Biochem Notes

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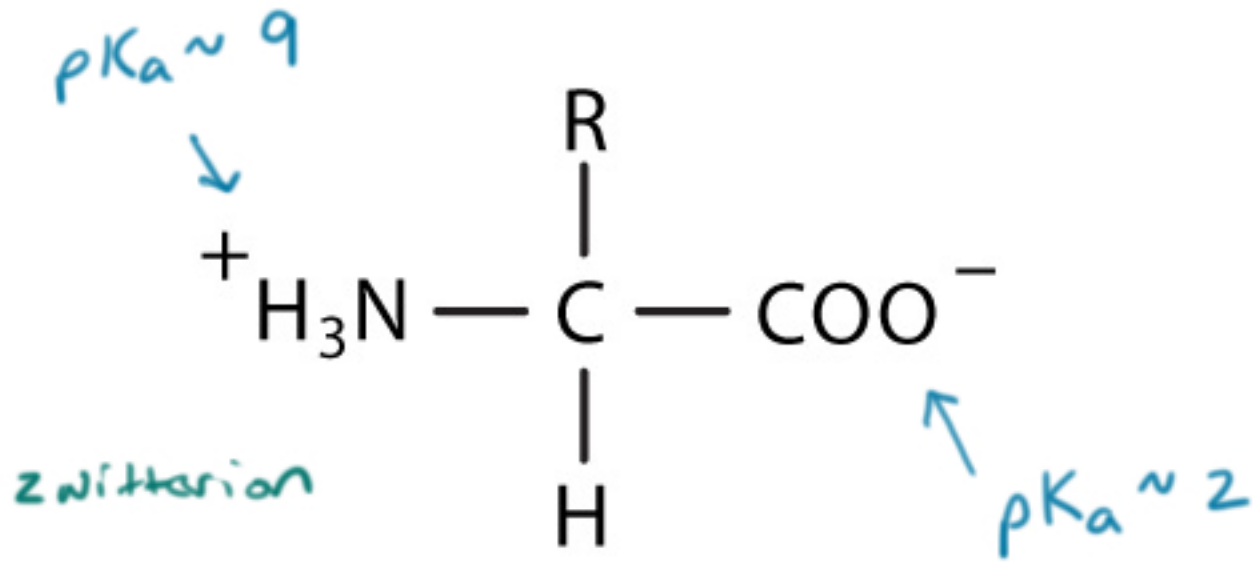
amino acids



L amino acid

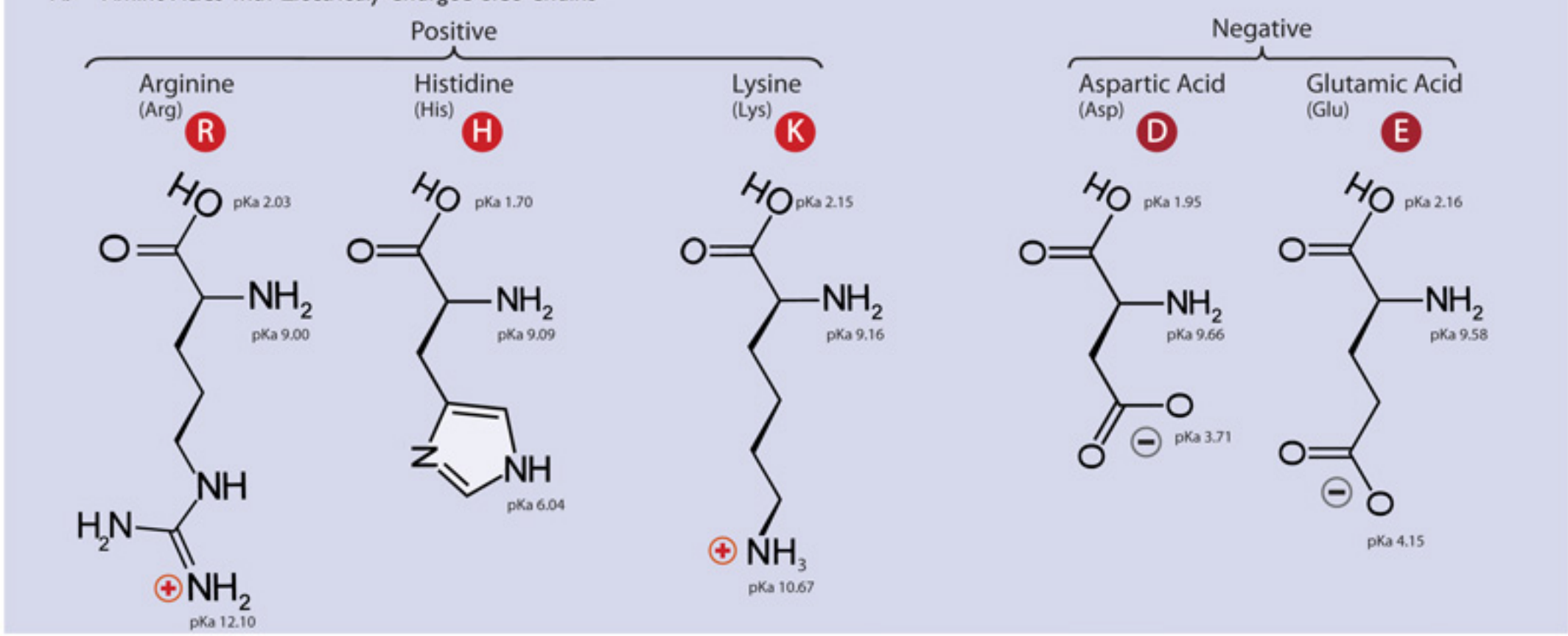
$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

$$7 \qquad 2 + \log\left(\frac{100{,}000}{1}\right)$$

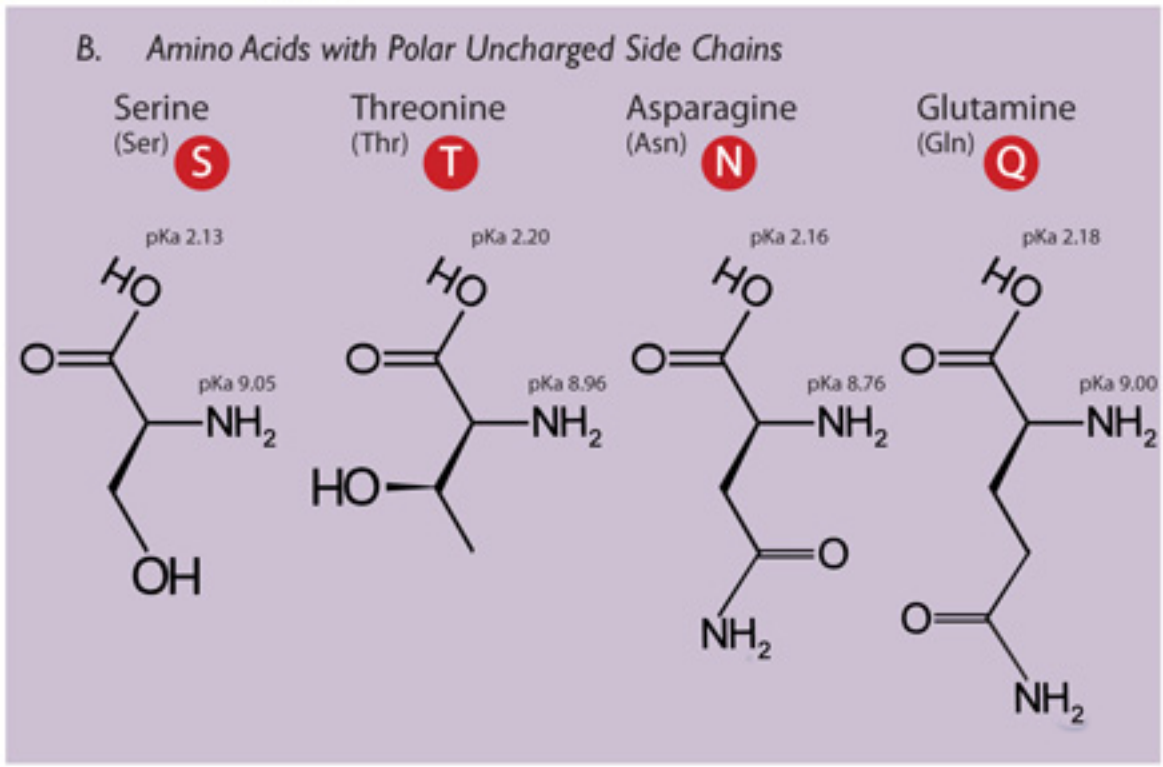
Twenty-One Amino Acids

⊕ Positive ⊖ Negative
• Side chain charge at physiological **pH 7.4**

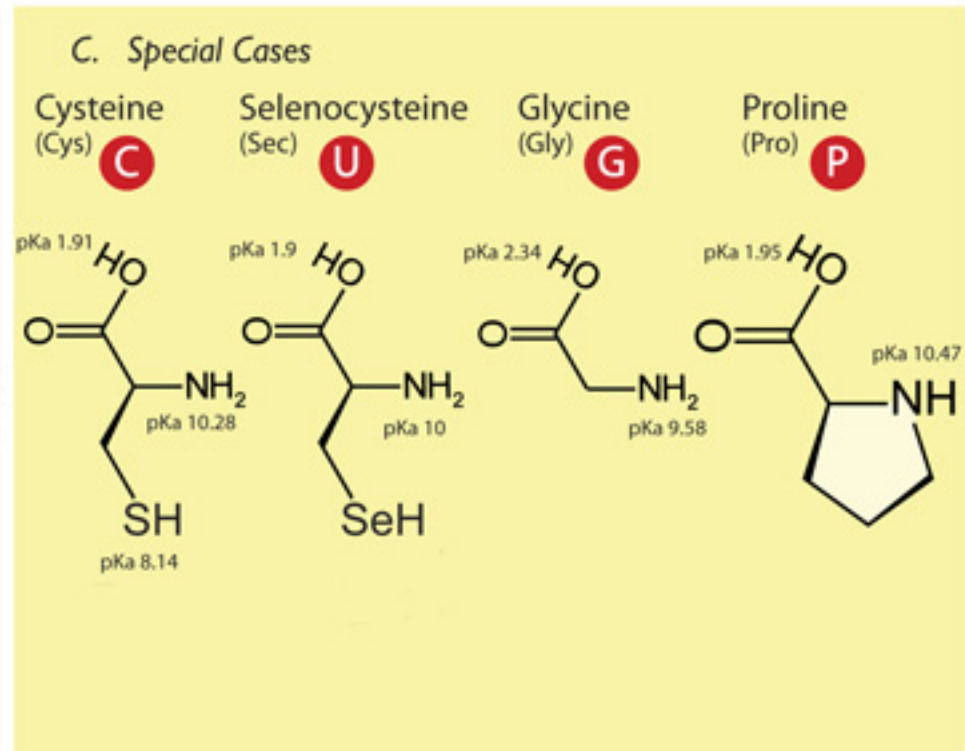
A. *Amino Acids with Electricaly Charged Side Chains*



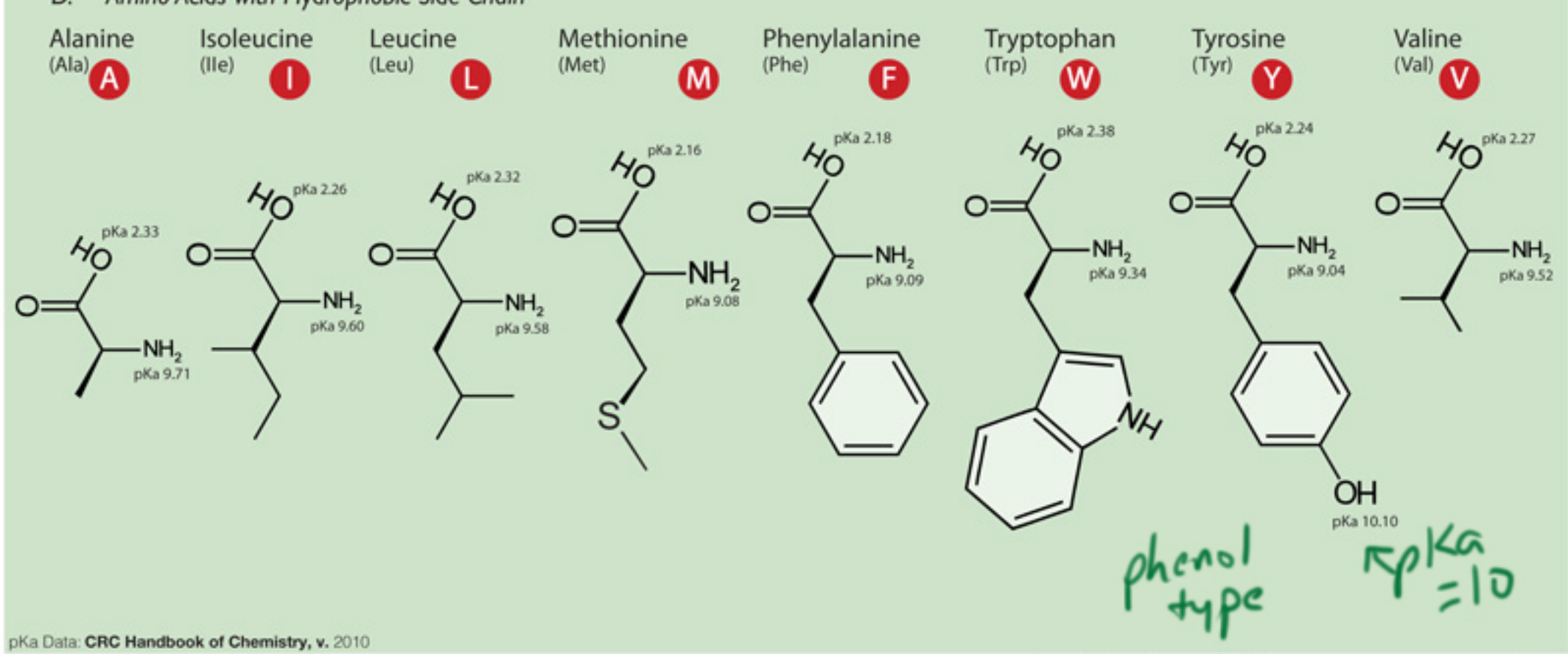
B. *Amino Acids with Polar Uncharged Side Chains*



C. *Special Cases*

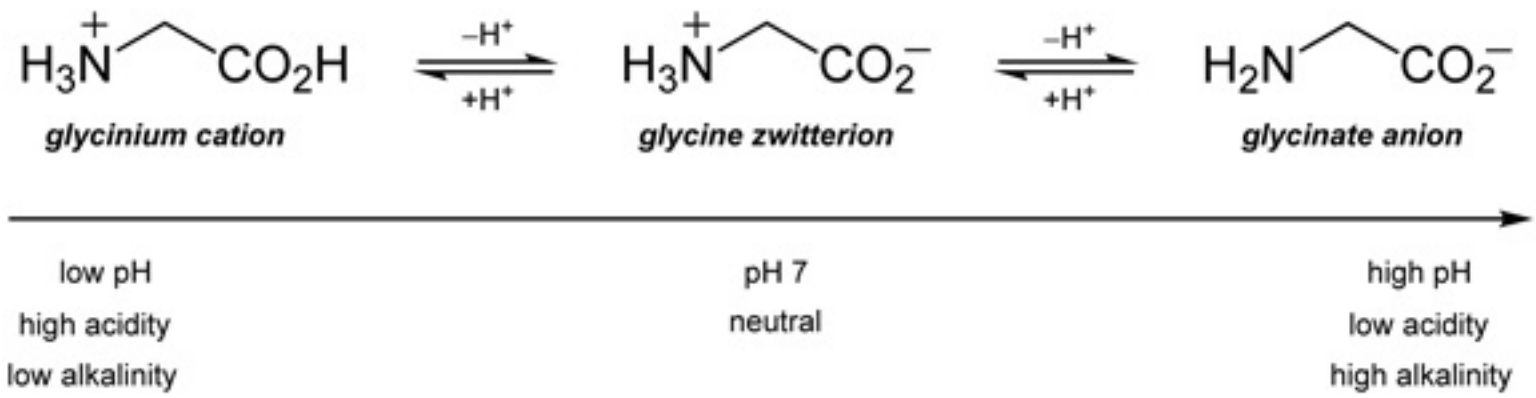


D. *Amino Acids with Hydrophobic Side Chain*



pKa Data: **CRC Handbook of Chemistry, v.** 2010

pH < 2.3 | 2.3 < pH < 9.6 | pH > 9.6

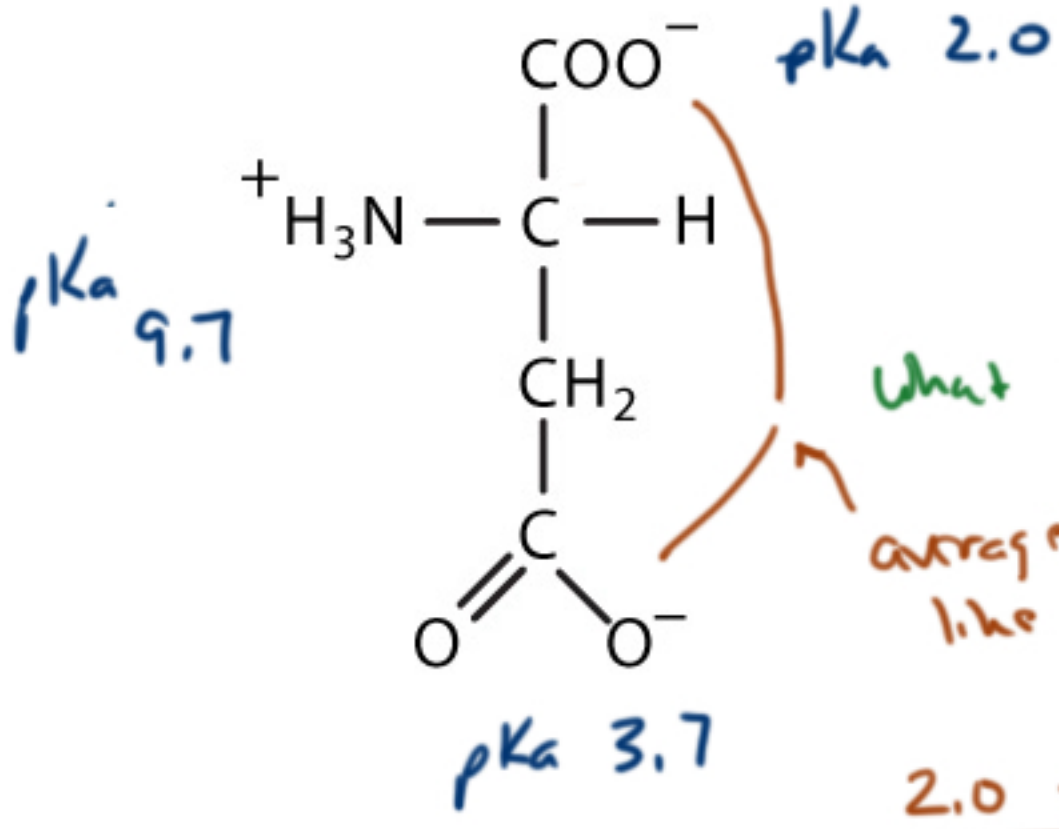


Isoelectric Point

$$\frac{2.3 + 9.6}{2} = 6.2$$

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

Aspartate

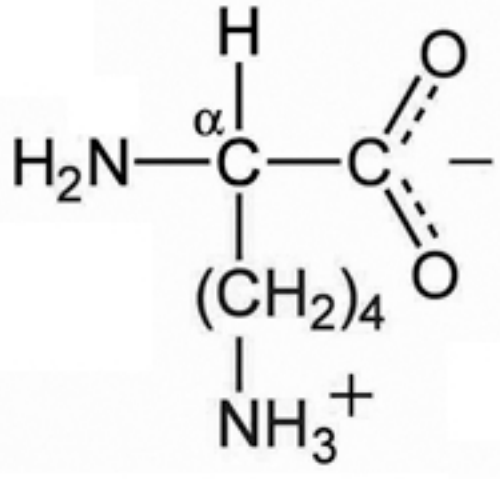


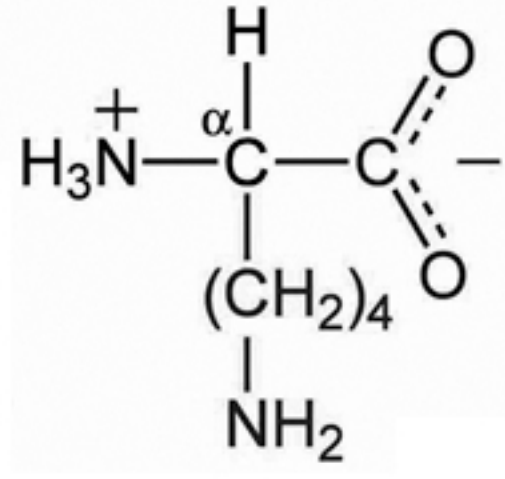
What is pI?

average the like groups

$$\frac{2.0 + 3.7}{2} = 2.9$$

Amino Acid	pKa
Asp (D)	3.9
Glu (E)	4.3
Arg (R)	12.0
Lys (K)	10.5
His (H)	6.08
Cys (C)	8.28 (-SH)
Tyr (Y)	10.1


H
O
α
H_2N
C
C
–
O
$(CH_2)_4$
NH_3^+


H
O
+
α
H_3N
C
C
–
O
$(CH_2)_4$
NH_2

hydrophobic

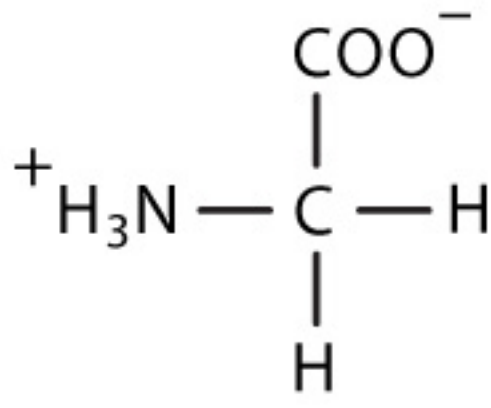
hydrophilic

Kyte and Doolittle (1)	Rose, et al (2)	Wolfenden, et al (3)	Janin (1979) (4)
Ile	Cys	Gly,Leu,Ile	Cys
Val		Val,ala	Ile
	Phe,Ile		Val
Leu	Val	Phe	Leu,Phe
	Leu,Met,Trp	Cys	Met
Phe		Met	Ala,Gly,Trp
Cys			
Met,Ala	His	Thr,Ser	
	Tyr	Trp,Tyr	His,Ser
Gly	Ala		Thr
Thr,Ser	Gly		Pro
Trp,Tyr	Thr		Tyr
Pro			Asn
		Asp,Lys,Gln	Asp
His	Ser	Glu,His	Gln,Glu
Asn,Gln	Pro,Arg	Asp	
Asp,Glu	Asn		
Lys	Gln,Asp,Glu		
			Arg
Arg	Lys	Arg	Lys

Glycine

G

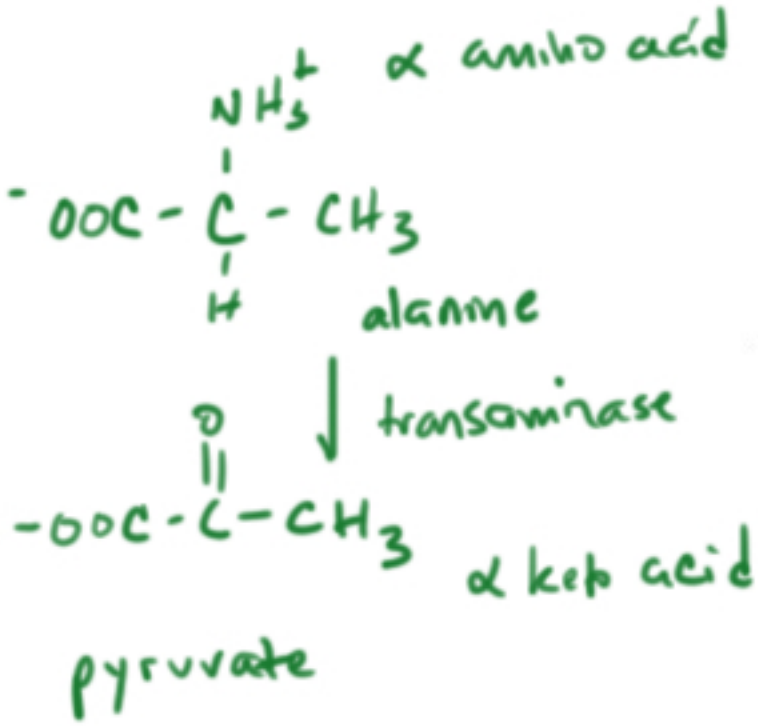
- like a missing tooth
  - oxianion hole in serine protease
  - 30% of collagen - interior of triple helix
  - backbone H bonding



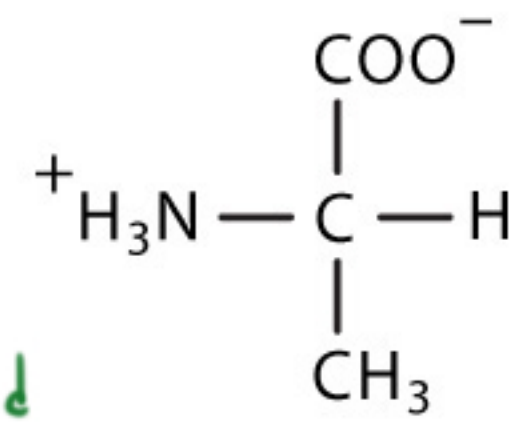
- achiral
- important precursor such as with porphyrin
  ↑
  organic component of heme

Alanine

A



Hence alanine is glucogenic.



- prototype boring amino acid in proteins
- K293A

  Lysine at 293 replaced with alanine

  ↑ isn't a confound

- AAAAAAHHHHH

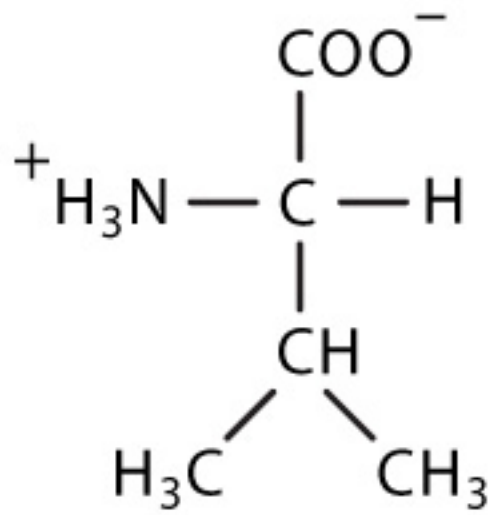
  ↗ alanine spacers

  ↑ polyhistidine for affinity chromatography with nickel column

Valine

V

- hydrophobic



example data memorize!

- breaks down to succinyl CoA

↓

succinate

↓

fumarate

↓

malate

↓

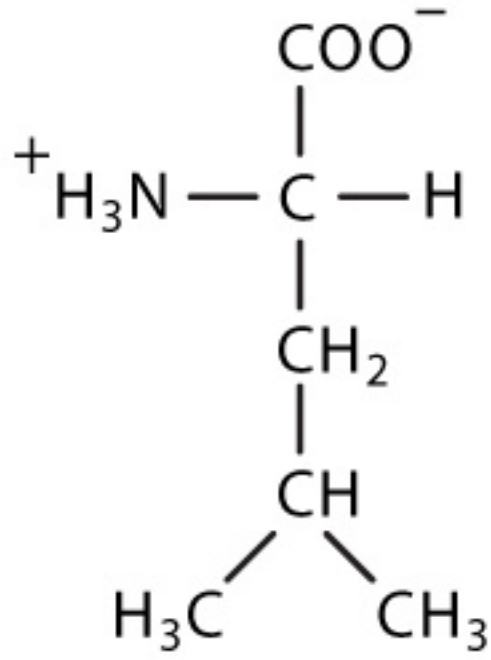
oxaloacetate

It is glucogenic.

Leucine

L

- example of an essential amino acid (don't synthesize)



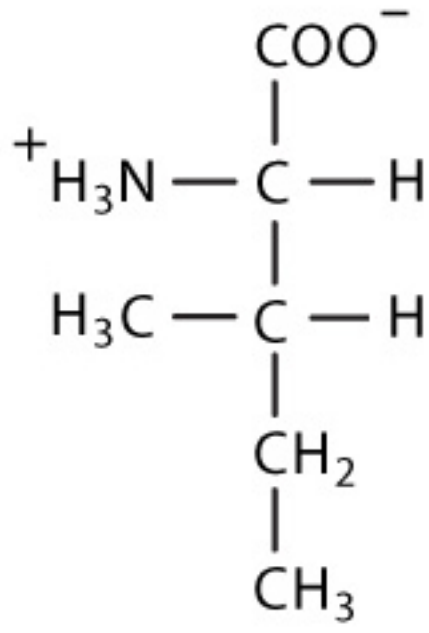
- breaks down to acetyl CoA and acetoacetate

There's no path from acetyl CoA to glucose.

Leucine is ketogenic.

Isoleucine

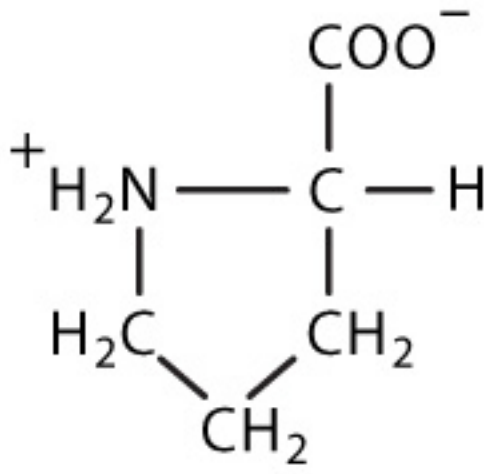
I



2 chiral centers
(like threonine)

Proline

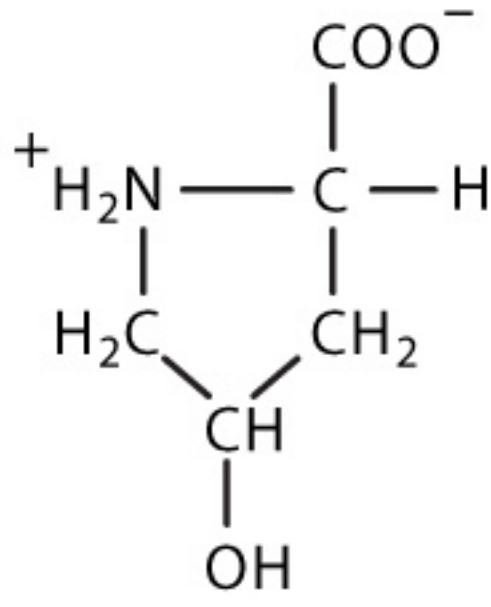
P



- Plays a big role in protein structure
- locks ϕ at 60° in polypeptide
  - reduces configurational entropy
- α helix disruptor and β turns

(essential prolines bond α helix)

Hydroxyproline



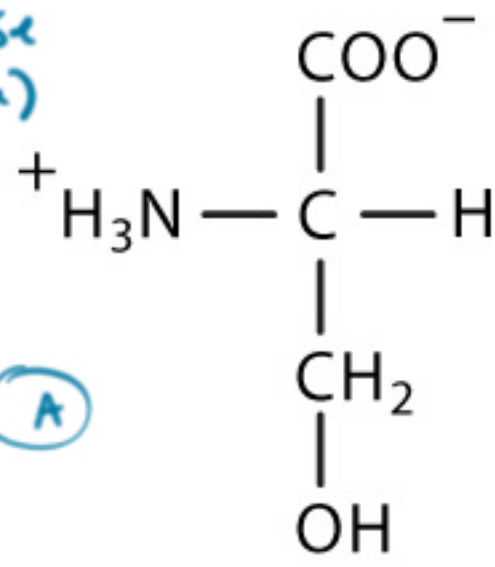
- hydroxyproline in collagen
- prolyl hydroxylase requires ascorbate (vitamin C)

P Serine S

- Often the target of phosphorylation by kinase (also threonine or tyrosine)

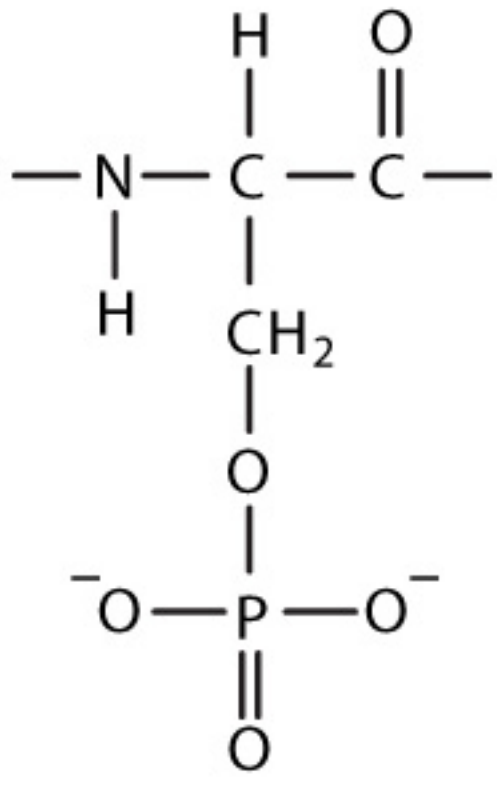
Kinase

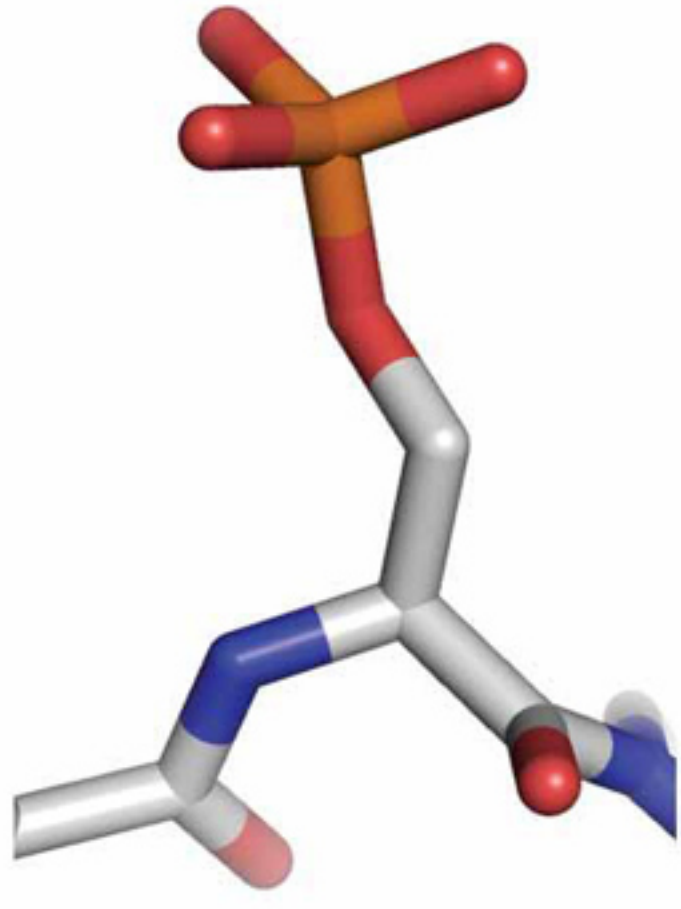
like SN2 across the phosphorus

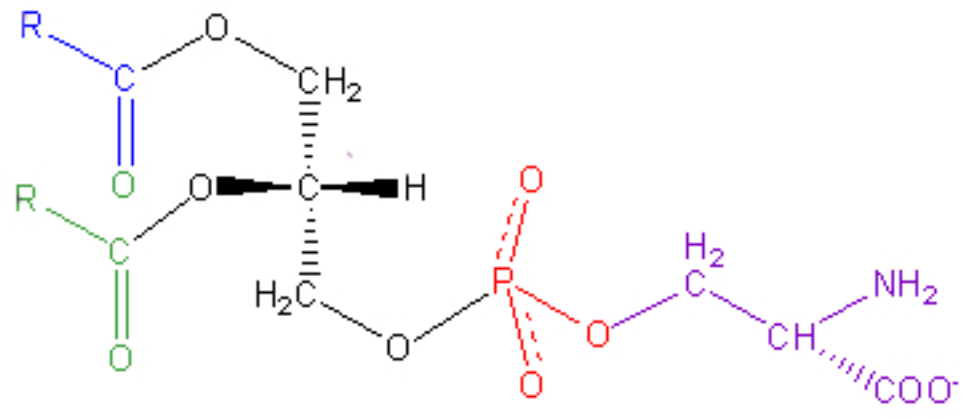


- also target of O-linked glycosylation
- precursor - part of phosphatidyl serine also sphingosine
- nucleophile in serine protease

Phosphorylated Serine

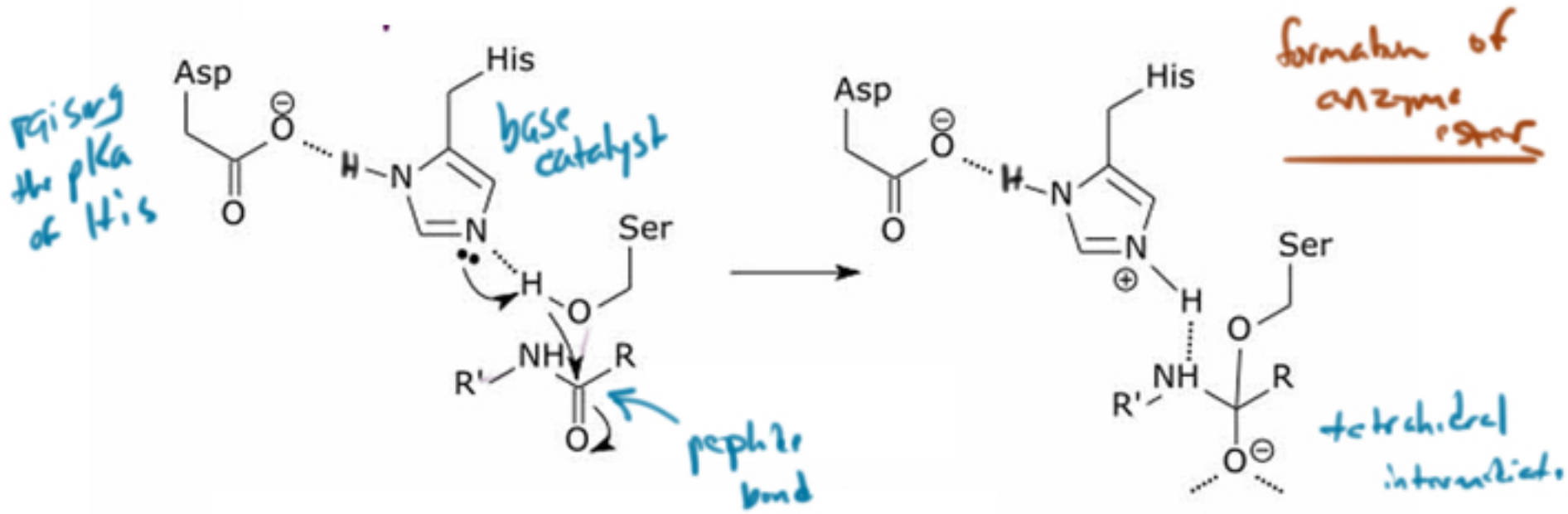


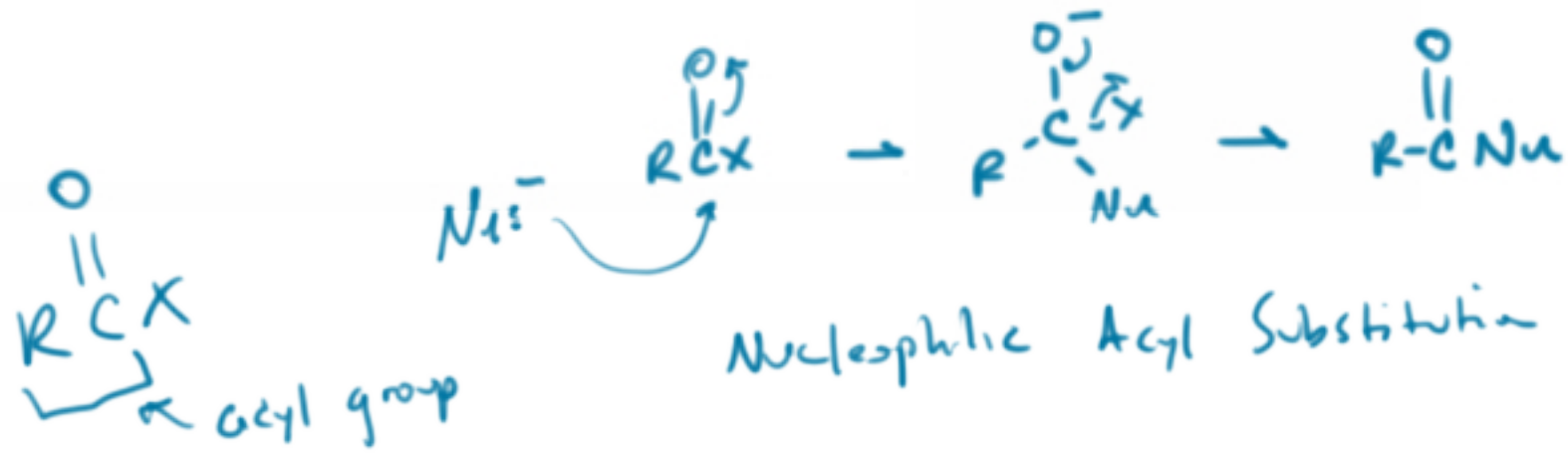




1 phosphatidate 1 serine 1

Serine protease such as chymotrypsin



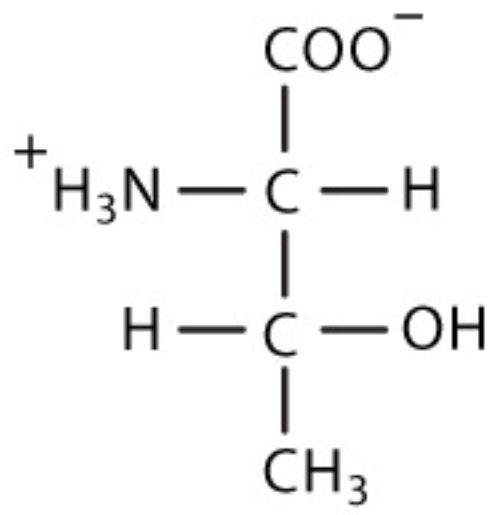


Nucleophilic Acyl Substitution

Threonine

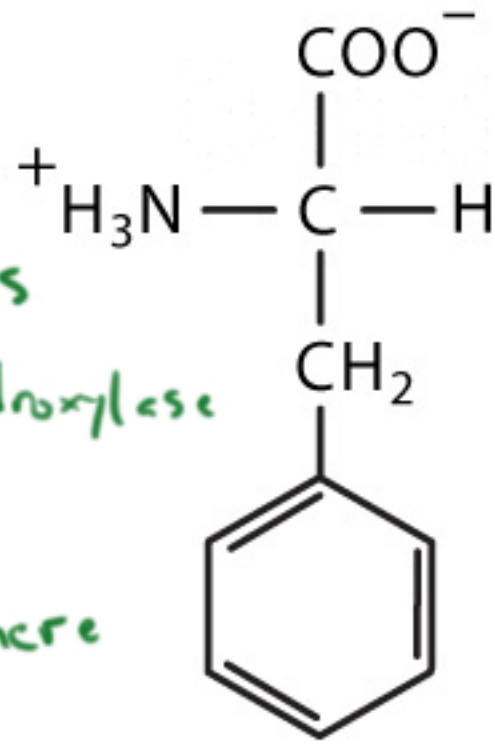
T

- Target of kinase
- Target of O-linked glycosylation



Phenylalanine

F



- Phenylalanine degradation - a few notable enzymes
  - phenylalanine hydroxylase
    F → Y
    genetic lesion here
    - homozygous - phenylketonuria
  - homogentisate oxidase
    - lesion leads to alcaptonuria
    - Archibald Garrod - one gene one enzyme

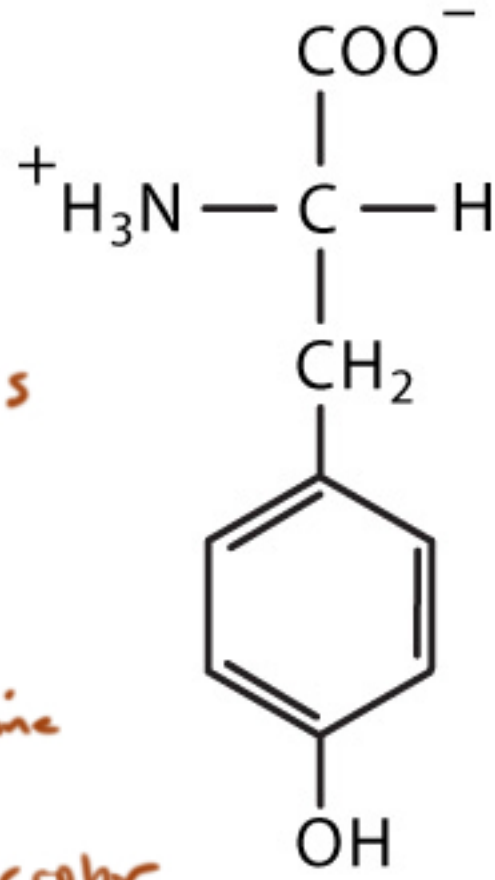
- Hydrophobic
- Membrane anchor

Phenylalanine
Hydroxylase

Tyrosine

Y

- Target of kinase
  - tyrosine kinase
  - catalytic receptors
    - growth factor receptors
      - intrinsic receptor tyrosine kinase
    - cytokine receptors
      - nonreceptor tyrosine kinase



- Hydrophobic (a bit of a gray area)
- Precursor of catecholamines epinephrine, norepinephrine dopamine

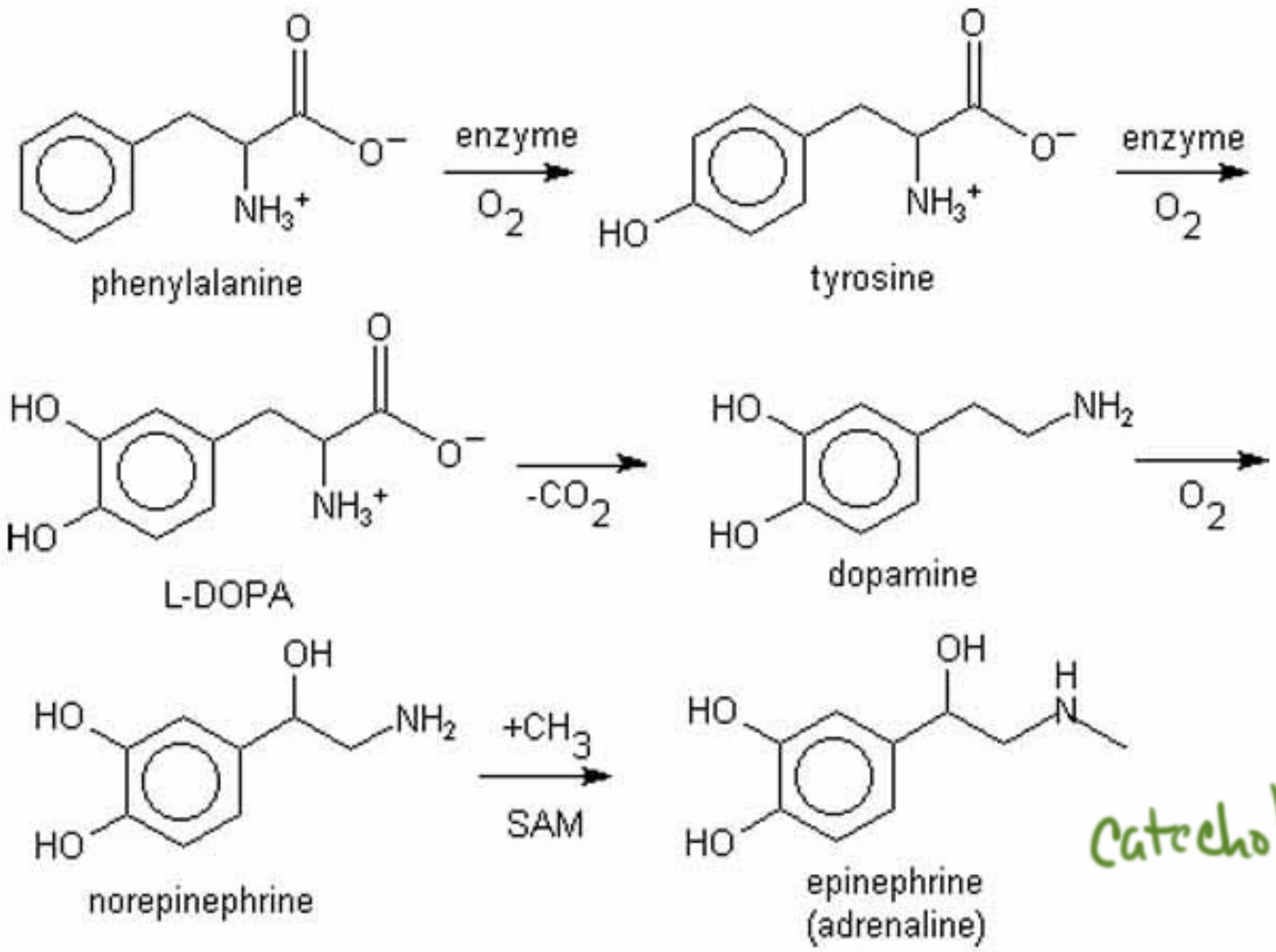
pKa ~10

GFR

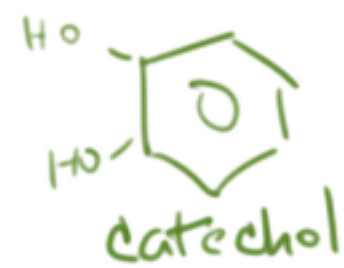
1) ligand binding leads to receptor dimerization

transautophosphorylation

- Phosphorylated tyrosines serve as docking sites for signaling proteins activating them.



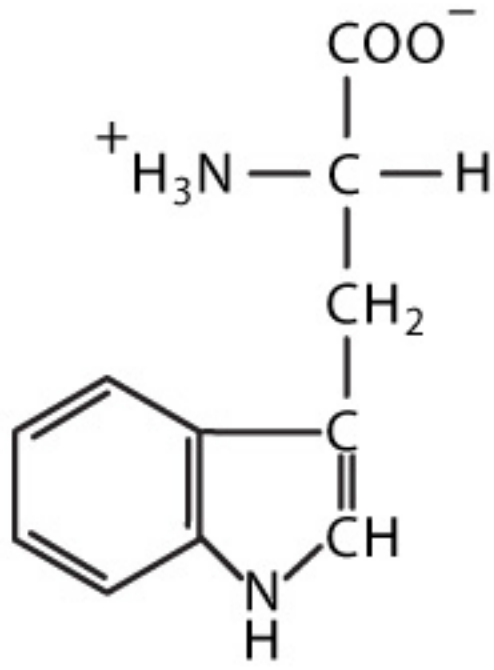
catecholamines



Tryptophan

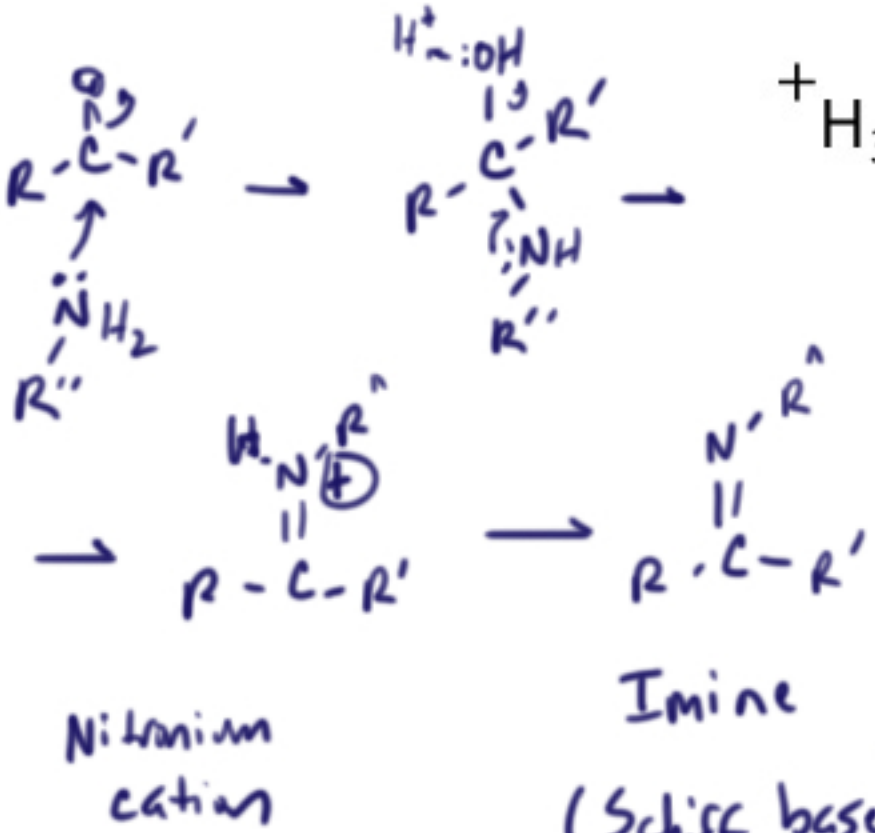
W

- Precursor of serotonin, melatonin



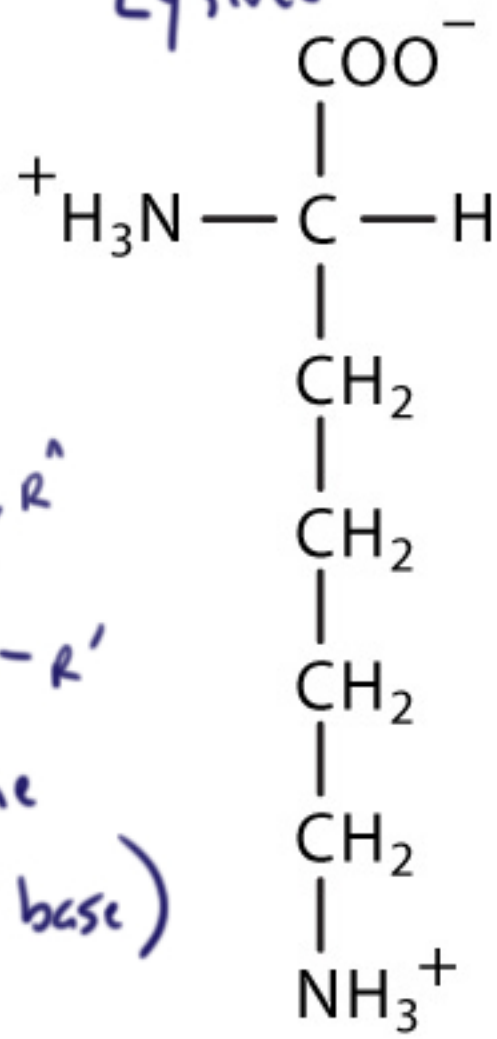
- Hydrophobic
- Membrane Anchor

Imine Formation

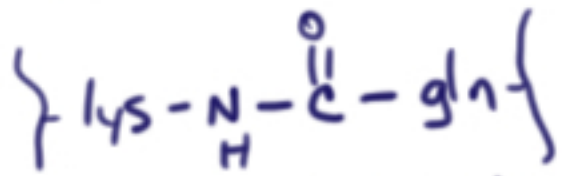


Lysine

K



- Reliable ⊕
- often seen forming Schiff base with substrate.
- can also form amide linkages



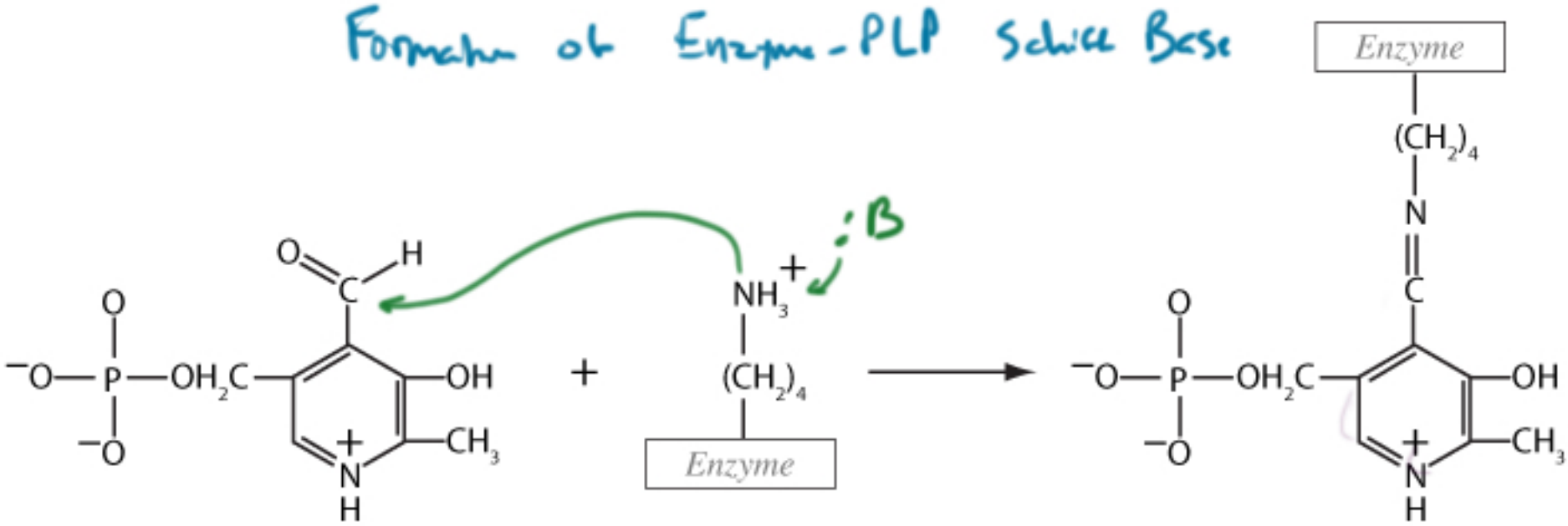
- amide linkage in a hard clot

pKa ~ 10.5

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

7.4 $\quad 10.5 + \log\left(\frac{1}{1000}\right)$

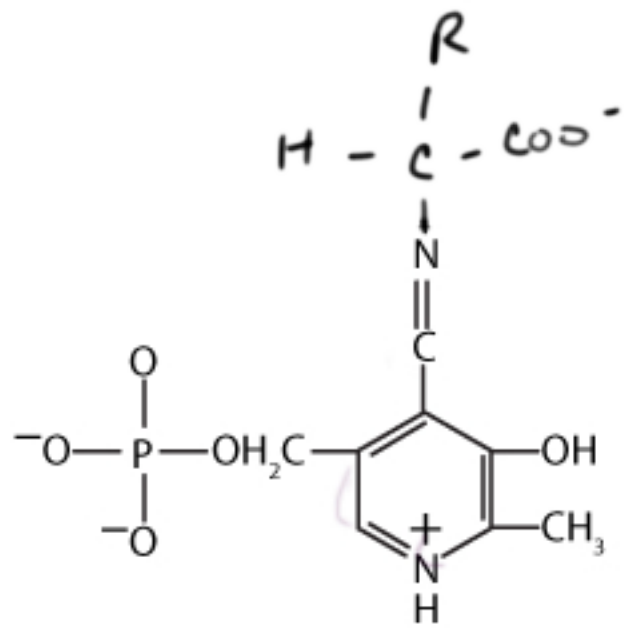
Formation of Enzyme-PLP Schiff Base



PLP (vitamin B6)

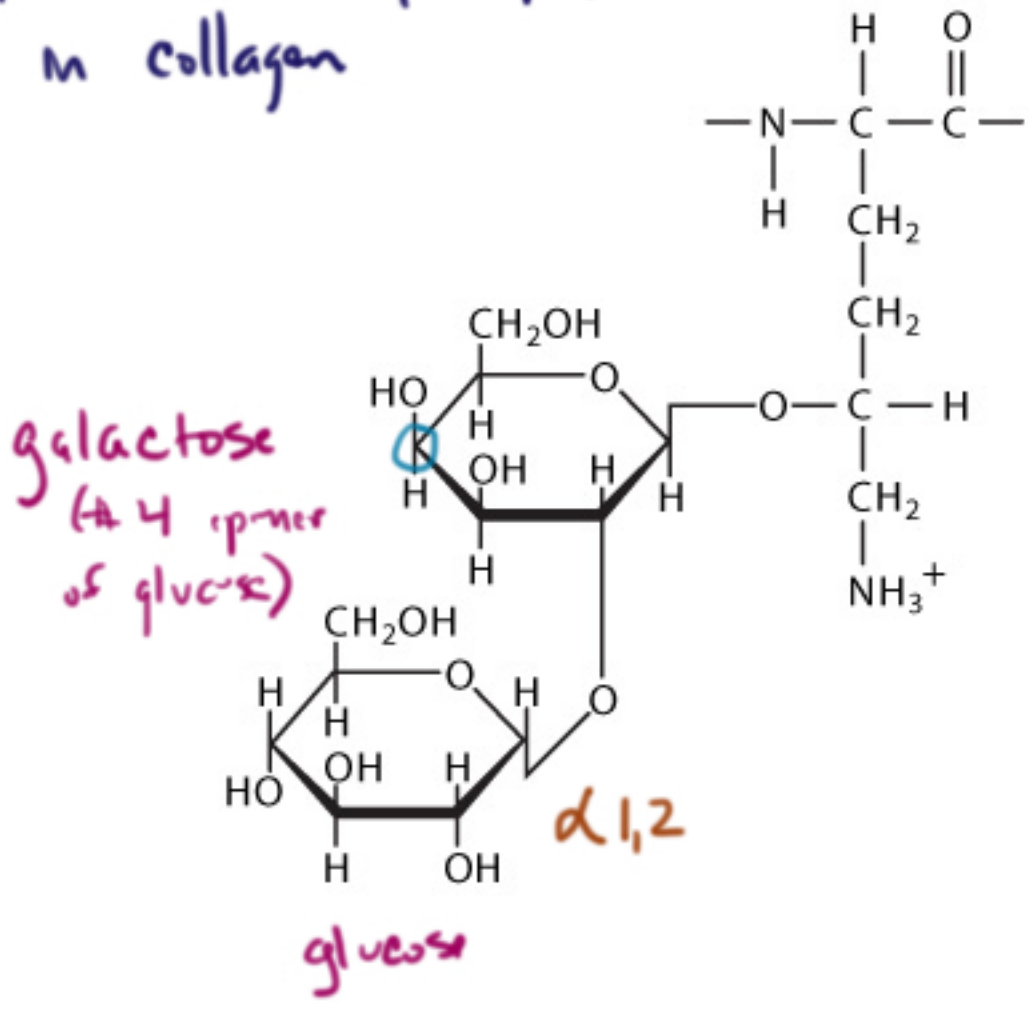
pyridoxyl phosphate

"the amino acid chemistry coenzyme"



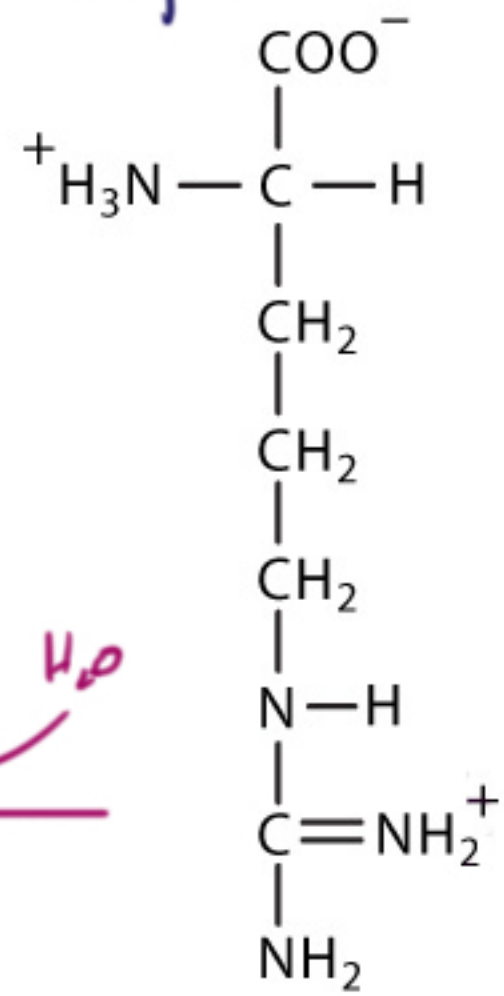
PLP-amino acid Schiff base

Glycosylated Hydroxylysine
in collagen



Arginine

R



- reliable ⊕
- salt bridges and substrate binding

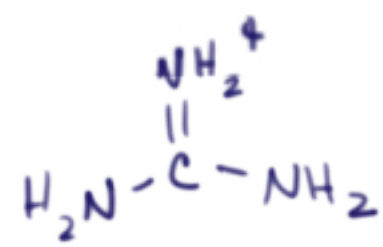
H_2O

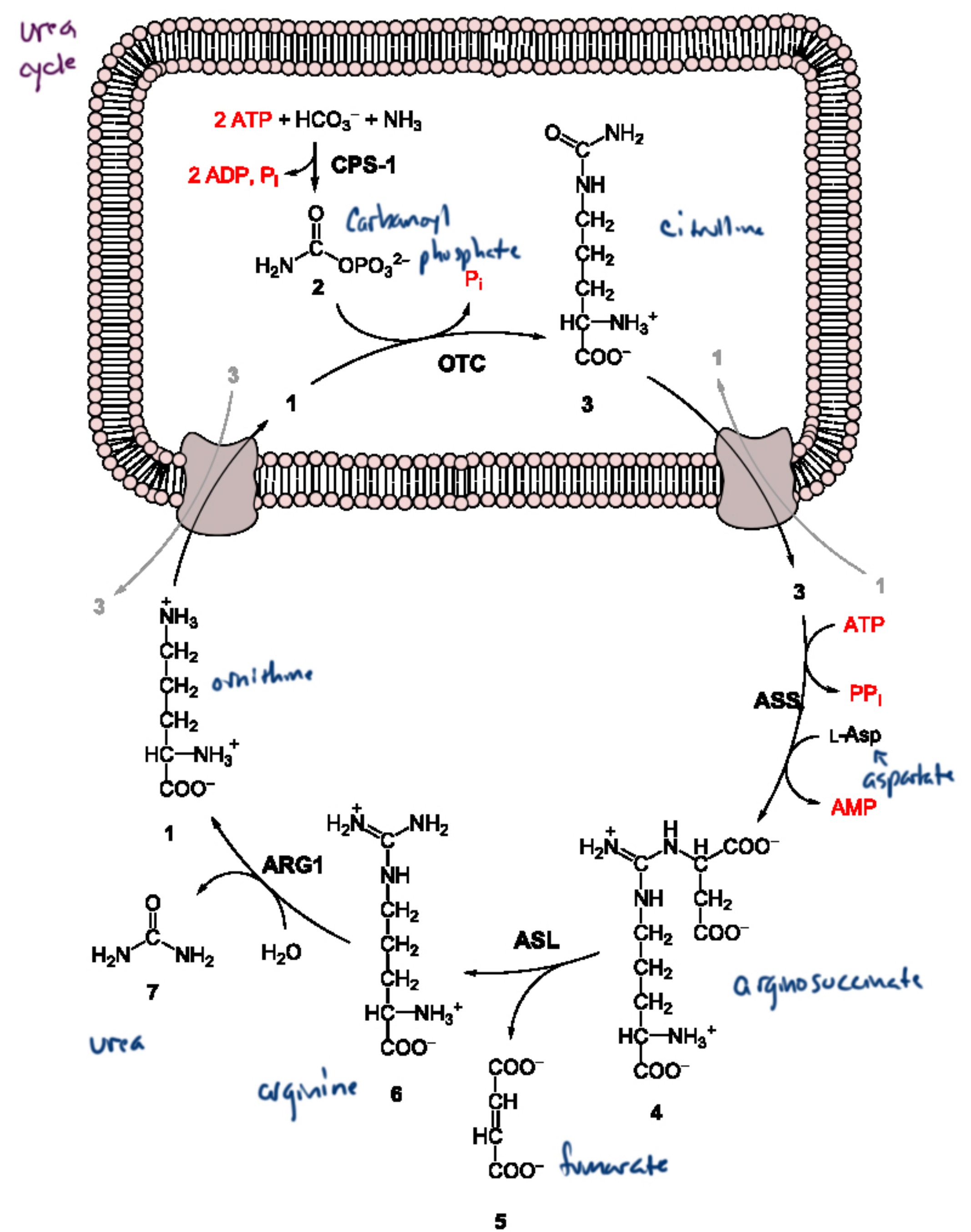
ornithine + $H_2N-C(=O)-NH_2$

urea

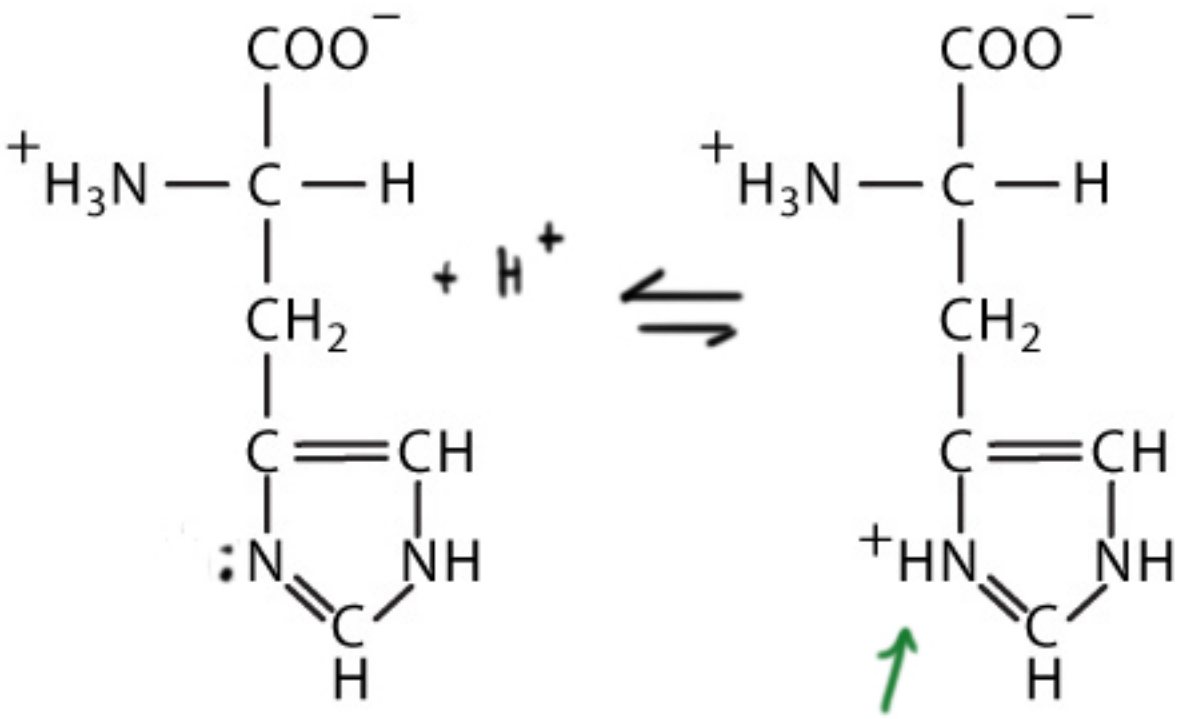
pKa ~ 12

guanadinium group




urea cycle
2 ATP + HCO3⁻ + NH3
2 ADP, Pi
CPS-1
Carbamoyl phosphate
2
Pi
OTC
1
3
citrulline
3
1
3
1
ATP
PPi
ASS
L-Asp
aspartate
AMP
ornithine
1
ARG1
H2O
7
urea
arginine
6
ASL
5
fumarate
4
argininosuccinate

Histidine



$pK_a \sim 6.0$

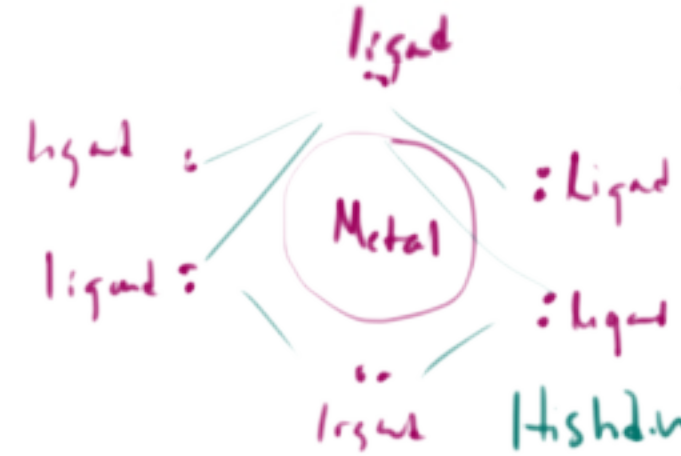
- at physiological pH about one in twenty five are protonated

asp is raising the pKa of His

- important acid-base catalyst

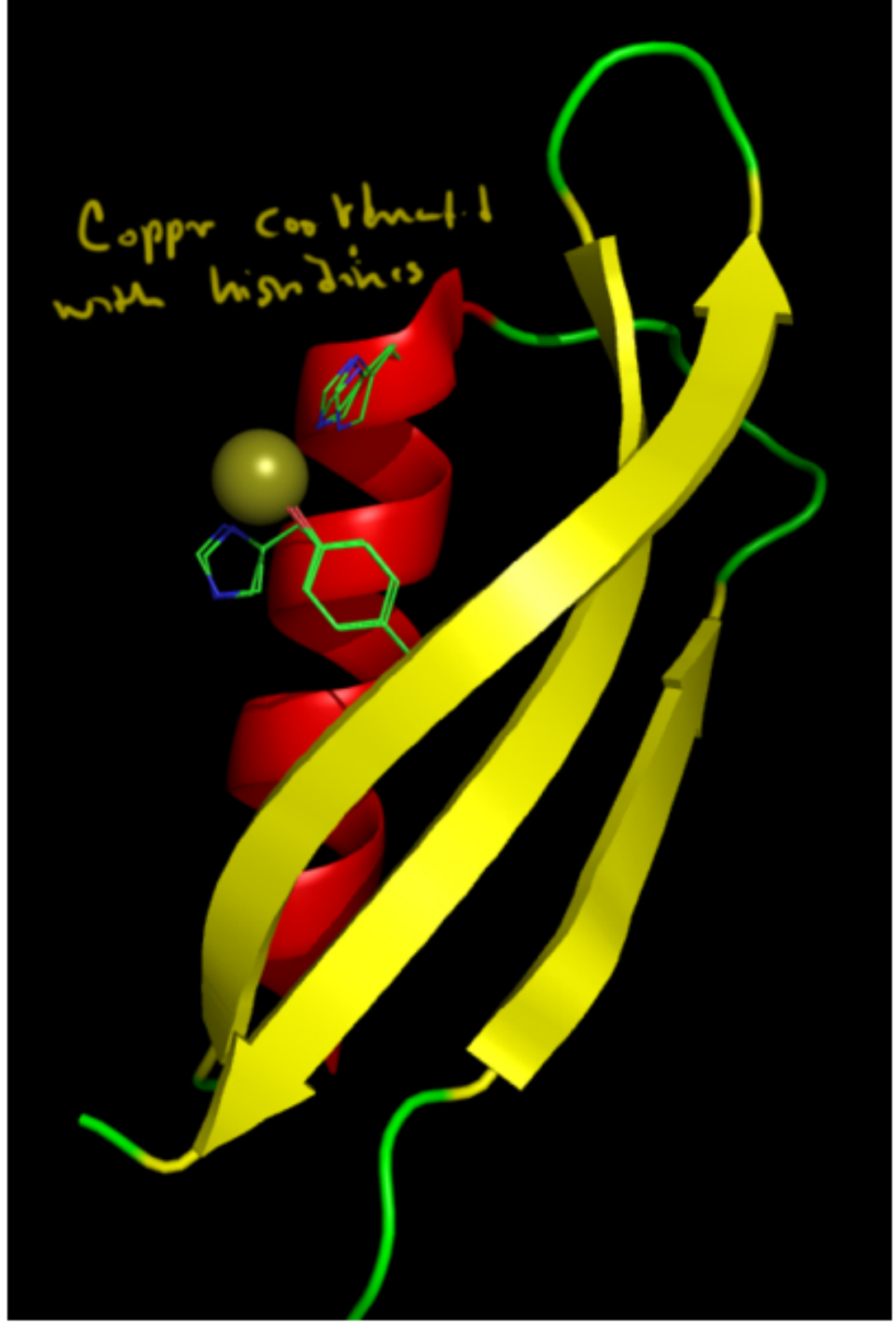
$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

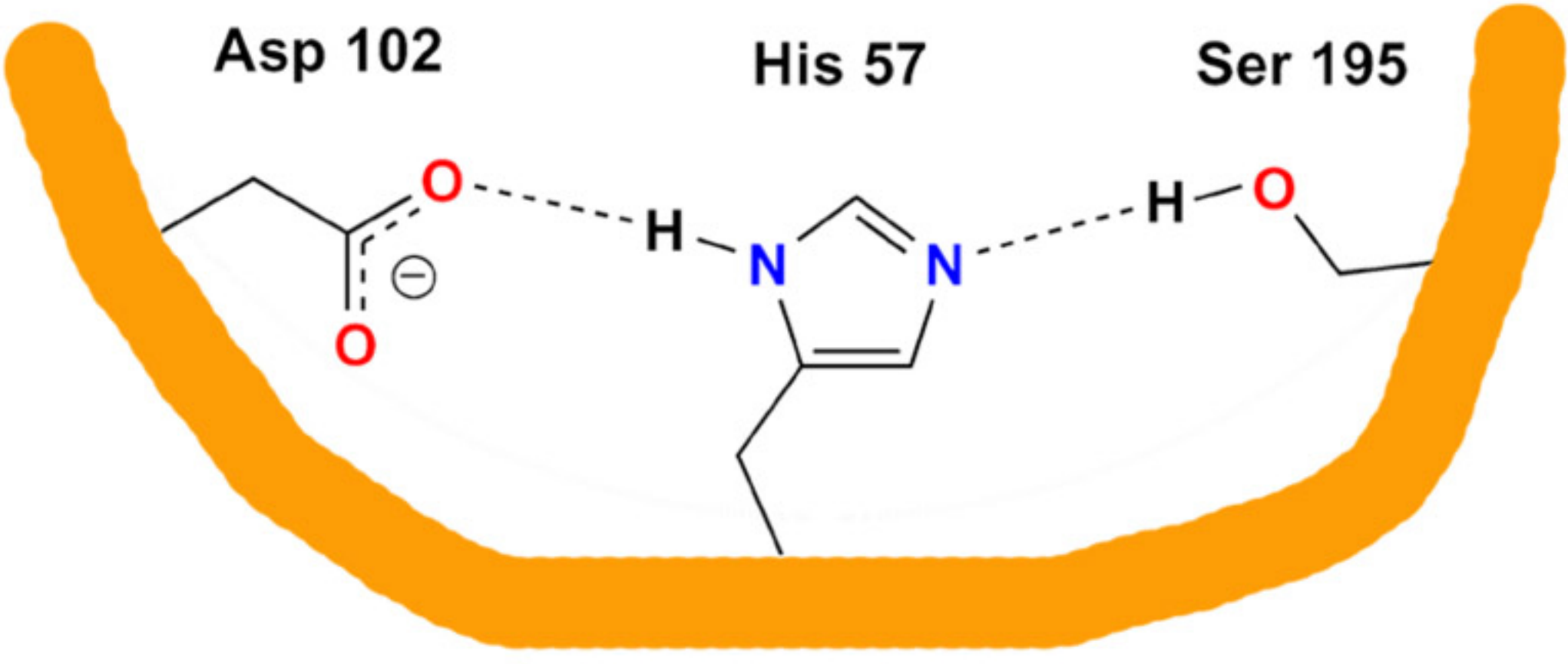
$$7.4 = 6.0 + \log\left(\frac{25}{1}\right)$$



- coordination complex

Histidine binds metals

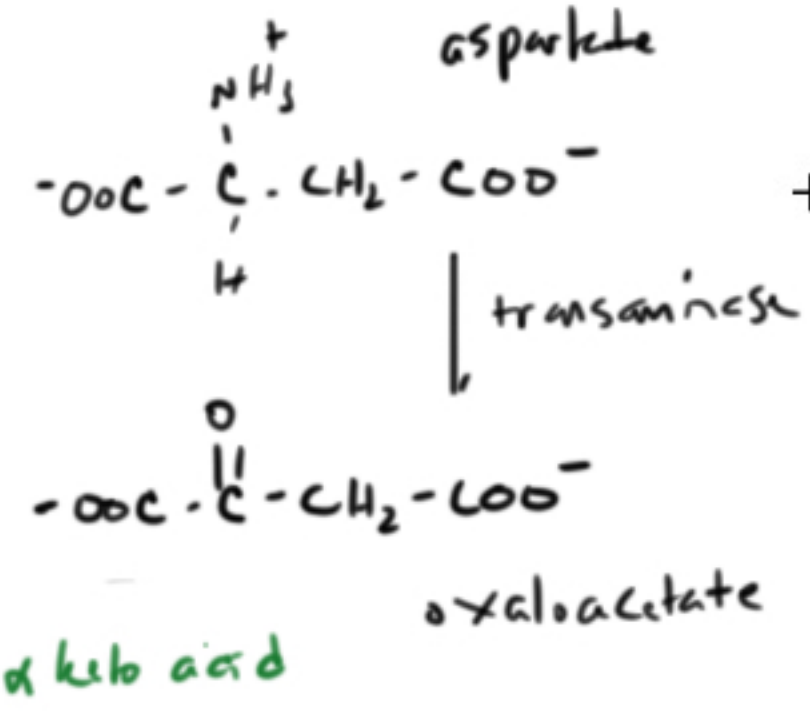

Copper coordinated
with histidines

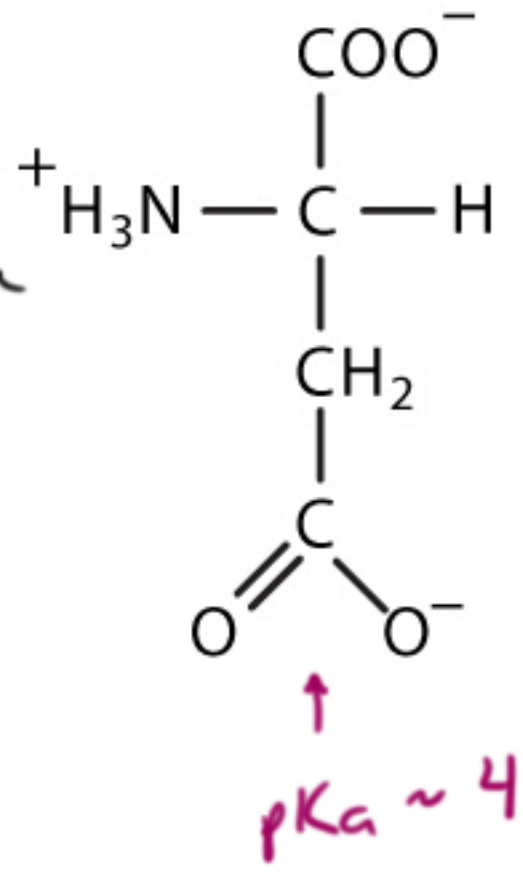

Asp 102
His 57
Ser 195
O
O
H
N
N
H
O

Aspartate

D

α amino acid



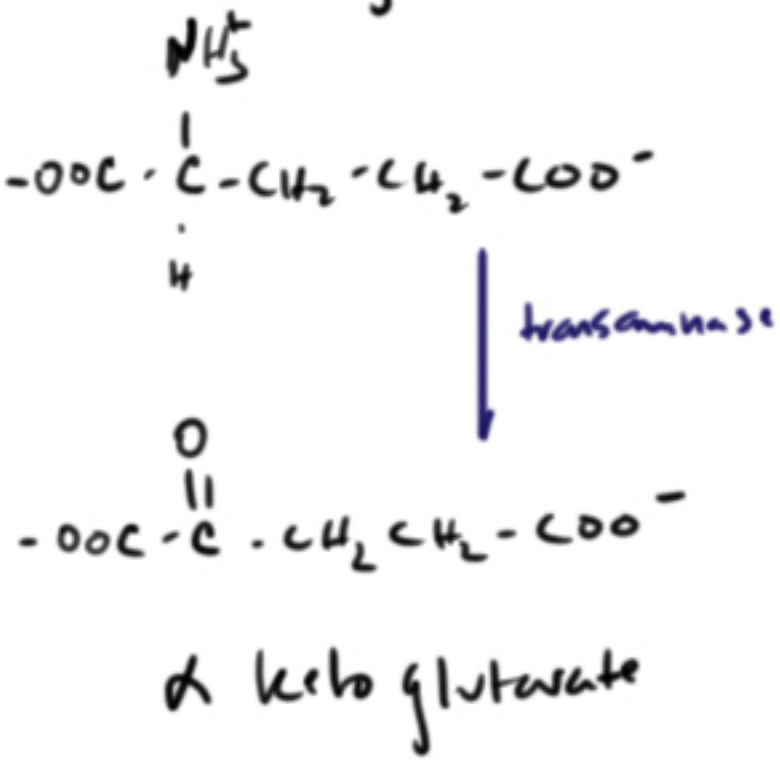


- reliable ⊖
- acid base catalyst
- carboxylate nucleophile
- salt bridges

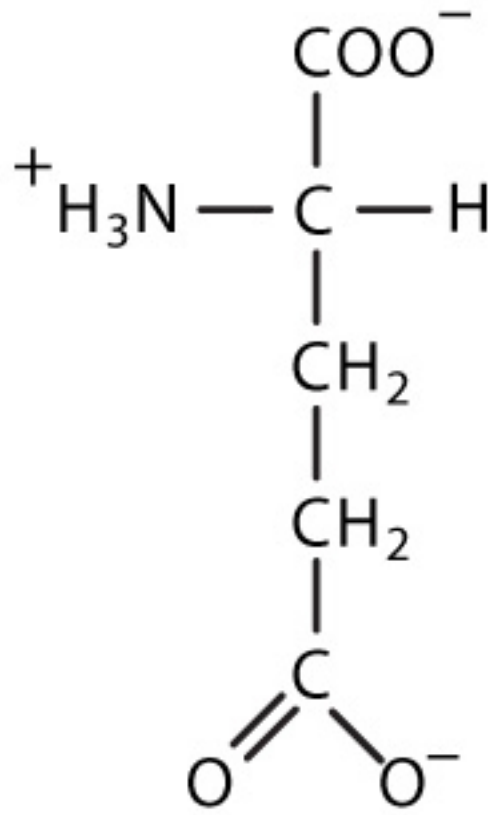
E

Glutamate

glutamate



α keto glutarate



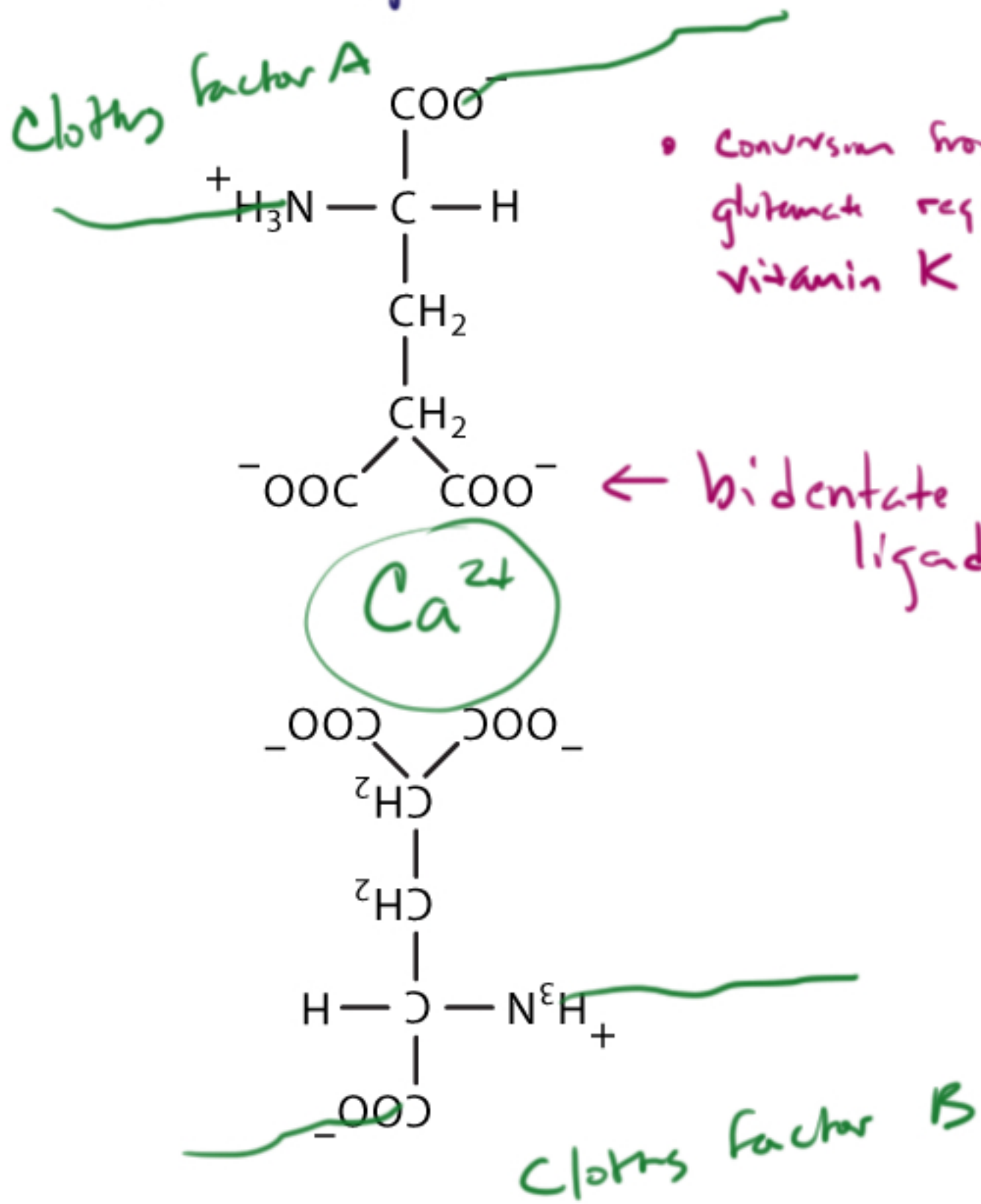
• reliable ⊖

pKa ~ 4

L amino acid$_a$ ⟶ α keto acid$_a$

α keto acid$_b$ ⟶ L amino acid$_b$

γ carboxyglutamate

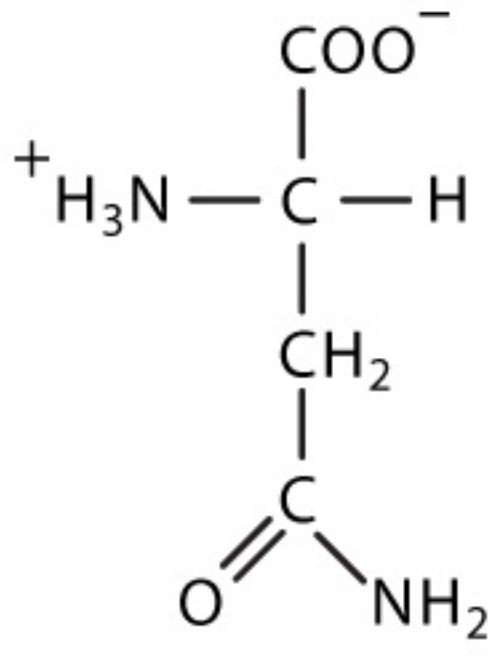


- conversion from glutamate requires vitamin K

← bidentate ligand

Asparagine

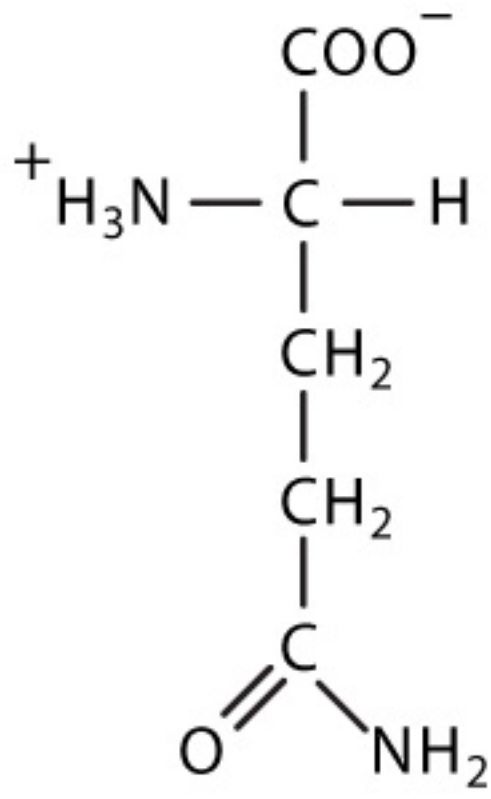
N



- H bonding - often to backbone is important for protein structure

Glutamine

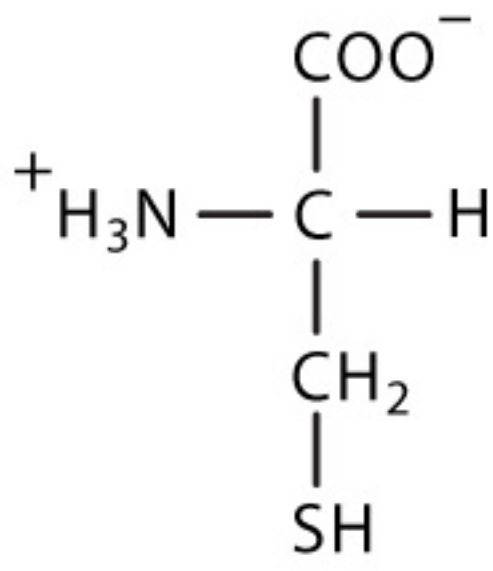
Q



- H bonding
- N source in biosynthesis

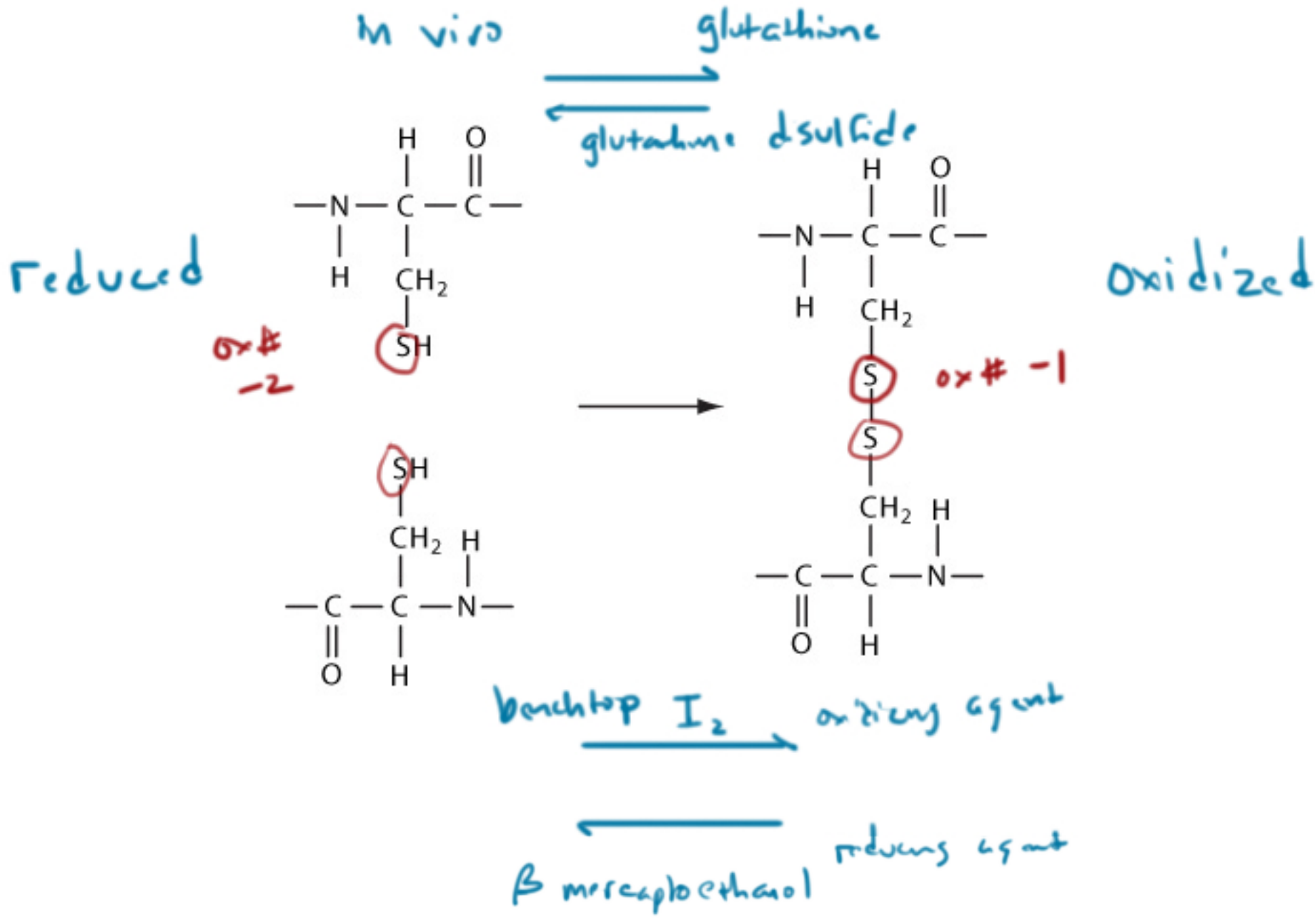
Cysteine

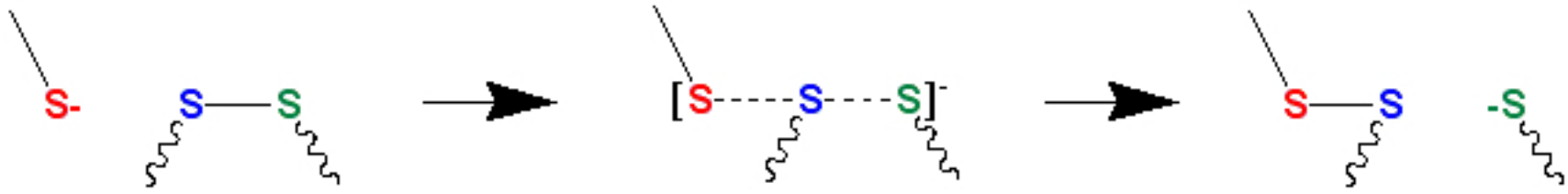
C



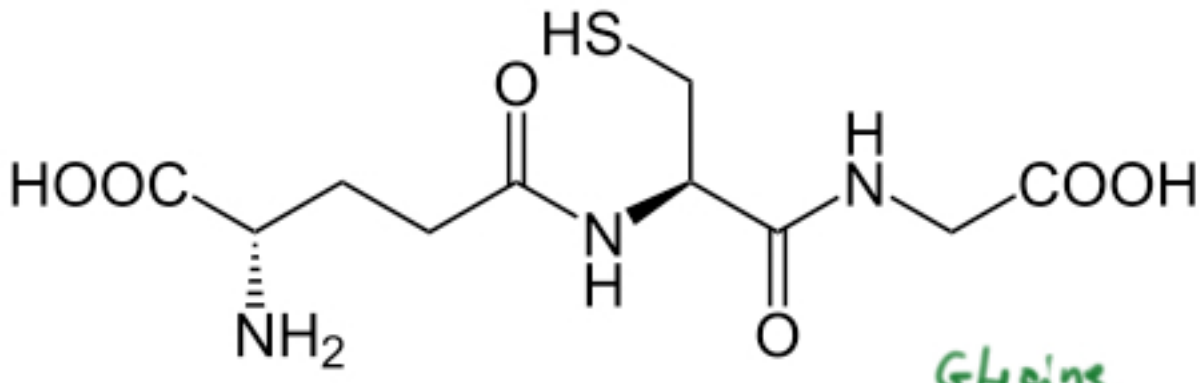
pKa ~ 8.1

- Forms disulfide bridges (covalent)
- Nucleophile
- Ligand in coordination complexes


in vivo
glutathione
glutathione disulfide
reduced
oxidized
ox # -2
ox # -1
benchtop I_2
oxidizing agent
reducing agent
β mercaptoethanol


S-
S
S
[S
S
S]-
S
S
-S

Glutathione



γ glutamate

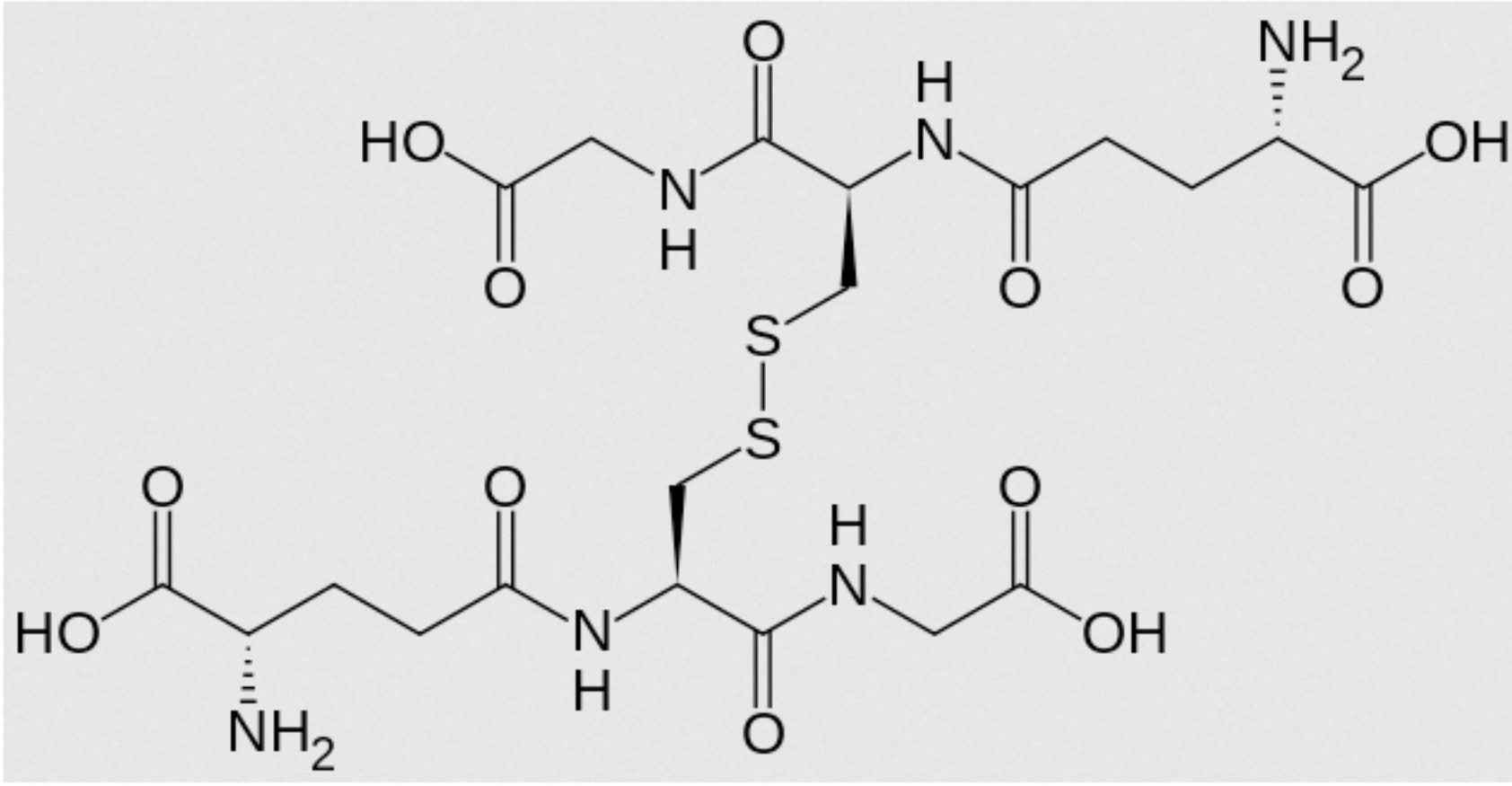
cystine

Glycine

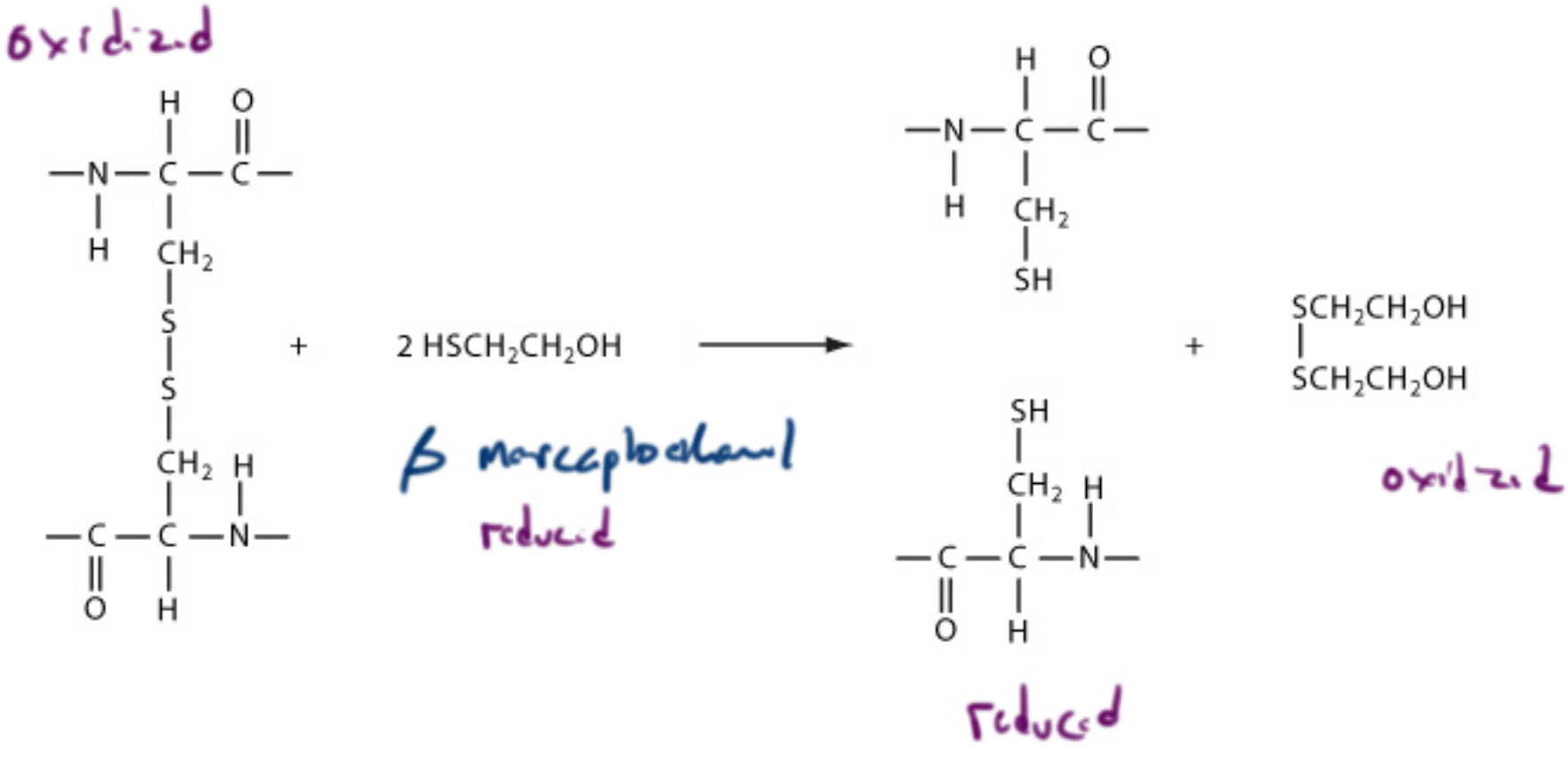
(Reduced)

- the most important redox buffer in the blood.

Glutathione Disulfide



(oxidized)

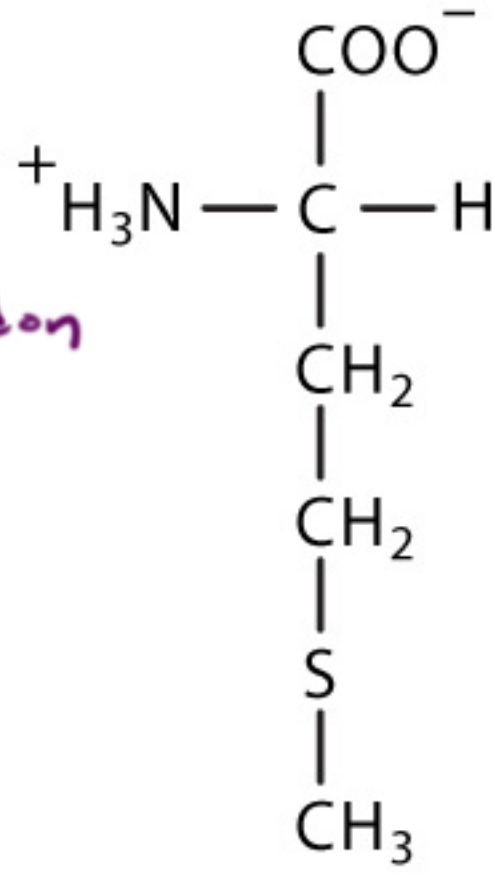

oxidized
2 HSCH2CH2OH
β mercaptoethanol
reduced
oxidized
reduced

Methionine

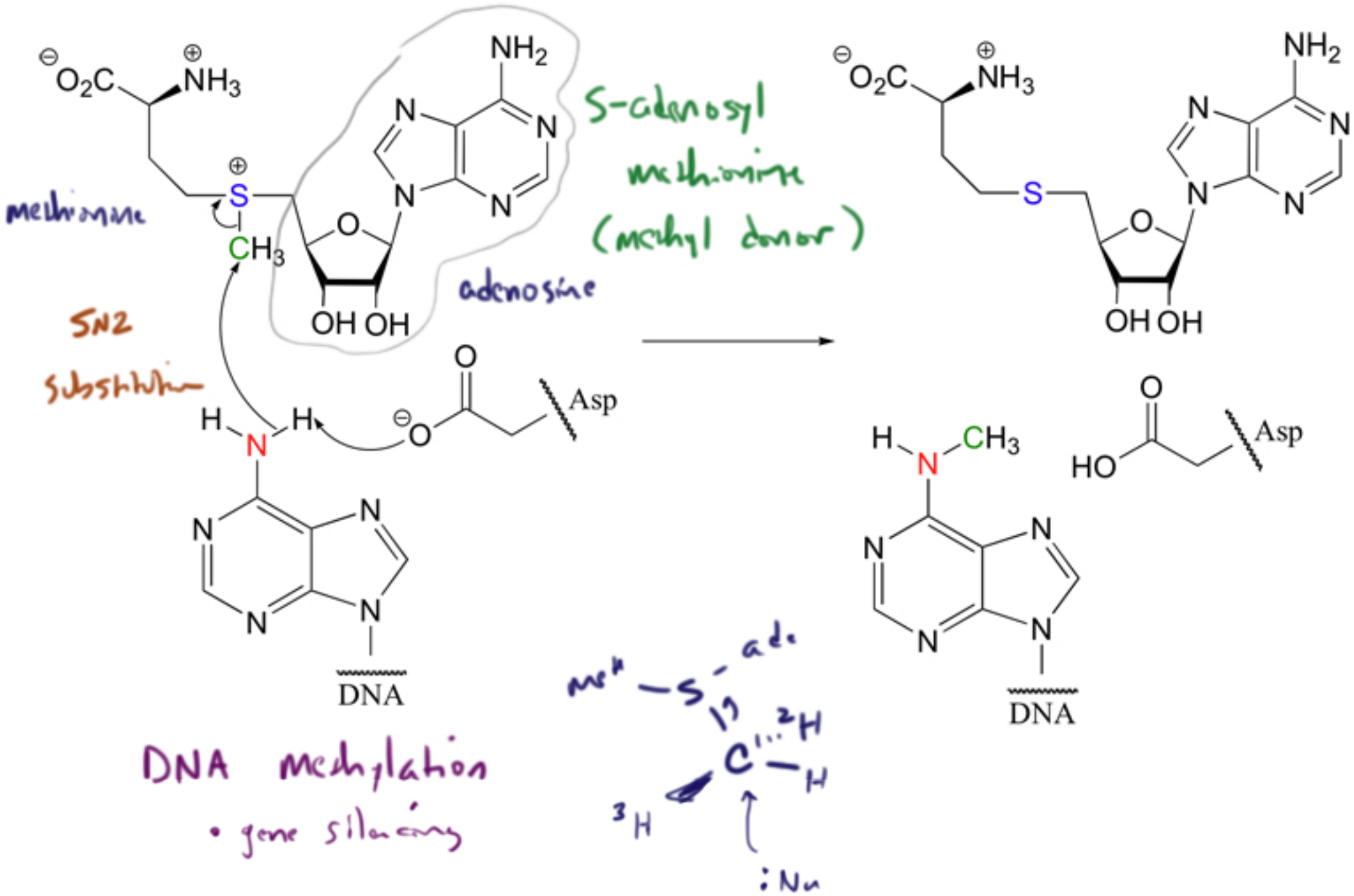
M

- AUG

↑
start codon
is the methionine codon

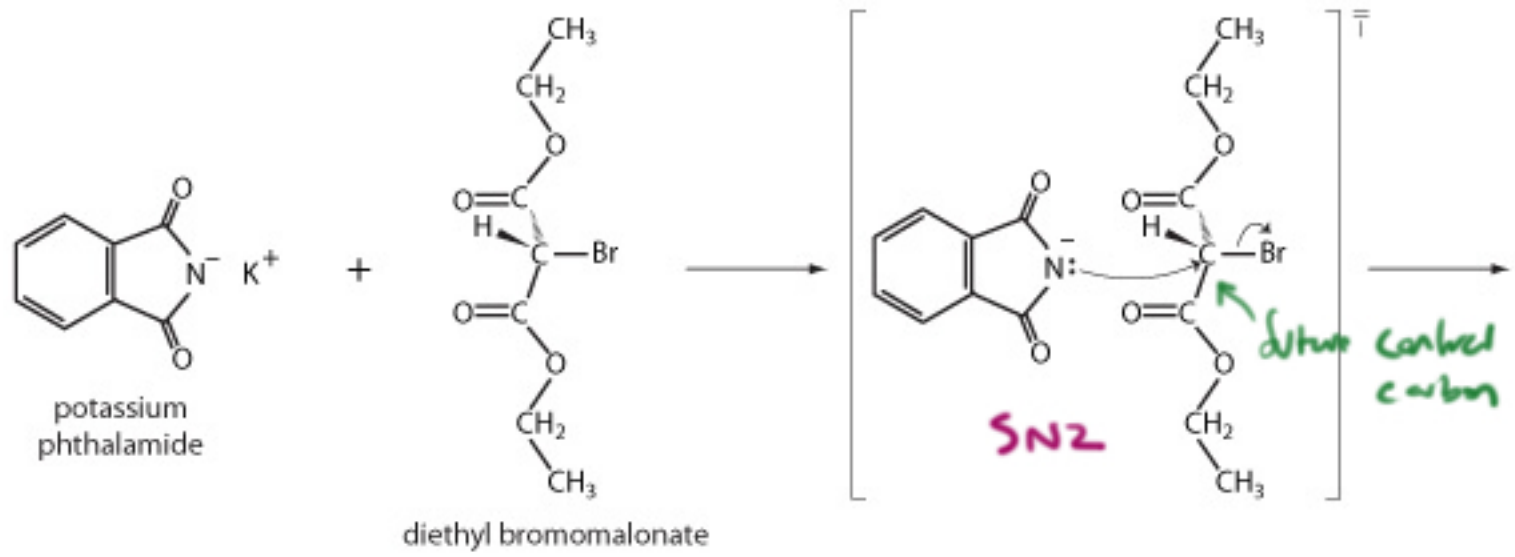


- nonpolar
- hydrophobic


S-adenosyl methionine (methyl donor)
methionine
adenosine
SN2 substitution
DNA methylation
• gene silencing
DNA
DNA

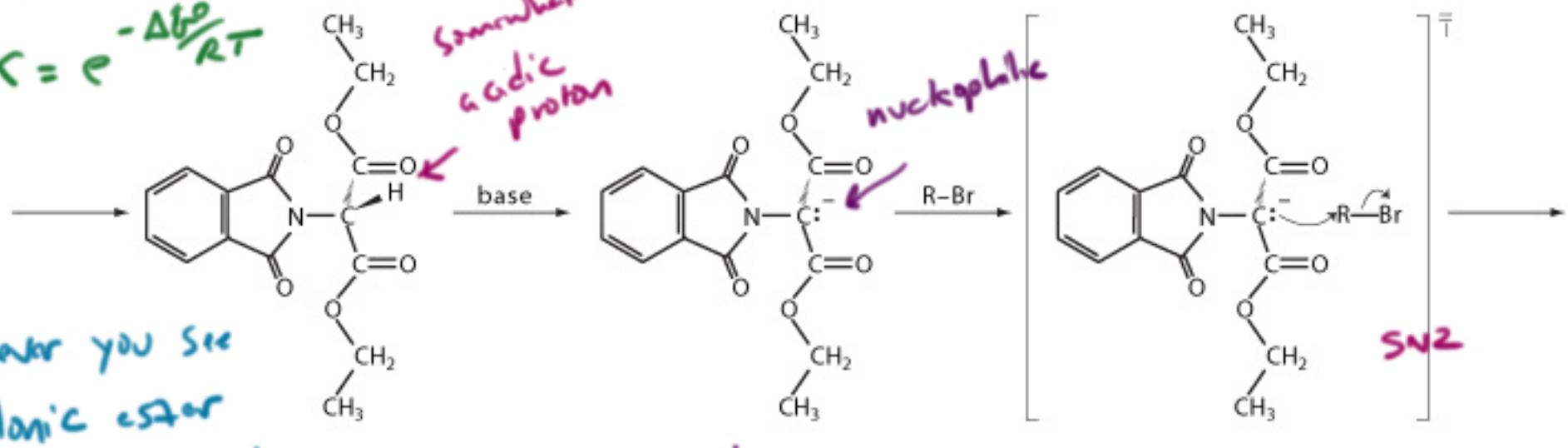
Gabriel Synthesis

- benchtop amino acid synthesis

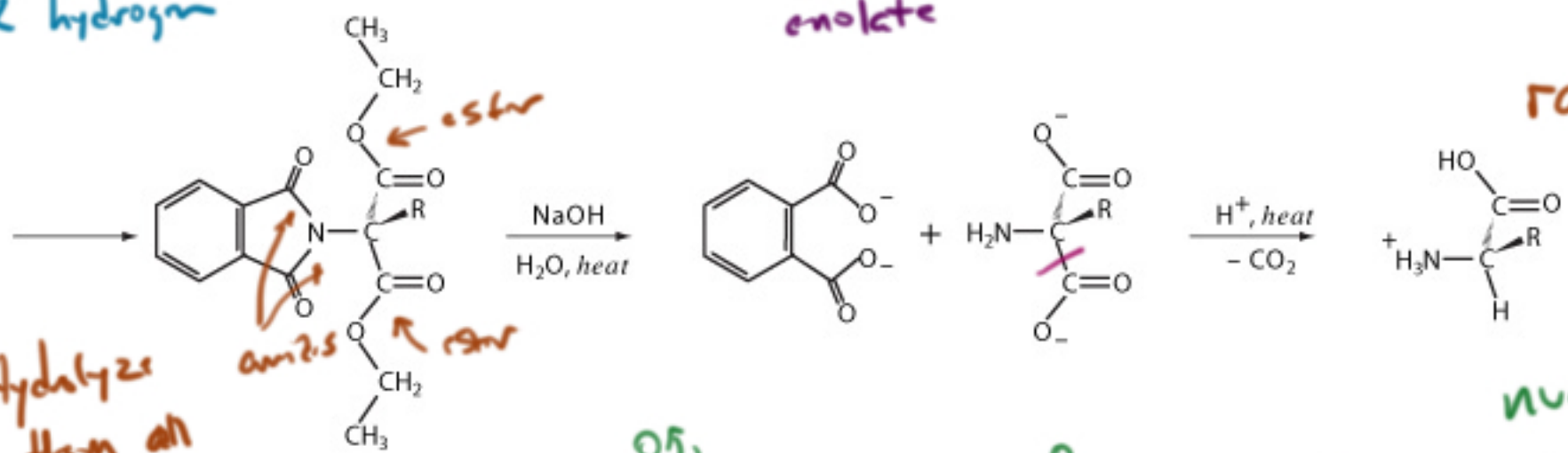


$HA \rightleftharpoons A^- + H^+$

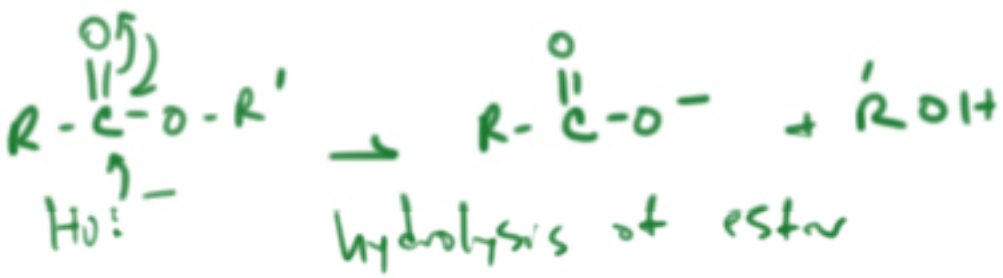
$K = e^{-\Delta G^\circ / RT}$



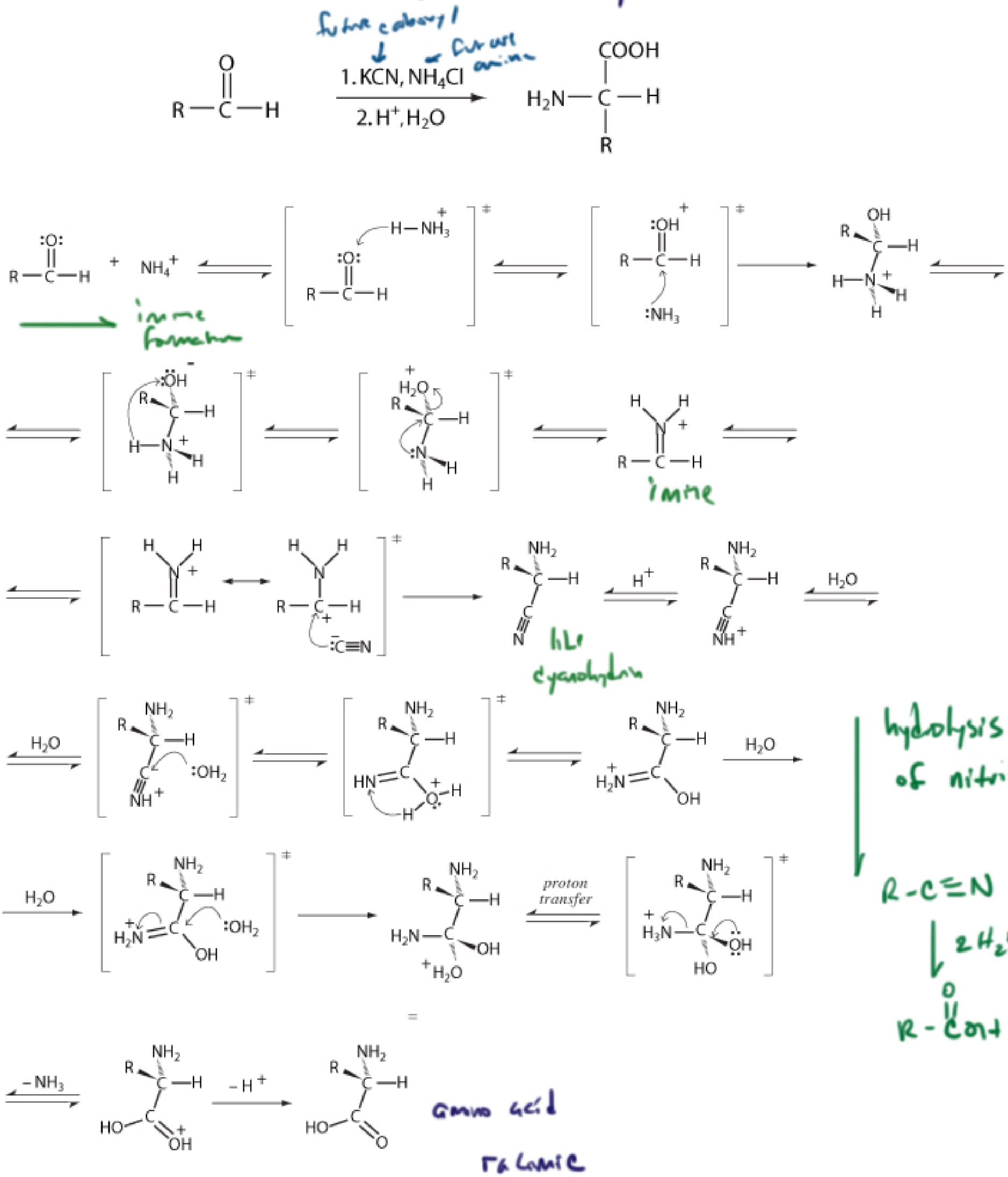
whenever you see malonic ester you have a great α hydrogen

