

3198-Plat**Syntaxin Clustering in Membranes**

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Syntaxin-1A and SNAP25 form the receptor component of the SNARE complex, which has been shown to be the minimal machinery for membrane fusion. In vivo studies have revealed that syntaxin-1A exists in cholesterol-dependent clusters that are distinct from lipid rafts. Additionally, SNARE-mediated membrane fusion has been shown to be stimulated by regulatory lipids, such as phosphatidylinositol 4,5-bisphosphate (PI-4,5-P2). An appreciation of the lipid-protein interactions which define syntaxin clustering dynamics is essential to understand the membrane role in organization of SNARE-mediated membrane fusion.

We determined that syntaxin exists in cholesterol-dependent clusters, from which it may be released by as little as 1-5 mole percent PI-4,5-P2 by in vitro fluorescence assays. Lipid-protein fluorescence resonance energy transfer reveals that the phosphoinositide interaction is direct and mediated by electrostatics. To investigate the dynamics of clustering, single-molecule fluorescence quenching microscopy was developed. The observation of syntaxins in single vesicles allows the step-wise statistical analysis of discrete syntaxin-syntaxin interactions, and determination of their dependence on concentration and membrane composition. These in vitro results help explain the mechanisms of dynamic clustering of syntaxin in cell membranes, and the activation of fusion by PI-4,5-P2. Moreover, they suggest a working model for cell membrane regulation of syntaxin clustering.

3199-Plat**A Fast, Single-Vesicle Fusion Assay Mimics Physiological SNARE Requirements**Erdem Karatekin^{1,2}, Jérôme Di Giovanni^{3,4}, Cécile Iborra^{3,4}, Jeff Coleman¹, Ben O'Shaughnessy⁵, Michael Seagar^{3,4}, James E. Rothman¹.

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SNARE proteins play a central role in nearly all intracellular fusion reactions; fusion is thermodynamically driven by formation of trans-SNARE complexes (SNAREpins) through pairing of vesicle-associated v-SNAREs with complementary t-SNAREs on target membranes. However, the number of SNARE complexes required for fusion is unknown and there is controversy about whether additional proteins may be required to account for the speed with which fusion can occur in cells. Small unilamellar vesicles containing the synaptic/exocytic v-SNAREs VAMP/syntaxin fuse rapidly with planar, supported bilayers containing the synaptic/exocytic t-SNARE syntaxin-SNAP25, with single fusion events occurring in ~10 ms to seconds. However, in previous reports the SNAP25 subunit of the t-SNARE was not required, or an artificial peptide was needed, raising questions about the physiological relevance of these results. We now include a lipid-linked polymer chain in both bilayers whose size and density mimic that of bulk membrane proteins that need to be cleared before fusion in physiological settings. The result is that SNAP25 dependence is restored but rapid fusion (mean delay after docking of 130 ms) remains. A dramatic drop in the overall fusion rate occurs as the number of v-SNAREs per vesicle is reduced below a threshold of 5-10 externally-oriented v-SNAREs per vesicle, directly establishing this as the minimum number required for rapid fusion. The distribution of delay times for fusion following docking by the first SNAREpin appears to be limited by the time required for additional t-SNAREs to diffuse to the docking site, and implies that 5-10 t-SNAREs must be recruited to achieve fusion, closely matching the v-SNARE requirement.

3200-Plat**Atomic-Resolution Simulations Yield New Insight into Vesicle Fusion and Fusion Protein Mechanisms**Peter Kasson¹, Erik Lindahl², Vijay Pande¹.

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Membrane fusion is critical to both cellular vesicle trafficking and infection by enveloped viruses. While the fusion protein assemblies that catalyze fusion are readily identifiable, the specific activities of the different proteins involved and nature of the membrane changes they induce remains unknown. Here, we report many atomic-resolution molecular dynamics simulations of both fusion by a pair of vesicles and assemblies of influenza fusion peptides in planar bilayers. The mechanism of fusion in our simulations is roughly consistent with the stalk hypothesis for fusion, but we observe several new features that help explain the mechanism of fusion proteins. Our high-resolution simulations yield new

structural intermediates that differ substantially from continuum models of fusion and give specific structural details for the membrane-altering effects of fusion proteins. These results may yield a common mechanistic pathway for structurally diverse classes of fusion proteins.

3201-Plat**Docking is the Rate-Limiting Step in Reconstituted Neuronal SNARE-Mediated Liposome Fusion**

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Over the past ten years, great efforts have been made to reconstitute SNARE-mediated vesicle fusion in vitro by monitoring the degree of lipid mixing between populations of vesicles in solution. The reconstitution approach is powerful because the vesicles have well-defined protein and lipid components and the effects of adding or subtracting a specific component can be assessed directly. In accord with previous work, we find that proteoliposomes composed of full-length SNAREs alone are capable of docking and fusing very slowly in solution. We have developed a FRET-based, single-vesicle fusion assay in which tethered v-SNARE vesicles containing synaptobrevin-II interact with solution-phase t-SNARE vesicles containing syntaxin-1A + SNAP-25. This enables separation of individual docking and fusion events. Our data clearly indicate that the rate-limiting step in this reaction is the docking step. Adding a peptide comprised of synaptobrevin-II(57-92) into the reaction significantly increases the efficiency of fusion by increasing the efficiency of docking. Once docking is achieved, fusion is very rapid, occurring on a time scale of ~0.3 s or less. The data strongly suggest that the synaptobrevin binding site in the t-SNARE complex is badly occluded in the absence of the syb(57-92) peptide. It seems possible that many added components that accelerate fusion in bulk assays act primarily to mitigate t-SNARE entanglements that prevent SNARE complex formation and enhance docking efficiency, not to accelerate the fusion step itself.

3202-Plat**Single Molecule Content Mixing Analysis of SNARE-Mediated Membrane Fusion**Jiajie Diao¹, Zengliu Su², Yuji Ishitsuka^{1,3}, Yeon-Kyun Shin², Taekjip Ha^{1,3}.

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A single liposome fusion assay has been developed in our lab to unambiguously detect different stages of fusion including docking, hemi and full fusion via fluorescence resonance energy transfer. [1, 2] While this assay provides the extent of lipid mixing, the presence of the fusion pore opening cannot be detected. In order to further push the technique and to reveal the minimal machinery of SNARE-mediated membrane fusion, we have developed a single molecule assay based on content mixing of a large DNA probe. It has been confirmed that yeast SNARE proteins are enough to produce large and stable fusion pores for content mixing. For the neuronal case, our results show that SNARE complexes are not enough to expand fusion pores, and the fusion regulator protein synaptotagmin is necessary for the completion of content mixing via pore expansion.

References

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Platform AY: Molecular Mechanics & Force Spectroscopy**3203-Plat****Domain Insertion Effectively Regulates the Mechanical Unfolding Hierarchy of Elastomeric Proteins: Toward Engineering Multifunctional Elastomeric Proteins**

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The architecture of elastomeric proteins controls fine-tuned nanomechanical properties of this class of proteins. Most elastomeric proteins are tandem modular in structure, consisting of many individually folded domains of varying stability. Upon stretching, these elements unfold sequentially following a strict hierarchical pattern determined by their mechanical stability, where the weakest element unfolds first and the strongest unfolds last. Although such a hierarchical architecture is well-suited for biological functions of elastomeric proteins, it may become incompatible with incorporating proteins of desirable functionality in order to construct multifunctional artificial elastomeric proteins, as many of these desired proteins are not evolved for mechanical purpose.