Forced polymers

1. *Pulling polymers:* Using optical tweezers, it is now possible to pull on the two ends of a single molecule. (Actually the tweezers pull on latex balls that are attached to the ends of the polymer; a complication that we shall ignore.) In the presence of the force $\vec{F}$ pulling on the ends of the polymer, there is an additional energy term

$$\delta E = -\vec{F} \cdot \vec{R},$$

where $\vec{R} = \vec{r}_N - \vec{r}_1$ is the end–to–end distance (between the first and N^{th} monomers) of the chain.

(a) For an ideal polymer, the number of configurations with an end–to–end distance of $\vec{R}$ is given by the usual Gaussian formula

$$\Omega_N\left(\vec{R}\right) = \frac{g^N}{(2\pi N a^2/3)^{3/2}} \exp\left(-\frac{3R^2}{2Na^2}\right).$$

By integrating the Boltzmann weight over all $\vec{R}$, calculate the (Gibbs) partition function $Z(N, F, T)$ at a temperature T. Using this result, obtain the mean extension $R_F = k_B T \partial \ln Z/\partial F$ along the direction of the force $\vec{F}$.

• The usual random walk partition function function is now modified by the external force to

$$Z = \int d^3\vec{R} \frac{g^N}{(2\pi\sigma^2)^{3/2}} \exp\left(-\frac{R^2}{2\sigma^2}\right) \exp\left(\beta\vec{F}\cdot\vec{R}\right)$$

where we have introduced $\sigma^2 = Na^2/3$. Evaluating the integrals yields

$$\begin{aligned}
Z(N,F,T) &= \frac{2\pi g^N}{(2\pi\sigma^2)^{3/2}} \int_0^\infty R^2 dR \exp\left(-\frac{R^2}{2\sigma^2}\right) \int_{-1}^{1} d\mu \exp\left(\beta F R \mu\right) \\
&= \frac{2\pi g^N}{(2\pi\sigma^2)^{3/2}} \int_0^\infty \frac{R dR}{\beta F} \exp\left(-\frac{R^2}{2\sigma^2}\right) \left[e^{\beta F R} - e^{-\beta F R}\right] \\
&= \frac{g^N}{(2\pi\sigma^2)^{3/2}} \frac{2\pi}{\beta F} \exp\left(\frac{\sigma^2\beta^2F^2}{2}\right) \int_0^\infty R dR \left[e^{-(R-\sigma^2\beta F)^2/2\sigma^2} - e^{-(R+\sigma^2\beta F)^2/2\sigma^2}\right] \\
&= \frac{g^N}{(2\pi\sigma^2)^{3/2}} \frac{2\pi}{\beta F} \exp\left(\frac{\sigma^2\beta^2F^2}{2}\right) \int_{-\infty}^\infty R dR \exp\left(-\frac{(R-\sigma^2\beta F)^2}{2\sigma^2}\right) \\
&= \frac{g^N}{(2\pi\sigma^2)^{3/2}} \frac{2\pi}{\beta F} \exp\left(\frac{\sigma^2\beta^2F^2}{2}\right) \sqrt{2\pi\sigma^2}(\sigma^2\beta F) \\
&= g^N \exp\left(\frac{\sigma^2\beta^2F^2}{2}\right) = g^N \exp\left(\frac{\beta^2F^2Na^2}{6}\right).
\end{aligned}$$

(The above result could of course be obtained more simply from the product of three one dimensional Gaussian integrals.) The the partition function, the mean extension is calculated as

$$R_F = k_B T \partial \ln Z / \partial F = \frac{N a^2 F}{3 k_B T} = \frac{\langle R^2 \rangle F}{3 k_B T}.$$

(b) For other cases in which Ω_N does not have a simple form (such as for self-avoiding polymers), it is still possible to obtain the *linear response* of the polymer to small force. To this end, expand the Boltzmann weight $\exp\left(\vec{F} \cdot \vec{R}/k_B T\right)$ to second order in $\vec{F}$, and hence show that

$$R_F = \frac{1}{3 k_B T} \left\langle R^2 \right\rangle_0 F + \mathcal{O}(F^3),$$

where $\langle R^2 \rangle_0$ is the mean end–to–end squared distance of the polymer in the absence of the force.

• Because of the rotational symmetry of the problem Ω_N still depends only on the $|\vec{R}| = R$, and

$$\begin{aligned} Z(N,F,T) &= \int d^3\vec{R} \Omega_N(R) \exp(\beta \vec{F} \cdot \vec{R}) \\ &= 2\pi \int_0^\infty R^2 dR \Omega_N(R) \int_{-1}^1 d\mu \left[1 + \beta F R \mu + \frac{1}{2}(\beta F R \mu)^2 + \frac{1}{6}(\beta F R \mu)^3 + \mathcal{O}(F^4) \right] \\ &= Z_0 \left[1 + \frac{\beta^2 F^2 \langle R^2 \rangle_0}{6} + \mathcal{O}(F^4) \right] = Z_0 \exp\left(\frac{F^2 \langle R^2 \rangle_0}{6 (k_B T)^2} + \mathcal{O}(F^4) \right), \end{aligned}$$

where we have introduced $Z_0 = Z(N, F = 0, T) = 4\pi \int_0^\infty R^2 dR \Omega_N(R)$ and linear and cubic terms are 0 after integration over μ. The mean extension is:

$$R_F = k_B T \partial \ln Z / \partial F = \frac{\langle R^2 \rangle_0 F}{3 k_B T} + \mathcal{O}(F^3).$$

