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Stretching DNA to quantify nonspecific protein binding

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Nonspecific binding of regulatory proteins to DNA can be an important mechanism for target search and storage. This seems to be the case for the lambda repressor protein (CI), which maintains lysogeny after infection of *E. coli*. CI binds specifically at two distant regions along the viral genome and induces the formation of a repressive DNA loop. However, single-molecule imaging as well as thermodynamic and kinetic measurements of CI-mediated looping show that CI also binds to DNA nonspecifically and that this mode of binding may play an important role in maintaining lysogeny. This paper presents a robust phenomenological approach using a recently developed method based on the partition function, which allows calculation of the number of proteins bound nonspecific to DNA from measurements of the DNA extension as a function of applied force. This approach was used to analyze several cycles of extension and relaxation of λ DNA performed at several CI concentrations to measure the dissociation constant for nonspecific binding of CI (~ 100 nM), and to obtain a measurement of the induced DNA compaction ($\sim 10\%$) by CI.

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I. INTRODUCTION

Nonspecific binding of proteins to DNA is a ubiquitous phenomenon in genomes and is likely to be involved in several biological processes. Some representative examples are facilitated target finding via hopping mechanisms, DNA protection by coating, DNA cross-linking, and compaction and packaging, among others [1–9]. Experiments have shown that the rate at which proteins bind to their target site increases when nonspecific binding increases (for a review see [10]). In transcriptional regulation, nonspecific binding plays a role, for example, in the control of transcriptional noise, in facilitating protein-protein interactions, and in regulating the activity of motor proteins along the DNA. Notable examples are those of histones in eukaryotes, which display weakly sequence-dependent affinity [11,12], and of nucleoid-associated proteins in prokaryotes [13,14]. Genetic switches based on protein-mediated DNA looping may also be heavily influenced by nonspecific binding. This may indeed shorten the effective loop length if proteins bend the DNA upon binding, or interact cooperatively, and may further stabilize the loop in case nonspecifically bound proteins form clusters at the loop closure site. Therefore,

quantitative determination of the level of nonspecific binding of a protein to DNA is relevant to the understanding of the molecular mechanism of several biological processes. However, while specific DNA binding is easily characterized with a variety of established biochemical techniques, such as gel electrophoresis, nonspecific binding is more difficult to characterize. Pulling experiments on single DNA molecules by magnetic tweezers yield “extension versus force” curves that are different for naked DNA and DNA bound by bending proteins. However, quantitative determination of the number of bound proteins was not possible due to the lack of a versatile model that allows for an unbiased interpretation of these data.

An important first step in this direction was taken by Zhang *et al.* [15], who proposed a thermodynamic approach, employing the partition function and Maxwell relations. Recently, we implemented the Maxwell relations to determine the *change* in the number of λ repressor (CI) proteins bound nonspecifically to a 16-kbp-long fragment of λ DNA from pulling experiments performed in our laboratory [16]. The λ repressor, also known as CI protein, binds specifically to DNA. Specific cooperative binding mediates a 2.3-kbp loop in λ DNA between the oL region (containing specific binding sites oL1, oL2, and oL3) and the oR region (containing specific binding sites oR1, oR2, and oR3) [17]. CI-mediated DNA looping provides stability to the quiescent (lysogenic) state of the λ bacteriophage after infection of its host, *E. coli*, and simultaneously guarantees efficient switch to virulence (lysis) when triggered by environmental conditions [18,19]. However, single-molecule imaging [20] as well as thermodynamic and kinetic measurements [9,17] show that CI also binds to DNA nonspecifically, and that this mode of binding may facilitate

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loop formation by shortening the effective loop length and may also provide further loop stabilization by enhancing the long-range interaction. CI nonspecific binding has been inferred to have a substantial free energy of 4.1 kcal/mol, [21] which suggests that most, if not all, of the CI protein available in the cell is DNA bound and supports the hypothesis of the biological relevance of nonspecific binding. Our previous work confirmed a significant amount of nonspecific binding of CI [9,16,17,22]. More recently, Xiao *et al.* [23] also used Maxwell relations to quantify the *change* in nonspecific binding of HU and Fis proteins from DNA extension versus force data.

