Chapter 1

Figures And Captions

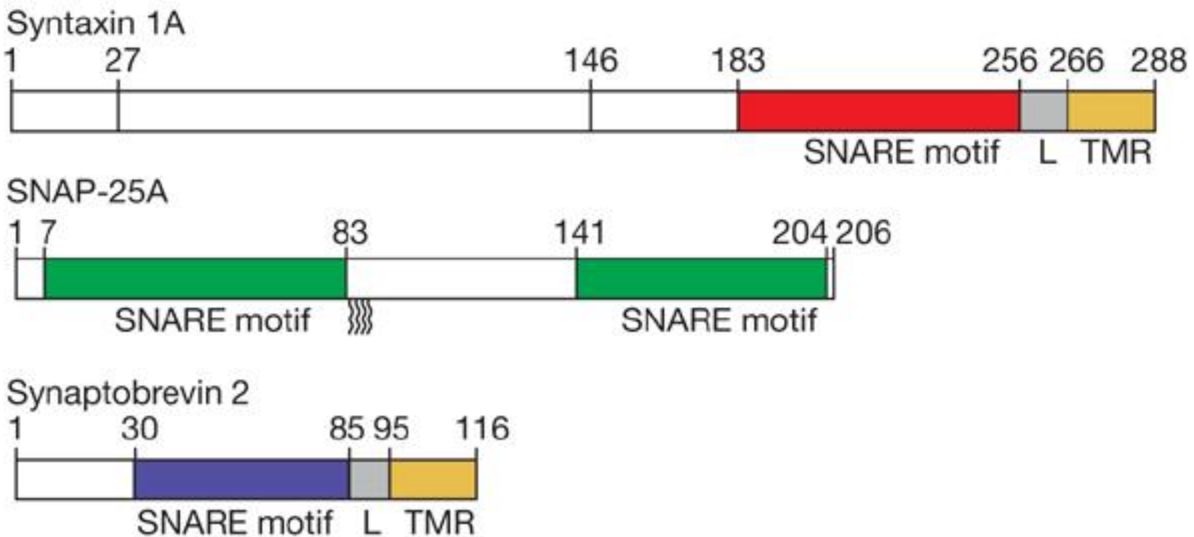

Fig 1. Primary structure diagram of neuronal SNARE proteins TMR, transmembrane region is located at the C-terminal of syntaxin 1A and VAMP2. The SNARE motifs are defined through the 16 layers as found in the crystal structure of the neuronal SNARE core complex. The N-terminal domain of syntaxin 1A is named Habc which can bind with the SNARE core domain or Munc18. SNAP-25A contributes two SNARE motifs to the core complex, which are SN1 and SN2, the four palmitoylation sites (cysteine 85, 88, 90 and 92) are indicated by lines. L, linker region is located between SNARE motif and transmembrane region.

[26] *Stein A et al*. Helical extension of the neuronal SNARE complex into the membrane. *Nature*. Jul 23; 460(7254):525-8 (*2009)*

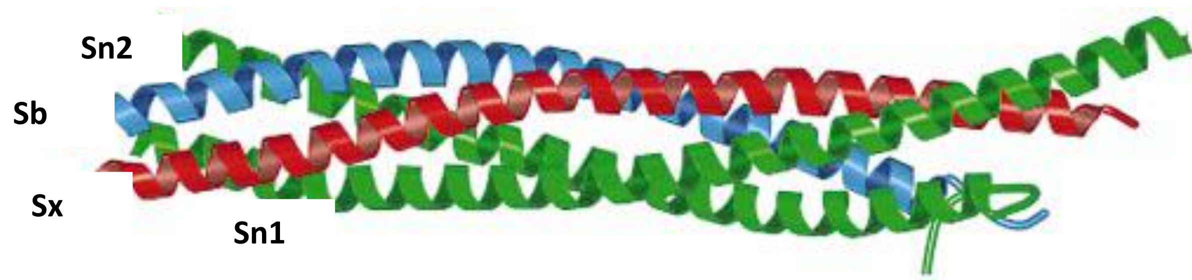

Fig 2. Crystal structure of neuronal SNARE complex. The four-helix bundle. Sn1 and Sn2 are two 'SNARE motifs' of SNAP-25. Sx is Syntaxin and Sb is Synaptobrevin.

[27] Sutton, R.B., Fasshauer, D., Jahn, R., and Brunger, A.T. (1998). Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 A resolution. Nature. 395, 347-353.

(a)

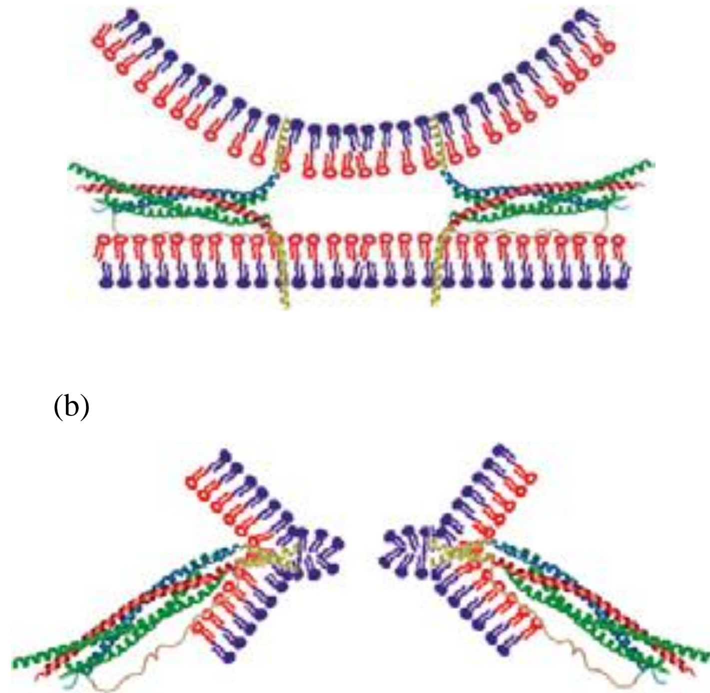

Fig 3. Trans- and Cis-SNARE complex (a) Partially assembled trans-SNARE Structure (b) Fully assembled cis-SNARE complex.

[28] Brunger, AT. (2005). Structure and function of SNARE and SNARE-interacting proteins. *Q Rev Biophys*.38, 1-47.

