Supporting Membrane Shape Instability in the Presence of Strongly Adsorbed Flexible Polymers

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Strongly adsorbed polymers are shown to undergo an isotropic−nematic surface-ordering transition as a consequence of interactions mediated by the supporting elastic membrane. Depending on the elastic coupling ratio between the membrane and polymer elasticity, the supporting membrane can concurrently undergo a transition into either preferred saddle or tubular shapes. It is argued that this scenario is relevant for the initial stages of the DNA−cationic liposome interactions.

Introduction

Though we have approached reasonable separate understandings of the behavior of lipid bilayer membranes and that of biological polyelectrolytes, most notably DNA, in terms of their effective elastic Hamiltonian and macromolecular interactions with the surrounding electrolyte solution, we are just beginning to grasp the intricacies of their respective interactions. Apart from its fundamental aspects, the problem of DNA−lipid membrane interactions has proved crucial to the understanding of the colloidal state of the genosomes (DNA−cationic liposome complexes) used to translocate DNA across cellular membranes in vivo.[1]

There have been quite a few proposals as to the alleged structure of the genosomes, but consensus has yet to be reached on the most appropriate model.[2] The most probable scenario emerging from numerous experimental studies is that a cationic lipid induces condensation of DNA while DNA induces liposome restructuration and its encapsulation into elongated bilayer liposomes. DNA thus becomes shielded from intercalating agents and digestive enzymes but available for translocation across the cellular membrane. These DNA−lipid complexes can later aggregate into higher order assemblies, creating stacked lipid−DNA multilayers.[3] It appears that the shuttling capabilities of these supramolecular complexes in vivo strongly depend on their colloidal state.

Even the primary stages of DNA−cationic lipid interactions can hardly be interpreted within the standard models of polymer adsorption. AFM studies of adsorption of DNA to constrained cationic lipid bilayers, deposited on a mica substrate, reveal that DNA under low salt screening conditions adsorbs to the extent of close packing.[4] No dangling ends or loose loops, usually present in strongly adsorbed polymers, have been observed in the case of DNA. The adsorbed DNA is typically found in a 2-D nematic phase (in 2-D this is equivalent to a smectic phase) with ordered domains of variable size. The energetics of the DNA-adsorbed state for immobilized charged substrates has been rationalized in terms of attractive interactions generated by local curvature changes as DNA comes into close vicinity of the oppositely charged bilayer,[5] as well as repulsive forces between DNA helices in close apposition, known to exist in the collapsed state in the bulk.[8]

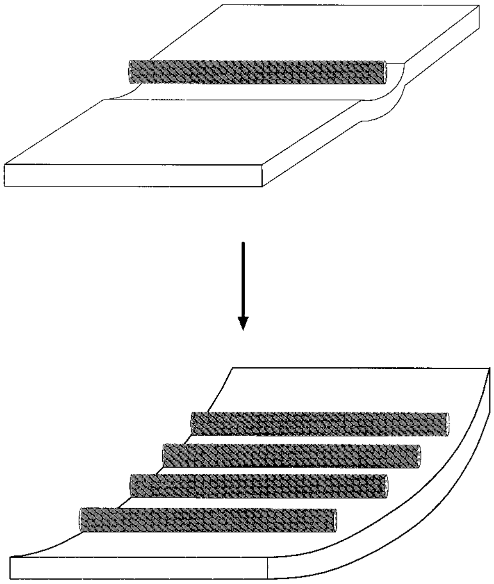

Figure 1. Schematic representation of tight adsorption of DNA to a cationic lipid-supporting membrane. Locally DNA induces a curvature on the membrane in the transverse direction with respect to its long axis (above). If the adsorbed DNA is not orientationally ordered, this local curvature does not amount to a net membrane curvature. If however DNA is orientationally ordered, then this local curvature stress is relaxed by inducing a net membrane curvature that encapsulates the adsorbed DNA (below).

