Gaussian fluctuations in tethered DNA chains

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In a recent work [Gao *et al.*, Appl. Phys. Lett. **134**, 113902 (2007)], we reported a novel DNA separation method by tethering DNA chains to a solid surface and then stretching the DNA chains with an electric field. The anchor is such designed that the critical force to detach a DNA chain is independent of its length. Because the stretching force is proportional to the DNA net charge, a gradual increase of the electric field leads to size-based removal of the DNA strands from the surface and thus DNA separation. Originally proposed for separation of long double-stranded DNA chains (>10 000 bps), this method has been proven useful also for short single-stranded DNA fragments (<100 bases) for which the fluctuation force induced by the solvent becomes significant. Here we show that the fluctuation force can be approximately represented by a Gaussian model for tethered DNA chains. Analytical expressions have been derived to account for the dependence of the fluctuation force on the surface confinement, the polymer chain length, and the DNA tethering potential. The theoretical predictions are found to coincide with experiment. © 2011 American Institute of Physics. [doi:10.1063/1.3548887]

I. INTRODUCTION

DNA separation by size is a cornerstone of biotechnology for DNA sequencing, manipulation, bioinformatics analysis, and cloning. Motivated by the broad applications of DNA separation, we have been developing a novel DNA separation method based on tethering DNA strands to a solid surface through precise end-hybridization and sequentially pulling DNA strands off the surface by an electric field E .¹ The anchor is such designed that the critical force to detach a DNA chain is independent of the chain length. Because the electrical force is proportional to the DNA net charge

$$\vec{f}_e = Nq\vec{E}, \quad (1)$$

where q is the net charge per nucleotide and N is the total number of nucleotides, a gradual increase of the electric field leads to perfectly linear size-based selection of the DNA strands from the surface. With appropriate end labeling, DNA chains removed from the surface can be detected from their fluorescence signals, which can be used to determine the sequence. This new DNA fractionation method is much more efficient than the electrophoresis-based technologies for separation of DNA strands in different lengths produced from the Sanger reaction.

In a previous work,¹ we demonstrated the effectiveness of this method to separate long DNA fragments. However, separation of short single-stranded (ss) DNA fragments, which is required for DNA sequencing, by this method has been challenging. In that case, the surface force induced by the substrate² (e.g., glass slide or metal surface) and the solvent-induced fluctuation force on DNA fragments would become

significant in comparison with the incremental electric force and the fluctuation severely compromises the separation efficiency. Furthermore, uploading short ssDNA fragments onto a surface requires an extremely large electric field, resulting in various problems such as Joule heating and side electrochemical reactions in the buffer solution. Despite those challenges, we show that, by a careful design of experiment, the stretching method is also applicable to separation of short ssDNA.³ Specifically, we employ a microfluidic platform with narrow channels filled with low conductivity buffer. This device provides very high electric field strength while reducing the Joule heating. When a dc electric field (E) is applied along the length of the microchannel, the DNA chains tethered to the surface through a short probe are experienced with a static electrical force. The total stretching force $\vec{f}_s$ on the tethered chain consists of the static electrical force and a fluctuation force $\vec{f}$

$$\vec{f}_s = Nq\vec{E} + \vec{f}. \quad (2)$$

When the stretching force f_s (in the following, force variable without arrow superscript refers to its magnitude) is equal to the tethering force f_t , the DNA chain detaches from the surface. This threshold stretching force to detach the DNA chain is called the critical force. Because the tethering force is independent of the DNA length, at a given electric field longer DNA chains are loaded with greater stretching forces. As a result, DNA fragments can be sequentially detached from the surface as the strength of the electric field increases gradually. The DNA fragments detached from the surface can be detected by the fluorescence labeling.

Experimental detection of DNA separation is monitored by normalized fluorescence intensity $\alpha(E)$ of given DNA fragments at the tethering surface. This quantity reflects the

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FIG. 1. Differential fluorescence intensity $-\frac{d\alpha(E)}{dE}$ vs electric field E for three short ssDNAs. From left to right the lines are corresponding to ssDNA chains with 90, 80, and 60 nucleotides.

probability of a single DNA chain to remain tethered on the surface under electric field E . Its negative derivative to the electric field, i.e., $-\frac{d\alpha}{dE}$, indicates the efficiency of detaching DNA chains away from the surface. Figure 1 shows $-\frac{d\alpha}{dE}$ versus E for three ssDNA chains observed in experiment. Here, the numbers of nucleotides (nt) are 90-nt, 80-nt, and 60-nt. From Fig. 1, several perspectives can be concluded: (a) experimental results show that detachment of ssDNA happens within a certain range of electric field instead of at a single critical point, which confirms that fluctuation force f becomes notable during the separation of short DNAs and thus interferes with the separation efficiency; (b) except at large electric field, the dependence of $-\frac{d\alpha}{dE}$ versus E presents Gaussian analog distributions; and (c) the expected values of such distributions vary with the DNA chain length. All those perspectives require the understanding of the fluctuation forces on tethered chains.

