Origin Assignment 3
Due: Friday, March 2, noon

Protein Molecular Weight Determination by Sedimentation Equilibrium

A single domain protein was analyzed in a sedimentation equilibrium experiment. Details of the experiment are:

rotor speed = 40,000 rpm
Temperature = 20 °C
Buffer density = 1.026 g/mL

Determine the molecular weight of the protein from the experimental data given below.

The equilibrium distribution of the protein concentration versus radial position in the sample cell was determined at three different initial (i.e. uniform) protein concentrations (0.2, 0.5 and 1.0 mg/mL). It may be assumed that the protein did not engage in a monomer-dimer equilibrium, and that the protein solution exhibited ideal behavior.

The procedure:

(1) Plot the protein concentration (in units of 'interference fringes') versus the radius squared (x variable). (These data sets are found in Origin_Assign_3_AUC_Data.xlsx at the course website.)

(2) Evaluate the quality of the data to decide whether some portion of it can be reasonably excluded from the analysis, *e.g.* data points near the ends of the distributions.

(3) Fit the concentration versus the square of the radial position to the 'ExpGrow1' function in Origin:

$$y = y_0 + A_1\exp((x-x_0)/t_1)) \quad (1)$$

(or you may write your own). Conduct a global fit of the three data sets, where some variables are shared and some are not. y_0 and A_1 can be treated as independent among the three data sets (0.1, 0.5 and 1 mg/mL of protein 'X') and are allowed to adjust during the fitting procedure. x_0 is fixed and shared among the three sets of data. t_1 is also shared and allowed to adjust during the fitting procedure.

(4) *Calculate the Molecular Weight*. The molecular weight is determined from an analysis of t_1 from the perspective of the sedimentation equilibrium experiment. (In other words, the best-fit value of t_1 determined from the fit, can be used to calculate the molecular weight.) The form of the exponential function that describes the protein concentration as a function of the radial position, and it's dependence on molecular weight, is discussed in the textbook (Cooper, Ch. 4), the (supplemental) textbook by Berg, Random Walks in Biology (Ch. 5) and in the booklet *Introduction to Analytical Ultracentrifugation* posted on the course website.