Stretching short biopolymers by fields and forces

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We study the mechanical properties of semiflexible polymers when the contour length of the polymer is comparable to its persistence length. We compute the exact average end-to-end distance and shape of the polymer for different boundary conditions, and show that boundary effects can lead to significant deviations from the well-known long-polymer results. We also consider the case of stretching a uniformly charged biopolymer by an electric field, for which we compute the average extension and the average shape, which is shown to be trumpetlike. Our results also apply to long biopolymers when thermal fluctuations have been smoothed out by a large applied field or force.

The mechanical properties of semiflexible biopolymers are important for their biological function. For example, DNA is tightly packed in eukaryotic chromosomes and in viruses, while actin and microtubules provide the scaffolding and structure for most animal cells [1]. In these cases the length scales at which the polymer properties of these macromolecules are of biological interest are comparable to their persistence length. Namely DNA packing typically involves loops of diameter less than its persistence length of 50 nm, and so does looping induced by DNA bound proteins such as the lac repressor, while actin is present in cells in the form of bundles and networks in which the typical length of the participating polymers is shorter than its 15 μm persistence length. Stretching short strands of DNA is also relevant for single molecule experiments involving tethered molecules [2].

Mechanical properties of semiflexible polymers are well-described by the wormlike chain model [3,4], which treats the polymer as a space curve with a bending energy quadratic in the curvature. On the basis of this model, the extension of a molecule in response to an applied force can be calculated [4], and has been shown to agree with experiments to a high level of accuracy. Typically, the method of solution has been to map the calculation of the partition function of the wormlike chain to solving a differential equation, either numerically [4], or analytically as in the two-dimensional case [5]. Since experiments have usually probed the force response of *long* DNA molecules, theoretical treatments in the past have typically taken the long chain limit, in which case boundary conditions play no role. However, this is not appropriate for molecules whose length is of the order of a persistence length. In fact, the discrepancy between the long chain results and the behavior of short molecules is glaringly obvious when we look at the force extension curves themselves. All force extension curves for long polymers pass through the origin in the limit as force drops to zero, but it has been known for a long time that the short wormlike chain has a finite extension at zero force [6].

Since the use of short DNA strands as a "force ruler" is becoming routine in force spectroscopy experiments, understanding the entropic elasticity of such strands is of considerable experimental importance [2,7]. For example, using the formula appropriate for long molecules while fitting force-extension measurements done on short molecules leads to incorrect estimates of the persistence length that could be 2-5 times smaller than the accepted value [7].

It is interesting to note that similar considerations apply even to long molecules, when the molecule in question is being stretched by large forces, assuming that the entropic limit is maintained and structural changes to the molecule are not induced. For example, if a long molecule is stretched while keeping the tangents at the two ends perpendicular to the direction in which the force is exerted, the molecule will bend away from the direction of the force at the ends. The stored length in these bends will make the force-extension behavior of this molecule different from when it is attached with the tangents parallel to the direction of the applied force. It has been proposed that such a mechanism is responsible for the observed deviations from ideal force-extension behavior when polymers are stretched by an atomic force microscope (AFM) tip [8].

The short chain and the large force limit discussed above are both characterized by a small probability of large deviations from the straight polymer configuration. Throughout this paper we refer to this situation as the "fluctuating rod" limit of a semiflexible polymer. In this limit we need only take into account small, quadratic fluctuations around the energy-minimizing configuration. This leads to a considerable simplification of the mathematics of the wormlike chain, and allows for some elegant analytical results, as we demonstrate below. Note that an alternative numerical treatment of small-chain entropic properties is provided by accounting for the appropriate boundary conditions and finite chain length in the series expansion of the partition function for the wormlike chain [7].

Previously, short chains were explicitly discussed by Wilhem and Frey in [9], who also used a harmonic approximation to obtain the probability distribution function of the end-to-end vector for free polymers in a series expansion. An interesting contribution was made by Keller, Swigon, and Bustamante [10], who discussed the difference in the applicability of thermodynamics to short and long polymers, and

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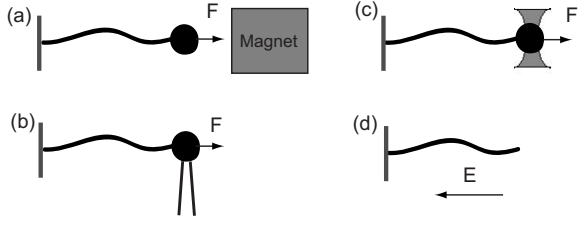
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FIG. 1. A diagram of different ways to stretch single molecules. (a) Magnetic tweezer apparatus. The bead is paramagnetic, and the force exerted is proportional to the spatial gradient of the magnetic field. (b) Stretching using a micropipette. The tip is imaged, and its bending is precalibrated with the corresponding force exerted. (c) Laser tweezers and (d) stretching using a uniform electric field.

derived a formula for stretching short polymers. Reference [11], along with Monte Carlo results, rederived this result for stretching short polymers, and also calculated distribution functions and the effect of walls. The effect of stored length due to boundary conditions on long polymers stretched by large forces was calculated by Kulic *et al.* [8], who also considered the effect of internal loops on force-extension relations in this regime. In this paper we recover prior results for stretching a short polymer by an applied force, using the generating functional method. We find this field-theoretic method particularly well-suited for calculating statistical properties of fluctuating rods. To demonstrate this we derive a number of formulas, including the hitherto unsolved problem of the average extension of a charged polymer in an electric field.

This paper is organized as follows. In Sec. I we present the general formalism for calculating the force-extension curves and rms fluctuations of a fluctuating rod under tension, using the generating functional method. In Sec. II we apply this formalism to a polymer stretched by a constant force. However, single molecule experiments that stretch semiflexible polymers by laser tweezers, magnetic tweezers, or a micropipette [4] differ in the way they treat the ends of the molecule, which may be free or constrained in several ways (Fig. 1). These experimental conditions affect the entropy of the molecule, and thereby the force-extension relation. We demonstrate this for fluctuating rods by showing that the effect of axis-clamping at the two ends of the molecule leads to a measurable effect on force-extension curves.

