DNA Localization and Stretching on Periodically Microstructured Lipid Membranes

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We study the conformational behavior of DNA molecules adsorbed on cationic-lipid membranes that are supported on grooved, one-dimensionally periodic microstructured surfaces. We reveal a striking ability of these periodically structured membranes to stretch DNA coils. We elucidate this DNA stretching phenomenon in terms of surface curvature dependent potential energy attained by the adsorbed DNA molecules. Because of it, DNA molecules undergo a localization transition causing them to stretch by binding to highly curved sections of the supported membranes.

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Biomacromolecular systems, such as the DNA-cationic-lipid complexes used in gene therapy applications [1–3], are inherently capable of forming interesting condensed matter structures. These complexes have exemplified novel and unusual liquid crystalline states of matter [2–5]. A prominent role in this area is played by the cationic-lipid membranes supported on solid surfaces [6–8], due to the fact that naturally anionic DNA adsorbed on such membranes is highly laterally mobile [7,8]. In this Letter, we explore the behavior of DNA molecules adsorbed on cationic-lipid membranes prepared on grooved, periodically structured substrates. We reveal a striking ability of these periodically microstructured membranes to stretch DNA coils. We elucidate this phenomenon in terms of an interesting DNA localization transition prone to play a significant role in future biophysical and biotechnological investigations. As a contribution to an ongoing quest to unfold the coiled and, therefore, inaccessible state of DNA in its natural 3D environment [9], our study initiates a new venue for controlling conformations of semiflexible biopolymers by employing their interactions with specially structured biocompatible surfaces.

Here we address a basic physics question: What happens with the large-scale conformations of semiflexible polymers, such as DNA, once they are adsorbed on *curved* surfaces, such as archetypical periodically structured surfaces? Previous experimental studies were concerned with the DNA behavior on planar surfaces and have exemplified some of the fundamental statistical physics laws that apply to polymers confined to a plane [7,8]. Little if anything is known (both experimentally and theoretically) on how DNA molecules behave on curved surfaces under realistic situations involving common binding forces, e.g., the screened electrostatic forces. To address these questions, here we study the behavior of DNA adsorbed on cationic-lipid membranes supported on periodically structured surfaces of the cyclic olefin copolymer (COC). 1D periodic arrangements of long grooves (grids) with the period $L = 1.2\ \mu$m (see Fig. 1) were imprinted into COC foils [10], using a silicon master produced by holographic methods and reactive ion etching [11]. To imprint grooves, the master was pressed into COC foil (with an 80 g weight) for 10 hours under 150 °C. We covered the grooved COC surfaces by fluid membranes prepared from mixtures of neutral-lipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cationic-lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), Avanti Polar Lipids, by the sol-

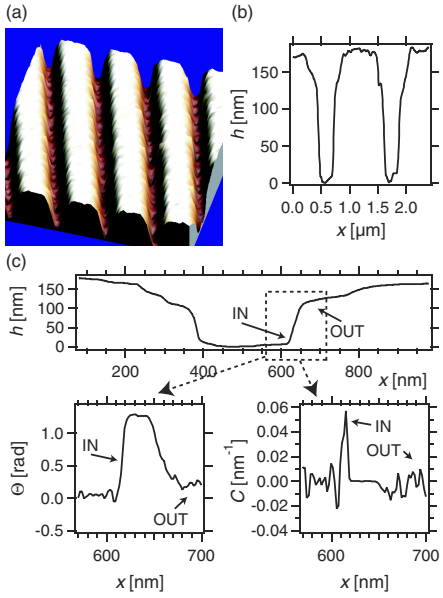

FIG. 1 (color online). AFM images of small subsections of the periodic COC surface: (a) 3D view and (b) cross-sectional profile of two consecutive grooves. (c) To-scale AFM cross-sectional image of the surface profile $h(x)$ of a single groove, perpendicular to the grooves. We also give close views on the right-hand side, in terms of the local surface slope angle $\Theta(x) = \tan^{-1}(dh/dx)$, and $C = d\Theta/ds = (d^2h/dx^2)/[1 + (dh/dx)^2]^{3/2}$, the local surface curvature (along x). Across the inward (IN) curved edge, $\Theta(x)$ increases rapidly by $\Delta\Theta \approx 1$ rad, yielding a very strong peak (with $C > 0$) in the C vs x plot, corresponding to $1/C = R = w/\Delta\Theta = 20$–$30$ nm (or even smaller, as this size $\approx$ AFM tip radius). In contrast, the outward (OUT) curved edge has a much smaller curvature, and its would-be peak in the C vs x plot is depressed by a background of curvature variations (of *both* signs) due to local surface roughness.