In this work, we examine the statistical behavior of tethered DNA chains by using a Gaussian chain approximation and study the dependence of entropic and fluctuation forces on the DNA chain length and tethering conditions. We show that the results observed in our ssDNA separation experiments can be reasonably explained with the simple theoretical model.

II. STATISTICS OF A TETHERED GAUSSIAN CHAIN

We use a coarse-grained model for ssDNA chains tethered at an electrode surface. In this model, we ignore the molecular details such as the excluded volume effects and electrostatic interactions. The system is represented by a Gaussian chain tethered at a planar surface as shown schematically in Fig. 2. We consider two scenarios by which the DNA chains are tethered near a surface. In the first scenario, one end of the chain is located at a fixed position near the surface; we name this as a grafted chain. In the second scenario, one end of the chain is tied to the surface through a harmonic spring that mimics the DNA probe; we mark this as a semigrafted chain. In both cases, a flat hard wall is placed at

FIG. 2. Schematic representation of (a) grafted Gaussian chain and (b) semigrafted Gaussian chain. In both cases, the Gaussian chain is constrained in $z > 0$ half space with one end fixed at $(0, 0, z_0)$ (for grafted chain) or anchored through a harmonic spring (for semigrafted chain). The planar wall is located at $z = 0$.

$z = 0$ so that the chain is constrained within the half space $z > 0$. While the grafted chain in the first scenario allows us to study the surface force due to the hard wall, by using the second scenario we may derive an analytical expression to link the fluctuation force with the distribution of the tethering point of the semigrafted chain.

Obviously, the Gaussian-chain model is insufficient to represent microscopic details of a single-strained DNA tethered at an electrode surface. Nevertheless, it provides useful information to understand the surface force on tethered DNA chains and the thermal fluctuation force induced by chain fluctuations. Besides, the Gaussian-chain model allows us to derive analytical expressions that may be useful for future experimental design. Because of its simplicity, the Gaussian chain model has been extensively used to address the structure and thermodynamic properties of confined polymers.⁴⁻¹³

A. Grafted Gaussian chain

The configurational partition function for a tethered Gaussian chain is given by

$$Q = \int D\mathbf{r}(s) \exp\{-\beta U_0[\mathbf{r}(s)] - \beta \Psi[\mathbf{r}(0)]\}, \quad (3)$$

where $\int D\mathbf{r}(s)$ denotes a functional integral over all polymer configurations, $\mathbf{r}(s)$ represents space curves describing the chain configuration, $\beta = 1/(k_B T)$ is defined by the Boltzmann constant k_B and absolute temperature T , $U_0[\mathbf{r}(s)]$

$= 3k_B T/2a^2 \int ds |d\mathbf{r}(s)/ds|$ is the configurational potential energy, and $\Psi[\mathbf{r}(0)]$ stands for a tethering potential applying to the polymer end. In Eq. (3), Kuhn length a is equivalent to the effective bond length of a randomly connected chain or the step length in the random flight model¹⁴

$$\langle \mathbf{r}^2 \rangle = Na, \quad (4)$$

where N is the number of steps. To avoid numerical subtlety in transferring continuous description of the Gaussian chain to discrete one, we use a reduced partition function

$$Z \equiv \frac{Q}{Q_0}, \quad (5)$$

where Q_0 is the partition function of the same polymer far from the surface. The reduced (or normalized) partition function can be expressed in terms of a propagator function (or Green function) $G(\mathbf{r}, \mathbf{r}', N)$,

$$Z = \frac{1}{V} \int d\mathbf{r} d\mathbf{r}' G(\mathbf{r}, \mathbf{r}', N), \quad (6)$$

where V stands for the system volume. $G(\mathbf{r}, \mathbf{r}', N)$ represents the statistical weight of a Gaussian chain of N Kuhn segments with the ends fixed at positions $\mathbf{r}$ and $\mathbf{r}'$. If the segment number N is treated as a continuous variable, $G(\mathbf{r}, \mathbf{r}', N)$ satisfies the diffusion equation¹⁴

$$\frac{\partial}{\partial N} G(\mathbf{r}, \mathbf{r}', N) = \frac{a^2}{6} \Delta G_N(\mathbf{r}, \mathbf{r}'), \quad (7)$$

where Δ represents the Laplacian operator. With a suitable boundary condition, Eq. (7) can be solved analytically.

