Problem Set 4: Oxidative Phosphorylation and Photosynthesis

This Problem Set is due before 5pm on Thursday December 7. There are eight questions for a total of 70 points.

<u>Question 1</u> (12 points). Complex I in the electron transport chain oxidizes NADH to NAD^+ and reduces UQ to UQH_2 in a 2-electron process.

(a) Calculate the free energy of the process at 37 °C and pH8. Standard reduction potentials for the half-reactions at pH7 can be found in Table 20.1
(b) Based on your answer to (a), calculate how many moles of protons can be translocated across the inner mitochondrial membrane by complex I if translocation of 1 mole of protons requires 23 kJ.
(c) Experimentally it is observed that oxidation of 1 NADH is coupled to the translocation of 4 protons. If the concentrations of UQ and UQH_2 are approximately equal, what is the ratio of NADH to NAD^+ necessary to accomplish this?

(a) $UQ + 2H^+ + 2e^- \rightarrow UQH_2$ $\quad$ $E°' = 0.06$ V

$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$ $\quad$ $E°' = -0.32$ V

The NAD^+ reaction must be reversed, so $\quad$ $\Delta E°'_{(reaction)} = 0.38$ V

Converting the standard reduction potentials to free energies using $\Delta G°' = -nF\Delta E°'$:

$\Delta G°' = -73.33$ kJ/mol

Correcting this for pH1 at 298 K (standard state) using $\Delta G°' = \Delta G° - RT\ln[H^+]$

$\Delta G° = -113.26$ kJ/mol

And recalculating for 310 K and pH8 $\quad$ $\Delta G = -65.79$ kJ/mol

(b) 65.79 kJ/mol will translocate 2.9 moles of protons

(c) Translocation of 4 moles of protons requires 92 kJ

$NADH + UQ + H^+ = NAD^+ + UQH_2$ $\quad$ $\Delta G° = -113.26$ kJ/mol

Using $\Delta G = \Delta G° + RT\ln[NAD^+][UQH_2]/[NADH][UQ][H^+]$

$-92{,}000 = -113{,}260 + RT\ln[NAD^+]/[NADH][H^+]$

At pH8, $[NAD^+]/[NADH] = 3.8 \times 10^{-5}$, so to pump 4 protons $NADH:NAD^+ = 26{,}000:1$

<u>Question 2</u> (12 points). Complex III in the electron transport chain oxidizes UQH_2 to UQ and reduces cytochrome *c* from Fe^{3+} to Fe^{2+} via the Q-cycle. Calculate the redox potentials for each of the 4 electron transfer steps at pH7. Standard reduction potentials for the half-reactions at pH7 can be found in Table 20.1

Step 1: UQH_2 is oxidized to UQ at the Q_P site in a 2 electron process. UQ is reduced to $UQH^{\cdot}$ At the Q_N site.

(i) UQH_2 reduces cytochrome *c* in a 1-electron process:

$UQH_2 \rightarrow UQH^{\cdot} + H^+ + e^-$	$E°' = -0.19$ V
cyt *c* (Fe^{3+}) + e^- $\rightarrow$ cyt *c* (Fe^{2+})	$E°' = 0.254$ V
	$\Delta E°' = 0.064$ V

(ii) 1 electron is transferred from $UQH^{\cdot}$ to UQ at the Q_N site:

$UQH^{\cdot} \rightarrow UQ + H^+ + e^-$	$E°' = -0.03$ V
$UQ + H^+ + e^- \rightarrow UQH^{\cdot}$	$E°' = 0.03$ V
	$\Delta E°' = 0$ V

Step 2: UQH_2 is oxidized to UQ at the Q_P site in a 2 electron process. $UQH^{\cdot}$ is reduced to UQH_2 At the Q_N site.

(i) UQH_2 reduces cytochrome *c* in a 1-electron process:

$UQH_2 \rightarrow UQH^{\cdot} + H^+ + e^-$	$E°' = -0.19$ V
cyt *c* (Fe^{3+}) + e^- $\rightarrow$ cyt *c* (Fe^{2+})	$E°' = 0.254$ V
	$\Delta E°' = 0.064$ V

(ii) 1 electron is transferred from $UQH^{\cdot}$ to UQ at the Q_N site:

$UQH^{\cdot} \rightarrow UQ + H^+ + e^-$	$E°' = -0.03$ V
$UQH^{\cdot} + H^+ + e^- \rightarrow UQH_2$	$E°' = 0.19$ V
	$\Delta E°' = 0.16$ V

Question 3 (12 points). Normally ATP synthesis is tightly coupled to electron transfer through the electron transport chain. Under these conditions, the ratio of ATP produced per 2 electrons transferred to oxygen (P/O ratio) is about 2.5.

