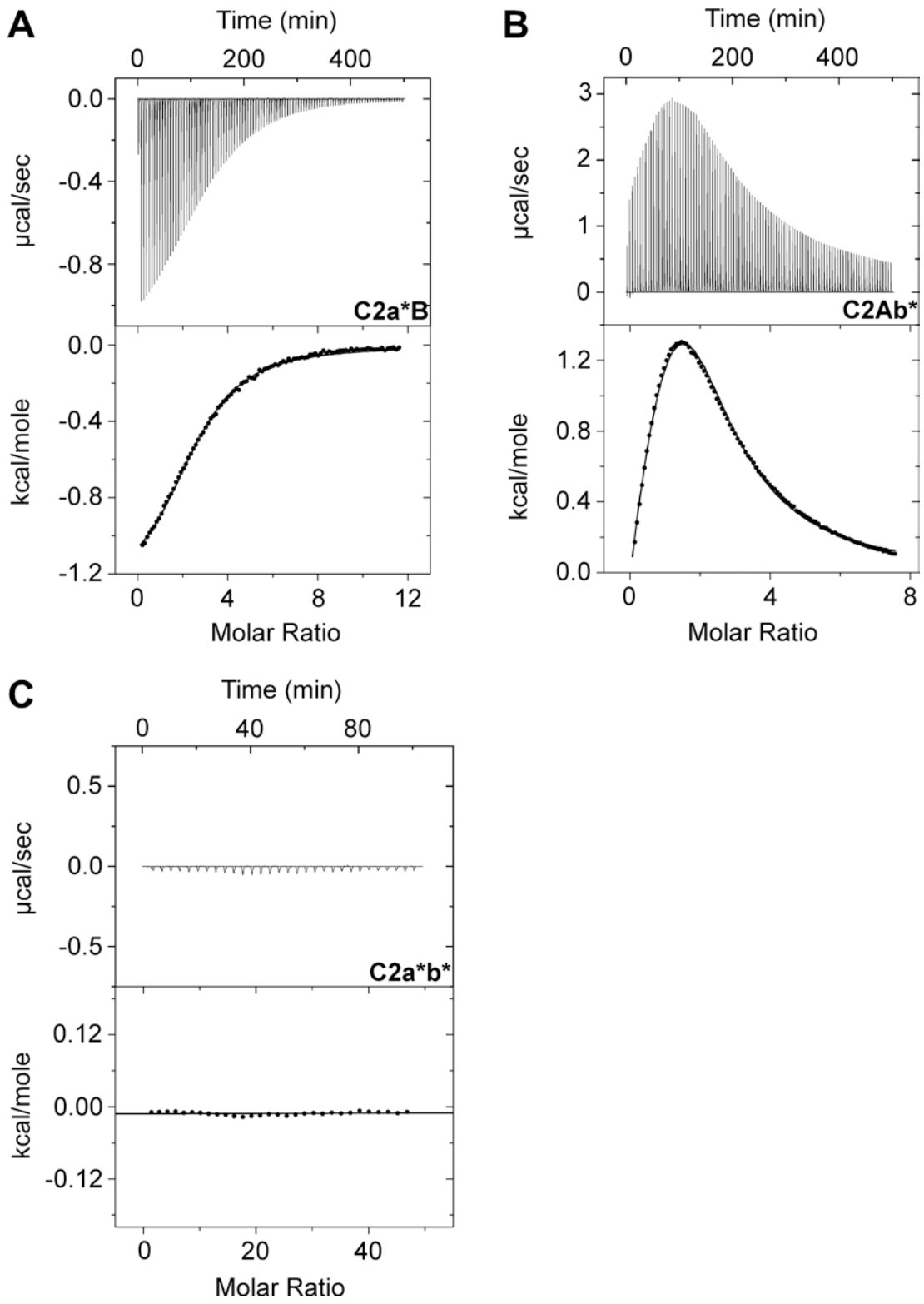
Supplementary Material

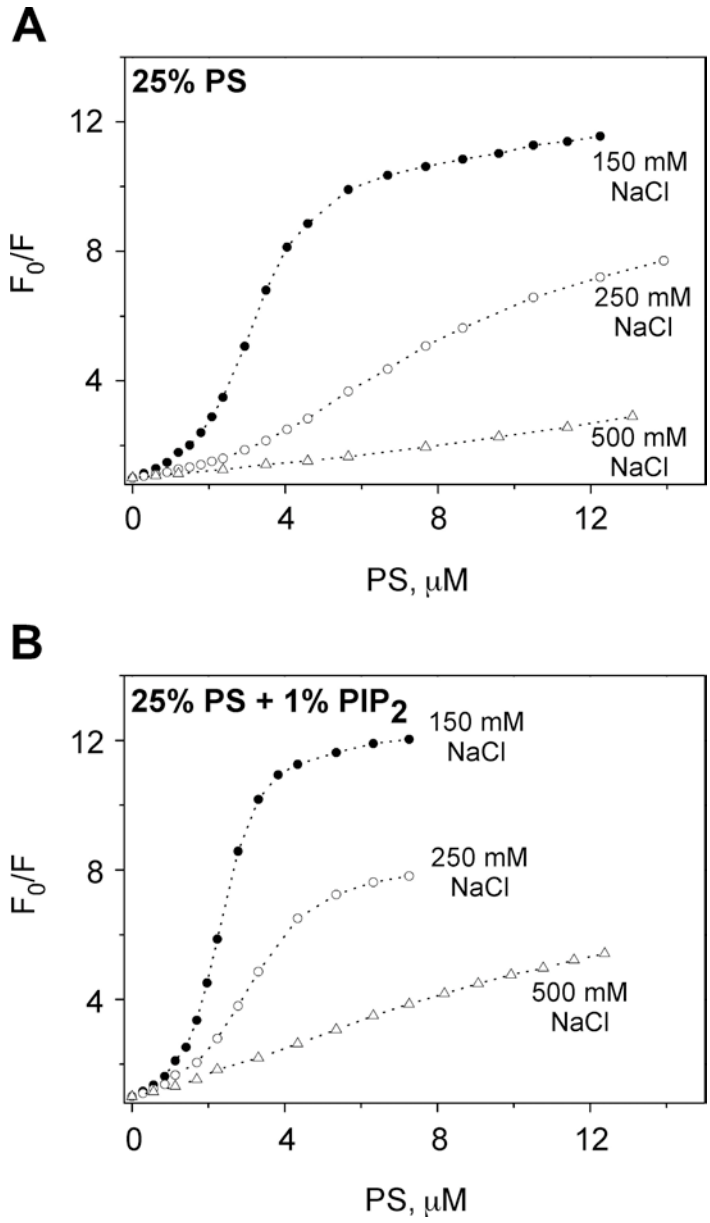
THE CA^{2+} AFFINITY OF SYNAPTOTAGMIN 1 IS MARKEDLY INCREASED BY A SPECIFIC INTERACTION OF ITS C2B DOMAIN WITH $PI(4,5)P_2$