For a grafted Gaussian chain with one end located at $\mathbf{r}_0 = (0, 0, z_0)$, the polymer configuration is restricted by an impermeable and nonabsorptive wall. In that case, the boundary condition for the diffusion equation [Eq. (7)] is

$$\begin{cases} G(\mathbf{r}, \mathbf{r}'; N) \equiv 0 & \text{if } z \leq 0 \\ \lim_{N \rightarrow 0} G(\mathbf{r}, \mathbf{r}'; N) = \delta(\mathbf{r} - \mathbf{r}') & \end{cases} \quad (8)$$

An analytical solution has been derived for Eq. (7) with the image principle originally introduced by Smoluchowski¹⁵. The allowed configuration number $G(\mathbf{r}, \mathbf{r}', N)$ of Gaussian chain with N segments is just the total configuration number $G_{\text{tot}}(\mathbf{r}, \mathbf{r}', N)$ in bulk (that without constraint) with same fixed ends minus the forbidden configuration number $G_{\text{forbidden}}(\mathbf{r}, \mathbf{r}', N)$. The forbidden configurations are those crossing the wall at least twice, and have one-to-one correspondence to the “image” configurations $G(\mathbf{r}, -\mathbf{r}'; N)$. With the image principle, we can obtain an analytical solution for Green function,^{16,17}

$$\begin{aligned} G(\mathbf{r}, \mathbf{r}'; N) = & \left(\frac{3}{2\pi Na^2} \right)^{3/2} \exp \left(-\frac{3(x-x')^2}{2Na^2} \right) \\ & \times \exp \left(-\frac{3(y-y')^2}{2Na^2} \right) \left[\exp \left(-\frac{3(z-z')^2}{2Na^2} \right) \right. \\ & \left. - \exp \left(-\frac{3(z+z')^2}{2Na^2} \right) \right]. \end{aligned} \quad (9)$$

By integrating the Green function from one end to the other end of the chain, we obtain the reduced partition

FIG. 3. Confinement free energy per segment for a grafted Gaussian chain in terms of chain length N . The solid line, the dashed-dotted line, and the dotted line correspond to $z_0/a = 1, 2$, and 3 , respectively.

function

$$\begin{aligned} Z &= \int d\mathbf{r} d\mathbf{r}' G(\mathbf{r}, \mathbf{r}'; N) \delta(\mathbf{r} - \mathbf{r}_0) \\ &= \int d\mathbf{r}' G(\mathbf{r}_0, \mathbf{r}'; N) \\ &= \int_{-\infty}^{+\infty} dx' \int_{-\infty}^{+\infty} dy' \int_0^{+\infty} dz' G(\mathbf{r}_0, \mathbf{r}'; N). \end{aligned} \quad (10)$$

Here the integral interval $z' > 0$ accounts for the hard-wall effect. Substituting Eq. (9) into Eq. (10) yields^{12,16}

$$\begin{aligned} Z &= \left(\frac{3}{2\pi Na^2} \right)^{1/2} \int_0^{\infty} dz \left[\exp \left(-\frac{3(z-z_0)^2}{2Na^2} \right) \right. \\ &\quad \left. - \exp \left(-\frac{3(z+z_0)^2}{2Na^2} \right) \right] \\ &= \left(\frac{3}{2\pi Na^2} \right)^{1/2} \left(\int_{-z_0}^{\infty} dz \exp \left(-\frac{3z^2}{2Na^2} \right) \right. \\ &\quad \left. - \int_{z_0}^{+\infty} dz \exp \left(-\frac{3z^2}{2Na^2} \right) \right) \\ &= \text{erf} \left(z_0 \sqrt{\frac{3}{2Na^2}} \right) = \text{erf} \left(\frac{z_0}{2R_g} \right), \end{aligned} \quad (11)$$

where $\text{erf}(x)$ stands for the error function and $R_g = \sqrt{Na^2/6}$ is the gyration radius of the Gaussian chain. From the partition function, we derive the tethering free energy, i.e., the free energy difference between the grafted chain and a free chain is given by

$$\begin{aligned} \Delta F &= -k_B T \ln Z \\ &= -k_B T \ln [\text{erf}(z_0/2R_g)]. \end{aligned} \quad (12)$$

Figure 3 shows the tethering free energy per segment, i.e., $\Delta F/N$, versus the chain length N and tethering distance z_0 . Here the positive value of tethering free energy is due to the entropic repulsion caused by the presence of wall. At small chain length N , the separation of the polymer from the surface is larger than the gyration so that the surface effect