In Sec. III we show that a uniformly charged polymer in a constant electric field behaves as if it is being stretched by a force that varies linearly along the contour. Calculating the force-extension relationship in this case, using the standard method of obtaining the partition function by solving the partial differential equation it satisfies, is not practical, since the differential equation in this case is not separable. Previous work in this area has relied on either phenomenological arguments [4,12], or on linear response approximations for weak fields [13]. However, the general method we outline in Sec. I can easily handle this situation as well, and we present analytical results for the force-extension relation and the shape of a uniformly charged fluctuating-rod polymer stretched by an electric field. The calculated average shape is in accord with observations [12]. Our results are applicable to stiff polymers like actin for a wide range of field strengths

and can be used to measure the effective charge density of actin for different salt conditions.

I. STATISTICAL MECHANICS OF FLUCTUATING RODS

Since stretching experiments can be performed in both two and three dimensions, we work in general *d*-dimensions. The objective is to calculate the average extension of the polymer in the direction of the applied force or field. We start by making the small-fluctuation approximation, which transforms the partition function of the wormlike chain into a Gaussian path integral. Using this partition function we obtain the tangent-tangent correlation function, from which we derive the average extension of the molecule.

The Hamiltonian of a wormlike-chain polymer of contour length L, which is stretched by a constant force $\boldsymbol{F}$, is

$$\beta H[\boldsymbol{t}(s)] = \int_0^L ds \left\{ \frac{\xi}{2} \left(\frac{d\boldsymbol{t}}{ds} \right)^2 - \beta \boldsymbol{F} \cdot \boldsymbol{t}(s) \right\}, \quad (1)$$

where $\beta = 1/k_B T$, s is the arclength, and ξ is the bending stiffness and is simply related to the persistence length, $l_p = 2\xi/(d-1)$, in *d*-dimensions. For double-stranded DNA $\xi \sim 50$ nm and for an actin filament, $\xi \sim 15$ μm. The unit tangent vector $\boldsymbol{t}(s)$ specifies the conformation of the chain. The polymer is stretched by tethering the $s=0$ end to a fixed support, usually a bead held by a micropipette, and pulling on the other end. The usual procedure is to attach either a magnetic or a polystyrene bead to the other end of the polymer, and to exert a constant force $\boldsymbol{F}$ using magnetic tweezers, optical tweezers, or another micropipette (Fig. 1). We assume that $\boldsymbol{F}$ is along the $\boldsymbol{e}_1$ direction in *d*-dimensional space. The components of the tangent vector are $\boldsymbol{t}(s) = \{\pm\sqrt{1 - \sum_{i=2}^d t_i(s)^2}, t_2(s), \ldots, t_d(s)\}$. In the limit of small fluctuations, the tangent vector makes only very small deviations away from the direction of the applied force, so that its coordinate along the $\boldsymbol{e}_1$ direction can be approximated as

$$t_1(s) = 1 - \frac{1}{2}\sum_{i=2}^d t_i(s)^2. \quad (2)$$

In this limit $(d\boldsymbol{t}/ds)^2 \approx \sum_{i=2}^d (dt_i/ds)^2$. Inserting these expressions in the Hamiltonian, Eq. (1), and integrating the bending energy term by parts yields

$$\beta H[t_i(s)] = \frac{1}{2}\sum_{i=2}^d \left[\int_0^L ds\, t_i(s) O(s) t_i(s) + B_i(L,0) \right], \quad (3)$$

where we have left out an unimportant constant term. Here the term $B_i(L,0) \equiv \frac{\xi}{2} t_i(s) \frac{dt_i}{ds}\big|_0^L$ depends on the boundary conditions, while

$$O(s) \equiv -\xi \frac{d^2}{ds^2} + \beta F(s) \quad (4)$$

is a differential operator. We consider the general case of an *s*-dependent force, $\boldsymbol{F}(s)$, which includes the case of stretching by an electric field discussed in Sec. III.

The boundary term $B_i(L,0)$ depends in general on the tangent vectors at the two ends of the polymer, and for the cases discussed here it vanishes. Namely, the tangent vector at the end is either unconstrained, in which case $d\mathbf{t}/ds=0$ for the end-tangent vector, or aligned with the force, and then $t_i=0$ for $i=2,3,\ldots$.

The partition function of the fluctuating rod, $\mathcal{Z}$, is a path integral of the Boltzmann factor, $\exp\{-\beta H[t_i(s)]\}$, over all possible conformations of the polymer, where $H[t_i(s)]$ is given by Eq. (3) with the above boundary conditions. To compute the tangent-tangent correlation function we employ the generating functional, $\mathcal{Z}(J_i)$, which is obtained by adding a source term $J_i(s)$ to the Hamiltonian:

$$\mathcal{Z}(J_i) = \int \mathcal{D}[\{\mathbf{t}(s)\}] \exp[-\beta H[\{t_i(s)\}] + J_i(s)t_i(s)]. \quad (5)$$

The correlation function, $\langle t_i(s)t_i(s')\rangle$ is then obtained by taking functional derivatives [14],

$$\langle t_i(s)t_i(s')\rangle = \lim_{J_i\to 0} \frac{\delta^2 \log[\mathcal{Z}(J_i)]}{\delta J_i(s)\delta J_i(s')} = G(s,s'), \quad (6)$$

where $G(s,s')$ is the Green's function of the operator $\mathcal{O}(s)$ defined by

$$O(s)G(s,s') = \left[-\xi\frac{d^2}{ds^2} + \beta F(s)\right]G(s,s') = \delta(s-s'). \quad (7)$$

Note that, by symmetry considerations, fluctuations transverse to the applied force are equivalent for all transverse directions, hence we need to calculate this Green's function for only one of the $d-1$ directions.

