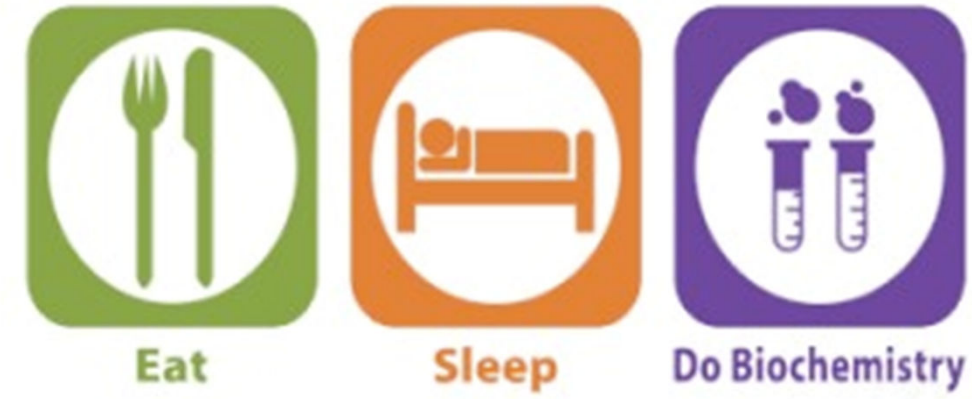
153A Winter 2011
Review session for Final Exam

Thursday, March 10

5-7 pm in Dodd 147

Theresa and Megan

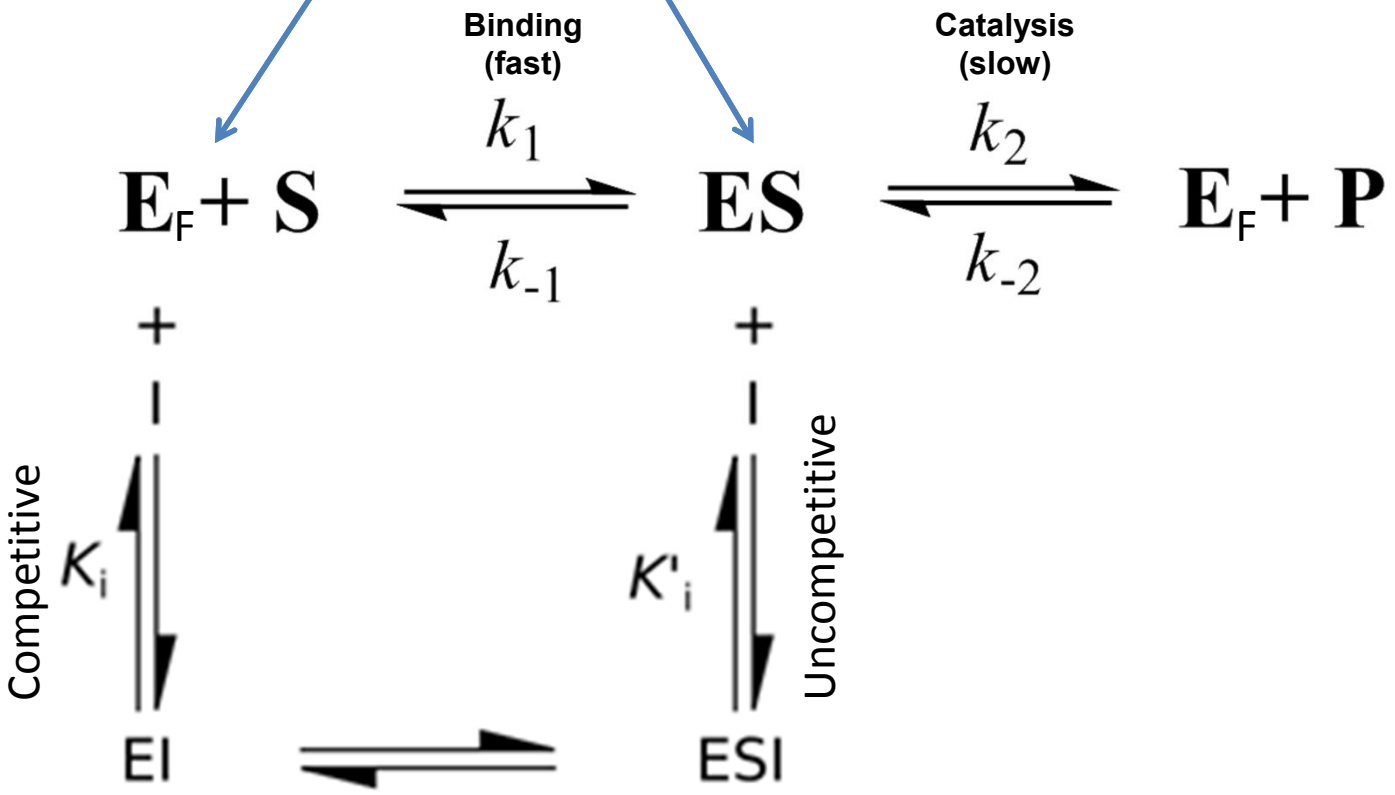
- Inhibitor kinetics
  - Mixed inhibition
- Enzyme Regulation
  - Allosteric regulation
  - Covalent modification
- Metabolism overview
- Glycolysis
- Fates of Pyruvate
  - Gluconeogenesis and anapleurotic reactions
  - Homolactic and alcohol fermentation
  - PDH complex



- TCA Cycle
- Reduction potentials
- Electron Transport Chain
- ATP Synthase
- Metabolic ATP yield
- Old exam questions
- Q&A!

Inhibitor Kinetics

Mixed inhibitors can bind both to E_F and ES



Inhibitor binds active site on E_F

Inhibitor binds ES complex

Inhibitor Kinetics

What is K_m?

Definition of K_m (Units = M)

1. $K_m = \frac{k_{-1} + k_2}{k_1}$ or the $\frac{breakdown\ of\ ES}{formation\ of\ ES}$, or the ratio of rate constants describing an enzyme's affinity for its substrate. Enzyme affinity for substrate is inversely related to K_m:
   Low K_m = high affinity
   High K_m = low affinity.

2. K_m = [S] when $\frac{V_{max}}{2}$, or the substrate concentration at one half of maximal velocity.

K_m is characteristic of the enzyme and is not affected by [E] or [S].
In the presence of enzyme inhibitors, K_m may look to increase (competitive inhibition) or decrease (uncompetitive inhibition) but this is only an *apparent* change.

Inhibitor Kinetics

What is V_{max}?

Definition of V_{max} (Units = M/s)

1. V_{max} is the velocity of catalysis at which reaction goes as fast as it can to make product.
   a. When [S] >>> K_m so that Vo approaches V_{max}, all enzymes are virtually bound in a E-S complex, or $[E_T]$ = [E-S]. Since $V_o = k_2$ [E-S] (see Assumptions (1) and (2) in Dr. Nelson's handout), we can substitute to get $V_{max} = k_2[E_T]$.

2. $V_{max} = [E] \times k_{cat}$, where k_{cat} (turnover number, units = s^{-1}) is the rate at which the enzyme is capable of catalyzing the reaction at infinite [S]. V_{max} is a function of k_{cat} and is therefore dependent on [E].

V_{max} depends on [E], so increasing [E] will increase V_{max}.
In uncompetitive inhibition, not all E will become E-S; you will always have some small amount of E-S-I even at high [S] that does not make product. Therefore, you will be a little lower than true $V_{max.}$ It will look like V_{max} has an *apparent* decrease in uncompetitive inhibition, and also mixed inhibition (mixed inhibitors have some affinity for E-S, will make unproductive E-S-I)

.

Inhibitor Kinetics

Inhibitor terminology

- **α and α'** describe how competitive (α) or how uncompetitive (α') an inhibitor is. The higher the α or α', the higher the [substrate] must be to overcome inhibition. (Arbitrary units?)
- **K_I or $K_{I'}$** describe the affinity of inhibitor to enzyme. Like K_M and K_d and p_{50} and $K_{0.5}$, K_I or $K_{I'}$ are inversely related to enzyme affinity. (Units: M)
    - Low K_I or $K_{I'}$ = high affinity of enzyme to inhibitor
    - High K_I of $K_{I'}$ = low affinity of enzyme to inhibitor

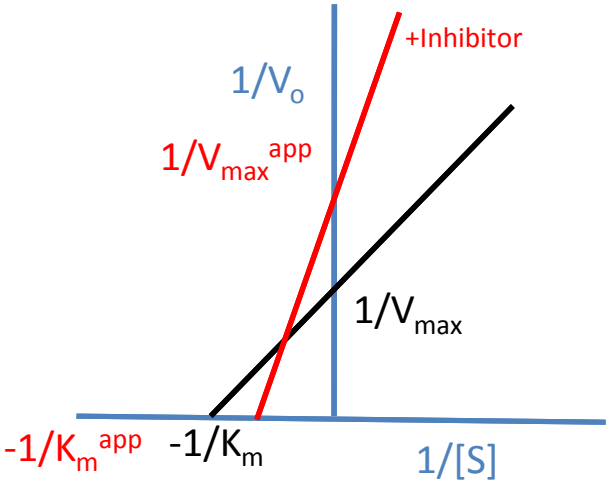
$$K_i = \frac{[E][I]}{[EI]} \qquad K_i' = \frac{[ES][I]}{[ESI]}$$

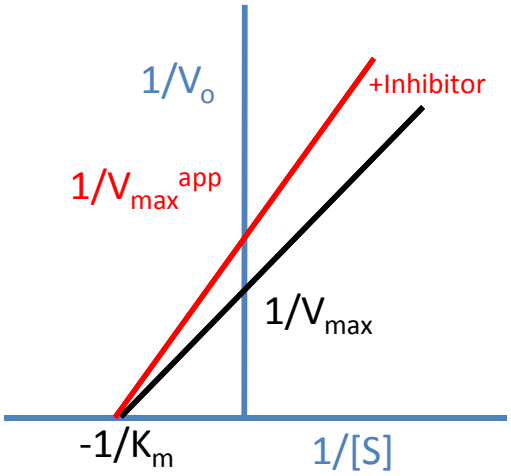
Low K_I or $K_{I'}$ comes from having a large [EI] or large [ESI]

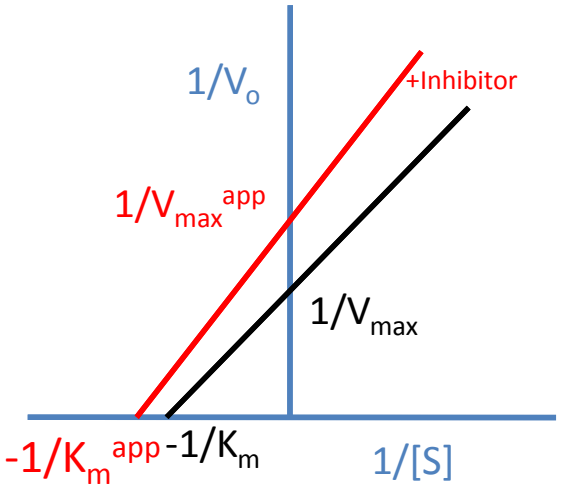
Inhibitor Kinetics

Mixed inhibition kinetics can be observed as a spectrum of competitive and uncompetitive properties

Mixed Inhibition		
More competitive	Equal Noncompetitive	More uncompetitive
$\alpha' < \alpha$	$\alpha' = \alpha$	$\alpha' > \alpha$
$K_I < K_{I'}$	$K_I = K_{I'}$	$K_I > K_{I'}$
K_M^{app} increases	K_m stays same	K_m^{app} decreases
V_{max}^{app} decrease	V_{max}^{app} decrease	V_{max}^{app} decrease



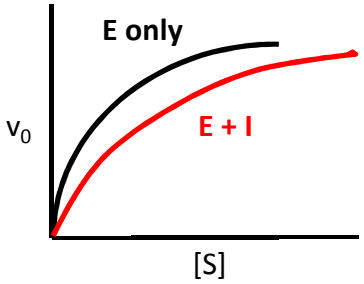


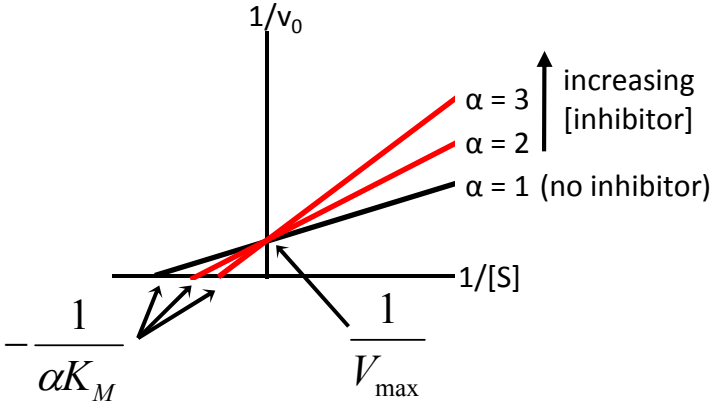


Inhibitor Kinetics

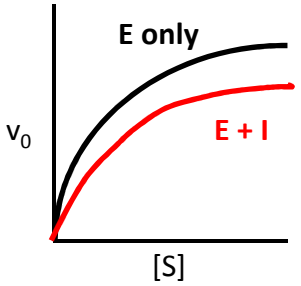
From Review Session 2 with Dan and Han:
Summary of kinetic effects of enzyme inhibition: M-M & Lineweaver-Burk plots from Exam 2

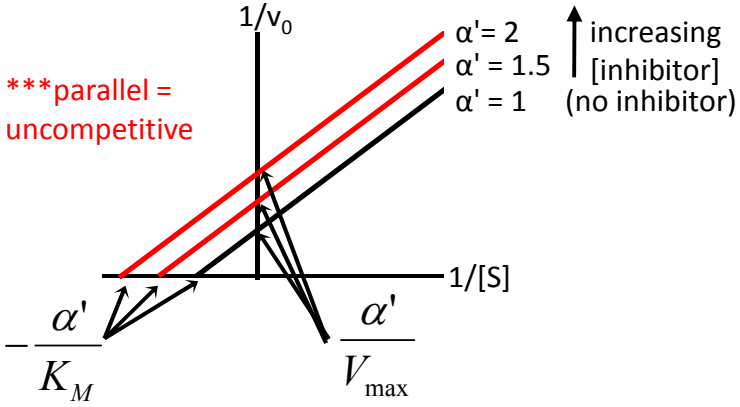
Competitive inhibition (α' =1)





Unccompetitive inhibition (α=1)



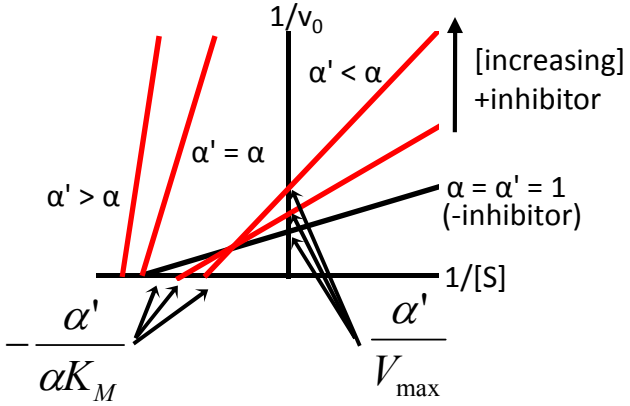


Mixed inhibition

α' < α +inhibitor and
-inhibitor lines cross
K_M^{app} increases

α' > α lines do not cross
K_M^{app} decreases

α' = α lines intersect at x-int
K_M^{app} no change



Regulation

How can enzymes be regulated?

- Control the concentration of enzyme
  - Genetic repression or activation of synthesis of enzyme
- Control availability of substrate
  - Production, degradation or compartmentalization of substrate
  - Production of competitive inhibitors that limit substrate availability by binding to enzyme active site
- Control activity of enzyme
  - Allosteric regulation **(Review session example: PFK-1 from glycolysis)**
  - Covalent modification
    - Irreversible (e.g. serine proteases' zymogens)
    - Reversible (e.g. glycogen phosphorylase's phosphorylation and dephosphorylation; **Review session example: eukaryotic PDH complex**)

Regulation

Example of allosteric enzyme regulation

PFK-1 in glycolysis is regulated by multiple positive and negative effectors/modulators

Regulation

Allosteric effectors can regulate enzymes

Because we love hemoglobin, every time we hear "allosteric" we think of…

1. More than one binding site
2. Binding induces conformational change (e.g. positive effector CO or negative effector Cl^-)
3. T state = inactive, deoxy

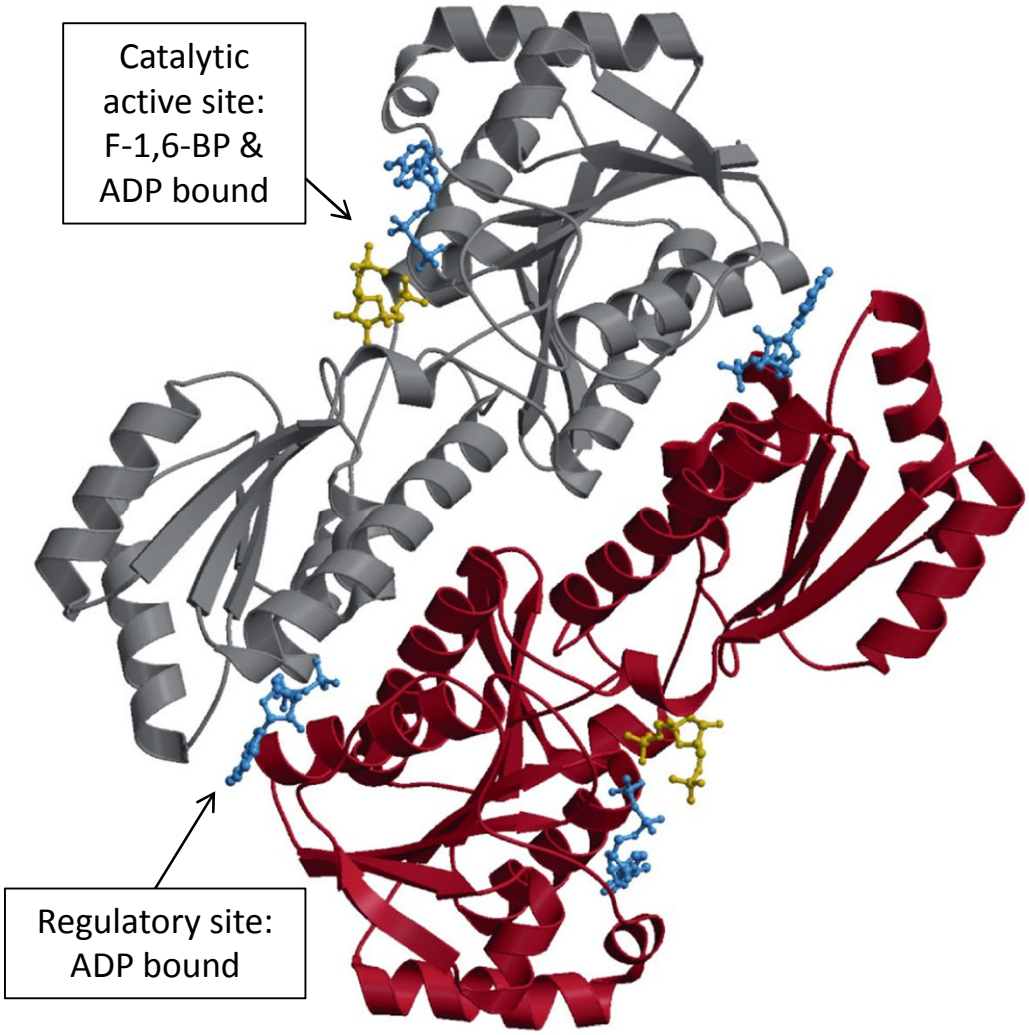
   R state = active, oxy
4. Binding curves are sigmodial (<u>not</u> like myoglobin's hyperbolic) to show that Hb is great at picking up and dropping off O_2

Allosteric regulatory enzymes are analogous to allosteric proteins!

