C483 Exam 3
Summer 2017

Name Key Seat Number __________

Student ID ____________________

Page 11 of this exam contains equations and other helpful information. Page 12 is scratch paper; nothing on this page will be graded.

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. In the glycolysis pathway, starting from glucose and ending at pyruvate, a total of 2 ATP are invested and 4 ATP are produced so that the overall net production in the pathway is 2 ATP.

B. F-2,6-bP is the most potent allosteric effector of phosphofructokinase (PFK) in humans.

C. Increasing the concentration of citric acid cycle intermediates increases flux through the pathway.

D. NADH is reoxidized to NAD$^+$ by yeast under anaerobic conditions by reaction with pyruvate. (acetaldehyde)

E. Glycogen phosphorylase doesn't release glucose from glycogen by hydrolysis, but rather by phosphorolysis

F. A large percentage of glucose is metabolized in the liver through the pentose phosphate pathway to produce NADPH.

G. Complex I catalyzes transfer of high energy electrons from NADH to Q through a series of redox centers.

H. Complex II, from the citric acid cycle, adds to the Q pool but does not transport any protons across the membrane.

I. ATP synthase is a molecular motor embedded in the inner membrane of the mitochondria that utilizes the protonmotive force to make ATP.

J. Reciprocal regulation allows one pathway to be turned on when its metabolically opposing pathway is simultaneously turned off.

2. 10 pts. True or false (1 points each)

A. True The reaction catalyzed by hexokinase is made thermodynamically irreversible through the breaking of one high energy phosphoanhydride bond.

B. True The net cost for bring glucose into a liver cell, storing it in glycogen, and then releasing it back to the bloodstream is 2 ATP.

C. False Chemically, the reaction catalyzed by α-ketogluterate dehydrogenase is more like isocitrate dehydrogenase than pyruvate dehydrogenase.

D. False Although not possible in mammals, the glyoxylate pathway in plants allows pyruvate to be made into net glucose.

E. True Transketolases stabilize acyl anions that allow for 2-carbon transfers in the nonoxidative phase of the pentose phosphate pathway.

F. True Two of the irreversible steps in the citric acid cycle are catalyzed by dehydrogenases.

G. False The citrate transport system is utilized to carry acetyl CoA out of the matrix so that it can be made into glucose.

H. False A negative change in standard reduction potential means that a reaction will spontaneously transfer electrons under standard conditions.

I. False Oxidative phosphorylation is another term for substrate-level phosphorylation.

J. False The near equilibrium reactions of glycolysis all have ΔG°' values close to zero kJ.

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

A. Write the net reaction of glycolysis, starting from glucose and ending at pyruvate (ignore water and protons.) Underneath, write the net reaction of gluconeogenesis, starting from pyruvate and ending with glucose. What is the net reaction for a molecule of glucose being transformed into pyruvate and then back into glucose?

(+2) Glucose + 2 NAD^+ + 2ADP + 2 P_i → 2 pyruvate + 2ATP + 2NADH

(+2) 2 pyruvate + 2NADH + $6\,\underset{(eq)}{ATP}$ → Glucose + 2NAD^+ + 6 ADP + 6 P_i

(+1) 4 ATP → 4 ADP + 4 P_i

B. Write the name of each cofactor. In one case, write "none."

Q/QH_2 Organic redox reagent that carries two electrons but accepts and donates one at a time

TPP Catalytic cofactor required in decarboxylation of α-ketoacids

$NAD^+/NADH$ Stoichiometric redox cofactor in the pyruvate dehydrogenase complex

CoA Hydrolysis releases this cofactor in formation of citrate

none Utilized by transaldolase enzyme

C. What is the *in vivo* P:O ratio for NADH oxidation in mammals? Show how you came to this number.

$$\left(\frac{3\ ATP}{8\ H^+}\right)\left(\frac{10\ H^+}{NADH}\right)\left(\frac{NADH}{\frac{1}{2}O_2}\right) = 3.7\ \frac{ATP}{\frac{1}{2}O_2}$$

D. Show a calculation of change in standard reduction potential that explains why succinate is not oxidized to fumarate using NAD^+ as a cofactor.

