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Supplemental Information

Bilayer Properties of Lipid A from Various Gram-negative Bacteria

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S1. Area per lipid estimation from the polymer brush model

Here, we derive a simple expression for the area per lipid (APL) in a bilayer based on the polymer brush model (PBM) (1). In the PBM, the energy functional for a monolayer is given as the sum of the interfacial energy and the free energy of chain extension,

$$F = \gamma a + \epsilon \left(\frac{a_c}{a}\right)^2 \tag{S1}$$

where γ is the interfacial energy density for hydrophobic interactions, a is the area per chain at the water-hydrocarbon interface, $\epsilon = 3k_B T n_s/2$, k_B is the Boltzmann constant, T is the temperature, $n_s = L/2b$ is the number of statistical segment per chain, L and a_c are the chain length and the APL in fully extended state, and b is the persistence length of the chain, respectively. Here, we assume that the lipid is incompressible (i.e., a constant molecular volume). By minimizing the energy functional (Eq. S1) and assuming the identical area per chain in a lipid molecule, the APL in PBM can be expressed as

$$\mathrm{APL} = a \cdot N_{\mathrm{CHAIN}} = \left(\frac{3k_B T a_c^2}{2\gamma b}\right)^{1/3} \cdot N_{\mathrm{CHAIN}} \cdot L^{1/3} \tag{S2}$$

The APL in Eq. S2 increases with L, which we attribute to the negligence of van der Waals interactions in the PBM. Substituting L with $\langle L_{\mathrm{CHAIN}} \rangle_2$, we get a fitting equation for APL

$$\mathrm{APL} \propto N_{\mathrm{CHAIN}} \cdot \langle L_{\mathrm{CHAIN}} \rangle_2^{1/3} \tag{S3}$$

Other expressions for APL and corresponding fitting equation can be obtained, for example, by decomposing then APL into the chain and saccharide contributions, which did not change the major behavior of APL shown in Eqs. S2 and S3.

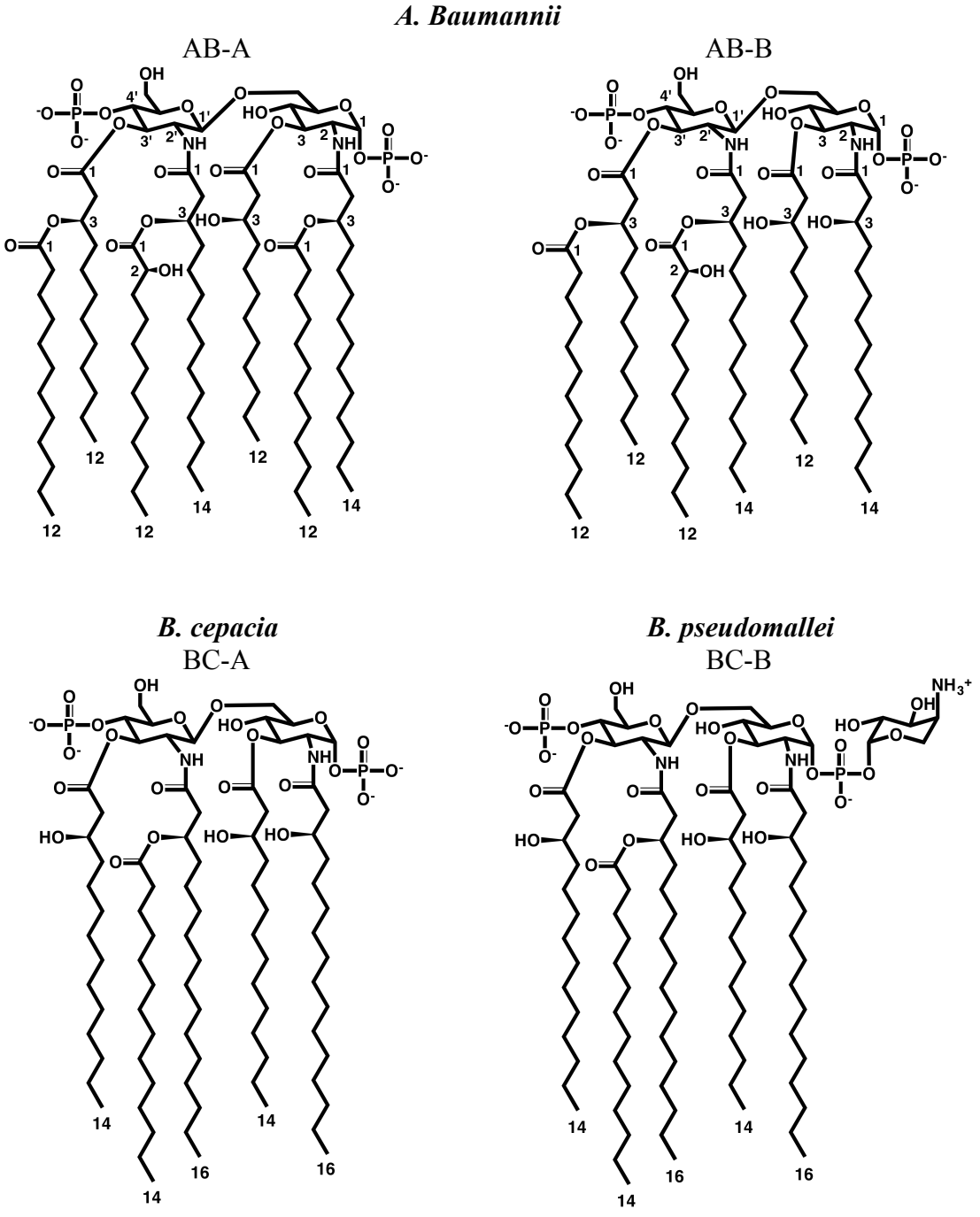
Table S1 APL, APL/N_{CHAIN}, and T_{MEMB} of all lipid A bilayer systems with the standard errors over three replicates.

