Coiling of Cylindrical Membrane Stacks with Anchored Polymers

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We study experimentally a coiling instability of cylindrical multilamellar stacks of phospholipid membranes, induced by polymers with hydrophobic anchors grafted along their hydrophilic backbone. We interpret our experimental results in terms of a model in which local membrane curvature and polymer concentration are coupled. The model predicts the occurrence of maximally tight coils above a threshold polymer concentration. Indeed, only maximally tight coils are observed experimentally. Our system is unique in that coils form in the absence of twist and adhesion.

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The coil motif is ubiquitous in a wide range of natural contexts. One-dimensional filaments of mutant bacteria [1], supercoiled DNA molecules [2], and tendrils of climbing plants [3] all exhibit a writhing instability as a result of forcing or interaction with an external agent. Such systems are dominated by elastic properties, and the appearance of coils is a result of the relief of twist. In this paper we show that coiling can also be effected in cylindrical multilamellar tubes of phospholipid bilayers, by anchoring hydrophilic polymers with hydrophobic side groups grafted along the backbone. This system is unique in that, in contrast with the above examples, fluid membranes cannot support any twist. Yet coils are formed in the system, and are stable for a very long time.

Our system is representative of a wide class of systems of membranes with embedded inclusions, such as biological cells with membrane-associated proteins. Other examples include erythrocyte ghosts incorporating amphipathic drugs [4] and liposomes with covalently attached polymers used for drug delivery [5–7] (for additional examples see [8]). Our experiments give us a unique opportunity to study a wide variety of phenomena induced by such inclusions in a relatively simple and controlled environment.

The cylindrical multilamellar tubes, called *myelin figures*, consist of a large number of bilayers reaching almost to the core [9,10]. Adjacent bilayers are separated by thin hydration layers. Coiling of myelin figures has already been observed in phospholipid binary mixtures [11] when the constituents undergo a phase separation process triggered by the addition of Ca^{++}. Coiled myelin figures of egg-yolk phosphatidylcholine (egg-pc) have also been reported [12,13]. In both cases [11–13] coiling was attributed to surface adhesion. The novelty of our work is that coiling occurs in the absence of both adhesion and twist. Our experiments clearly show that surface adhesion is negligible in our system.

We believe that in our system coiling results from a coupling between the polymer concentration and local membrane curvature, induced by anchoring of the polymer in the membrane. We show below that a high enough polymer density together with the constraints imposed by the cylindrical geometry of the tube destabilizes the straight tube. Coiling as a manifestation of this instability is proposed here for the first time.

The coupling between membrane curvature and polymers has been considered both theoretically [14–17] and experimentally [7,8,18–21]. The theoretical studies emphasize the polymer backbone and its effect on the elastic properties of the membrane. They do not consider the mobility of anchors embedded in the bilayers. We believe this effect is important in our system, and attempt to capture it in the model presented below.

In our experiments, tubular membrane stacks were made of stearoyl-oleoyl-phosphatidylcholine (SOPC), with C_{18} alkyl chains. The polymer we used is hydrophilic dextran (MW 162 000 g/mol) functionalized both with C_{16} alkyl chains and dodecanoicnitrobenzoxadiazole (NBD) chains as fluorescent markers. The hydrophobic anchors, distributed statistically along the backbone (about 1 alkyl chain for 25 glucose units) are C_{16} long. On average there are 4 persistence lengths between consecutive anchors. Therefore, the extension of each polymer molecule on the two-dimensional membrane is much larger than its extension into the third dimension. Samples were prepared by drying a 0.5–1.0 μl droplet of SOPC dissolved in a 4:1 chloroform-methanol solution (7.35 mg/ml) on a glass slide. The sample was then closed, and hydration was effected by injecting a polymer solution of known concentration, c_p, into the cell. The development of myelin structures and their coiling were followed using phase contrast microscopy and recorded on video. Our experiments were conducted at room temperature, well above the solid-liquid transition for SOPC.

For small values of c_p we observe myelin figures, which display a clear tendency to straighten over lengths many times larger than their diameter. As c_p is increased,

myelin figures become more floppy and curved. For large enough values of c_p, a writhing instability sets in and tubes bend, forming irregular structures, single coils (Fig. 1), and double helices (Fig. 2). We emphasize that all the coiled structures we observe are maximally tight as they form and do not tighten up gradually (see Fig. 2); no loose coils have been found (unlike Sakurai *et al.* [12,13]). In quantitative terms, this means that the curvature of the tube central line, C, is $C \approx \frac{1}{r_0}$, where r_0 is the radius of the tube. Figure 2 also demonstrates that there is no adhesion between membrane surfaces in our system; tube segments that are in contact in Fig. 2b are separated in Fig. 2c.