1. In addition to catalytic subunits, allosteric enzymes have additional **<u>regulatory subunits</u>**
2. Binding of **<u>effectors</u>** to regulatory subunit changes conformation to promote or inhibit catalysis
3. **<u>T state</u>** = inactive
   <u>R state</u> = active
4. **<u>Kinetic curves are sigmodial</u>** (<u>not</u> like Michaelis-Menten's hyperbolic) to show that enzyme can be activated and inactivated

Regulation

Allosteric effectors can regulate enzymes



1. In addition to active sites, allosteric enzymes have additional **regulatory sites**
2. Binding of **effectors** to regulatory subunit changes conformation to promote or inhibit catalysis
3. **T state** = inactive
   R state = active
4. **Kinetic curves are sigmodial** (not like Michaelis-Menten's hyperbolic) to show that enzyme can be activated and inactivated

Regulation

Allosteric effectors can regulate enzymes

Positive effectors increase enzyme activity:

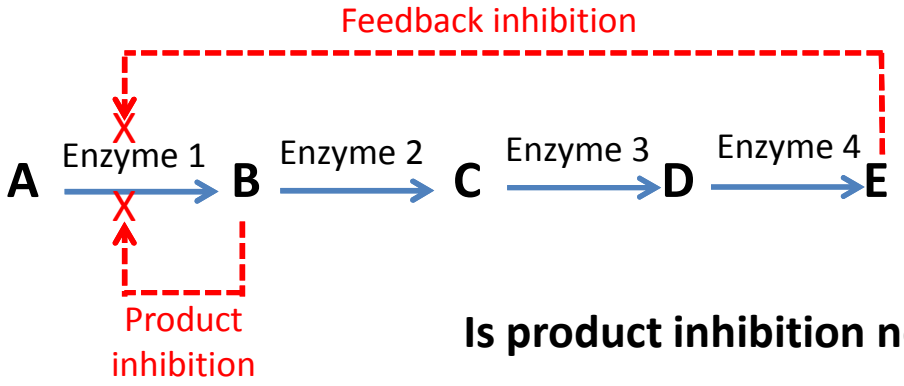
- F6P (substrate)
- ADP, AMP
- F-2,6-BP

Negative effectors decrease enzyme activity:

- ATP (feedback inhibition)
- Citrate (feedback inhibition)

1. In addition to active sites, allosteric enzymes have additional **regulatory sites**
2. Binding of **effectors** to regulatory subunit changes conformation to promote or inhibit catalysis
3. **T state** = inactive
   R state = active
4. **Kinetic curves are sigmodial** (not like Michaelis-Menten's hyperbolic) to show that enzyme can be activated and inactivated

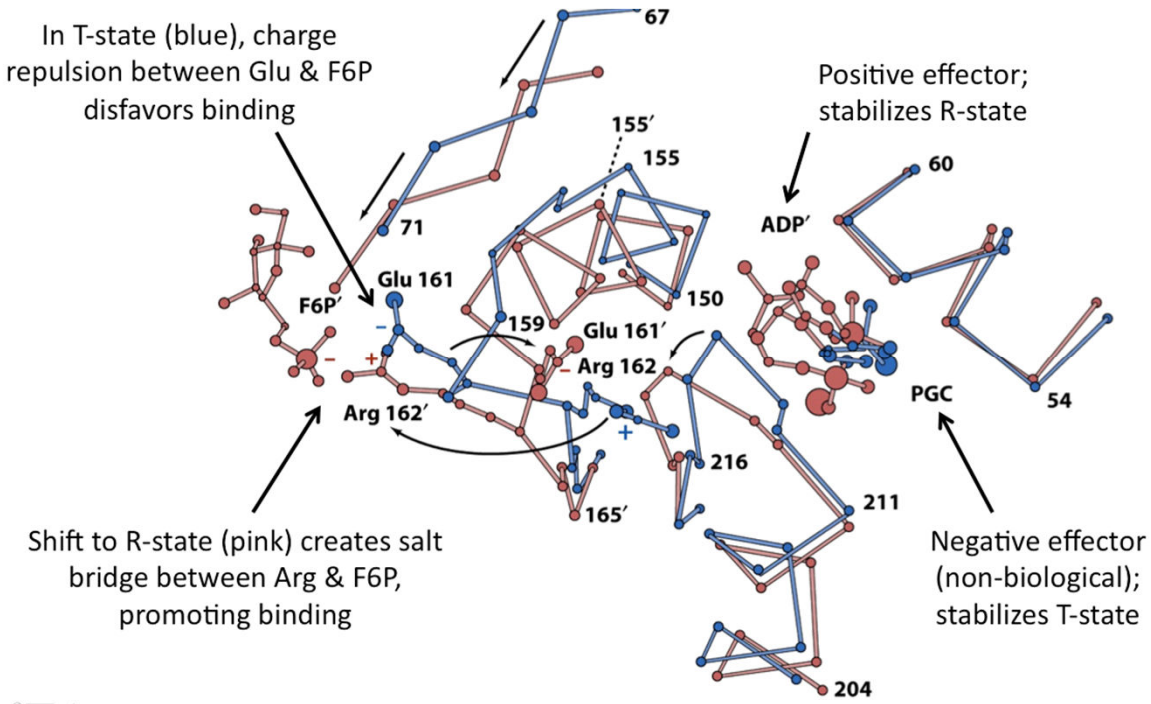
What are possible negative effectors of Enzyme 1?



Is product inhibition necessarily allosteric regulation?

Regulation

Allosteric effectors can regulate enzymes



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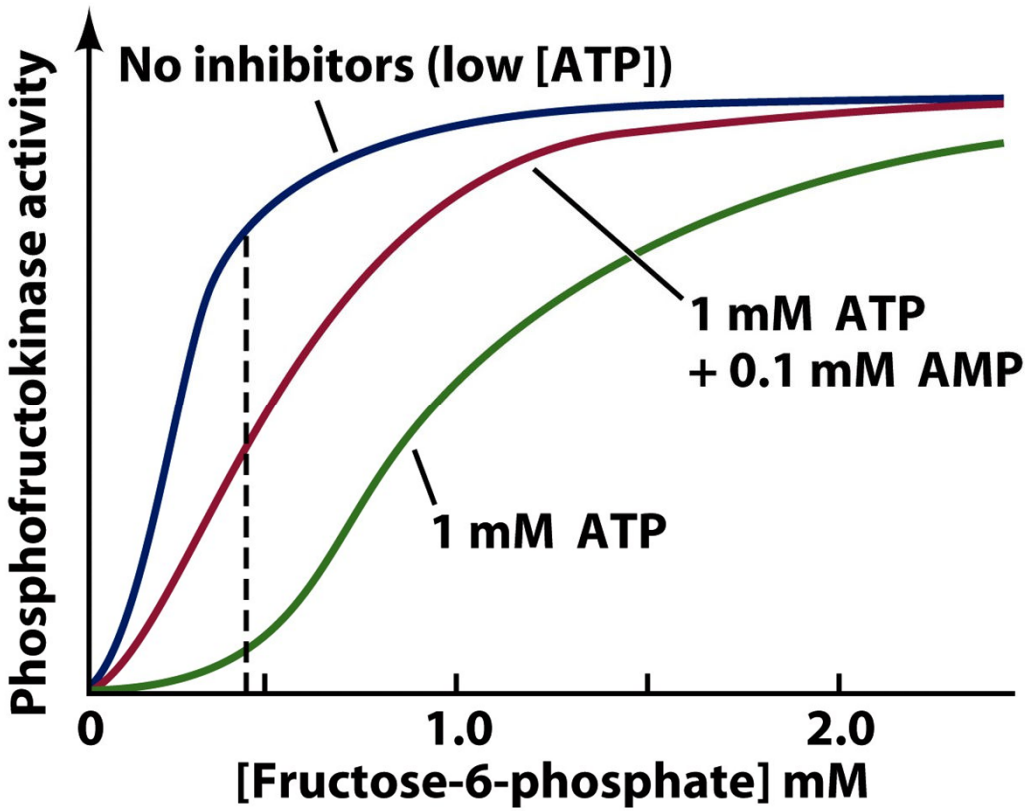
Regulation

Allosteric effectors can regulate enzymes



1. In addition to active sites, allosteric enzymes have additional **regulatory sites**
2. Binding of **effectors** to regulatory subunit changes conformation to promote or inhibit catalysis
3. **T state** = inactive
   R state = active
4. **Kinetic curves are sigmodial** (not like Michaelis-Menten's hyperbolic) to show that enzyme can be activated and inactivated (not called a K_m but a $K_{0.5}$)

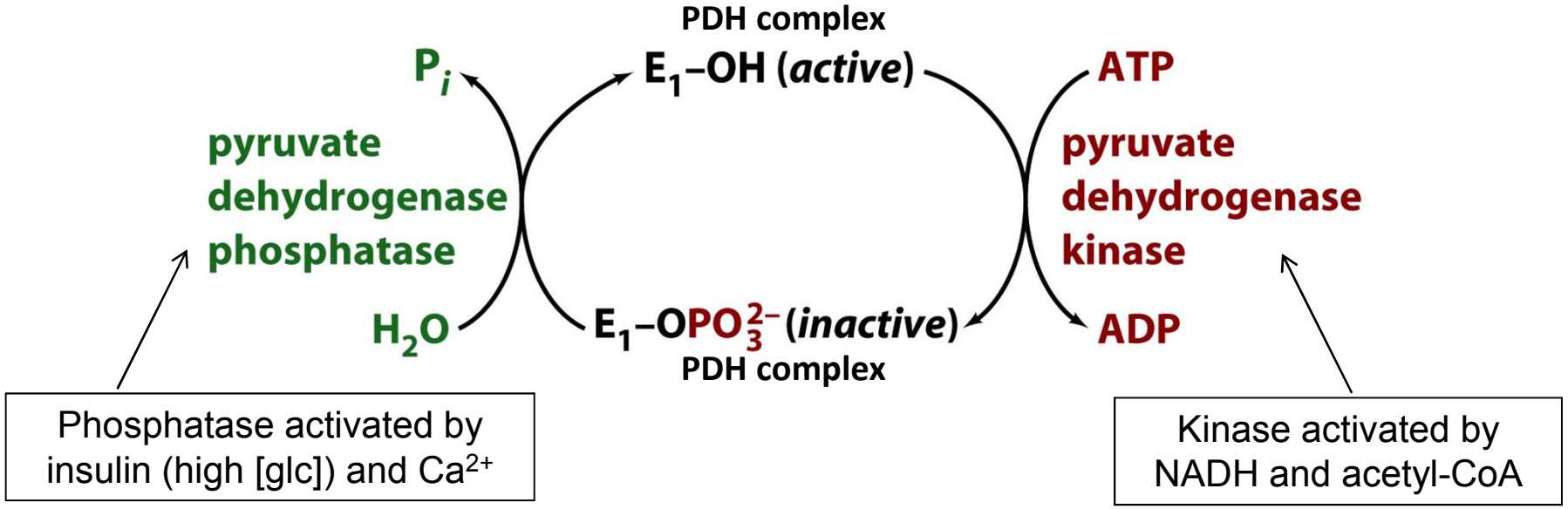
Regulation

Example of covalent modification for enzyme regulation

Pyruvate dehydrogenase complex is regulated by phosphorylation and dephosphorylation

Regulation

Covalent modification only in Eukaryotic PDH complex



What kind of effector are Ca^{2+} and glucose?

They are allosteric positive effectors of pyruvate dhase **phosphatase,** not the PDH complex. They do not act on PDH complex directly, but on the phosphatase that turns on PDH complex. Always ask yourself, "which enzyme is the effector affecting?" If you sort out which enzyme, you will understand the activity and overall effect.

Does dephosphorylation activate all enzymes?

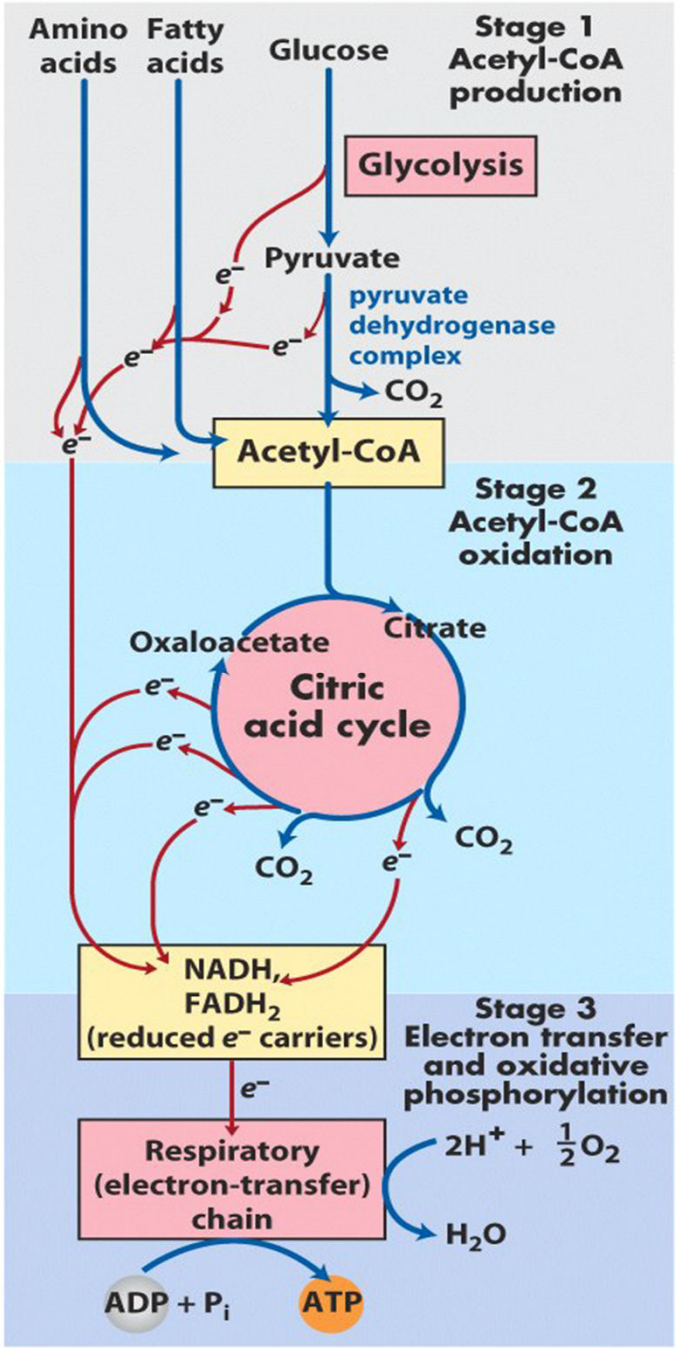
No! Activation/deactivation by covalent modification varies in each regulatory enzyme. Read the context carefully! E.g., dephosphorylation turned off glycogen phosphorylase

Metabolism Overview

Living cells and organisms must preform work to stay alive, to grow, and to produce

Thus, the necessity to harness energy and channel it into biological work is a fundamental property of ALL living organisms

These organisms have evolved countless interconnected pathways that combine biosynthetic (anabolic) and degradative (catabolic) pathways with complex multilayered regulatory mechanisms all in the effort to obtain a dynamic steady state of life



Metabolism Overview

What to study for metabolic pathways

Thermodynamics

- Which direction is favored (ΔG°' vs. ΔG)? Why?
  - Coupling by pushing and pulling of reactions (**Q** or [products]/[reactants]) or coupling by hydrolysis of high energy compound?
  - Is it reversible or irreversible?

Energy currencies (e.g. ATP, NADH, $FADH_2$)

- What currency is used? What currency is made? How is that currency made?

Regulation

- Is the step a good point of regulation?
  - Why? First committed step, slow step, initial step?
  - How? Allosteric effectors, covalent modification, non-allosteric product inhibition?

Mechanism

- Know the basic, general mechanism and not arrow-pushing
  - If mechanism includes covalent attack, what is the nucleophile and what is the electrophile?
- Know unique aspects of mechanisms
  - E.g. Are there metal ions, cofactors, unique intermediates, important residues (e.g. TCA's succinyl-CoA synthetase and glycolysis's phosphogycerate mutase (PGM) phospho-His ?

Enzyme

- What is the class (and, if possible, subclass)?
- Know unique aspects of enzymes

Carbon tracing

- If you label carbons from glucose, where does it end up after each step in glycolysis? Each step in TCA cycle? Each round in the TCA cycle?
  - Requires knowledge of reactants' and products' structure

Metabolism Overview

Thermodynamics in metabolism

	ΔG	Direction
$Q < K_{eq}$	Negative	Forward; to the right; spontaneous
$Q = K_{eq}$	0	Can go either way, equilibrium
$Q > K_{eq}$	Positive	Backwards; to the left; not spontaneous

$$Q = \frac{[\text{products}]}{[\text{reactants}]}$$

$$K_{eq} = \frac{[\text{products}]_{eq}}{[\text{reactants}]_{eq}}$$

Equilibrium is reached when the **rate of conversion** is equal.

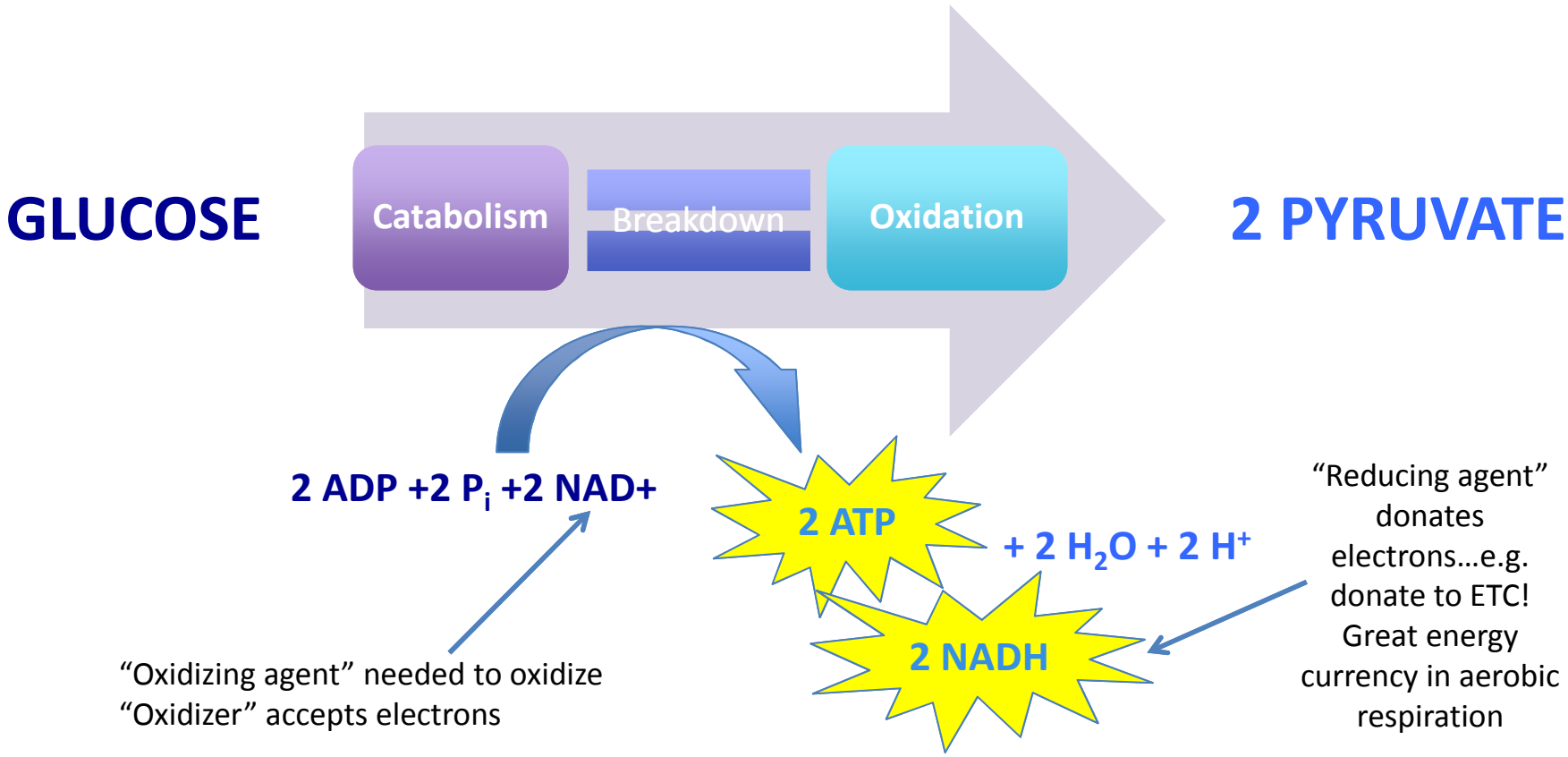
Irreversible (Far from equilibrium) = $|\Delta G| > 10$ kJ/mol

Reversible (Near equilibrium) = $|\Delta G| < 10$ kJ/mol

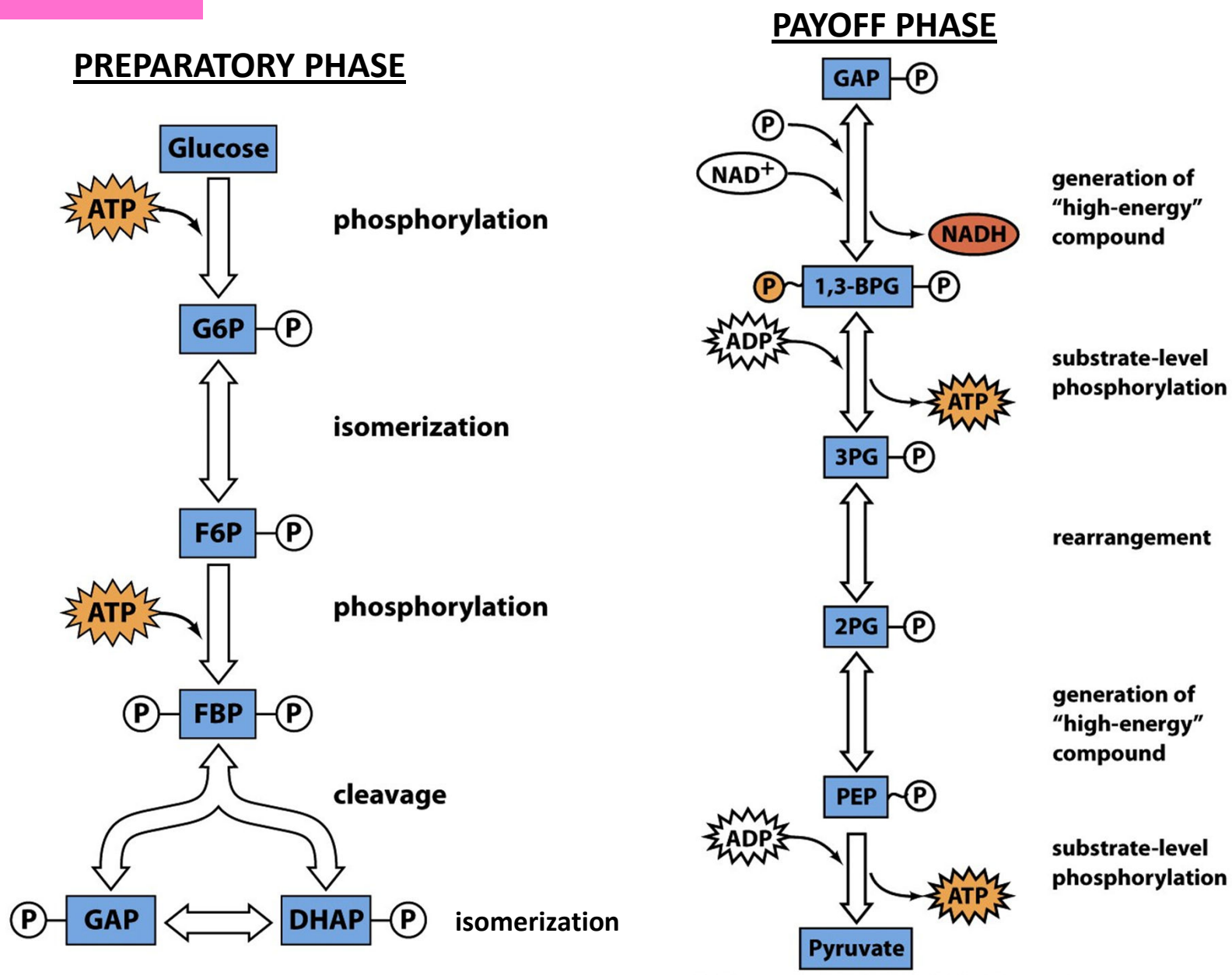
Coupling refers to the additive property of ΔG or ΔG'° for successive reactions and allows high ΔG'° reactions to occur biologically:

