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SUPPLEMENTARY INFORMATION

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A molecular mechanism for calcium-mediated synaptotagmin-triggered exocytosis

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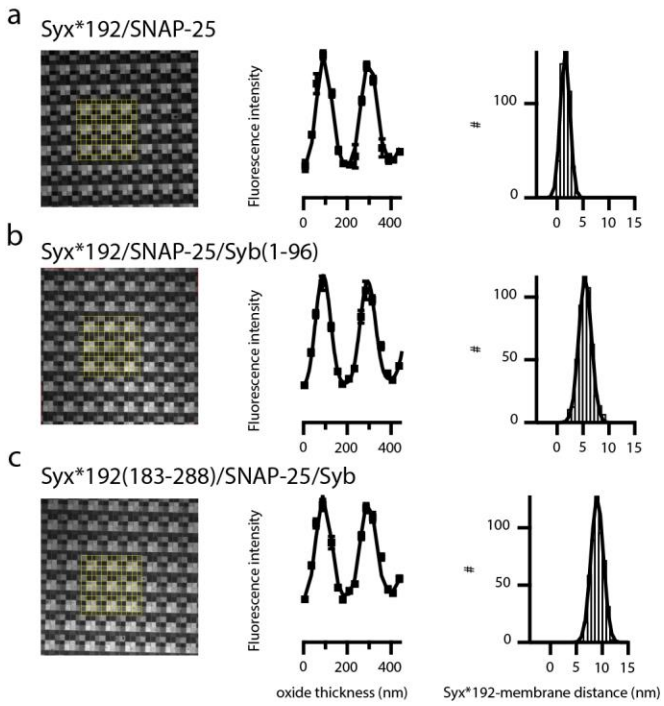
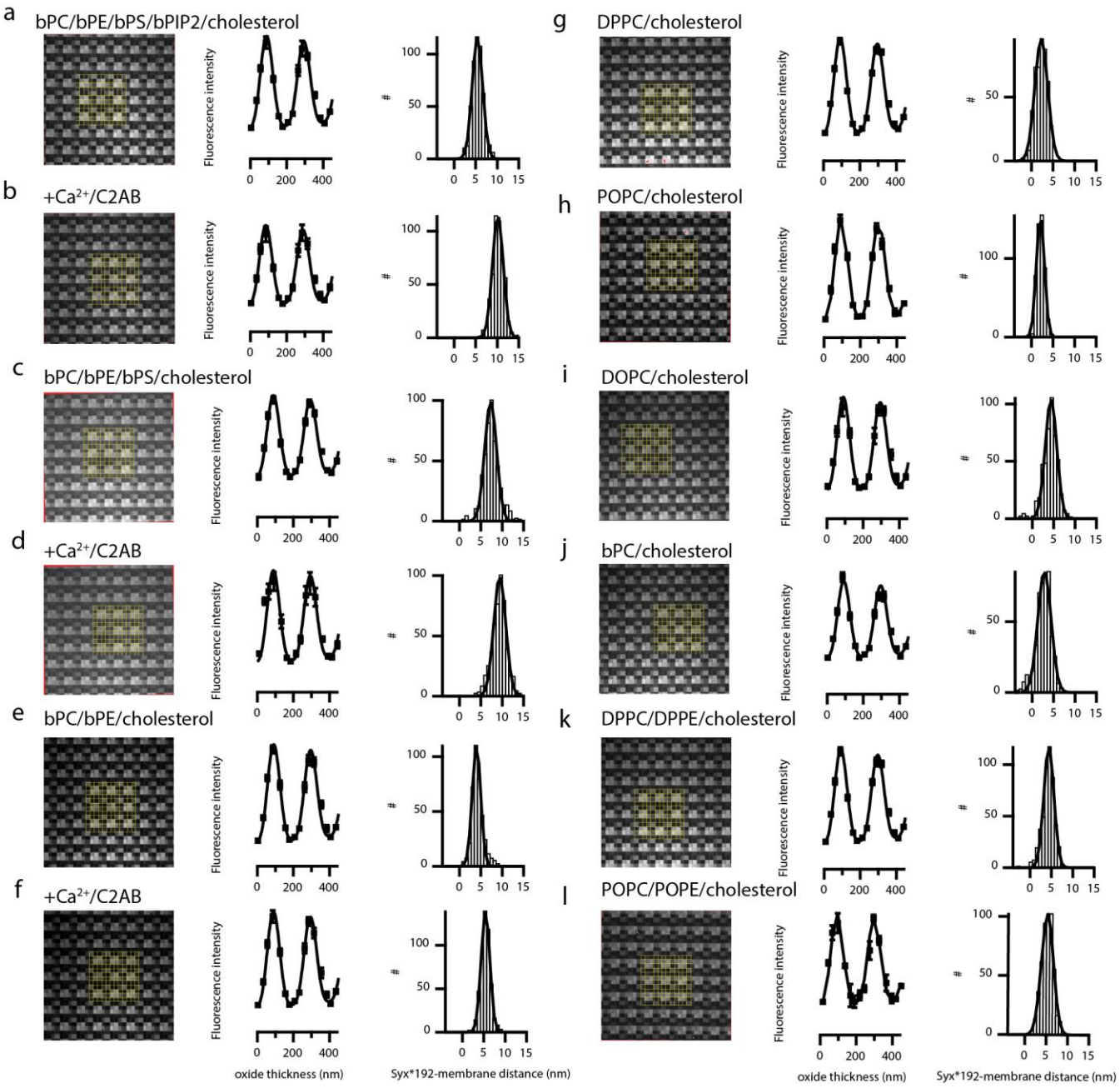


