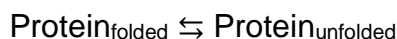


## How does temperature affect protein folding?

The folded and unfolded states of a protein exist in equilibrium in aqueous solutions:



You are exploring this folding and unfolding process, using a total of  $2.75 \times 10^{-3}$  M protein. You have collected concentration data of the folded and unfolded states at various temperatures.

Temperature (°C)	Unfolded Concentration (M)	Folded Concentration (M)
5	$1.9 \times 10^{-3}$	$6.3 \times 10^{-4}$
30	$3.4 \times 10^{-4}$	$2.1 \times 10^{-3}$
60	$2.2 \times 10^{-3}$	$5.6 \times 10^{-4}$

- A. At each temperature, which state is favored? Support your argument with numerical equilibrium constants. *Show your work.*

*Having trouble? Review questions from Chapter 13: 52 and 53.*

$$K = \frac{[\text{unfold}]}{[\text{folded}]}$$

$$5^{\circ}\text{C} = \frac{1.9 \times 10^{-3}}{6.3 \times 10^{-4}} = 3.01$$

$$30^{\circ}\text{C} = \frac{3.4 \times 10^{-4}}{2.1 \times 10^{-3}} = 0.61$$

$$60^{\circ}\text{C} = \frac{2.2 \times 10^{-3}}{5.6 \times 10^{-4}} = 3.93$$

At  $5^{\circ}\text{C}$  and  $60^{\circ}\text{C}$ ,  $K > 1$  indicating a higher concentration of the unfolded protein. At  $30^{\circ}\text{C}$ ,  $K < 1$  indicating a higher concentration of folded protein.

B. For each temperature, if you started with a sample of all folded protein, is the unfolding process spontaneous? Support your argument with numerical free energy values. Show your work.

*Having trouble? Review questions from Chapter 12: 40 and Chapter 13: 89.*

$$\Delta G = -RT \ln K$$

$$\begin{aligned} 5^{\circ}\text{C} \rightarrow \Delta G &= - (8.314 \text{ J/mol}\cdot\text{K}) (278) \ln(3.01) \\ &= - 2.55 \text{ kJ/mol} \end{aligned}$$

$$\begin{aligned} 30^{\circ}\text{C} \rightarrow \Delta G &= - (8.314 \text{ J/mol}\cdot\text{K}) (303 \text{ K}) \ln(0.16) \\ &= 1.01 \times 10^{-4} \text{ kJ/mol} \end{aligned}$$

$$\begin{aligned} 60^{\circ}\text{C} \rightarrow \Delta G &= - (8.314 \text{ J/mol}\cdot\text{K}) (333 \text{ K}) \ln(3.93) \\ &= - 3.79 \text{ kJ/mol} \end{aligned}$$

At  $5^{\circ}\text{C}$  and  $60^{\circ}\text{C}$  unfolding is spontaneous as indicated by the negative value of  $\Delta G$ .  
At  $30^{\circ}\text{C}$  unfolding is NOT spontaneous as indicated by the positive value of  $\Delta G$ .

C. At each temperature, is the favored process (folding or unfolding) driven by entropy or enthalpy? Justify your answer.

*Having trouble? Review questions from Chapter 12: 45, 49, and 50.*

$$\Delta G = \Delta H - T\Delta S$$

At 5°C the unfolding process is entropy driven.  $\Delta G$  is negative, however to break the IMFs holding the folded protein together.  $\Delta H$  will need to be a positive value. The low  $T$  requires a large positive (favorable) value for  $\Delta S$ .

At 30°C folding is the favored process and it needs to be driven by enthalpy. Energy is released from the formation of IMFs in folding, leading to a large negative  $\Delta H$  value.  $\Delta S$  is also negative as folding increases order.

At 60°C unfolding is favored but the process is driven by both entropy and enthalpy. The disorder is increasing and the high temperature adds enough thermal energy to overcome the energy needed to break the IMFs.

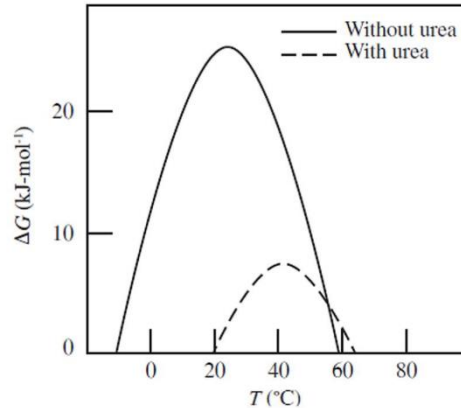
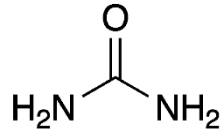
D. Can protein unfolding happen at very low temperatures (below  $-15^{\circ}\text{C}$ ). Why or why not? Explain your answer.

*Having trouble? Review material from Unit 1.*

No, protein folding cannot occur. Below  $15^{\circ}\text{C}$  the solvent ( $\text{H}_2\text{O}$ ), will be a solid and therefore no movement can occur. Protein unfolding requires the ability to move.

- E. Urea is a small molecule (shown below) that can be used to cause protein unfolding. Using the data below and your answers to parts A through C, does the addition of urea affect the folding and unfolding process at low temperatures (0 °C to 25 °C), moderate temperatures (30 °C to 55 °C) or high temperatures (60 °C and above)? Justify your argument.

*Having trouble? Review questions from Chapter 12: 36 and 37.*



We know unfolding is favored at low temps (5 °C) and high temps (60 °C) as seen by the  $K$  values and  $\Delta G$  values calculated. As seen in the graph above the cold temperature denaturation shifted more dramatically (-5 °C  $\rightarrow$  20 °C) than the warm temperature. We also know the cold temp has an enthalpic cost. Urea can replace ~~the~~ some of HMFs in the folded protein.