

membrane. Simulations show that a minimally-sized cluster ring expands outward, driven principally by electrostatic and steric repulsions between the SNARE complexes. Ring expansion thereby pulls the curved vesicle surface toward the target membrane. Addition of new complexes to the cluster further expands the ring, thus reducing the membrane separation and elevating the pressure between the membranes. We find a cluster of ~5-10 complexes docking a 50-nm vesicle exerts local pressures of tens of atmospheres, similar to the threshold pressures required for fusion measured in planar bilayer systems [Wong et al, *Biophys J.*, 1999]. Thus, the SNARE cluster-generated pressure may be sufficient for fusion. This is consistent with the recently reported 5-11 complex requirement. In addition, our model makes the testable prediction that fusion of smaller (higher curvature) vesicles requires fewer SNAREs.

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Direct Observation of Dual Pathways of Yeast Minimal-Machinery-SNARE Driven Vesicle Fusion

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Solution Single Vesicle Fusion Assay Reveals PIP₂ Mediated Sequential Actions of Synaptotagmin-1 onto SNAREs

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Synaptotagmin-1 (Syt1) is a major Ca²⁺ sensor for fast synchronous neurotransmitter release, which requires vesicle fusion mediated by SNAREs. Syt1 is known to interact with target membrane (-) SNARE, ternary SNARE complex, and anionic phospholipids. However, how Syt1 utilizes its diverse interactions to regulate vesicle fusion remains illusive. To dissect the functions of Syt1, we apply a single-molecule technique, alternating-laser excitation (ALEX), which is capable of sorting out all subpopulations of fusion intermediates in bulk solution, particularly the docking stage before lipid mixing. The results show that membrane-anchored Syt1 undergoes at least three distinct steps prior to lipid mixing. First, in the absence of Ca²⁺, Syt1 mediates vesicle tethering by directly binding to t-SNARE, which requires PIP₂. Second, synaptobrevin-2 binding to t-SNARE to form the ternary complex displaces Syt1 from the SNARE complex. Third, in the presence of Ca²⁺, Syt1 rebinds to the SNARE complex, which again requires PIP₂. Thus in the absence of Ca²⁺, Syt1 may bring vesicles to the plasma membrane in proximity via binding to t-SNARE/PIP₂ to help ternary SNARE complex formation and then, upon Ca²⁺-influx, it may rebind to the ternary complex with the aid of PIP₂, which may trigger fast synchronous fusion.

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Structural Model of the Juxtamembrane Region of the Trans-SNARE Complex with EPR

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Neuronal SNARE proteins play a central role in mediating the fusion of synaptic vesicles with the nerve cell plasma membrane, which is necessary for neurotransmitter release. Plasma membrane SNARE Syxtaxin 1A and vesicle

SNARE Synaptobrevin 2, anchor to respective membranes and the juxtamembrane regions connect their SNARE motifs to the respective transmembrane domains. SNARE complex formation, *in trans*, brings vesicle and membrane closely together to the pre-fusion state. The zippering may continue to the linker regions and extend the helical structure all the way through the transmembrane domain (*cis*-SNARE complex). Although the *trans*-SNARE core complex and *cis*-SNARE complex structures are known, it is not known what the linker region structure should be act in the *trans*-complex, which is believed to be a force transducer that plays a role in membrane merging. Here, we investigated SNARE complexes containing linker region truncated version of Syxtaxin 1A or Synaptobrevin 2 to mimic the *trans*-complex using spin labeling electron paramagnetic resonance (EPR). We will present the new EPR results that might shed lights on the structure of the *trans*-SNARE complex.

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Single Vesicle Fusion System for Content Mixing and SNARE Complex Formation

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SNARE proteins drive membrane fusion by SNARE complex formation. However, it is hard to study SNARE complex formation dynamics and fusion kinetics with single molecule resolution in realtime. We developed new single-molecule FRET analyze mechanism and adapted single vesicle content dequenching assay to observe content mixing and SNARE complex formation simultaneously. Our result reveals existence of two different kinetic components in SNARE complex formation which was inaccessible in previous studies.

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Insights into Membrane Fusion from Molecular Dynamics Simulations of SNARE Proteins

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The SNARE proteins family affects numerous intra-cellular fusion processes. Synaptobrevin (Sb), Syntaxin-1 (Sx) and both SNAP25 (Sn) chains are involved during synaptic vesicular exocytosis. They form a bundle of four alpha-helices. Both Sb and Sx have a trans-membrane domain (TMD) used to anchor the bundle between the vesicle and cell membranes. It has been shown experimentally that these proteins trigger the fusion process *in-vitro* as well as *in-vivo*. Yet the molecular mechanisms of this process are not elucidated. Molecular dynamics simulation (MD) approaches offer very detailed insights into such systems and can describe the behavior of each component. We performed several simulations of a membrane-embedded SNARE complex between two mixed POPC/POPS membranes. Despite a simulation artefact initially moving the membranes away from each other, we observe strong deformations around the TMDs and a decreasing distance between them. If the link between the TMDs and the bundle is severed, both membranes go back to a flat state. This observation can be explained by very robust inter-helical interactions that prevent the bundle from breaking away. In addition, the TMD composition allows them to be strongly anchored to the membranes. Electrostatic interactions between the proteins and the membrane further seem to help accelerate this process.

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HIV gp41 Trans-Membrane Domain Promotes both Stalk and Fusion Pore Formation in Poly(Ethylene-) Glycol Mediated Membrane Fusion

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The trans-membrane domain (TMD) of gp41 is essential for efficient fusion between HIV-1 and its host cell *in vivo*. HIV virus with gp41 mutated by R696L is reported to be defective in infectivity and fusion (Helseth, *J Vir*, 1990, 6314), so we have examined both native and R696L gp41 TMDs' effects on PEG-mediated fusion of PC/PE/SM/CH (35/30/15/20) SUVs. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to a 3-state sequential model (Weinreb & Lentz, *BJ*, 2007, 4012), from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state. The WT peptide increased the rates of stalk (k1) and fusion pore (k3) formation in a cooperative fashion