In order to understand the properties of the DNA−cationic lipid complexation, one first has to understand what happens when a flexible polymer adsorbs to a flexible surface. The process of adsorption itself is a separate problem[9] that I shall not analyze here, but will concentrate instead on the state of DNA when it is already adsorbed tightly to the surface. Assuming that DNA in close association with a flexible bilayer surface will locally modify its curvature in the direction perpendicular to its long axis (Figure 1), one can derive an effective anisotropic attraction between different polymer segments mediated by the membrane elasticity. Even if there is no other anisotropic interaction between DNA segments of the

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general type described in ref 6, this interaction alone is capable of inducing nematic ordering of adsorbed DNA. Nematic order coupled to the preferred curvature of the bilayer membrane in close proximity to the DNA could furthermore lead to a tubulization transition, similar to the curvature instability from anisotropic membrane inclusions,[78] and encapsulation of DNA within a lipid bilayer tubule (Figure 1). This I believe to be an effective triggering mechanism for encapsulation of DNA and later restructuration of the complete genosome. What I intend to investigate are the details and limitations of this process.

If I consider the supporting lipid bilayer to be described in Monge parameterization as $z = \zeta(x,y) = \zeta(\vec{\rho})$, where obviously $\vec{\rho} = (x,y)$ is a 2-D radius vector, then the Hamiltonian of the adsorbed polymer described by a position vector of the nth monomer $\mathbf{r}(n) = (x(n),y(n),z(n))$ with $\dot{\vec{\mathbf{r}}}(n) = \mathrm{d}\vec{\mathbf{r}}(n)/\mathrm{d}n$, has the form

$$\beta H_{\mathrm{P}}(\vec{\mathbf{r}}(n)) = {}^1/_2\beta\epsilon\int_0^N \ddot{\vec{\mathbf{r}}}(n)^2\,\mathrm{d}n = {}^1/_2\beta\epsilon\int_0^N (\ddot{\vec{\rho}}(n)^2 + \ddot{\zeta}(n)^2)\,\mathrm{d}n \quad (1)$$

where N is the total length of the polymer, ϵ is its curvature modulus, and β is the inverse thermal energy. This form of the polymer elastic free energy already assumes that the polymer chain is so tightly adsorbed to the surface that there is no difference between adsorption and total embedding. This is the lesson of DNA adsorption experiments performed by Yang et al.[4]

For the level of our theory (mean-field) it is irrelevant whether we deal with a single polymer chain or many polymer chains, and the results for the two cases are closely connected. In fact if I have M chains, the monomer density simply gets multipied by M. To the polymer Hamiltonian (eq 1) I add first of all the Helfrich−Canham−Evans elastic energy of the supporting membrane, which in the limit of small deformations assumes the form

$$\beta H_{\mathrm{M}}(\zeta(\vec{\rho})) = {}^1/_2\beta K_{\mathrm{c}}\int d^2\vec{\rho}\ H^2 - \beta\bar{\kappa}\int^2\vec{\rho}\ K \qquad (2)$$

where $H = {}^1/_2\ \mathrm{Tr}\ K_k^i = \nabla_\perp^2\zeta(\vec{\rho})$ is the local mean curvature, $K = \det K_k^i$ is the Gaussian curvature, and $K_k^i(\vec{\rho})$ is the curvature tensor of the supporting membrane. I assume that the bare membrane prefers planar conformations, thus setting $\bar{\kappa} \sim K_{\mathrm{c}}$.

The last term in the overall energy expression is the coupling between the membrane curvature and the embedded polymer. I assume that the polymer adsorbs only to one side of the membrane, thus breaking its transverse up−down symmetry. The appropriate coupling has to be of the general form (Model I in the language of ref 10)

$$\beta H_{\mathrm{MP}}(\vec{\rho}(n),\zeta(\vec{\rho})) = -\beta K_{\mathrm{c}}c_0\int d^2\vec{\rho}\ S^{ik}(\vec{\rho})\ K_{ik}(\zeta(\vec{\rho})) \qquad (3)$$

where $S^{ik}(\vec{\rho})$ is a traceless 2-D tensor connected with the orientational state of the surface-embedded polymer. Since we assume that only orientationally ordered polymer states couple with the supporting surface curvature, we have to set