(c) Dimensional analysis suggests that quite generally the extension–force curve for polymers should have the form

$$\frac{R_F}{\sqrt{\langle R^2 \rangle_0}} = \Phi\left(\frac{F \sqrt{\langle R^2 \rangle_0}}{k_B T} \right).$$

The left hand side is a dimensionless extension; on the right hand side a dimensionless combination involving the force appears as the argument of an unknown function Φ. At large forces F, the polymer becomes stretched such that $R_f \propto N$. For self-avoiding polymers $\sqrt{\langle R^2 \rangle_0} \approx a N^\nu$ with $\nu \approx 0.59$. Use these facts to deduce a non-linear behavior $R_F \propto F^\lambda$ for the extension at large force, and give the value of the exponent λ.

• For large arguments x we expect that Φ scales as $\Phi(x) \propto x^\lambda$. For large forces F, the left hand side scales as $N^{1-\nu}$, while the right hand side scales as $N^{\lambda\nu}$. This implies

$$\lambda = \frac{1-\nu}{\nu} \approx 0.69\,,$$

such that for large forces F:

$$R_F \approx \sqrt{\langle R^2 \rangle_0} \left(\frac{F\sqrt{\langle R^2 \rangle_0}}{k_B T} \right)^{\lambda} .$$

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2. *Denaturing DNA by force:* Obtain the phase diagram of DNA pulled by a force $\vec{F}$, by generalizing the Poland–Scheraga model as follows:
(a) By integrating over the position vectors, show that the (Gibbs) partition function of DNA of length N can be decomposed into products of contributions from double-stranded rods and single stranded bubbles, as

$$Z(N,F) = \sum_{\ell_1,\ell_2,\ell_3,\cdots} R(\ell_1)B(\ell_2)R(\ell_3)\cdots , \qquad \text{with} \qquad \ell_1+\ell_2+\ell_3+\cdots = N.$$

- It is obvious that partition function can be decomposed in this way:

$$Z(N,F) = \sum_{\ell_1,\ell_2,\ell_3,\cdots} \int d^3\vec{r}_1 \mathcal{R}(\ell_1,\vec{r}_1) \int d^3\vec{r}_2 \mathcal{B}(\ell_2,\vec{r}_2-\vec{r}_1) \int d^3\vec{r}_3 \mathcal{R}(\ell_3,\vec{r}_3-\vec{r}_2)\cdots ,$$

where $\vec{r}_i$ represent end point locations of double-stranded rods ($\mathcal{R}$) and single stranded bubbles ($\mathcal{B}$). Each part is almost independent of the next one, they are only connected with the positions where rods and bubbles are joined together. Integrals can be evaluated one by one from right to left and this is equivalent to

$$\begin{aligned} Z(N,F) &= \sum_{\ell_1,\ell_2,\ell_3,\cdots} \int d^3\vec{s}_1 \mathcal{R}(\ell_1,\vec{s}_1) \int d^3\vec{s}_2 \mathcal{B}(\ell_2,\vec{s}_2) \int d^3\vec{s}_3 \mathcal{R}(\ell_3,\vec{s}_3)\cdots \\ &= \sum_{\ell_1,\ell_2,\ell_3,\cdots} R(\ell_1)B(\ell_2)R(\ell_3)\cdots . \end{aligned}$$

(b) Treat the double stranded segments as rigid rods of fixed length $a\ell$. By integrating over all orientations in three dimensions show that

$$R(\ell) = w^{\ell} \times \frac{\sinh(\beta F a \ell)}{\beta F a \ell},$$

where $w = e^{-\beta\varepsilon}$, and ε is the energy gain of forming the double strand.

- Integrating over all possible directions of rod yields

$$R(\ell) = \frac{1}{2}\int_{-1}^{1} d\mu \; e^{-\beta\epsilon\ell} e^{\beta F a \ell \mu} = w^{\ell} \times \frac{\sinh(\beta F a \ell)}{\beta F a \ell}.$$

As usual we have ignored the unimportant factors of 4π.

