

micromanipulation setup is mounted on a spinning disc confocal microscope, simultaneous monitoring of fluorescence from labeled membranes can also be used to probe vesicle docking and fusion. Our preliminary results show increasing membrane tension increases the fusion rate.

2057-Pos Board B194

Collective Action of SNAREpins Exerts Forces between Membranes that Activate Fusion

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SNARE proteins mediate most intracellular membrane fusion processes such as exocytosis. It has been established that vesicle associated v-SNAREs and target-membrane associated t-SNAREs, assemble into a parallel four-helix bundle (SNAREpin) in a zipper like fashion, which brings the membranes close together. However, the detailed mechanisms whereby SNAREs drive fusion remains unclear. It is also unknown whether several SNAREpins cooperate to induce fusion, and widely varying SNARE requirements for fusion are reported, 2-15 in vivo (Montecucco et al., Trends Biochem Sci, 2005) and 1-11 in vitro (van den Bogaart et al., NSMB, 2010; Karatekin et al., PNAS, 2010). Here, we developed a mathematical model of SNAREpins connecting a vesicle to a planar membrane, quantifying inter-membrane, inter-SNAREpin and membrane-SNAREpin interactions, and taking account of the zipper/unzipping of the SNAREpins. Monte Carlo simulations showed that SNAREpins assemble through the SNARE motifs and self-organize into a ring. The ring tends to expand, driven by inter-SNAREpin and SNAREpin-membrane interactions, reducing membrane separation by geometrical coupling. Assuming an energy criterion for fusion, we determined the waiting times for fusion from the distributions of inter-membrane energies. Our data show that although one SNAREpin can induce membrane fusion, fusion waiting times decrease rapidly with the number of SNAREpins. Applying the model to single-vesicle fusion assays, we predict that the dependency of docking-to-fusion delay times on the number of v-SNAREs reaches a plateau, in agreement with experiments (Karatekin et al., PNAS, 2010). We also find that waiting times increase with vesicle size and with insertion of flexible segments into the SNARE linker domains, in qualitative agreement with experiments (McNew et al., Mol Cell, 1999). Our results suggest that fully zippered SNAREs work in concert to trigger fusion, and explain the wide range of reported SNARE requirements for fusion.

2058-Pos Board B195

Cholesterol Modulates SNARE Mediated Hemi- and Full-Fusion

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Cholesterol is essential for exocytosis in secretory cells, but distinguishing contributions from lateral organization and dynamics of membrane proteins and stabilization of fusion pores by intrinsic curvature and other mechanical effects of cholesterol have been elusive. The direct effect of cholesterol on fusion pore formation was examined between synaptobrevin 2 (VAMP 2) containing proteoliposomes and an acceptor SNARE complex containing planar supported bilayer using both membrane and content fluorescent markers. This revealed that increasing cholesterol in either the planar supported bilayer or in the synaptobrevin proteoliposome decreases the amount of hemi-fusion and increases the amount of full-fusion with minimal effects on the fusion kinetics.

2059-Pos Board B196

Chasing the Functional Asymmetry between C2A and C2B in Full-Length Synaptotagmin 1 during Ca²⁺-Dependent Membrane Binding

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Synaptotagmin 1 (Syt1) acts as the major calcium sensor in neuronal exocytosis. Syt1 is a synaptic vesicle-anchored membrane protein that contains two tandem Ca²⁺ binding C2 domains, named C2A and C2B. There is evidence that Syt1 interacts with membrane lipids and SNARE proteins simultaneously to facilitate two key steps during Ca²⁺-triggered membrane fusion: vesicle binding and content release. Although the overall function of Syt1 has been extensively investigated, the detailed molecular behavior of the single C2A and C2B domains in the neural regulatory process remains unclear. In particular, the differential function of the two C2 domains and how they cooperate in membrane fusion has not been determined.

Here we employed EPR spectroscopy, fluorescence interference contrast (FLIC) microscopy and total internal reflection fluorescence (TIRF) microscopy to dissect the state of C2A and C2B domains in full-length Syt1 during Ca²⁺-dependent membrane binding. CW-EPR lineshapes and power saturation of spin-labeled positions in calcium binding loops of C2A in full-length Syt1 (1-421) and truncated Syt1 (1-266) without C2B domain suggest that C2A domain alone in Syt1 has a stronger membrane binding ability. A TIRF liposome capture assay further reveals that truncated Syt1 has a significant higher initial rate and extent in trans-binding with liposomes than full-length Syt1. Our data indicate that the C2B domain might be involved in some cis-binding thereby reducing total liposome binding. FLIC microscopy validates the strong involvement of C2A during Ca²⁺-dependent liposome binding.

Our detailed structural and functional information thus provides a clue to differential regulatory mechanisms employed by C2A and C2B domains in full-length Syt1 interacting with Ca²⁺ and key membrane lipids, such as PS and PIP₂.

2060-Pos Board B197

Mechanical Model for Self-Assembly of Synaptotagmin on a Lipid Membrane

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Synaptotagmin (Syt) is a calcium-sensor that is responsible for the action-potential-controlled fusion of synaptic vesicles to pre-synaptic membranes. Recent biochemical and structural studies show that Syt can form ring-like oligomers, which occasionally convert into tubular structures on monolayer or bilayer membrane with buckled membrane inside. This suggests certain mechanical interactions between the Syt and the lipid bilayer. To explore it in detail, we developed a coarse-grained mechanical model assuming that (i) Syt self-polymerizes into an elastic chain with a spontaneous curvature; (ii) Syt attracts the membrane through binding sites located at the inner side of the Syt molecule; and (iii) membrane is a uniform sheet with constant bending rigidity and tension. Using computer simulations, we have been able to estimate the spontaneous curvature and bending stiffness of the Syt chain. The model also allowed us to understand how the Syt oligomerization depends on the strength of Syt-membrane adhesion, the bending rigidity of the membrane, and the pressure on the membrane. These experimentally testable predictions from this modelling study will provide insight into the molecular mechanism of calcium-triggered membrane fusion at the synapse.

2061-Pos Board B198

Single Vesicle Assay to Study Membrane Tethering and Docking Factors Jiajie Diao.

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In vitro vesicle-vesicle fusion assays based on fluorescence resonance energy transfer (FRET) of lipid or content mixing are widely used to investigate the molecular mechanism of membrane fusion process. However, without FRET signals from lipid or content mixing, these ensemble assays are insensitive to early stages of fusion such as tethering and docking. We have developed a single vesicle assay to study protein factors involved in tethering and docking [1-3]. Through our assay, we have studied proteins inducing membrane aggregation [4] and enhancing SNARE-mediated vesicle docking [5-6].

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2062-Pos Board B199

Deficiency of HID-1 Leads to Impaired Proinsulin Processing

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Diabetes mellitus has been a major social problem due to its prevalence, which results from a combination of peripheral insulin resistance and insufficient insulin secretion. The whole process of insulin biosynthesis, transportation, maturation and secretion is largely understood, however, detailed mechanism remains to be uncovered. We reported here a highly conserved protein, HID-1, functions during the correct processing of insulin. We generated a beta cell-specific conditional knock out mouse model of hid-1 gene. The knock out mice showed significant glucose intolerance while normal response to insulin with no significant defect in islets' morphology. Further study revealed remarkable increase in proinsulin to insulin ratio and abnormal proinsulin accumulation, suggesting that HID-1 may function in the conversion process from proinsulin to insulin.