A model for protein equilibrium denaturation

In this text we derive an equation that describes the equilibrium denaturation experiment for two-state proteins, as measured by tryptophan (Trp) fluorescence. Shortly, the basis of the experiment is that the amount of denaturant modifies the folding equilibrium of the protein, such that the fluorescent signal decreases as the concentration of denaturant increases. The goal is to obtain an expression that describes the fluorescent intensity as a function of the denaturant concentration, so we are aiming for some function of the form:

$$I = f([\text{denaturant}])$$

We start by recalling the protein denaturation equilibrium, which we write as:

$$D \rightleftharpoons N$$

where N refers to the **N**ative state and D refers to the **D**enatured state. The result of each measurement depends on the amounts of protein populating each state. In this case, the native state is the source of fluorescence, so the signal increases as the fraction of native protein, f_N, grows.

$$f_N = \frac{I - I_D}{I_N - I_D} \tag{1}$$

where I is any given measurement, I_N and I_D are the *characteristic* values of the native state and denatured states, respectively. Simultaneously, f_N is related to the equilibrium constant K by the following expression:

$$K = \frac{N}{D} \Rightarrow f_N = \frac{N}{N + D} = \frac{K}{K + 1}$$

The expression above can be further manipulated so that K appears only once:

$$f_N = \frac{K}{K + 1} = \frac{1}{1 + \frac{1}{K}} \tag{2}$$

By virtue of the basic thermodynamic relationship $\Delta G = -2.3RT\log(K)$ we can rearrange eq. 2 as follows:

$$\begin{aligned} f_N &= \frac{1}{1+\frac{1}{10^{-\Delta G/RT}}} \\ &= \frac{1}{1+10^{\frac{\Delta G}{RT}}} \end{aligned} \tag{3}$$

Together with to eq. 1, eq. 2 allows us to connect I with ΔG, by equating the right-hand side of both expressions.

$$\frac{I - I_D}{I_N - I_D} = \frac{1}{1+10^{\frac{\Delta G}{RT}}}$$

This way, the intensity measurement (I) can be related to the Gibbs free energy change (ΔG) so it verifies that:

$$I = \frac{I_N - I_D}{1+10^{\frac{\Delta G}{RT}}} + I_D \tag{4}$$

That ΔG is not a thermodynamic constant, it is itself dependent of the concentration of denaturant. In fact, we know what that dependence look like: ΔG changes linearly with the concentration of denaturant—this is again analogous to what we did in the log-linear method.

$$\Delta G = \Delta G^0 + \gamma[\text{denaturant}]$$

We can more conveniently rewrite this expression by exploiting the fact that, at the midpoint, $K = 1 \implies \Delta G = 0$, so we can replace $\Delta G^0 = -\gamma[\text{denaturant}_{\frac{1}{2}}]$ and obtain:

$$\begin{aligned} \Delta G &= \gamma([\text{denaturant}] - [\text{denaturant}]_{1/2}) \\ &= \gamma(x - x_0) \end{aligned} \tag{5}$$

By substituting eq. 5 into eq. 4, we get for the first time an expression that connects I with [denaturant]:

$$\begin{aligned} I &= \frac{I_N - I_D}{1+10^{\frac{\gamma(x-x_0)}{RT}}} + I_D \\ &= \frac{I_N - I_D}{1+10^{m(x-x_0)}} + I_D \end{aligned} \tag{6}$$

where we have consolidated γ, R and T into a single constant $m = \gamma/RT$. For the sake of simplicity, we will rename $I_N - I_D = L$ and $I_D = I_0$, so our expression finally becomes:

$$\boxed{I = \frac{L}{1 + 10^{m(x-x_0)}} + I_0} \tag{7}$$

The meaning of the four parameters in that expression (eq. 7) should be clear:

I_0**:** is a reference value of the minimum fluorescence intensity seen during the experiment—often, but not always, that of the fully denatured protein.

L**:** is the amplitud of the signal intensity change between the minimum and the maximum—often, but again not always, between fully D and fully N.

x_0**:** is the concentration of denaturant at which we find the midpoint, at which $K = 1$.

m**:** determines the sharpness of the transition and is related to slope of the free-energy change as we add denaturant (γ).