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Buckling of Lipid Tubules in Shrinking Liquid Droplets

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ABSTRACT

Self-assembled hollow lipid tubules are interesting and potentially useful supramolecular structures. Here, we study the deformation of lipid tubules of 1,2-*bis*(*tricosa*-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) trapped inside liquid droplets on glass substrates. The interface tension of the shrinking liquid droplets exerts a compression force on the ends of the trapped lipid tubules, and causes them to buckle. This provides a method to measure their mechanical properties. The Young's modulus of the $DC_{8,9}PC$ lipid tubules is estimated to ~1.07 GPa. As the strain energy of the buckled tubules builds up, they poke through the interface of shrinking liquid droplets and then adhere onto glass substrates to form looplike shapes.

Self-assembled supramolecular structures are a subject of current interest.[1] This field is driven by not only a desire for understanding the formation of biological structures but also technological needs. As the push for the miniaturization of structures and devices continues, conventional fabrication techniques are struggling to keep up. Molecular self-assembly is becoming increasingly popular as an alternative approach to synthesize technologically useful nanostructures.[2] Self-assembled hollow lipid tubules represent interesting and potentially useful supramolecular architectures.[3] It has been shown that a number of synthetic lipids with modified head groups or acyl chains are able to self-assemble into tubule structures in solutions.[4−5] The diameters of self-assembled lipid tubules span the range between 10 nm and 2.0 μm depending on the nature of the lipid molecules and the condition under which self-assembly occurs.

Recently, self-assembled hollow lipid tubules of 1,2-*bis*(*tricosa*-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}$PC) have been extensively studied. The $DC_{8,9}PC$ is a chiral molecule with eight methylenes between the ester and the diacetylenic group and nine methylenes between the diacetylenic and the terminal methyl groups. The tubule structures can be easily formed by cooling the $DC_{8,9}PC$ bilayer from the fluid L_α phase, where the chains are disordered, into the ordered L_β gel phase.[6] The diameter of $DC_{8,9}PC$ tubules formed in ethanol/water solutions is ~0.5 μm with little variation, but the length of $DC_{8,9}PC$ tubules varies from 5 to 100 μm. Fourier-transform infrared spectroscopy[7] and X-ray diffraction[8] have shown that the acyl chains of $DC_{8,9}PC$ molecules are highly ordered and tilted in the tubule walls. The chirality of $DC_{8,9}PC$ tubules is reflected by large peaks and is visible in their circular dichroism (CD) spectra.[9] The molecular tilt ordering[10] and the tilt-induced helical ripples[11] in $DC_{8,9}PC$ tubules have been observed using liquid-crystal optical amplification and atomic force microscopy, respectively. The technological applications of $DC_{8,9}PC$ lipid tubules in inorganic cylinder synthesis[12−13] and drug delivery[14−15] rely on two essential properties. First, they have hollow cylindrical shapes with crystalline bilayer walls. Second, they have relatively rigid structures. The rigidity of lipid tubules is evident from their straight shapes in optical and electron microscopy observations. However, the mechanical properties such as bending rigidity and Young's modulus of $DC_{8,9}PC$ tubules have not been characterized explicitly.

It is known that the solid−liquid−air triple contact line is associated with a number of surface processes including wetting, dewetting, coating, painting, and lubrication.[16] Recently, the moving contact line of liquid droplets during wetting and dewetting on surfaces has been used in nanofluidic technology and nanofabrication. For example, the contact line of a receding meniscus has been used to stretch and align chain molecules such as DNA molecules,[17−20] peptide tapes,[21] lipid tubules,[22] and tobacco mosaic virus.[23] Such a technique, referred as "molecular combing", is based on the surface tension of the liquid−air interface that pulls the molecules along the moving direction of the contact line. As a result, the molecules are aligned along the direction of motion of the meniscus. The contact line of a receding meniscus has also been used to bend DNA molecules, which

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are grafted at both ends on a vinyl-terminated silane monolayer into a looplike shape.[24] In this paper, we study the deformation of $DC_{8,9}PC$ lipid tubules trapped inside shrinking liquid droplets on glass substrates. The interface tension of the lipid droplets exerts a compression force on the ends of the trapped tubules and causes them to buckle. This provides a simple method to study the bending rigidities of lipid tubules. The Young's modulus of $DC_{8,9}PC$ tubules is estimated to be ~1.074 GPa. As the strain energy of buckled tubules builds up, they poke through the liquid droplet interface and then adhere to glass substrates to form looplike shapes.