System	N_{CHAIN}	$\langle L_{CHAIN} \rangle_2$	APL [Å^2]	APL/N_{CHAIN} [Å^2]	T_{MEMB} [Å]
AB-A	7	13.86	176.78 ± 0.55	25.25	23.23 ± 0.07
AB-B	6	13.67	161.65 ± 0.29	26.94	21.89 ± 0.03
BC-A	5	15.40	146.57 ± 1.39	29.31	24.43 ± 0.20
BC-B	5	15.40	147.51 ± 0.29	29.50	24.21 ± 0.03
CJ-A	6	15.67	160.37 ± 1.12	26.73	26.09 ± 0.18
EC-A	6	14.67	160.68 ± 0.10	26.78	23.79 ± 0.01
HP-A	4	18.25	100.12 ± 0.92	25.03	33.11 ± 0.25
HP-B	6	17.67	155.10 ± 0.57	25.85	30.92 ± 0.11
KP-A	7	15.57	177.66 ± 0.64	25.38	26.65 ± 0.09
KP-B	6	15.00	161.68 ± 1.19	26.95	24.43 ± 0.15
NG-A	6	13.67	158.77 ± 0.31	26.46	22.01 ± 0.04
PA-A	6	12.33	163.75 ± 0.64	27.29	18.41 ± 0.05
PA-B	5	12.80	147.81 ± 0.40	29.56	17.82 ± 0.05
ST-A	7	15.29	176.18 ± 1.19	25.17	26.16 ± 0.16
ST-B	7	15.29	178.06 ± 0.89	25.44	25.98 ± 0.10
ST-C	7	15.29	176.59 ± 0.94	25.23	26.16 ± 0.12
VC-A	6	14.00	164.02 ± 0.20	27.34	22.03 ± 0.04
VC-B	6	14.00	176.50 ± 0.98	29.42	20.74 ± 0.08
VC-C	6	14.00	179.70 ± 0.98	29.95	20.51 ± 0.13
YP-A	6	15.00	165.51 ± 1.11	27.59	23.68 ± 0.16
YP-B	4	14.00	138.70 ± 0.48	34.68	20.38 ± 0.12

Table S2 Diffusion coefficients, residence times, and compressibility modulus of the six lipid A systems with the chemical modifications. The errors are the standard errors over three replicates. The residence times of Ca^{2+} in ST-B could not be measured because of zero net charge for this lipid A, and thus neutralization ion was not needed.

