High throughput monitoring of single vesicle fusion using free-standing membranes and automated analysis

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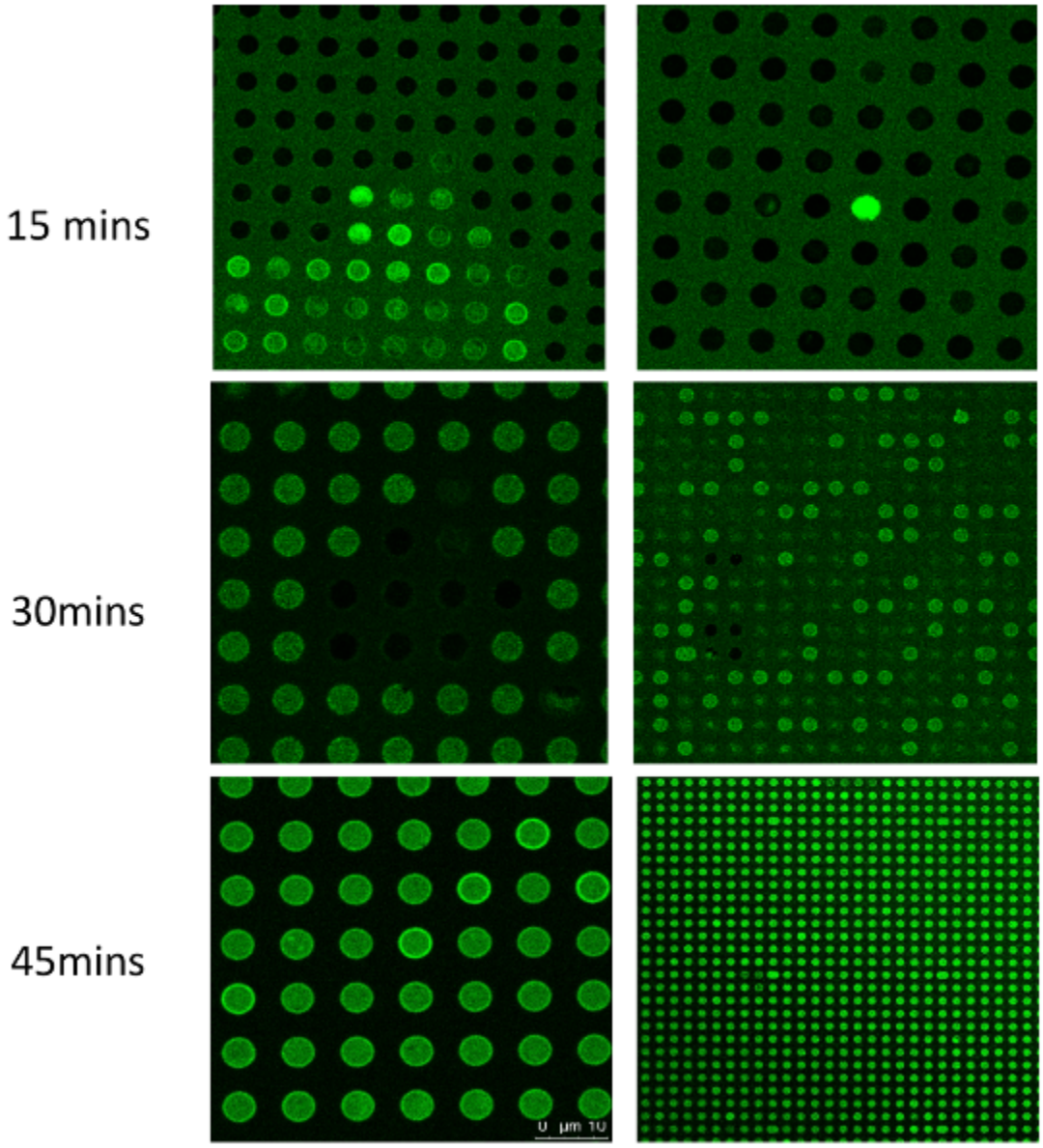

Figure S1: Time-dependent proteo-GUV formation and freestanding bilayer coverage. Proteo-GUVs containing Alexa488 labeled t-SNAREs that are formed after 45 minutes incubation exhibit maximum coverage when being spread on the chip. (Size of each hole = 5 μm)