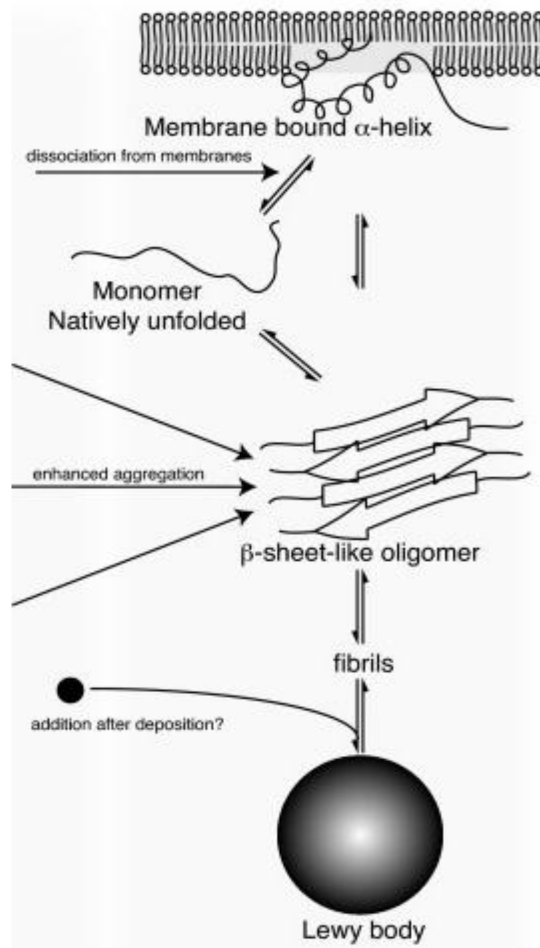

Fig 4. α-Syn aggregation pathway. Monomeric α-Syn is natively unfolded in solution. Upon binding to membranes, it adopts an α-helical structure in the N-terminal region. The unfolded monomer can also aggregate first into small oligomeric species that is stabilized by β-sheet-like interactions. Fibrils and further Lewy body can be formed after further aggregation into higher molecular weight.

[29] Cookson *Molecular Neurodegeneration* 2009 **4**:9 doi:10.1186/1750-1326-4-9

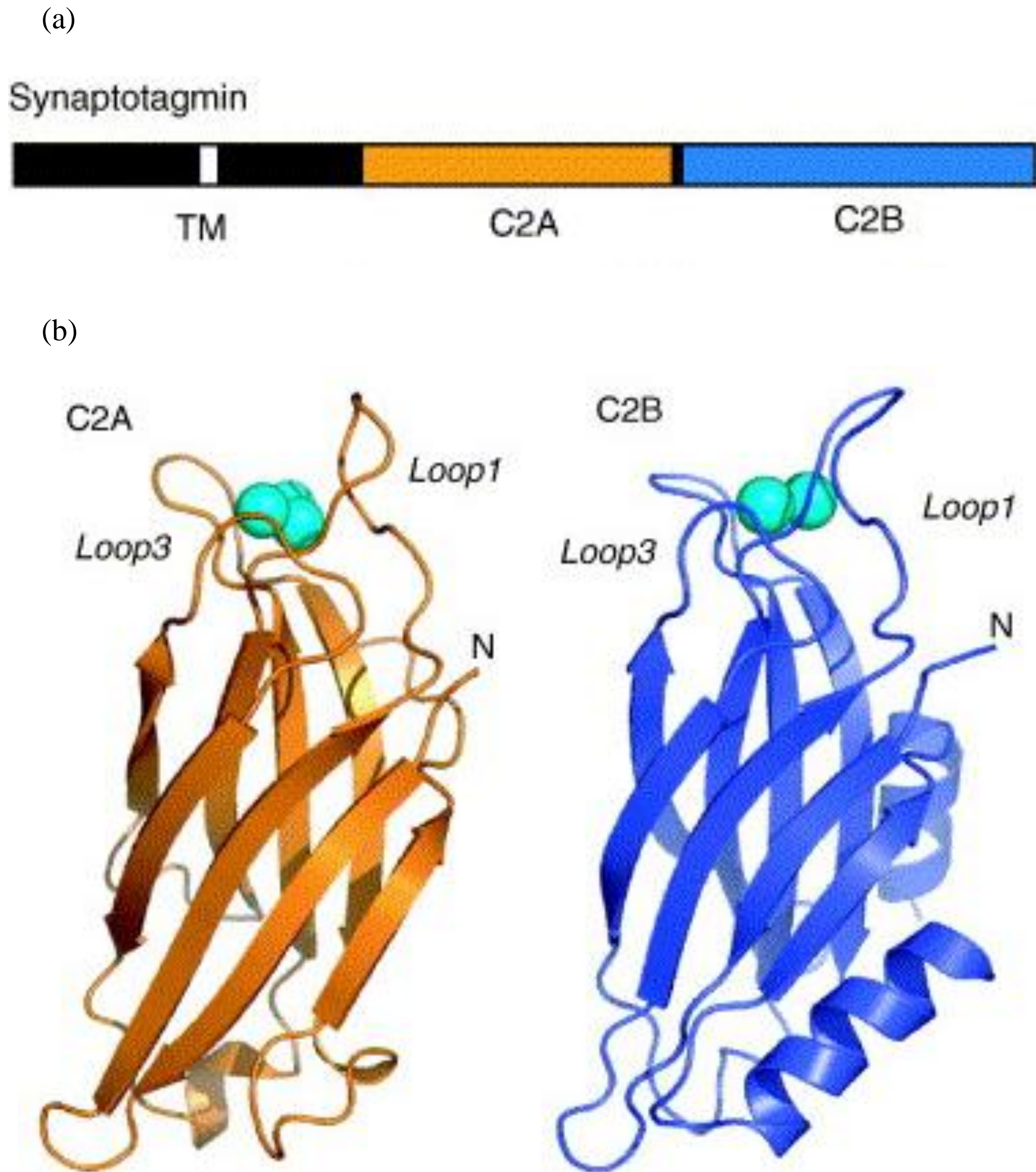

Fig 5 Structure of Syt I (a) The primary domains structure of Syt 1, of which a single transmembrane domain(TM) near its N terminus helps anchor to the vesicle membrane (b) The NMR structure of the C2 domains, C2A and C2B. Blue spheres represent multiple calcium ions bound to loops 1 and 3.

[30]Rizo J, Chen X and Arac D. Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. Trends Cell Biol. 16, 339-50 (2006)

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Figures And Captions

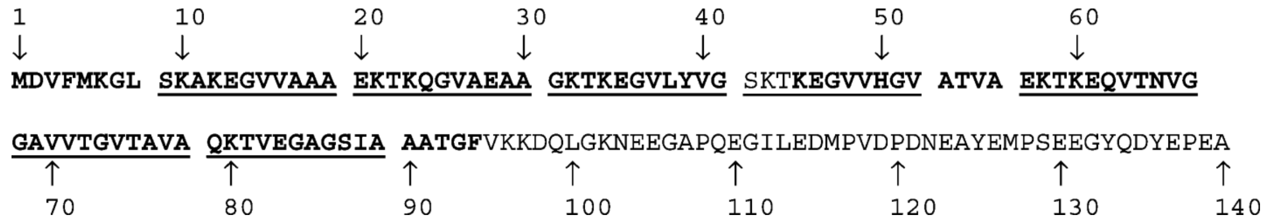


Fig 1. The sequence of human α-Syn. Upon binding to membranes, the two helical regions of α-Syn are indicated in bold. The underlined region shows the imperfect 11-mer repeats.

