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Supplemental information

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Article

Supplementary Material for Lpp positions peptidoglycan at the AcrA-TolC interface in the AcrAB-TolC multidrug efflux pump

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Table S1: Lipids used in the IM and OM.

Lipid	membrane	Lipid (full name)
PMPE	IM	1-palmitoyl-2-cis-9,10-methylene-hexadecanoic-acid-sn-glycero-3-phosphoethanolamine
POPE	IM	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine
QMPE	IM	1-pentadecanoyl-2-cis-9,10-methylene-hexadecanoic-acid-sn-glycero-3-phosphoethanolamine
PMPG	IM	1-palmitoyl-2-cis-9,10-methylene-hexadecanoic-acid-sn-glycero-3-phosphogycerol
PYPG	IM	1-palmitoyl-2-palmitoleoyl-sn-glycero-3-phosphoglycerol
YOPE	IM	1-oleoyl-2-palmitoleoyl-sn-glycero-3-phosphoethanolamine
PVCL2	IM/OM	1,1'-palmitoyl-2,2'-vacenoyl cardiolipin
PPPE	OM	1-palmitoyl(16:0)-2-palmitoleoyl(16:1 cis-9)-phosphoethanolamine
PVPG	OM	1-palmitoyl(16:0)-2-vacenoyl(18:1 cis-11)-phosphoglycerol

Table S2: Simulations run.

System	# atoms	replicas/windows	time/(replica/window) (ns)	total time (ns)
Full pump	1,450,799	4	300	1200
Full (REUS)	1,450,799	55	25	1375
Lpp x 2 (REUS)	147,523	36	30	1080

Table S3: Rates of diffusion of lipids in the inner leaflet of the OM. Lpp is for the three acyl chains attached to Lpp's N-terminus. All rates are given in units of 10^{-8} cm^2/s.

Lipid	replica 1	replica 2	replica 3	replica 4
PPPE	7.5	6.8	7.0	7.2
PVPG	7.5	7.4	6.8	7.6
PVCL2	5.3	4.2	4.2	4.8
Lpp	5.4	4.5	4.8	5.3

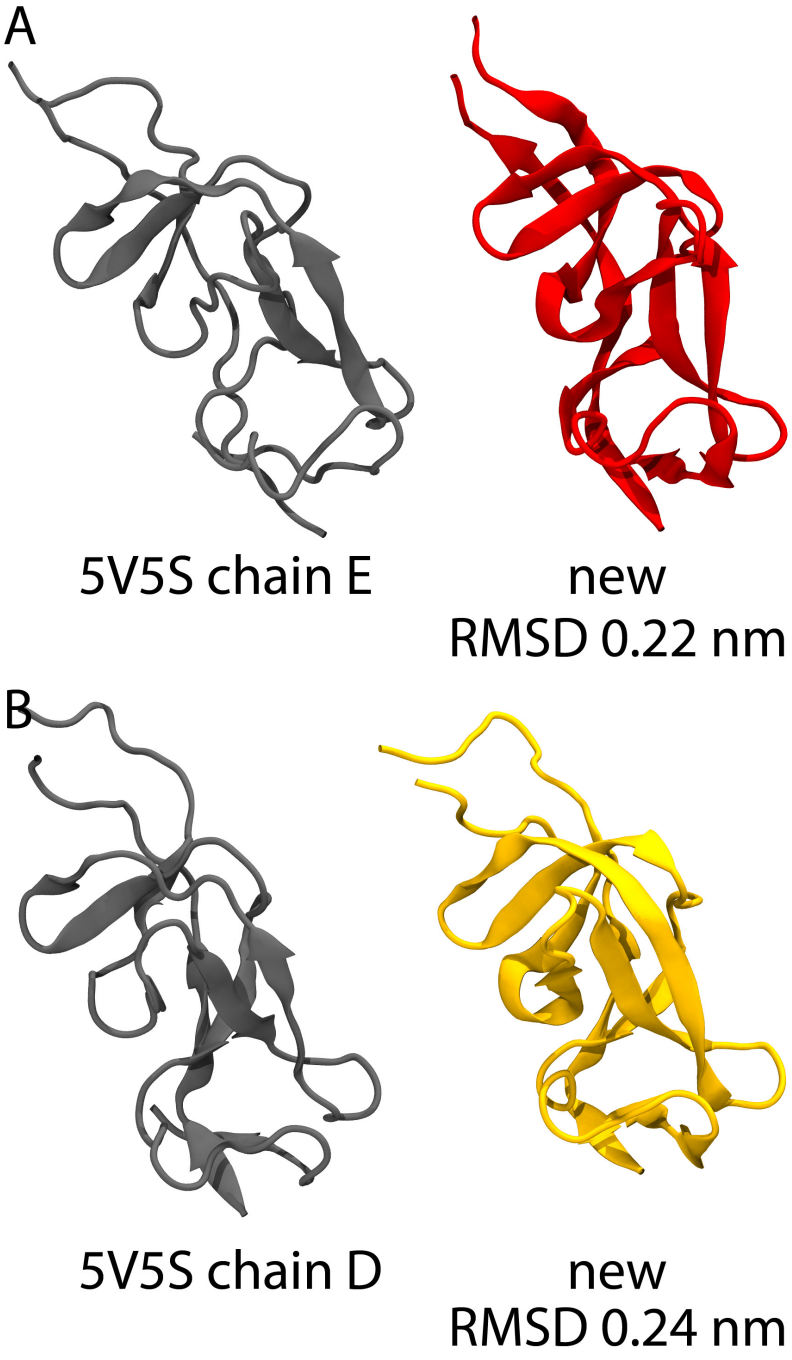

Figure S1: Comparison between AcrA MP domains from PDB 5V5S and the newly modeled ones. (A) AcrA in site 1. (B) AcrA in site 2. The root-mean-square deviation (RMSD) between the model and the original is shown for each.