Lipid tubules used in our experiments were prepared by cooling a 5 mg/mL suspension of 1,2-*bis*(*tricosa*-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$) (Avanti Polar Lipids, Alabaster, AL) in ethanol/water (70:30 v/v) from 60 °C to room temperature at a rate of ~0.5 °C/min. The polymerization of $DC_{8,9}PC$ tubule suspension was performed with UV irradiation (254 nm) for 20 min at room temperature. Glass slides were used as a hydrophilic substrate with a water contact angle of ~10°. 1-Dodecanethiol (DDT) (Aldrich) was dissolved on ethanol. Self-assembled DDT monolayers prepared on Au-coated mica (Molecular Imaging Inc.) through absorption from 1 mM DDT ethanol solution provided a hydrophobic substrate with a water contact angle of ~105°. An optical microscope (BX 40 Olympus) with a digital camera (Olympus C2020 Zoom) was used to image $DC_{8,9}PC$ lipid tubules on these substrates. Optical microscopy image analysis was performed with MATLAB software. Lipid tubules in optical microscopy images were digitized into pixels, producing the spatial coordinates of each point along their long axis and then traced with a morphological operator. The traced contour length and the end-to-end distance were measured directly from optical images. Transmission electron microscopy (TEM) measurements of lipid tubules dried on carbon-coated grids were performed on a Tecnai F30 microscope with an accelerating voltage of 300 kV at room temperature.

In our experiments, a drop of a dilute tubule solution was applied to a glass substrate and then allowed to dry in air at room temperature. We note that the drop first wets the glass surface to form a thin liquid film. Dewetting from the glass surface causes holes to open up in the film, leading to the formation of a number of small liquid droplets. Figure 1 shows optical microscopy images of tubules trapped in a shrinking lipid droplet. As the solvent continuously evaporates, the radius of the droplet base decreases. The long tubule that is circled in Figure 1a remains straight until it encounters the shrinking contact line. In this case, the gas−liquid interface applies a compressive load on the ends of the tubule and causes it to buckle (Figure 1b). As the strain energy builds up in the buckled tubule, it pokes through the interface (Figure 1c) and adheres to the glass substrate instead of snapping back to form a straight shape (Figure 1d). As can be seen, the short tubule, which is circled in Figure 1c, directly pokes the interface without buckling. The competition between the elasticity of the tubules and the compressive force determines whether the tubule remains straight or buckles.

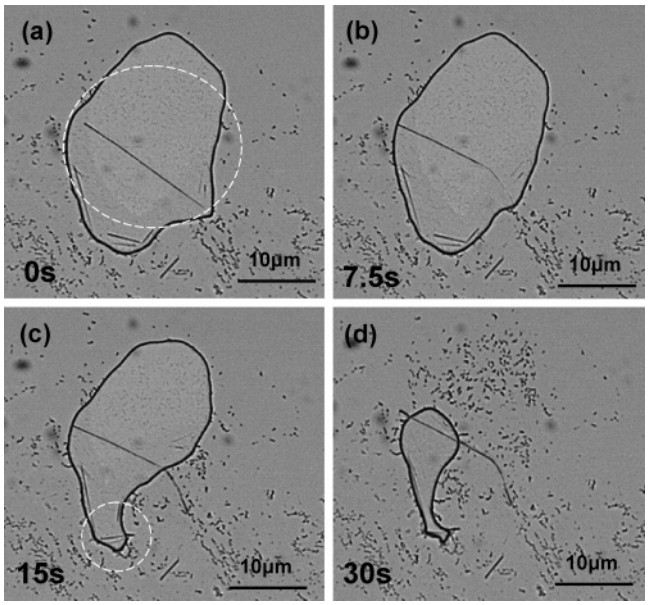

Figure 1. Optical microscopy images of the deformation of lipid tubules trapped in a shrinking liquid droplet on a glass substrate. The liquid droplet was dried in air at ~23°. The radius of the droplet base decreases with the solvent evaporation. The long lipid tubule circled in (a) remains straight until it encounters the shrinking contact line. The interface tension causes the lipid tubule to buckle (b). As the strain builds up in the bent tubule, it pokes through the interface (c) and adheres on the glass substrate (d). The short lipid tubule circled in (c) directly pokes the interface without buckling.

The buckling of tubules trapped in droplets allows their bending rigidity to be estimated. The critical force for tubule buckling can be written as follows:[25]

$$F_{\mathrm{crit}} = \frac{YI\pi^2}{l^2} \tag{1}$$

where l is the length of unbent tubules and YI, the bending rigidity, is the product of the Young's modulus (Y) and the area moment (I) of inertia. For a lipid tubule trapped in a liquid droplet, the compression force that the liquid interface exerts on the tubule ends is related to the interface tension:[26−27] $F_{\mathrm{comp}} = 2\pi r_e \gamma \cos\theta$, where r_e is the external radius of the lipid tubule, γ is the tension of the interface, and θ is the contact angle at the interface (Figure 2). If $F_{\mathrm{comp}} > F_{\mathrm{crit}}$, the lipid tubule buckles, otherwise, it remains straight. The balancing of the two forces yields a critical length (l_c). Below l_c, the lipid tubule remains straight, and above l_c, it buckles. The Young's modulus (Y) is given by:

$$E = \frac{2r_e\gamma l_c^{\,2}\cos\theta}{I\pi} \tag{2}$$

For a water droplet on a hydrophilic glass surface, interface tension (γ) is ~72 mN/m at room temperature. The lipid tubules terminated by the phosphochloline groups are hy-

