

# Macromolecules

## 7. Macromolecular Mechanics

Forces are present everywhere in biology. Near equilibrium, these exist as local fluctuating forces that induce thermally driven excursions from the free-energy minimum, and non-equilibrium problems for small perturbations from equilibrium are commonly described using linear response theory. We also encounter forces far from equilibrium, including forces derived from external energy sources (such as ATP). One example of this limit involves the directed motion of molecular motors along actin and microtubules.

Our focus in this section is on experiments that allow careful application and measurement of forces on single macromolecules. These are being performed to understand mechanical properties and stress/strain relationships. They can also be unique reporters of biological function involving the strained molecules. Examples:

	Force Range (pN)	Displacement (nm)	Loading rate (pN/sec)	
Optical Tweezers:	0.1–100 pN	0.1–10 <sup>5</sup>	5–10	Near equilibrium
AFM:	10–10 <sup>4</sup>	0.5–10 <sup>4</sup>	100–1000	Non-equilibrium!
Stretching under flow:	0.1–1000 pN	10–10 <sup>5</sup>	1–100	Steady state force
MD simulations:	Arb.	<10 nm	10 <sup>5</sup> –10 <sup>7</sup> !	

Remember:  $k_B T$ : 4.1 pN nm

### Force and Work

Here we will focus on the stretching and extension behavior of macromolecules. The work done on the system by an external force to extend a chain is

$$w = -\int \vec{f}_{ext} \cdot d\vec{x}$$

Work ( $w$ ) is a scalar, while force ( $f$ ) and displacement ( $x$ ) are vectors. On extension, the external force is negative, leading to a positive value of  $w$ , meaning work was done on the system. Classical mechanics tells us that the force is the negative gradient of the potential one is stretching against ( $\vec{f} = -\partial U / \partial x$ ), but we will have to work with free energy and the potential of mean force since the configurational entropy of the chain is important. Since the change in free energy for a process is related to the reversible work needed for that process, we can relate the force along a *reversible path* to the free energy through

$$\vec{f}_{rev} = -\left(\frac{\partial G}{\partial x}\right)_{p,T,N} = -\left(\frac{\partial A}{\partial x}\right)_{V,T,N}$$

This describes the reversible process under which the system always remains at equilibrium, although certainly it is uncomfortable relating equilibrium properties ( $G$ ) to nonequilibrium ones such as pulling a protein apart. For an arbitrary process,  $\Delta G \leq w$ .

### **Jarzynski Equality**

A formal relationship between the free energy difference between two states and the work required to move the system from initial to final state has been proposed. The Jarzynski equality states

$$e^{-\Delta G/kT} = \left\langle e^{-w/k_B T_{\text{in}}} \right\rangle_{\text{path}}$$

Here one averages the Boltzmann-weighted work in the quantity at right over all possible paths connecting the initial and final states, setting  $T$  to the initial temperature ( $T_{\text{in}}$ ), and one obtains the Boltzmann weighted exponential in the free energy. This holds for irreversible processes! Further, since one can show that  $\left\langle e^{-w/k_B T} \right\rangle \geq e^{-\langle w \rangle / k_B T}$ , we see that the average work done to move the system between two states is related to the free energy through  $\langle w \rangle \geq \Delta G$ . This reinforces what we know about the macroscopic nature of thermodynamics, but puts an interesting twist on it. Although the *average* work done to change the system will equal or exceed the free-energy difference, for any one microscopic trajectory, the work may be less than the free-energy difference. This has been verified by single molecule force/extension experiments.

### **Statistical Mechanics of Work**

Let's relate work and the action of a force to changes in statistical thermodynamic variables:<sup>1</sup>

The internal energy is

$$U = \langle E \rangle = \sum_j P_j E_j$$

and therefore the change in energy in a thermodynamic process is

$$dU = d\langle E \rangle = \sum_j E_j dP_j + \sum_j P_j dE_j$$

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1. T. L. Hill, *An Introduction to Statistical Thermodynamics*. (Addison-Wesley, Reading, MA, 1960), pp. 11-13, 66-77.

