Structure, Volume *32*

Supplemental Information

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Supplementary Information

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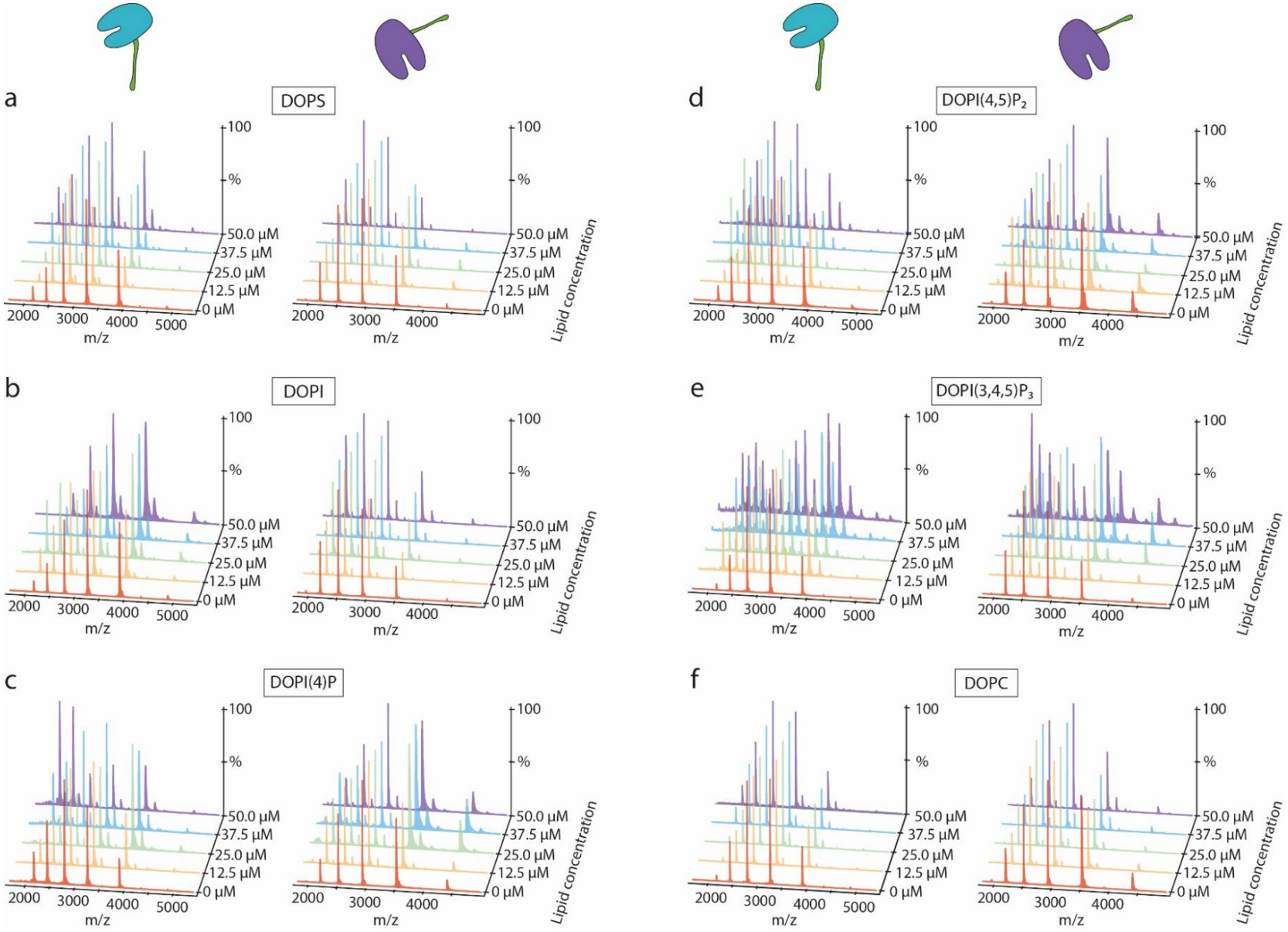
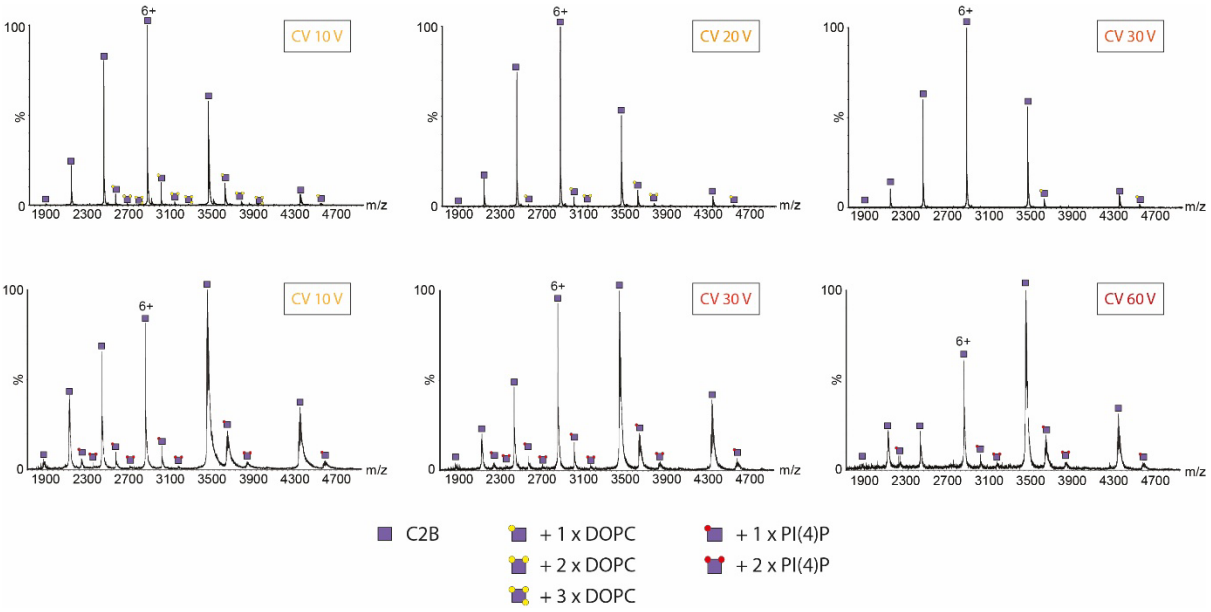
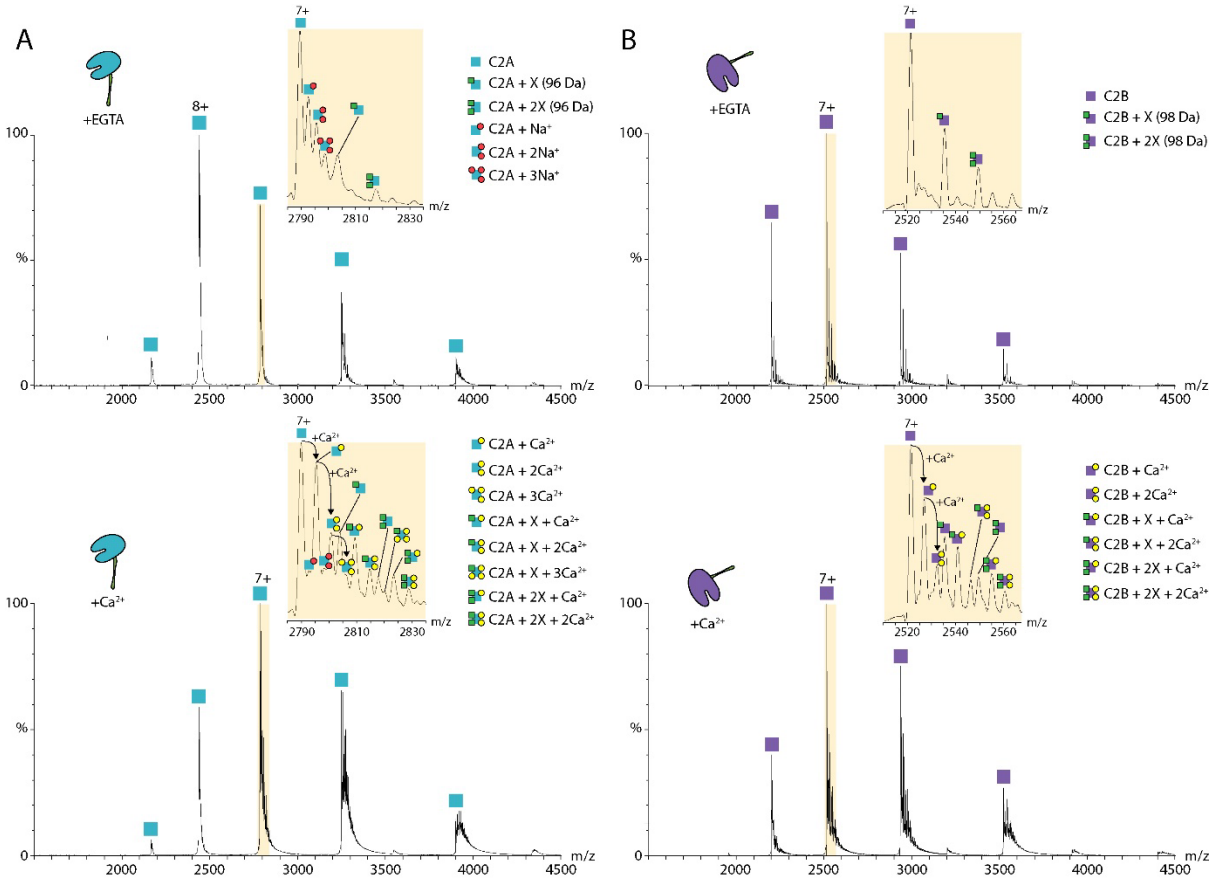
Supplementary Figures



