C483 Exam 3
Fall2016

Name Key Seat Number _____

Student ID _____

The last page of this exam contains equations and other helpful information.

This exam contains 110 points. The highest score you may earn on this exam is 100 points.

1 _____/20pts

2. _____/10pts

3. _____/20pts

4. _____/10pts

5. _____/10pts

6. _____/10pts

7. _____/10pts

8. _____/10pts

9. _____/10pts

Total:

Regrading: All requests for regrades must be submitted in writing within 48 hours of the return of the exam. You must explicitly state what has been misgraded and why it is an error. The entire exam will be regraded, which could result in points being added or deducted overall.

Section 1: Reading guides (50 points)

1. 20 pts. Fill in the blanks (2 points each.)

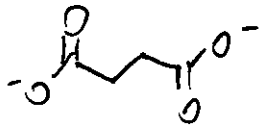
A. Anaplerotic reactions, such as the conversion of pyruvate to oxaloacetate, can serve to increase flux of acetyl CoA through the citric acid cycle.

B. NADH is a cofactor that serves as the major allosteric inhibitor of the citric acid cycle.

C. According to experimental determination, how many ATP can be made using the energy stored in cytoplasmic NADH going through the malate/aspartate shuttle? 2.5

D. FAD/$FADH_2$ (FMN) is an organic cofactor that can accept or donate either 1 or 2 electrons at a time.

E. Hydrolysis of a high energy bond in succinyl CoA leads to formation of this compound: (Draw the structure below.)



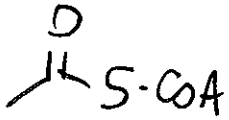
F. The imbalance of protons across the mitochondrial membrane represents a source of free energy, also called the protonmotive force, which can drive the activity of the ATP synthase.

G. The pentose phosphate pathway is used to produce NADPH, which can be used in biosynthesis or detoxification.

H. Ketone bodies are water-soluble fuels made when levels of acetyl CoA rise to high concentrations in the mitochondrial matrix.

I. Cholesterol biosynthesis begins with the synthesis of isopentenyl pyrophosphate from the starting material acetyl CoA.

J. The highly regulated pyruvate dehydrogenase complex requires 5 cofactors to convert pyruvate into this molecule: (Draw structure below.)



2. 10 pts. Write True or False (1 points each)

A. False Transsfer of three carbon atoms from a seven carbon sugar to a three carbon sugar to make a six carbon and four carbon sugar is catalyzed by a transketalase.

B. True Converting glucose to pyruvate through glycolysis involves ten reactions, seven of which are near-equilibrium reactions.

C. True All of the irreversible reactions of glycolysis are catalyzed by kinases.

D. False In glycolysis, the chemical purpose of isomerizing glucose-6-phosphate to fructose-6-phosphate is to allow an oxidation to take place in the next step.

E. False Fermentation reacitons occur so that NADH can be regenerated under anaerobic conditions.

F. True Both the citric acid cycle and β-oxidation of fatty acids have a step in which a membrane-bound enzyme reduces Q to QH_2.

G. True Humans cannot make net glucose starting from fatty acids or ketone bodies.

H. False In the electron transport chain, Compexes I and II pump 4 protons across the inner membrane of the mitochondrion, but Complex IV only pumps 2 protons.

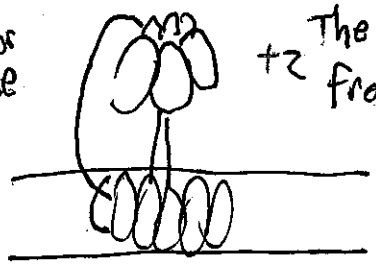
I. True A vitamin B_{12} deficiency would cause an individual to be unable to completely oxidize odd-chain fatty acids.

J. False Genetic abnormalities causing a deficiency in HDL receptors can lead to early death from cardiovascular problems caused by high cholesterol.

3. 20 pts. Short answer (5 points each)

A. Draw a simple schematic of the ATP synthase that accounts for the binding-change mechanism of ATP production:

+1 The stator holds the knob still.



+2 The knob has 3 αβ subunits that change conformation from open to loose to tight to make ATP.