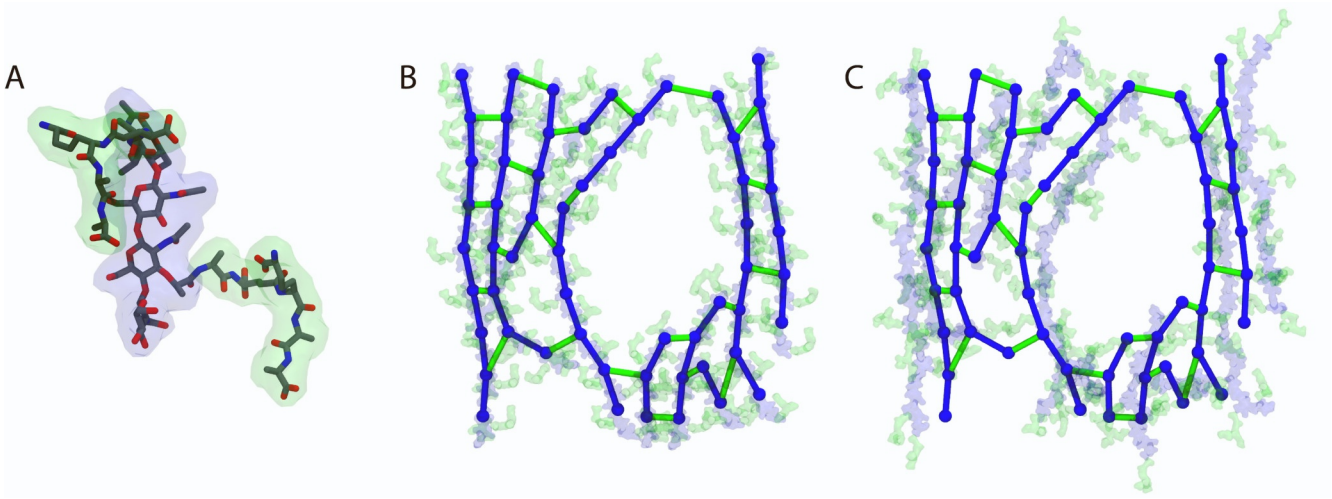

Figure S2: Reverse-coarse-graining of PG. (A) Unit of PG used in in the coarse-grained (CG) models in Nguyen et al. (**?**), which represents two disaccharides (transparent blue outline) and two peptide stems (transparent green outline). (B) Initial organization of the atomistic PG (transparent) that was reverse-coarse-grained from the CG model extracted from whole-sacculus simulations. (C) Result of equilibration of the atomistic model compared to the same CG starting model.

