Nanomechanics of membrane tubulation and DNA assembly

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(Received 24 September 2002; accepted 21 January 2003)

We report an interesting regime of tubule formation in multilamellar membrane vesicles. An optically trapped bead is used to apply a localized subpicoNewton force on a cationic vesicle to form a membrane tubule. The force extension curves reveal a saturation phase, with the tubule length extending up to tens of microns, beyond a threshold force 0.6 ± 0.2 pN. We then use the tubule as a sensor for monitoring the dynamics of charge induced DNA integration on cationic membrane vesicles. Our results may also have applications in the development of nanowires and nanofluidic devices. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1559632]

Membrane vesicles and tubules have importance in cell biology,[1] nonviral DNA-membrane gene delivery systems,[2] and as conduits for nanolithography.[3] Such conduits may enable the realization of three-dimensional (3D) networks of membrane channels for nanofluidic and template assisted nanowire applications.[4] Micromanipulation experiments, on both artificial and biological membranes, have been used to characterize physical properties of membranes.[5] Networks of membrane tubules have also been observed in biological cells by fluorescence microscopy and are thought to be intermediates of intracellular transport.[6] Recent *in vitro* experiments have shown that localized picoNewton forces exerted by molecular motor proteins induce tubule formation.[7]

In this letter, we study the mechanics of membrane tubulation from multilamellar cationic lipid vesicles using an optical tweezer. The experimentally measured force extension curves reveal a sharp increase in the force required to pull out a tubule of length 5 μm from the vesicle of diameter in the range of 5–10 μm. Beyond a threshold force $\sim 0.6 \pm 0.2$ pN, the tubule length can be increased to a few tens of microns with <0.2 pN increase in the force. The diameter of the tubule is below the diffraction limited optical resolution. The tubule can be pulled out of the same vesicle any number of times with the force extension curves being the same. The curves show no hysteresis during extension and retraction. We also measure the tubule recoil time scales to quantify its elastic parameters. We further show an application of these tubules as sensors for DNA integration. Although DNA-cationic lipid complexes have been studied through bulk experiments,[8] the dynamics of such integration has not been monitored in real time. We find that the integration of DNA molecules on the tubule results in the inelastic deformation of the membrane as a function of time. Within about 5 min of addition of DNA, the integration of the negatively charged DNA polymer on the membrane vesicle leads to formation of lipid-DNA complexes.

Figure 1(a) is a schematic of our experiment. An optical tweezer is constructed, using an Nd–yttritium–aluminum–garnet (YAG) laser, on an inverted optical microscope.[9] A visible laser backscattering method is used to probe the displacement of the 2 μm latex particle from the center of the trap and hence measure the forces acting on the bead.[10] Multilamellar vesicles of didodecyl dimethyl ammonium bromide (DDAB) cationic surfactant molecules are prepared by the hydration method.[11] This method of preparation[12] gives mostly multilamellar vesicles. 50 μl of vesicle solution is transferred onto a clean glass coverslip with a sample well made using an O-ring fixed with paraffin. The sample is then mounted on a piezoelectric (PZT) stage (PhysiK Instruments; 1 nm precision) fixed on the microscope. The sample well is washed [ten times with phosphate buffer saline (PBS) *p*H 6.0, 1× buffer] to remove free vesicles. 2 μm carboxyl coated polybeads (Polysciences) suspended in PBS *p*H 6.0 buffer are added to the sample well (10^6 beads/100 μl sample volume). For the experiments we usually choose an isolated vesicle adhered on the coverslip (diameter in the range of 5–10 μm). The trapped bead and the vesicle are brought in proximity to enable their nonspecific electrostatic adhesion. The PZT stage is then moved in steps of 10 nm away from the trapped bead to form the tubule and the force-extension curve is recorded. Typical recording time is ~ 10 s for 50 μm extension.

In Fig. 1(b) we plot the force-extension curve, which reveals a structural change in tubule formation. The force increases sharply to about 0.4 pN where the tubule length is about 5 μm and then saturates. The increase in force for extending the tubule length from 5 μm to more than 80 μm is less than 0.2 pN. We observe no hysteresis in these highly reproducible curves. We find that the force extension curves do not depend on the rate of pulling (maximum rate in our setup being 5 μm/s). The threshold force varies between 0.4 and 0.8 pN for different vesicles. In 30% of the vesicles, we also find that beyond the threshold force, there is a negative slope in the curve before the onset of tubulation [Fig. 1(b) inset].