FIG. 4. Surface force for a grafted Gaussian chain in terms of chain length N . The solid line, the dashed-dotted line, and the dotted line correspond to $z_0/a = 1, 2$ and 3 , respectively.

becomes negligible. Therefore, the tethering free energy per segment has a small value. As the chain length increases, the polymer gyration radius grows and the chain configuration becomes more significantly limited by the surface, leading to an increase of the tethering free energy. For a very long chain, the free energy per segment becomes equivalent to the end segment chemical potential, which is not influenced by the boundary effect. As a result, the tethering free energy approaches to zero as $N \rightarrow \infty$. When the tethering point shifts away from the wall (or equivalently z_0 increases), the boundary effect becomes less significant thus the tethering free energy per segment decreases.

The mean surface force on the polymer is calculated by taking a derivative of the free energy difference with respect to z_0

$$f_w(z_0) = -\frac{d\Delta F}{dz_0}. \quad (13)$$

Here the direction of force $f_w(z_0)$ is along the z axis, i.e., in the direction perpendicular to the surface. Substituting Eq. (12) into Eq. (13) yields

$$\begin{aligned} f_w(z_0) &= k_B T \frac{1}{\text{erf}(z_0/2R_g)} \frac{d}{dz_0} \text{erf}(z_0/2R_g) \\ &= \frac{k_B T}{\sqrt{\pi}} [R_g \text{erf}(z_0/2R_g)]^{-1} \exp\left[-(z_0^2)/(2R_g^2)\right]. \end{aligned} \quad (14)$$

Equation (14) provides an analytical expression for the surface force averaged over all possible configurations of Gaussian chain with one end located at $(0, 0, z_0)$; it indicates that surface force depends only on tethering position z_0 and gyration radius of the chain R_g .

Figure 4 plots the surface force in terms of chain length N , in which z_0/a is fixed at three positions. As discussed before, the surface effect is insignificant when the gyration radius of the tethered chain is smaller than z_0 . While the surface force increases linearly with N for short chains, it reaches a plateau as the chain length further increases. As expected, the mean force is magnified when the tethering position is closer

to the wall. The limiting value for the surface force can be estimated with the aid of a very accurate approximation for the error function

$$\text{erf}(x) \approx \frac{x}{|x|} \sqrt{1 - \exp\left(-x^2 \frac{4/\pi + 0.14x^2}{1 + 0.14x^2}\right)}. \quad (15)$$

Substituting Eq. (15) into Eq. (14) gives

$$\begin{aligned} f_w(z_0; N \rightarrow \infty) &= k_B T \frac{1}{\sqrt{1 - \exp\left(-z_0^2 \frac{3}{2Na^2} \frac{4}{\pi}\right)}} \\ &\times \sqrt{\frac{6}{\pi Na^2}} \exp\left[-\frac{3z_0^2}{2Na^2}\right] \\ &= k_B T \frac{1}{\sqrt{\frac{6z_0^2}{\pi Na^2}}} \sqrt{\frac{6}{\pi Na^2}} \exp\left[-\frac{3z_0^2}{2Na^2}\right] = k_B T/z_0. \end{aligned} \quad (16)$$

Equation (16) affirms that, when the tethered chain is very long, the surface force becomes irrelevant to the chain length.

B. Semigrafted Gaussian chain

We now consider the second scenario when one end of the Gaussian chain is anchored to a hard wall through a spring with nature length l_0 and modulus k . Comparing to the grafted chain discussed above, the tethered end is not fixed at one position but subjected to the spring potential $k(r - l_0)^2/2$, where r stands for the spring length, i.e., the distance of the polymer tethered end to the spring anchor point $(0, 0, 0)$. Because the external potential applies only to one end of the Gaussian chain rather than to all polymer segments, both the diffusion equation and the same boundary condition [i.e., Eqs. (7) and (8)] remain valid for the semigrafted Gaussian chain. Consequently, the Green function still takes the form of Eq. (9), and the partition function reads

$$Z = \int d\mathbf{r} \int d\mathbf{r}' G(\mathbf{r}; \mathbf{r}'; N) \exp[-\beta k(r - l_0)^2/2]. \quad (17)$$

