

Quantitative Biology and Biophysics – 2020

Lab 1 - Conformational Equilibrium (2-State)

The goal of this lab is to gain a working knowledge and of, and the ability to use, simulation as a tool to understand biological systems. This exercise introduces you to simulation, asking you to simulate the Gibbs energy and probabilities for a two-state transition with different parameters.

Here are some additional notes and helpful clues to moving forward. I told you in class that in order to simulate a two-state transition, you need two parameters (ΔH and ΔS), which are constant and therefore are the same at every temperature. This is technically not true in most cases. Both entropy and enthalpy are often temperature dependent, which is determined by the heat capacity difference (ΔC_p), obeying the following relationships;

$$\Delta H(T) = \Delta H(T_{ref}) + \Delta C_p \cdot (T - T_{ref}) \quad (1)$$

$$\Delta S(T) = \Delta S(T_{ref}) + \Delta C_p \cdot \ln\left(\frac{T}{T_{ref}}\right) \quad (2)$$

This just means that instead of writing the Gibbs energy at any temperature (i.e., $\Delta G(T)$) in terms of two constant parameters (yellow), as we did in class and as reproduced here;

$$\Delta G(T) = \Delta H - T \cdot \Delta S \quad (3a)$$

Equation 3a becomes

$$\Delta G(T) = \Delta H(T) - T \cdot \Delta S(T) \quad (3b)$$

where the $\Delta H(T)$ and $\Delta S(T)$ vary with temperature according to eqs. 1 and 2. This means that to get an expression for the Gibbs energy at any temperature (i.e., $\Delta G(T)$) expressed in terms of constants, you need just to substitute eqs. 1 & 2 into eq. 3b and you get;

$$\Delta G(T) = \Delta H(T_{ref}) + \Delta C_p \cdot (T - T_{ref}) - T \cdot \left(\Delta S(T_{ref}) + \Delta C_p \cdot \ln\left(\frac{T}{T_{ref}}\right) \right) \quad (3c)$$

which has four constants (yellow), the enthalpy and entropy at some reference temperature, T_{ref} (i.e., $\Delta H(T_{ref})$, $\Delta S(T_{ref})$) and ΔC_p .

The next important issue, which we will discuss in a future class, regards rewriting equation 3a in terms of the transition temperature (T_m) and the enthalpy at the T_m (i.e., $\Delta H(T_m)$) instead of in terms of ΔH and ΔS . The reason is entirely practical; it allows you to specify which temperature is the midpoint of the transition between the two states (i.e., where $\Delta G = 0$). We can get this by noting that at the transition temperature (i.e., $T = T_m$), equation 3a gives;

$$\Delta G(T_m) = \Delta H(T_m) - T_m \cdot \Delta S(T_m) = 0$$

which, when rearranged, yields;

$$\Delta S(T_m) = \frac{\Delta H(T_m)}{T_m} \quad (3d)$$

That allows us to rewrite eq. 3a as

$$\Delta G(T) = \Delta H(T_m) - T \cdot \frac{\Delta H(T_m)}{T_m} = \Delta H(T_m) \left(1 - \frac{T}{T_m}\right) \quad (3e)$$

Note, this is exactly equivalent to eq. 3a. Thus, to simulate a two-state transition when $\Delta C_p = 0$, you still need just two parameters, the enthalpy difference at T_m (i.e., $\Delta H(T_m)$) and the transition temperature (T_m), both of which are constant (yellow).

In the case when $\Delta C_p \neq 0$, you need to start with eq. 3c, which after substituting eq. 3d in place of $\Delta S(T_{ref})$ becomes;

$$\Delta G(T) = \Delta H(T_m) + \Delta C_p \cdot (T - T_m) - T \cdot \left(\frac{\Delta H(T_m)}{T_m} + \Delta C_p \cdot \ln \left(\frac{T}{T_m} \right) \right) \quad (4a)$$

And upon rearranging gives;

$$\Delta G(T) = \Delta H(T_m) \left(1 - \frac{T}{T_m}\right) + \Delta C_p \cdot \left[T \cdot \left(1 - \ln \frac{T}{T_m}\right) - T_m \right] \quad (4b)$$

where you can see that there are just 3 constants (yellow), and that when there is no heat capacity difference (i.e., $\Delta C_p = 0$), the second part of the equation (i.e. after the '+' sign) goes to 0, and equation 4b reduces to eq. 3e. Thus eq. 4b is the general expression for the energy difference between two states in a two-state transition. It is the expression you will use in every simulation where the temperature dependence of a conformational transition is modeled.

Assignment:

1. **Free Energy Simulations I (Exploring Enthalpy):** For a 2-state transition, write the expression for the statistical weights and the partition function. On one graph plot ΔG vs T (in Kelvin) for the following three cases,

- Case 1: $\Delta H = 50$ kcal/mol, $T_m = 50^\circ\text{C}$, $\Delta C_p = 0$
- Case 2: $\Delta H = 100$ kcal/mol, $T_m = 50^\circ\text{C}$, $\Delta C_p = 0$
- Case 3: $\Delta H = 150$ kcal/mol, $T_m = 50^\circ\text{C}$, $\Delta C_p = 0$

By inspecting the graph, what's the difference between these cases?

2. Population Simulations I:

- a. For each case from Question 1, make a plot of probability of the folded and unfolded states vs T (between 0 and 100C (i.e., 273 to 373K)).
- b. On one graph, plot the folded probability vs T for each case. What do you observe?

3. Free Energy Simulations II (Exploring Heat Capacity):

On one graph plot ΔG vs T (in Kelvin) for the following three cases,

- a. Case 1: $\Delta H = 100 \text{ kcal/mol}$, $T_m = 50^\circ\text{C}$, $\Delta C_p = 0 \text{ cal/(mol}\cdot\text{K)}$
- b. Case 2: $\Delta H = 100 \text{ kcal/mol}$, $T_m = 50^\circ\text{C}$, $\Delta C_p = 1500 \text{ cal/(mol}\cdot\text{K)}$
- c. Case 3: : $\Delta H = 100 \text{ kcal/mol}$, $T_m = 50^\circ\text{C}$, $\Delta C_p = 3000 \text{ cal/(mol}\cdot\text{K)}$
- d. By inspecting the graph, what's the difference between these cases?

4. Population Simulations:

- a. For each case from Question 3, make a plot of probability of the folded and unfolded states vs T (between -50 and 100C (i.e., 223 to 373K)).
- b. On one graph, plot the unfolded probability vs T for each case.
- c. What do you observe? How do you interpret it?