FDE 303
FOOD CHEMISTRY
WEEK-6

Amino Acids, Peptides and Proteins

---Continued---

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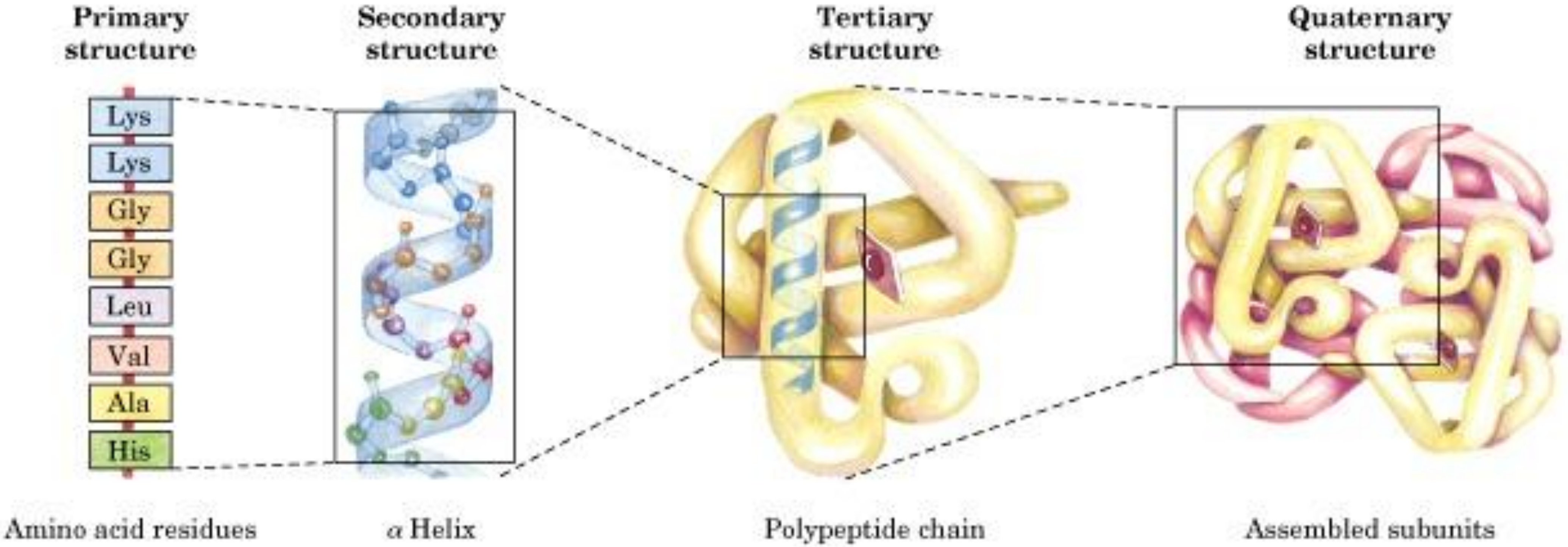
PROTEINS-structure

- The differences in structure and function arise from the sequence in which the amino acids are linked together via amide bonds.

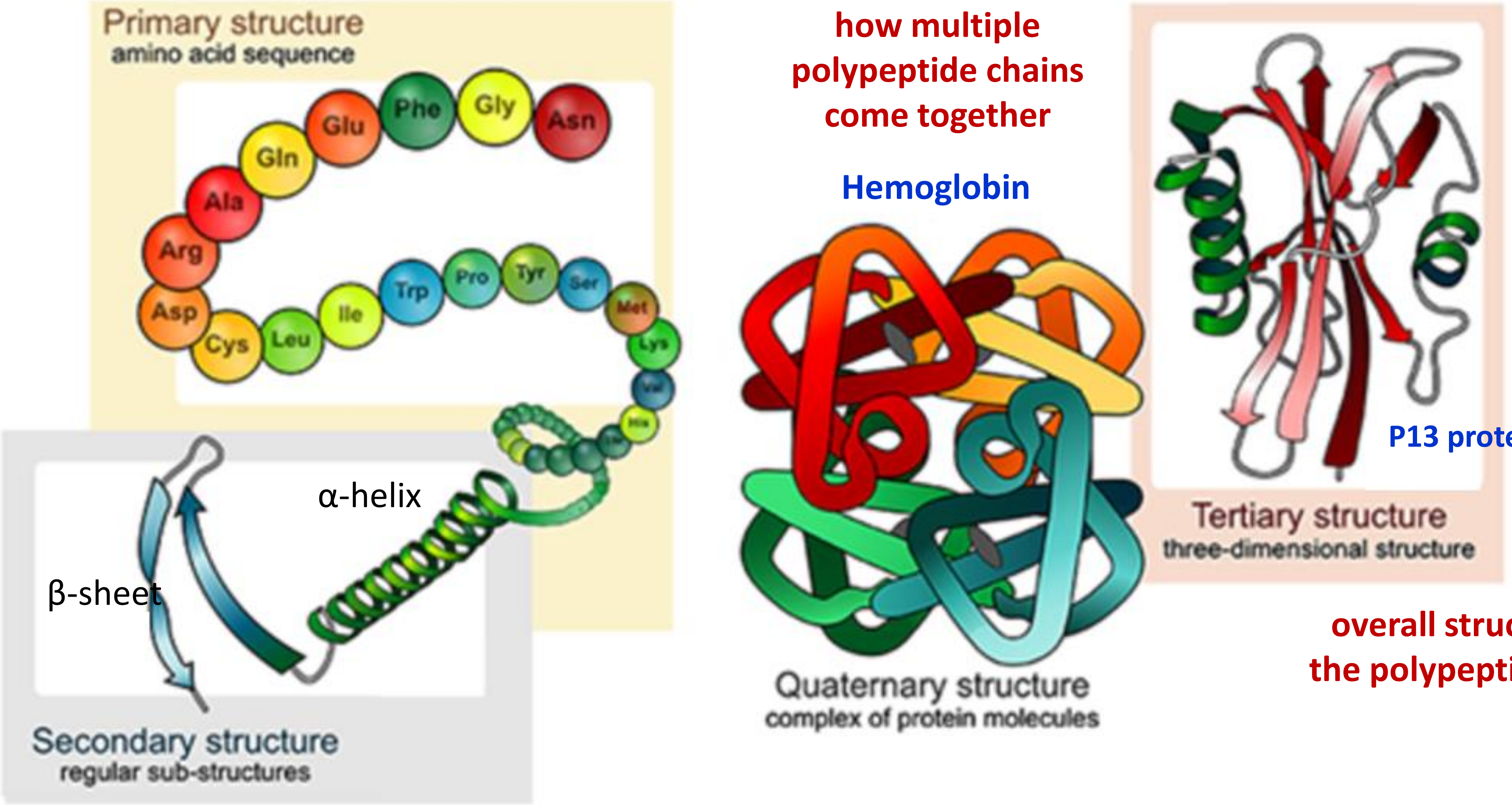


PROTEINS-structure (General)

- Primary structure: the sequence of amino acids in the polypeptide chain
- Secondary structure: structural elements found in many proteins
  - These are energetically favorable primarily because of hydrogen bonds between backbone atom
- Tertiary structure: the overall three-dimensional (3D) structure of a polypeptide chain
- Quaternary structure: the arrangement of multiple polypeptide chains in a larger protein
- Domains: Large proteins often consist of multiple compact 3D structures called domains – Many contacts with a domain. Few contacts between domains.
  - One polypeptide chain can form multiple domains

Levels of protein structure

sequence of amino acids



α-helix

β-sheet

local structural elements

how multiple polypeptide chains come together

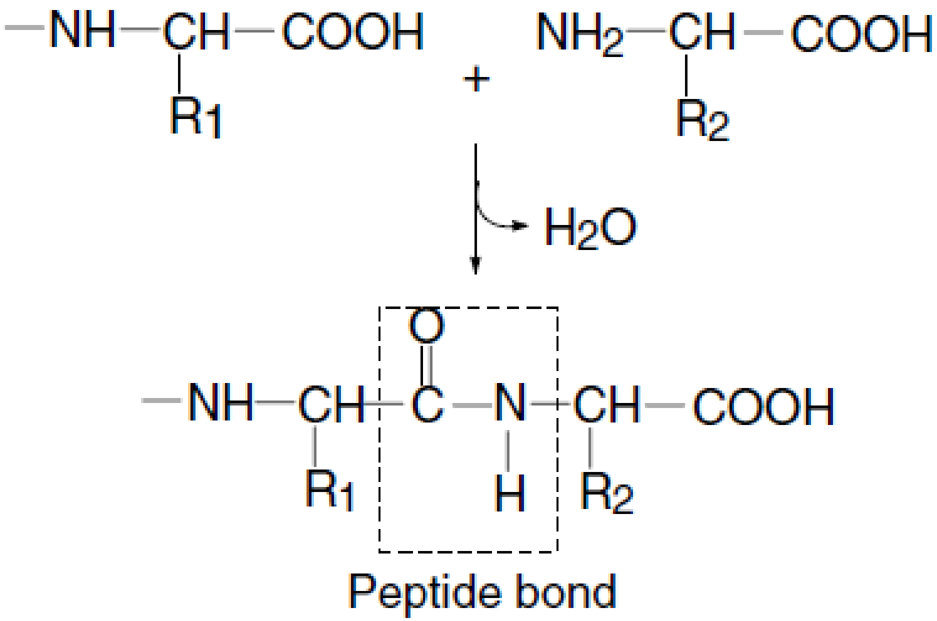
Hemoglobin

P13 protein

overall structure of the polypeptide chain

Primary structure

- Linear sequence in which the constituent amino acids are covalently linked through amide bonds, also known as peptide bonds.
- During their RNA-mediated polymerization process, the amine group of one amino acid reacts with the carboxylic acid group of another, and water is eliminated.
- The dipeptide formed still has a reactive amino and carboxylic acid group, which can continue to react to form sequentially larger polypeptides and eventually a complete protein.