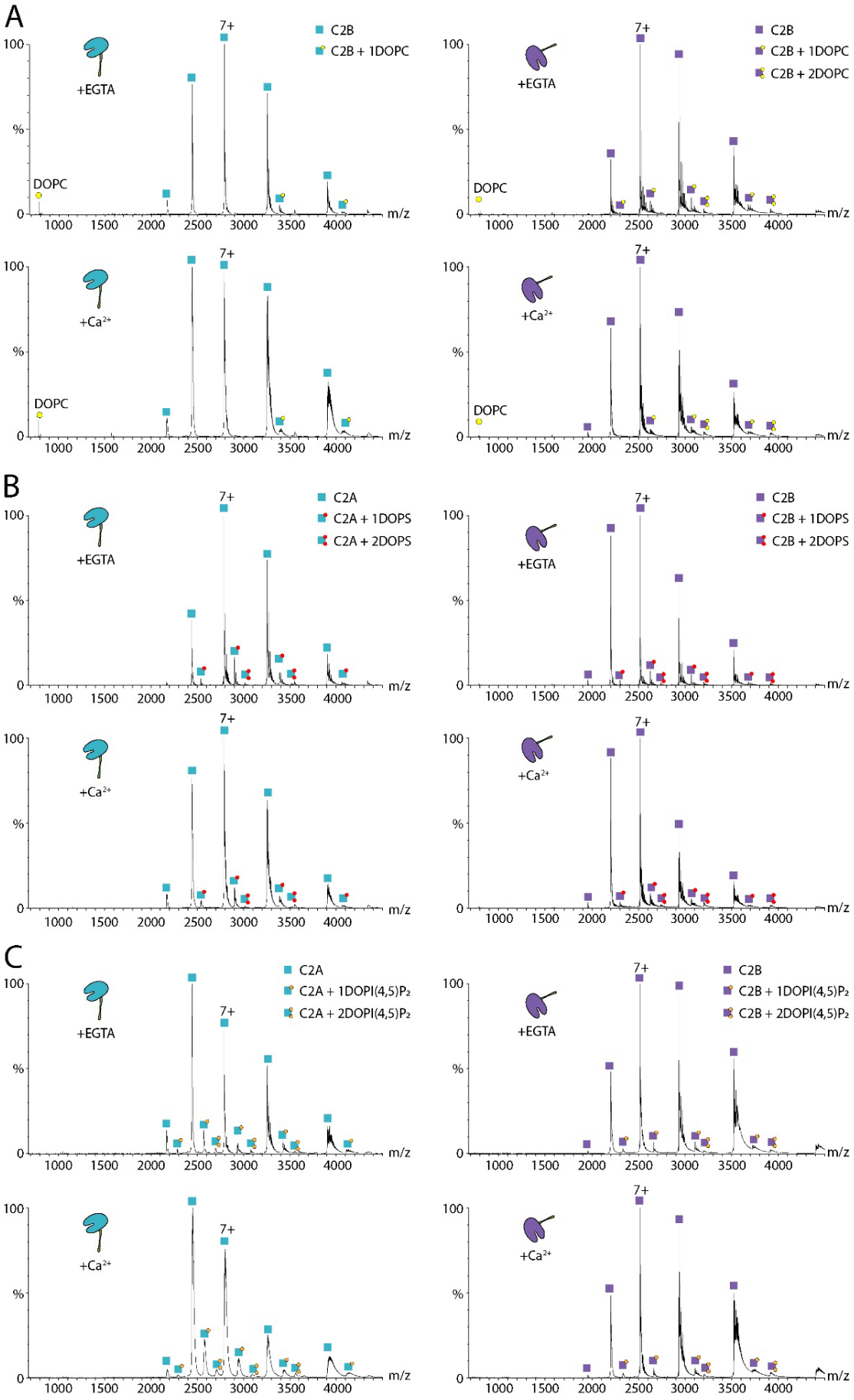
Supplementary Figure 1. Lipid binding of C2A (cyan) and C2B (purple) explored by native mass spectrometry. Related to Figure 2. Mixed detergent-lipid micelles containing increasing lipid concentrations were prepared and mixed with the two C2 domains. Lipid binding was then analysed by native mass spectrometry. **(a)** DOPS, **(b)** DOPI, **(c)** DOPI(4)P, **(d)** DOPI(4,5)P_2, **(e)** DOPI(3,4,5)$_3$, **(f)** DOPC.



