

①

CHEM141 Midterm 1 Review

 $\Delta G^\circ = \text{free energy at biological standard state}$

$[H^+] = 10^{-7}, T = 298K, [H_2O] = 55.5M$

$\Delta G^\circ = -RT \ln(K_{eq})$

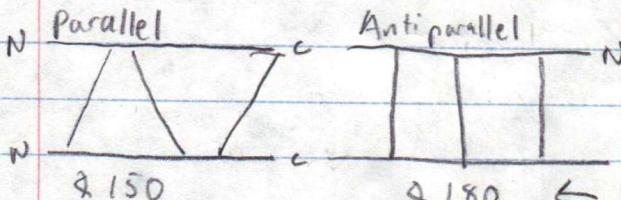
$\Delta G^\circ = -nFE^\circ + -nFE^\circ \rightarrow \text{one for each half reaction}$

 $K_{eq} = \text{make to adjust for } \Delta G^\circ \text{ conditions i.e. } \underline{\quad} \text{ or for } \underline{OH^-/H^+}$ Amide rotational barrier = 18.21 kcal/mol $1m$ $10^{-3}m$ at isolectric point (pI) = AAs crash out of solution↳ equal $+/-$ charges = AAs overcome solvation and attract, sticking togetherCalculate pI

1) ID all pKa's

2) Compute pKa ranges

3) Tally up charges at each range

4) (lower, upper) at $pI \rightarrow (\text{upper} + \text{lower}) / 2 = pI$ Protein StructureSecondary: α -helix = H bond stabilized β -sheets phi $\phi = \text{btw } \text{C=O groups}$ psi: $\psi = \text{btw } N \text{ groups}$ 3^o struct: 3D all 2^o features4^o struct = multiple chainsHydrophobic effect allows protein folding:
less surface area = more free H_2O molecules

↳ all elements /

termini in same spot

Salt bridges = dipole-dipole interactions btw O^- and O^+ $\epsilon = 20$ hydrophilic $\epsilon = 80$ H_2O - more hydrophobic - less charge distribution by H_2O

Coulomb's Law = $E = \frac{q_1 q_2}{4\pi\epsilon_0 \epsilon r} \cdot \frac{6.02 \times 10^{23}}{1000} \text{ converts to kcal/mol}$ $\epsilon' = 3.7 \times 10^{-11}$

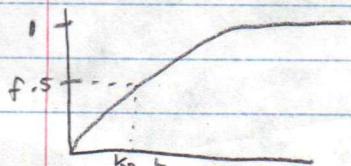
 $VdW = \text{induced dipole interactions btw atoms}$ Given $[L]$ (PL)Ligand Bindingdisplacement of $H_2O = \text{makes } \curvearrowright \text{ favorable}$  $\frac{PL}{P} \text{ use } \frac{PL}{P_{\text{tot}}} \text{ to find}$

$K_A = \frac{k_{on}}{k_{off}}, K_D = \frac{k_{off}}{k_{on}}$

$f = \frac{[L]}{K_D + [L]}, f_D = [L] \text{ when } K_D = [L] \text{ when half of } [L]$

$\Delta G^\circ = RT \ln K_D \text{ smaller } K_D = \text{more binding affinity}$

$\Delta \Delta G^\circ = \text{difference between affinities} = \text{specificity} = -RT \ln \left(\frac{K_{D1}}{K_{D2}} \right) \text{ for bound } \rightarrow P$



(2)

CHEM141 Midterm Review

Kinetics

6 kinds of enzymes

$$k = \frac{k_B T}{h} e^{-\Delta G^*/RT}$$

hydrolyse - hydrolyze

$$\frac{k_{cat}}{k_{uncat}} = x \log(x) \cdot 10 = \Delta \Delta G^\circ \text{ approx}$$

mono substrate

isomerase - change isomeric

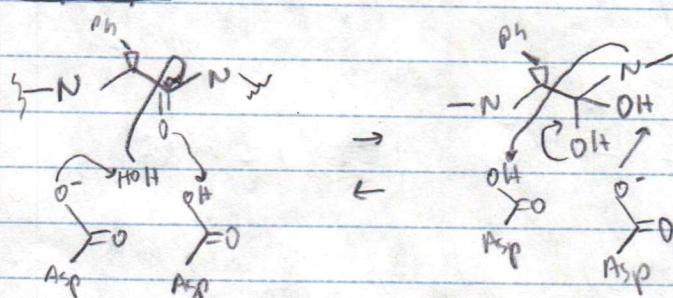
ligase - form bonds - couples w/ $\Delta G < 0$ rxn

lyase - break bonds w/o hydrolysis/redox

oxidoreductase - redox

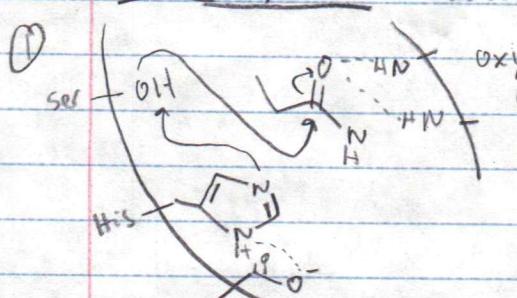
transferase - transfer group

Hydrolases: Proteases:

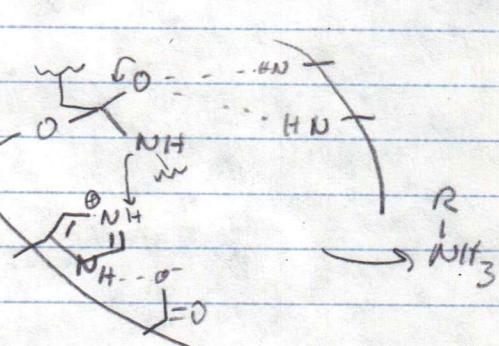
Aspartyl

NO covalent intermediate
where Asp attached to peptide

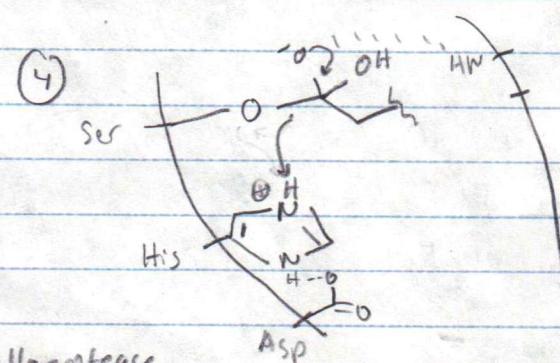
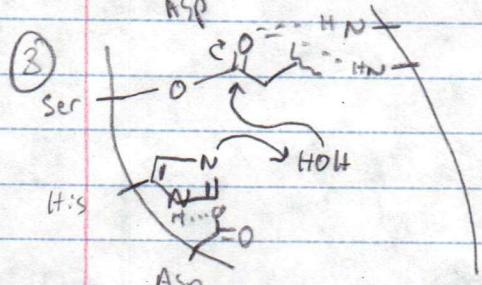
Dependent on Asp = pH 5-6 so 50:50 H₂O⁺

Serine / cysteine

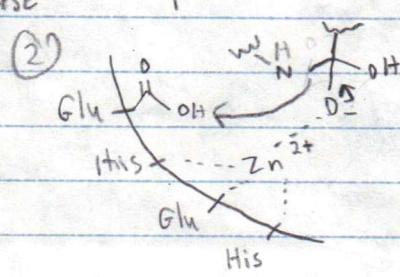
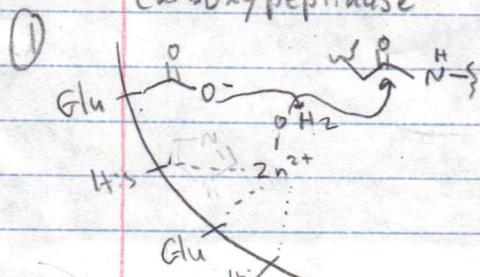
Selectivity - asp. creates salt bridges w/ arg / lys.
oxyanion hole cleaves PAs ↓



- 1) Serine attack
- 2) LG leaves
- 3) H₂O cleaves Serine



Carboxypeptidase - metalloprotease



CHEM 141 Midterm 1 Review

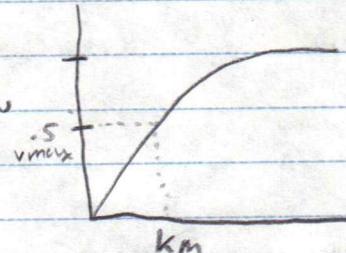
Kinetics cont.

$$\text{Amide Rate} = k_2 \left(\frac{k_1 [\text{Amide}]}{k_1 + k_2} \right)$$

Law

MM Assumptions

- 1) $\text{ES} \rightarrow \text{EP}$ irreversible
- 2) EP release not RDS
- 3) $\frac{d[\text{ES}]}{dt} = 0$
- 4) $[\text{L}] \sim [\text{L}]_{\text{Total}}$



$$\text{Efficiency} = \frac{k_{\text{cat}}}{k_m}$$

$$V_{\text{max}} = k_{\text{cat}} [E]_{\text{tot}}$$

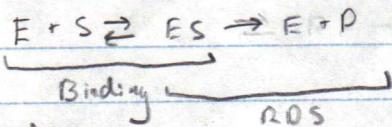
$$v = \frac{V_{\text{max}} [S]}{K_m + [S]}$$

smaller k_m = stronger binding

$$\text{Diffusion limited} = \frac{k_{\text{cat}}}{k_m} = 10^9$$

$$\frac{k_{\text{cat}} [E]_{\text{tot}} [S]}{K_m}$$

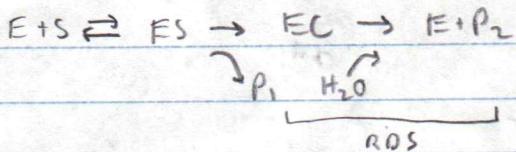
when $[S] \ll K_m$ = 1st order
= k rate constant



