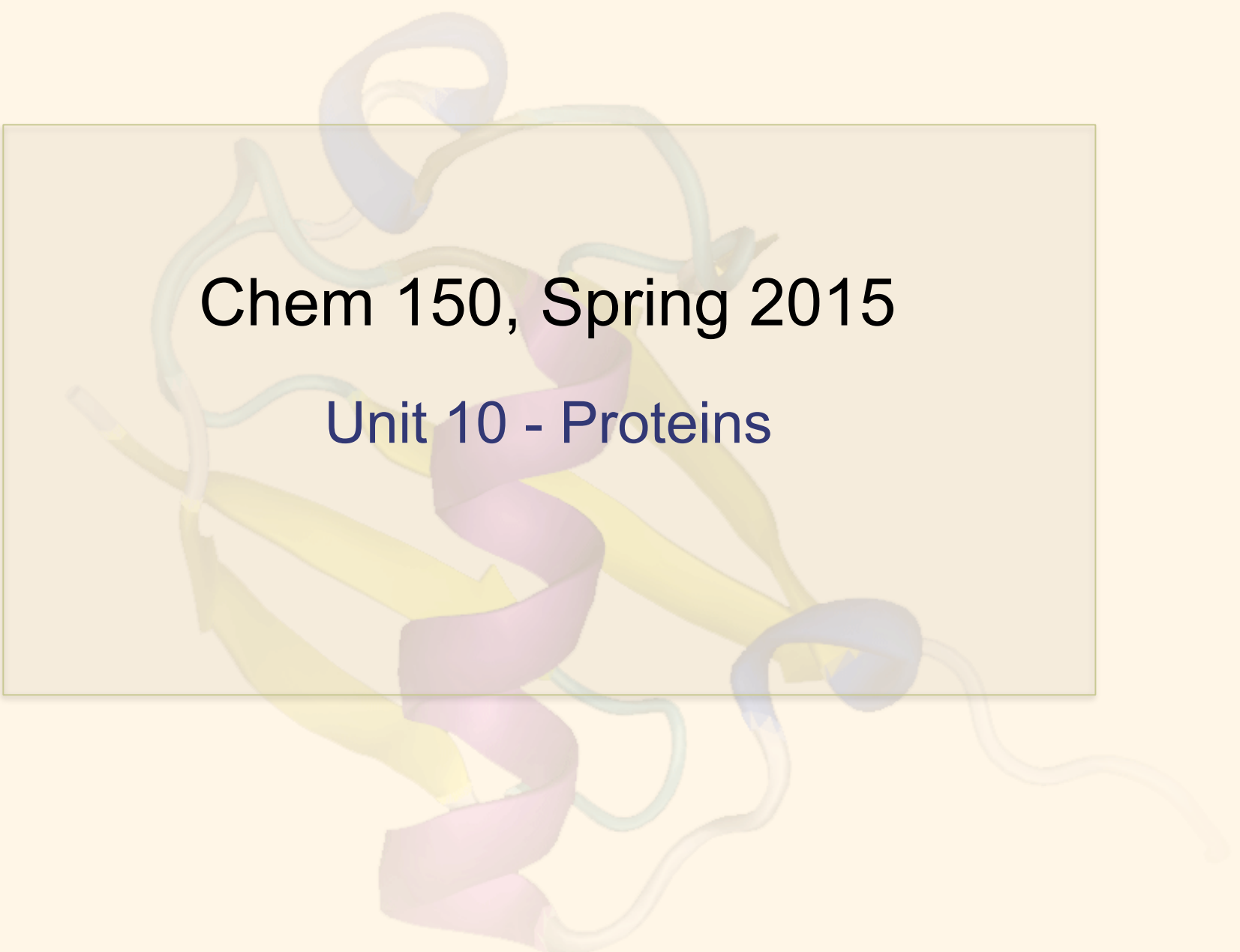




Chem 150, Spring 2015

Unit 10 - Proteins

Introduction

- Biomolecules include
 - ✦ Proteins
 - ✦ Nucleic acids
 - ✦ Carbohydrates
 - ✦ Lipids
- Some can be quite large and complex, but comprise a limited number of elements (C,H,O,N,S,P) arranged in predictable patterns.

Introduction

- Human cells are estimated to contain 30,000 different proteins



- **Proteins**

- ✦ Play many important functional roles:
 - Catalysts (enzymes)
 - Transporters (*e.g.* hemoglobin)
 - Receptors (*e.g.* Insulin receptor)
 - Hormones (*e.g.* Insulin)
 - Mechanical transducers (*e.g.* muscles)
 - Defence (*e.g.* Antibodies)
 - *et al.*

- **Proteins**

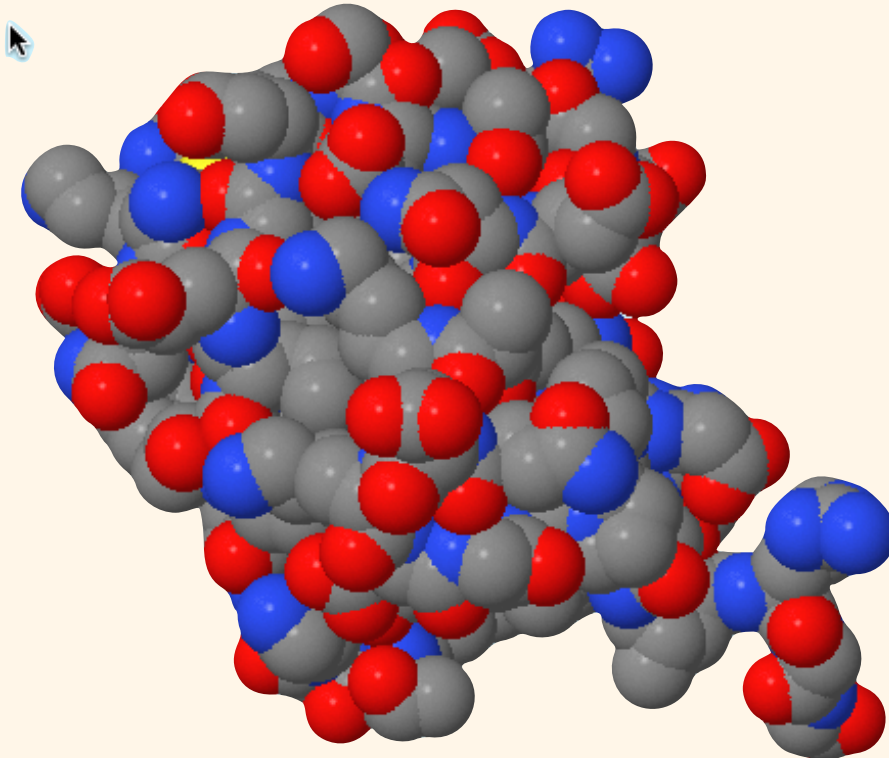
- ✦ Are made from linear polymers (chains) of 20 different amino acids.
- ✦ The sequence of amino acids is determined by the genes

DNA (genes) → mRNA → protein

- ✦ The chain must usually adopt a well-defined, three-dimensional structure, in order to function.
- ✦ The folding of the chain into its proper three-dimensional structure is determined by non-covalent interactions.

- **Proteins**

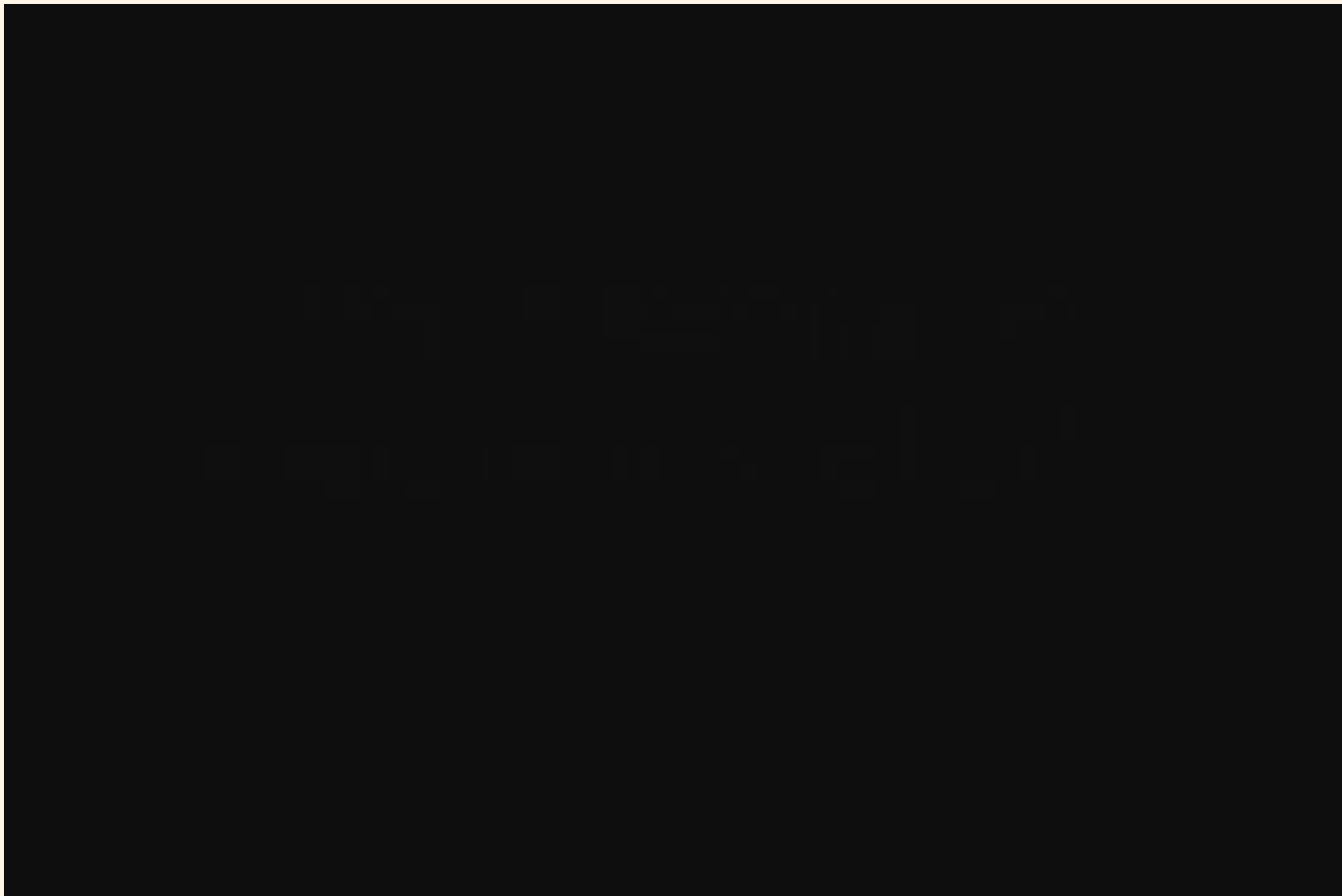
- ✦ The chain must usually adopt a well-defined, three-dimensional structure, in order to function.



Introduction

- **Proteins**

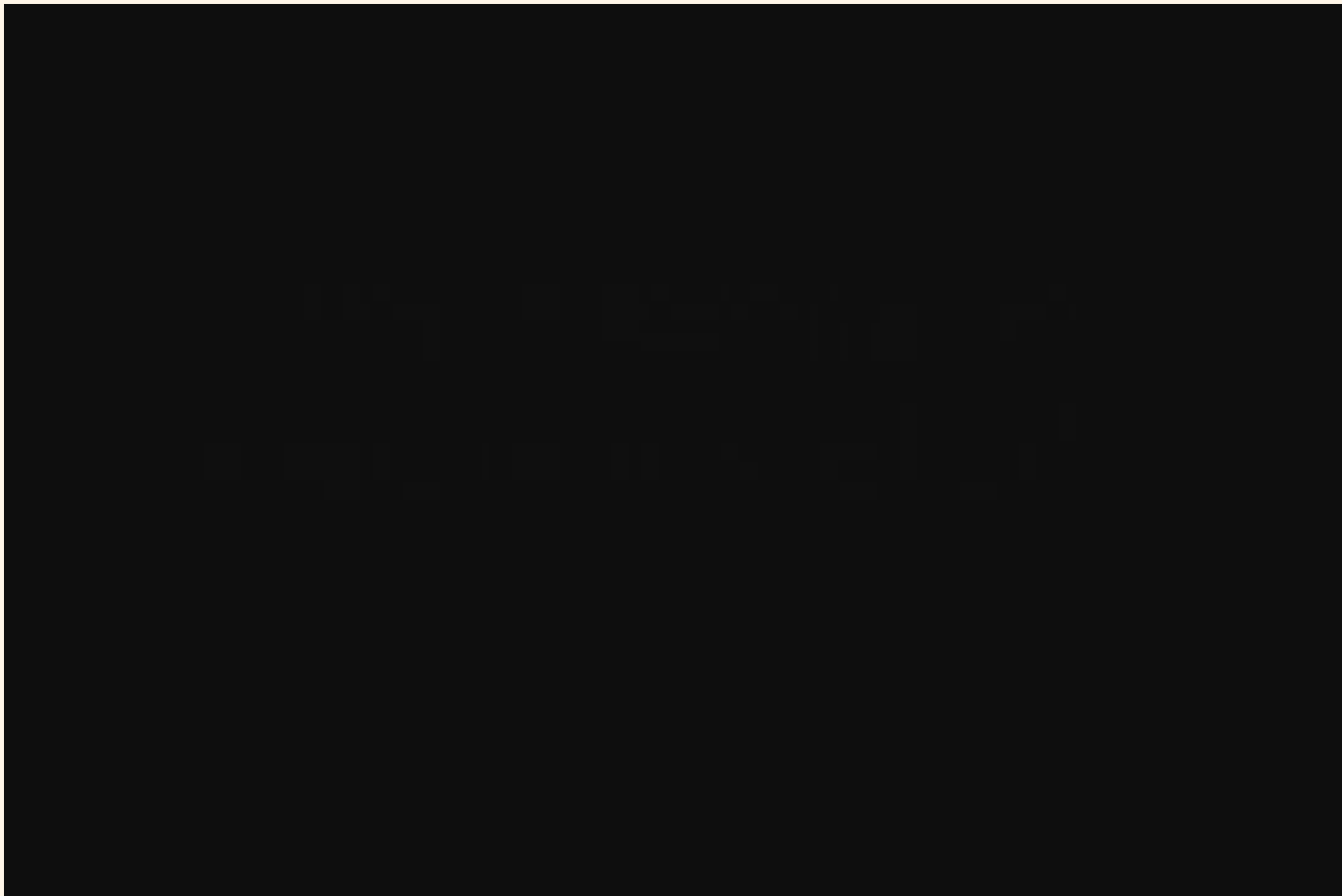
- ✦ Play a big role in the machinery of life