[1] Bussell R Jr, Eliezer D. A structural and functional role for 11-mer repeats in alpha-Synuclein and other exchangeable lipid binding proteins. *J Mol Biol* 329(4):763-78. (2003)

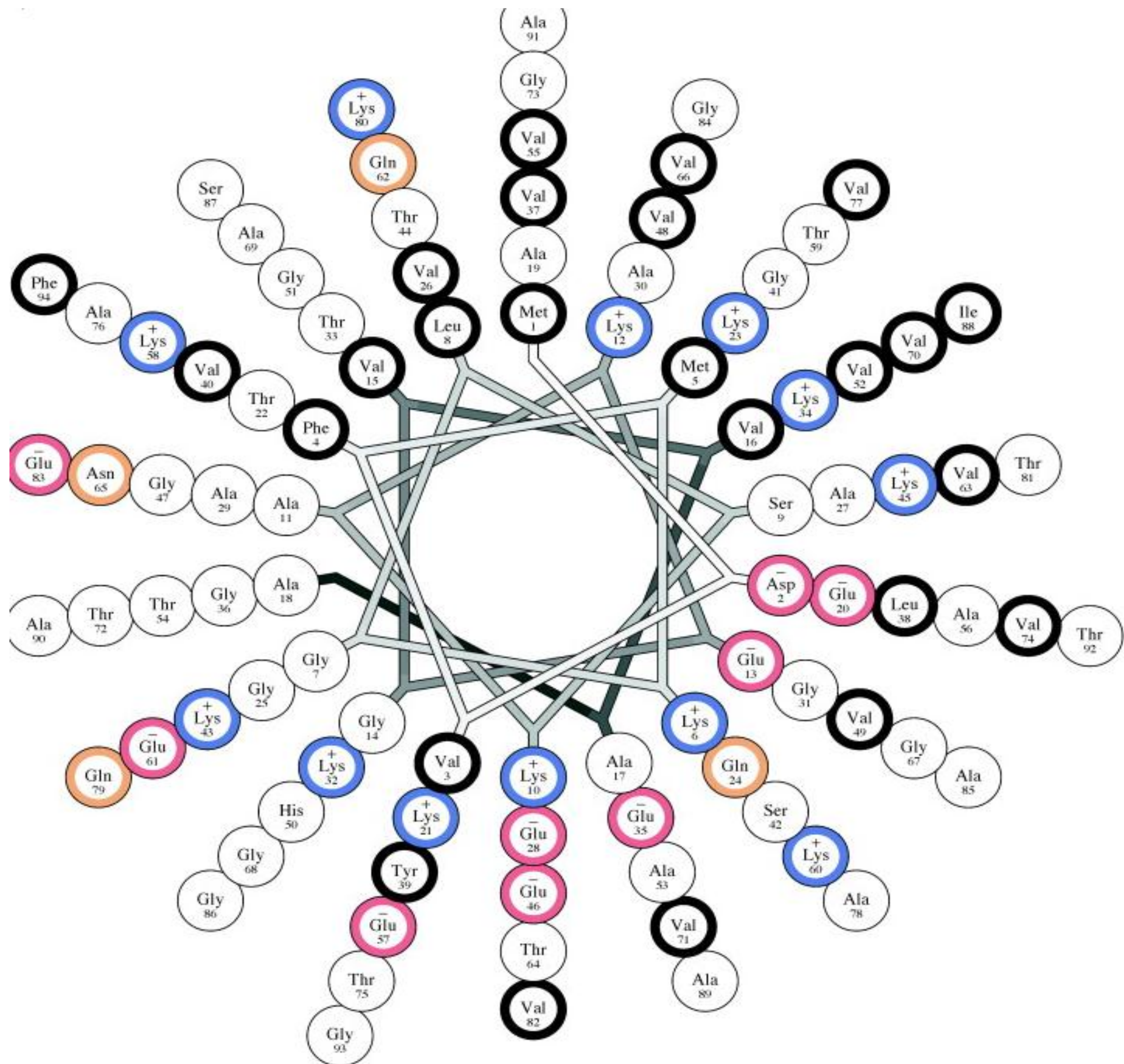


Fig 2. (a) Helical pinwheel plot and (b) space filling representation of the micelle-bound region of αS (residues 1–94) as an ideal α-helix. Hydrophobic residues are in black, positively charged residues in red, negatively charged residues in blue and polar residues in yellow. Basic residues are in blue and acidic residues are in red.

[1] Bussell R Jr, Eliezer D. A structural and functional role for 11-mer repeats in alpha-Synuclein and other exchangeable lipid binding proteins. *J Mol Biol* 329(4):763-78. (2003)