We stress that while the polymer concentration in solution, c_p, is known, we do not control the surface concentration on the bilayers. The slow evolution of some of the structures we observe is consistent with a possible variation of this concentration over time. As a first step towards a theoretical understanding of this system, we neglect this slow evolution.

We now make the following assumptions: (i) anchors penetrate the membrane to a depth of about half a bilayer. This is because the anchor length is comparable to that of a lipid, and the large hydrophilic backbone to which anchors are attached cannot penetrate a bilayer. We assume that these anchors and the polymer backbone induce a local spontaneous curvature, H_0 [4,8,22]. (ii) Polymer molecules are present everywhere in the system, including inner regions of the myelin figures. We have carried out fluorescence microscopy experiments and found that the polymer is present between layers of the stack in significant concentrations (additional details of the experiments will be published elsewhere). (iii) Given that bilayers are in a liquidlike state, polymer molecules can diffuse on the membrane. Thus, the energy of the system is lowered if they migrate to regions where the mean surface curvature H is closer to H_0. (iv) Bilayers in a myelin figure maintain a constant area and are strongly constrained by the geometry of the stack, which prevents them from buckling. We therefore regard the myelin figures as flexible cylinders having a fixed circular cross section everywhere along their axis. These assumptions are strongly supported by our experimental observations. Note that the central line of the tube can bend. However, as a consequence of the above assumptions, its length remains fixed.

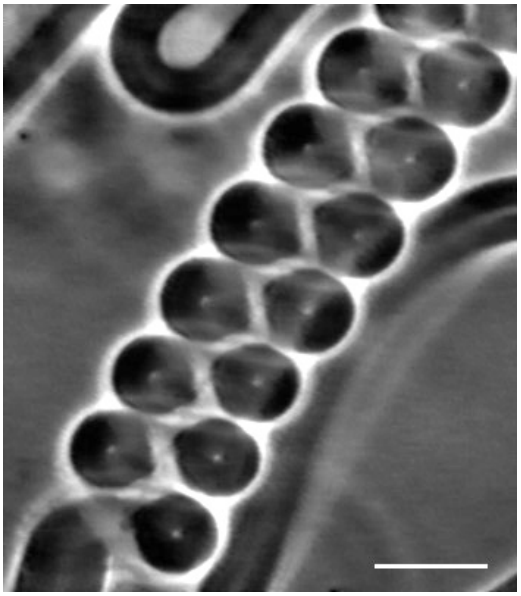

FIG. 1. A single-stranded coil. This structure formed one hour after hydration in a solution containing 0.02 mg/ml polymer, at a rate of 0.15 pitch/min. Phase contrast images a slice of the coil, showing bilayers filling up almost the whole tube, though a narrow water-filled core can still be observed along the tube axis. The bar represents 10 μm.

Based on these assumptions, we present a simple model which captures the essential physical features of the experimental system, and accounts for many of the experimental observations. We represent each bilayer as two square lattices (in the spirit of lattice-gas models), corresponding to the outer and inner monolayers. We associate 2 degrees of freedom with each lattice site, which corresponds to a membrane patch of area a^2. The first is the local mean surface curvature, H. The second is a binary occupation variable, which takes the values 1 or 0 when the site is occupied or unoccupied, respectively, by an anchored molecule. This molecule induces a local spontaneous curvature, H_0. The area of a site, a^2, and the value of H_0 depend on the specific mechanism responsible for the spontaneous curvature. Thus, if H_0 is induced by individual anchors, the area of a site is microscopic ($a^2 \sim 60$ Å^2). If, on the other hand, the spontaneous curvature is induced by the polymer backbone, the area of a site is mesoscopic ($a^2 \sim 6 \times 10^5$ Å^2).

By convention, the curvatures of the inner and outer layers have opposite signs at the same position. Within the model, the energy of the system is a sum of the curvature energies of the individual area patches: $2\kappa H^2$

