Supporting Information

CHARMM-GUI Supports Hydrogen Mass Repartitioning and Different Protonation States of Phosphates in LPS

Ya Gao[1†], Jumin Lee[2†], Iain Peter Shand Smith[3†], Hwayoung Lee[2†], Seonghoon Kim[2,4], Yifei Qi[5], Jeffery B. Klauda[6], Göran Widmalm[7], Syma Khalid[3], and Wonpil Im[2*]

[†]These authors contributed equally to this work.

[1]School of Mathematics, Physics and Statistics, Shanghai University of Engineering Science, Shanghai 201620, China

[2]Department of Biological Sciences, Department of Chemistry, Department of Bioengineering, and Department of Computer Science and Engineering, Lehigh University, Bethlehem, PA 18015, USA

[3]School of Chemistry, University of Southampton, Southampton, S017 1BJ, UK

[4]School of Computational Sciences, Korea Institute for Advanced Study, Seoul 02455, Republic of Korea

[5]Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, Shanghai, 200062, China

[6]Department of Chemical and Biomolecular Engineering and the Biophysics Program, University of Maryland College Park, Maryland 20742, USA

[7]Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-10691 Stockholm, Sweden

*Correspondence: wonpil@lehigh.edu

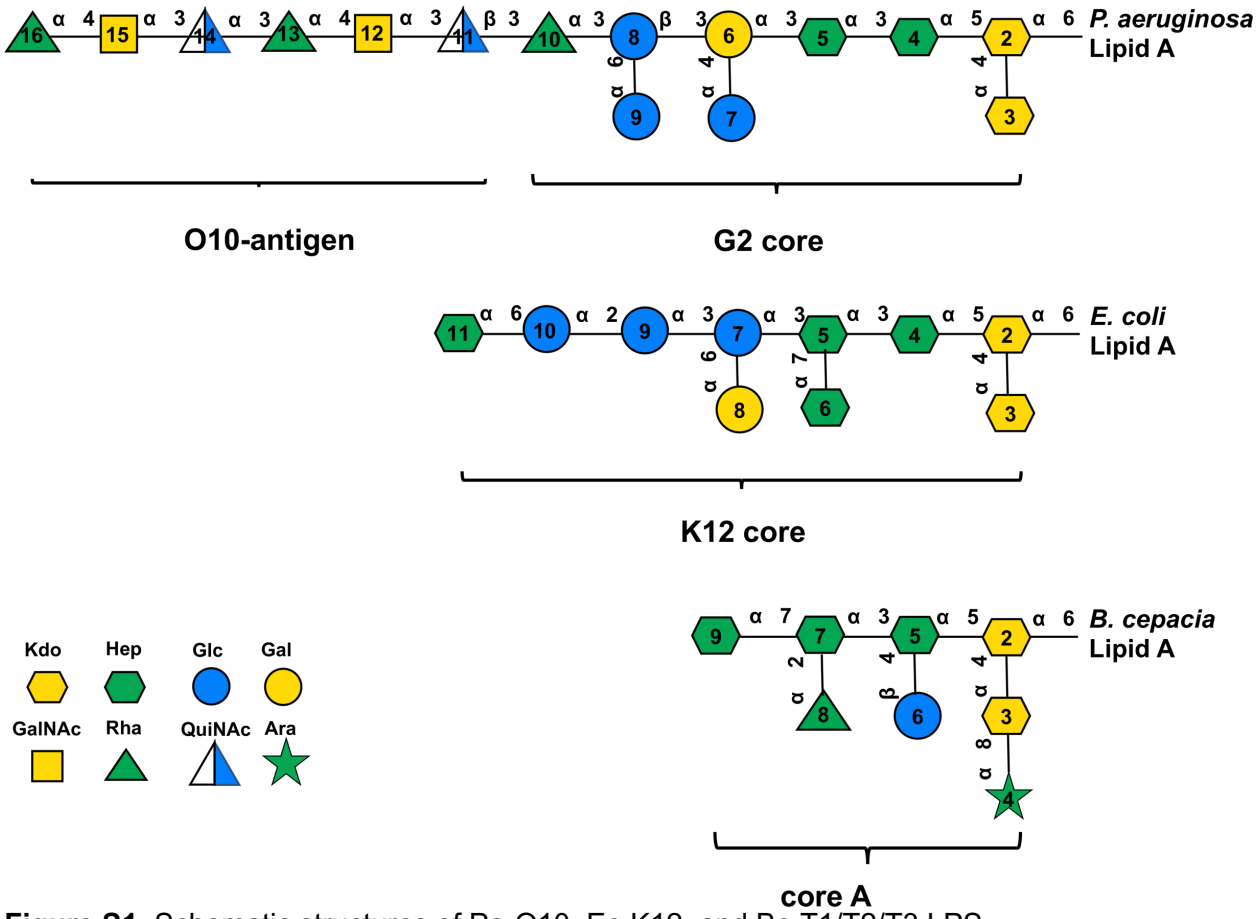


Figure S1. Schematic structures of Pa-O10, Ec-K12, and Bc-T1/T2/T3 LPS.