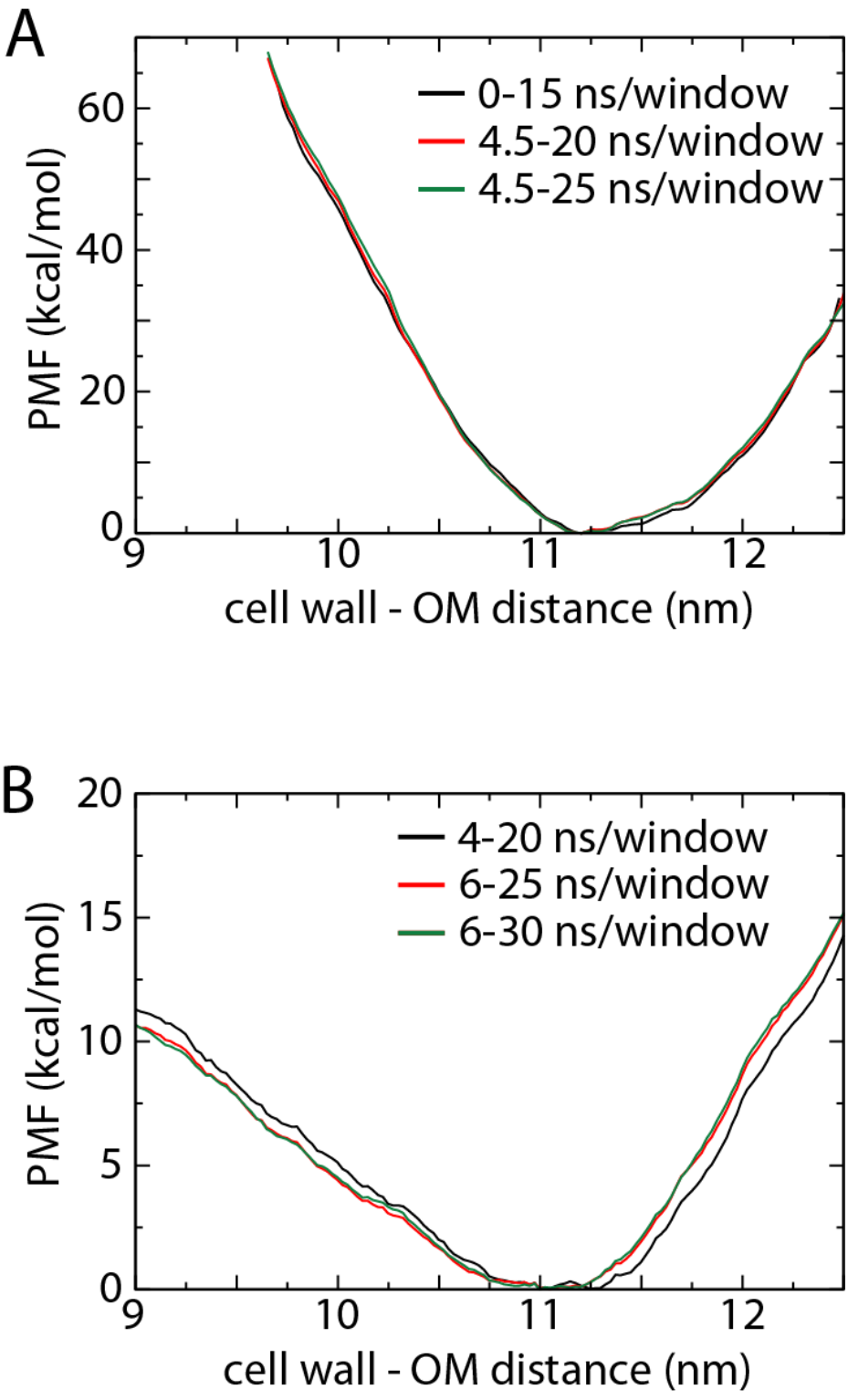

Figure S3: Comparison of the PMFs calculated from different sampling lengths. (A) PMFs for PG-OM distance in the efflux-pump-containing system after 15, 20, and 25 ns/window. Note that for the latter two, the first 4.5 ns is discarded as equilibration. (B) PMFs for the PG-OM distance in the two-Lpp-trimer-only system after 20, 25, and 30 ns/window. For the first one, the first 4 ns is discarded, while for the latter two, the first 6 ns is discarded.

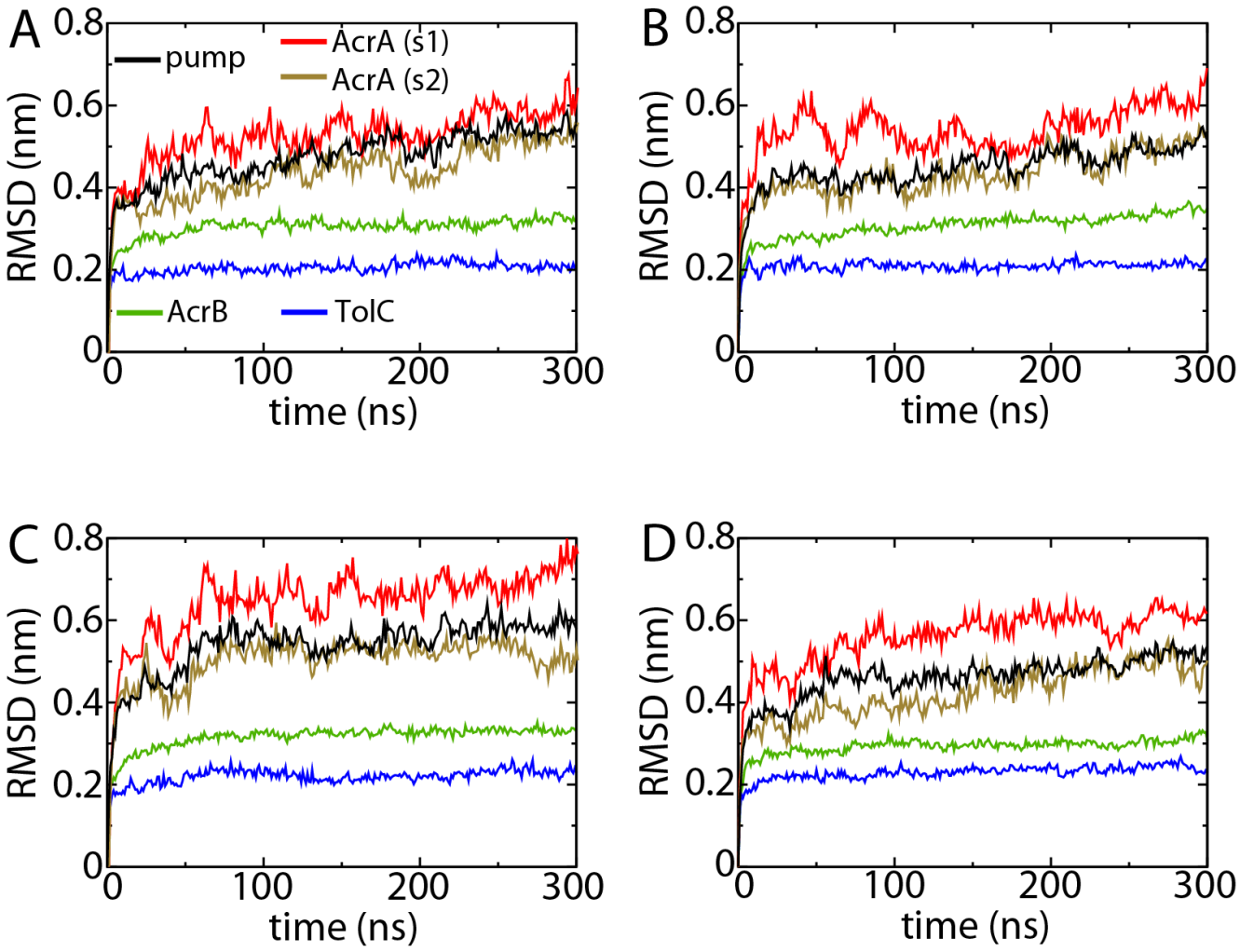

Figure S4: Root mean-square deviation (RMSD) of the pump and its components over four 300-ns replica simulations (A-D). AcrA (s1) and (s2) reference the three copies bound in site 1 and in site 2, respectively.

