

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 a~~ 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)  $\Delta G$  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



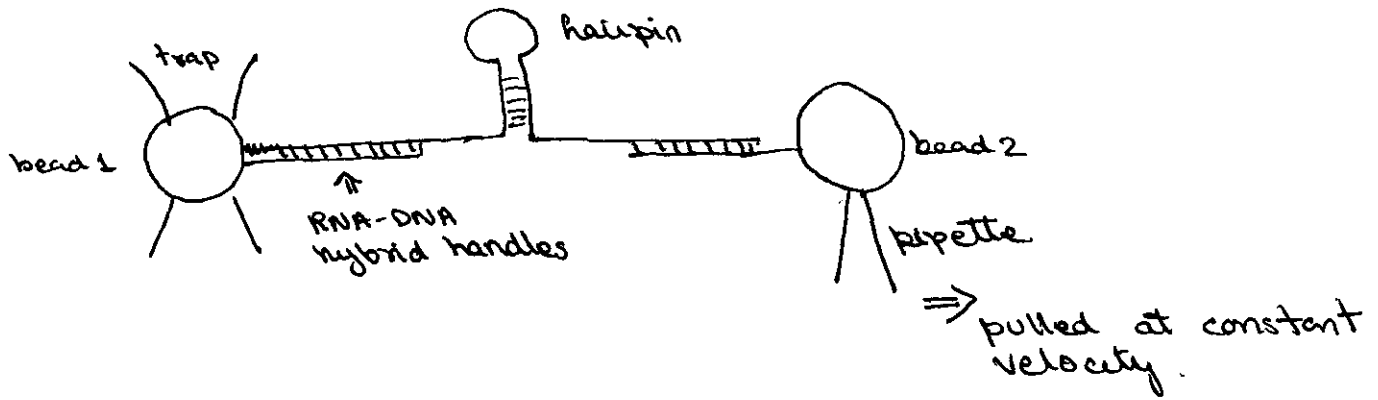
folded  $\rightleftharpoons$  unfolded

mechanical co-ord: end-to-end distance

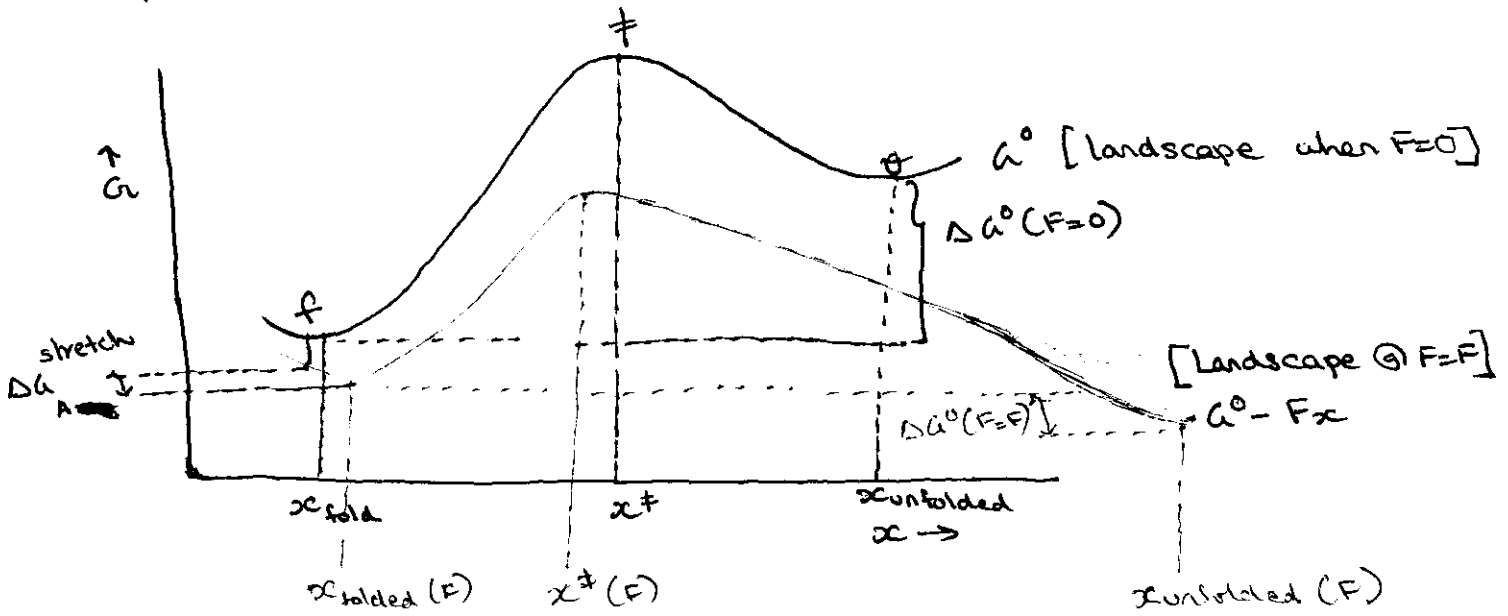
(measured as dist  
between the two  
beads)