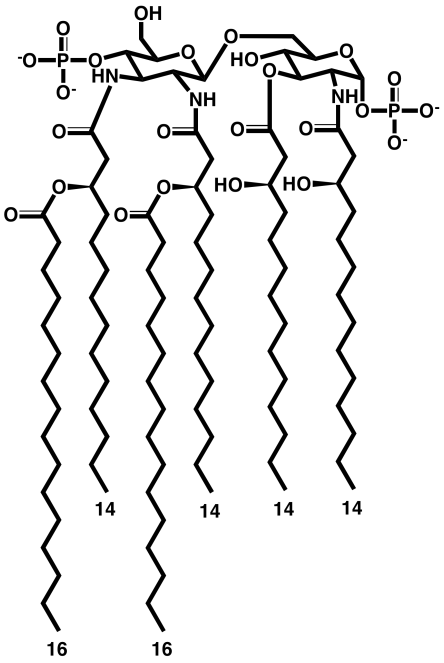
Neutralizing Ion	System	Diffusion coefficient ($\mu m^2/s$)	Residence time of Ca^{2+} (ns)	Compressibility modulus (dyn/cm)
Ca^{2+}	ST-A	1.01 ± 0.15	57 ± 10	32 ± 8
	ST-B	1.11 ± 0.09	–	26 ± 4
	ST-C	0.94 ± 0.12	19 ± 3	37 ± 5
	VC-A	0.96 ± 0.13	73 ± 11	29 ± 10
	VC-B	1.09 ± 0.14	58 ± 4	24 ± 4
	VC-C	1.05 ± 0.13	71 ± 3	17 ± 2

Figure S1 Lipid A molecular structures of Gram-negative bacteria studied in this work.



