

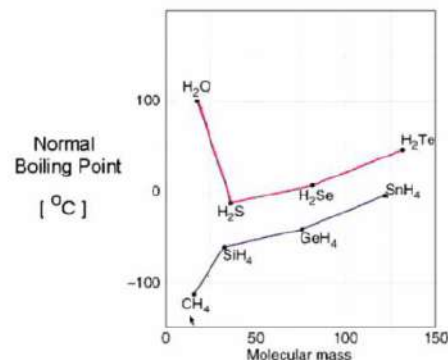
# FOOD30010 – Functional Foods

## PROTEINS

### Lecture 1 – Water

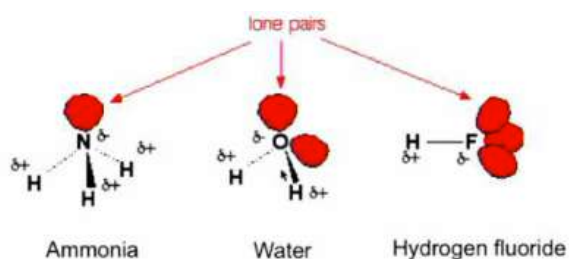
#### Unique Physical Properties of Water

- Exhibits unusually large values for:
  - 1) Heats of phase transition → Heats of fusion (melts), vaporization (boils) and sublimation (Solid to vapor)
  - 2) Surface tension → Strength of water-water interaction at liquid surface
  - 3) Dielectric constant → A dimensionless constant that indicates how easy a material or solvent can be polarized (aligned) by an electric field
  - 4) Heat capacity → Amount of heat required to change a body's temperature by a given amount
  - 5) Heat conductivity → Transfer of thermal energy between neighboring molecules



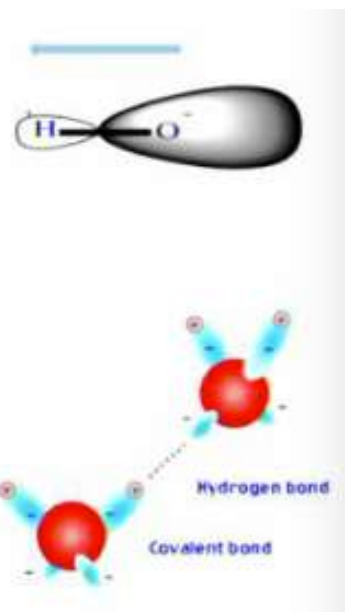
#### The Water Molecule

- Oxygen atom has 2 sp<sup>3</sup> bonding + 2 sp<sup>3</sup> non-bonding orbital
- Two hydrogen atoms form 2 covalent bonds with the 2 sp<sup>3</sup> bonding orbital
- Two lone pairs of electrons occupy the 2 sp<sup>3</sup> non-bonding orbital
- The 4 pairs of electrons surrounding the oxygen atom form a tetrahedral shape with the oxygen atom at the center



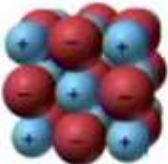


#### Polarity & Hydrogen Bonding

- Water is a polar molecule → Has partial negative charges (delta negative) near the oxygen atom due to the lone pair electrons and electronegativity of oxygen atom. Water has partial positive charges (delta positive) near the hydrogen atoms due to electronegativity of oxygen atom. Therefore, the H-O bond is a Dipole
- Water molecules can interact with each other through hydrogen bonding
- The electrostatic attraction between the hydrogen (delta positive) with the oxygen (delta negative) between water molecules resulted in the formation of very strong hydrogen bonds








- Nitrogen and Oxygen have large electronegativity values, therefore when other molecules are bonded to them, especially Hydrogen, there will be a dipole and the electrons will be closer to the electron clouds of these molecules → H (2.1), N (3.0), O (3.5). Carbon has a value of 2.5 so therefore the electronegativity difference between it and H is small, therefore does not contribute to Hydrogen bonding

### Bonding Intermolecular Forces

Force	Model	Basis of Attraction	Energy (kJ/mol)	Example
<b>Bonding</b>				
Ionic		Cation–anion	400–4000	NaCl
Covalent		Nuclei–shared e <sup>-</sup> pair	150–1100	H–H
Metallic		Cations–delocalized electrons	75–1000	Fe

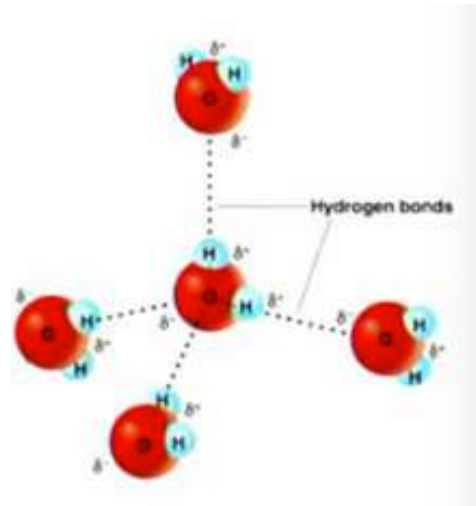
### Non-Bonding Intermolecular Forces

#### Nonbonding (Intermolecular)

Ion-dipole		Ion charge–dipole charge	40–600	$\text{Na}^+ \cdots \text{O} \begin{matrix} \text{H} \\ \text{H} \end{matrix}$
H bond	$\delta^- \quad \delta^+ \quad \delta^-$ –A–H $\cdots$ :B–	Polar bond to H–dipole charge (high EN of N, O, F)	10–40	$\begin{matrix} \text{:}\ddot{\text{O}}\text{--H} \\   \\ \text{H} \end{matrix} \cdots \begin{matrix} \text{:}\ddot{\text{O}}\text{--H} \\   \\ \text{H} \end{matrix}$
Dipole-dipole		Dipole charges	5–25	$\text{I--Cl} \cdots \text{I--Cl}$
Ion-induced dipole		Ion charge–polarizable e <sup>-</sup> cloud	3–15	$\text{Fe}^{2+} \cdots \text{O}_2$
Dipole-induced dipole		Dipole charge–polarizable e <sup>-</sup> cloud	2–10	$\text{H--Cl} \cdots \text{Cl--Cl}$
Dispersion (London)		Polarizable e <sup>-</sup> clouds	0.05–40	$\text{F--F} \cdots \text{F--F}$