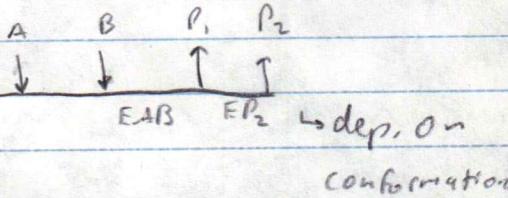
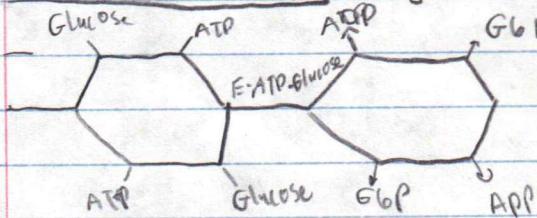
Problem Solving

- 1) Assume steady state / find intermediate rates for $[\text{ES}]$ and $[\text{EC}]$
- 2) Mass Balance

Covalent Intermediate MM



B: Substrate Kinetics - Ligases, Transferases, Oxo-reductases

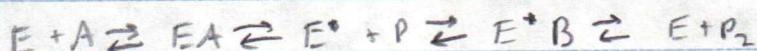


$$v = \frac{k_{\text{cat}} [E]_{\text{tot}} [A][B]}{K_A K_B + K_B [A] + K_A [B] + [A][B]}$$

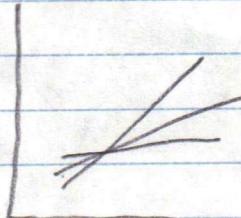
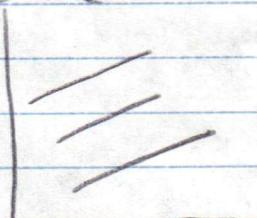
$$K_A / K_B = K_m \text{ for } A \text{ and } B$$

Change

Substrate \rightarrow Product \rightarrow Substrate \rightarrow Product



$$v = \frac{k_{\text{cat}} [E]_{\text{tot}} [A][B]}{K_B [A] + K_A [B] + [A][B]}$$



Ping Pong

Sequential

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

(3)

↳ k_{cat}

quantity

$$\rightarrow \Delta \Delta G^\circ = -RT \ln(\text{specificity})$$

$$\text{specificity} = \frac{k_{\text{cat}}}{k_m} = 10^{\frac{\Delta \Delta G^\circ}{RT}}$$

$$10^{\frac{\Delta \Delta G^\circ}{RT}}$$

$$10^{\frac{-\Delta G^\circ}{RT}}$$

(4)

Pset Notes

Identifying oxidation state

count oxidation numbers for each atom

↳ compare w/ products

- → + donor - lost

+ → - acceptor - gained

Add up $\Delta G^\circ_{\text{red}}$ + $\Delta G^\circ_{\text{ox}}$ - divide

by # of mols of product

Inhibition

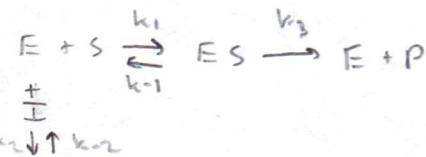
irreversible - covalent bond w/ active site

reversible - noncovalent interactions

- competitive, noncompetitive, uncompetitive

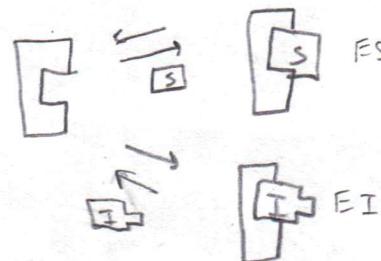
Competitive

Structure similar to substrate - "competes" for active site



$$V = \frac{V_{max}[S]}{K_m d + [S]}$$

$$V = \frac{k_{cat}[E]_{\text{Total}}[S]}{K_m \left(1 + \frac{[I]}{K_I}\right) + [S]}$$



$$\text{rate} = k_2 [ES] \quad \textcircled{1} \text{ Rate law}$$

$$v = \frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

$$0 = d[EI] = k_2[E][I] - k_{-2}[EI] \quad \textcircled{2} \text{ steady state}$$

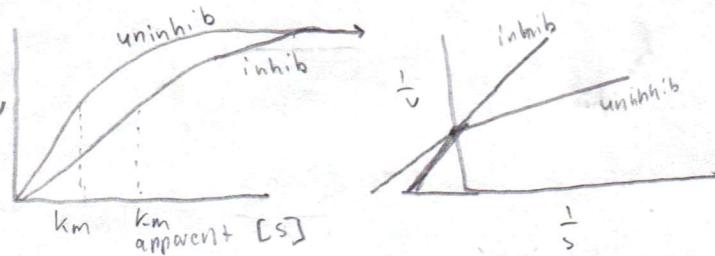
K_m ↓ Binding ↑K_I ↓ Binding Inhibitor ↑

Mass Balance

$$[E]_{\text{Total}} = [E]_{\text{free}} + [ES] + [EI]$$

$$[E]_{\text{Total}} = [E]_{\text{free}} + \frac{[E][I]}{K_I} + \frac{[E][S]}{K_m}$$

$$\frac{1}{V_{\text{inhib}}} = \frac{K_m d + [S]}{V_{max}[S]}$$

Overcome inhibition by saturating substrate
[S] > [I] will outcompete [I]net effect = increase apparent K_m of substrate
- as more I binds instead of S, it will make it appear the substrate has a weaker binding affinity

slope changes
y intercept stays
the same

Problem

$$K_m = 2.00 \text{ mM} \quad k_{cat} = 150 \text{ s}^{-1} \text{ for A} \quad [S] = 2.00 \text{ mM}$$

5.00 mM of I - rate decreases by 50%.

Find K_I

$$0.5 = \frac{V_{\text{inhib}}}{V_{\text{uninhib}}} = \frac{V_{max}[S]}{K_m d + [S]} = \frac{V_{max}[S]}{\frac{V_{max}[S]}{K_m} + [S]} = \frac{V_{max}[S]}{\frac{V_{max}[S]}{K_m} + \frac{V_{max}[S]}{K_I}}$$

$$0.5 \left(K_m d + [S] \right) = K_m + 0.5[S]$$

$$0.5 K_m d + 0.5[S] = K_m + 0.5[S]$$

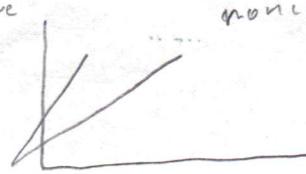
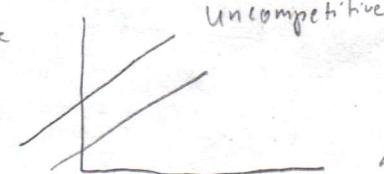
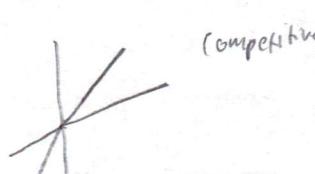
$$0.5 K_m d = K_m$$

$$d = \frac{K_m}{0.5 K_m} = \frac{K_m}{0.5 K_m} = \frac{K_m}{0.5 K_m} = \frac{K_m}{0.5 K_m}$$

$$0.5(K_m d + [S]) = K_m + 0.5[S]$$

$$0.5 K_m d + 0.5[S] = K_m + 0.5[S]$$

$$d = \frac{K_m}{0.5 K_m} = \frac{K_m}{0.5 K_m} = \frac{K_m}{0.5 K_m}$$

Summary

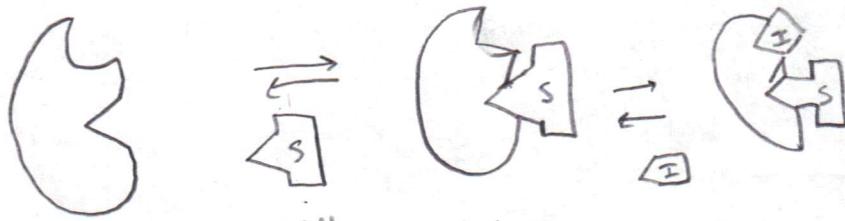
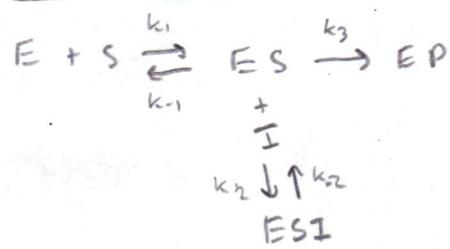
noncompetitive

$$d = 3 \text{ M}$$

$$1 + \frac{[I]}{K_I}$$

$$K_I = 2.5 \times 10^{-3} \text{ M}$$

Uncompetitive



Substrate binds first
I can't bind w/o conformational change from S

$$\text{rate} = k_3 [ES]$$

$$\frac{d[ES]}{dt} = k_1 [E][S] - k_{-1}[ES] - k_3 [ES] - k_2 [ES] + k_{-2}[ESI] = 0$$

$$\frac{d[ESI]}{dt} = k_2 [ES] + k_{-2}[ESI] = 0$$

cannot be overcome by increased [S]

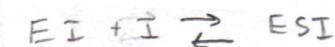
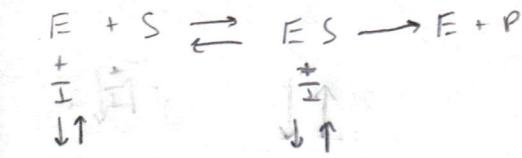
$$V = \frac{\frac{V_{max}}{d} [S]}{\frac{k_m}{d} + [S]}$$

↳ ESI removes ES, meaning increasing S would just make more ESI

$$\frac{1}{V} = \frac{k_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{d}{V_{max}}$$