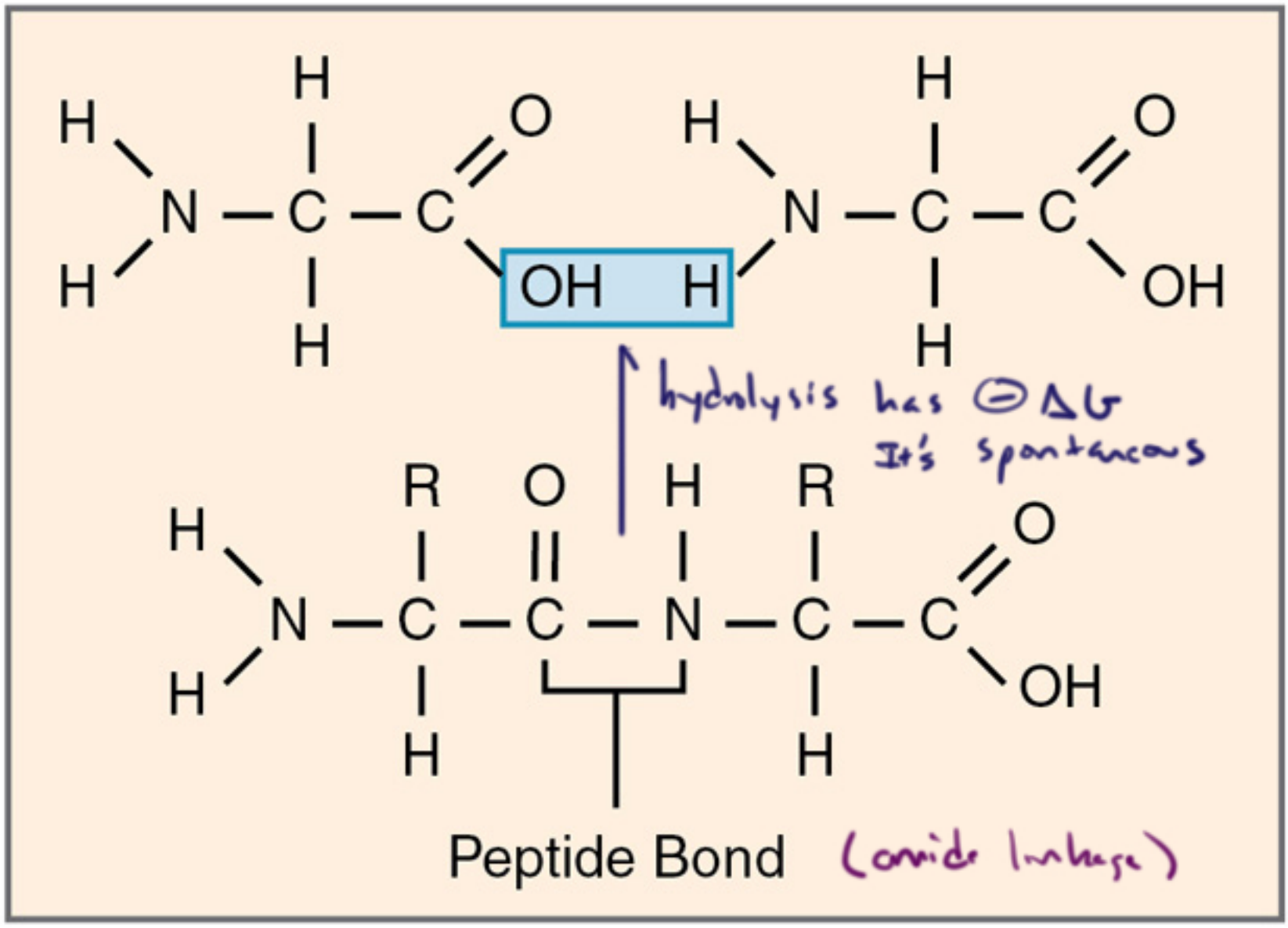


Hydrolyze them all

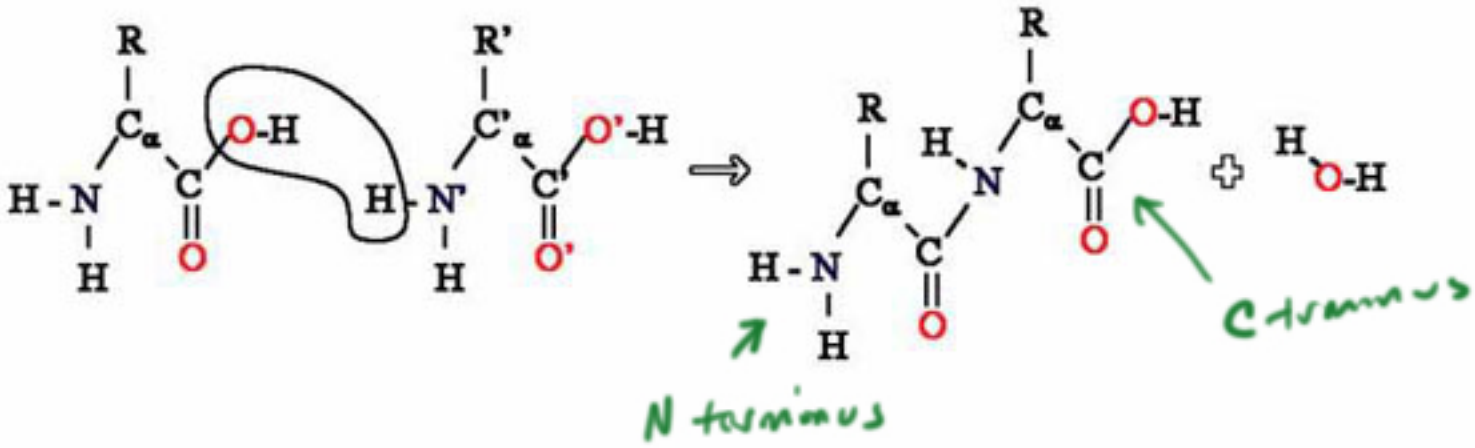


Strecker Synthesis





Proteins are not thermodynamically stable
but they are kinetically stable



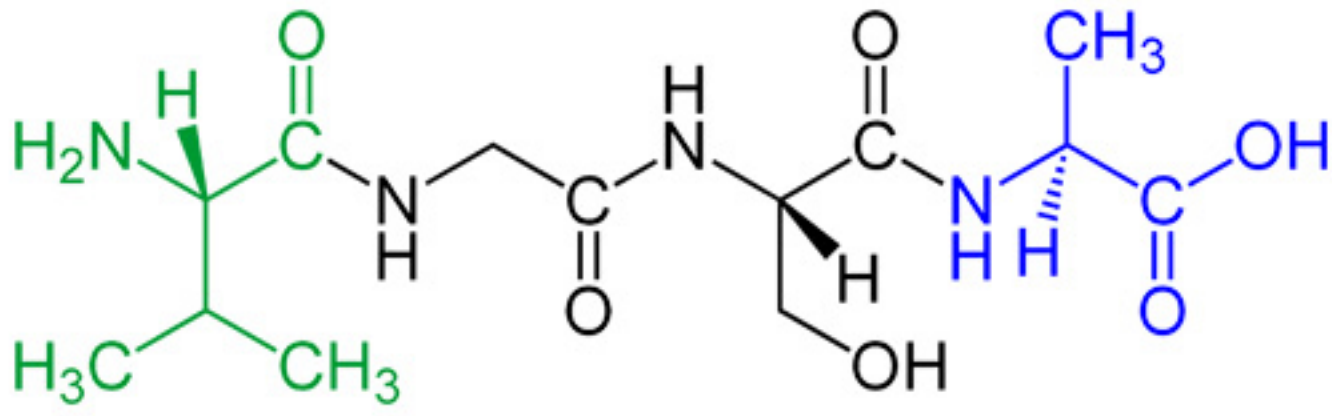
Orienting a polypeptide

1) Find the N terminus and C terminus

2) Continue backbone
N-C-C-N-C-N

3) identify side chains

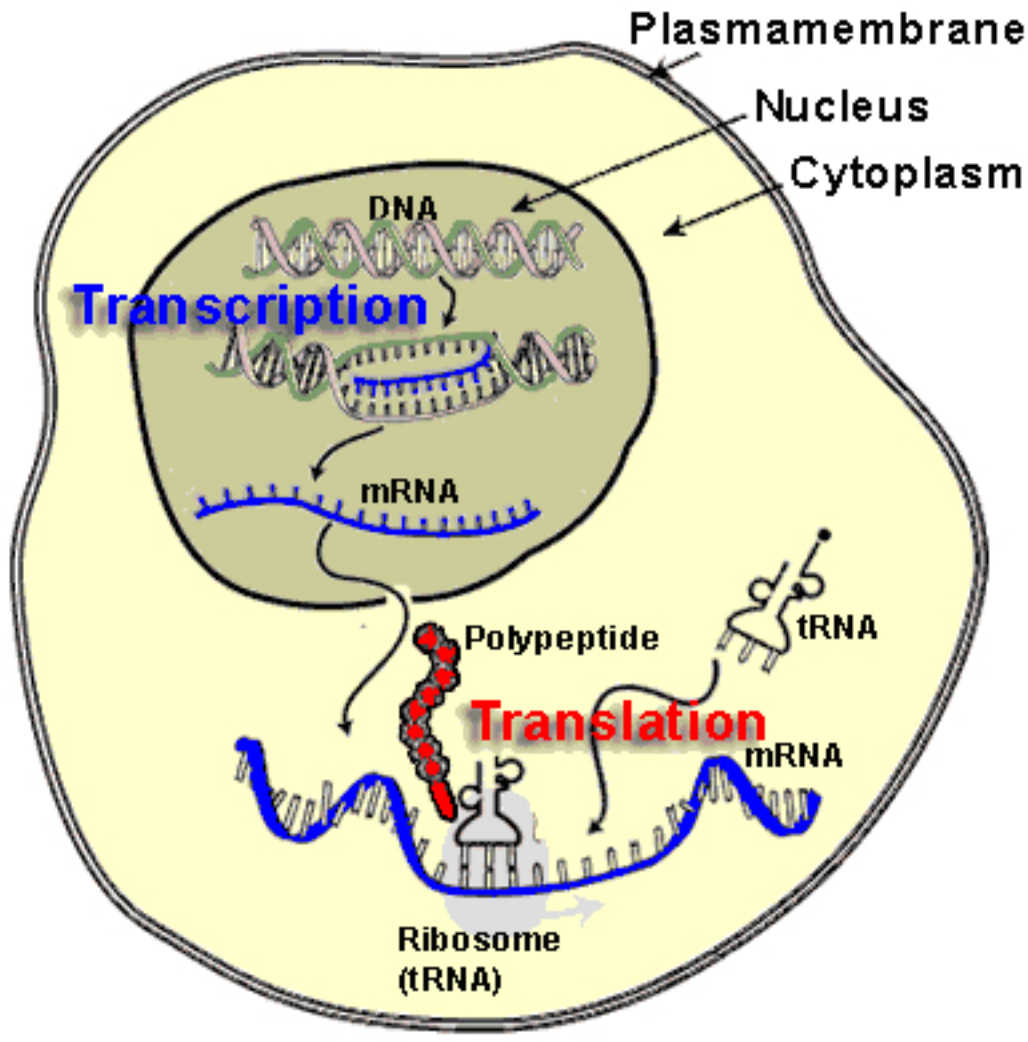
Tetrapeptide

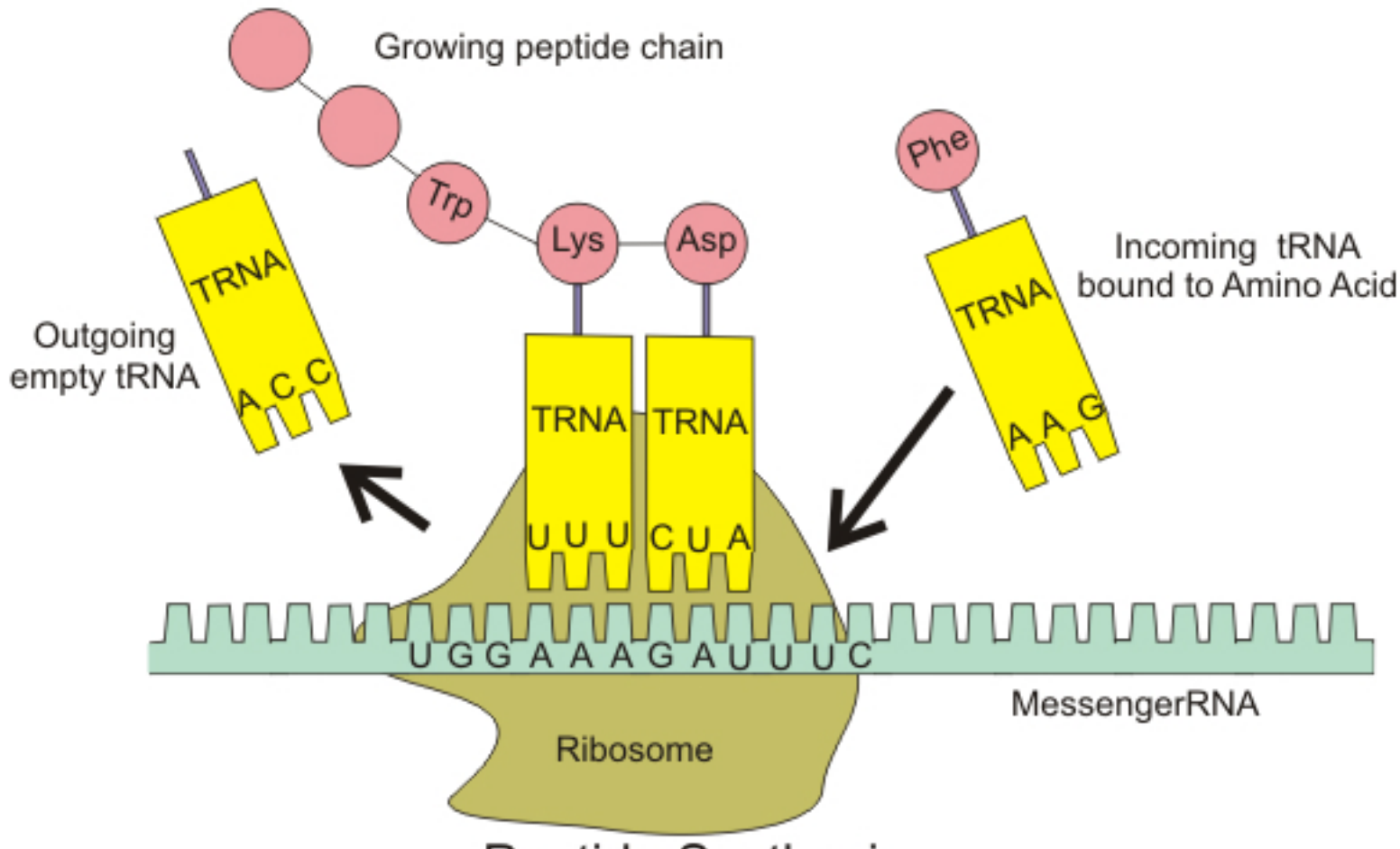


V G S A

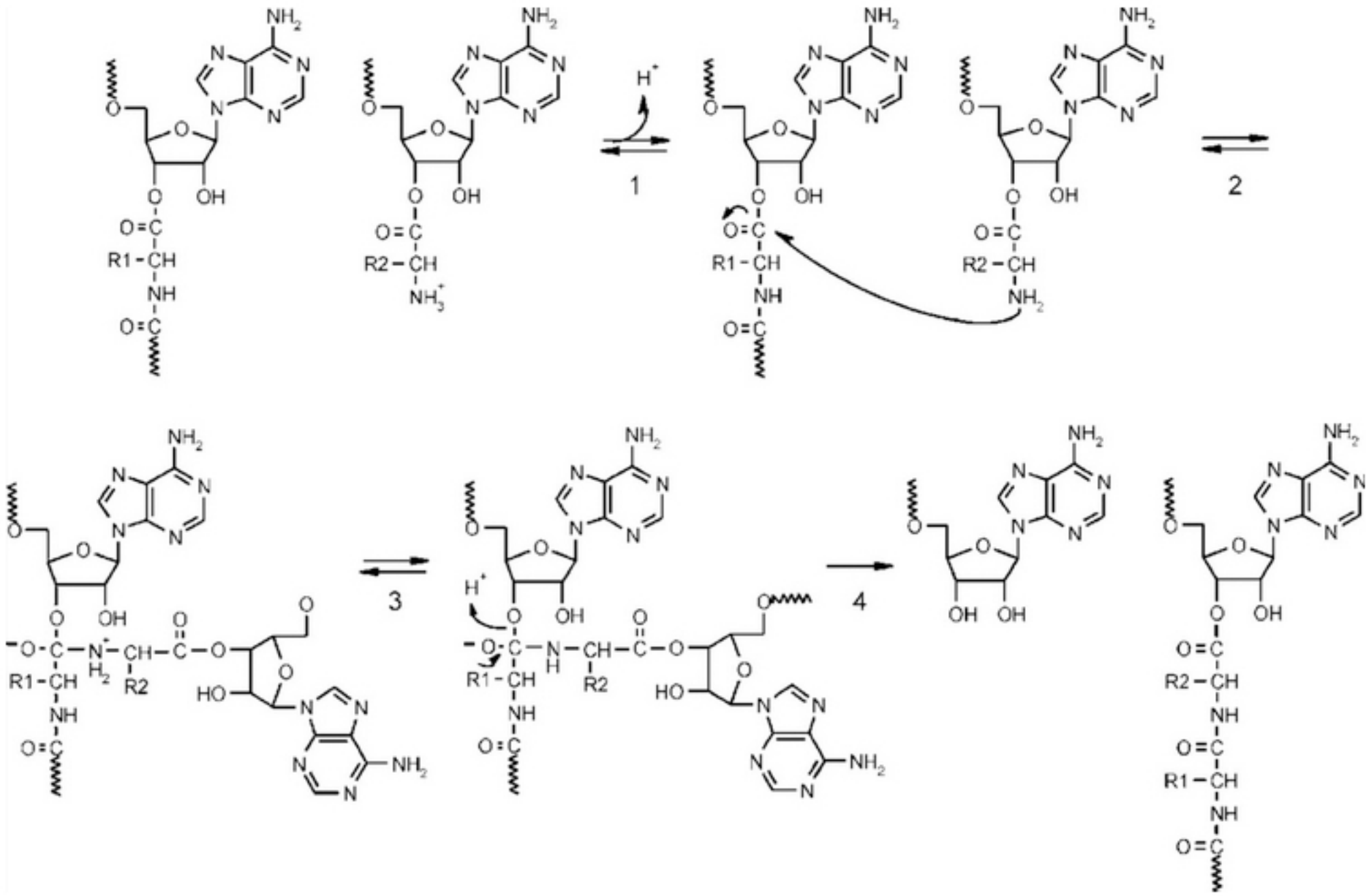
Central Dogma

Transcription
&
Translation

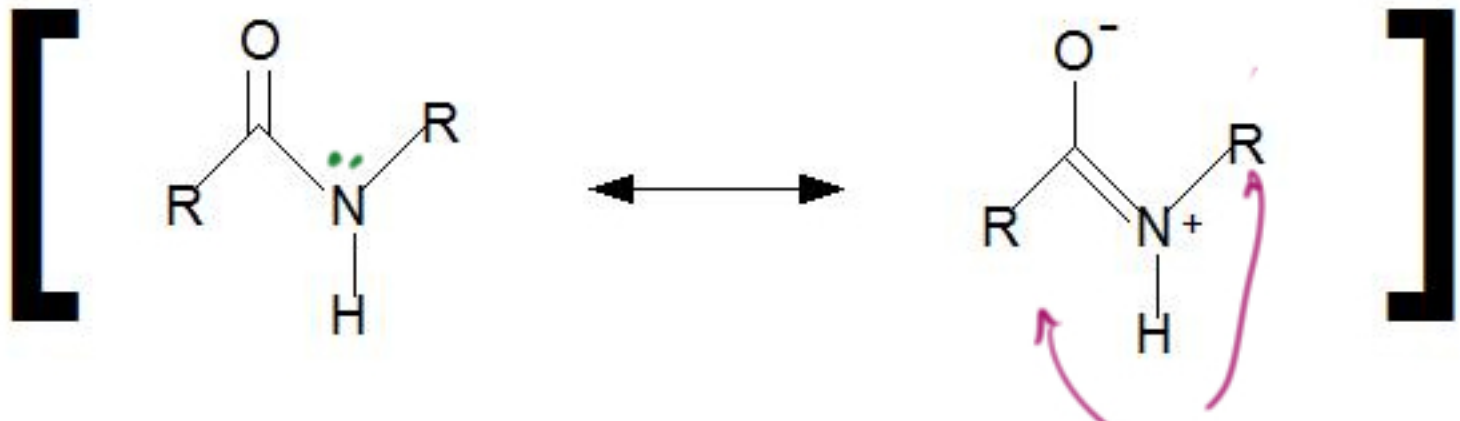




Peptide Synthesis

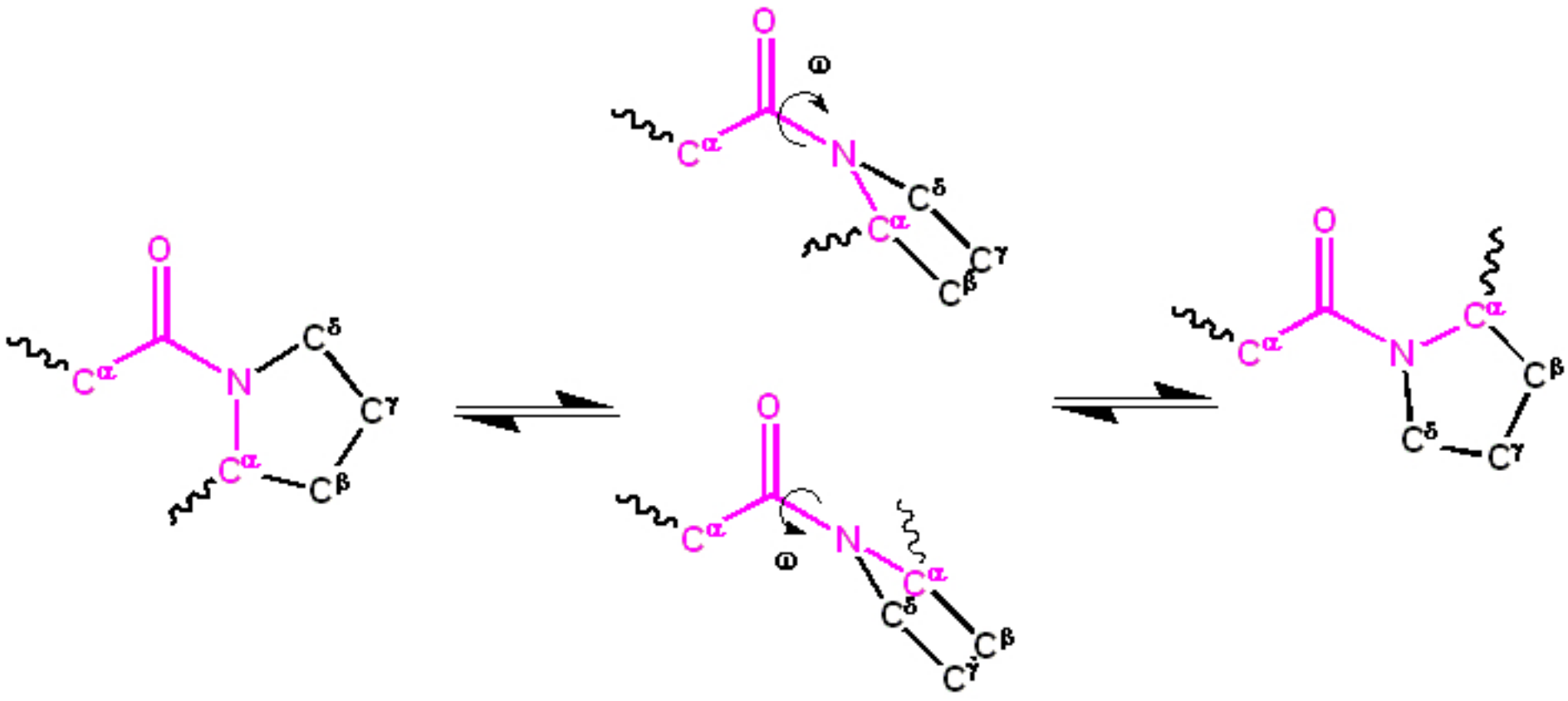

1
2
3
4

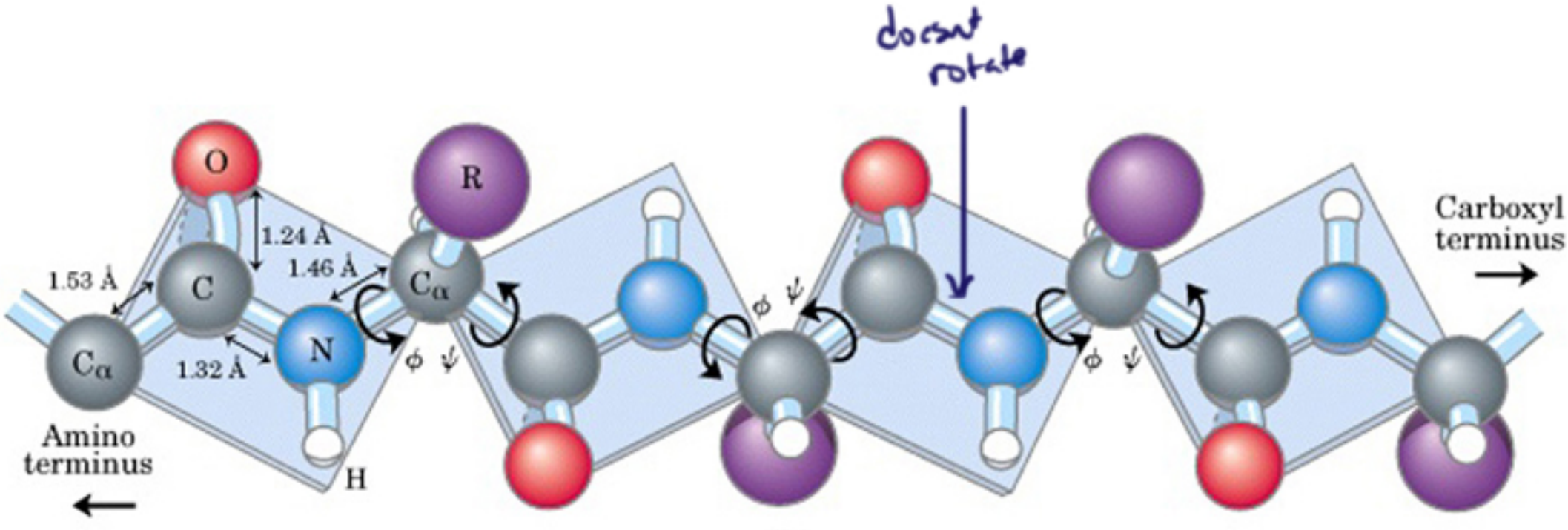
Peptide bond is rigid and planar



Peptide bond has partial double bond character

these 2 central carbons are always trans (except with proline where its 50:50)

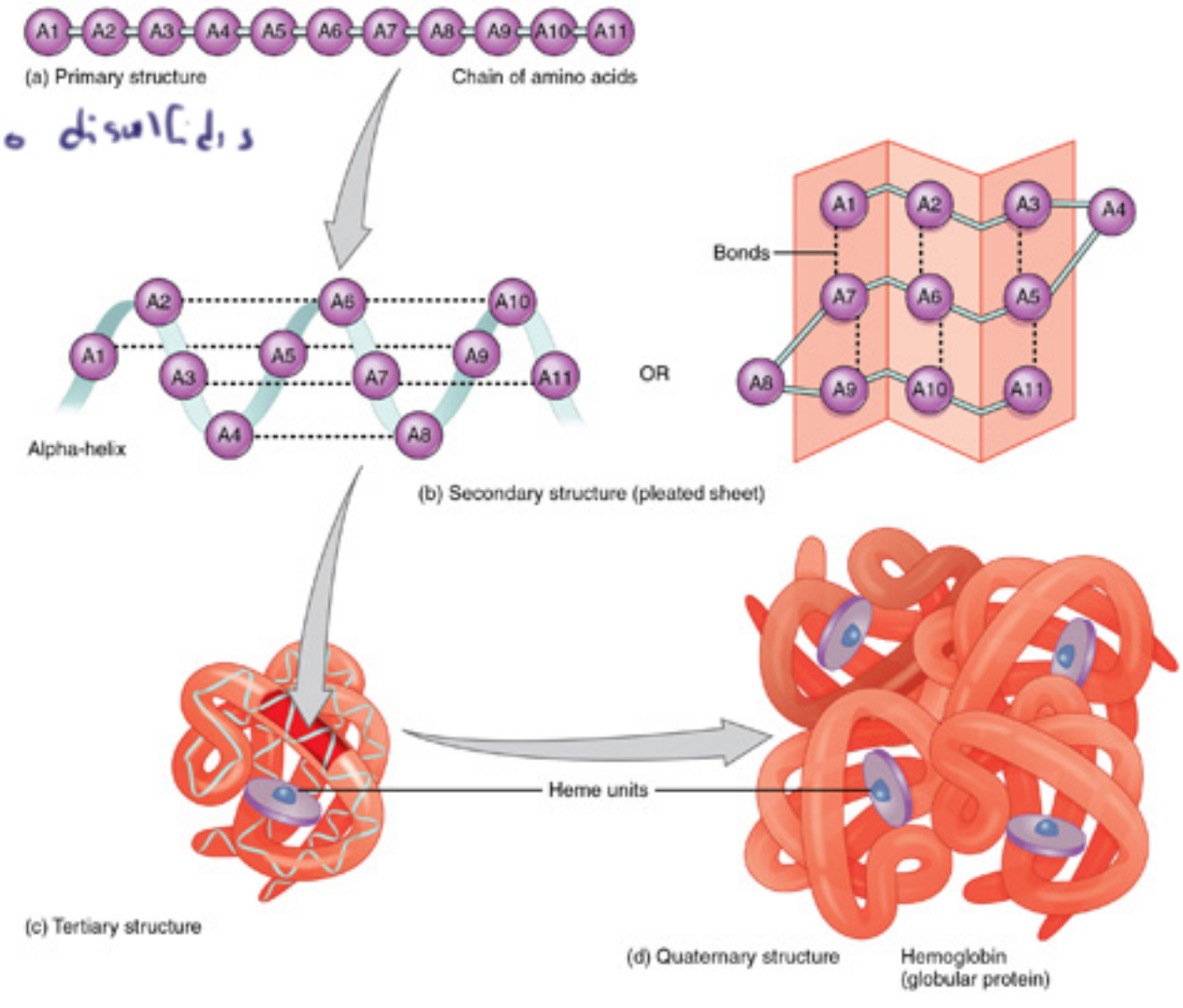

O
Cα
N
Cδ
Cγ
Cβ
Cα
Cα
N
Cδ
Cγ
Cβ
Cα
O
Cα
N
Cα
Cβ
Cδ
Cγ
O
Cα
N
Cα
Cδ
Cβ
Cγ


doesn't rotate
O
R
1.24 Å
1.46 Å
1.53 Å
C
Cα
Cα
N
1.32 Å
H
φ ψ
φ ψ
φ ψ
Amino terminus
Carboxyl terminus

(b)

covalent bonds

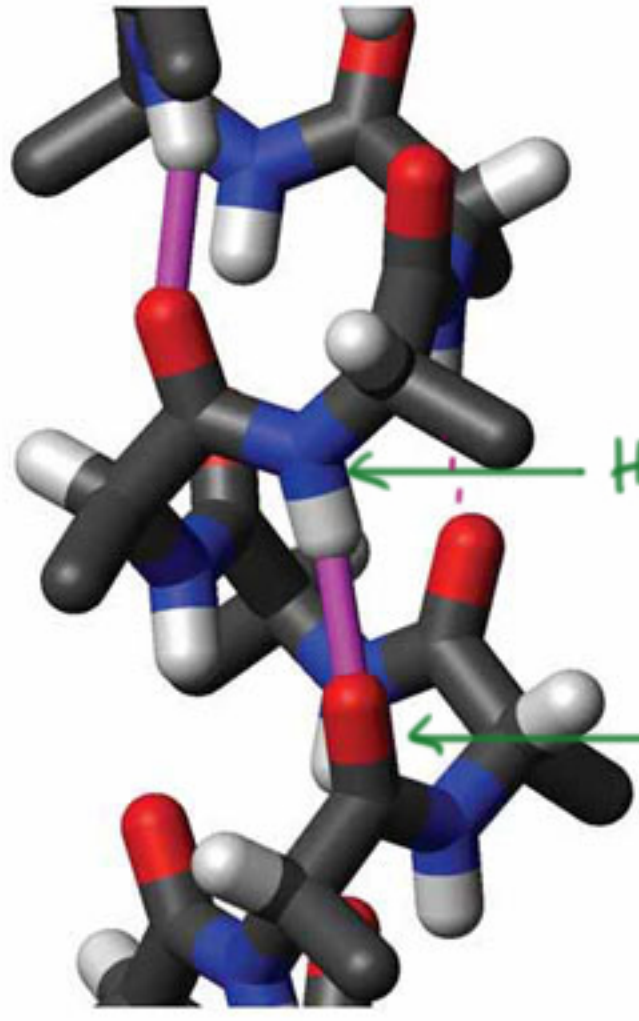
also disulfides



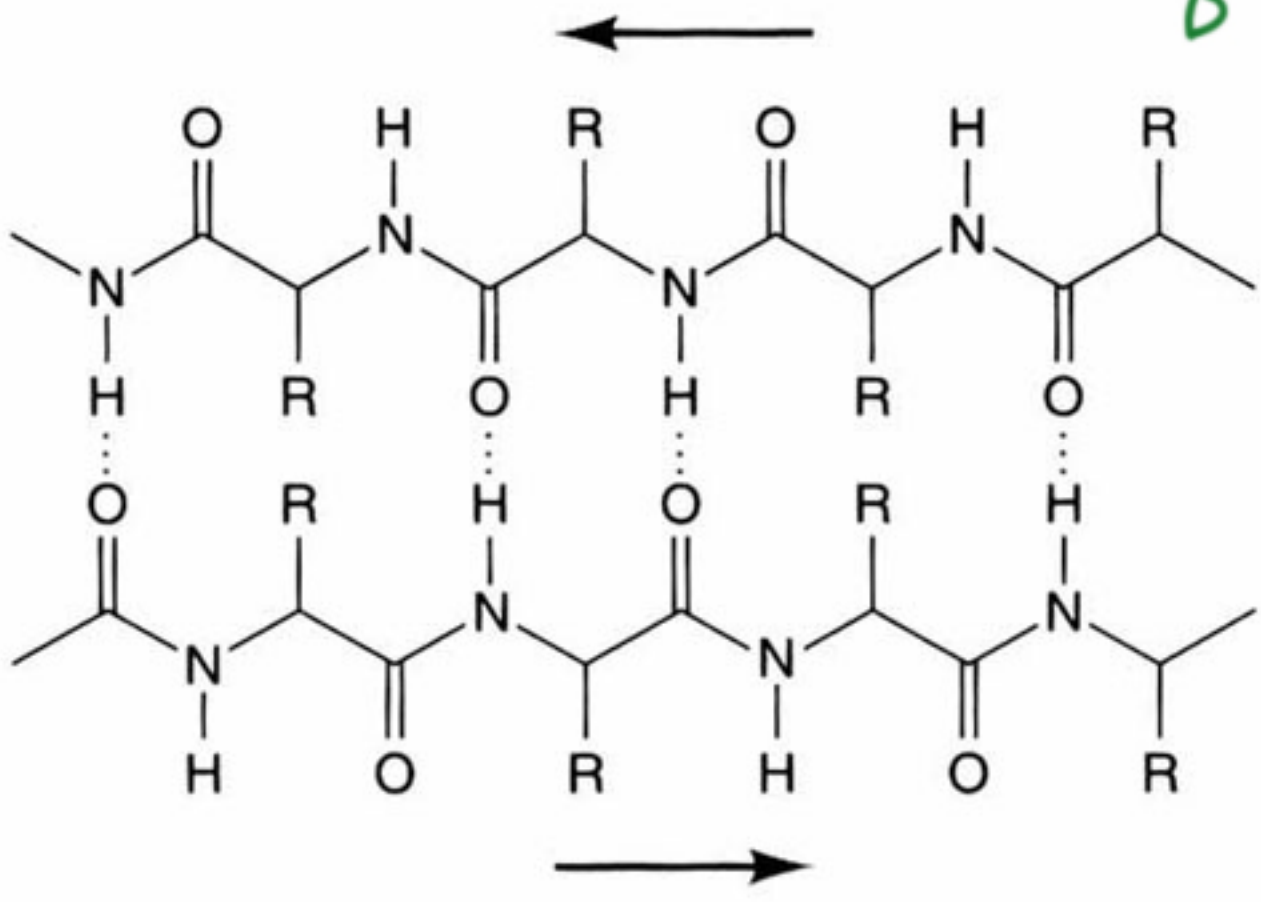
α helix

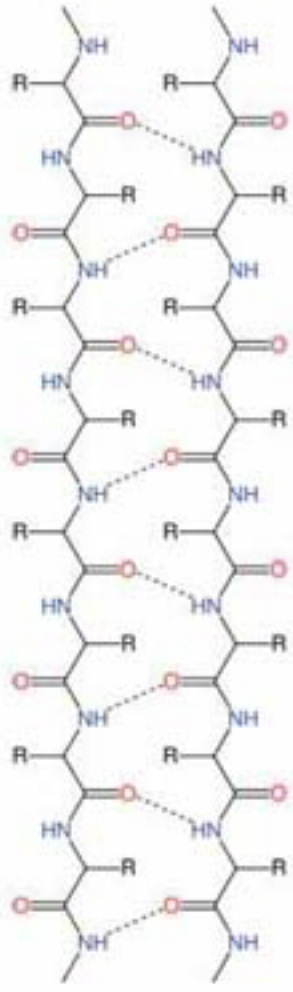
- disrupted by proline

(also glycine)

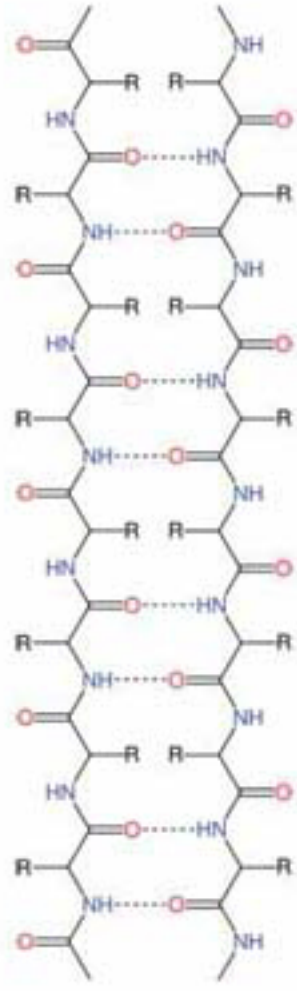


β pleated sheet

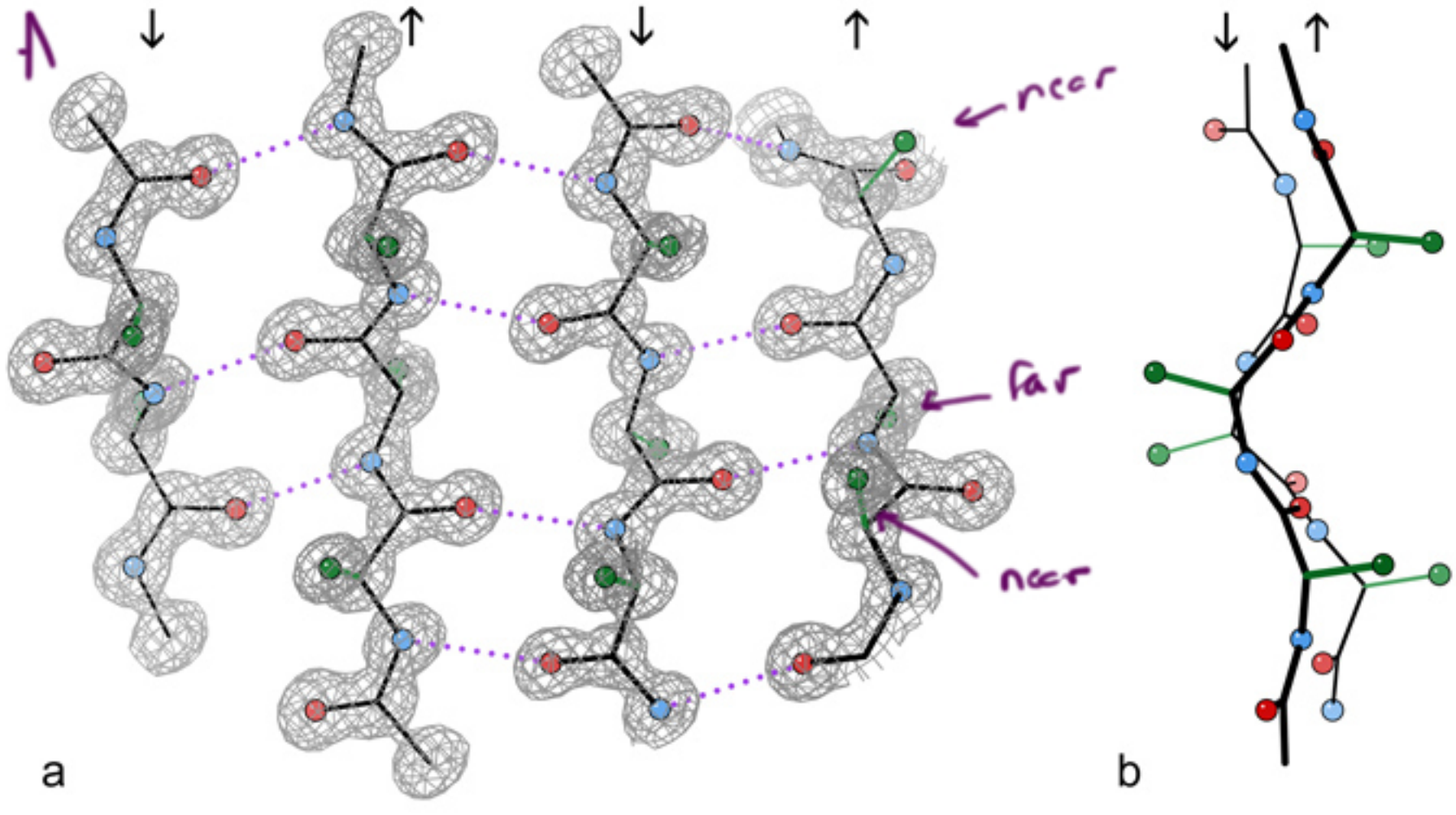




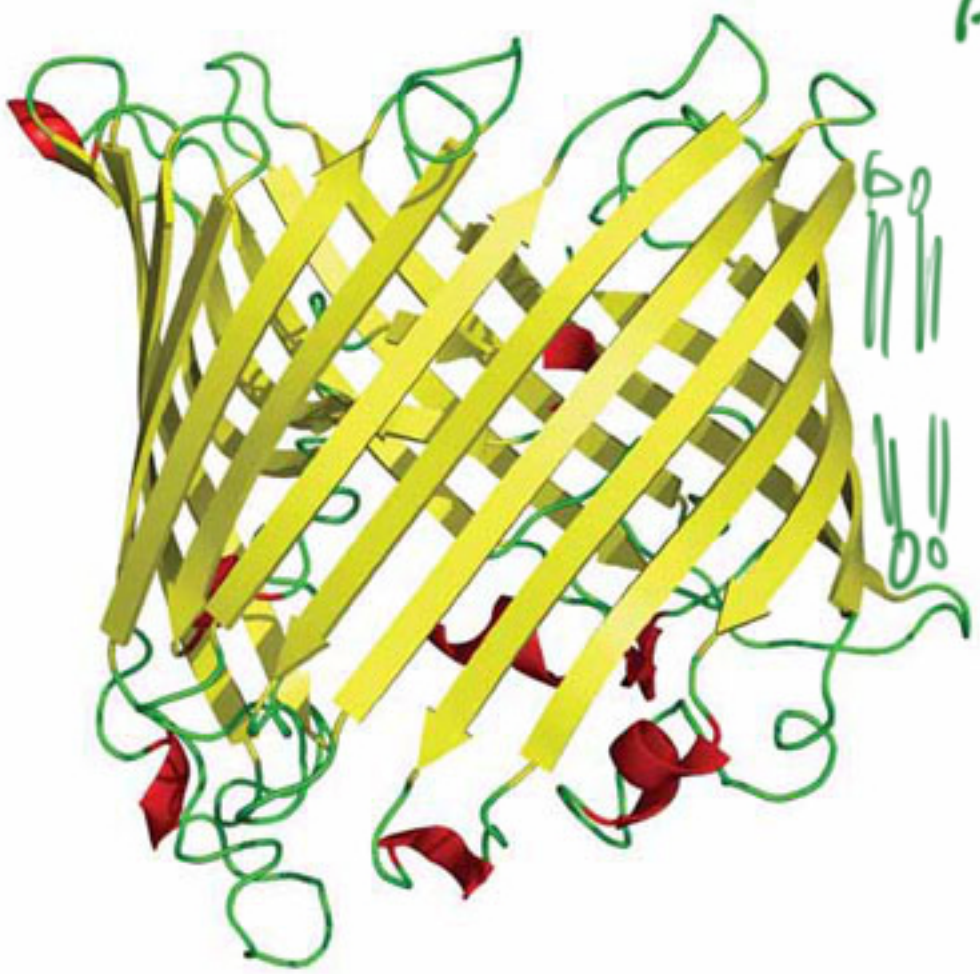
parallel



antiparallel


near
far
near
a
b

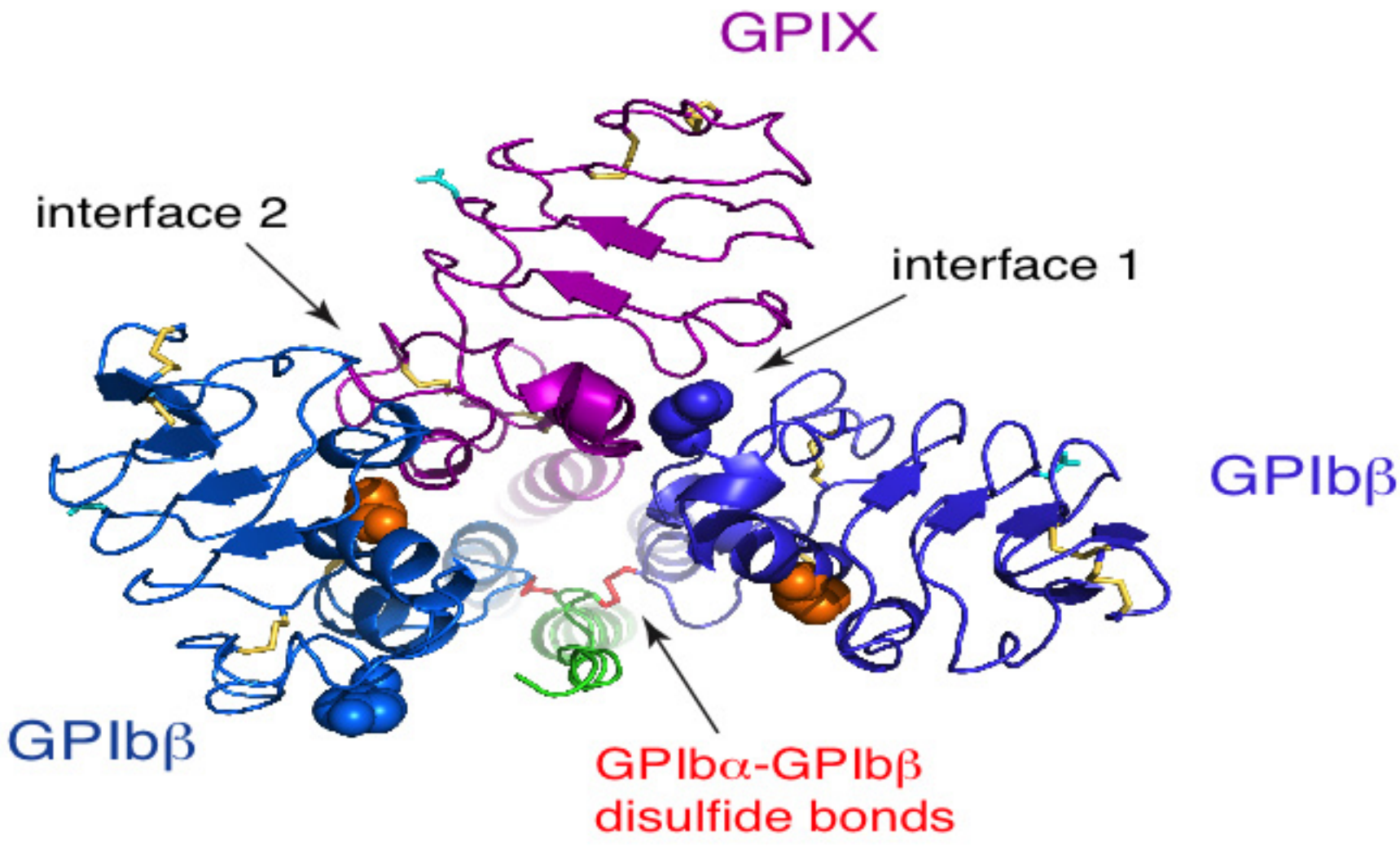
β Barrels



Porin

Hydrophobic outside
Hydrophilic inside

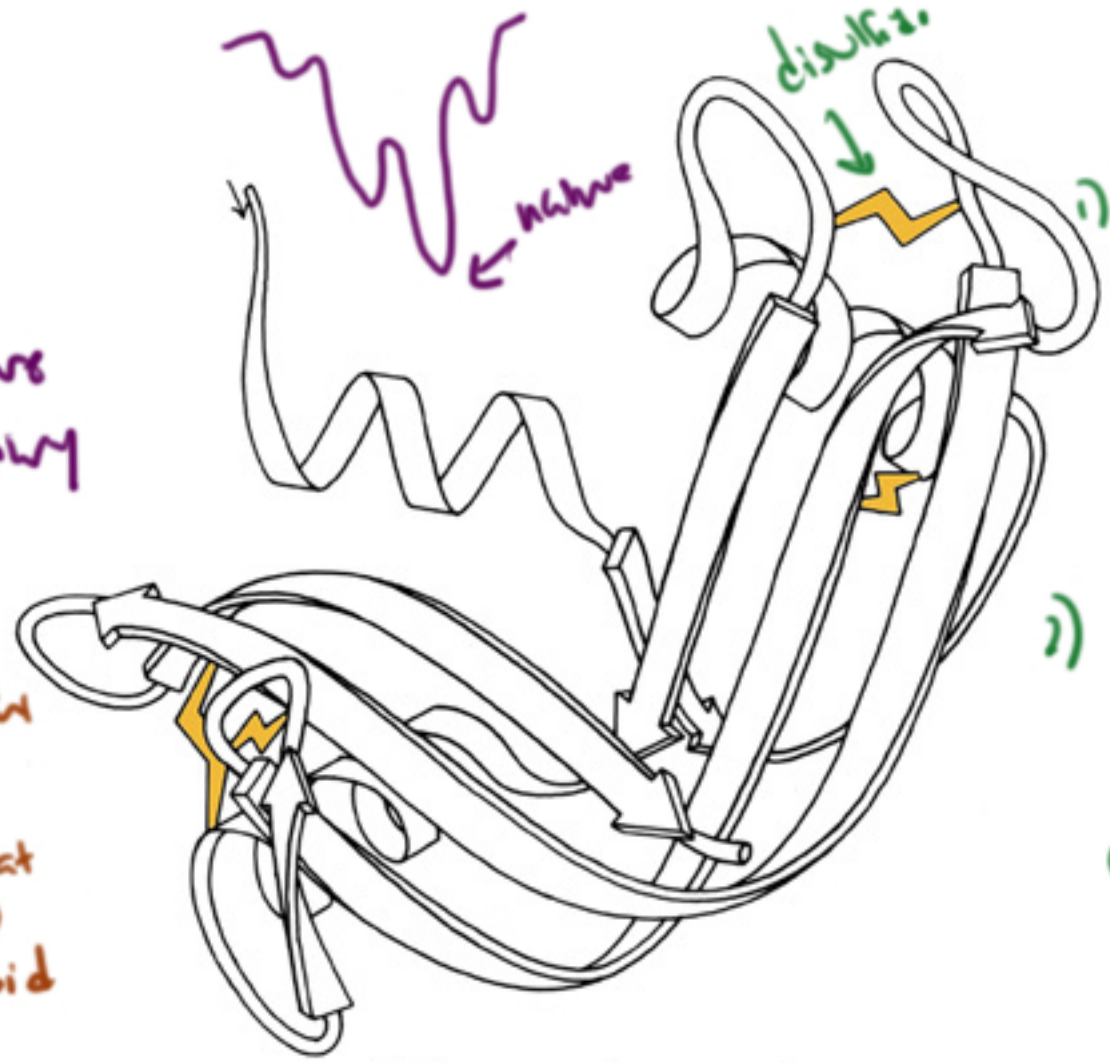
Retinal
Binding
Protein


GPIX
interface 2
interface 1
GPIbβ
GPIbβ
GPIbα-GPIbβ
disulfide bonds

Anfinsen's Dogma

1) Primary structure determines tertiary structure.

| exceptions-
- often proteins don't refold
- chaperones (heat shock proteins)
- prions & amyloid



Ribonuclease A

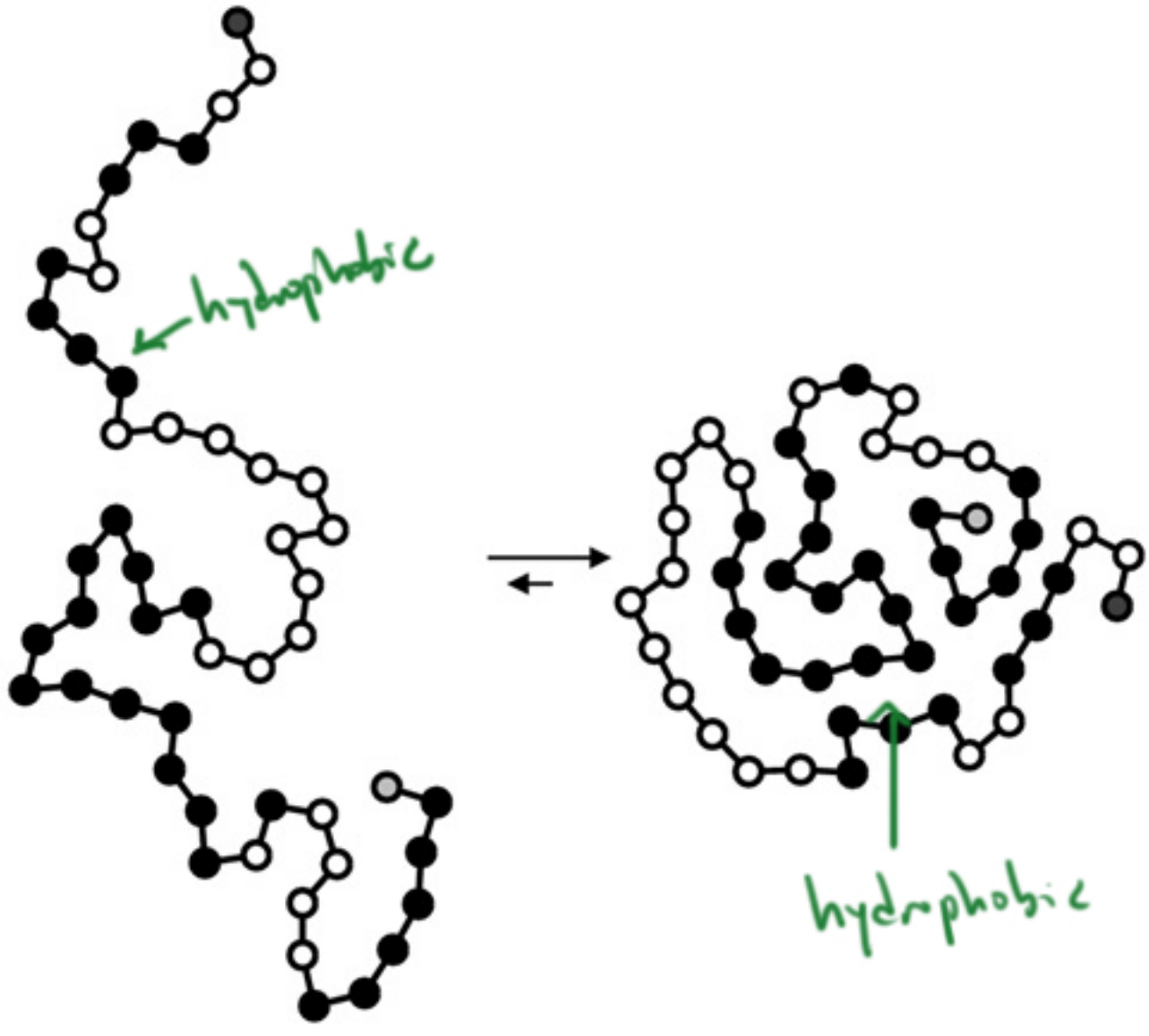
cleaves RNA

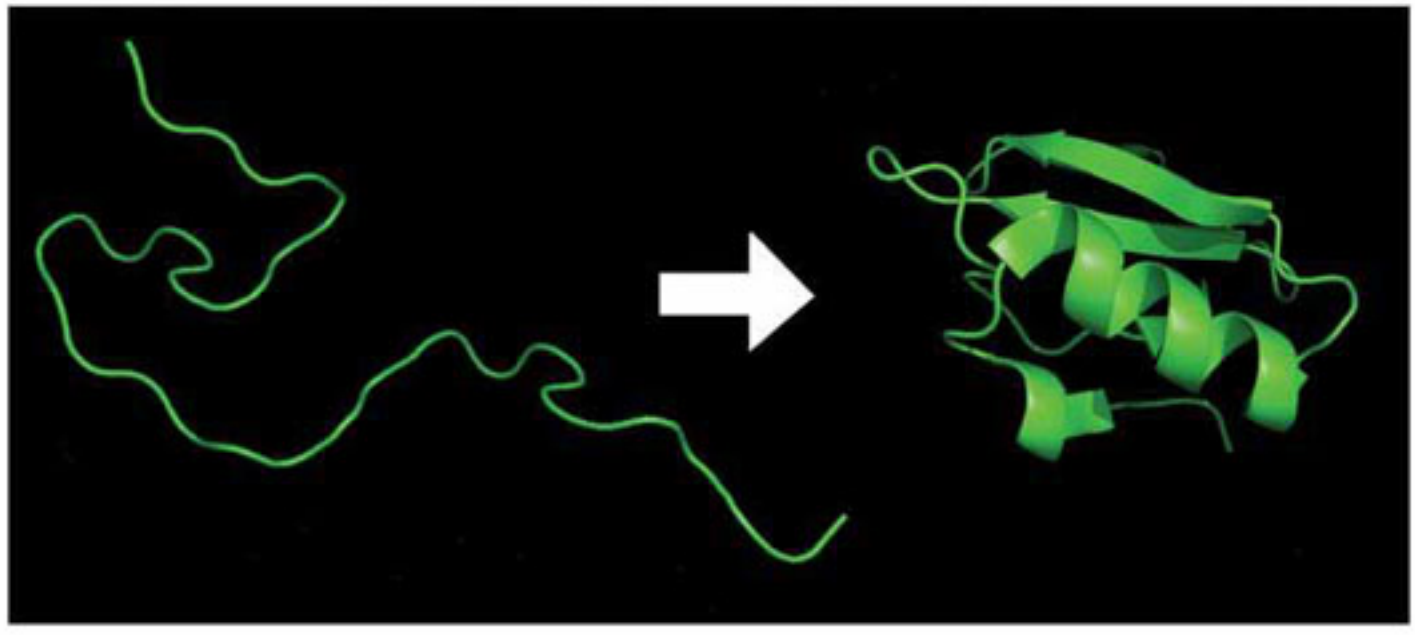
1) Added urea and β mercaptoethanol - enzyme lost activity

2) Removed these by dialysis - activity was regained

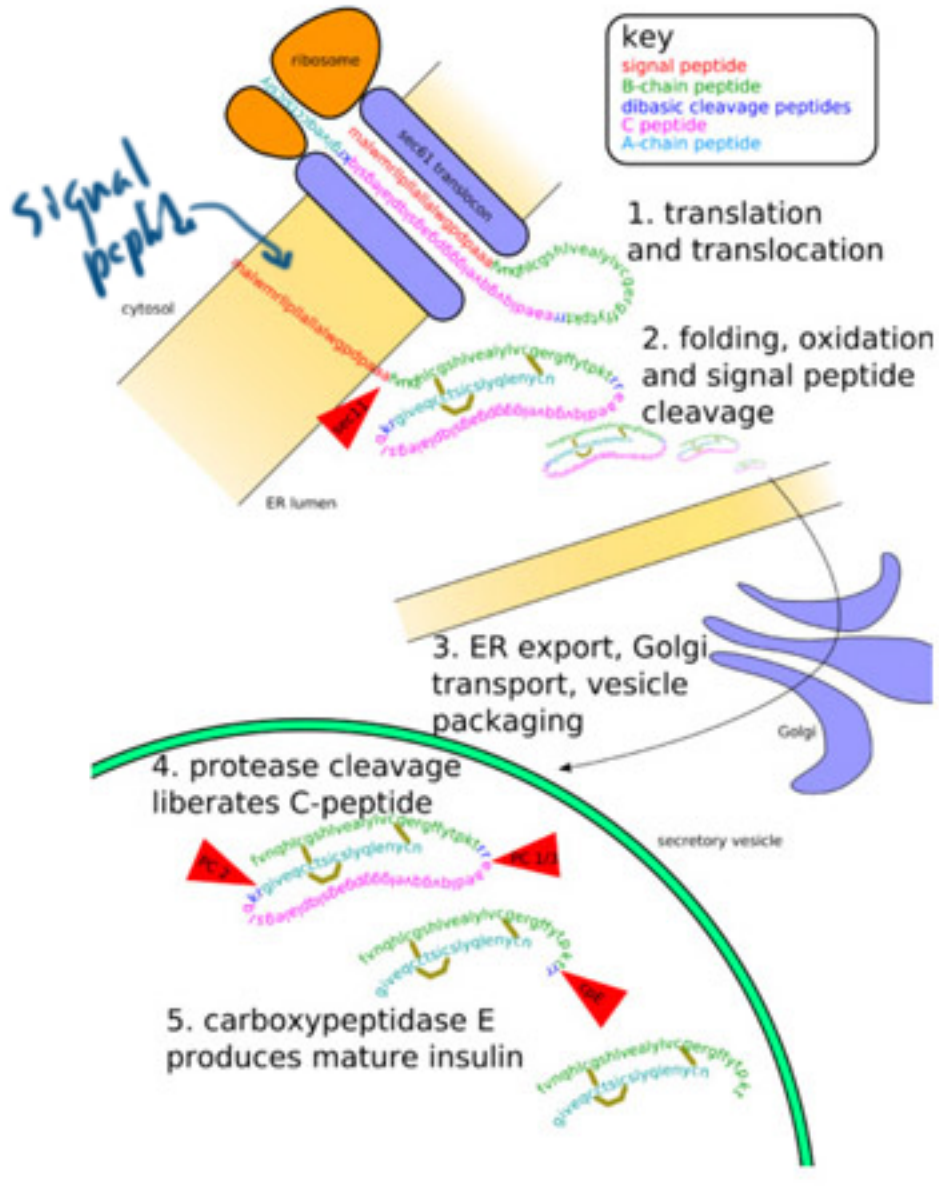
$H_2N\overset{O}{\overset{\|}{C}}NH_2$

urea is the king of H bonding

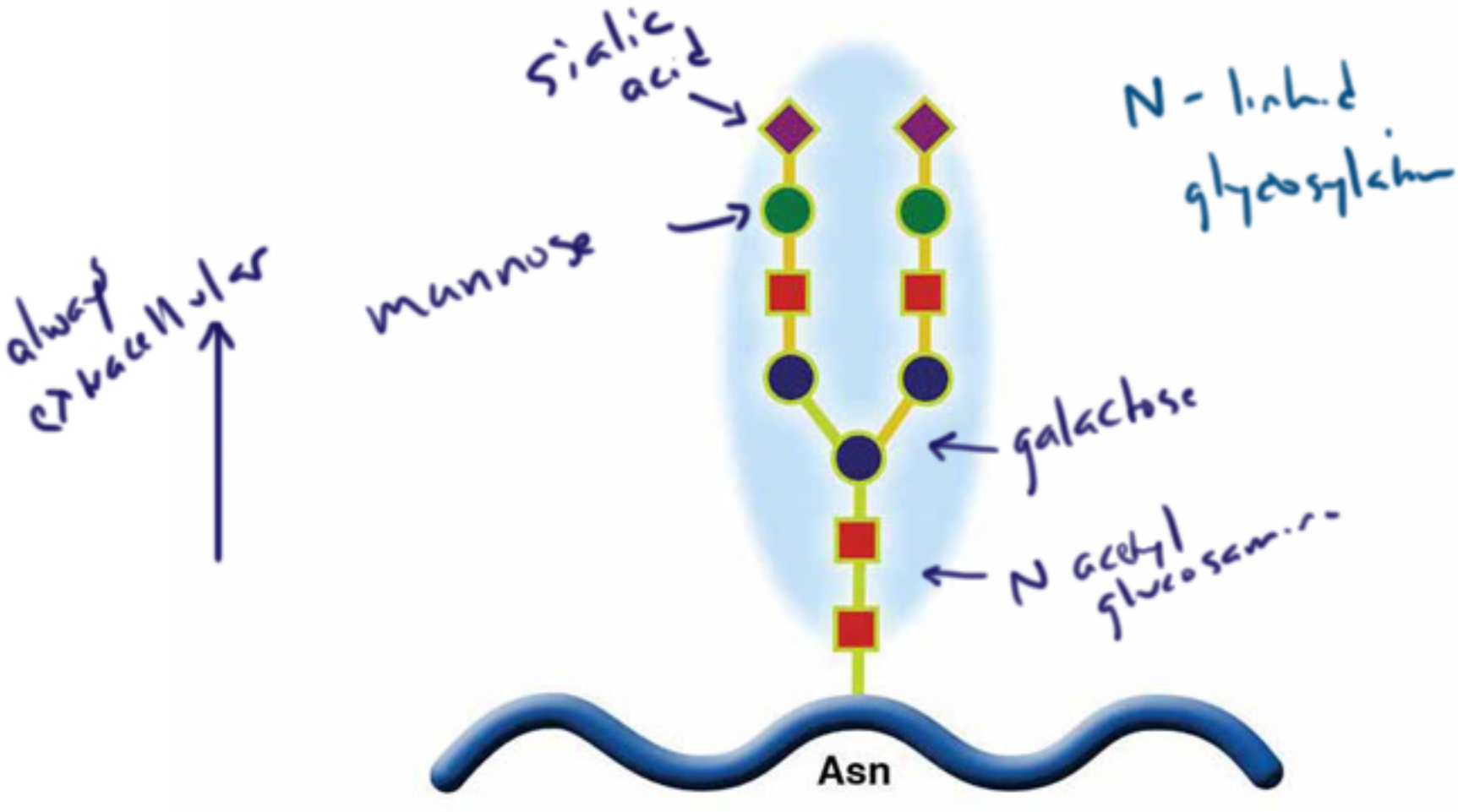

hydrophobic
hydrophobic



insulin synthesis



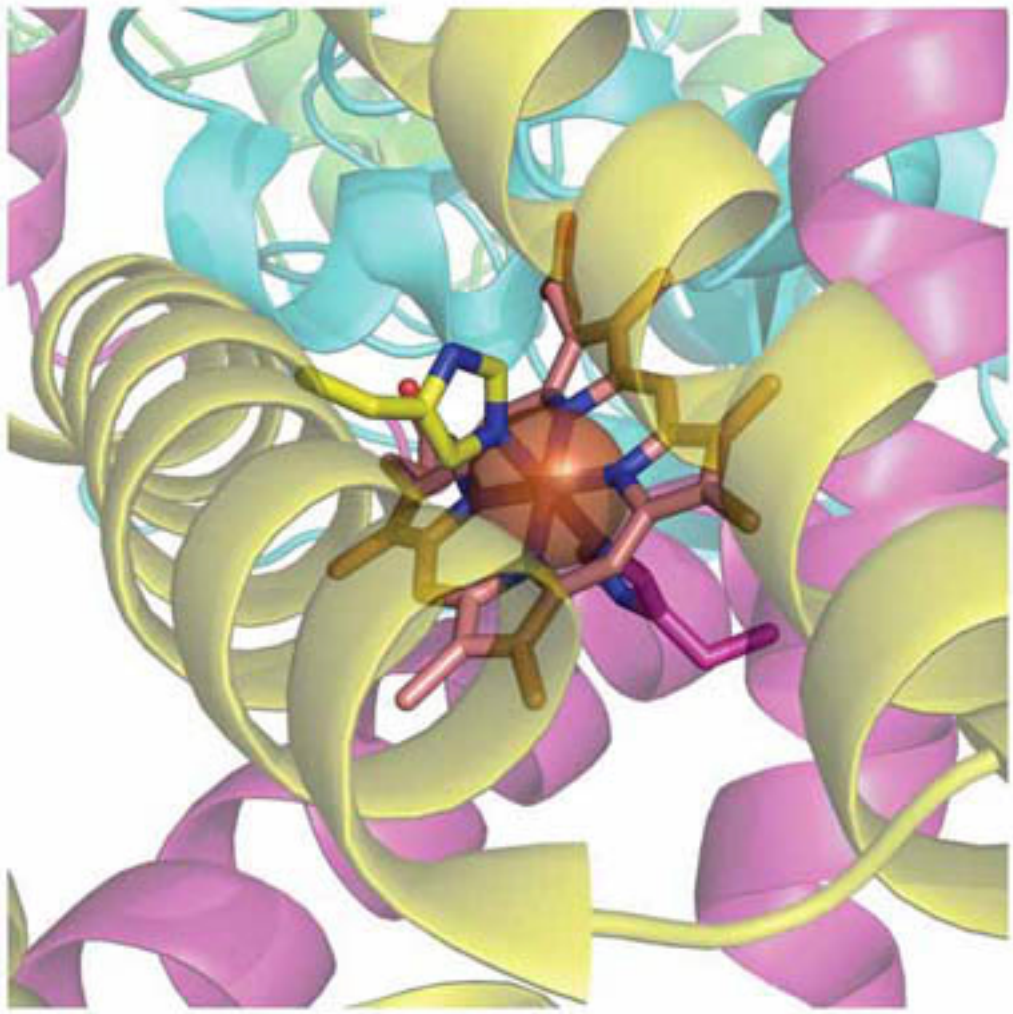
Post translational modification


sialic acid
N - linked glycosylation
always extracellular
mannose
galactose
N acetyl glucosamine
Asn

Cofactors

organic cofactor is called a coenzyme

a permanently attached cofactor is a prosthetic group.



Heme prosthetic group in Complex II (succinate dehydrogenase)

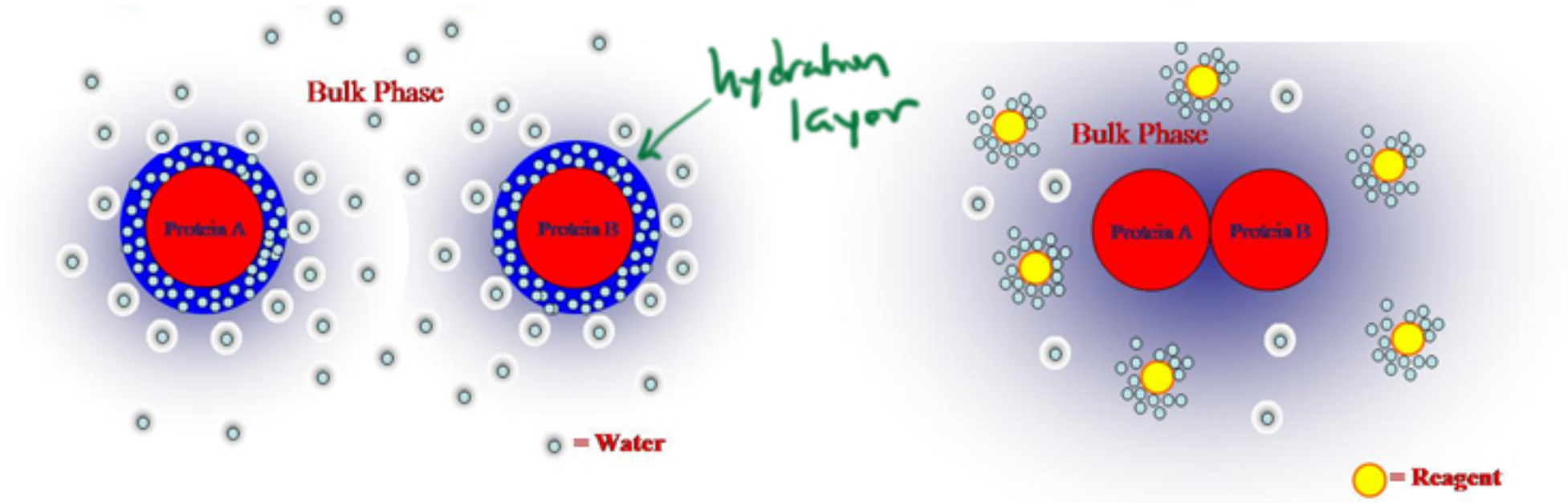
The Protein Lab

- separation & purification
- preparative or analytical

centrifugation

- low speed - nuclei
- medium mitochondria ribosomes
- higher speed membranes

Protein Precipitation



Hofmeister Series

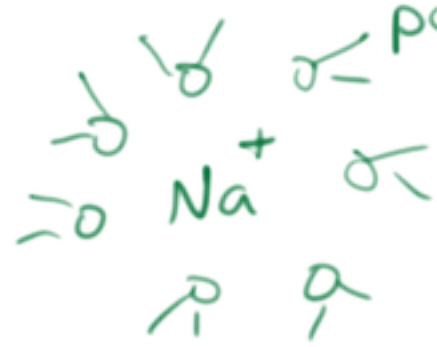
$$F^- \approx SO_4^{2-} > HPO_4^{2-} > \text{acetate} > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$

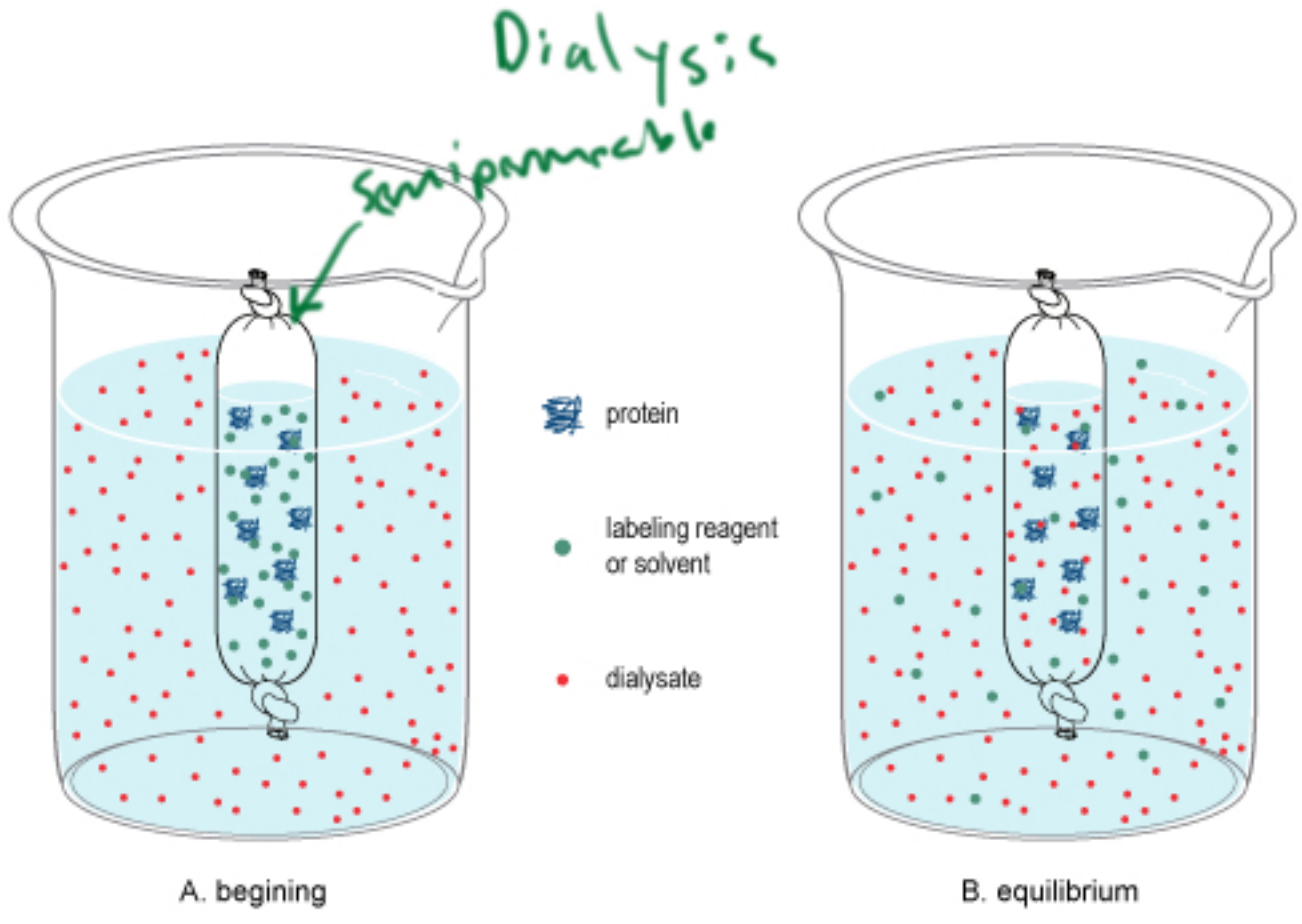
$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > \text{guanidinium}$$

← chaotropic

$(NH_4)_2SO_4$

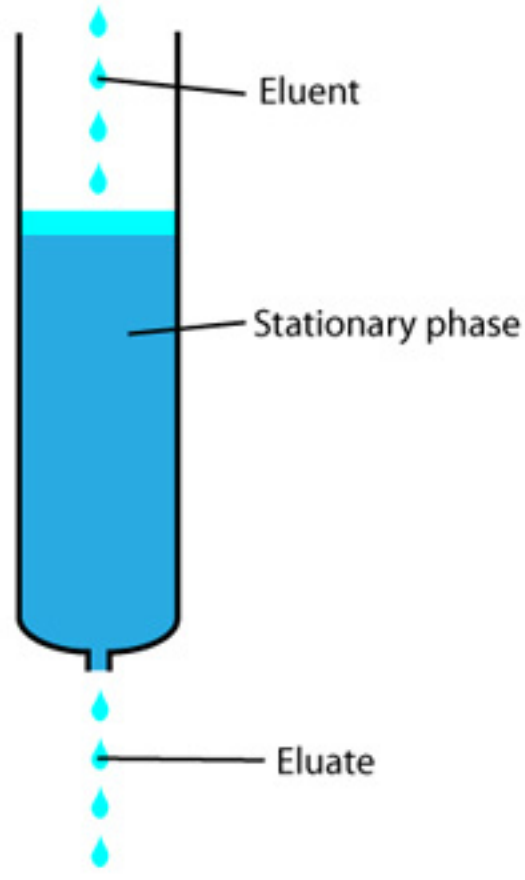
- enthalpy of hydration
- → entropic penalty




Dialysis
Semipermeable
protein
labeling reagent
or solvent
dialysate
A. begining
B. equilibrium

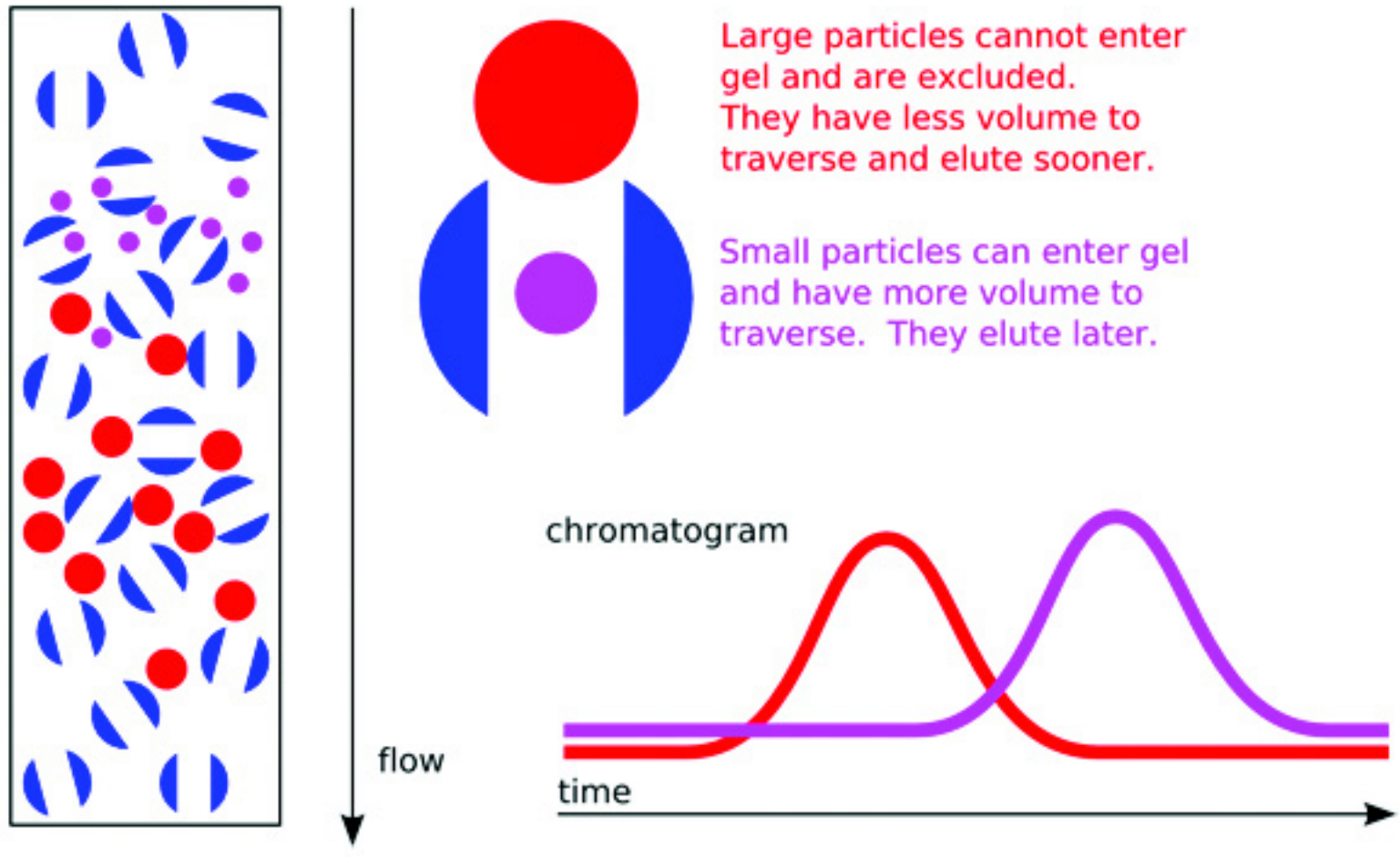
Chromatography

- mobile phase and a stationary phase

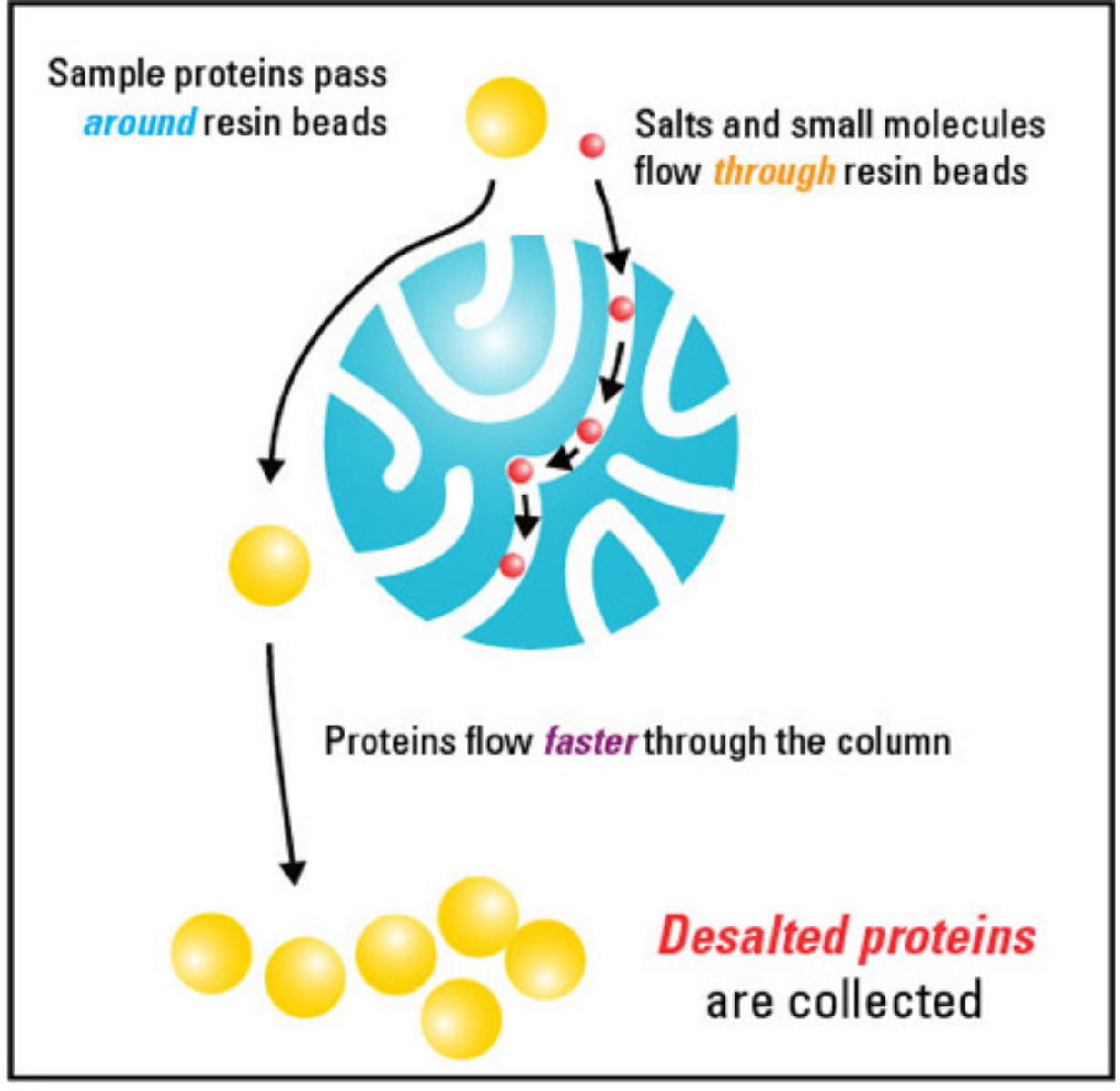


- Column chromatography
  - size exclusion (gel filtration)
  - ion exchange
  - affinity chromatography
- TLC - thin layer
  and paper
  TLC - silicate stationary phase (hydrophilic)
  mobile phase - most often hexane
- HPLC

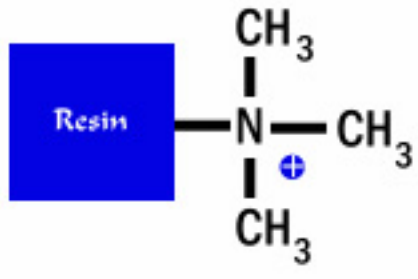
size exclusion chromatography



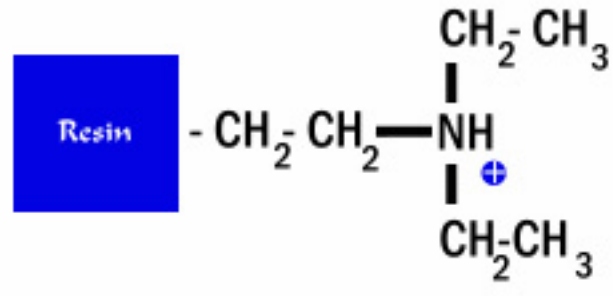
Large samples have a lower retention time.


Sample proteins pass
around resin beads
Salts and small molecules
flow through resin beads
Proteins flow faster through the column
Desalted proteins
are collected

ion exchange



Q-anion exchanger



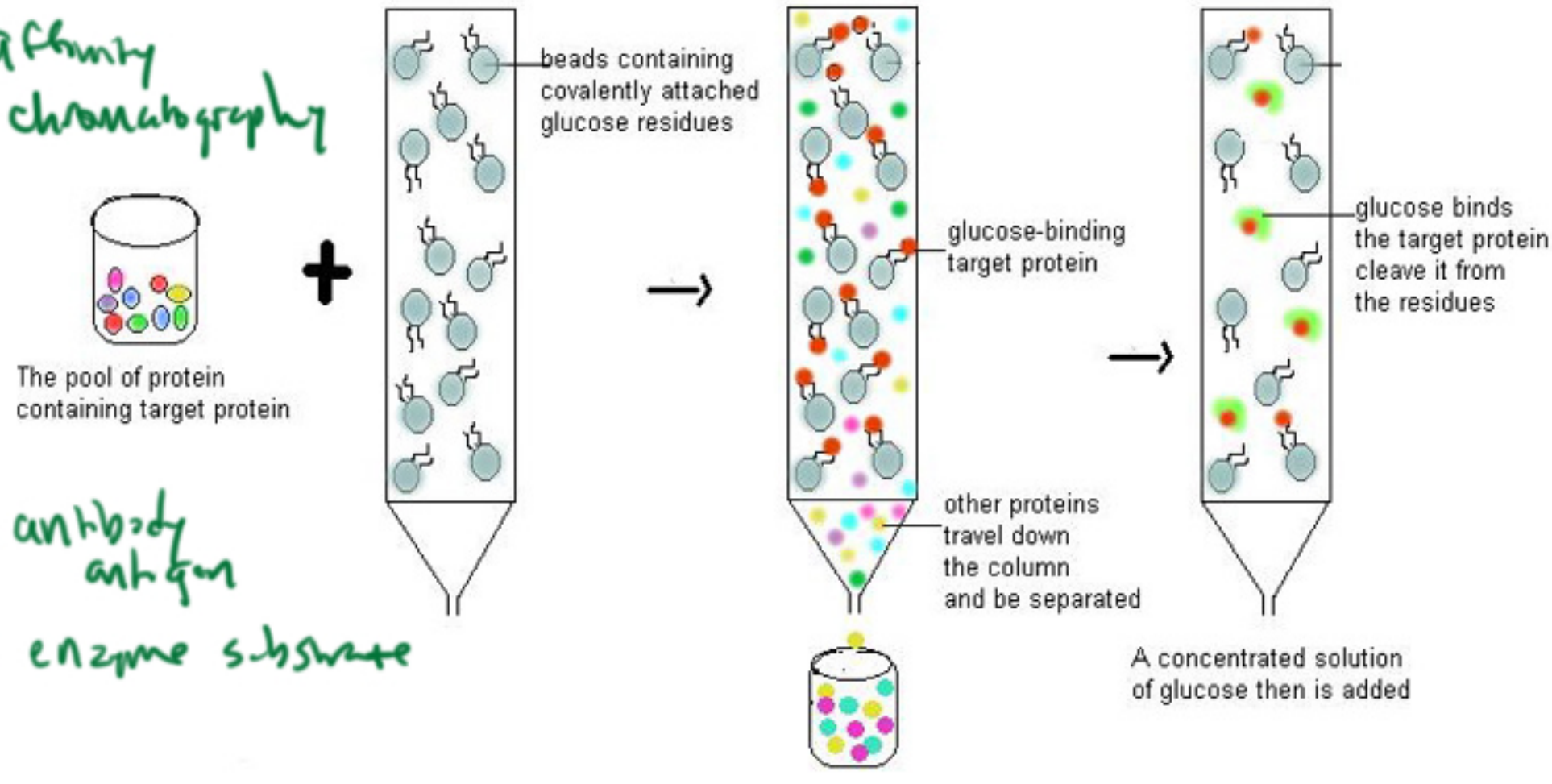
DEAE-anion exchanger

an anion exchange resin is positively charged

use with a gradient elution buffer

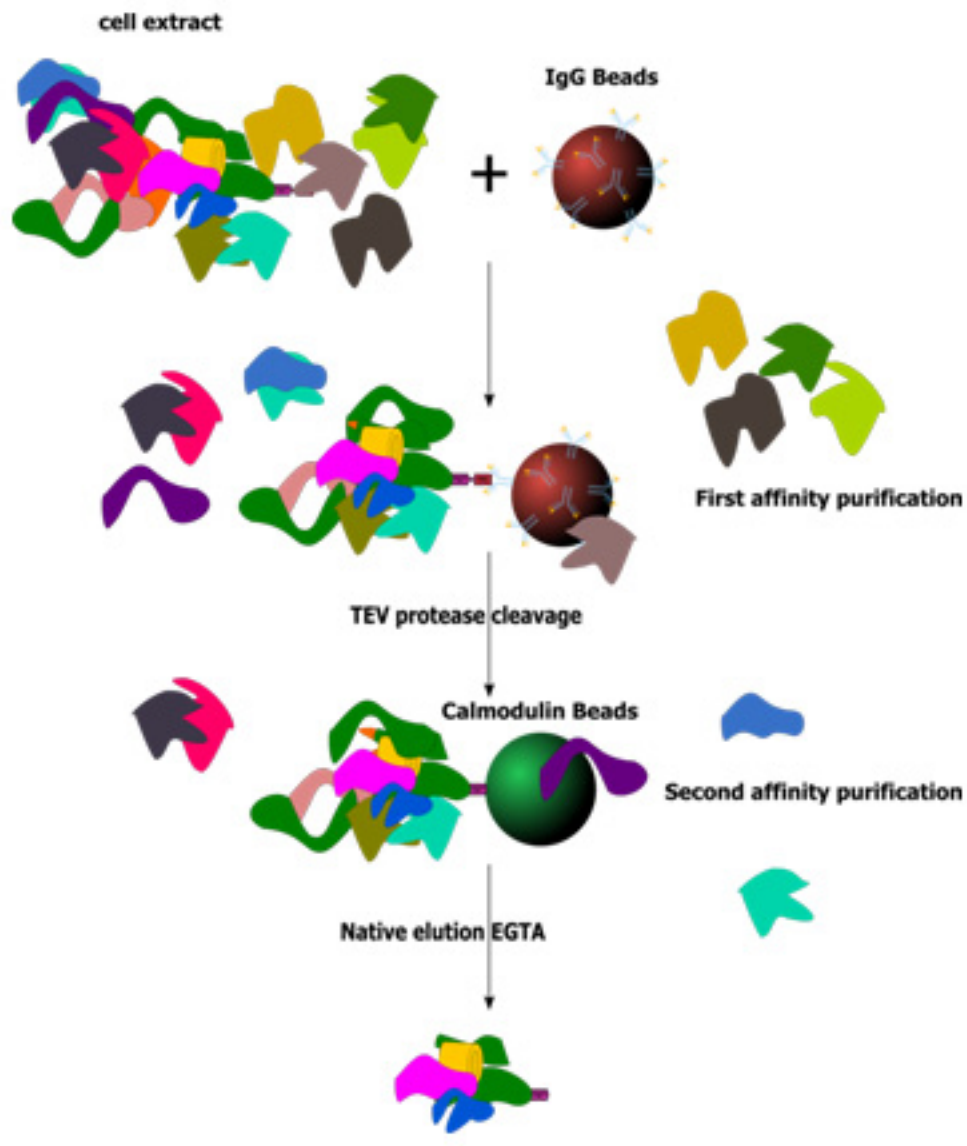
such as 0.01 M NaCl ⟶ 1.0 M

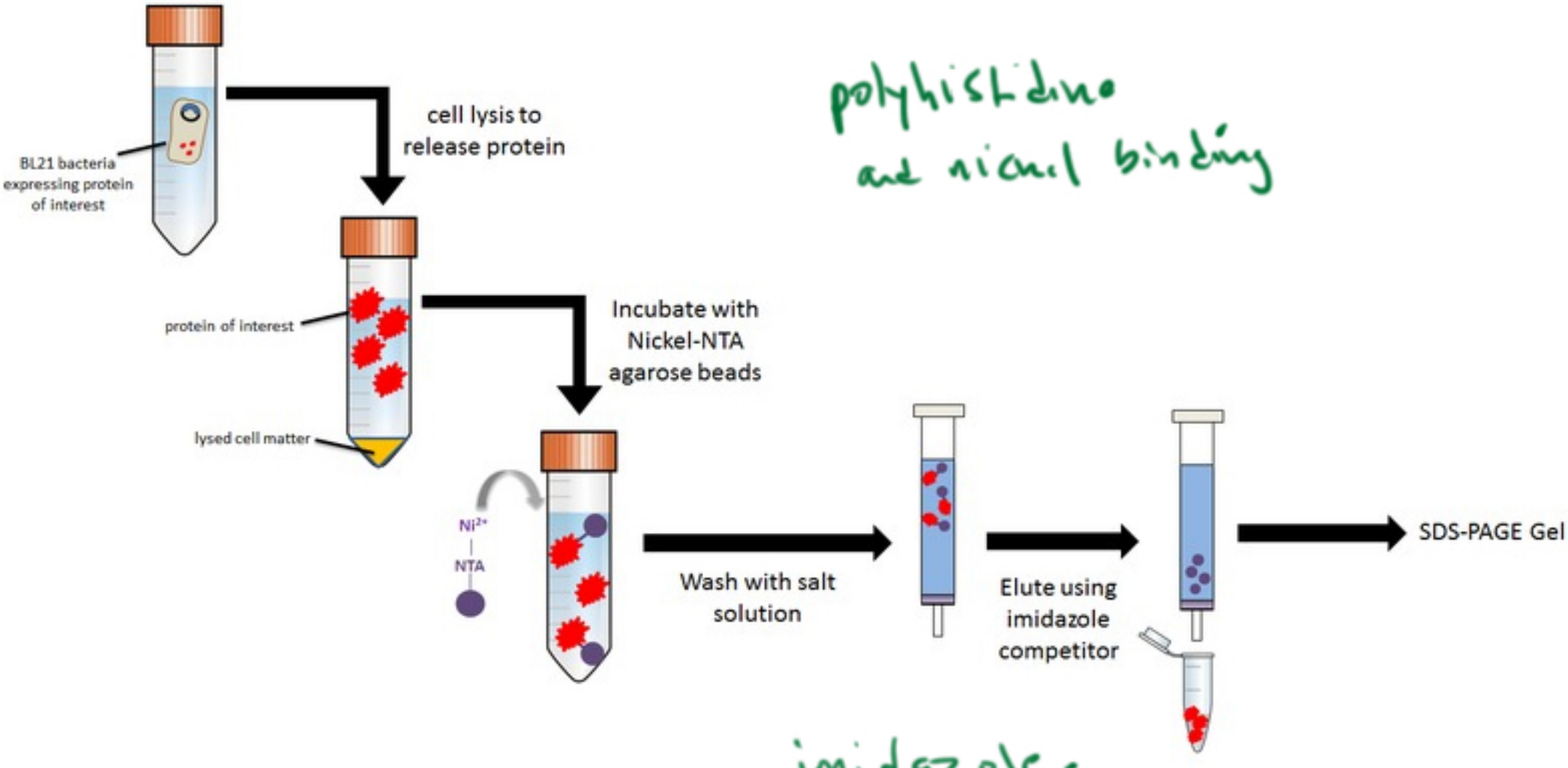
affinity chromatography



The demonstration of the steps in Affinity Chromatography

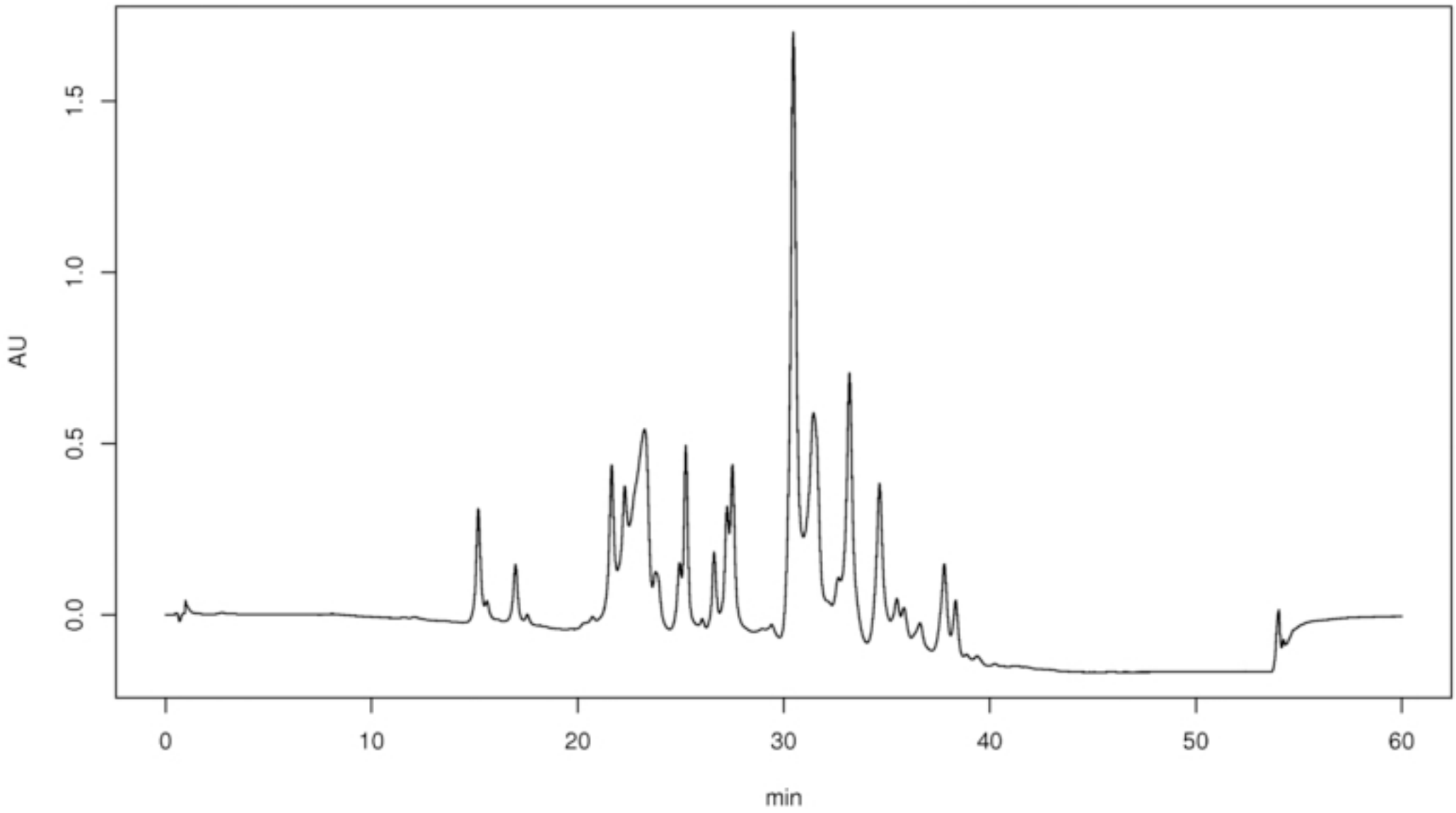
- antibody antigen
- enzyme substrate
- polyhistidine tail with nickel coated beads
- biotinylation with avidin coated beads


cell extract
IgG Beads
First affinity purification
TEV protease cleavage
Calmodulin Beads
Second affinity purification
Native elution EGTA



polyhistidine and nickel binding

imidazole - histidine side chain



HPLC - high performance liquid chromatography

- often using a gradient such as

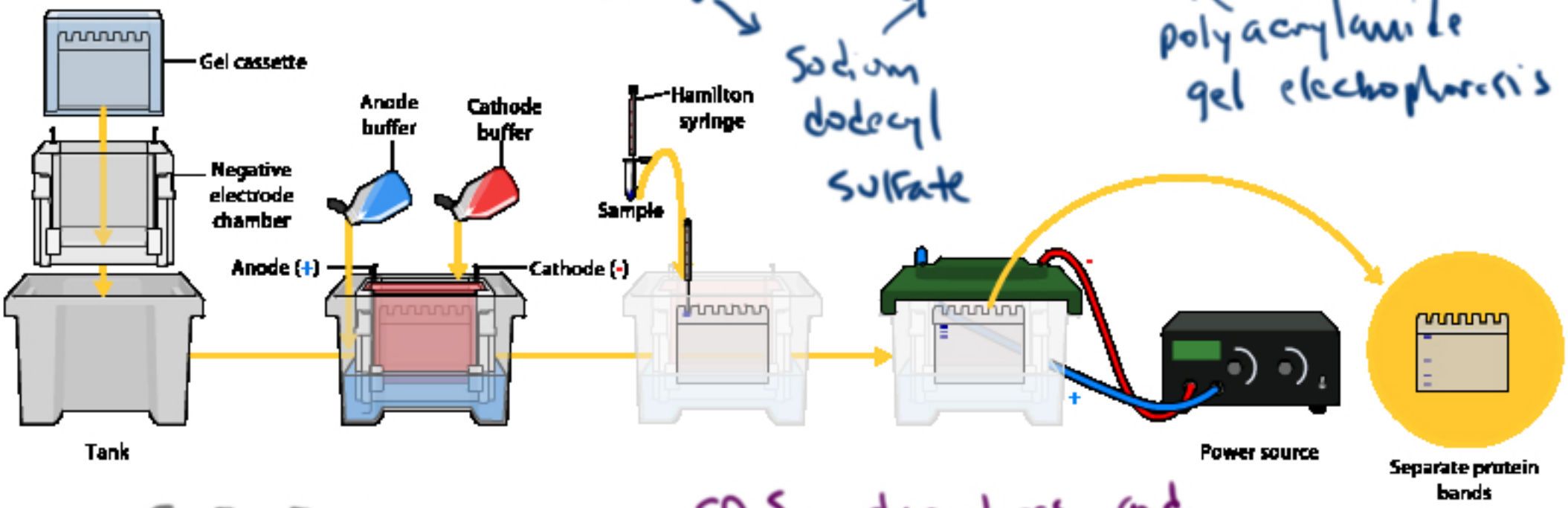
100% acetonitrile → 100% water

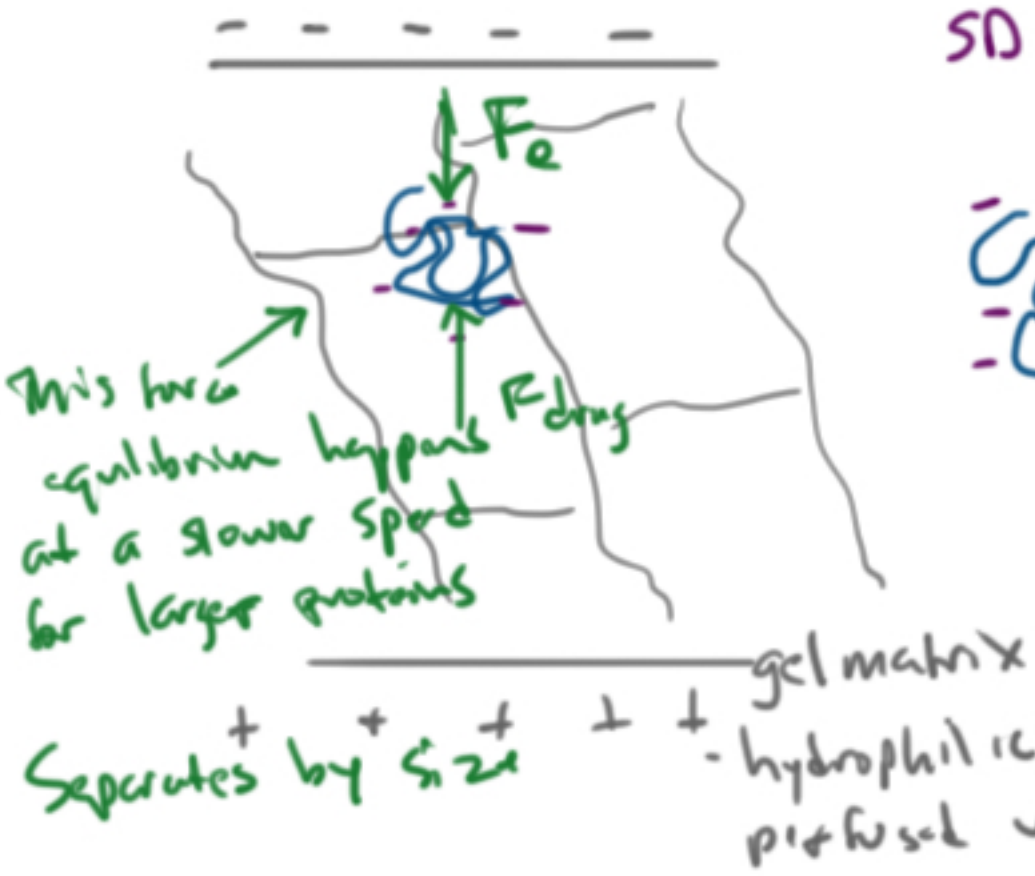
!

SDS - PAGE

detergent → sodium dodecyl sulfate

polyacrylamide gel electrophoresis





SDS - denatures and disrupts noncovalent interactions

SDS imparts a uniform charge to mass ratio

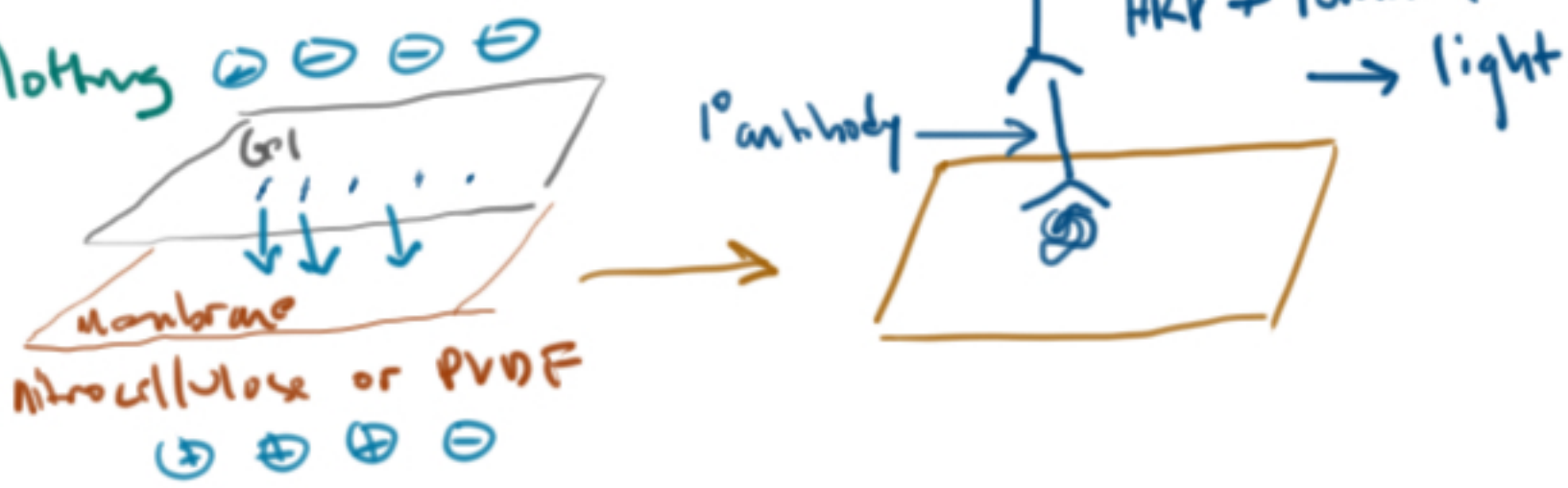
- denaturing gel - with SDS
- native gel - without SDS
- ALSO - reducing gel uses β mercaptoethanol in the loading buffer

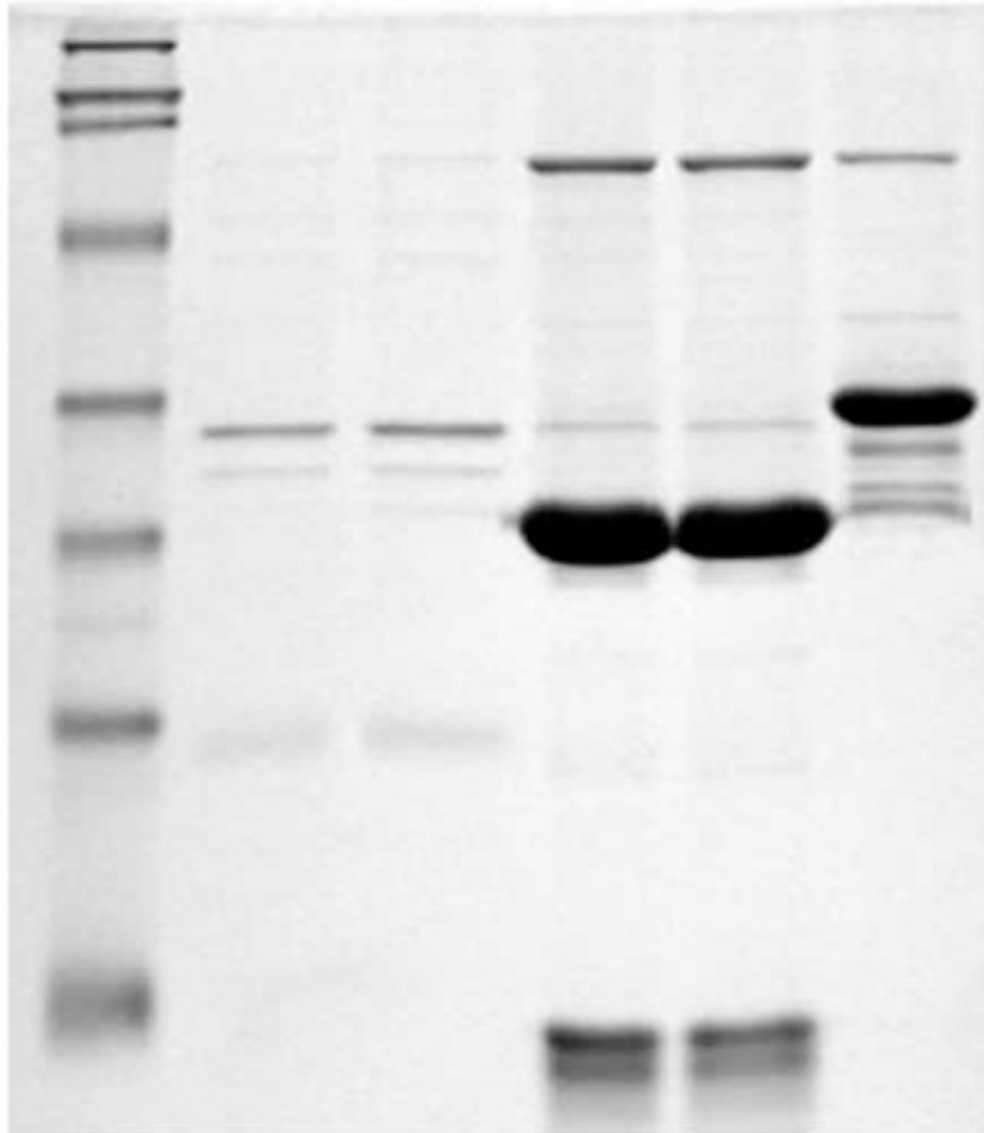
---

Post electrophoresis - staining or sample recovery - crush and soak
electroelution

Blotting - Western - Protein
Southern - DNA
Northern - RNA

Western Blotting



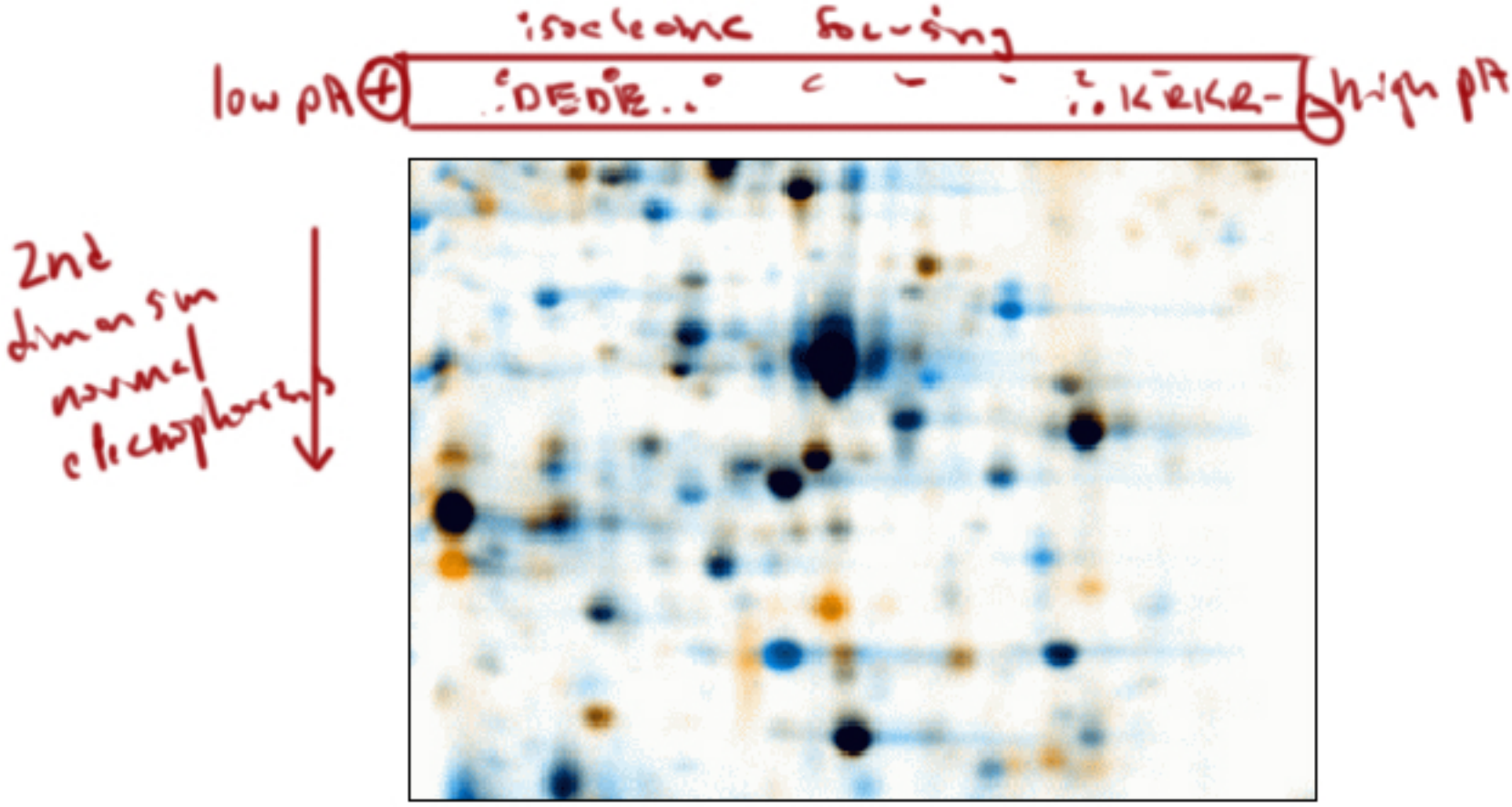


MW ladder

1 Dalton = 1 amu

1 kD = 1000 Daltons

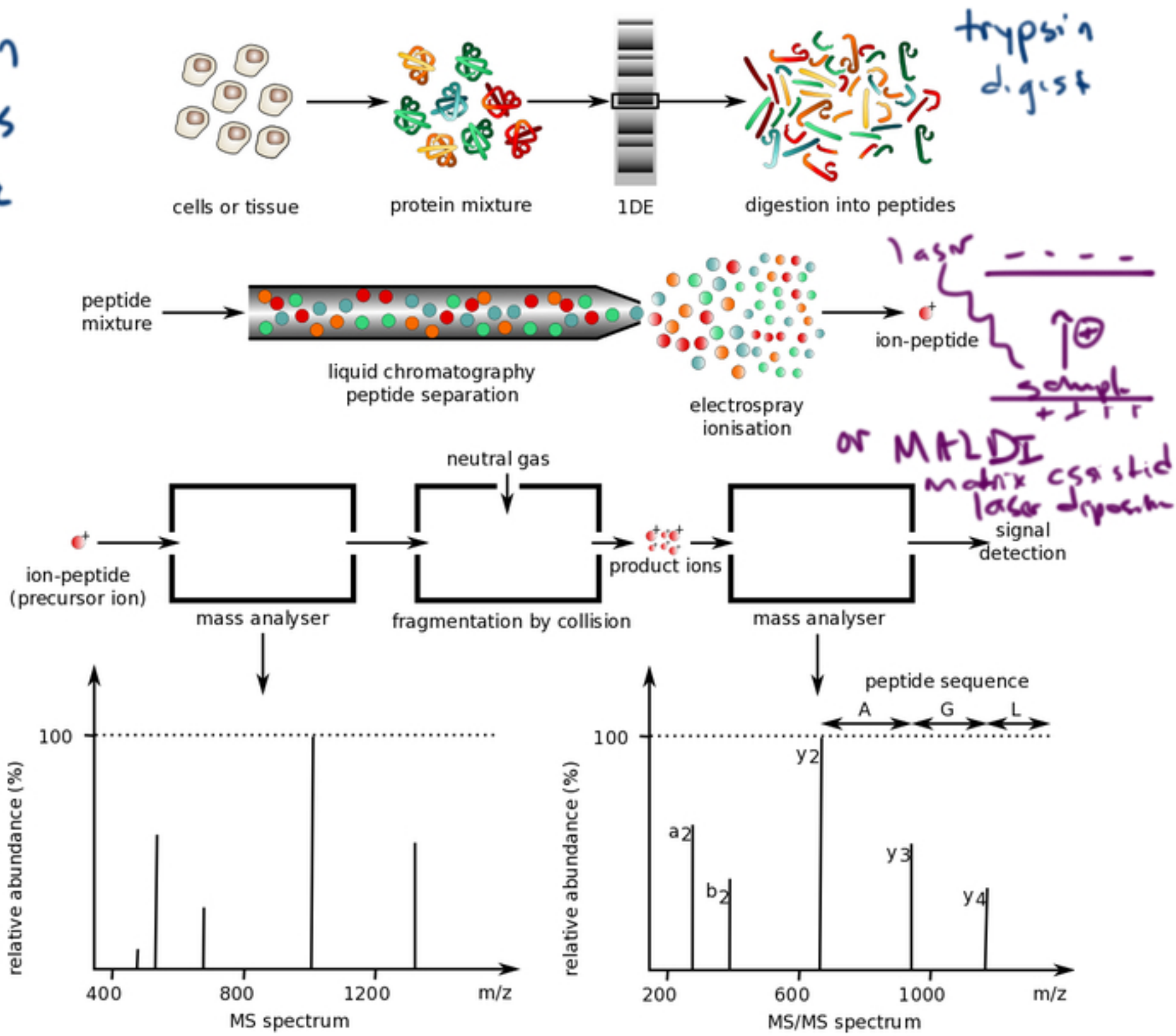
1 kD ~ 9 residues



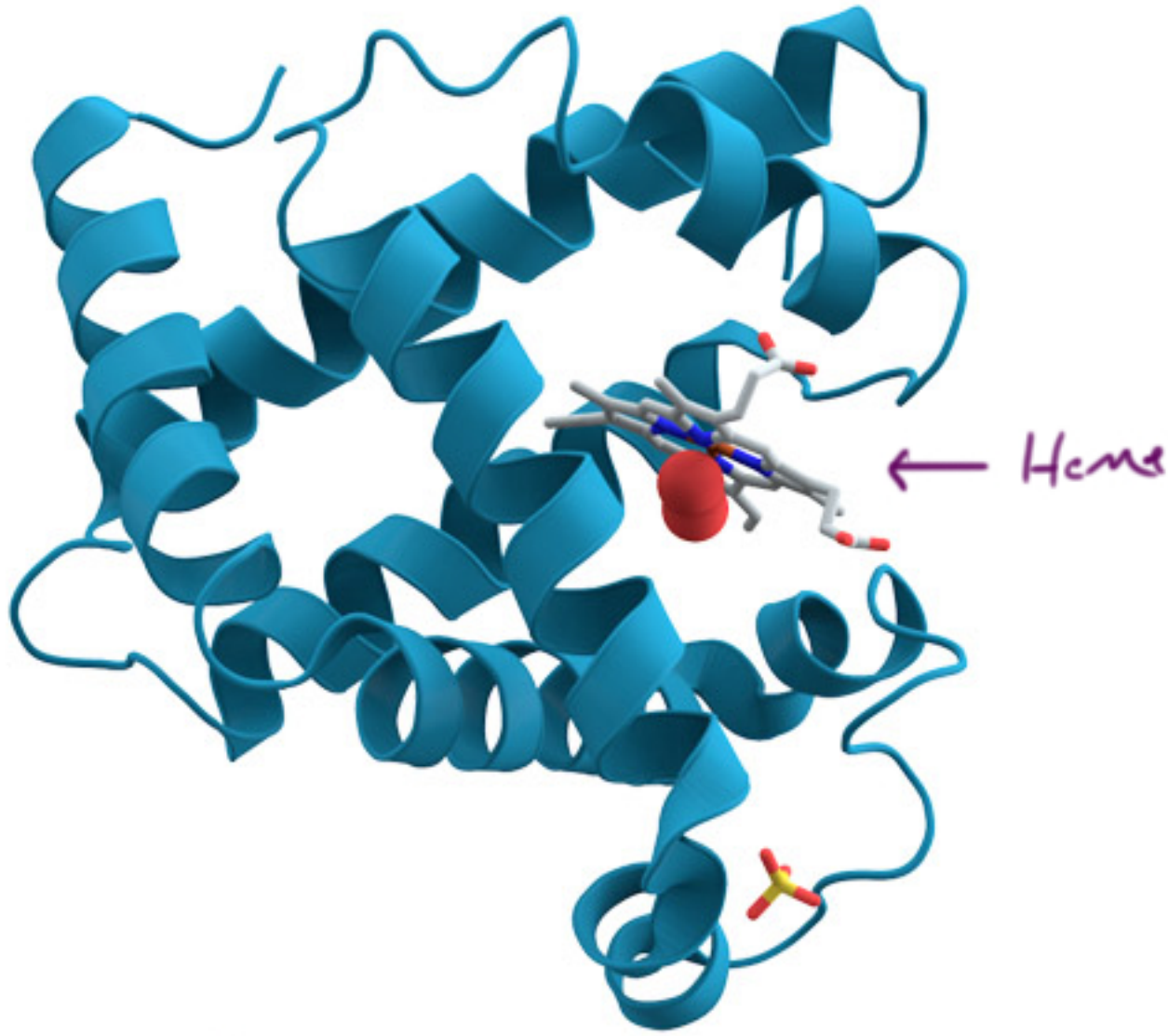
Two Dimensional Electrophoresis

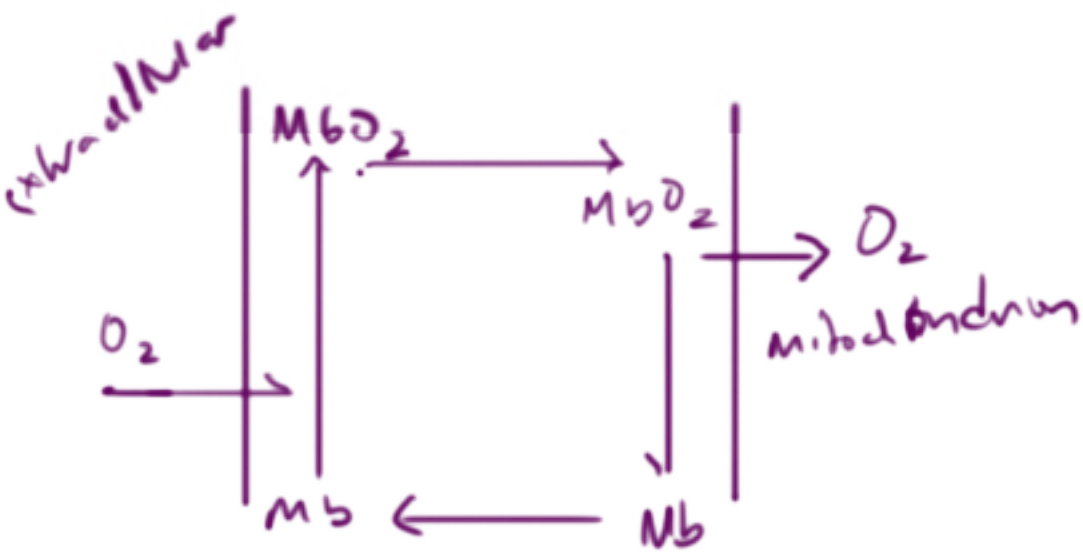
(actually two 2-D gels superimposed one stained blue, one stained orange)

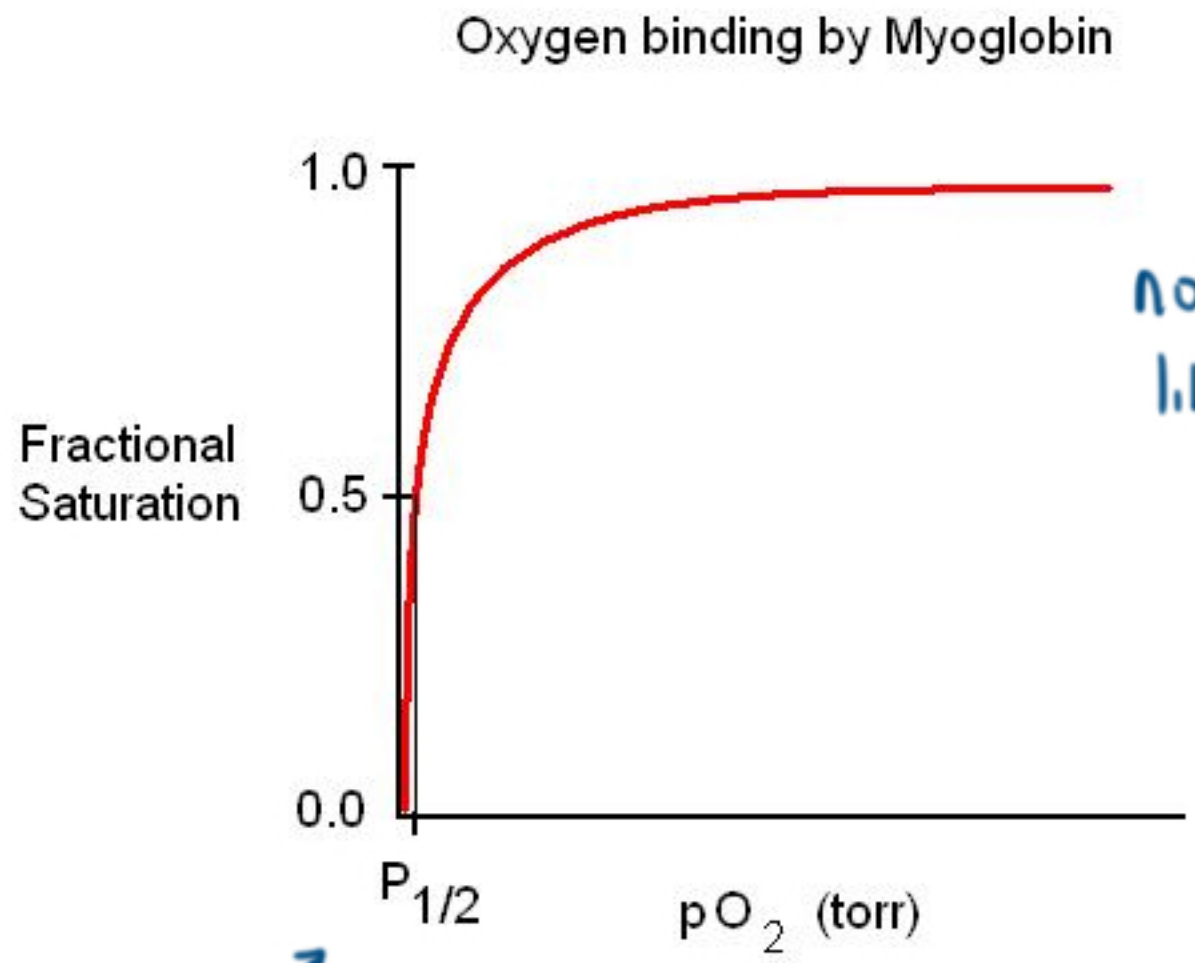
Protein Mass Spec



Myoglobin





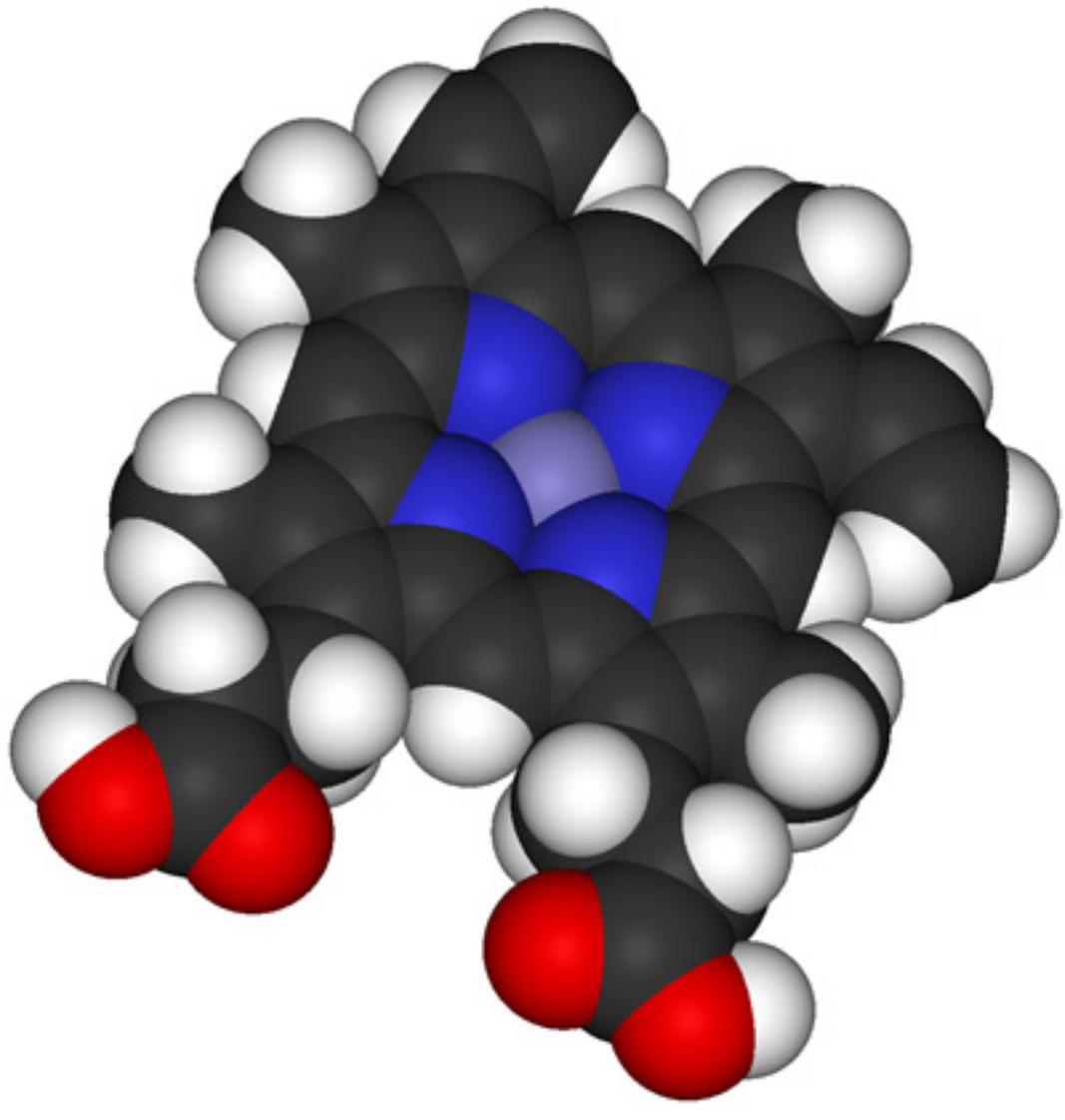


not sigmoidal like hemoglobin

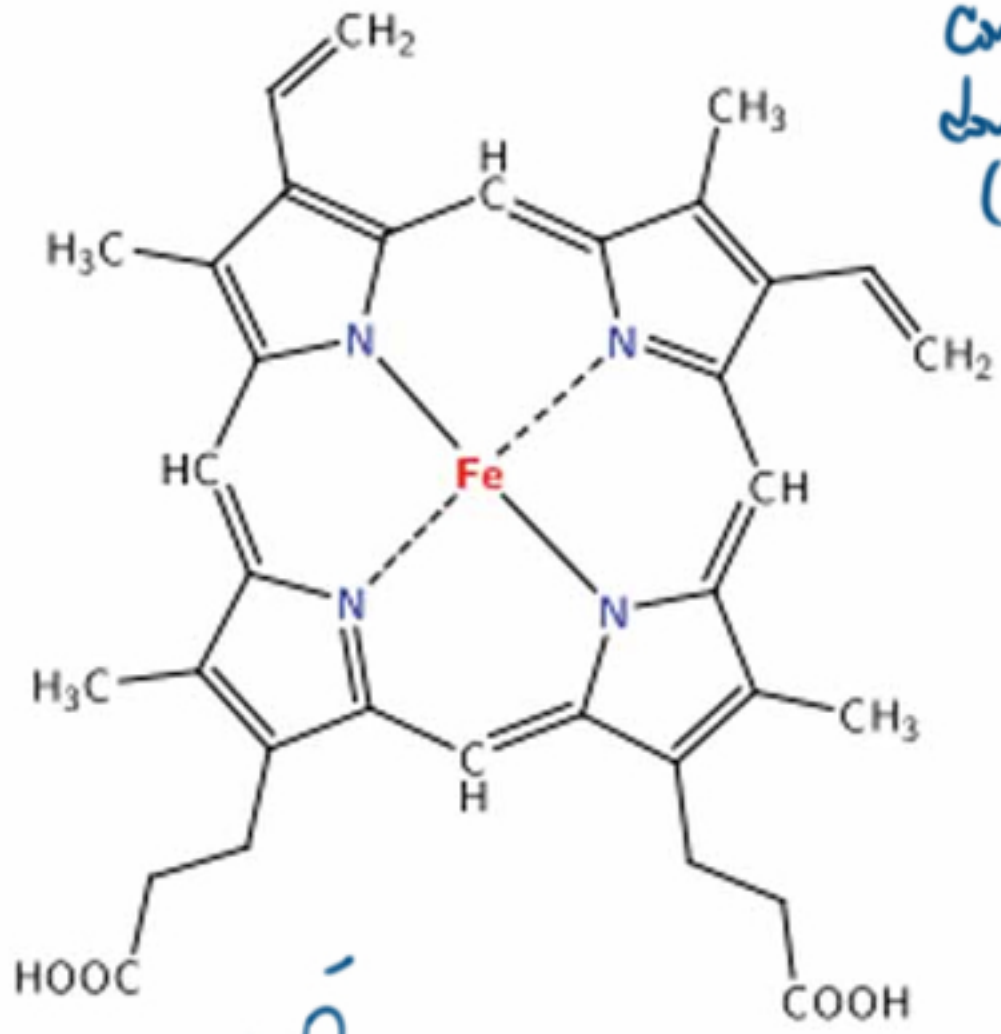
2.75 torr

P_{50} ← concentration at half saturation

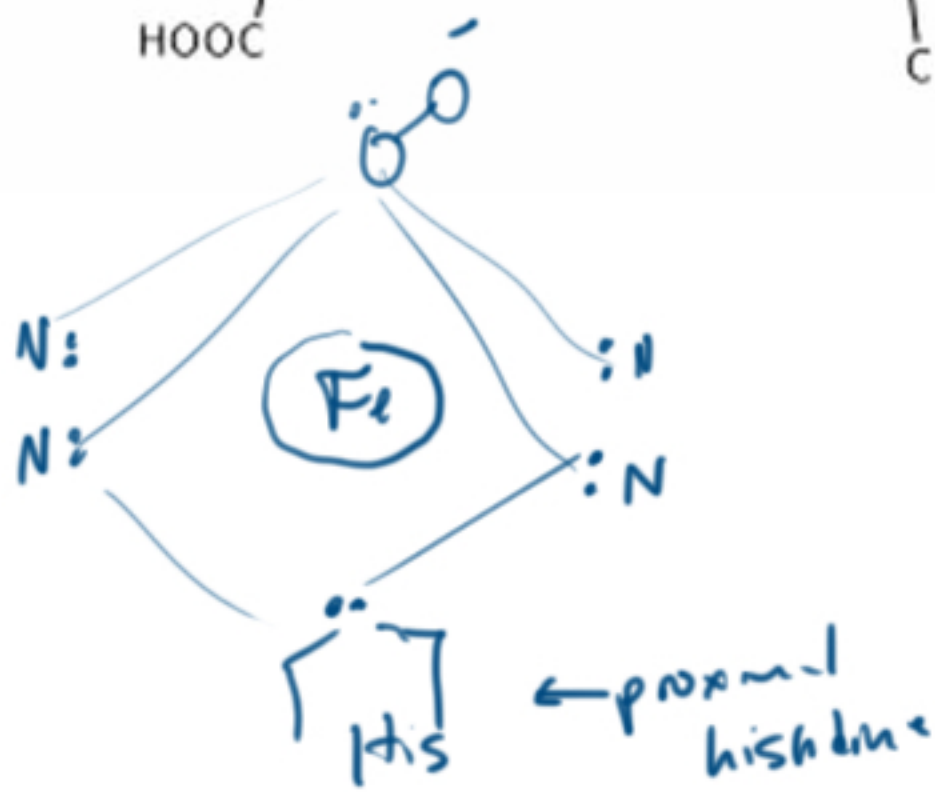
with hemoglobin $P_{50} \approx 27$ torr



