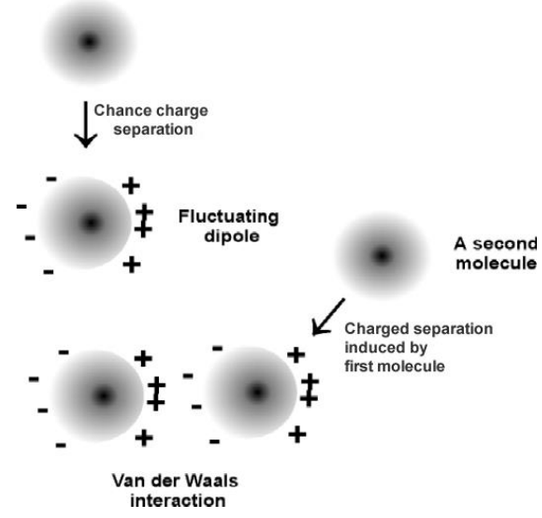
Lecture 2
Protein Structure and Function, Carbohydrates and Lipids

- Protein Structure and Stability
- Ligand Binding
- Proteins as enzymes (PKU disease)
- Carbohydrates
- Lipids & Cholesterol regulation

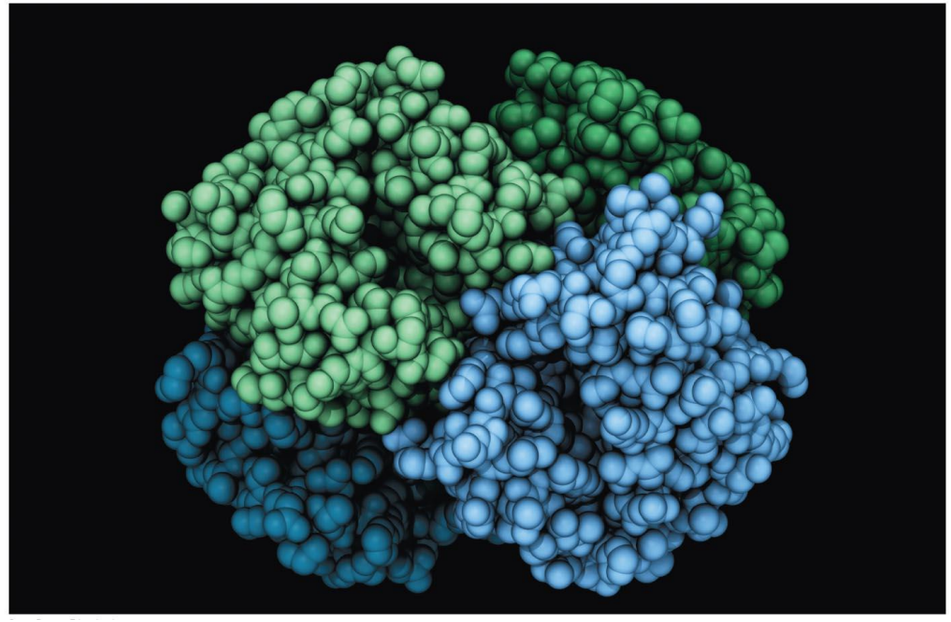
Prior to next week's lecture (lecture 3), please review the lecture material on introduction to nucleic acids (slides and video link posted on course web site)

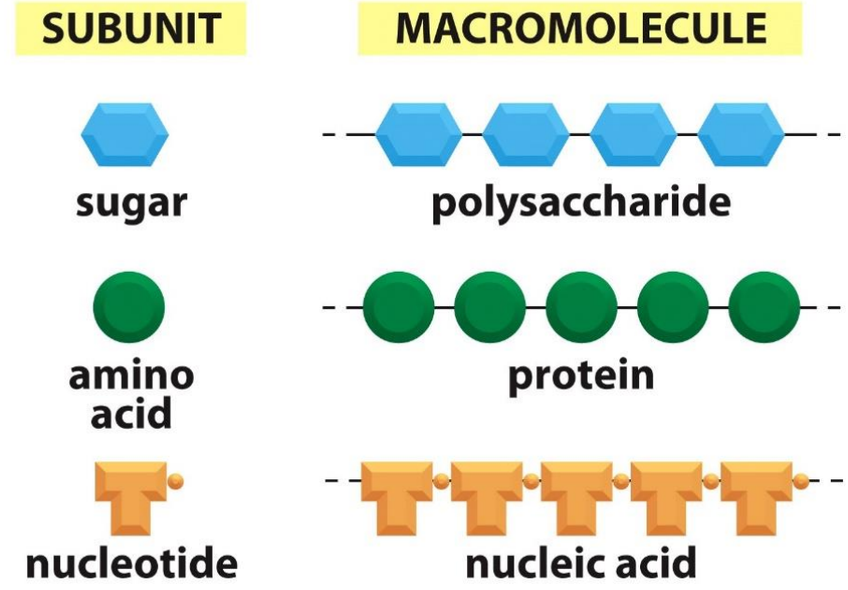
Relative Energy of Interactions

Interaction	Interaction	Energy (kJ/mol)
Covalent Bond	Electron sharing	200-400 kJ/mol
Electrostatic interactions (in water)	Full charges	~5 kJ/mol/single interaction
VdW - Dipole-dipole (Keesom)	Perm. partial charges	~0.05 kJ/A^2 x 100 A^2 = 5 kJ/mol for 100 A^2
VdW – Induced dipole (London)	Induced partial charges	~0.02 kJ/A^2 x 100 A^2 = **2 kJ/mol for 100 A^2**
H-Bonds	Electrostatic + e sharing	~20 kJ/mol gross, **~5 kJ/mol net**



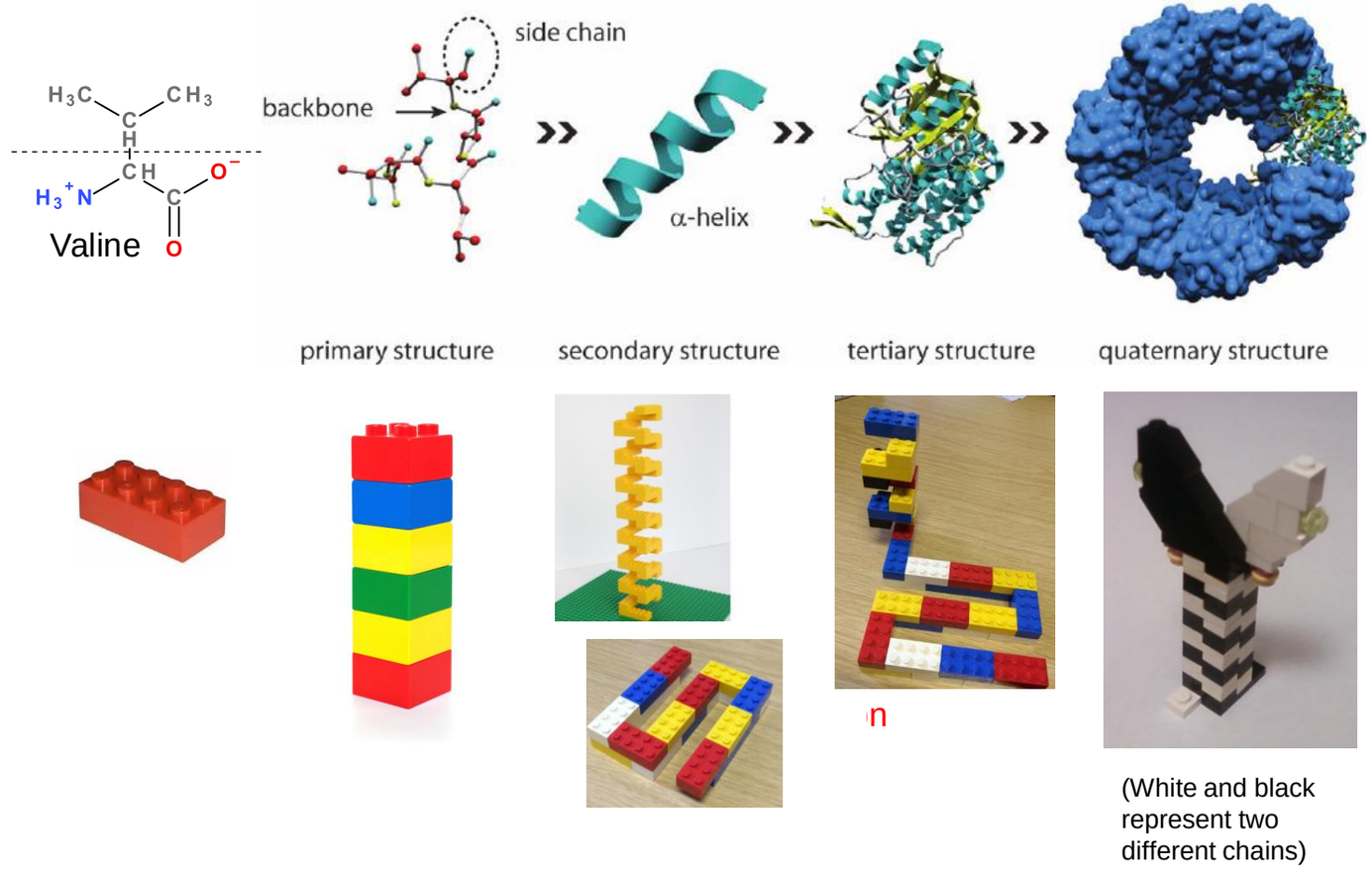
Proteins and Amino Acids



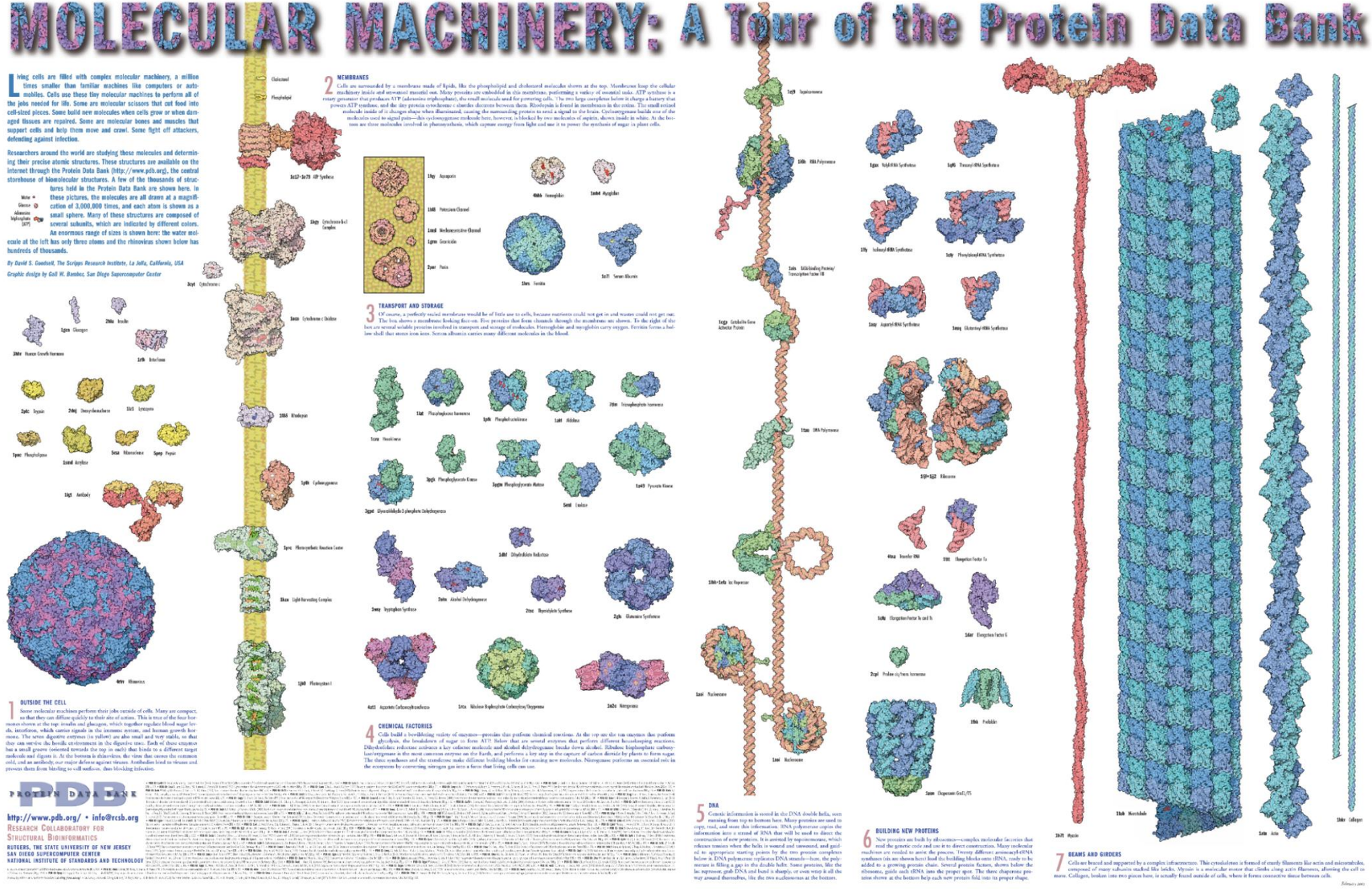


Structural Hierarchy of Proteins

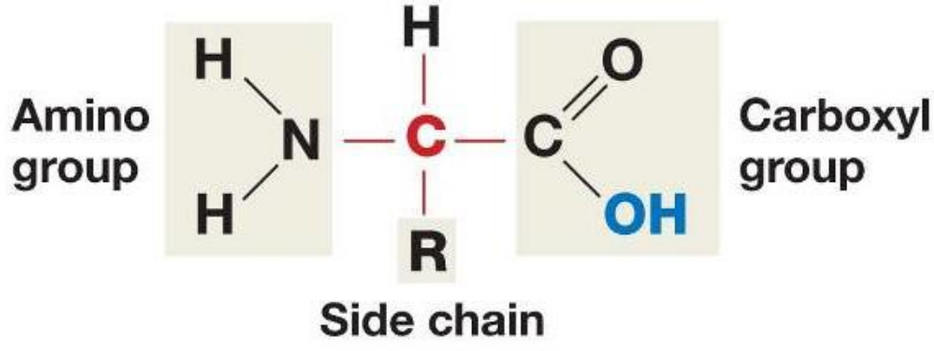
- Primary - sequence of amino acids, no 3D structural information
- Secondary - local structural elements, only mainchain atoms involved
- Tertiary - 3D position of ***all*** atoms, functional form of many proteins.
- Quaternary - multiple chains – multiple chains often required for function.



(White and black represent two different chains)

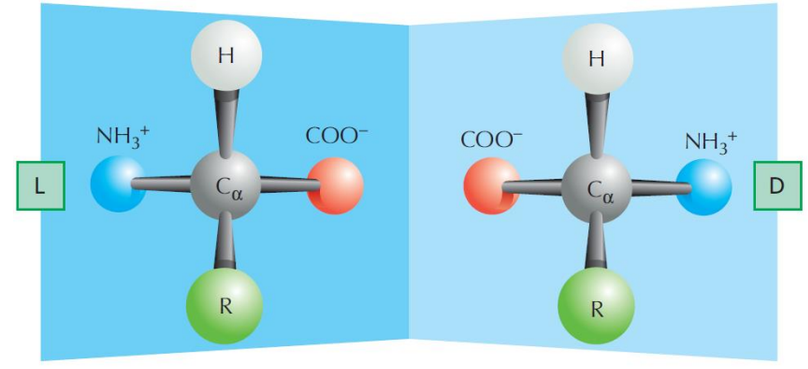

MOLECULAR MACHINERY: A Tour of the Protein Data Bank
Living cells are filled with complex molecular machinery, a million times smaller than familiar machines like computers or automobiles. Cells use these tiny molecular machines to perform all of the jobs needed for life. Some are molecular scissors that cut food into cell-sized pieces. Some build new molecules when cells grow or when damaged tissues are repaired. Some are molecular bones and muscles that support cells and help them move and crawl. Some fight off attackers, defending against infection.
Researchers around the world are studying those molecules and determining their precise atomic structures. These structures are available on the internet through the Protein Data Bank (http://www.pdb.org), the central storehouse of biomolecular structures. A few of the thousands of structures held in the Protein Data Bank are shown here. In these pictures, the molecules are all drawn at a magnification of 3,000,000 times, and each atom is shown as a small sphere. Many of these structures are composed of several subunits, which are indicated by different colors. An enormous range of sizes is shown here: the water molecule at the left has only three atoms and the rhinovirus shown below has hundreds of thousands.
Water
Glucose
Adenosine triphosphate (ATP)
By David S. Goodsell, The Scripps Research Institute, La Jolla, California, USA
Graphic design by Gail W. Bamba, San Diego Supercomputer Center
1 OUTSIDE THE CELL
Some molecular machines perform their jobs outside of cells. Many are compact, so that they can diffuse quickly to their site of action. This is true of the four hormones shown at the top: insulin and glucagon, which together regulate blood sugar levels, interferon, which carries signals in the immune system, and human growth hormone. The seven digestive enzymes (in yellow) are also small and very stable, so that they can survive the hostile environment in the digestive tract. Each of these enzymes has a small groove (oriented towards the top in each) that binds to a different target molecule and digests it. At the bottom is rhinovirus, the virus that causes the common cold, and an antibody, our major defense against viruses. Antibodies bind to viruses and prevent them from binding to cell surfaces, thus blocking infection.
2 MEMBRANES
Cells are surrounded by a membrane made of lipids, like the phospholipid and cholesterol molecules shown at the top. Membranes keep the cellular machinery inside and unwanted material out. Many proteins are embedded in this membrane, performing a variety of essential tasks. ATP synthase is a rotary generator that produces ATP (adenosine triphosphate), the small molecule used for powering cells. The two large complexes below it charge a battery that powers ATP synthase, and the tiny protein cytochrome c shuttles electrons between them. Rhodopsin is found in membranes in the retina. The small retinal molecule inside of it changes shape when illuminated, causing the surrounding protein to send a signal to the brain. Cyclooxygenase builds one of the molecules used to signal pain—this cyclooxygenase molecule here, however, is blocked by two molecules of aspirin, shown inside in white. At the bottom are three molecules involved in photosynthesis, which capture energy from light and use it to power the synthesis of sugar in plant cells.
Cholesterol
Phospholipid
3 TRANSPORT AND STORAGE
Of course, a perfectly sealed membrane would be of little use to cells, because nutrients could not get in and wastes could not get out. The box shows a membrane looking face-on. Five proteins that form channels through the membrane are shown. To the right of the box are several soluble proteins involved in transport and storage of molecules. Hemoglobin and myoglobin carry oxygen. Ferritin forms a hollow shell that stores iron ions. Serum albumin carries many different molecules in the blood.
4 CHEMICAL FACTORIES
Cells build a bewildering variety of enzymes—proteins that perform chemical reactions. At the top are the ten enzymes that perform glycolysis, the breakdown of sugar to form ATP. Below that are several enzymes that perform different housekeeping reactions. Dihydrofolate reductase catalyzes a key cofactor molecule and alcohol dehydrogenase breaks down alcohol. Ribulose bisphosphate carboxylase/oxygenase is the most common enzyme on the Earth, and performs a key step in the capture of carbon dioxide by plants to form sugar. The three synthases and the transferase make different building blocks for creating new molecules. Nitrogenase performs an essential role in the ecosystem by converting nitrogen gas into a form that living cells can use.
5 DNA
Genetic information is stored in the DNA double helix, seen running from top to bottom here. Many proteins are used to copy, read, and store this information. DNA polymerase copies the information into a strand of RNA that will be used to direct the construction of new proteins. It is assisted by topoisomerase, which releases tension when the helix is wound and unwound, and guided to appropriate starting points by the two protein complexes below it. DNA polymerase replicates DNA strands—here, the polymerase is filling a gap in the double helix. Some proteins, like the lac repressor, grab DNA and bend it sharply, or even wrap it all the way around themselves, like the two nucleosomes at the bottom.
6 BUILDING NEW PROTEINS
New proteins are built by ribosomes—complex molecular factories that read the genetic code and use it to direct constructions. Many molecular machines are needed to assist the process. Twenty different aminoacyl-tRNA synthases (six are shown here) load the building blocks onto tRNA, ready to be added to a growing protein chain. Several protein factors, shown below the ribosome, guide each tRNA into the proper spot. The three chaperone proteins shown at the bottom help each new protein fold into its proper shape.
7 BEAMS AND GIRDERS
Cells are braced and supported by a complex infrastructure. This cytoskeleton is formed of sturdy filaments like actin and microtubules, composed of many subunits stacked like bricks. Myosin is a molecular motor that climbs along actin filaments, allowing the cell to move. Collagen, broken into two pieces here, is actually found outside of cells, where it forms connective tissue between cells.
PROTEIN DATA BANK
http://www.pdb.org/ • info@rcsb.org
RESEARCH COLLABORATORY FOR STRUCTURAL BIOINFORMATICS
RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
SAN DIEGO SUPERCOMPUTER CENTER
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY

The Structure of Amino Acids and Proteins

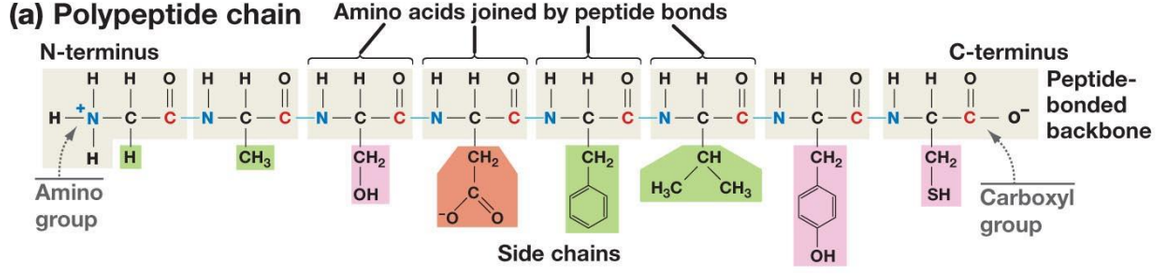


Is there a chiral carbon on amino acids?

- *The amino group, Cα (and one hydrogen), and the carbonyl group are common to all amino acids*
- *The N-Cα-C=O are the mainchain of the protein polymer.*
- *The R groups are different –there are 20 common R groups they are the sidechain of the protein polymer – their* ***sequence*** *defines the properties of the protein.*



Proteins consist exclusively of L-amino acids.
(as does the ribosome that make them)



Primary Structure

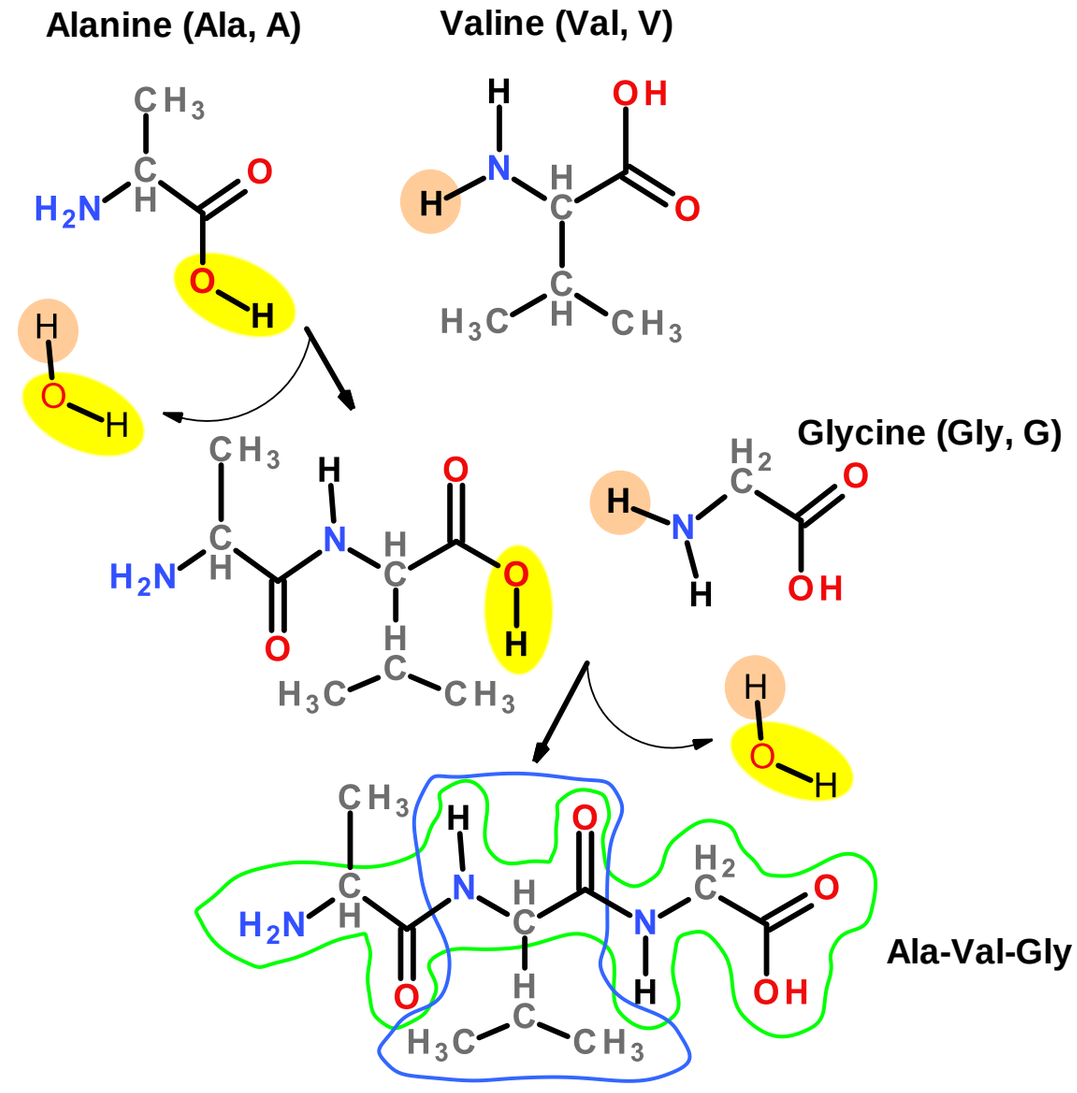
- Amino acids are joined together to form linear polymers by the formation of a **peptide bond** between the carboxyl of one amino acids and the amino group of the next.
- This reaction releases water: a **dehydration** reaction.
- The peptide bond can be broken (*lysis*) by the addition of water = **hydrolysis**.

Incorporated amino acid = *residue* (atoms are lost when the peptide bond is formed).

Polarity of chain direction – amino (N) terminus to carboxy(C) terminus = order of amino acids = *sequence* = *primary structure*

Mainchain (or backbone) – linear atoms of the polymer

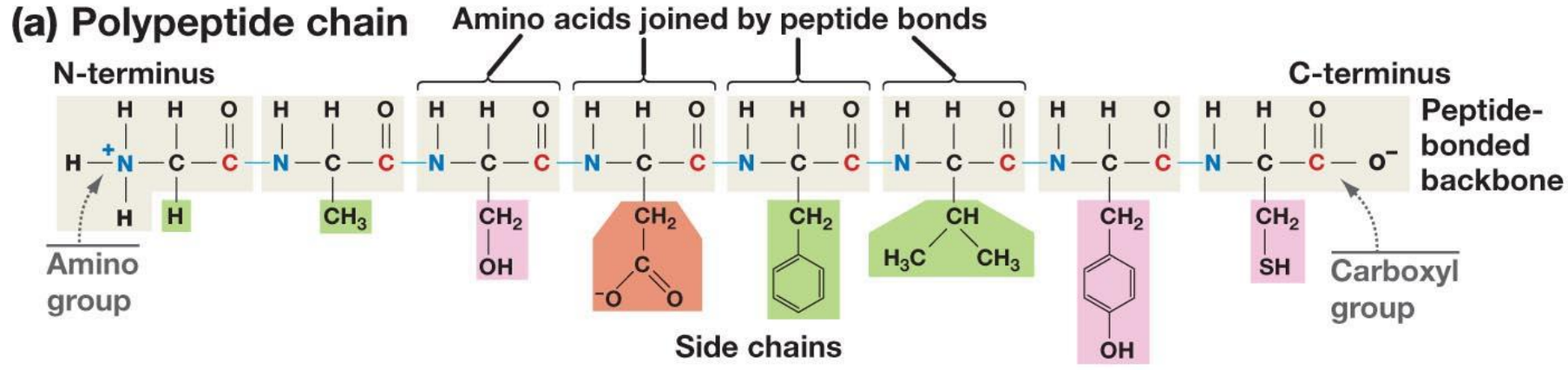
Sidechain – atoms off the Ca carbon



Primary Structure – Expectations

- Draw chemical structure given the sequence.
- Determine the seq. from chemical structure.
- Distinguish/identify:
  - **Mainchain** & **Sidechain** atoms,
  - **Residue** = aa in polymer,
  - N & C terminus,
  - Peptide bond(s).

Sidechain *Functional* Groups Affect Behavior (and the order is important)

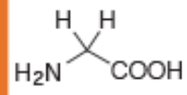


- Sidechains (R-groups) differ in their size, shape, reactivity, and interactions with water.
  1. Nonpolar Sidechains: **hydrophobic**; do not form hydrogen bonds; coalesce in water - typically form the core of folded proteins.
  2. Polar Sidechains: **hydrophilic**; form hydrogen bonds; readily dissolve in water
  3. **Ionizable** Sidechains: Can be charged at certain pH values. Interact strongly with water.

