Persistence length (l_p)

$\langle u(s)\cdot u(s')\rangle = \exp(-|s-s'|/l_p)$

The value of l_p

l_p *(ds-DNA)-50 nm,*

l_p *(ss-DNA)-1-2 nm,*

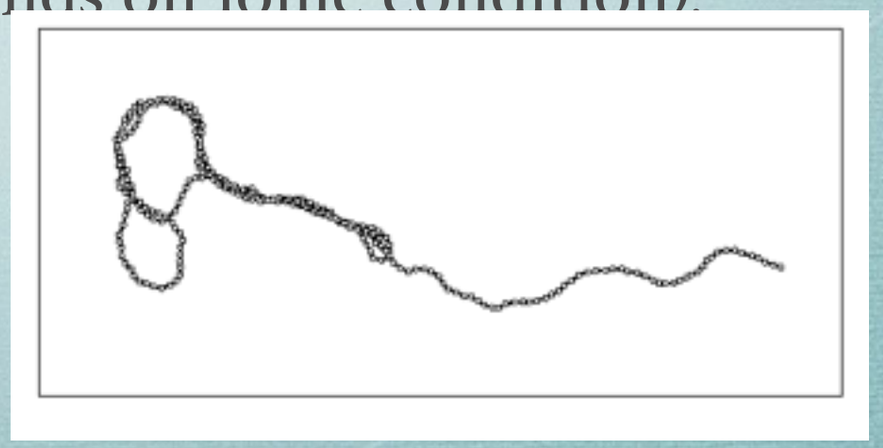
l_p *(RNA)-1-2 nm,*

l_p *(polypeptides)-0.4 – 0.7 nm,*

l_p *(actin)-15* μ*m,*

l_p *(microtubule)-0.5-8mm (Compare this length with cell dimension - 12* μ*m)*

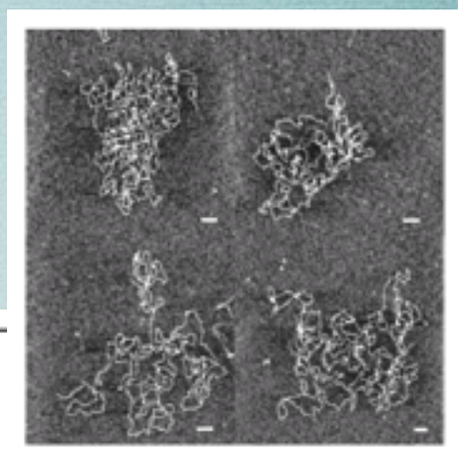
(l_p *of polyelectrolyte* strongly depends on ionic condition).

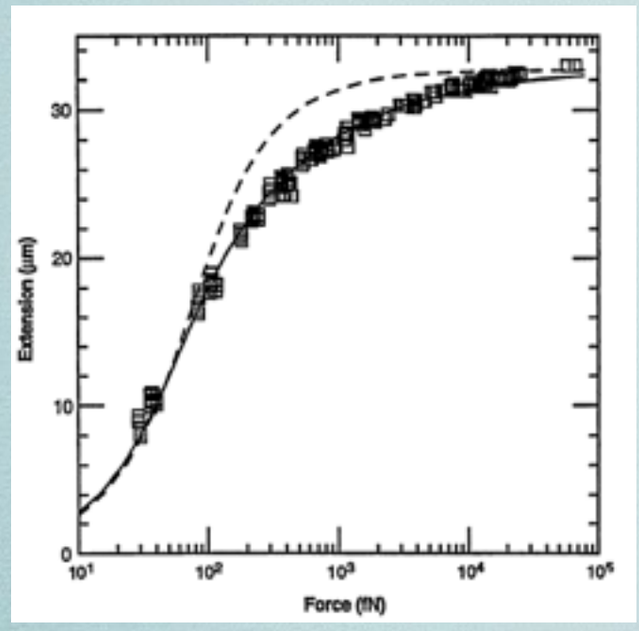


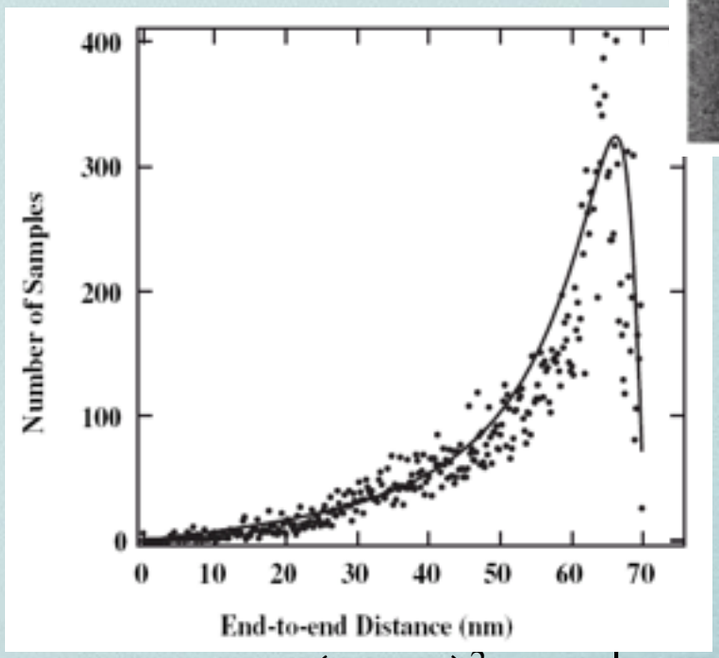
Measurement of persistence length

Wormlike-chain (WLC) model suggests the analytical expression of force-extension curve or end-to-end distance distribution function P(R). The persistence length is usually obtained by fitting the experimental data to these expressions. (e.g. lp of ds-DNA has been measured to give lp~50nm)

$$\Psi_{WLC}[\vec{u}(s)] \propto \exp\left[-\frac{l_p}{2}\int_0^L ds\left(\frac{\partial\,\vec{u}}{\partial\,s}\right)^2\right] \times \prod_{0\le s\le L}\delta\left(\vec{u}^{\,2}(s)-1\right)$$







$$f_{WLC} = \frac{k_B T}{l_p}\left[\frac{1}{4(1-x/L)^2} + \frac{x}{L} - \frac{1}{4}\right]$$

Bustamante, Marko, Siggia, and Smith, Science 265 1599-1600

$$P_{WLC}(R) = \frac{4\pi\, C(R/L)^2}{\left(1-(R/L)^2\right)^{9/2}} \exp\left[-\frac{3(L/l_p)}{4\left(1-(R/L)^2\right)}\right]$$

Valle et al (2005) PRL vol 95 158105; Hyeon & dt (2006)

Single Molecule Force Spectroscopy

Do not waste clean thoughts on dirty experiments

Introduction to RNA Folding

RNA Hairpin

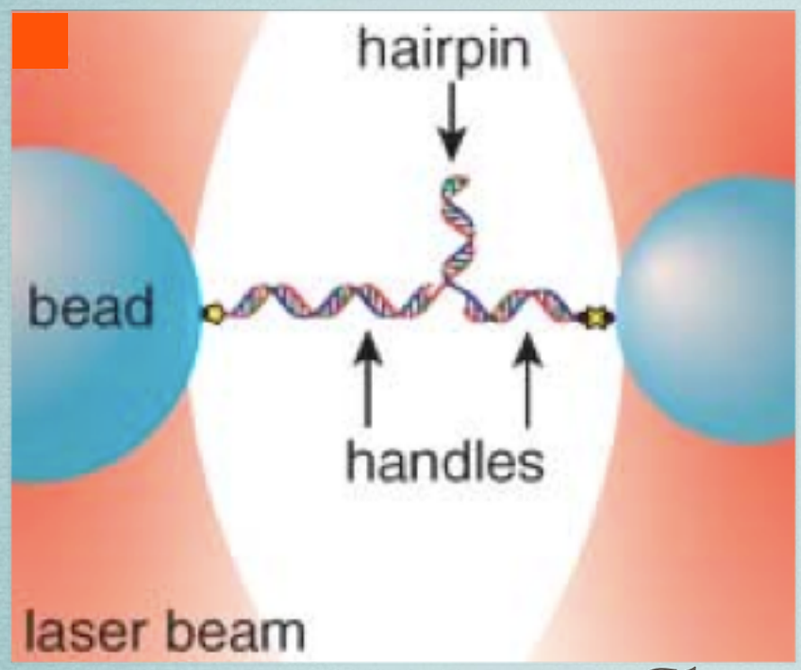
Generalized Rouse Model (GRM)

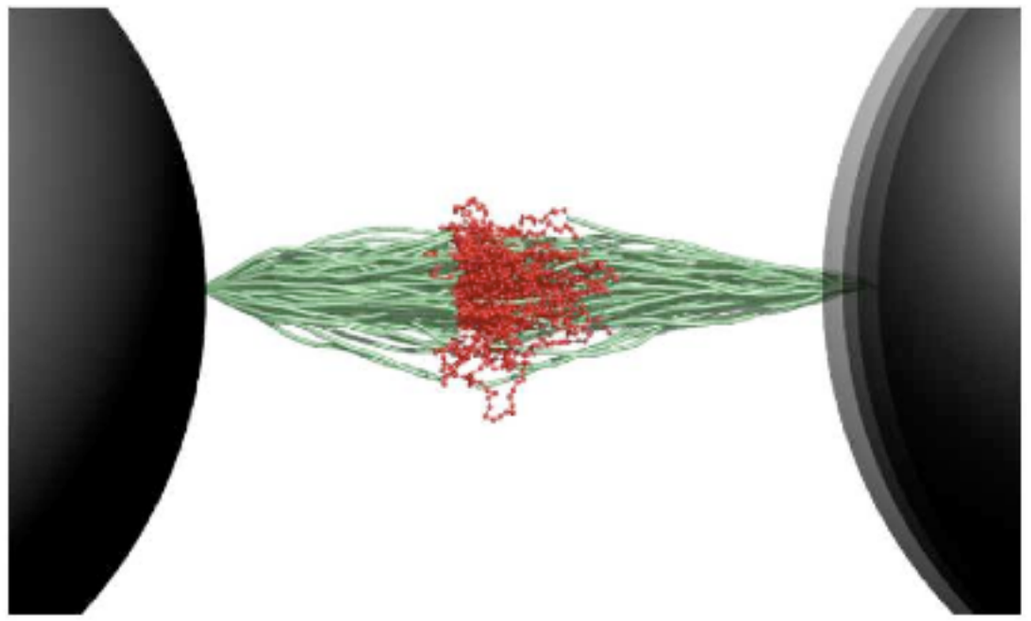
Uses of constant force or constant x

RNA folding (Bustamante, Block, Williams..)
Protein Folding + ribosome (Rief, Fernandez, Marqusee..)
Motors (Block, Yangida, Vale, Goldman, Valentine, Neuman,..)

RNA hairpin Folding landscape : SMFS

Woodside + Block (2006)



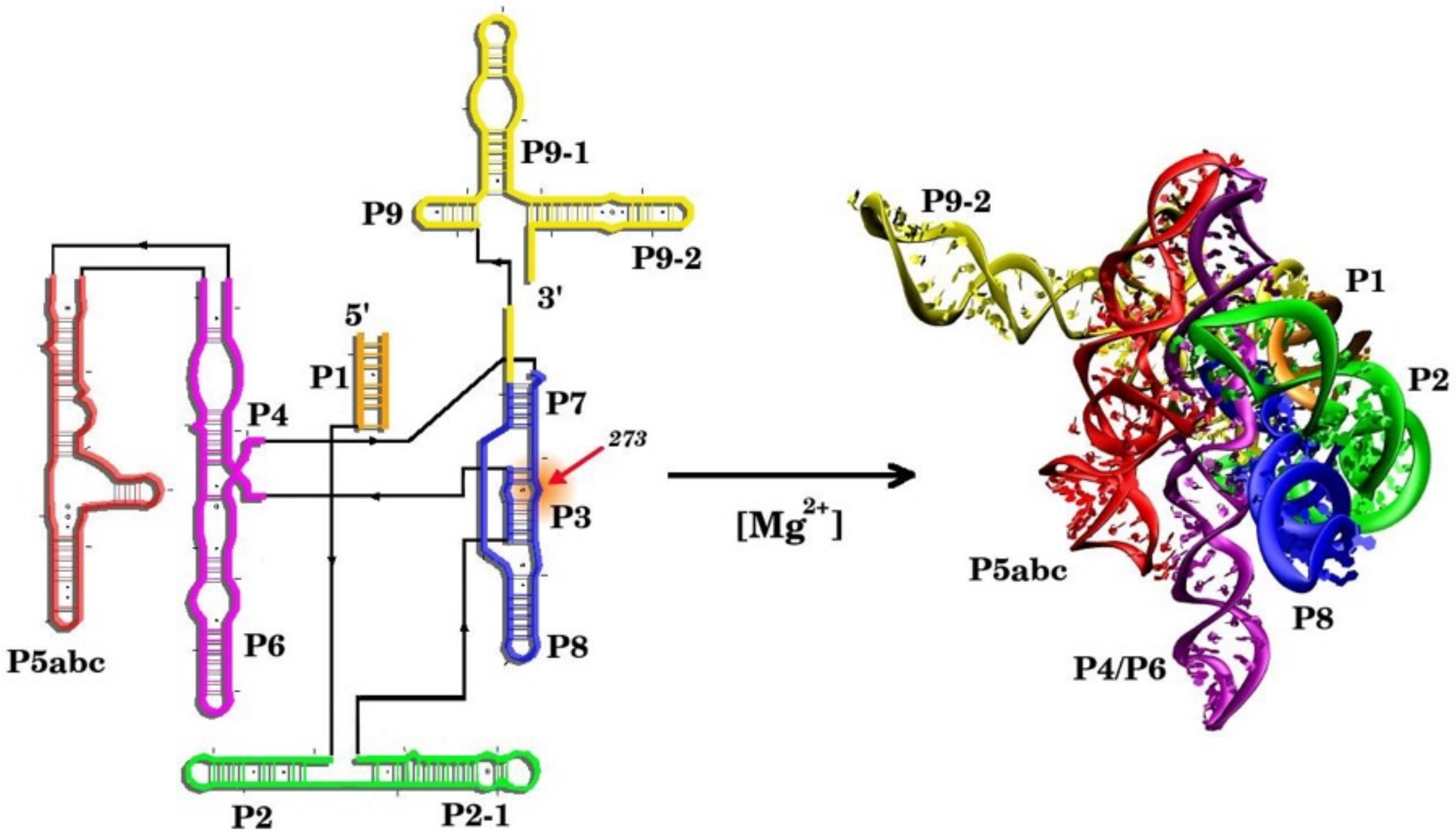


Changbong Hyeon, Greg Morrison
& dt PNAS **105,** 9607 (2008)

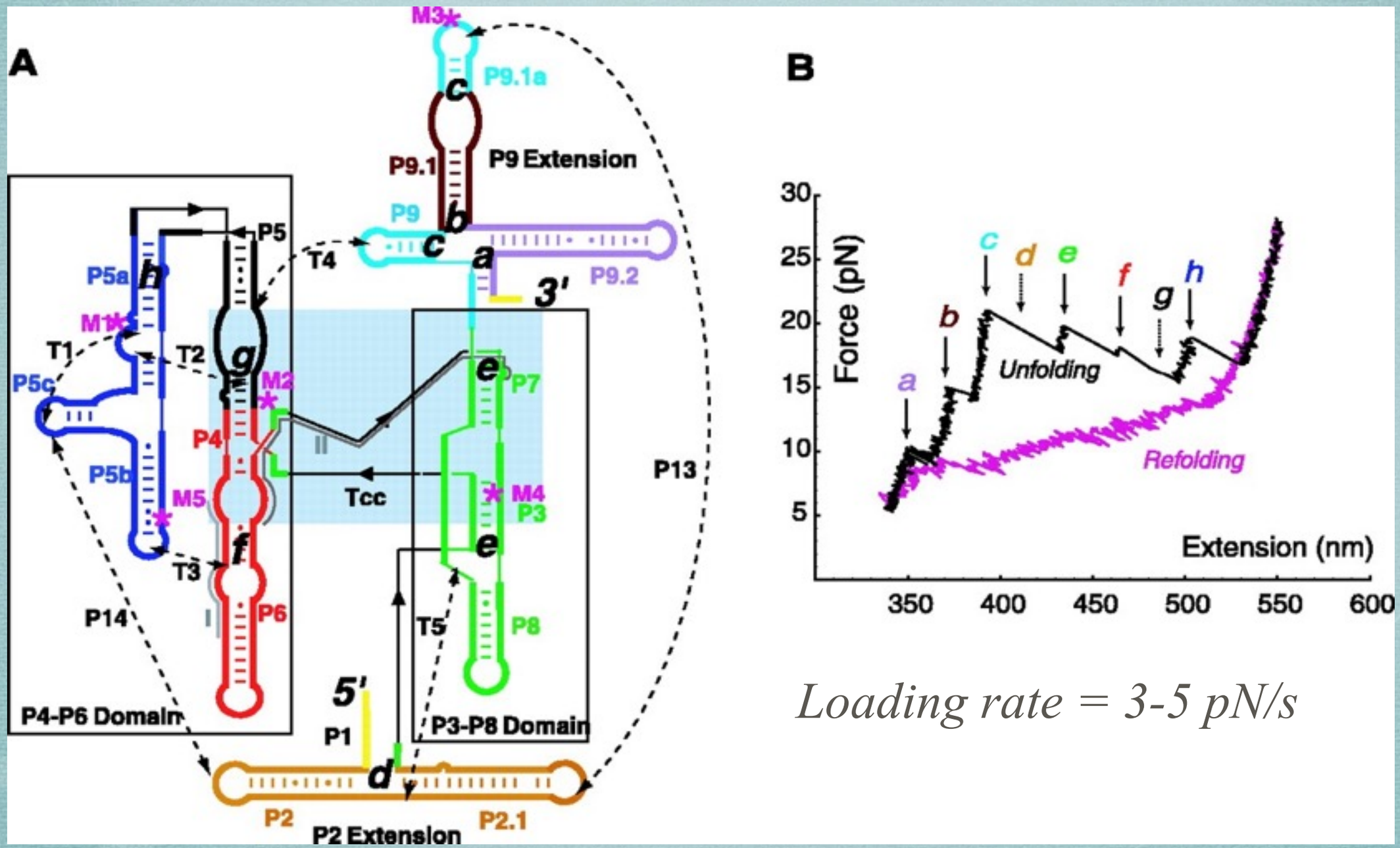
Riboswitch:
J. C. Lin &dt JACS **130,** 14080 (08)

Force Quench Refolding:
C. Hyeon, G. Morrison, D. Pincus & dt PNAS (09)

