HW 7: Synthetic transcriptional circuits.

(Dated: November 17, 2010)

1. DNA SEQUENCE DESING TOOLS.

(a) Write a random sequence generator: The input to the sequence generator should be a string of characters 'N', 'W' and 'S'. Here, 'N' can take any of the four values 'A', 'G', 'C' or 'T' while 'S' can take the values 'C' or 'G' (the strongly binding bases) and 'W' is either 'A' or 'T' (the weakly binding bases). The length of the string should be variable (i.e. you can design sequences of arbitary length).

(b) Write tools for generating complementary and inverted sequences. The input should be an arbitrary DNA sequence (e.g. 'AATAGCTCGA') and the output should be (i) the complement of that sequence (i.e. 'TCGAGCTATT'), or (ii) the inverse (i.e. 'AGCTCCGATAA'). Note that both input and output need to be written in the same orientation, i.e. $5' \rightarrow 3'$. Check your tool using Nupack.

(c) Use your tools to design strands that assemble into a four-arm junction where each arm has length 15bp.

2. DNA AND GATE.

(a) Hsa-mir-133a and hsa-mir-1 are two human microRNAs that are highly expressed in muscle tissue. You can find the sequences of the mature microRNAs at www.mirbase.org. Design the sequences for a DNA AND gate (see example (4) from 11/15) that takes these two microRNA as inputs. Make the toe-holds 6 nt long. Using NUPACK, test all four possible input combinations and show that the gate works as expected. Assume that all concentrations are 100nM. Assume that the output is a fluorescent signal.

(b) Write a reaction model for the DNA AND gate and use MATLAB to solve the resulting differential equations. Assume that the gate is at 100nM and that all rate constants are the same, i.e. $k = 10^5/\text{M/s}$. Test a few different input combinations where the inputs concentrations can be different from the gate concentration (this is not really a digital system).

3. TWO-STATE MODEL FOR HYBRIDIZATION.

DNA hybridization is often modeled as a two state process

$$S + S^* \underset{k_d}{\overset{k_k}{\rightleftharpoons}} SS^* \tag{1}$$

where k_d is the dissociation rate constant and k_h is the hybridization rate constant. The equilibrium constant for this reaction is defined as

$$K_{eq} = \frac{k_h}{k_d} = \frac{[SS^*]}{[S][S^*]} = \mathrm{e}^{-\Delta G/RT}. \tag{2}$$

In the last equation we have related the free energy of the reaction ΔG to the equilibrium constant. Furthermore, R is the ideal gas constant and T is the absolute temperature in Kelvin. The hybridization rate constant for two short strand of DNA at 25°C and in 1M NaCl is around $10^6/\text{M/s}$. Furthermore, assume that the free energy released in a hybridization reaction is approximately $-2RT/\text{base/Mole}$.

(a) Calculate the dissociation constant for (i) a 6-mer and (ii) a 20-mer. From that, estimate the time it will take for a duplex at that length to fall apart.

(b) Strand displacement (examples (1) or (2) on 11/15) proceeds at a rate almost as fast as hybridization if the single-stranded toe-hold (domains 1 and 1^*) is of length 6 and the double-stranded region is 20 bp (domains 2 and 2^*). Is this compatible with a two-state model and if not, why does strand displacement work?

(c) Can you propose a more realistic model for strand displacement and can you estimates the rates of the different steps in your model based on the information provided here?