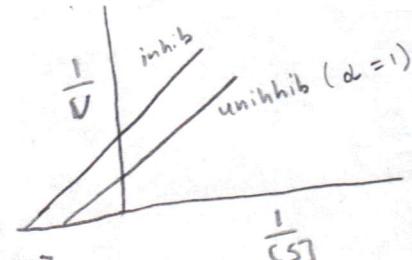
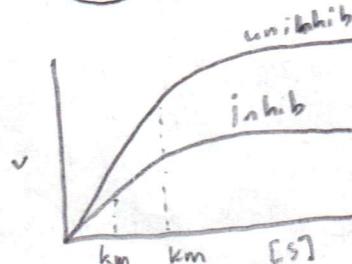
Noncompetitive

inhibitor / substrate bind at different sites
both can bind at anytime



binding affinities for k_2/k_m do not change depending on the other molecule

$$\frac{1}{V} = \frac{k_m d}{V_{max}} \cdot \frac{1}{[S]} + \frac{d}{V_{max}}$$



same slope
y intercept
change

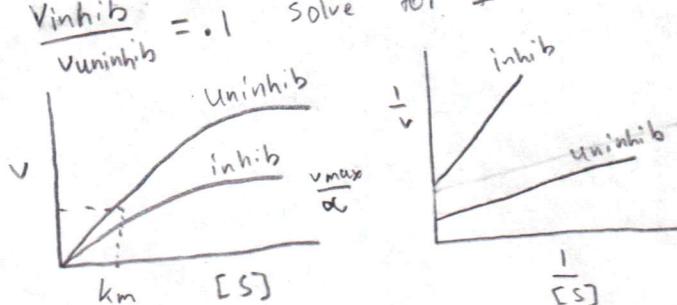
Both
km and
Vmax affected
slopes are same

net affect is both V_{max} and k_m appear to be reduced

Noncompetitive Problem

I has k_I of .00029 M. How much [I] needed to give 90% inhibition.

$$\frac{V_{inhib}}{V_{uninhib}} = .1 \quad \text{solve for } I$$



$$\frac{V_{inhib}}{V_{uninhib}} = \frac{V_{uninhib} - V_{inhib}}{V_{uninhib}} = 1 - \frac{V_{inhib}}{V_{uninhib}}$$

Both slope and y intercept are different

net affect is a decrease in V_{max} , k_m unaffected

noncompetitive inhibitors = allosteric ligands = binding distorts active site altering interaction w/ substrate often regulate enzymatic reactions

cannot be overcome w/ increased substrate

$$V = \frac{\frac{V_{max}}{d} [S]}{\frac{k_m}{d} + [S]}$$

$$\frac{V_{max}}{d} < \frac{V_{max}}{1}$$

Discriminating random vs ordered

Mix I_1, I_2 to mimic S_1, S_2
Randomly bind = I_1 competitive S_1 , noncompetitive S_2

S_1 must bind = I_2 comp. to S_2 , uncomp. to S_1

Metabolism

catabolism - breakdown of molecules

anabolism - build molecules

irreversible / exergonic steps targeted

↳ allows easy control

↳ hexokinase, phosphofructokinase, pyruvate kinase in glycolysis

Regulatory Mechanisms

- Inhibition

- Post translational modification - modify active site AA

- Translational modification - alter $[E]$

Cooperativity

allosteric activation involving multimeric enzymes

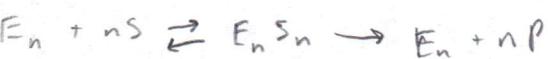
Binding of 1 substrate unit increasing binding

of other subunits

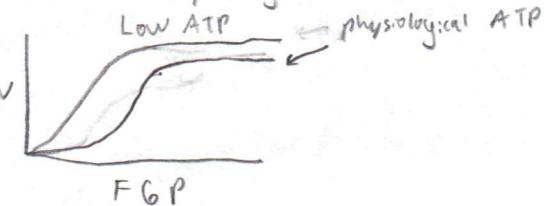
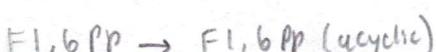
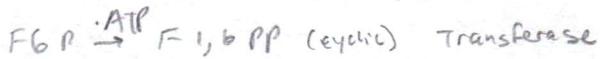
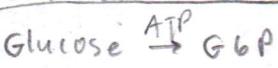
Dissociation also works cooperatively

$$V = \frac{V_{max} [S]^n}{k_{50} + [S]^n} \quad k_{50} = \frac{k_{off}}{k_{on}}$$

n = hill coefficient
= measure of cooperativity



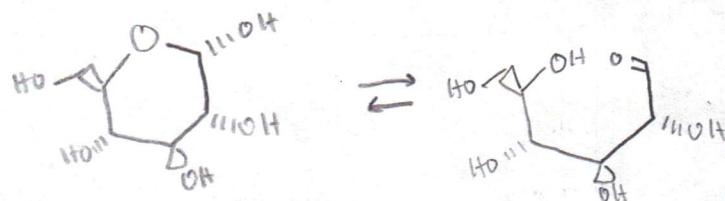
indicated by sigmoidal

Aerobic RespirationGlycolysis Overview

Transferase

Isomerase

} Energy investment

Glucose Metabolism Regulation

ATP binds to PFK = lowers binding affinity

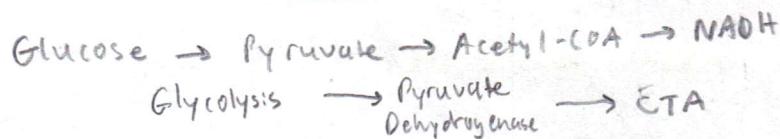
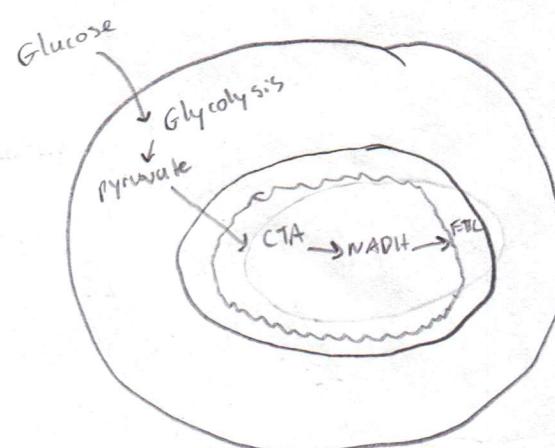
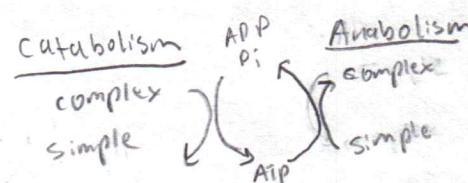
ADPLAMP have reverse effect

ATP = tense

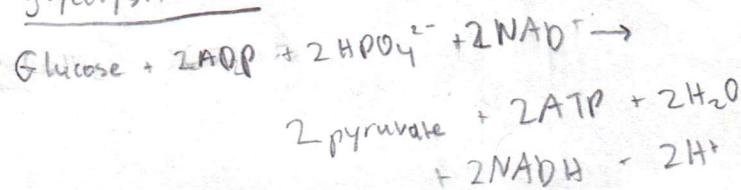
AMP / ADP = relaxed

F1,6PP activates PFK to ↑ rxn rate

Hexokinase, phosphofructokinase, pyruvate kinase major regulatory points



substrate-level phosphorylation - transfer of P: from substrate to ADP

Glycolysis Net:

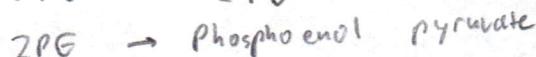
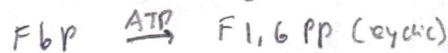
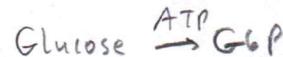
(4)

Glycolysis

- breakdown glucose into pyruvate

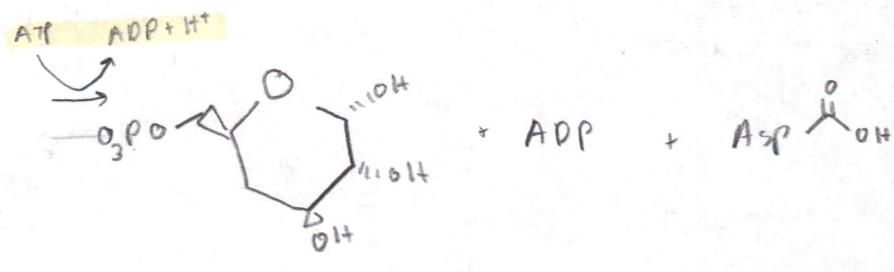
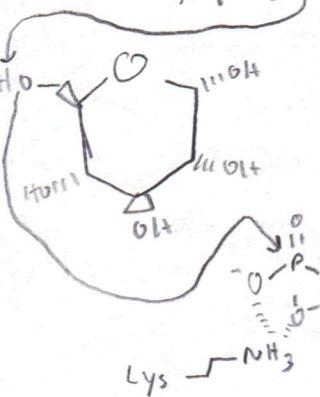
Steps

- 1) Add P_i groups to glucose in energy investment
- 2) fragment glucose into smaller pieces
- 3) Produce high energy phosphorylated intermediates



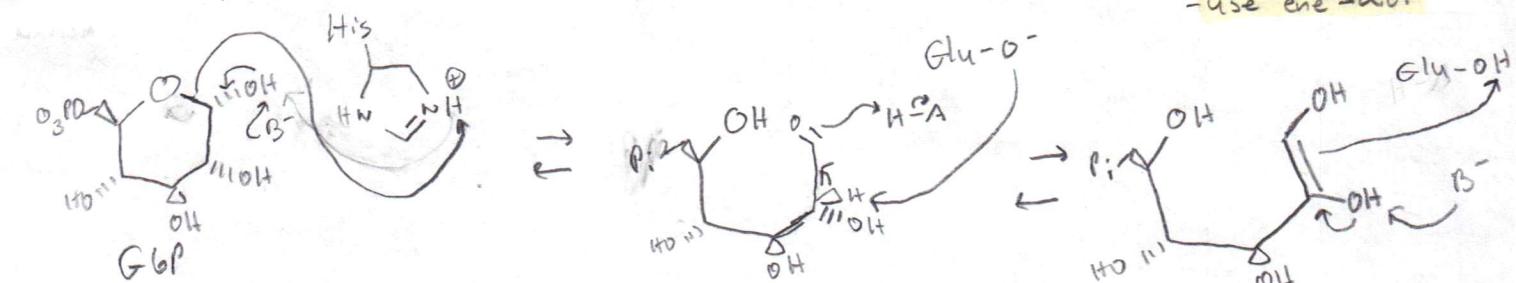
(1) Hexokinase - Transfer P_i to trap glucose in cell

Asp-O⁻ \hookrightarrow Induced fit binding
prevents water from being
in active site



