Supplementary Material (ESI) for Soft Matter

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Title: "Formation and Release of Circular Lipid Nanotubes"

Circular lipid nanotubes and phase diagrams.

In the paper we assume that the total surface area and volume of a nanotube is preserved during the course of an experiment. We also assume that there is no flip-flop of molecules across the bilayer membrane, this fixes the difference in surface area of the inner and outer monolayers of the membrane, which are separated by a distance D ~2.5 nm. These assumptions lead to the bilayer-couple model,[1-3] where the bending energy of a vesicle is given by $F = (\kappa/2)\oint dA(c_1 + c_2)^2$, where c_1 and c_2 are the principle curvatures of a surface with an area A , and the area difference $\Delta A = A^{ex} - A^{in} \approx 2DM$ between inner and outer monolayers is constant. ΔA can be calculated from the total mean curvature $M = (1/2)\oint dA(c_1 + c_2)$. The local mean curvature of a nanotube with radius r is $(1/2)(c_1 + c_2) \approx (1/2)c_1 = 1/(2r)$, the total mean curvature for a nanotube of length L is $M = 2\pi rL/2r = \pi L$.

A circular nanotube can also be considered as a special case of a toroidal vesicle, which also have topology g=1. Equilibrium shapes of toroidal vesicles have been calculated and presented in phase diagrams.[4, 5] In general, the vesicle shape phase diagram is described by two dimensionless parameters: reduced volume $v = V/((4\pi/3)R_0^3)$ and reduced total mean curvature $m = M/R_0$, where $R_0 = (A/4\pi)^{1/2}$ is the radius of a sphere with area A . The reduced volume v usually varies between 0 and 1, and $m/4\pi$ between 0 and 2, with the geometric shapes well described in the central part of the diagram.[4] Due to the high aspect ratio, our lipid nanotubes have rather unusual estimated values of $v \approx 0.07$ and $m/(4\pi) = (1/2)\sqrt{L/(2r)} \approx 10$. These values fall outside the phase diagram of known stationary shapes of toroidal vesicles.

Formation of a nanotube-vesicle network.

The method used in the present work for constructing a nanotube-vesicle network is based on a micromanipulation and microelectroinjection technique.[7] The liposomes are prepared in such a way that a giant unilamellar liposome (mother liposome) is attached to a multiamellar vesicle (Fig. 1 a). The multilamellar vesicle is required as a source of lipid material for building the network. The liposomes have a diameter of tens of micrometers and they are immobilized on a surface. During the course of the experiment, constant adhesion of the vesicles to the surface is assumed.[8]

An experiment begins with placing a microelectrode and a micropipette filled with buffer solution and containing a counter electrode, close to the surface of a mother liposome. By applying an electric pulse (~ 40-80 V/cm during 1-5 ms[9]), and piercing the unilamellar liposome with the micropipette, it is possible to penetrate the membrane so that the pipette tip enters the vesicle. The membrane then seals around the micropipette tip. By pulling the micropipette away from the mother liposome, a lipid nanotube is created which connects the pipette tip and the vesicle (nanotube ***I*** in Fig. 1 b). Next, a positive pressure is applied through the micropipette. This leads to injection of a buffer solution, and a small (daughter) vesicle is formed at the pipette tip (Fig. 1 c). The size of the daughter vesicle is controlled by the amount of injected liquid. The newly formed vesicle ***1*** is then positioned on the surface (Fig. 1 d), and the pipette can be detached from the vesicle by pulling the pipette away from the daughter vesicle and by applying electric pulses at the same time.

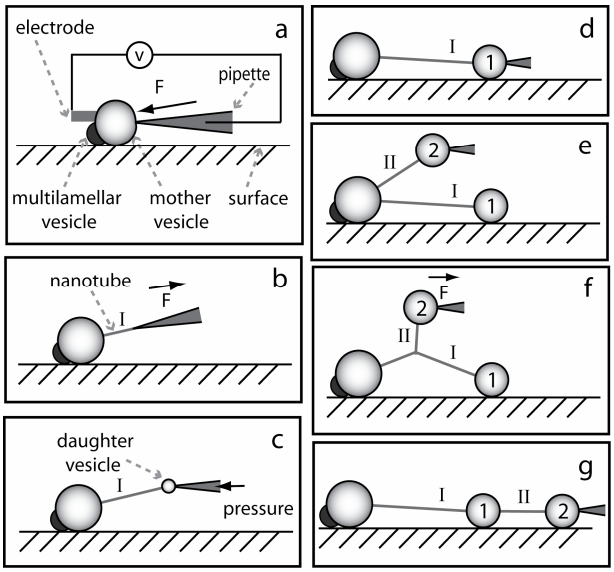

Fig.1. Formation of a nanotube-vesicle network. (a) Electroporating and penetrating a vesicle membrane with a micropipette. (b) The nanotube connecting the mother vesicle and the micropipette tip is formed. (c) Inflating of the daughter vesicle. (d) Placing of the daughter vesicle ***I*** on the surface. (e) Formation of the second daughter vesicle. (f) Translating vesicle ***2*** over the nanotube ***I*** and vesicle ***1***. (g) Placing daughter vesicle ***2*** on the surface behind the vesicle **1**.

In a similar way, another daughter vesicle (***2***) can be formed (Fig. 1 e). Initially, the newly formed vesicle ***2*** is attached to the micropipette and directly connected to the mother vesicle by nanotube ***II***. By moving the micropipette with attached vesicle ***2*** along the nanotube ***I*** and then over the vesicle ***1*** (Fig. 1 f), vesicle ***2*** is translated over the network and placed behind vesicle ***1***. Finally, vesicle ***2*** is put on the surface (Fig. 1 g). All the vesicles are consequently conjugated and arranged in the following order: mother vesicle - nanotube ***I*** - vesicle ***1*** - nanotube ***II*** - vesicle ***2***. The liposomes in the network are adhered on the surface and the nanotubes are suspended between them (*i.e.* not attached to the surface). Such an adhesion of the vesicles allows for maintaining a stable nanotube-vesicle configuration. It should be noted that the whole network is made of continuous lipid bilayer.[9] The described procedure can be repeated to build rather complex nanotube-vesicle networks.

Persistence length of lipid nanotubes.

In order to estimate the persistence length circular nanotubes were analyzed at different time points during thermal fluctuations. The most curved parts of the lipid nanotube were chosen to estimate the persistence length as the distance along the nanotube at which orientation in the direction of the tangent appears uncorrelated. Fig. 2 shows one of such images. The arrows point at the curved regions of the nanotube that where chosen to measure the persistence length, which was found to be approximately 8μm (in this particular figure).

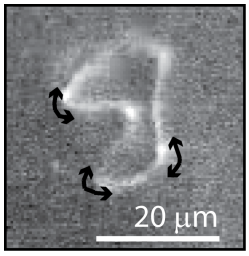

Fig.2. Fluorescence micrograph of fluctuating circular nanotube. Black arrowed lines are pointing at the curved regions where the persistence length was measured.

Notes and references

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