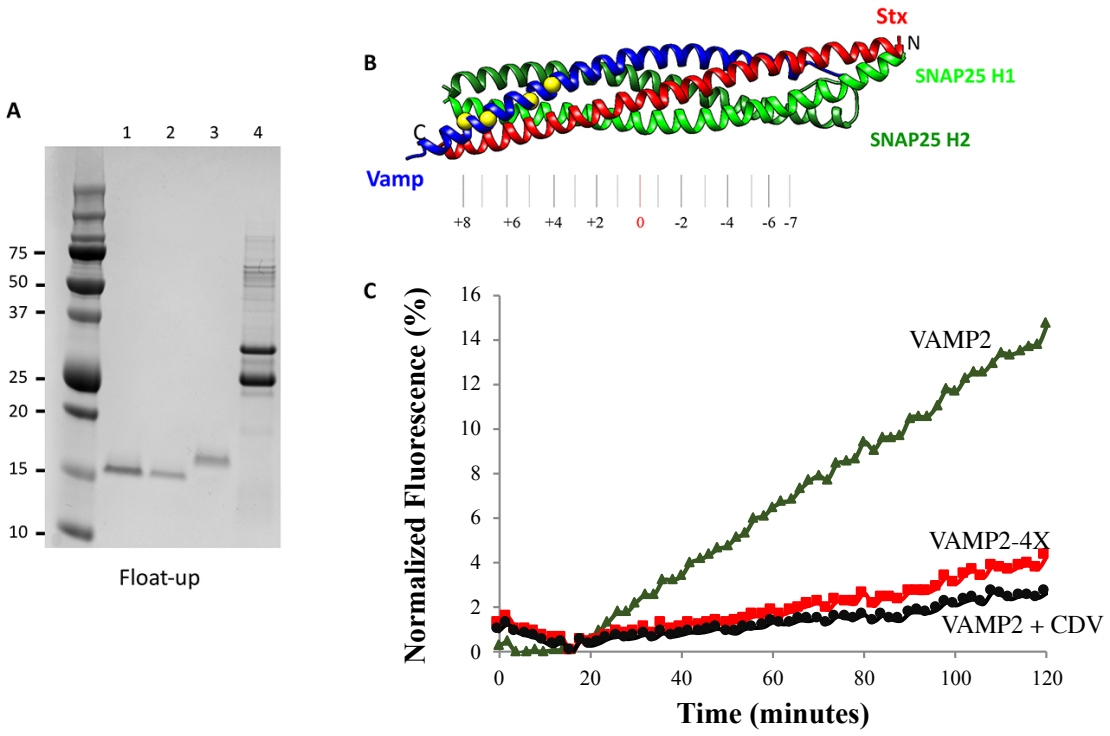

Figure S2: A) Coomassie-stained sodium dodecyl sulfate (SDS)-PAGE gel shows the incorporation of SNARE proteins liposomes. Lanes: 1: VAMP2 (1:200); 2: VAMP2 (1:400); 3: VAMP2-4X (1:400); 4: t-SNARE (1:400); migration of size marker (Precision Plus Protein Standards, Bio-Rad) in kilodaltons is denoted B) Image shows the mutated residues in VAMP2-4x protein (yellow balls). Mutations were made after +4 layer helps V-SNAREs to zipper partly with the t-SNARE. C) SNARE protein fusogenic activity was verified by lipid dequenching assay using VAMP2 (Green) and VAMP2-4X (Red) liposomes (labeled with 1.5 mol% NBD and Rhodamine) together with label free liposomes containing t-SNAREs. Normalized fluorescence intensities are plotted versus time. The cytoplasmic domain of VAMP2 (CDV) (19 μM) was added to the VAMP2 fusion reaction as a control (Black).

