

Biology 5357

Chemistry & Physics of Biological Molecules

Examination #2

Nucleic Acids
Membranes

December 15, 2011

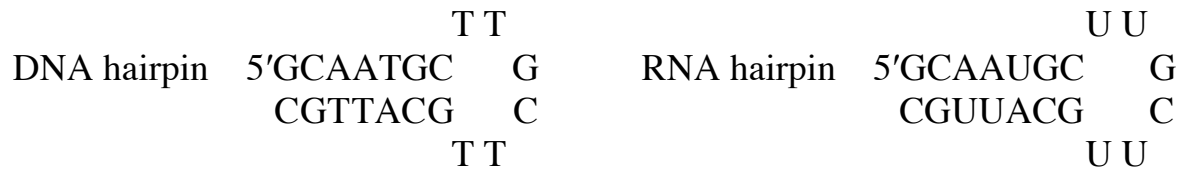
1. (15 points) Graph the predicted thermal denaturation plot (melting curve) of these two duplexes at 0.1 and 10 μM duplex concentration in 1 M NaCl, 10 mM buffer pH 7.0.

DNA duplex 5'GCAATGC
CGTTACG

RNA duplex 5'GCAAUGC
CGUUACG

- A.** Indicate the T_M on each curve.
- B.** Write the equilibrium expression for the reaction.
- C.** For RNA and DNA duplexes at the same concentration, will the free energy (ΔG°) of duplex formation be the same? Why or why not?

2. (15 points) For these hairpins, graph the expected melting curves at 0.1 and 10 μM strand concentration in 1 M NaCl, 10 mM buffer pH 7.0.

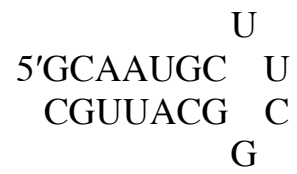


A. Indicate the T_M on each curve.

B. Write the equilibrium expression for the reaction.

C. Will the folding free energy of the hairpins be more or less favorable than for the duplex alone? Explain your answer.

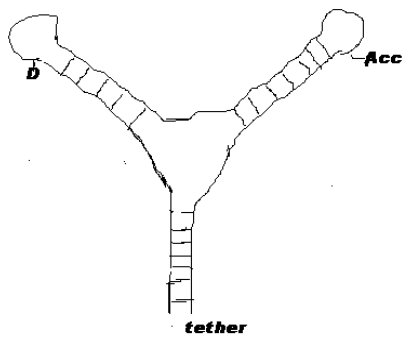
3. (15 points) Would the properties of this RNA hairpin differ from those of the one in Question 2? Explain your answer.



4. (15 points) This RNA aptamer (drawn in the absence of its Ligand) has been labeled with a Donor (D) and Acceptor (Acc) fluorophore as shown. Assume that the FRET pair has been optimized for the distance between sites (its R_0 is appropriate). The RNA is tethered to a surface for observation in a single molecule experiment that simultaneously measures donor and acceptor fluorescence intensity. Draw a single molecule FRET trace as a function of time for the aptamer RNA at 22° C.

- A.** in 150 mM KCl
- B.** in 150 mM KCl, 2 mM MgCl₂
- C.** in 150 mM KCl, 2 mM MgCl₂, 1 mM Ligand

State all your assumptions regarding binding sites, then interpret your data in terms of binding/structure/dynamics.



5. (10 points) It is the year 2150 and the mean temperature of the Earth has increased 4°C. Although the bipedal mammals have developed their own mechanisms of compensation, it has been noticed that bacteria and other unicellular organisms appear to have evolved to meet this challenge. Membrane composition is one of the cellular systems in bacterial that has been modified, but interestingly there appear to be two distinct types of changes. Suggest what these changes might be and explain how they would make the affected organisms more viable at increased temperatures. How will other macromolecular species be forced to change in order to accommodate the membrane alterations?

6. (10 points) The fusion of membranes is critical to cellular function. Membrane fusion and the protein-regulated membrane fusion found in cells has been studied intensively for thirty years. Recent studies on the fusion of single vesicles can be time-resolved (almost) by microscopy. Under these conditions vesicle docking is slow with the following steps being kinetically indistinguishable in the microscope. Docking to membranes requires surmounting an energy barrier, U_D , which defines the probability to surmount this barrier as $P_D = \exp(-U_D/k_B T)$. The U_D can be parsed into activating (P_A) and inhibiting (P_I) components that are independently determined. Using this experimental system please suggest how you would address the following issues:

A. The effect of membrane bilayer composition on the activation and inhibition of the docking.

B. Genetic analysis has identified proteins that are important in the fusion-based physiology of cells. Propose studies to clarify how these proteins are influencing membrane docking.

C. The role and/or importance of membrane curvature in membrane docking.

7. (10 points) The membranes of cells are well-identified as barriers to the diffusion of soluble components in and out of cells. However perfect isolation is not appropriate for cells. Membranes in cells can be shown to develop holes that allow the passage of ions or larger molecules across the membrane barrier.

A. Considering the lamellar properties of lipids suggest a structure for a membrane pore that might be thermodynamically possible. Consider the full range of lipid structures and explain your choices of lipids and how they might arrange themselves to stabilize the pore structure.

B. Proteins can also change the ability of soluble molecules to cross membrane barriers. Describe the classes of proteins that perform this function, and briefly describe how each influences or adapts to membrane structure.

8. (10 points) In a two lipid mixture (lipid A and B) the enthalpy change for the liquid-gel phase transition depends upon composition:

$$\Delta H(T) = x_B \times \Delta H_B + (1 - x_B) \times \Delta H_A$$

where x_B is the mole fraction of lipid B in the system.

A. If $\Delta H_A < \Delta H_B$ and $T_A < T_B$ draw a proposed phase diagram for this system as it depends upon composition.

B. How would the plot of specific heat capacity against temperature change in going from $x_B = 0.25$ to $x_B = 0.75$ (increasing the higher melting lipid)?

C. At a constant temperature of $T = \frac{T_A + T_B}{2}$ what will be the predicted comparative effect of introducing 0.2 mole% cholesterol to each of the binary mixtures, $x_B = 0.25$ and $x_B = 0.75$?