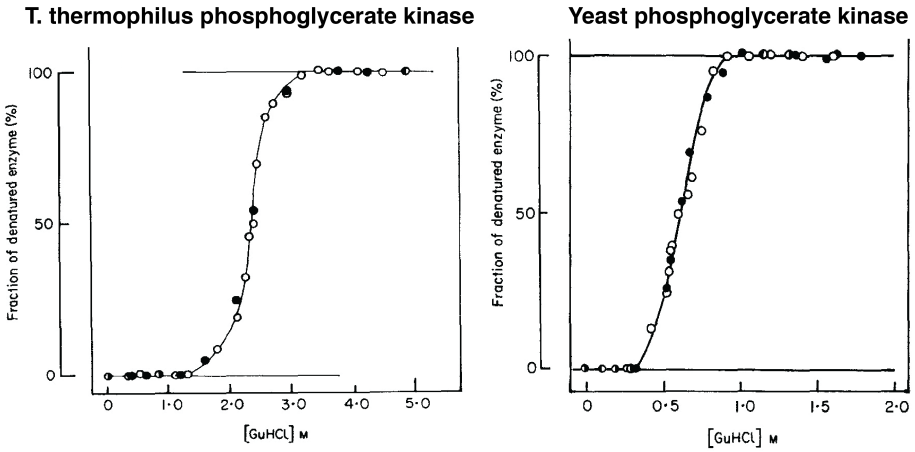
Problem set 3
(Discussion on May 14)

Problem 1

Two-state protein folding, continued. In Problem 2 of the last problem set (PS 2), we have derived some basic relationships regarding two-state protein folding. Here, we will apply the expressions for protein folding equilibria to real data. Nojima *et al.* (*J. Mol. Biol.*, 1977) have investigated the unfolding of phosphoglycerate kinase (PGK) by guanidinium hydrochloride (GdnHCl). They have studied two variants of PGK: One from *T. thermophilus*, an extremely thermophilic bacterium, and one from yeast. Below are plots of the fraction unfolded as a function of GdnHCl concentration from their paper, determined by circular dichroism spectroscopy (open symbols) or fluorescence intensity (black symbols).



a) Have a look at the data shown above. Which of the two PGK variants do you think is more stable in the absence of denaturant? Why?

b) In the last problem set, we saw that the effect of denaturants on the free energy of folding is often in good approximation linear: $\Delta G_{\text{fold}}([D]) = \Delta G_0 + m \cdot [D]$, where ΔG_0 indicates the thermodynamic stability of the protein in the absence of denaturant and m is a coefficient that describes the dependence of the free energy on the denaturant concentration $[D]$. I have digitized the black data points from the two panels shown above, which are available as text files for download on the class website. Fit the expression for the fraction unfolded (or denatured) as a function of denaturant concentration that we derived on the last problem set to the data by Nojima *et al.*; treat ΔG_0 and m as free fitting parameters. You can use any software you like (matlab and python are likely good choices). Show a plot of your fit to the data.

c) From your fit: What do you find for ΔG_0 and m for *T. thermophilus* and yeast PGK? What concentrations correspond to the midpoint of the transition ($[GdnHCl]_{1/2}$)?

d) Compare the results from your fit to your initial assessment in part a) and to the values of ΔG_0 and m reported in the paper.

Problem 2

Humpty-Dumpty binding cooperativity. You do a series of experiments with a protein called Humpty. It binds a ligand called Dumpty. In your experiments you measure the amount of binding of Humpty with different concentrations of Dumpty. 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 Humpty and Dumpty from the fits to the Hill equation?

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

Problem 3

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) Maeda *et al.*, *Nucleic Acids Research* (2000) (`https://www.ncbi.nlm.nih.gov/pmc/articles/PMC110723/`), measured binding curves for different σ subunits binding to the RNA polymerase core enzyme. The 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.