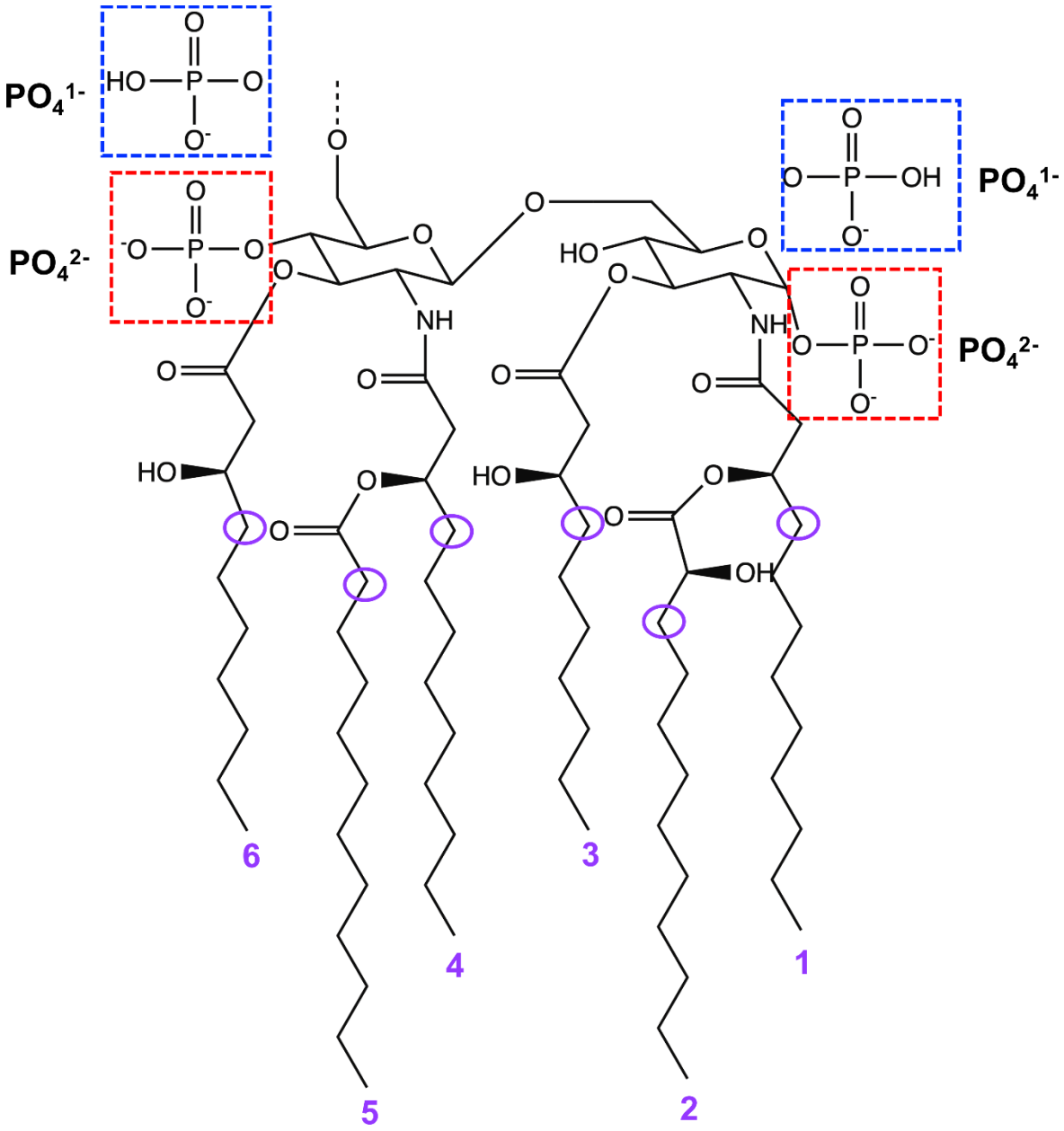
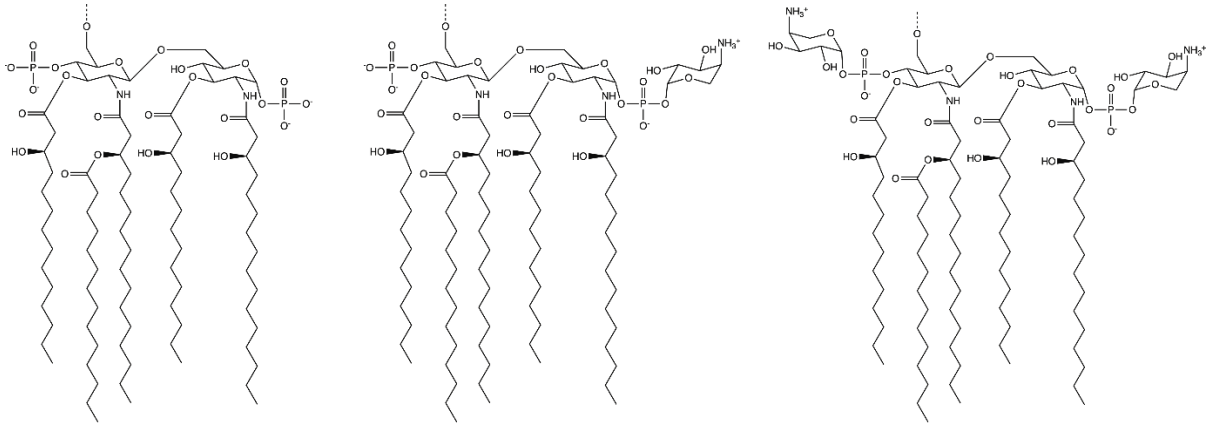


Figure S2. Chemical structures of *P. aeruginosa* lipid A. Carbons in the purple ovals are used to calculate the hydrophobic thickness.



Type 1 **Type 2** **Type 3**

Figure S3. Chemical structures of three types of *B. cepacia* lipid A.

