DNA Hairpin Melting Experiment

In this experiment, you will monitor the order-disorder transition between a folded (hairpin) state of single-stranded DNA, and the unfolded (disordered) state and learn about the strength of the interactions that hold the hairpin together.

Background

At the simplest level of description, the folding-unfolding transition can be described in terms of a two-state chemical reaction:

\[ \text{Unfolded} \xrightleftharpoons[k_c]{k_o} \text{Hairpin} \tag{1} \]

where \( k_c \) is the rate coefficient for the closing (folding) step and \( k_o \) is the rate coefficient for the opening (unfolding) step (see Figure 1). The ratio of the equilibrium populations at temperature \( T \) is given by

\[ \frac{f_h}{f_u} = \frac{k_c}{k_o} = K_{eq} = \exp\left(-\frac{\Delta G}{RT}\right) \tag{2} \]

where \( \Delta G \) is the free energy difference between the hairpin and the unfolded states \( (\Delta G = G_H - G_U) \), \( R \) is the universal gas constant, and \( f_h \) and \( f_u \) are the fraction of molecules in the hairpin and the unfolded states, respectively, at equilibrium.

In a two-state description, Eq. 2 can be rearranged to yield the fraction of molecules in the unfolded conformation as a function of temperature, as:

\[ f_u = \frac{1}{1 + K_{eq}} = \frac{1}{1 + \exp\left[-\frac{\Delta H}{R} \left(\frac{1}{T} - \frac{1}{T_m}\right)\right]} \tag{3} \]

where we have expressed \( \Delta G = \Delta H - T \Delta S = \Delta H (1 - T/T_m) \). Here, \( \Delta H \) is the enthalpy difference and \( \Delta S \) is the entropy difference between the hairpin and the unfolded state, and \( T_m \) is the melting temperature at which half the population is in the unfolded state, \( f_u(T_m) = 1/2 \) (see Figure 2).
Experiment

You will monitor the fraction of unfolded population as a function of temperature, by monitoring the fluorescence of the sample versus temperature $F(T)$. The sample provided to you is a single-strand of DNA with sequence 5'-CGGATXA(T16)TTATCCG-3', where X indicates 2-aminopurine (2AP), which is a fluorescent analog of the adenine base. The fluorescence of 2AP is partially quenched when it is incorporated in single-stranded DNA, and is further quenched when 2AP is base-paired to form duplex DNA. Thus, for this sample, the folded, hairpin structure, in which 2AP forms a Watson-Crick base-pair with thymine to form the stem of the hairpin, is characterized by low fluorescence, while the unfolded structure is characterized by high fluorescence. The melting transition appears as a change in the fluorescence over a narrow temperature range.

In this experiment, you will measure the fluorescence emission spectra of the hairpin as a function of temperature. Define $F(T)$ as the maximum of the fluorescence spectrum at each temperature. The fraction of molecules in the unfolded conformation can then be obtained from

$$f_U(T) = \frac{(F(T) - F_L(T))}{(F_U(T) - F_L(T))}$$

where $F_L(T)$ and $F_U(T)$ are the lower and upper baselines, respectively, and are parameterized as linear functions of temperature.

To fit theory to experiment, you can rearrange Eq. 4 to give:

$$F(T) = f_U(T)[F_U(T) - F_L(T)] + F_L(T)$$

Therefore, the thermodynamic parameters in Eq. 3, $\Delta H$ and $T_m$, can be determined by fitting the experimentally obtained $F(T)$ with Eq. 5, using Eq. 3 to describe $f_U(T)$, and four additional parameters to describe the lower and upper baselines $F_L(T)$ and $F_U(T)$ as straight lines.
General Questions

1) Describe the layout of your experiment.

2) Explain what is meant by fluorescence.

3) What is 2-aminopurine, and how is it different from the adenine base?

4) Explain how you could have used absorbance measurements instead of fluorescence to obtain the thermodynamic parameters for this hairpin, if your DNA strand did not contain 2AP?

5) Explain why the conformation of the molecule changes from an ordered hairpin state to the disordered state as you raise the temperature.

6) Do you expect the molecule to go back to the hairpin state as you lower the temperature? How can you check for this reversibility?

7) Why is the hairpin melting transition called an order-disorder transition, and not a first-order phase transition?

Specific Questions

1) What is the melting temperature of your hairpin?

2) What can you say about the opening (\(k_o\)) and closing (\(k_c\)) rates at (i) \(T < T_m\), (ii) \(T = T_m\), and (iii) \(T > T_m\).

3) How would you expect the melting temperature to change when you change the salt concentration?

4) If the stem of the hairpin was made up of all G-C pairs, would you expect the melting temperature to increase or decrease? Explain.

5) For the same stem, if you were to decrease the size of the loop, would you expect the melting temperature to increase or decrease? Explain.

6) At \(T_m\), what is the equilibrium constant, and what is the value of the free energy difference \(\Delta G\) between the hairpin state and the unfolded state.

7) What is the value of the free energy difference \(\Delta G\) at room temperature?

8) From the best fit values of the parameters in your fitting function, evaluate the difference in entropy between the hairpin state and the unfolded state.
9) Using the relation \( S = k_B \ln \Omega \), where \( k_B \) is the Boltzmann constant, and \( \Omega \) is the number of accessible configurations, calculate by what factor the accessible configurations increase in the unfolded state relative to the hairpin state.

10) Use the program mfold available at the website http://www.idtdna.com/Scitools/Applications/mFold/ to predict the thermodynamic parameters of your hairpin, and compare with your experimental values. Discuss the most likely sources for any discrepancies.

Suggested reading