In Fig. 2 we plot the recoil time of a tubule, which is measured by switching off the trap at a tubule extension of

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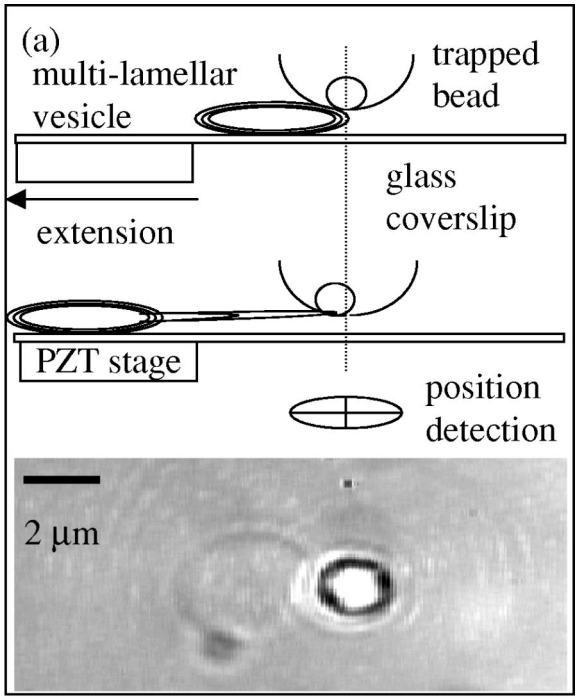

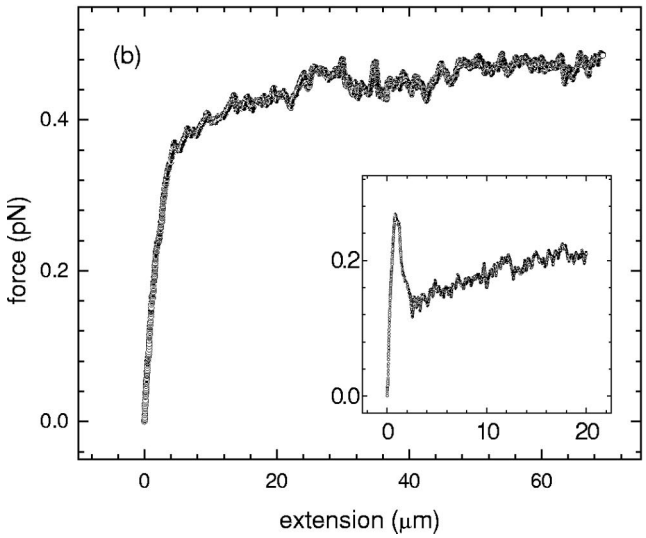

FIG. 1. (a) Schematic of the experiment with the photograph of a bead adhered to a vesicle. The optical trap is used to trap the bead and pull out a membrane tubule by moving the PZT stage, in the direction marked, while monitoring the force on the bead using position sensing quadrant photodiode. (b) Force extension curve of membrane tubulation. Typical recording time is ~10 s for 50 μm extension. The threshold forces required for tubule formation on different vesicles are in the range of 0.4–0.8 pN. Inset shows the force extension curve obtained in about 30% of the vesicles, where a sharp decrease in the force is observed beyond the threshold value. The saturation region, which is the same as in the previous case, is plotted only up to 20 μm.

34 μm (limited by the imaging window of our setup). The images recorded at video rate are digitized to get the recoil time of the tubule-tethered bead. Typical recoil times for different tubules are of the order of 6 s. A linear regime is observed in the initial phase of the recoil, which tapers of as the tubule approaches the vesicle. The terminal velocity ($v = 8.8$ μm/s) is estimated using the linear regime by approximating the bead recoil movement to be in quasistatic equi-

