

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 ~~is~~ along a particular direction, we can study force induced conformational changes along a well defined mechanical reaction co-ordinate

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

- These experiments often yield information about the free energy landscape of a reaction. Some of the obtainable information are 1) ΔG 2) distance to the transition state \leftarrow 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



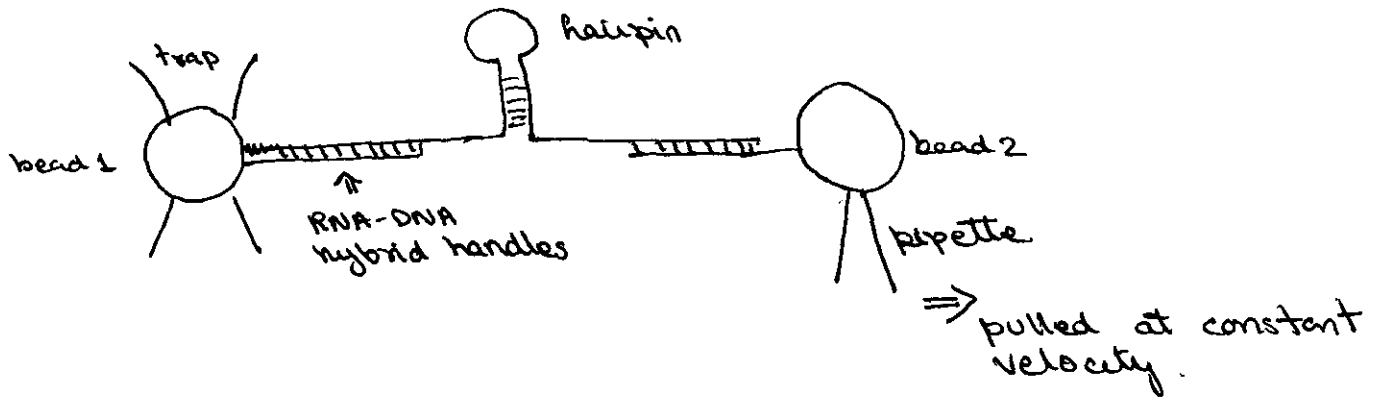
folded \rightleftharpoons unfolded

mechanical co-ord: end-to-end distance

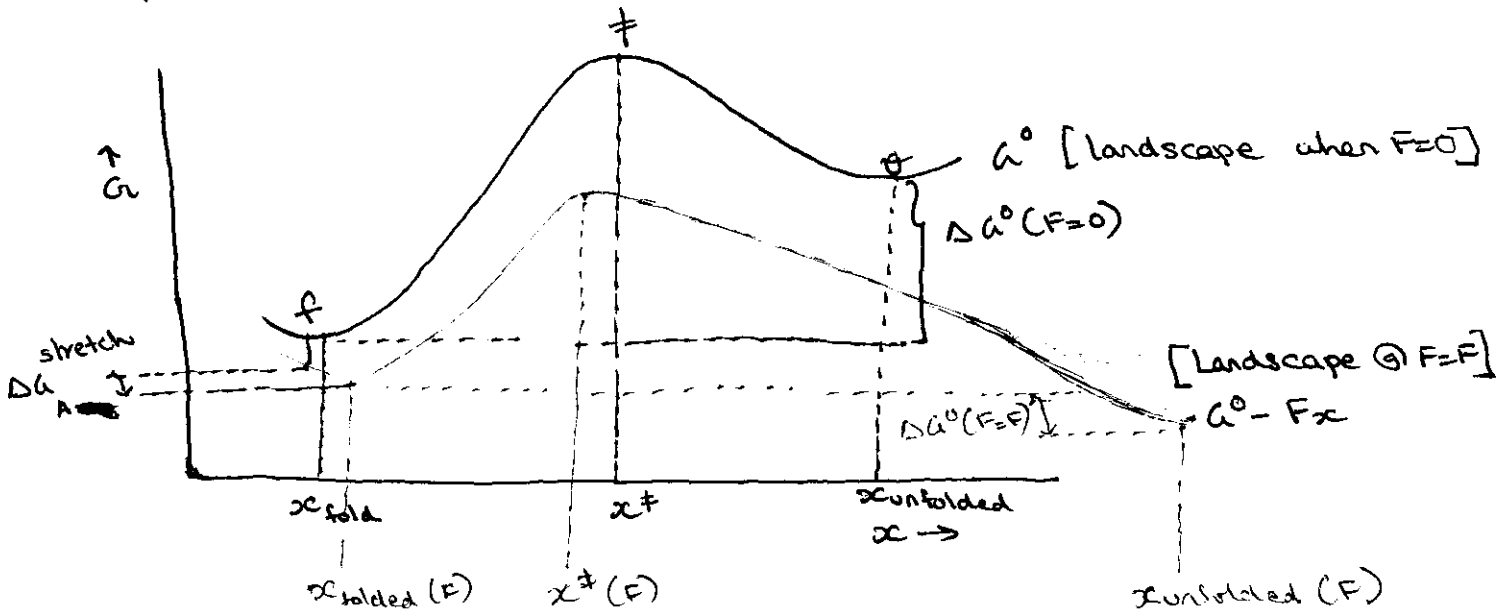
(measured as dist
between the two
beads)

