Synaptotagmin 1 and Ca^{2+} drive trans SNARE zippering

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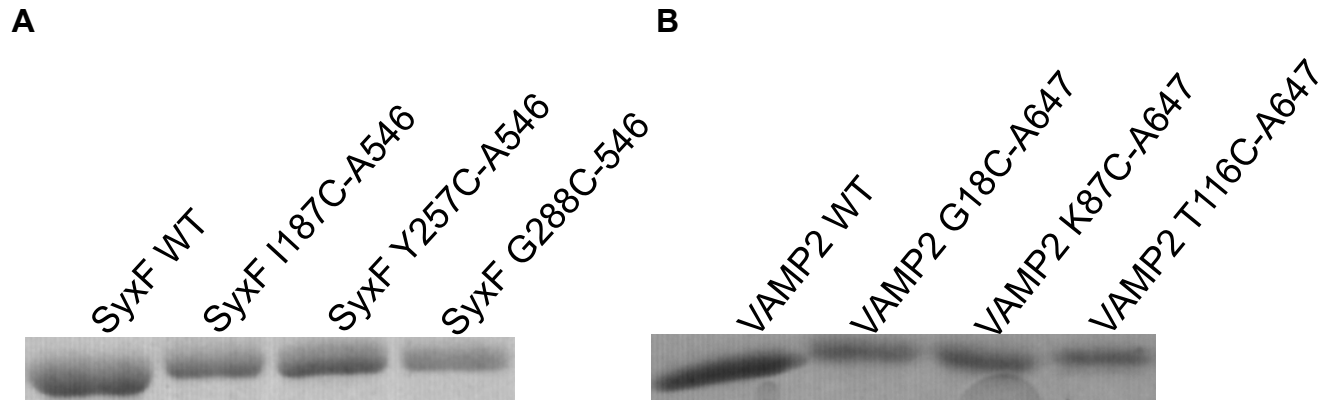
Running title: Role of Synaptotagmin 1 in SNARE sequential zippering

