Online Supplementary Information for Vasquez, et al. *Biochemistry*

Lateral Diffusion of Proteins on Supported Lipid Bilayers: Additive Friction of Synaptotagmin 7 C2A-C2B Tandem Domains

Table S1. Diffusion of Syt7 C2A is independent of fluorophore.

species[a]	D ($\mu m^2/s$)[b]
C2A-AF488	2.12 ± 0.08
C2A-AF555	2.11 ± 0.06
C2A-AF647	2.10 ± 0.04

[a]The three different fluorescent-tagged species of Syt7 C2A were added to the same supported bilayer and imaged in the three different colors.

[b]Mean ± S.D. among 4-5 movies from the same supported bilayer are reported. Due to sample-to-sample and day-to-day variability, values may differ from the average values reported in Table 1.

Table S2. Diffusion of Syt7 C2B before and after addition of unlabeled C2A.

	D ($\mu m^2/s$)[a]			
	C2B alone	C2B + unlabeled C2A	unlabeled C2A added (pM)	unlabeled C2A added (molecules/μm^2)[b]
Bilayer 1	1.87 ± 0.07	1.75 ± 0.05	50	50
Bilayer 2	1.65 ± 0.12	1.74 ± 0.04	50	50
Bilayer 3	1.77 ± 0.04	1.74 ± 0.04	130	120
Mean ± S.D.[c]	1.76 ± 0.11	1.74 ± 0.01		

[a]Mean ± S.D. among 4-5 movies from each supported bilayer are reported. Due to sample-to-sample and day-to-day variability, values may differ from the average values reported in Table 1.

[b]Calculated based on dimensions of perfusion chambers, assuming 100% of added protein binds to bilayer. Due to incomplete lipid binding and/or nonspecific protein loss, the actual density of bound protein is likely lower.

[c]Mean ± S.D. of the three samples listed above.