- If ΔG'° is small positive value:
  - Coupling by adjusting concentration of products and reactants (Q) to push and pull reactions to change Q
    - Can make reaction favorable by quick utilization of product (pulling)
    - Can make reaction favorable by having a lot of reactant feed into reaction (pushing)
- If ΔG'° is large positive value:
  - Coupling by hydrolysis of high-energy compound, like ATP or thioester

Glycolysis

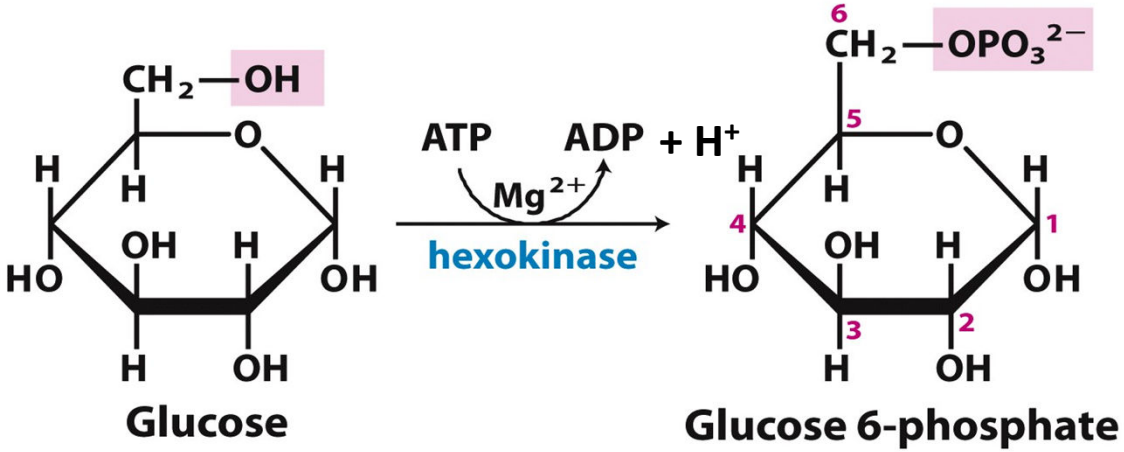

GLUCOSE
Catabolism
Breakdown
Oxidation
2 PYRUVATE
2 ADP +2 P_i +2 NAD+
2 ATP
+ 2 H_2O + 2 H^+
2 NADH
"Oxidizing agent" needed to oxidize
"Oxidizer" accepts electrons
"Reducing agent" donates electrons…e.g. donate to ETC! Great energy currency in aerobic respiration

Glycolysis


PREPARATORY PHASE
Glucose
ATP
phosphorylation
G6P
P
isomerization
F6P
P
ATP
phosphorylation
P
FBP
P
cleavage
P
GAP
DHAP
P
isomerization
PAYOFF PHASE
GAP
P
P
NAD+
NADH
generation of "high-energy" compound
P
1,3-BPG
P
ADP
ATP
substrate-level phosphorylation
3PG
P
rearrangement
2PG
P
generation of "high-energy" compound
PEP
P
ADP
ATP
substrate-level phosphorylation
Pyruvate

Glycolysis

1. Hexokinase



$\Delta G'^{\circ} = -16.7$ kJ/mol

Why expend 1 ATP to phosphorylate glucose?

To keep [glucose] levels low, so glucose moves down its gradient into cell. Also phosphorylated glucose cannot leave the cell

What is the Mg^{2+} ion for?

Mg^{2+} forms an electrostatically stable complex with negative ATP

What good is a "Pac-Man" enzyme?

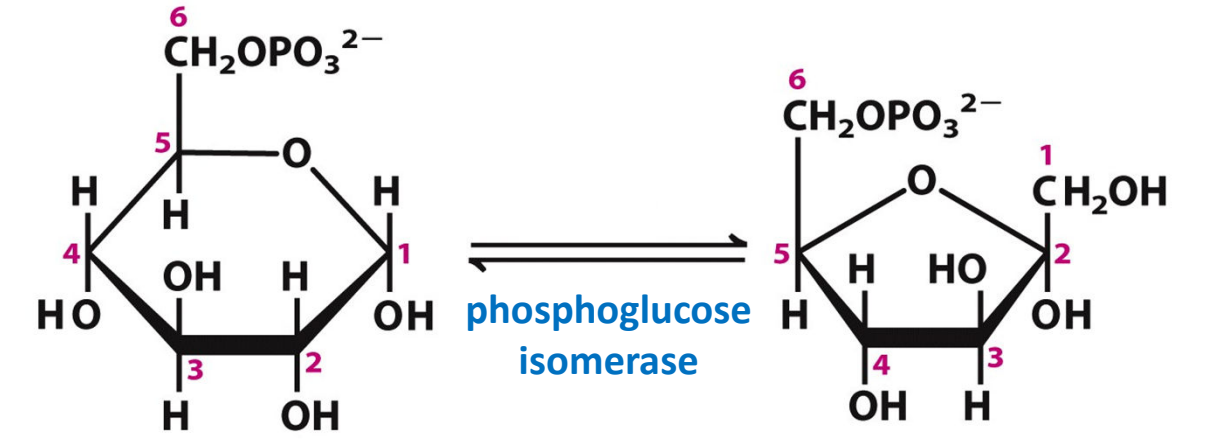
Induced fit when both substrates bind, also excludes water.

What would the products look like if a hydrolase were performing the reaction?

Water would be doing the nucleophilic attack, so water would accept the phosphoryl group.

Glycolysis

2. Phosphofructose Isomerase (PGI)



Glucose 6-phosphate **Fructose 6-phosphate**

$\Delta G'^{\circ} = 1.7$ kJ/mol

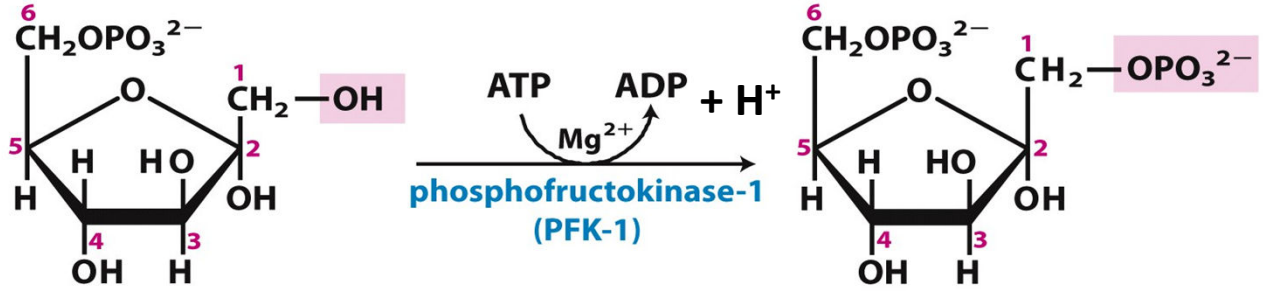
What's the purpose of this isomerization?

Going from aldose (Glc) to ketose (Frc) prepares for steps 3 and step 4

- Alcohol at C1 is better for PFK phosphorylation in step 3
- C2 carbonyl and C4 alcohol is better for aldol cleavage at C3-C4 in step 4

Glycolysis

3. Phosphofructose Kinase (PFK-1)



Fructose 6-phosphate

Fructose 1,6-bisphosphate

$\Delta G'^{\circ} = -14.2$ kJ/mol

Why is this step considered the rate-determining step of glycolysis?

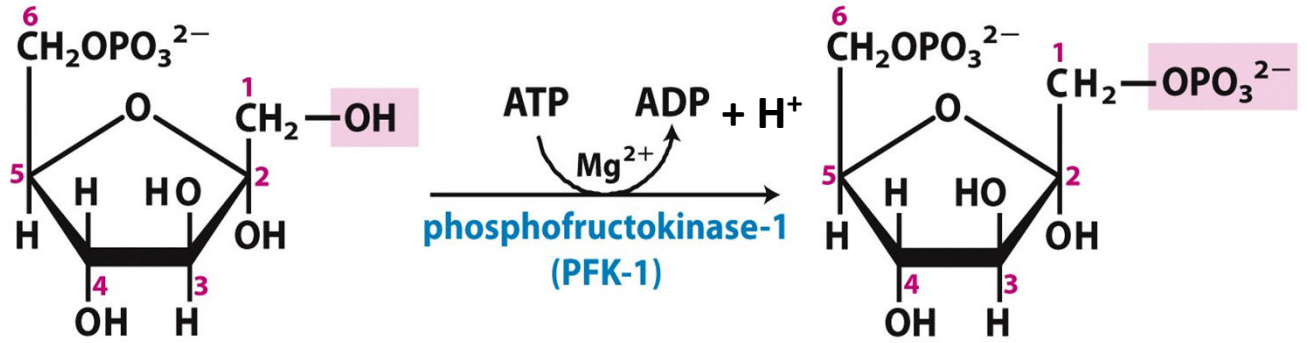
F1,6BP is a dedicated glycolysis intermediate, so its production commits initial glucose to finish pathway

What is the nucleophile in this mechanism? The electrophile? Why?

The nucleophile is the C1 alcohol that attacks the electrophilic gamma phosphorus of ATP. We know the *sugar* is the nucleophile because the reaction is to transfer a phosphoryl group onto the *sugar*.

Glycolysis

3. Phosphofructose Kinase (PFK)



Fructose 6-phosphate

Fructose 1,6-bisphosphate

$\Delta G'^{\circ} = -14.2$ kJ/mol

Which enzyme(s) of glycolysis is/are regulated?

PFK-1! See slides on Regulation: Allosteric at the beginning of the review. In brief:

Positive effectors

Signal ATP supply is low, thereby increasing PFK-1 activity

- (+) ADP
- (+) AMP
- (+) F6P
- (+)F2,6BP

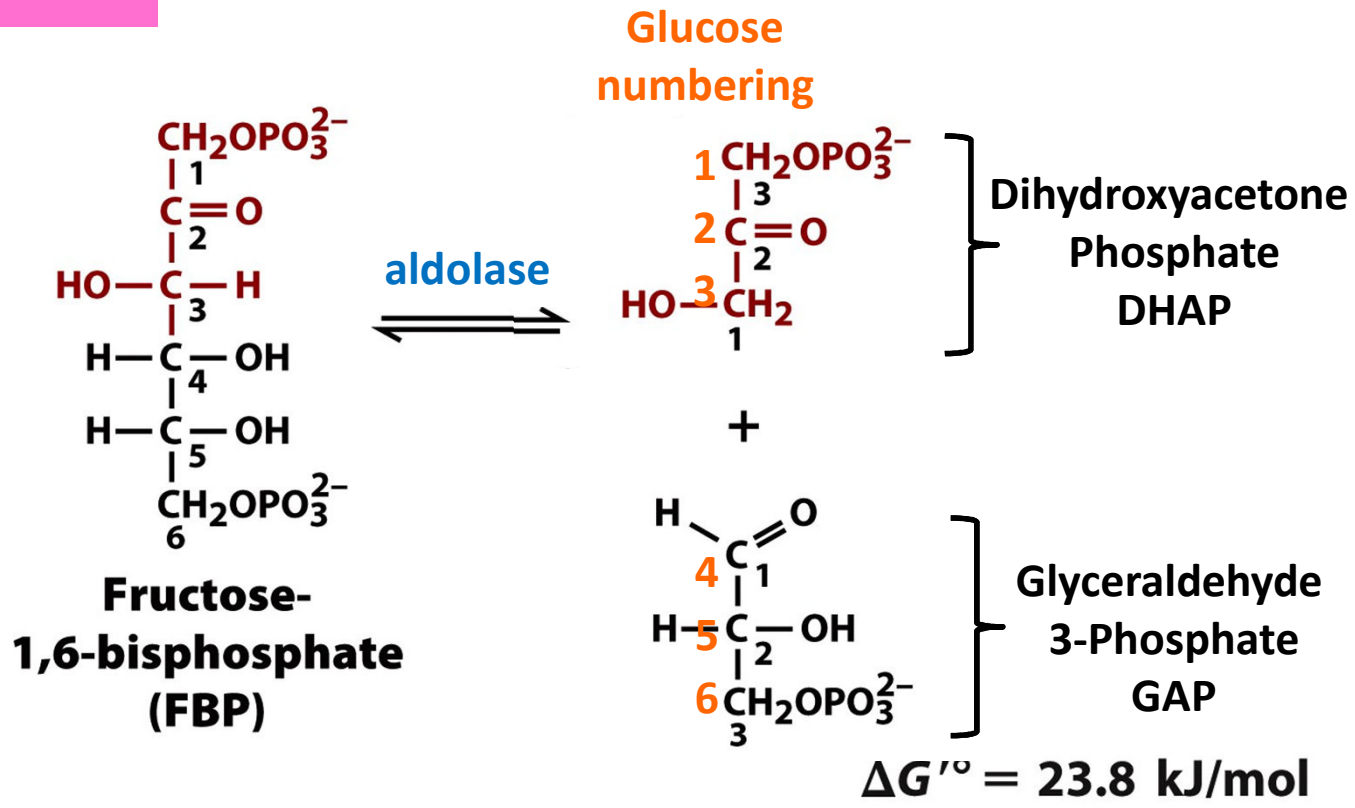
Negative effectors

Signal ATP supply is high, thereby decreasing PFK-1 activity

- (-) ATP
- (-) Citrate

Glycolysis

4. Aldolase

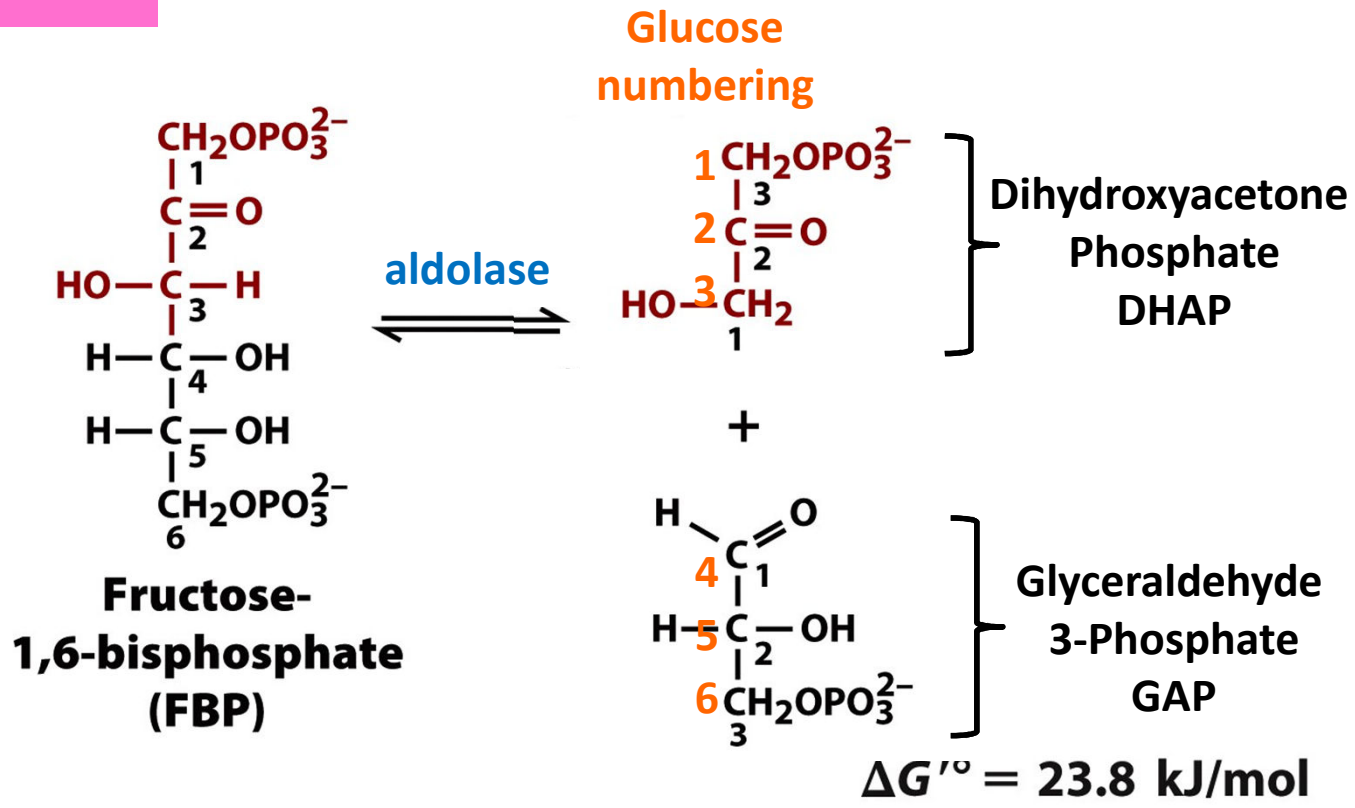


The ΔG°' is very large and positive, but ΔG is negative. How?

Reaction proceeds because cellular [F-1,6-BP] is cleaved into two products that are utilized very quickly (low Q). Therefore, the dG is slightly negative under physiological conditions as long as the concentrations of products [DHAP] and [G3P] is kept low by utilization (coupling)

Glycolysis

4. Aldolase



What is the purpose of a Schiff base intermediate?

Nucleophile Lys on enzyme attacks C2 carbonyl and forms Schiff base, which stabilizes the carbanion (negative carbon, C^-) formed when the first triose GAP (**C4-C5-C6**) is released.

What other mechanisms stabilize carbanions?

TPP, the prosthetic group cofactor of decarboxylation enzymes like pyruvate decarboxylase, pyruvate dehydrogenase complex (E1) and a-KG dehydrogenase complex (E1). Both TPP and Schiff base intermediate act as electron withdrawing sinks, stabilizing the carbanion

Glycolysis

4. Aldolase

Which triose is released first?

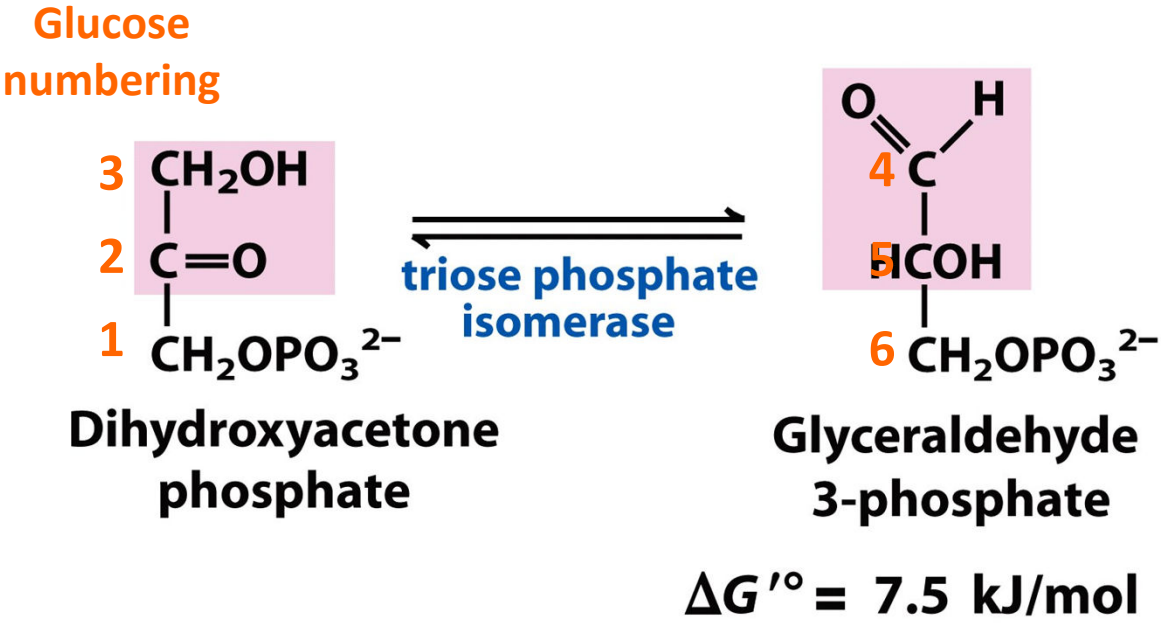
GAP! Nucleophile Lys on enzyme attacks C2 carbonyl of ketose, forming Schiff base intermediate. General base then general acid catalysis releases first triose, GAP, which is the bottom 3 carbons of glucose (**C4-C5-C6**)

Correction on Glycolysis handout highlighted in green!

4	Aldolase *lyase*	**Fructose-1,6-bisphosphate** ⟷ **Dihydroxyacetone phosphate + Glyceraldehyde-3-Phosphate** *Nucleophilic NH_2 of Lys attacks the electrophilic carbonyl of F-1,6-BP and forms a Schiff base intermediate. Schiff base acts as electron sink (e-withdrawing) and stabilizes negative charge of enolate to become an enamine. General base catalysis releases first triose, GAP. General acid catalysis liberates second triose, DHAP.*	+23.8	-5.9	Reversible	**Enzyme**: Cleavage catalysis occurs because the enamine intermediate formed is more stable than the enolate intermediate in the base-catalyzed aldol cleavage **Thermodynamics:** Reaction proceeds because cellular [F-1,6-BP] is cleaved into two products that are utilized very quickly (low Q, or low [products]/[reactants])Therefore, the dG is slightly negative under physiological conditions as long as the concentrations of products [DHAP] and [G3P] is kept low by utilization (coupling) **Carbon tracing:** DHAP = original glucose's [C1-C2-C3] GAP = original glucose's [C4-C5-C6]

Glycolysis

5. Triose Phosphate Isomerase (TIM)



What is k_{cat} of this enzyme?