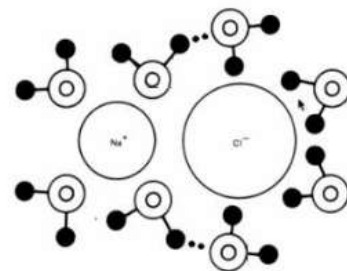
### Structure of Water

- The unusual properties of water arise from its angled V-shape and the 4 hydrogen bonds that it can form with other molecules
- The oxygen atom of a water molecule is the hydrogen acceptor for 2 hydrogen atoms, and each O-H group serves as a hydrogen donor
- Since each water molecule has an equal number of hydrogen-bond donor (H) and receptor (O) sites that are arranged to permit 3D hydrogen bonding, the attractive forces among water molecules are unusually large
- Many of the unique physical properties of water (high melting and boiling points and expansion upon freezing) are due to its ability to engage in 3D hydrogen bonding
- Water is a structured fluid that can flow → The uninterrupted 3D network arrangement is dynamic and present throughout the whole water sample. Therefore, water molecules can easily exchange H-bonds and hence rapidly alter the bonding arrangement of individual molecules
- The average lifetime of a hydrogen bond is short (about  $10^{-11}$  sec). Individual molecules can alter their hydrogen bonding relationships with neighbouring molecules within the time frame of nanoseconds ( $10^{-9}$ ) to picoseconds ( $10^{-12}$ ), thereby enhancing molecular mobility and fluidity



### Water Interaction with Ions and Ionic Groups

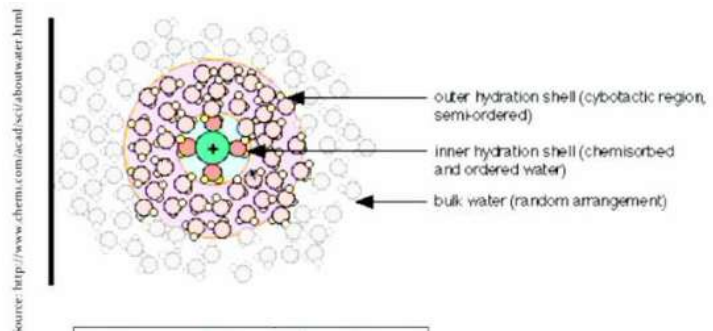
- Water interacting with ions and ionic groups represents some of the most tightly bound water in foods → Charge-dipole Interactions
- The normal structure of pure water (hydrogen-bonded tetrahedral structure) is disrupted by dissociable solutes ( $\text{Na}^+$   $\text{Cl}^-$ ) → Oxygen attracted to cation, Hydrogen attracted to anion
- Some ions in dilute aqueous solution have a net structure-breaking effect, where the solution more fluid than pure water → Net structure breakers have weak electric fields e.g.  $\text{K}^+$ ,  $\text{Rb}^+$ ,  $\text{Cs}^+$ ,  $\text{NH}_4^+$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$
- Other ions have a structure forming effect, where the solution is less fluid than pure water → Net structure formers have strong electric fields:  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{F}^-$ ,  $\text{OH}^-$
- Ability of a given ion to alter net structure is related closely to its polarizing power (charge divided by radius) or the strength of its electric field



### The Multilayer or Association Theory

- The fixed charges on macromolecules and their associated counter ions constrain much of the cellular water to form a matrix of polarized multilayers having restricted motion compared with pure water → The charge restricts mobility of water

- This matrix contains 3 layers:  
Inner hydration shell (chemisorbed and ordered water), Outer hydration shell (cybotactic region, semi-ordered) and Bulk water (random arrangement)

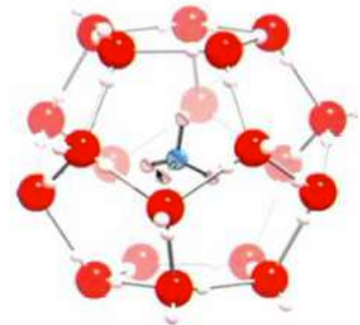


### Water Interaction with Neutral Groups

- Hydrogen bonding of the water can occur with various neutral functional groups such as hydroxyl, amino, carbonyl and amide → These water-neutral group interactions (dipole-dipole) are weaker than water-ion (dipole-charge) interactions
- Neutral solutes capable of hydrogen bonding DO NOT usually disrupt the normal structure of pure water

### Water Interaction with Non-Polar Groups

- Water clathrate or cage is a structure forming response of water to non-polar entities → Water clathrate around non-polar amino acids in protein contributes to protein stability and folding
- E.g. Water surrounds the non-polar methane molecule, but since it is hydrophobic, there is no direct interaction between water and methane



## Lecture 2 – Proteins

### Use of Protein Ingredients in Food

- Liquid (Milk, soy drink), Viscous (Mayonnaise), Semi-solid (Yogurt, Tofu), Foamed texture (Ice cream, mousse, cappuccino) and Solid-like (Breakfast bars)
- Protein ingredients contribute to the attributes of all these classes of food
- The functional properties of proteins influence the quality and physical attributes of a food product
- Structural properties of proteins influence their functional properties

### Types of Protein Ingredients

- Animal sources → Meat, dairy, fish, egg proteins
- Vegetable sources → Soy protein, wheat protein, legume protein

- Newer types of protein products → Protein hydrolysates and bioactive peptides: Casein, whey, wheat, soy/fish hydrolysates. Also microalgal protein source such as Spirulina

### **Protein Types – Simple Proteins**

- Yield only amino acids on hydrolysis

#### Albumins

- Soluble in neutral, salt free water
- Usually these are proteins of relatively low molecular weight
- Examples are egg albumin, lactalbumin, and serum albumin in the whey proteins of milk, leucosin of cereals and legumelin in legume seeds

#### Globulins

- Soluble in neutral salt solutions and almost insoluble in water
- Examples are serum globulins and B-lactoglobulin in milk, myosin and actin in meat, and glycinin in soybeans

#### Glutelins

- Soluble in very dilute acid or base and insoluble in neutral solvents
- These proteins occur in cereals, such as glutenin in wheat and oryzenin in rice