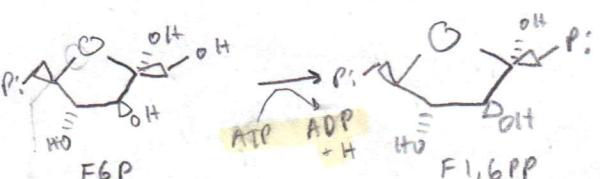
(2) Glucose-6-phosphate isomerase - convert to fructose so 1° can attack P_i

- use ene-diol



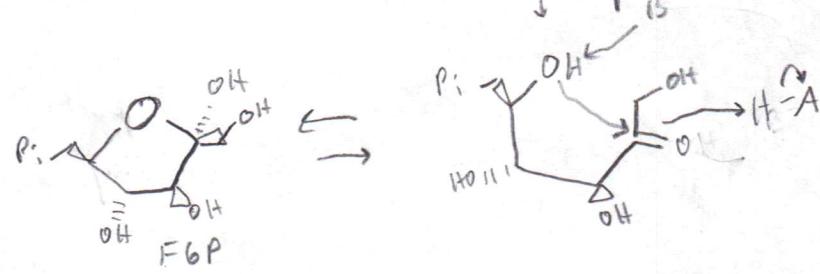
(3) Phosphofructokinase - Transfer P_i to 2^o alcohol from (2)

\hookrightarrow Now cannot revert back



Same Mechanism as Hexokinase

\downarrow F1,6PP uncycles w/o Enzyme



(4)

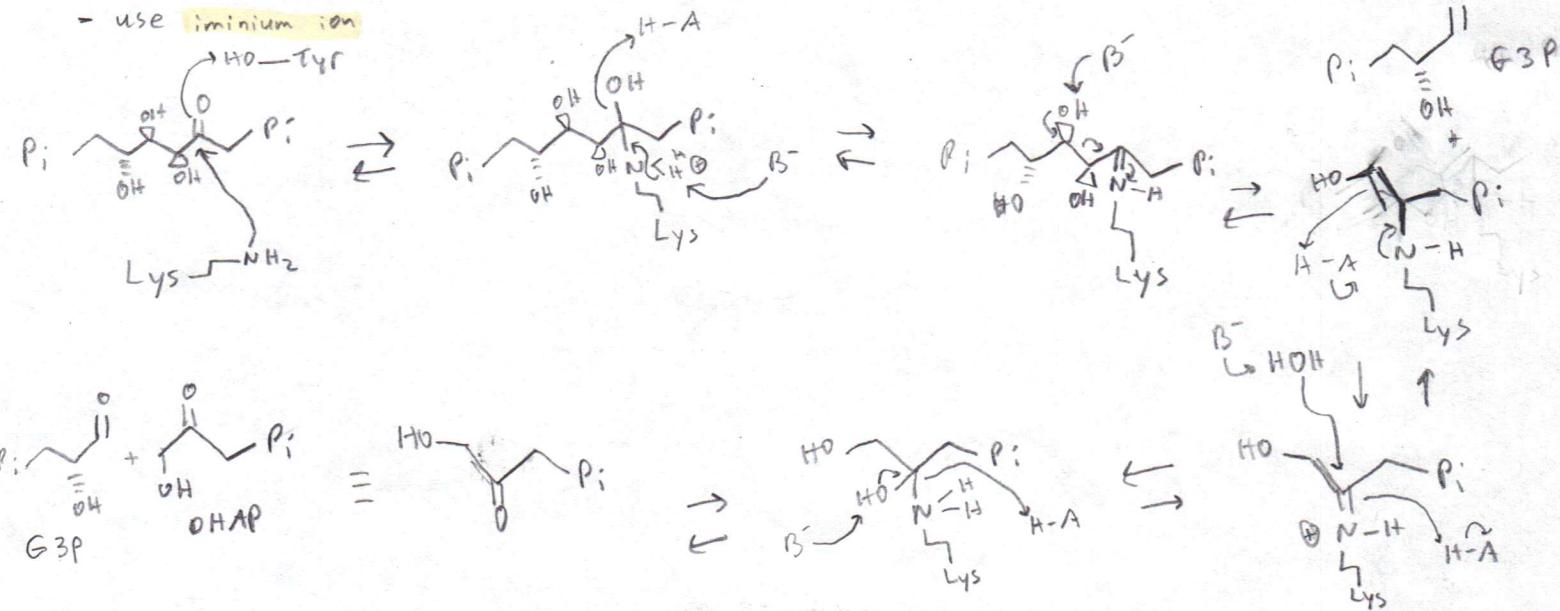
see next page

Glycolysis cont.

(5)

④ Fructose - 1,6 - Phosphate aldolase (lyase)

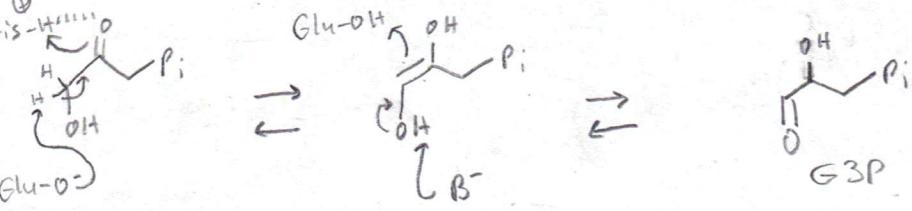
- Convert F1,6PP to G3P + DHAP → produce NADH later
- use iminium ion



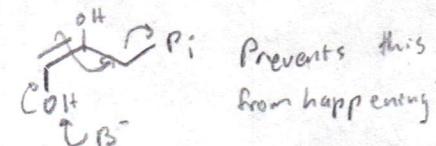
⑤ Triose Phosphate Isomerase END ENERGY INVESTMENT

- Convert DHAP to G3P
- more efficient for all enzymes to work w/ G3P

Enediol intermediate

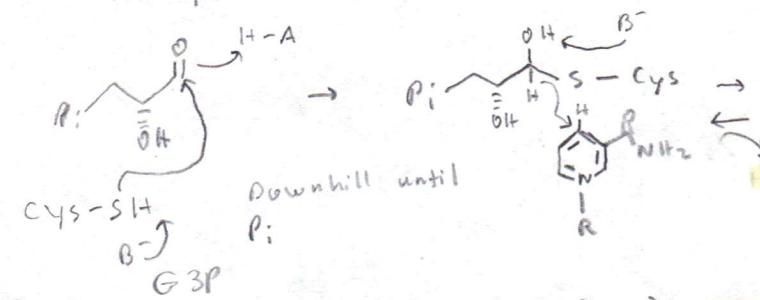


Pi not kicked out as it is locked into place in the active site

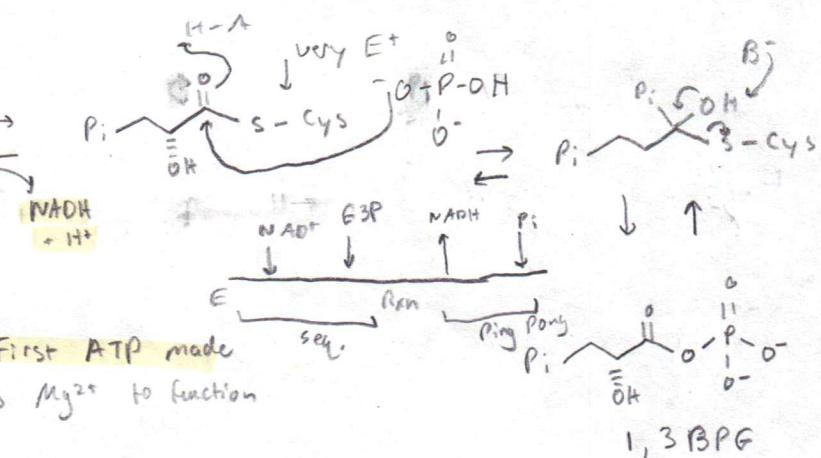


⑥ Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

- Take away H₂ = oxidize
- Aldehyde → carboxylic acid derivative

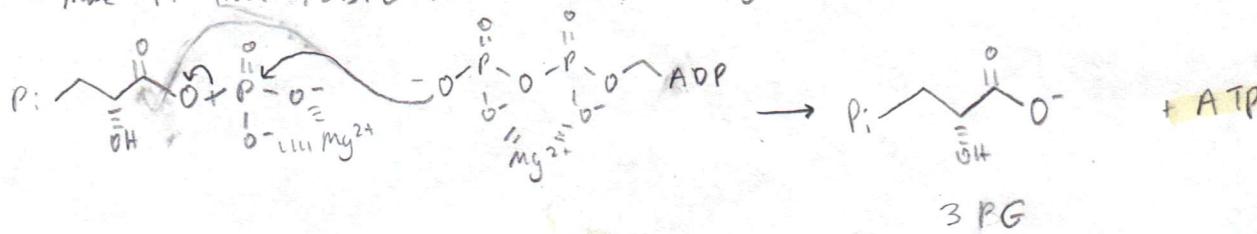


BEGIN ENERGY GENERATION



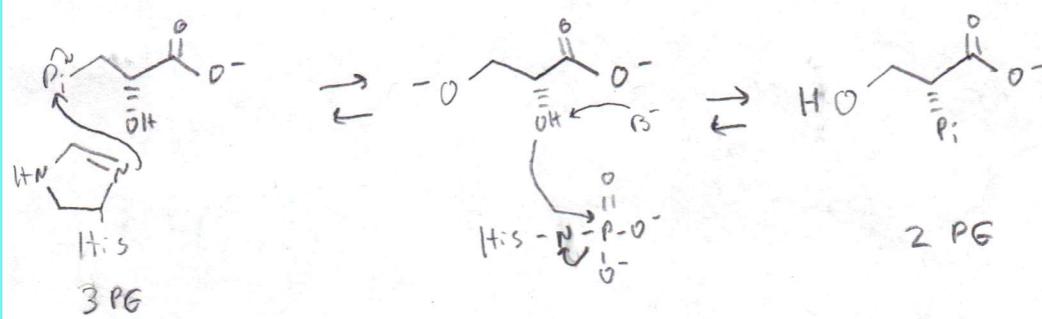
⑦ Phosphoglycerate kinase (transferase)

- First ATP made
- move Pi from 1,3BPG to ATP - requires Mg²⁺ to function



Glycolysis cont.