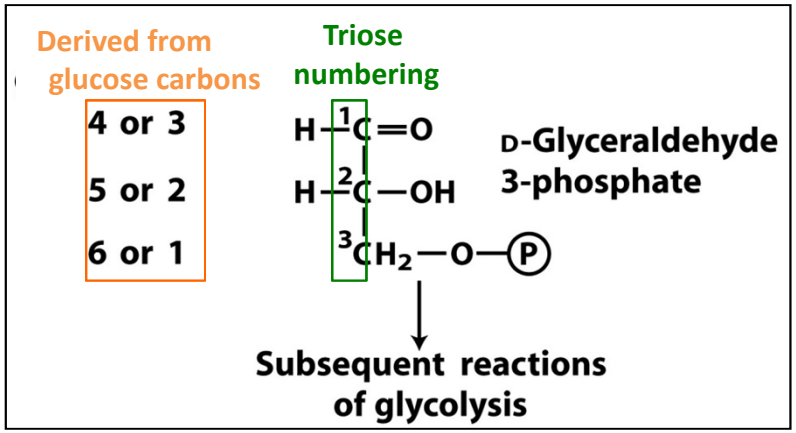
Very high because it's "catalytically perfect"—every binding results in product

Where is the isomeration occurring?

Move Glucose C2 carbonyl to C3. Produce 2 GAPs, where **C1** and **C6** are now triose carbon **number 3**

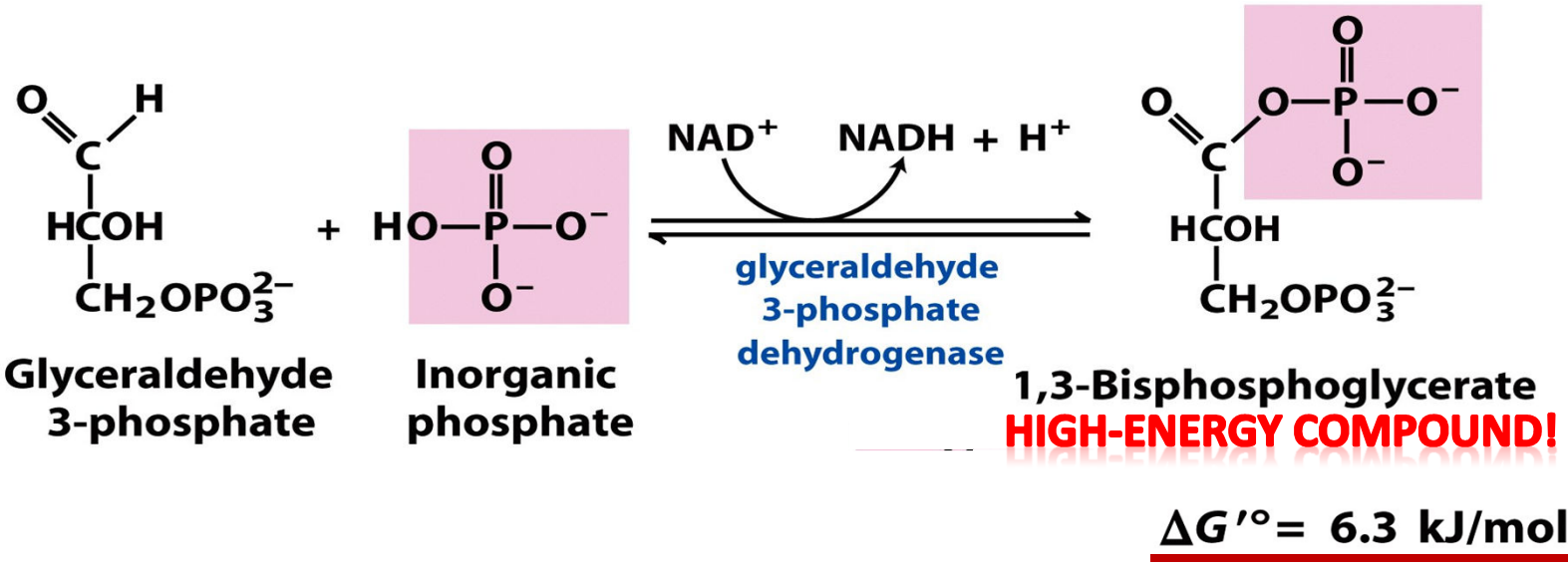
Why do we want two of the same compounds?

More efficient pathway



Glycolysis

6. Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)

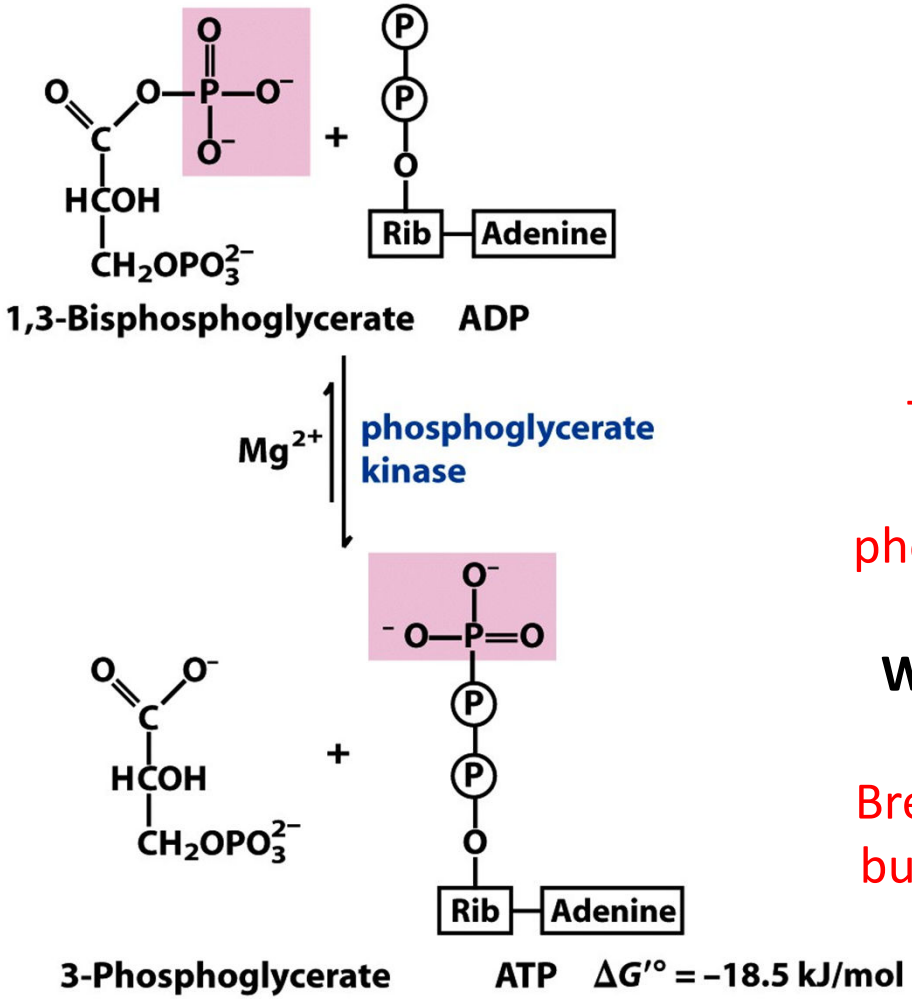


How is the high-energy 1,3BPG formed?

Energy from aldehyde oxidation is conserved in synthesis of thioester. Because thioester hydrolysis releases much energy, O^- of Pi can attack thioester and phosphorylate the triose making high-energy 1,3BPG. The normally unfavorable phosphorylation is *coupled* to hydrolysis of thioester (analogous to Hexokinase, PFK-1, except oxidation power and not ATP is used to drive reaction)

Glycolysis

7. Phosphoglycerate Kinase (PK)



Since high-energy 1,3BPG was made in step 6, the immediate next step is to cash in!

1 ATP made per GAP

Why is this called "substrate-level phosphorylation"?

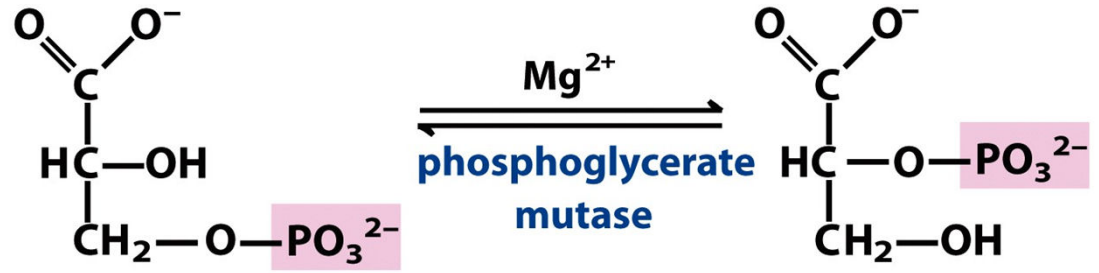
The *substrate* transfers its phosphoryl group to *ADP.* Compare to "oxidative phosphorylation" where H^+ gradient drives Pi to directly combine with ADP.

Why is the ΔG°' so negative, but the ΔG near equilibrium?

Breaking of high-energy cmpd is favorable, but is coupled to pull step 6 forward. Also, Q is large ($[ATP]_{high}/[1,3\text{-}BPG]_{low}$)

Glycolysis

8. Phosphoglycerate Mutase (PGM)



3-Phosphoglycerate **2-Phosphoglycerate**

$\Delta G'^{\circ} = 4.4$ kJ/mol

Why is the isomerization necessary?

It is easier to make the high-energy compound PEP in step 9 with 2-PG than with 3-PG

We've seen isomerases that move carbonyls, so how do you move phosphates?

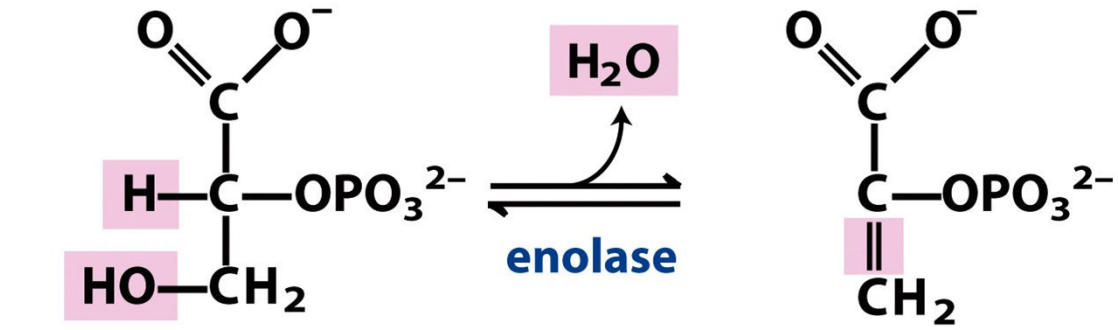
Substrate takes on extra phosphate from the enzyme at its carbon 2 to become 2,3-BPG. Then substrate gives phosphoryl group on carbon 3 back to enzyme. Swapping phosphate groups!

Where does the enzyme get the phosphate in the first place?

From 2,3-BPG—the negative effector of hemoglobin ☺

Glycolysis

9. Enolase

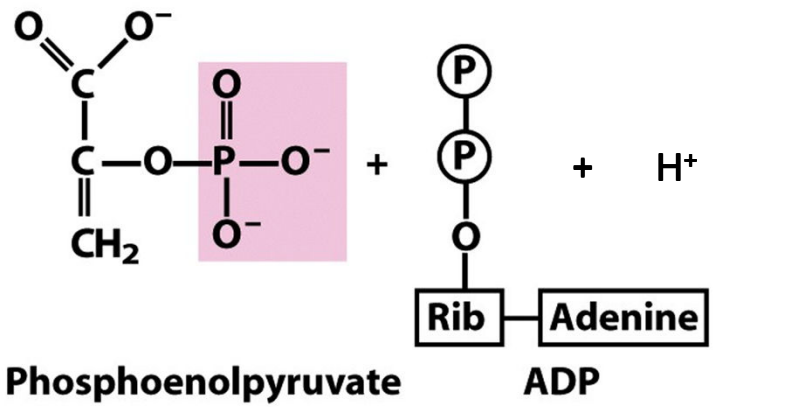


How is high-energy PEP formed?

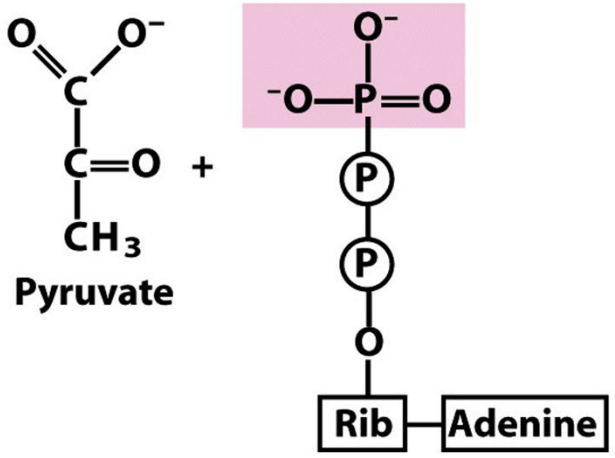
Enolase is a lyase, and removal of water increases the standard free energy of hydrolysis of the phosphate

Glycolysis

10. Pyruvate Kinase (PK)



Mg^{2+}, K^+ | pyruvate kinase



$\Delta G'^{\circ} = -31.4$ kJ/mol

Since high-energy PEP was made in step 9, the immediate next step is to cash in!

What is the nucleophile and the electrophile in this substrate-level phosphorylation step?

Just like in step 7, the nucleophile is the O^- on the ADP that attacks the phosphoryl group on the triose, PEP.

What is Mg^{2+} and K^+ doing?

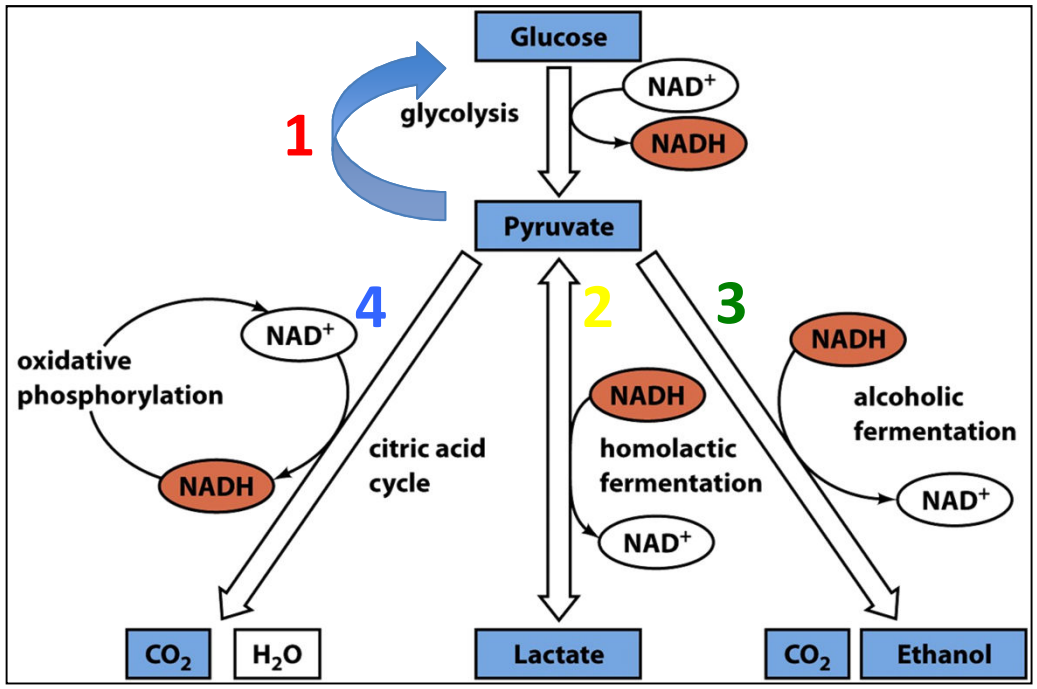
Mg^{2+} stabilizes the O^- on PEP and ADP

K^+ stabilizes carbonyl of PEP

Note: Hydrolysis of PEP is not sufficient to drive transfer of PEP's phosphate onto ADP. However, the tatutomerization of enolpyruvate (one C=O, one C=C bond) to ketopyruvate (two C=O) can power substrate-level phosphorylation.

Immediate fates of pyruvate

1. **Gluconeogenesis and Anapleuorotic reaction**: Pyruvate carboxylase
   - first step in remaking glucose via gluconeogenesis
   - Anapleurotic reaction to replenish OAA for TCA
2. **Homolactic Fermentation** (e.g. in mammals): Lactate dehydrogenase
   - Reduce and Regenerate NAD+
3. **Alcohol Fermentation** (e.g. in yeast): Pyruvate decarboxylate
   - Decarboxylate and then reduce into ethanol, regenerating NAD+

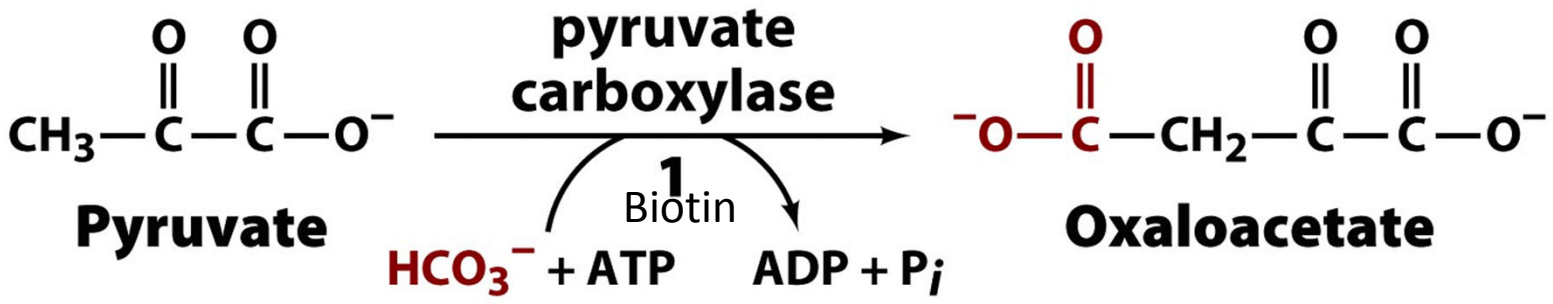


4. **Preparation for TCA Cycle**: Pyruvate dehydrogenase complex

   To make into acetyl-CoA to feed into TCA cycle

Pyruvate in
Anapleurotic reactions

Pyruvate can be carboxylated to replenish OAA pools



What are anapleurotic reactions?

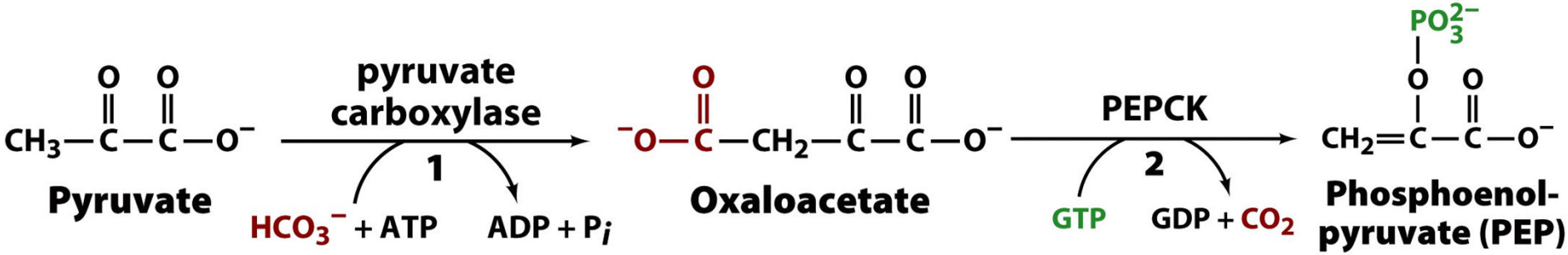
Reactions that replenish intermediates of TCA cycle. We know all acetyl-CoA put into TCA cycle is oxidized off as CO_2—there is not net gain of carbons. So need pyruvate carboxylase to replenish supply of OAA via pyruvate, or transaminases to replenish supply of alpha-KG via amino acid degradation.

We saw a lot of taking off CO_2 using the cofactor TPP. Here, we add on CO_2. How?

Pyruvate carboxylase uses the prosthetic cofactor biotin. ATP activates CO_2 and biotin's N attacks carboxyl and holds CO_2 for deprotonated pyruvate to attack and take CO_2 .

Pyruvate in Gluconeogenesis

Pyruvate can start the gluconeogenesis to make glucose



Why are we carboxylating Pyr and then decarboxylating OAA?