#### Prolamins

- Soluble in 50 to 90 percent ethanol and insoluble in water
- These proteins have large amounts of proline and glutamic acid
- These occur in cereals such as zein in corn, gliadin in wheat and hordein in barley

#### Scleroproteins

- Insoluble in water and neutral solvents and resistant to enzyme hydrolysis
- These are fibrous proteins serving structural and binding purposes
- Examples are collagen of muscle tissue (as is gelatin which is derived from it), elastin (a component of tendons) and keratin (a component of hair and hoofs)

#### Histones

- Soluble in water
- Basic proteins, as defined by their high content of lysine and arginine

#### Protamines

- Strongly basic proteins of low molecular weight (4000 to 8000) rich in arginine
- Examples are clupein from herring and scrombrin from mackerel

### **Protein Types – Conjugated Proteins**

- Contain an amino acid part combined with a non-protein material such as a lipid, nucleic acid, or carbohydrate

#### Phosphoproteins

- An important group that includes many major food proteins

- Phosphate groups are linked to the hydroxyl groups of serine and threonine
- This group includes casein of milk and the phospho-proteins of egg yolk

#### Lipoproteins

- These are combinations of lipids with protein and have excellent emulsifying capacity
- Lipoproteins occur in milk and egg yolk

#### Nucleoproteins

- These are combinations of nuclei acids with protein
- These compounds are found in cell nuclei

#### Glycoproteins

- These are combinations of carbohydrates with protein
- Usually the amount of carbohydrate is small, but some glycoproteins have carbohydrate contents of 8 to 20 percent
- Example of such a mucoprotein is ovomucin of egg white and proteoglycans from cartilage

#### Chromoproteins

- These are proteins with a colored prosthetic group
- There are many compounds of this type, including hemoglobin and myoglobin, chlorophyll and flavoproteins

#### **Protein Types – Derived Proteins**

- Protein products obtained by chemical or enzymatic digestions of proteins

#### Primary Derivatives

- These are slightly modified and are insoluble in water
- Rennet coagulated casein is an example of a primary derivative

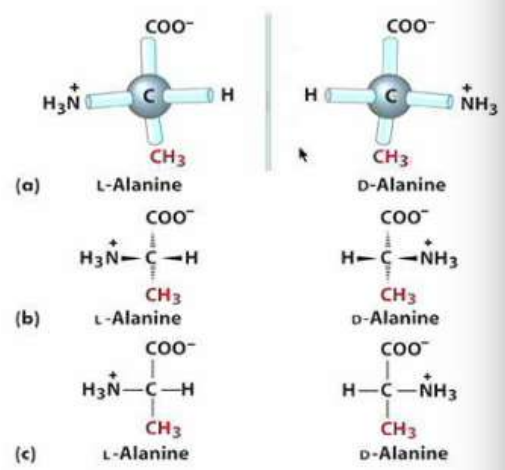
#### Secondary Derivatives

- These more extensively changed and include proteoses, peptones and peptides
- The difference between these breakdown products is in size and solubility
- All are soluble in water and not coagulated by heat, but proteoses can be precipitated with saturated ammonium sulfate solution
- Peptides contain two or more amino acid residues
- These breakdown products are formed during the processing of many foods, for example, during ripening of cheese

## Lecture 3 – Proteins cont.

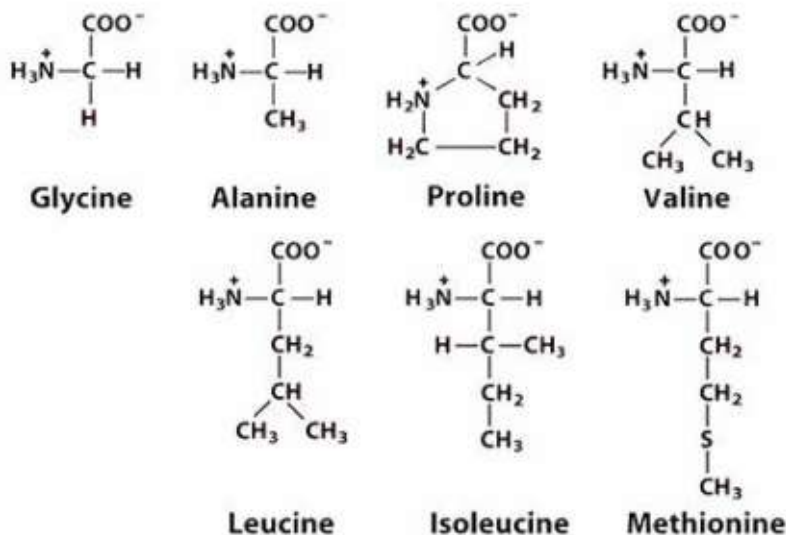
### Amino Acids

- L-stereoisomers found in most proteins, whilst D-Stereoisomers only found in some small peptides from bacterial cell walls and peptide antibiotics
- L and D stereoisomers are non-superimposable mirror images of each other
- They can be differentiated by how they rotate plane-polarized light



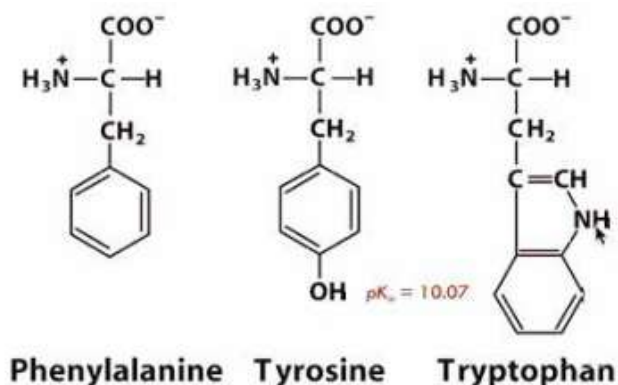
### Aliphatic Non-Polar Amino Acids

- Hydrophobic
- C-H bond does not have polar character
- Relatively unreactive
- Performs structural functions in proteins



