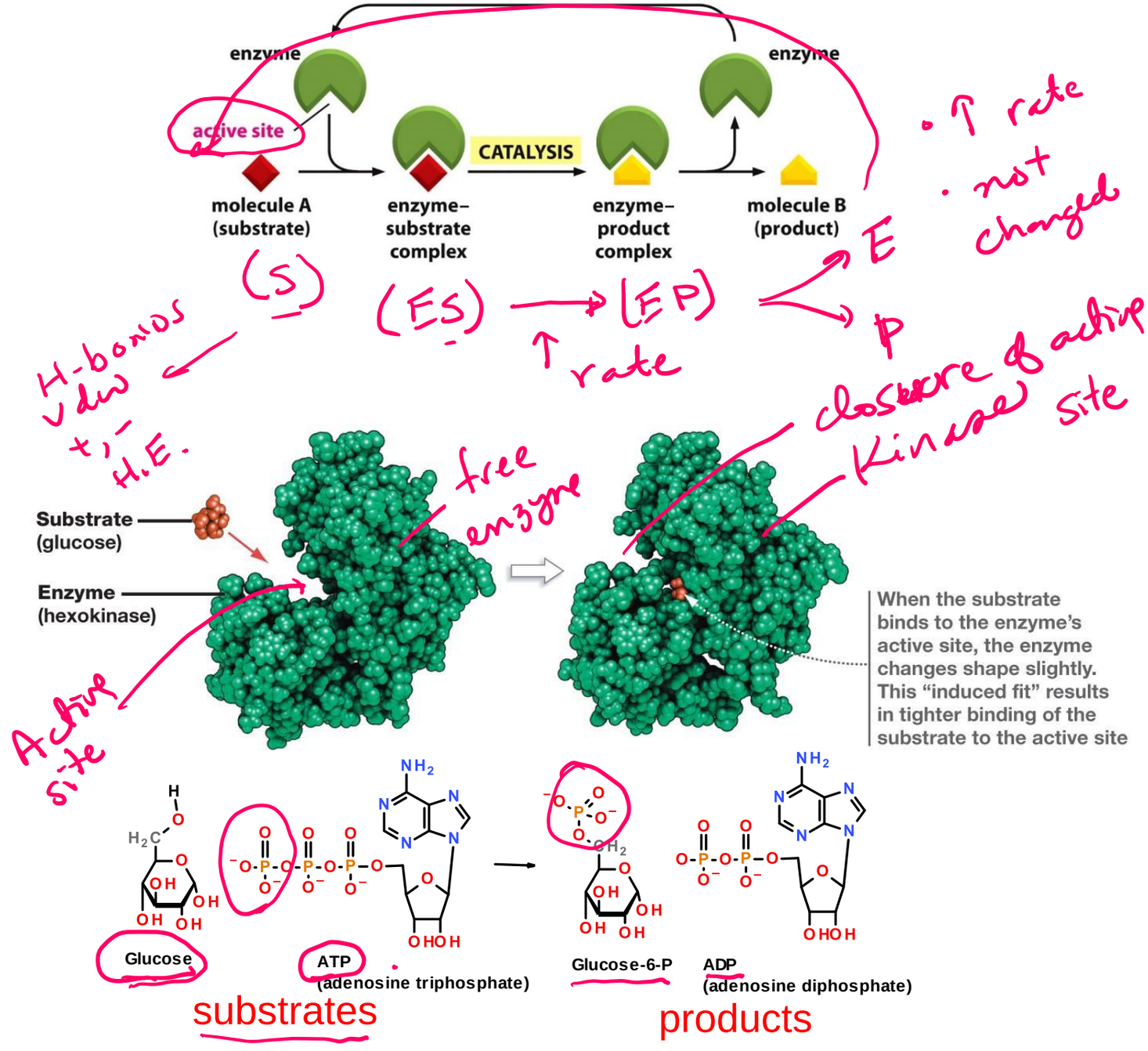
Lecture 3
Protein Function, Carbohydrates, Lipids, DNA Technologies

- Proteins as enzymes (PKU disease)
- Carbohydrates (Lactose intolerance)
- Lipids & Cholesterol regulation
- Review of DNA and DNA polymerases
- DNA Sequencing
- Polymerase chain reaction (PCR) & Applications

Enzymes

- **Enzymes** are protein or RNA catalysts. They increase the rate of the reaction.
- They bind "substrates" and convert them to "products". 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

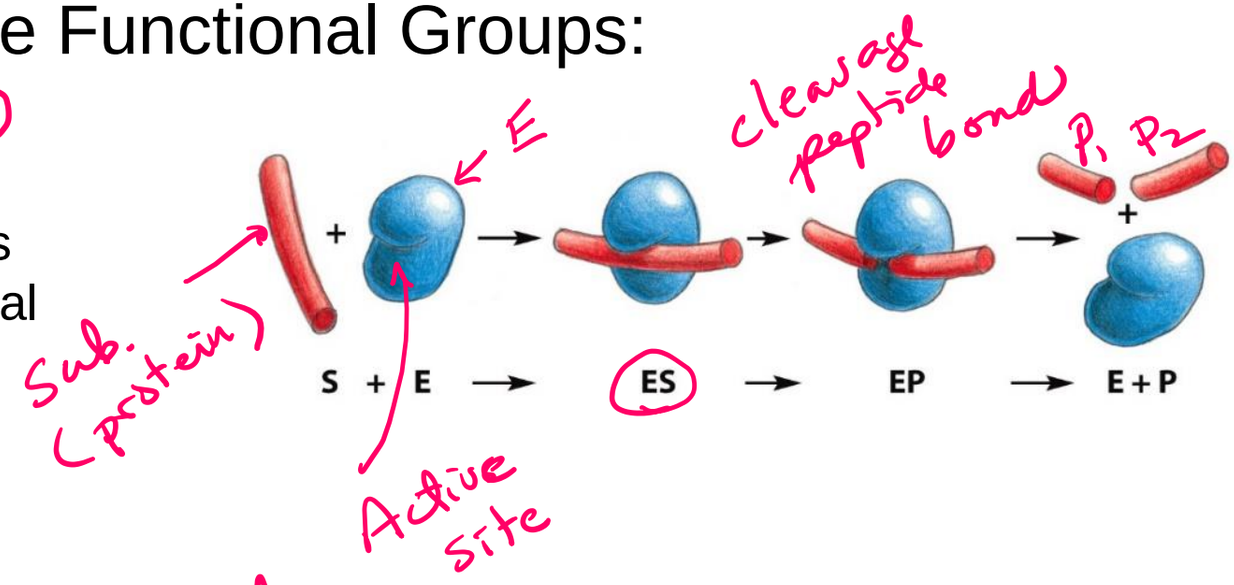
TABLE 4–1 SOME COMMON FUNCTIONAL CLASSES OF ENZYMES

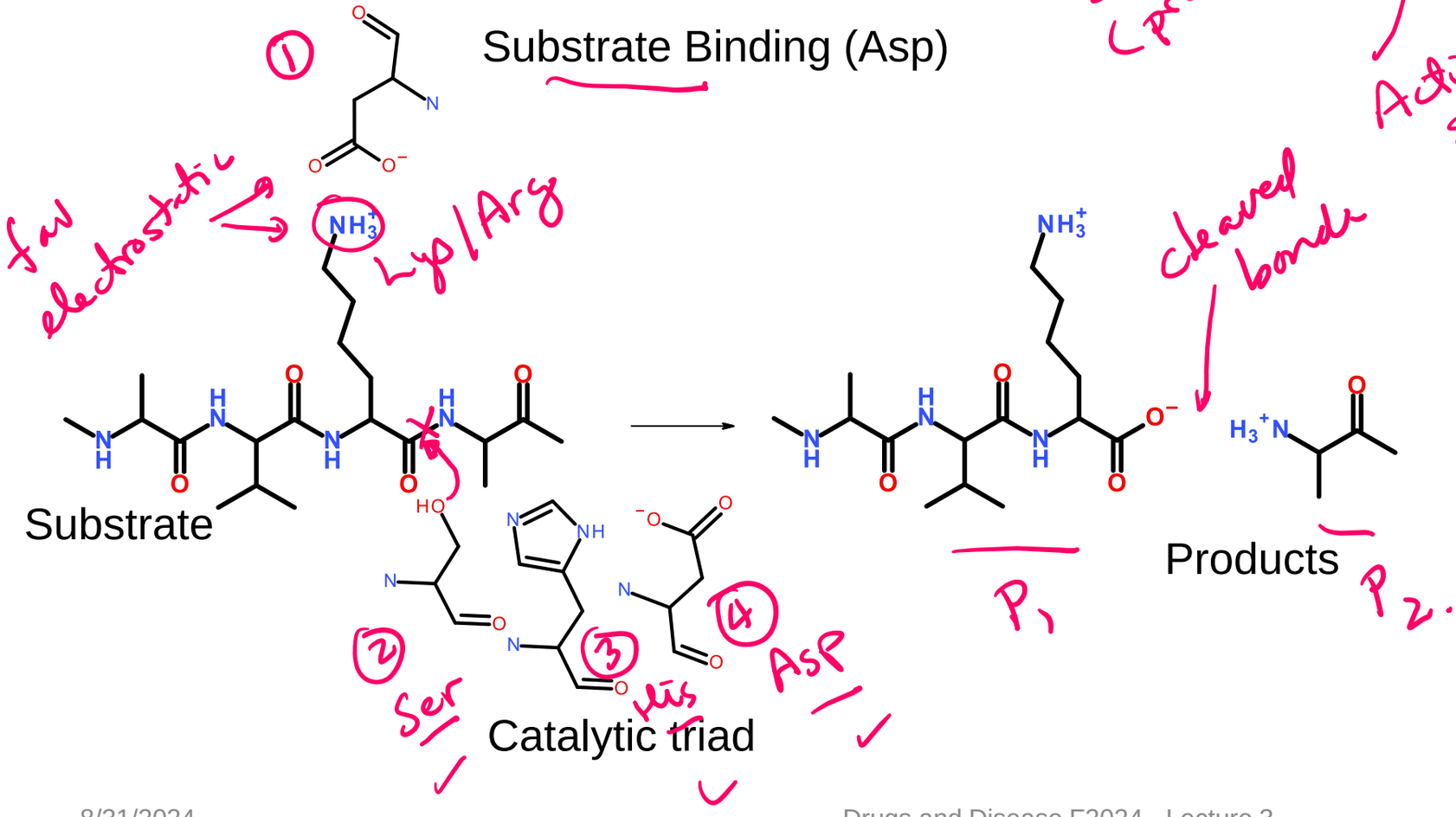
ENZYME CLASS	BIOCHEMICAL FUNCTION
Hydrolase	General term for enzymes that catalyze a hydrolytic cleavage reaction.
Nuclease	Breaks down nucleic acids by hydrolyzing bonds between nucleotides.
Protease	Breaks down proteins by hydrolyzing peptide bonds between amino acids.
Synthase	General name used for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

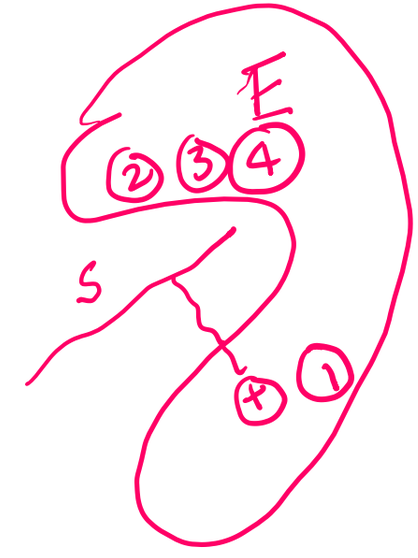
- Most enzyme names end in “-ase”
- Usually named by their substrates and the reactions they catalyse, i.e. glucose kinase

Example of Active Site Functional Groups:

- Catalytic triad (Asp, His, Ser) in Protease Trypsin cleaves the peptide bond.
- More active with Lys and Arg containing substrates because of a favorable interaction with an additional Asp residues in the enzyme.





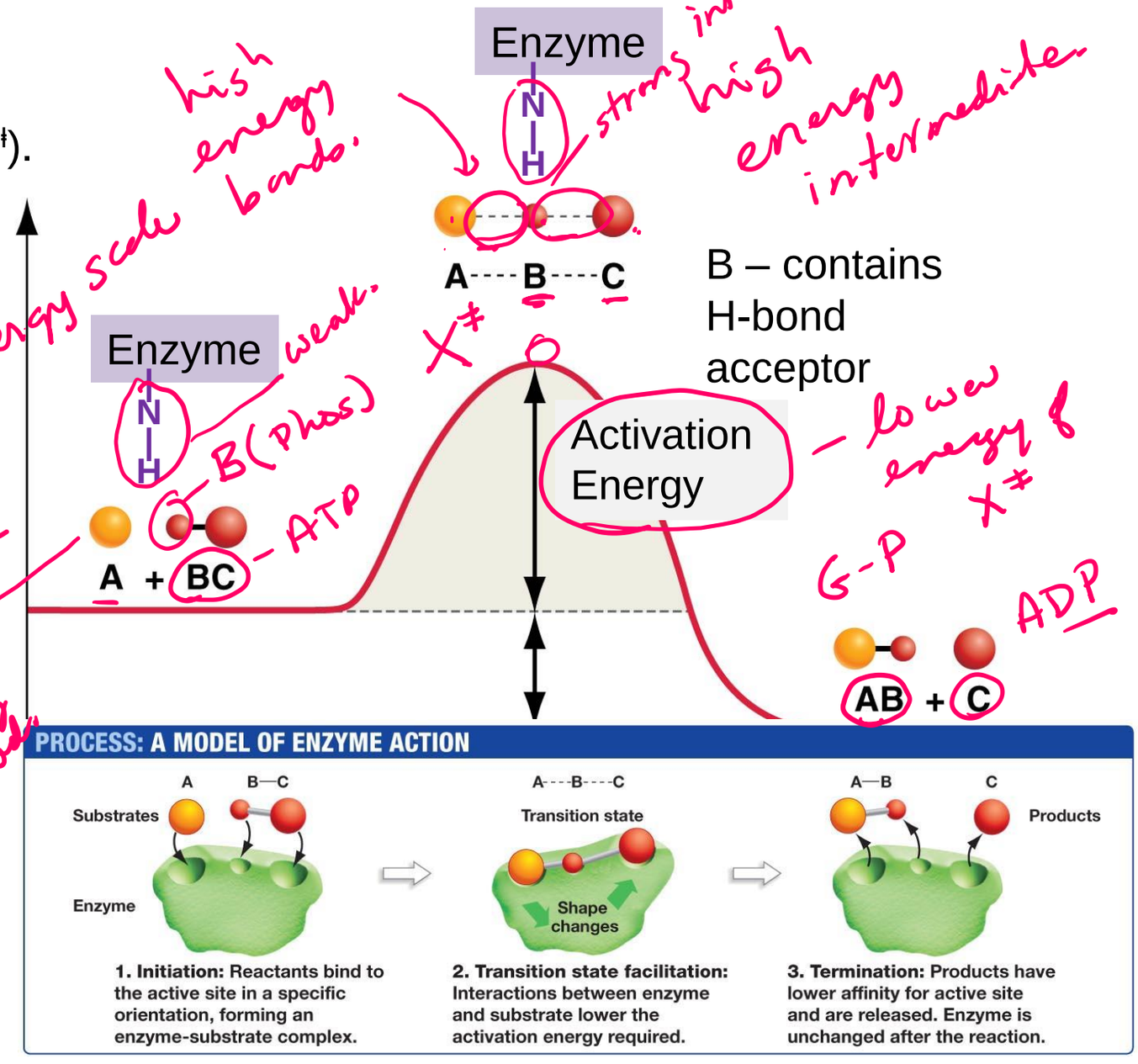


How Do Enzymes Increase Rates?

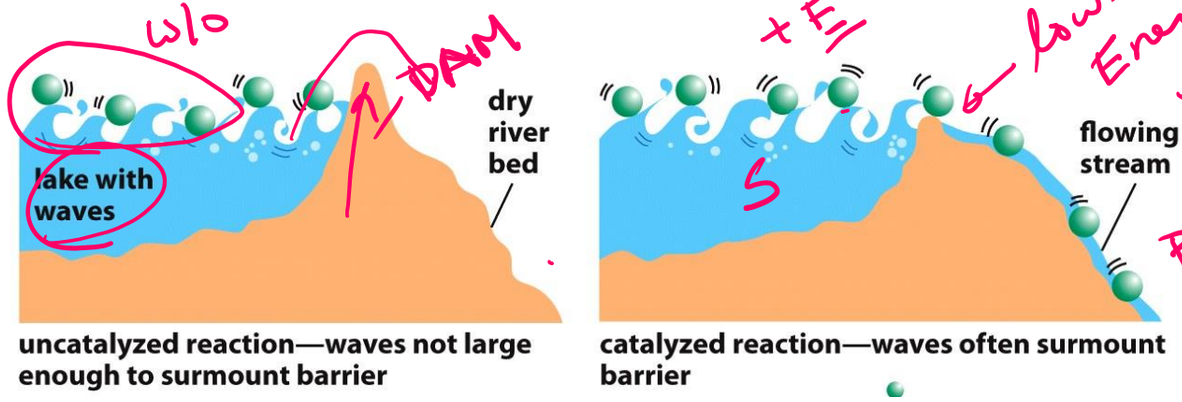
- **Transition state** = high energy intermediate that occurs during the reaction.
- Energy barrier is called the activation energy ($\Delta G^{\ddagger}$).