[see next pg for  
envo set-007

# 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  $\Delta G$  :-

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

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  $\Delta G$  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  $F \cdot x$

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

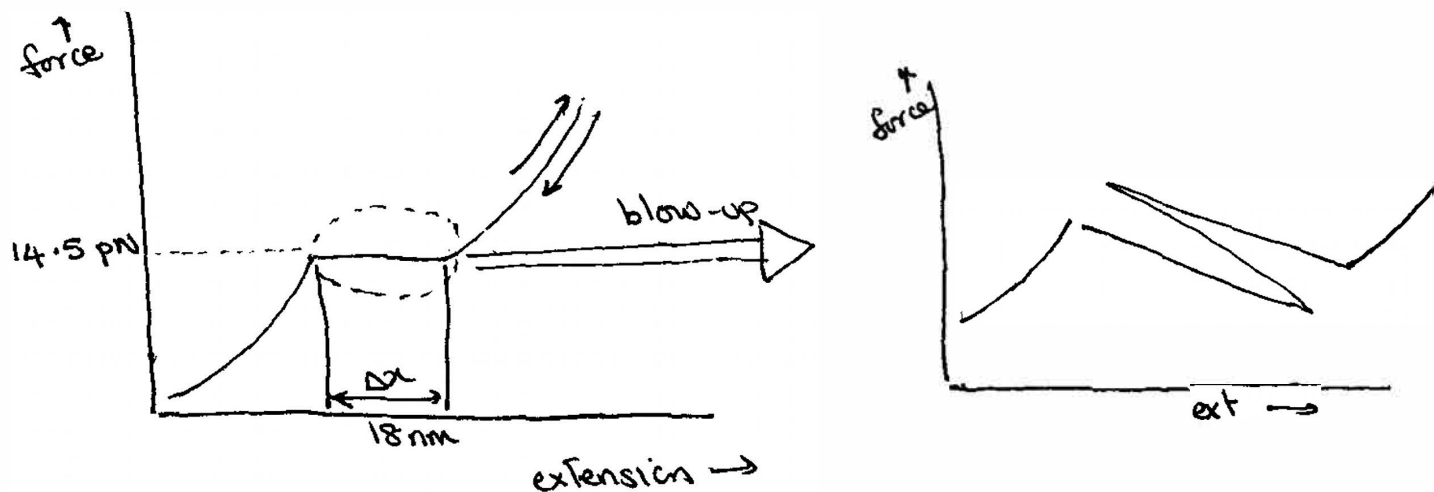
- This results in the unfolded state being stabilized greater than the folded state
- A more extended molecule is stabilized with force such that  $\Delta x_{A-B}(F) > \Delta x_{A-B}(F=0)$
- Rate of the unfolding reaction increases as the transition state is stabilized more relative to the folded state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

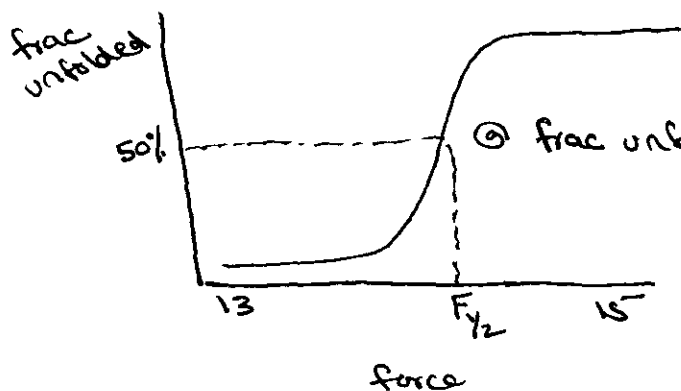
→ Experiment in which the micropipette attached bead is pulled at a constant velocity thus increasing the force on the molecule at a constant rate