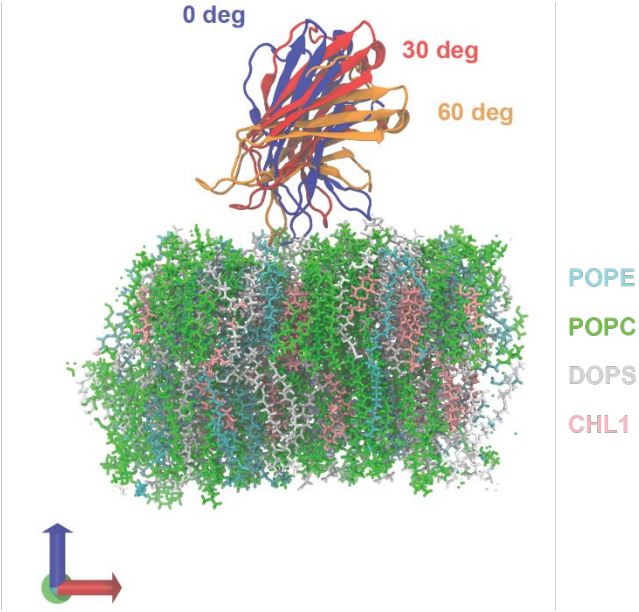
Supplementary Figure 2. Dissociation of C2B-lipid complexes. Related to Figure 2. Increasing the collisional voltage from 10 to 30 V caused loss of all associated DOPC molecules (upper row), while PI(4)P binding to C2B was still observed at 60 V (lower row).