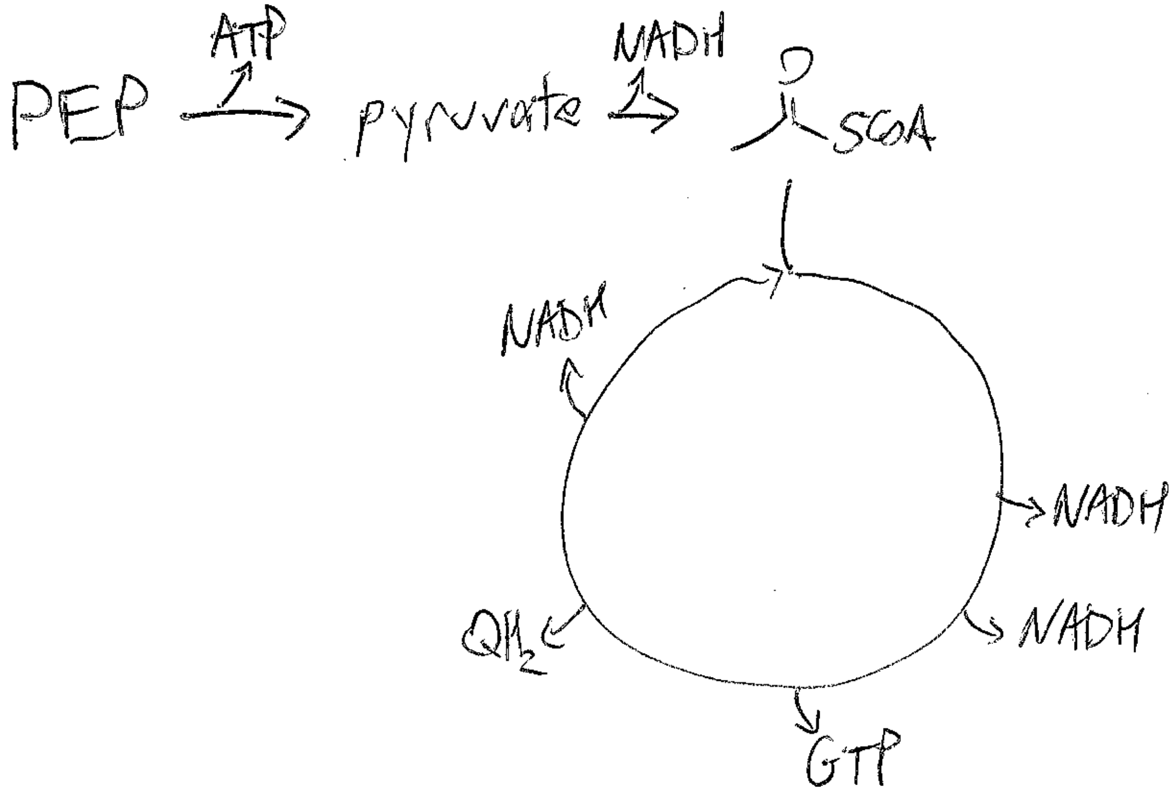
$$\text{Succinate} \longrightarrow \text{Fumarate} \qquad \Delta E^{\circ\prime} = +0.031$$

$$NAD^+ \longrightarrow NADH \qquad E^{\circ\prime} = -0.315$$

$$\text{Succinate} + NAD^+ \longrightarrow \text{fumarate} + NADH \qquad \Delta E^{\circ\prime} = -0.284$$

Section 2: Problems (10 points each)

4. What is the net number of NTP produced when one molecule of PEP is completely oxidized? (Assume fully active citric acid cycle.) For full credit, outline the pathway necessary for this transformation/storage and indicate which steps require NTP input.