(c) Treat the double stranded loop as two random walks of length ℓ connected at the two end points. Integrating over all separations of the two end points show that

$$B(\ell) = \frac{s}{\ell^{3/2}} \left[g^2 \exp\left(\frac{\beta^2 F^2 a^2}{12}\right)\right]^\ell .$$

- This is similar problem to the problem 1

$$B(\ell) \;=\; \int d^3\vec{R} \left[\frac{g^\ell}{2\pi\sigma^2} \exp\left(-\frac{R^2}{2\sigma^2}\right)\right]^2 \exp(\beta \vec{F}\cdot\vec{R}),$$

where $\sigma^2 = \ell a^2/3$. Introducing $\sigma^2 = 2\tilde{\sigma}^2$, we obtain the same integral as in problem 1

$$\begin{aligned} B(\ell) &= \frac{1}{(8\pi\tilde{\sigma}^2)^{3/2}} \int d^3\vec{R} \frac{(g^2)^\ell}{(2\pi\tilde{\sigma}^2)^{3/2}} \exp\left(-\frac{R^2}{2\tilde{\sigma}^2}\right) \exp\left(\beta\vec{F}\cdot\vec{R}\right) \\ &= \frac{1}{(8\pi\tilde{\sigma}^2)^{3/2}} g^{2\ell} \exp\left(\frac{\tilde{\sigma}^2\beta^2F^2}{2}\right) \\ &= \frac{s}{\ell^{3/2}} \left[g^2 \exp\left(\frac{\beta^2 F^2 a^2}{12}\right)\right]^\ell . \end{aligned}$$

In the last step we have inserted the value of $\tilde{\sigma}^2 = \ell a^2/6$ and combined all unimportant constants in s.

(d) Examine the problem in a (grand canonical) ensemble with variable DNA lengths N, additionally weighted by a factor of z^N. Give the expressions for the (Laplace) transformed $\tilde{B}(z)$ and $\tilde{R}(z)$ in this ensemble in terms of the (Bose) sums $f_m^+(x) = \sum_{\ell=1}^\infty x^\ell/\ell^m$.

- As in class we introduce Laplace transformation:

$$\begin{aligned} \tilde{B}(z) &= \sum_{\ell=1}^\infty z^\ell B(\ell) = \sum_{\ell=1}^\infty \frac{s}{\ell^{3/2}} \left[z g^2 \exp\left(\frac{\beta^2 F^2 a^2}{12}\right)\right]^\ell = s f^+_{3/2}\left(z g^2 \exp\left(\frac{\beta^2 F^2 a^2}{12}\right)\right), \\ \tilde{R}(z) &= \sum_{\ell=1}^\infty z^\ell R(\ell) = \sum_{\ell=1}^\infty \frac{z^\ell w^\ell}{\beta F a \ell} \left[e^{\beta F a \ell} - e^{-\beta F a \ell}\right] = \frac{1}{\beta F a} \ln\left(\frac{1 - z w e^{-\beta F a}}{1 - z w e^{\beta F a}}\right) \end{aligned}$$

Laplace transform of partition function is the sum over all bubbles:

$$\Gamma(z) = \tilde{R}(z) + \tilde{R}(z)\tilde{B}(z)\tilde{R}(z) + \cdots = \frac{1}{\tilde{R}^{-1}(z) - \tilde{B}(z)} .$$

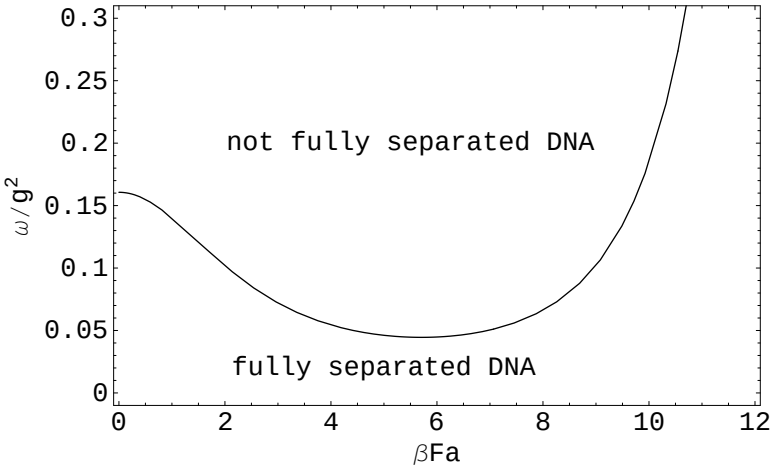
The following discussion is similar to the one in class where we analyzed force-less case. $\tilde{B}(z)$ starts at zero for $z = 0$ and monotonically increases with z until $z^* = 1/\left(g^2 \exp\left(\beta^2F^2a^2/12\right)\right)$, where $B(z^*) = s\zeta_{3/2}$. For $z > z^*$, $\tilde{B}(z)$ diverges. $\tilde{R}^{-1}(z)$ starts at infinity for $z = 0$ and then monotonically decreases to zero at $z = 1/(we^{\beta Fa})$.

(e) Show that the strands become fully separated at a critical point satisfying $\tilde{R} = \tilde{B}^{-1} = \left(s\zeta_{3/2}\right)^{-1}$, where $\zeta_{3/2} \equiv f^+_{3/2}(1) \approx 2.612$.

