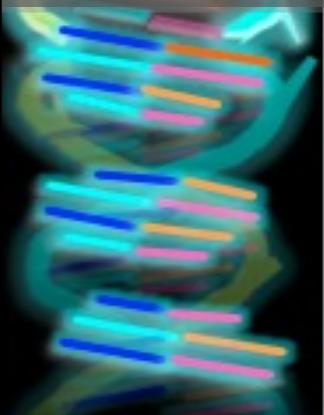
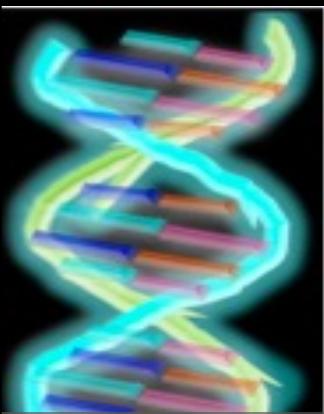


- **Alternative splicing** is a process by which exons or coding sequences of pre-mRNA produced by transcription of a gene are combined in different ways during RNA splicing.
- Resulting mature mRNA give rise to different protein products.
- Therefore, a single gene can give rise to multiple protein products.

Genes to Proteins (2)



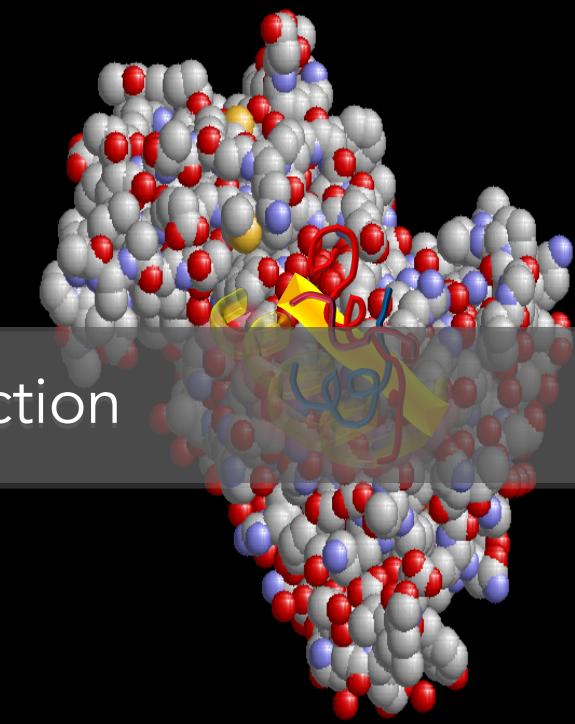
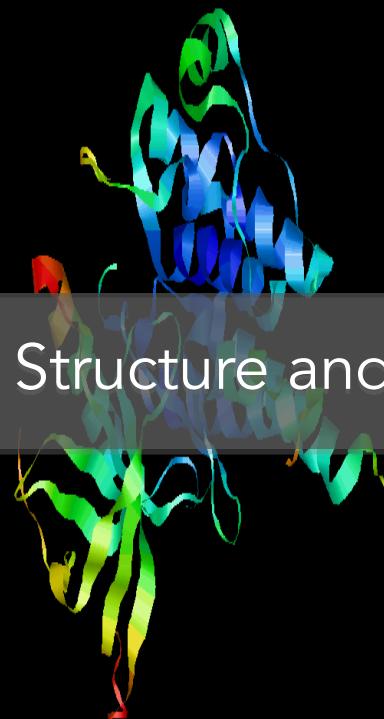
- Many proteins undergo PTM at some of their amino acid residues after synthesis process.
- Hydroxylation, methylation, alkylation, acylation are commonly observed modifications.



T
A
T
A

C
A
G

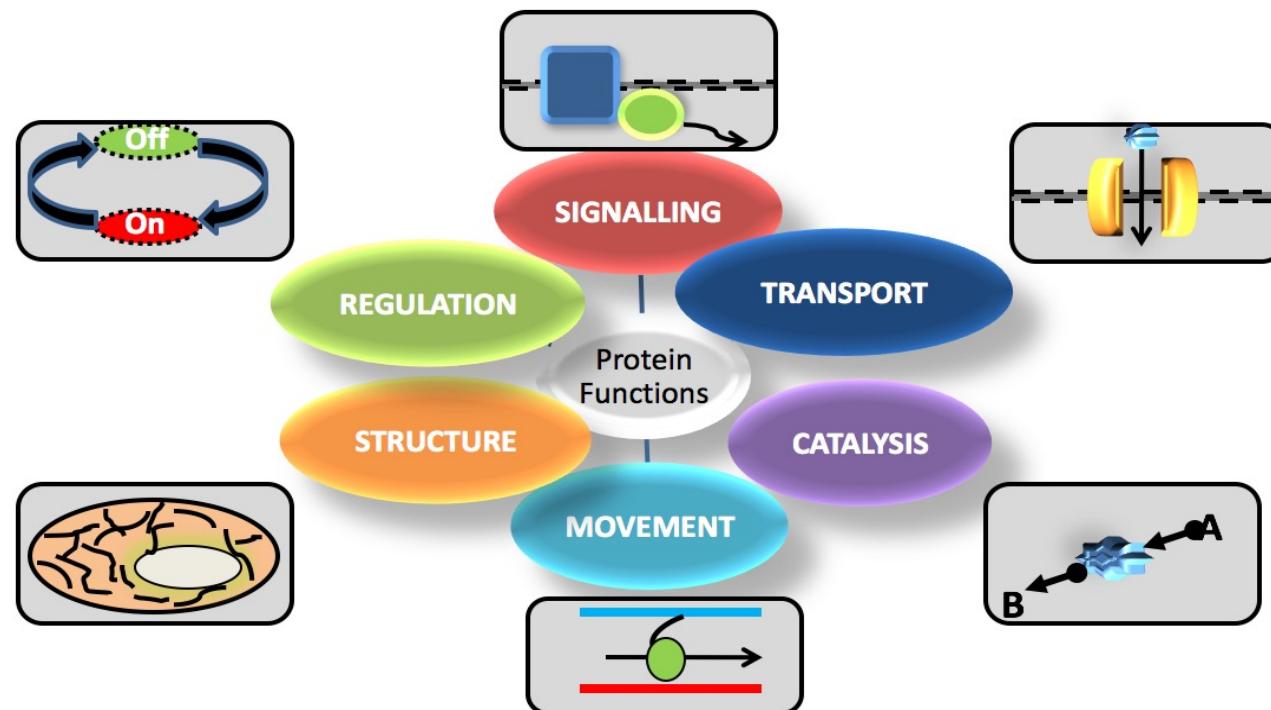
C
G
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Basics of Protein Structure and Function

Proteins

- Linear polymers built of monomers (amino acids)
- Wide range of functional groups accounts for various protein function

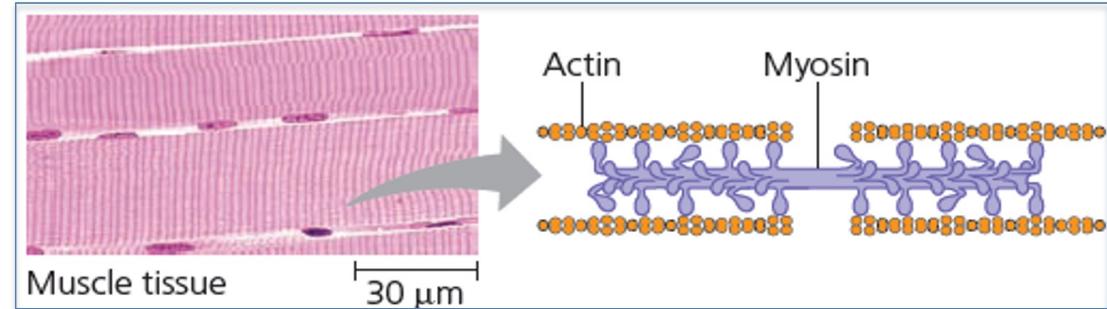


Understanding Protein Function is Key to Biology (1)

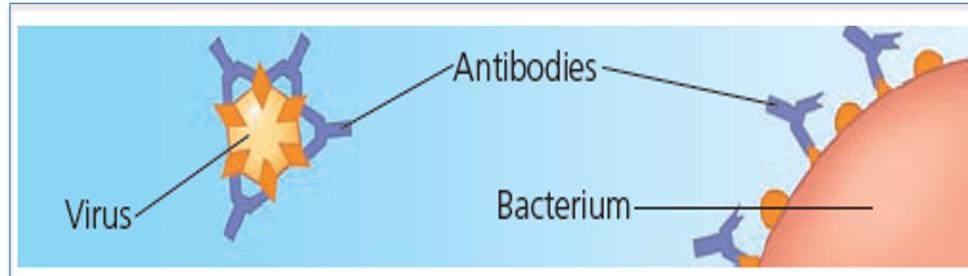
Enzymatic Proteins



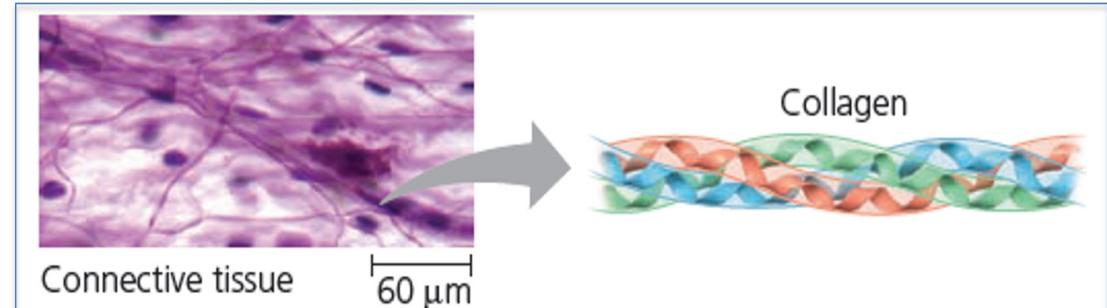
Contractile and Motor Proteins



Defensive Proteins

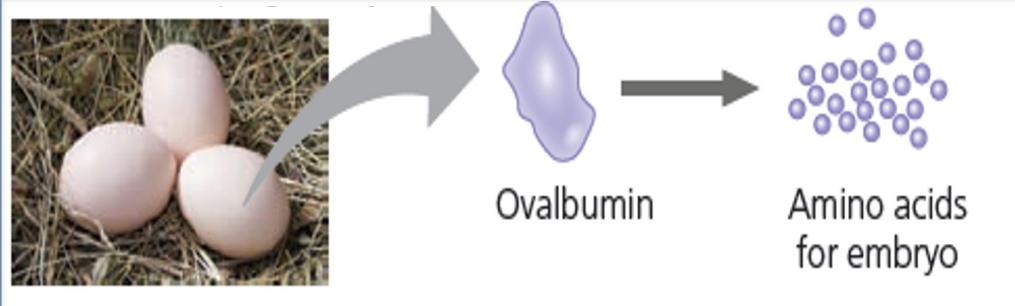


Structural Proteins

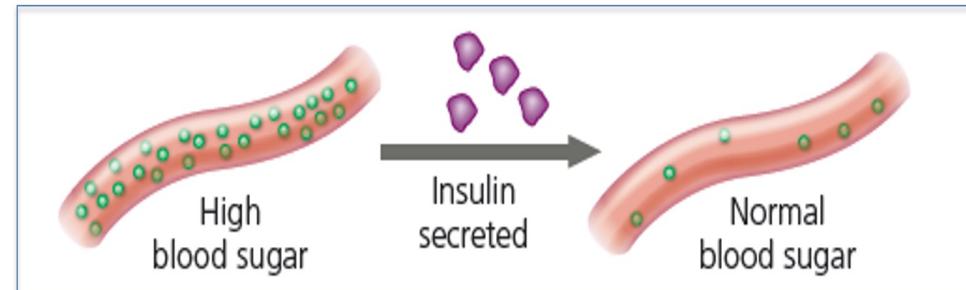


Understanding Protein Function is Key to Biology (2)

