Abstract

Methods were developed to produce microscopic networks of vesicles (5 to 50 micrometers in diameter) conjugated by lipid nanotubes (50 - 200 nanometers in diameter). The networks are constructed from a continuous lipid bilayer membrane held together by non-covalent interactions and since it has thickness of only 5 nm, it can be viewed as a two-dimensional liquid. The membrane is very hard to stretch, but at the same time very easily bent and therefore it is possible to create complex nanoscale structures from it, for example, lipid nanotubes. Networks were produced through forced shape transformations of vesicles into nanotube-vesicle geometries, using micromanipulation-based protocols. The first technique was based on mechanical fission of vesicles, using a micromanipulator-controlled carbon fiber (5 μm in diameter). This technique was preferentially used for multilamellar vesicles with possibilities of constructing templates for solid state structures, which could be used in microfluidics or microelectronics applications. Another method for creation of networks of unilamellar vesicles and interconnecting lipid nanotubes was developed, which was based on a micropipette-assisted electroinjection technique. A microinjection pipette was inserted into a unilamellar vesicle using the electroinjection technique in which a mechanical pushing force was combined with the application of an electric field that destabilized the membrane. The membrane was allowed to reseal around the tip, whereby the pipette was pulled away to create a lipid nanotube. Buffer was injected into the nanotube (with a typical velocity of femtoliters per second) and a new vesicle was formed at the injection tip taking material from the originating vesicle. When the vesicle had reached the desired size it was allowed to adhere to the surface and the procedure could be repeated to create new vesicles. By changing the solution contained in the micropipette it was possible to differentiate the contents of the vesicle containers in a network either during or after formation. The networks have controlled connectivity, container size, nanotube-length and angle between nanotubes connected to the same vesicle.

A method to transport lipid material, fluids and particles through the nanotubes between vesicle containers was developed. The transport is based on creating a surface tension difference across nanotubes which results in a moving lipid wall having velocities ranging between 20 – 60 μm/s. Due to the small dimensions of the lipid nanotube, fluids and particles confined inside the nanotube were dragged along the lipid flow through viscous coupling. A nanofluidic system, which is capable of working with volumes down to the femtoliter (10^{-15} L) or even the attoliter range (10^{-18} L), can therefore be realized. Fluid control in nanometer-sized channels open up possibilities, for example, to perform single-molecule studies in confined spaces.

To be able to route material and fluids in larger networks, a two-point perturbation technique was developed. In this technique, the membrane tension of the vesicle from which material was to be transported was decreased, while the membrane tension of the target vesicle was simultaneously increased. Transport, therefore took place mainly through the nanotube interconnecting the two selected vesicles, without affecting the rest of the network. This way, a molecule can be shuttled throughout a complex network between selected containers, making it possible to expose it to different chemical or physical environments.

A method was developed to create and transport micrometer-to-submicrometer-sized vesicles integrated onto the interconnecting nanotubes of larger surface-adhered vesicles. The two-point perturbation technique was used to momentarily destroy the energy balance and the shape of the system, subsequently leading to formation of micrometer-sized vesicles integrated onto the nanotube. These vesicles were mobile and since they were integrated onto the lipid nanotube they could be transported to a larger target vesicle, using the principle of tension-driven lipid flows, where they could release their material.

A vesicle model system for exocytosis consisting of a pipette-attached vesicle inside another larger vesicle was constructed. The vesicles were interconnected by a lipid nanotube, making the system resemble the late stages of exocytosis, in which a synaptic vesicle ready for release of transmitter substance is fused to the outer membrane of the cell. This way, it was possible to study the last stage before release, to see how this system behaved without the controlling protein units normally present in a living cell.

Keywords: Lipid, membrane, bilayer, vesicle, biomimetics, lipid nanotube, nanotube-integrated vesicles, networks, tension-driven lipid flow, transport, nanofluidics, vesicle cell-models

ISBN 91-628-5870-X