Supplementary Materials

Membrane Tension, Lipid Adaptation, Conformational Changes, and Energetics in MscL Gating

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Tabel S1. The average system and protein dimensions in *XY* for $\delta\tau = 1°$ and 30°.

$\delta\tau$	$L_X = L_Y$[#]	L_{top}[$]	L_{bot}[%]	$(L_X - L_{top}) / 2$	$(L_X - L_{bot}) / 2$
1°	96.3	55.1	51.2	20.6	22.6
30°	109.1	74.1	64.3	17.5	22.4

[#]The average system size in *XY*. [$]The maximum protein extent ($Z > 0$) in *XY*. [%]The maximum protein extent ($Z < 0$) in *XY*.

TABLE S2. The free energy difference (in kcal/mol) between $\delta\tau = 1°$ and $30°$ for TM1 and TM2 in all Tb-MscL subunits.

	TM1	TM2
A	7.6 ± 1.4	6.4 ± 2.5
B	-2.8 ± 1.5	7.3 ± 1.2
C	7.9 ± 1.9	12.4 ± 1.8
D	-4.6 ± 1.3	5.3 ± 1.9
E	5.7 ± 1.3	12.7 ± 1.4

TABLE S3. Decomposition of ΔG_{TM1} or ΔG_{TM2} into $\Delta G_{TM\text{-}TM}$, $\Delta G_{TM\text{-}MEMB}$, and $\Delta G_{TM\text{-}SOLV}$ (in kcal/mol) at $\delta\tau = 30°$.

	TM1				TM2			
	$\Delta G_{TM\text{-}TM}$	$\Delta G_{TM\text{-}MEMB}$	$\Delta G_{TM\text{-}SOLV}$	ΔG_{TM1}	$\Delta G_{TM\text{-}TM}$	$\Delta G_{TM\text{-}MEMB}$	$\Delta G_{TM\text{-}SOLV}$	ΔG_{TM2}
A	-1.8±2.8	0.0±0.2	9.4±1.6	7.6	7.8±2.0	-0.7±0.2	-0.7±0.6	6.4
B	4.3±1.9	-1.0±0.2	-6.1±0.8	-2.8	7.9±1.3	-0.3±0.1	-0.2±0.4	7.3
C	-0.1±1.3	-0.2±0.1	8.1±1.8	7.9	13.4±1.5	0.0±0.1	-1.0±0.3	12.4
D	-4.2±1.0	-0.5±0.1	0.1±1.3	-4.6	5.0±1.3	-0.3±0.2	0.6±0.9	5.3
E	-1.7±1.5	-0.4±0.3	7.7±2.0	5.7	13.7±1.7	-0.3±0.1	-0.7±0.4	12.7

