Supplementary Note

The structure of ~13nm MSP1E2 was modeled based on crystal structure 1AV1 of the lipid binding domain of ApoA-I[26]. To generate the smaller 6 nm disc helix 4 to helix 6 were deleted using Modeller[27]. The lipids were chosen based on the synaptic vesicle lipid composition[28] (12 nm disc: 154 CHOL, 13 PPCS, 69 POPC, 89 POPE and 25 POPS; 6 nm disc: 50 CHOL, 5 DPSM (sphingomyelin), 23 POPC, 30 POPE, 9 POPS). Lipid numbers were chosen based on previous simulation results showing that MSP1E2 nanodisc contains ~125 DMPC lipids/leaflet[29]. DMPC has an area per lipid (APL) of 0.61 nm^2. Considering the average APL of 0.44 nm^2 for the multicomponent planar bilayer, total number is ~173 lipids/leaflet. The appropriate lipids from a pre-equilibrated self-assembled asymmetric bilayer were placed in the empty nanodisc. A short equilibration (50 ns) was carried out with the head groups restrained along Z-direction (normal to bilayer) followed by an unrestrained 500 ns simulation.

SNARE complex structures were taken from 3HD7 pdb files[30]. The missing C terminal residues T116 of Syb2, F287/G288 of Stx1, and K201-G206 of SNAP-25, and part of the SNAP-25 linker K83-H100 (KFCGLCVCPCNKLKSSDA) were added using Modeller. The SNAP-25 palmitoyl lipid anchors were added to C85, C88, C90 and C92 based on the lipid tail definition for the DPPC CG model. The atomistic structures of Syb2 and the Stx1/SNAP-25 t-SNARE complex were separately converted to respective martini coarse-grained representation using the standard martinize script. To generate a SNARE complex trans configuration that can bridge vesicle and plasma membrane, the Syb2 and Stx1 TM domains were first separated by pulling on Syb2 T116 relative to G288 of Stx1. Subsequently the pulling was continued placing the Stx1 TM domain in a DPPC bilayer. The pulling was performed at constant velocity of 0.2 nm/ns until the +5 layers in the SNARE domains were separated. Four copies of such SNARE complexes were placed in a simulation box with the SNARE domains pointing radially away from each other. This protein CG structure was then inserted into an asymmetric bilayer with the Stx1 TM domains using a recently developed self-assembly method[31]. Plasma membrane lipid composition was based on ref. 32: CHOL 1142, DBSM 8 (sphingomyelin), DPG1 52 (ganglioside GM1), DPG3 52 (ganglioside GM3), DPPC 437, DPSM 120 (sphingomyelin), DUPE 367, DXSM 3 (sphingomyelin), PNSM 3 (sphingomyelin), PAP2 68 (PIP2), PAPC 99, PAPE 296, PAPS 39, POP2 16 (PIP2), POPC 486, POPE 112, POPS 52, PUP2 8 (PIP2), PUPC 60, PUPE 114, PUPS 252. The nanodsic shown in Fig. 1B was then placed such that the syb2 TM domains were properly located in the nanodisc lipid bilayer. The system was subjected to several rounds of energy minimization with removing severe clashes manually at each round. After successful minimization, 20 ns long restraint free equilibrations were done at successive time steps of 0.005 ps, 0.01 ps and 0.02 ps. The production simulation was run with 0.02 ps time steps.

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