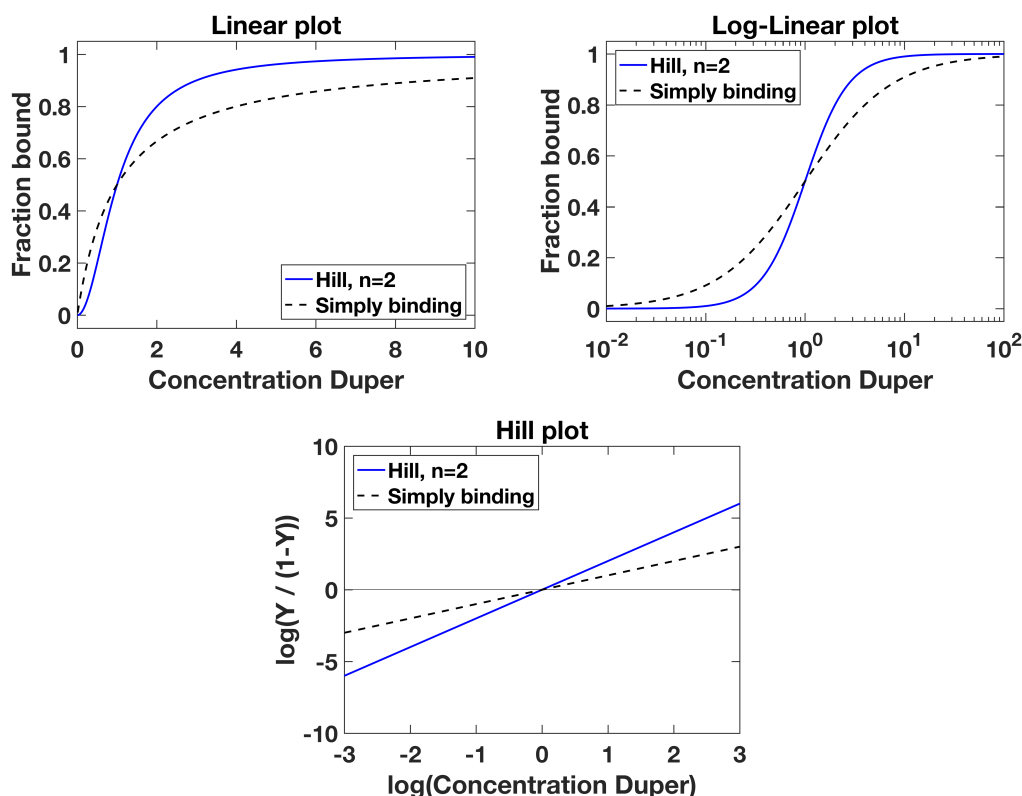


Solutions to problem set 4

Problem 1

Super-Duper binding cooperativity.

- a) Sketches of a Hill binding curve with $n = 2$ (and binding constant = 1, in the “arbitrary” concentration units of the plots) are shown below. The linear plot is fine, but often not very informative. Most commonly, you will see the plot of fraction bound vs. log-concentration, where the “sigmoidal” shape of the binding curve is readily apparent. In the old days, before it was easy to do non-linear fits on a computer, one would often transform the data to in the end fit a straight line. This is what the Hill plot of $\log(Y/(1 - Y))$ vs. $\log([Duper])$ accomplishes. The slope is equal to the Hill coefficient and the concentration where the x-axis is intercepted is the (log of) the midpoint concentration.



- b) From the data, you can conclude that the binding of Duper to Super is cooperative. The data suggest that there are 2 binding sites that are bound in a “all-or-nothing” fashion; however, there could be more binding sites that binding with less than perfect cooperativity.

- c) From the crystal structure with 4 binding sites, one can conclude that the binding exhibit cooperativity, but that the binding is not completely “all-or-nothing”, since the Hill coefficient is less than 4.

Problem 3

Free vs. bound ligand. This problem follows essentially Problem 6.3 or 6.4, respectively, from the first or second edition of *Physical Biology of the Cell* by Phillips *et al.*

- a) To derive an expression for the fraction bound as a function of $[L]_{tot}$ and $[R]_{tot}$, we can start with the definition

$$Y = \frac{[RL]}{[R]_{tot}} = \frac{[RL]}{[R] + [RL]} = \frac{[L]/K_d}{1 + [L]/K_d} \quad (1)$$

where we have used the definition of the dissociation constant

$$K_d = \frac{[L] \cdot [R]}{[RL]} \Rightarrow [RL] = \frac{[L] \cdot [R]}{K_d} \quad (2)$$

in the last step in equation 2.

We now need to find expression of Y in terms of $[L]_{tot}$, $[R]_{tot}$, and K_d only. Total ligand concentration is the sum of free and bound ligand:

$$[L]_{tot} = [L] + [RL] \Rightarrow [L] = [L]_{tot} - [RL] \quad (3)$$

Similarly, total receptor is the sum of free and bound receptor:

$$[R]_{tot} = [R] + [RL] \Rightarrow [R]_{tot} = \frac{K_d[RL]}{[L]} + [RL] \quad (4)$$

In the last step, we have again used the definition of the dissociation constant. Now we plug in the last expression in equation 4 into 5:

$$[R]_{tot} = \frac{K_d[RL]}{[L]_{tot} - [RL]} + [RL] \quad (5)$$

The last expression can be rearranged to be a quadratic equation for $[RL]$, by multiplying through with the denominator and rearranging terms

$$[R]_{tot}[L]_{tot} - [R]_{tot}[RL] = K_d[RL] + [L]_{tot}[RL] - [RL]^2 \quad (6)$$