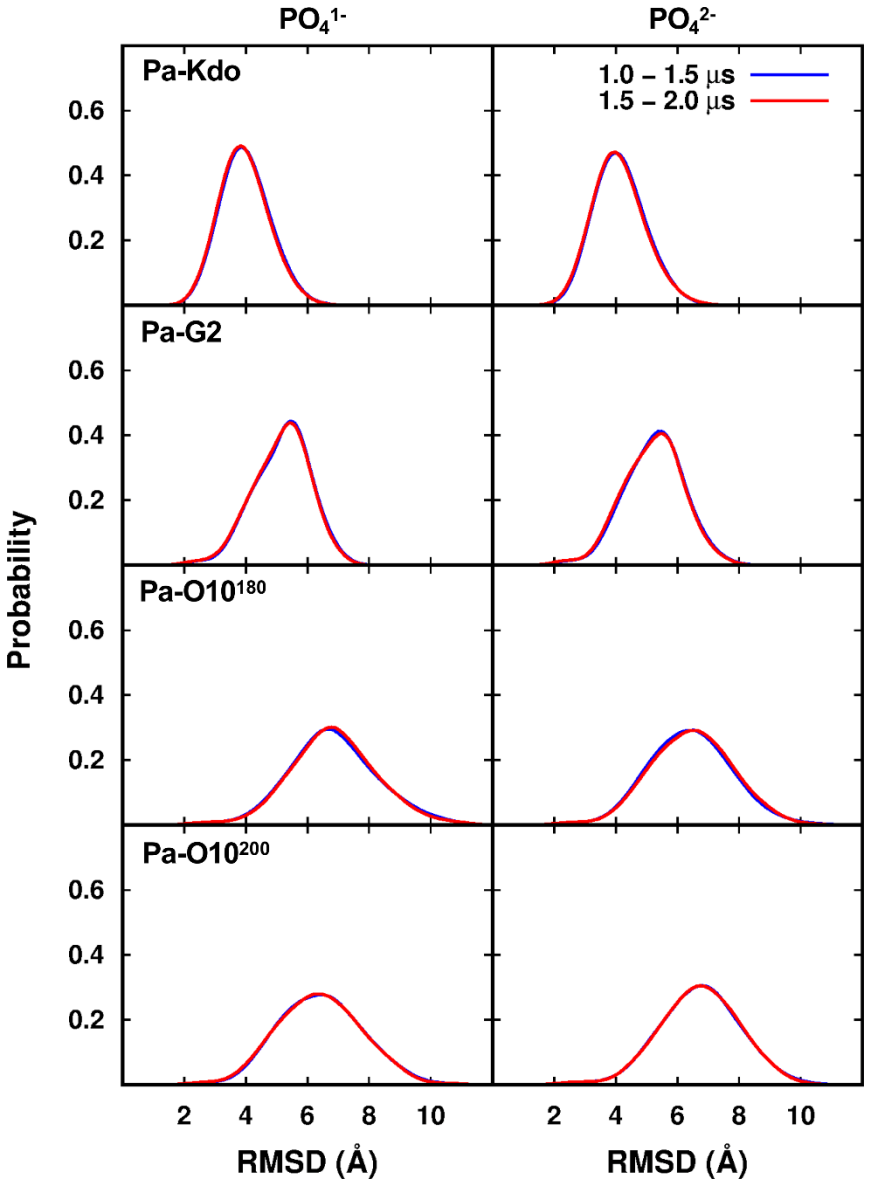


Figure S4. Comparisons of pairwise RMSD distributions (of the entire LPS) with two phosphate protonation states between 1.0 – 1.5 μs and 1.5 – 2.0 μs standard simulations for each Pa system.

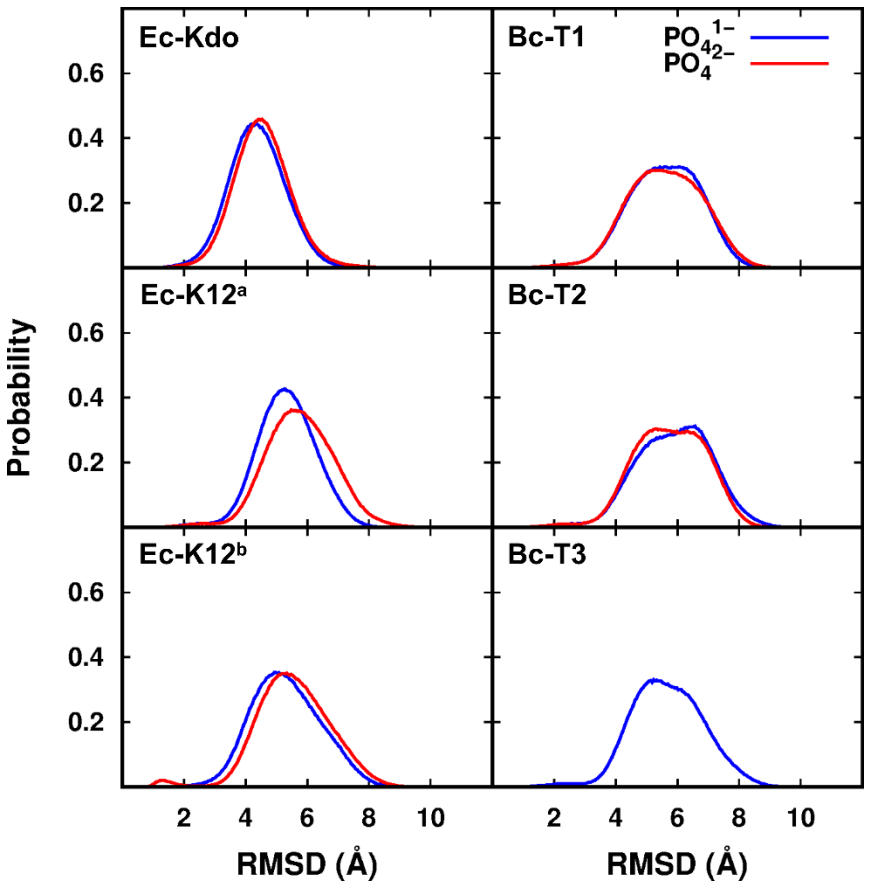


Figure S5. Comparisons of pairwise RMSD distributions (of the entire LPS) between two phosphate protonation states for each Ec and Bc systems. Ec-K12^{a} and Ec-K12^{b} are for Hep with -2*e* or -1*e* phosphate group, respectively.