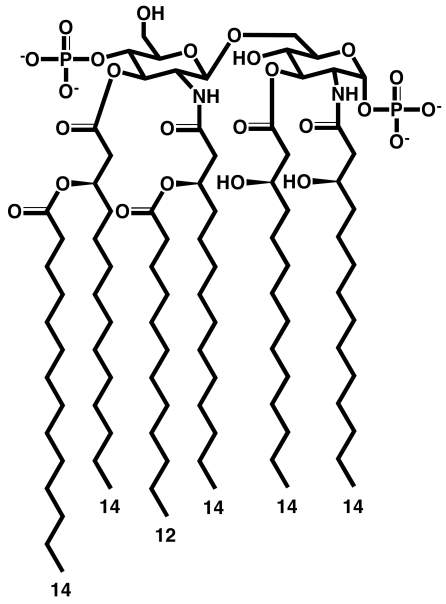
C. jejuni

CJ-A



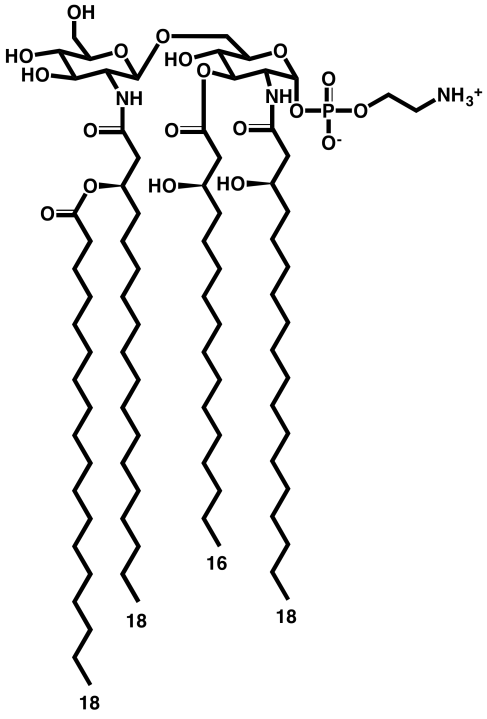
E. coli

EC-A

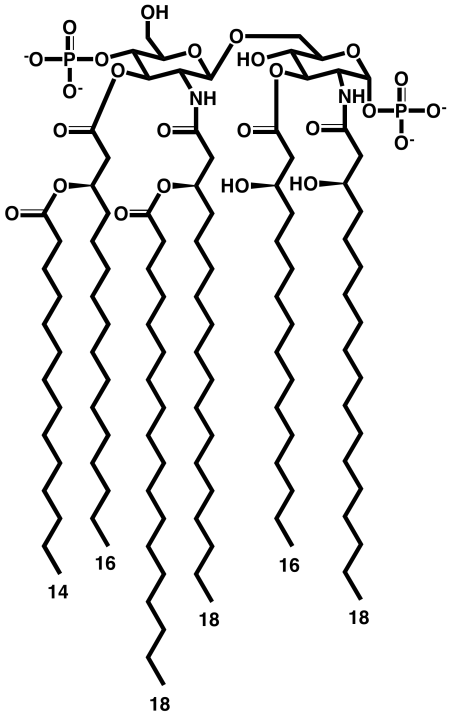


H. pylori

HP-A

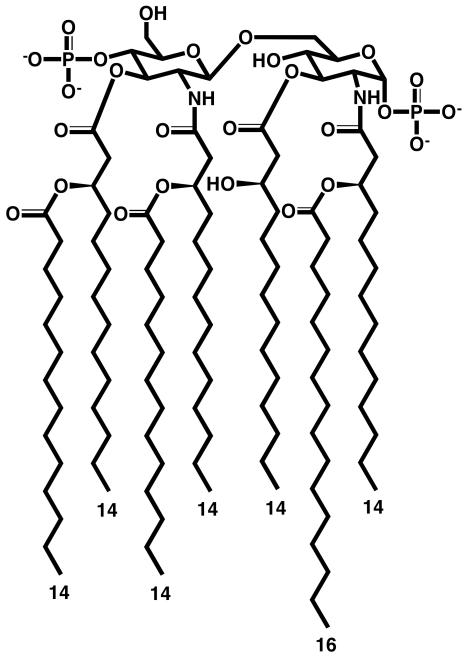


HP-B

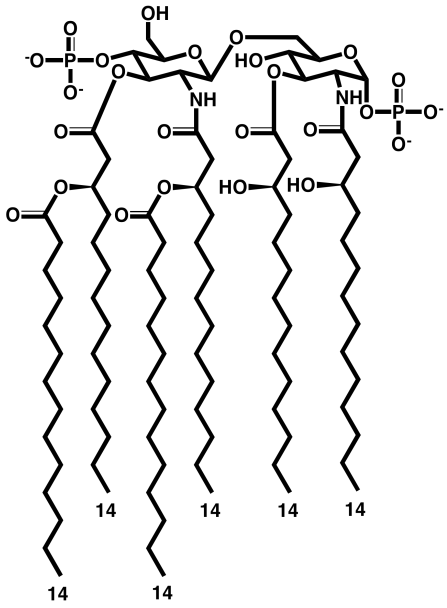


K. pneumoniae

KP-A

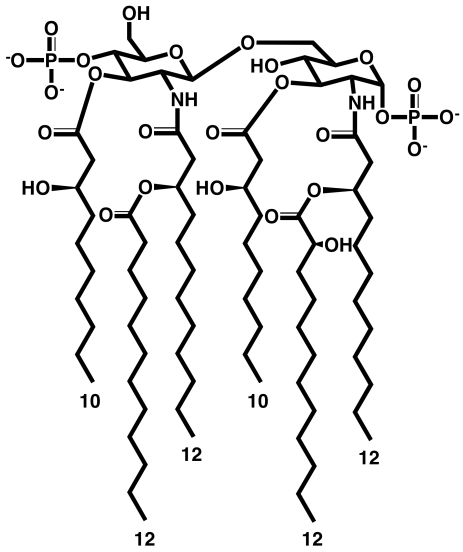


KP-B

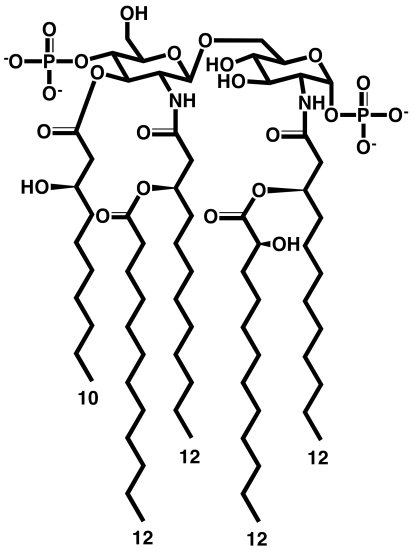


P. aeruginosa

PA-A

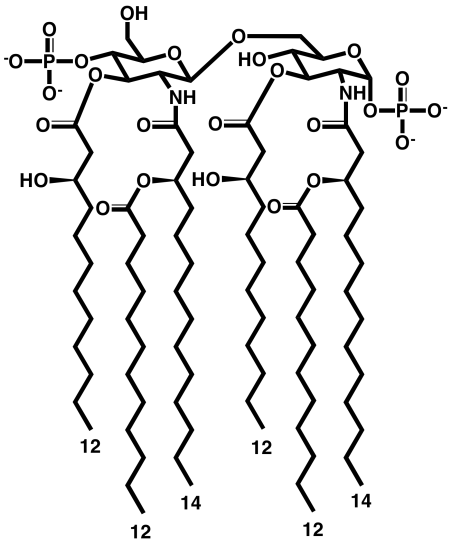