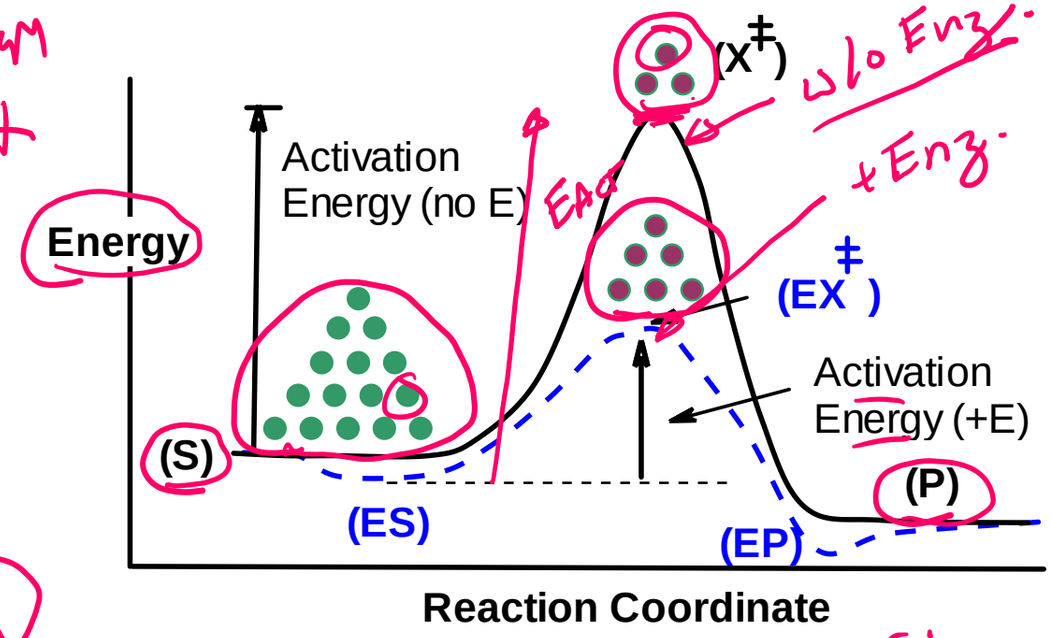
- Interactions between the enzyme and the substrate stabilize the **transition state** (X) and lower the activation energy required for the reaction to proceed.

- Stabilization can include:
  1. – Pre- alignment of key groups in the active site, reducing entropy cost of organizing groups.
  2. – 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.

Rate of product formation depends on the concentration of the transition state

- Low [X] = Slow reaction

  Higher [EX] = Faster reaction

[S] = 15

[X] = 3

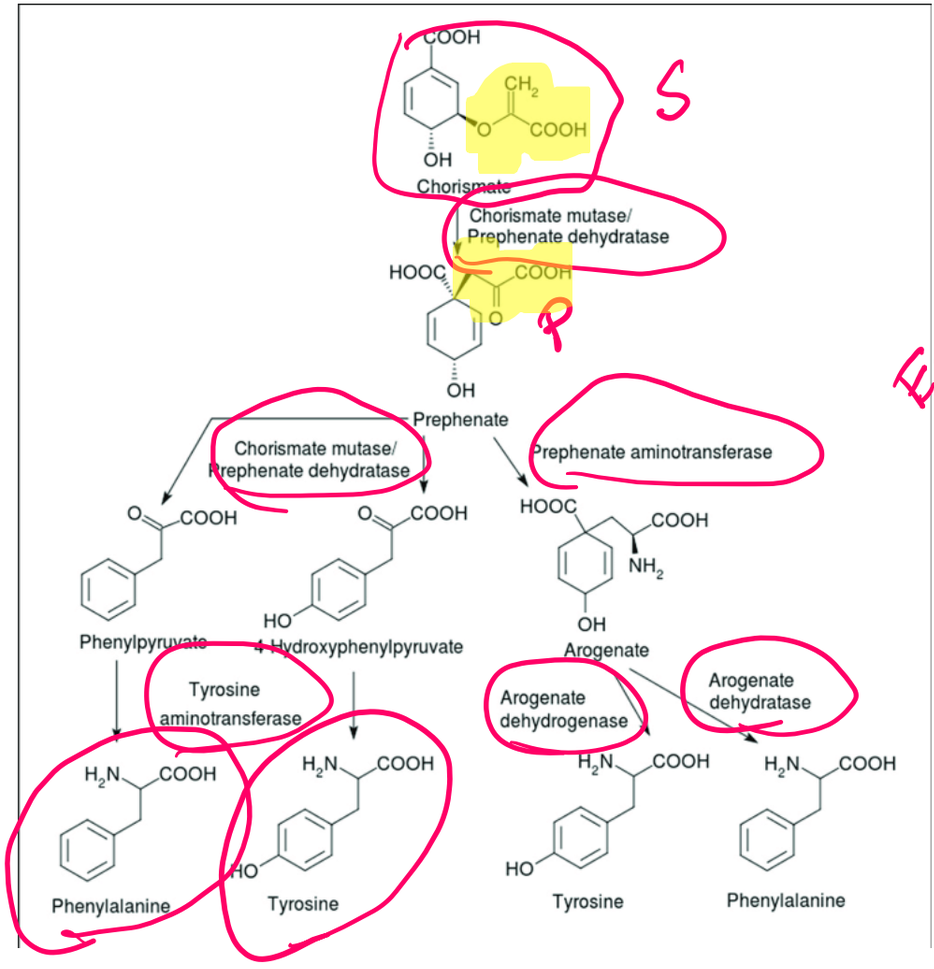
[EX] = 6

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

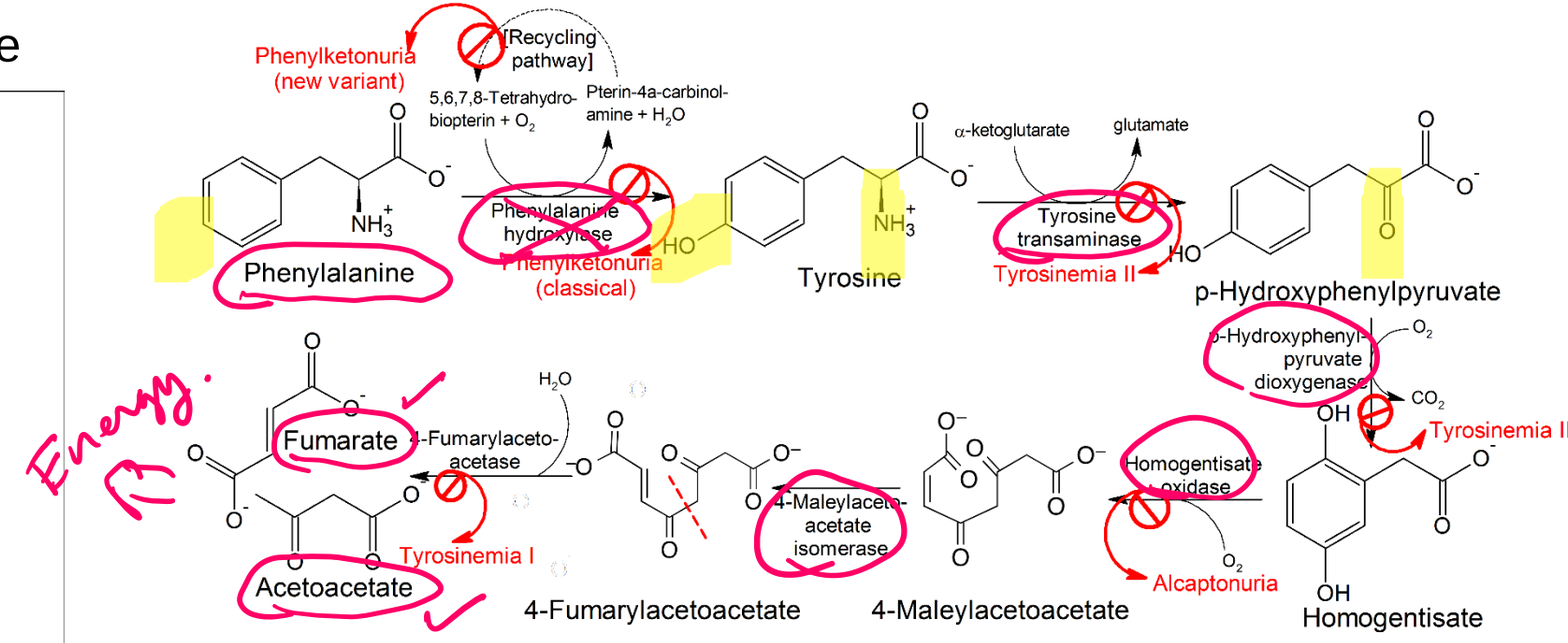
Enzymes, Metabolic Pathways, and Diseases

Synthetic Pathway for Phe, Tyr (beginning with chorismite)

- Each step catalyzed by an enzyme



Pathway for Degradation of Phenylalanine



Energy.

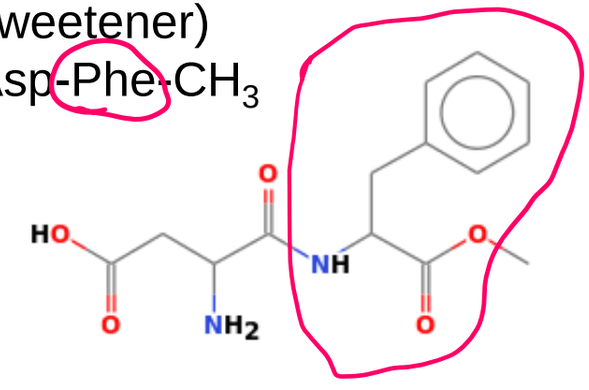
PKU Disease:

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

Treatment - low phe diet:

Aspartame (artificial sweetener)

Asp-Phe-CH_3

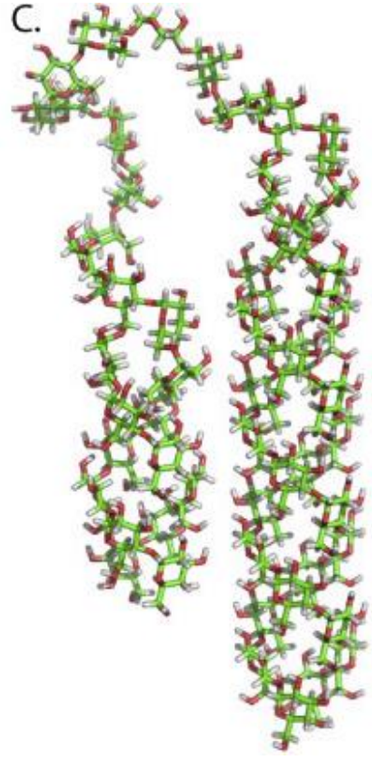


Key Points:

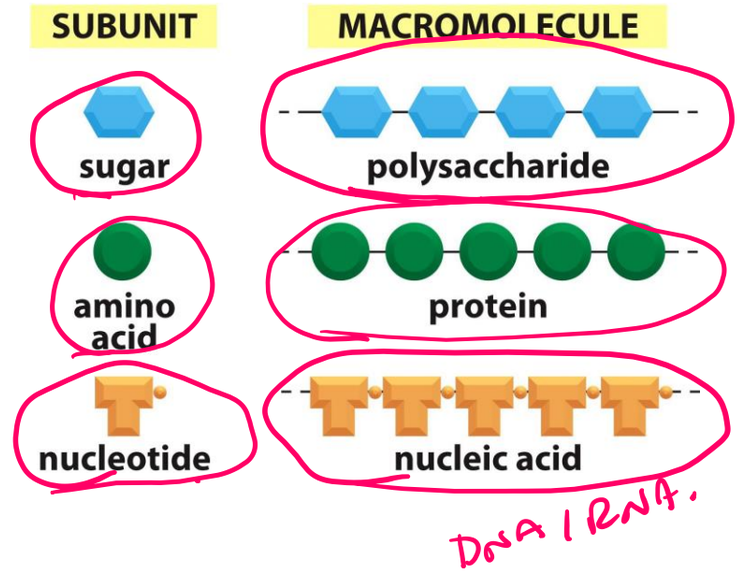
Enzymes:

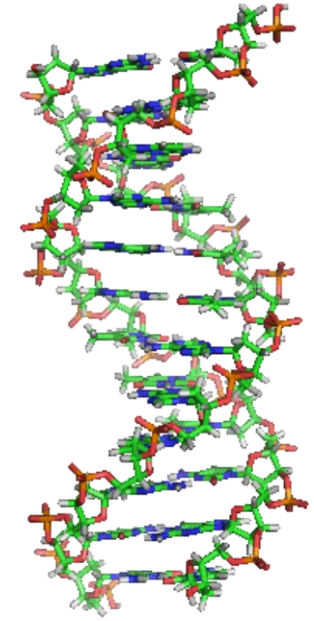
- Enzymes bind substrates (S), forming (ES) complex in active site, converting to P, releasing P.
- Rate enhancement since the transition state complex (EX) forms more readily with enzymes due to:
  - Bringing substrates and functional groups on the enzyme together by binding (less entropy change)
  - 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



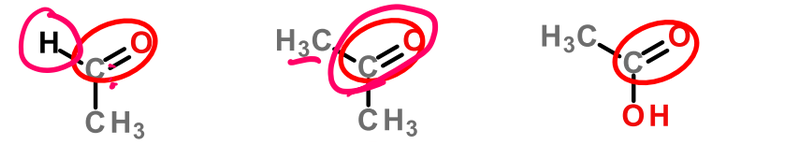


DNA (Nucleic Acid)

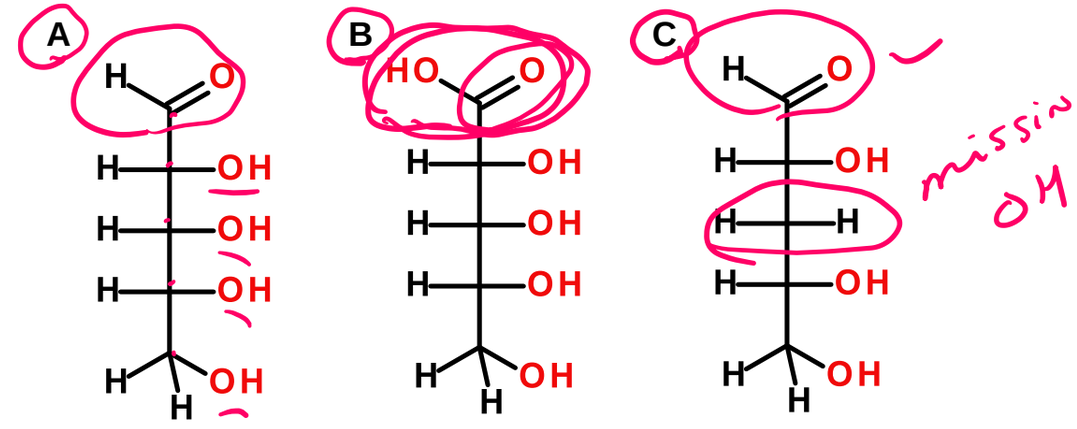
Carbohydrates

- 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:



Carbonyl group –> C=O



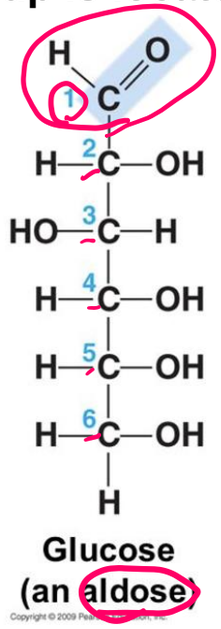
Only one of these is a carbohydrate, which one?

A *B* *C*

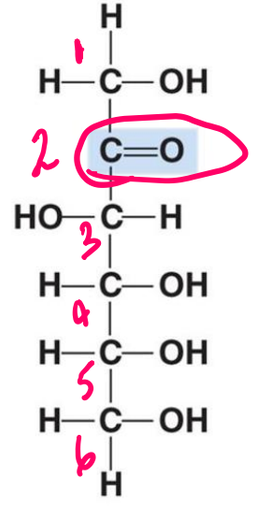
3 ways simple sugars (monosaccharides) differ from each other

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)

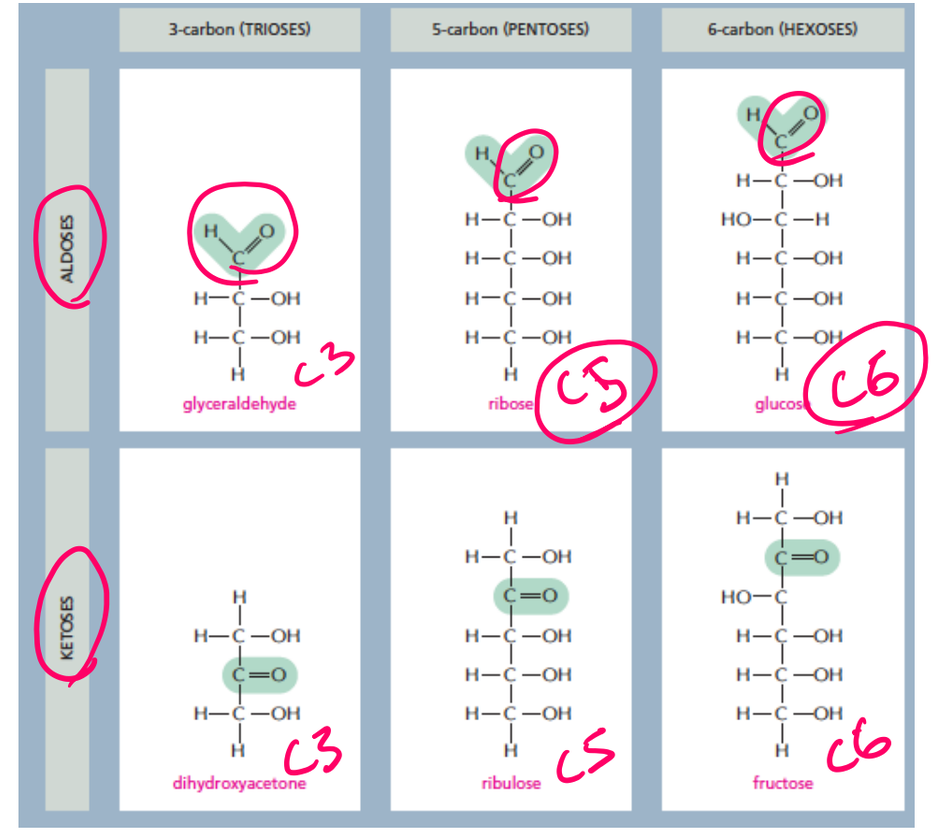


Fructose (a ketose)

What carbon is the carbonyl?

