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Single Neuronal Snare Complexes Zipper in Three Distinct Stages
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SNARE proteins drive membrane fusion by assembling into a four-helix bundle in a zippering process. Here we used optical tweezers to observe in real time a long-sought SNARE assembly intermediate in which only the membrane-distal N-terminal half of the bundle is assembled. Our finding supports the zippering hypothesis, but suggests that zippering proceeds through three sequential binary switches, not continuously, in the N- and C-terminal halves of the bundle and the linker domain. The half-zippered intermediate was stabilized by externally applied force which mimicked the repulsion between apposed membranes being forced to fuse. This intermediate then rapidly and forcefully zippered, delivering free energy of 36 kBT to mediate fusion.

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Mechanical Unzipping and Rezipping of a Single SNARE Complex Reveals Large Hysteresis as the Force Generating Mechanism
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SNARE proteins are thought to provide energy required for membrane fusion through formation of a parallel four-helix bundle, called the SNARE complex. However, the molecular mechanism by which the SNARE-complex formation is translated into mechanical thrust is still unclear. By using magnetic tweezers, we observed dynamic mechanical behavior of single neuronal SNARE complexes under constant force levels. Single neuronal SNARE complexes could be unzipped by applying a 34 pN force. This unzipping process, starting from the C-terminal end, showed frequent stops near the ionic layer, implying the C-terminal half of SNARE motifs was selectively disassembled. Remarkably, this partially-assembled state could be stably maintained even under an 11 pN force, which was more than 20 pN less than the unzipping force. When the force was further lowered by only a few pN from stably-clamping 11 pN, assembly of the C-terminal half could be efficiently triggered. Our observations suggest large hysteresis in unzipping and rezipping of single neuronal SNARE complexes as a function of applied force. This mechanical hysteresis prevents unzipping in the partially-assembled state, permitting robust one-way zippering toward transmembrane domains. Therefore, the mechanical hysteresis rectifies the direction of SNARE complex formation, making the SNARE complex a faithful force-generating machine.

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Dynamic Ca^{2+}-Dependent Activity of Membrane-Anchored Synaptotagmin1 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. Therefore, Syt1 seems to become a far versatile fusion regulator when it is in a form of membrane protein.

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IFITM Proteins Block the Creation of Hemifusion
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Interferon-inducible transmembrane (IFITM) proteins have previously been shown to potently reduce infection caused by several highly pathogenic viruses, but the mechanisms of inhibition were not identified. There are three known functional IFITM members that are widely expressed in human tissues and cell lines. The extent of inhibition of infectivity varies among the functional IFITM-members. We have found that the presence of IFITM proteins blocks viral-protein-induced fusion in a pattern that is dependent on the cell type expressing the fusion protein. IFITM1 blocks fusion and IFITM2 does not, independent of cell line. The IFITM3 block does depend on the cell line expressing the fusion protein. The pattern of blockage was the same for viral fusion proteins of the three structural classes of fusion proteins, indicating that the block occurs through a physical mechanism, rather than being dependent on specific structural or chemical properties. We performed cell-cell fusion experiments for low-pH-requiring proteins to determine the intermediate point at which inhibition occurs. We acidified at low temperature to create a state of hemifusion and found that subsequent raising of temperature or addition of chlorpromazine (CPZ) did not cause fusion for IFITM1 target cells. We conclude that the interferon-inducible protein blocks fusion at a step prior to hemifusion. Experiments were performed in which oleic acid (OA) was added prior to or subsequent to creating the hemifusion intermediate to determine whether the negative spontaneous curvature conferred by OA could relieve the block caused by IFITM. OA promoted fusion only if present prior to creating the hemifusion intermediate, showing that this amphiphile facilitated the occurrence of hemifusion. We conclude that the physical state of the lipid bilayer is altered by IFITM proteins, and this change underlies the inhibition of fusion and infectivity.

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Multiple Effects of Cholesterol on Influenza Membrane Fusion Kinetics
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Influenza and other enveloped viruses differ in their envelope lipid composition from the cellular plasma membrane from which they bud. Viruses also appear to fuse preferentially to specific membrane compartments, suggesting that the lipid environment may play a selective role in permissiveness for fusion. Cholesterol in particular is enriched in the viral membrane and known to exert effects on fusion kinetics and efficiency in a number of model systems for viral infection. Cholesterol can affect membranes in multiple ways, both impacting materials properties such as membrane bending energy and lateral organization of the membrane.
To probe the different effects of cholesterol, we have measured viral fusion kinetics as we vary the cholesterol concentration in both live influenza virus and target liposomes. The liposomal membrane composition was selected not to support lateral membrane heterogeneity. We observe an increase in fusion rate with increasing liposomal cholesterol concentration, consistent with a model where cholesterol promotes stalk formation and stalk formation is the rate-limiting step of the fusion reaction we measure. We observe a more complex dose-response relationship as cholesterol is extracted from influenza virions. Since hemagglutinin is known to have cholesterol-dependent lateral organization, we postulate that the altered kinetics with changes to viral sterol content reflect both changes to hemagglutinin organization in the membrane and changes to the material properties of the membranes. We further characterize this dose-response relationship to help resolve these disparate effects of cholesterol in mediating viral entry.

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The Role of Lipid Composition in the Fusion of Dengue Virus with Liposomes
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Pathogenic membrane-enveloped viruses including flaviviruses, arenaviruses, and paramyxoviruses cause devastating infectious diseases (e.g., hemorrhagic fever, encephalitis, etc.) and pose major biodefense threats. For these viruses, fusion of the viral membrane with the cellular endosomal membrane is essential for viral replication and infectivity. In flaviviruses, fusion of the two membranes is facilitated by pH-dependent conformational changes of a dedicated envelope (E) protein. Specifically, we have been studying this fusion process in Dengue virus. Dengue is endemic in more than 100 countries with an estimated 100 million cases of infection reported per year. After binding to a cell-surface receptor, the virus is taken up into an endosome compartment where the pH gradually decreases. A low pH environment induces conformational changes in the E protein that cause the viral membrane to fuse with the endosomal membrane, resulting in the release of viral RNA into the cytosol, followed by replication. Recently, it has been reported that full viral fusion only occurs with membranes containing anionic lipids, such as those found in late endosomes. The origin of this very strong dependence on lipid composition