PA-B



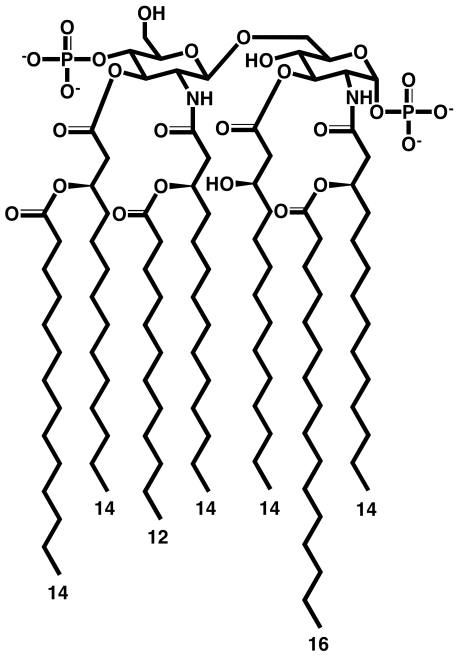
N. gonorrhoeae

NG-A



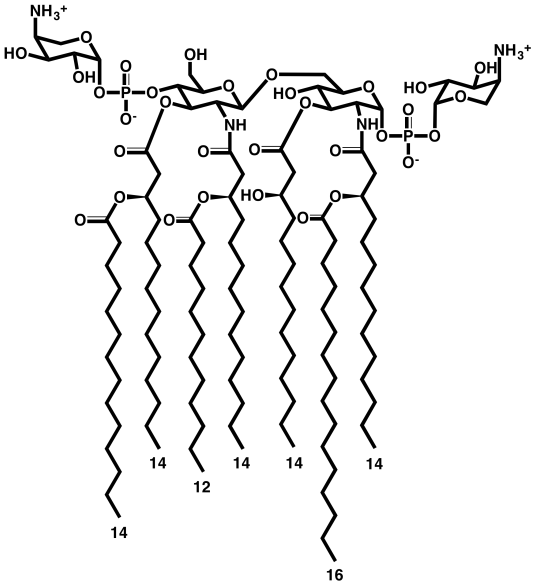
S. typhimurium

ST-A

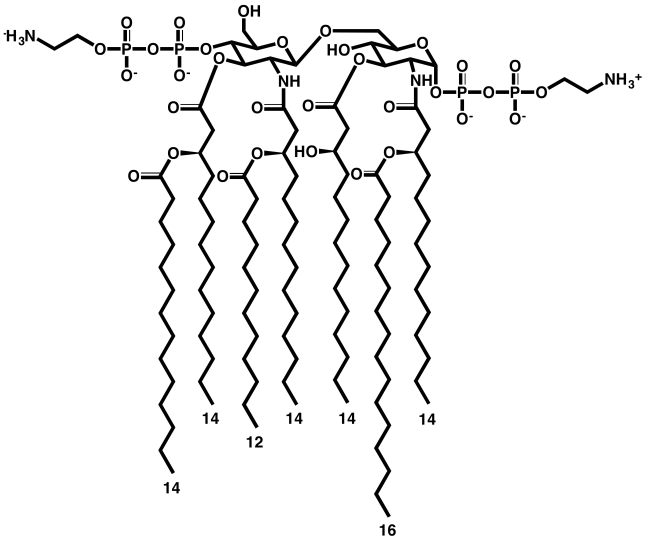


S. typhimurium

ST-B

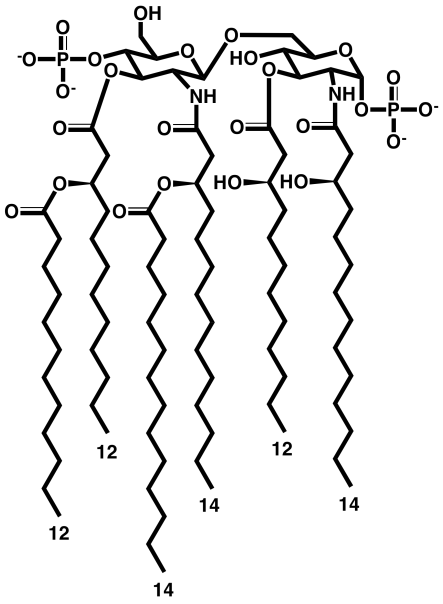


ST-C

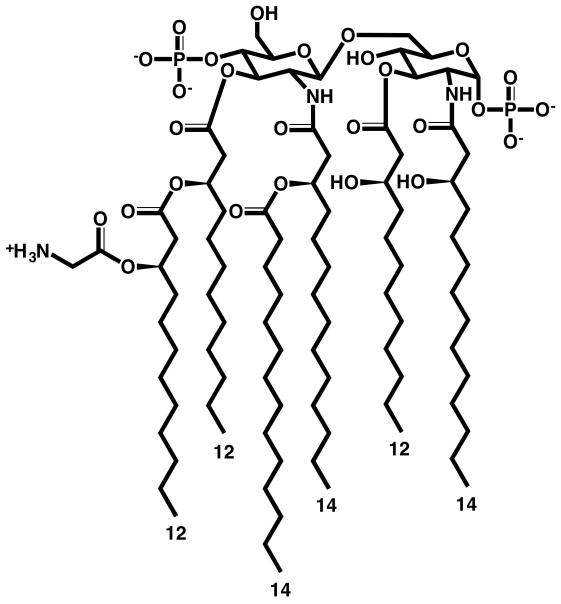


V. cholerae

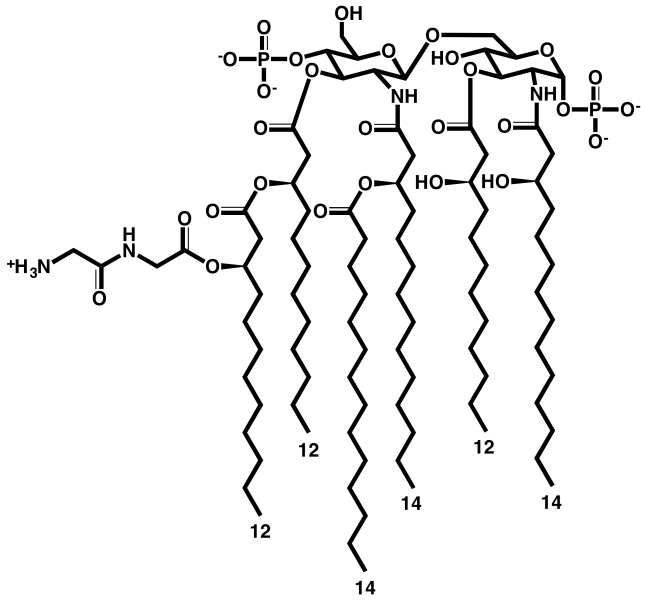
VC-A



VC-B

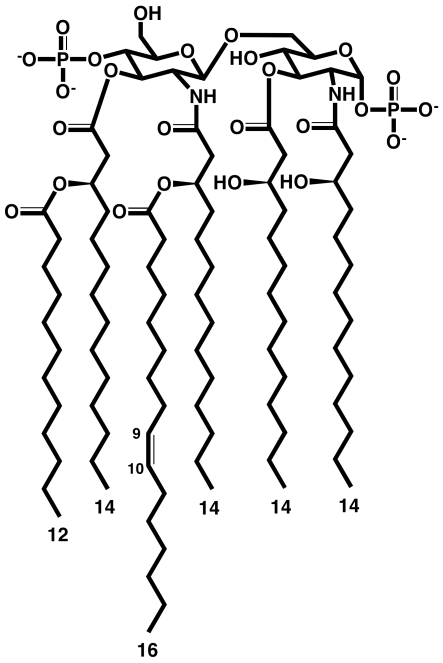


VC-C



Y. pestis

YP-A



YP-B

