

## Instability of myelin tubes under dehydration: Deswelling of layered cylindrical structures

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We report experimental observations of an undulational instability of myelin figures. Motivated by this, we examine theoretically the deformation and possible instability of concentric, cylindrical, multilamellar membrane structures. Under conditions of osmotic stress (swelling or dehydration), we find a stable, deformed state in which the layer deformation is given by  $\delta R \propto r \sqrt{B_A / (hB)}$ , where  $B_A$  is the area compression modulus,  $B$  is the interlayer compression modulus, and  $h$  is the repeat distance of layers. Also, above a finite threshold of dehydration (or osmotic stress), we find that the system becomes unstable to undulations, first with a characteristic wavelength of order  $\sqrt{\xi d_0}$ , where  $\xi$  is the standard smectic penetration depth and  $d_0$  is the thickness of dehydrated region.

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The swelling of bulk phospholipid by water at a temperature higher than the chain melting temperature can lead to the growth of multilayer lipid tubules from the interface of bulk lipid and water [1–5]. These tubules are of mesoscopic dimensions with a diameter about 20–40  $\mu\text{m}$ . They have the symmetry of a smectic-A liquid crystal and a strong optical anisotropy. Both electron microscopy [6] and x-ray diffraction show a concentric multilayer structure of these lipid tubes with a layer spacing about 60  $\text{\AA}$  [7]. Due to the similarity of their structural features to those of nerve myelin sheaths, these tubules are often referred to as “myelin tubes” or “myelin figures.” The morphological features of myelin tubes have been classified into two steps according to the time of growth [3]. During the first step, simple tubes grow into bulk water with an initial growth rate in length about 1  $\mu\text{m}/\text{sec}$ . The diameter and number of layers of tubes appear to remain constant during this process and the growth rate is inversely proportional to the square root of growth time [2,4]. The growth almost stops in the second step, which is characterized by the formation of complicated morphologies of myelin tubes, such as helical and coiling forms, apparently in order to maximize their intermembrane attraction [8].

In our experiments, we have observed an instability of myelin tubes under dehydration by isolating a single myelin tube from others (during the first step, as shown in Fig. 1) and allowing the bulk water to evaporate in a controlled open chamber. Figure 1 is a low resolution view of dimyristoyl phosphatidylcholine (DMPC) lipid tubes using differential interference contrast microscopy. During this dehydration step, periodic bumps with a wavelength about 1  $\mu\text{m}$  are observed on the surface of myelin tubes as shown in Fig. 2(a). As water further evaporates, these bumps grow into arms, as shown in Figs. 2(b) and 2(c), and a similar instability can occur for these long arms. As far as we know, this is the first reported observation of the instability of myelin tubes under dehydration. After the bulk water dries out, the tubular structure of myelins disappear and a mosaic structure is observed. Although the full structural change of myelin

tubes under dehydration is very complicated and highly non-linear, the initial instability of myelin tubes under dehydration poses an interesting deswelling of layered structures in cylindrical geometry, which we examine theoretically.

Here, we suggest an explanation for the initial morphological change of myelin tubes observed in the dehydration step based on energetic arguments. We first demonstrate and examine a stable configuration of myelin under conditions of osmotic stress, in which layer bending does not occur. We then examine the limit of stability of this to undulations, which relieve in-plane compression. We predict the appearance of undulations with a finite wavelength that can be of order micrometers. We then speculate on the kinetic aspects of the deswelling process. We note that, unlike the pearling instability [9], the compression and bending energies already favor finite wavelength undulations in this case, and the appearance of such an instability need not be due to hydrodynamic effects. These, we argue will not significantly change our results for slow drying rates.

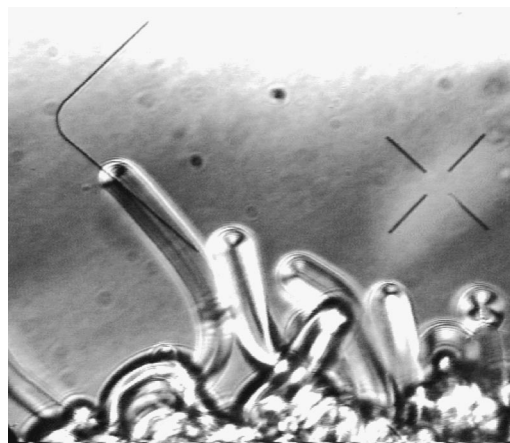


FIG. 1. A low resolution view of myelin tubes during the first step. The diameter of tubes is about 20–40  $\mu\text{m}$ . An isolated myelin tube can be obtained by introducing a water flow across the chamber to disintegrate the bulk lipid.