• As in class we introduce the average length $\langle L\rangle = z\partial \ln\Gamma/\partial z$ of the DNA and the average number of bound pairs $\langle N_B\rangle = w\partial \ln\Gamma/\partial w$. We are interested in the limit of the very large lengths $\langle L\rangle$, which is equivalent to condition $\tilde{R}^{-1}(z) = \tilde{B}(z)$. The critical point is met, when $\tilde{R}^{-1}(z_c) = \tilde{B}(z_c) = s\zeta_{3/2}$ and $z_c = 1/\left(g^2 \exp\left(\beta^2F^2a^2/12\right)\right)$. These conditions also set the critical value of w_c. For $w < w_c$ DNA is fully separated. From expression for $\tilde{R}(z)$ we determine w_c

$$\begin{aligned}
\frac{1 - z_c w_c e^{-\beta F a}}{1 - z_c w_c e^{\beta F a}} &= \exp\left(\frac{\beta F a}{s\zeta_{3/2}}\right) = C, \\
z_c w_c &= \frac{C-1}{Ce^{\beta F a} - e^{-\beta F a}} \\
\frac{w_c}{g^2} &= \left\{ \frac{\exp\left(\frac{\beta F a}{s\zeta_{3/2}}\right) - 1}{\exp\left(\frac{\beta F a}{s\zeta_{3/2}}\right) e^{\beta F a} - e^{-\beta F a}} \right\} \exp\left(\beta^2 F^2 a^2/12\right) .
\end{aligned}$$

(f) For $s = 1$, plot the phase diagram of the model in the coordinates (w/g^2) and $(\beta F a)$.
• The phase diagram is plotted below



The horizontal axis is a dimensionless force $\beta F a$; the vertical axis represents the effective binding weight w/g^2. We see that for large forces, $\beta F a \gg 1$, DNA is fully separated. w_c/g^2 reaches minimum value ≈ 0.045 at $\beta F a \approx 5.71$.

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3. (Optional) *Denaturing RNA by force:* By pulling on the ends of RNA, the hydrogen bonds can be broken to yield a stretched polymer. Let us model the partially denatured state as a sequence of linear segments with no hydrogen bonds and 'blobs' which are hydrogen bonded (opposite to the case of DNA). Assume that the force carrying backbone of the molecule is made up of the linear segments, and that the RNA blobs carry no force (similar to the loop in problem 2). After integrating over the position vectors, the (Gibbs) partition

function of an RNA of length N can be written as

$$Z(N,F) = \sum_{\ell_1,\ell_2,\ell_3,\cdots} P(\ell_1)R(\ell_2)P(\ell_3)\cdots, \qquad \text{with} \qquad \ell_1+\ell_2+\ell_3+\cdots = N.$$

The contributions of linear and blob segments are respectively

$$P(\ell) = g^\ell \exp\left(\frac{F^2a^2\ell}{6k_B^2T^2}\right), \quad \text{and} \quad R(\ell) = f^\ell \frac{A}{\ell^{3/2}},$$

where f and g are constant entropic factors.

(a) Exploit the mathematical similarity to the Poland–Scheraga model to evaluate the grand partition function of the model.

- As in the Poland-Scheraga model the grand partition function is:

$$\begin{aligned}
\Gamma(z) &= \sum_N z^N Z(N,F) = \frac{1}{\tilde{P}^{-1}(z) - \tilde{R}(z)}, \\
\tilde{P}(z) &= \sum_\ell z^\ell P(\ell) = \frac{zg\exp\left(\frac{F^2a^2}{6k_B^2T^2}\right)}{1 - zg\exp\left(\frac{F^2a^2}{6k_B^2T^2}\right)}, \\
\tilde{R}(z) &= \sum_\ell z^\ell R(\ell) = A f_{3/2}^+(fz)\ .
\end{aligned}$$

(b) Identify the force F_c at which denaturation starts.

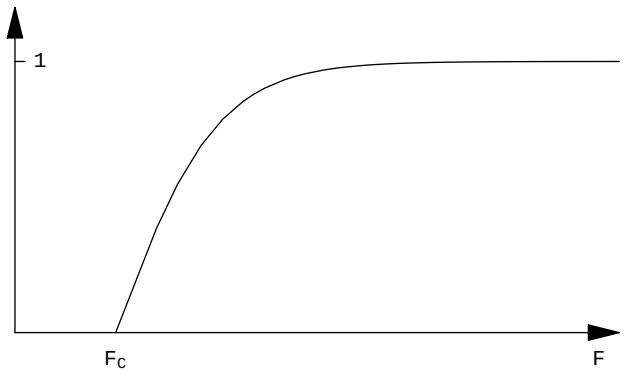
- As in the Poland-Scheraga model, the critical point is located at $z_c = 1/f$ and $\tilde{R}(z_c) = \tilde{P}^{-1}(z_c)$, resulting in

$$\begin{aligned}
A\zeta_{3/2} &= \frac{f}{g\exp\left(\frac{F_c^2a^2}{6k_B^2T^2}\right)} - 1, \\
F_c &= \frac{k_BT}{a}\sqrt{6\ln\left(\frac{f}{g(1+A\zeta_{3/2})}\right)}.
\end{aligned}$$

This is true only for $f > g(1 + A\zeta_{3/2})$. If this condition is not met, then $F_c = 0$.