Supplementary Figure 1

SdFLIC example images and evaluation of different SNARE complexes in bPC/bPE/bPS/bPIP2/cholesterol.

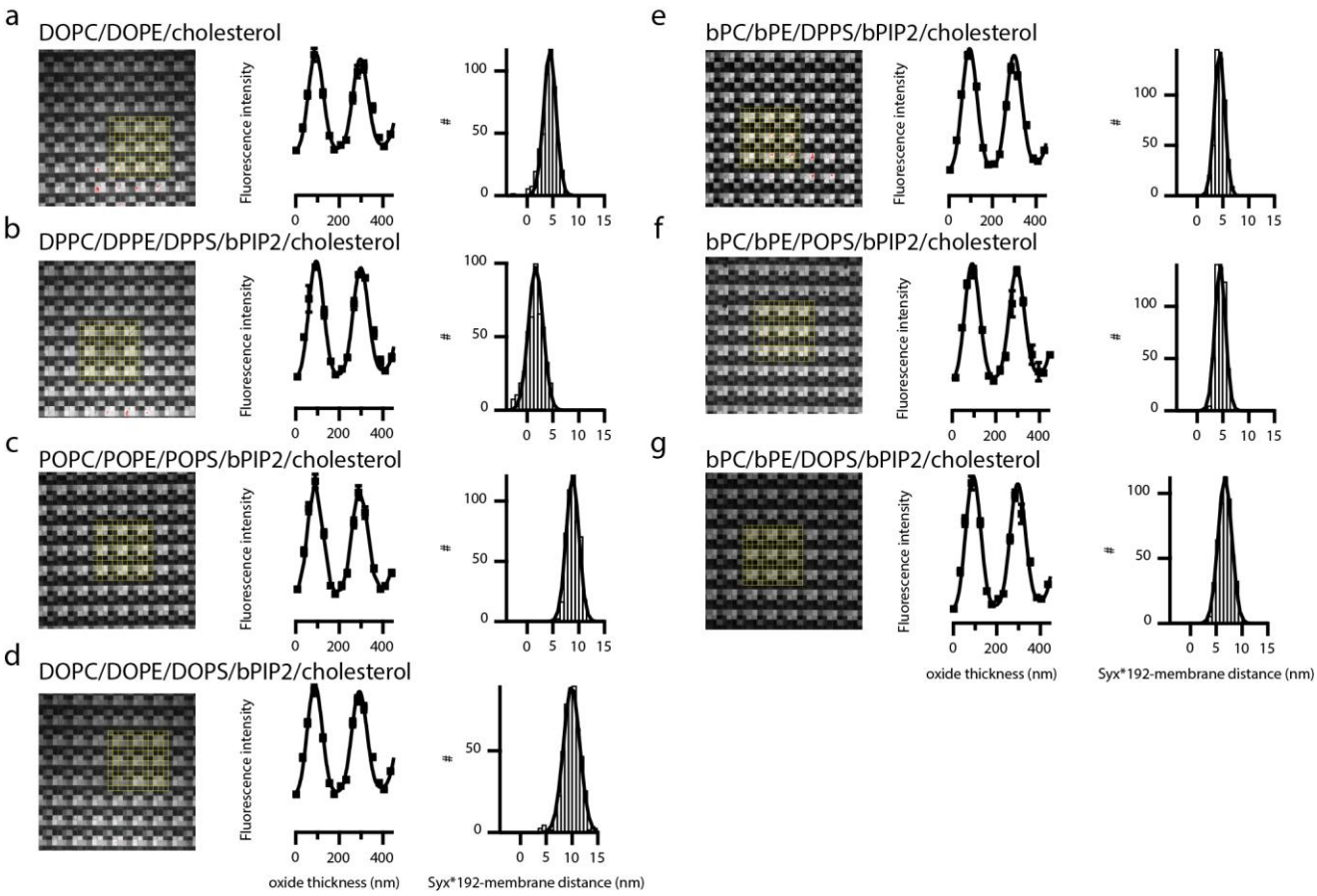
a–**c**, Example sdFLIC images (second column) with a yellow grid of 12 × 12 oxides that were evaluated, extracted intensity data from one set of 16 oxides and FLIC fit curve (third column), and example histogram of fit results (fourth column) obtained from one sample under one condition for Syx*192/SNAP-25 (**a**), Syx*192/SNAP-25/Syb(1-96) (**b**), and Syx*192(183–288)/SNAP-25/Syb (**c**). Images were rotated and scaled for printing and show an area of 201 × 201 μm^2.



