Spontaneous Shape Transformation of Free-Floating Lipid Membrane Nanotubes

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Electronic Supporting Information (ESI)

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Experimental section

Surface preparation

To prolong sample live time cover slide were covered with SU-8 before use: Microscope cover slips slides #1 (VWR, Sweden) were coated with SU-8 photoresist to attain well adhered vesicles while minimizing lipid spreading. Firstly, a 5vol% SU-8 2002 (MicroChem, Newton, MA) solution in cyclopentanone (Sigma-Aldrich, Sweden) was spin-coated onto each coverslip at 2000rpm for 60s. These were baked at 100ºC for 2-3min, then flood exposed with 254nm UV light for 45s at 12-15mW/cm^2. Each coverslip was then post exposure baked at 150ºC for 10min in an oven, resulting in a crosslinked photopolymer surface. The SU-8 coated coverslips were washed with ethanol and deionized water prior to use.

Vesicle preparation

Complexes of a multilamellar vesicle (MLV) connected to a giant unilamellar vesicle (GUV) were prepared from Soybean Polar Lipid Extract (SPE) (Avanti Polar Lipids, USA) using the dehydration/rehydration method [1-3]. A 1.7mM SPE solution was prepared in chloroform (Sigma-Aldrich, Sweden) and dried in a rotary evaporator (Büchi R-144) for 5 hours (-80kPa at room temperature). The obtained lipid cake was rehydrated using 1ml of phosphate buffer (5mM Trizma, 30mM K_3PO_4, 30mM KH_2PO_4, 1mM $MgSO_4$, 0.5mM Na_2EDTA at pH7.8) containing 10µl of glycerol (Sigma-Aldrich, Sweden). This was left at 4ºC overnight, to ensure adequate hydration. The resulting suspension was brought up to room temperature and sonicated for 45s, and either used immediately or stored at -20ºC. Before each experiment a 10µl sample of the lipid suspension was dried onto a SU-8 coated coverslip in a vacuum desiccator for 15min. A rectangular poly(dimethylsiloxane) (PDMS) frame was positioned on the glass defining a well [4], into which 2-3ml of phosphate buffer was introduced, rehydrating the sample prior to use.

Fluorescence labeling

Texas Red labelled vesicles (SPE-TR) were prepared by addition of 1wt% of Texas Red 1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine (DHPE) (Life Technologies) to the SPE lipid mixture in chloroform. N-(3-triethylammoniumpropyl)-4-(4-(dibutylamino) styryl) pyridinium dibromide (FM1-43) (Life Technologies) labelled vesicles (SPE-FM) were prepared through addition of FM1-43 solution (20µg/ml, 40µl) to the sample after the final rehydration step.

Formation and release of nanotubes

Nanotubes were formed and released from MLV-GUV complexes using electroporation and micromanipulation [1,5]. Briefly, two glass micropipettes were pulled from borosilicate glass tubes (GC100TF-10, Harvard apparatus) with a capillary puller (P-2000, Sutter Instrument). The pipettes were filled with phosphate buffer, fitted with electrodes and positioned near an MLV-GUV complex with fine hydraulic micromanipulators MC-35A/MHW-3 (Narishige, Japan). The counter electrode was placed into the well and submerged into the solution. One of the pipettes was inserted through the GUV membrane by applying several short electrical impulses (6ms, 60mV) with a rectangular pulse generator (DS2A, Digitimer, Welwyn Garden. City, UK), and pushing the pipette tip into the vesicle. A nanotube of approximately 300μm long was formed by withdrawing the pipette tip out and away from the GUV.

Both ends of the nanotube were released in sequence, by the application of a localized electric field. The tip of the second pipette was placed near the nanotube-GUV junction, and long electrical pulses (60ms, 60mV) were applied until the nanotube disconnected from the vesicle. Immediately following this disconnection, long electrical pulses were applied through the first pipette tip, releasing the other end of the nanotube. Nanotubes displaying shape and fluorescence intensity irregularities directly after release were discharged from analysis, due to the obvious defects induced by the electric field during cutting.

Imaging and data collection

A DM IRB inverted microscope (Leica, Germany) coupled with an N PLAN L 40x/0.55 objective (Leica, Germany) was used to perform all the experiments. FM1-43 dye was exited at 488nm using a Sapphire 488-150CW laser (Coherent, Germany), Texas Red DHPE dye was exited at 532nm using an MLG-III532-200mW (Changchun New Industries Optoelectronics Tech., China). Fluorescence images were recorded at 8fps rate with Prosilica GX camera (Allied Vision Technologies, Germany), using a custom Labview (National Instruments) program.

A low numerical aperture was used to obtain quantitative intensities, as the Rayleigh length is longer than the diameter of the tube for the majority of the transformation. This leads to direct proportionality between the thicknesses of tube, amount of material in the crossection and the integral fluorescence intensity across the tube image. Having such a long Rayleigh length also reduces inaccuracies induced by thermal fluctuation of the tube, as the movement out of focus is reduced. The recorded images were processed with a custom Labview program, to extract lengths and intensities across tube segments.