Total Net NTP:

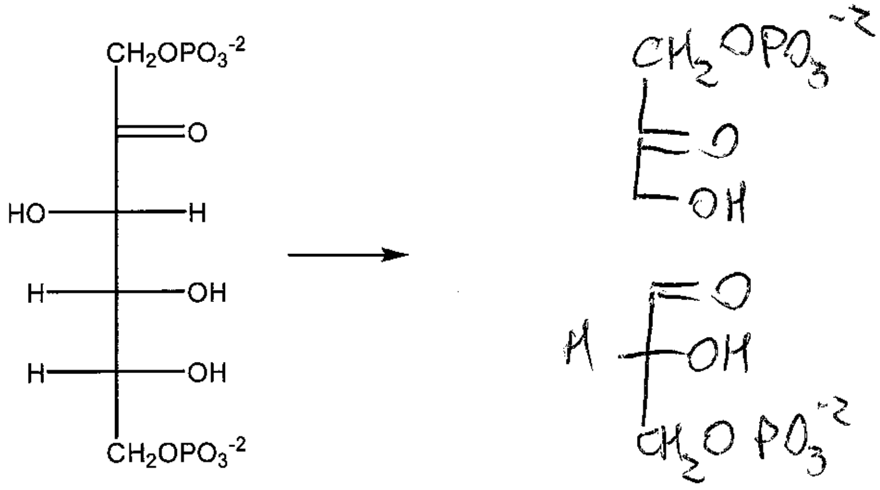
2 substrate level = 2

4 NADH $\left(2.5 \frac{ATP}{NADH}\right)$ = 10

1 QH_2 $\left(1.5 \frac{ATP}{QH_2}\right)$ = 1.5

$\boxed{13.5 \text{ net}}$

5. A. Write the products of the reaction catalyzed by aldolase:



+ 4

B. Drawn an enzyme-mediated reaction for the aldolase reaction. What is the role of lysine in this catalyzed reaction?

Based on Fig. 13.4

+6

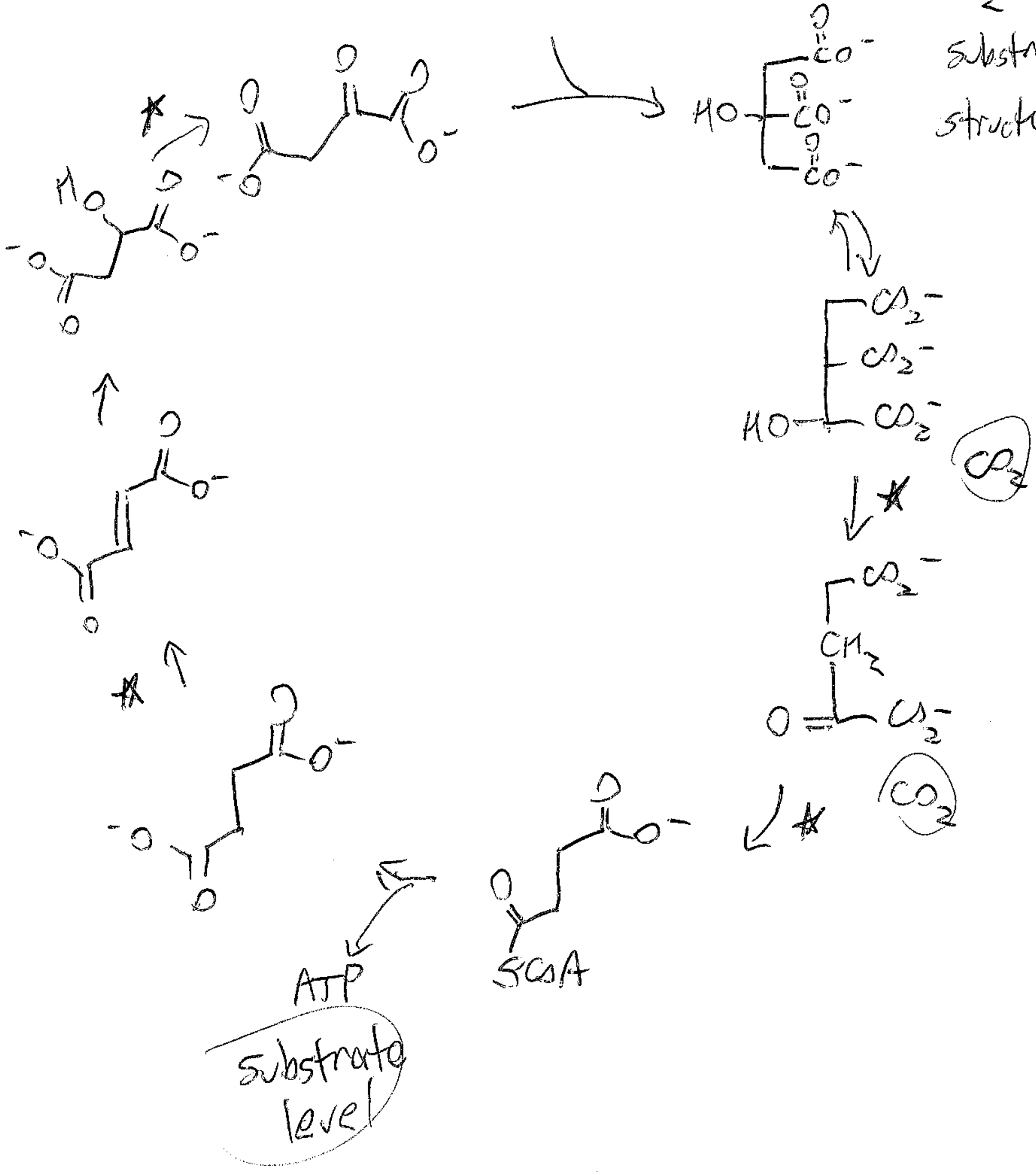
6. Draw the structures of the eight intermediates of the citric acid cycle. Mark the reaction(s) that produce carbon dioxide. Mark reaction(s) that require redox cofactors. Mark the reaction(s) that do substrate level phosphorylation.