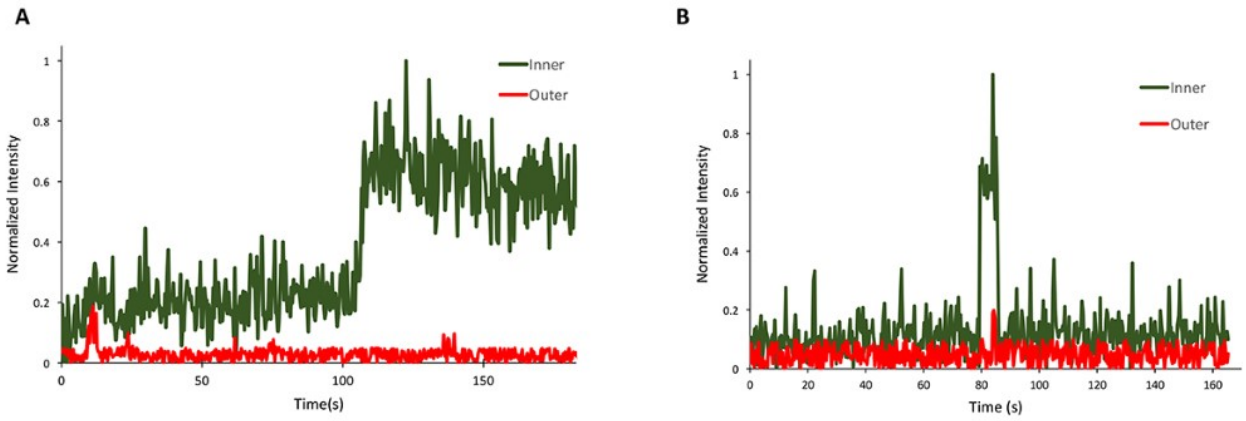

Figure S3: A) Fluorescent intensity plot of a v-SNARE vesicle docked on the membrane. Almost 80% of the fusion process happens in a few seconds, we considered any vesicle docked on the membrane for more than 30 seconds as docking but not fusing. These did show no fluorescence increase in the outer ROI B) Plot shows a short-docked state of a vesicle followed by undocking.

Protein Diffusion Coefficient Estimation

FRAP was done by bleaching with an elliptical mask (width = 2 μm and height = 5 μm) with a part covering silicon chip and part by the hole. Fig S4 shows the fluorescence from Alexa-488 labeled t-SNAREs get bleached over time. Since there is no incoming proteins diffusion from neighboring holes, we employ a pseudoquantitative approach to roughly estimate protein diffusion constant. Using elliptical mask, we bleached the hole for about 50 frames with an acquisition time of 871 ms. We estimated characteristic time (t_c) of fluorescence intensity decay by fitting an exponential function. Finally, diffusion coefficient can be roughly estimated by dividing the area of the hole by t_c; the result ~2.2 $\mu m^2/s$. Clearly, the relationship between this ratio and the precise diffusion coefficient will depend on the actual bleached pattern (here, the intercept of an ellipse and a circle) and is extremely difficult to evaluate. Nevertheless, they must be of the same order of magnitude. Hence, our final rough estimate for the protein diffusion coefficient is 1 $\mu m^2/s$.

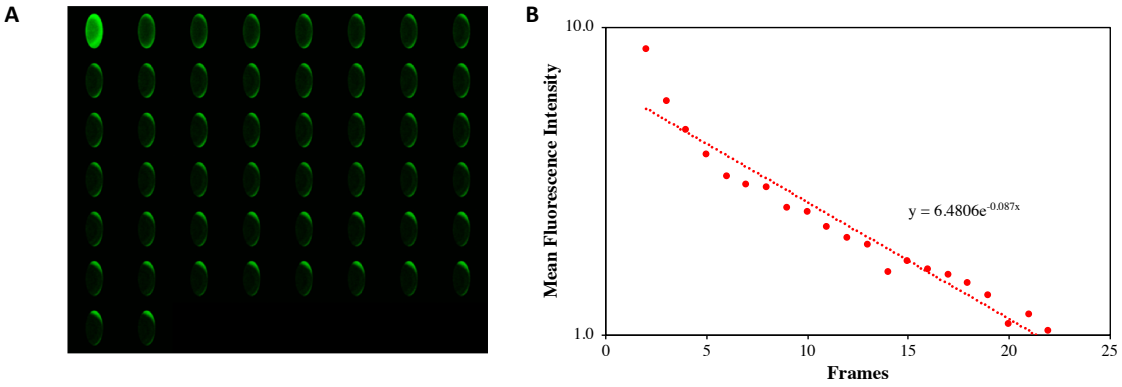

Figure S4: A) Representative snapshots of FRAP bleaching series of Alexa-488 labeled t-SNAREs in the hole using an elliptical mask (width = 2 μm and height = 5 μm). B) The plot shows the exponential fitting to the mean fluorescence intensity to obtain the half-life time (t_c = 11.5 s).