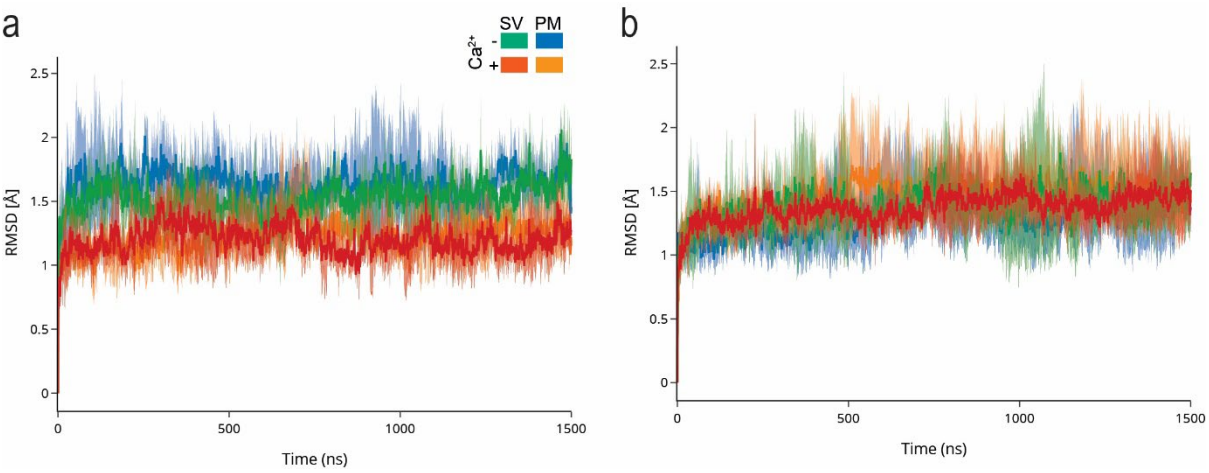
Supplementary Figure 3. Ca^{2+} binding of C2A and C2B domains. Related to Figure 2. C2A and C2B were incubated with EGTA (upper panels) or $CaCl_2$ (lower panels) and analysed by native mass spectrometry. **(A)** In the presence of EGTA, binding of sodium ions (red circles) to C2A (cyan) was observed (upper panel). Upon incubation with Ca^{2+}, binding of three Ca^{2+} ions (yellow circles) to C2A was observed (lower panel). Note that binding of additional adducts (+96 Da each, green squares) was observed in the absence (+EGTA, upper panel) and in the presence of Ca^{2+} (lower panel). This additional adduct likely corresponds to phosphate. Binding of Ca^{2+} ions was also observed to the phosphate-bound states. **(B)** Binding of two Ca^{2+} ions (yellow circles) to C2B was observed. Again, additional adducts (+98 Da each, green squares) were observed in the absence (+EGTA, upper panel) and in the presence of Ca^{2+} (lower panel). Binding of Ca^{2+} ions was also observed to the phosphate-bound states.


A
B
C
+EGTA
+Ca2+
7+
DOPC
C2B
C2B + 1DOPC
C2B + 2DOPC
C2A
C2A + 1DOPS
C2A + 2DOPS
C2B + 1DOPS
C2B + 2DOPS
C2A + 1DOPI(4,5)P2
C2A + 2DOPI(4,5)P2
C2B + 1DOPI(4,5)P2
C2B + 2DOPI(4,5)P2
100
%
0
1000 1500 2000 2500 3000 3500 4000
m/z

Supplementary Figure 4. Phospholipid binding of C2A and C2B domains in the presence of EGTA or Ca^{2+}. Related to Figure 2. C2A (cyan) and C2B (purple) were pre-incubated with EGTA or Ca^{2+} and binding of **(A)** DOPC (yellow), **(B)** DOPS (red) and **(C)** DOPI(4,5)P_2 (orange) was subsequently analysed by native mass spectrometry.



Supplementary Figure 5. Starting orientation of the three starting simulations. Related to Figures 4, 5, 6 and 7. The C2A domain of Synaptotagmin-1 is placed on the synaptic vesicle membrane in the absence of calcium. Structures corresponding to the different starting orientations (0°, blue; 30°, red; 60°, orange) and membrane lipids (POPE, cyan; POPC, green; DOPS, silver; cholesterol (CHL1), pink) are colour coded.



Supplementary Figure 6. Cα Root-mean-square deviations (RMSDs) of the C2 domains with respect to the first frame of the simulation. Related to Figures 4, 5, 6 and 7. RMSDs are shown for each C2 domain interacting with the SV membrane in the presence (red) and in the absence (green) of Ca^{2+} ions as well as the plasma membrane in the presence (orange) and in the absence (blue) of Ca^{2+}. The mean RMSD (solid line) and the range of RMSDs per time step of the three simulations with different starting angles (transparent area) are shown. **(a)** C2A (residues 141-263) **(b)** C2B (residues 272-418).