RNA Folding (Mg^{2+}) problem



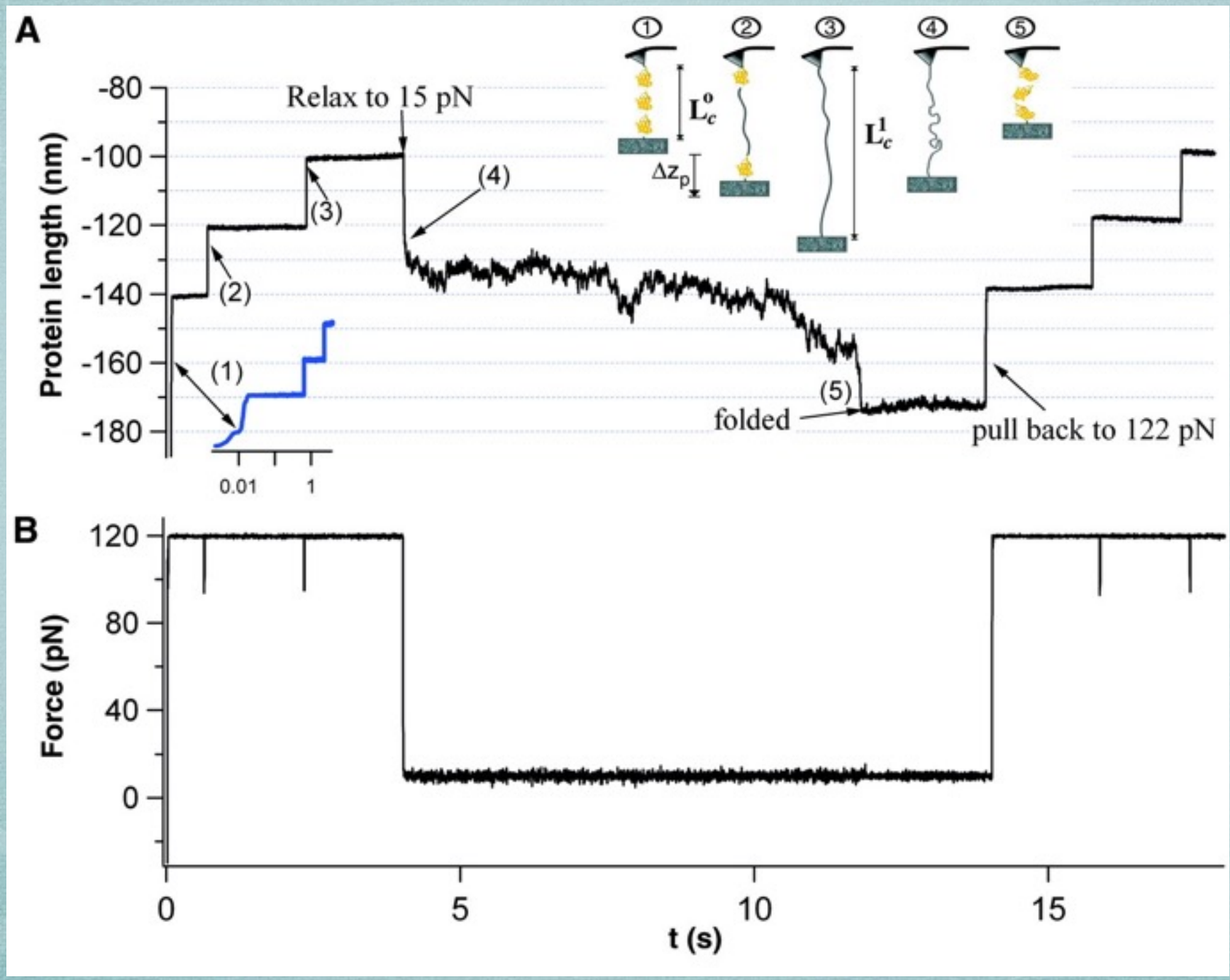
Forced-unfolding Tetrahymena Ribozyme Energy landscape from LOT FEC



Loading rate = 3-5 pN/s

Onoa et. al. Science Vol 299, 1892 (2003)

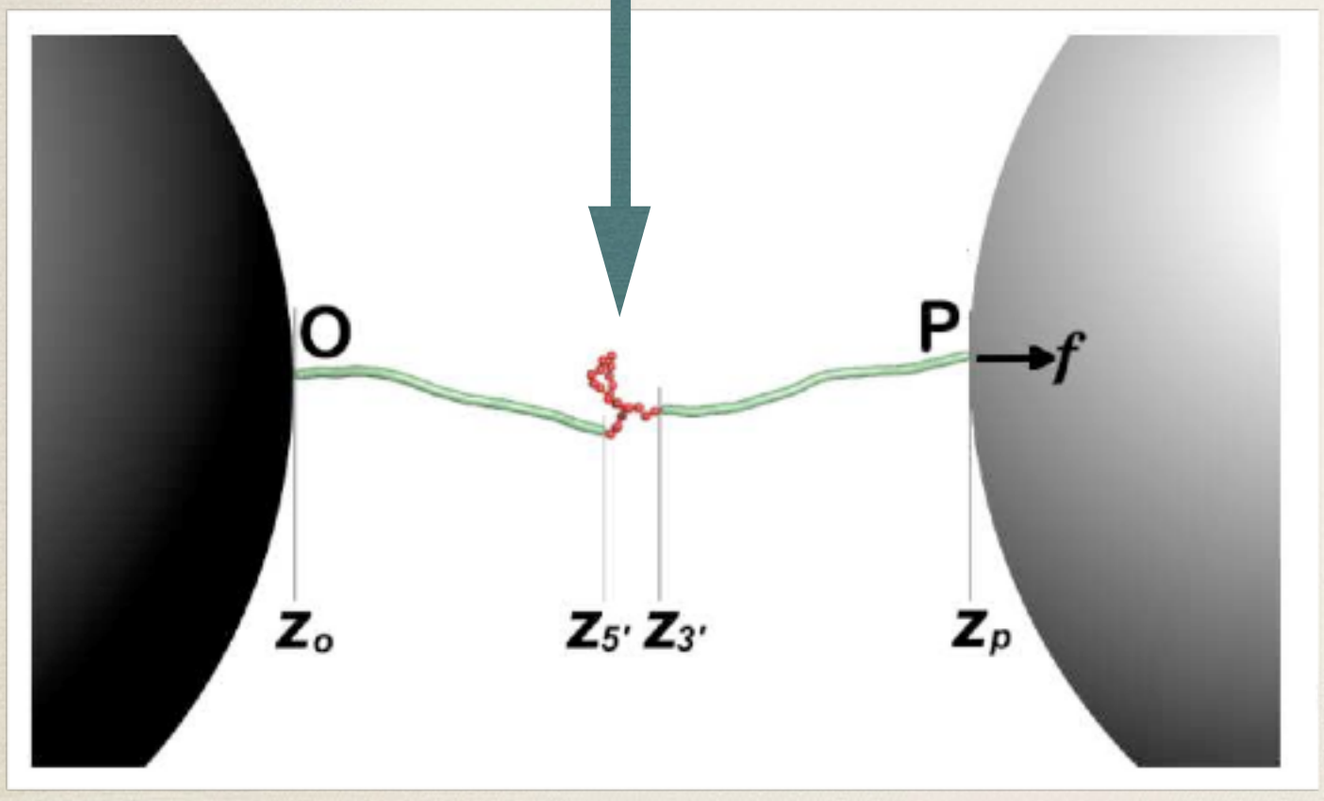
Folding Trajectory of Poly-Ub using force clamp



Fernandez and Li Science vol 303, 1674-1678 (2004)

LOT Experimental Setup

RNA Hairpin



Free Energy profiles $F(z_m)$ and hopping kinetics from $z_{sys}(t)$

$L \approx (1\text{-}10)\mu m$

$l_P \approx 50nm$

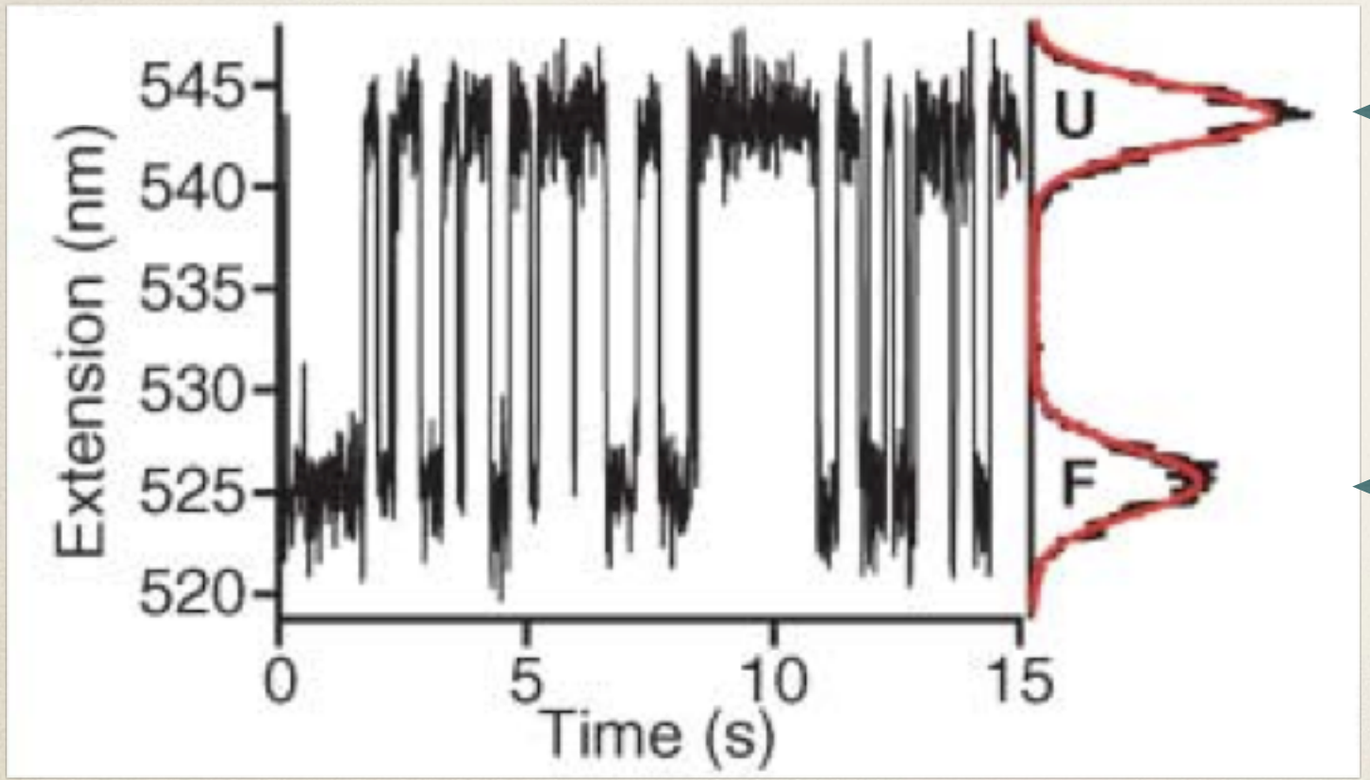
$RNA \approx 2nm$

$b = 500nm$

Effect of Attaching handles on the hairpin

Experimental Measurements

In a LOT experiment, the extension as a function of time is measured ($f \approx f_m$).



Unfolded state (larger extension)

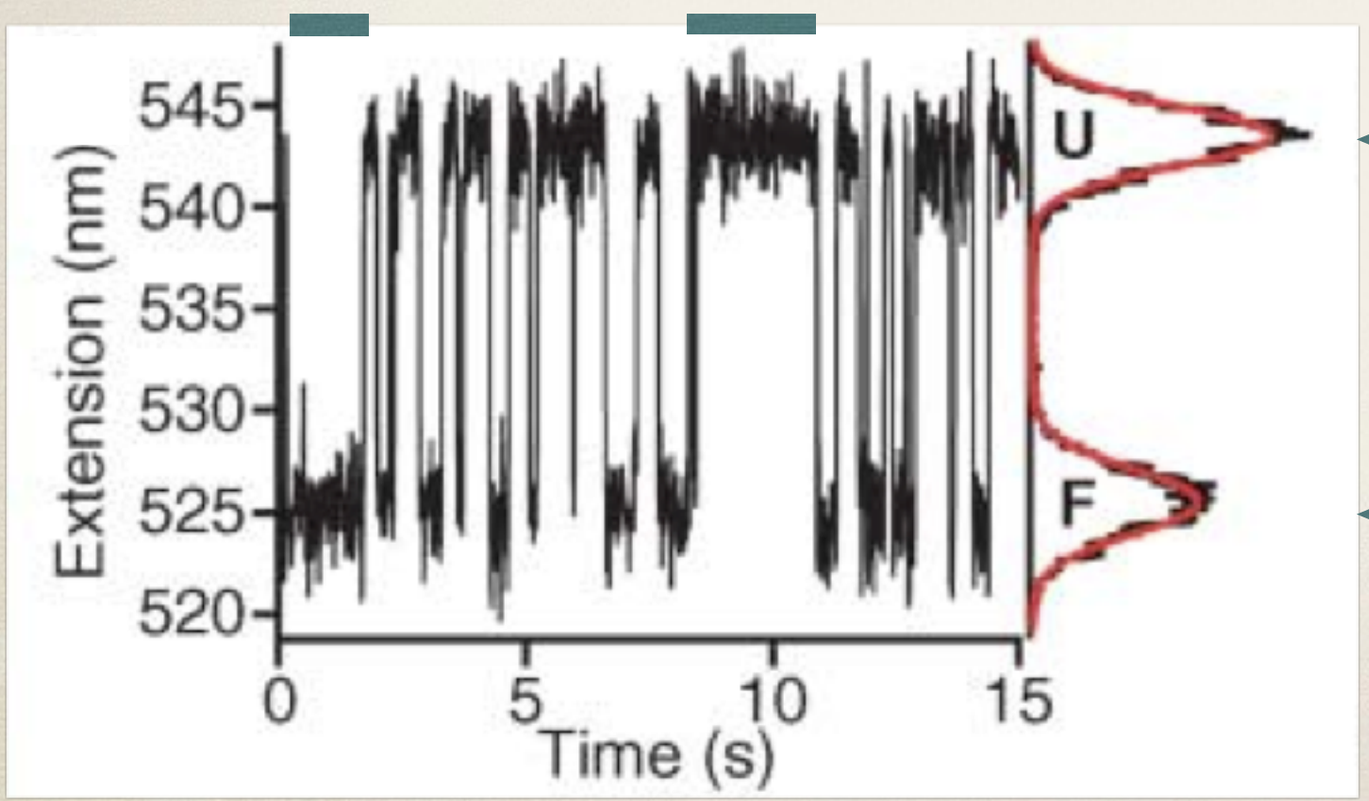
Folded state (smaller extension)

The distribution of extensions can be computed.
This gives the free energy $\beta F_{eq}(z) = -\log[P_{eq}(z)]$

Experimental Measurements

We can determine the lifetimes of the folded and unfolded states as the average of individual dwell times.

$\tau_U^{(i)}$ $\tau_F^{(j)}$ $\tau_{U(F)} = \frac{1}{N}\sum_i \tau_{U(F)}^{(i)}$



Unfolded state

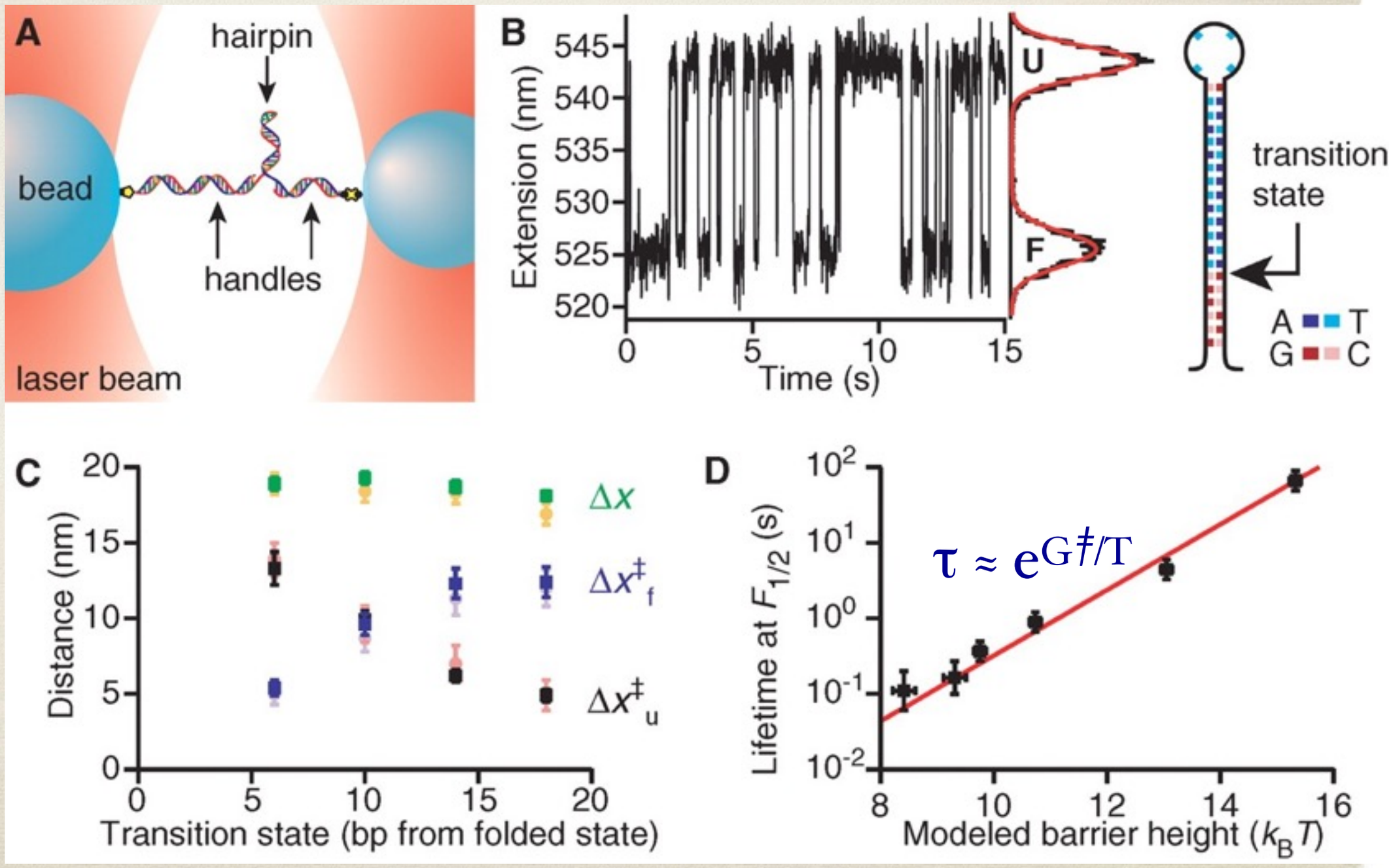
Folded state

Woodside, Science **314** 1001 (2006)

Sequence determines energy landscape of hairpins

Woodside et. al.
Science, 314, 1001 (06)

$$\tau_F = \tau_0 exp(-\Delta x_F^{\ddagger} F / k_B T)$$



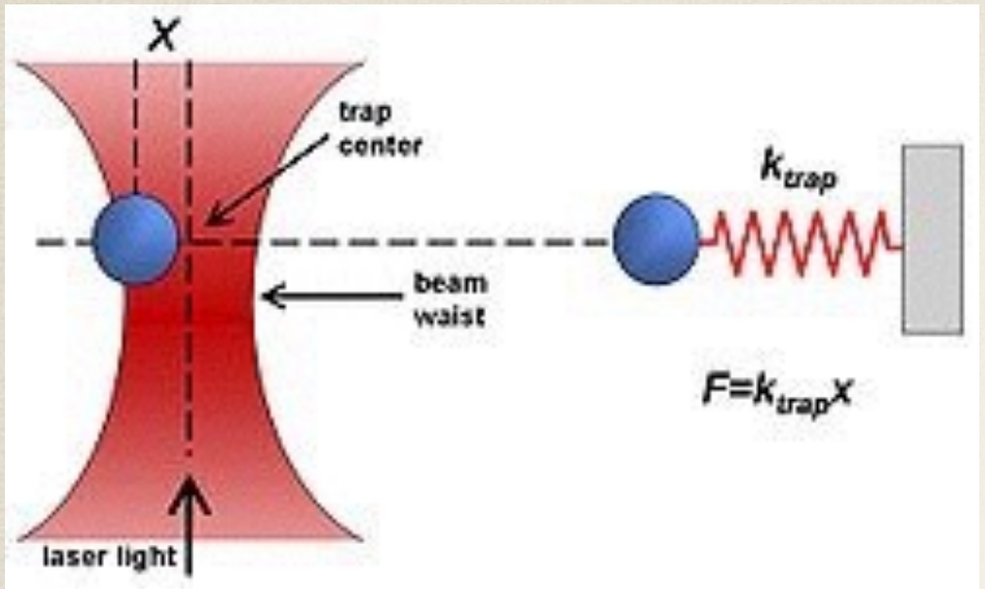
$\Delta x_u^{\ddagger} + \Delta x_f^{\ddagger}$

$\approx \Delta x$

What can we learn from SMFS?

