# A Highly Tilted Membrane Configuration for the Pre-Fusion State of Synaptobrevin

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Table S1: Average TM2 Tilt angle from equilibrated systems

| System | Initial Tilt (°)[a] | TM2 Tilt (°)[b] |
|---|---|---|
| WT20′ | 22.2 | $23.1 \pm 8.7$ |
| WT10′ | 13.2 | $25.4 \pm 7.3$ |
| WT0 | 3.3 | $28.5 \pm 9.6$ |
| WT20 | 16.8 | $28.5 \pm 10.7$ |
| WT40 | 36.8 | $24.1 \pm 7.9$ |
| GG | 13.2 | $22.8 \pm 7.0$ |
| KK | 13.2 | $28.6 \pm 6.8$ |
| EE | 13.2 | $27.9 \pm 6.6$ |
| POPC | —[c] | $22.2 \pm 2.5$ |
| Avg | — | $25.7 \pm 2.7$ |

*a*. The initial tilt is taken as the initial tilt of the truncated synaptobrevin helix, as synaptobrevin was modeled as a continuous helix. *b*. Average tilt over the last 20 ns of the simulation. The tilt is measured for residues 100–115 relative to the membrane normal. *c*. As the starting configuration for the POPC simulation was the final snapshot from the WT simulations with the kink already present, the initial tilt is not presented.

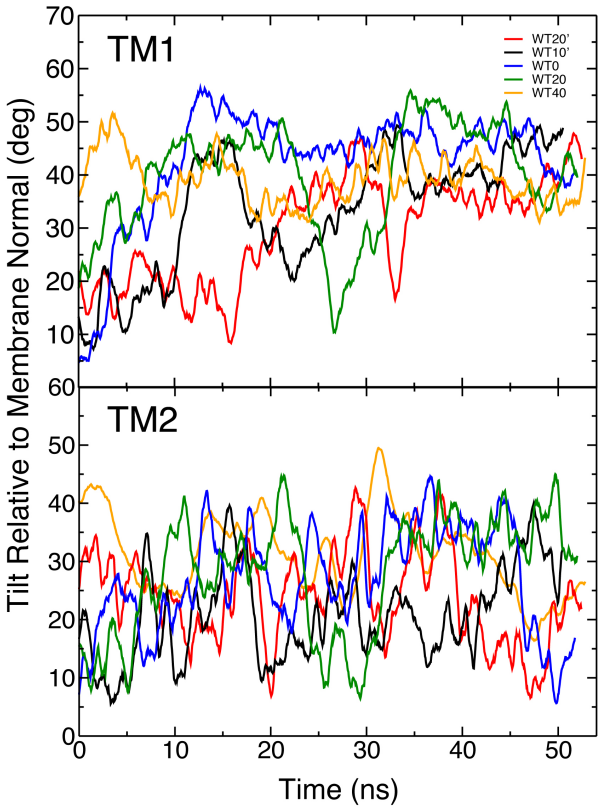


Figure S1: Plots of the TM1 (top) and TM2 (bottom) angles with respect to the membrane normal using the method of Åqvist (36), a least-squares definition of the helical axis.

