Estimating the Persistence Length of a Worm-Like Chain Molecule from Force-Extension Measurements

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ABSTRACT We describe a simple computation of the worm-like chain model and obtain the corresponding force-versus-extension curve. We propose an improvement to the Marko and Siggia interpolation formula of Bustamante et al (*Science* 1994, 265:1599–1600) that is useful for fitting experimental data. We apply it to the experimental elasticity curve of single DNA molecules. Finally, we present a tool to study the agreement between the worm-like chain model and experiments.

INTRODUCTION

Stretching individual biomolecules is now achieved by a variety of techniques including flow stress (Smith et al., 1992), AFM (Rief et al., 1997), micro-needles (Cluzel et al., 1996), optical tweezers (Wang et al., 1997), and magnetic tweezers (Strick et al., 1996) that allow measurement of forces from 10 femtonewtons to hundreds of piconewtons. Different biological molecules have now been analyzed (Kellermayer et al., 1997) and the accuracy of these techniques has sufficiently improved so that the theoretical models used to analyze force-extension curves must be refined. In particular, Bustamante et al. (1994) have shown that the force-extension diagram of a DNA molecule is well described by a worm-like chain (WLC) model. In this note, we present a simple derivation of the WLC model whose solution can easily be implemented on a personal computer. Furthermore, we propose an improvement to the Marko and Siggia (Bustamante et al., 1994) interpolation formula with a relative error in force of 0.01% for any given extension. This new interpolation scheme is easy to introduce into fitting algorithms and allows for accurate estimation of the persistence length. We propose and discuss the possibility of taking into account the enthalpic elasticity contribution that is observed experimentally in force-extension curves.

EXACT SOLUTION OF THE WLC MODEL

The WLC model was first treated numerically by Fixman and Kovac (1973), after which a preliminary analytical approach was performed by Kovac and Crabb (1982). Its complete treatment was achieved by Marko and Siggia and reported by Bustamante et al. (1994).

The energy, E, of a stretched DNA molecule is given in the WLC model by a line integral of two terms. The first term, $e_b(s)$, describes the resistance of the chain to bending and is proportional to the inverse square of the radius of curvature, R. One writes R^{-2} as the square of the derivative with respect to the arc length, s, of the unit tangent vector, $\boldsymbol{t}(s)$, and gets

$$e_b(s) = \frac{A}{2}\left|\frac{dt(s)}{ds}\right|^2 = \frac{A}{2R^2}.$$

The second term, $e_f(s)$, gives the stretching energy resulting from the application of a force, F, to the end of the molecular chain. Taking the force along the z axis, one arrives at the final expression for E:

$$E_{WLC} = \int_0^{L_0} ds\left(\frac{A}{2}\left|\frac{d\mathbf{t}(s)}{ds}\right|^2 - F\cos\theta(s)\right), \qquad (1)$$

where $\cos\theta(s)$ is the angle between $\mathbf{t}(s)$ and the z axis, and L_0 is the contour length of the molecule. Experimental data are usually analyzed in terms of the persistence length, L_p, (sometimes denoted by ξ) which is related to the quantity A by $L_p = A\,\beta$ where $\beta = 1/k_BT$.

The partition function, $Z(L_0, F, \mathbf{t}_0, \mathbf{t}_1)$, is given by the path integral:

$$Z(L_0, F, \mathbf{t}_0, \mathbf{t}_1) = \int \mathcal{D}(\mathbf{t})\exp(-(\beta E_{WLC}). \qquad (2)$$