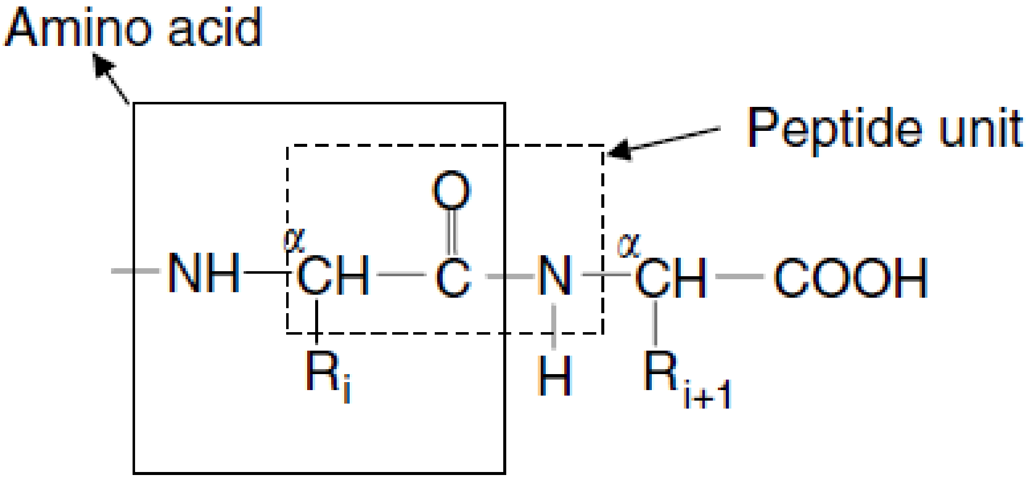


Primary structure

- The peptide linkage results from condensation of the α-carboxyl group of *i*th amino acid and the α-amino group of *i*+1th amino acid with removal of a water molecule.

- In this linear sequence, all the amino acid residues are in the L-configuration.

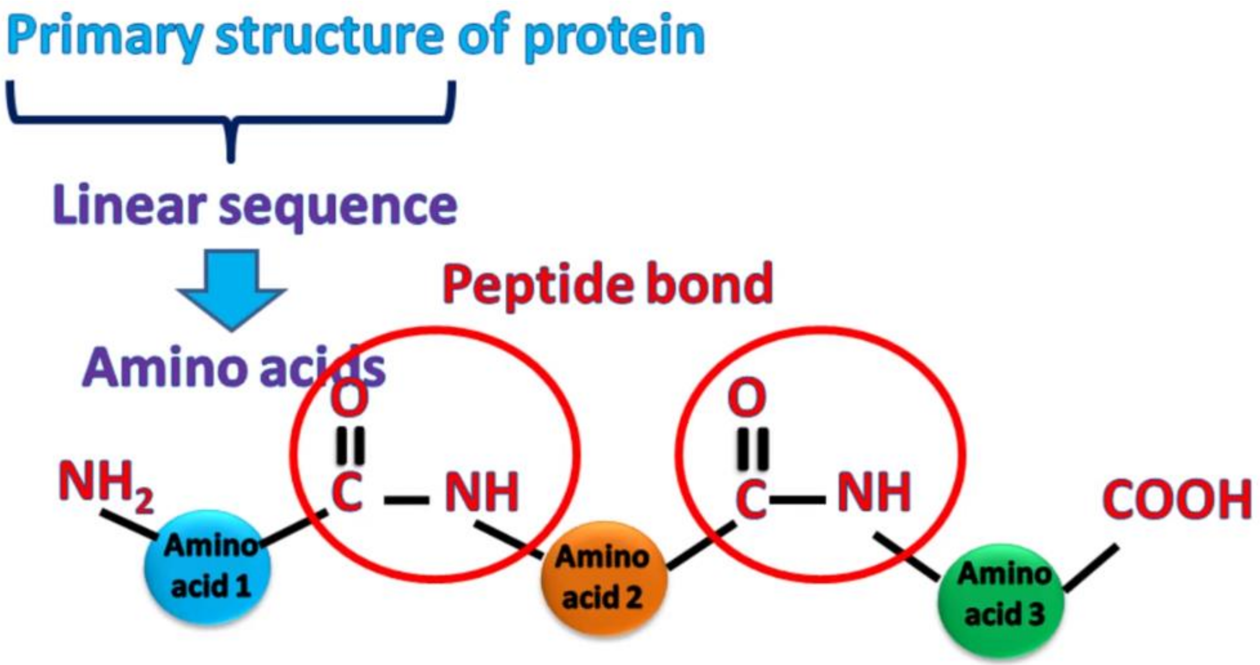
- A protein with *n* amino acid residues contains *n* – 1 peptide linkages.

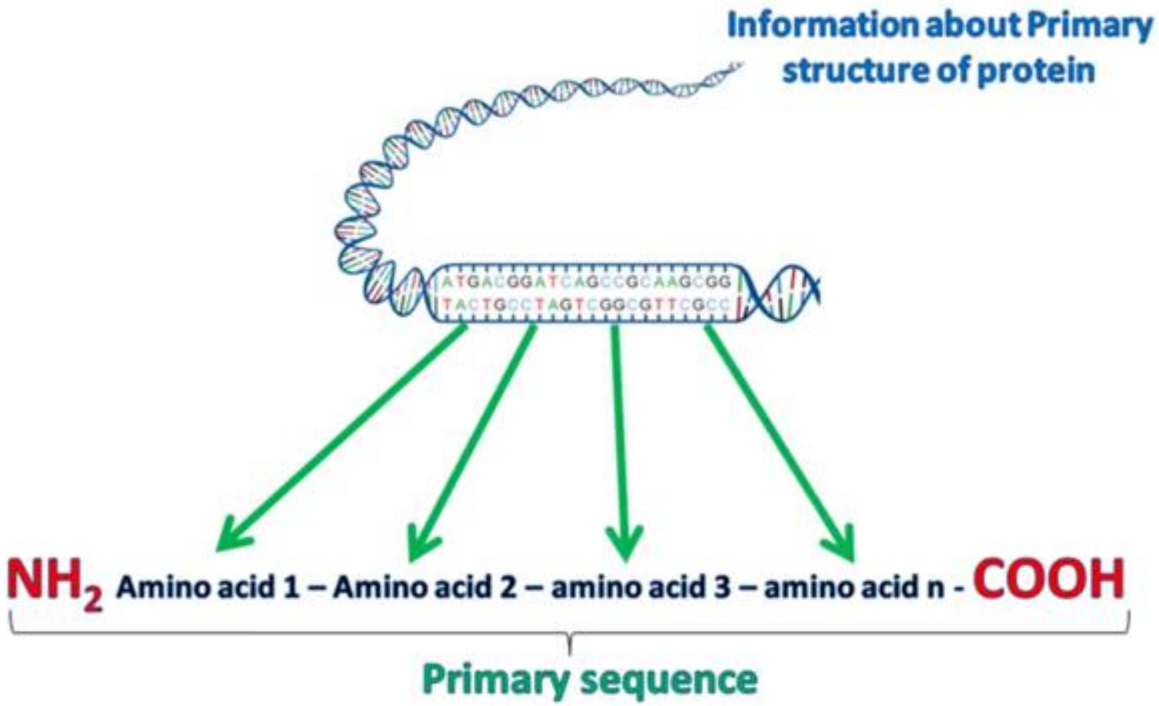


Primary structure depends on:

- Amino acid type
- Amino acid number
- Amino acid sequence
- Amino acid group bonding

Primary structure





Every protein has a unique primary structure that is coded in its genetic code and leads to a specific biological function

Secondary structure

- Secondary structure refers to the periodic spatial arrangement of amino acid residues at certain segments of the polypeptide chain.