A drawback of using Maxwell relations alone, in our previous work [16], was that we could solve only for the *change* in the number of proteins bound to DNA as a function of force. Furthermore, the implementation of Maxwell relations involves numerical differentiation of the DNA extension with respect to protein concentration, which is a challenging computational problem [24]. In [16], this problem was circumvented because pulling measurements were performed only at one protein concentration to estimate the numerical derivative.

An alternative approach that employs a discrete persistent-chain model has recently been proposed to compute the protein occupancy from DNA extension versus force data [25]. This approach, however, assumes that both the naked DNA and the DNA fully saturated with proteins follow the WLC model. It further makes assumptions about the length of the binding site for the discretization of the persistent chain and about how proteins deform the binding site.

Here we present a phenomenological approach that does not make such assumptions, and is easy to implement. Our approach is based on the theory of Zhang *et al.* [15], predicts the absolute value of protein occupancy (not the change) from DNA extension versus force data, and is applicable to any protein that binds to DNA nonspecifically.

For completeness, in Sec. II, we briefly summarize the formulation of the partition function from [15]. Section III describes a scheme for the computation of protein occupancy from DNA extension versus force data. Section IV presents our measurements of DNA extension as a function of force at several CI concentrations using magnetic tweezers and the results of our computational analysis. Finally, Sec. V presents a discussion of the method and its applicability.

II. FORM OF THE PARTITION FUNCTION

The microscopic energy function of a DNA molecule of finite length in the absence of a twisting moment is defined as

$$E = -fX - \sum_i \frac{n_i}{\beta} \ln \frac{c}{K_D} + E_{\text{int}}, \quad (1)$$

where $\beta = 1/k_B T$, k_B is the Boltzmann constant, T is the absolute temperature, and f is the external stretching force coupled to the tether extension X . Protein binding is accounted for by the finite sum over the binding positions i , each with a (nonspecific) dissociation constant K_D coupled with the binding state n_i , which is 1 when the position i is occupied and is 0 otherwise. The concentration of the protein is denoted by c , and the last term E_{int} is the internal energy due to conformational fluctuations of the DNA molecule and of the

bound proteins. Notice that, here the entropic term in the free energy discussed by Lam [26] is included in E_{int} and does not enter the calculations of protein occupancy.

The partition function is $Z(f, \hat{c}) = \sum e^{-\beta E}$, where $\hat{c} = c/K_D$ is the dimensionless concentration. Assuming a two-state model with and without bound proteins, the partition function becomes [15]

$$Z = [e^{\beta \hat{g}d} + \hat{c}e^{\beta \hat{h}d'}]^{L/d}, \quad (2)$$

where $\hat{g}(f)$ is the free energy per unit length without bound protein, $\hat{h}(f)$ is the free energy per unit length with a bound protein, d is the length of the binding site and d' is the length of the binding site as changed by the binding of the protein, and L is the tether contour length. Equation (2) will be used in Sec. III to derive an expression for the expected DNA extension as a function of protein concentration and force. Note that nonspecific binding can change the free energy not only by changing the length of the binding site, but also by changing the stiffness of DNA. For example, a protein sheath around the DNA at high HU concentrations has been observed to stiffen the apparent characteristics of the fiber [27–30].

III. RELATIONSHIP BETWEEN PROTEIN OCCUPANCY AND DNA EXTENSION

From Eq. (1) and the definition of partition function, the number of proteins bound nonspecifically to DNA can be calculated as

$$N = \sum_i n_i = \frac{\partial \{\ln Z\}}{\partial \{\ln \hat{c}\}}. \quad (3)$$

The number of bound proteins is normalized to the maximum number that can bind to a DNA molecule, $N_{\text{max}} = \frac{L}{d}$. The normalized quantity $\gamma(f) = \frac{N(f)}{N_{\text{max}}}$ is the protein occupancy, which represents the fraction of tethered DNA occupied by proteins.