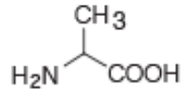
Amino Acids – Structure and Properties

Polar sidechains

SMALL

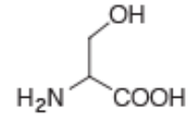


Glycine (Gly, G)
MW: 75.07

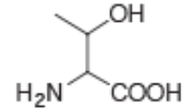


Alanine (Ala, A)
MW: 89.09

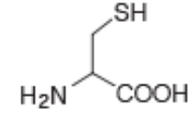
NUCLEOPHILIC



Serine (Ser, S)
MW: 105.09, $pK_a \sim 16$



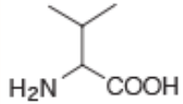
Threonine (Thr, T)
MW: 119.1, $pK_a \sim 16$



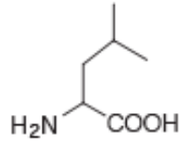
Cysteine (Cys, C)
MW: 121.2, $pK_a = 8.18$

Non-polar sidechains

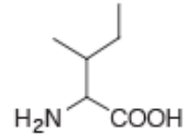
HYDROPHOBIC



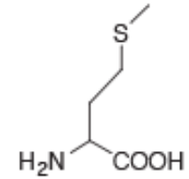
Valine (Val, V)
MW: 117.1



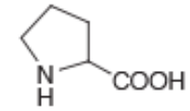
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2



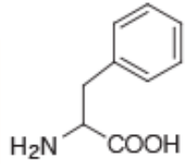
Methionine (Met, M)
MW: 149.2



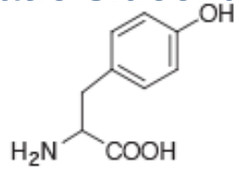
Proline (Pro, P)
MW: 115.1

Aromatic sidechains

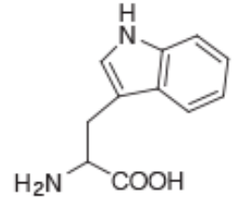
AROMATIC



Phenylalanine (Phe, F)
MW: 165.2



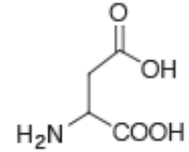
Tyrosine (Tyr, Y)
MW: 181.2, $pK_a = 10.46$



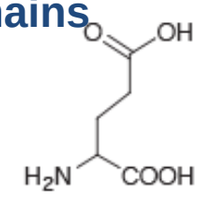
Tryptophan (Trp, W)
MW: 204.2

Ionizable sidechains

ACIDIC



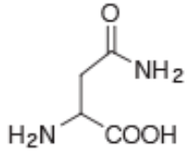
Aspartic Acid (Asp, D)
MW: 133.1, $pK_a = 3.9$



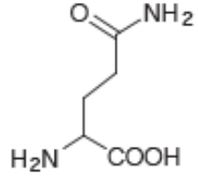
Glutamic Acid (Glu, E)
MW: 147.1, $pK_a = 4.07$

Polar sidechains

AMIDE



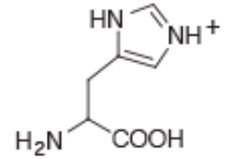
Asparagine (Asn, N)
MW: 132.1



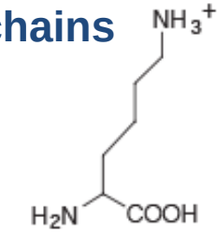
Glutamine (Gln, Q)
MW: 146.1

Ionizable sidechains

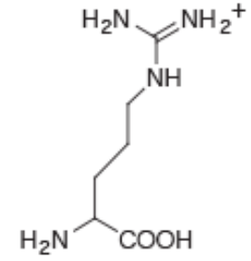
BASIC



Histidine (His, H)
MW: 155.2, $pK_a = 6.04$

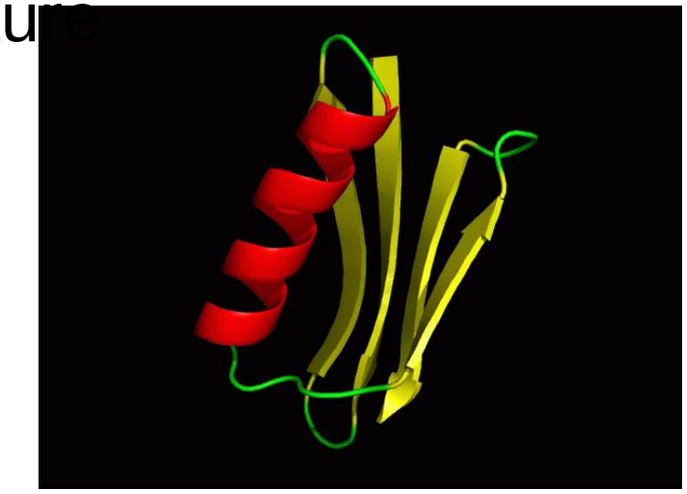


Lysine (Lys, K)
MW: 146.2, $pK_a = 10.79$



Arginine (Arg, R)
MW: 174.2, $pK_a = 12.48$

Secondary Structure



"Building blocks of proteins"

- **Hydrogen bonds** between the *mainchain* carbonyl group of one amino acid and the *mainchain* amino group of another form a protein's **secondary structure**.
  - A polypeptide must bend to allow this hydrogen bonding, forming:
    - **α-helices**
    - **β-pleated sheets**

- The large number of hydrogen bonds in a protein's secondary structure increases its stability - each hydrogen bond that is formed releases some energy.
- All amino acids can be incorporated into either secondary structure

  (However, some are found more frequently in one structure)

General Rule for Hydrogen Bonds:

X-H Y

X & Y are electronegative (N and O usually)

X-H = *Donor* of the hydrogen bond

Y = *Acceptor* of the hydrogen bond

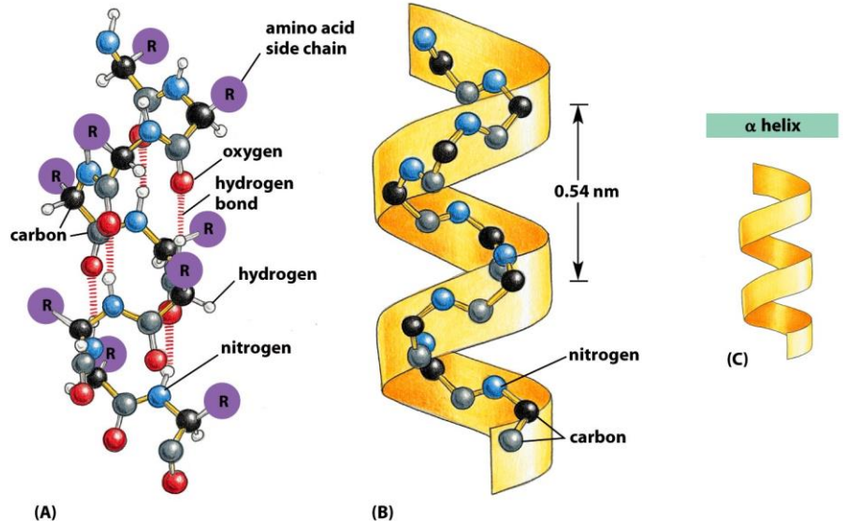
Mainchain hydrogen bonds

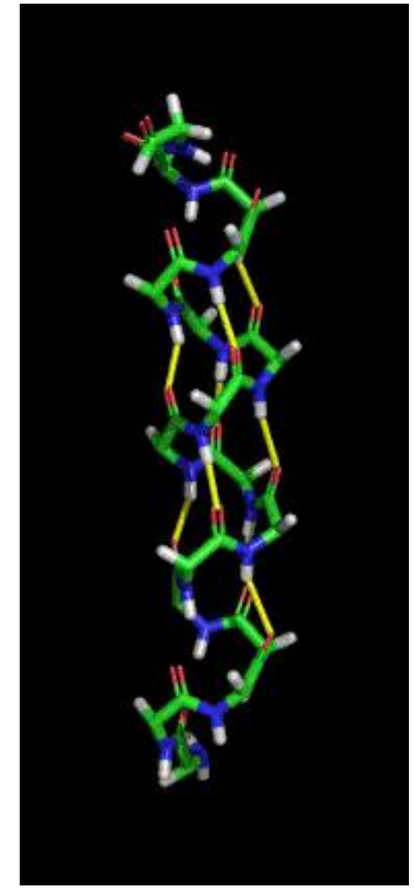
N-H O=C

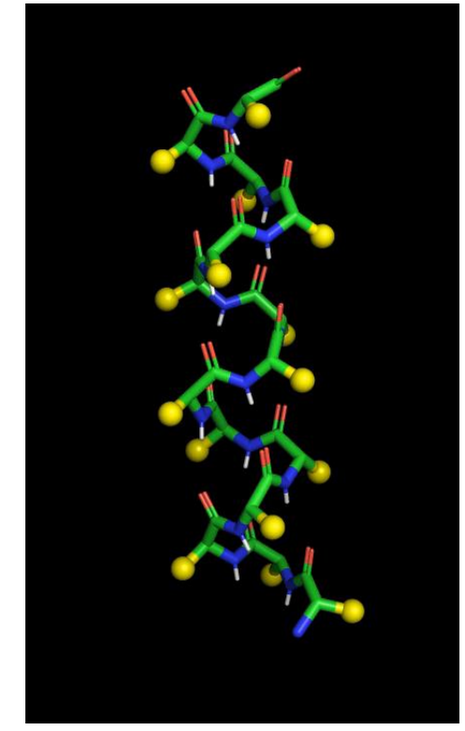
The NH is the hydrogen bond_____.

The C=O is the hydrogen bond _____.

Alpha Helix





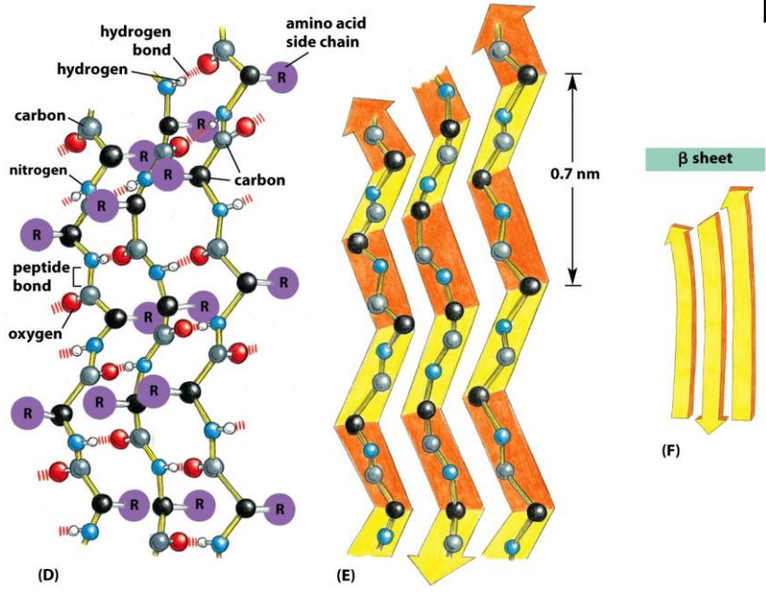


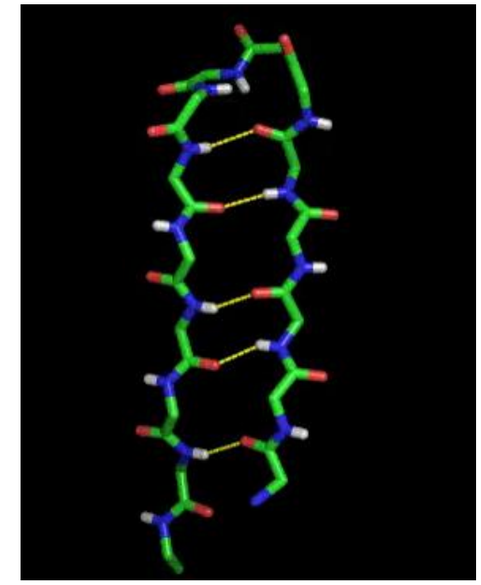
Spiral conformation (*helix*) in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of the amino acid four residues earlier:

Intra-strand H-bonds, parallel to helix axis.

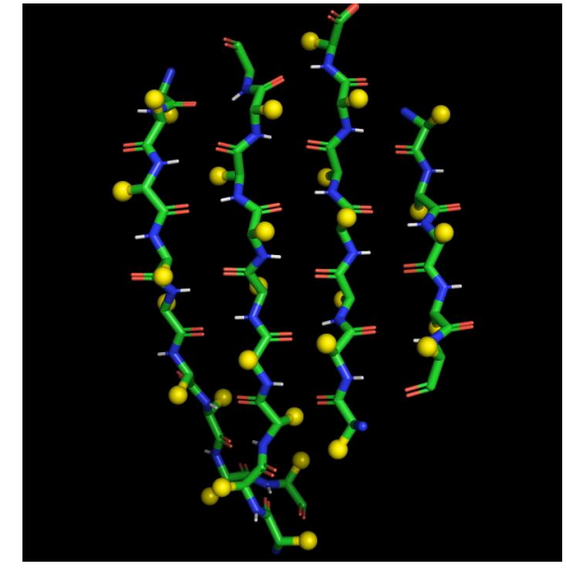
Side-chains project outwards.

Beta Sheet

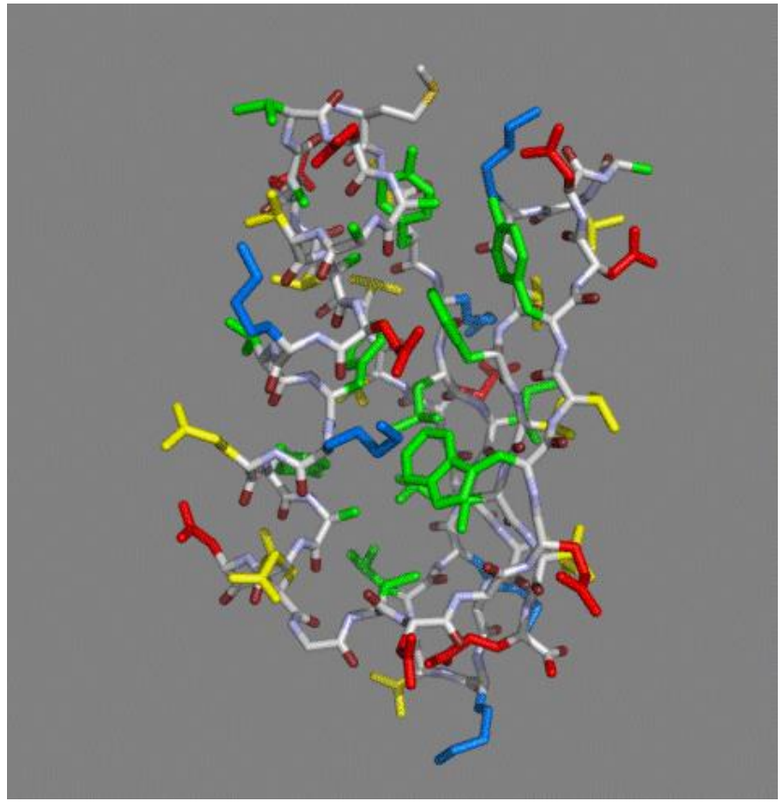




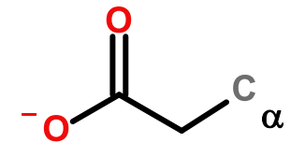
- Beta-Strands connected laterally by backbone hydrogen bonds that are perpendicular to the strand, forming a generally twisted, pleated sheet.
- Sheets can have two or more strands
- Side-chains:
  - project up and down along a strand.
  - project in the same direction going from strand to strand across the sheet.



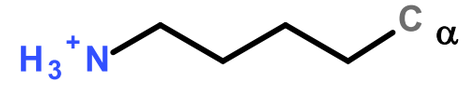
Tertiary Structure - Location of Residues in Globular Proteins



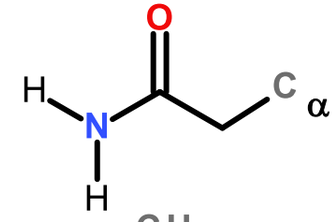
Red - amino acids with neg. sidechains (e.g. Asp)



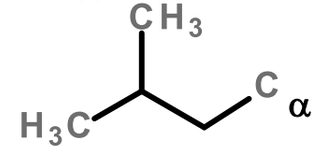
Blue - amino acids with pos. sidechains (e.g. Lys)

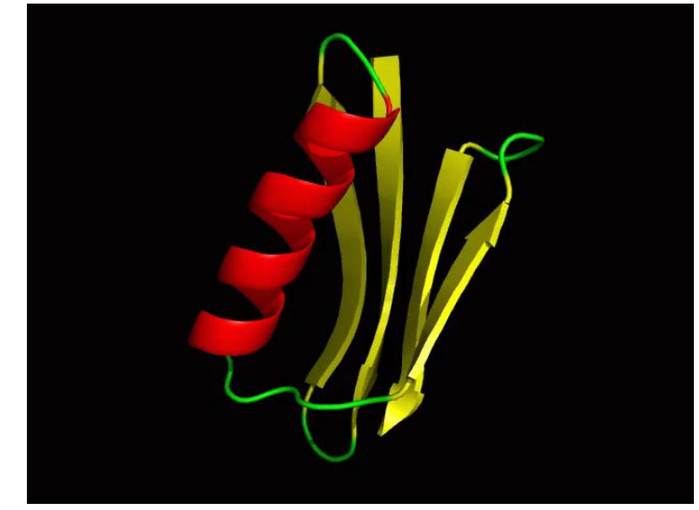


Yellow – amino acids with polar sidechain (e.g. Asn)

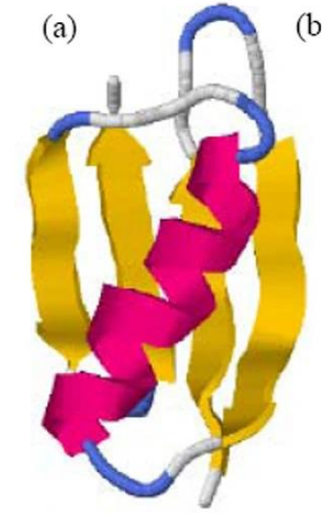


Green - amino acids with hydrophobic side chains (e.g. Leu)





Amino Acid Type		
	Inside (I)	Surface (S)
Charged		
Polar		
Non-polar		



Protein Stability:

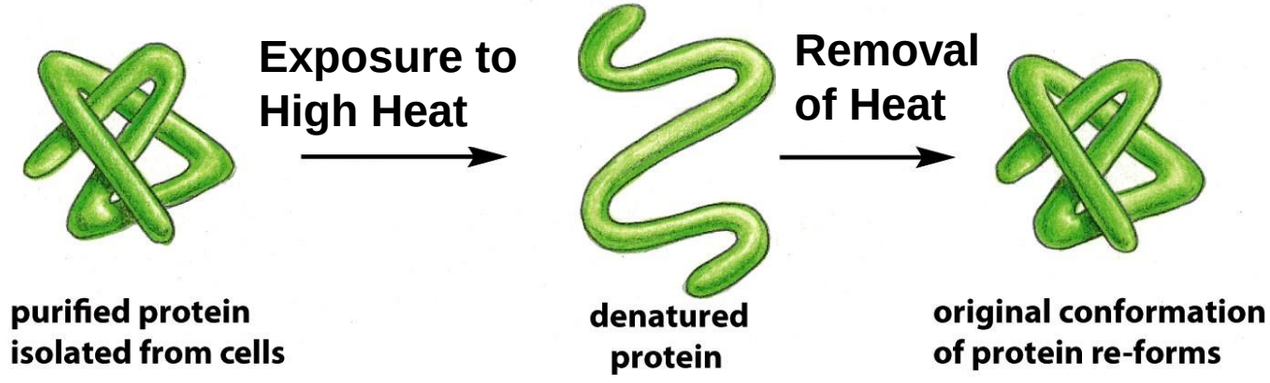
H-bonds
van der Waals
Hydrophobic effect

Native

Chain disorder

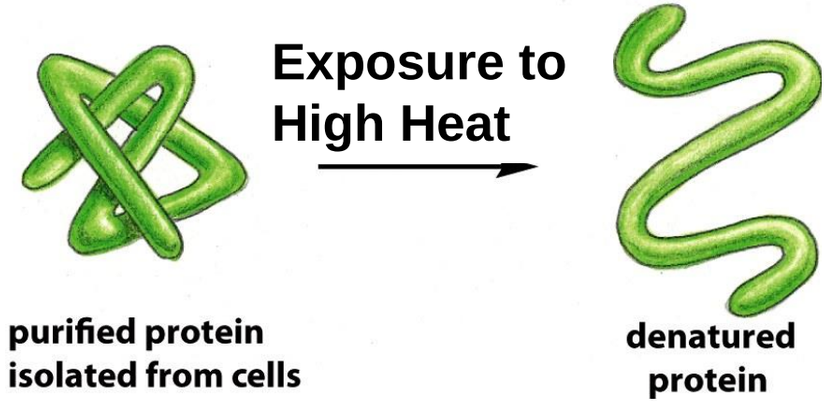
Unfolded

Protein Denaturation

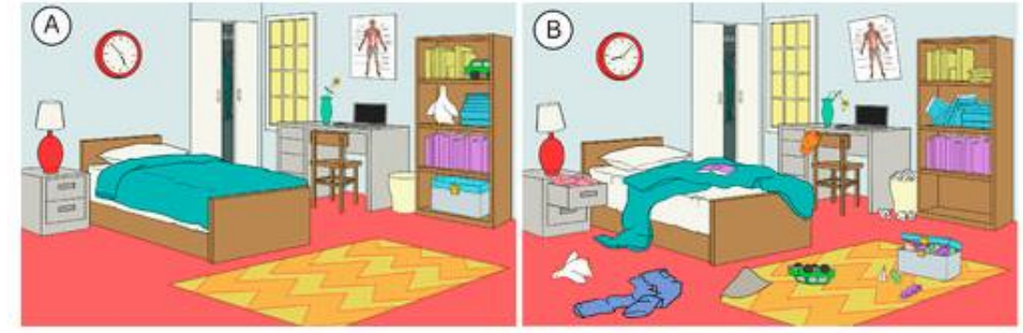


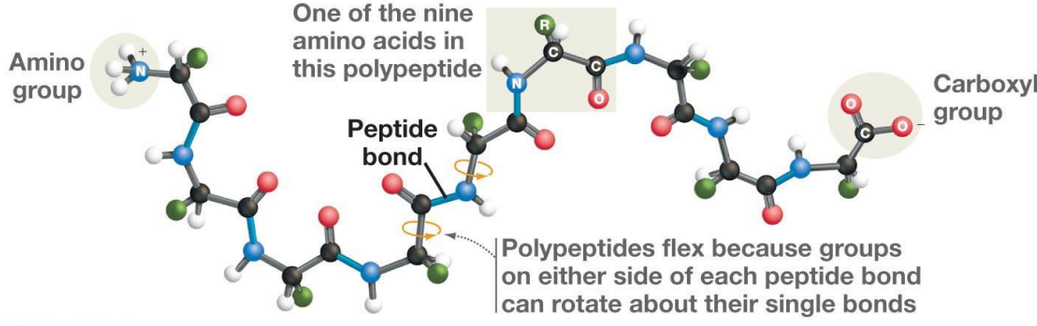
- Often, unfolded protein aggregate, which prevents refolding.

Unfolded Polypeptides Are Flexible – High Entropy stabilizes the Unfolded state

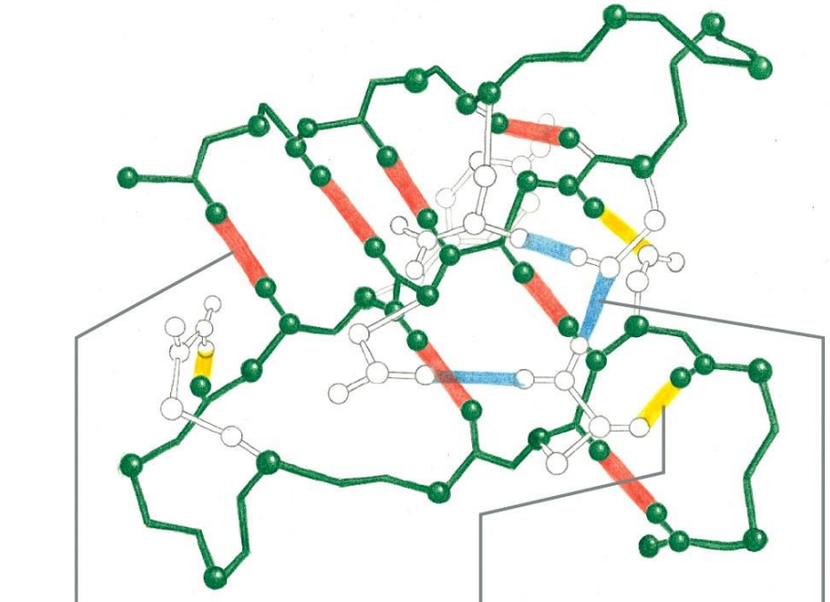


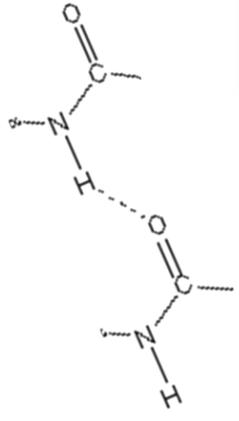
Energy and Entropy



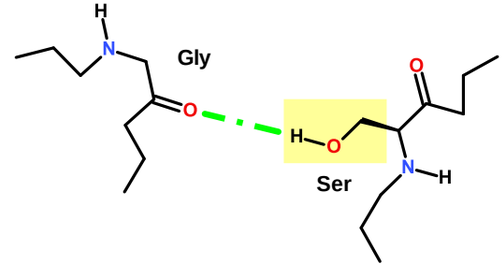


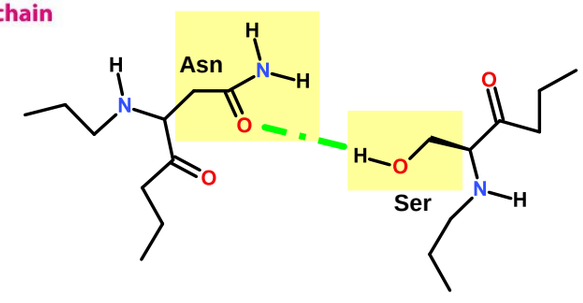
Hydrogen Bonding Stabilizes the Tertiary Structure





hydrogen bond between atoms of two peptide bonds	hydrogen bond between atoms of a peptide bond and an amino acid side chain	hydrogen bond between two amino acid side chains
backbone to backbone	backbone to side chain	side chain to side chain

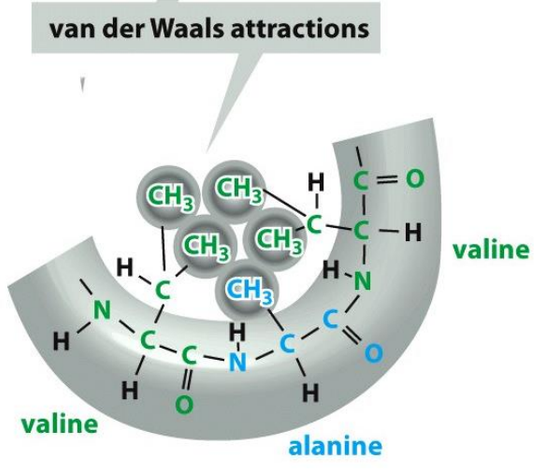


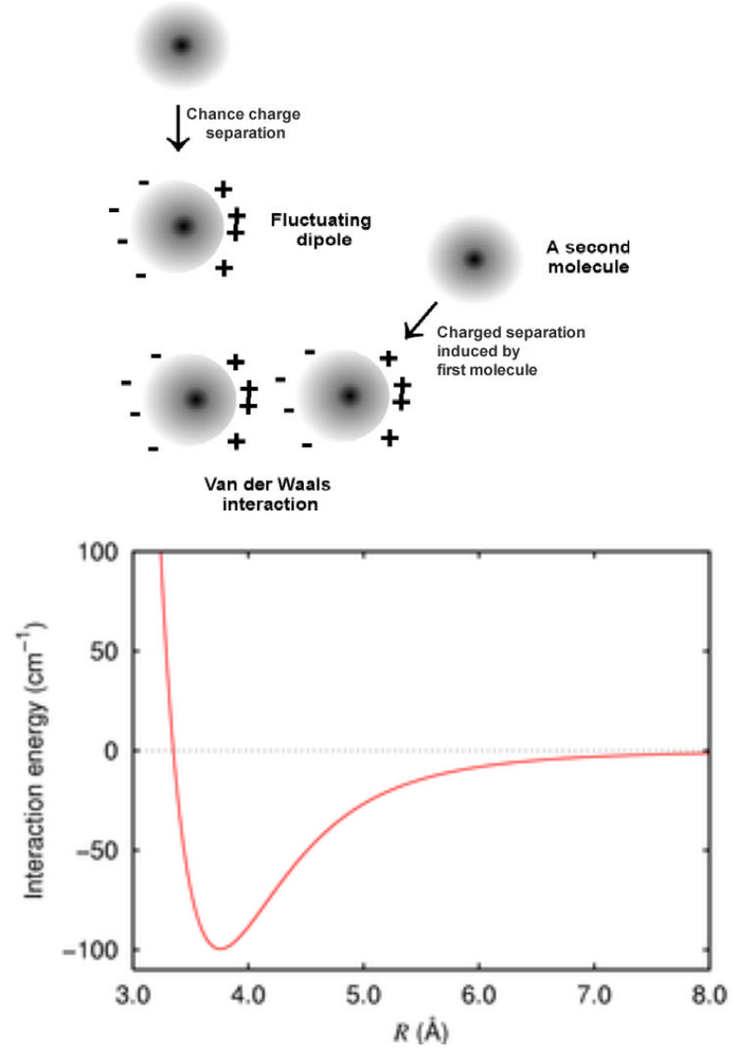


- **Hydrogen bonds** form between hydrogen atoms and the carbonyl group in the peptide-bonded backbone – secondary structure
- Hydrogen bonds are also found between hydrogen and electronegative atoms in side chains (sidechain-sidechain)
- Sidechains can form hydrogen bonds to the mainchain too.

Van der Waals (VdW) interactions Stabilize the Folded State

- VdW are weak electrostatic interactions between side chains due to temporary (fluctuating) charges.
- Attractive from long distance
- Distance at lowest energy is at the van der Waals radii of the atoms.
- Optimized in the core of folded proteins by “knobs fitting into holes”
- Strength proportional to contact area.

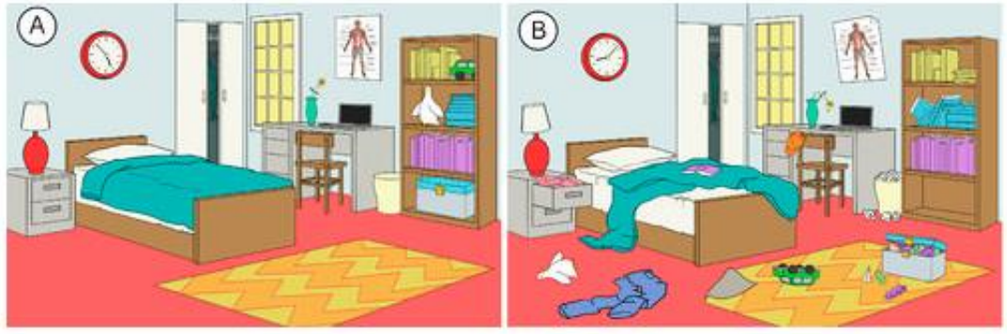




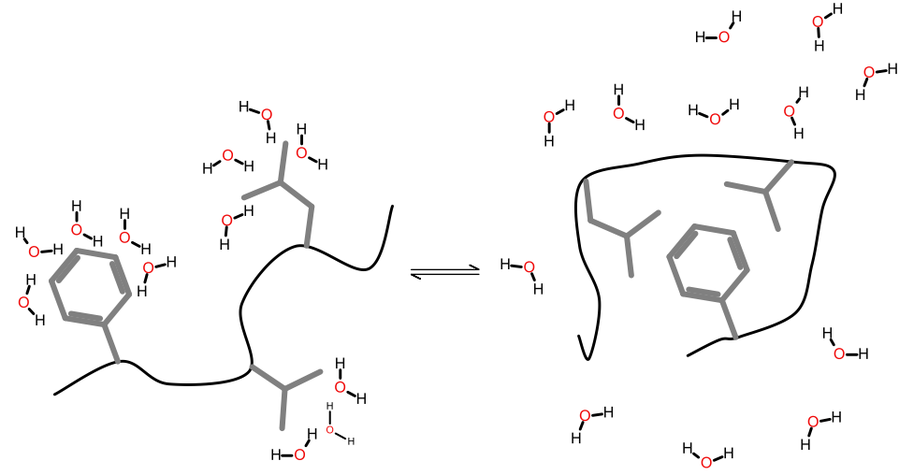
Strength of Van der Waals Depends on the Surface Area

Hydrophobic Interactions are Critical for Stabilizing the Folded Structure

Energy and Entropy



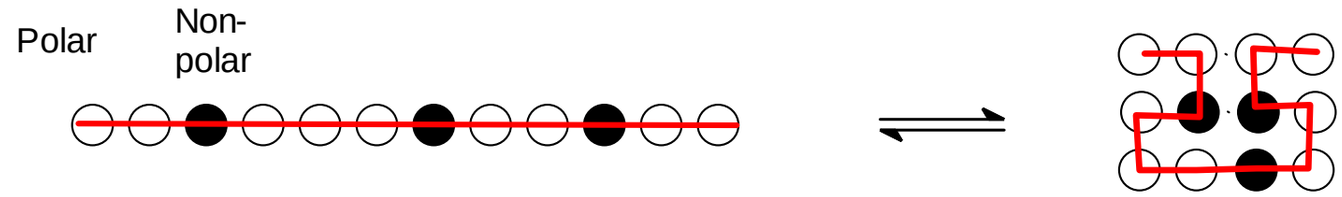
Ordered water hydrating a non-polar group



Hydrophobic interactions within a folded protein increase stability of the folded protein by releasing the ordered water that surrounded exposed non-polar groups in the unfolded protein. *Folding increases the entropy of the water – favorable.*

Fold Depends on Amino Acid Sequence

The *position* of non-polar residues (filled circles) mostly affects the final fold:



Which is more stable fold?