To derive radial dimensions of tube segments from membrane surface area conservation law, we considered two models: Swelling, which leads to single walled structures throughout the whole

transformation; and Folding, where both the bright tube parts and final shapes were assumed to always have a double wall.

Estimation of membrane permeability, required for nanotube transformation by swelling

We consider a simple model where nanotube transforms by swelling into thicker tube (Fig. SI 1) and show that this transformation mechanism implies very high membrane permeability for ions.

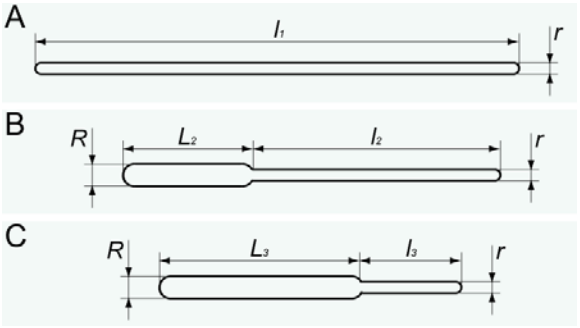

Figure SI 1. Swelling nanotube. Original nanotube of radius *r* (A) transforms and swelled part with uniform radius *R* appears (B, C). Length of nanotubular part *l* (l_1, l_2, l_3) is decreasing, providing lipid material for swelled part growth in length *L* (L_2, L_3) while *r* and *R* stay the same.

Linear membrane transport, in the most general form, is given by the Kedem-Katchalsky equations [6]

$$J_v = L_p(\Delta P - \sigma \Delta\Pi)$$

$$J_s = \omega \Delta\Pi + \bar{c}_s(1-\sigma)J_v$$

where $J_v = J_s\bar{v}_s + J_{water}\bar{v}_{water}$ is the bulk volume and J_s solute flux. ω is the solute permeability, σ the reflection coefficient, L_p the filtration coefficient, ΔP the hydrostatic pressure jump over the membrane, and $\Delta\Pi = k_B T \Delta c_s$ the osmotic pressure jump. These equations were originally developed for non-electrolytes, however assuming zero electric current across the membrane gives the same equations for ion transport [7]. We shall consider a simple two-ion model (cation-anion), where c_s denotes the sum of the ion-concentrations. This model is clearly an oversimplification since there are several different ion species present in the buffer, the purpose here is to give an estimate of the permeability.

The dissipation per unit membrane area $T\dot{S}$ associated with the KK-equations is [6]

$$T\dot{S} = J_V \Delta P + J_D \Delta\Pi$$

where $J_D = J_s / \bar{c}_s - J_{water} / c_{water}$ is the solute exchange flow. The bending pressure [8] in a tube of radius 25nm is $P = \frac{\kappa}{2r^3} \approx 10^3 Pa$, small compared with the osmotic pressure $\Pi \approx 2 \cdot 10^5 Pa$, therefore bending can only induce a weak osmotic pressure difference across the membrane. Thickening of the nanotube would imply a large volume change, the flow across the membrane must therefore be dominated by bulk volume flow, since the solute exchange flow would modify the osmotic balance. Zero exchange flow gives dissipation

$$T\dot{S} = \left(\frac{1}{L_p} + \frac{\bar{c}_s \sigma^2}{\omega} \right) J_V^2$$

For a given volume change, the lowest possible dissipation is obtained by a homogeneous flux, which gives a lower bound on the total dissipation

$$\int T\dot{S}\, dA > \left(\frac{1}{L_p} + \frac{\bar{c}_s \sigma^2}{\omega} \right) \frac{\dot{V}^2}{A}$$

where A is a total area of the membrane. $\dot{V}$ is the volume growth rate. As tension relaxes rapidly, the total tube energy is given by the bending energy [8-10]

$$E = \frac{\pi \kappa L}{R} + \frac{\pi \kappa l}{r}$$

where L and l are length of the swelled and nanotubular part.

Balancing dissipation and energy gain $\frac{dE}{dt}$, gives a lower bound on the transport coefficients

$$\left(\frac{1}{L_p} + \frac{\bar{c}_s \sigma^2}{\omega} \right)^{-1} > \frac{Rr^2}{\kappa} \frac{1}{A} \frac{dV}{dt}$$

Assuming that transport is limited by ion permeability gives

$$\omega > \frac{Rr^2}{\kappa} \frac{1}{A} \frac{dV}{dt} \bar{c}_s \sigma^2$$

It is common to express permeability as a velocity $P = \omega k_B T$. From the estimated volume growth we obtain value for *P* between 0.02 and 0.5μm/s, much higher than ion permeability previously measured for bilayers (~10^{-6}μm/s) [11].

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