+2 The c-ring has half channels that allow H^+ flow to drive the rotor around inside the knob.

B. Researchers isolated a yeast mutant that was deficient in the enzyme phosphofructokinase. The mutant yeast was able to grow on glycerol as an energy source, but not glucose. Explain why.

+2 With no PFK, glucose cannot be made into F-1,6-bP and then through glycolysis. Glycerol can be made directly into

+3 glyceraldehyde-3-P, though, which bypasses PFK and allows glycolysis to continue.

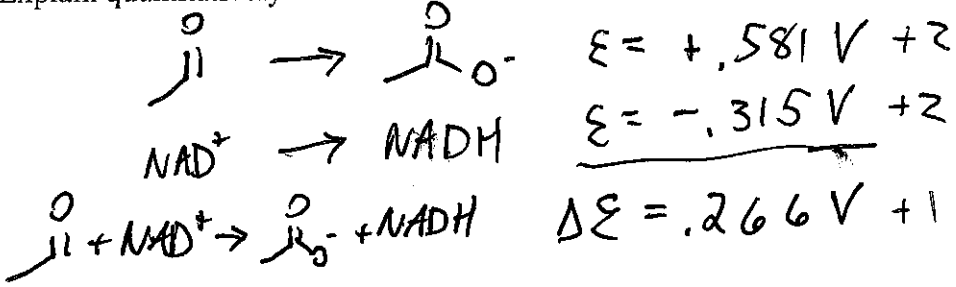
C. Glucose-1,6-bisphophate is an allosteric effector that has the overall effect in muscle of limiting entry of blood glucose into the cell and blocking the pentose phosphate pathway, but activating the breakdown of glycogen for stored glucose to be used in glycolysis. Indicate whether glucose-1,6-bP is an inhibitor or activator of each of these enzymes:

+1 each

Hexokinase: Inhibitor PFK: Activator pyruvate kinase: Activator

Phosphoglucomutase: Activator 6-phosphogluconate DH inhibitor

D. Acetaldehyde may be oxidized to acetate. Would NAD^+ be an effective oxidizing agent? Explain quantitatively.



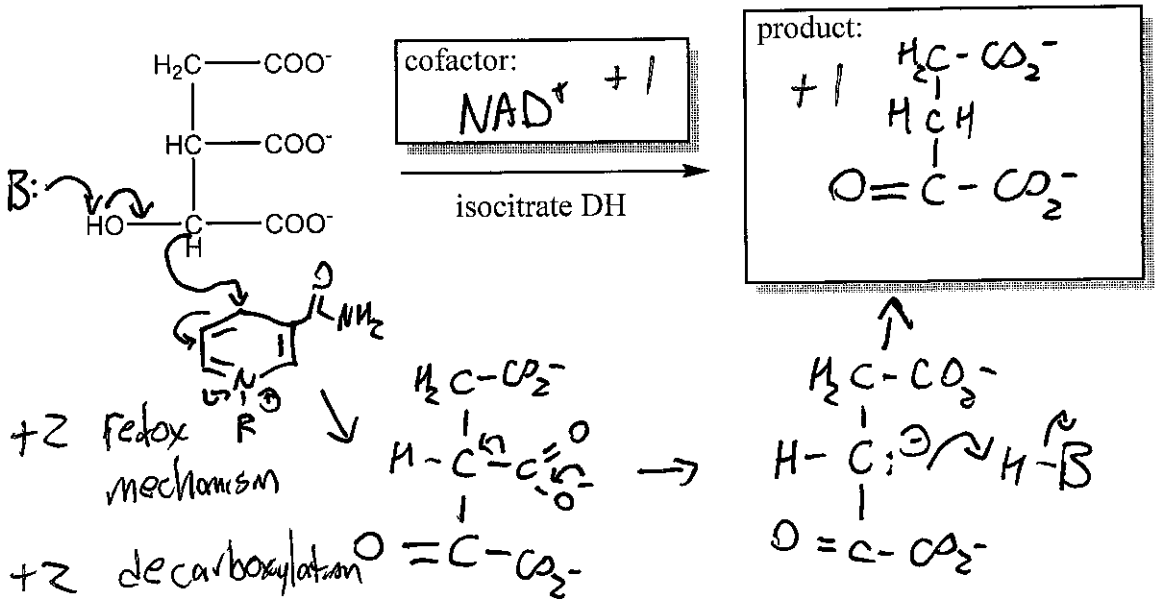
$\varepsilon = +.581\ V$ +2

$NAD^+ \rightarrow NADH$ $\varepsilon = -.315\ V$ +2 yes, This is Spontaneous.

