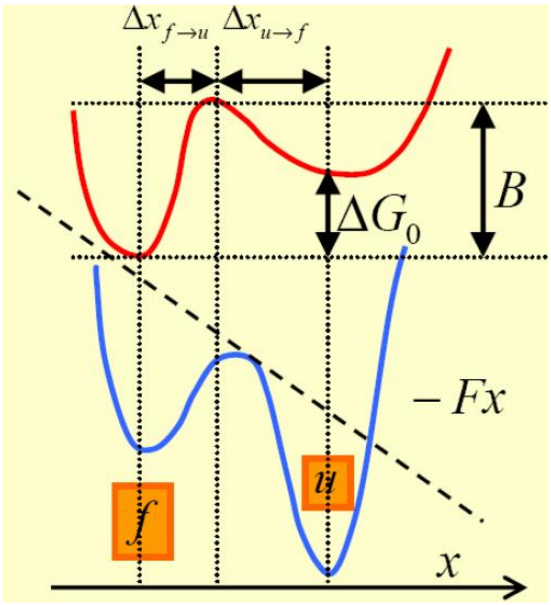
(530190) Methods in Single Molecule Biophysics, Fall 2011

Exercise 4: Wednesday 30.11.2011 at 12:15 in D116



Kramers-Bell theory for unfolding and re-folding

Your friend has done some measurements on an RNA-hairpin of unknown origin. Your job is to help him analyze the data and explain what is happening using an energy-landscape model (see fig).

The data is available from the homepage in the following format ASCII files:

Time (s) X (nm)

NB! The time-step and the duration of the different experiments vary a lot, so be careful! The instrument is running in constant-force mode, and the filenames indicate the force at which the experiment is done:
7 pN 7.5 pN 8 pN 8.5 pN 9 pN

1. Determine dX ($x_{f\text{-}u}$ + $x_{u\text{-}f}$ in the figure)

Find out what the change in length of the hairpin construct is as it unfolds. As the measured signals are noisy, this is probably best done by histogramming the time-series, and fitting Gaussians to the peaks in the histogram. Use a time-series where the peaks are well-defined (the molecules spends roughly 50% in each state)

2. Determine the average state-lifetimes as a function of force

Write a MATLAB-program that reads in the time-series and computes the average lifetime in the folded (shorter) and unfolded (longer) state. You can do this by starting the ‘clock’ for one up-state for example when the signal crosses the average value. Plot the log of the lifetimes (or reaction rates, reaction rate = 1/ lifetime) as a function of force. They should lie roughly on a line.

3. Determine the position of the barrier (xf-u in the figure)

In your semilog plot from prob. 2, the reaction rates (1/lifetime) should be given by the Kramers-Bell expressions:

$$k_{f\to u}(F) = k_{eff}\exp\left[-\frac{(B - F\Delta x_{f\to u})}{k_B T}\right]$$

$$k_{u\to f}(F) = k_{eff}\exp\left[-\frac{(B - \Delta G_0 + F\Delta x_{u\to f})}{k_B T}\right]$$

If you take the logarithm of these expressions you get an equation you can fit to your data in prob. 2 and determine the position of the transition state. Do you get the same value for the barrier position using both the f-u and the u-f formula? Is the barrier closer to the U state or the F state?

4. Determine the $F_{1/2}$, the force at which the molecule spends 50% of its time in the U state and 50% of its time in the F state.

This is also the point at which the reaction rates are equal. So you should look for the point in your prob. 2 graph where the two curves cross.

5. Extrapolate both lines to zero force. If you now subtract the intercepts from each-other, can you determine dG?

6. If, by some other method we are given the fact that k_{eff} is ca. 10^{10} (1/s), then can you determine B?