Introduction

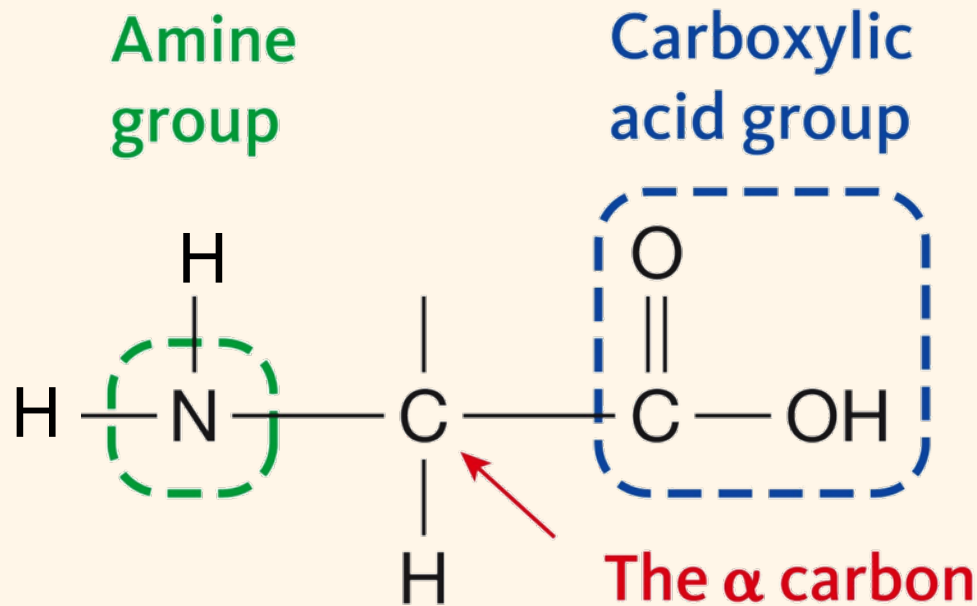
- **Proteins**

- ✦ Play a big role in the machinery of life



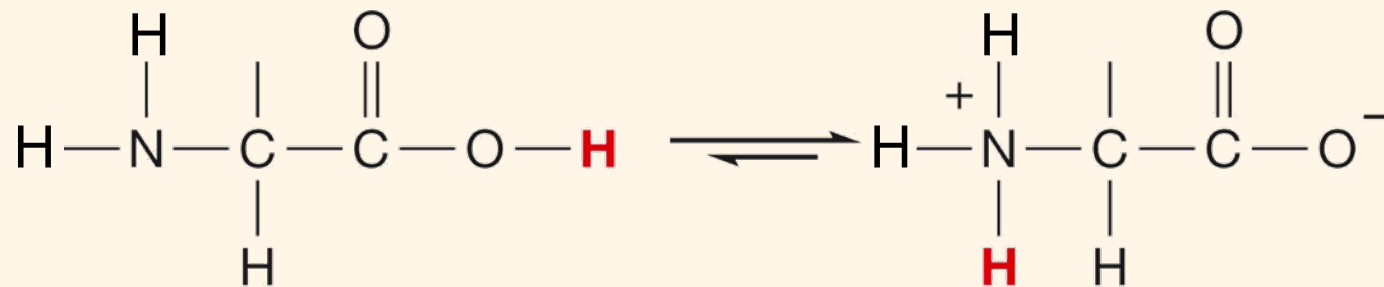
14.1 Amino Acids

- Amino acids are compounds that contain both an **amine** and a **carboxylic acid**.
- The **alpha carbon** of the carboxylic acid is attached to an amine, a hydrogen and some other group (**side chain**) that makes each amino acid unique.



Zwitterion

- When a compound contains both an amine and an acid, the hydrogen ion will move from the acid to the amine, resulting in a structure with one positive charge and one negative charge, which is called a **zwitterion**.
- Although this is an equilibrium, the vast majority of the molecules appear in the ionized (zwitterion) form, so that is how amino acids are typically drawn.

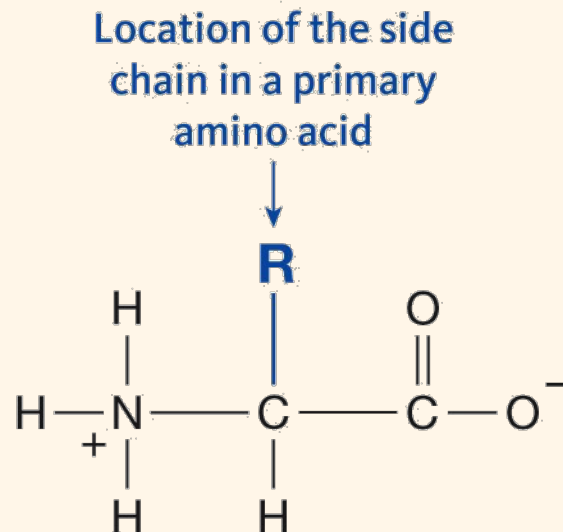


Unionized form

Zwitterion form

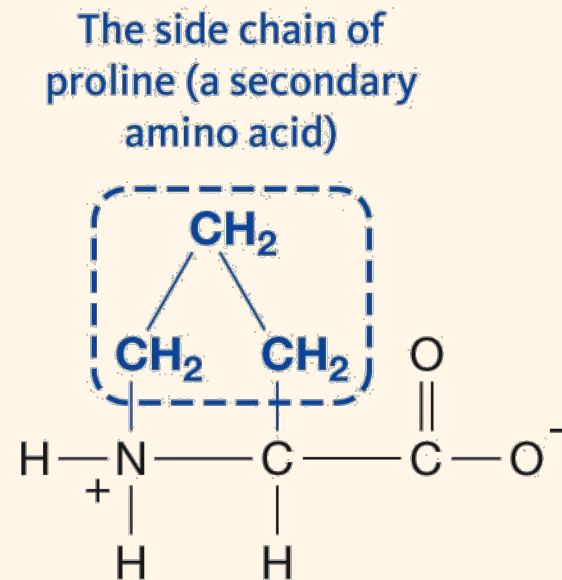
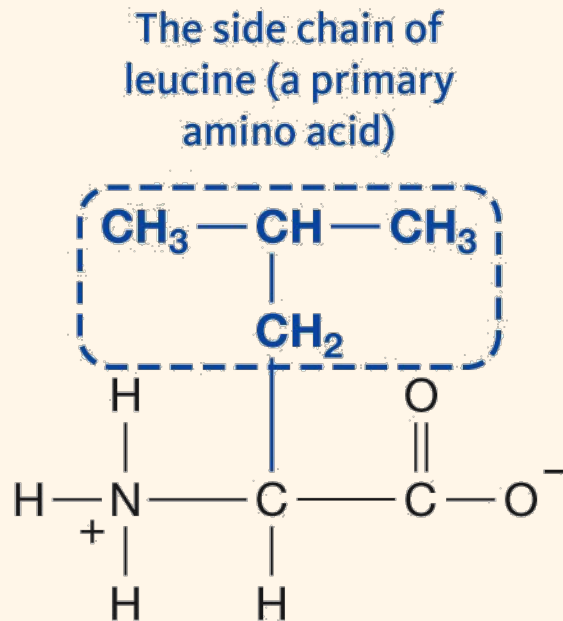
Side Chains

- Most proteins are built from a combination of 20 different amino acids.
- These amino acids are unique because they have differing side chains attached to the alpha carbon.
- The side chain is often denoted as “R”

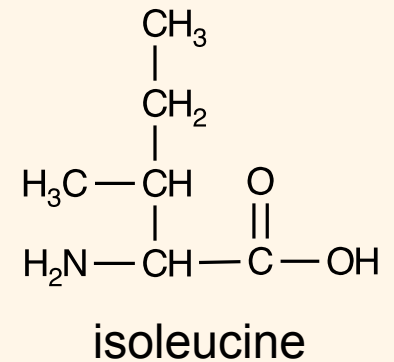
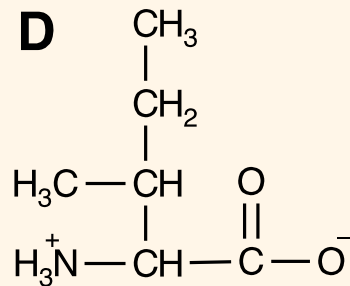
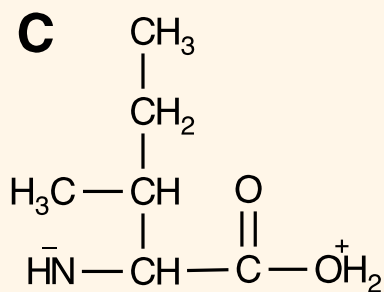
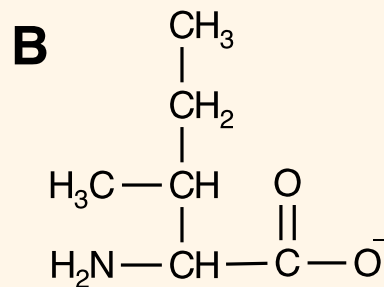
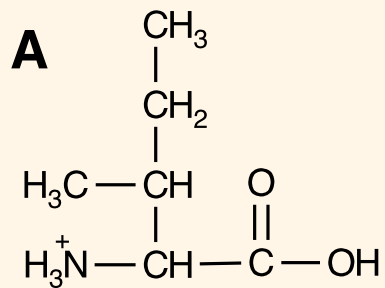


The Side Chain for Proline

- For all except proline, the side chain of each amino acid is only attached to the alpha carbon.
- Proline is made of a ring that connects the alpha carbon to the amine nitrogen.



Try It!



Classification of Amino Acids

- Amino acids are classified based on the side chain.
- **Hydrophobic (nonpolar)** amino acids: side chains are nonpolar and thus not attracted to water
- **Hydrophilic (polar)** amino acids: side chains contain, OH, SH, an amide, a carboxylic acid or an amine.
 - ✦ Side chains with OH, SH, or an amide are considered **polar neutral**
 - ✦ Side chains with a carboxylate groups that are **negatively charged at pH 7** are also classified as **acidic**.
 - ✦ Side chains with an ammonium groups that are **positively charged at pH 7** are also classified as **basic**.

Hydrophobic (Nonpolar) Amino Acids

- Hydrocarbon side chains (on board)

Hydrophilic (Polar) Amino Acids: Neutral

- Side chains contain polar functional groups (on board)

Hydrophilic (Polar) Amino Acids: Acidic

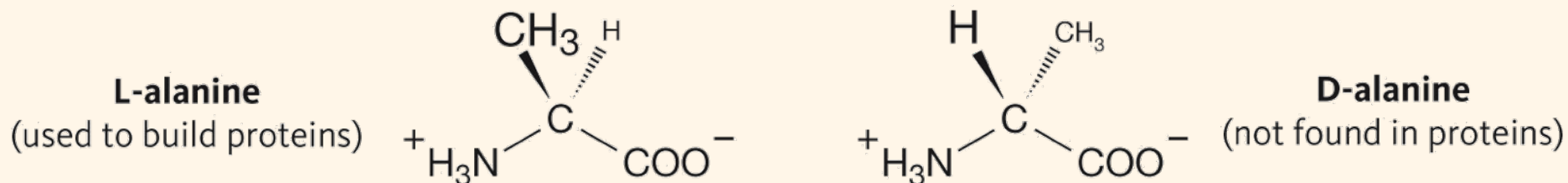
- Aspartic Acid and Glutamic Acid are both polar acidic because their side chains contain a carboxylic acid group (on board)

Hydrophilic (Polar) Amino Acids: Basic

- Lysine, Arginine, and Histidine are polar basic because their side chains contain amines (on board).

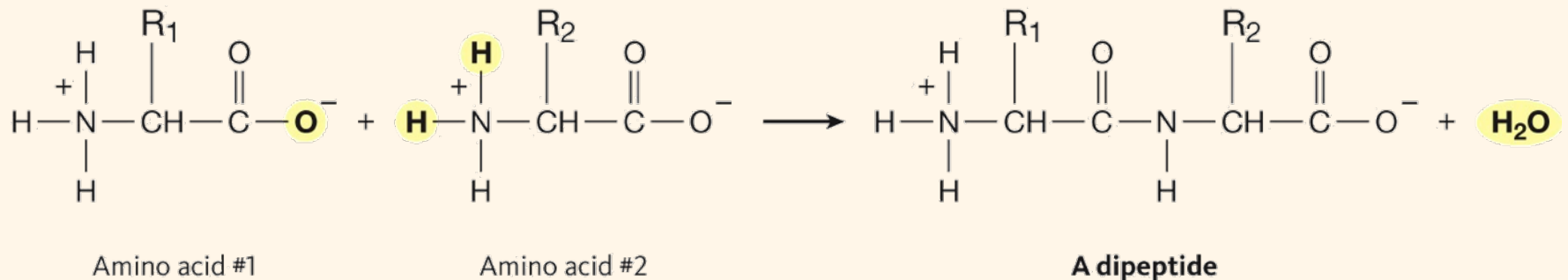
Chirality of Amino Acids

- Except for glycine, all of the amino acids contain a chiral alpha carbon.
- Therefore all amino acids have two possible forms, called **enantiomers**.
- Naturally occurring amino acids are all left-handed, L-amino acids.
- The enantiomer of L-amino acids rarely in nature and are called D-amino acids.



14.2 Peptide Bonds and the Secondary Structure of a Protein

- Amino acids can condense to form chains of amino acids.
- The amide bond that is formed is called a **peptide bond**, and two amino acids linked together is called a **dipeptide**.

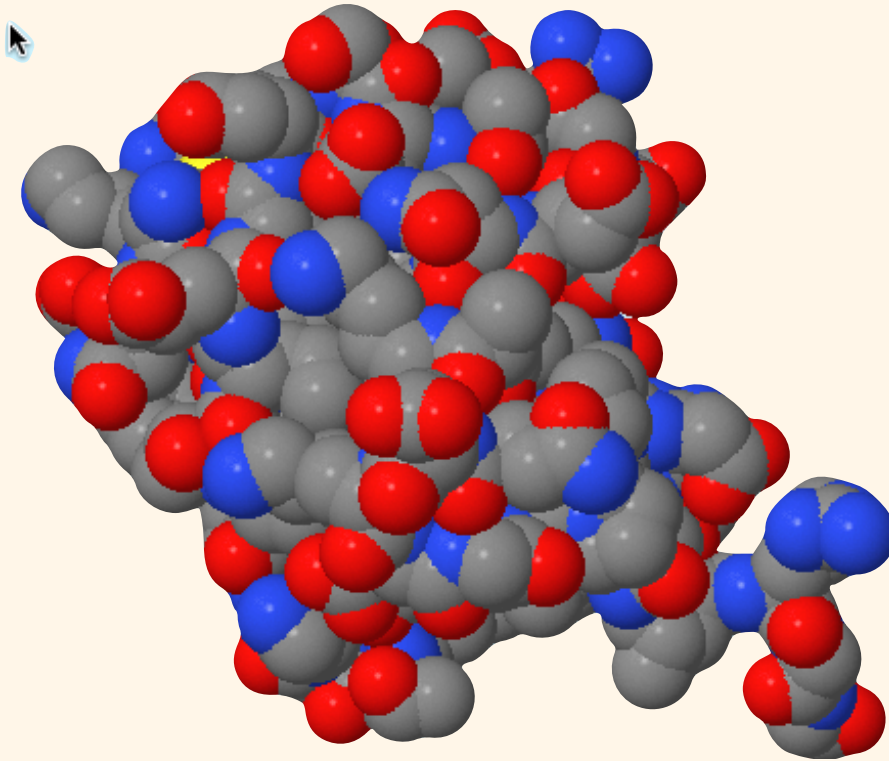


Peptides and Proteins

- More amino acids can be added on to a dipeptide forming a tripeptide, tetrapeptide, etc...
- The prefixes indicate how many amino acids are present in the structure.
- A large number of amino acids linked together is known as a **polypeptide**.
- Proteins are polypeptides typically with 100-500 amino acids.

Peptides and Proteins

- The protein we looked at before, which is called ubiquitin, contains only 76 amino acid residues.



Primary Structure

- The **primary structure** of a protein is specific order order the amino acids in the protein that is determined by the gene for that protein
- It is given beginning with the amino acid that has the free amino group (the **N-terminal amino acid**) ending with the amino acid with the free carboxylate group (the **C-terminal amino acid**)

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Primary Structure

- The primary structure of a protein is a specific order of amino acids that determines the protein's function.