(a) Predict the effect of a low concentration of an uncoupling agent on the P/O ratio

Uncouplers depolarize the inner mitochondrial membrane, and ATP synthesis is compromised. The electron transport chain remains unaffected, so protons are still pumped by complexes I, III and IV. Protons pumped from the matrix to the intermembrane space are rapidly returned to the matrix by the membrane-soluble uncoupler, so energy that would normally be used to activate ATP synthase is ultimately expended as heat. Low concentrations of uncouplers dissipate some of the proton gradient necessary for ATP synthesis. Overall, more protons have to be transferred across the membrane for each ATP synthesized. This requires more NADH per ATP synthesized, so the P/O ratio will decrease.

(b) Predict the effect of DCCD on the P/O ratio (DCCD irreversibly blocks asp61 of the c subunit of F_1F_o ATP synthase)

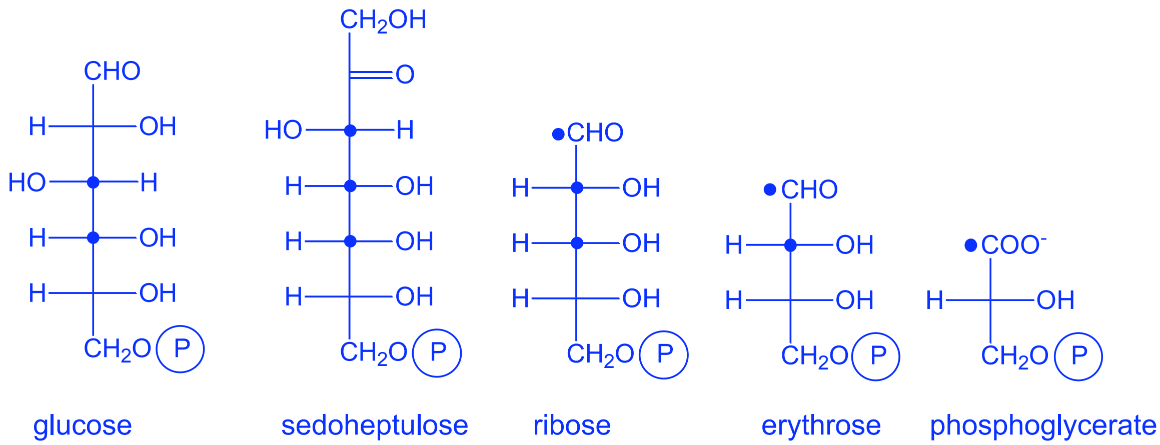
With the proton channel through F_1F_o ATP synthase blocked, ATP synthesis will be reduced and the proton gradient will not dissipate through the synthase. Increasing $[H^+]$ in the intermembrane space will stop proton pumping by Complexes I, III, and IV and so will reduce delivery of electrons through the ETC to O_2. The decrease in ATP synthesis will be exactly matched by a decrease in O_2 reduction so the P/O ratio will be unaffected.

Question 4 (6 points) Which will make more use of cyclic photophosphorylation, a C-3 plant or a C-4 plant? Briefly explain your reasoning.

In order to minimize the deleterious effects of photorespiration, C-4 plants transfer CO_2 from mesophyll cells at the surface to bundle sheath cells in the interior. This is accomplished by converting PEP to oxaloacetate, then reducing (NADPH) oxaloacetate to malate which can cross the cell membranes. Oxaloacetate is regenerated ($NADP^+$) and the CO_2 used for Calvin cycle glucose synthesis, leaving pyruvate. Pyruvate is transferred back to the mesophyll cells where it is converted to PEP using ATP.

Cyclic photophosphorylation makes ATP, but not NADPH. C-4 plants have a higher demand for ATP, and so make more use of cyclic photophosphorylation than C-3 plants.

Question 5 (10 points). $^{14}CO_2$ is administered to a green plant in a closed system. After a short time (one pass through the Calvin-Benson cycle), the plant is harvested, and the following substances are isolated: glucose, sedoheptulose-7-phosphate, ribose-5-phosphate, erythrose-4-phosphate and 3-phosphoglycerate. Draw the structures of these five molecules and indicate which atoms will be ^{14}C labeled.



After equilibrium is reached, all the carbons of all the molecules will be labeled.

Question 6 (8 points). List four similarities and four differences between photosynthesis and oxidative phosphorylation.

Similarities: ATP synthase (F_o/F_1 ATP synthase/CF_oCF_1 ATP synthase), chemiosmotic mechanism (electron transport coupled to proton translocation), lipophilic electron trasport pool (ubiquinone/plastoquinone), dedicated organelles (mitochondria/chloroplasts), small, water-soluble 1-electron transfer proteins (cytochrome *c*/plastocyanin).

Differences: Source of chemical reduction (light/NADH), ATP/ADP transport (passive/active), products (photosynthesis produces NADPH), coupling to TCA (photosynthesis has no analog of complex II), oxygen evolution (photosynthesis) vs. oxygen consumption (oxidative phosphorylation).