To calculate the average extension in the x_1-direction at any contour position s, we now make use of the relation

$$\langle x_1(s)\rangle = \int_0^s ds'\left(1 - \frac{d-1}{2}\langle t_i(s')^2\rangle\right), \quad (8)$$

which is valid in the small fluctuation approximation regime given by Eq. (2). The end-to-end extension is $X=\langle x_1(L)\rangle$. The average of each of the other $d-1$ orthogonal coordi-

nates, $x_i(i=2,\ldots,d)$, is zero, while their root-mean-square (rms) value is

$$\langle x_i(s)^2\rangle^{1/2} = \left(\int_0^s ds' \int_0^s ds'' \langle t_i(s')t_i(s'')\rangle\right)^{1/2}. \quad (9)$$

A parametric plot of the mean extension against the root-mean-square value of $x_i(s), (i=2,\ldots,d)$ using Eqs. (8) and (9) describes the average shape of the polymer.

An important question is, under what conditions does the fluctuating rod approximation apply? For this we employ the tangent-tangent correlation function which yields a self-consistency condition for the small-fluctuation assumption. In two dimensions, the small fluctuation approximation, Eq. (2), implies that $t_x\approx 1-t_y^2/2$ for all s, which is accurate to within 1% when $t_y^2<1/2$. In d-dimensions this suggests the condition that the sum of all mean-squared fluctuations at any contour position is at most 1/2. In other words, we require that

$$\langle t_i(s)^2\rangle \leq 1/(2d-2), \quad (10)$$

for all s. Below we repeatedly make use of this condition in order to estimate the range of experimental parameters (polymer length, magnitude of force, etc.) for which the fluctuating rod model is applicable.

II. STRETCHING BY A CONSTANT FORCE

To illustrate the method described above, we calculate explicitly the force-extension relation and the rms fluctuations of a fluctuating rod stretched by a force applied at one of its ends. The experimental setup is as shown in Fig. 1(a). The correlation function, $\langle \mathbf{t}(s)\mathbf{t}(s')\rangle$ is given by the Green's function of the differential operator, Eq. (4). Note that the assumption that the tangent vectors at the two ends of the polymer are both aligned with the direction of force makes the Green's function vanish at the boundaries.

The differential equation is solved separately in the domains $s<s'$ and $s>s'$, in which the delta function vanishes, and then the solutions are matched at the boundary $s=s'$. This yields

$$G(s,s') = \begin{cases} \mathcal{N} \sinh[\kappa_F s]\sinh[\kappa_F(L-s')] \equiv G_<(s,s'), & \text{when } s < s' \\ \mathcal{N}' \sinh[\kappa_F(L-s)]\sinh[\kappa_F s'] \equiv G_>(s,s'), & \text{when } s > s', \end{cases} \quad (11)$$

where

$$\kappa_F \equiv \sqrt{\frac{F}{\xi k_B T}} \quad (12)$$

is a force-dependent inverse length. Requiring the continuity of $G(s,s')$ at $s=s'$, we find $N=\mathcal{N}'$. Also, by integrating both sides of Eq. (7) over the interval $s \in (s-\epsilon, s+\epsilon)$ and taking

the limit $\varepsilon\to 0$ gives the final boundary condition on $G(s,s')$:

$$\left.\frac{\partial G_>}{\partial s}\right|_{s=s'} - \left.\frac{\partial G_<}{\partial s}\right|_{s=s'} = -\frac{1}{\xi}. \quad (13)$$
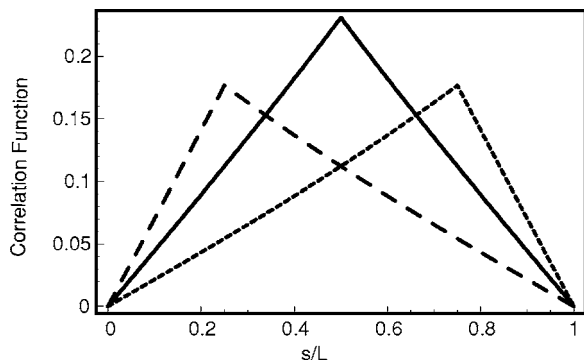
Putting it all together, we get

FIG. 2. The Green's function $G(s,s')$, with $\kappa_F=1$, plotted for $s'/L=0.25$ (long dashes), 0.5 (solid line), and 0.75 (small dashes).

$$G(s,s') = \begin{cases} \dfrac{\sinh[\kappa_F s]\sinh[\kappa_F(L-s')]}{\kappa_F \xi \sinh[\kappa_F L]}, & \text{when } s \leq s' \\[4mm] \dfrac{\sinh[\kappa_F(L-s)\sinh][\kappa_F s']}{\kappa_F \xi \sinh[\kappa_F L]}, & \text{when } s \geq s'. \end{cases}$$

$$(14)$$

This Green's function is plotted in Fig. 2.

When $s=s'$, the correlation function yields the mean-square fluctuations of the tangent vector coordinate, $\langle t_i(s)^2 \rangle$. Plugging this into Eq. (8), we obtain

$$\frac{\langle x_1(s) \rangle}{L} = \frac{s}{L} - (d-1)$$

$$\times \frac{2\kappa_F s \cosh[\kappa_F L] - \sinh[\kappa_F L] + \sinh[\kappa_F(L-2s)]}{8\kappa_F^2 \xi L}.$$

$$(15)$$

Now we set $s=L$ in Eq. (15) to get the force extension relation for a fluctuating rod polymer,

$$\frac{X}{L} = 1 - \frac{d-1}{2} \frac{\kappa_F L \cosh[\kappa_F L] - \sinh[\kappa_F L]}{2\kappa_F^2 \xi L \sinh[\kappa_F L]}. \qquad (16)$$

A plot of this relation, and its comparison with the long-polymer result, is shown in Fig. 3. For $d=3$, Eq. (16) agrees
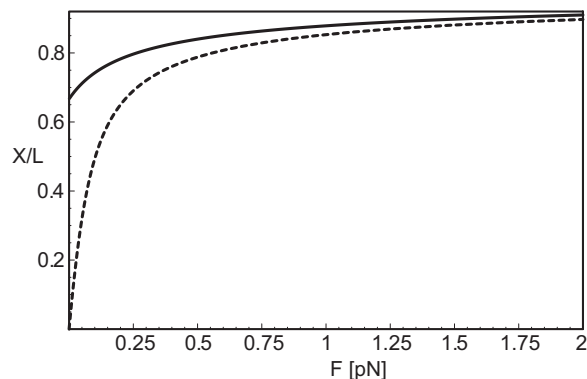

FIG. 3. The force-extension curve for a 100 nm strand of DNA, plotted against force in piconewtons (solid line) using Eq. (16). The dashed line shows the force-extension curve for a long polymer, plotted using the approximate interpolation formula of [4]

with the force-extension relation computed in [10].