Now, we bring this into the standard form for quadratic equations:

$$[RL]^2 - ([R]_{tot} + [L]_{tot} + K_d)[RL] + [R]_{tot}[L]_{tot} = 0 \quad (7)$$

This can be solved by quadratic completion (known in German as the “p-q-formula”) and gives:

$$[RL] = \frac{([R]_{tot} + [L]_{tot} + K_d) - \sqrt{([R]_{tot} + [L]_{tot} + K_d)^2 - 4[R]_{tot}[L]_{tot}}}{2} \quad (8)$$

Note that the other solution (with a + in front of the square root) gives physically meaningless results (you obtain negative dissociation constants from the fit). Finally, we recall that $Y = \frac{[RL]}{[R]_{tot}}$, the starting point of our derivation, and write

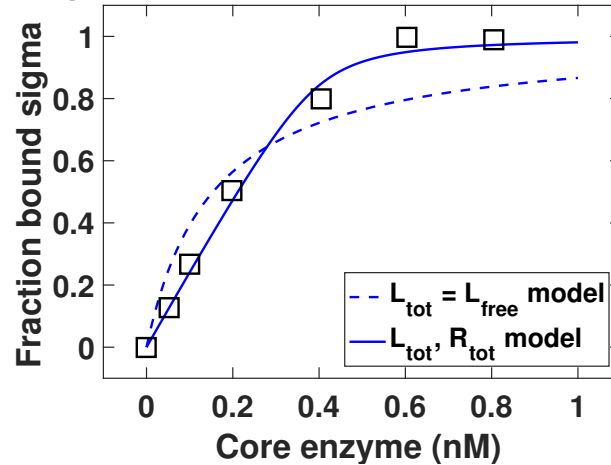
$$Y = \frac{([R]_{tot} + [L]_{tot} + K_d) - \sqrt{([R]_{tot} + [L]_{tot} + K_d)^2 - 4[R]_{tot}[L]_{tot}}}{2[R]_{tot}} \quad (9)$$

As desired, this gives us an expression of Y as a function of $[L]_{tot}$, $[R]_{tot}$, and K_d only. This expression can be used to fit K_d to the data, see the separate matlab code for part c.

- b) Briefly, in the limit where $[L]_{tot} \gg [R]_{tot}$, we also have $[L]_{tot} \gg [RL]$ and thus $[L]_{tot} \approx [L]$ and we do not need to distinguish free and bound ligand concentration and can use equation 2.
- c) Inspecting the data of Maeda *et al.*, *Nucleic Acids Research* (2000), for core enzyme binding to the σ^{70} subunit, we see that the core enzyme concentrations (the ligand, in this case) are in the range of 0 to 0.8 nM, while the receptor concentration (σ^{70}) is at a concentration of 0.4 nM; clearly we do not have $[L]_{tot} \gg [R]_{tot}$ and need to fit our modified model.

If we fit the standard expression (dashed line below), we obtain a relatively poor fit and an apparent dissociation constant of 0.15 nM. Fitting the correct model, we obtain a much better fit (solid line) and a significantly lower dissociation constant of 0.012 nM, corresponding to higher affinity. This is to be expected: Given the high receptor concentration, at the same total ligand concentration the free ligand concentration is always going to be lower than it would be for a lower receptor concentration. Therefore, the true affinity needs to be higher than it would have to be in the $[L]_{tot} \gg [R]_{tot}$ case to see e.g. half maximal binding at a certain concentration.

Simple Kd = 0.15 nM; Correct Kd = 0.012 nM



The matlab code used for fitting the two models and plotting the results is available on the course website.

Problem 3

Mean-first-passage-time analysis. The dissociation constant k_{off} can be calculated as follows: Let's assume a stability constant per base pair of $s = k_+/k_- = \exp(-\Delta G_{bp}/k_B T)$ and the mean first passage time $t \approx s^{n-N} \cdot (2k_+(n-N))^{-1}$ with n being the number of base pairs in a duplex and N the lower bound of stable base pairs in a duplex (here we assume $N = 3$). This leads to a dissociation constant $k_{off} = 1/t = s^{-(n-N)} \cdot 2k_+(n-N)$. If we now assume $-\Delta G_{bp} \approx 3k_B T$ per base pair and a base reformation rate of $k_+ = 10^7$ per second we can calculate the dissociation rate in a straightforward fashion:

- a) For a 9-mer duplex: $k_{k_{off}} = 1.8$ per second
- b) For a 10-mer duplex: $k_{off} = 0.1$ per second

Problem 4

Folding DNA and proteins.

- a) The minimum and maximum RMS values are 1.122112 nm and 5.755623 nm, respectively.
- b) Single layer origami structures are based on parallel DNA double helices that are connected to each other by inter-strand crossovers every 32 bp. As discussed in class, this value was chosen to be approximately commensurable with the helical periodicity of double-stranded DNA in its B form, which is 10.5 bp per helix turn. The difference in 'linking number' between origami DNA and relaxed B-DNA is therefore $\Delta Lk = Lk - Lk_0 = 1/10.67 - 1/10.5 = -0.00152$ per bp, which means that each helix in a flat origami structure is slightly underwound. In the absence of writhe, this should result in a right-handed superhelical twist with a period of approximately $|1/\Delta Lk| \approx 658$ bp = 224 nm, which relieves the strain of the local underwinding.
- c) In order to diminish the global twist observed in the structures, one can introduce so-called targeted deletions to reduce the initially imposed double-helical twist density to 10.5 bp/turn. This can be implemented by removing a single bp from all helices in a cross section of the structure every 64 bp (i.e. approximately every 6 turns).