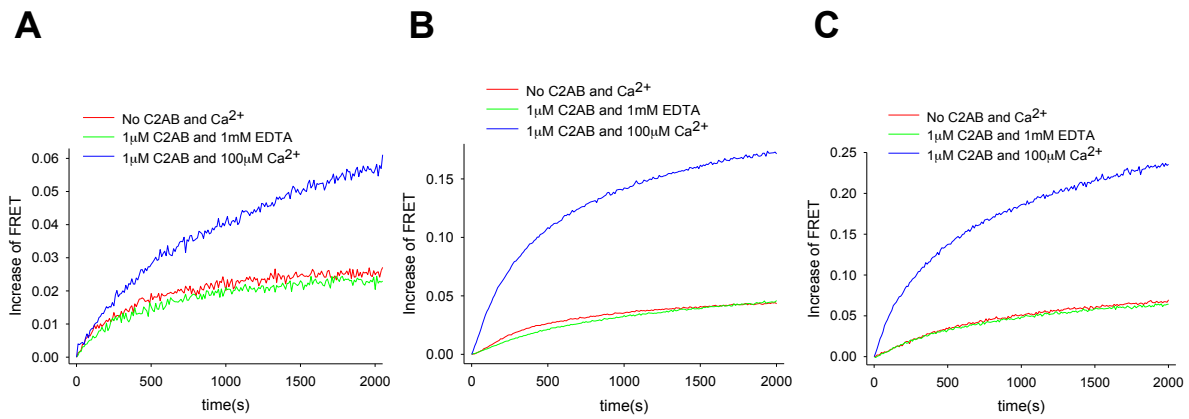
Supporting information

Supplementary Figure S1



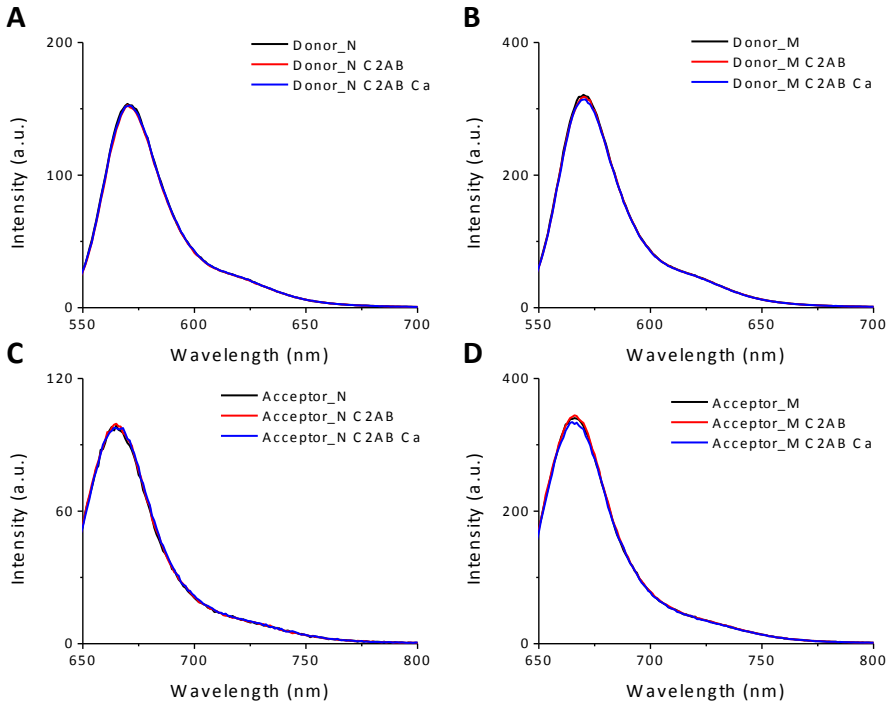
Supplementary Figure S1 Purified labeled SNARE proteins analyzed by the coomassie blue stained SDS-polyacryamide gel. Labeled proteins run a little slower than the wild-type (WT) proteins. (**A**) SyxF WT (cysteine free version) and mutants labeled with A546. (**B**) VAMP2 WT (cysteine-free version) and mutants labeled by A647.

Supplementary Figure S2



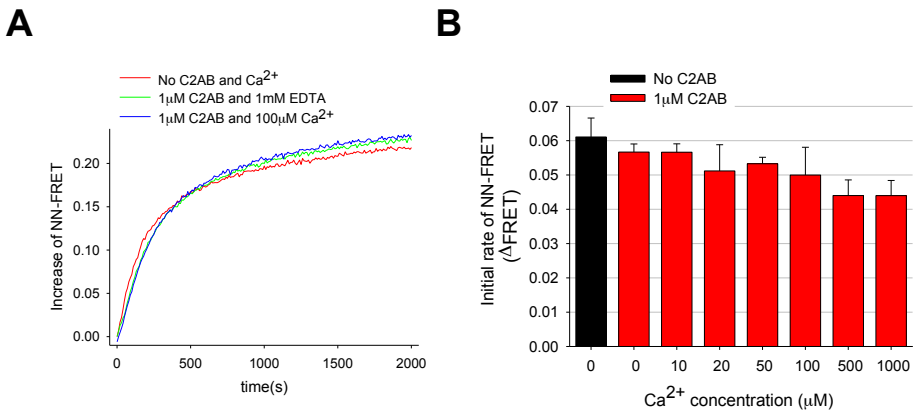
Supplementary FigureS2 Lipid mixing and stimulation of lipid mixing by C2AB-Ca^{2+} for fluorescently labeled SNAREs. The FRET signals according to the time were recorded. The green line represents the lipid mixing with 1 μM C2AB and 1 mM EDTA, while the blue line represents the lipid mixing with 1 μM C2AB and 100 μM Ca^{2+}. The red line is the control with SNAREs only. (**A**) NN-FRET pairs (SyxF I187C-A546 and SNAP-25 in t-vesicles and VAMP2 G18C-A647 in v-vesicles). (**B**) MM-FRET pairs (SyxF Y257C-A546and SNAP-25 as t-SNARE and VAMP2 K87C-A647 as v-SNARE). (**C**) CC-FRET pairs (SyxF G288C-A546 and SNAP-25 as t-SNARE and VAMP2 T116C-A647 as v-SNARE). A protein-to-lipid molar ratio of 1:200 was used in all experiments.

