Microteaching topics

Department of Biological Engineering TA Training

1. Free energy and ATP hydrolysis

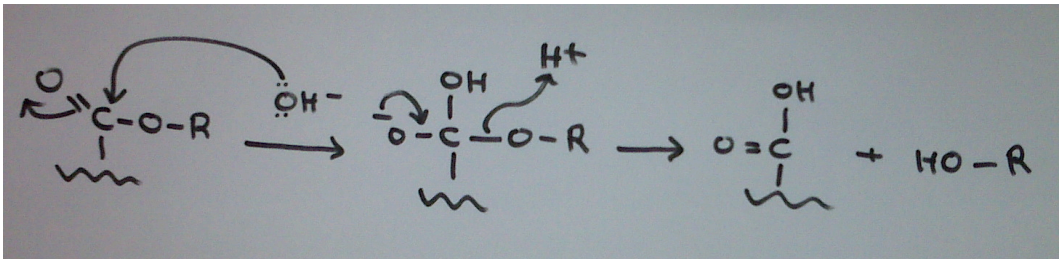
The hydrolysis of ATP to ADP and inorganic phospate has a standard reaction free energy of about -30 kJ/mole at body temperature.

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, approximating it as 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. To do so, explicitly consider how the L:K_D ratio affects the magnitude of y.
b) The curve from part a) can 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. Draw both proteins' binding curves on the same plot and discuss them.

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 change 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).

7. Protein mutant structure and thermodynamics

Consider a protein-protein interaction A+B <-> C, where C is the complex. The ΔΔG values for the binding reactions of three mutants of protein A are shown below. The wild-type protein A has Leu at position 100 and Ile at position 110.

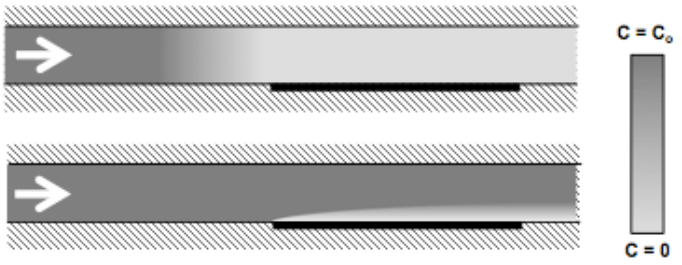
Amino Acid Position		$\Delta\Delta G = \Delta G_{mut} - \Delta G_{wt}$ (kcal/mol)
100	110	
Asp	Ile	0.5
Leu	Lys	0.4
Asp	Lys	-2.0

a) Assume that the mutations have a negligible effect on the free energy of protein A. Compute the ΔΔG values for the following transitions, where (X,Y) indicates X at position 100 and Y at position 110:
(Leu,Lys) to (Asp,Lys)
(Asp,Ile) to (Asp,Lys)

b) The mutations occur in close proximity to the binding site of B on A. Propose an explanation for why the double mutant has a negative value ΔΔG and the single mutants have positive values. Your answer should make a prediction for the molecular properties of the surface of B in the vicinity of these mutations.

8. Interplay between convection, reaction, and diffusion

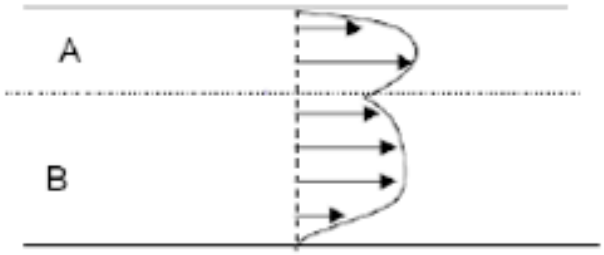
(a) A perfectly adsorbing sensor is placed in an infinitely sized bath of molecules and the steady-state binding current is measured. Next, a stir bar is added to the system in order to create flow past the adsorber. Does the steady-state binding rate increase or remain the same? Explain why.
(b) A perfectly adsorbing sensor is placed in the microchannels below under different flow conditions. The top sensor collects every molecule that flows into the channel; the bottom sensor does not. Channel/sensor dimensions are identical for each case. Explain which sensor has the greater binding rate, or if they have equal binding rates, or if there is not enough information to tell.



