

Biochemistry – Van't Hoff plots and protein folding.

Consider a protein and a single point mutation in two states, native (folded) and denatured (unfolded).
Native $\rightleftharpoons$ Denatured.

The equilibrium is defined like any other reaction: $K_{eq} = [\text{denatured protein}] / [\text{native protein}]$

If one uses plane polarized light or fluorescence to determine the fraction of protein folded or unfolded at different temps a protein-melting curve (see graph) can be produced.

Both the Enthalpy (ΔH) and entropy (ΔS) can be calculated using a curve of a fraction (%) of protein in folded and unfolded state. The S and H values can be calculated using the van't Hoff equation and plot. The Van't Hoff equation informs about the temperature dependence of the equilibrium constant.

**** To use this equation, the protein must refold – this is an equilibrium problem!**

The van't Hoff equation is derived from Gibbs Free energy equation: $\Delta G = \Delta H - T \Delta S$

Then also understanding that for this reaction of folding and unfolding: $\Delta G = -RT \ln K_{eq}$

We can create the van't Hoff equation to relate equilibrium constant to temperature by substituting the two equations and rearranging to generate the van't Hoff equation:

$$\ln K_{eq} = -\Delta H/R(1/T) + \Delta S/R$$

Note: The equation is a straight line $Y=mx+B$. Therefore a plot of $\ln K_{eq}$ vs $1/T$, known as the van't Hoff plot, yields a straight line of slope $-\Delta H/RT$ and Y intercept $= \Delta S/R$.

Using both the melting or transition curve and Van't Hoff's plot and equation, we can determine the thermodynamic functions of protein stability (the fraction of protein at a given temperature that is native or denatured).

- Determine the fraction of protein folded from the transition curve where $[\text{native protein}] = [\text{denatured protein}]$ and convert that information to K_{eq} for each temperature.
- From this data you can create a van't Hoff plot and...
- Calculate the ΔH and ΔS from the slope and Y intercept.

Ex – for our wild-type protein, determine G, S and H.

1. **Create a van't Hoff plot** - Start by using the conversion of the transition graph to a van't Hoff plot
2. **Calculate ΔH** . The slope of van't Hoff plot will give: ΔH
for a test you would be given this information or the slope and intercept of a van't Hoff plot).
3. **Calculate ΔG** : Using the melting point (T_m) to determine ΔG
 - a. $K_{eq} = [\text{denatured protein}] / [\text{native protein}] = 1.0$. AND
 - b. $\Delta G = -RT \ln K_{eq}$ because, $\ln 1.0$ is 0.0 . Therefore, at the T_m $\Delta G = 0$
4. **Calculate ΔS** : Again, using and rearranging the van't Hoff plot/equation: $\Delta S = \Delta H/T_m$
5. Now you have ΔS and ΔH for this protein and **can determine** the protein stability at **any other temperature** using $\Delta G = \Delta H - T \Delta S$

To determine the stability of a protein with and without some change (ligand binding, protein interaction or mutation), determine the ΔG for each protein $\Delta \Delta G = \Delta G_{\text{wild-type}} - \Delta G_{\text{mutant}}$. A POSITIVE value indicates that the unfolding of the wild type is LESS favorable than the mutant by the calculated value (that the mutation decreases the stability of the native protein). A NEGATIVE value indicates the unfolding of the wild type is More favorable than the mutant (or that the mutation stabilizes the native structure)

