1. (12) Definitions. In the context of this course, distinguish between the following:

**NAD⁺ vs. FAD**

NAD⁺ is a freely diffusible electron carrier that is used often in fermentation and in respiration, where it carries 2 electrons to the membrane-bound electron transport chain. FAD is always enzyme bound, often to membrane proteins, has a lower (more positive) midpoint potential for reduction and is used where reduction of NAD⁺ would be non-favorable.

Succinate dehydrogenase vs. Fumarate reductase

\[ \text{`OOC-CH=CH-COO` (fumarate) + 2e⁻ + 2H⁺} \rightarrow \text{`OOC-CH₂CH₂-COO` (succinate)} \]

SDH is used aerobically during respiration in the TCA cycle, transferring electrons to FAD and into the quinone pool. FRD is expressed only anaerobically, and accepts electrons from menaquinone when fumarate is being used as an electron acceptor.

Glycerol-3-phosphate dehydrogenase vs. Glyceraldehyde-3-phosphate dehydrogenase

Glycerol-3-P DH is used during the fermentation (cytoplasmic) or respiration (membrane-bound) of glycerol; in both cases, electrons are transferred to FAD. Glyceraldehyde-3-P DH is a glycolytic enzyme the couples electron transfer to NAD⁺ to the incorporation of inorganic phosphate : substrate-level phosphorylation.

**ΔG vs. ΔG⁰**

ΔG⁰ is the free energy of a chemical reaction at standard conditions, which include 25 °C, 1 molar or 1 atmosphere concentration of reactants. ΔG⁰ is the free energy of a chemical reaction under non-standard conditions, which often include varying concentrations of reactants. One may think of a ΔG as a ΔG⁰ corrected for the physiological concentrations of reactants.
2. (10 Points). Quinones. For words in parentheses, circle the correct answer and cross out the incorrect answer. Fill in the blanks with a valid name or number. IN some cases, more than one answer may be valid. In other cases, not so much.

A.
During a full quinine cycle, the molecular ubiquinone can be (reduced) by 2__ electrons and binds 2__ H⁺ ions to form ubiquinol at the membrane bound enzyme __e.g., NADH dehydrogenase_____ on the (cytoplasmic) side of the membrane. Ubiquinol diffuses through the lipid bilayer where it can (reduce) the enzyme _____e.g., Cytochrome oxidase_____. There, 2__ electrons are donated and 2__ H⁺ ions are released into the (periplasm).

Considering the same pair of enzymes as electron donors and recipients, the semiquinone can be (reduced) by 1__ electrons and bind 2__ H⁺ ions to form ubiquinol. Ubiquinol diffuses through the lipid bilayer where 1__ electrons are donated and 2__ H⁺ ions are released.

B.
Semiquinone carries a charge of __________-1___________
Ubiquinone carries a charge of __________0_____________
Ubiquinol carries a charge of __________0_____________
Menaquinone carries a charge of __________0_____________
3. (10 points) Lactate. In the fermentation of lactate, D-lactate is oxidized to form acetate, allowing ATP synthesis. L-lactate is dehydrated to form acrylyl-CoA. Why was this dehydration (loss of water) performed? To balance the fermentation of 1 mole of lactate to start with, how many moles of acetate are formed?

The dehydration forms a compound that can accept the electron pairs generated by lactate oxidation.

Since 2 oxidations occur to allow acetyl-CoA to form acetate, 2 reductions must occur. 1/3 mole of acetate is produced from 1 mole lactate, while 2/3 mole of the substrate is used as an electron acceptor.
4. (5 points) $\Delta \mu$. Describe the relative contributions of $\Delta \Psi$ and $\Delta p$H towards $\Delta \mu$ under (a) Acidic conditions and (b) Alkaline conditions.

Under acidic conditions, $\Delta p$H contributes strongly to creating $\Delta \mu$; $\Delta \Psi$ either provides a small contribution or detracts from $\Delta \mu$ if $\Delta p$H is too large, whereby the energy in $\Delta \mu$ would exceed the energy of ATP hydrolysis. Under alkaline conditions the opposite is true, where $\Delta \Psi$ contributes entirely to creating $\Delta \mu$. Since the external pH is > 7.0 under alkaline conditions, $\Delta p$H can only detract from $\Delta \mu$.

5. (10 points) Butyrate Fermentation. In precise physiological and thermodynamic terms, explain exactly, step-by-step, why a butyrate fermenter can not grow at pH 4.5. Consider the first step as follows:

- In the cytoplasm, at pH 6.5, butyrate is formed as an anion (a negatively charged molecule)
- Butyrate is excreted out of the cell.
- Since the pKa of butyrate is > 4.5, the molecule becomes protonated and uncharged.
- As an organic molecule, uncharged butyrate is free to diffuse through the lipid bilayer and into the cytoplasm.
- In the cytoplasm, the pH is > pKa of butyrate, and a hydrogen ion is released into the cytoplasm.
- Charged butyrate is excreted as above and the cycle repeats.
- The influx of hydrogen ions caused a depletion in membrane potential ($\Delta \mu$)
- Therefore, the $F_0F_1$ ATPase will hydrolyze ATP to export hydrogen ions since the positive $\Delta G$ of $H^+$ efflux through this enzyme is smaller than the energy released by ATP hydrolysis.
- ATP pools will be consumed until the concentration of ATP is so low that $H^+$ ions cannot be extruded
- The cell will lack energy and perish.
6. (10 points) Kryptonite.