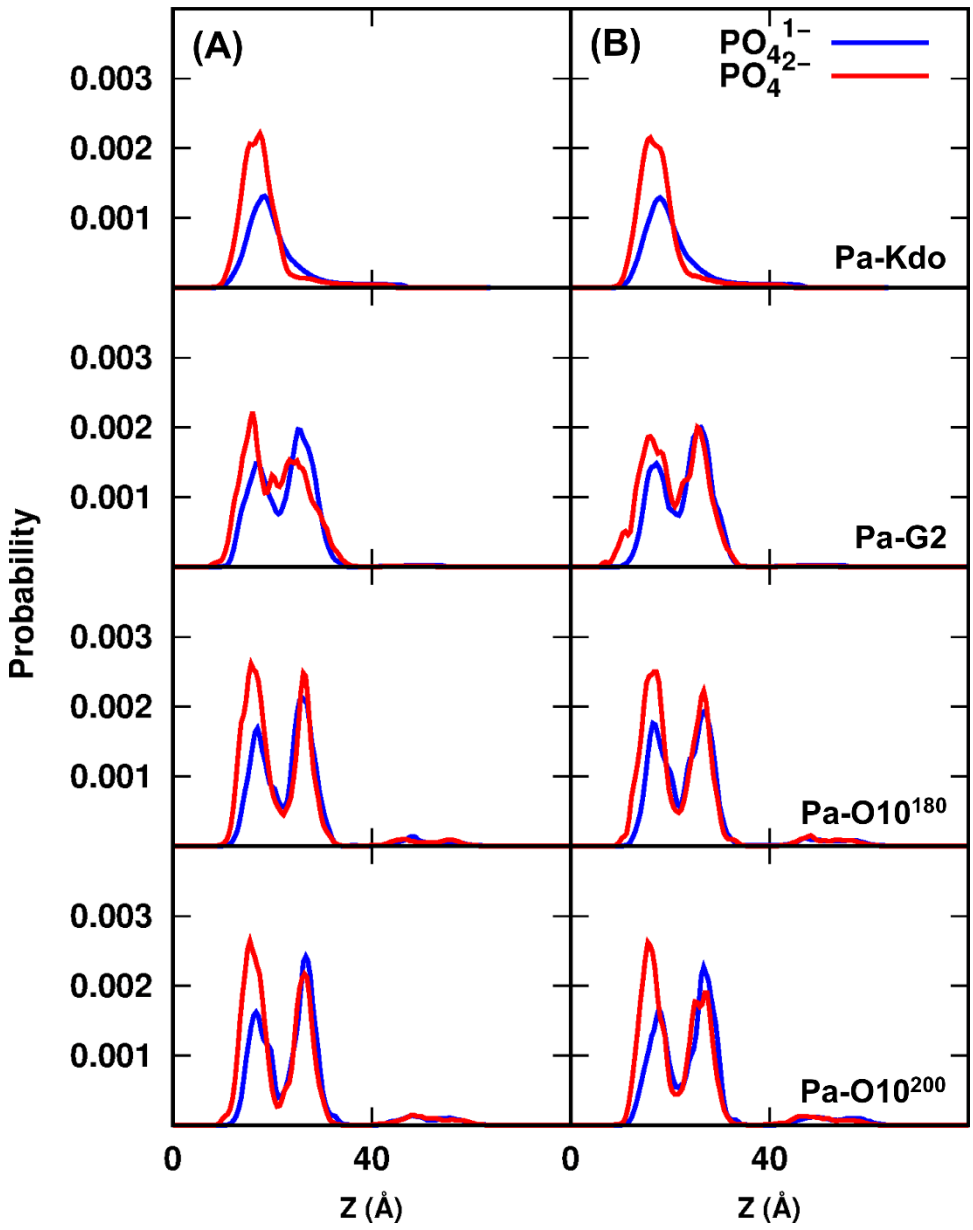


Figure S6. Comparisons of distributions of Ca^{2+} ions along the Z-axis (i.e., the membrane normal) between two phosphate protonation states for each Pa systems in (A) standard and (B) HMR simulations. In the distributions, only the Z > 0 membrane portion up to Z = 80 Å is shown after symmetrization.

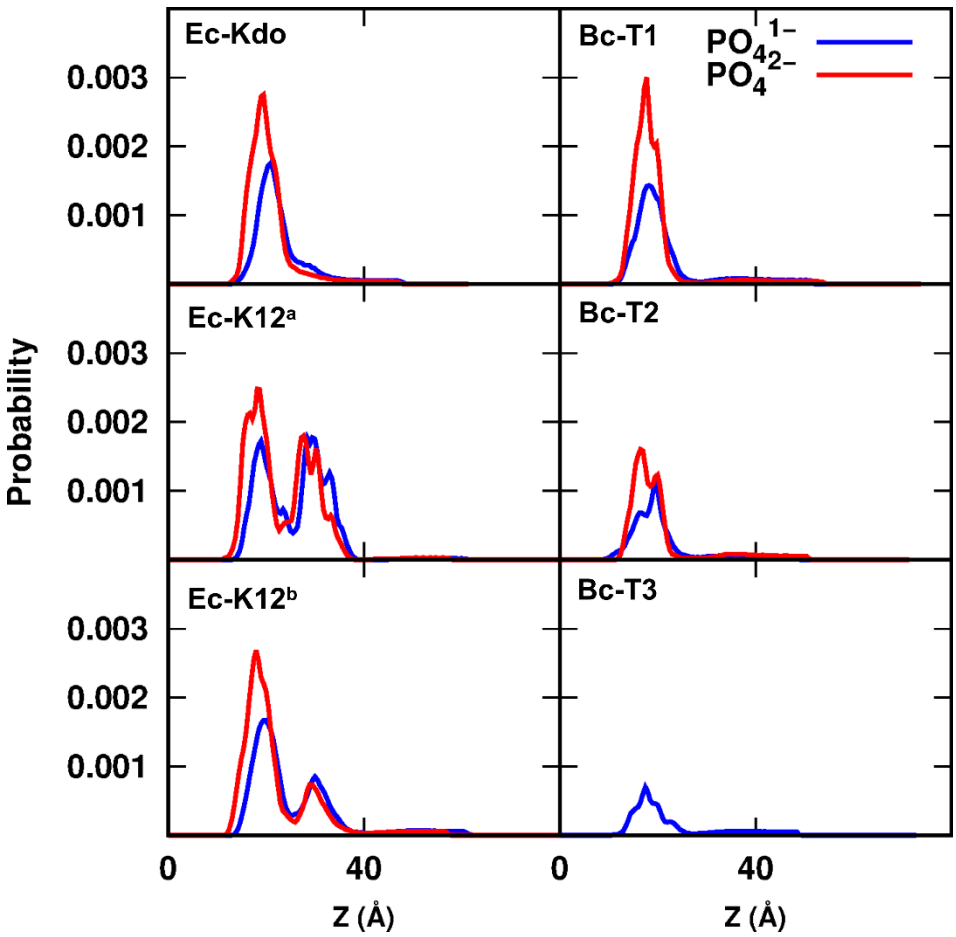


Figure S7. Comparisons of distributions of Ca^{2+} ions along the Z-axis (i.e., the membrane normal) between two phosphate protonation states for each Ec and Bc systems. Ec-K12[a] and Ec-K12[b] are for Hep with -2*e* or -1*e* phosphate group, respectively. In the distributions, only the Z > 0 membrane portion up to Z = 80 Å is shown after symmetrization.

