Formation of Ordered Domains in Membrane-Bound DNA

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ABSTRACT The interactions between DNA molecules adsorbed on fluid membranes are calculated. The adsorbing DNA perturbs the equilibrium packing of the lipids, thereby giving rise to membrane-induced, attractive interactions. These balance the direct repulsive interactions between DNA molecules. As a result, DNA adsorbed on membranes is predicted to form ordered domains characterized by a finite spacing, which varies with the membrane characteristics and the solution Debye screening length. Comparing the model predictions to recent experiments (Yang et al. 1996) yields excellent agreement with only one free (i.e., experimentally unknown) parameter.

INTRODUCTION

Membrane-DNA complexes have been shown to play an important role in prokaryotic DNA replication and segregation (Gennis, 1989; Firshein, 1989). Although many studies have investigated these complexes (Firshein, 1989), evaluation of the role of membrane-DNA interactions in DNA replication is difficult. This is due to the complexity of the system and the large number of components involved. Various questions regarding the role of the membrane in the replication process remain, therefore, unanswered, including determination of the type of membrane-DNA binding, the role (if any) of the complex in the replication process, and the effect of binding on the DNA superhelix structure.

Examining the interactions between DNA and model membranes could provide some clues. However, although it is clear that adsorption of DNA on membranes is not the same as adsorption on solids, most studies investigate the latter (see, for example, Jing et al., 1993). The difference between the two cases is significant, stemming from the self-assembled nature of bilayers; whereas a solid surface remains unchanged by adsorption, the structure of a membrane is distorted by the addition of large molecules such as proteins (Gennis, 1989), or long, semiflexible ones such as DNA.

Recently Yang et al. (1996) investigated DNA adsorbed on a supported dipalmitoyl-dimethylammonium-propane (DPDAP) lipid bilayer, using atomic force microscopy. They found that the DNA adsorbed strongly on the membrane, forming ordered domains. These were characterized by a regular interaxial spacing of approximately 5 nm (Yang et al., 1996). The ordered domains did not cover the entire membrane surface. However, because of experimental limitations it could not be determined whether a preferred domain size existed.

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The formation of these domains is quite surprising, because the interactions between DNA molecules are known to be strongly repulsive at such distances (Podgornik et al., 1994). Moreover, similar phases were not observed in DNA adsorbed on solid surfaces (Jing et al., 1993). It seems clear, then, that the DNA arrays are due to some membrane-induced mechanism.

In this paper we calculate the interactions between DNA molecules adsorbed on fluid membranes and use them to predict the preferred spacing between molecules in ordered domains. To this end we utilize the theoretical approach, developed by Dan et al. (Dan et al., 1993, 1994; Dan and Safran, 1995; Aranda-Espinoza et al., manuscript submitted for publication) for embedded membrane proteins: the adsorbed DNA perturbs the local packing of the bilayer lipids in a manner similar to that imposed by embedded proteins. Attractive, membrane-induced interactions between adjacent molecules thus arise that balance the direct repulsive interactions. (The membrane-induced interactions are attractive only when the spontaneous curvature (Safran, 1994) of the lipids is zero. In systems where that is not the case, membrane-induced interactions can be repulsive (Dan et al., 1993; Aranda-Espinoza et al., manuscript submitted for publication). The adsorbed DNA will then form a dilute, random phase on the membrane surface.) A preferred spacing in the domains of DNA adsorbed on a fluid membrane surface is the result.

MODEL AND RESULTS

Because of symmetry, a uniform, fluid bilayer of zero spontaneous curvature is locally flat. It can be characterized by an equilibrium thickness and surface density, i.e., area per molecule (Safran, 1994). The membrane energy has two contributions. The first is due to changes in area, and the other to the "bending energy," which is the energy associated with local shape changes (Safran, 1994). Clearly, both contributions are minimal when the membrane is in the equilibrium, flat state. However, the interaction between adsorbing DNA and the heads changes the equilibrium density in regions of the top layer, as sketched in Fig. 1, thereby increasing the membrane energy.