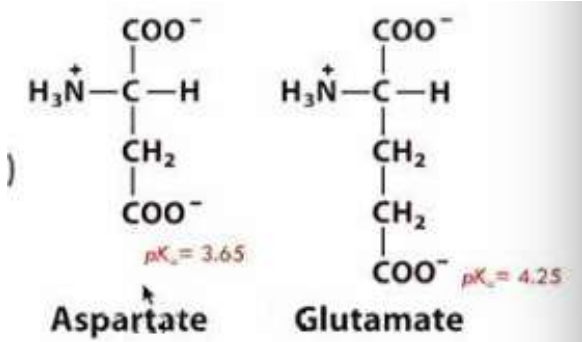
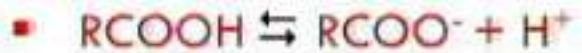
### Aromatic Non-Polar Amino Acids

- Hydrophobic
- OH group of Try (not ionized) is neutral at pH 7
- However, hydrogen of the –OH group of Tyr and –NH of tryptophan can form hydrogen bonds and thus, interact with water



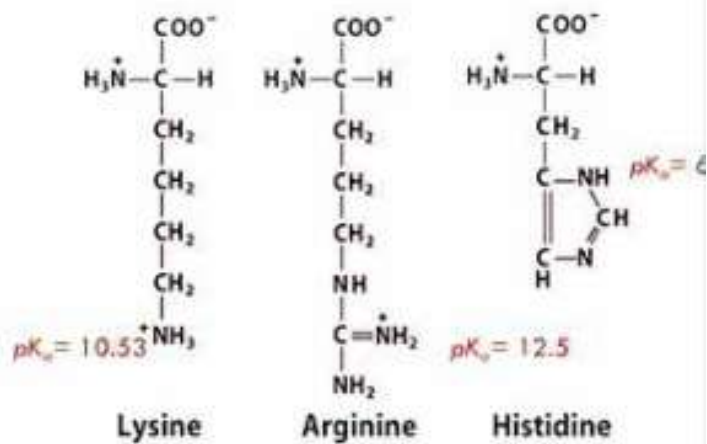
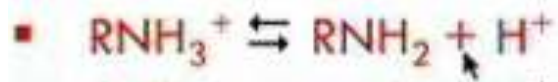
### Acidic Polar Amino Acids

- Hydrophilic
- Carboxyl group is ionized and negatively charged at pH 7
- Carboxyl group is largely neutral in solution pH below its pKa and largely ionized in a solution pH above its pKa
- Participates in Charge-charge interactions (COO<sup>-</sup>/NH<sub>3</sub><sup>+</sup>) and Charge-dipole interactions (COO<sup>-</sup>/H<sub>2</sub>O)



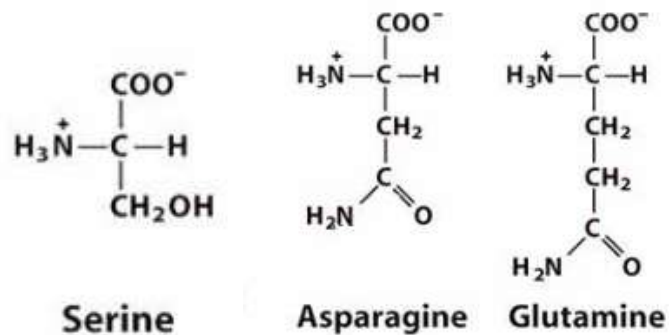
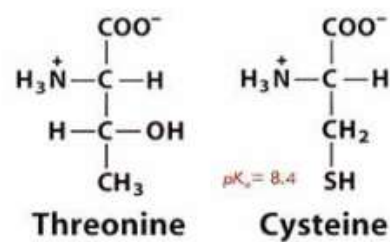
### Basic Polar Amino Acids

- Hydrophilic
- Neutral amine participates in hydrogen bonding due to H bonded to electronegative nitrogen atoms
- Amines in lysine and arginine are protonated and positively charged at pH 7
- Amino group is mostly protonated in solution pH below its pKa and mostly neutral in solution pH above its pKa
- Participates in charge-charge (NH<sub>3</sub><sup>+</sup>/COO<sup>-</sup>) and charge-dipole interactions (NH<sub>3</sub><sup>+</sup>/H<sub>2</sub>O)
- Only 10% of the secondary amine in histidine is protonated at pH 7



### Neutral Polar Amino Acids

- Hydrophilic
- Participates in H-bonding due to electropositive H and electronegative O or N
- Cysteine is the odd one out but can form covalent S-S (Disulfide) linkages





- In summary of all the amino acids, the hydrogen is the hydrogen bond donor and the oxygen is the hydrogen bond acceptor

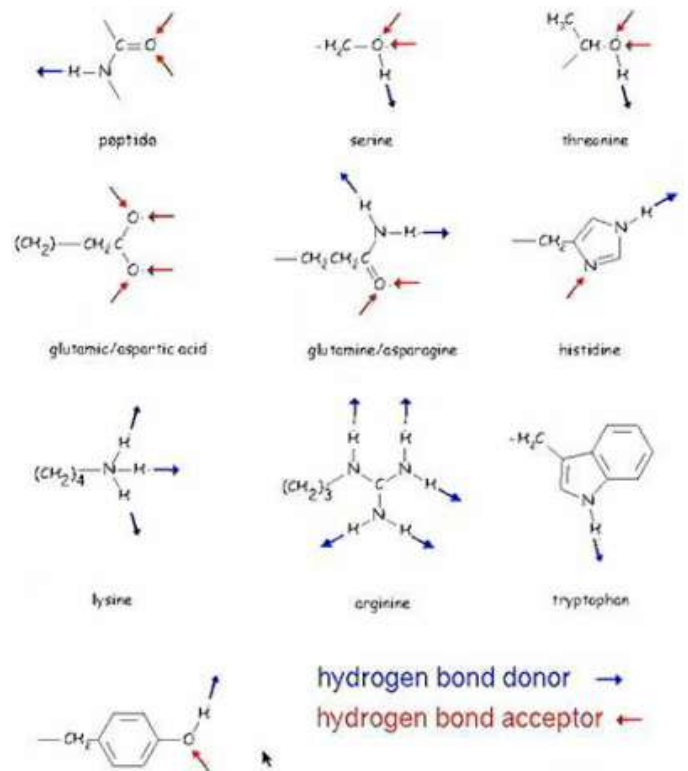
### Protein Structures

#### Primary

- The linear sequence of amino acids

#### Secondary

- The localized organization of parts of a polypeptide chain
- The different shapes can take place at different parts of a polypeptide. The nature of the amino acid interactions (primary structure) can help us predict what shapes occur at this secondary structural level e.g. Small amino acids favor the beta sheet because the small neutral R groups does not interfere with how it folds