Experiments that involve stretching short strands of DNA, or other semiflexible molecules, should use Eq. (16) in place of the force-extension relation of [4], which is appropriate in the long chain limit [15]. As we have remarked earlier, use of the long chain formula leads to erroneous results when fitting data from stretching short polymers [7]. It is reassuring to note that, as we shall show below, Eq. (16) reproduces known results in the limits of high force and zero force.

It should be also noted that in the long-polymer limit, the statistical properties of a semiflexible polymer under an applied force depend on one dimensionless ratio, $F\xi/(k_B T)$, which also delineates the limits of high and low force. In the case of fluctuating rods, however, both of the two independent dimensionless ratios that can be formed by the three lengths, $L, \xi,$ and κ_F^{-1}, are present in the functions describing the statistical quantities of interest. Also, in this case the high force limit is governed by a different dimensionless number, $\kappa_F L \equiv \sqrt{FL^2/\xi k_B T}$.

We can also calculate the root-mean-square fluctuations of the polymer in the transverse directions, using Eq. (9),

$$\frac{\langle x_i(s)^2 \rangle^{1/2}}{L} = \left(\frac{2\kappa_F s \sinh[\kappa_F L] - 3\cosh[\kappa_F L] + 4\cosh[\kappa_F(L-s)] - \cosh[\kappa_F(L-2s)]}{2\kappa_F^3 L^2 \xi \sinh[\kappa_F L]} \right)^{1/2}, \qquad (17)$$

for the transverse directions $i=2,\ldots,d$. A parametric plot of $\langle x_1(s) \rangle$ given by Eq. (15) against $\sqrt{\langle x_i(s)^2 \rangle}$, $i=2,\ldots,d$ defines the average shape of the polymer.

The self-consistency assumption, Eq. (10), is a necessary condition for the validity of Eqs. (16) and (17). It is clear from Eq. (14) that the maximum fluctuations of the tangent vector are at the center of the chain, $s=L/2$. We therefore require that

$$\langle t_i(s)^2 \rangle \leq \left\langle t\left(\frac{L}{2}\right)^2 \right\rangle \leq \frac{1}{2(d-1)}. \qquad (18)$$

Taking $d=2$ for illustration, this yields the condition that

$$\frac{1}{2\kappa_F \xi} \tanh\left[\frac{\kappa_F L}{2}\right] \leq \frac{1}{2}. \qquad (19)$$

As the hyperbolic tangent is never greater than one, Eq. (19) always holds when

$$\kappa_F \xi \geqslant 1. \tag{20}$$

However, even when the force is weak, Eq. (16) can apply, provided the polymer is short enough compared to its persistence length. In other words, even when $\frac{1}{2\kappa_F\xi} > 1$, the small fluctuation assumption can be satisfied, provided the hyperbolic tangent is small enough. In this limit Eq. (19) can be rewritten as

$$\frac{L}{\xi} \leqslant \frac{1}{\kappa_F\xi} \log\left[\frac{1 + \kappa_F\xi}{1 - \kappa_F\xi}\right]. \tag{21}$$

Conditions (20) and (21) are summarized graphically by the shaded region in Fig. 4.

It is an interesting exercise to examine the limits of Eq. (16) in the two cases above, described by Eqs. (20) and (21). When the polymer is long, so that $L/\xi \gg 1$, the strong force condition, Eq. (20), ensures that the product $\kappa_F L \gg 1$. Then the force-extension curve in Eq. (16) reduces to

$$\frac{X}{L} \simeq 1 - \frac{d-1}{4}\sqrt{\frac{k_B T}{F\xi}}, \tag{22}$$

which, for $d=3$, is the well-known result for high force stretching [4]. Our result in Eq. (16) shows, in fact, that even for a short chain satisfying $L/\xi \leqslant 4$, as long as the force is strong enough, such that the product $\kappa_F L \gg 1$, we recover a force-extension relation of the form Eq. (22).

The other limit is the case when the force is weak such that $\kappa_F L \leqslant 1$, then the force extension curve in Eq. (16) becomes

$$\frac{X}{L} \simeq 1 - \frac{d-1}{2}\left[\frac{1}{6}\left(\frac{L}{\xi}\right) - \frac{F\xi}{90k_B T}\left(\frac{L}{\xi}\right)^3\right]. \tag{23}$$

This is different from the behavior of long wormlike-chain polymers under weak forces, in which case the force-extension relation is proportional to the force [4], with no constant term. Note also that when the force goes to zero, the relative extension of the polymer tends to $1 - \frac{d-1}{2}\left(\frac{1}{6}\right)\left(\frac{L}{\xi}\right)$, which is a result one finds in Landau and Lifshitz for $d=3$ [6].

What about the case when $1/(2\kappa_F\xi) > 1$? Then we must have $\tanh(\kappa_F L/2) < \kappa_F\xi$, or in other words, to a first approximation,

$$L < 2\xi, \tag{24}$$

which can be confirmed by a look at Fig. 3. Hence polymers up to one persistence length in two dimensions, or two persistence lengths in three dimensions, can be regarded as satisfying the small fluctuation approximation for any applied force.

A. Effects of axis clamping

We now show how axis clamping affects the force extension for a fluctuating rod. If the free end of the polymer is held rigidly, such that the end-to-end separation vector of the polymer is constrained to be collinear with the direction of the force, there is an additional constraint,

$$\int_0^L ds\, t_i(s) = 0 \quad \text{for } i = 2, 3, \dots, d. \tag{25}$$

Note that this constraint depends upon the entire conformation of the chain, hence it is not *a priori* clear when it can be ignored.