Using the expression of the partition function Z from Eq. (2) in Eq. (3), the expression for protein occupancy takes a form similar to that of Langmuir adsorption,

$$\gamma = \frac{N}{L/d} = \frac{\hat{c}}{\hat{c} + \eta}, \quad (4)$$

where the function

$$\eta(f) = e^{\beta(\hat{g}d - \hat{h}d')} \quad (5)$$

is a characteristic of the protein and does not depend on its concentration. Knowledge of $\eta(f)$ permits the calculation of the protein occupancy at any force and concentration, and can be derived from DNA extension versus force measurements as follows.

The tether extension $X_c(f)$ at a given protein concentration \hat{c} is a measurable quantity. From Eq. (1) and the definition of partition function,

$$X_c = \frac{1}{\beta} \frac{\partial \{\ln Z\}}{\partial f}. \quad (6)$$

Substituting the partition function Z from Eq. (2) in Eq. (6) above, the normalized extension becomes

$$\frac{X_c}{L} = \frac{\partial \hat{g}}{\partial f} + \frac{d' \frac{\partial \hat{h}}{\partial f} - \frac{\partial \hat{g}}{\partial f}}{\left(1 + \frac{\eta}{\hat{c}}\right)}. \quad (7)$$

As \hat{c} tends to 0,

$$\frac{X_0}{L} = \frac{\partial \hat{g}}{\partial f}, \quad (8)$$

while as \hat{c} tends to ∞ ,

$$\frac{X_\infty}{L'} = \frac{\partial \hat{h}}{\partial f} \quad \text{and} \quad L' = L \frac{d'}{d}. \quad (9)$$

The latter corresponds to the situation where the concentration of protein is so high that DNA remains saturated with protein at all force values. In this scenario, L' will be the maximum possible entropic extension of the DNA.

Substituting Eqs. (8) and (9) into Eq. (7), the expression for the tether extension becomes

$$X_c = X_0 + \frac{X_\infty - X_0}{\left(1 + \frac{\eta}{\hat{c}}\right)}. \quad (10)$$

Then, a form of $\eta(f)$ is sought for which Eq. (10) fits the measured extension versus force data. To choose the appropriate function $\eta(f)$, it is convenient to express it in terms of the extensions X_0 and X_∞ . To do so, Eqs. (8) and (9) can be integrated to yield the free energy densities,

$$\hat{g}(f) = \int_0^f \frac{X_0}{L} df' \quad (11)$$

and

$$\hat{h}(f) = \int_0^f \frac{X_\infty}{L'} df'. \quad (12)$$

Equations (11) and (12) can be substituted in (5) to obtain

$$\eta(f) = e^{\frac{\beta}{L'a} \int_0^f (X_0 - X_\infty) df'}. \quad (13)$$

Note that as the force f increases, $[X_0(f) - X_\infty(f)]$ tends to $(L - L')$, which is a constant. Therefore, according to Eq. (13) and assuming that each protein that binds to DNA reduces the extension by a fixed amount, the function $\eta(f)$ must behave as an exponential function, as explained in Appendix. So, the following function can be fitted to the DNA extension versus force data at a given concentration \hat{c} :

$$X_c = X_0 + \frac{X_\infty - X_0}{\left(1 + \frac{\eta}{\hat{c}}\right)} = X_0 + \frac{k_2}{\left(1 + \frac{K_D e^{k_1 f}}{c}\right)}, \quad (14)$$

where k_1 , k_2 , and K_D are the fitting parameters, and c is the bulk protein concentration (in physical units). Once the parameters k_1 and K_D are determined, the protein occupancy can be calculated from (4) by setting $\frac{\eta(f)}{\hat{c}} = \frac{K_D e^{k_1 f}}{c}$.

IV. CI PROTEIN OCCUPANCY FROM EXPERIMENTAL MEASUREMENTS

A. Materials and methods

λ -DNA tethers were produced using plasmid pKLJ12wt provided by the Yang group [31]. This 15981-bp-long plasmid was digested with SacII and PspOMI restriction enzymes, and only the 6838-bp-long DNA fragment without specific binding sites for the CI protein was used in the experiments. This fragment was then ligated at each end to biotin- and digoxigenin-labeled fragments, respectively. These ‘‘tails’’

were approximately 1 kbp long, and were produced by PCR from pBluKSP and cut with SacII and PspOMI for the ligation [32]. Single molecules of this DNA were attached at one end to the antidigoxigenin-coated glass surface of a flow chamber and at the other end to a 2.8- μm streptavidin-coated, super-paramagnetic bead (Invitrogen, Carlsbad, CA, USA). Note that since the tails may not be attached to the glass or to the bead along their full length, the effective tether may include part of the tails.