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

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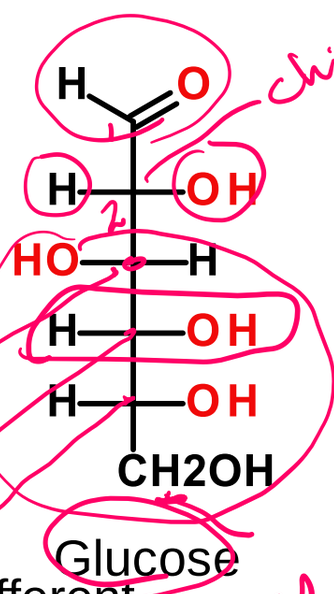
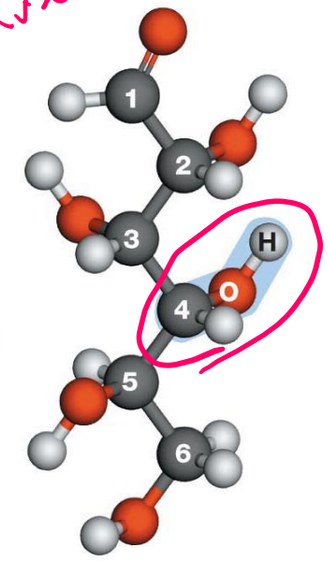
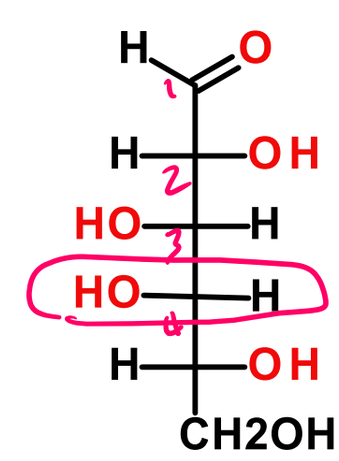
3. **Spatial arrangement of atoms (the position of the OH groups)**

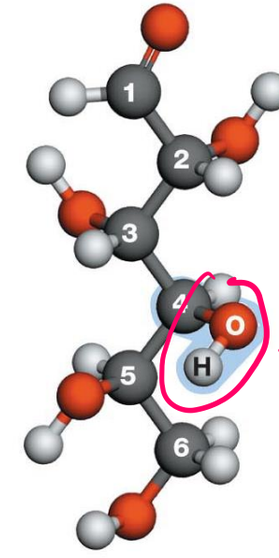


Glucose





Galactose

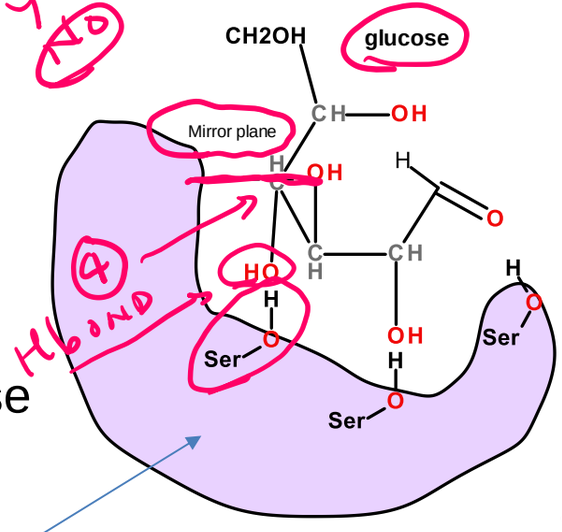


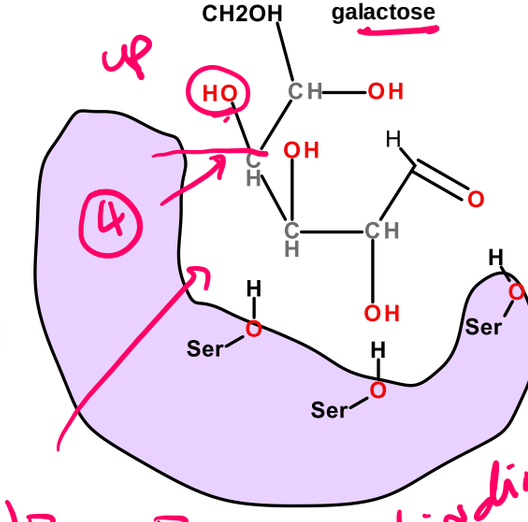
Both have the same chemical formula $C_6H_{12}O_6$. Both are aldose sugars with 6 carbons.
Yet their functions are different.

- 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.

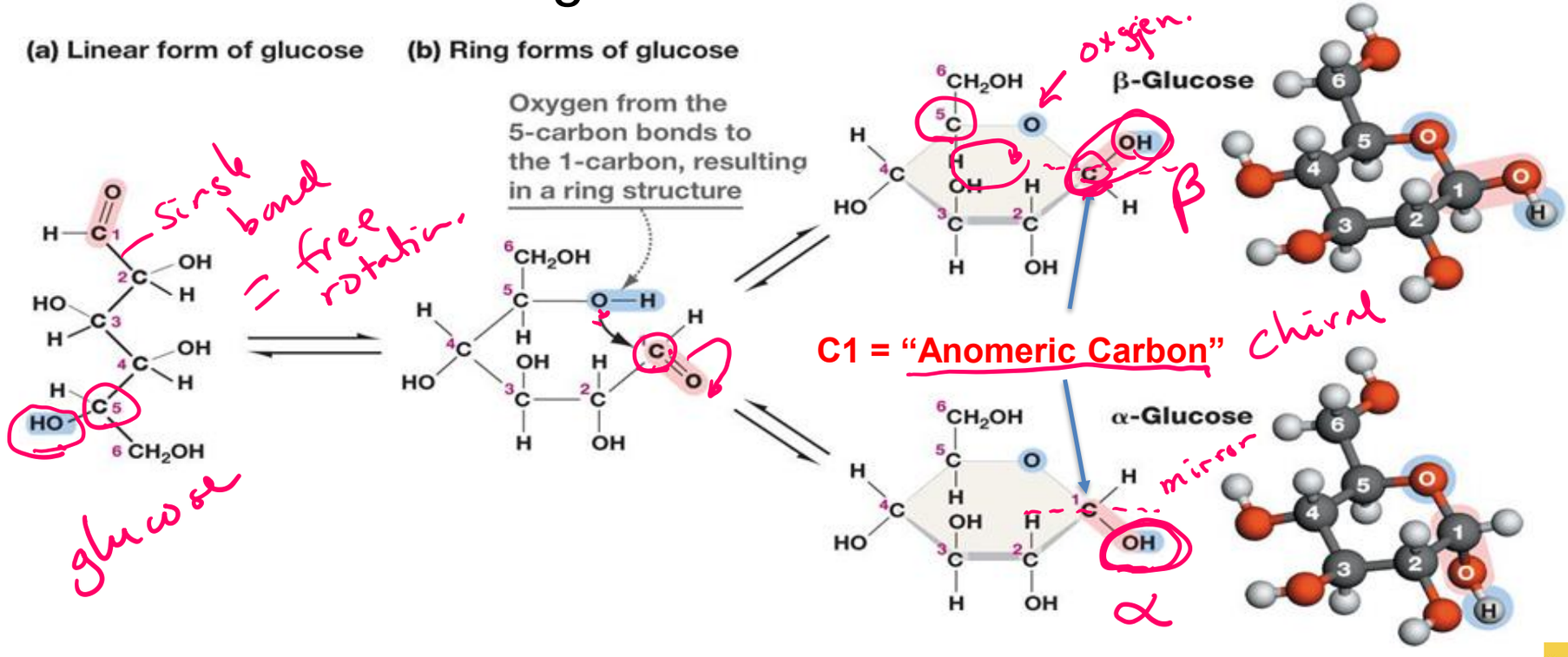
- OH is down in glucose
- OH is up in galactose





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)"*

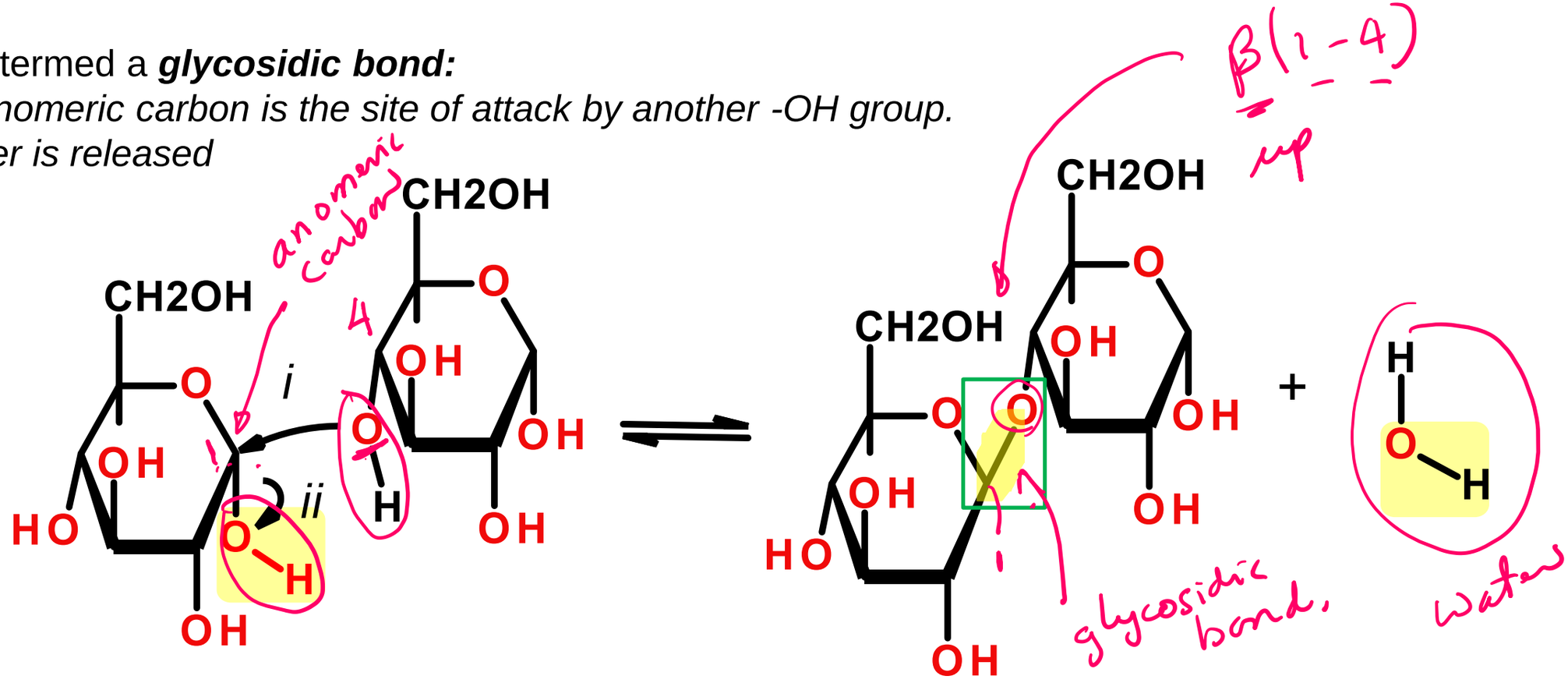
Disaccharides

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

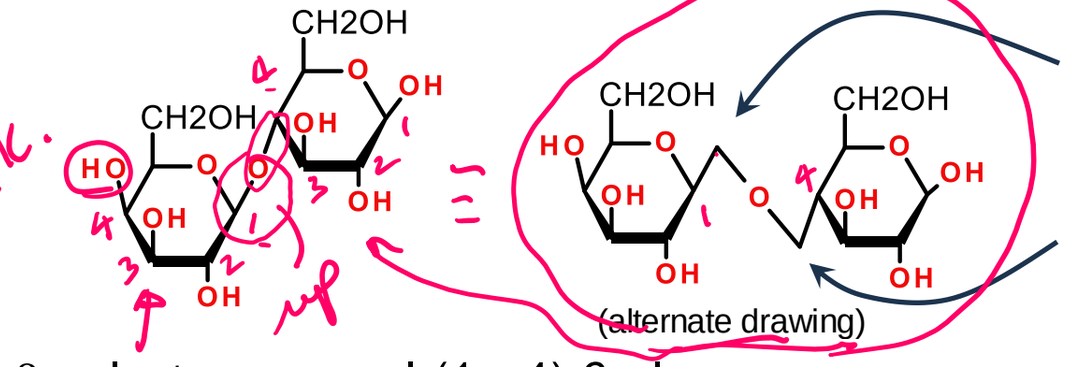


Nomenclature rules for linkage:

- 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)



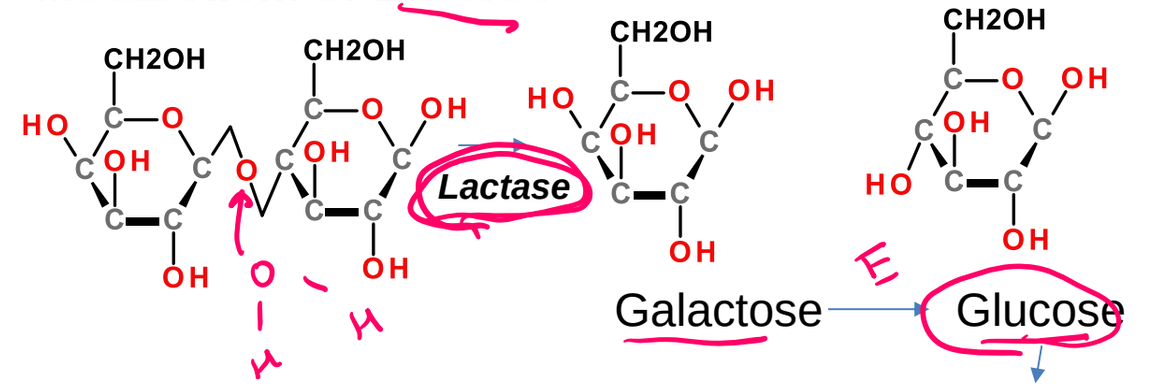
β-galactopyranosyl-(1→4)-β-glucopyranose

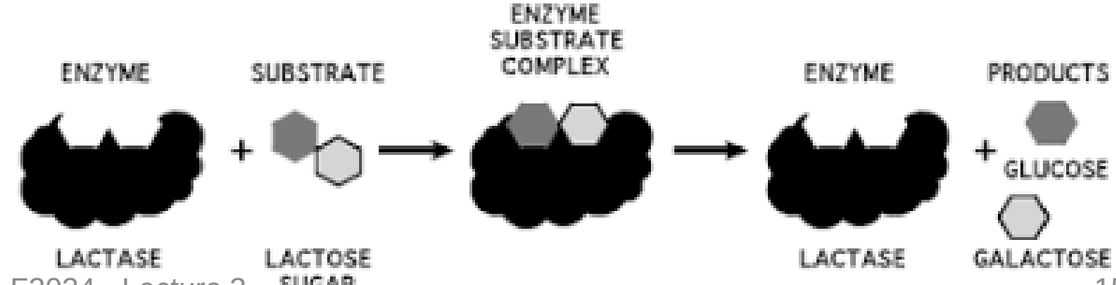
These kinks are not carbons but are drawn in this way to indicate that the chirality of the anomeric is beta (pointing up). The kinks allow the line to reach the downward pointing –OH on C4 in glucose.

Lactose is the major sugar in mammalian milk.