Note the close relationship between this expression and the First Law:

$$dU = dw + dq$$

We can draw parallels between the two terms in these expressions:

$$dq_{\text{rev}} = TdS \quad \leftrightarrow \quad \sum_j E_j dP_j$$

$$dw \cong pdV \text{ or } f dx \quad \leftrightarrow \quad \sum_j P_j dE_j$$

Heat is related to the ability to change populations of energetically different states, whereas work is related to the ability to change the energy levels with an external force.

### Continuum Mechanics<sup>2</sup>

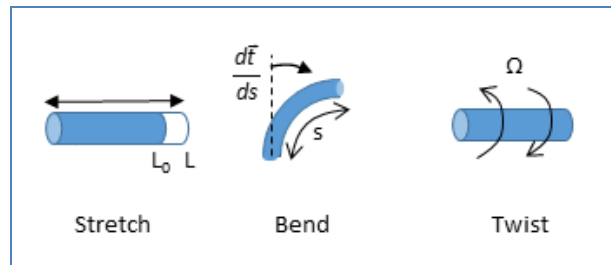
Summary of forces on a rod, as commonly applied to dsDNA

$s$ : position along contour of rod

$L_0$ : unperturbed length of rod

$\vec{t}$ : tangent vector.  $d\vec{t}/ds$ : curvature

$\Omega$ : local twist



The energy for distorting the rod is

$$U = U_{st} + U_b + U_{tw}$$

In the harmonic approximation for the restoring force, we can write these contributions as

$$U = \frac{1}{2} \int_{L_0}^L \kappa_{st} s ds + \frac{1}{2} \int_{L_0}^L \kappa_b \left( \frac{d\vec{t}}{ds} \right)^2 ds + \frac{1}{2} \int_{L_0}^L \kappa_{tw} \Omega^2 ds$$

2. D. H. Boal, *Mechanics of the Cell*, 2nd ed. (Cambridge University Press, Cambridge, UK, 2012).

The force constants are:

Stretching:  $\kappa_{st} = \kappa_{st-entropic} + \kappa_{st-enthalpic}$

$$\kappa_{st-entropic} \approx 3k_B T / \ell_p L_c$$

where  $\ell_p$  is the persistence length and  $L_c$  is the contour length.

Bending:  $\kappa_b$


$$\kappa_b \approx 205 \text{ pN nm}^2 \quad \text{for dsDNA}$$


Twisting:  $\kappa_{tw}$

$$\kappa_{tw} \approx (86\text{nm})k_B T = 353 \text{ pN nm}^2 \quad \text{for dsDNA}$$



Writhe is coupled bending and twisting, most commonly found in DNA supercoiling. The writhe number refers to the number of complete loops. Writhe can be positive or negative.

positive: 

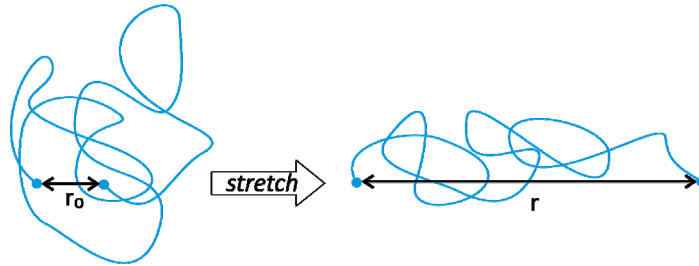
negative: 

## Polymer Elasticity: Force–Extension Behavior

### The Entropic Spring

To extend a polymer requires work. We calculate the reversible work to extend the macromolecule from the difference in free energy of the chain held between the initial and final state. This is naturally related to the free energy of the system as a function of polymer end-to-end distance:

$$w_{\text{stretch}} = A(r) - A(r_0) = - \int_{r_0}^r \vec{f}_{\text{rev}} \cdot d\vec{r}$$



For an ideal chain, the free energy depends only on the entropy of the chain:  $A = -TS$ . There are fewer configurational states available to the chain as you stretch to larger extension. The number of configurational states available to the system can be obtained by calculating the conformational partition function,  $Q_{\text{conf}}$ . For stretching in one dimension, the Helmholtz free energy is:

$$\begin{aligned} dA &= -p dV - S dT + f dx \\ &= -k_B T \ln Q_{\text{conf}} \\ S_{\text{conf}} &= k_B \ln Q_{\text{conf}} \\ f &= - \left( \frac{\partial A}{\partial x} \right)_{V,T,N} = -k_B T \frac{\partial \ln Q_{\text{conf}}}{\partial x} = -T \frac{\partial S_{\text{conf}}}{\partial x} \end{aligned} \quad (1)$$

When you increase the end-to-end distance, the number of configurational states available to the system decreases. This requires an increasingly high force as the extension approaches the contour length. Note that more force is needed to stretch the chain at higher temperature.