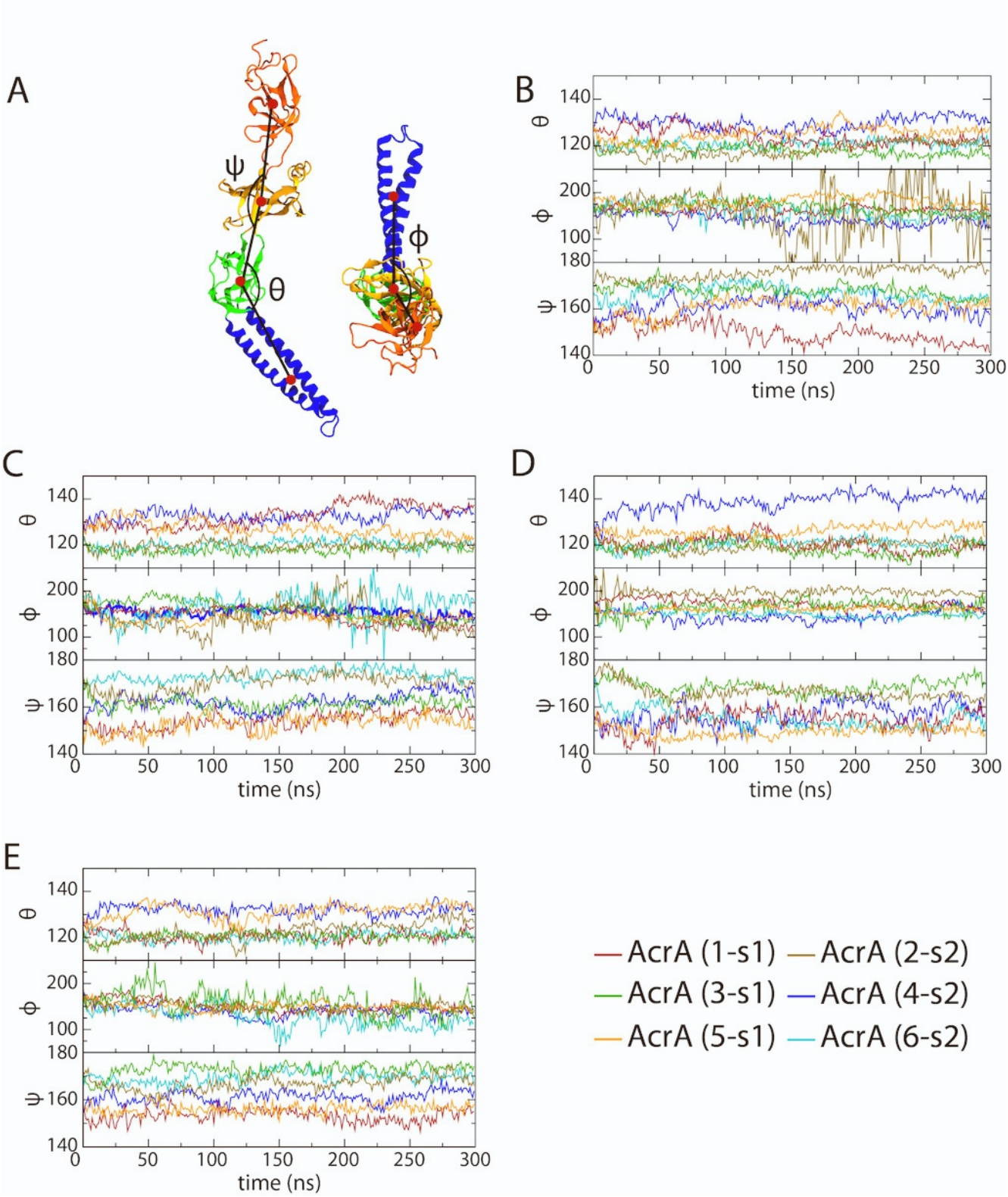

Figure S5: AcrA angles over time. (A) Two angles (θ and ψ) and one dihedral angle (ϕ) between AcrA's membrane proximal (orange), β-barrel (yellow), lipoyl (green), and α-helical (blue) domains used to define AcrA's conformation. (B-E) Three angles over time in the four replica simulations. AcrA copies labeled 1, 3, and 5 are bound to AcrB in site 1, while those labeled 2, 4, and 6 are bound in site 2.