(c) Sketch the fraction of denatured sites as a function of force, clearly indicating the nature of the singularity at F_c.

- As in the Poland-Scheraga model denaturated sites (linear segments) start forming for $F > F_c$ and the fraction of denatured sites scales as $(F - F_c)^{\frac{2-c}{c-1}}$, where $c = 3/2$. This means that the fraction of denaturated sites scales linearly with $(F - F_c)$ near F_c. Note that the fact that we have $\exp(F^2 \ldots)$ factor in the $\tilde{P}(z)$ does not affect the scaling behavior, because we are interested in small changes around F_c. Scaling behavior around F_c is completely determined by the scaling behavior of $f_c^+(x) \propto (1-x)^{c-1}$ near $x = 1$.



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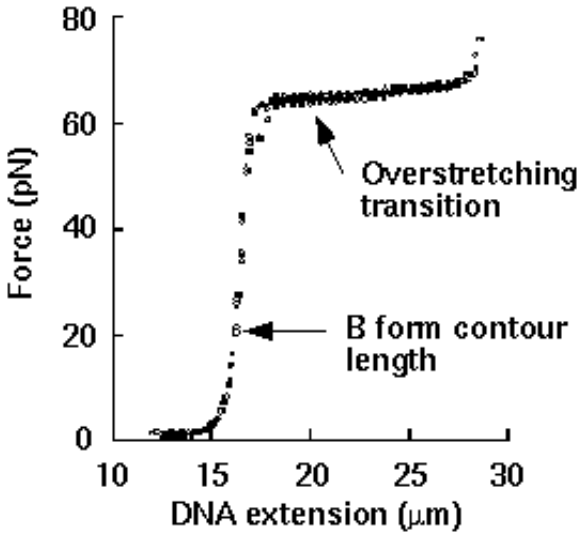
4. *Over-stretching DNA:* In standard (B–from) DNA the basepairs stack in spiral fashion at separation of 3.4Å. As indicated in the following figure [from S. B. Smith, Y. Cui, C. Bustamante, Science **271**, 795 (1996), and *http://alice.berkeley.edu/˜ steve/DNAstr.html*], pulling on DNA with optical tweezers causes it to greatly stretch at forces of around 65±5pN.



(a) One interpretation is that this represents a transition to a new structure of over-stretched DNA, in which the separation of bases has increased to 5.8Å. As a very simple model of this putative state consider DNA as a one dimensional chain in which each unit can either be in the regular form of size 3.4Å, or in the stretched form of size 5.8Å. Assume that a free energy difference U per basepair is required to change the regular B–form to the stretched form. For this part of the problem ignore the three dimensional orientations of each segment, and assume that the state of each element is independent of its neighbors. Calculate the length $L(F,T)$ for this model when pulled by a force F at a temperature T.

- Partition function of each unit is

$$Z_0(F,T) = e^{\beta Fa} + e^{\beta(Fb-U)},$$

where $a = 3.4$Å is size of the regular form, $b = 5.8$Å is size of the stretched form and U is energy needed to change the regular to stretched form. The length of the DNA is thus:

$$L(F,T) = N\frac{\partial \ln Z_0(F,T)}{\partial(\beta F)} = L_0\ \frac{e^{\beta Fa} + (b/a)e^{\beta(Fb-U)}}{e^{\beta Fa} + e^{\beta(Fb-U)}},$$

where N is number of units and $L_0 = Na$ is contour length of the DNA in the regular form.

(b) Compare the result from part (a) to the experimental figure, and thus estimate the parameter U from the data in the above model from experiments. Is the width of the transition region in F consistent with the assumptions of the model.

- At the transition force $F_0 = 65$pN contributions of regular and stretched form are equal in partition function. This allow us to estimate the parameter U as

$$U = F_0(b-a) = 1.6 \times 10^{-20} J = 0.10eV\,.$$

Experiment suggests that transition occurs over a very narrow force range ($\Delta F \sim 2$pN). From the partition function we identify the ratio of units in stretched forms to units in the regular form as $\exp[\beta(F(b-a)-U)]$. To estimate the typical width of the transition region in force F, we take the transition to occur between ratios 0.1 and 10 of the numbers of units in stretched and regular forms. Estimated width of the transition region in F is then

$$\Delta F = \frac{k_B T \ln 100}{b-a} = 80pN\,.$$

This leads to a width of $\Delta F = 80$pN which is much larger than experimentally measured 2pN.

(c) Now consider a more realistic model in which neighboring elements tend to be in the same state. Would this lead to a sharpening or widening of the transition region in F?

