Supporting Information

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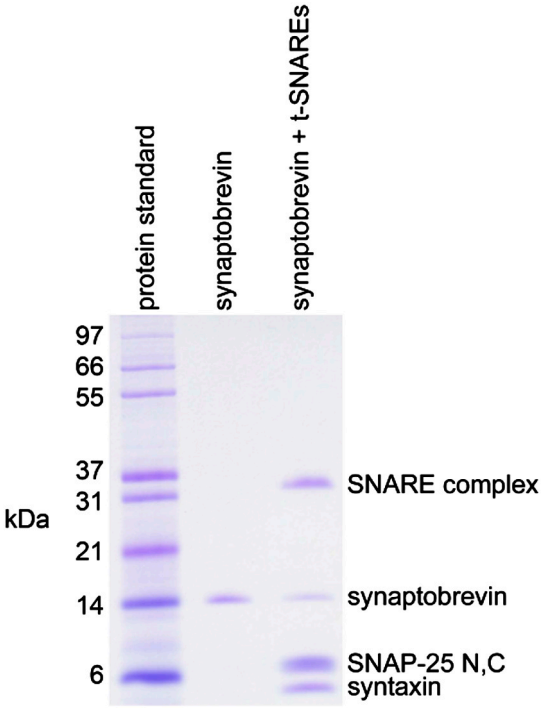

Fig. S1. The reconstituted synaptobrevin can readily form SNARE complexes. A sample of synaptobrevin reconstituted into proteoliposomes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC):1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS) 85:15 (molar ratio) (10 μM protein, 5 mM lipid) was incubated overnight with excess SNARE motifs from the t-SNAREs syntaxin-1 (residues 191–253; labeled syntaxin) and SNAP-25 (residues 11–82 and 141–203; labeled SNAP-25 N,C), and analyzed by SDS-PAGE (right lane). The middle lane corresponds to a control sample without addition of the t-SNAREs. The SDS-resistant band corresponding to the SNARE complex shows that the reconstituted synaptobrevin forms SNARE complexes efficiently. The unreacted synaptobrevin can be attributed to protein that has the cytoplasmic region oriented toward the lumen of the vesicles and hence is not accessible to the s-SNAREs. Molecular mass markers are on the left.

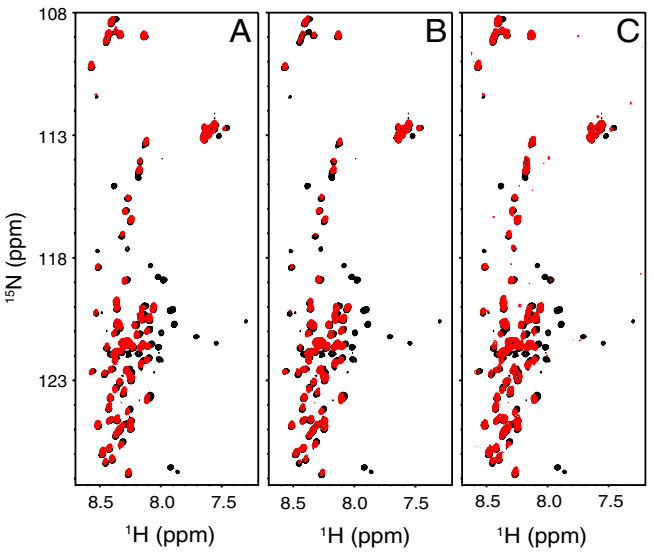

Fig. S2. Superposition of ^{1}H-^{15}N heteronuclear single quantum coherence spectra of the soluble synaptobrevin(1–96) fragment (black contours) and full-length synaptobrevin (red contours) reconstituted into liposomes composed of POPC:DOPS 85:15 (molar ratio) as in Fig. 3*A* but in the presence of 1 mM Mg^{2+} (*A*), liposomes composed of only POPC (*B*), or liposomes composed of POPC, 1,2-dipalmitoyl-sn-glycero-3- phosphoethanolamine, DOPS, and cholesterol 45:20:15:20 (molar ratio) (*C*).