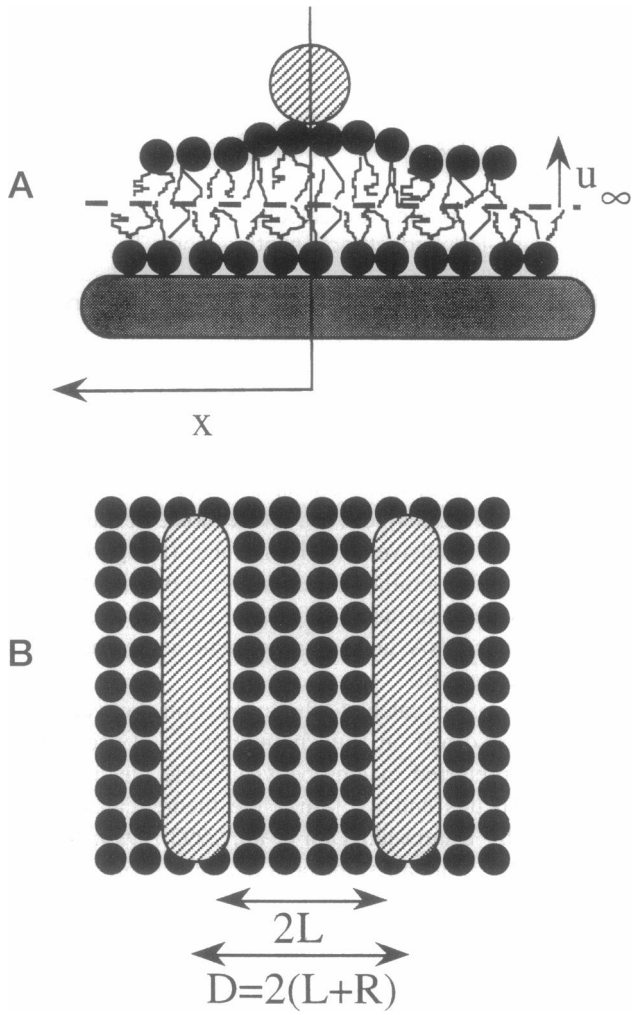

FIGURE 1 DNA adsorbed on a supported membrane. (*A*) The attraction between the DNA and the lipids perturbs the equilibrium thickness, u_∞, of the top monolayer. This perturbation decays with distance from the DNA boundary, x. (*B*) The proteins are taken to be long cylinders, distributed uniformly on the membrane with an interaxial distance D (or an edge-to-edge distance of $2L$).

Membrane fluidity implies that the two monolayers composing the bilayer can be decoupled, so that transporting the membrane onto a solid surface does not perturb the top monolayer (if the surface is molecularly smooth), which remains at equilibrium. The top monolayer equilibrium thickness, u_∞, is coupled to a surface density Σ_∞ by an equation of state, which we take to be the condition of incompressibility (Dan et al., 1993, 1994). Because we are interested in interactions over relatively short distances, we neglect the wormlike nature of DNA and model the molecules as long, rigid cylinders of radius R. The perturbation of the top monolayer varies, therefore, only with the distance from the cylinder center, x (see Fig. 1). We define the local perturbation by a deviation of the local thickness or surface density, $u(x)$ or $\Sigma(x)$, from the equilibrium value; $\Delta(x) \equiv \{u(x) - u_\infty\}/u_\infty \approx \{\Sigma(x) - \Sigma_\infty\}/\Sigma_\infty$. Because of the relative stiffness of the hydrocarbon tails, Δ must be small; however, it may be positive or negative, depending on the type of DNA-membrane binding. The membrane energy, per adsorbed molecule (per unit length), can be written as (Dan et al., 1993; Dan and Safran, 1995)

$$F_m = \int_0^L \frac{1}{\Sigma_\infty}\left\{B\Delta^2 + u_\infty^4 K\left(\frac{d^2\Delta}{dx^2}\right)^2\right\}dx, \qquad (1)$$

