Extracting thin membrane tubules with optical tweezers

Application Note

Introduction

Thin membrane tubules with diameters as small as 50 nanometers (also called "membrane tethers" or "membrane nanotubes") are one of the basic membrane structures in living cells. For example, they play an important role in the intracellular trafficking (e.g., in the Golgi apparatus) and in the cellular response to external stimuli (e.g., in leucocyte response to inflammation). In vitro, the membrane tubules can be formed by applying a several pN external force perpendicularly to the membrane. Typically, the force is applied by optical tweezers via a microsphere that is chemically attached to the membrane. Coupled with the well-established theoretical models of membrane elasticity, the measurement of the force needed for the tubule extraction provides an invaluable experimental probe into physical properties of membranes and cells.

Optical tweezers

All experiments shown here are performed on the Aresis Tweez 250 system. This is an advanced analytical turn-key laser tweezers system. It uses a 1060 nm, 5 W fiberlaser. Beam steering is controlled using a set of acousto optic deflectors (AOD). The AOD technology allows the generation of practically unlimited number of traps which can be positioned independently. Trap to trap switching rate of 100 kHz ensures quasistationary conditions for each trap. Linear response and spatial uniformity of this setup is calibrated to ensure uniform trap stiffness over the field of view of ca. 150x150 μm^2.

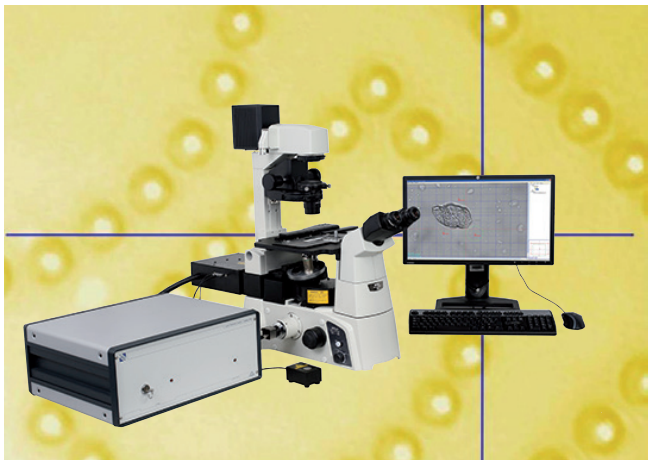

Fig. 1 Aresis Tweez 250 optical tweezer mounted on Nikon Eclipse inverted microscope.

Further details of the experimental set-up can be found at: Vrhovec S., M. Mally, B. Kavčič, J. Derganc. 2011. "A microfluidic diffusion chamber for reversible environmental changes around flaccid lipid vesicles." Lab Chip. 11:4200-4206.

Pulling tethers from vesicles

Giant unilamellar vesicles are accepted as a simplified model of a cell membrane and therefore are widely used in experiments simulating biological processes involving membrane deformation, fusion or remodeling (e.g. exocytosis, endocytosis, cell division). The mechanical properties of these vesicles, the so called surface bending elasticity, are influenced by the phospholipid chemistry, temperature and others. The characterisation of the curvature modulus or bending rigidity is often done using a micropipette technique analysing the relative change in area under aspiration pressure.

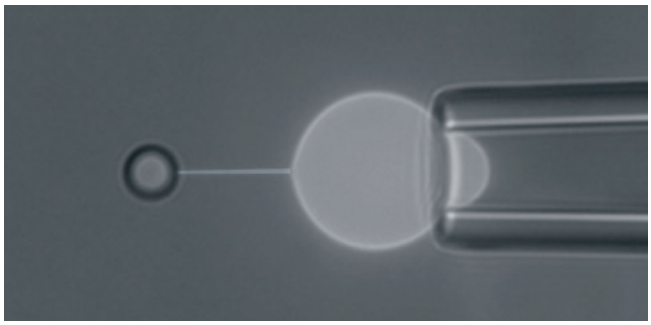

Fig.2 Membrane tubule extracted from a lipid vesicle aspirated into a micropipette

Fig. 2 shows a thin membrane tubule extracted from a synthetic lipid vesicle aspirated into a micropipette. First a microsphere is chemically attached to the membrane. In the next step the microsphere is trapped by the optical tweezers and pulled away from the membrane. The membrane tension is controlled by the micropipette aspiration pressure and the tubule extraction force is determined from the microsphere displacement from the center of the optical trap. The diameter of the microsphere is 5.4 micrometers. The image is a composite of a brightfield and an EPI fluorescence image (the membrane is fluorescently labeled).

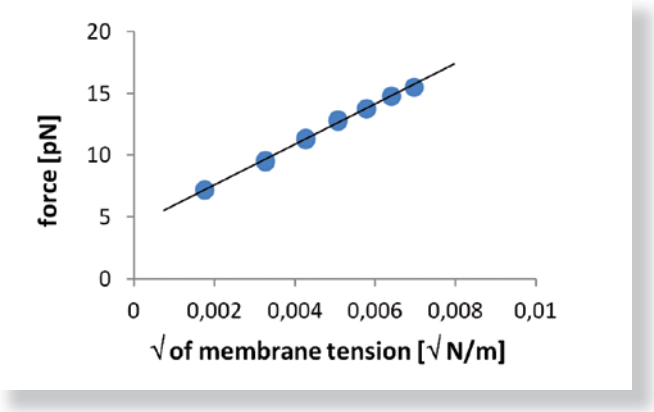

Fig. 3 Membrane tubule extraction force vs. membrane tension

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The membrane tubule extraction force versus membrane tension is plotted in Fig. 3.The extraction force is proportional to the square root of the membrane tension. The slope in this diagram is directly related to the membrane bending modulus.