- Formed by hydrogen-bond interactions of adjacent amino acids.

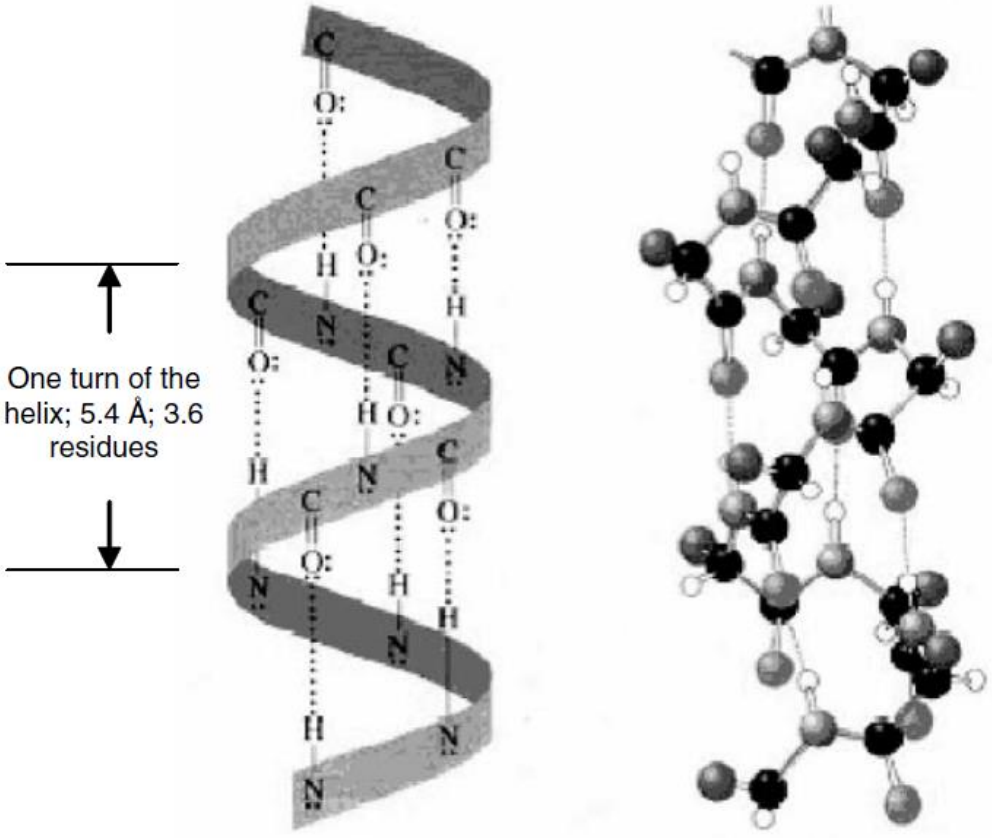
- Two forms of periodic (regular) secondary structures are found in proteins.
  - helical structures and extended sheet-like structures

- Large numbers of such local interactions form alfa-helices and beta-pleated sheets

Secondary structures

Helical structures:

- Among the three helical structures, the α-helix is the major form found in proteins and it is the most stable
- *α*-Helix is stabilized by hydrogen bonding. In this structure, each backbone N–H group is hydrogen bonded to the C==O group of the fourth preceding residue.
- Polypeptide chain coils like a spring, with one turn every 3.6 amino acids
- Most of the α-helical structure found in proteins is amphiphilic in nature
  - one-half of the helix’s surface is occupied by hydrophobic residues and the other half by hydrophilic residues.



One turn of the helix; 5.4 Å; 3.6 residues

Secondary structures

β-Sheet structure:

- The β-sheet is an extended structure with specific geometries
- C==O and N−−H groups are oriented perpendicular to the direction of the chain
  - therefore hydrogen bonding is possible only between segments (i.e., intersegment), and not within a segment (i.e., intrasegment).
- The β-strands are usually about 5–15 amino acid residues long.

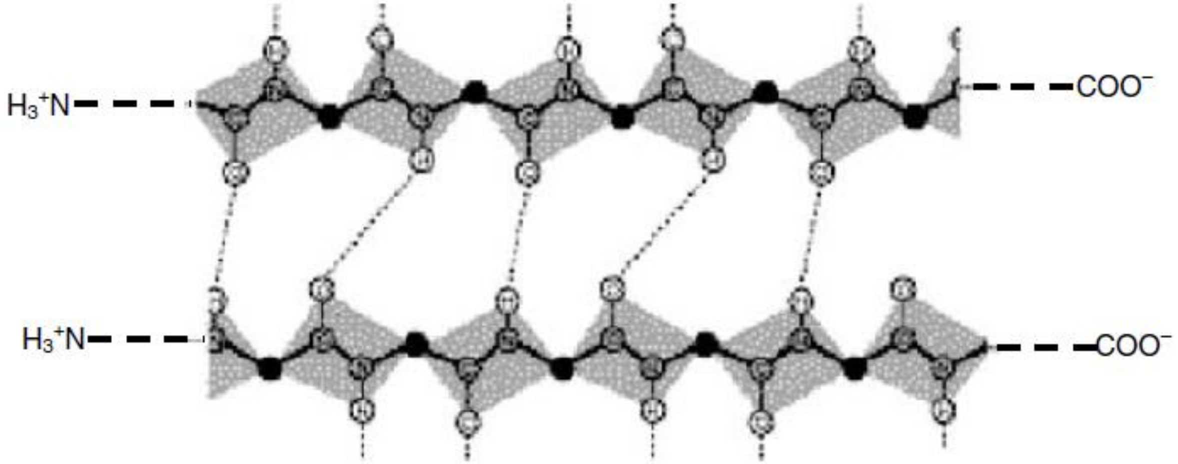
Secondary structures

β-Sheet structure:

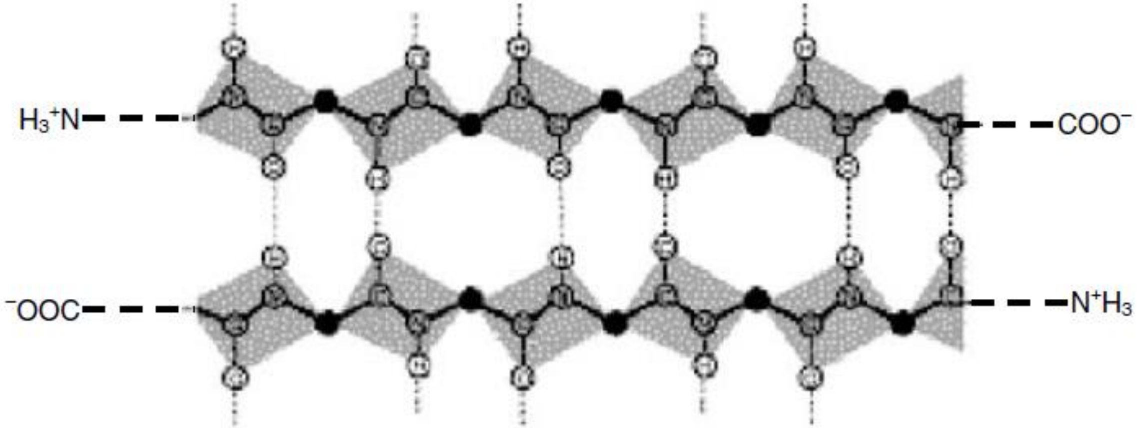
- The β-strands are usually about 5–15 amino acid residues long.
- In proteins, two *β*-strands of the same molecule interact via hydrogen bonds, forming a sheetlike structure known as *β*-pleated sheet.
- In the sheet-like structure, the side chains are oriented perpendicular (above and below) to the plane of the sheet.
- Depending on the N→C directional orientations of the strands, two types of *β*-pleated sheet structures are;
    - *parallel β-sheet*
    - *antiparallel β-sheet*

Secondary structures

β-sheets



parallel β-sheet



antiparallel β-sheet

In parallel β-sheets;

- the directions of the *β*-strands run parallel to each other
- the N−−H· · ·O atoms lie at an angle, which reduces the stability of the hydrogen bonds.