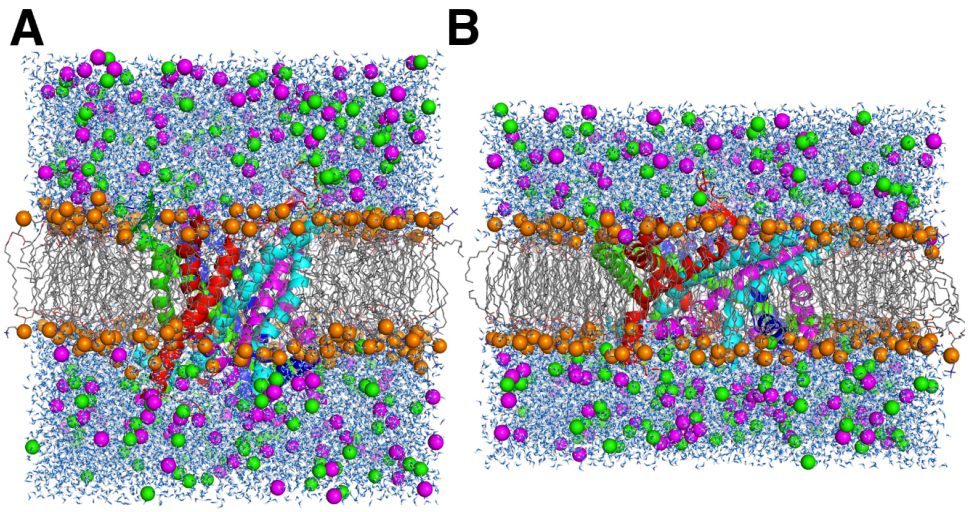

FIGURE S1. Molecular representation of (*A*) the final snapshot in system $\delta\tau_1$ and (*B*) the final snapshot in system $\delta\tau_{30}$. The Tb-MscL pentamer (*red*, *green*, *blue*, *magenta*, and *cyan*) is embedded in a DMPC bilayer (*grey*). The water molecules (*marine*) are in line representation. K^+ (*magenta*) and Cl^- (*green*) ions and phosphate atoms (*orange*) are represented by spheres.

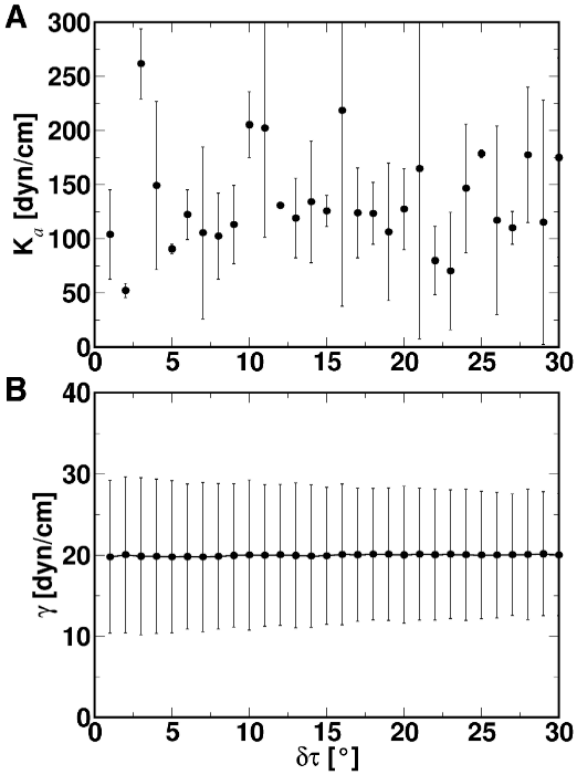

FIGURE S2. (*A*) The area compressibility (K_a) of all the systems. (*B*) Membrane tension (γ) calculated from pressure tensors: $\gamma = < L_z(P_{zz} - (P_{xx} + P_{yy})/2) > /2$, where L_z is the length of the simulation cell along the *Z*-axis and P_{xx}, P_{yy}, and P_{zz} are the *X*, *Y*, and *Z* components of the pressure tensor. The coefficient of 1/2 arises from the two identical interfaces in the system. γ is a constant surface tension applied on the membrane to reproduce the correct equilibrium area per-lipid in simulations and its value is held at 20 dyn/cm. It is also different from the excess tension γ_{ex}, which is derived based on area expansion and fluctuation and is not present in the system.