Since this is a freely joined chain and all microstates have the same energy, we can equate the conformational partition function of a chain at a particular extension  $x$  with the probability density for the end-to-end distances of that chain

$$Q_{\text{conf}} \rightarrow P_{\text{fjc}}(r)$$

Although we are holding the ends of the chain fixed and stretching with the ends restrained along one direction ( $x$ ), the probability distribution function takes the three dimensional form to properly account for all chain configurations:  $P_{\text{conf}}(r) = P_0 e^{-\beta^2 r^2}$  with  $\beta^2 = 3k_B T / 2n\ell^2$  and  $P_0 = \beta^3 / \pi^{3/2}$  as constants. Then

$$\ln P_{\text{conf}}(r) = -\beta^2 r^2 + \ln P_0$$

The force needed to extend the chain can be calculated from eq. (1) after substituting  $r^2 = x^2 + y^2 + z^2$  which gives

$$f = -2\beta^2 k_B T x = -\kappa_{st} x$$

So we have a linear relationship between force and displacement, which is classic Hooke's Law spring with a force constant  $\kappa_{st}$  given by

$$\kappa_{st} = \frac{3k_B T}{n\ell^2} = \frac{3k_B T}{\langle r^2 \rangle_0}$$

Here  $\langle r^2 \rangle_0$  refers to the mean-square end-to-end distance for the FJC in the absence of any applied forces. Remember:  $\langle r^2 \rangle_0 = n\ell^2 = \ell L_C$ . In the case that all of the restoring force is due to entropy, then we call this an entropic spring  $\kappa_{ES}$ .

$$\kappa_{ES} = \frac{T}{2} \left( \frac{\partial^2 S}{\partial x^2} \right)_{N,V,T}$$

This works for small forces, while the force is reversible. Notice that  $\kappa_{ES}$  increases with temperature—as should be expected for entropic restoring forces.

### Example: Stretching DNA<sup>3</sup>

At low force:

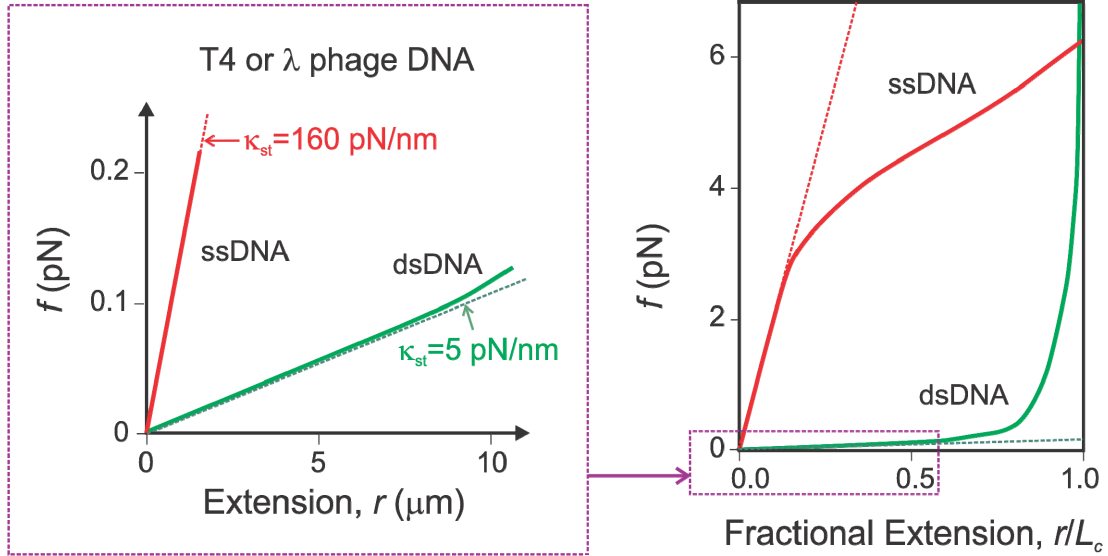
$$\text{dsDNA} \rightarrow \kappa_{st} = 5 \text{ pN/nm}$$

$$\text{ssDNA} \rightarrow \kappa_{st} = 160 \text{ pN/nm} \rightarrow \text{more entropy/more force}$$

At higher extension you asymptotically approach the contour length.

