Problem set 4
(Hand in by June 1)

Problem 1

Super-Duper binding cooperativity. *Thanks to Steve Block for inspiration for this problem.* You do a series of experiments with a protein called Super. It binds a ligand called Duper. In your experiments you measure the amount of binding of Super with different concentrations of Duper. When you plot your data appropriately and fit them with the Hill equation, you find a Hill coefficient $n = 2$.

a) (Roughly) Sketch your plot.

b) What can you conclude about Super and Duper from the fits to the Hill equation?

c) A few days later, a colleague publishes a crystal structure of Super with bound Duper. Her structure reveals four binding sites, each occupied by a molecule of Duper. With this additional information, can you conclude anything else about the cooperative binding of Super by Duper?

Problem 2

Free vs. bound ligand. In class, we covered the basic expression for the probability or "fraction bound" of a ligand L binding to a receptor R (i.e. a protein), as a function of the *free* ligand concentration $[L]$, i.e. for the reaction

$$L + R \leftrightarrow RL \tag{1}$$

we find the fraction bound as

$$Y = \frac{[L]/K_d}{1 + [L]/K_d} \tag{2}$$

where K_d is the dissociation constant. Experimentally, however, the free ligand concentration can be difficult to determine; much more readily available is the *total* ligand concentration $[L]_{tot}$ and the total receptor concentration $[R]_{tot}$.

a) Derive an expression for the fraction bound Y as a function of $[L]_{tot}$ and $[R]_{tot}$. Hint: $[L]_{tot} = [L] + [RL]$ and $[R]_{tot} = [R] + [RL]$; you should find a quadratic equation for $[RL]$ and solve it using the standard formulae for quadratic equations.

b) Take the limit where $[L]_{tot} \gg [R]_{tot}$ and show how the expression given in Equation 1 is recovered.

c) Maeda *et al.*, *Nucleic Acids Research* (2000), measured binding curves for different σ subunits binding to the RNA polymerase core enzyme. The paper and data are available on the course website. Fit the data from their Figure 1A to the binding model derived in subproblem a), assuming a total receptor (σ^{70}, in this case) concentration of 0.4 nM. Compare this to the result obtained by fitting the standard expression, Equation 1.

Problem 3

Mean-first-passage-time analysis applied to DNA Melting. In the lecture, we have discussed how to apply mean-first-passage-time analysis to estimate dissociation rates k_{off} of short DNA duplexes using essentially a 1D random walk model with a reflecting and an absorbing boundary. For short duplexes of length n, dissociation starts from the ends, where thermal fluctuations lead to the breaking of terminal base-pairs ('fraying'). After the first bp is broken, it may either reform or the adjacent bp may also break, and so on. The duplex is assumed to completely dissociate, when a critical duplex length, e.g. $N = 3$, is reached. The mean first passage time for this random process is approximately given by $t \approx s^{n-N} \cdot (2k_+(n-N))^{-1}$. Using typical values of $-\Delta G_{bp} \approx 3k_b T$ and $k_+ \approx 10^7$ per second, calculate the dissociation constant for:

a) A 9-mer DNA duplex

b) A 10-mer DNA duplex

Problem 4

fold.it for fun. Folding DNA and Proteins - *in silico*.

a) Download the .json file from the course website of the 2D DNA origami structure we've designed in class. Use the MIT web service CanDo (www.cando-dna-origami.org) to perform an FEM analysis of the structure. What is the minimum and maximum RMS fluctuation observed in the model?

b) Estimate the amount of 'twist' (periodicity to perform a full turn in a polymerized structure) introduced in the structures due to local underwinding of the DNA double helices in the DNA origami.

c) At which distance would you introduce base deletions in the design in order to relieve the twist and obtain a flat (non-twisted) structure?

d) We've briefly had a look at the science game foldit (www.fold.it) in class. Download the game to your computer and create an account using the following rule: [first two letters of your first name][first two letter of your last name][bom17] (mine would be rajubom17) and start to play around and check out the tutorial to familiarize yourself with the game. We might have a small competition in one of the upcoming problem sets. Also check out the paper behind the game: Cooper *et al.*, *Nature* (2010) (available on the course website).