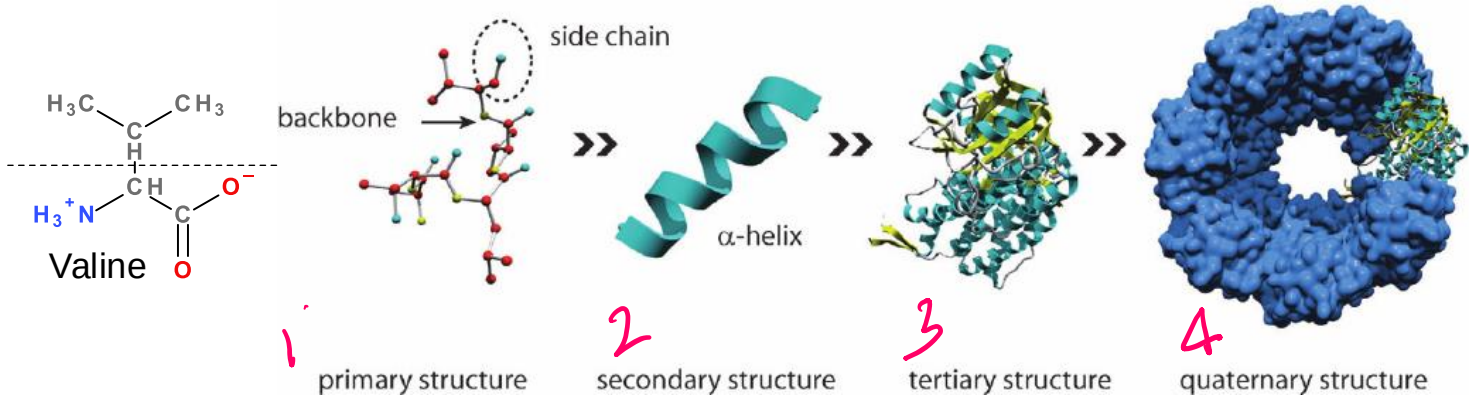
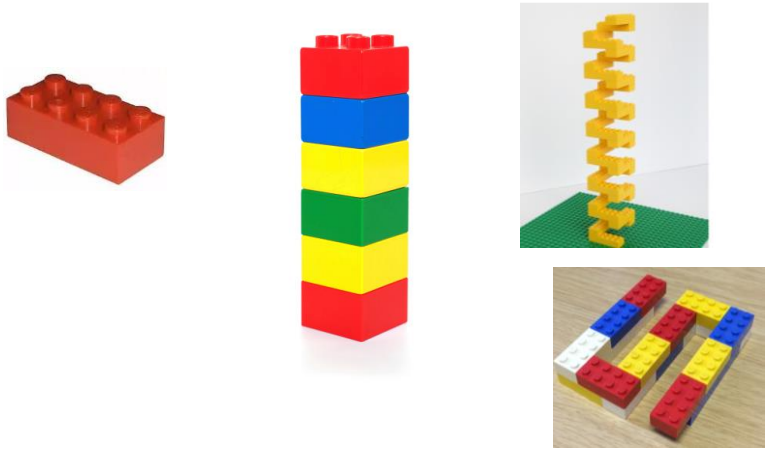
Lecture 2:
Biology Fundamentals

- Review of Protein Structure and Stability
- Ligand Binding
- Proteins as enzymes
- Carbohydrates

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.

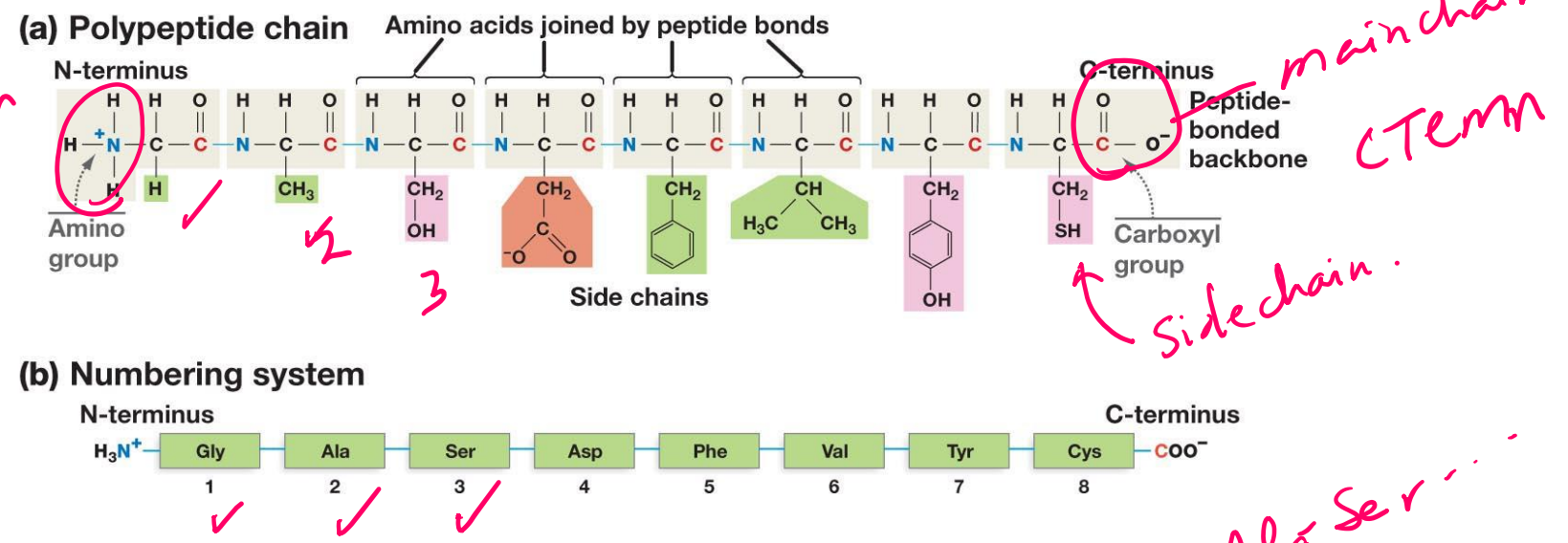




(White and black represent two different chains)

Primary Structure = Protein Sequence

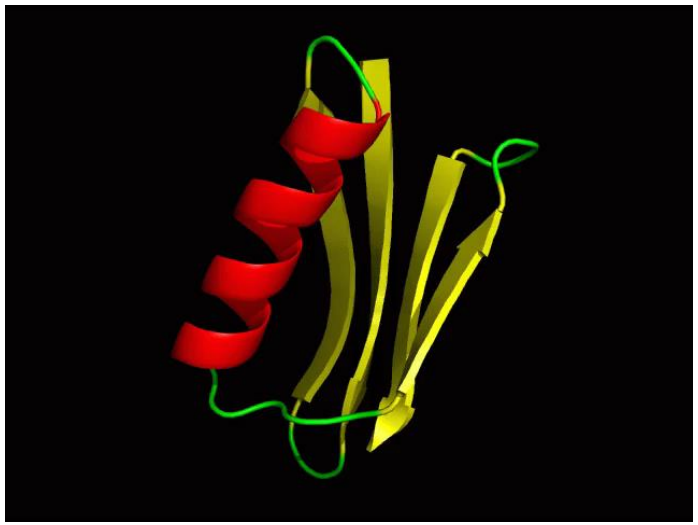
Order of amino acids, from the amino terminus to the carboxy terminus, e.g. Gly-Ala-Ser-Asp…..

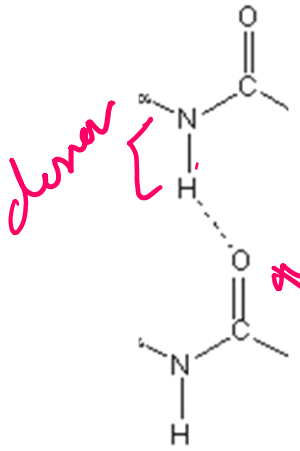


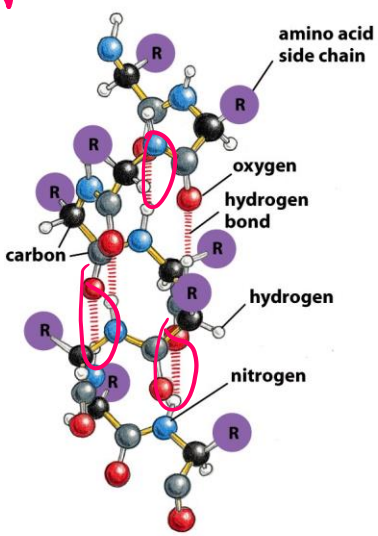
- Within the polypeptide, the "backbone" has three key characteristics:
  1. **Directionality**
     - Free amino group, on the left, is called the **N-terminus.**
     - Free carboxyl group, on the right, is called the **C-terminus.**
  2. **R-group orientation**
     - Side chains can interact with each other or water.
  3. **Flexibility**
     - Single bonds on either side of the peptide bond can rotate, making the entire structure flexible.

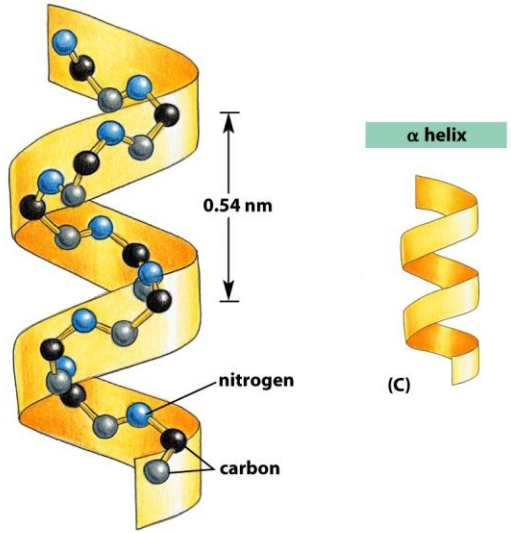
Secondary Structure

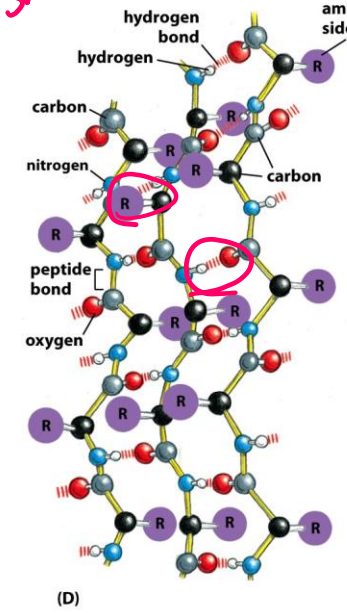
"Building blocks of proteins"

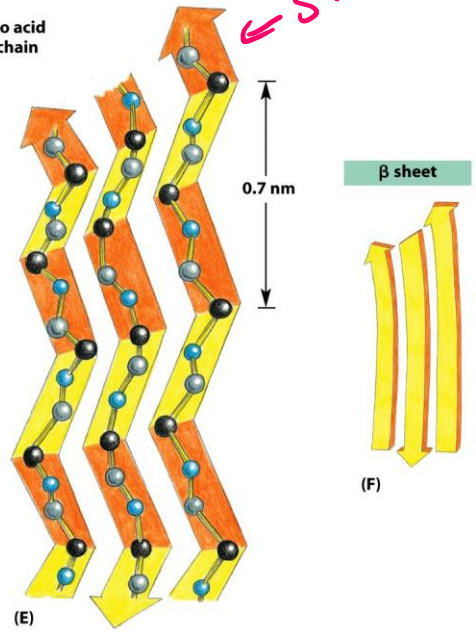




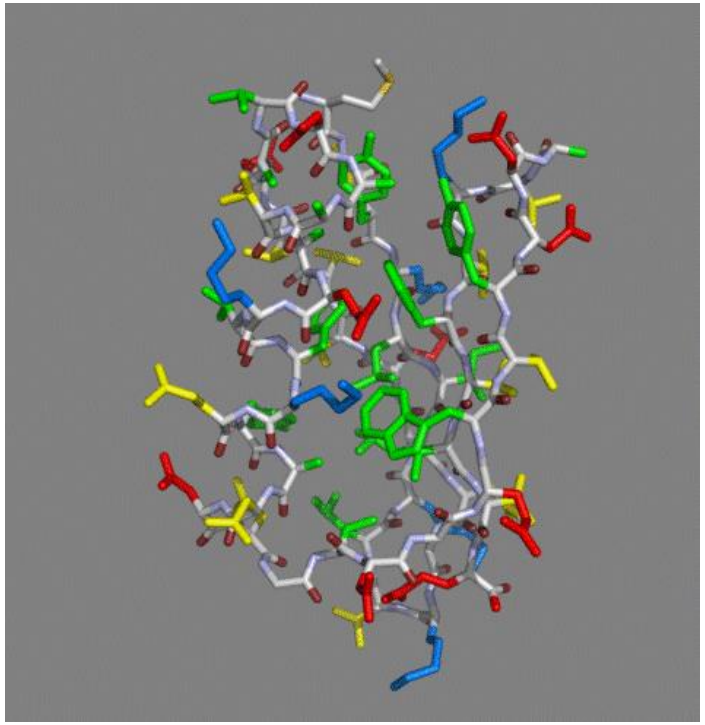








Location of Residues in Globular Proteins

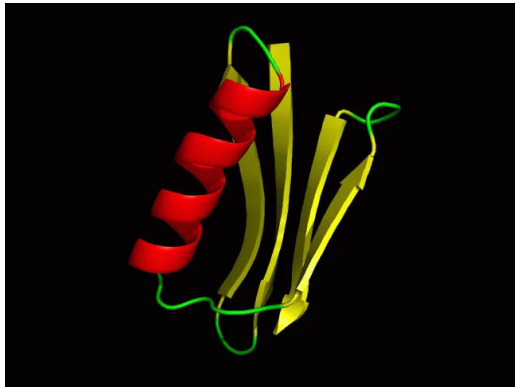


Red - amino acids with neg. sidechains

Blue - amino acids with pos. sidechains

Yellow – amino acids with polar sidechains.

Green - amino acids with hydrophobic side chains



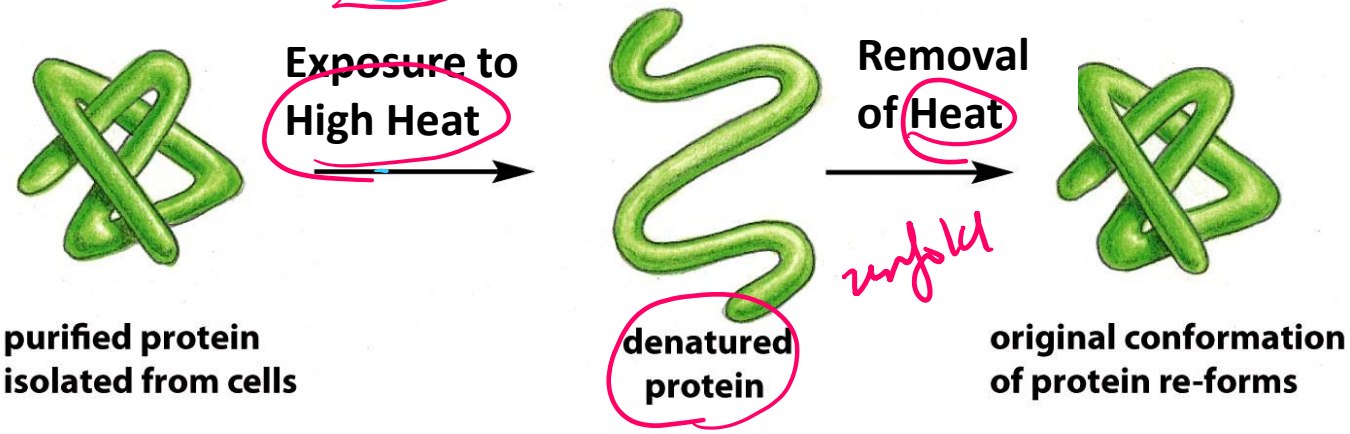
Amino Acid	Inside (core)	Surface
Charged		✓
Polar		✓
Non-polar	100%	✓

Protein Stability

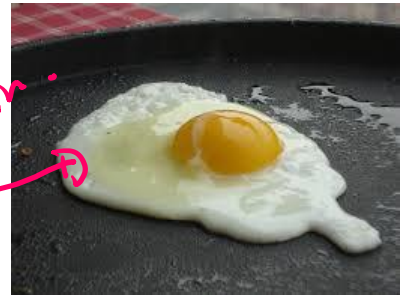
- H-bonds
- van der Waals
- Hydrophobic effect

Chain disorder

Protein Denaturation



- Often, unfolded protein aggregate, which prevents refolding.