A

B

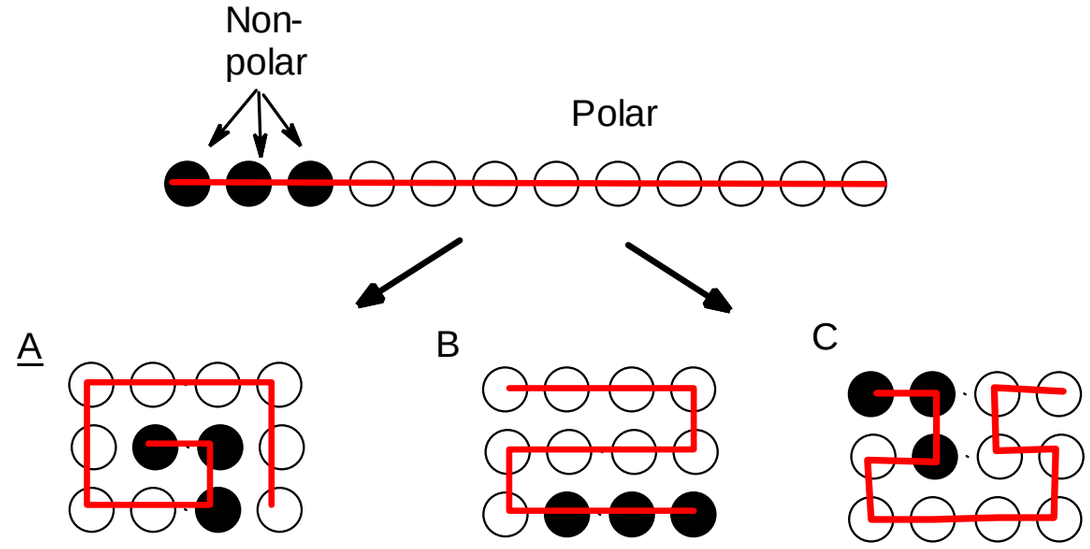
C

Which is the least stable fold?

A

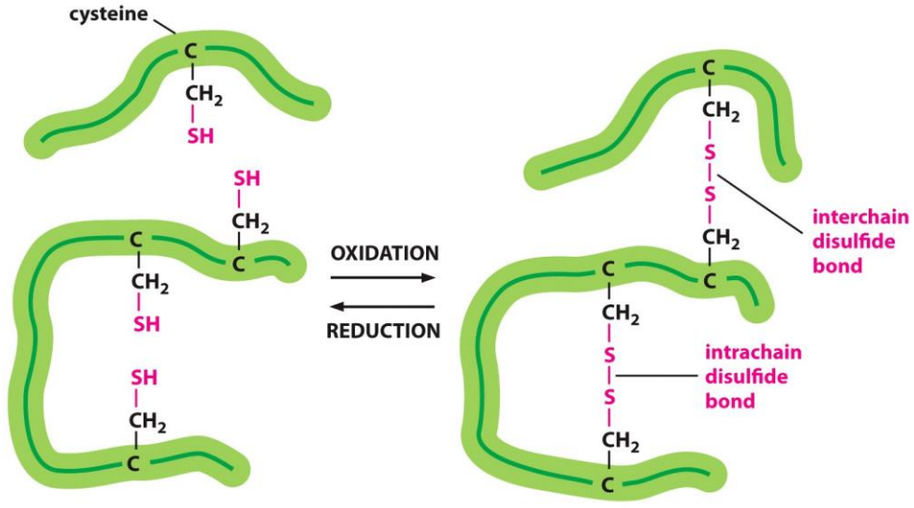
B

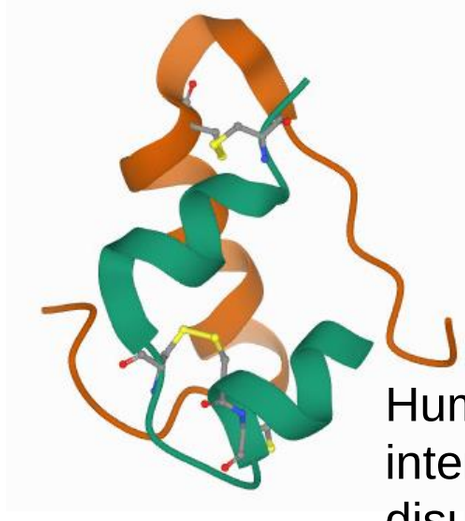
C



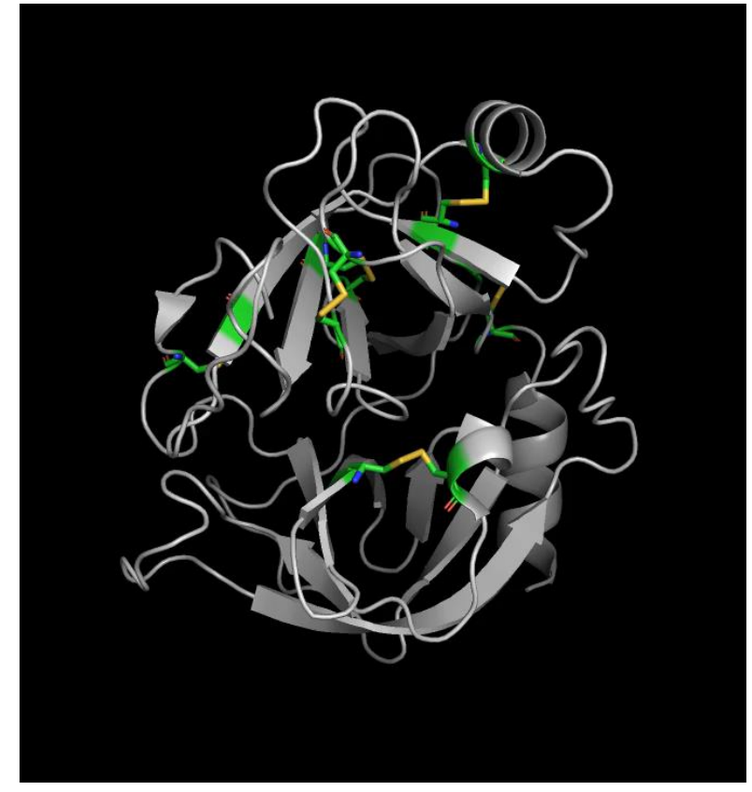
Why?

Disulfide Bonds Stabilize Some Proteins Outside the Cell (and body)





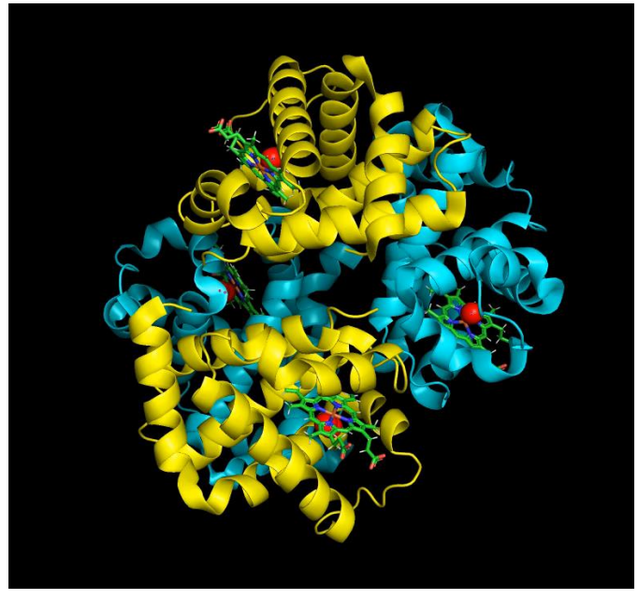
Human Insulin – interchain disulfide bonds



Trypsin – a digestive enzyme produced in the pancreas, exported to the small intestine – disulfide bonds within a single chain.

Quaternary Structure

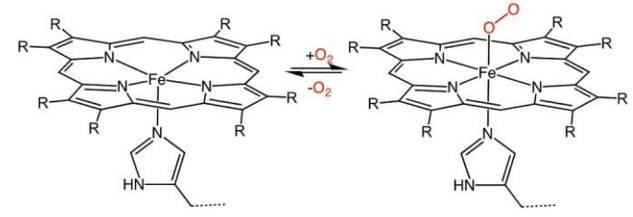
- Combinations of polypeptide subunits (combinations of tertiary structures).
- May be held together by covalent bonds (disulfide), but usually non-covalent interactions between amino acids on the different chains.
- Proteins can be a dimer, a tetramer, etc.
- If the chains are the same, called homo_____. If chains are different, hetero_____



Quaternary structure of hemoglobin (oxygen transport protein):

- two α chains
- two β chains

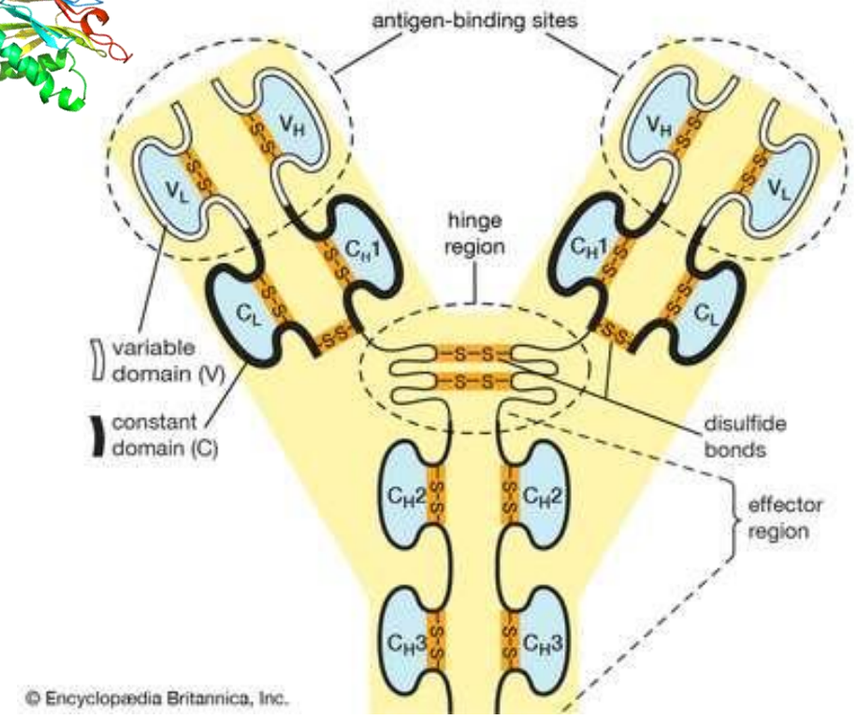
Oxygen is carried on Fe^{2+} within heme groups:

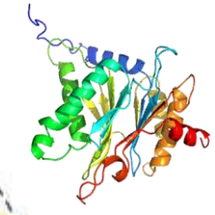


Antibodies – Produced by the Adaptive Immune system to Fight Pathogens.

Antigen

Antigen = ligand that binds to an antibody.





Antigen

Properties of Antibodies:

- 4 chains – two identical light (200 aa), two identical heavy (400 aa).
- Bind two identical antigens (pathogens, toxins)
- Chains crosslinked with disulfide bonds, increasing stability.

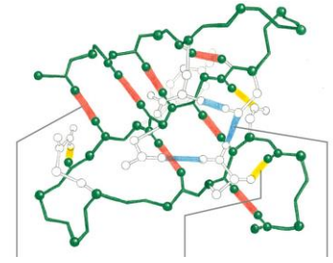
Summary - Interactions that Stabilize Folded Proteins.

- **Hydrogen bonds** form between hydrogen atoms (NH) and the carbonyl group in the peptide backbone (mainchain), and between and donors and acceptors on sidechains. *Mainchain-mainchain H-bonds are responsible for secondary structures.*
- **Hydrophobic interactions** within a protein increase stability of the folded state by *increasing entropy due to the release of water that was ordered by the exposed non-polar groups in the unfolded protein.*
- **van der Waals interactions** are *optimized in the well packed core of the protein*.

- **Covalent disulfide bonds** form between sulfur-containing cysteine residues stabilizing them (usually only exported, secreted proteins).

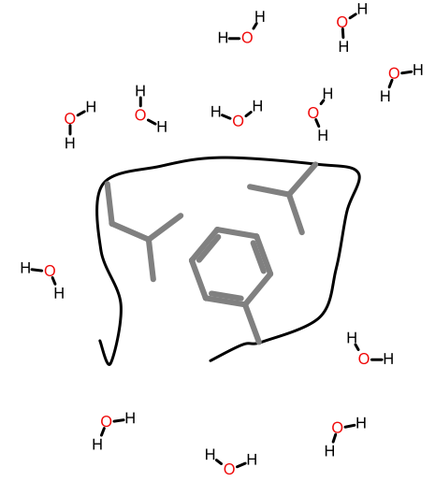
H-bonds
van der Waals
Hydrophobic effect

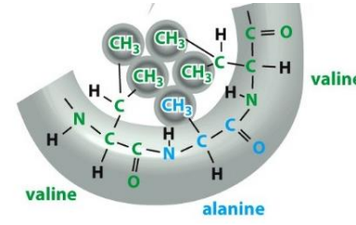
Chain disorder

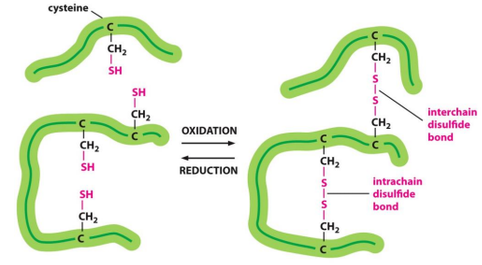


Native

Unfolded







A single change in the amino acid sequence can change the function of a protein, and often affecting how it folds – Producing Inactive Proteins.

frontiers in Molecular Biosciences

ORIGINAL RESEARCH
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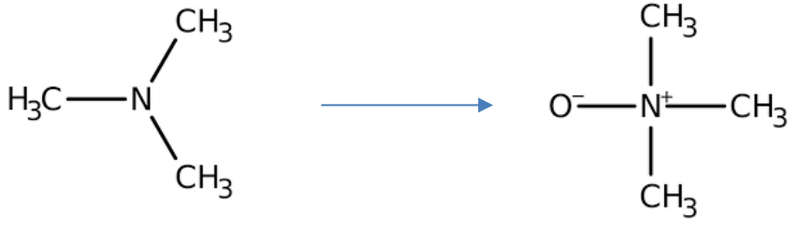
Solvent Accessibility of Residues Undergoing Pathogenic Variations in Humans: From Protein Structures to Protein Sequences

Castrense Savojardo [1], Matteo Manfredi [1], Pier Luigi Martelli [1] and Rita Casadio [1,2]*

[1] Biocomputing Group, Department of Pharmacy and Biotechnologies, University of Bologna, Bologna, Italy, [2] Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies of the National Research Council, Bari, Italy

Mutations in dimethylaniline monooxygenase 3 cause trimethylaminuria (high levels of trimethylamine)



dimethylaniline monooxygenase 3

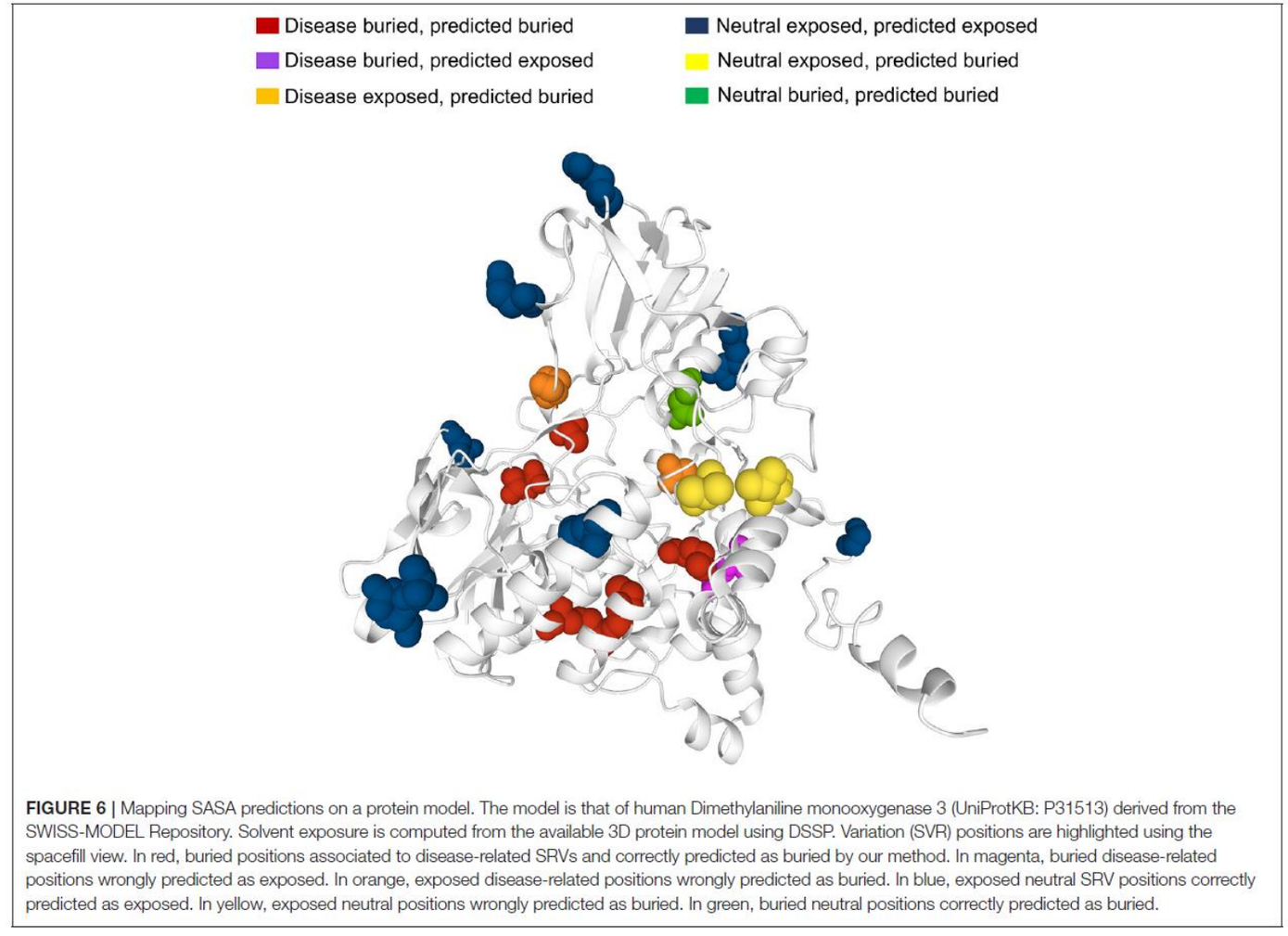
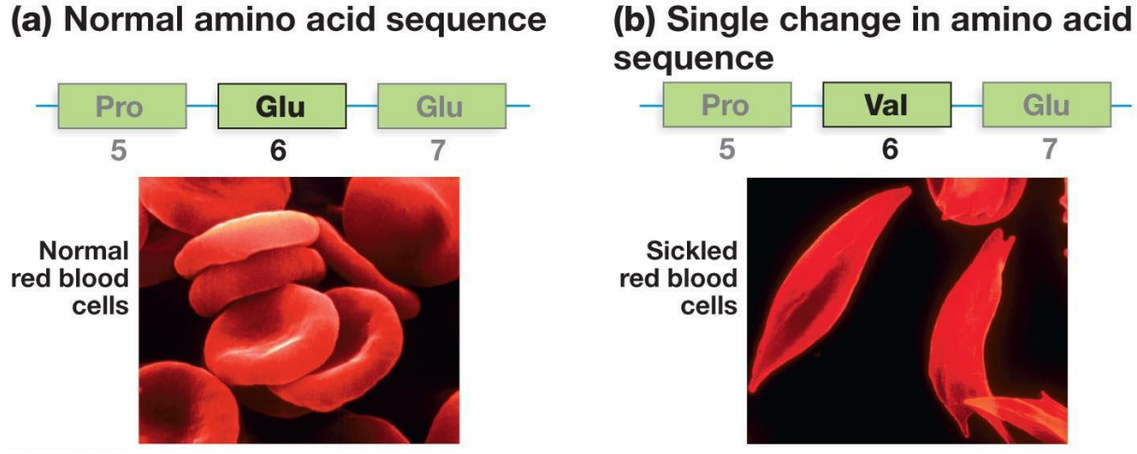


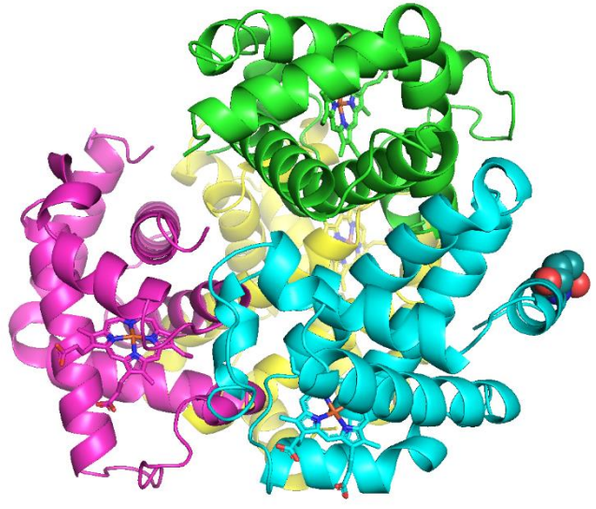
FIGURE 6 | Mapping SASA predictions on a protein model. The model is that of human Dimethylaniline monooxygenase 3 (UniProtKB: P31513) derived from the SWISS-MODEL Repository. Solvent exposure is computed from the available 3D protein model using DSSP. Variation (SVR) positions are highlighted using the spacefill view. In red, buried positions associated to disease-related SRVs and correctly predicted as buried by our method. In magenta, buried disease-related positions wrongly predicted as exposed. In orange, exposed disease-related positions wrongly predicted as buried. In blue, exposed neutral SRV positions correctly predicted as exposed. In yellow, exposed neutral positions wrongly predicted as buried. In green, buried neutral positions correctly predicted as buried.

Surface Mutations May Also Lead to Disease

Effect of mutations on protein folding – sickle cell anemia



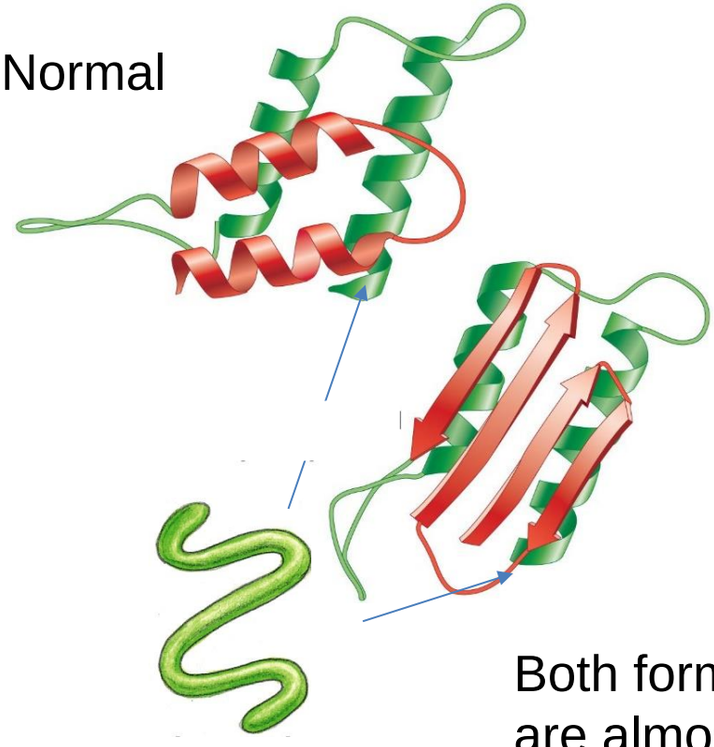
A single change in the amino acid sequence can change the function of a protein



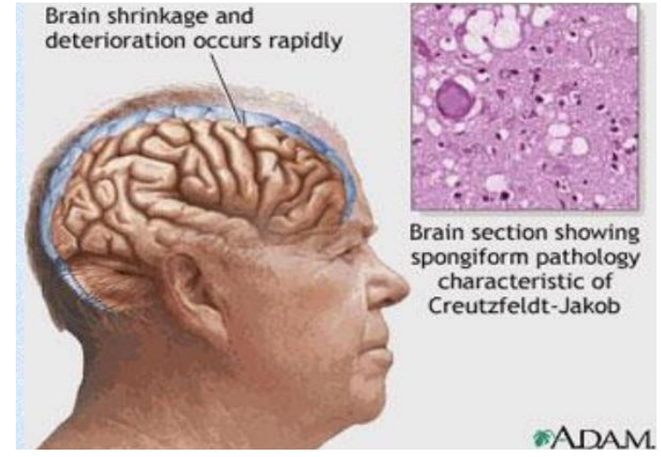
Glu6

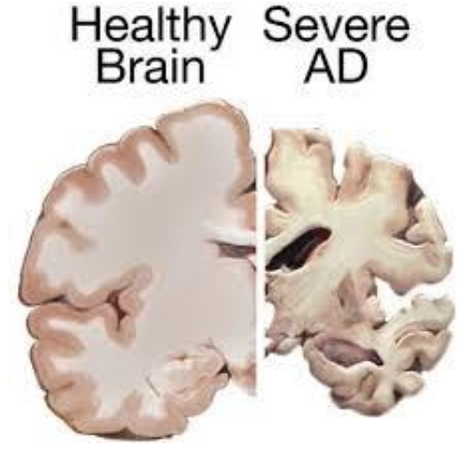
What Happens When Proteins Fold Into Different Structures?

Prions are improperly folded proteins that cause neurodegenerative diseases



Both forms are almost equally stable.





What is the effect on the brain?

Unfolded protein response (UPR): The presence of unfolded proteins can trigger the UPR, which can turn off protein synthesis in the cell, leading to cell death.

Why do the brain cells die?

Protein Structure - Summary and Expectations

Primary Structure:

- Can you describe the mechanism of peptide bond formation
- Can you draw structure of peptides.
- Can you identify amino terminus and give the sequence of amino acids, N -> C

Secondary structure:

- Identify helical and sheet secondary structures,
- know that they are stabilized by **mainchain** hydrogen bonds between N-H and O=C.
- Location of H-bonds and sidechains

Tertiary Structure:

- Can you describe and identify role of the following in stabilizing the folded state.
  - H-bonds,
  - van der Waals,
  - hydrophobic effect
- Can you predict, based on sidechain, which amino acids are found in the core of the protein and which are found on the surface.

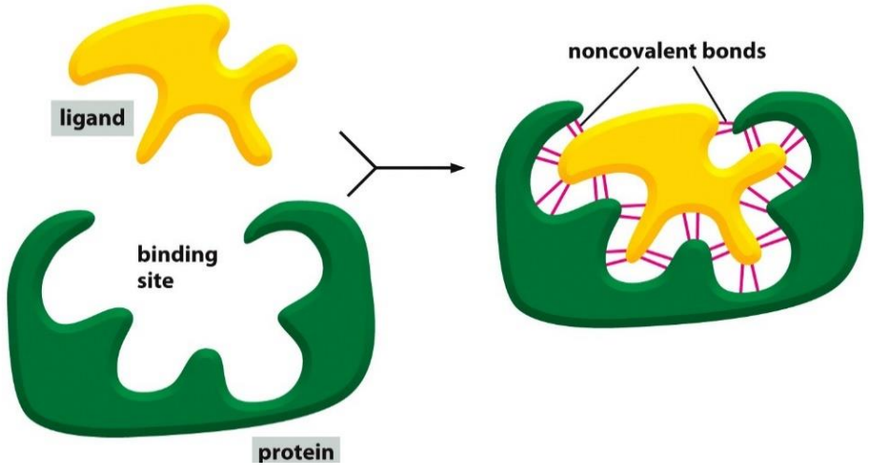
Quaternary Structure:

- Multiple chains, stabilized by non-covalent and covalent (disulfide bonds) interactions.

Diseases related to protein structure:

- Core mutations - affect folding
- Surface mutations - affect protein-protein (and Protein-DNA) interactions
- Stable isoforms – toxic to the cell

Ligand Binding: Most Proteins Bind to Other Molecules in Biological Interactions:



Ligand: Something that binds to a protein, usually small molecules (e.g. cyclicAMP, cAMP).

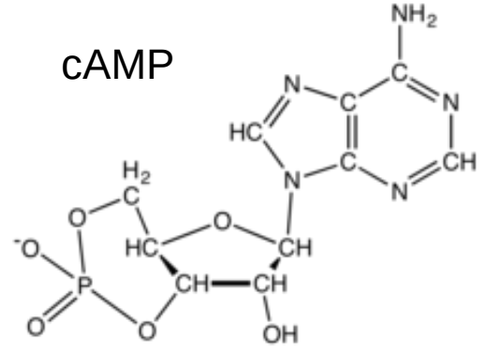
Binding site allow a protein to interact with specific **ligands**

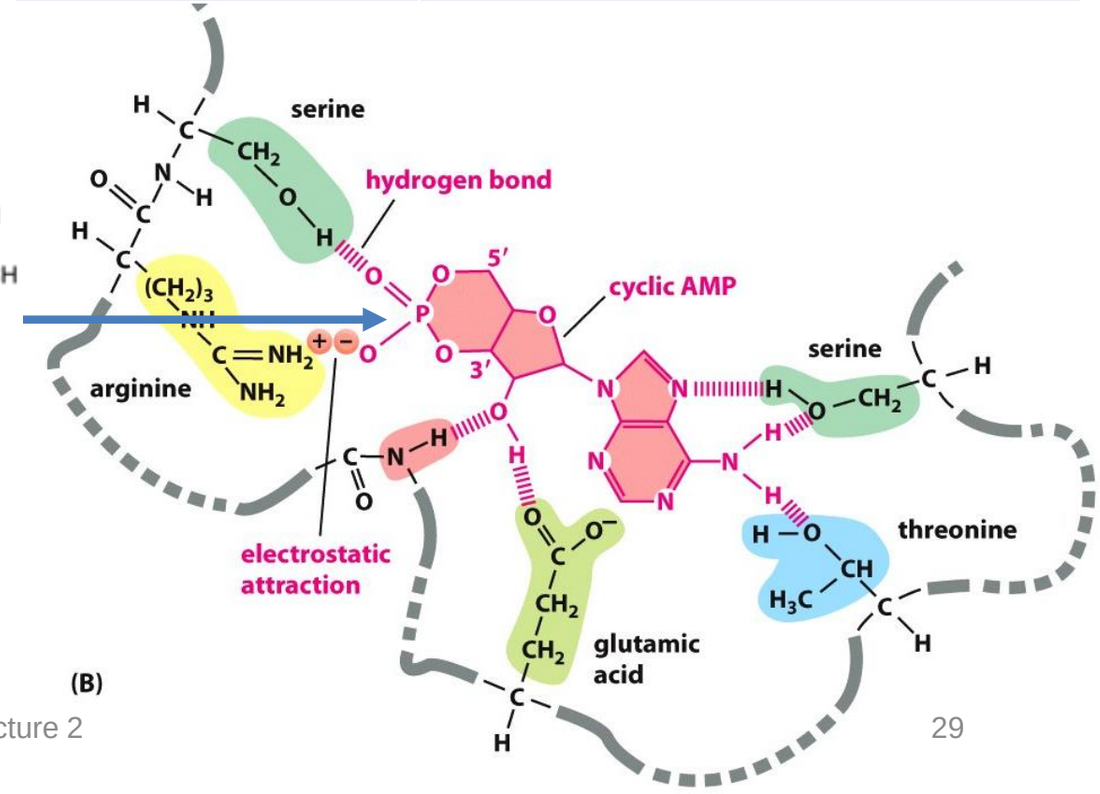
Binding site is generated by the **folded** form of the protein.