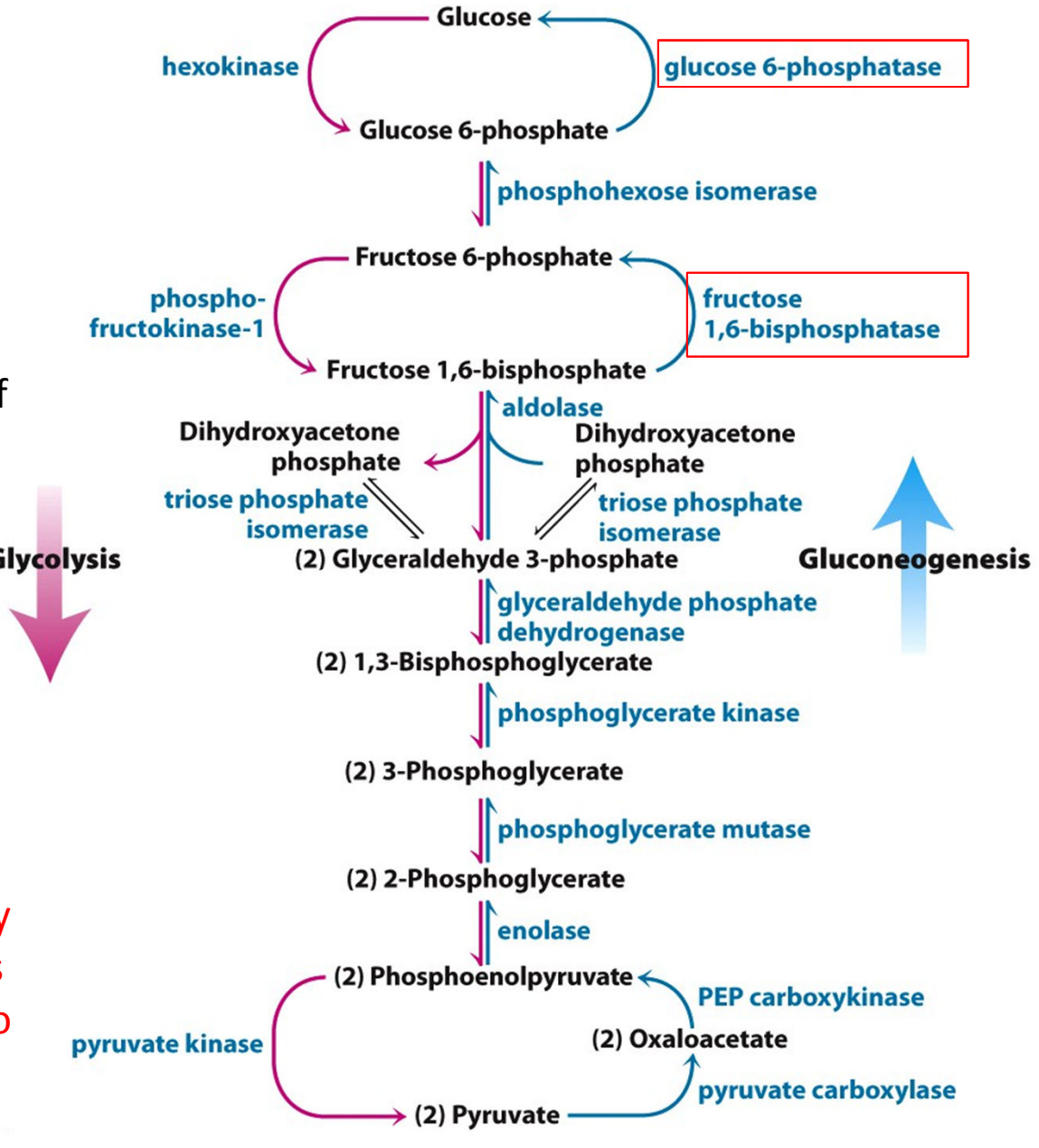
The end goal is to make PEP, a high-energy compound. We carboxylate to prime Pyr for PEP carboxylkinase (PEPCK) and also to make more OAA (see anapleurotic rxn). The decarboxylation catalyzed by PEP carboxylkinase does release energy, but we still need two energy currencies to make PEP (1 ATP, 1 GTP)—these first two reactions are that expensive!

Gluconeogenesis

Side note: Gluconeogenesis is the reverse of glycolysis.
The 7 reversible enzymes are the same, but 4 gluconeogeneic enzymes are needed to catalyze the opposite of the 3 irrevesible glycolytic enzymes.

To get back to glucose, cells liberate 2 phosphate groups. Why use hydrolyases (glucose-6-phosphatase and fructose 1,6-bisphosphotase) instead of kinases? Can't we make ATP via substrate-level phosphorylation?

Substrate-level phosphorylation is only possible with high-energy compounds like 1,3-BPG and PEP. F1,6BP or G6P do not have high hydrolysis potential, therefore we cannot make ATP.



Pyruvate in Homolactic Fermentation

Pyruvate can be reduced to regenerate NAD^+

Why do we need NAD+?

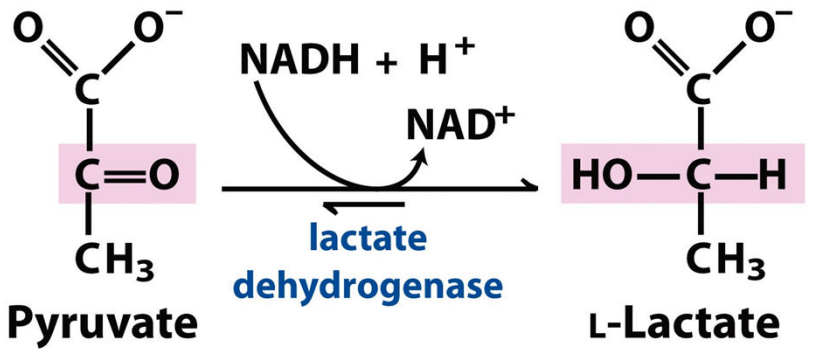
To use as oxidizer for glycolysis

Isn't it better to completely oxidize pyruvate through TCA cycle? That is, isn't aerobic respiration is better than anaerobic respiration?

While we do get more energy from aerobic respiration than anaerobic (30-32 ATP vs. 2 ATP!), aerobic respiration is much slower. Additionally, O_2 needs to be present to be the final e^- acceptor. If our tissues are depleted in O_2 (because of rigorous activity), pyruvate from glycolysis is made into lactate to replenish NAD+ for more glycolysis.

What happens to all that lacate?

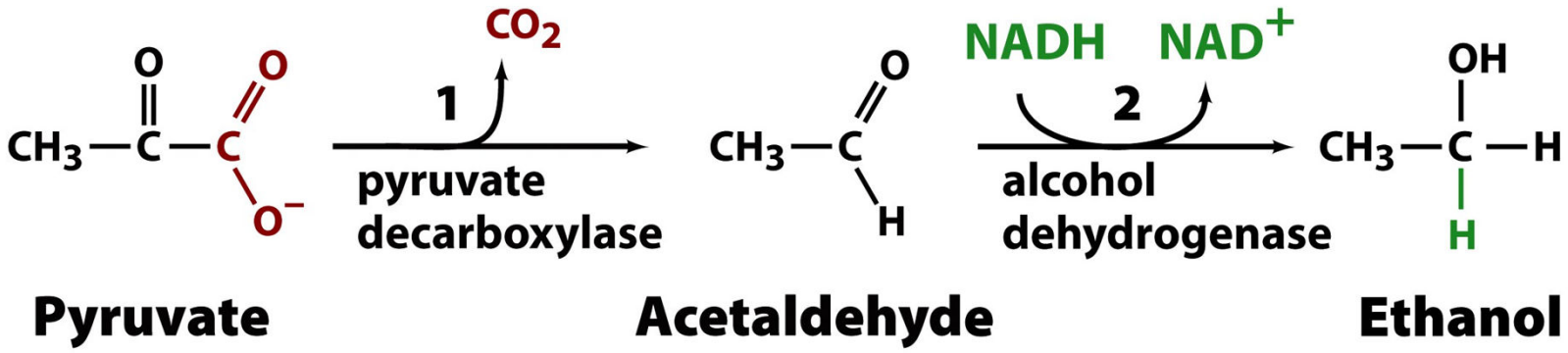
Does it really cause muscle soreness? Hmm, check out: http://en.wikipedia.org/wiki/Lactic_acid#Exercise_and_lactate. But certainly lactate can be reversed into pyruvate in liver to make glucose via gluconeogenesis.



$\Delta G'^\circ = -25.1$ kJ/mol

Pyruvate in
Alcohol Fermentation

Pyruvate can be decarboxylated and then reduced to form Ethanol and NAD^+



What other enzymes decarboxylate?

PEPCK, PDH complex, isocitrate Dhase, and a-KG Dhase.

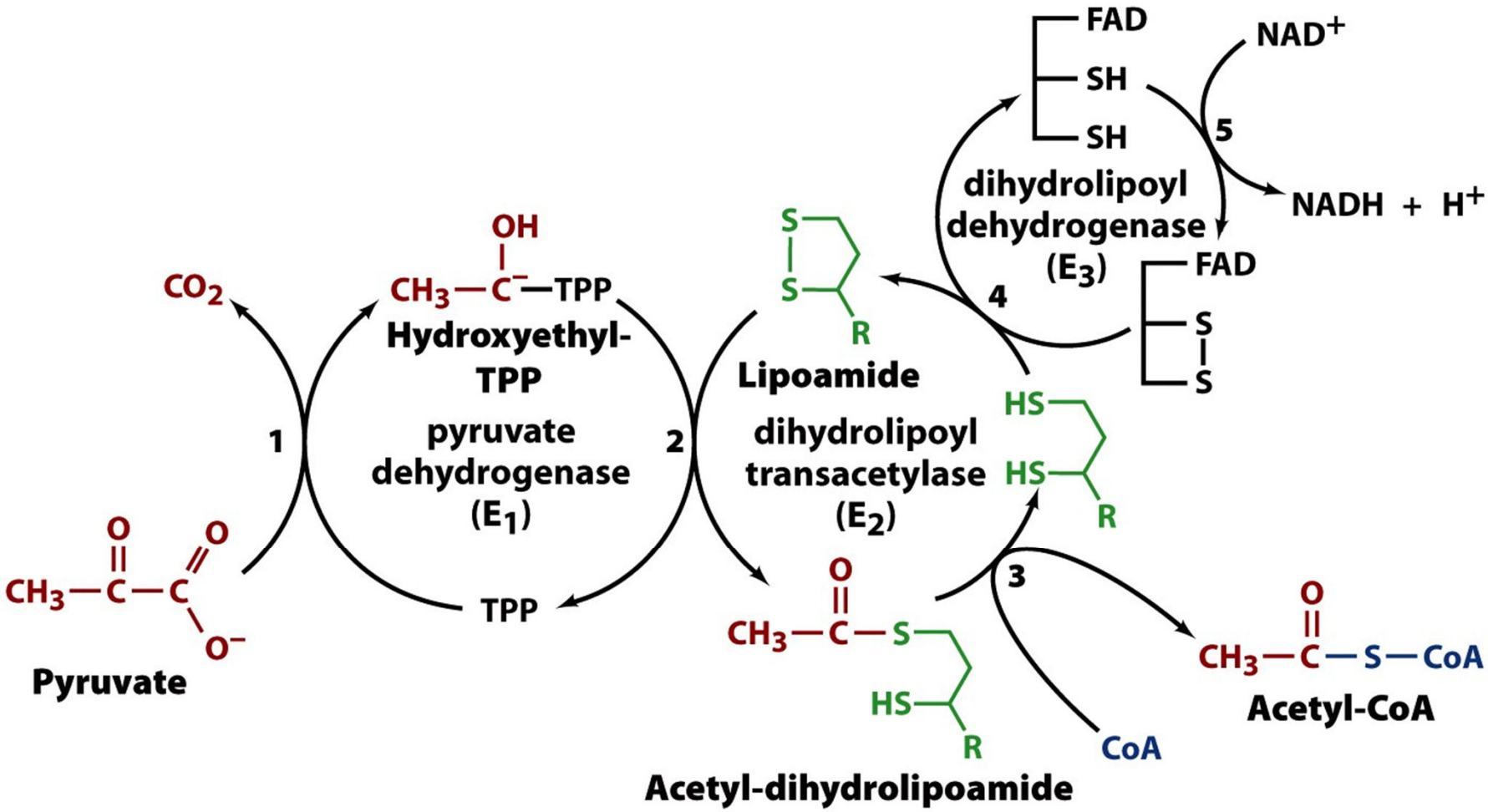
Are their mechanisms exactly the same?

No, but pyruvate decarboxylase, PDH complex and a-KG Dhase all use TPP (but pyruvate decarboxylase catalyzes only decarboxylation, nothing fancy with CoA).

Again, TPP is the prosthetic cofactor that covalent attacks to kick off CO_2 and then because of its great resonance, acts as electron withdrawing sink to stabilize the carbanion formed when a-keto acids like a-KG or pyruvate are decarboxylated.

Pyruvate in
Pyruvate Dehydrogenase Complex

Pyruvate can converted into Acetyl-CoA by the PDH complex, the bridge from glycolysis to TCA cycle



Pyruvate Dehydrogenase Complex

Pyruvate can decarboxylated and given a CoA group for preparation to be furthered oxidized as CO_2

Enzyme	Name	Cofactors (*cosubstrates)	Reactions	Product
E1	Pyruvate DH	TPP	1. Decarboxylate pyruvate with TPP 2. Transfer substrate to lipoamide, which oxidizes substrate into acetyl group and reduces lipoamide into dihydrolipamide	CO_2
E2	Dihydrolipoyl transacetylase	Lipoic acid, Coenzyme A*	3. $CoAS^-$ attacks acetyl substrate, product is released	Acetyl-CoA
E3	Dihydrolipoyl DH	FAD, NAD^+*	4. Reoxidize dihydrolipoamide into lipoamide with FAD 5. Reoxidize $FADH_2$ into FAD by NAD^+	NADH

Is Acetyl-CoA considered a high-energy compound?

Yes, it has a thioester that has a high hydrolysis potential

Is PDH complex apart of glycolysis or TCA cycle?

Neither, it is the bridge between the two and is located in the mito matrix

How is it regulated?

By phos/dephos of E_1 (see review slides Regulation: covalent modification) and by product inhibition: Acetyl-CoA binds and inhibits E_2 and NADH binds and inhibits E_3 at active sites (not allosteric)

TCA Cycle

Overall Reaction:

Acetyl-CoA + 3 NAD^+ + FAD +GDP + P_i + 2 H_2O → 2 CO_2 + 3 NADH + $FADH_2$ + GTP + 3 H^+ + CoASH

Function:

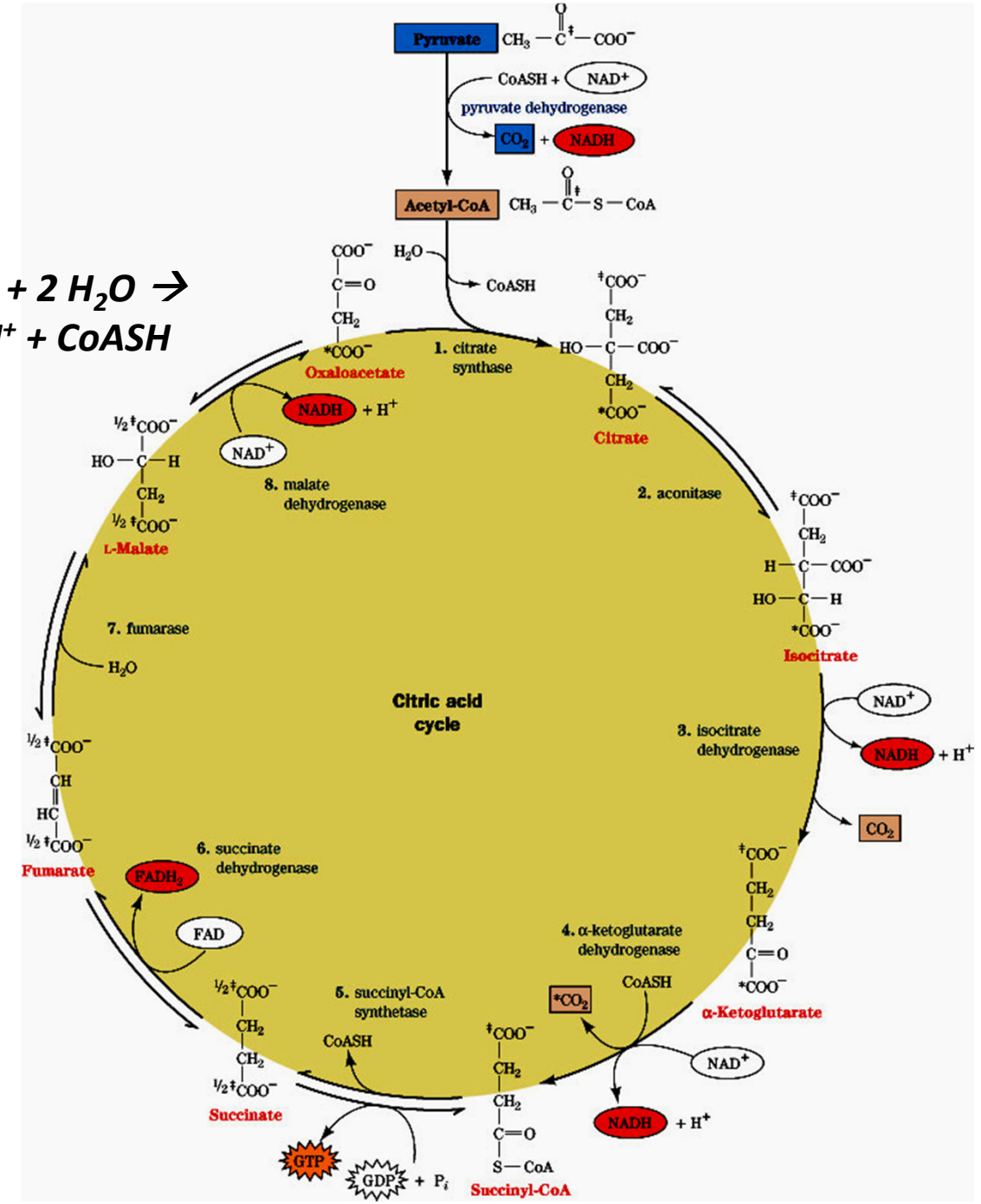
Oxidizes Acetyl-CoA into CO_2 releasing energy, which is harnessed in the reduction of NAD^+ and FAD to become NADH and $FADH_2$, respectively

Location:

Mitochondrial matrix (eukaryotes) or cytoplasm (bacteria)

Rate Limiting Step:

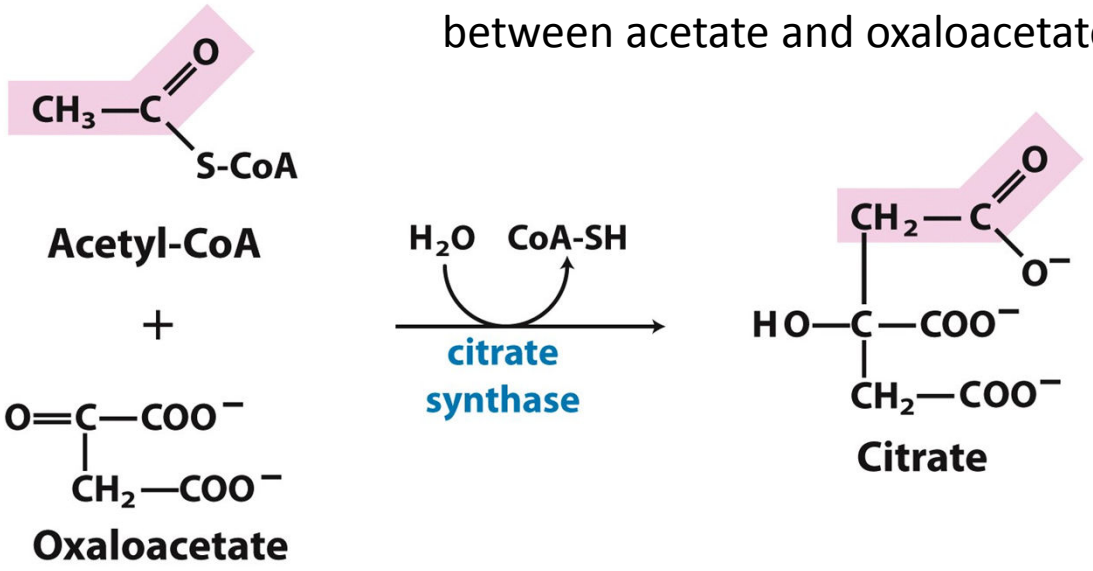
Acetyl-CoA + OAA → CoA-SH + Citrate (Catalyzed by Citrate Synthase)



TCA Cycle

1. Citrate Synthase

Catalyzes the C-C bond formation (condensation reaction) between acetate and oxaloacetate to form **citrate**



Positive effector: ADP
Negative effectors: ATP, NADH, Citrate, Succinyl CoA

$\Delta G'^{\circ} = -32.2$ kJ/mol **ΔG = -8.0 kJ/mol**

Why is this enzyme regulated?

Hydrolysis of high energy thioester intermediate, citroyl CoA, make the forward reaction highly exergonic (Large negative $\Delta G'^{o}$)

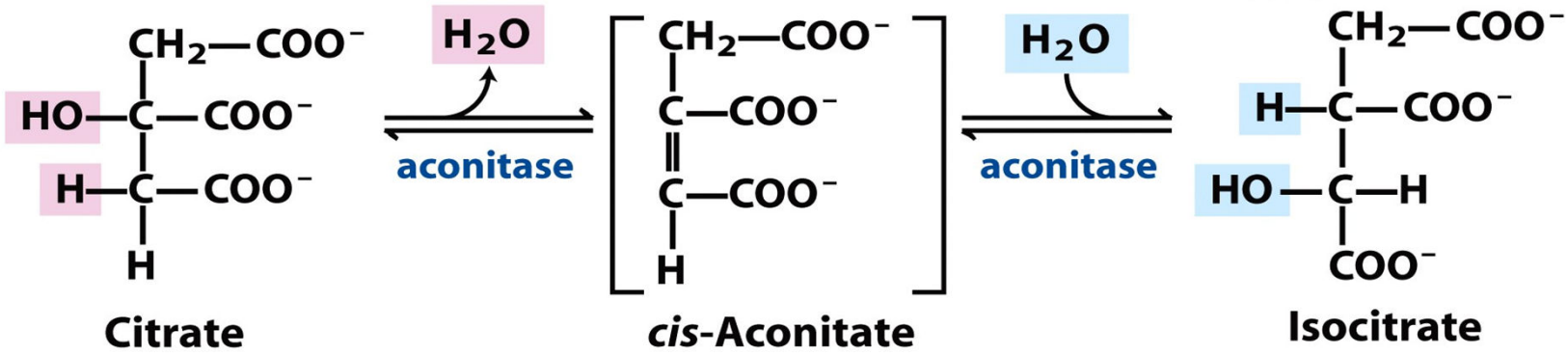
The large neg. $\Delta G'^{o}$ is needed to keep the TCA cycle going, Why?