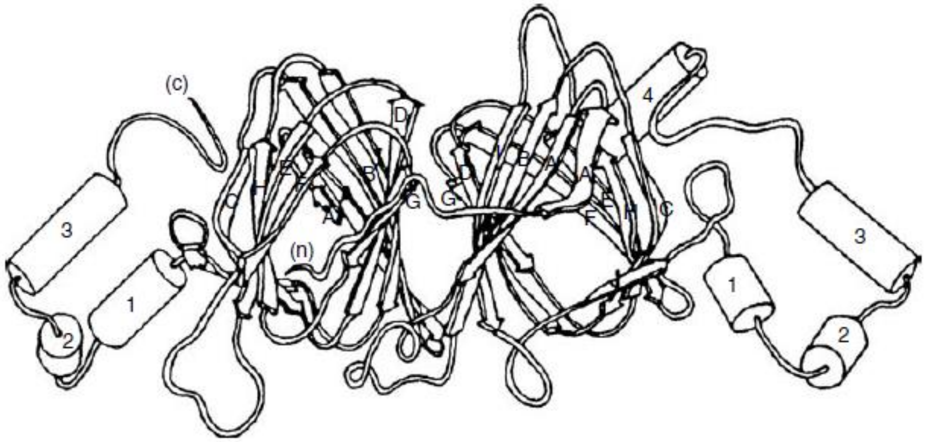
In antiparallel β-sheets;

- the directions of the *β*-strands run opposite to each other
- the N−−H· · ·O atoms lie in a straight line (zero H-bond angle), which enhances the stability of the hydrogen bond

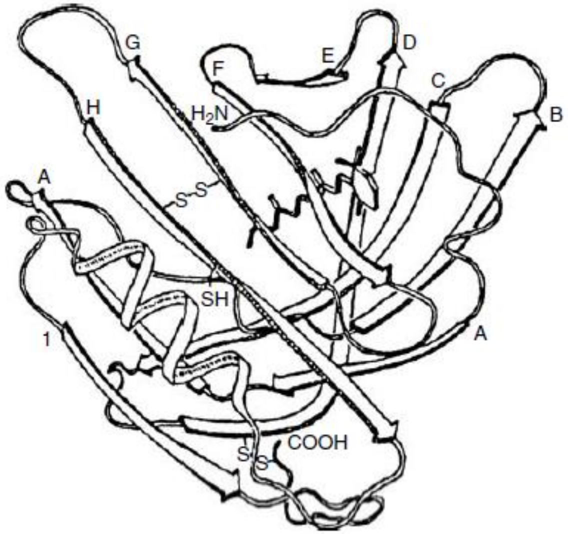
Antiparallel β-sheets are more stable than parallel β-sheets.

Tertiary structure

- The spatial arrangement attained when a linear protein chain with secondary structure segments folds further into a compact 3D form



The tertiary structure of β-lactoglobulin



The tertiary structure of phaseolin (the storage protein in kidney beans)

Tertiary structure can be nucleated by the pattern of polar and nonpolar amino acids in a polypeptide chain, which plays a central role in determining the protein's final conformation.

- Proteins can be divided into two large groups on the basis of conformation:
  - (a) fibrous or scleroproteins
  - (b) globular or folded proteins

Tertiary structure

- Tertiary structures of several single polypeptide proteins are made up of domains.
  - Domains: regions of the polypeptide sequence that fold up into a tertiary form independently.
    - In essence, miniproteins within a single protein.
- The number of domains in a protein usually depends on its molecular weight.
- Small proteins (e.g., lysozyme, *β*-lactoglobulin, and *α*-lactalbumin) with 100–150 amino acid residues usually form a single domain tertiary structure.
- Large proteins, such as immunoglobulin, contain multiple domains.

Forces involved in the stability of protein structure

- From a thermodynamic viewpoint, formation of tertiary structure involves
  - optimization of various interactions between various groups in protein
  - the conformational entropy of the polypeptide chain

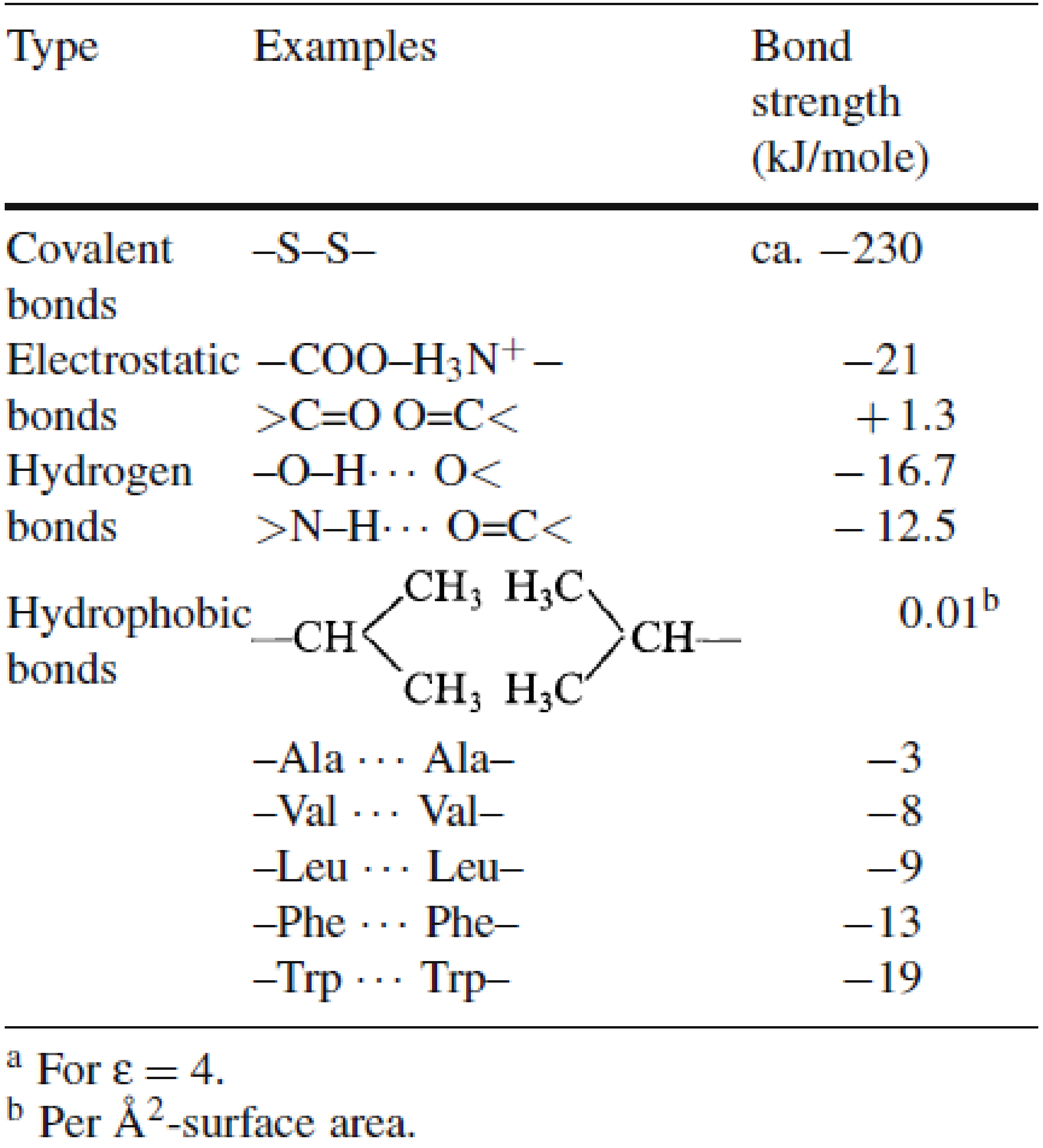
- Folding of a random polypeptide chain into three-dimensional structure
  - Transformation of an unfolded state to a folded state is slow but spontaneous
  - This process is faciliated by intramolecular noncovalent interactions.

- The forces involved in protein folding:

1. Intramolecular interactions emanating from forces intrinsic to the protein molecule:
   - ➢ van der Waals and steric interactions

2. Intramolecular interactions affected by surrounding solvent
   - ➢ hydrogen bonding, electrostatic, and hydrophobic interactions

Bond-types in proteins



Type	Examples	Bond strength (kJ/mole)
Covalent bonds	–S–S–	ca. –230
Electrostatic bonds	$-COO^{-}H_3N^{+}-$	–21
	$>C{=}O\ O{=}C<$	+1.3
Hydrogen bonds	$-O-H\cdots O<$	–16.7
	$>N-H\cdots O{=}C<$	–12.5
Hydrophobic bonds	$-CH\begin{matrix}CH_3 & H_3C\\ CH_3 & H_3C\end{matrix}CH-$	0.01^{b}
	–Ala ··· Ala–	–3
	–Val ··· Val–	–8
	–Leu ··· Leu–	–9
	–Phe ··· Phe–	–13
	–Trp ··· Trp–	–19