$\mathcal{D}(\mathbf{t})$ is the integration measure in functional space of the paths drawn on the unit sphere starting at the point $\mathbf{t}(s = 0) = \mathbf{t}_0$ and ending at $\mathbf{t}(s = L_0) = \mathbf{t}_1$. By performing an analytic continuation of the s integral in E_{WLC} toward the imaginary axis, one gets the action integral relative to a fictitious particle of unit charge moving upon a sphere under the influence of an electric field, F, with the time variable $t \propto \mathrm{Im}(s)$. The above analytic continuation of the partition function, $Z(L_0, F, \mathbf{t}_0, \mathbf{t}_1)$, maps to the Feynman transition probability amplitude $\langle \mathbf{t}_1 t_f \,|t = 0\mathbf{t}_0\rangle$ for the fictitious particle, initially localized at the coordinate $\mathbf{t}_0$, at the final time t_f at the point of coordinate $\mathbf{t}_1$. This amplitude is given, accord-

Received for publication 23 January 1998 and in final form 30 September 1998.

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0006-3495/99/01/409/05 $2.00

ing to the usual rules of quantum mechanics, by the matrix element:

$$\langle \mathbf{t}_1 t_f | t = 0 \mathbf{t}_0 \rangle = \left\langle \mathbf{t}_1 \left| \exp\left(-\frac{i t_f}{\hbar} \hat{H} \right) \right| \mathbf{t}_0 \right\rangle, \quad (3)$$

where $\hat{H}$ is the Hamiltonian operator.

In the present case, the Hamiltonian $\hat{H}_{WLC}$, written in a dimensionless reduced form, reads as follows:

$$\hat{H}_{WLC} = -\frac{1}{2 \sin \theta} \frac{\partial}{\partial \theta} \sin \partial \frac{\partial}{\partial \theta} - \alpha \cos \theta, \quad (4)$$

where we have introduced the dimensionless quantity $\alpha = \beta L_p F$. Going back to the partition function, a simple adaptation of Eq. 3 gives:

$$Z(L_0, F, \mathbf{t}_0, \mathbf{t}_1) = \left\langle \mathbf{t}_1 \left| \exp\left(-\frac{L_0}{L_p} \hat{H}_{WLC} \right) \right| \mathbf{t}_0 \right\rangle. \quad (5)$$

Introducing the eigenvalues, ϵ_n, and eigenwavefunctions, $\Psi_n(\theta)$, of the WLC Hamiltonian: $\hat{H}_{WLC}\Psi_n(\theta) = \epsilon_n \Psi_n(\theta)$, the partition function given by Eq. 5, can be written as a series of eigenstates, each term being of the order of $\exp(-L_0/L_p(\epsilon_n))$. In practice $L_0 \gg L_p$, so it is legitimate to retain only the ground state contribution. The logarithm of the partition function is given by the simple expression:

$$\ln(Z(L_0, F, \mathbf{t}_0, \mathbf{t}_1)) = -\frac{L_0}{L_p} \epsilon_0(\alpha) + O(1)), \quad (6)$$

where $\epsilon_0(\alpha)$ is the ground state energy of $\hat{H}_{WLC}$ and $O(1)$ stands for a term of order unity associated with a prefactor involving the ground state wave function, $\Psi_0(\theta)$. We are now in a position to compute the relative extension of the chain:

$$\frac{\langle z \rangle}{L_0} = \frac{1}{\beta L_0} \frac{\partial \ln Z}{\partial F} = -\frac{\partial \epsilon_0}{\partial \alpha}(\alpha) = \langle \Psi_0 | \cos \theta | \Psi_0 \rangle, \quad (7)$$

where the second part of the above equation follows from first order perturbation theory applied to the infinitesimal Hamiltonian resulting from a small variation of α.

We must now solve the eigenvalue problem, $\hat{H}_{WLC}\Psi_0(\theta) = \epsilon_0 \Psi_0(\theta)$. Instead of using linear algebra methods to diagonalize large matrices, we solve the ordinary differential equation associated with the eigenvalue equation:

$$\frac{1}{2 \sin \theta} \frac{\partial}{\partial \theta} \left(\sin \theta \frac{\partial}{\partial \theta} \Psi_0(\theta) \right) + (\alpha \cos \theta + \epsilon_0) \Psi_0(\theta) = 0. \quad (8)$$

We look for a solution which satisfies regularity conditions for both $\theta = 0$ and $\theta = \pi$. These requirements are necessary in order for $\hat{H}_{WLC}$ to be a self-adjoint operator, and can be fulfilled if and only if the reduced energy, ϵ, belongs to a discrete set of values.