Hydrogen Bonding Stabilizes the Folded Form

General Pattern:

X-H Y

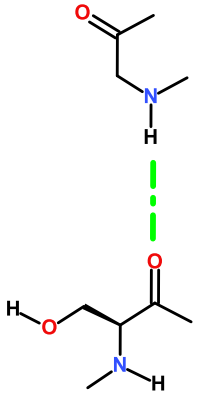
Possible Donors:

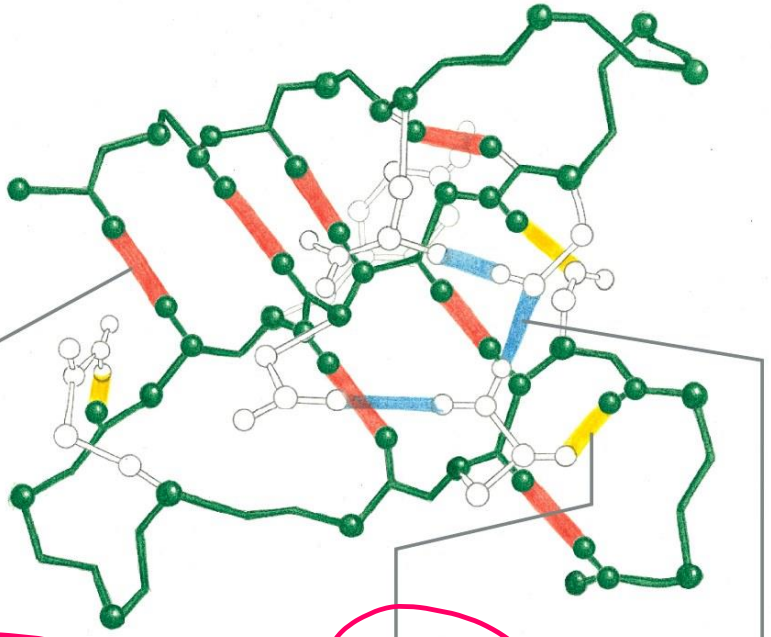
N-H or O-H

Possible Acceptors

C=O or -O-H or

N in amino group (Lysine)

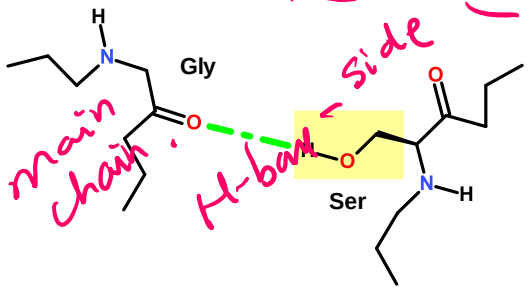


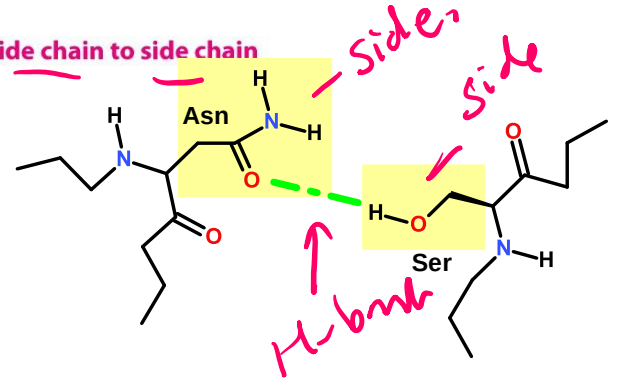


2ndy Structure

3ry 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

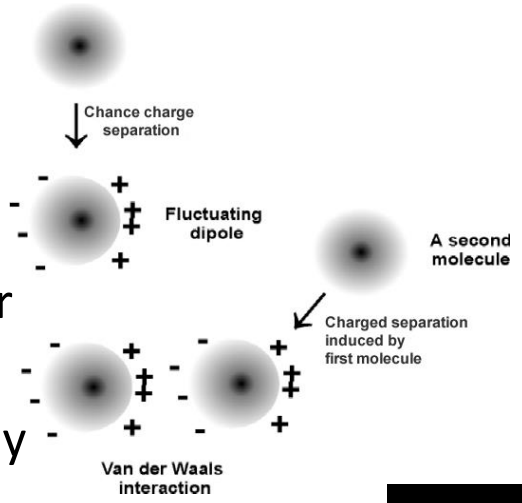


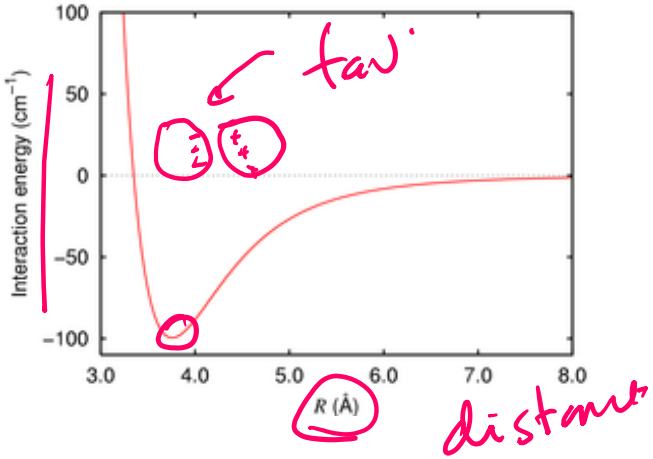


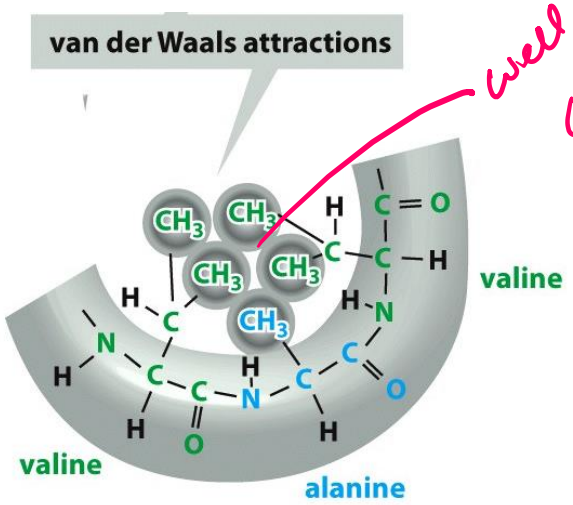
- **Hydrogen bonds** form between hydrogen atoms and the carbonyl group in the peptide-bonded backbone = secondary structure
- Hydrogen bonds are also found between hydrogen and electronegative atoms on side chains (sidechain-sidechain)
- Sidechains can form hydrogen bonds to the mainchain too.

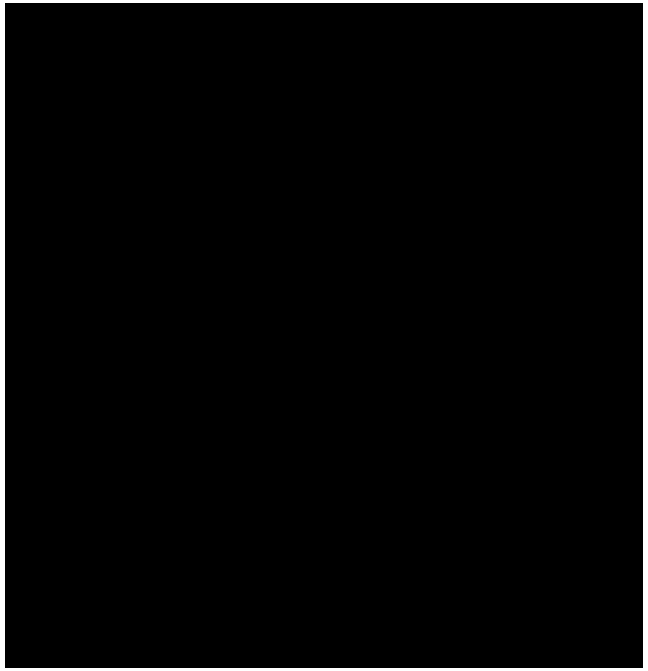
Van der Waals Interactions Stabilize the Folded State

- 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"









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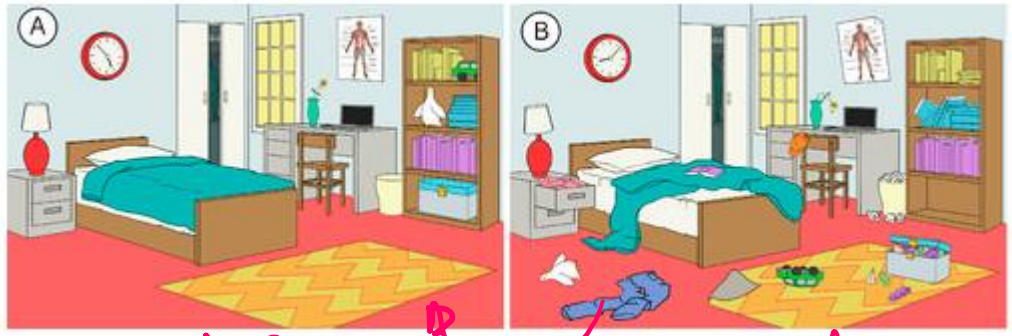
Hydrophobic Interactions are Critical for Stabilizing the Folded Structure

Ordered water hydrating a non-polar group

Energy and Entropy → disorder



order

add energy

disorder.

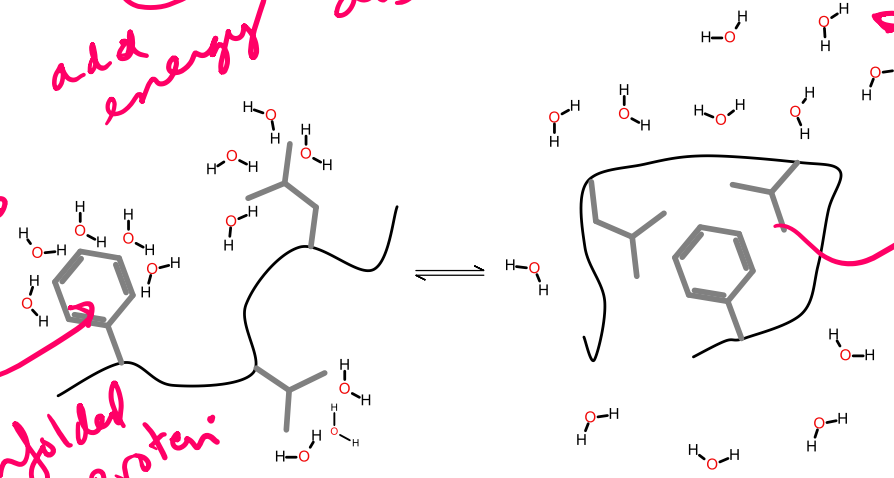
- because energy is added, disorder is favoured
- disordered
increase in entropy of water stabilizes folded form.
non-polar core. (no water)

Folded.

H.E. ordered water b

exposed to H_2O

unfolded protein



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.*

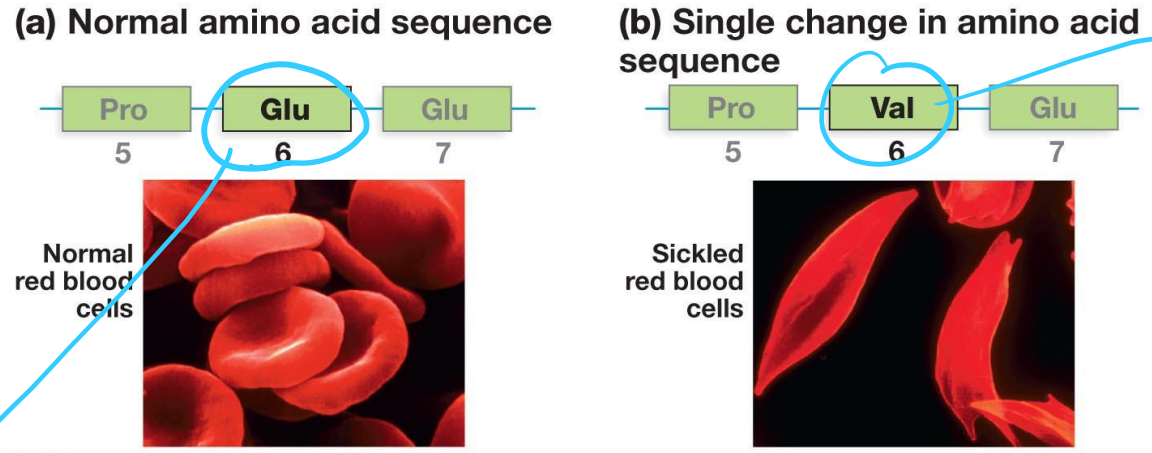
TABLE 3.1 **How Amino Acids Interact with Water**

20 amino acids are ranked according to how likely they are to interact with water. Color codes are based on Figure 3.3.

Isoleucine	Highly hydrophobic
Valine	
Leucine	
Phenylalanine	
Methionine	Moderately hydrophobic
Alanine	
Glycine	
Cysteine	
Tryptophan	
Tyrosine	Mildly hydrophobic
Proline	
Threonine	
Serine	
Histidine	Mildly hydrophilic
Glutamate	
Asparagine	
Glutamine	
Aspartate	
Lysine	
Arginine	Highly hydrophilic

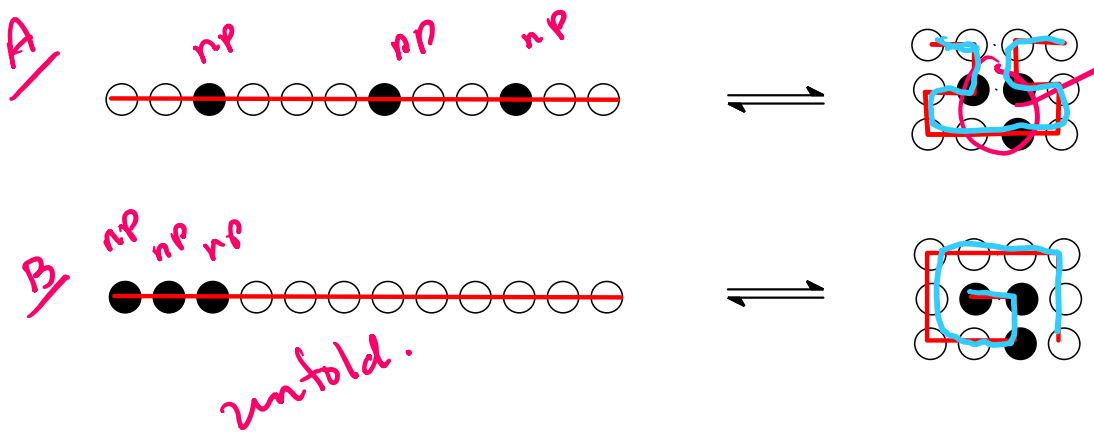
Fold Depends on Amino Acid Sequence

Effect of mutations on protein folding -- sickle cell anemia



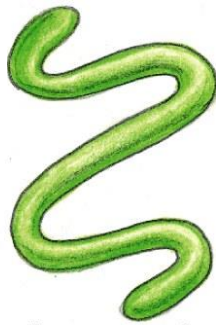
A single change in the amino acid sequence can change the function of a protein, and often affecting how it folds.