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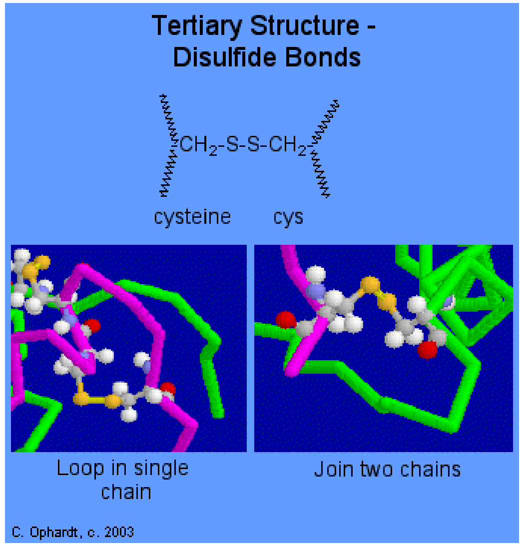
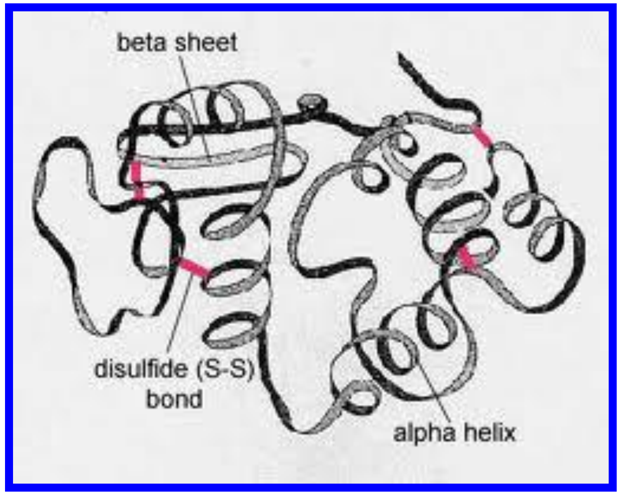
[a] For ε = 4.

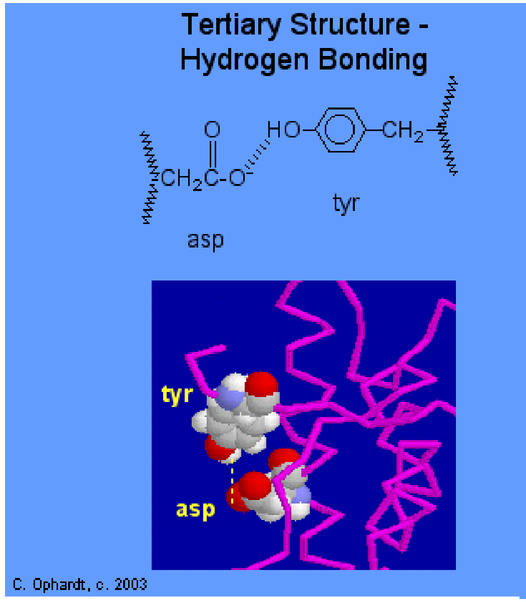
[b] Per $Å^2$-surface area.



Tertiary Structure - Disulfide Bonds

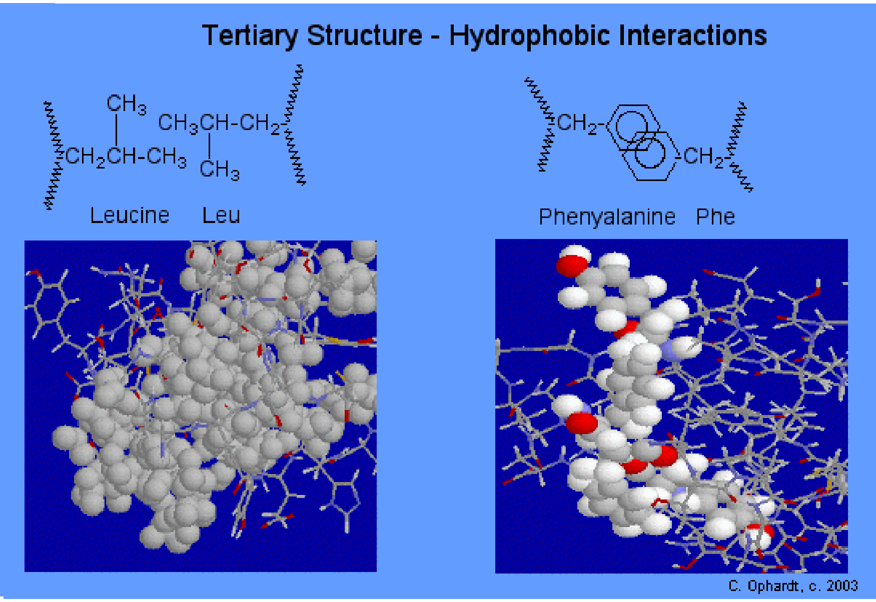
C. Ophardt, c. 2003





Tertiary Structure - Hydrogen Bonding

C. Ophardt, c. 2003



Tertiary Structure - Hydrophobic Interactions

C. Ophardt, c. 2003

Quaternary structure

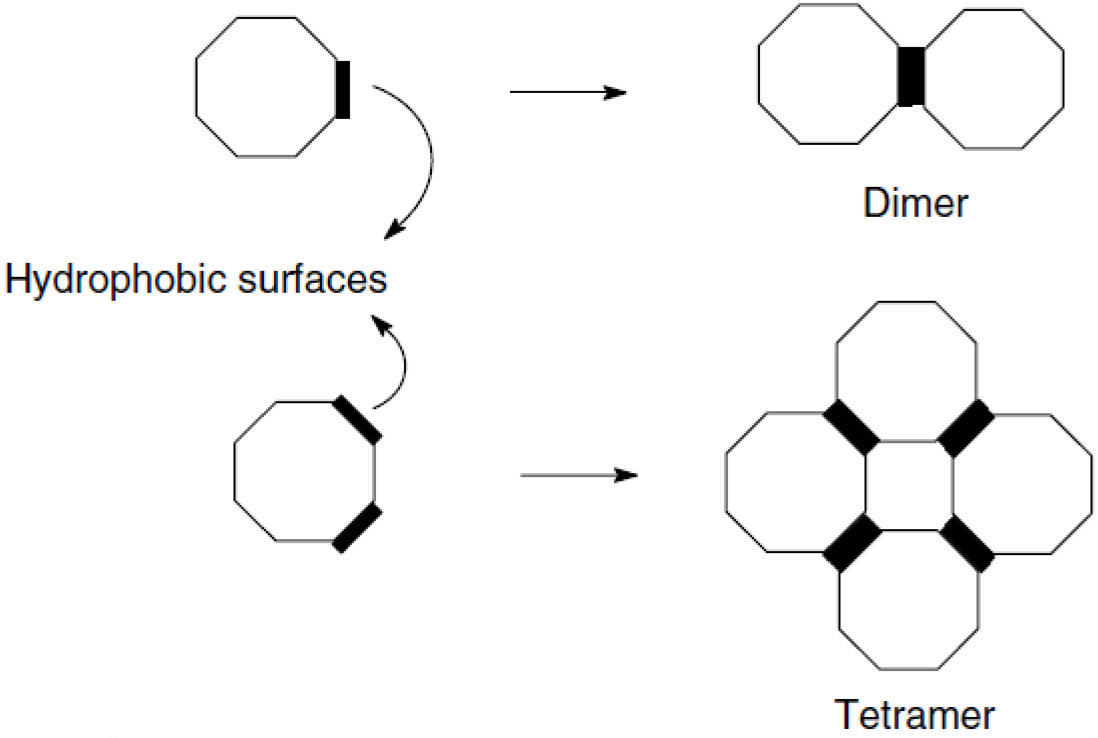
- Formation of oligomeric structures is the result of specific protein–protein interactions.
  - primarily driven by noncovalent interactions such as hydrogen bonding, hydrophobic and electrostatic interactions.
- The fraction of hydrophobic amino acids appears to influence the tendency to form oligomeric proteins.
  - Proteins that contain >30% hydrophobic amino acid residues exhibit a greater tendency to form oligomeric structures than do those that contain fewer hydrophobic amino acid residues.