[see next pg for
env set-up]

Exp. design



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



- Effect of force on ΔG :-

$$\Delta G_{A \rightarrow B}(F) = \Delta G_{A \rightarrow B}(F=0) - F(x_B - x_A) + \Delta G_{A \rightarrow B}^{stretching}(F)$$

A \equiv folded
B \equiv unfolded

where, $\Delta G_{A \rightarrow B}(F=0) = \Delta G_{A \rightarrow B}^0 + k_B T \ln \frac{[B]}{[A]}$

$\Delta G_{A \rightarrow B}^{\text{stretch}}$ arises from the shifting of the minima of the ΔG unfolded & folded states to the right x is given by :-

$$\Delta G_{A \rightarrow B}^{\text{stretch}}(F) = \int_{x_B(0)}^{x_B(F)} F dx - \int_{x_A(0)}^{x_A(F)} F dx$$

Summary points :-

- Force "tilts" the free energy landscape of a reaction by $F \cdot x$

$$G(F) = G(0) - F \cdot x$$

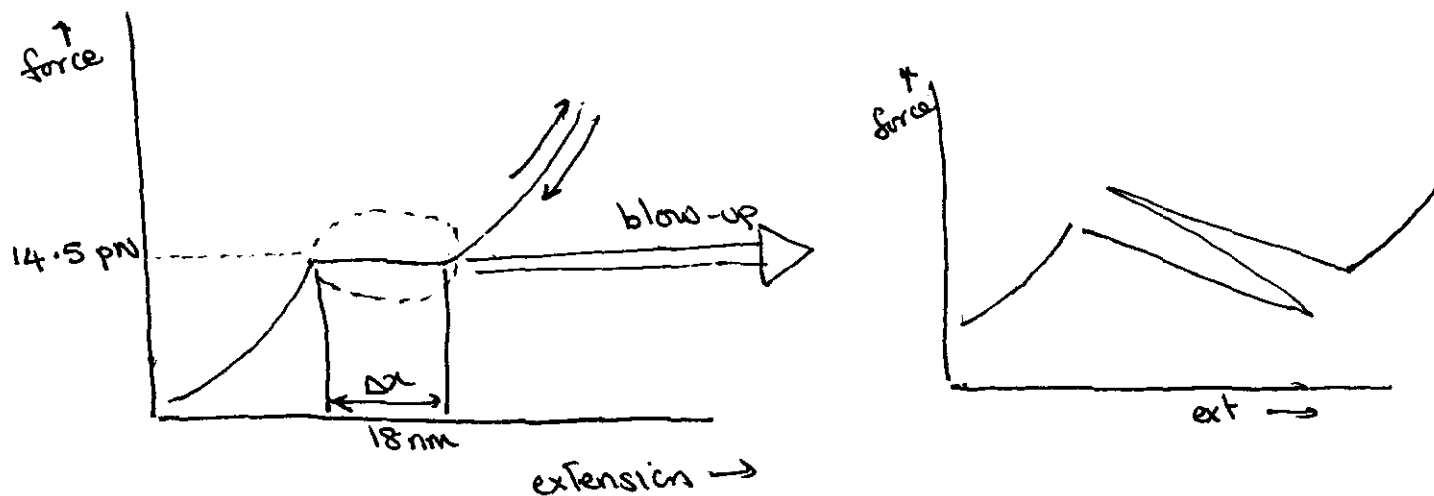
- This results in the unfolded state being stabilized greater than the folded state
- A more extended molecule is stabilized with force such that $\Delta x_{A-B}(F) > \Delta x_{A-B}(F=0)$
- Rate of the unfolding reaction increases as the transition state is stabilized more relative to the folded state
- At equilibrium $\Delta G_{A \rightarrow B}(F) = 0$