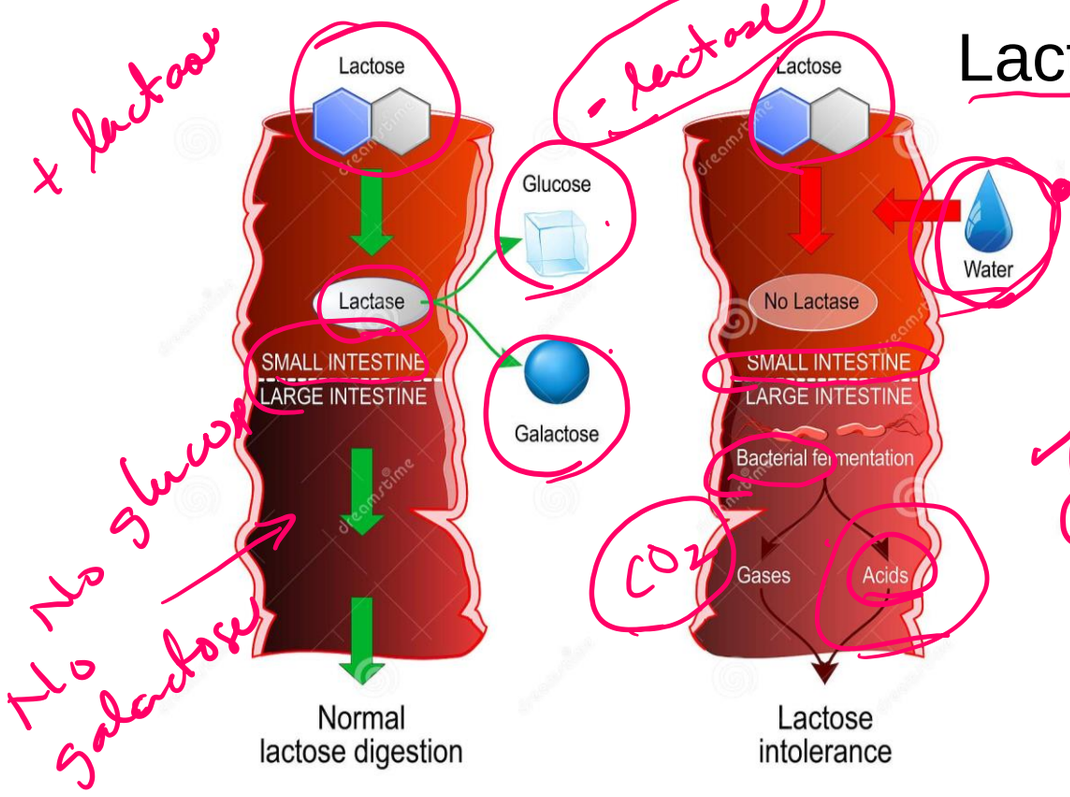
- Infants produce the enzyme ***lactase*** to hydrolyze the disaccharide to monosaccharides.
- Lactase expression is turned off in some adults, depending on their genetic background.

Metabolism of Lactose



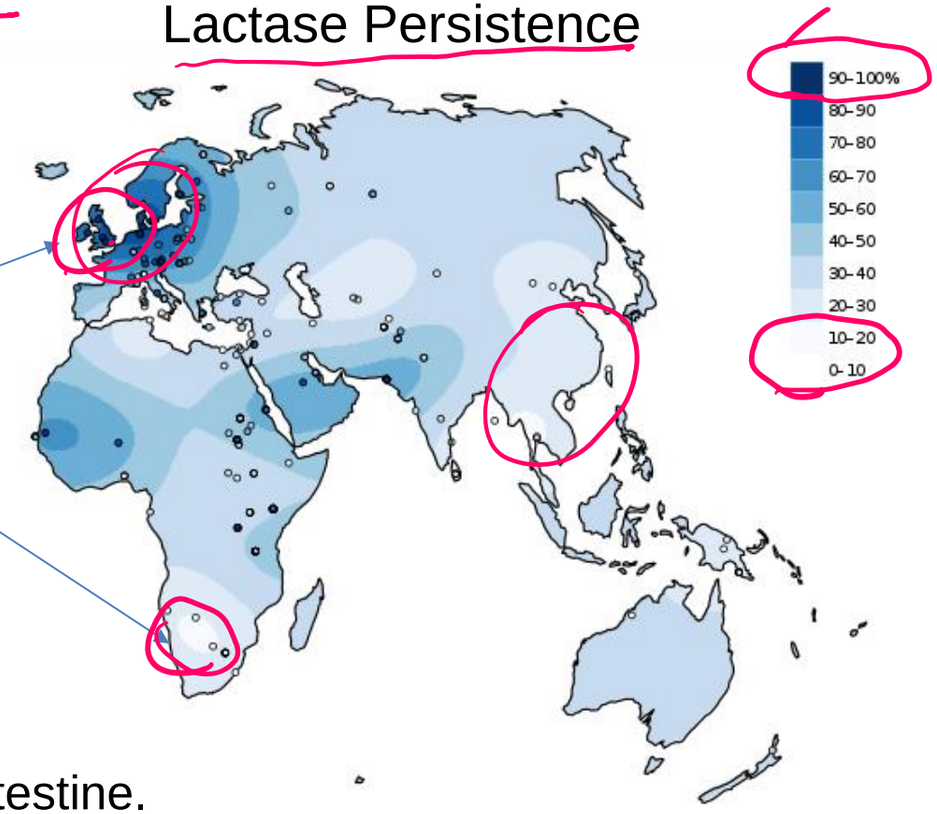


Lactose Intolerance



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

A) UK
B) South Africa



In an infant (lactase +):

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

In a lactose intolerant individual (lactase -)

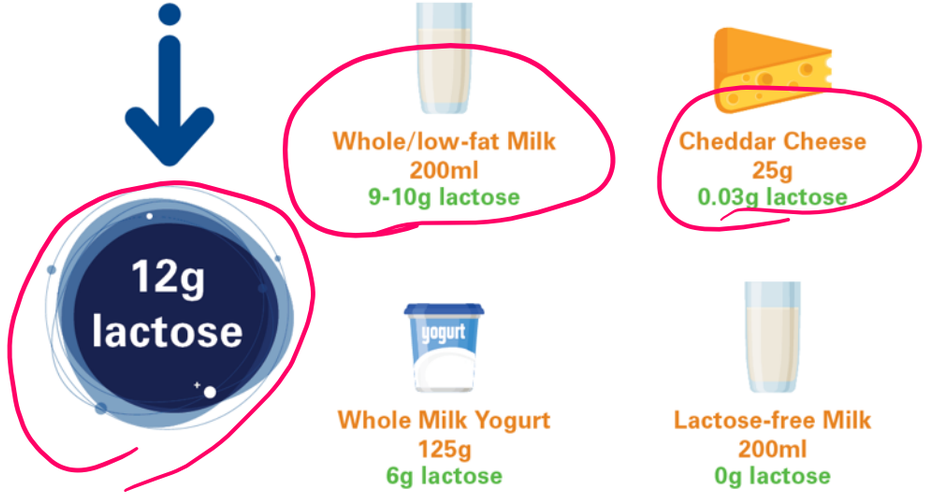
- The lactose is not absorbed in the small intestine, but instead draws water into the intestine due to osmosis – leading to bloating and potentially diarrhea.
- Lactose enters the large intestine where gut bacteria use it as a carbon source, generating gas.

Lactose Intolerance

What to do if you are lactose intolerant:

A. Consume less lactose

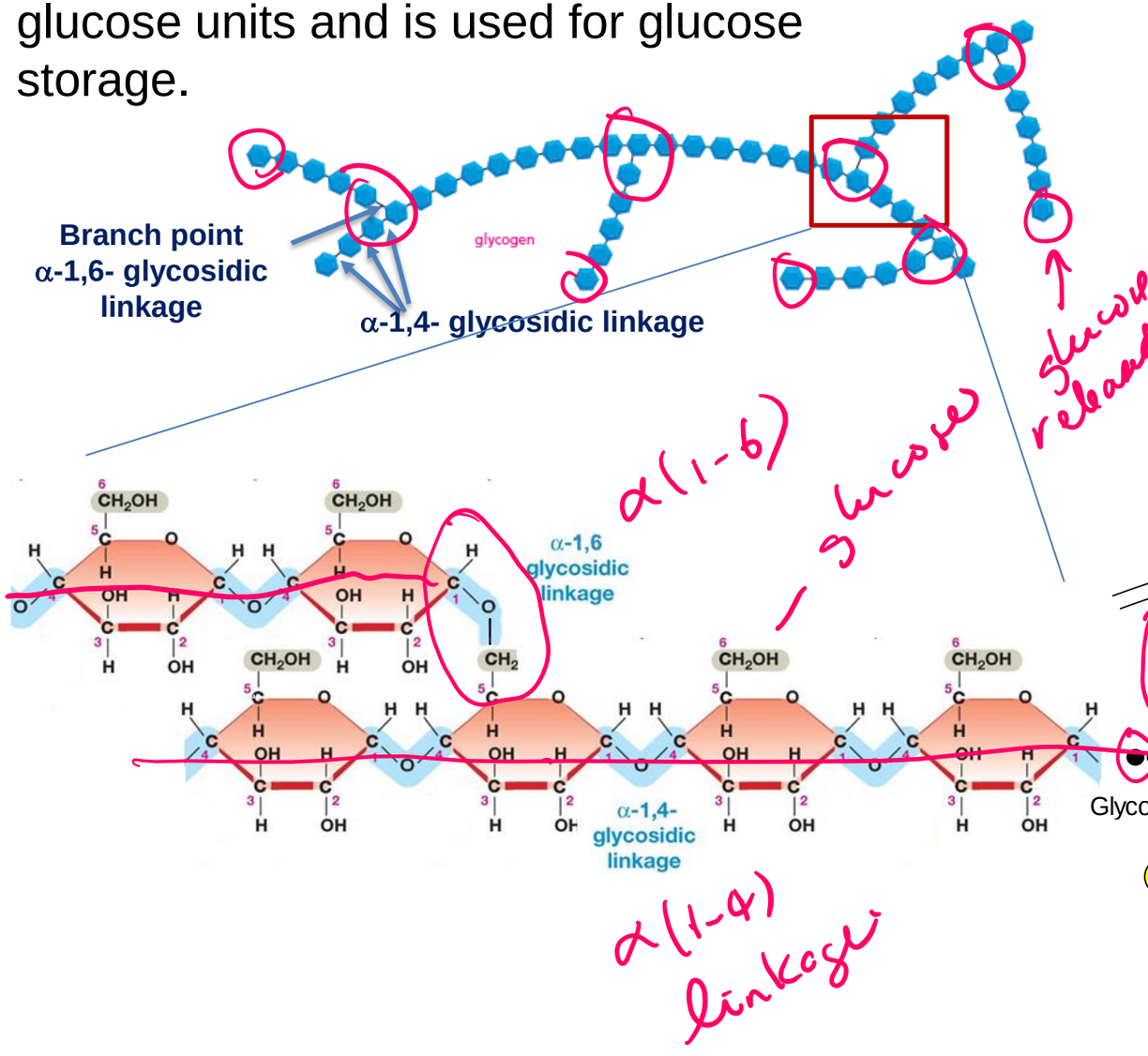
Most individuals with lactose maldigestion can tolerate up to 12g of lactose as a single dose with no, or minor, symptoms
The European Food Safety Authority (EFSA)



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.

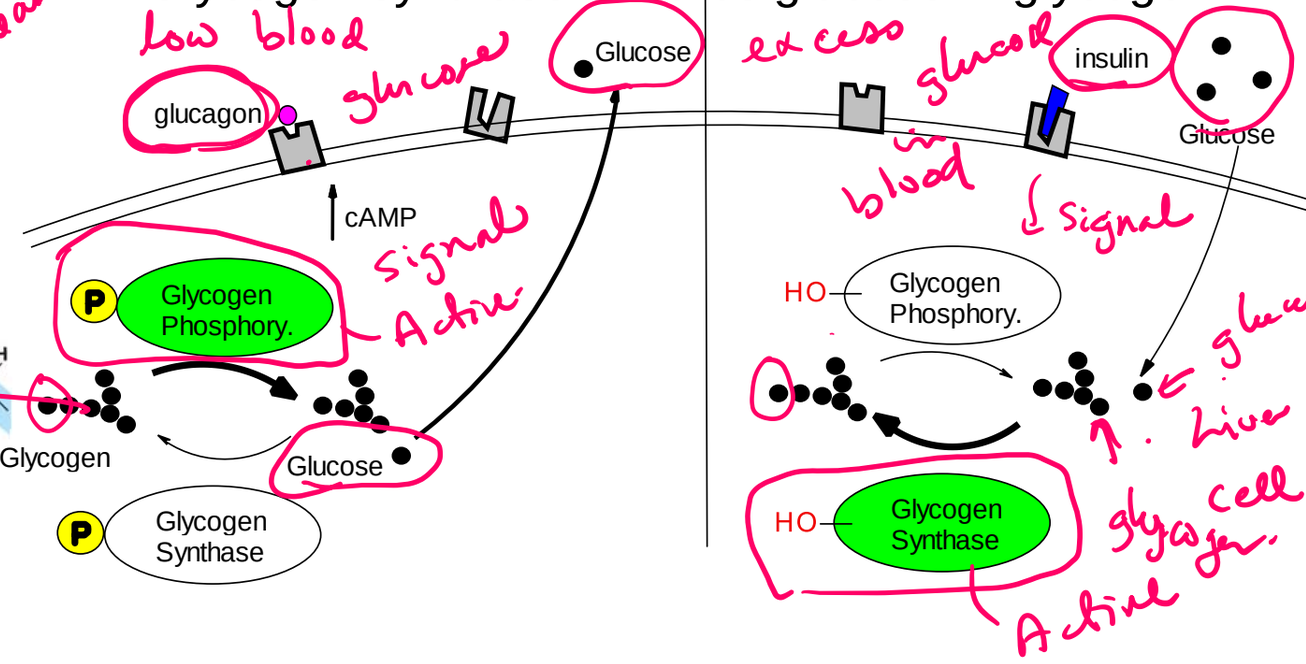


Glycogen Levels are regulated by hormones secreted due to blood glucose levels.

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

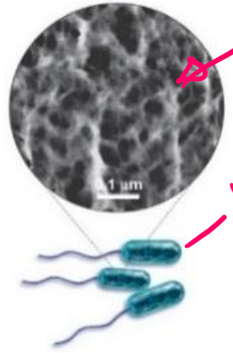
Two enzymes degrade or synthesize glycogen

- 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.

9:09

9:14.

Lipids

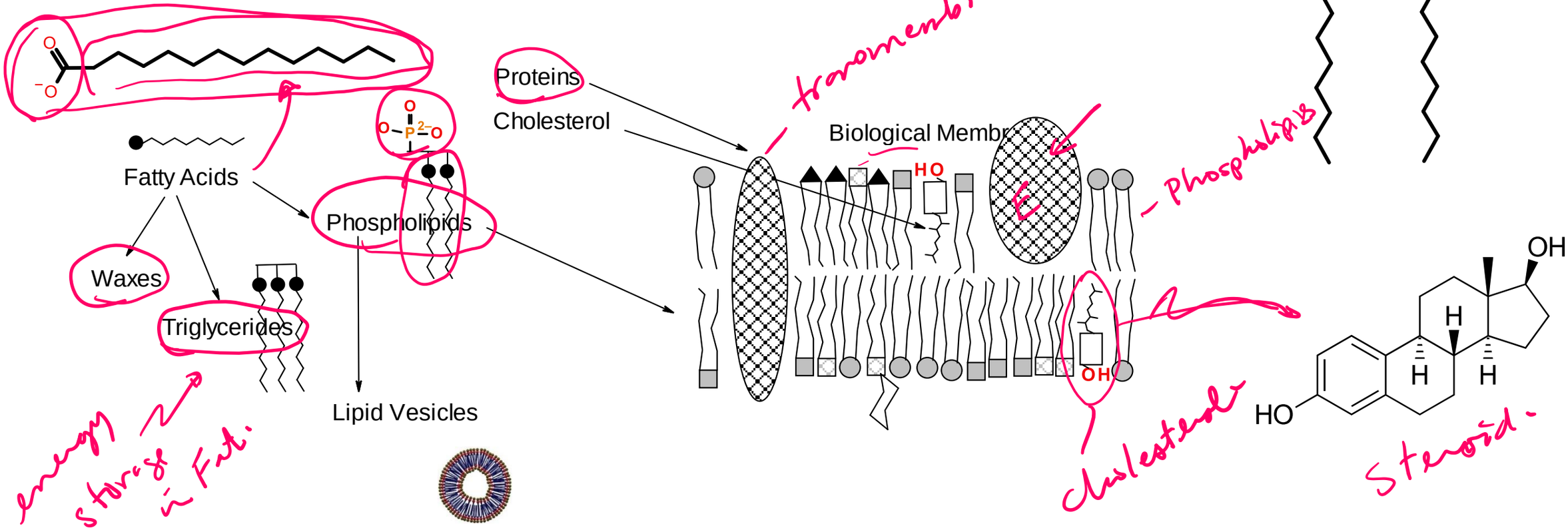
Lipids

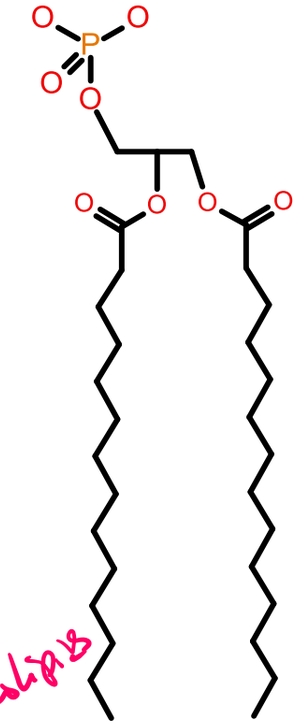
A chemically diverse group of molecules that are generally insoluble in water.

