Abstract

This thesis is based on the development of techniques to use liposomes and lipid nanotubes as micro- and nanoscopic reaction containers. In brief, miniaturized versions of electroporation and electrofusion techniques were developed and used to initiate chemical reactions inside single liposomes. Electroporation and electrofusion was performed by application of one or several short (typically 10-40 μs) and intense (typically 20-50 kV/cm) electric pulses, by the use of micromanipulator-controlled carbon fiber electrodes (5 μm in diameter). Chemical reactions could, for example, be induced by electrofusion of two liposomes each containing a reagent. This was demonstrated by fusion of a Ca^{2+}-containing liposome with a Fluo-3 containing liposome, which resulted in an increase of the fluorescence, upon chelation. Product formation of the enzymatic reaction between alkaline phosphatase and the substrate fluorescein diphosphate, which resulted in the product fluorescein, was measured using sensitive fluorescence microscopy and the use of a single-photon avalanche diod (SPAD) detector. Due to the small size of the liposomes, the surface-to-volume ratio is very high, making surface interactions very probable and simple computer simulations of single enzyme-single substrate encounters inside a spherical cavity revealed high collision frequency between molecules and the container wall as opposed to molecule-molecule collisions. A device consisting of sample containers connected to a common fusion chamber through microfluidic channels, consisting of ~3 mm long borosilicate glass capillaries (10-30 μm i.d., 30-100 μm o.d.) was constructed to facilitate pair-wise electrofusion of single liposomes, which could be applied to combinatorial approaches to synthesise a vast amount of different types of product liposomes with respect to membrane composition and interior contents from a small set of starting liposomes. Optical tweezers allowed selection and transport of single liposome from the sample containers into the fusion chamber.

Micromanipulator-based methods to construct two-dimensional lipid bilayer network structures of nanotubes (100-300 nm diameter) and containers (5-50 μm diameter) immobilised on surfaces were developed. The networks have controlled connectivity, container size, nanotube-length and angle between nanotube extensions emanating from a single liposome. The first method was based on simple mechanical fission of multilamellar and unilamellar liposomes using a 5 μm diameter carbon fiber as a cutting tool. A micropipette-assisted technique to create unilamellar networks of liposomes and nanotubes was also developed. A pulled microinjection pipette was inserted into a unilamellar liposome using a combination of mechanical force and an electric field. After resealing of lipid around the pipette-tip, the pipette was pulled away to create a lipid nanotube. Pressure-controlled injection of buffer (typically femtoliters per second) expanded the nanotube at the injection tip into a new liposome and when the desired size was reached, the new liposome was allowed to adhere to the surface. This technique also enables easy differentiation of interior contents in the liposomes through exchange of the fluid contained in the microinjection pipette.

Methods to control and handle fluid and material transport between the nanotube interconnected unilamellar liposomes were developed. Transport was induced by a membrane tension difference, resulting in a flow of lipids across the nanotube to diminish this difference, which dragged along the liquid column and any dispersed particles inside the lipid nanotube through viscous coupling, thus offering means of transporting and trapping of molecules in nanoscale channels. Lipid velocities usually ranged between 20-30 μm/s, however, much higher velocities, up to 60 μm/s, were occasionally observed. The nanotube segment could also function as a nanoscale flow channel, which could be combined with single-molecule-sensitive detection techniques, such as laser induced fluorescence (LIF). Also, a novel method to create, load and transport nanotube-integrated liposomes (500 nm-5 μm) between interconnected liposomes was developed. These nanotube-integrated vesicles were created by addition of excess membrane material to one of the surface-attached liposomes in a network. This was performed by merging of a nanotube-connected liposome, which resulted in a drop of membrane tension. The interconnecting lipid nanotube was subsequently destabilised and attained an asymmetric funnel-like shape, which was transformed into nanotube-integrated vesicles. As a continuation of this work, a method to route these nanotube-integrated liposomes in large networks was also developed by using a two-point perturbation technique, where the tension was increased in one liposome and at the same time decreasing the tension in another liposome that was connected to it, thus creating a larger tension-difference between these target containers, than between any other containers in the network.

Finally, network formation technology was combined with chemical reaction initiation techniques. Networks of liposomes and nanotubes were used to create two liposomes with differentiated contents with a connecting nanotube. The fluid character enables movement of nanotube attachment point across the entire network surface. Through this, two differentiated network liposomes can be created, interlinked with a nanotube. When the two compartments were brought together, *i.e.* when the nanotube length approached zero to provide a single fluid contact point, the liposome compartments spontaneously merged and their contents were mixed and the reaction could therefore be initiated. An enzymatic reaction between alkaline phosphatase and the substrate fluorescein diphosphate was used to demonstrate mixing of reagents and product formation, using this technique.

Keywords: Lipid, membrane, bilayer, liposome, biomimetic, lipid nanotube, nanotube-integrated vesicles, networks, transport, nanofluidics, electroporation, electrofusion, microinjection, reaction, initiation
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