$$S^{ik}(\vec{\rho}) = \sigma^{ik}(\vec{\rho}) - {}^1/_2\delta_{ik}\ \mathrm{Tr}\ \sigma^{ik}(\vec{\rho}) \qquad (4)$$

where

$$\sigma_{ik}(\vec{\rho}) = \int_0^N \mathrm{d}n\ \dot{\vec{\rho}}_i(n)\ \dot{\vec{\rho}}_k(n)\ \delta(\vec{\rho}-\vec{\rho}(n)) \qquad (5)$$

is the orientational tensor of the polymer chain,[11] with $\delta(\vec{\rho})$ being the 2-D Dirac delta function. In the case of many chains this expression simply changes with $\int_0^N \mathrm{d}n \rightarrow \sum_{i=1}^M \int_0^N \mathrm{d}n_i$. In this way disordered polymer states with $\sigma_{ik} \sim \delta_{ik}$ are truly uncoupled from the membrane curvature. c_0 is on the order of the microscopic curvature induced by a single polymer molecule in the direction perpendicular to its long axis (Figure 1).

As noted in our previous work,[12] $\sigma_{ik}(\vec{\rho})$ is also one of the collective variables that can simplify an approximate evaluation of the partition function for an embedded polymer. If one ignores fluctuations, one can demonstrate that $\sigma_{ik}(\vec{\rho})$ satisfies the equality $\mathrm{Tr}\ \sigma_{ik}(\vec{\rho}) = \rho(\vec{\rho})$,[11] valid standardly in 3-D, where $\rho(\vec{\rho})$ is now the polymer surface density, defined as

$$\rho(\vec{\rho}) = \int_0^N \mathrm{d}n\ \delta(\vec{\rho}-\vec{\rho}(n)) \qquad (6)$$

Again for many chains I have $\int_0^N \mathrm{d}n \rightarrow \sum_{i=1}^M \int_0^N \mathrm{d}n_i$. On the mean-field level this simply means that the monomer density gets multiplied by M.

The evaluation of the partition function for the system defined with $H_{\mathrm{P}} + H_{\mathrm{M}} + H_{\mathrm{MP}}$ now proceeds through the collective variables $\sigma_{ik}(\vec{\rho})$ and $\rho(\vec{\rho})$. In the mean-field limit where all the collective variables and physical fields are constant along the surface, the free energy (F) can be derived approximately in the form of an extremum of[12]

$$\frac{F}{A} \approx \mathrm{Extremum}\left[\frac{F_{\mathrm{P}}(\psi_{ik},\phi)}{A} + \frac{1}{2}\beta K_c(\mathrm{Tr}\ K_{ik}^2) + \frac{1}{2}\frac{\beta\epsilon}{\rho}(\sigma_{ik}K_{ik})^2 + \beta K_{\mathrm{c}}c_0\left(K_{ik}\sigma_{ik} - \frac{1}{2}(\mathrm{Tr}\ K_{ik})(\mathrm{Tr}\ \sigma_{ik})\right) - \rho\phi - \sigma_{ik}\psi_{ik}\right] \quad (7)$$

where A is the total area of the supporting surface. Here the second term is the membrane elastic energy while the third term is due to embedding or strong adsorption of the polymer chains to the membrane. It stems from the term $\ddot{\zeta}(n)^2$ in the elastic energy of the polymer chains (eq 1); see ref 12 for details. The next term in eq 7 is the lowest order coupling term between the membrane curvature and the polymer orientation, both described through the respective tensors K_{ik} and σ_{ik}, allowed by symmetry.[10] The fields ψ_{ik} and ϕ are actually Legendre functions that permit the transformation from the original variables $\dot{\vec{\rho}}_i(n)$ and $\vec{\rho}_i(n)$ to the collective variables $\sigma_{ik}(\vec{\rho})$ and $\rho(\vec{\rho})$. Quantities without the explicit coordinate dependence are mean-field values. The above free energy has to be extremized with respect to all the Legendre fields as well as K_{ik}, σ_{ik}, and ρ. In the above formalism $F_{\mathrm{P}}(\psi_{ik},\phi)$ is simply the free energy of a single polymer chain subjected to the external fields ψ_{ik} and ϕ. It can be derived in an explicit form only within the *mean-field* ansatz as[11,13]

