COUPLED FOLDING AND BINDING (THERMODYNAMIC LINKAGE)

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The "question of the day" in class today asked how one could represent coupled folding and binding using the table of states approach. In this situation, we have a protein that can exist in the folded (N) and unfolded (U) states, and we have a small molecule (L) capable of binding only the folded state (NL). The relevant equilibria are:

$$\mathrm{N} \rightleftarrows \mathrm{U} \qquad \mathrm{K_{eq}} = K_U = \frac{[U]}{[N]}$$

$$\mathrm{N + L} \rightleftarrows \mathrm{NL} \qquad \mathrm{K_{eq}} = K_A = \frac{[NL]}{[N][L]}$$

Given this situation, our protein can adopt three different states. We can write the table of states, assuming that U is the reference state when calculating our weights:

State	Weight	Weight Expression
U	$\frac{[U]}{[U]}$	$w_U = 1$
N	$\frac{[N]}{[U]}$	$w_N = ?$
NL	$\frac{[NL]}{[U]}$	$w_{NL} = ?$

We can use some algebra to determine the empty tables above. For example, if $K_U = \frac{[U]}{[N]}$, then we know that $\frac{[N]}{[U]} = K_U^{-1}$. Similarly, we can show that $\frac{[NL]}{[U]} = \frac{K_A}{K_U}[L]$ by combining the equilibrium expressions above. Thus:

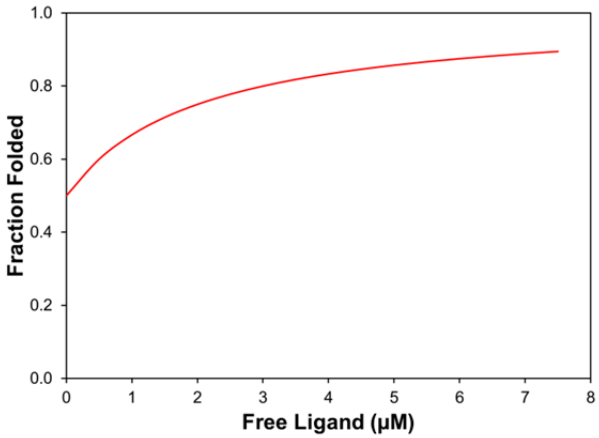
State	Weight	Weight Expression
U	$\frac{[U]}{[U]}$	1
N	$\frac{[N]}{[U]}$	K_U^{-1}
NL	$\frac{[NL]}{[U]}$	$K_A K_U^{-1}[L]$

Now, suppose we want to calculate the fraction of protein folded as a function of free ligand concentration. It makes sense from Le Chatelier's principle that, if a protein is marginally stable, we should be able to "drive" it to the folded state by adding enough L. The fraction of folded protein is measurable for many systems, and it is given by:

$$f = \frac{\text{folded protein}}{\text{total protein}} = \frac{[N] + [NL]}{[U] + [N] + [NL]} = \frac{\frac{[N]}{[U]} + \frac{[NL]}{[U]}}{\frac{[U]}{[U]} + \frac{[N]}{[U]} + \frac{[NL]}{[U]}} = \frac{w_N + w_{NL}}{w_U + w_N + w_{NL}}$$

$$f = \frac{K_U^{-1} + K_U^{-1}K_A[L]}{1 + K_U^{-1} + K_U^{-1}K_A[L]}$$

The expression above isn't pretty, but it makes sense. Suppose in the absence of ligand a protein is marginally stable, i.e. $K_U = 1$, but assume that the ligand has a relatively strong equilibrium constant of $K_A = 1 \times 10^6$. Plotting the expression above gives the following plot:



As we expect, the addition of ligand drives the folding reaction to the folded state.

Does the Reference State Matter?

Alternatively, we could imagine a system where we chose the native state as the reference state. In class, I argued that the observable (fraction folded) cannot depend on the choice of the reference state. This simple system allows us to test this idea. Constructing the same table as above, but choosing N as my reference, I get:

State	Weight	Weight Expression
U	$\frac{[U]}{[N]}$	K_U
N	$\frac{[N]}{[N]}$	1
NL	$\frac{[NL]}{[N]}$	$K_A[L]$

The weights are clearly different, but what happens when I calculate the fraction of folded protein?

$$f = \frac{w_N + w_{NL}}{w_U + w_N + w_{NL}} = \frac{1 + K_A[L]}{K_U + 1 + K_A[L]}$$

This seems different, but if I multiply both the numerator and denominator by K_U^{-1}, I get:

$$f = \frac{K_U^{-1} + K_A K_U^{-1}[L]}{1 + K_U^{-1} + K_A K_U^{-1}[L]}$$

This is identical to the expression we derived assuming that the U state was the reference state. Thus, the choice of reference state makes no difference on the observable expression.