Heme :
porphyrin
+
iron



Conjugated double bonds (extensive) means it is a pigment



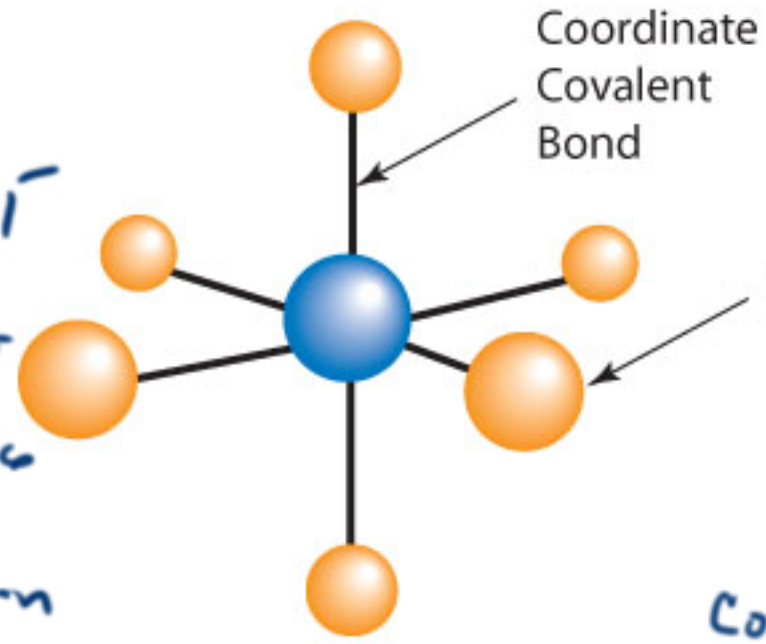
Coordination Complex

brief coordination chemistry primer

$CoCl_3 \rightleftharpoons Co^{3+} + 3Cl^-$

$Co^{3+} + 6NH_3 \rightleftharpoons Co(NH_3)_6^{3+}$

complex ion

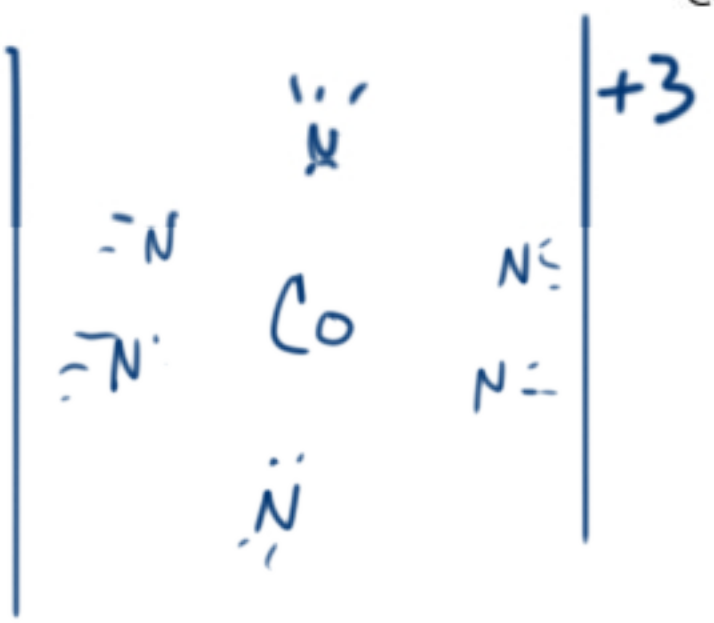


← Lewis base

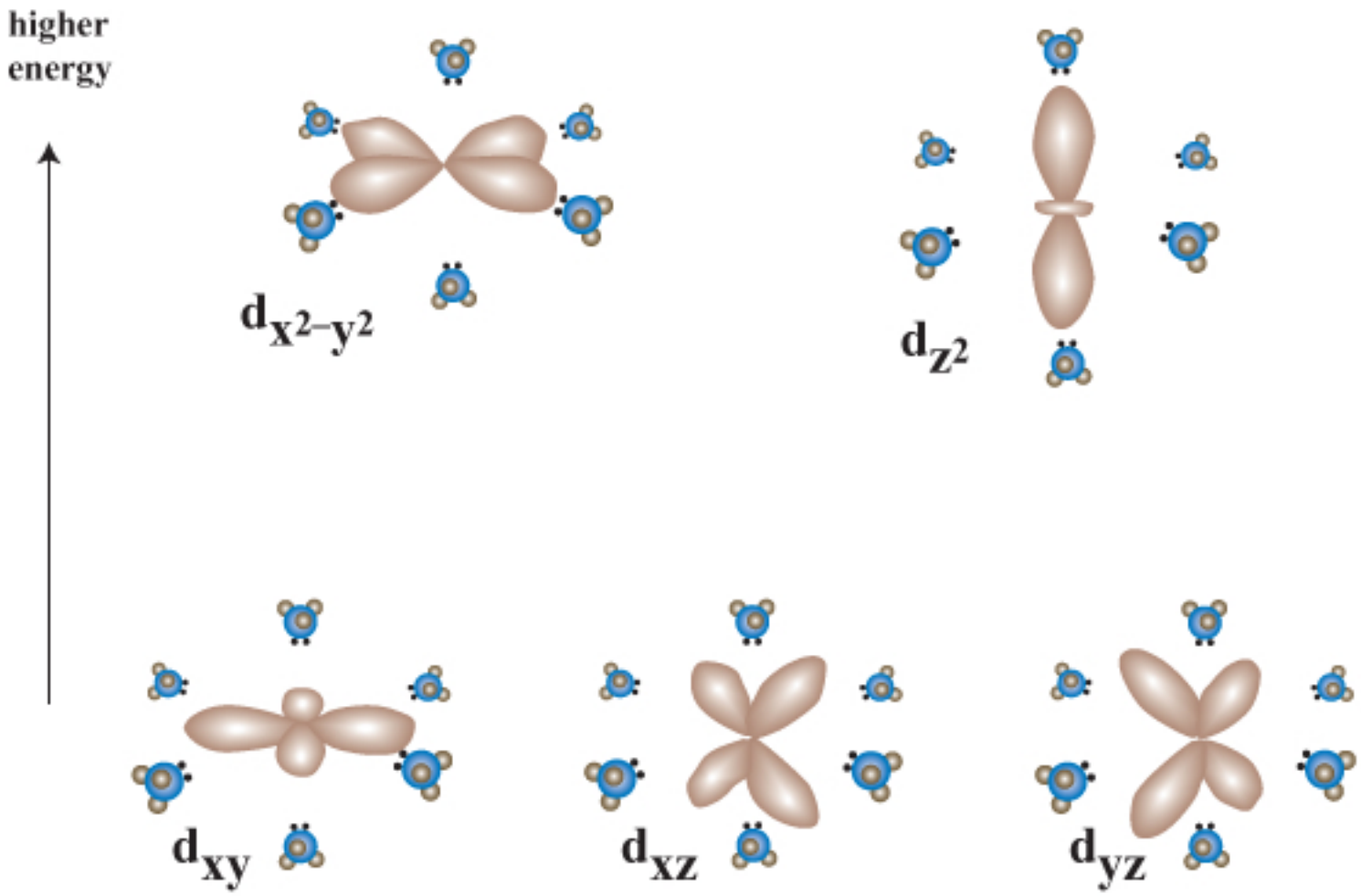
H_2O, NH_3, Cl^- etc

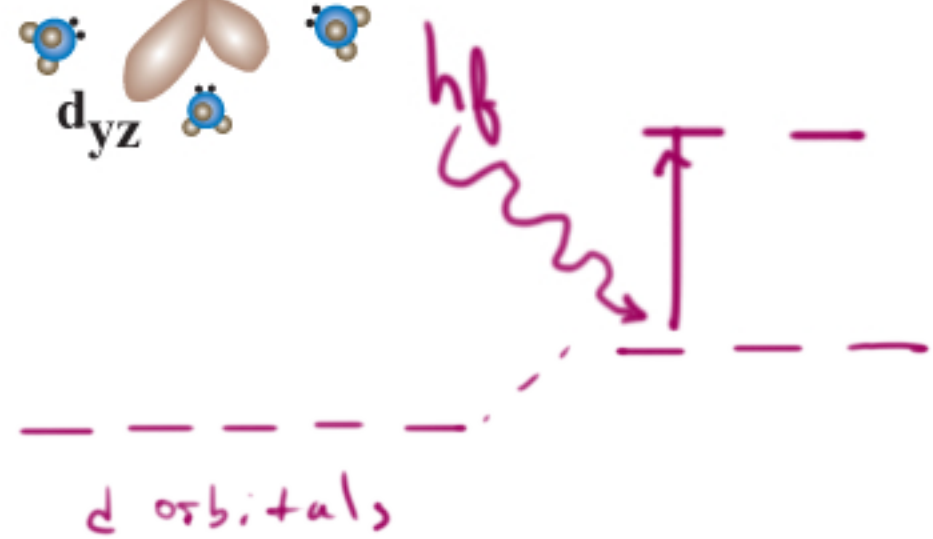
Coordination Complex

Coordination # is the # of electron pairs involved. (may be polydentate ligands)



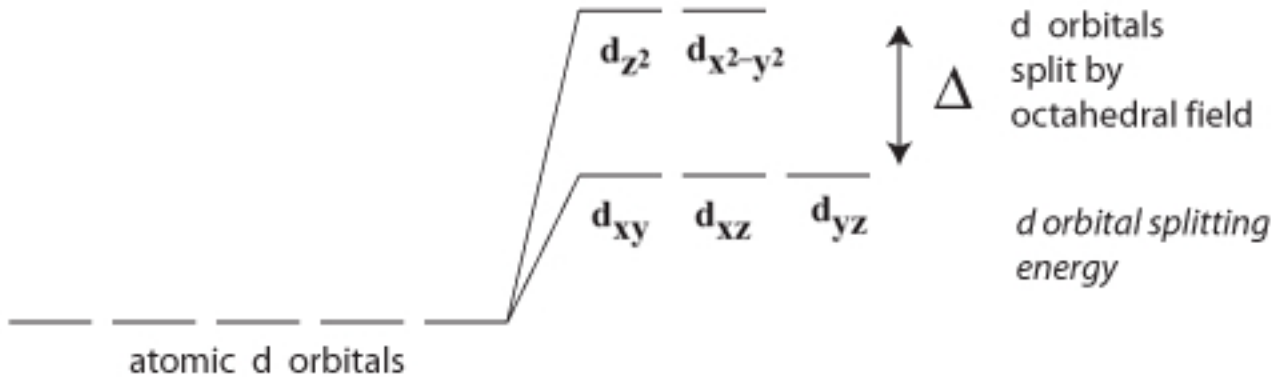
Complex ion

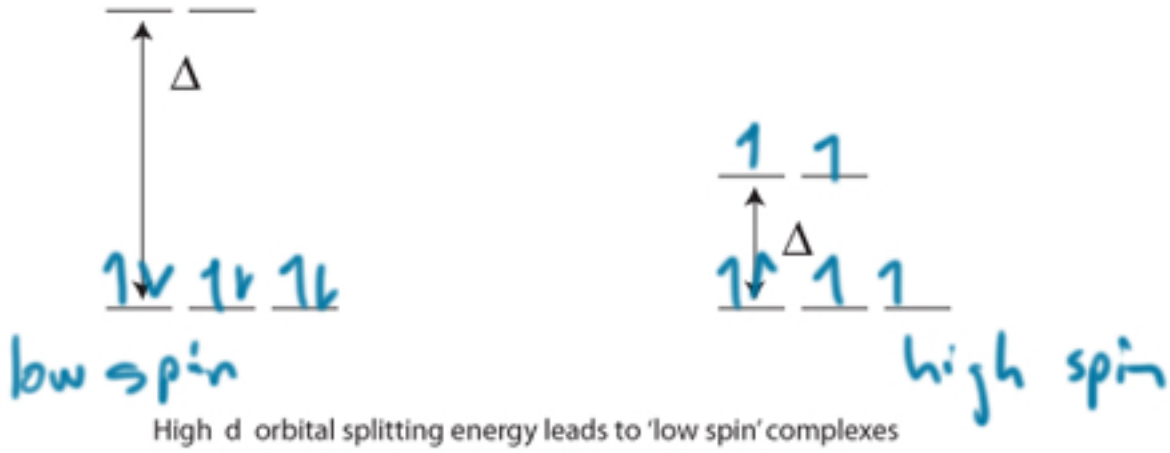




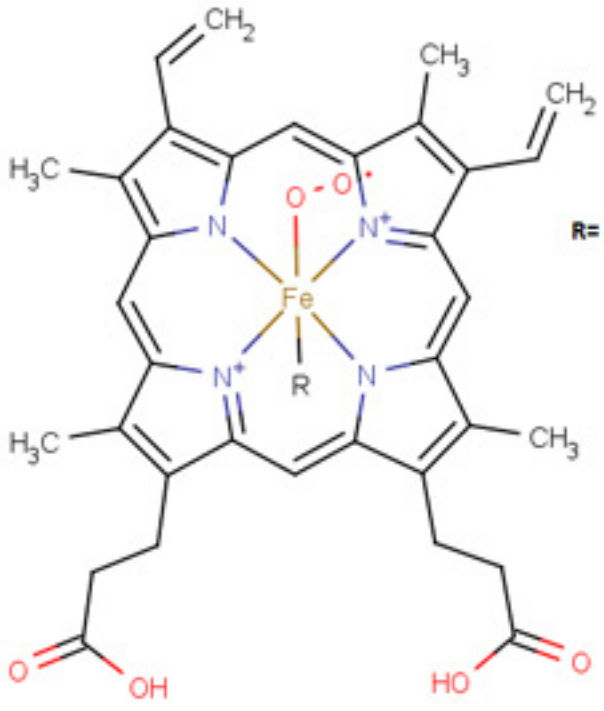
Coordination complexes are often brightly colored

crystal field splitting





High d orbital splitting energy leads to 'low spin' complexes



R= globin protein

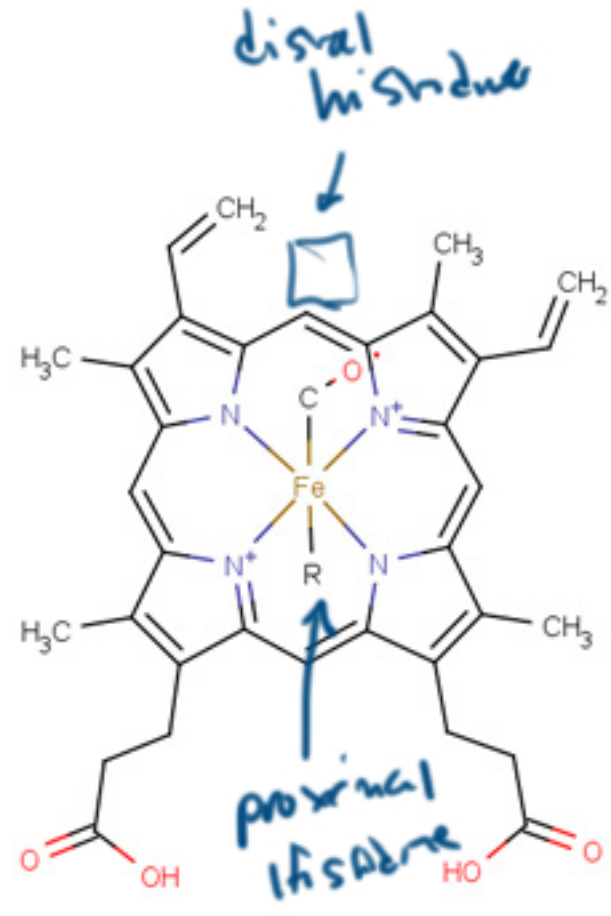
Oxyhemoglobin

— — superoxide electron coupling

↑↓ ↑↓ ↑↓

in oxyhemoglobin
low spin Fe^{III}

- yet HbO_2 is diamagnetic

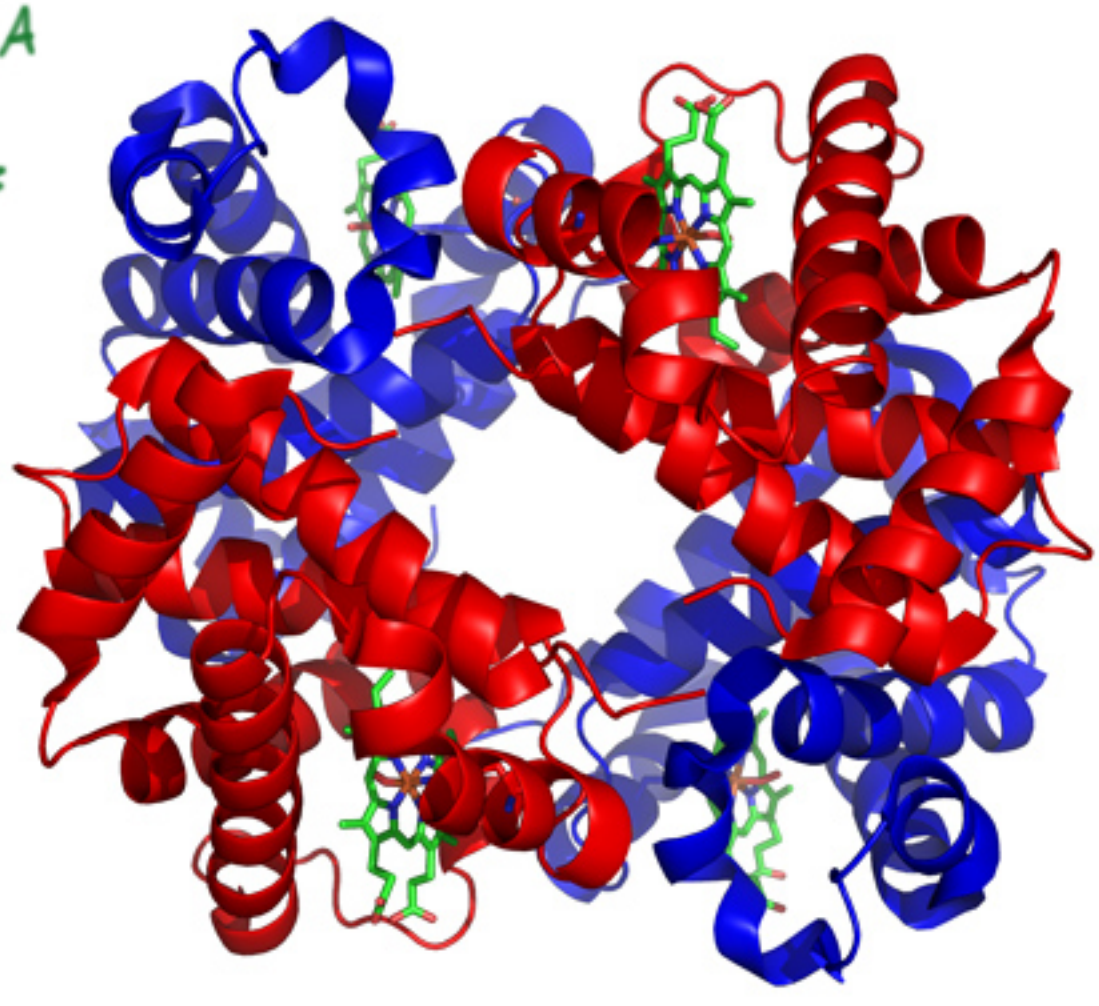


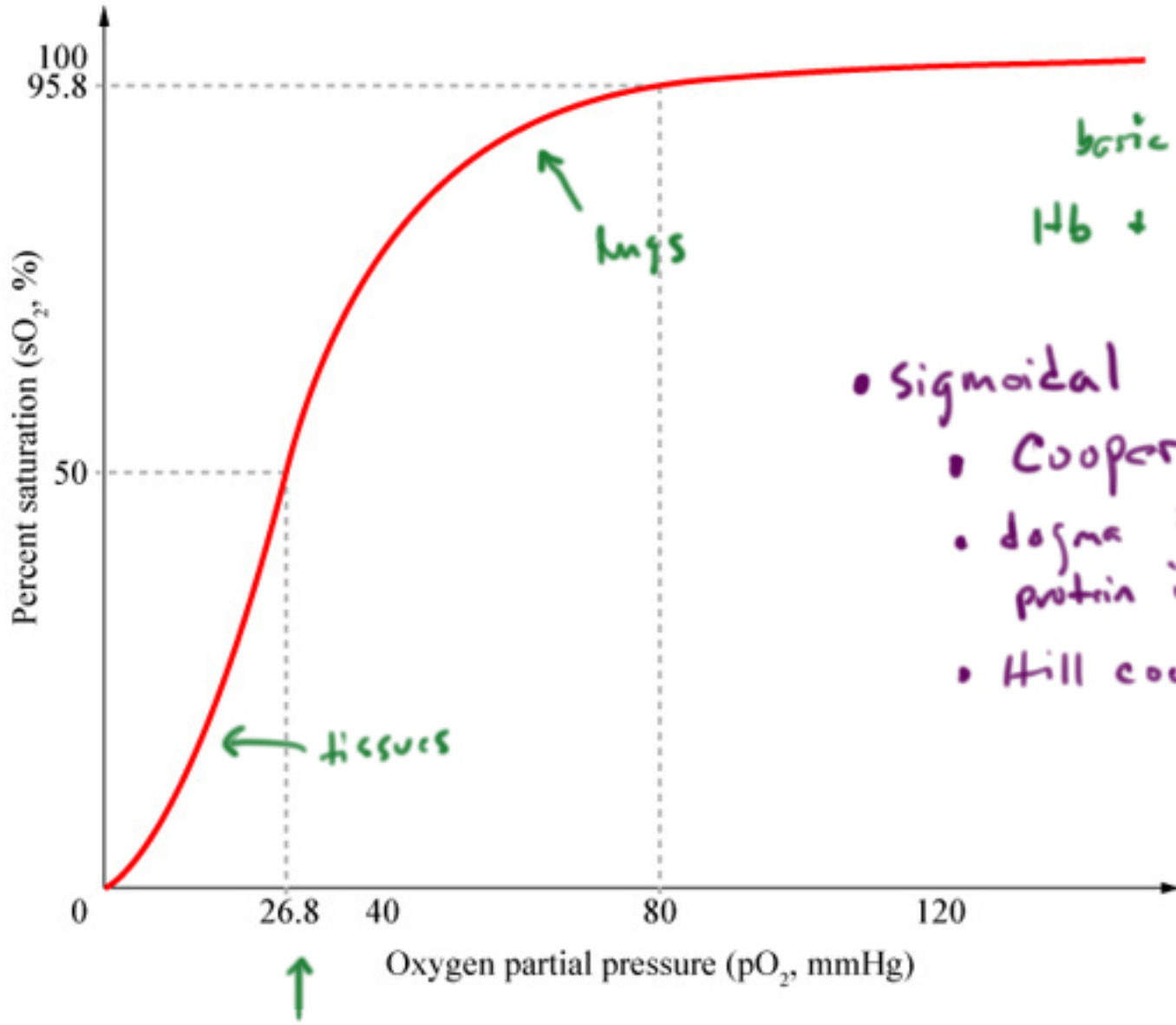
Carboxyhemoglobin

$\alpha_2\beta_2$ HbA

$\alpha_2\gamma_2$ HbF

Hemoglobin



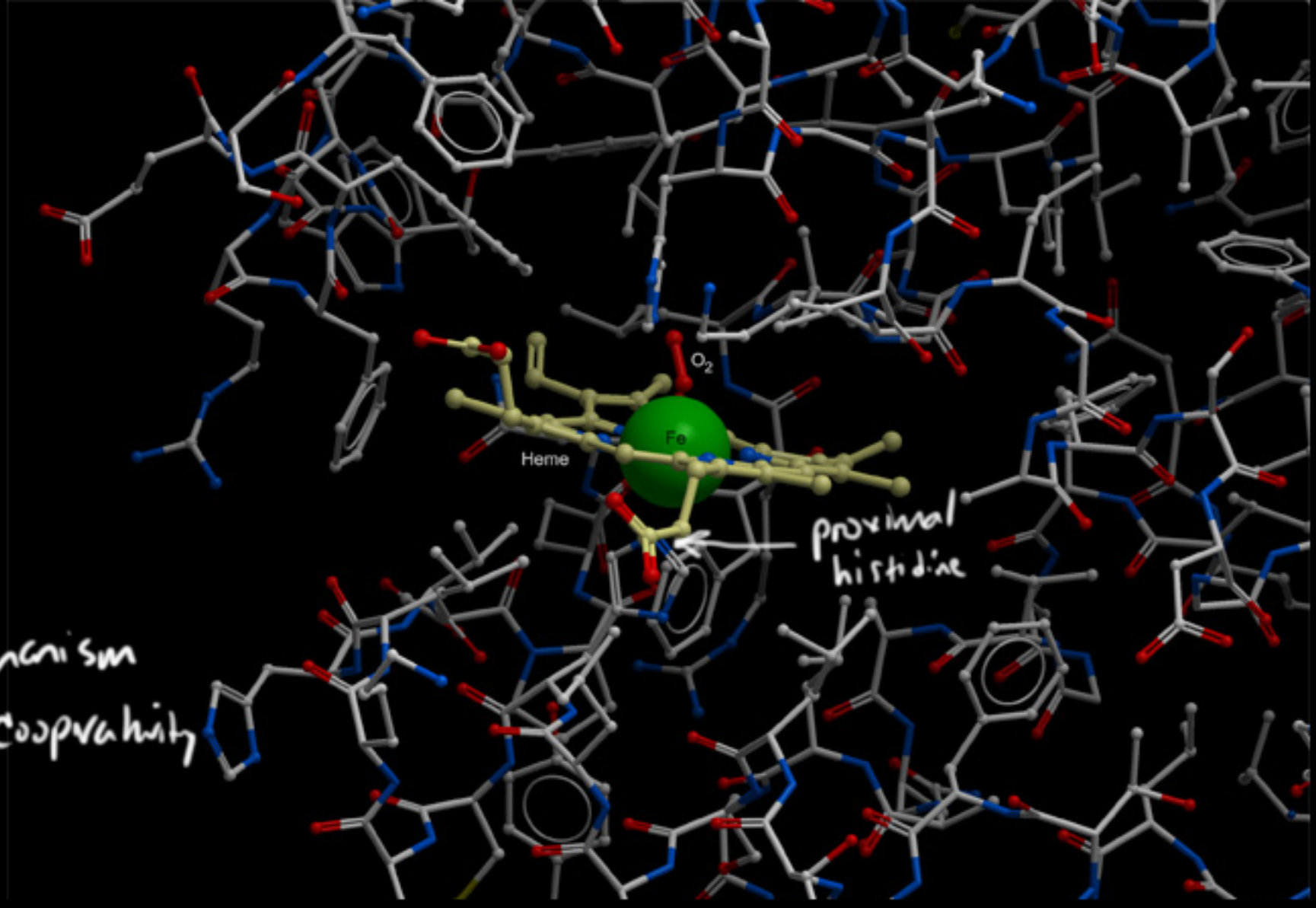


basic picture

$Hb + O_2 \rightleftharpoons HbO_2$

- sigmoidal
  - cooperativity
  - dogma is that the protein is multisubunit
  - Hill coefficient > 1

p^{50}

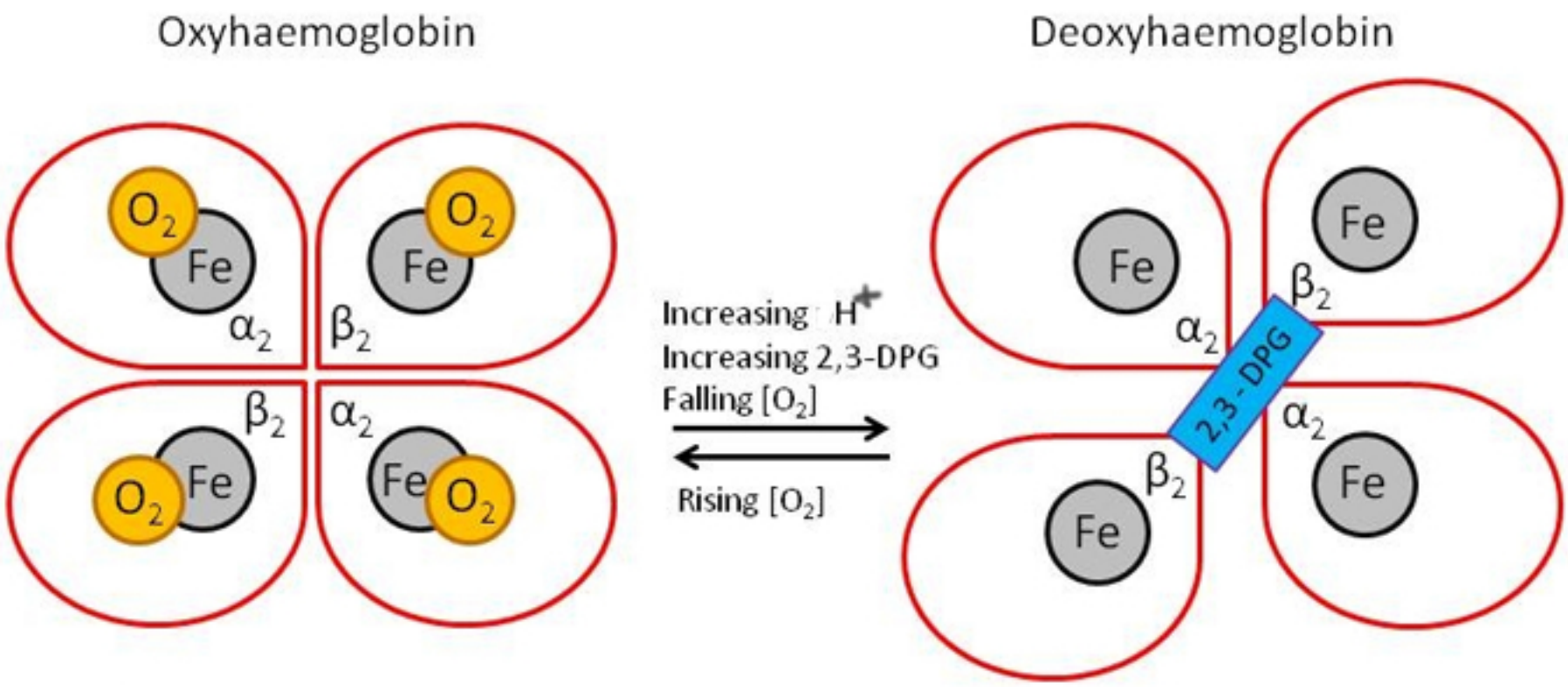


Mechanism of cooperativity

When O_2 binds, the Fe shifts into the porphyrin plane, pulling the proximal histidine. This triggers a conformational change. One $\alpha\beta$ shifts 15° to the other. All the other binding sites are now more facilitative.

Oxygen Binding and Unloading

Monod Jacob Model



$$R \rightleftharpoons T$$

Bohr effect - H^+ stabilizes the tight structure. (also CO_2, DPG)

carbonic acid

$$\xrightarrow{\text{tissues}} \quad CO_2 + H_2O \overset{\text{carbonic anhydrase}}{\rightleftharpoons} H_2CO_3$$

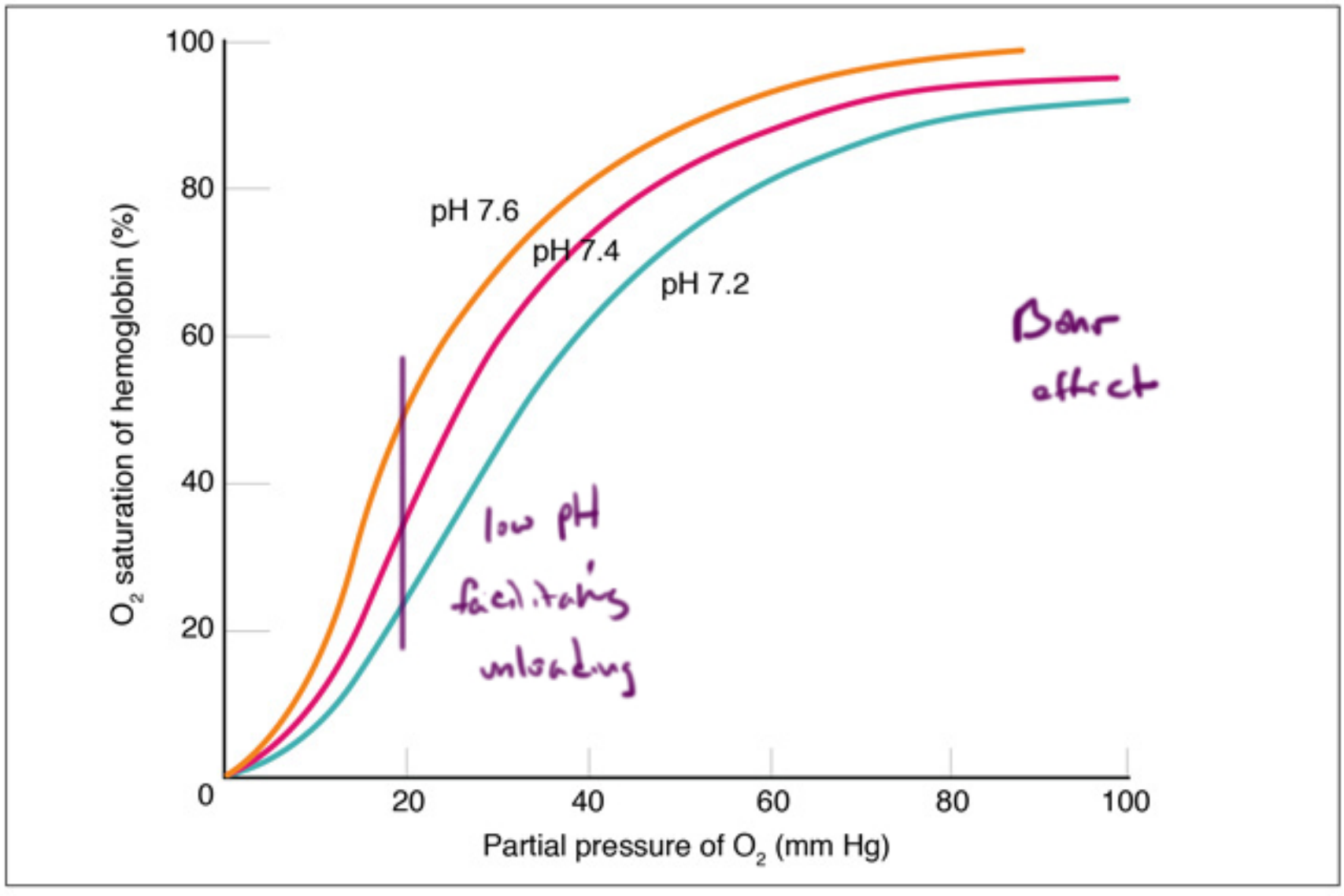
$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

$$H^+ + HbO_2 \rightleftharpoons HbH^+ + O_2 \quad \leftarrow \text{lungs}$$

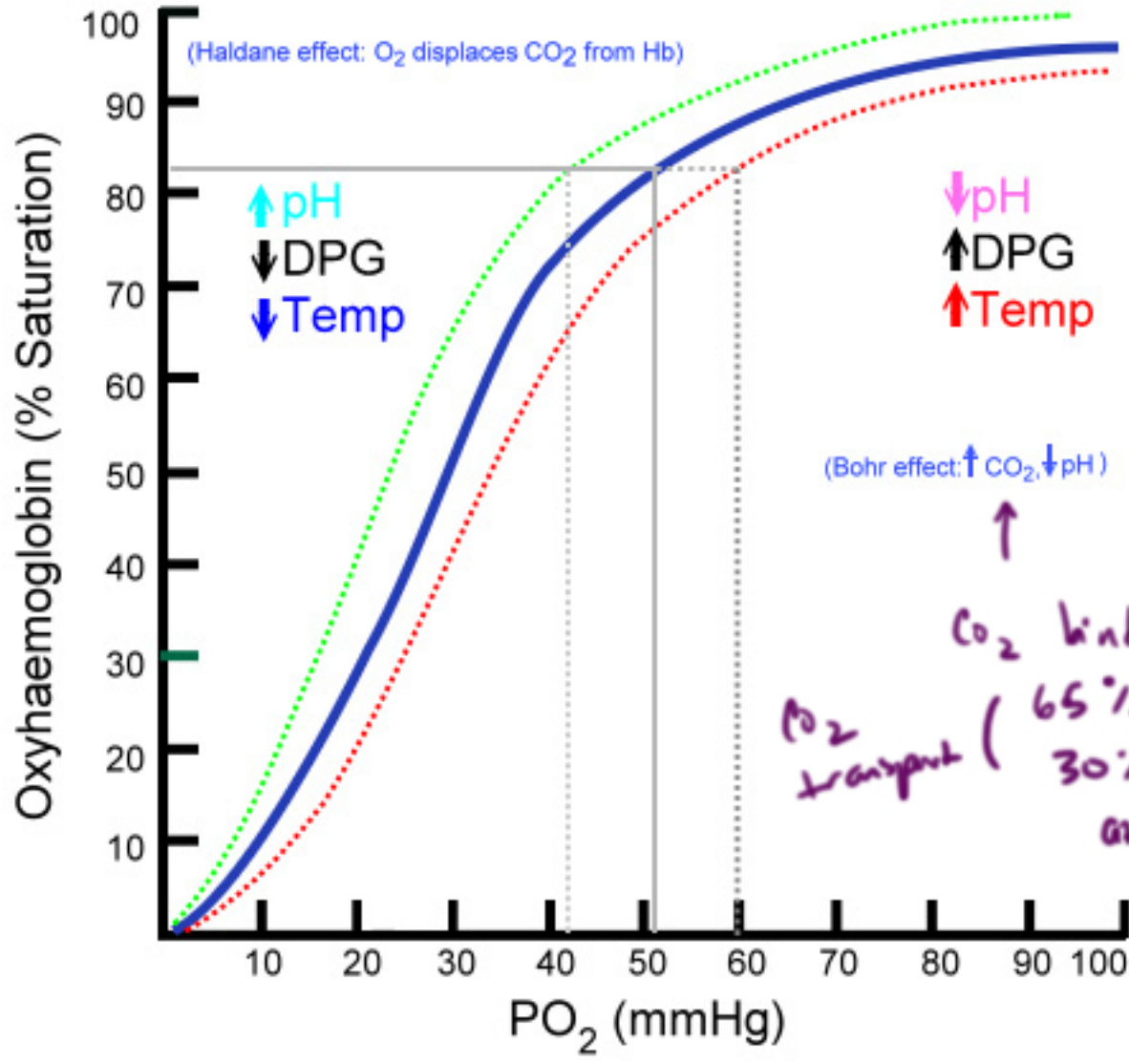
$pKa_{H_2CO_3} = 6.3$

$$pH = pKa + \log\left(\frac{[A^-]}{[HA]}\right)$$

$$7.3 = 6.3 + \log\left(\frac{10}{1}\right)$$

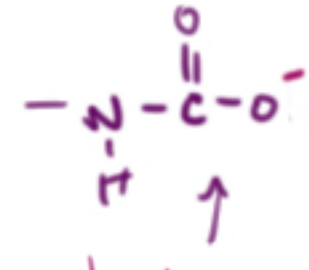


(b) Effect of pH



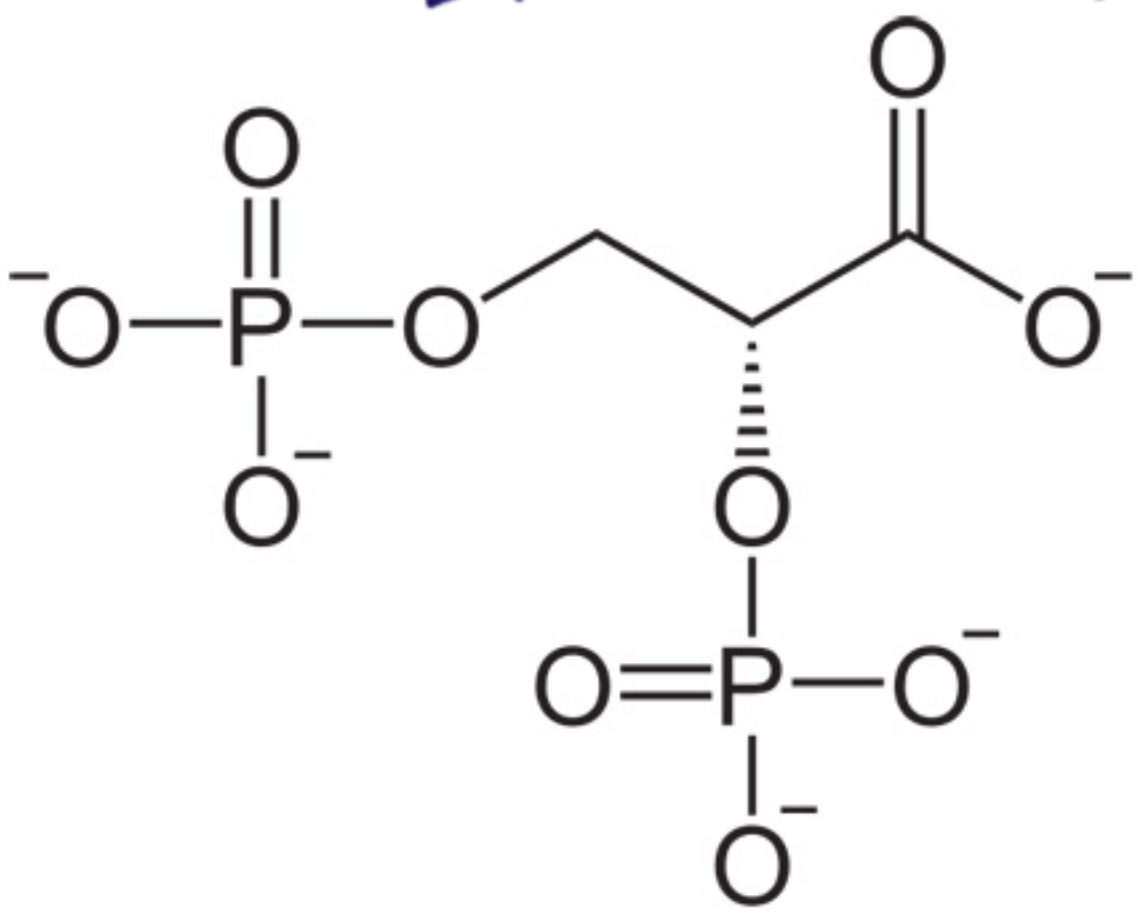
CO_2 binds Hb

CO_2 transport (65% as $^-HCO_3$
30% bound directly
as N termini:

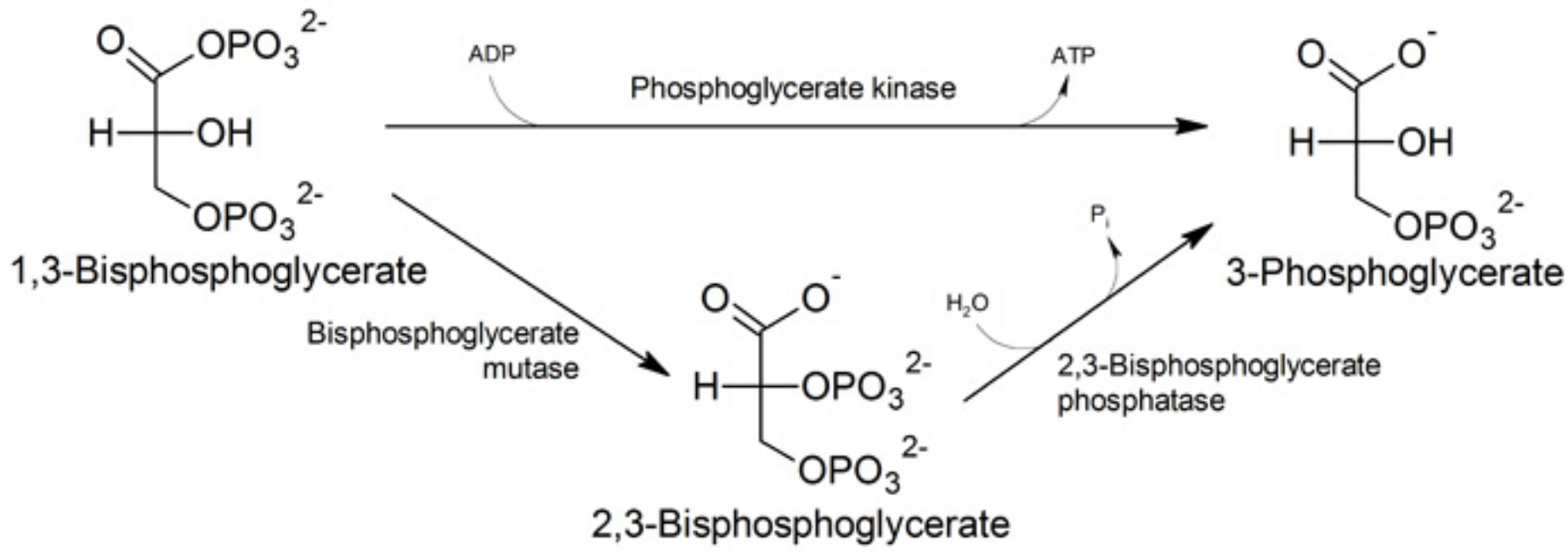


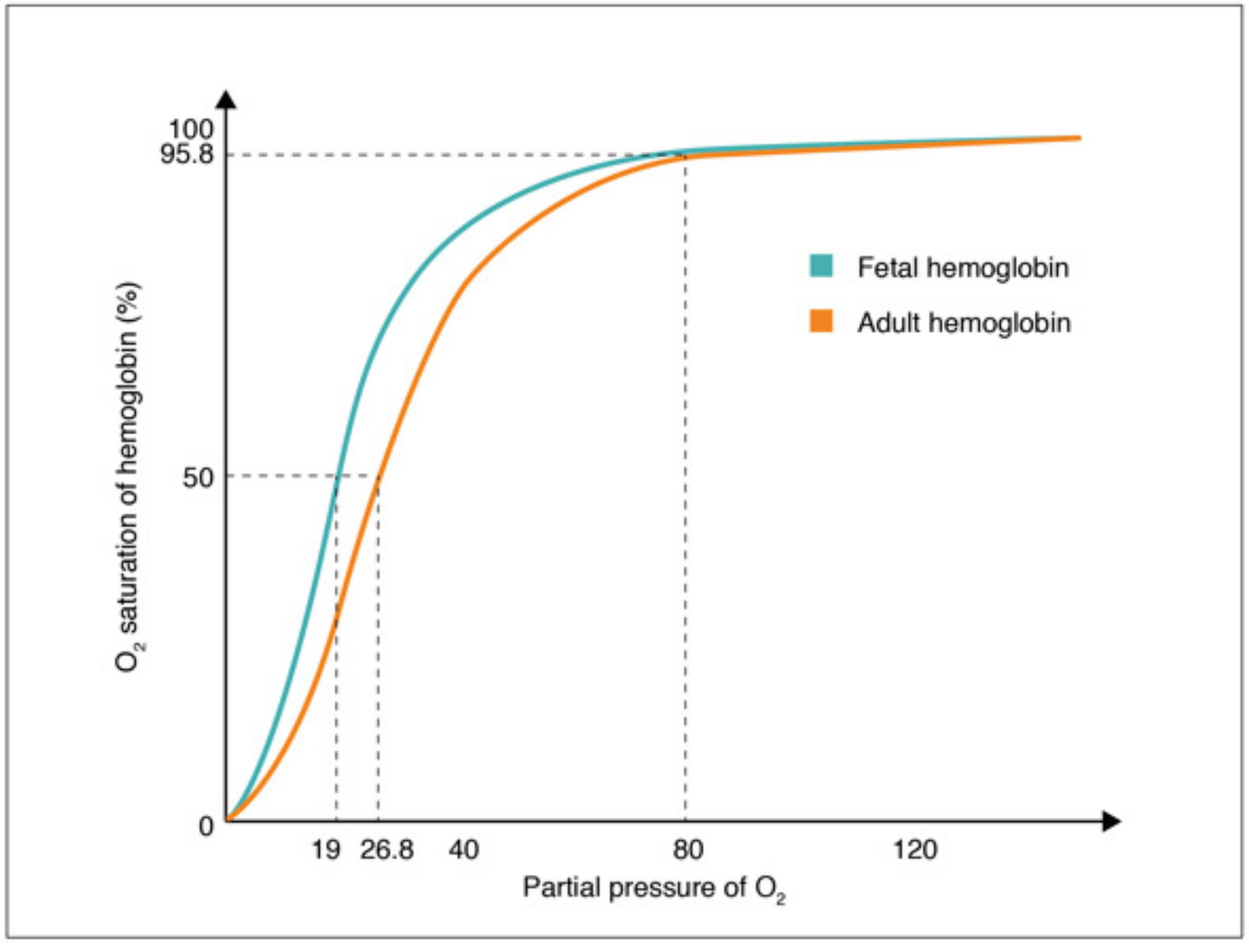
bound carbamide

DPG

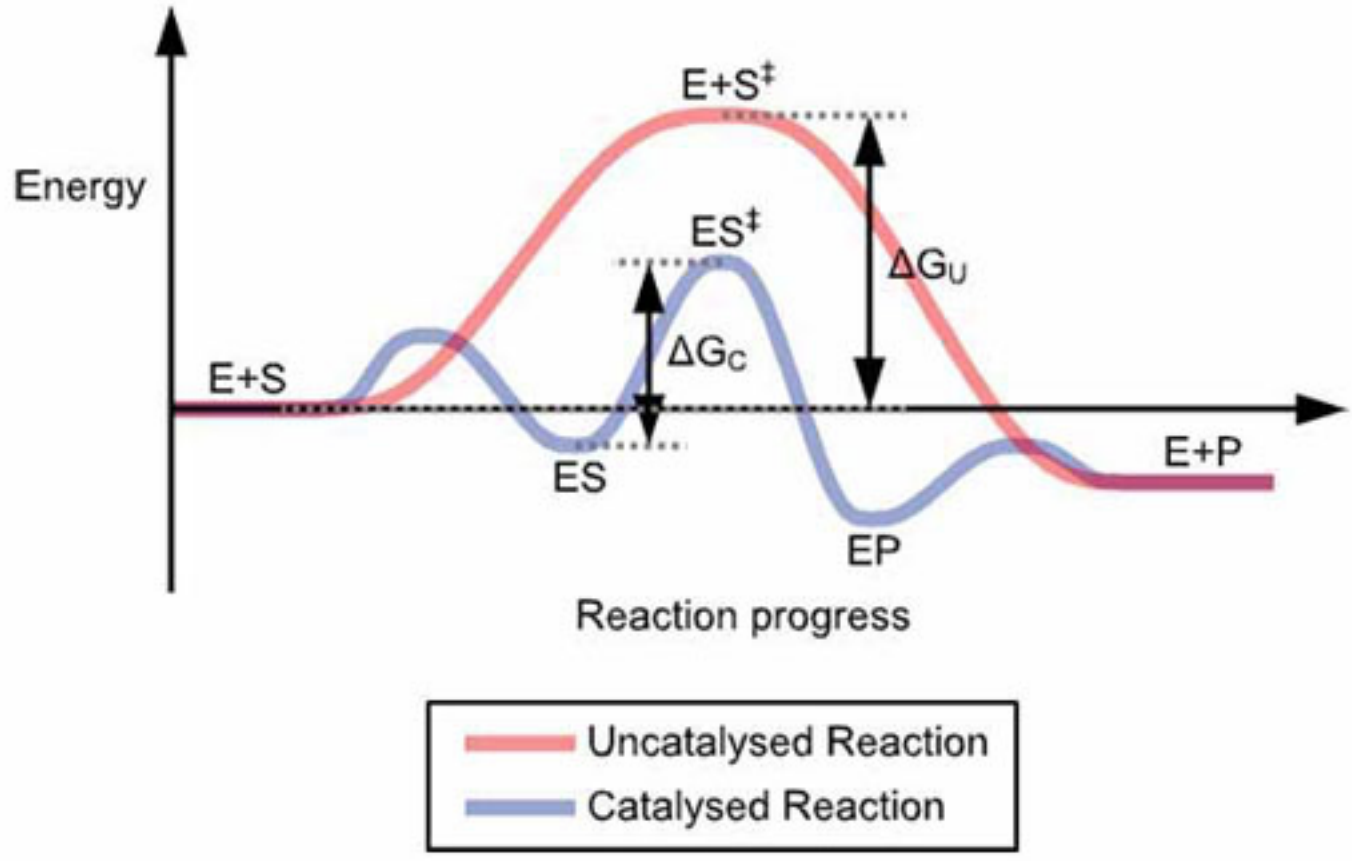


- stabilizes the tight structure
- reduces the O_2 affinity
- facilitates unloading

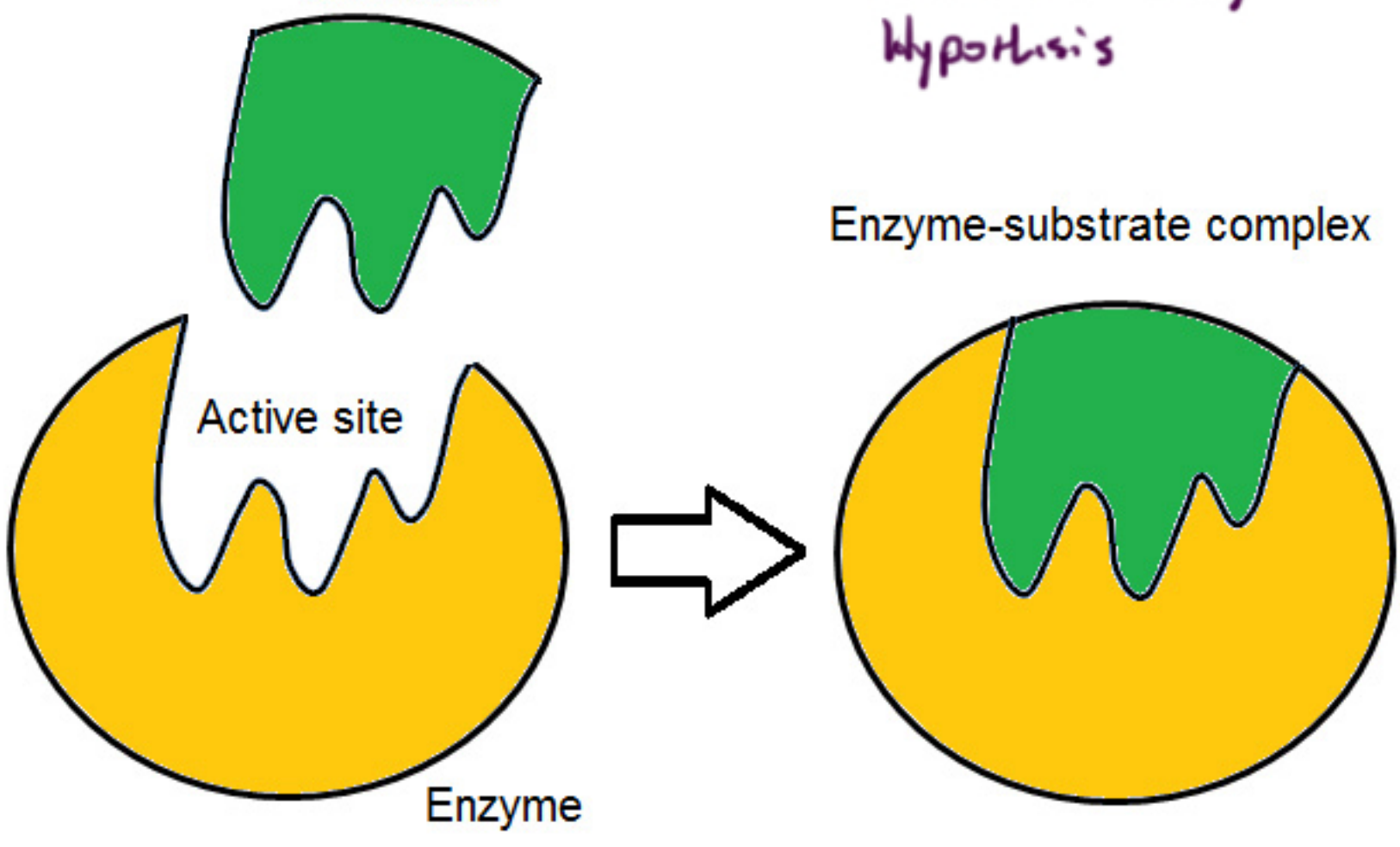

ADP
Phosphoglycerate kinase
ATP
1,3-Bisphosphoglycerate
3-Phosphoglycerate
Bisphosphoglycerate mutase
2,3-Bisphosphoglycerate
H_2O
P_i
2,3-Bisphosphoglycerate phosphatase


100
95.8
50
0
O_2 saturation of hemoglobin (%)
Fetal hemoglobin
Adult hemoglobin
19
26.8
40
80
120
Partial pressure of O_2

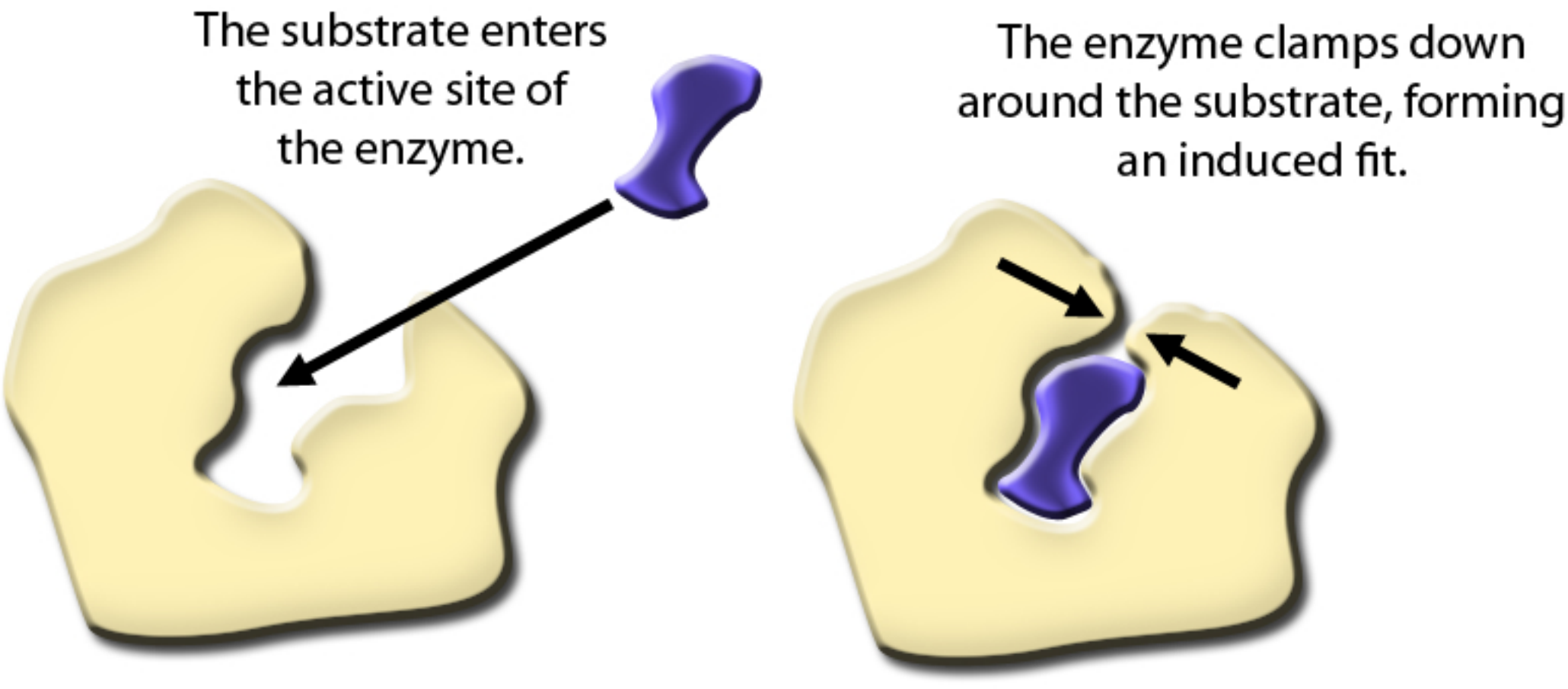
Enzyme Activity



Lock and Key Hypothesis

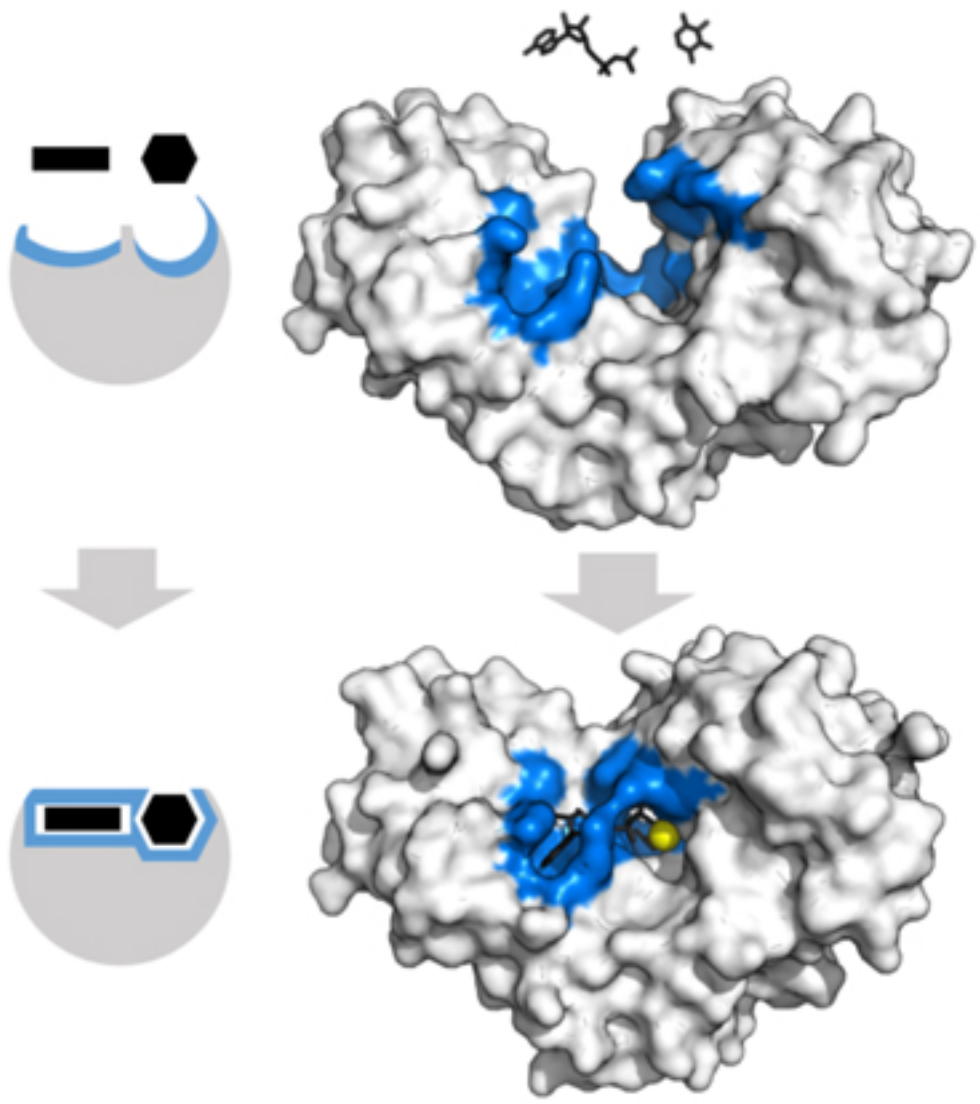


Induced Fit



not only binding but also turnover

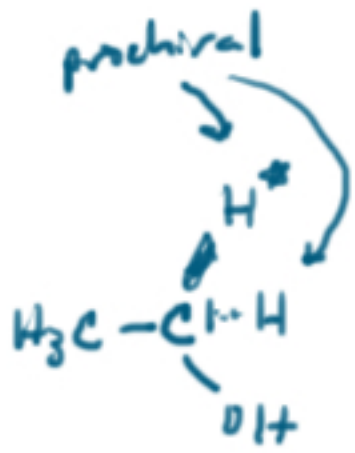
Induced Fit
in Hexokinase



glucose + ATP ⟶ G6P + ADP

Enzymes may distinguish prochiral substituents.

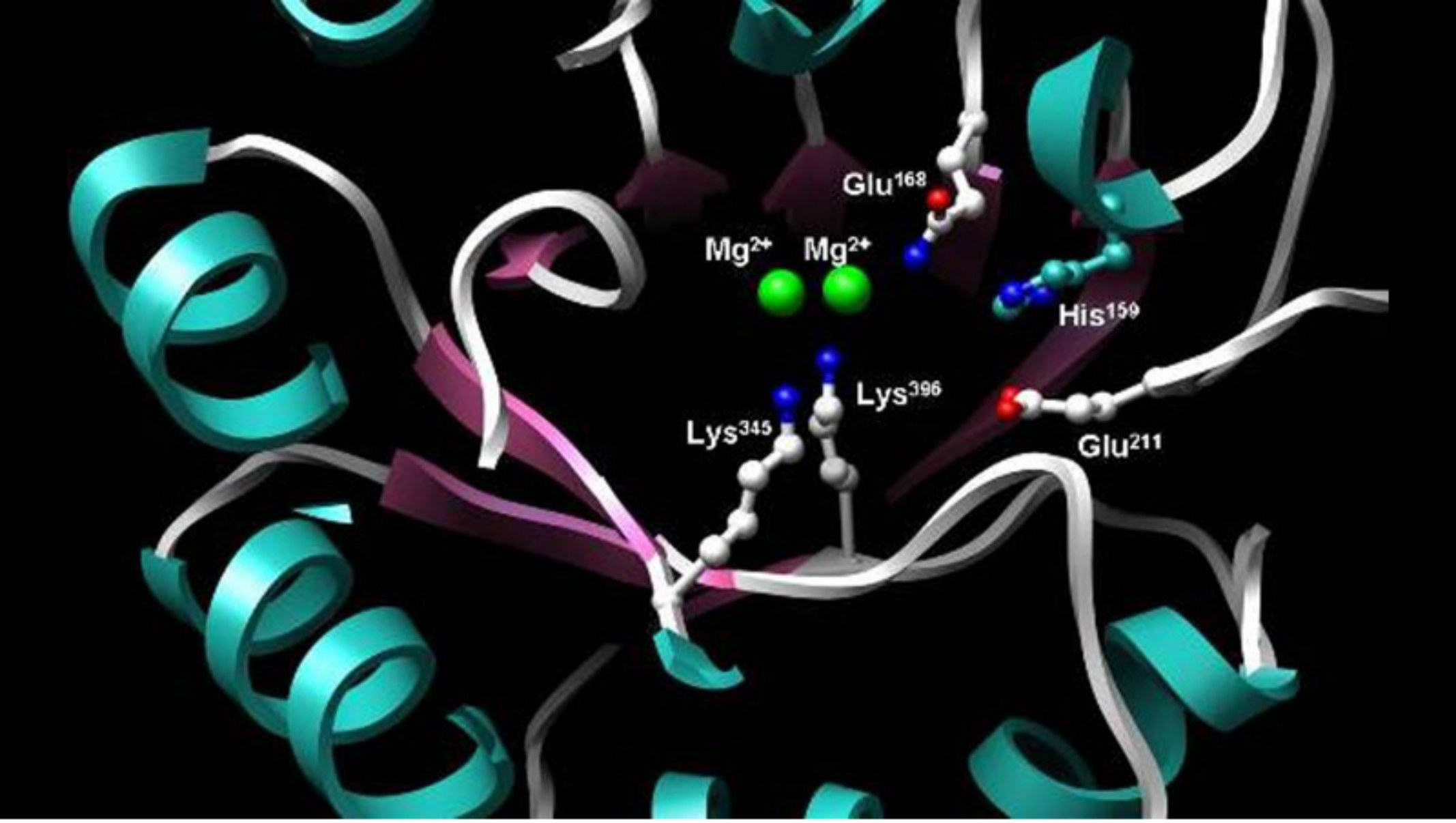
$$CH_3CH_2OH + NAD^+ \underset{\text{Yeast alcohol dehydrogenase}}{\rightleftharpoons} CH_3\overset{O}{\overset{\|}{C}}H + NADH + H^+$$



← If one H were ^2H (deuterium) is it possible that the NADH product is all NADH* or must it be 50:50?

On the benchtop the two H's would be equivalent.

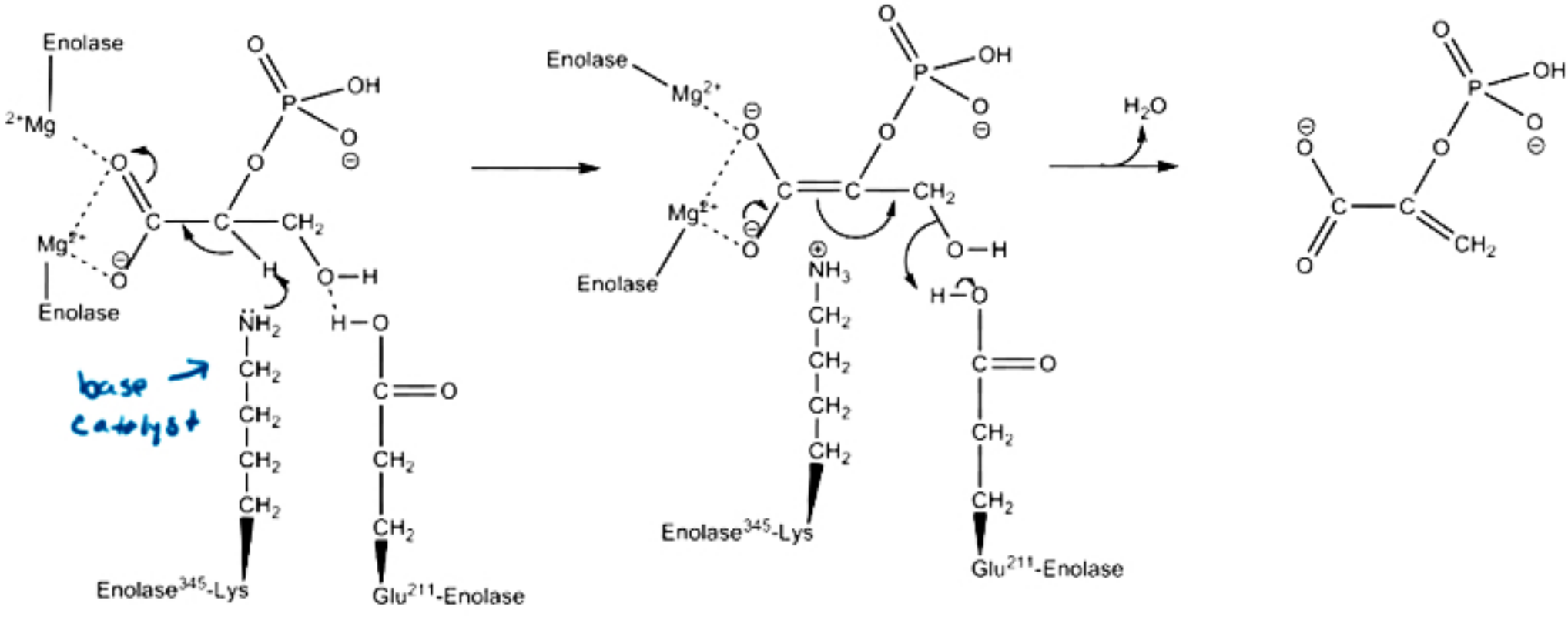
But an enzyme can distinguish prochiral substituents.

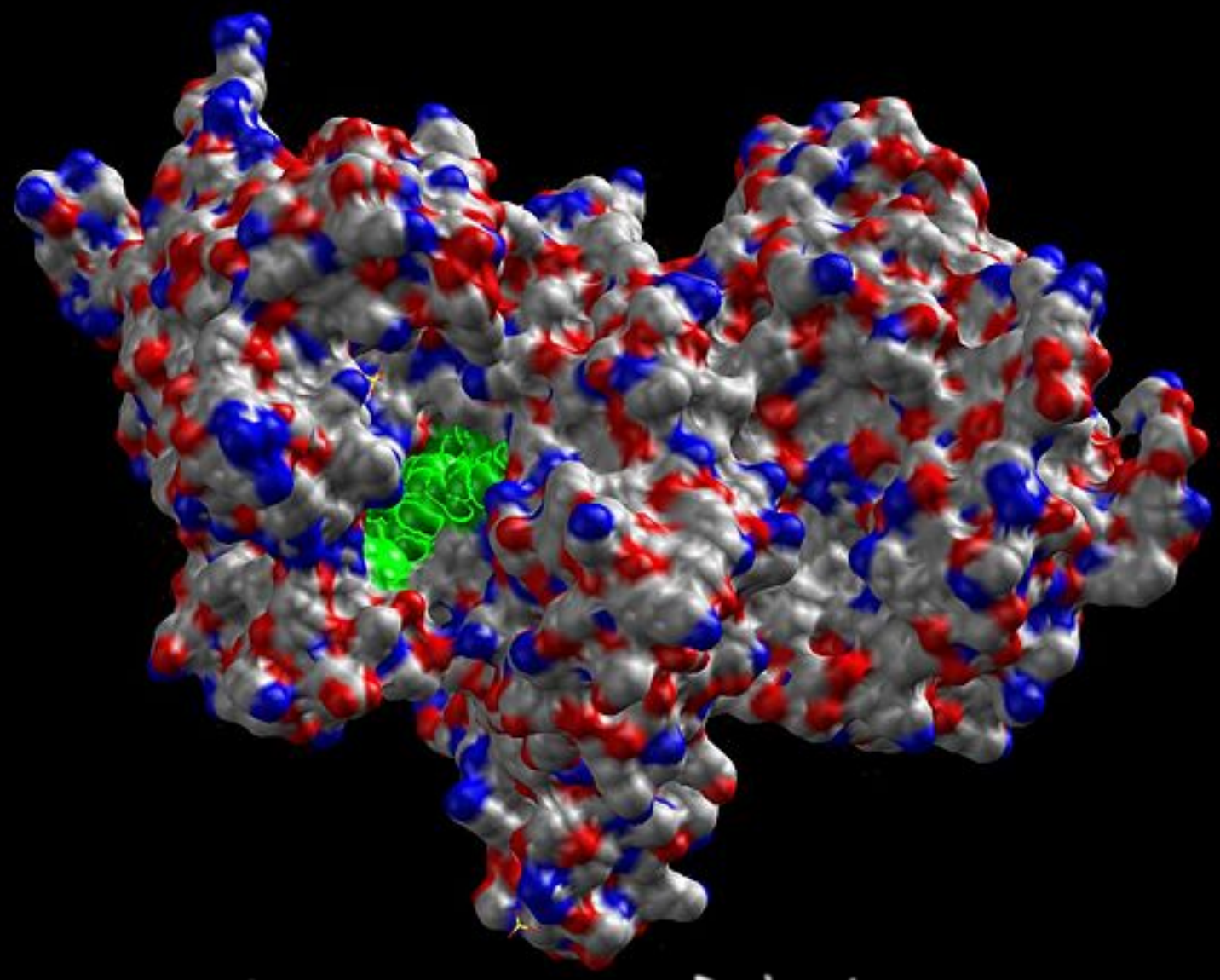


Enolase:

2-phosphoglycerate ⟶ phosphoenolpyruvate

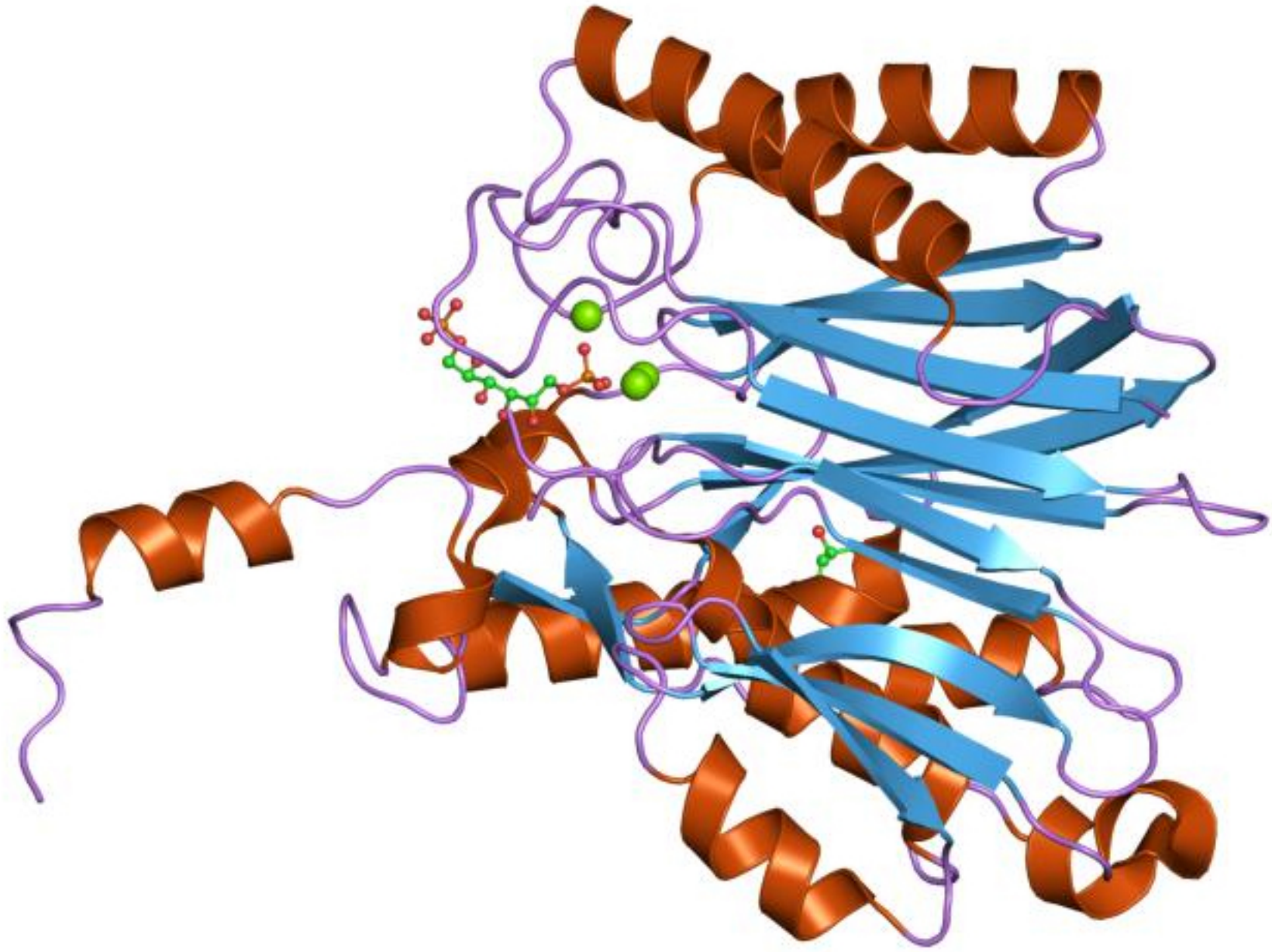
Enolase Mechanism





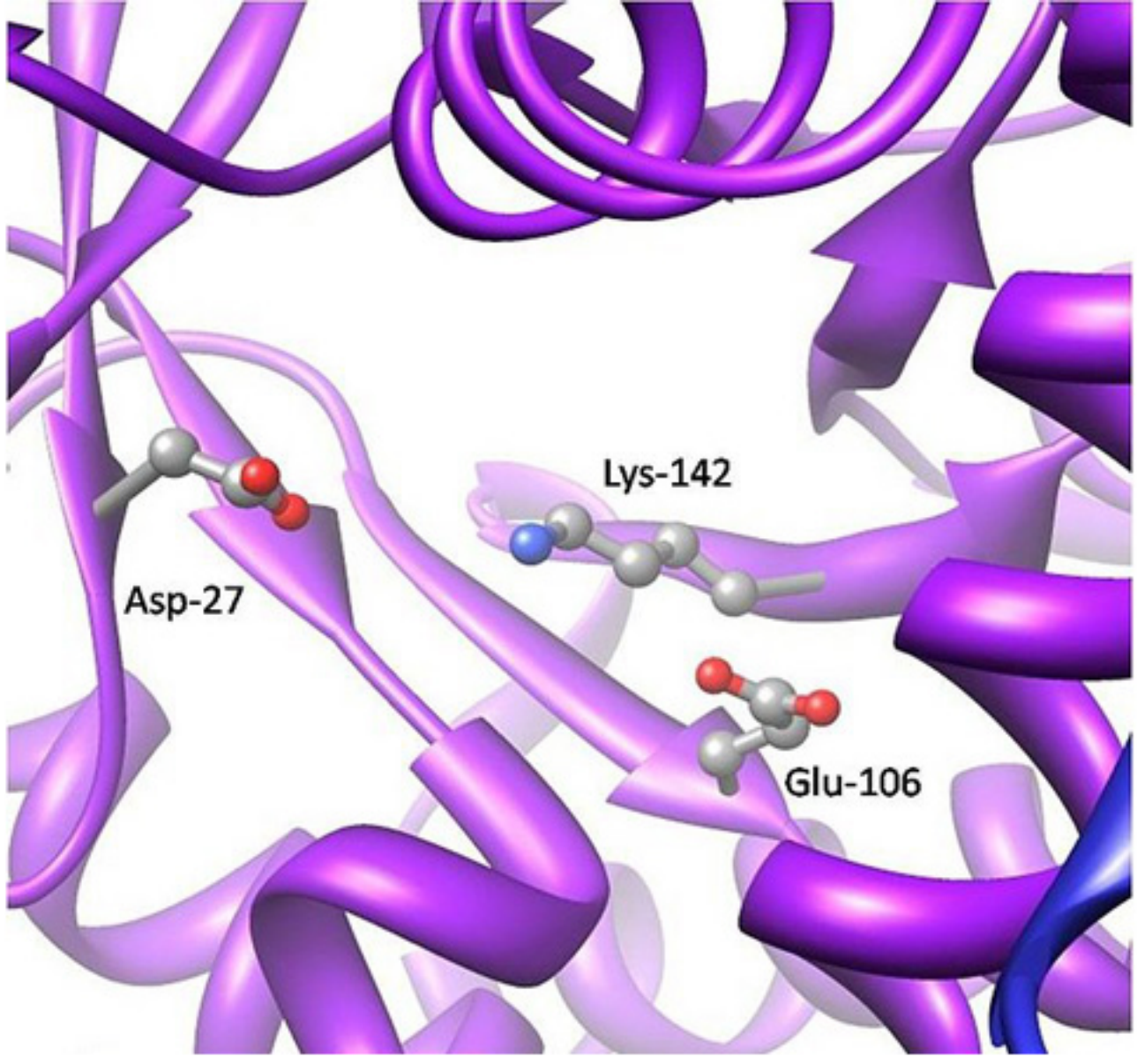
Isocitrate Dehydrogenase

$$\text{Isocitrate} \xrightarrow[NAD^+ \rightarrow NADH]{} \text{Oxalosuccinate} \xrightarrow[CO_2]{} \alpha\text{-ketoglutarate}$$



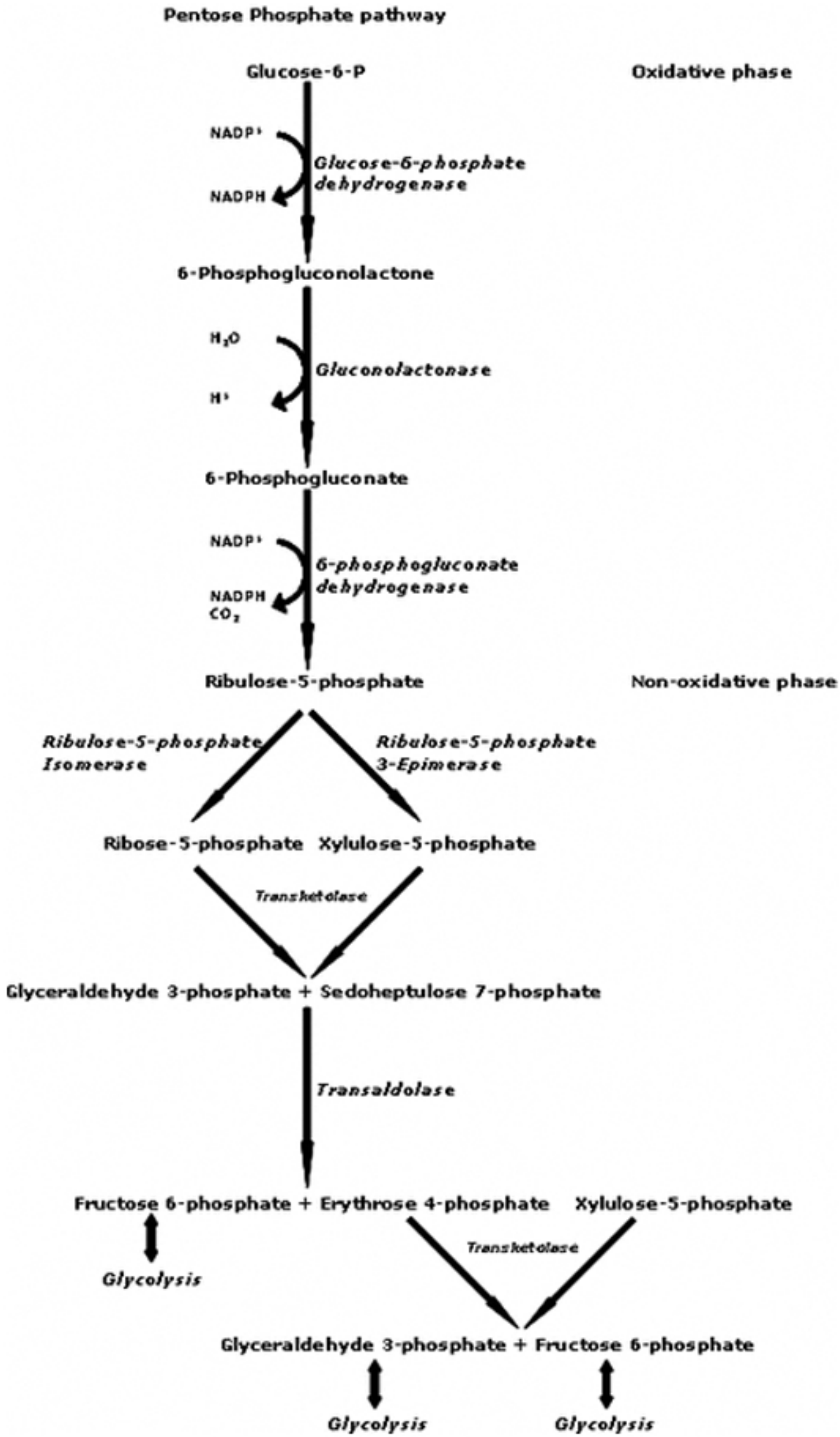
Fructose 1, 6 Bis phosphatase

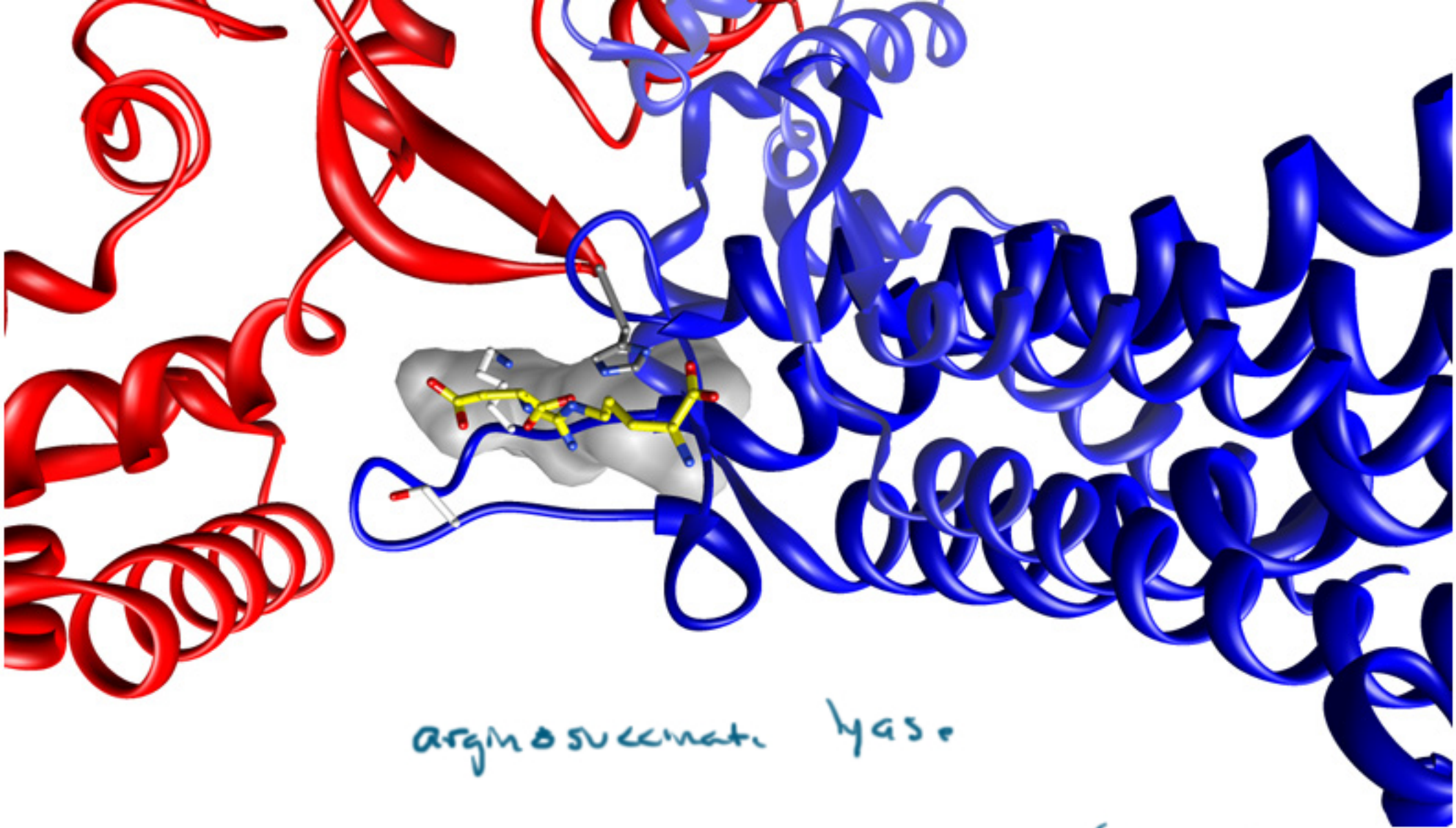
$$F\,1,6\,P \longrightarrow F\,6\,P + P_i$$



Transaldolase

S7P + G3P ⟶ E4P + F6P


Pentose Phosphate pathway
Glucose-6-P
Oxidative phase
NADP+
NADPH
Glucose-6-phosphate dehydrogenase
6-Phosphogluconolactone
H2O
H+
Gluconolactonase
6-Phosphogluconate
NADP+
NADPH
CO2
6-phosphogluconate dehydrogenase
Ribulose-5-phosphate
Non-oxidative phase
Ribulose-5-phosphate Isomerase
Ribulose-5-phosphate 3-Epimerase
Ribose-5-phosphate
Xylulose-5-phosphate
Transketolase
Glyceraldehyde 3-phosphate + Sedoheptulose 7-phosphate
Transaldolase
Fructose 6-phosphate + Erythrose 4-phosphate
Xylulose-5-phosphate
Glycolysis
Transketolase
Glyceraldehyde 3-phosphate + Fructose 6-phosphate
Glycolysis
Glycolysis



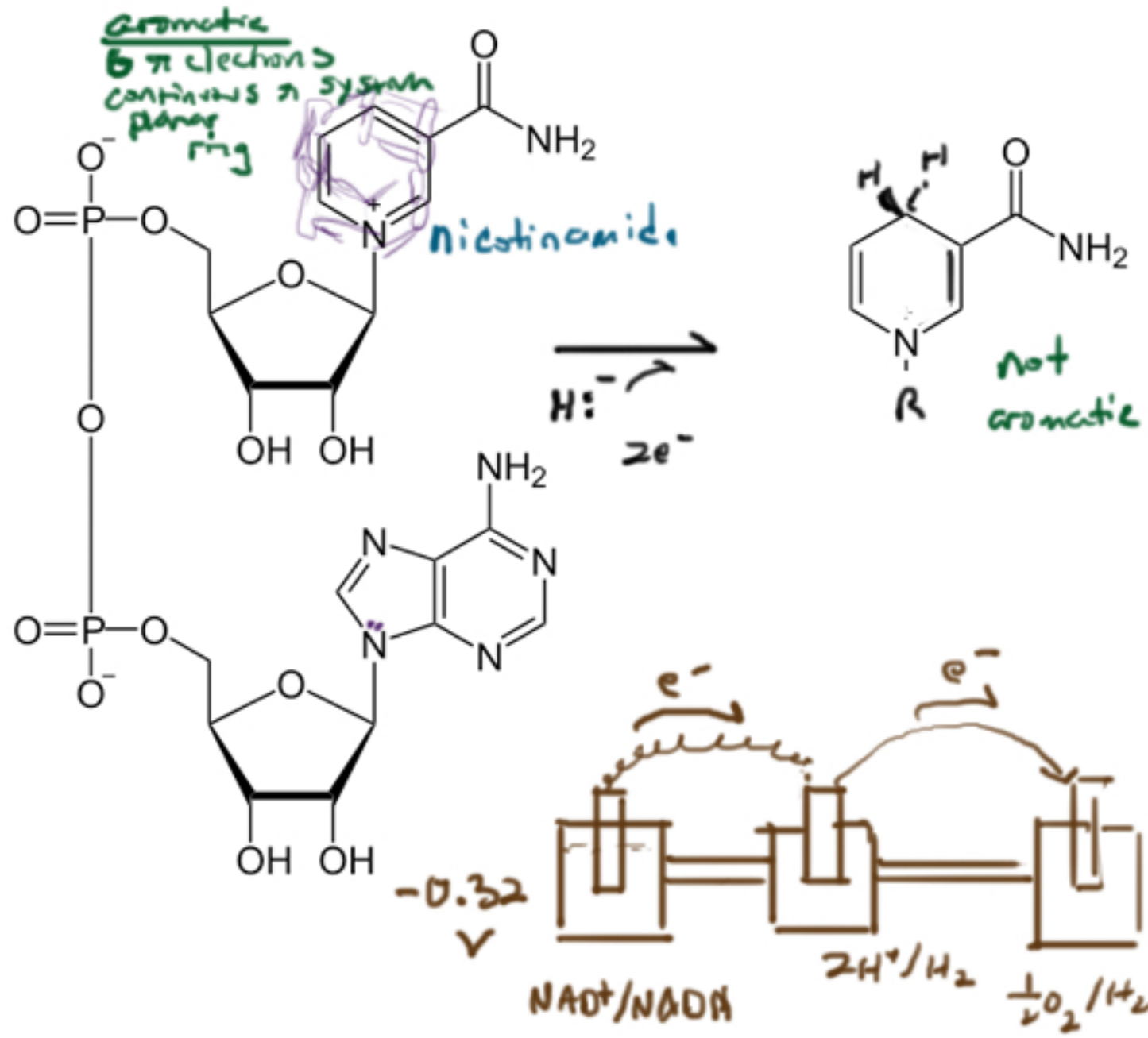
argininosuccinate lyase

argininosuccinate ⟶ arginine and fumarate

H_2O

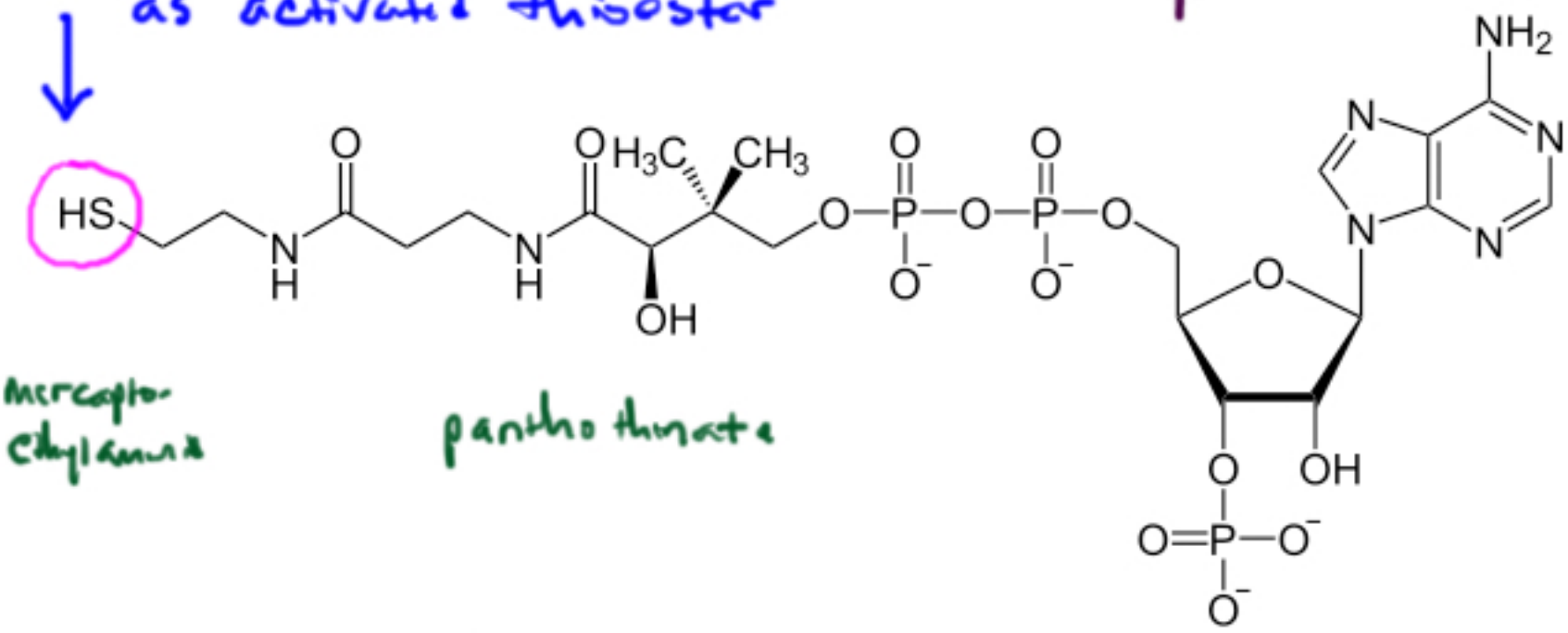
urea and ornithine

NAD^+


aromatic
6 π electrons
continuous π system
planar ring
nicotinamide
NH2
O
N
OH OH
H:⁻
2e⁻
not aromatic
R
-0.32 V
NAD+/NADH
2H+/H2
½O2/H2O
+0.82 V

carries acyl groups here as activated thioester

CoEnzyme A

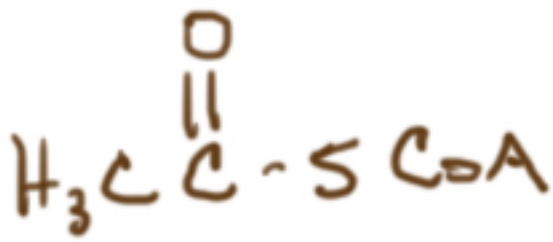


mercapto-ethylamine

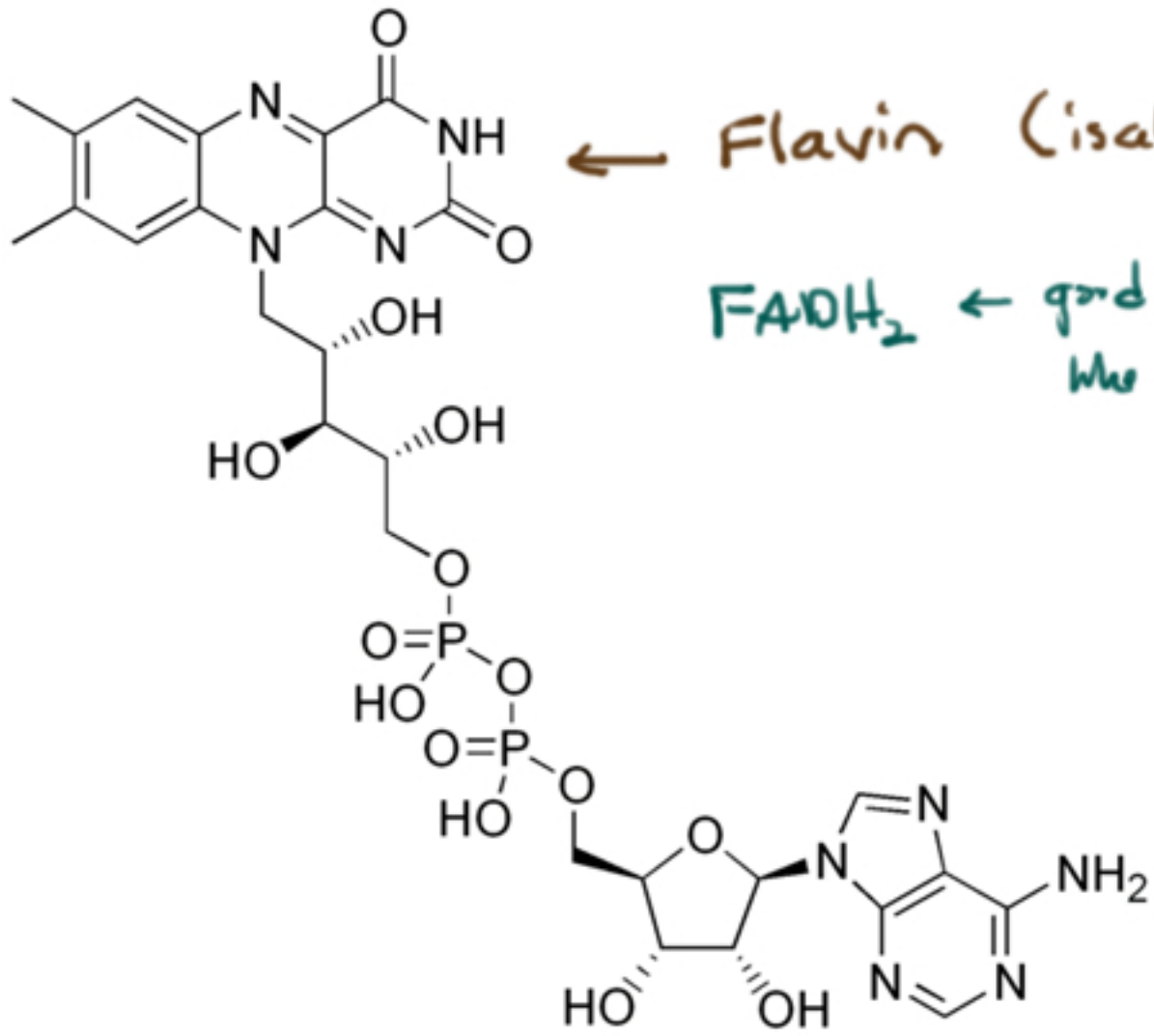
pantothenate

adenosine

thioester



FAD

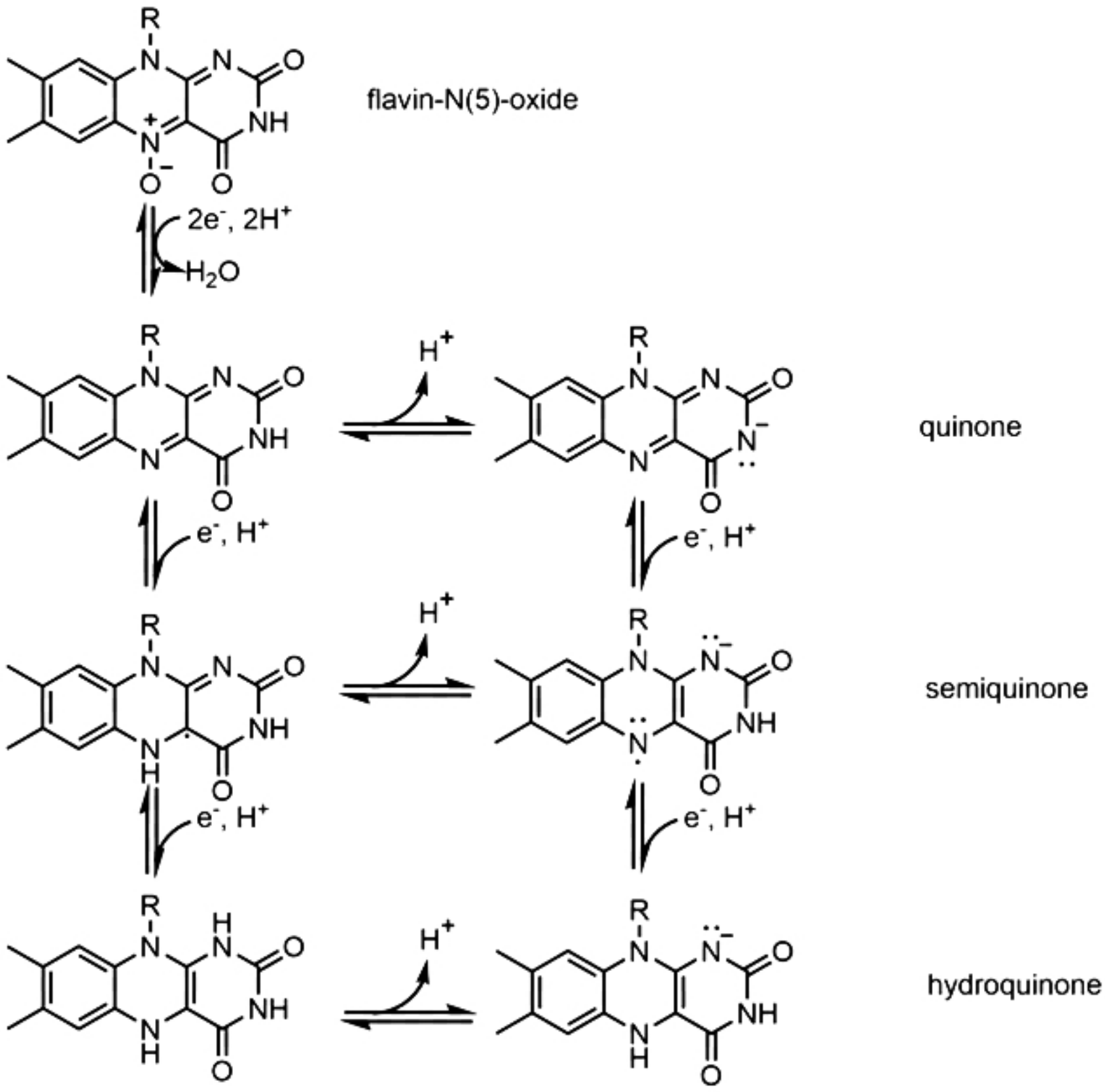


← Flavin (isalloxazine)

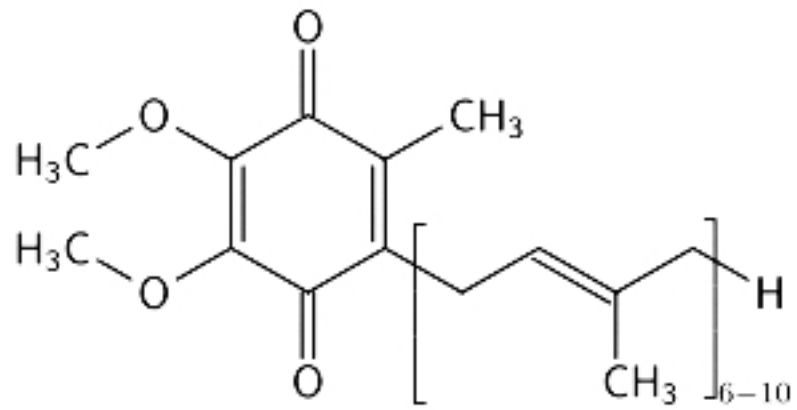
$FADH_2$ ← good electron carrier like NADH

Flavin oxidized $\xrightleftharpoons{2e^-}$ Flavin reduced

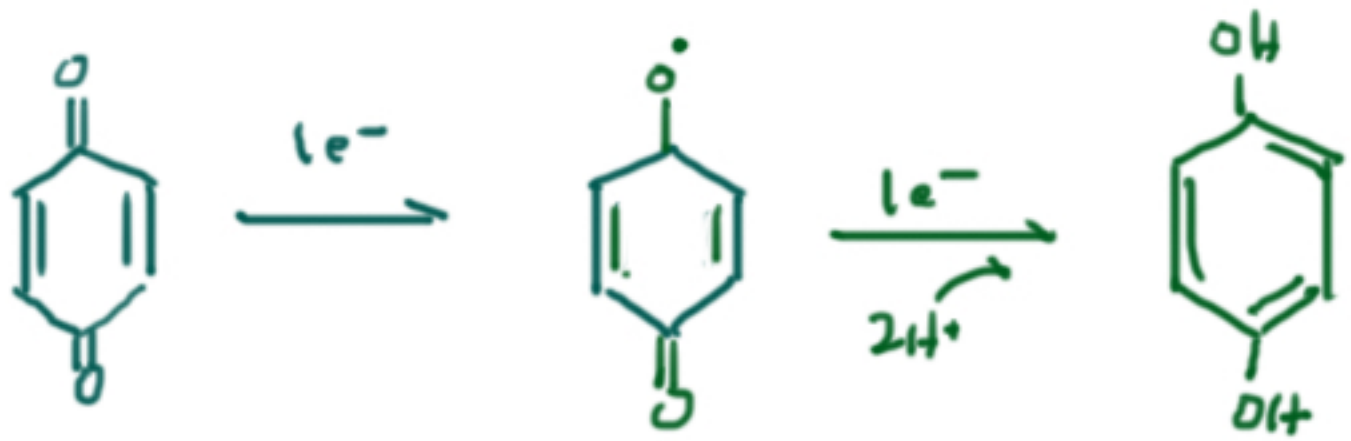
$1e^-$ ⇌ Flavin semiquinone ⇌ $1e^-$


flavin-N(5)-oxide
2e⁻, 2H⁺
H₂O
H⁺
quinone
e⁻, H⁺
semiquinone
hydroquinone

Ubiquinone - CoQ

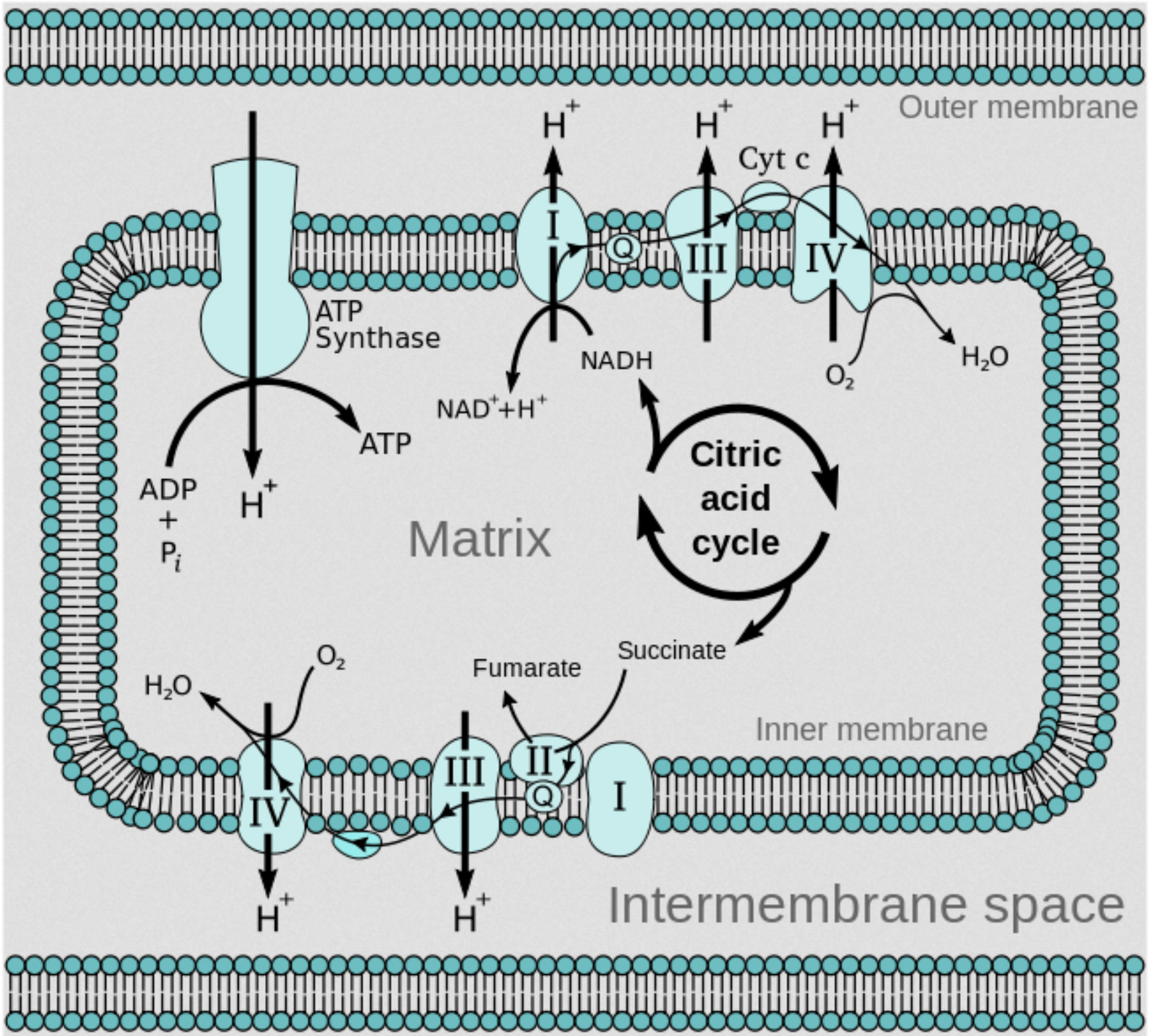


isoprene unit

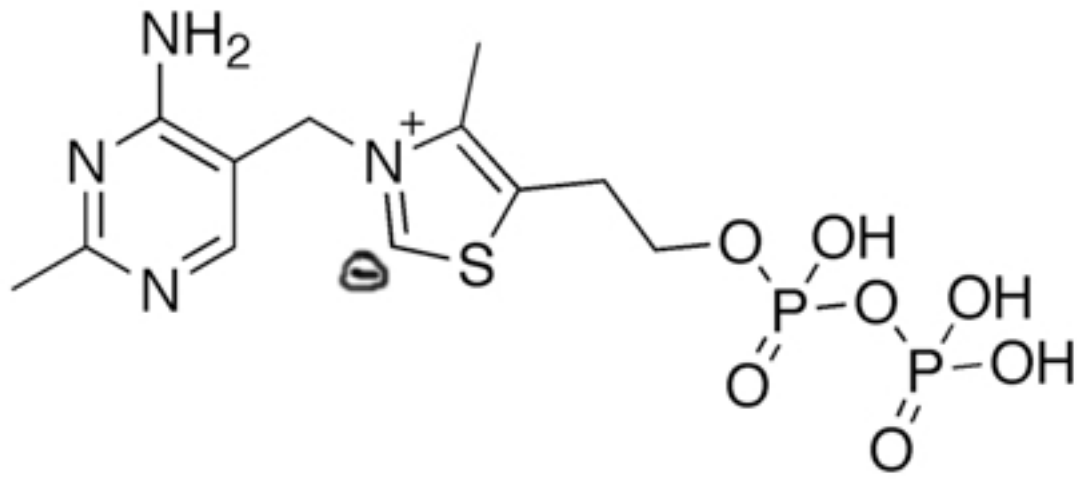


Benzoquinone — Semiquinone — Quinol

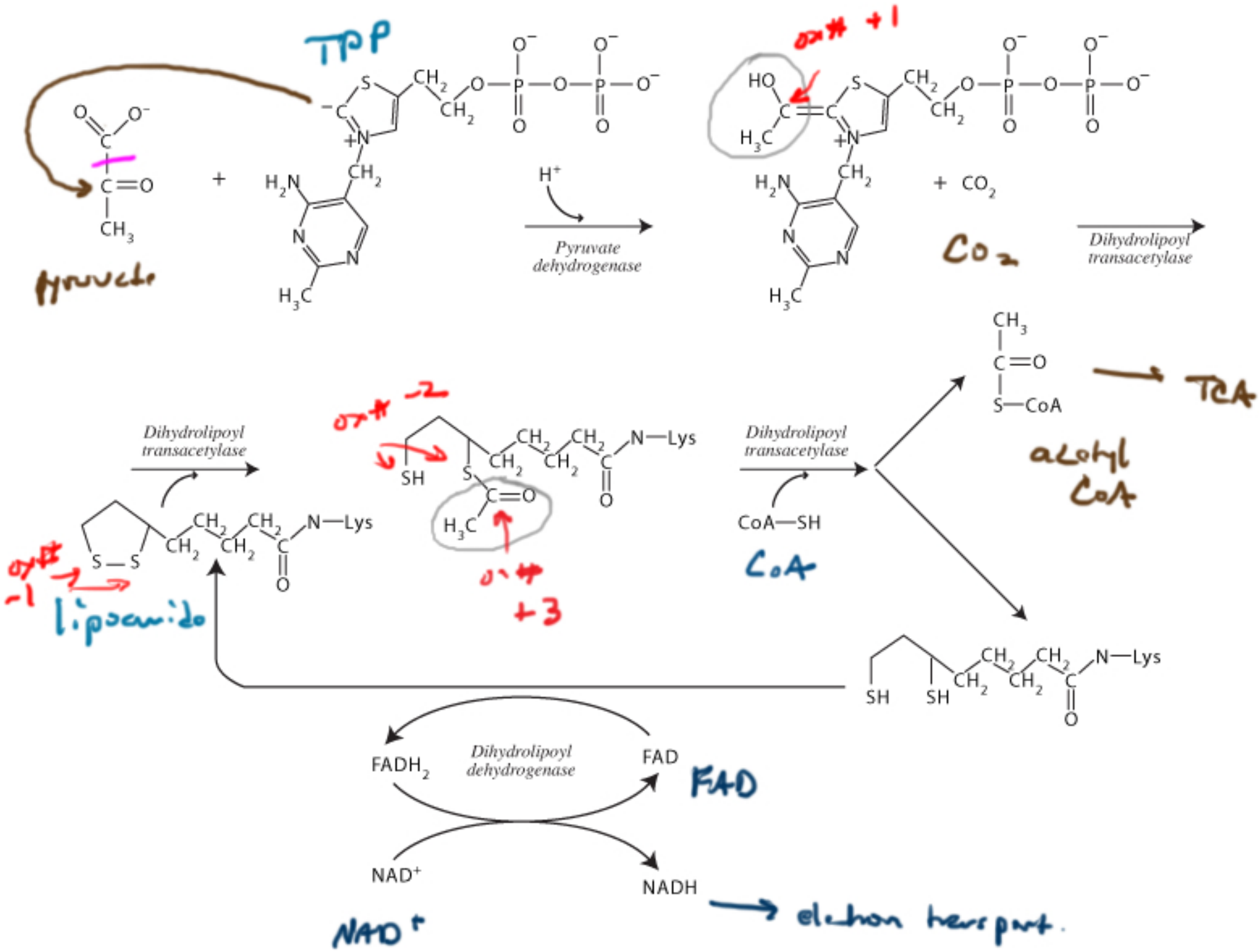
stable radical


Outer membrane
H+
H+
H+
Cyt c
I
Q
III
IV
ATP
Synthase
NADH
H2O
O2
NAD++H+
ATP
ADP
+
Pi
H+
Citric
acid
cycle
Matrix
Succinate
O2
Fumarate
H2O
Inner membrane
IV
III
II
Q
I
H+
H+
Intermembrane space

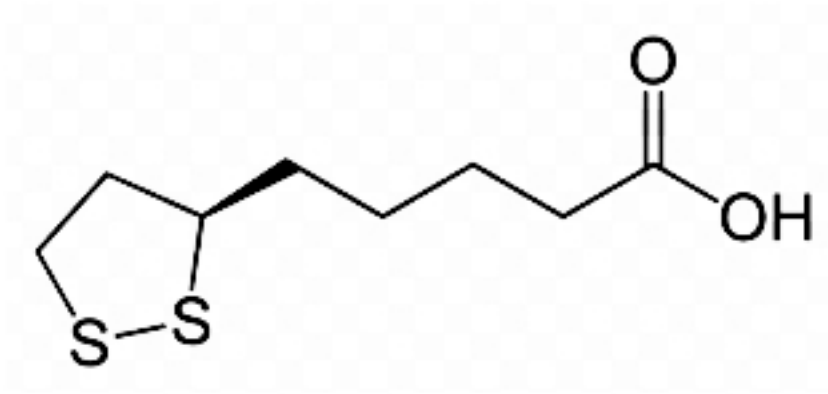
TPP · Thiamine Pyrophosphate



- pyruvate dehydrogenase
- α ketoglutarate dehydrogenase
- transketolase

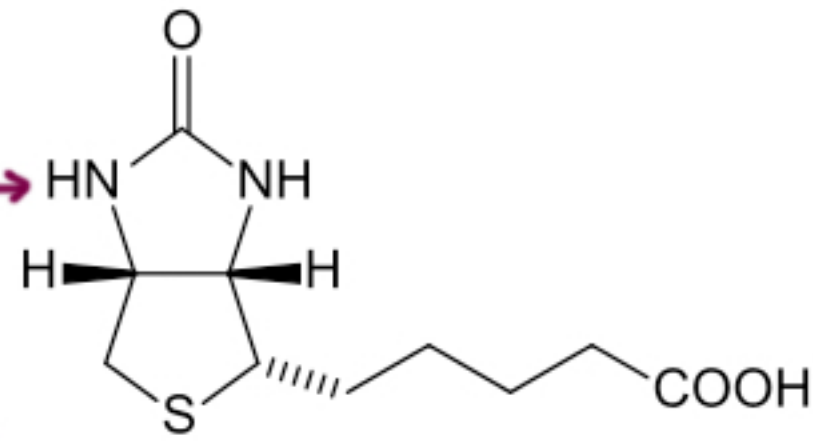

TPP
ox# +1
Pyruvate
H^+
Pyruvate dehydrogenase
CO_2
Dihydrolipoyl transacetylase
ox# -2
Dihydrolipoyl transacetylase
ox# -1
lipoamide
ox# +3
Dihydrolipoyl transacetylase
CoA—SH
CoA
acetyl CoA
TCA
$FADH_2$
Dihydrolipoyl dehydrogenase
FAD
FAD
NAD^+
NADH
NAD^+
electron transport.

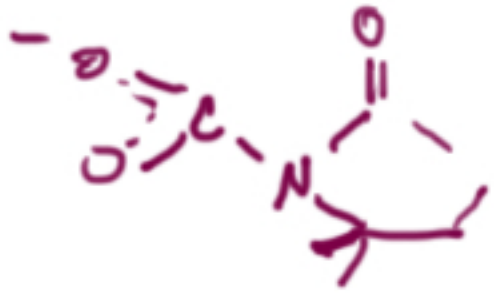
Lipoic Acid



Biotin

carries activated CO_2



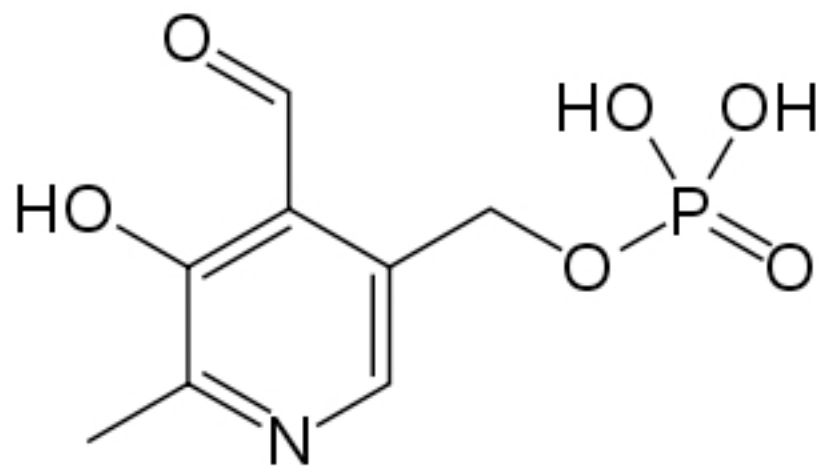


Bench top application

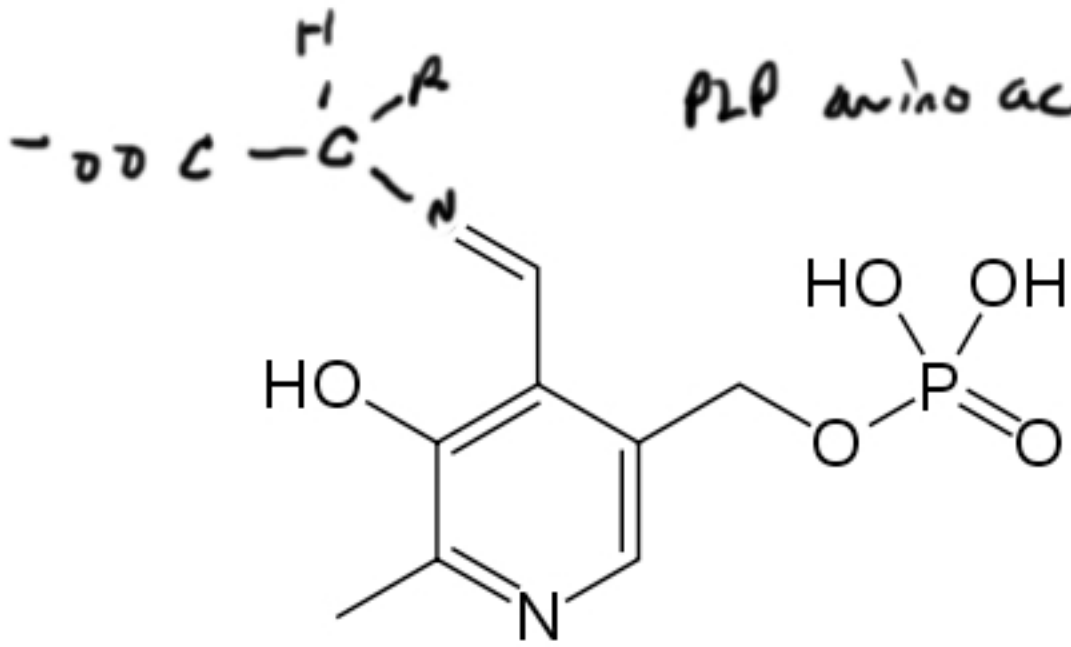
Biotinylation

- adding biotin for binding to avidin in affinity chromatography

PLP

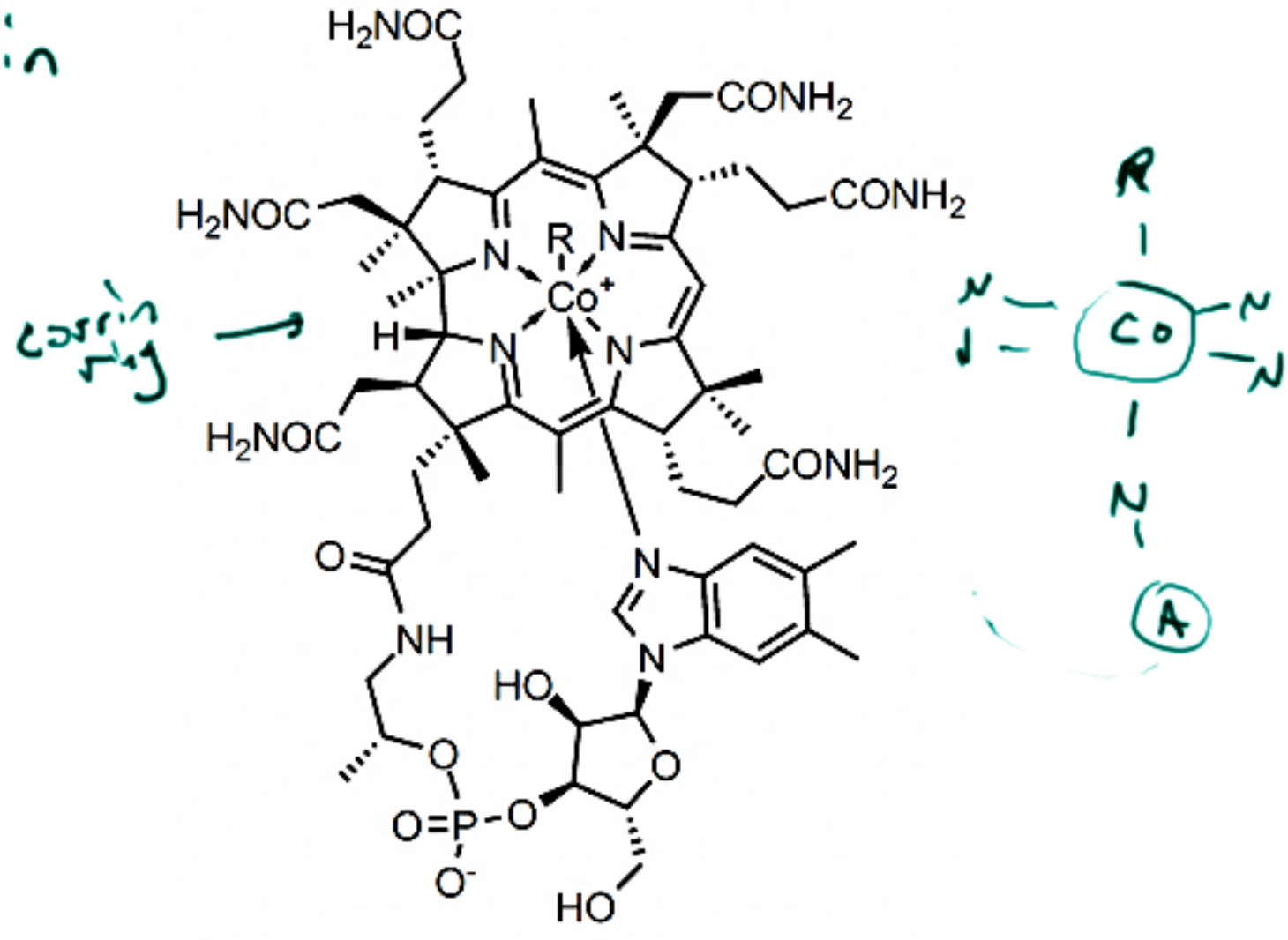


" the amino acid chemistry coenzyme "



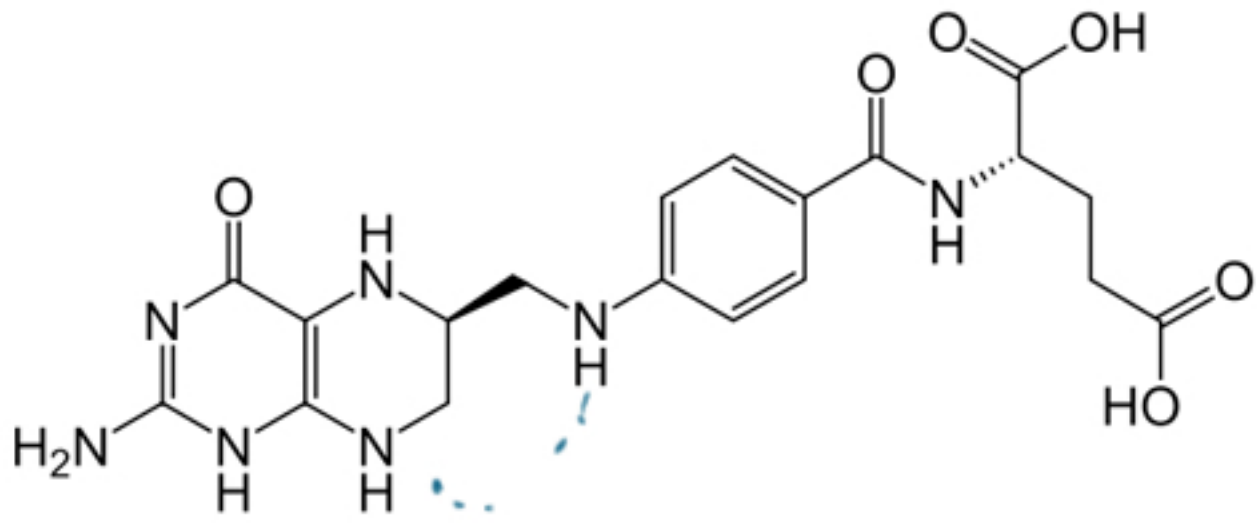
PLP amino acid

cobalamin



R = 5'-deoxyadenosyl, Me, OH, CN

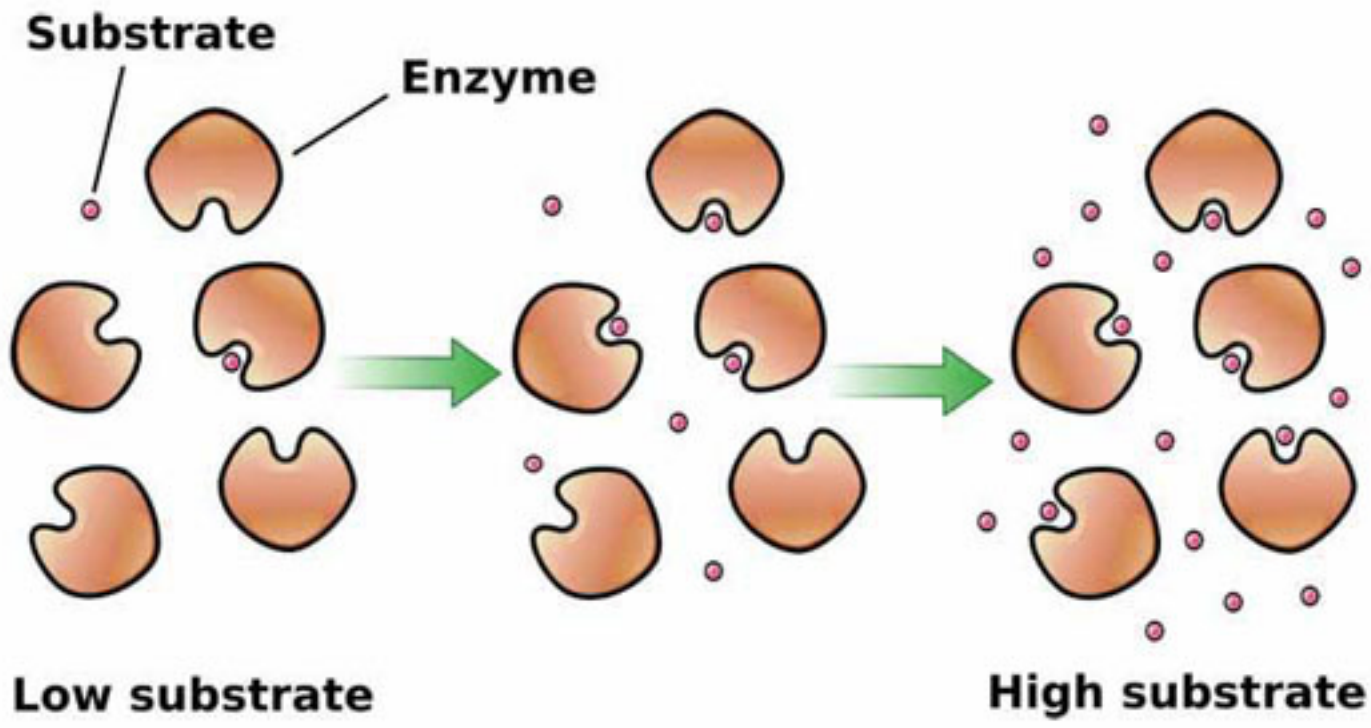
Folate



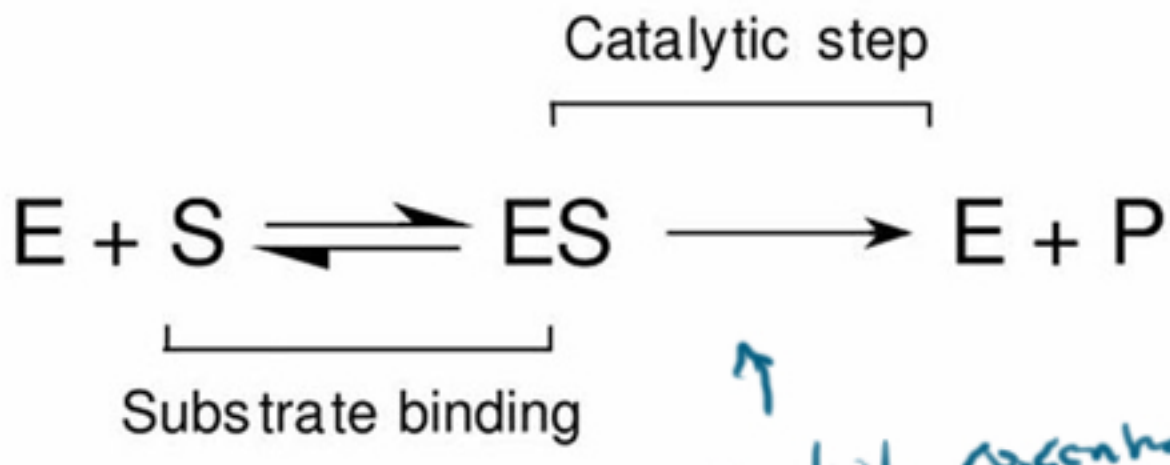
donor of single carbon units (methyl, formyl.

a variety of types.