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

Expectations:

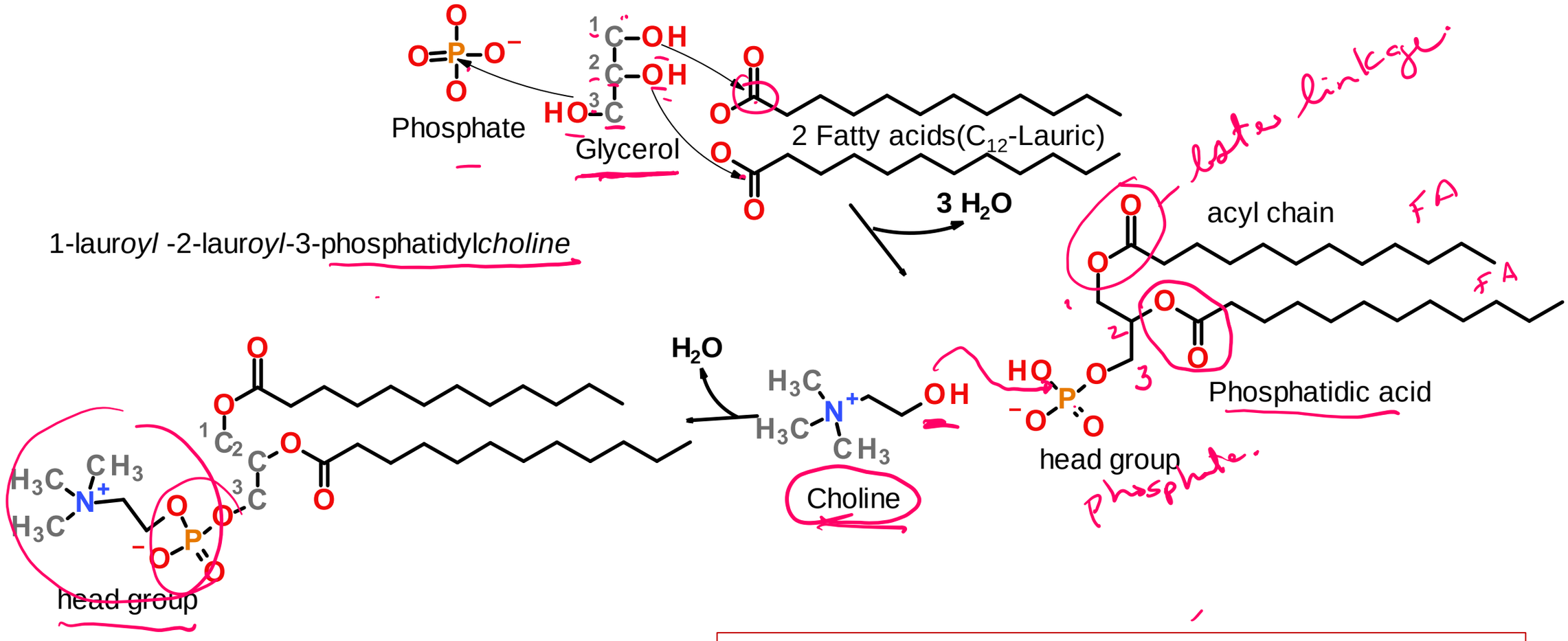
- Recognize chemical structure of steroids and phospholipids.
- Usage of liposomes in drug delivery
- Effect of cholesterol on fluidity of phospholipid membranes.





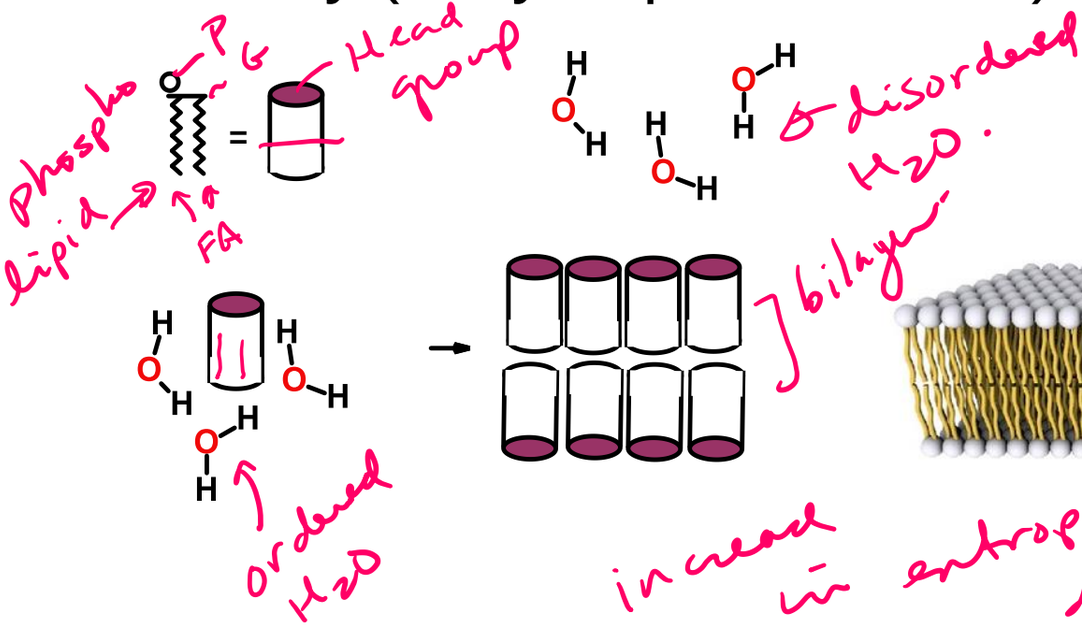
Phospholipids - Glycerophospholipids:

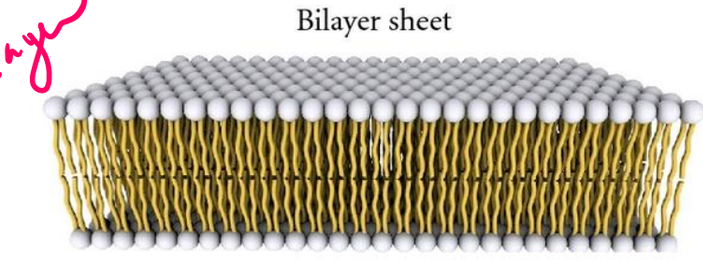
1. Head group + phosphate + glycerol + two fatty acids (**acyl chains**) of *various* types form a phospholipid.
2. Various **head groups** are attached to the phosphate, giving a diverse set of lipids.



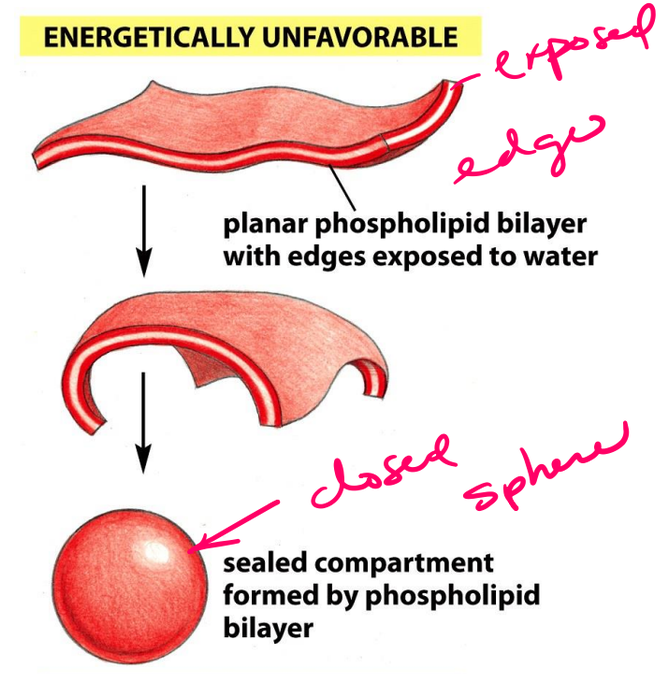
Expectations: Know the overall structure.

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



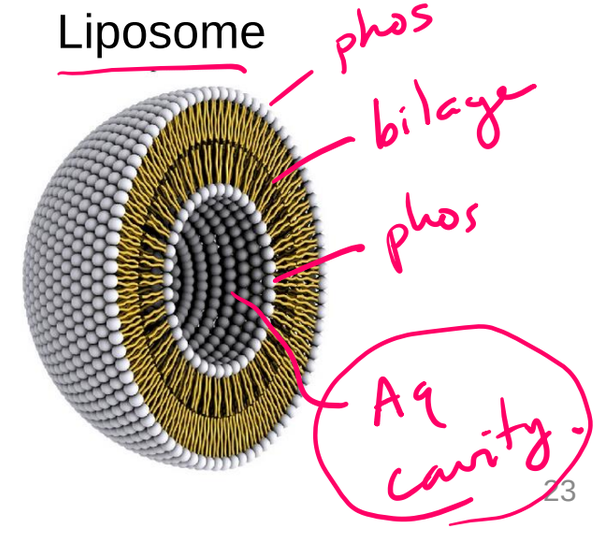


exposed N. polar group.

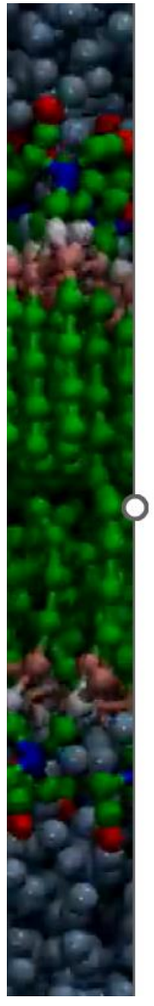


Physical Properties of Pure Lipid Bilayers:

- Phospholipids self-assemble in water to form **bilayers** (two opposing layers of phospholipids). This assembly is driven by the hydrophobic effect.
- Ordered water is released from the non-polar fatty acid tails when the phospholipids form the bilayer.
- 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.***



Spontaneous Assembly of the Phospholipid Bilayer:

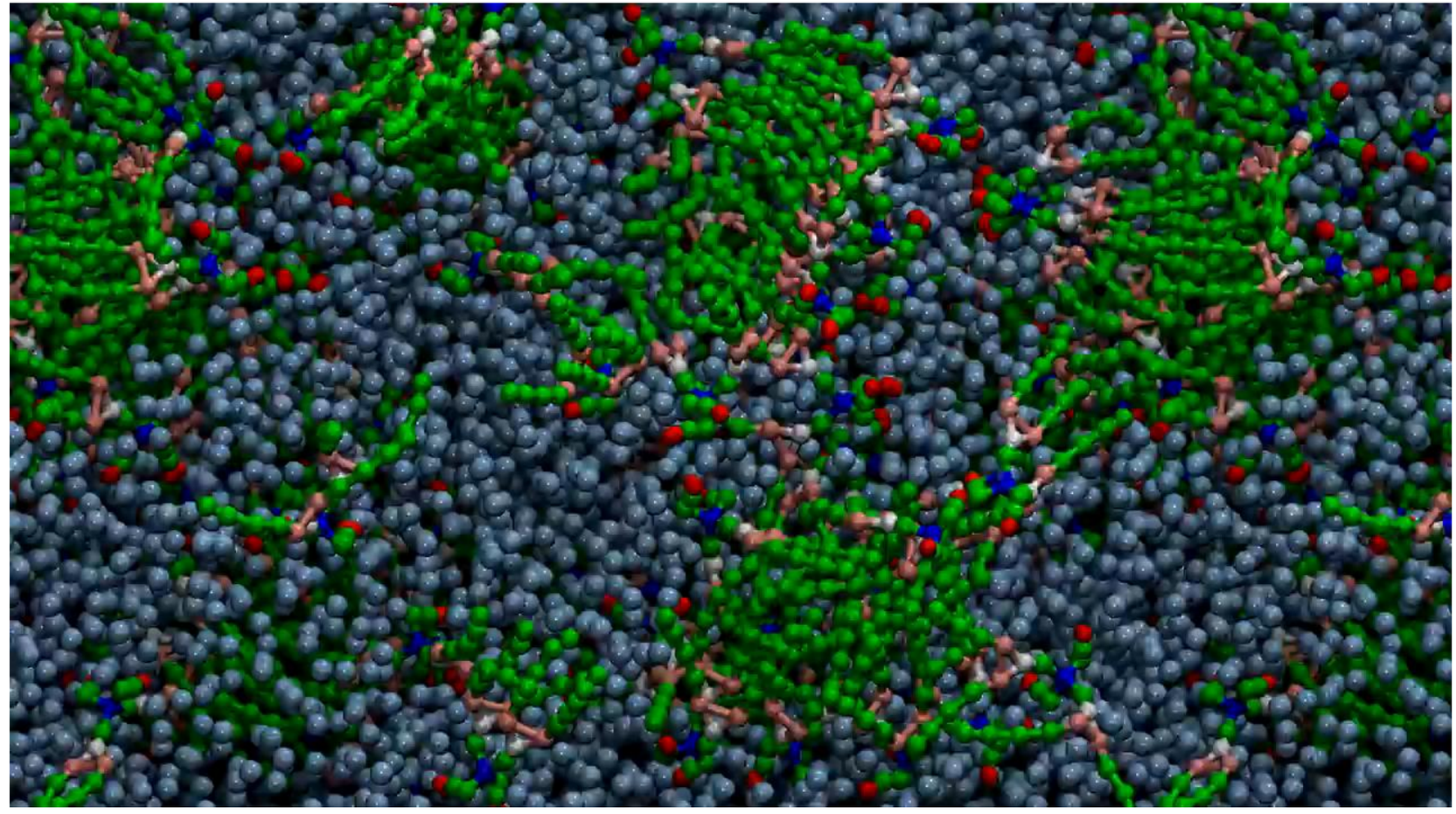


Gray spheres
= water

P. Headgroup

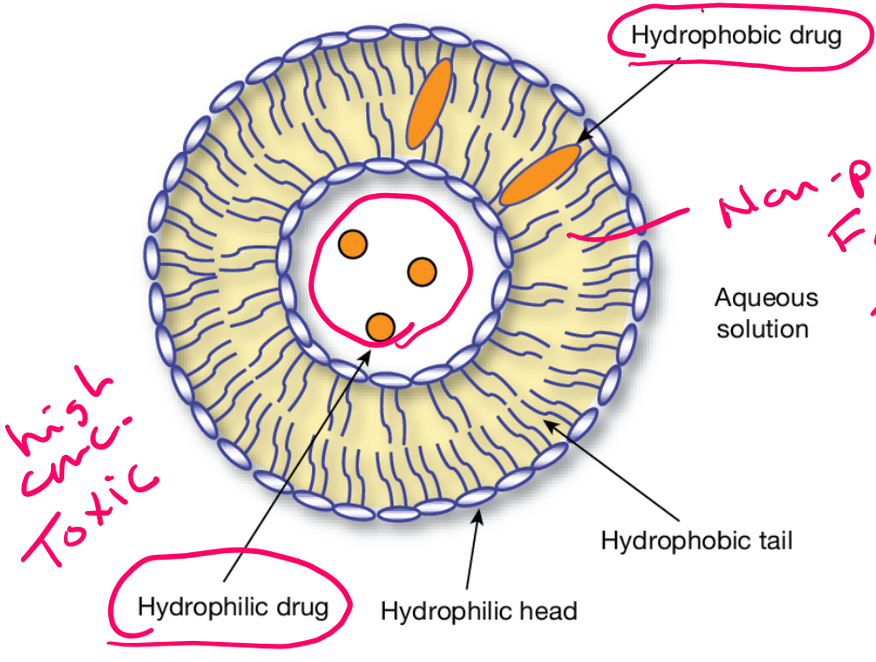
One
phospholipid

NP. Tail

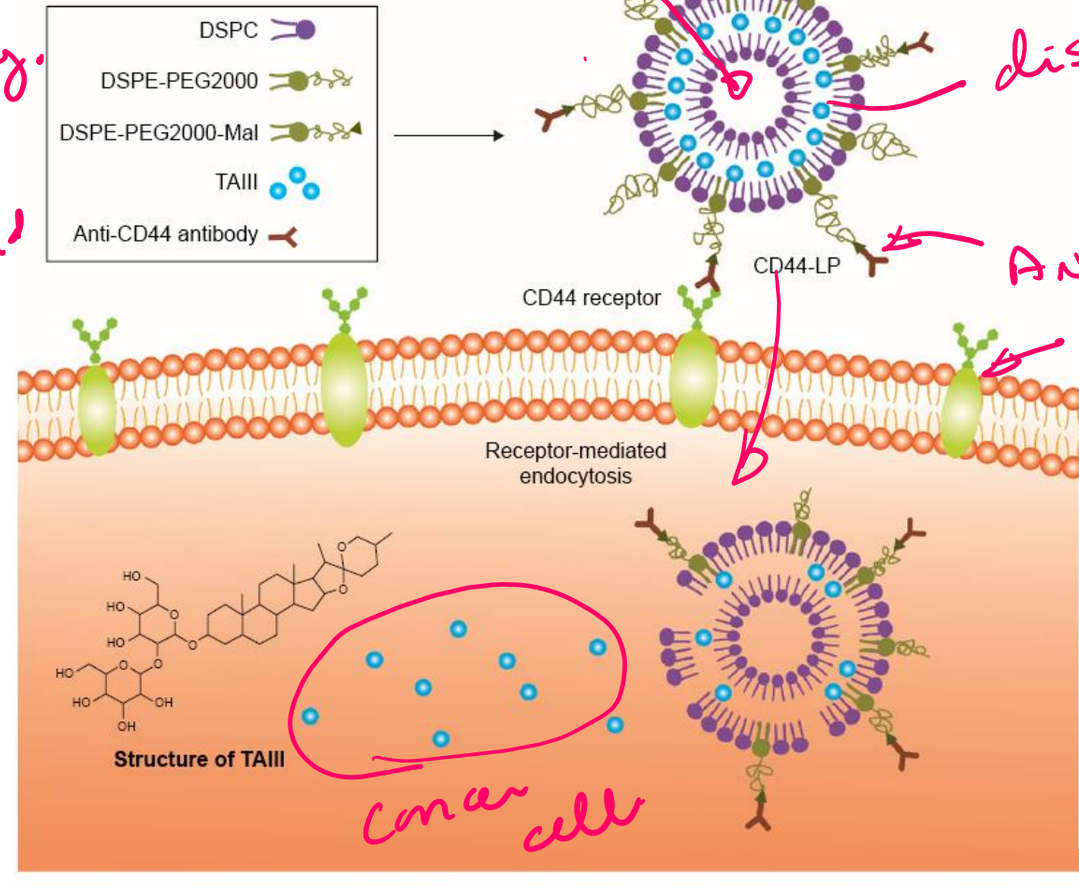


Liposomes (pure lipid vesicles) can be used for Drug Delivery

1. Drug delivery.
- Non-polar drugs dissolve in the lipids, ***increasing their solubility***
- Highly toxic water-soluble drugs can be encapsulated, reducing the exposure to healthy cells.