Why Does pH Unfold Proteins? (Bonus Material – Not on Your Exam)

We can extend the treatment above to handle pH effects in protein folding. It is known that many proteins will unfold at extremes of pH. Suppose we have a simple system where both the folded state and the unfolded state bind a proton, but they have different affinities for proton binding. In this situation:

$$\begin{aligned}
\mathrm{N} &\rightleftarrows \mathrm{U} && \mathrm{K_{eq}} = K_U = \frac{[U]}{[N]} \\
\mathrm{N} + \mathrm{H} &\rightleftarrows \mathrm{NH} && \mathrm{K_{eq}} = K_{NH} = \frac{[NH]}{[N][H]} \\
\mathrm{U} + \mathrm{H} &\rightleftarrows \mathrm{UH} && \mathrm{K_{eq}} = K_{UH} = \frac{[UH]}{[U][H]} \\
\mathrm{NH} &\rightleftarrows \mathrm{UH} && \mathrm{K_{eq}} = K_U \cdot \frac{K_{UH}}{K_{NH}} = \frac{[UH]}{[NH]}
\end{aligned}$$

Remember that, for the last expression, we know the equilibrium constant because these equations form a complete thermodynamic cycle. If the sum of all $\Delta\bar{G}^0$'s must be zero around the thermodynamic cycle, then the product of all the K's is equal to one.

As before, we can set up a table of states:

State	Weight	Weight Expression
U	$\frac{[U]}{[U]}$	1
N	$\frac{[N]}{[U]}$	K_U^{-1}
NH	$\frac{[NH]}{[U]}$	$K_{NH}K_U^{-1}[H]$
UH	$\frac{[UH]}{[U]}$	$K_{UH}[H]$

The fraction folded is determined as before:

$$f = \frac{w_N + w_{NH}}{w_U + w_{UH} + w_N + w_{NH}} = \frac{K_U^{-1} + K_{NH}K_U^{-1}[H]}{1 + K_{UH}[H] + K_U^{-1} + K_{NH}K_U^{-1}[H]}$$

$$f = \frac{(1 + K_{NH}[H])}{K_U(1 + K_{UH}[H]) + (1 + K_{NH}[H])}$$

$$f = \frac{1}{K_U \cdot \frac{1 + K_{UH}[H]}{1 + K_{NH}[H]} + 1} = \frac{1}{K_{app} + 1}$$

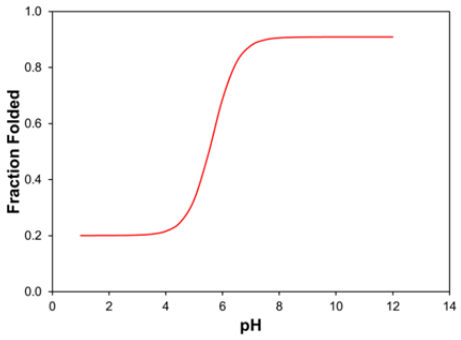
Remember from your homework assignment that the fraction of folded protein in the absence of any pH effects is given by:

$$f = \frac{1}{K + 1}$$

When pH effects are present, the expression has a similar functional form, but the observed equilibrium constant (K_{app}) includes additional terms that depend on the proton concentration. Keeping in mind that proton concentration is given by $pH = -\log[H]$ (alternatively, $[H] = 10^{-pH}$) and that the acid dissociation constants in the bound and unbound form are $K_{UH}^{-1} = K_A^U$ and $K_{NH}^{-1} = K_A^N$ respectively, we can write the apparent equilibrium constant in more conventional terms:

$$K_{app} = K_U \cdot \frac{1 + 10^{(pK_A^U - pH)}}{1 + 10^{(pK_A^N - pH)}}$$

Now suppose that $K_U = 1 \times 10^{-1}$, $pK_A^U = 6.6$, and $pK_A^N = 5$. This corresponds to a histidine residue that has shifted its pK_A in the folded state, possibly because the local environment is somewhat less solvent exposed (and thus the uncharged state is preferred). In this case:



The result here makes sense; as the pH increases, the neutral state of His is favored, which in turn favors folding. This is very complex behavior, but our simple "table of states" approach is nevertheless able to reproduce it.