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



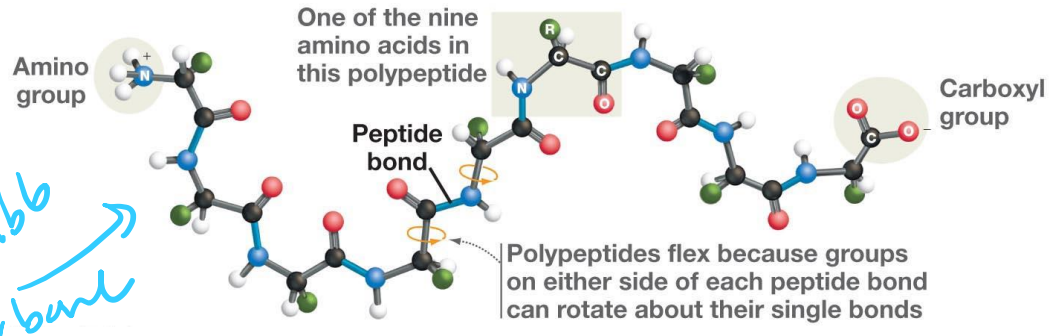
Unfolded Polypeptides Are Flexible – High Entropy stabilizes the Unfolded state

Exposure to High Heat

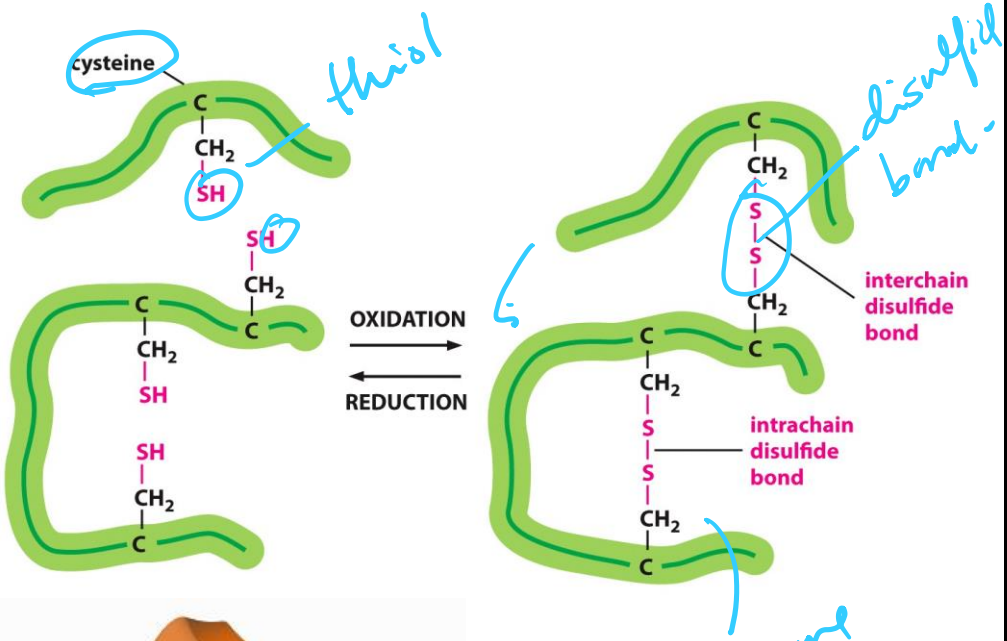


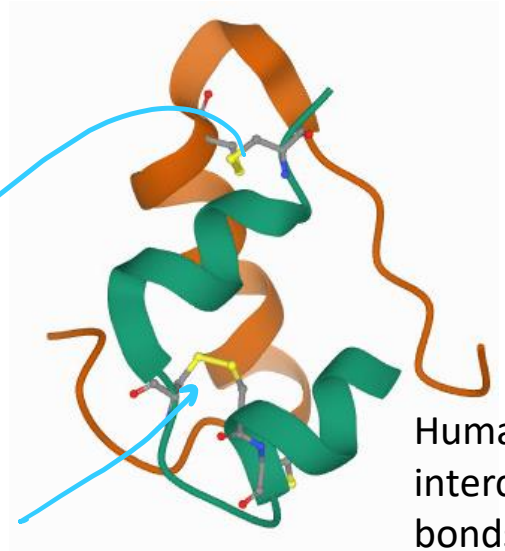
purified protein isolated from cells

denatured protein



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.

Summary - Interactions that Stabilize Folded Proteins.

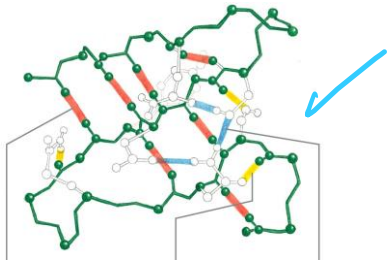
- **Hydrogen bonds** form between hydrogen atoms (NH) and the carbonyl group in the peptide backbone (mainchain), and between and donors and acceptors on sidechains. *Mainchain-mainchain H-bonds are responsible for secondary structures.*
- **Hydrophobic interactions** within a protein increase stability 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).

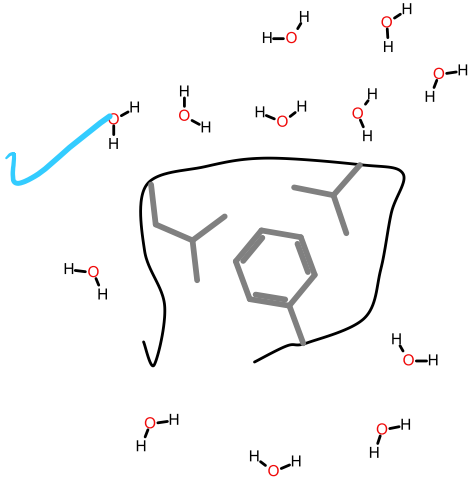
H-bonds
van der Waals
Hydrophobic effect

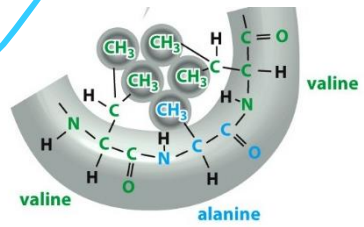
Chain disorder

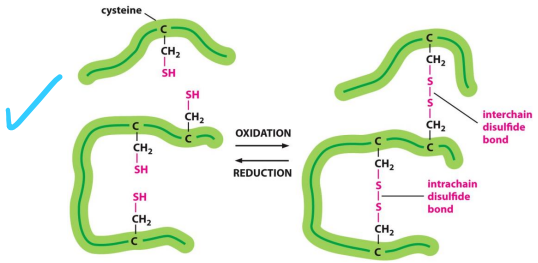
Native

Unfolded



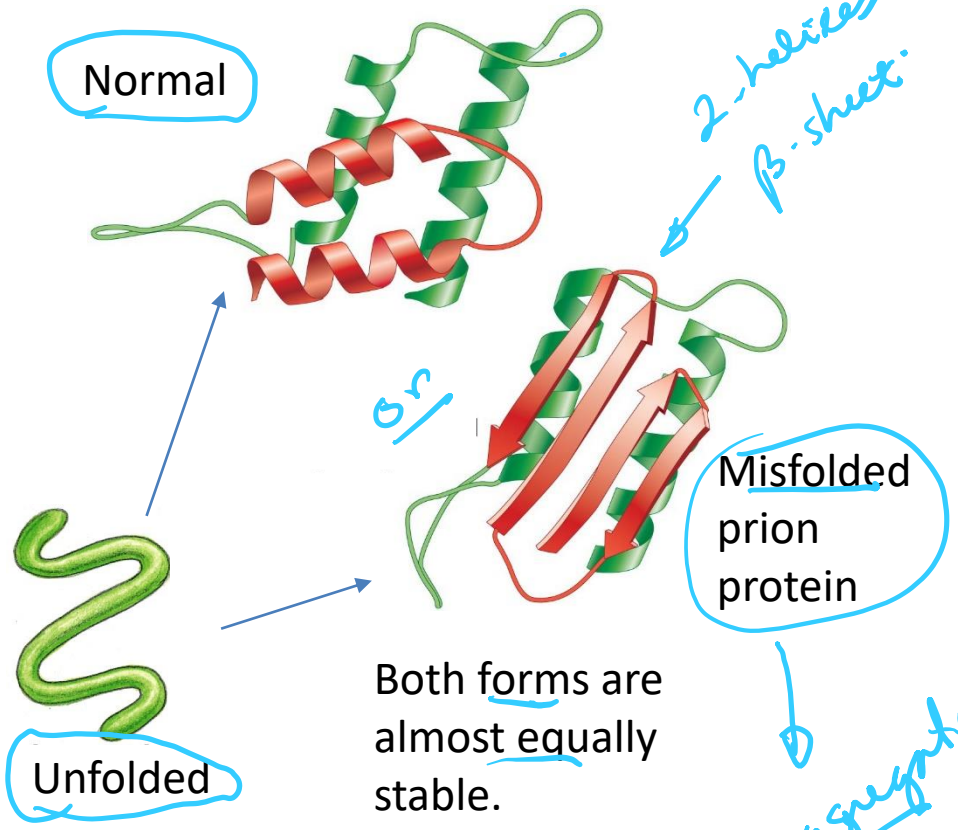






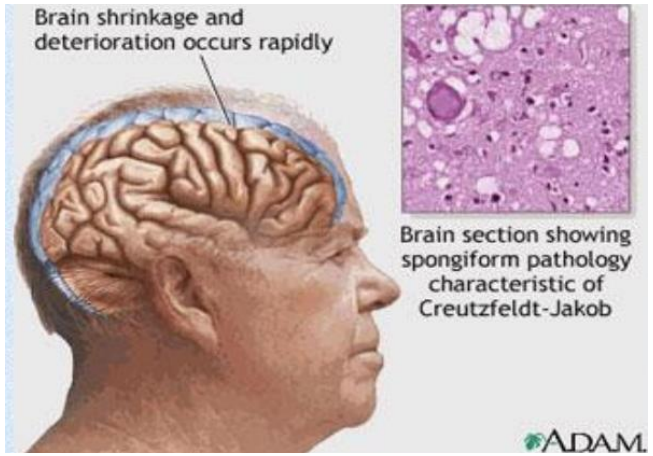
What happens when proteins don't fold properly?

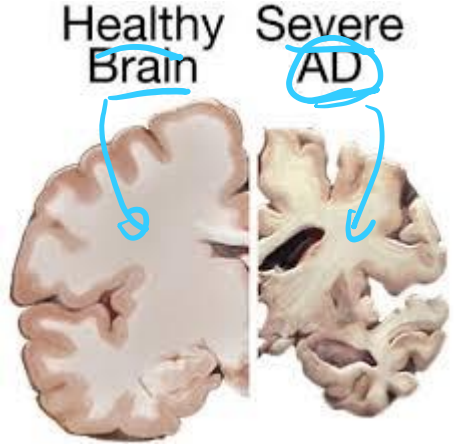
Prions are improperly folded proteins that cause neurodegenerative diseases



Both forms are almost equally stable.

Why does this occur?



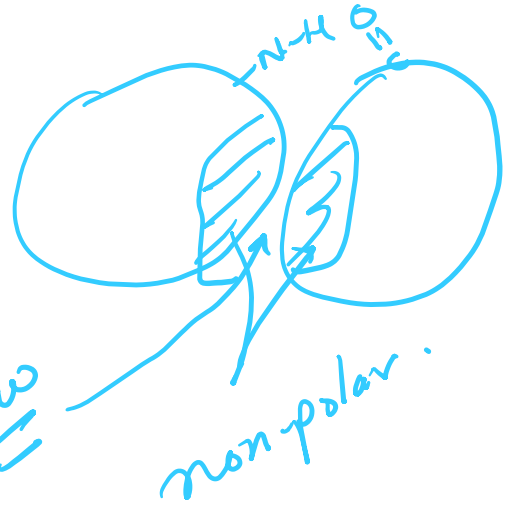


What is the effect on the brain?

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?

Quaternary Structure



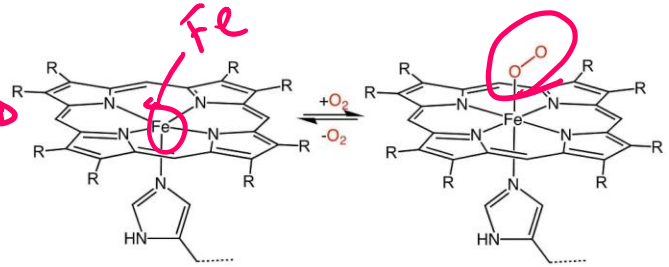
- Combinations of polypeptide subunits (combinations of tertiary structures).
- May be held together by covalent bonds (disulfide), but usually non-covalent interactions between R groups on the different chains.
- Proteins can be a dimer, a tetramer, etc.
- If the chains are the same, called homo dimer.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

Quaternary structure of hemoglobin (oxygen transport protein):

- two α chains
- two β chains

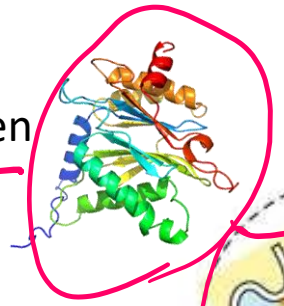
hetero tetramere

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

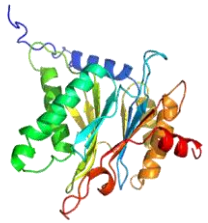


Antibodies – Produced by the Adaptive Immune system to Fight Pathogens.

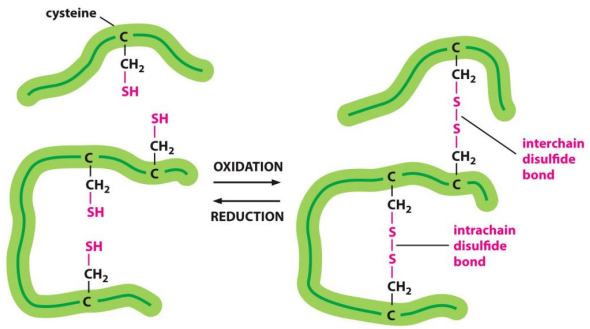
Antigen



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.

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

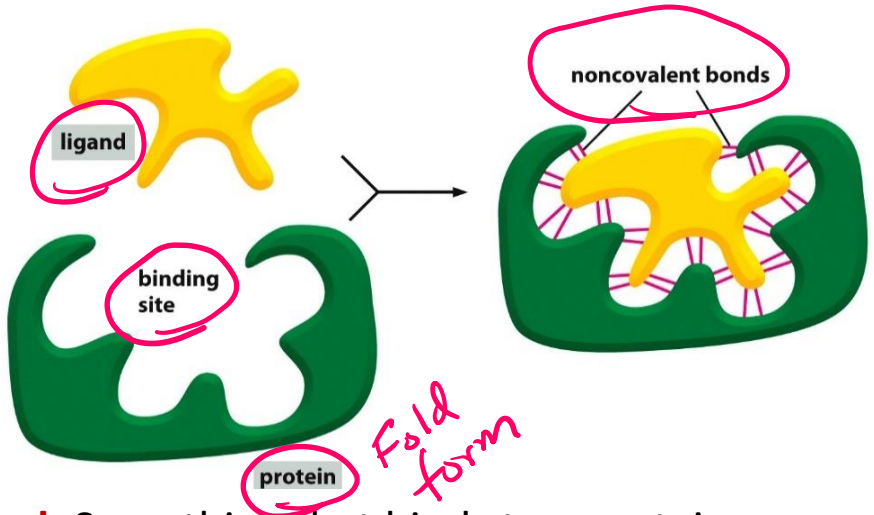
Tertiary Structure:

- 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 a protein, and which are found on the surface.

Quaternary Structure:

- Multiple chains, stabilized by non-covalent and covalent (disulfide bonds) interactions.
- What is the quaternary structure of an antibody?

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



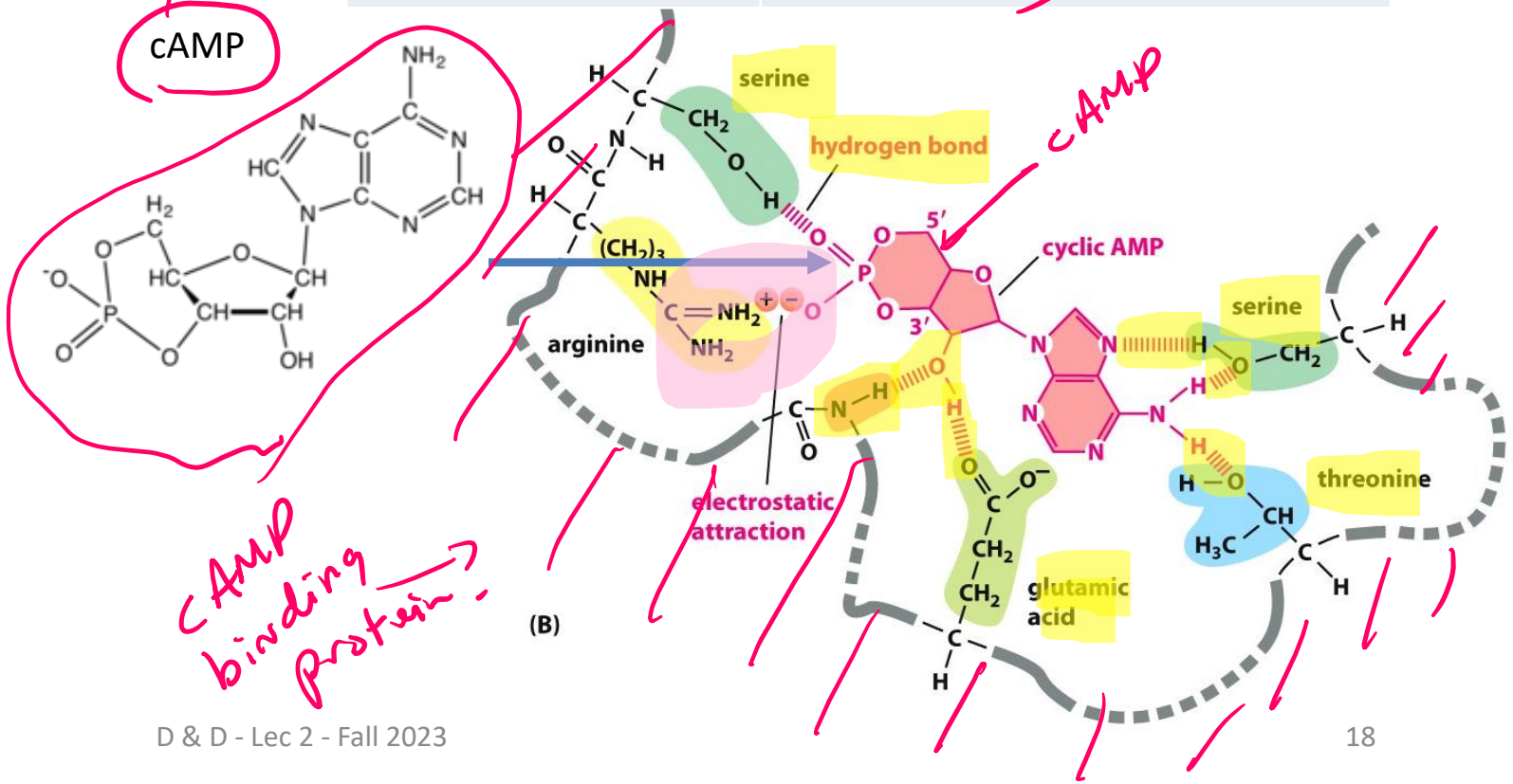
Ligand: Something that binds to a protein, usually small molecules (e.g. cyclicAMP, cAMP).