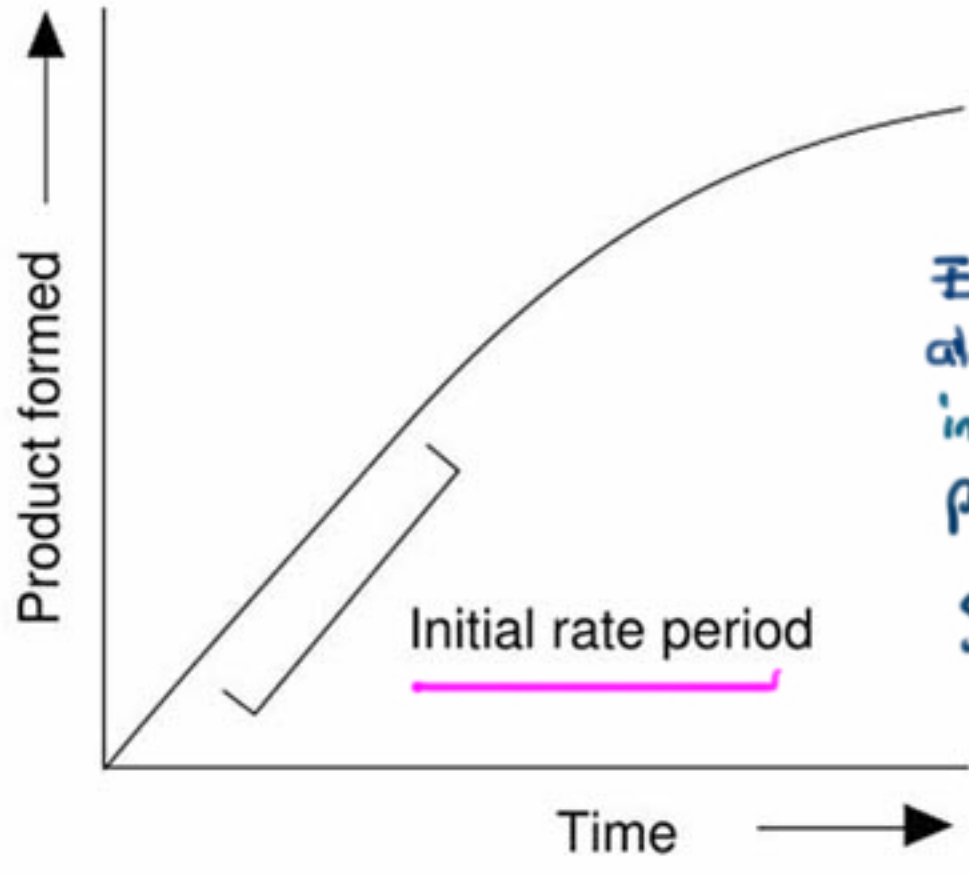
Enzyme Kinetics



at high [S] the enzyme becomes saturated and the rate no longer increases with [S]



at high concentration
the 2nd step becomes
rate limiting.



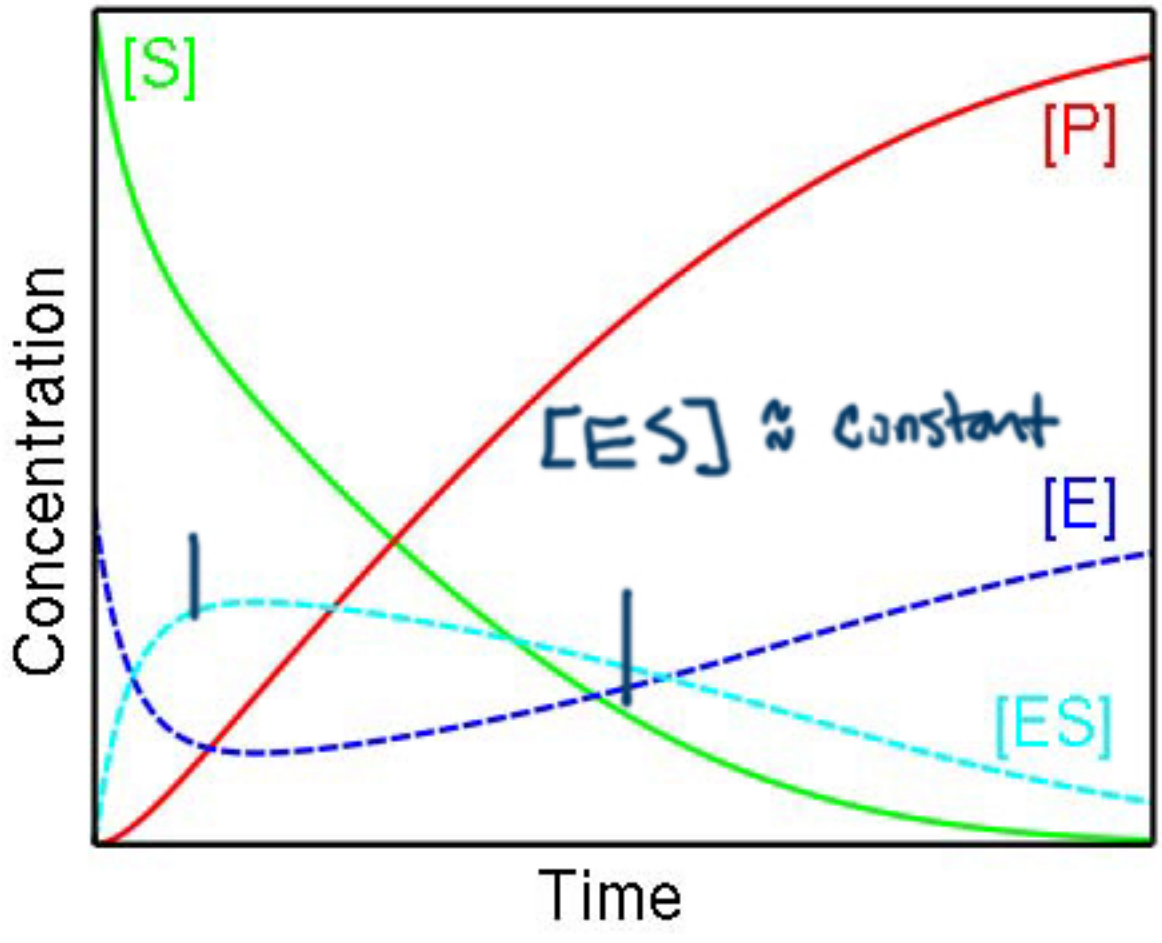
Enzyme measurements are almost always done in the initial rate period.

Sometimes you'll see

V_0

Sometimes, just V

They're the same.



underlies the steady state assumption

- foundation of Michaelis Menton kinetics.

k_2 - turnover number
(k_{cat})

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

If [ES] is constant

formation = breakdown

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad v = k_2[ES]$$

1) Assuming a steady state where [ES] is constant:

formation breakdown

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$ steady state

$$[ES] = \frac{[E][S]}{(k_{-1} + k_2)/k_1}$$

$$K_M = \frac{k_{-1} + k_2}{k_1}$$ Michaelis constant

$$[ES] = \frac{[E][S]}{K_M}$$

↑

The bigger K_M the harder to saturate the enzyme.

2) Assuming total enzyme doesn't change:

$$[E] = [E_T] - [ES]$$

$$[ES] = \frac{([E_T] - [ES])[S]}{K_M}$$

$$[ES] = [E_T]\frac{[S]}{[S] + K_M}$$ $\frac{[S]}{[S]+K_M}$ ← % saturation

$$v = k_2[E_T]\frac{[S]}{[S] + K_M}$$

$$\boxed{v = V_{max}\frac{[S]}{[S] + K_M}}$$

$\frac{V}{V_{max}}$ = % saturation = $\frac{[S]}{[S]+K_M}$

$$V_0 = \frac{V_{max}[S]}{[S] + K_M}$$

$$v = V_{max}\frac{[S]}{[S] + K_M}$$

- If $[S] >> K_M$

  $V \approx V_{max}$

- If $[S] << K_M$

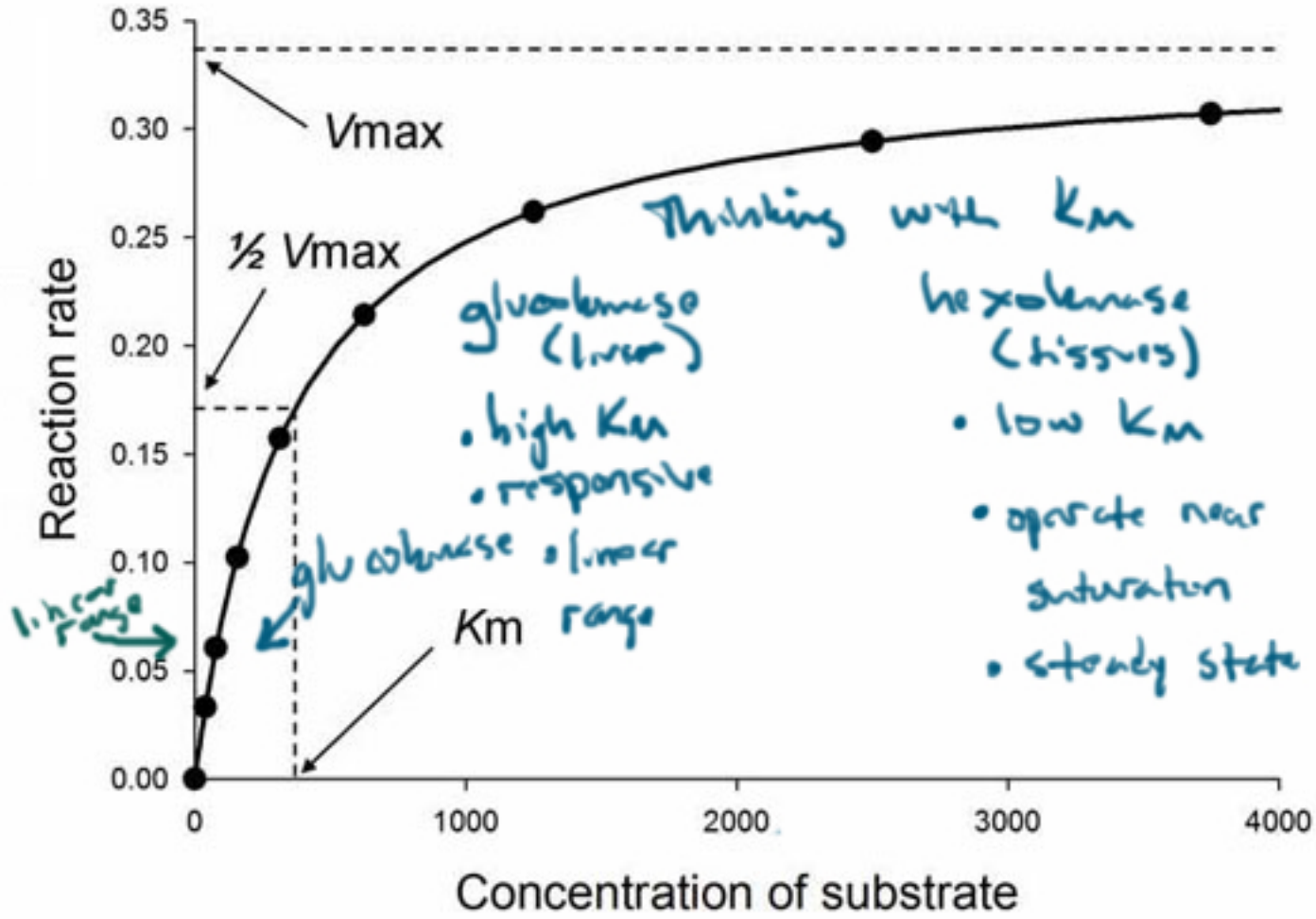
  $V \approx \frac{V_{max}}{K_M}[S]$

  (linear range)

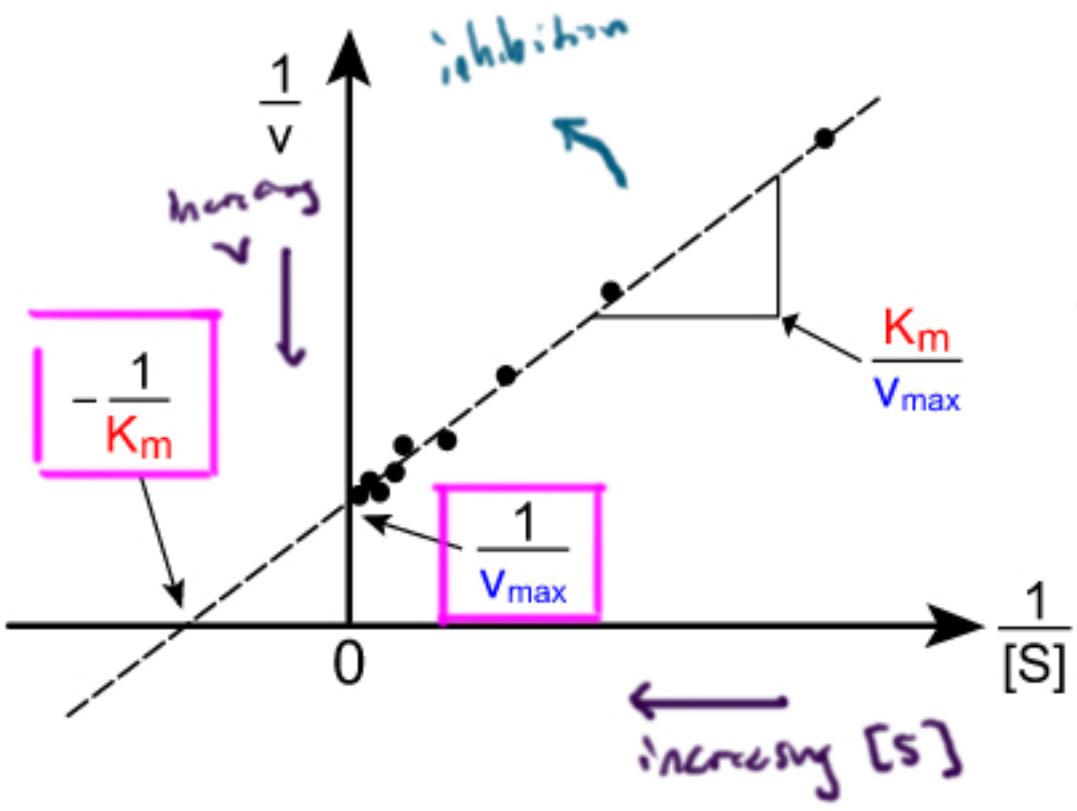
- If $[S] = K_M$

  $V = \frac{1}{2}V_{max}$

K_M is the [S] needed to half saturate the enzyme.



Lineweaver Burke Plot



$$\frac{1}{v} = \frac{1}{V_{max}} + \left(\frac{K_M}{V_{max}}\right)\left(\frac{1}{[S]}\right)$$

$$y = b + m x$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Catalytic efficiency : $\frac{k_2}{K_M}$ or $\frac{k_{cat}}{K_M}$

Enzyme	K_m (M)	k_{cat} (1/s)	k_{cat}/K_m (1/M*s)
Chymotrypsin	1.5×10^{-2}	0.14	9.3
Pepsin	3.0×10^{-4}	0.50	1.7×10^{3}
Tyrosyl-tRNA synthetase	9.0×10^{-4}	7.6	8.4×10^{3}
Ribonuclease	7.9×10^{-3}	7.9×10^{2}	1.0×10^{5}
Carbonic anhydrase	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7} ★
Fumarase	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8} ★

when it's high the enzyme binds substrate, doesn't fall backwards, and turns over right away.

Is there a natural limit?

$$\frac{k_2}{K_M} = \frac{k_2 k_1}{k_{-1} + k_2}$$

Yes. Diffusion limits k_1.

Catalytic efficiency is limited to 10^8 to 10^9 $M^{-1} s^{-1}$

- to get close means enzymatic perfection ★

Hexokinase catalyzes the phosphorylation of both glucose and fructose. K_m for hexokinase with glucose is 0.15mM. K_m for fructose is 1.5mM. Assuming that V_{max} is the same for both enzymes, calculate the normalized initial velocity (v_0 / V_{max}) when the initial substrate concentration is 0.15mM.

glucose

$K_M = [S]$

$\frac{V_0}{V_{max}} = 50\%$

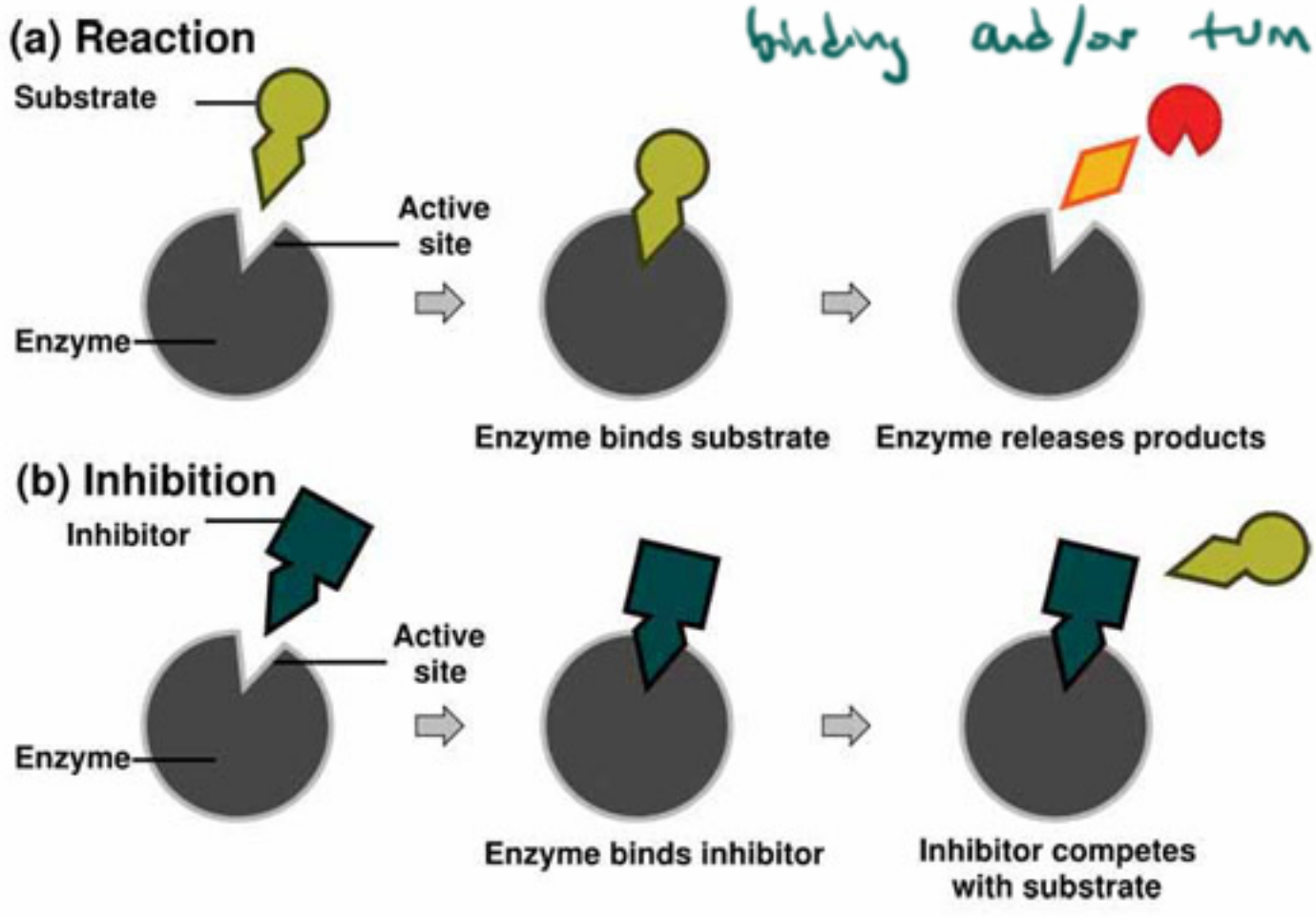
fructose

$\sim 9\%$

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

$$\frac{V_0}{V_{max}} = \frac{[S]}{[S] + K_M}$$

Enzyme inhibitors affect binding and/or turnover



Competitive inhibition

Competitive Inhibition

$$v = \frac{V_{max}[S]}{\alpha K_M + [S]}$$

apparent K_M

You can still saturate the enzyme

You can still get back to V_{max}

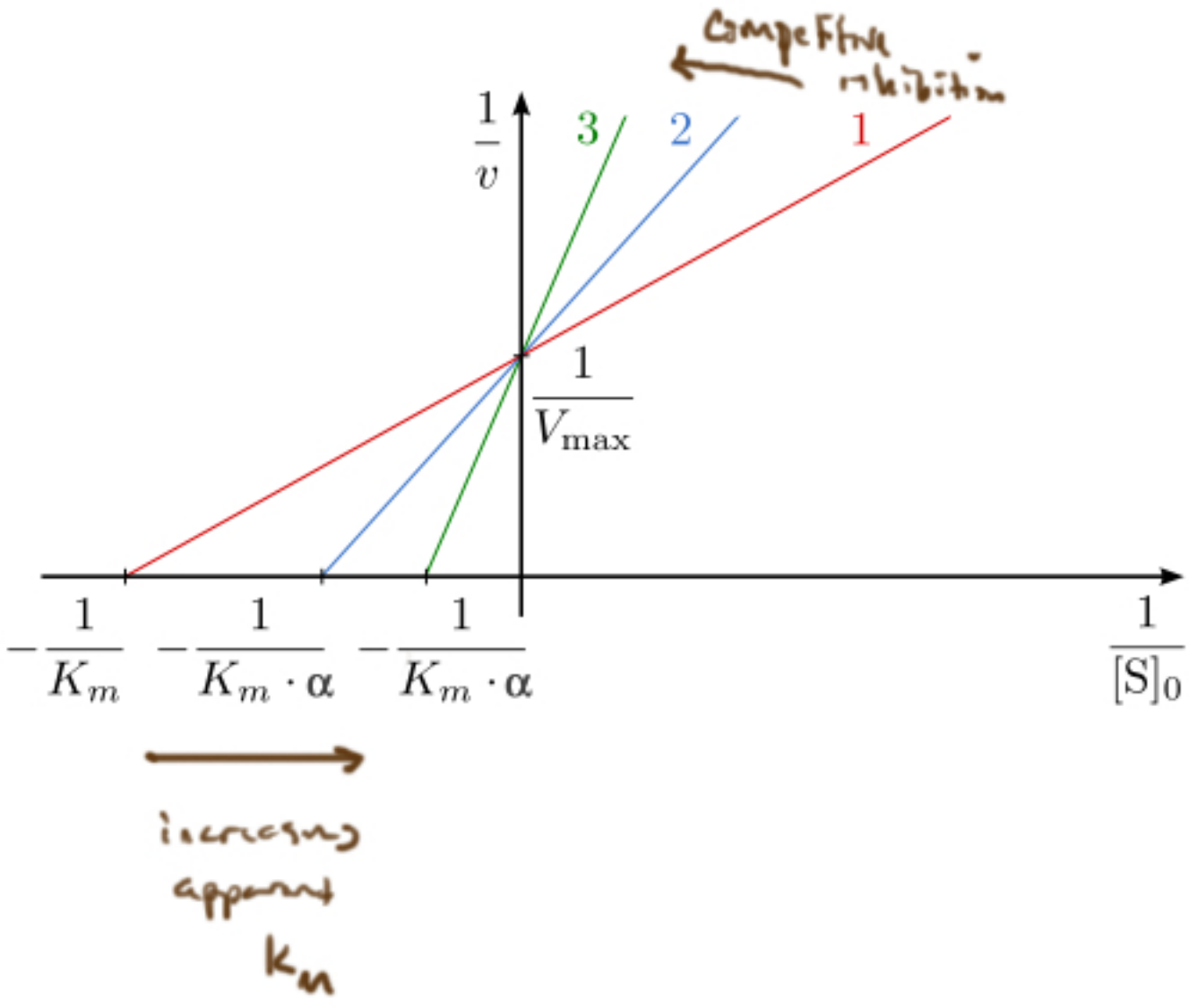
But it's harder to saturate the enzyme.

(Apparent) K_M has increased.

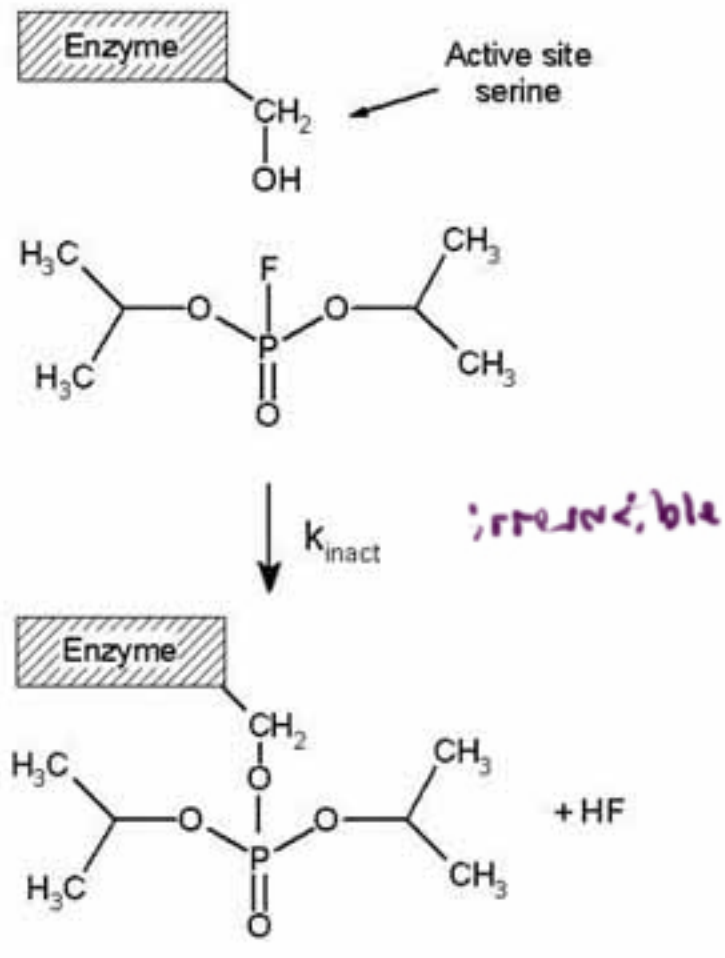
Competive - V_{max} unchanged

K_M^* increased

* apparent

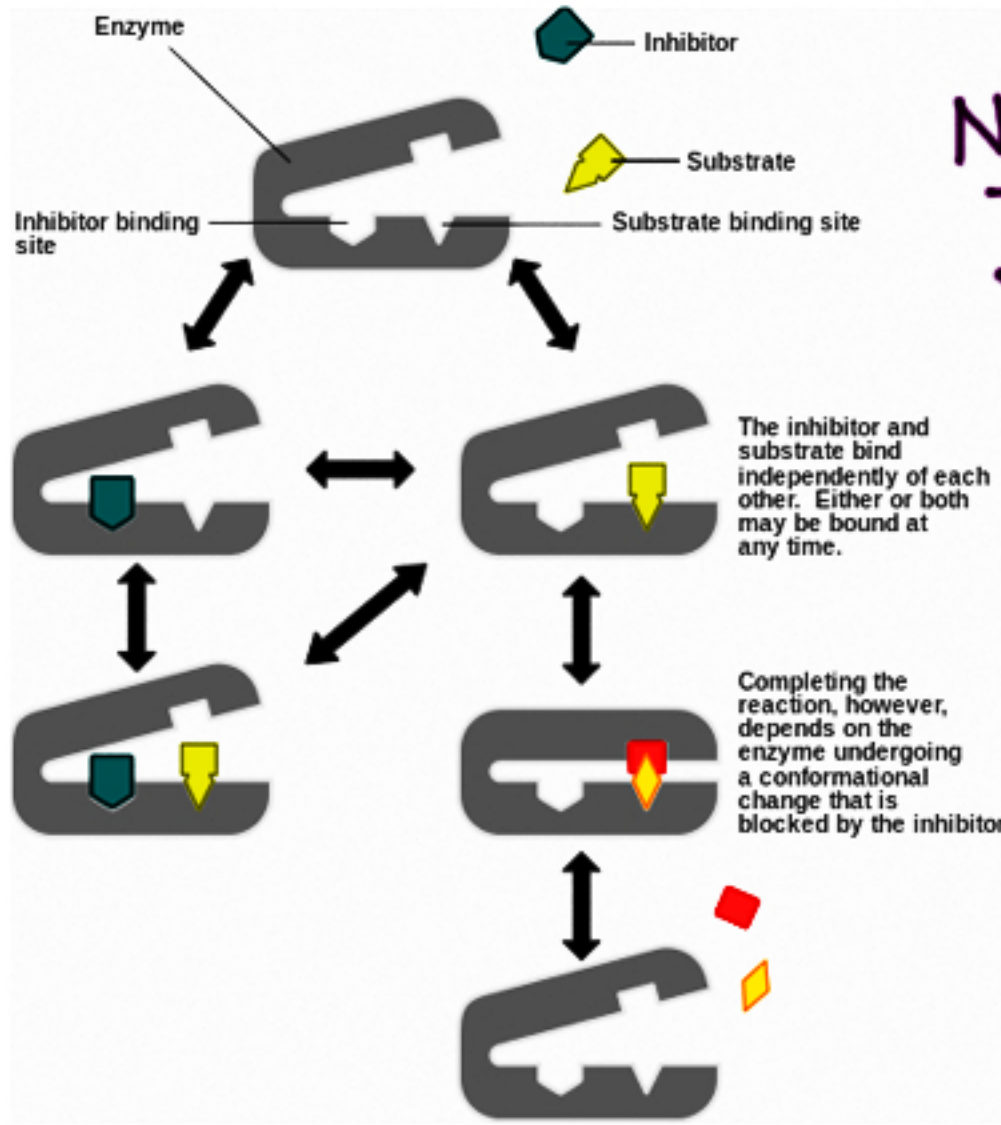

competitive inhibition
$\frac{1}{v}$
3
2
1
$\frac{1}{V_{\max}}$
$-\frac{1}{K_m}$
$-\frac{1}{K_m \cdot \alpha}$
$-\frac{1}{K_m \cdot \alpha}$
$\frac{1}{[S]_0}$
increasing apparent K_m

acetylcholine esterase →



suicide inhibitor

(not a form of competitive inhibition)



cannot turn over

Noncompetitive

- substrate binds just as easily
- enzyme is as easy to saturate as before

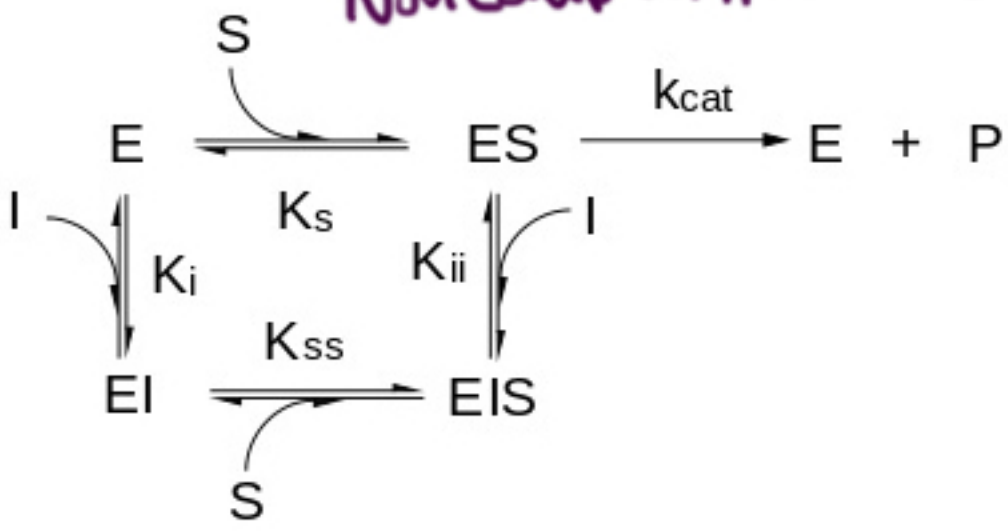
K_M is unchanged

- V_{max} decreased

Mixed

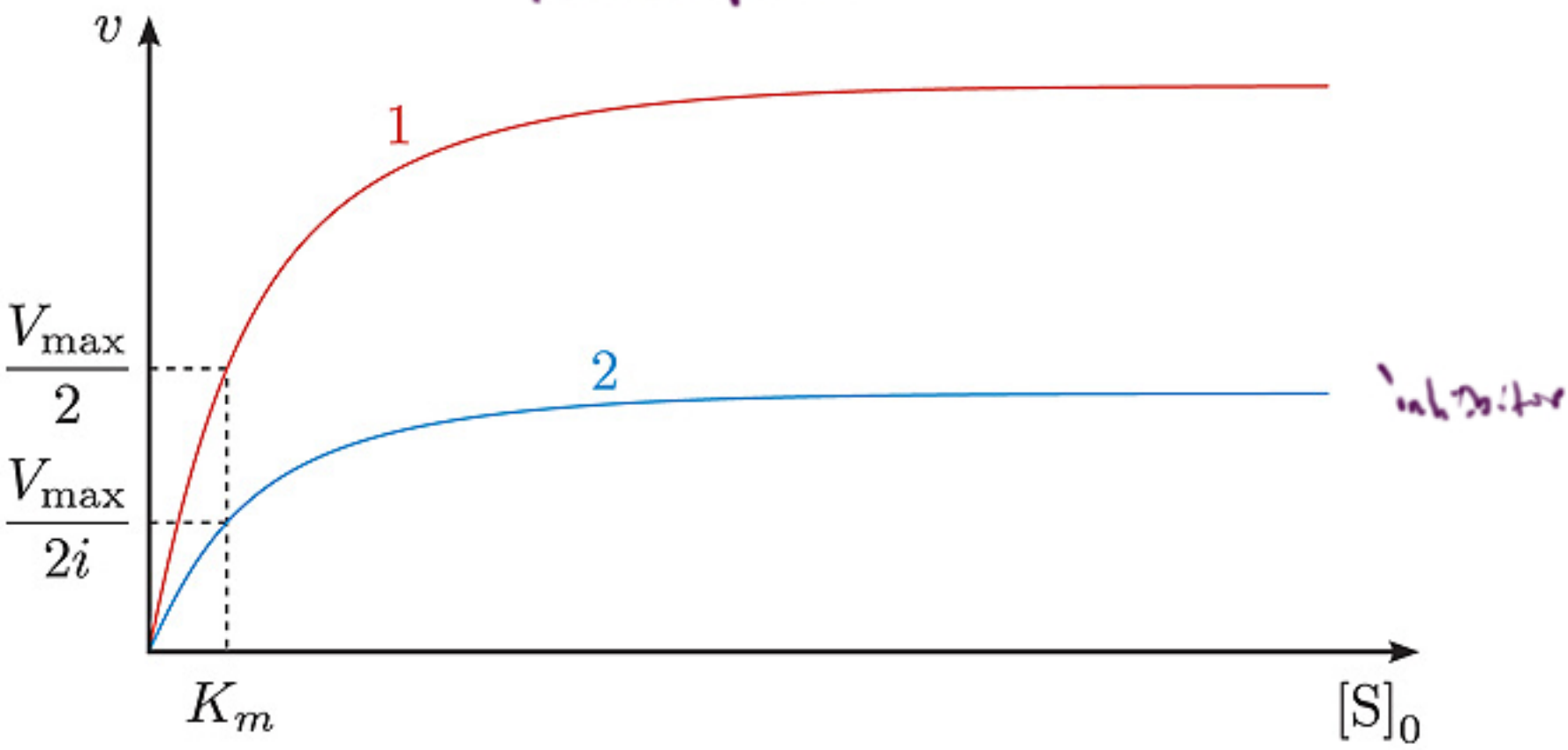
when inhibitor has some effect on binding both K_M and V_{max} change

Noncompetitive (or mixed)



$$v = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]}$$

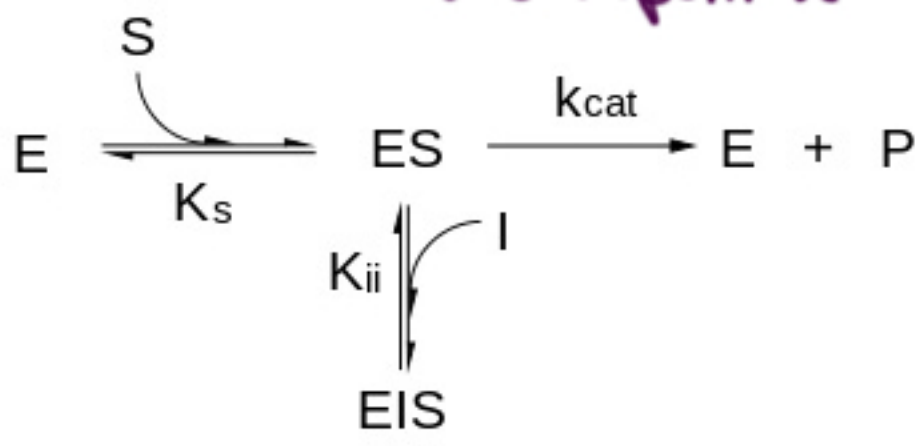
Noncompetitive



V_{max} decreased

K_M unchanged.

uncompetitive

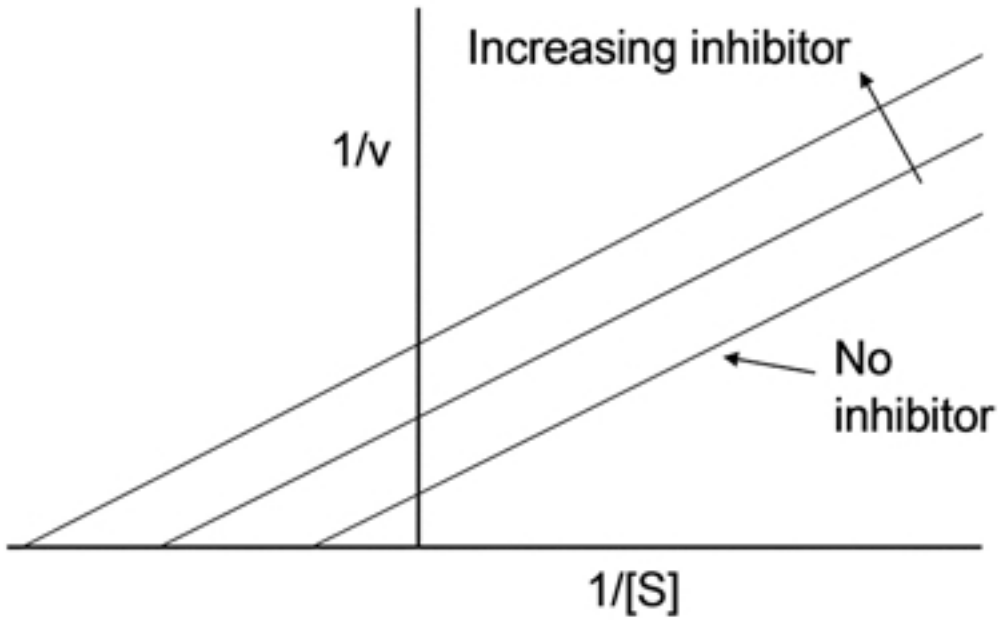


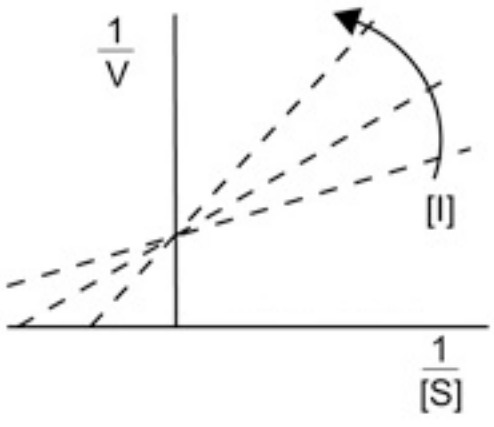
$$v = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

V_{max} decreased

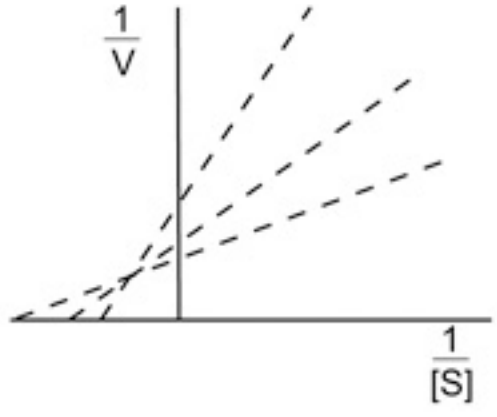
K_M decreases also (proportionally)

uncompetitive

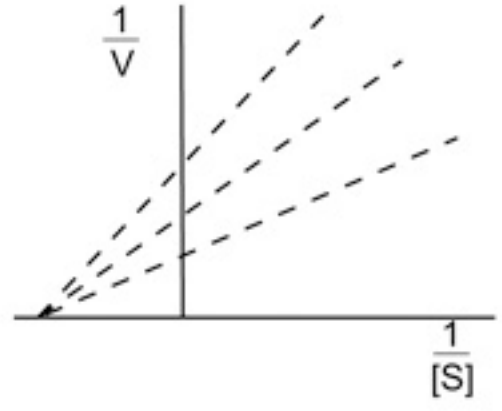




Competive



mixed



noncompetitive

Type of inhibition	K_m apparent	V_{max} apparent
competitive	$K_m\alpha$	$V_{\max}$
uncompetitive	$\frac{K_m}{\alpha'}$	$\frac{V_{\max}}{\alpha'}$
non-competitive	K_m	$\frac{V_{\max}}{\alpha'}$
mixed	$\frac{K_m\alpha}{\alpha'}$	$\frac{V_{\max}}{\alpha'}$

Bisubstrate Mechanisms

$$\text{Sucrose} + \text{Phosphate} \underset{\text{sucrose phosphorylase}}{\rightleftharpoons} \text{Glucose-1-phosphate} + \text{Fructose}$$

$$\text{Maltose} + \text{Phosphate} \underset{\text{maltose phosphorylase}}{\rightleftharpoons} \text{Glucose-1-phosphate} + \text{Glucose}$$

With sucrose phosphorylase, incubate with sucrose and isotopically labelled fructose* in the absence of phosphate - the label passes to sucrose.

Also, incubation with labelled glucose-1-phosphate* and phosphate, the label passes to phosphate.

The analagous process does not happen with maltose phosphorylase.

Sucrose Phosphorylase - Ping Pong Mechanism

$$\text{Enzyme} + \text{Sucrose} \longrightarrow \text{Enzyme-glucose} + \text{Fructose}$$

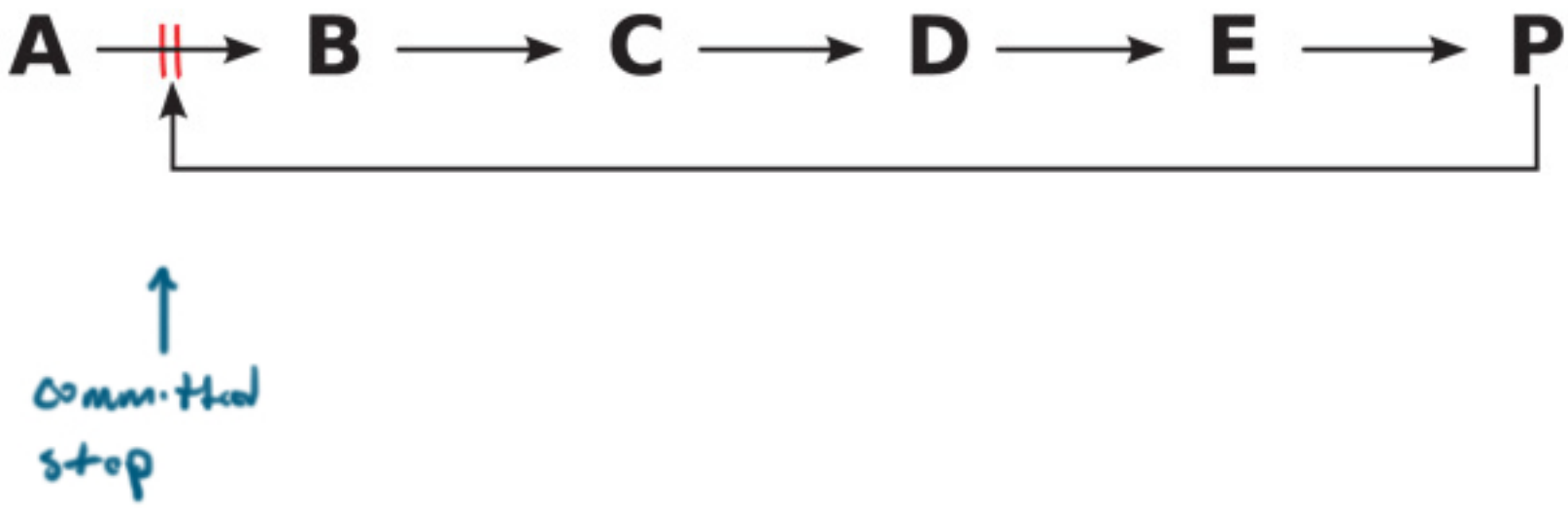
$$\text{Enzyme-glucose} + P_i \longrightarrow \text{Enzyme} + \text{G1P}$$

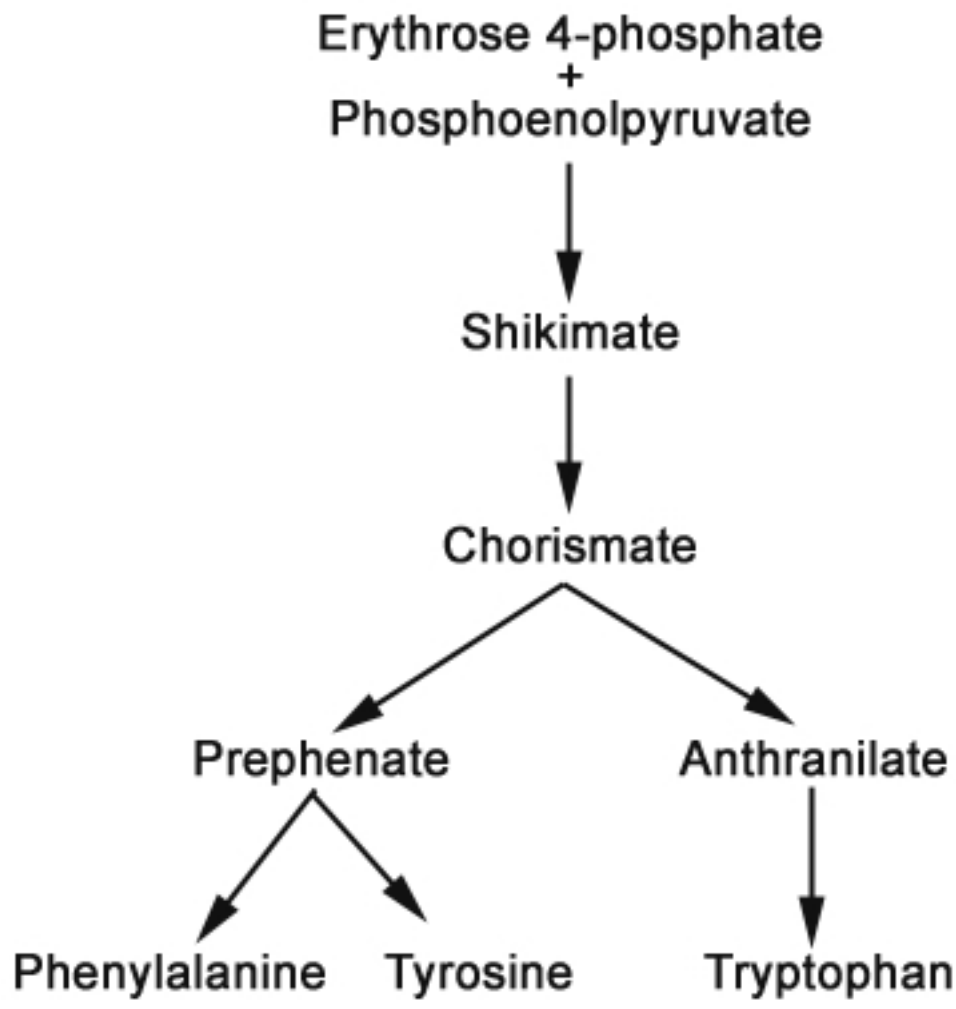
Maltose Phosphorylase - Sequential Mechanism

$$\text{Enzyme} + \text{Maltose} + P_i \longrightarrow \text{Enzyme} + \text{G1P} + \text{Glucose}$$

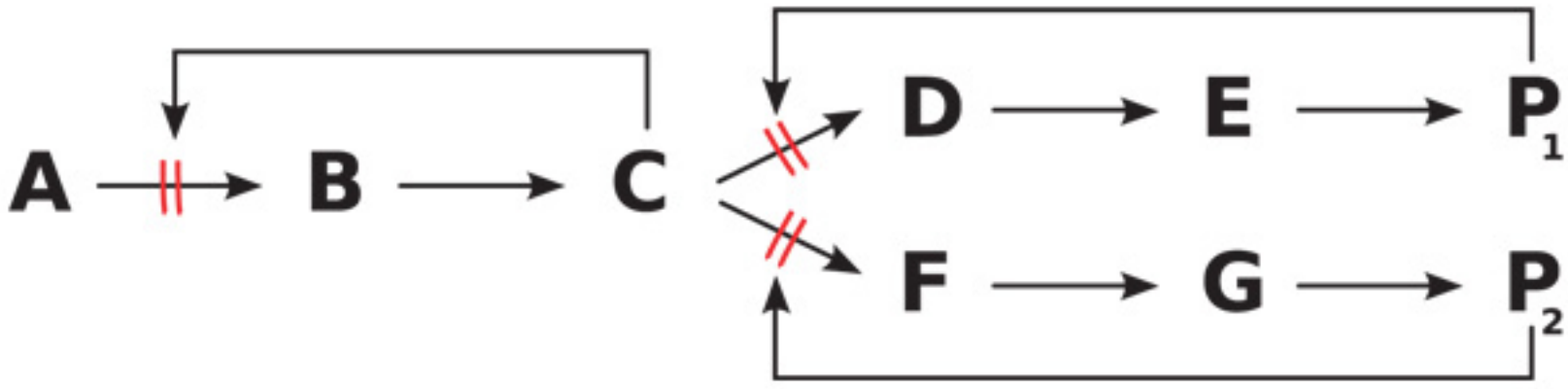
- Sequential mechanisms may be ordered or random.

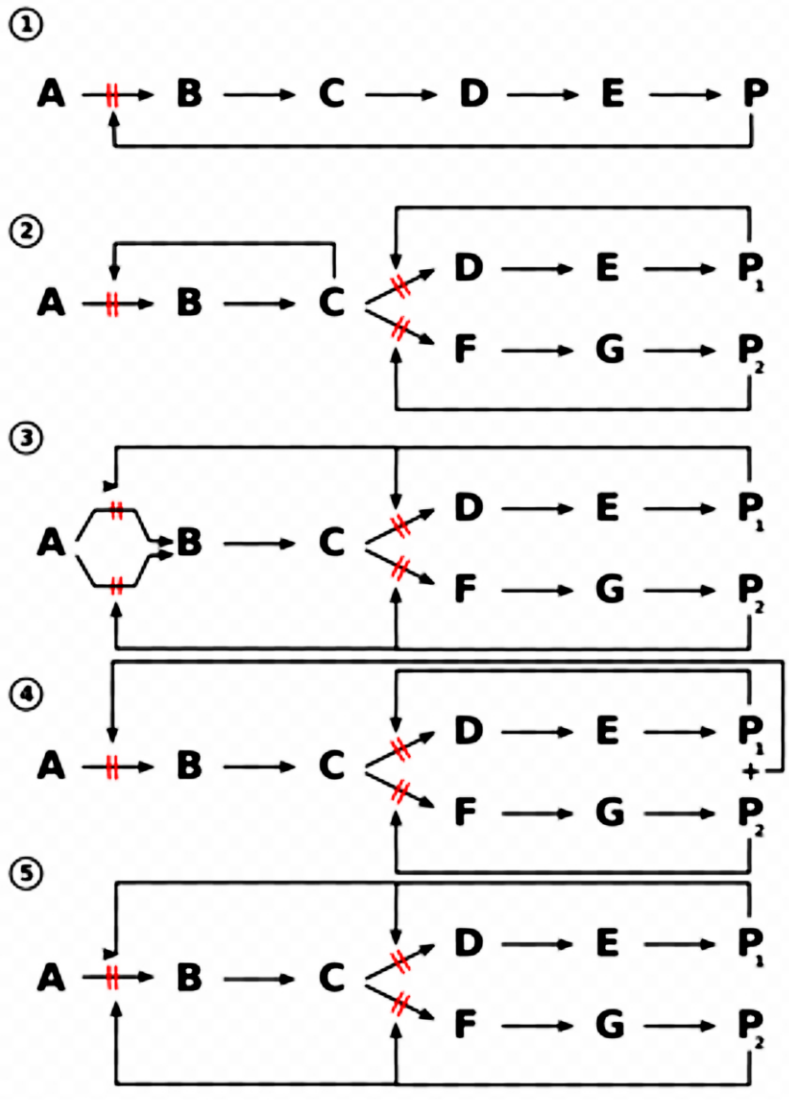
Basic Feedback




Erythrose 4-phosphate
+
Phosphoenolpyruvate
Shikimate
Chorismate
Prephenate
Anthranilate
Phenylalanine
Tyrosine
Tryptophan

Sequential Feedback





1. The basic feedback inhibition mechanism, where the product (P) inhibits the committed step (A→B).

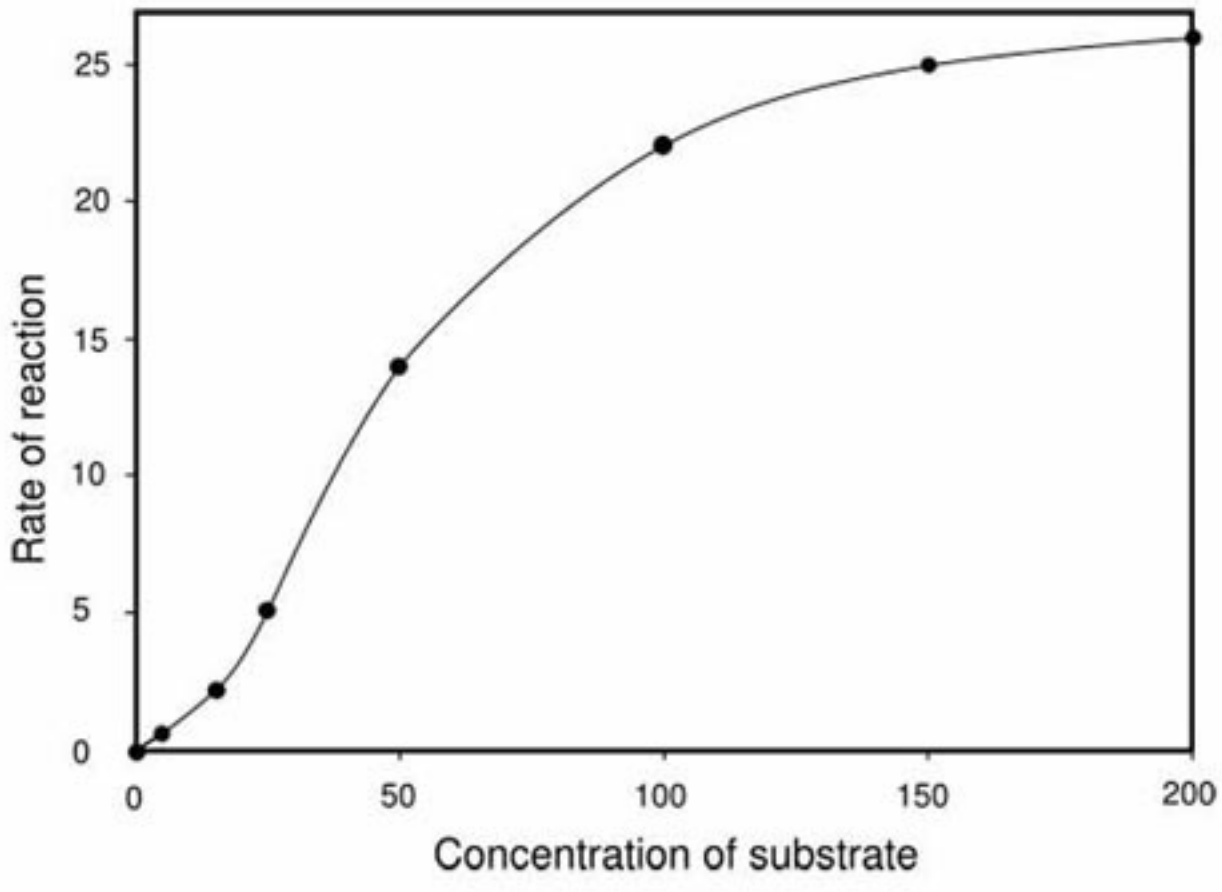
2. *Sequential feedback inhibition*. The end products P_1 and P_2 inhibit the first committed step of their individual pathway (C→D or C→F). If both products are present in abundance, all pathways from C are blocked. This leads to a buildup of C, which in turn inhibits the first common committed step A→B.

3. *Enzyme multiplicity*. Each end product inhibits both the first individual committed step and one of the enzymes performing the first common committed step.

4. *Concerted feedback inhibition*. Each end product inhibits the first individual committed step. *Together*, they inhibit the first common committed step.

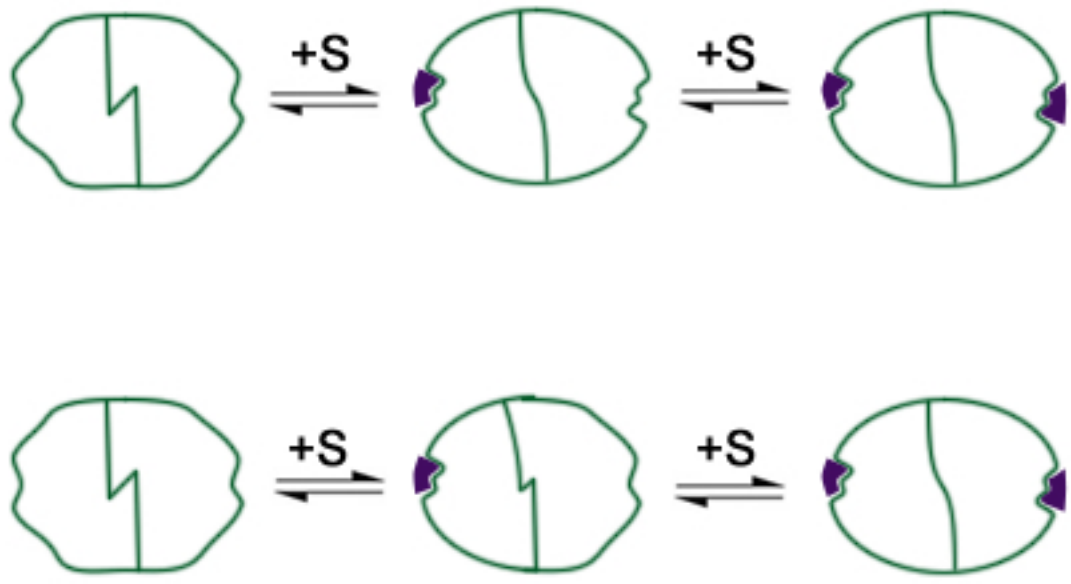
5. *Cumulative feedback inhibition*. Each end product inhibits the first individual committed step. Also, each end product *partially* inhibits the first common committed step.

Supplamental

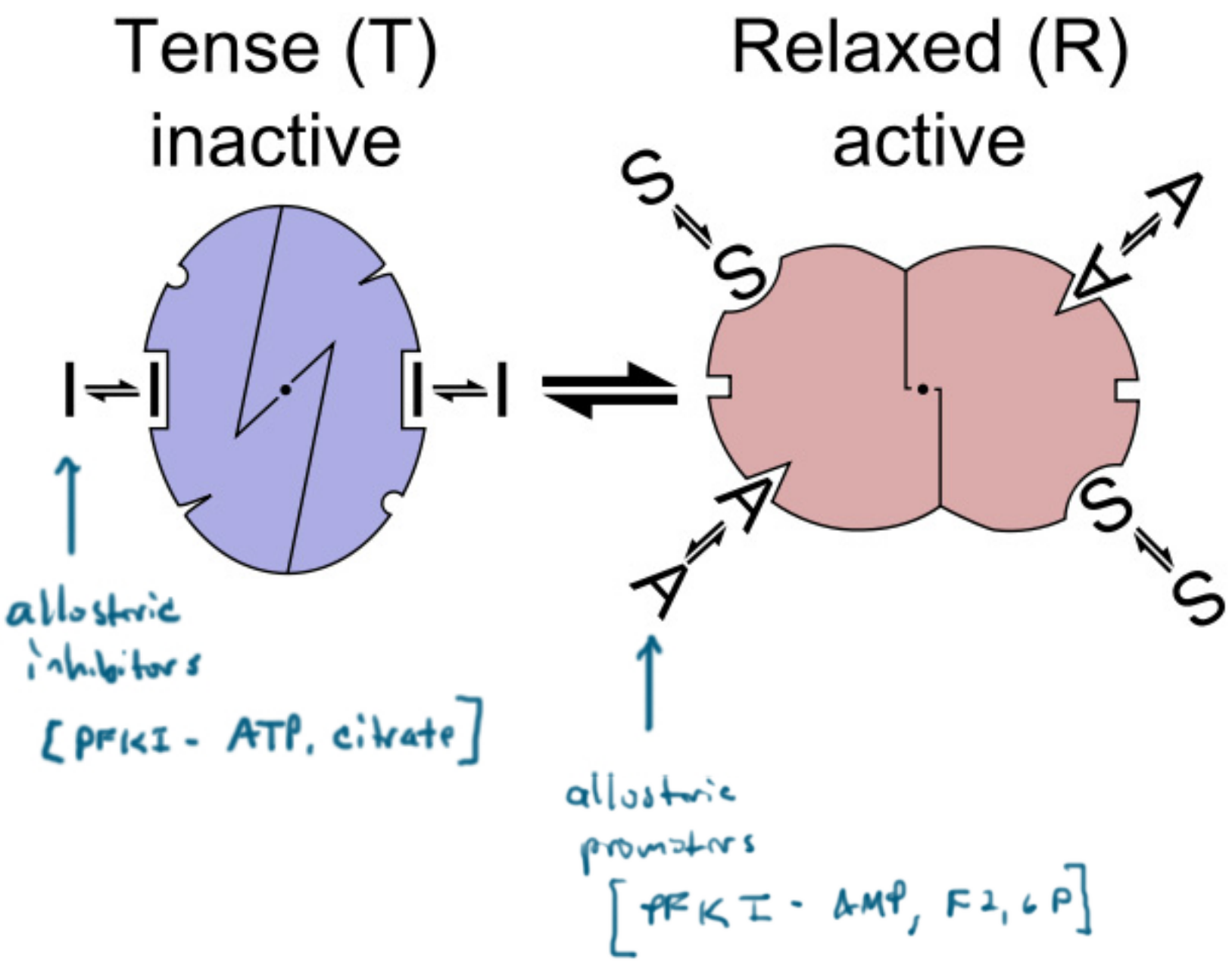


sigmoidal

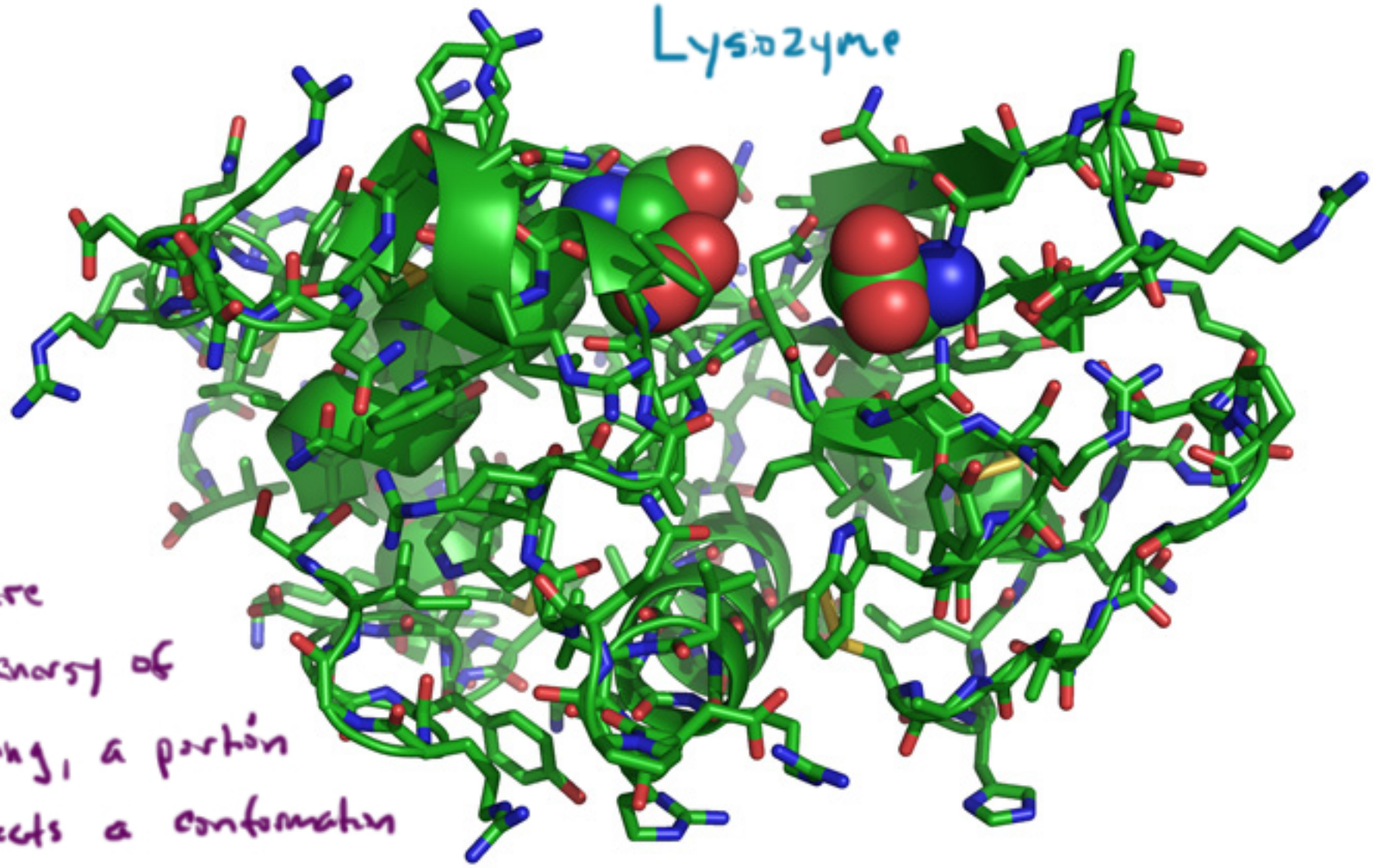
- positive cooperativity
- multisubunit (almost certainly)
- Hill coefficient > 1


+S
+S
+S
+S

Monod Jacob Model



Lysozyme

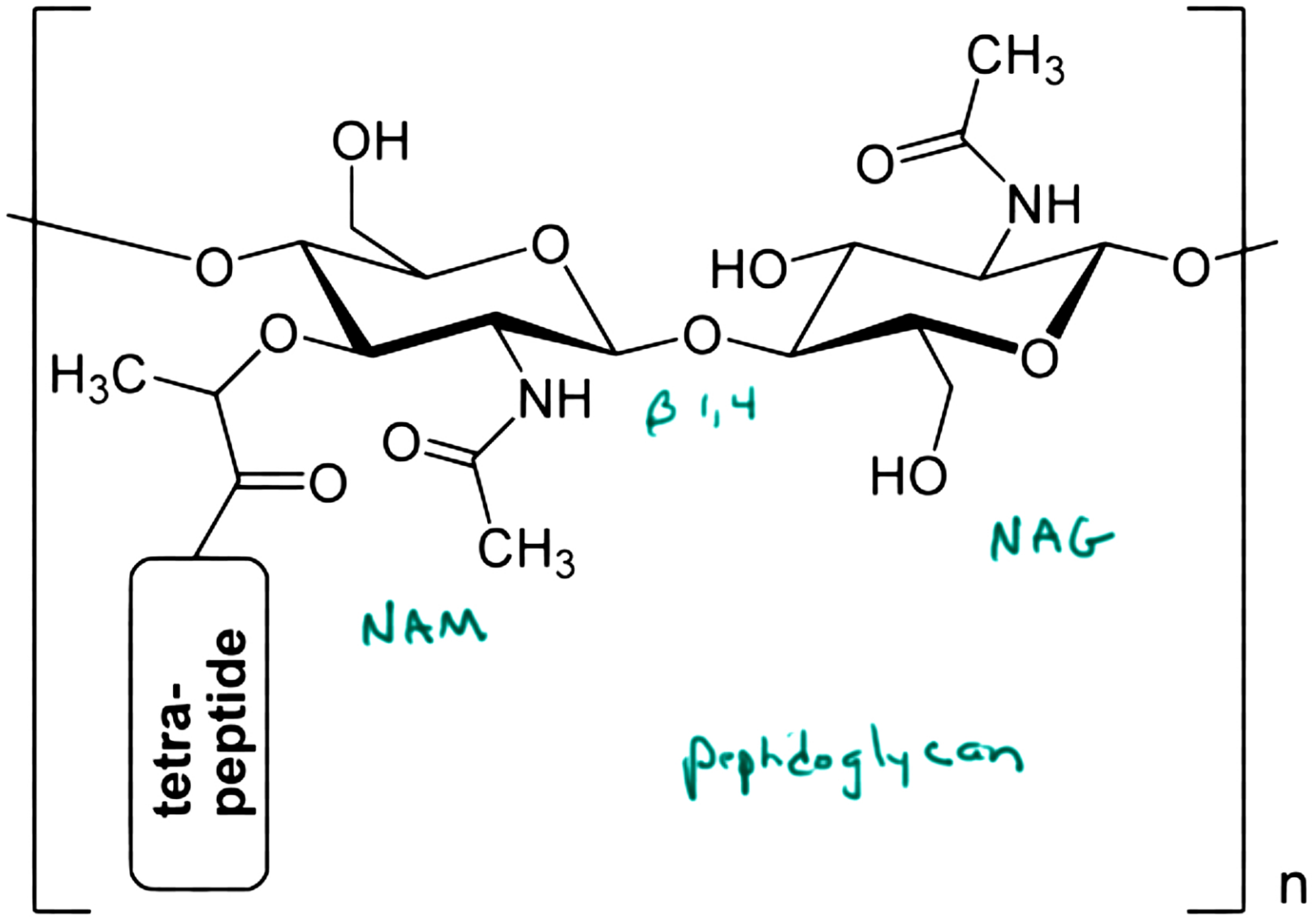


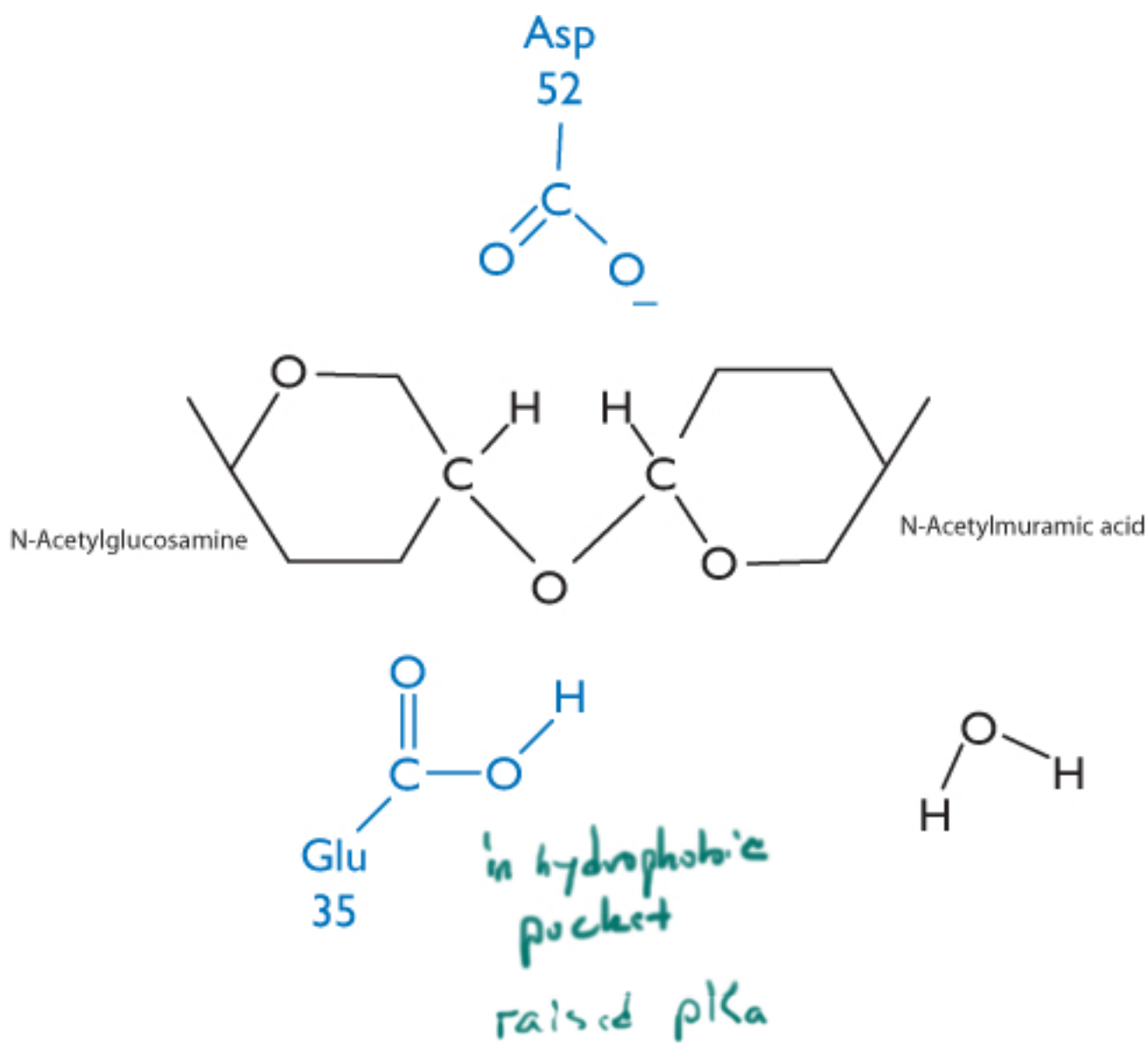
- where free energy of binding, a portion affects a conformation change in the substrate

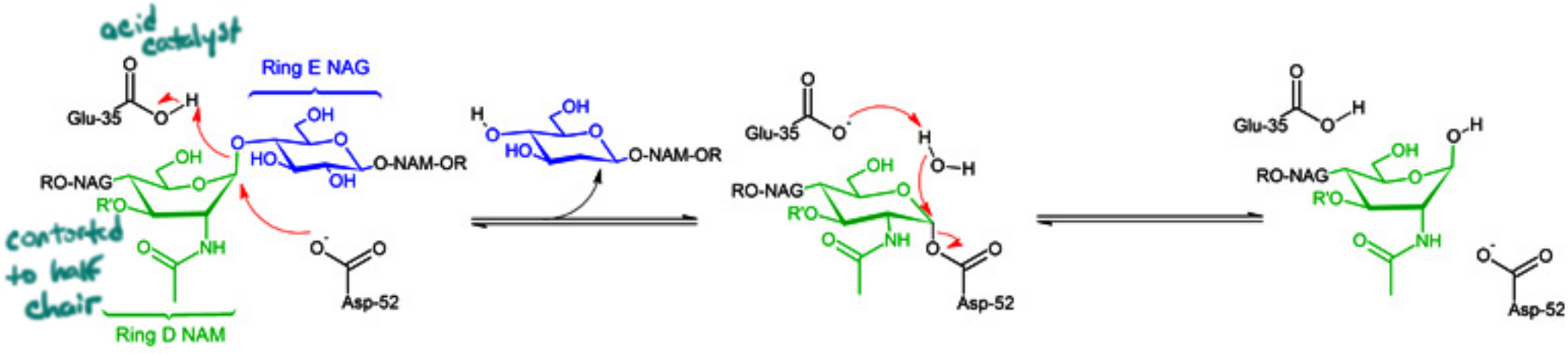
Cleaves peptidoglycan
↑
bacterial cell well

peptidoglycan - long carbohydrate chains
- NAG - N-acetyl glucosamine
- NAM - N-acetyl muramic acid

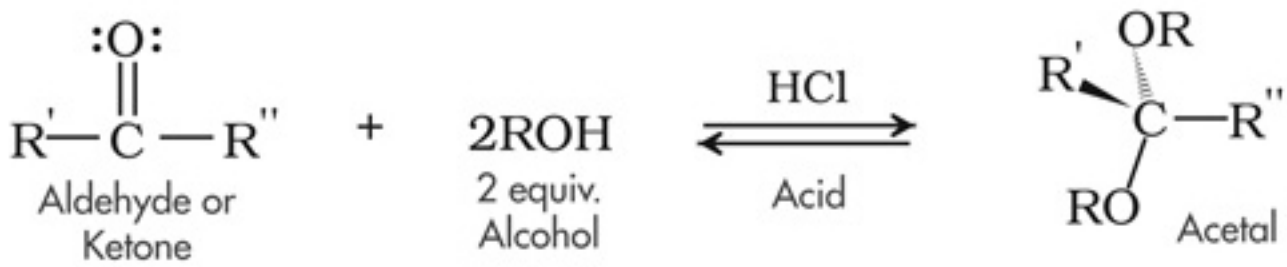
crosslinked with tetra peptides


CH3
OH
O
NH
O
O
HO
O
O
O
O
H3C
NH
β 1,4
O
HO
O
CH3
NAG
NAM
tetra-
peptide
peptidoglycan
n

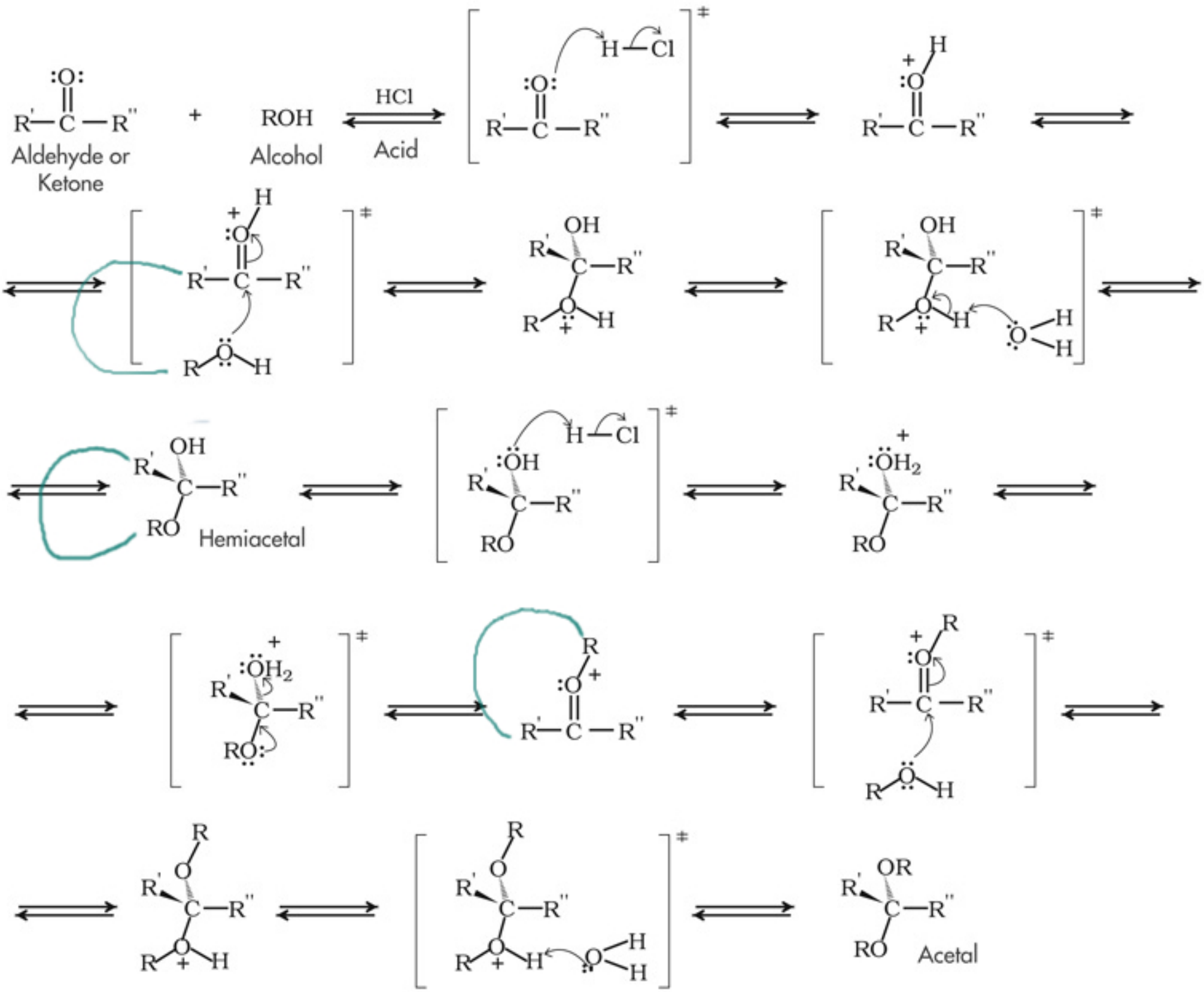

Asp
52
N-Acetylglucosamine
N-Acetylmuramic acid
Glu
35
in hydrophobic
pocket
raised pKa

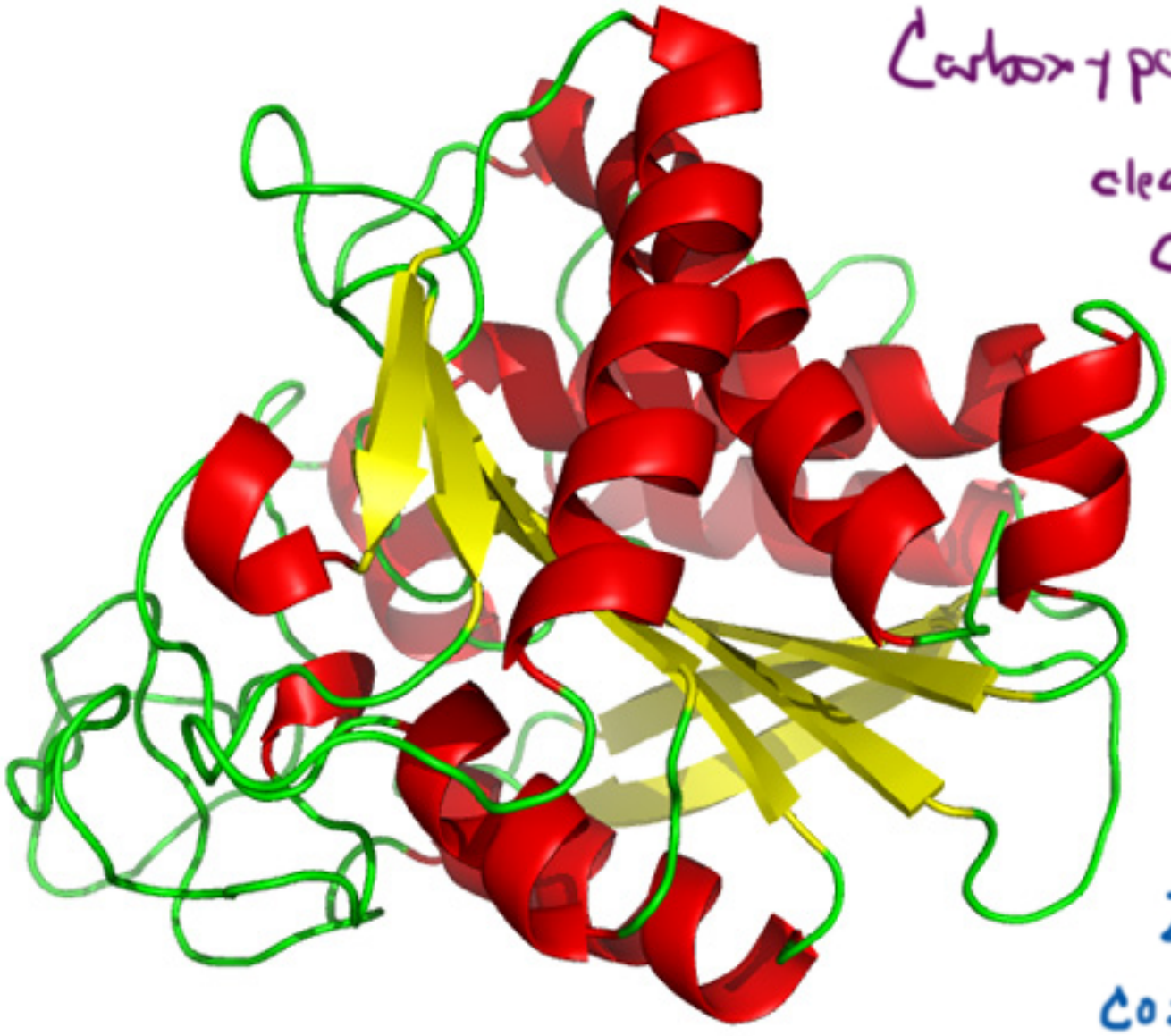


R = cell wall oligosaccharide chain
R' = cell wall peptide side chain



acetal formation





Carboxypeptidase

cleaves the C-terminal peptide

Zinc protease

Zn cofactor

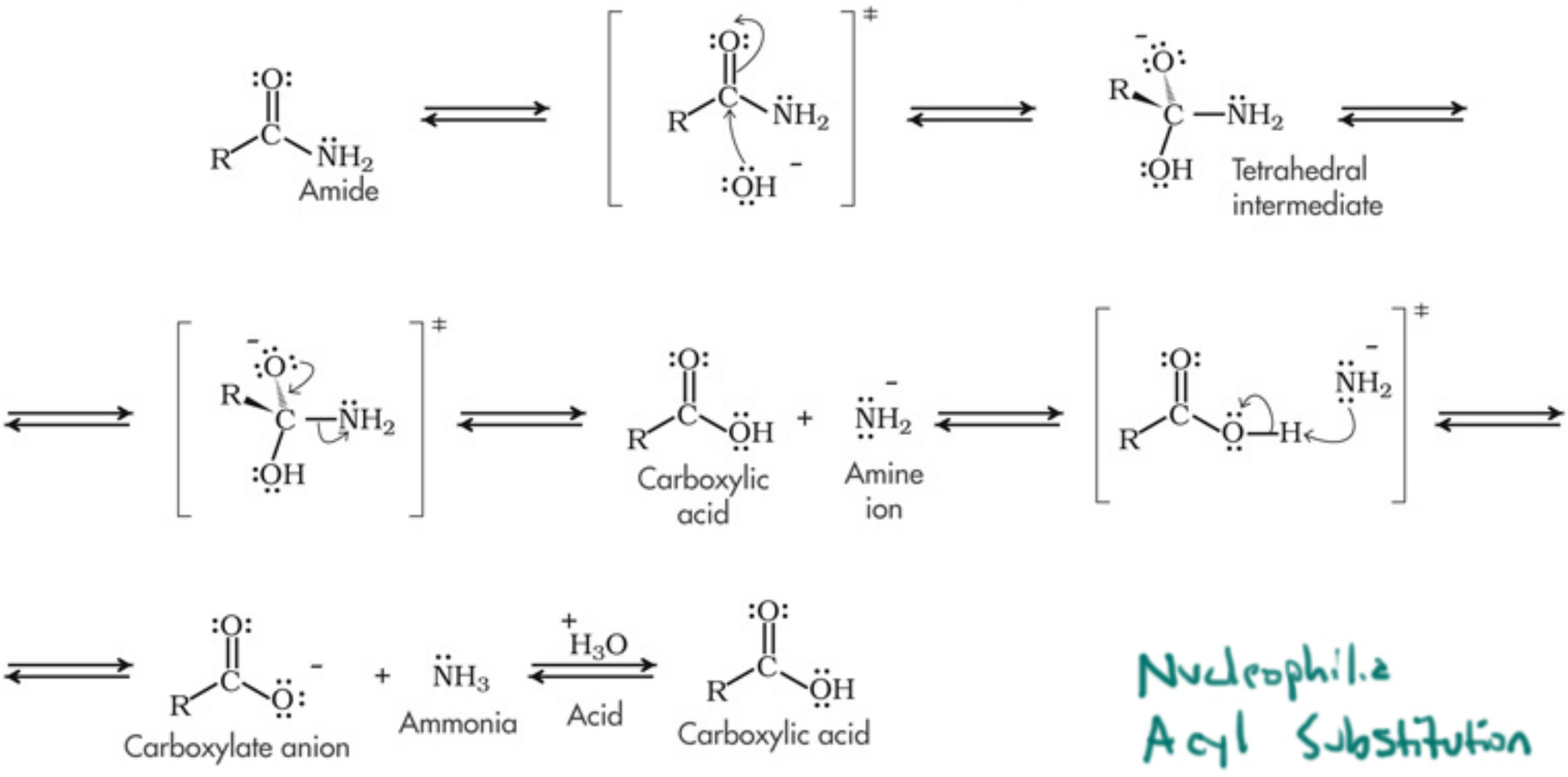
coordinated tetrahedrally

2 His

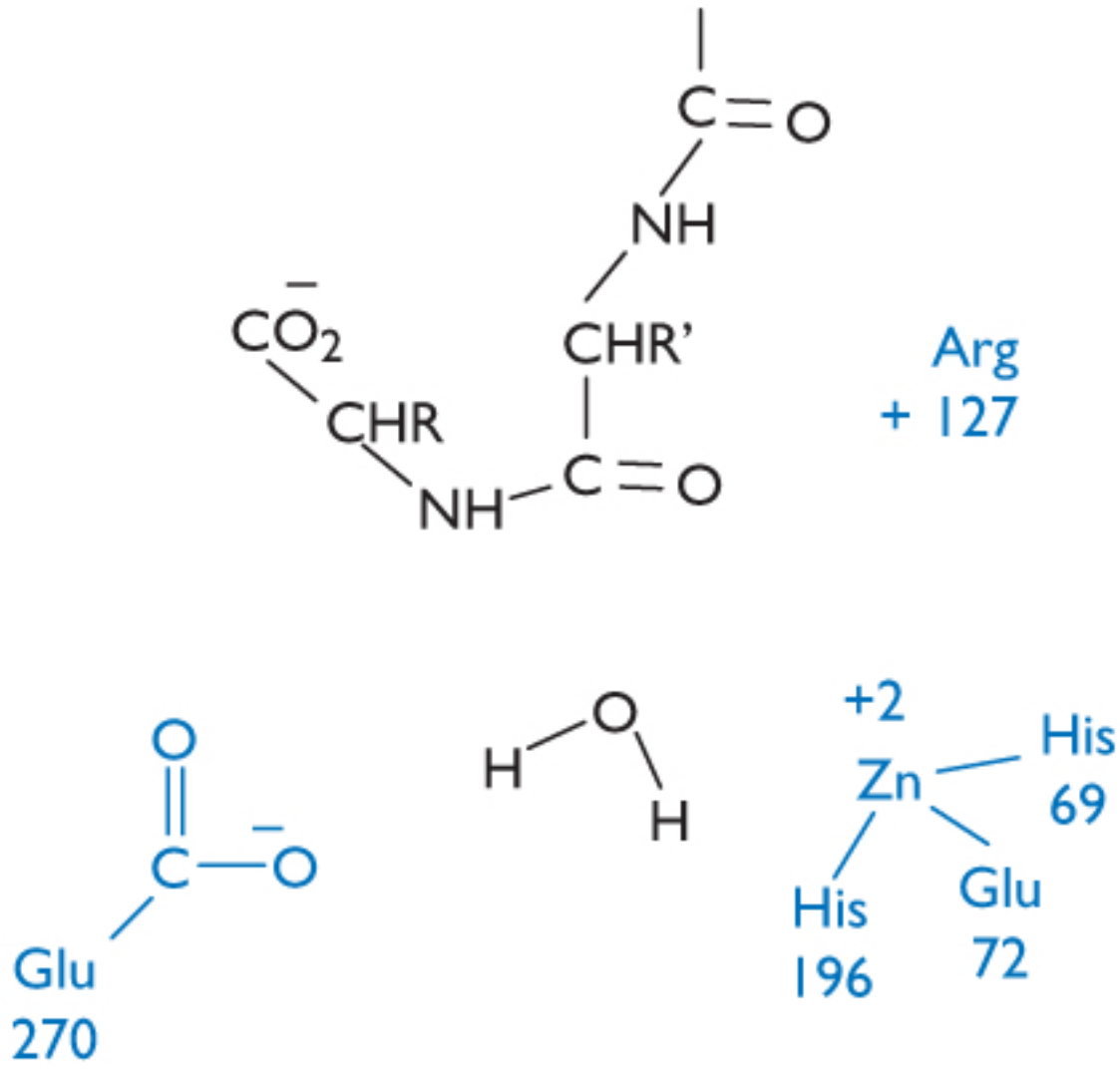
1 Glu

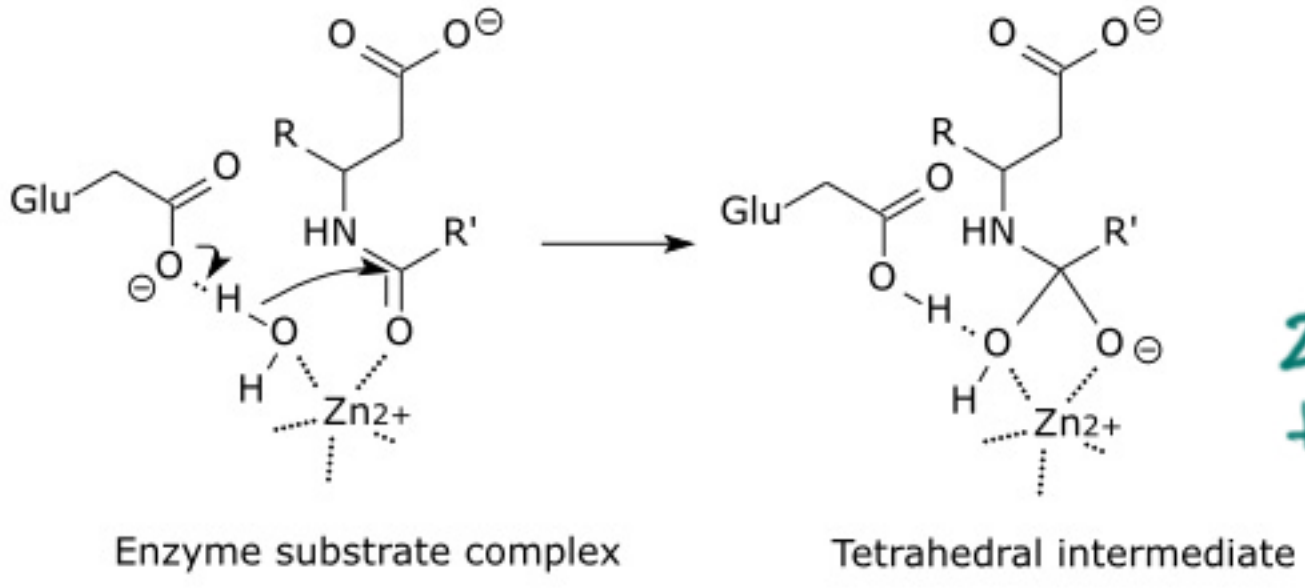
H_2O

Hydrolysis of Amide



Nucleophilic Acyl Substitution

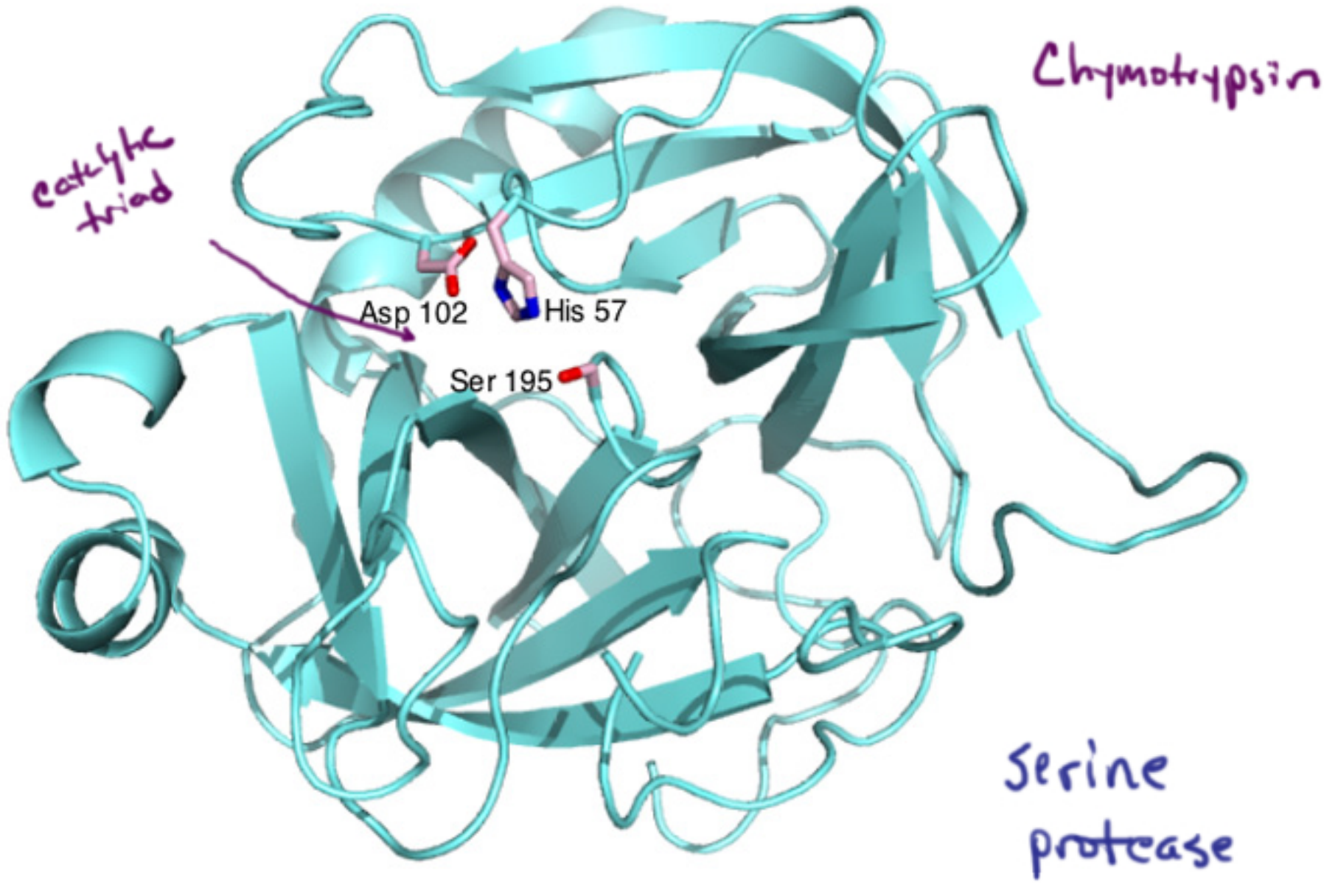

C=O
NH
CO2
CHR'
Arg
+ 127
CHR
NH
C=O
O
C
O
Glu
270
H
O
H
+2
Zn
His
69
His
196
Glu
72



Enzyme substrate complex

Tetrahedral intermediate

Zn stabilizes tetrahedral intermediate


Chymotrypsin
catalytic triad
Asp 102
His 57
Ser 195
serine protease

Chymotrypsin

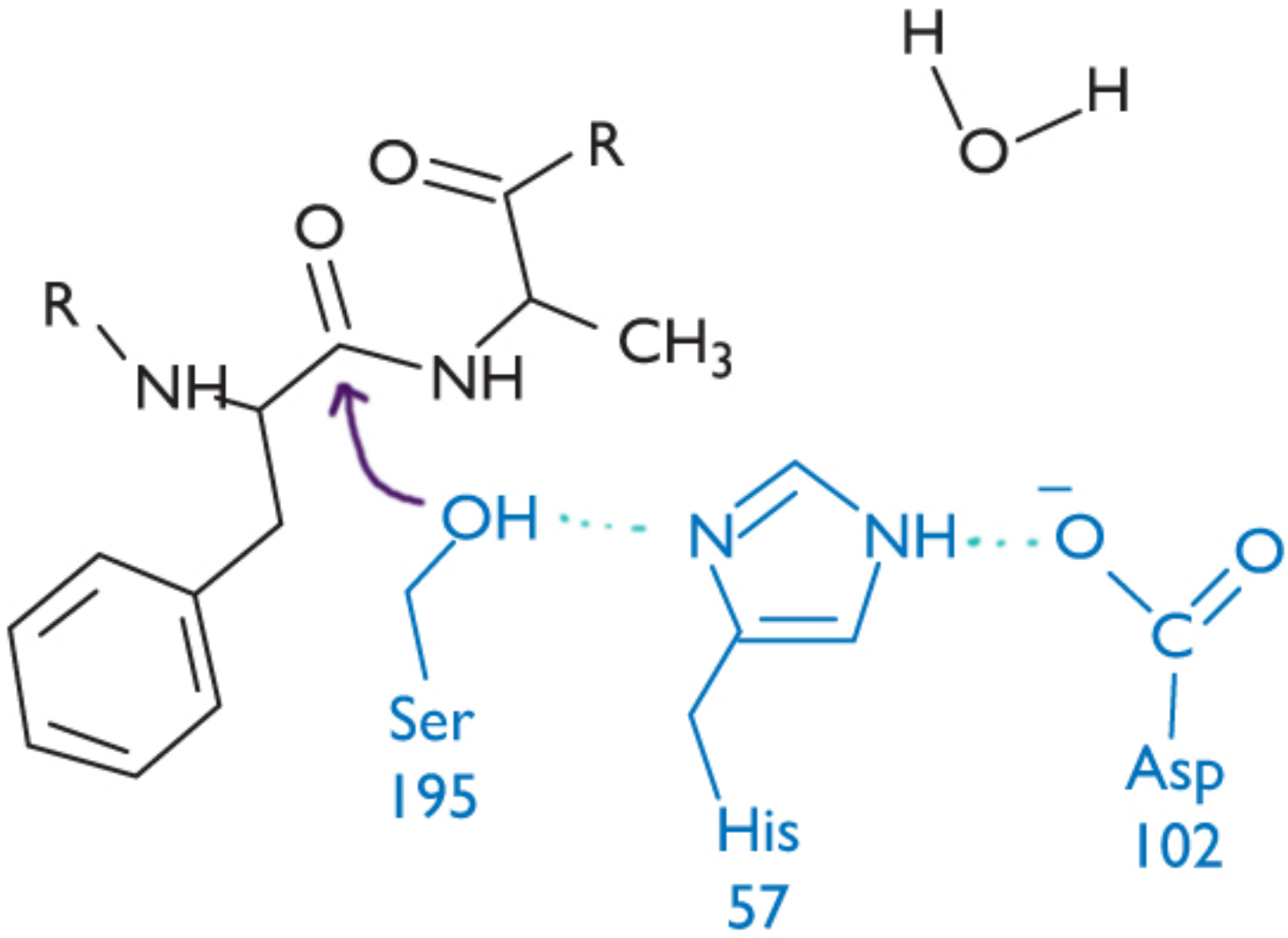
aromatic, bulky residue

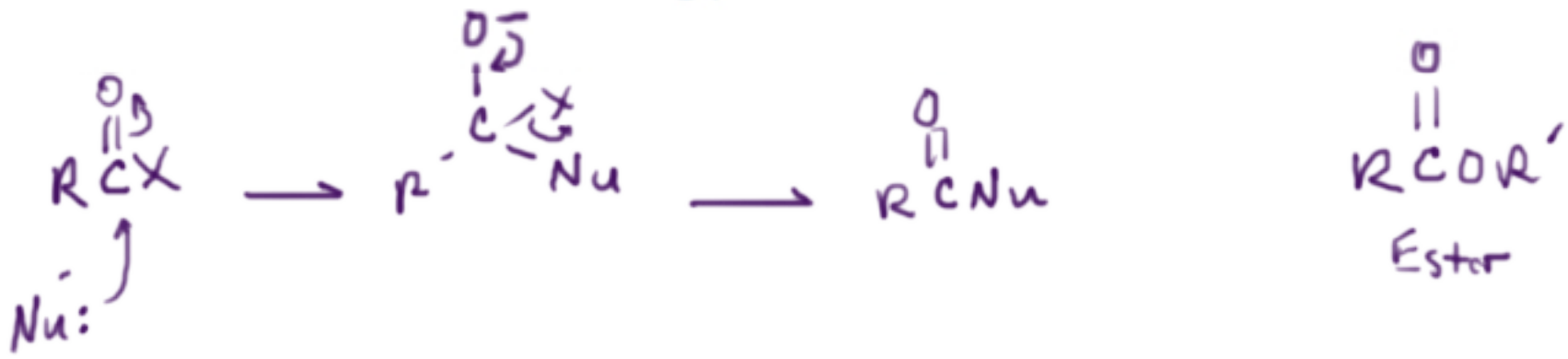
Trypsin

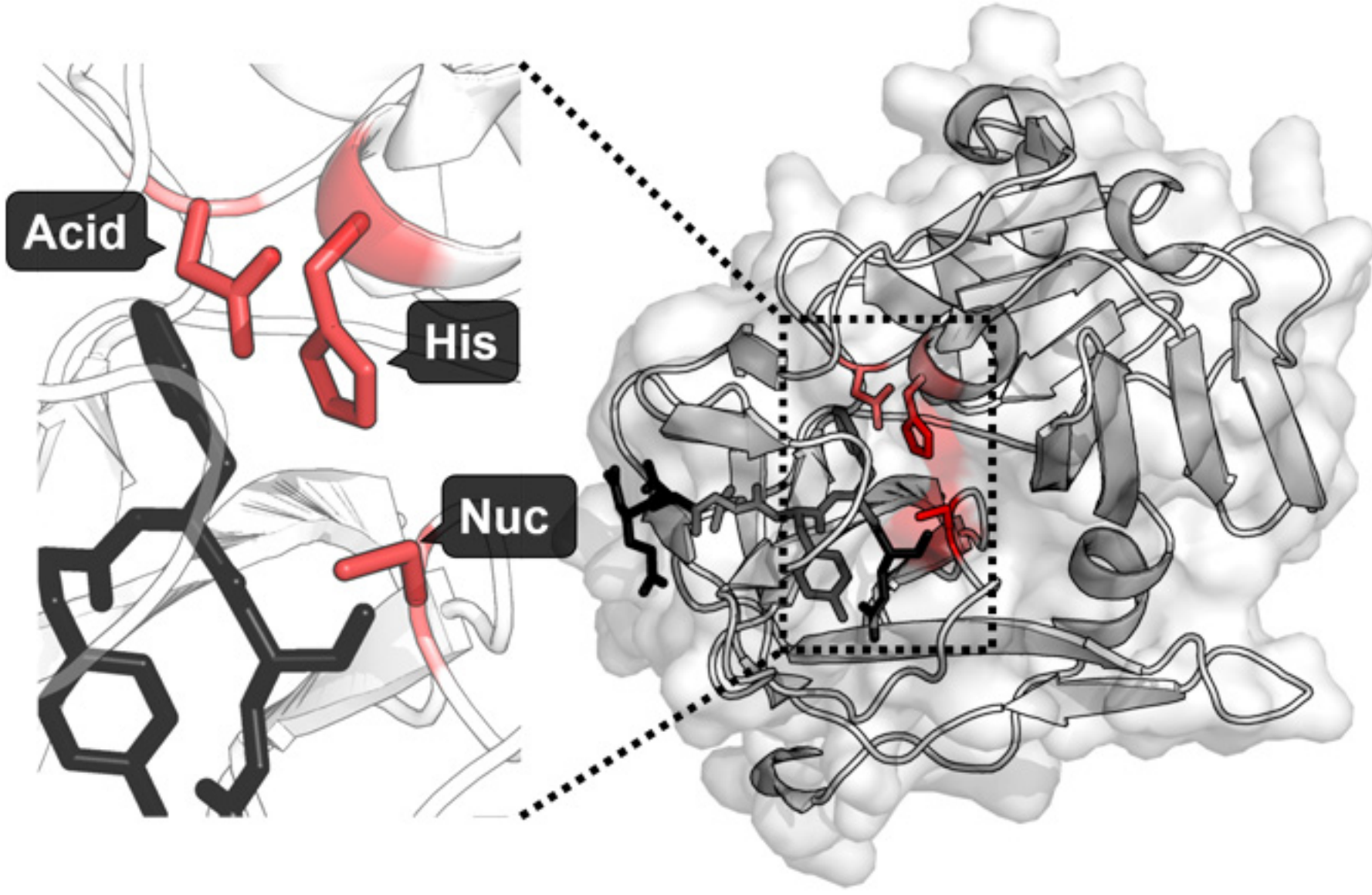
lysine or arginine

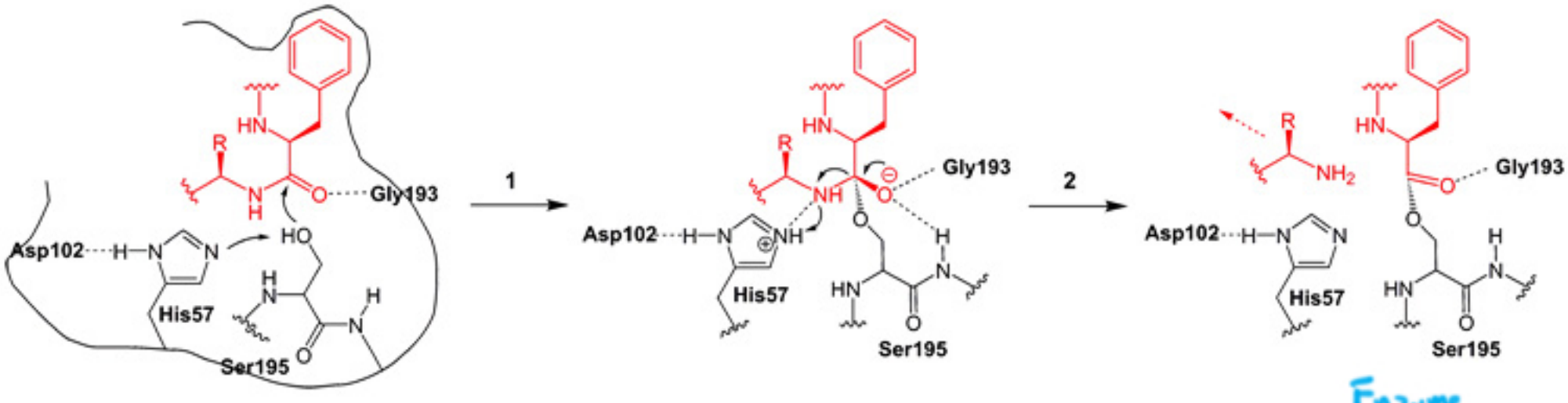
Elastase

smaller, uncharged residue

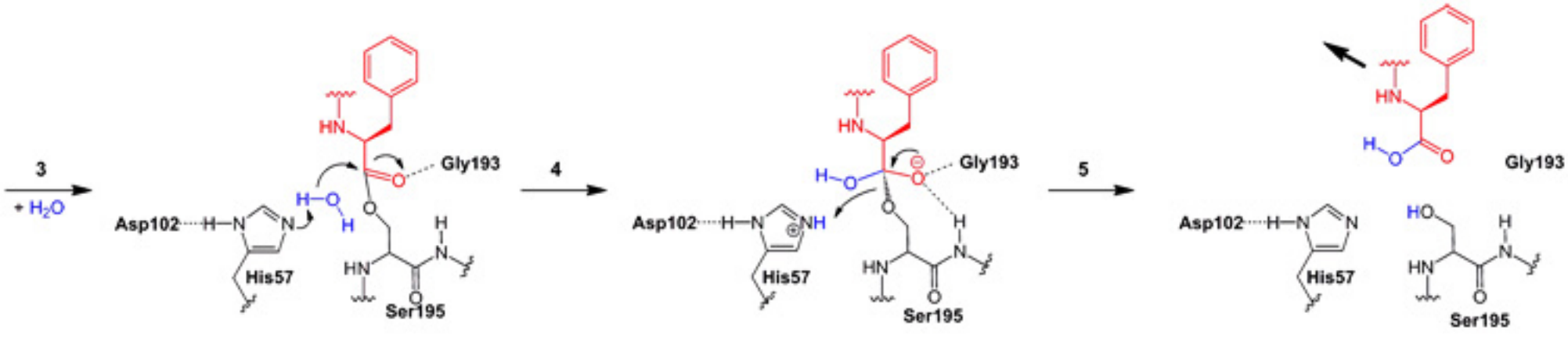

H
H
O
O
R
R
NH
NH
CH3
OH
N
NH
O
O
C
Ser
195
His
57
Asp
102


RCX
Nu:
Nu
RCNu
RCOR'
Ester


Acid
His
Nuc

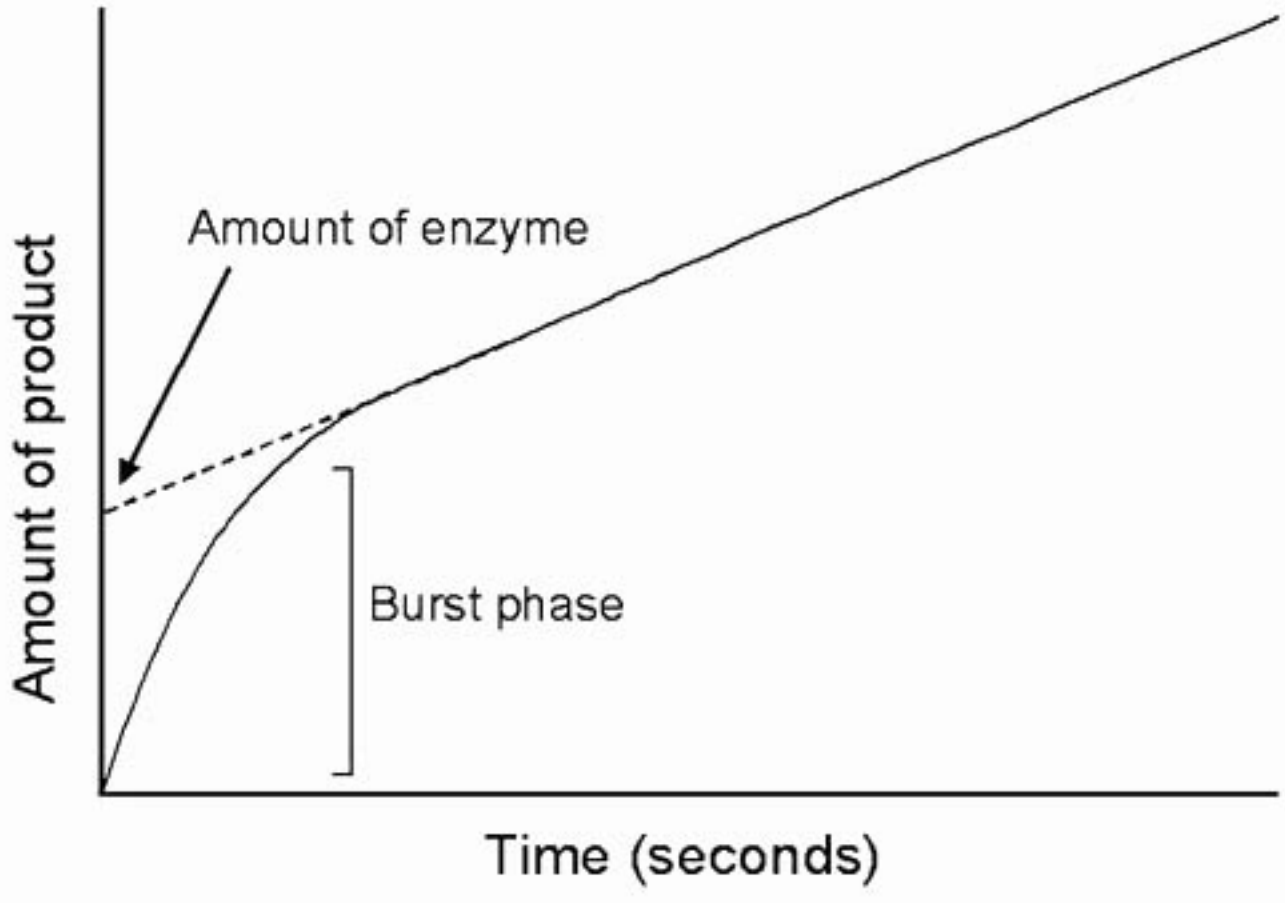


1st nucleophilic acyl
substitution
formation of enzyme ester

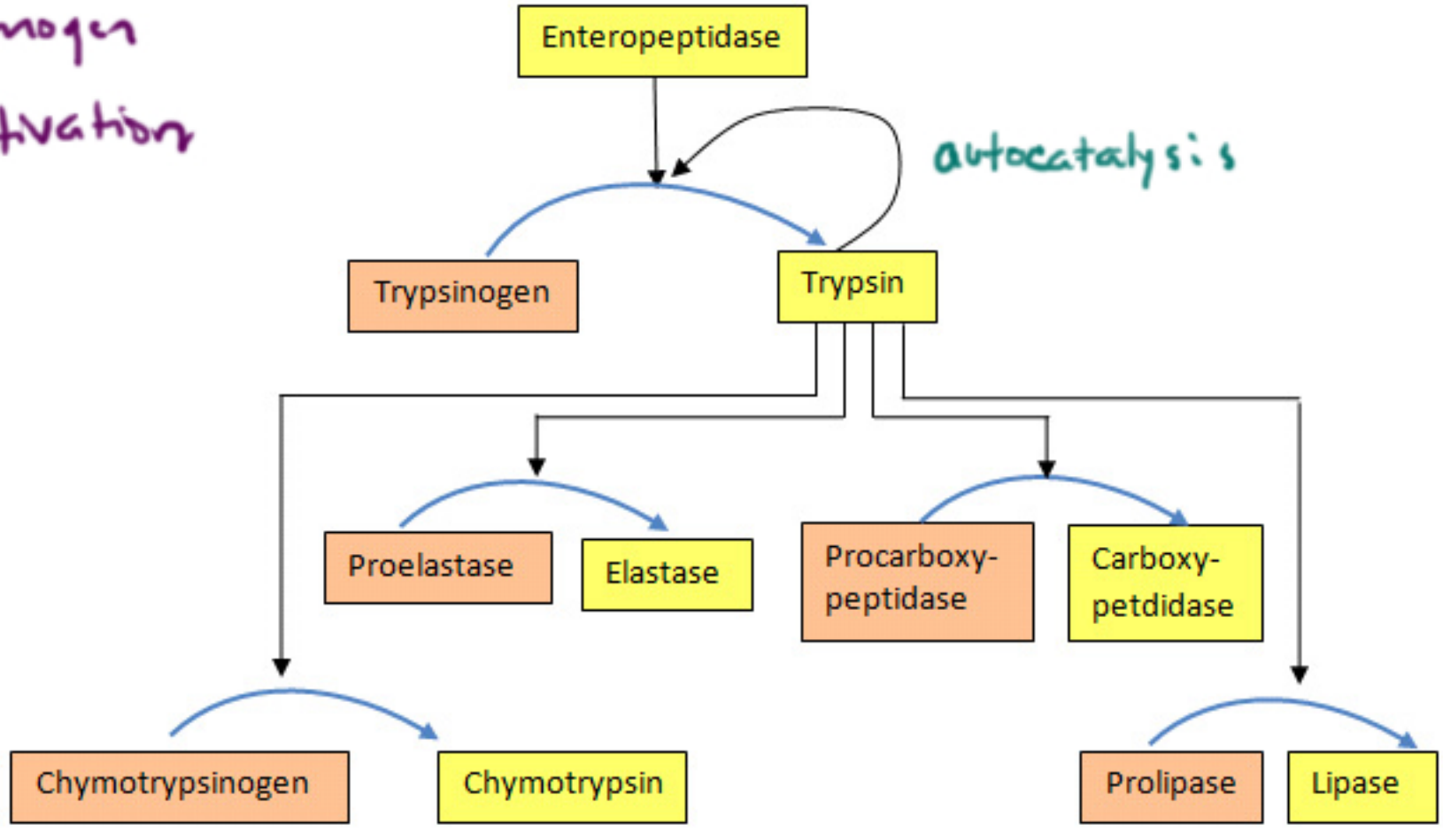


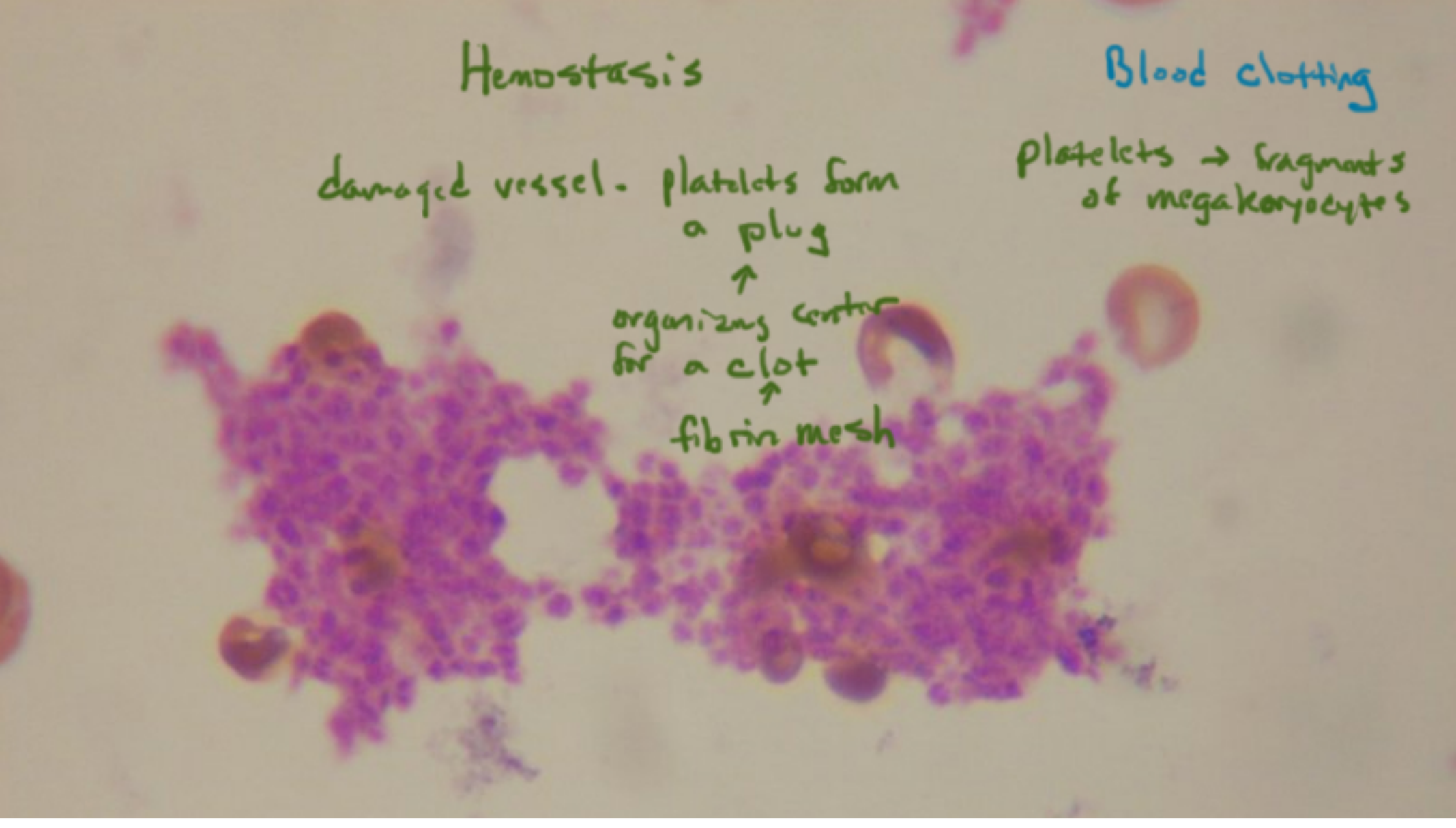
2nd nucleophilic acyl substitution

Hydrolysis of enzyme ester


Amount of enzyme
Amount of product
Burst phase
Time (seconds)

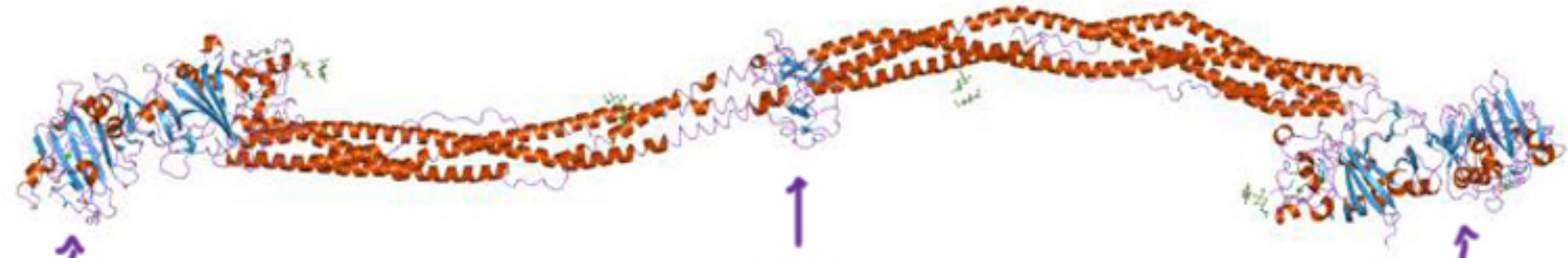
Zymogen activation




Hemostasis
Blood clotting
damaged vessel. platelets form a plug
↑
organizing center for a clot
↑
fibrin mesh
platelets → fragments of megakaryocytes

Fibrinogen - 2% - 3% of plasma protein

↑ soluble precursor



-4 charge

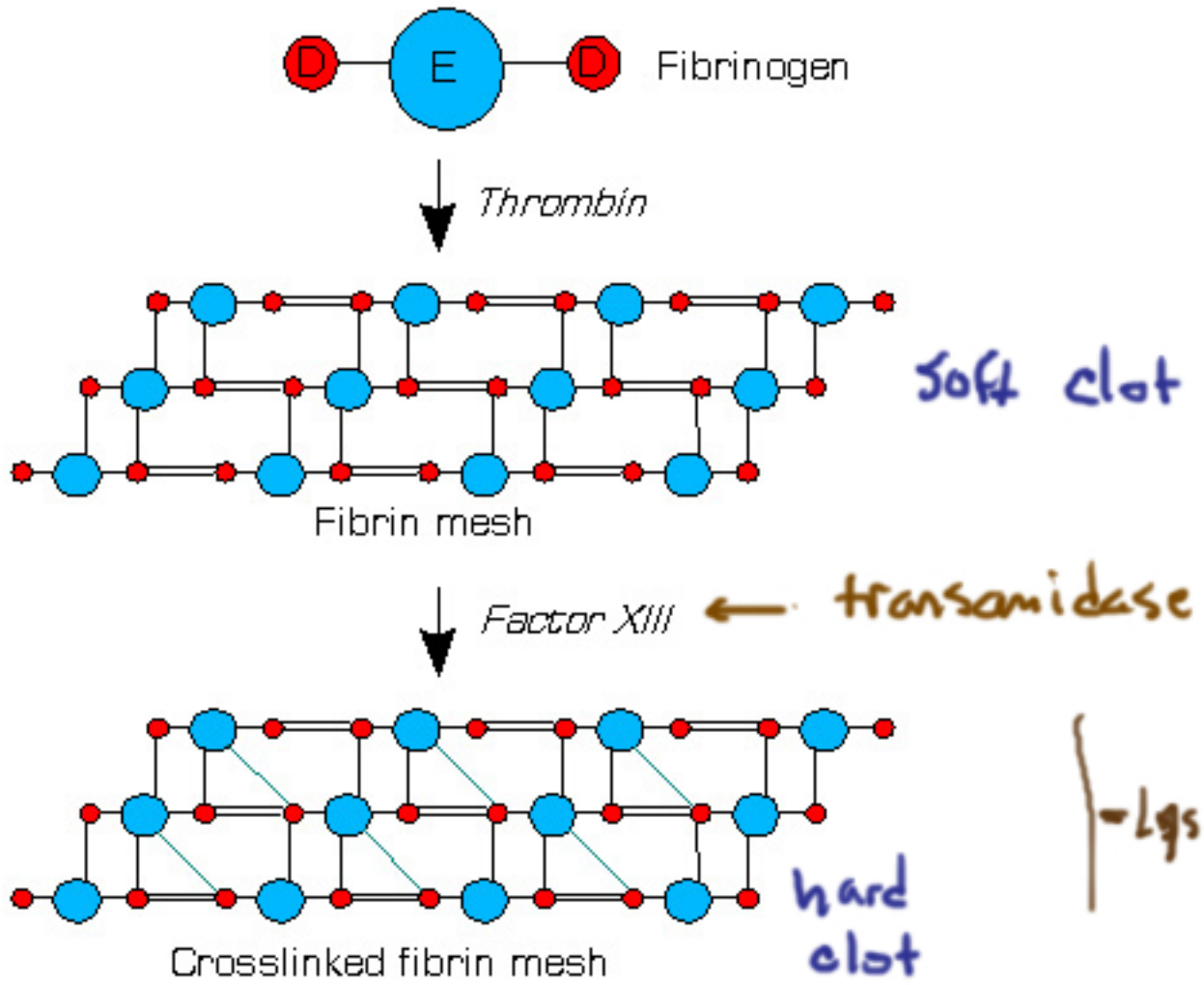
central region

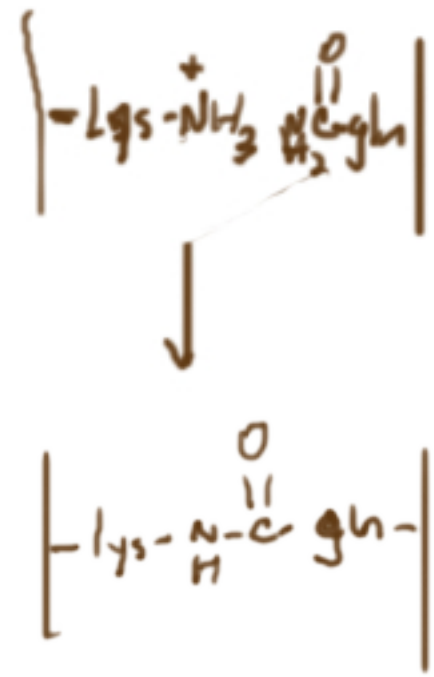
-4 charge

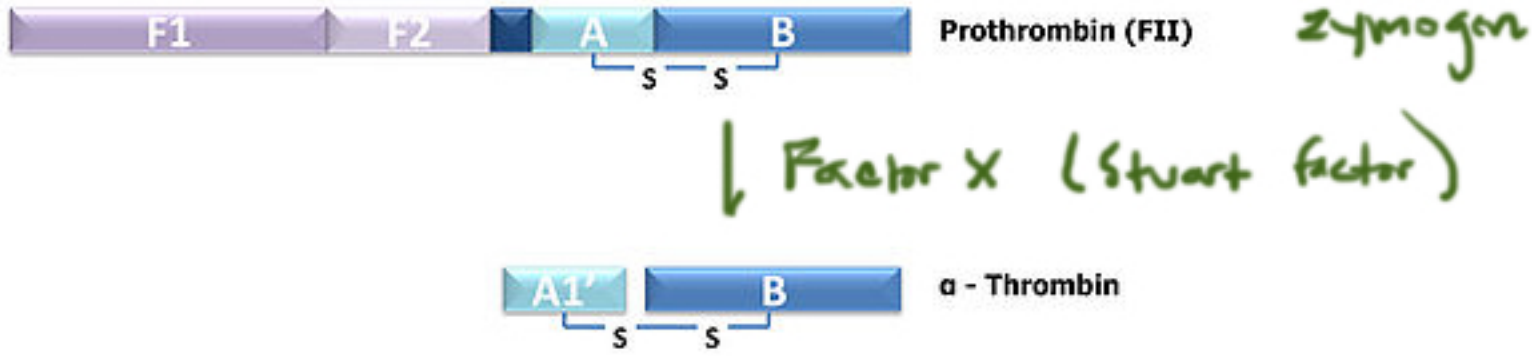
-8 charge with fibrinopeptides

+5 charge without thrm

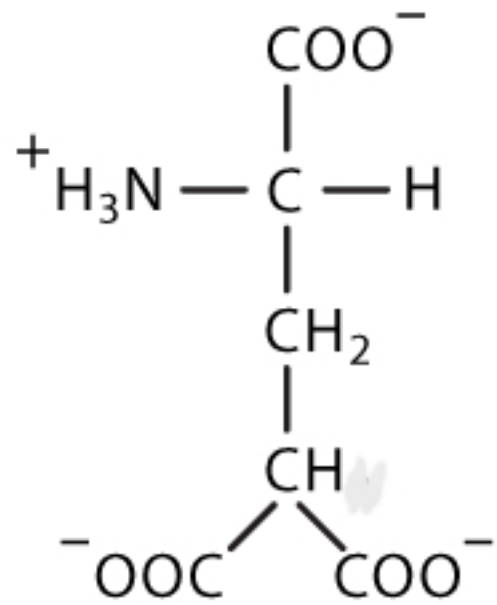
cleaved by thrombin


D
E
D
Fibrinogen
Thrombin
Fibrin mesh
soft clot
Factor XIII
transamidase
Crosslinked fibrin mesh
hard clot

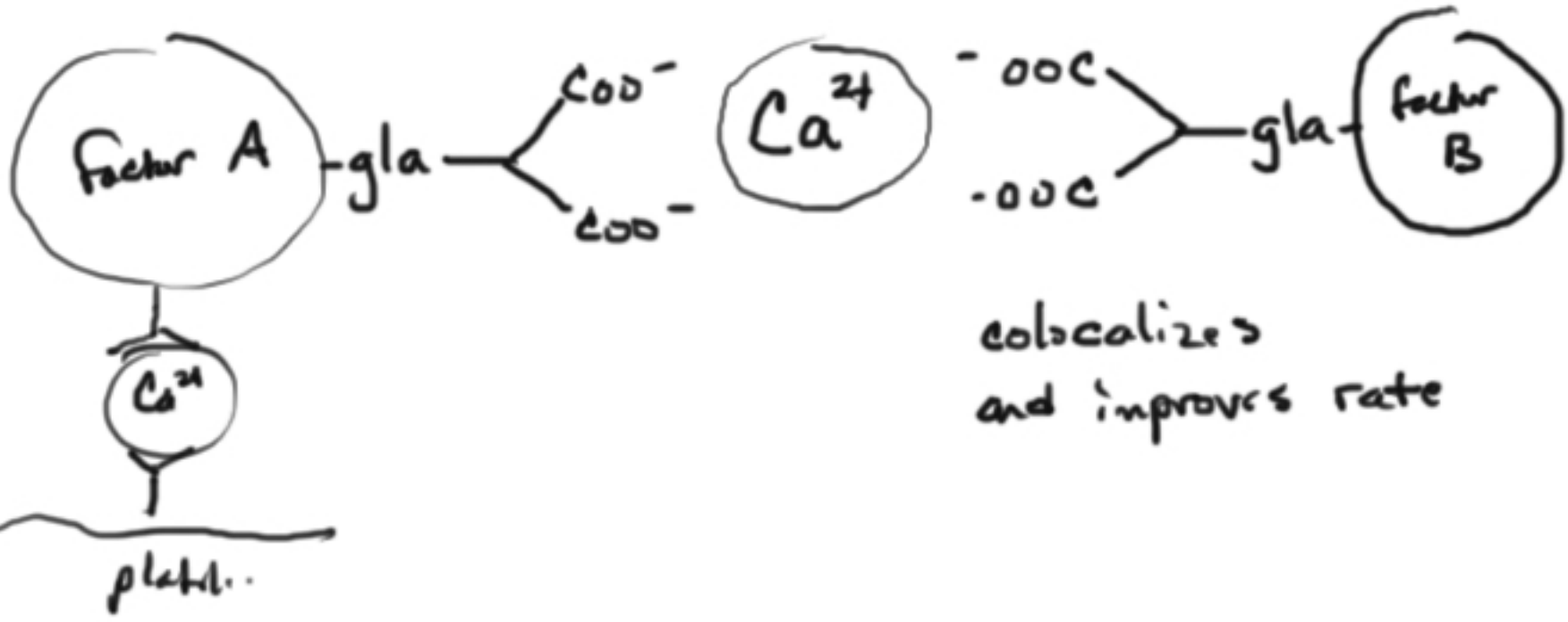

lys
gln
lys
gln


F1
F2
A
B
S
S
Prothrombin (FII)
zymogen
Factor X (Stuart factor)
A1'
B
S
S
α - Thrombin