We can compute the correlation function, $\langle t_i(s)t_i(s')\rangle$, for all $i = 2, 3, \dots, d$, by forcing the constraint in Eq. (25) using the Dirac delta function,

$$\langle t_i(s)t_i(s')\rangle = \frac{\int \mathcal{D}t_i(s)\, t_i(s)t_i(s')\, \delta\left(\int_0^L ds\, t_i(s)\right)\exp\left[\int_0^L ds\left\{-\frac{1}{2}t_i(s)O(s)t_i(s)\right\} + \beta FL\right]}{\int \mathcal{D}t_i(s)\, \delta\left(\int_0^L ds\, t_i(s)\right)\exp\left[\int_0^L ds\left\{-\frac{1}{2}t_i(s)O(s)t_i(s)\right\} + \beta FL\right]}. \tag{26}$$

We use the integral representation of the delta function in Fourier space, add the source terms as before, and compute the Gaussian integrals by completing the square. This gives us an additional term in the correlation function,

$$\langle t_i(s)t_i(s')\rangle = G(s,s') - \frac{\left(\int_0^L ds_1\, G(s,s_1)\right)\left(\int_0^L ds_2\, G(s',s_2)\right)}{\int_0^L ds_1 \int_0^L ds_2\, G(s_1,s_2)}, \tag{27}$$

where $G(s,s')$ is the Green's function of the operator $\mathcal{O}(s)$ shown in Eq. (14). Now using Eq. (27) in Eq. (8), we obtain the average extension,

$$\frac{X}{L} = 1 - \frac{(d-1)(\kappa_F L\cosh[\kappa_F L] - \sinh[\kappa_F L])}{4\kappa_F^2\xi L\sinh[\kappa_F L]} + \frac{(d-1)(\kappa_F L\cosh[\kappa_F L] - 3\sinh[\kappa_F L] + 2\kappa_F L)}{2\kappa_F^3\xi L^2 + 2\kappa_F^3\xi L^2\cosh[\kappa_F L] - 4\kappa_F^2\xi L\sinh[\kappa_F L]}. \tag{28}$$
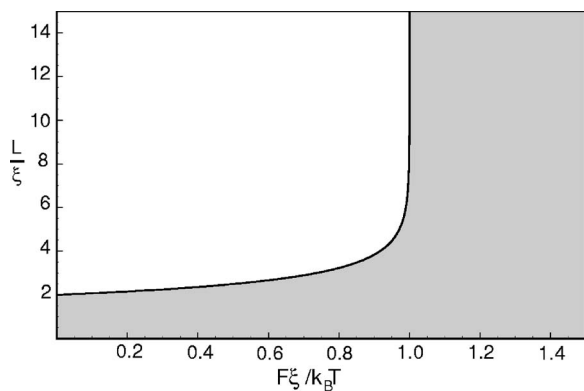
FIG. 4. The set of parameter values that satisfy the small fluctuation approximation is shown by the shaded area. The x-axis is the reduced force, $\kappa_F^2 \xi^2 \equiv F\xi/k_BT$. The y-axis is the polymer length in terms of the $3d$-persistence length, L/ξ.

The first two terms in Eq. (28) are identical to Eq. (16) derived above. Therefore we find that the loss in entropy due to axis clamping of the two ends of the polymer leads to a small but measurable correction to the average extension. This is plotted in Fig. 5 for $d=2$. We can also use Eq. (9) and calculate the average shape of the polymer, defined by Eqs. (8) and (9), by plotting $\langle x_1(s) \rangle$ against $\sqrt{\langle x_2(s)^2 \rangle}$ parametrically, as shown in Fig. 5(b).

We calculate the range of validity of Eq. (28) by imposing Eq. (10) as before. The parameter values that satisfy this condition are displayed in the inset of Fig. 5. It can be shown that when $L/\xi \lesssim 6$, Eq. (28) holds for all F, while for long polymers axis clamping has no effect on the force-extension relationship, and for $\kappa_F L \gg 1$ we again recover the well-known result [4], Eq. (22).

We can expand Eq. (28) in the limit of small forces to obtain an equation analogous to Eq. (23) for the case of axis clamping. Interestingly, for $d=3$ we obtain

$$\frac{X}{L} = 1 - \frac{1}{30}\left(\frac{L}{\xi}\right) + \frac{11F\xi}{25\,200k_BT}\left(\frac{L}{\xi}\right)^3. \qquad (29)$$

Note that as $F \to 0$, X/L approaches $1 - L/(30\xi)$, while Eq. (23) shows that without the entropic constraint, X/L approaches $1 - L/(6\xi)$ in the limit of zero force. Hence we see a different behavior at low forces due to the entropic constraint at the end.

An experimentally relevant situation is illustrated in Fig. 1(b), when the polymer is stretched using a micropipette and the force exerted is calculated by the bending of the micropipette tip. An equivalent setup uses a bead attached to a glass fiber and measures the bending of the fiber. These methods have been used earlier, for example, to stretch chromosomes [16] and long DNA molecules [17]. Stretching using an AFM also involves axis clamping, though here the applicability of our formulas depends on the other boundary condition, i.e., the collinearity of the first and last tangent vectors with the force, being satisfied. In principle, the entropic effect of axis-clamping may also be observed in stretching with a laser tweezer since the optical bead is held in a three-dimensional trap formed by the laser beam [Fig. 1(c)]. In practice, how-
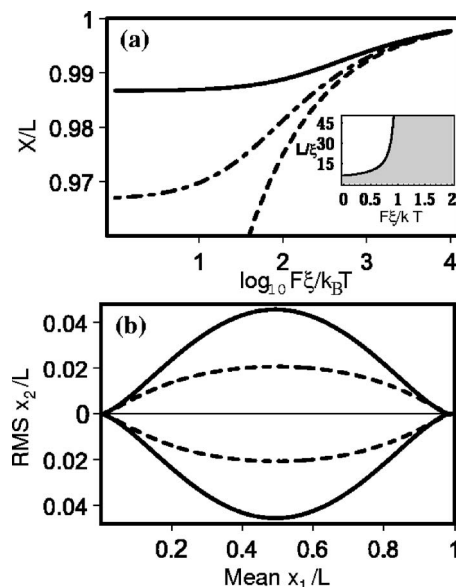