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:

Interaction	Which stabilize cAMP Binding?
van der Waals	✓
H-Bonding	Side & mainchain.
Electrostatic	✓
Hydrophobic effect	No



Ligand Binding & Saturation:

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

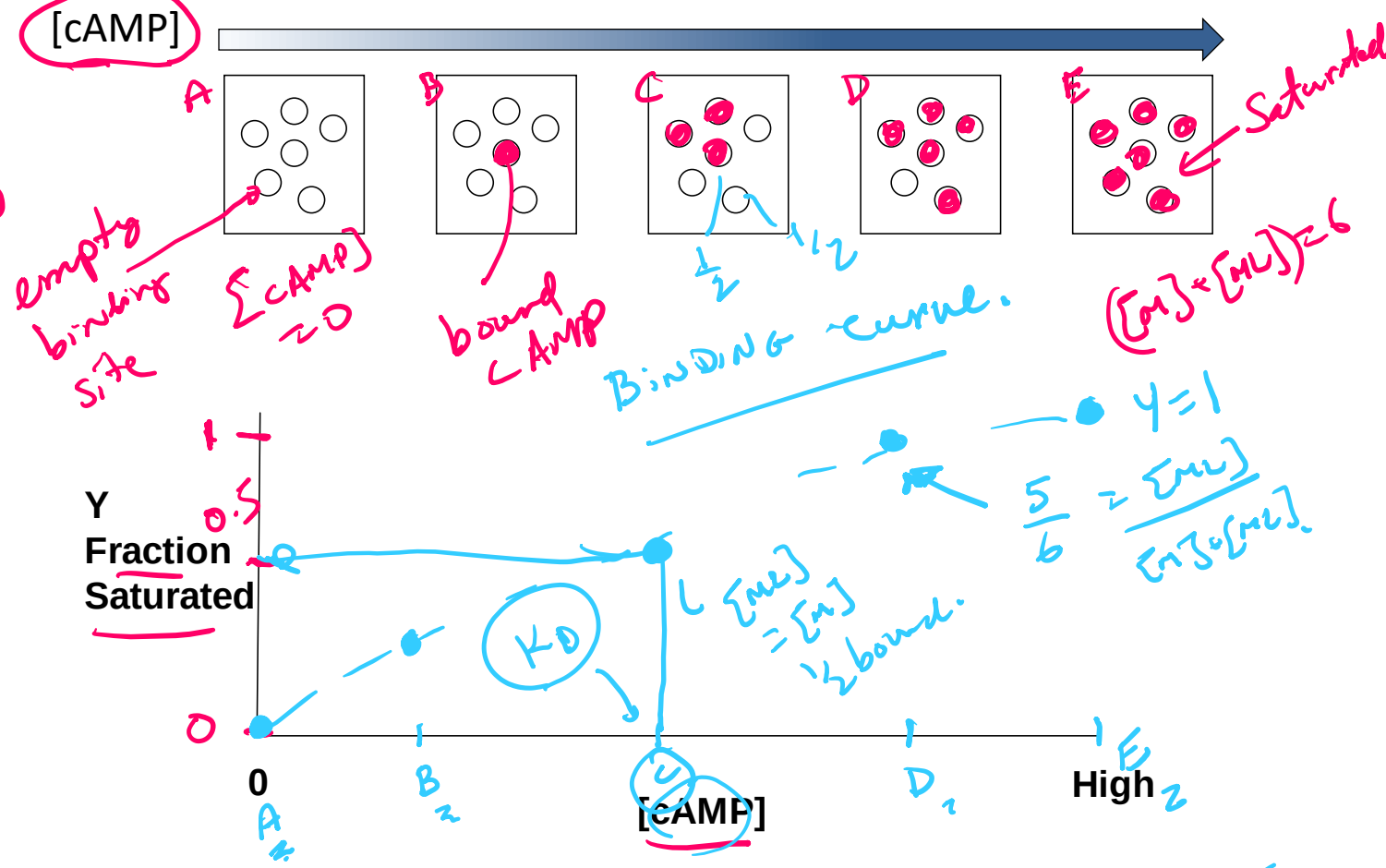
[M] = free macromolecule (e.g. antibody with no antigen).

[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 location on the fraction saturated curve for each box.*



Key Points:

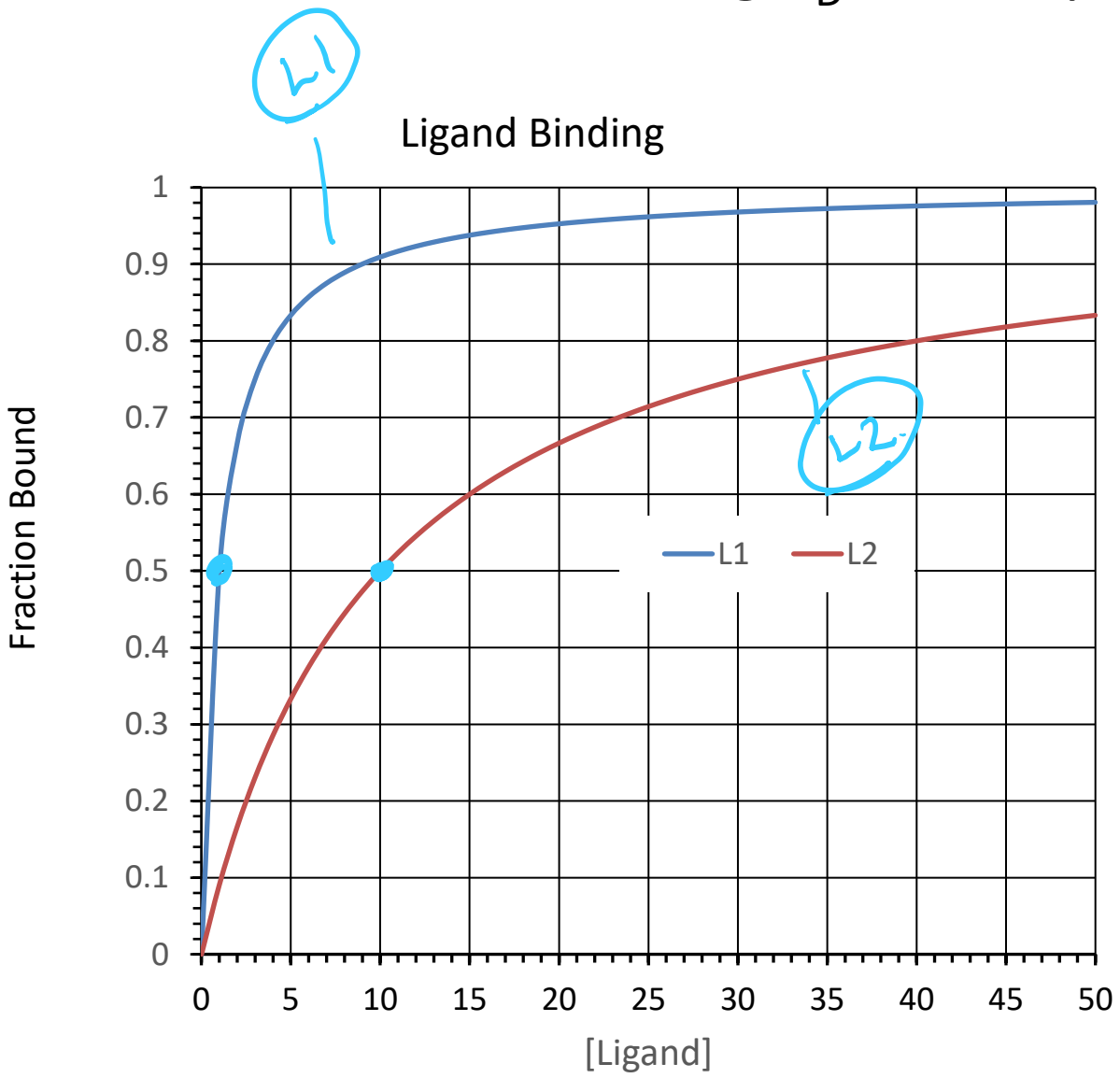
1. The binding sites saturate, when all are full no more ligand can bind.
2. There is a ligand concentration, [L], where ½ the sites are full. This [L] is K_D.
3. K_D is the equilibrium constant for ligand dissociation:

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

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

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

Using K_D to Compare Ligand Binding



same protein

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?

L1

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

L2

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

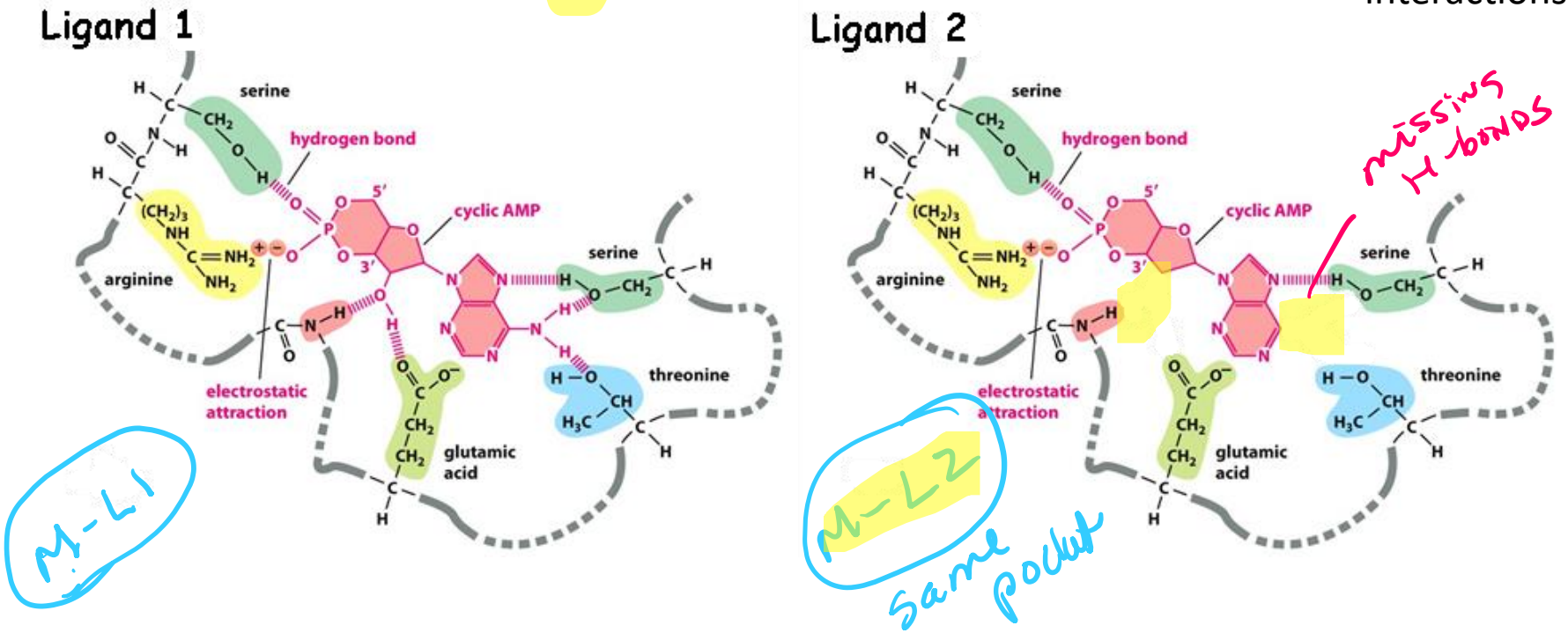
higher fraction bound? L1 or L2

lower KD ⇒ higher affinity, more bound.

Why does L1 bind more tightly (higher affinity)?

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

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



3. How do the differences affect K_D?

loss H-bonds
increased KD
(lower affinity)
KD L1 → KD L2

Key Points:

Binding:

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

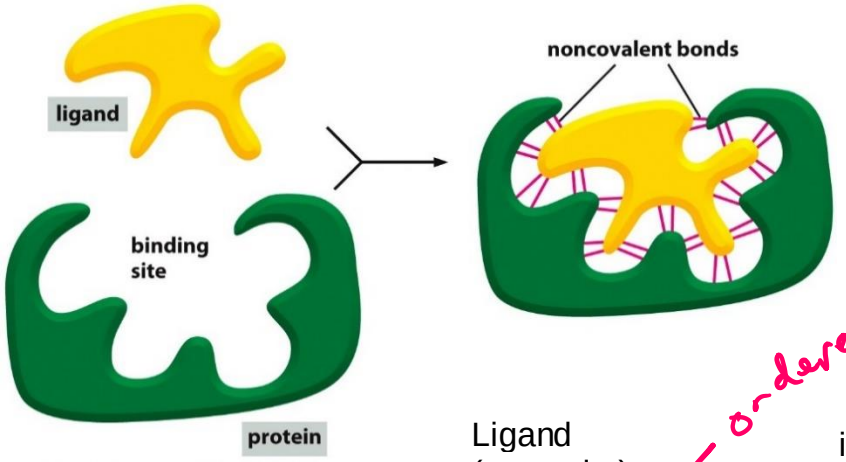
- H-bonds,
- van der Waals,
- Electrostatic,
- Non-polar interactions (hydrophobic)

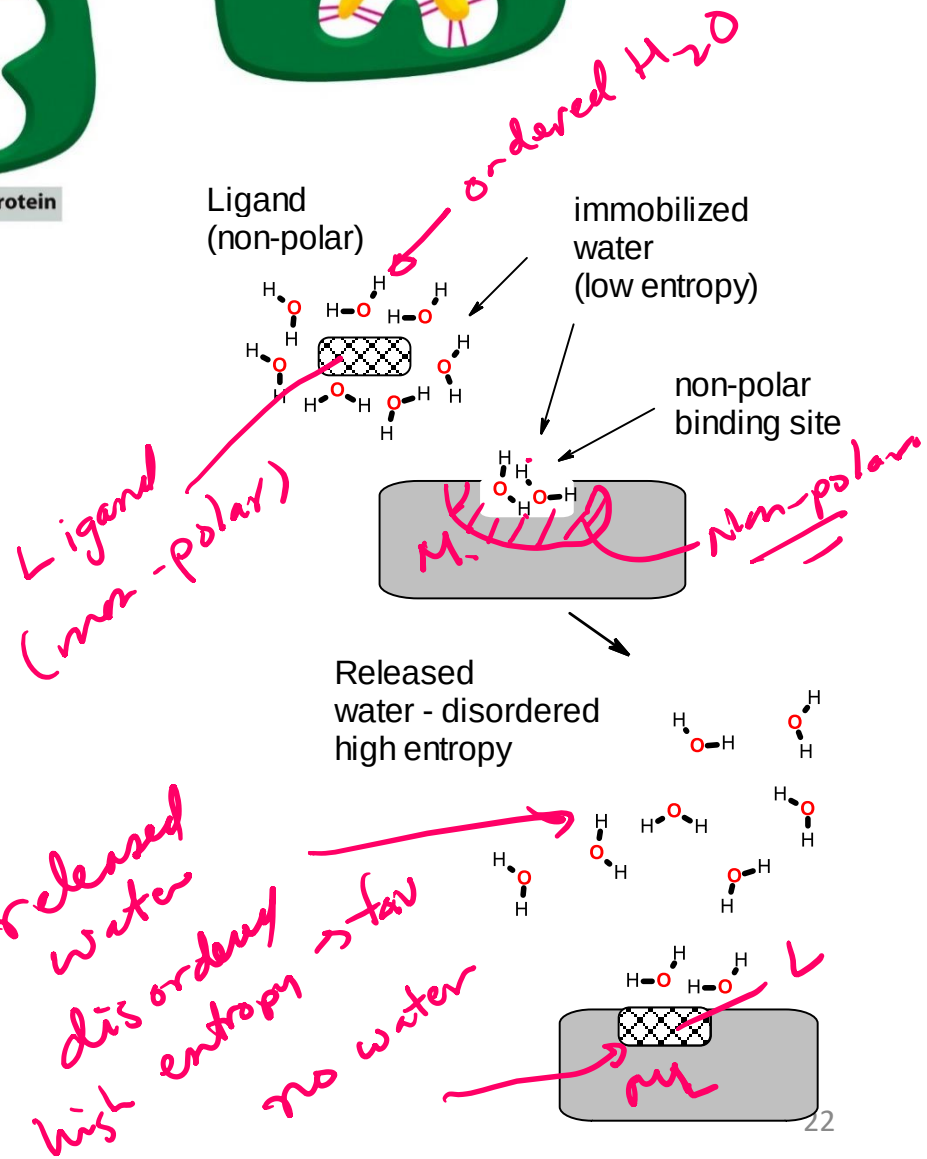
Binding is **reversible**

Binding is **saturable**

Binding ½ point (Y=0.5) occurs at K_D

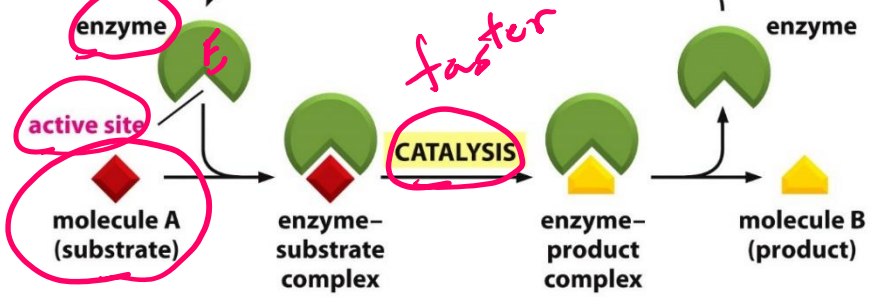
The higher the affinity (strength of interaction), the lower the K_D