Magnetic tweezers (MT) consisted of a pair of magnets (Invitrogen, Carlsbad, CA, USA) placed on a mount above the microscope stage that can be both translated along or rotated around the optical axis of the microscope. The magnetic field attracts a DNA-tethered superparamagnetic bead and stretches the DNA tether. Therefore, the distance of the magnets above the stage of the microscope from the sample can control the tension on the DNA tethers. Individual λ DNA molecules were stretched in the presence of several different concentrations of CI in λ buffer (10 mM Tris-HCl pH 7.4, 200 mM KCl, 5% dimethyl sulfoxide (DMSO), 0.1 mM ethylenediamine tetra-acetic acid (EDTA), 0.2 mM dithiothreitol (DTT), and 0.1 mg/ml α -casein). At each concentration, after their tether contour length was determined at high forces (10 pN), molecules were relaxed and stretched three times and their extension was recorded as a function of force during each relaxation and stretching cycle. At each magnet position, the data were acquired at a rate of 20 frames/s for 10 s before moving to the next magnet position. The extension of the DNA tether was determined with 1-s averaging using three-dimensional, video-rate tracking of the bead [33]. The tension in the molecule was determined using the horizontal motion of the bead $\langle \Delta x^2 \rangle$ via the equipartition theorem $f = \frac{k_B T X}{\langle \Delta x^2 \rangle}$ with 10% accuracy. The WLC fit suggested a systematic error of 0.5 pN in the force measurement from our apparatus. We have accounted for this offset in the force data presented in the next section. Mechanical drift in the data was eliminated using differential tracking of a second bead stuck on the surface. Data were analyzed as described in [34].

B. Data analysis

Figure 1 shows a set of DNA extension versus force data averaged over three stretching and relaxation cycles on a single ~ 7 -kbp-long λ -DNA tether at several different CI concentrations. The extension is normalized to the tether contour length. The points represent the experimental data. The upper solid black curve represents the WLC fit of the control data (stars) in the absence of CI using a persistence length of 50 nm. The lower solid black curve (red online) is obtained by fitting the data at 950 nM CI (diamonds) with Eq. (14). The fit corresponds to $k_1 = 1.2206 \text{ pN}^{-1}$, $K_D = 98 \text{ nM}$, and $k_2 = -0.1084 L$, where L is the tether contour length approximated by the extension at 10 pN. The fitted values of the three parameters, k_1 , k_2 , and K_D were validated with the data at two more concentrations, 190 and 380 nM CI. In particular, the upper dashed black and gray curves (blue and green online) shown in Fig. 1 represent the DNA extension versus force curves at 190 and 380 nM CI, respectively, predicted from Eq. (14) using the three fitted parameters. Finally, the lower