- Coupling of states of neighboring elements can be taken into account by considering independent blocks of M units, where each unit inside the block is in the same state. The stronger the coupling the larger the blocks M and in the limit of very strong coupling we have only one block of all units. Partition function for each block is:

$$Z_M(F,T) = e^{\beta MFa} + e^{\beta M(Fb-U)},$$

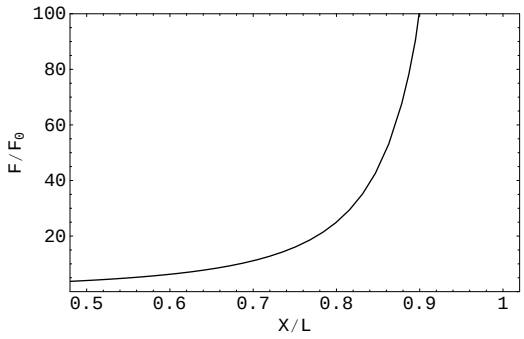
leading to a mean length of

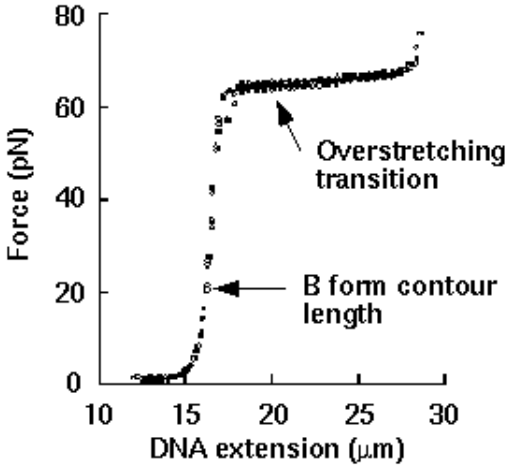
$$L(F,T) = \frac{N}{M}\ \frac{\partial \ln Z_M(F,T)}{\partial(\beta F)} = L_0\ \frac{e^{\beta MFa} + (b/a)e^{\beta M(Fb-U)}}{e^{\beta MFa} + e^{\beta M(Fb-U)}}.$$

Estimating the width ΔF of the transition with the same procedure as before now leads to

$$\Delta F = \frac{k_B T \ln 100}{M(b-a)}.$$

Thus coupling of states of neighboring units leads to sharpening of the transition region in F. Comparison with the experimental width $\Delta F \sim 2$pN is obtained for block size of $M = 40$ units. On the force-length plot, we see that transition region is described quite well with this model ($L_0 = 16.4\mu$m, $M = 40$).





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5. (Optional) *Force–extension curve for stretched DNA:* Configurations of a worm-like chain (WLC) in two dimensions can be characterized by variations of the angle $\theta(s)$ of the tangent at (monomer) position s along the curve. In the presence of a large force $\vec{F}$, variation of the angle (measured with respect to $\vec{F}$) are small, and the energy cost can be approximated as

$$H = \frac{1}{2}\int_0^L ds \frac{\kappa}{R^2(s)} - \int_0^L \vec{ds}\cdot\vec{F} \approx -FL + \frac{1}{2}\int_0^L ds \left[\kappa\left(\frac{d\theta}{ds}\right)^2 + F\theta^2(s)\right].$$

(a) Rewrite the WLC Hamiltonian as a sum over harmonic modes of the DNA "string" $\tilde{\theta}_q$.

• Inserting Fourier modes $\theta(s) = \sum_q e^{iqs}\tilde{\theta}_q$ in Hamiltonian and using standard procedures transforms Hamiltonian to:

$$H = -FL + L\sum_q \frac{1}{2}\left(\kappa q^2 + F\right)|\tilde{\theta}_q|^2,$$

where we used the fact that $\tilde{\theta}_{-q} = \tilde{\theta}_q^*$.

(b) Equipartition dictates that each quadratic mode will have an average energy of $k_B T$. Write the expression for $\langle|\tilde{\theta}_q|^2\rangle$, and hence calculate $\langle\theta^2(s)\rangle$.

- From Hamiltonian in the previous part we find:

$$\left\langle |\tilde{\theta}_q|^2 \right\rangle = \frac{k_B T}{L(\kappa q^2 + F)} = \frac{k_B T}{L\kappa \left(q^2 + \ell_0^{-2}\right)},$$

where factor 1/2 goes away from the fact that $\tilde{\theta}_q$ and $\tilde{\theta}_{-q}$ produce the same energetic term. Next we calculate $\langle \theta^2(s) \rangle$:

$$\left\langle \theta^2(s) \right\rangle = \sum_{q,q'} e^{i(q+q')s} \left\langle \tilde{\theta}_q \tilde{\theta}_{q'} \right\rangle = \sum_{q,q'} \delta_{q',-q} \left\langle |\tilde{\theta}_q|^2 \right\rangle = \sum_q \left\langle |\tilde{\theta}_q|^2 \right\rangle,$$

where we used the fact that different modes are independent. Replacing sum with an integral gives:

$$\begin{aligned} \left\langle \theta^2(s) \right\rangle &= L \int \frac{dq}{2\pi} \frac{k_B T}{L\kappa \left(q^2 + \ell_0^{-2}\right)} \\ \left\langle \theta^2(s) \right\rangle &= \frac{k_B T}{2\pi\kappa} \times \pi \ell_0 = \frac{k_B T}{2\sqrt{\kappa F}} \end{aligned}$$

(c) Calculate the extension X of the DNA as a function of force F. Invert the relation and plot the function $F(X)$. Use $k_B T$ and persistence length ξ_p in your answer.