Supplementary Figure 2

SdFLIC example images and evaluation for Syx*192/SNAP-25/Syb(1-96) in different lipid environments.

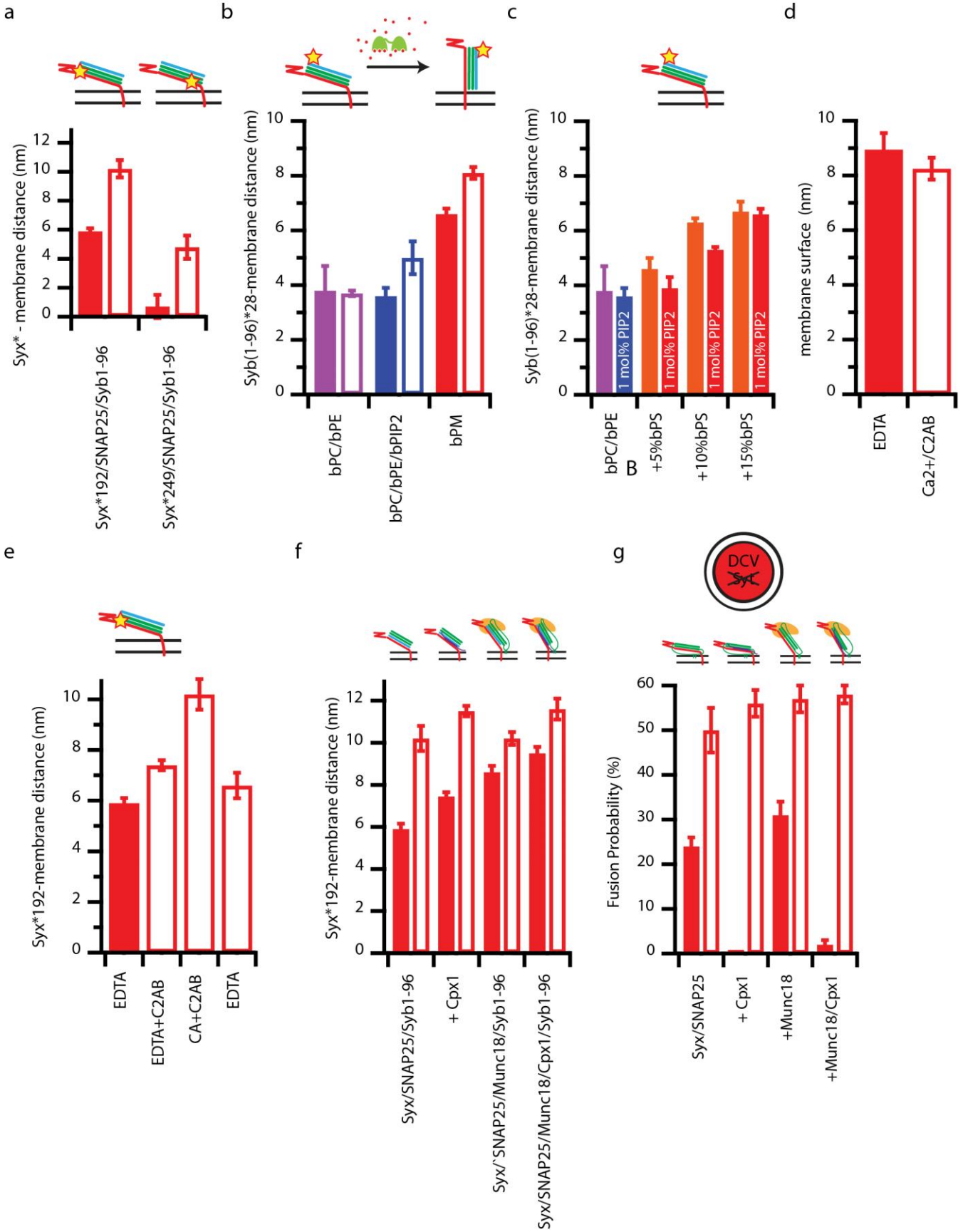
a–**l**, Example sdFLIC images with a yellow grid of 12 × 12 oxides that were evaluated, extracted intensity data from one set of 16 oxides and FLIC fit curve, and example histogram of fit results obtained from one sample under one condition for Syx*192/SNAP-25/Syb(1-96). Images in **a**–**f** were taken before and after the addition of Ca^{2+}/C2AB in different lipid headgroup environments, as described in Fig. 1d and indicated in each panel. Images in **g**–**l** were taken in different lipid environments, as described in Fig. 2a and indicated in each panel. Images were rotated and scaled for printing and show an area of 201 × 201 μm^2.



Supplementary Figure 3

SdFLIC example images and evaluation for Syx*192/SNAP-25/Syb(1-96) in different lipid environments (continued).