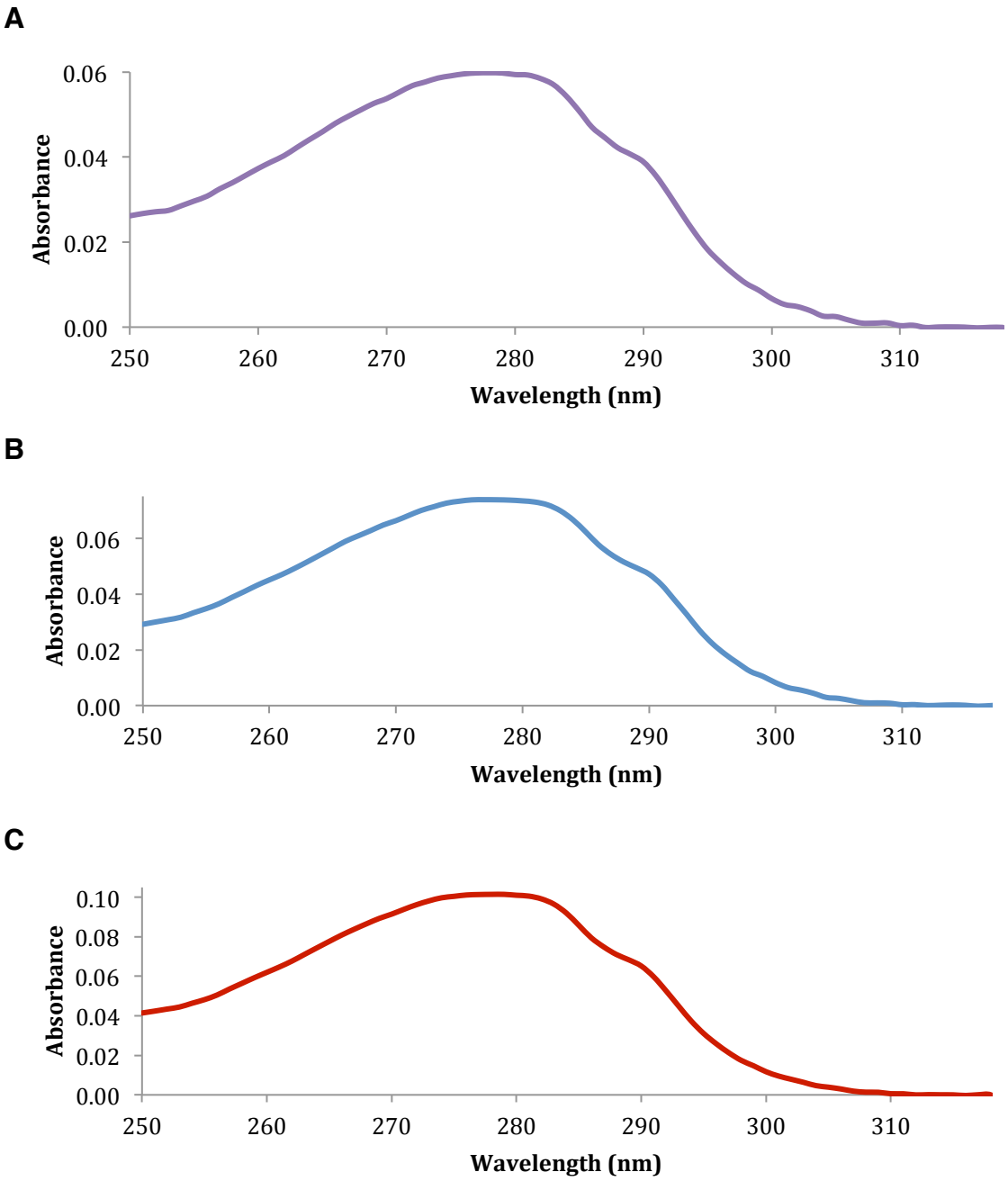

Figure S1: Syt7 C2AB absorbance data. UV/Vis absorbance data after elution from a glutathione column and before AF488 labeling. **A.** C2AB-WT (260/280 ratio is 0.63). **B.** C2AB-FE4 (260/280 ratio is 0.61). **C.** C2AB-FE16 (260/280 ratio is 0.61). The low 260/280 ratios indicate very low levels of nucleic acid contamination.