- Construct free energy profiles F(R) vs R
- Is this correct?
- Extract dynamics at F other than F_m ($F_{1/2}$ in Woodside)?
- All require sound theory beyond clean data!

Effect of bead dynamics



$$\tau_r = \gamma/k$$

$\gamma = 6\pi\eta a, a = 1\mu\text{m}, \eta \approx 1cP, k_p \approx 0.01\,\text{pN/nm}$ $k_m \approx 0.1\,\text{pN/nm}$

$k = k_p + k_m.$ we find $\tau_r \lesssim 1\,\text{ms}.$

cf. RNA hairpins (Bustamante and coworkers) or DNA hairpins (Block and coworkers)

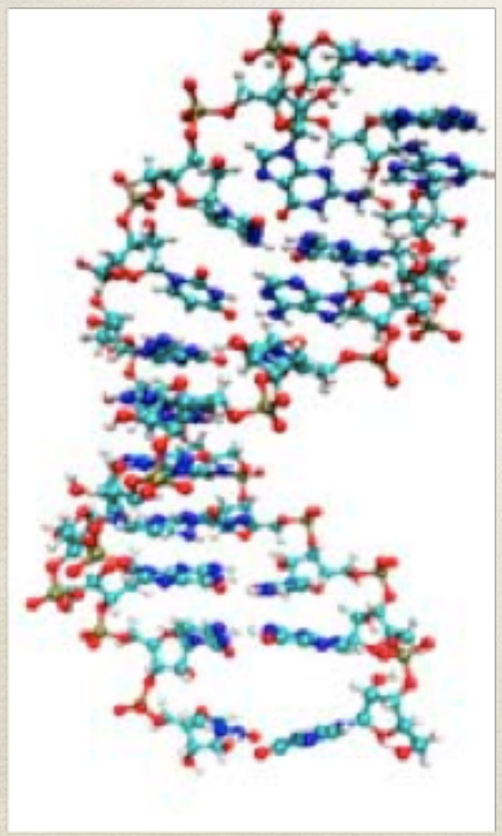
Hopping time ~ 0.1-1 sec.

- Folding/Unfolding time >> Bead relaxation time

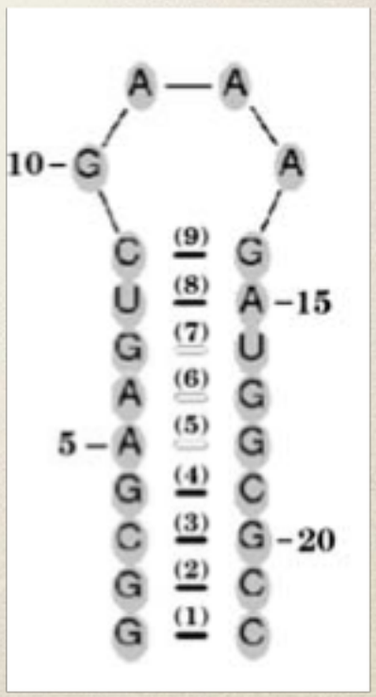
$\tau_U^o \approx \tau_F^o \gg \tau_r$ at $f \approx f_m$.

Role of linkers in modulating the Energy landscape & Kinetics

- Equilibrium free energy profiles $F(z_m)$
- Force-dependentHopping kinetics

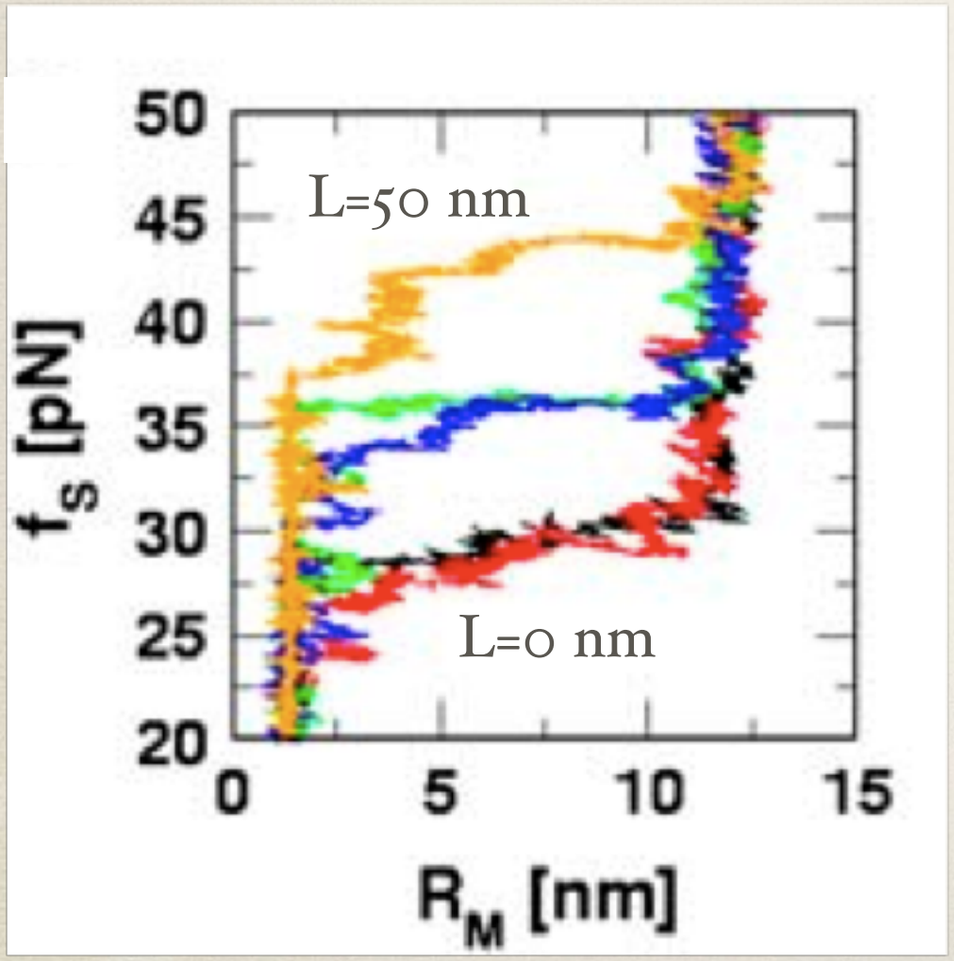


22-mer P5GA in the simulation.



Some Simulation Results on Kinetics

Hyeon: Hopping kinetics in a constant loading rate simulation of the P5GA RNA hairpin depends on the handle length.



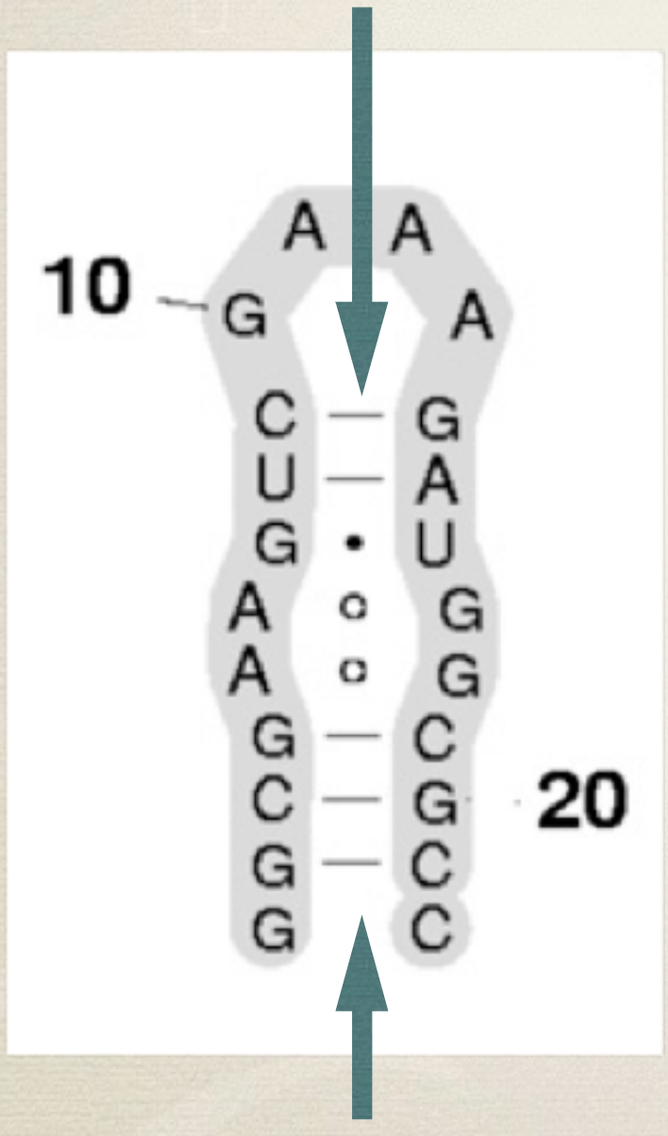
Increasing handle length increases the force required for rupture.

Stiffer handles have a greater effect on the rupture forces.

Hyeon and dt, Biophys J **90** 3410 (2006)

Simulations of the P5GA Hairpin

Native Contact



Native Contact

P5GA is a 22-mer RNA, which forms a hairpin.

The hairpin structure stabilized by Stacking interactions and h-bonds

Simulations show P5GA's midpoint force (f_m) is about 15.4 pN.

C.Hyeon and dt, Biophys J **92** 731 (2007)

Simulation Details

P5GA modeled using the Self-Organizing Polymer (SOP) Model

$$H = -\sum_{i=1}^{N-1} \frac{k}{2} R_0^2 \log\left(1 - \frac{(r_{i,i+1} - r_{i,i+1}^o)^2}{R_0^2}\right)$$ (Connectivity of the backbone)

$$+ \sum_{i=1}^{N-3} \sum_{j=i+3}^{N} \epsilon_h \left[\left(\frac{r_{ij}^o}{r_{ij}}\right)^{12} - 2\left(\frac{r_{ij}^o}{r_{ij}}\right)^6\right] \Delta_{ij}$$ (Native Interactions)

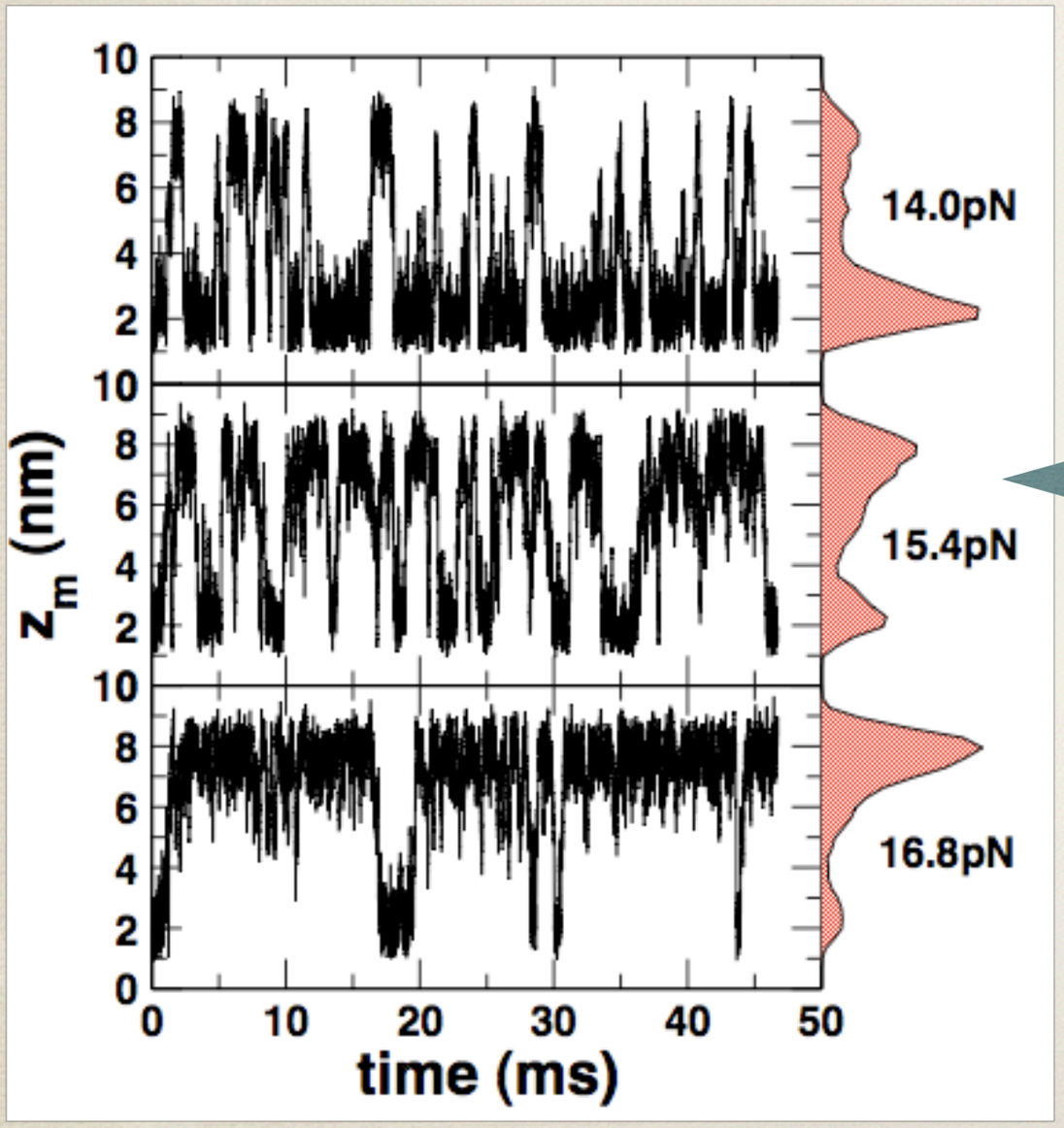
$$+ \sum_{i=1}^{N-2} \epsilon_l \left(\frac{\sigma^*}{r_{i,i+2}}\right)^6 + \sum_{i=1}^{N-3} \sum_{j=i+3}^{N} \epsilon_l \left(\frac{\sigma}{r_{ij}}\right)^6 (1 - \Delta_{ij}).$$ (Non-Native Excluded Volume)

Overdamped Langevin dynamics is used

Hyeon & dt, Biophys J **92** 731 (2007)

The Midpoint Force

Accurate profiles and kinetics require $f \approx f_m$

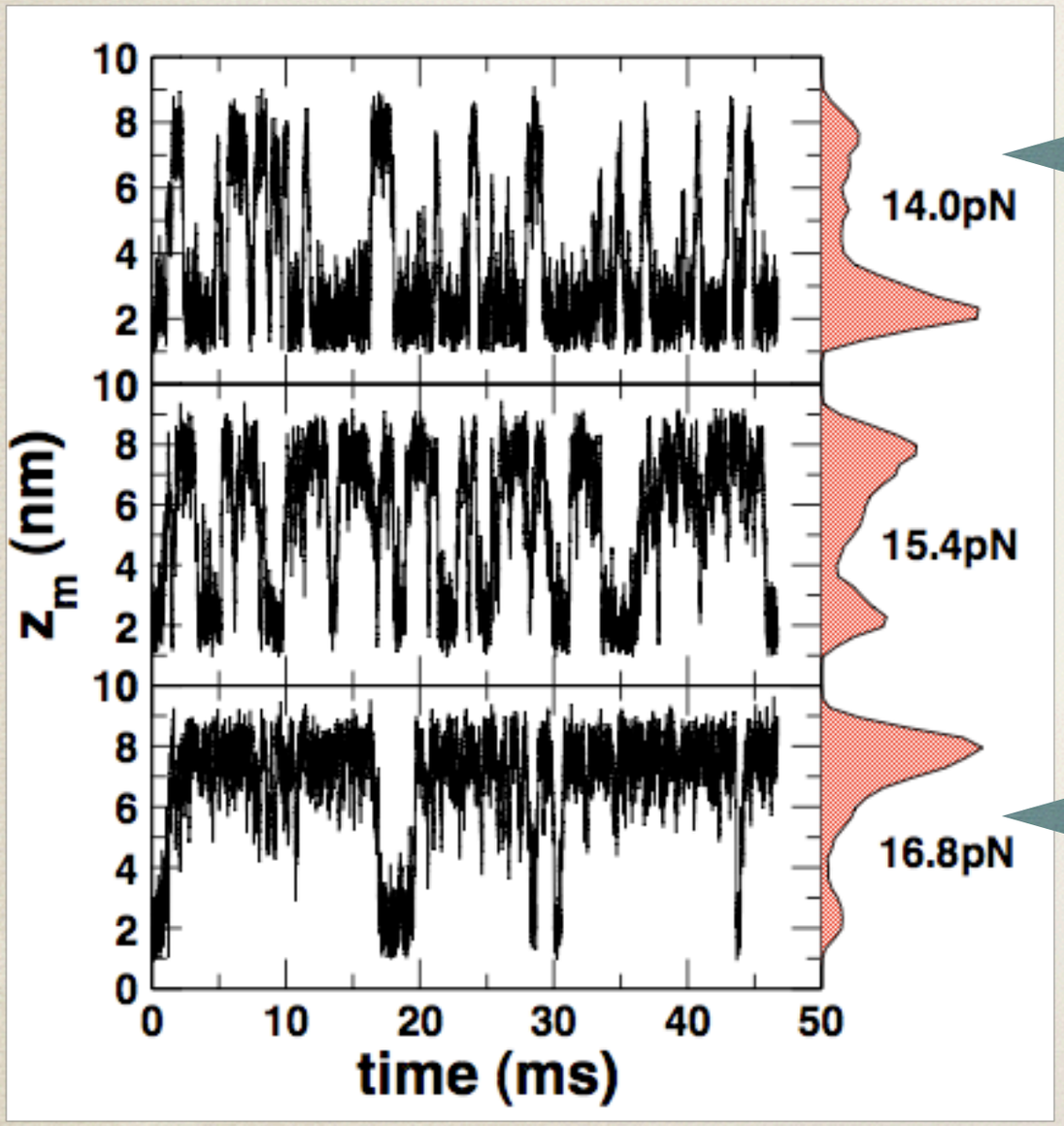


At $f \approx f_m$
the hairpin samples both basins of attraction.