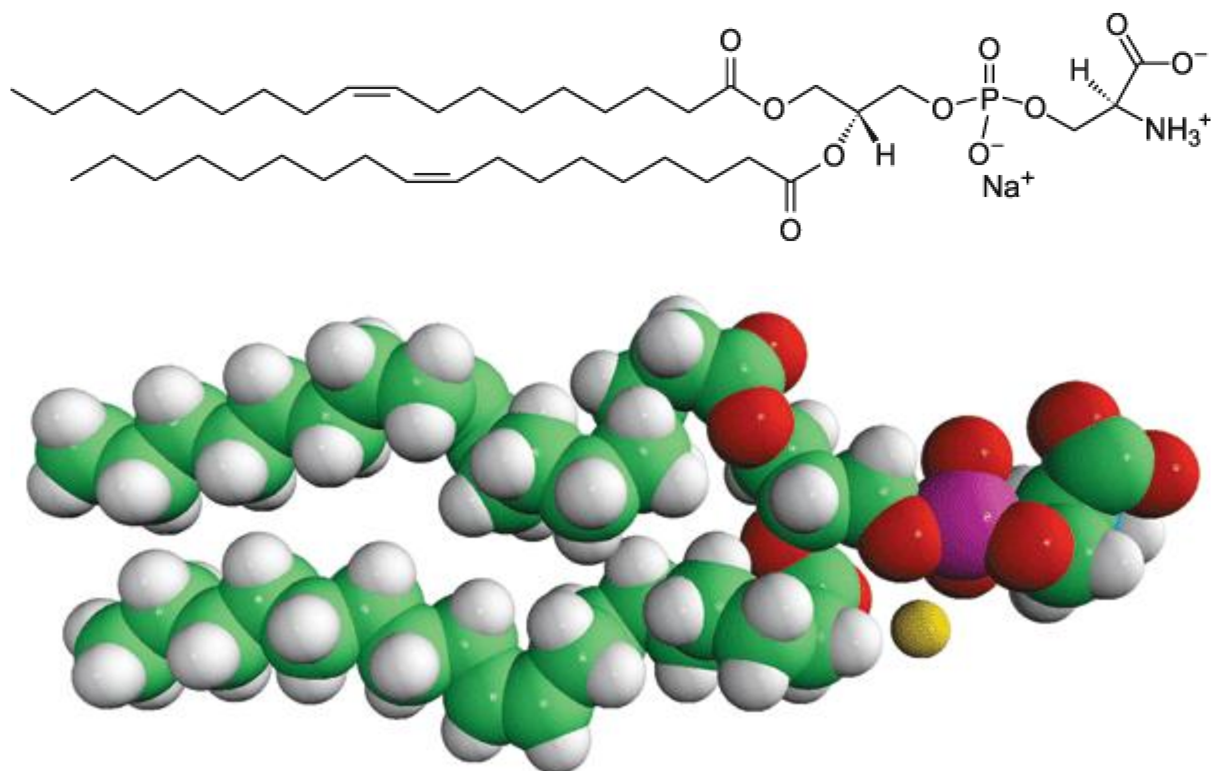


Fig 3. Structure of DOPS. Phosphatidylserine (PS) is the most abundant negatively charged phospholipid in eukaryotic membranes, which has three ionizable groups.

Avanti Polar Lipids, Inc.

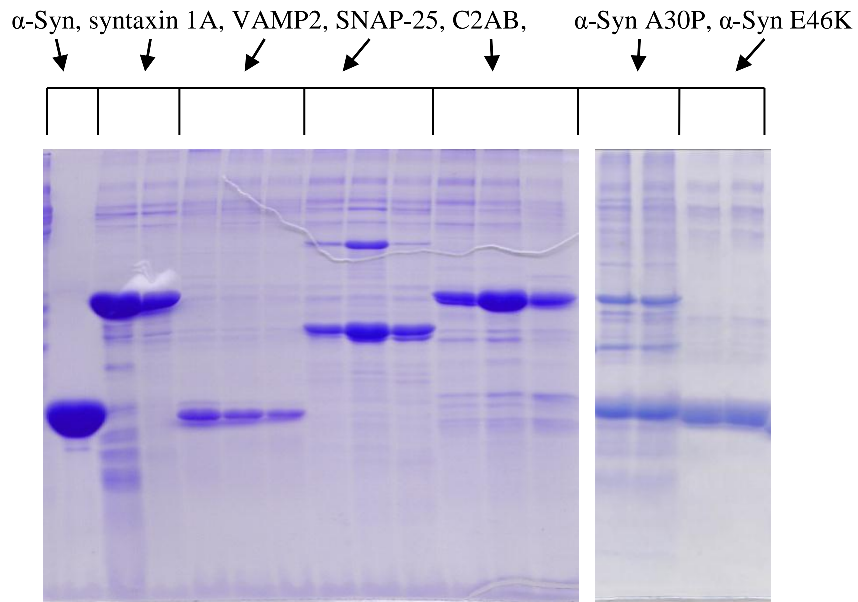


Fig 4. SDS gel of purified recombinant proteins. From left to right: α-Syn, syntaxin 1A(two lanes), VAMP2(three lanes), SNAP-25(three lanes), C2AB(three lanes), α-Syn A30P and E46K mutants(two lanes each).

(a)

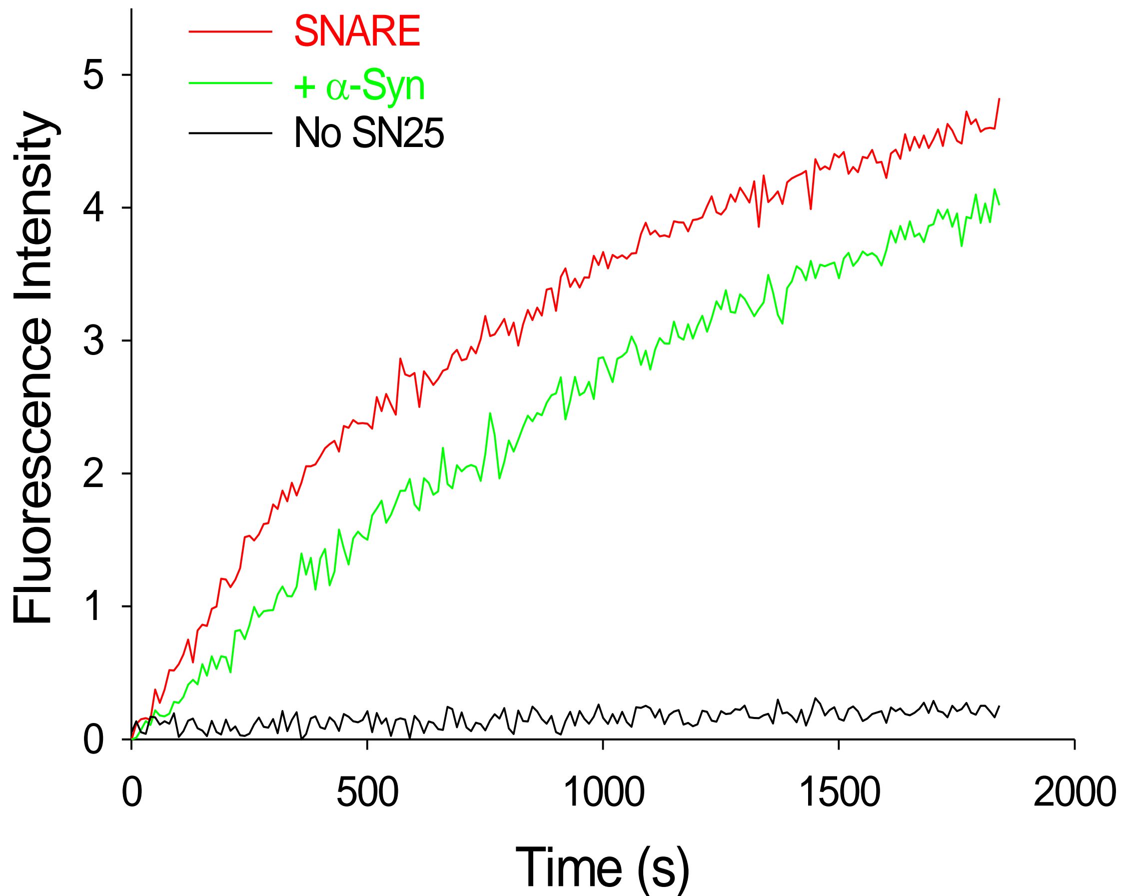
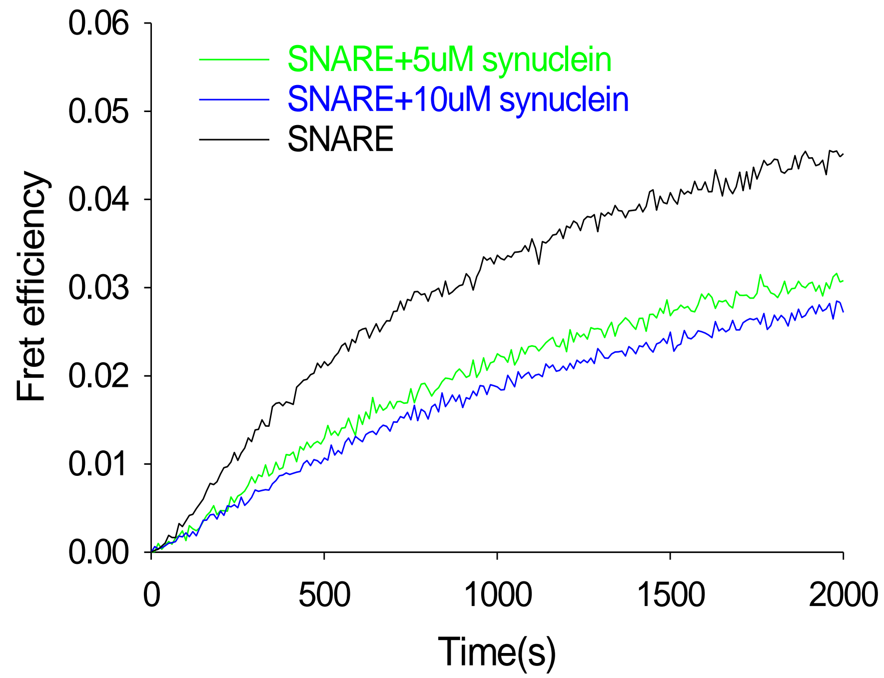