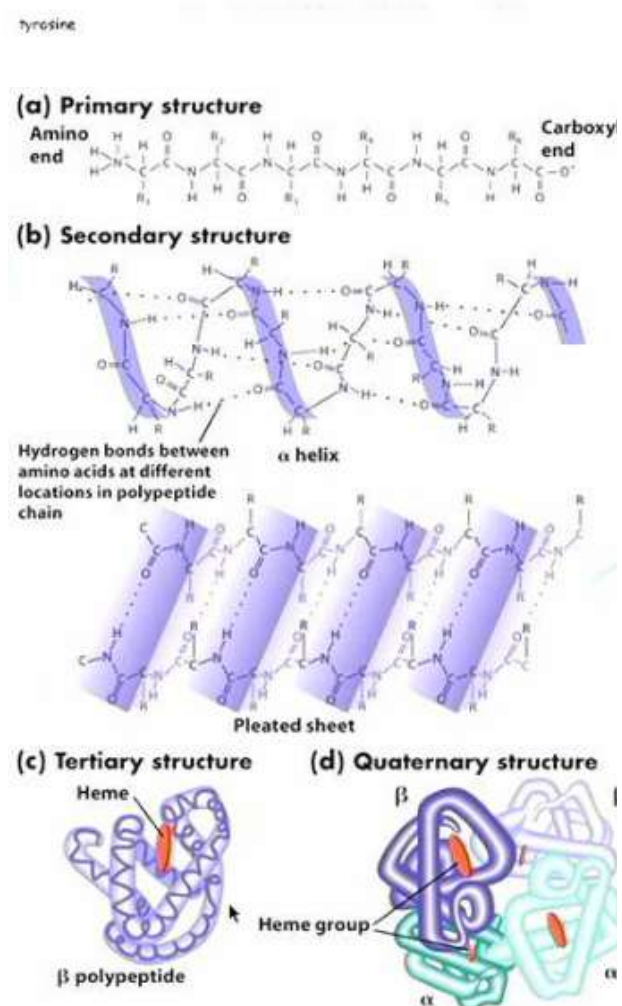
- Alpha helixes: Right handed  $\alpha$ -helix predominates. Stability favored by intra-chain hydrogen bonding. Interaction of R groups (size, charge, H-bonding) can be stabilize/destabilize the  $\alpha$ -helix, for example, Proline (too rigid) and Glycine (too small) unfavorable for the helix structure
- Beta Sheets: Stretches of small neutral R-group amino acids such as glycine and alanine favored B-sheet in proteins
- Random coil

#### Tertiary

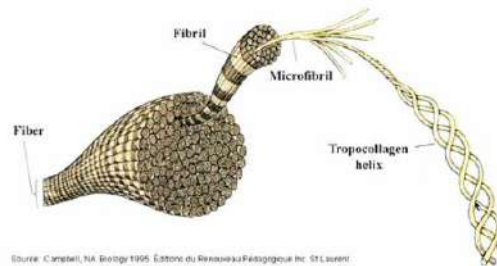
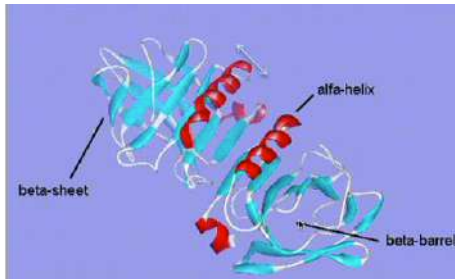
- The overall 3D arrangement of the polypeptide chain

#### Quaternary

- The association of two or more polypeptides into a multi-subunit complex
- An example of a protein quaternary structure in foods is B-Lactoglobulin in milk → It is a dimer (two polypeptides) driven by hydrophobic interactions. Contains beta-sheets, alpha-helixes, beta-barrels (empty space surrounded by hydrophobic amino acids) and random coils

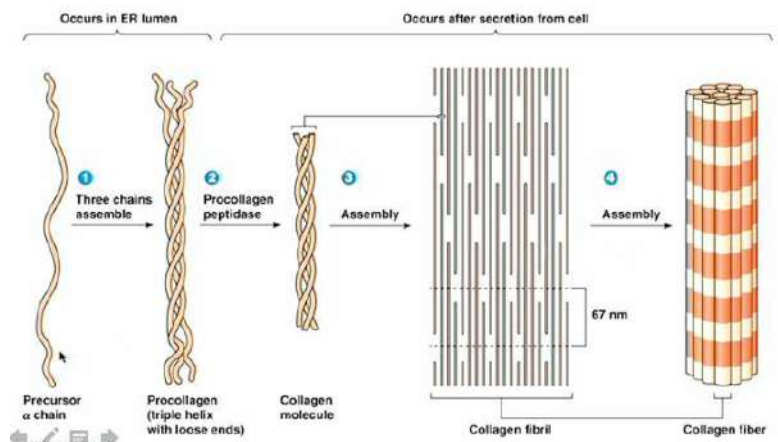


- Another higher structural order is Protein Fiber → Consists of fibers, which ultimately are made up of fibrils, to microfibrils to Tropocollagen helices. Each Tropocollagen helix is made up of 3 polypeptides intertwining with each other



### Collagen

- Three precursor  $\alpha$ -chains assemble into a procollagen (Triple helix with loose ends). This occurs in the ER lumen
- It is then secreted from the cell to be modified and matured. The enzyme procollagen peptidase cuts off the loose ends to form a mature collagen molecule, which then undergoes series of assembly to form a collagen fibril and then a collagen fiber



### Structural Properties of Animal Proteins

- The pI represents the Isoelectric pH, which is the pH for which the protein is neutral/zero charge
- Normal food conditions are at pH 7, so most proteins are negatively charged apart from Collagen