Supplementary Figure S3



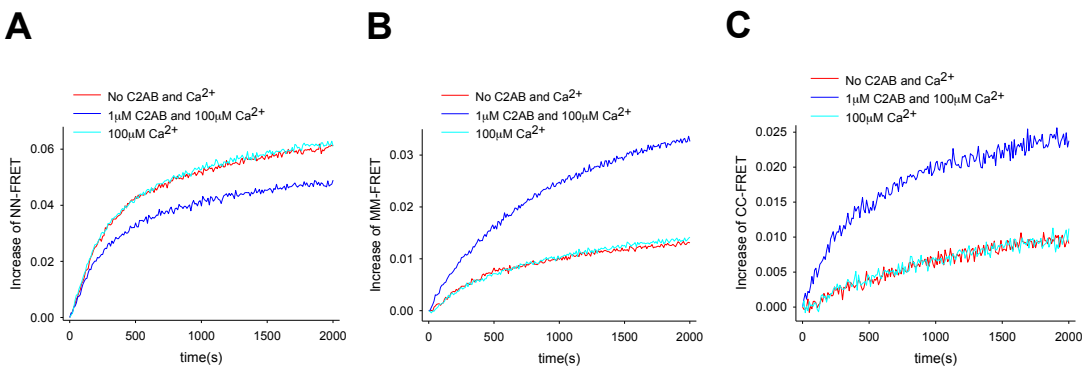
Supplementary Figure S3 C2AB or C2AB/Ca^{2+} alone do not affect the individual spectrum of t-vesicle reconstituted with donor N: SyxF I187C-A546/SNP (**A**), t-vesicle reconstituted with donor M: SyxF Y257C-A546/SNP (**B**), v-vesicle reconstituted with acceptor N: VAMP2 G18C-A647 (**C**), and v-vesicle reconstituted with acceptor M: VAMP2 K87C-A647. The scanning spectrum were monitored with the excitation wavelength of 530 nm and 630 nm for A546 and A647, respectively. The black lines show the emission spectra of vesicles reconstituted with labeled Syx1A or VAMP2, the red lines show the emission spectra of reconstituted vesicles with C2AB, and the blue lines show the emission spectra of reconstituted vesicles with C2AB and Ca^{2+}.

Supplementary Figure S4



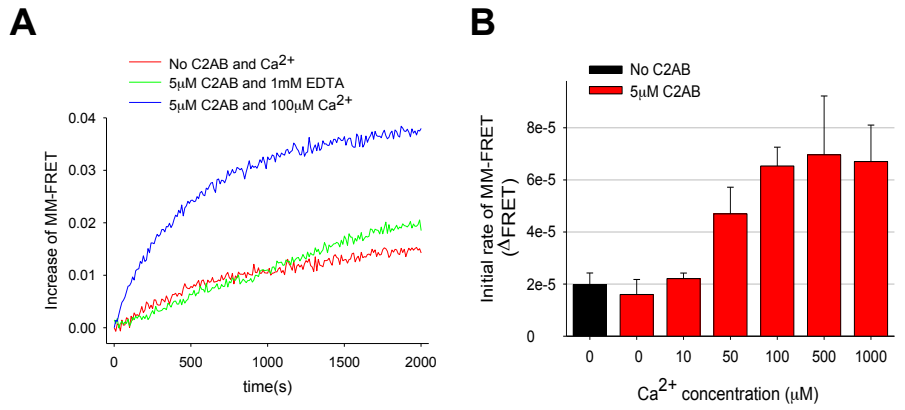
Supplementary Figure S4 NN-FRET assay with C2AB with AmershamCy dyes. (**A**) Changes of the FRET signals in time after mixing t- and v-vesicles are shown. The red line is the control with t-vesicle reconstituted with SyxF I187C-Cy3 and SNAP-25 and v-vesicle reconstituted with VAMP2 G18C-Cy5. The green line represents the change of the fluorescence intensity with 1 μM C2AB and 1 mM EDTA, while the blue line represents the NN-FRET with 1 μM C2AB and 100 μM Ca^{2+}. (**B**) The initial rates of NN-FRET at different Ca^{2+} concentrations. The error bars, which represent standard deviation, were obtained from 3 independent measurements from 3 different preparations.