⑧ Phosphoglycerate Mutase - Move Pi to middle carbon via His mediated transfer



End result Glycolysis

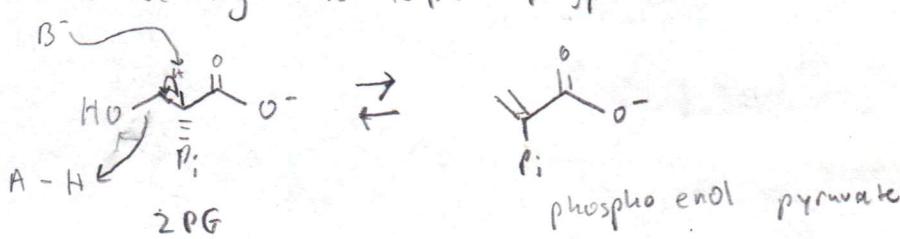
2 pyruvate made
for each glucose

2 ATP - make 4 ATP
total (2 per G3P)
but 2ATP put
in during
investment phase

once G3P stage is hit,
2 of everything
pyruvate critical for
NAD⁺ generation - needed
by GAPDH

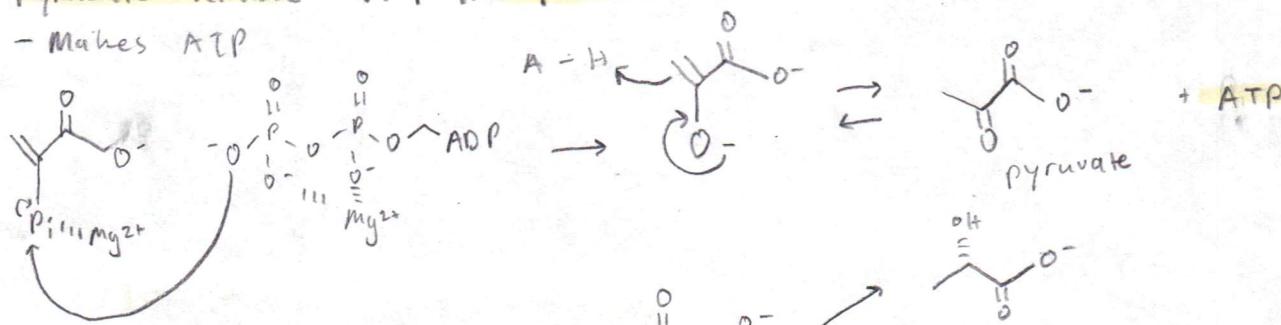
⑨ Enolase (lyase)

- α, β elimination to create phosphoenol pyruvate
- Need 2 Mg²⁺ to temper phosphate



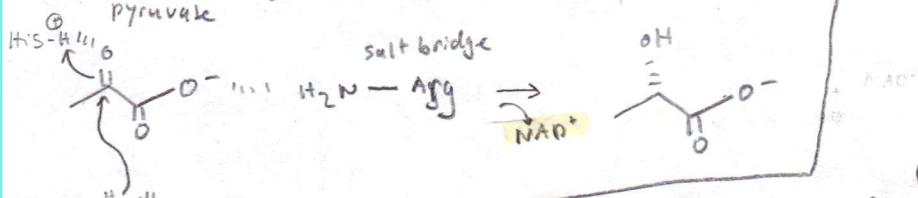
⑩ Pyruvate kinase - ATP H2 produced

- Makes ATP

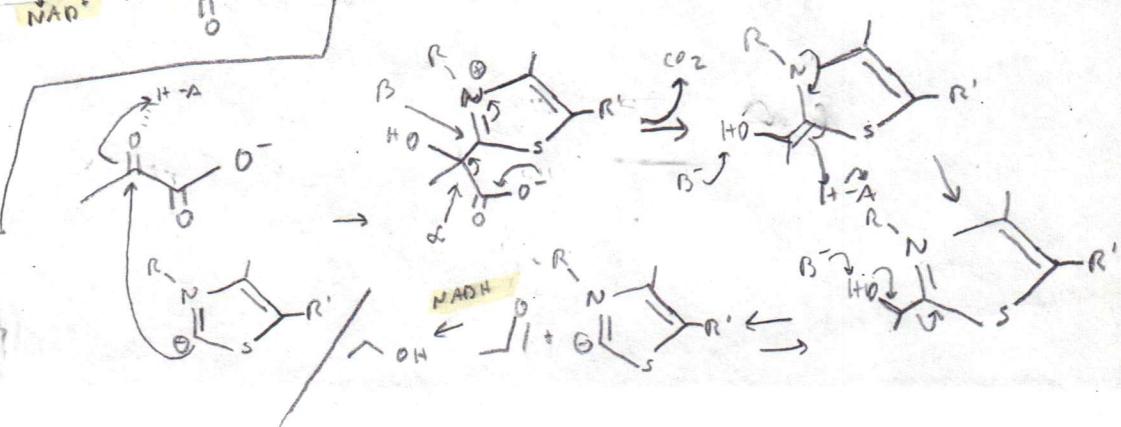
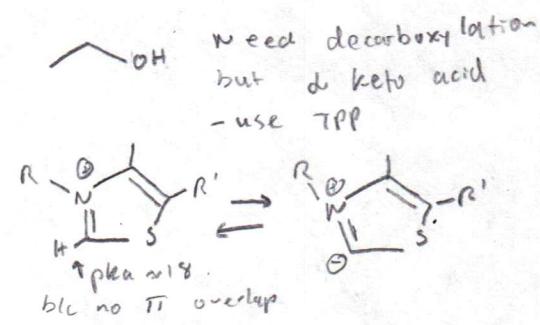
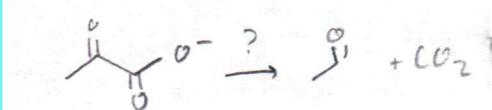
Fermentation (Anaerobic Respiration)

- Lactic Acid Fermentation
- Ethanol Fermentation

Lactate Dehydrogenase - in cytosol - reduce pyruvate



Ethanol Fermentation w/ TPP

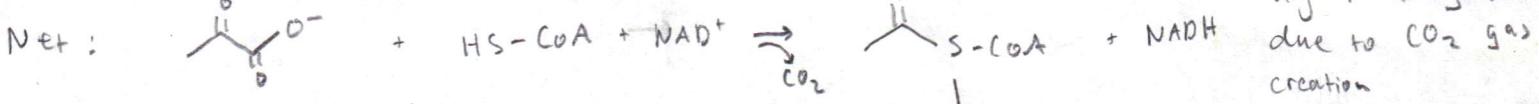


Pyruvate Dehydrogenase Complex (PDH)

(7)

Cofactors:

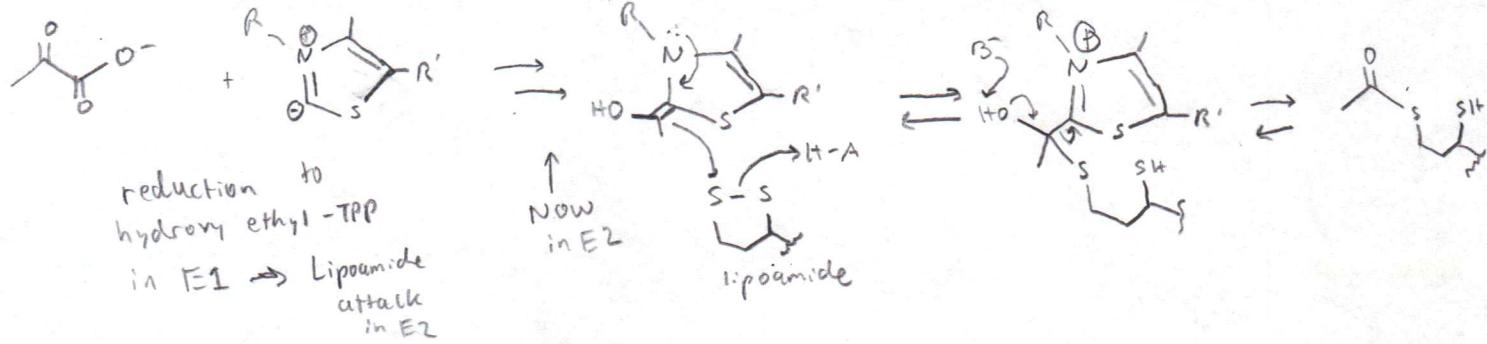
- 1) TPP
 - 2) Lipoamide
 - 3) Coenzyme A
 - 4) FAD/FADH₂
 - 5) NAD⁺
- converts pyruvate into acetyl-CoA in mitochondria's cytosol
3 subunits:
- 1) E1 - TPP, Lipoamide (Pyruvate Dehydrogenase) - oxidate pyruvate
 - 2) E2 - CoA (Dihydrolipoyl Trans acetylase) - bind lipoamide, CoA
 - 3) E3 - Lipoamide, FAD, NAD⁺ (Dihydrolipoamide Dehydrogenase) - regenerate lipoamide