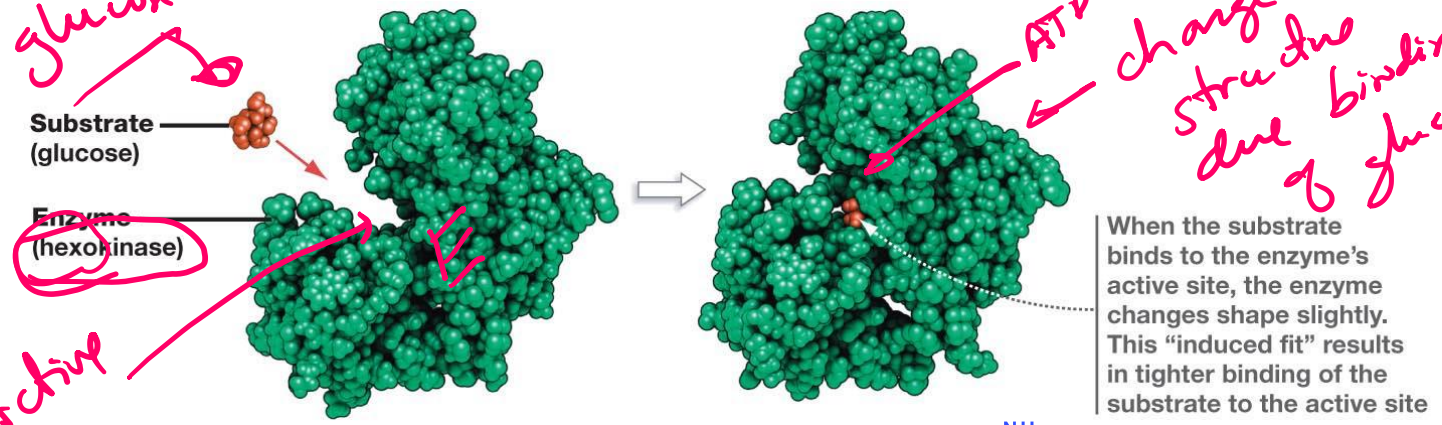


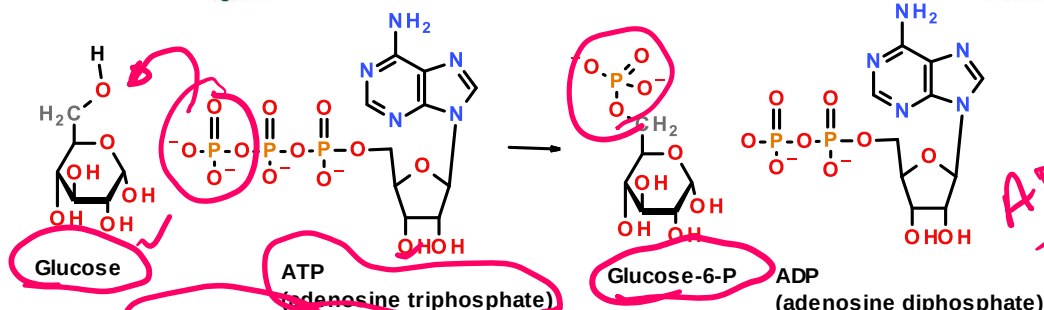
Enzymes

- **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**.



$$E + S \rightleftharpoons (ES) \rightarrow (EP) \rightleftharpoons E + P$$



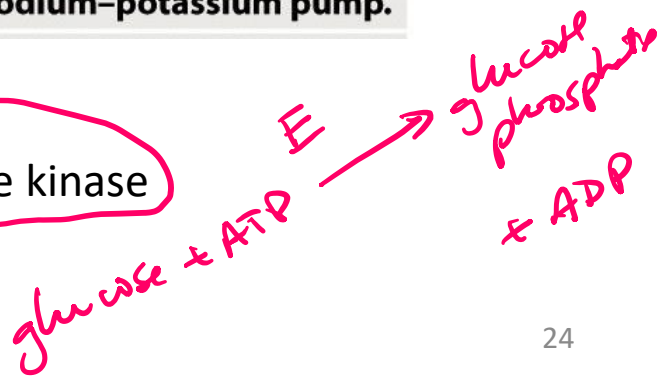


Enzyme – Chemical Diversity

TABLE 4–1 SOME COMMON FUNCTIONAL CLASSES OF ENZYMES

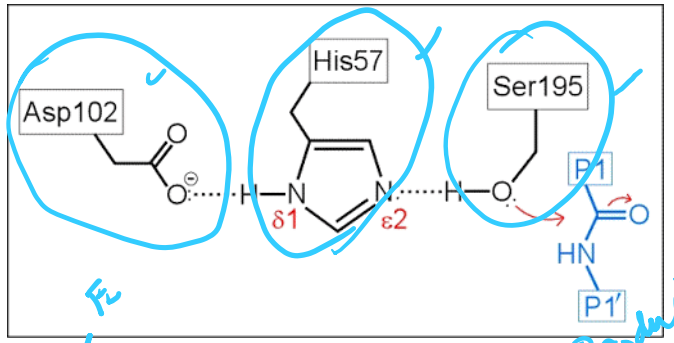
ENZYME CLASS	BIOCHEMICAL FUNCTION
Hydrolase	General term for enzymes that catalyze a hydrolytic cleavage reaction.
Nuclease	Breaks down nucleic acids by hydrolyzing bonds between nucleotides.
Protease	Breaks down proteins by hydrolyzing peptide bonds between amino acids.
Synthase	General name used for 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.

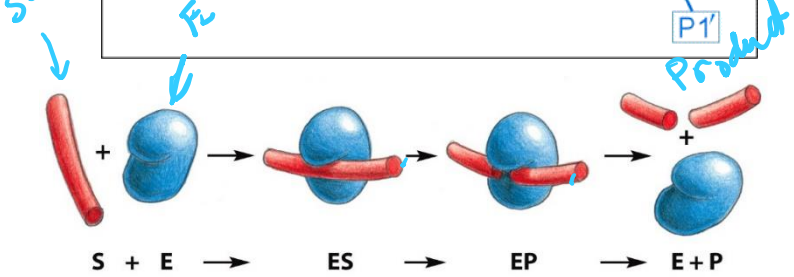
- Most enzyme names end in "-ase"
- Usually named by their substrates and the reactions they catalyse, i.e. glucose kinase

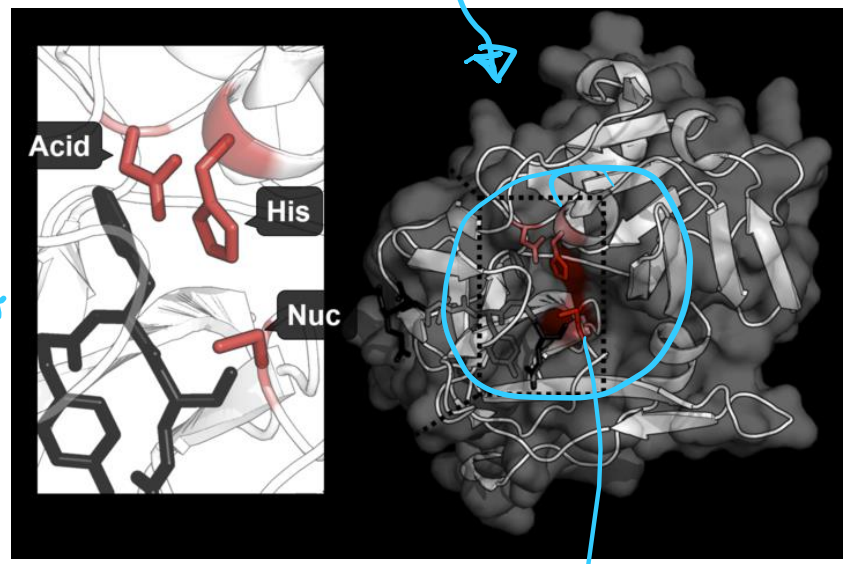


Example of Active Site Functional Groups:
Catalytic triad (Asp, His, Ser) in Protease Trypsin cleaves after Lys Residues

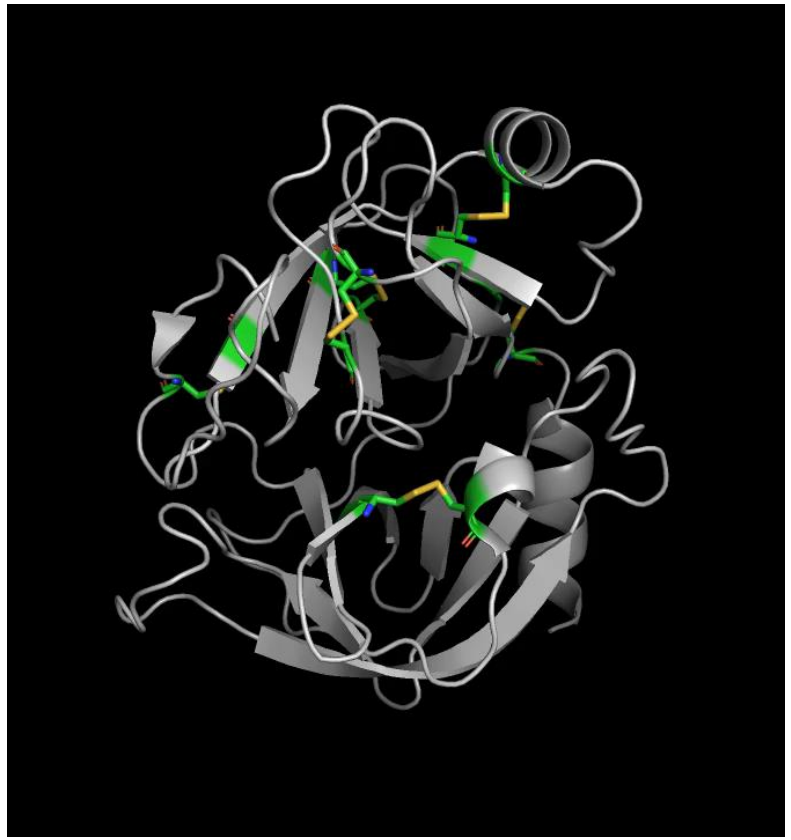
Catalytic triad





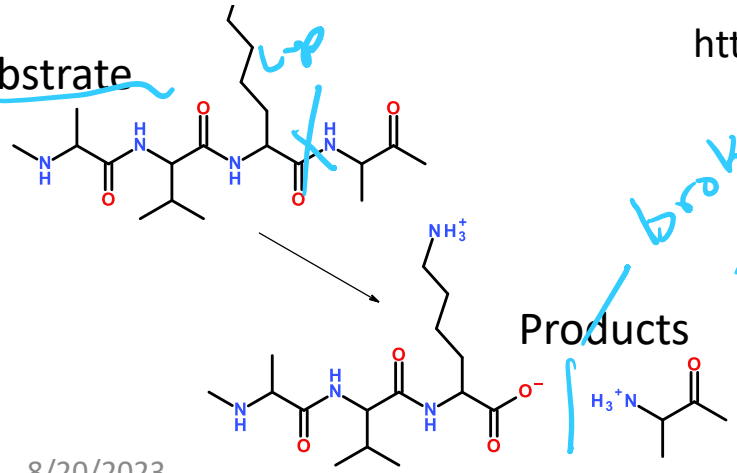


https://shirleychemproject.weebly.com/



Disulfide bonds in trypsin

Substrate



Products

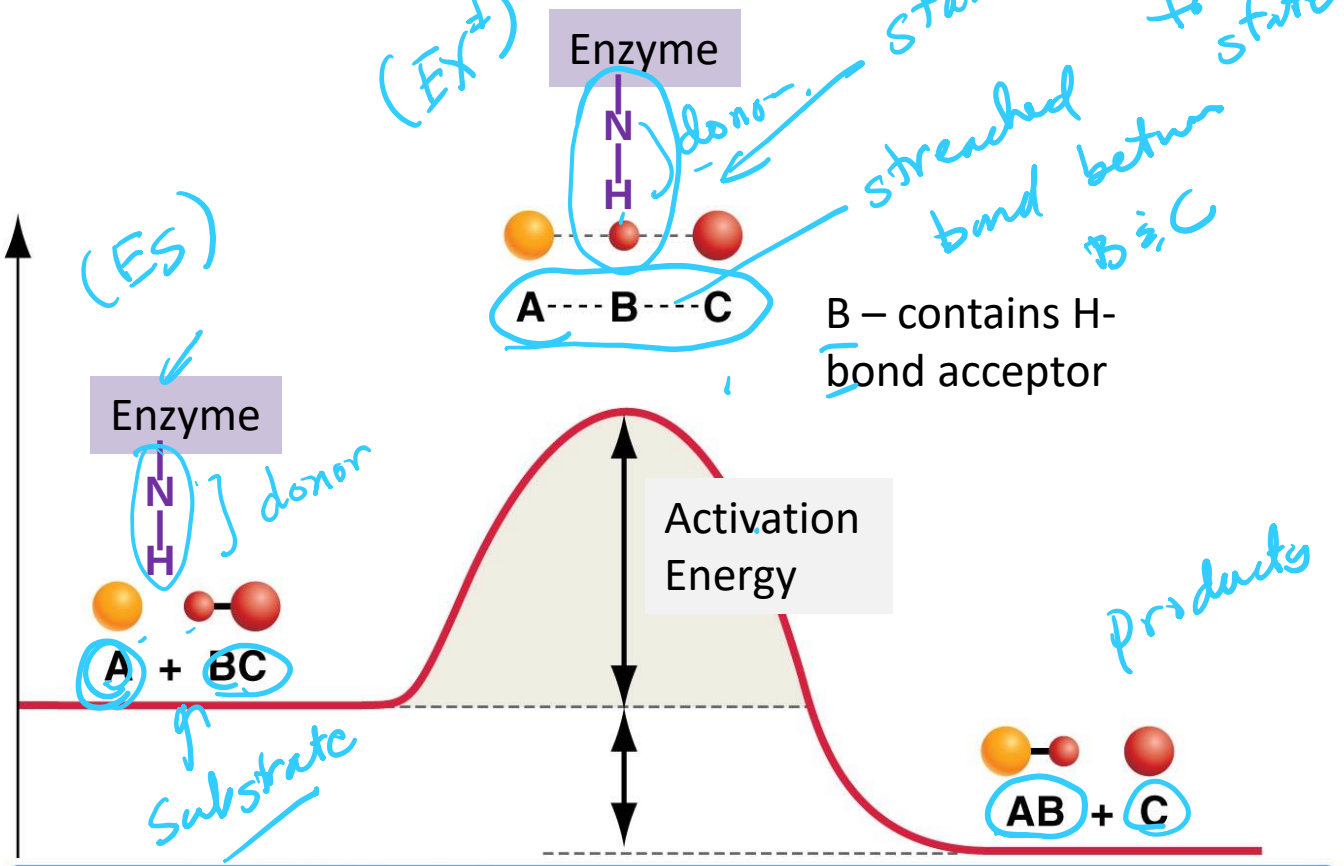
How Do Enzymes Increase Rates?