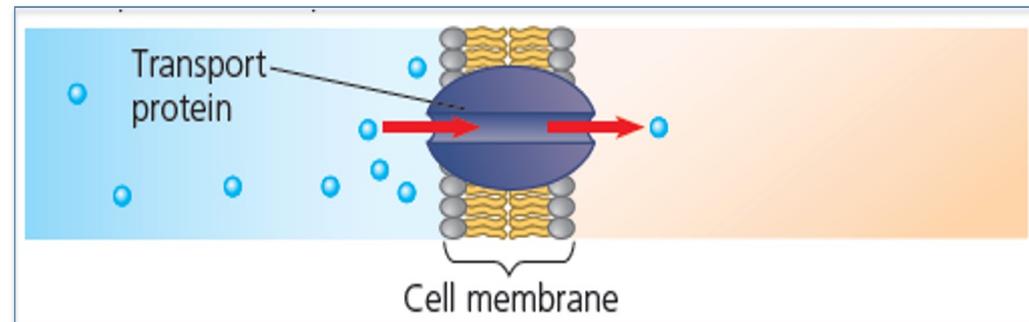
Storage Proteins



Hormonal Proteins



Transport Proteins



Receptor Proteins

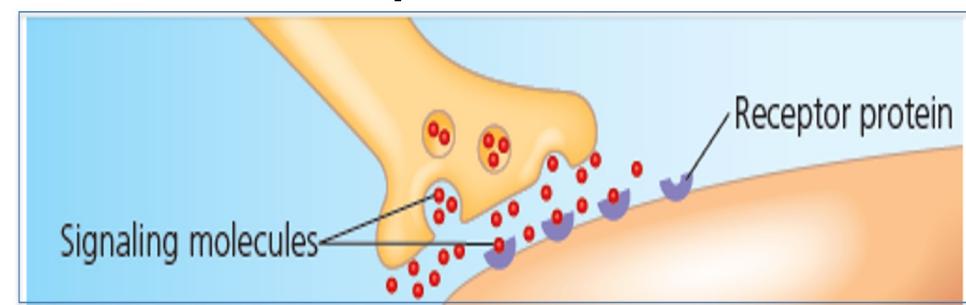
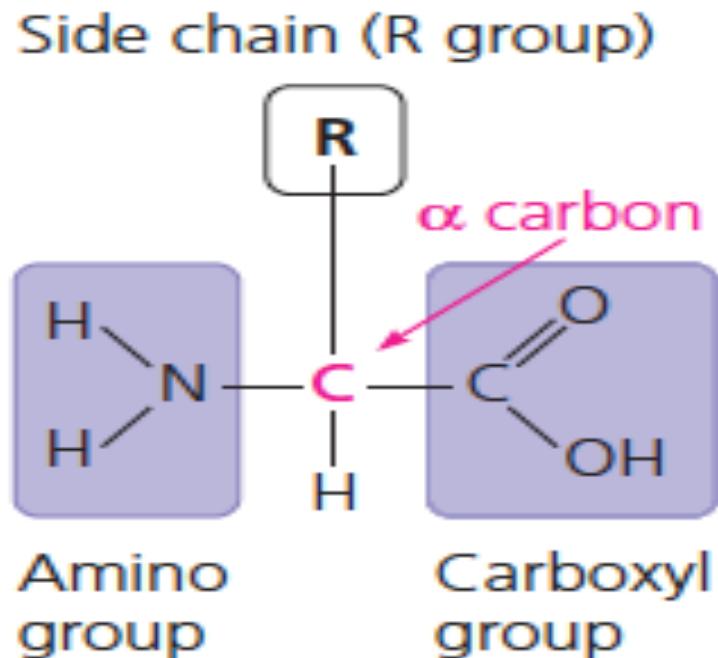


Figure 5.13

Amino Acids: Building Blocks of Proteins

- Proteins are assembled using 20 amino acids
- Peptide bonds link amino acids to form polypeptide proteins



Peptide Bond and Polypeptide Chain

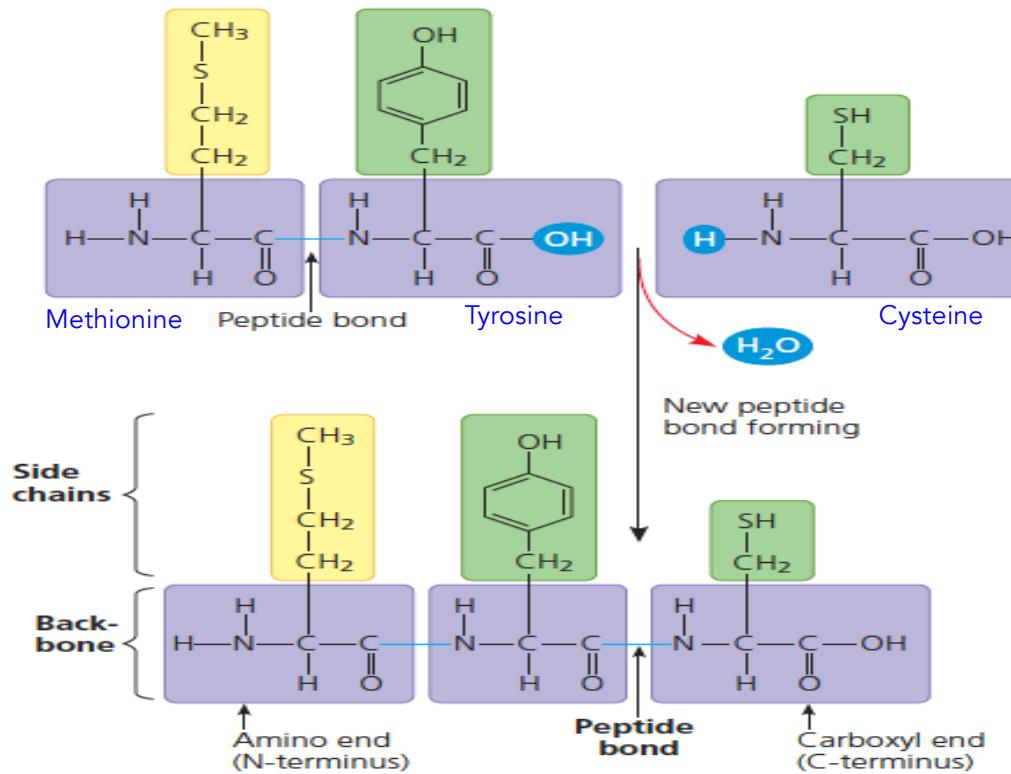
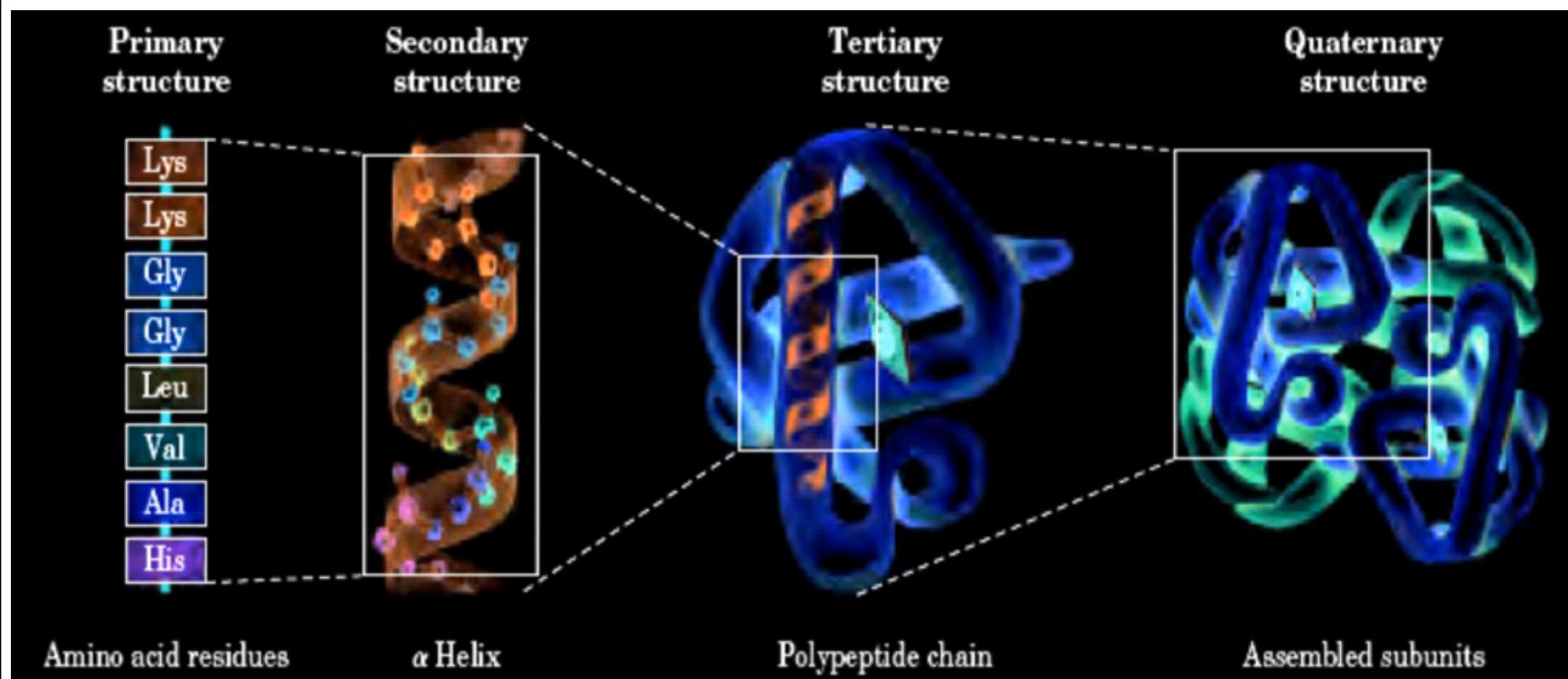


Figure 5.15

- Peptide bond link amino acids to form polypeptide protein
 - α -carboxylic group of one amino acid linked to α -amino group of another amino acid
 - peptide bond formation accompanies loss of water