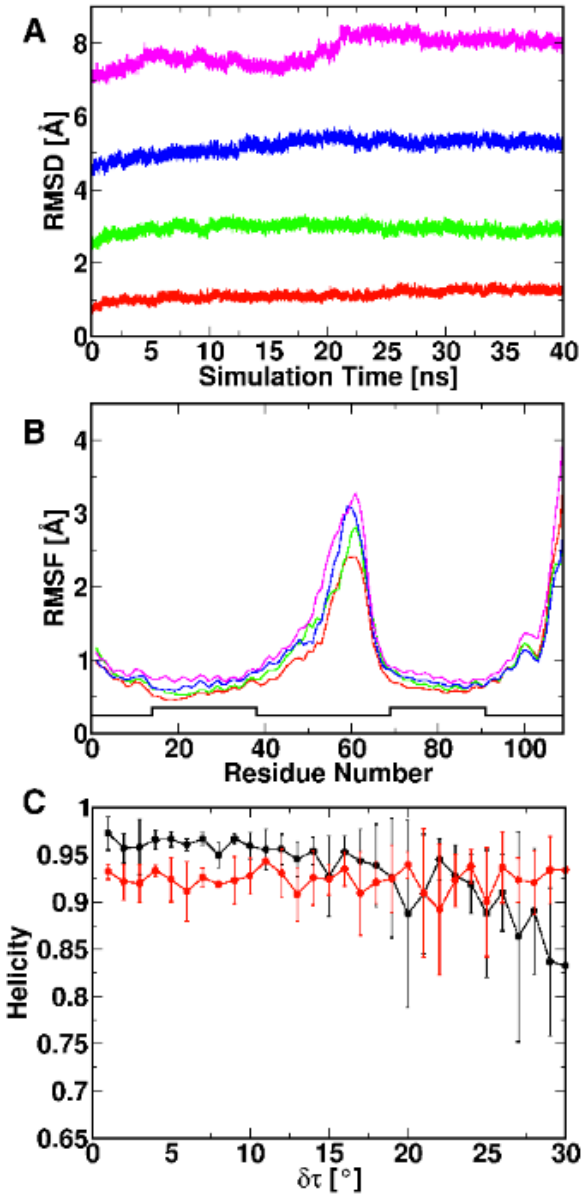

FIGURE S3. (*A*) RMSD of TM1 and TM2 backbone atoms as a function of simulations time in the $\delta\tau_1$ (*red*), $\delta\tau_{10}$ (*green*), $\delta\tau_{20}$ (*blue*), and $\delta\tau_{30}$ (*magenta*) systems. (*B*) Residual backbone RMSF in $\delta\tau_1$ (*red*), $\delta\tau_{10}$ (*green*), $\delta\tau_{20}$ (*blue*), and $\delta\tau_{30}$ (*magenta*). (*C*) The average helicity of TM1 (*black*) and TM2 (*red*) for all systems. The standard error calculated from the averages of the five subunits is also plotted. The helicity is characterized as the ratio between the number of instantaneous and total possible H-bonds in a given TM helix.