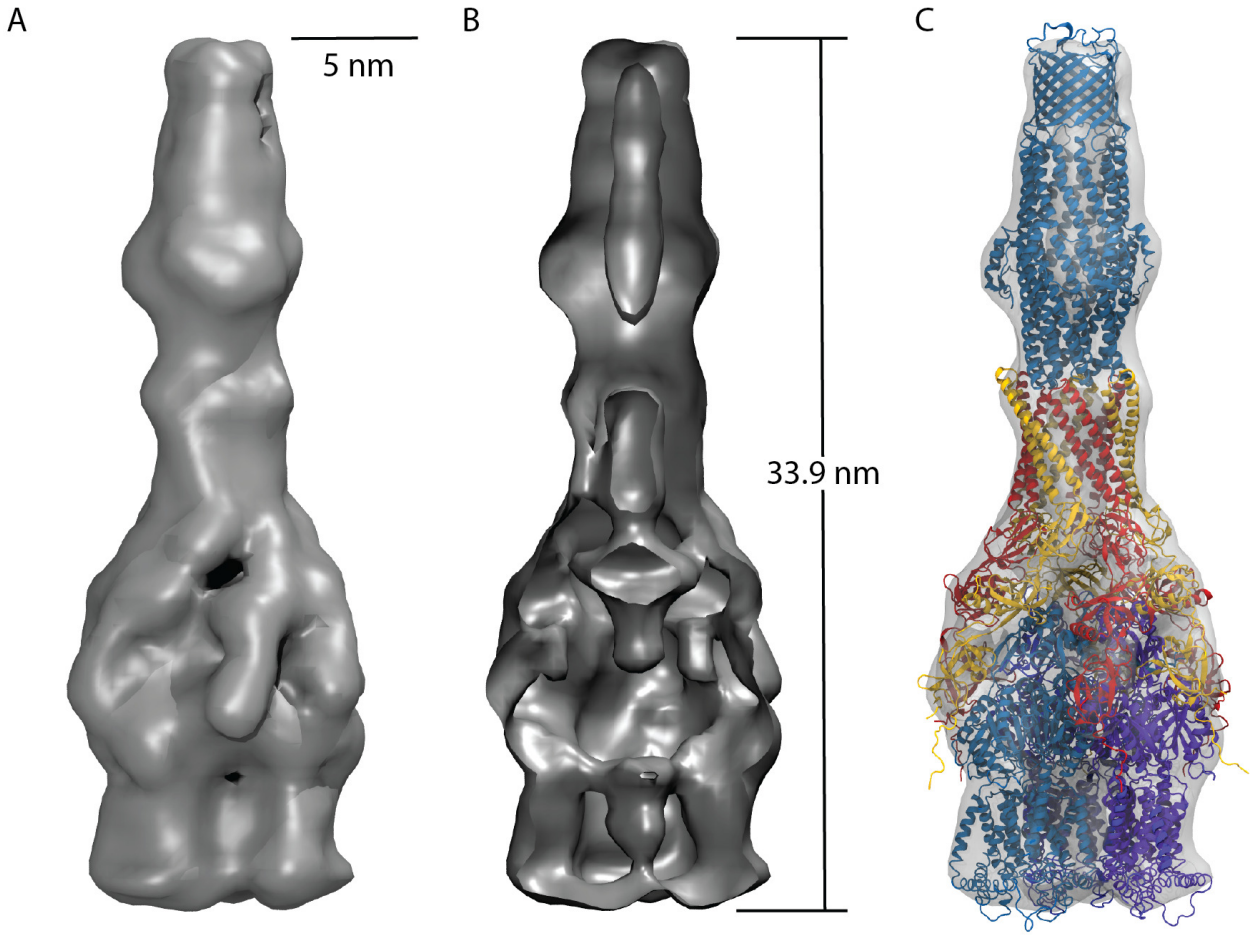

Figure S6: Simulated map. A 15-Å-resolution density map of the efflux pump was created from the the last 200-ns of each of the four equilibration runs. (A) Map. (B) Cut through the center of the map showing the pores and the constriction at the AcrA-TolC junction, as expected. (C) Map outline with atomistic structure, colored as in Fig. 1.