where $2L$ is the distance between neighboring DNA molecules; B is the monolayer compressibility, namely, the energetic cost associated with perturbation of the surface density from Σ_∞; and K is the bending modulus of the head/tail interface (Safran, 1994). Minimizing the free energy with respect to the perturbation profile, we find that the top layer thickness profile is (see Appendix)

$$\Delta(x) = \sum_{j=1}^{4} A_j e^{k_j \rho x}, \qquad (2)$$

where the characteristic bilayer correlation length is given by $1/\rho = \beta u_\infty (K/B)^{1/4}$, and k_j are the four roots of $(-1)^{1/4}$. The coefficients A_j are determined by the boundary conditions (see Appendix). It is interesting to note that, because ρ is a real number, the membrane thickness will always oscillate with distance from the perturbation boundary, although the magnitude of the perturbation decays exponentially. The membrane-mediated energy, per unit width, is given by (see Appendix)

$$F_m = -\frac{u_\infty^2 K}{\Sigma_\infty}\{\Delta'\Delta'' - \Delta\Delta'''\}_{L=0} = \frac{\sqrt{2}\rho^3\Delta_0^2 u_\infty^2 K}{\Sigma_\infty} J(\tilde{L}), \qquad (3)$$

where $\tilde{L} \equiv 2(-1)^{1/4}\rho L$, $i \equiv 2^{1/2}(-1)^{1/4}$, Δ_0 is the magnitude of the imposed perturbation at the cylinder boundary, and

$$J = \frac{(I + e^{\tilde{L}} - e^{I\tilde{L}} - Ie^{i\tilde{L}}}{(1 + e^{\tilde{L}})(1 + e^{I\tilde{L}})}. \qquad (3.a)$$

In Fig. 2 we plot F_m as a function of the dimensionless spacing, ρL. As expected, the membrane-induced interactions between adsorbing molecules are attractive. However, the energy barrier at $L\rho \approx 1$ may prevent aggregation, even in the absence of other forces. The value of the membrane energy, in the limit of large spacing between adsorbed molecules ($L \to \infty$), denotes the membrane-induced "adsorption energy," per unit length of DNA (Dan and Safran, 1995). This energetic penalty reduces the "inherent" molecular adsorption energy that arises from the energetic difference between free molecules in solutions and those on the surface. The decrease in the effective adsorption energy imposed by the membrane reduces, therefore, the concentration of adsorbed DNA, when compared to an equivalent solid surface.

The direct, noncontact interactions between DNA molecules are due to both electrostatic and hydration forces. The former dominate long-range interactions, and the latter determine short-range ones. Although the long-range interac-

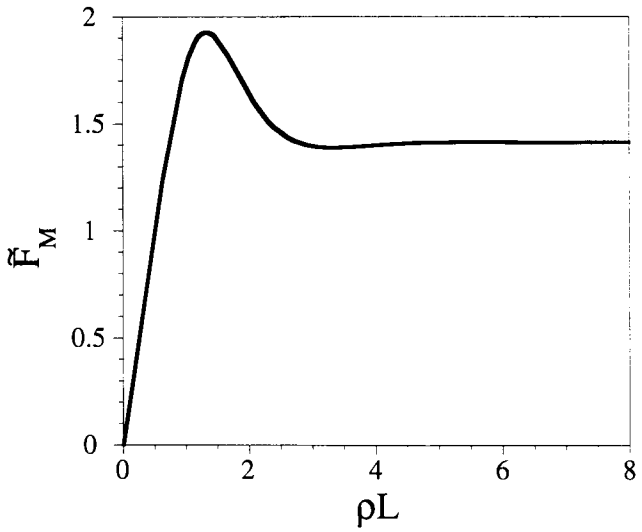

FIGURE 2 The membrane-induced interactions between adsorbed DNA (Eq. 3): $\tilde{F}_M = F_m/\{\sqrt{2}\rho^3\Delta_0^3 u_\infty^2 K/\Sigma_\infty\} \equiv J$.

tions cannot be described by a straightforward electrostatic double-layer model, Podgornik et al. (1994) have shown that they can still be written in an effective exponential form:

$$F_d = \frac{1}{2}(2R)qe^{-D/\lambda}, \tag{4}$$

where $D = 2(L + R)$ is the interaxial separation. The exponential decay length, λ, is approximately equal to twice the solution Debye screening length due to chain fluctuations. We use this form for λ, rather than the standard Debye length, because the adsorbed DNA can still fluctuate in two dimensions on the surface (as the membrane is fluid). Obviously, the true value of the screening length will be somewhere lower. However, the results presented here will be largely unaffected. The effective energy per unit area is given by q; the prefactor of $2R$ is added to convert F_d to the dimensions of per unit length.