Anand Radhakrishnan, Alexander Stein, Reinhard Jahn, and Dirk Fasshauer



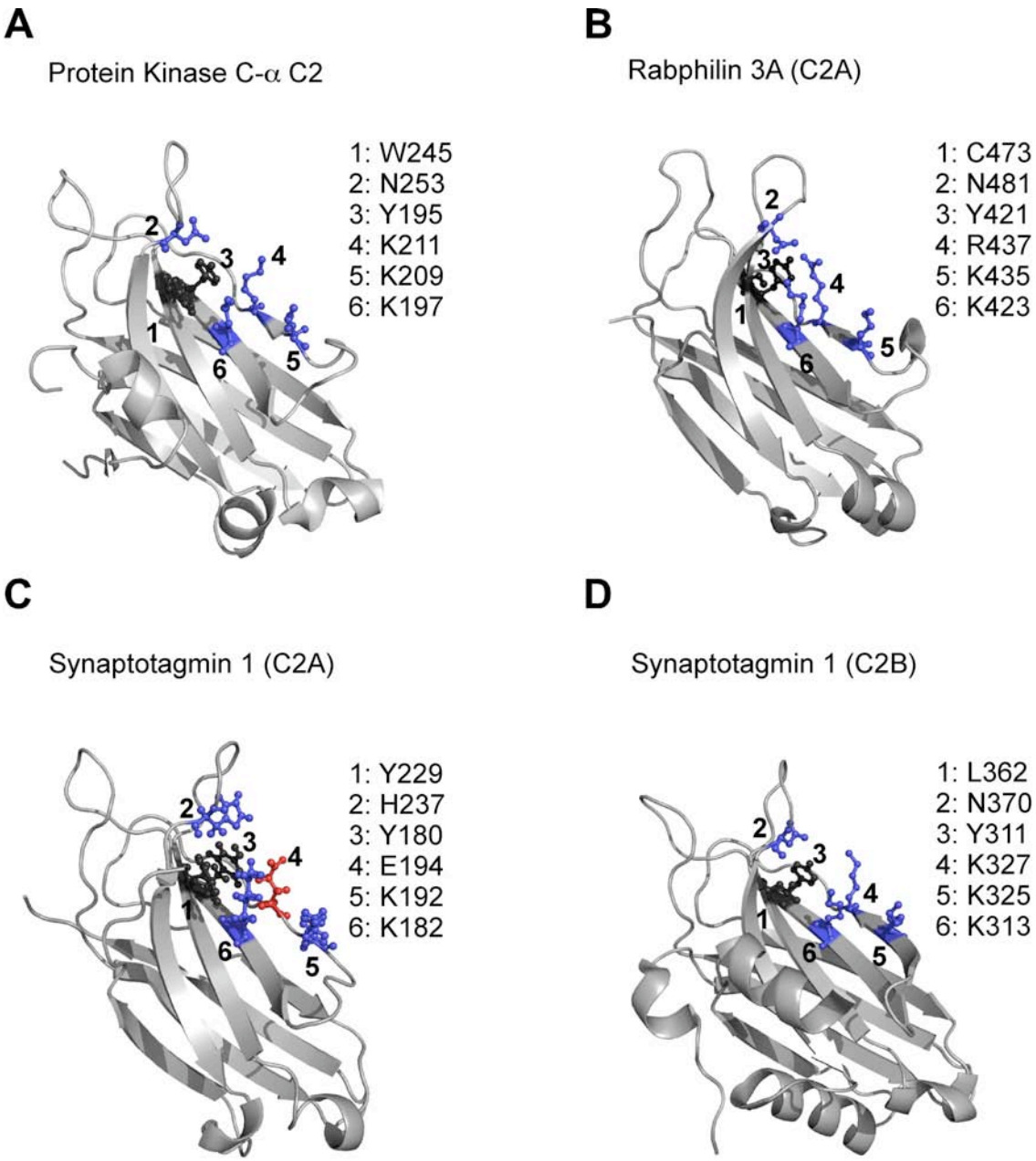
Suppl Fig. 1: Intrinsic calcium binding properties of the calcium mutants.

The cooperativity of the two C2 domains was investigated using ITC at 25°C. Calcium chloride solution at concentrations of 10 mM, 20 mM and 10 mM were titrated to 202 µM C2a*B (**A**), 619 µM C2Ab* (**B**) and 50 µM C2a*b* (**C**) protein solutions respectively. The double mutant, C2a*b* exhibited no detectable calcium binding. The single domain mutations, C2a*B and C2Ab* bound calcium with a similar profile and affinites to the isolated C2B or C2A domain respectively confirming the independence of the two domains in calcium binding. The thermodynamic parameters are summarised in Table 1.