vent exchange method [12]. We varied the strength of DNA-membrane electrostatic interactions by using membranes with different relative fractions of DOTAP. As discussed in Ref. [13], the membranes are formed as lipid bilayers supported on COC, with a lipid diffusion constant $= 0.8 \pm 0.1\ \mu\text{m}^2/\text{sec}$. In our study, we used λ-phage DNA with Toto-1 fluorescent labels (Molecular Probes) at 1:5 fraction per base pair (added ratio) having a contour length of 22 μm [14]. Our experimental strategy was the following: The anionic DNA molecules were deposited on the supported cationic membranes from a salt-free water solution (10 mM Hepes, *p*H 7.0). The absence of salt provides a rapid deposition of the DNA molecules, yet it makes them practically immobile after they deposit. To activate DNA dynamics, the electrostatic interactions are weakened (screened) by exchanging the solvent with a NaCl water solution (10 mM Hepes, 30 mM NaCl, 5 mM vitamin C, *p*H 7.0).

The postdeposition dynamics and conformations of the DNA molecules adsorbed onto the membranes supported on the grooved 1D periodic solid surfaces were visualized by an inverted fluorescence microscope (FM), Axiovert 100 M, Carl Zeiss (resolution 0.4 μm). Initially, the adsorbed DNA molecules form as globules sitting on the membranes. We find a strong dependence of the subsequent DNA dynamics and shapes on the amount of the charged lipid; see Fig. 2. We reveal a striking conformation behavior, the DNA coil stretching phenomenon, best seen on membranes with 5% of DOTAP, at 30 °C [Figs. 2(a) and 2(b)]: DNA molecules from the globules get efficiently sucked into nearby grooves, on a 25–50 min time scale [*stage 1*, see Fig. 2(a)]. During this dynamics stage, DNA globules evolve into configuration typically including two long DNA sections (arms) sucked into the surface grooves, which are connected by about 1 μm long DNA *crossing* from one to another groove. See Fig. 2(a), in which some DNA molecules are still globular whereas others have already assumed the arms-and-crossing structure (in the figure, the grooves run vertically, and the DNA crossings are horizontal). After stage 1, the DNA dynamics slows down. It assumes the character of the one-dimensional reptationlike motion of the DNA molecule, which moves along its contour directed by the grooves (*stage 2*). This motion eventually leads to a pullout of one of the two DNA arms in the grooves, so the entire DNA molecule eventually slips into a single groove. After this apparently irreversible extinction of the DNA crossing between the grooves, which typically occurs on the time scale of several hours, the entire DNA molecule gets completely adsorbed into a single long groove. Stage 2 of the DNA dynamics is captured in Fig. 2(b), in which some DNA molecules still exhibit the two arms connected by crossings between grooves, whereas other DNA molecules have already slipped into single grooves and, thus, stretched.

The periodically structured charged membranes are thus capable to stretch long DNA molecules. We proceed with physical elucidation of these novel phenomena and discuss

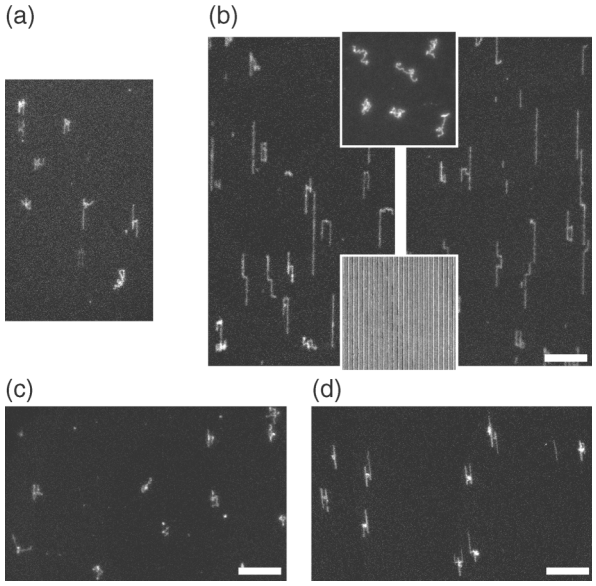

FIG. 2. FM images of DNA on a periodically structured membrane with 5% of DOTAP: (a) from stage 1 ($t = 48$ min after exchanging the solvent with the salt solution, see the text) and (b) from stage 2 (two different surface sections, left at $t = 264$ min, right at $t = 369$ min; scale bar $= 10\ \mu$m). The grooves (invisible) form a vertical grid with 1.2 μm period, seen in the to-scale scanning electron microscopy image of the COC surface [lower inset in (b)]. The average DNA projected length along the grooves $\approx 10.5\ \mu$m, at $t \approx 5$ h. In the upper inset in (b), DNA on the planar 5% DOTAP membrane (here at $t = 140$ min; qualitatively the same images are obtained at any $t > 25$ min). In (c), DNA on the periodically structured membranes with 3% of DOTAP ($t = 180$ min) and, in (d), with 7% of DOTAP ($t = 185$ min).