You are working at *Metropolis Biochemicals*, a huge multinational corporation that specializes in microbial detoxification of chemical spills. As a side project, you have been looking for mutants of *Bacillus subtilis* that oxidize kryptonite, rendering it harmless as kryptonate. You have discovered that the half reaction for kryptonite oxidation is:

\[
\text{Kryptonite} \rightarrow \text{Kryptonate} + 2e^- + 2H^+ \quad E_{m,7} = +380 \text{ mV}
\]

A. Will NADH serve as a good electron donor (that is, will this reaction have a negative \(\Delta G\))? Show the balanced equation and calculation of the \(\Delta E\) value.

**NADH as a donor**

\[
\text{NADH} \rightarrow \text{NAD}^+ + 2 \text{ electrons} + 2 \text{ H}^+ : E = +320 \text{ mV}
\]

\[
\text{K'ate} + 2 \text{ electrons} + 2 \text{ H}^+ \rightarrow \text{K'ite} : E = -380 \text{ mV}
\]

**Sum:** \(\Delta E = -60 \text{ mV} : \text{negative} \Delta E \text{ corresponds to positive} \Delta G : \text{NOT FAVORABLE}

**NAD^+ as an acceptor**

\[
\text{NAD}^+ + 2 \text{ electrons} + 2 \text{ H}^+ \rightarrow \text{NADH} : E = -320 \text{ mV}
\]

\[
\text{K'ite} \rightarrow \text{K'ate} + 2 \text{ electrons} + 2 \text{ H}^+ : E = +380 \text{ mV}
\]

**Sum:** \(\Delta E = +60 \text{ mV} : \text{positive} \Delta E \text{ corresponds to negative} \Delta G : \text{FAVORABLE}

B. Which compound would serve as a better electron donor than NADH, FADH\(_2\) or Ferredoxin, and why?

**As a donor:** Ferredoxin, since \(E = +432 \text{ mV for oxidation would allow} \Delta E = +152 \text{ mV}

**As an acceptor:** FAD, since \(E = -220 \text{ mV for reduction would allow} \Delta E = +160 \text{ mV}

<table>
<thead>
<tr>
<th>Couple</th>
<th>(E_{m,7} \text{ (mV)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferredoxin(<em>{oxidized}) + 2e(^-) → Ferredoxin(</em>{reduced})</td>
<td>- 432</td>
</tr>
<tr>
<td>CO(_2) + 2e(^-) + 2H(^+) → HCOOH (formate)</td>
<td>- 432</td>
</tr>
<tr>
<td>2H(^+) + 2e(^-) → H(_2)</td>
<td>- 410</td>
</tr>
<tr>
<td>NAD(^+) + 2e(^-) + 2H(^+) → NADH + H(^+)</td>
<td>- 320</td>
</tr>
<tr>
<td>FAD + 2e(^-) + 2H(^+) → FADH(_2)</td>
<td>- 220</td>
</tr>
<tr>
<td>H(_3)C-CHO (acetylaldehyde) + 2e(^-) + 2H(^+) → H(_3)C-CH(_2)OH (ethanol)</td>
<td>- 197</td>
</tr>
<tr>
<td>FMN + 2e(^-) + 2H(^+) → FMNH(_2)</td>
<td>- 190</td>
</tr>
<tr>
<td>MQ (Menaquinone(_{oxidized})) + 2e(^-) + 2H(^+) → MQH(<em>2) (Menaquinol(</em>{reduced}))</td>
<td>- 74</td>
</tr>
<tr>
<td>‘OOC-CH=CH-COO’ (fumarate) + 2e(^-) + 2H(^+) → ‘OOC-CH(_2)-CH(_2)-COO’ (succinate)</td>
<td>+ 33</td>
</tr>
<tr>
<td>Q (Ubiquinone(_{oxidized})) + 2e(^-) + 2H(^+) → QH(<em>2) (Ubiquinol(</em>{reduced}))</td>
<td>+ 100</td>
</tr>
<tr>
<td>(\frac{1}{2} \text{O}_2) + 2e(^-) + 2H(^+) → H(_2)O</td>
<td>+ 815</td>
</tr>
</tbody>
</table>
7. (10 Points) Pentoses. Lactate heterofermenters make 2 ATP per mole pentose consumed. What is the maximum molar yield of ATP from a mixed-acid fermenter using 1 (ONE) mole pentose as a substrate? Metabolism in *Escherichia coli* is shown on the last page. Show your work and consider the following pathway to introduce pentoses into the glycolytic pathway:

\[
3 \text{ Pentose} + 3 \text{ ATP} \rightarrow 3 \text{ Pentose-5-P} + 3 \text{ ADP}
\]