$$\Delta G_{A \rightarrow B}(F) = \Delta G^{\circ} - F \Delta x + k_B T \ln \frac{[B]}{[A]} \rightleftharpoons \Delta G_{A \rightarrow B}^{\text{stretch}}(F) = 0$$

$$\Delta G^{\circ} = F \Delta x - k_B T \ln \frac{[B]}{[A]} + \Delta G_{A \rightarrow B}^{\text{stretch}}(F)$$

Experimental results from RNA hairpin unfolding experiments :-

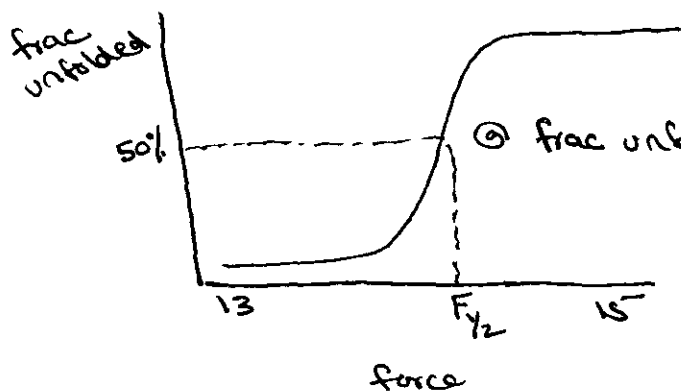
→ Experiment in which the micropipette attached bead is pulled at a constant velocity thus increasing the force on the molecule at a constant rate



- 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

$$W = \int_{x_0}^{x_0 + \Delta x} F \cdot dx = \Delta G^\circ$$

- Another way to replot the data



⊙ frac unfolded = 50% , $K_{eq} = 1$

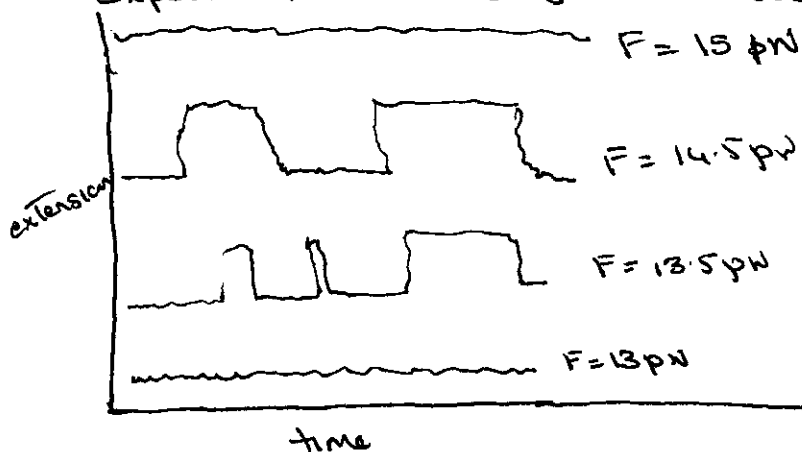
$$\Delta G^\circ = F_{1/2} \Delta x$$

⇓

(Also has stretch component)

ΔG° is 150 kJ/mol, compared well with predicted value

- Experiment at constant force



- From these curves, 2 rate constants for folding & unfolding rcs can be calculated from the lifetimes in the two states as a function of force.