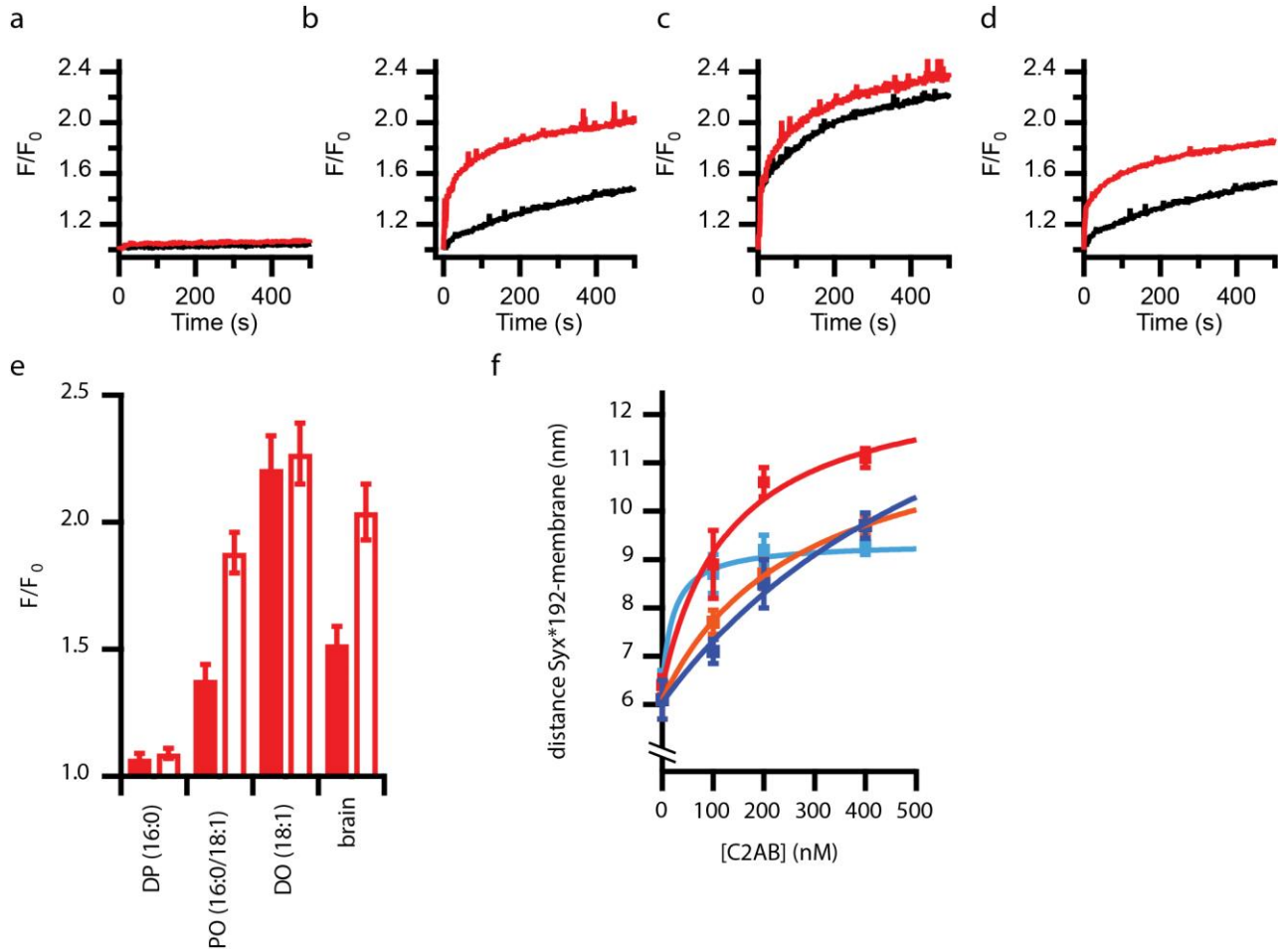
a–**g**, Example sdFLIC images with a yellow grid of 12 × 12 oxides that were evaluated, extracted intensity data from one set of 16 oxides and FLIC fit curve, and example histogram of fit results obtained from one sample under one condition for Syx*192/SNAP-25/Syb(1-96). Images were taken in different lipid environments, as described in Fig. 2a (**a**–**d**) and Fig. 2c (**e**–**g**), and as indicated in each panel. Images were rotated and scaled for printing and show an area of 201 × 201 μm^2.


a
b
c
d
Syx* - membrane distance (nm)
Syx*192/SNAP25/Syb1-96
Syx*249/SNAP25/Syb1-96
Syb(1-96)*28-membrane distance (nm)
bPC/bPE
bPC/bPE/bPIP2
bPM
1 mol% PIP2
+5%bPS
+10%bPS
+15%bPS
membrane surface (nm)
EDTA
Ca2+/C2AB
e
f
g
Syx*192-membrane distance (nm)
EDTA
EDTA+C2AB
CA+C2AB
EDTA
Syx/SNAP25/Syb1-96
+ Cpx1
Syx/SNAP25/Munc18/Syb1-96
Syx/SNAP25/Munc18/Cpx1/Syb1-96
DCV
Syt
Fusion Probability (%)
Syx/SNAP25
+ Cpx1
+Munc18
+Munc18/Cpx1

Supplementary Figure 4

SdFLIC and DCV fusion results obtained with different SNARE complex constructs and supported membrane control.