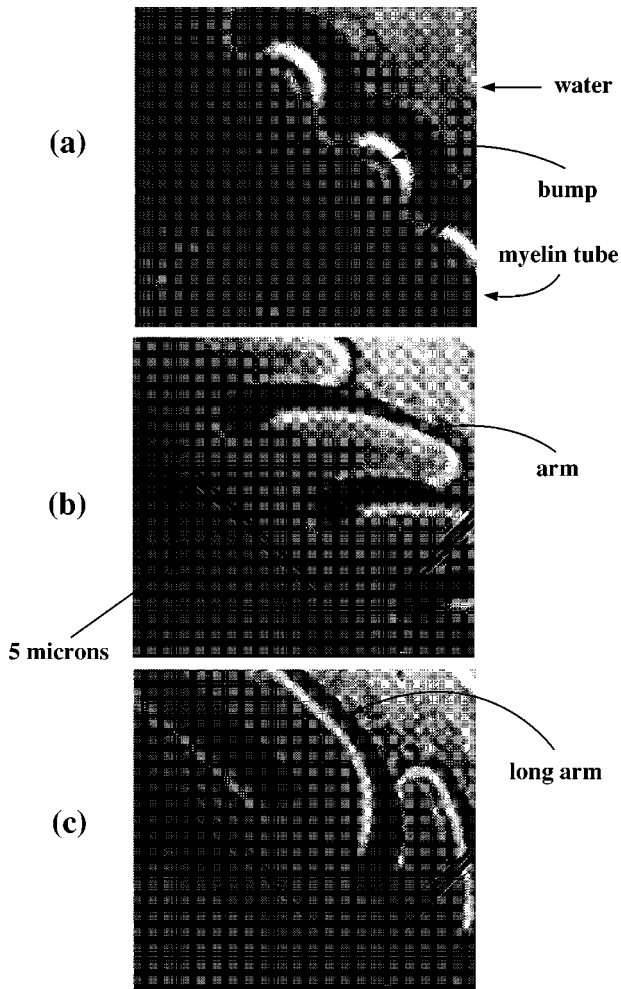


FIG. 2. A series of photographs of a myelin tube showing the instability under dehydration at different times. The wavelength of undulation is about  $1 \mu\text{m}$ . At later times, the amplitude of undulation increases and a bump becomes an arm.

For a unilamellar tube, dehydration tends to decrease its radius but increase its length. Surface undulation is disfavored because it increases tube's bending energy. For a multilamellar tube, in addition to the bending energy of each layer, interlayer and in-plane compression energies need to be considered. It can be shown that, in the limit of long cylinders, a change in length becomes energetically prohibitive. We show below that undulation of dehydrated layers occurs only for finite dehydration of a multilamellar tube in the presence of an undisturbed core, while in-plane compression occurs (without undulations) below this threshold. Although a one-dimensional undulation along the long axis of myelin tubes is reported in this paper, we cannot exclude the possibility of a general two-dimensional undulation along the surface of the tube.

We begin our discussion by considering the dehydration of a myelin tube in bulk water. The length  $L$  of the myelin tube is assumed to be much larger than its radius  $R_0$ . As water evaporates, the osmotic pressure changes due to the increased impurity concentration in water. In response to the drop in chemical potential outside, water molecules in the

outer layers of the myelin tube will diffuse out to the bulk and the myelin tube will deform. This process is relatively fast, as the membranes are permeable to water. Migration of lipids will be ignored here, since this is a much slower process due to their low solubility in water. The removal of water thus results in a radial deformation  $\delta R < 0$  of a cylinder of initial radius  $R$ . Through second order in this deformation, the area change of this cylinder is given by  $\delta A = 2\pi \int dz (\delta R + R |\nabla \delta R|^2 / 2)$ . Here, the gradient operator is taken to act in the plane of the membrane.