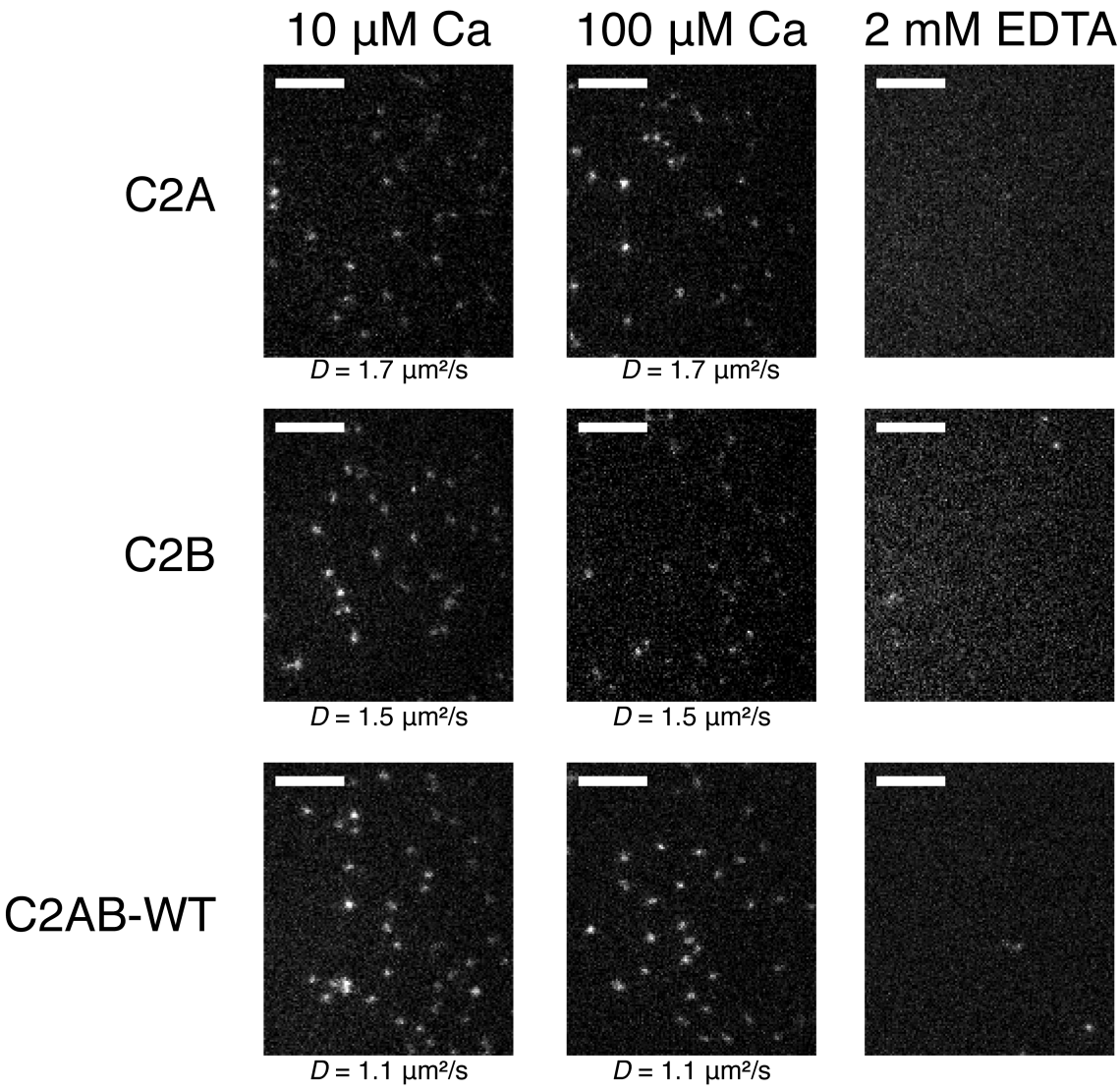

Figure S2: Ca^{2+} dependence of lipid binding and diffusion. Above are representative images of Alexa Fluor 555 labeled Syt7 C2A, C2B, and C2AB on 3:1 PC:PS imaged under the Ca^{2+} concentrations shown. Each sample was imaged first in 10 µM Ca^{2+}, then following addition to 100 µM Ca^{2+}, then finally upon addition of 2 mM EDTA. Particles visible in EDTA were generally short-lived, although there were enough remaining to show that the bilayer remained intact. Neither particle density nor diffusion constant was observed to differ significantly between 10 µM and 100 µM Ca^{2+} conditions. Diffusion constants listed are for individual movies and may not correspond exactly to those in Table 1 due to sample-to-sample differences. These samples were not included in averaging to obtain the values shown in Table 1, due to the different fluorophore and acquisition used. The images above were captured using a home-built TIRF microscope as described previously (Knight et al, *Biophys J* 2010). The authors thank J. Falke and A. Pardi for instrument access. Scale bars: 5 µm.