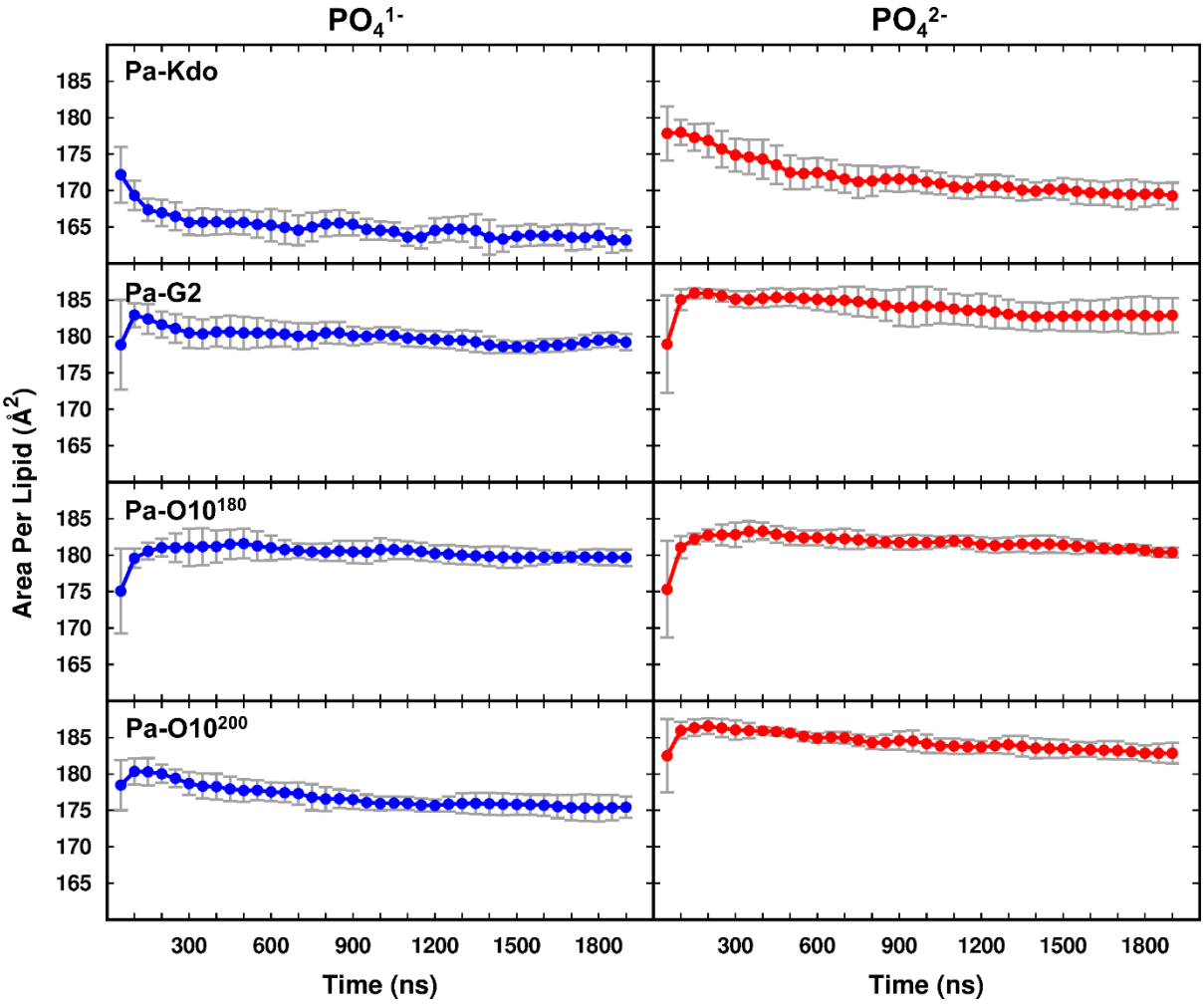


Figure S8. Time series of averaged APL every 100 ns for each Pa system in both protonation states and its corresponding standard errors.