We can now write the total interaction energy, per unit length of DNA, as

$$F_T = Rqe^{-D(L)/\lambda} + \frac{\sqrt{2}\rho^3\Delta_0^2 u_\infty^2 K}{\Sigma_\infty} J(\tilde{L}). \tag{5}$$

Minimization of F_T with respect to L gives an implicit expression for the spacing of adsorbed DNA, as a function of the membrane correlation length ($1/\rho$), the strength of the membrane-protein coupling (Δ_0), DNA characteristics (q and R), and the salt concentration (κ or λ). (The rigorous way to obtain the composition of the equilibrium phases from the free energy is by using the conditions of equality of the chemical potential and the osmotic pressure (Safran, 1994). However, implementing it in this case is mathematically complex. Using the minimization condition implies no conservation constraint, namely, the number of adsorbed DNA molecules is not conserved. Because the condensed-phase minima obtained are relatively narrow, the equilibrium spacing obtained by this method is within 5 Å of the one obtained by the rigorous calculation.) Assuming that $L\rho \ll 1$, we find that the equilibrium spacing can be written using the following simple analytical expression:

$$D_{eq} \equiv 2(L_{eq} + R) \approx \lambda \ln\left[\frac{qR\Sigma_\infty}{2\lambda B\Delta_0^2}\right]. \tag{6}$$

Somewhat unexpectedly, we see that D_{eq} does not vary linearly with $\lambda/2$, the electrostatic screening length, implying that the spacing between adsorbed species will not decrease monotonically with increasing salt concentration. Taking all other parameters to be constant, D_{eq} is minimal when $\lambda = (qR\Sigma_\infty e/2B\Delta_0^2)$.

To test this model, we estimate the spacing in the case of DNA adsorbed on a DPDAP membrane (Yang et al., 1996). From Podgornik et al. (1994), in a solution of 20 mM NaCl, $q \approx 0.02$ erg/cm^2, and λ, which is twice the Debye length, is 43 Å. (q was shown (Podgornik et al., 1994) to vary with the salt concentration. We calculate the value of q at 20 mM NaCl by extrapolation of the data in Table 1 of Podgornik et al. (1994).) The radius of DNA is 10 Å. Yang et al. (1996) measured the values of $u_\infty \approx 22.5$ Å and $\Sigma_\infty \approx 36$ Å^2 for the DPDAP bilayer. We do not have any data on the compressibility and bending energy of this specific lipid system. However, the experiments of Evans and Rawicz (1990) provide a good estimate of these quantities. They show that for various lipids, $B \approx 7.5 \times 10^{-13}$ ergs and $K \approx 5 \times 10^{-13}$ ergs, so that $1/\rho \approx u_\infty/1.1 \approx 20.5$ Å.

The equilibrium spacing, as a function of the membrane perturbation Δ_0, is plotted in Fig. 3 *A*. In general, the phase diagram is reminiscent of liquid/gas equilibrium as a function of either temperature or pressure. The analytical solution (Eq. 6) is seen to yield a reasonably good estimate up to ρL_{eq} values of approximately 1/2. However, the range of membrane perturbation values at which an ordered, condensed phase can be obtained is quite narrow. This can be understood when examining the overall energy, F_T, as a function of distance between DNA molecules (Fig. 3 *B*)—when Δ_0 is large (in this case, greater than ~0.36%) the attractive, membrane-induced interactions dominate. The DNA will then form an aggregated phase where $L_{eq} = 0$, at equilibrium with an infinitely dilute phase where $L \to \infty$. When Δ_0 is small (in this case, less than ~0.29%) the electrostatic repulsion is overwhelming, and an infinitely dilute phase will form. Between these limits, a condensed phase, characterized by a finite spacing, is obtained at equilibrium with the infinitely dilute phase.