The bound ligand can be stabilized by any and all of the following interactions:

Interaction	Which stabilize cAMP Binding?
Electrostatic	
van der Waals	
H-Bonding	
Hydrophobic effect	

cAMP



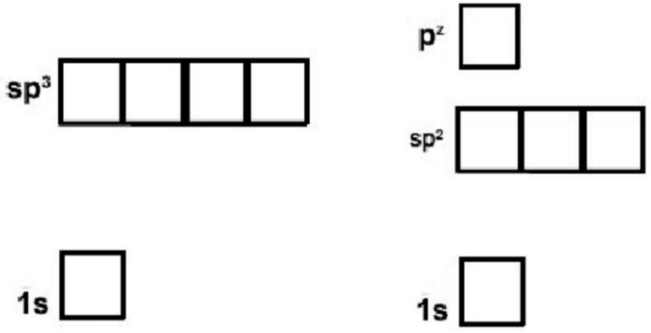


How to Identify Hydrogen Bond Donor and Acceptors

O-H and N-H are always donors

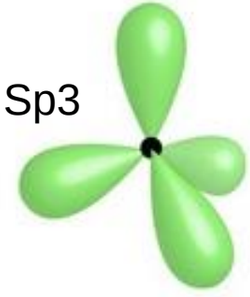
Exceptions, N in a delocalized system:

- Will not accept from above or below the plane of the system, because the lonepair is delocalized.
- Can accept in the plane of the ring if there is no attached hydrogen, via lone pair in sp2 orbital

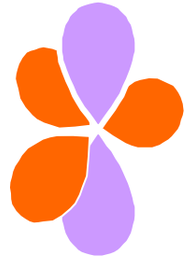


N – 7 electrons

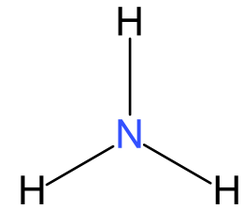
Sp3

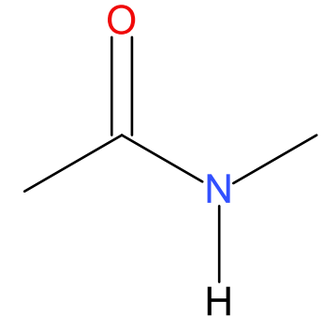


Sp2

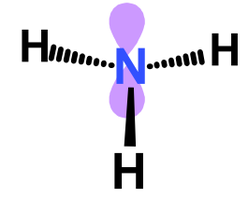


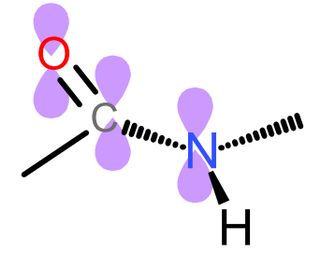
Nitrogen can form two types of hybrid orbitals, sp3 (tetrahedral geometry) or sp2 (planer) + pz. (Hybrid orbitals are combinations of atomic orbitals.)



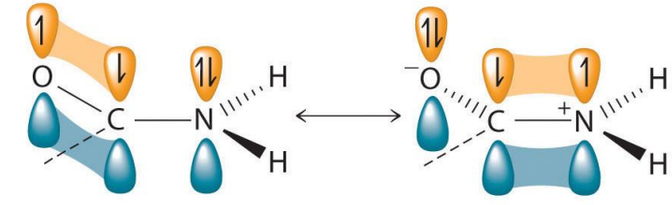


- Sp2 is used in amides, allowing favorable overlap of the full pz orbital with the pz on C and O
- The lone pair in the pz is shared with the pz electrons on carbon and oxygen.
- Due to electron sharing, there is only a slight neg. charge and the group does not accept an H-bond.





- Sp3 is used in ammonia, keeping the three hydrogen atoms as far from each other as possible. The fourth sp3 orbital is full with two electrons (lone pair).
- The lone pair is an excellent acceptor.

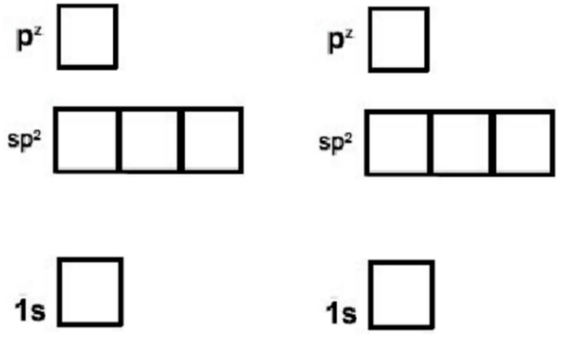


(a)

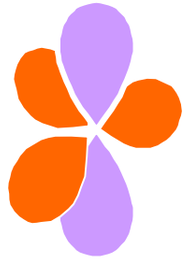
How to Identify Hydrogen Bond Donor and Acceptors

Exception, N in a delocalized system:

- Will not accept from above or below the plane of the system, because the lonepair is delocalized.
- Can accept in the plane of the ring if there is no attached hydrogen, via lone pair in sp2 orbital



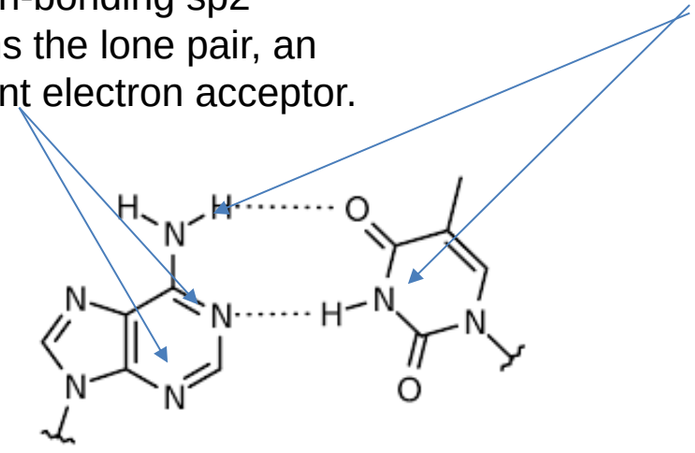
N – 7 electrons



Sp2

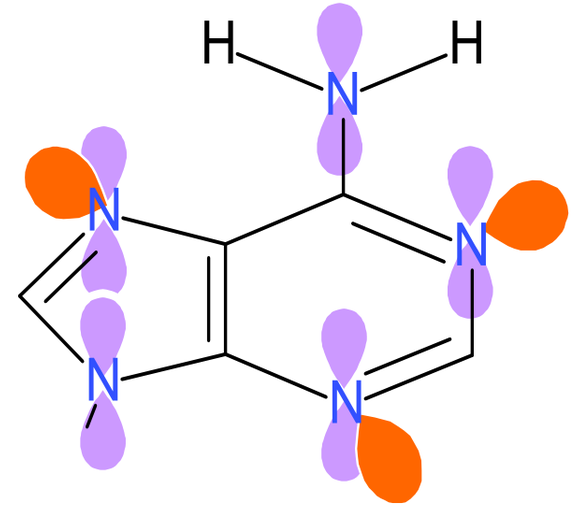
In planer aromatic rings the nitrogen must use sp2

- The pz orbital holds one electron, used to form double bonds
- The non-bonding sp2 contains the lone pair, an excellent electron acceptor.

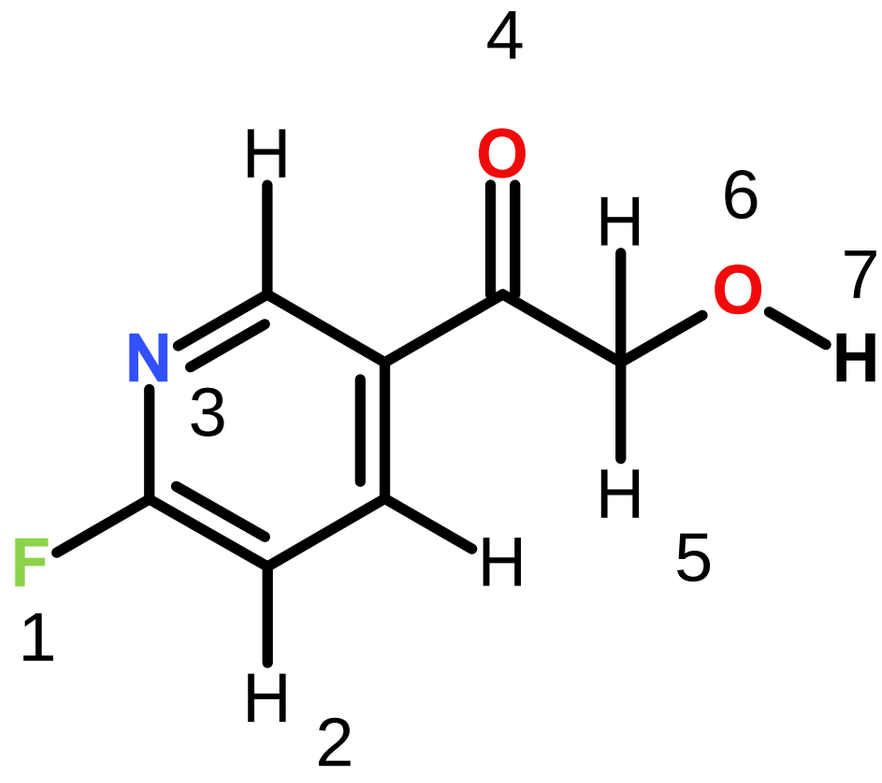


Adenine Thymine

- The pz orbital holds two electrons since each sp2 contains one electron to share in forming the single bonds.
- Although the pz orbital contains two electrons (lone pair), these are delocalized (shared) over the ring. Therefore not an acceptor.



1. *Indicate which atoms are donors and which are acceptors.*
2. *If appropriate, draw a water molecule interacting with the donor/acceptor.*

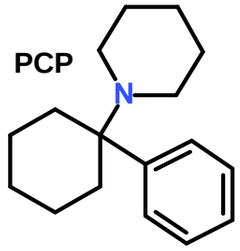


ATOM	Donor?	Acceptor?	Neither
1 (F)			
2 (C_{aro}-H)			
3 (N)			
4 (C=O)			
5 (C-H)			
6 (O)			
7 (O-H)			

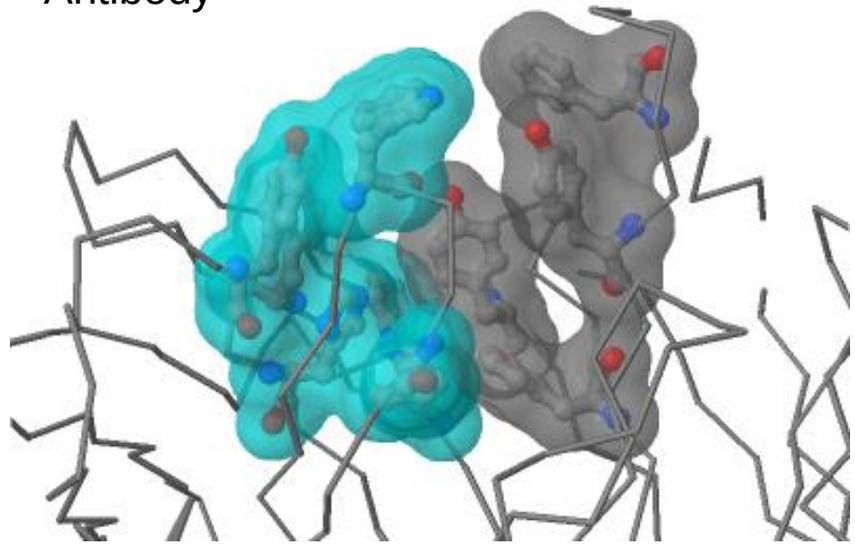
Can you?

- Identify groups that can donate or accept hydrogen bonds?

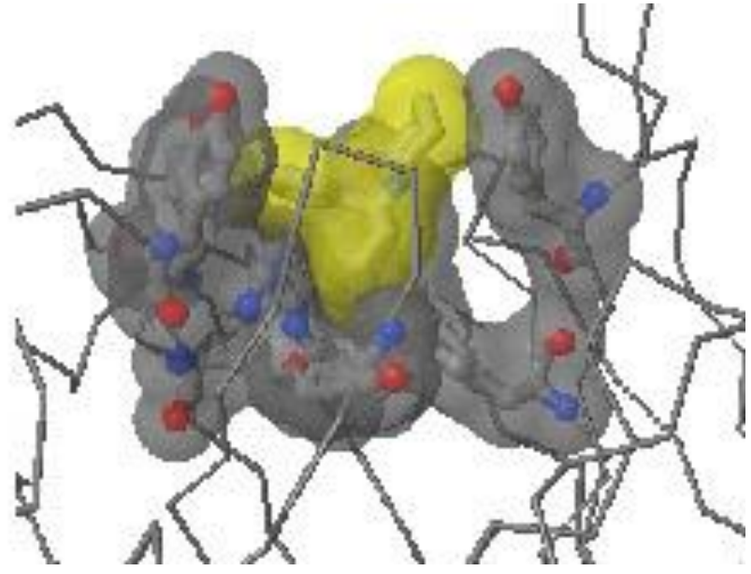
Hydrophobic Effect and Ligand Binding

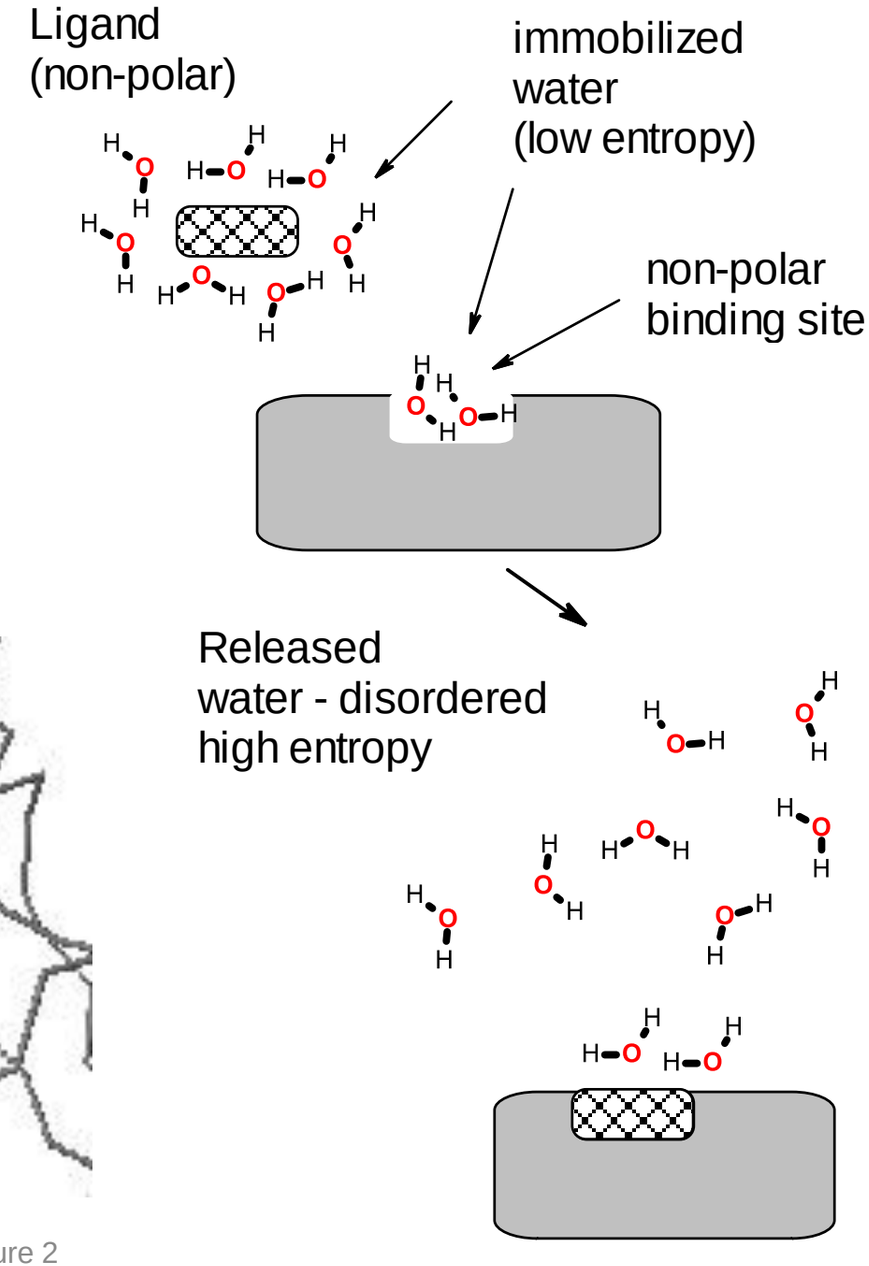


Antibody



Antibody-PCP complex

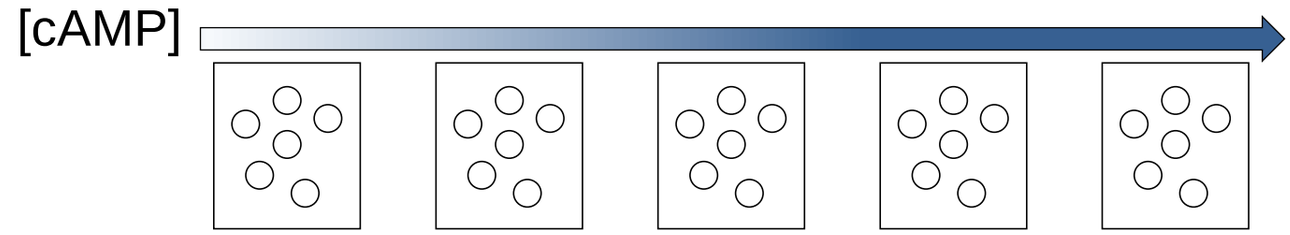


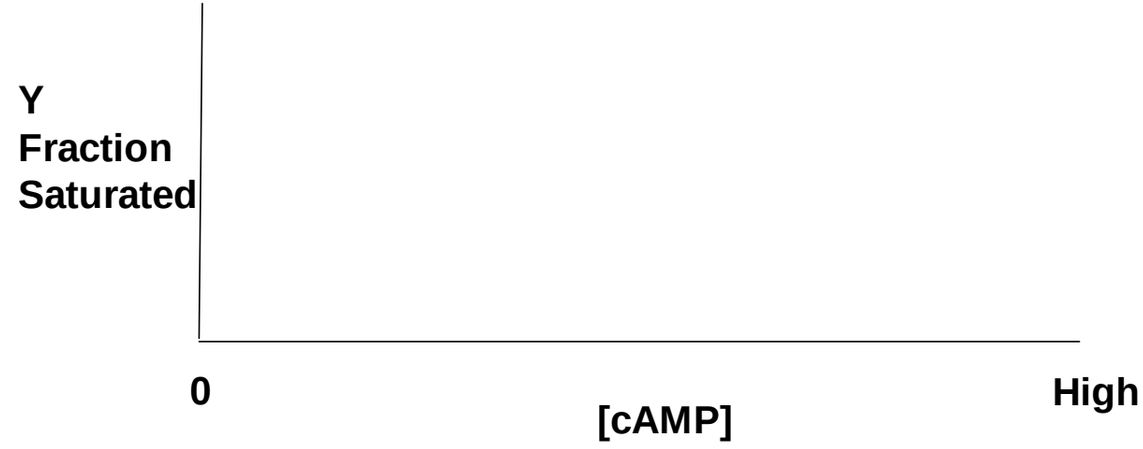


Ligand Binding & Saturation:

Define fraction saturated: $Y = \frac{[ML]}{[M] + [ML]}$

[M] = free macromolecule (e.g. antibody with no antigen).
[ML] = macromolecule with ligand bound (e.g. antibody with antigen bound).





The boxes with circles represent proteins with no cAMP bound, each box (left to right) is at a higher [cAMP]. Filled circles indicate bound ligand.

1. How will the number of filled circles depend on the cAMP concentration?

2. Plot the fraction saturated data point for each box.

Key Points:

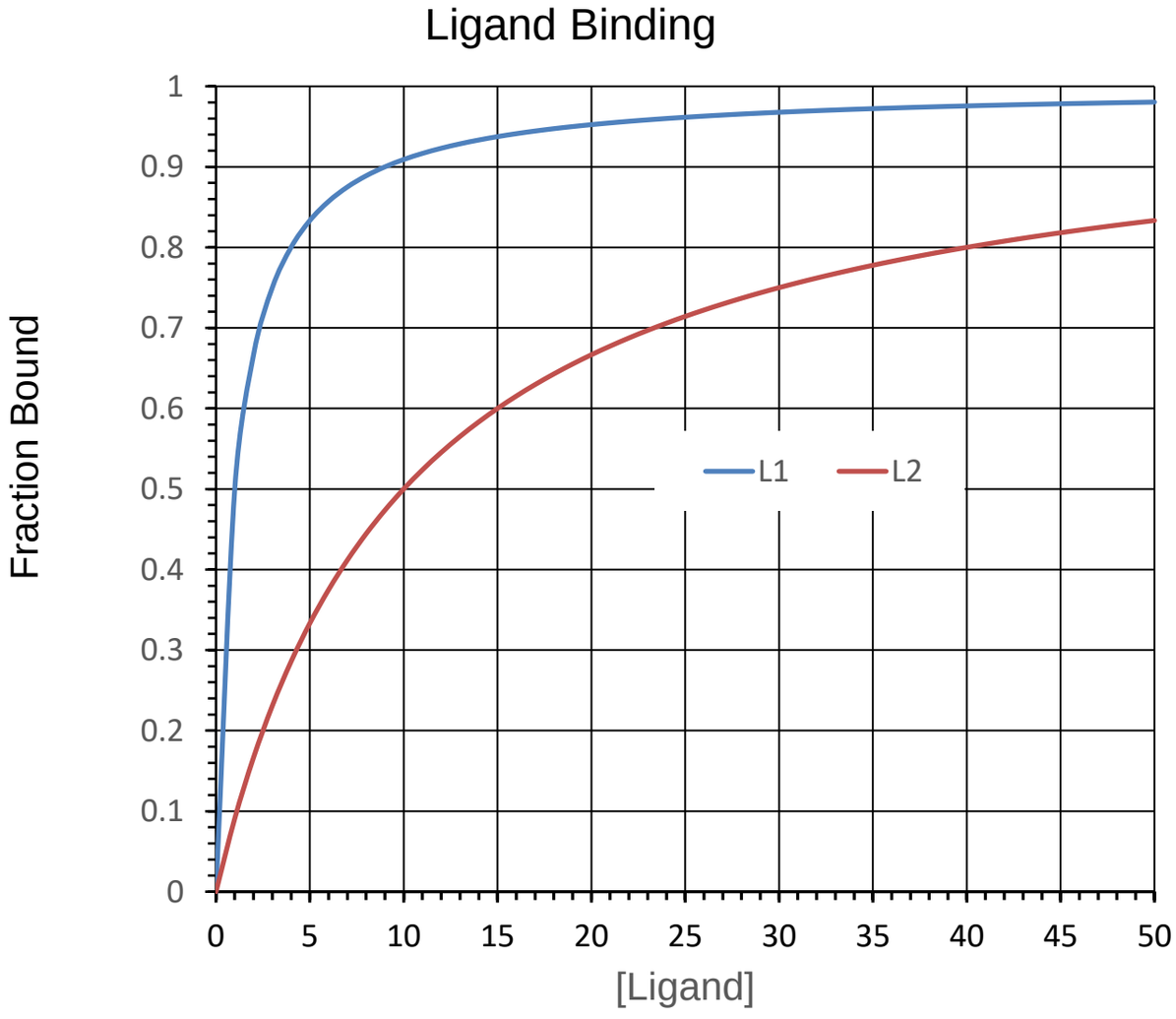
1. The binding sites saturate, when all are full no more ligand can bind.
2. There is a ligand concentration, [L], where ½ the sites are full. This [L] is K_D
3. K_D is the equilibrium constant for ligand dissociation:

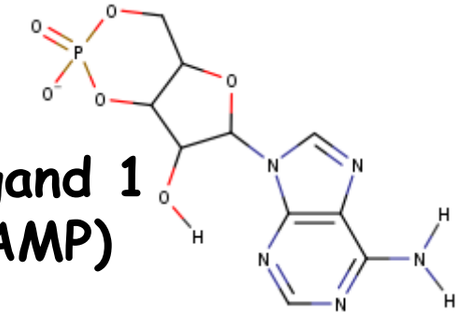
$$K_{Eq} = \frac{[products]}{[reactants]}$$

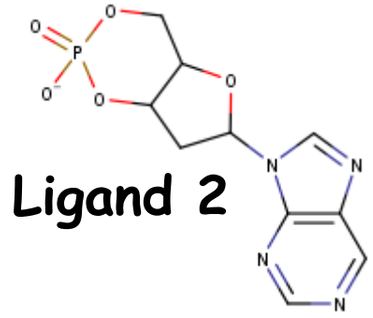
$$(ML) \rightleftharpoons (M) + (L)$$

$$K_D = \frac{[M][L]}{[ML]}$$

Using K_D to Compare Ligand Binding







The binding of two different molecules to the same protein was measured and the data is shown on the right. L1 is cAMP, L2 is similar to cAMP

Which ligand has a K_D of 1? L1 or L2?

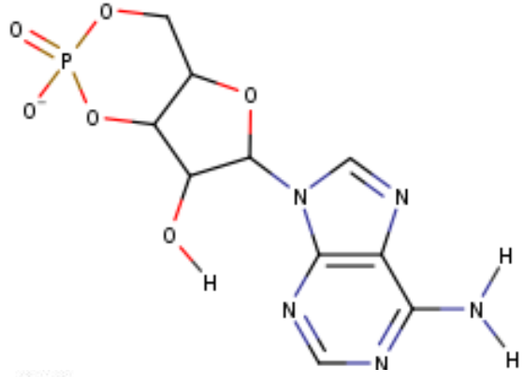
Which ligand has a K_D of 10? L1 or L2?

Which ligand binds more tightly to the protein (higher affinity)? L1 or L2?

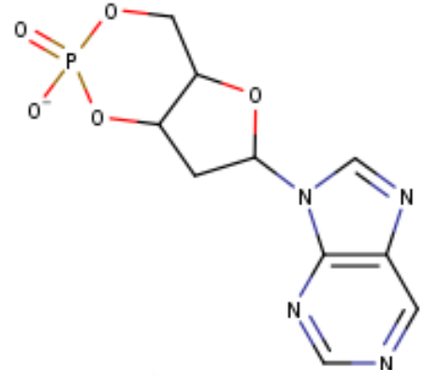
__Why__ does L1 bind more tightly (higher affinity)?