Different Levels of Protein Structure



Primary Structure of Proteins

- Linear sequence of amino acids constitutes primary structure

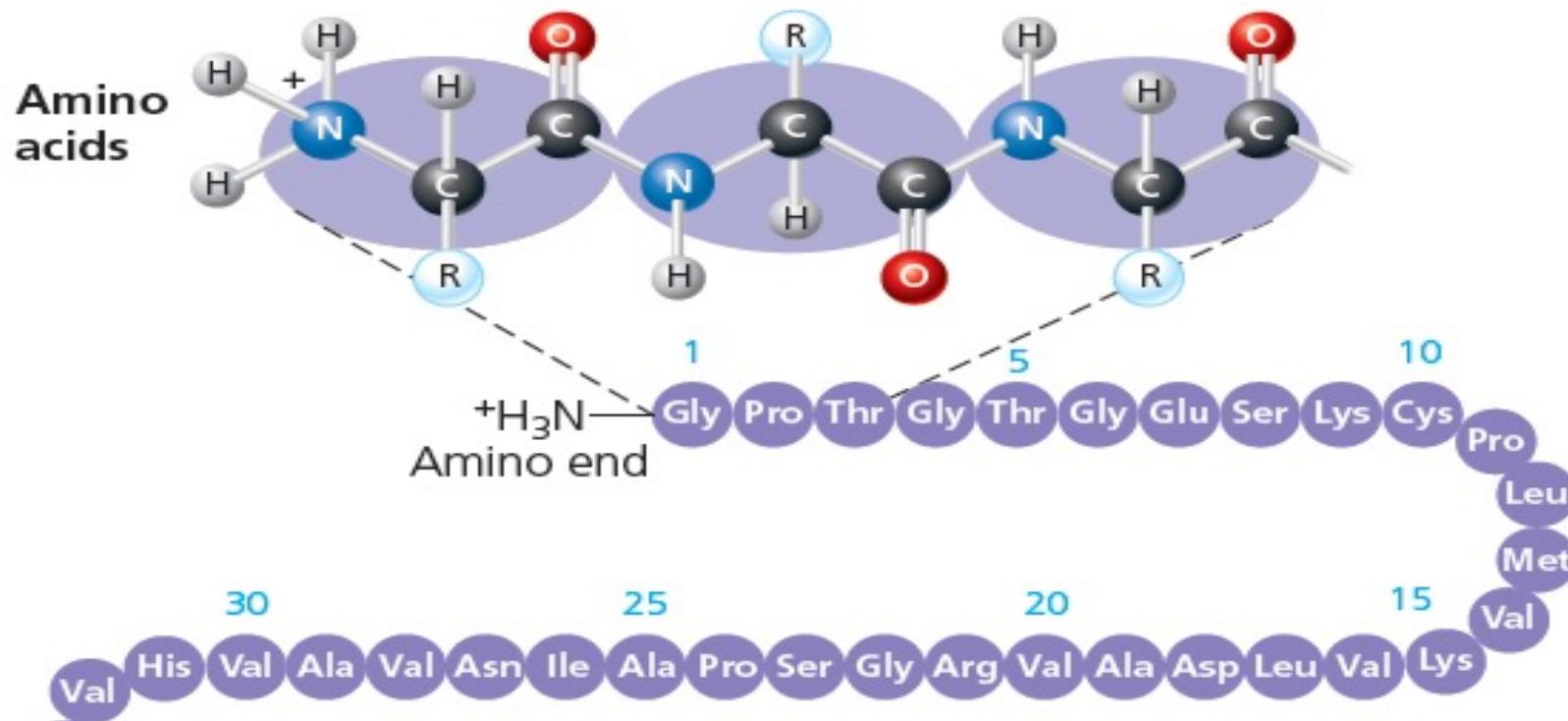


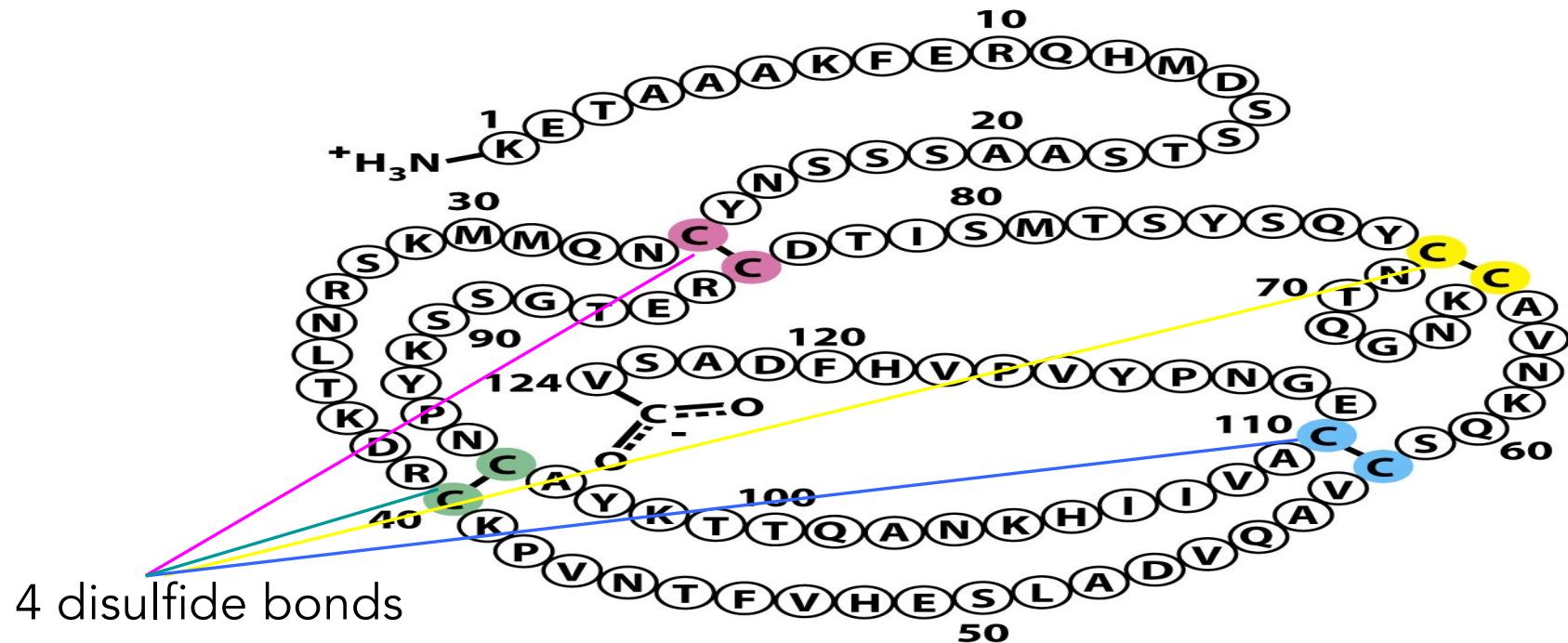
Figure 5.18

Sequence structure relationship

- Amino acid sequence dictates the conformation adopted by polypeptide chain at secondary & tertiary levels
 - Anfinsen's experiment
 1. Denaturation
 2. Refolding

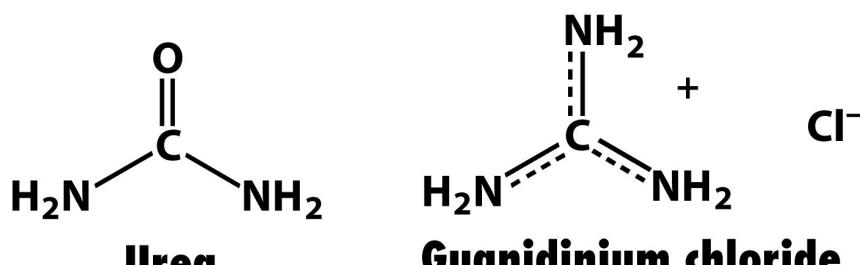
Amino acid sequence of bovine Ribonuclease

- Christian Anfinsen (1950s) used Ribonuclease
 - 124 amino acids, 4 disulfide bonds



1. Denaturation of ribonuclease

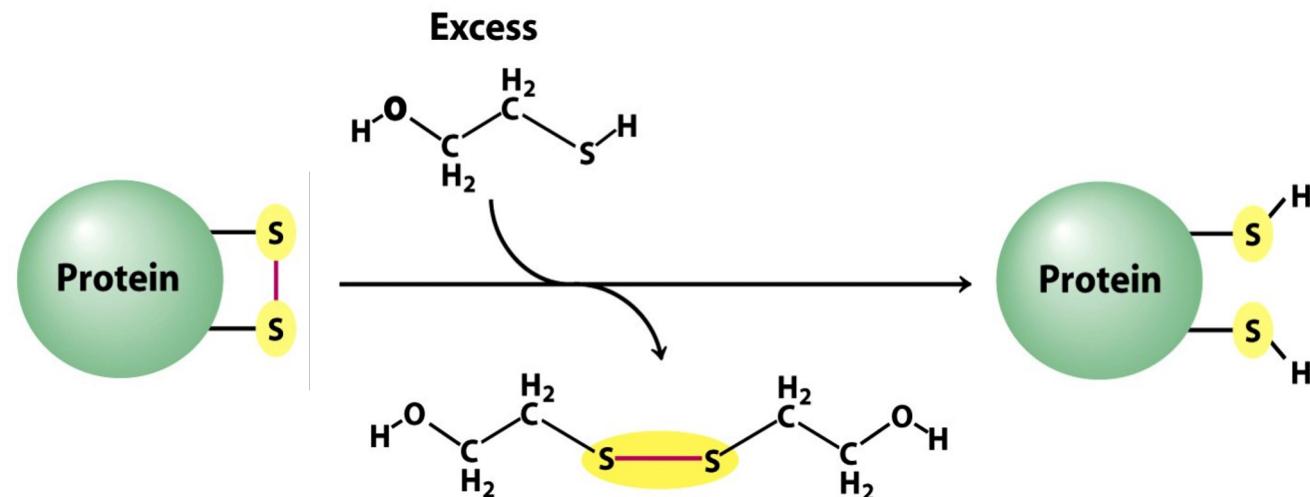
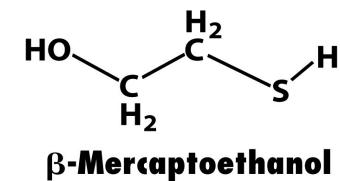
- Used urea, guanidine HCl
 - denaturants
- β -mercaptoethanol
 - breaks disulfide bonds
- these chemicals denatured ribonuclease



