Sample questions for the bioenergetics quiz, MIBO 8610

1. Lefti Morgani, the prominent Mongolian microbiologist, reported the isolation of the first dinitrogen-oxidizing bacterium at the International Union of Microbiological Societies (IUMS) in Australia last year. Use your knowledge of bioenergetics to decide if Lefti's claim is possible or is she just another loser. The reaction is:

$$N_2 + {}^{5}/_{2}O_2 + H_2O \rightarrow 2NO_3^- + 2H^+$$

2. You have been recently be hired as a Research Microbiologist at a small contract research company in the Bay area. On your first day of work, the president of the company, a prominent cell biologist, asks you to respond to a request for proposals (RFP) from NASA concerning the use of microbial bioreactors to generate food for astronauts on long range space missions. The president says, "Look, my boyfriend had an idea that can't miss. We set up a thermophilic bioreactor with an acetogenic bacterium and a little H_2 and CO_2. The acetogen generates acetate from the H_2 and CO_2. We also add a syntrophic bacterium that oxidizes the acetate back to H_2 and CO_2. Both organisms keep growing and the biomass just accumulates (see below). All we have to do is just harvest the cells occassionally."

acetogen

$$4H_2 + 2CO_2 \quad \longrightarrow \quad \text{acetate} + 2H_2O$$

$$\longleftarrow$$

syntroph

Use your knowledge of bioenergetics to evaluate the merits of this proposal.

3. I was talking to this guy yesterday, and he said that the high fluxes of ultraviolet light on the surface of Mars would generate significant amounts of carbon monoxide (CO) and oxygen (O_2) from atmospheric CO_2. In fact, the atmospheric concentration of CO and O_2 are believed to be about 490 ppmv and 1300 ppmv (parts per million by volume), respectively. All other considerations aside, are the concentrations of these gases high enough to support life similar to that found on Earth? Support your answer with the appropriate calculations. [Note that the E_o' for CO_2/CO = -530 mV and for ${}^{1}/_{2}O_2/H_2O$ = +820 mV.]

4. An anaerobe was isolated from marine sediments and found to oxidize glycine to acetate and leucine to isovalerate using a variety of sulfur compounds as electron acceptors. Compare the energetics for the oxidation of each of these amino acids with sulfate (SO_4^{2-}), elemental sulfur (S) or thiosulfate ($S_2O_3^{2-}$) as the electron acceptor. Which reaction do you think would dominate if all these substrates were present at the same time? Why? Which reaction do you think would dominate in nature? Why? [For each potential reaction, write a balanced equation. Pay attention to your units when comparing potential reactions.]

5. Geochemists argue that before O_2 became a major component in the earth's atmosphere, large amounts of reduced iron were first oxidized. Thus, it is possible that early photosynthetic organisms may have used Fe^{+2} as an electron donor instead of H_2O. Is this possible?

To answer this question, calculate the shortest wavelength of light a photosynthetic organism would need to reduce $NAD(P)^+$ using Fe^{+2} as the electron donor. What if only 60 % of the light energy was conserved in the process? Make the same calculations for the reduction of menaquinone.

6. For many organisms the $NADH/NAD^+$ ratio in the cell varies between 2 and 10. For an organism using acetogenic substrates, is it likely that pyruvate could be formed by the reaction: acetyl-CoA + CO_2 + NADH + H^+ $\Leftrightarrow$ pyruvate + HS-CoA + NAD^+?

To answer this question, calculate the ratio of pyruvate/acetyl-CoA that could be formed assuming the $NADH/NAD^+$ ratios above. The total CoA pool (free + thioesters) in cells is usually on the order of 0.1 mM, the $[CO_2]$ is on the order of 10 %, and the total NADH + NAD^+ pool is on the order of 1 mM. What affinities would the enzyme have to have for its substrates? Are these reasonable, ie. do you know of any other enzymes with affinities in this range?

7. In the supernatant of a complex microbial community growing on a complex substrate of unknown composition, large amounts of acetate, lactate, propionate, and CO_2 are found. Using the Gibbs free energies of formation for these compounds (at 25 C), calculate what reactions might be catalyzed by living organisms using any combination of these compounds as either substrates or products.

To answer this question, write as many balanced reactions as possible. Note, that any proposed reaction must be balanced, and you may include H_2O or H^+ as either a substrate or product. Calculate the change in free energy for each of these reactions. Only those reactions where the change in free energy is negative could be used to support growth.

8. Assuming that a marine organism maintains a low intracellular concentration of Na^+ of 10 mM (the extracellular $[Na^+]$ is 0.4 M), could this Na^+ gradient be used to make ATP? If ATP was made by a Na^+-dependent F_1F_0 ATP synthase, how many Na^+ ions would have to be translocated for synthesis of one ATP?

9. While studying bacteria in coal, Dr. Goose noticed that some cells contained very bright inclusion bodies that looked like diamonds. In a fit of inspiration, he wrote the following equation for growth of a diamond-producing bacterium:

$$2H_2 + CO_2 \rightleftharpoons C(\text{graphite}) + 2H_2O$$

Has Dr. Goose laid a golden egg?

10. Meanwhile his graduate research assistant Fry D. Egg was studying hydrogen oxidation by a microaerophilic organism that he had isolated from the stomach of a pig. This organism grew

well with 5 % O_2. In the pig stomach, the H_2 concentration was about 4 nM or 0.5 x 10^{-5} atm, which presumably represented the levels of H_2 available during normal growth of this organism in nature. Prof. Goose recommended that Mr. Egg look for an NAD^+-dependent hydrogenase enzyme from this organism. [This enzyme catalyzes the following reversible reaction: $H_2 + NAD^+ \leftrightarrow NADH + H^+$.] Is Mr. Egg likely to complete his dissertation in the forseeable future? (Support your answer with the appropriate calculations and your general knowledge in biology.)

11. Dr. Hen, Dr. Goose's postdoctoral associate, believed that she isolated a marine denitrifying bacterium that could grow using sulfide as the electron donor. Is this possible?

12. The reaction centers of photosynthetic bacteria are the pigment-protein complexes that convert light energy to chemical energy. The reaction centers of the purple nonsulfur photosynthetic bacteria *Rhodopseudomonas palustris* and *Rhodospeudomonas viridis* absorb light at 860 nm and 1020 nm, respectively.

A. Is there sufficient energy in the light that each organism absorbs to make ATP? Show the calculations that allow you to form this conclusion.

B. Assume that the light energy is used to change the midpoint potential of an electron donor. What would the maximum change in midpoint potential possible in each organism.

13. Not so recently, evidence for an organism called *Candidatus* Brocadia anammoxidans has been obtained in mixed culture (hence the candidatus status). This organism oxidizes ammonium ion anaerobically to form dinitrogen, called the anammox reaction:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

For this reaction, calculate the change in free energy. Is this energy sufficient to support growth? In this reaction, what is the electron donor and what is the electron acceptor?

14. The oxidation of lactate to pyruvate has a positive ΔG under standard conditions, yet this reaction is common in nature. Show by calculations conditions where this reaction might occur in the natural environment.

$$\text{lactate}^- \rightarrow \text{pyruvate}^- + H_2 \qquad \Delta G^{o\prime} = + 43.1 \text{ kJ/mol}$$