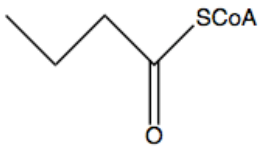
Exam II **Name:**_____

Complete five of the six following problems. One problem will not be graded. Indicate which problem you do not want graded by crossing it off this point list.

1. _____/20 pts
2. _____/20 pts
3. _____/20 pts
4. _____/20 pts
5. _____/20 pts
6. _____/20 pts

1. (20 pts) If it were possible to label glucose with the traceable radioisotope ^{14}C at any position or combination of positions, which labeled carbon or carbons would result in the *most rapid* appearance of label in CO_2 if it were metabolized aerobically? Draw out (using chemical structures) the reaction where this CO_2 is produced.

2. (20 pts) Imagine that a cycle similar to the citric acid cycle exists that completely oxidizes the following molecule (instead of acetyl-CoA) to CO_2:



i) How many steps of this similar cycle would produce CO_2?

ii) How many steps of this similar cycle would produce GTP?

iii) How many steps of this similar cycle would be oxidation/reduction rxns?

3. (20 pts) Draw out (using chemical structures; and naming reactants/products/enzymes) an example of a reaction from the citric acid cycle that produces each of the following:

CO_2

NADH

(3 con't)
CoASH

$FADH_2$

4. (20 pts) Oligomycin inhibits mitochondrial ATP synthase. Cyanide inhibits mitochondrial Complex IV. Imagine that you treated two mitochondrial preparations with these inhibitors (one with oligomycin and one with cyanide). Unfortunately, you did not label the test tubes and are not certain which tube contains which inhibitor. You decide that it would be awesome fun to experimentally determine which tube is which! Besides the inhibited mitochondrial preparations, you also have stock solutions of succinate and dinitrophenol (DNP, an uncoupler). With **only** these solutions and any readily available lab instrument (i.e. thermometer; UV-Vis spectrophotometer; oxygen sensor; water bath, automatic pipettes), design an experiment to unambiguously determine the identity of the inhibitor in each test tube. Be very explicit about what the expected result for each inhibitor is in your design.

5. (20 pts) Indicate the direction which protons are pumped across the following "membrane" cartoons by drawing an arrow. Indicate the driving force(s) behind each pumping process by adding to each cartoon.

Outer membrane _____
_____

Intermembrane
space

Inner membrane_____
_____

Stroma

Thylakoid membrane_____
_____

Thylakoid lumen/matrix

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periplasm

plasma membrane_____
_____

cytoplasm

6. (20 pts) Determining the concentration of intermediates in biochemical pathways (metabolites) within a cell can be very difficult. Imagine that you isolate the cytoplasm and remove all endogenous proteins. You would like to determine the concentration of glucose-6-phosphate using the OceanOptic UV spectrophotometers ($\varepsilon_{340\text{ nm}}^{NADH} = 6.22x10^{3}\ \text{M}^{-1}\ \text{cm}^{-1}$) and the following reaction catalyzed by glucose-6-phosphate dehydrogenase:

$$\text{glucose-6-phosphate} + \text{NAD}^{+} \Leftrightarrow \text{gluconate-6-phosphate} + \text{NADH}.$$

You allow these solutions to come to equilibrium and take the following observations:

#1

125 uL	5.0 mM glucose-6-phosphate
25 uL	1xTBE, pH 8.0
725 uL	dH_2O
25 uL	4.0 mM NAD^+
100 uL	glucose-6-phosphate dehydrogenase

$A_{340\text{ nm}}^{\text{equilibrium}} = 0.22$

#2

125 uL	Cytoplasm isolated solution
25 uL	1xTBE, pH 8.0
725 uL	dH_2O
25 uL	4.0 mM NAD^+
100 uL	glucose-6-phosphate dehydrogenase

$A_{340\text{ nm}}^{\text{equilibrium}} = 0.14$

What is the concentration of isocitrate in the cytoplasm?