- It is given by the sequence of amino acids, starting from the free amino group (the N-terminus) and ending with the carboxylate group (the C-terminus).

primary structure of the enzyme phosphofructokinase

```
MET ILE LYS LYS ILE GLY VAL LEU THR SER GLY GLY ASP  
ALA PRO GLY MET ASN ALA ALA ILE ARG GLY VAL VAL ARG  
SER ALA LEU THR GLU GLY LEU GLU VAL MET GLY ILE TYR  
ASP GLY TYR LEU GLY LEU TYR GLU ASP ARG MET VAL GLN  
LEU ASP ARG TYR SER VAL SER ASP MET ILE ASN ARG GLY  
GLY THR PHE LEU GLY SER ALA ARG PHE PRO GLU PHE ARG  
ASP GLU ASN ILE ARG ALA VAL ALA ILE GLU ASN LEU LYS  
LYS ARG GLY ILE ASP ALA LEU VAL VAL ILE GLY GLY ASP  
GLY SER TYR MET GLY ALA MET ARG LEU THR GLU MET GLY  
PHE PRO CYS ILE GLY LEU PRO GLY THR ILE ASP ASN ASP  
ILE LYS GLY THR ASP TYR THR ILE GLY PHE PHE THR ALA  
LEU SER THR VAL VAL GLU ALA ILE ASP ARG LEU ARG ASP  
THR SER SER SER HIS GLN ARG ILE SER VAL VAL GLU VAL  
MET GLY ARG TYR CYS GLY ASP LEU THR LEU ALA ALA ALA  
ILE ALA GLY GLY CYS GLU PHE VAL VAL VAL PRO GLU VAL  
GLU PHE SER ARG GLU ASP LEU VAL ASN GLU ILE LYS ALA  
GLY ILE ALA LYS GLY LYS LYS HIS ALA ILE VAL ALA ILE  
THR GLU HIS MET CYS ASP VAL ASP GLU LEU ALA HIS PHE  
ILE GLU LYS GLU THR GLY ARG GLU THR ARG ALA THR VAL  
LEU GLY HIS ILE GLN ARG GLY GLY SER PRO VAL PRO TYR  
ASP ARG ILE LEU ALA SER ARG MET GLY ALA TYR ALA ILE  
ASP LEU LEU LEU ALA GLY TYR GLY GLY ARG CYS VAL GLY  
ILE GLN ASN GLU GLN LEU VAL HIS HIS ASP ILE ILE ASP  
ALA ILE GLU ASN MET LYS ARG PRO PHE LYS GLY ASP TRP  
LEU ASP CYS ALA LYS LYS LEU TYR
```

Primary Structure

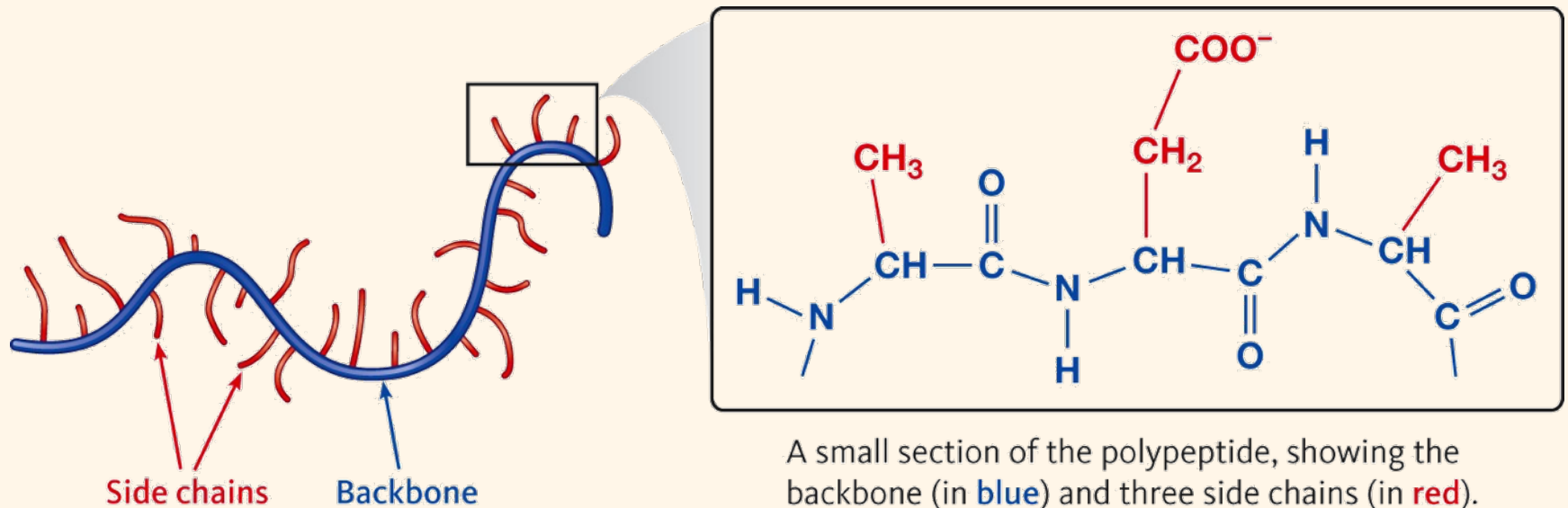
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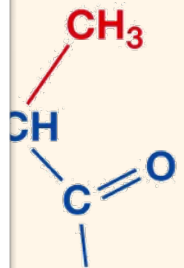
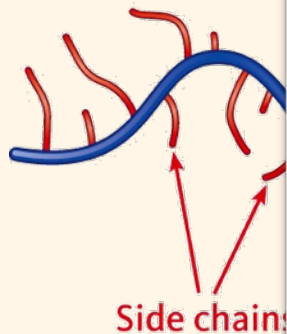
Protein Backbone

- Peptide groups form the backbone of all polypeptides.
- Side chains extend away from the backbone.



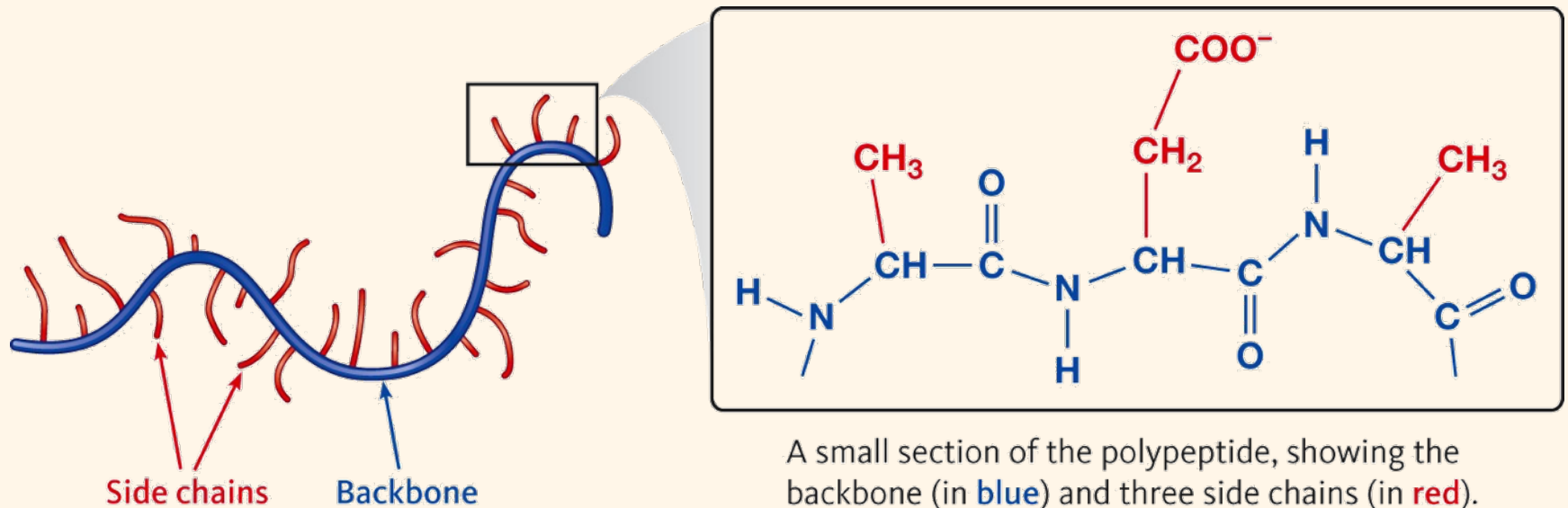
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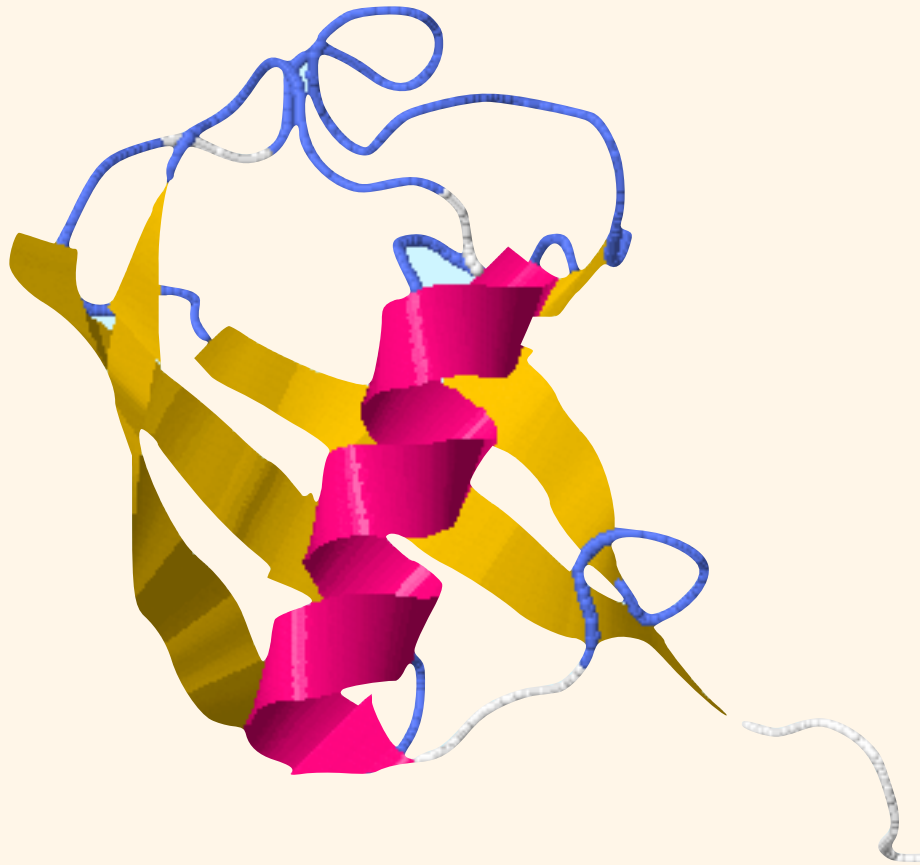
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Secondary Structures

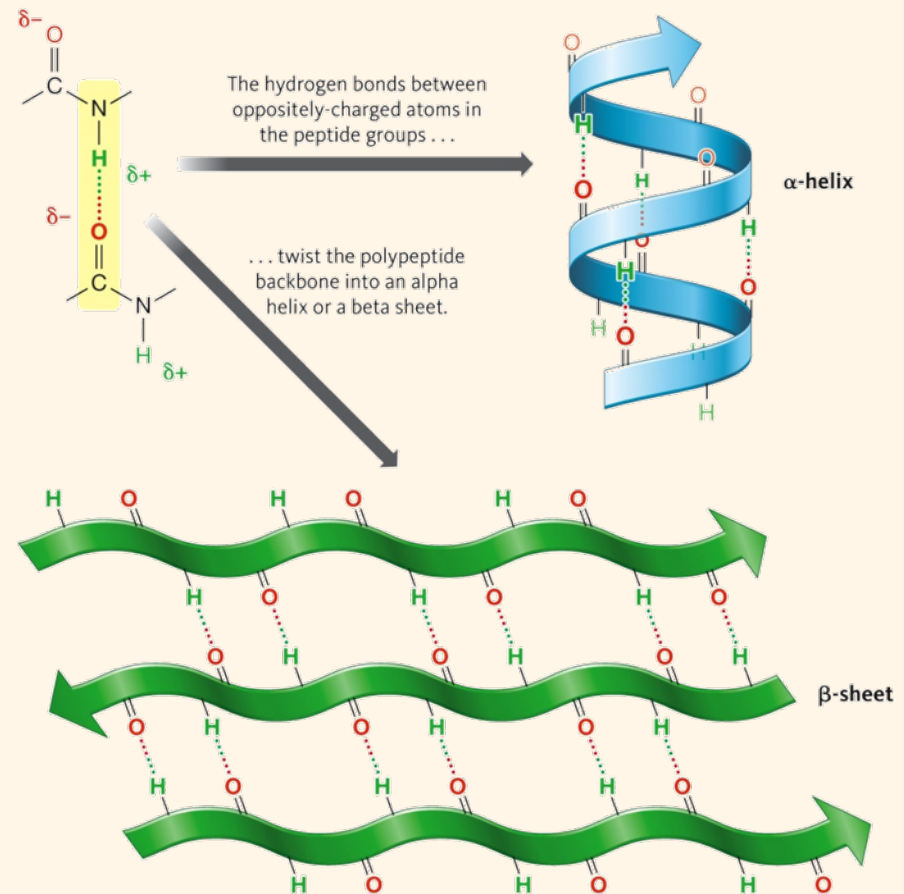
- The polypeptide backbone can produce regular, repeating structures called **secondary structures**.



Secondary Structures

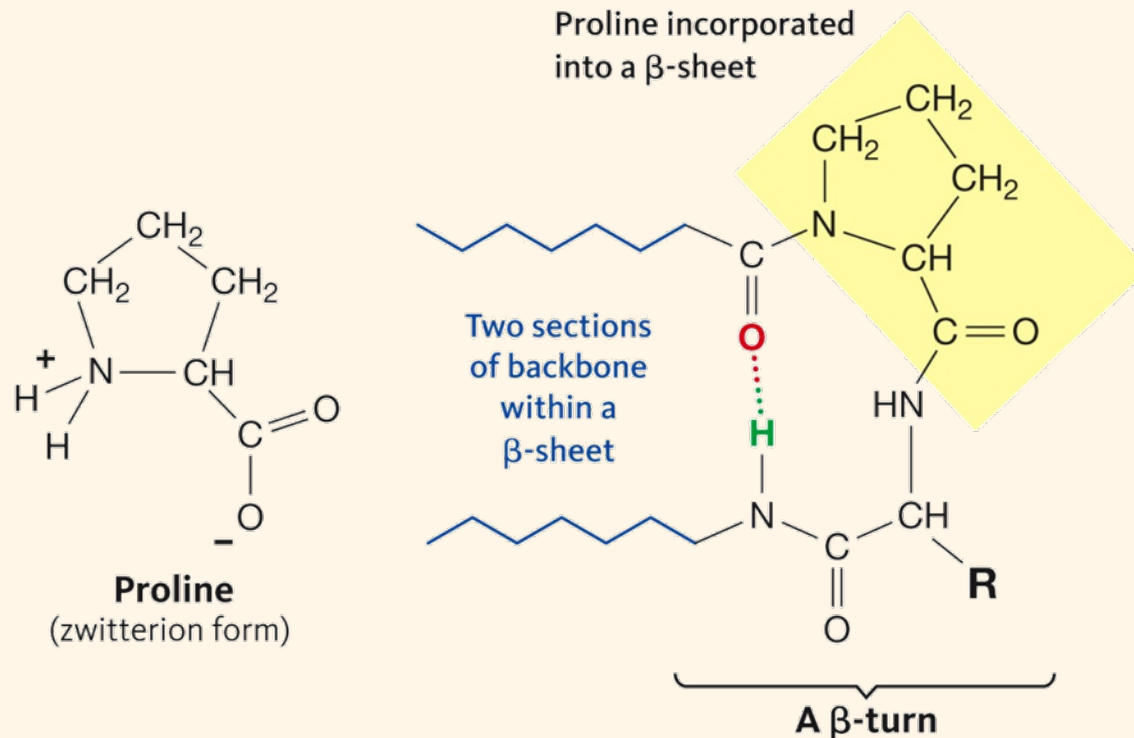
- There are two primary types of secondary structures; **alpha helices** and the **beta sheets**.