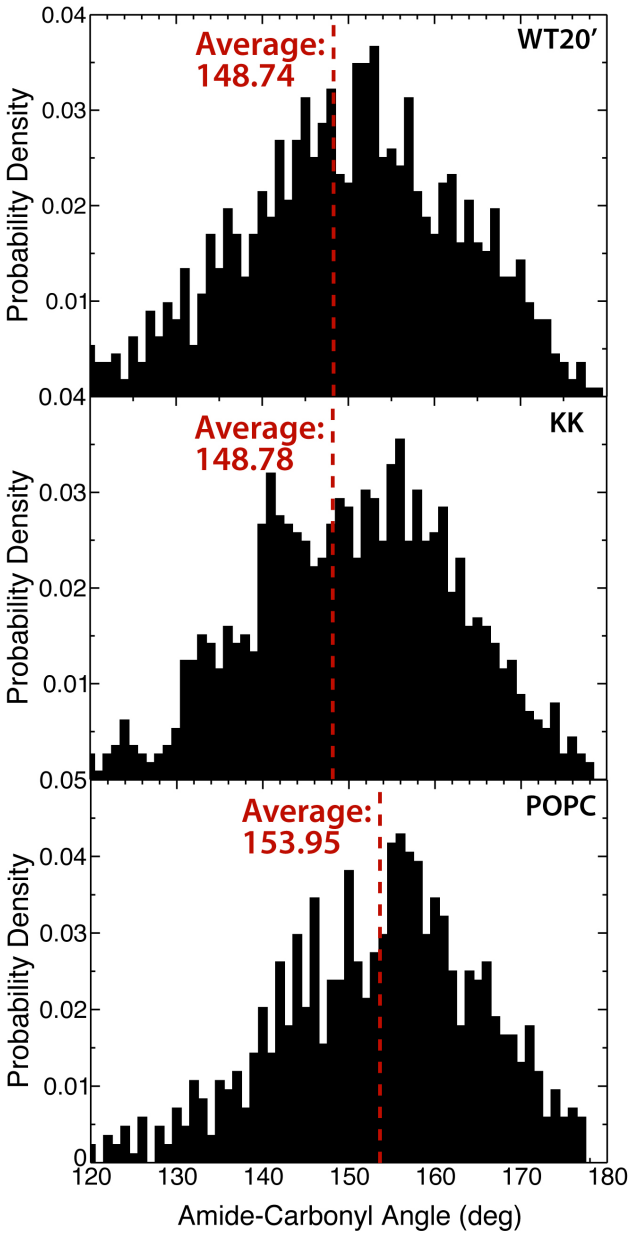


Figure S2: Plot of the angle between the amide of G100 and carbonyl of M96 for representative simulations WT20' (top), KK (middle), and POPC (bottom).