The equilibrium and kinetic properties can be reliably measured.

The Midpoint Force

Accurate profiles and kinetics require $f \approx f_m$



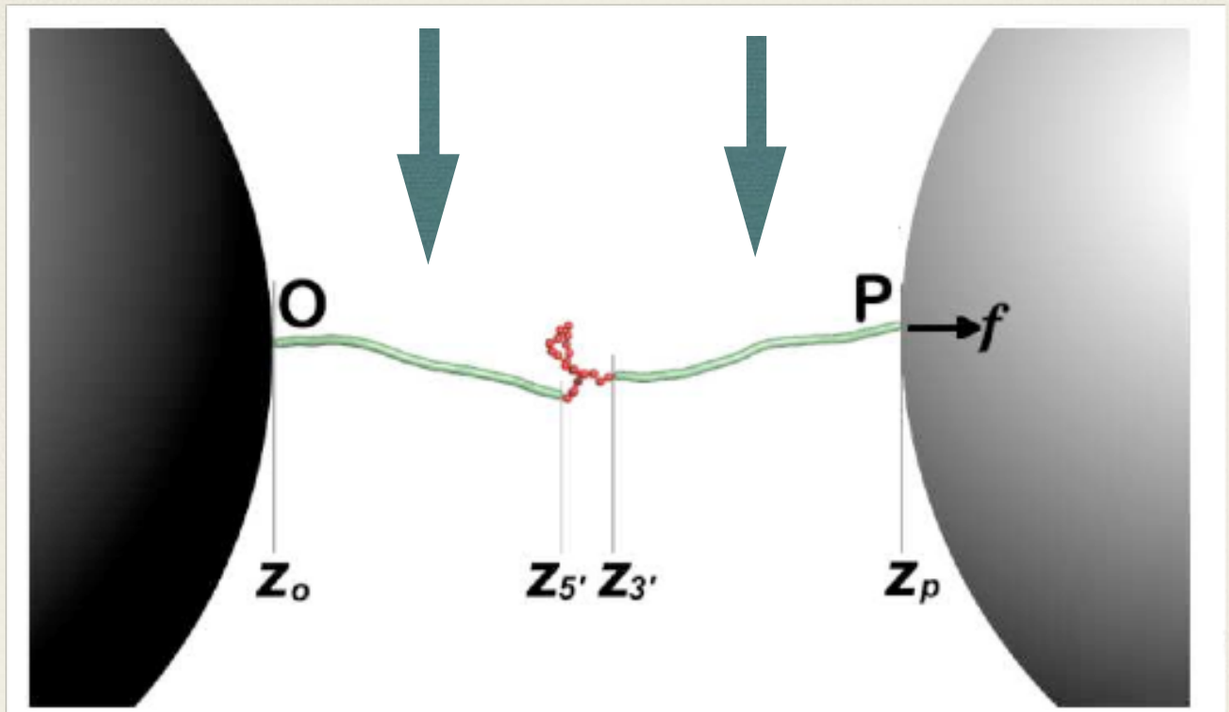
For $f \ll f_m$ or $f \gg f_m$

the hairpin is trapped in one basin only.

Accurate statistics require a prohibitively long time.

Simulation Details

Handles can be stiff or flexible, depending on bending rigidity.



$$H_{handles} = \sum_{i=1}^{N-1} \frac{k_B}{2}(r_{i,i+1} - b)^2 - \sum_{i=1}^{N-2} k_A \hat{r}_{i,i+1} \cdot \hat{r}_{i+1,i+2}.$$

Connectivity of the handles

Resistance to bending

Simulation Details

$$H_{handles} = \sum_{i=1}^{N-1} \frac{k_B}{2}(r_{i,i+1} - b)^2 - \sum_{i=1}^{N-2} k_A \hat{r}_{i,i+1} \cdot \hat{r}_{i+1,i+2}.$$

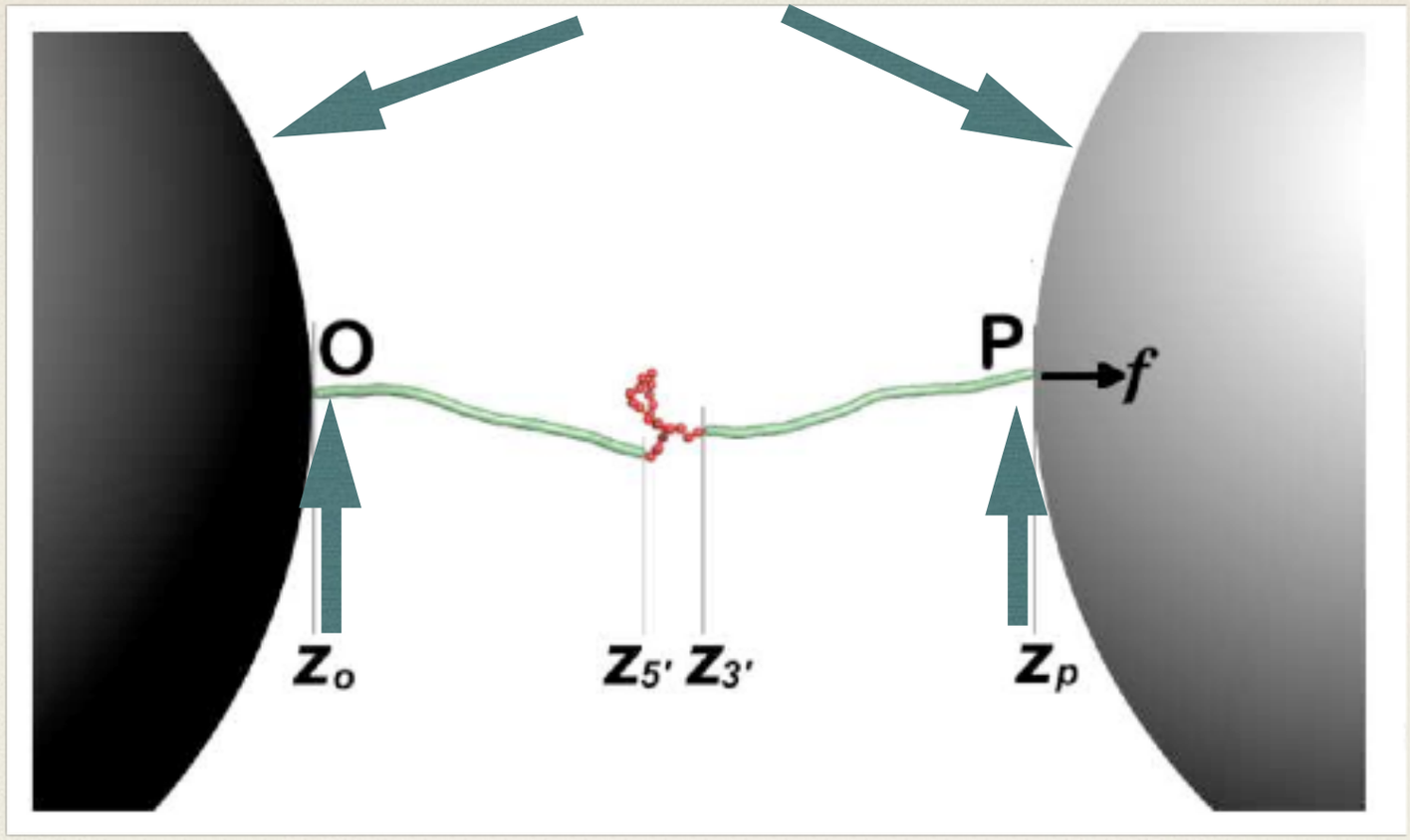
The Wormlike Chain model characterizes stiffness with the persistence length l_p

We choose the monomer spacing $b = 0.5$ nm

$k_A = 7\text{pN nm}$ gives $l_p = 0.6$ nm (flexible chain)

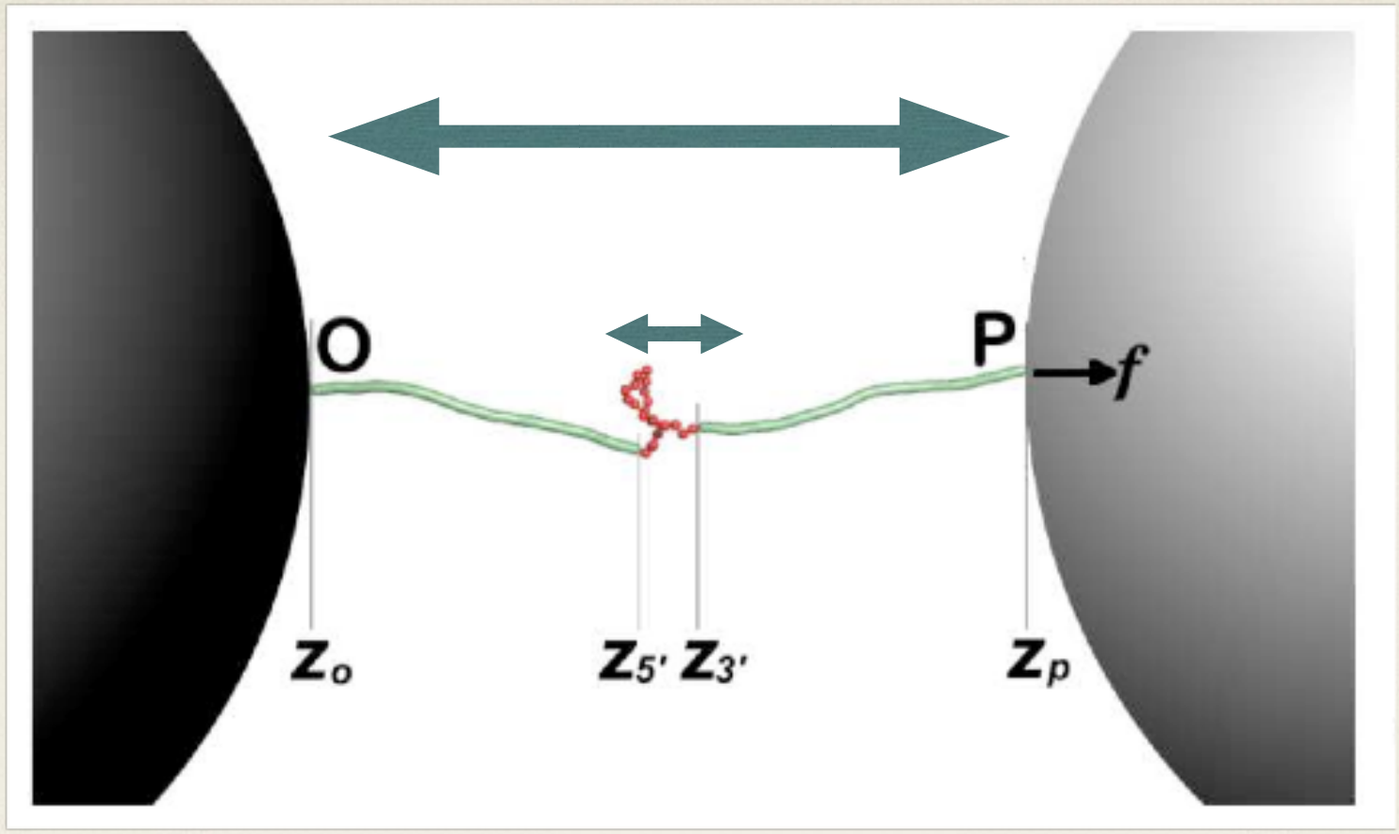
$k_A = 561\text{pN nm}$ gives $l_p = 70$ nm (very stiff chain)

Simulation Details



- z_0 is harmonically bound to the origin
- A force f =15.4 pN applied at z_P in the z-direction
- Transverse fluctuations in z_0 and z_P are suppressed

What we measure



$$z_m(t) = z_{3'} - z_{5'}$$

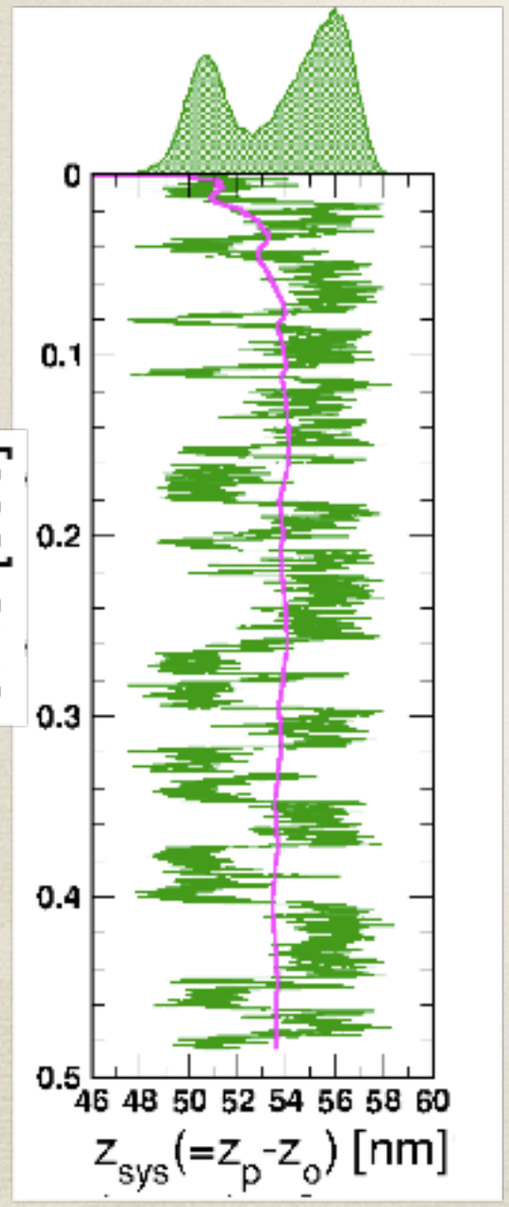
The extension of the RNA
(the quantity of interest)

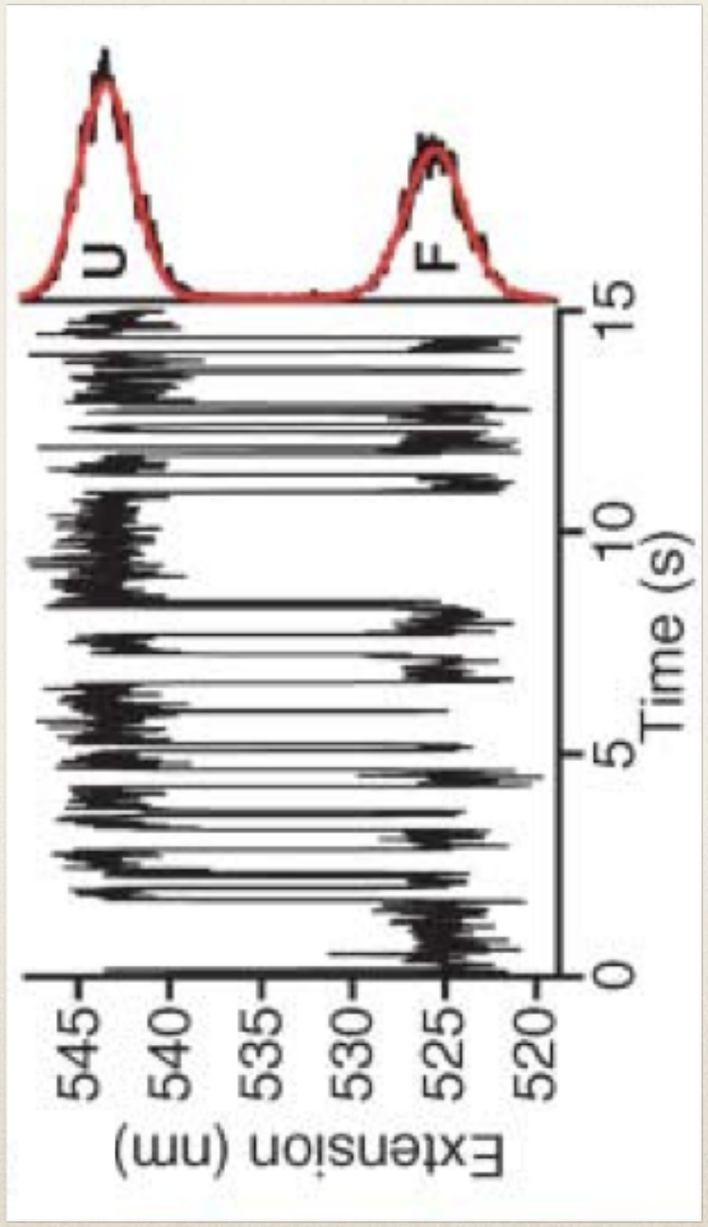
$$z_{sys}(t) = z_P - z_0$$

The distance between the beads
(the experimental observable)

A Typical Time Trace for our Simulation

P5GA Simulation ssDNA experiment





The SOP model for RNA behaves similar to experiments on other hairpins.