FIG. 5. (a) The relative extension of a polymer X/L pulled by a constant force in the presence of axis-clamping is plotted against the reduced force $F\xi/(k_BT)$, for a short polymer (solid line) for $d=2$. The dot-dashed line is the extension without the axis-clamping condition, Eq. (16), while the dashed curve is the long chain result [5]. The reduced polymer length is $L/\xi=0.4$ (≈ 20 nm for DNA and ≈ 6 μm for actin). In terms of force, $F\xi/(k_BT)=1$ means 0.082 pN for DNA, and 0.27 fN for actin. Inset shows the range of allowed parameter values (shaded area). (b) The shape of a polymer stretched with a laser tweezer is plotted parametrically in units of polymer contour length L for a short polymer ($L/\xi=0.4$). The solid curve is for $F\xi/(k_BT)=1$, and the dotted curve is for $F\xi/(k_BT)=1000$. RMS stands for root mean square.

ever, the trap is not a perfect clamp. First, it is significantly weaker in the z-direction, so some fluctuations do occur. Second, it is very hard to control where on the surface of the bead the polymer binds, so collinearity may be only approximately satisfied, since the bead is about three orders of magnitude larger than the polymer thickness. Finally, the bead is free to rotate in the trap, to the extent allowed by the twist elasticity of the polymer. Experimentally the effect of axis clamping might be relevant for stretching very short DNA strands—a 0.1 pN force will stretch a 300 nm strand of DNA to 0.86 of its contour length if stretched with axis-clamping, compared to 0.81 without it. The difference might also be observable when pulling on actin of about a persistence length in size, but in the range of femtonewton forces.

III. FLUCTUATING ROD IN AN ELECTRIC FIELD

Next we consider stretching of a charged polymer by a constant electric field. Here, one end of the polymer is tethered, and when the field is switched on, the polymer is observed to extend [12,18]. Because the polymer is in a charged aqueous environment, the molecular mechanism of the electrophoretic stretch is complicated [18,19]. Here we abstract from these difficulties and assume that the polymer responds to the field E as a uniformly charged rod with charge per unit length λ.

As in the previous section the contour length along the polymer is denoted by s, with $s=0$ denoting the tethered end. If the position vector of the segment s along the contour is denoted by $\mathbf{r}(s)$, the interaction potential of the polymer with the field can be written as

$$H_I[\mathbf{r}(s)] = -\lambda \mathbf{E} \int_0^L ds[\mathbf{r}(s) - \mathbf{r}(0)]. \tag{30}$$

Note that we are not only assuming that the effective charge density is constant, but that it also remains unchanged during the course of stretching. We can express Eq. (30) in terms of tangent vectors,

$$H_I[\mathbf{t}(s)] = -\lambda \mathbf{E} \int_0^L ds \int_0^s ds' \mathbf{t}(s'). \tag{31}$$

We now change the order of integration. Instead of integrating first over s' from 0 to s, and then over s from 0 to L, we can equivalently first integrate over s, from s' to L, then integrate over s', from 0 to L. Hence we get

$$H_I = -\lambda \mathbf{E} \int_0^L ds' \int_{s'}^L ds \mathbf{t}(s') = -\int_0^L ds' \lambda \mathbf{E}(L-s') \mathbf{t}(s'), \tag{32}$$

Thus a constant electric field stretches on a uniformly charged polymer as though it were subjected to a contour

dependent force, $\mathbf{F}(s) \equiv \mathbf{E}\lambda(L-s)$. This force is zero at the free end, $s=L$, and reaches a maximum of $|\mathbf{E}|\lambda L$ at the tethered end.

The s-dependent potential H_I makes the application of spectral methods to evaluating the partition function not practical. Namely, in this case the calculation of the eigenfunctions for the spectral representation of the partition function maps to a Schrodinger-like equation with a time dependent potential, making the analysis quite complicated. The generating functional formalism, on the other hand, can be employed without much difficulty.

The boundary conditions appropriate for this situation are $t_i(0)=0$, $i=2,3,\ldots,d$ at the tethered end, and $\frac{dt_i(s)}{ds}\big|_{s=L}=0$, for all i at the free end. The boundary term in Eq. (3) once again vanishes. The correlation function $\langle \theta_i(s)\theta_i(s')\rangle$ is now the Green's function, $G(s,s')$, of the operator

$$O(s) \equiv -\xi \frac{d^2}{ds^2} + \beta\lambda E(L-s), \tag{33}$$

with the boundary conditions above.

The solution to Eq. (7) that satisfies the boundary conditions is calculated in the same way as for the constant force case discussed above, but now in terms of Airy functions, Ai and Bi, and their derivatives, Ai′ and Bi′,

$$G(s,s') = \frac{\pi\{\text{Ai}[\kappa_E(L-s)]\text{Bi}'[0] - \text{Ai}'[0]\text{Bi}[\kappa_E(L-s)]\}\{\text{Ai}[\kappa_E(L-s')]\text{Bi}[\kappa_E L] - \text{Ai}[\kappa_E L]\text{Bi}[\kappa_E(L-s')]\}}{\kappa_E \xi(\text{Ai}[\kappa_E L]\text{Bi}'[0] - \text{Ai}'[0]\text{Bi}[\kappa_E L])}. \tag{34}$$

Equation (34) is the solution for $s \geq s'$, while the case $s < s'$ follows from symmetry.

The relevant, electric-field dependent length scale is given by κ_E^{-1}, where

$$\kappa_E = (\beta\lambda E/\xi)^{1/3}. \tag{35}$$

It is interesting to note that this length scales as $E^{-1/3}$, which is different than the $-1/2$ power that characterizes the dependence of κ_F^{-1} on the force. This could not have been predicted by dimensional arguments. In fact, since $E\lambda L$ is a force, one might have guessed the same scaling for κ_E^{-1} as for κ_F^{-1}.