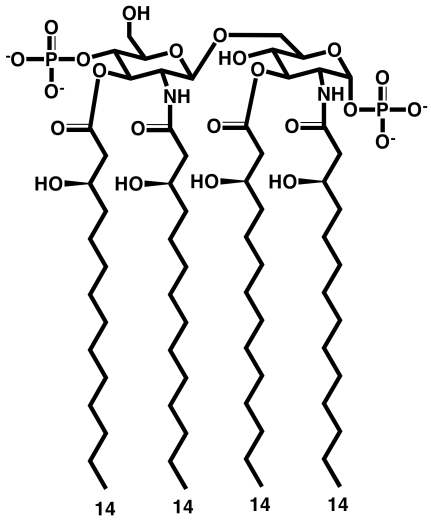
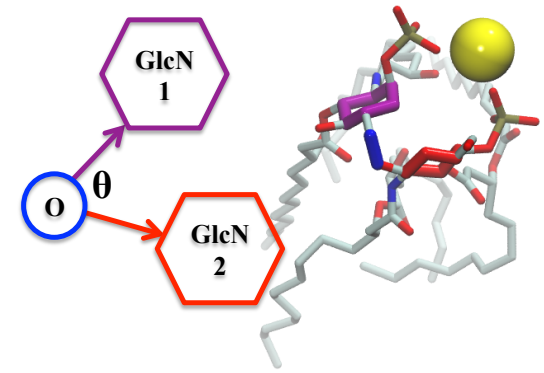


Figure S2 (A) A schematic representation of the angle between two sugars in lipid A, and a snapshot of Ca^{2+} (yellow sphere) tightly bound to lipid A phosphate oxygen atoms. (B) Lennard-Jones parameter R_{min} for the relevant pairs by the Lorentz-Berthelot combination rule and modified by NBFIX. (C) Time-series of the number of acute angles in AB-B three