The SOP shows the expected two-state behavior

Equilibrium Properties of Interest

- $\beta F_{eq}(z_m) = -\log[P_{eq}(z_m)]$

The free energy, measured using z_m

- $\beta F_{eq}(z_{sys}) = -\log[P_{eq}(z_{sys})]$

The free energy, measured using z_{sys}

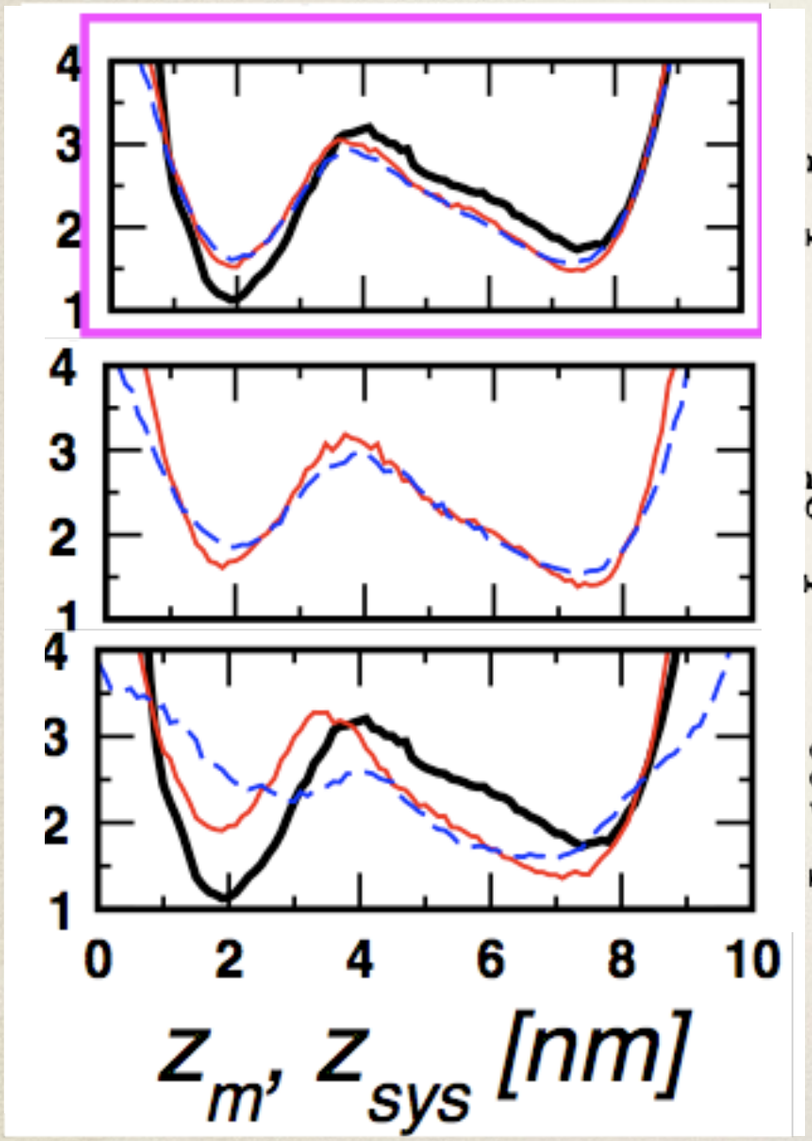
- $\beta F^0_{eq}(z_m)$

The intrinsic free energy, with no handles attached

All of these quantities are computed using the distribution of time traces

Free Energy Profiles

$l_p = 70$ nm (stiff)



Solid Black: $\beta F^o_{eq}(z_m)$

Solid Red: $\beta F_{eq}(z_m)$

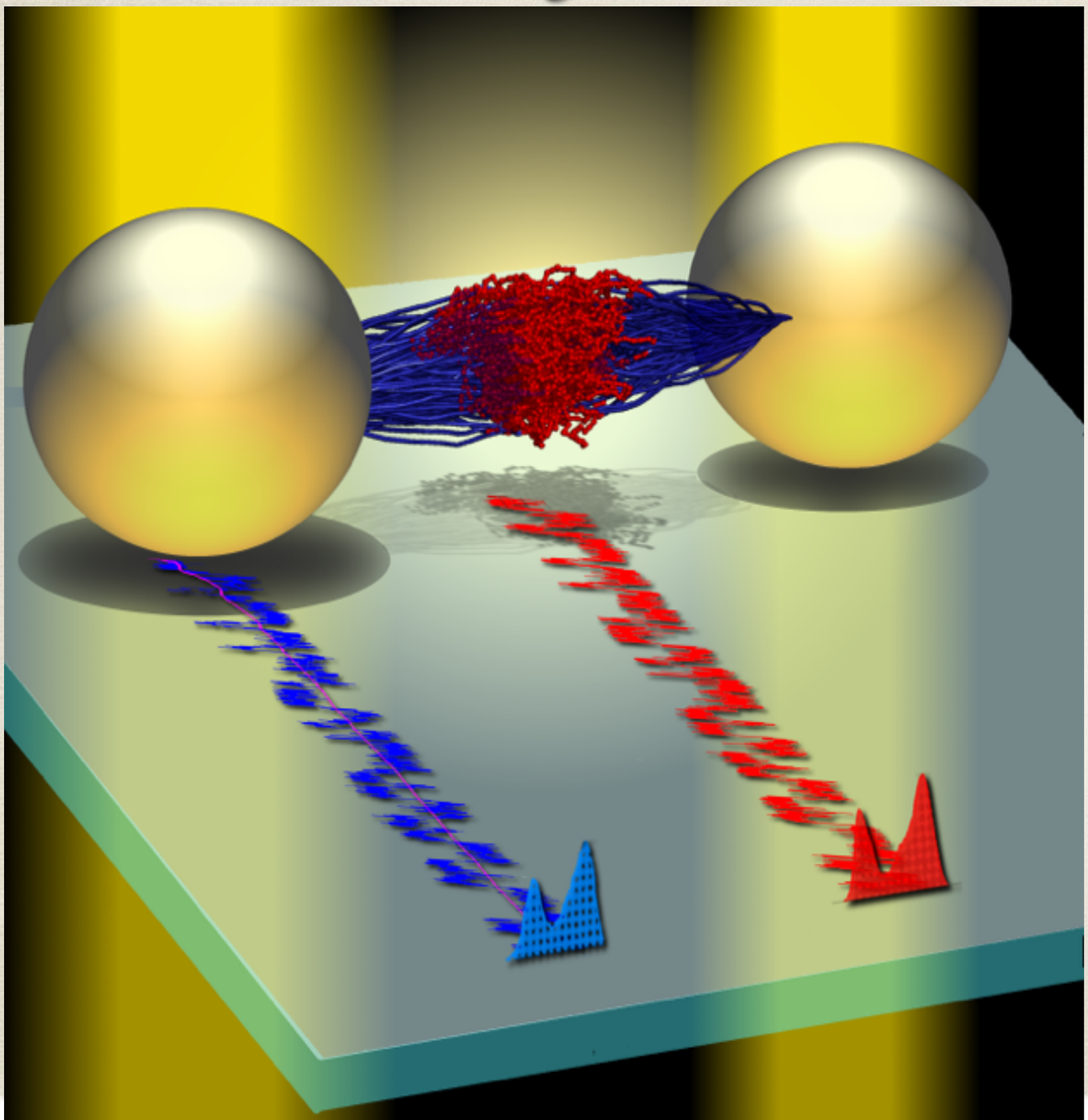
Dashed Blue: $\beta F_{eq}(z_{sys})$

Attaching stiff handles does change the free energy, but $\beta F_{eq}(z_m) \approx \beta F^o_{eq}(z_m)$

Short, stiff handles give good agreement between $\beta F_{eq}(z_{sys})$ and $\beta F_{eq}(z_m)$

Longer handles give worse agreement

Importance of transverse Fluctuations



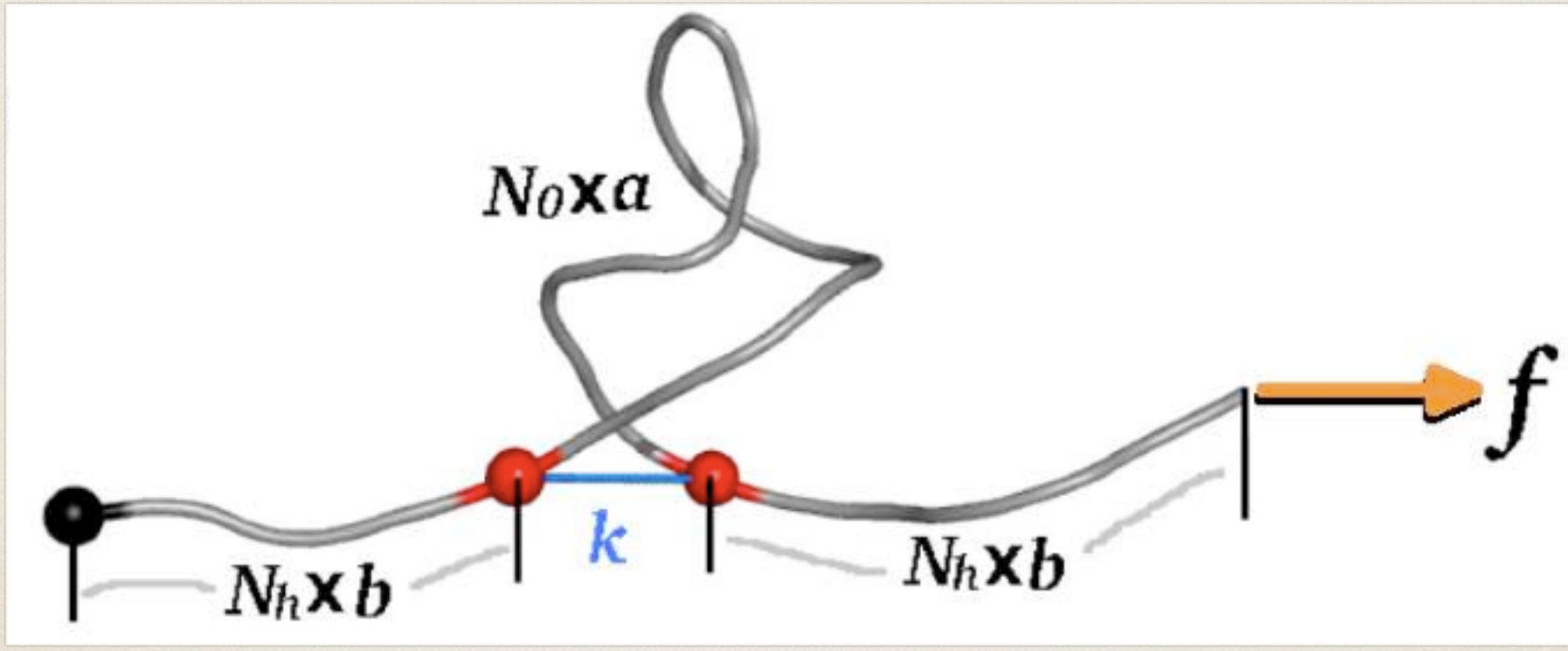
Minimize transverse fluctuations;

$L/l_p < 10$

Exps it is Roughly 6 -12

Toy Model

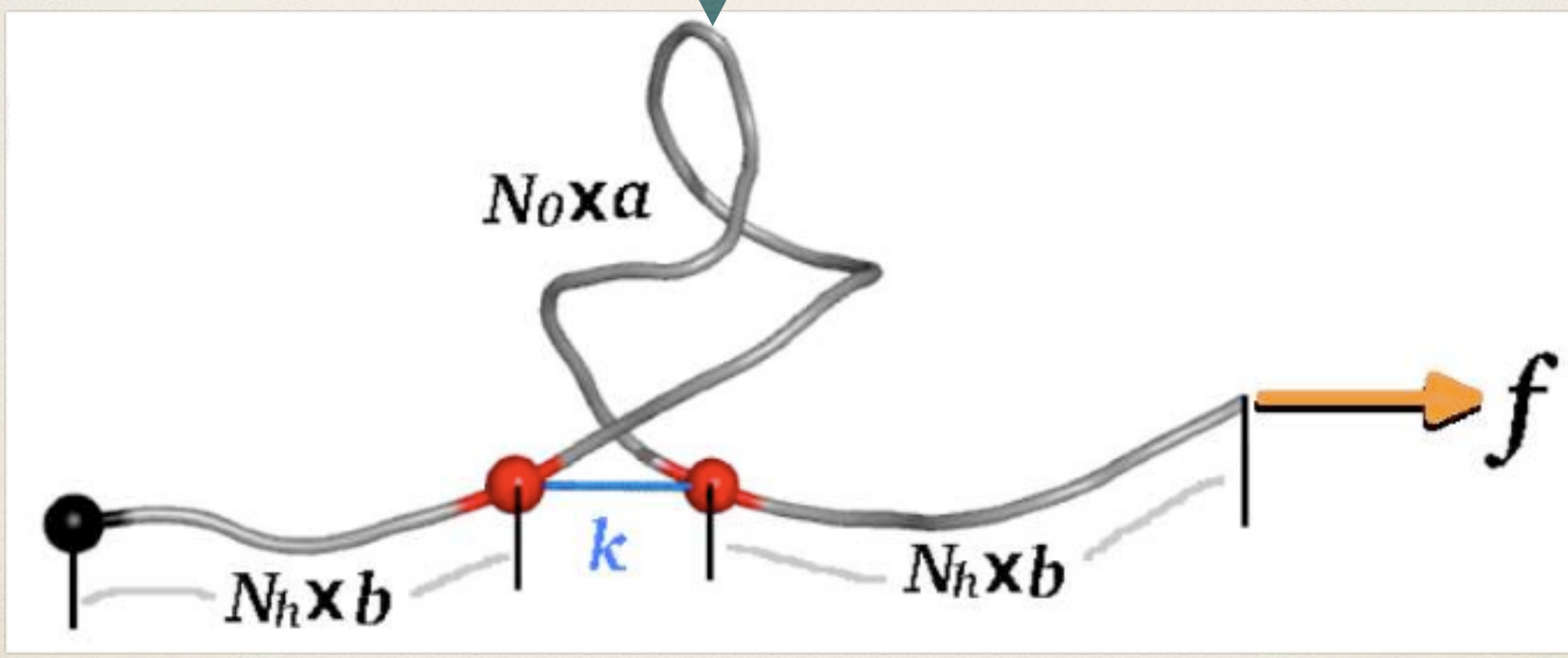
It is useful to understand the physics of the RNA + handles system by studying a simpler problem, which minimally represents the system.



A Generalized Rouse Model, which captures the essential physics of the problem.

Generalized Rouse Model (GRM)

Model RNA as Gaussian, with spacing a and with N_0 bonds.

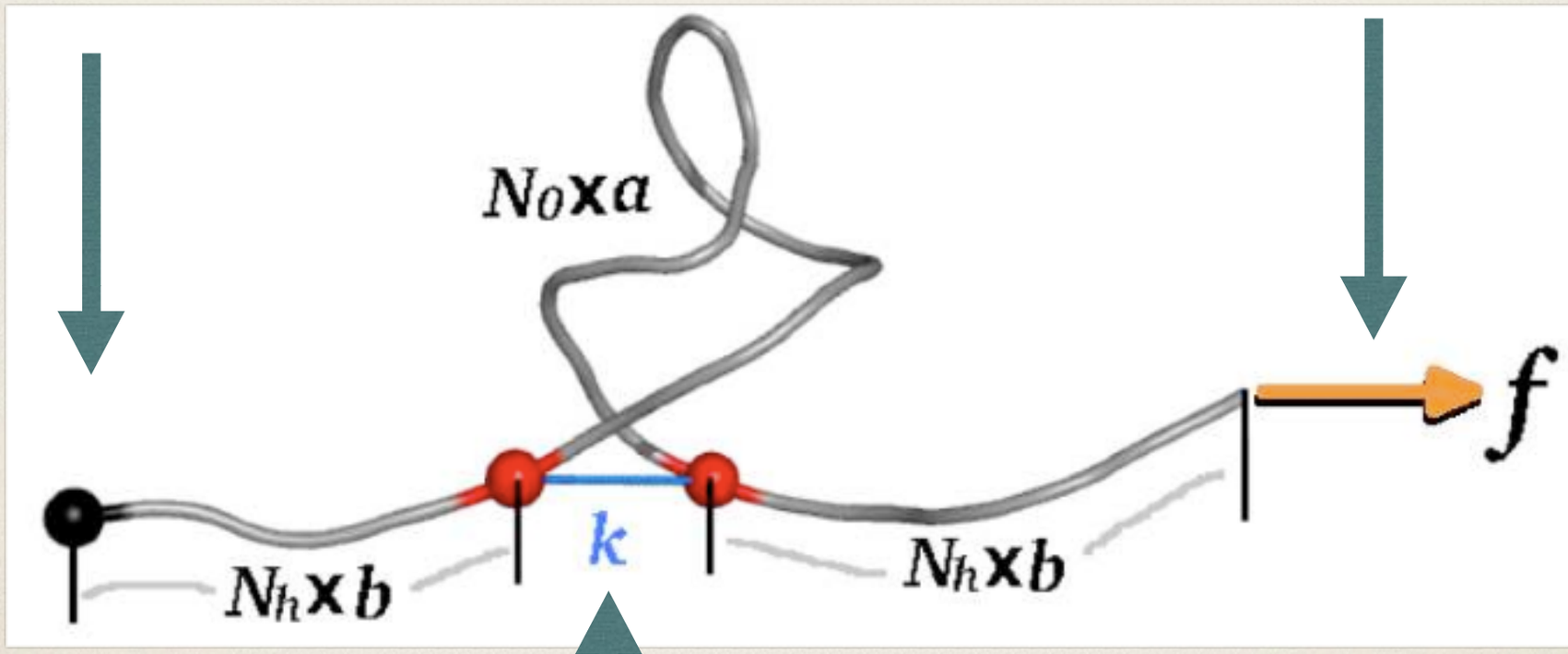


Model handles as Gaussian, with spacing b and with N_h bonds

A Generalized Rouse Model

Fix one endpoint

Pull the other with the unfolding force



Model two-state nature with a harmonic bond that has a cutoff

Modeling 'stiffness' with a Gaussian chain

Under tension, a Wormlike (stiff) chain has longitudinal fluctuations

$$\langle \delta R_{||}^2 \rangle \sim \frac{L}{\sqrt{l_p}} \left(\frac{k_B T}{f} \right)^{\frac{3}{2}}$$