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$$F_{\mathrm{P}}(\psi_{ik},t_{ik},\phi) = \frac{N}{\sqrt{2}}(\sum_{\alpha})\sqrt{\left(\frac{\psi_{\alpha}}{\beta\epsilon}\right)} + N\phi \qquad (8)$$

where the index α refers to the eigenvalues, so that ψ_{α} is the indexed eigenvalues of ψ_{ik}. The reader will find all the relevant details of the above calculation in, ref 12, eq 7 being just a simplified ($\lambda = 0$, $u = 0$) version of eq 30 in ref 12.

Ignoring the fluctuations in the mean-field approximation has the effect of leaving the curvature modulus of the membrane at its bare value. Their inclusion would simply renormalize the effective value of K_c, as well as the strength of the anisotropic attraction between polymer segments (see below). Qualitatively none of the conclusions for the surface nematic ordering transition reached on the mean-field level would be invalidated.

The free energy (eq 7) is not complete but can be shown to be a reasonable approximation in the case where both the polymer and as the supporting membrane are stiff, i.e. $\beta\epsilon$, $\beta K_c >> 1$.

The extremal Euler–Lagrange (EL) equations that now follow from the above free energy essentially describe two types of coupling between the membrane curvature and orientational order of the embedded polymers, one local and the other one nonlocal. Firstly locally the membrane curvature is directly proportional to the polymer orientational order at that same position. Secondly polymer order at two different positions along the membrane is coupled through membrane elasticity *via* a nematic-like interaction. The two together account for the ordering transition of the adsorbed polymer as well as the associated change in the membrane shape.

Starting from the Euler–Lagrange equations, one can derive explicit forms of the two couplings in the following form. The first type of coupling is expressed formally as

$$K_{ik} = c_0\,({}^{1}/_{2}\delta_{ik}(\mathrm{Tr}\ \sigma_{ik}) - \sigma_{ik}) - c_0\left(\frac{\epsilon}{K_c\rho}\right)\frac{{}^{1}/_{2}(\mathrm{Tr}\ \sigma_{ik})^2 - \sigma_{ik}^2}{1 + (\epsilon/K_c\rho)(\mathrm{Tr}\ \sigma^2{}_{ik})}\cdot\sigma_{ik} \qquad (9)$$

the curvature tensor of the membrane being thus proportional to the orientational tensor of the polymers. The effect of membrane elasticity-mediated nematic interactions between polymer order at two different positions along the membrane can be deduced from the second EL equation in the form

$$(\sigma_{\alpha} - \sigma_{\beta})\left(\frac{\rho_2}{8\beta\epsilon}\,\frac{\mathrm{Tr}\ \sigma_{ik}}{\sigma_{\alpha}^2\sigma_{\beta}^2} - \beta K_c c_0^2 f\left(\frac{\epsilon}{K_c\rho},\sigma_{ik}\right)\right) = 0 \qquad (10)$$

α and β index the two eigenvalues, while the function $f(x,\sigma_{ik})$ is defined as

$$f(x,\sigma_{ik}) = x\,\frac{(\mathrm{Tr}\ \sigma_{ik})^2}{2} + \frac{1}{1 + x(\mathrm{Tr}\ \sigma_{ik}^2)} + \frac{\frac{1}{2}(\mathrm{Tr}\ \sigma_{ik})^2 - \mathrm{Tr}\ \sigma ik^2}{(1 + x(\mathrm{Tr}\ \sigma_{ik}^2))^2}x \qquad (11)$$

Though it is difficult to see the effect of membrane-mediated nematic interactions from the above formalism, comparison with a theory that would take into account an explicit intersegment contact potential of a nematic form and strength u_{a}

$$V(\vec{\mathbf{r}}(n),\vec{\mathbf{r}}(m)) = {}^{1}/_{2}\int_0^N\int_0^N \mathrm{d}n\ \mathrm{d}m\ (\dot{\vec{\mathbf{r}}}(n) \times \dot{\vec{r}}(m))^2 u_a\delta(\vec{\mathbf{r}}(n) - \vec{\mathbf{r}}(m)) \qquad (12)$$

leads to the conclusion that in the mean-field approximation the membrane-mediated interactions leading to eq 10 are essentially equivalent to such a potential with $u_{\mathrm{a}} \sim c_0(1 + 1/[\gamma(1 + \gamma)])^{1/2}$. Here and below, $\gamma = \epsilon\rho/2K_c$, which is a dimensionless quantity considering the definition of the polymer elastic energy in eq 1.