Substitute Eq. (9) into the above equation and carry out the integrations

$$\begin{aligned} Z &= \int d\mathbf{r} \int d\mathbf{r}' \exp[-\beta k(r - l_0)^2/2] \left(\frac{3}{2\pi Na^2}\right)^{3/2} \\ &\times \exp\left(-\frac{3(x - x')^2}{2Na^2}\right) \exp\left(-\frac{3(y - y')^2}{2Na^2}\right) \\ &\times \left[\exp\left(-\frac{3(z - z')^2}{2Na^2}\right) - \exp\left(-\frac{3(z + z')^2}{2Na^2}\right)\right] \\ &= \int d\mathbf{r} \exp[-\beta k(r - l_0)^2/2] \text{erf}(z\sqrt{3/2Na^2}) \\ &= \int_0^\infty r^2 dr \int_0^{2\pi} d\phi \exp[-\beta k(r - l_0)^2/2] \end{aligned}$$

$$\begin{aligned}
& \times \int_0^1 d \cos \theta \operatorname{erf} \left(r \cos \theta \sqrt{\frac{3}{2Na^2}} \right) \\
& = 2\pi \int_0^\infty r^2 dr \exp[-\beta k(r-l_0)^2/2] \left[\operatorname{erf} \left(\frac{r}{2R_g} \right) \right. \\
& \quad \left. - \frac{2R_g}{r\sqrt{\pi}} + \frac{2R_g}{r\sqrt{\pi}} \exp \left(-\frac{r^2}{(2R_g)^2} \right) \right]. \quad (18)
\end{aligned}$$

In deriving Eq. (18), we have used the formula $\int \operatorname{erf}(x)dx = x \cdot \operatorname{erf}(x) + \exp[-x^2]/\sqrt{\pi}$. As before [Eq. (12)], the tethering free energy is related to the partition function by $\Delta F = -k_B T \ln Z$.

Figure 5(a) plots the tethering free energy per segment in terms of chain length N at three spring natural lengths ($l_0/a = 1, 2$, and 3) and a fixed spring constant $k\beta a^2 = 1$. Due to the harmonic attraction from the spring at the tethering point, the tethering free energy per segment is negative. In other words, the harmonic potential introduces an effective attraction for the polymer that may overcompensate the entropic effect introduced by the surface. For a short Gaussian chain, the surface repulsion is small and the harmonic attraction dominates the total external potential. In this case, the affinity of the harmonic spring requires a positive work to remove the polymer from the surface. Such surface attraction is absent for the grafted chain, in which the free energy of the Gaussian chain vanishes far away from the wall. As the chain length increases, the attraction due to the harmonic potential is cancelled by the repulsion due to the surface restriction of the chain configurations. Unlike the grafted chain, the free energy per segment for the case of harmonic tethering shows no maximum at an intermediate chain length. A similar conclusion can be obtained from Fig. 5(b), in which different spring constants $k\beta a^2$ are considered while the natural length is fixed at $l_0 = a$. A comparison of Figs. 5(a) and 5(b) indicates that the effect of the spring constant on the tethering free energy is similar to that of the natural length of the spring, i.e., an increase of the spring constant is nearly equivalent to decrease of the spring natural length.

Tethering a polymer to a harmonic spring allows us to examine the fluctuation force explicitly. From the partition function, we may calculate the average spring extension $\bar{r}_t$, i.e., the average distance of the polymer tethered end to the spring anchor point $(0, 0, 0)$

$$\begin{aligned}
\bar{r}_t &= \frac{2\pi}{Z} \int_0^\infty r^3 dr \exp \left[-\beta k \frac{(r-l_0)^2}{2} \right] \\
& \times \left[\operatorname{erf} \left(\frac{r}{2R_g} \right) - \frac{2R_g}{r\sqrt{\pi}} + \frac{2R_g}{r\sqrt{\pi}} \exp \left(-\frac{r^2}{(2R_g)^2} \right) \right]. \quad (19)
\end{aligned}$$

According to Hooke's law, the mean spring extension is related to the mean fluctuation force through

$$\bar{f} = k(\bar{r}_t - l_0). \quad (20)$$

Figures 6(a) and 6(b) present the average force in terms of chain length N when we fix the reduced spring constant $k\beta a^2$ or the reduced spring natural lengths l_0/a , respectively. Both figures indicate that for short chains, the mean fluctua-

FIG. 5. Confinement free energy per segment for a semigrafted Gaussian chain as a function of chain length N . (a) spring constant $k\beta a^2 = 1$ and natural length $l_0/a = 1$ (solid line), 2 (dashed-dotted line), and 3 (dotted line); (b) $l_0/a = 1$ and $k\beta a^2 = 1$ (solid line), 2 (dashed-dotted line), and 3 (dotted line).

tion force increases with the natural spring length but when the polymer chain is sufficiently long, the mean fluctuation force approaches a plateau. The limiting force is magnified as the natural length decreases or as the spring constant increases.