♦ Hydrogen bonds between peptide groups of the backbone help to stabilize these structures.



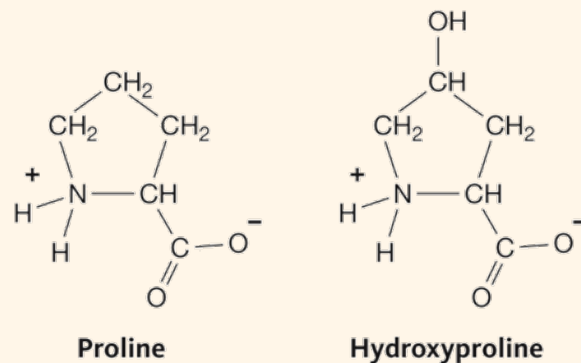
Proline in Secondary Structures

- Secondary structures form regardless of the primary structure.
- However, proline has a unique rigidity, and typically appears in the turn (β -turn) of a beta sheet.



The Triple Helix and Collagen

- A triple helix is a secondary structure in collagen.
- It is composed of three chains wrapped around each other and held together by hydrogen bonding of peptide groups on adjacent chains.
- Glycine, proline, and hydroxyproline (a modified proline) are the primary amino acids in collagen.

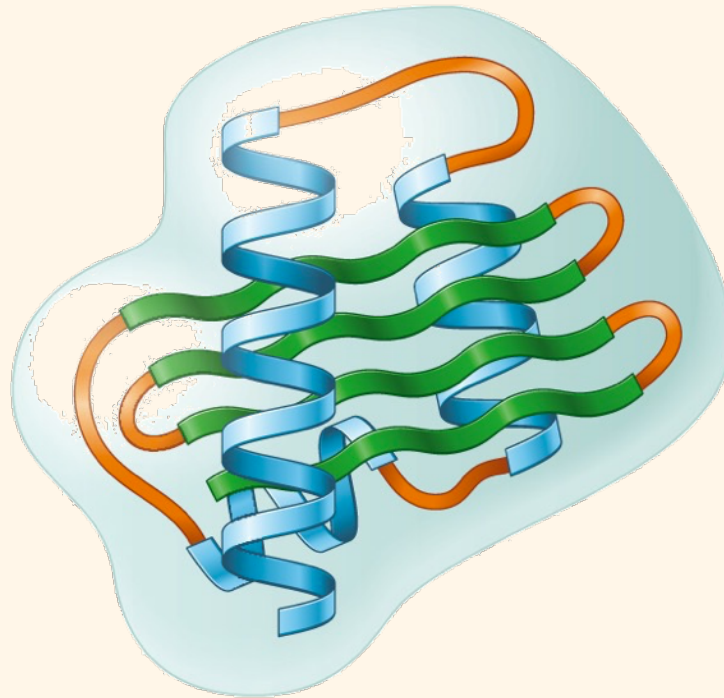


The polypeptide
backbones in
the triple helix
of collagen

14.3 Side Chain Interactions and Tertiary Structures

- The folded, 3-dimensional structure is called the **tertiary structure**:

This protein has:



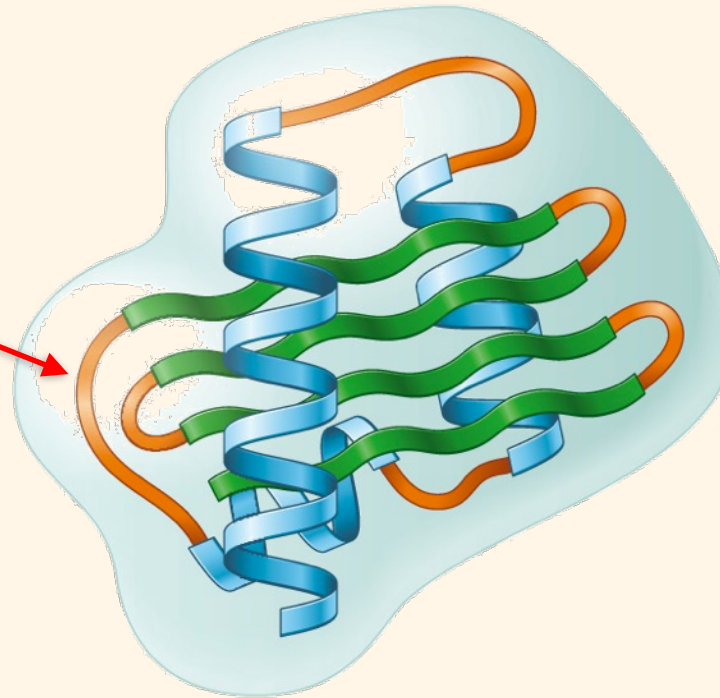
14.3 Side Chain Interactions and Tertiary Structures

- The folded, 3-dimensional structure is called the **tertiary structure**:

This protein has:

A Primary structure

(the polypeptide chain, which all proteins have)



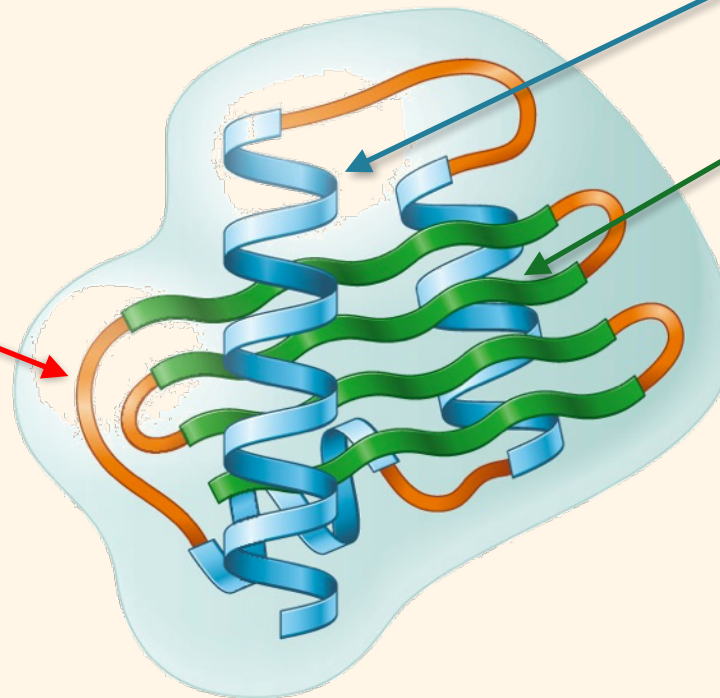
14.3 Side Chain Interactions and Tertiary Structures

- The folded, 3-dimensional structure is called the **tertiary structure**:

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Secondary structure
(the α -helices and β -sheets)



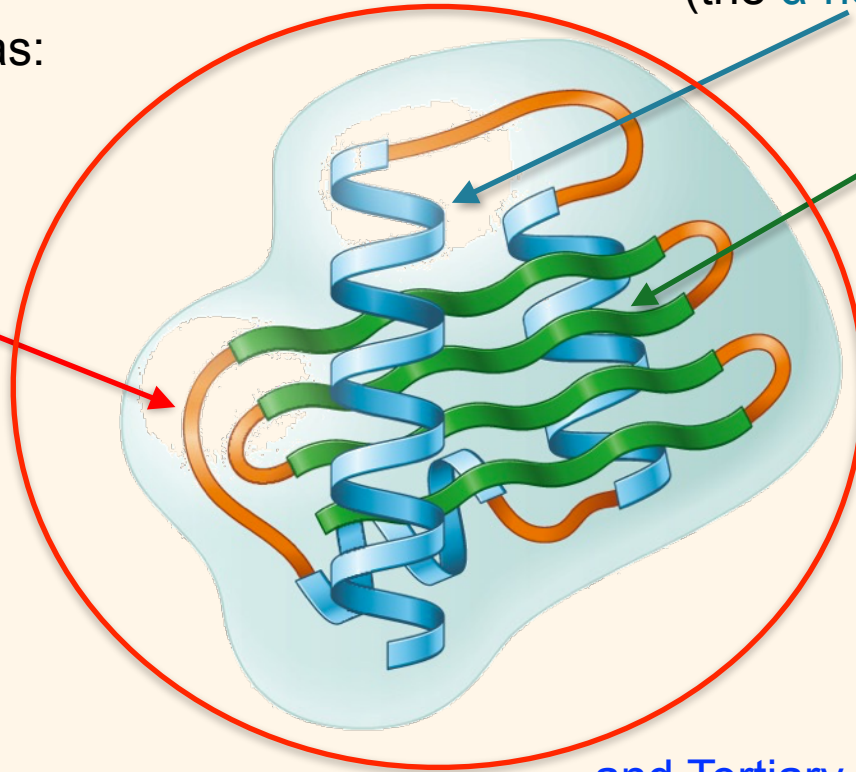
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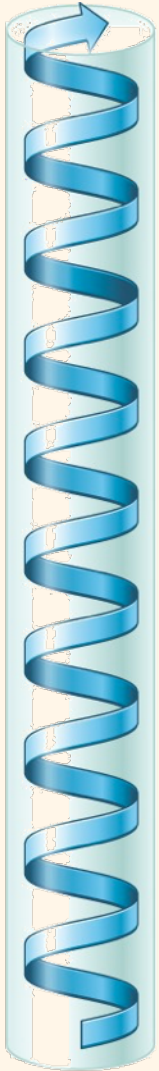


and Tertiary structure
(the 3-dimensional fold)

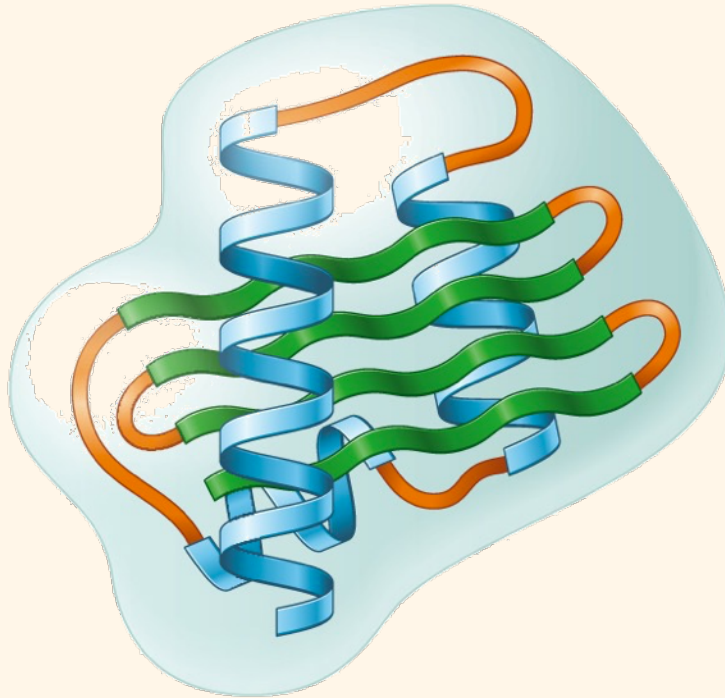
Protein Classification

- Proteins are classified based on how they fold and what elements of structure they contain
 - ✦ **Globular**: protein is folded into a compact shape. Most water soluble proteins are globular
 - ✦ **Fibrous**: long narrow water insoluble proteins. Hair, silk, tendons and ligaments are made by fibrous proteins.
 - ✦ **Membrane**: proteins anchored in membranes.

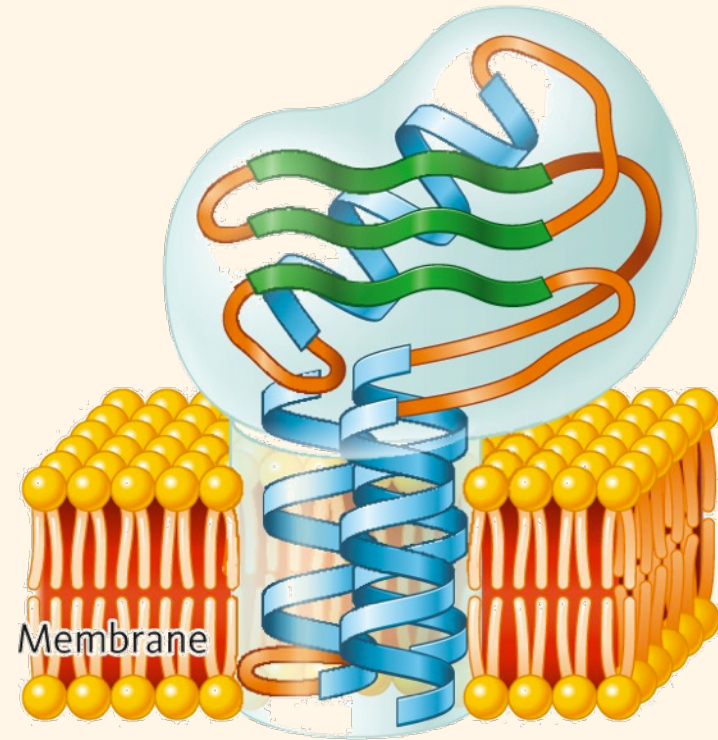
Classes of Proteins



Fibrous protein



Globular protein



Membrane protein

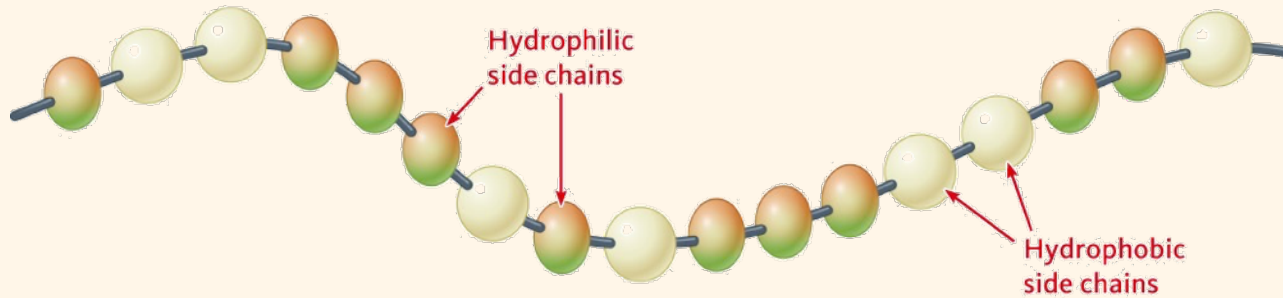
Tertiary Structures

- Interactions **between side chains** of the amino acids in the proteins primarily determine the final shape of a protein.
- We will focus here on globular proteins.
 - ✦ Hydrophobic amino acids tend to cluster together in the interior of a folded protein.
 - ✦ Hydrophilic amino acids tend to be on the surface of a folded protein.
 - ✦ Ionized acidic and basic amino acids are often found near each other
 - ✦ A cysteine side chain can form a disulfide bond with another cysteine side chain.