Assuming now that the mean-field equations can be solved by means of the ansatz

$$\sigma_{\alpha} = \frac{\rho}{2}(1 + S) \qquad (13)$$

where S is the orientational order parameter, with $S = 0$ corresponding to a completely disordered case and $S = 1$ to a perfect nematic order, I obtain from eq 10

$$S\left[(1 - S^2)^2 - \frac{4}{(\beta\epsilon\rho c_0)^2}\,\frac{\gamma(1 + \gamma(1 + S^2))^2}{1 + 2\gamma + 2\gamma^2(1 + S^2) + \gamma^3(1 + S^2)^2}\right] = 0 \qquad (14)$$

where the elastic coupling parameter γ weights the relative strengths of polymer vs membrane elastic moduli. The solutions of eq 14 describe a second-order transition between an orientationally disordered state ($S = 0$) and a nematic state ($S \neq 0$) if the condition $4/[(\beta\epsilon\rho c_0)^2] \leq 1 + 1/[(1 + \gamma)]$ is satisfied (Figure 2). In the above analysis this transition is driven purely by nonlocal membrane-mediated nematic interactions between polymer segments. In case there are other anisotropic forces,[6] they would simply displace the transition point.

The consequences of the ordering of the polymers for the supporting surface are profound. If one evaluates the membrane curvature through eq 9, it is obviously vanishing in the disordered state of the polymers. In the nematic phase two different things can happen, depending on the magnitude of the elastic coupling γ. From eq 9 I obtain the following eigenvalues for the curvature tensor

$$K_{\alpha} = c_0\frac{\rho}{2}\,\frac{S(1 + \gamma) + \gamma S^2}{1 + \gamma(1 + S^2)} \qquad (15)$$

which lead to the following two limits in the case of membrane-dominated and/or polymer-dominated elastic coupling

$$\lim_{\gamma << 1} K_{\alpha} = \left\{\begin{matrix} -\dfrac{c_0\rho}{2}S \\ +\dfrac{c_0{}^2\rho}{2}S \end{matrix}\right\} \qquad (16)$$

and

$$\lim_{\gamma >> 1} K_{\alpha} = \left\{\begin{matrix} -\dfrac{c_0\rho}{2}S\ \dfrac{S - 1}{1 + S^2} \\ +\dfrac{c_0\rho}{2}S\ \dfrac{S + 1}{1 + S^2} \end{matrix}\right\} \qquad (17)$$

In the limit of perfect nematic ordering of the embedded polymer, i.e. $S \rightarrow 1$, saddle-like shapes of the supporting membrane would be promoted in the case of membrane elasticity-dominated elastic coupling, while tubular or cylindrical membrane shapes would be preferred in the opposite limit. The dimensionless elastic coupling ratio γ is thus a crucial parameter leading to a bifurcating