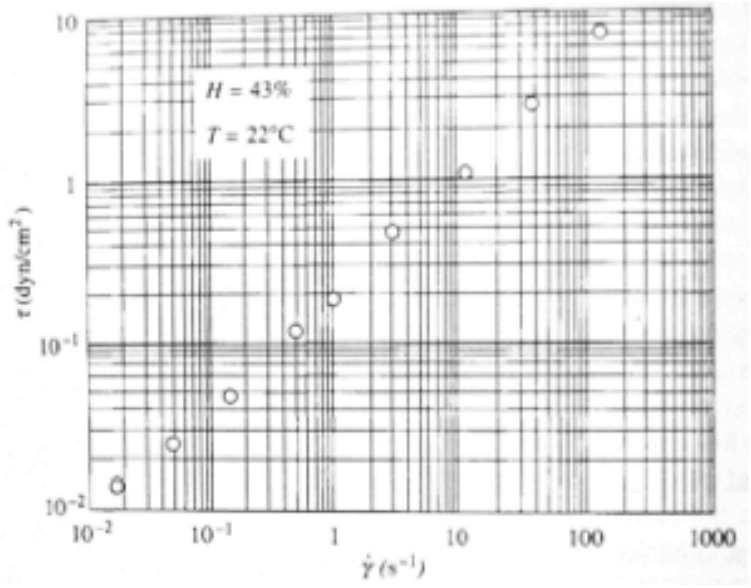
9. Three short answer, independent questions about fluid dynamics

a) A metal bead is dropped in a large tank filled with glycerin and its velocity is measured as it falls. The same experiment is then repeated, but this time the bead is dropped near the wall of the tank (within a distance comparable to bead's radius). In this experiment, do you expect the falling velocity of the bead to be high, lower, or the same? Briefly explain.

b) Two immiscible liquids, A and B, are flowing in a laminar flow between two parallel plates. Is it possible for the velocity profiles to have the form shown below? Explain.

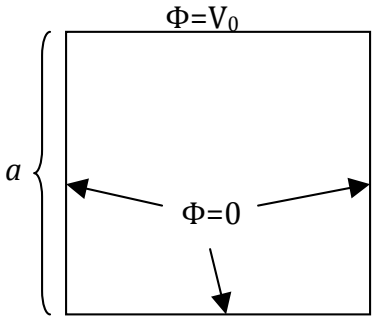


c) Shear stress – shear rate data for blood, as measured by rotational viscometry, is shown below where the hematocrit H is the volume percentage of red bloods cells (from Thurston, *Biorheology* 31, 179 1994). Based on this data, is the question, "What is the viscosity of blood?" a well defined question? Explain.

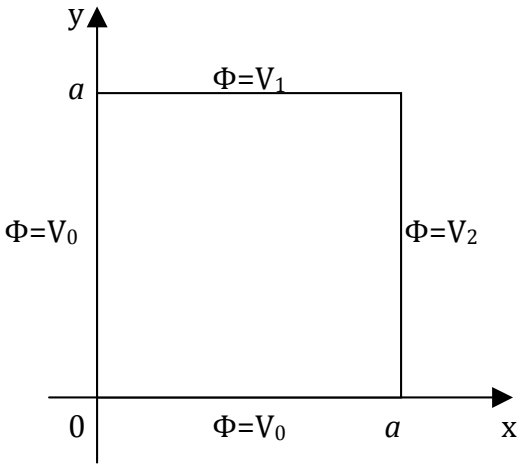


10. Superposition approach for complex potentials

We will have already covered the solution of the Laplace's equation for the geometry shown in below figure (If we didn't, it is explained in the H&M textbook Chapter 5).



Now get the solution of Laplace's equation for a more general case, shown below. It is the same square two-dimensional space, but on each side different potential values are applied. (Hint: Superposition!)



11. Short answer biomechanics questions, 1

a) The cellular tensegrity model is often described as "action-at-a-distance effect," such that force application or mechanical deformation at one end of the cell can result in cytoskeletal or nuclear displacements in the center of the cell or the other end of the cell more than 10–20 micrometers away. Under what circumstances would you expect this model to hold true? Describe both general model assumptions and a specific biological example.
b) How does this "action at a distance" compare to the deformation behavior we would expect from scaling analysis (force or energy) for a homogeneous elastic linear isotropic (HILE) continuum mechanics model of a cell?

12. Short answer biomechanics questions, 2

a) In the biopolymer model for cell mechanics and filament networks, why does the bulk elastic modulus depend both on the polymer/filament density (i.e. solid volume fraction) and the cross-link density (as reflected in the distance between cross-links or entanglements)?
b) How would you expect the hydraulic permeability of a material (e.g., collagen gel) to change as the solid fraction increases? Why?
c) Although we only spoke in class of *isotropic* poroelastic materials, i.e., those having the same permeability for flow in every direction, many biological materials are anisotropic. Give one example of a tissue that you expect would have anisotropic permeability, and explain why.

Problems submitted by: John Essigmann, Ernest Fraenkel, Jay Han, Roger Kamm, Scott Manalis, and Agi Stachowiak.