2. Delivery can be ***targeted to cancer cells*** by antibodies that recognize tumor specific antigens.



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

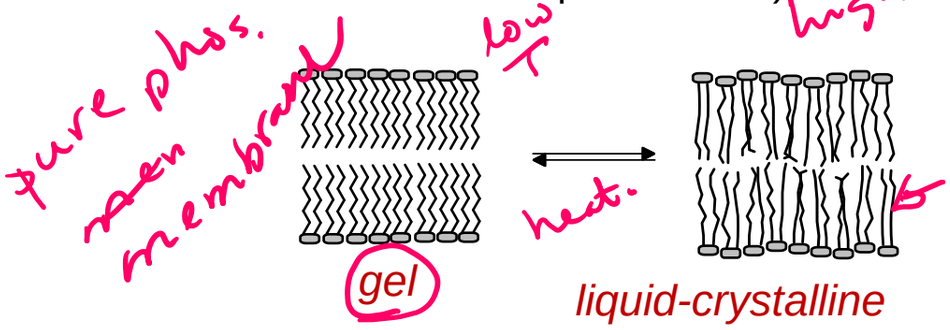
Figure 1 Illustration of CD44-LP for active CD44-targeting TAIII delivery and enhancing antitumor activity against CD44-overexpressing HepG2 cells.
Note: Anti-CD44 antibody was conjugated to LP through the reaction of sulfhydryl residues on the antibodies with the C-terminal maleimide groups of the PEG 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 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***. ***A fluid membrane is required for biological function.***

(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)



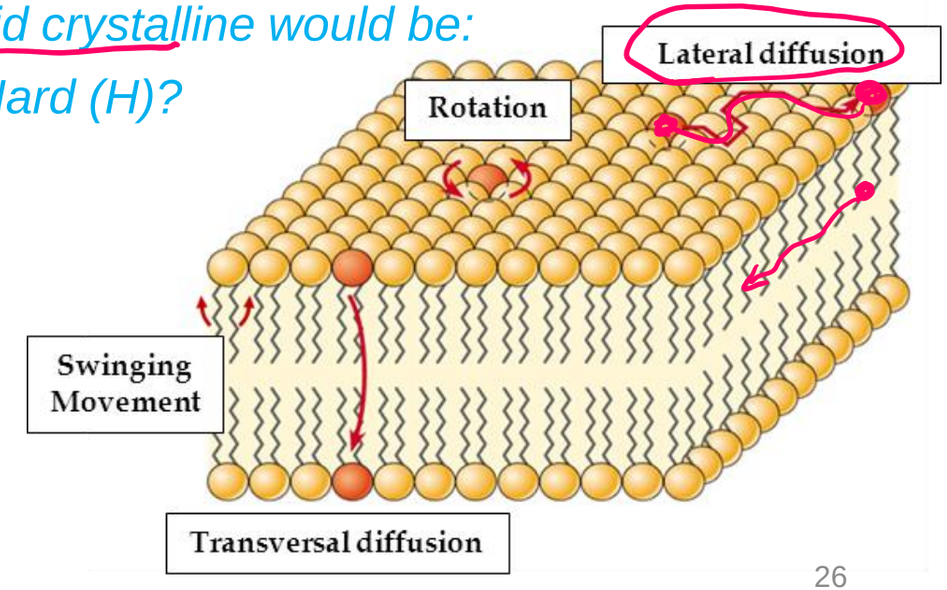
Lateral diffusion *of molecules within the plane of the membrane is an important biological function.*

A. Moving through gel phase would be:

Easy (E) or Hard (H)?

B. Moving through liquid crystalline would be:

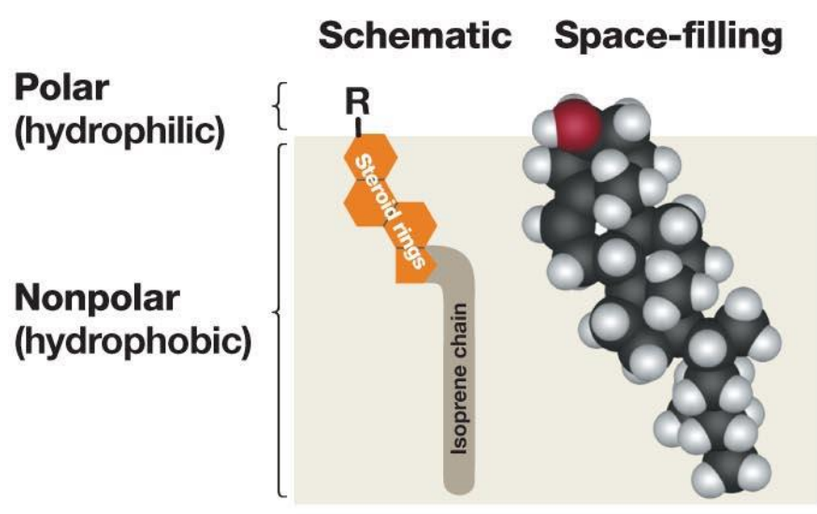
Easy (E) or Hard (H)?



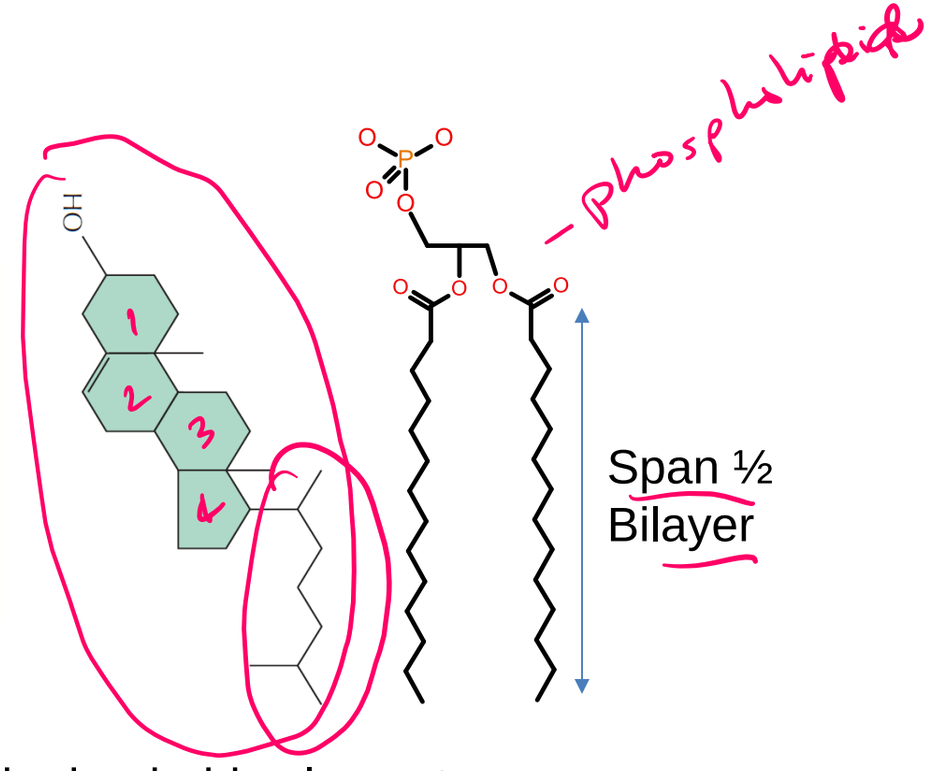
Steroids

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

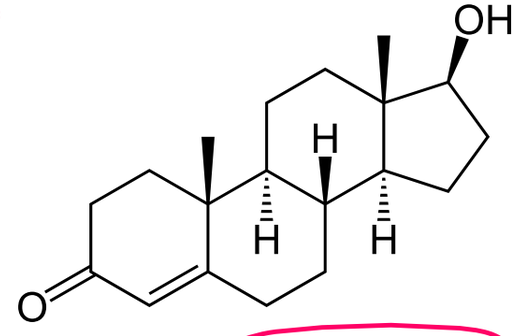
- Cholesterol - example of steroid molecule; essential function in plasma membrane
- All steroids (*testosterone, estrogen, progesterone…)* are derived from cholesterol!



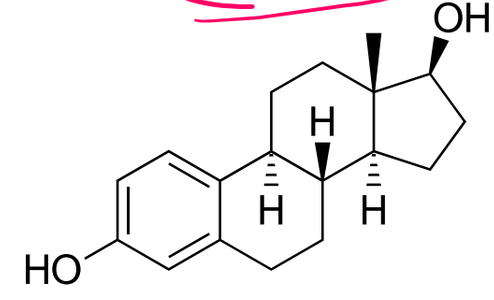
R= OH group



phospholipid



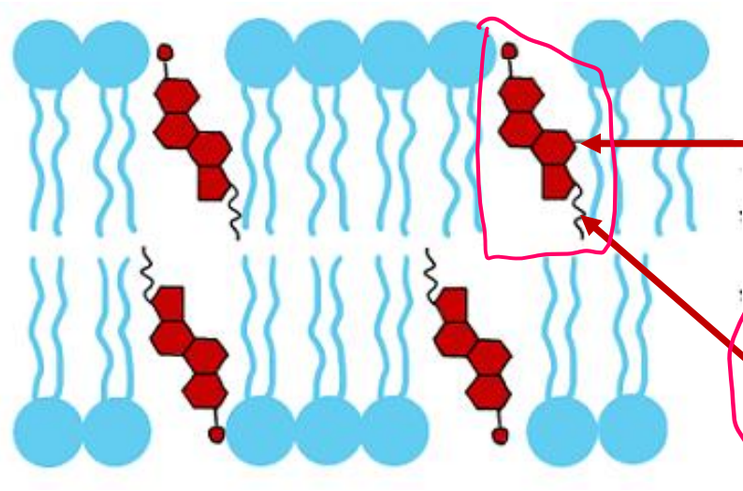
Testosterone ♂



Estrogen ♀

Amphipathic - contains hydrophilic and hydrophobic elements

Cholesterol Affects Fluidity



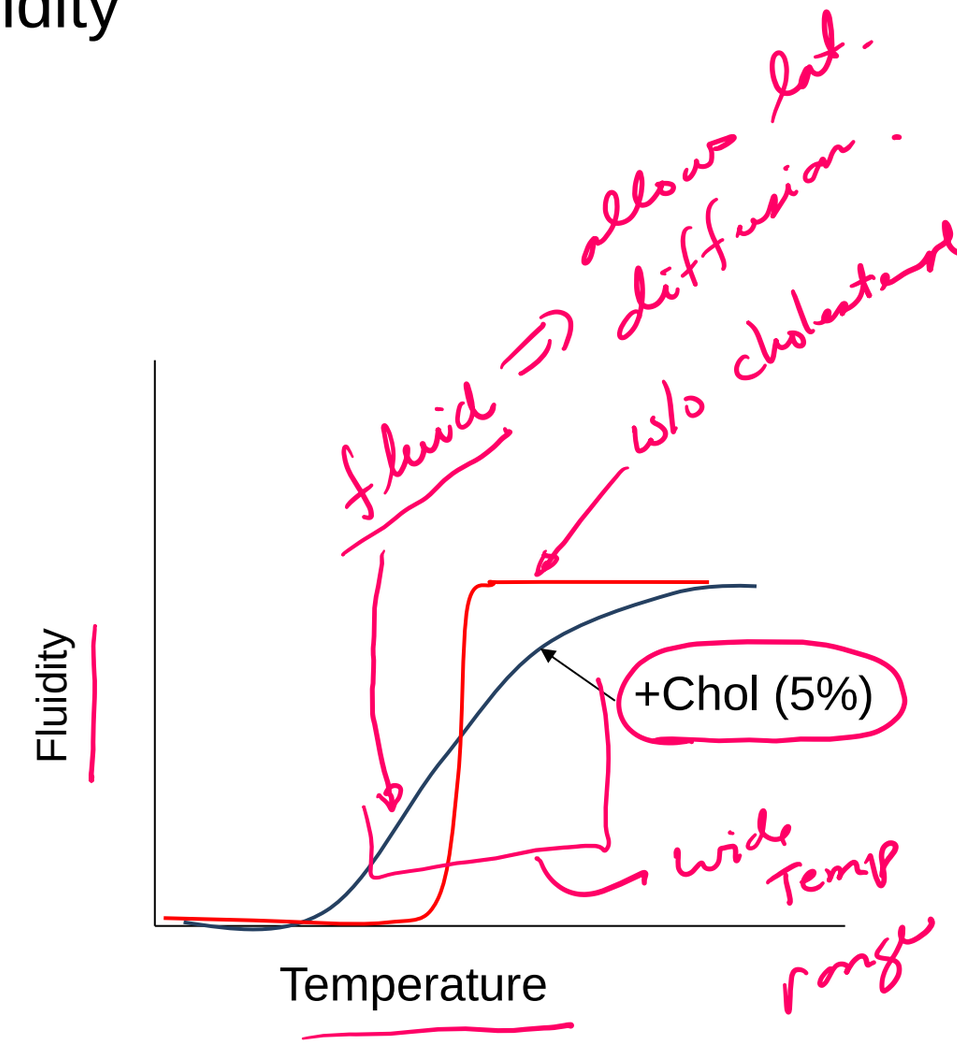
Rigid ring decreases flexibity at high temperatures

Flexible tail increases flexibility at low temps.

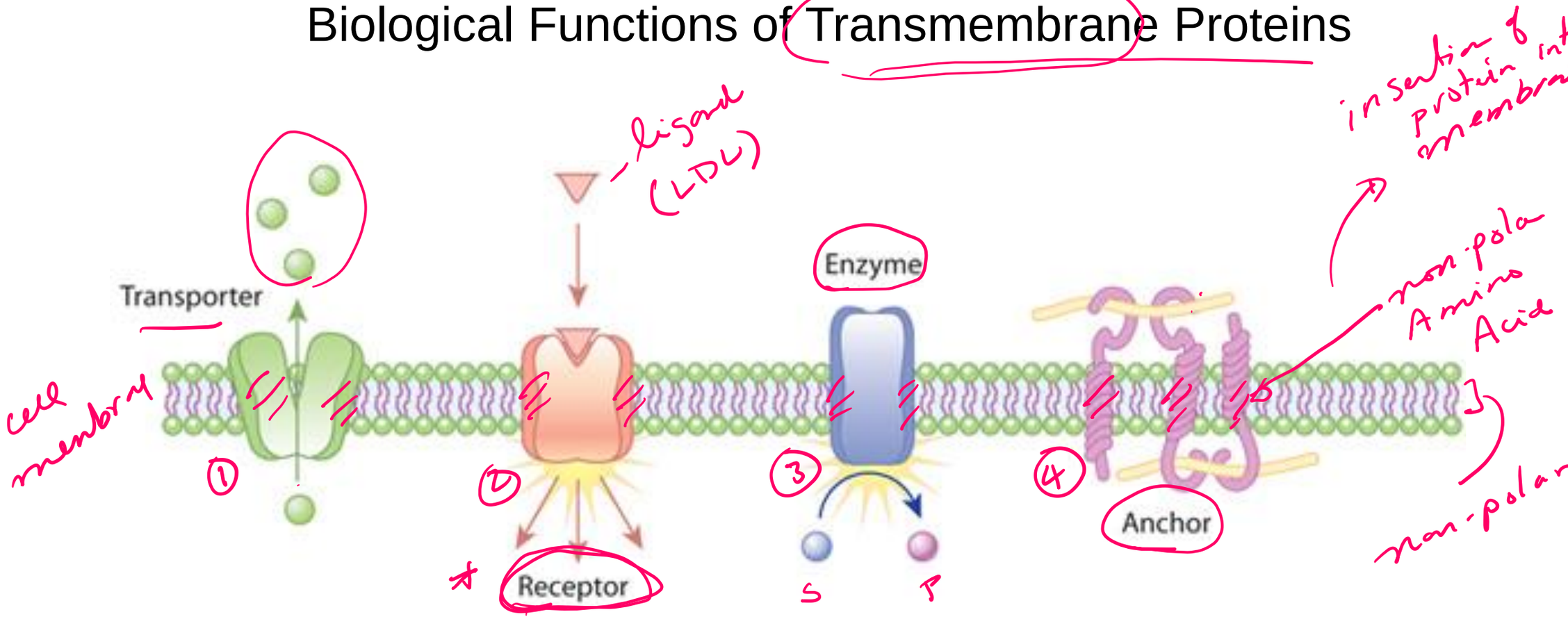
At cooler temperatures, cholesterol maintains fluidity by preventing tight packing of phospholipids due to flexible tail.