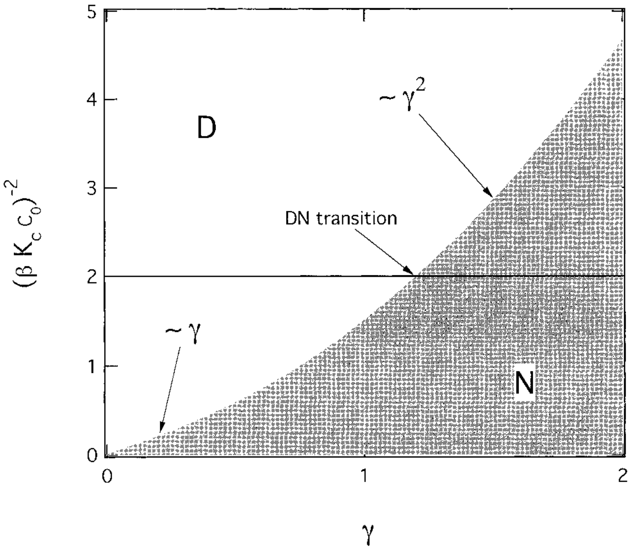

Figure 2. Schematic presentation of the solutions to eq 14. The phase diagram has disordered (D) and nematic (N) regions. The phase boundary follows a linear dependence on γ for $\gamma << 1$ and quadratic dependence on γ for $\gamma >> 1$. Changing the adsorbed DNA density ρ has the effect of transversing the phase diagram horizontally, i.e. $\gamma = (\epsilon/2K_c)\rho$. An example of the iso-$(\beta K_c c_0)$ line is shown on the figure. The value of $(\beta K_c c_0)$ determines if in the anisotropic DNA state the supporting membrane will prefer cylindrical $\gamma >> 1$ or saddle-like $\gamma << 1$ shapes.

scenario for the membrane shape after the adsorbed polymer has undergone the ordering transition.

Right at the disordered–nematic (DN) transition point the scale of the typical induced curvature radius R of the membrane changes with the nematic order parameter as

$$\lim_{\gamma >> 1} \frac{\partial}{\partial S}\left(\frac{1}{R}\right)_{\mathrm{DN}} \sim \epsilon^{-1}$$

$$\lim_{\gamma << 1} \frac{\partial}{\partial S}\left(\frac{1}{R}\right)_{\mathrm{DN}} \sim (c_0 \epsilon K_c)^{-1} \tag{18}$$

In the first limit it is thus set only by the persistence length of the polymer, while in the second limit it depends on both polymer and membrane parameters. One concludes that at the transition point for $\gamma >> 1$ typical induced membrane curvatures would be $R \sim L_p$, where L_p is the polymer persistence length, $L_p \sim 500$ Å in the case of DNA. The final curvature of the cylindrical DNA–membrane aggregate would be set by the surface DNA density as $R \sim (c_0\rho)^{-1}$ and would thus be quite small, assuming that $c_0 \sim D_{\mathrm{DNA}}$, where D_{DNA} is on the order of the radius of DNA ~ 20–30 Å, as indeed observed in experiments.[2]

If one assumes now nematic order along the surface, with polymers lying parallel at a distance $l_\perp$ apart, then the elastic coupling for the case of DNA on a cationic lipid membrane is obtained as $\gamma = \epsilon\rho/2K_c \sim kTL_p/2K_c l_\perp \sim \frac{1}{2}(kT/K_c)(500/30)$, where the value for $l_\perp$ can be estimated experimentally.[3,4] It appears that for cationic lipids γ would turn out to be on the order of ~1, meaning there is no clear cut preference for any of the two scenarios. However, different, though not completely conclusive, experiments by Gershon et al., Sternberg et al., and Felgner et al.[2,14] show that DNA encapsulation leads to elongated complexes, which, translated into the language developed in this contribution, would mean that nevertheless $\gamma >> 1$. The larger than expected value of γ is probably connected with the effect of the coupling between the elastic properties of the lipid membrane and the ionic strength of the surrounding medium on the encapsulation ability of the cationic membrane. This fine tuning of γ, which was not explicitly considered in this contribution, could make it sufficiently large in order to favor the scenario of tubular encapsulation.

The theory presented in this paper is of course approximate. The main limitation of the above analysis is the mean-field assumption. Relaxing this constraint would change the effective anisotropic forces acting between polymer segments. Most notably elastic fluctuations of the underlying membrane would contribute additional Casimir-like interactions of a general nematic type.[6] These interactions would tend to displace the DN transition point but would not modify the qualitative features of the scenario of the DNA encapsulation proposed here.

In this short Letter we have established and analyzed a close connection between the orientational order of strongly adsorbed (=embedded) polymers on an elastic membrane surface and the equilibrium shape of the supporting membrane. The main conclusion is that orientational ordering of the polymers promotes curving of the supporting surface, leading eventually to complete encapsulation of the embedded polymers.

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