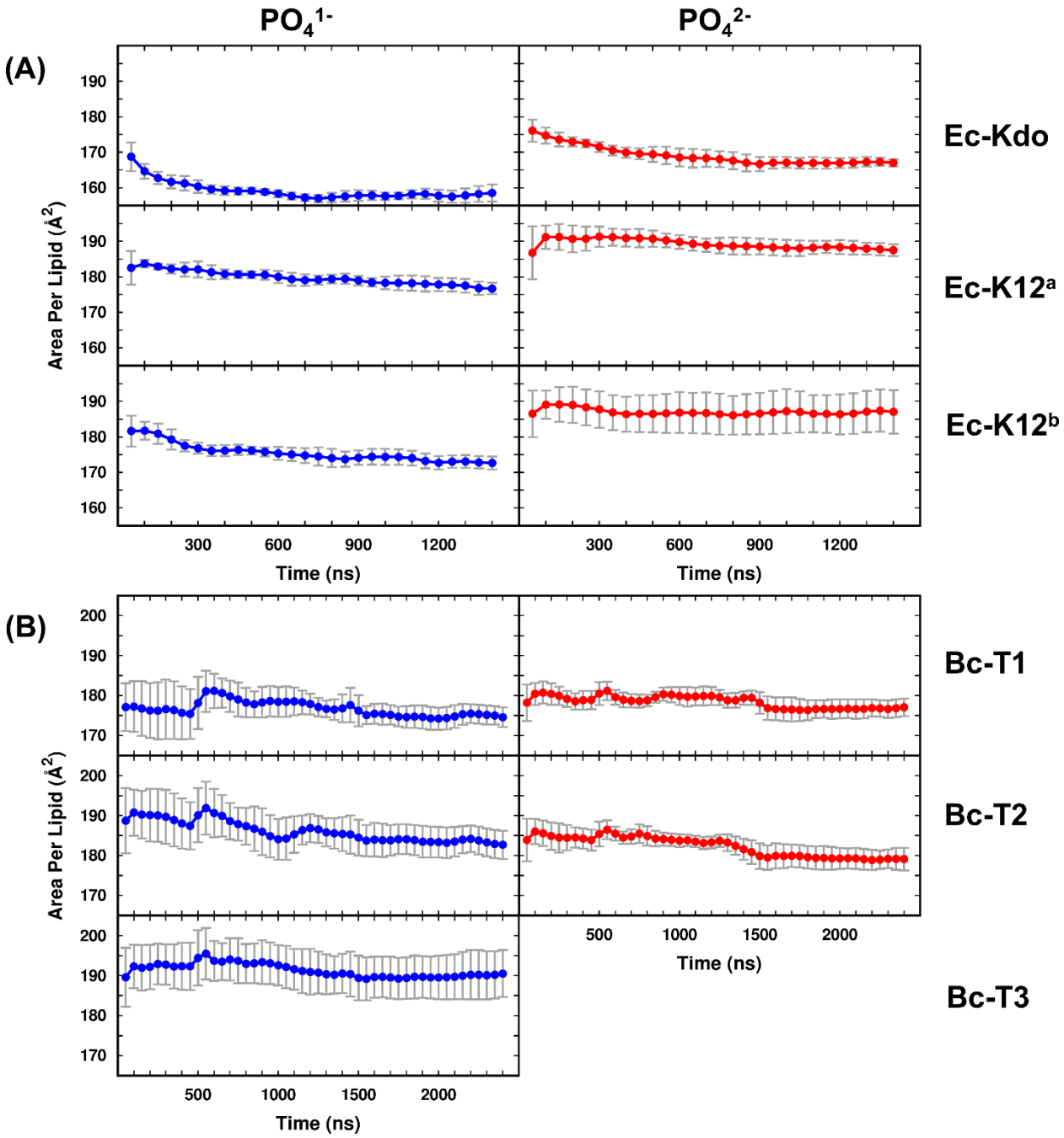


Figure S9. Time series of area per lipid for each Ec (A) and Bc (B) systems in both protonation states.

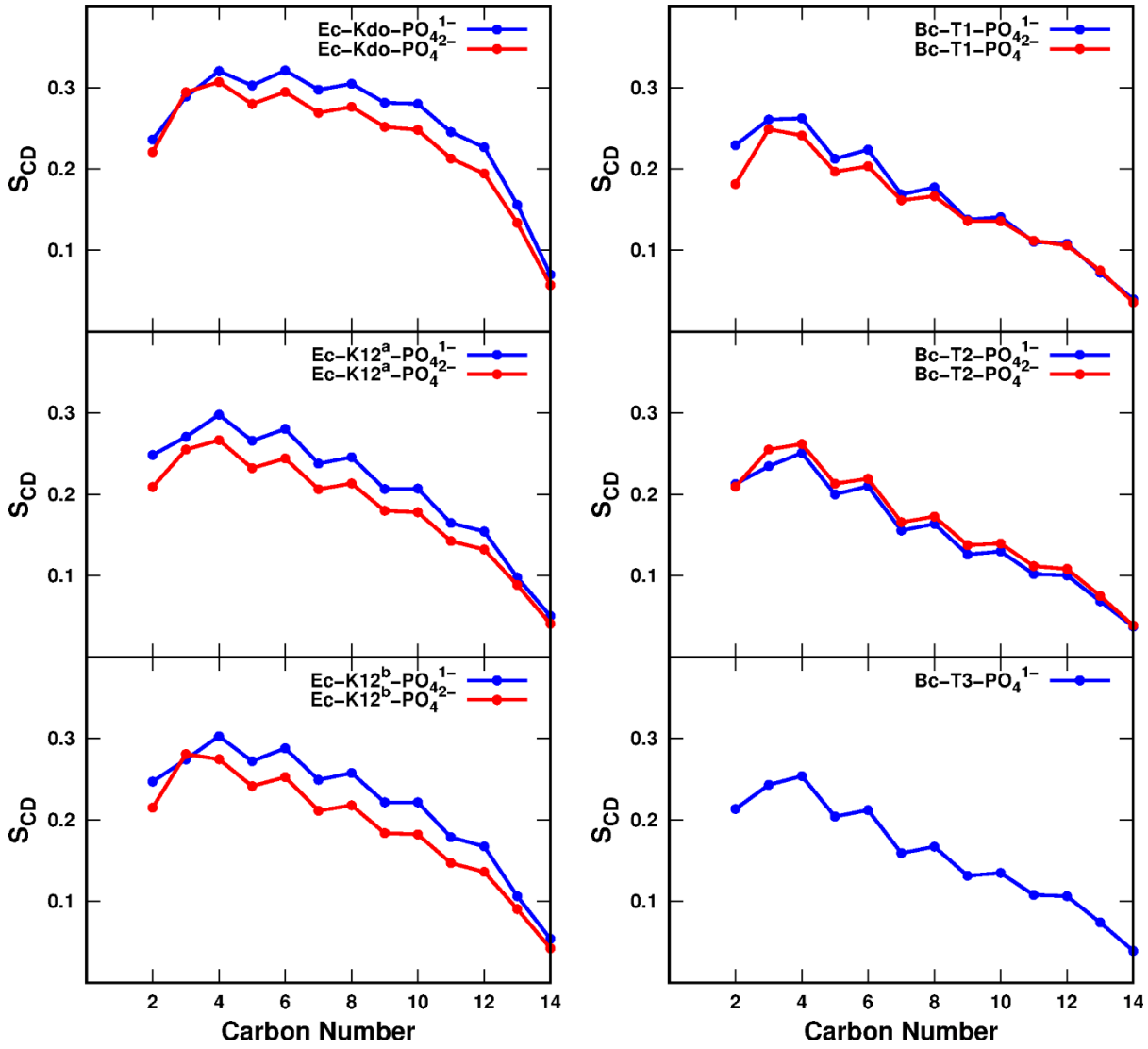


Figure S10. Calculated chain order parameters for acyl chain 2 of lipid A for each Ec and Bc system with two phosphate protonation states.

