Neurotransmitter vesicles are triggered to fuse with the plasma membrane within milliseconds. The vesicles are prepositioned adjacent to the membrane to facilitate fast synchronized fusion, but fusion itself relies on the helical transmembrane SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptor). The vesicle (v-) or target (t-) SNAREs are in the vesicle or plasma membranes, respectively, bundled in their pre-fusion trans-conformations. In this conformation, only their N-terminal helices are assembled into four-helix bundles. The clamping protein complexin prevents further assembly into a full four-helix coiled-coil. Complexin is released upon activation, and the SNAREs then progressively assemble into full coiled-coil four-helix bundles. This ‘zippering’ provides the energy for membrane fusion, resulting in a single membrane with the SNAREs in the post fusion cis-conformation. Ca\(^{2+}\) influxes activate fusion through synaptotagmin1 (Syt1), which trigger the t-SNARE syntaxin and synaptosomal-associated protein 25 (SNAP25) to complete zippering with the v-SNARE vesicle associated membrane protein 2 (VAMP2). Syt1, which is inserted in the vesicle membrane, has two cytoplasmic domains, C2A and B, which interact in the presence of Ca\(^{2+}\) with PIP\(_2\) in the plasma membrane. Recently, a crystal structure of a pre-fusion SNARE complex was solved. A C-terminal truncated VAMP2 prevented the formation the full coiled-coil structure, thus capturing the SNARE complex in the pre-fusion trans-conformation with complexin.

Two models have been proposed for the coupling of Ca\(^{2+}\) sensing to fusion. In one, the two C2 domains of Syt1 rearrange on the surface of the SNAREs, resulting in the SNARE rearrangement. Alternatively, a rigid Syt1 may insert in the membrane, perturbing the clamped complexin-SNARE complex. To resolve these models, purified SNAREs were reconstituted in nanodiscs, circular lipid bilayers enclosed by two helical amphipathic proteins. The nanodiscs contained syntaxin/SNAP25 or VAMP2, wild type or a mutant in which the C-terminus cannot form the full four-helix coiled-coil, thus resembling the trans-SNARE pre-fusion state. Föster resonance energy transfer (FRET) fluorophores were attached to the helix of SNAP25 and the C2 domains of Syt1. The C2B domain of Syt1 interacted with SNAP25 in both pre- and post-fusion conformations (with mutant or wild type VAMP2, respectively), independently of complexin. The fluorophore NBD was next attached to the C2 domains. NBD is strongly fluorescent in hydrophobic environments such as membranes, but its fluorescence is quenched by rhodamine. The fluorescence of C2A or B increased within milliseconds after Ca\(^{2+}\) addition, and was equally quenched by rhodamine in t- or v-nanodiscs. Adding PIP\(_2\) to the t-discs, however, resulted in preferential quenching at them. Lastly, Ca\(^{2+}\) did not change the interaction between Syt1 and the SNAREs, as evaluated by FRET at millisecond scale.

In conclusion, Syt1 is a rigid molecule that interacts with the SNAREs before and after Ca\(^{2+}\) activation. A model is proposed in which in presence of Ca\(^{2+}\) the Syt1 C2 domains rapidly insert into the target membrane containing PIP\(_2\). This insertion provides the force required to dislodge the complexin clamp from the SNARE complex, such that ‘zippering’ of the SNAREs can proceed.


Supporting article: Kümmel, D. et al., Complexin cross-links pre fusion SNAREs into a zigzag array. Nat. Struct. Mol. Biol. 18, 927–33 (2011)