Supplementary Figure S5



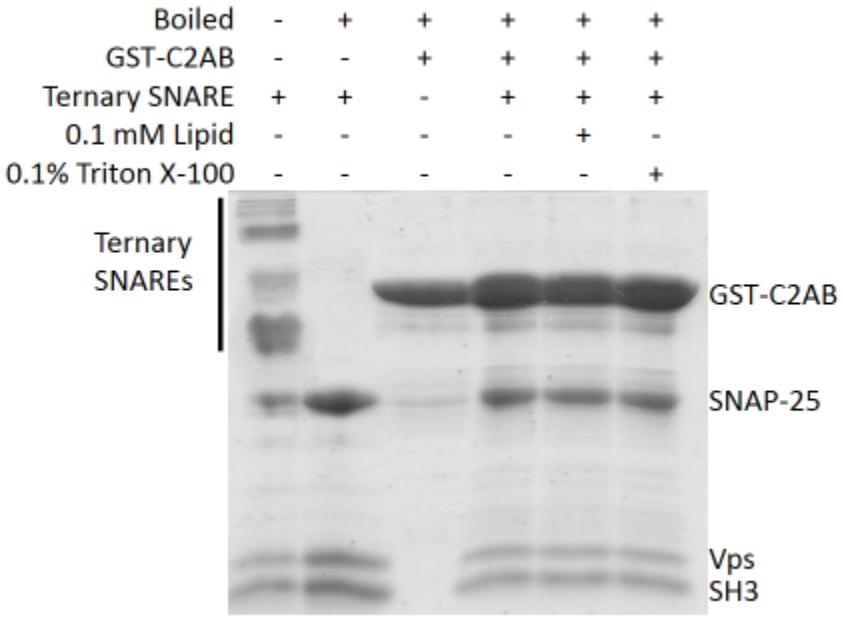
Supplementary Figure S5 Ca^{2+} alone in the absence of C2AB does not affect FRET assay. Changes of the FRET signals in time after mixing t- and v-vesicles are shown. The cyan lines represent the control group with only 100 μM Ca^{2+}, while the blue lines represent the control group with 1 μM C2AB and 100 μM Ca^{2+}. The red lines are NN-FRET (**A**), MM-FRET (**B**), and CC-FRET (**C**).

Supplementary Figure S6



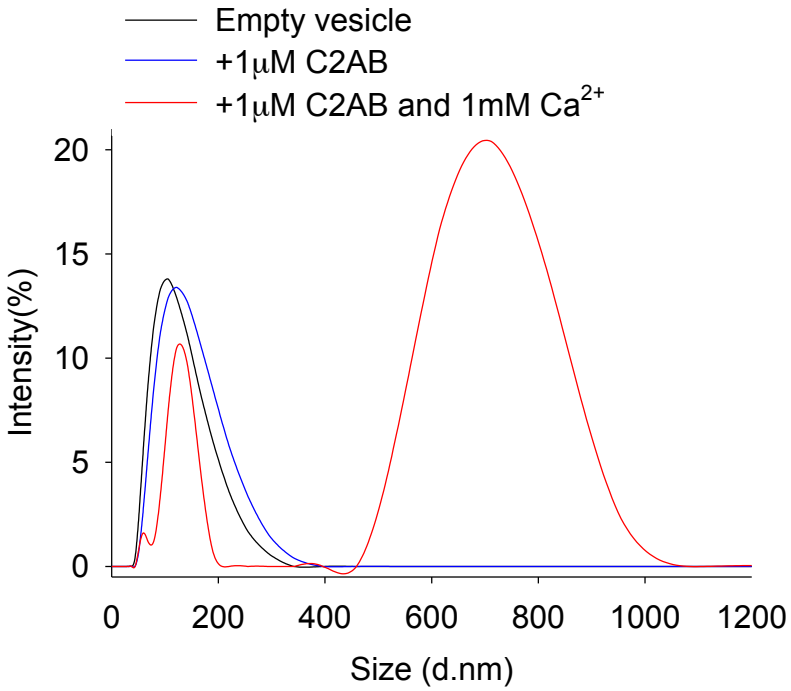
Supplementary Figure S6 Ca^{2+}-dependent stimulation of SNARE complex zippering with 5 μM C2AB. (**A**) The experimental design is the same as that in **Figure 2**. The red line is MM-FRET with SNAREs only. The green line represents the MM-FRET with 5 μM C2AB and 1 mM EDTA, while the blue line represents the MM-FRET with 5 μM C2AB and 100 μM Ca^{2+}. (**B**) The initial rate of MM-FRET at different Ca^{2+} concentrations. The error bars, which represent standard deviation, were obtained from 3 independent measurements from 3 different preparations.