As a first step, one constructs by a series expansion two solutions of Eq. 8, $\Psi_a(\theta)$ and $\Psi_b(\theta)$, which are respectively regular for $\theta = 0$ and $\theta = \pi$. One then proceeds with an outward numerical integration of $\Psi_a(\theta)$ and inward numerical integration of $\Psi_b(\theta)$ up to an intermediate value of $\theta = \theta_0$. The energy eigenvalue is obtained as a matching condition for the two wave functions, which ensures the regularity of the eigenfunction for the whole physical domain of θ. One requires, for $\theta = \theta_0$, equality of logarithmic derivatives of $\Psi_a(\theta)$ and $\Psi_b(\theta)$:

$$\frac{\partial \Psi_a}{\partial \theta} \Big/ \Psi_a - \frac{\partial \theta_b}{\partial \theta} \Big/ \Psi_b = 0. \quad (9)$$

Eq. 9 is then solved by a standard iteration method. The construction of Table 1 giving the elongation $\langle z \rangle / L_0$ as a function of α is started with a small value of α: one uses $-\alpha^2/3$ as the ground state energy approximation given by the lowest order perturbation theory.

Because the present method used to solve the eigenvalue problem automatically yields the eigenfunction $\Psi_0(\theta)$, one uses Eq. 7 in order to get $-(\partial \epsilon_0 / \partial \alpha) = (\langle z \rangle / L_0)$. One can then proceed to the neighboring value $\alpha + \Delta\alpha$, using $\epsilon_0 + \Delta\alpha(\partial \epsilon_0 / \partial \alpha)$ as a starting eigenvalue. In this way we have easily obtained, using numerical methods, the ground state energy eigenvalues for the complete range of experimental interest ($0.06 \leq \langle z \rangle / L_0 \leq 0.97$), with a precision in $\langle z \rangle / L_0$ better than 10^{-5} (for a given force). These results are used in the next section to obtain an analytical interpolation of the force-extension formula of the WLC model, which turns out to be somewhat more precise than the interpolation formula proposed by Bustamante et al. (1994).

AN IMPROVED FIT TO THE WLC MODEL

In this section we shall use the notation $z = \langle z \rangle$. Marko and Siggia (Bustamante et al., 1994) proposed the following

TABLE 1 Normalized force $\alpha = FL_p/k_BT$ versus extension of the exact worm-like chain

$\langle z \rangle / L_0$	$\alpha = FL_p/k_B$
0.066343	0.1
0.130807	0.2
0.191795	0.3
0.248177	0.4
0.345160	0.6
0.421692	0.8
0.527686	1.2
0.594418	1.6
0.672583	2.4
0.717901	3.2
0.754505	4.2
0.801653	6.4
0.828069	8.5
0.855420	12
0.878595	17
0.899926	25
0.915441	35
0.929264	50
0.942251	75
0.949991	100
0.959170	150
0.964642	200

interpolation formula as a useful approximation to the WLC model:

$$F = \frac{k_B T}{L_p}\left[\frac{1}{4(1 - z/L_0)^2} - \frac{1}{4} + \frac{z}{L_0}\right]. \qquad (10)$$

Their expression has the nice property of reducing to the exact solution as either $z \to 0$ or $z \to L_0$. In between it reproduces the general behavior of the exact solution but may differ by as much as 10% for $z/L_0 \sim 0.5$ (Bustamante et al., 1994). Using this interpolation in the fitting procedure leads to a small overestimate in the value of L_p: the error is typically of the order of 5%, but will actually depend upon the range of extensions spanned by an experiment and by the statistical error for each point.