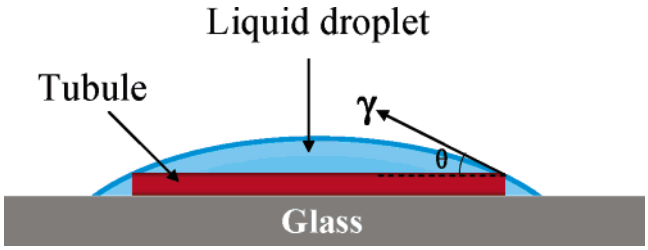

Figure 2. Schematic of a lipid tubule with both ends trapped a liquid droplet on a glass substrate, where γ is the interface tension of liquid droplet, and θ is the contact angle of water with the hydrophilic tubule. The force (F_{comp}), which is applied on the tubule end, is related to the interface tension: $F_{comp} = 2\pi r_e \gamma \cos\theta$, where r_e is the external radius of the lipid tubule.

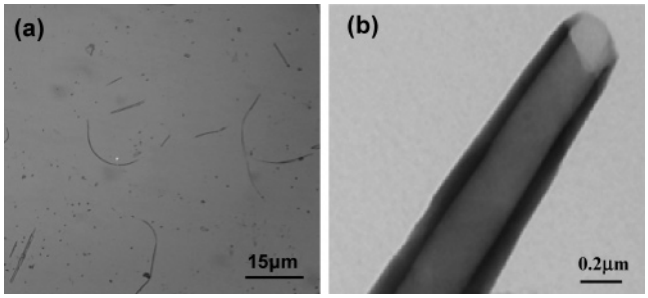

Figure 3. (a) Optical microscopy image of lipid tubules with different lengths on a glass substrate. Dilute tubule solution was applied on the glass substrate. This image was taken after the tubule solution was completely dried in air at ~23°. (b) TEM image of a straight lipid tubule dried on carbon coated grids. The TEM image was taken at room temperature.

drophilic. The contact angle (θ) of water with the hydrophilic lipid tubules is expected to be very low. Given the small diameter of lipid tubules, it is difficult to measure the water contact angle on the lipid tubules. It is reasonable to assume $\cos\theta \sim 1$. Figure 3a shows an optical microscopy image of the tubules with different lengths on a glass substrate. As can be seen, longer tubules are semiflexible and bent into looplike shapes, while shorter tubules are stiff and remain straight. On the basis of the observation of tubules bent on glass substrates, we found that the critical length (l_c) was 15 $\pm$ 0.2 μm. The $DC_{8,9}PC$ tubules formed in ethanol/water typically have 10 bilayers in tubule walls.[28] Figure 3b is a transmission electron microscopy image of a typical $DC_{8,9}PC$ lipid tubule. The external radius (r_e) and internal radius (r_i) of the hollow tubules are found to be ~0.25 and ~0.17 μm, respectively. The area moment (I) of inertia for a hollow lipid tubule can be written as: $I = (\pi(r_e^4 - r_i^4))/4$. Given these parameters, we estimate that the Young's modulus (Y) of the $DC_{8,9}PC$ lipid tubule to be ~1.074 GPa.

We now compare the estimated Young's modulus for the $DC_{8,9}PC$ tubules with those of other self-assembled supramolecular structures. Frusawa et al.[29] used an optical tweezer to bend a lipid nanotube of cardanyl-β-D-gluocopyranoside, which was immobilized on a glass slide. The nanotube had a diameter of 50 nm and a wall thickness of 40 nm. By ignoring the interaction between the nanotube and the glass surface, they estimated the Young's modulus of the nanotube to be ~720 MPa. The Young's modulus of tubelin microtubules, which were measured by observing their thermal fluctuations, was ~1 GPa.[30] Recently, de Pablo et al.[31] used atomic force microscope indentation to measure the local mechanical properties of tubelin microtubules and found that their Young's modulus is ~800 MPa based on a finite element calculation.

We note that Elbaum et al.[26] carried out a similar approach in measuring the mechanical properties of tubelin microtubules. They studied the deformation of tubelin microtubules in a lipid vesicle with micropipette aspiration. The tubelin microtubule buckled as aspiration increased the tension in the membrane of the lipid vesicle. The IY of the tubelin microtubules was estimated to be about 8.6×10^{-23} Nm.[2] In their approach, tubulin polymerized inside the lipid vesicles to form a microtubule. But not all self-assembled supramolecular tubes and fibers can be easily synthesized in lipid vesicles. From this point of view, our approach is more simple and practical in studying mechanical properties of preformed soft tubes and fibers.