PROTEIN	MW (daltons)	TYPE	pI	S-S and SH (Number)	Prosthetic group
Myosin (bovine)	475,000	Fibrous	4-5	0 and 40	Phosphate
Actin (bovine) (1-300 sub-units)	42,000	Globular → Fibrous	4-5	0 and 5-6	-
Collagen (bovine)	300,000	Fibrous	~9 (positively charged at pH7)	-	-
Ovalbumin (Hen's egg)	45,000	Globular	4.6	1-2 and 4	Sugar Phosphate

### Structural Properties of Plant Proteins

- No isoelectric pH means that there is not enough charge of the proteins to have one
- Having a sugar prosthetic group means it is a glycoprotein, whilst having a phosphate prosthetic group means it is a phosphoprotein

PROTEIN	MW (daltons)	TYPE	pI	S-S and SH (Number)	Prosthetic group
Gliadin (wheat)	30,000-45,000	Globular → Fibrous	-	2-4 and 0	-
Glutenins (wheat) (15 sub-units)	>1,000,000	Fibrous	-	50 and 0	-
Glycinin (soybean)	350,000	Globular	4.6	23 and 2	-
Conglycinin (soybean)	200,000	Globular	4.6	2 and 0	Sugar

### Structural Properties of Milk Proteins

PROTEIN	MW (daltons)	TYPE	pI	S-S and SH (Number)	Prosthetic group
$\alpha$ S <sub>1</sub> -casein B (bovine)	23,500	Random coil	5.1	0 and 0	Phosphate
$\beta$ -casein A (bovine)	24,000	Random coil	5.3	0 and 0	Phosphate
$\kappa$ -casein B (bovine)	19,000	Random coil	4.1-4.5	0 and 2	Sugar Phosphate
$\beta$ -lactoglobulin A (bovine)	18,400	Globular	5.2	2 and 1	
$\alpha$ -lactalbumin B (bovine)	14,200	Globular	5.1	4 and 0	
Serum albumin (bovine)	69,000	Globular	4.8	17 and 1	

## Lecture 4 – Protein Hydration, Solubility & Stability

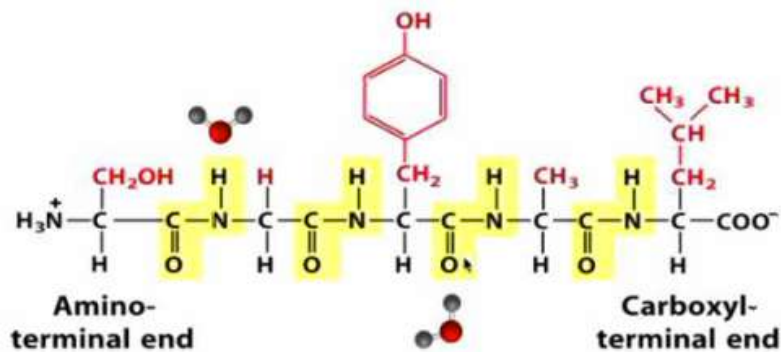
### Protein Hydration

- Proteins possess a number of sites that interacts with water
- Protein-water interactions cause loosening of protein structure as it breaks up forces holding protein in various conformation states
- Water acts as a plasticizer by influencing internal motions of atoms in proteins
- Many functional properties of proteins are dependent on the ability of the protein to hydrate and binds water

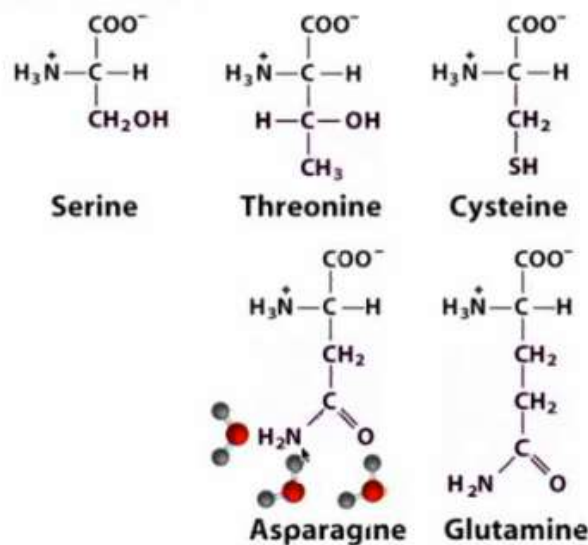
- Water sorption → Water holding by protein provides moisture content in foods
- Viscosity modulation → Water holding by protein affects viscosity of solution (thickness)
- Gelling → Water immobilization by protein 3D network produce semi-solid structure
- Emulsion → Denatured protein at water-oil interface stabilizes emulsion particles
- Foam → Denatured protein at water-air interface stabilizes foam

### Specific Protein-Water Interaction Sites

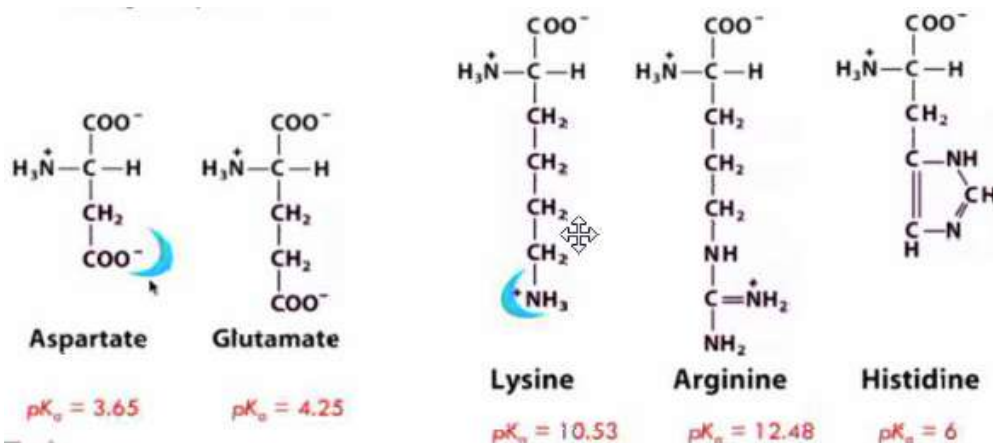
- 1. Peptides bonds → Hydrogen bonding with water through electropositive hydrogen and electronegative oxygen (And lone pair on oxygen)



