Rapid Fusion of Synaptic Vesicles with Reconstituted Target SNARE Membranes

Volker Kiessling,[†] Saheeb Ahmed,[‡] Marta K. Domanska,[†] Matthew G. Holt,^{‡§} Reinhard Jahn,[‡] and Lukas K. Tamm[†]*

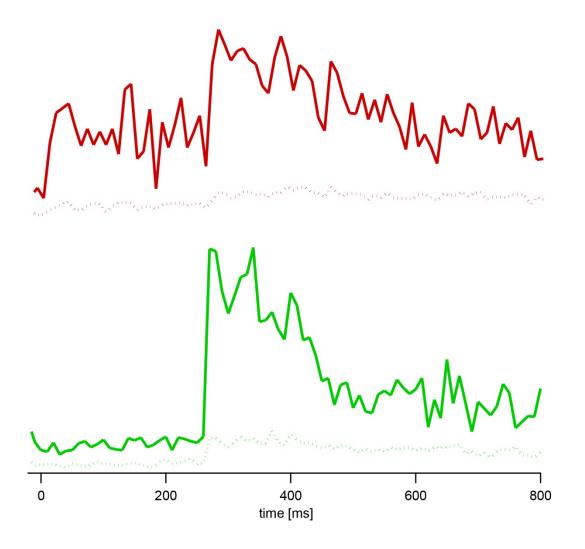
[†]Center for Membrane Biology and Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, Virginia; [‡]Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany; and [§]VIB Center for the Biology of Disease, K.U. Leuven Center for Human Genetics, Leuven, Belgium

Kiessling et al.

Rapid Fusion of Single Synaptic Vesicles

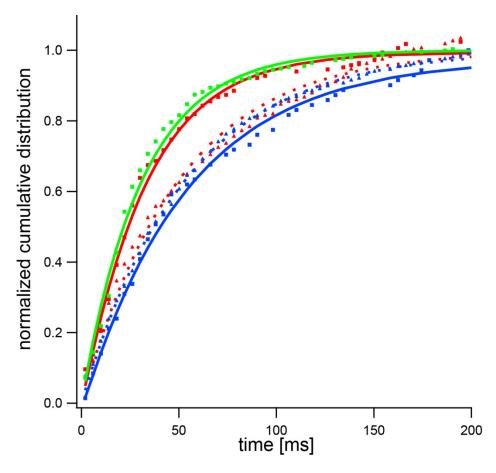
Submitted January 25, 2013, and accepted for publication March 22, 2013.

*Correspondence: <u>lkt2e@virginia.edu</u>



Supplemental Figure 1. Membrane and content fluorescence signals during proteoliposome docking and fusion event to acceptor SNARE complex-containing supported membrane.

Peak (solid) and mean (dotted) fluorescence from membrane dye DiD (red) and content dye sulforhodamine (green) of $1.9 \times 1.9 \, \mu m^2$ regions around a proteoliposome were plotted. Time point zero was set to the time of docking characterized by a sharp increase of DiD fluorescence. The onset of fusion is characterized by a second sharp increase of the DiD intensity followed by an immediate decrease of the peak intensity and a delayed decrease of the mean intensity due to diffusion of the membrane labels out of the observed region. At the same time point, dequenching of rhodamine causes a sharp increase of fluorescence intensity which is followed by a decrease over several hundred milliseconds due to diffusion within the small cleft between membrane and quartz.



Supplemental Figure 2. Single vesicle fusion kinetics for different salt and lipid conditions. Normalized cumulative distribution functions of fusion lag times from single synaptic vesicles in the presence of 1 mM Ca²⁺ (red), 1 mM Mg²⁺ (green) and 1 mM EDTA (blue). The lipid composition of the supported membrane consists of either 32% bPC, 30% bPE, 15% bPS, 3% bPIP₂ and 20% cholesterol (data: squares, best fit: solid lines), or 80% POPC and 20% cholesterol (data: triangles, best fit: dashed lines).