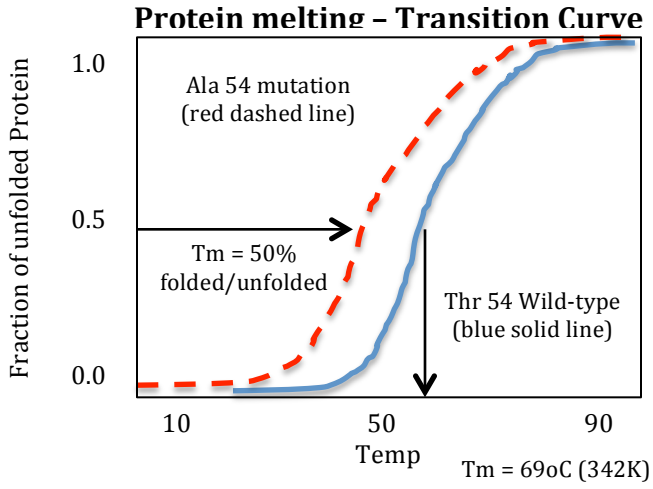
Biochemistry – Van't Hoff plots and protein folding.

Consider a protein and a single point mutation in two states, native (folded) and denatured (unfolded).

Native ⇔ Denatured.

The equilibrium is defined like any other reaction: Keq = [denatured protein] / [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!

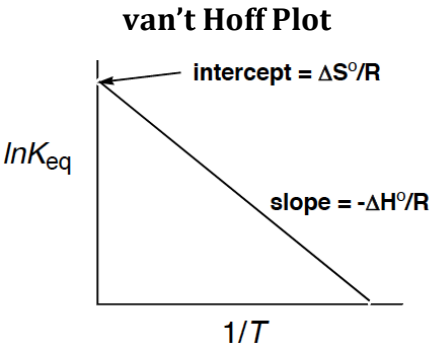
The van't Hoff equation is derived from Gives Free energy equation: $\Delta G = \Delta H - T\,\Delta S$

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

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:

$$\ln Keq = -\Delta H/R(1/T) + \Delta S/R$$

Note: The equation is a straight line Y=mx+B. Therefore a plot of lnKeq vs 1/T, known as the van't Hoff plot, yields a straight line of slope -ΔH/RT and Y intercept = ΔS/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 [native protein] = [denatured protein] and convert that information to Keq 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 an 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 (Tm) to determine ΔG
   a. Keq = [denatured protein] / [native protein] = 1.0. AND
   b. $\Delta G = -RT\ \ln Keq$ because, ln1.0 is = 0.0. Therefore, at the Tm ΔG= 0
4. **Calculate ΔS**: Again, using and rearranging the van't Hoff plot/equation: $\Delta S = \Delta H/Tm$
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 ΔΔG = ΔG wild-type - ΔG mutant. A POSTIVE 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 he native structure)