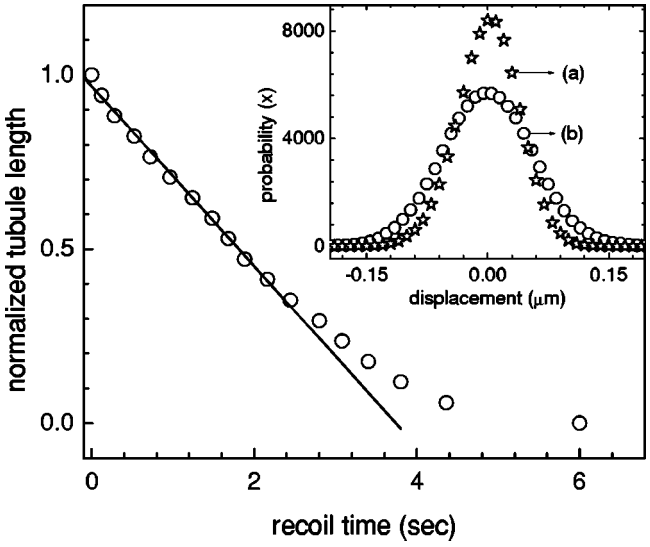

FIG. 2. Recoil dynamics of the extended tubule is recorded by switching off the trap. The linear part in the normalized length vs recoil time plotted for a tubule extension of 34 μm, gives a terminal recoil velocity of 8.8 μm/s. Using this the tubule extension force is estimated to be 0.17 pN. Inset: Fluctuations of the trapped bead (a) fixed on a vesicle (standard deviation 37 nm; variance 13×10^2 nm^2, $k_{\text{eff}}=3\times10^{-6}$ N/m) and (b) free (standard deviation 54 nm; variance 29×10^2 nm^2, $k_{\text{trap}}=1.4\times10^{-6}$ N/m).

librium. The recoil force, $F_{\text{recoil}}=F_{\text{drag}}=6\pi\eta a v=0.17$ pN (where η is the viscosity, a is the bead radius, and v is the terminal velocity) is consistent with the tubule extension force. The membrane tension of vesicles was characterized by monitoring the fluctuations of the trapped bead attached to the vesicle (Fig. 2 inset). This is equivalent to two springs in series, one due to the membrane and other due to the trap. The effective spring constant is given by, $(1/k_{\text{eff}}=1/k_{\text{trap}}+1/\sigma)$. From the measured $k_{\text{eff}}=3\times10^{-6}$ N/m and $k_{\text{trap}}=1.4\times10^{-6}$ N/m, the membrane tension $\sigma=2.6\times10^{-6}$ N/m. The free energy (E) of the membrane bilayer is proportional to the square of its curvature ($E=\frac{1}{2}Bc^2A$), where B is the bending rigidity modulus and A is the surface area.[13,14] Using such a description in our experiment, the tubule radius and the rigidity modulus can be calculated. The tubule radius, $r=f/\pi\sigma=80$ nm, where $f=0.6$ pN is the threshold tubulation force and $\sigma=2.6\times10^{-6}$ N/m is the membrane tension. From the tubule radius $r=80$ nm and the tubulation force $f\sim0.6$ pN, we can also estimate the membrane bending rigidity modulus $B=fr/2\pi\sim8\times10^{-21}$ N m. We use the tubule extension force ($f\sim0.17$ pN), the extension length $\delta L=75$ μm, the initial length $L=5$ μm, and the radius of the tubule ($r\sim80$ nm) to calculate its Young's modulus $Y=fL/\pi r^2\delta L\sim0.5$ Pa.

We now show that the membrane tubules can be used to study DNA self-assembly on cationic membrane bilayers. The negatively charged DNA molecules integrate onto the tubule, with the cationic surfactant molecules acting as counterions. Previous studies in bulk have shown that DNA molecules form interesting structures with cationic lipids under different DNA to lipid charge ratio.[8] Liquid crystalline phases like the DNA intercalated lamellar phases and the inverted hexagonal phases are known to form depending on the DNA and lipid concentrations. Using the tubule as a handle, we directly monitor the change in its elastic parameters due to DNA integration. 5 μl of DNA (Lambda DNA

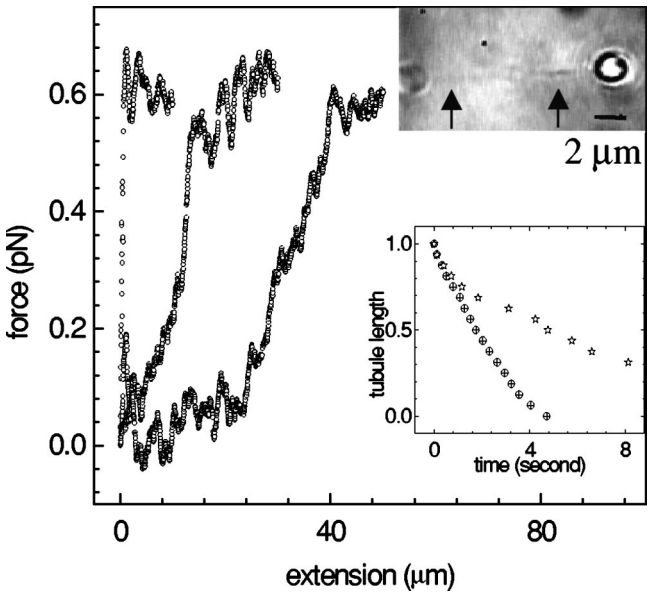

FIG. 3. Force extension curves recorded with DNA assembled membrane tubule showing the onset of entropic response. The three curves show the time evolution (~2 min interval) of DNA integration on the tubule. Inset: the recoil of the membrane tubule before and after DNA integration. The recoil curves with DNA are taken after the tubule becomes optically visible. A photograph of the DNA integrated tubule is also shown.