Remember that this pathway is **CYCLICAL**. The previous reaction (#8) from *malate* to *oxaloacetate* is so endergonic that [OAA] are low. The high exergonic nature of this rxn allows citrate to be formed even at low [OAA]

TCA Cycle

2. Aconitase

The stereospecific conversion of citrate to **isocitrate**



$\Delta G'^{\circ}$ = 13.3 kJ/mol

ΔG = +1.5 kJ/mol

At pH 7 and 25°C, the equilibrium mixture is < 10% isocitrate. Why is this reaction pulled in the forward direction?

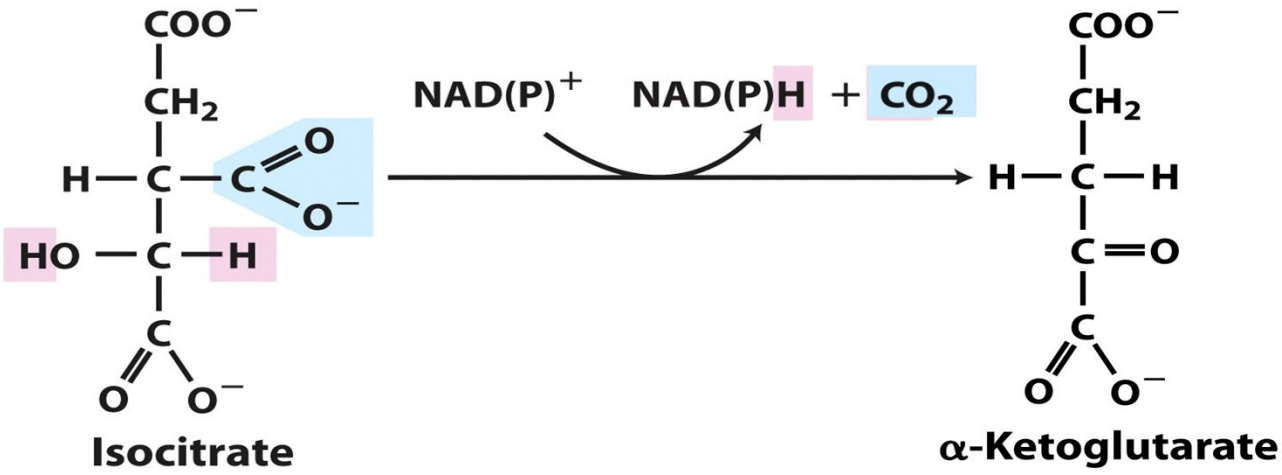
Isocitrate is rapidly used in the next step (#3 isocitrate DH). Thus its steady state concentration is lowered.

REMEMBER: KNOW THE DIFFERENCE BETWEEN STEADY STATE AND EQUILIBRIUM!

TCA Cycle

3. Isocitrate Dehydrogenase

Catalyzes the oxidative decarboxylation of isocitrate to release CO_2 and form **α-Ketogluterate**



Positive effectors:
ADP, NAD^+, Ca^{2+}
Negative effectors:
ATP, NADH

$\Delta G'° = -21$ kJ/mol

$\Delta G = -1.7$ kJ/mol

What is the purpose of Mn^{2+} is the active site?

The Mn^{2+} interacts with the newly formed carbonyl (C=0) intermediate (oxalosuccinate) to facilitate decarboxylation by e^- withdrawing. It also stabilizes the enol formed transiently before finally α-KG is formed

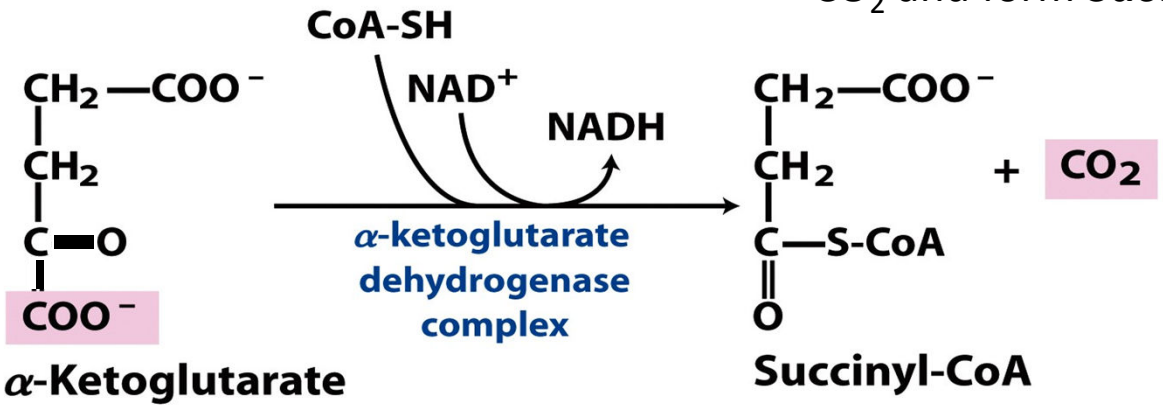
Where is the released CO_2 is from?

Oxaloacetate NOT Acetyl-CoA

TCA Cycle

4. α-Ketogluterate Dehydrogenase

Catalyzes the oxidation of α-Ketogluterate to release CO_2 and form **Succinyl-CoA**



Positive effector:
Ca^{2+}
Negative effectors:
ATP, NADH, Succinyl-CoA

$\Delta G'° = -33.5$ kJ/mol

$\Delta G = -8.0$ kJ/mol

What enzyme that we've seen before has a virtually identical mechanism to *α-KG DH?*

Pyruvate Dehydrogenase
(uses TPP, lipoamide, CoA-SH, FAD, NAD^+)

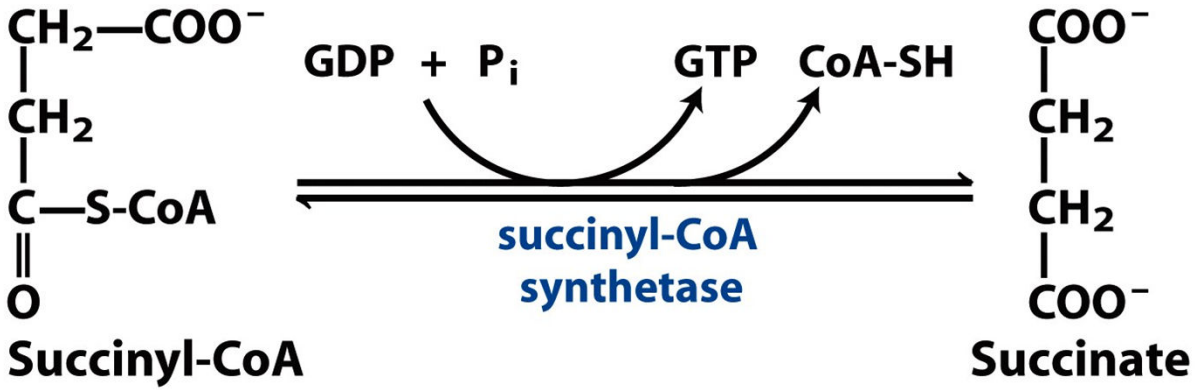
What substance serves as the last e^- acceptor? NAD^+

Is the CO_2 released from the 1st cycle of Acetyl-CoA? No

TCA Cycle

5. Succinyl-CoA Synthetase

Catalyzes the conversion of succinyl-CoA to **succinate** and the **substrate-level phosphorylation of GTP**



$\Delta G'^{\circ} = -2.9$ kJ/mol **$\Delta G = -0.8$ kJ/mol**

Why is the conversion of Succinyl-CoA to succinate coupled to the formation of GTP?

Succinyl-CoA is a thioester, which is a high energy intermediate with its hydrolysis having a $\Delta G'^{o} \approx -36$ kJ/mol. GTP is an ATP equivalent with its synthesis having a $\Delta G'^{o} \approx +33$ kJ/mol. By coupling these steps the overall $\Delta G'^{o} = -2.9$ kJ/mol making the forward direction favored

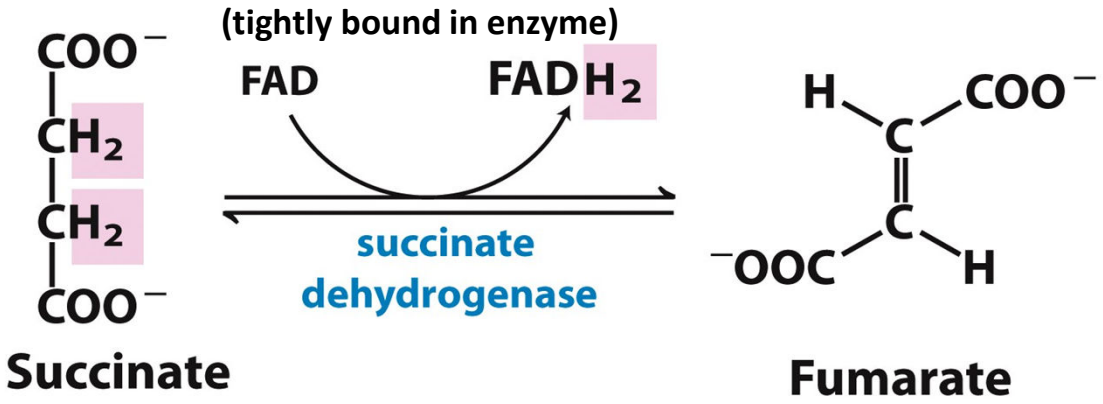
What is the order in which these processes are coupled?

Succinyl-CoA + P_i → CoA-SH + Succinyl-phosphate + His_{enzyme} →
Succinate + phospho-His_{enzyme} + GDP → His_{enzyme} + **GTP**

TCA Cycle

6. Succinate Dehydrogenase

Catalyzes the oxidation of succinate to **fumerate**



$\Delta G'° = 0$ kJ/mol $\Delta G \approx 0$

Where is this enzyme located in the cell and what other process is it a part of?

Succinate DH is tightly-bound to the inner mitochondrial membrane (eukaryotes) or plasm membrane (bacteria). It is also **Complex II** in the electron transport chain and is involved in oxidative phosphorylation

What type of inhibitor is malonate, which only differs from succinate by a -CH_2?

Competitive

How would the addition of malonate affect the V_{max} and K_m of succinate DH?

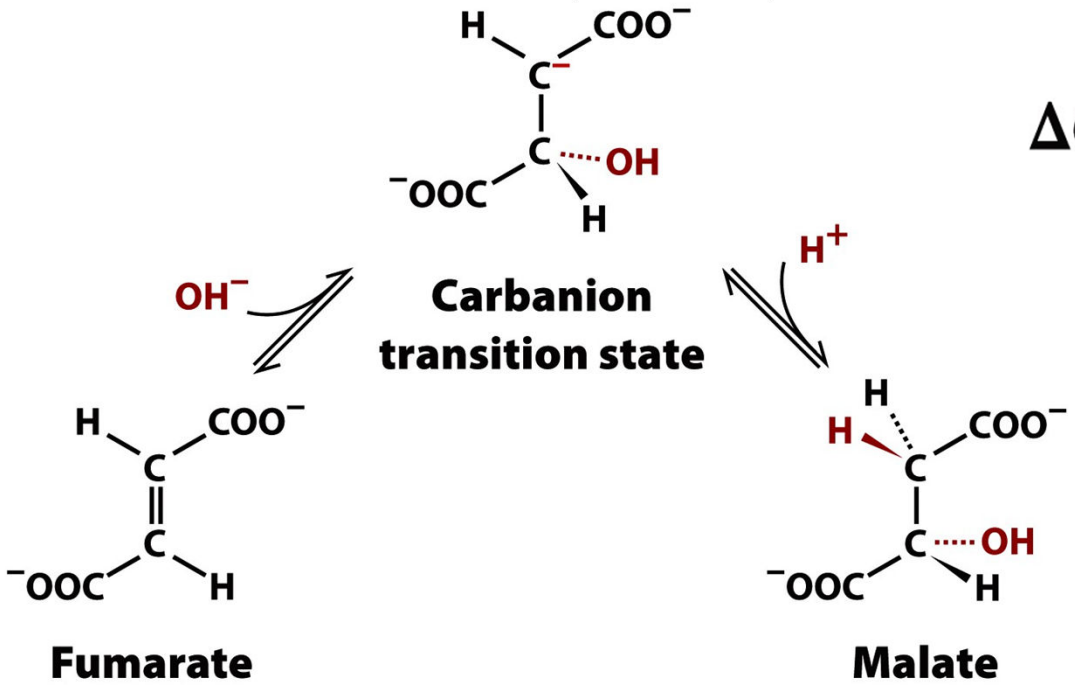
The V_{max} would be unchanged ($V_{max} = V_{max}^{app}$), while the K_m increases with [I]

$K_m < K_m^{app}$

TCA Cycle

7. Fumerase

Catalyzes the hydration of fumerate to **L-Malate**



$\Delta G'^{\circ} = -3.8$ kJ/mol

$\Delta G = -0.9$ kJ/mol

What does the transition state look like?

The transition state has a carbanion

True or False:

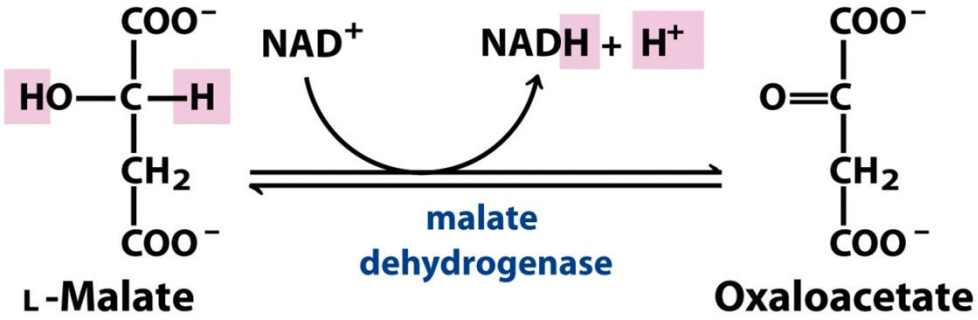
Fumerase cannot differentiate between *cis* or *trans* isomers

FALSE. Fumerase is highly stereospecific. It catalyzes the hydration of the *trans* isomer (fumerate) and NOT the *cis* isomer (maleate). In the reverse direction, D-malate is NOT a substrate while L-malate is.

TCA Cycle

8. L-Malate Dehydrogenase

Catalyzes the oxidation of L-Malate to **oxaloacetate**



$\Delta G'^{\circ} = 29.7$ kJ/mol

$\Delta G = +7.0$ kJ/mol

At standard conditions, the equilibrium of this reaction lies far to the left. Then why, in a cell, does this reaction proceed forward?

In intact cells, oxaloacetate is being continually removed by the highly exergonic reaction of citrate synthase (step #1). This keeps the [OAA] low and thus the equilibrium is shifted toward the formation of oxaloacetate

What does this teach you about $\Delta G'^{o}$ vs. ΔG?

The standard free energy change, **$\Delta G'^{o}$**, is a **characteristic** of a reaction that shows which direction a reaction proceeds and how far a reaction must go to reach equilibrium (remember @ equilibrium $\Delta G'^{o} = 0$), therefore it's a **constant**. The actual free energy change, **ΔG**, is **function** of the reactant and product concentrations (and temp), which may or may not by the same as standard conditions, therefore it is **variable**.

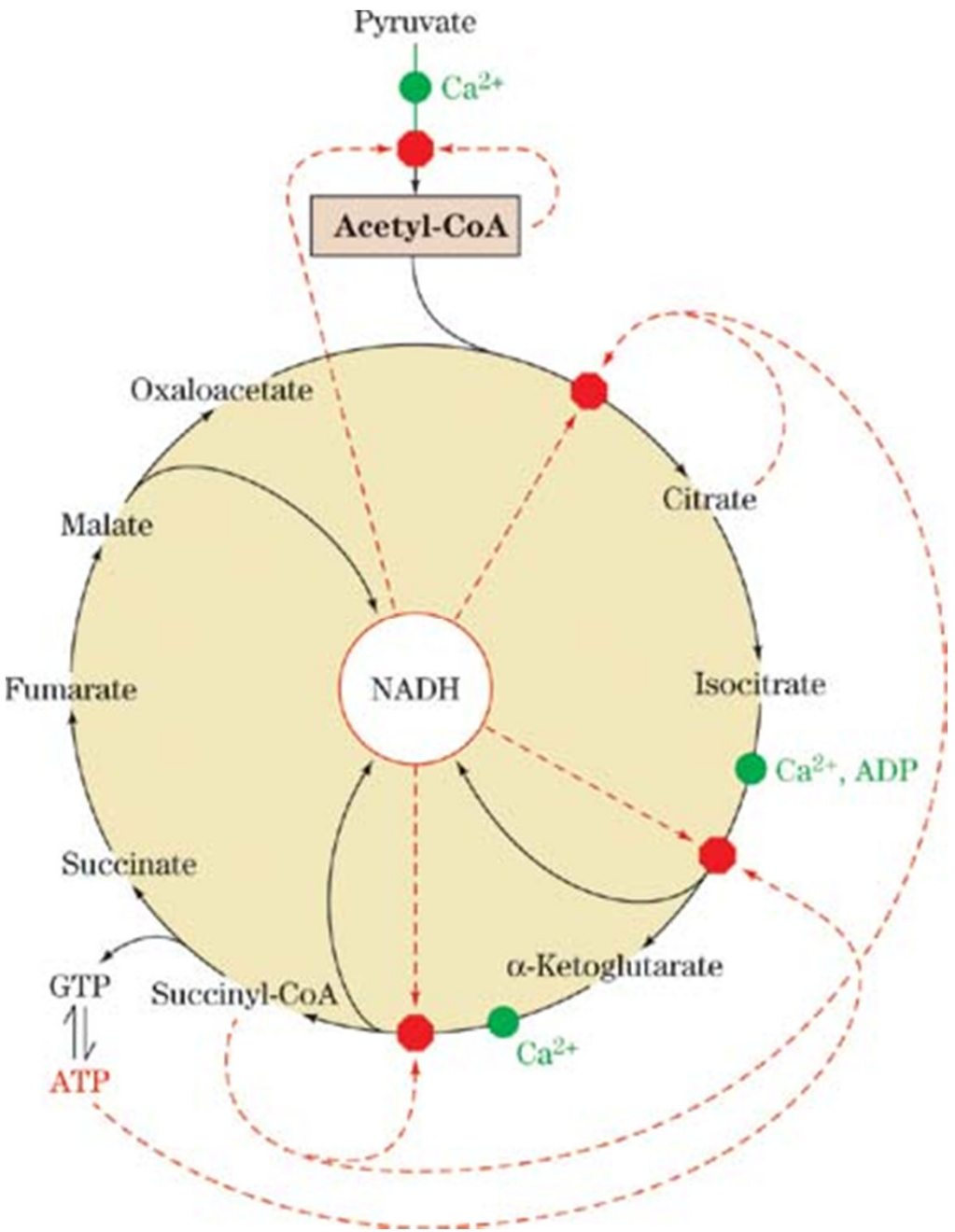
TCA Cycle Regulation

Under normal conditions, the rates of glycolysis (PDH) and TCA cycle are integrated so that only as much glucose is metabolized as needed to supply the TCA cycle with its fuel.

Basically this is an efficiency game and you can think of ATP and NADH as global regulators of metabolism. They reflect metabolic flux as well as the energy status of the cell.

Other factors in TCA cycle Regulation:

- Product Inhibition
- Substrate Availability
- Allosteric feedback inhibition of enzyme that catalyze early steps in cycle



TCA Cycle Regulation

With that said….

Other than acting like a "global regulator," how does NADH regulate the TCA cycle?

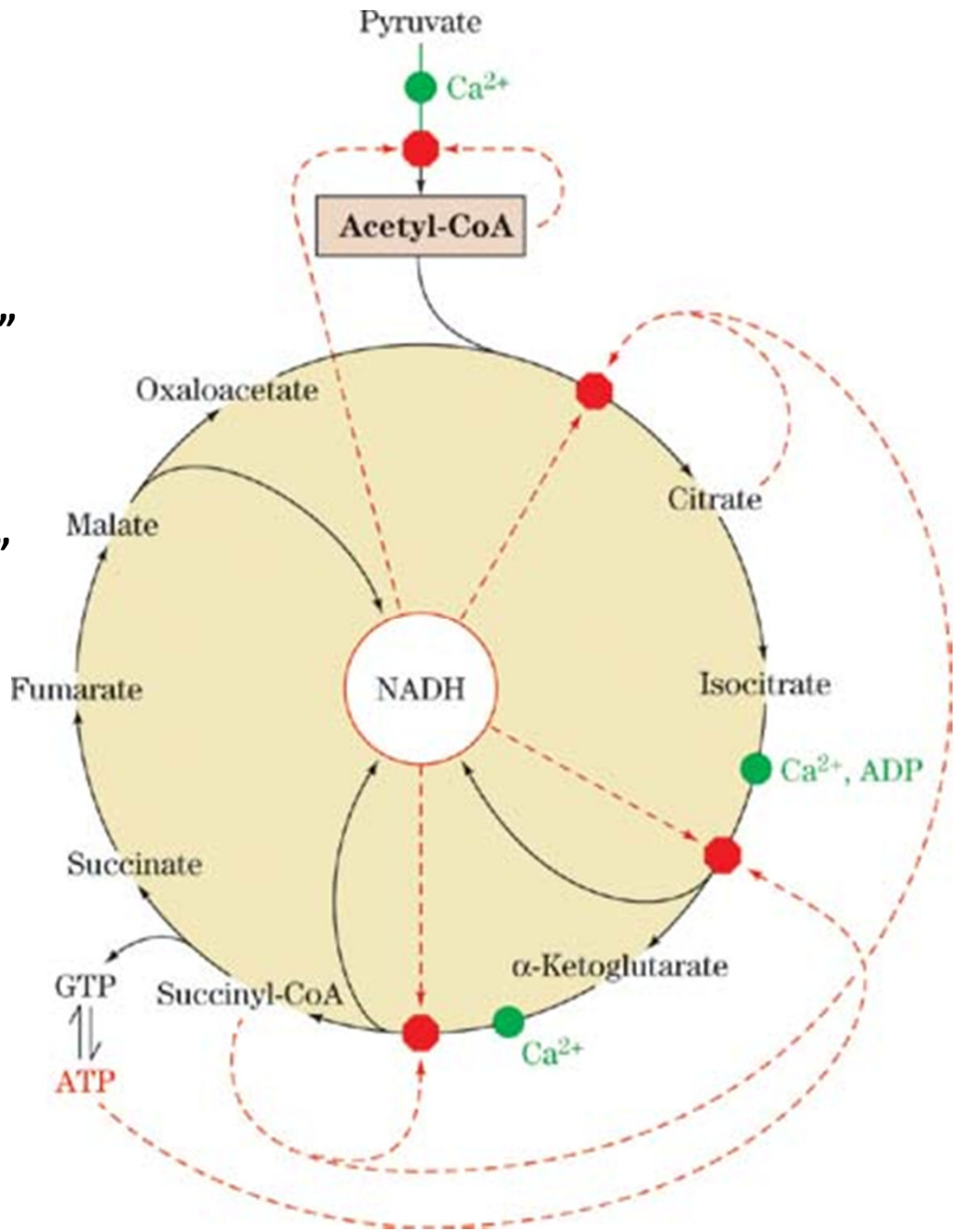
For isocitrate and α-KG dehydrogenases, it is a product inhibitor.