1. What are the chemical differences between L1 and L2 (Upper diagram)

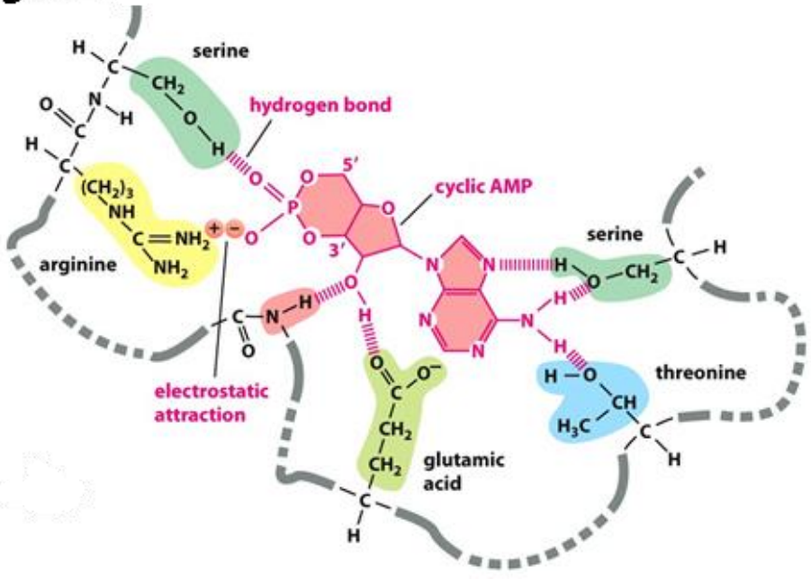
Ligand 1 (cAMP)



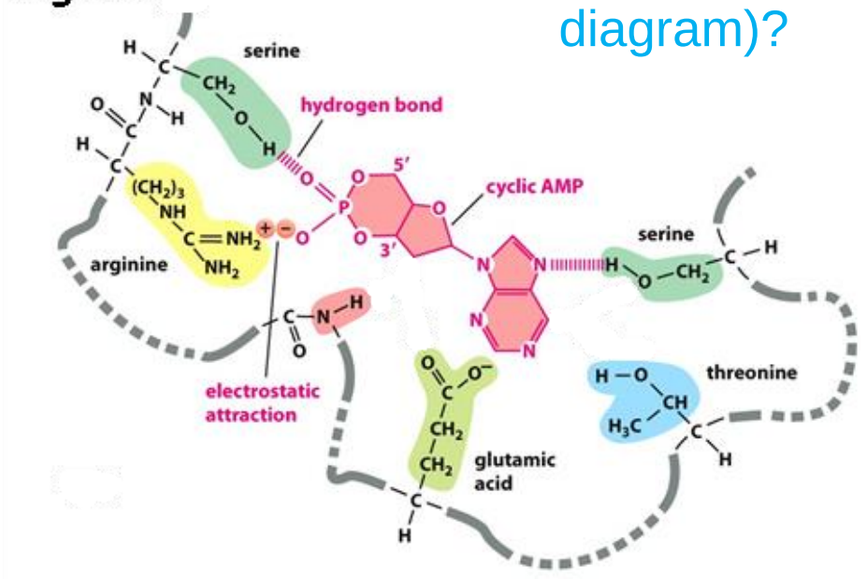
Ligand 2



Ligand 1



Ligand 2



2. How do these differences affect the interactions with the protein (lower diagram)?

3. How do the differences affect K_D?

Key Points:

Binding:

Folded proteins have ***binding sites*** that recognize other molecules (***ligands***) using ***any and all*** of the following:

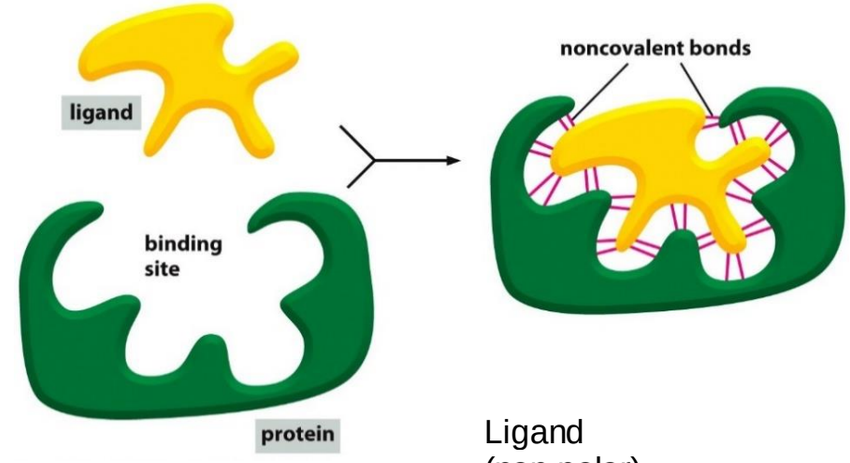
- H-bonds,
- van der Waals,
- Electrostatic,
- Non-polar interactions (hydrophobic)

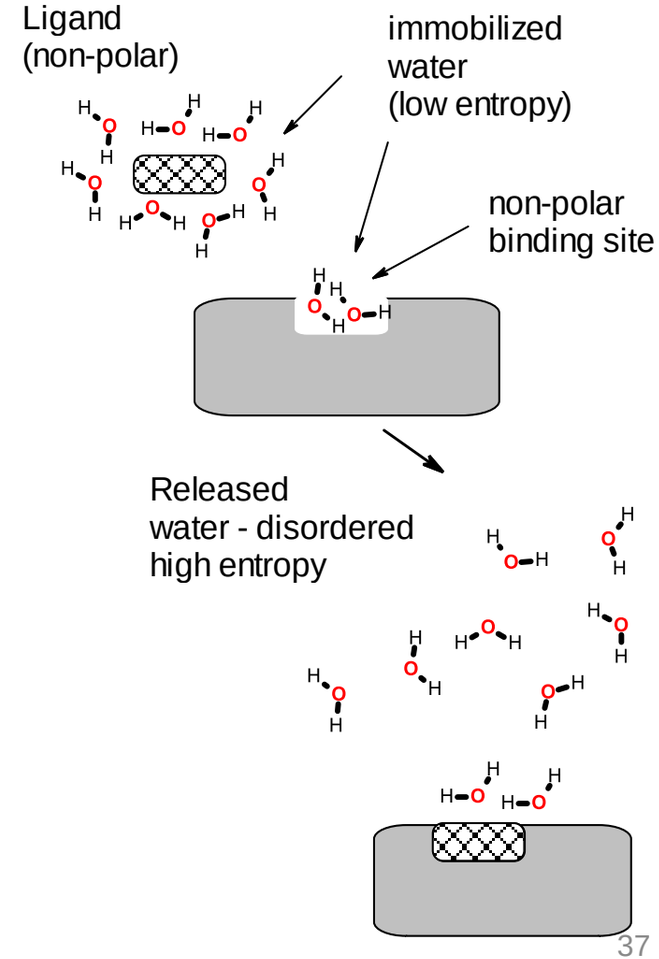
Binding is **reversible**

Binding is **saturable**

Binding ½ point (Y=0.5) occurs at K_D

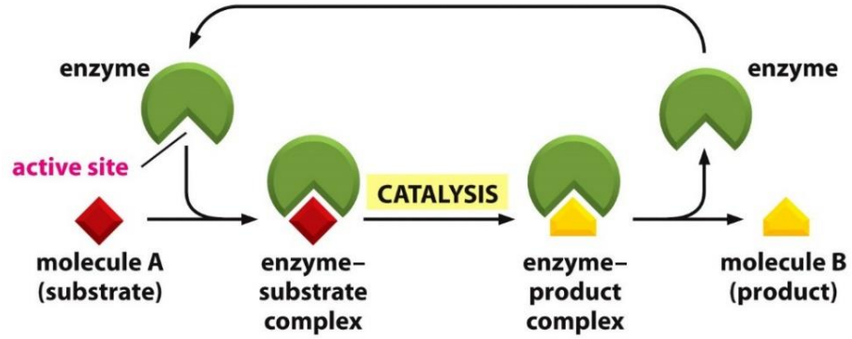
The higher the affinity (strength of interaction), the lower the K_D

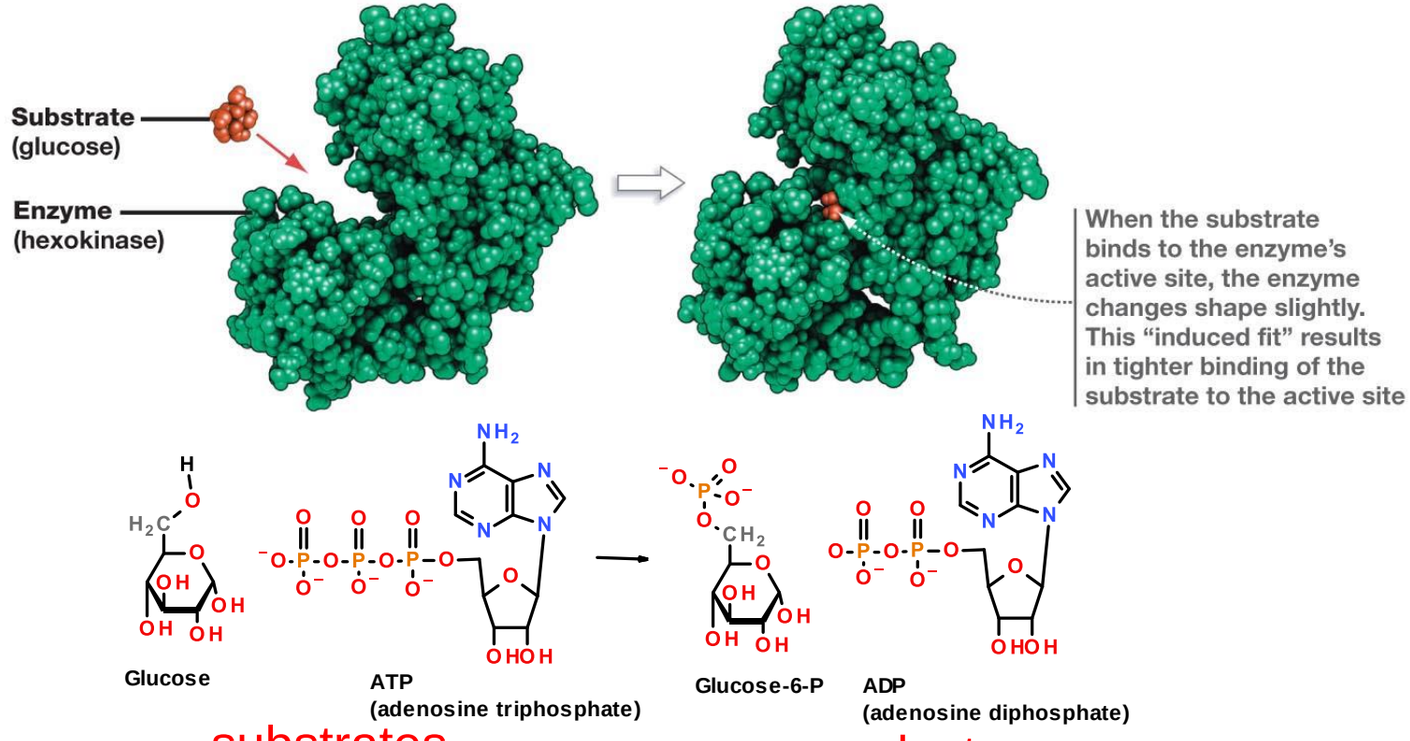




Enzymes

- **Enzymes** are protein or RNA catalysts. They increase the rate of the reaction.
- They bind “substrates” and convert them to “products”. Usually, the substrate undergoes a chemical reaction and is changed in its structure.
- Most biological chemical reactions occur at meaningful rates only in the presence of an enzyme.
- Substrates bind specifically to the enzyme’s **active site,** interacting with amino acid side chains (or RNA bases). Usually a single enzyme binds one substrate.
- The chemical change caused by the enzyme is catalyzed by additional functional groups in the active site.
- Many enzymes undergo a conformational change when the substrates are bound to the active site; this change is called an **induced fit**.





Enzyme – Chemical Diversity

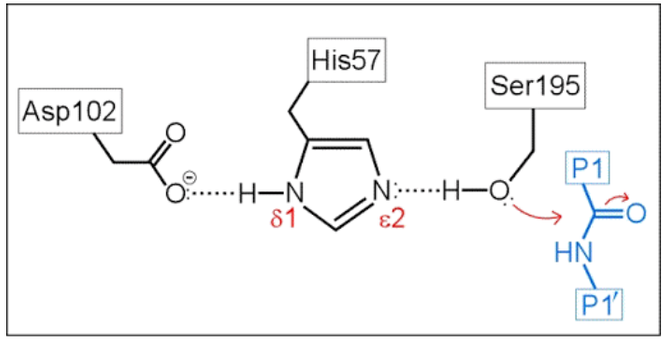
TABLE 4–1 SOME COMMON FUNCTIONAL CLASSES OF ENZYMES	
ENZYME CLASS	**BIOCHEMICAL FUNCTION**
Hydrolase	General term for enzymes that catalyze a hydrolytic cleavage reaction.
Nuclease	Breaks down nucleic acids by hydrolyzing bonds between nucleotides.
Protease	Breaks down proteins by hydrolyzing peptide bonds between amino acids.
Synthase	General name used for enzymes that synthesize molecules in anabolic reactions by condensing two molecules together.
Isomerase	Catalyzes the rearrangement of bonds within a single molecule.
Polymerase	Catalyzes polymerization reactions such as the synthesis of DNA and RNA.
Kinase	Catalyzes the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins.
Phosphatase	Catalyzes the hydrolytic removal of a phosphate group from a molecule.
Oxido-reductase	General name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often called oxidases, reductases, or dehydrogenases.
ATPase	Hydrolyzes ATP. Many proteins with a wide range of roles have an energy-harnessing ATPase activity as part of their function, including motor proteins such as myosin and membrane transport proteins such as the sodium–potassium pump.

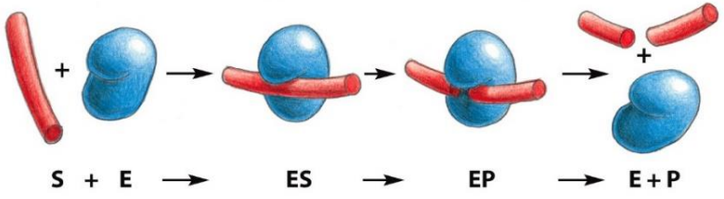
- Most enzyme names end in “-ase”
- Usually named by their substrates and the reactions they catalyse, i.e. glucose kinase

Example of Active Site Functional Groups:

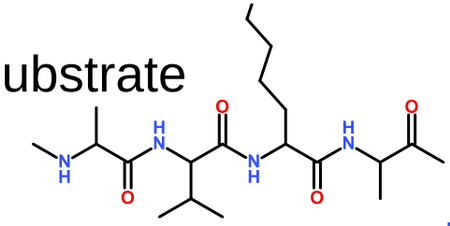
Catalytic triad (Asp, His, Ser) in Protease Trypsin cleaves after Lys Residues

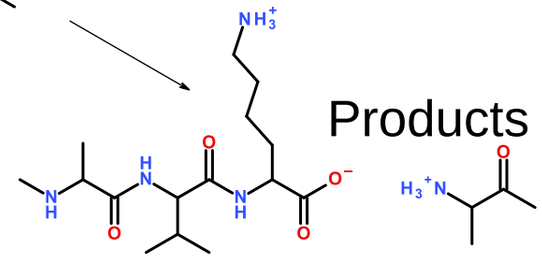
Catalytic triad



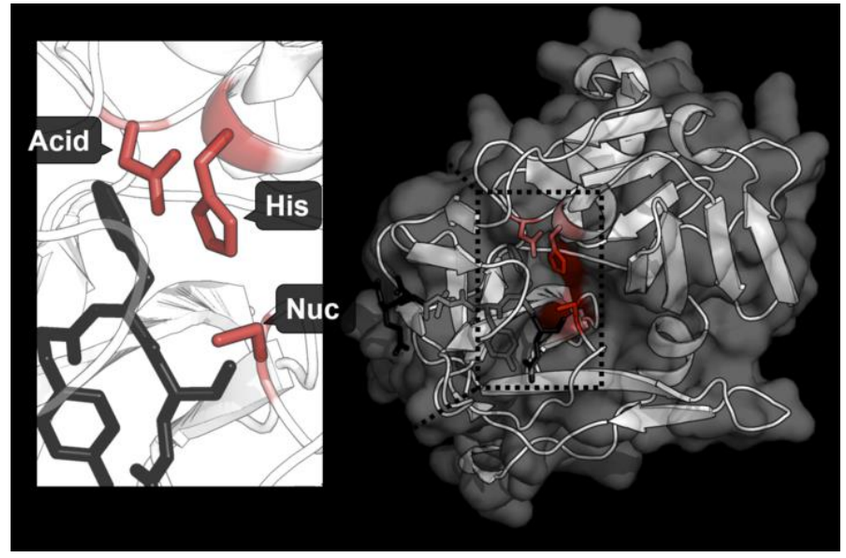


Substrate

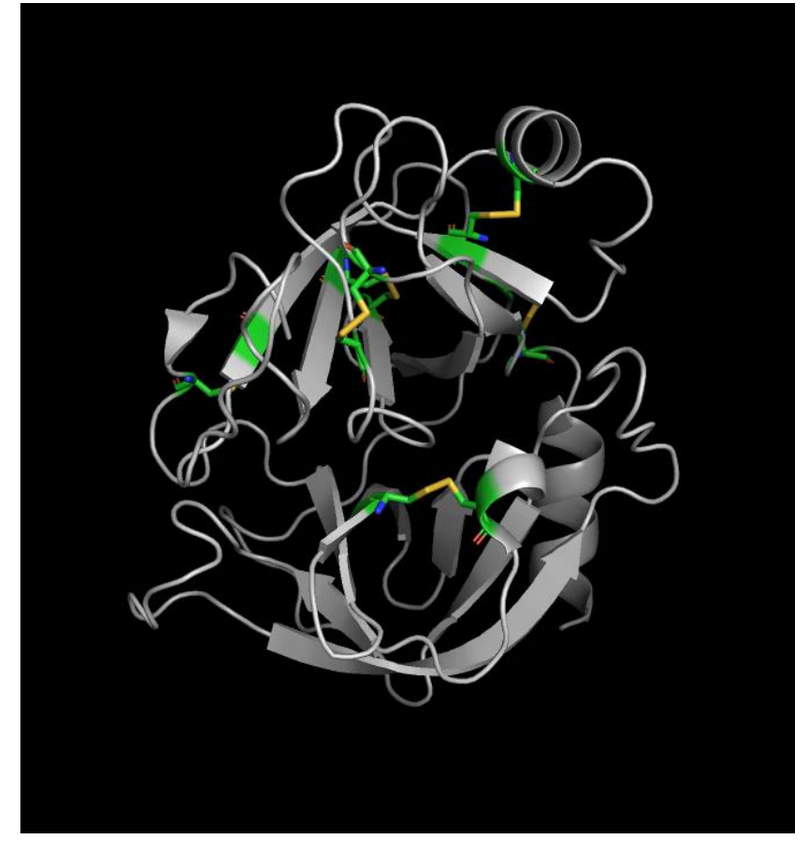




Products



https://shirleychemproject.weebly.com/



Disulfide bonds in trypsin

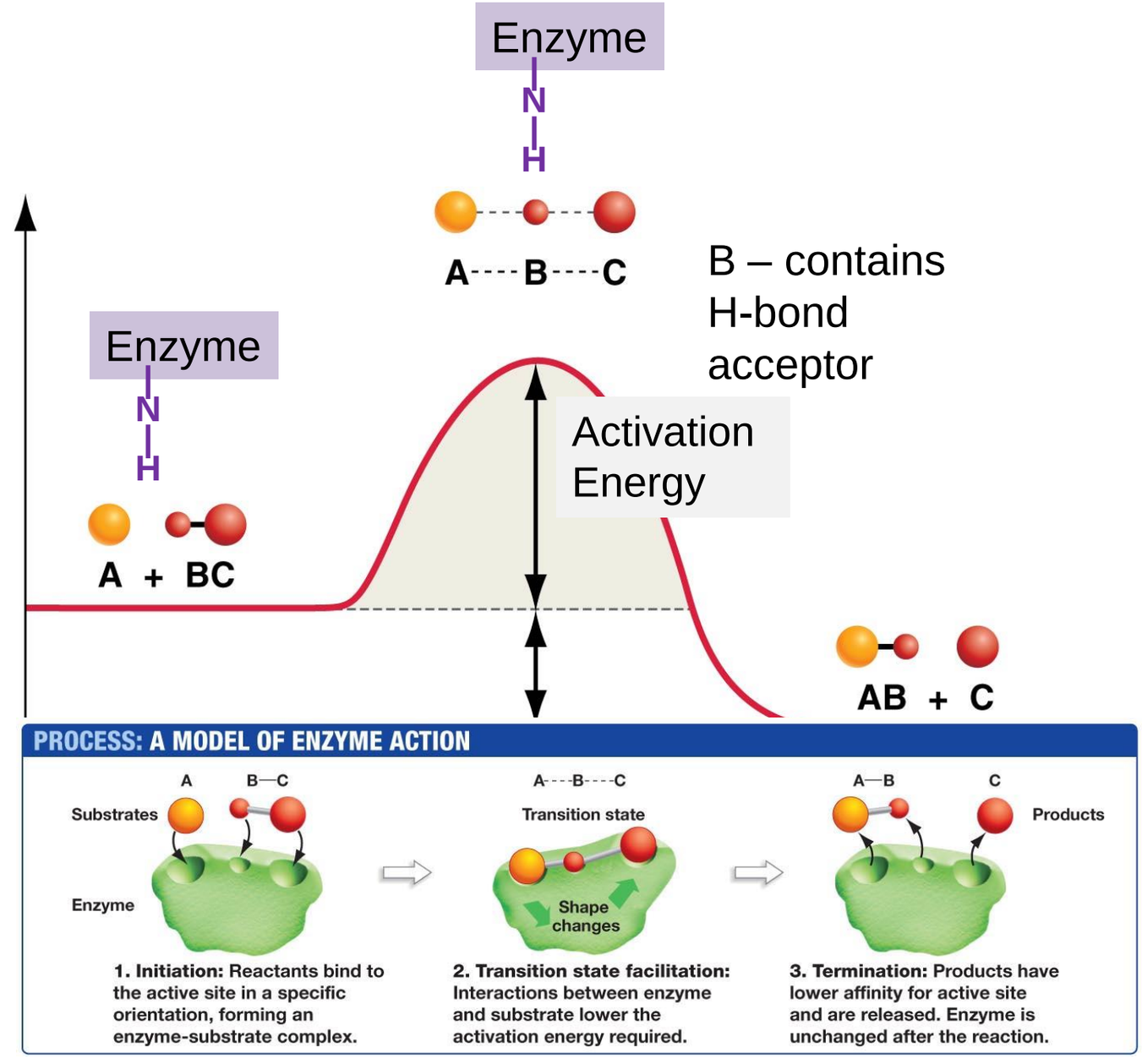
How Do Enzymes Increase Rates?

- **Transition state** = high energy intermediate that occurs during the reaction.
- Energy barrier is called the activation energy.
- Rate of product formation depends on the concentration of the transition state.

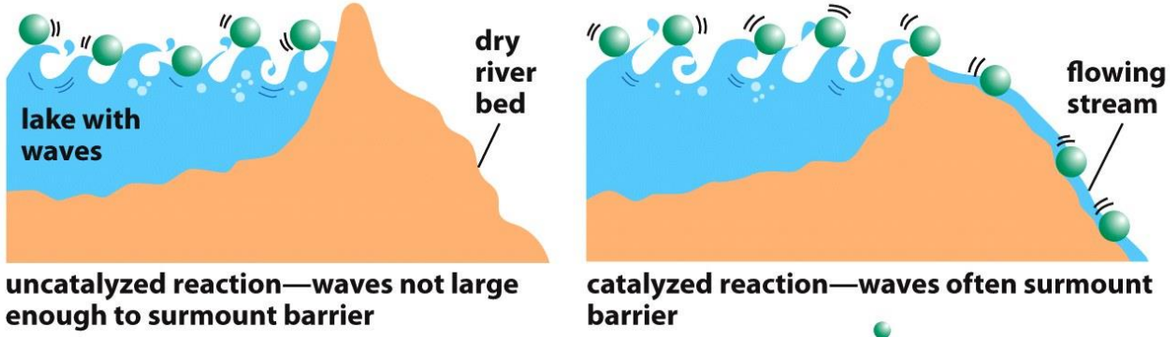
Low [X] = Slow reaction

Higher [EX] = Faster reaction

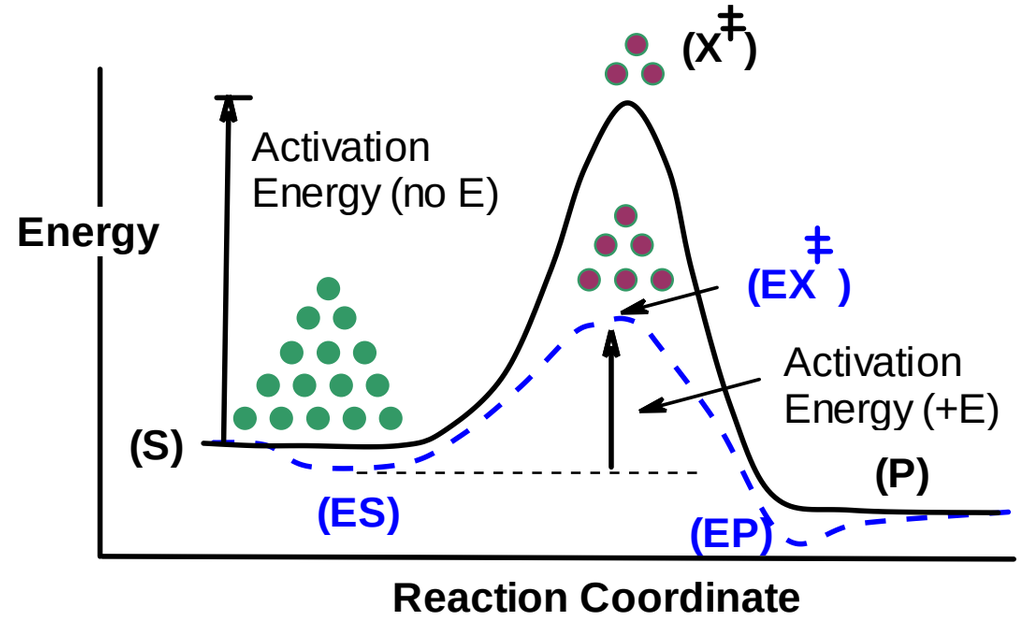
- Interactions between the enzyme and the substrate stabilize the **transition state** (X) and lower the activation energy required for the reaction to proceed.
- Stabilization can include:
  - Pre- alignment of key groups in the active site, reducing entropy cost of organizing groups.
  - Direct interactions with the transition state (see diagram, N-H group interacts more favorably with the transition state)



A model of transition state stabilization.



Lower energy of transition state allows more substrates to reach transition state due to their thermal energy.



[S] = 15

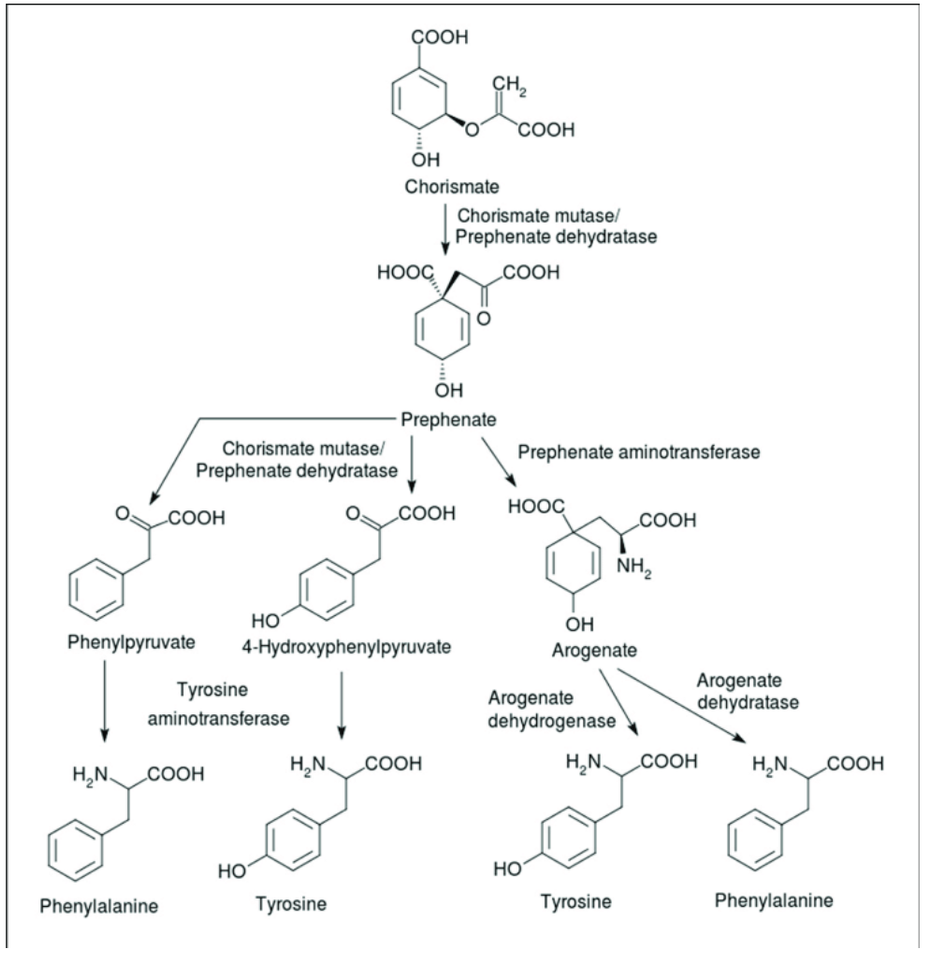
[X] = 3
[EX] = 6

How much faster will the rate be when the enzyme is present?

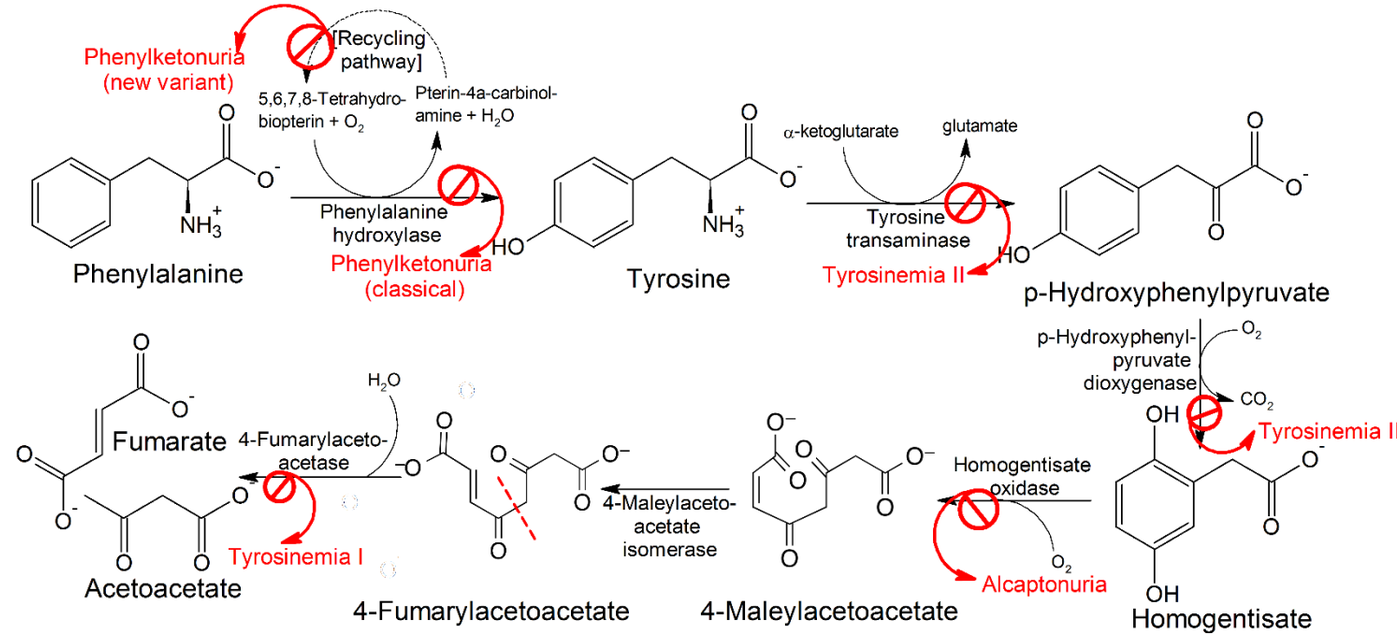
Enzymes, Metabolic Pathways, and Diseases

Synthetic Pathway for Phe, Tyr (beginning with chorismite)

- Each step catalyzed by an enzyme



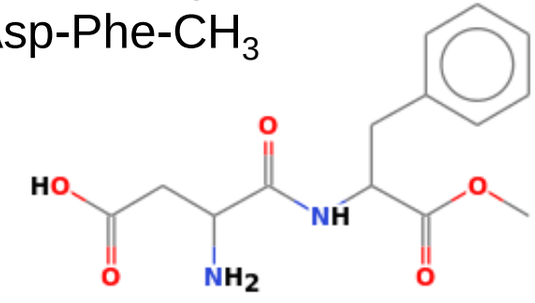
Pathway for Degradation of Phenylalanine



PKU Disease:

- Inactive phenylalanine hydroxylase
- Phe levels become toxic:
- Neurological problems
  - Intellectual disability
  - Developmental delays
  - Mental health disorders.

Aspartame (artificial sweetener)
Asp-Phe-CH_3

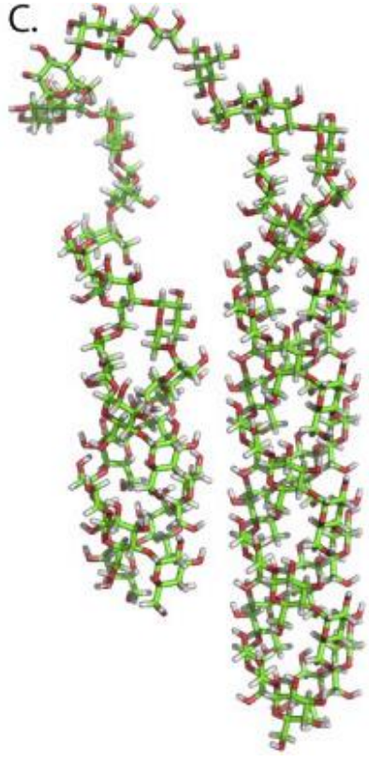


Key Points:

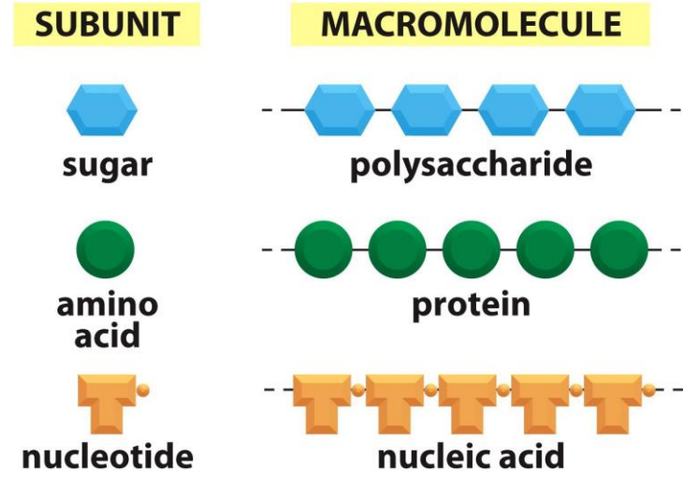
Enzymes:

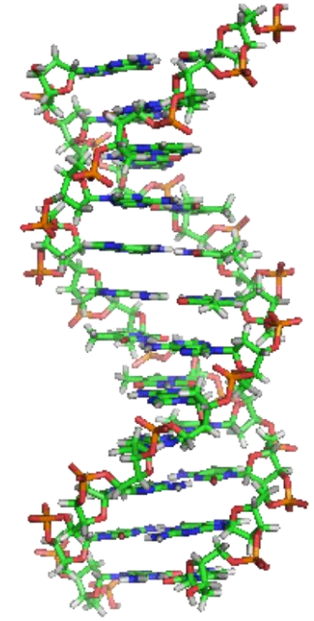
- Enzymes bind substrates (S), forming (ES) complex in active site, converting to P, releasing P.
- Rate enhancement since the transition state complex (EX) forms more readily with enzymes due to:
  - Bringing substrates and functional groups on the enzyme together by binding (less entropy change)
  - Directly lowering energy of transition state (X) through favorable interactions that are unique to the transition state, such as forming unique hydrogen bonds.
- Genetic diseases that lead to inactive metabolic enzymes can cause disease due to the build-up of toxic intermediates.