E1

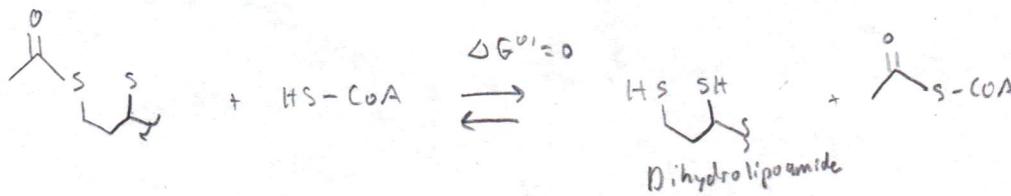
- Periphery - Pyruvate \rightarrow Hydroxyethyl-TPP
Lipoamide used to decarboxylate pyruvate

used as starting material for many products



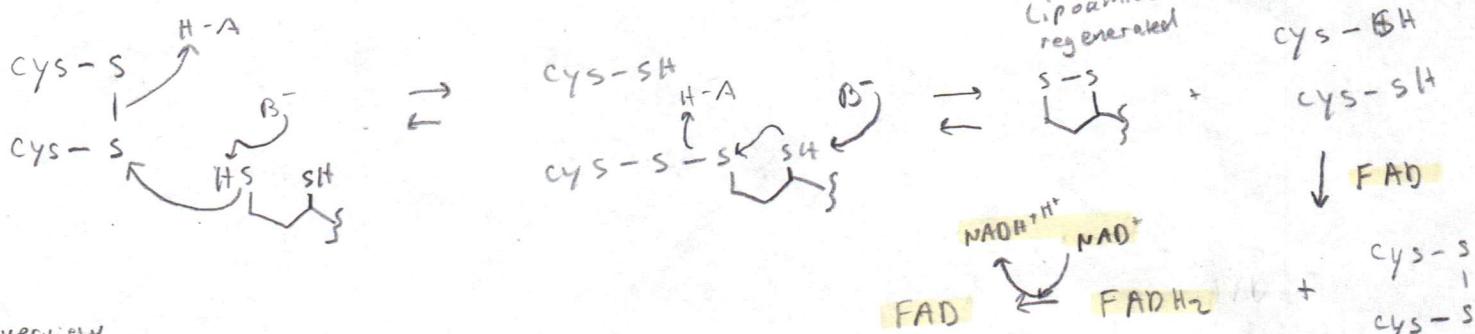
E2

Regenerate TPP and make acetyl-CoA

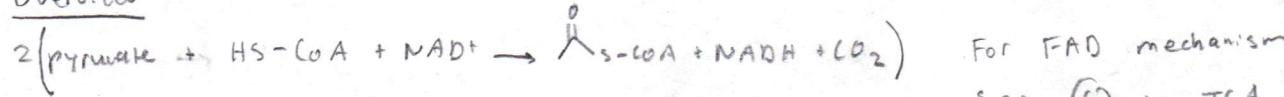


Now we must regenerate lipoamide

E3 - Regenerate Lipoamide and make NADH



Overview



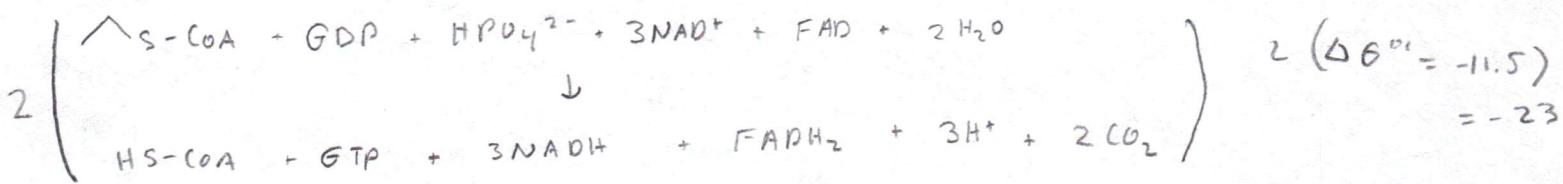
For FAD mechanism see (6) in TCA.

Regulation

Acetyl-CoA / NADH act as competitive inhibitors

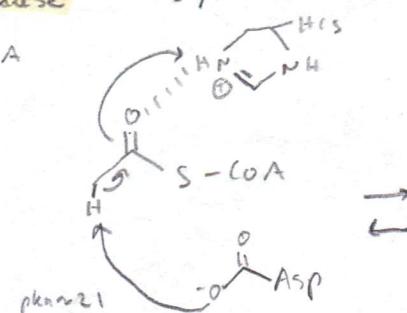
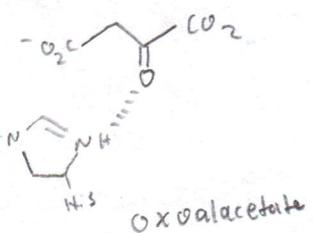
Citric Acid Cycle

- Produce NADH for ETC
- Oxidize acetyl-CoA $\rightarrow \text{CO}_2$ coupled w/ reduction NAD^+/FAD

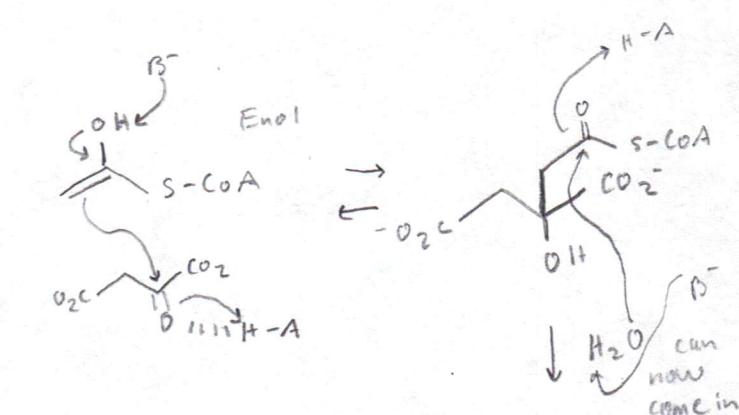


① Citrate Synthase

- Regenerate CoA



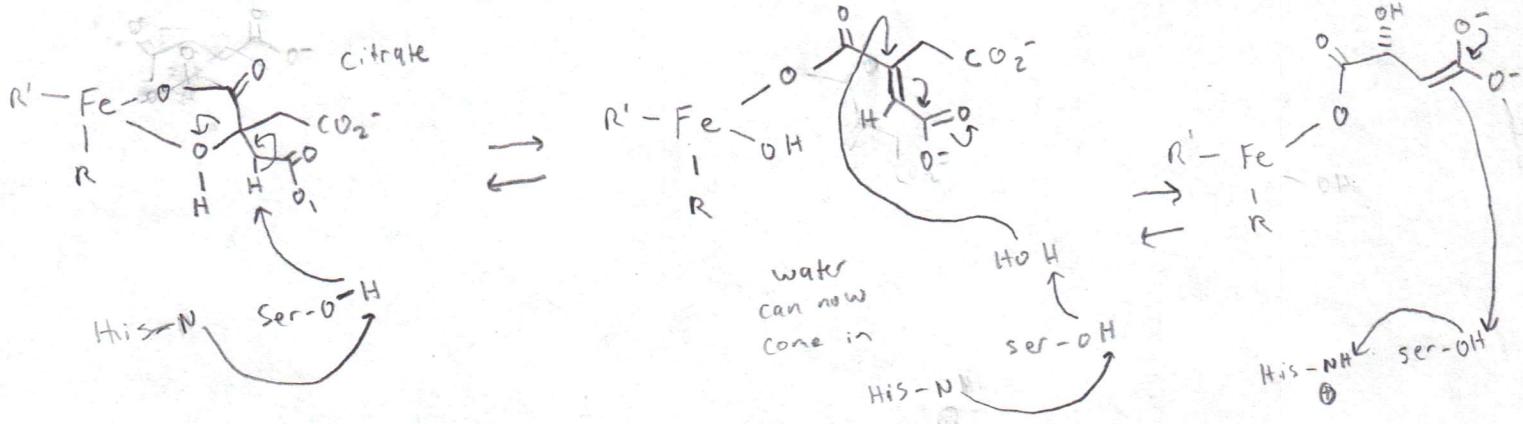
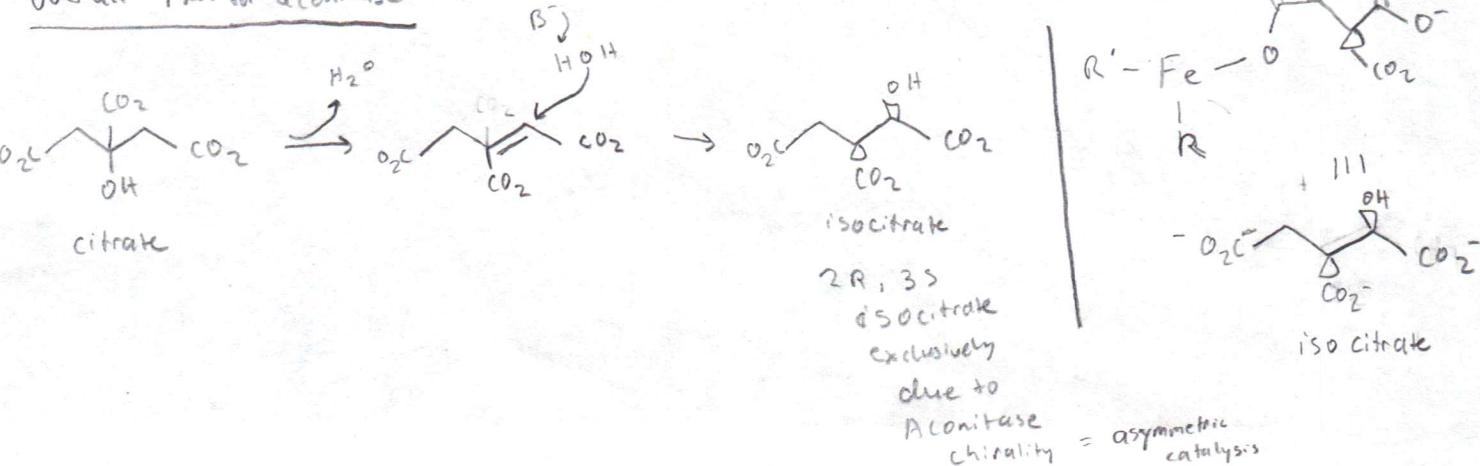
Synthetase - build bond w/ ATP



4 carbons

② Aconitase (isomerase)