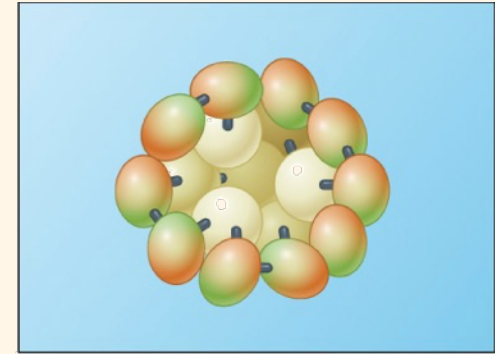
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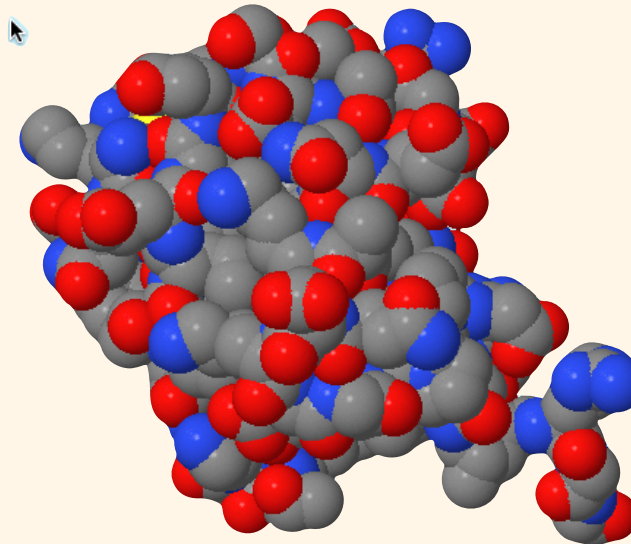
Hydrophilic and Hydrophobic Interactions



Polar (hydrophilic) and nonpolar (hydrophobic) amino acids are arranged randomly in a polypeptide.



When the polypeptide is surrounded by water, the polar side chains are pulled to the exterior and the nonpolar side chains cluster in the interior.

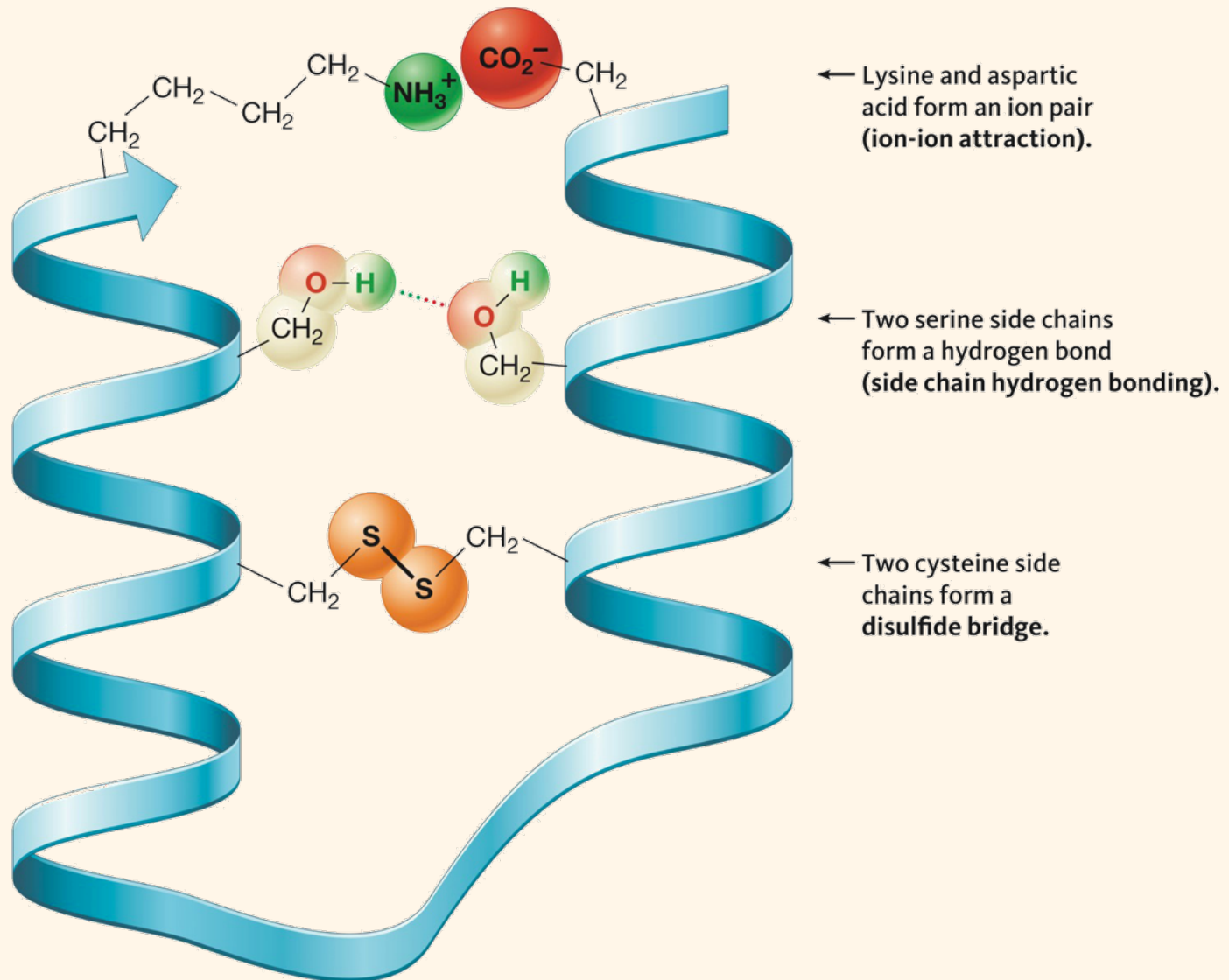


Other Side Chain Interactions

- If buried, hydrophilic side chains will look for ways to **hydrogen bond** with other side chains or the backbone.
- Ionized acidic and basic side chains will look for ways to form **ion-ion attractions (salt bridges)**.
- If two cysteine side chains are buried next to one another, they can react to form a **disulfide bridge**.
 - ✦ Disulfide bridge formation is an oxidation reaction that requires either O_2 or a redox coenzyme like NAD^+ .



Side Chain Interactions

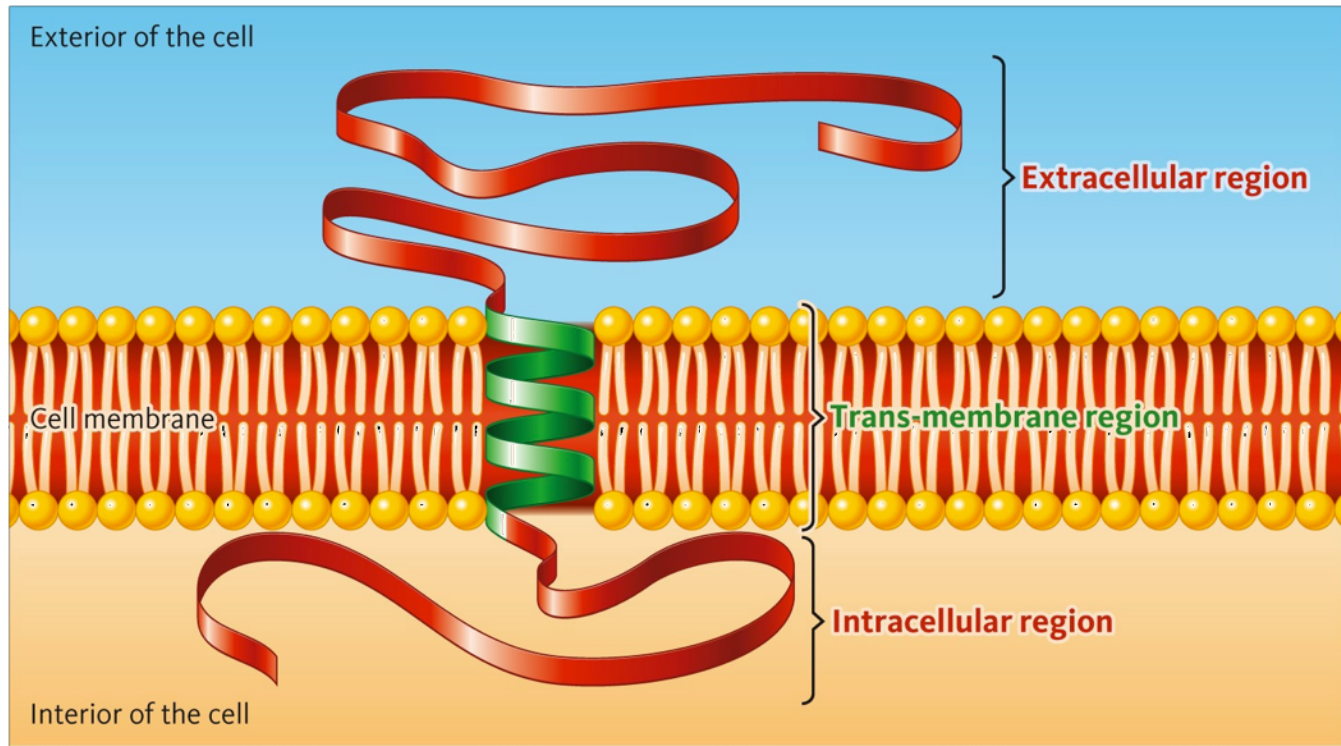


Amino Acids in Membrane Proteins

- Membranes are composed of nonpolar compounds, so the parts of the membrane protein that traverse or embed into the membrane are primarily hydrophobic, inside and out.
- Extracellular and intracellular areas of the cell are aqueous, so the region of the membrane protein that contacts these areas are primarily hydrophilic on the outside.

Amino Acids in Membrane Proteins

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Region	Number of nonpolar amino acids	Number of polar amino acids	Number of charged (acidic or basic) amino acids
Intracellular	16	12	11
Trans-membrane	17	2	0
Extracellular	21	27	22

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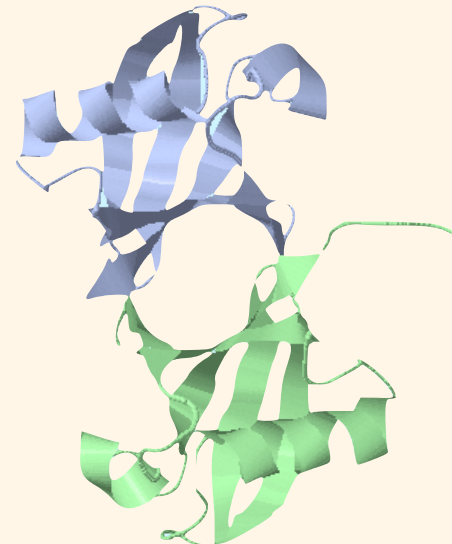
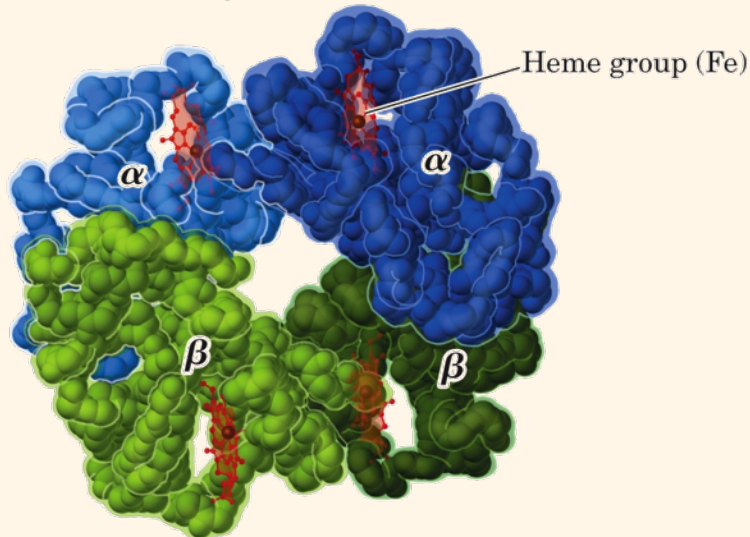
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Amino Acids in Membrane Proteins

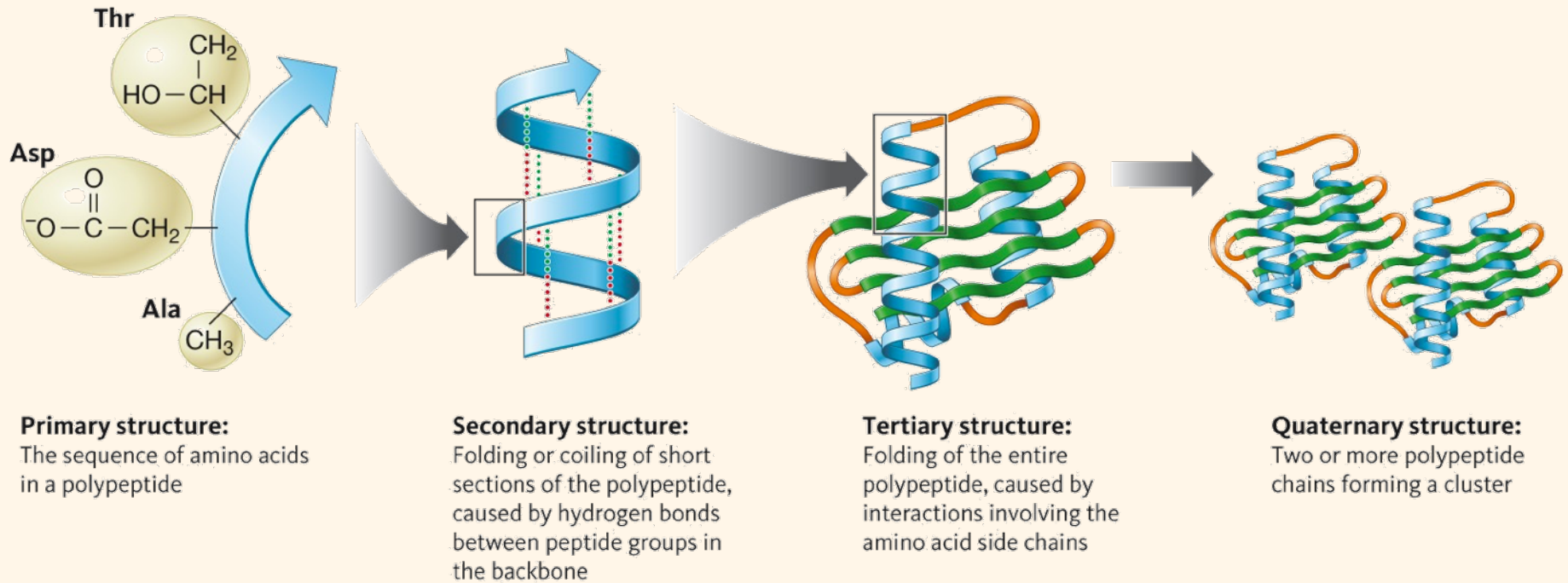
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- Extracellular and intracellular areas of the cell are aqueous, so the region of the membrane protein that contacts these areas are primarily hydrophilic on the outside.