A Gaussian chain

$$\langle \delta R_{||}^2 \rangle \sim Lb$$

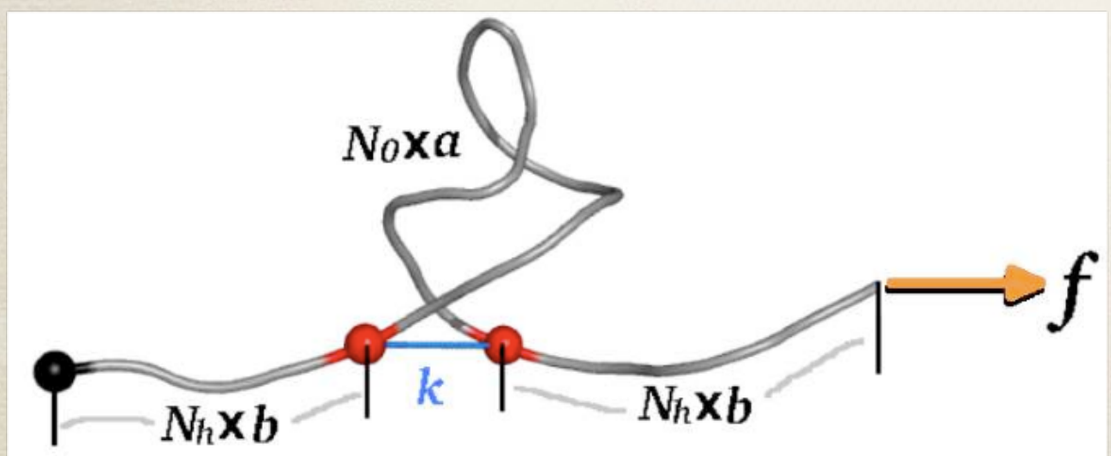
Smaller spacing mimics stiffer handles

$$l_p^{eff} \sim b^{-2}$$

A Generalized Rouse Model

Toy model Hamiltonian is

$$\begin{aligned}\beta H &= \frac{3}{2b^2}\int_0^{N_h} ds\ \dot{\mathbf{r}}^2(s) + \frac{3}{2a^2}\int_{N_h}^{N-N_h} ds\ \dot{\mathbf{r}}^2(s) + \frac{3}{2b^2}\int_{N-N_h}^{N} ds\ \dot{\mathbf{r}}^2(s) \\ &\quad -\beta\mathbf{f}\cdot\int_0^N ds\ \mathbf{r}(s) + \beta V[\mathbf{r}(N-N_h)-\mathbf{r}(N_h)]\end{aligned}$$



with

$$V[\mathbf{x}] = \begin{cases} k\mathbf{x}^2/2 & |\mathbf{x}| \le c \\ kc^2/2 & |\mathbf{x}| > c \end{cases}$$

Free Energy Profiles

The equilibrium distribution functions are

$$P(z_m) = \frac{z_m}{\mathcal{N}} e^{-3z_m^2/2N_0a^2 - \beta V[z_m]} \sinh(f z_m)$$

$$P(z_{sys}) = \sqrt{\frac{3}{\pi b^2 N_h}} \frac{\sinh(z_{sys})}{\mathcal{N}} \int_0^\infty dy\ y \sinh\left(\frac{3yz_{sys}}{N_h b^2}\right) e^{-3y^2/2N_0a^2 - 3(y-z_{sys})^2/4N_h b^2 - \beta V[y]}$$

$$\mathcal{N} = \int_0^\infty dy\ y e^{-3y^2/2N_0a^2 - \beta V[y]} \sinh(fy)$$

$N_0 = 20$

$a = 0.5$ nm

$f = 15.4$ pN

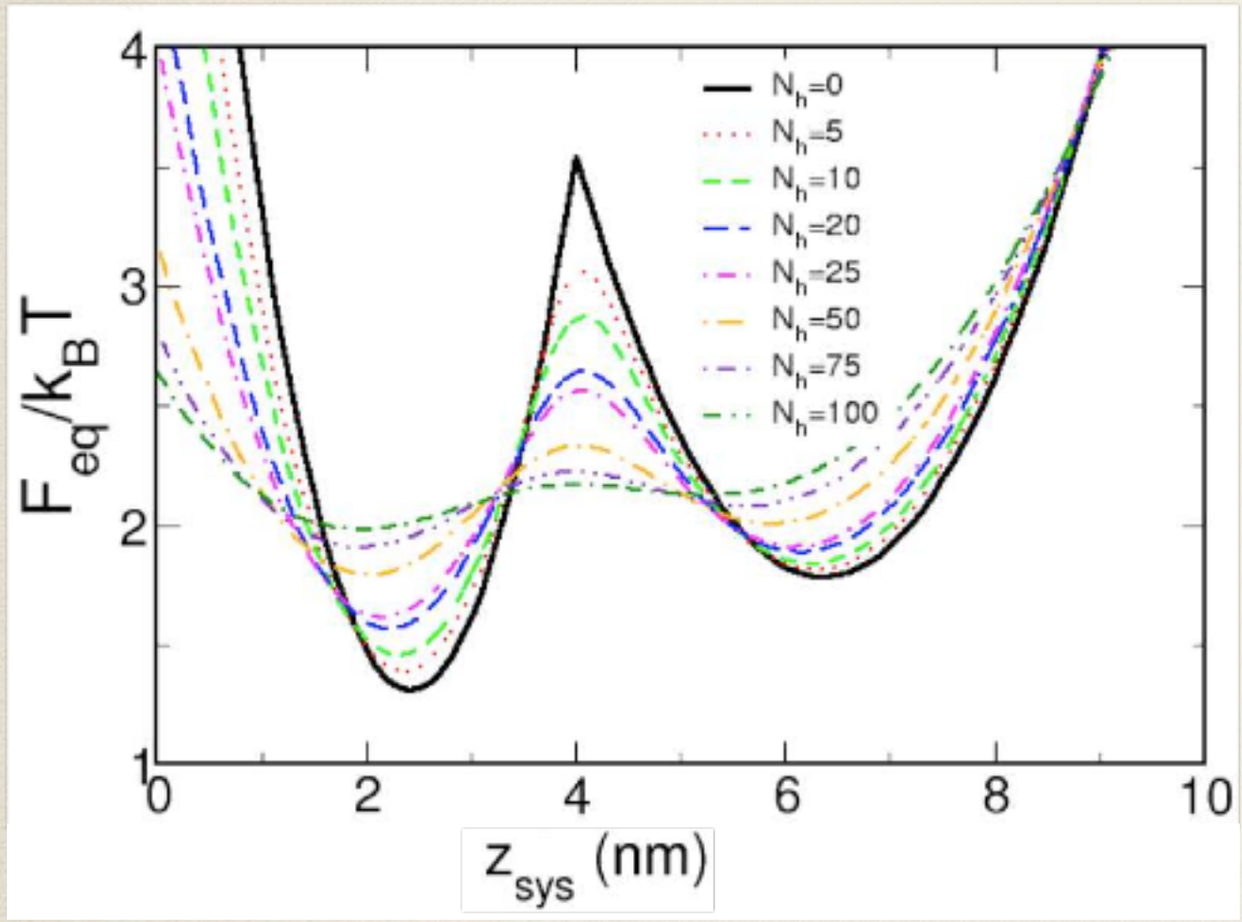
$k = 0.54$ pN/nm

$c = 4$ nm

These choices qualitatively reproduce the handle-free Free Energy Profile of the SOP RNA model

Free Energy Profiles

$$b = a/3$$

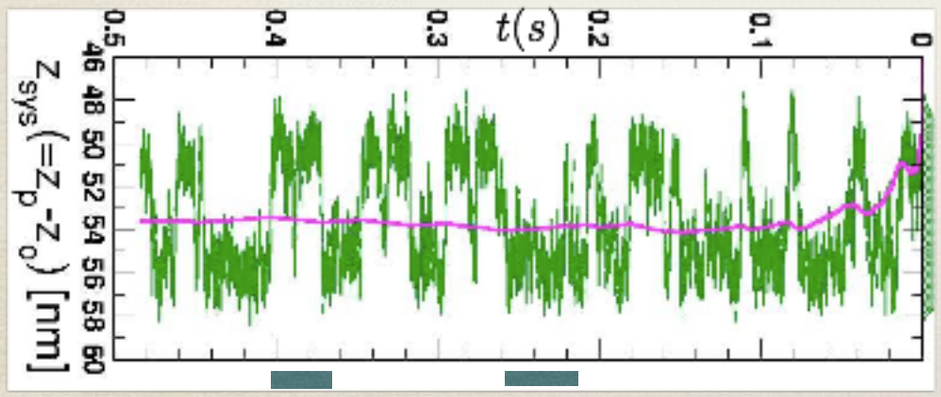


The free energy profile for the GRM gives consistent results compared to the SOP simulation.

- Increasing the handle length causes disagreements between $F_{eq}(r_m)$ and $F_{eq}(r_{sys})$

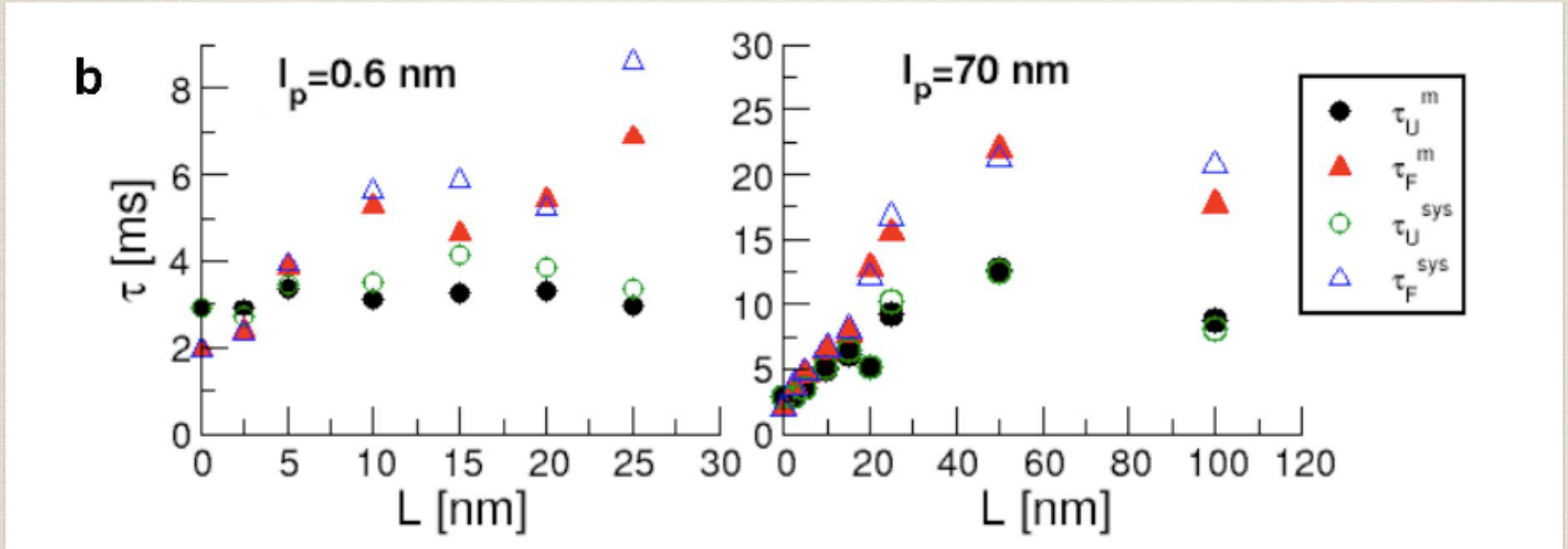
Kinetic Properties of Interest

- $\tau_{U(F)}^{m}$ The unfolding (folding) rate, measured using z_m
- $\tau_{U(F)}^{sys}$ The unfolding (folding) rate, measured using z_{sys}
- $\tau_{U(F)}^{0}$ The intrinsic unfolding (folding) rate, with no handles atatched



$$\tau_{U(F)} = \frac{1}{N} \sum_i \tau_{U(F)}^{(i)}$$

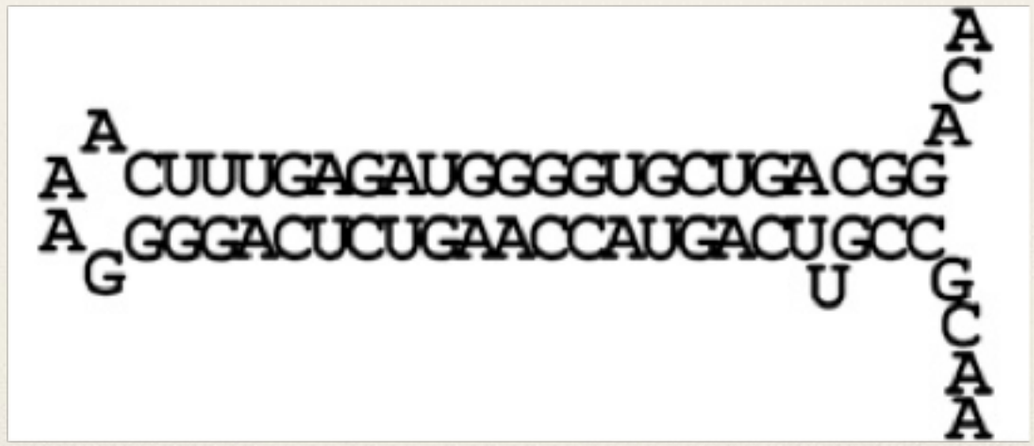
Folding and Unfolding Kinetics



- Handles of any type will increase folding and unfolding times
- Stiffer handles change the times more than flexible handles
- Observed times are almost identical between z_m and z_{sys}

Some Experimental Results

Manosas et. al. studied the effect of multiple experimental parameters on the hopping kinetics of the 56nt P5ab RNA hairpin, using both simulations and experiments.

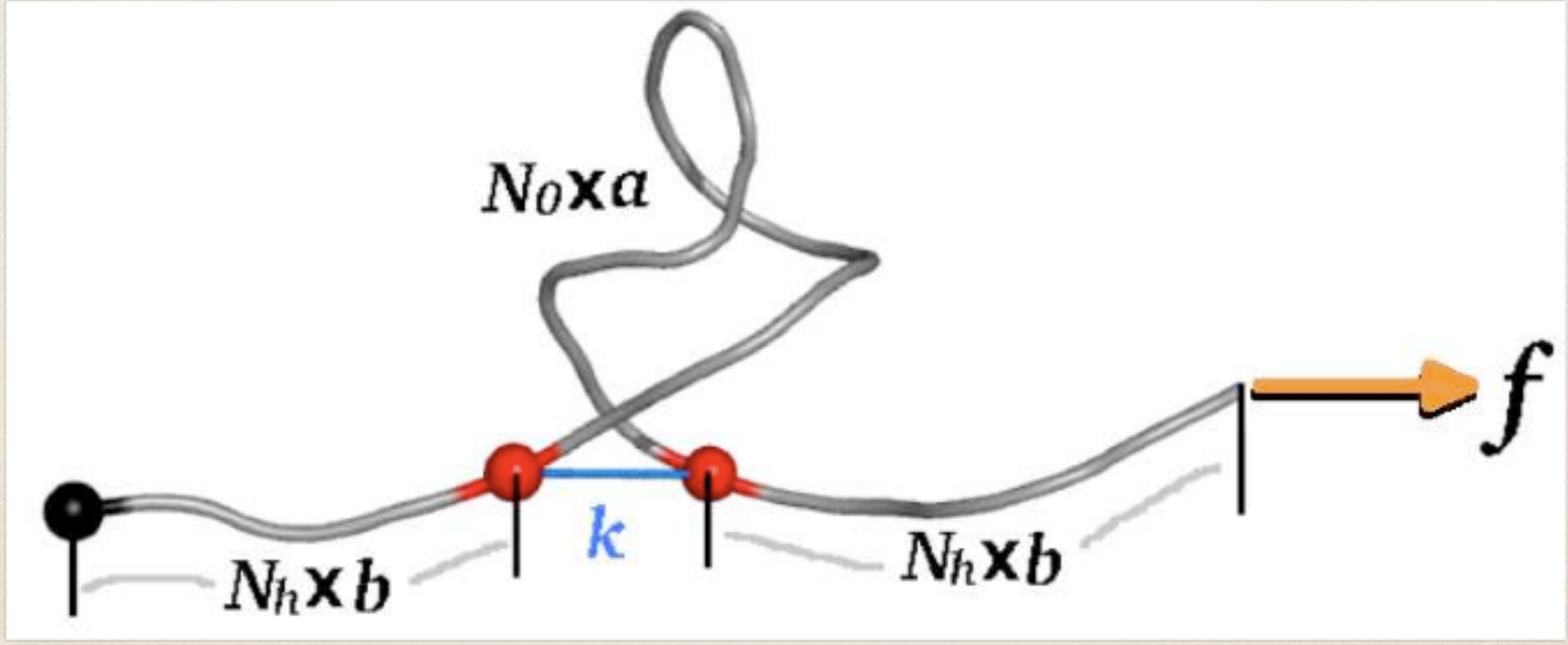


They find flexible handles change the kinetics of the hairpin less than stiff handles.

Manosas, Biophys J **92** 3010 (2007)

Hopping kinetics Toy Model

The simple two-state Gaussian chain can capture the essential physics of the kinetic effects of the handles.



Modeling the Folding Kinetics

If we start in an unfolded state, our single harmonic interaction is cut off, so it does not contribute to the folding kinetics.

The folding of the RNA is purely entropic
(no interaction energy).

$$H = \frac{3}{2a^2}\mathbf{R}\mathbf{M}\mathbf{R}^T - \beta \mathbf{f}\mathbf{R}^T$$

with $\mathbf{R} = (\mathbf{r}_1, \ldots, \mathbf{r}_{N+1})^T$ and $\mathbf{F} = (-\mathbf{f}, 0, \ldots, 0, \mathbf{f})^T$.

M is a Rouse-like matrix, that includes the handles with spacing b and the "RNA" with spacing a

$$\mathbf{M}(b=a) = \begin{pmatrix} 1 & -2 & 0 & 0 & 0 \\ -2 & 2 & -2 & 0 & 0 \\ 0 & \ddots & \ddots & \ddots & 0 \\ 0 & 0 & -2 & 2 & -2 \\ 0 & 0 & 0 & -2 & 1 \end{pmatrix}.$$

Modeling the Folding Kinetics

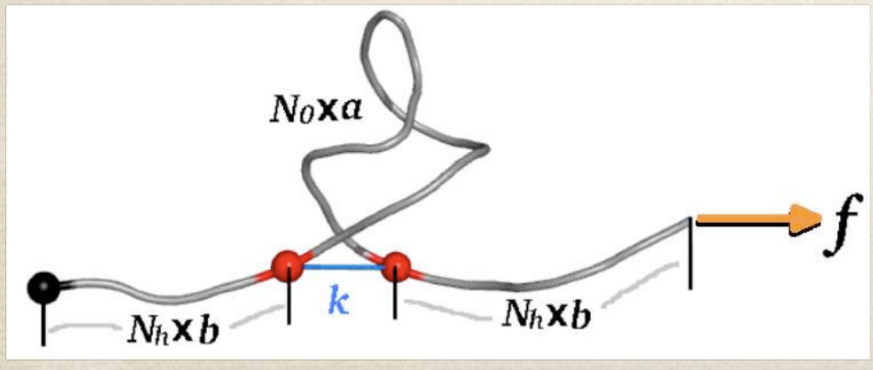
If we start in an unfolded state, our single harmonic interaction is cut off, so it does not contribute to the folding kinetics.