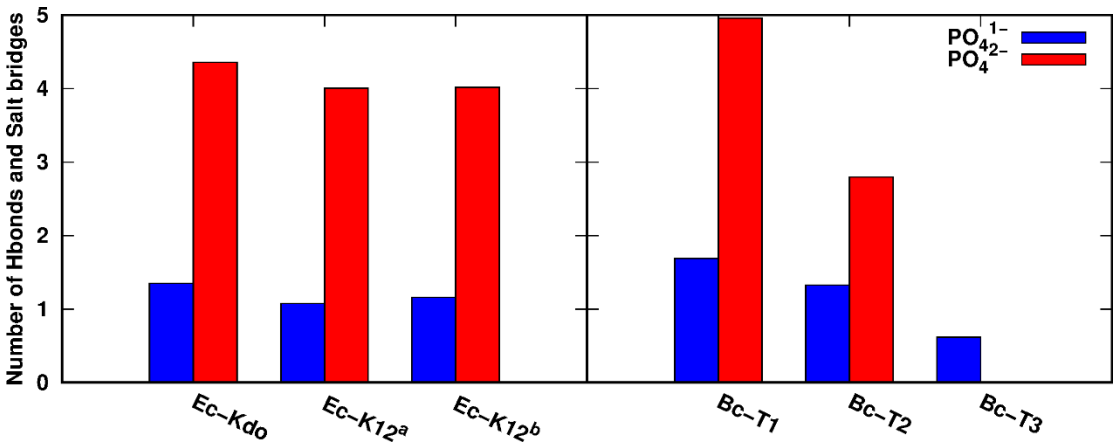


Figure S11. Sum of the per-LPS average number of inter-lipid A hydrogen bonds and the per-LPS average salt bridges between Ca^{2+} and phosphate groups for each Ec and Bc system with two phosphate protonation states. A hydrogen bond is counted when the distance between the donor and acceptor is less than 3 Å and the angle is larger than 120º. A salt bridge is counted when the distance between Ca^{2+} and any atom on phosphate groups of lipid A is less than 4 Å.