Effectively disrupts non-covalent bonds of proteins

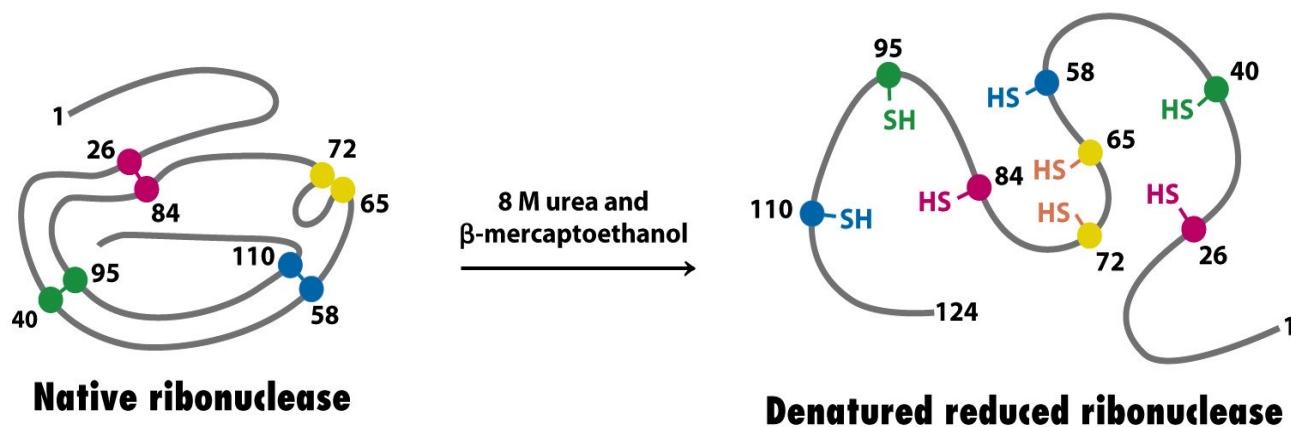
Role of β – mercaptoethanol

- Cleavage of disulfide bonds
 - Large excess converts disulfides to sulfhydryls



Reduction and denaturation of ribonuclease

- 8 M urea & β -mercaptoethanol treatment
 - converted native protein to fully reduced, randomly coiled polypeptide “denatured”
 - lacked enzymatic activity



2. Re-establishment of correct folding

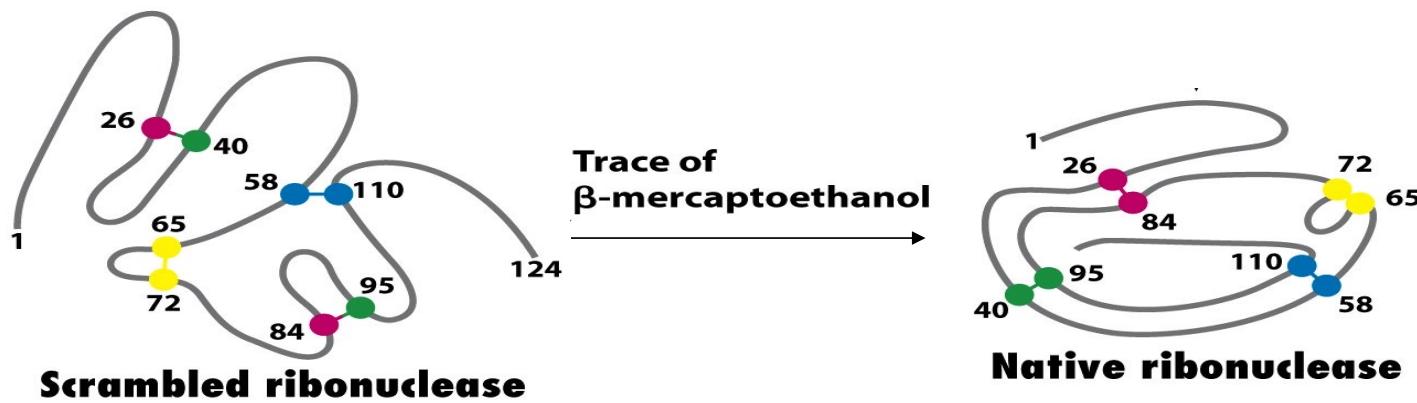
- Urea and β -mercaptoethanol removed by dialysis
- Denatured ribonuclease regained activity
 - Enzyme refolded into active form
 - Sulfhydryl groups became oxidized by air

Information required for specific catalytically active structure of ribonuclease is contained in its amino acid sequence

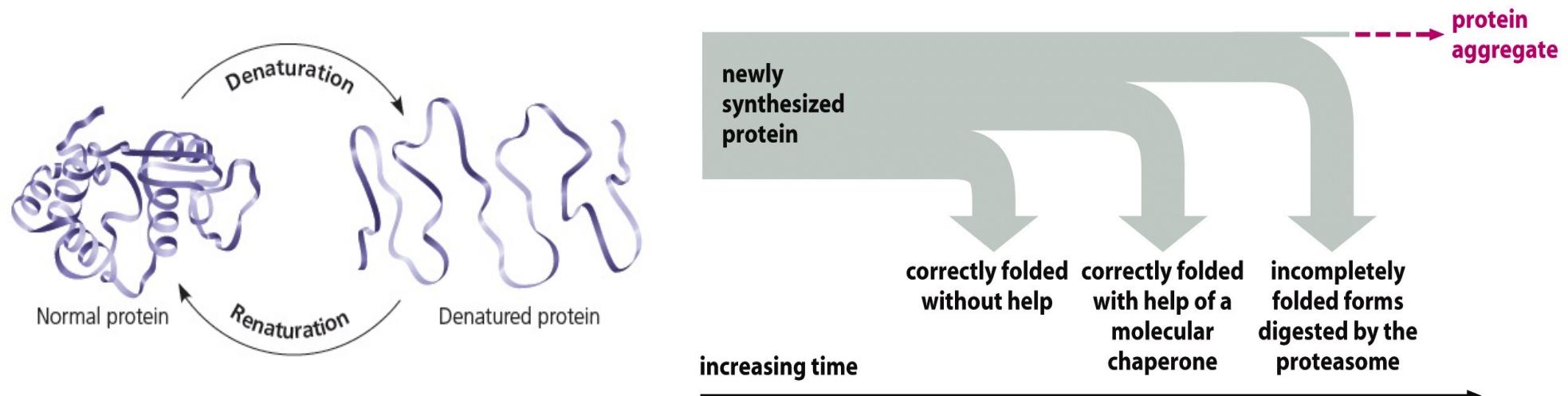
Re-establishment of correct folding

- Trace amount of β -mercaptoethanol addition helped to convert scrambled ribonuclease to active form
 - Scrambled – wrong pairings, 104
 - Trace amount of β -mercaptoethanol catalyzed rearrangement of disulfide pairing

Re-establishment of correct folding



Denaturation/ Renaturation of a Protein & Protein Misfolding

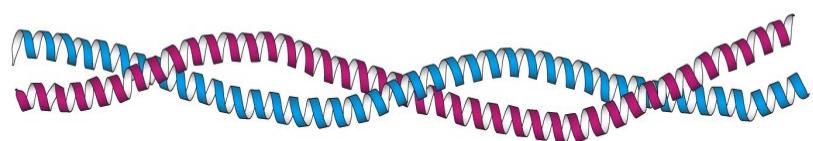


- Protein folds into a single, energetically favorable conformation, specified by its amino acid sequence
- A protein may fold into alternative 3D structure due to mutations, inappropriate covalent modifications
 - Misfolding leads to loss of normal protein function

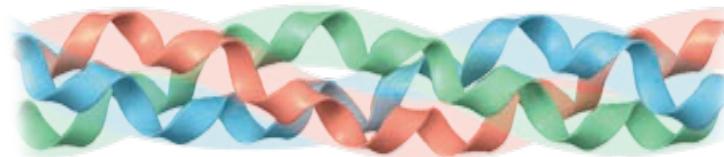
Figure 5.20

Secondary Structures

- Refers to the locally folded regions
- Folding of polypeptide/protein chain into regular structures like α -helices, β -sheets, turns and loops
- Conformation of polypeptide chain could be predicted if properties of its components are precisely known



Keratin



Collagen – triple helix

Tertiary Structures

- Refers to overall folded structure
- Three dimensional compactly folded structure of proteins
- Overall organization of secondary structural elements in 3-D space
- Numerous interactions stabilize tertiary structure of proteins

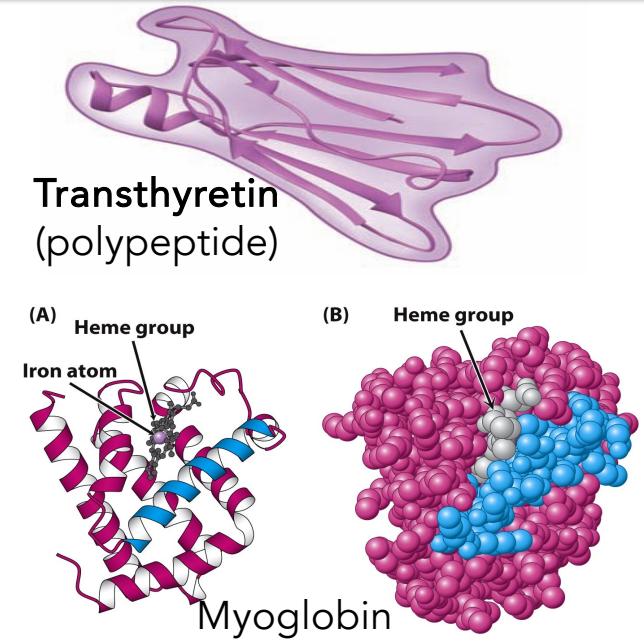
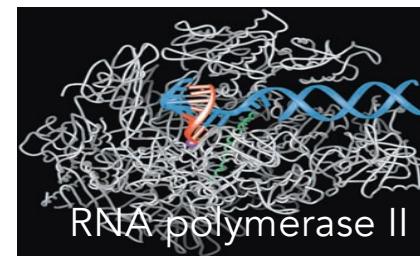
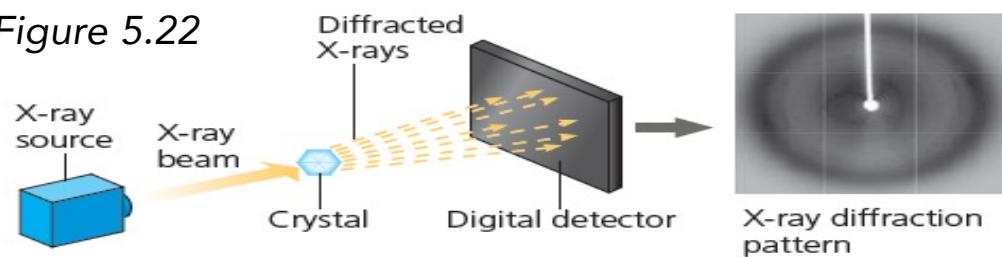


Figure 5.22



NMR and X-ray crystallography provided detailed 3D structures

Quaternary Structures

- Refers to interaction between individual protein subunits in a multi-subunit complex
- Final level of protein structure
- Spatial arrangement of subunits and their interactions
- Polypeptide chains assemble to form multi-subunit structure