We have subtracted the Marko-Siggia interpolation formula from the exact numerical solution of the WLC model and expressed the residuals as a seventh-order polynomial, which provides all the correction terms. We therefore write:

$$F = \frac{k_B T}{L_p}\left[\frac{1}{4(1 - z/L_0)^2} - \frac{1}{4} + \frac{z}{L_0} + \sum_{i=2}^{i\le 7} \alpha_i \left(\frac{z}{L_0}\right)^i\right], \qquad (11)$$

with $a_2 = -0.5164228$, $a_3 = -2.737418$, $a_4 = 16.07497$, $a_5 = -38.87607$, $a_6 = 39.49944$, and $a_7 = -14.17718$. It can be verified that the above formula is compatible with the properties of F as an analytic function of z.

The order of the polynomial is arbitrary; lower or higher order could also be used. We choose the seventh order since it leads to an accuracy better than 0.01% over the useful extension range (which is more accurate than required for experiments but useful inasmuch as the derivative $\partial F/\partial z$ is often needed in the fitting procedure).

Vologodskii has also proposed an interpolation formula (Eq. 4 of Vologodskii, 1994) which describes the force-versus-extension curve of a WLC model obtained by Monte Carlo simulation. This formula is neither valid at small extension $z/L_0 < 0.1$ nor at large extension $z/L_0 > 0.97$, yet in between the agreement with the exact WLC model is good, the relative error in force being smaller than 5%. Recently Moroz and Nelson (1997) also proposed the following approximation for the extension-versus-force curve valid for forces larger than 0.1 pN:

$$\frac{\langle z\rangle}{L_0} = 1 - \frac{1}{2}\left[\frac{L_o F}{k_B T} - \frac{1}{32}\right]^{-1/2} + \frac{F}{K_0}. \qquad (12)$$

A finite size effect may be added to this equation in the form $k_B T/L_0 F$.

ENTHALPIC CORRECTIONS AT HIGH FORCE

As shown by Smith et al. (1996) and Wang et al. (1997), the B-DNA structure displays a linear stretching behavior at high force. Indeed, the chemical bonds are slightly modified by the strong stretching, leading to a small increase in the length of the molecule. This behavior is evident in the range of 5–50 pN, but above 60 pN a drastic structural change occurs leading to an abrupt increase in length (the so-called "overstretch transition"). So far, no exact model has been derived to incorporate the latter effect in the WLC model. An appropriate model for the behavior below the overstretch transition was proposed by Wang et al. (1997) and inspired by Odijk (1995); it consists of replacing z/L_0 by $z/L_0 - F/K_0$. Analogously we obtain:

$$F = \left(\frac{k_B T}{L_p}\right)\left[\frac{1}{4(1 - l)^2} - \frac{1}{4} + l + \sum_{i=2}^{i\le 7} a_i (l)^i\right] \qquad (13)$$

with $l = z/L_0 - F/K_0$.

In practice, with K_0 ranging from ~500 pN to ~1500 pN (which covers the existing experimental measurements), the enthalpic correction will not affect the estimation of the persistence length within 1% accuracy, provided that only the data below 1–2 pN are fit. Failing to account for the enthalpic correction for forces as small as 5–10 pN leads to underestimation of L_p. However, nonlinear elasticity is also expected and the domain of validity of Eq. 13 is not known.

As may be seen in Fig. 1, we used this more elaborate approach to fit the data of Fig. 5 of Wang et al. (1997) and found slightly different values for the fit parameters (see Fig. 1) as compared with the values found in Wang et al. (1997) ($L = 1314\ nm \pm 1$, $L_p = 43.3\ nm \pm 0.5$ $K_0 = 1246 \pm 10\ pN$, $p = 0.18$). The fit is somewhat improved using the exact WLC model, the value of L_p being smaller by 3 nm.