- Extension-force relation is

$$X = L(1 - \left\langle \theta^2(s) \right\rangle /2) = L \left(1 - \frac{k_B T}{4\sqrt{\kappa F}}\right)$$

and inverted form:

$$F(X) = \frac{(k_B T)^2}{16\kappa(1 - X/L)^2} = \frac{k_B T}{16\xi_p(1 - X/L)^2} = \frac{F_0}{(1 - X/L)^2},$$

where we used the relation $\kappa = k_B T \xi_p$ and introduced typical force $F_0 = k_B T/16\xi_p$.

(d) Use the high-force worm-like chain (WLC) asymptotic

$$F(X) = \frac{k_B T}{16\,\xi_p} \frac{1}{\left(1 - X/L\right)^2},$$

to estimate the fractional extension X/L of double-stranded DNA under a force $F = 10$ pN. Take $k_B T \simeq 4.1$ pN $\cdot$ nm at room temperature and $\xi_p = 50$ nm. (If you want an absolute length, use the contour length from the overstretching example $L = 16.4$ μm.)

- Invert the high-force WLC relation:

$$1 - \frac{X}{L} = \sqrt{\frac{k_B T}{16\,\xi_p\, F}}.$$

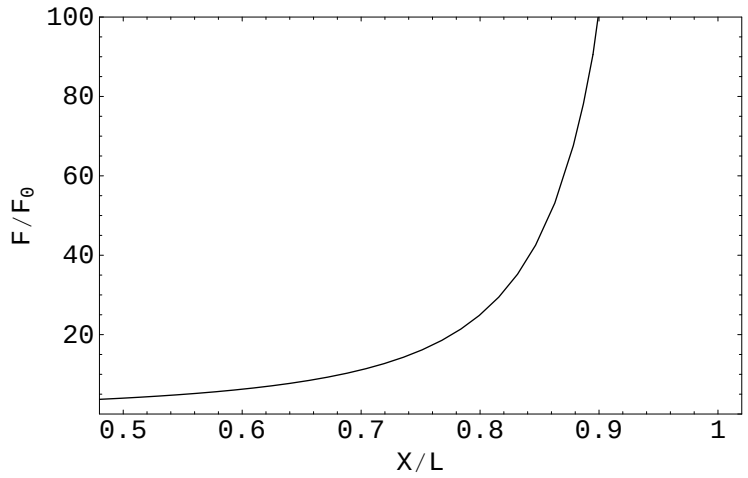

Figure 1: Force-extansion curve has singularity near $X/L = 1$, where $F/F_0 = (1 - X/L)^{-2}$ and $F_0 = k_B T/16\xi_p$.

Insert numbers:

$$1 - \frac{X}{L} = \sqrt{\frac{4.1}{16 \times 50 \times 10}} = \sqrt{\frac{4.1}{8000}} \approx \sqrt{5.125 \times 10^{-4}} \approx 2.26 \times 10^{-2}.$$

Hence

$$\boxed{\frac{X}{L} \approx 0.977}$$

i.e., at 10 pN dsDNA is already 97.7% of its contour length. If $L = 16.4$ μm, the absolute extension shortfall is

$$\Delta L = L\,(1 - X/L) \approx 16.4\ \mu\text{m} \times 0.0226 \approx 0.37\ \mu\text{m},$$

so $X \approx 16.0$ μm.

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6. (Optional) *Pulling RNA:* The server at http://bioserv.mps.ohio-state.edu/rna/ gives force extension curves for RNA based on secondary structure calculations. Use this server to examine force extension curves for: (a) a uniform sequence; (b) an alternating sequence of G and C; (c) an alternating sequence of A and U; and (d) an actual RNA sequence. (Choose sequences of roughly the same length.) Comment on the general characteristics of these curves. Does any of them resemble the theoretical result from the problem on 'denaturing RNA by force'?