We shall find below that there is an initial uniform (in  $z$ ) deformation  $\delta \bar{R}$  that, beyond a finite threshold, drives an undulatory instability. We take this undulatory contribution  $\delta R'$  to be sinusoidal. Thus, in order to determine the onset of this instability, we shall keep terms only through second order in  $\delta R'$ . With this in mind, we take the area strain to be  $\epsilon = \delta \bar{R} / R + |\nabla \delta R'|^2 / 2$ . The first term in  $\epsilon$  is the area deformation due to the average radius change in a cylinder, while the second term is due to layer undulation [10]. This strain is actually averaged over one period along the cylinder axis. For fluid membranes, it would not be correct to integrate the square of the *local* area strain, as molecules are free to migrate within each layer. Rather, the integrated square of  $\epsilon$  above properly accounts for the energy associated with area compression, through second order in  $\delta R'$ .

We parametrize the structure by the radial position  $r$  before dehydration, and the position  $z$  along the axis [11]. Near the onset of the instability, the displacement field  $\delta R(r, z) = \delta \bar{R} + \delta R'$  is expected to consist of a displacement  $\delta \bar{R}(r)$  that is uniform along the cylinder axis plus a sinusoidally varying displacement  $\delta R'(r, z) \propto \cos(qz)$ . For such a deformation, the associated energy density in our model can be expressed as

$$f = \frac{1}{2} \frac{B_A}{h} \left( \frac{\delta \bar{R}}{R} + \frac{1}{2} |\nabla \delta R'|^2 \right)^2 + \frac{1}{2} B \left( \frac{\partial \delta R}{\partial r} \right)^2 + \frac{1}{2} K (\nabla^2 \delta R')^2, \quad (1)$$

where  $B_A$  is the in-plane compression modulus,  $B$  is the interlayer compression modulus,  $K$  is the bulk bending rigidity, and  $h$  is the repeat distance of layers before dehydration.

Before discussing possible undulatory instabilities of the myelin tubes, we first consider deswelling of the structure in the absence of undulations (i.e.,  $\delta R' = 0$ ). Here, we ignore the bending contributions. (Note the small curvatures, with radii of order several micrometers, involved in especially the outer layers of the myelin, where the deswelling is greatest.) In this case, the shift in chemical potential of water molecules in a layer at radius  $r'$  due to layer deformation can be calculated as

$$\begin{aligned} \mu(r') - \mu(r'') &\equiv \frac{\delta F}{\delta N(r')} - \frac{\delta F}{\delta N(r'')} \\ &= v_0 \int_{r'}^{r''} \left( \frac{B_A}{h} \frac{\delta R}{r^2} - \frac{B}{r} \frac{\partial \delta R}{\partial r} - B \frac{\partial^2 \delta R}{\partial r^2} \right) dr, \end{aligned} \quad (2)$$

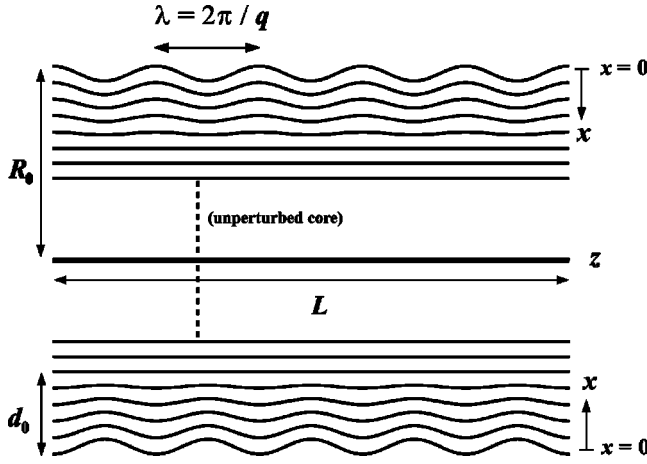


FIG. 3. Schematic representation of the undulation profile of a myelin tube: a cross-sectional view showing different layers.