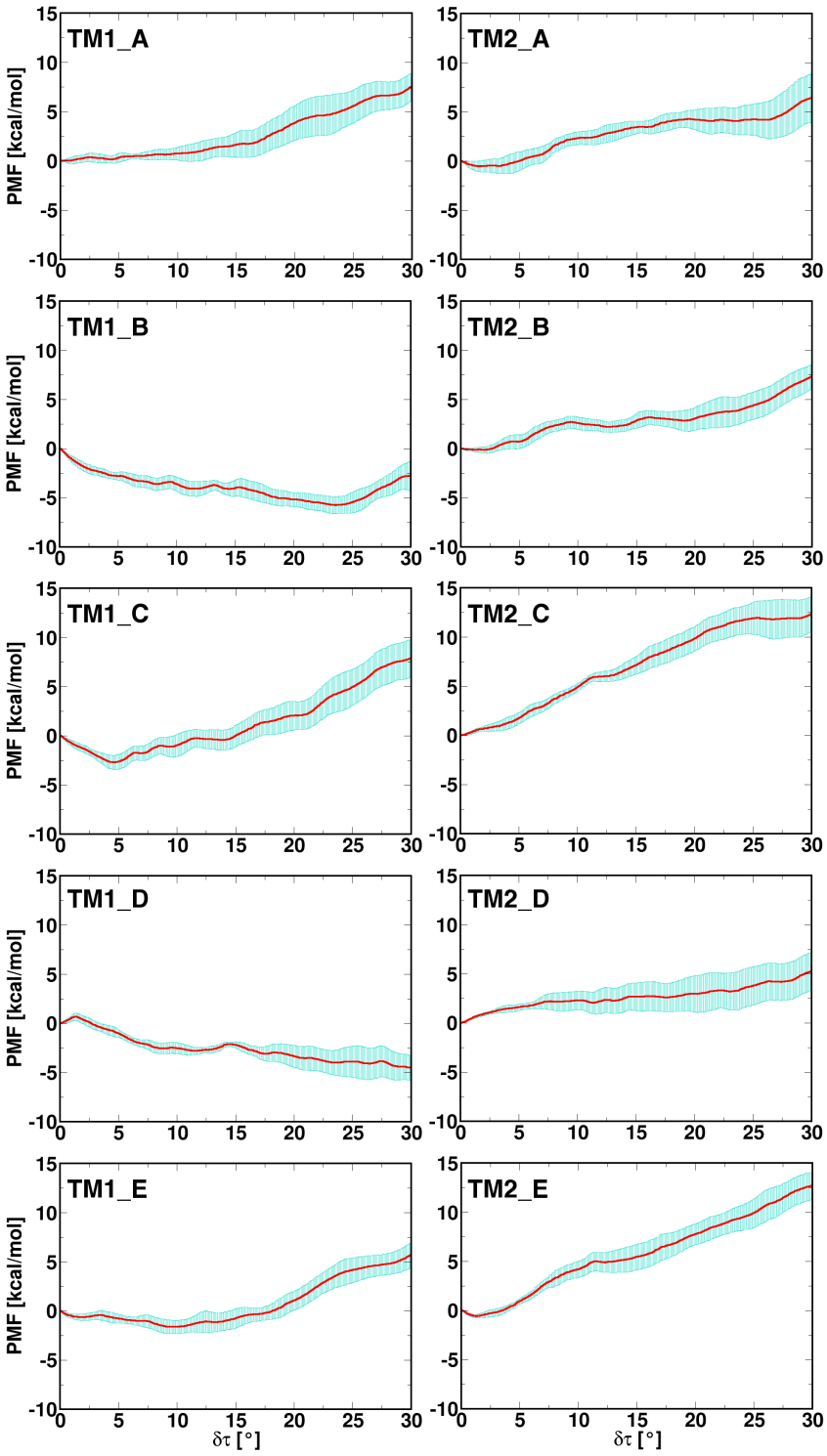

FIGURE S4. The PMFs of TM1 and TM2 in each subunit as a function of δτ. The standard deviation (cyan) is calculated by sequentially dividing the 30-ns trajectory in each δτ window into 6 pieces of 5-ns duration.