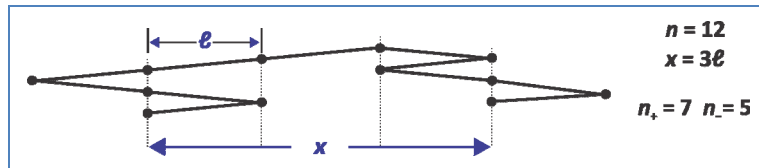
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3. A. M. van Oijen and J. J. Loparo, Single-molecule studies of the replisome, *Annu. Rev. Biophys.* **39**, 429-448 (2010).



### Force/Extension of a Random Walk Polymer

Let's derive force extension behavior for a random walk polymer in one dimension. The end-to-end distance is  $r$ , the segment length is  $\ell$ , and the total number of segments is  $n$ .



For any given  $r$ , the number of configurations available to the polymer is:

$$\Omega = \frac{n!}{n_+!n_-!}$$

This follows from recognizing that the extension of a random walk chain in one dimension is related to the difference between the number of segments that step in the positive direction,  $n_+$ , and those that step in the negative direction,  $n_-$ . The total number of steps is  $n = n_+ + n_-$ . Also, the end-to-end distance can be expressed as

$$r = (n_+ - n_-)\ell = (2n_+ - n)\ell = (n - 2n_-)\ell \quad (2)$$

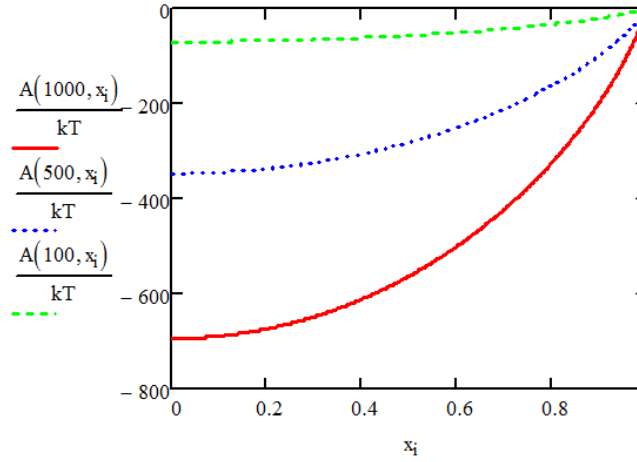
$$n_{\pm} = \frac{1}{2} \left( n \pm \frac{r}{\ell} \right) \quad \frac{\partial n_{\pm}}{\partial r} = \pm \frac{1}{2\ell}$$

Then we can calculate the free energy of the random walk chain that results from the entropy of the chain, i.e., the degeneracy of configurational states at any extension. This looks like an entropy of mixing calculation:

$$\begin{aligned}
A &= -k_B T \ln \Omega \\
&= -k_B T (n \ln n - n_+ \ln n_+ - n_- \ln n_-) \\
&= nk_B T (\phi_+ \ln \phi_+ + \phi_- \ln \phi_-) \\
\phi_{\pm} &= \frac{n_{\pm}}{n} = \frac{1}{2}(1 \pm x)
\end{aligned}$$

Here the fractional end-to-end extension of the chain is

$$x = \frac{r}{L_C} \quad (3)$$



Next, we can calculate the force needed to extend the polymer as a function of  $r$ :

$$f = -\frac{\partial A}{\partial r} \rightarrow \frac{\partial A}{\partial \phi_{\pm}} \frac{\partial \phi_{\pm}}{\partial r} \quad \frac{\partial \phi_{\pm}}{\partial r} = \pm \frac{1}{2L_C}$$

