Coarse-Grain Simulations Reveal Movement of the Synaptobrevin C-Terminus in Response to Piconewton Forces

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Supporting Material

Table S1

wt Force constant (kJ mol^{-1} nm^{-2}) / Pull Position (Å)	60	250	1000
0.0	IF-I	IF-I	IF-I
5.0	IF-I	IF-I	IF-I
10.0	IF-I	IF-I	IF-I
22.5	IF-I	IF-I	IF-I
30.0	IF-I	IF-I	IF-I
37.5	IF-I	IF-I	IF-I
45.0	IF-I	IF-I	IF-I
49.0	IF-I	IF-I -> IF-E	IF-E
52.5	IF-I	IF-E	IF-E
56.0	IF-I	IF-E	IF-E
60.0	IF-I -> IF-E	IF-E -> W	

WA Force constant (kJ mol^{-1} nm^{-2}) / Pull Position (Å)	60	250	1000
0.0	IF-I	IF-I	IF-I
5.0	IF-I	IF-I	IF-I
10.0	IF-I	IF-I	IF-I
22.5	IF-I	IF-I	IF-I
30.0	IF-I	IF-I	IF-I
37.5	IF-I	IF-I	IF-I
45.0	IF-I	IF-I	IF-I
49.0	IF-I	IF-I -> IF-O	IF-O
52.5	IF-I	IF-O	IF-O
56.0	IF-I	IF-O	IF-O
60.0	IF-I -> IF-O ->	IF-O -> W	

KK Force constant (kJ mol^{-1} nm^{-2}) / Pull Position (Å)	60	250	1000
0.0	IF-I	IF-I	IF-I
5.0	IF-I	IF-I	IF-I
10.0	IF-I	IF-I	IF-I
22.5	IF-I	IF-I	IF-I
30.0	IF-I	IF-I	IF-I
37.5	IF-I	IF-I	IF-I
45.0	IF-I	IF-I	IF-I
49.0	IF-I	IF-I	IF-I
52.5	IF-I	IF-I	IF-I -> IF-O
54.0			IF-O
56.0	IF-I	IF-I	IF-O -> W
58.0			W
60.0	IF-I	IF-I -> W	

Table S1. Positioning of the syb2 C terminus in syb2 **wt**, syb2 **WA** and syb2-**KK** umbrella sampling simulations with the indicated pull positions and harmonic force constants applied to the WW or AA group. IF-I indicates interactions of the C terminus with the intravesicular membrane-water interface as in Fig. 3A,B. IF-E indicates interactions of the C terminus with the extravesicular membrane-water interface as in Fig. 3C. W indicates a fully extracted state such that the whole fragment is in the water phase, including its C terminus. IF-I -> IF-E indicates that during the analyzed simulation time a transition occurred from the IF-I state to the IF-E state. IF-E -> IF-W indicates that during the analyzed simulation time a transition occurred from the IF-E state to the fully extracted (W) state. Simulations with transition were split in two parts, excluding the time during which the actual transition occurred. (**wt**) Syb2 wt simulations, all trajectories were used to construct the free energy profile of Fig. 4N, green line. (**WA**) Syb2 WA mutant simulations, the part marked in red was not included in the data set to construct the free energy profile of Fig. 3N, red line, because in this simulation the section of the trajectory in the IF-I state showed a non-zero slope and was very short, indicating that it was not in a proper local minimum. (**KK**) Syb2-KK simulations, the part marked in red was not included in the data set to construct the free energy profile of Fig. 3N, blue line, because the section of the trajectory in the IF-I state was too short producing a histogram with mostly one or zero counts. To produce sufficient overlap, two additional simulations marked in green were performed for this construct with harmonic forces centered at 54 Å and at 58 Å, both with force constants of 1000 kJ mol^{-1} nm^{-2}.