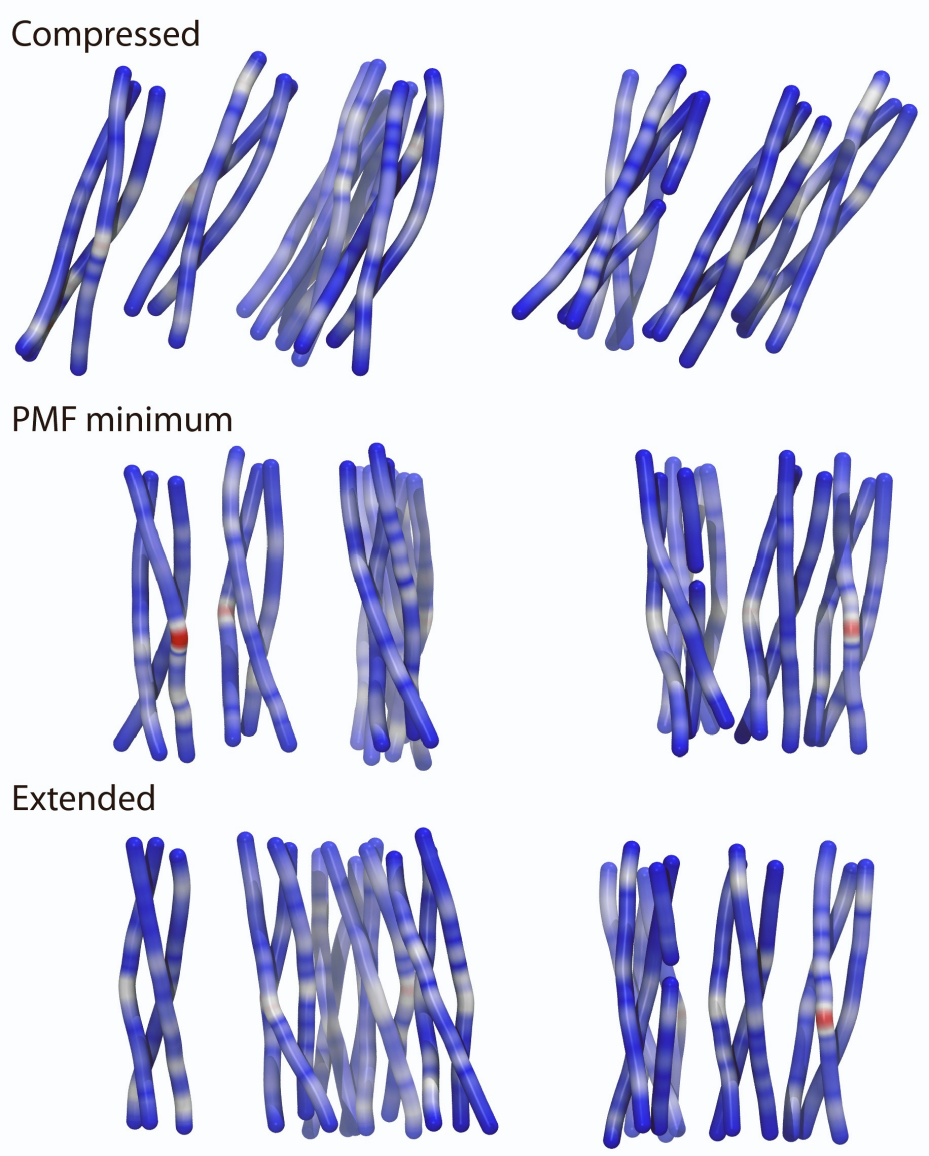

Figure S7: Bend angles of Lpp helices at different OM-PG distances from the end of REUS simulations. The most compressed state represents a distance of 9.7 nm, the PMF minimum is 11.2 nm, and the most extended one is 12.5 nm. The color scale ranges from blue (no kink) to white (15°) to red (30°). The top is the N-terminus (near the OM) and the bottom is the C-terminus (connected to PG).

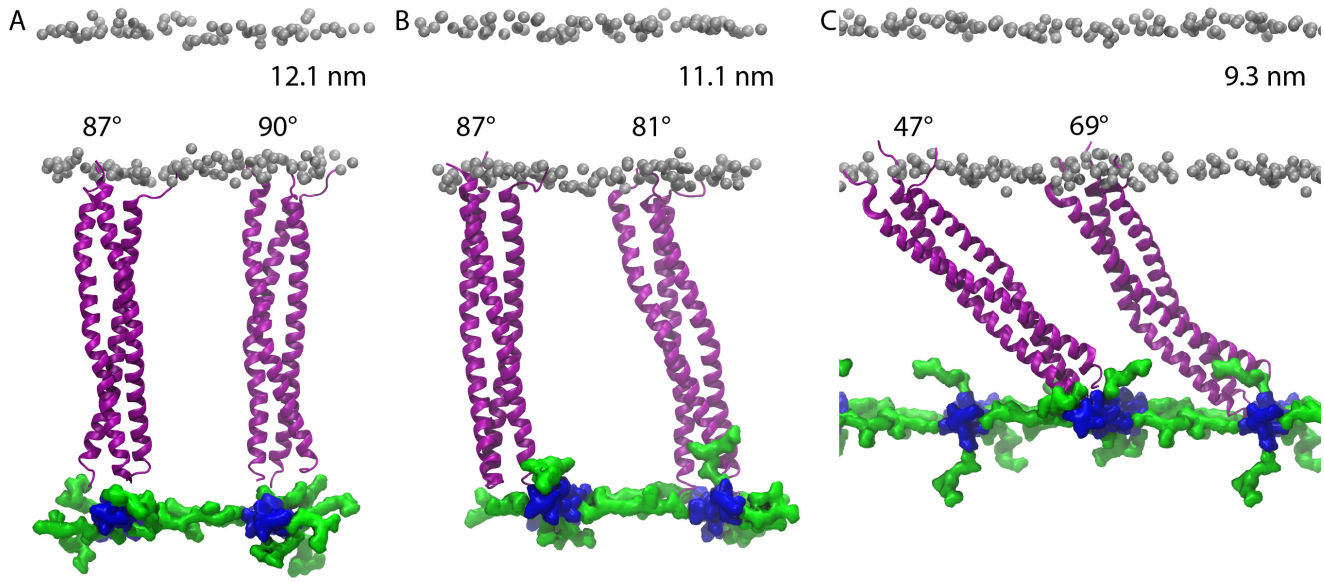

Figure S8: Example states from steered MD simulations of the OM-PG system with two Lpp trimers (purple). Glycan strands of the PG are in blue and peptide chains are in green. Phosphorus atoms of the OM are shown as grey spheres (note that core sugars, which extend above, are not shown). (A) PG-OM distance of 12.1 nm, which is at 10 kcal/mol in the PMF. The Lpp trimers are at 87° and 90°, respectively. (A) PG-OM distance of 11.1 nm, which is near the minimum of the PMF. The Lpp trimers are at 87° and 81°, respectively. (C) PG-OM distance of 9.3 nm, which is at 9 kcal/mol in the PMF. The Lpp trimers are at 47° and 69°, respectively.