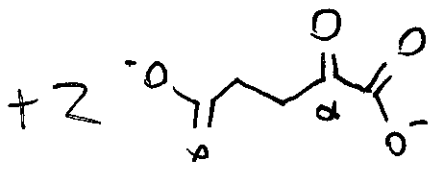
$\Delta\varepsilon = .266\ V$ +1

Section 2: Problems (10 points each)

4. Isocitrate dehydrogenase is a key reaction in the citiric acid cycle. Fill in the necessary cofactor and product of the reaction, then provide a full arrow mechanism for the reaction. Refer to the end of the exam for cofactors. (You may use any necessary general acid/general base.)



Draw the structure of α-ketoglutarate below. Use the structure of the compound to explain why isocitrate dehydrogenase and α-ketoglutarate dehydrogenase have such different mechanisms of action even though they both catalyze an oxidative decarboxylation reaction.



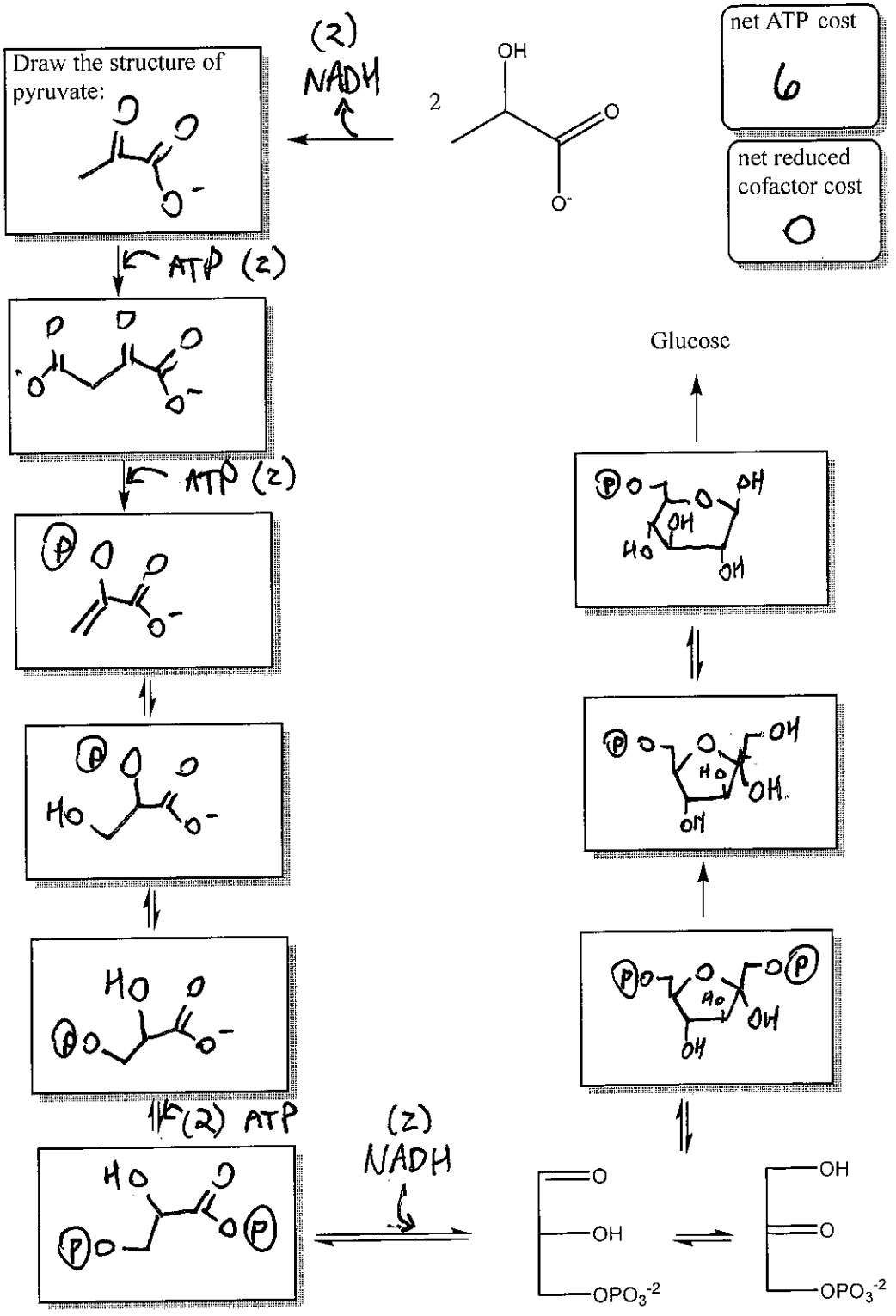
αKG is an α keto acid, so the decarboxylation requires the cofactor TPP.