where  $F \equiv \int (2\pi r f) dr$  is the free energy per unit length,  $N(r')$  represents the number of water molecules per unit length in a layer at radius  $r'$ , and  $v_0$  is the molecular volume. In Eq. (2) we have used the relation  $2\pi r [\delta R(r)] = v_0 \delta N(r') \theta(r-r')$  resulting from simple geometry, where the Heaviside step function  $\theta(r-r')$  ensures that  $\delta R(r)$  can only be changed by changing  $N$  at  $r' < r$ . Uniformity of the chemical potential in equilibrium (at least over time scales for which lipid transport is negligible) requires the integrand in Eq. (2) to vanish. The solution to this differential equation is

$$\delta R \propto r^\alpha, \quad (3)$$

where  $\alpha = \sqrt{B_A / (Bh)}$ . This result applies for both swelling ( $\delta R > 0$ ) and dehydration ( $\delta R < 0$ ), where the amplitude of the displacement field is determined by the value of the chemical potential outside the cylinder. The result is valid near the surface where bending contributions are negligible, and is sensible in that for large  $B_A$  there will be little penetration of the dehydration profile in from the surface, while for large  $B$ , the profile is more uniform toward the outside of the cylinder.

Upon changing the osmotic pressure in the bulk water a dehydration profile will extend throughout the cylindrical structure. The initial stage is a smooth deswelling with only a change in the radius of each layer as calculated above. This also involves bending energy, as the dehydration profile extends deeper and becomes more pronounced. For a finite degree of deswelling, however, the layers can bend to relieve the intra and interlayer compression energies. Hence, we now show that incorporating the bending degrees of freedom leads to an instability to a surface undulation. For simplicity, we assume a single mode approximation and a linear deformation profile that extends to a depth  $d_0$  below the outer surface  $R_0$  of the myelin, i.e.,  $\delta R(r=R_0-x) = (1-x/d_0)(\bar{a} + a' \cos(qz))$ , where  $\bar{a}$  and  $a'$  are the uniform and undulatory deformation amplitudes,  $x$  is the depth below the outermost layer, and  $q$  is the wave number (as shown in Fig. 3). This

approximates the state of the system at an instant in time during dehydration, rather than an equilibrium profile.

In this case, the modulated part of the total free energy per unit length can then be calculated from Eq. (1) as

$$\Delta F_{\text{mod}} \approx \pi d_0 \left[ \left( \frac{K}{6R_0^3} + \frac{BR_0}{2d_0^2} \right) - \frac{B_A q^2 \bar{a}}{8h} + \frac{KR_0 q^4}{6} \right] a'^2. \quad (4)$$

Minimizing Eq. (4) with respect to the wave number  $q$ , the optimal wave number is given by  $(q^*)^2 \approx 3B_A \bar{a} / 8hKR_0$ . Such an undulation is favored only when  $\Delta F_{\text{mod}} \leq 0$ . This results in a threshold

$$\bar{a}_c = \sqrt{\frac{1}{3} \frac{8hR_0 \sqrt{KB}}{B_A d_0}}, \quad (5)$$

below which the system is stable. The corresponding reduction in volume of the cylinder is then given by  $\Delta V_c = 2\pi R_0 L \bar{a}_c$ . At the onset of instability, we find that the wavelength of the instability is

$$\lambda \approx 5 \sqrt{\xi d_0}, \quad (6)$$

which depends only on material parameters and on the depth of the deswelling profile. Note that any finite range dehydration profile would lead to a similar result, with different prefactors. For lipid bilayers, the standard smectic penetration depth  $\xi = \sqrt{K/B}$  is about  $0.01 \mu\text{m}$ . Therefore the undulation wavelength is of order  $1 \mu\text{m}$  if  $d_0$  is a few microns. We have considered a one-dimensional undulation along the long axis of the myelin tube. Additional undulation along the  $\varphi$  direction might exist. However, our calculation shows that such an undulation is disfavored if the undulation amplitude and wavelength are isotropic in both directions. We also note that a change in layer spacing due to surface area compression is much smaller than that due to dehydration (their ratio is less than  $d_0/R_0$ ) and can be neglected.

The discussion thus far has been based on energetic considerations. A dynamic calculation would incorporate the energetic driving force for the instability and dissipative damping, and conservation of water in a given layer during the instability should shift the wavelength  $\lambda$  to a smaller value [12]. Moreover, there is the possibility that large effluence of water induced by a severe dehydration could induce a dynamic, rather than quasiequilibrium instability, through nonlinear convective couplings [13].