the DNA behavior both at visible long scales and at short scales ($<0.4\ \mu$m FM resolution). On planar charged membranes, a DNA molecule has a random walk shape fluctuating in time [see Fig. 2(b) (upper inset) and Refs. [7,8]], whereas on the present curved membranes it stretches. Why? Unlike planar membranes, the surfaces of curved membranes are (generally) not equipotentials. Due to this, a macro-ion adsorbed on a curved charged surface attains a position dependent free energy, which depends on the *local curvature of the surface C*. Thus, in particular, a charged rod (DNA) sliding along our one-dimensionally modulated membrane surface, with its axis along the grooves direction, acquires the potential energy of the form

$$U_{\text{ul}}(C) = -\Gamma C, \tag{1}$$

per unit length (ul) of DNA. Curvature potential in (1), with $\Gamma > 0$, exhibits a Mullins-type change of the binding free energy of an adsorbed object due to nonzero C: Surface sections with $C > 0$ (curved inward) approach the object more closely and, thus, bind it *more* strongly than flat surface sections with $C = 0$ [15]. By Eq. (1), Γ has the dimension of energy. Γ ranges up to $\approx 5k_BT$ in the present system [16], and, importantly, it can be tuned by changing the fraction of charged lipids. For the present

one-dimensionally modulated surface, Eq. (1) yields an interesting potential landscape for DNA, a periodic sequence of potential traps ($C > 0$) and barriers ($C < 0$); see Fig. 3. Note that there are actually two traps inside of each groove. They occur at highly curved sections of the surface, the inward curved *surface edges* across which the surface slope angle increases quasidiscontinuously by an amount $\Delta\Theta$ over a narrow edge width w; see Figs. 1(c) and 3. From the atomic force microscope (AFM) images in Fig. 1(c), the surface curvature within the edge $C = \Delta\Theta/w \approx 0.035$–$0.045\ \mathrm{nm}^{-1}$, and $\Delta\Theta \approx 1$ rad, corresponding to the edge width $w \approx 20$–30 nm. By Eq. (1), such an edge yields a potential trap (binding well in Fig. 3) with the depth $-U_0 = -\Gamma\Delta\Theta/w$. A *single* long edge trap can permanently localize a DNA molecule along it, provided U_0 is big enough to overcome the molecule's positional entropy tending to unbind it from the trap. To elucidate this, we consider the free energy difference between the bound and the unbound polymer, $\Delta F_{\mathrm{ul}} \approx -U_0 + F_{\mathrm{st}}$ (per unit length). Here F_{st} is the (steric) free energy of confinement, $F_{\mathrm{st}} = 1.103 k_B T/[(\xi_{\mathrm{pers}}/2)^{1/3} w^{2/3}]$, needed to confine a long polymer within a stripe (2D "tube") of the width w [17]; here ξ_{pers} is the polymer persistence length ($\approx$60 nm for our 1:5 labeled DNA [14]). Thus, $\Delta F_{\mathrm{ul}} \approx -U_0(1-k)$. Here $k = F_{\mathrm{st}}/U_0 = 1.103(k_B T/\Gamma\Delta\Theta)(2w/\xi_{\mathrm{pers}})^{1/3} \approx 0.96 k_B T/\Gamma$, with $\Delta\Theta \approx 1$ rad and $w \approx 20$ nm. For $k < k_c \approx 1$, i.e., $\Delta F_{\mathrm{ul}} < 0$, the binding energy wins over the entropy ($U_0 > F_{\mathrm{st}}$), and the polymer remains localized inside the edge [18]. Conversely, for $k > k_c \approx 1$, i.e., $\Delta F_{\mathrm{ul}} > 0$, the entropy wins over binding energy ($F_{\mathrm{st}} > U_0$), and the polymer unbinds from the edge (while *still* bound to the membrane) to eventually assume the shape of a 2D self-avoiding random walk. In the present system, $k \approx k_B T/\Gamma \approx 0.2$ at high surface charge densities, and it increases with a decreasing amount of charged lipids [16], allowing to cross the DNA unbinding transition at $k_c \approx 1$ [18]. Indeed, our experiments show that on 5% DOTAP membranes the DNA still binds to the edges [Fig. 2(b)], while on 3% DOTAP membranes we find that the deposited DNA does not enter the edges [see Fig. 2(c)]. To the best of our knowledge, this is the first observation of such semiflexible polymer unbinding phenomena *on a 2D manifold*.