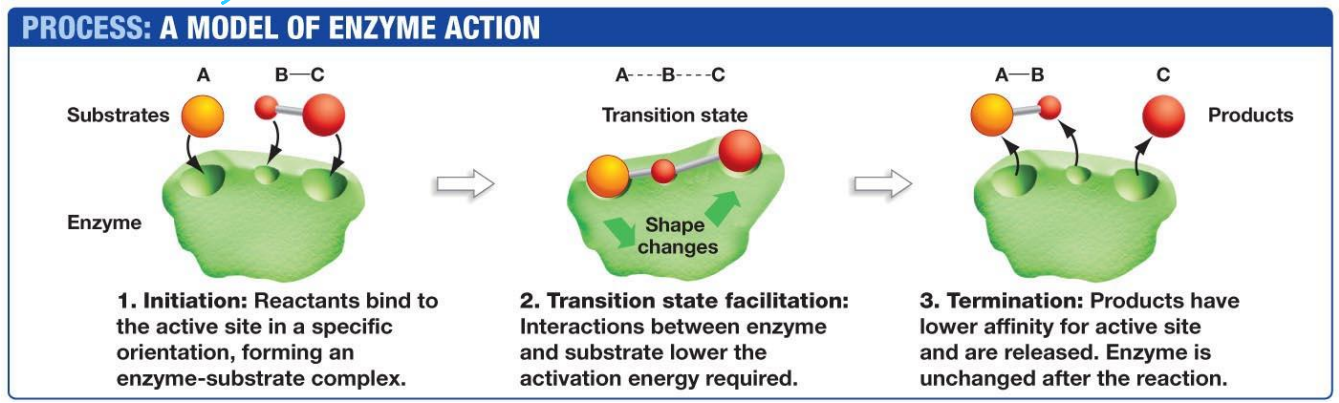
- **Transition state** = high energy intermediate that occurs during the reaction.
- Energy barrier is called the activation energy.
- Rate of product formation depends on the concentration of the transition state.

Low [X] = Slow reaction

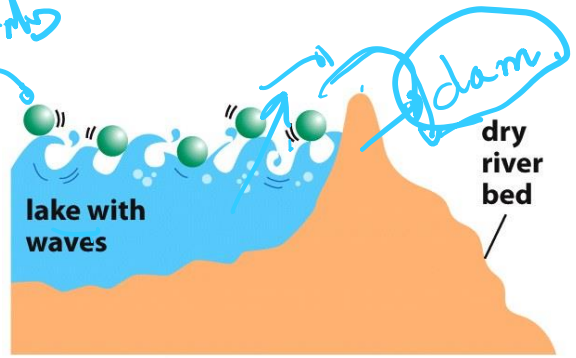
Higher [EX] = Faster reaction

- 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)

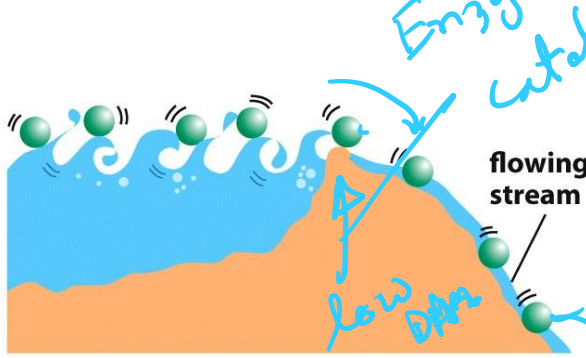




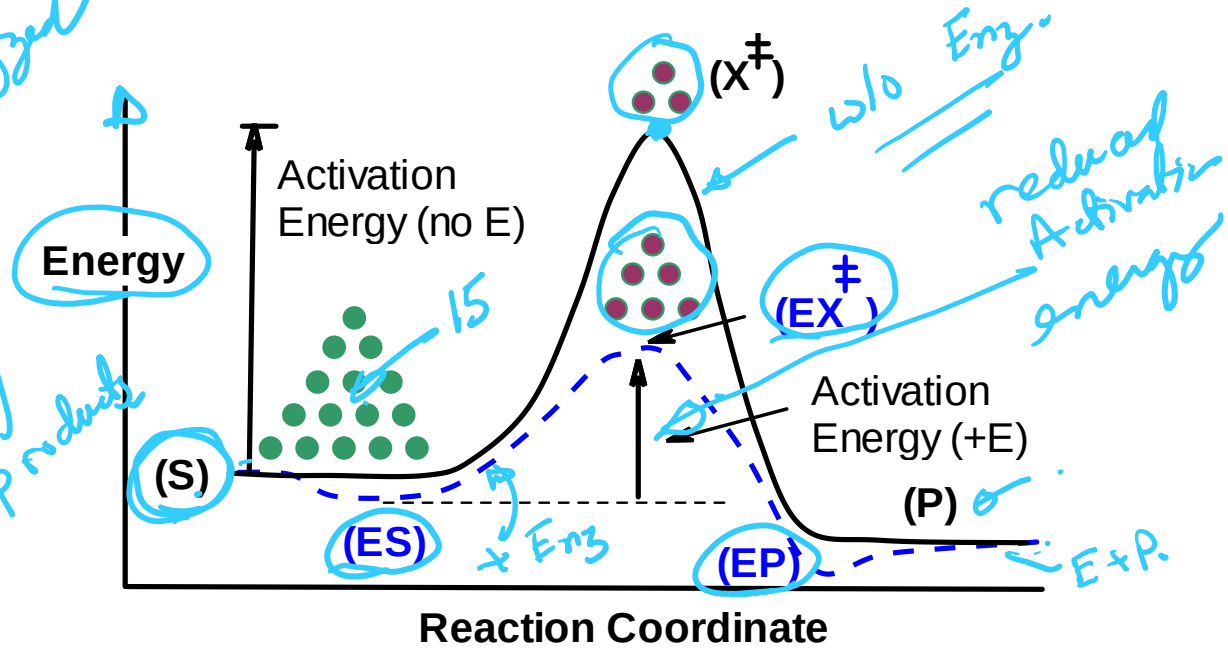
A model of transition state stabilization.



uncatalyzed reaction—waves not large enough to surmount barrier



catalyzed reaction—waves often surmount barrier



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?

Key Points:

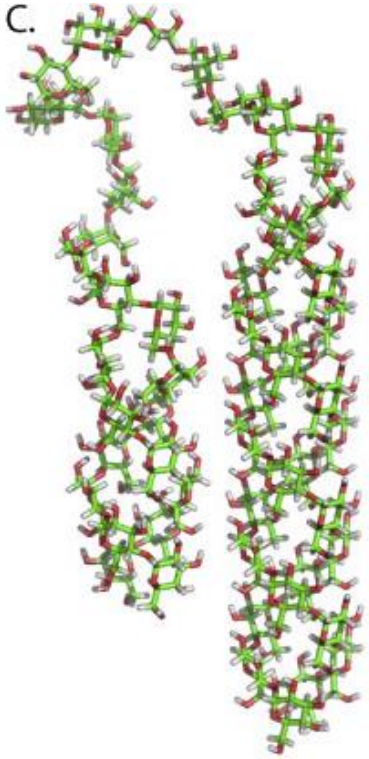
Enzymes:

Enzymes bind substrates (S), forming (ES) complex in active site, converting to P, releasing P.

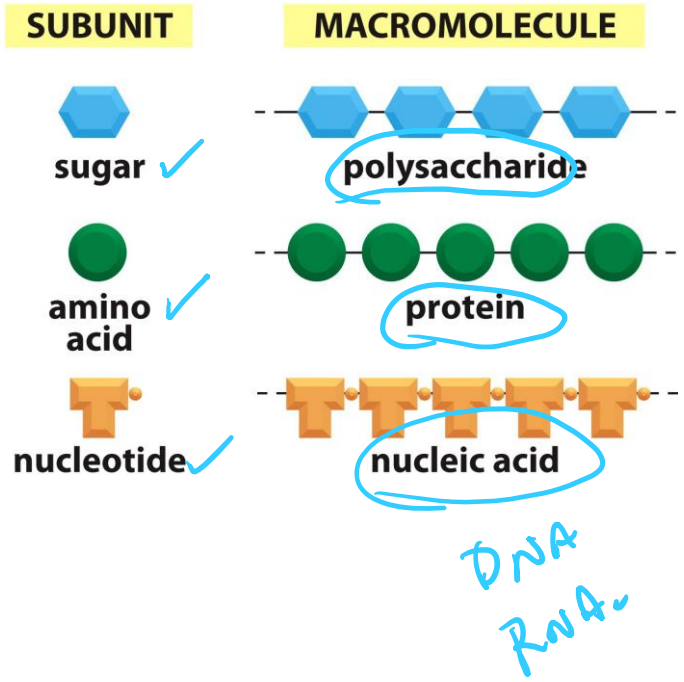
Rate enhancement since the transition state complex (EX) forms more readily with enzymes due to:

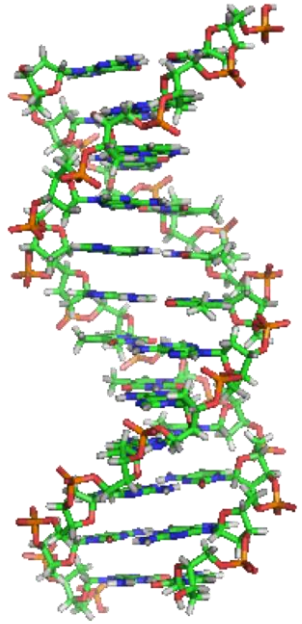
- Bringing substrates and functional groups on the enzyme together by binding (less entropy change)
- Directly lowering energy of transition state (X) through favorable interactions that are unique to the transition state, such as forming unique hydrogen bonds.

Carbohydrates



Polysaccharide



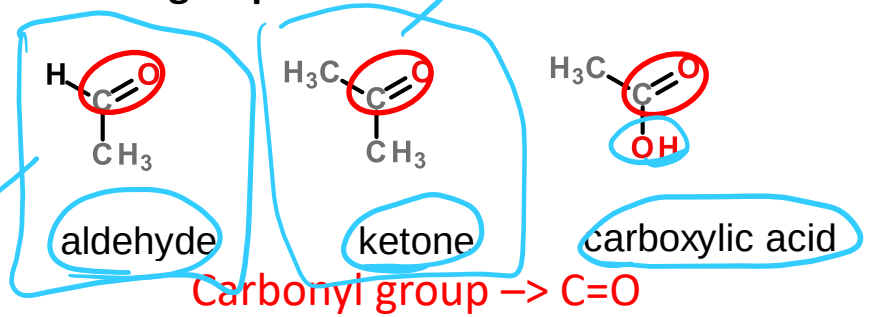


DNA (Nucleic Acid)

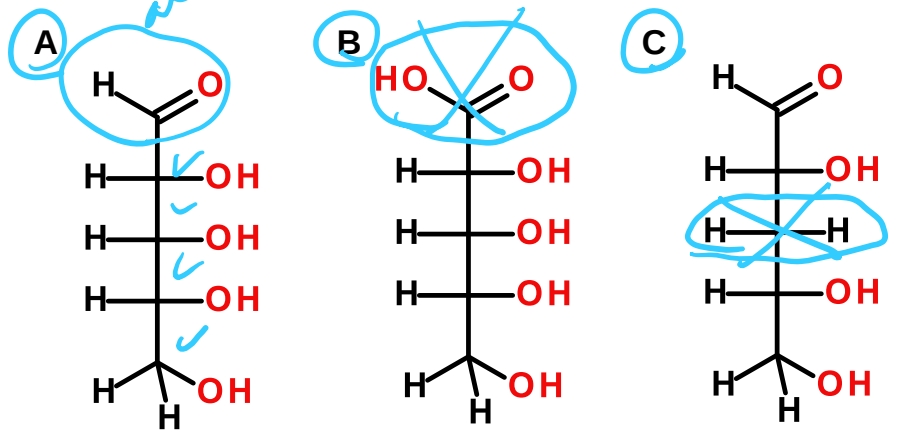
Carbohydrates

- 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:



Carbonyl group –> C=O

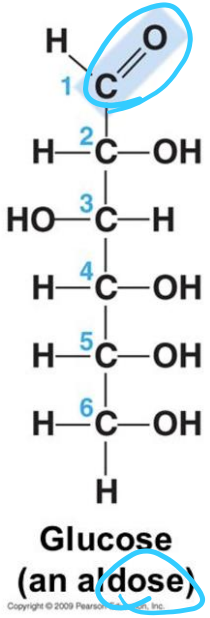


Only one of these is a carbohydrate, which one?

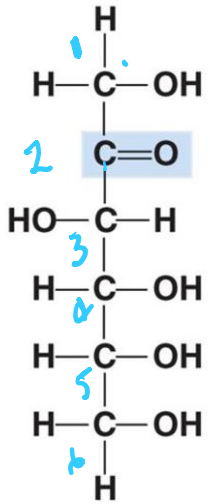
3 ways simple sugars (monosaccharides) differ from each other

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

Carbonyl group is located on C_1



Glucose (an aldose)

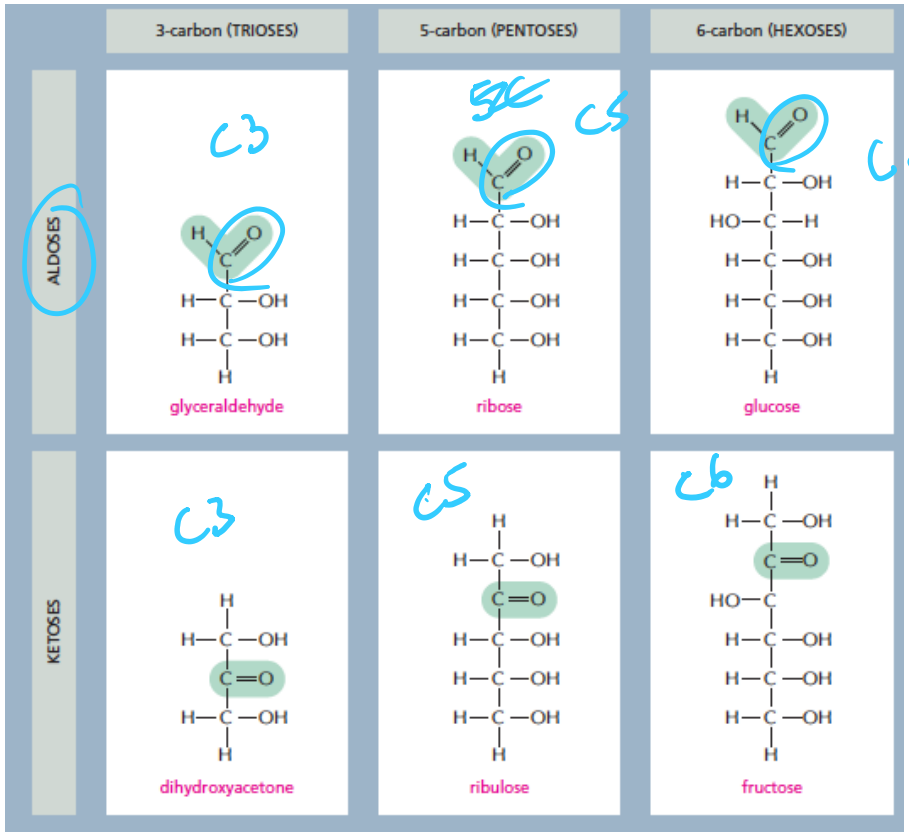


Fructose (a ketose)

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

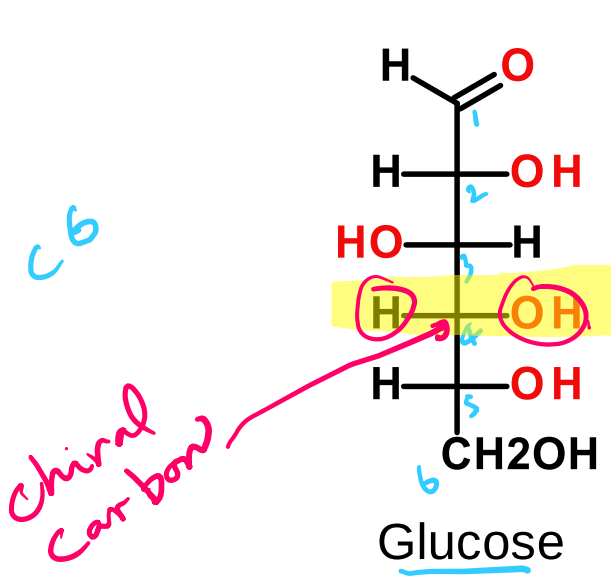
What carbon is the carbonyl?