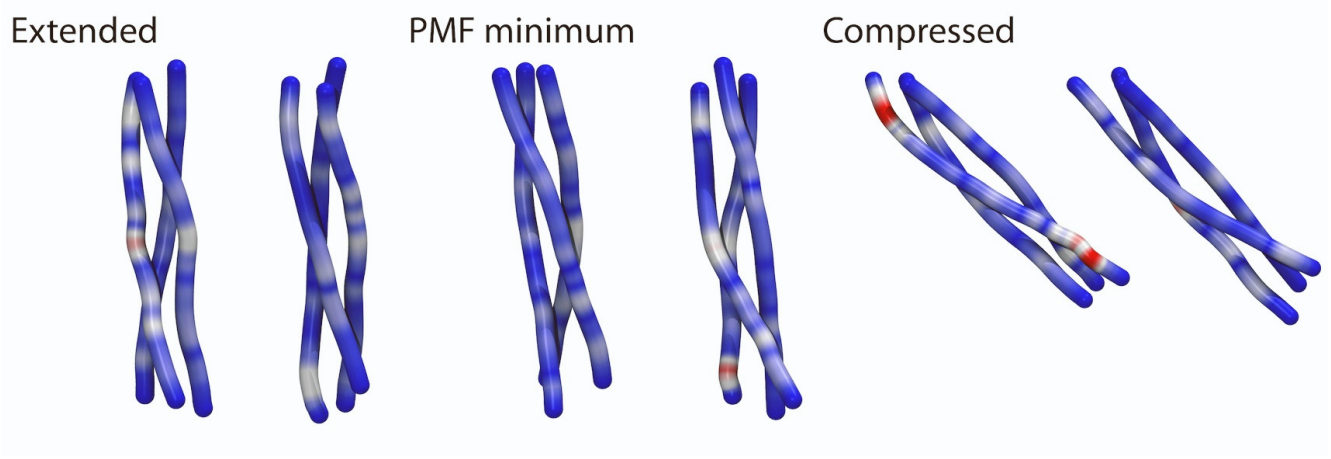

Figure S9: Bend angles of Lpp helices at different OM-PG distances from the end of the Lpp-only REUS simulations. The states are taken from Fig. S8. The color scale ranges from blue (no kink) to white (15°) to red (30°).