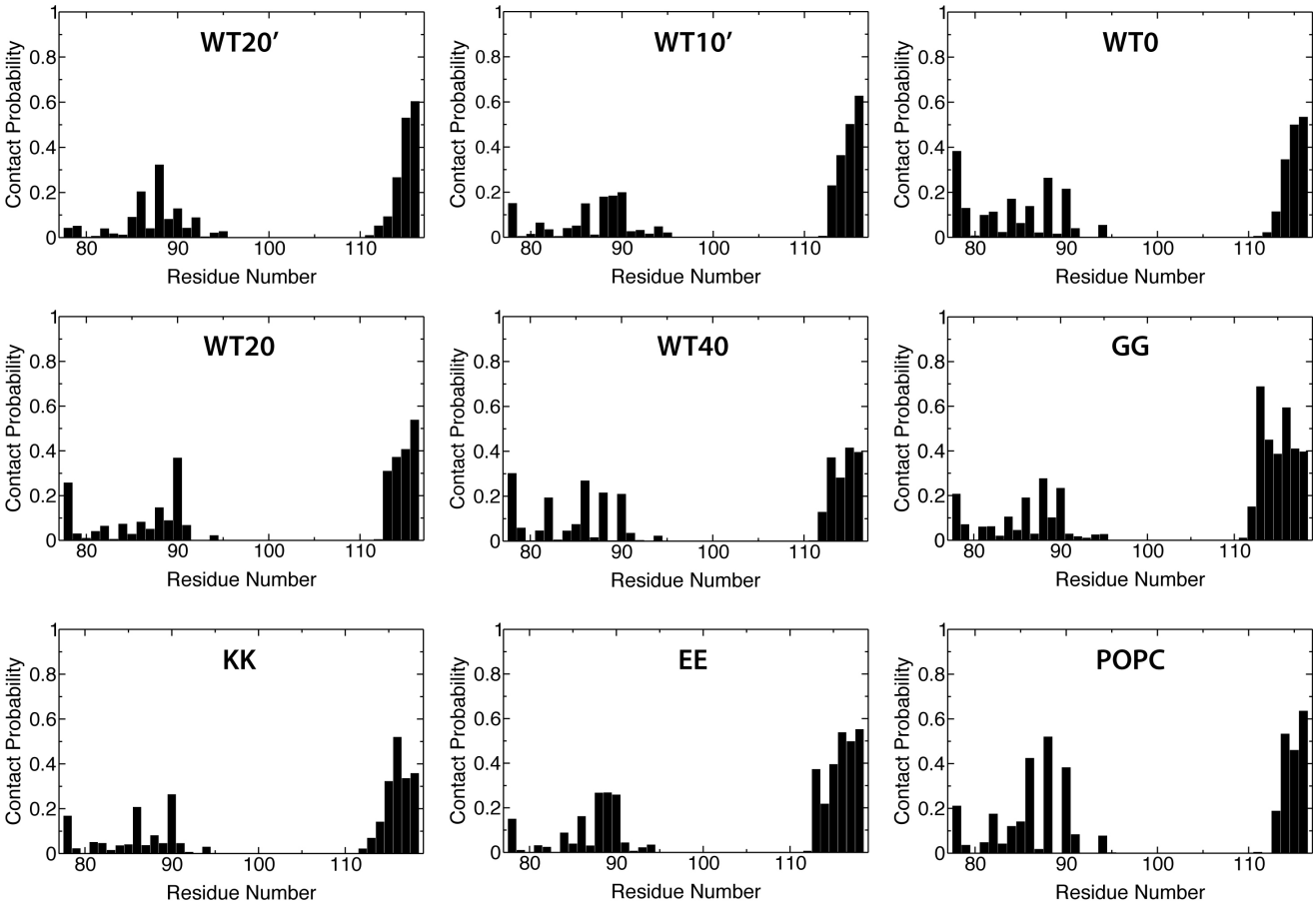


Figure S3: Plots of the contact probability of each residue of the truncated synaptobrevin construct with the choline headgroups of the PC lipids. The contact probability was calculated over the last 20 ns of each trajectory. The contact probability was measured by counting the number of frames in which the sidechain of the residue was within 4.0 Å of the $N(CH_3)_3^+$ of choline and normalizing by the number of frames over which the probability was measured, with each frame representing 20 ps.