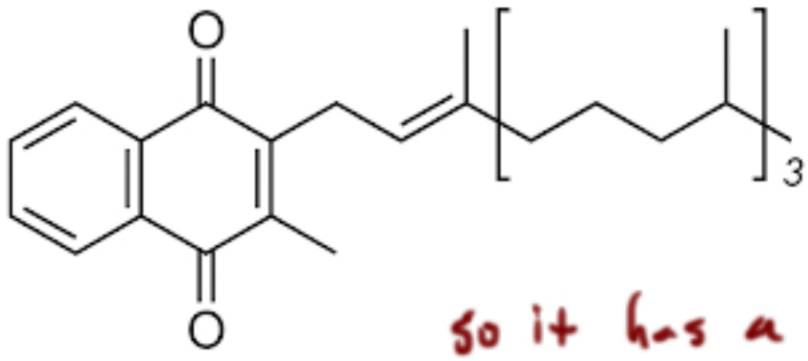
Many clotting factors possess γ carboxy glutamate



synthesis requires vitamin K

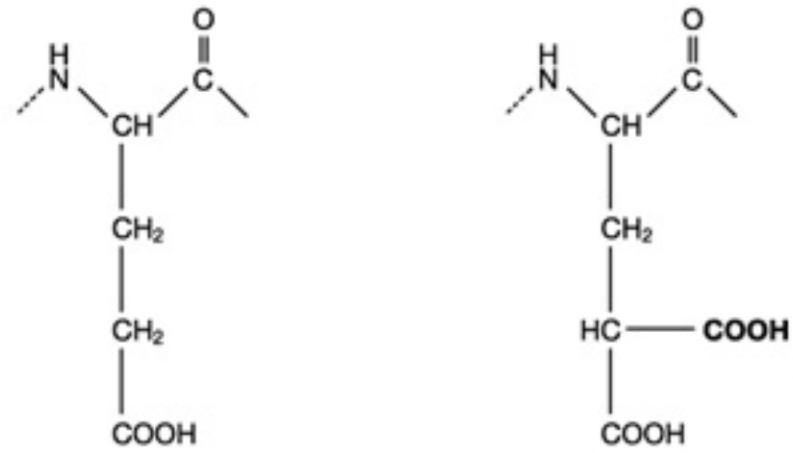


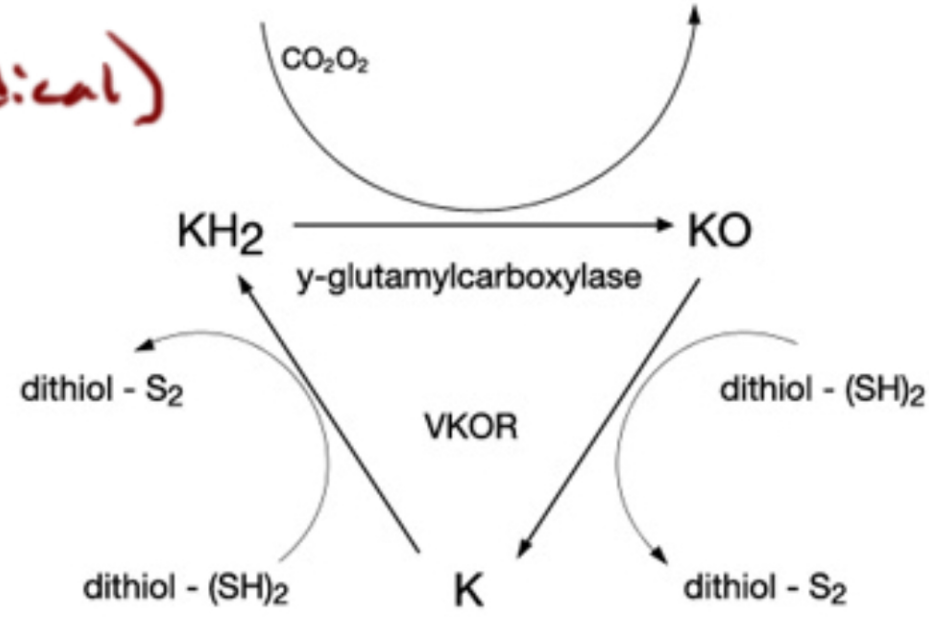
colocalizes and improves rate



↑ benzoquinone like CoQ

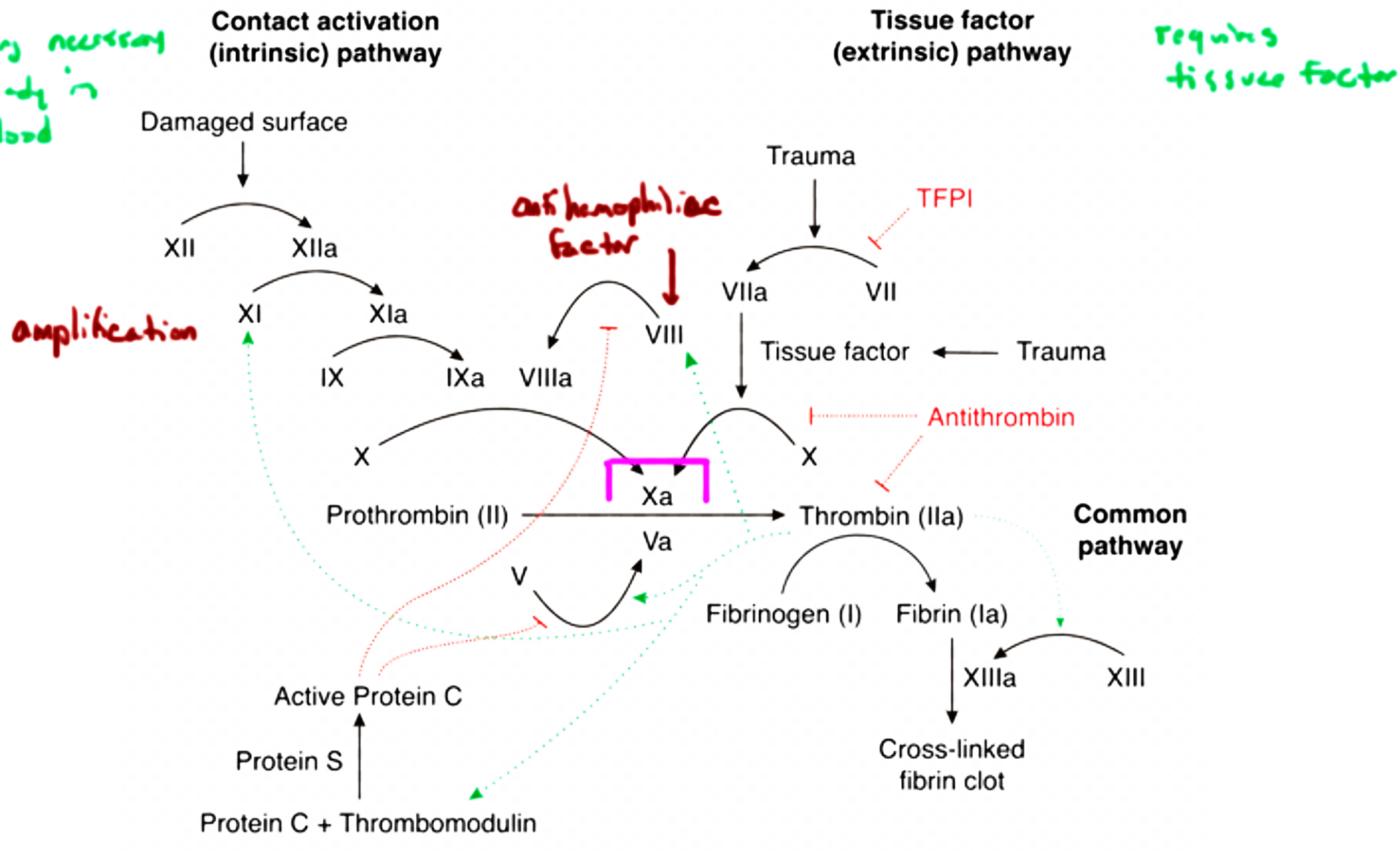
so it has a semiquinone (stable radical) form



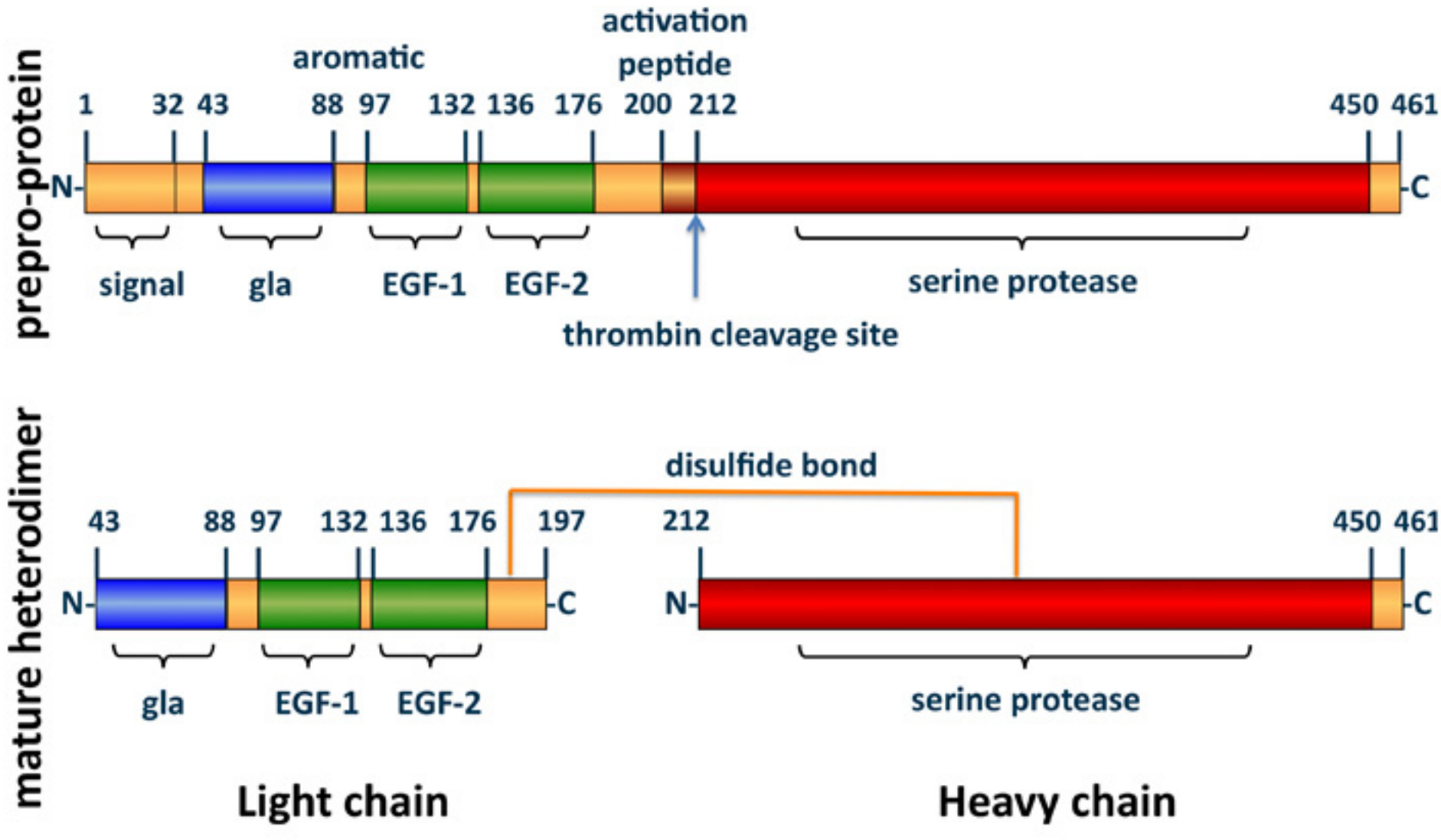


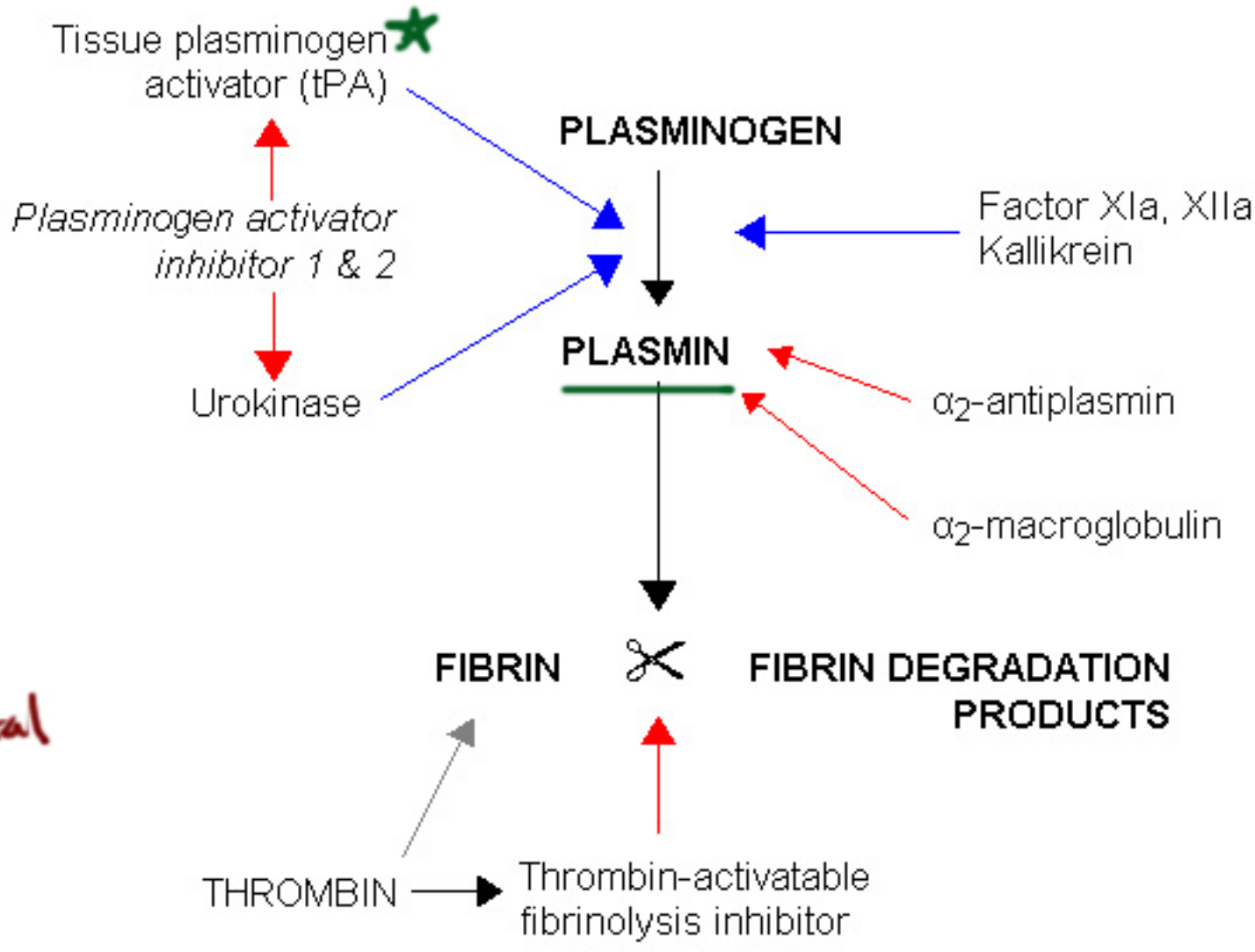
Warfarin and Coumadin are vitamin K competive inhibitors

everything necessary is already in the blood

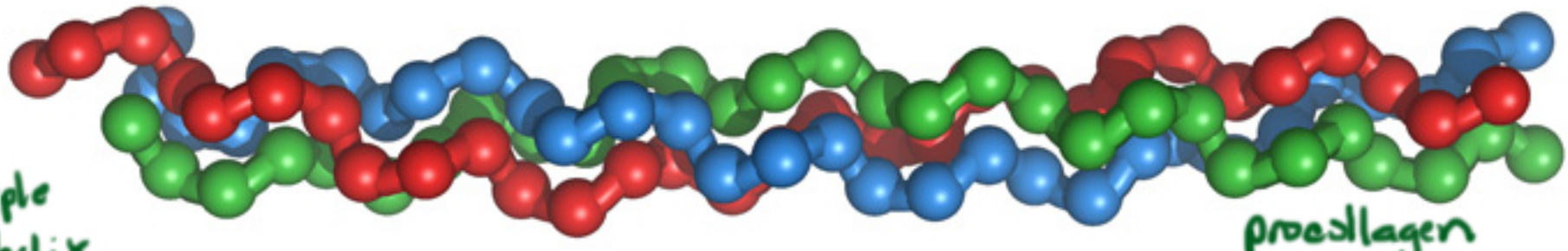


Don't worry about nitty gritty details


prepro-protein
activation
peptide
aromatic
1
32
43
88
97
132
136
176
200
212
450
461
N-
-C
signal
gla
EGF-1
EGF-2
serine protease
thrombin cleavage site
mature heterodimer
disulfide bond
43
88
97
132
136
176
197
212
450
461
N-
-C
N-
-C
gla
EGF-1
EGF-2
serine protease
Light chain
Heavy chain

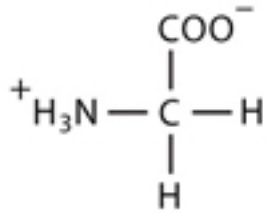


Supplamental



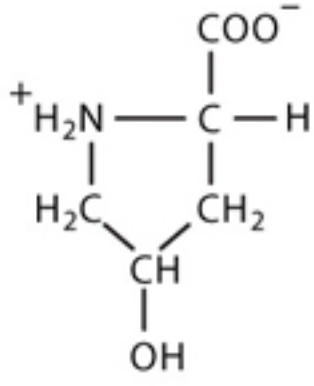
triple helix

procollagen

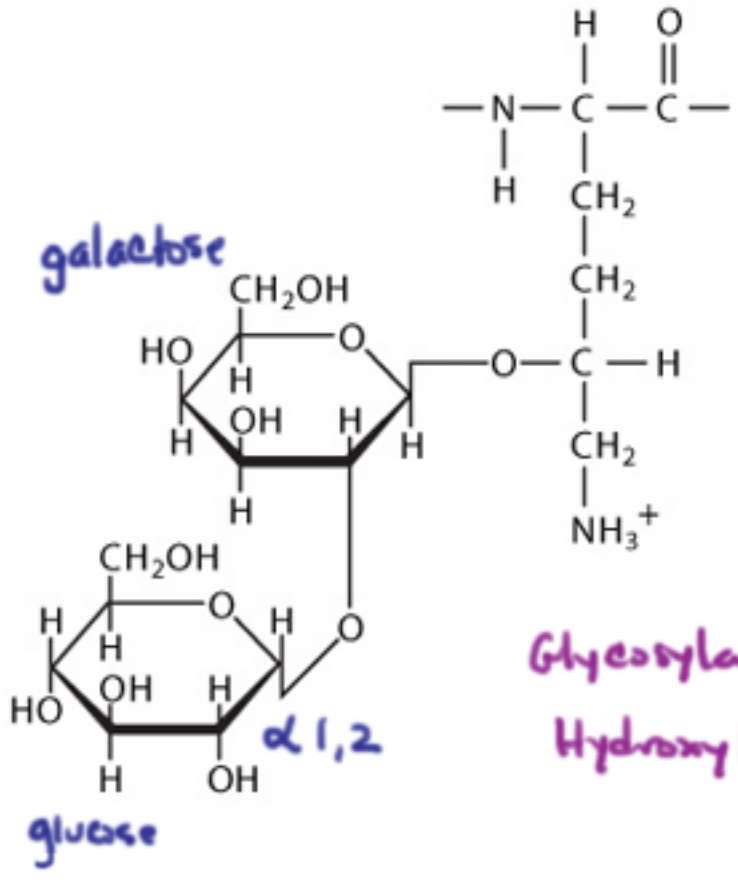


30% glycine

- interior of triple glycine



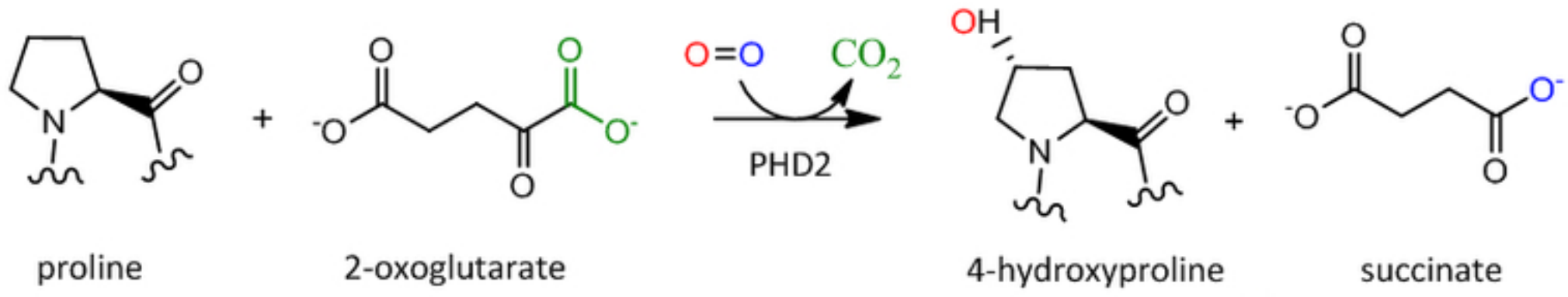
Hydroxy proline



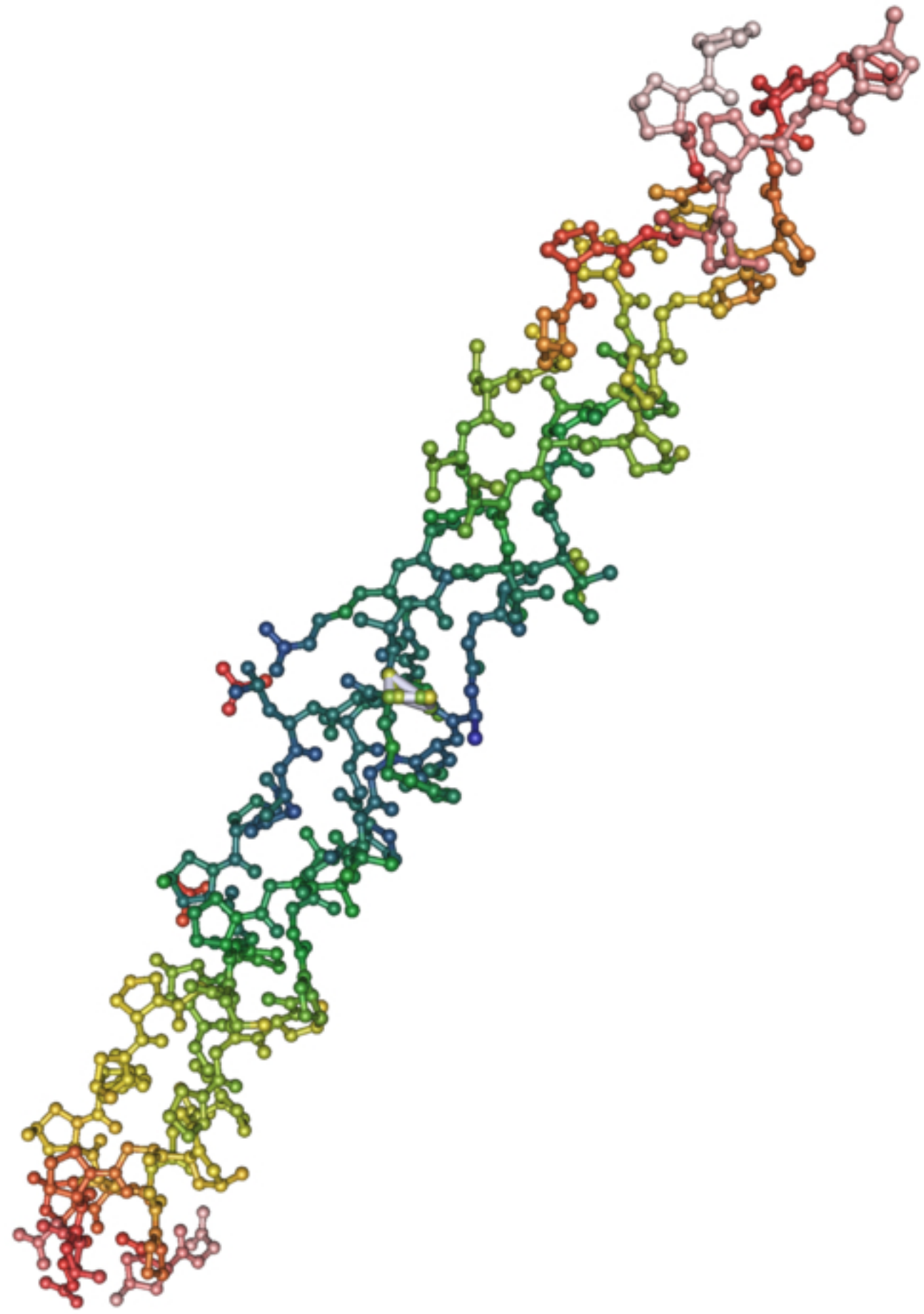
Glycosylate Hydroxylysine

Connective tissue proteins

Synthesis of Hydroxyproline and Hydroxylysine

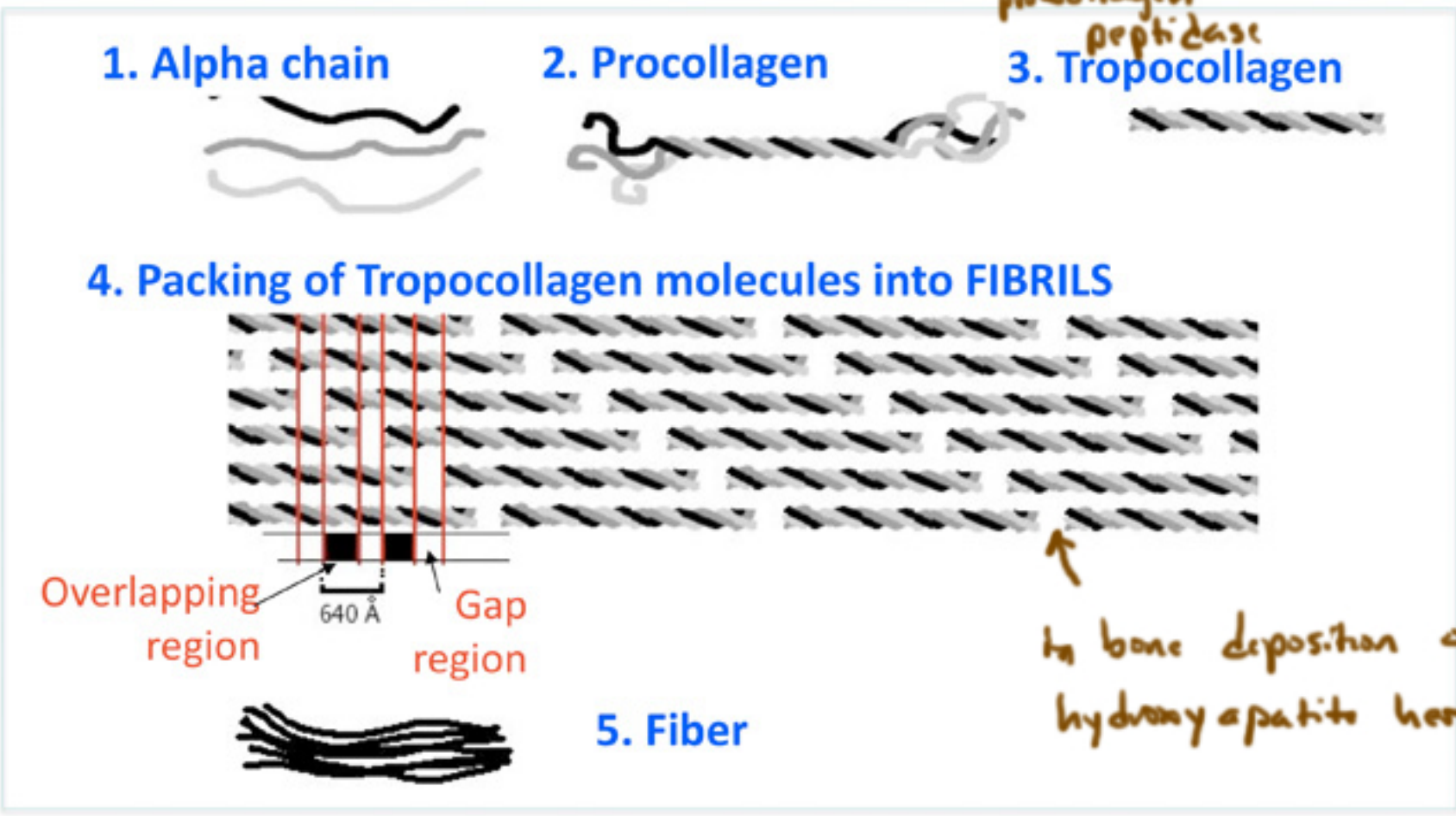


- requires ascorbate (vitamin C)
- returns ferric → ferrous

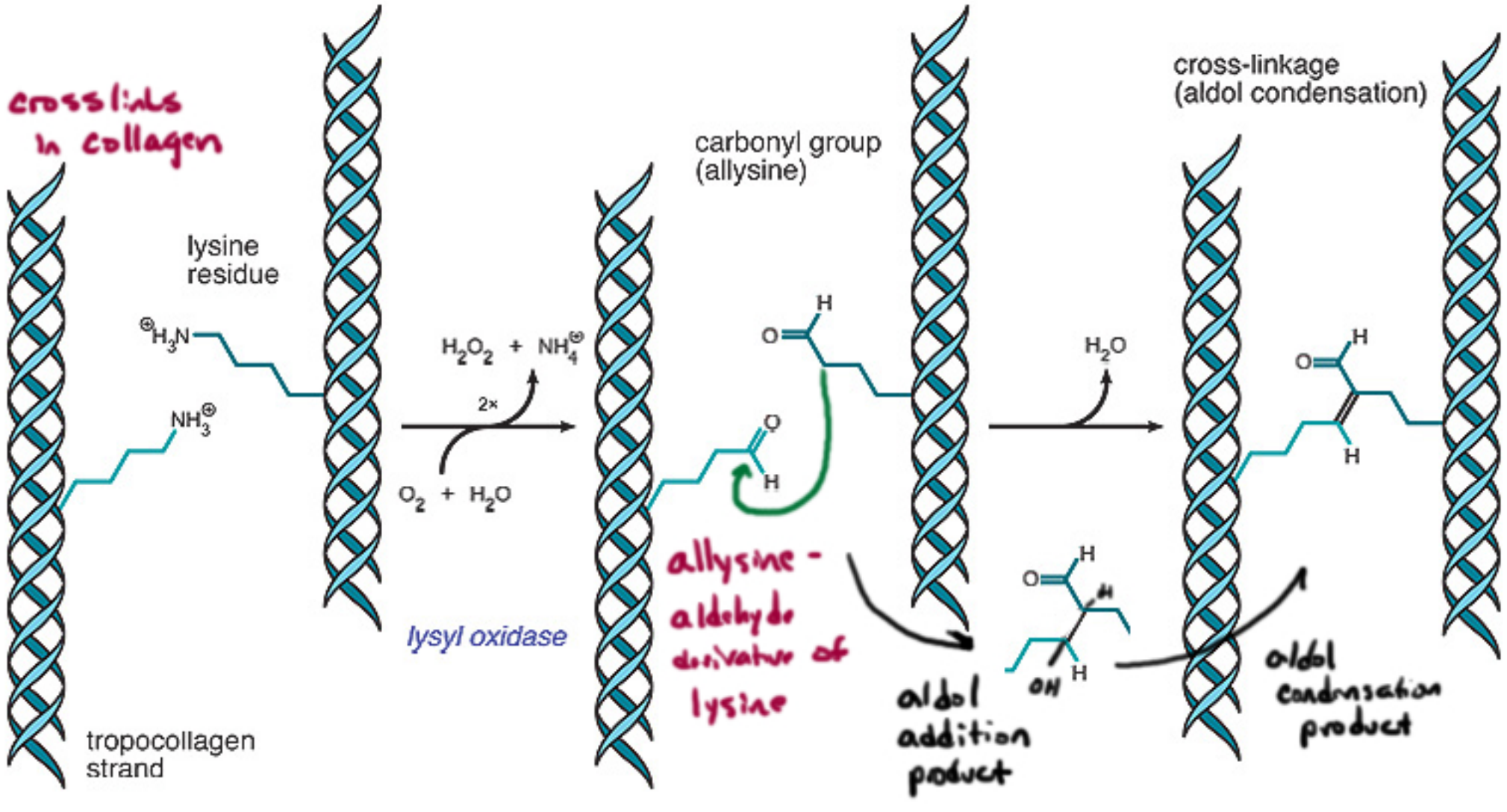


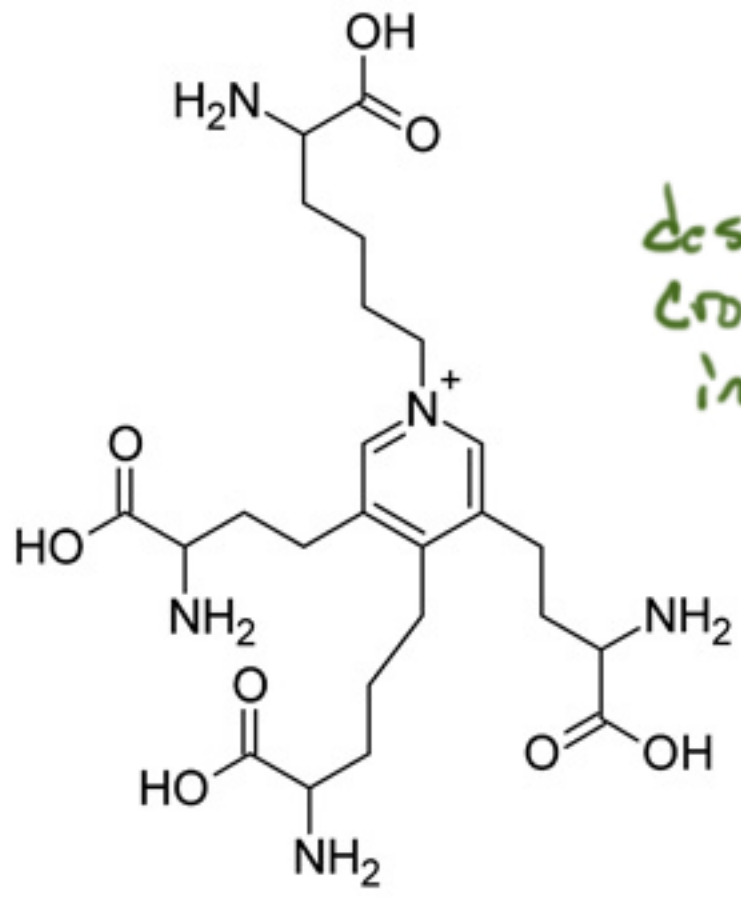
procollagen $\xrightarrow[\text{peptidase}]{\text{procollagen}}$ tropocollagen

c



to bone deposition of hydroxyapatite here


crosslinks in collagen
lysine residue
$^{\oplus}H_3N$
$NH_3^{\oplus}$
tropocollagen strand
H_2O_2 + $NH_4^{\oplus}$
2×
O_2 + H_2O
lysyl oxidase
carbonyl group (allysine)
allysine - aldehyde derivative of lysine
aldol addition product
H_2O
cross-linkage (aldol condensation)
aldol condensation product

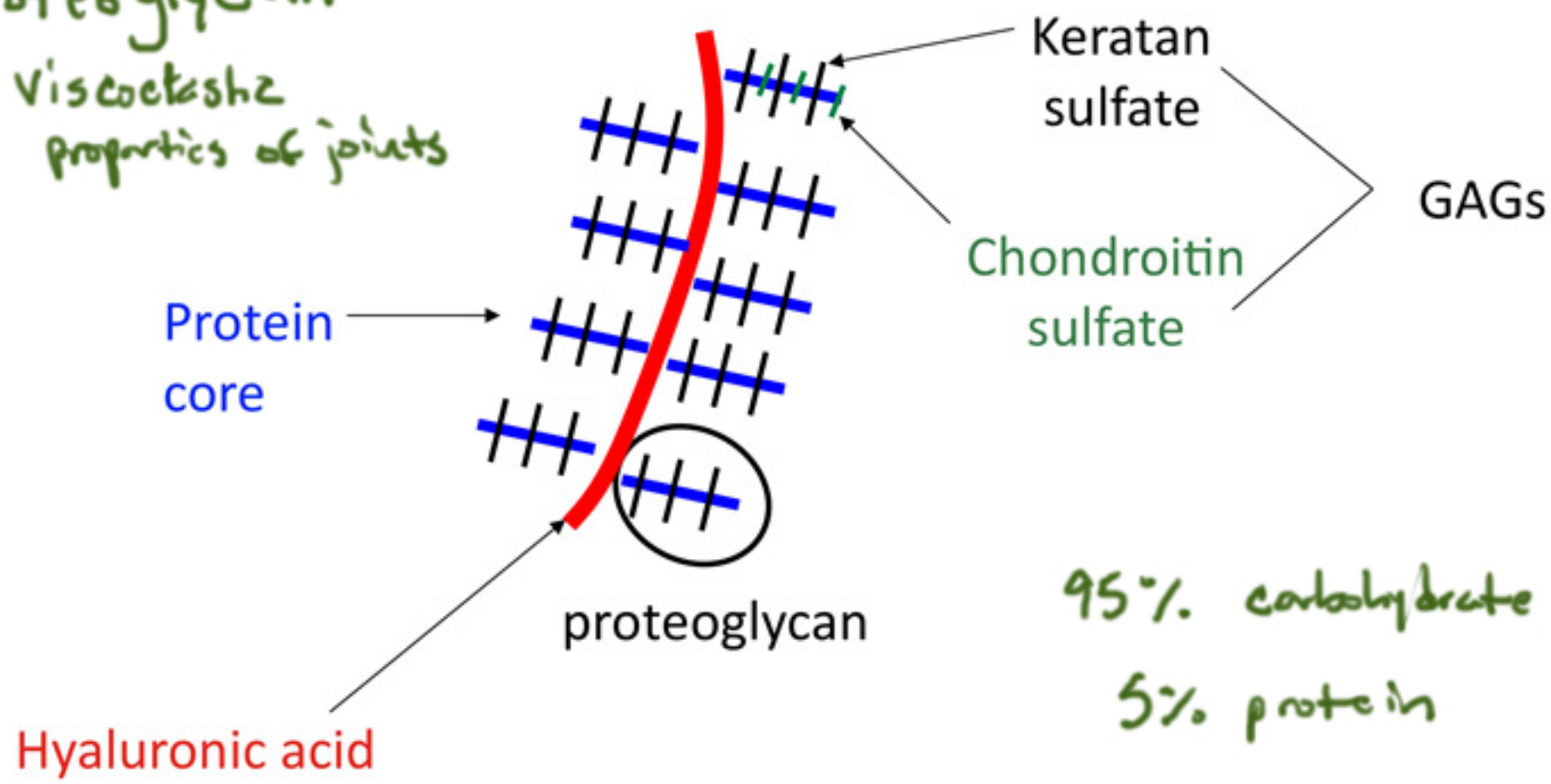


desmosine
crosslink
in elastin

3 allysines
+ lysine

Proteoglycan

- viscoelastic properties of joints



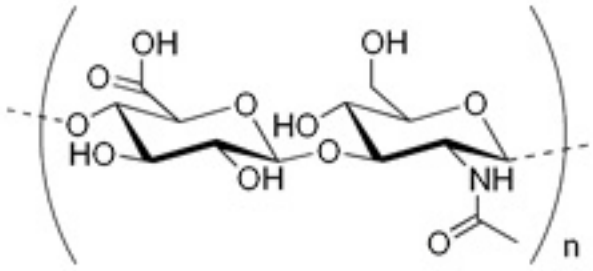
95% carbohydrate

5% protein

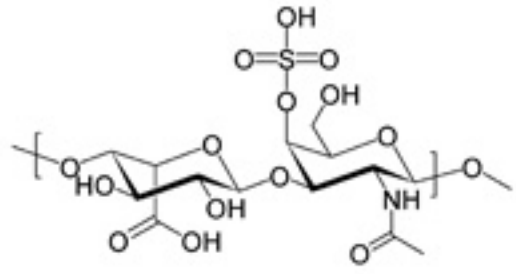
sugar monomers are acidic derivatives of N-acetyl glucosamine

↖ carboxyl and/or sulfates

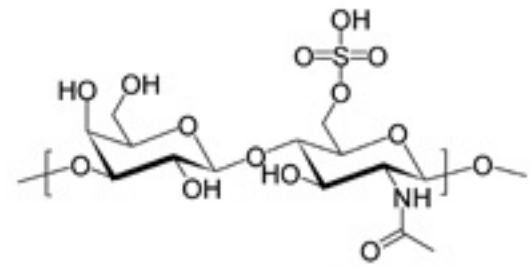
- lots of ⊖ charge
  - very hydrophilic
  - very slippery (repulsion)



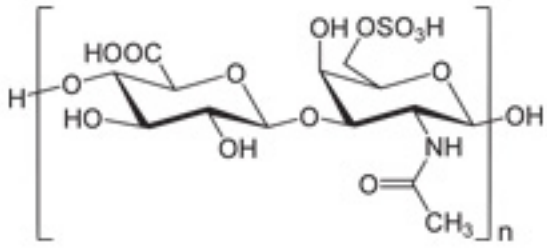
Hyaluronate



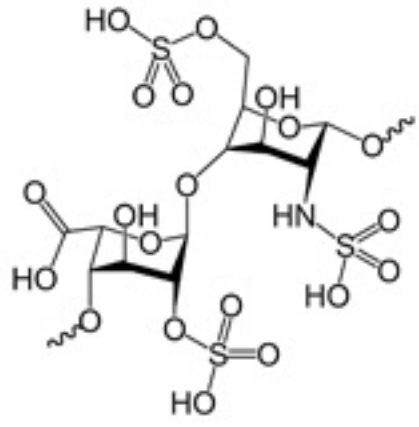
Dermatan sulfate



Keratan sulfate



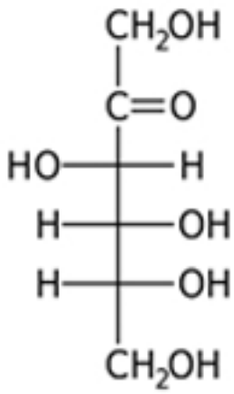
Chondroitin 6-sulfate



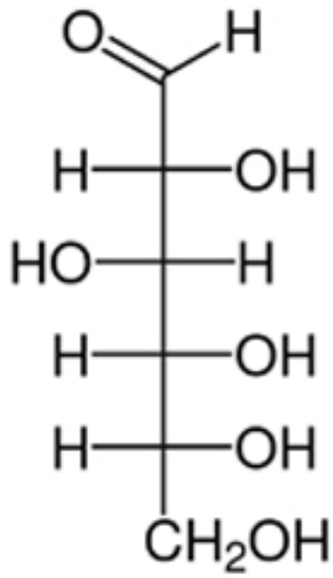
Heparin

Sugar monomers in proteoglycan

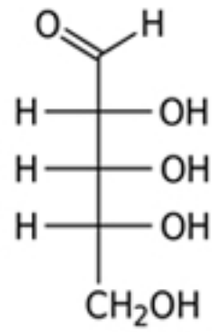
Carbohydrates



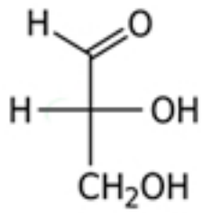
Fructose



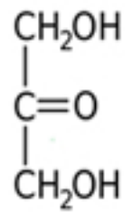
Glucose



Ribose



Glyceraldehyde

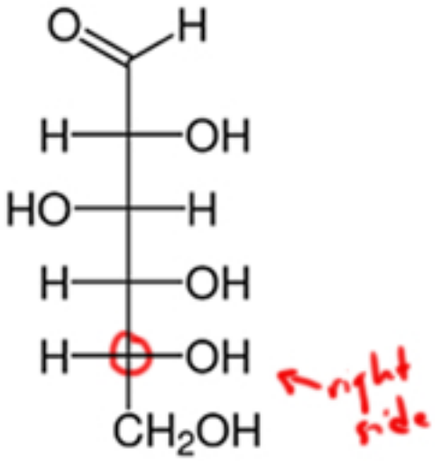


Dihydroxyacetone

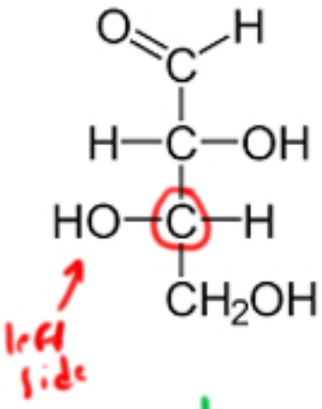
simple sugars

$(CH_2O)_n$

carbohydrates $C_m(H_2O)_n$


O
H
H
OH
HO
H
H
OH
H
OH
CH2OH
right side

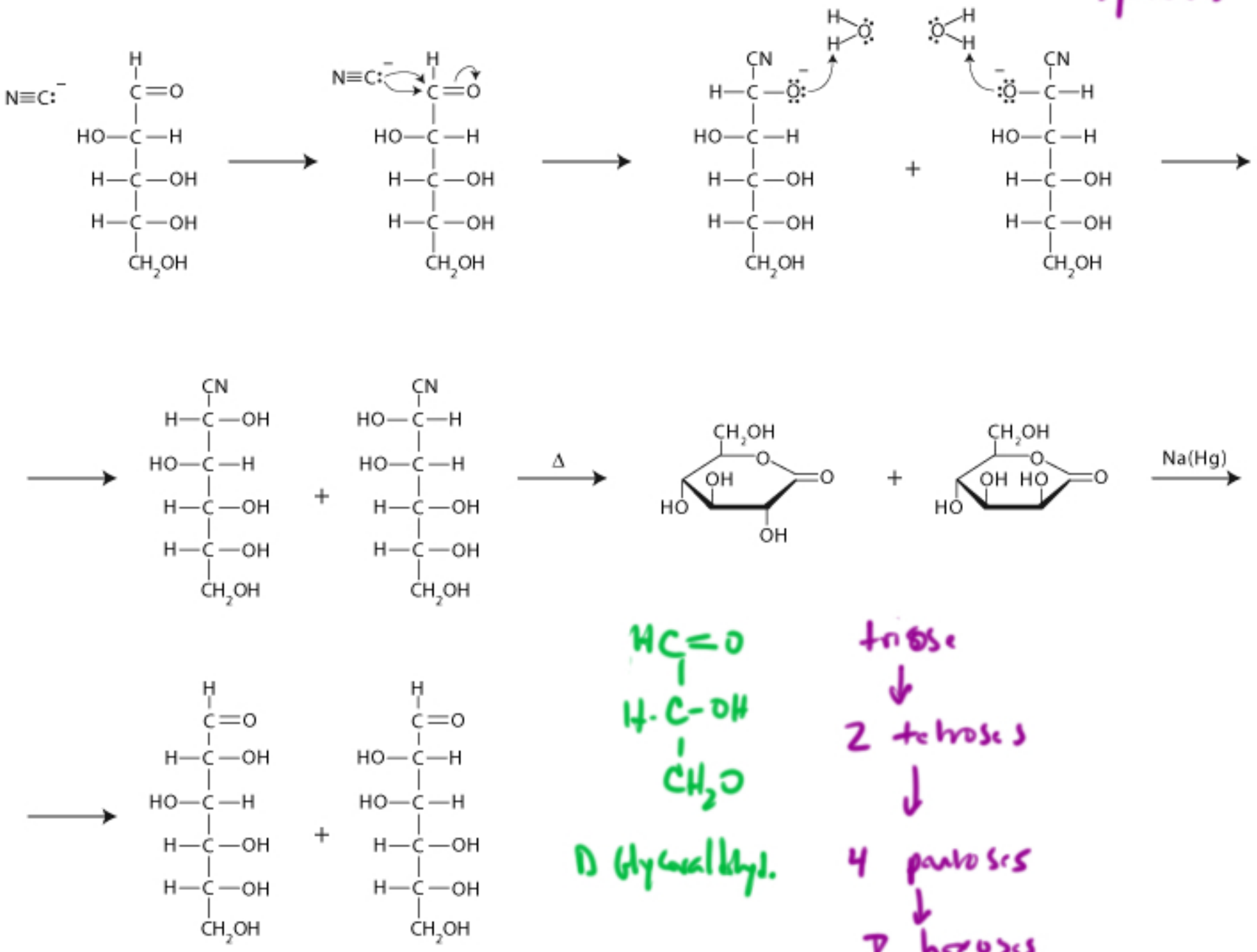
D


O
H
C
H
C
OH
HO
C
H
CH2OH
left side

L

(not d or l)

Kiliani Fischer synthesis



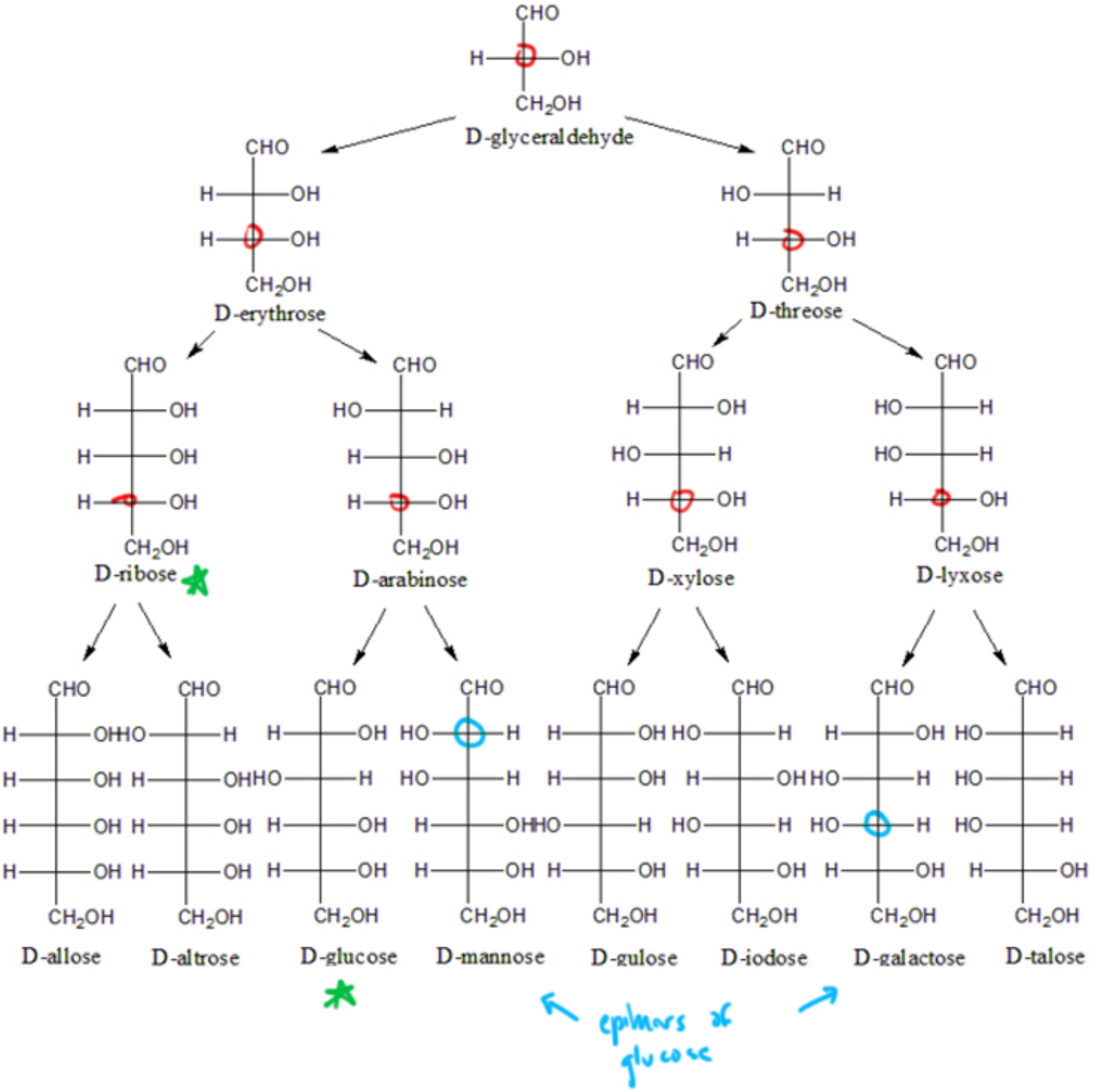
HC=O
H-C-OH
CH_2O

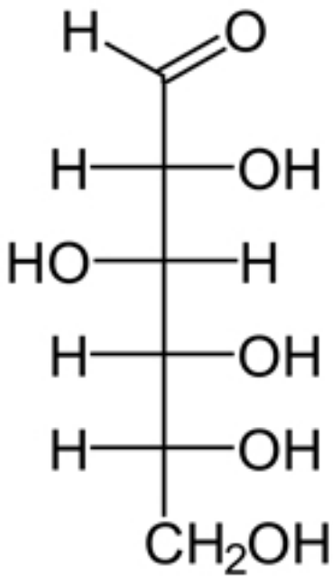
D Glyceraldehyd.

triose
↓
2 tetroses
↓
4 pentoses
↓
7 hexoses

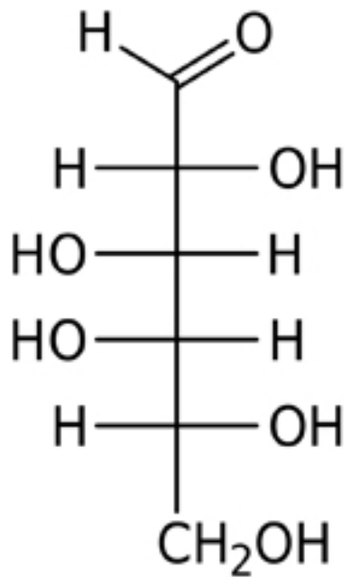
Steps of the Kiliani-Fischer synthesis of D-glucose and its C-2 epimer, D-mannose, from D-arabinose

Fischer Projection of D- Aldoses.

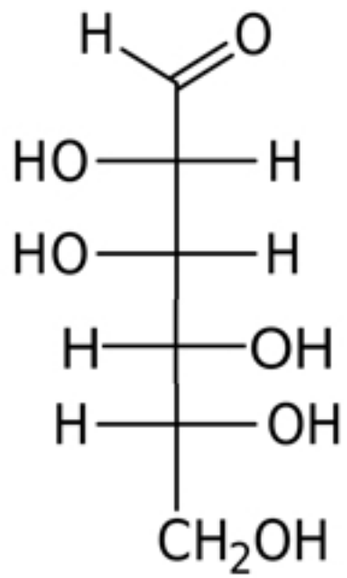




Glucose

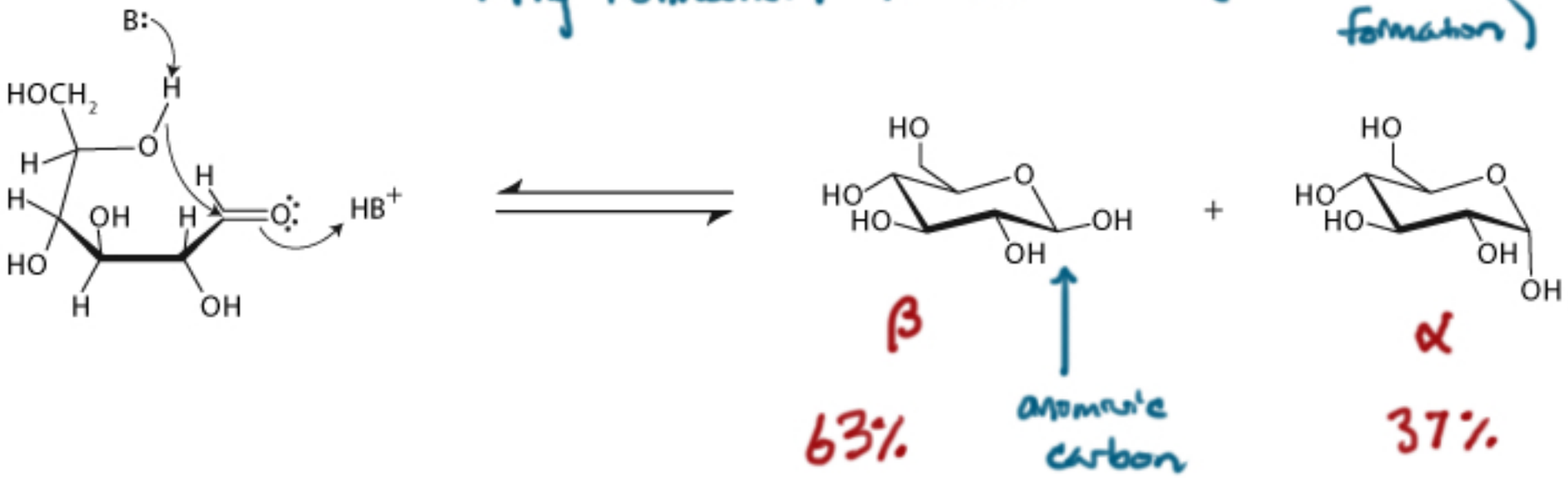


Mannose



Galactose

Ring Formation in Glucose (Hemiacetal formation)



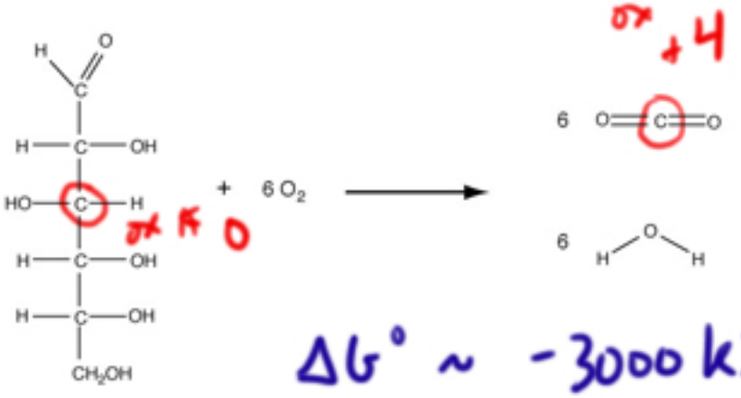
$$\alpha \rightleftharpoons \beta \qquad K = \frac{2}{1}$$

$$\Delta G^\circ = -2.3RT \log K$$

$$= (-2.3)(8)(300)(0.3)$$

$$\sim -1600\ J$$

$$\sim -1.6\ kJ$$

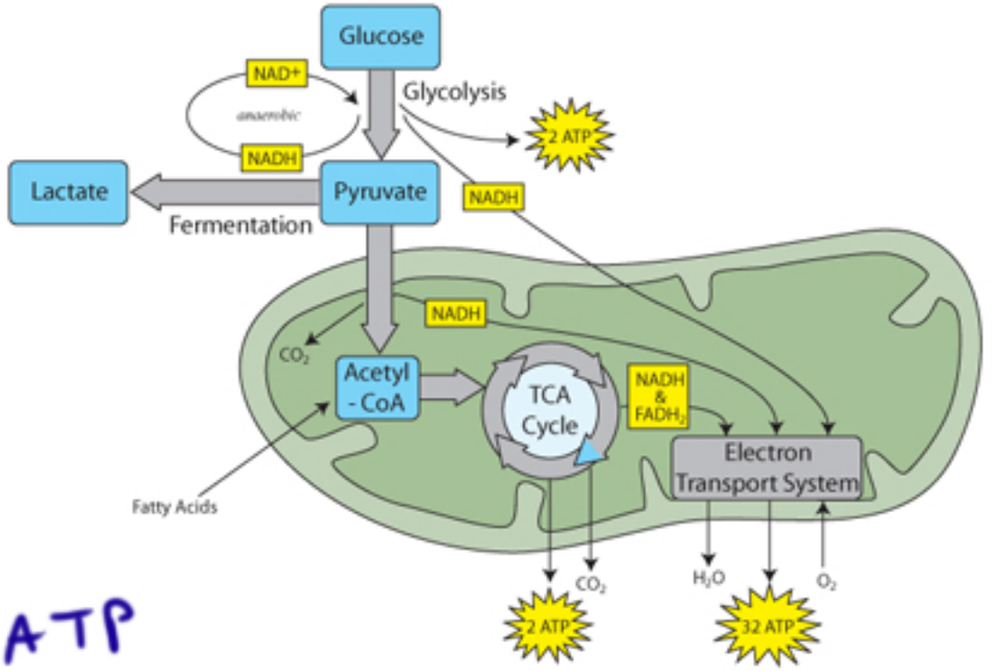


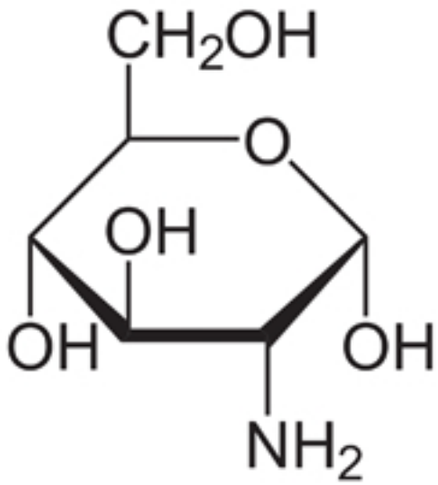
$\Delta G^\circ \sim -3000$ kJ

coupled with

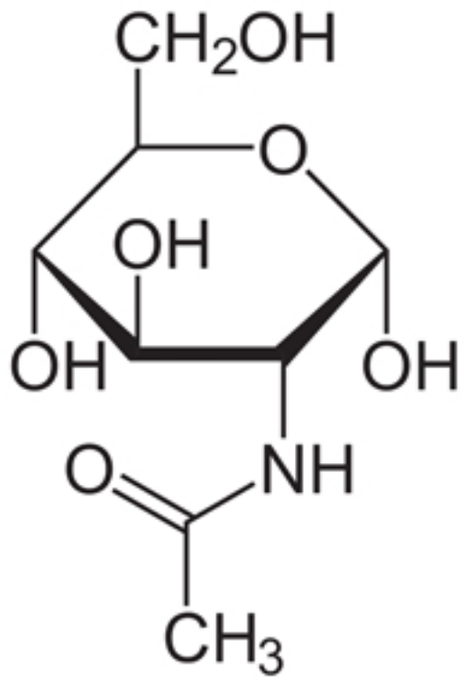
$36\ ADP + 36\ P_i \rightarrow 36\ ATP$

$+1100$ kJ

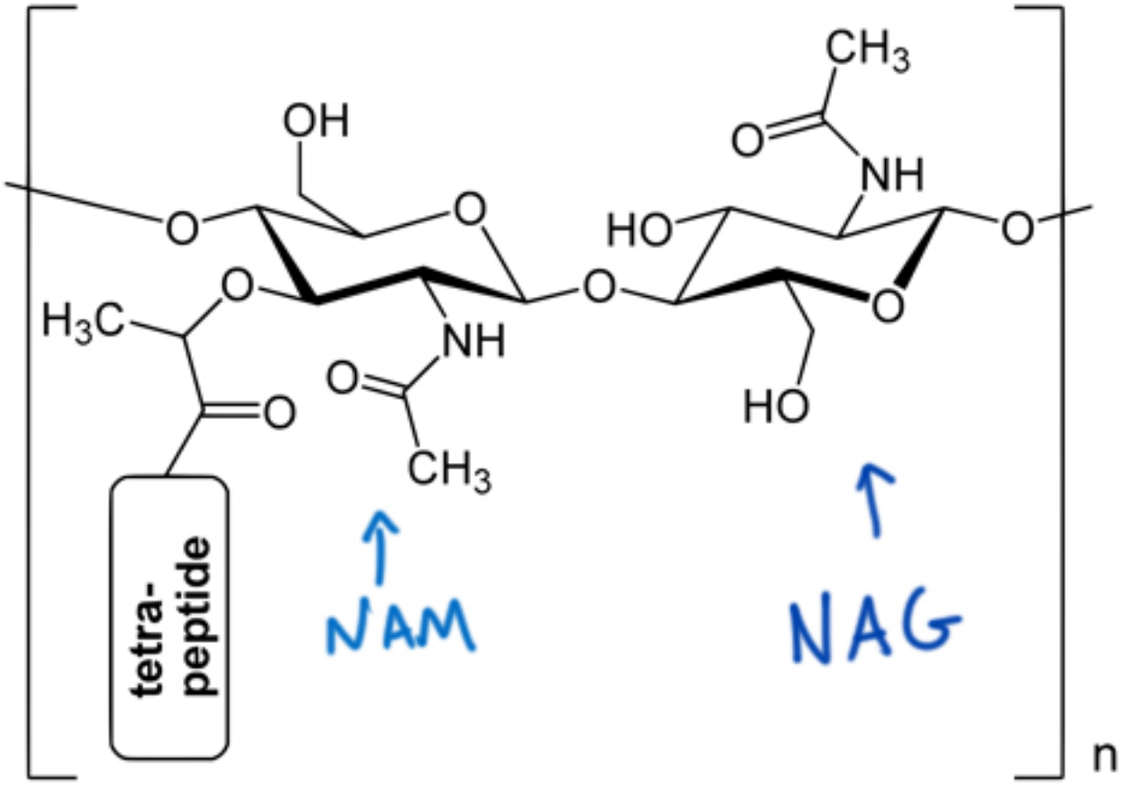




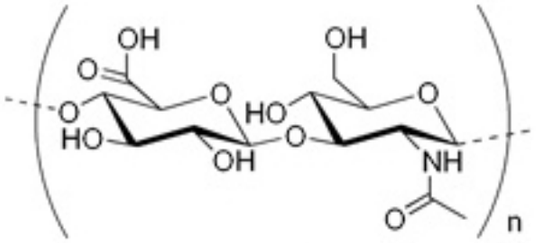
Glucosamine



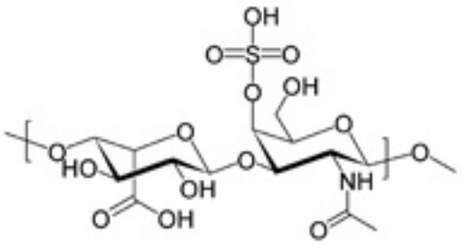
N-acetyl glucosamine



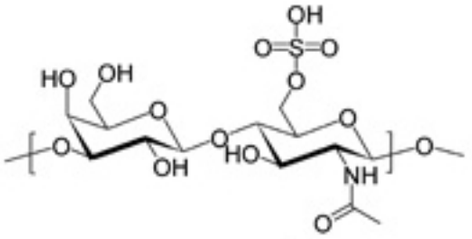
Peptidoglycan



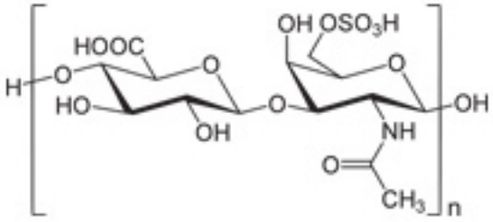
Hyaluronate



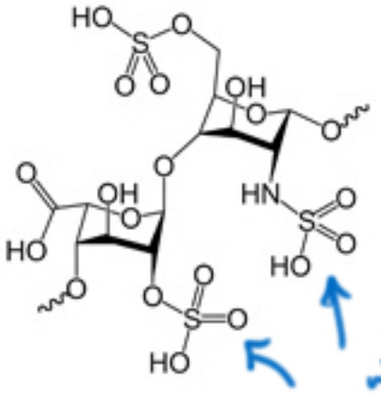
Dermatan sulfate



Keratan sulfate



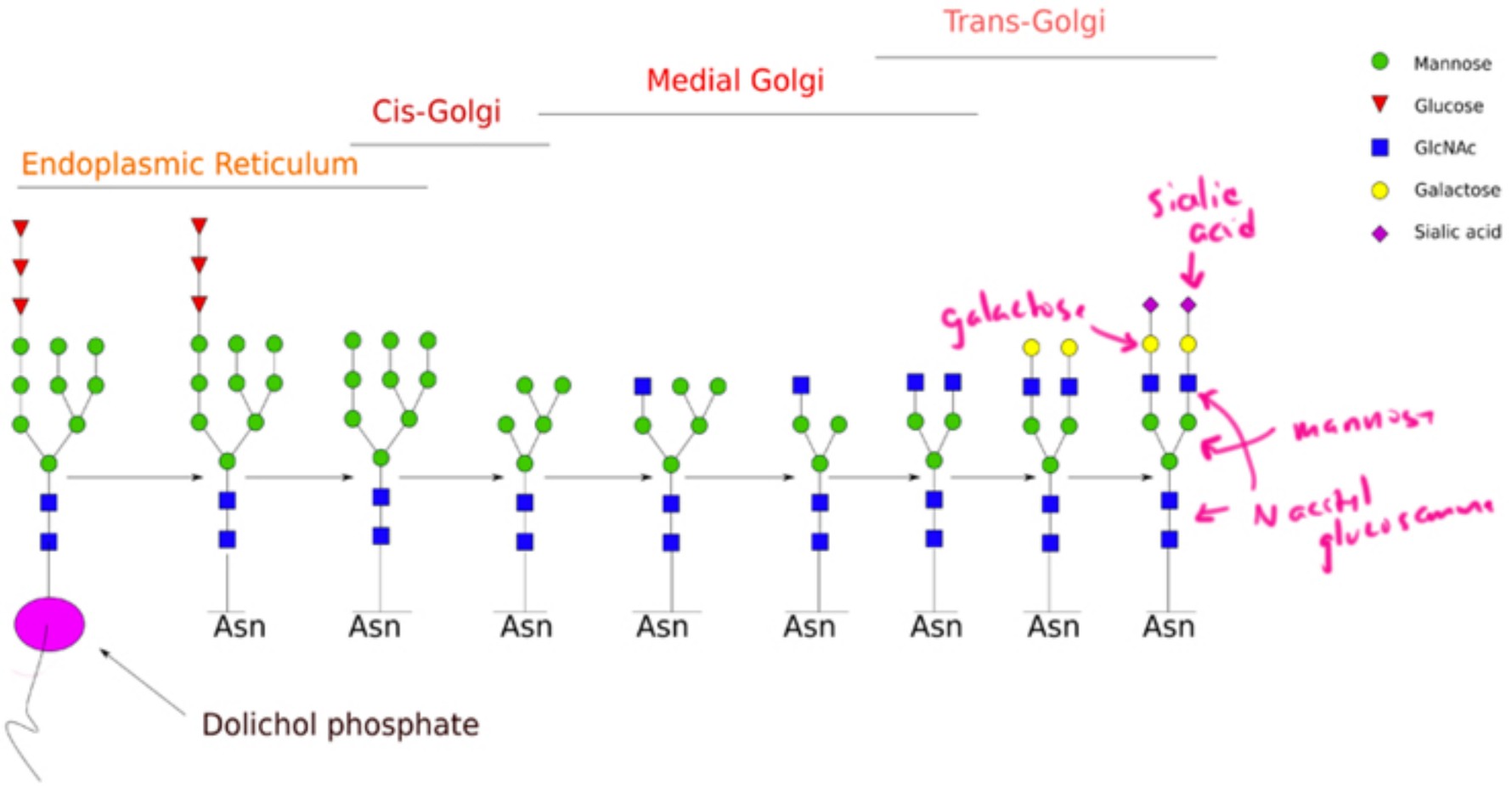
Chondroitin 6-sulfate



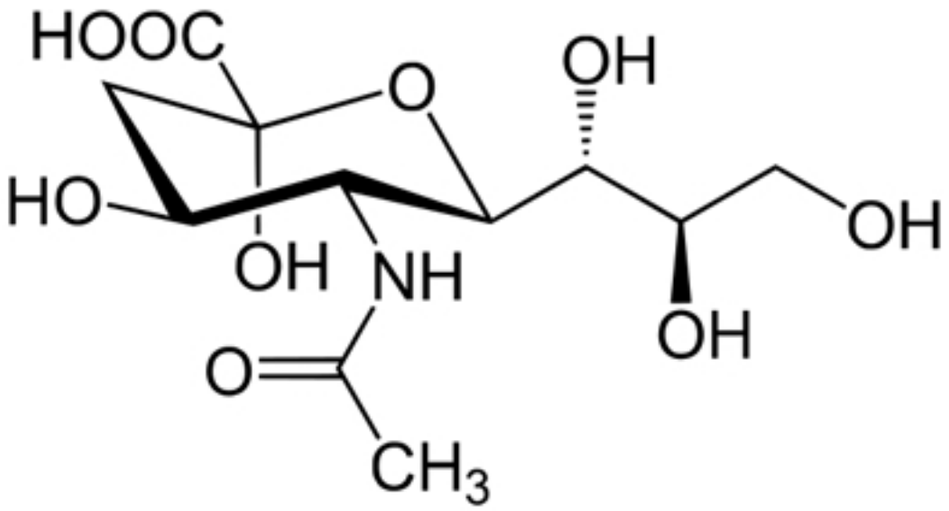
Heparin these will be ⊖

acidic derivatives of N-acetyl glucosamine in proteoglycan

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$



N-linked glycosylation
to form glycoproteins



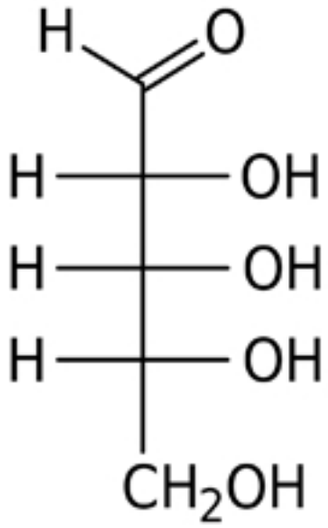
a sialic acid

glycoproteins

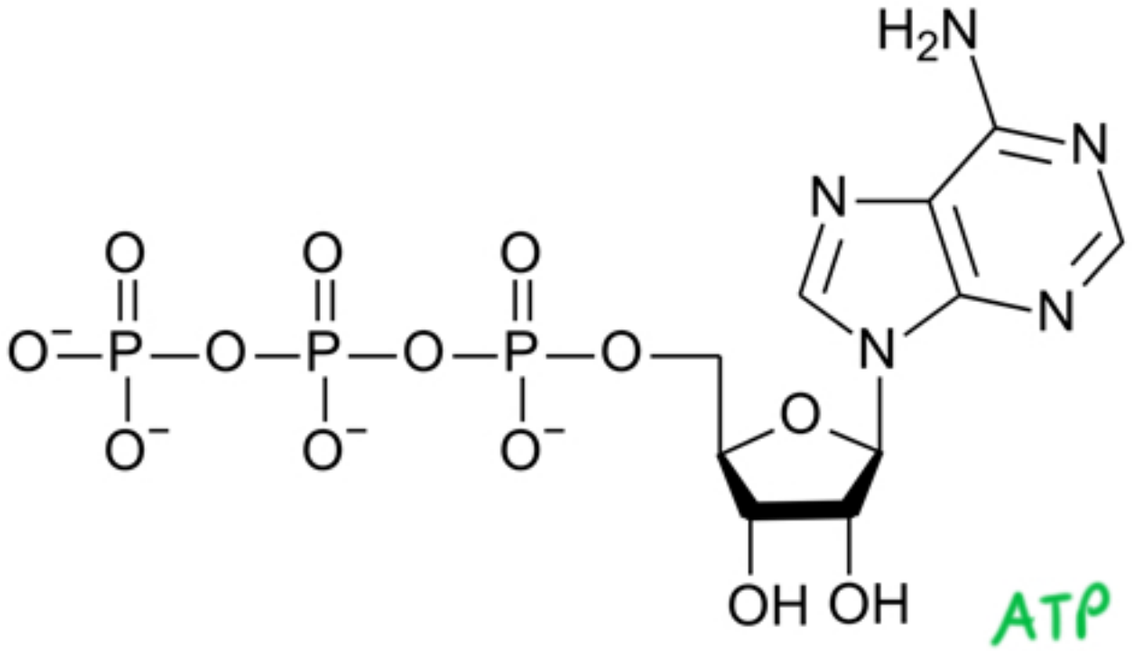
and gangliosides

↑

a kind of sphingolipid

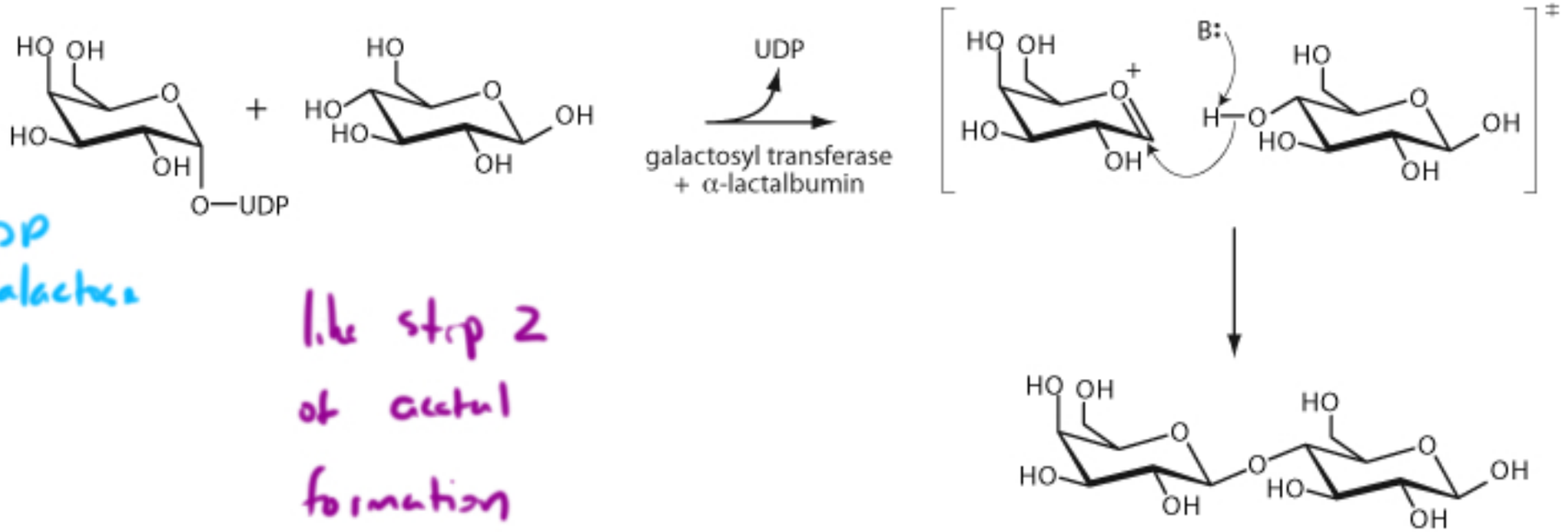


ribose



ATP

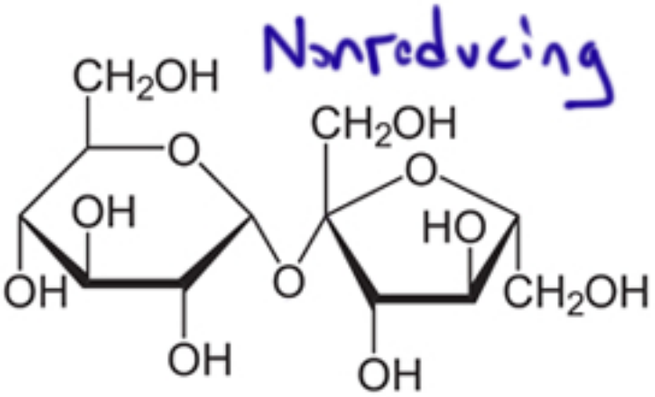
Glycosidic Bond Formation



UDP Galactose

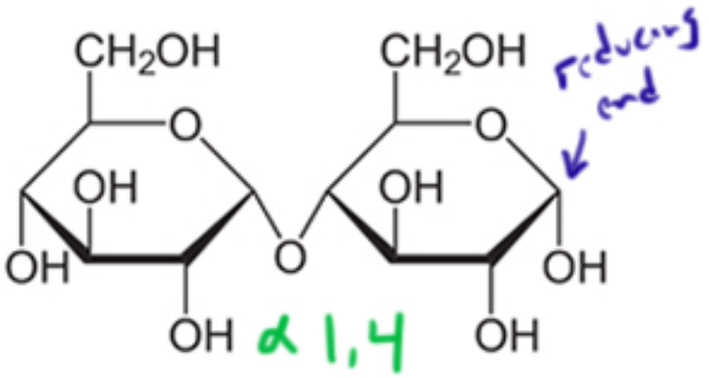
like step 2 of acetal formation

β 1,4 Lactose



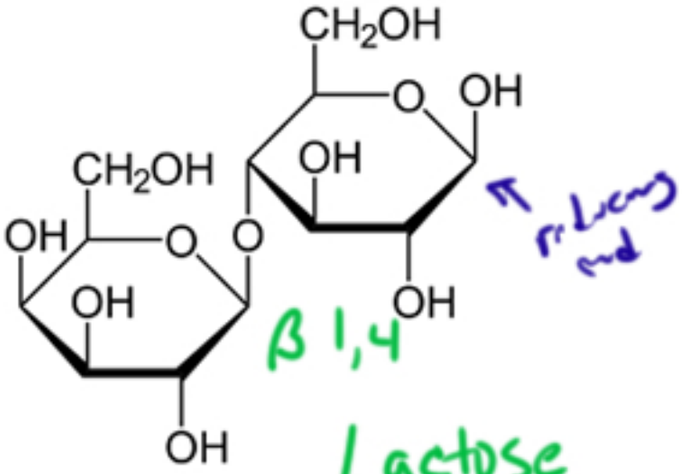
Sucrose

glucose + fructose



Maltose

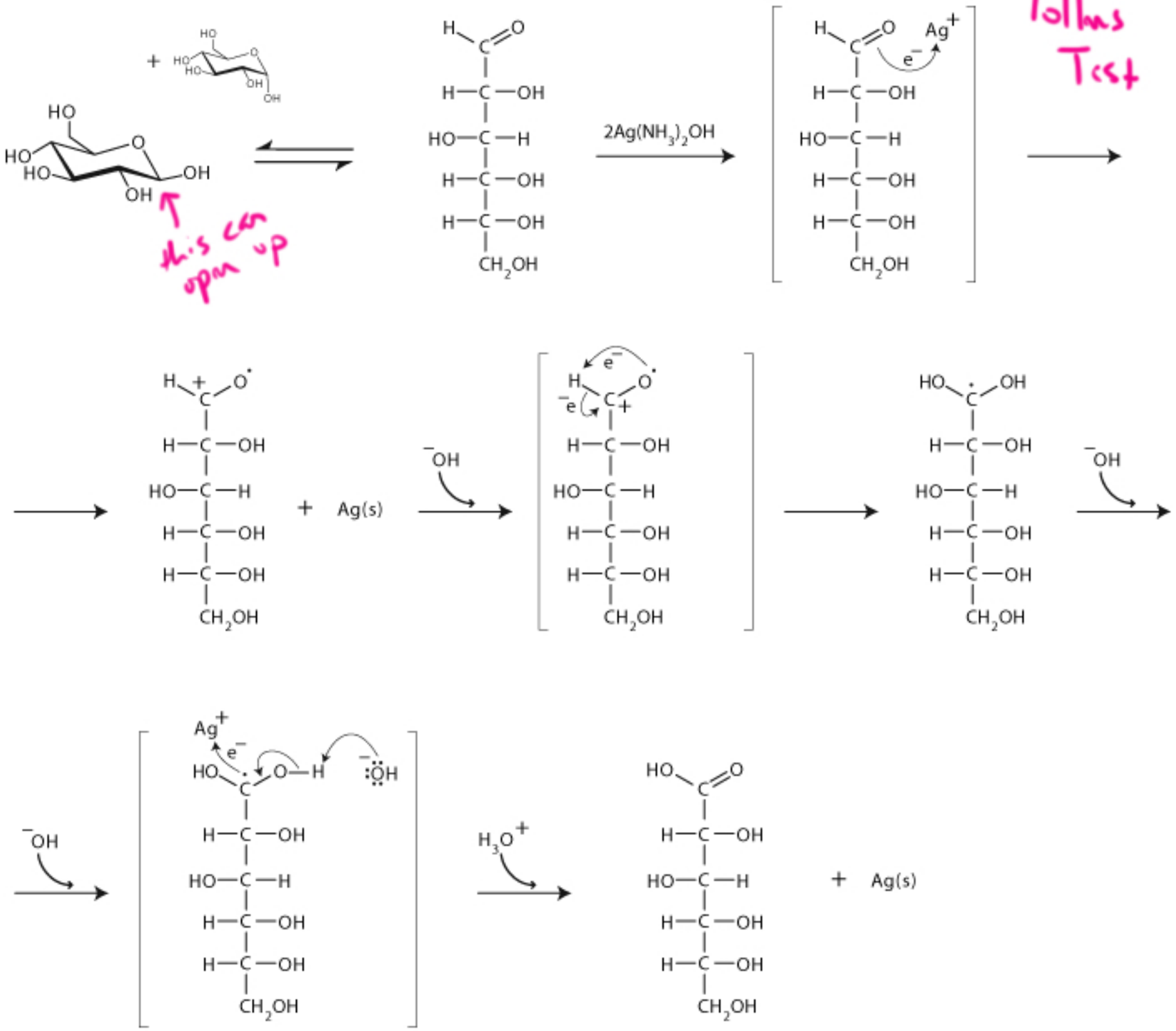
glucose + glucose

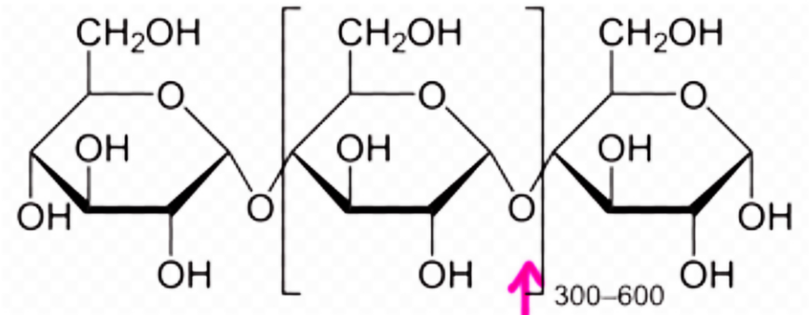


Lactose

glucose + galactose

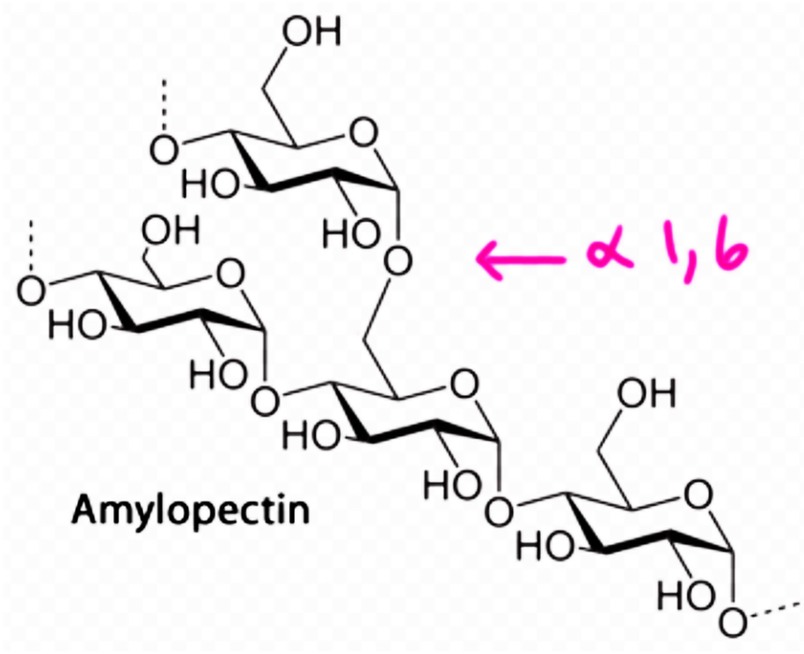
Tollens Test





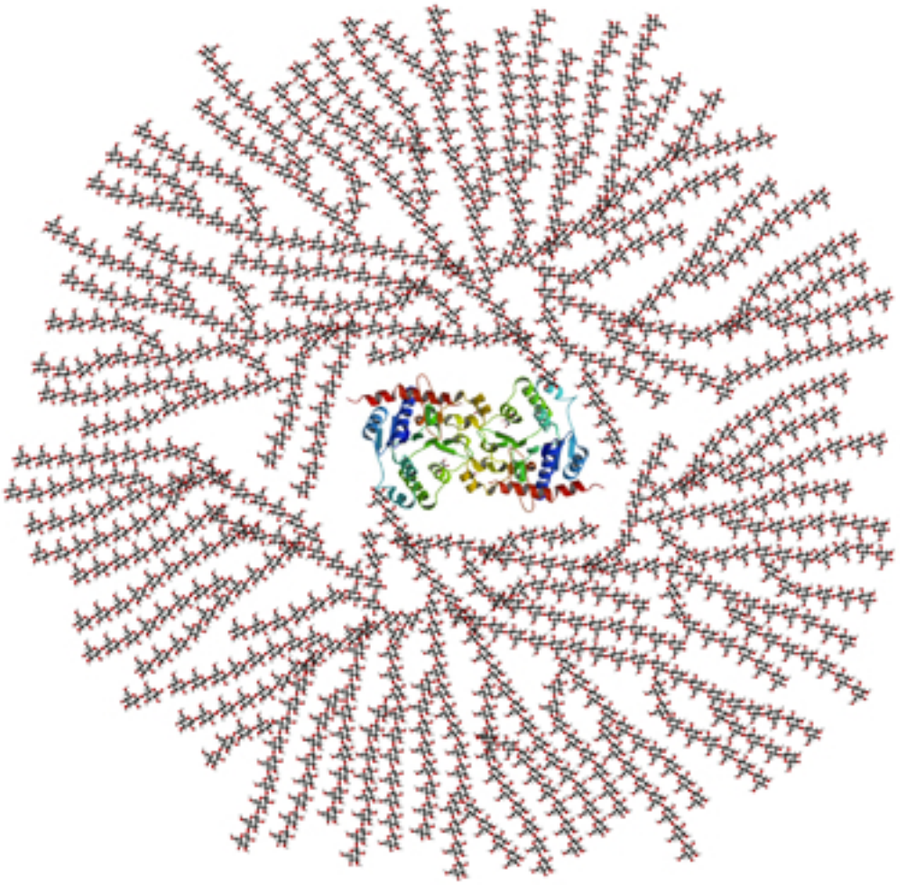
Amylose

α 1,4

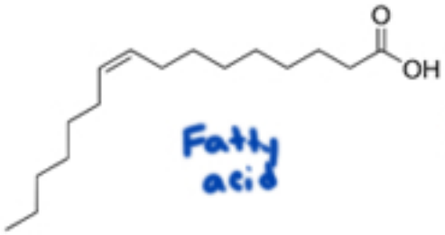


Amylopectin

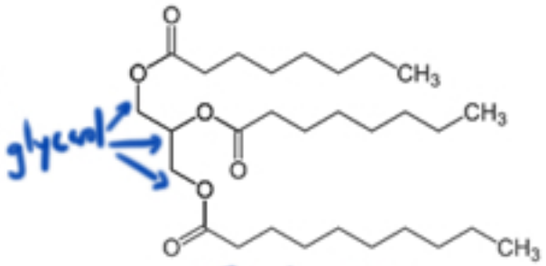
α 1,6


Glycogen
nonreducing ends

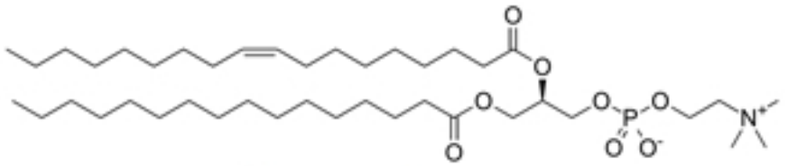
Lipids



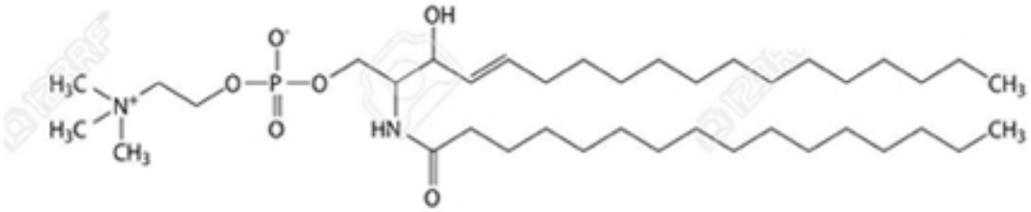
Fatty acid



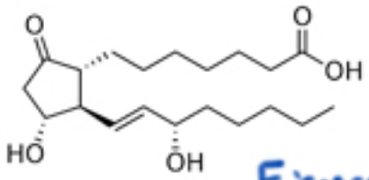
Triglyceride



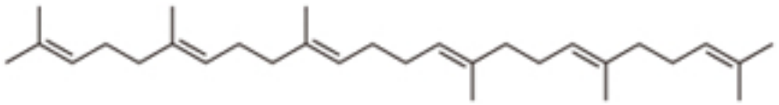
Phosphoglyceride



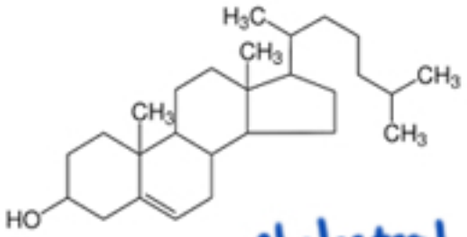
Sphingolipid



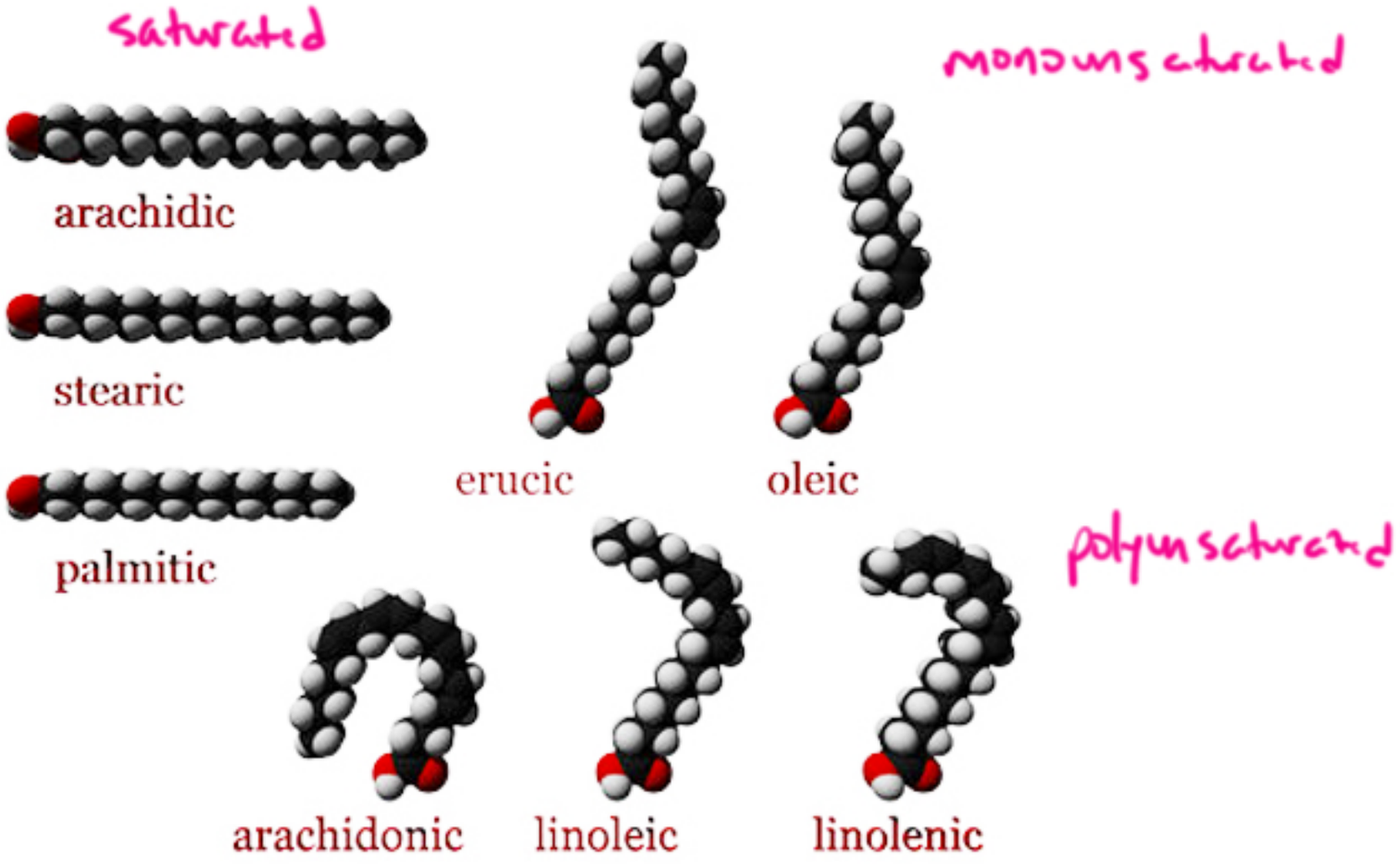
Eicosanoid signaling lipids

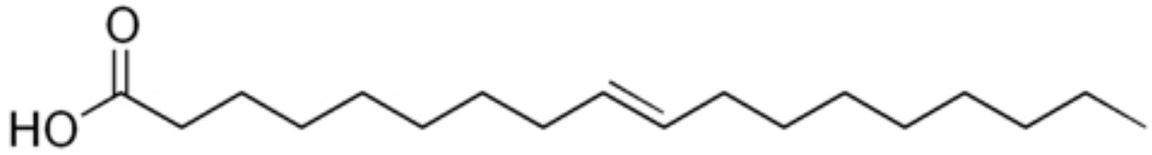


Isoprenoid Lipids

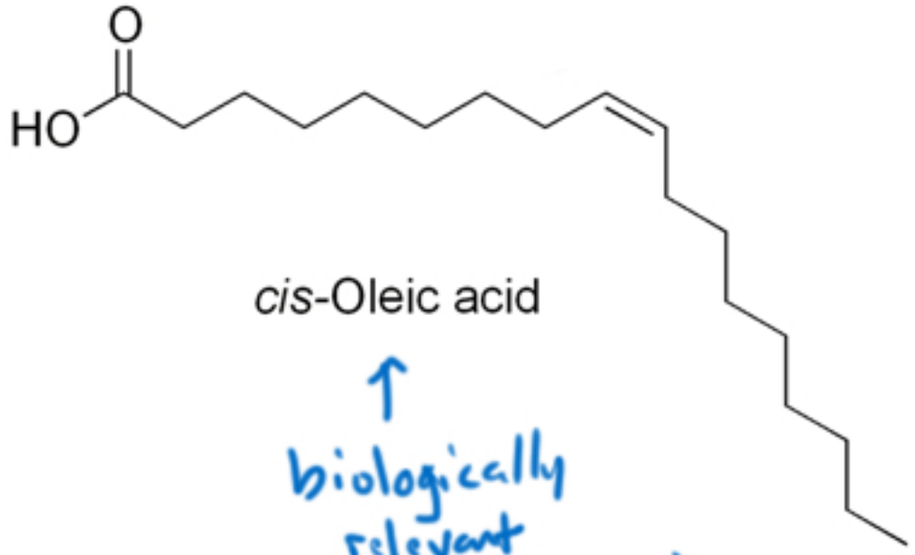


cholesterol


saturated
monounsaturated
polyunsaturated
arachidic
stearic
palmitic
erucic
oleic
arachidonic
linoleic
linolenic



trans-Oleic acid



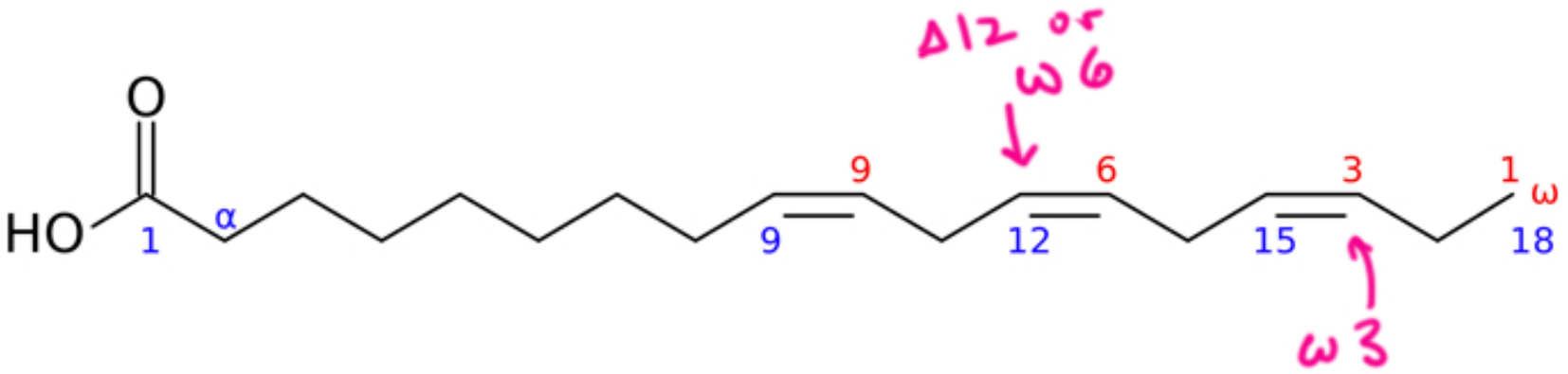
cis-Oleic acid

↑

biologically relevant (for the most part)

higher melting point

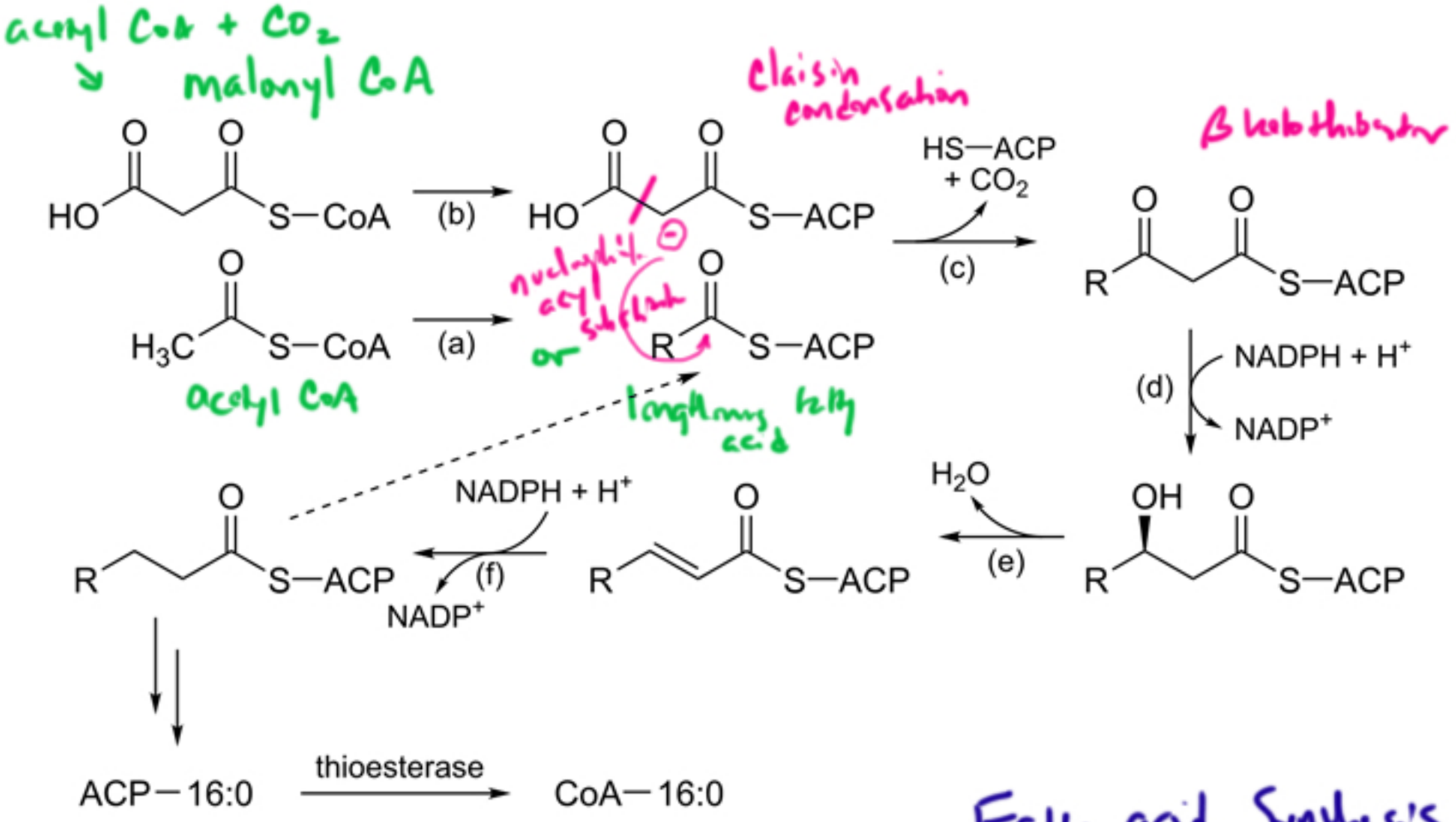
α-linolenic acid ← an essential fatty acid



Lipid Number 18:3 (n3)

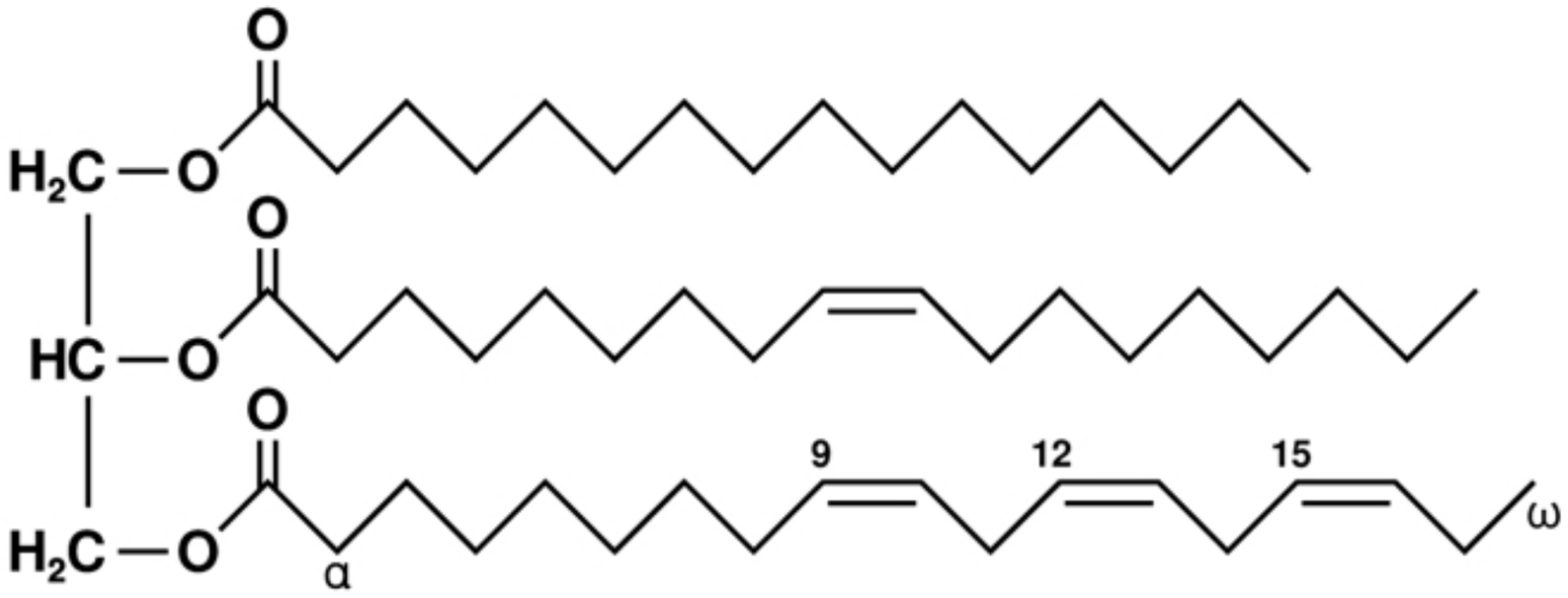
3 double bonds

starting at ω3



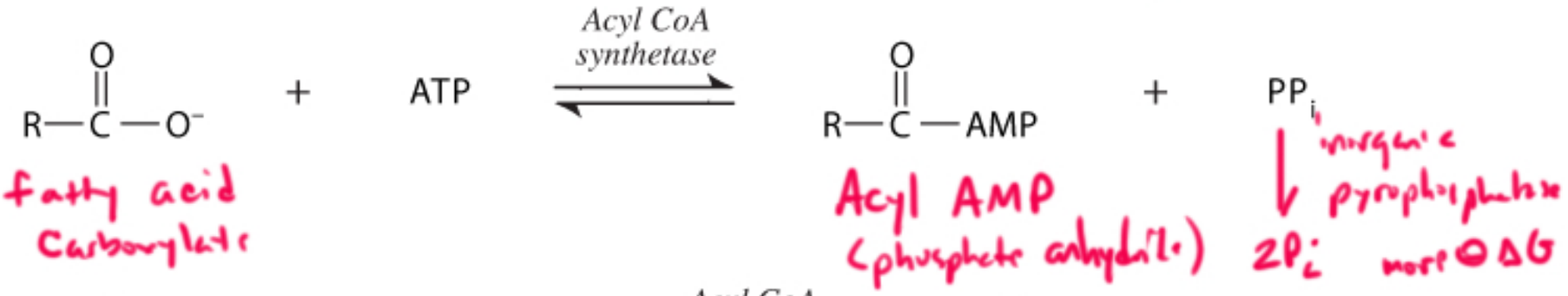
Fatty acid Synthesis
(supplemental)

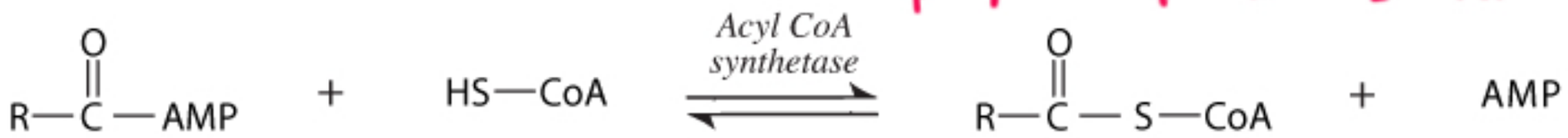
Triglycerides



Fatty Acid Activation

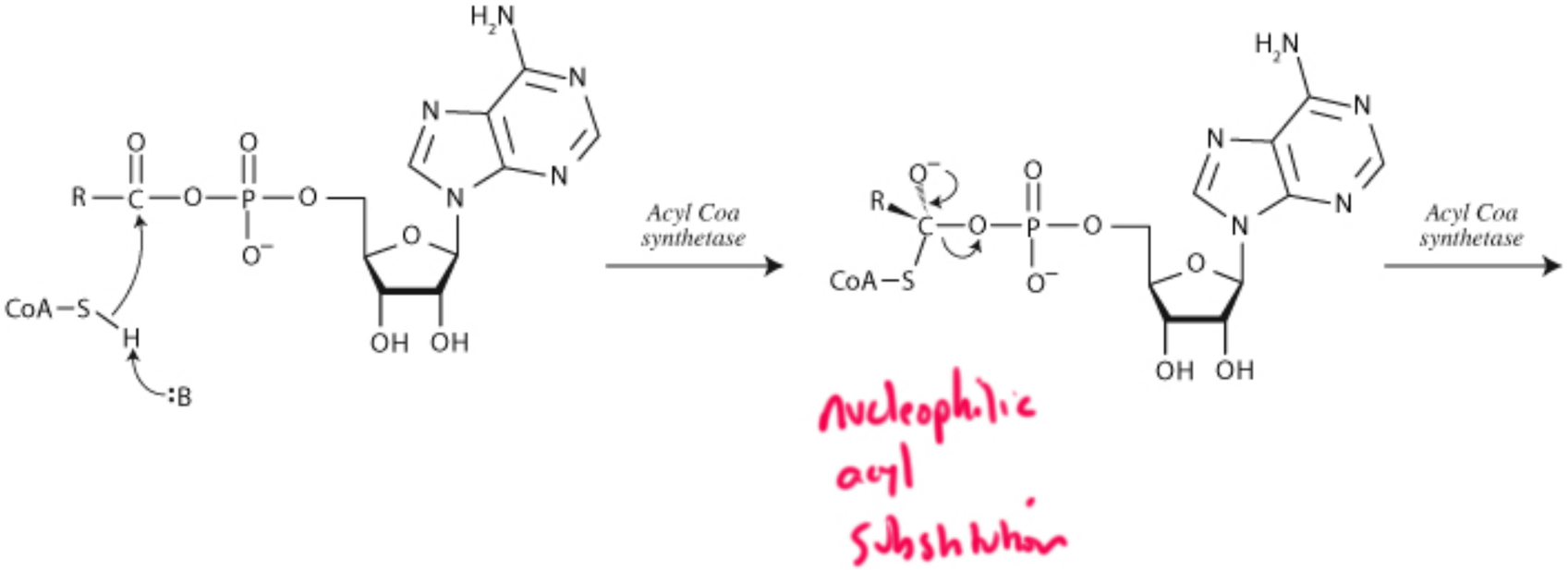
Triglyceride Synthesis

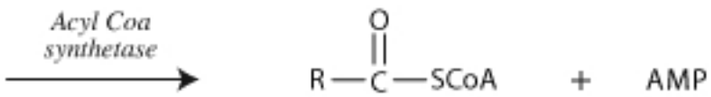


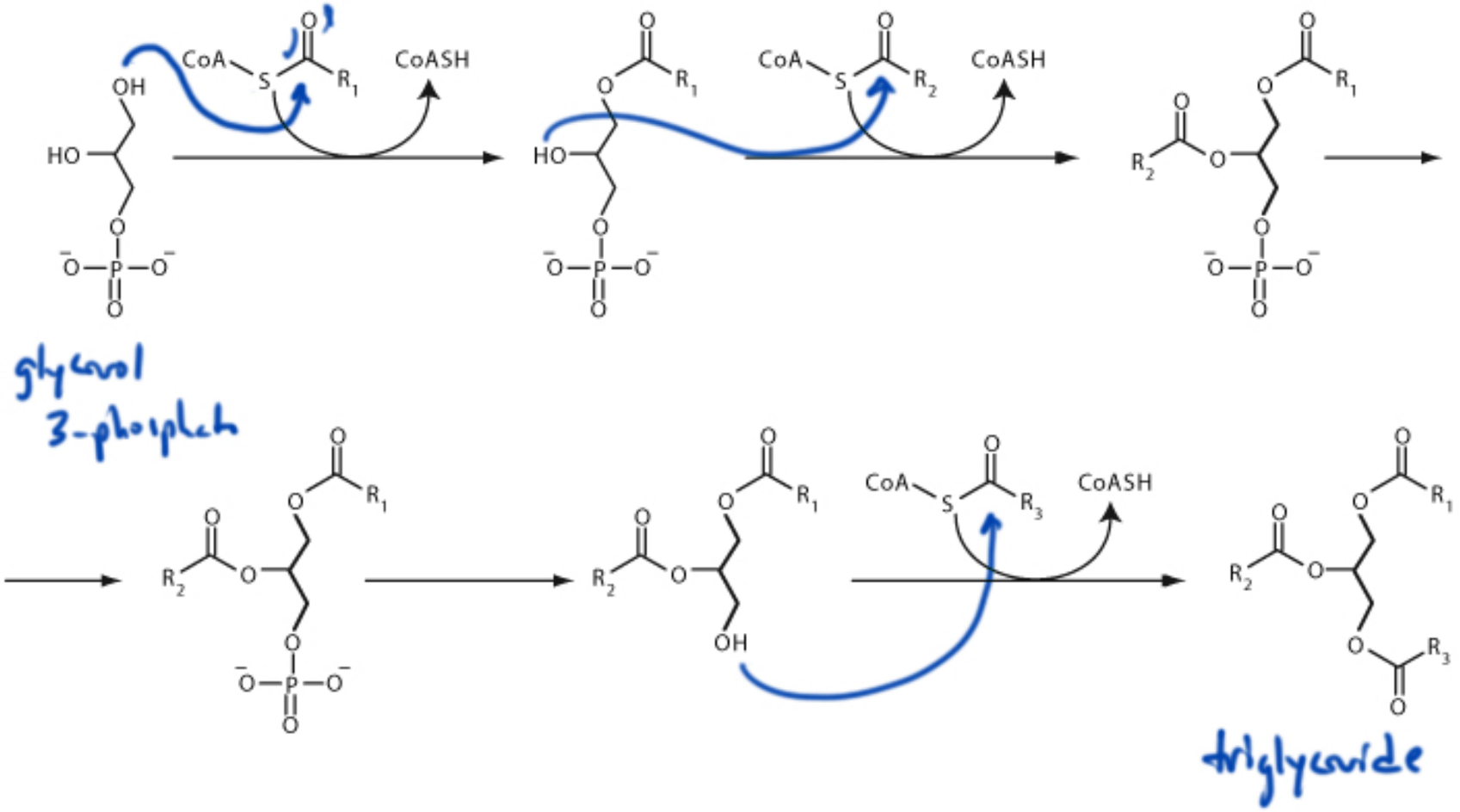


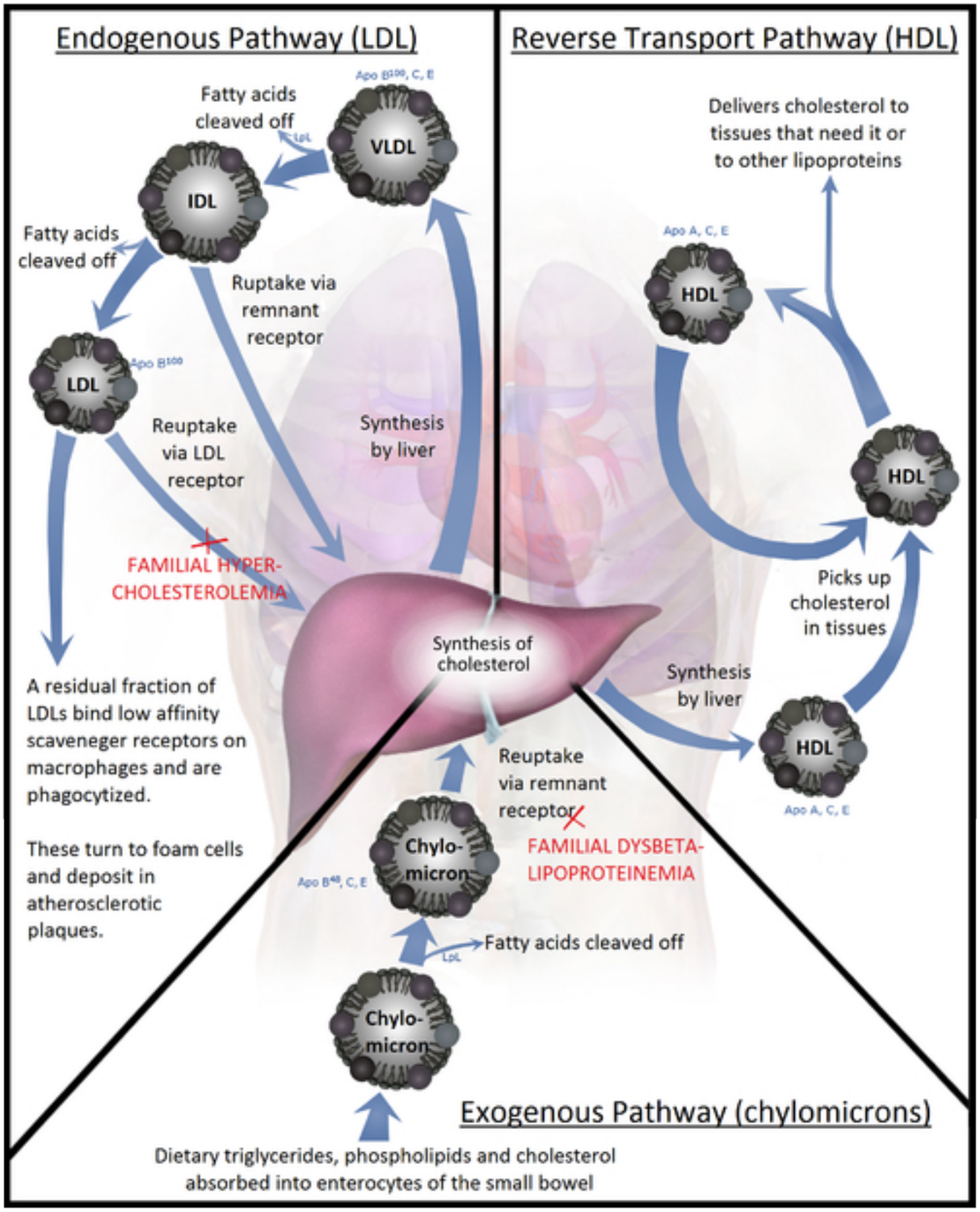
acyl CoA

(thioester)

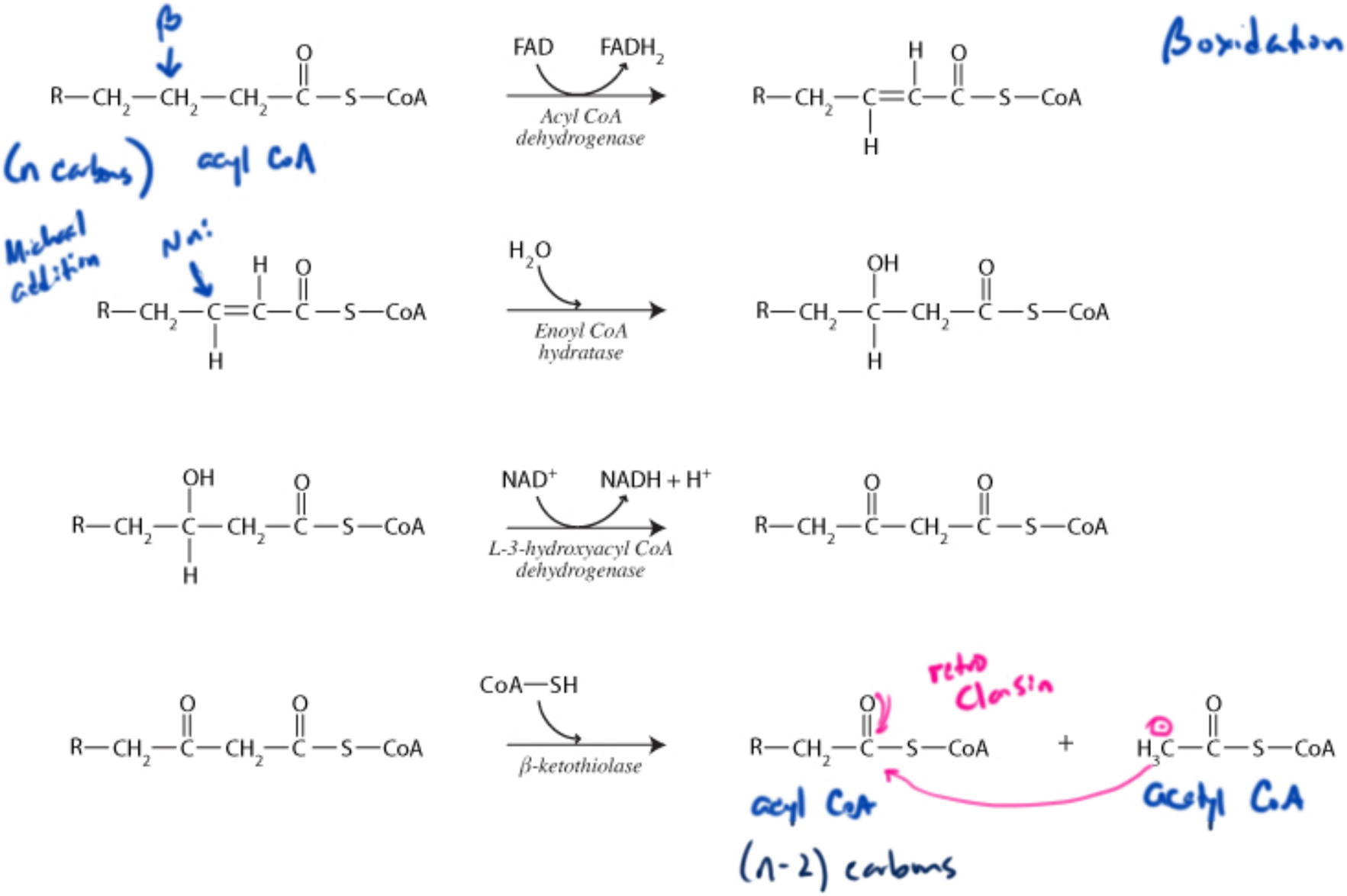

Acyl Coa synthetase
Acyl Coa synthetase
Nucleophilic acyl substitution


Acyl Coa synthetase
R—C—SCoA
+
AMP

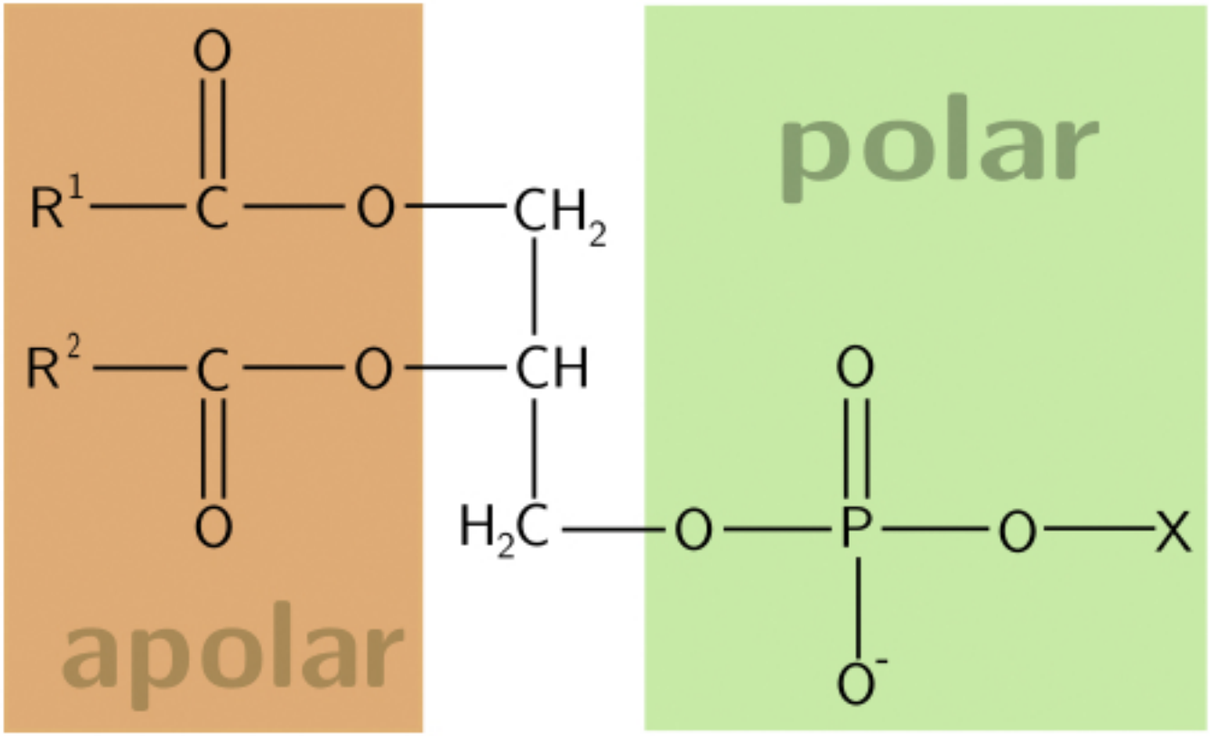

CoA
S
O
R1
CoASH
OH
HO
O
O
P
O
O
R1
R2
R3
glycerol
3-phosphate
triglyceride

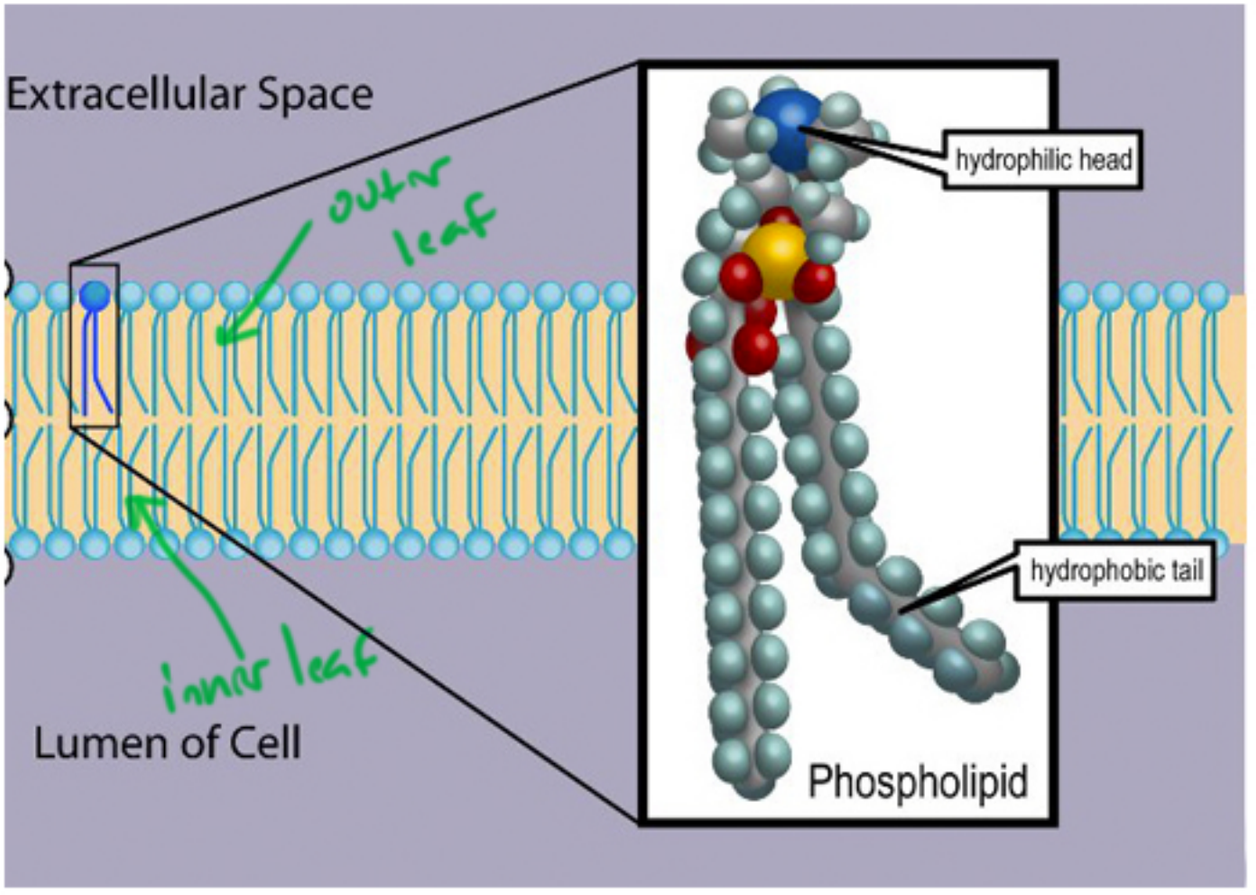

Endogenous Pathway (LDL)
Reverse Transport Pathway (HDL)
Fatty acids cleaved off
VLDL
IDL
Fatty acids cleaved off
Ruptake via remnant receptor
LDL
Reuptake via LDL receptor
Synthesis by liver
FAMILIAL HYPER-CHOLESTEROLEMIA
A residual fraction of LDLs bind low affinity scaveneger receptors on macrophages and are phagocytized.
These turn to foam cells and deposit in atherosclerotic plaques.
Delivers cholesterol to tissues that need it or to other lipoproteins
HDL
HDL
Picks up cholesterol in tissues
Synthesis by liver
HDL
Synthesis of cholesterol
Reuptake via remnant receptor
FAMILIAL DYSBETA-LIPOPROTEINEMIA
Chylo-micron
Fatty acids cleaved off
Chylo-micron
Exogenous Pathway (chylomicrons)
Dietary triglycerides, phospholipids and cholesterol absorbed into enterocytes of the small bowel

β-oxidation

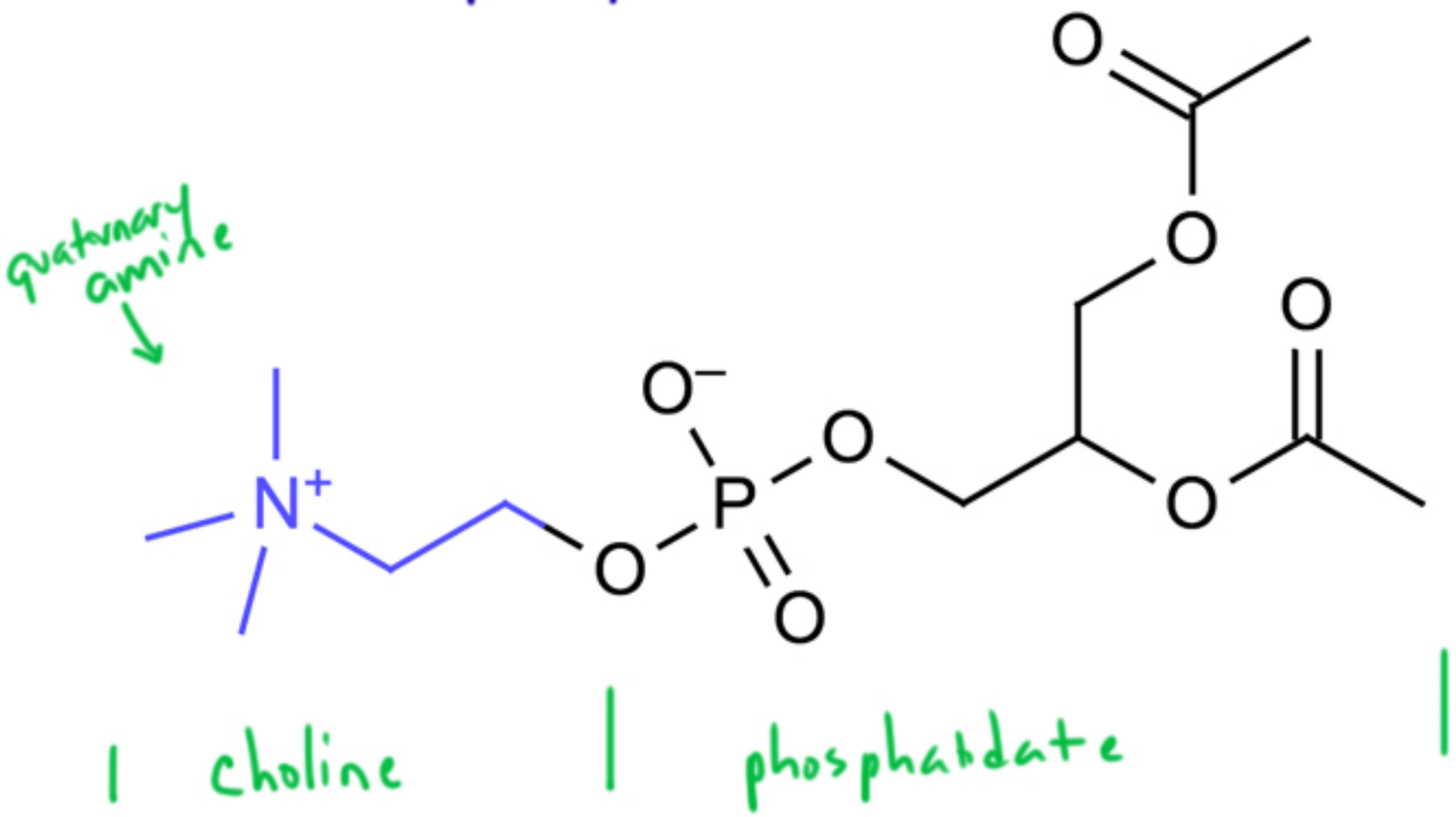

β
FAD
FADH2
Acyl CoA dehydrogenase
(n carbons) acyl CoA
Michael addition
Nu:
H2O
Enoyl CoA hydratase
NAD+
NADH + H+
L-3-hydroxyacyl CoA dehydrogenase
CoA—SH
β-ketothiolase
retro Claisen
acyl CoA
(n-2) carbons
Acetyl CoA