Pellagra and beriberi are both diseases caused by vitamin deficiencies leading to insufficient cofactors. Pellagra results in inactiveation of both isocitrate DH and α-ketoglutarate DH, but beriberi only inactivates α-ketoglutarate DH. Propose an explanation for this.

+1 Pellagra - deficiency in NAD^+, needed for both enzymes.

+1 Beriberi - deficiency in TPP, only needed in αKG DH.

5. Gluconeogenesis occurs when lactate from the muscle arrives at the liver. In the boxes below, fill in the structures of the compounds on the way from lactate to glucose. Indicate steps that use or produce ATP/GTP and NADH. What is the total cost of ATP equivalents for gluconeogenesis starting with 2 lactate? What is the total cost of reduced cofactors?



+2 (with correct placement in scheme)

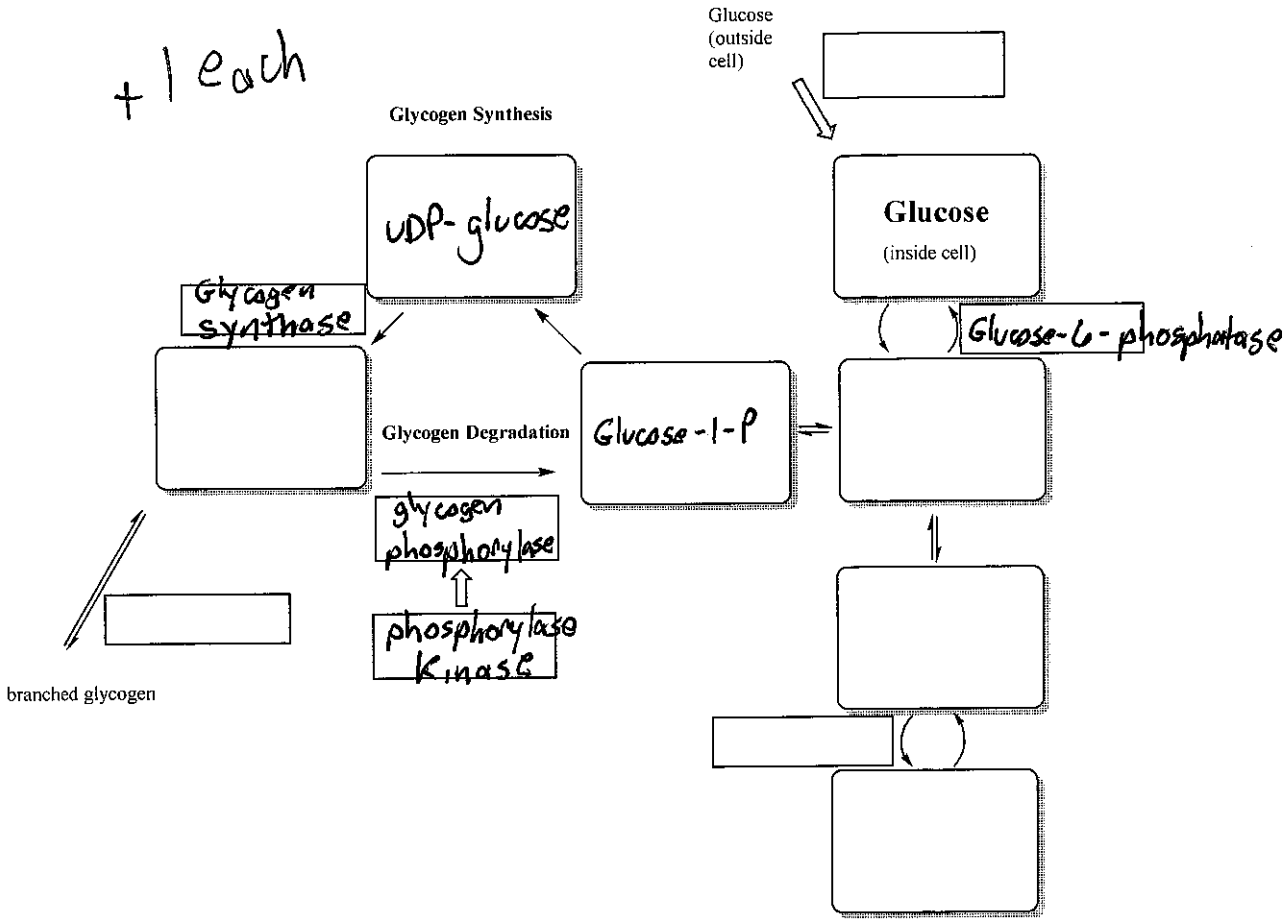
+2 (with correct placement in scheme)