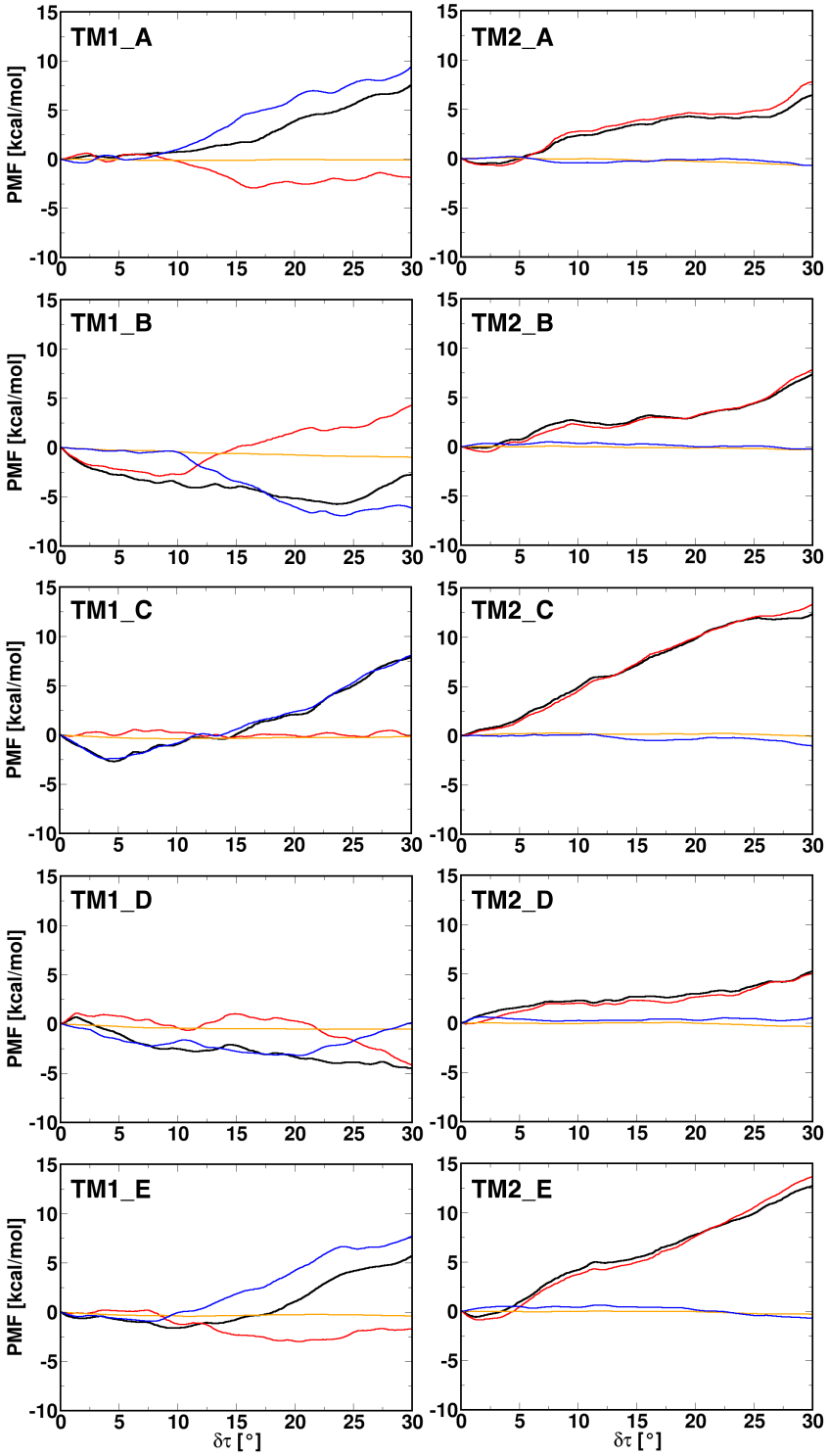

FIGURE S5. The PMF decomposition of TM1 and TM2 in each subunit as a function of δτ. The total PMF (*black*) of each TM helix is decomposed into three contributions: $\Delta G_{TM\text{-}TM}$ (*red*), $\Delta G_{TM\text{-}MEMB}$ (*orange*), and $\Delta G_{TM\text{-}SOLV}$ (*blue*).

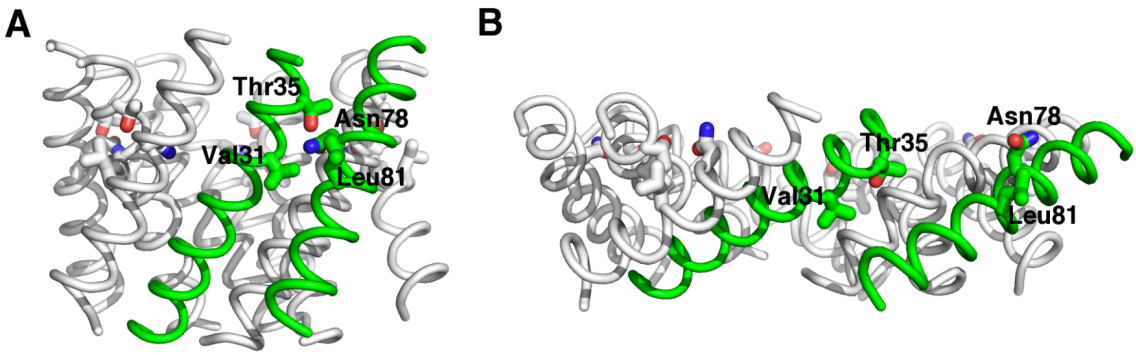

FIGURE S6. Molecular representation of subunit B (green) in (*A*) the X-ray structure of Tb-MscL and (*B*) system $\delta\tau_{30}$. Val31, Thr35, Leu81, and Asn78 are shown in stick representation.