The strain energy of a rod confined in a liquid droplet can be written as follows:[27]

$$E = \frac{YI\pi^2}{3l}\left(1 - \frac{d}{l}\right)\left(3 + \sqrt{1 - \frac{d}{l}}\right) \qquad (3)$$

where l is the contour length of the rod, and d is the diameter of the droplet. For a bent tubule with the d/l ratio of 0.86 trapped inside a shrinking liquid droplet (Figure 1b), the elastic energy of the tubule is calculated to be $\sim 1.67 \times 10^{-11}$ Nm. The elastic energy continuously increases as the d/l ratio decreases. If the strain energy of the buckled tubule is larger than the interface energy, it pokes through the interface (Figure 1c). As can be seen in Figure 3a, there are no cracks and breaks observed for the bent lipid tubules on glass substrates.

The $DC_{8,9}PC$ lipid tubules, which are terminated by the phosphochloline groups, are hydrophilic. By exposing patterned substrates with alternating hydrophilic and hydrophobic stripes to a tubule solution, we found that the $DC_{8,9}PC$ tubules selectively adsorb onto the hydrophilic stripes,[22] suggesting that the interaction of the $DC_{8,9}PC$ lipid tubules with the hydrophilic surfaces is stronger than that with the hydrophobic surfaces. In a previous publication,[32] we showed that lipid tubules could also be bent into looplike shapes on hydrophobic DDT monolayers by shrinking liquid droplets. By digitizing the trace of these bent lipid tubules from optical microscopy images into pixels to give the spatial coordinates of each position along these bent tubules with MATLAB software, we measured the contour lengths (L_c) and end-to-end distances (D_e) of these bent lipid tubules on hydrophilic glass surfaces and hydrophobic DDT monolayers. Their distribution histograms are shown in Figure 4a and b, respectively. It is clear from these histograms that there is almost no difference in the extension of these bent tubules on the glass surface and the DDT monolayer. The average extension, defined as $(L_c - D_e)/L_c$, is ~14% on both substrates. This suggests that the interaction of the lipid tubules with the substrates inside liquid droplets does not

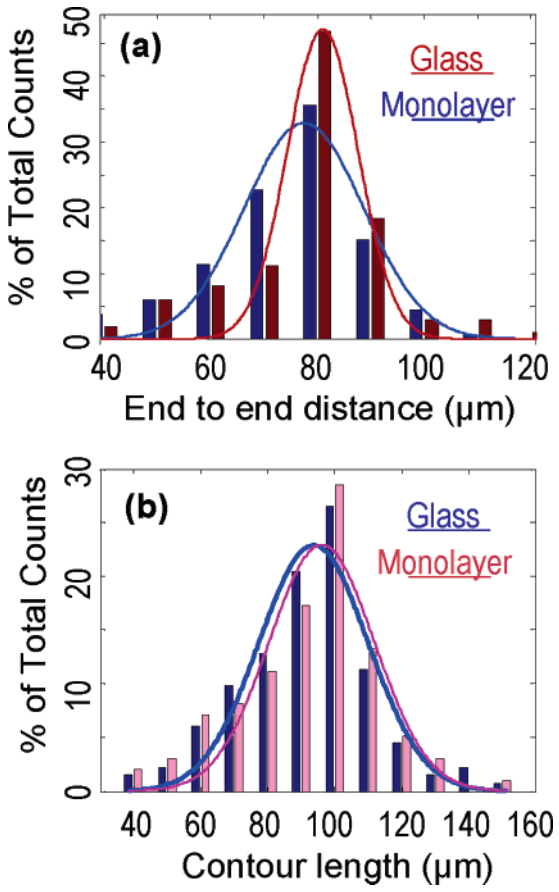

Figure 4. Histograms of the end-to-end distances (a) and the contour lengths (b) of bent lipid tubules on hydrophilic glass substrates and hydrophobic DDT monolayers with Gaussian distributions. The contour length and end-to-end distance were measured by digitizing the trace of these bent lipid tubules from optical microscopy images into pixels to give the spatial coordinates of each position along the tubules with MATLAB software.

affect their buckling. So we can ignore the substrate effect in calculating the Young's modulus (Y) of lipid tubules.

In conclusion, we have studied the deformation of lipid tubules trapped in shrinking liquid droplets. The interface tension of the liquid droplets exerts a compression force on the ends of trapped lipid tubules and causes them to buckle. This allows the Young's modulus of lipid tubules to be estimated. As the elastic energy builds up, the buckled lipid tubules poke through the interfaces and immobilize on the substrates to form looplike shapes. It is believed that the approach can also be applied to other soft tubes and fibers, which opens up a simple way to estimate their mechanical properties.

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