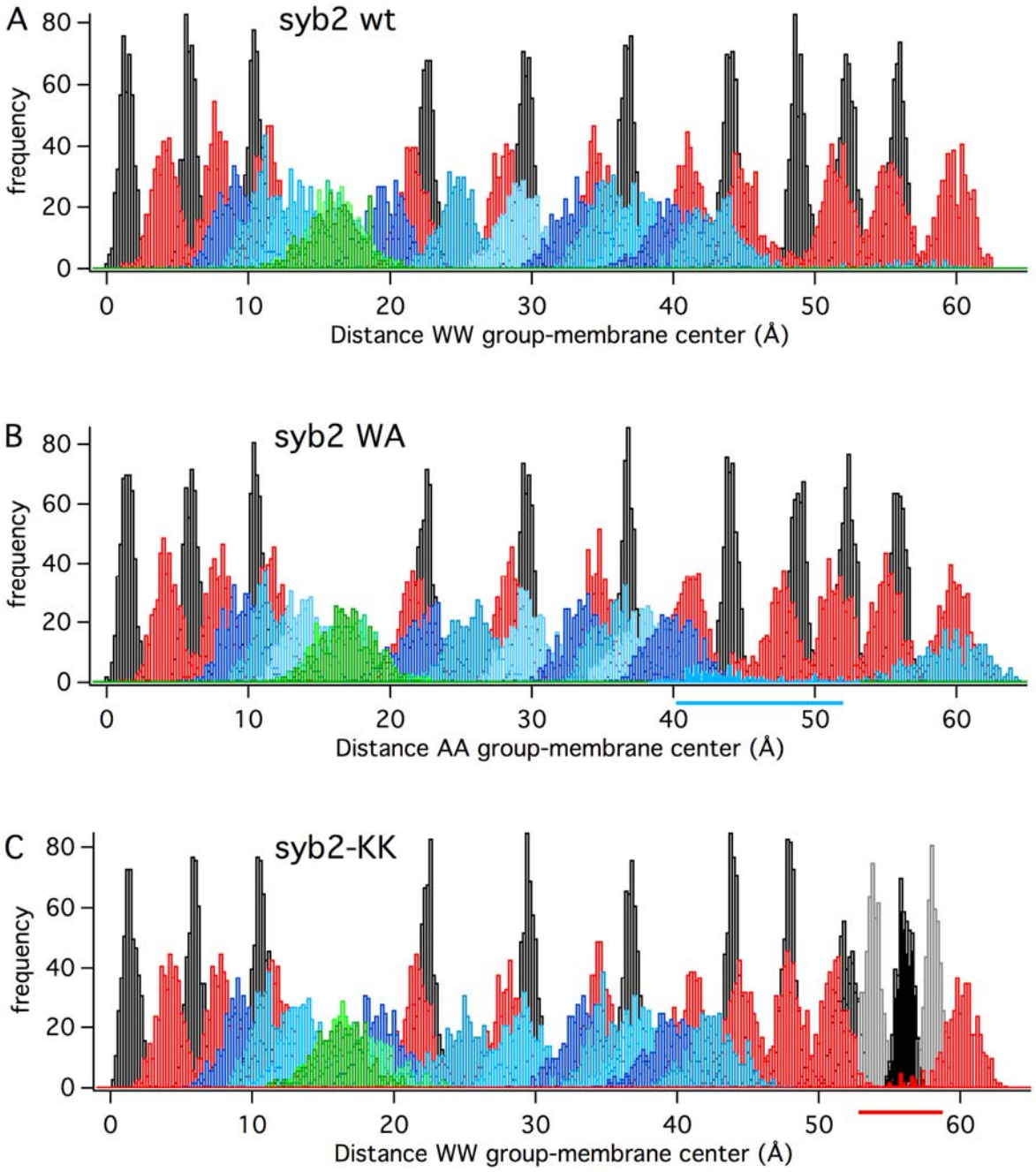

Figure S1. WW/AA position histograms from simulation trajectories for umbrella sampling of syb2 wt (A), syb2 WA (B), and syb2-KK (C). Colors indicate harmonic force constants: 1000 kJ mol^{-1} nm^{-2} (black), 250 kJ mol^{-1} nm^{-2} (red), and 60 kJ mol^{-1} nm^{-2} (blue). The green histograms are from simulations without restraining force (zero force constant). Note that for easier viewing the histograms in this figure are from whole trajectories (16-200 ns) and were not split into partial histograms as indicated in table S1 and as used for WHAM. The solid histogram bars above the colored horizontal lines were excluded due to inappropriate sampling. The gray histograms in (C) are from simulation with 1000 kJ mol^{-1} nm^{-2} force constant centered at 54 Å and 58 Å, which were added to produce sufficient overlap for WHAM (see Table S1, KK).

Force constant (kJ mol^{-1} nm^{-2}) / Pull Position (Å)	30	60	120	250	375	1000
-9.0		184				
0.0	984	984	184	184	984	184
3.0				972		
6.0		984*		984		
10.5		152				
15.0		984				
20.0		184				

Table S2. Force constants and pull positions applied to syb2 S115 T116 group for umbrella sampling simulations.
* In this simulation a metastable state near -4 Å was initially attained followed by a transition to +10 Å after ~300 ns.

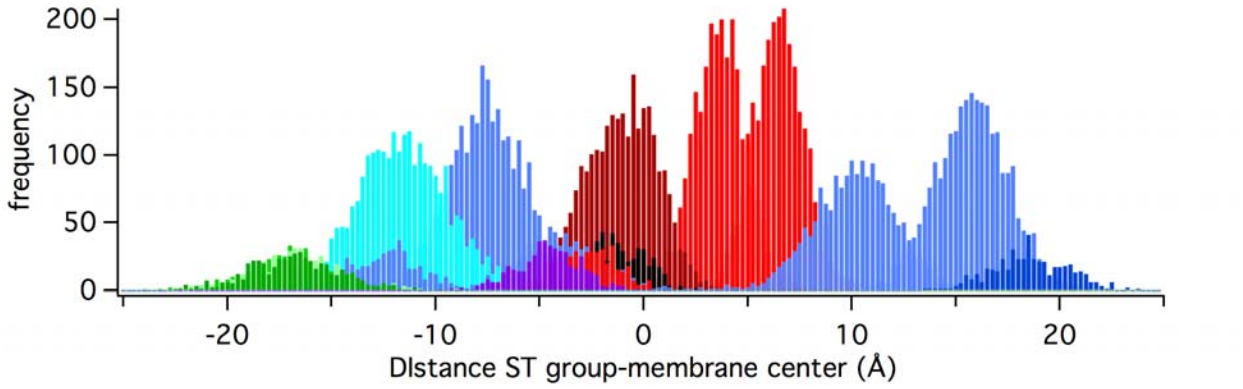

Figure S2. ST group position histograms from simulation trajectories for umbrella sampling of syb2 wt. Colors indicate harmonic force constants: 1000 kJ mol^{-1} nm^{-2} (black), 375 kJ mol^{-1} nm^{-2} (brown), 250 kJ mol^{-1} nm^{-2} (red), 150 kJ mol^{-1} nm^{-2} (purple), 60 kJ mol^{-1} nm^{-2} (blue), and 30 kJ mol^{-1} nm^{-2} (turquoise). The green histograms are from simulations without restraining force (zero force constant).