Quaternary structure

- The spatial arrangement of a protein when it contains more than one polypeptide chain.
  - three-dimensional organization of multiple protein chains

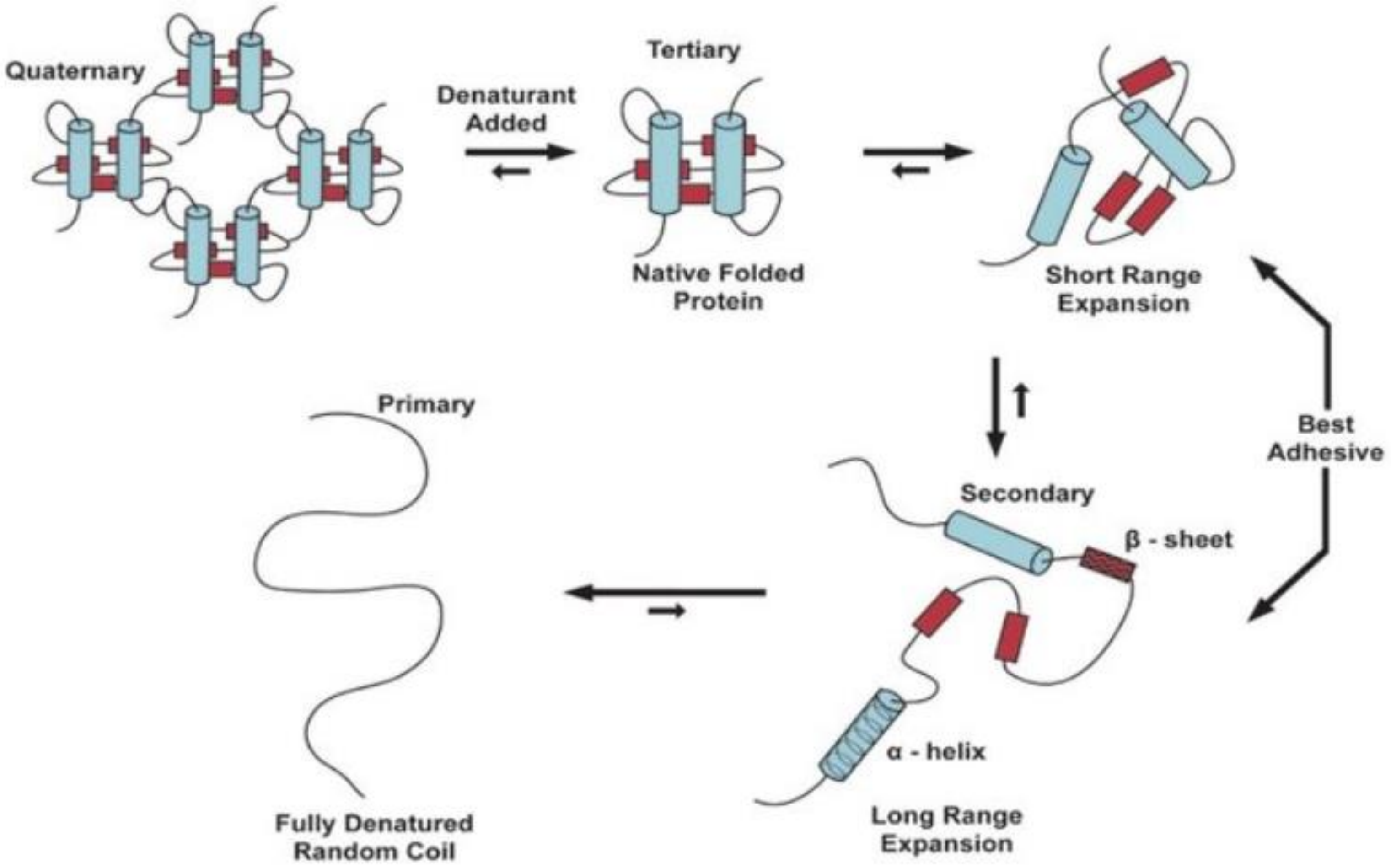
- Several biologically important proteins exist as dimers, trimers, tetramers, and so forth.

- Oligomers can be made up of protein subunits (monomers) that are the same (homogeneous) or different (heterogeneous).



PROTEINS
Denaturation, Functionality

Protein denaturation

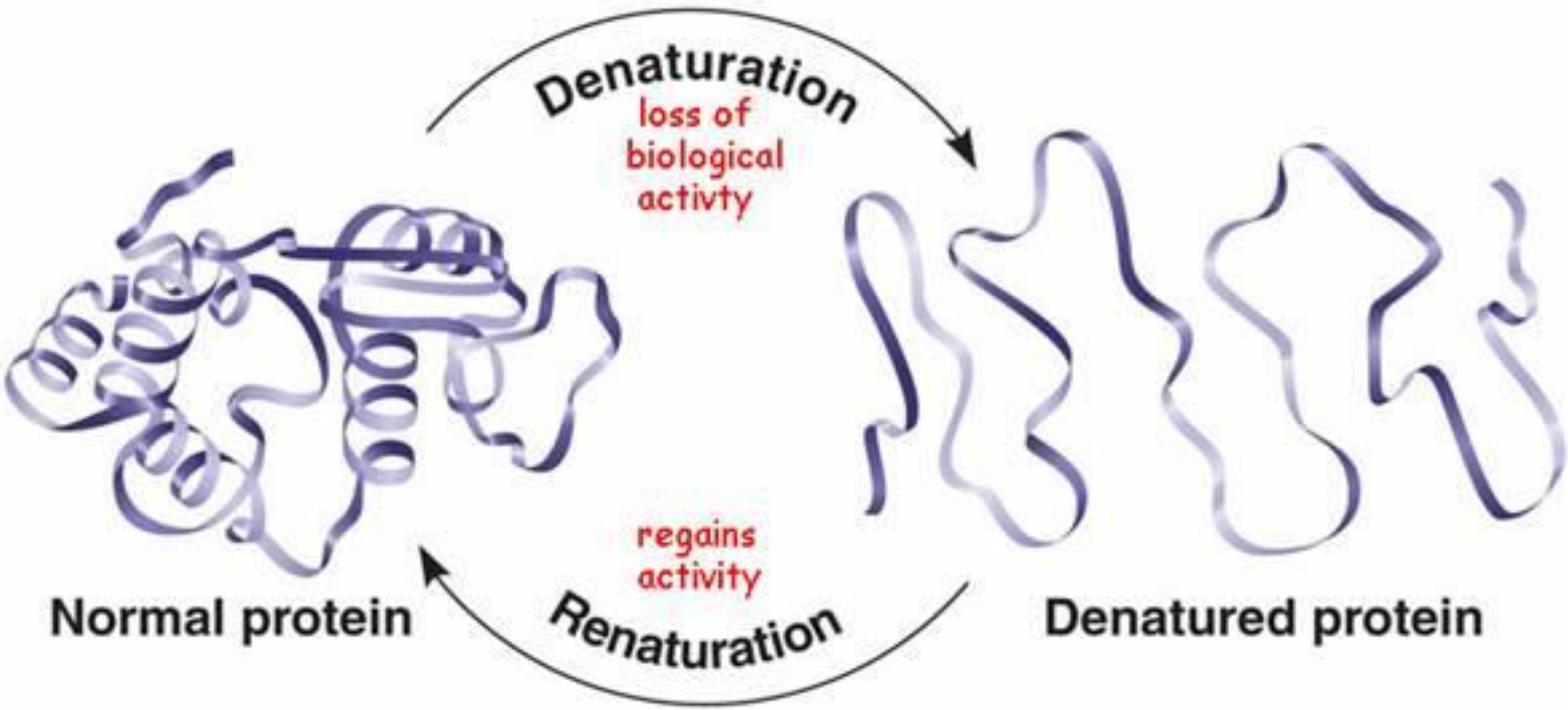


Protein denaturation

- Denaturation is the process of modifying the conformation of the protein structures without rupturing the native peptide linkages.
- This inactivates the functionality of the protein molecules, decreases its solubility, decreases/destroys its biological activity, improves digestibility and alters the water binding ability of the molecule.
- Denaturation of proteins is achieved by disrupting the hydrogen bonding in the peptide linkage by applying external stress.
- It can be carried out by applying heat, treatment with alcohols, heavy metals, or acids/bases.
- Protein denaturation is widely used in food processing and dairy industries and a simple example is the cooking of egg white for making an omelets.

- Unfolding of native proteins

- Breakage of hydrogen bonds, disulfide bonds, hydrophobic interactions, van der Waals forces.



There is no breakage of peptide bonds during denaturation.

Protein denaturation

- A reversible or irreversible change of native conformation (tertiary structure) without cleavage of covalent bonds (except for disulfide bridges).
- Denaturation is possible with any treatment that cleaves hydrogen bridges, ionic or hydrophobic bonds.
- This can be accomplished by;
  - changing the temperature
  - adjusting the pH
  - increasing the interface area
  - adding organic solvents, salts, urea, guanidine hyrochloride or detergents such as sodium dodecyl sulfate

Protein denaturation

- Denaturation is generally reversible when the peptide chain is stabilized in its unfolded state by the denaturing agent
  - the native conformation can be reestablished after removal of the agent.

- Irreversible denaturation occurs when the unfolded peptide chain is stabilized by interaction with other chains
  - Example: egg proteins during boiling

- During unfolding reactive groups, such as thiol groups, that were buried or blocked, may be exposed.

- Their participation in the formation of disulfide bonds may also cause an irreversible denaturation.

Protein denaturation in foods

- Denaturation of food proteins, usually causes loss of solubility and some functional properties.