We see from Fig. 3 *A* that the 50 Å equilibrium interaxial spacing, observed by Yang et al. (1996), can be obtained at a reasonable perturbation value of 0.292%. This value is, perhaps fortuitously, on the edge of the stable condensed phase region. Assuming that the ρ value we use for this membrane is reasonable, this means that manipulation of the DNA-membrane coupling, while keeping the salt concentration constant, will not increase this spacing.

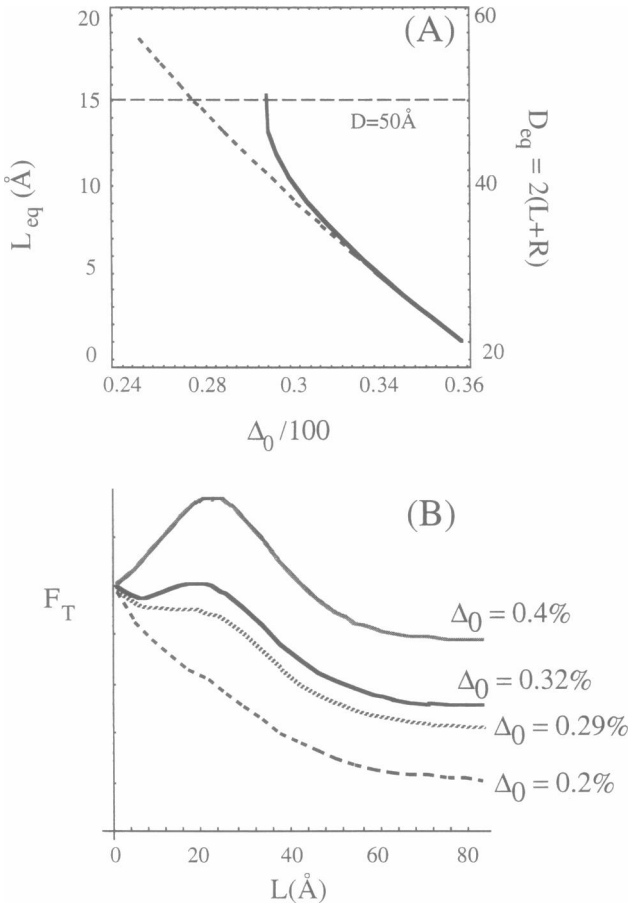

FIGURE 3 (*A*) The equilibrium edge-to-edge distance (L_{eq}), or interaxial spacing (D_{eq}), of adsorbed DNA molecules as a function of membrane perturbation. The solid line denotes the full numerical solution (from Eq. 7); the dashed line is the approximate solution of Eq. 6. The numerical values of the various parameters are given in the discussion of Eq. 7. (*B*) The overall DNA free energy (Eq. 7), as a function of spacing and perturbation. At large perturbations (Δ_0 = 0.4%) the energy is minimal when the DNA aggregates ($L = 0$) or disperses ($L \to \infty$). At more moderate perturbations (Δ_0 = 0.32%) the energy is minimal at a finite spacing ($L \approx$ 10 Å) or in the dispersed phase. At lower perturbations ($\Delta_0 < 0.292\%$) the minimum at the finite spacing disappears, and only a dispersed phase can be obtained.