Redox +2

CO_2 +1

substrate +1

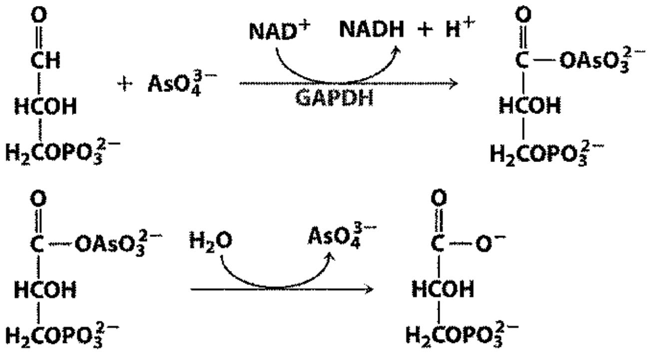
structures +6



problem 13.17

8. Arsenate acts as a phosphate analog and can replace phosphate in the GAPDH reaction. The product of this reaction is 1-arseno-3-phsophoglycerate. It is unstable and spontaneously hydrolyzes to form 3-phosphoglycerate as shown below. What is the effect of arsenate on cells undergoing glycolysis?

+6



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- Anaerobic glycolysis would net zero ATP.

- This step is typically the production of the high energy bond necessary for substrate phosphorylation, but now 1,3-bP glycerate Kinase gets bypassed.

Problem 13.21

Vanadate (VO_4^{-3}) inhibits GAPDH, not be acting as a phosphate analog, but by interacting with essential –SH groups on the enzyme. What happens to cellular levels of phosphate and ATP when red blood cells are incubated with vanadate?

+4

This inhibits the pathway that catalyzes the overall rxn

$$ADP + P_i \rightarrow ATP$$

so $[ATP] \downarrow$ and $[P_i] \uparrow$.

problem 13.63

Section 3: Case study (10pts) Several studies have shown that the metabolite glucose-1,6-bisphosphate (G16bP) regulates several pathways of carbohydrate metabolism by inhibiting or activating key enzymes. The effect of G16bP on several important enzymes is summarized in the table below. (Phosphoglucomutase catalyzes the interconversion of glucose-1-P and glucose-6-P.) What pathways are active when G16bP is present? What pathways are inactive? What is the overall effect on the cell?