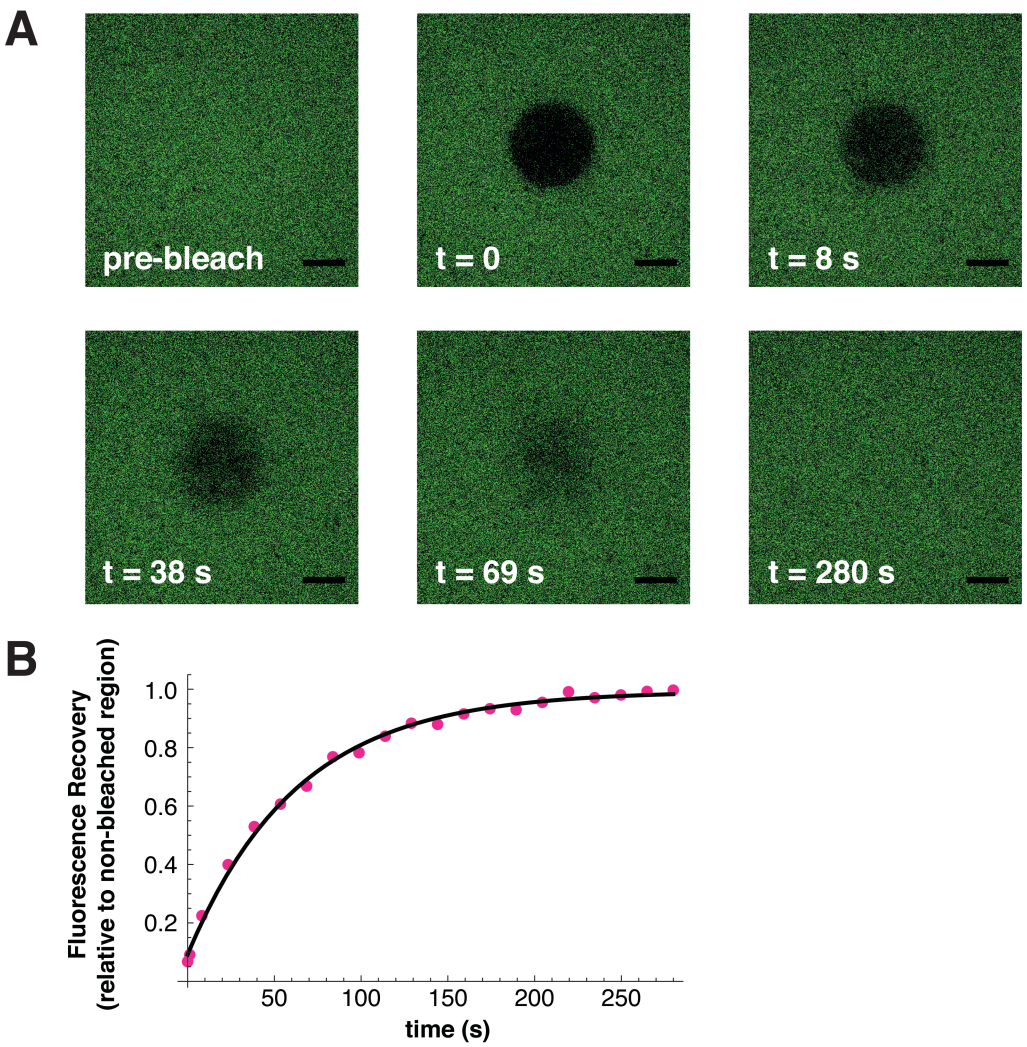

Figure S3: FRAP analysis of supported lipid bilayers. Bilayers containing 1 mol% 1-oleoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-sn-glycero-3-phosphocholine (NBD-PC) were prepared as described in the main text. FRAP was performed using a Zeiss Observer Z1 confocal microscope equipped with a 488-nm laser and LSM-700 camera. **A:** A 10-μm radius spot was bleached at full laser power until a maximum of 20% fluorescence remained (total bleach time ~6 s) and then imaged during recovery at 500-fold lower laser power. **B:** The average intensity within the bleached spot was normalized to a non-bleached region and fit to a single-exponential recovery profile. Diffusion constants were calculated as $D = {\omega^2}/{4t_{1/2}}$, where ω is the bleach radius and $t_{1/2}$ is the half-time of recovery from the fit. Five measurements each were performed on 4 supported bilayers, and produced a diffusion constant of 0.6 ± 0.2 μm^2/s. This value is interpreted as the approximate diffusion constant of the lower leaflet, proximal to the glass, as the upper leaflet is expected to recover on a much faster timescale comparable to the bleach pulse itself. Attempted fits to double exponentials produced inconsistent parameters. Representative data shown. Scale bar: 10 μm.

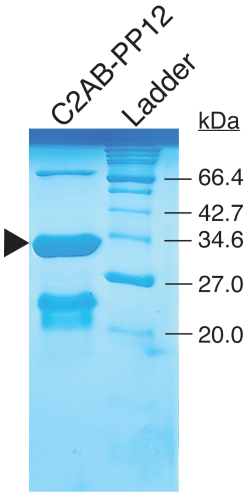

Figure S4: Impurity in C2AB-PP12. SDS-PAGE (14%) shown with Broad Range Protein Marker (New England Biolabs). The triangle indicates the expected mass of the C2AB-PP12 tandem domain. The high molecular weight impurity is sometimes present in protein stocks and has not been observed to interfere with membrane binding. The low-MW impurities seen here are likely cleavage products.