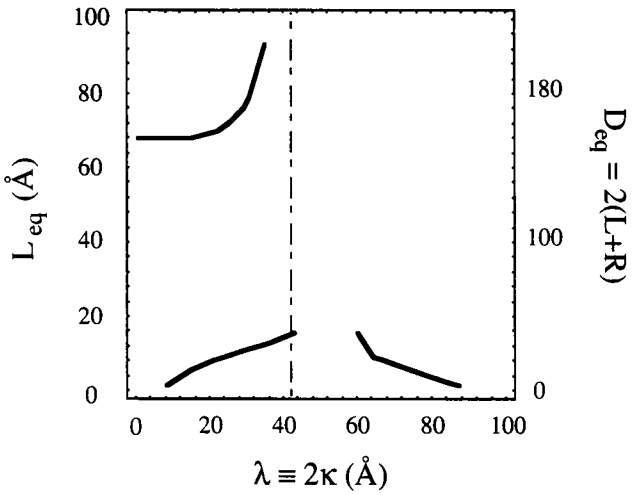

FIGURE 4 The full numerical solution for the equilibrium edge-to-edge distance (L_{eq}), or interaxial spacing (D_{eq}), of adsorbed DNA molecules as a function of the solution Debye decay length κ or the DNA screening length λ. The dashed line denotes λ = 43 Å. The numerical values of the various parameters are given in the discussion of Eq. 7. $\Delta_0 \approx 0.292\%$.

It is quite difficult to determine experimentally, or manipulate, the membrane/DNA coupling Δ_0. However, the solution salt concentration (and thus, the effective decay length λ) can be easily controlled. In Fig. 4 we plot the equilibrium spacing as a function of λ, for a given value of the membrane perturbation: the Δ_0 value chosen is the one corresponding to the experimentally observed 50-Å interaxial spacing at λ = 43 Å, namely, 0.292%. (We assume that, because the interactions between the bilayer heads and the DNA molecules are close-range, the membrane perturbation Δ_0 is unaffected by changes in the salt concentration.) We see that there are regions where ordered phases are unstable (in this system, when 44 Å $< \lambda <$ 52 Å), or where only an infinitely dilute phase will exist (for $\lambda >$ 90 Å). However, when the screening length is small, we see that two types of condensed phases can coexist—one with relatively small spacing, and one with relatively large spacing. (The calculation we use is more accurate at predicting small spacing. To obtain the properties of the more dilute phase, fluctuations should be taken into account (Dan and Safran, manuscript in preparation).) It is encouraging to note that the experiments by Yang et al. (1996), which find only one type of spacing, fall in the one-phase region.

CONCLUSIONS

We present a simple model that predicts the equilibrium spacing in dense arrays of DNA adsorbed on membranes. This spacing is determined by a balance between the direct DNA interactions, which are repulsive, and the membrane-induced ones, which are attractive.

We find that there are regions in the phase diagram (as a function of the membrane perturbation, Δ_0, and salt concentration, λ or κ) where a stable phase of adsorbed DNA can form, in which the interaxial distance between molecules is finite. When the salt concentration is high (i.e., λ is small) two different equilibrium spacings might be obtained. It is premature to speculate whether these predictions can be taken to describe an equilibrium between two types of domains, each characterized by a different spacing. To determine that, a rigorous calculation of the full-phase diagram should be done (Dan and Safran, manuscript in preparation).

The value of the spacing we predict, based on this simplified model, agrees quantitatively with that observed for DNA adsorbed on supported membranes by Yang et al.

(1996) when taking a reasonably low value of membrane perturbation. Furthermore, we find that the system parameters in this experiment are such that only one condensed phase is expected, as indeed observed.

The model we propose here also seems to be the only mechanism that can explain, in principle, the formation of isolated domains of regularly spaced DNA. A high enough value of the DNA adsorption energy can very well overcome the inherent molecular repulsion and lead to dense adsorption. However, in that case the whole surface will be uniformly covered. Moreover, the spacing between neighboring molecules will vary from region to region, and experiment to experiment—a function of the DNA concentration in solution and the incubation time. If the cationic membrane mitigates the electrostatic repulsion between the negatively charged DNA, we expect a random adsorption pattern. An equilibrium spacing, in isolated domains, can only occur as the result of a balance between two opposing forces.