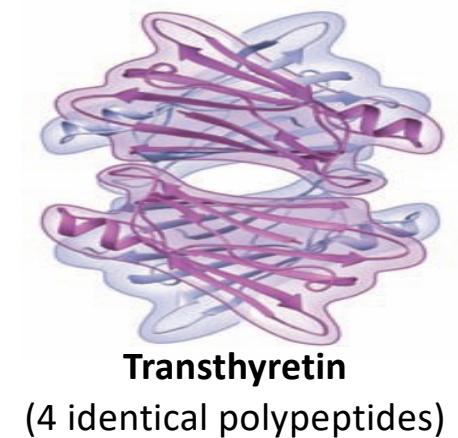
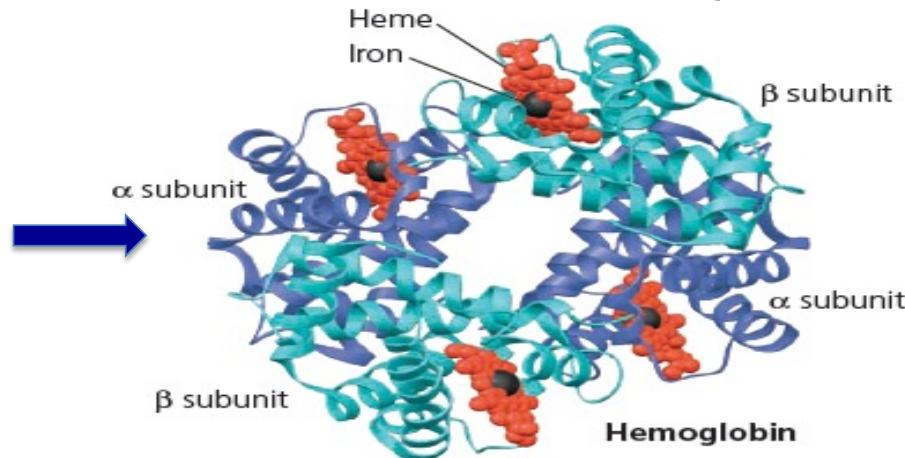
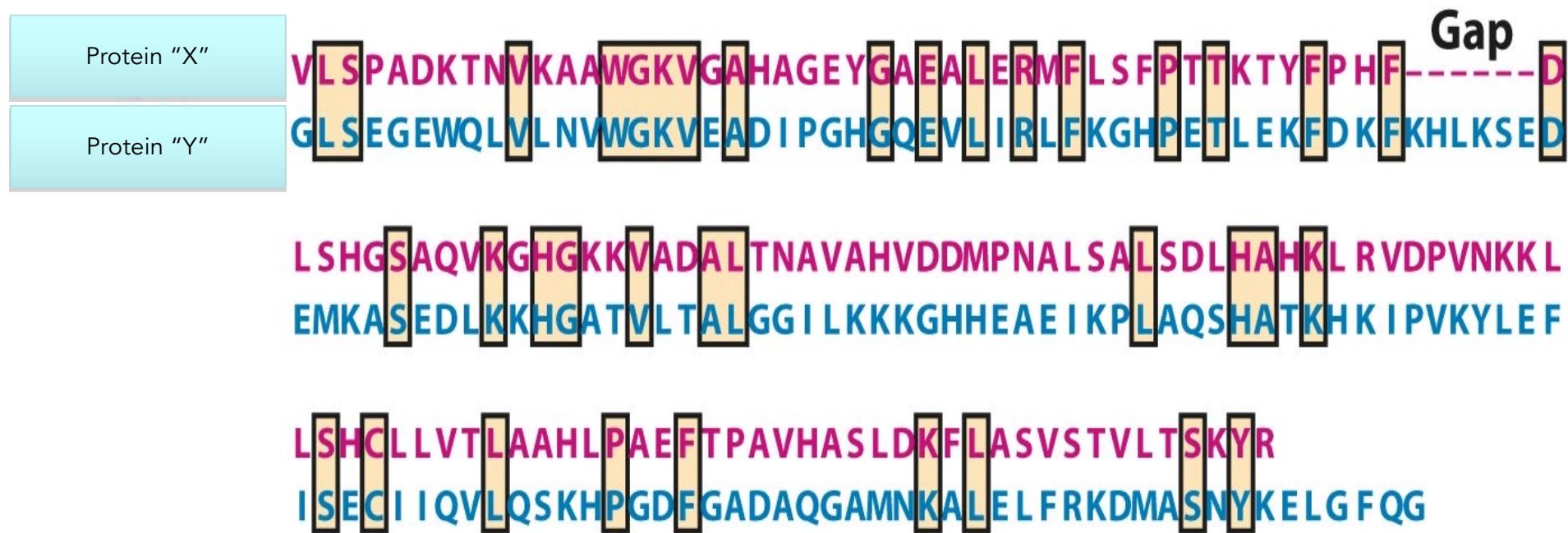


Figure 5.18

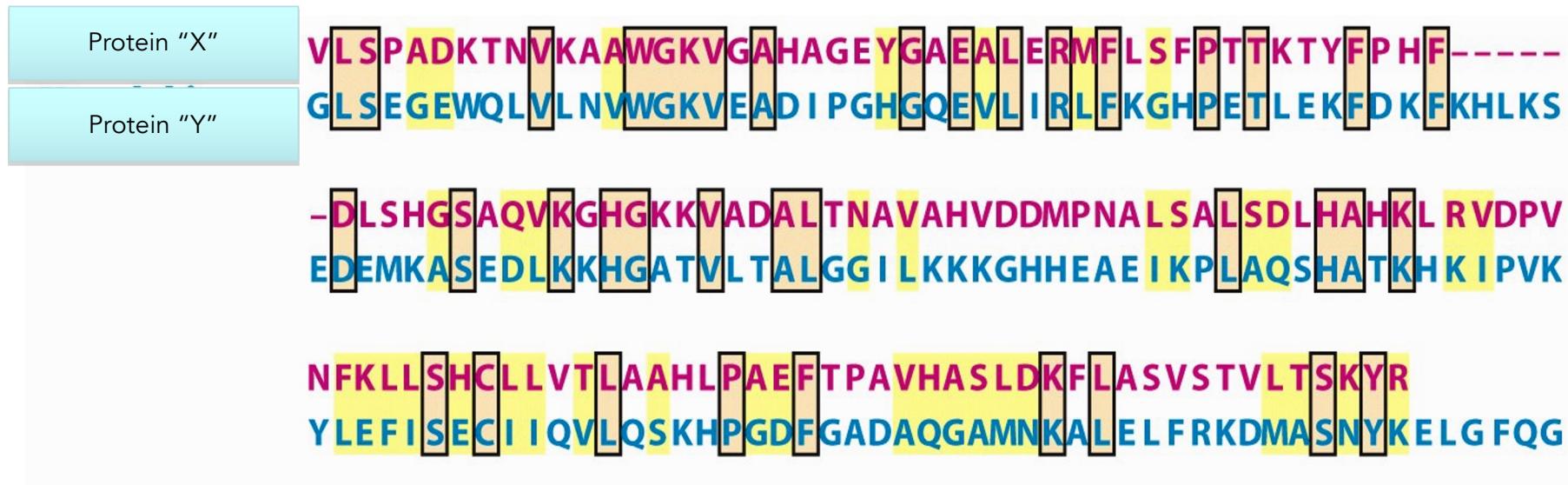
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Sequence alignment



- Sequence alignment of protein "X" & protein "Y"
 - gaps indicate insertion or deletion of nucleotides

Conservative substitutions



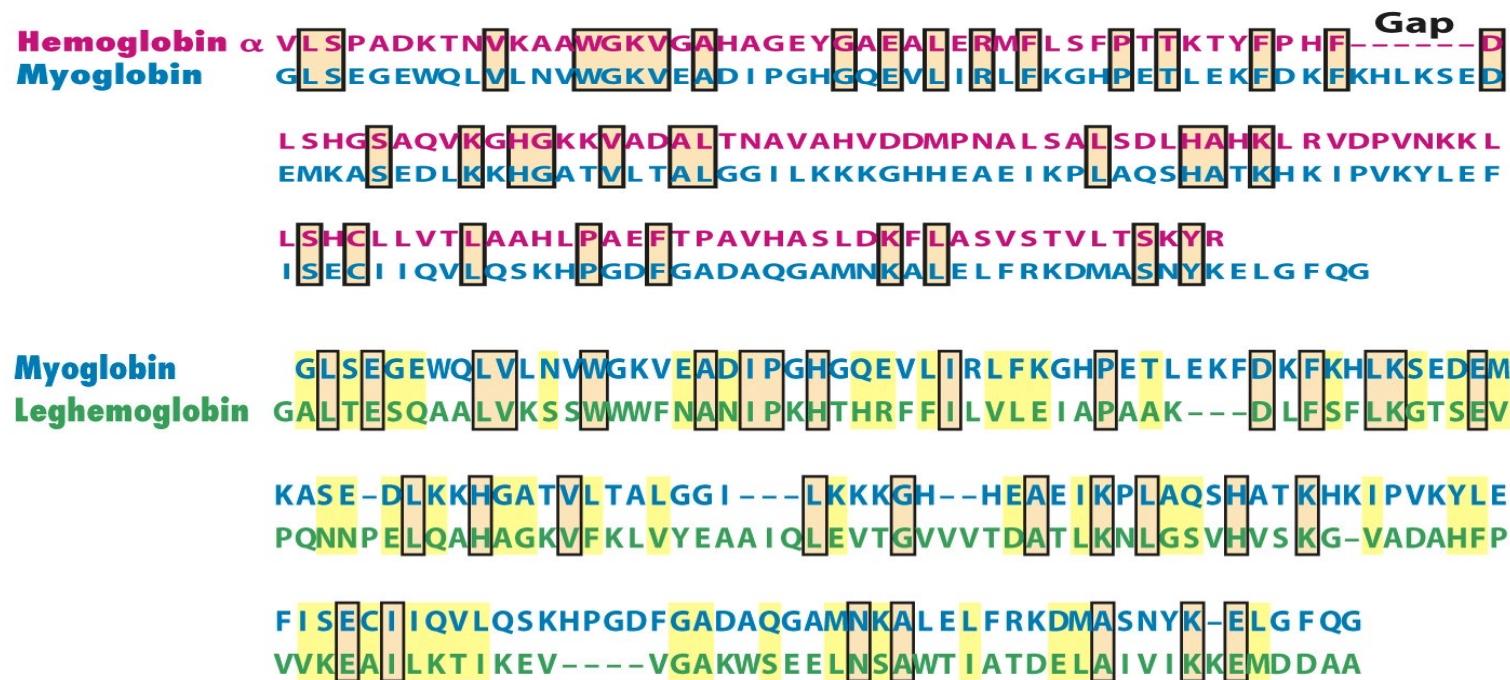
- Conservative amino acid substitutions - replacement of one amino acid with another
 - Similar in size and chemical properties
 - minor effect on protein structure and function

