2009 TA Training: Microteaching topics

Department of Biological Engineering

1. Free energy and ATP hydrolysis

The hydrolysis of ATP to ADP and inorganic phospate has a standard reaction free energy of about -30 kJ/mole at body temperature.

a) If the reaction goes to equilibrium, what is the ratio of ADP:ATP at a 1 mM phosphate concentration? What does this result imply about hydrolysis in the body?

b) Often ATP hydrolysis is coupled to a needed cellular reaction (e.g., sugar processing) with a positive free energy change, making the overall paired reaction spontaneous. Physically, how might an enzyme work to couple two reactions, i.e., to ensure that they only proceed simultaneously?

c) If ATP hydrolysis is *not* coupled to a cellular process, what happens to the released free energy?

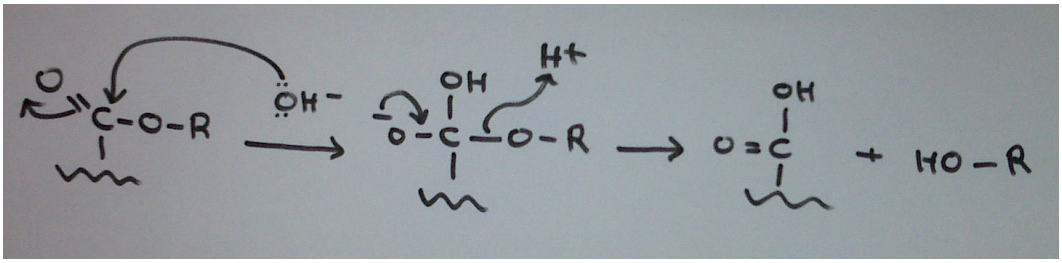
2. Proton availability in a cell

How many free protons are there in a cell? A billion, a million, a hundred? We often take protons for granted because they are abundantly available to organic chemists, who are able to contrive reaction conditions to suit their purposes (see Figure). In a cell, however, the situation may be different. This problem involves calculation of the number of protons in an *E. coli* cell – that is, free protons available to participate in chemical reactions.

a) Please calculate the volume of the cell, which is a cylinder 1 micron wide and 2 microns long.

b) Given the volume of the cell, at pH 7 how many protons are contained within it?

c) A bacterial cell contains thousands of macromolecules such as proteins and nucleic acids that have ionizable groups. What does this calculation tell you about the role of the H+ and OH- in the chemistry of acid-base reactions in the cell?



3. Protein-ligand binding parameters

A simple model for a ligand L binding to a protein can be written $y = L/(L+K_D)$, where y is the fraction of the protein that is bound, and K_D is the dissociation constant of the reaction.

a) Sketch the curve y vs. L (consider how the ratio of $L:K_D$ affects the binding fraction).

b) The curve from part a) might for example represent the oxygen-binding protein myoglobin. Whereas myglobin has one site for binding oxygen, hemoglobin has four sites that are strongly positively cooperative. It also has a lower affinity for oxygen than does myoglobin. On the same plot, draw both proteins' binding curves.

c) What would the plot for the imaginary protein sortaglobin look like, if sortaglobin has a lower cooperativity than hemoglobin, but is otherwise similar?

d) The compound 2, 3-bisphosphoglycerol (DPG) causes the affinity of hemoglobin for oxygen to decrease. Do you think people living in high or low altitudes have higher blood concentrations of DPG?

4. Biologically relevant redox

Thermodynamically, we know that oxidation of NADH to NAD^+ gives us three ATPs whereas oxidation of $FADH_2$ to FAD gives only two.

a) Using a chart of standard reduction potentials and the relevant equation(s), explain this stoichiometry (3 vs. 2 ATPs, respectively).

b) Professor Eric Alm discovers a new organism that lives deep underground, far from a sufficient pO_2 to allow oxygen to be used a terminal electron acceptor. There is, however, abundant nitrate and nitrite in the niche occupied by the new organism. How many moles of ATP would be generated from the reduced molecules of part a)?

5. Conformational entropy

Discuss the Boltzmann formulation of entropy ($S = k \ln W$), and apply it to a simple biological example. For example, you might talk about protein folding.

a) If you model the native protein as having precisely one state, what is its entropy? How realistic is this model?

b) What is the entropy of a protein with N amino acids and 2 available orientations per bond (bond angles), if it samples all possible conformations? Does sampling this many conformations seem physically feasible?

c) Consider the protein as a system of interest. If its entropy decreases on folding, what must happen to the entropy of the surroundings, and by what processes might this occur?

6. Metabolic network adaptation

Describe the role of Hif-1α in the mechanism by which metabolic networks adapt at the gene expression level to varying pO_2. Consider cells that are near a source of oxygen (e.g., a blood vessel; call this situation zone A) and cells that are remote from it (call this zone B).

If you do problem 6, you should attempt to improve on John's presentation!