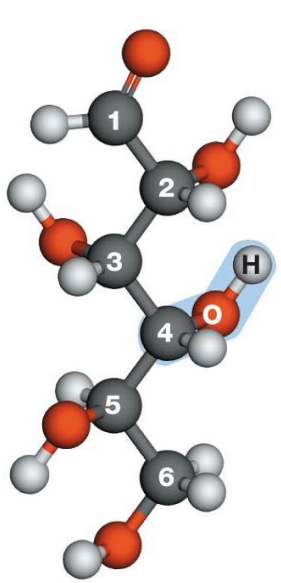
1. Location of the carbonyl group
2. **Number of carbons**
3. Spatial arrangement of atoms (the position of the OH groups)

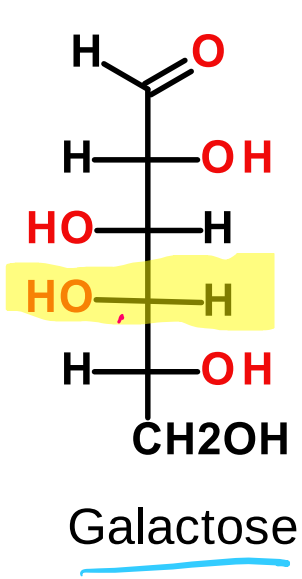


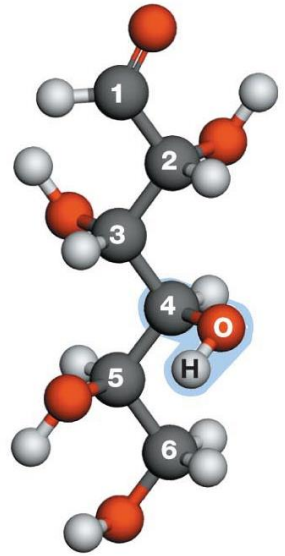
3 ways simple sugars (monosaccharides) differ from each other

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









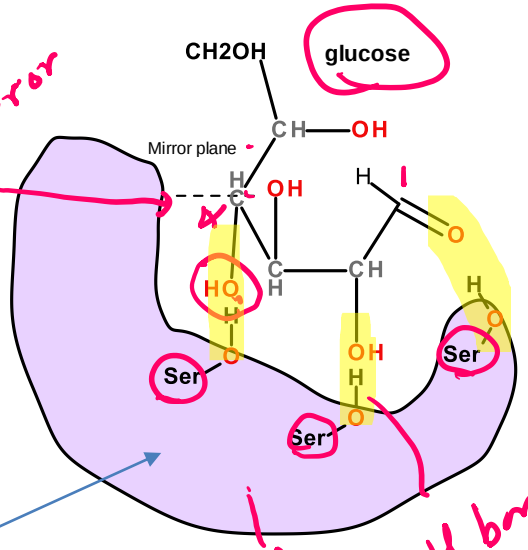
Both have the same chemical formula $C_6H_{12}O_6$.
Both are aldose sugars with 6 carbons.
Yet their functions are different.

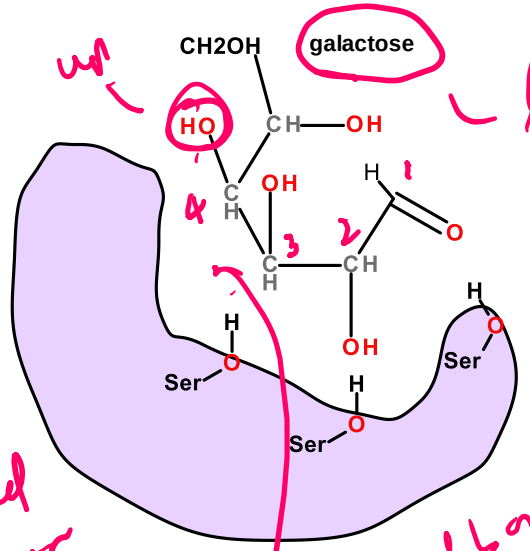
- Glucose can be used for energy immediately.
- Galactose has to be converted to glucose before it can be used for energy.

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

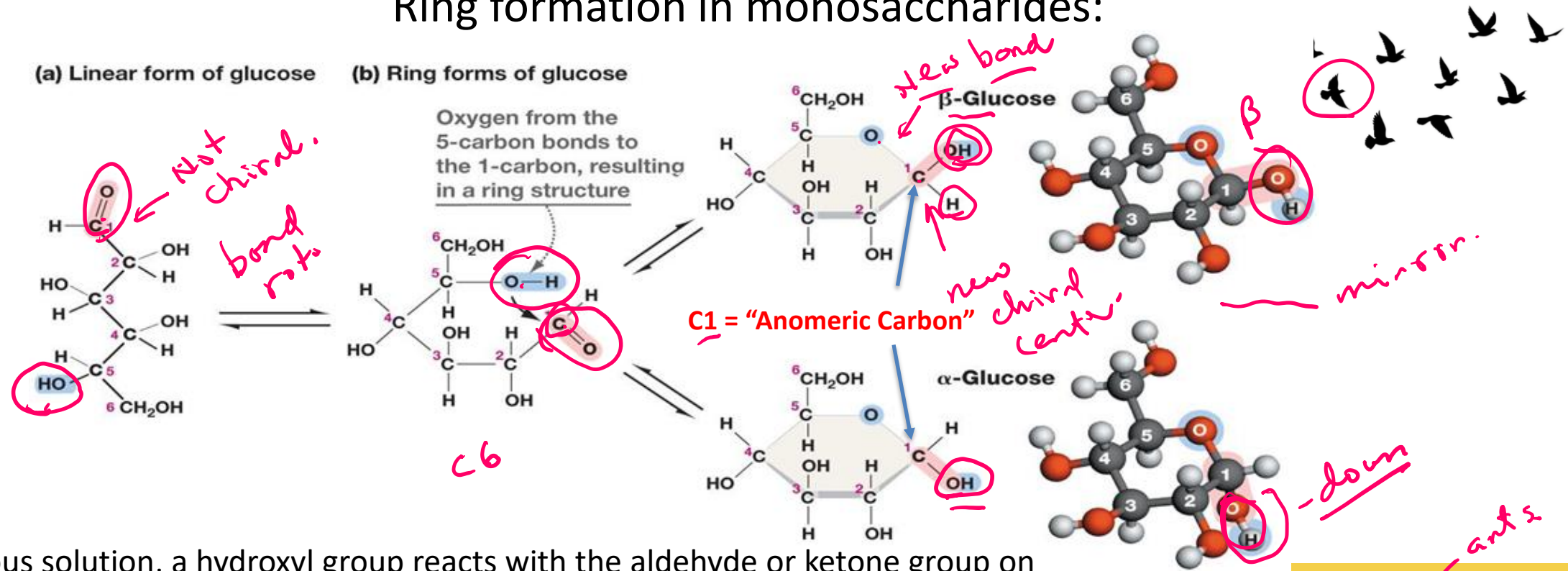
- OH is down in glucose
- OH is up in galactose

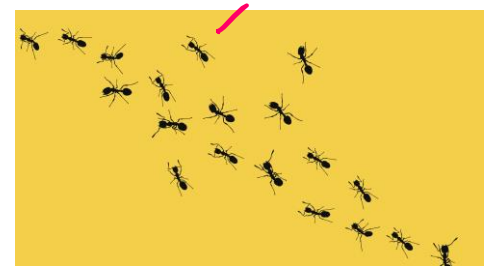
Enzyme specific for a-glucose





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
- Stable ring sizes are 5 atoms or 6 ato
- It is usually the 2nd to last -OH group, i.e. C5 in glucose, C4 in ribose.
- 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:

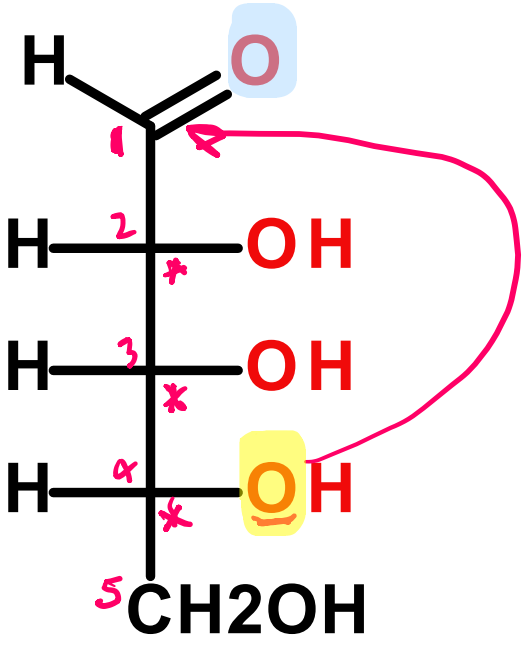
  α (new OH is down), β (new OH is up)

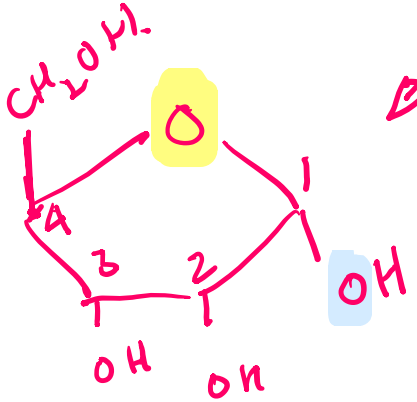
  "(ants are down, birds are up)"

Example Problem:

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

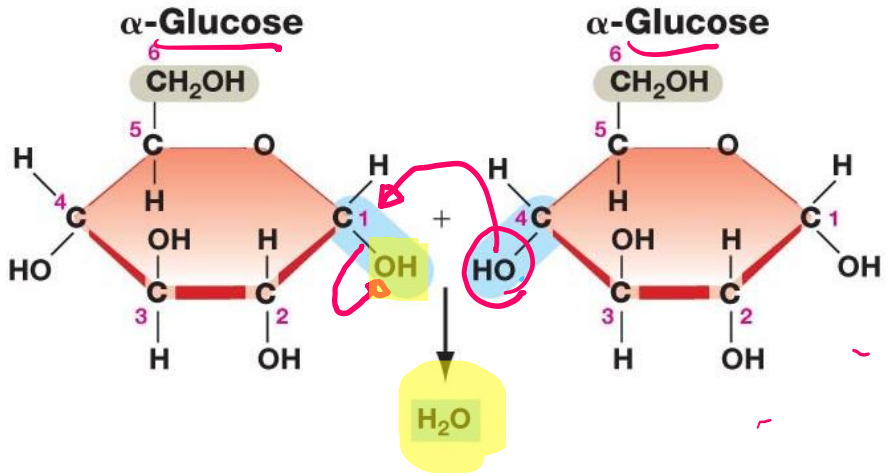
1. Number the carbons.
2. Which carbons are chiral? Mark them with a *.
3. Draw the cyclic form of α-ribose

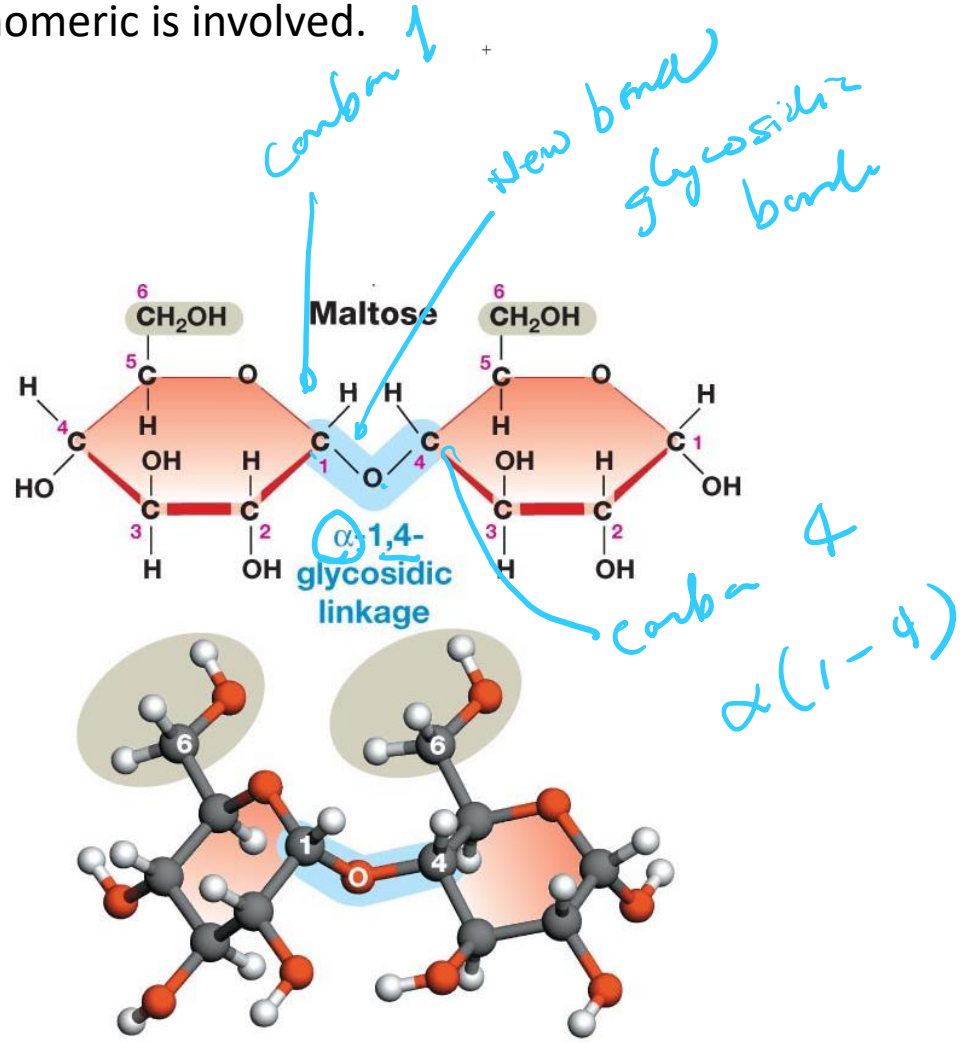




Glycosidic Linkages - Disaccharides

- **Glycosidic** bond formed between **any** -OH of one sugar and the anomeric carbon of another (e.g. at least one anomeric is involved.
- Water released (dehydration reaction).



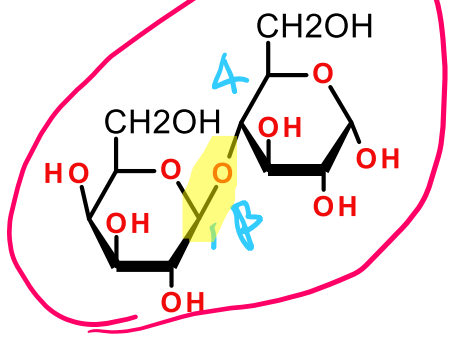


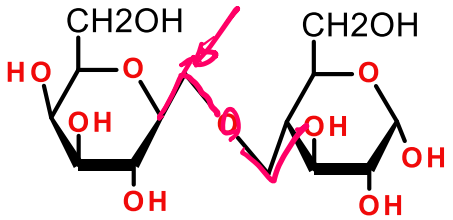
Nomenclature rules for linkage:

- 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

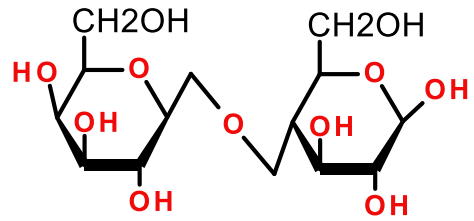
Lactose (milk sugar)





(alternate drawing)

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

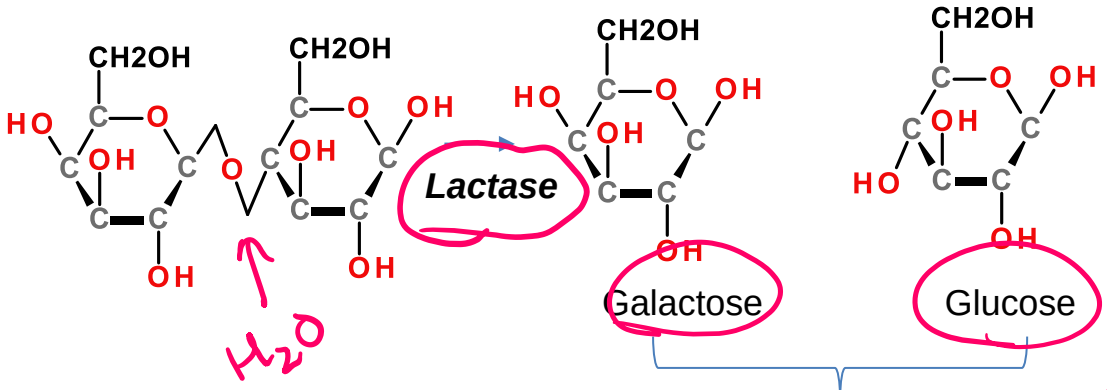


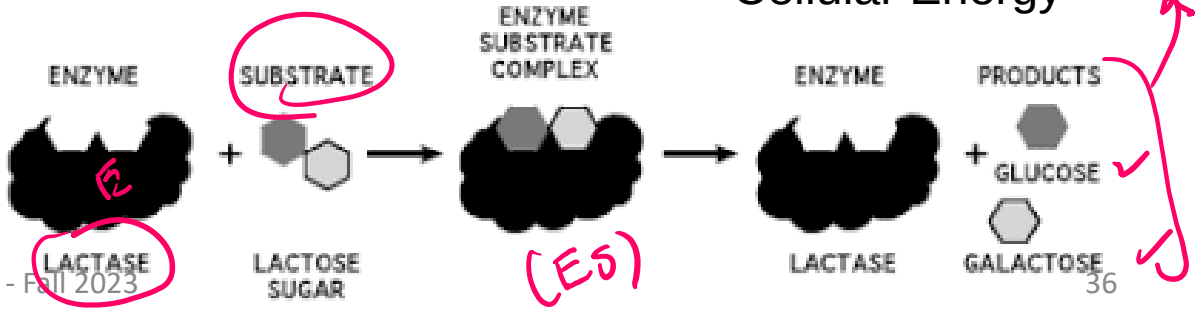
β-galactopyranosyl-(1→4)-β-glucopyranose

Lactose is the major sugar in mammalian milk.