Sequence alignment



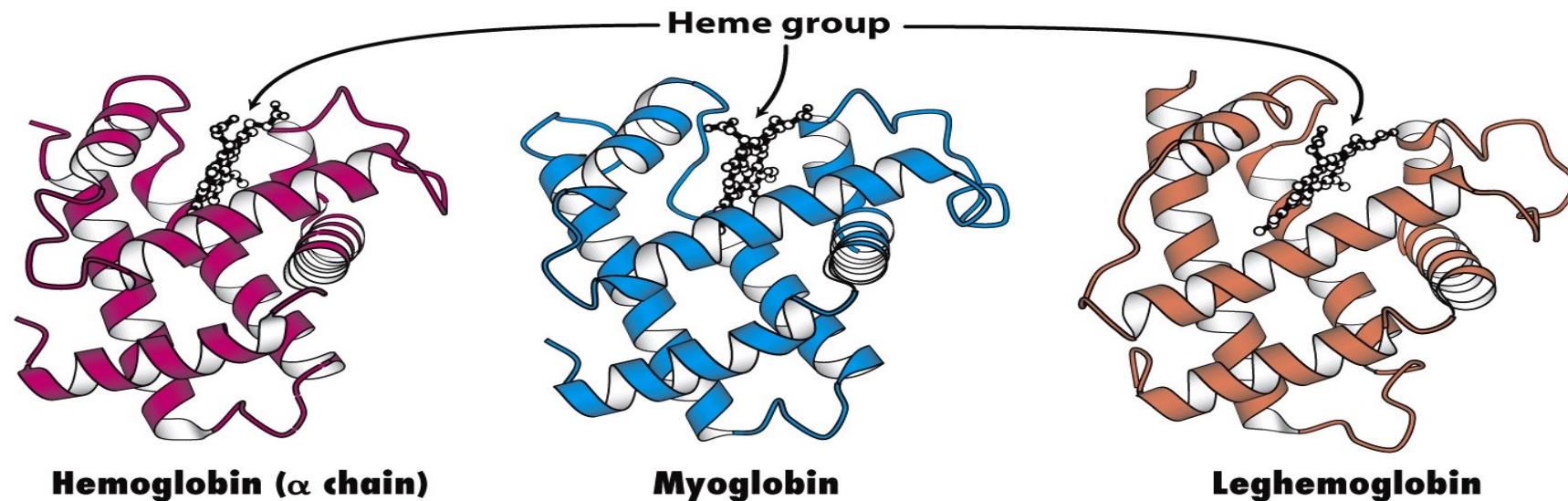
- Sequence alignment of α – hemoglobin & myoglobin
 - reveals a gap in hemoglobin sequence
 - gaps indicate insertion or deletion of nucleotides

Globins: primary sequence alignment

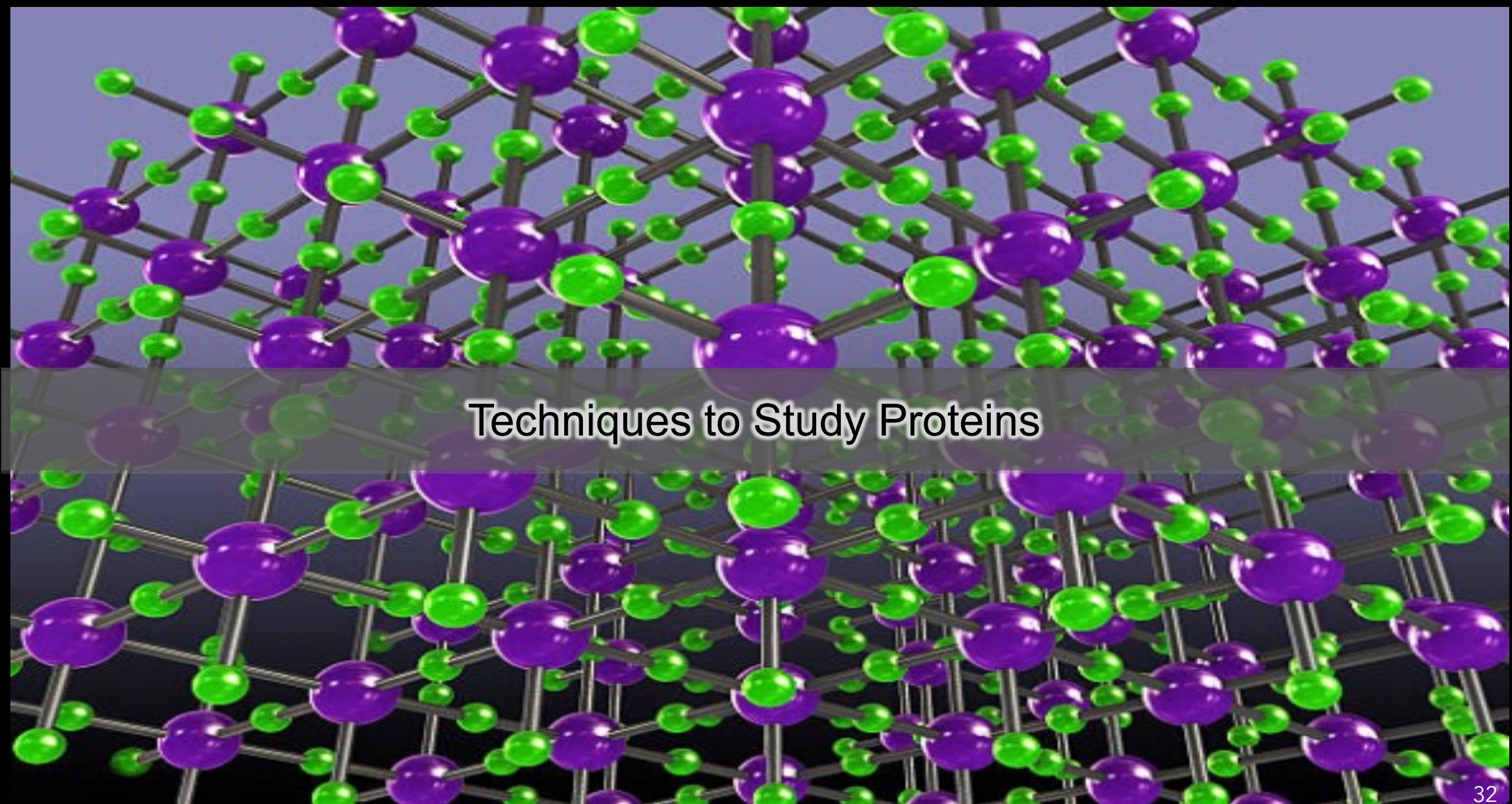


- Similarity between myoglobin & hemoglobin is high
- Similarity between myoglobin & lupine leghemoglobin is less
- Similarity between lupine leghemoglobin & hemoglobin is statistically insignificant

Tertiary structures evolutionary more conserved



- Tertiary structures are evolutionary highly conserved
 - more than primary sequence structure
- 3D structure reveals more functional association
 - evolutionarily, all of these proteins bind heme group & facilitates reversible binding of oxygen

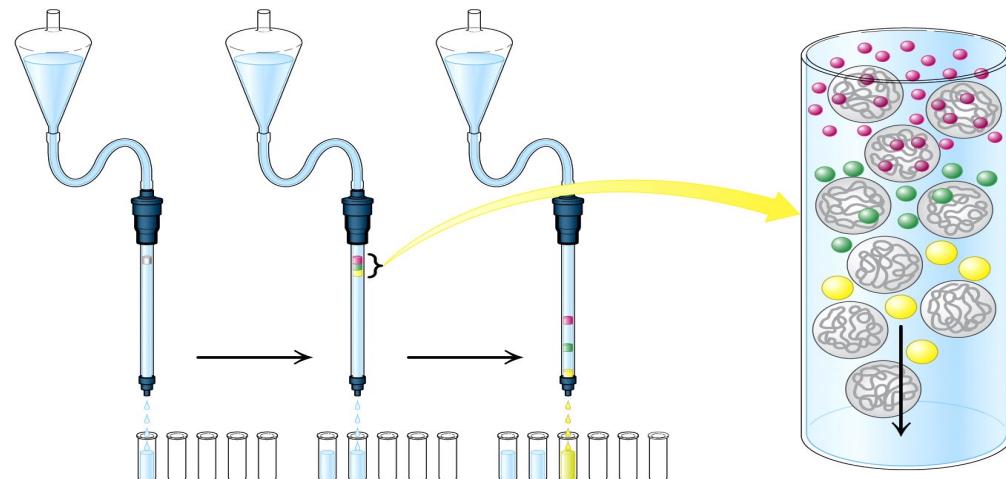


Techniques to Study Proteins

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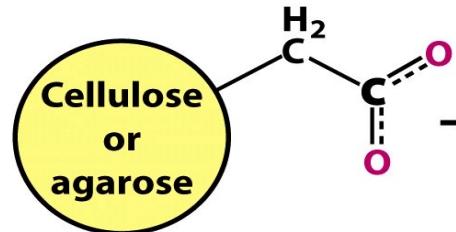
Gel Filtration Chromatography

- Gel filtration column composed of porous beads
 - made from polyacrylamide, dextran or agarose
- Size exclusion chromatography
 - proteins are separated according to their size
 - small molecules (including salt): retained longer
 - larger protein molecules elute first

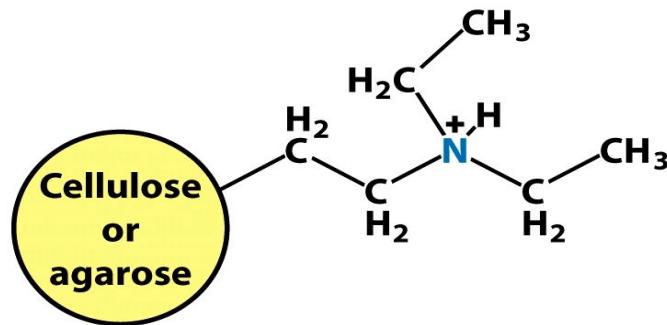


Ion Exchange Chromatography

- Proteins are separated based on charge difference
- Proteins with overall negative charges will interact with positive charges or vice versa
 - varying amounts of positive/negative amino acids
 - pH influences net charge on proteins



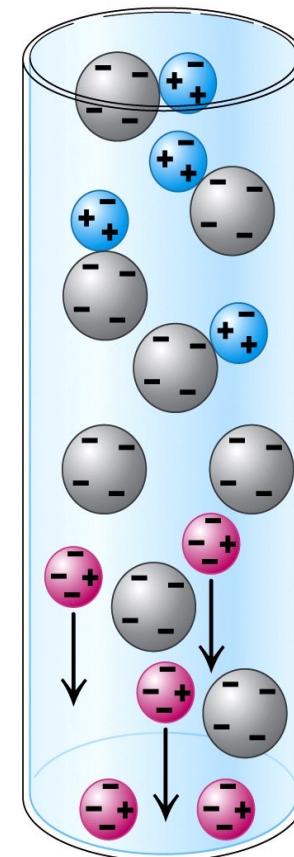
**Carboxymethyl
(CM) group
(ionized form)**



**Diethylaminoethyl
(DEAE) group
(protonated form)**

Ion Exchange Chromatography

- Proteins “adsorbed” to ion-exchange column
- Desorbed: increasing salt
 - Na^+ and Cl^- ions compete
- Desorption: altering the pH of buffer
 - changes charge on protein