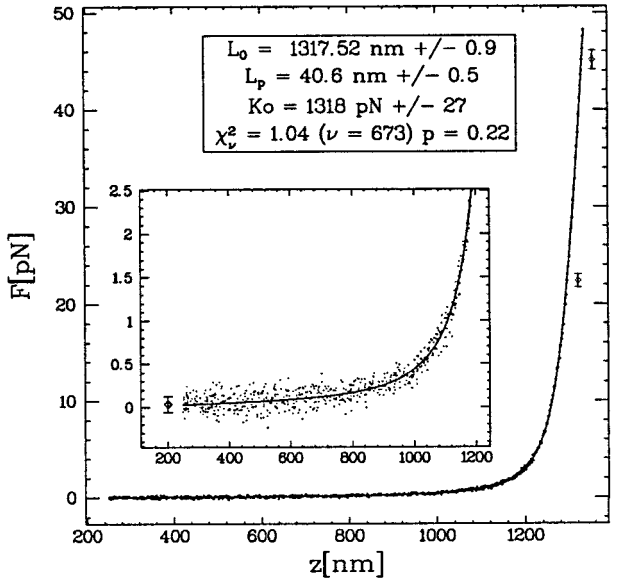

FIGURE 1 Experimental data (*points*) and fit (*solid line*) corresponding to figure 5 of Wang et al. (1997). The inset is a blow-up of the small force regime. The optical tweezers used here allow for quick measurements with roughly a constant noise level when the force is <6 pN (see the inset) and an increasing error above 6 pN as indicated by the two error bars (shifted by 25 nm from the data). The exact model leads to a better overall fit in terms of χ^2_ν.

In the same spirit, in Fig. 2 we have analyzed another DNA stretching experiment using a different measurement technique (Strick et al. 1996). The very good fit demonstrates that the WLC model applies over a large range of forces.

COMPARISON WITH EXPERIMENTS

How well does the WLC work?

The WLC's force-versus-extension curve is a highly nonlinear function: at low extension, force grows linearly with x with a slope $3k_BT/2L_pL_0$, whereas at high extension, the force diverges as $(k_BT/L_p)\ [4(1 - z/L_0)^2]^{-1}$. For moderate extensions, a crossover occurs between these two regimes. Thus various approaches may be tried to determine L_p: concentrating on the low extension regime, considering the molecule near its full extension, or considering it over the entire domain. An interesting test of the WLC model's validity is given by the equality between the values of L_{pl} and L_{ph} obtained for low and high extension, respectively.

So far, this test has been partially completed: Marko and Siggia carefully analyzed data of Smith et al. (1992) and plotted the effective persistence length versus the applied force, which should remain constant if the WLC applies exactly (Marko and Siggia, 1995). They have shown that the WLC model correctly describes the experiment performed in a 10-mM salt buffer. In a low-salt buffer, the long range interaction added by electrostatic effects (Barrat and Joanny, 1993) induces significant departures from the WLC model. To be more specific, in a 1-mM buffer, Marko and Siggia show that Smith et al.'s data leads to $L_{pl} = 85 \pm 10$ *nm*, whereas $L_{ph} \sim 50$ *nm*. This result contrasts with the new one published by the same experimental group (Baumann et al., 1997) using a different technique, namely $L_{ph} \sim 95 \pm 6$ *nm*.