a, sdFLIC results from Syx*/SNAP-25/Syb(1-96) in bPC/bPE/bPS/bPIP2/cholesterol, labeled at residues 192 and 249 for comparison in EDTA (solid bars) and after addition of Ca^{2+}/C2AB (open bars). **b**, sdFLIC results from Syb*28(1-96) after it was added to reconstituted Syx1a/SNAP-25 acceptor complex[30] in different lipid environments as indicated in EDTA (solid bars) and after addition of Ca^{2+}/C2AB (open bars). **c**, sdFLIC results from Syb*28(1-96) after binding to reconstituted Syx1a/SNAP-25 acceptor complex at different bPS concentrations with and without 1 mol% PIP_2 as indicated. **d**, Distance of the lipid-bilayer surface from substrate as determined by FLIC in EDTA and after addition of Ca^{2+}/C2AB. **e**, sdFLIC results from Syx*192/SNAP-25/Syb(1-96) in bPC/bPE/bPS/bPIP2/cholesterol in EDTA (solid bar) and after addition of C2AB/EDTA, Ca^{2+} and EDTA in successive order. **f**, sdFLIC and **g**, fusion probabilities when complexin, Munc18, or both have been added to form the 'trigger-ready' primed prefusion state[19]. When complexin is added to Syx*/SNAP-25/Syb(1-96), the complex moves slightly further away from the membrane. Adding Ca^{2+}/C2AB straightens the complex in the same way as without complexin. When complexin is added to acceptor complex in the supported membrane, it inhibits Syb binding and DCV docking in the absence of Ca^{2+} (ref. [22]). In the presence of Ca^{2+}/C2AB, Syt-deficient DCVs dock and proceed to fusion with the supported membrane, similar to the WT DCV fusion in the presence of Ca^{2+} (ref. [19]). When Munc18 and soluble Syb2 (Syb1-96) is added to reconstituted Syx*192/SNAP-25 (Fig. 1c), the measured distance of Syx*192 within this complex increases by more than 6 nm. When Ca^{2+}/C2AB is added to this in the membrane assembled complex, its structure becomes more upright although the distance does not increase to the same height as in the isolated preassembled SNARE complex. Fusion of SytKD-DCVs in the absence of Ca^{2+} is increased by Munc18 and is further stimulated by Ca^{2+}/C2AB. The height of Syx*192 within a complex that has been assembled by adding Munc18, complexin, and Syb1-96 to Syx*192/SNAP-25, is raised to 9.5 nm. When Ca^{2+}/C2AB is added, the distance of Syx*192 from the membrane increases further to 11.6 nm, about the same as that of the cis-SNARE complex. Adding Munc18 and complexin at the same time to Syx1a/SNAP-25 in the supported membrane results in a 'trigger competent' prefusion state that allows DCV docking in the absence of Ca^{2+} without significant fusion[19]. Adding Ca^{2+}/C2AB to the docked DCVs after a 15-min incubation time increases the fusion probability of the SytKD-DCVs from almost zero to 58%. Note that when multiprotein complexes are assembled on the membrane, the measured distance of Syx*192 most likely represents an average originating from different multiprotein assemblies. All data represent means from at least five independent experiments. Error bars represent s.e.m.



Supplementary Figure 5

SNARE and Ca^{2+}/C2AB-dependent fusion in proteo-liposome/DCV fusion assay and Syx*192-membrane distance changes induced by C2AB-WT or the C2AB-KAKA mutant with and without PIP_2.

a–d, Example (NBD) fluorescence dequenching curves after WT-DCVs have been added to Syx/SNAP-25-containing proteoliposomes in EDTA (black curves) or in the presence of Ca^{2+}/C2AB. Syx/SNAP-25 is reconstituted into DP (**a**), PO (**b**), DO (**c**), or brain lipids (**d**). **e**, Average fluorescence increase due to NBD dequenching after proteoliposome/DCV fusion in EDTA (solid bars) and in the presence of Ca^{2+}/C2AB. The lipid headgroup composition is PC/PE/PS/PIP2/cholesterol (34/30/15/1/20) in all cases, and the acyl chain composition is as indicated. Averages represent means from three repeats, and error bars represent s.d. **f**, Syx*192-membrane distance changes in response to C2AB-WT with (red) and without (orange) 1 mol% PIP_2 and C2AB-KAKA with (light blue) and without (dark blue) 1 mol% PIP_2. Data points represent means from at least five independent experiments. Error bars represent s.e.m.