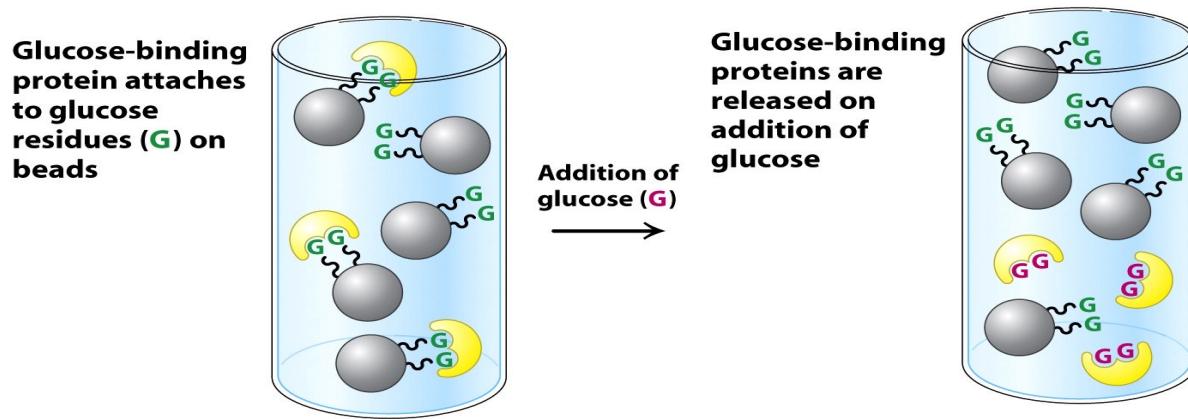


Positively charged protein binds to negatively charged bead

Negatively charged protein flows through

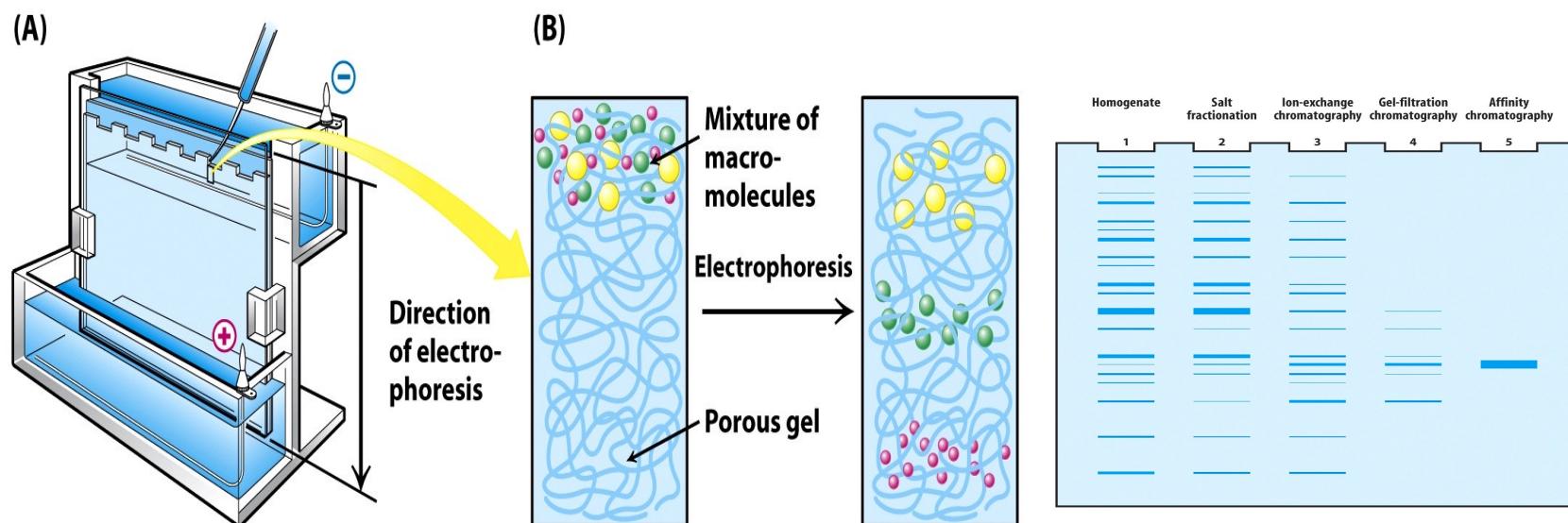
Affinity Chromatography

- Based on affinity of protein to other molecules
 - substrates, products, cofactors, antibodies, metal
- Matrix beads are chemically coupled to ligand
- Protein binds through a specific interaction
 - all other proteins do not bind
- Protein desorbed by excess ligand in solution

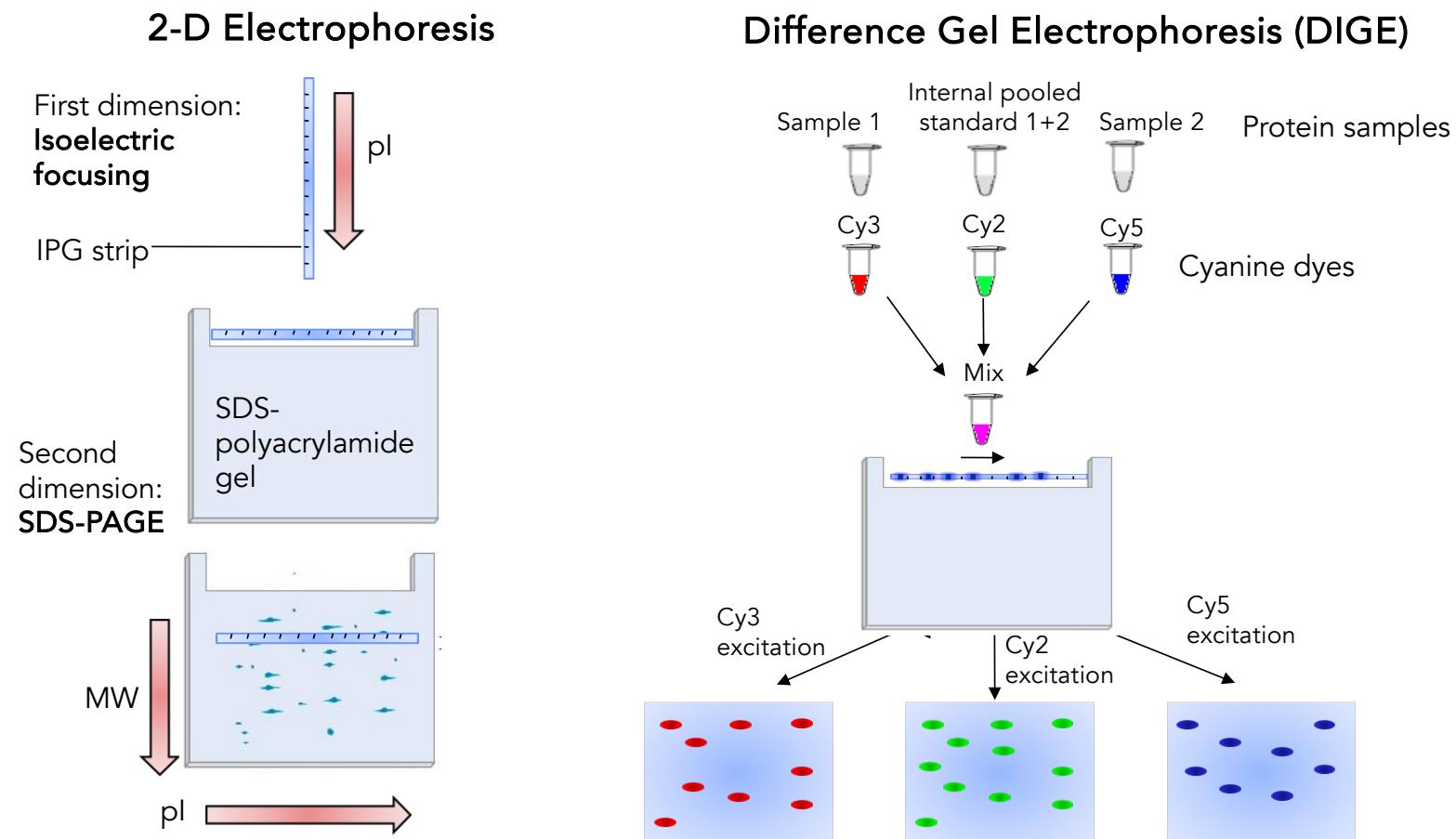


SDS-PAGE Gel Electrophoresis

- Widely used electrophoretic technique
- Sodium dodecyl sulfate (SDS) is negatively charged
- Boil proteins in SDS and thiol agent (β -ME)
 - Denatures proteins, breaks S-S bonds