From the partition function for the polymer end distribution [Eq. (18)], we can readily derive the probability density for the distribution of the tethered polymer end

$$\begin{aligned}
\rho_t(r') &= \frac{2\pi}{Z} \int_0^\infty dr r^2 \delta(r' - r) \exp[-\beta k(r-l_0)^2/2] \\
& \times \left\{ \operatorname{erf}(r/2R_g) - \frac{2R_g}{r\sqrt{\pi}} [1 - \exp(-r^2/4R_g^2)] \right\} \\
& = \frac{2\pi}{Z} r'^2 \exp[-\beta k(r'-l_0)^2/2] \\
& \times \left\{ \operatorname{erf}(r'/2R_g) - \frac{2R_g}{r'\sqrt{\pi}} [1 - \exp(-r'^2/4R_g^2)] \right\}. \quad (21)
\end{aligned}$$

As indicated above, r' represents the separation from the tethered polymer end to the anchor site of the spring $(0, 0, 0)$. Because the fluctuation force is linearly proportional to r' , i.e.,

FIG. 6. The mean fluctuation force for a semigrafted Gaussian chain as a function of the chain length N . (a) spring constant $k\beta a^2 = 1$ and natural length $l_0/a = 1$ (solid line), 2 (dashed-dotted line), and 3 (dotted line); (b) $l_0/a = 1$ and $k\beta a^2 = 1$ (solid line), 2 (dashed-dotted line), and 3 (dotted line).

$f = k(r' - l_0)$, we have $r' = l_0 + f/k$ and $dr' = df/k$. As a result, the probability density of the polymer tethered end is linearly proportional to that for the fluctuation force, and Eq. (21) can be rewritten as

$$\rho(f) = \frac{2\pi}{Zk} (l_0 + f/k)^2 \exp\left(-\frac{\beta f^2}{2k}\right) \times \left[\operatorname{erf}\left(\frac{kl_0 + f}{2kR_g}\right) - \frac{2kR_g}{(kl_0 + f)\sqrt{\pi}} \times \left(1 - \exp\left[-\left(\frac{kl_0 + f}{2kR_g}\right)^2\right]\right) \right]. \quad (22)$$

In deriving Eq. (21), we have taken an average over all orientations. As a result, f in Eq. (22) is the averaged fluctuation force (over orientations) with effective direction toward z axis; f is different from $\vec{f}_f$ in Eq. (2) which depends on both the magnitude of extension and orientations. Given electric field E along z direction, the integrated fluctuation force, i.e., f , along the same direction is of our interest.

FIG. 7. The probability density of the fluctuation force for $N = 90$ predicted with three sets of spring parameters.

Figure 7 shows $\rho(f)$ for a polymer with $N = 90$ calculated from three different sets of spring parameters ($k\beta a^2$, l_0/a), corresponding to different conditions of tethering. As expected, an increase of the spring constant or a decrease of the spring nature length magnifies the mean fluctuation force. A reduction of either k or l_0 narrows the distribution of the fluctuation force.

C. Fluctuation force in a tethered DNA

As stated before, the normalized fluorescence intensity (α) measures the amount of DNA chains remaining at the surface under an electric field (E). This quantity is directly connected to the probability of a single DNA chain detached from the surface, or equivalently, the probability of the stretching force exceeding the critical force of DNA departure [Eq. (2)]. Given an electric field and a tethering condition, the critical fluctuation force to remove a DNA chain is given by

$$f_c = f_t - NqE. \quad (23)$$

If we let $\alpha = 0$ for the DNA departure from the surface and $\alpha = 1$ otherwise, the probability of a DNA chain remaining at the surface can be obtained by integrating the probability density for $f < f_c$, i.e.,

$$\alpha(E) = \int_{-\infty}^{f_c} \rho(f) df, \quad (24)$$

where $\rho(f)$ stands for the probability density for the distribution of fluctuation force. A derivative of Eq. (24) with respect to E leads to

$$-\frac{d\alpha(E)}{dE} = Nq\rho(f_t - NqE) \equiv Nq\rho(f_c). \quad (25)$$

Equation (25) is formally exact, independent of any specific DNA model; it indicates that for a given DNA chain length (N), $-d\alpha(E)/dE$ is proportional to the probability density of the critical fluctuation force $\rho(f_c)$. We further point out that at different external field E , $\rho(f)$ could be different, i.e., the probability of fluctuation force is correlated with the external field.