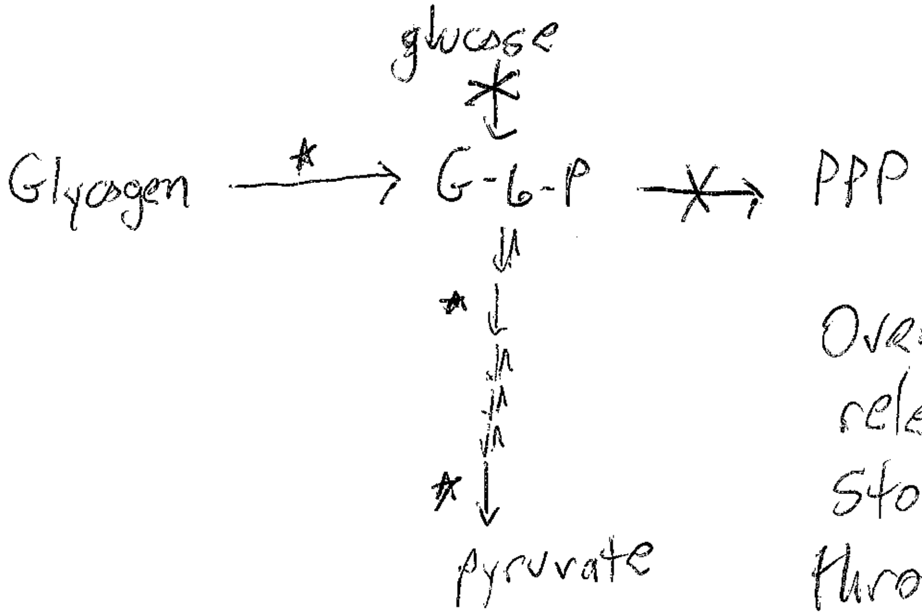
Enzyme	**Effect of G16bP**
Hexokinase	Inhibits
PFK	Activates
Pyruvate kinase	Activates
Phosphoglucomutase	Activates
Glucose-6-P dehydrogenase	Inhibits

① Hexokinase inhibition - shuts down process that brings extracellular glucose into cell

② Glycolysis is activated (PFK, pyruvate kinase)

③ Glycogen metabolism activated

④ PPP shut down (G-6-P DH)



Overall, glucose is released from storage and run through glycolysis

Data Tables and equations:

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	$\mathcal{E}^{\circ\prime}$(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).

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

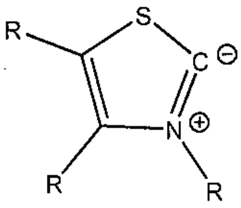
$$R = 8.314\ \text{J/ mol}\cdot\text{K} \qquad \mathcal{E} = \mathcal{E}^{\circ\prime} - \frac{RT}{nF}\ln\frac{A(reduced)}{A(oxidized)}$$

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

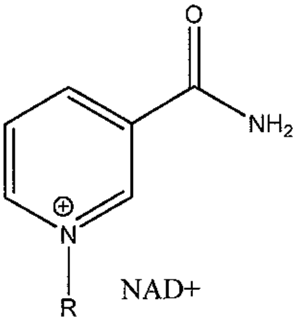
$$\Delta G^{\circ\prime} = -RT \ln K_{eq}$$

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

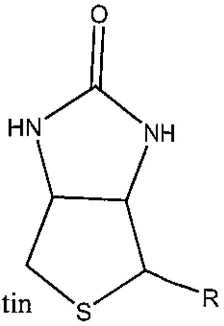
Cofactor Structures:



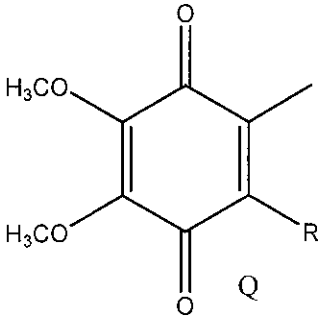
TPP



NAD+



biotin



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