The folding of the RNA is purely entropic
(no interaction energy).

The discrete representation of the Hamiltonian is

$$\beta H = \frac{3}{2b^2}\sum_{i=1}^{N_h}(\mathbf{r}_{i+1}-\mathbf{r}_i)^2 + \frac{3}{2a^2}\sum_{i=N_h+1}^{N-N_h}(\mathbf{r}_{i+1}-\mathbf{r}_i)^2 + \frac{3}{2b^2}\sum_{i=N-N_h+1}^{N}(\mathbf{r}_{i+1}-\mathbf{r}_i)^2$$

$$-\beta\mathbf{f}\cdot(\mathbf{r}_N-\mathbf{r}_1)+\beta k_0\mathbf{r}_1^2$$



An Exact Solution to the Langevin Equation

From the Hamiltonian, we can find the overdamped Langevin equation for each coordinate:

$$\zeta\dot{\mathbf{X}}(t) = -\frac{3\beta}{a^2}\mathbf{M}\mathbf{X} + \beta\mathbf{f}^T\mathbf{X} + \mathbf{N}(t)$$

This has the exact solution

$$\mathbf{X}(t) = \mathbf{A}(t)\mathbf{X}(0) + \frac{a^2}{3}\mathbf{M}^{-1}\Big(\mathbf{I} - \mathbf{A}(t)\Big) + \int_0^t dt'\ \mathbf{A}(t-t')\mathbf{N}(t')$$

$\mathbf{A}(t) = \exp(-3Dt\mathbf{M}/a^2)$, and $\mathbf{N}(t)$ a white noise force

An Exact Solution to the Langevin Equation

The propagator for any internal or end-to-end distance can be found by averaging over noise and coordinates

$$G(\mathbf{R}_{nm}, t|\mathbf{R}^0_{nm}) = \left(\frac{3}{2\pi\langle\mathbf{R}^2_{nm}\rangle\sqrt{1-\varphi^2(t)}}\right)^3 \exp\left(\frac{3}{2\langle\mathbf{R}^2_{nm}\rangle(1-\varphi^2(t))}\right.$$
$$\left.\times\left[\bar{\mathbf{R}}^2_{nm} + (\bar{\mathbf{R}}^0_{nm})^2 - 2\varphi(t)\bar{\mathbf{R}}_{nm}\cdot\bar{\mathbf{R}}^0_{nm}\right]\right)$$

with

$$\langle\mathbf{R}^2_{nm}\rangle = (\mathbf{M}^{-1})_{nn} + (\mathbf{M}^{-1})_{mm} - 2(\mathbf{M}^{-1})_{nm}$$

$$\langle\mathbf{R}^2_{nm}\rangle \times \varphi(t) = (\mathbf{M}^{-1}\mathbf{A}(t))_{nn} + (\mathbf{M}^{-1}\mathbf{A}(t))_{mm} - 2(\mathbf{M}^{-1}\mathbf{A}(t))_{nm}$$

$$\bar{\mathbf{R}} = \mathbf{R} - \langle\mathbf{R}_{nm}\rangle = \mathbf{R} - \beta f\langle\mathbf{R}^2_{nm}\rangle/3$$

Modeling the Folding Kinetics

The theory of Wilemski and Fixman relates the loop closure time (folding time) to the propagator as

$$\tau_F \approx \int_0^\infty dt \left(\frac{C(t)}{C(\infty)} - 1 \right)$$

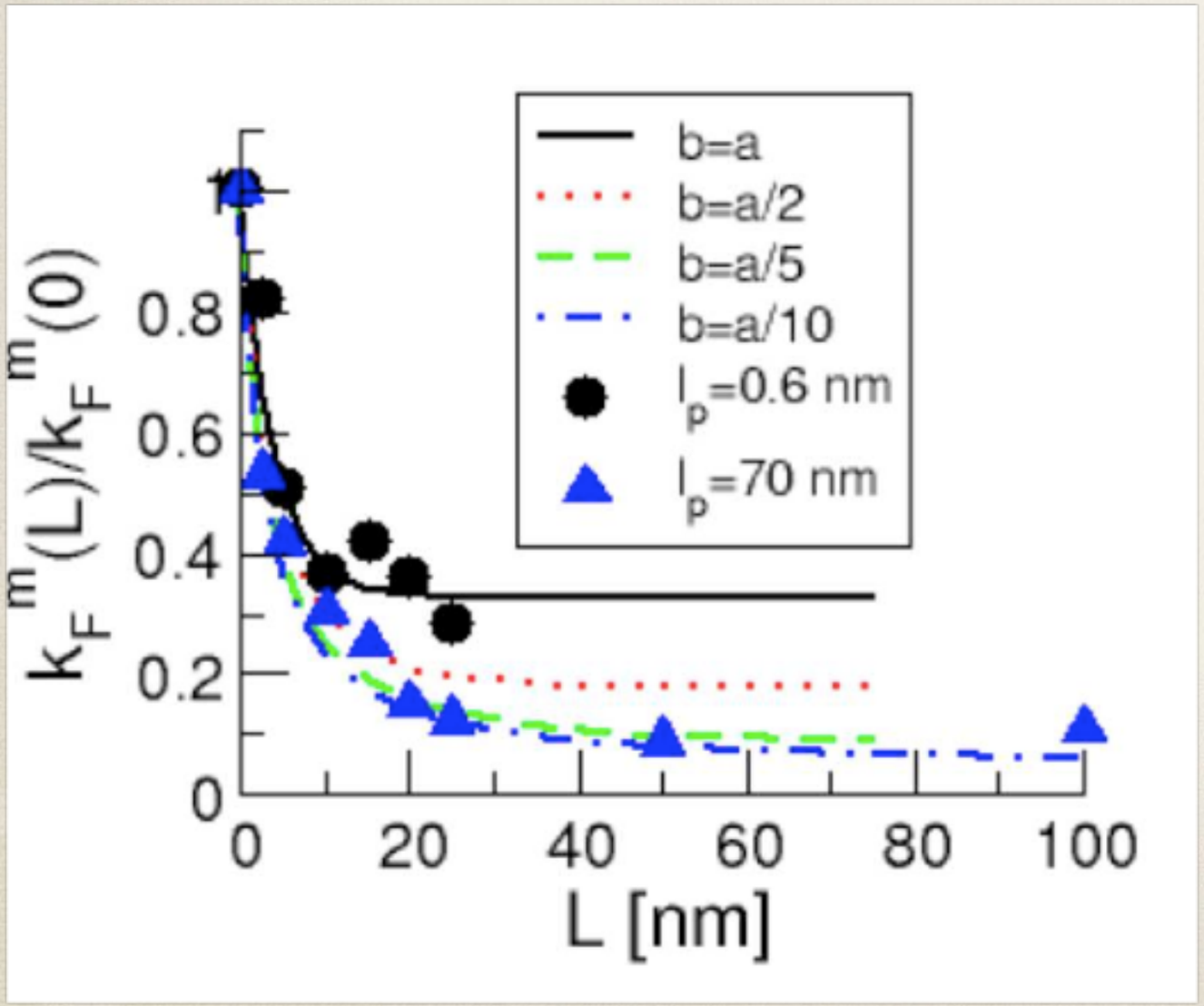
$$C(t) = \int d^3\mathbf{R} d^3\mathbf{R}_0 S(\mathbf{R}) G(\mathbf{R}, t|\mathbf{R}_0) S(\mathbf{R}_0) P_{eq}(\mathbf{R}_0) \qquad S(\mathbf{R}) = \delta(|\mathbf{R}| - c)$$

For high tension, the system is almost one-dimensional.

$$C(t) \approx G_z(c, t|c) P_z^{eq}(c)$$

Modeling the Folding Kinetics

The GRM gives surprisingly good agreement with the SOP simulations for the folding rates



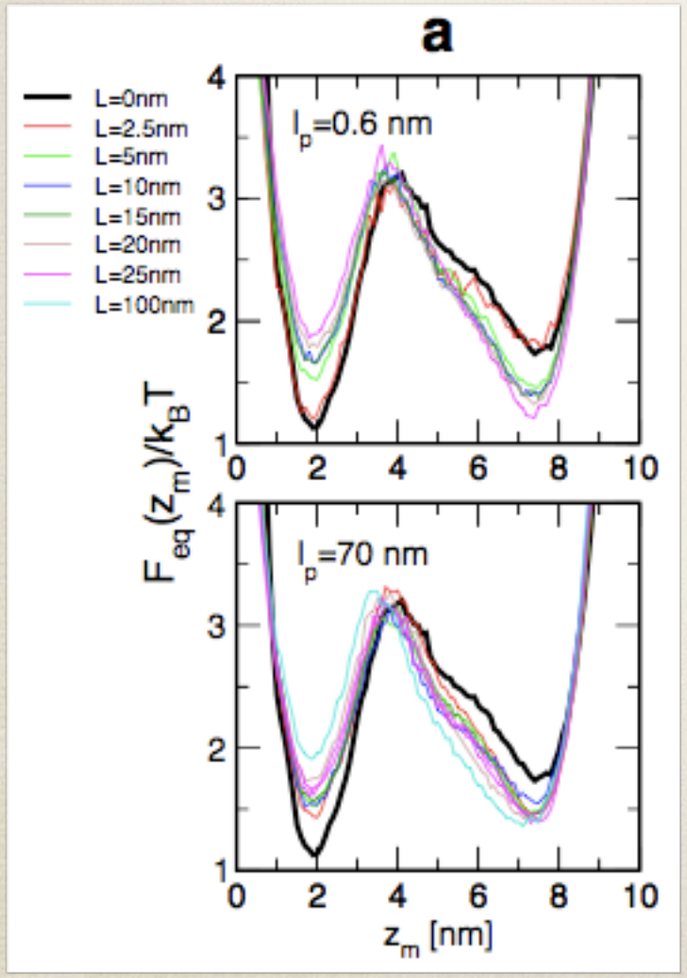
All handles decrease the folding rate

Stiffer handles have a much larger effect on the folding rate

The GRM can reproduce the folding rate accurately

Determining the Intrinsic Rates

If attaching handles always changes the hopping times, how can one determine the intrinsic rates?



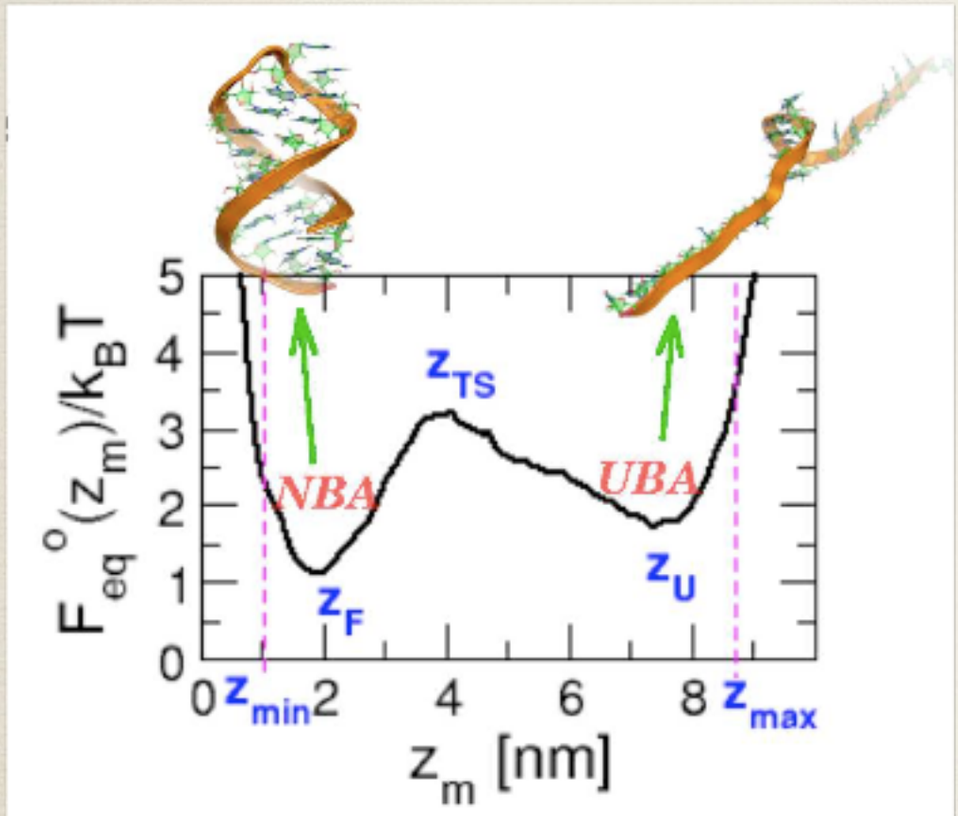
Attaching handles to the RNA slightly perturbs the free energy as measured by z_m

$$F_{eq}(z_m) \approx F^o_{eq}(z_m)$$

Both stiff and flexible handles cause similar perturbations.

How good is z_m as a reaction coordinate?

Accurately measuring the free energy allows
the determination of the rates (first passage)



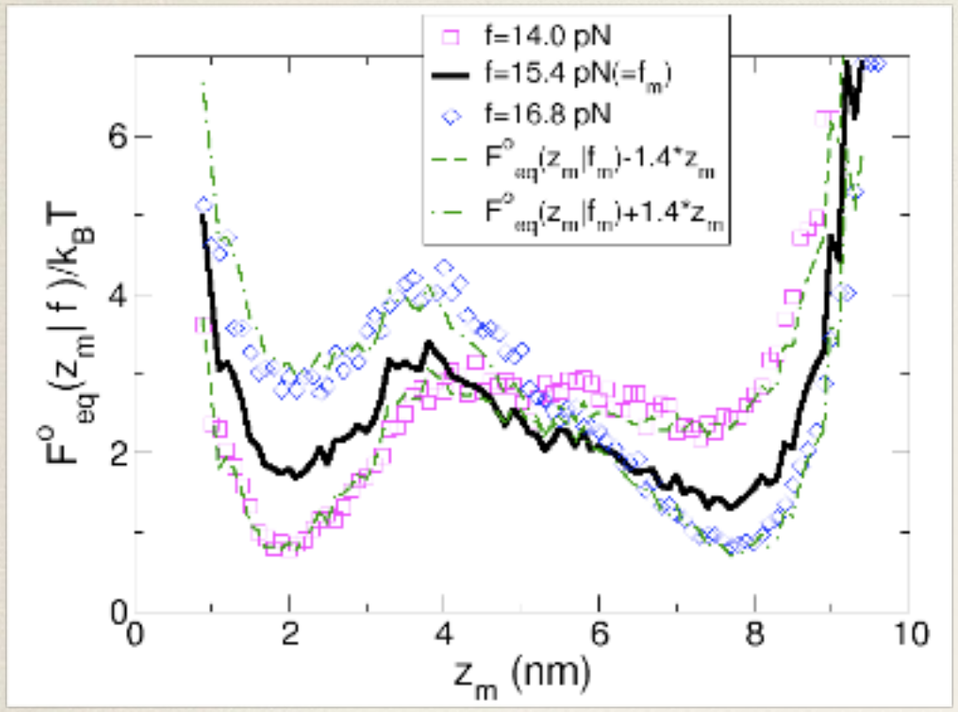
$$\tau_U^{KR}(z) = \int_z^{z_U} dy e^{\beta F_{eq}(y)} \frac{1}{D_U} \int_{z_{min}}^{y} dx e^{-\beta F_{eq}(x)}$$

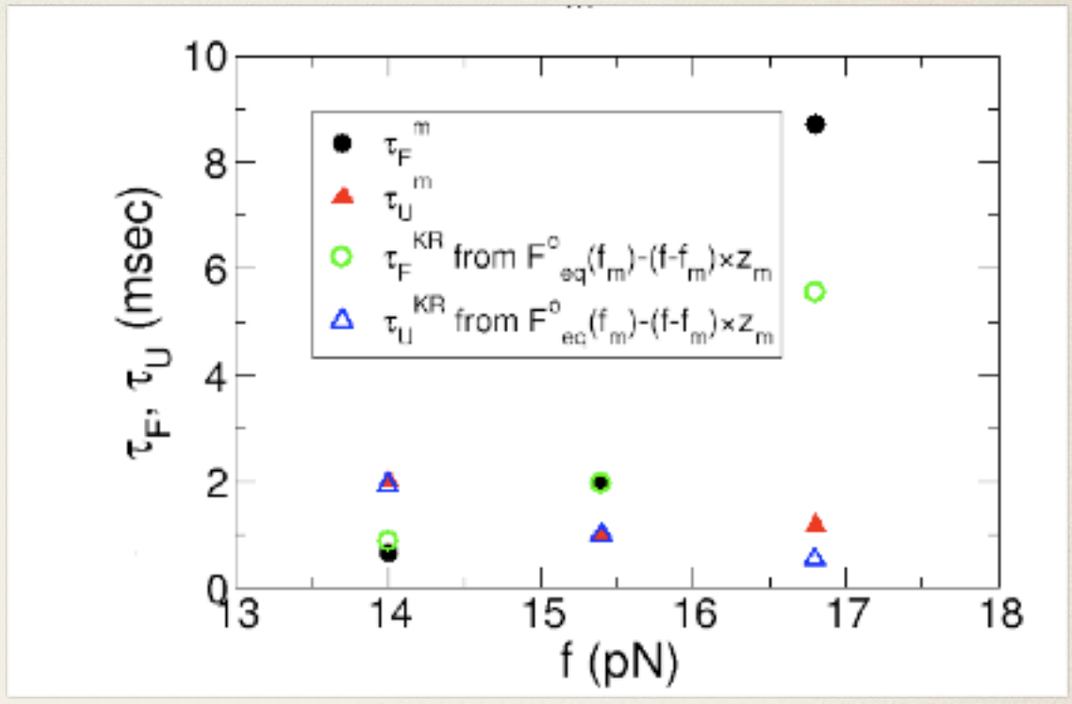
$$\tau_F^{KR}(z) = \int_{z_F}^{z} dy e^{\beta F_{eq}(y)} \frac{1}{D_F} \int_{y}^{z_{max}} dx e^{-\beta F_{eq}(x)}$$