Fig 5. α-Syn inhibits SNARE-mediated lipid mixing. It shows the change of DiD signal strength. The change of fluorescence intensity is due to t- and v- vesicle lipid mixing. (a)The red line is the fusion kinectics of lipid mixing between t-vesicle reconstituted with Syntaxin 1A/SNAP-25 and v-vesicle reconstituted with VAMP2. The green line is lipid mixing with 20uM α-Syn added to the assay. The black line is a control, which is in the absence of SNAP-25 on t-vesicle.

(b)



(b) With the increasing concentration of α-Syn, the inhibition effect is stronger.

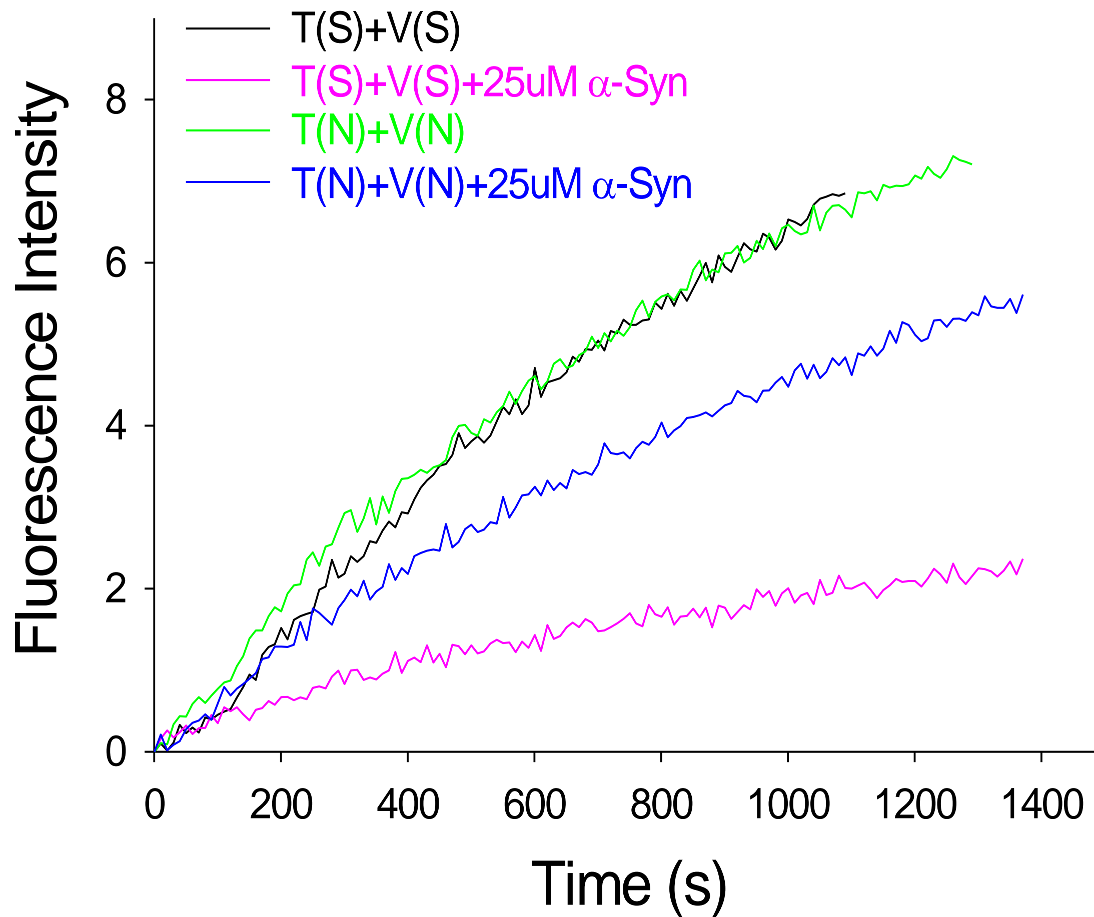


Fig 6. α-Syn's effect on lipid mixing with neutral and PS lipids. T(S) and V(S) are vesicles with PS. T(N) and V(N) are vesicles without PS. Pink line and blue line are lipid mixing with 25uM α-Syn. The green line and black line are SNARE-only mediated lipid mixing. It shows that without PS on the vesicles, α-Syn's inhibition effect was attenuated.