We can now calculate the relative extension of the polymer in the direction of the field using Eq. (8):

$$\frac{X}{L} = 1 - \frac{(d-1)(\text{Ai}'[\kappa_E L]\text{Bi}'[0] - \text{Ai}'[0]\text{Bi}'[\kappa_E L])}{2\kappa_E^2 \xi L(\text{Ai}[\kappa_E L]\text{Bi}'[0] - \text{Ai}'[0]\text{Bi}[\kappa_E L])}. \tag{36}$$

Equation (36) is one of the main results of this paper. It provides an exact expression for the field-extension relation for a charged semiflexible polymer in an electric field.

Since the quadratic Hamiltonian, Eq. (3), implicitly assumes that the fluctuations of the tangent vector are small, we require $\langle t_i(s)^2\rangle$ to be small for our analysis to be self-consistent. Once again we impose the self-consistency condition, Eq. (10), on the variance of the fluctuations, $\langle t_i(s)^2\rangle$. It is clear from the boundary conditions that the tangent vector is fixed at the $s=0$ end and completely free at the $s=L$ end and that the maximum fluctuations of the tangent vector occur at $s=L$. Thus the self-consistency requirement can be written as

$$\langle t_i(L)^2\rangle = \frac{\text{Ai}[0]\text{Bi}[\kappa_E L] - \text{Ai}[\kappa_E L]\text{Bi}[0]}{\phi^{1/3}(\text{Ai}[\kappa_E L]\text{Bi}'[0] - \text{Ai}'[0]\text{Bi}[\kappa_E L])} \leq \frac{1}{2(d-1)}. \tag{37}$$

When the argument of Airy function is large, Ai converges to 0 whereas Bi diverges to $+\infty$ exponentially as

$$\text{Ai}[z] \simeq \frac{\exp\left[-\frac{2}{3}z^{3/2}\right]}{2\sqrt{\pi}z^{1/4}},$$

$$\text{Bi}[z] \simeq \frac{\exp\left[\frac{2}{3}z^{3/2}\right]}{\sqrt{\pi}z^{1/4}}. \tag{38}$$

Thus when $\kappa_E L \gg 1$, the condition in Eq. (37) reduces to

$$\langle t_i(L)^2 \rangle \simeq -\frac{\text{Ai}[0]}{\xi \kappa_E \text{Ai}'[0]} \leq \frac{1}{2(d-1)}. \tag{39}$$

Again we get a ratio of length scales, i.e., the strong field condition is equivalent to

$$\kappa_E \xi \gtrsim 2.74(d-1), \tag{40}$$

where we have used $\text{Ai}[0]/\text{Ai}'[0] \approx 1.37$. The small fluctuation approximation therefore requires that the length scale κ_E^{-1} be less than about one-third of the persistence length.

On the other hand, when $\kappa_E L \ll 1$, we can Taylor expand $\text{Ai}[\kappa_E L]$ around 0 as $\text{Ai}[\kappa_E L] \simeq \text{Ai}[0] + \kappa_E L\,\text{Ai}'[0]$ and similarly for Bi. Then, Eq. (37) becomes

$$\frac{L}{\xi} \leq \frac{1}{2(d-1)}. \tag{41}$$

Thus Eq. (36) is valid for all field strengths for polymers less than $\xi/2$ in two dimensions, and $\xi/4$ in three dimensions. For longer polymers, stronger fields are needed for its applicability. For example, Eq. (36) is applicable to a molecule of actin that is about 15 μm in size, and is being stretched by an electric field of at least 0.02 V/cm in strength, but it is not useful for a molecule of DNA longer than about 25 nm; the minimum field required for a 50 nm DNA molecule for the small fluctuation approximation to be appropriate is of the order of 10^2 V/cm. The inset of Fig. 6 shows the region of parameter space, where in $d=2$ Eq. (37) holds for the whole range of $\kappa_E L$.

When the chain is long and the applied field is strong such that $E \gg (\beta\lambda\xi^2)^{-1}$, the expression of extension reduces to

$$\frac{X}{L} = 1 - \frac{d-1}{2}\sqrt{\frac{k_B T}{\lambda E \xi L}}. \tag{42}$$

It should be noted that it is possible to derive this equation using approximate phenomenological arguments [4,12]. A comparison with the constant force case here is very interesting. As we have shown, the length scale κ_F^{-1} in the constant force case scales as $F^{-1/2}$ in contrast with κ_E^{-1} that scales as $E^{-1/3}$. In the high field limit, however, the dependence of the relative extension on the electric field becomes $E^{-1/2}$, just as the dependence of extension on force in the high force limit.

In the other limit, when the polymer is short so that $L/\xi \leq 1/(2d-2)$, and the electric field is small, $E \ll (\beta\lambda\xi^2)^{-1}$, Eq. (36) yields the limiting linear response,

$$\frac{X}{L} = 1 - \frac{d-1}{4}\left(\frac{L}{\xi}\right) + \frac{(d-1)\lambda E\xi^2}{40 k_B T}\left(\frac{L}{\xi}\right)^4. \tag{43}$$

Equation (43) has been derived for $d=3$ using different methods in [13].

Again we can compute the average shape, defined as before by Eqs. (8) and (9), of the polymer under stretch. In
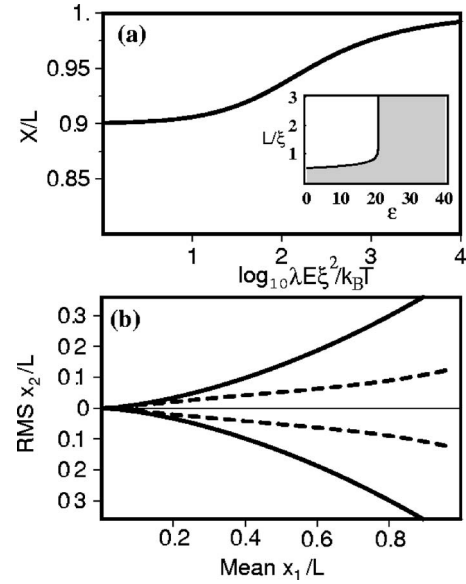

