toxicity and enhanced TE in comparison to lipoplexes stabilized with pH-stable PEG-lipids. Live-cell images show that both pH-sensitive and pH-stable PEG-lipoplexes were internalized to quantitatively similar particle distributions within the first 2 h of incubation. Thus, the increased TE of the HPEG-lipoplexes can be attributed to efficient endosomal escape, enabled by the novel HPEG-lipid. Funded by NIH-GM-59288 and NSF-DMR-1101900.

[1] Ewert, K. K. *et al*, *Topics Curr. Chem.* **2010**, 296, 191-226

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Dynamic Ca^{2+}-Dependent Activity of Membrane-Anchored Synaptotagmin 1 Observed at the Content Mixing Level

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Synaptotagmin 1 (Syt1) is thought to be the main Ca^{2+} switch for the presynaptic vesicle fusion. Although in vitro fusion assays importantly contributed to understanding the molecular mechanism of Syt1, the results was largely restricted to truncated Syt1 that retained only soluble C2AB domains. Using the single-vesicle fluorescence assay, we have recently shown the strong fusogenic activity of membrane-anchored Syt1 at physiological Ca^{2+} levels (Science 328, 760 (2010)). Moreover, Syt1 shows a biphasic activity that Syt1 activity is observed to diminish at extraordinarily high Ca^{2+} concentrations. By developing ability to detect content mixing in single vesicle fusion events, we here show that such dynamic Ca^{2+} -dependent Syt1 activity is also observed at the content mixing level. In addition, we report point mutations in the linker, which was previously thought as a dull unstructured region, critically modulates the Syt1 activity. Therefore, Syt1 seems to become a far versatile fusion regulator when it in a form of membrane protein.

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Real-Time Imaging of Retrovirus-Endosome Fusion followed by Release and Trafficking of the Viral Core

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Binding to a cognate receptor renders the Avian Sarcoma and Leukosis Virus (ASLV) Env glycoprotein competent for undergoing low pH-dependent refolding which lead to fusion with acidic endosomes. Here we took advantage of the ASLV's ability to enter endosomes and maintain infectivity in the presence the NH_4Cl, which raises the endosomal pH. This feature allowed capturing receptor-primed viruses in neutral compartments in the presence of NH_4Cl and synchronous triggering of fusion by removing the weak base. Such "on-demand" fusion offered several advantages, including the quick and uniform reduction of endosomal pH in all intracellular compartments and precise duration of low pH exposure. The capture/release protocol resulted in a very fast release of a small viral content marker into the cytosol, as visualized by real-time single particle imaging. Moreover, this protocol favored the viral nucleocapsid release which was visualized as spatial separation of the GFP-labeled core from a virus-harboring endosome. Mean square displacement analysis of the core trajectories in the cytosol revealed that these sub-viral particles became much more motile compared to endosomes from which they were released. Importantly, we detected statistically significant differences between the diffusion coefficients of cores released from endosomes in host cells expressing two distinct receptor isoforms. In addition to the difference in motility of sub-viral particles, we also found that the rate of fusion pore dilation (a lag between the loss of a small content marker and release of the viral core) was faster in cell expressing the full-length receptor. Collectively, our data imply that, all things being equal, early endosomes are more conducive to ASLV fusion compared to late endosomes. This work has been supported by the NIH AI053668 grant.

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Whole Cell Electrical Access Obtained by Electroporation of a Synthetic Bilayer Approximating the Plasma Membrane

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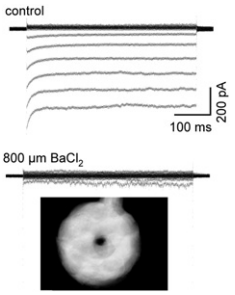
In classical patch clamping, the whole cell configuration the cytoplasmatic membrane under the pipette tip is disrupted by suction, by electroporation ("zapping") or by pore-formers. We here report that access can also be obtained by electroporating a synthetic lipid bilayer in close proximity to the cell membrane. On a microelectrode cavity array bilayers were formed by painting and dissociated rat basophilic leukemia cells (RBL-1 or RBL-2H3) were seeded thereon at high density. Bilayers were then electroporated using voltage pulses. While in the majority of cases cavity resistance decreased to 1-5 MOhm, in 21 of 88 cavities tested it remained elevated between 0.5 and 2 GOhm. In these cases, where microscopic images invariably showed an RBL cell centered on the cavity, the characteristic inwardly rectifying current responses of RBL cells to a series of voltage pulses from a holding potential of 0 mV (symmetrical [K]=135 mM) was observed. As expected for a Kir-channel, the inward current was abolished by application of BaCl2 (0.8 mM) (B). Our working hypothesis is that electroporation of a close-by bilayer may lead to fusion with the cell membrane.



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Engulfment of Model Membranes by Alveolar Macrophages

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In order to investigate the role of lipid membranes on the clearance of apoptotic cells by alveolar macrophages (AM), we use lipid vesicles containing phosphatidylserine (PS), phosphatidylcholine (PC) and ceramide (Cer) lipids. We find that model vesicle engulfment is in proportion to the PS content of the vesicle and that this engulfment is inhibited by the presence of Cer in either model vesicles or within macrophages themselves. Furthermore, we investigate how changes in physical properties of model lipid vesicles and inhibition of phosphatidylserine receptors in AM affect engulfment. Engulfment experiments are performed using rat alveolar macrophages obtained via bronchoalveolar lavage. AM were treated with various ceramide species and engulfment was quantified by flow cytometry. The physical properties of model vesicles, including molecular organization and electrostatic interactions were measured by small-angle X-ray scattering and solid state ^2H NMR. These studies can help understand the physical mechanisms involving lipid interactions responsible for apoptotic cell clearance.

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Microfluidic Construction of Synthetic Cellular Structures

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Among the multitudes of chemical structures in the cell, the membrane is the most difficult to synthesize. Microfluidic technology uniquely allows us to control the synthesis of cell membranes in a layer-by-layer fashion, and furthermore allows us control over the cargo of resultant vesicles. Vesicles were formed first by dispersing aqueous droplets into a phospholipid-containing oil phase, trapping the droplets in microfabricated cups, and finally flushing aqueous solution over the trapped droplets. In-plane valves were developed for computer control over the complex fluidic programming and capture cup geometry was optimized for droplet capture and complete phase exchange around trapped droplets. Membrane lamellarity was confirmed with α-hemolysin (αHL), an integral membrane protein that inserts into and selectively permeabilizes bilayers. The membranes were successfully permeabilized using purified αHL, as observed in confocal imaging microscopy. To demonstrate compartmentalized metabolism, the gene encoding αHL was expressed by an in-vitro transcription translation (IVTT) system within synthetic membranes. Activity was observed as before. After insertion of the gene and IVTT, only small-molecule cargo (fluorescein) dissipated from the interior of the vesicle, whereas large macromolecular cargo was retained. The lack of leakage of large cargo demonstrates both the internal metabolism as well as the presence of a only a single bilayer membrane. The computer-controlled membrane prototyping platform is now being used to reconstitute integral membrane protein signaling complexes (e.g. receptor kinases) and confirm their in vitro pharmacological behavior.