In the early stage of dehydration, the dominant process limiting diffusion is the compression energy between layers, and the associated diffusion equation for water molecules to cross bilayers near the surface  $r=R_0$  is

$$\frac{\partial \phi}{\partial t} \approx BD \frac{\partial^2 \phi}{\partial r^2}, \quad (7)$$

where  $\phi$  is a coarse-grained composition variable representing the relative change in water composition (ratio of interlayer spacing after dehydration to spacing before dehydration), and  $D$  is a mobility proportional to the membrane

permeability. As dehydration proceeds the intralayer compression elasticity, controlled by  $B_A$ , will eventually slow this process. In our calculation above, we have approximated the profile  $\phi(r,t)$  by a piecewise linear profile of depth  $d_0$ , but other bounded profiles lead to similar results. At a time  $t$  after the external osmotic pressure has been quenched, the penetration of the dehydration layer will thus scale as  $d_0(t) \approx \sqrt{BDt}$ . Using the piecewise-linear approximation above for  $\delta R$ , the total volume change of the outermost cylinder is given by  $\Delta V \approx \pi LR_0 d_0 (1 - \phi_0)$ . The change in layer spacing of the outermost layer is  $\delta R(r) - \delta R(r-h) = h(\phi_0 - 1)$ .

Our instability condition, Eq. (5), assumes a given profile and dehydration depth. Assuming further the local equilibrium assumption, that the chemical potential of the outermost layer is equal to the chemical potential  $\mu_{ext}$  specified by the bulk, leads to the relation  $\mu_{ext} \approx -v_0 B(1 - \phi_0)$ . [Corrections to this involve terms that go as  $1/R_0$  and  $1/R_0^2$  from Eq. (2)]. A typical scenario would involve sealing the chamber after a rapid dehydration to maintain, for a large water-to-lipid ratio, a constant osmotic pressure  $\Pi = \mu_{ext}/v_0$  outside, and hence, the relation  $(1 - \phi_0) \sim \Pi/B$  at the outermost layer. In this case the critical size of the dehydrated region is  $d_0^c \approx \sqrt{10h\xi R_0 B^2 / (B_A \Pi)}$ . Equivalently, the critical time scale for the instability to occur after the dehydration is  $\tau \approx h\xi B R_0 / (DB_A \Pi)$ , which increases linearly with  $R_0$ . The initial undulation wavelength is proportional to  $R_0^{1/4}$ .

One could envision another extreme of imposing a constant dehydration flux  $j$  by, for example, exposing the chamber to an unsaturated vapor that continually increases the impurity concentration  $c_{imp}$  in the bulk water, hence increasing the osmotic pressure and the driving force for dehydration. In this case the details of the timescale necessary for the instability would depend on comparing the rate of change of  $\phi_0$  to the time for diffusion out of the multilamellar state, although the relation between outer composition and overall

volume change,  $\Delta V_c = 2\pi R_0 L \bar{a}_c$ , would still hold. For the outermost layer, the stress of layers must balance the osmotic pressure difference to the bulk water. In this case  $\phi_0(t) \sim 1 - jt$ , for a constant flux  $j \sim kT \dot{c}_{imp}/B$  where  $kT$  is the thermal energy, and the critical value of  $\phi_0$  is  $\phi_0^c \approx 1 - 10Bh\xi R_0 / (B_A d_0^2)$ . The delay time before undulation is  $\tau \approx \sqrt{h\xi R_0 / (B_A D j)} \propto \sqrt{R_0/j}$ . The initial undulation wavelength is proportional to  $(R_0/j)^{1/8}$ . At a higher drying rate, the thickness of the dehydrated region is smaller at the onset of instability, which corresponds to a smaller onset undulation wavelength. Therefore, in an experiment with controllable humidity, the undulation wavelength can be varied by controlling the relative humidity. Nevertheless, we do not have quantitative measurements yet.

To conclude, we have studied the deformation of myelin tubes under dehydration by curvature elasticity. The cylindrical geometry is stable for infinitesimal dehydration, and the surface area of dehydrated layers is reduced due to an in-plane compression. If an equilibrium is achieved at this stage, the equilibrium radius deformation profile can be described by  $\delta R \propto r \sqrt{B_A / (hB)}$ . For finite dehydration, the cylindrical geometry becomes unstable and undulation occurs to reduce the in-plane compression energy. The critical volume change for this instability is estimated to be  $\Delta V_c \approx 30h\xi L R_0^2 B / (d_0 B_A)$ . The undulation wavelength is predicted to be about  $5\sqrt{\xi d_0}$  and is comparable to the observed undulation wavelength.

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