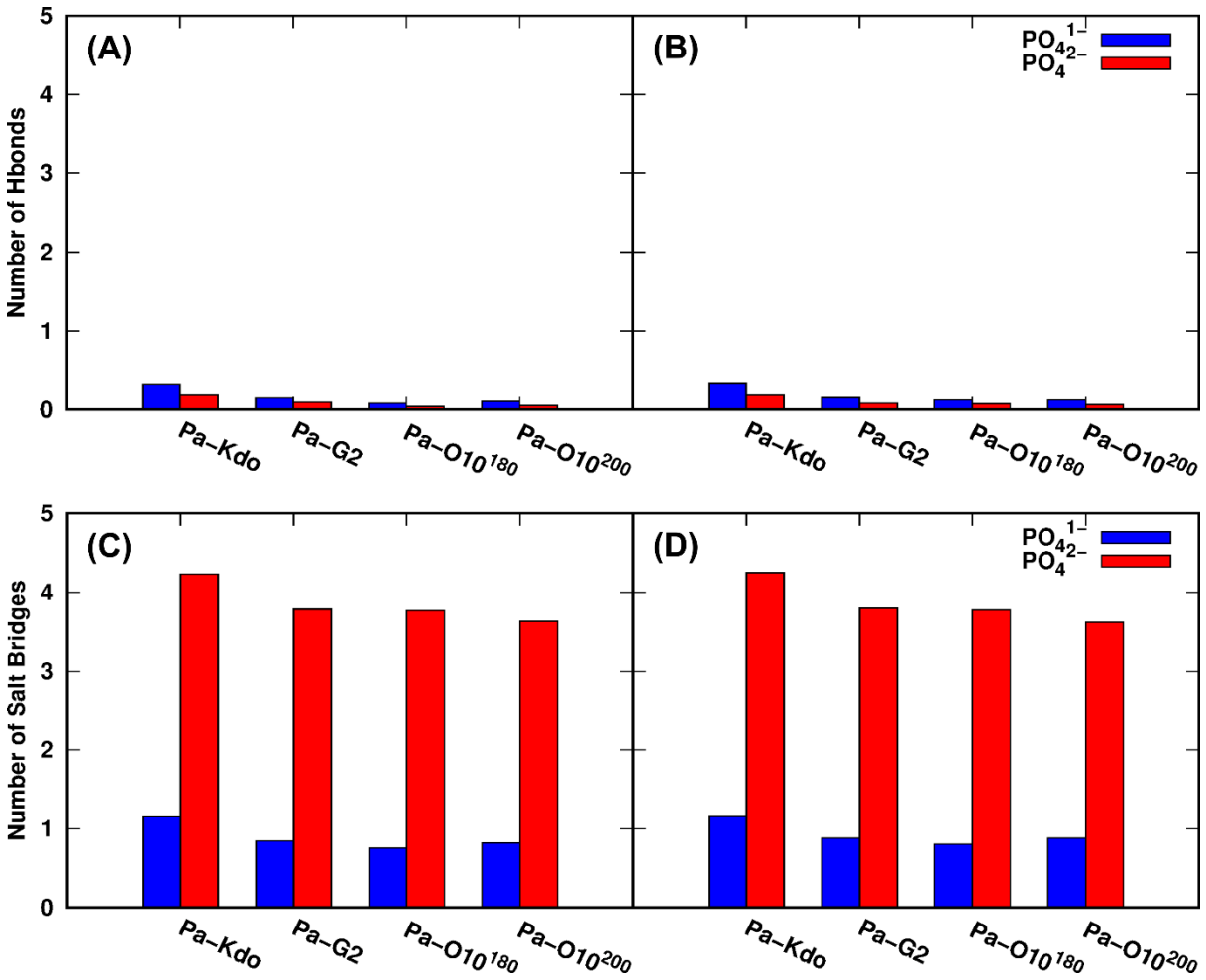


Figure S12. Per-LPS average number of (A, B) inter-lipid A hydrogen bonds and (C, D) salt bridges between Ca^{2+} and any atom on phosphate groups of lipid A for each Pa system: (A, C) standard and (B, D) HMR simulations.

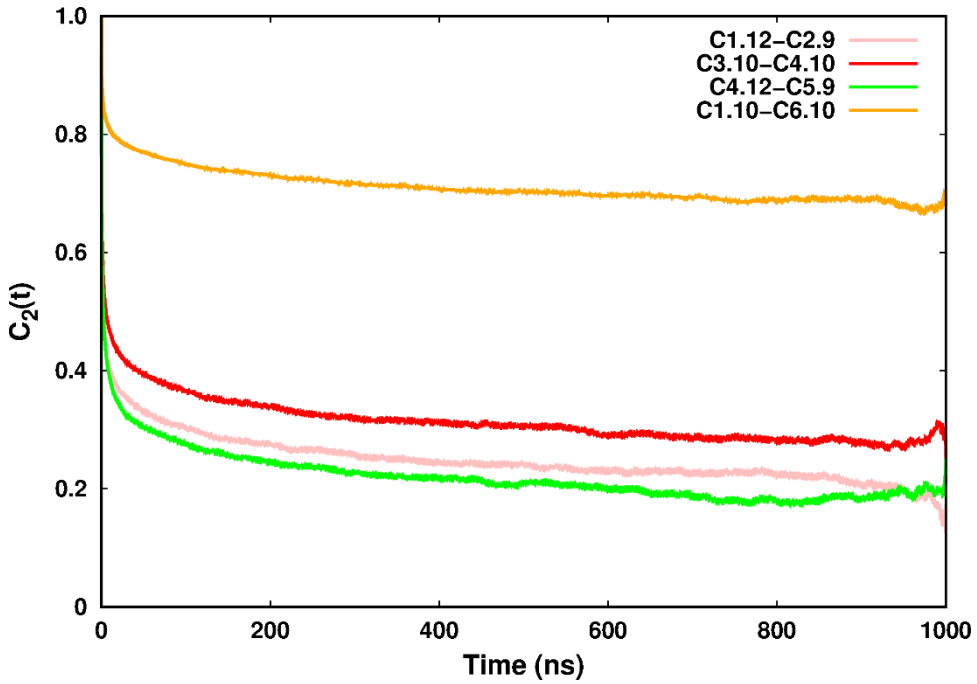


Figure S13. Correlation function $C_2(t)$ for the cross acyl chains in Pa-O10 system.

Table S1. Averaged area per lipid and hydrophobic thickness with standard errors for each Pa system in standard and HMR simulations with two phosphate protonation states.

System	Protonation State	Area Per Lipid (Å^2)		Hydrophobic Thickness (Å)	
		OpenMM 2-fs	OpenMM HMR	OpenMM 2-fs	OpenMM HMR
Pa-Kdo	PO_4^{1-}	163.2 ± 1.4	163.2 ± 1.7	18.6 ± 0.2	18.6 ± 0.2
	PO_4^{2-}	169.3 ± 1.8	169.8 ± 1.3	17.9 ± 0.2	17.9 ± 0.2
Pa-G2	PO_4^{1-}	179.2 ± 1.1	178.5 ± 1.3	17.0 ± 0.1	17.0 ± 0.2
	PO_4^{2-}	182.9 ± 2.4	181.2 ± 1.5	16.6 ± 0.2	16.8 ± 0.2
Pa-O10^{180}	PO_4^{1-}	179.7 ± 1.1	177.8 ± 1.1	16.9 ± 0.1	17.1 ± 0.1
	PO_4^{2-}	180.4 ± 0.7	178.9 ± 1.5	16.8 ± 0.1	16.9 ± 0.2
Pa-O10^{200}	PO_4^{1-}	175.4 ± 1.5	175.8 ± 0.5	17.3 ± 0.2	17.2 ± 0.1
	PO_4^{2-}	182.9 ± 1.4	182.1 ± 1.6	16.5 ± 0.1	16.7 ± 0.1

Table S2. Averaged area per lipid and hydrophobic thickness with standard errors for each Ec/Bc system with two phosphate protonation states.

System	Protonation State	Area Per Lipid ($Å^2$)	Hydrophobic Thickness (Å)
Ec-Kdo	PO_4^{1-}	158.6 ± 2.4	24.3 ± 0.3
	PO_4^{2-}	167.0 ± 1.1	23.2 ± 0.2
Ec-K12[a]	PO_4^{1-}	176.7 ± 1.6	21.8 ± 0.3
	PO_4^{2-}	187.5 ± 1.7	20.7 ± 0.3
Ec-K12[b]	PO_4^{1-}	172.7 ± 1.8	22.3 ± 0.3
	PO_4^{2-}	187.0 ± 6.2	20.8 ± 0.7
Bc-T1	PO_4^{1-}	174.6 ± 2.5	20.0 ± 0.3
	PO_4^{2-}	177.1 ± 2.2	19.7 ± 0.3
Bc-T2	PO_4^{1-}	182.7 ± 3.5	19.1 ± 0.3
	PO_4^{2-}	179.1 ± 2.8	19.6 ± 0.3
Bc-T3	PO_4^{1-}	190.5 ± 5.9	18.4 ± 0.6