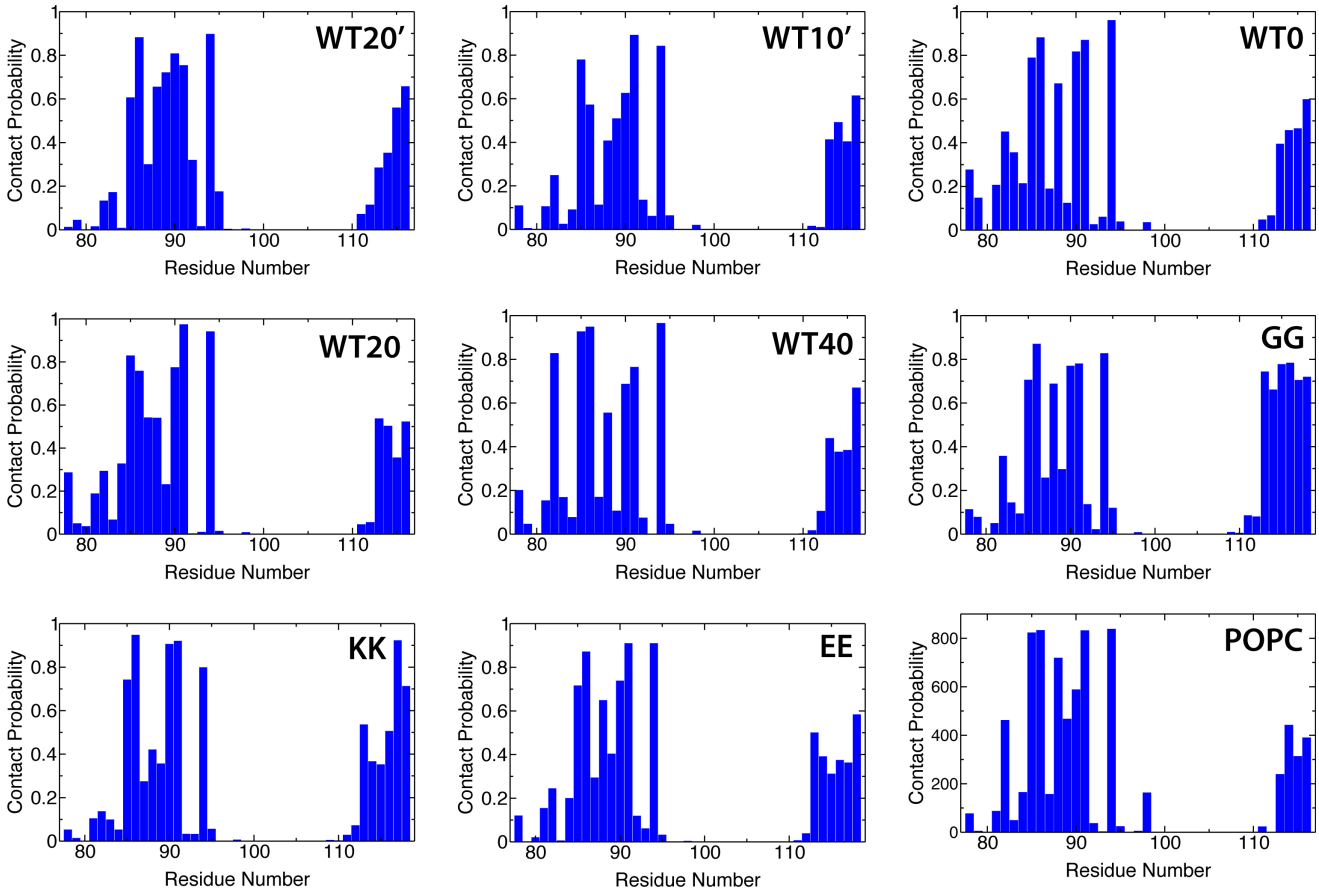


Figure S4: Plots of the contact probability of each residue of the truncated synaptobrevin construct with the phosphate group of the PC lipids. The contact probability was calculated over the last 20 ns of each trajectory. The contact probability was measured by counting the number of frames in which the sidechain of the residue was within 4.0 Å of the $PO_4^-$ of the phosphate and normalizing by the number of frames over which the probability was measured, with each frame representing 20 ps.