Glycerophospholipids




Extracellular Space
outer leaf
inner leaf
Lumen of Cell
hydrophilic head
hydrophobic tail
Phospholipid

Phosphatidyl Choline



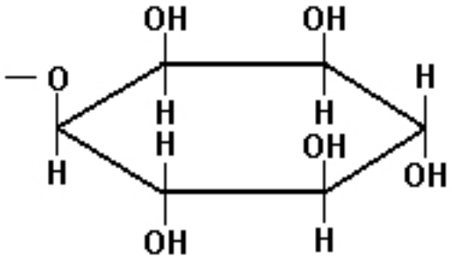
Glycerophospholipids

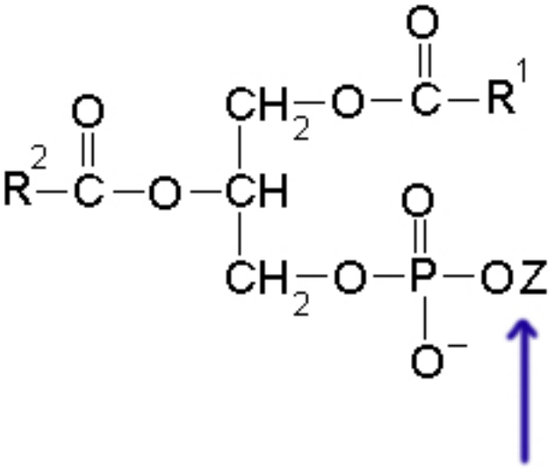
Z = $-H$ Phosphatidic acid

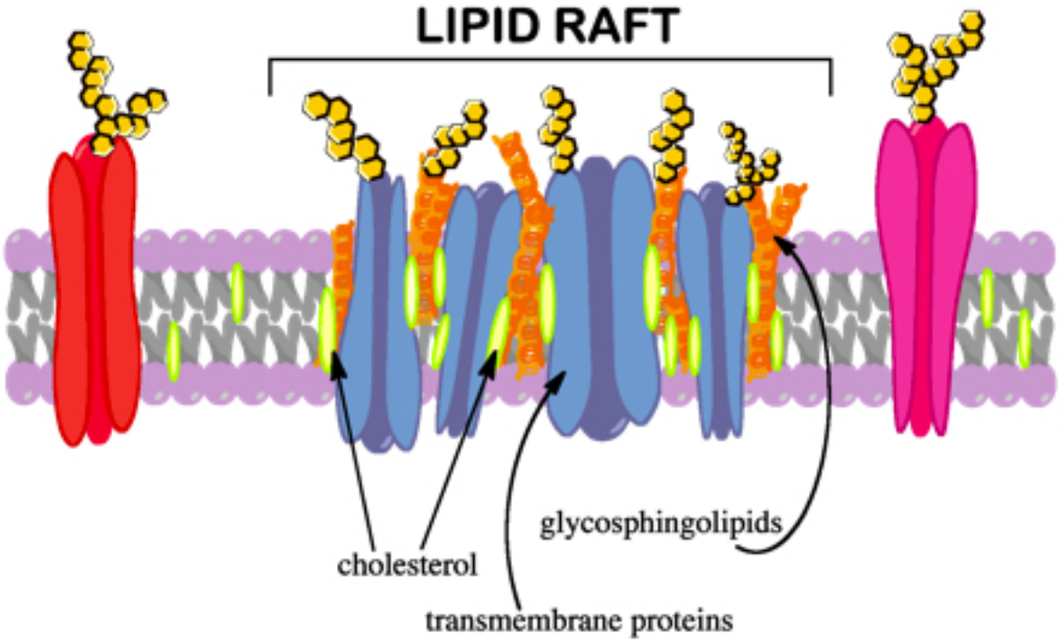
Z = $-CH_2-CH(NH_2)-COOH$ Phosphatidylserine

Z = $-CH_2-CH_2-\overset{+}{N}H_3$ Phosphatidylethanolamine

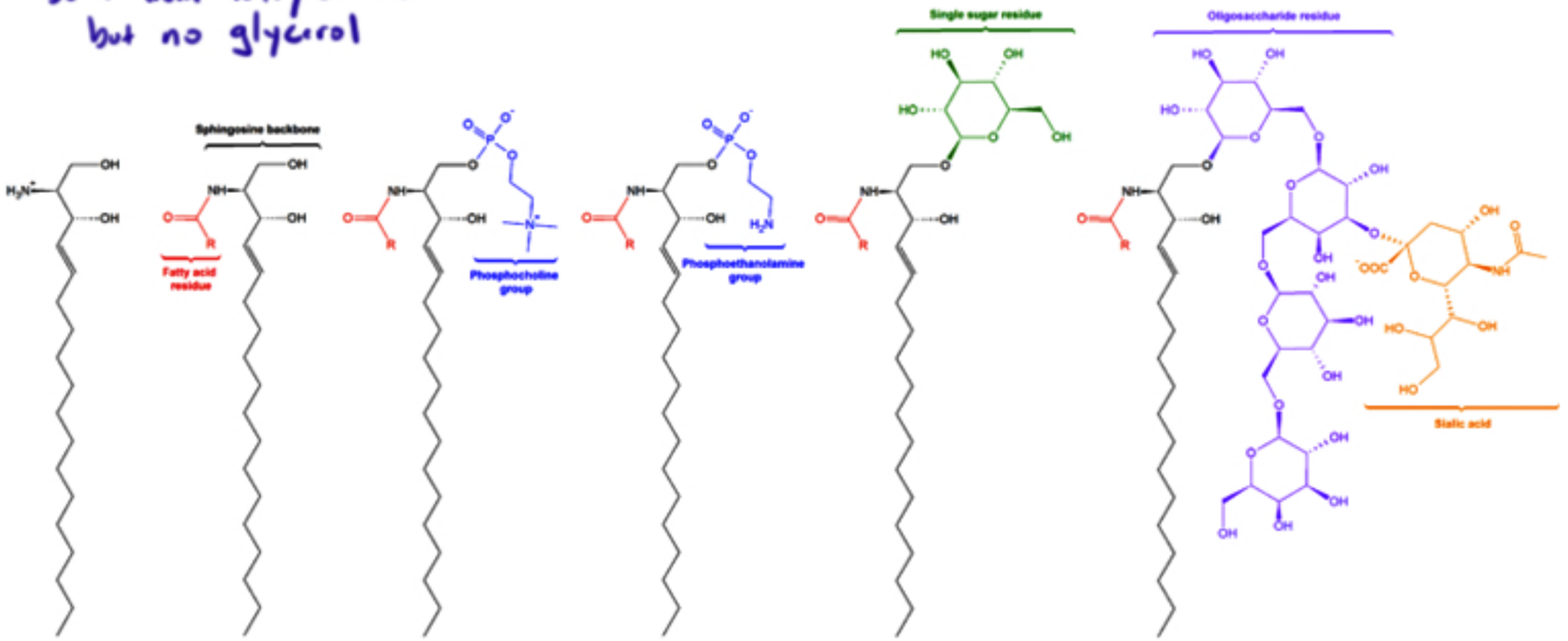
Z = $-CH_2-CH_2-\overset{+}{N}(CH_3)_3$ Phosphatidylcholine

Z =  Phosphatidylinositol




LIPID RAFT
glycosphingolipids
cholesterol
transmembrane proteins

built from fatty acids
but no glycerol

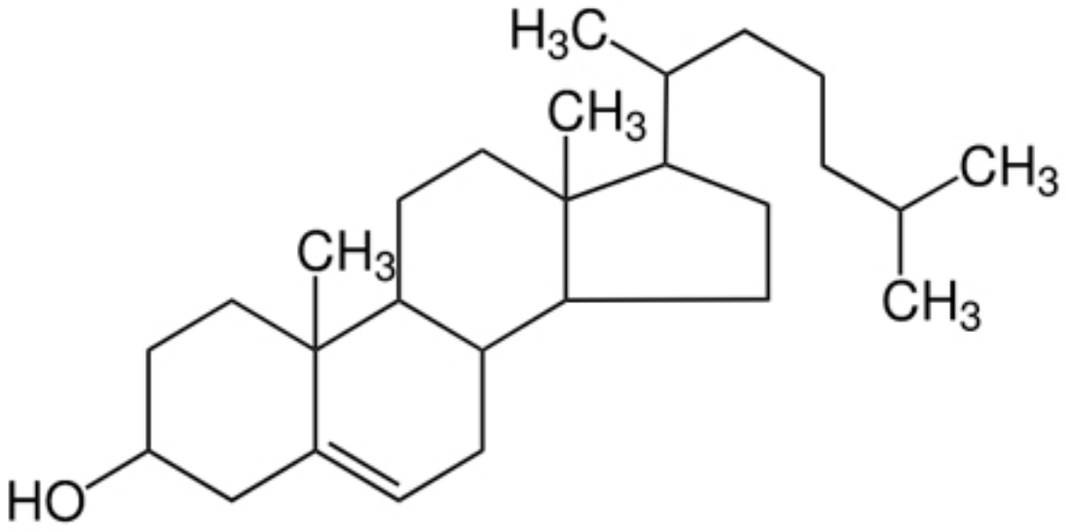


Sphingosine | A Ceramide | A Sphingomyelin | A Sphingomyelin | A Cerebroside | A Ganglioside

common membrane lipids

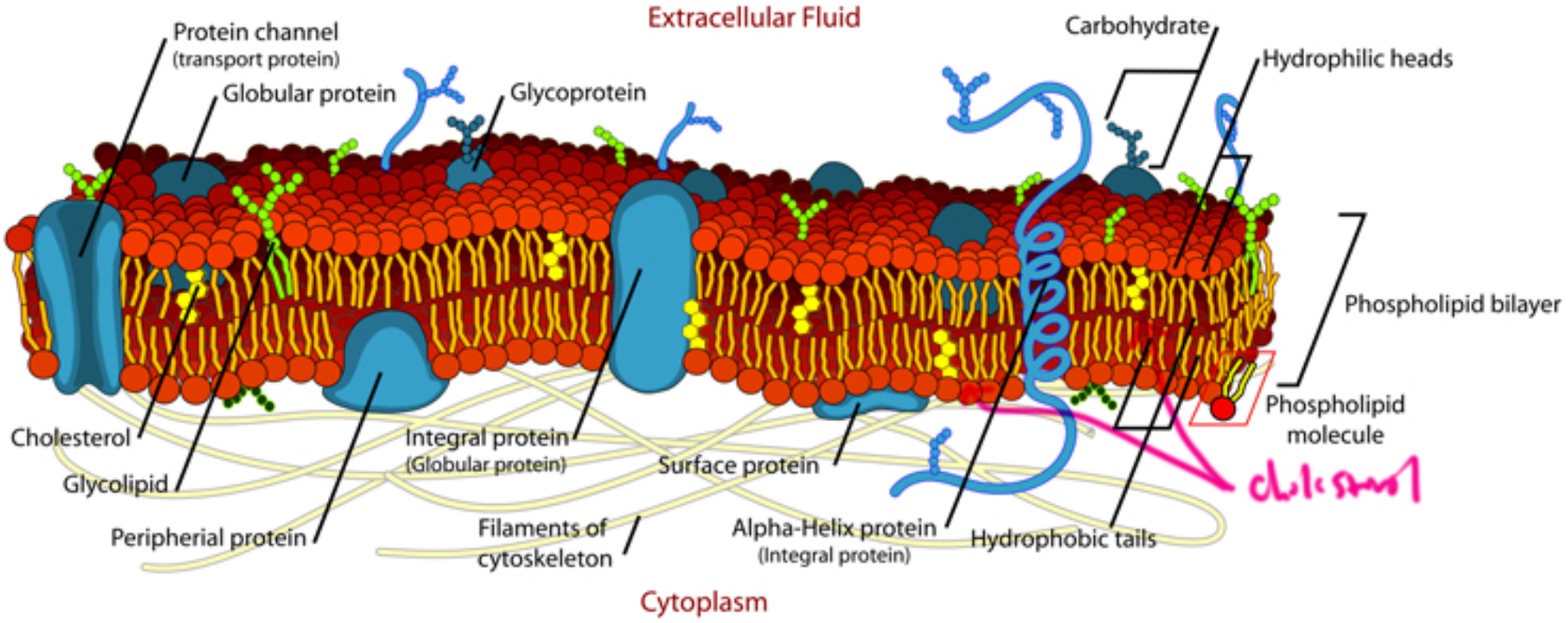
palmityl CoA
+ serine → CO_2 + NADPH + FAD

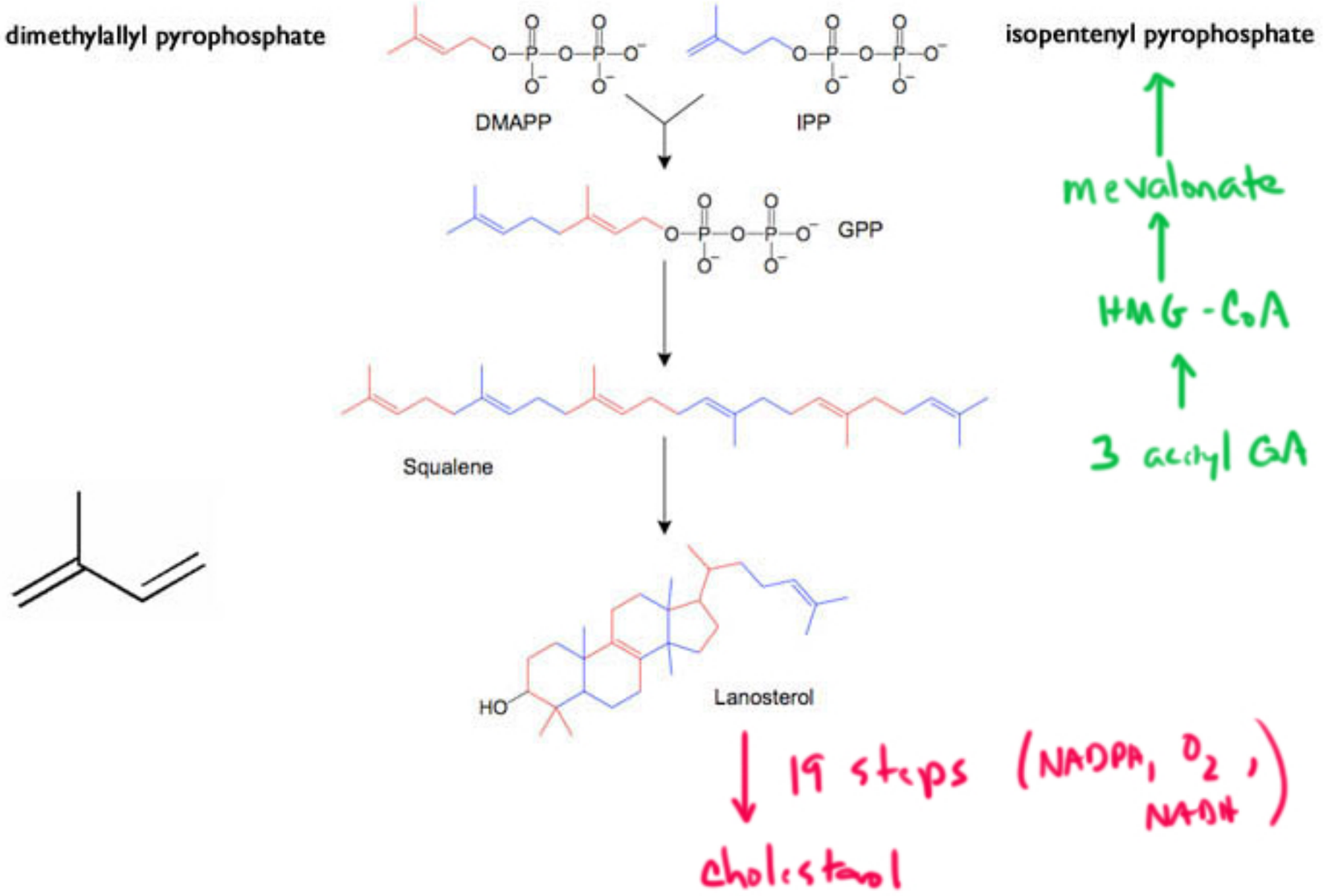
Cholestarol



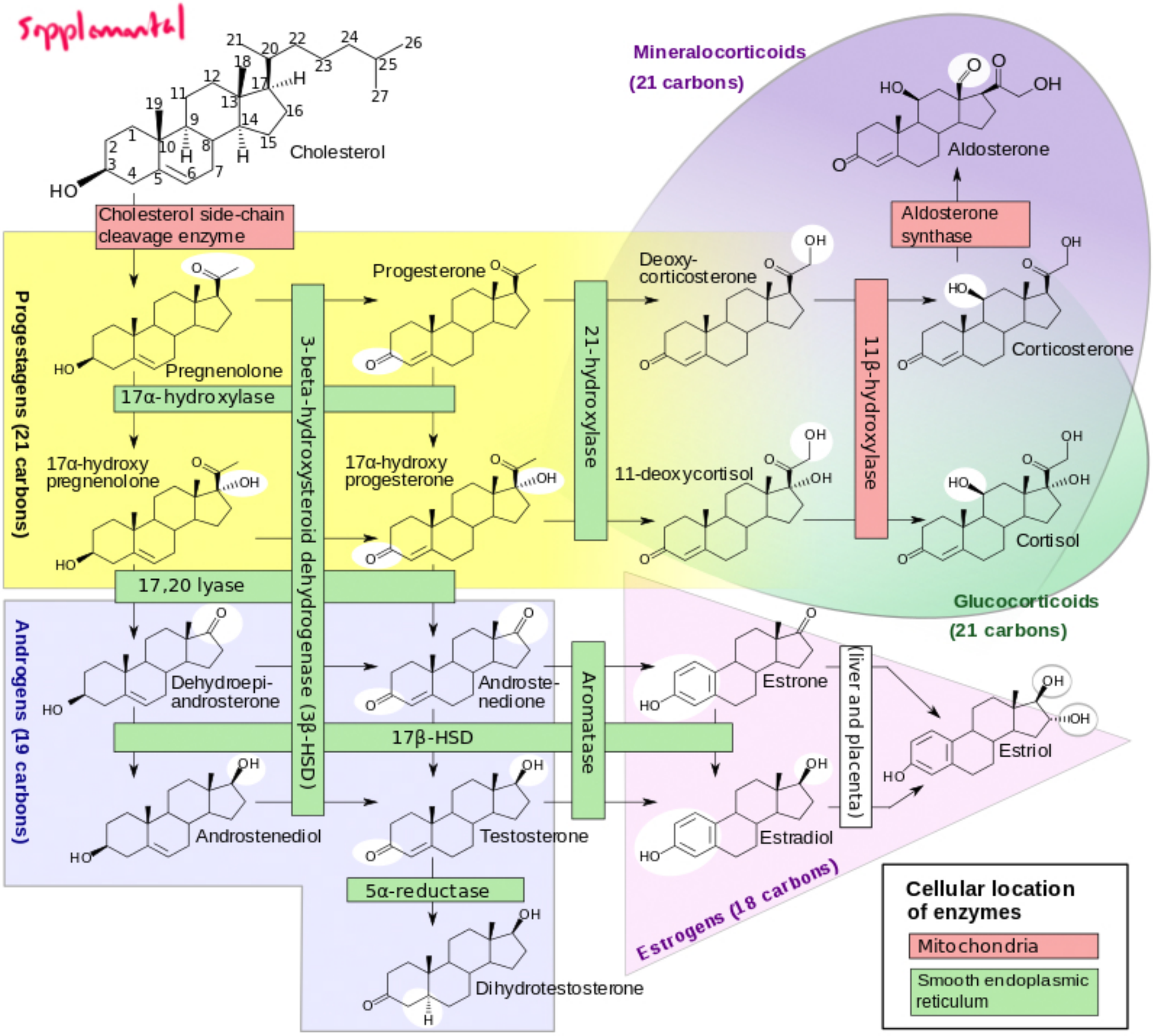
- lipid raft

- serves as a fluidity buffer
- low T - prevents crystallisation
- high T - increases viscosity

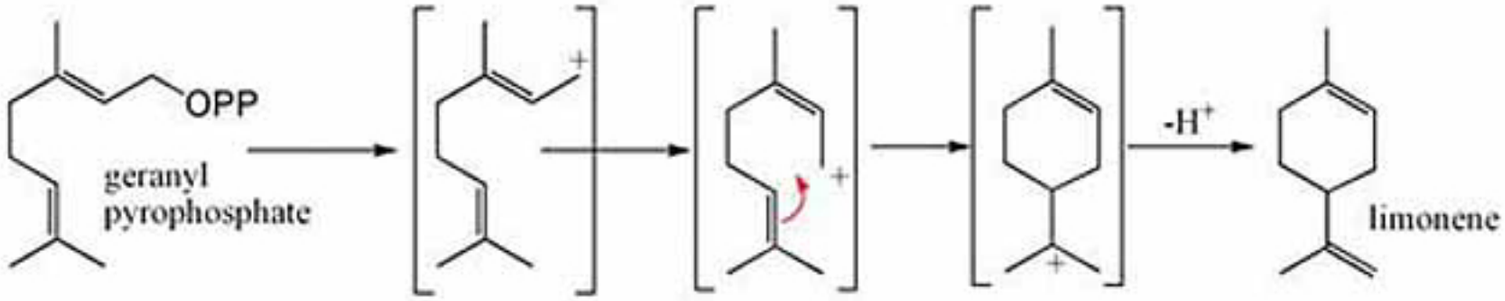

Extracellular Fluid
Protein channel
(transport protein)
Globular protein
Glycoprotein
Carbohydrate
Hydrophilic heads
Phospholipid bilayer
Phospholipid
molecule
Cholesterol
Glycolipid
Integral protein
(Globular protein)
Surface protein
Peripherial protein
Filaments of
cytoskeleton
Alpha-Helix protein
(Integral protein)
Hydrophobic tails
Cytoplasm


dimethylallyl pyrophosphate
isopentenyl pyrophosphate
DMAPP
IPP
GPP
Squalene
Lanosterol
HO
mevalonate
HMG-CoA
3 acyl CoA
19 steps (NADPH, O2, NADH)
cholestanol

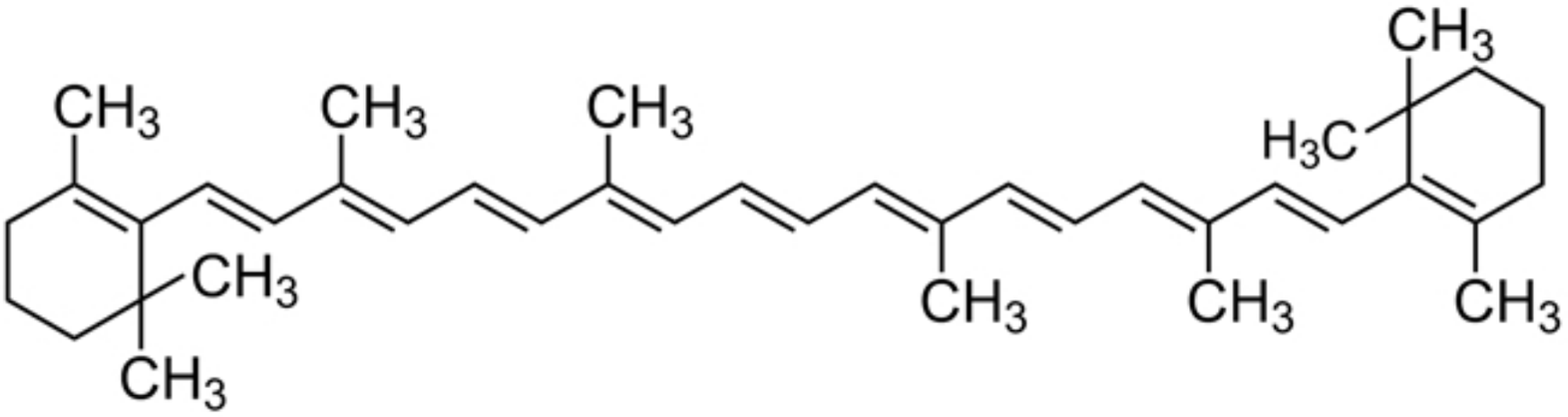
Supplamental



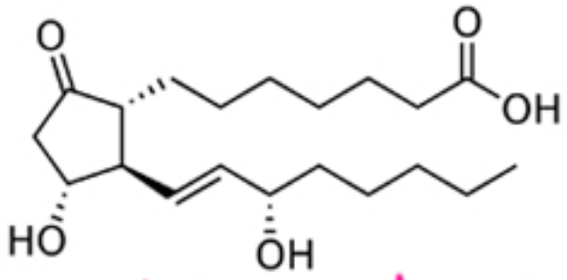
terpenes (plant essential oils)



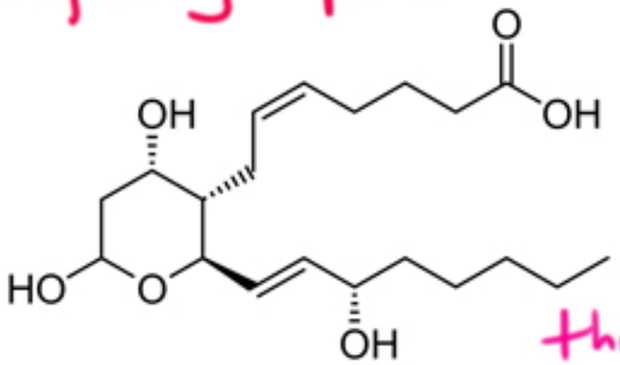
β carotene - precursor of retinal



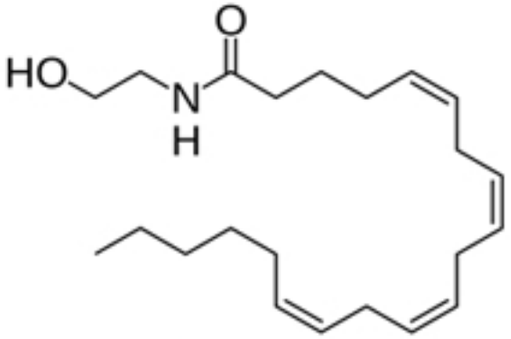
eicosanoid signaling lipids



prostaglandin - alprostadil
(vasodilation, inflammation)

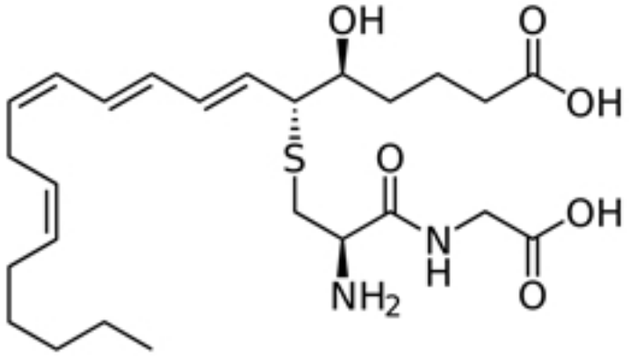


thromboxane
(vasoconstrictor)



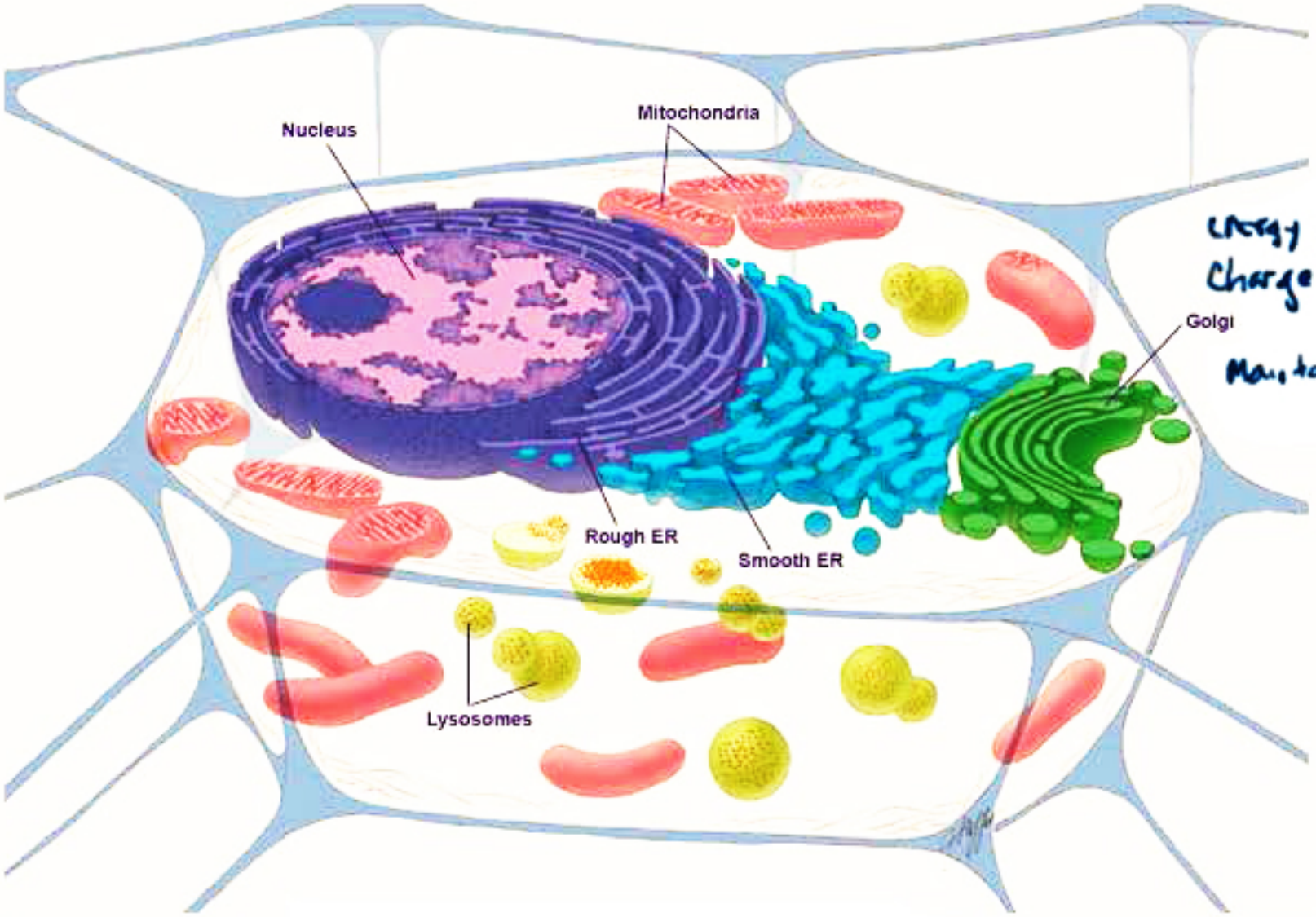
anandamide

(neurotransmitter)



a cysteinyl
leukotriene

Energy Metabolism



Energy Charge : $\frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$

Maintained 0.85 - 0.90

- substrate level phosphorylation

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \qquad \Delta G^\circ \sim -3000\,kJ$$

$$36\,ADP + 36\,P_i \longrightarrow 36\,ATP \qquad \Delta G^\circ \sim +1100\,kJ$$

- oxidative phosphorylation

$$ATP \xrightarrow{H_2O} ADP + P_i \qquad \Delta G^\circ \sim -30\,kJ$$

$$A \rightleftharpoons B$$

$$K = \frac{[B]}{[A]}$$

$$K = e^{-\Delta G^\circ/RT}$$

$$\Delta G^\circ = -RT \ln K$$

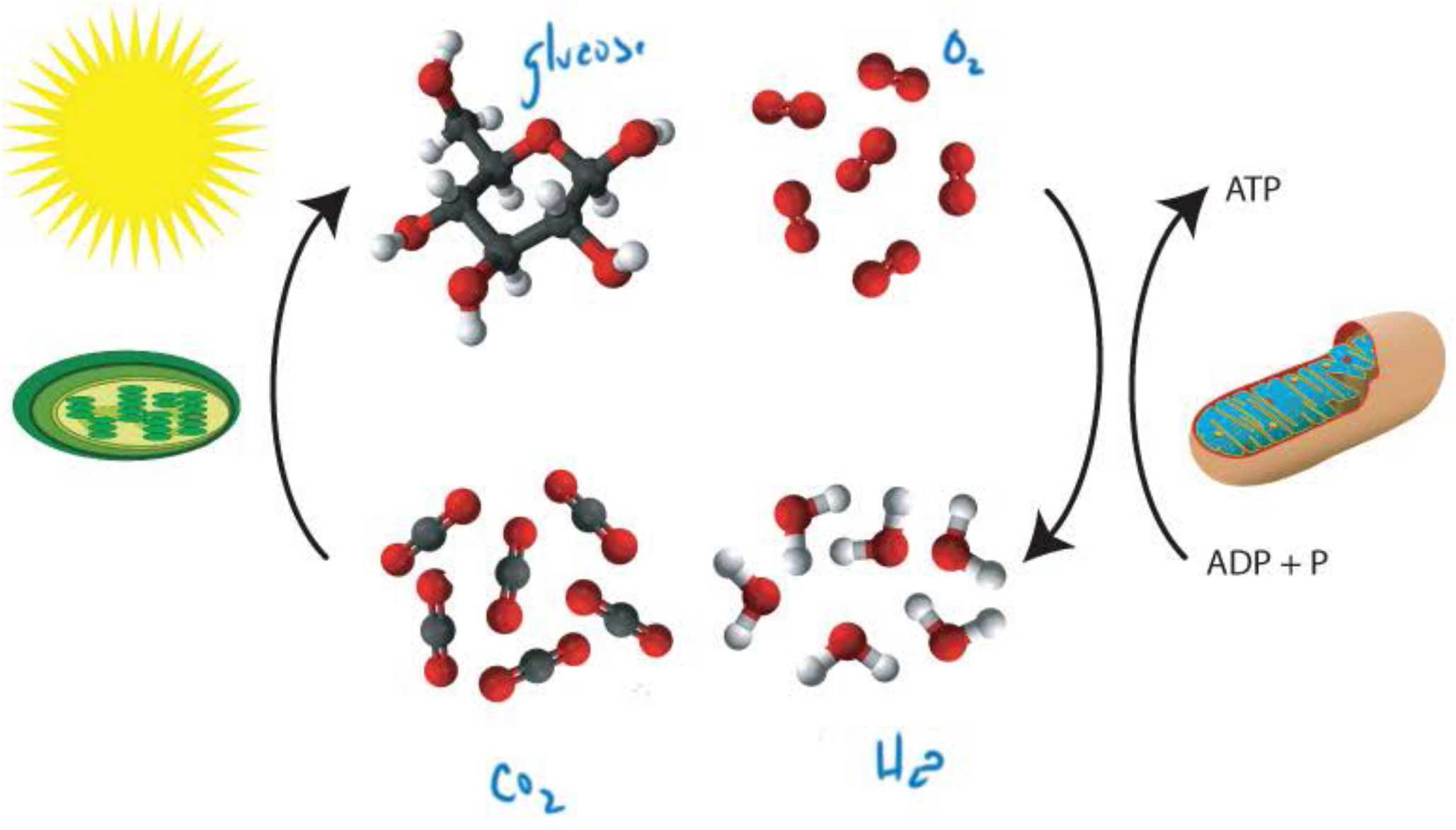
nonspontaneous $K_x = e^{-\Delta G^\circ_x/RT}$

couple with ATP cleavage

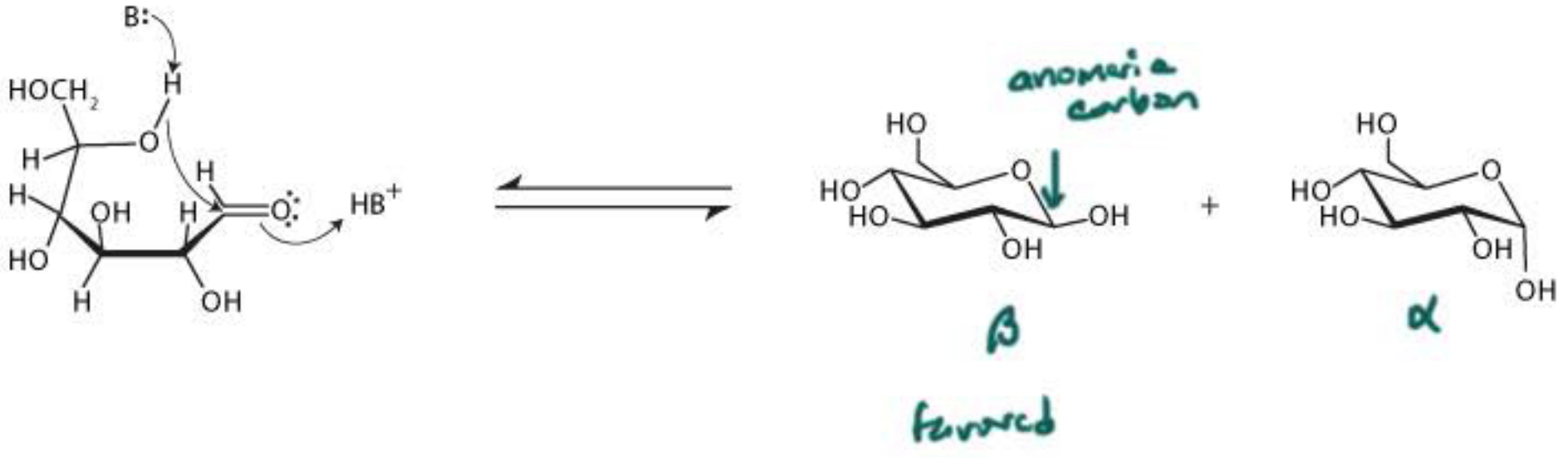
coupled process $K_y = e^{-(\Delta G^\circ_x + \Delta G^\circ_{ATP})/RT}$

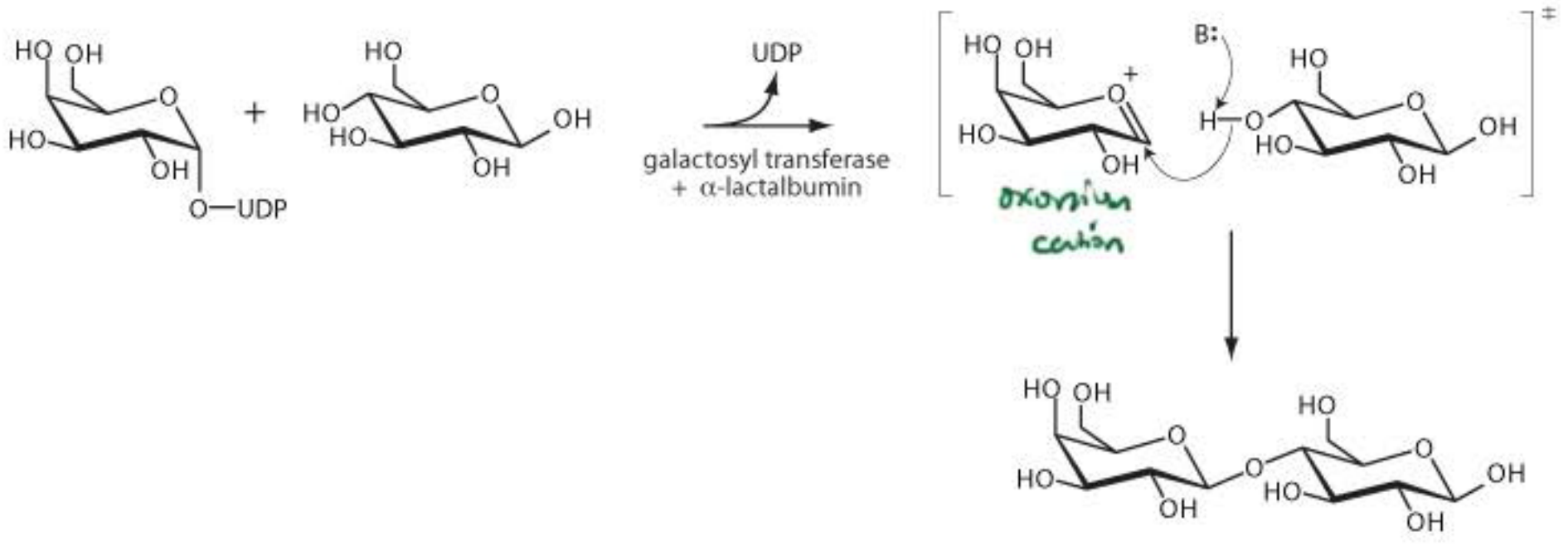
$$K_y = K_x K_{ATP}$$

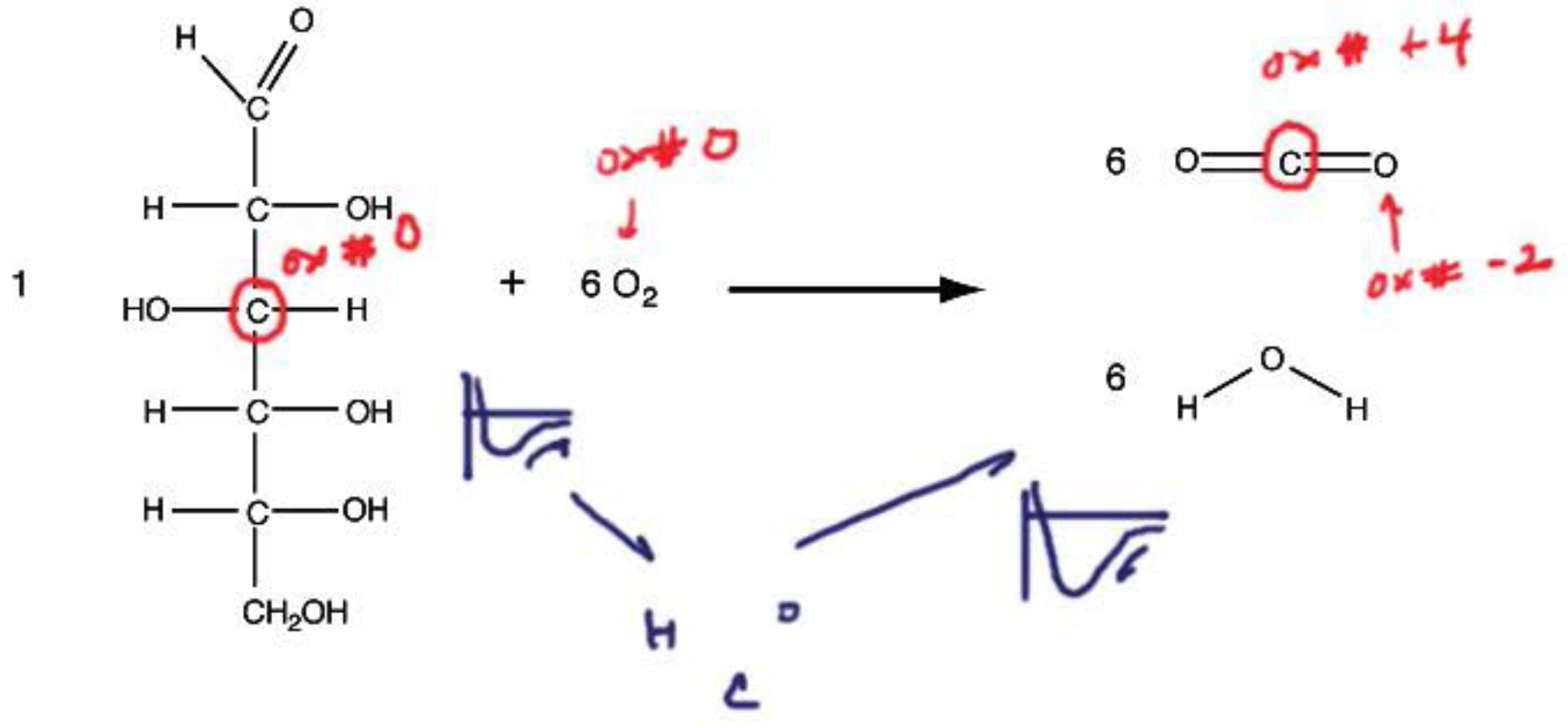
coupling with ATP cleavage shifted K about 10^8 in favor of products

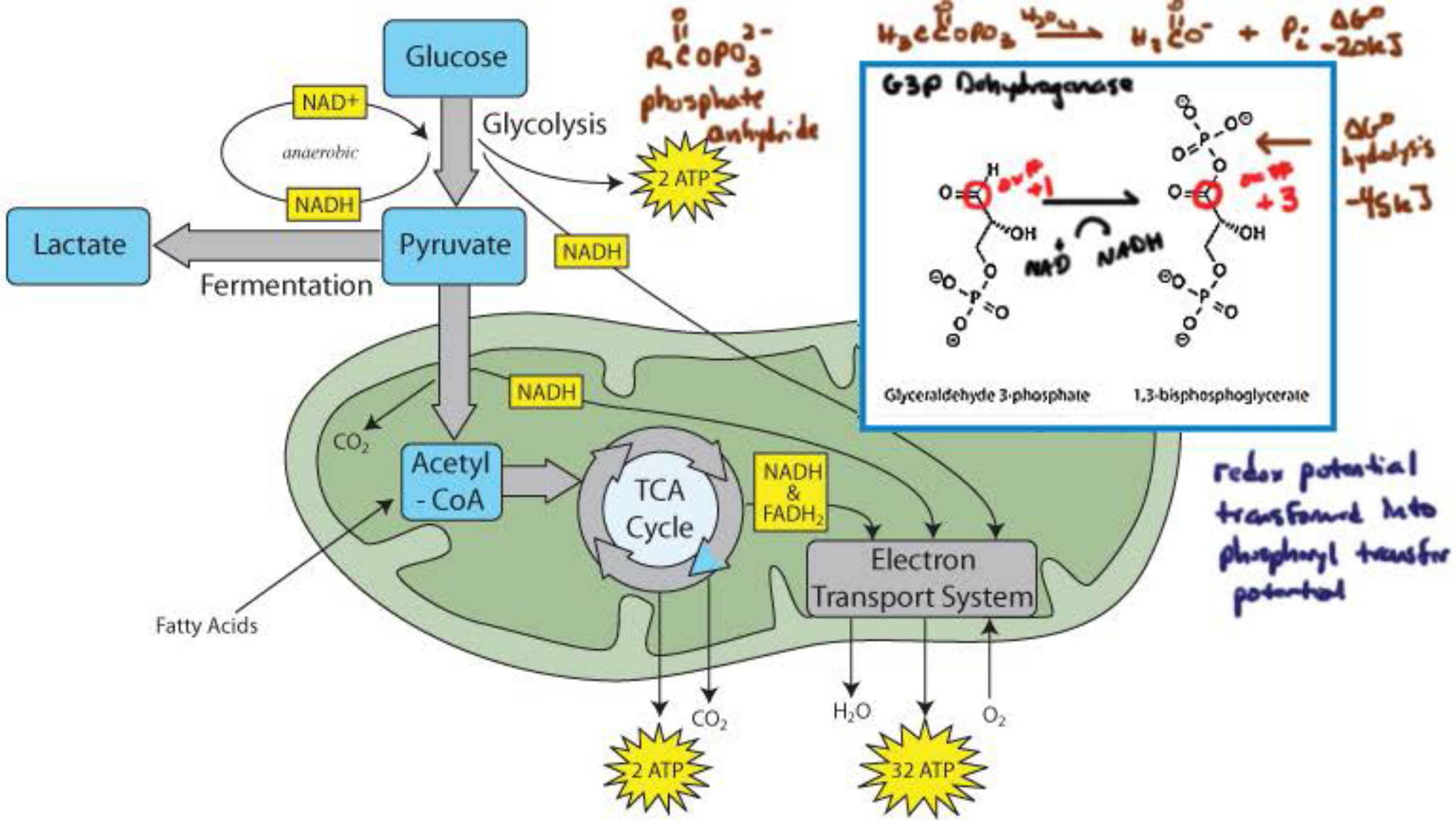

glucose
O_2
ATP
ADP + P
CO_2
H_2O

Glucose Ring Formation (hemiacetal formation)

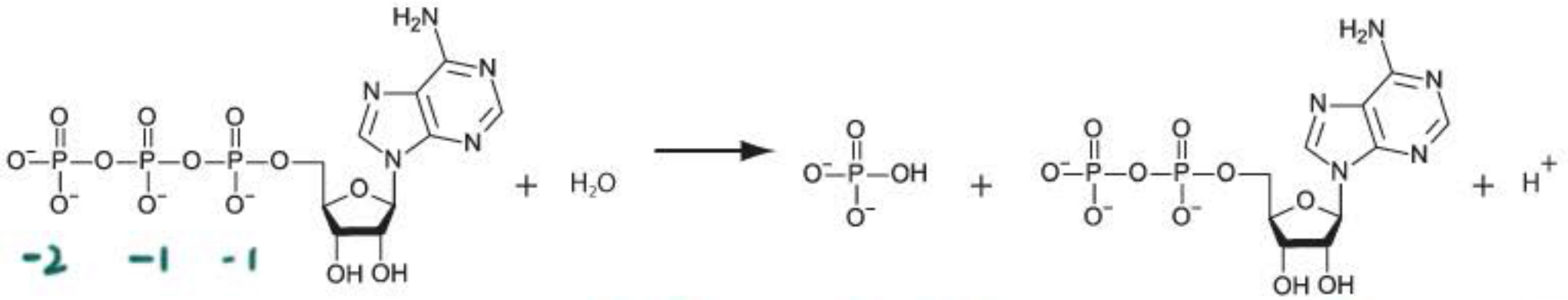



UDP
galactosyl transferase
+ α-lactalbumin
B:
oxonium
cation


1
+
6 O_2
6
6
ox # 0
ox # 0
ox # +4
ox # -2


Glucose
NAD+
anaerobic
NADH
Glycolysis
2 ATP
Lactate
Pyruvate
Fermentation
NADH
NADH
CO_2
Acetyl - CoA
TCA Cycle
NADH & $FADH_2$
Electron Transport System
Fatty Acids
CO_2
H_2O
O_2
2 ATP
32 ATP
G3P Dehydrogenase
Glyceraldehyde 3-phosphate
1,3-bisphosphoglycerate
NAD
NADH
phosphate anhydride
-20kJ
-45kJ
redox potential transformed into phosphoryl transfer potential

Hydrolysis of ATP

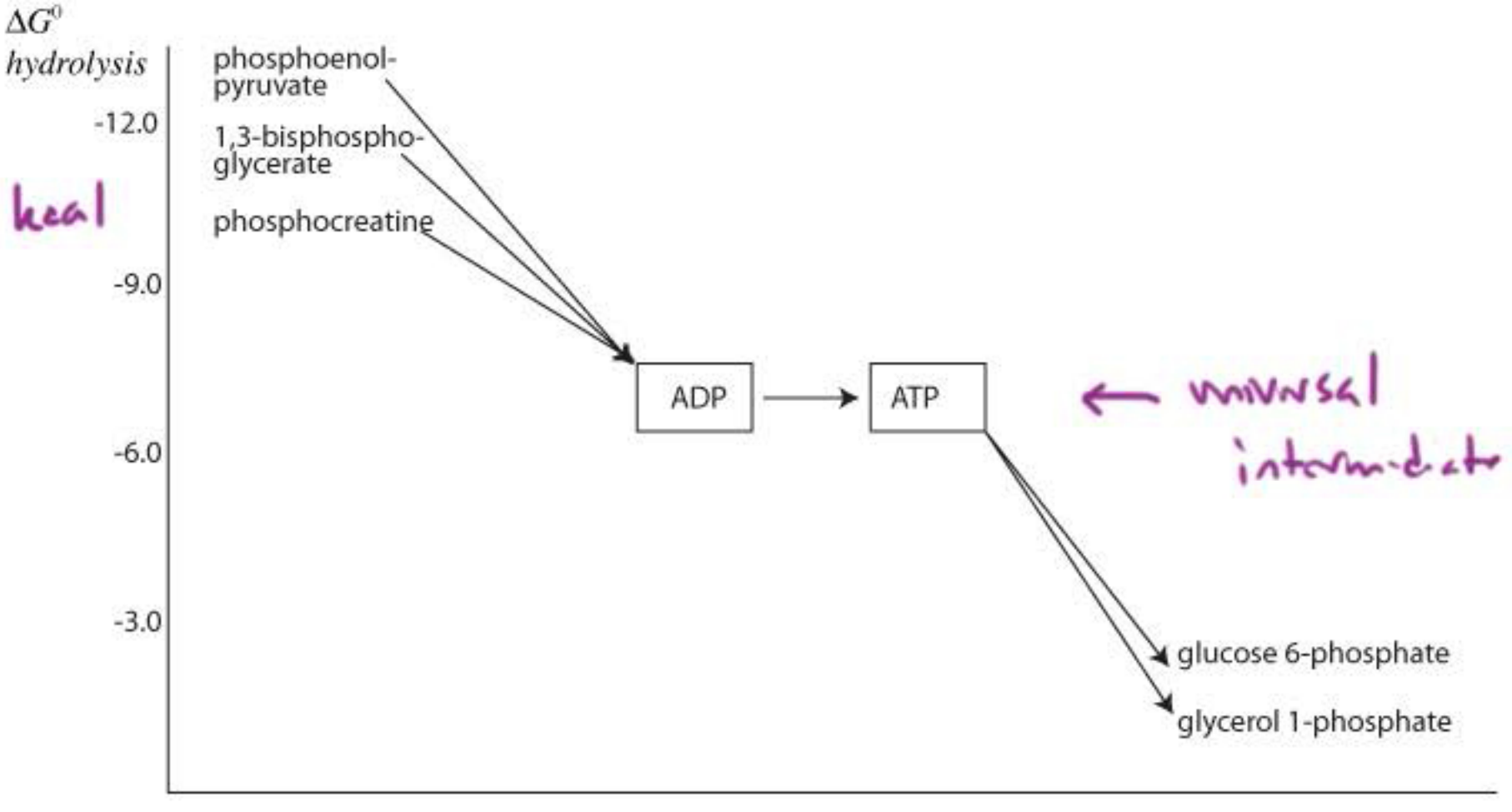


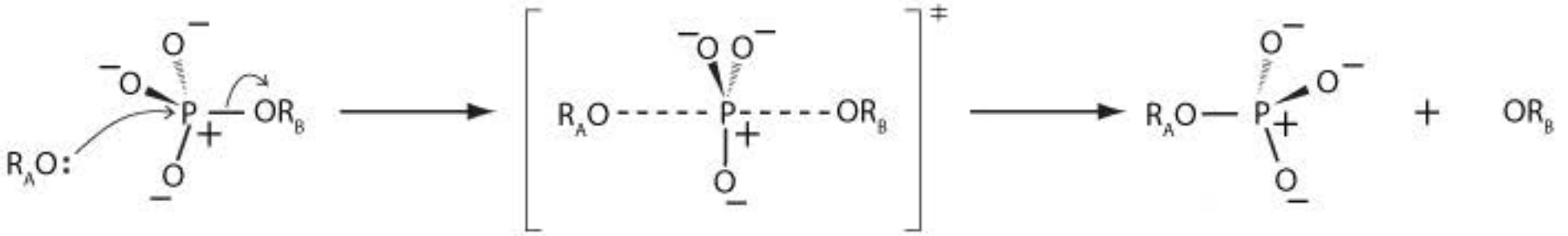
$\Delta G^\circ \sim -30\,kJ$

$\Delta U \Rightarrow \Delta H \Rightarrow \Delta G$

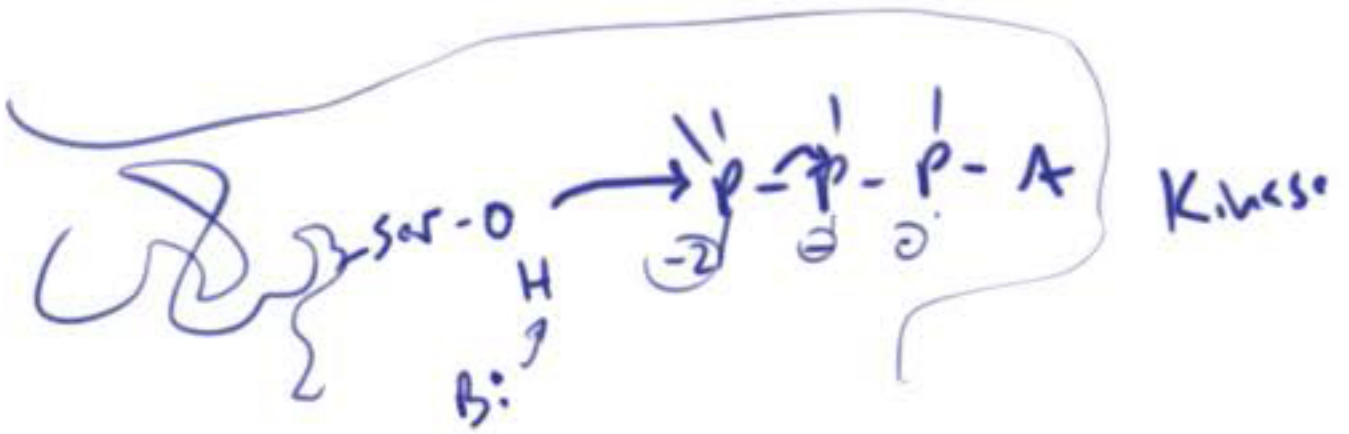
$U = \frac{k q_1 q_2}{r}$

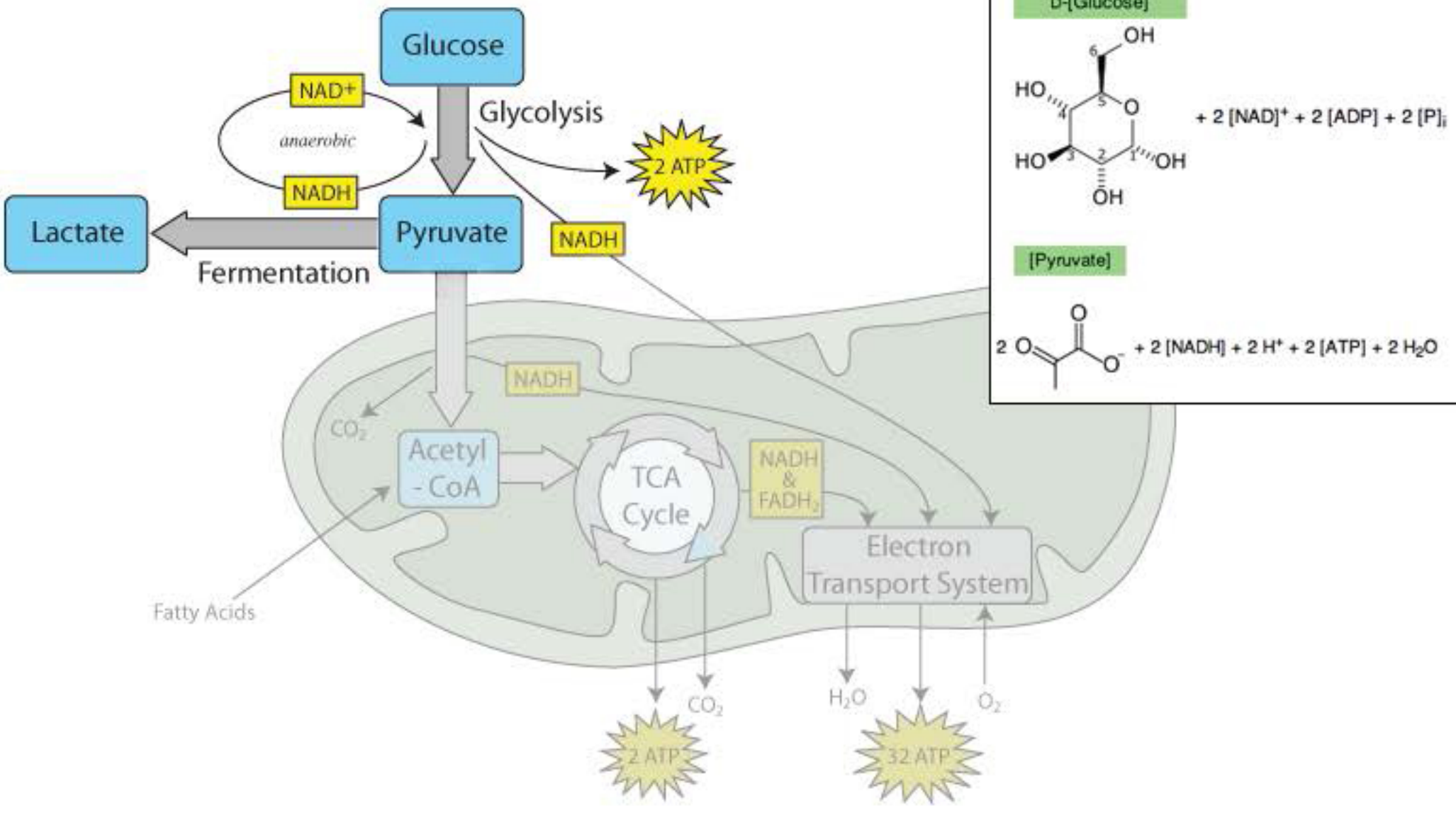
- electrostatic repulsion ★
- resonance
- hydration


ΔG^0
hydrolysis
-12.0
kcal
-9.0
-6.0
-3.0
phosphoenol-
pyruvate
1,3-bisphospho-
glycerate
phosphocreatine
ADP
ATP
← universal
intermediate
glucose 6-phosphate
glycerol 1-phosphate



like SN2
across the
phosphate



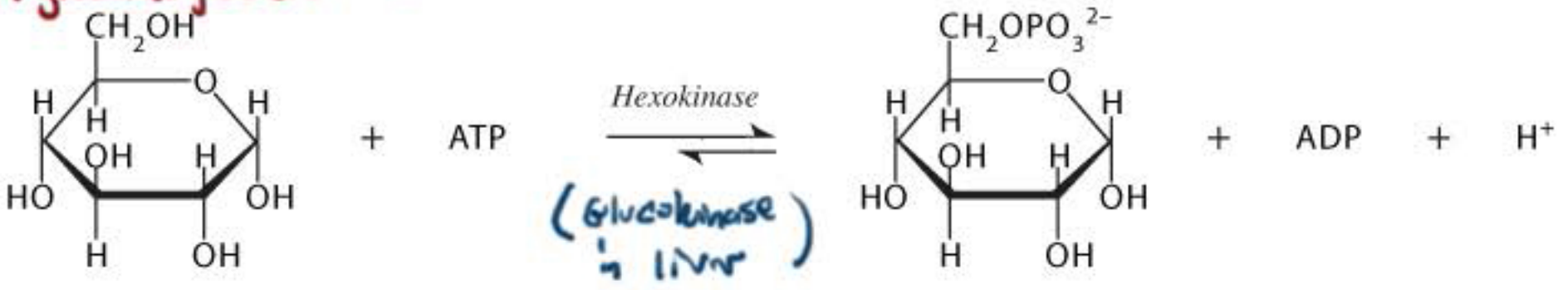

Glucose
NAD+
anaerobic
NADH
Glycolysis
2 ATP
Lactate
Pyruvate
Fermentation
NADH
NADH
CO2
Acetyl - CoA
TCA Cycle
NADH & FADH2
Electron Transport System
Fatty Acids
CO2
H2O
O2
2 ATP
32 ATP
D-[Glucose]
+ 2 [NAD]+ + 2 [ADP] + 2 [P]i
[Pyruvate]
+ 2 [NADH] + 2 H+ + 2 [ATP] + 2 H2O

$-\Delta G$

[Glucose 6 phosphatase in gluconeogenesis]

Hexokinase

• inhibited by G6P



Glucose

Glucose-6-Phosphate

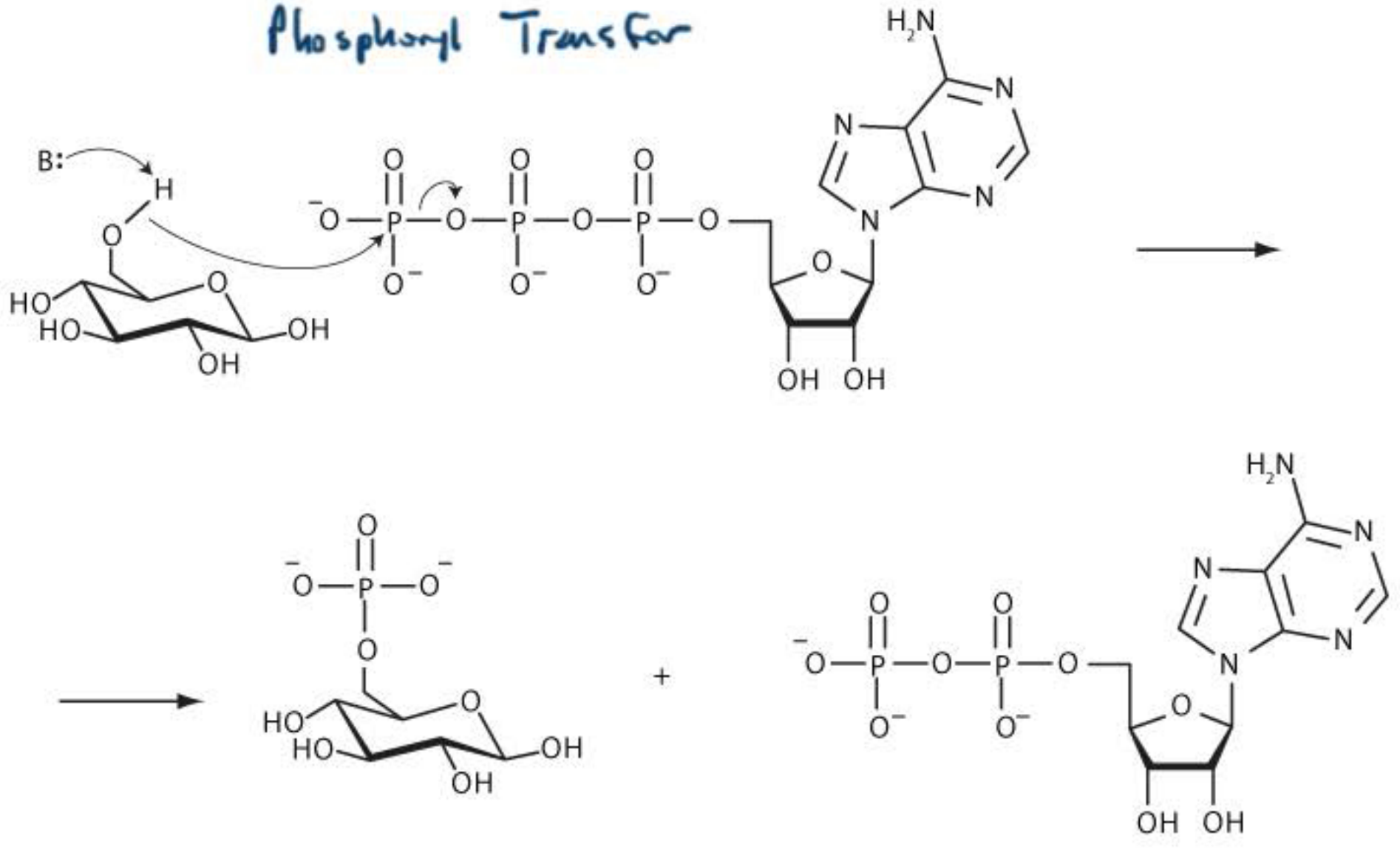
PPP

glycolysis

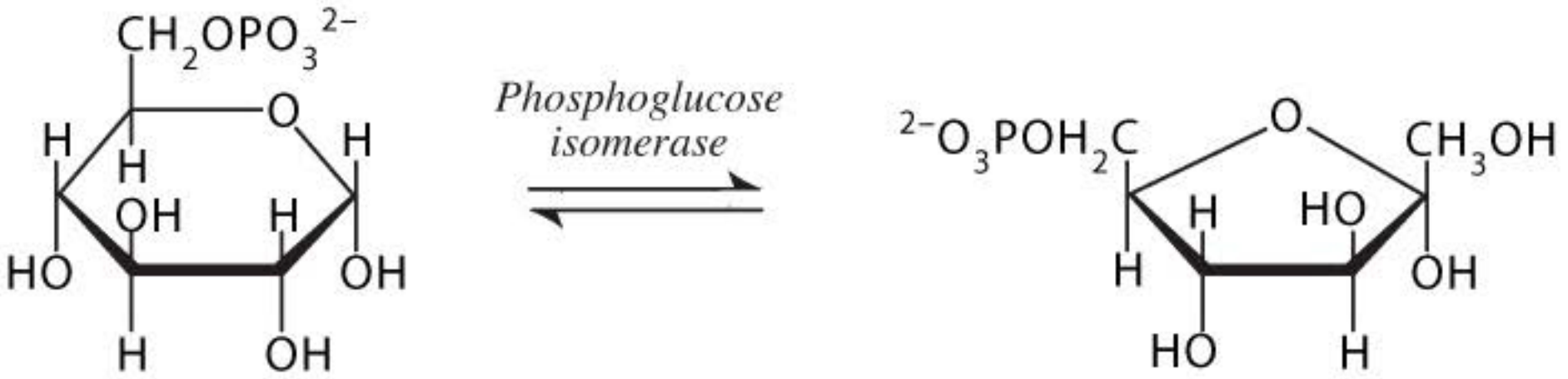
G1P

glycogen synthesis

Phosphoryl Transfer

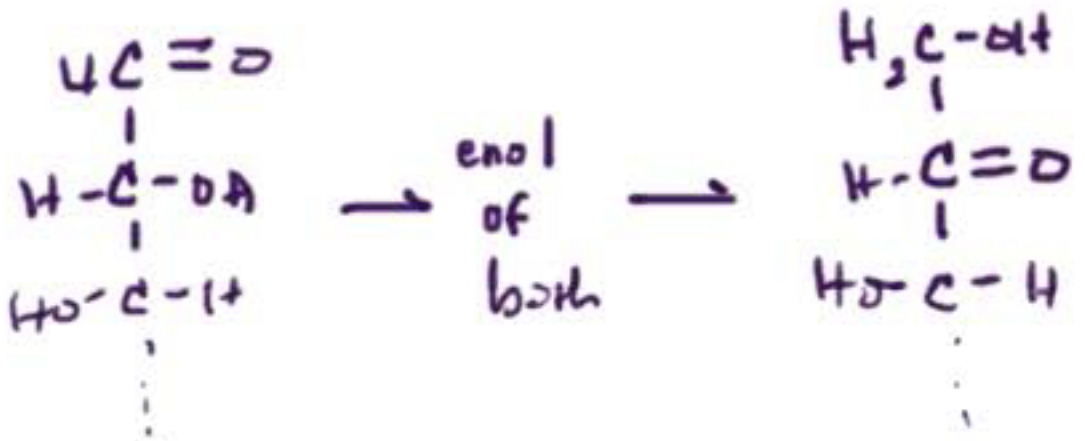


Phosphoglucose Isomerase



G6P

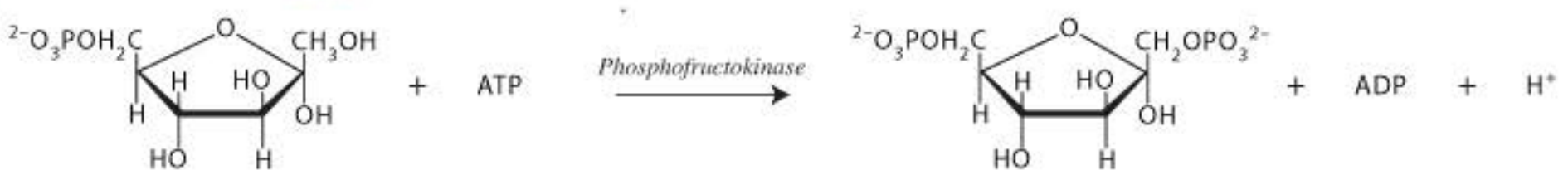
F6P



ΔG°

Fructose 1,6 Bisphosphatase

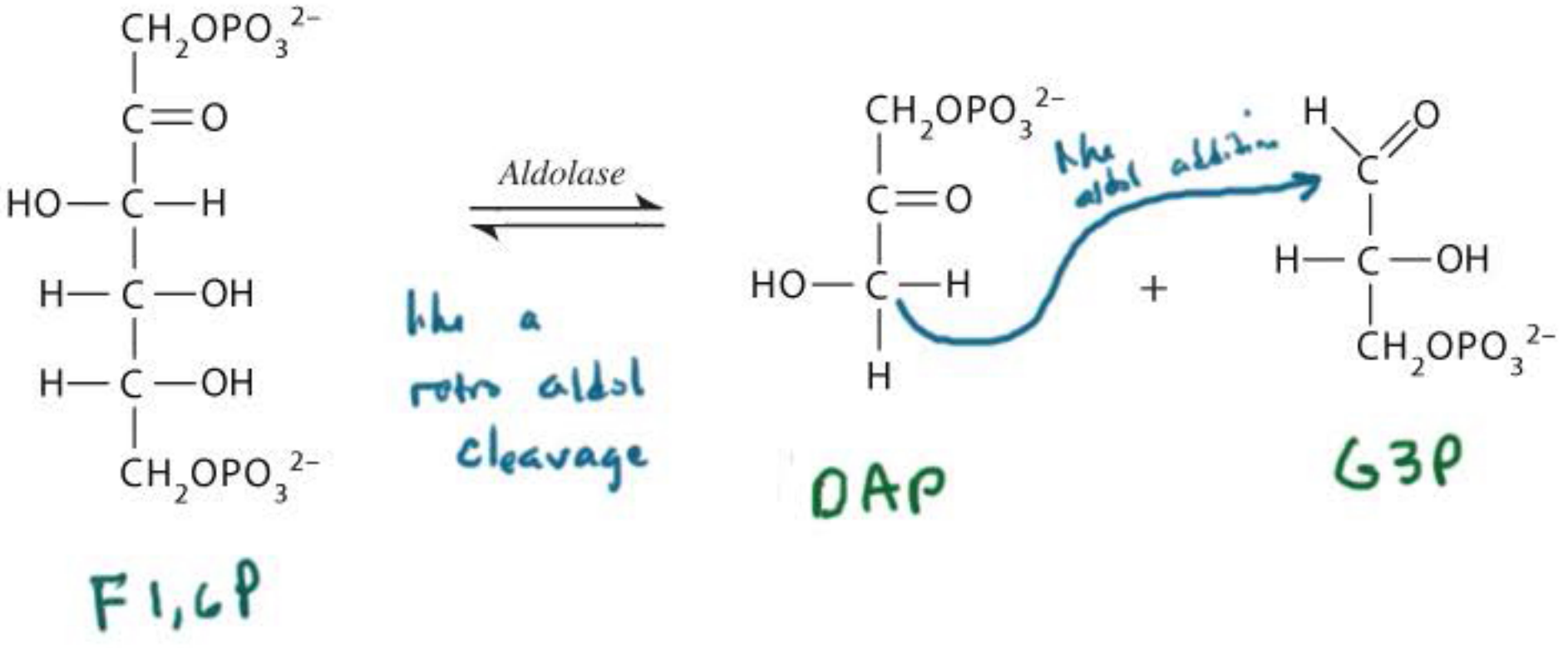
Phosphofructokinase. PFK I



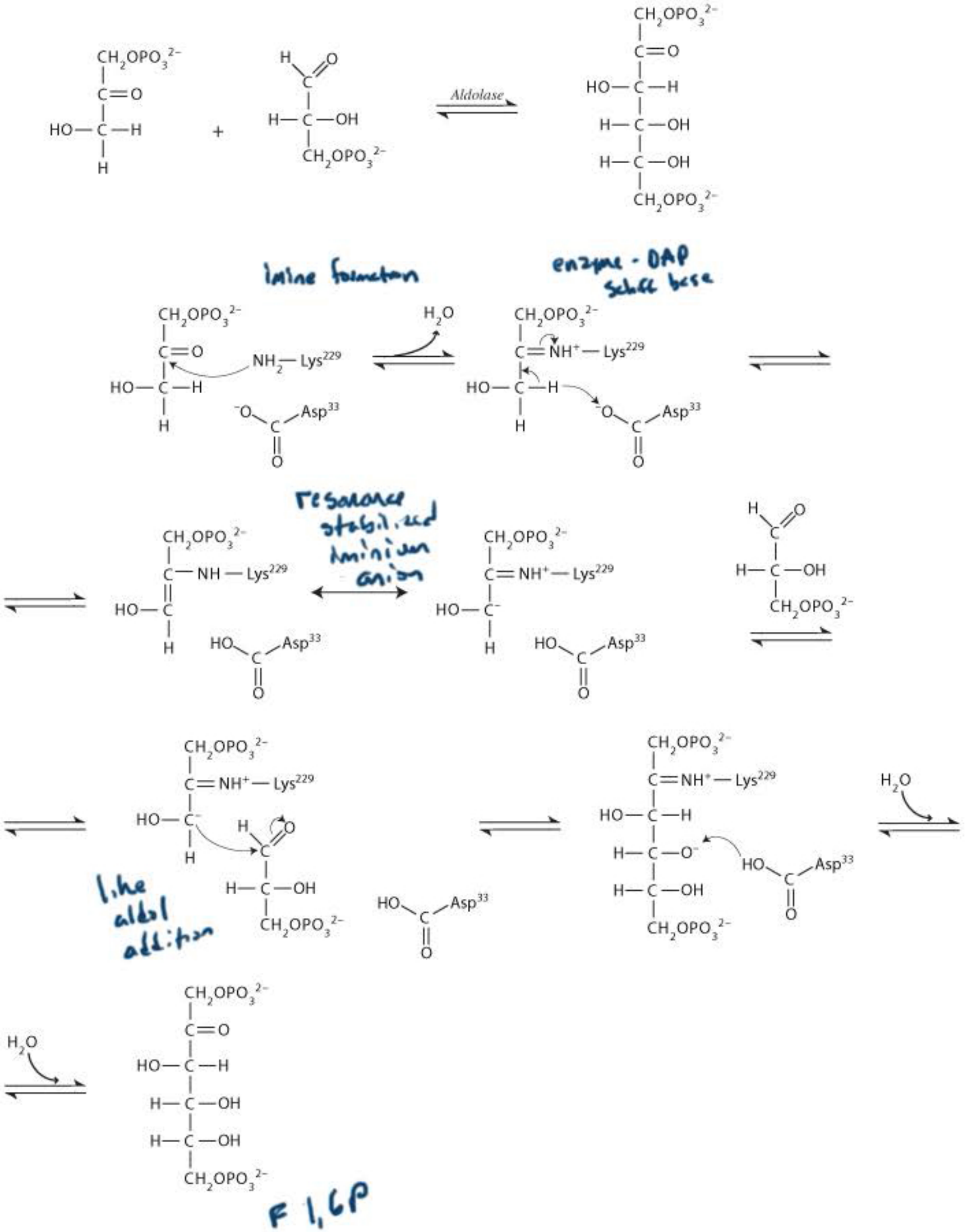
F6P

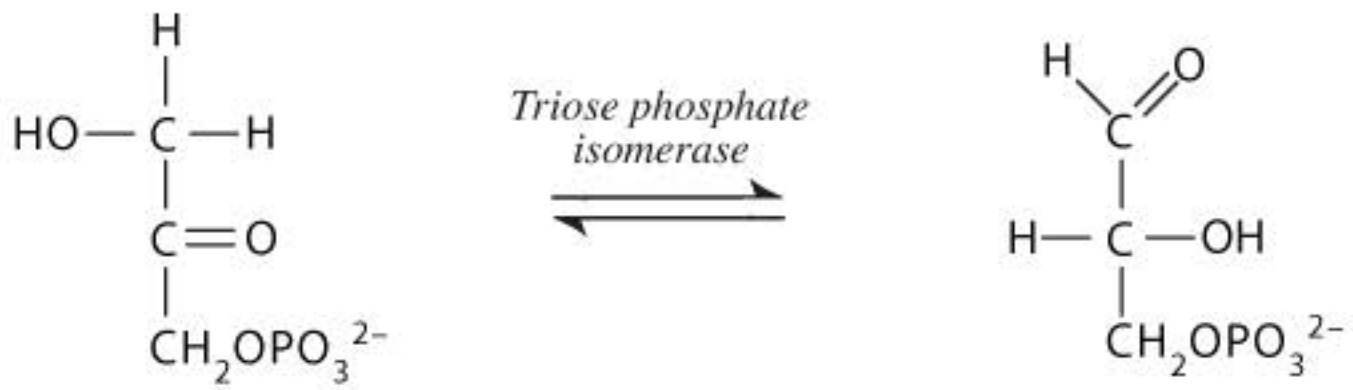
Fructose 1,6 Bis phosphate

- Committed step
- multisubunit allosteric enzyme
- cooperativity
- allosteric promoters and inhibitors
  - AMP ↑ • ATP ↓
  - Citrate ↓ ← signal of adequate precursors
  - Fructose 2,6 Bisphosphate ← strong activator of glycolysis. formed by PFK II
    - mechanism by which signaling can affect metabolic profile.


Aldolase
like a retro aldol cleavage
like aldol addition
F1,6P
DAP
G3P

Gluconeogenesis Direction





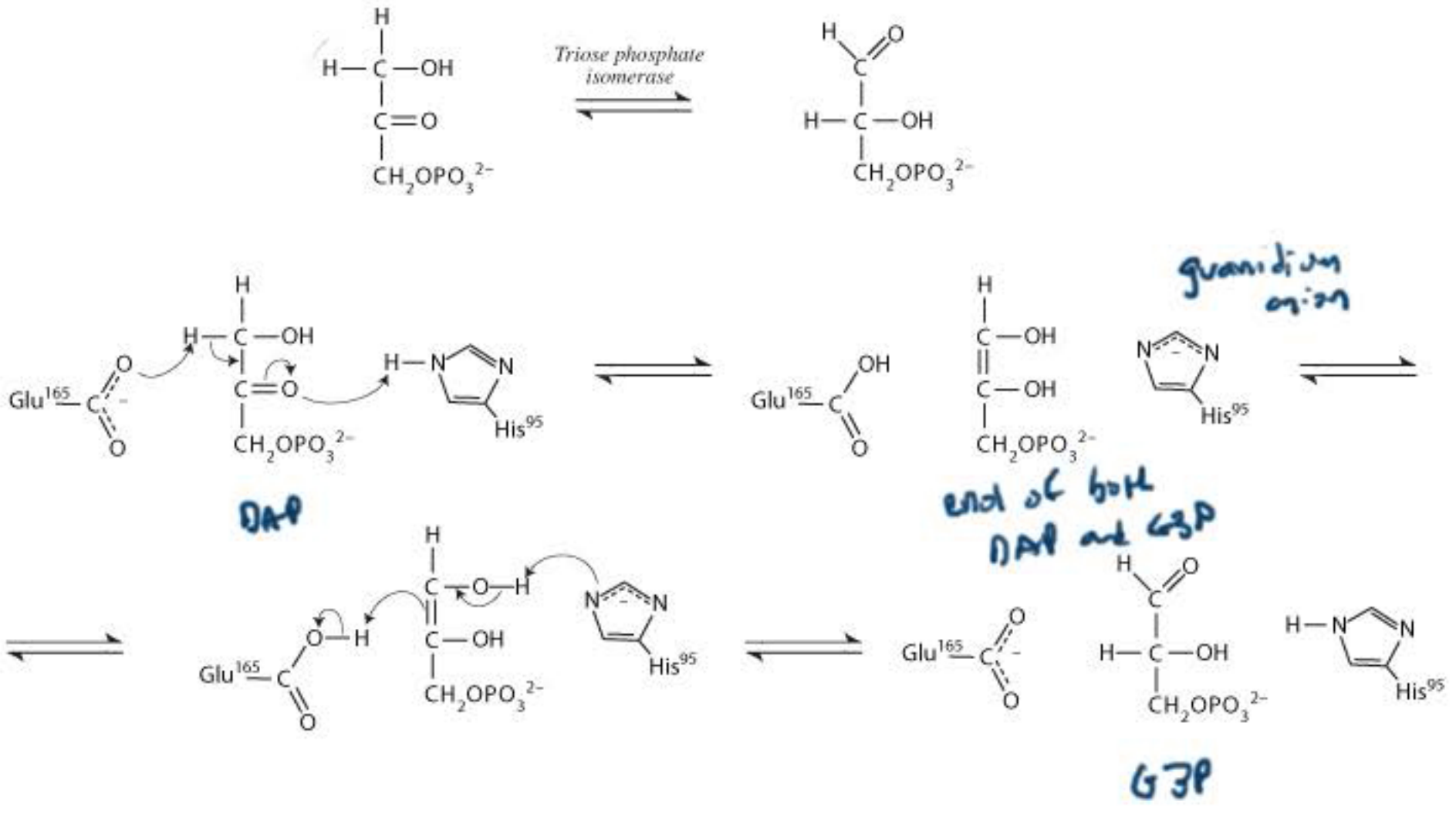
DAP G3P

activity has $+\Delta G^\circ$

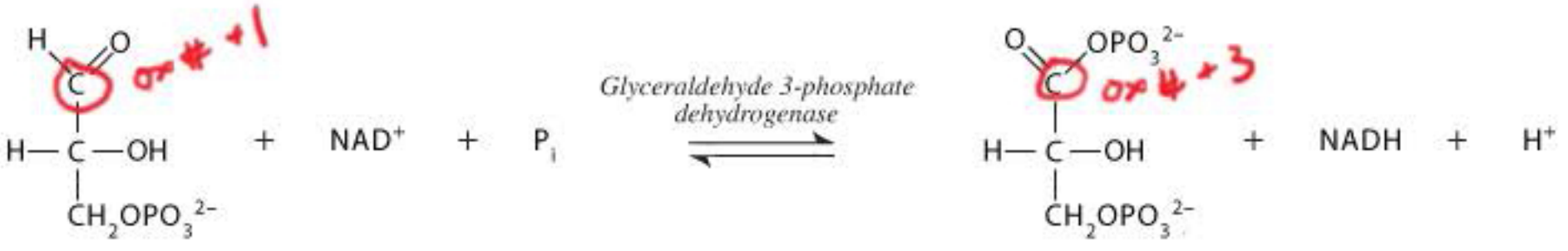
$$\Delta G = \Delta G^\circ + 2.3RT \log Q$$

$$Q \sim \frac{1}{30}$$

$$Q = \frac{[G3P]}{[DAP]}$$


Triose phosphate isomerase
Glu165
His95
DAP
guanidium cation
end of both DAP and G3P
G3P

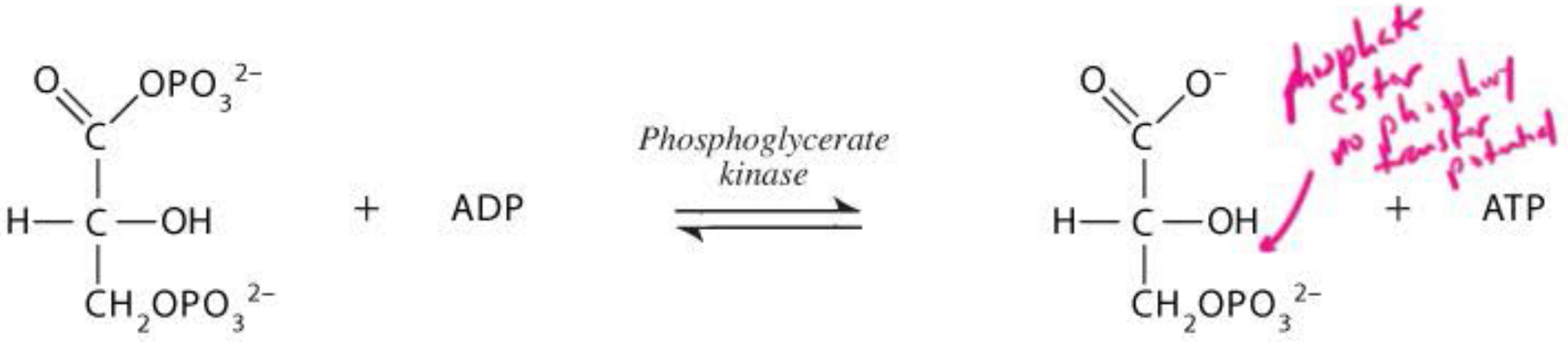
G3P Dehydrogenase



G3P

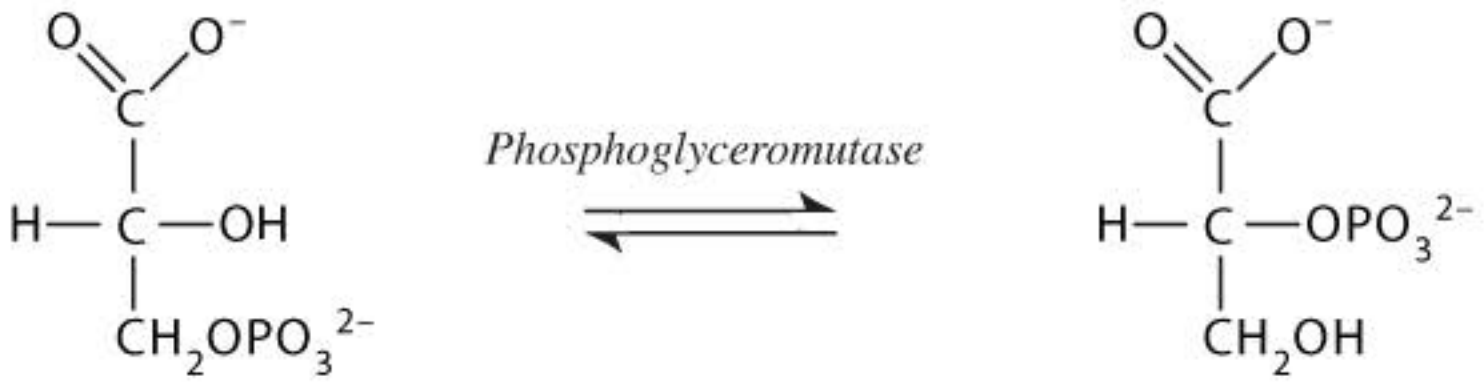
1,3 BPG

redox potential transformed into phosphoryl transfer potential



1,3 BPG

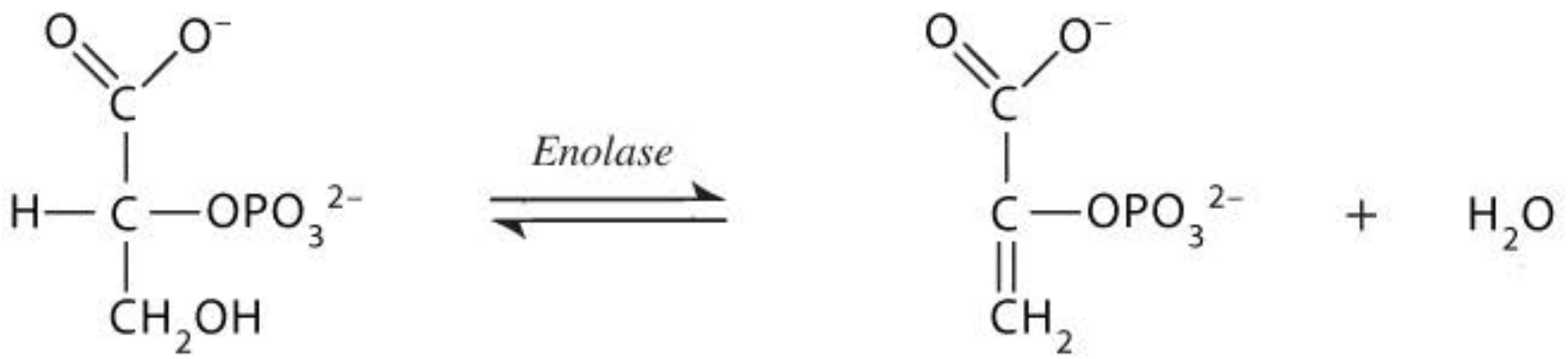
3 Phosphoglycerate

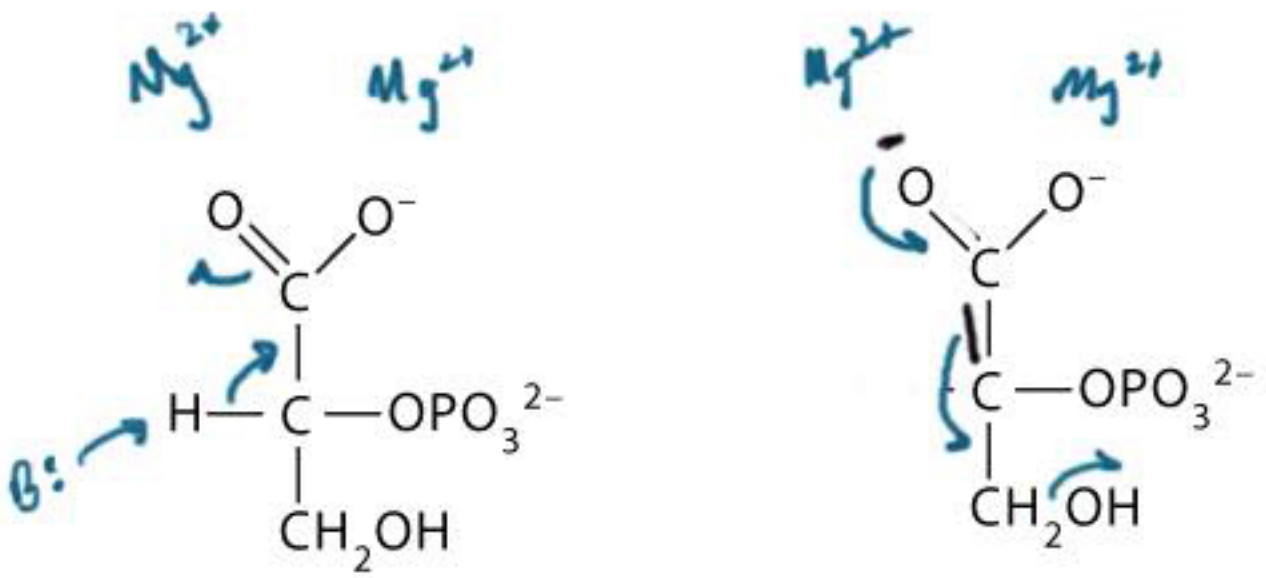


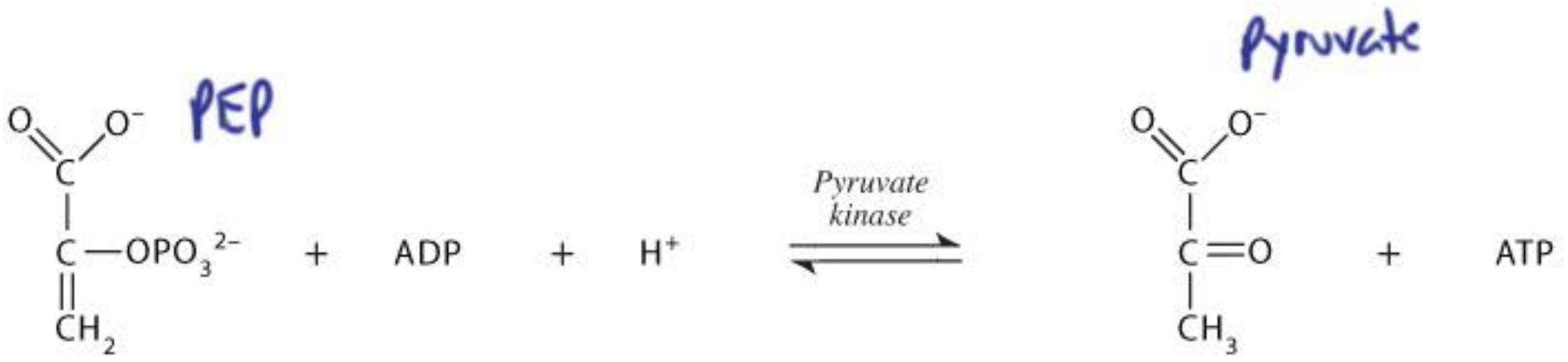
3 Phosphoglycerate

2-Phosphoglycerate

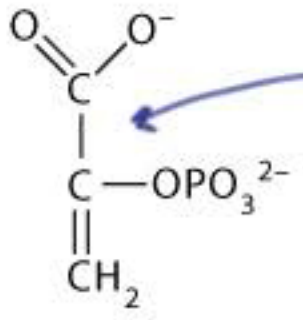
Enolase


Enolase
2-Phosphoglycerate
Phosphoenolpyruvate

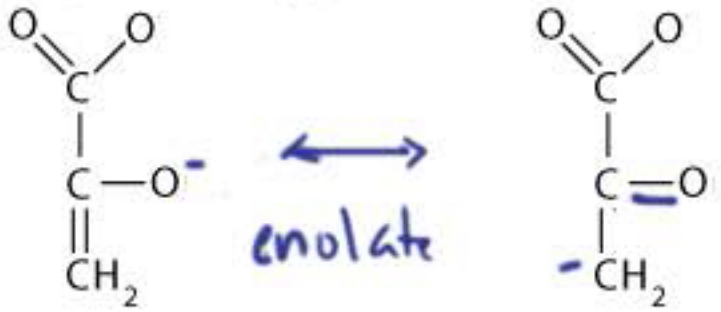

Mg2+
Mg2+
B:

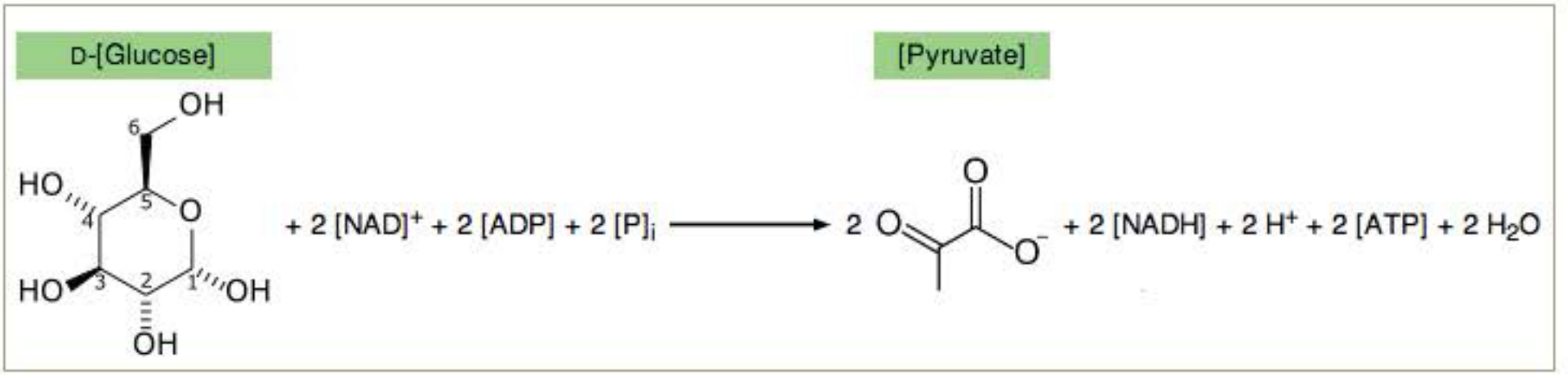


Why does PEP have so much phosphoryl transfer potential?



- partial double bond character
  shortened bond - compressed ⊖ charges
- phosphoryl transfer leaves behind an enolate

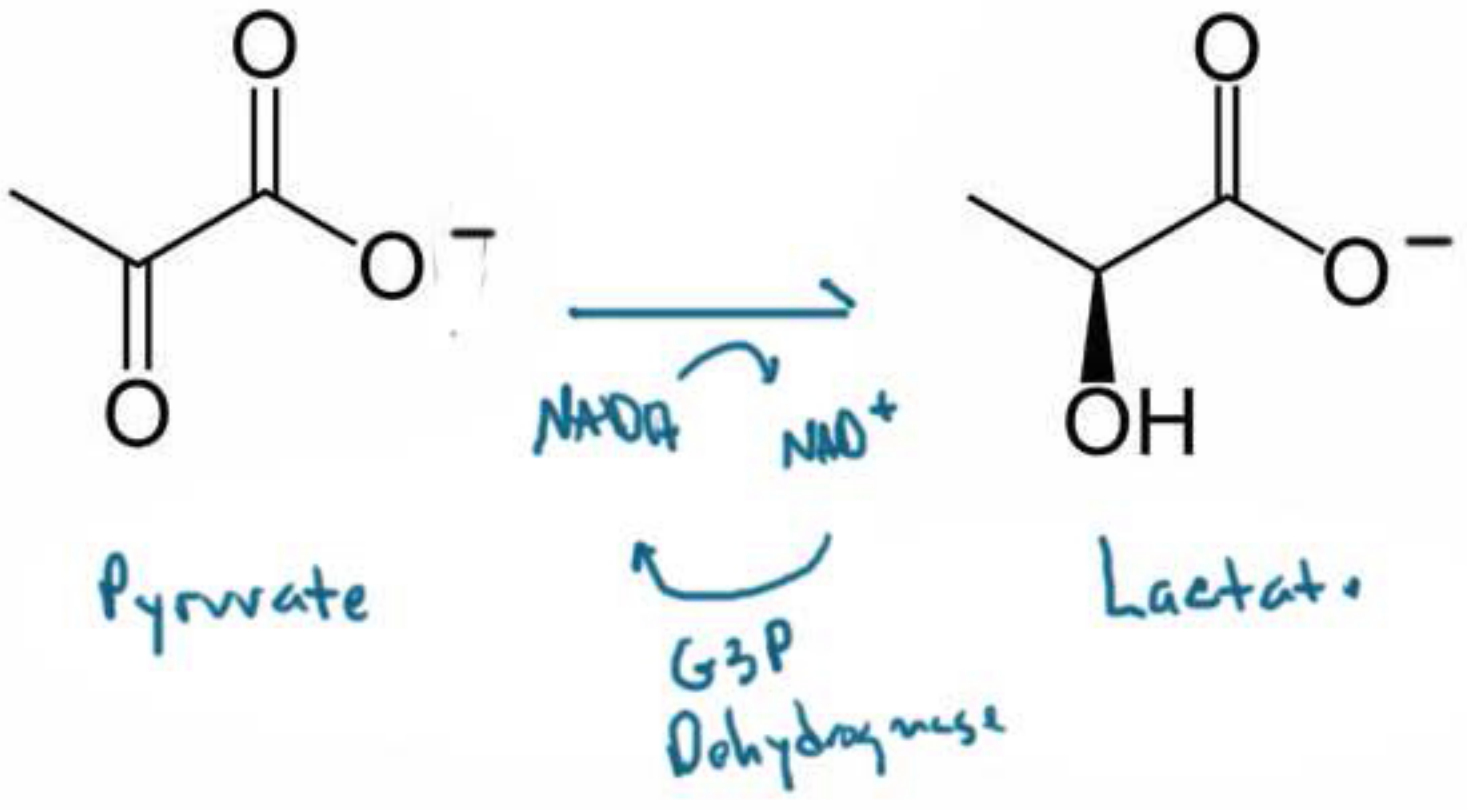



D-[Glucose]
[Pyruvate]
OH
6
HO
5
O
4
HO
3
2
1
OH
OH
+ 2 [NAD]+ + 2 [ADP] + 2 [P]i
2 O
O
O−
+ 2 [NADH] + 2 H+ + 2 [ATP] + 2 H2O

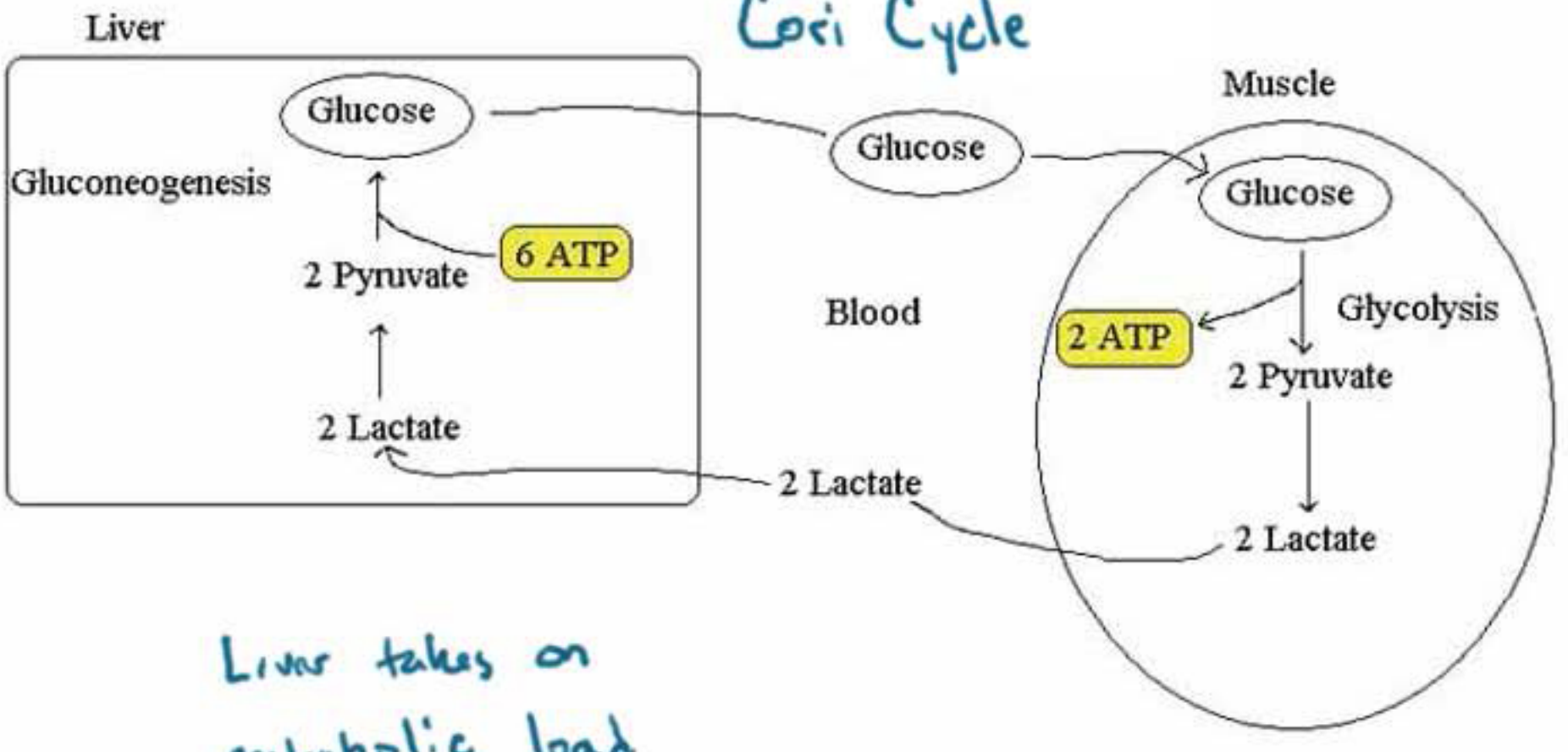
Change in free energy for each step of glycolysis

Step	Reaction	$\Delta G^{\circ\prime}$ / (kJ/mol)	ΔG / (kJ/mol)
1	glucose + ATP^{4-} → glucose-6-phosphate^{2-} + ADP^{3-} + H^+	-16.7	-34
2	glucose-6-phosphate^{2-} → fructose-6-phosphate^{2-}	1.67	-2.9
3	fructose-6-phosphate^{2-} + ATP^{4-} → fructose-1,6-bisphosphate^{4-} + ADP^{3-} + H^+	-14.2	-19
4	fructose-1,6-bisphosphate^{4-} → dihydroxyacetone phosphate^{2-} + glyceraldehyde-3-phosphate^{2-}	23.9	-0.23
5	dihydroxyacetone phosphate^{2-} → glyceraldehyde-3-phosphate^{2-}	7.56	2.4
6	glyceraldehyde-3-phosphate^{2-} + P_i^{2-} + NAD^+ → 1,3-bisphosphoglycerate^{4-} + NADH + H^+	6.30	-1.29
7	1,3-bisphosphoglycerate^{4-} + ADP^{3-} → 3-phosphoglycerate^{3-} + ATP^{4-}	-18.9	0.09
8	3-phosphoglycerate^{3-} → 2-phosphoglycerate^{3-}	4.4	0.83
9	2-phosphoglycerate^{3-} → phosphoenolpyruvate^{3-} + H_2O	1.8	1.1
10	phosphoenolpyruvate^{3-} + ADP^{3-} + H^+ → pyruvate$^-$ + ATP^{4-}	-31.7	-23.0

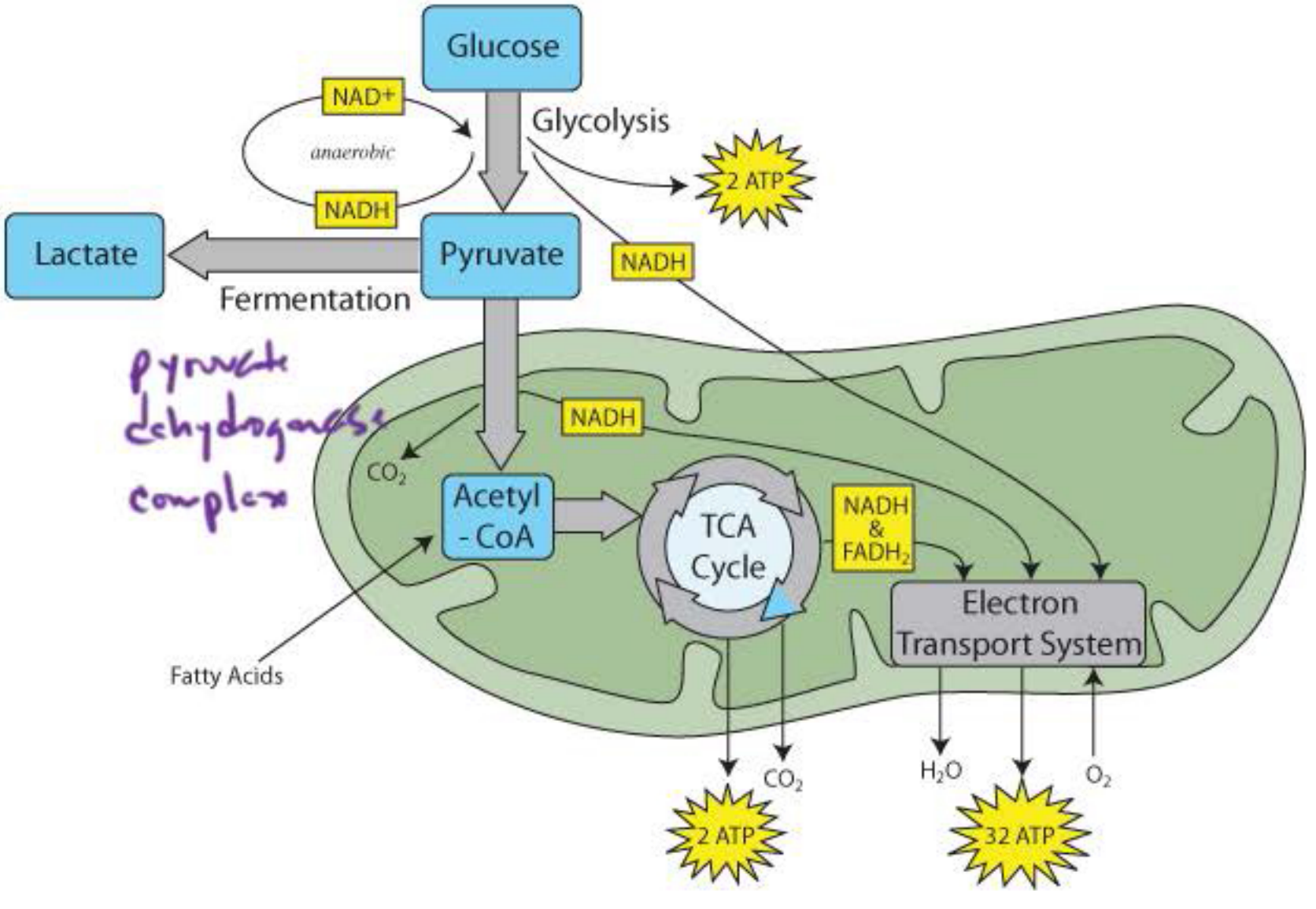
$$\Delta G = \Delta G^{\circ} + RT \ln Q$$


O
O
O⁻
NADH
NAD+
OH
O
O⁻
Pyruvate
Lactate
G3P
Dehydrogenase

Cori Cycle



Liver takes on metabolic load


Glucose
NAD+
anaerobic
NADH
Glycolysis
2 ATP
Lactate
Pyruvate
NADH
Fermentation
pyruvate dehydrogenase complex
NADH
CO_2
Acetyl - CoA
TCA Cycle
NADH & $FADH_2$
Electron Transport System
Fatty Acids
CO_2
H_2O
O_2
2 ATP
32 ATP

pyruvate

acetyl CoA

Coenzymes

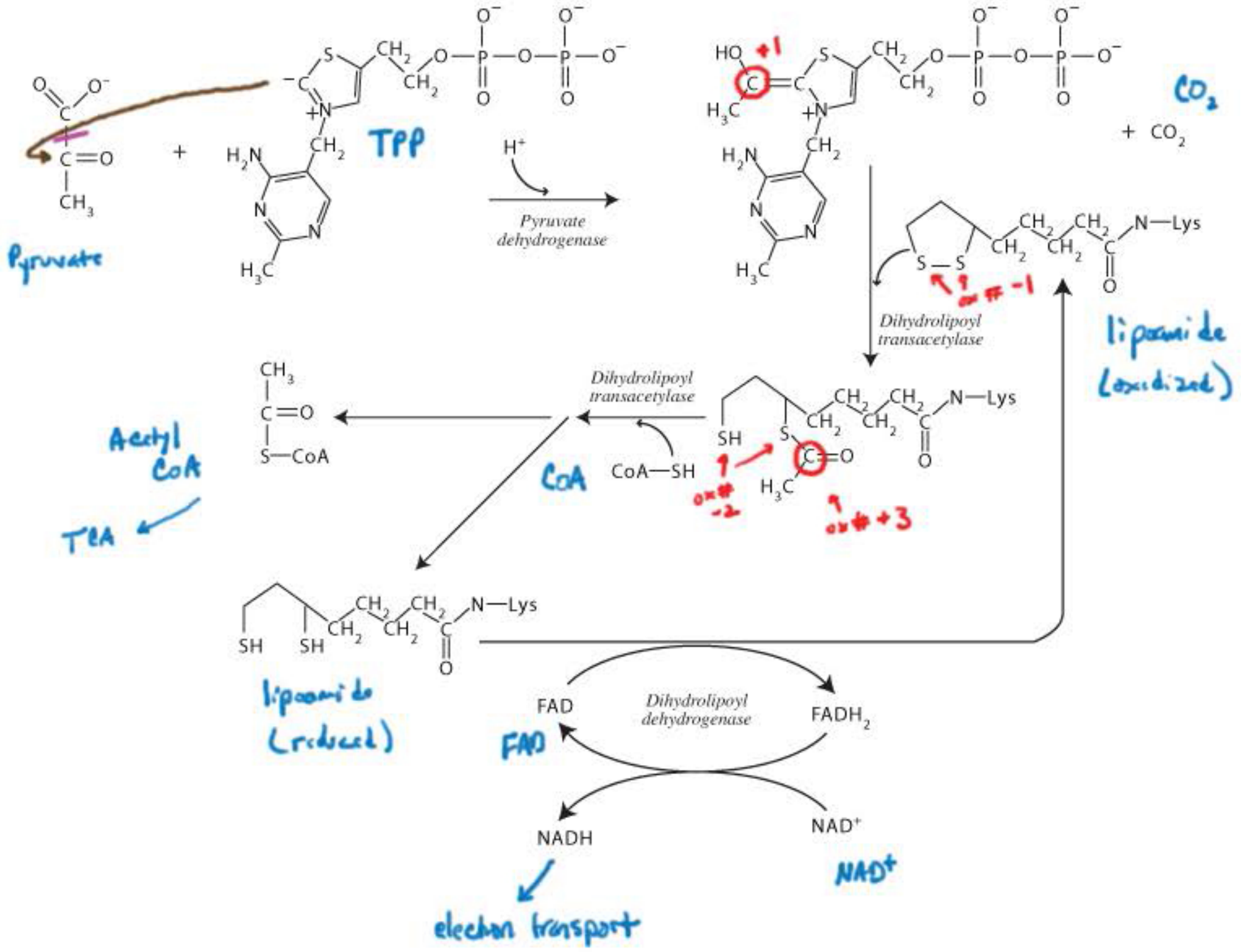
TPP

lipoamide

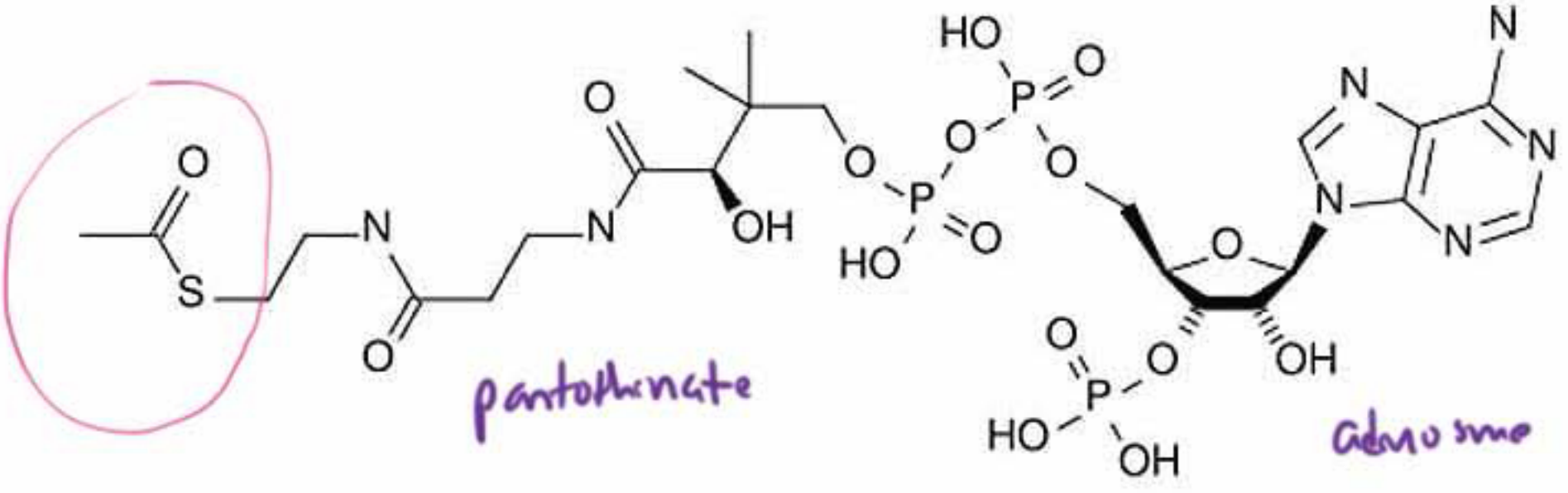
CoA

FAD

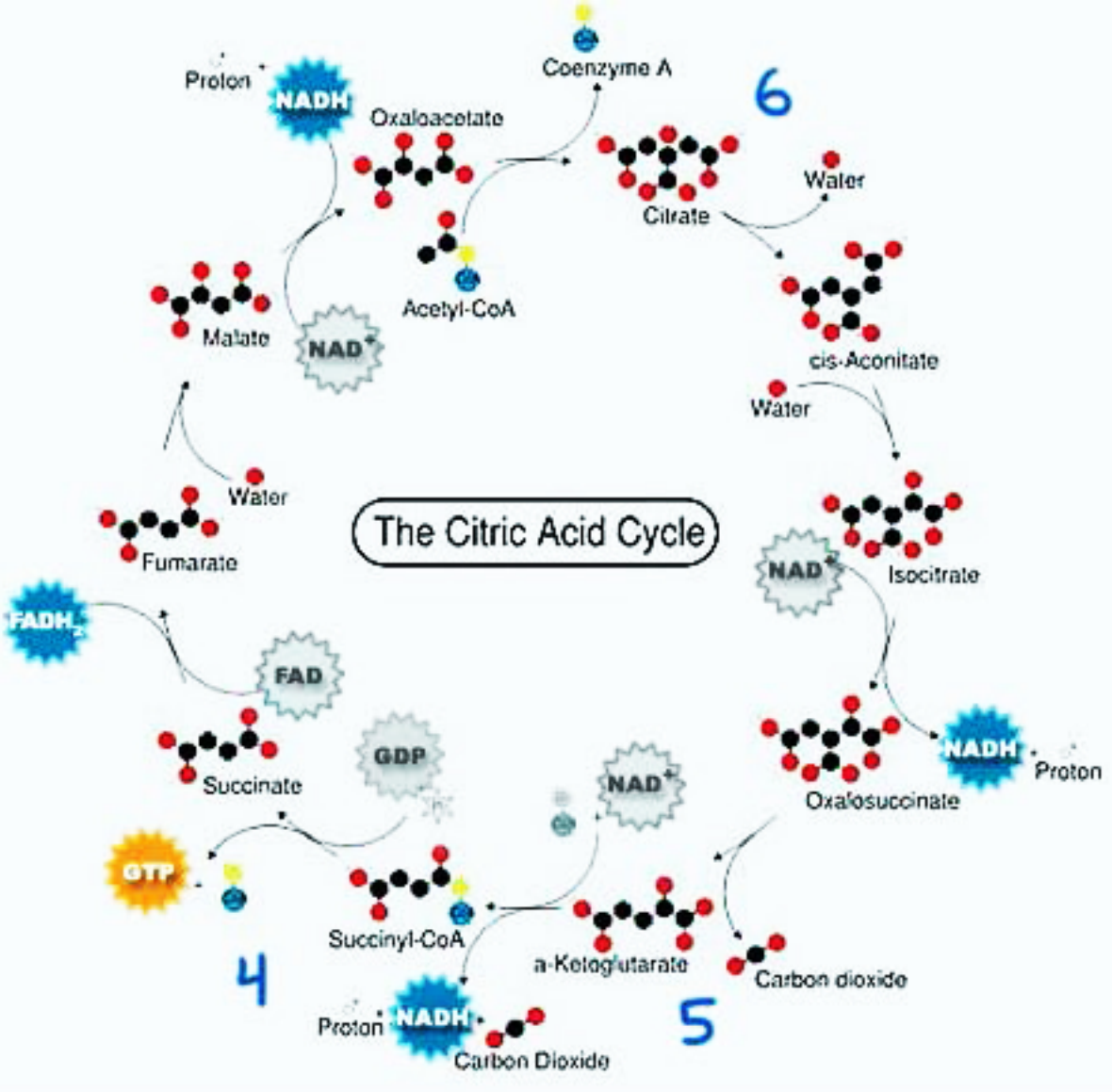
NAD+


Pyruvate
TPP
Pyruvate dehydrogenase
CO_2
Dihydrolipoyl transacetylase
lipoamide (oxidized)
Acetyl CoA
TCA
CoA
lipoamide (reduced)
FAD
Dihydrolipoyl dehydrogenase
$FADH_2$
NADH
NAD^+
electron transport

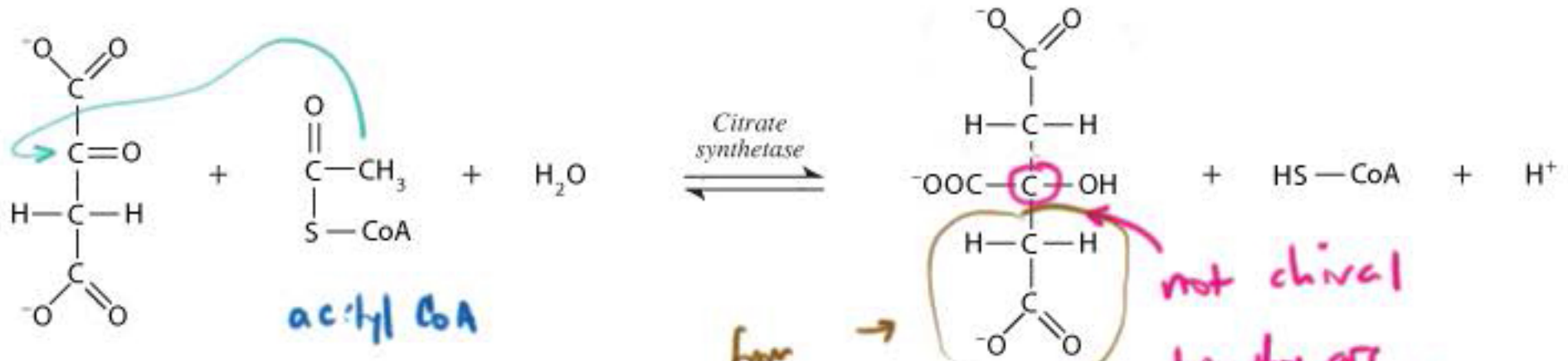
acetyl CoA



carrier of activated acyl groups

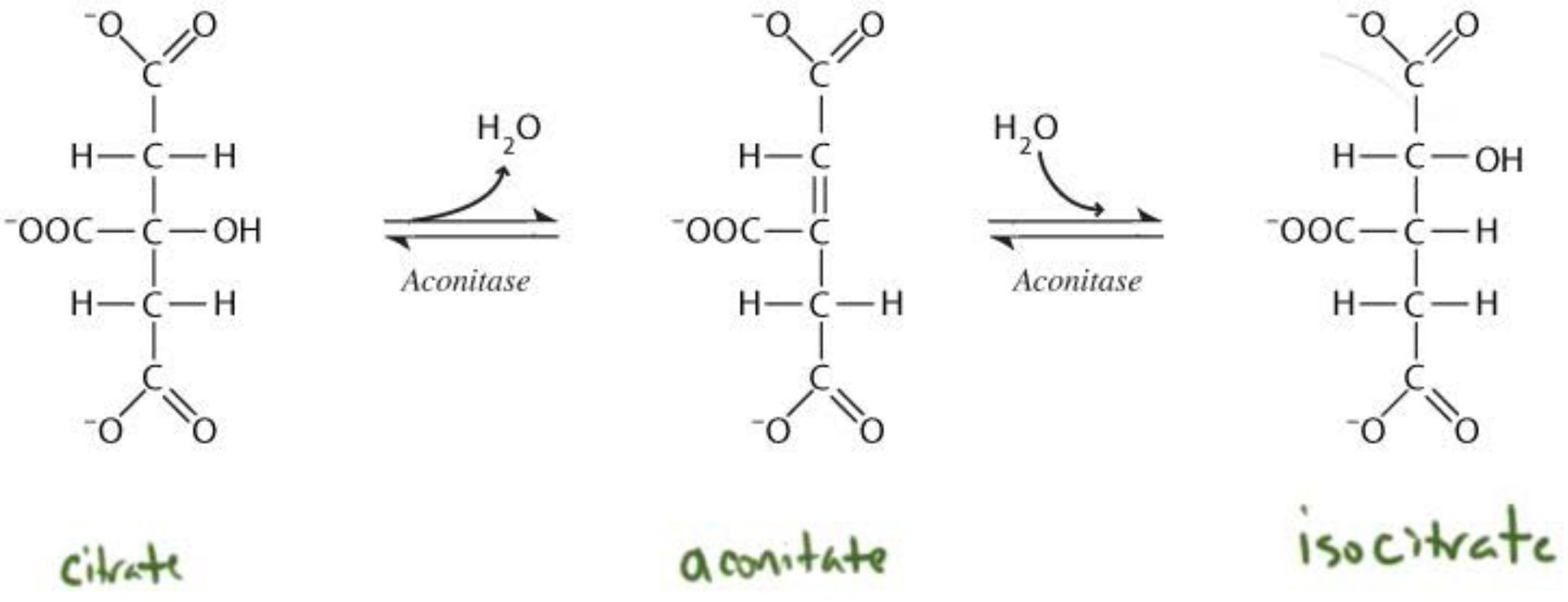


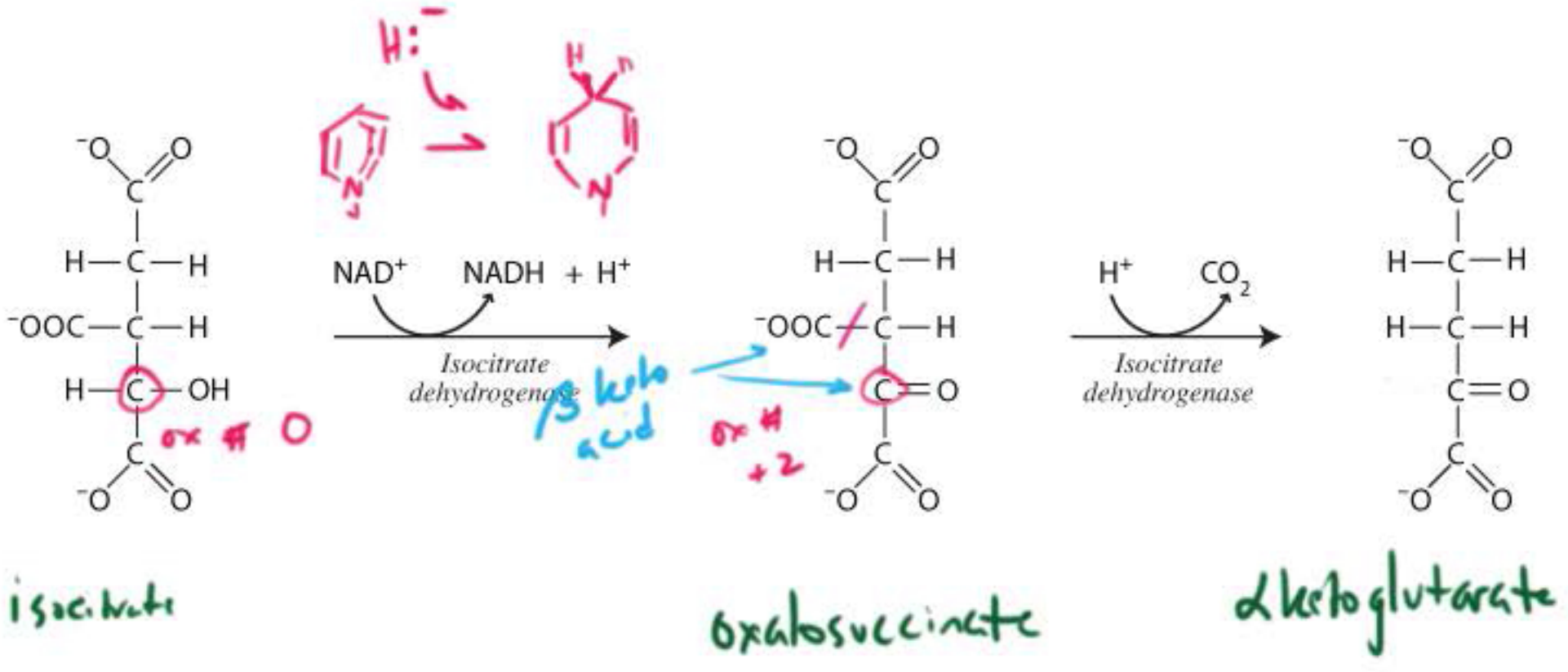
The plot is oxidative phosphorylation

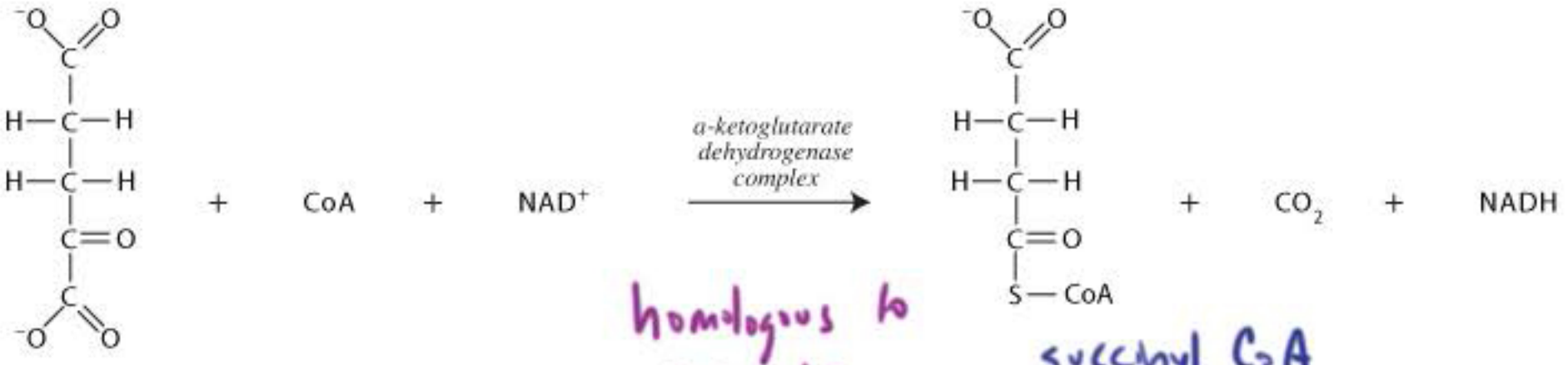


ΔG is from hydrolysis of CoA

The CO_2 produced is never from the acetyl CoA end.


