Measurement of Protein-Protein Interactions by Fluorescence Anisotropy

The experiments described by Silversmith described in the accompanying research article (Silversmith, 2005) measured the binding interaction between two proteins by following the change in the polarization of fluorescence from the labeled CheZ protein upon binding. Key facts:

- The protein, CheZ, is a dimer. Each subunit in the CheZ dimer binds one molecule of CheY (1:1 binding stoichiometry).
- A fluorescent organic molecule 'chromophore' or 'fluorophore' was attached to CheZ at various places. In this study, the chromophore was fluorescein.
- The polarization anisotropy of the fluorescence emitted from labeled CheZ was measured as a function of the added CheY concentration.
- The polarization anisotropy changed in proportion to the amount of CheY bound to CheZ.

Using the data in the associated Excel file (Origin_Assign_4_LigBind_Data.xlsx), determine the equilibrium binding, (K_D), constant between CheZ and CheY. Different function will be evaluated for describing the binding interaction.

Procedure

1. Plot the data for Fl-CheZ$_{180}$ in Origin. What do you observe? Why?

2. Fit the data to a single-set-of-sites binding model. You can start by assuming the [CheY] = [CheY]$_{tot}$. What is the expression for the anisotropy as a function of the CheY concentration (Y for short)?

$$\mathrm{An}(\mathrm{Y}) \propto \frac{K_A[\mathrm{Y}]}{1+K_A[\mathrm{Y}]} \qquad \mathrm{An}(\mathrm{Y}) = \frac{CK_A[\mathrm{Y}]}{1+K_A[\mathrm{Y}]} = \frac{C[\mathrm{Y}]}{K_D+[\mathrm{Y}]}$$

3. Can you find a suitable function in Origin? Use it to fit the data. Did it work? If not, what did you do to figure this out?

4. Introduce a new function for the anisotropy:

An(Y) = A0 + A1*K_A*[Y]/(1 + K_A*[Y])

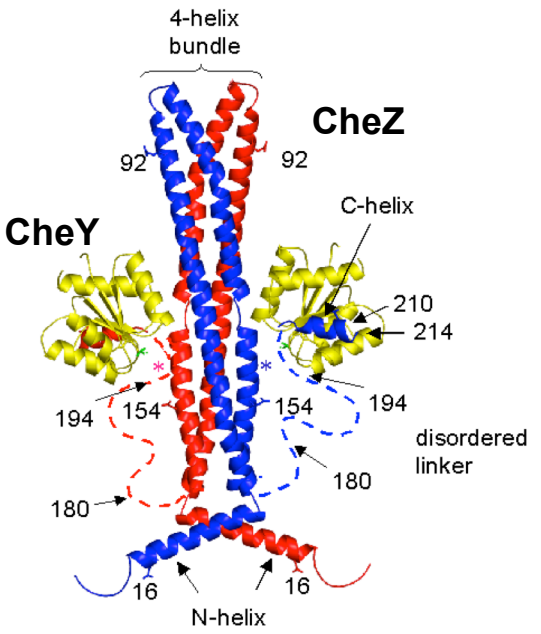
5. Does this function fit the data better? Re-examine the assumption that the free concentration of CheY is equivalent to the total concentration of CheY, *i.e.* [Y] = [Y]$_{tot}$. Is it safe to make this assumption? What do you need to do to change this? ([CheZ]$_{total}$ = 0.2 μM).

To fit the binding isotherm data without assuming [Y] = $[Y]_{tot}$, express [Y] as a function of $[Y]_{tot}$, $[Z]_{tot}$ and K_A. (This still requires an assumption about the underlying equilibrium.) Use the expression for [Y] = $f([Y]_{tot}, [Z]_{tot}, K_A)$ in the above equation to find better estimates of K_A.

$An(Y) = A0 + A1*f_B([Y]_{tot}, [Z]_{tot}, K_A)$

Here, f_B is the fraction of CheZ with CheY bound, not to be confused with f, the function for the free concentration of CheY ([Y]).

6. Fit all the data sets to the model (individually and globally) that does ***not*** assume $[Y]_{free} = [Y]_{total}$. "Share" the value of K among all the appropriate data sets. *In the report, tabulate the values of K obtained from individual fits with K obtained in the global fit and briefly discuss the merits of global fitting in this situation (if there are any).*



(Silversmith. 2005. *Biochemistry* **44**, 7768-76.)

The crystal structure of the CheZ dimer:CheY complex. The CheZ dimer is a four helix bundle generated by the association of two coiled-coil hairpins (red and blue). The dotted lines represent unstructured regions of CheZ. One CheY (yellow) protein binds to each CheZ subunit. This unstructured region connects the main part of the bundle to a c-terminal helix that forms a binding interaction with CheY. Numbers along the CheZ polypeptide are locations at which the fluorescein chromophore was attached to individual site-directed cysteine mutants.