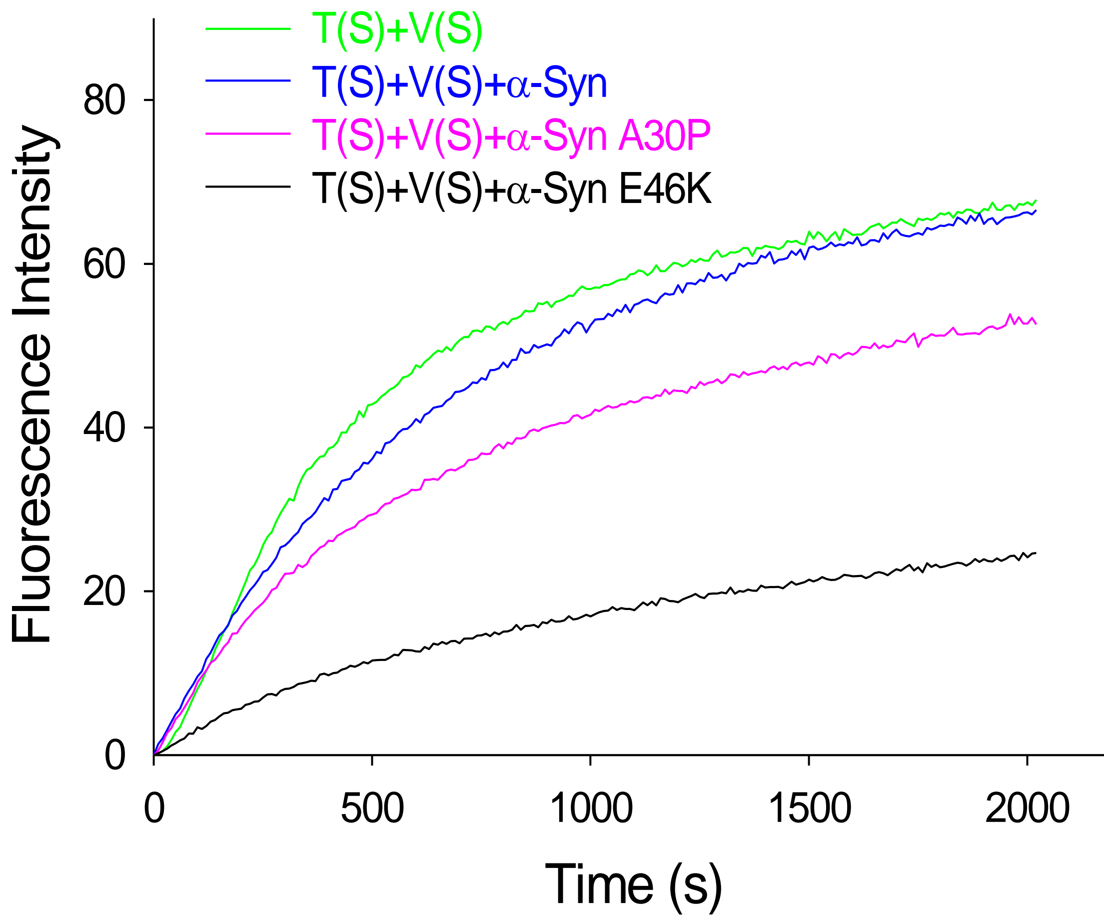


Fig 7. α-Syn mutants A30P, E46K associated with PD exhibit stronger inhibition effect on SNARE-mediated lipid mixing than wild-type

Chapter 3

Figures And Captions

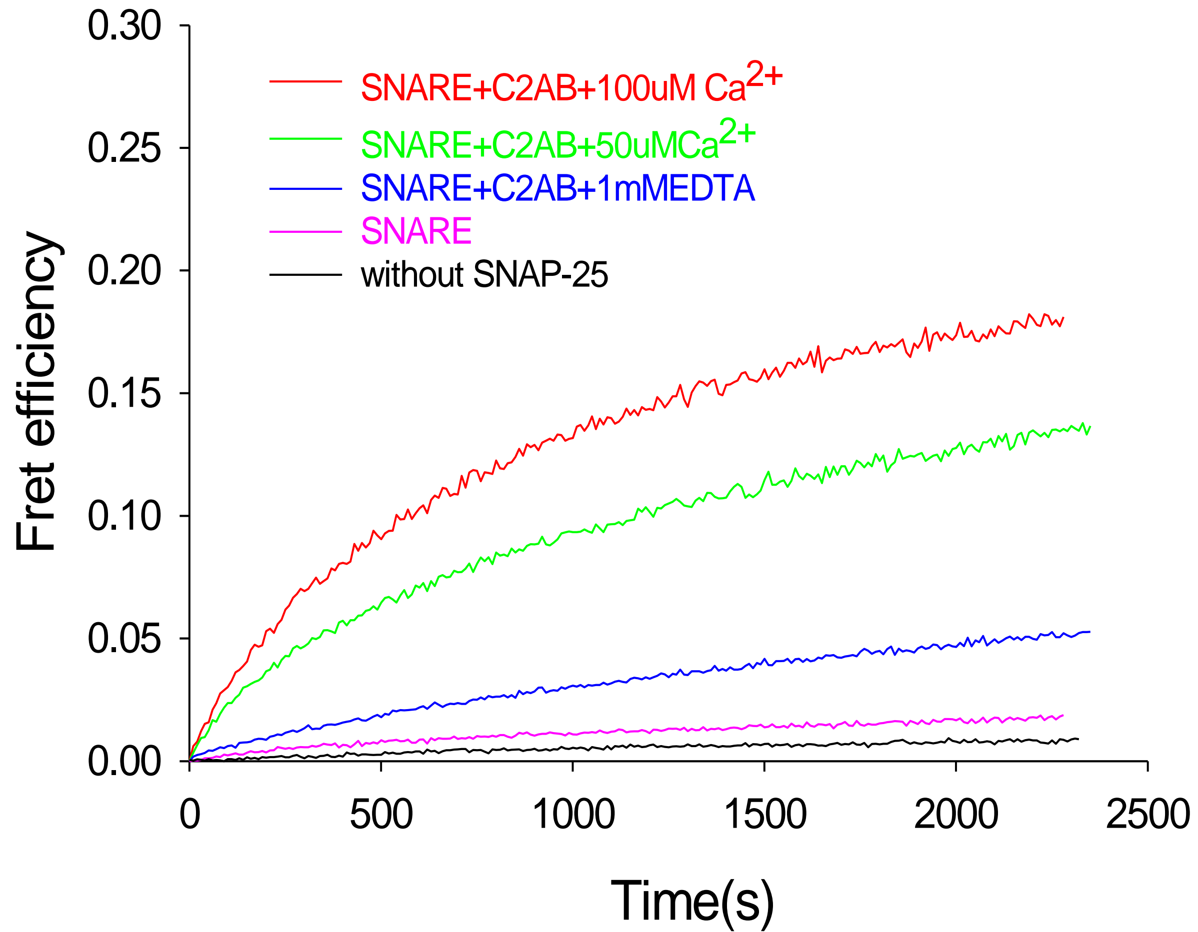


Fig 1. C2AB and Ca^{2+} can stimulate the SNARE-mediated lipid mixing. 1uM C2AB was added to all the assays. With the increasing concentration of Ca^{2+}, from 0 (blue line) to 50uM (green line) to 100uM (red line), C2AB's shows higher stimulatory effect on the SNARE-mediated lipid mixing.