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.

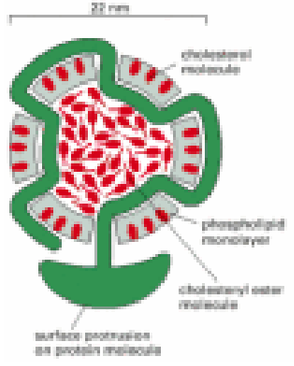


Biological Functions of Transmembrane Proteins



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

Cholesterol Regulation & Endocytosis



LDL particle

- Protein
- Triglycerides
- Cholesterol

2. LDL and its receptor are internalized in vesicles

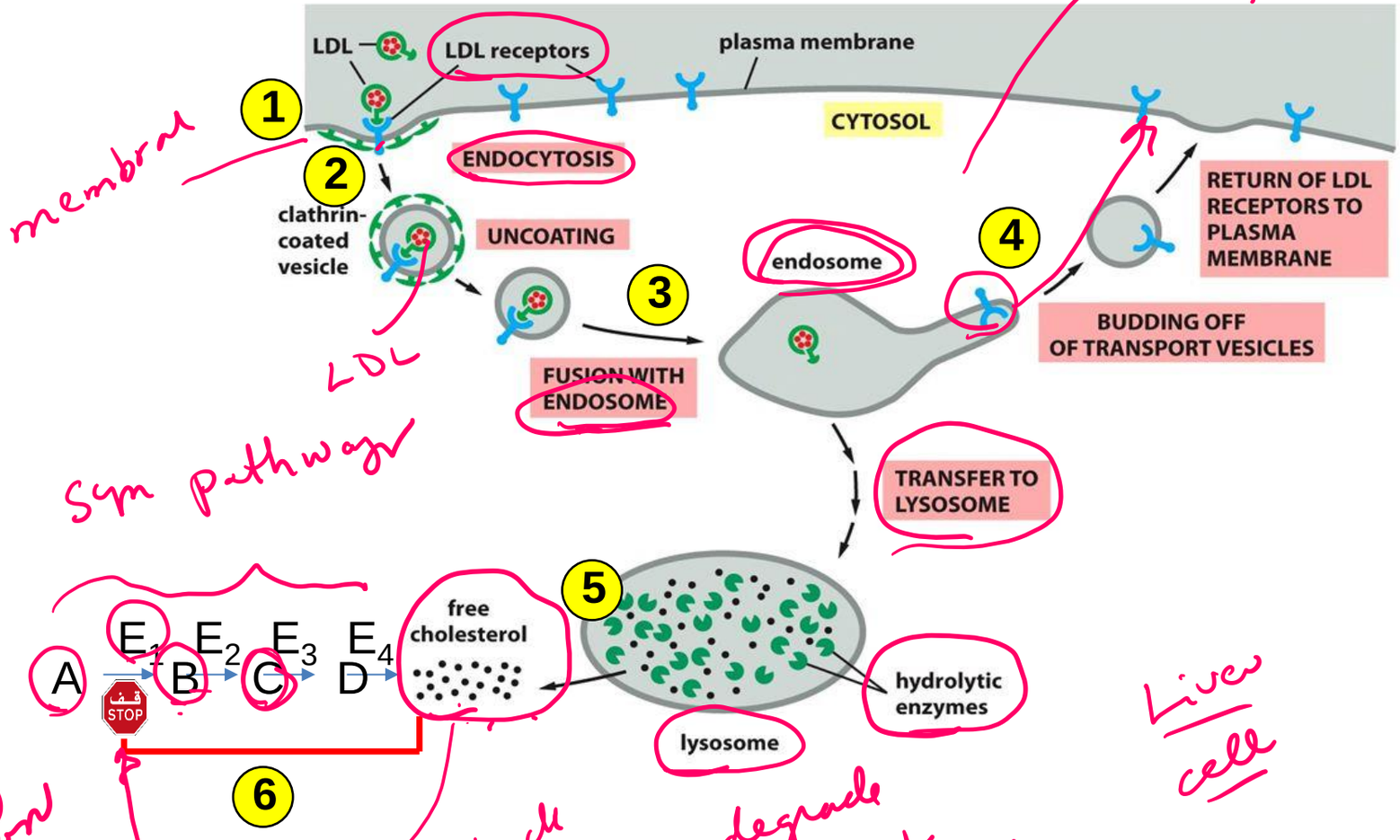
3. The vesicles fuse with endosomes

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

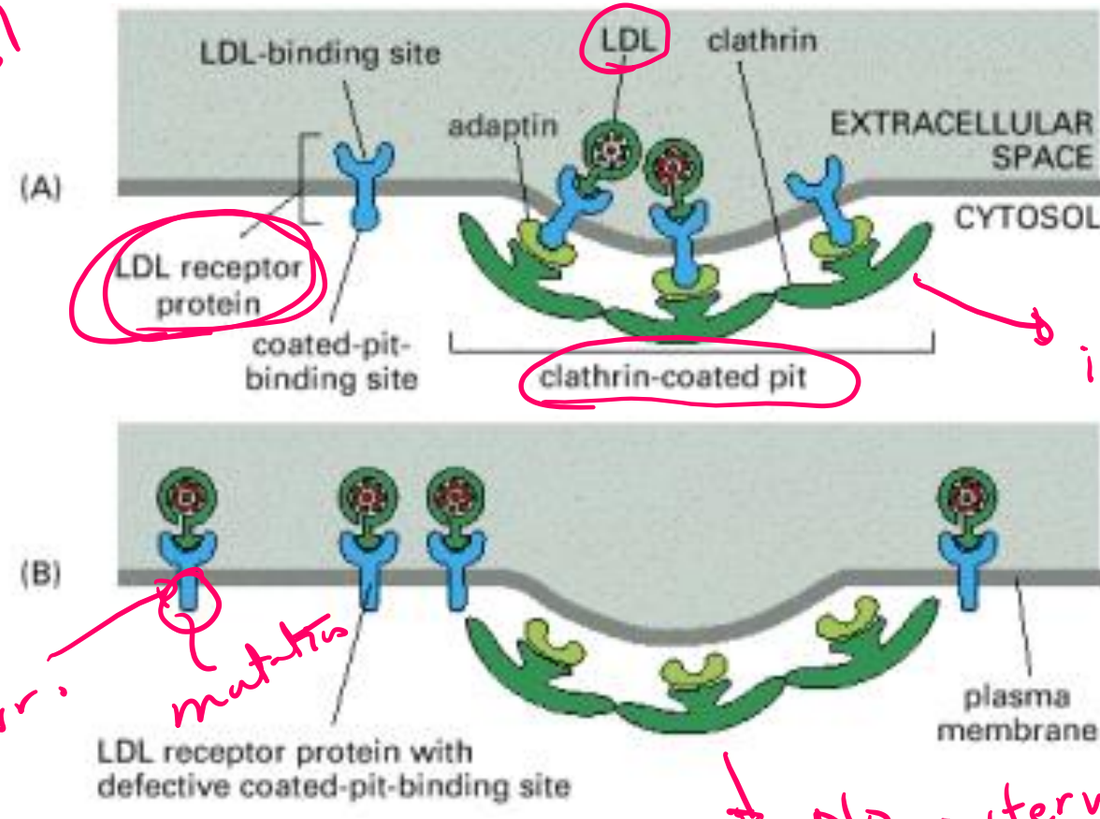
5. LDL is degraded and free cholesterol is released.

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

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



Some Individuals Inherit a Defective Gene Encoding the LDL receptor



Normal functioning LDL receptor

Receptor is a transmembrane protein:

- N-terminus outside the cell, binds LDL
- C-terminus Inside the cell, required for internalization

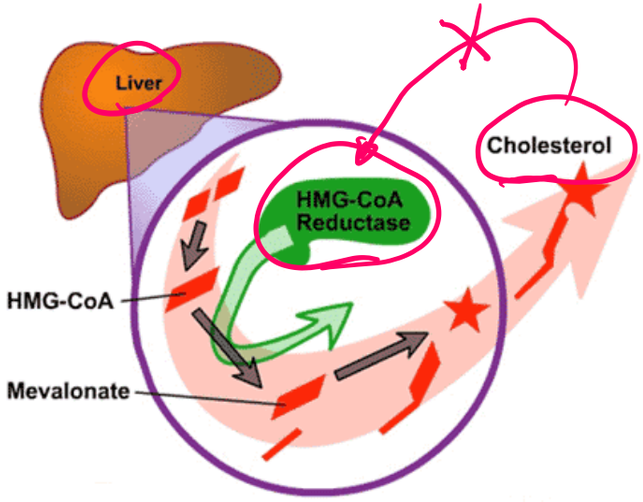
Non-functional LDL receptor:

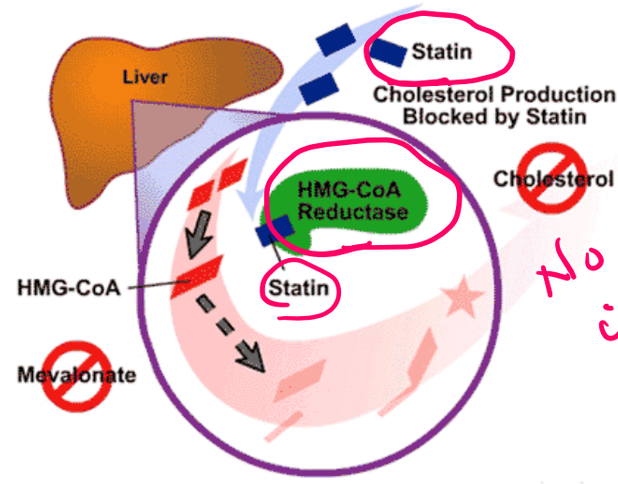
- Mutation in C-terminus that causes loss of interaction with Catherin coated pits.
- The coated pits are required for endocytosis of the LDL-receptor.

The altered receptors lack the cytoplasmic domain that enables them to be internalized.

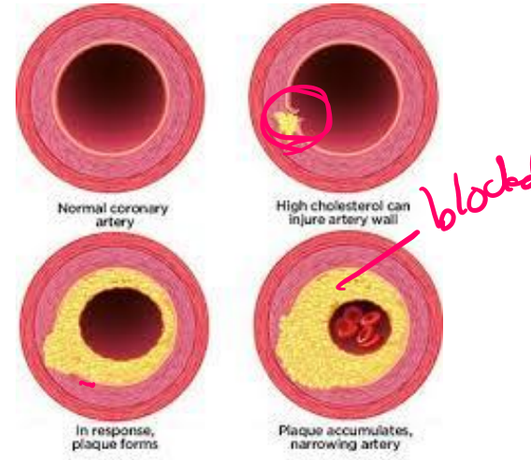
Such cells can bind LDL but cannot internalize it, leading to dysregulation of cholesterol production due to lack of feedback inhibition, *the liver cell continues to produce cholesterol.*

Cholesterol Metabolism and Regulation

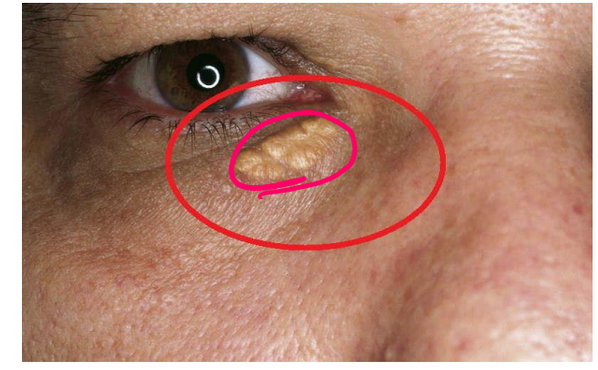




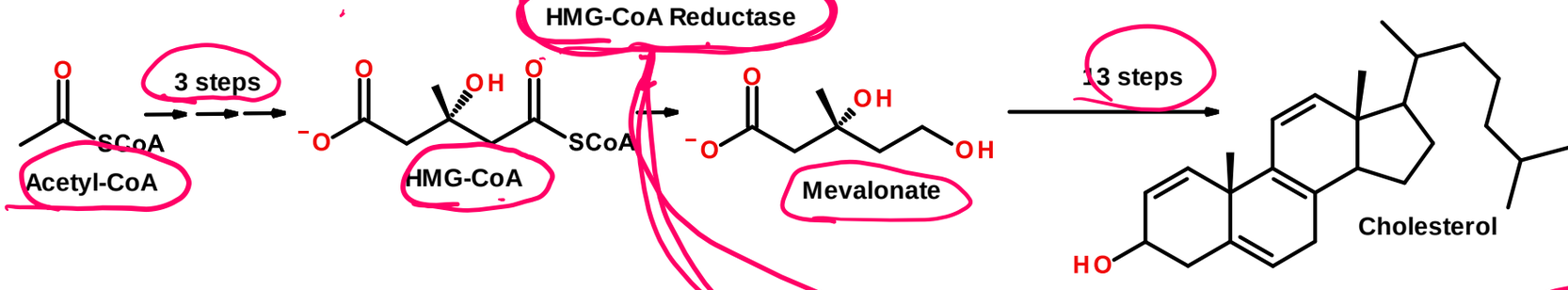
No cholesterol is made.



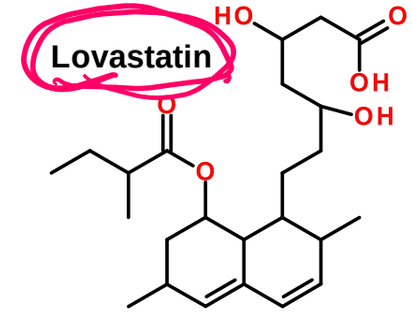
blocked



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



- **Statins** are competitive inhibitors that inhibit one of the enzymes (HMG-CoA Reductase) that is required to make cholesterol

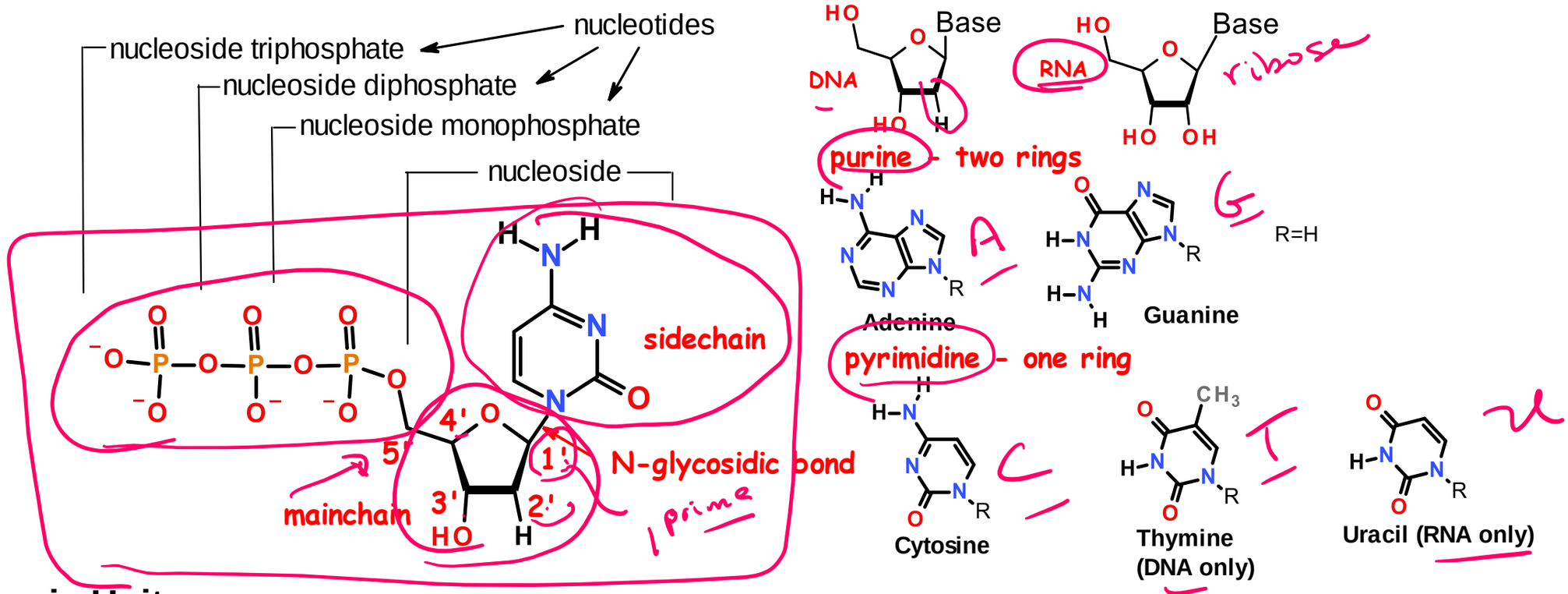


→ binds to Active site. blocks Substrate binding.