Supplemental Figure S7



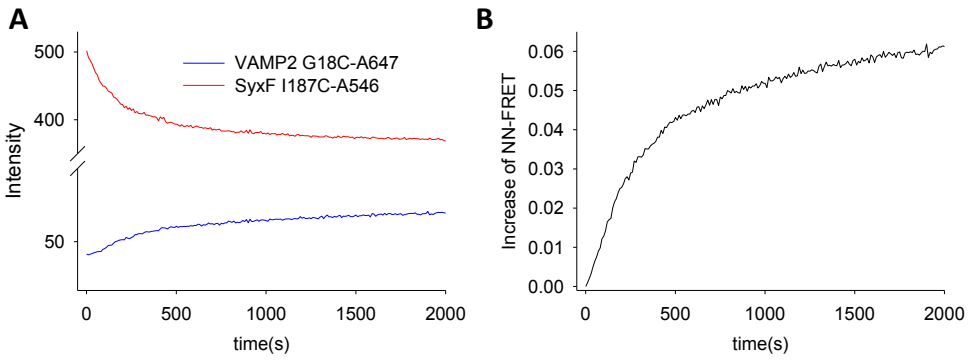
Supplemental Figure S7 Lipid or detergent does not affect C2AB-SNARE interaction. The GST Pull-down assay followed by 15% SDS-PAGE with coomassie blue staining shows that lipid or detergent does not change the SNAREs binding ability of GST-C2AB. To assemble ternary complex, soluble VAMP2 (Vps, 1-96), soluble Syntaxin (SH3, 191-266), and His-tagged SNAP-25 (1-206) were first premixed at 1:1:1 ratio and incubated with Ni-NTA beads overnight, and then eluted by 500 mM Imidazole. SDS-resistant ternary complex was confirmed with or without boil.

Supplemental Figure S8



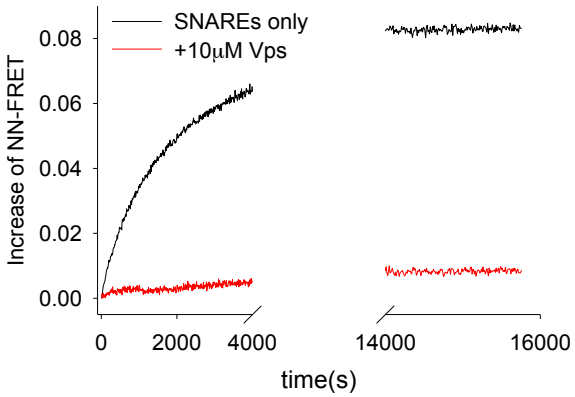
Supplemental Figure S8 C2AB/Ca^{2+} binding to synaptic vesicle. Dynamic light scattering measurement of particle sizes in samples containing phospholipid vesicles in HEPES buffer only (black line), 1μM C2AB only (blue line), and 1μM C2AB and 1mM Ca^{2+} (red line). All dispersions analyzed in a HEPES buffer background.

Supplementary Figure S9



Supplementary Figure S9 Bulk NN-FRET donor and acceptor signal. (**A**) Fluorescence intensities of donor dye A546 and acceptor dye A647 with the time course. (**B**) The increase of NN-FRET, calculated by the formula: E = Ia/(Ia+Id), where Ia is the fluorescence intensity of the acceptor A647 and Id is that of the donor A546.

Supplementary Figure S10



Supplementary Figure S10 Steady state of bulk NN-FRET assay. The negative control (the red line) was 90% inhibited by 10μM Vps and can not achieved to the same steady state level as NN-FRET induced by SNAREs (the black line).