Other than acting like a "global regulator," how does ATP regulate the TCA cycle?

For citrate synthase and isocitrate DH it is a feedback inhibitor because it is the final product from GTP catalyzed by *nucleoside diphosphate kinase*. For citrate synthase, ATP inhibition is relieved by ADP, an allosteric activator

Citrate is a product inhibitor for citrate synthase. What other type of metabolic inhibitor is citrate? Where in metabolism does this occur?

Citrate is a feedback inhibitor of phosphofructokinase (PFK-1) in glycolysis



RECAP

So far glycolysis and the TCA cycle have achieved…

- Carbons of glucose has been completely oxidized to CO_2
- Substrate-level phosphorylation has conserved some of the energy released from oxidation
- However, most of the energy is conserved (temporarily) in the reducing power of NADH and $FADH_2$

RECAP

So what's next?

- NADH and $FADH_2$ accept e^- from catabolic intermediates and transfer them, via a series of protein complexes to the final e^- acceptor, O_2
- The energy released from this series of transfers drives the translocation of H^+ across mitochondrial membrane
- These H^+ flow back across membrane via channels provided by **ATP synthase**, which is an enzyme complex that synthesizes ATP, in a process known as **oxidative phosphorylation**

Reduction Potentials

- Reduction potential, *E*, measures how well a compound becomes reduced (accepts e^-)
- E'^o is the reduction potential of a substance at standard biochemical state
- A high E'^o = a high affinity for e^-
- e^- move from a lower reduction potential to a higher reduction potential
- The change in reduction potential is calculated:

$$\Delta E = E_{(\text{e- acceptor})} - E_{(\text{e- donor})}$$

- The change in free energy, can be related to the reduction potential:

$$\Delta G^{0\prime} = -nF\Delta E^{0\prime} \quad \text{or} \quad \Delta G = -nF\Delta E$$

where: n = # of e^- transferred (in biochemistry usually but not always 2)
F = Faraday's constant (96.5 kJ V^{-1} mol^{-1})

- The actual reduction potential can be calculated by:

$$E = E^{0\prime} + \frac{RT}{nF} ln \frac{[e^- acceptor]}{[e^- donor]}$$

Reduction Potentials

Why is NADH and $FADH_2$ able to accept e^- from catabolic intermediates and donate them ultimately to O_2?

Electrons flow from lower to higher reduction potentials (can think lower affinity to higher affinity) and from the table to the right you can see that both NADH and $FADH_2$ have intermediate reduction potentials. Therefore they are able accept e^- from intermediates that have lower E'^o and donate them to O_2 that has the highest E'^o (via protein complexes)

Table 14-5 Standard Reduction Potentials of Some Biochemically Important Half-Reactions

Half-Reaction	$\mathscr{E}'^o$ (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$	0.815
$NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O$	0.42
Cytochrome a_3 (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome a_3 (Fe^{2+})	0.385
$O_2(g) + 2H^+ + 2e^- \rightleftharpoons H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome *c* (Fe^{2+})	0.235
Cytochrome c_1 (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + e^- $\rightleftharpoons$ cytochrome *b* (Fe^{2+}) (*mitochondrial*)	0.077
Ubiquinone + $2H^+ + 2e^-$ $\rightleftharpoons$ ubiquinol	0.045
Fumarate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ succinate$^-$	0.031
FAD + $2H^+ + 2e^-$ $\rightleftharpoons$ $FADH_2$ (*in flavoproteins*)	~0.
Oxaloacetate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ malate$^-$	−0.166
Pyruvate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^-$ $\rightleftharpoons$ ethanol	−0.197
FAD + $2H^+ + 2e^-$ $\rightleftharpoons$ $FADH_2$ (*free coenzyme*)	−0.219
S + $2H^+ + 2e^-$ $\rightleftharpoons$ H_2S	−0.23
Lipoic acid + $2H^+ + 2e^-$ $\rightleftharpoons$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^-$ $\rightleftharpoons$ NADH	−0.315
$NADP^+ + H^+ + 2e^-$ $\rightleftharpoons$ NADPH	−0.320
Cysteine disulfide + $2H^+ + 2e^-$ $\rightleftharpoons$ 2 cysteine	−0.340
Acetoacetate$^-$ + $2H^+ + 2e^-$ $\rightleftharpoons$ β-hydroxybutyrate$^-$	−0.346
$H^+ + e^-$ $\rightleftharpoons$ $\frac{1}{2}H_2$	−0.421
$SO_4^{2-} + 2H^+ + 2e^-$ $\rightleftharpoons$ $SO_3^{2-} + H_2O$	−0.515
Acetate$^-$ + $3H^+ + 2e^-$ $\rightleftharpoons$ acetaldehyde + H_2O	−0.581

Reduction Potentials

Why doesn't NADH and $FADH_2$ directly donate their e^- O_2?

The direct e^- transfer from $NADH/FADH_2$ to O_2 in a single redox reaction would be highly spontaneous but not efficient in order to maximize ATP production

Alternatively, the e^- are passed through a series of protein complexes, containing multiple redox centers, with increasingly higher and higher reduction potentials until finally to O_2. This is process is called the **electron transport chain (ETC)** and it releases energy in small increments to maximize ATP production

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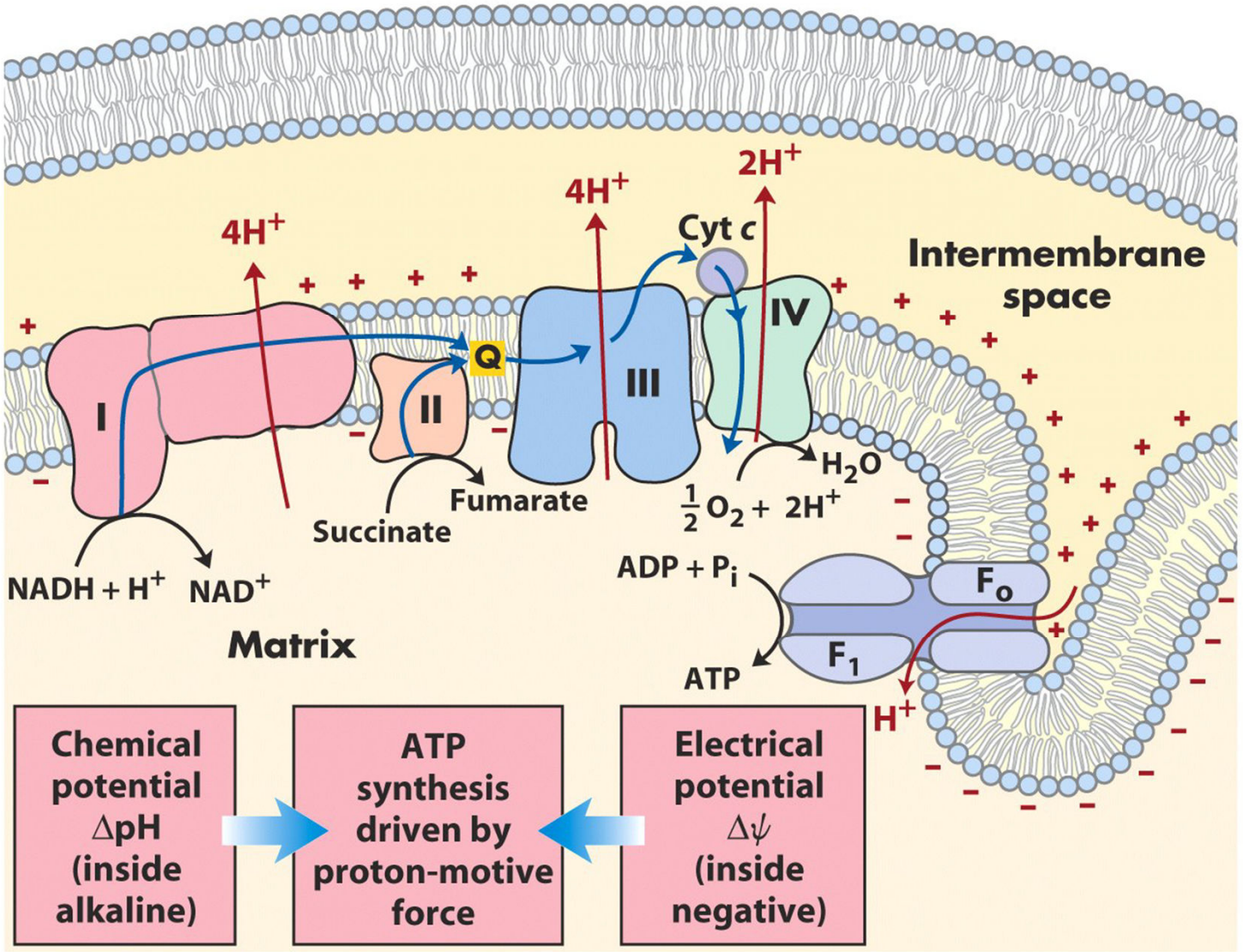
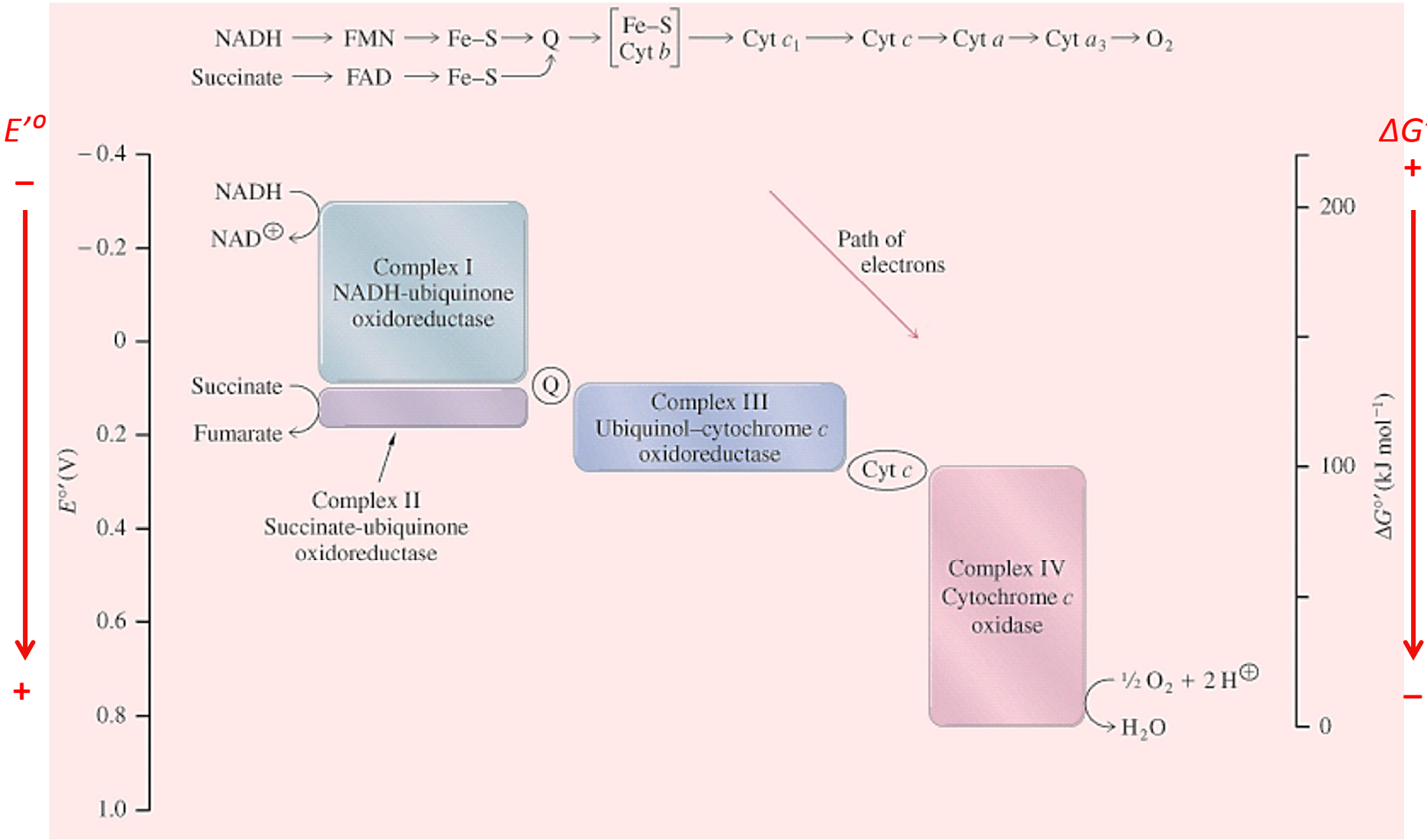
Electron Transport Chain



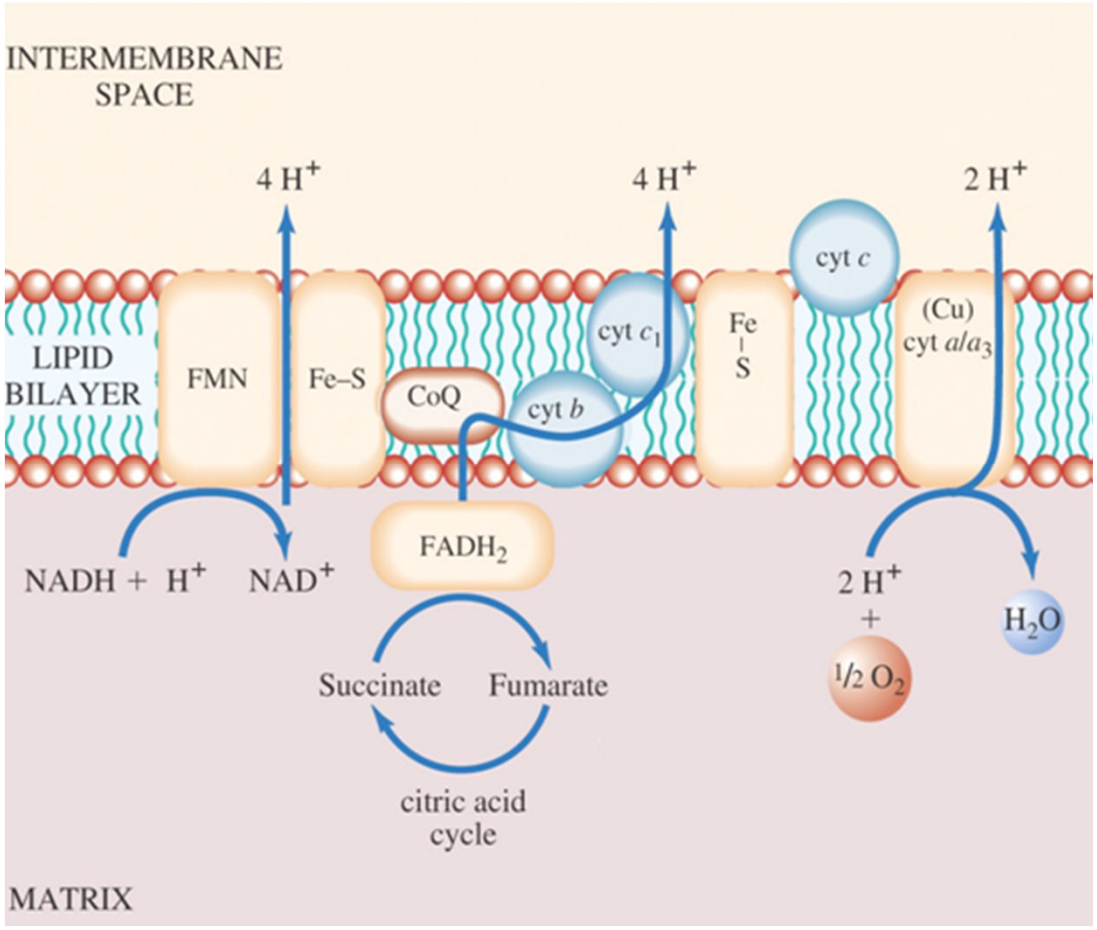
Electron Transport Chain

Electrons flow from lower reduction potential to increasingly higher reduction potentials with the change in free energy becoming more favorable by decreasing in + value



Electron Transport Chain

Overview



Complex I:

$NADH + H^+ + Q \rightarrow NAD^+ + CoQH$

- 2 e^- from NADH are transferred to CoQ
- QH_2 diffuses in membrane <u>from comp I to comp III</u>
- 4 H^+ are pumped from matrix to IM space

Complex II

$Succinate + Q \rightarrow Fumerate + QH_2$

- 2 e^- from $FADH_2$ are transferred to CoQ
- Does <u>NOT</u> pump any protons!

Complex III

$QH_2 + 2\ cyt\ c_1 \rightarrow Q + 2\ cyt\ c_1$

- Transfers e^- from CoQ to cyt c <u>one e^- at a time</u>
- 4 H^+ are pumped to IM space

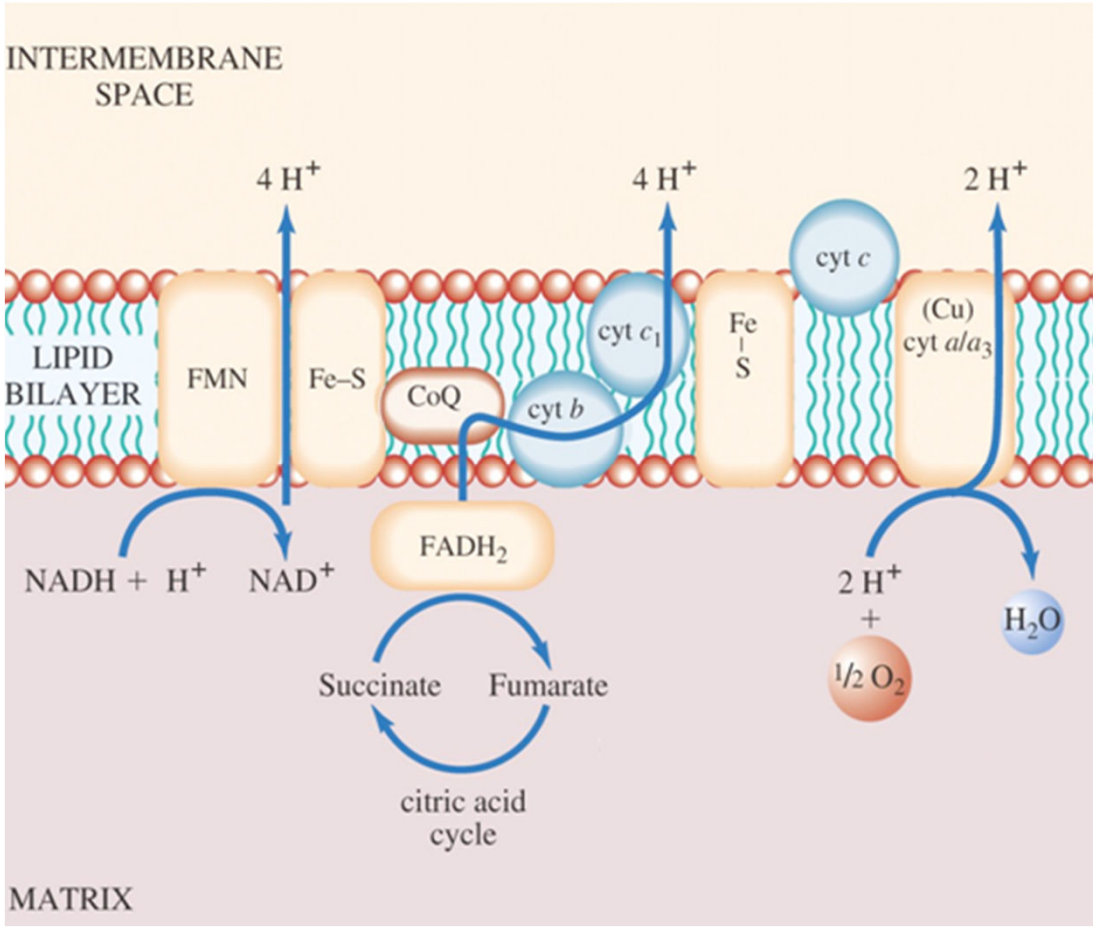
Complex IV

$2\ cyt\ c_1 + 2H^+ + ½\ O_2 \rightarrow 2\ cyt\ c_1 + H_2O$

- Accepts <u>one e^- at a time</u> from cyt c
- Donates a total of 4 electrons per O_2 molecule
- 2 H^+ are pumped to IM space

Electron Transport Chain

Overview



What is special about the FMN's ability to transfer e^- in Complex I?