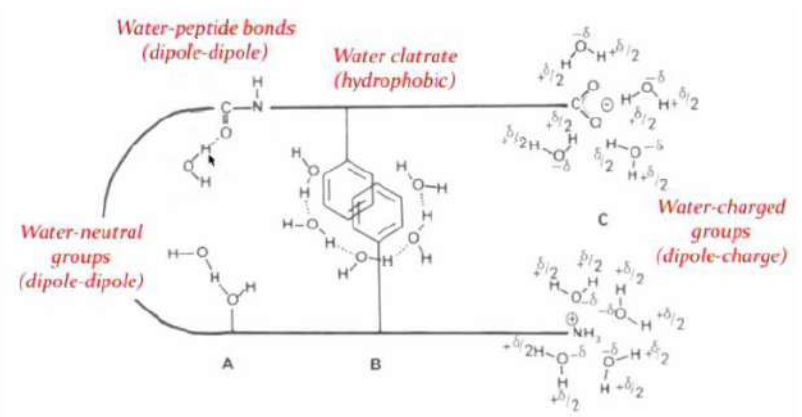
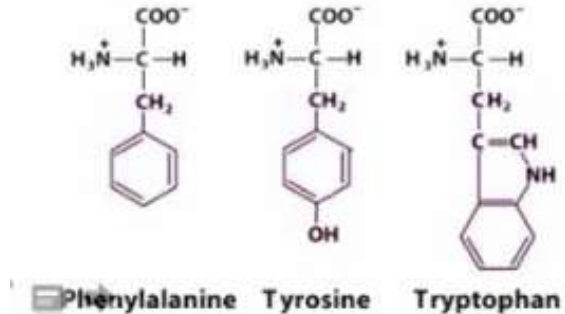
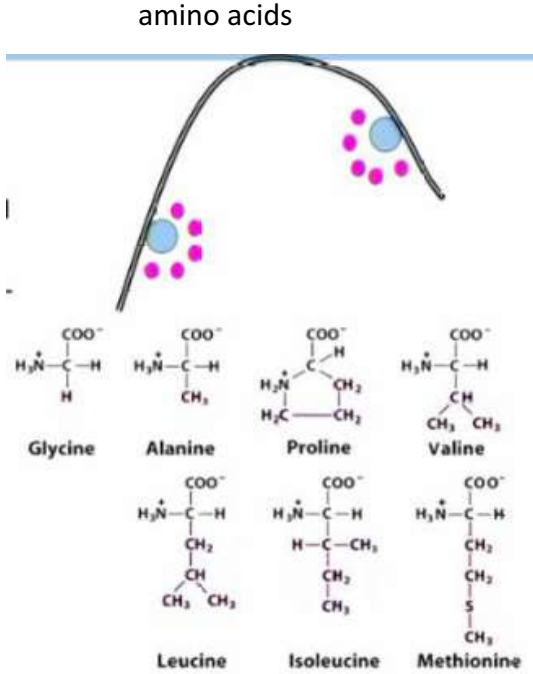
- 2. Polar neutral amino acids → Hydrogen bonding with water through R-groups



- 3. Polar acidic and basic amino acid → Charge-dipole water. This is a stronger interaction than hydrogen bonding therefore, highly charged proteins are highly soluble



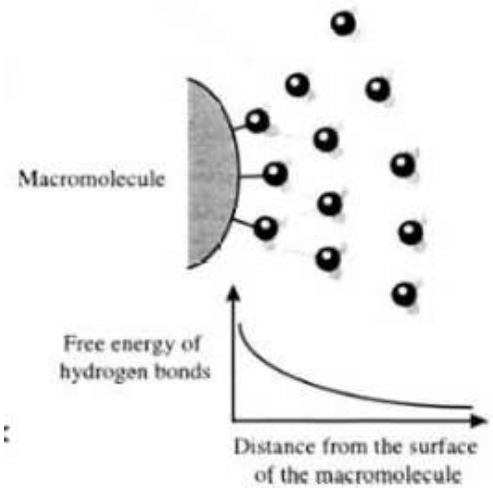
- 4. Non-polar aliphatic and aromatic amino acids → Water clathrate formation around a polar group. Water induced association of non-polar groups (Hydrophobic)
- No physical electrostatic interaction, but “cage water” occupies the space and surrounds the aliphatic or aromatic amino acids



**Water Hydration Layers**

1. Monolayer (Or constitution) water
  - Structural hydration water → H-bonded to protein groups and peptide bonds; Stabilizes protein structure (Higher free energy of hydrogen bonds)
  - Hydrophobic “hydration” water → Water arrays in “cage-like” structures around non-polar groups. Also stabilizes protein structure but the driving force derived from an increase in water entropy

**• Monolayer, constitutional**

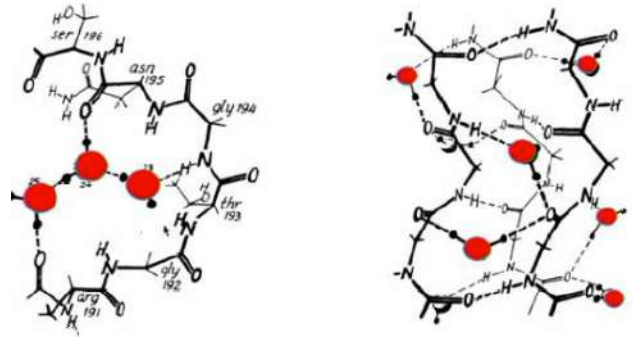


2. Multilayer (Or solvation) water
  - Layers of water with progressively decreasing structure (increasing mobility) that surround monolayer water
3. Hydrodynamic hydration water
  - Surrounds and in the crevices of protein
  - Moves with protein but is not bounded to protein