Using eq. (2)

$$\begin{aligned}
f &= -nk_B T (\ln \phi_+ - \ln \phi_-) \left( \frac{1}{2L_C} \right) \\
&= -\frac{nk_B T}{2L_C} \ln \left( \frac{1+x}{1-x} \right) \\
&= -\frac{k_B T}{\ell} \frac{1}{2} \ln \left( \frac{1+x}{1-x} \right) \\
f &= -\frac{k_B T}{\ell} \tanh^{-1}(x) \quad (4)
\end{aligned}$$

where I used the relationship:  $\ln\left(\frac{1+x}{1-x}\right) = 2 \tanh^{-1}(x)$ . Note, here the forces are scaled in units of  $k_B T / \ell$ . For small forces  $x \ll 1$ ,  $\tanh^{-1}(x) \approx x$  and eq. (4) gives

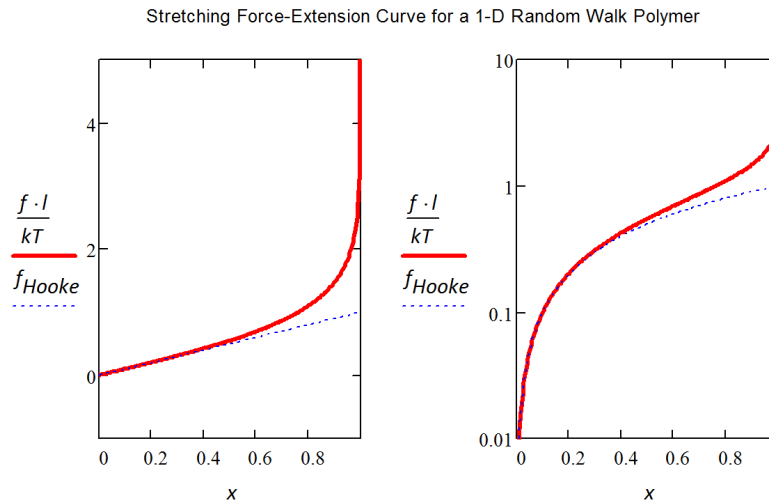


$$f \approx \frac{k_B T}{\ell L_C} r$$

This gives Hooke's Law behavior with the entropic force constant expected for a 1D chain. For a 3D chain, we would expect:

$$f \approx \frac{3k_B T}{\ell L_C} r$$

The spring constant scales with dimensionality.



### The relationship between position, force, and the partition function

Now let's do this a little more carefully. From classical statistical mechanics, the partition function is

$$Q = \iint dr^{3N} dp^{3N} \exp(-H / k_B T)$$

Where  $H$  is the Hamiltonian for the system. The average value for the position of a particle described by the Hamiltonian is

$$\langle x \rangle = \frac{1}{Q} \iint dr^3 dp^3 x \exp(-H / k_B T)$$

If the Hamiltonian takes the form

$$H = -f \cdot x$$

Then

$$\langle x \rangle = \frac{k_B T}{Q} \left( \frac{\partial Q}{\partial f} \right)_{V, T, N} = k_B T \left( \frac{\partial \ln Q}{\partial f} \right)_{V, T, N}$$

This describes the average extension of a chain if a force is applied to the ends.

### Force/Extension Behavior for a Freely Jointed Chain

Making use of the expressions above and  $Q = q^N$

$$q_{\text{conf}} = \iint dr^3 dp^3 e^{-U/kT} e^{\vec{f}\cdot\vec{r}/k_B T} \quad \langle r \rangle = N k_B T \left( \frac{\partial \ln q_{\text{conf}}}{\partial f} \right)_{U, r, n}$$

Here we also inserted a general Hamiltonian which accounts for the internal chain interaction potential and the force exerted on the chain:  $H = U - \vec{f} \cdot \vec{r}$ . For  $N$  freely jointed chains with  $n$  segments, we set  $U \rightarrow 0$ , and focus on force exerted on every segment of the chain.

$$\vec{f} \cdot \vec{r} = \sum_{i=1}^n \vec{f} \cdot \vec{\ell}_i = f \ell \sum_{i=1}^n \cos \theta_i$$

Treating the segments as independent and integrating over all  $\theta$ , we find that

$$q_{\text{conf}}(f) = \frac{2\pi \sinh \varphi}{\varphi}$$

$$\langle r \rangle = n\ell \left[ \coth \varphi - \frac{1}{\varphi} \right] \quad (5)$$

where the unitless force parameter is

$$\varphi = \frac{f\ell}{k_B T} \quad (6)$$

As before, the magnitude of force is expressed relative to  $k_B T / \ell$ . Note this calculation is for the average extension that results from a fixed force. If we want the force needed for a given average extension, then we need to invert the expression. Note, the functional form of the force/extension curve in eq. (5) **Error! Reference source not found.** is different than what we found for the 1D random walk in eq. (4). We do not expect the same form for these problems, since our random walk example was on a square lattice, and the FJC propagates radially in all directions.