Coalescence of membrane tubules

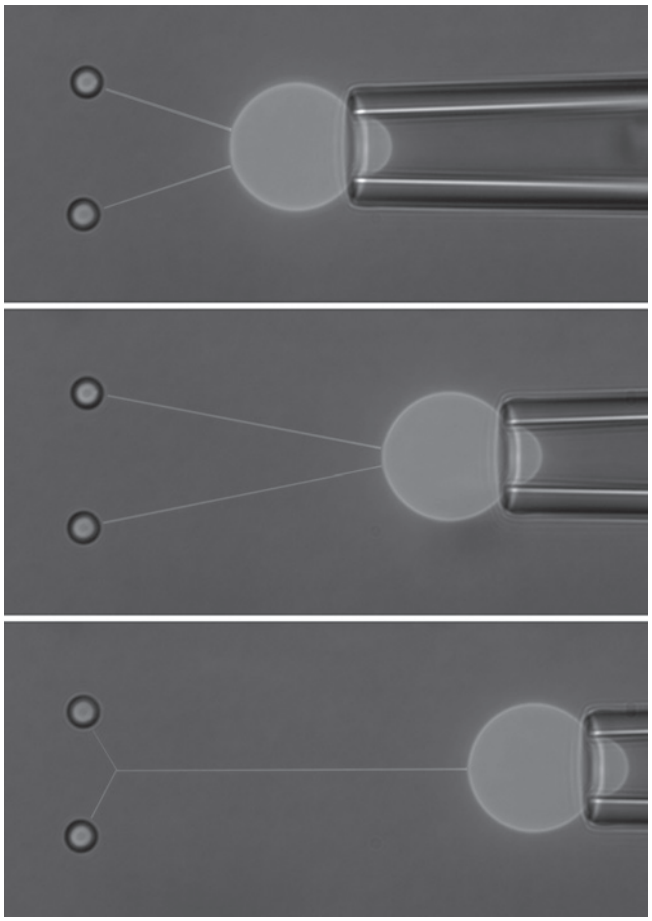

Fig. 4 Coalescence of membrane tubules

In further experiments two membrane tubules are extracted from a synthetic lipid vesicle aspirated into a micropipette. As the micropipette is retracted, the angle between the two tubules decreases. When the angle reaches a critical value, the two tubules coalesce and form a Y-shaped tubular junction. The diameter of the microsphere is 5.4 micrometers. The image is a composite of a brightfield and an EPI fluorescence image (the membrane is fluorescently labeled).

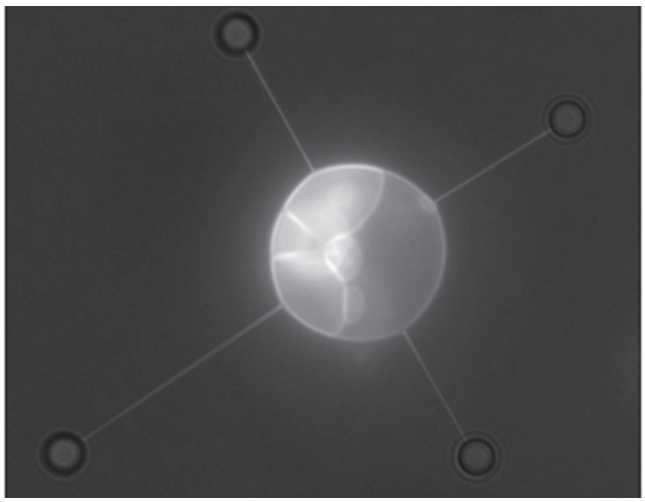

Fig. 5 Extraction of multiple membrane tubules from a synthetic lipid vesicle. The image is a composite of a brightfield and an EPI fluorescence image (the membrane is fluorescently labeled).

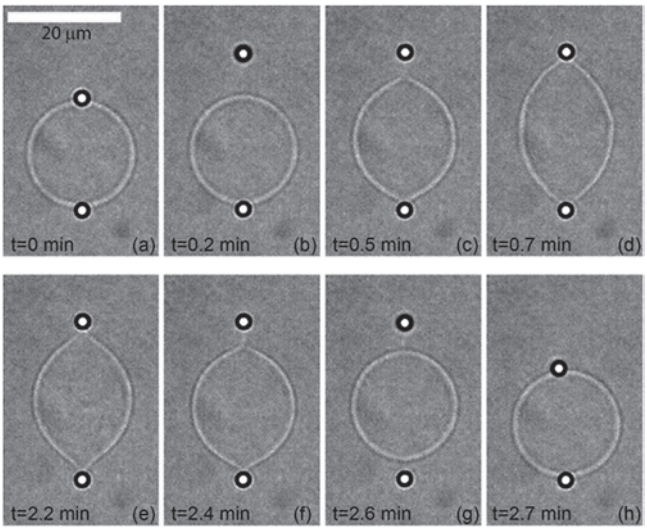

Fig. 6 shows a synthetic lipid vesicle in a microfluidic diffusion chamber suspended on two membrane tubules. The vesicle is deflated by osmotic effects and then inflated back to the initial volume. During this process, the vesicle undergoes a reversible shape change via the lemon shapes which are characteristic for vesicles under an axial force.

Conclusion

In this work we demonstrated the power of using optical tweezers to study properties of cellular membranes. The complexity of the experiments increases from single to multi trap experiments pulling up to four tethers from a single vesicle. Linearisation of the optical tweezer response enables precise force measurements.

Acknowledgement

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