Carbohydrates



Polysaccharide



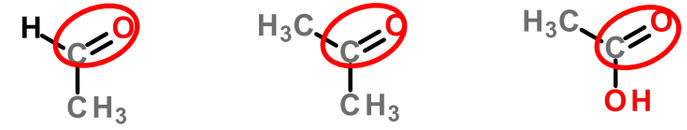


DNA (Nucleic Acid)

Carbohydrates

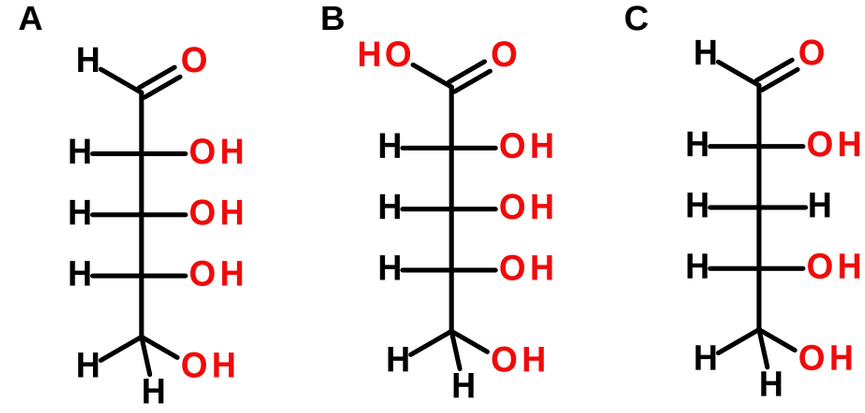
- Monosaccharides (one sugar),
- oligosaccharides (few sugars)
- polysaccharides (many sugars)
- Chemical formula is $(CH_2O)_n$ (e.g. hydrated carbon)
- They are molecules with:
  - one aldehyde or ketone group, on 1st or 2nd carbon
  - -OH group on *all* other carbons, leading to a chiral carbon for most carbons.

Functional groups:



aldehyde ketone carboxylic acid

Carbonyl group –> C=O



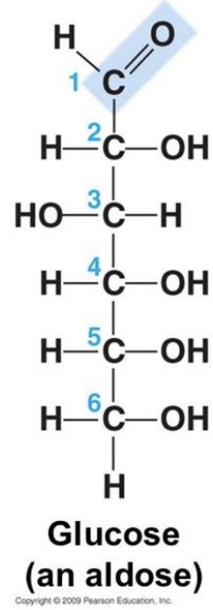
Only one of these is a carbohydrate, which one?

A B C

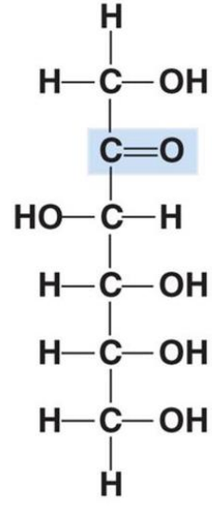
3 ways simple sugars (monosaccharides) differ from each other

1. Location of the carbonyl group
2. Number of carbons
3. Spatial arrangement of atoms (the position of the OH groups)

Aldose: Carbonyl group is located on C_1



Glucose (an aldose)

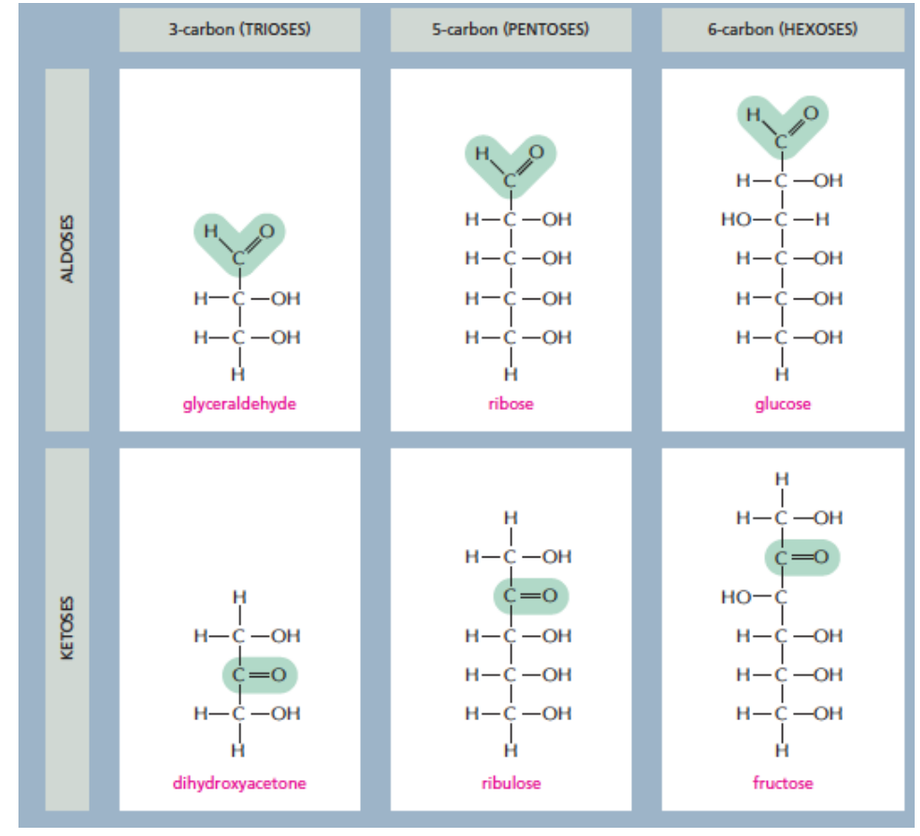


Fructose (a ketose)

Numbering carbons: Carbon 1 is at the end closest to the C=O group.

What carbon is the carbonyl?

1. Location of the carbonyl group
2. Number of carbons
3. Spatial arrangement of atoms (the position of the OH groups)



3 ways simple sugars (monosaccharides) differ from each other

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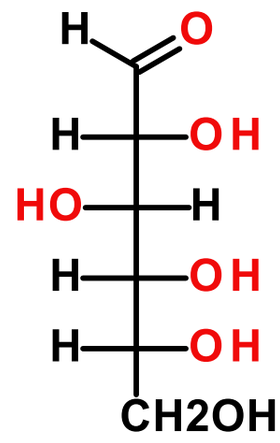
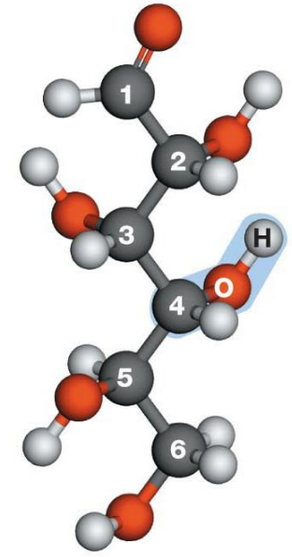
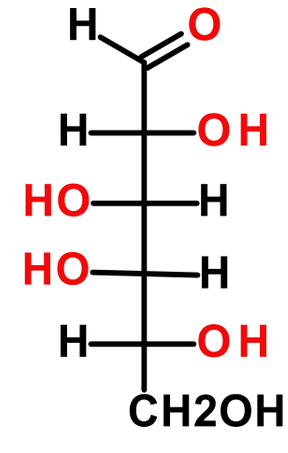
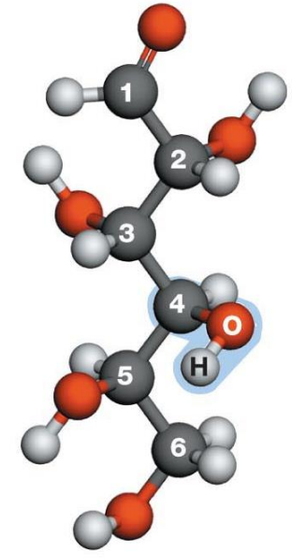
3. **Spatial arrangement of atoms (the position of the OH groups)**





Glucose





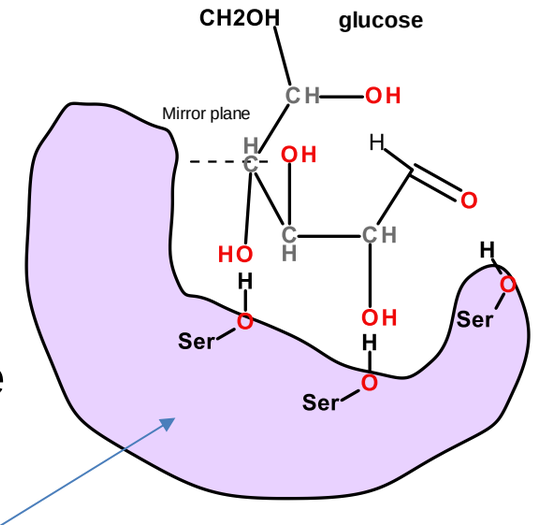
Galactose

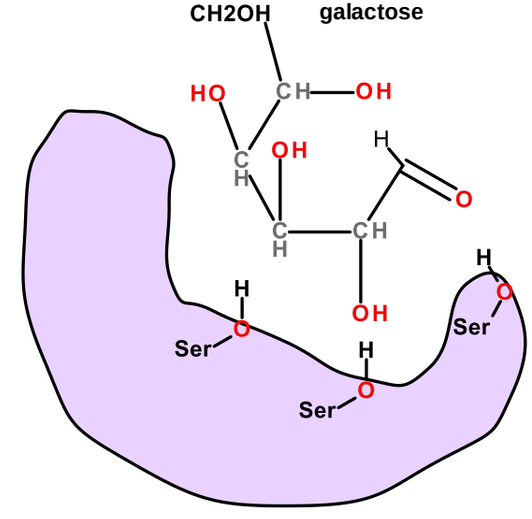
Both have the same chemical formula $C_6H_{12}O_6$. Both are aldose sugars with 6 carbons.
Yet their functions are different.

- Glucose can be used for energy immediately.
- Galactose has to be converted to glucose before it can be used for energy.

They have different interactions with enzymes due to the different chirality at carbon 4.

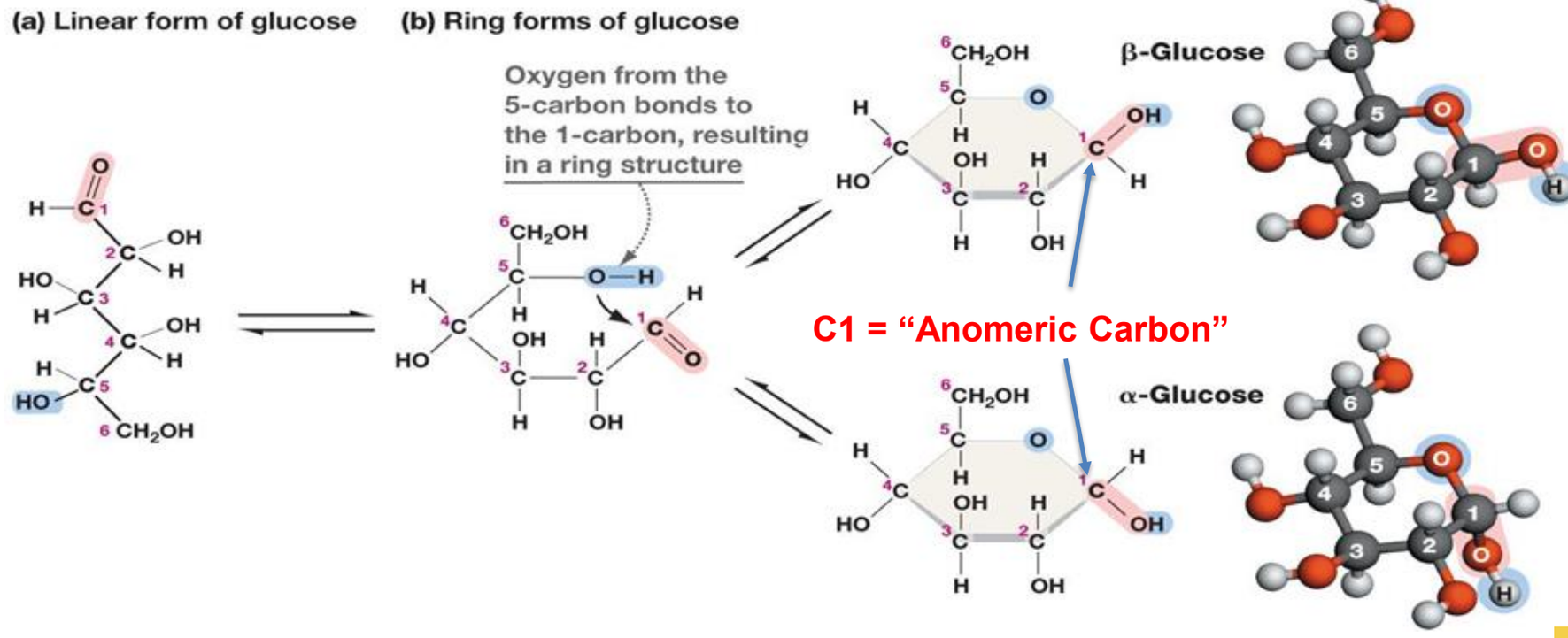
- OH is down in glucose
- OH is up in galactose





Enzyme specific for a-glucose

Ring formation in Monosaccharides:



- In aqueous solution, a hydroxyl group reacts with the aldehyde or ketone group on the same molecule, closing the molecule into a ring, with a bridging oxygen
- It is usually the 2nd to last -OH group, i.e. C5 in glucose, C4 in ribose.
- Stable ring sizes are 5 atoms or 6 atoms
- No atoms are lost or gained in this reaction.
- The carbonyl carbon becomes chiral and is called the ***anomeric carbon*****.**
- The rings with different chirality at C1 are different:

  α (new OH is down), β (new OH is up) *"(ants are down, birds are up)"*

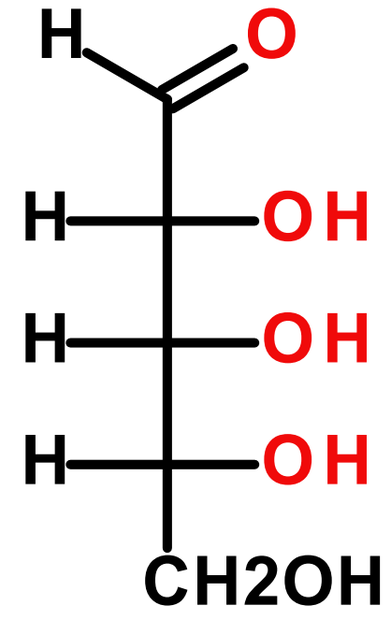
Example Problem:

The linear form of ribose, a 5 carbon aldose is shown on the right. This sugar is found in RNA (ribonucleic acid).

1. Number the carbons.

2. Which carbons are chiral? Mark them with a *.

3. Draw the cyclic form of α-ribose



Disaccharides

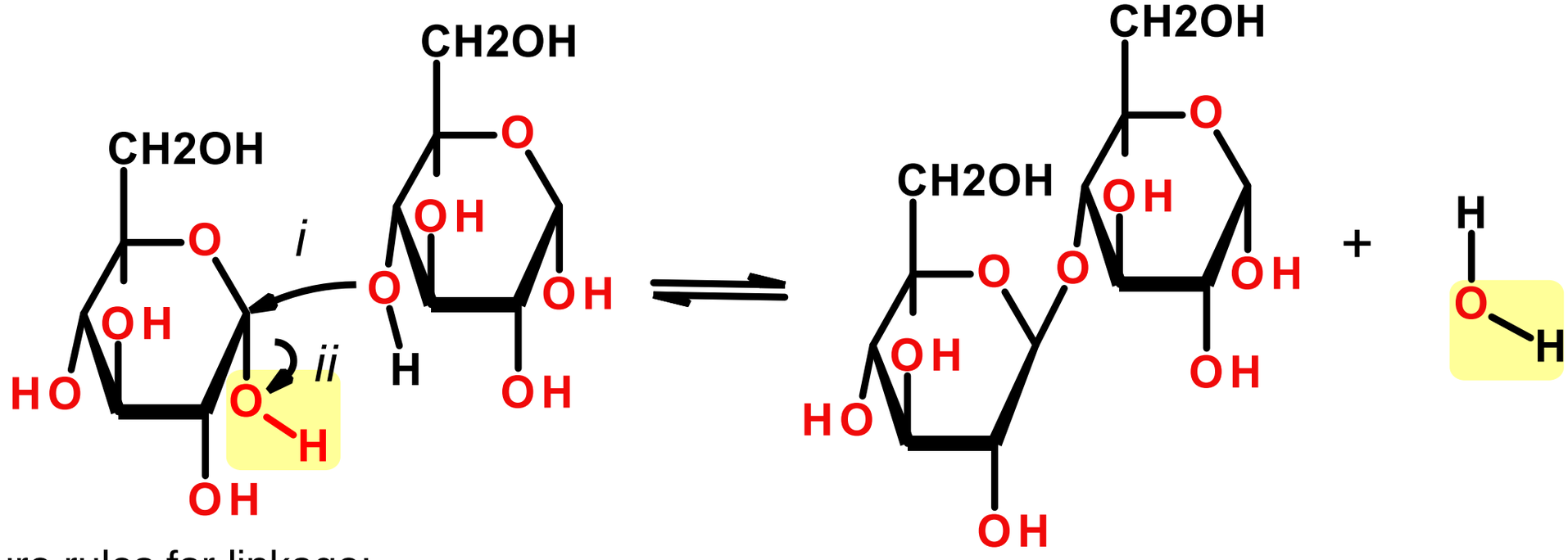
Linkage of the anomeric carbon of one monosaccharide to the OH of another monosaccharide via a *condensation* reaction.

The bond is termed a ***glycosidic bond:***

i) The anomeric carbon is the site of attack by another -OH group.

ii) A water is released

Why is the anomeric carbon the preferred site for nucleophilic attack?



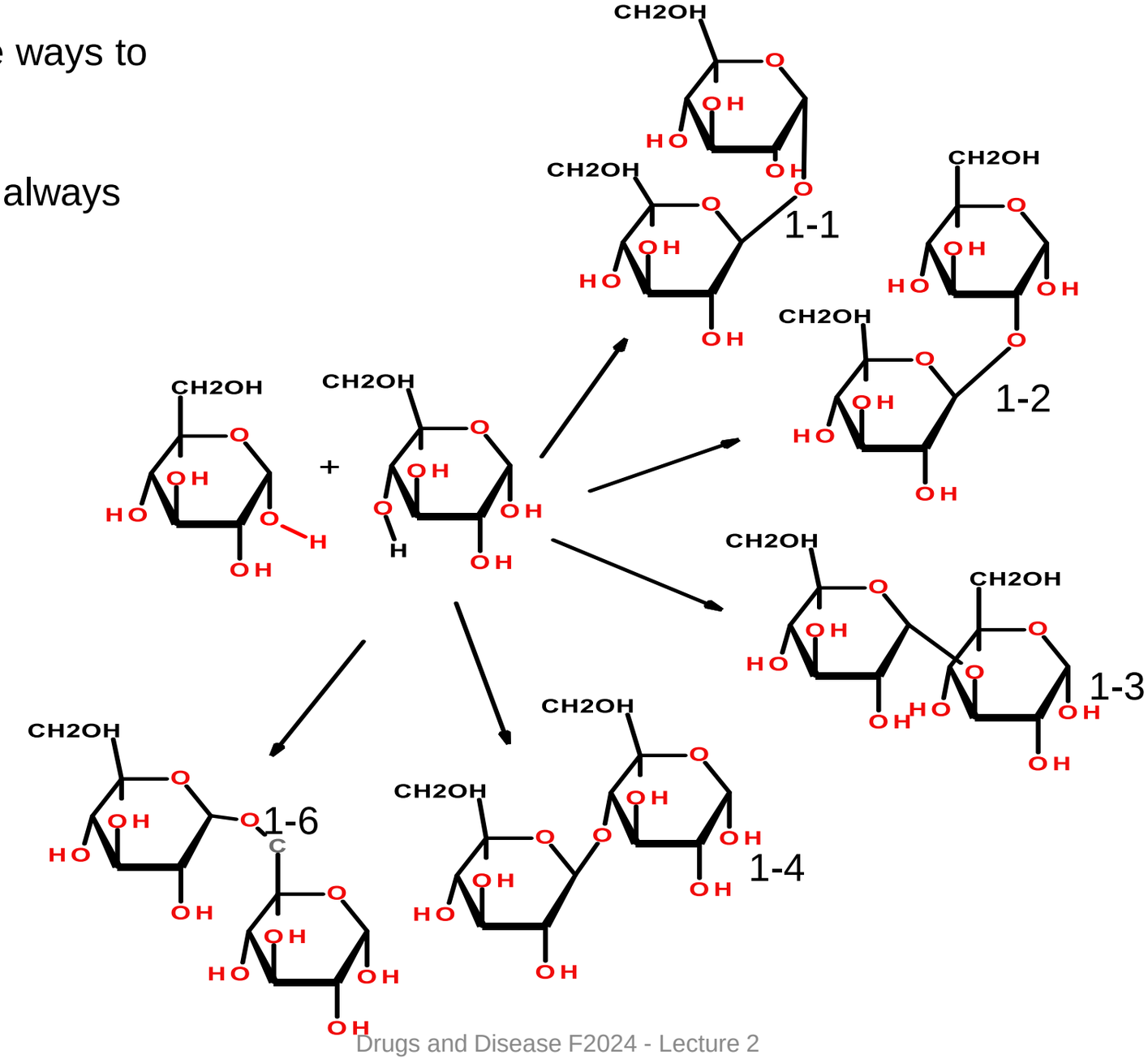
Nomenclature rules for linkage:

- Orientation of the **anomeric** involved in the linkage (α oxygen is down, β oxygen is up)
- Carbons involved in the linkage (e.g. 1-4)

Disaccharides

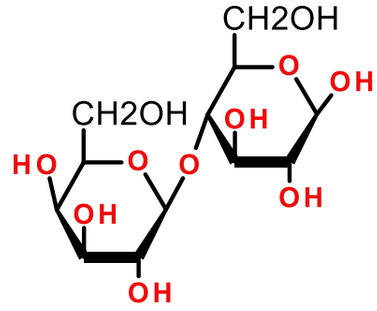
There are many possible ways to connect two sugars.

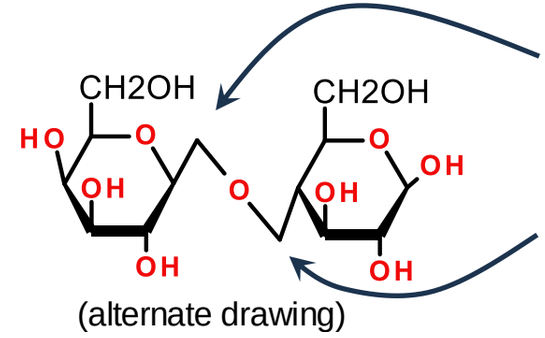
At least one anomeric is always involved.



Lactose (milk sugar)

Disaccharides





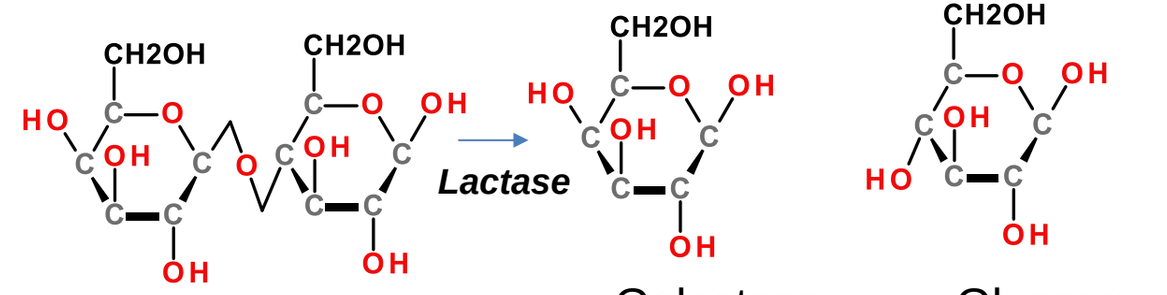
These kinks are not carbons but are drawn in this way to indicate that the chirality of the anomeric is beta (pointing up). The kinks allow the line to reach the downward pointing –OH on C4 in glucose.

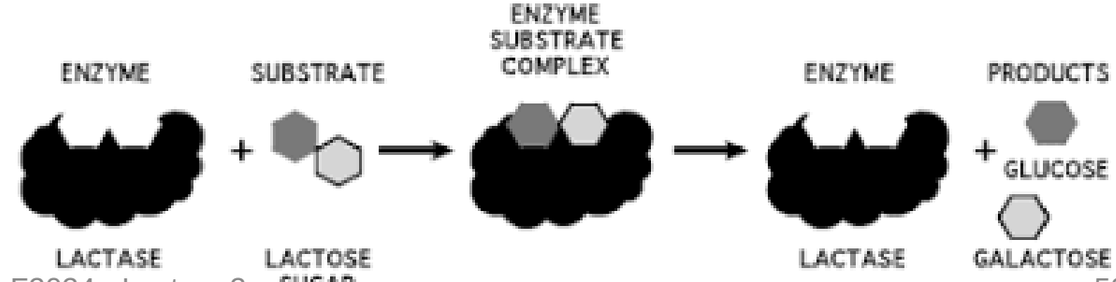
β-galactopyranosyl-(1→4)-β-glucopyranose

Lactose is the major sugar in mammalian milk.

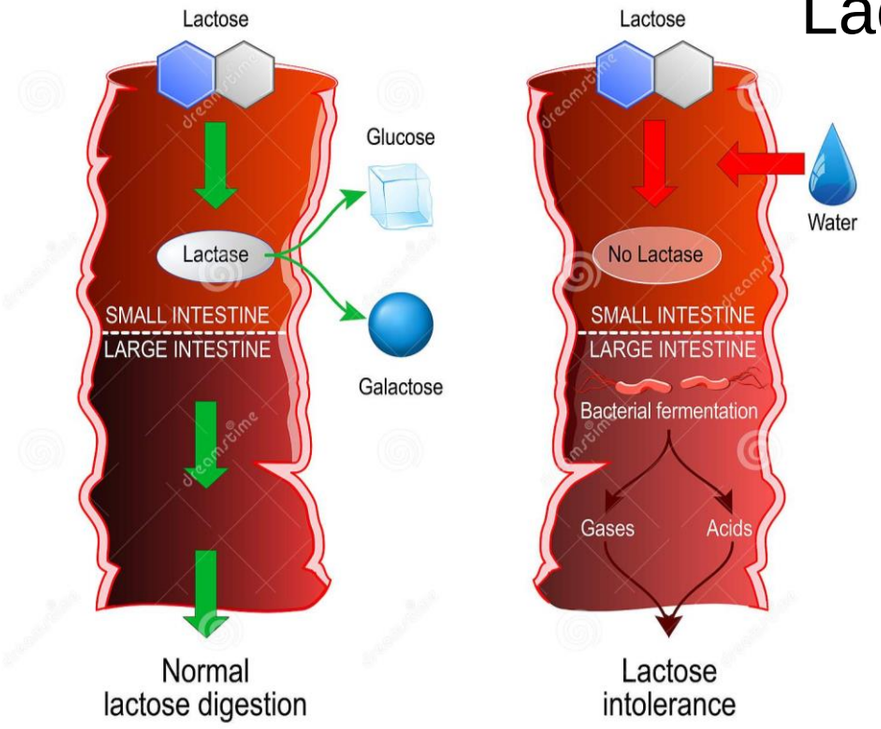
- Infants produce the enzyme ***lactase*** to hydrolyze the disaccharide to monosaccharides.
- Lactase expression is turned off in some adults, depending on their genetic background.

Metabolism of Lactose





Lactose Intolerance

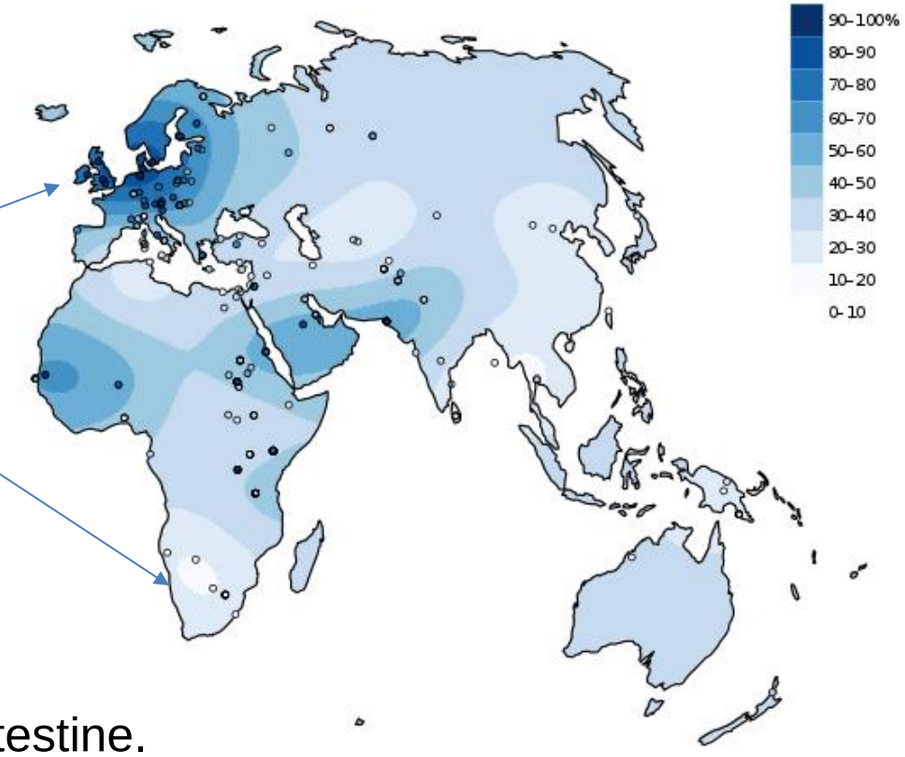


In which region do most of the people still produce lactase as adults?