4. Bulk Water
  - Totally free water that is available only in dilute protein solutions

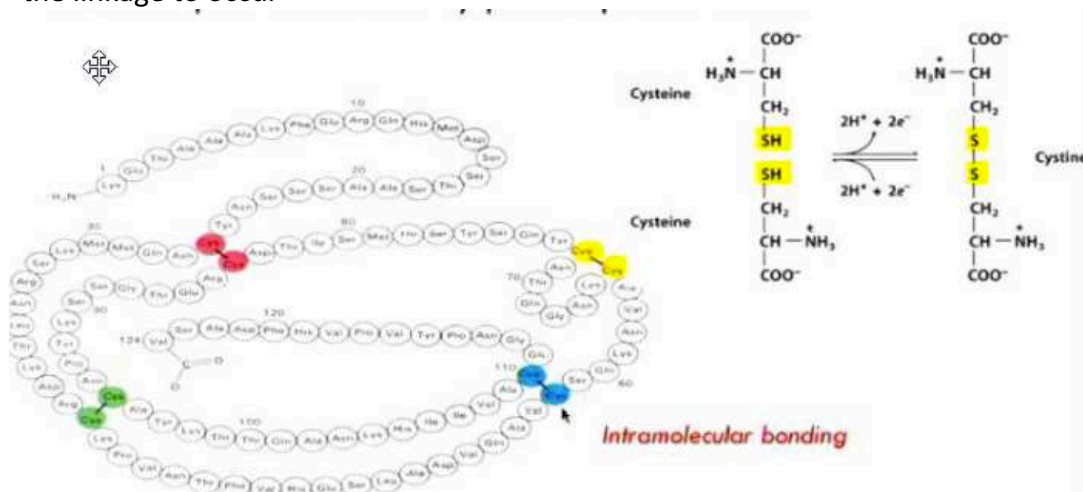
### Additional Interactions: Water Bridge

- Stabilizes protein structure by protein-water-protein interactions
- Water binding contributes to the final folding pattern and stability of papain and collagen → Structural water, part of the protein. Although the interactions are not covalent, they are strong enough to stabilize the protein structure



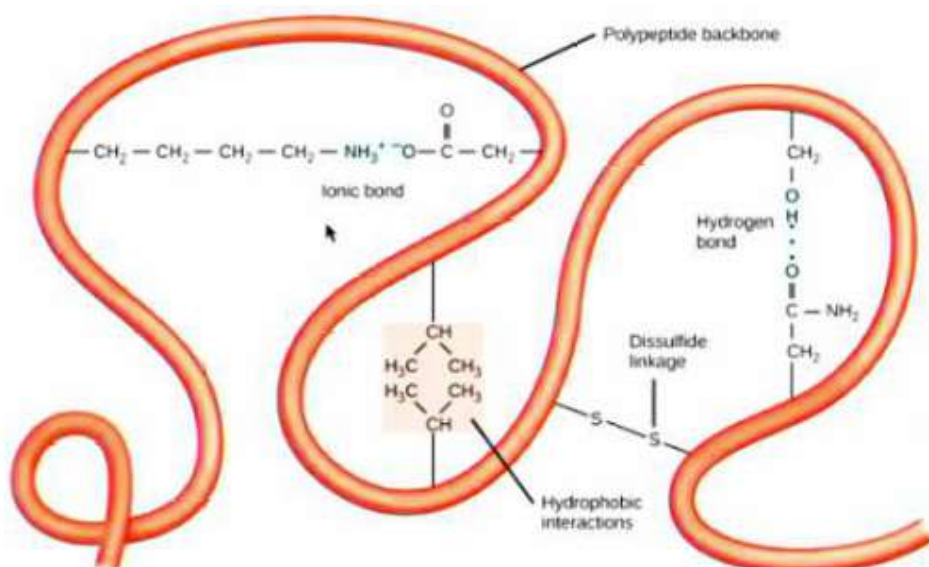
### Additional Interactions; Disulfide Covalent Bonds

- Stabilizes protein structure by protein-protein bonds
- Intramolecular bonding between the sulfides of Cysteine
- The folding pattern must allow the Cysteines to be close enough together to drive the linkage to occur



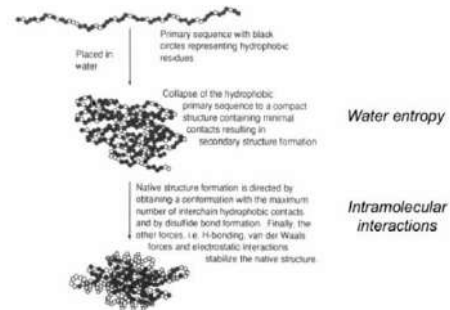
### Protein Folding

- Various interactions contribute to stabilizing the folded protein
- E.g. Ionic bonds, Hydrophobic interactions, Disulfide linkage and hydrogen bonds



## Folding Events

- When a primary sequence is placed with water, collapse of the hydrophobic primary sequence to a compact structure occurs, containing minimal contacts resulting in secondary structure formation → Folding driven by Water Entropy
- Native structure formation is directed by obtaining a conformation with the maximum number of inter-chain hydrophobic contacts and by disulfide bond formation. Finally, the other forces i.e. H bonding, van der Waals forces and electrostatic interactions, stabilize the native structure → Folding driven by Intramolecular interactions



## Protein Denaturation

- Involves the destruction of both the secondary and tertiary structure while the primary structure (sequence of amino acids) remains the same
- It is the reverse of protein folding which produce order in the form of those structures

## Denaturation Effects

1. Denaturation of globular proteins decrease solubility
  - Native globular proteins are water soluble because in the folded state, most hydrophobic groups are buried inside protein, while most hydrophilic groups are exposed on the surface. Denaturation exposes hydrophobic groups, creating high surface hydrophobicity that lower protein-water interaction
2. Denaturation of protein causes loss of biological activity
  - Biological information resides in tertiary structure
  - Denaturation destroys tertiary structure
3. Denaturation of protein increases susceptibility to proteolytic attack
  - Proteases binds to specific amino acids residues of polypeptides in order to cleave peptide bonds
  - Denaturation exposes amino acids sequence of proteins for proteases binding

## Enthalpy Change

- Chemical reaction involved enthalpy change
- Unit = KJ per mol
- $\Delta H = H(\text{Products}) - H(\text{Reactants})$
- $\Delta H$  negative → Exothermic reaction that releases heat. Enthalpy driven changes
- $\Delta H$  positive → Endothermic reaction that absorbs heat. Needs input of energy for change to occur