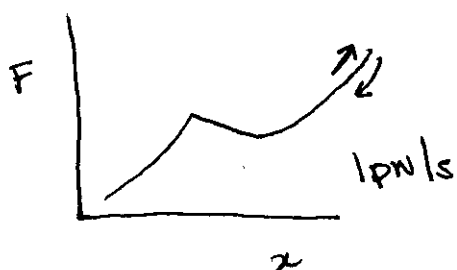
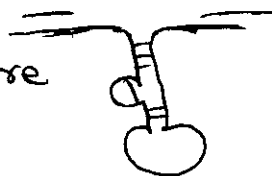
- This gives us information about the distance to the transition state

$$K_{eq} = \frac{k_{fold, B \rightarrow A}}{k_{unfold, A \rightarrow B}}$$

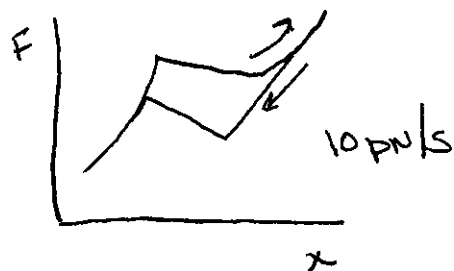
$$k_{unfold, A \rightarrow B}(F) = k_0 e^{-F \Delta x^\ddagger / k_B T} ; \Delta x^\ddagger \Rightarrow \text{dist to transition state}$$

- Reactions not occurring at equilibrium.

For the hairpin with a bulge in the structure



reversible, equilibrium process. $\Delta U = W_{rev}$



$r \neq$ not at equilibrium, not reversible

$$W_{irrev} > \Delta U$$

Hysteresis indicates that the reaction is irreversible & not occurring at equilibrium. This results when the loading rate ($r = dF/dt$) 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 @ each force is short

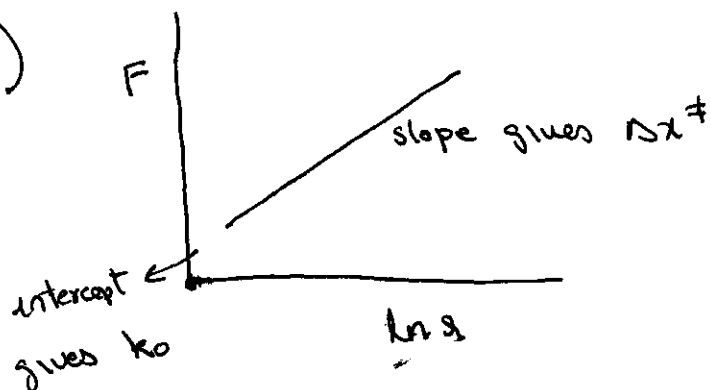
Probability of unfolding as a function of force & loading rate is given by:-

$$P_u(F, r) = 1 - e^{-\frac{k_0 k_B T}{r \Delta x^\ddagger} (e^{F \Delta x^\ddagger / k_B T} - 1)}$$

where, Δx^\ddagger = distance to the transition state
 k_0 = unfolding rate constant at zero force

The most probable unfolding force is given by:-

$$F^* = \frac{k_B T}{\Delta x^\ddagger} \ln \left(\frac{\gamma \Delta x^\ddagger}{k_0 k_B T} \right)$$



For a complex landscape with multiple energy barriers



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



A break in the curves suggests two
 different transitions

This illustrates why " k_0 " is not a
 very meaningful quantity as the
 intercept of the two curves gives
 different values neither of which may
 be the true " k_0 " at $F=0$

Discussion points:-

- Mechanical versus chemical unfolding results
 - Thermodynamic quantities like ΔG should be comparable
 - Kinetic quantities are path dependent & should be compared with a lot of caution.
 - Mechanical ~~versus~~ versus thermodynamic stabilities

\Downarrow	\Downarrow
F^*	ΔG
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 ΔG
- 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.