H2O
Aconitase
H2O
Aconitase
citrate
aconitate
isocitrate


H:⁻
NAD⁺
NADH + H⁺
Isocitrate dehydrogenase
β keto acid
ox # 0
ox # +2
H⁺
CO₂
Isocitrate dehydrogenase
isocitrate
oxalosuccinate
α ketoglutarate

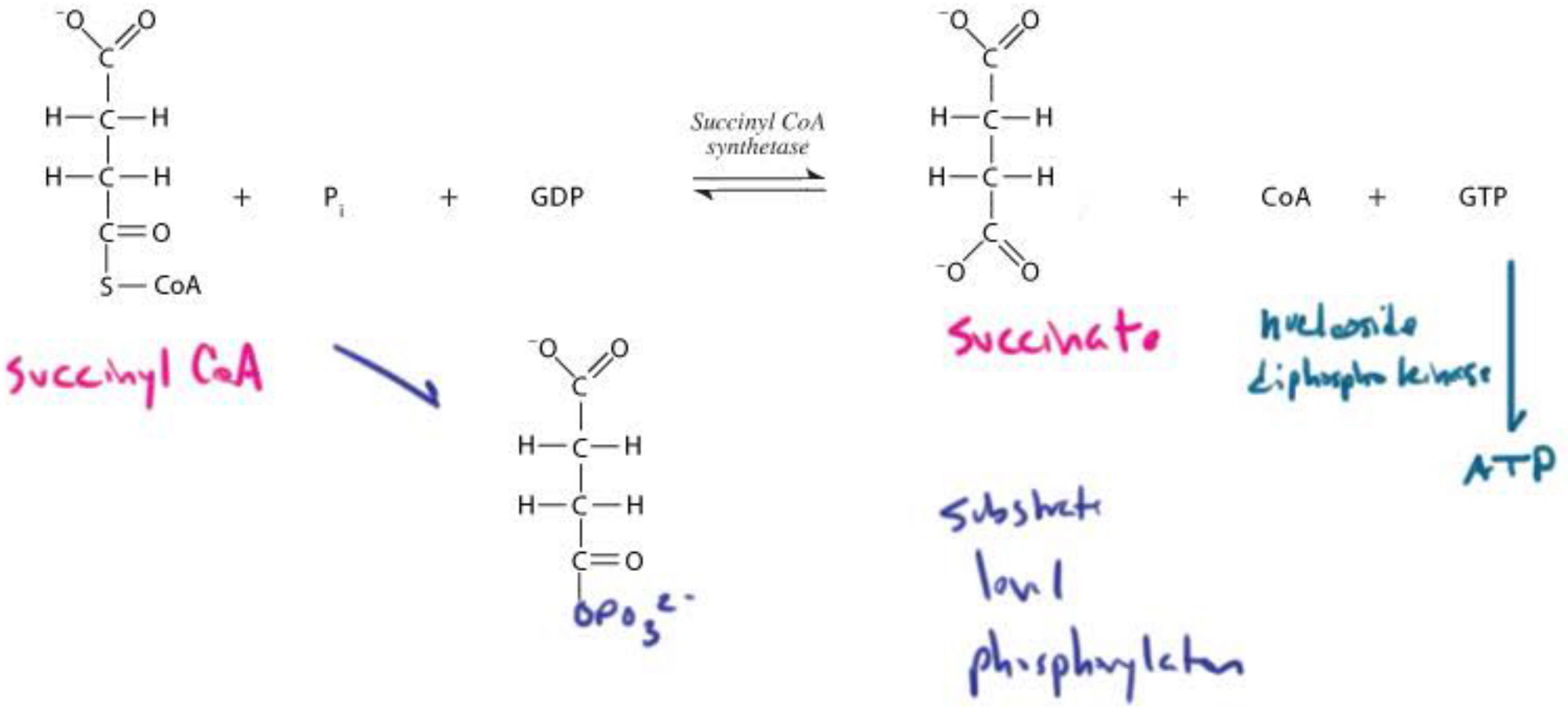


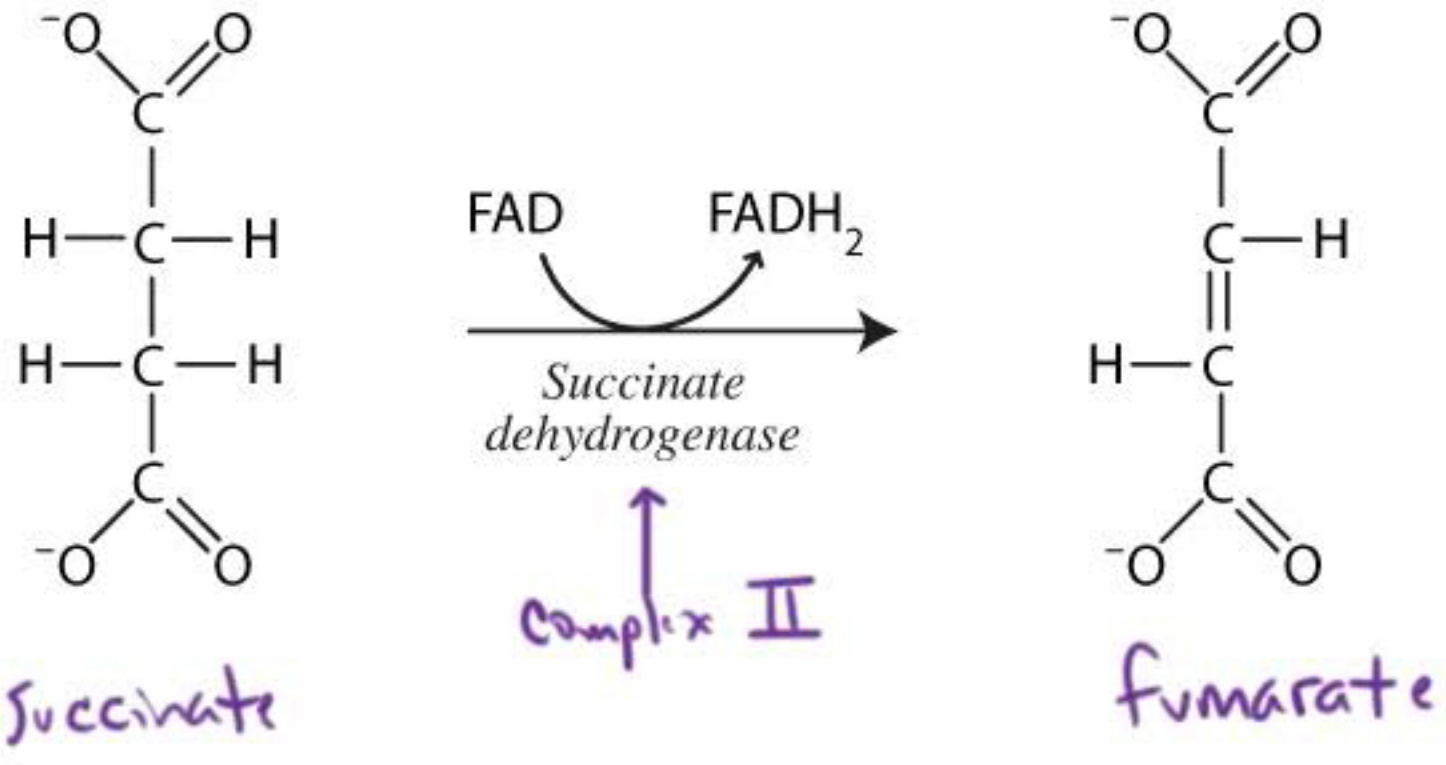
α ketoglutarate

homologous to pyruvate dehydrogenase

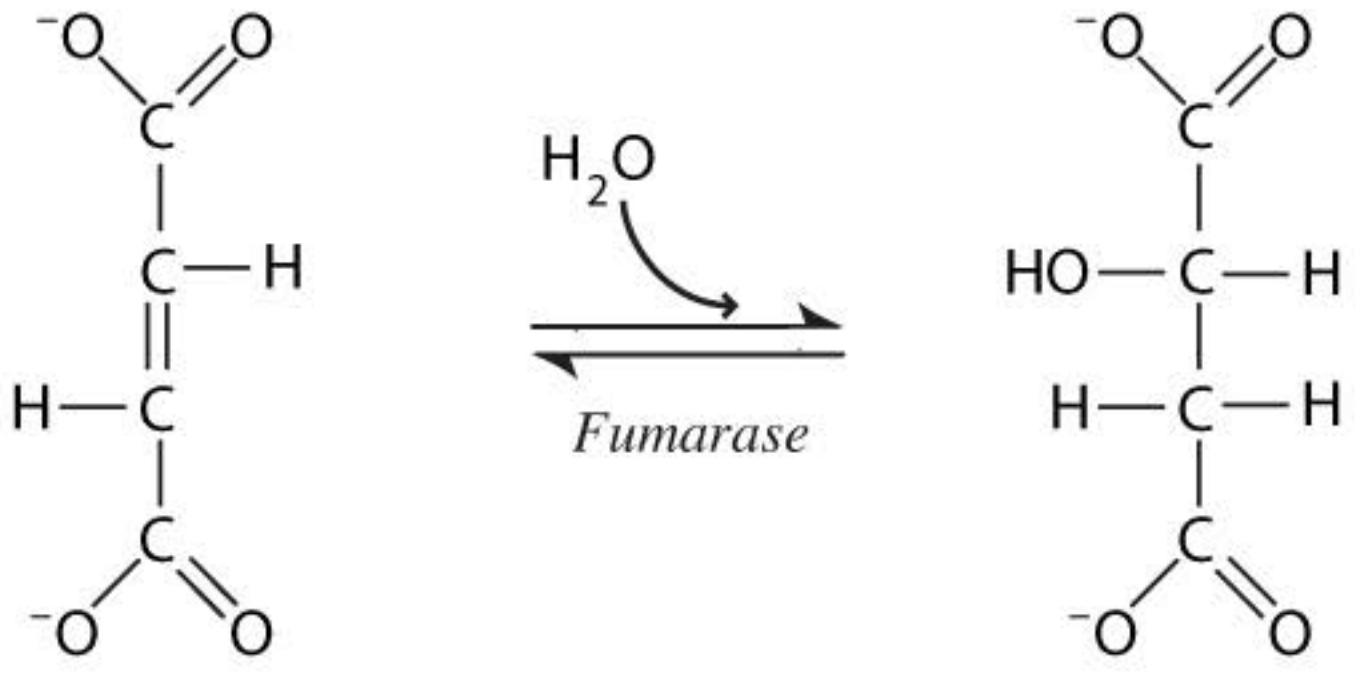
succinyl CoA

TPP
lipoamide
CoA
FAD
NAD^+


Succinyl CoA synthetase
+ P_i + GDP
+ CoA + GTP
Succinyl CoA
Succinate
nucleoside diphosphokinase
ATP
OPO_3^{2-}
substrate level phosphorylation



The step in TCA intersects with the electron transport chain.



fumarate

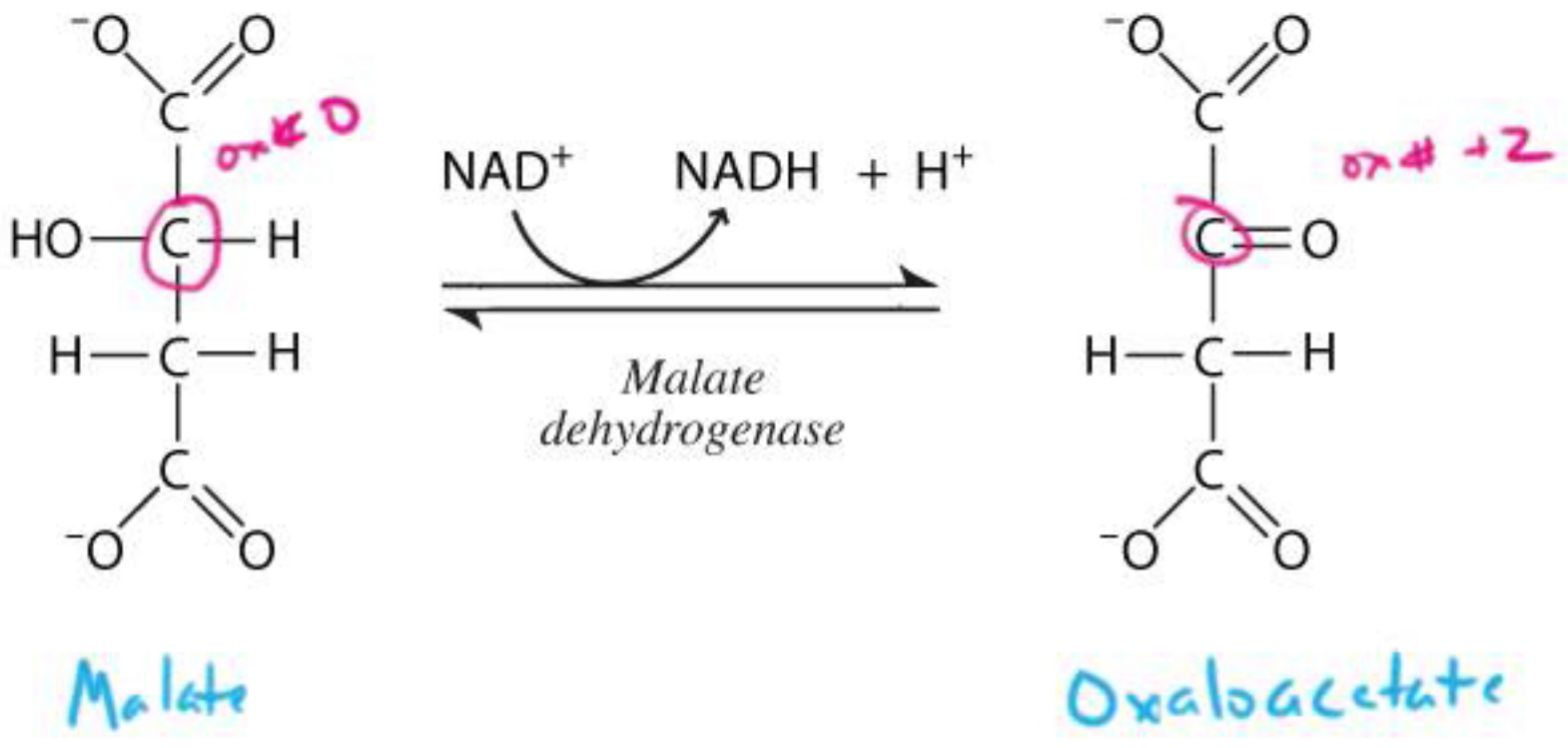
malate

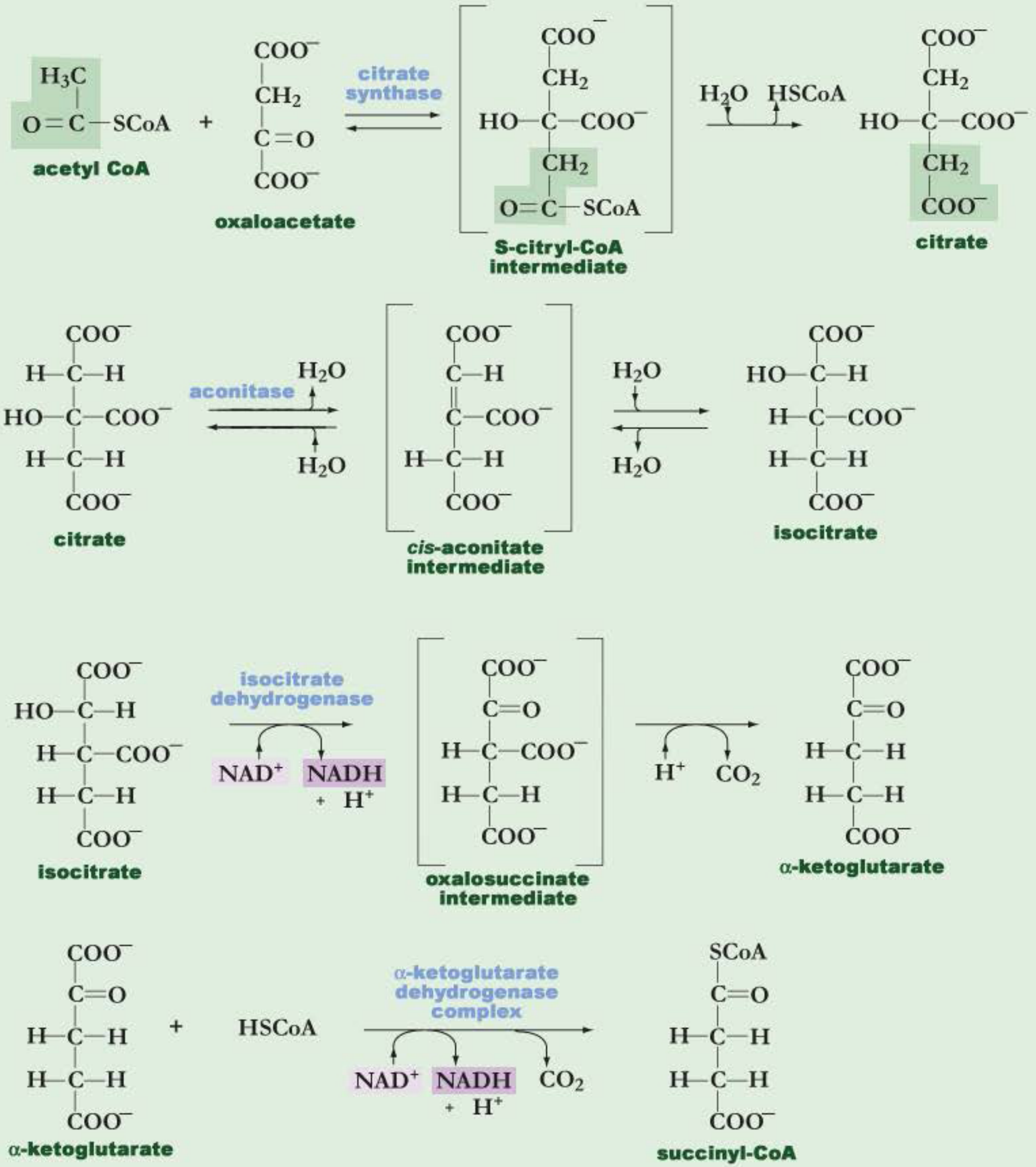
$k_M = \frac{k_2 + k_{-1}}{k_1}$

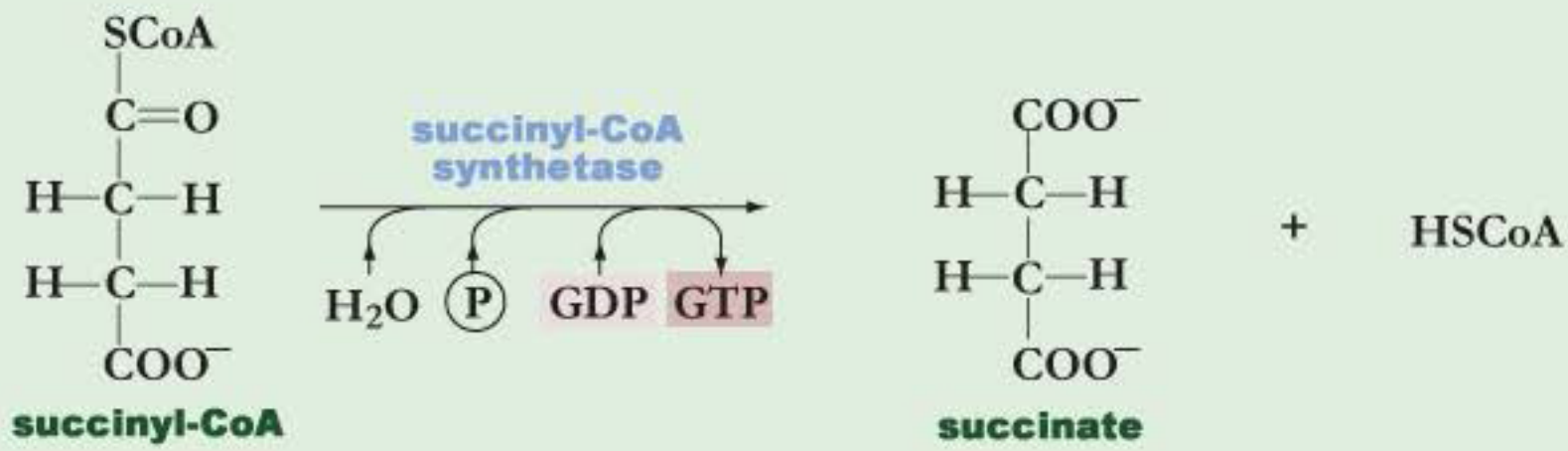
• has achieved enzymatic perfection

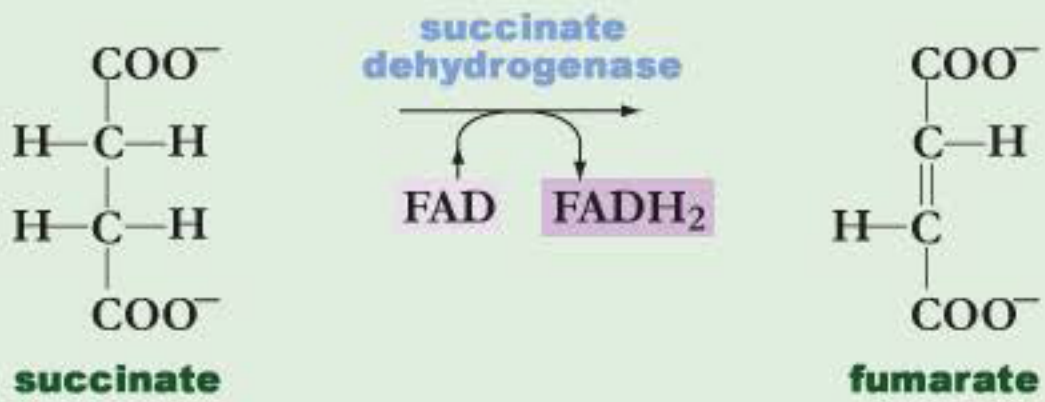
$k_2 = k_{cat}$

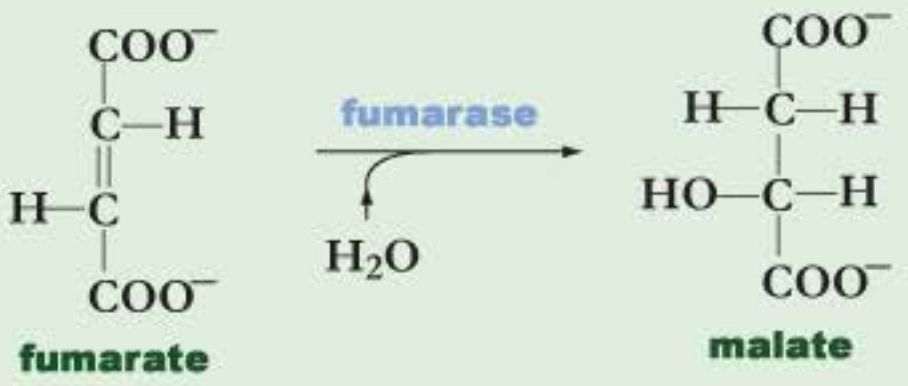
catalytic efficiency : $\frac{k_{cat}}{K_M}$ as high as possible $= \frac{k_1 k_{cat}}{k_{cat} + k_{-1}}$

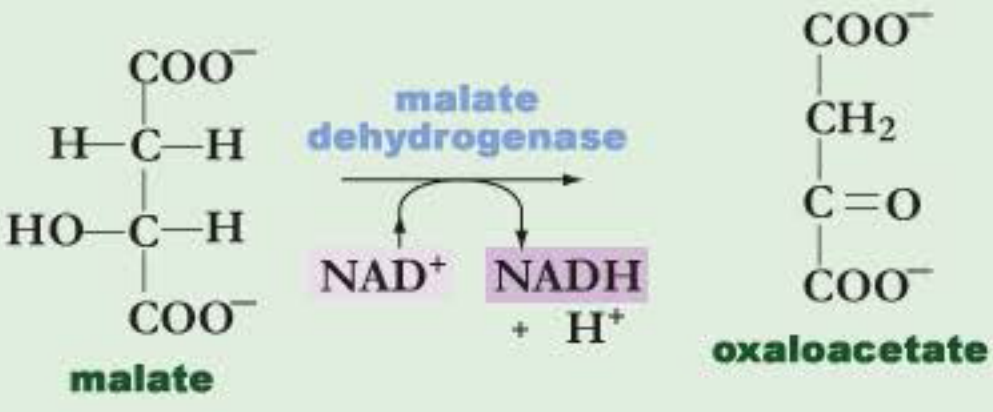

NAD+
NADH + H+
Malate dehydrogenase
Malate
Oxaloacetate

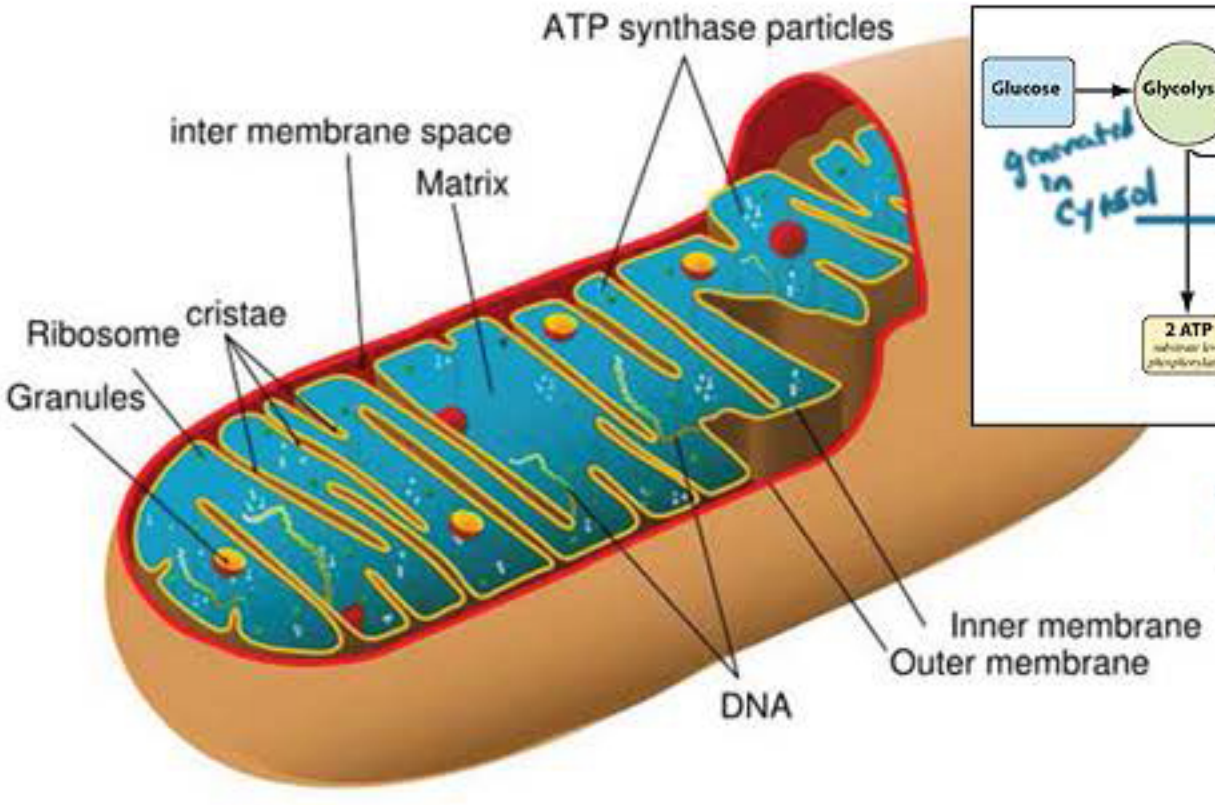

acetyl CoA
oxaloacetate
citrate synthase
S-citryl-CoA intermediate
H2O
HSCoA
citrate
citrate
aconitase
H2O
H2O
cis-aconitate intermediate
H2O
H2O
isocitrate
isocitrate
isocitrate dehydrogenase
NAD+
NADH + H+
oxalosuccinate intermediate
H+
CO2
α-ketoglutarate
α-ketoglutarate
HSCoA
α-ketoglutarate dehydrogenase complex
NAD+
NADH + H+
CO2
succinyl-CoA

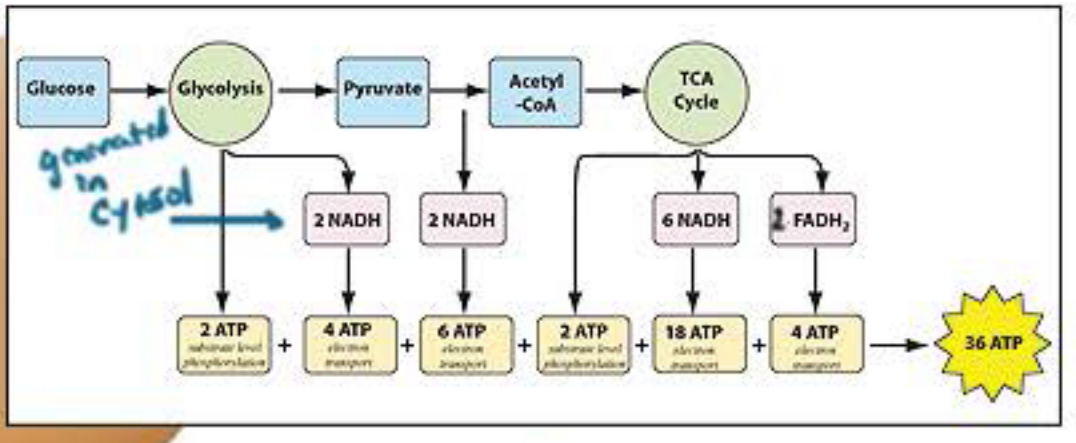

SCoA
C=O
H—C—H
H—C—H
COO⁻
succinyl-CoA
succinyl-CoA synthetase
H2O
P
GDP
GTP
COO⁻
H—C—H
H—C—H
COO⁻
succinate
+
HSCoA


COO⁻
H—C—H
H—C—H
COO⁻
succinate
succinate dehydrogenase
FAD
FADH2
COO⁻
C—H
H—C
COO⁻
fumarate


COO⁻
C—H
H—C
COO⁻
fumarate
fumarase
H2O
COO⁻
H—C—H
HO—C—H
COO⁻
malate


COO⁻
H—C—H
HO—C—H
COO⁻
malate
malate dehydrogenase
NAD+
NADH
+ H+
COO⁻
CH2
C=O
COO⁻
oxaloacetate





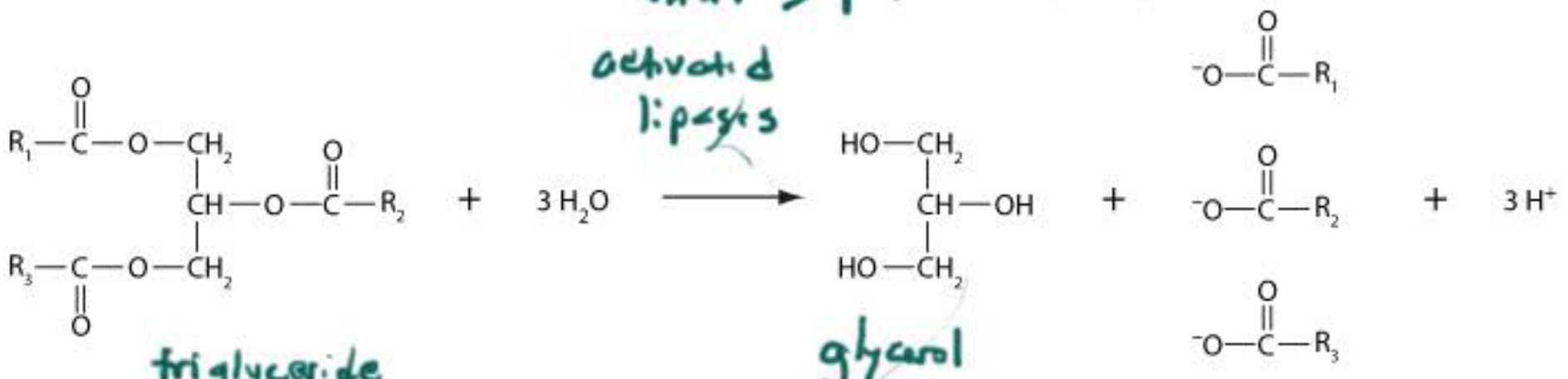
NADH from glycolysis, electrons are shuttled.

Aerobic bacteria yield 38 ATP

Boxidation

hydrolysis by
cAMP ⇒ protein kinase A

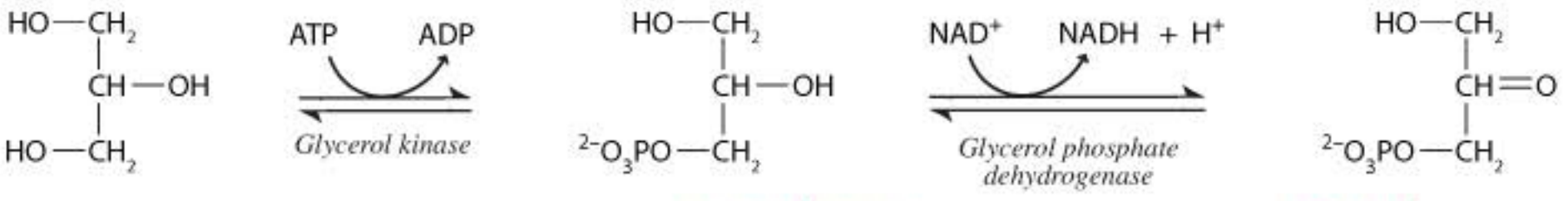
activated
lipases



triglyceride

glycerol
(gluconeogenic)

Fatty acids
(ketogenic)



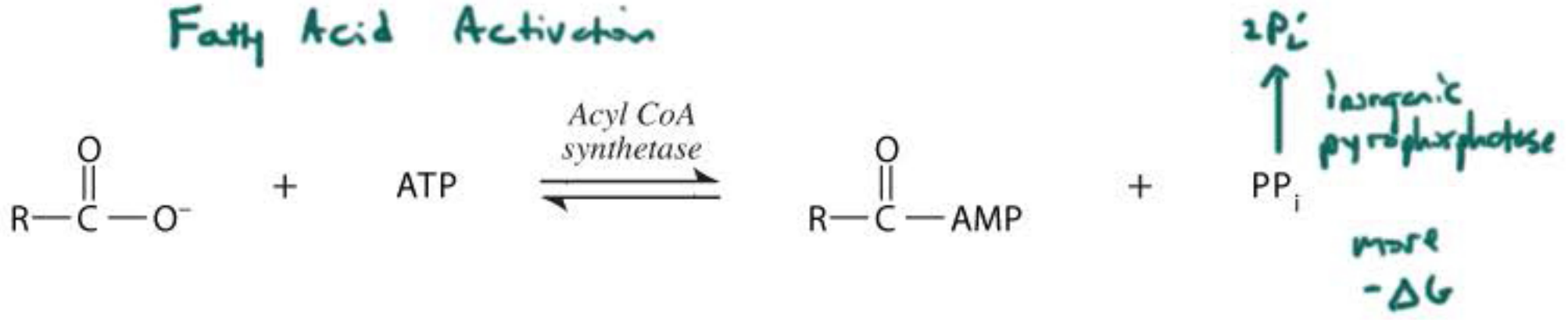
glycerol 3-phosphate

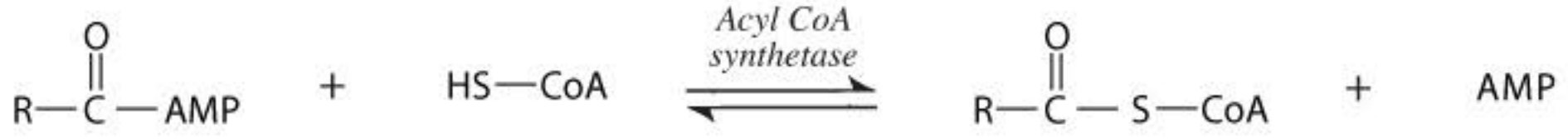
DAP

↓

glycolysis or gluconeogenesis

Fatty Acid Activation



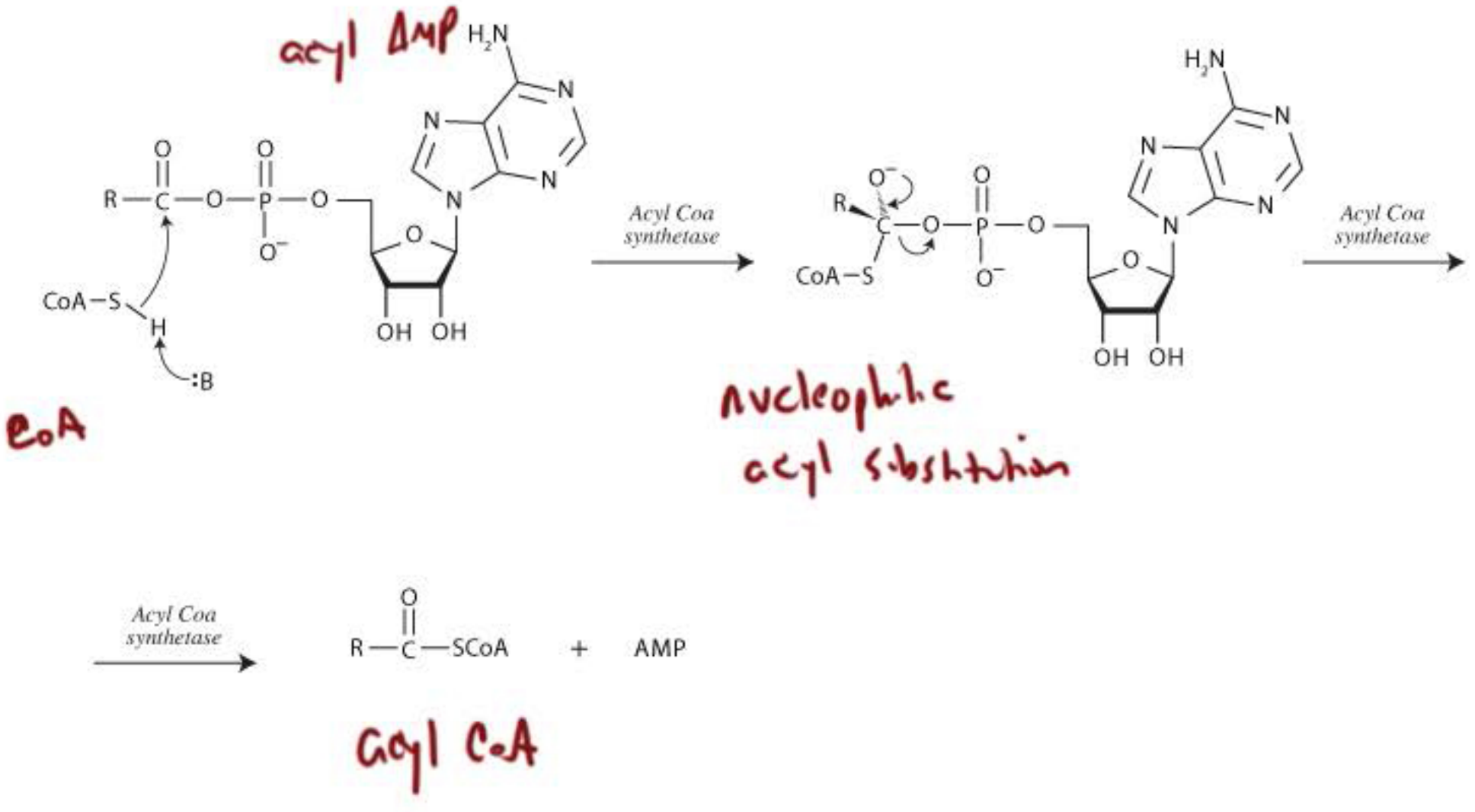


acyl AMP

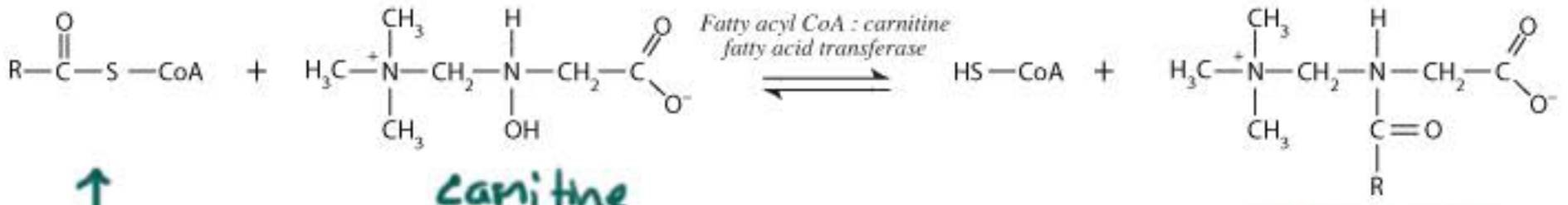
activated (phosphate anhydride)

acyl CoA

activated (thioester)


acyl AMP
CoA
nucleophilic acyl substitution
Acyl Coa synthetase
Acyl Coa synthetase
Acyl Coa synthetase
R—C—SCoA + AMP
acyl CoA

Getting acyl CoA into the mitochondrion for β oxidation



in cytosol

carnitine

shuttle

acyl carnitine

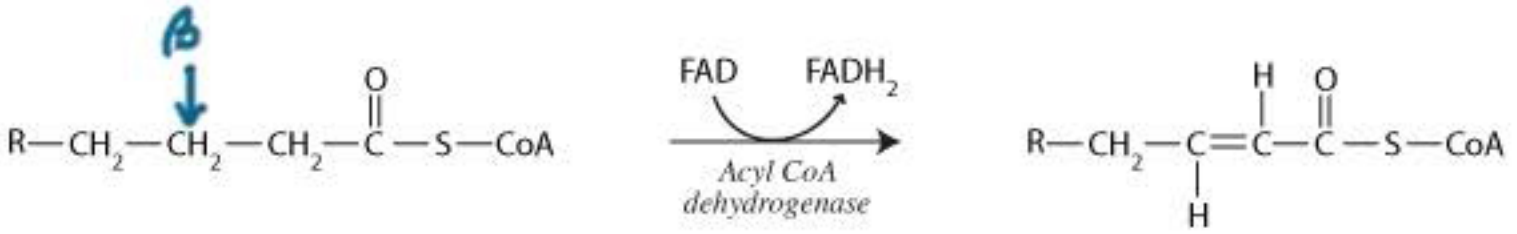
mitochondrion

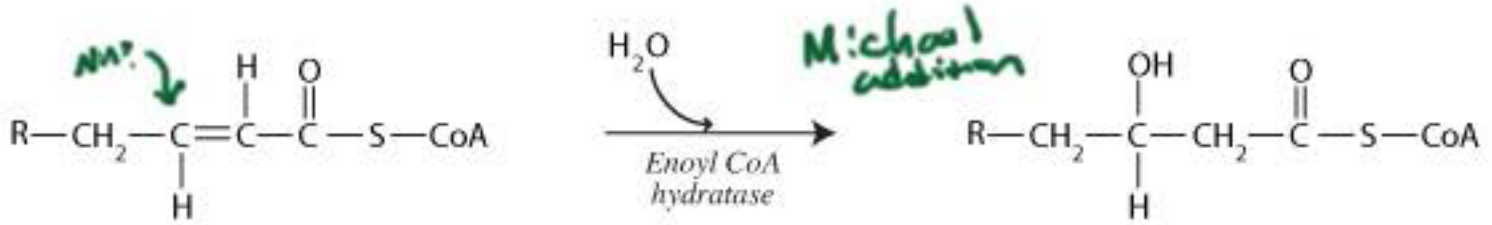
CoA

acyl CoA

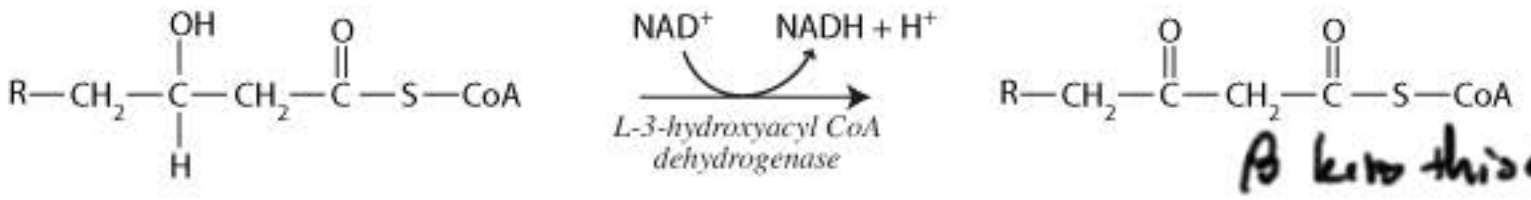
β oxidation

carnitine

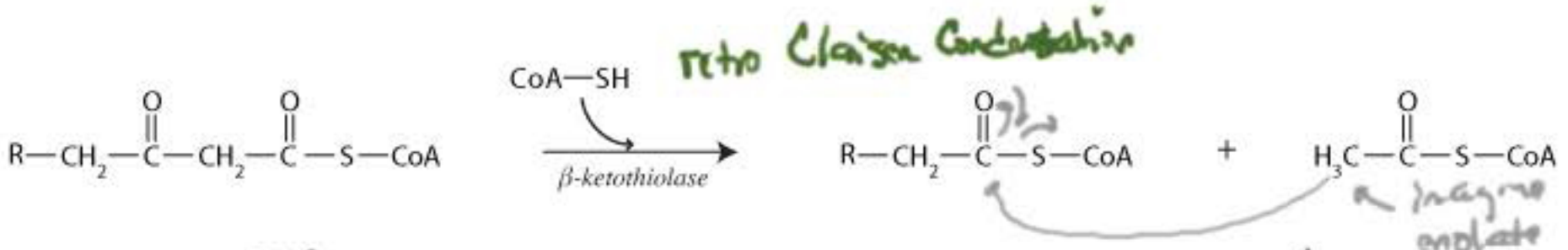


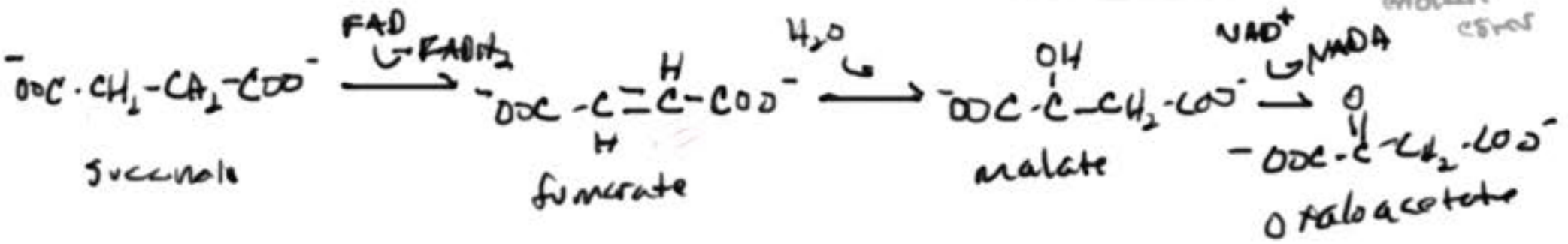


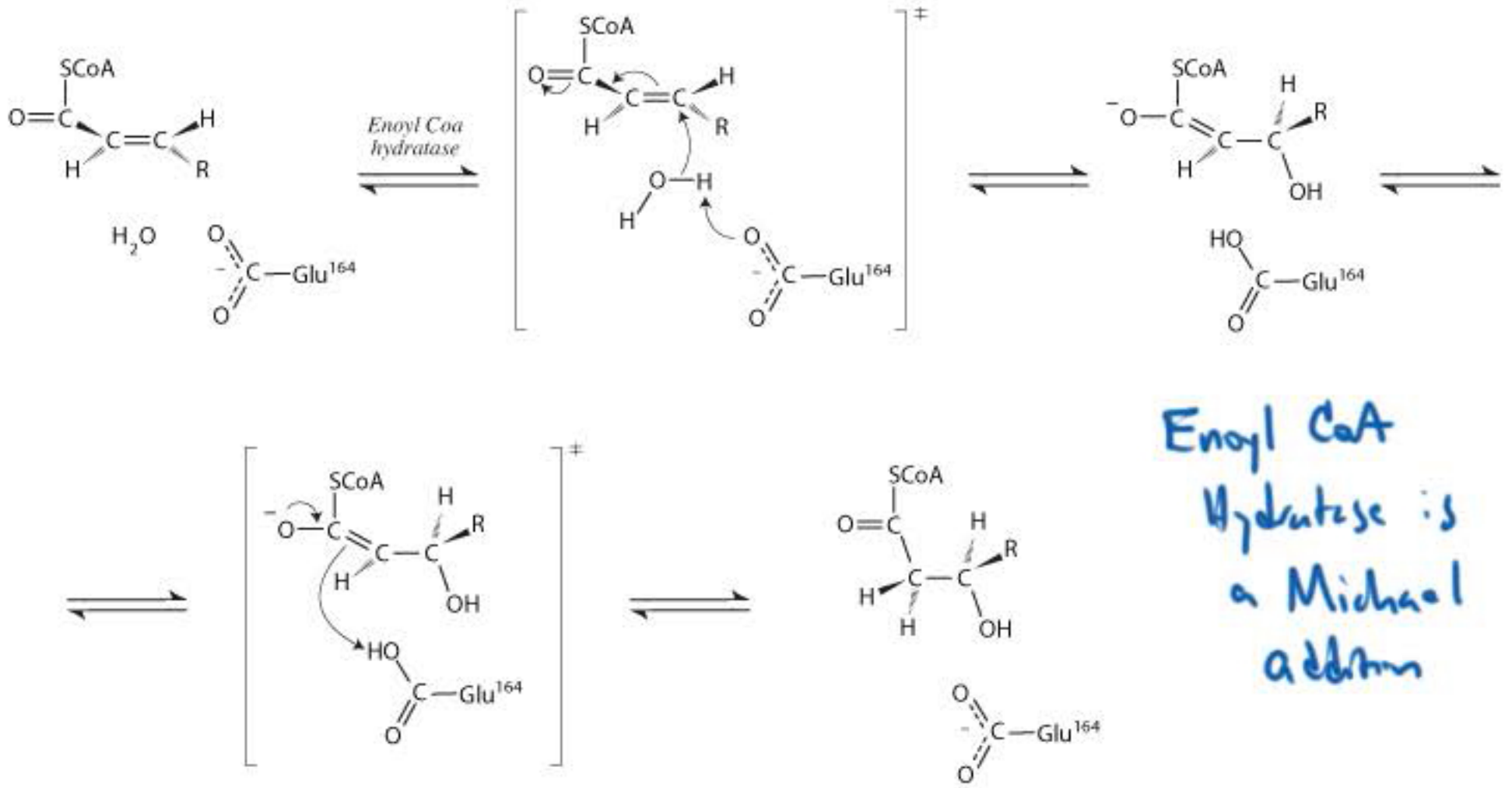
(Michael addition - nucleophiles add at β position of α,β unsaturated carbonyl compounds)



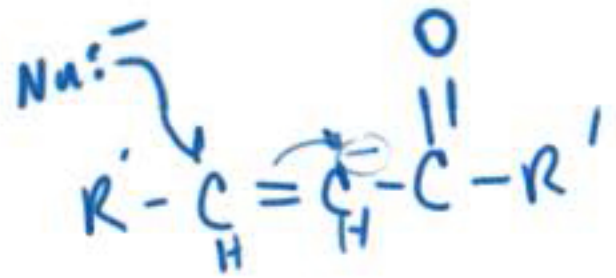
β keto thioester



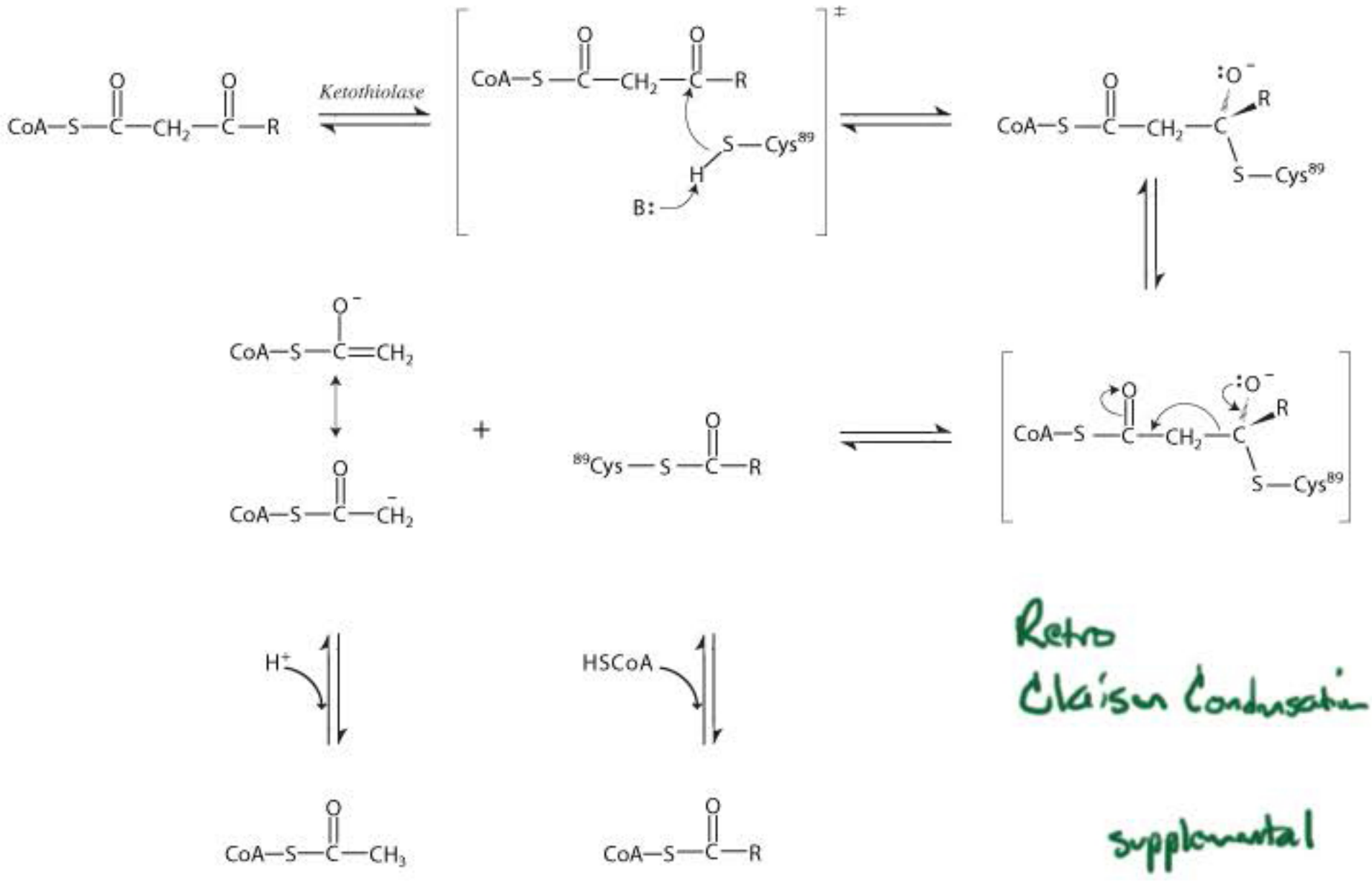




Enoyl CoA Hydratase is a Michael addition



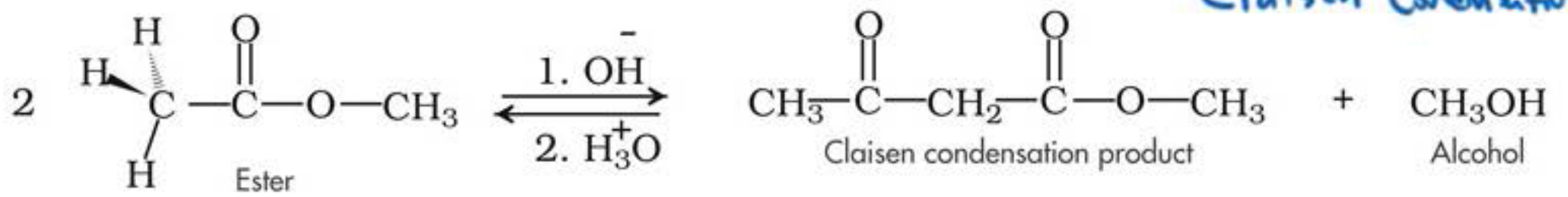
Nucleophiles add to the β carbon of α-β unsaturated carbonyl compounds.



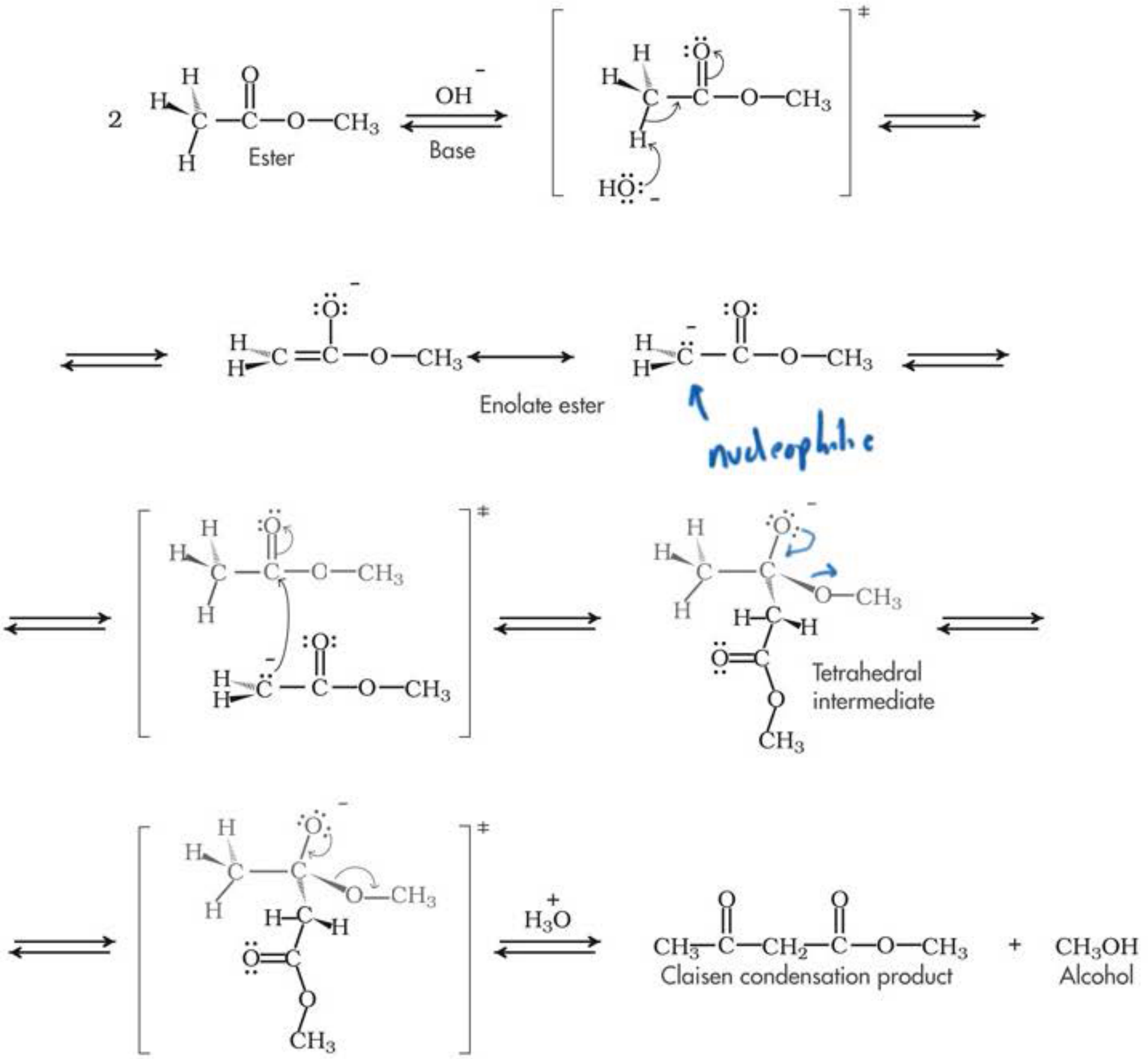
Retro
Claisen Condensation

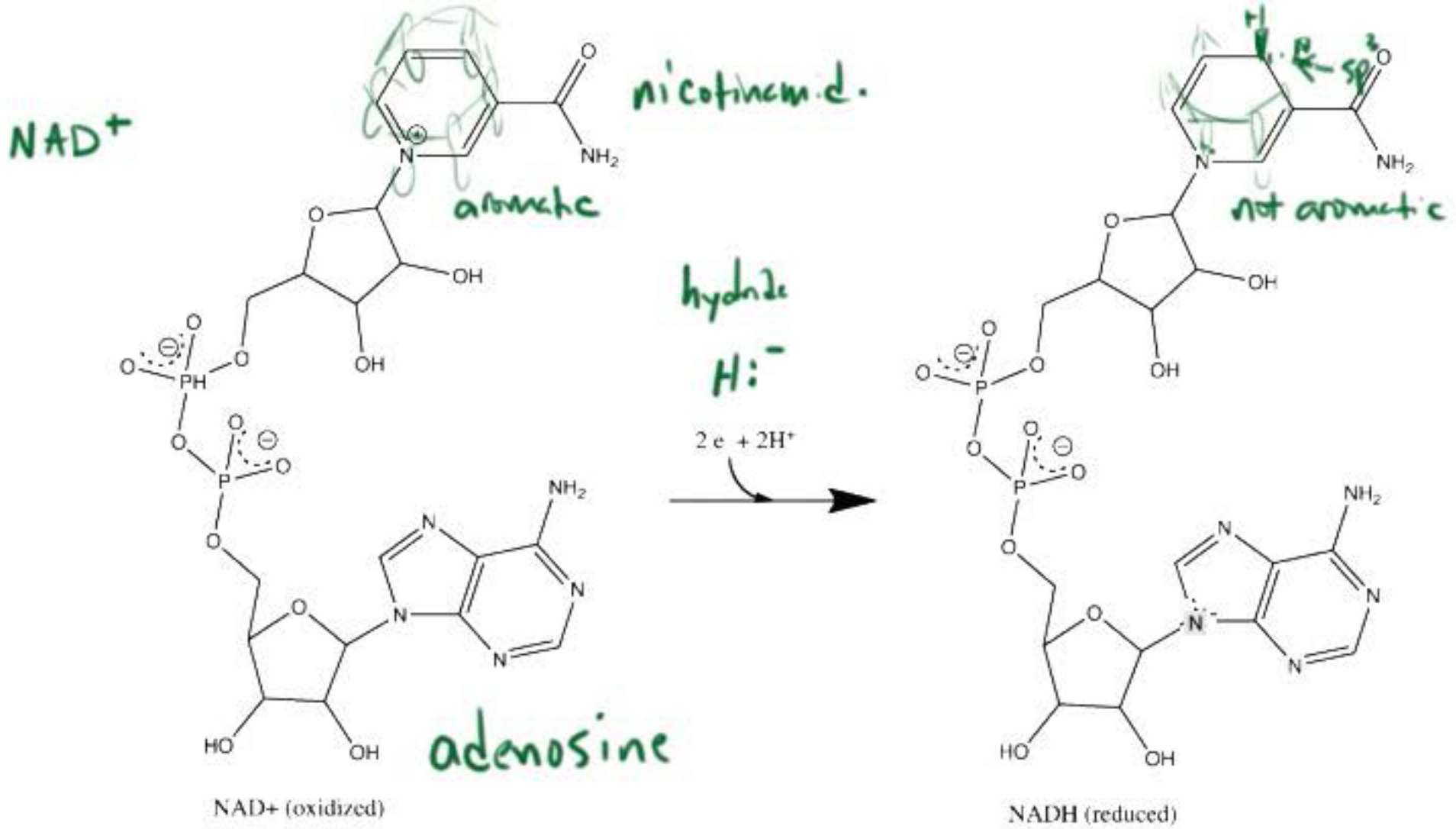
supplemental

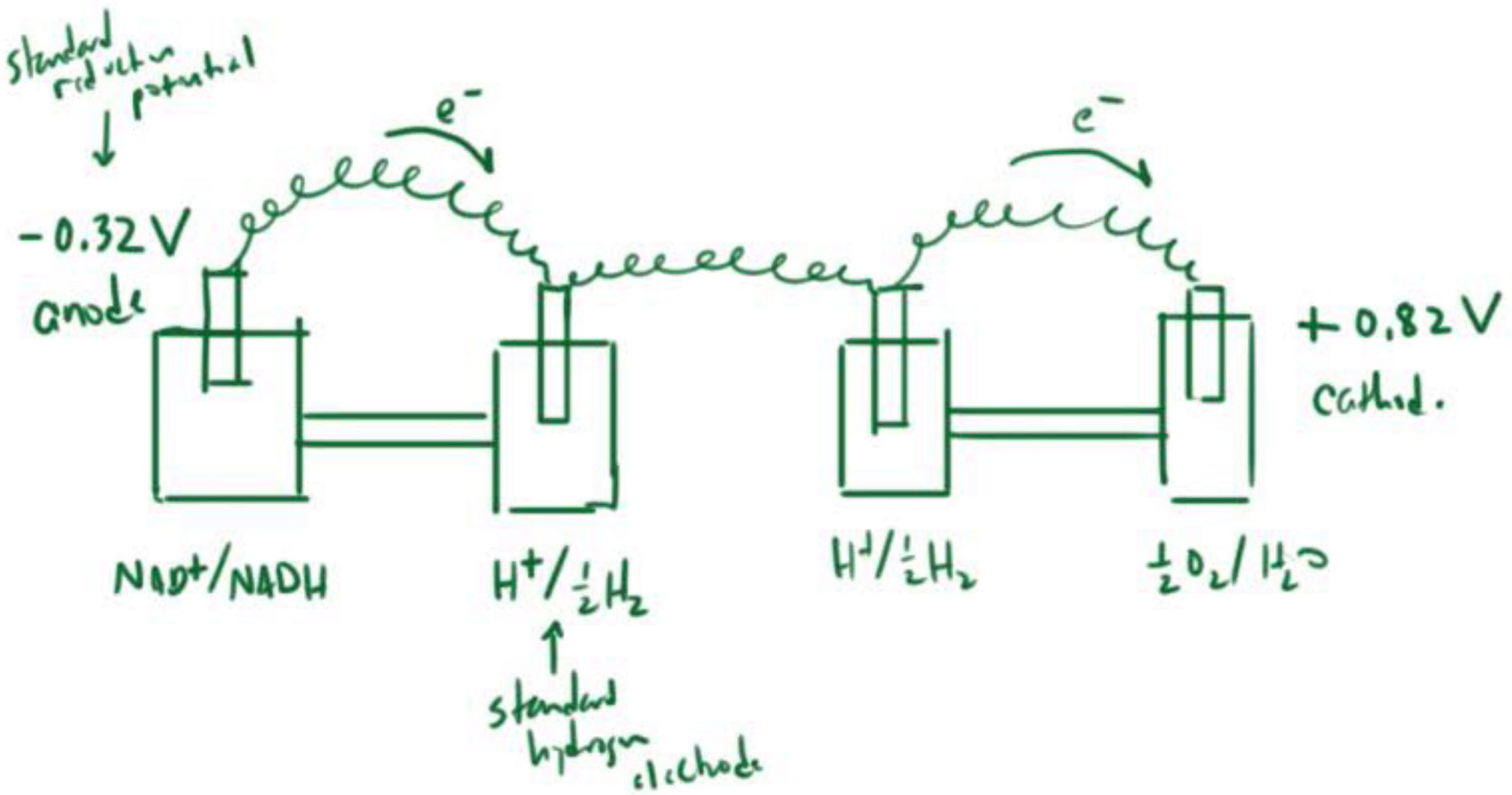
Claisen Condensation



like aldol addition but following the nucleophilic acyl substitution pattern

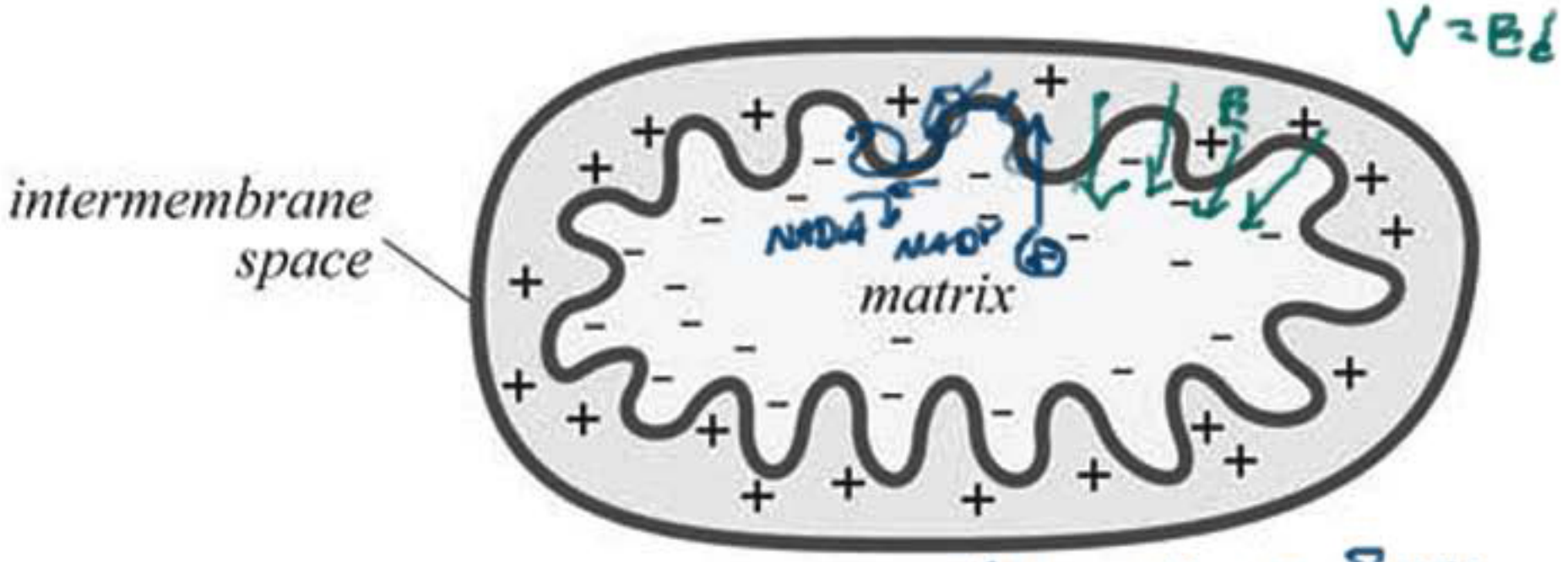






$$E_{cell} = E^{\circ}_{cathode} - E^{\circ}_{anode}$$

$$(0.82) - (-0.32) = 1.14\,V$$

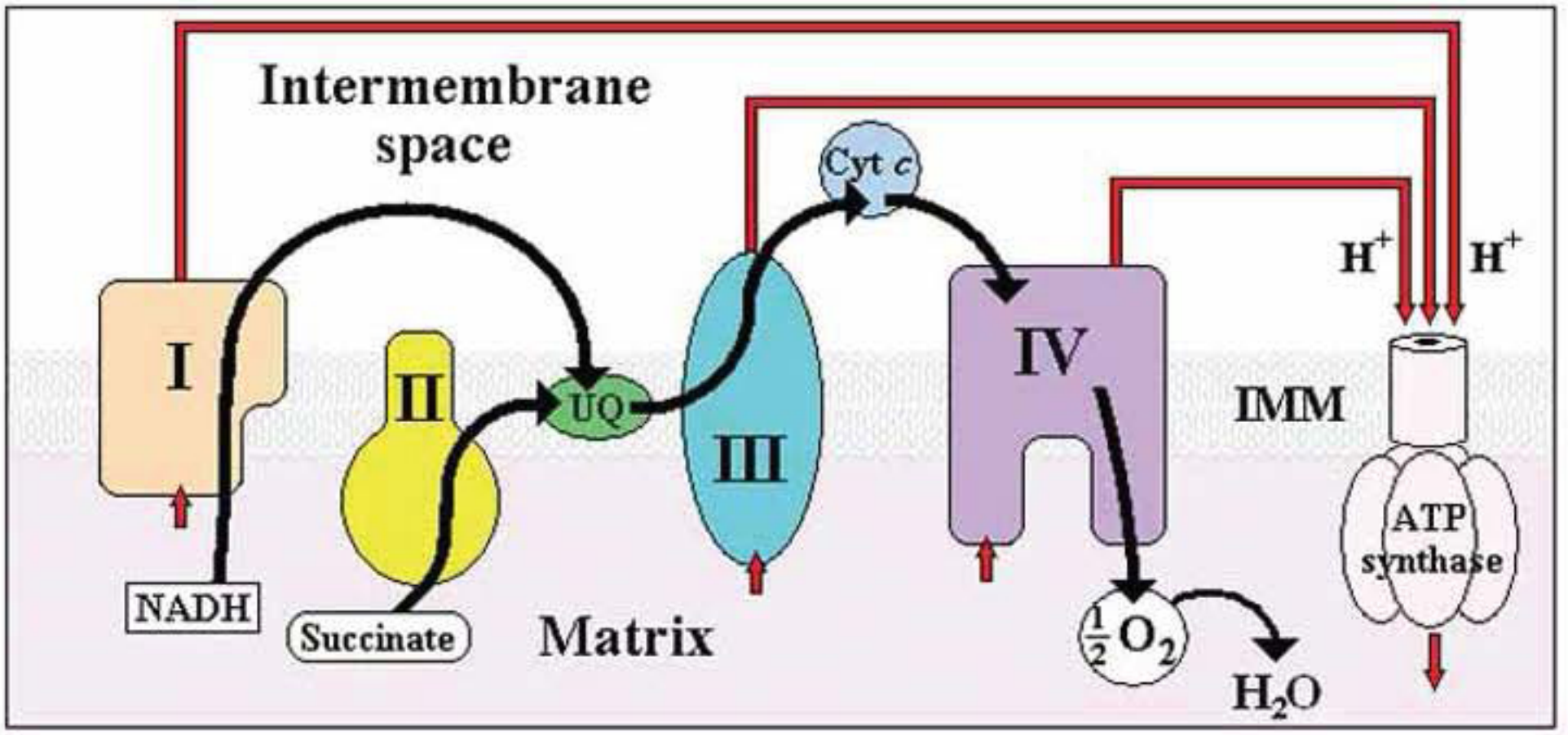


Proton Motive Force

electrostatic potential : 0.16 V

diffusion gradient : 0.05V

210 mV



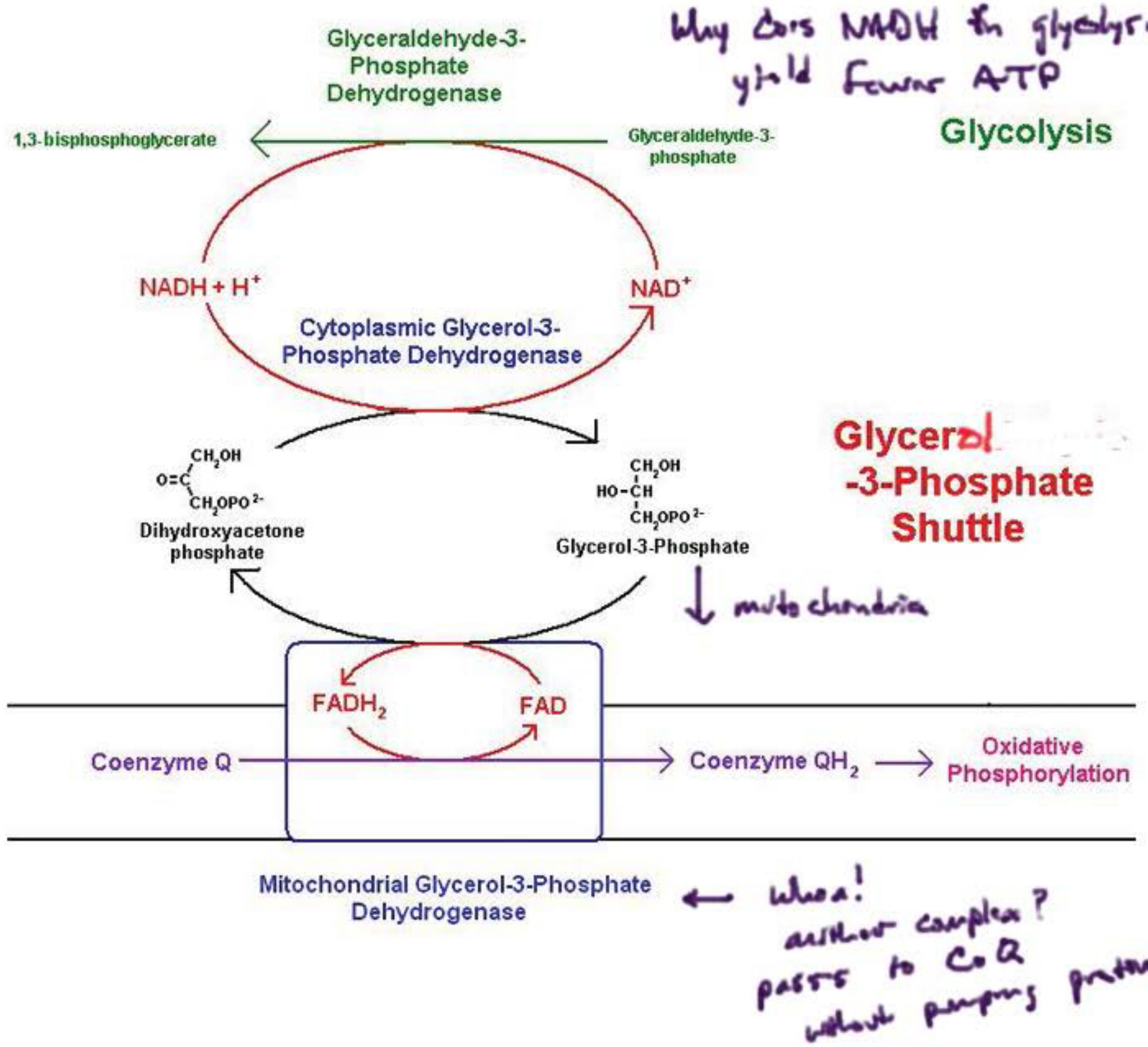
I - NADH ubiquinone oxido reductase

II - Succinate dehydrogenase

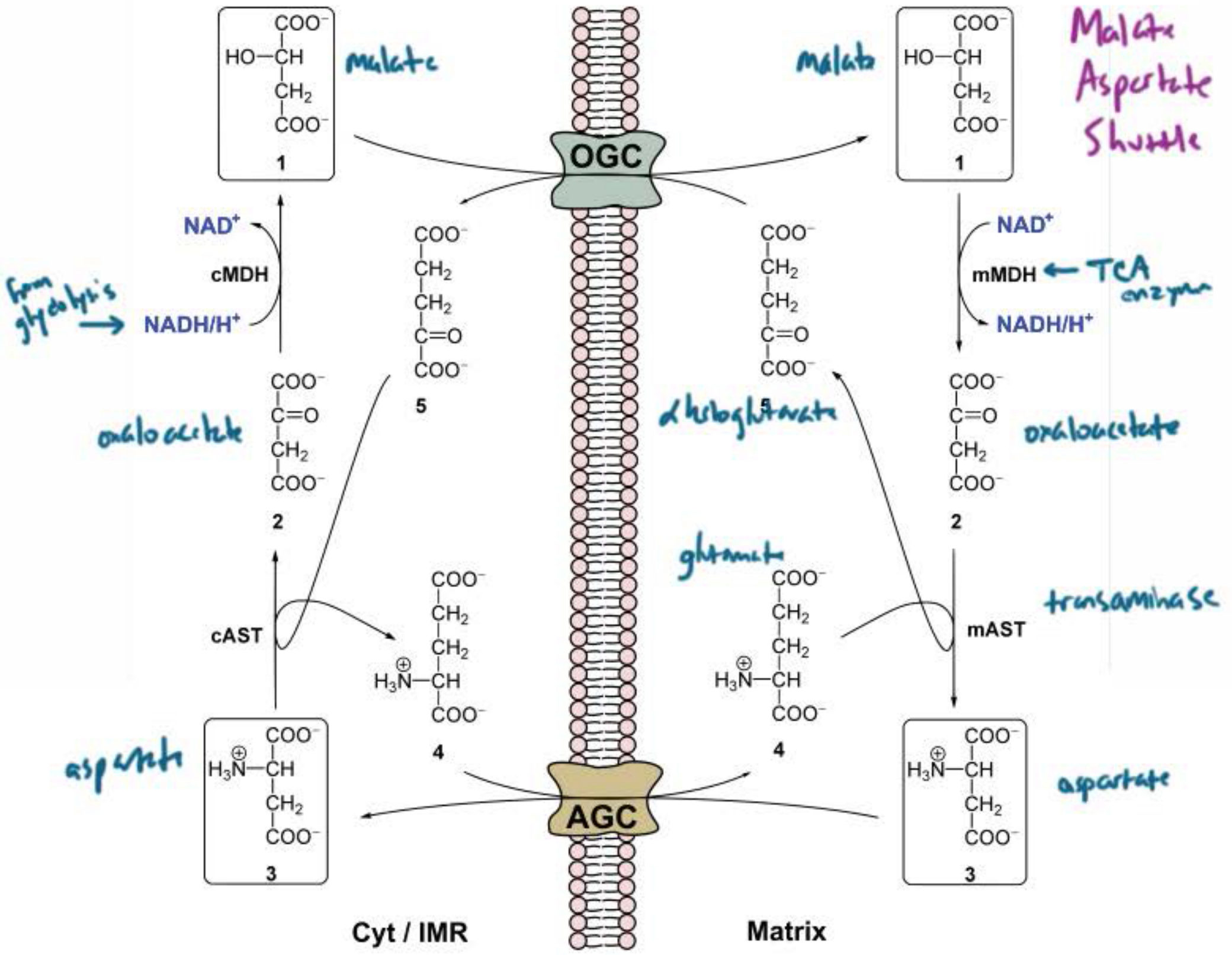
III - Cytochrome c reductase

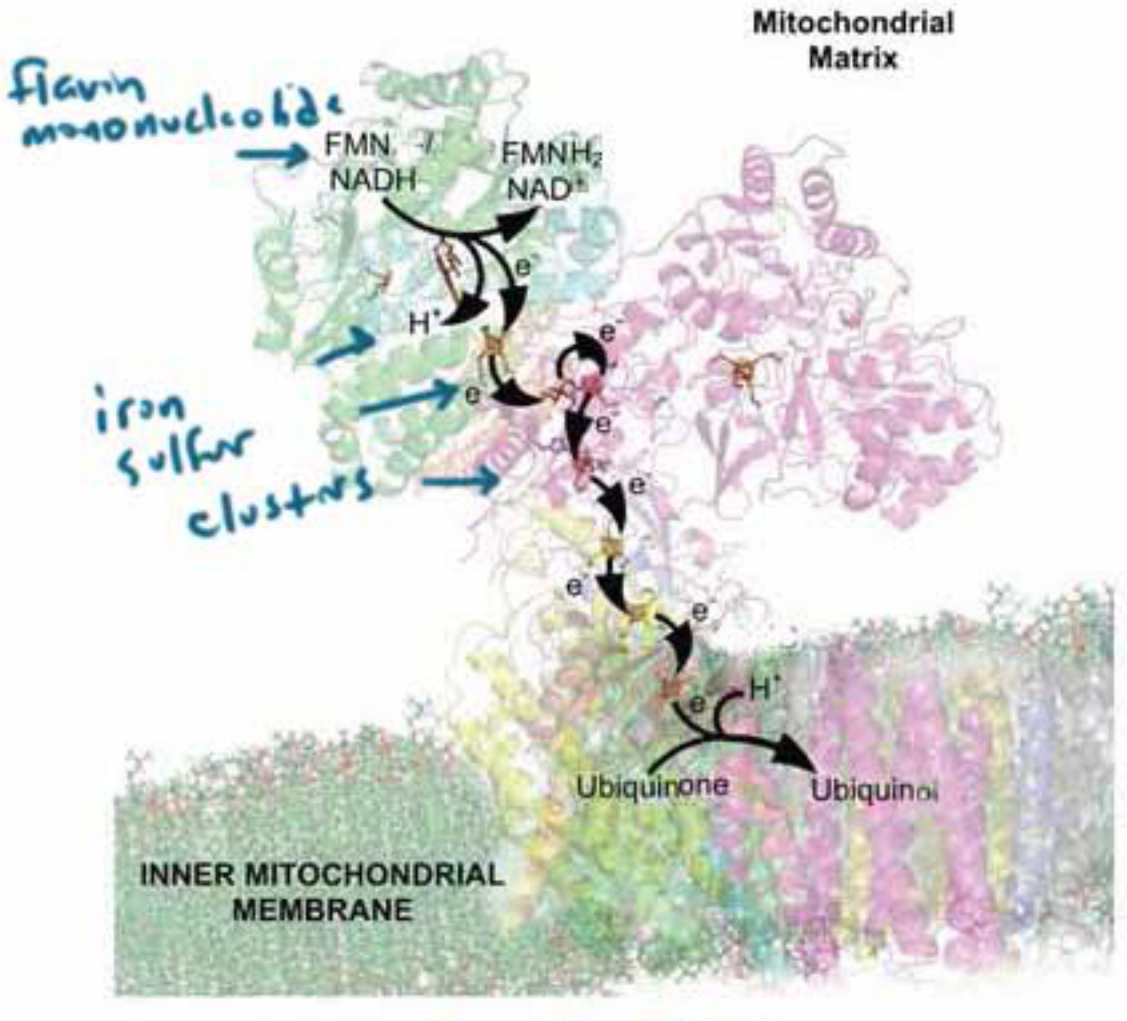
IV - Cytochrome c oxidase

Why does NADH from glycolysis yield fewer ATP

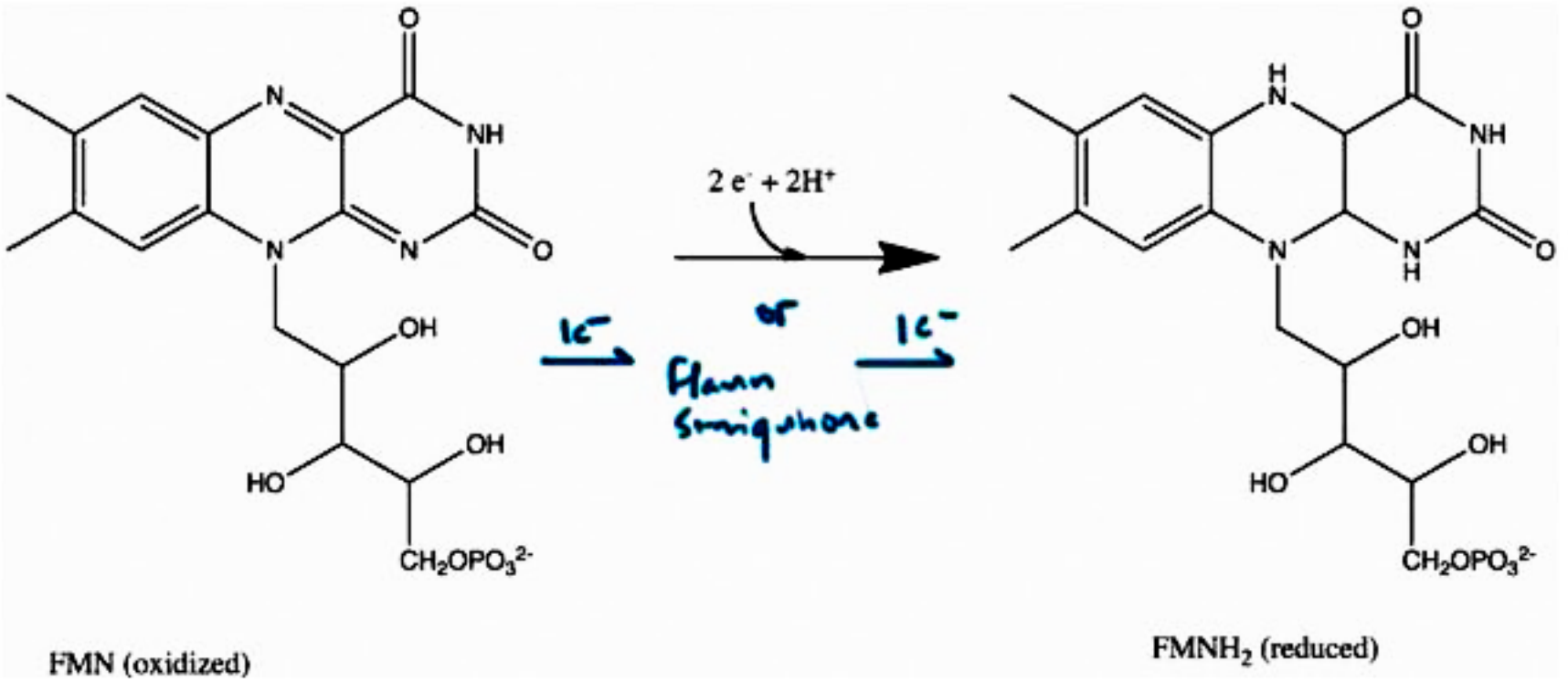


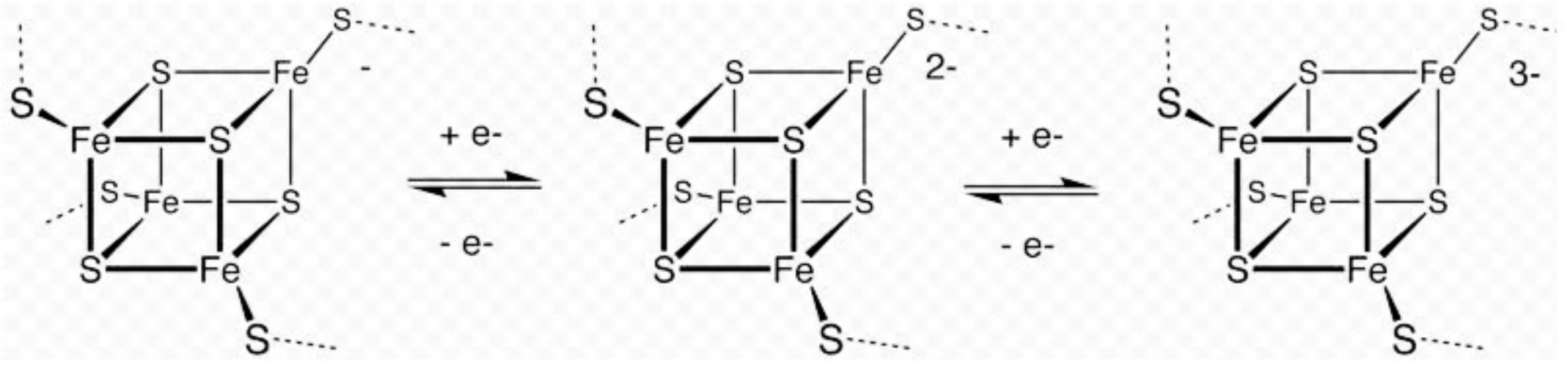
Whoa! another complex? passes to CoQ without pumping protons


Malate Aspartate Shuttle
OGC
AGC
malate
malate
1
1
NAD+
cMDH
NADH/H+
from glycolysis
NAD+
mMDH
NADH/H+
TCA enzyme
5
5
α ketoglutarate
oxaloacetate
oxaloacetate
2
2
glutamate
cAST
mAST
transaminase
4
4
aspartate
aspartate
3
3
Cyt / IMR
Matrix


Mitochondrial Matrix
flavin mononucleotide
FMN
NADH
FMNH2
NAD+
e-
H+
iron sulfur clusters
Ubiquinone
Ubiquinol
INNER MITOCHONDRIAL MEMBRANE

Complex I

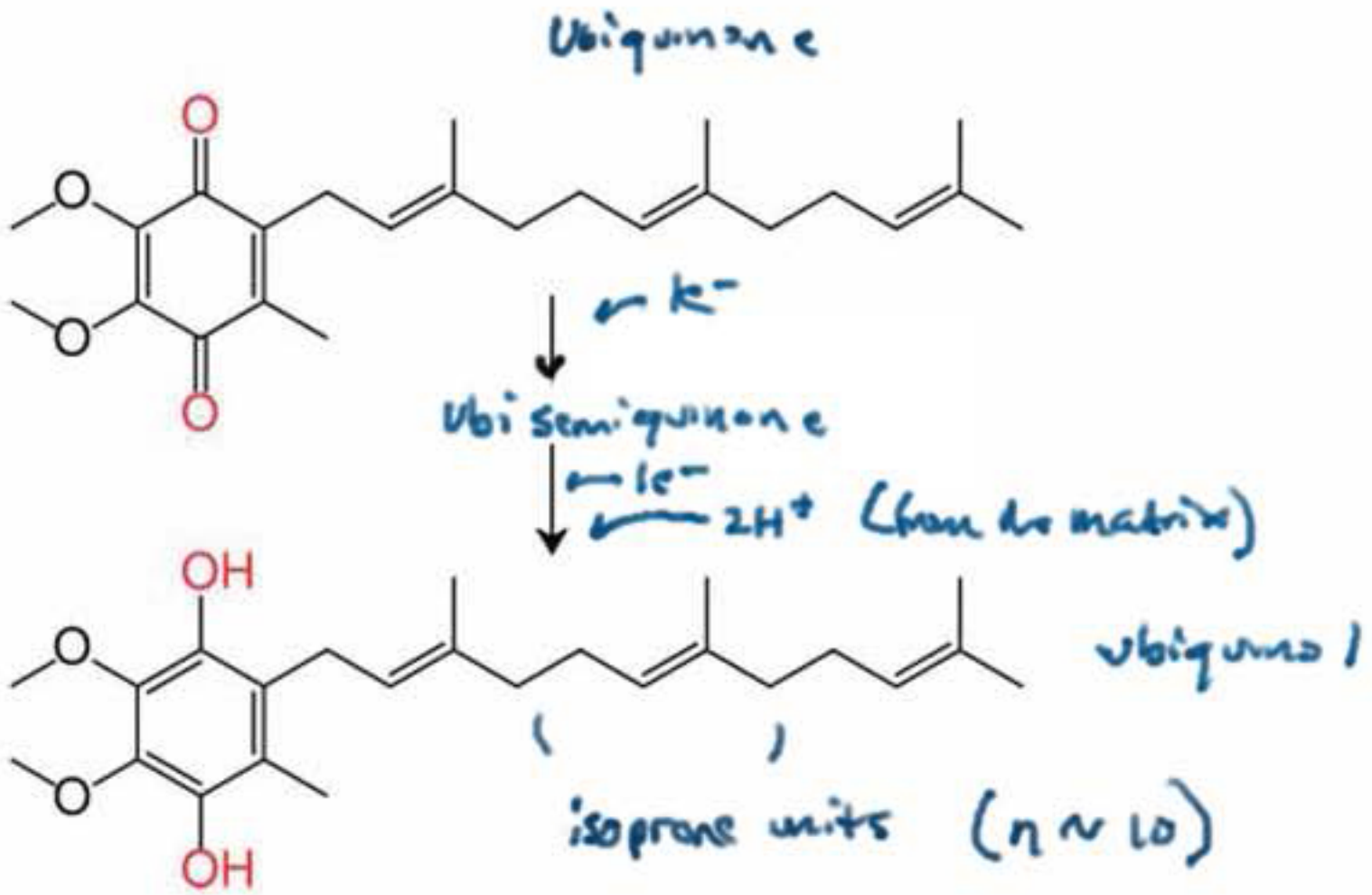

O
N
NH
N
N
O
OH
HO
OH
$CH_2OPO_3^{2-}$
$2\,e^- + 2H^+$
$1e^-$
or
Flavin
Semiquinone
$1e^-$
H
N
NH
N
N
H
O
OH
HO
OH
$CH_2OPO_3^{2-}$
FMN (oxidized)
$FMNH_2$ (reduced)



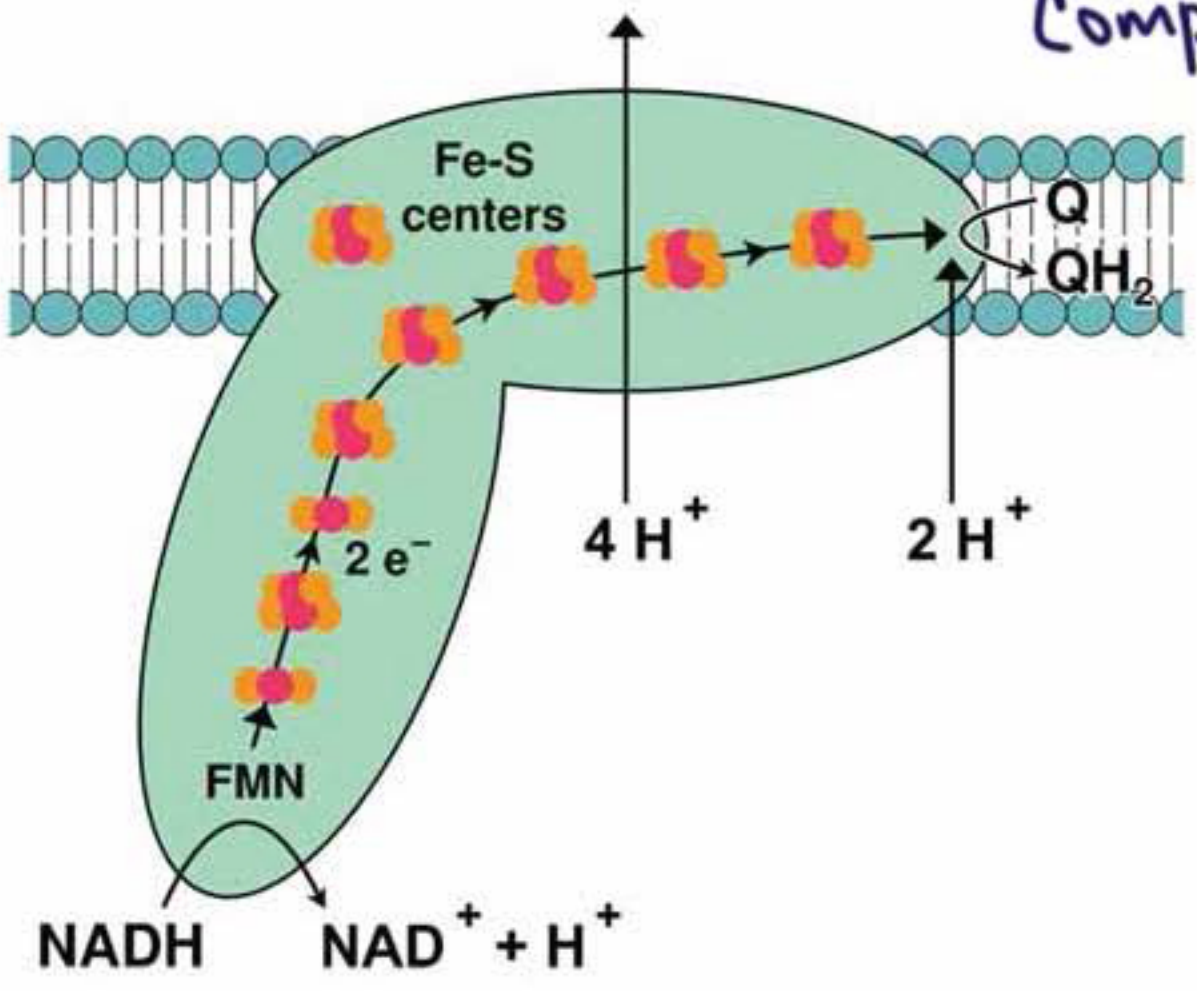
Iron Sulfur Clusters Fe_4S_4

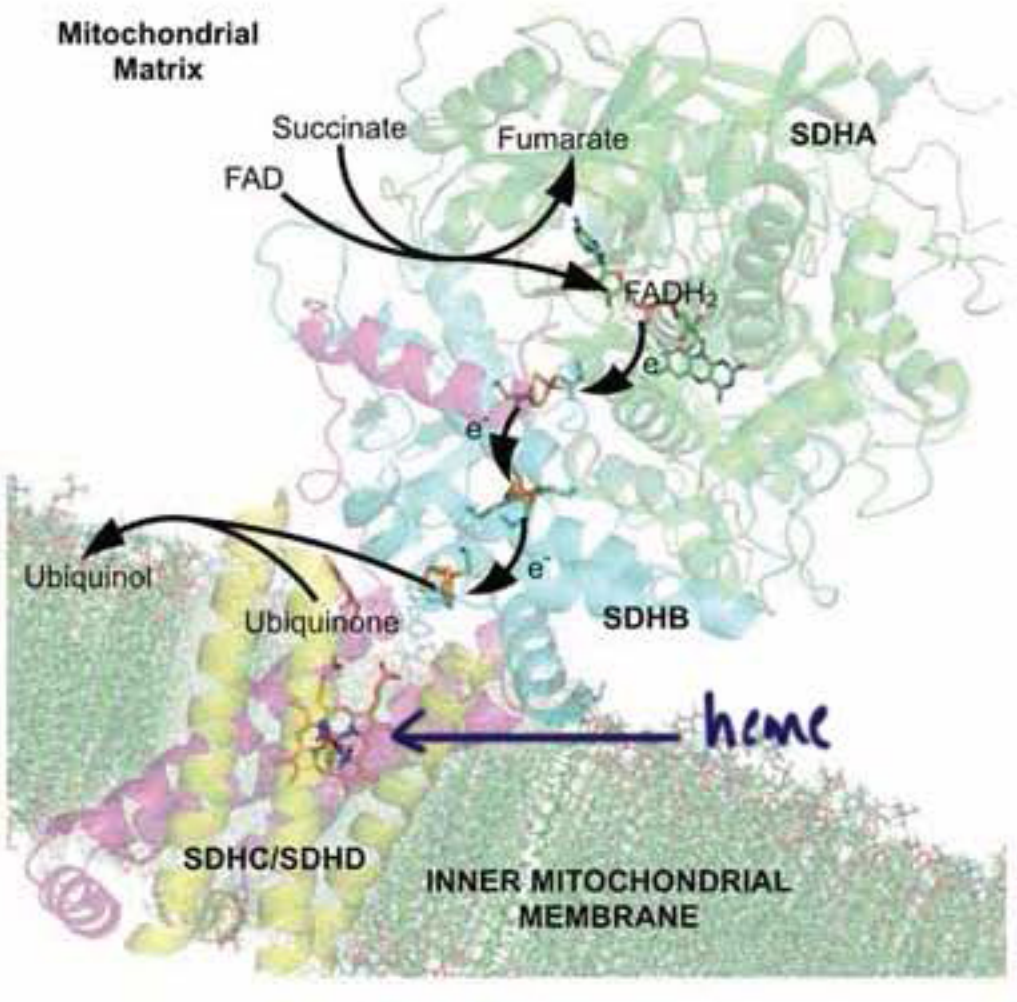
also FeS

Fe_2S_2


Ubiquinone
O
O
O
O
e^-
Ubisemiquinone
$1e^-$
$2H^+$ (from the matrix)
OH
OH
O
O
ubiquinol
isoprene units (n ~ 10)

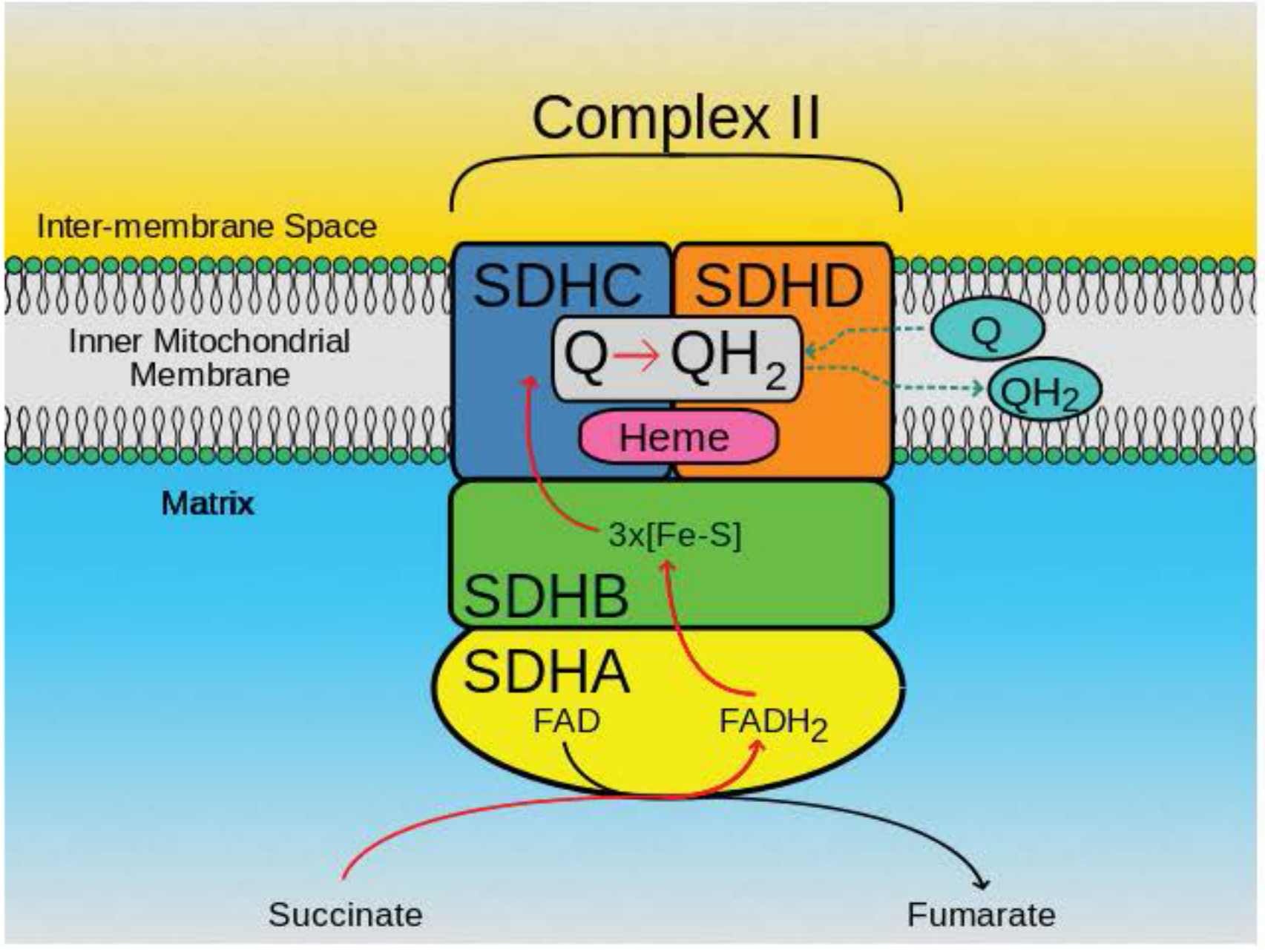
Complex I

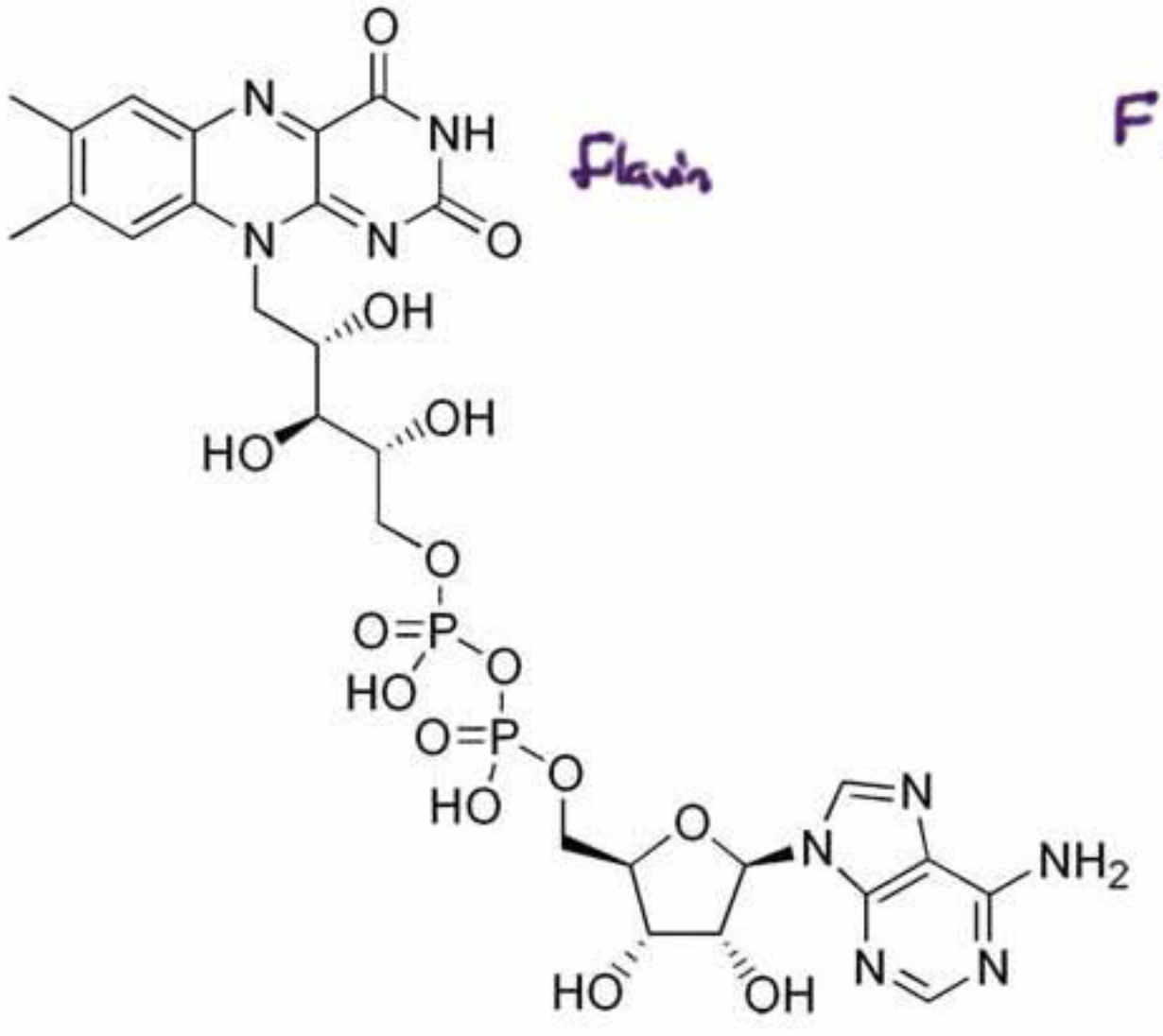




does not pump protons

Complex II
succinate dehydrogenase

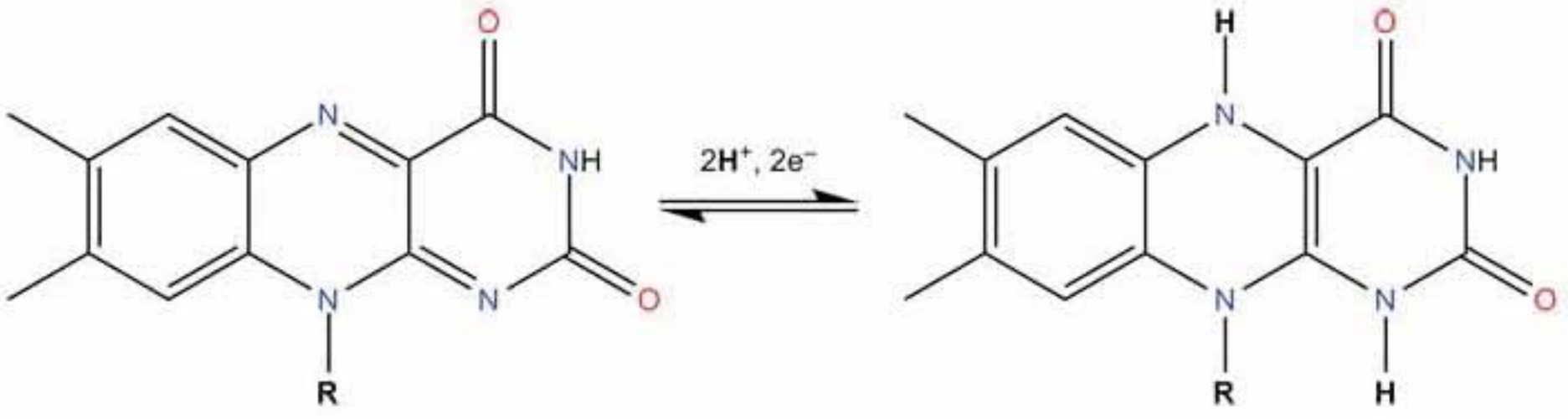

Complex II
Inter-membrane Space
Inner Mitochondrial Membrane
Matrix
SDHC
SDHD
Q → QH2
Heme
Q
QH2
3x[Fe-S]
SDHB
SDHA
FAD
FADH2
Succinate
Fumarate



flavin

FAD

adenosine

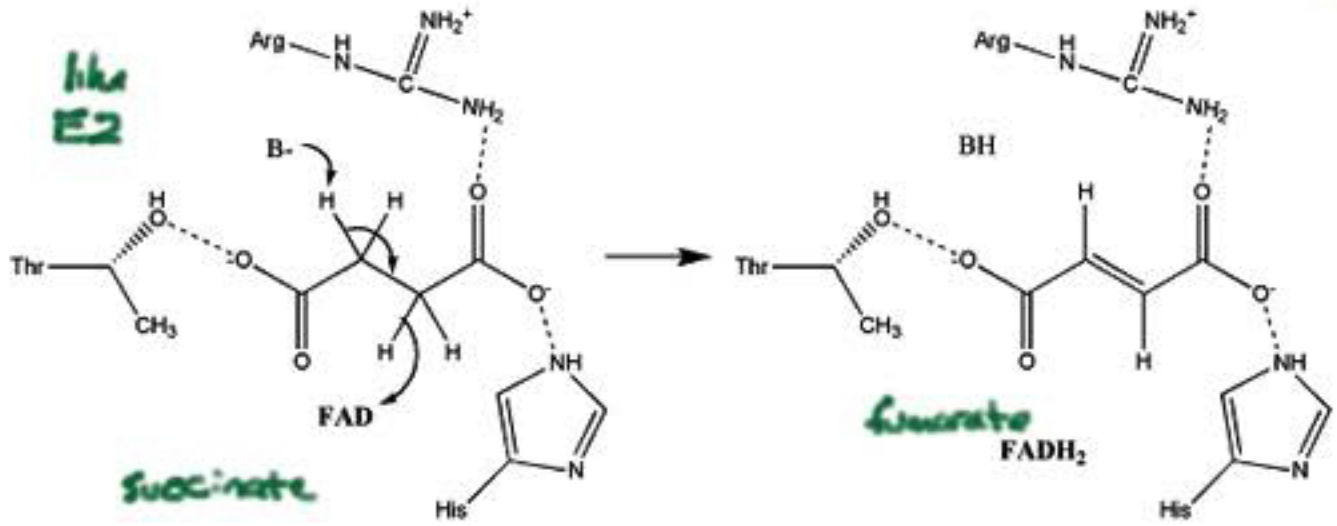


FAD

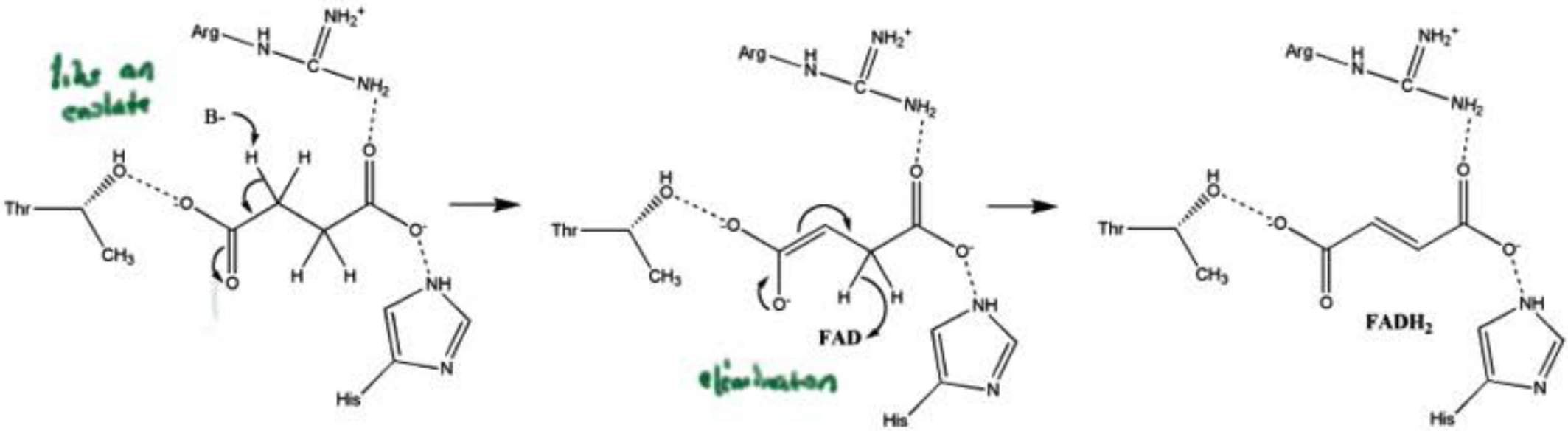
FADH$_2$

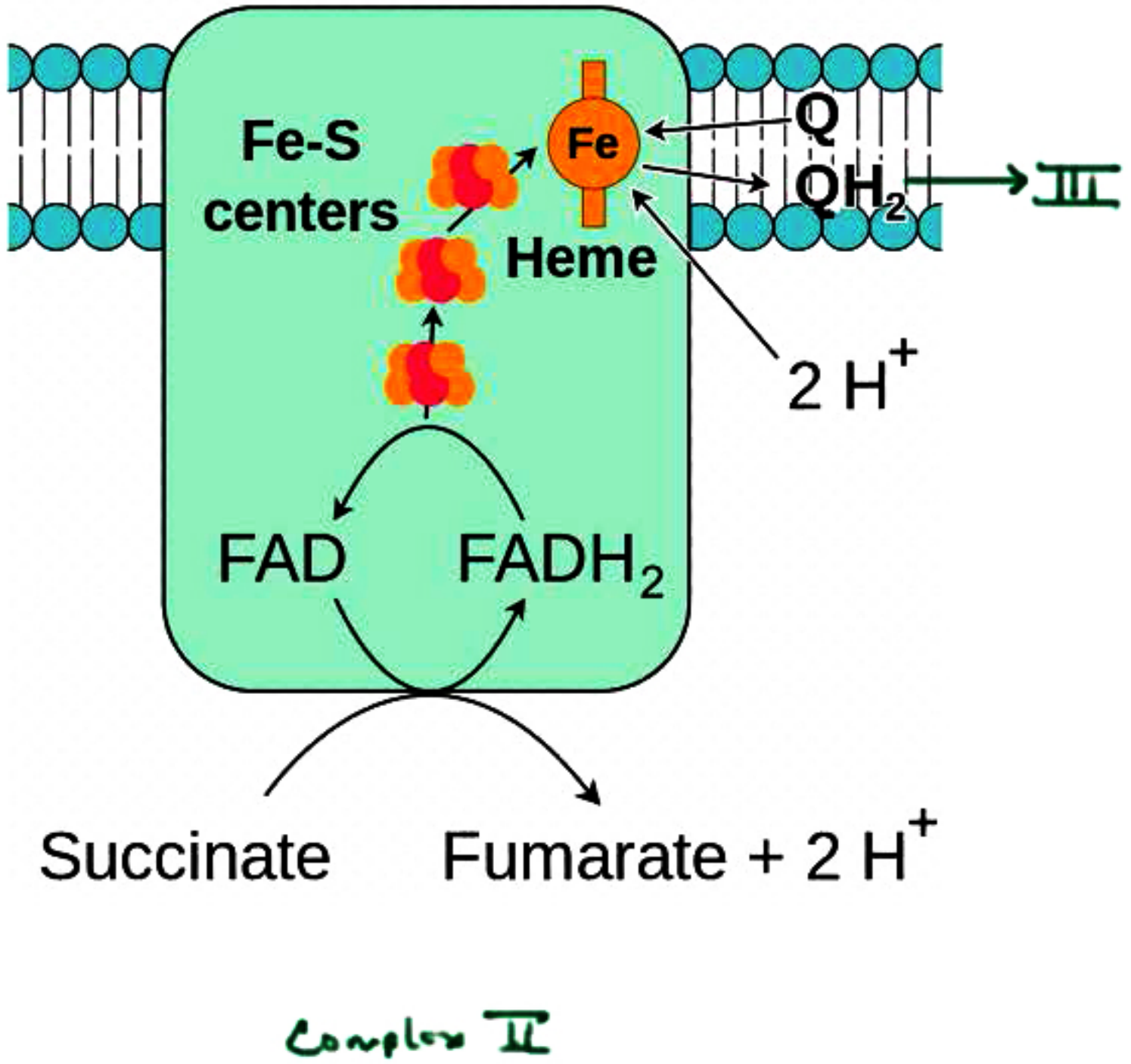
Two alternative proposed mechanisms for succinate dehydrogenase

like E2

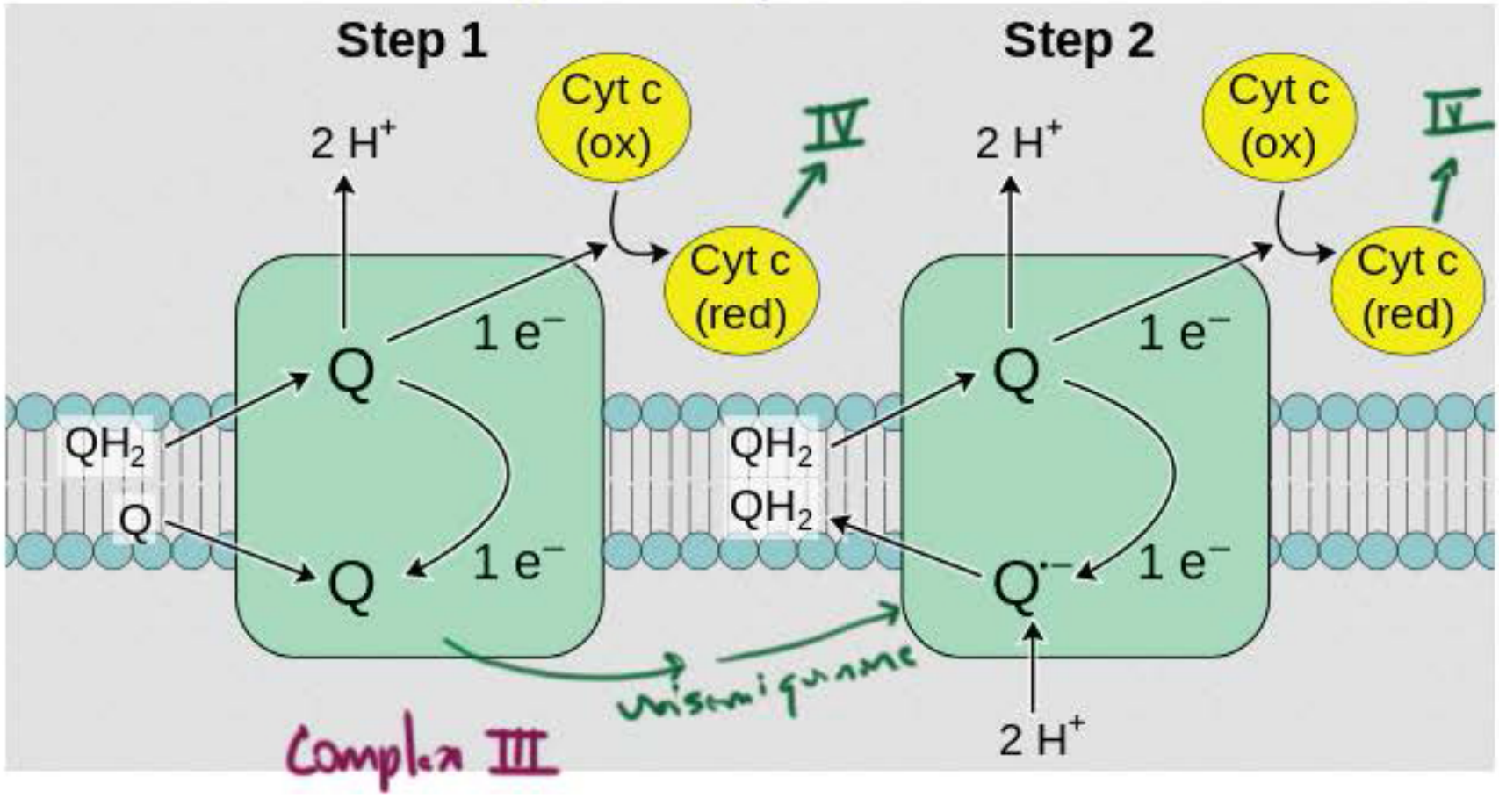


like an enolate




Fe-S
centers
Fe
Q
QH2
III
Heme
2 H+
FAD
FADH2
Succinate
Fumarate + 2 H+
Complex II

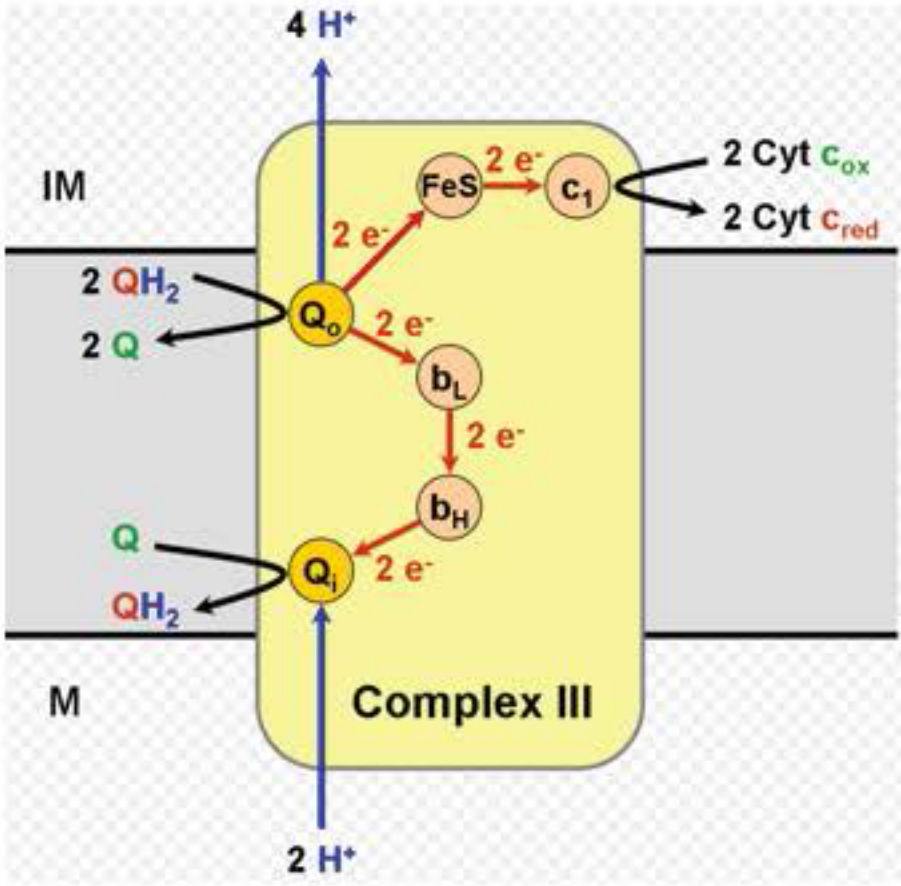
Ubiquinone Cycle



- allows two extra protons to be pumped per ubiquinol

Round 1:

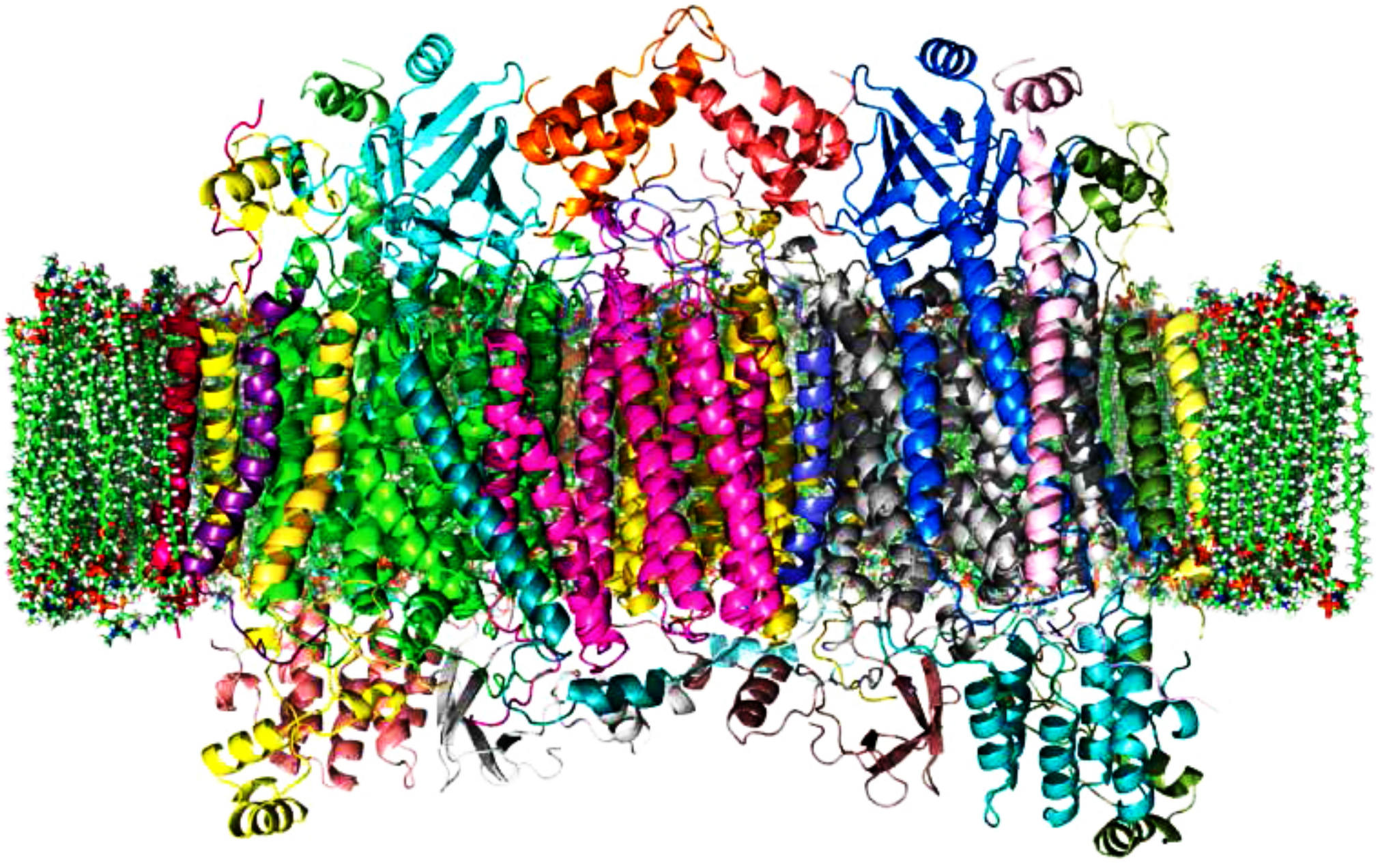
1. Cytochrome b binds a ubiquinol and a ubiquinone.
2. The 2Fe/2S center and B_L heme each pull an electron off the bound ubiquinol, releasing two hydrogens into the intermembrane space.
3. One electron is transferred to cytochrome c_1 from the 2Fe/2S centre, while another is transferred from the B_L heme to the B_H Heme.
4. Cytochrome c_1 transfers its electron to cytochrome c (not to be confused with cytochrome c_1), and the B_H Heme transfers its electron to a nearby ubiquinone, resulting in the formation of a ubisemiquinone.
5. Cytochrome c diffuses. The first ubiquinol (now oxidized to ubiquinone) is released, whilst the semiquinone remains bound.



Round 2:

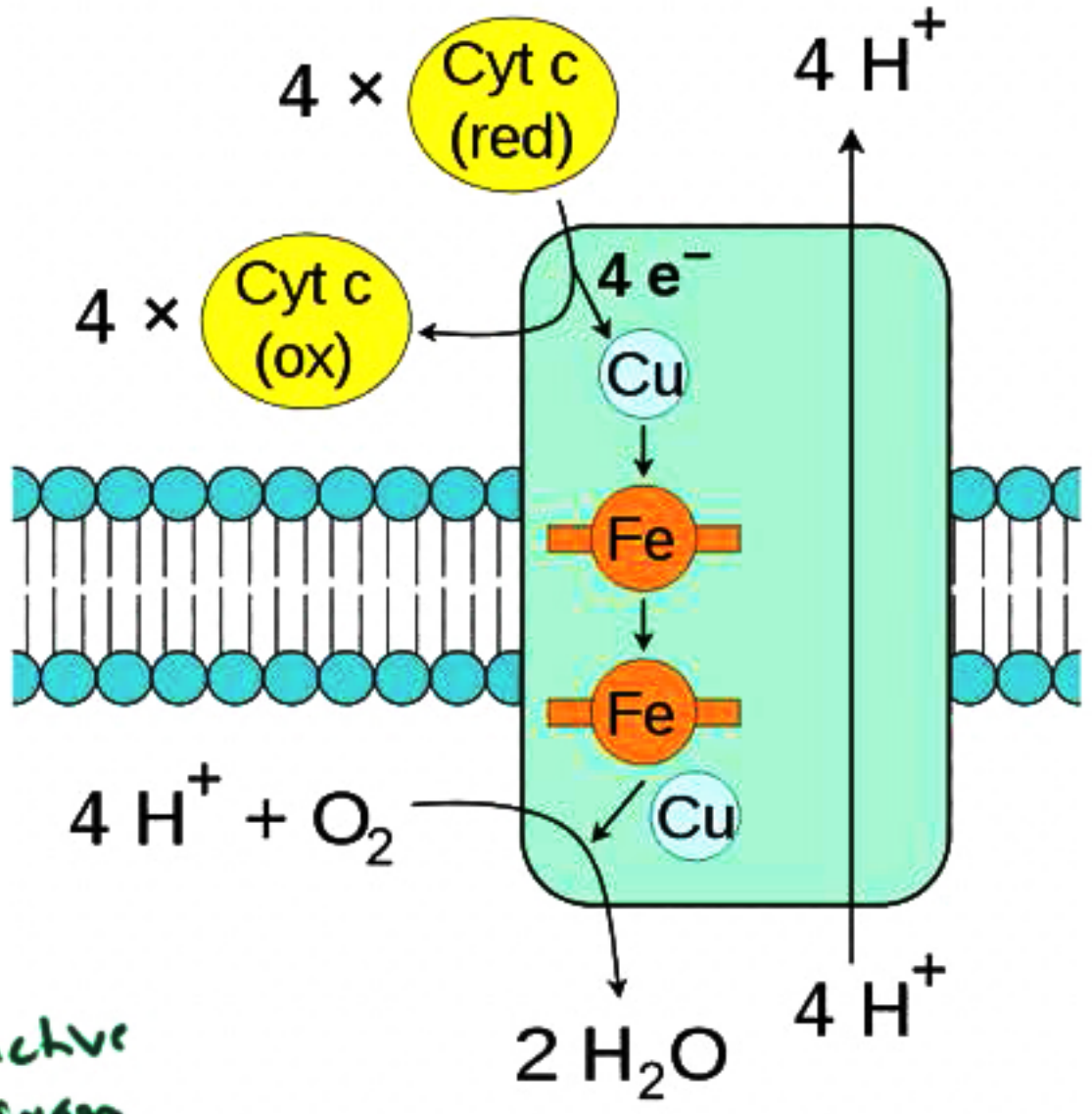
1. A second ubiquinol is bound by cytochrome b.
2. The 2Fe/2S center and B_L heme each pull an electron off the bound ubiquinol, releasing two hydrogens into the intermembrane space.
3. One electron is transferred to cytochrome c_1 from the 2Fe/2S centre, whilst another is transferred from the B_L heme to the B_H Heme.
4. Cytocrome c_1 then transfers its electron to cytochrome c, while the nearby semiquinone picks up a second electron from the B_H heme, along with two protons from the matrix.
5. The second ubiquinol (now oxidised to ubiquinone), along with the newly formed ubiquinol are released.