#### Derivation

For a single polymer chain

$$q = \iint dr^3 dp^3 e^{U/k_B T} e^{-f \cdot r/k_B T}$$

$$P(r) = \frac{1}{q} e^{-U/k_B T} e^{f \cdot r/k_B T}$$

$$\langle r \rangle = \frac{k_B T}{q} \left( \frac{\partial \ln q}{\partial f} \right)_u$$

In the case of the freely jointed Chain, set  $U \rightarrow 0$ .

$$\vec{f} \cdot \vec{r} = \vec{f} \cdot \sum_{i=1}^n \vec{\ell}_i = f \ell \sum_{i=1}^n \cos \theta_i$$

Decoupled segments:  $q \approx \int dr^3 \exp\left(\sum_i \frac{f \ell}{k_B T} \cos \theta_i\right)$

$$= \left( \int_0^{2\pi} \int_0^\pi \exp[\varphi \cos \theta] \sin \theta d\theta d\phi \right)^n$$

$$= \left( \frac{2\pi \sinh(\varphi)}{\varphi} \right)^n$$

$$\langle r \rangle = k_B T \frac{\partial}{\partial f} \ln q$$

$$= n k_B T \frac{\partial}{\partial f} \left[ \ln \left\{ \frac{2\pi \sinh(\varphi)}{\varphi} \right\} \right]$$

$$\coth(x) = \frac{e^x + e^{-x}}{e^x - e^{-x}}$$

$$\langle r \rangle = n \ell [\coth(\varphi) - \varphi^{-1}]$$

or  $\langle x \rangle = \coth(\varphi) - \varphi^{-1}$       The average fractional extension:  $\langle x \rangle = \langle r \rangle / L_C$

Now let's look at the behavior of the expression for  $\langle x \rangle$ —also known as the Langevin function.

$$\langle r \rangle = n \ell [\coth(\varphi) - \varphi^{-1}] \quad (7)$$

Looking at limits:

- Weak force ( $\varphi \ll 1$ ):  $f \ll k_B T / \ell$

Inserting and truncating the expansion:

$$\coth \varphi = \frac{1}{\varphi} + \frac{1}{3} \varphi - \frac{1}{45} \varphi^3 + \frac{2}{945} \varphi^5 + \dots$$

we get

$$\langle x \rangle = \frac{\langle r \rangle}{L_C} \approx \frac{1}{3} \varphi$$

$$\langle r \rangle \approx \frac{1}{3} \frac{n \ell^2}{k_B T} f$$

$$\text{or } f = \frac{3 k_B T}{n \ell^2} \langle r \rangle = \kappa_{ES} \langle r \rangle$$

Note that this limit has the expected linear relationship between force and displacement, which is governed by the entropic spring constant.

- Strong force ( $\phi \gg 1$ ).  $f \gg k_B T / \ell$  Taking the limit  $\coth(x) \rightarrow 1$ .

$$\langle r \rangle \approx n\ell \left[ 1 - \frac{1}{\phi} \right] \leftarrow \lim_{f \rightarrow \infty} = \lim_{\alpha \rightarrow \infty} = L_C \text{ Contour length}$$

$$\text{or } f = \frac{k_B T}{\ell} \frac{1}{1 - \langle x \rangle} \text{ where } \langle x \rangle = \frac{\langle r \rangle}{L_C}$$

For strong force limit, the force extension behavior scales as,  $x \sim 1 - f^{-1}$ .

So, what is the work required to extend the chain?