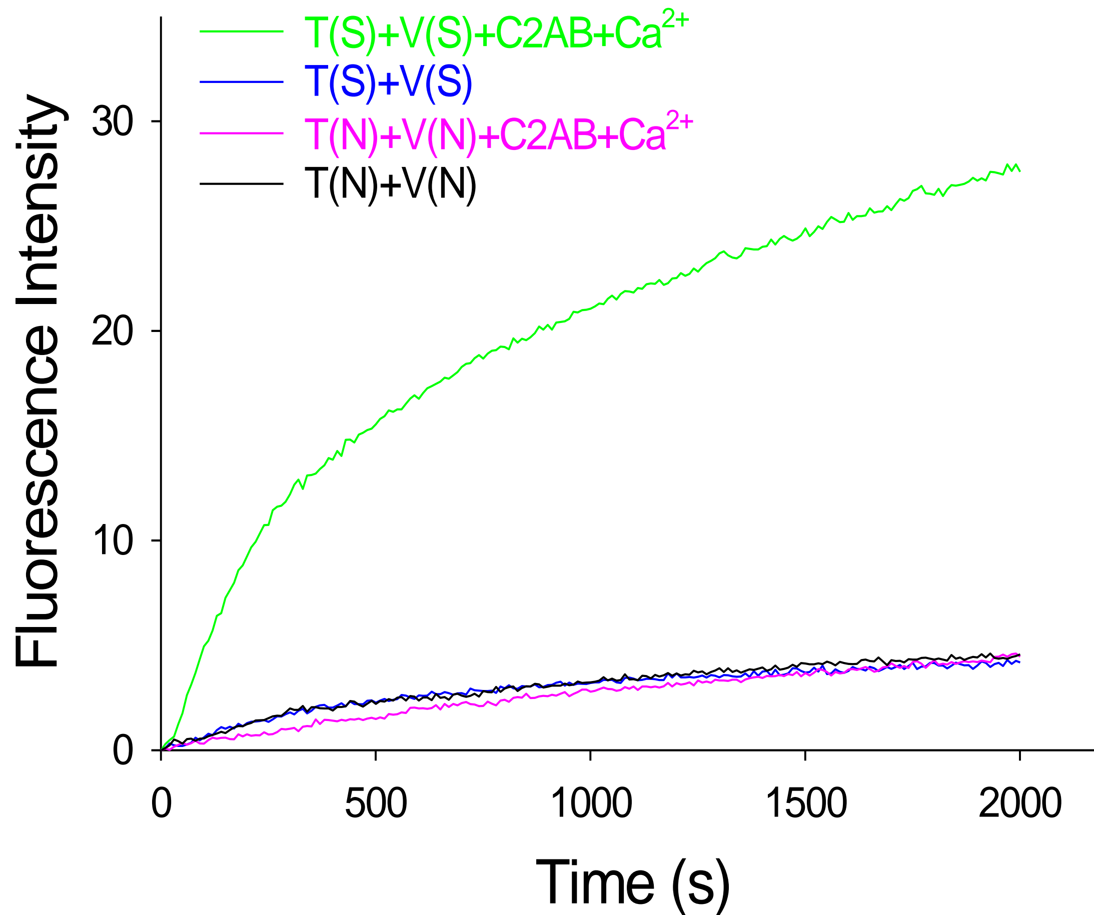


Fig 2. C2AB and Ca^{2+}'s stimulatory effect on SNARE-mediated membrane fusion requires PS on the vesicles. Green and blue lines were assays with vesicles containing PS. Pink and black lines were assays with neutral vesicles. With the presence of PS, C2AB and Ca^{2+} shows a much higher stimulatory effect on the lipid mixing.

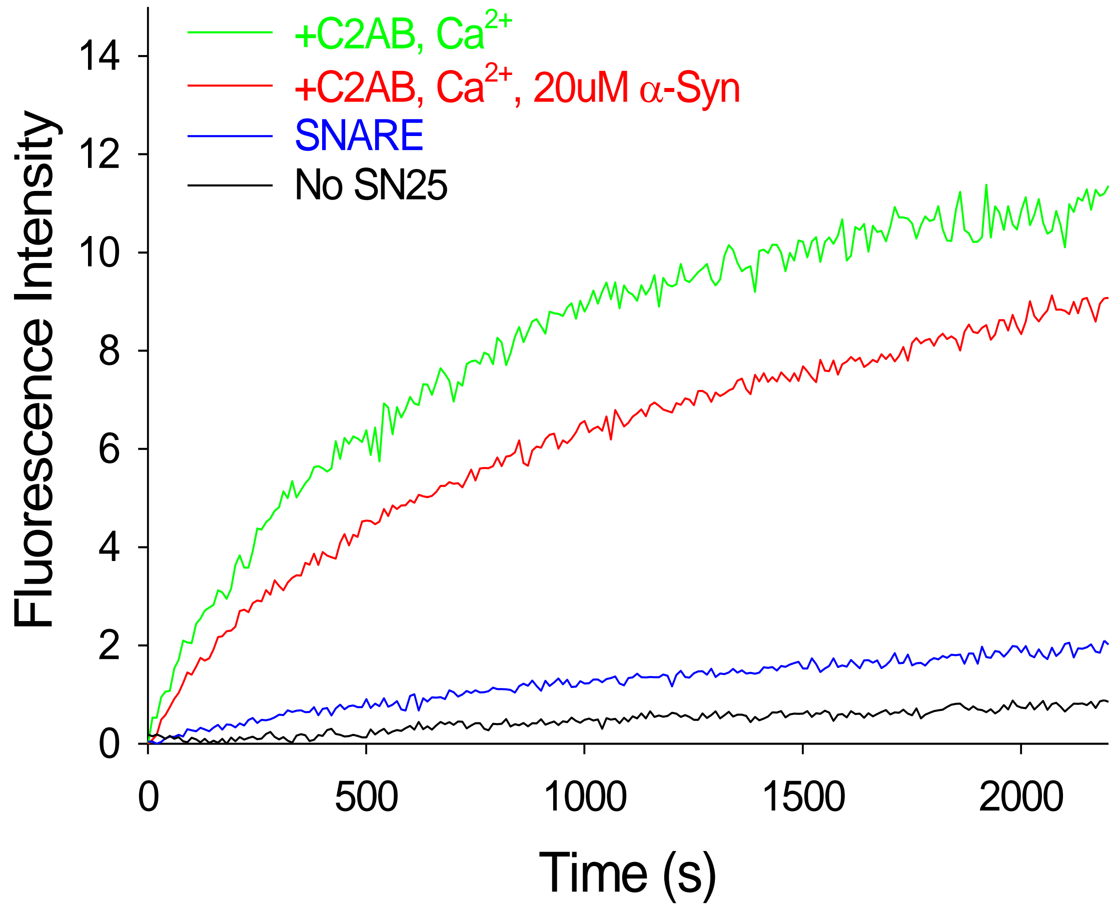


Fig 3. α-Syn inhibits C2AB and Ca^{2+}'s promotion effect on SNARE-mediated lipid mixing. 1uM C2AB and 100uM Ca^{2+} were added. Green line shows the stimulatory effect of C2AB and Ca^{2+}. The red line shows a decreased lipid mixing rate inhibited by α-Syn.