Nucleic Acid Technologies

- Review of DNA Structure
- Review of DNA Polymerase activity
- Nucleic Acid Technologies – PCR & Sequencing

Nucleic Acid Structure



Monomeric Units

a) Nucleoside triphosphates are the building blocks of nucleic acids (**dNTP = dATP, dGTP, dCTP, dTTP**)

b) The base ("sidechain") is connected to the C1' of the sugar ("mainchain") by an **N-linked glycosidic** bond.
   Base + sugar = **nucleoside**.
   Base + sugar + n-phosphates = **nucleotide**

c) The carbon atoms on the sugar are numbered 1' to 5'. The primes distinguish the atoms on the sugar from those on the base.

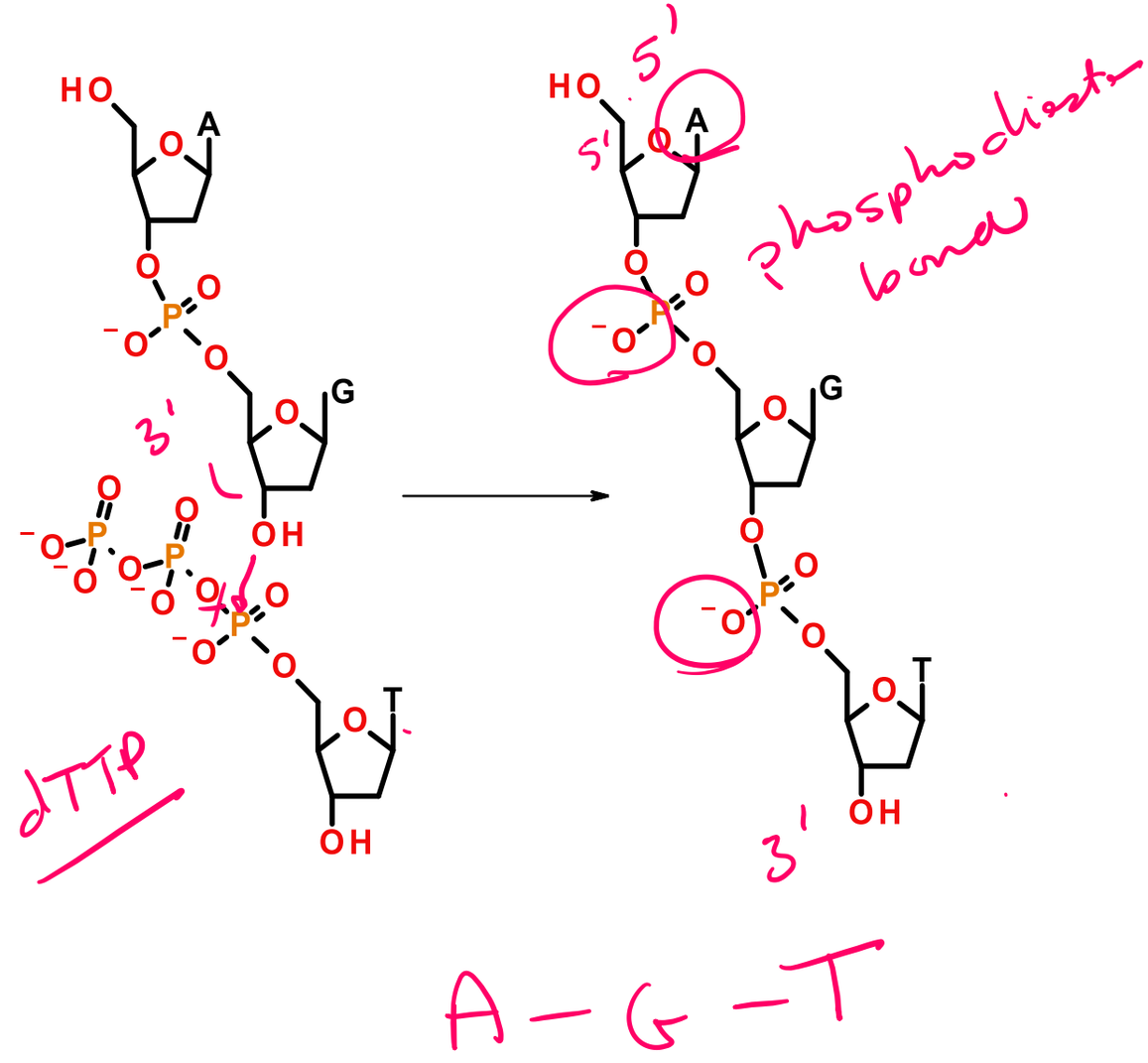
d) DNA differs from RNA in the sugar (deoxyribose versus ribose) and one base.

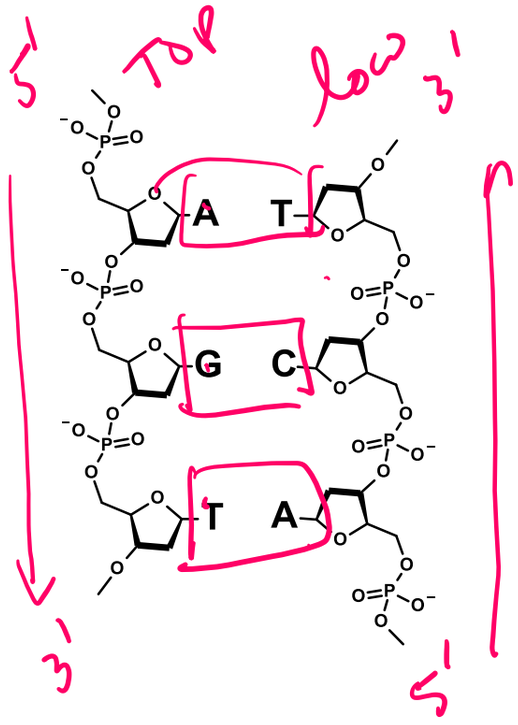
e) Four different monomers, A, G, C, T in DNA. U replaces T in RNA.

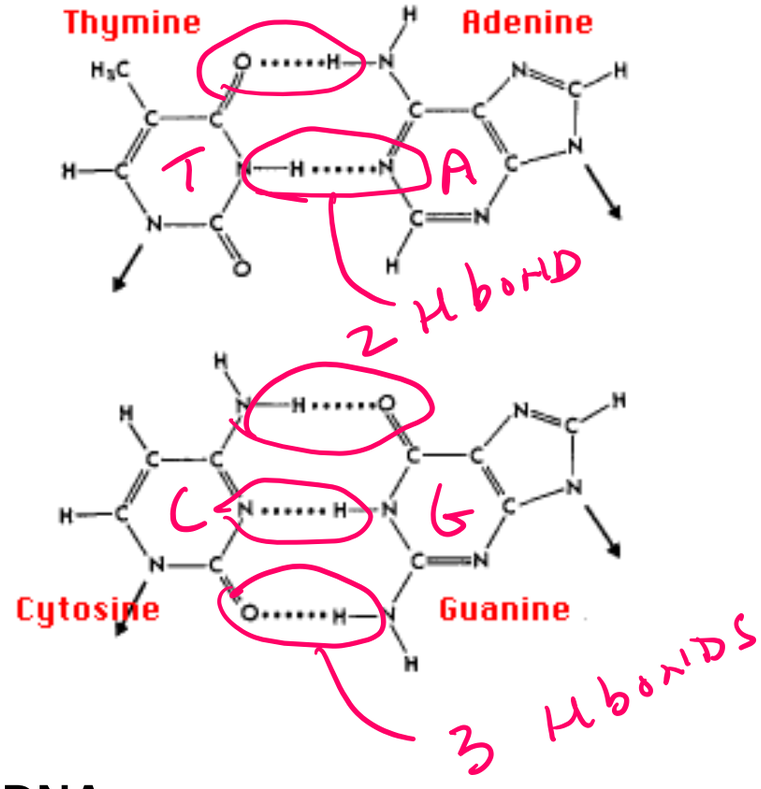
DNA and RNA are Polynucleotides:

- Two phosphates are lost during polymer formation.
- The **phosphodiester** backbone is comprised of deoxyribose (DNA) or ribose (RNA) sugars bridged by one phosphate between the **3' and 5'** positions of the sugars. *Be able to draw this structure.*
- The phosphates are always ionized (pK_a~1), nucleic acids are **polyanions**. The negative charge is important for protein interactions (and electrophoresis).
- Note the polarity: 5' → 3'. *Be able to identify the 5' and 3' ends:*
  - Start at the end atom and move down the chain. The first carbon you find defines the end.

Sequence of nucleotide bases is written in the 5'-3' direction.



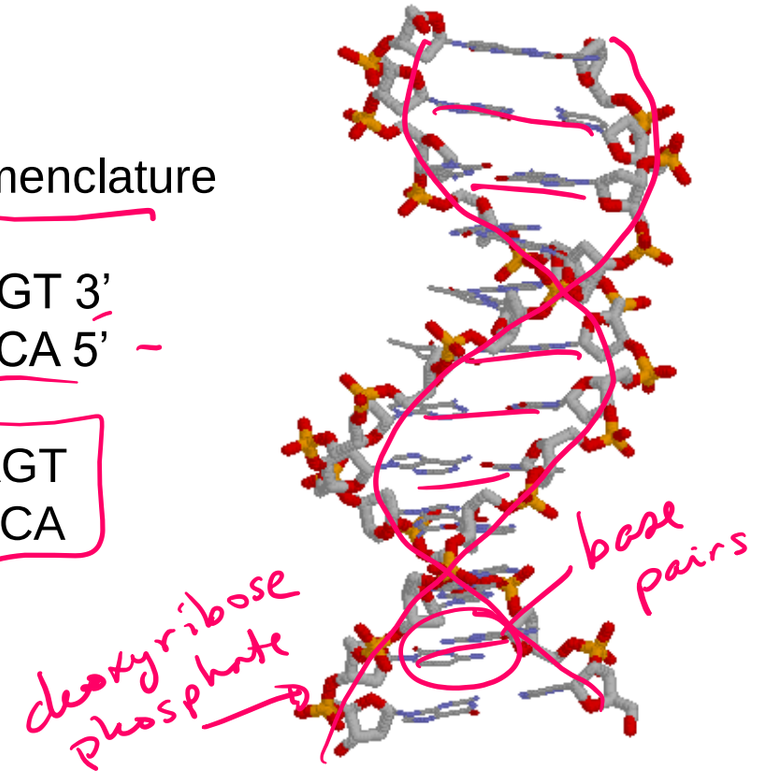




Nomenclature

5' AGT 3'
3' TCA 5'

= AGT
TCA



https://www.andrew.cmu.edu/user/rule/jsmol/nucleic.html

Double Helical Structures: B-DNA

a) The helix is right-handed; the chains are **antiparallel**.
b) **10 bp/turn**.
c) The helix interior is filled with stacked base, phosphates and deoxyriboses on the outside.
d) T pairs with A via two "Watson-Crick H-bonds"
e) C pairs with G via three "Watson-Crick hydrogen bonds"
f) Opposite strand termed “complimentary strand”. Top strand is always written 5’->3’, lower strand 3’ -> 5’.

Introduction to Central Dogma

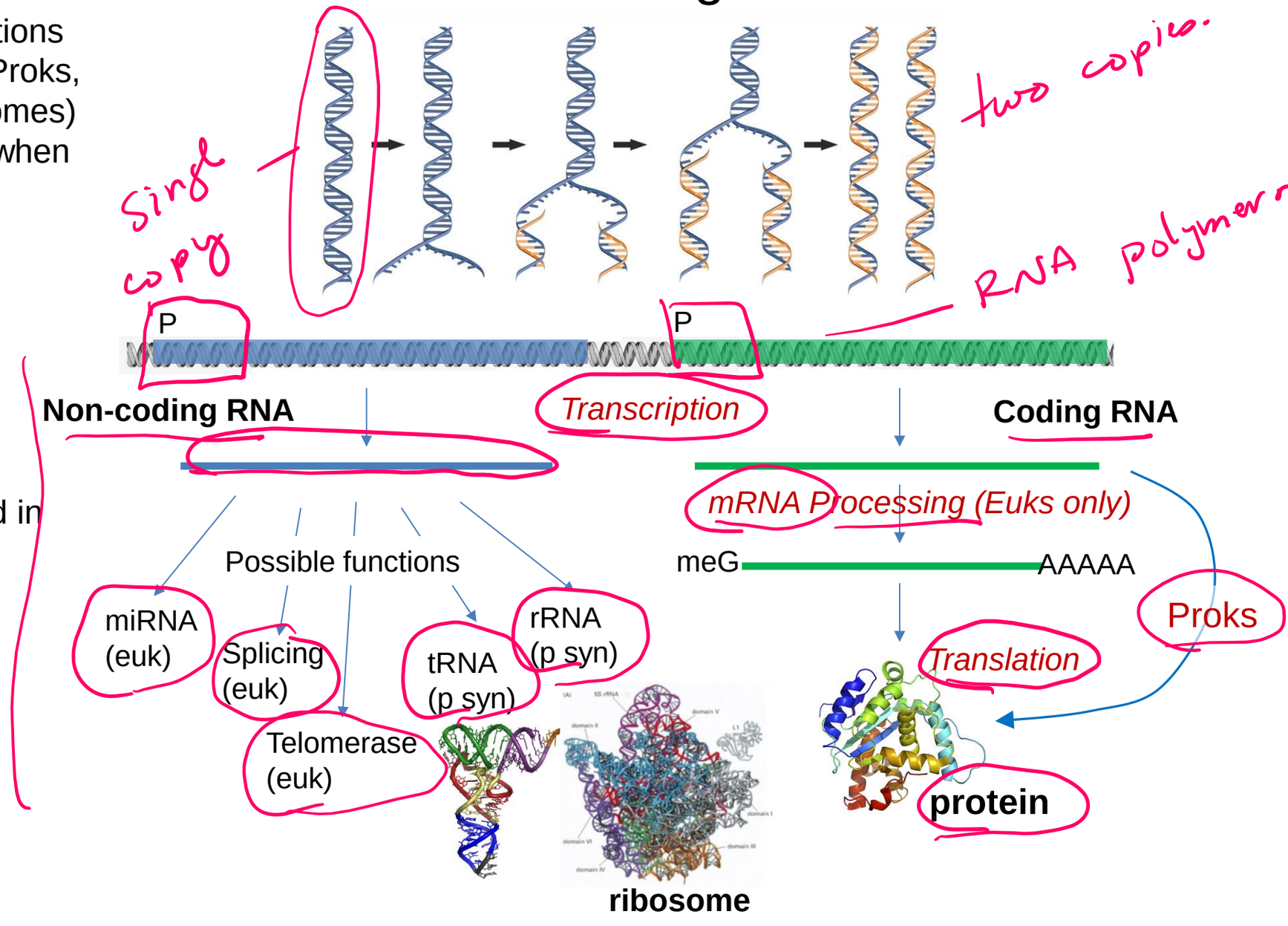
Genome: Entire DNA content of an organism, contains all of the instructions for life. Single circular molecule in Proks, multiple linear molecules (chromosomes) in Euks. The genome is *replicated* when cells divide.

Gene – a segment of DNA that is converted (*transcribed*) to RNA. A *promoter* (P) sequence on the DNA is the minimal requirement for the production of RNA.

RNA molecules are often processed in **Eukaryotic cells** before they are functional

Many RNAs are functional on their own

mRNA are *translated* to a protein.



The Genetic Code – Converting a DNA/RNA Sequence to a Protein

First base	Second base: U	Second base: C	Second base: A	Second base: G	Third base
U	UUU Phenylalanine	UCU Serine	UAU Tyrosine	UGU Cysteine	U
	UUC Phenylalanine	UCC Serine	UAC Tyrosine	UGC Cysteine	C
	UUA Leucine	UCA Serine	UAA Stop codon	UGA Stop codon	A
	UUG Leucine	UCG Serine	UAG Stop codon	UGG Tryptophan	G
C	CUU Leucine	CCU Proline	CAU Histidine	CGU Arginine	U
	CUC Leucine	CCC Proline	CAC Histidine	CGC Arginine	C
	CUA Leucine	CCA Proline	CAA Glutamine	CGA Arginine	A
	CUG Leucine	CCG Proline	CAG Glutamine	CGG Arginine	G
A	AUU Isoleucine	ACU Threonine	AAU Asparagine	AGU Serine	U
	AUC Isoleucine	ACC Threonine	AAC Asparagine	AGC Serine	C
	AUA Isoleucine	ACA Threonine	AAA Lysine	AGA Arginine	A
	AUG Methionine (start codon)	ACG Threonine	AAG Lysine	AGG Arginine	G
G	GUU Valine	GCU Alanine	GAU Aspartic acid	GGU Glycine	U
	GUC Valine	GCC Alanine	GAC Aspartic acid	GGC Glycine	C
	GUA Valine	GCA Alanine	GAA Glutamic acid	GGA Glycine	A
	GUG Valine	GCG Alanine	GAG Glutamic acid	GGG Glycine	G

Codon = 3 bases that code for an amino acid

Promoter

...ATATGCCCATGTGGTAA..

(DNA Sequence)

RNA polymerase

...AUAUGCCCAUGUGGUAA..

(mRNA Sequence)

Start codon — Bind to ribosome — STOP

..U-AUG-CCC-AUG-UGG-UAA

Codon

(Punctuated RNA sequence – how the ribosome interprets the sequence)

- Each codon codes for one amino acid.
- Many amino acids are coded by more than one codon.
- Most organisms use the same codon table – some codons have different meanings in some organisms.

Special Codons:

AUG = Is used to begin almost all proteins that are synthesized on the ribosome, codes for methionine when found internally.

UAA, UAG, UGA = stop codons, terminate synthesis

Met - pro - met - Trp

(Protein Sequence)

met-pro-met-Trp