Pathway +6

* one free error
* -1 for every error after that

6. A. Write the following molecule/enzyme names in the appropriate boxes in the glycogen metabolism figure below: UDP-glucose, glycogen phosphorylase, glucose-1-phosphate, glucose-6-phosphatase, phosphorylase kinase, fructose-6-phosphate.

+1 each



B. How many net ATP are made/used in each of these transformations?

From glucose outside the cell to glucose stored in branched glycogen: 2 (made or used?) +1

From glucose stored in branched glycogen to glucose outside the cell: 0 (made or used?) +1

C. Would an individual with a glucose-6-phosphatase deficiency be able to access glycogen stores as a fast fuel source (first 30 seconds) for an anaerobic burst to run from the tiger? Explain.

Yes. Because muscle has its own glycogen storage, a deficiency of this enzyme would not effect the path of retreival to make G-6-P then running G-6-P through glycolysis. +2

7. How many net ATP can be generated in a human muscle cell from the complete oxidation (aerobic conditions) of oleate? (Oleate is an 18-carbon, mono-unsaturated fatty acid commonly found in cooking oil.) Show a full accounting, including all reduced cofactors and substrate level phosphorylations.

★ For 18 carbon fatty acid, 8 rounds of β-oxidation (+1)

8 QH_2 (minus one for unsaturation) (+1) = 7 QH_2 (1.5 ATP) (+1)
8 NADH (2.5 ATP) (+1) > 30.5 ATP

★ Nine (+1) acetyl CoA through CAC

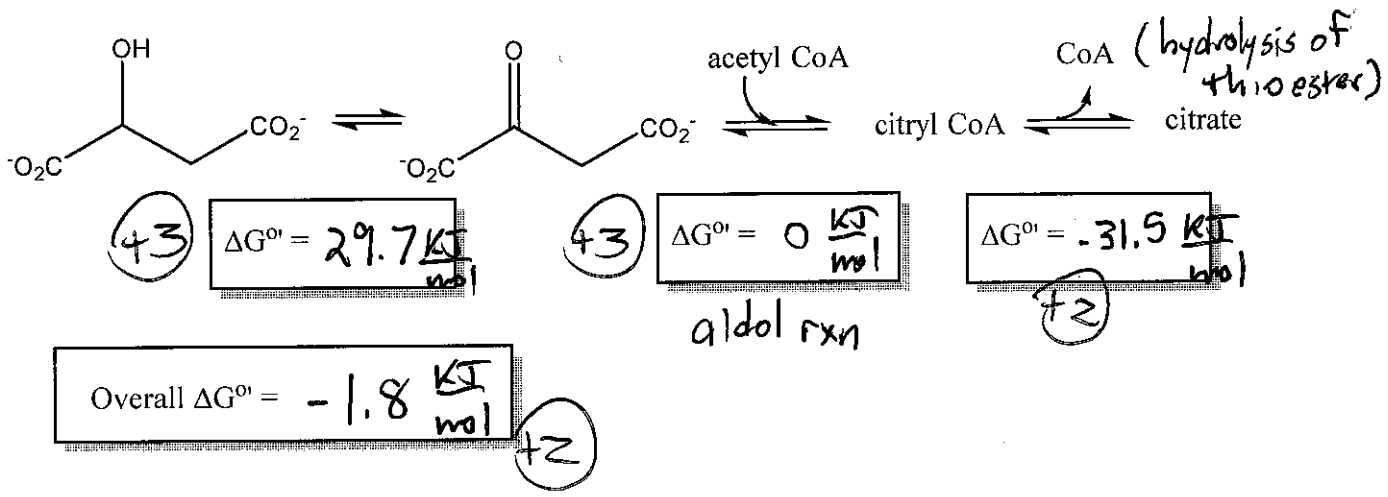
3 NADH (2.5) (+1)
1 QH_2 (1.5) (+1) = 10 ATP (9) = 90 ATP
1 substrate level (+1)

★ Activation of fatty acid = -2 ATP equivalents (+2)

Net = 90 + 30.5 - 2 = **118.5 ATP**