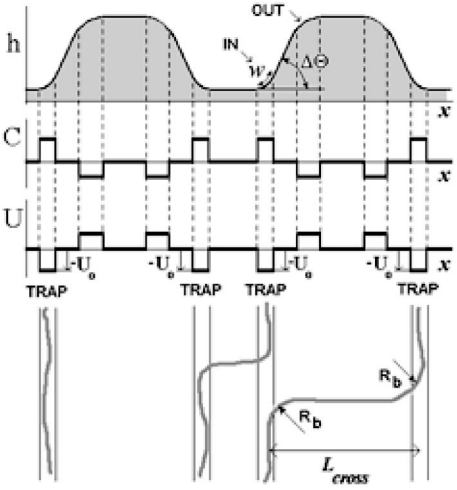

FIG. 3. Surface curvature dependent potential energy of DNA, Eq. (1), along the periodic surface, is a sequence of potential traps ($C > 0$) and barriers ($C < 0$). DNA may form crossings between two edge traps. On exiting the traps, the DNA makes small nearly circular bends, with estimated radius $R_b \approx (\pi/4(\pi-2))^{1/2}(l_\sigma \xi_{\mathrm{pers}})^{1/2} \sim 30$ nm, ($l_\sigma \sim w \sim 20$ nm). Their bending elasticity contributes to the total crossing energy $\Delta F_{\mathrm{cross}}$ the amount $\approx k_B T(\pi(\pi-2))^{1/2}(\xi_{\mathrm{pers}}/l_\sigma)^{1/2} \sim 3.5 k_B T$ (from the two bends per each crossing). It is much smaller than the extraction tension contribution to the crossing energy $= k_B T(L_{\mathrm{cross}}/l_\sigma) \sim 50 k_B T$ for the long crossings between grooves, with $L_{\mathrm{cross}} \approx 1\ \mu$m.

Above, we focused on the effects of a single edge potential trap, whereas on our surfaces we have a periodic sequence of such edge traps competing to capture DNA (Fig. 3). Because of this, a very long polymer may still maintain an *anisotropic* random walk shape even for $k < k_c$ by developing crossings between different attractive edges (see Fig. 3). In our experiments, we indeed observe that such crossings form initially but then tend to disappear at long times, so that DNA eventually stretches in equilibrium. See the images in Fig. 2(b), taken within stage 2 of the DNA evolution, in which some long DNA crossings between the grooves, with the length $L_{\mathrm{cross}} \approx 1\ \mu$m ($\approx$distance between grooves), are still visible. What ensures the experimentally observed extinction of these crossings and the resulting DNA stretching? To elucidate this effect, we note that the presence of such a crossing along the polymer introduces an extra free energy cost (relative to the polymer configuration completely bound to a single edge) of the form $\Delta F_{\mathrm{cross}} = \sigma L_{\mathrm{cross}}$. Here the *line extraction tension* $\sigma = -\Delta F_{\mathrm{ul}}$ is the free energy cost to unbind a unit length of the polymer from the edge trap. It is positive for $k < k_c$. In thermal equilibrium, the crossings between traps *must* form along a *long enough* polymer: By analogy to thermally activated domain walls in 1D systems [19], the equilibrium separation between the crossings along the polymer contour behaves as $\xi_{\mathrm{cross}} \sim \exp(\Delta F_{\mathrm{cross}}/k_B T) = \exp(L_{\mathrm{cross}}/l_\sigma)$. Here l_σ is a capillary length given by $l_\sigma = k_B T/\sigma \approx w(k_B T/\Gamma\Delta\Theta)/(1-k)$. Unless very close to the DNA unbinding transition at $k = k_c \approx 1$, the capillary length $l_\sigma < w \approx 20$–30 nm, so l_σ is 50–30 times smaller than the length L_{cross} of the 1000 nm long DNA crossings between the grooves. Thanks to this large separation between the length scales L_{cross} and $l_\sigma \sim w =$ edge width, the average *equilibrium* distance between such crossings $\xi_{\mathrm{cross}} \sim \exp(L_{\mathrm{cross}}/l_\sigma)$ is large. For a realistic finite size polymer, this means there will be no such crossings between grooves once the thermal equilibrium is reached. Because of this, the 1 μm long crossings in Fig. 2(b) irreversibly disappear at long times, during stage 2 over which DNA moves into single grooves and, thus, stretches, as best seen at the 5% DOTAP fraction [20].