- During processing it is not always undesirable. In fact, in some cases it is highly desirable.

- **Example:** partial denaturation of proteins at the air–water and oil–water interfaces improves their foaming and emulsifying properties, whereas excessive thermal denaturation of soy proteins diminishes their foaming and emulsifying properties.

- Thermal denaturation markedly improves digestibility of legume proteins as a result of inactivation of trypsin inhibitors.

Protein denaturation in foods

- In general, partially denatured proteins are more digestible than native proteins.
- In protein beverages, where high solubility and dispersibility of proteins is required, even partial denaturation of protein during processing may cause flocculation and precipitation during storage and thus may adversely affect the sensory attributes of the product.
- Thermal denaturation is also a prerequisite for heat-induced gelation of food proteins.
- In order to develop appropriate processing strategies, it is imperative to have a basic understanding of the environmental and other factors that affect structural stability of proteins in food systems.

Protein denaturation

- An aggregation of the peptide chains caused by the folding of globular proteins is connected with reduced solubility or swellability.
- In the case of fibrous proteins, denaturation, through destruction of the highly ordered structure, generally leads to increased solubility.
  - Example: the thermally caused collagen-to-gelatin conversion, which occurs when meat is cooked
- Denaturation of biologically active proteins is usually associated with loss of activity.
- Denatured proteins are more readily digested by proteolytic enzymes.

Protein Functionality

- The sensory attributes of a food such as texture, flavor, color, and appearance are the net effect of complex interactions among various minor and major components of the food.

- Proteins generally have a great influence on the sensory attributes of foods.

- For example;
  - the sensory properties of bakery products are related to the viscoelastic and dough-forming properties of wheat gluten
  - the textural and succulence characteristics of meat products are largely dependent on muscle proteins (actin, myosin, actomyosin, and several soluble meat proteins)
  - the textural and curd-forming properties of dairy products are due to the unique colloidal structure of casein micelles
  - the structure of some cakes and the whipping properties of some desert products depend on the properties of egg-white proteins

- Functional properties of food proteins refers to the physical and chemical properties that influence the performance of proteins in food systems during processing, storage, preparation, and consumption.

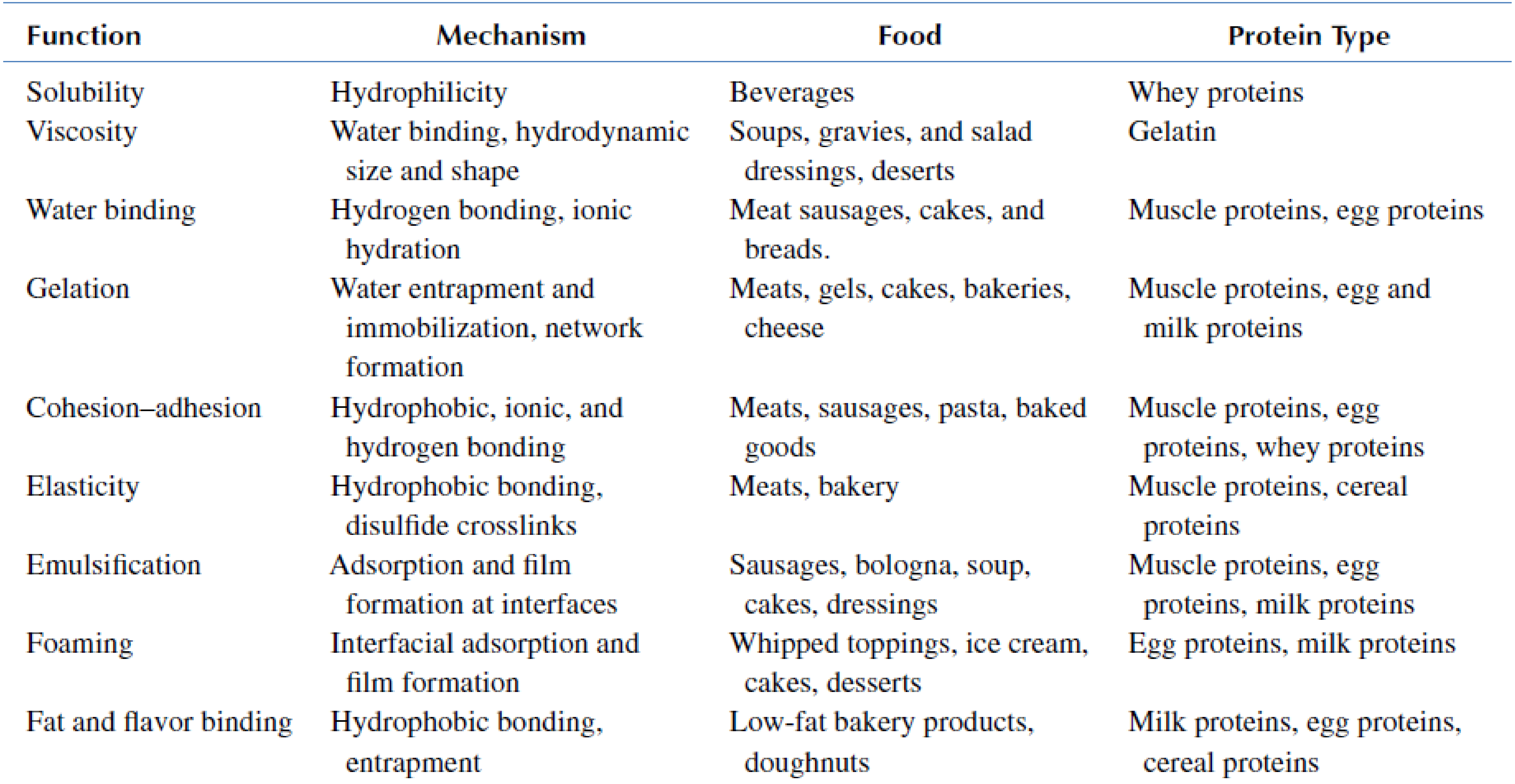
Protein Functionality

- In proteins, for example, unavoidable association of water with certain hydrophobic groups has an important influence on protein functionality

- The functional properties of proteins in foods are related to their structural and other physicochemical characteristics.

- The physical and chemical properties that govern protein functionality:
  - size; shape; amino acid composition and sequence; net charge and distribution of charges; hydrophobicity/hydrophilicity ratio; secondary, tertiary, and quaternary structures; molecular flexibility/rigidity; and ability to interact/react with other components.

Functional Roles of Food Proteins in Food Systems



Function	Mechanism	Food	Protein Type
Solubility	Hydrophilicity	Beverages	Whey proteins
Viscosity	Water binding, hydrodynamic size and shape	Soups, gravies, and salad dressings, deserts	Gelatin
Water binding	Hydrogen bonding, ionic hydration	Meat sausages, cakes, and breads.	Muscle proteins, egg proteins
Gelation	Water entrapment and immobilization, network formation	Meats, gels, cakes, bakeries, cheese	Muscle proteins, egg and milk proteins
Cohesion–adhesion	Hydrophobic, ionic, and hydrogen bonding	Meats, sausages, pasta, baked goods	Muscle proteins, egg proteins, whey proteins
Elasticity	Hydrophobic bonding, disulfide crosslinks	Meats, bakery	Muscle proteins, cereal proteins
Emulsification	Adsorption and film formation at interfaces	Sausages, bologna, soup, cakes, dressings	Muscle proteins, egg proteins, milk proteins
Foaming	Interfacial adsorption and film formation	Whipped toppings, ice cream, cakes, desserts	Egg proteins, milk proteins
Fat and flavor binding	Hydrophobic bonding, entrapment	Low-fat bakery products, doughnuts	Milk proteins, egg proteins, cereal proteins

The Linkage Between the Physicochemical Aspects of Proteins and Their Impact on Functionalities in Foods

General Property	Functions Affected
Hydration	Solubility, dispersibility, wettability, swelling, thickening, water absorption, water-holding capacity
Surface activity	Emulsification, foaming, flavor binding, pigment binding
Hydrodynamic/Rheological	Elasticity, viscosity, cohesiveness, chewiness, adhesion, stickiness, gelation, dough formation, texturization

- On an empirical level, the various functional properties of proteins can be viewed as manifestations of three molecular aspects of proteins

Texturization

- Transformation of a protein from a globular state to a fibrous physical structure that has meat-like mouthfeel characteristics.

- The various functional properties that texturized protein products are expected to possess include chewiness, elasticity, softness, and juiciness.

- Vegetable proteins are often the preferred protein source for texturization, primarily because they lack other desirable functional properties that proteins of animal origin display.

- Textured vegetable proteins are manufactured using two different processes, namely *spun-fiber texturization* and *extrusion texturization*.

Dough Formation

- When a mixture of wheat flour and water (about 3:1 ratio) is kneaded, it forms a viscoelastic dough.