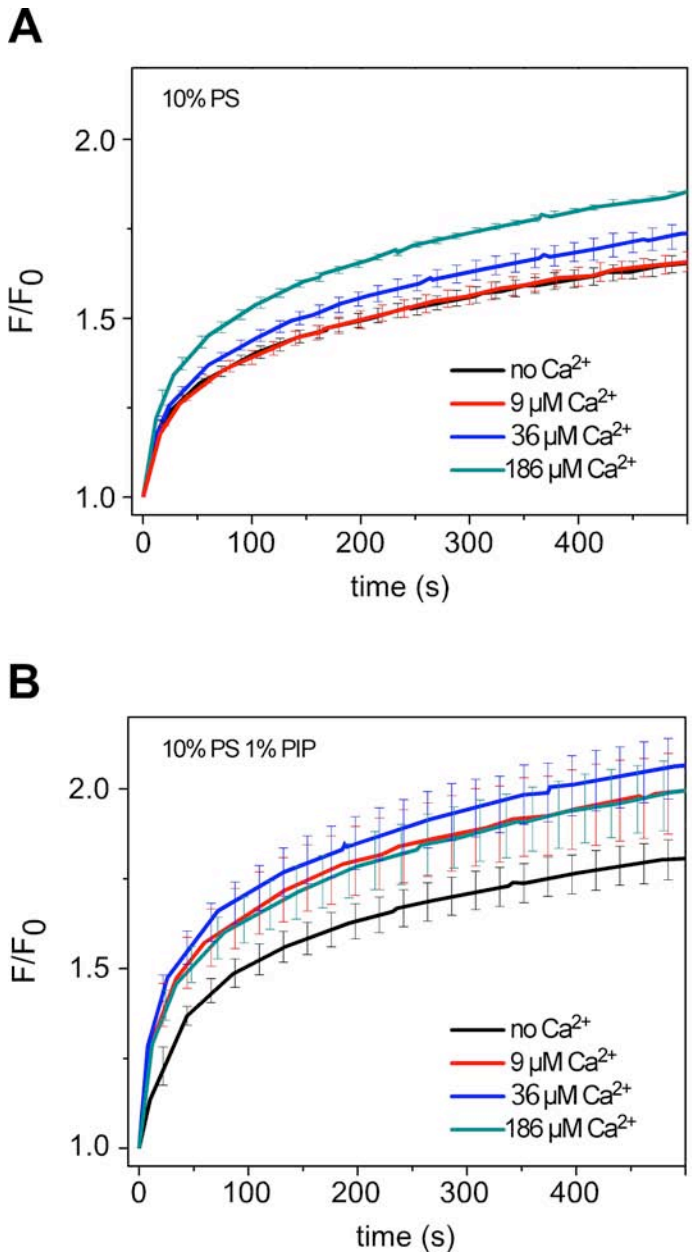
Suppl. Fig. 2: Increasing ionic strength reduces the binding strength of synaptotagmin binding to membranes.

Liposomes labelled with Texas Red PE, with (**B**) or without (**A**) 1% $PI(4,5)P_2$, were titrated to wild-type synaptotagmin C2AB labelled with Alexa 488 in either 150 mM (solid circles, ●), 250 mM (open circles, ○) or 500 mM NaCl (open triangles, Δ). When the salt concentration was increased, the binding affinity of te liposomes to synaptotagmin seem to reduce in its affinity indicating that the measured binding in the FRET assay is due to an electrostatic association between these molecules.



Suppl. Fig. 3: Structural comparison of the Lys-rich patches of different C2 domains.

The residues of the C2 domain PKC-α (Guerrero-Valero et al. 2009, PNAS 106: 6603-7) involved in binding to $PI(4,5)P_2$ are shown in sticks (**A**), the homologus residues were mapped on the C2A domain of rabphilin (Montaville et al. 2008, Protein Sci 17: 1025-34 & Coudevyille et al. 2008, JBC 283: 35918-28) (**B**) as well as on the C2A (**C**) and on the C2B (**D**) domains of synaptotagmin. With the exception of C2A domain of synaptotagmin, which contains an acidic amino acid (E194) in this region, the $PI(4,5)P_2$-binding site is well conserved. In the KAKA mutant only one of the residues involved in $PI(4,5)P_2$-binding was mutated (K327A), possibly explaining why the KAKA mutation did not exhibit a strong phenotype. Additionally, it seems that one of the residues (N253 on PKC-α, N481 on Rabphilin C2A domain and N370 on the C2B domain of synaptotagmin) that is involved in $PI(4,5)P_2$ binding is directly on the calcium binding loop, possibly influencing the loop geometry and the calcium binding affinities.



Suppl. Fig. 4: Faster liposome fusion rates in the presence of PI(4,5)P_2.

In the presence of PI(4,5)P_2 membrane-bound synaptotagmin increases the efficiency of SNARE mediated liposome fusion at lower calcium concentrations. The Q-SNARE liposome membrane contained 10 % PS in the absence (**A**) or presence (**B**) of 1% PI(4,5)P_2, whereas the liposomes containing synaptobrevin did not contain PS nor PI(4,5)P_2. Fusion between syntaxin 1a:SNAP-25-containing liposomes and synaptobrevin-containing liposomes was measured by a standard lipid dequenching assay. Fluorescence values were normalized to the initial fluorescence measured (denoted as F/F_0). Individual fusion reactions were carried out at different calcium concentrations and repeated three times, each time using freshly prepared liposomes. Selected kinetic traces are shown.