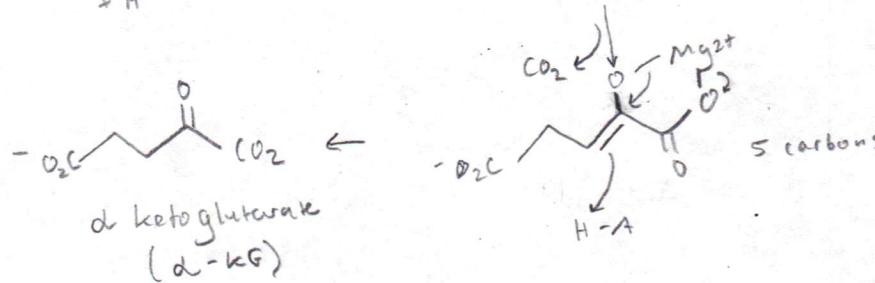
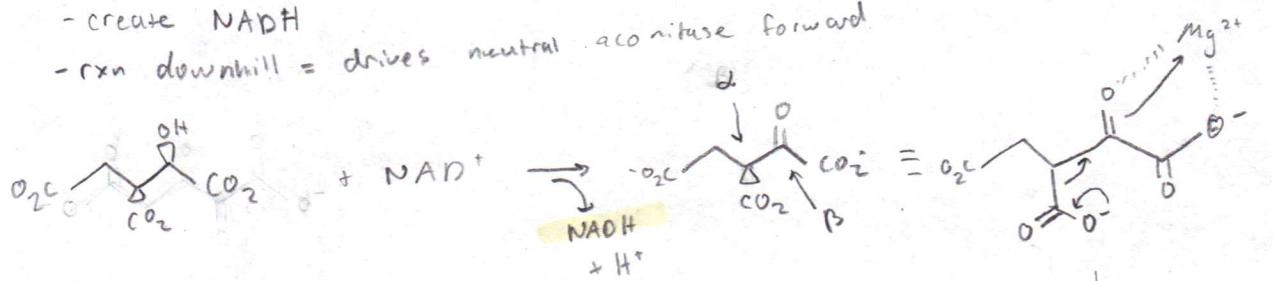
- Citrate too stable for oxidation
- Transfer Alcohol to enable decarboxylation

Overall rxn for aconitase

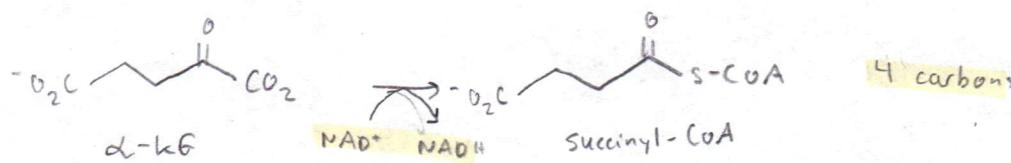
Citric Acid Cycle cont.

3) Isocitrate Dehydrogenase (3)

- oxidize new 2° alcohol from aconitase
- use 2° alcohol to decarboxylate
- create NADH
- rxn downhill = drives neutral aconitase forward

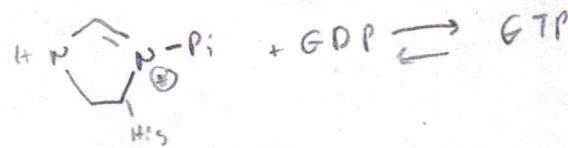
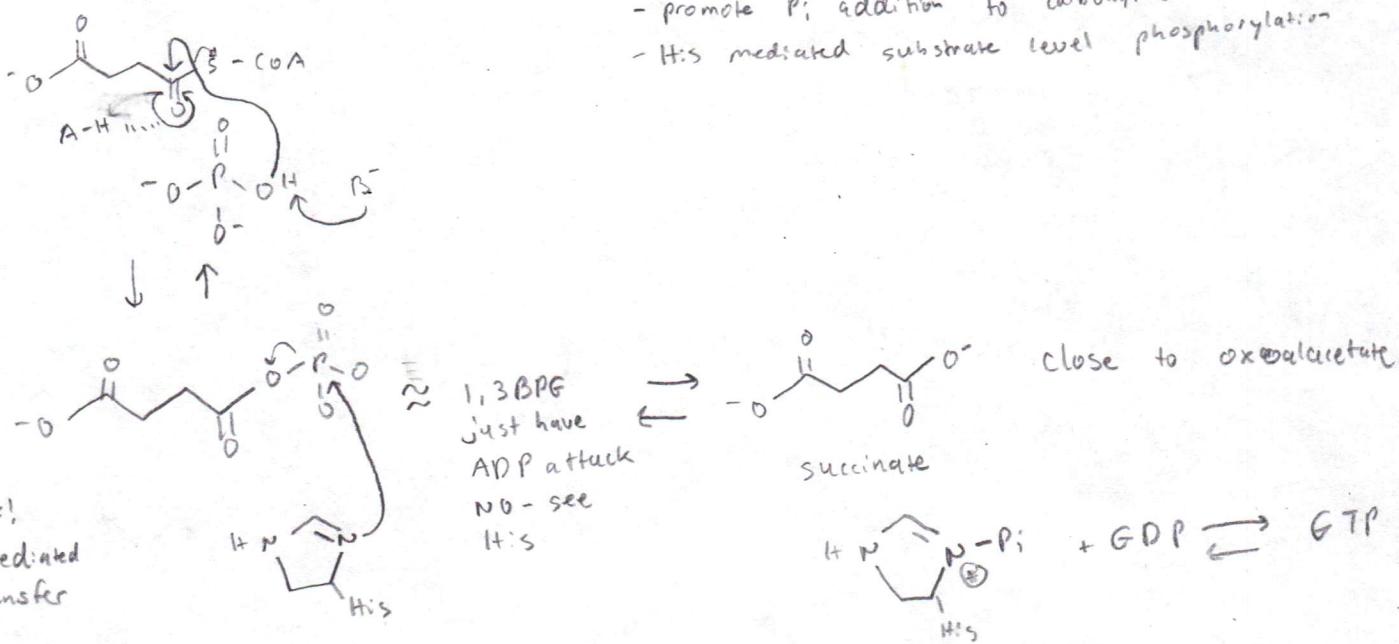
4) $\alpha\text{-KG}$ Dehydrogenase complex

- Analogous to PDH
- Decarboxylate via TPP right end CO_2 , replace w/ HS-COA - follow E1 \rightarrow E2 \rightarrow E3 of PDH



5) Succinyl-CoA Synthetase (ligase)

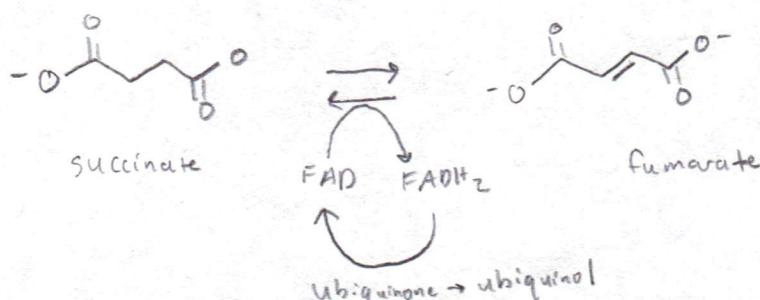
- use energy rich thioester to create GTP
- promote Pi addition to carbonyl
- His mediated substrate level phosphorylation



substrate level phosphorylation!

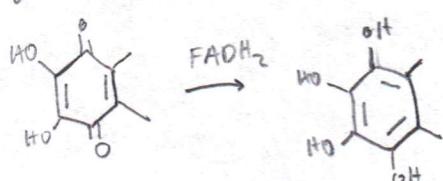
(6) Succinate Dehydrogenase

- Need to regenerate oxaloacetate
- must add C=O in middle of succinate
- create alkene \rightarrow C=O



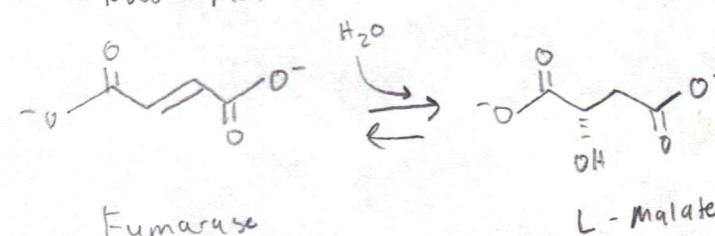
FAD oxidizes succinate to fumarate
but FAD used over NAD⁺ as oxidizing
to an alkene is more difficult
FADH₂ shuttles e⁻ to ubiquinone
to regenerate FAD

Ubiquinone reduced as it makes ring aromatic

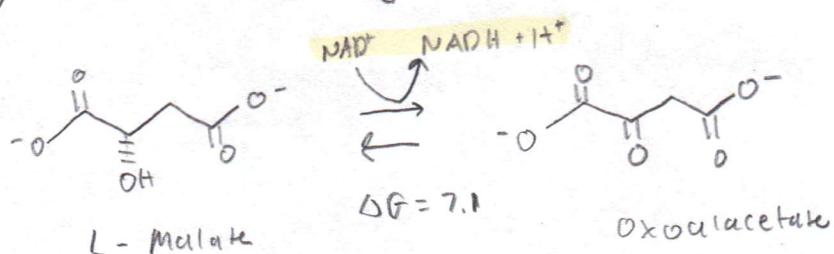


(7) Fumarase (Lyase)

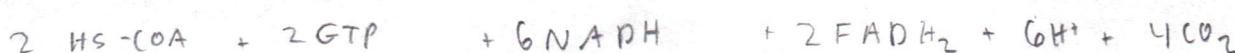
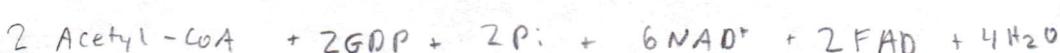
- Now must convert alkene \rightarrow alcohol so we can oxidate to C=O



(8) Malate Dehydrogenase - Regenerate Oxaloacetate



Very endergonic
driven by extremely low [oxaloacetate]

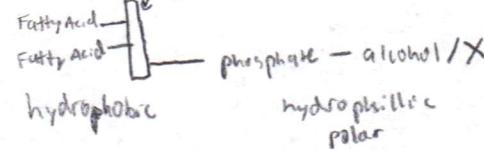
CTA Summary

Fatty Acids

Saturated fats more solid / higher mp

↳ better packing

glycerol

Membranes

ATP needed for assembly / maintenance

of membranes

$$\Delta G = -RT \ln K_c + RT \ln \frac{S_{\text{in}}}{S_{\text{out}}} + zF\Delta V$$

↑
of mols
moving across
must be multiplied

Transition Metals

Lewis Acid Metal
Lewis Base Ligand

Fe [Ar] $4s^2 3d^6$

Fe^{2+} $3d^6$

Fe^{3+} $3d^5$

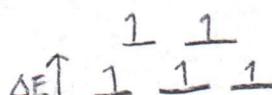
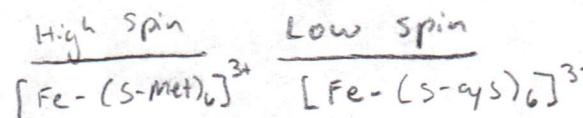
High spin vs low spin