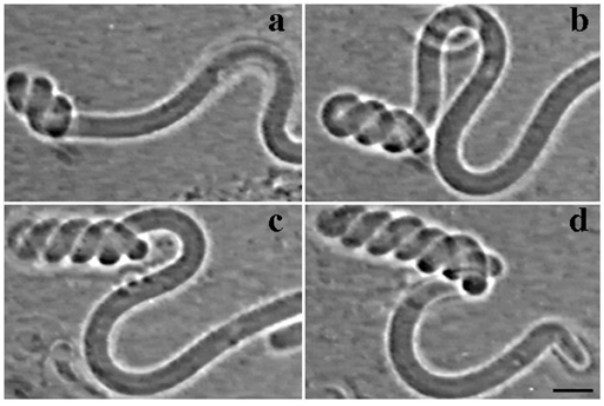

FIG. 2. Formation of a double helix. After bending, the tip slides along the tube, coiling at the same time. The four frames were taken at times (a) 0, (b) 20, (c) 30, and (d) 40 min after the double helix was first seen. The bar represents 20 μm.

for a vacant site, and $2\kappa'(H - H_0)^2$ for an occupied site. κ and κ' are the local bending rigidities of a single layer without and with an anchored molecule, respectively. We suppose $\kappa' > \kappa$; this is consistent with models of composite membranes [16,17,22] (although the systems these models describe are different from ours).

In order to find the equilibrium state of a tube, we have to calculate its free energy. This free energy depends on the curvature of its central line, C, and on ρ, the average of the occupation variable (ρa^2 is the average density of the anchored molecules). If the spontaneous curvature, H_0, is large enough, the free energy of a bent tube is lower than that of a straight one. To show this we evaluate separately the energy and the entropy of the system.

Consider one cylindrical bilayer of length l and circular cross section of radius r, with the same average occupancy, ρ, on both sides. Let us calculate the energy cost of bending the bilayer into a portion of a coil with central line curvature C in two steps. First, the energy of a bent cylindrical bilayer with a *homogeneous* distribution of anchored molecules is $E_{\rm hom} = E_{\rm hom}^{\rm out} + E_{\rm hom}^{\rm in}$, where $E_{\rm hom}^{\rm out,in}$ are the energies of the outer and inner monolayers. According to our model

$$E_{\rm hom}^{\rm out,in}(C) = 2\rho\kappa' \int dA[H(C) - H_0]^2 + 2(1 - \rho)\kappa \int dA[H(C)]^2, \qquad (1)$$

where $H(C)$ is the local membrane curvature, and is known for a cylindrical geometry. The expressions for $E_{\rm hom}^{\rm out}$ and $E_{\rm hom}^{\rm in}$ are different since the curvatures of the inner and outer monolayers have opposite signs. Note that when $C \neq 0$, $H(C)$ varies around the bent cylinder. For our geometry the total mean curvature $\int dA\, H(C) = 2\pi l$ for the outer monolayer, while for the inner one $\int dA\, H(C) = -2\pi l$, independent of the central line curvature C. Thus the cost of bending the cylindrical membrane, keeping the distribution of anchored molecules homogeneous is $\Delta E_{\rm hom} = 4[\rho\kappa' + (1 - \rho)\kappa] \int dA[H(C)]^2$, independent of the value of H_0. Note that the term containing H_0^2 is independent of C and therefore does not contribute to the cost of bending.

Next, we take into account inhomogeneities in the distribution of anchored molecules around the tube. Such inhomogeneities reduce the energy if these molecules move to regions of membrane curvature closer to H_0 in both the outer and inner monolayers. The full calculation shows that the energy gain, $\Delta E_{\rm inhom}(C, H_0)$, depends linearly on the spontaneous curvature and can become arbitrarily large for large values of H_0.

As for the entropy of the system, we assume that the dominant contribution is the entropy of mixing of occupied and vacant sites. This entropy is larger when the distribution of anchored molecules around the cylindrical bilayer is homogeneous, favoring a straight tube. However, it does not depend on the spontaneous curvature. Therefore, if H_0 is large enough, the energy gain due to $\Delta E_{\rm inhom}(C, H_0)$ is larger than the free energy cost coming from $\Delta E_{\rm hom}$ and the entropy of mixing. In this case, the tube is bent at equilibrium. It remains to be shown that such an equilibrium state can occur for reasonable and physical values of the model parameters.

We have carried out the full calculation of the free energy as a function of the central line curvature, the average occupancy and the spontaneous curvature (details will be published elsewhere). In the limit of a thick tube (a realistic case), the calculation can be done analytically, and the free energy of the entire tube (summing over all the bilayers) reads

$$F(C, \rho) = \frac{2l\kappa_{\rm tube}(\rho)}{r_0} \cdot \ln\left[\frac{2}{1 + \sqrt{1 - (Cr_0)^2}}\right], \qquad (2)$$

where $\kappa_{\rm tube}(\rho)$ is the effective bending rigidity of the entire tube, and l is its length.