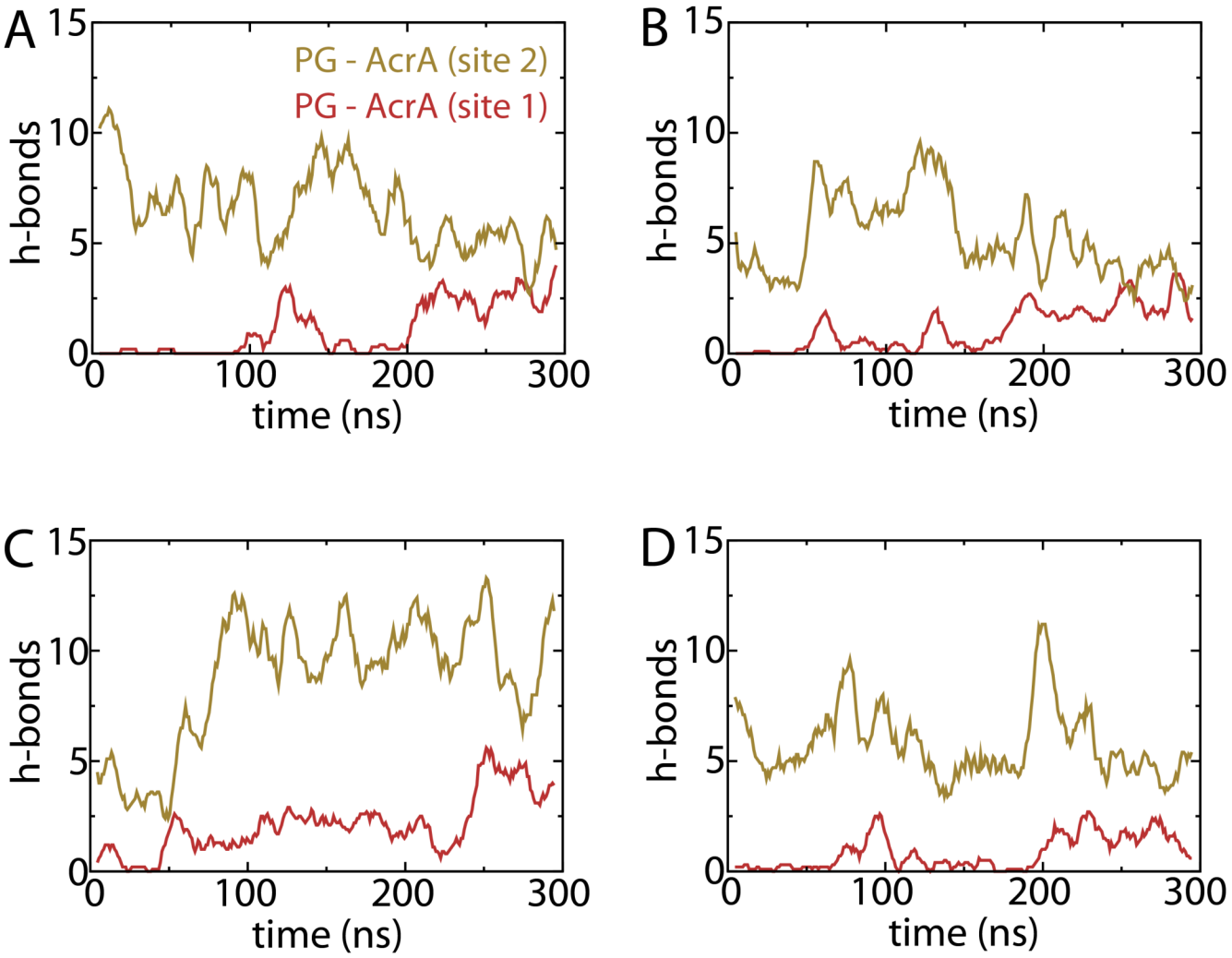

Figure S10: Hydrogen bonds between AcrA and PG over time. The colors represent three copies of AcrA in site 1 (red) and three in site 2 (gold) as in (A) and in Fig. 1 in the main text. The four panels A-D represent the four independent replica simulations.

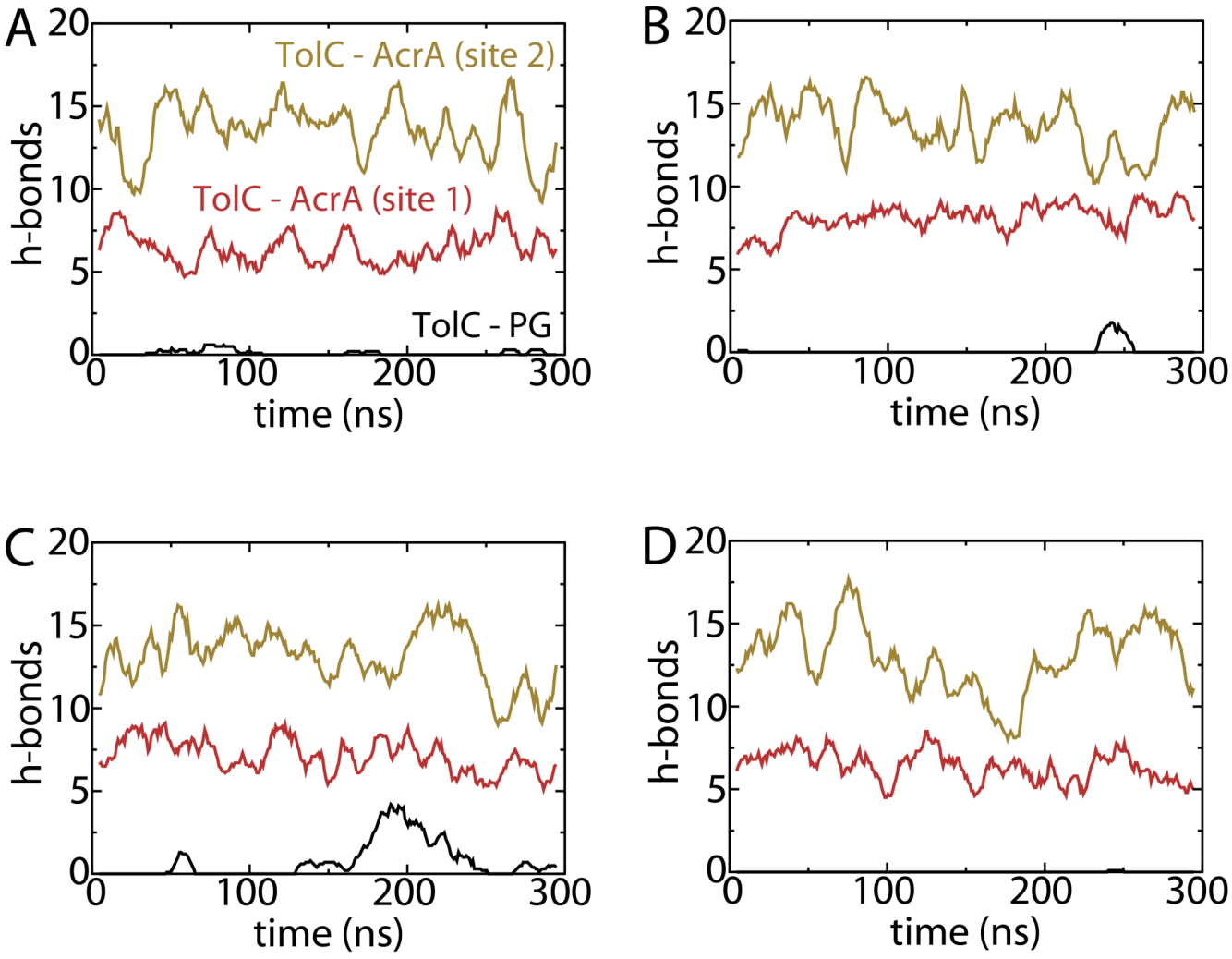

Figure S11: Hydrogen bonds between AcrA and TolC as well as PG and TolC over time. The four panels A-D represent the four independent replica simulations.