

Once we have information about the thermodynamics of a process, we can calculate the thermodynamics at other temperatures. This, in turn, can help us understand the molecular mechanisms of biological processes. The general thermodynamic relationships we use are:

$$\Delta H(T) = \Delta H(T_m) + \Delta C_p(T - T_m)$$

$$\Delta S(T) = \Delta S(T_m) + \Delta C_p \ln(T/T_m)$$

where  $T_m$  is the melting temperature in  $K$ ,  $\Delta H_m$  is the change in enthalpy at the melting temperature,  $\Delta S_m$  is the change in entropy at the melting temperature, and  $\Delta C_p$  is the heat capacity change of the reaction.

The following table shows measured values for these thermodynamic parameters for the enzyme RNaseH taken from two bacterial species that grow at radically different temperatures. By convention, studies of protein folding describe reactions as *unfolding* reactions ( $F \rightarrow U$ ), so these terms describe the enthalpy change etc. on *unfolding*:

species	$T_{growth}$ (K)	$T_m$ (K)	$\Delta C_p$ ( $kJ \cdot mol^{-1} \cdot K^{-1}$ )	$\Delta H_m$ ( $kJ \cdot mol^{-1}$ )	$\Delta S_m$ ( $kJ \cdot mol^{-1} \cdot K^{-1}$ )
<i>E. coli</i>	310	341	9.8	513	1.51
<i>T. thermophilus</i>	341	361	7.6	564	1.56

- What is the enthalpy change, entropy change, and free energy to unfold the *E. coli* protein at its growth temperature?
  - What makes the greatest contribution to the unfolding free energy?
  - Does this support, contradict, or say nothing about the statement: “protein folding is driven by the hydrophobic effect”?
- Plot  $\Delta H(T)$  between 200  $K$  and 400  $K$  for the *E. coli* protein. Can you rationalize the slope in molecular terms?
- Plot  $-T\Delta S(T)$  between 200  $K$  and 400  $K$  for the *E. coli* protein. Can you rationalize the curve in molecular terms?
- Plot  $\Delta G(T)$  as a function of temperature.
  - Does the shape of this curve surprise you?
  - Where is the  $T_m$  of the protein on this curve? What is the temperature of maximal thermodynamic stability? Where is the *growth* temperature of *E. coli* on this curve?
  - What happens, molecularly, that causes the protein to unfold at high temperature?
  - What happens, molecularly, that causes the protein to unfold at low temperature?
- Plot  $\Delta G(T)$  for *E. coli* and *T. thermophilus* RNaseH on the same curve.
  - What is  $\Delta G(T_{growth})$  for each protein?
  - What would the stability of the *E. coli* protein be if placed at the *T. thermophilus* growth temperature, and the stability of the *T. thermophilus* protein if grown at the *E. coli* growth temperature?
  - Can you speculate about what evolution “cares about” based on these studies?