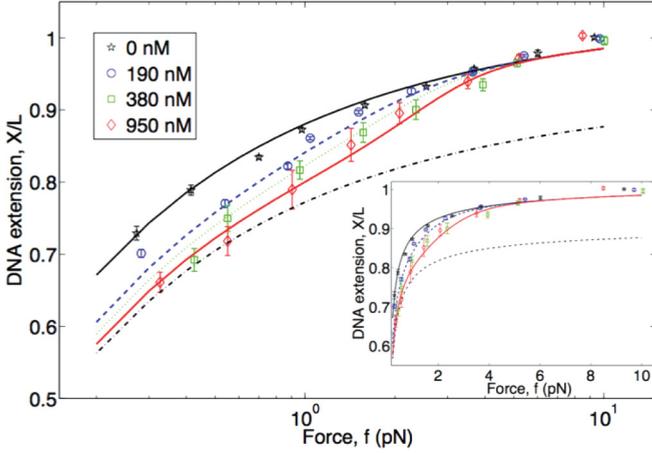


FIG. 1. (Color online) DNA extension versus force at several CI concentrations. The extension is normalized to the contour length L , which is approximated by the extension at 10 pN. The experimental data are averages over three stretching and relaxation cycles on the same tether and the error bars indicate the standard error. The solid black curves are fits to the data at 0 nM CI [upper, WLC fit with 50-nm persistence length] and 950 nM CI [lower; red online, Eq. (14) fit]. The upper dashed black and gray curves are the predicted trends at 190 nM CI (blue online), 380 nM CI (green online), and at \hat{c} tending to ∞ (lower dashed black), based on the fits. The inset shows the same figure using a linear force scale. Note that the data were fitted using a least-squares error minimization method. Thus the fitting curves do not necessarily intersect at $X/L = 1$ for $F = 10$ pN or any other common point.

dashed black curve shows the lower bound $X_\infty(f)$ of the DNA extension versus force curves, which is predicted from the fit as

$$X_\infty = X_0 + (X_c - X_0) \left(1 + \frac{\eta}{\hat{c}} \right) = X_0 + k_2. \quad (15)$$

Note that the data were fitted using a least-squares error minimization method; thus the fitting curves do not necessarily intersect at $X/L = 1$ for $F = 10$ pN or any other common point.

The data clearly shows that as CI concentration increases, the tether contracts monotonically. However, at any concentration and in the absence of protein, as force increases, the tether approaches its contour length L . Note that the fact that for all values of c , $X_c(f)$ converges to $X_0(f)$ as f increases, also follows from Eqs. (10) and (13) and is fulfilled *a priori* by Eq. (14).

Finally, the protein occupancy γ was calculated based on the fitted $\eta(f)$ using Eq. (4) at all the three experimental concentrations of CI, as well as extrapolated at several other concentrations (see Fig. 2). Note that at all concentrations, the protein occupancy diminishes to 0 as the force increases. This behavior is directly related to the fact that $X_c(f)$ converges to $X_0(f)$ as f increases and is in agreement with the previously published observation that tension drives proteins off DNA [35].

Figure 2 shows the dependence of protein occupancy on applied force as predicted by the theoretical framework described above. Below 10 nM CI, protein occupancy is hardly detectable, while above 1 M CI, the tether is fully saturated with protein almost up to 10 pN.

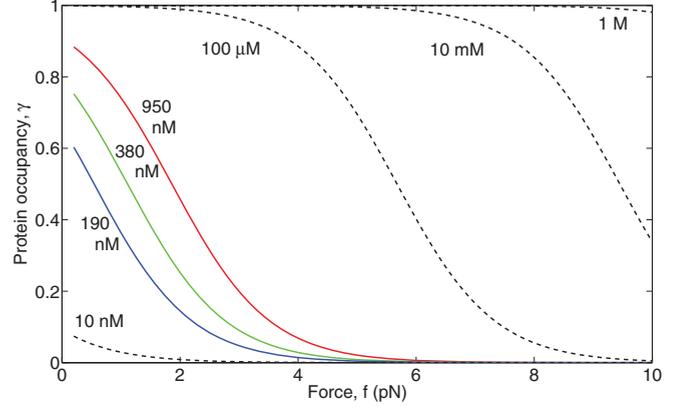


FIG. 2. (Color online) Calculated protein occupancy, $\gamma = N/N_{\max}$, versus force at 190 nM (blue online), 380 nM (green online), and 950 nM (red online) CI, as well as its extrapolation at several other concentrations (dashed curves).

Figure 3 shows the expected DNA extension versus force at several concentrations using Eq. (14). Note that the behavior of the DNA extension versus force curves are bound by the $X_0(f)$ and $X_\infty(f)$ such that at 10 nM CI, the curve is very close to that for $X_0(f)$, while at 1 M CI, the curve is very close to that for $X_\infty(f)$ up to 10 pN.