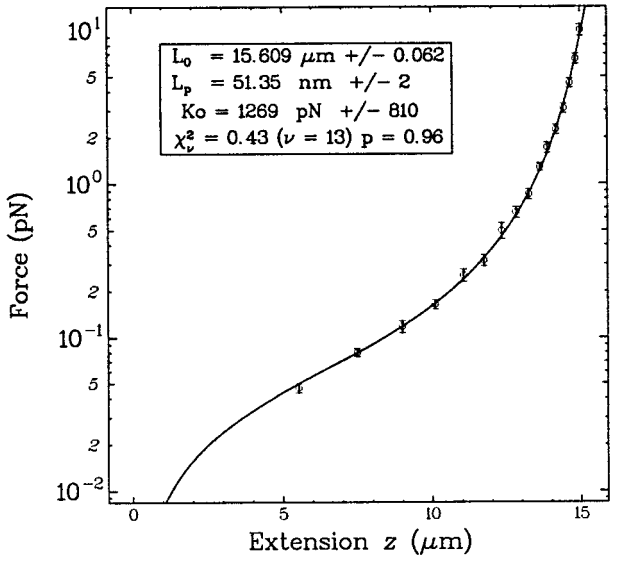

FIGURE 2 Experimental data and fit corresponding to the extension of a λ-DNA molecule in 10 mM PB buffer obtained using the Brownian motion method (Strick et al., 1996). This method is particularly well suited to measuring small forces. Moreover, the relative error in the force (*vertical error bars*) may be kept constant by adjusting the acquisition time. The semilogarithmic scale facilitates visualization of the large force range. The WLC model (*solid line*) nicely fits the experimental data. Note that the forces are too small to allow an accurate measurement of K_0. The error bars correspond to statistical errors, but the systematic ones are more difficult to evaluate. We estimate the calibration errors in the range of 5%.

We cannot resolve this discrepancy here but we emphasize that the fits done by Barrat and Joanny (1993) as well as the analysis of the effective persistence length (Marko and Siggia, 1995) were based on the interpolation formula (Eq. 10), which is less accurate in the crossover regime. We propose the following expression, derived from Eq. 13, to provide an improved effective persistence length test:

$$L_{peff} = \left\langle \frac{k_BT}{F} \right\rangle \left[\frac{1}{4(1-l)^2} - \frac{1}{4} + l + \sum_{i=2}^{i\le 7} a_i (l)^i \right] \qquad (14)$$

with $l = z/L_0 - F/K_0$.

In Fig. 3 *A* we have applied this analysis to the interpolation formulae of Marko and Siggia, Vologodskii, and Moroz and Nelson, and finally in Fig. 3*B* to the data of Fig. 2. This figure should be compared with Fig. 4 of Marko and Siggia (1995). Clearly the WLC model works remarkably well for this experiment performed in a 10-mM phosphate buffer (pH 8). This agreement is even better than one should expect from figure 4 of Marko and Siggia (1995). Repeating this test for low-salt buffer (as in Figure 5 of Marko and Siggia (1995)) and performing a careful error analysis should allow one to determine whether electrostatic stiffening leads to a departure of the experimental curve from the WLC model.

Although Fig. 1 corresponds to very accurate measurements, the effective persistence length (data not shown) is meaningful only at high force. Thus measuring the persistence length using a polymer's force-versus-extension curve requires accurate force measurements over a large range of forces. Moreover, the determination of a persistence length's value should be associated with the force regime or extension domain explored. We propose to define the mean extension corresponding to the χ^2 fit of L_p:

$$\frac{z_{mean}}{L_0} = \frac{\sum_i (z_i/L_0)\ F^2(z_i, L_0, L_p, K_0)/\sigma_i^2}{\sum_i F^2(z_i, L_0, L_p, K_0)/\sigma_i^2}, \qquad (15)$$

where σ_i represents the error in force for the experimental point i and $F^2(z_i, L_0, L_p, K_0)$ is the estimated force using Eq. 13 with the measured extension z_i and L_0, L_p, and K_0 (the parameters of the best fit). In the same spirit, the mean force may be defined by replacing z_i/L_0 with F_i. Applying this to the data presented in Fig. 1 leads to $z_{mean}/L_0 = 0.945$ and $F_{mean} = 6.95$ pN; applied to the data in Fig. 2 it leads to $z_{mean}/L_0 = 0.77$ and $F_{mean} = 0.37$ pN.