Quaternary Structures

- Some proteins contain more than one polypeptide chains.
- These chains are held together with a variety of the interactions previously discussed.
- The **quaternary structure** of a protein is formed when two or more polypeptides join to form an active protein, like in hemoglobin



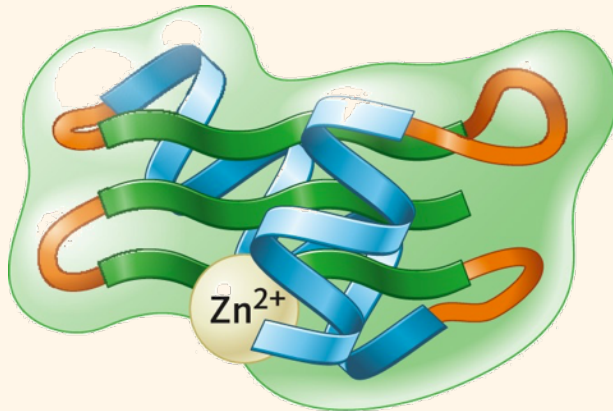
Summary of Structures



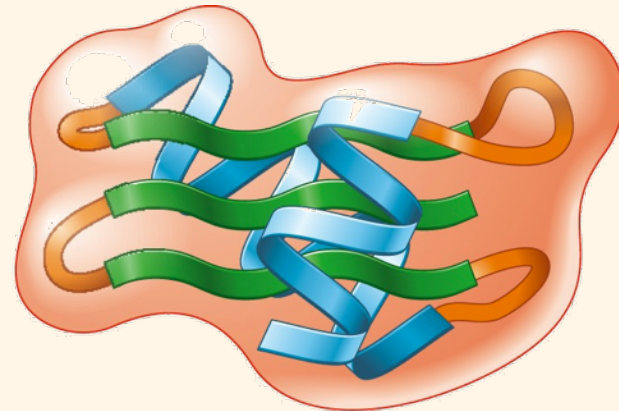
Enzymes and Cofactors

- Enzymes all contain at least one chain of amino acids. Some require an additional molecule to be active, known as a **cofactor**.
- There are two types of cofactors
 - ✦ Metal ions, such as Fe^{2+} , Fe^{3+} , Mg^{2+} , Zn^{2+} or Cu^{2+}
 - ✦ Organic compounds known as **coenzymes**, such as NAD^+ and FAD .
- ✦ Some cofactors are covalently bound to the enzyme and others are only temporarily bonded to the enzyme.
 - Most metal ions and FAD are covalently bonded to an enzyme that requires them.

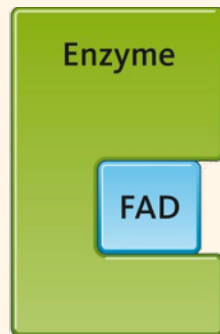
Cofactors



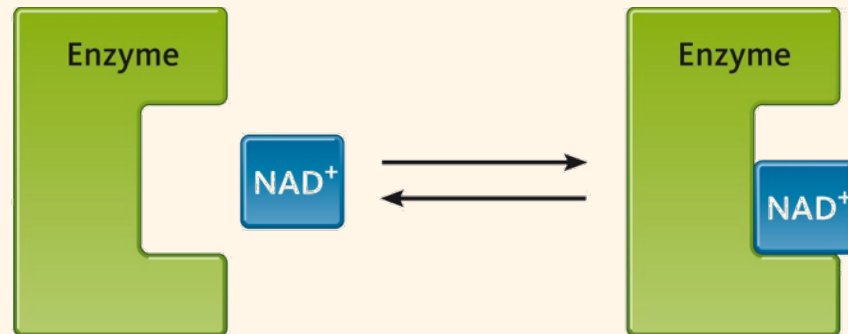
The active form of carbonic anhydrase contains a polypeptide chain and a zinc ion (the cofactor).



The polypeptide chain of carbonic anhydrase has no activity.



FAD is permanently bonded to the enzymes that require it.



NAD^+ binds temporarily to enzymes that require it.

Coenzymes

- Many of the coenzymes are derived from the vitamins we need to take in in our diet.

TABLE 7.2 Major coenzymes

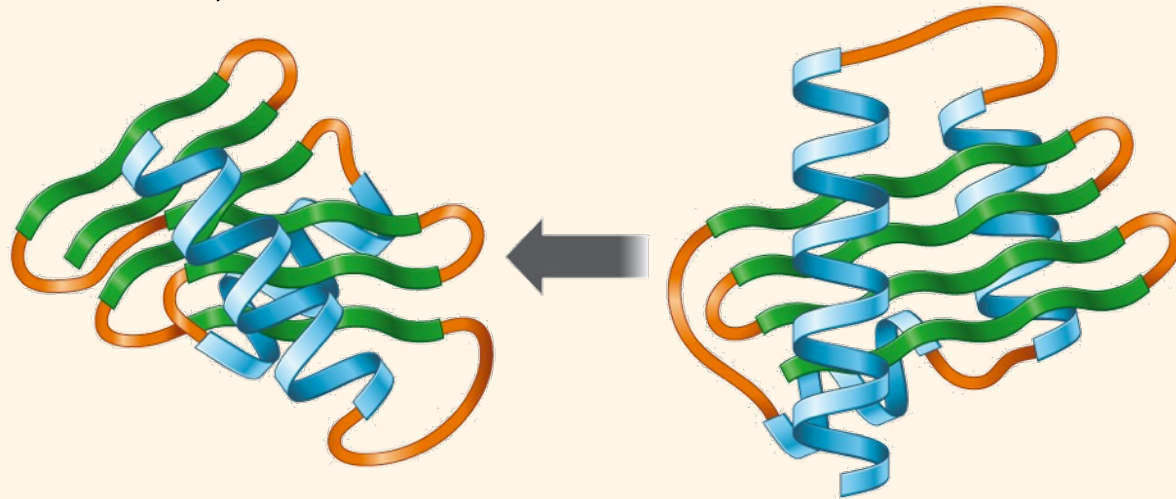
Coenzyme	Vitamin source	Major metabolic roles	Mechanistic role
Adenosine triphosphate (ATP)	—	Transfer of phosphoryl or nucleotidyl groups	Cosubstrate
<i>S</i> -Adenosylmethionine	—	Transfer of methyl groups	Cosubstrate
Uridine diphosphate glucose	—	Transfer of glycosyl groups	Cosubstrate
Nicotinamide adenine dinucleotide (NAD ⁺) and nicotinamide adenine dinucleotide phosphate (NADP ⁺)	Niacin	Oxidation-reduction reactions involving two-electron transfers	Cosubstrate
Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)	Riboflavin (B ₂)	Oxidation-reduction reactions involving one- and two-electron transfers	Prosthetic group
Coenzyme A (CoA)	Pantothenate (B ₃)	Transfer of acyl groups	Cosubstrate
Thiamine pyrophosphate (TPP)	Thiamine (B ₁)	Transfer of two-carbon fragments containing a carbonyl group	Prosthetic group
Pyridoxal phosphate (PLP)	Pyridoxine (B ₆)	Transfer of groups to and from amino acids	Prosthetic group
Biotin	Biotin	ATP-dependent carboxylation of substrates or carboxyl-group transfer between substrates	Prosthetic group
Tetrahydrofolate	Folate	Transfer of one-carbon substituents, especially formyl and hydroxymethyl groups; provides the methyl group for thymine in DNA	Cosubstrate
Adenosylcobalamin	Cobalamin (B ₁₂)	Intramolecular rearrangements	Prosthetic group
Methylcobalamin	Cobalamin (B ₁₂)	Transfer of methyl groups	Prosthetic group
Lipoamide	—	Oxidation of a hydroxyalkyl group from TPP and subsequent transfer as an acyl group	Prosthetic group
Retinal	Vitamin A	Vision	Prosthetic group
Vitamin K	Vitamin K	Carboxylation of some glutamate residues	Prosthetic group
Ubiquinone (Q)	—	Lipid-soluble electron carrier	Cosubstrate

Coenzymes

- Many of the coenzymes are derived from the vitamins we need to take in in our diet.

14.4 Protein Denaturation

- A disruption of the interactions in the protein keeps it folded can cause it to become inactive
 - ✦ This is known as **protein denaturation**.
- A denatured protein may unfold completely or shift into a different, non-active fold.



Protein folded in an
incorrect fashion
(inactive)

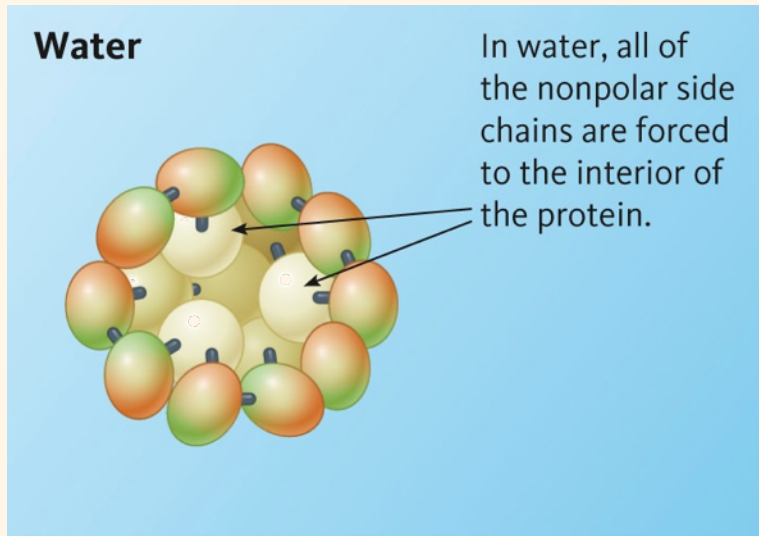
Original protein
(active)

Methods of Denaturation

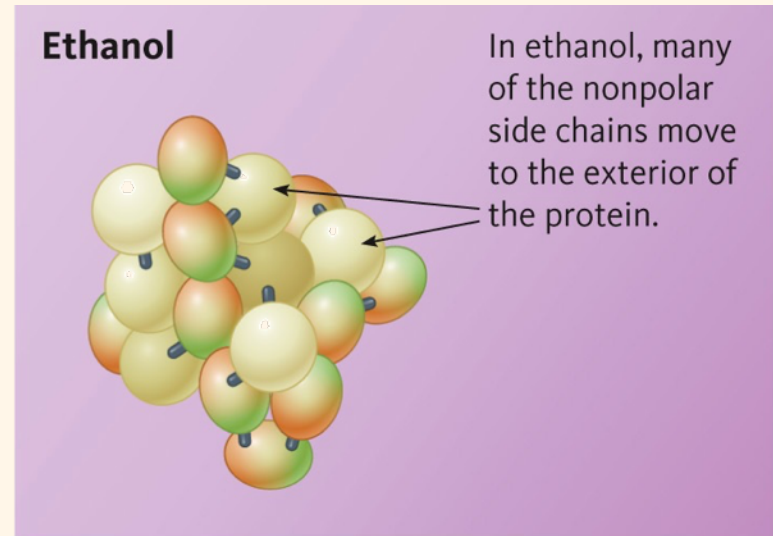
- Changing the solvent
- Changing the pH
- Raising the temperature
- Violent agitation
- Adding ionic substances

Changing the Solvents

- Organic solvents will shift the positions of hydrophobic and hydrophilic side chains.
- Hydrophobic side chains will move to the exterior to interact with the nonpolar solvent.
- Hydrophilic side chains will cluster in the interior.



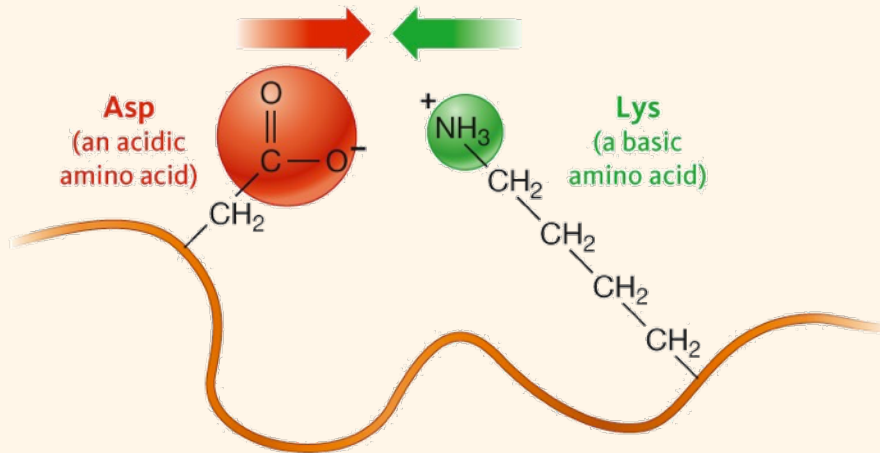
Protein dissolved in water (active form)



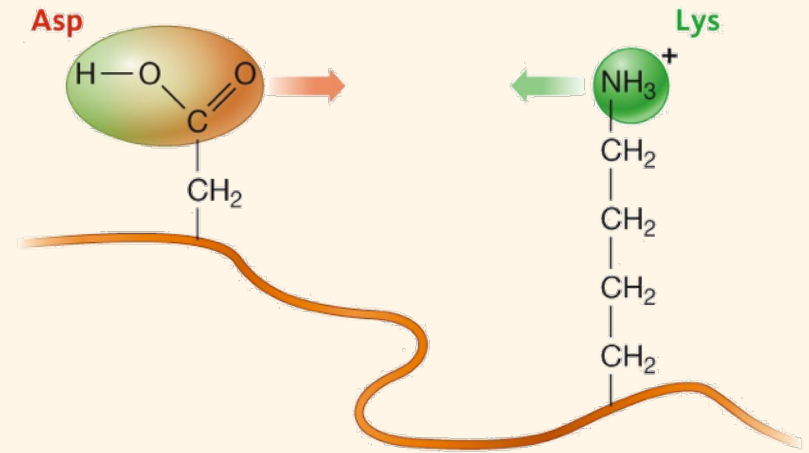
Protein dissolved in ethanol (denatured)

Changing the pH

- If the pH of the solvent is changed, the ionization of the acidic and basic side chains will change and ion-ion attraction will be disrupted.
- Lowering the pH will protonate carboxylic acid side chains. Increasing the pH will deprotonate the amine side chain.



At pH 7, aspartic acid and lysine have opposite charges and form an ion pair.



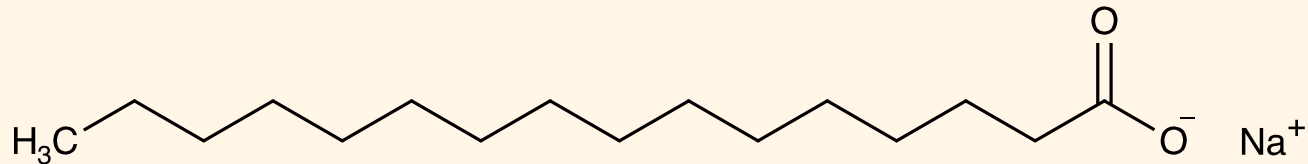
At pH 2, aspartic acid and lysine attract one another, but they cannot form an ion pair.

Raising the Temperature and Violent Agitation

- As temperature increases, molecules speed up.
- These vibrations will denature most proteins between 50 °F and 70 °F.
 - ✦ Denaturation of egg proteins is apparent when cooking, as the soluble egg white proteins change to a white insoluble tangle of proteins.
- Vigorous whipping of a protein solution will denature a protein.
 - ✦ Whipping cream converts soluble proteins into a stiff insoluble form.