- These unusual dough characteristics are mainly attributable to the proteins in wheat flour.

- Wheat flour contains several soluble and insoluble protein fractions.

- The soluble proteins
  - about 20% of the total proteins
  - primarily albumin and globulin type enzymes and certain minor glycoproteins
  - do not contribute to the dough-forming properties of wheat flour

- The major storage protein of wheat is gluten.
  - Gluten is a heterogeneous mixture of proteins, mainly gliadins and glutenins, with limited solubility in water.
  - When mixed with water, gluten forms viscoelastic dough capable of entrapping gas during fermentation.

Protein Hydration

- The rheological and textural properties of foods depend on the interaction of water with other food constituents, especially with macromolecules, such as proteins and polysaccharides.
- Water modifies the physicochemical properties of proteins.
- Many functional properties of proteins, such as dispersibility, wettability, swelling, solubility, thickening/viscosity, water-holding capacity, gelation, coagulation, emulsification, and foaming depend on water–protein interactions.
- In low and intermediate moisture foods, such as bakery and comminuted meat products, the ability of proteins to bind water is critical to the acceptability of these foods.
- The ability of a protein to exhibit a proper balance of protein–protein and protein–water interactions is critical to their thermal gelation properties.

Solubility

- The functional properties of proteins are often affected by protein solubility and those most affected are thickening, foaming, emulsifying, and gelling.
- Insoluble proteins have very limited uses in food.
- At pH values below and above the isoelectric pH, proteins carry a net positive or a net negative charge, respectively.
- Electrostatic repulsion and hydration of charged residues promote solubilization of the protein.
- Minimum solubility occurs at about the isoelectric pH of proteins.

Interfacial Properties of Proteins

- Proteins are amphiphilic molecules and they migrate spontaneously to an air/water interface or an oil–water interface.
- Unlike small molecular-weight surfactants, proteins form a highly viscoelastic film at an interface
- This has the ability to withstand mechanical shocks during storage and handling.
- Thus, protein-stabilized foams and emulsions are more stable than those prepared with small molecule surfactants
  - because of this, proteins are extensively used for these purposes.

Flavor Binding

- Proteins themselves are odorless.
- However, they can bind flavor compounds, and thus affect the sensory properties of foods
- The flavor-binding property of proteins also has desirable aspects, because they can be used as flavor carriers or flavor modifiers in fabricated foods.
- This is particularly useful in meat analogues containing plant proteins, where successful simulation of a meat-like flavor is essential for consumer acceptance.

Gelation

- Protein gelation refers to transformation of a protein from the “sol” state to a “gel-like” state.
- Heat, enzymes, or divalent cations under appropriate conditions facilitate this transformation.
- Most food protein gels are prepared by heating a moderately concentrated protein solution.
- In this mode of gelation, the protein in a “sol” state is first transformed into a “progel” state by denaturation.
- In the “sol” state, the number of noncovalent bonding groups available in proteins for network structure formation is limited.

Gelation

- The progel state however is usually a viscous liquid state in which some degree of protein denaturation and polymerization has already occurred.
-
- The conversion of sol to progel is irreversible because many protein–protein interactions occur between the unfolded molecules.

- When the progel is cooled to ambient or refrigeration temperature, the decrease in the thermal kinetic energy facilitates formation of stable noncovalent bonds among exposed functional groups of the various molecules and this constitutes gelation.

- The interactions involved in network formation are primarily hydrogen bonds, and hydrophobic and electrostatic interactions.

Protein Functionality

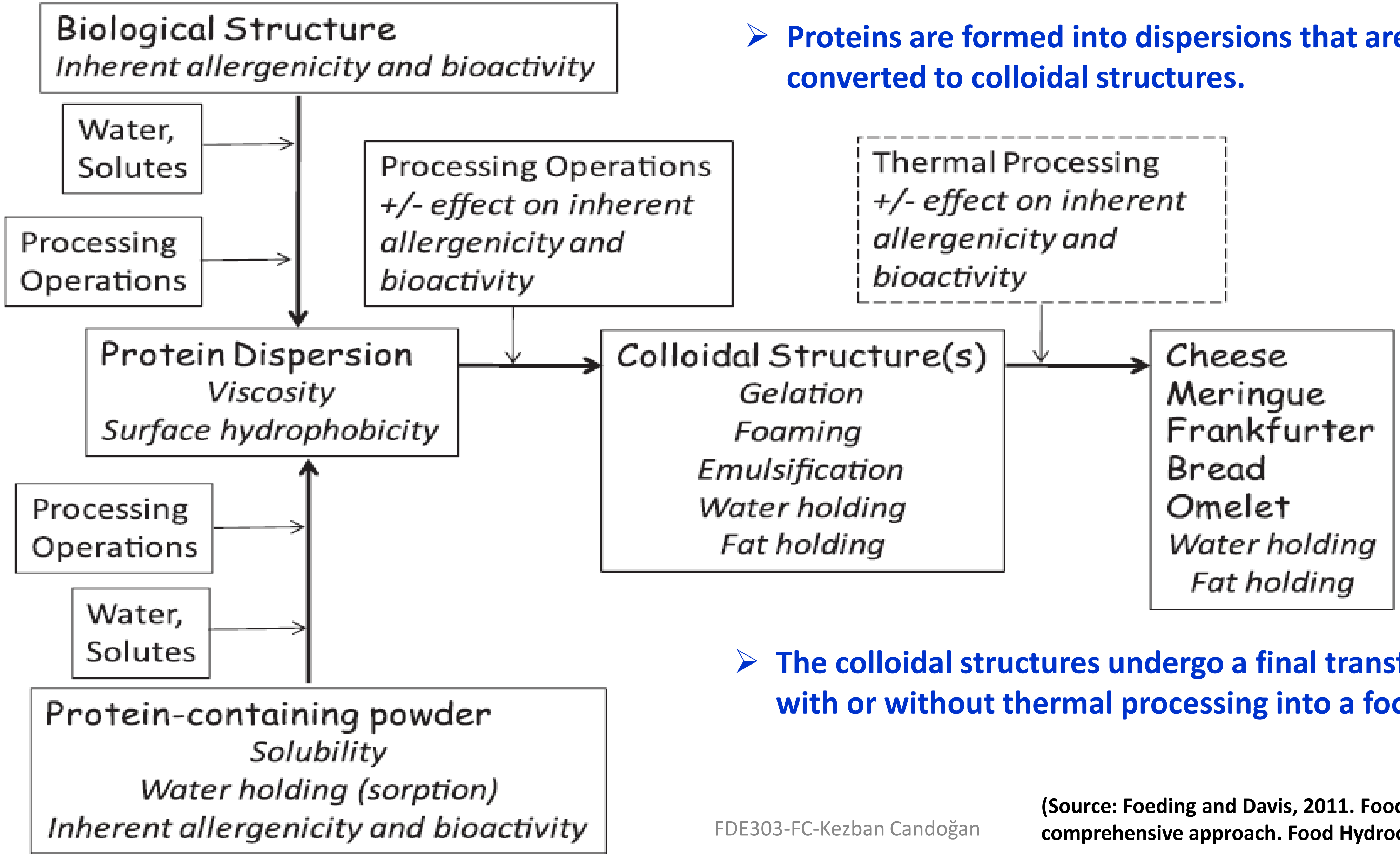
- Proteins are also used to stabilize structures in foods, such as emulsions and foams.

- The ability to form and/or stabilize networks (gels and films), foams, emulsions and sols are the main classically viewed “functional properties” of protein.

- What is usually minimally addressed is how these structures alter nutritional properties and biological activity of proteins.

- The general contribution of proteins/peptides to nutrition and health, including specific bioactivities, is of increasing importance prompting development of food products based on protein-related health issues

Processes in manufacturing of food with protein-based colloidal structures -Effects on functional properties and allergenity-


Biological Structure
Inherent allergenicity and bioactivity

Water, Solutes

Processing Operations

Processing Operations
+/- effect on inherent allergenicity and bioactivity

Thermal Processing
+/- effect on inherent allergenicity and bioactivity

Protein Dispersion
Viscosity
Surface hydrophobicity

Colloidal Structure(s)
Gelation
Foaming
Emulsification
Water holding
Fat holding

Cheese
Meringue
Frankfurter
Bread
Omelet
Water holding
Fat holding

Processing Operations

Water, Solutes

Protein-containing powder
Solubility
Water holding (sorption)
Inherent allergenicity and bioactivity

- **Proteins are formed into dispersions that are converted to colloidal structures.**
- **The colloidal structures undergo a final transformation with or without thermal processing into a food product.**

(Source: Foeding and Davis, 2011. Food protein functionality: A comprehensive approach. Food Hydrocolloids, 25, 1853-1864)