Abstract

Fusion of biomembranes occurs in many biological processes, *e.g*. fertilization, viral infection, mitosis and meiosis as well as endo- and exocytosis. The highly specialized fusion proteins, soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptors (SNAREs), are responsible for the release of neurotransmitters into the synaptic cleft *via* the fusion of neurotransmitter-filled vesicles with the presynaptic membrane. Analogues of SNAREs were used in the last decade to fuse liposomes with each other. Liposomes are another term for vesicles in a non-biological context. The SNARE analogues so far were either limited in programmability or in efficiency.

Here, we describe in Chapter 1 the development of an efficient fusion of liposomes mediated by lipidated peptide nucleic acids (liPs) (Figure I). Compared to the state of the art systems in the field, the liposome fusion occurred with high content mixing (31%) and a low level of content leaking (5%) at 50 °C, shown by fluorescence-based assays. Peptide nucleic acids are a DNA analogue and enable therefore a sequence-specific labeling of liposomes resulting in programmability of liposome fusion events. In addition, liPs are designed for a spontaneous incorporation into the lipid bilayer. Agarose gel electrophoresis showed that 15 min incubation of liposomes with liPs was sufficient for a quantitative anchoring to the membrane. This is a convenient feature of liPs, because liposomes can easily be labeled after their formulation.

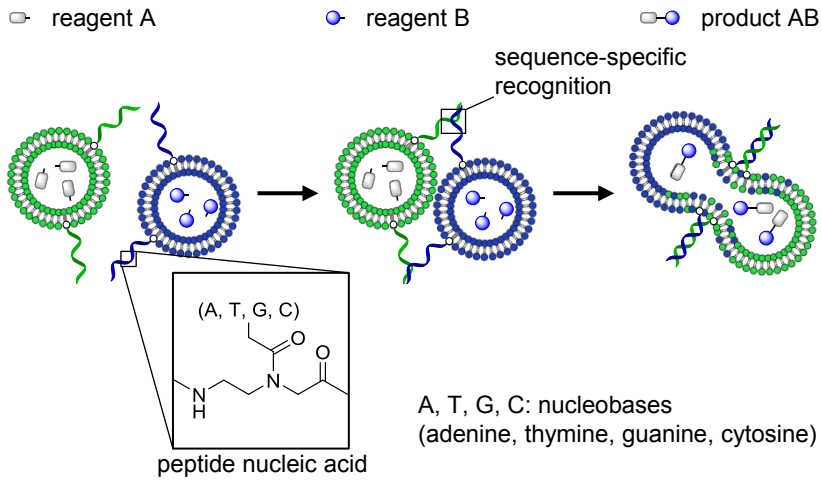

Figure I. Schematic representation of liP-mediated fusion of liposomes. Here, the generic application of liposome fusion for mixing reagents is shown.

The application of liposomes is very broad and not only limited to simulate fusion processes in nature. Another important application of liposomes is the usage as nanoreactors. Liposomes are especially well suited for enzymatic reactions but also a number of organic and inorganic reactions can be carried out in the cavity and in the lipid bilayer of liposomes. In parallel to the liP-mediated liposome fusion, our group established efficient fusion of liposomes with even higher content

mixing (93% after three fusion events) and also a low level of leakage (≤5%) by using lipidated DNA (liNA) in a programmed liposome fusion cascade. We describe in Chapter 2 studies of liNA binding to immobilized liposomes by surface plasmon resonance spectroscopy and research towards the application of liposome fusion for reagent mixing in nanoreactors. Using a strain-promoted alkyne-azide cycloaddition, we showed that mixing reagents by the fusion of liposomes resulted in higher reaction yields than adding one reagent *via* diffusion across the lipid bilayer. Furthermore, liNA-mediated fusion of immobilized liposomes and free liposomes was observed and compared to assembly of liposomes by surface plasmon resonance spectroscopy.

Liposomes can also be used for drug delivery. Liposomal-encapsulated drugs exhibit often increased *in vivo* stability and cellular uptake over the free drug. Liposomes can be modified with radiolabels and ligands of specific receptors. We describe in Chapter 3 the development of neutral liposomes, which turned into positively charged liposomes upon UV light irradiation. Using zebrafish as a model system for the human body, we showed that neutrally charged liposomes were freely circulating in the vascular system, but they sticked to endothelial cells after treatment with light. This enables specific binding of the liposomes to cells in light-treated tissue and a subsequent cellular uptake.