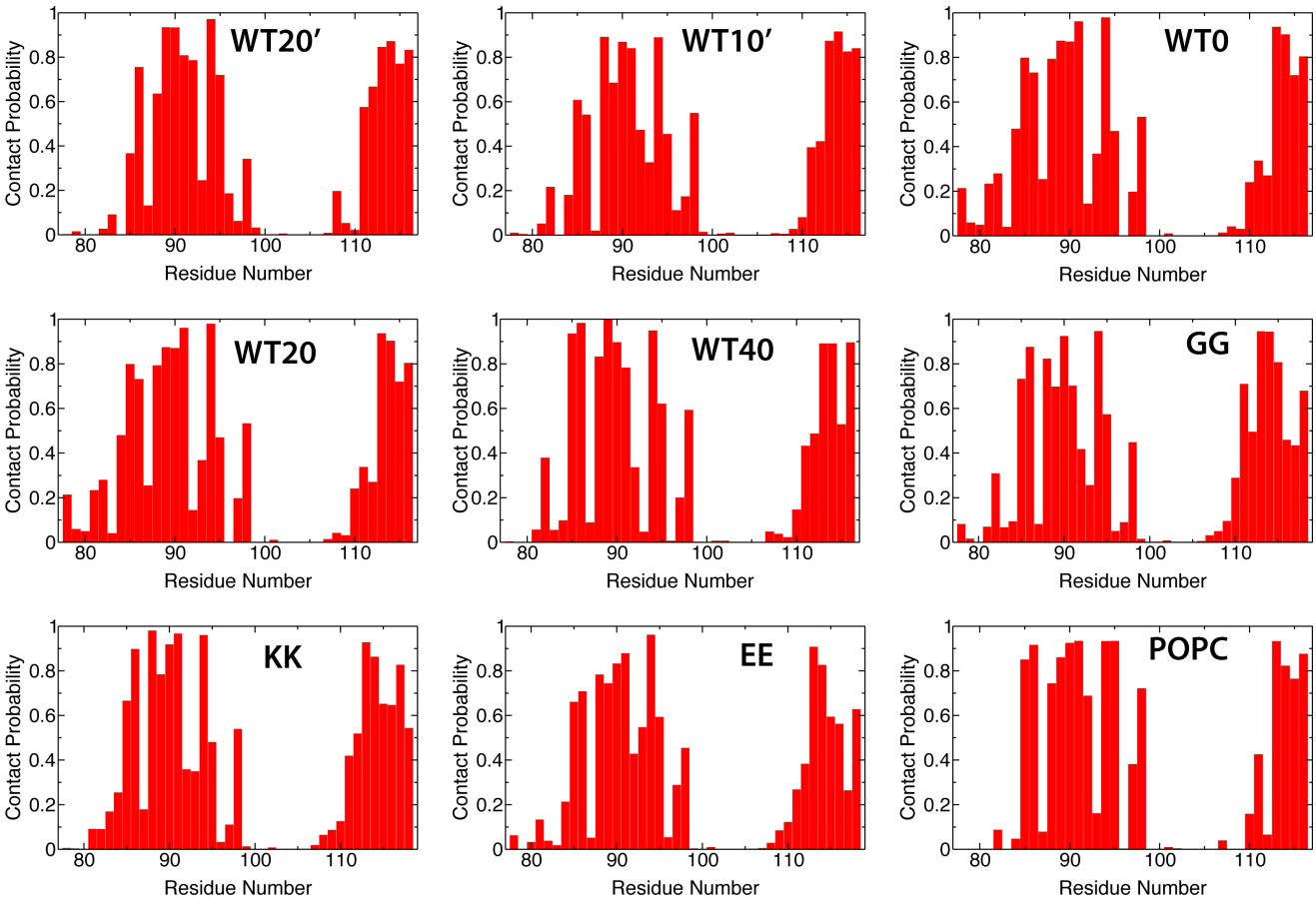


Figure S5: Plots of the contact probability of each residue of the truncated synaptobrevin construct with the glycerol backbone of the PC lipids. The contact probability was calculated over the last 20 ns of each trajectory. The contact probability was measured by counting the number of frames in which the sidechain of the residue was within 4.0 Å of the ester in the lipid and normalizing by the number of frames over which the probability was measured, with each frame representing 20 ps.

