Graduate Topics in Biophysical Chemistry – CH 8990 03
Assignment 2

Due Monday, February 10

To complete this assignment, you will need to use the program Gnuplot to perform nonlinear least squares fitting. You can find a tutorial for Gnuplot on the course web page; it is highly recommended that you read the tutorial before beginning with the computational portions of this assignment.

Of course, if you want to use Matlab, Kaleidagraph, or any other program for performing the analysis, you are welcome to do so, as long as they can provide estimates of the uncertainties and the reduced chi-square value.

1. On the course web page you will find some sample steady-state enzyme kinetics data (`kinetics.txt`). According to the standard Michaelis-Menten model, steady state kinetics data should fit the following curve:

$$v_0 = \frac{v_{max}}{\frac{K_M}{[S]} + 1}$$

In this model, v_0 is the initial velocity of substrate production (concentration per unit time), K_M is the Michaelis constant, v_{max} is the maximum velocity, and [S] is the substrate concentration.

a.) Using Gnuplot, find the v_{max} and K_M values for this particular enzyme. Do not linearize the plot. Submit the values for the reduced chi-square, as well as the parameters with their standard error. Submit a plot of your fit along with the plot of the residuals. In a few sentences, write whether you believe this is a good fit and why.

b.) Traditionally, scientists have linearized kinetics data to a form called the "Lineweaver Burk plot." The form of this plot is below:

$$\frac{1}{v_0} = \frac{K_M}{v_{max}}\frac{1}{[S]} + \frac{1}{v_{max}}$$

This plot has the advantage of being linear: by plotting $1/v_0$ vs. $1/[S]$, one can easily determine values for the parameters from the slope and intercept. Unfortunately, the uncertainties are much less certain because of the transformations made to the data.

Perform a detailed analysis of fitting with the Lineweaver-Burk plot and the kinetic data from part (a). You may assume that there is no uncertainty in [S], but you will have to transform the uncertainties for v_0 using error propagation formulas. In Gnuplot, fit a generic line ($f(x) = mx + b$) to your data (do not try to explicitly model the ratio of K_M and v_{max}, as this will make your uncertainties worse). Then, using error propagation formulas again, you will have to calculate uncertainties for K_M and v_{max} from the slope and intercept of the line.

Submit the results of your fitting, including: (1) how you obtained uncertainties for $1/v_0$, (2) A copy of the transformed data (it's okay to use an Excel printout), (3) Your derivation for uncertainties in K_M and v_{max} given the uncertainties in the slope and intercept, (4) The output of the fitting routine from Gnuplot, and (5) a plot of your fit along with the residuals.

c.) Comparing your results in parts (a) and (b), what are your observations? Considering the accuracy, precision, and amount of effort, which method is better?

2. Many proteins unfold according to the following two-state chemical reaction.

$$N \rightarrow U$$

As discussed in class, this reaction has an equilibrium constant, which (for true two-state unfolding) is related to the standard state molar free energy ($\Delta\bar{G}^0$). Unfolding is unfavorable for most proteins, so we expect $\Delta\bar{G}^0$ to be positive (approximately 5-10 kcal/mol)

One frequently-used method for determining $\Delta\bar{G}^0$ is to perturb equilibrium using a chemical denaturant such as urea or guanidinium chloride. It has been found that, to a very good approximation, $\Delta\bar{G}^0$ changes linearly with added denaturant. In that case, the following equation applies (for more information, see Santoro and Bolen. (1992) *Biochemistry.* **31**: 4901).

$$\Delta\bar{G}^0 = \Delta\bar{G}^0(H_2O) - m[Urea]$$

In this equation, $\Delta\bar{G}^0$ is the observed unfolding free energy at a given urea concentration, and $\Delta\bar{G}^0(H_2O)$ is the free energy in the absence of urea (presumably, this represents physiological conditions). *m* is a protein-specific positive constant, on the order of 1-5 kcal mol^{-1} M^{-1}.

As we will discuss later in the semester, CD spectroscopy can often be used to assess how folded a protein is. In the CD spectra, each state (N or U) makes a contribution to the overall signal:

$$\theta_f = a_f[Urea] + b_f$$
$$\theta_u = a_u[Urea] + b_u$$

Where θ_f and θ_u are the ellipticities of the folded and unfolded states, respectively. The observed CD signal is then the weighted average of the ellipticities, like so:

$$\theta_{obs} = f_f\theta_f + f_u\theta_u$$

Where f_f is the fraction of protein that is folded, and f_u is the fraction of protein that is unfolded.

a.) Using basic physical chemistry relationships and the information given above, write an equation for the observed CD signal as a function of urea concentration for a protein

unfolding experiment. Your expression should contain [*Urea*], a few physical constants, the temperature, and the model parameters a_f, b_f, a_u, b_u, m, and $\Delta\bar{G}^0(H_2O)$.

b.) The file `folding.txt` on the course website contains some sample folding data. How many degrees of freedom are there for fitting the model in part (a) to your data. You may assume that the temperature is 298K with negligible uncertainty.

c.) Using Gnuplot, determine the best-fit parameters for this dataset. Submit the script you used to perform the fit, along with your parameters (the output of the fit in human-readable form), the reduced chi-square, and a plot of the fit and residuals.

d.) What is your assessment of the fit? Given the value of the reduced chi-square, do the data fit well to your model? Use the table in Appendix D of Taylor to assess the significance of your reduced chi-square value.

3. Briefly explain how the hydrophobic interaction is different at low temperature vs. high temperature. If you're stuck, you may want to take another look at Dill's "Dominant Forces in Protein Folding" review.

4. For a certain protein, a grad student measures thermodynamic parameters for unfolding. She determines that $\Delta\bar{H}^0$ is 100 kcal mol^{-1}, and $\Delta\bar{S}^0$ is 300 cal mol^{-1} K^{-1}.

a.) Explain (in plain English) the physical reasons why temperature denatures proteins. Do not discuss thermodynamics – instead give you answer in terms of the atomic forces themselves.

b.) Is this reaction endothermic or exothermic?

c.) Assuming $\Delta\bar{H}^0$ and $\Delta\bar{S}^0$ are temperature-independent, calculate $\Delta\bar{G}^0$ and K_{eq} at 35 ^{o}C. Make sure your units agree!

d.) What is the fraction of proteins that are folded at this temperature?