Hind III, 2 No. SM101 MBI Fermentas; 0.5 mg/ml, preheated at 95 °C for 5 min) are added into the sample well containing the vesicle adhered to the trapped bead.[15] Force extension curves are then recorded on the same tubule, as a function of time, as shown in Fig. 3. We observe characteristic changes in the tubule formation, as the DNA molecules assemble onto the membrane tubule. The two distinct features of the force extension curve (without DNA as shown in Fig. 3), the initial sharp rise in the force and its saturation are altered. As a function of DNA integration, the tubule behaves more as a random coil (similar to that of a DNA polymer[10]). The surfactant molecules are recruited as counter ions to neutralize the charge on the assembled DNA, hence, disrupting the fluid like nature of the membrane and also making them optically visible (Fig. 3). In Fig. 3 inset, we plot the recoil of the optically resolved DNA assembled tubule. The long recoil time scales, suggest its entropic nature, which is also revealed in the force extension curves. The tubule and the vesicle eventually rupture forming DNA-lipid complex, at sufficiently high DNA concentration on the membrane.

In conclusion, we have observed an interesting regime in membrane tubulation from multilamellar vesicles. An optical tweezer was used to apply localized subpicoNewton forces to pinch off tubules of radius 80 nm. Similar forces are indeed applied by molecular motors within biological cells and are implicated in the formation of membrane tubule networks. From the data, we have estimated the membrane tension, its Young's modulus and the membrane rigidity modulus. In our experiment, it is still unclear, how such long tubules can emanate from micron size vesicles for applied subpicoNewton forces. An estimate of the volume fraction of the tubule, suggests that inner layers may be contributing to the tubule elongation. A possible mechanism is the flow of material from the inner layers to the outer layer, due to nucleation of defects resulting from applied localized forces and our observation may present an interesting model system to study such flow in soft matter physics.[16]

In our experiment, the tubules formed using vesicles containing fluorescent molecules are easily observed using fluorescence microscopy. Multiple tubules can be branched out either from an existing tubule or from the vesicle, suggesting interesting paradigms for creating two-dimensional and 3D network of tubules on various semiconductor substrates. We propose that such networks, can present applications in nanofluidics and nanowires. The integration of DNA onto the tubules, presents an approach to incorporate any desired conducting polymers or metal nanoparticles for various applications. We are exploring the possibility of creating nanofluidic networks using vesicles immobilized on electrical pads as reservoirs and the tubules as interconnecting channels.

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[9] Optical trap calibration: The trap is constructed on an inverted microscope (Olympus IX70) by focusing an infrared beam (Nd–YAG LASER, 1064 nm wavelength; 200 mW; Coherent) into a diffraction limited spot by a high numerical objective NA=1.4. The displacement of the bead in the trap is detected by focusing the backscattered RED LASER (635 nm wavelength) on to a Quadrant Photodiode (SPOT4D; UDT Instruments & Model 431 X-Y Position Indicator; UDT Instruments). The variance in the fluctuations of the trapped bead is used to calibrate the trap stiffness, $k_{trap}=k_BT/var(x)$. In our case, with 200 mW laser power input, the standard deviation is 54 nm for a 2 μm bead and, hence, $k_{trap}=1.4\times10^{-6}$ N/m. Data acquisition and analysis is done using DAQ card (National Instruments PC-MIO-16XE-10) and LabVIEW.
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[12] Vesicle preparation: DDAB is mixed in ethanol at a concentration of 20 mM. 100 μl of this solution is spread on a 1 in.×1 in. clean and dry teflon piece and incubated in the oven at a temperature of 50° over 8 h. 500 μl of PBS (2× buffer) at *p*H 7.4 is added to this so that the final lipid concentration is 4 mM. The solution is sonicated for 1 h to get vesicles with sizes ranging from 4 to 20 μm. The vesicles may be stored at 4 °C and used over two weeks. For fluorescence imaging of the membrane tubules Fluorescein Isothiocynate (excitation 497 nm and emission 521 nm) is mixed to PBS *p*H 7.4 at 2 nM concentration before sonication.
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