A) UK

B) South Africa

Lactase Persistence



In an infant (lactase +):

- lactose is broken down to glucose and galactose in the small intestine.
- The two sugars are absorbed and used for energy

In a lactose intolerant individual (lactase -)

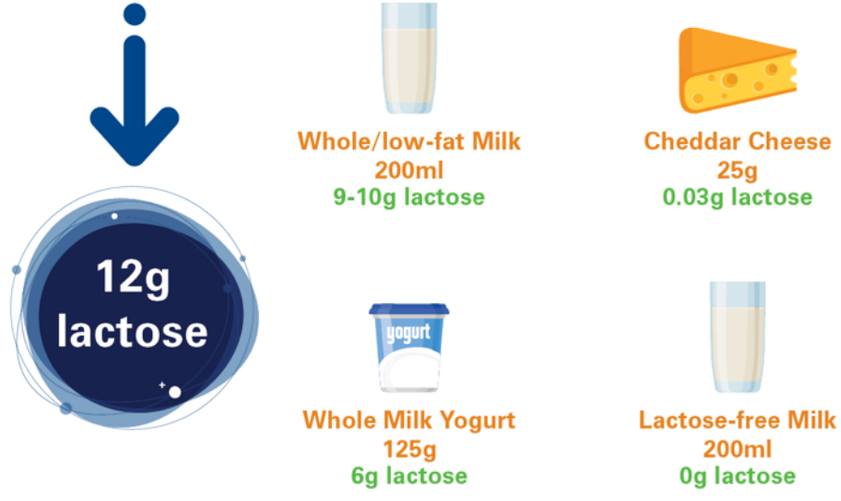
- The lactose is not absorbed in the small intestine, but instead draws water into the intestine due to osmosis – leading to bloating and diarrhea.
- Lactose enters the large intestine where gut bacteria use it as a carbon source, generating gas.

Lactose Intolerance

What to do if you are lactose intolerant:

A. Consume less lactose

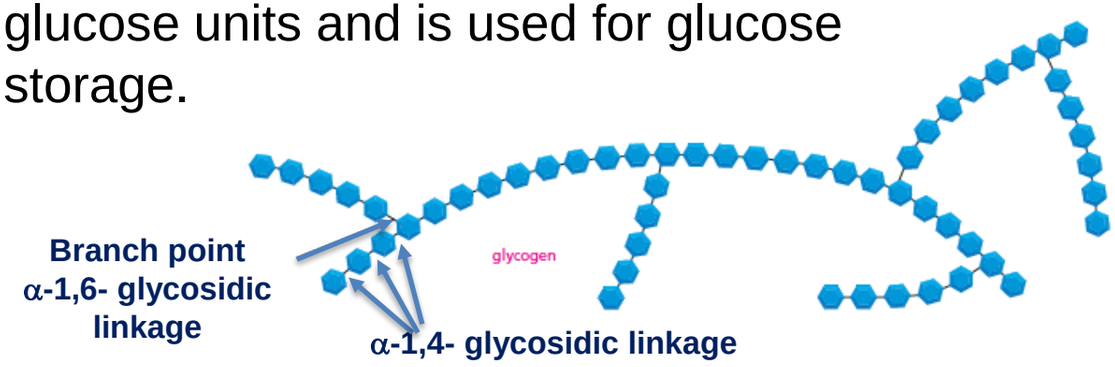
Most individuals with lactose maldigestion can tolerate up to 12g of lactose as a single dose with no, or minor, symptoms
The European Food Safety Authority (EFSA)

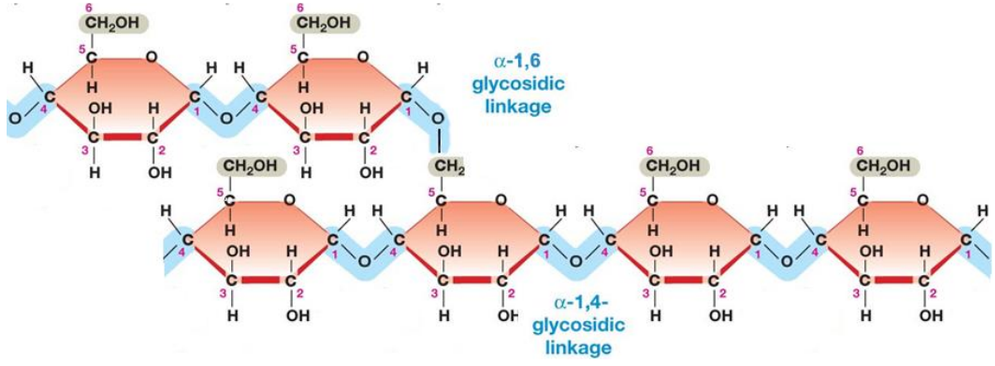


B. Hydrolyze the lactose to glucose and galactose before consumption.

Polysaccharides as Energy Storage – Glycogen Storage Disease

Glycogen and is made entirely of glucose units and is used for glucose storage.



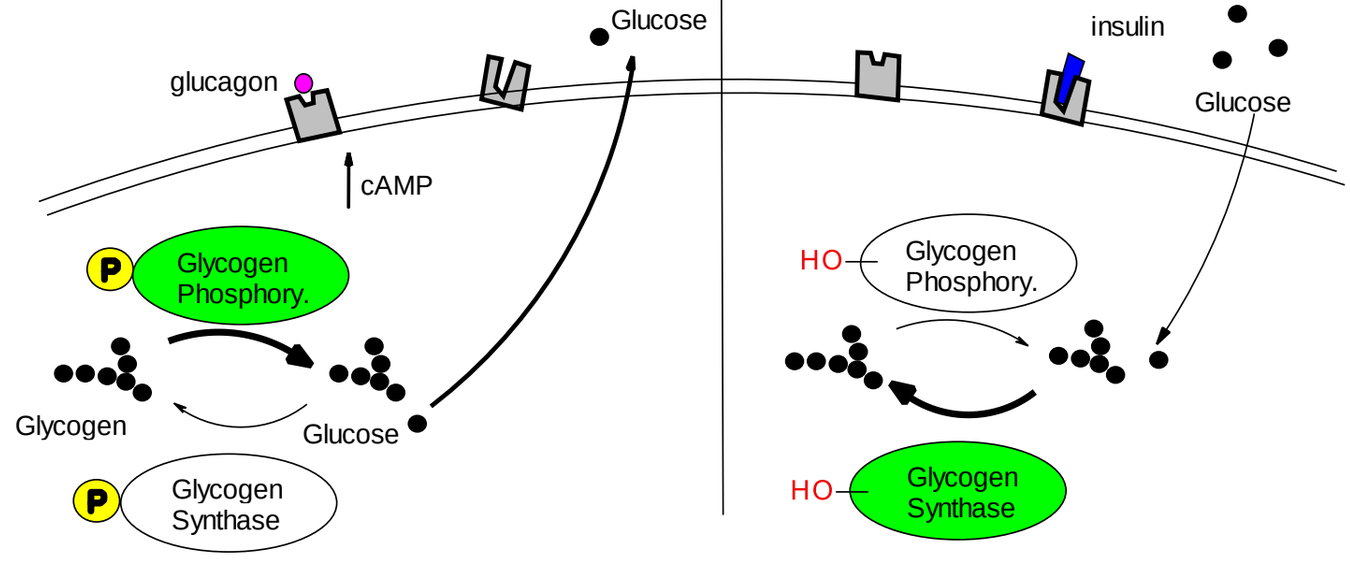


Glycogen Levels are regulated by hormones secreted due to blood glucose levels.

- Glucagon – low blood sugar
- Insulin – high blood sugar

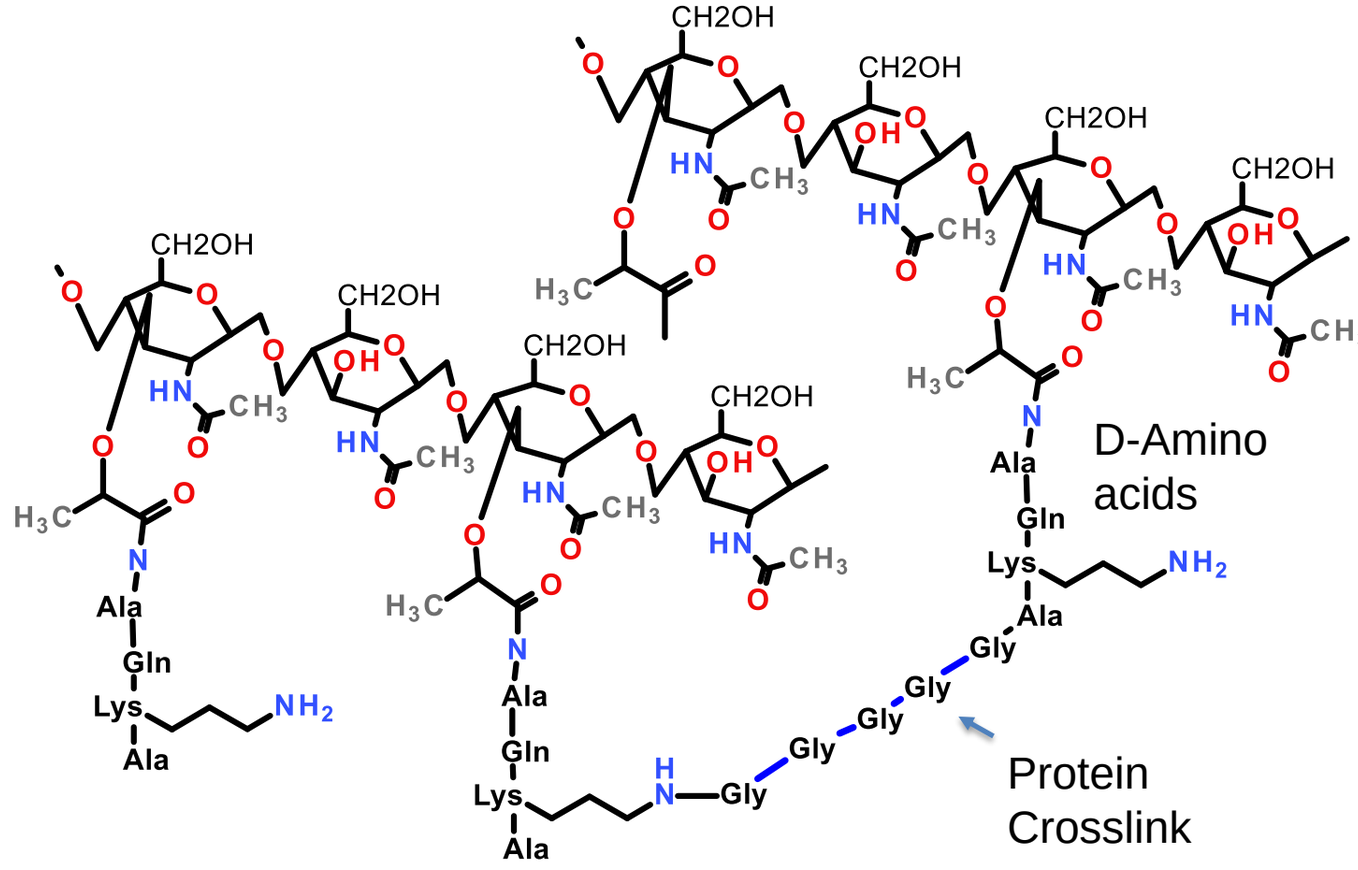
Two enzymes degrade or synthesize glycogen

- Glycogen phosphorylase – releases glucose from glycogen
- Glycogen synthase – stores glucose in glycogen



Polysaccharides as Structural Molecules

Peptidoglycan (protein + sugar) in bacterial cell wall



Peptidoglycan (Bacterial Cell Wall)

Many antibiotics interfere with cell wall synthesis (e.g. penicillin)

Summary and Expectations for Carbohydrates

Key Points:

- General structure of monosaccharides - be able to distinguish between aldose and ketose (and identify compounds that are not sugars).
- Know how to number carbons on aldoses and ketoses
- Be able to describe the linkage between two monosaccharides (configuration at the anomeric carbon, atoms linked)
- Treatments for lactose intolerance
- Be able to describe the linkage between glucose molecules in:
  - Glycogen (glucose storage)
- Be able to describe the overall structure of the peptidoglycan in bacterial cell walls.

Lipids

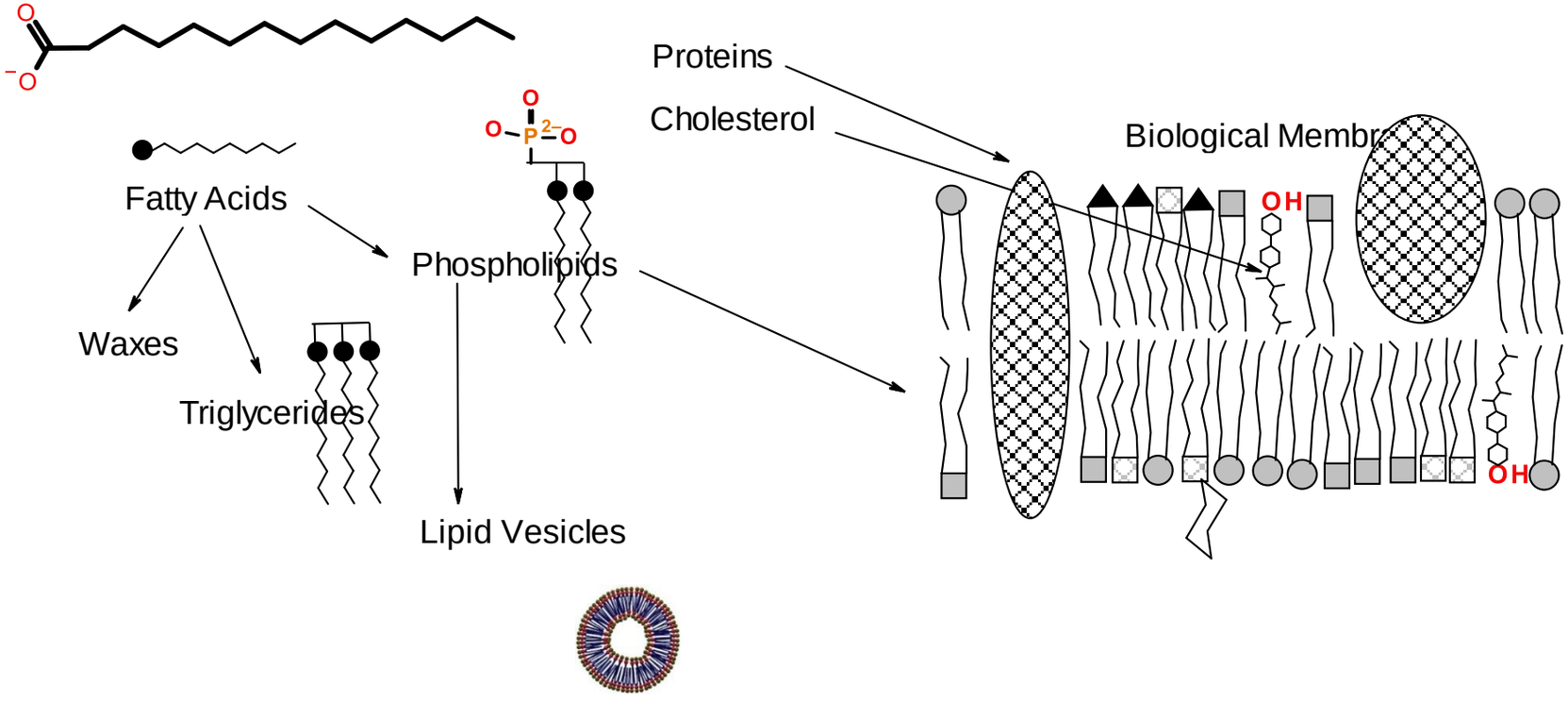
Lipids

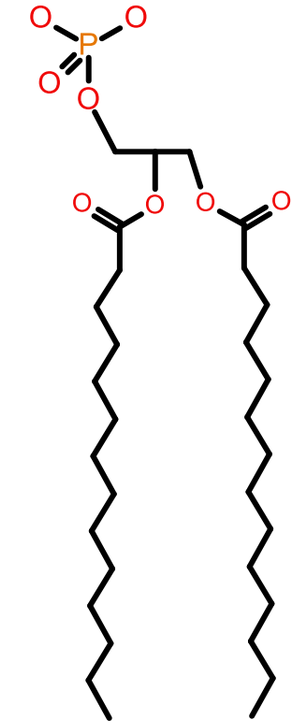
A chemically diverse group of molecules that are generally insoluble in water.

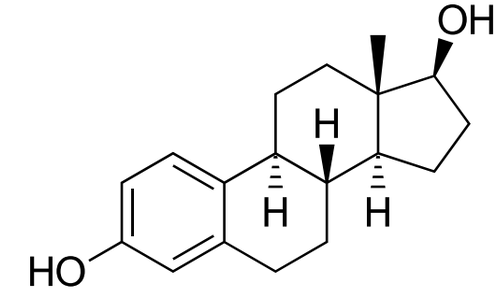
- Mostly hydrocarbon with a small number of polar functional groups.
- Self-assembly of larger structures *without* the formation of covalent bonds.

Expectations:

- Recognize chemical structure of steroids and phospholipids.
- Usage of liposomes in drug delivery
- Effect of cholesterol on fluidity of phospholipid membranes.

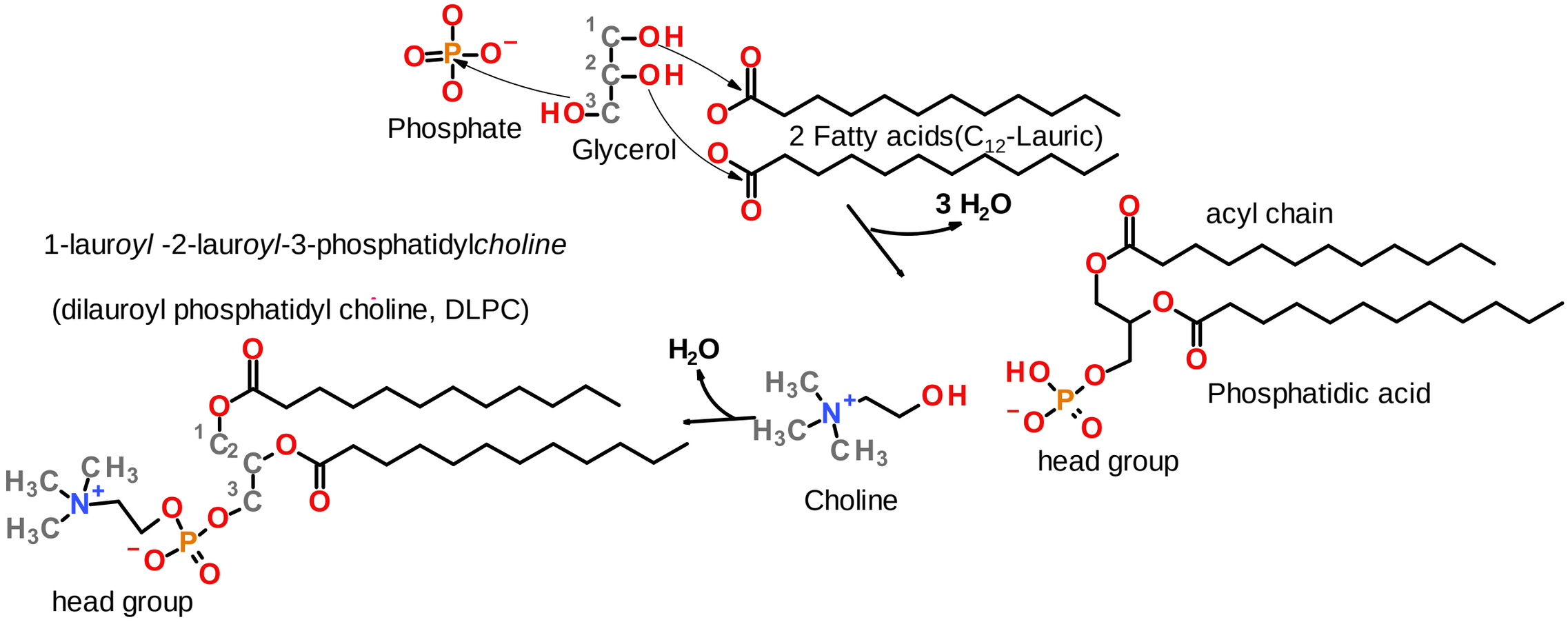






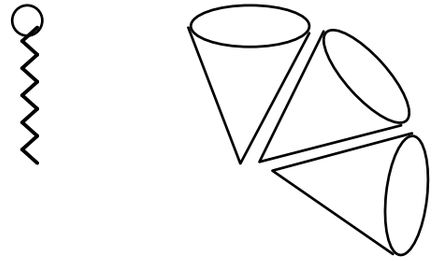
Phospholipids - Glycerophospholipids:

1. Head group + phosphate + glycerol + two fatty acids (**acyl chains**) of *various* types form a phospholipid.
2. Various **head groups** are attached to the phosphate, giving a diverse set of lipids.

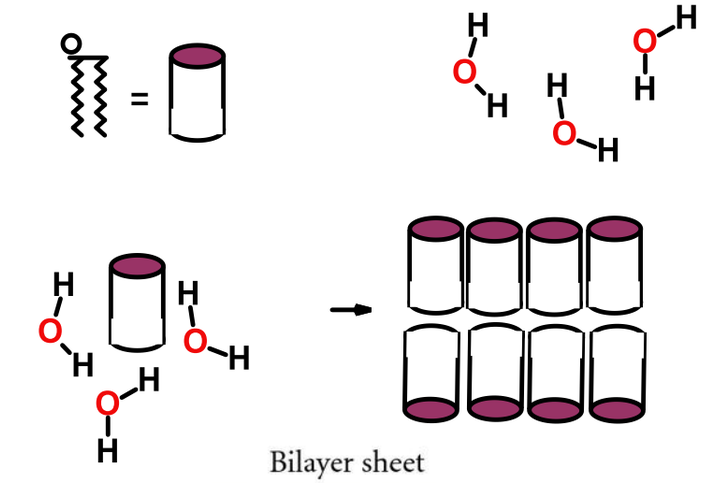


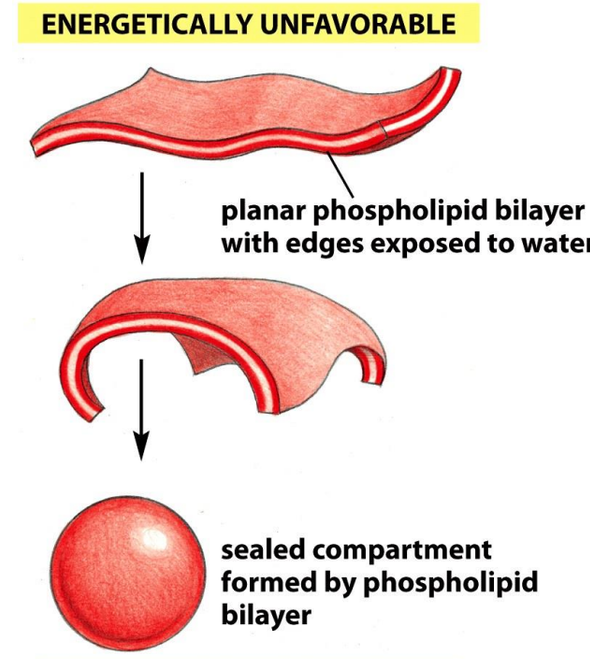
Expectations: Know the overall structure.

Geometry (& Hydrophobic Effect) Determines Macrostructures of Lipids in Water



Micelle

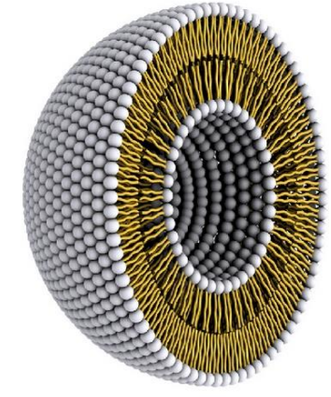




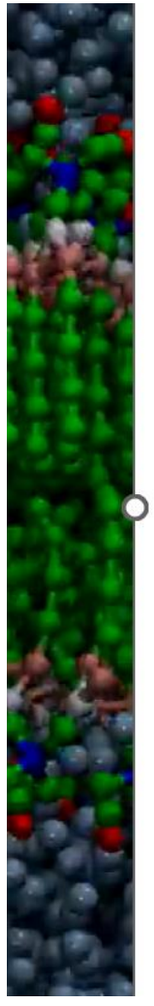
Physical Properties of Pure Lipid Bilayers:

- Phospholipids self-assemble in water to form **bilayers** (two opposing layers of phospholipids). This assembly is driven by the hydrophobic effect.
- Bilayers are formed instead of micelles because the cross section of the head group is roughly equal to the cross section of the 2 fatty acid chains found in phospholipids; phospholipids are cylindrical in shape.
- To remove the non-polar edges, the bilayers form closed, water filled, vesicles with a 40-50 Å thick wall. The non-polar acyl chain width is about 30 Å. These are called ***liposomes*** or ***lipid vesicles*****.**

Liposome



Spontaneous Assembly of the Phospholipid Bilayer:

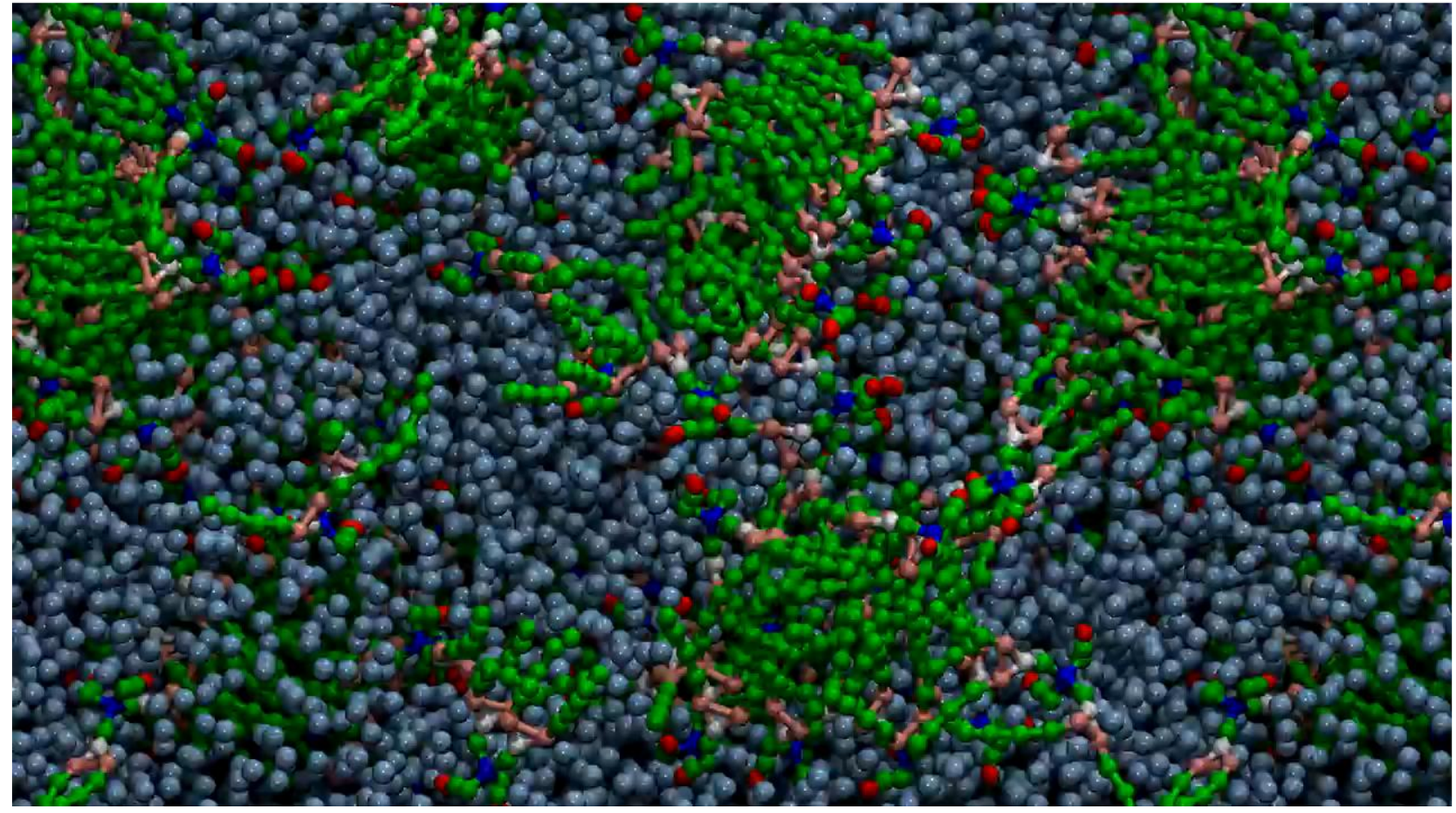


Gray spheres
= water

P. Headgroup

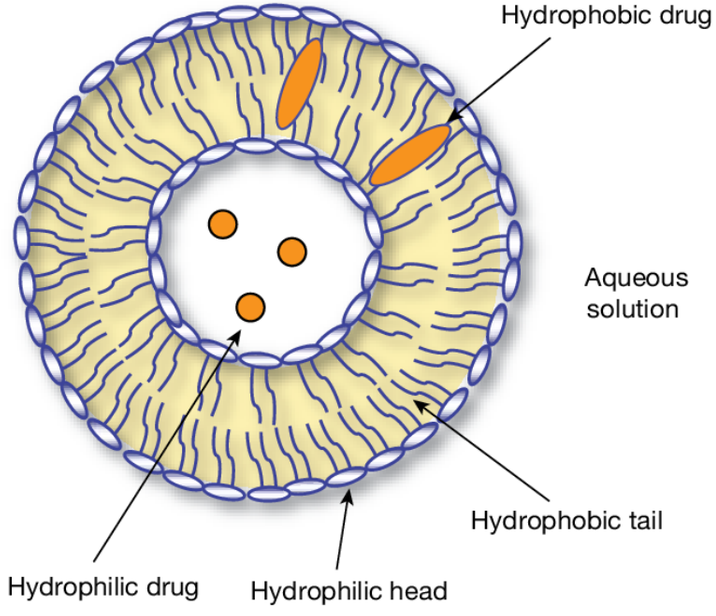
One
phospholipid

NP. Tail

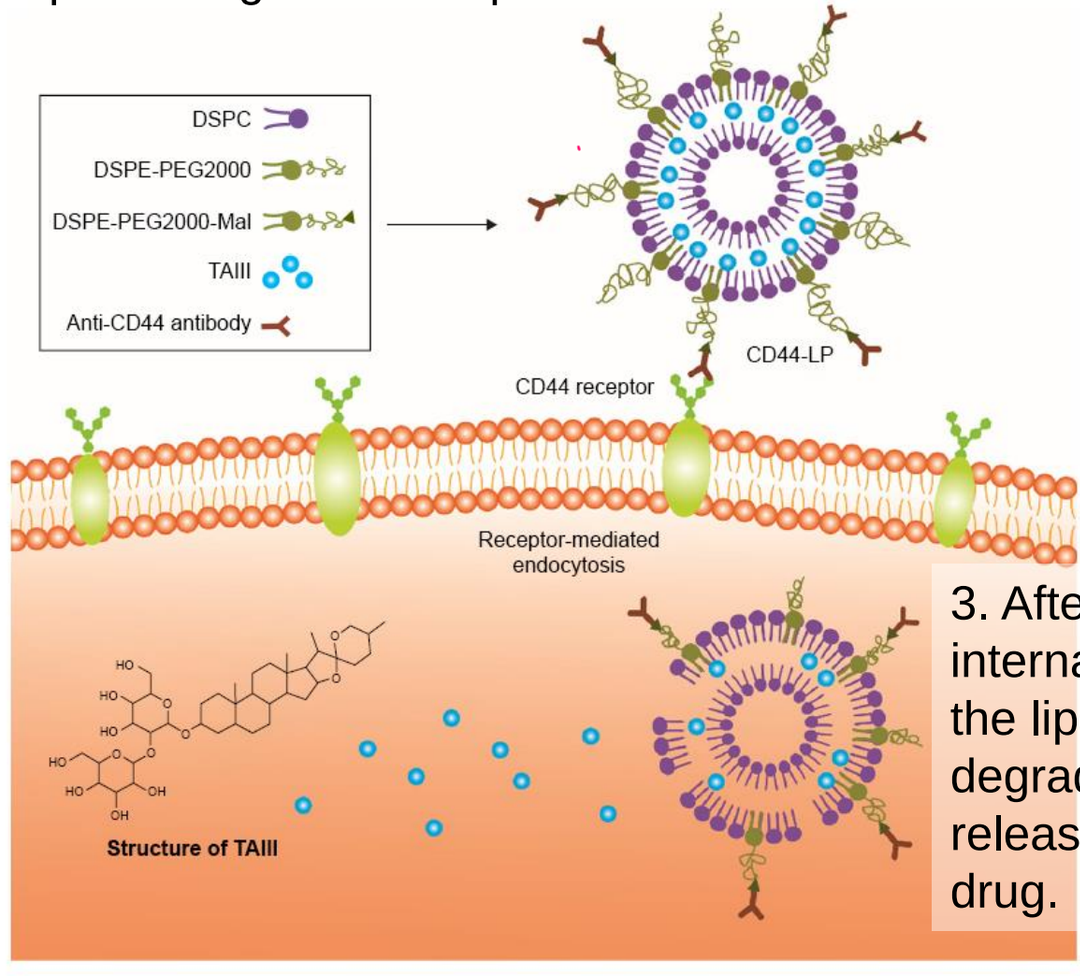


Liposomes (pure lipid vesicles) can be used for Drug Delivery

1. Liposomes can be used for drug delivery.
- Non-polar drugs dissolve in the lipids, ***increasing their solubility***
- Water soluble drugs can be encapsulated



2. Delivery can be ***targeted to cancer cells*** by antibodies that recognize tumor specific antigens.
Toxic polar drugs are encapsulated can be delivered to cancer cells.