Adding Ionic Substances

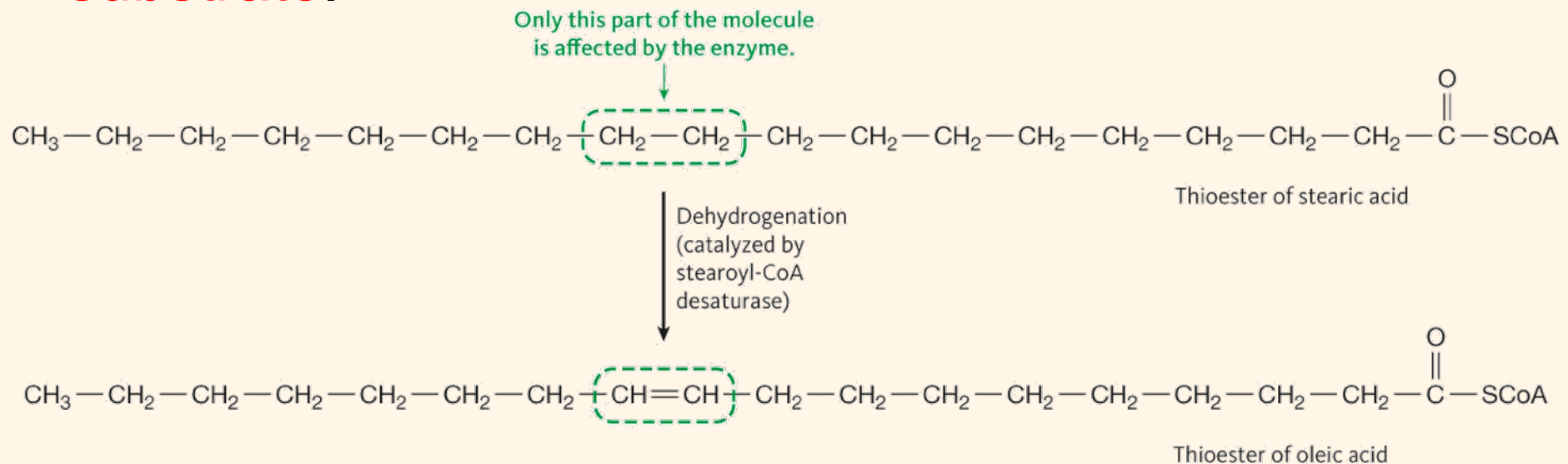
- Addition of high concentrations of ionic compounds will interrupt ion-ion attractions.
- Soaps will interrupt ion-ion attractions and hydrophilic and hydrophobic interactions.



- Heavy metal ions such as the ions of lead and mercury are strongly attracted to sulfur and will disrupt disulfide bridges.

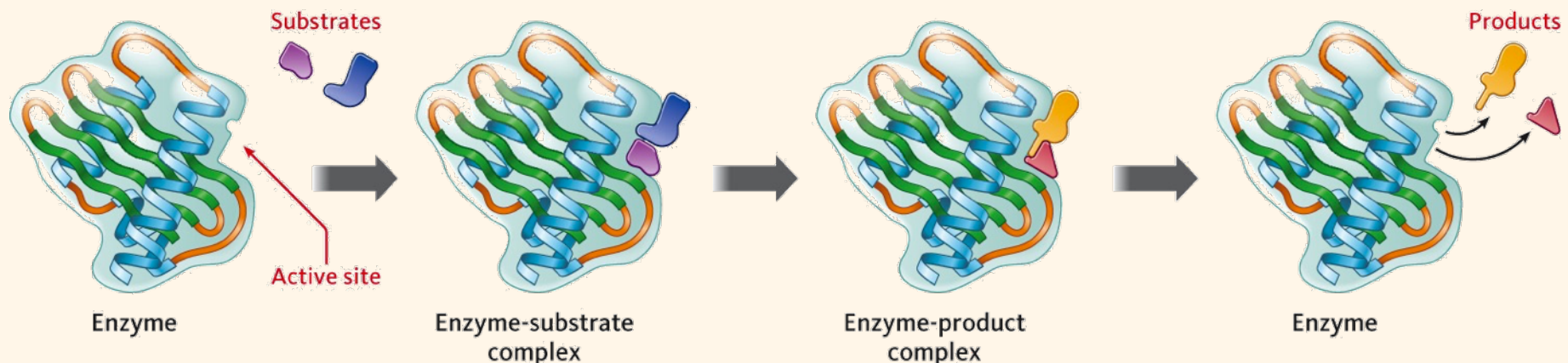
14.5 Enzymes Structure and Function

- Enzymes are protein catalysts that speed up biological reactions.
- Enzymes are often highly specific and will only allow one of many possible products form.
- The reactant in a biochemical reaction is known as a **substrate**.



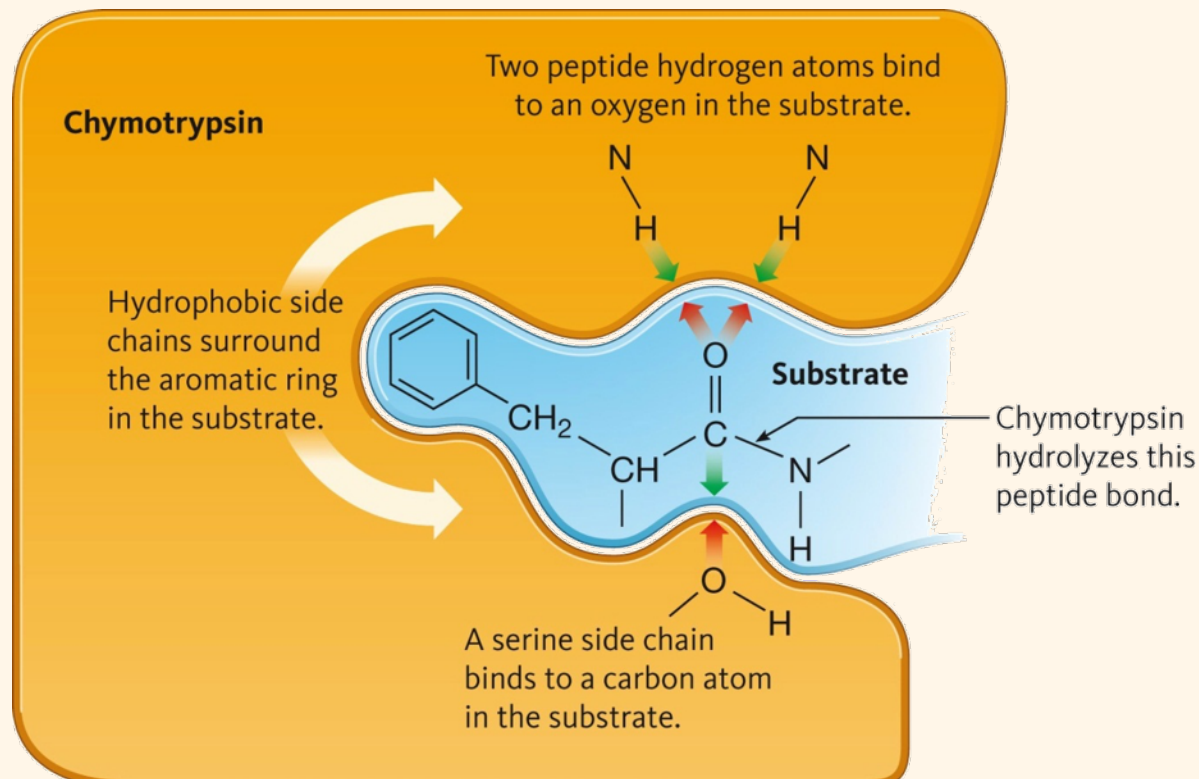
Three Steps of Enzyme Catalysts

- The enzyme binds to the substrate(s).
 - ✦ The substrate will bind to the **active site** of the enzyme forming an **enzyme-substrate complex**.
- The enzyme converts the substrate to product(s).
 - ✦ This occurs in the **enzyme-product complex**.
- The enzyme releases the product(s).



The Enzyme-Substrate Complex

- Most enzymes can only bind to a limited number of possible substrates.
- The active site of the enzyme is shaped to match a certain type of substrate.

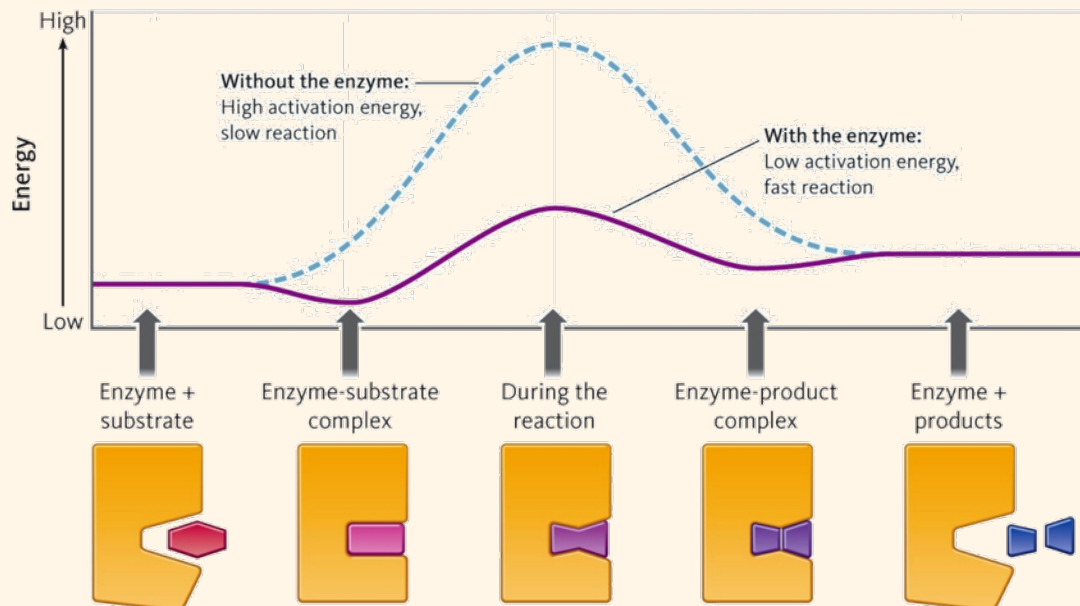


The Enzyme-Product Complex

- The reaction will occur in the active site, producing the enzyme product complex.
- The product can then exit the enzyme and the enzyme is free to react with other substrate molecules.
- **Activity** of an enzyme is measured by the number of reaction cycles per second.
- Most enzymes will have 10-1000 reaction cycles per second.

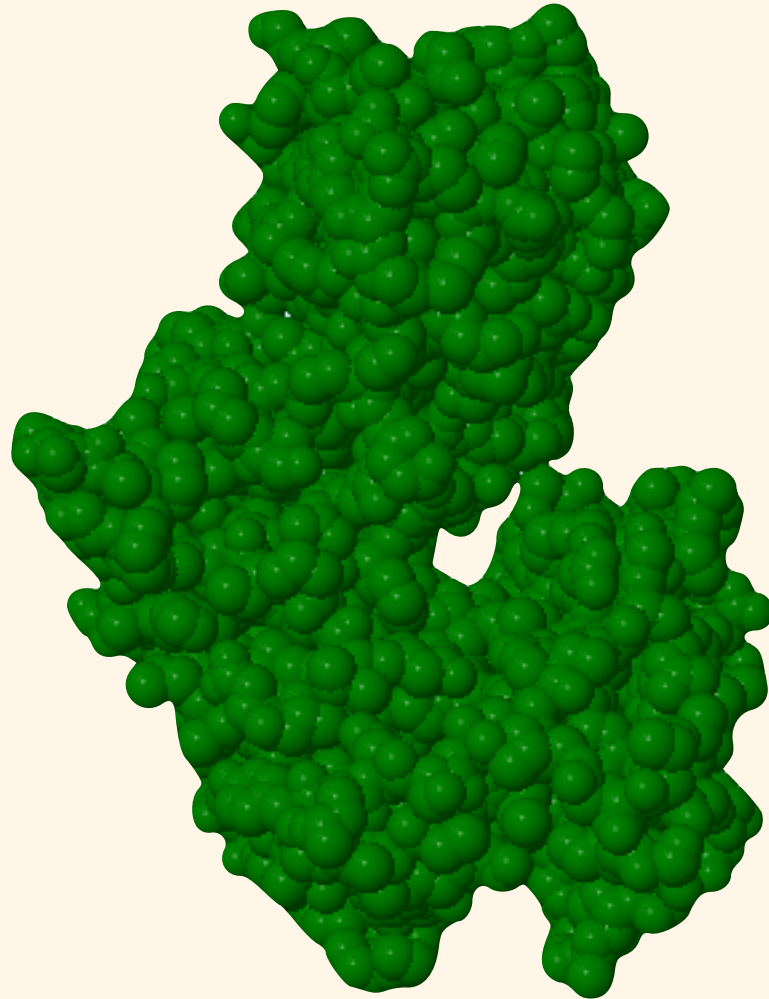
Activation Energy

- Like any catalyst, enzymes lower the activation energy of a reaction.
- The enzyme-substrate complex and the enzyme-product complex are also more stable (lower energy) together than when they are individuals.



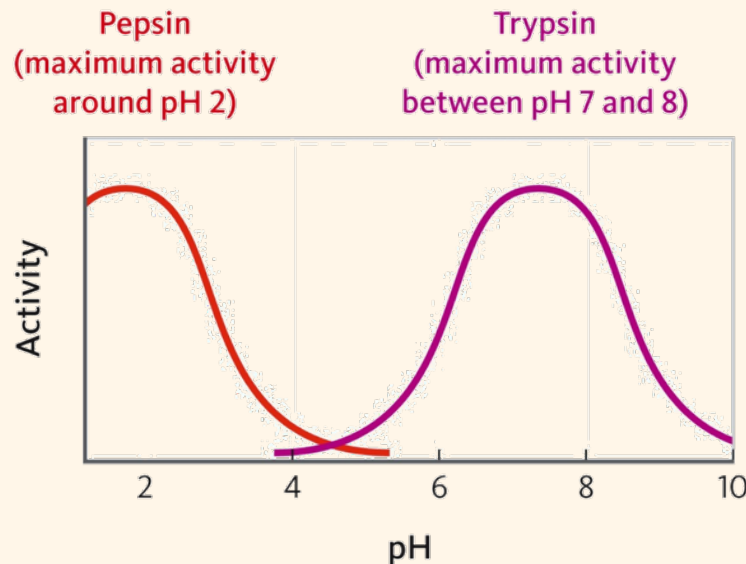
Three Steps of Enzyme Catalysts

- Hexokinase



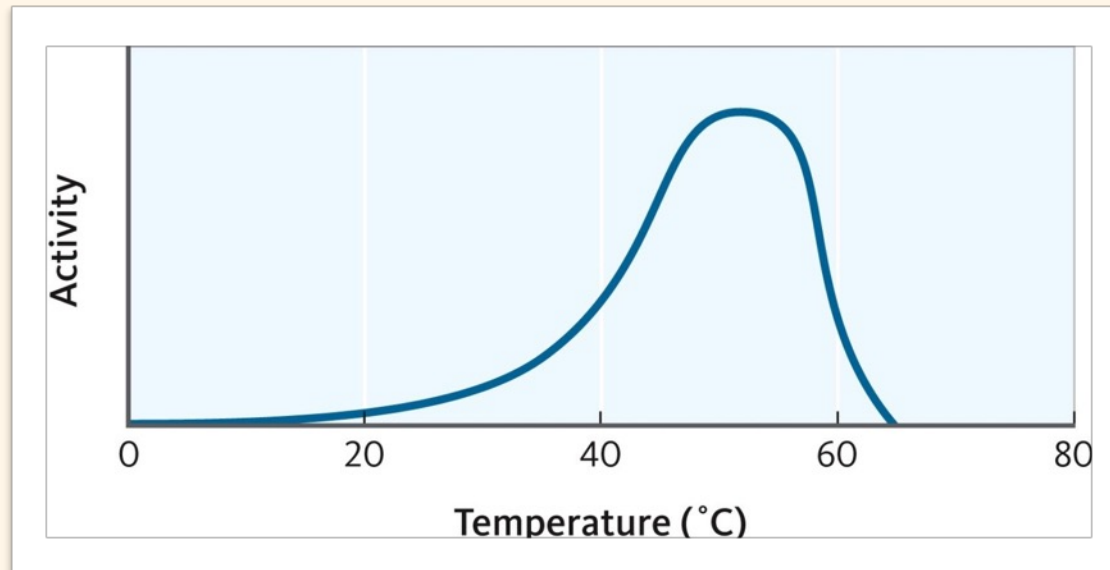
Activity and Surroundings

- Activity of enzymes are strongly influenced by the environment. Most enzymes function at a very tight pH range.
- Pepsin is active in the stomach (acidic environment) and trypsin functions in the pancreas (basic environment).