In practical terms, the revealed DNA stretching effect relies also on the kinetic ability of DNA to reach its thermal equilibrium shape within experimentally accessible time scales. Interestingly, we find that increasing the charged lipid fraction above 5% *increases* the stretching time scale and may even obstruct the DNA to ever enter the stretching stage 2. Indeed, on 7% DOTAP membranes, we find that stage 2 is preempted by a halt of the stage 1 process [see Fig. 2(d)]: The initially formed small DNA globules release arms growing into grooves, but then the arm growth is halted. This halt appears to be caused by energy barriers clogging the edges for the DNA advance. The barriers can be produced by the surface curvature along the direction of edge traps, i.e., longitudinal imperfections of (otherwise straight) grooves; see Fig. 1(a). Like the curvature potential of the (more curved) edge traps, a weaker curvature potential contributed by the (less curved) longitudinal imperfections also increases with increasing DOTAP percentage, which eventually closes the edges for the DNA passage. Because of this, by increasing the DOTAP percentage above the critical value for the DNA unbinding-from-edges transition (at $\approx$3% of DOTAP), the stretching effect enhances only initially, up to 5% of DOTAP (at 30 °C), which turns out to be the *optimum* charged lipid fraction, providing the minimum DNA stretching time of a few hours time scale. Further increase of DOTAP percentage only slows down (and eventually halts) the DNA stretching.

In summary, the statistical physics of semiflexible polymers adsorbed on periodic membranes encompasses the conceptually and practically interesting phenomena of polymer localization and coil stretching revealed in our experiments with DNA. We highlighted an unanticipated significant role of surface curvature potentials in governing biomacromolecules. On the practical side, in contrast to presently employed microfluidic methods [9], our new approach to stretch DNA coils avoids any use of fluid flows and the throughput limitations due to the difficulties in entering DNA into microfluidic channels. Indeed, the DNA can be easily brought in large amounts onto our periodic membranes. Importantly, to be stretched by our approach, DNA need not be confined into small spaces of microfluidic channels. Rather, the stretched DNA molecules are freely exposed to a larger surrounding water medium and all the molecules dissolved in it. Because of this feature, our new way to stretch polyelectrolytes may facilitate more direct, high throughput protocols and experimental studies of fundamental biological interactions between DNA and other biomolecules.

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[16] The curvature free energy in Eq. (1) is the difference between the binding free energy (per unit length) of DNA adsorbed along a charged cylindrical surface *subsection* of a *large* radius $R = 1/C = w/\Theta$ [$\gg$Debye length = 1.755 nm here vs $R \approx 20$ nm for inward curved edges in Fig. 1(c)] and the binding free energy of DNA adsorbed on a charged plane. Using this, and the Poisson-Boltzmann theory (with *mobile* surface and spatial charges), we estimate the Γ constant in Eq. (1) to range up to ~ 5 $k_B T$ in the present system, depending on the charged lipid fraction. In addition to the electrostatic effects, the known packing differences between DOTAP and DOPC head groups are also significant. They tend to enhance positive surface charge (DOTAP) density in the inward curved edges, making Γ in Eq. (1) even more positive.
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[18] Semiflexible polymers attracted by a single potential well exhibit a true second order unbinding transition. For rectangular wells (as in Fig. 3), recent Monte Carlo simulations [L. Gao and L. Golubovic (unpublished)] show that the critical value of k is $\cong 1.5$. In the bound phase (in which the DNA localizes and, thus, stretches), the simple stat-mech picture employed here is (asymptotically) exact for $k < 1$.
[19] See L. D. Landau and E. M. Lifshits, *Statistical Physics* (Pergamon, Oxford, 1969).
[20] Note that, on our surface, DNA can also make short crossings between the two edge traps in the *same* groove (see Figs. 1 and 3), with $L_{\text{cross}} \approx 200$ nm, about 5 times shorter than the length of the long DNA crossings between *different* grooves, with $L_{\text{cross}} \approx 1000$ nm. Therefore, nonequilibrium and equilibrium densities of the short crossings can be significant and responsible for the fact that the DNA projected length (along the grooves direction) is smaller than the total DNA contour length of 22 μm, even for the DNA completely adsorbed into single grooves [see Fig. 2(b)].