8. The citric acid cycle is driven forward thermodynamically by the coupling of the last step of the cycle back to the first step, which is catalyzed by citrate synthase in two phases. The overall coupled reaction is shown below. Indicate the standard free energy of each step in the process, and then calculate the standard free energy of this coupled reaction. Write your final answers in the boxes with the figure, but show all work for full credit.

A. The equilibrium ration of malate to oxaloacetate under standard conditions is 1.61×10^5.
B. Under standard conditions hydrolysis of a thioester has a change in free energy of about -31.5 kJ/mol.
C. The aldol reaction has an equilibrium constant of about 1.



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

$$= -8.314 \frac{J}{mol\,K} (298\,K) \ln \frac{1}{1.61 \times 10^5} = +29.7 \frac{KJ}{mol}$$

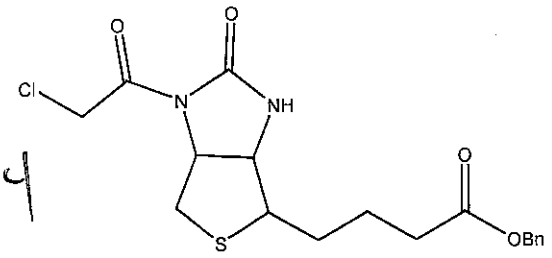
9. Section 3: Case study (10pts) A research team is proposing a series of strategies for treating obesity. Explain the biochemical basis of how each strategy would work.

A. Small doses of p-nitrophenol, a known uncoupler, would increase the amount of fatty acids consumed to meet daily energy needs.

An uncoupler acts to dissipate the proton gradient created by the e⁻ transport chain with no ATP production. Therefore, more fuel would need to be oxidized to make the same amount of ATP.

+3

B. The cofactor mimic (shown below) would decrease fatty acid synthesis.



+4

This is a biotin mimic. It would inhibit acetyl CoA carboxylase, which is the activation step of Fatty acid Synthesis. If no malonyl CoA is made, no fats can be synthesized.

C. Gene therapy to introduce a mutant ATP synthase with a c-ring with 12 subunits.

+3 This c-ring is less efficient. 12 H^+ would be needed to make three ATP rather than 8 H^+ for a typical mammalian cell. Therefore, more fuel would need to be oxidized to make the same amount of ATP.