- 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% ,  $K_{eq} = 1$

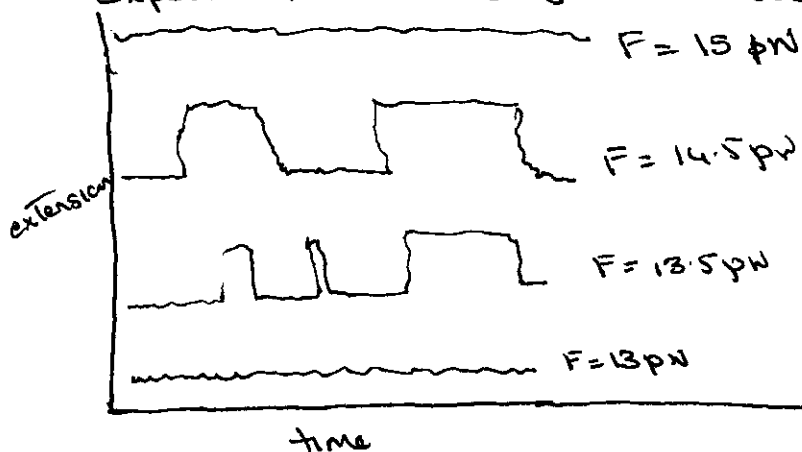
$$\Delta G^\circ = F_{1/2} \Delta x$$

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(Also has stretch component)

$\Delta G^\circ$  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.

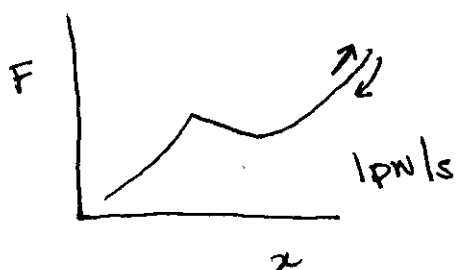
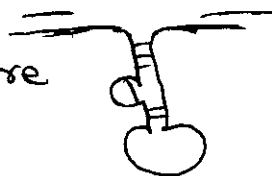
- This gives us information about the distance to the transition state

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

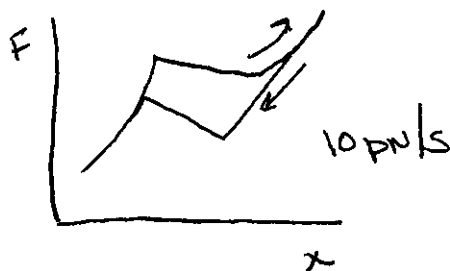
$$k_{unfold, A \rightarrow B}(F) = k_0 e^{-F \Delta x^\ddagger / k_B T} ; \Delta x^\ddagger \Rightarrow \text{dist to transition state}$$

- Reactions not occurring at equilibrium.

For the hairpin with a bulge in the structure



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



$r \ll \tau$  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:-

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

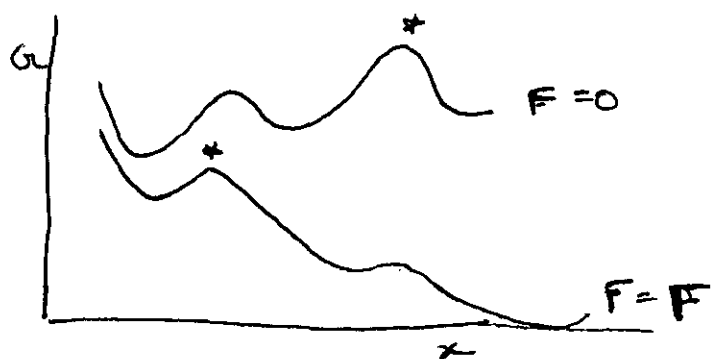
where,  $\Delta x^\ddagger$  = distance to the transition state  
 $k_0$  = unfolding rate constant at zero force

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  $\Delta G$  should be comparable
  - Kinetic quantities are path dependent & should be compared with a lot of caution.
- Mechanical ~~verses~~ versus thermodynamic stabilities
  - $\Downarrow$
  - $F^*$   $\Delta G$
  - 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.