Pentose-5-P (#1) + Pentose-5-P (#2) \rightarrow Sedoheptulose-7-P + Glyceraldehyde-3-P
Sedoheptulose-7-P + Glyceraldehyde-3-P \rightarrow Fructose-6-P + Erythrose-4-P
Erythrose-4-P + Pentose-5-P (#3) \rightarrow Glyceraldehyde-3-P + Fructose-6-P

**In sum:**

\[
3 \text{ Pentose} + 3 \text{ ATP} \rightarrow 2 \text{ Fructose-6-P} + \text{Glyceraldehyde-3-P} + 3 \text{ ADP}
\]

\[
2 \text{ Fructose-6-P} + 2 \text{ ATP} \rightarrow 2 \text{ Fructose-1,6-Bis-P} + 2 \text{ ADP}
2 \text{ Fructose-1,6-Bis-P} \rightarrow 4 \text{ Glyceraldehyde-3-P}
\]

Sum:

3 Pentose (15 carbons) + 5 ATP \rightarrow 5 Glyceraldehyde-3-P (15 carbons) + 5 ADP

5 Glyceraldehyde-3-P + 5 NAD\(^+\) + 10 ADP \rightarrow 5 \text{ Pyruvate} + 10 \text{ ATP} + 10 \text{ NADH} + 10 \text{ H}^+

Balance 5 NADH optimally by creating ethanol, not lactate; this will allow maximum acetate production:

5 Pyruvate + 5 HS-CoA \rightarrow 5 \text{ Acetyl-CoA} + 5 \text{ Formate}

2 ½ Acetyl-CoA + 5 NADH + 5 H\(^+\) \rightarrow 2 ½ Ethanol + 5 NAD\(^+\) : \text{NAD}^+ \text{ pools balanced}

2 ½ Acetyl-CoA + 2 ½ Pi + 2 ½ ADP \rightarrow 2 ½ Acetate + 2 ½ ATP + 2 ½ HS-CoA

Carbon Check: 2 ½ Ethanol (5 carbons) + 2 ½ Acetate (5 carbons) + 5 formate (5 carbons)

ATP Pools:
5 used to create 5 Glyceraldehyde-3-P
10 generated in glycolysis
2 ½ generated for acetate production

Total: 7 ½ mol ATP for 3 pentoses
Total: 2 ½ mol ATP for 1 pentose, or ½ mole ATP per carbon

Recall, mixed acid fermentation for glucose (6 carbons) yielded 3 ATP, also ½ ATP per carbon.

For lactate homofermentation, Glucose yields 2 ATP and pentose yields 1 2/3 ATP, an efficiency of 1/3 mol ATP per carbon:

5 Pyruvate + 5 NADH + 5 H\(^+\) \rightarrow 5 \text{ Lactate} + 5 \text{ NAD}^+

ATP : (-5) + 10 = 5 for 3 pentoses, or 5/3 for 1 pentose.
9. (8 Points). Pot Luck. Answer one of the following questions. Circle the letter for the question you will be answering. If you answer both questions, the first answer will be graded.

A. In precise thermodynamic terms, explain why the use of the Cyd cytochrome oxidase allows for the synthesis of less ATP than does the Cyo cytochrome oxidase during aerobic respiration. When would the Cyd enzyme be employed?

B. Describe the mechanism by which the Escherichia coli F_{0}F_{1} ATPase couples the influx of exactly 3 H^{+} into the cell to the synthesis of exactly 1 ATP. Include in your answer an explanation of how this ratio is obtained, and why ATP would be synthesized and not hydrolyzed.

A. The Cyd enzyme lacks the direct hydrogen ion pump that the Cyo enzyme has; Cyo can extrude 1 H^{+} for each electron transferred through this pore. Therefore, for the same electron transport chain will extrude fewer H^{+} ions, thereby created a smaller increase in DM, which results in less ATP production via the F_{0}F_{1} ATPase. The Cyd enzyme would be used when O_{2} concentration is low, and the negative ΔG of electron transport to O_{2} would be too small to allow for Cyo to extrude the additional 2 H^{+} ions.

B. The F_{0} subunit has 9 c subunits which, when rotated 360 degrees, carry 9 H^{+} ions into the cell. The F_{1} subunit has 3 αβ dimers, each with a catalytic center. The γ subunit of F_{1} is connected to F_{0}. A full rotation allows each active center to (a) bind ADP and Pi, (b) synthesize ATP, and (c) release ATP. Therefore, the influx of 9 H^{+} allows for the synthesis of 3 ATP, or 1 ATP per 3 H^{+}. ATP is synthesized since H^{+} influx occurs with the energy released by 3 H^{+} ions crossing with their electrochemical gradient is greater than the energy required to synthesize 1 ATP. Rotation of the γ subunit upon H^{+} influx forced the order of reactions to be ADP binding, ATP synthesis and ATP release. H^{+} ions cannot enter the cell and rotate F_{1} in the opposite direction since a centrally-placed arginine residue changes the pKa of the aspartate residue carrying the H^{+} ion, preventing H^{+} transport directly between the cytoplasmic and periplasmic wells of the F_{0} subunit.
Central Metabolism in *Escherichia coli*

**Aerobic dissimilation of pyruvate**

**Anaerobic dissimilation of pyruvate**