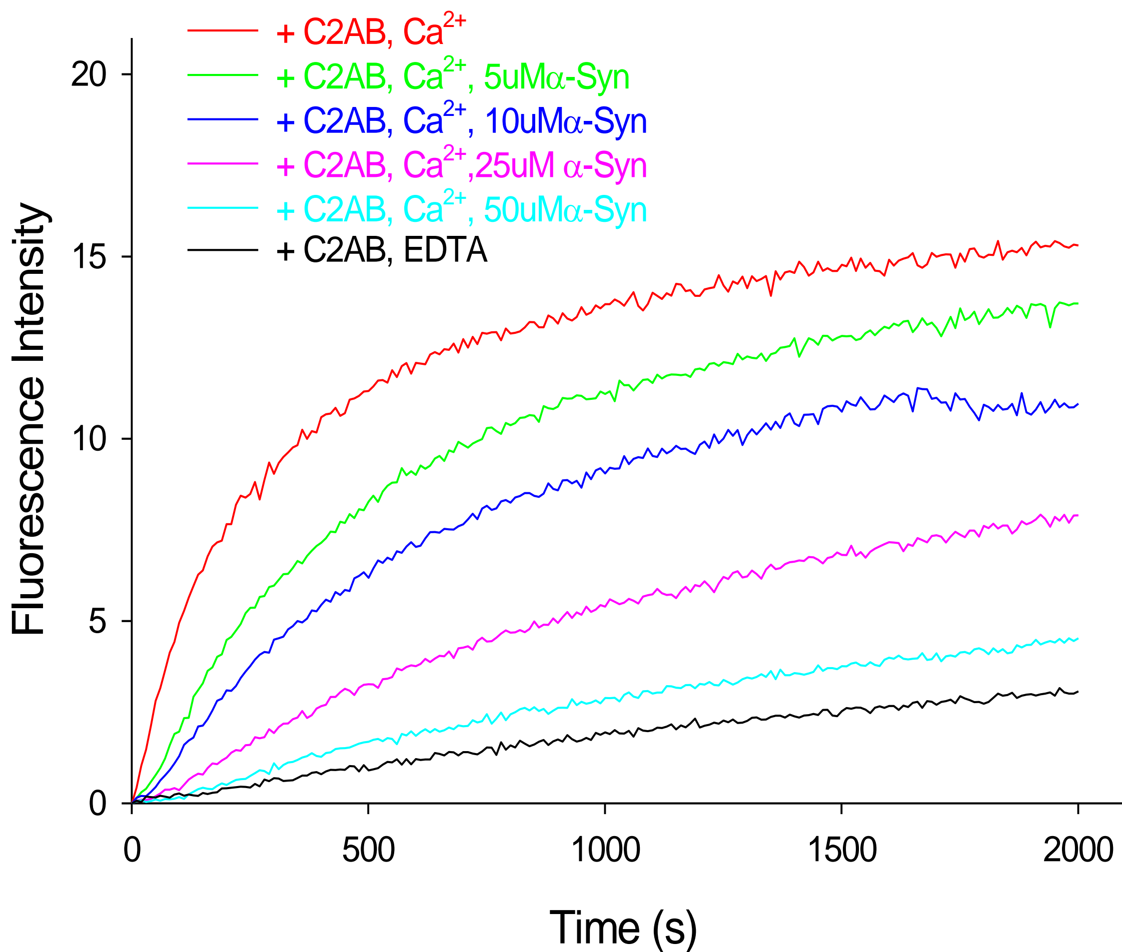
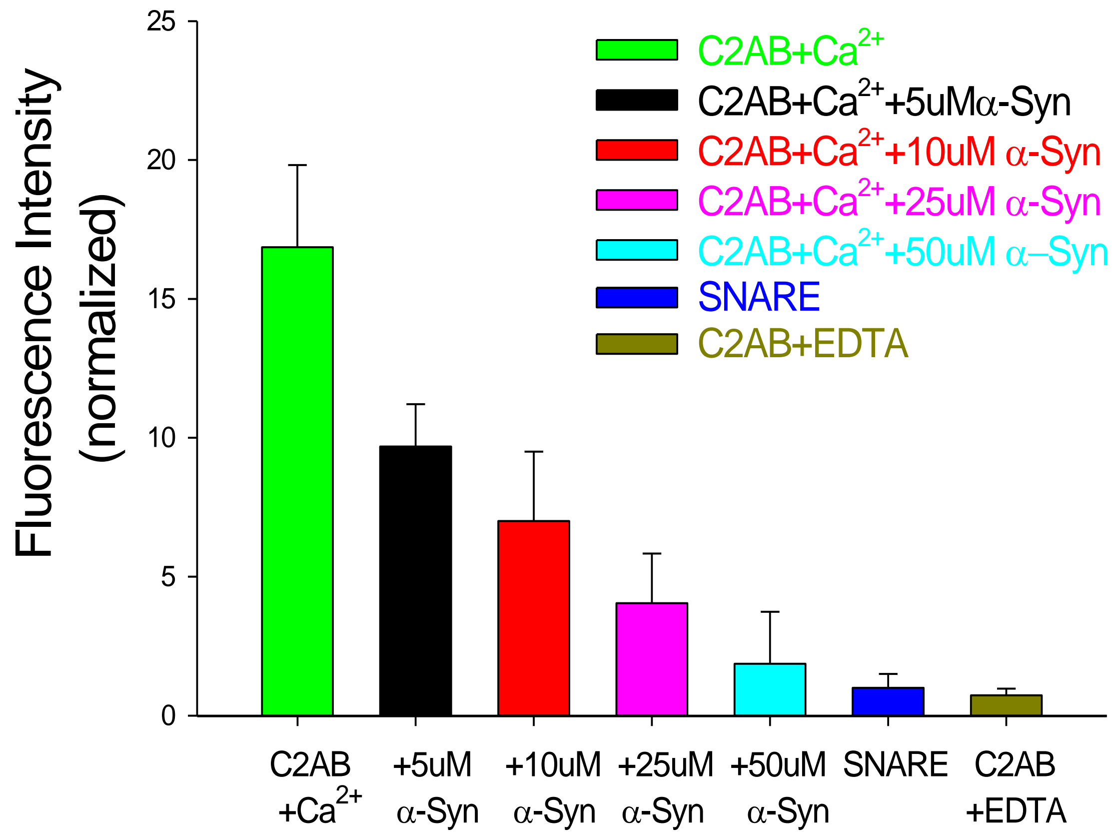


Fig 4. (a) α-Syn inhibits C2AB and Ca^{2+}'s promotion effect on SNARE-mediated lipid mixing. The increase of fluorescence intensity reflects lipid mixing. The red line is lipid mixing with 1uM C2AB, 100uM Ca^{2+}. The green line is lipid mixing with 1uM C2AB, 100uM Ca^{2+}, and 5uM α-Syn, the blue line (10uM α-Syn), the pink line (25uM α-Syn), and the cyan line (50uM α-Syn). The black line is the control, which is lipid mixing with 1uM C2AB, and 1mM EDTA.



(b) Normalized initial rates of the lipid mixing assays. Error bars were obtained from measurements of 3 independent assays.