The high force regime has been quite well investigated (Baumann et al., 1997; Wang et al., 1997). In this regime the

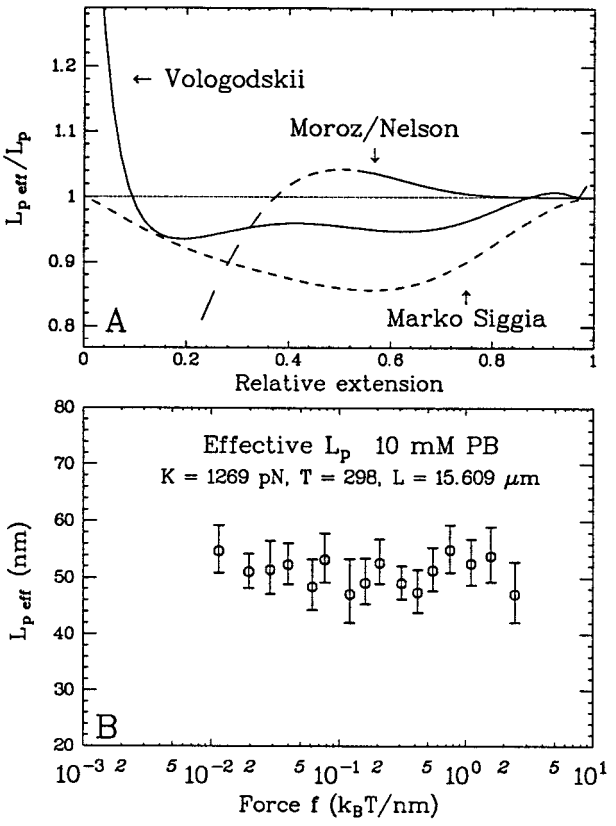

FIGURE 3 Effective persistence length versus relative extension (*A*) or normalized force (*B*). For each point of a force-versus-extension curve, the effective persistence length is computed using Eq. 14. A perfect WLC model leads to an effective persistence length independent of the extension and the force. In (*A*) we characterize the interpolation formula (Eq. 10) of Marko and Siggia (*dashed line*) (Bustamante et al., 1994), the Vologodskii interpolation formula (Vologodskii, 1994) (*solid line*), and the Moroz and Nelson (1997) formula (drawn in solid line in its validity domain ($(F > 0.1$ pN) and in dashed line at lower forces). (*B*) Analysis of the experimental of Fig. 2 shows no significant variation of L_{peff} with either extension or force. This confirms the experimental validity of the WLC model. Compare *B* with figure 4 of Marko and Siggia (1995).

enthalpic elasticity cannot be neglected and electrostatic effects may lead to departures from the WLC model (Marko and Siggia, 1995). We are definitely missing the very small force regime (with better resolution than in Smith et al., 1992), which should allow one to measure L_{pl}.

Finally, the WLC model has been used to describe the stretching of titin protein (Kellermayer et al., 1997; Rief et al., 1997; Tskhovrebova et al., 1997), yielding a persistence length equal to a few times the monomer size. This is somewhat surprising since the Kratky-Porod model leads asymptotically to the WLC only when the number of monomers per persistence length is large (Kratky and Porod, 1949). Using the concept of effective persistence length should show how well the WLC works in this case.

We supply an improved method to analyze the force-versus-extension curve of a stretched polymer using the WLC model. We hope that this method will allow more accurate determination of the properties of biological and other polymers. Furthermore, it may help to identify any experimental departures from the ideal WLC and reveal if electrostatic effects are their major cause, as originally proposed by Marko and Siggia (1995).

We thank D. Bensimon and M. Mézard for helpful discussions and CNRS, Université Paris VI and VII for financial support. M. D. Wang is supported by the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation Fellowship DRG-1326. S. M. Block is supported by grants from National Institutes of Health, the National Science Foundation, and the W. H. Kech Foundation.

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