FIG. 6. (a) Relative extension X/L is plotted against the reduced electric field $\beta\lambda E\xi^2$ for a polymer of size $L/\xi = 0.4$ for $d=2$. Inset shows parameter values allowed by self-consistency (shaded area). For actin, if $\lambda \approx 1e/$nm, $\beta\lambda E\xi^2 = 1$ is about 10^{-3} V/cm, for DNA with $0.6e/$nm it is about 170 V/cm. Note that we are assuming that the screened charge density is about one-tenth of the bare charge density. This is consistent with experiments on DNA [12]. (b) The shape of a short polymer stretched by a constant electric field in two dimensions is plotted parametrically in units of polymer contour length $L/\xi = 0.4$. The solid curve is for $\beta\lambda E\xi^2 = 1$, and the dotted curve is for $\beta\lambda E\xi^2 = 1000$.

experiments on long DNA molecules, it has been reported that the polymer assumes a trumpetlike shape, reflecting the fact that force is stronger towards the grafted end and tends to vanish at the free end [12]. The integral in Eq. (8) can be calculated analytically, while Eq. (9) needs to be calculated numerically. A trumpetlike shape emerges naturally as the result of our computation, and is shown in Fig. 6.

In experiments that stretch single molecules by electric fields, the polymer molecule is in an ionic solution, and an electric field will drive a net current [4]. The current creates a flow in the system, and the net deformation of the molecule, it has been argued, is therefore a combination of the flow and the field [4,18,19]. In experiments performed with long DNA molecules of different lengths, it has been shown that Eq. (42) fits the data only with different effective charge densities for different lengths of the molecule, thus questioning whether the effective charge density is a physically meaningful parameter [18]. However, we feel that interpretation of the results has been hampered by the lack of precise formulas for the electric field induced stretching case. While this continues to be the case for long DNA molecules, Eq. (36) provides a means of testing these questions using actin molecules. Quantitative comparison of our results with experiments would be relatively easy for actin polymers, since the required electric fields for the fluctuating-rod assumptions to hold are small, and easily produced in the laboratory. Assuming that charge screening reduces bare actin charge to about 1 electrons/nm, our results are valid for all actin
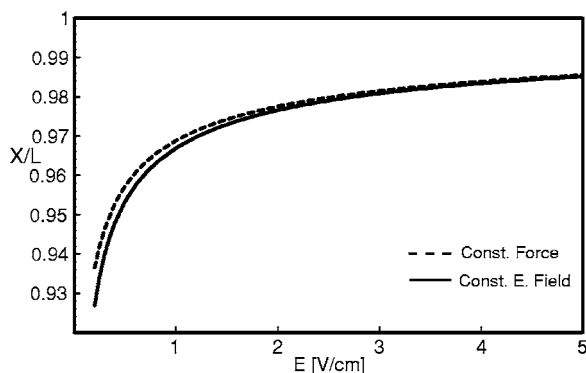
FIG. 7. The relative extension of a 15 μm actin molecule in a constant electric field compared to the case when it is stretched by a constant force due to a single charge on the free tip of the polymer. When this charge is chosen to be exactly 1/4 of the total charge on the polymer, the two curves agree well over most of the range of applicability.

lengths at electric field strengths starting from 0.03 V/cm upwards. From force-extension measurements in such experiments, the effective charge density of actin of different lengths and under different ionic conditions can be extracted.

In view of the nonuniform nature of the effective force a charged polymer experiences in a constant electric field, it may be useful to ask what is the effective constant force, which would lead to the same relative extension as a given electric field strength. Equation (42) appears to suggest that at high forces the polymer behaves as if it is being stretched by a constant force equivalent to that produced by one-fourth of the total charge concentrated at the end of the chain. It turns out that over most of the regime of applicability of our result, that is a reasonable approximation, as demonstrated in Fig. 7.

IV. CONCLUSIONS

Biopolymers like DNA are fast emerging as tools—rulers and templates—for a number of biophysical applications, such as measuring DNA-protein interactions. Some of these uses are due to the remarkable fact that a simple physical model, the wormlike chain, explains DNA entropic elasticity with great accuracy, and small deviations from this behavior, say due to bound proteins, can be detected. However, entropic effects are subtle and may be affected by experimental conditions in ways that are not obvious. For example, it was recently pointed out that tethered bead experiments, that use a short DNA strand attached to a bead, need to take into account entropic exclusion forces of the bead with the wall

[20]. Similarly, experiments involving entropic elasticity need to account for the influence of experimental conditions on the entropy of the molecule that may quantitatively change the force-extension relation.

In this paper we have theoretically examined entropic effects of boundary conditions on force-extension curves. We demonstrated that the force-extension response of fluctuating rods is qualitatively different from that of long polymers, for which the contour length is much greater than its persistence length. We also derived analytic expressions for the force-extension relation and root-mean-square fluctuations of such a polymer stretched by a constant force. Finally, we showed that in the fluctuating rod limit the constraint of axis-clamping, which might be imposed by single-molecule stretching techniques such as laser tweezers, can lead to a measurable effect on force-extension curves. More importantly, blindly employing the long-polymer formulas to data obtained for short chains might lead to erroneous conclusions [7].

Single molecule experiments using electric fields to stretch molecules may also become a useful tool, but progress is hampered by the lack of analytic expressions for the force-extension curves in this case. We examine the case of a polymer stretched by an electric field, and show that it behaves as if it is being stretched by a nonuniform force, which increases linearly from the free end of the chain. We derive an analytic expression for the field-extension relation and average shape of the polymer stretched by an electric field, in the fluctuating rod limit.

In all cases considered here, the force extension formulas, Eqs. (28) and (36), are functions of three length scales, two of which are the contour length L and the 3d-persistence length ξ. For stretching by a constant force, the third length scale is $\kappa_F^{-1} \equiv \sqrt{k_B T \xi / F}$, while for a constant electric field it is $\kappa_E^{-1} \equiv (k_B T \xi / E \lambda)^{1/3}$. It is noteworthy that these length scales do not follow from dimensional arguments alone due to the presence of dimensionless combinations of polymer parameters. Since these length scales are related to the decay of fluctuations in the directions transverse to the force, and become smaller as the polymer becomes more straight, they may be interpreted as confinement length scales, pointing towards deeper analogies between stretched and confined polymers [21,22] that warrant further exploration.

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