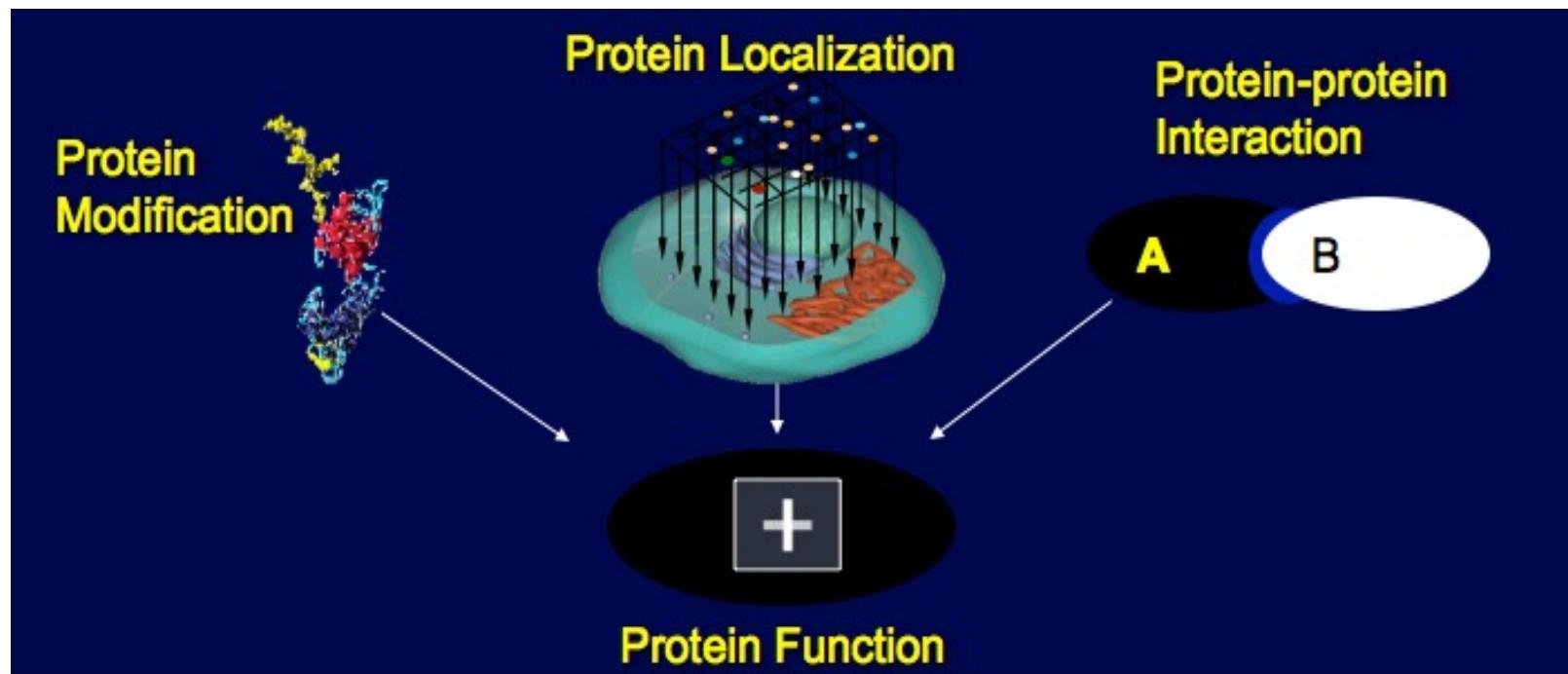


Two Dimensional Electrophoresis

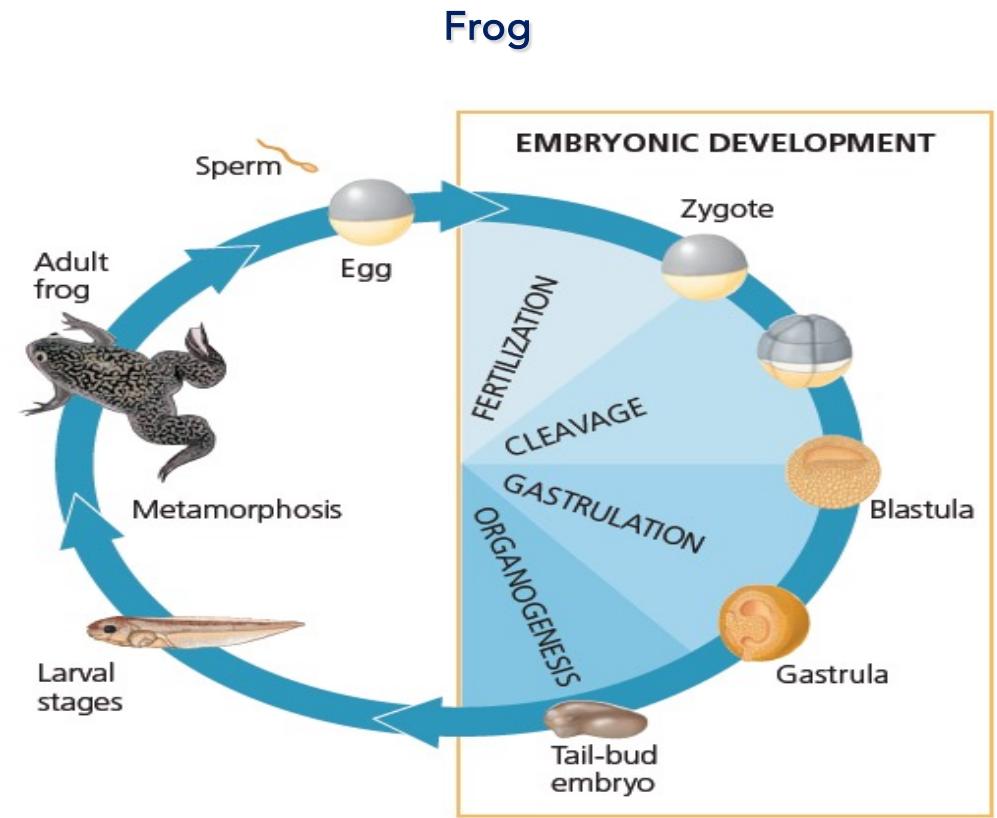
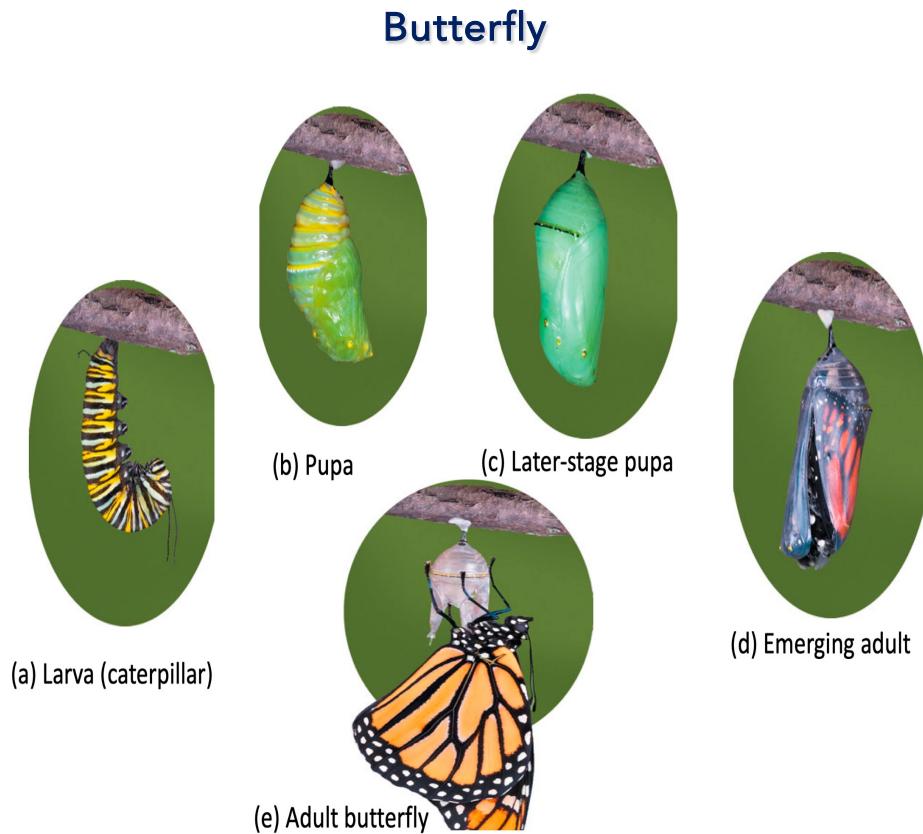


Proteomics

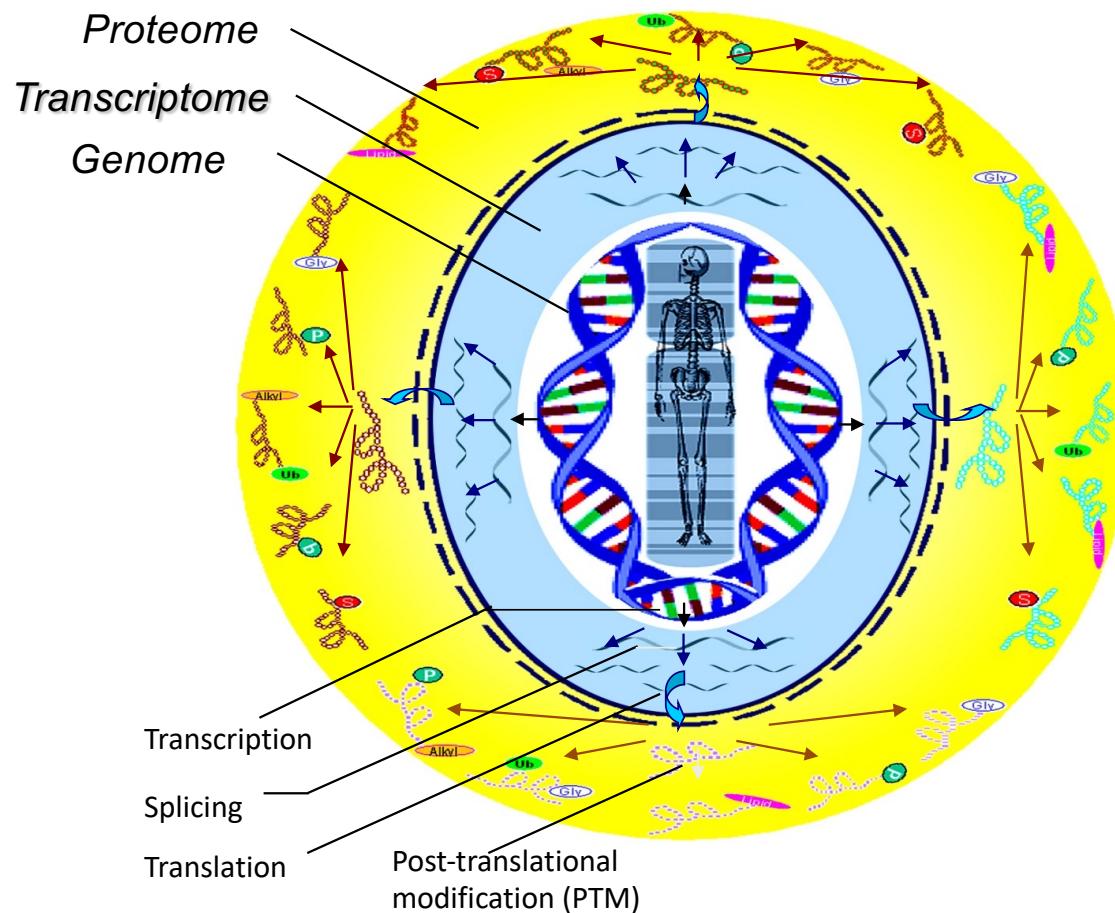
- Proteome: set of all the proteins expressed by a genome
- Proteomics: study of proteins and their properties to provide an integrated view of cellular processes



Genomics vs. Proteomics



Complexity of Human Proteome



- Number of genes ~20,500,
- No clear estimate of number of proteins (1- several million)

Draft Human Proteome Maps



Mass spectrometry-based approaches used to decipher human proteome

Human Proteome Map



A high-stringency blueprint of the human proteome



Summary

- Understanding protein function is vital in biology
- Proteins are composed of amino acids, serving as their building blocks
- Protein structure is hierarchical: primary, secondary, tertiary, and quaternary
- Sequence alignment techniques aid in functional analysis
- Techniques to study proteins include chromatography and electrophoresis
- Chromatography methods: gel filtration, ion exchange, affinity chromatography
- Gel electrophoresis techniques: SDS-PAGE, two-dimensional electrophoresis
- Proteomics, the study of an organism's entire complement of proteins, contributes significantly to biology

References

- Campbell Biology - Reece, Urry, Cain, Wasserman, Minorsky, Jackson
10th Edition, Pearson
- Wilmut, I., et al., Nature, 385: 264-267 (1997)
- *Acknowledgment*
 - Cover images – getty images

5

The Structure and Function of Large Biological Molecules

KEY CONCEPTS

- 5.1 Macromolecules are polymers, built from monomers.
- 5.2 Carbohydrates serve as fuel and building material
- 5.3 Lipids are a diverse group of hydrophobic molecules
- 5.4 Proteins include a diversity of structures, resulting in a wide range of functions
- 5.5 Nucleic acids store, transmit, and help express hereditary information
- 5.6 Genomics and proteomics have transformed biological inquiry and applications

The Molecules of Life

Given the rich complexity of life on Earth, it might surprise you that the most important large molecules found in all living things—from bacteria to elephants—can be sorted into just four main classes: carbohydrates, lipids, proteins, and nucleic acids. On the molecular scale, members of three of these classes—carbohydrates, proteins, and nucleic acids—are relatively few, while the fourth class, lipids, includes many more molecules consisting of thousands of atoms that form a molecular colossus with a mass well over 100,000 daltons. Considering the size and complexity of macromolecules, it is noteworthy that biochemists have determined the detailed structure of so many of them. The image in Figure 5.1 is a molecular model of a protein called alcohol dehydrogenase, which breaks down alcohol in the body.

The architecture of a large biological molecule plays an essential role in its function. Like water and simple organic molecules, large biological molecules exhibit unique emergent properties arising from the orderly arrangement of their atoms. In this chapter, we'll first consider how macromolecules are built. Then we'll examine the structure and function of all four classes of large biological molecules: carbohydrates, lipids, proteins, and nucleic acids.

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Next Lecture... *Metabolism*