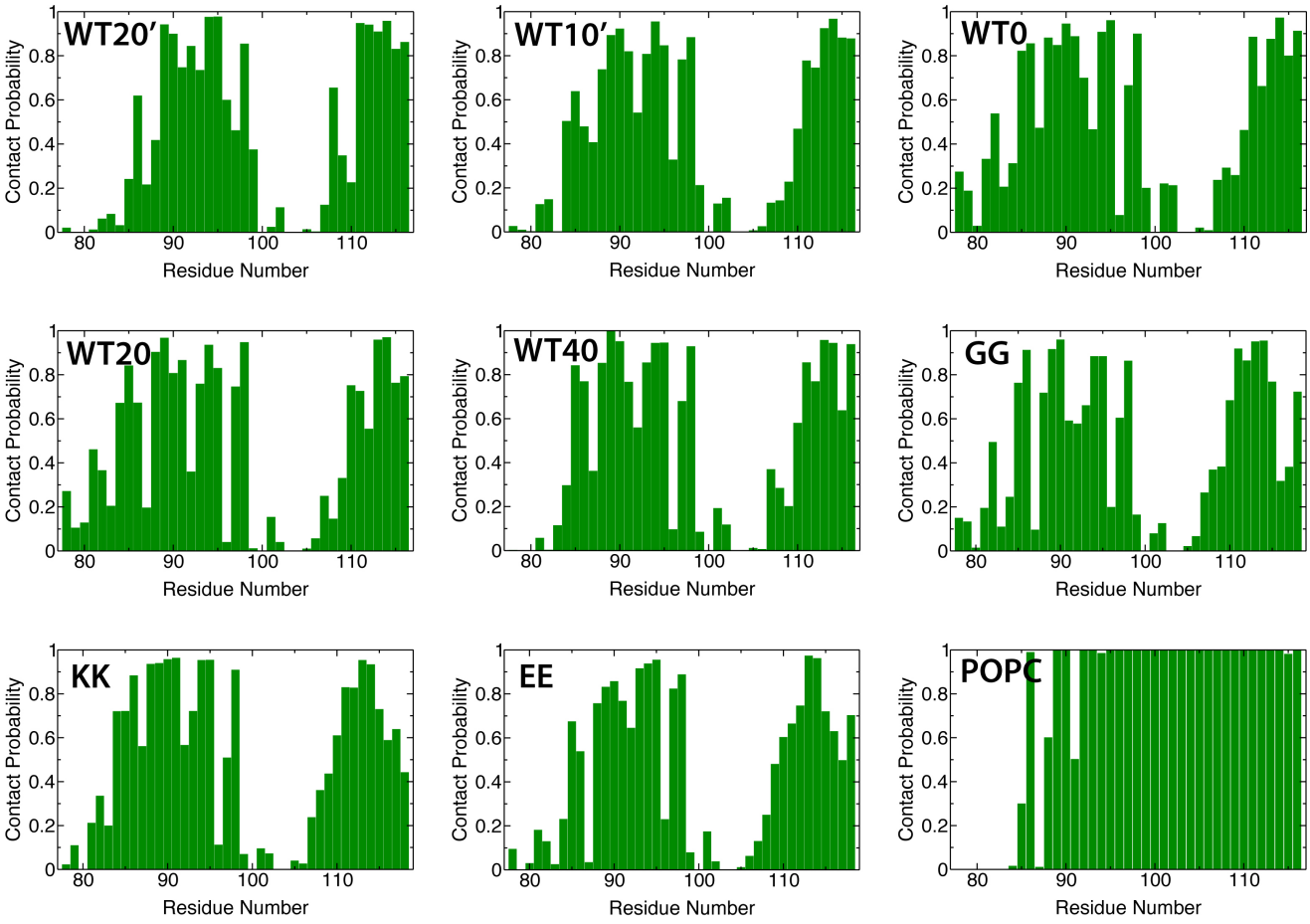


Figure S6: Plots of the contact probability of each residue of the truncated synaptobrevin construct with the aliphatic tails of the PC lipids. The contact probability was calculated over the last 20 ns of each trajectory. The contact probability was measured by counting the number of frames in which the sidechain of the residue was within 4.0 Å of the any aliphatic carbon in the lipid tail and normalizing by the number of frames over which the probability was measured, with each frame representing 20 ps. There is a discrepancy between the HMMM and POPC results due to the fact that in the HMMM, the tails of the lipids do not extend through the width of the membrane. Therefore, the center of the membrane is void of aliphatic carbons and we expect a lack of contact probability in this region.

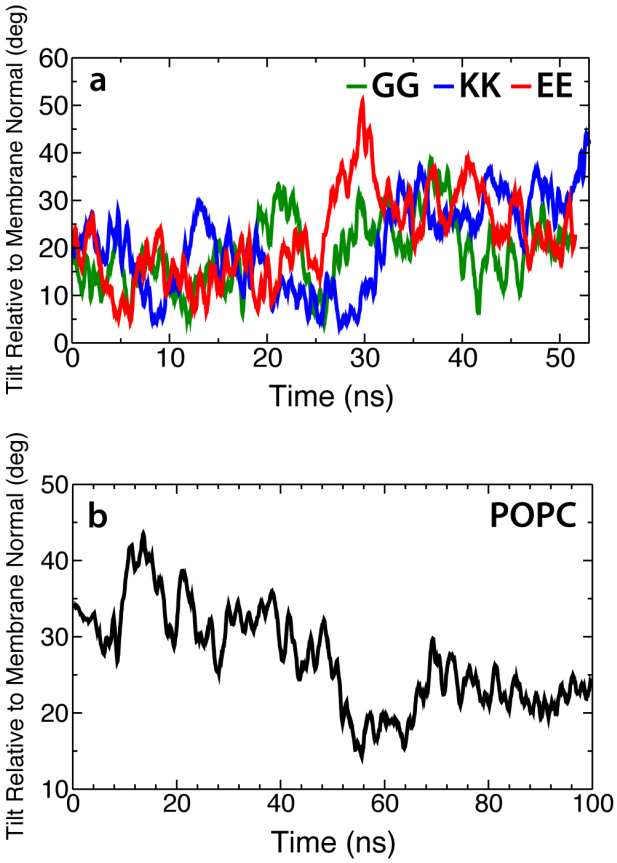


Figure S7: Running average of the tilt angle between TM2 and the membrane normal for the C-terminal addition (a) and full membrane (b) simulations, similar to Fig. 3.