It has the ability to transfer 1 or 2 e^- allowing it to mediate between accept 2 e^- from NADH and donating them one at a time to the Fe/S centers, which only accept 1 e^-

Where do the protons on QH_2 come from?

CoQ binds to complexes I and II near the interface of the matrix and the inner mitochondrial membrane, therefore it picks up its protons from the **matrix**

Would raising the pH of the fluid in the intermembrane space result in ATP synthesis in the matrix?

No. Raising the pH would eliminate the H^+ gradient, which is the essential driving force of ATP synthase.

* Remember that in a gradient substances flow from higher concentration to lower concentration. Raising the pH would decrease the $[H^+]$ and they would no longer flow back toward the matrix ($pH_{inside} \approx pH_{outside}$)

Electron Transport Chain

$E^{0'}$

−

+

mitochondria membrane protein complex		Full name		redox center	oxidized	reduced	e^- passed	H^+ pumped
Complex I	Complex II	I	NADH-coenzyme Q oxidoreductase	FMN, ion-sulfer clusters	NADH	Q	2	4
		II	succinate-coenzyme Q oxidoreductase	FAD, ion-sulfer cluster	$FADH_2$	Q	2	0
Complex III		coenzyme Q-cytochrome c oxidoreductase		cytochrome c_1, b_L, and $b_{H,}$ ion-sulfer protein	QH_2	cyt c	2	4
Complex IV		cytochrome c oxidase		cytochrome a, cytochrome a_3, Cu_B, Cu_A	cyt c	O_2	2	2

ATP Synthase

Enzyme complex that catalyzes the formation of ATP

$ADP + P_i \rightarrow ATP + H_2O$

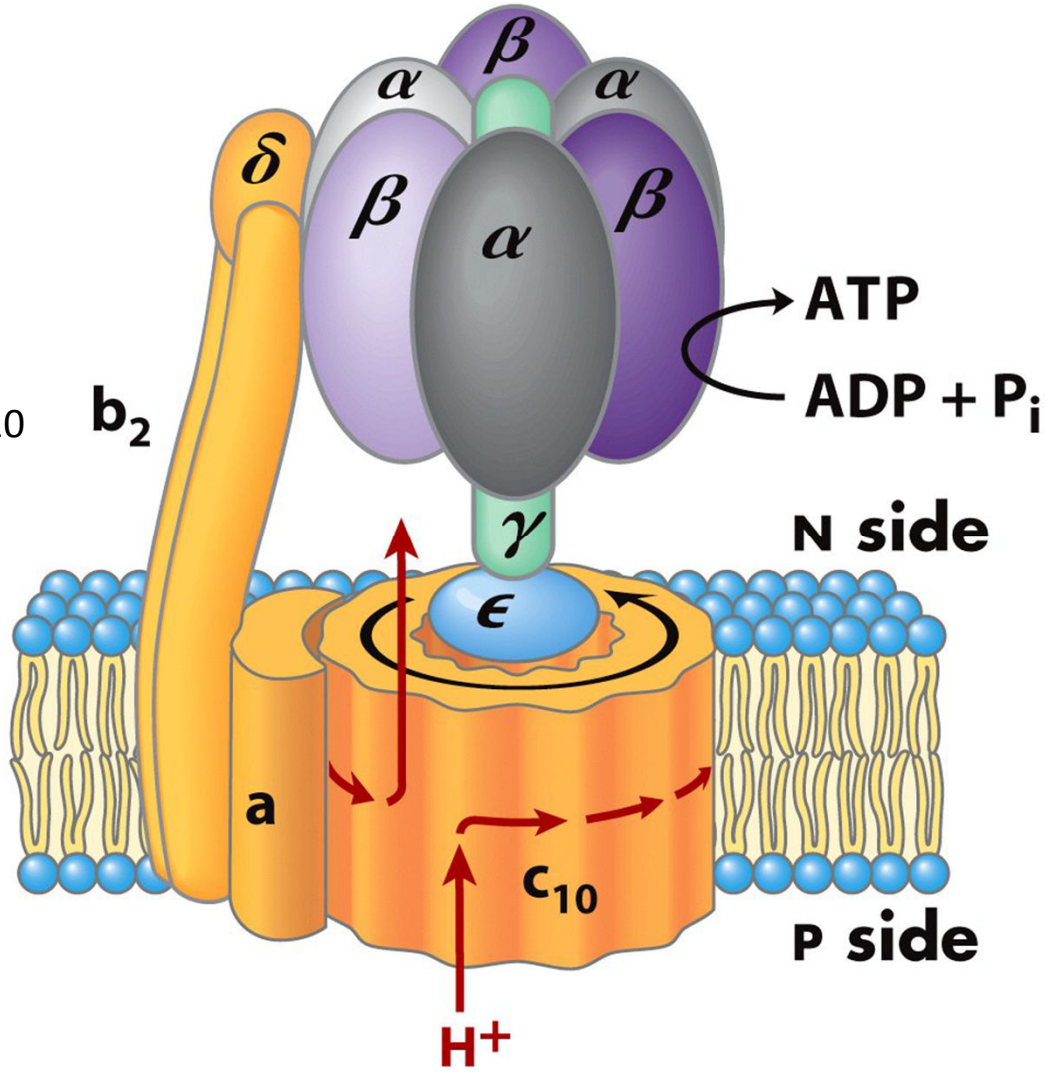
Overview

The F_o Complex

- Membrane-spanning
- 13 subunits total
  - 10 c subunits (c_{10}) form a ring in membrane and acts as a pore to carry H^+
  - 2 b subunits (b_2) act to stabilize the F_1 subunit relative to membrane and c+10 ring
  - 1 a subunit

The F_1 Complex

- Attached to F_o and protrudes out toward the matrix
- Where ATP synthesis occurs
- 5 different subunits ($\alpha_3\ \beta_3\ \gamma\ \delta\ \varepsilon$)
- 3 αβ dimers surround the central shaft, γ, each with a slightly different conformational state, which affect the affinity in which they bind ADP, P_i, and ATP
- Each β has one catalytic acitve site for ATP synthesis



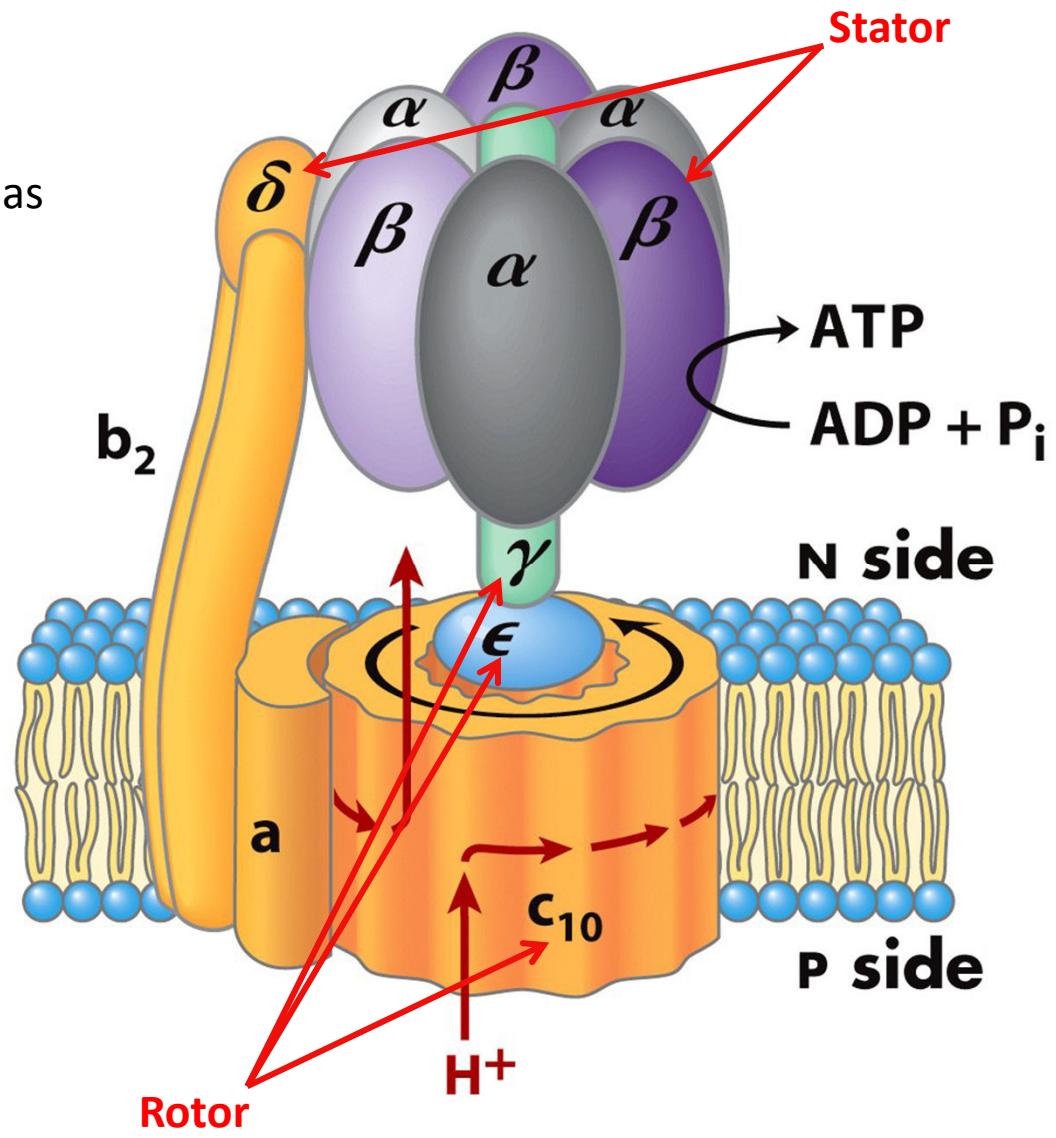
ATP Synthase

Enzyme complex that catalyzes the formation of ATP

$ADP + P_i \rightarrow ATP + H_2O$

"Binding change" Mechanism

- The c_{10} subunits in the F_o complex and the ε γ subunits in the F_1 complex act as the <u>rotor</u> (rotates)
- The α β_2 δ subunits in the F_1 complex act as the <u>stator</u> (remain stationary relative to the rotor)
- Every time 3 H^+ are bound and released in the c_{10} subunits, the rotor rotates 120°
- As the rotor moves past each αβ dimer, it induces a conformational change
- Each 120° rotation results in the synthesis of 1 ATP



ATP Synthase

Enzyme complex that catalyzes the formation of ATP

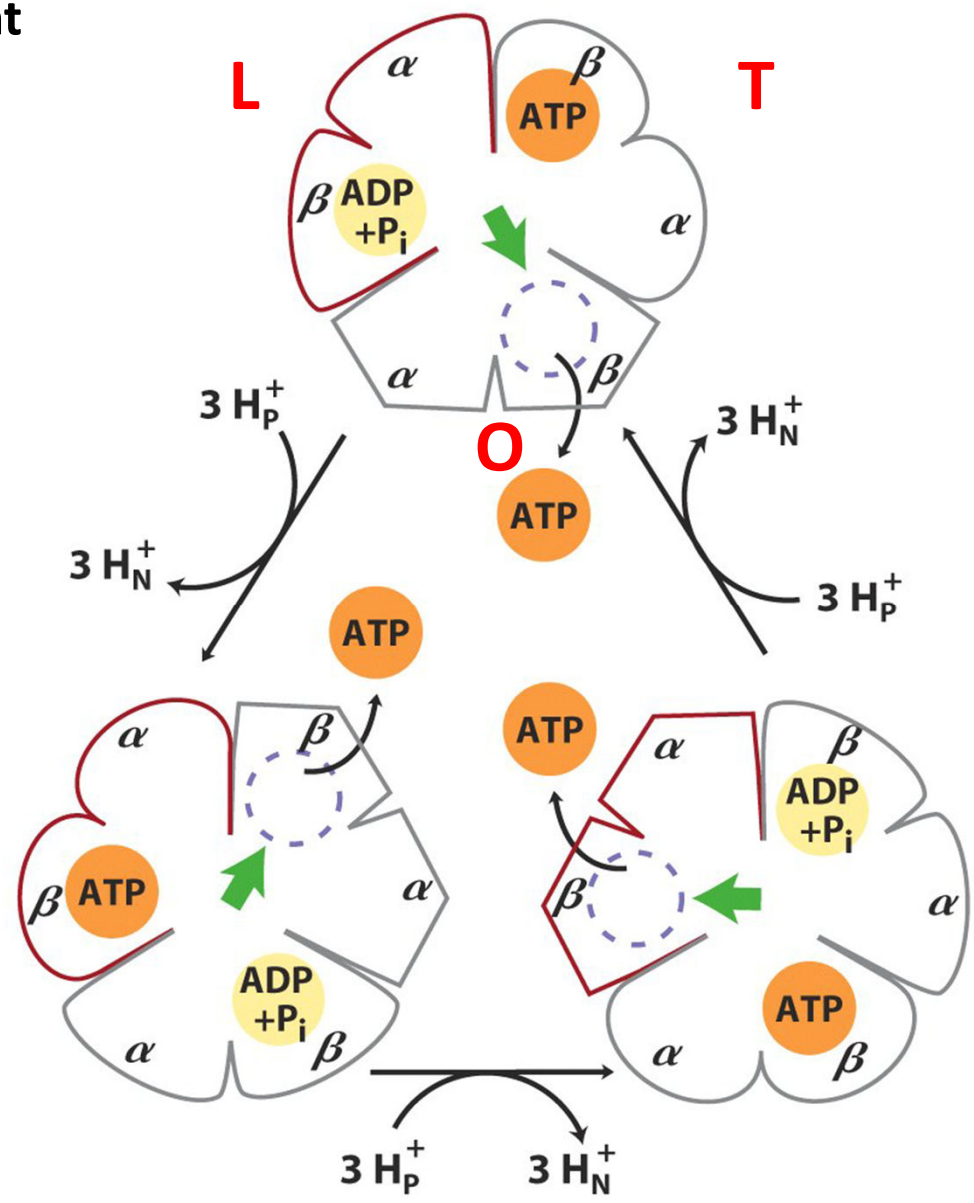
$ADP + P_i \rightarrow ATP + H_2O$

The αβ dimers participate in 3 different conformational states

- Loose (ADP and P_1 binding)
- Tight (ATP formation)
- Open (empty)

Paul Boyer (of Boyer Hall fame at UCLA) proposed a **rotational catalysis** mechanism is which each active site takes turns catalyzing the synthesis of ATP

The subunits act in such a way that when one adopts the open state, the one next to it MUST adopt the Loose state and the one next to that MUST adopt the Tight state and so forth



ATP Counts

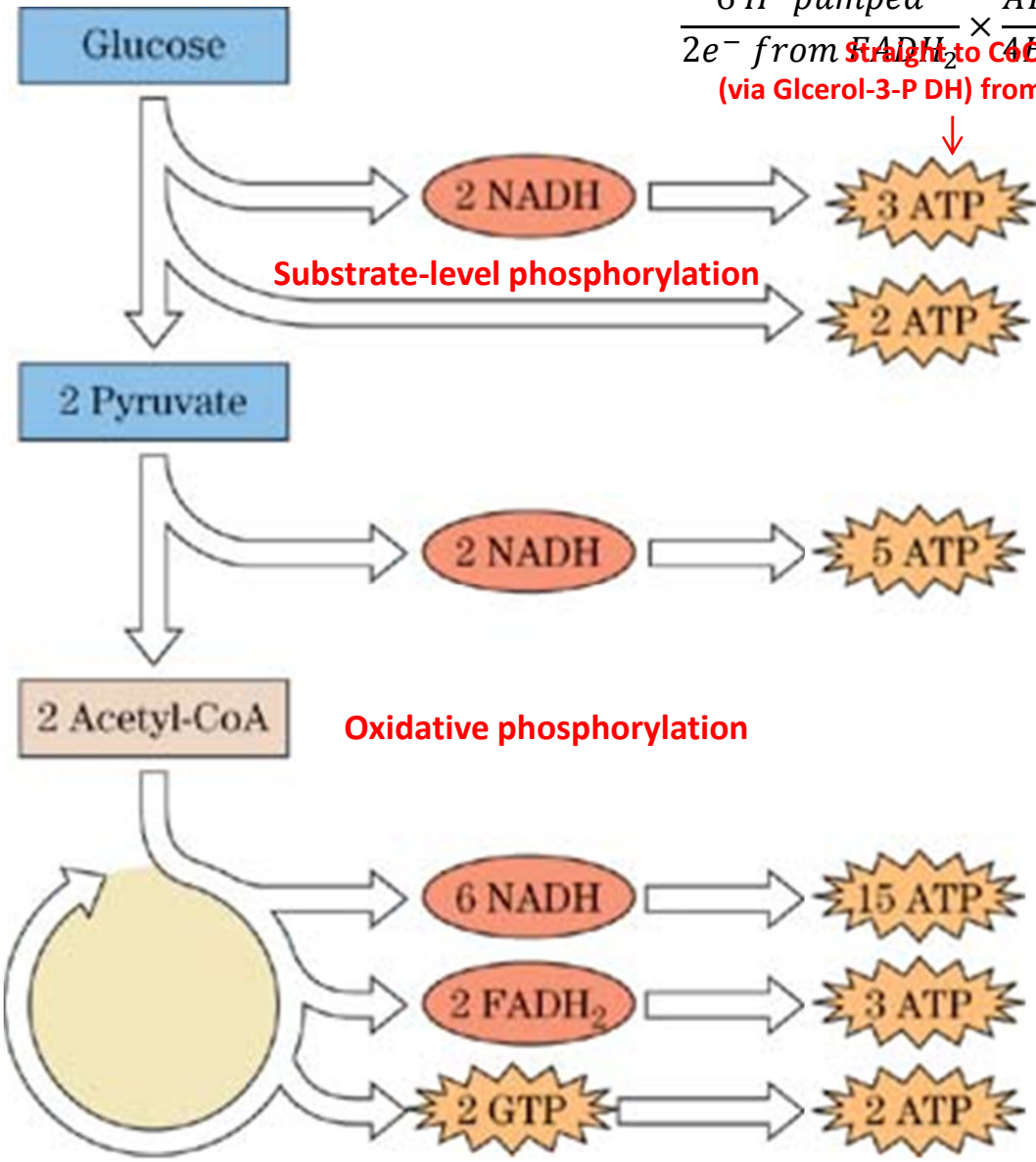
P/O ratios for $FADH_2$ and NADH

$$\frac{10\ H^+ pumped}{2e^-\ from\ NADH} \times \frac{ATP}{4H^+} = \mathbf{2.5}$$

$$\frac{6\ H^+ pumped}{2e^-\ from\ FADH_2} \times \frac{ATP}{4H^+} = \mathbf{1.5}$$

Straight to CoQ (via Glcerol-3-P DH) from IM Space

Transferred from IM space to matrix via malate



What is the total ATP count for an anarobic organism?

2
(substrate-level phosphorylation)

What is the total ATP count for an aerobic organism?

30 or 32
(substrate-level phosphorylation and oxidative phosphorylation)

ATP Synthase Animation

http://www.dnatube.com/video/104/ATP-synthase-structure-and-mechanism

Previous Exam Questions

9. (19 pts) You are a blossoming biochemist and wine enthusiast, and you're interested in studying the overlap of your two loves. You decide to try a carbon-tracing experiment with a batch of champagne. After the initial fermentation and bottling, you add yeast and labeled sugar (labeled at carbon 1 of glucose) to the bottle for a second fermentation, which will convert the bottle's contents to champagne. After the ageing period, you open the bottle and measure where the label ended up.

    a. (5) What compound contains the majority of the label? Give its name, draw its structure, and circle the labeled carbon.

    A bit of the label ended up in L-malate, which you find surprising since you know that no aerobic metabolism was occurring. After some reading, you learn that L-malate is responsible for tartness in some wines and can be produced under anaerobic conditions.

    b. (2) Complete the given equation for the net conversion of glucose to malate. (Hint: look at the differences in numbers of C, H, and O elements.)

    c. (7) Briefly explain how glucose would have been converted to L-malate in your champagne, naming the important pathways &/or enzymes (*Hint: there are three of these*). (30 words or fewer.)

    d. (5) Draw the structure of L-malate, and circle the carbon that was labeled in your experiment.

Previous Exam Questions

21. (29) Lactose is the major carbohydrate of milk. Its catabolism begins with hydrolysis of its glycosidic bond, then the monosaccharides are broken down via glycolysis. (Galactose is converted to glucose-6-phosphate through a series of reactions, one of which uses ATP and produces ADP.)

    a. (5) List the energy currencies produced as a result of the aerobic breakdown of lactose. Show your reasoning.

    b. (4) How many ATPs can be made from these energy currencies? Show your work.

    In the breakdown of proteins, different amino acids are catabolized to different intermediates of glycolysis &/or the citric acid cycle. For the dipeptide Ser-Thr, ser is converted to pyruvate in one step, and thr to pyruvate and acetyl-CoA in several steps:

    $$\text{Ser} \rightarrow \text{pyruvate} + NH_3$$
    $$\text{Thr} + NAD^+ + \text{CoA} \rightarrow \rightarrow \rightarrow \rightarrow \text{pyruvate} + \text{acetyl-CoA} + \text{NADH}$$

    c. (2) Name the class of enzyme that catalyzes the conversion of serine to pyruvate.

    d. (4) List the energy currencies produced as a result of the aerobic breakdown of Ser-Thr. Show your reasoning.

    e. (2) How many ATPs can be made from these energy currencies? Show your work.

    f. (4) Given the molecular masses of the two nutrient compounds, lactose = 342 g/mol; Ser-Thr = 206 g/mol, which is the more efficient energy source? Show your reasoning.

    g. (4) Suggest two different compounds that could regulate the breakdown of Ser-Thr (to pyruvate and acetyl-CoA): a likely product inhibitor, and a likely feedback inhibitor.

    h. (4) Of the two inhibitors you listed for part f, choose one, and briefly explain how its levels reflect the energy status of the cell (25 words or fewer).

Great luck studying!

Thanks for a great quarter, biochemists!

P.S. Yes, yes you can ☺