The hopping rates for varying force
can be determined by
tilting the free energy profile
at the transition force

$$F_{eq}(z_m|f) = F_{eq}(z_m|f_m) - (f - f_m) \times z_m$$

Kramer's Theory



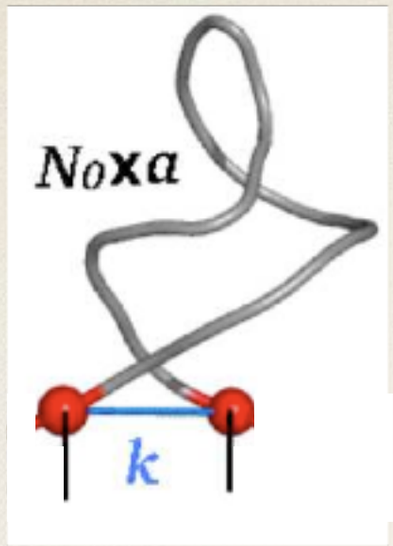


The SOP simulations of the P5GA verify that

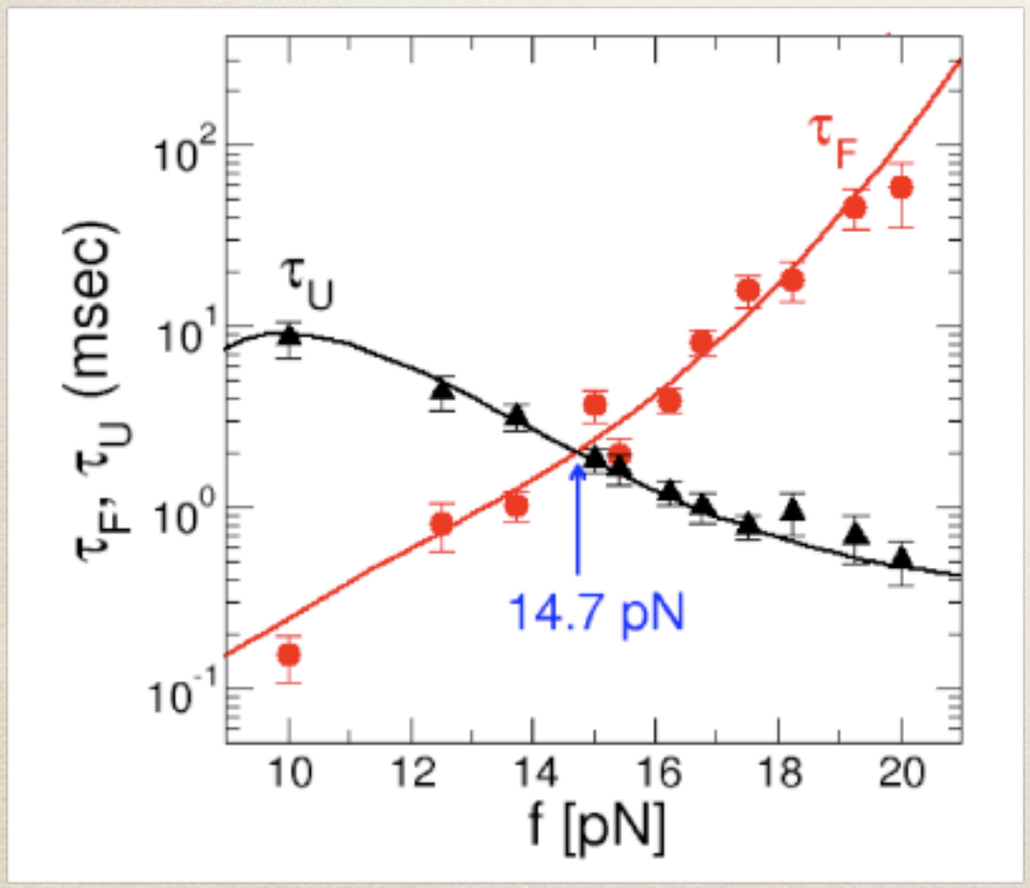
$$F_{eq}(z_m|f) = F_{eq}(z_m|f_m) - (f - f_m) \times z_m$$

Kramer's theory gives good estimates for the hopping times

Kramer's Theory

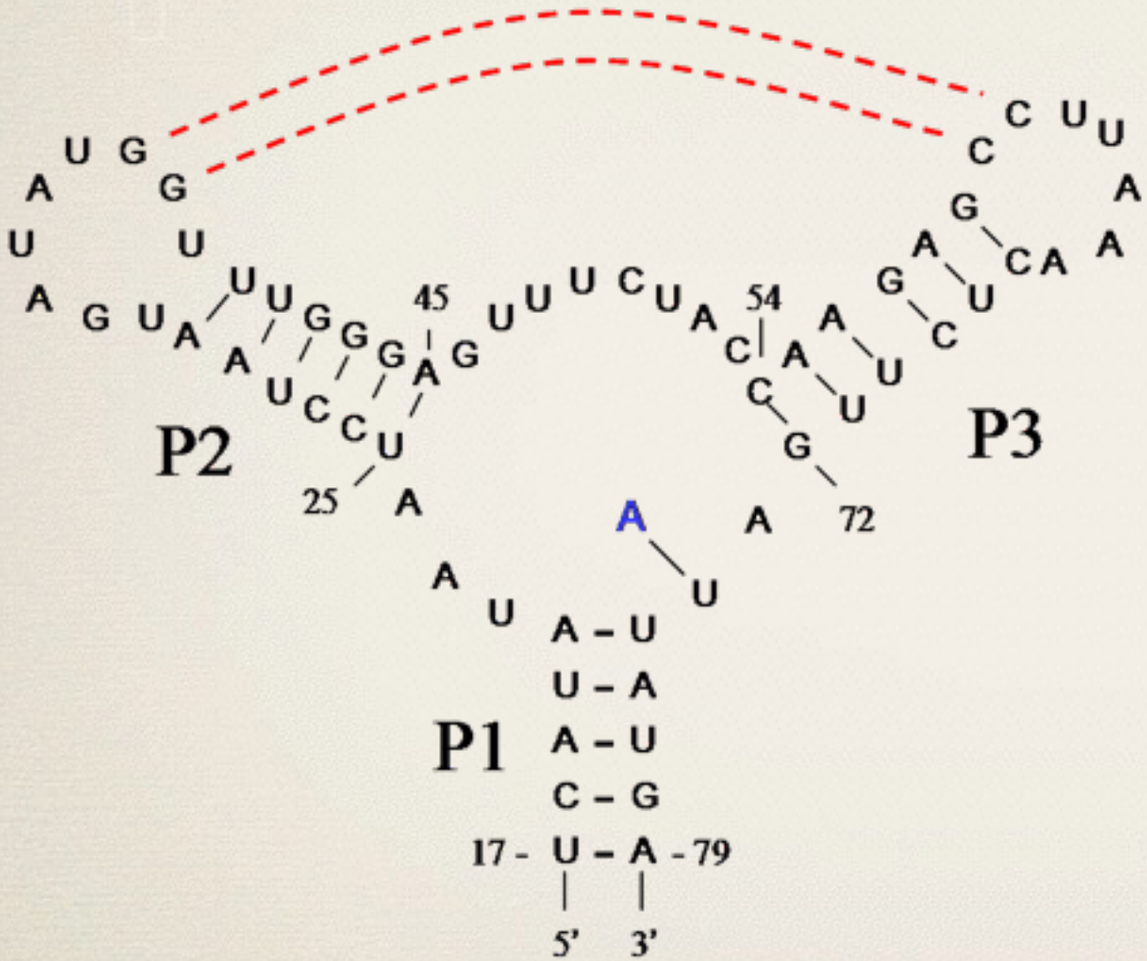


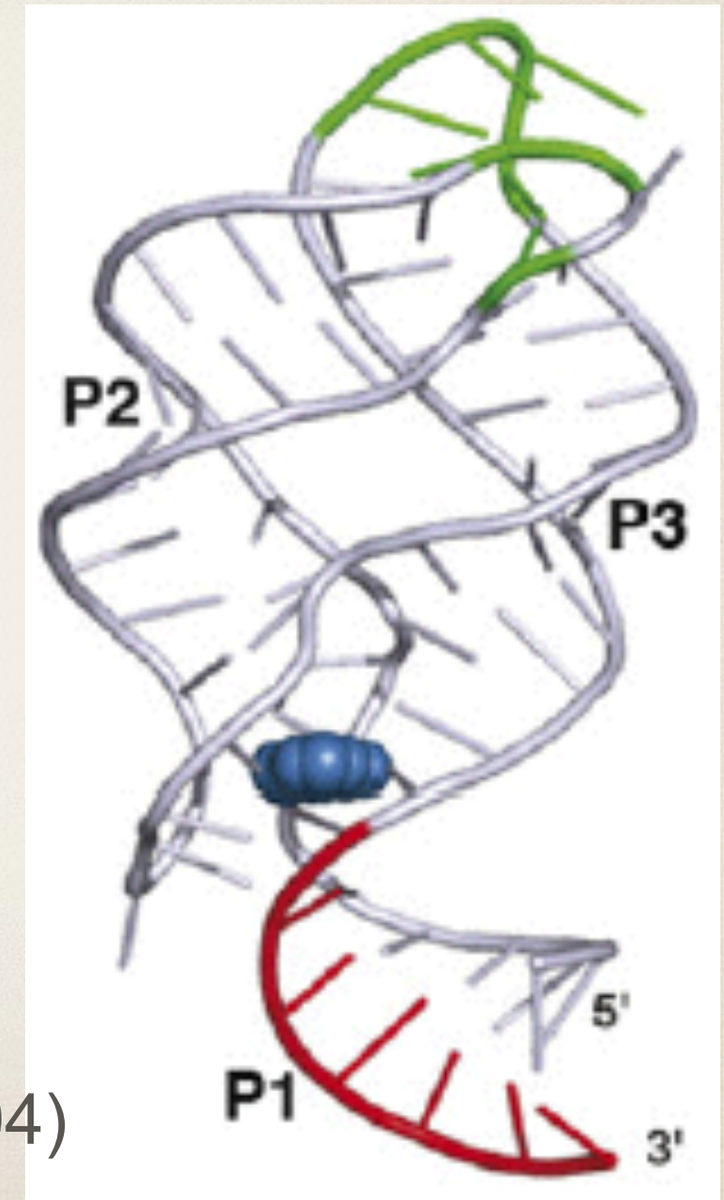
To study a wider range of forces, we can simulate the handle-free GRM directly



Kramer's theory gives excellent agreement over a wide range of forces

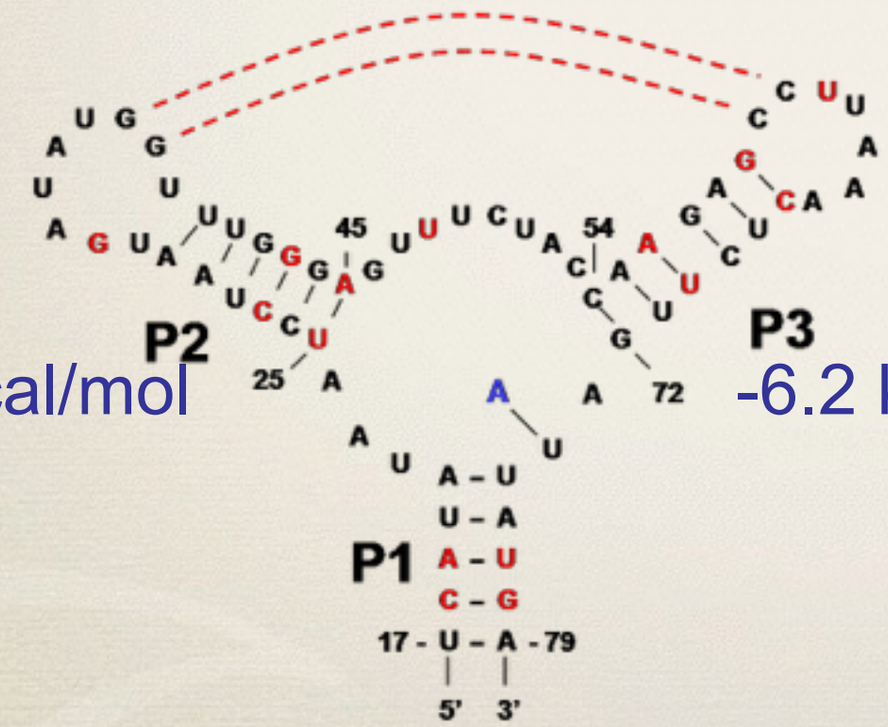
Add adenine riboswitch aptamer





(A. Serganov et al., *Chem. Biol*. 11, 1729, 2004)

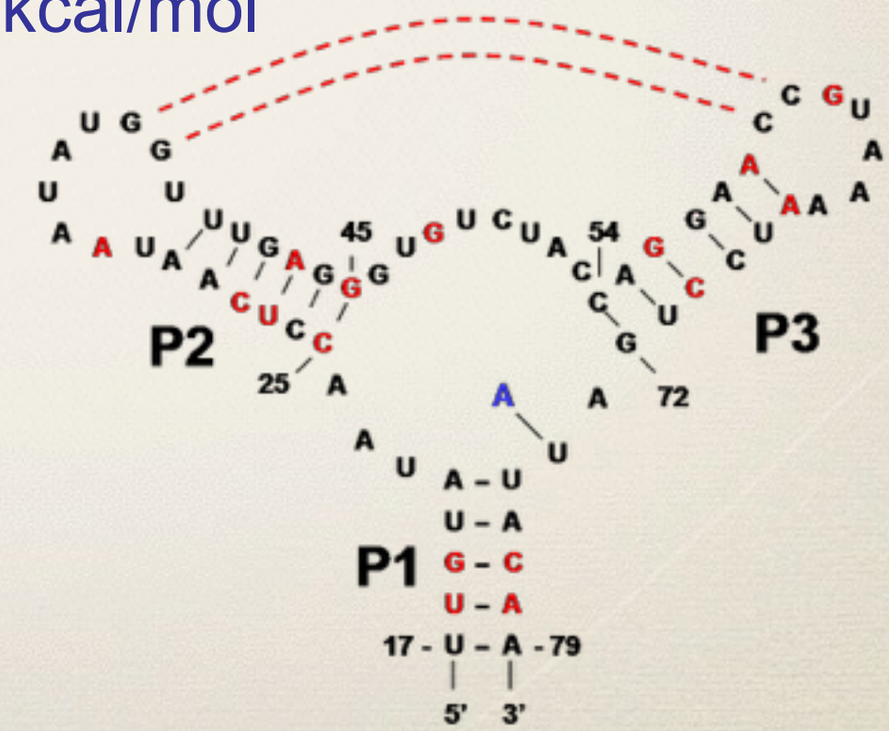
Folding pathway



-5.0 kcal/mol

-6.2 kcal/mol

-7.3 kcal/mol



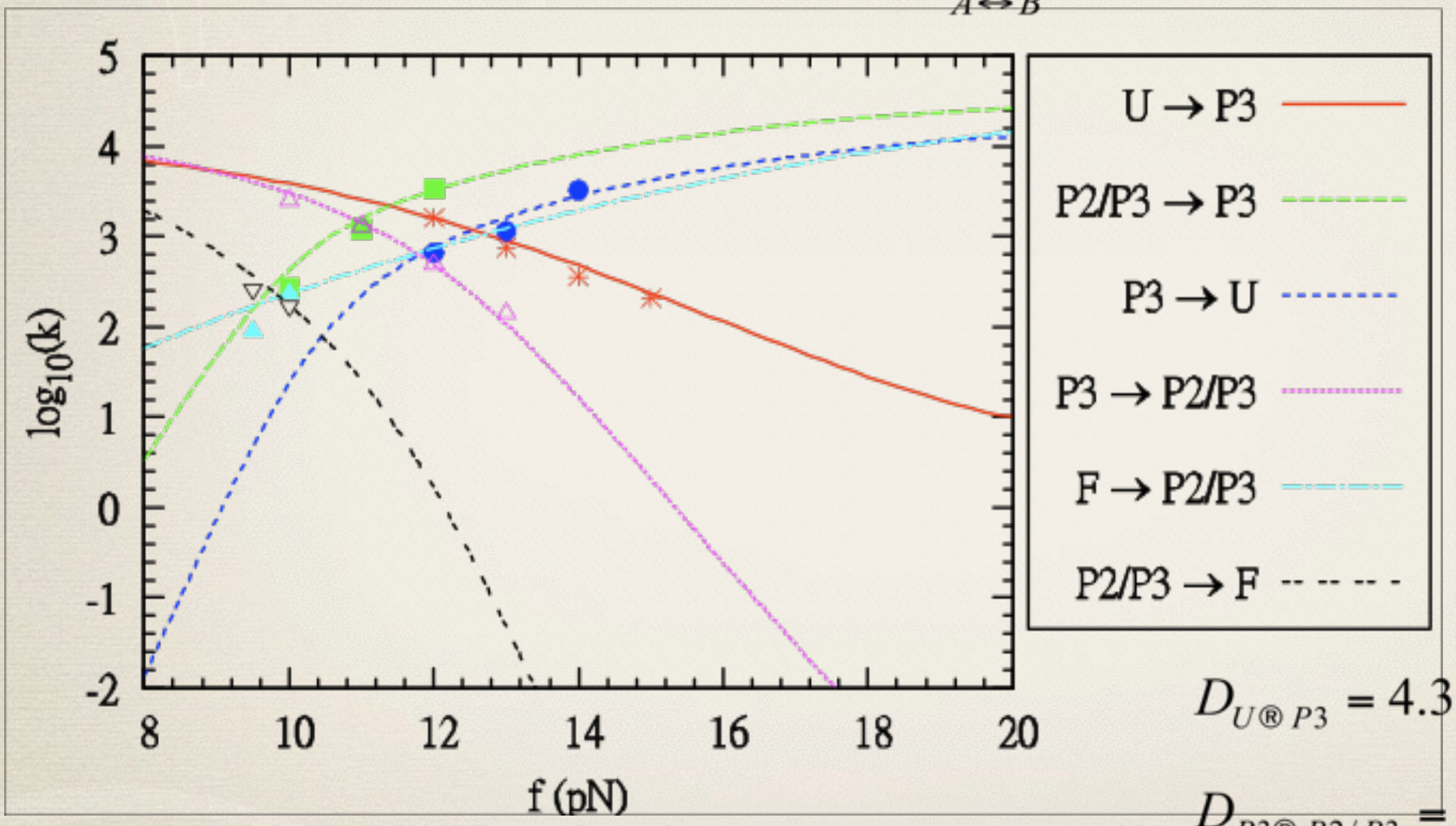
-5.2 kcal/mol

U → P3 → P2/P3 → F

U → P2 → P2/P3 → F

Transition rates

$$k^{-1}(f) = \tau(f) \approx \int_{x_A}^{x_B} dy e^{\beta G(y)} \frac{1}{D_{A \leftrightarrow B}} \int_{x_0}^{y} dz e^{-\beta G(z)}$$



$$D_{U \circledR P3} = 4.3 \times 10^4 \, \mathrm{nm}^2/\mathrm{s}$$

$$D_{P3 \circledR P2/P3} = 8.7 \times 10^4 \, \mathrm{nm}^2/\mathrm{s}$$

$$D_{P2/P3 \leftrightarrow F} = 2 \times 10^5 \, \mathrm{nm}^2/\mathrm{s}$$