TS1; it eliminates step 2 by destabilizing the second intermediate; and catalyzes step 3 by favoring correlated fluctuations of lipids from cis and trans leaflets of an unstable second intermediate. Supported by NIGMS grant 32707 to BRL.

458-Pos Board B227
A Content Indicator is Critical to Assess Fast Fusion
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In vitro reconstitution assays are commonly used to study SNARE-mediated membrane fusion. However, many ensemble and single vesicle experiments have been performed with lipid mixing, but not content mixing, indicators. The fundamental assumption of using lipid mixing to study membrane fusion is that there is a direct correlation between lipid and content mixing. Through simultaneous detection of lipid and small content indicators, we found that lipid mixing can occur seconds before content mixing, or without any content mixing during the observation period (50 secs), for calcium-triggered fusion with SNAREs, full-length synaptotagmin 1, and complexin [1]. Our study, as well as the result from a large content mixing assay [2,3], calls into question liposome assays that only rely on a lipid-mixing reporter to assess fast fusion. Our results suggest that liposome fusion experiments should always employ content-mixing indicators in addition to, or in place of, lipid-mixing indicators.
[1] Diao, J., Grob, P.,⋯, Brunger, A.T. (2012) Synaptic proteins promote calcium-triggered fast transition from point contact to full fusion. eLife, 1, in press.
[2] Diao, J., Su, Z.,⋯, Ha, T. (2010) A single vesicle content-mixing assay for SNARE-mediated membrane fusion. Nat. Commun. 1, 54.
[3] Diao, J., Ishitsuka, Y.,⋯, Ha, T. (2012) A single vesicle-vesicle fusion assay for in vitro studies of SNAREs and accessory proteins. Nat. Protoc. 7, 921.

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Properties of Self-Quenching Fluorescence Dye for Vesicle-Vesicle Content Mixing System
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Since the first in-vitro demonstration of lipid mixing system using purified neuronal components, there has been many attempts to build in-vitro system capable of observe content mixing because neurotransmitter release is main function of membrane fusion in synaptic terminal. To detect content mixing, usually a reporter molecule is encapsulated inside of vesicle. Water soluble self-quenching fluorescence molecules like calcein and sulforhodamine B have been most successful in content mixing experiments because of their high solubility and hydrophilicity contributing easy encapsulation and reduced membrane permeability respectively. Nevertheless, some issues including degree of self-quenching and leakage of dye remain unresolved. We investigated self-quenching property of sulforhodamine B up to 100mM concentration using microscopy system and conventional fluorometer system. We first measured self-quenching property of sulforhodamine B in free solution up to 100mM concentration. Because of excitation light depletion occurring at high concentration, conventional fluorometer measurement is not applicable in this concentration region. So we used TIR microscopy system to obtain correct fluorescence signal of high concentration solution to found that sulforhodamine B is 99.9%(1000X) quenched at 100mM concentration in free solution. Next we made sulforhodamine B encapsulating 50nm size vesicle using extrusion and found that sulforhodamine B is only 90%(10X) quenched when encapsulated inside of a 50nm diameter vesicle. In single vesicle fusion system, this self-quenching property of vesicle encapsulated sulforhodamine B gives increasing fluorescence signal only with occurrence of content mixing thereby enables straightforward fluorescence signal interpretation - signal increase is content mixing and signal decrease is leakage.

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VAMP2 Forms Ring Structures in Lipid Bilayers
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Intracellular membrane fusion events are mediated by the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs). At the nerve terminal, vesicle-associated membrane protein 2 (VAMP2) on the vesicle membrane (v-SNARE) interacts with a binary complex of syntaxin 1 and SNAP-25 on the target plasma membrane (t-SNAREs) to form a four-helix bundle that drives fusion. Although the SNAREs have been studied extensively, there is still little information about the spatial arrangement of these proteins in the vesicle and plasma membranes.
We analyzed the behavior of VAMP2 and the t-SNAREs reconstituted in lipid bilayers using atomic force microscopy (AFM) imaging under fluid. We found that VAMP2 formed ring-shaped structures in the lipid bilayers whereas the t-SNAREs existed as globular structures. Molecular volume analysis indicated that a typical VAMP2 ring contains about eight VAMP2 monomers. Interestingly, the VAMP2 structures were more prominent in bilayers containing cholesterol, indicating that cholesterol directly affects the arrangement of VAMP2 in the bilayer. Rings were not seen with the yeast v-SNARE Snc2p, suggesting that they may be a feature specific to the neuronal v-SNARE.
We propose that the ability of VAMP2 to arrange itself into ring-like assemblies might play a role in organizing SNARE complexes during synaptic vesicle fusion.

461-Pos Board B230
Fast Snare Mediated Synaptic Vesicle Fusion in Supported Membranes
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In vitro reconstitution experiments have played an essential role in a large body of research on SNARE-mediated membrane fusion. One of the open questions is how well reconstituted membranes with recombinant proteins approximate the physiological membrane.
Here we report experiments from a hybrid system consisting of purified synaptic vesicles from rat brain and Syntaxin 1a/SNAP25 containing supported membranes prepared from lipid extracts and recombinant protein. Using total internal reflection fluorescent microscopy (TIRFM), we observed membrane labeled single vesicles as they approached, docked and fused with the plasma membrane-mimicking supported bilayer with ms time resolution. Docking of vesicles could be inhibited by preincubating the acceptor SNARE complex containing supported membrane with the soluble Synaptobrevin fragment Syb2(1-96) or by using protein free membranes. Analyzing the time delay between docking and onset of fusion, we were able to determine the fusion kinetics under different salt conditions. Synaptic vesicle fusion in these hybrid systems showed first order kinetics with typical time constants between 35 ms and 70 ms.
We compare the data with results from experiments in which the synaptic vesicles were replaced by proteoliposomes containing recombinant Synaptobrevin2 and Synaptotagmin1. Docking and fusion was observed by following the fluorescence originating from membrane and content labels.

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SNARE-Mediated Fusion Pore Dynamics from Quantitative TIRF Microscopy
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Neurotransmitter and hormone release occurs when vesicles filled with these cargoes fuse with the plasma membrane. Electrophysiological recordings from neuroendocrine cells and neurons indicate the initial connection between the vesicular and the plasma membrane is a small, highly dynamic pore that can rapidly flicker between open and closed (unfused) states before either fully expanding or permanently closing (''kiss and run'' fusion). The dynamics of the fusion pore regulate the release process and have been proposed to play important physiological roles for different cell types. However, the molecular mechanisms governing the pore dynamics are not known. To examine this problem we have used a recently developed in vitro fusion assay in which fluorescent liposomes reconstituted with the neuronal v-SNAREs (v-SUVs) dock and fuse with planar bilayers containing cognate t-SNAREs (t-SBLs). Upon fusion, fluorescently labeled lipids transfer from the SUV to the SBL. Under appropriate conditions, this transfer results in a change in the total fluorescence due to the properties of TIRF excitation. Here, using a mathematical model for lipid release through a flickering pore we show that the quantitative analysis of the total intensity changes upon fusion allows extraction of the pore connectivity, C_pore, the mean fraction of time the pore is connected. The single-molecule sensitivity of the measurements allowed us to determine the sizes of individual fusing vesicles of sub-optical dimensions. We have studied how the fusion pore dynamics depend on the lipid composition and vesicle size. Cholesterol, when included at physiological quantities, increases the pore connectivity, whereas vesicle size has little influence on the pore dynamics and the fusion probability.