Force as a variable to study unfolding reactions

- Many biological reactions proceed by large conformational changes that may be force dependent.

- By "stretching" a molecule in a along a particular direction, we can study force induced conformational changes along a well defined mechanical reaction coordinate.

  \[ a \]

  These can be extension, position of a marker along its track etc.

- These experiments often yield information about the free energy landscape of a reaction. Some of the obtainable information are 1) \( \Delta U \) 2) distance to the transition state 3) number of intermediates.

Illustrative example: unfolding of a RNA hairpin

Liphardt et al. Science 2001 Apr 27; 292(5517)
733-737

Simple two-state system

\[ A \leftrightarrow B \]

folded \( \Rightarrow \) unfolded

mechanical coord.: end-to-end distance

(measured as dist)

between the two (see next pg for end set-up)
Exp. design

RNA-DNA hybrid handles

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How does force affect the landscape of an unfolding reaction? ⇒ A theoretical discussion before looking at experimental data

• Effect of force on ΔU:

\[ \Delta U_{A \rightarrow B}(F) = \Delta U_{A \rightarrow B}(F=0) - F(x_B-x_A) + ΔU_{A \rightarrow B}^{\text{stretchy}} \]

\[ A = \text{folded} \quad \frac{A \rightarrow B}{B = \text{unfolded}} \]

\[ \Delta U_{A \rightarrow B}(F=0) = ΔU_{A \rightarrow B}^0 + k_BT \ln \frac{[B]}{[A]} \]
\( \Delta u_{A\rightarrow B} \) arises from the shifting of the minima of the
folded states to the right \( \xi \) is given by:

\[
\Delta u_{\text{stretch}} (F) = \xi \Delta \bar{u} (F) = \bar{x} \Delta \bar{u} (F)
\]

Summary Points:

1. Force "tilts" the free energy landscape of a reaction by \( F_{\text{ext}} \)

\[
\Delta u (F) = \Delta u (0) - F_{\text{ext}}
\]

2. This results in the unfolded state being stabilized greater than the folded state.

3. A more extended molecule is stabilized with force such that \( \Delta x_{A\rightarrow B} (F) > \Delta x_{A\rightarrow B} (F=0) \)

4. Rate of the unfolding reaction increases as the transition state is stabilized more relative to the folded state.

5. At equilibrium \( \Delta u (F) = 0 \)

\[
\Delta u (F) = \Delta u (0) - F_{\text{ext}} + k_B T \ln \frac{[B]}{[A]} + \Delta u_{\text{stretch}} (F) = 0
\]

\[
\Delta u (0) = F_{\text{ext}} - k_B T \ln \frac{[B]}{[A]} + \Delta u_{\text{stretch}} (F)
\]
Experimental results from RNA hairpin unfolding experiments:

- Experiment in which the micropipette attached bead is pulled at a constant velocity thus increasing the force on the molecule at a constant rate.

![Graph showing force vs. extension]

- Stretching & relaxing curves superimpose indicating that the reaction is reversible & happening at equilibrium.

- Because we are seeing a process at equilibrium, "hopping" between folded & unfolded states is observed at the unfolding transition plateau.

- Average area under the unfolding plateau

\[ W = \int_{x_0}^{x_0 + \Delta x} F \, dx = \Delta G \]
Another way to replot the data

\[ \frac{\text{frac unfolded}}{\text{frac unfolded}} = 50\% , \quad K_{eq} = 1 \]

\[ \Delta \alpha = F_{1/2} \Delta x \]

(Also has stiffer component)

\[ \Delta \alpha \approx 150 \text{ kJ/mol} , \text{ compared well with predicted value} \]

Experiment at constant force

\[ F = 15 \text{ pN} \]
\[ F = 14.5 \text{ pN} \]
\[ F = 13.5 \text{ pN} \]
\[ F = 13 \text{ pN} \]

\[ K_{eq} = \frac{k_{\text{fold}, A \to B}}{k_{\text{unfold}, A \to B}} \]

\[ k_{\text{unfolding, } A \to B}(F) = k_o e^{-F \Delta x^*/k_o T} \]

\[ k_o = \text{rate constant @ } F=0 \]
• Reactions not occurring at equilibrium.

For the hairpin with a bulge in the structure:

- Reversible equilibrium process, \( \Delta u = \text{Wrev} \)
- Not at equilibrium, not reversible, \( \text{Wrev} > \Delta u \)

Hysteresis indicates that the reaction is irreversible and not occurring at equilibrium. This results when the loading rate \( (\gamma = \frac{dF}{dt}) \) is greater than the slowest relaxation rate of the system.

Unfolding force depends on loading rate:

- Higher loading rate \( \equiv \) higher unfolding force

As the time spent \( \gamma \) each force is short.

Probability of unfolding as a function of force and loading rate is given by:

\[
P_u(F, \gamma) = 1 - e^{-\frac{k_b k_B T}{\gamma \Delta x} \left( e^{\frac{F \Delta x}{k_B T}} - 1 \right)}
\]
where, $\Delta x^*$ = distance to the transition state
$k_o$ = unfolding rate constant at zero force

The most probable unfolding force is given by:

$$F^* = \frac{k_b T}{\Delta x^*} \ln \left( \frac{\gamma \Delta x^*}{k_o k_b T} \right)$$

For a complex landscape with multiple energy barriers

*significant energy barriers can change when reaction is occurring at different forces

A break in this curves suggests two different transitions

This illustrates why $k_o$ is not a very meaningful quantity as the intercept of the two curves gives different values neither of which may be the true $k_o$ at $F=0$
Discussion points:

- Mechanical versus chemical unfolding results
  - Thermodynamic quantities like $\Delta G$ should be comparable.
  - Kinetic quantities are path dependent & should be compared with a lot of caution.

- Mechanical versus thermodynamic stabilities

  $F^*$
  $\Delta G$
  most probable unfolding force
  free energy

  - These are not the same quantity
  - Mechanical stability depends on both the rate of pulling & the direction of pulling
  - Mechanical stability is the response of a molecule to force & depends on the distance to the transition state. This information is not contained in $\Delta G$

- Biological relevance of mechanical stability

  - Unfolding processes & rupture processes in biology are probably better described by mechanical rather than thermodynamic stabilities. However, it is important to consider direction/pulling rates in-vivo to make the measurement more biologically relevant.