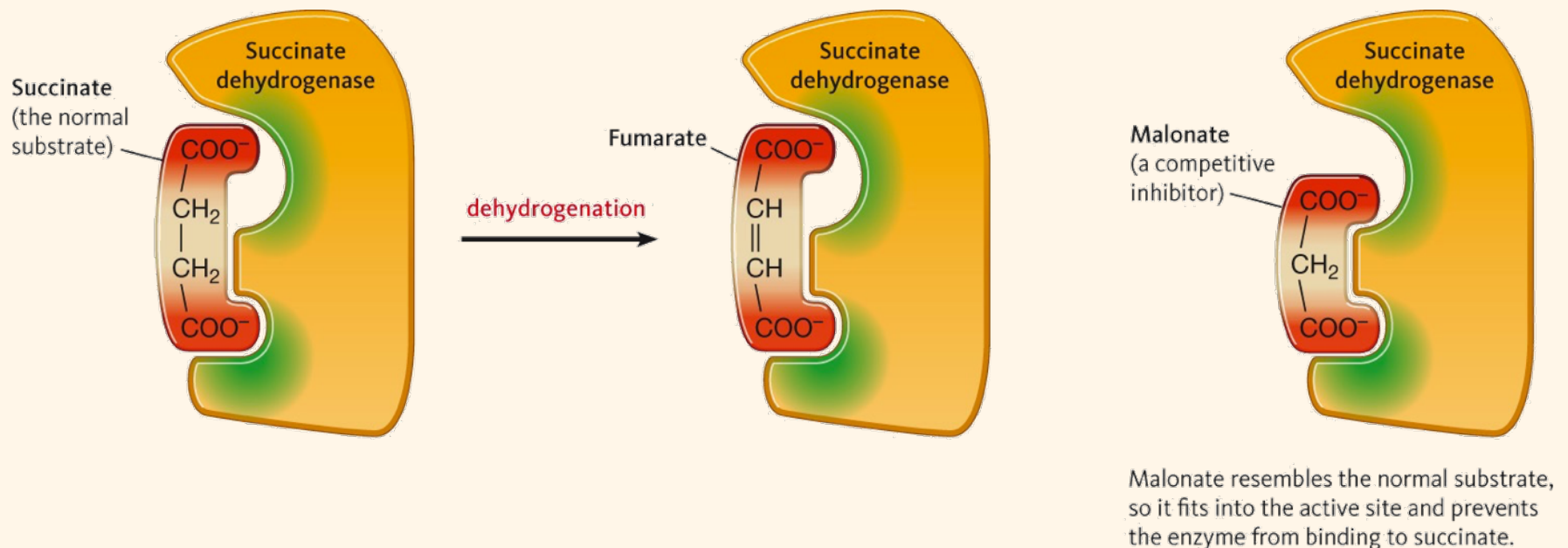
Temperature Affects Activity

- A small increase in temperature will increase enzyme activity.
- Enzyme function will cease if temperature is too low or too high.
- Above 50 °C, enzymes begin to denature and will not function.



Competitive Inhibitors

- **Competitive inhibitors** are molecules that block the active site but not react.
 - ✦ Many drugs are designed to be competitive inhibitors

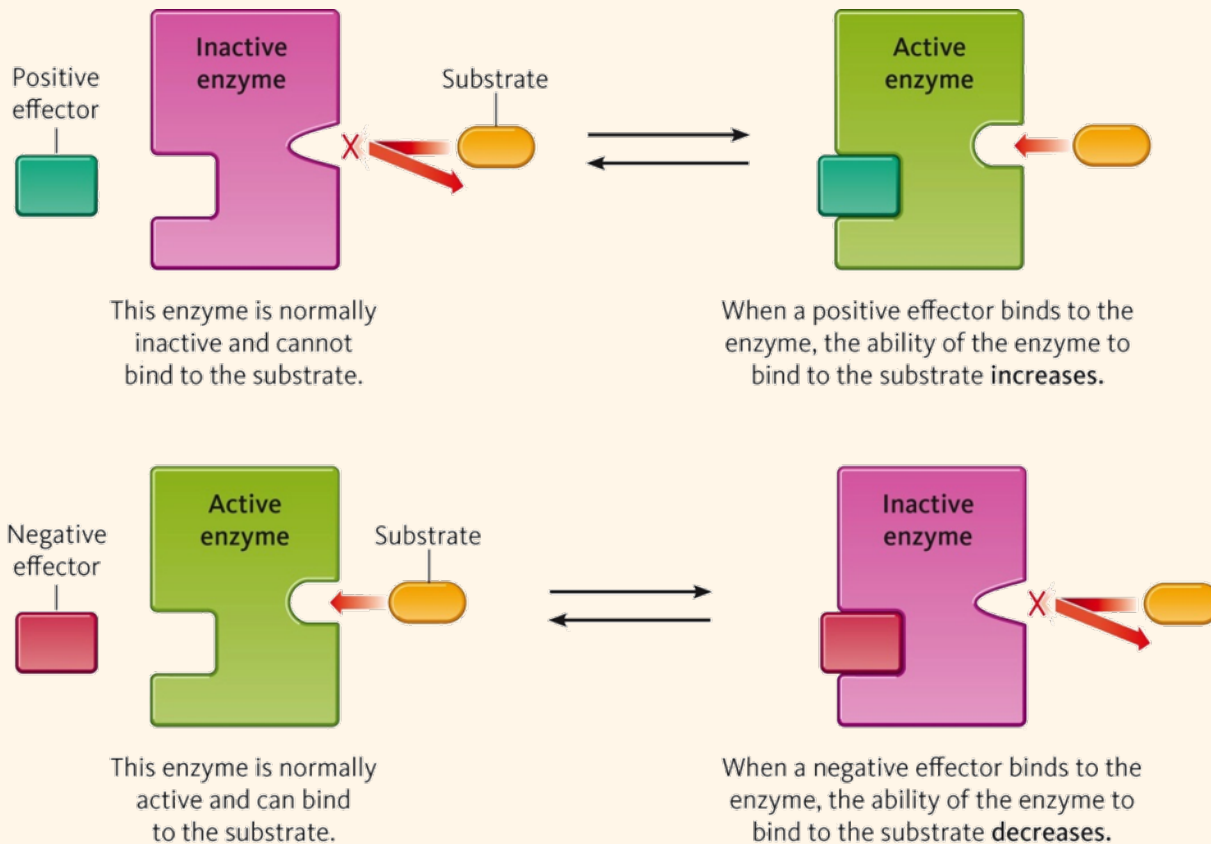


Effectors

- In the cell, effectors affect enzyme activity by binding to the enzyme somewhere other than the active site.
 - ✦ These are called **allosteric effectors**
- **Positive effectors** increase the enzymes ability to bind a substrate.
- **Negative effectors** decrease the enzymes ability to bind a substrate.
 - ✦ These are also known as **noncompetitive inhibitors** because they decrease activity but are not competing for the active site.

Effectors Help Control Enzyme Activity

- Cells use effectors to control the rate of enzymes.
- If a cell needs a product, a positive effector will be produced to speed up the reaction and vice versa.



14.6 Sources and Metabolism of Amino Acids

- Amino acids are not stored and thus need to be obtained from diet or synthesized by the cell when needed.
- In diet, amino acids are obtained by the digestion of proteins of animal products or plants.
- Proteins are digested into individual amino acids and do not retain any of their original activity.
 - ✦ This is why insulin, a small protein, must be injected and not ingested.

Essential and Nonessential Amino Acids

- Essential amino acids are those that cells have no or limited ability to produce, and must be obtained by diet.

*Arginine and Histidine are produced in limited amounts by children and some adults.

Essential Amino Acids	Nonessential Amino Acids
<i>Isoleucine</i>	<i>Alanine</i>
<i>Leucine</i>	<i>Arginine*</i>
<i>Lysine</i>	<i>Asparagine</i>
<i>Methionine</i>	<i>Aspartic Acid</i>
<i>Phenylalanine</i>	<i>Cysteine</i>
<i>Threonine</i>	<i>Glutamic Acid</i>
<i>Tryptophan</i>	<i>Glutamine</i>
<i>Valine</i>	<i>Glycine</i>
	<i>Histidine*</i>
	<i>Proline</i>
	<i>Serine</i>
	<i>Tyrosine</i>

Incomplete and Complete Proteins

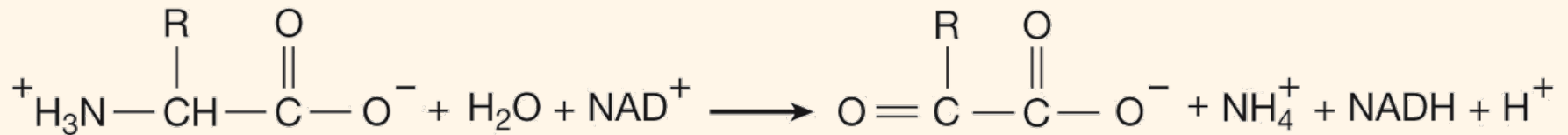
- Animal products are complete proteins as they contain all essential amino acids.
- Most grains are incomplete proteins, meaning they lack some essential amino acid.
 - ✦ Wheat and rice lack lysine, peas and beans lack methionine.
 - ✦ This is why it is essential that vegetarians eat a balanced diet of multiple protein sources to obtain all essential amino acids.
 - A meal of rice and beans provides all essential amino acids. Interestingly, this meal was established long before proteins were understood and probably discovered as a healthy meal by trial and error.

Amino Acids are a Nitrogen Supply

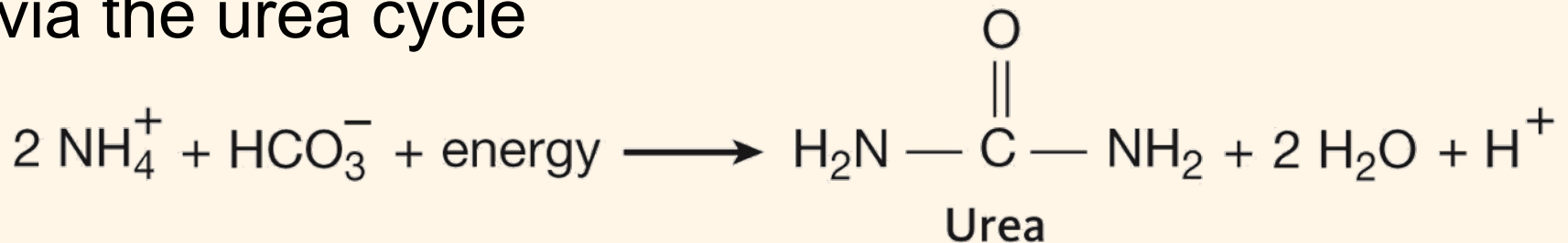
- To synthesize nonessential amino acids, the cell requires a nitrogen source.
- A diet must contain enough amino acids to provide nitrogen for nonessential amino acids and other necessary molecules that use nitrogen.
- A diet also need to provide the minimum amount of essential amino acids.
- Proteins can be used as fuel for energy if needed, so it is important that plenty of protein be eaten to provide for all needs.

Urea Cycle

- To remove nitrogen from amino acids, a reaction called an oxidative deamination occurs.



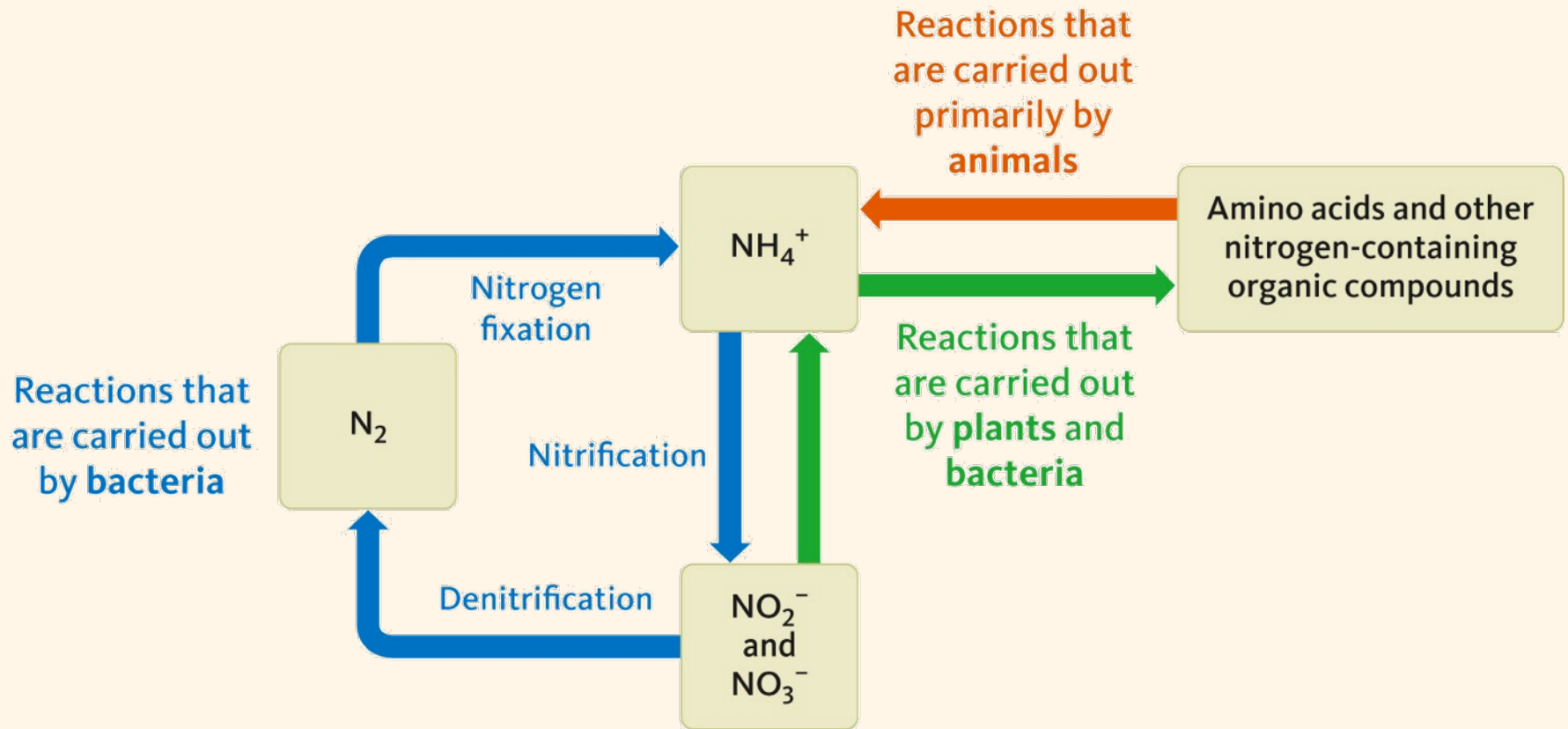
- Ammonium ions are toxic, so the liver rapidly absorbs them and converts them to non-toxic urea via the urea cycle



Nitrogen Fixation

- Unlike most organisms, bacteria and plants can obtain nitrogen from inorganic sources.
- Some bacteria can convert N_2 into NH_4^+ , a process called **nitrogen fixation**.
- NH_4^+ is absorbed by plants to produce amino acids, and **nitrifying bacteria** oxidize NH_4^+ into NO_2^- and NO_3^- .
- **Denitrifying bacteria** convert NO_2^- and NO_3^- into N_2 .

The Nitrogen Cycle



Next up

- Unit 11 - Carbohydrates
 - ✦ Unit 11 Assignments are due 28. April (deadline is 5. May)