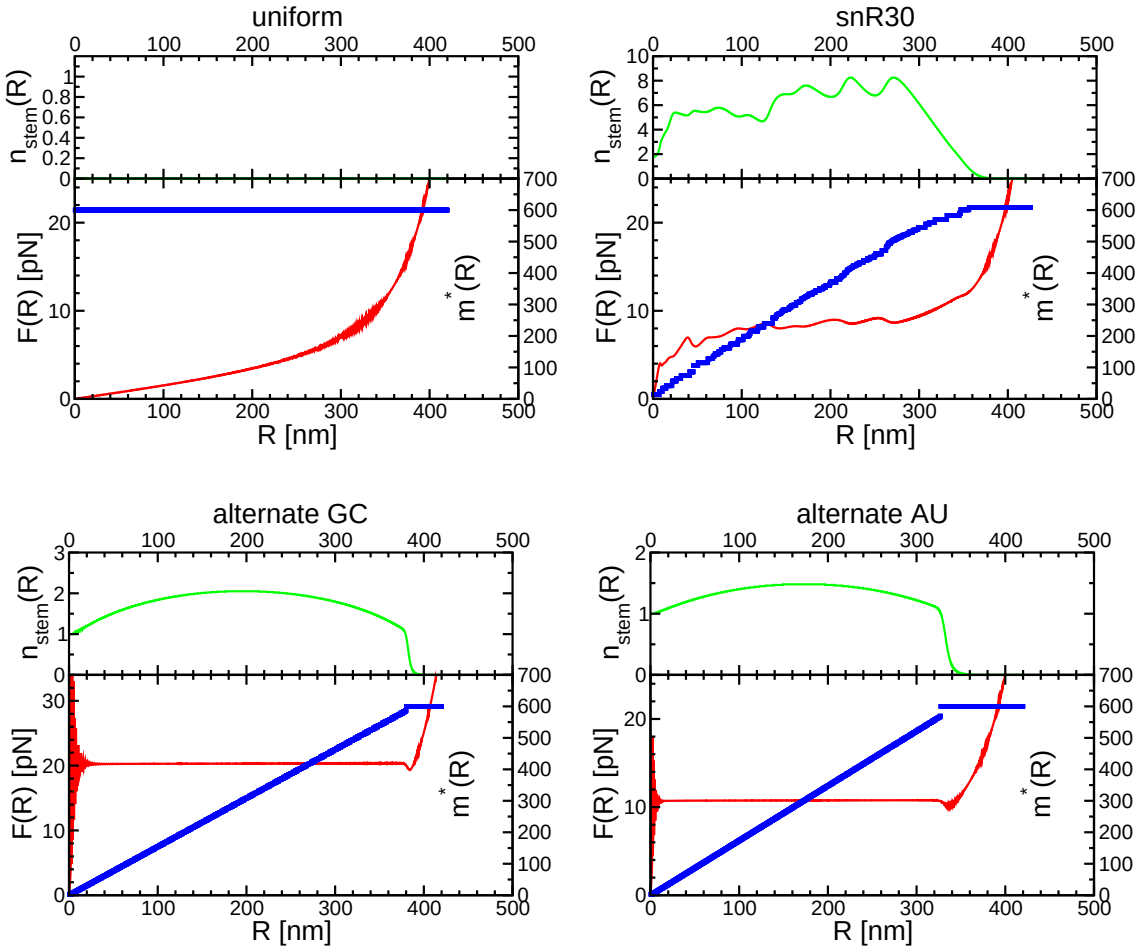
- Server will be tested with snR30 sequence with 608 nucleotides found on

`http://people.biochem.umass.edu/ sfournier/fournierlab/snornadb/snrs/snr30_ta.php`

Sequence:

1	aaccauaguc	ucgugcuagu	ucgguacuau	acagggaagg	gaagucacuc	gcauacgugu
61	gugugcauuu	cuugcuauug	cugcuuagcu	ucucuaaaac	acugggcuac	guuuuucaac
121	gcucgagagg	cagagucuca	aggagccucc	aaugggccuc	acguauucau	cuagauggcg
181	cuucggacaa	cggcaucaca	uaagagaugc	agcuccugac	uucuccucug	aucuucguga
241	ucagaguuuu	gagucgucag	acuacgagca	guuucucuua	gucguugcau	cgggugcugu
301	ugccuuaagc	auguguauau	gggguucggg	ggcuguugcc	augauauaua	uggaugagac
361	agaaguggcc	ccguugacga	guuuaacuua	gauuaaguag	gacgcaugau	cuugagcucu
421	uuuccuauac	uuuguccuau	ggccagcuuu	cuccuuauua	cgaagagauu	gcgggaugug
481	ggugcagagu	gggaaaaucu	gaguucgguc	aucuuuguug	uucguccuac	cgcaguauau
541	uccuaaacac	uaugaaauga	cccuaguugg	uccaugauca	uuuggguaaa	accauacugc
601	agacaucu					

None of the force extension curves on the next page matches particularly well with the theoretical model of the previous problem. The curve for a uniform sequence of 600 elements has $F_c = 0$, and apparently no secondary structure is formed in this case. In the case of an alternating sequence of G and C (A and U) we have to apply certain force $F_c = 20$pN ($F_c = 10$pN) to break the G–C (A–U) primary bonds. The dominant structure is one big line segment with G–C (A–U) primary bonds and one big blob. At critical force G–C (A–U) primary bonds starts braking at the contact of line segment and blob. Eventually all primary bonds are broken and after that extension starts linearly with increasing force $F > F_c$ as in the previous problem. For the real RNA sequence we still see some plateau in the force-extension diagram for forces ≈ 8pN when we start breaking G–C and A–U bonds. The last bond is broken at force $F_c \approx 14$pN and then we are again in the regime predicted in an earlier problem.


uniform
snR30
alternate GC
alternate AU
n_stem(R)
F(R) [pN]
m*(R)
R [nm]

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