Data Tables and scratch work

Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP → AMP + PP_i	−45.6
Phosphocreatine	−43.1
ATP → ADP + P_i	−30.5
Glucose-1-phosphate	−20.9
PP_i → 2 P_i	−19.2
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

[TABLE 15-1] Standard Reduction Potentials of Some Biological Substances

Half-Reaction	$E^{\circ\prime}$ (V)
	0.815
	0.48
	0.42
Cytochrome a_3 (Fe^{3+}) + e^- ⇌ cytochrome a_3 (Fe^{2+})	0.385
Cytochrome a (Fe^{3+}) + e^- ⇌ cytochrome a (Fe^{2+})	0.29
Cytochrome c (Fe^{3+}) + e^- ⇌ cytochrome c (Fe^{2+})	0.235
Cytochrome c_1 (Fe^{3+}) + e^- ⇌ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b (Fe^{3+}) + e^- ⇌ cytochrome b (Fe^{2+}) (*mitochondrial*)	0.077
Ubiquinone + 2 H^+ + 2 e^- ⇌ ubiquinol	0.045
Fumarate$^-$ + 2 H^+ + 2 e^- ⇌ succinate$^-$	0.031
FAD + 2 H^+ + 2 e^- ⇌ $FADH_2$ (*in flavoproteins*)	~ 0.
Oxaloacetate$^-$ + 2 H^+ + 2 e^- ⇌ malate$^-$	− 0.166
Pyruvate$^-$ + 2 H^+ + 2 e^- ⇌ lactate$^-$	− 0.185
Acetaldehyde + 2 H^+ + 2 e^- ⇌ ethanol	− 0.197
S + 2 H^+ + 2 e^- ⇌ H_2S	− 0.23
Lipoic acid + 2 H^+ + 2 e^- ⇌ dihydrolipoic acid	− 0.29
NAD^+ + H^+ + 2 e^- ⇌ NADH	− 0.315
$NADP^+$ + H^+ + 2 e^- ⇌ NADPH	− 0.320
Acetoacetate$^-$ + 2 H^+ + 2 e^- ⇌ 3-hydroxybutyrate$^-$	− 0.346
Acetate$^-$ + 3 H^+ + 2 e^- ⇌ acetaldehyde + H_2O	− 0.581

Source: Mostly from Loach, P. A., in Fasman, G. D. (ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. I, pp. 123–130, CRC Press (1976).

$$\Delta G = RT \ln\frac{[X]final}{[X]initial} + ZF\Delta\psi \qquad \Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$$

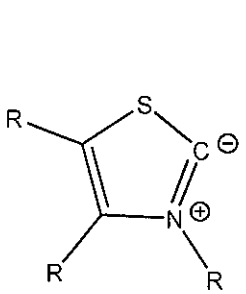
$R = 8.314\ J/\ mol \cdot K$

$F = 96{,}485\ J/V \cdot mol$

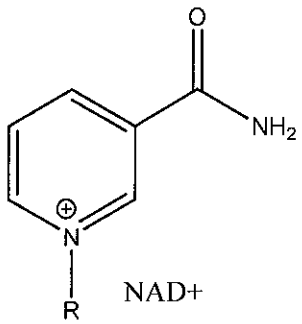
$\Delta G^{\circ\prime} = -RT \ln K_{eq}$

$$\Delta G_{reaction} = \Delta G^{\circ\prime}{}_{reaction} + RT \ln\frac{[products]}{[reactants]}$$

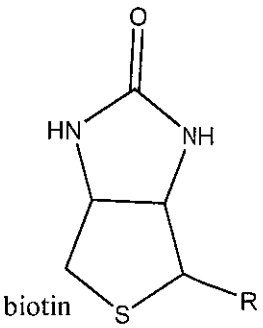
Cofactor Structures:



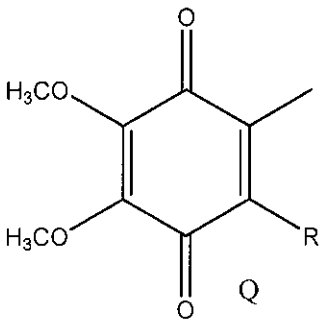
TPP



NAD+



biotin



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