Whereas the incremental electric field is relatively small, we assume $\rho(f)$ is decoupled with electric field E . With $\rho(f)$ derived from the Gaussian chain approximation [Eq. (22)], we plot in Fig. 8(a) $Nq\rho(f_i - NqE)$ versus E for three DNA chain lengths ($N = 60, 80$, and 90), corresponding to the experimental results shown in Fig. 1. In the theoretical calculations, the spring parameters are $k\beta a^2 = 2$ and $l_0/a = a$, and the tethering force is $f_i = 9/(\beta a)$. Although a Kuhn segment in the Gaussian chain model is different from a DNA nucleotide, qualitative similarity between the theory and the experimental results is apparent. While such similarity confirms the applicability of the Gaussian chain model to the distribution of the DNA fluctuation force, there are two noticeable differences between theory and experiments. First, the Gaussian chain model does not capture the small shoulder or a second peak at large electric field shown in experiments. The “abnormal” behavior is probably related to the polydispersity of the DNA chains. Second, the experimental results show that the peak of the curve for 80-nt ssDNA is significantly higher than those of other two curves (Fig. 1). However, the Gaussian chain model predicts that the peaks decrease monotonically with the chain length. Because the theoretical prediction of the peak value is sensitive to the spring parameters, we suspect that the nonmonotonic behavior is due to a different tethering probe for 80-nt ssDNA. To support our hypothesis, we plot in Fig. 8(b) $\rho(f_i - NqE)$ versus E for three different sets of the spring parameters ($k\beta a^2, l_0/a$). Here the polymer chain length is fixed at $N = 80$ and the tethering force remains $f_i = 9/(\beta a)$. As shown in Fig. 8(b), the distribution of the fluctuation force becomes narrower and the peak value gets higher as the spring constant k and/or natural length l_0 is decreased. Most likely, the tethering probe for 80-nt ssDNA is less rigid and/or shorter than those of other DNAs.

For efficient separation of two DNA chains close to each other, the critical electric field, i.e., the expected values of $-\alpha(E)/dE$, should be different. According to Eq. (25), we have

$$\begin{aligned}\bar{E}(N) &= - \int E \frac{d\alpha(E)}{dE} dE \\ &= - \int [f_i - (f_i - NqE)] \rho(f_i - NqE) dE \\ &= \frac{1}{Nq} \int [f_i - f] \rho(f) df \\ &= \frac{f_i}{Nq} - \frac{\bar{f}(N)}{Nq}.\end{aligned}\quad (26)$$

Like Eq. (25), Eq. (26) is formally exact; it indicates that $N\bar{E}$ depends on tethering force f_i and the mean fluctuation force $\bar{f}(N)$.

According to the Gaussian chain model (Fig. 6), $\bar{f}$ grows linearly with the chain length for short chains and independent of the chain length for large chains. Correspondingly, Eq. (26) predicts that $N\bar{E}$ linearly decreases with the chain length for short chains and independent of the chain length when N is sufficiently large. To verify this prediction, we calculate $N\bar{E}$ based on the experimental data for

FIG. 8. Distribution of $Nq\rho(f_i - NqE)$ in terms of electric field E : (a) Here the spring parameters are $k\beta a^2 = 2$, $l_0/a = 1$, and $f_i = 9/(\beta a)$; the chain lengths are $N = 60$ (dotted blue line), 80 (dashed-dotted red line), and 90 (solid black line). (b) The chain length is fixed at $N = 80$, while the reduced rigidity and the natural spring length ($k\beta a^2, l_0/a$) are $(1, 1)$, $(2, 1)$, and $(2, 2)$ for the solid line, the dashed-dotted line, and dotted line, respectively. The parameter f_i remains the same as in (a).

$-\alpha(E)/dE$ and the results are shown in Fig. 9. Here the dotted line is a linear connection between two points for $N = 60$ and 90 . The point for $N = 80$ is below such connection line. According to Eq. (26), $\bar{f}(N = 80)$ is unusually large in comparison with the others, again suggesting that the tethering probe for 80-nt ssDNA is more rigid and/or shorter than those of other probes. Figure 9 includes one additional point for $N = 70$ according to the data from supplementary experimental results. This point almost stands on the connection line, which confirms our prediction that for short chains, the $N\bar{E}$ is linearly proportional to N .

Finally, we point out that Eqs. (25) and (26) suggest an experimental route to measure the fluctuation force of a tethered DNA. With the experimental results for $-\alpha(E)/dE$, we are able to get $q\rho(f_i - NqE)$ as a function of electric field E from Eq. (25). To obtain $\rho(f)$ from $q\rho(f_i - NqE)$ to $\rho(f)$, we first fit the straight line in Fig. 9 ($\bar{E}$ in kV/cm).

$$N\bar{E} = 245.995 - 0.07471N. \quad (27)$$

FIG. 9. Dependence of $N\bar{E}$ on the chain length for four ssDNA chains studied in experiment. Solid triangle is the data from Fig. 1, while the squared solid triangle is from supplementary experiment data in Ref. 3. The dotted line connects the first point ($N = 60$) and the last point ($N = 90$).