In related, recent experiments, Barenholz et al. (1996), Safinya et al. (1996), and Lasic et al. (1996) examined DNA in free multilamellar arrays of cationic lipids. They found a peak in the x-ray scattering pattern that is attributed to the DNA ordering as a two-dimensional nematic between the lamellae (H. Strey, private communication). The spacing between DNA molecules was, in this case, 3.6 nm, which is about 1.6 nm larger than close packing. Although these results are preliminary and cannot be used to verify our model predictions (which applies for DNA adsorbed on freely suspended lamellae and on supported membranes), they do not contradict them. Safinya et al. (1996) found nematic-like order of the DNA.

A simple test of this theory would be to examine the effect of changes in salt concentration, and hence the strength of electrostatic interactions, on the DNA spacing. Another possible test would be to adsorb DNA on a membrane below and above the phase transition temperature; in the fluid membrane ordered domains will form. In the gel phase, the mechanism leading to membrane-induced attraction cannot take place (namely, perturbation of the surface density or the thickness). As a result, ordered domains should not form in nonfluid bilayers.

This model is obviously oversimplified and cannot be directly related to DNA replication in DNA-membrane complexes. However, it should be noted that our assumption that the DNA is strongly adsorbed on the membrane is in agreement with the results of experiments on *B. subtilis* complexes (Firshein, 1989). These have shown that DNA binding to the membrane is salt-resistant, namely, the coupling is strong. More intriguing, however, is the fact that unsaturated fatty acids were found to be indispensable for the initiation process (Firshein, 1989). It was argued that this is because they optimize the fluid phase of the membrane. Because our model predicts that ordered, condensed DNA phases cannot occur in nonfluid membranes, one might speculate that such phases play a role in the replication process.

APPENDIX

Minimization of the monolayer perturbation energy (Eq. 1) yields the Euler-Lagrange equation, defining the perturbation profile as

$$B\Delta + 2Ku_\infty^2\Delta'''' = 0 \tag{A.1}$$

where (′) denotes a derivative with respect to x. The top monolayer thickness profile is, therefore,

$$\Delta(x) = \sum_{j=1}^{4} Aje^{k_j\rho x}. \tag{2}$$

Four boundary conditions are required. The first one is defined by the magnitude of the perturbation at the boundary of the adsorbed molecule, namely,

$$\Delta(x = 0) = \Delta_0. \tag{A.2}$$

The second boundary condition ensures symmetry at the midpoint between adjacent adsorbed DNA molecules;

$$\Delta'(x = L) = 0. \tag{A.3}$$

The two remaining boundary conditions are the natural ones (Dan et al., 1993; Dan and Safran, 1995) and are given by

$$\Delta'''(x = L) = 0 \tag{A.4}$$

$$\Delta''(x = 0) = 0. \tag{A.5}$$

Combining these conditions yields

$$A_1 = \frac{\Delta_0}{2(1 + e^{k_1\rho L})} \tag{A.6}$$

$$A_2 = \frac{\Delta_0 e^{k_1\rho L}}{2(1 + e^{k_1\rho L})} \tag{A.7}$$

$$A_3 = \frac{\Delta_0}{2(1 + e^{k_1 I\rho L})} \tag{A.8}$$

$$A_4 = \frac{\Delta_0 e^{k_1 I\rho L}}{2(1 + e^{k_1 I\rho L})}, \tag{A.9}$$

where $k_1 = (-1)^{1/4}$. The free energy can be written as

$$F_M = \int_0^L \frac{1}{\sum_\infty}\left\{B\Delta^2 + u_\infty^4 K\left(\frac{d^2\Delta}{dx^2}\right)^2\right\} dx$$

$$= \int_0^\infty \frac{1}{\sum_\infty} \Delta\{B\Delta + u_\infty^4 K\Delta''''\}\, dx \tag{A.6}$$

$$+ \frac{1}{\sum_\infty}\left(Ku_\infty^4\Delta'\Delta''\Big|_0^L - Ku_\infty^4\Delta\Delta'''\Big|_0^L\right).$$

Integrating by parts, twice, and using the Euler-Lagrange equation and the boundary conditions,

$$Fm = -\frac{u_\infty^2 K}{\sum_\infty}\{\Delta'\Delta'' - \Delta\Delta'''\}_{L=0}. \tag{3}$$

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