High spin - Fill all

orbitals first, then pair

Low spin - fill low orbitals,

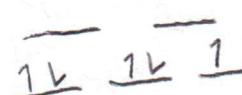
then pair before high



ΔE smaller
than the pairing
energy - move up
before pairing

All e^- spinning

$\Delta E > 0$



Only 1 e^- spinning
 ΔE larger than pairing
energy - pairs before σ^*

$\Delta E < 0$

$\Delta E \uparrow$

Stronger donor means larger ΔE

Fe^{2+} w/ 2 ligands
↳ Octahedral
↳ low spin
 $\text{Fe(OH}_2)_6^{3-}$
↳ high spin

Octahedral - low spin
Tetrahedral - high spin
↳ geometries given
to us

Anion = hard to reduce
b/c already Θ^-

tetrahedral structures high always

weaker donor = weaker bond = more likely to be reduced

stronger donor = stronger bond = bigger ΔE btw σ^* and nonbonding

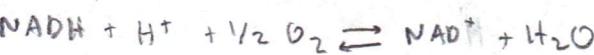
(2)

ETC

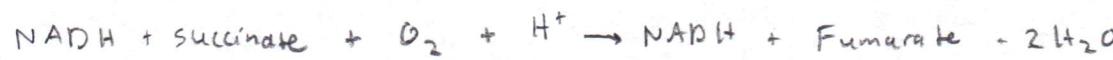
MM pH 7.7

IMS pH 6.8

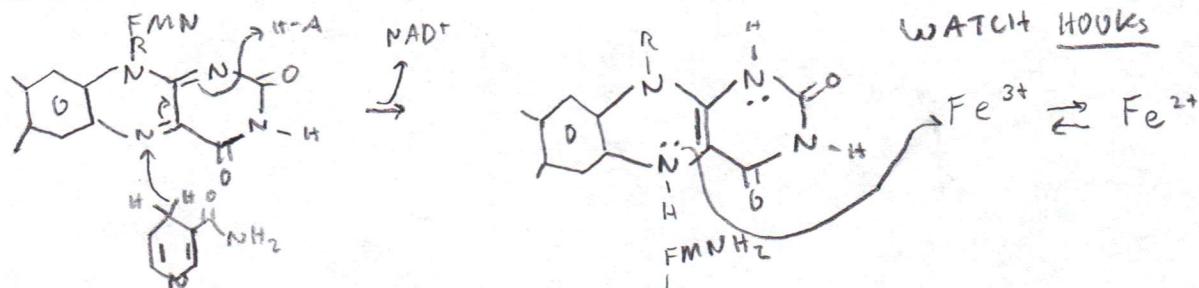
Goal - create H^+ concentration gradient
by expending NADH

Move 12 H^+

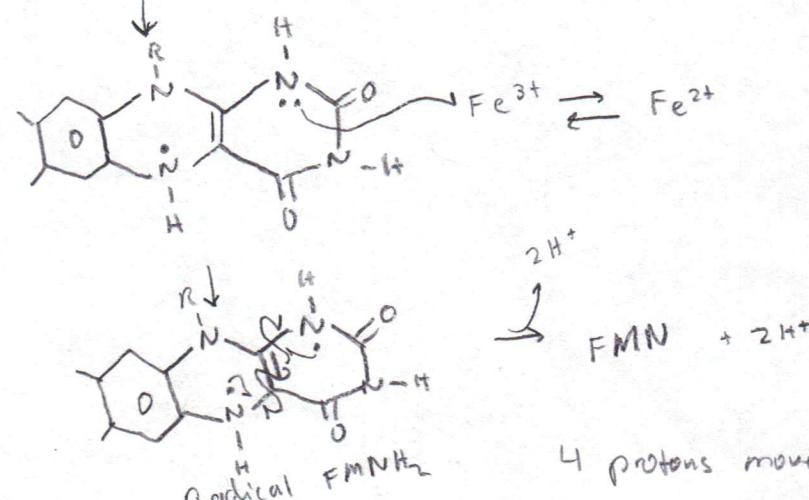
Full rxn:



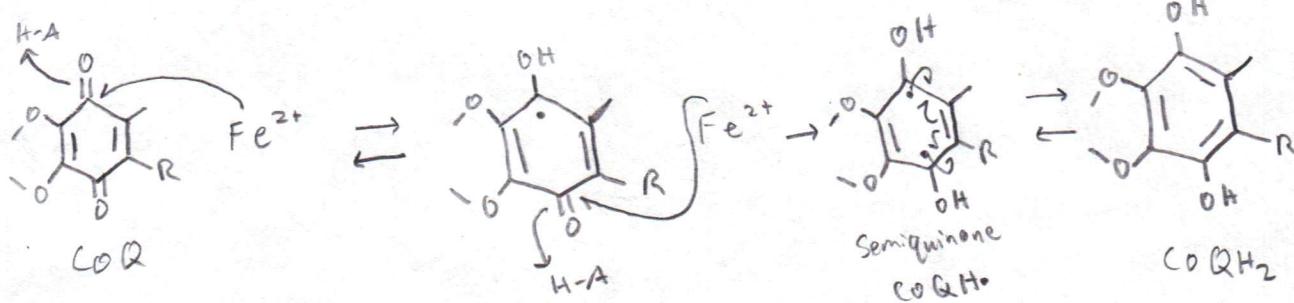
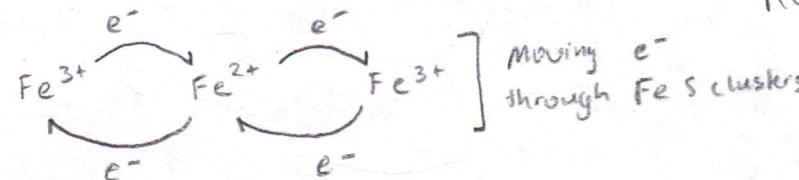
Complex I - Move e^- from NADH to Fe while translocating protons



Also Called NADH Hydrogenase



4 protons move
across membrane
due to conformational
changes

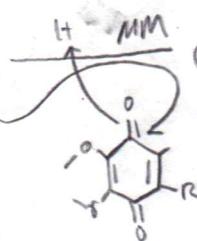
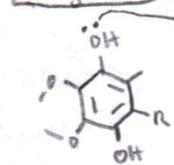
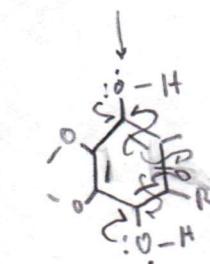
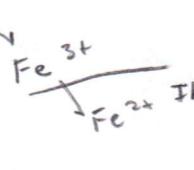
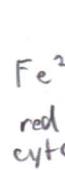
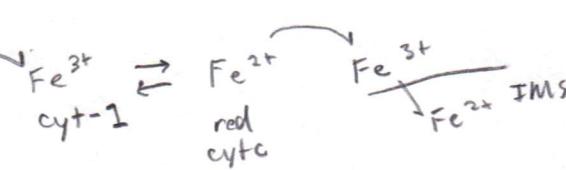
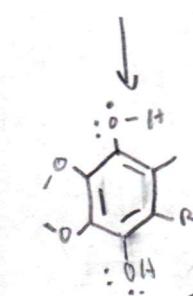
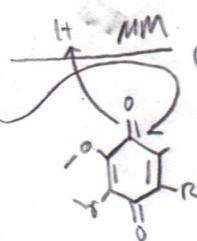
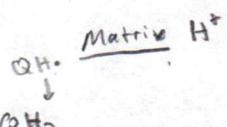
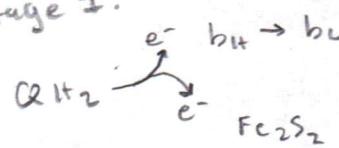
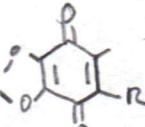
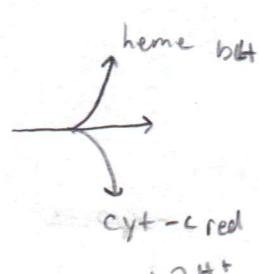
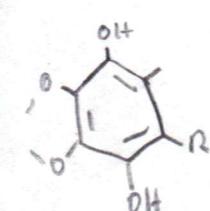


ETC cont.complex 2 - Succinate Dehydrogenase

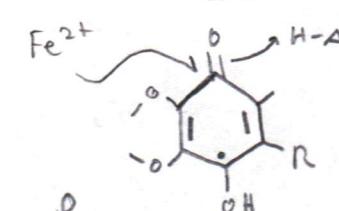
No protons shuttled

Deliver e^- to CoQ pool

Succinate oxidized to fumarate

Reduces $\text{FAD} \rightarrow \text{FADH}_2$ then oxidizedby $\text{CoQ} \rightarrow \text{CoQH}_2$ Complex III - Cytochrome b-c₁Move e^- from CoQH_2 to cyt-c↳ Move 1 e^- at a timeQ cycle - oxidize 2 CoQH_2 and generate 2 Cyt-cReleases protons into IMS w/ oxidation
of CoQH_2 Stage IStage II:Reform CoQ, translocate H^+ 's from $\text{CoQH}_2 \rightarrow \text{IMs}$ Stage 2

cyt-c red

 CoQH_2 from Stage I

$\rightarrow \text{CoQH}_2$
(intermediate)
steps
Complex
I

ETC cont.

complex 4 - cytochrome oxidase

Reduce O_2 to make H_2O

$Fe^{2+}, Fe^{3+}, Fe^{4+} \rightarrow$ low spin

inhibitors prevent binding $COQ/COQH_2$

Strong e^- donors like CN^- decrease
cytochrome favorability $\downarrow \Delta G$

ETC overview

uncharged - don't use $\pm FDV$

Cytochrome c moves e^- from
complex 3 \rightarrow complex 4

Q cycle generates cyt c red

$12 H^+_{MM} \rightarrow 12 H^+_{IMS}$

Problem Solving