A comparison of Eqs. (27) and (26) yields

$$\begin{cases} f_t/q = 245.995. \\ \bar{f}(N)/q = 0.07471N. \end{cases} \quad (28)$$

Equation (28) is consistent with the Gaussian chain analysis of $\bar{f}(N)$, i.e., when chain length is not extremely large, $\bar{f}(N)$ increases linearly with N . With an estimate of the effective net charge q per nucleotide, we can obtain the tethered force f_t and subsequently $\rho(f)$ from $q\rho(f_t - NqE)$ by using $f = f_t - NqE$. The procedure may be tested with additional experiment to estimate f_t independently.

III. CONCLUSION AND DISCUSSION

We have developed two complementary Gaussian chain models to examine the dependence of the fluctuation forces on the DNA chain length and tethering conditions. In the first model, the chain is completely fixed at a position near hard wall; in the second model, the chain is tethered near the wall through a harmonic spring. The first model allows us to check the surface force on the Gaussian chain with one end locating at a given point, and in this model no external potential but geometrical confinement (for one end) is introduced. The second model contains an additional harmonic potential, which supplies a route to study the fluctuation force explicitly.

Despite the simplicity of the Gaussian models, the analytical results capture a number of major physical points pertinent to the experiment: the density of fluctuation force takes Gaussian analog distribution, and for short chains, the average value of the fluctuation force increases linearly with the chain length. The qualitative agreement of the theoretical results with experimental data shows that the Gaussian chain approximation can be used to analyze the dependence of fluctuation force on the chain length.

In addition to the tethering free energy, we studied the fluctuation force varying with spring constant and natural length. This analytical study provides insights to optimize experimental design to separate short DNA fragments with high accuracy. Generally, if the spring constant is larger, i.e., the material of probe through which the DNA chains are tethered to surface is more rigid, the fluctuation force becomes sharper, which would improve the separation efficiency. For a long probe, the chain length dependence of the surface force becomes less responsive, but it extends the range of chain length for efficient separation. As a result, a careful design of probe length is essential for efficient separation of short DNA chains.

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- ¹L. Z. Gao, J. M. Wu, D. Gao, and J. Z. Wu, *Appl. Phys. Lett.* **91**, 113902 (2007).
- ²A. Gholami, J. Wilhelm, and E. Frey, *Phys. Rev. E* **74**, 041803 (2006).
- ³S. L. Zhao, J. M. Wu, L. Z. Gao, J. Z. Wu, and D. Gao (unpublished). Detailed experimental design and procedure, as well as the results for separating single-stranded DNAs with external field, are published elsewhere.
- ⁴S. M. Bhattacharjee and D. Marenduzzo, *J. Phys. A* **35**, L349 (2002).
- ⁵D. Marenduzzo, S. M. Bhattacharjee, A. Maritan, E. Orlandini, and F. Seno, *Phys. Rev. Lett.* **88**, 028102 (2002).
- ⁶D. K. Lubensky and D. R. Nelson, *Phys. Rev. E* **65**, 031917 (2002).
- ⁷H. Isambert and E. D. Siggia, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6515 (2000).
- ⁸H. X. Zhou, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 3569 (2002).
- ⁹U. Gerland, R. Bundschuh, and T. Hwa, *Biophys. J.* **81**, 1324 (2001).
- ¹⁰W. Sung and P. J. Park, *Phys. Rev. Lett.* **77**, 783 (1996).
- ¹¹K. K. Kumar and K. L. Sebastian, *Phys. Rev. E* **62**, 7536 (2000).
- ¹²M. Muthukumar, *J. Chem. Phys.* **118**, 5174 (2003).
- ¹³P. J. Park and W. Sung, *J. Chem. Phys.* **108**, 3013 (1998).
- ¹⁴M. Doi and S. F. Edwards, *The Theory of Polymer Dynamics* (Clarendon, Oxford, 1986).
- ¹⁵H. S. Carslaw and J. C. Jaeger, *Conduction of Heat in Solids*, 2nd ed. (Oxford University Press, Oxford, 1959).
- ¹⁶T. Ambjornsson, S. P. Apell, Z. Konkoli, E. A. Di Marzio, and J. J. Kasianowicz, *J. Chem. Phys.* **117**, 4063 (2002).
- ¹⁷T. Bickel, C. Jeppesen, and C. M. Marques, *Eur. Phys. J. E* **4**, 33 (2001).