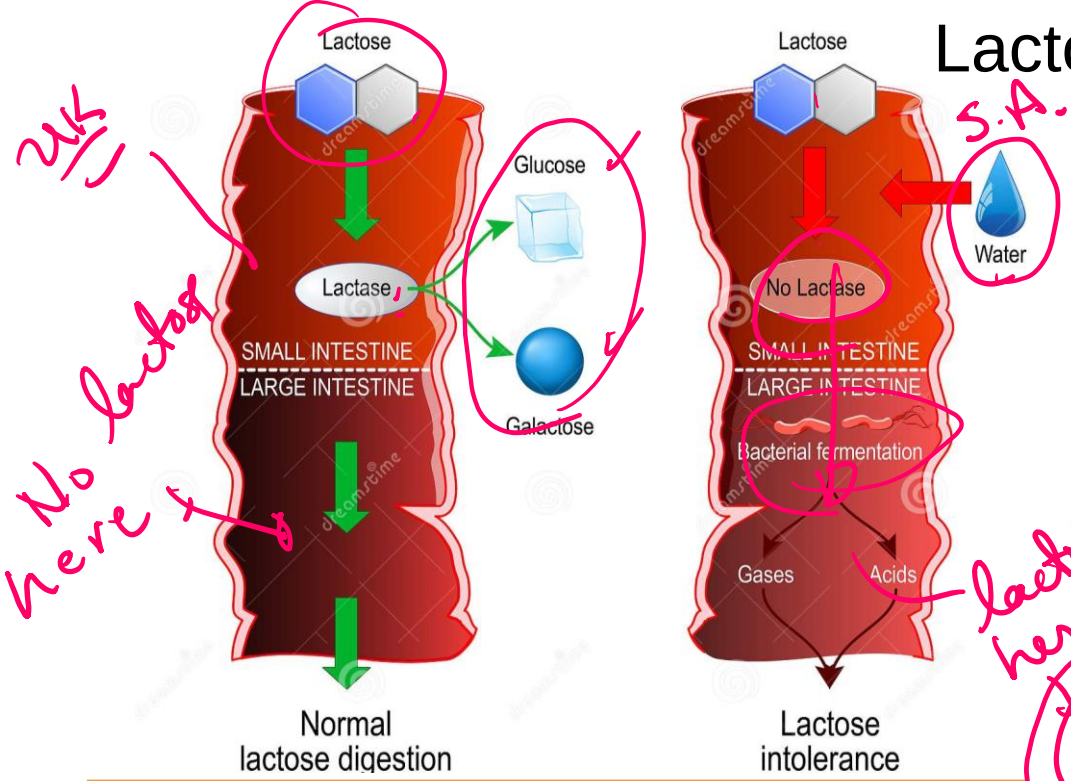
- Infants produce the enzyme ***lactase*** to hydrolyze the disaccharide to monosaccharides.
- Some adults have low levels of lactase (genetically programmed). This leads to *lactose intolerance*. The ingested lactose is not absorbed in the small intestine, but instead is fermented by bacteria in the large intestine, producing uncomfortable volumes of CO_2 gas.
- It is possible to purchase dairy products that have been treated with lactase, reducing the effects of lactose intolerance.

Metabolism of Lactose





Lactose Intolerance

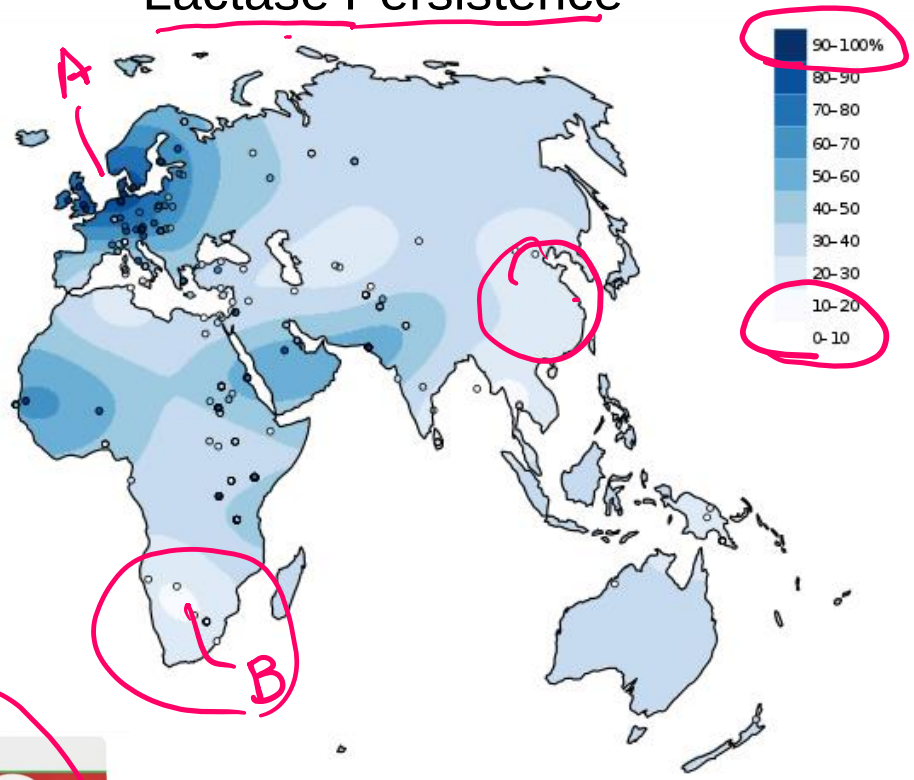


Which region can individuals consume lactose (High lactase persistence)?

A) UK

B) South Africa

Lactase Persistence



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)

Whole/low-fat Milk
200ml
9-10g lactose

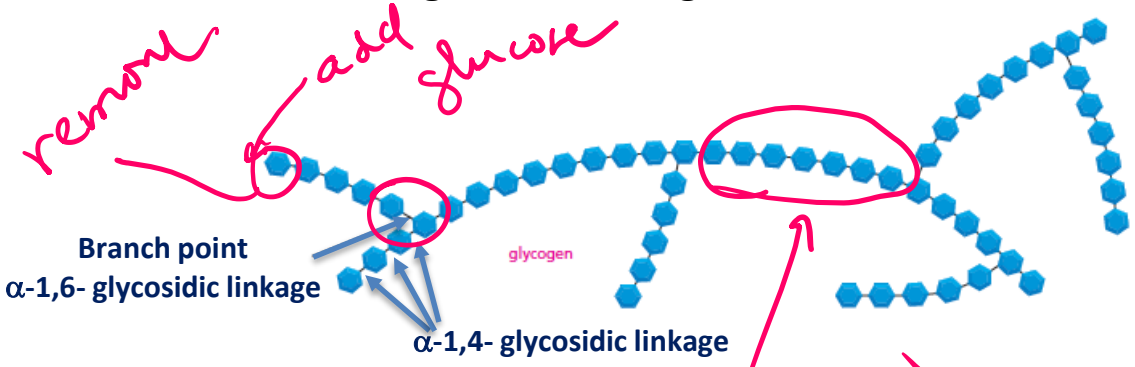
Cheddar Cheese
25g
0.03g lactose

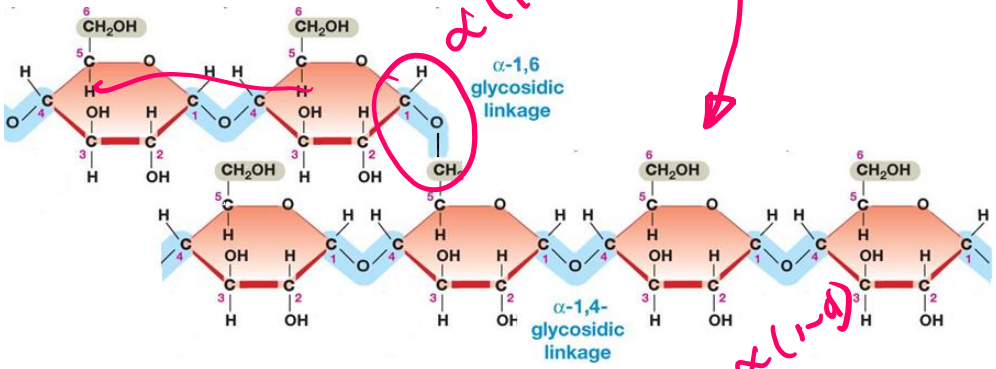
Whole Milk Yogurt
125g
6g lactose

Lactose-free Milk
200ml
0g lactose

Polysaccharides as Energy Storage – Glycogen Storage Disease

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



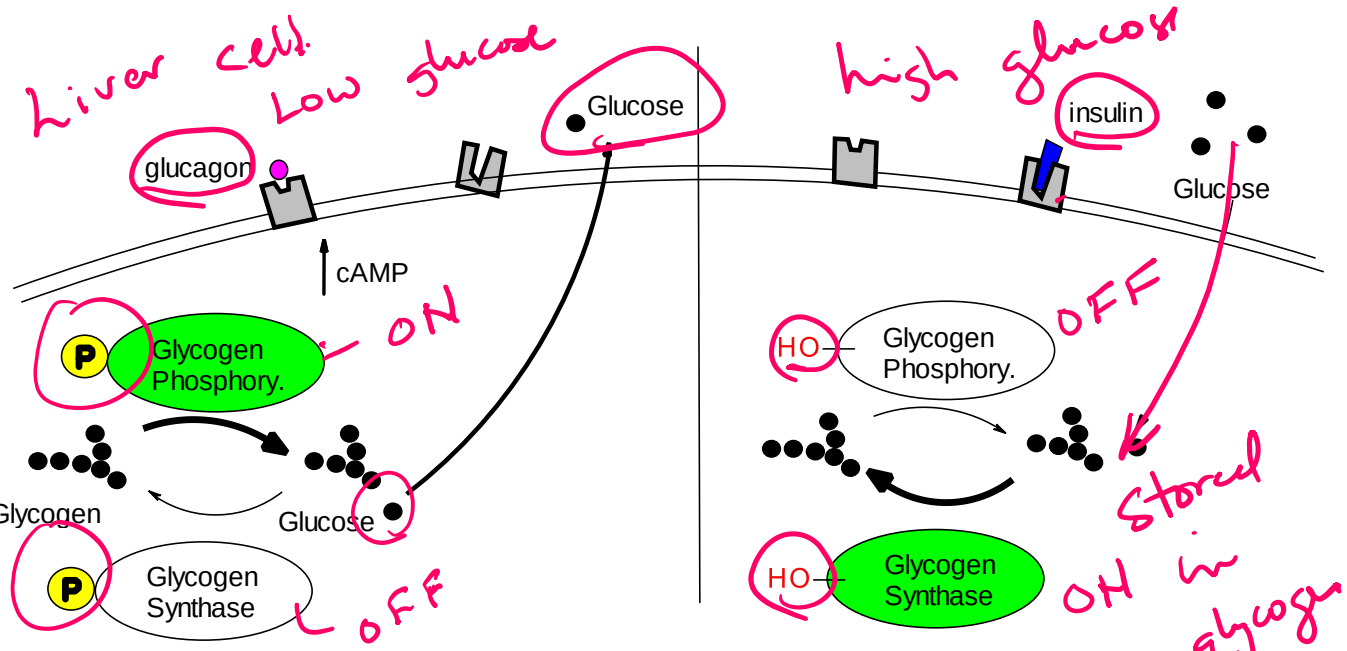


Glycogen Levels are regulated by hormones secreted due to blood glucose levels.

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

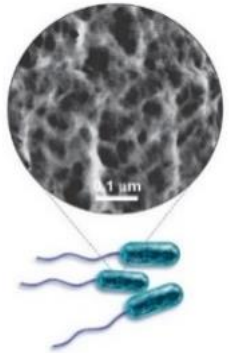
Two enzymes degrade or synthesize glycogen

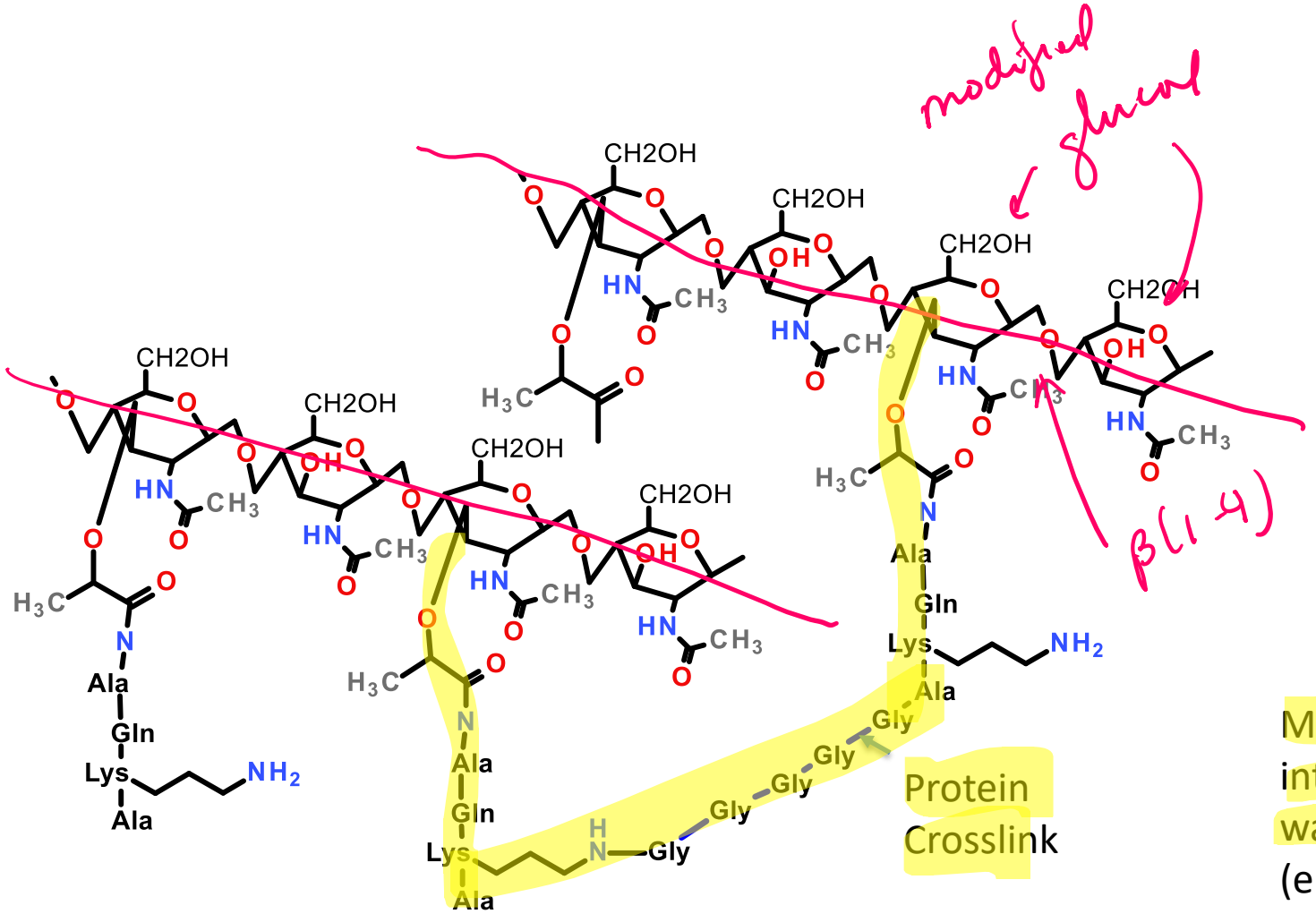
- Glycogen phosphorylase – releases glucose from glycogen
- Glycogen synthase – stores glucose in glycogen



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)

prevents protein cross linking

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)
- 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.

- lactose intolerance
- glycogen as glucose storage