At small forces, we can integrate over the linear force/extension behavior. Under those conditions, to extend from  $r$  to  $r + \Delta r$ , we have

$$w_{\text{rev}} = \int_0^{\Delta r} \kappa_{ES} r \, dr = \frac{3k_B T}{2n\ell^2} \Delta r^2$$

### Worm-like Chain

For the worm-like chain model, we found that the variance in the end-to-end distance was

$$\langle r^2 \rangle = 2\ell_p L_C - 2\ell_p^2 (1 - e^{-L_C/\ell_p}) \quad (8)$$

where  $L_C$  is the contour length, and the persistence length was related to the bending force constant as

$$\ell_p = \frac{\kappa_b}{k_B T}$$

The limiting behavior for eq. (8) is:

$$\begin{array}{lll} \text{rigid:} & \ell_p \gg L_C & \langle r^2 \rangle \propto L_C^2 \\ \text{flexible:} & \ell_p \ll L_C & \langle r^2 \rangle \sim 2L_C \ell_p \quad \therefore \text{for WLC} \\ & & = n_e \ell_e^2 \quad (2\ell_p = \ell_e) \end{array}$$

Following a similar approach to the FJC above, it is not possible to find an exact solution for the force/extension behavior of the WLC, but it is possible to show the force/extension behavior in the rigid and flexible limits.

Setting  $2\ell_p = \ell_e$ ,  $\phi = f\ell_e / k_B T$ , and using the fractional extension

$$\langle x \rangle = \frac{\langle r \rangle}{L_C}$$

1) Weak force ( $\phi \ll 1$ ) Expected Hooke's Law behavior

$$f \ell_e \ll k_B T \quad f = \frac{3k_B T}{\ell_e L_C} \langle r \rangle \longrightarrow \frac{f \ell_e}{k_B T} = 3 \langle x \rangle$$

For weak force limit, the force extension behavior scales as,  $x \sim f$ .

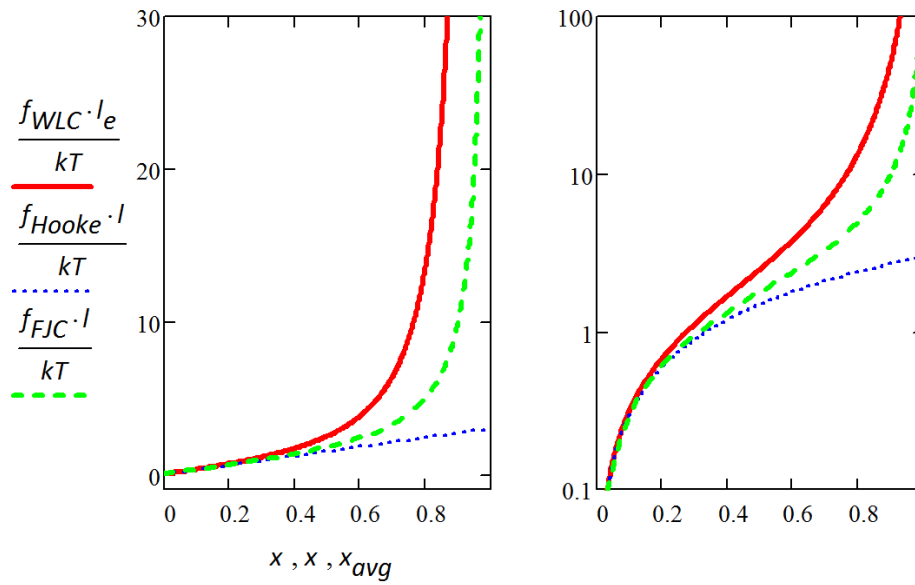
2) Strong force ( $\phi \gg 1$ )

$$f \ell_e \gg k_B T \quad \langle r \rangle = L_C \left( 1 - \frac{1}{2\sqrt{\phi}} \right) \longrightarrow \frac{f \ell_e}{k_B T} = \frac{1}{4(1 - \langle x \rangle)^2}$$

For strong force limit, the force extension behavior scales as,  $x \sim 1 - f^{-1/2}$ .

An approximate expression for the combined result (from Bustamante):

$$\frac{f \ell_p}{kT} = \frac{1}{4(1 - \langle x \rangle)^2} - \frac{1}{4} + \langle x \rangle \quad (9)$$



# Stretching regimes

Macromolecules 2010, 43, 4394–4400