Figure 3 shows the typical dependence of $\kappa_{\rm tube}$ on ρ for large enough values of H_0 (solid line). When $\rho < \rho_-$,

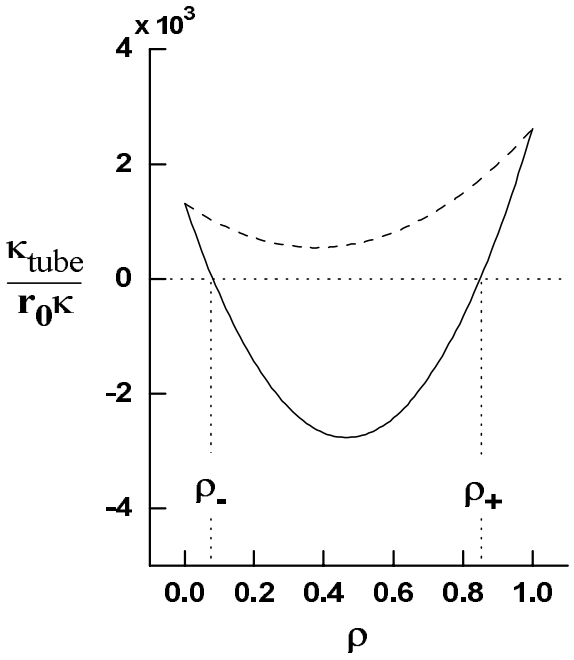

FIG. 3. The effective bending modulus of the tube, $\kappa_{\rm tube}$, is parabolic in the average occupancy, ρ. We have used the following values of the parameters: $\kappa = 10k_BT$, $\kappa' = 2\kappa$, and $r_0 = 5\ \mu$m. We find that $\kappa_{\rm tube}$ depends on a and H_0 only through the product aH_0. The solid curve represents $\kappa_{\rm tube}$ for $aH_0 = 0.3$. The physical meaning of this number depends on the specific mechanism responsible for the spontaneous curvature. For example, if the spontaneous curvature is induced by the polymer backbone, $a \sim 800$ Å. For the low polymer concentration in our experiments (i.e., in the vicinity of $\rho = \rho_-$), this corresponds to a membrane spontaneous curvature $\rho H_0 \sim 0.3\mu^{-1}$. When aH_0 is large enough ($aH_0 > 0.19$ for the values of κ and κ' we have used), $\kappa_{\rm tube} < 0$ between ρ_- and ρ_+. For smaller values of aH_0, $\kappa_{\rm tube} > 0$ for all values of ρ. The dashed curve corresponds to $aH_0 = 0.16$.

κ_{tube} is positive and decreases with ρ. In this regime the minimum of the free energy is at $C = 0$. Therefore, the tube is predicted to be straight on the average, but with enhanced fluctuations due to the smaller bending modulus. Although anchored molecules increase the local bending rigidity of the membrane ($\kappa' > \kappa$), their mobility makes it *easier* to bend the tube.

For $\rho_- < \rho < \rho_+$, κ_{tube} is negative, and the free energy decreases upon bending with its minimum at the maximally possible central line curvature $C = 1/r_0$ (in agreement with the qualitative argument outlined above). Hence, ρ_- is a threshold occupancy above which straight tubes are unstable and form *maximally tight* coiled structures. Above ρ_+ straight tubes become stable again; however, this regime is probably unreachable in our experiments, since too large a polymer concentration destroys the bilayers.

A salient feature of our model is that it precludes loose coiled structures; i.e., tubes are either uncoiled ($\rho < \rho_-$ and $\rho > \rho_+$) or *maximally tight* and coiled ($\rho_- < \rho < \rho_+$). This is consistent with our experimental observations. In addition, according to the model all the coils with $C = 1/r_0$ are equally probable. One should therefore expect to see irregular coils without a well defined chirality as well as regular helices, all of which we indeed observe.

A somewhat similar instability of a flat membrane due to coupling between membrane shape and local spontaneous curvature has been discussed by Leibler [23] and by Safran [24]. There is, however, a fundamental difference between these models and ours. They considered an unconstrained flat membrane, whereas our membranes are severely constrained by the cylindrical geometry of the tube. These geometrical constraints play a crucial role in determining the shapes of the observed structures, and the final state of the system.

We believe coiling occurs mainly due to the spontaneous curvature induced by the anchored molecules as well as their mobility. Our model emphasizes these aspects, and neglects others such as interactions between polymer molecules. We do not expect these effects to change the qualitative behavior of the system. So far, we have not been able to identify the precise mechanism by which the polymer induces spontaneous curvature. We intend to study polymer length effects on the coiling phenomenon in order to partially address this issue.

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