Supplemental reading



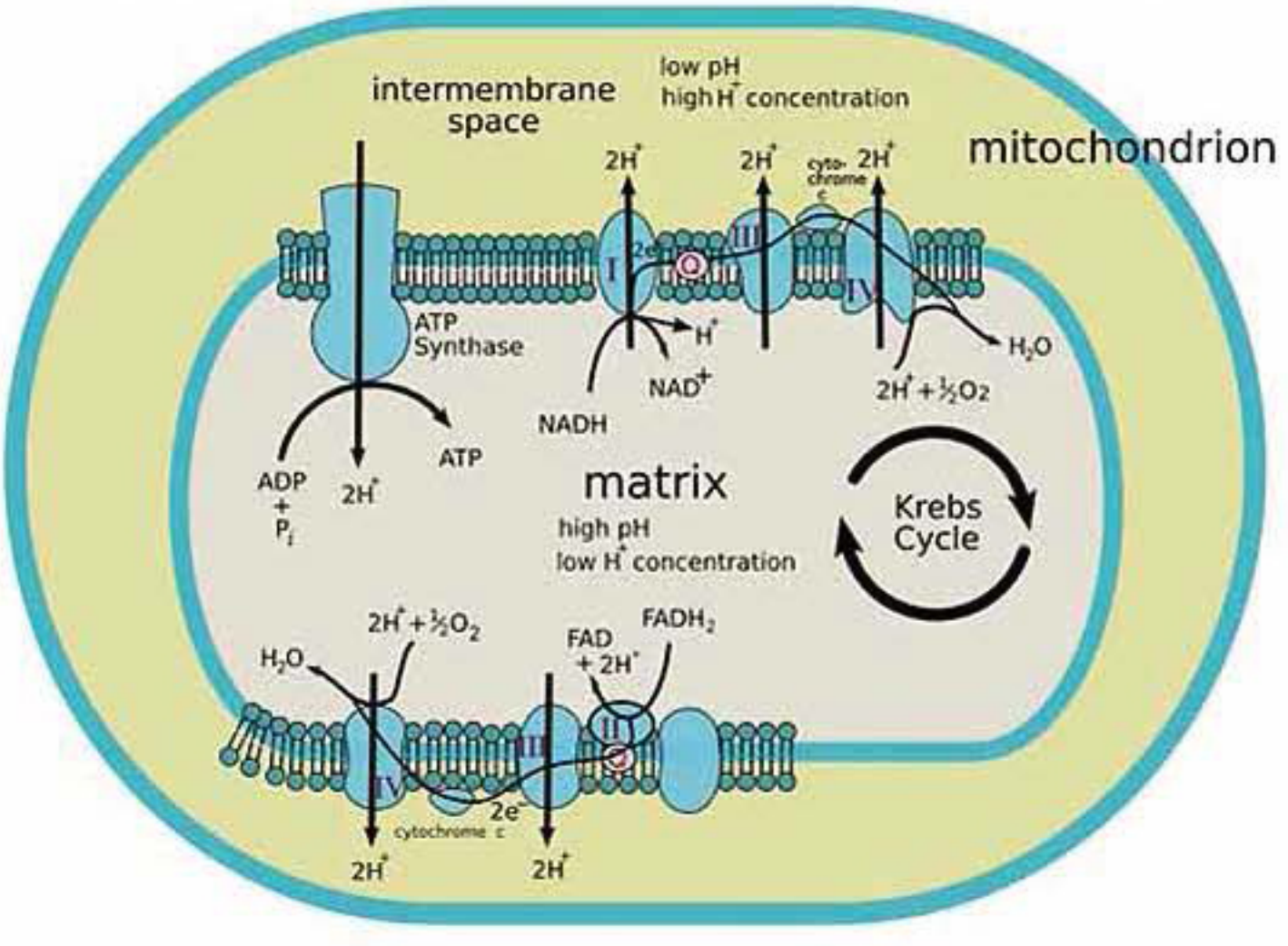
$$4\ Fe^{2+}\text{-cytochrome } c + 8\ H^{+}_{in} + O_2 \rightarrow 4\ Fe^{3+}\text{-cytochrome } c + 2\ H_2O + 4\ H^{+}_{out}$$

Complex IV

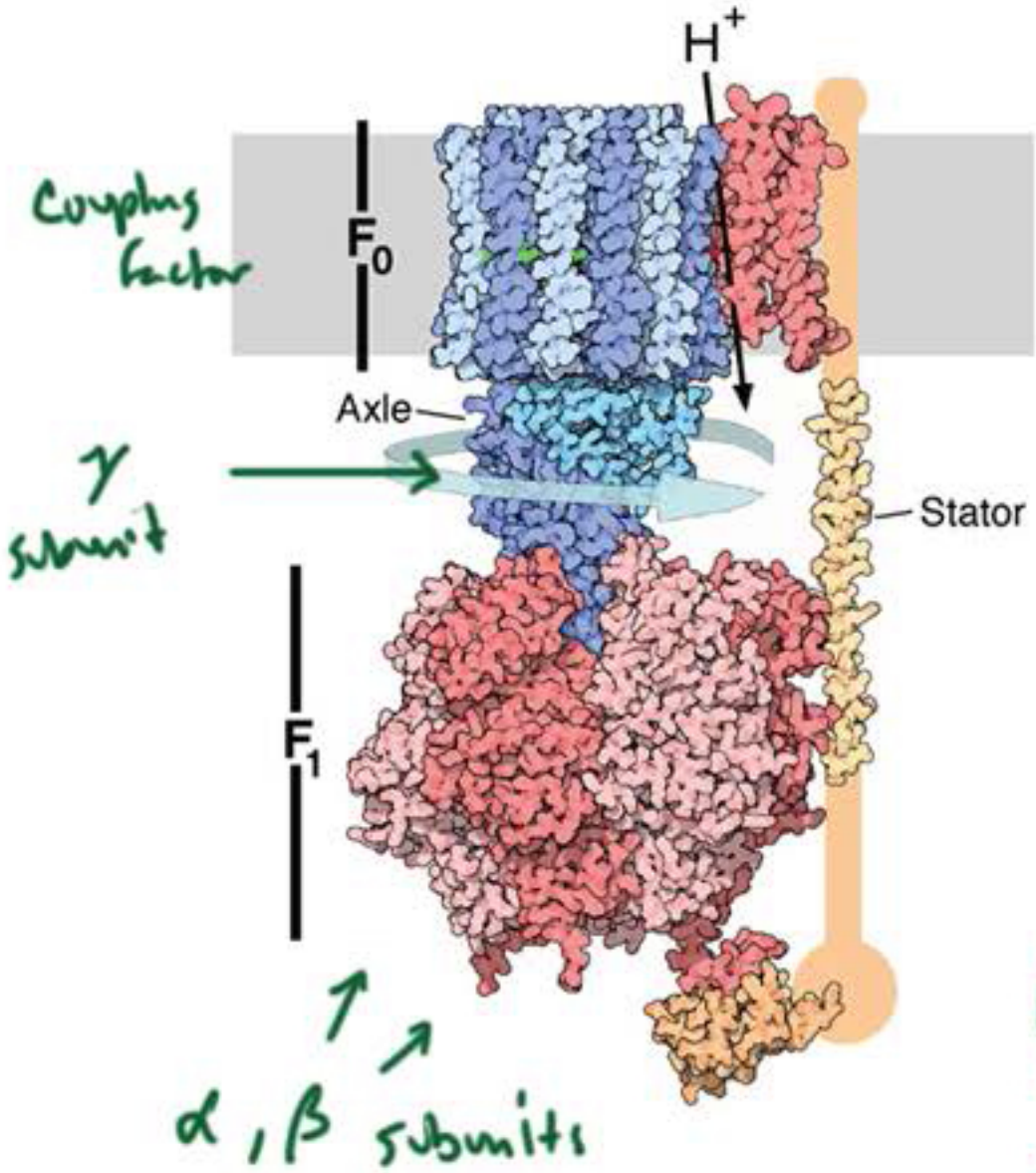


Reactive oxygen species may form here
R.O.S.

Mitochondrial Electron Transport Chain

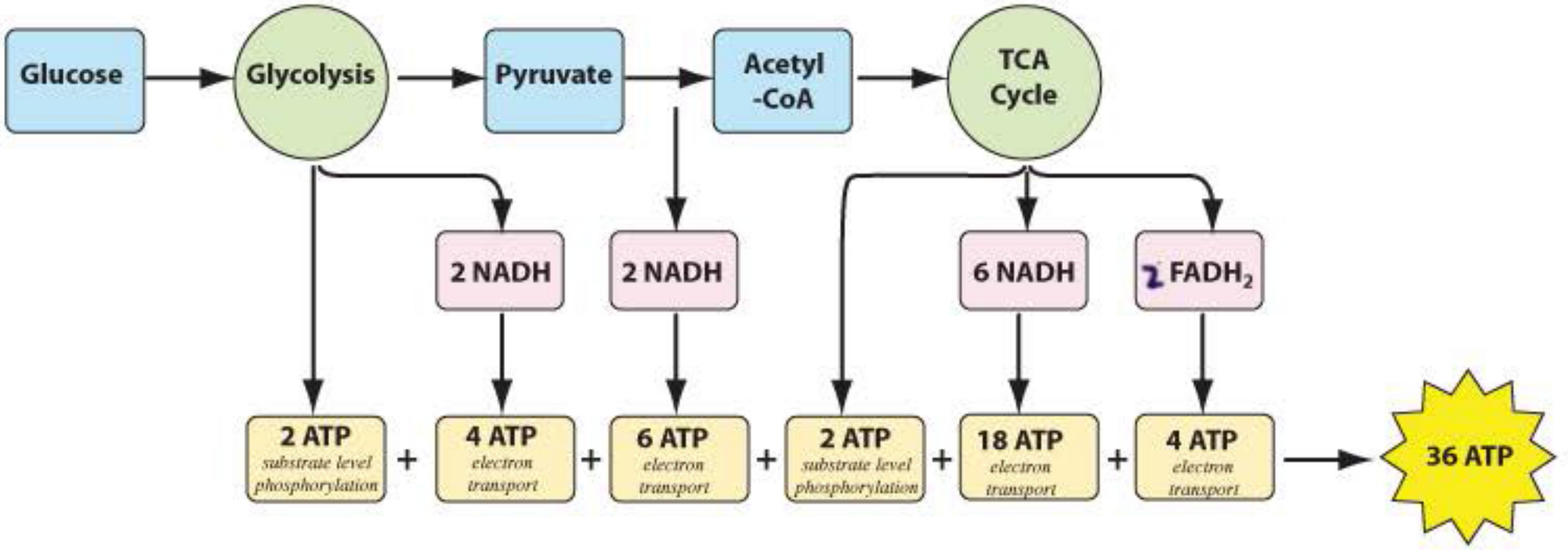


ATP Synthase

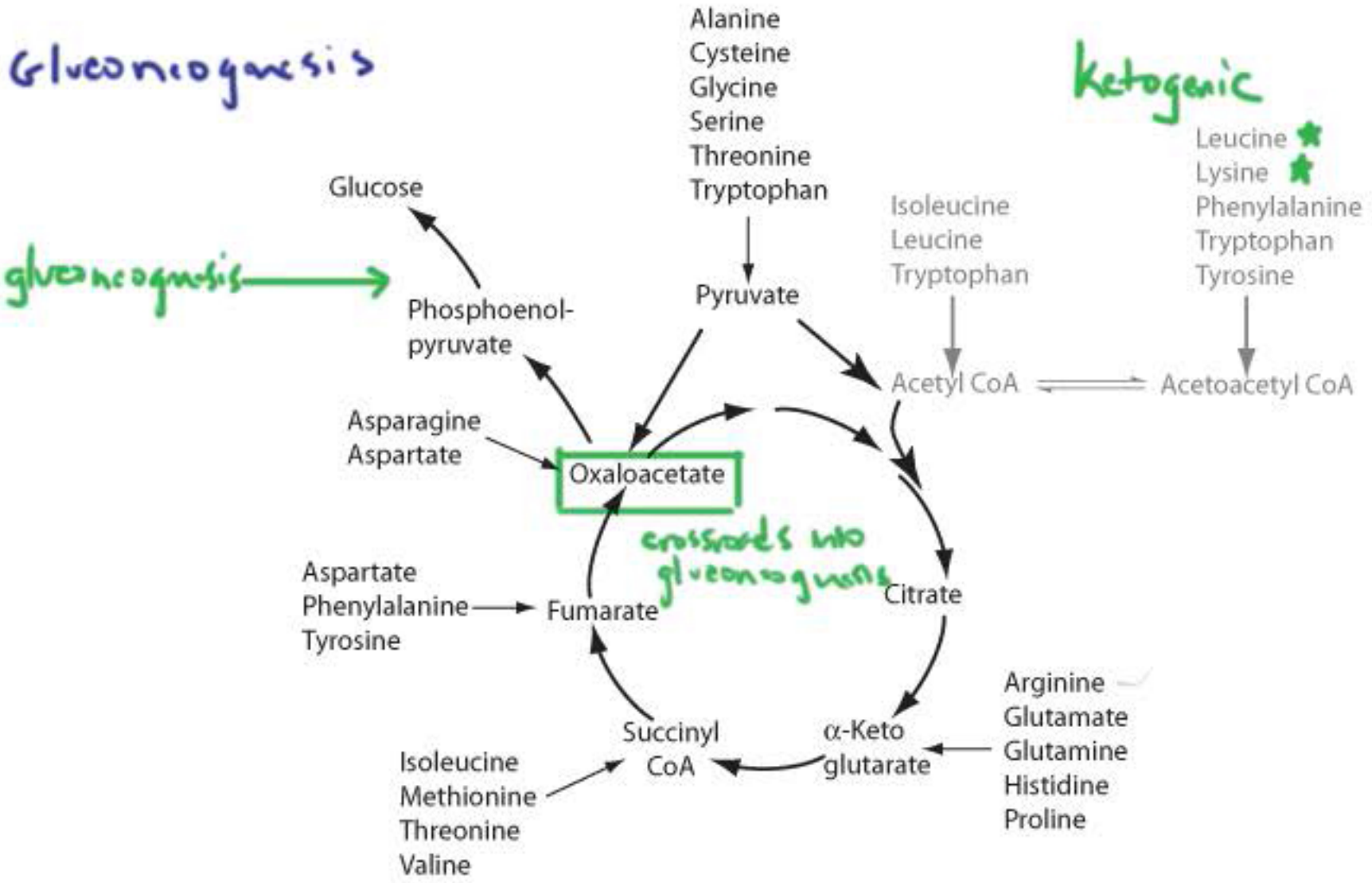


β subunits undergo conformational change

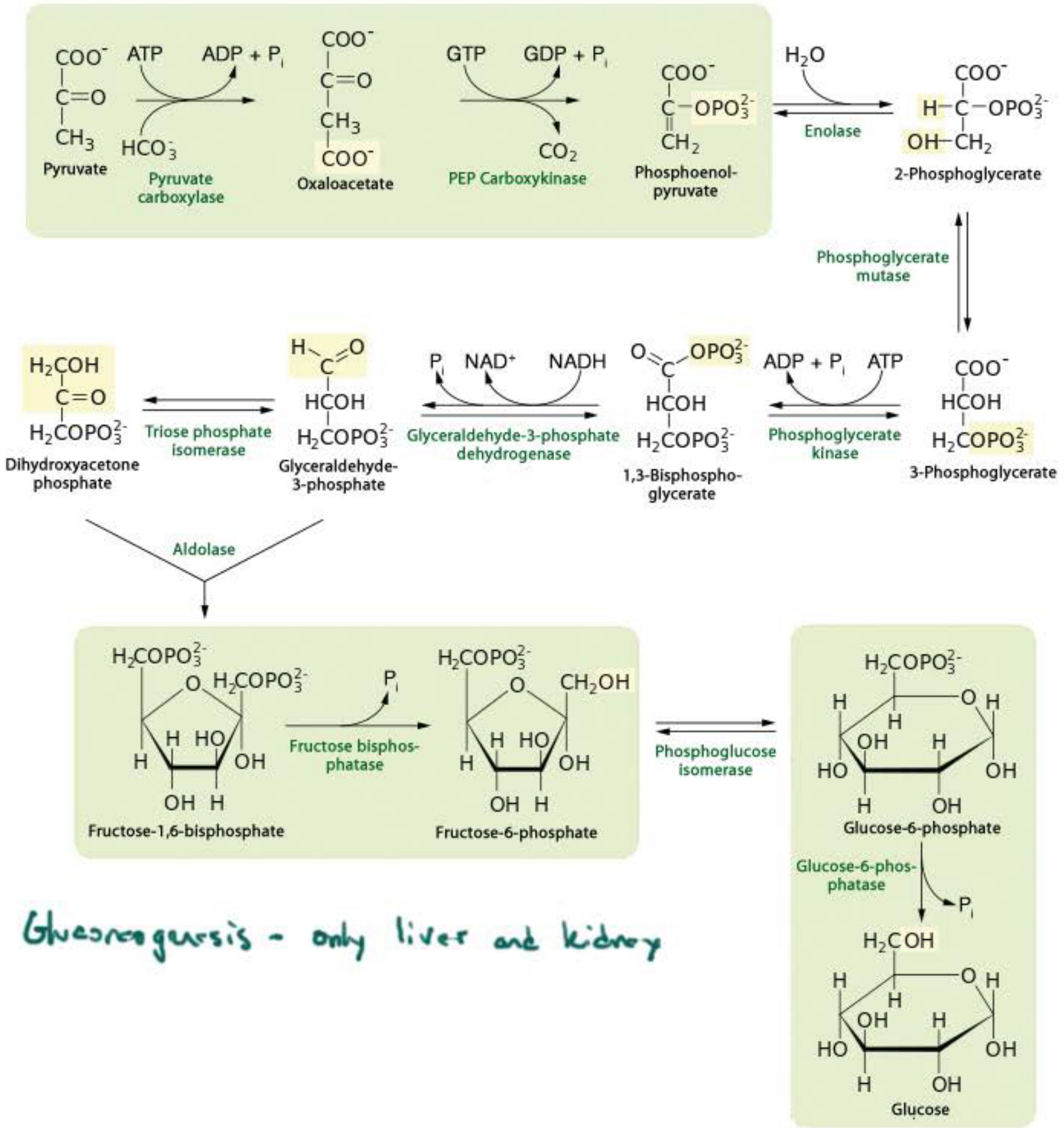
1) open - ADP & Pi to enter
2) loose - binds loosely
3) tight - forms ATP


Glucose
Glycolysis
Pyruvate
Acetyl -CoA
TCA Cycle
2 NADH
2 NADH
6 NADH
2 FADH2
2 ATP substrate level phosphorylation
+
4 ATP electron transport
+
6 ATP electron transport
+
2 ATP substrate level phosphorylation
+
18 ATP electron transport
+
4 ATP electron transport
36 ATP

Gluconeogenesis



most important precursors
- amino acids
- lactate
- glycerol

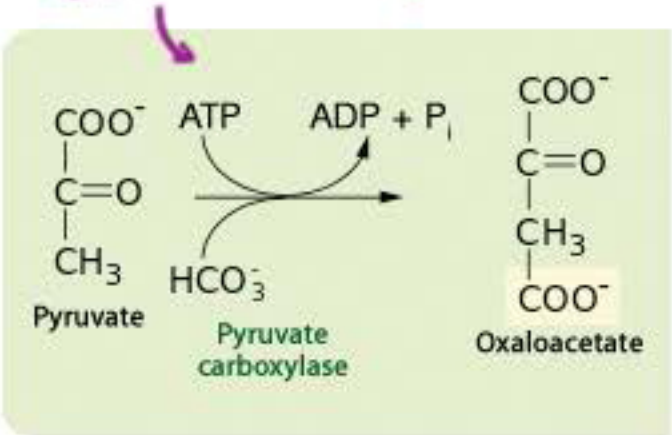


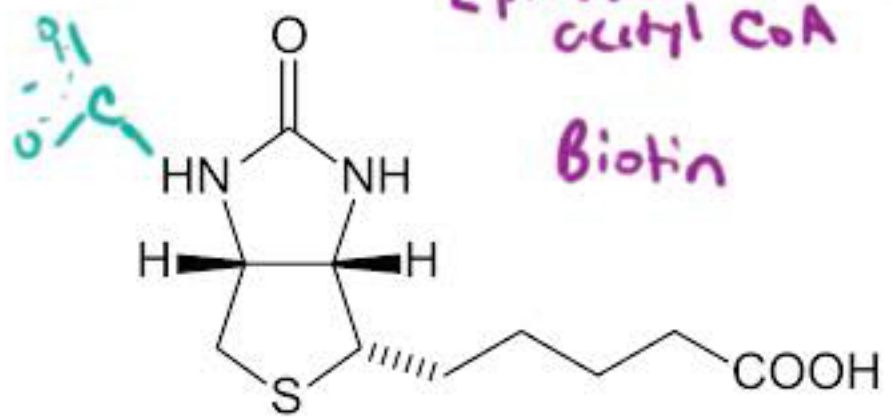
Gluconeogenesis - only liver and kidney

ATP activates biotin

Pyruvate Carboxylase

[enzyme activated by presence of acetyl CoA]





Biotin

pyruvate
↓ (presence of acetyl CoA)
oxaloacetate

high ATP → gluconeogenesis

low ATP → TCA

Biotin carries activated CO_2

- in mitochondria

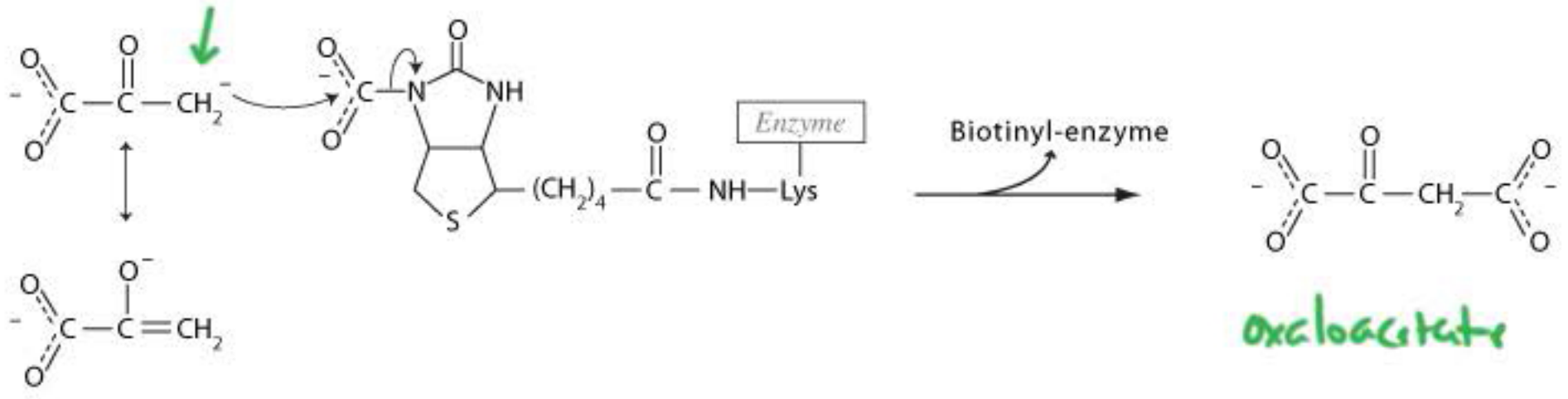
tangent benchtop application of biotin

(Note - remember biotinylation for affinity chromatography with avidin beads)

proton + substrate ⇌ bound complex

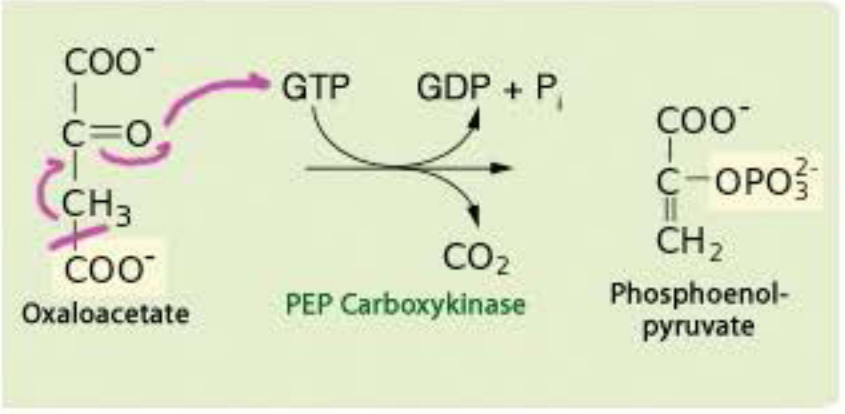
$$K_a = \frac{[\text{bound}]}{[\text{proton}][\text{substrate}]}$$

Pyruvate Carboxylase



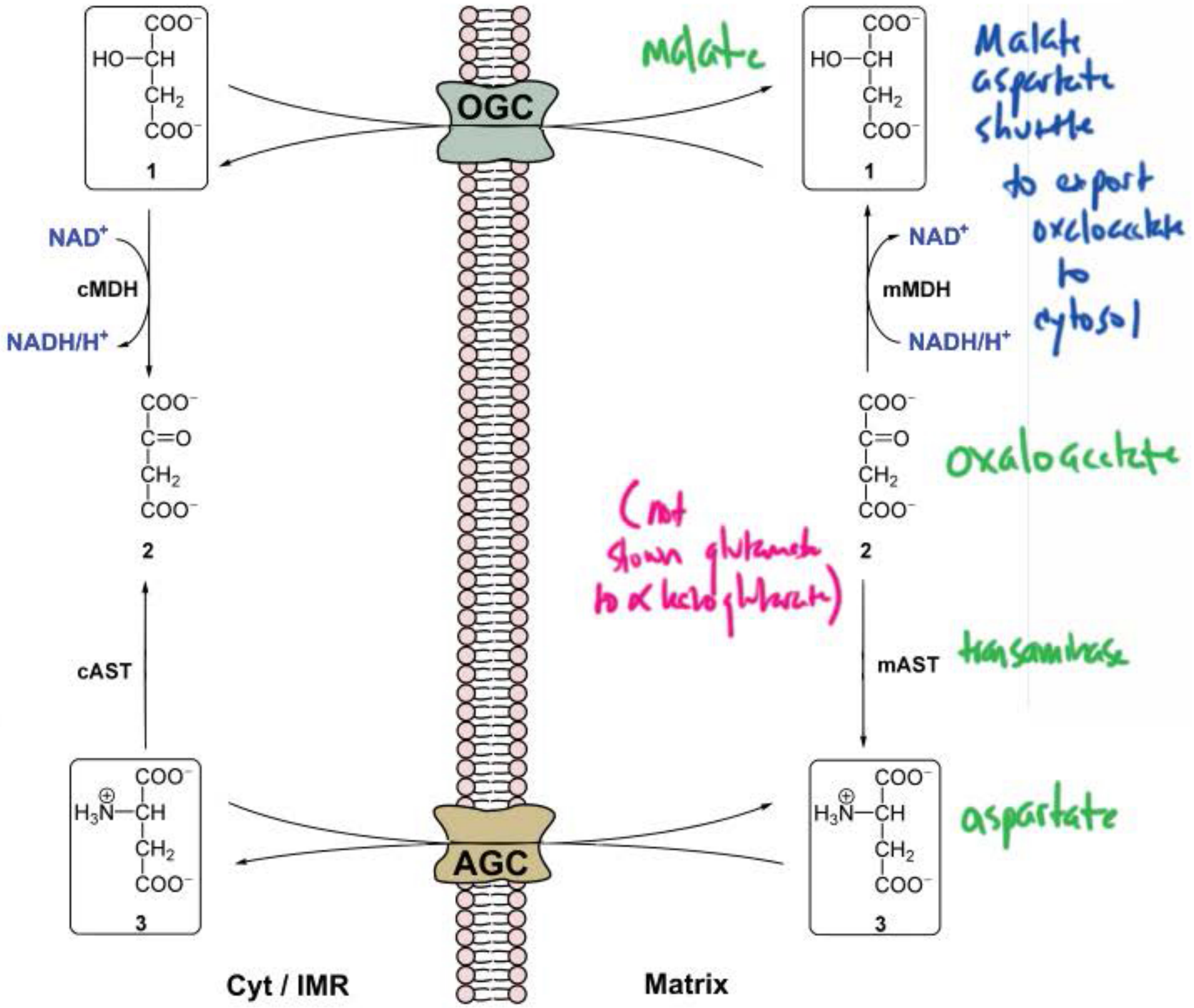
enolate of pyruvate

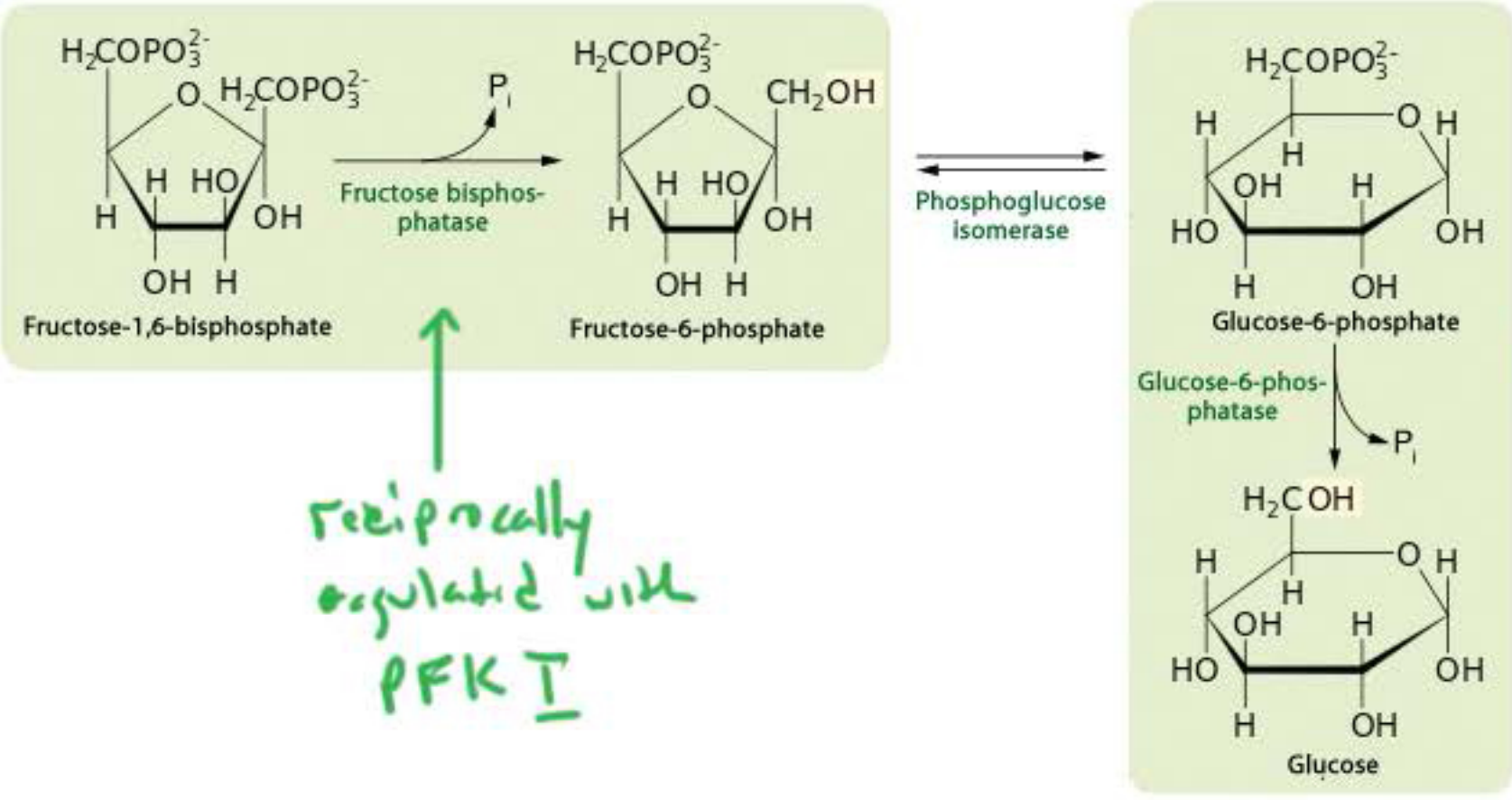
In cytosol



- PKA activates transcription factor

PEP-CK reaction may happen in either the cytosol or the mitochondrion, actually. It depends on the gluconeogenic precursor. If lactate is the precursor, PEP-CK happens in the mitochondrion. This is because lactate to pyruvate will generate the NADH gluconeogenesis needs later, so there is no need to move one to the cytosol. If the precursor is glycerol or an amino acid, on the other hand, the NADH (equivalent) is carried out to the cytosol before PEP-CK with the oxaloacetate being transported out as malate. This is just on the edge of the MCAT knowledge, probably.

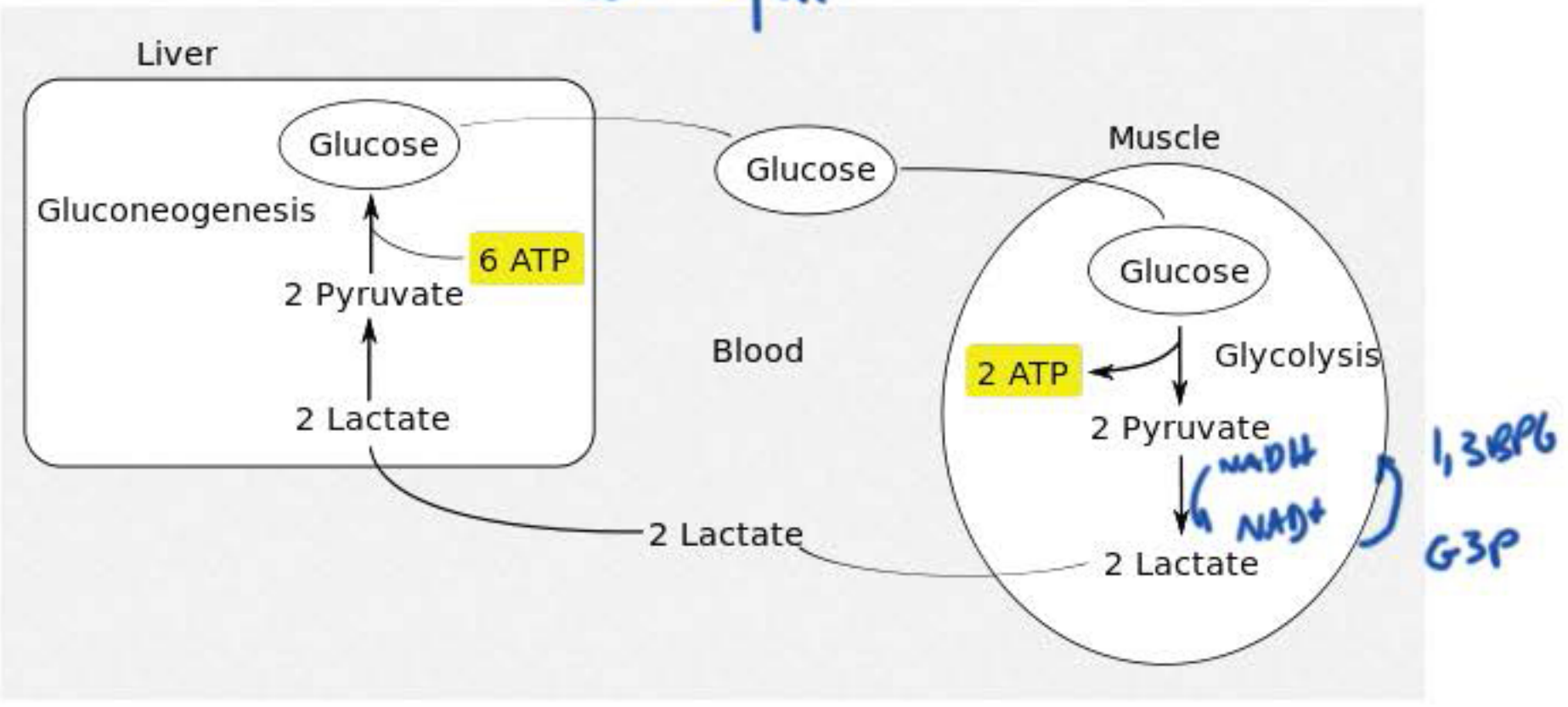

OGC
AGC
1
2
3
NAD+
NADH/H+
cMDH
mMDH
cAST
mAST
Cyt / IMR
Matrix
malate
Malate aspartate shuttle to export oxaloacetate to cytosol
oxaloacetate
(not shown glutamate to α ketoglutarate)
transaminase
aspartate



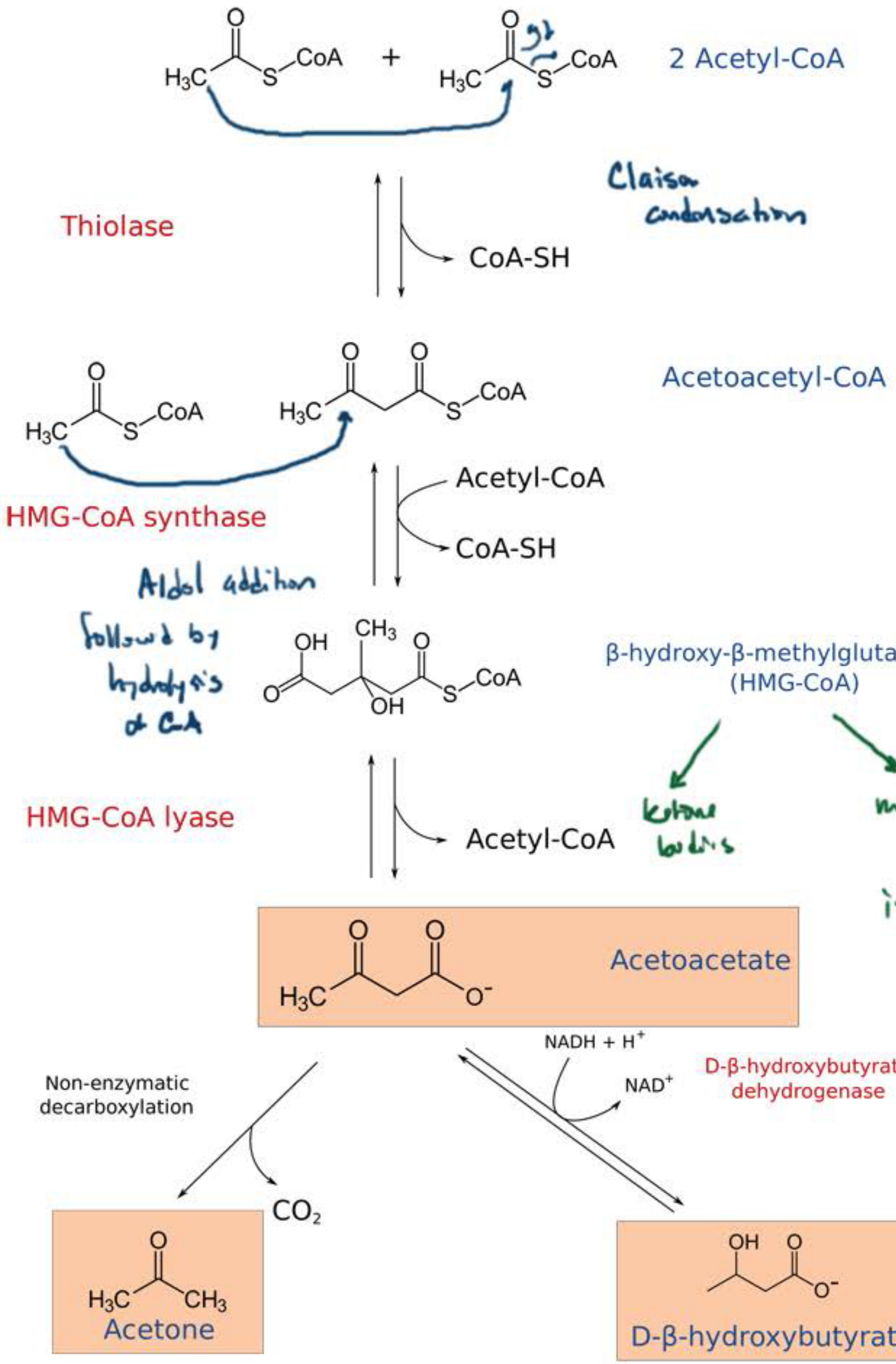
reciprocally regulated with PFK I

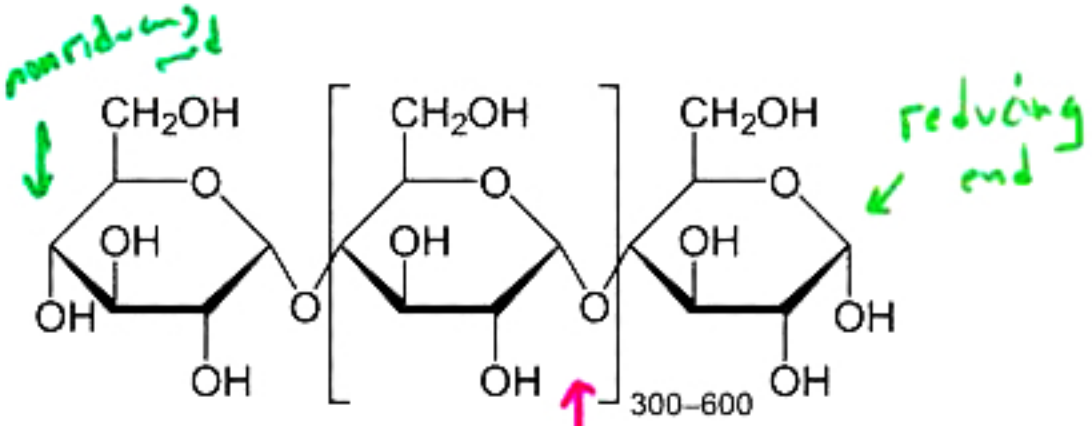
liver and kidney

Cori Cycle


Liver
Glucose
Gluconeogenesis
6 ATP
2 Pyruvate
2 Lactate
Glucose
Blood
2 Lactate
Muscle
Glucose
2 ATP
Glycolysis
2 Pyruvate
NADH
NAD+
2 Lactate
1,3BPG
G3P

ketosis

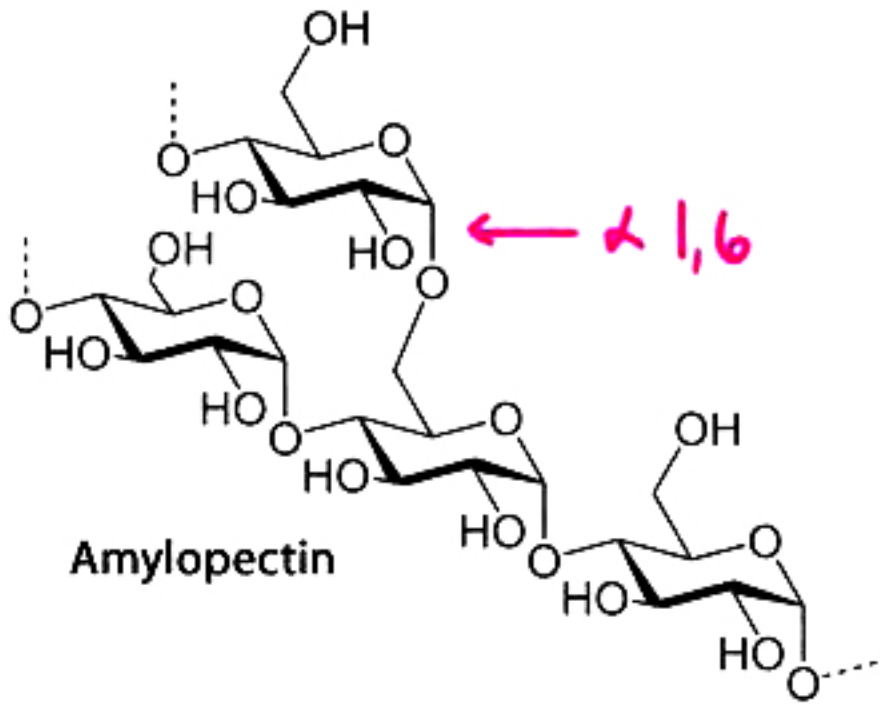

2 Acetyl-CoA
Claisen condensation
Thiolase
CoA-SH
Acetoacetyl-CoA
HMG-CoA synthase
Acetyl-CoA
CoA-SH
Aldol addition
followed by hydrolysis of CoA
β-hydroxy-β-methylglutaryl-CoA (HMG-CoA)
HMG-CoA lyase
Acetyl-CoA
ketone bodies
mevalonate
isopentenyl pyrophosphate
isoprenoid lipids
Acetoacetate
NADH + H+
NAD+
D-β-hydroxybutyrate dehydrogenase
Non-enzymatic decarboxylation
CO2
Acetone
D-β-hydroxybutyrate



Amylose

α 1,4

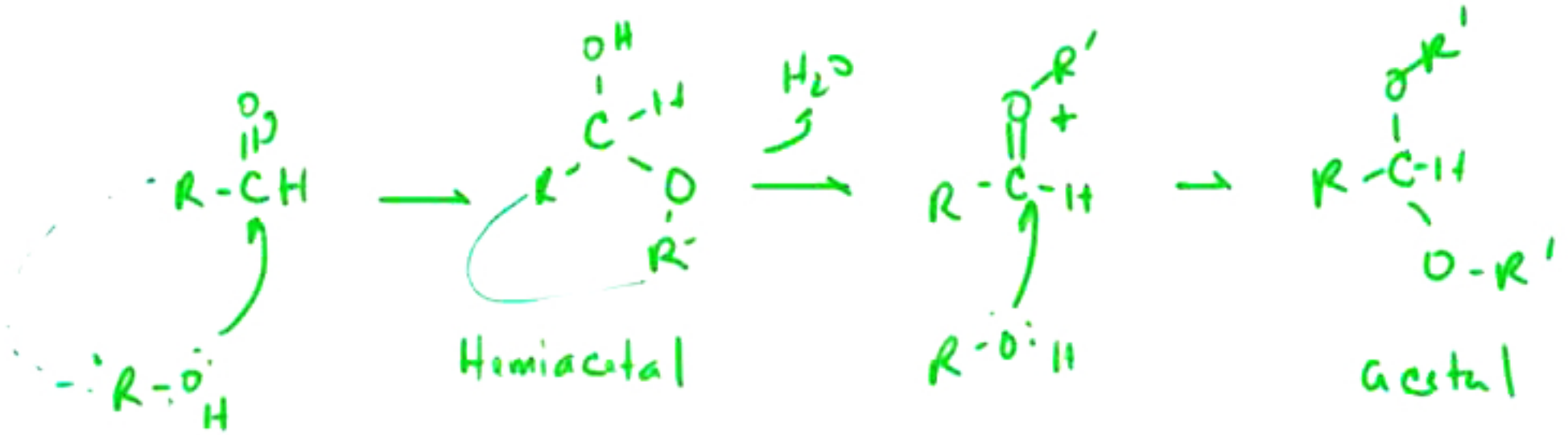
like glycogen



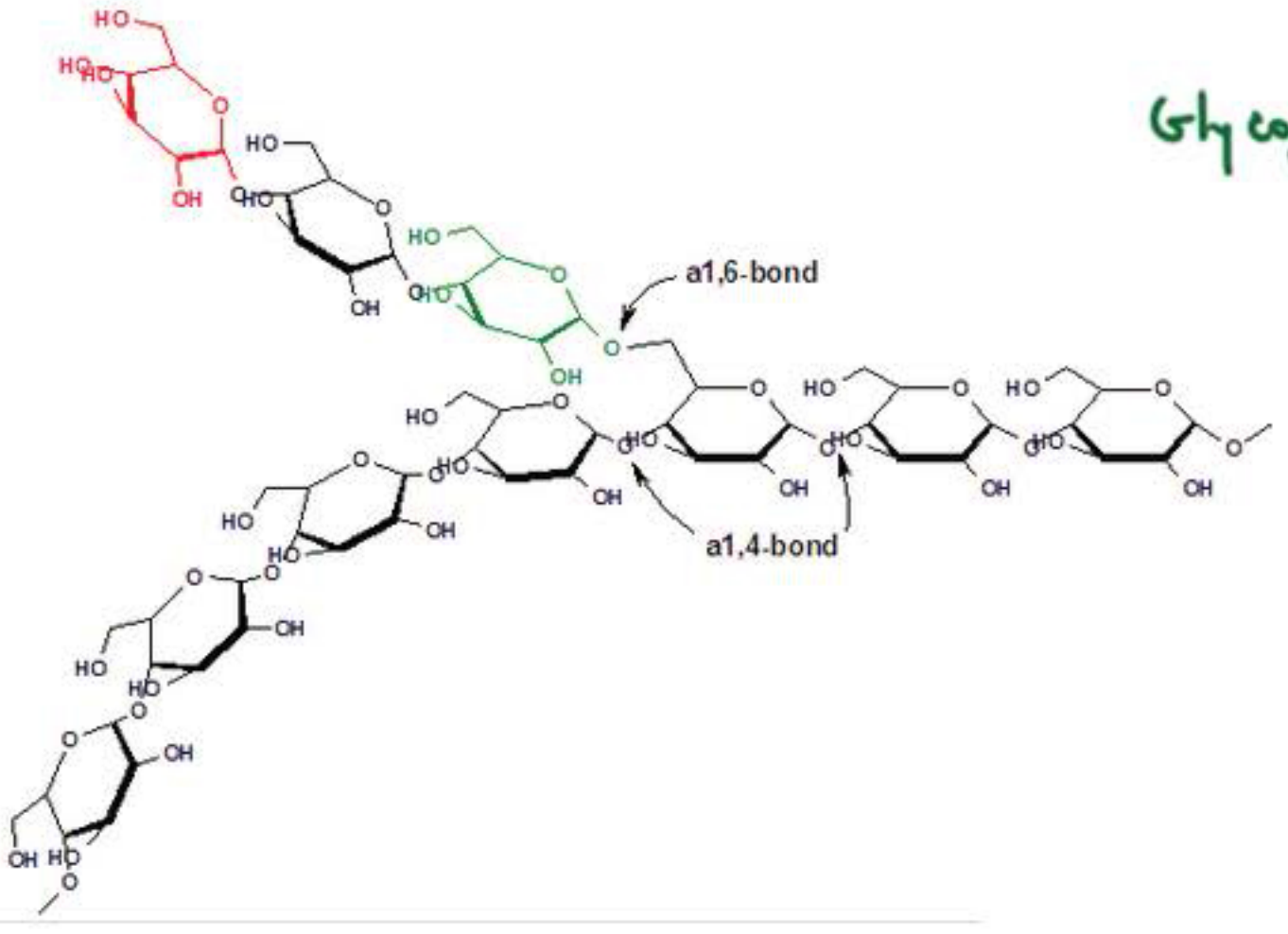
Amylopectin

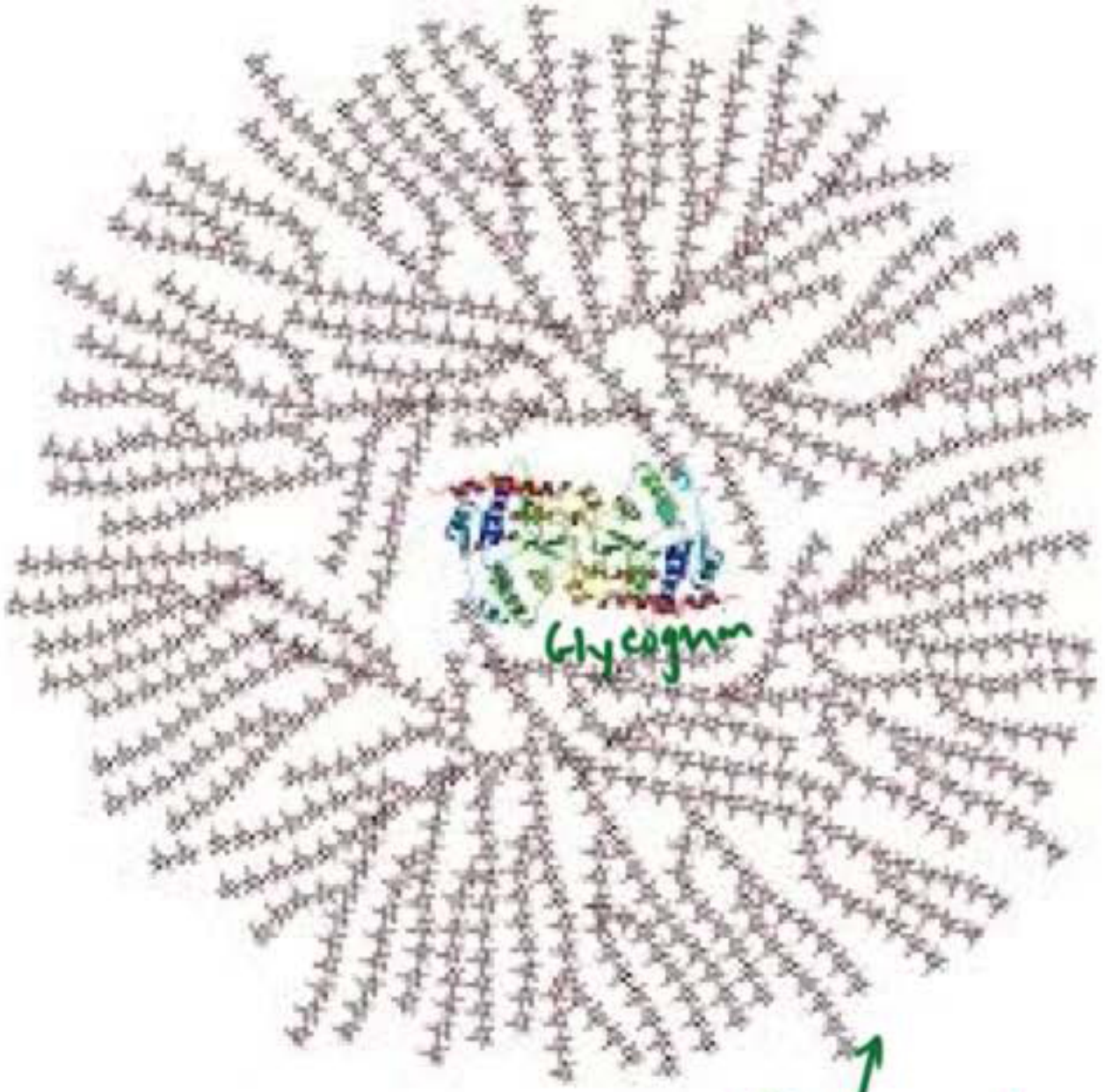
α 1,6

plant starches



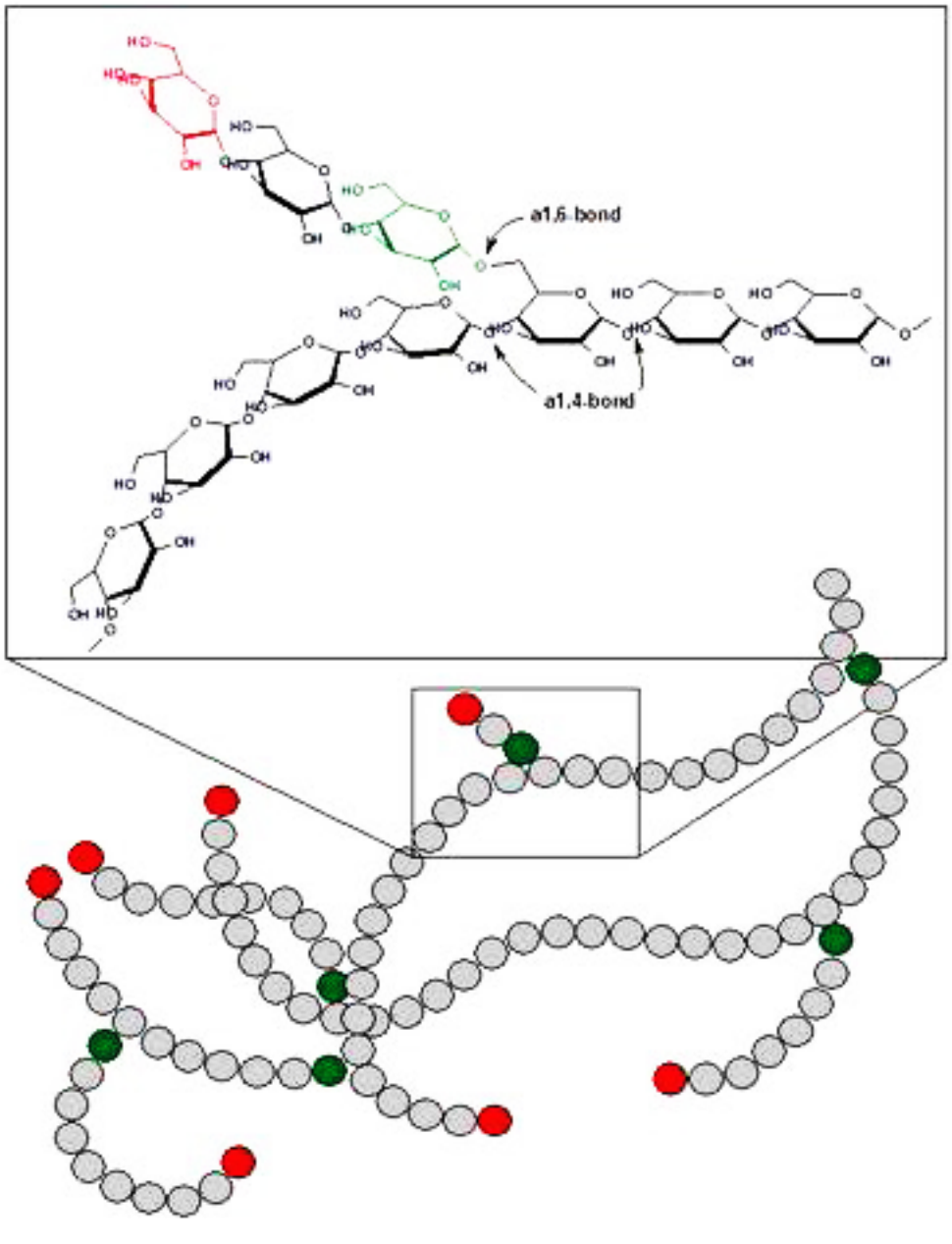
Glycogen

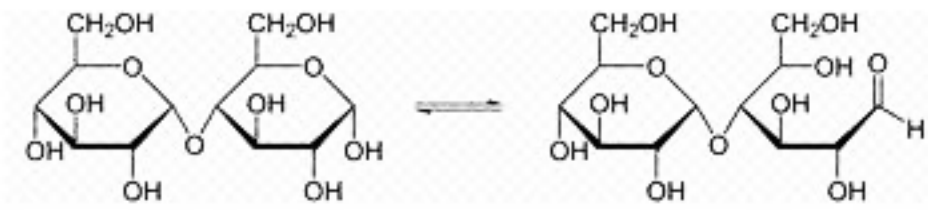




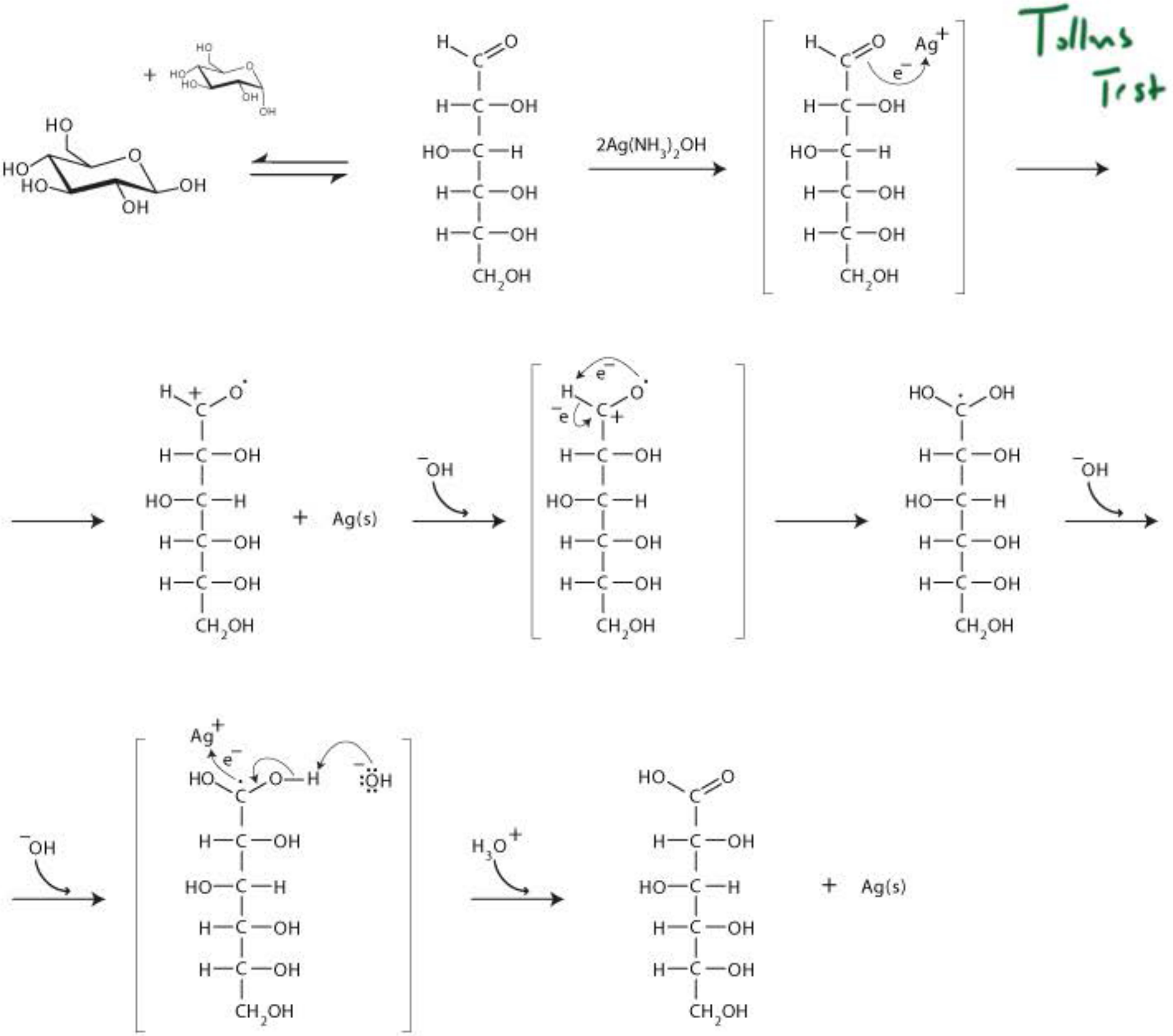
Liver and Muscle

nonreducing ends

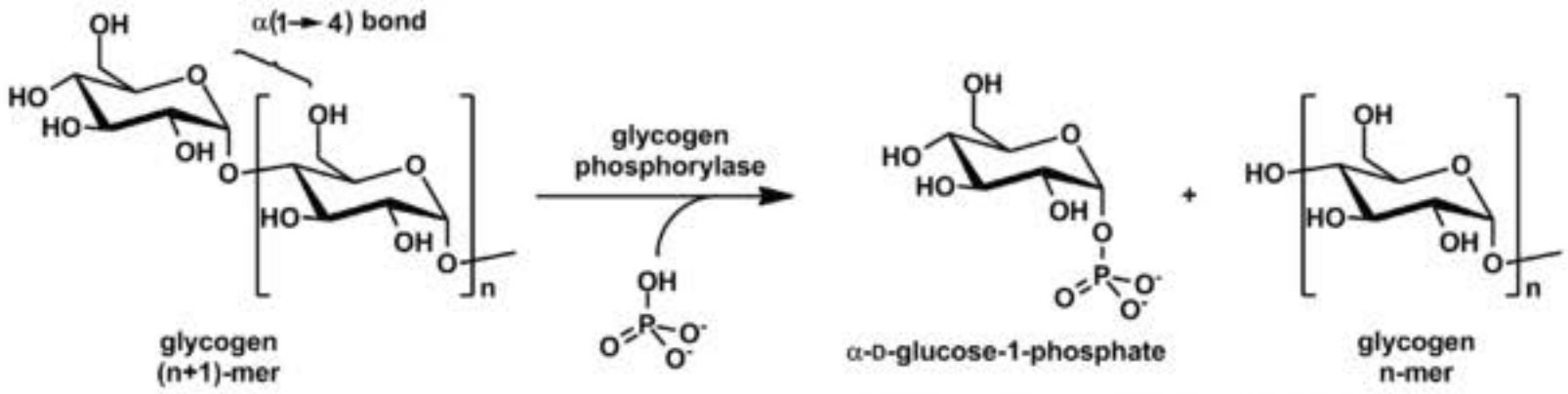

a1,6-bond
a1,4-bond


CH2OH
OH
O
H

Tollens Test



Glycogen Phosphorylase (multisubunit allostric enzyme



Glucose 1 Phosphate

phosphoglucomutase → glucose 6 phosphate

glucose 6 phosphatase → glucose

- also debranching enzyme and transferase

ΔG° is ⊕

$\Delta G = \Delta G^\circ + 2.3RT \log Q$

$\ominus \quad \oplus \quad \ominus$

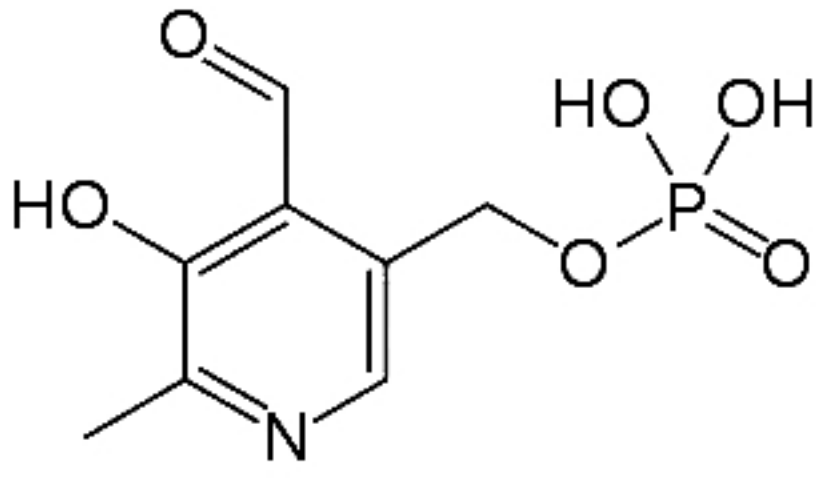
$\Delta G = \Delta G^\circ + RT \ln Q$

$\ln X$ ← natural logarithm (base e)

$\log X$ ← common logarithm (base 10)

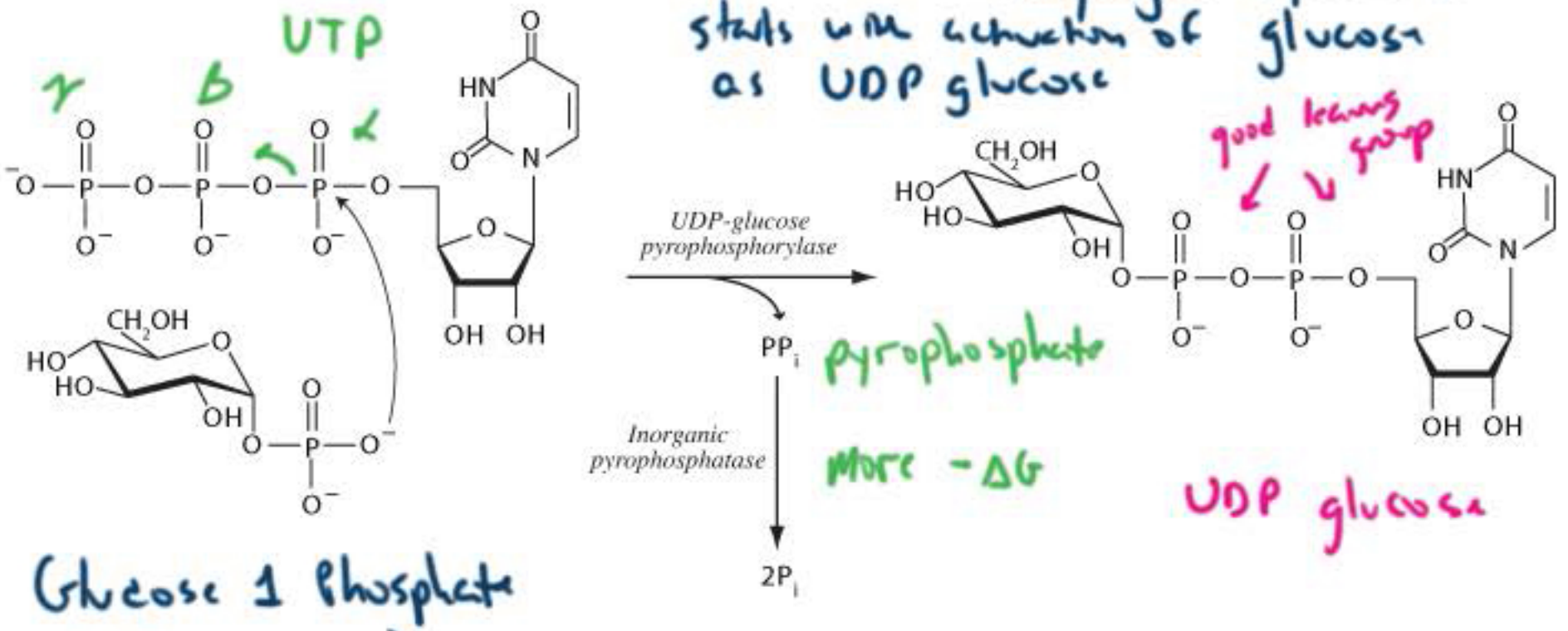
at physiology $Q = \frac{[G1P]}{[P_i]} \sim \frac{1}{1000}$

PLP



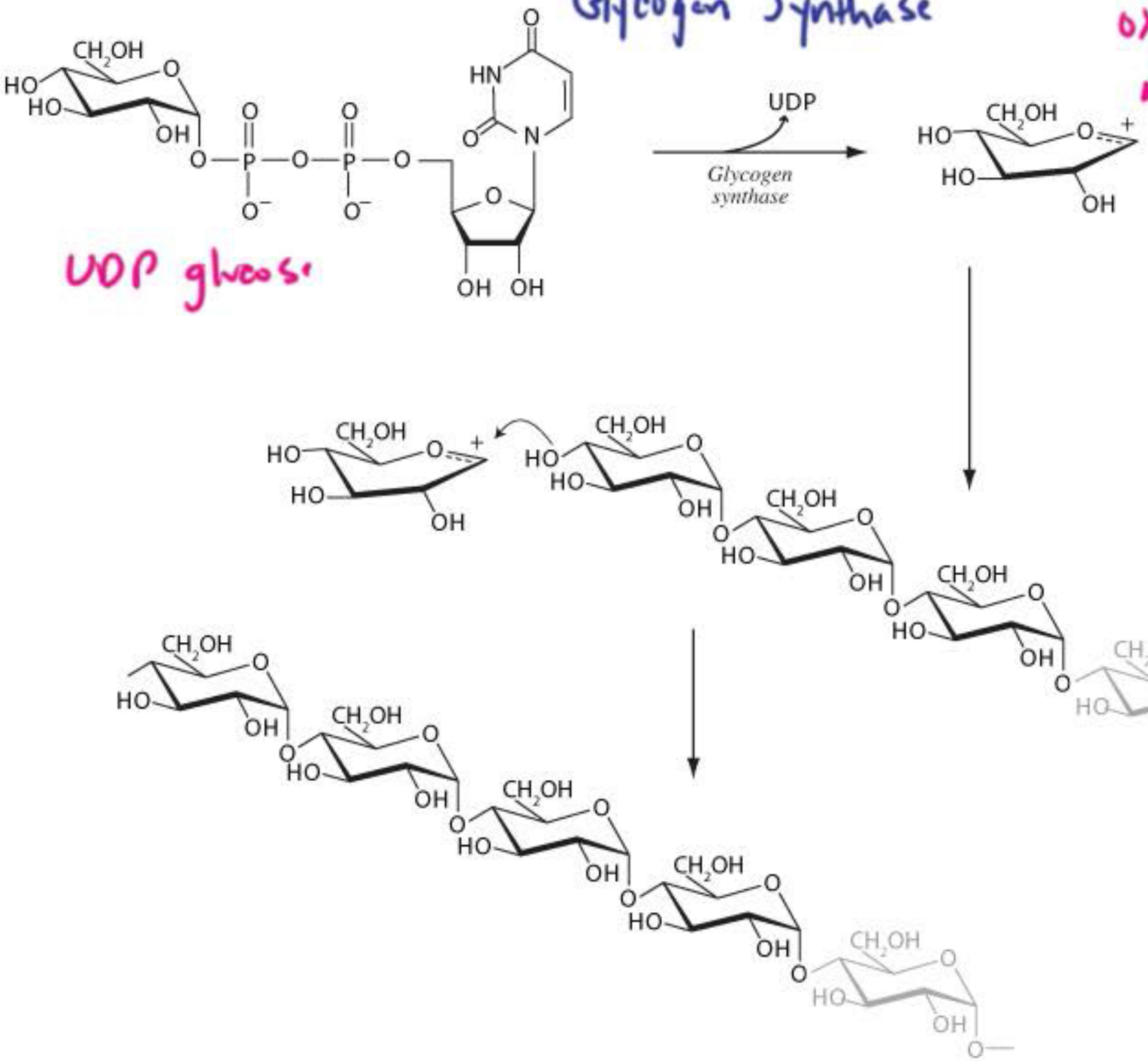
$$\text{glycogen}_{(n\ \text{residues})} + P_i \rightleftharpoons \text{glycogen}_{(n\text{-}1\ \text{residues})} + \text{glucose-1-phosphate}$$

Glycogen Synthesis starts with activation of glucose as UDP glucose

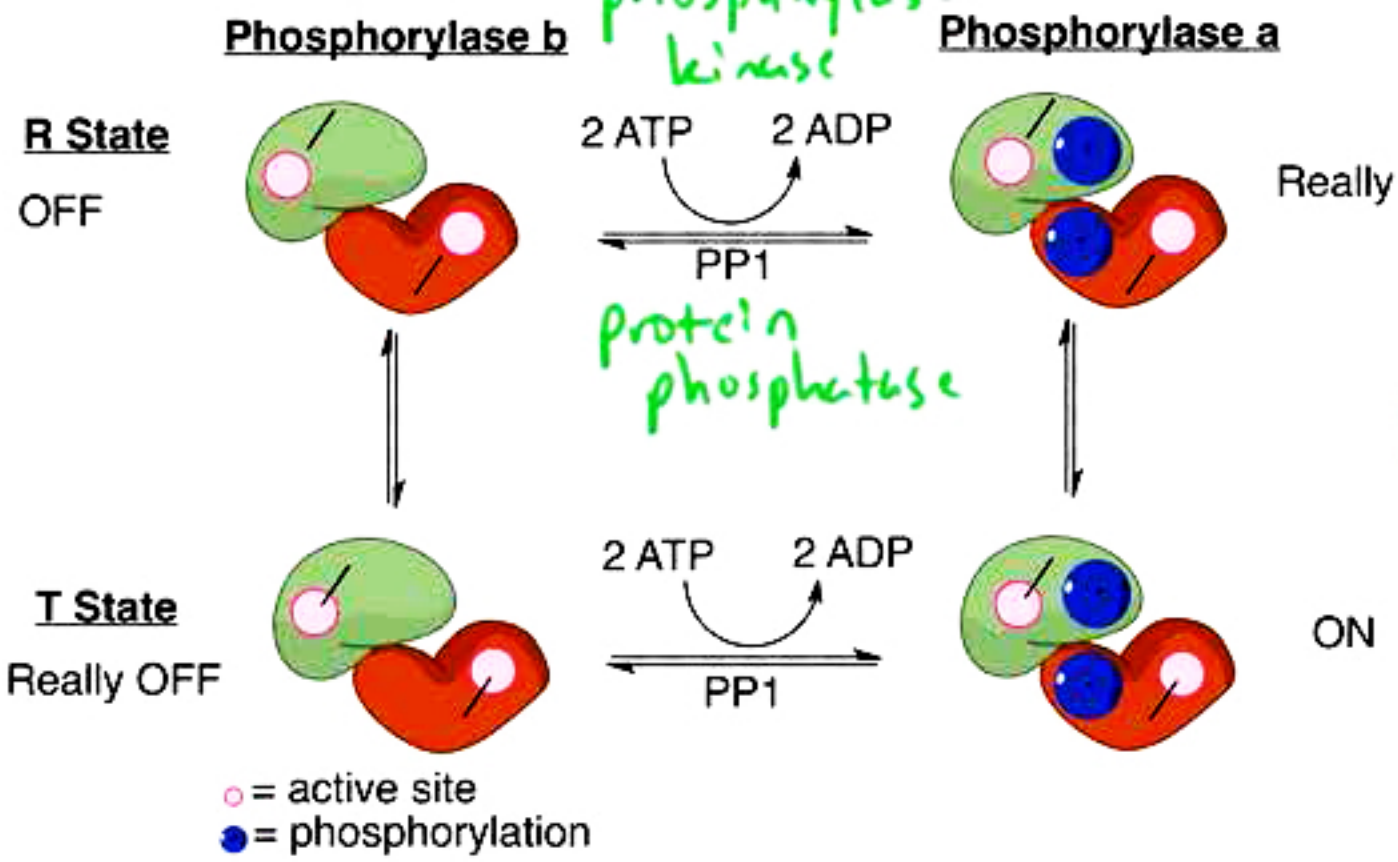


Glucose 1 Phosphate
(Cori ester)

Glycogen Synthase



Covalent modification by kinase



allostric regulation

AMP ↑

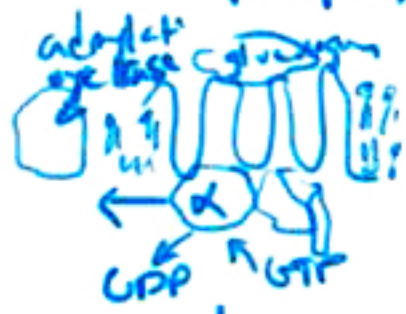
GCP ↓

glucagon

↓

GPCR

(g protein coupled receptor)



↓

α subunit activate adenylate cyclase

↓

2nd messenger →

cAMP

↓

Protein Kinase A

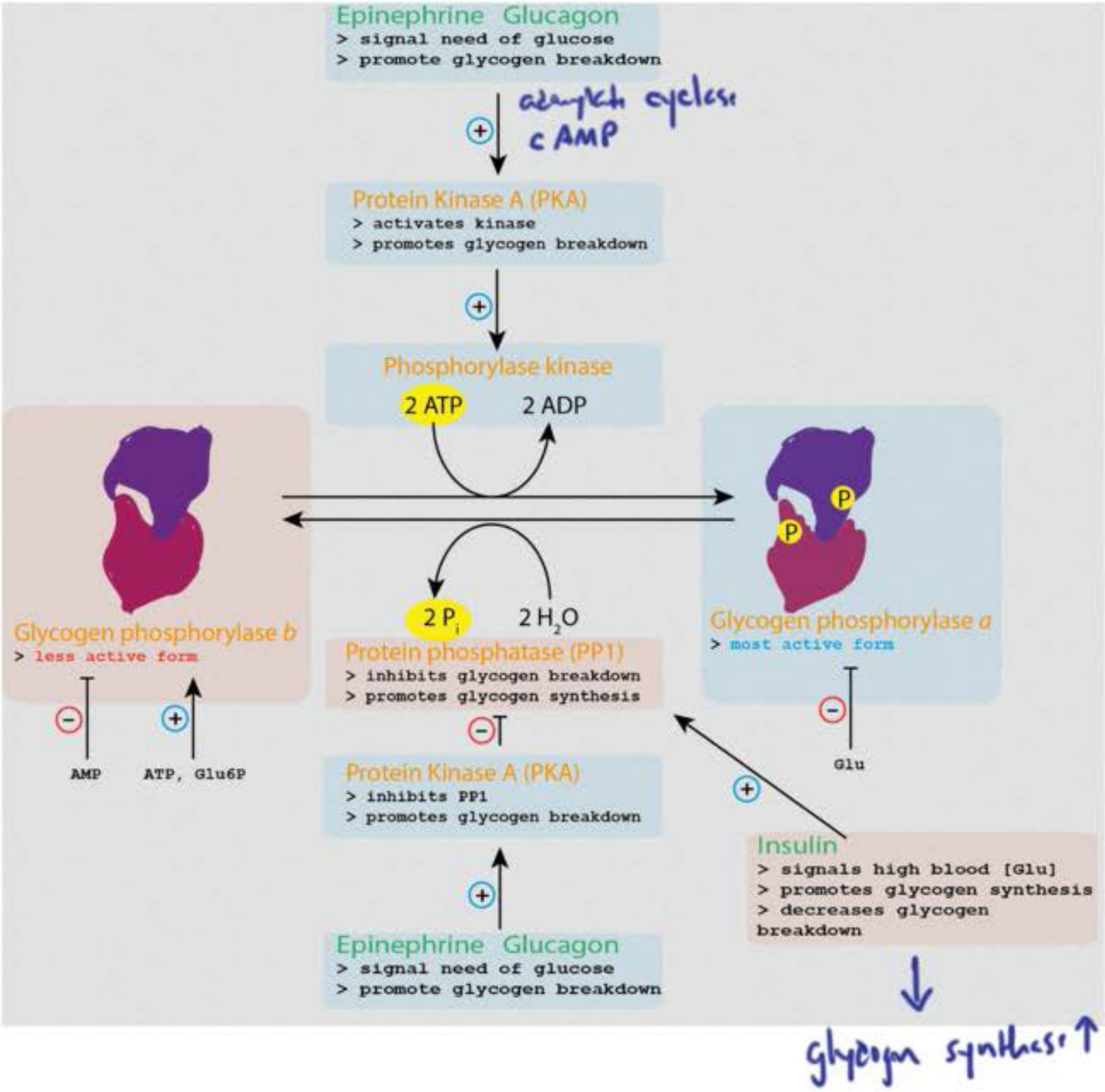
transcription factor for PEP carboxykinase ↑

↓ pyruvate dehydrogenase

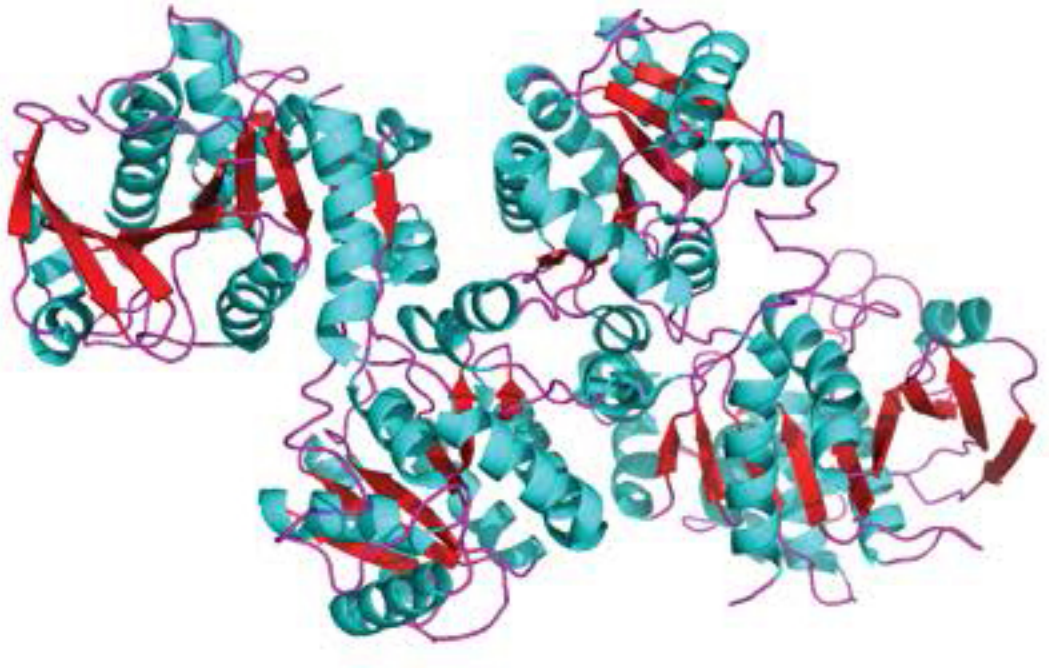
protein phosphatase ↓

phosphorylase kinase ↑

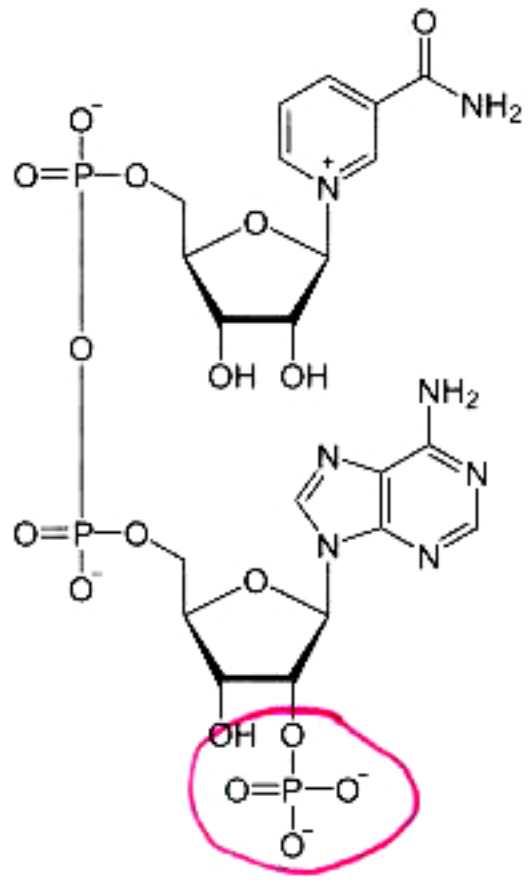
(etc.)


Epinephrine Glucagon
> signal need of glucose
> promote glycogen breakdown
adenylate cyclase
cAMP
Protein Kinase A (PKA)
> activates kinase
> promotes glycogen breakdown
Phosphorylase kinase
2 ATP
2 ADP
Glycogen phosphorylase b
> less active form
P
P
Glycogen phosphorylase a
> most active form
2 Pi
2 H2O
Protein phosphatase (PP1)
> inhibits glycogen breakdown
> promotes glycogen synthesis
AMP
ATP, Glu6P
Glu
Protein Kinase A (PKA)
> inhibits PP1
> promotes glycogen breakdown
Insulin
> signals high blood [Glu]
> promotes glycogen synthesis
> decreases glycogen breakdown
Epinephrine Glucagon
> signal need of glucose
> promote glycogen breakdown
glycogen synthase ↑

Glycogen Synthase



NADPH brings reducing equivalents to biosynthesis

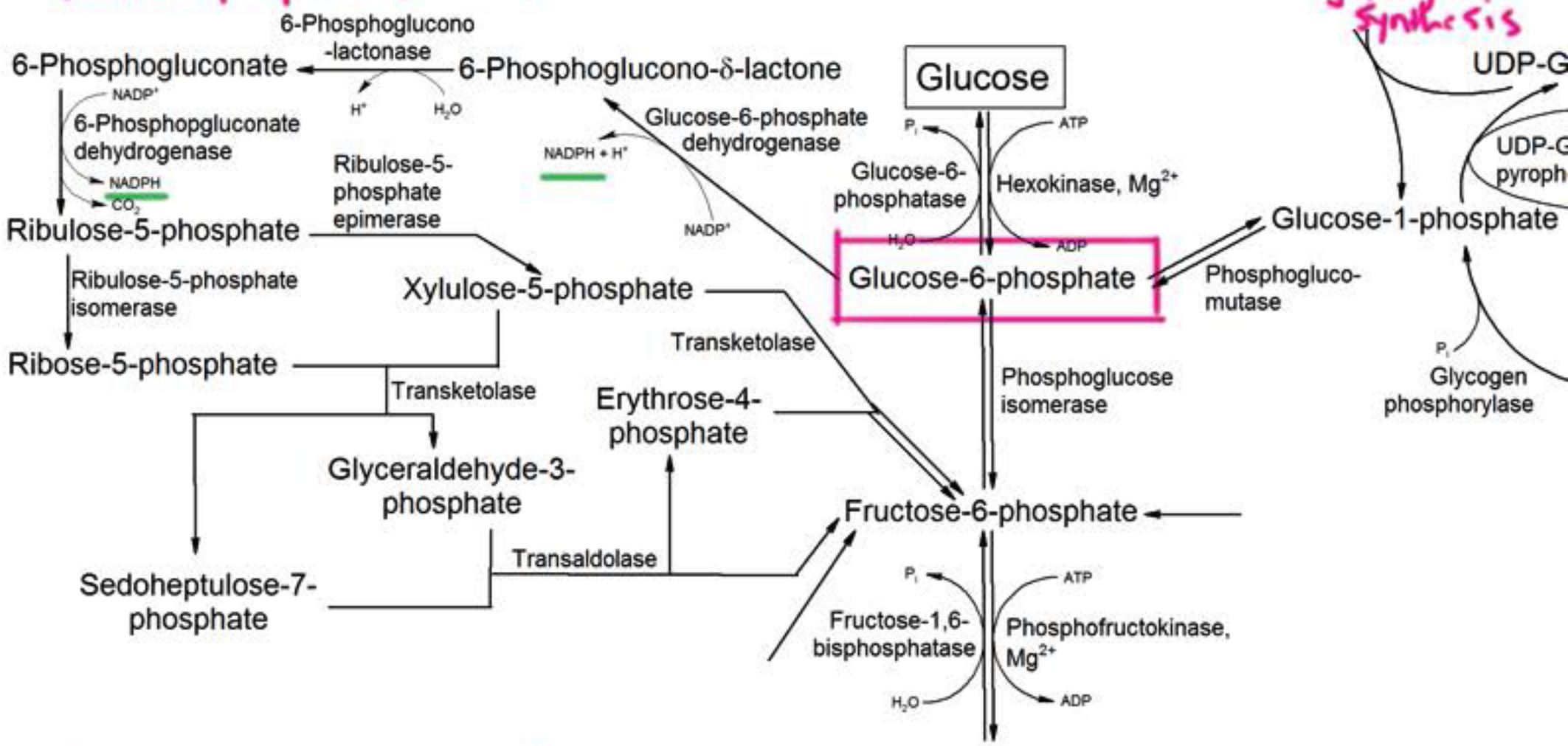


$NADP^+$

primary source is PPP

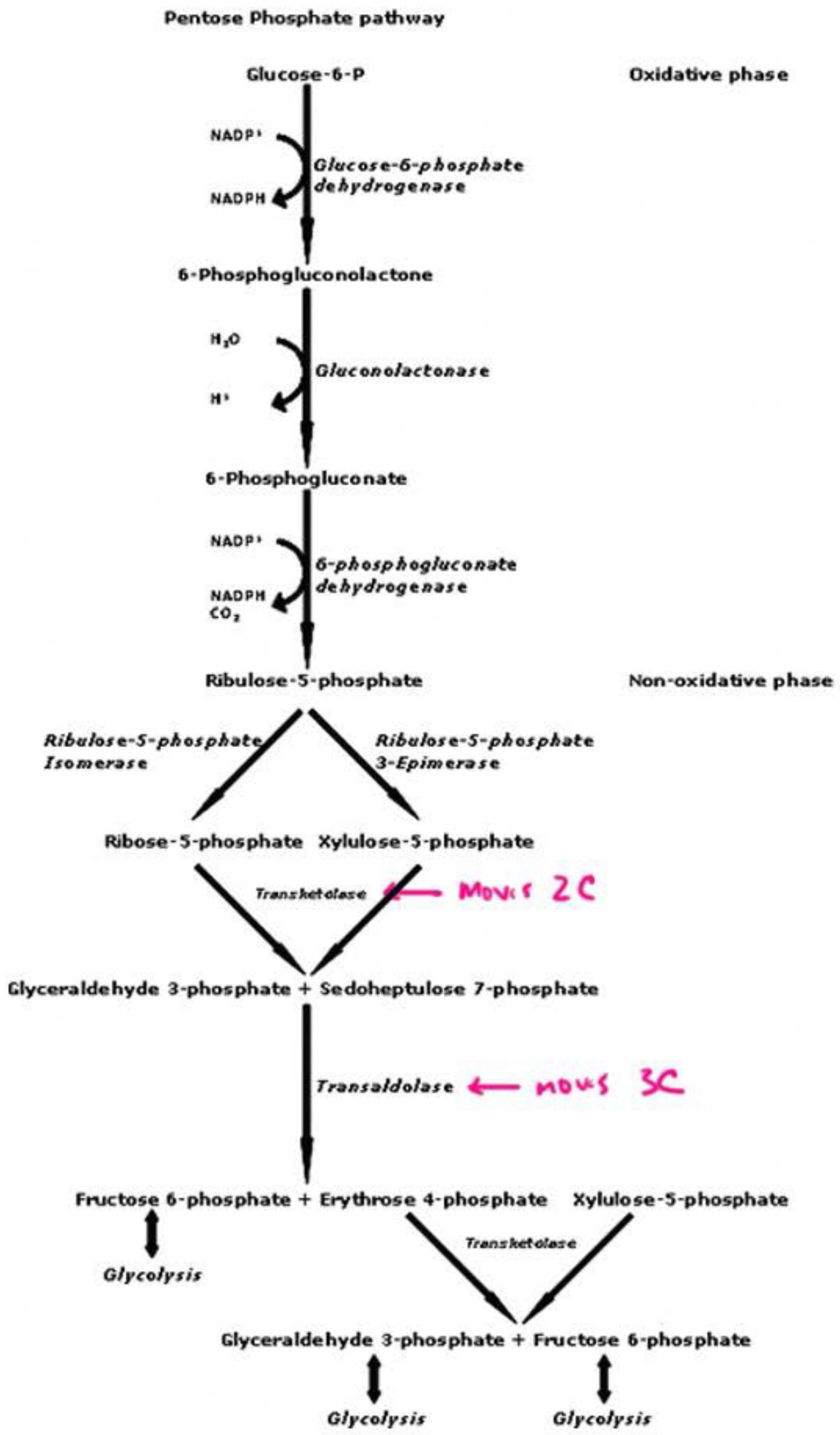
pentose phosphate pathway

glycogen synthesis



PPP - source of NADPH
and ribose

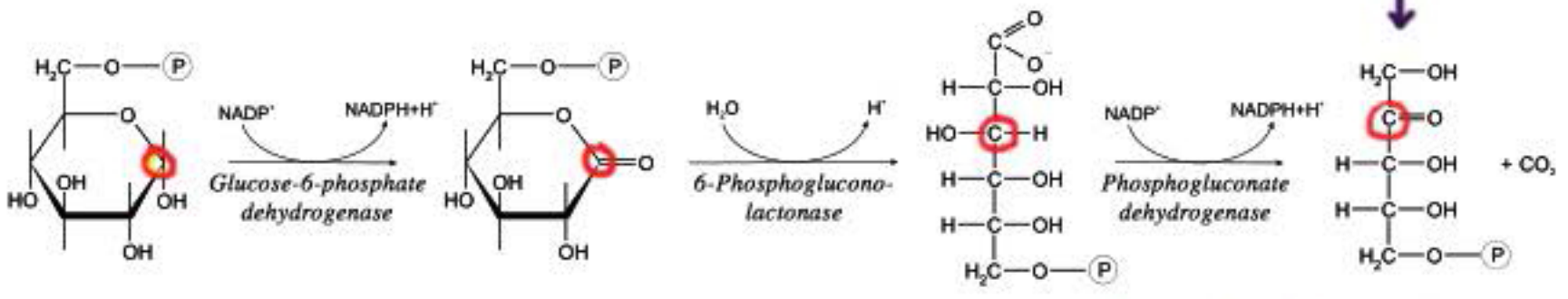
glycolysis



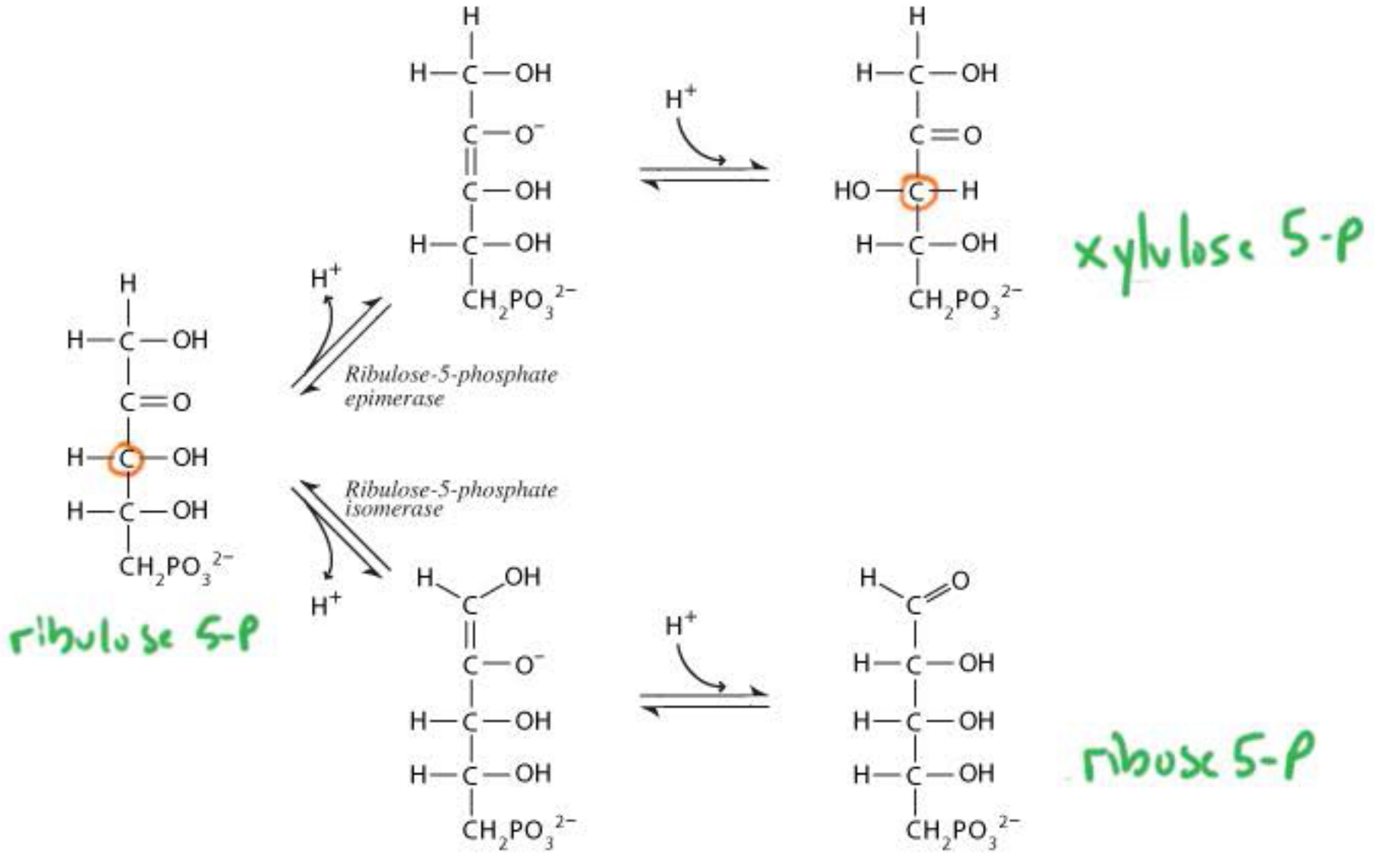
$$3G6P + 6NADP^+ + 3H_2O \rightleftharpoons 6NADPH + 6H^+ + 3CO_2 + 2F6P + GAP$$

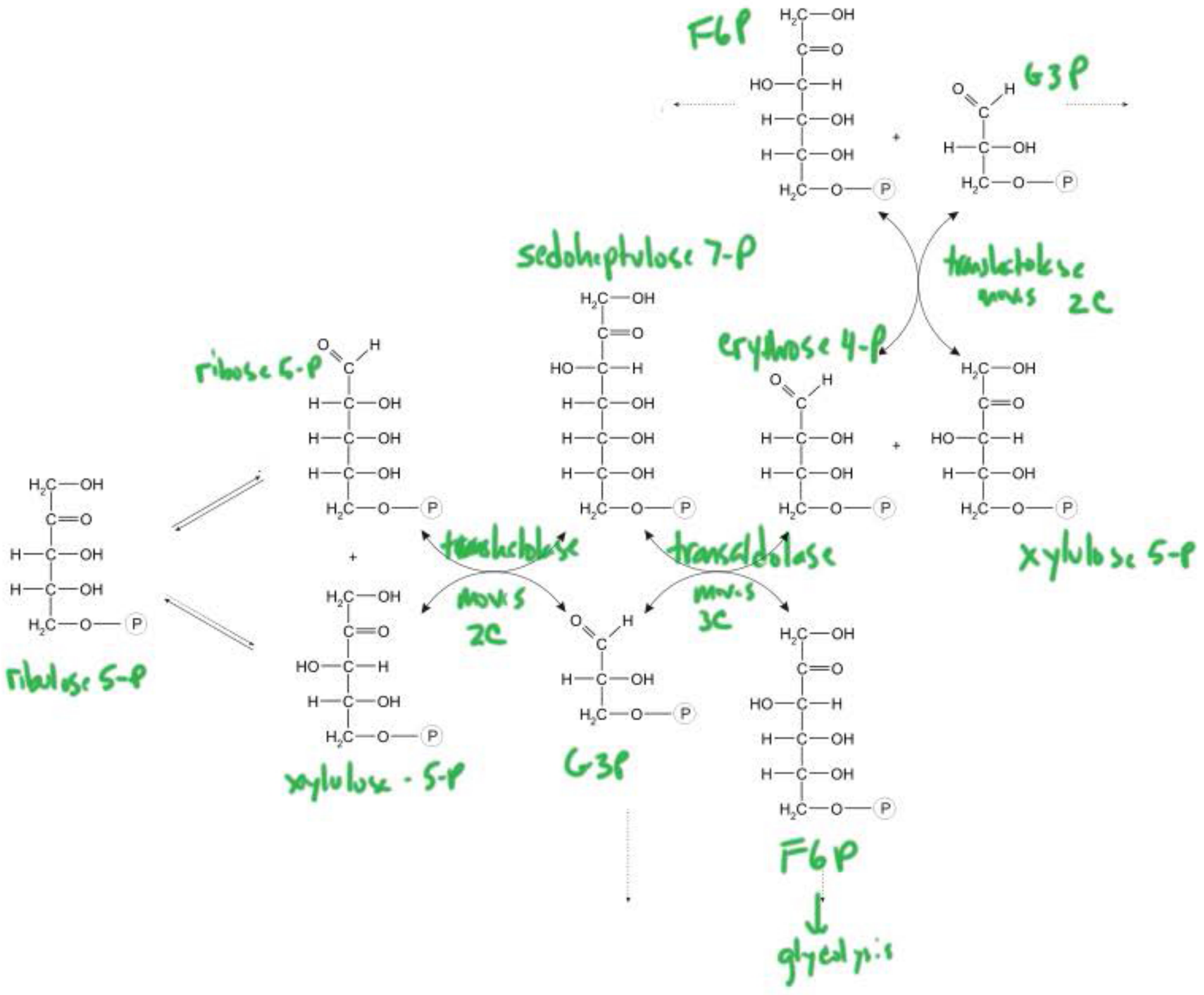
Oxidative portion

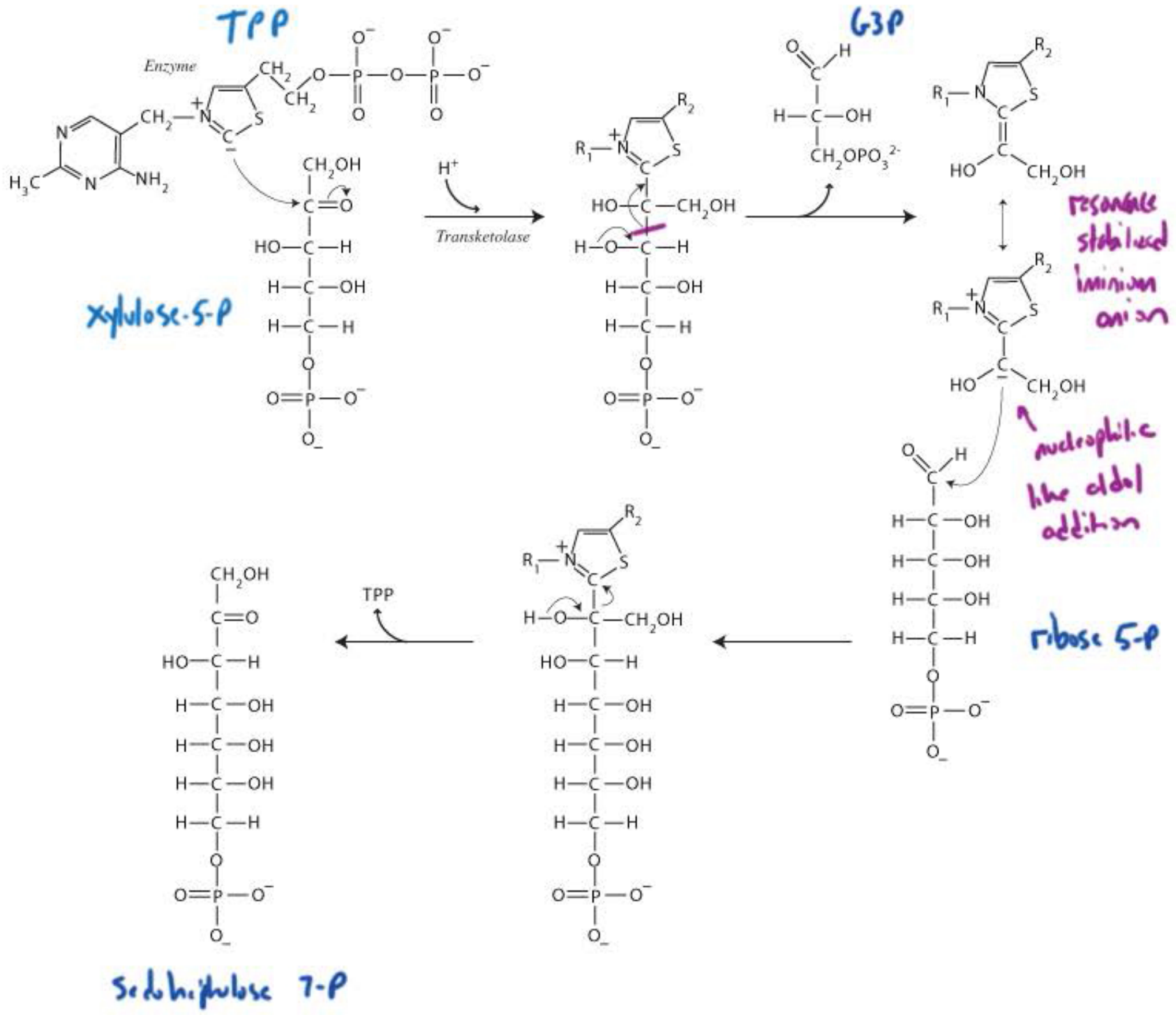
ribulose 5-P

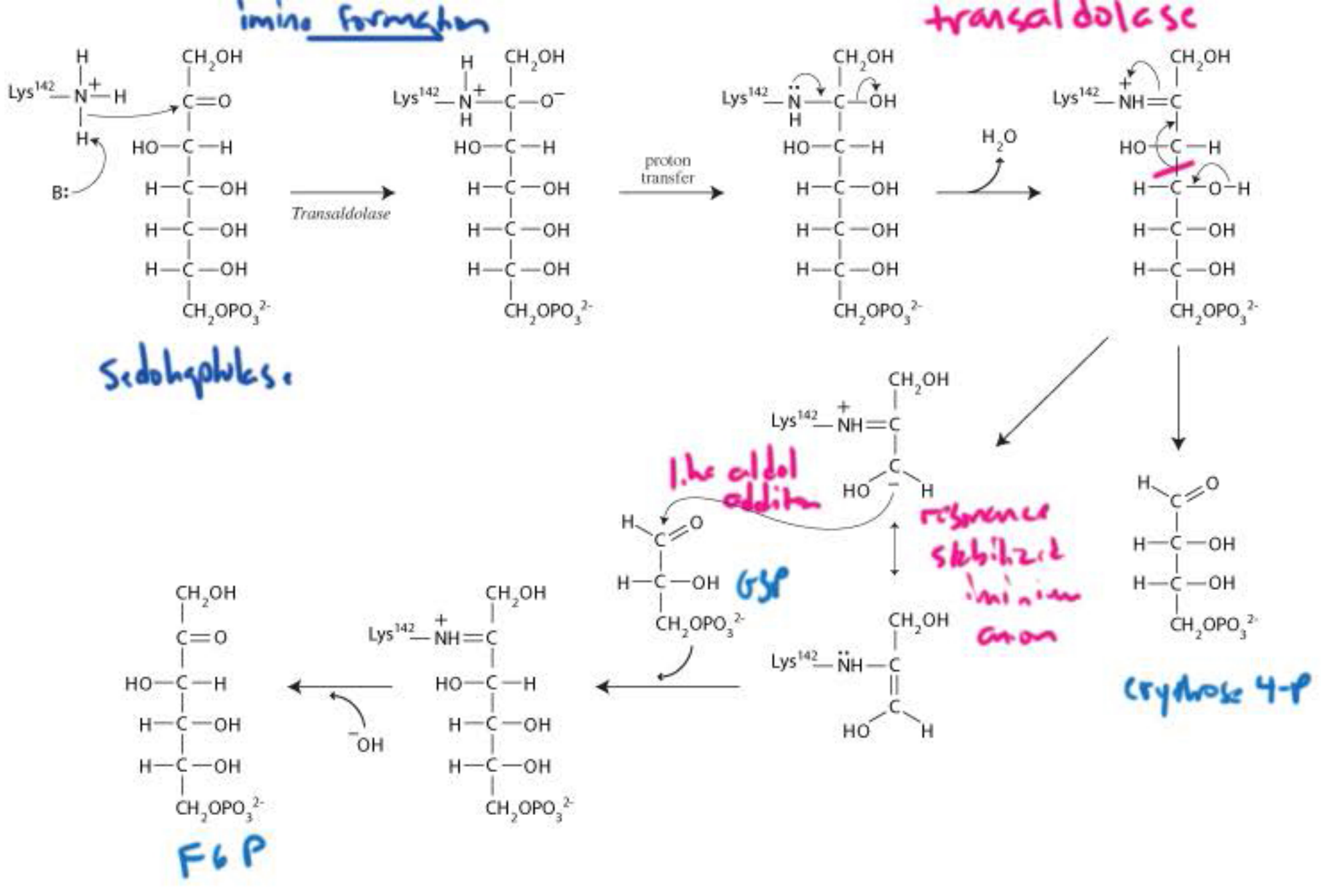


oxidative branch
β keto acid
which decarboxylates


Ribulose-5-phosphate epimerase
Ribulose-5-phosphate isomerase
ribulose 5-P
xylulose 5-P
ribose 5-P


F6P
G3P
sedoheptulose 7-P
transketolase
moves 2C
erythrose 4-P
ribose 5-P
transketolase
transaldolase
moves 2C
moves 3C
xylulose 5-P
ribulose 5-P
xylulose - 5-P
G3P
F6P
glycolysis


TPP
Enzyme
xylulose-5-P
Transketolase
G3P
resonance stabilized iminium anion
nucleophilic like aldol addition
ribose 5-P
TPP
Sedoheptulose 7-P


imine formation
transaldolase
Transaldolase
proton transfer
H_2O
Sedoheptulose
like aldol addition
resonance stabilized imine anion
GSP
Erythrose 4-P
F6P

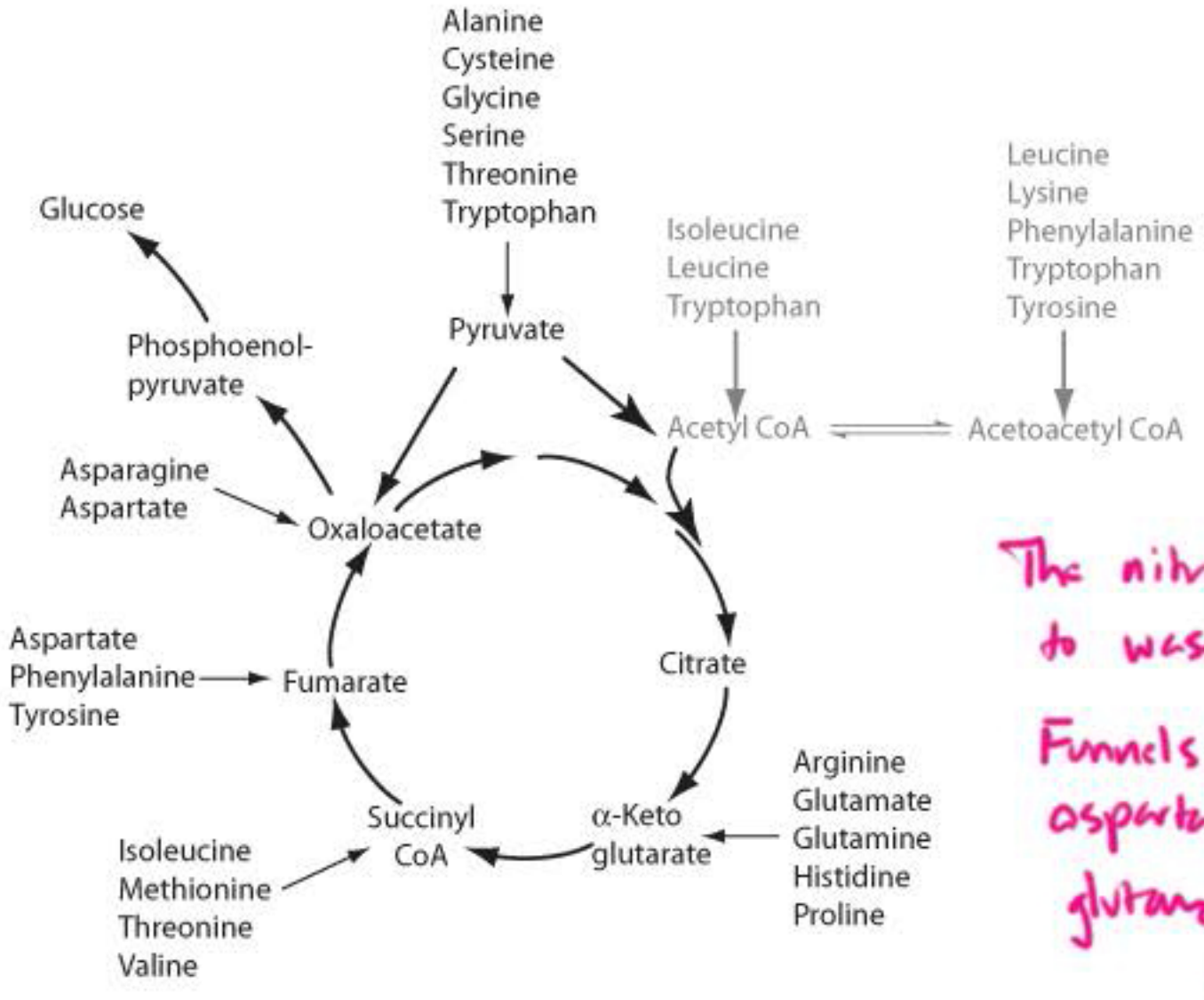
$$C_5 + C_5 \rightleftharpoons C_7 + C_3$$ transketolase

$$C_7 + C_3 \rightleftharpoons C_6 + C_4$$ transaldolase

$$C_5 + C_4 \rightleftharpoons C_6 + C_3$$ transketolase

---

sum $$3C_5 \rightleftharpoons 2C_6 + C_3$$



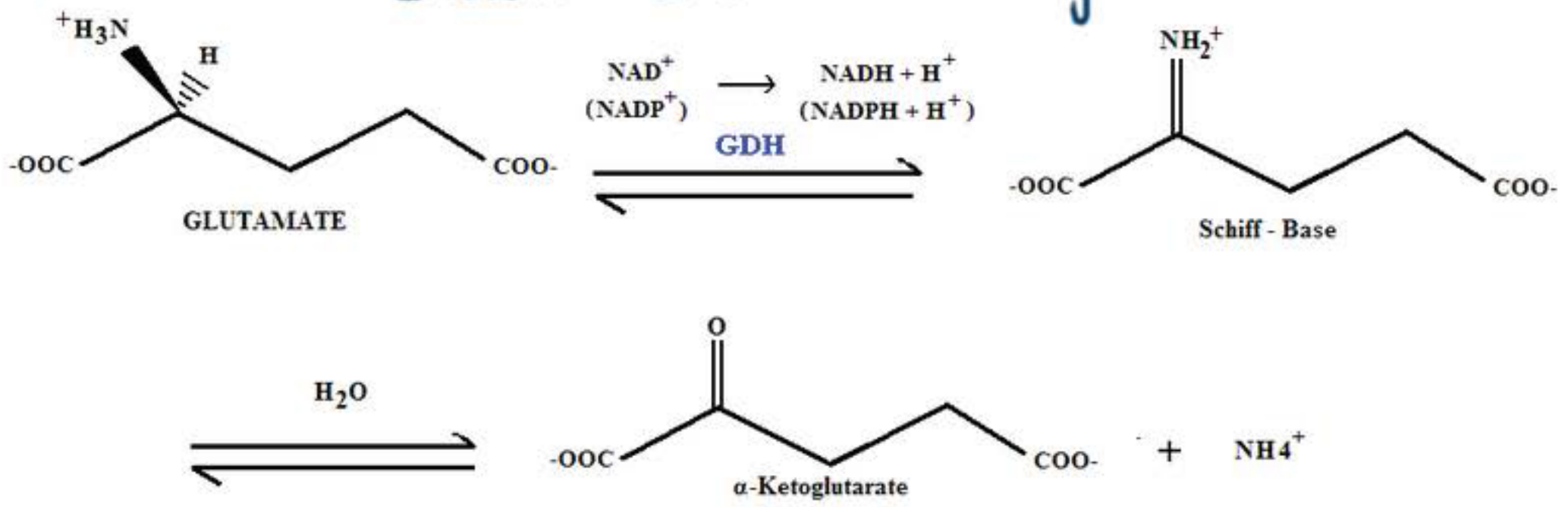
The nitrogen flows to waste.

Funnels into aspartate and glutamate

↓

urea cycle

oxidative deamination of glutamate

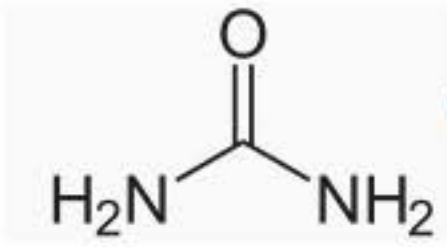


ΔG° ⊕

product removal

ΔG ⊖ at physiological $[NH_4^+]$

$$\Delta G = \Delta G^\circ + RT \ln Q$$

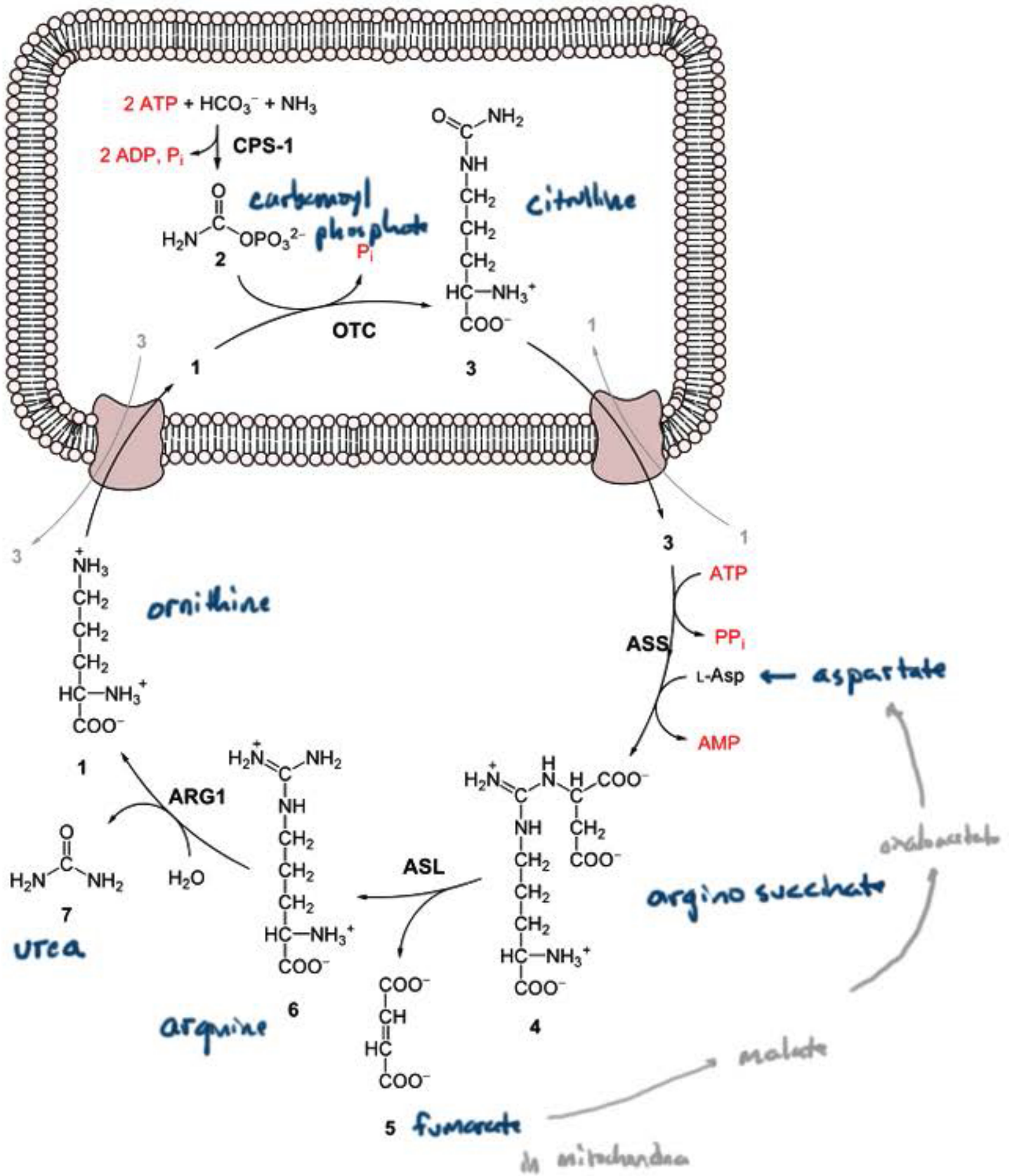


urea

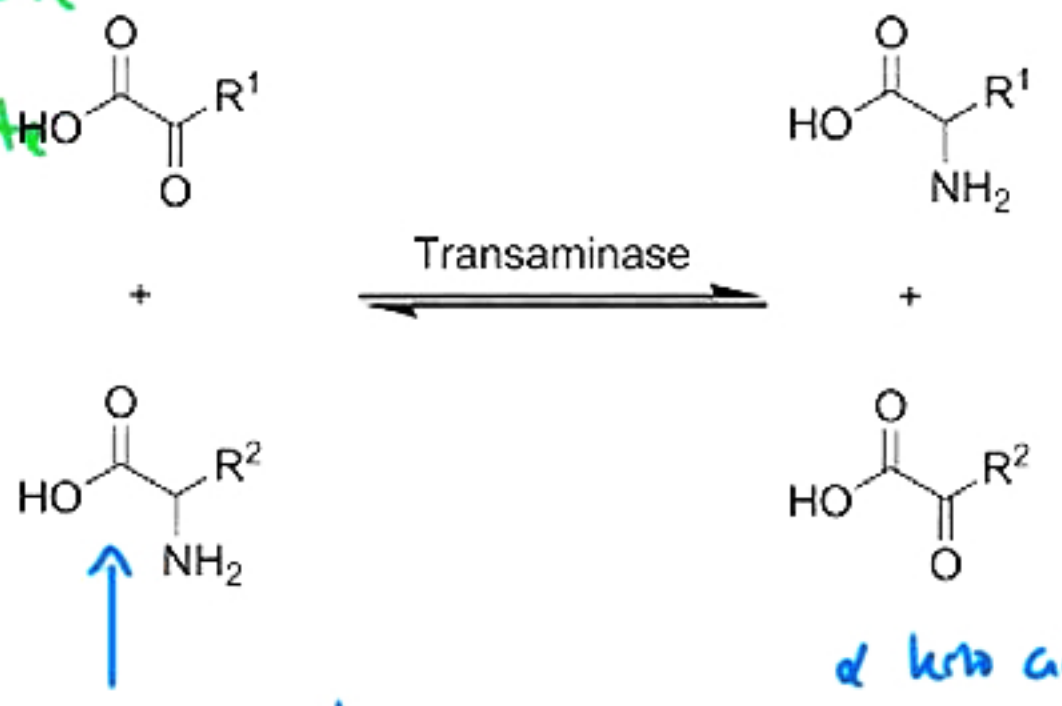
$$NH_3 + CO_2 + \text{aspartate} + 3\ ATP + 2\ H_2O \rightarrow \text{urea} + \text{fumarate} + 2\ ADP + 2\ P_i + AMP + PP_i$$

N #1 N #2

urea cycle


2 ATP + HCO3⁻ + NH3
2 ADP, Pi
CPS-1
carbamoyl phosphate
2
citrulline
Pi
OTC
1
3
3
1
3
1
ATP
PPi
ASS
L-Asp
aspartate
AMP
ornithine
1
ARG1
H2O
7
urea
6
arginine
ASL
4
argino succinate
oxaloacetate
5 fumarate
malate
in mitochondria

α keto glutarate
or
oxaloacetate



glutamate
or
aspartate

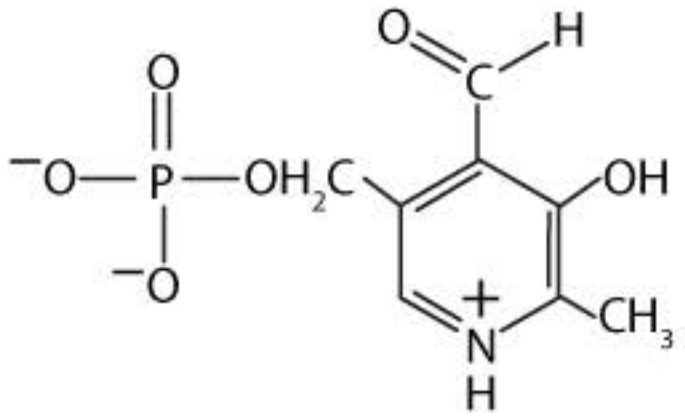
⇓

urea cycle

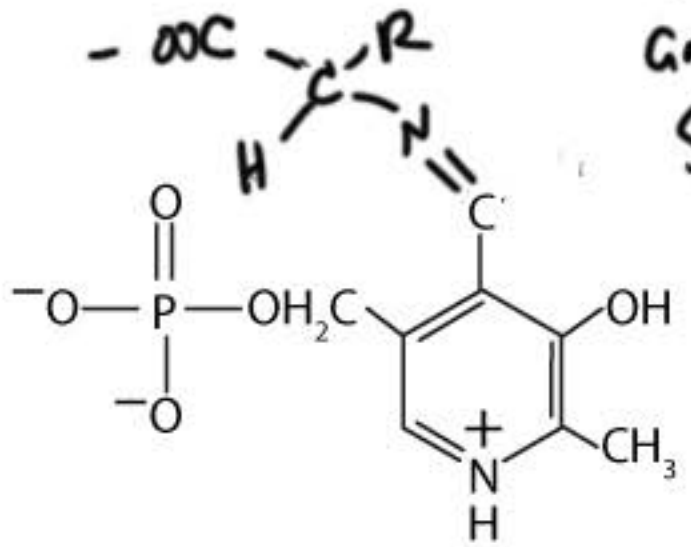
amino acid
for food

α keto acid

Transaminase is a PLP enzyme

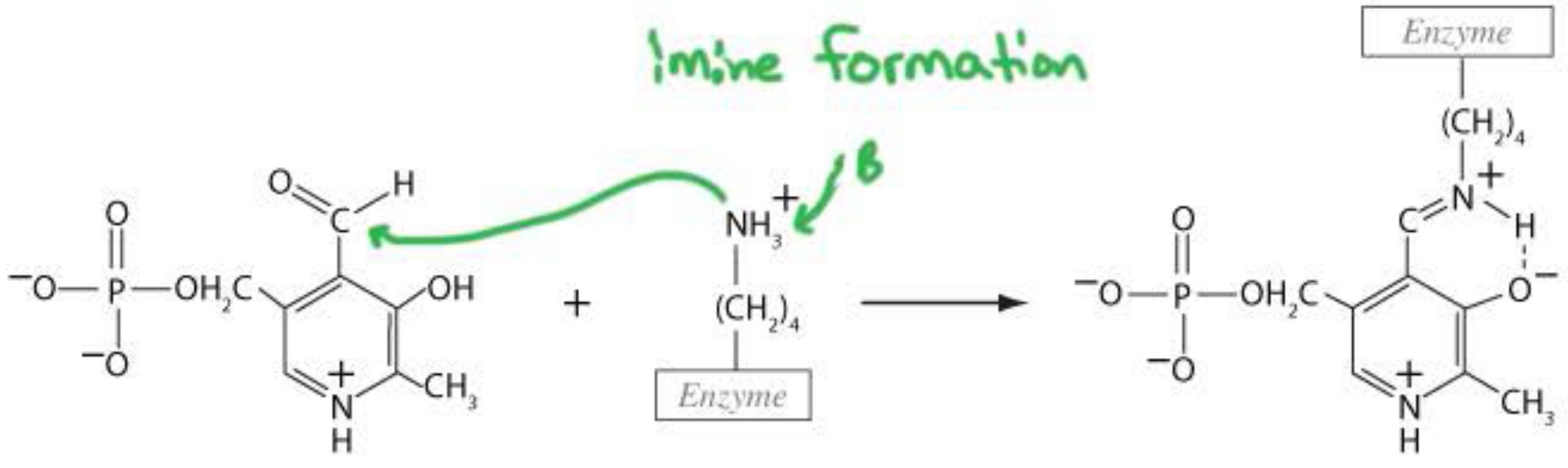


PLP
amino acid
Schiff base

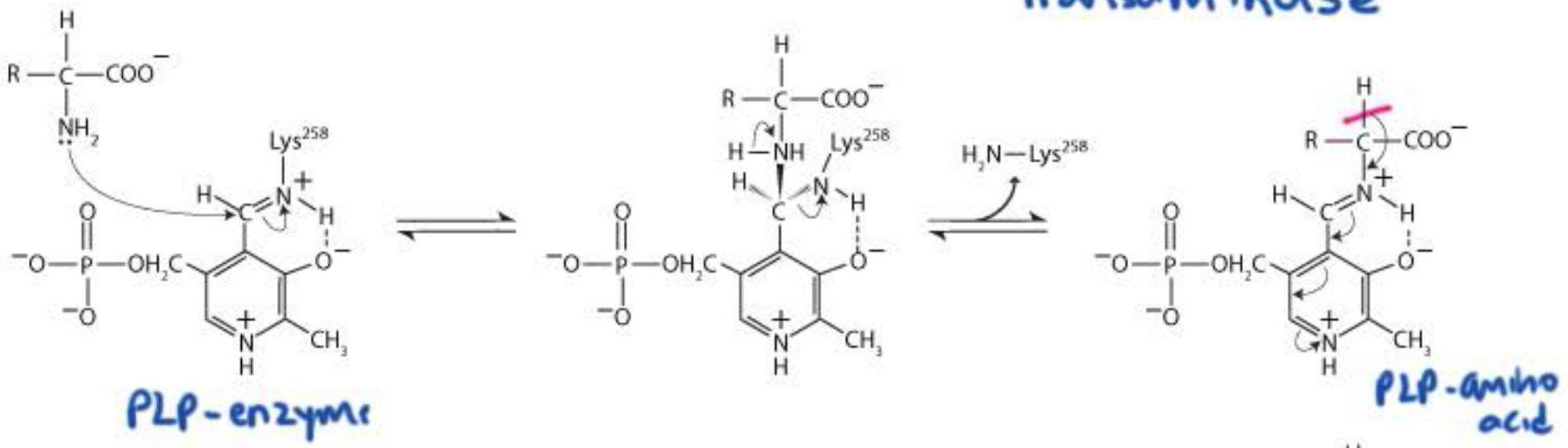


Formation of PLP - enzyme Schiff base

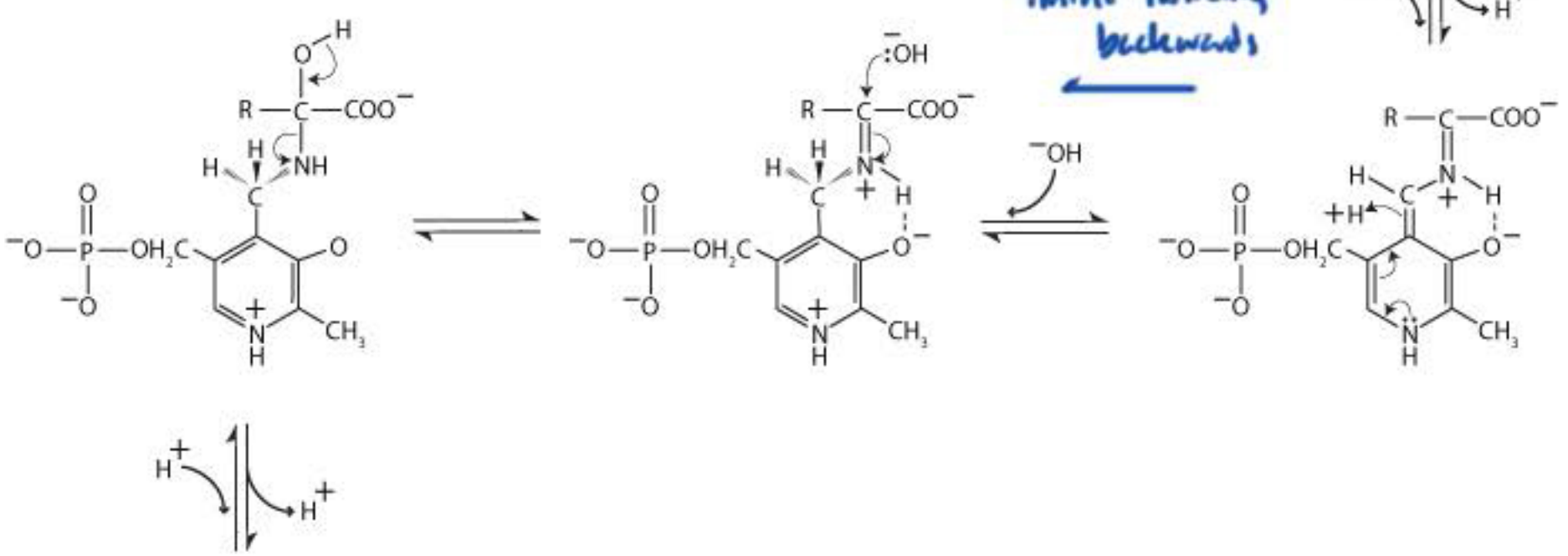
Imine formation

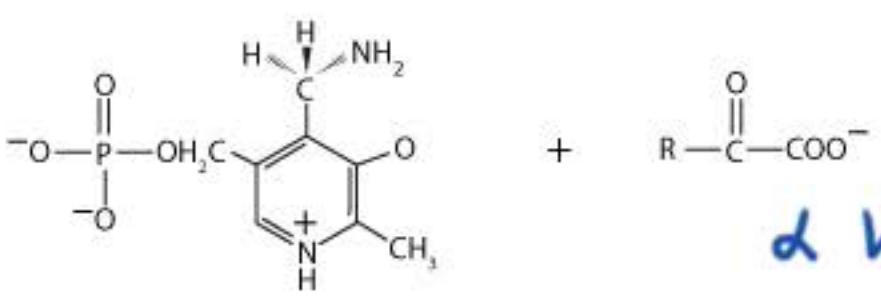


Transaminase



Imine formation backwards





PMP

α keto acid

Next, we go backwards with a different α keto acid