3. After internalization, the liposome is degraded, releasing the drug.

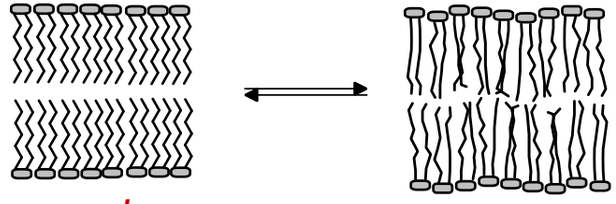
Lu L, Ding Yue, Zhang Y, Ho RJY, Zhao Y, Zhang T, Guo C. Antibody-modified liposomes for tumor-targeting delivery of timosaponin AIII. *Int J Nanomedicine*. 2018;13:1927-1944 https://doi.org/10.2147/IJN.S153107

Figure 1 Illustration of CD44-LP for active CD44-targeting TAIII delivery and enhancing antitumor activity against CD44-overexpressing HepG2 cells.
Note: Anti-CD44 antibody was conjugated to LP through the reaction of sulfhydryl residues on the antibodies with the C-terminal maleimide groups of the PEG chains.
Abbreviations: DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSPE-PEG2000, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(PEG)-2000]; DSPE-PEG2000-Mal, DSPE-PEG2000-maleimide; LP, liposomes; TAIII, timosaponin AIII; PEG, polyethylene glycol.

Lipid Phase Transition & Membrane Fluidity

Lipid bilayers undergo a *highly cooperative* (melt over a narrow temp range) phase transition with a defined T_m.

- Below T_m the lipids exist as a solid-like *gel*; the acyl chains are tightly packed, the membrane is ***solid.***
- Above T_m the lipids are in a liquid-like *liquid crystal phase*. The acyl chains are disordered, and the membrane is ***fluid***.
- Note that the bilayer remains a bilayer – due to the hydrophobic effect, the only thing that changes is the order/disorder of the non-polar chains.



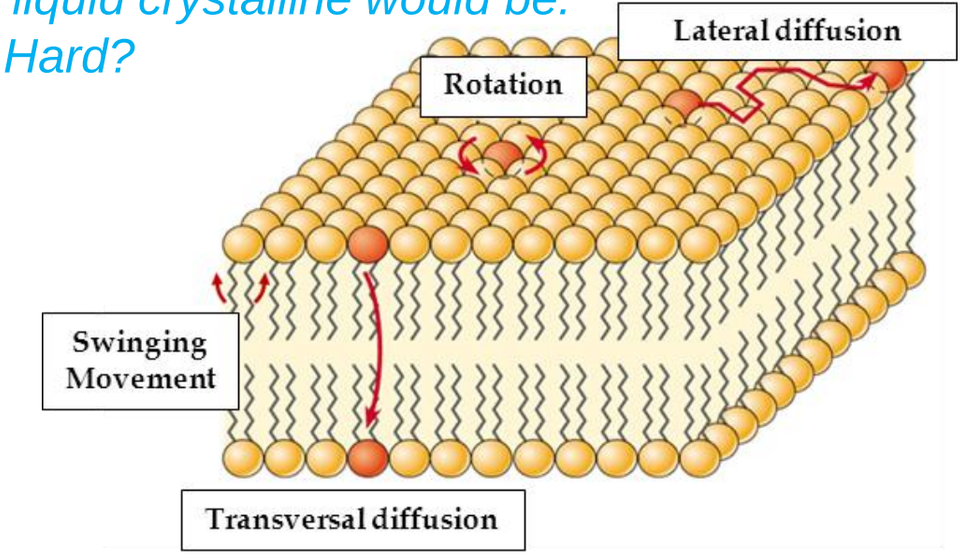
gel

liquid-crystalline

*How does the physical state of the membrane affect **lateral diffusion** within the plane of the membrane (lateral diffusion)?*

A. Moving through gel phase would be:
Easy or Hard?

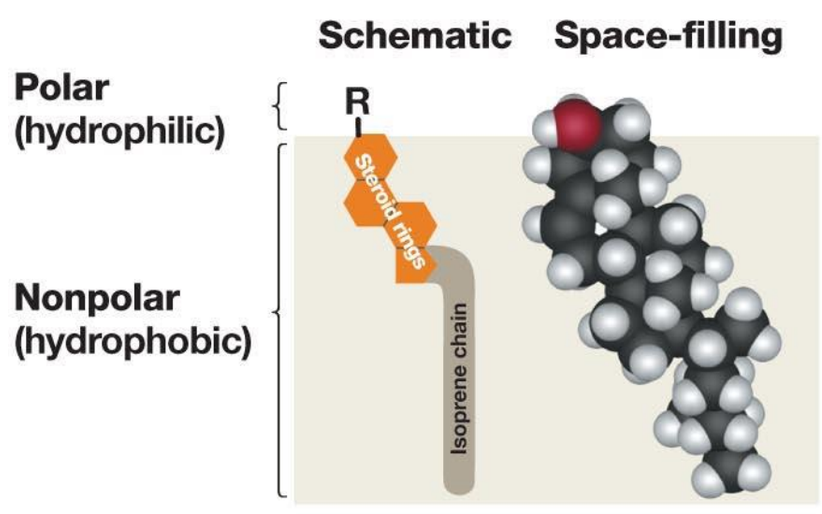
B. Moving through liquid crystalline would be:
Easy or Hard?



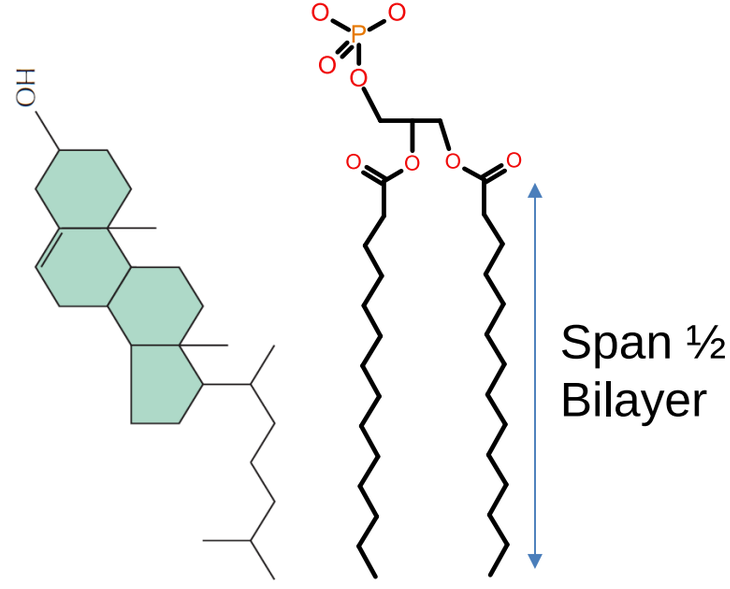
Steroids

Defined by four-ring structure + functional groups bound to ring structure

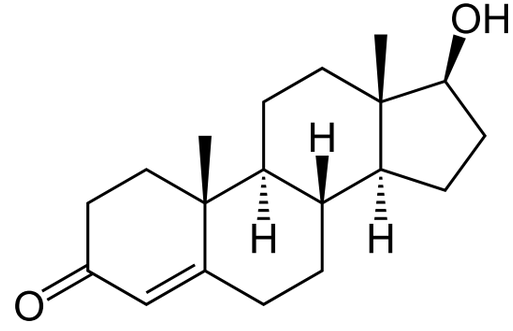
- Cholesterol - example of steroid molecule; essential function in plasma membrane
- All steroids (*testosterone, estrogen, progesterone…)* are derived from cholesterol!



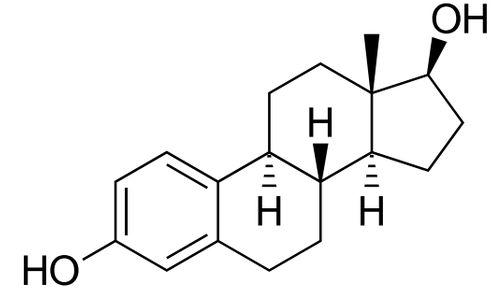
R= OH group



Span ½ Bilayer



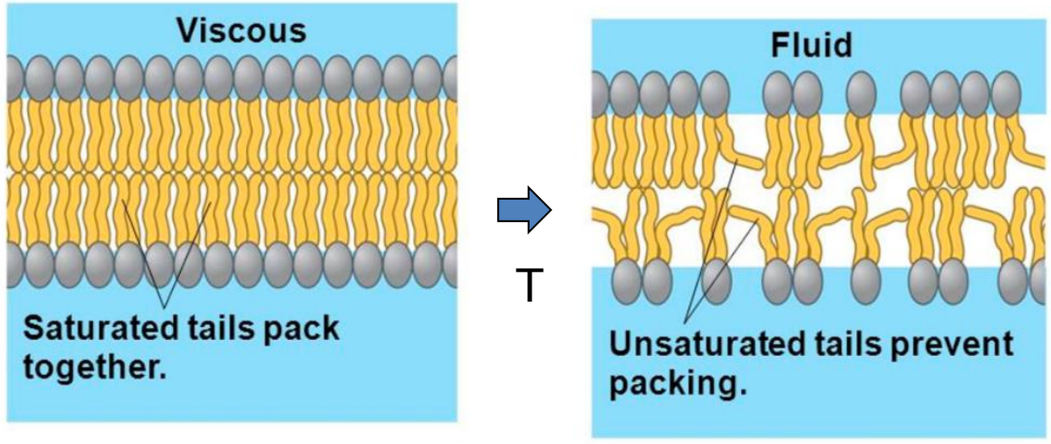
Testosterone ♂

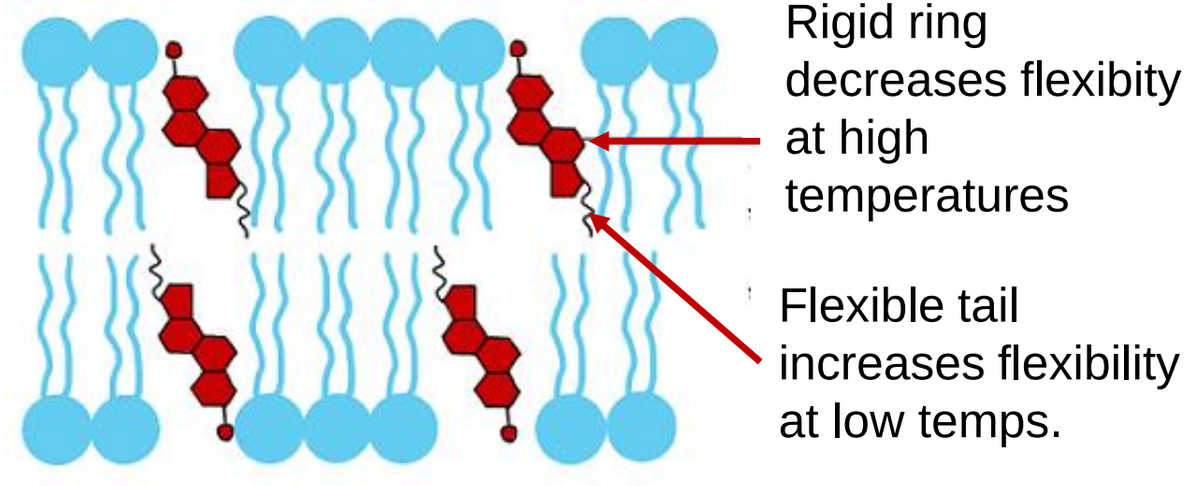


Estrogen ♀

Amphipathic - contains hydrophilic and hydrophobic elements

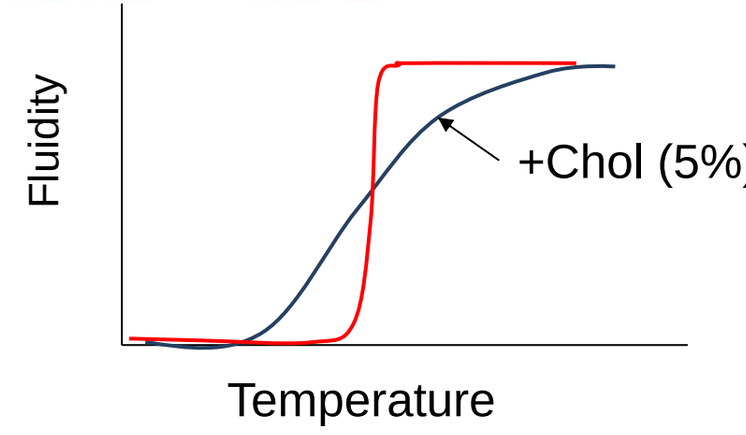
Cholesterol Affects Fluidity





At cooler temperatures, cholesterol maintains fluidity by preventing tight packing of phospholipids due to flexible tail.

At warmer temperatures, cholesterol constrains motion of acyl chains due to rigid ring, thus decreasing membrane fluidity

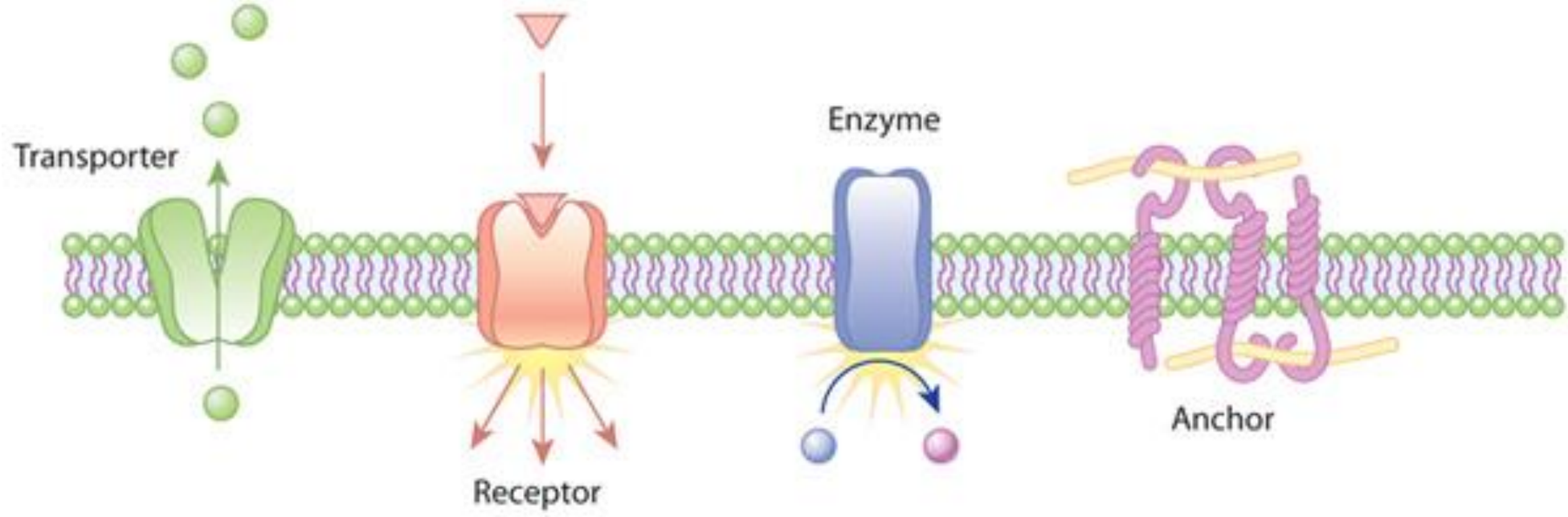


In mammals – cholesterol is required to maintain membrane fluidity at body temperature.

What interaction between the phospholipids is cholesterol affecting?

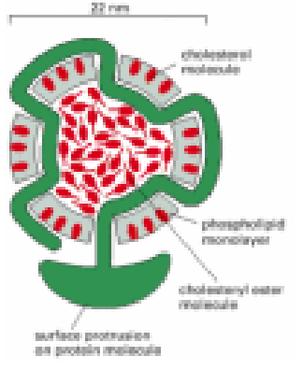
- ❑ H-bonds
- ❑ Electrostatics
- ❑ Hydrophobic effect
- ❑ van der Waals

Biological Functions of Transmembrane Proteins



- Many are potential drug targets
- Genetic defects can cause disease

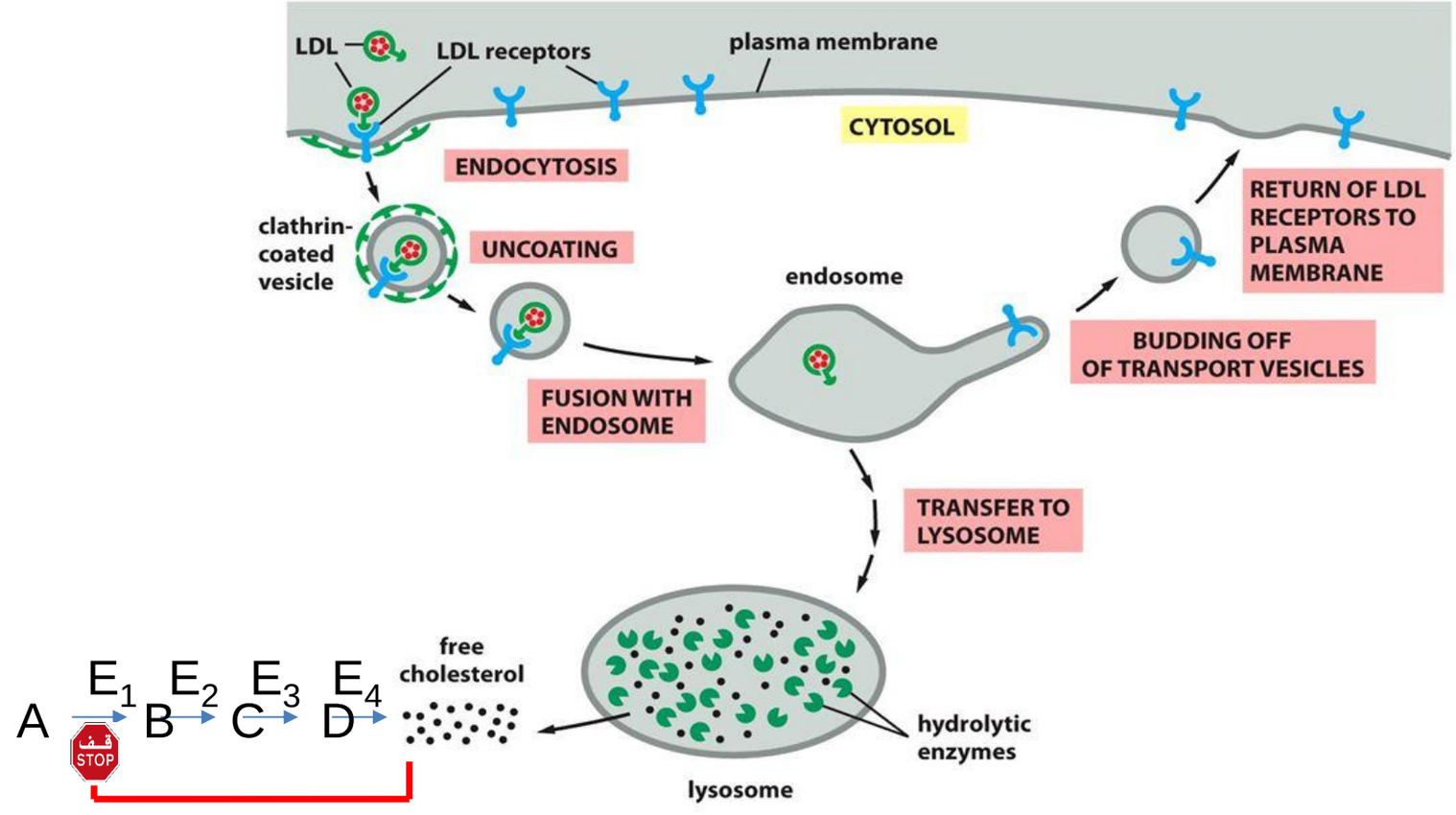
Cholesterol Regulation & Endocytosis



LDL particle

- Protein
- Triglycerides
- Cholesterol

1. Low density lipoprotein (LDL) enters via receptor-mediated endocytosis



2. LDL and its receptor are internalized in vesicles

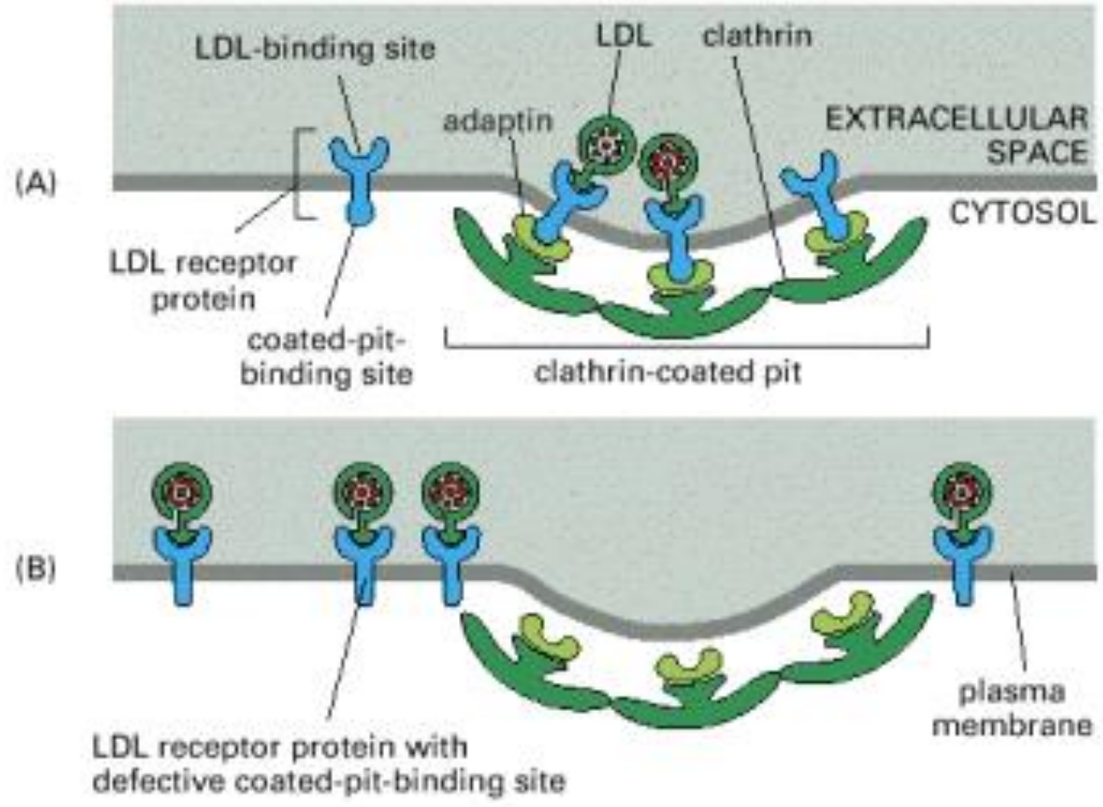
3. The vesicles fuse with endosomes

4. In the acidic environment, LDL dissociates from its receptor. Receptor is recycled back to plasma membrane and LDL ends up in lysosomes

5. LDL is degraded and free cholesterol is released.

6. Free cholesterol regulates biosynthesis in liver (feedback regulation)

Some Individuals Inherit a Defective Gene Encoding the LDL receptor



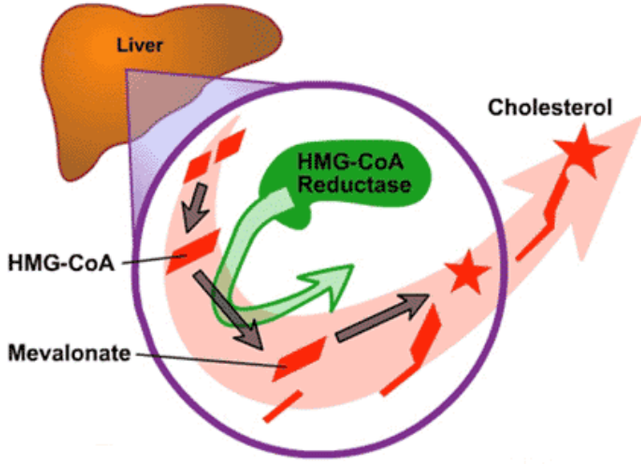
Normal functioning LDL receptor

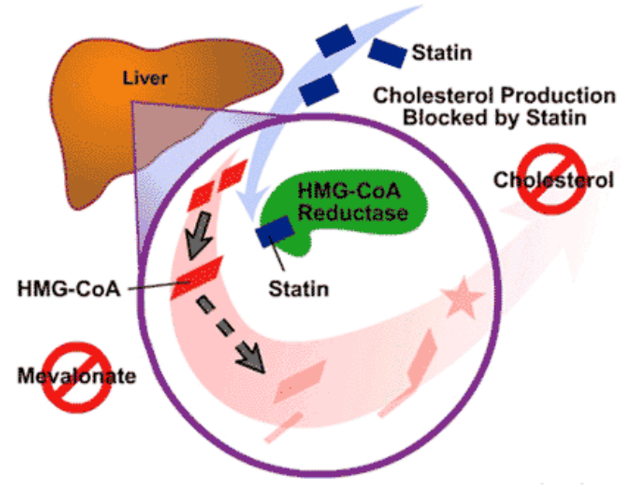
Non-functional LDL receptor due to mutation due to loss of interaction with Catherin coated pits. The coated pits are required for endocytosis of the LDL-receptor.

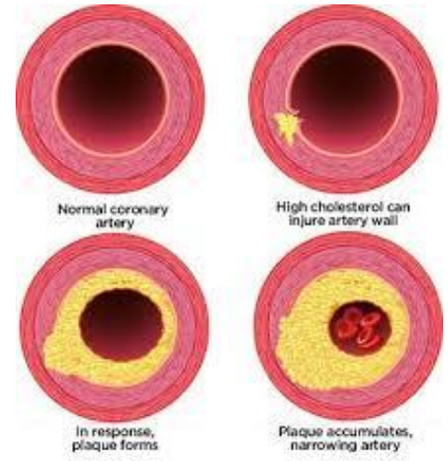
The altered receptors lack the cytoplasmic domain that enables them to bind to adaptins in coated pits

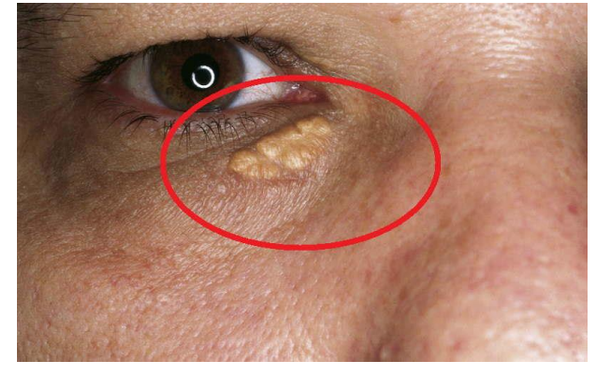
Such cells can bind LDL but cannot internalize it, leading to dysregulation of cholesterol production due to lack of feedback inhibition, *the liver cell continues to produce cholesterol.*

Cholesterol Metabolism and Regulation

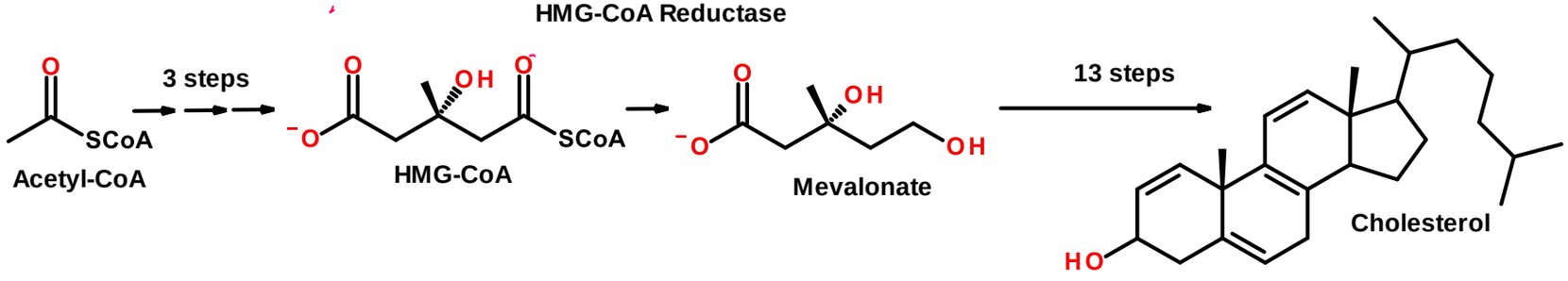


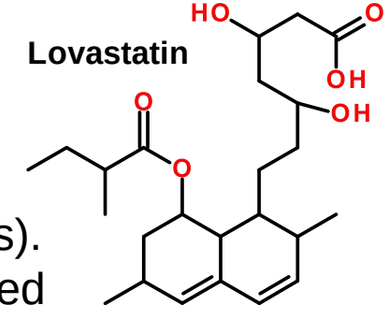






Cholesterol is produced by a series of steps, each catalyzed by an enzyme:





- In normal individuals, cholesterol production in the liver is tightly controlled by cholesterol levels in the blood by a feedback mechanism (the liver actively takes up cholesterol from the blood).
- A genetic disease causes this regulation to be dysfunctional in some individuals, leading to high cholesterol levels, leading to damage of the arterial walls and cholesterol deposits (often near the eyes).
- **Statins** are competitive inhibitors that inhibit one of the enzymes (HMG-CoA Reductase) that is required to make cholesterol