V. DISCUSSION AND CONCLUSIONS

We presented a simple and robust way to compute protein occupancy from measurements of DNA extension as a function of force. The method uses the partition function proposed in [15]. Unlike the computational scheme based on Maxwell relations alone [16,23], our approach neither requires numerical differentiation nor numerical integration, and yields the amount of nonspecific binding (protein occupancy), not its change as a function of force. Furthermore, unlike [25], this method does not require discretized modeling and does not require the protein-saturated DNA curve to obey the WLC model.

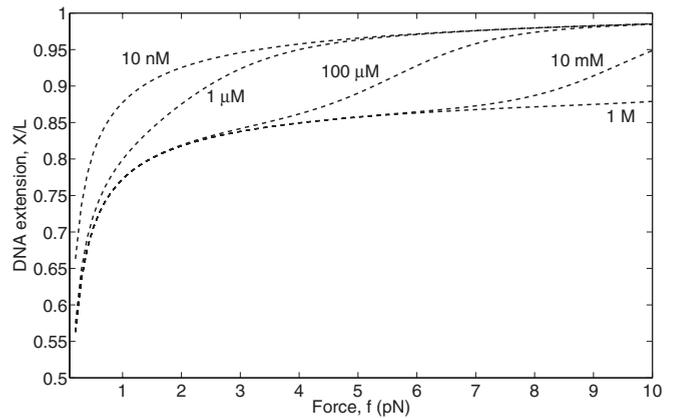


FIG. 3. Expected dependence of DNA extension on force from 10 nM to 1 M CI based on the fitted $\eta(f)$. At 10 nM CI, the curve is close to the one obtained in the absence of CI, while at 1 M CI, the curve is close to that of $X_\infty(f)$. The curves are shown on a linear scale to highlight the points of inflection, where the protein occupancy changes the most with force.

While in general protein binding may change DNA extension by variable amounts, our method assumes that each bound protein changes the extension of DNA by a fixed quantity, which does not vary significantly with the force as shown in Appendix. Therefore, the approach presented here will describe well those cases in which each protein binds to DNA in the same way, and causes small, yet detectible changes in its extension.

The method was tested on the DNA extension versus force data that were collected at several different CI concentrations using magnetic tweezers. We note that this new computational approach predicts DNA extension versus force curves that agree well with the measured data within the experimental error. The fitted parameter $k_2 = -0.1084 L$ suggests that each CI binding should decrease the extension of the binding site d' by about 10%, yielding an estimation of CI-induced DNA compaction. The fitted parameter $K_D = 98$ nM provides a firm number to a previous study, where by using gel-shift assays and fluorescence microscopy on short DNA fragments, it was estimated that CI had a nonspecific dissociation constant in the neighborhood of hundreds of nanomolar [36]. This value implies strong nonspecific binding, supporting the idea that CI nonspecific binding may have a physiological, regulatory role.

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APPENDIX

Assume that a protein bound to DNA changes its extension by a fixed amount Δx and the energy of the system by μ . Therefore, with an applied force f the change in total free energy due to each protein binding is

$$\Delta E = \mu + f \Delta x. \quad (\text{A1})$$

The equilibrium constant of the system is

$$K_{eq} = \frac{N}{(N_{\max} - N)c}, \quad (\text{A2})$$

where $(N_{\max} - N)$ is the number of unoccupied binding sites. Setting $K_{eq} = e^{-\beta \Delta E} = e^{-\beta(\mu + f \Delta x)}$ results in

$$\frac{N}{(N_{\max} - N)c} = e^{-\beta(\mu + f \Delta x)}. \quad (\text{A3})$$

Solving for N gives

$$N = \frac{N_{\max}}{1 + \frac{e^{\beta(\mu + f \Delta x)}}{c}}. \quad (\text{A4})$$

The extension of the DNA is then

$$X_c = X_0 + N \Delta x = X_0 + \frac{N_{\max} \Delta x}{1 + (e^{\beta(\mu + f \Delta x)}/c)}, \quad (\text{A5})$$

which is equivalent to Eq. (14) with $k_1 = \beta \Delta x$, $K_D = e^{\beta \mu}$, and $k_2 = N_{\max} \Delta x = L - L'$. We fitted Eq. (14) through the measured data assuming k_1 to be constant. This is a valid approximation when Δx , which is of the order of $(d' - d)$, does not vary significantly with f .

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