independent simulations. NBFIX was applied after 150 ns (black vertical line). (D) Time-series of per-lipid area and hydrophobic thickness averaged every 50 for with the standard fluctuations.

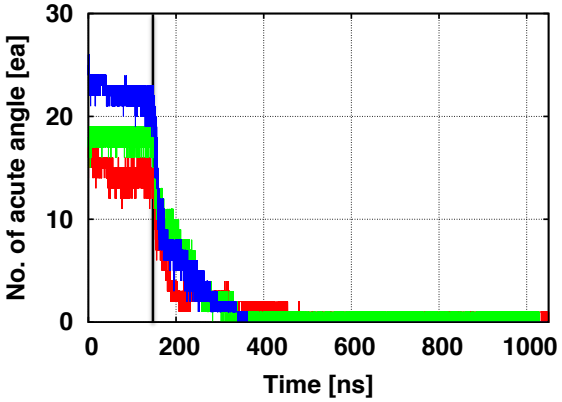
A



B

Ion pair	R_{min} (Å)	
	combination rule	NBFIX
Ca^{2+} – OCL	3.067	3.225
Ca^{2+} – O2L	3.067	3.304
Ca^{2+} – OC2DP	3.067	3.304
Ca^{2+} – OC2D2	3.067	3.225

C



D

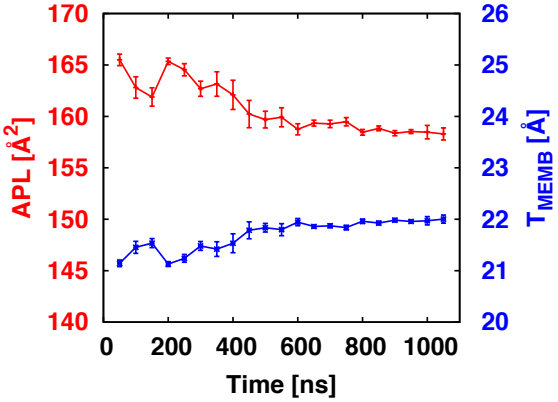
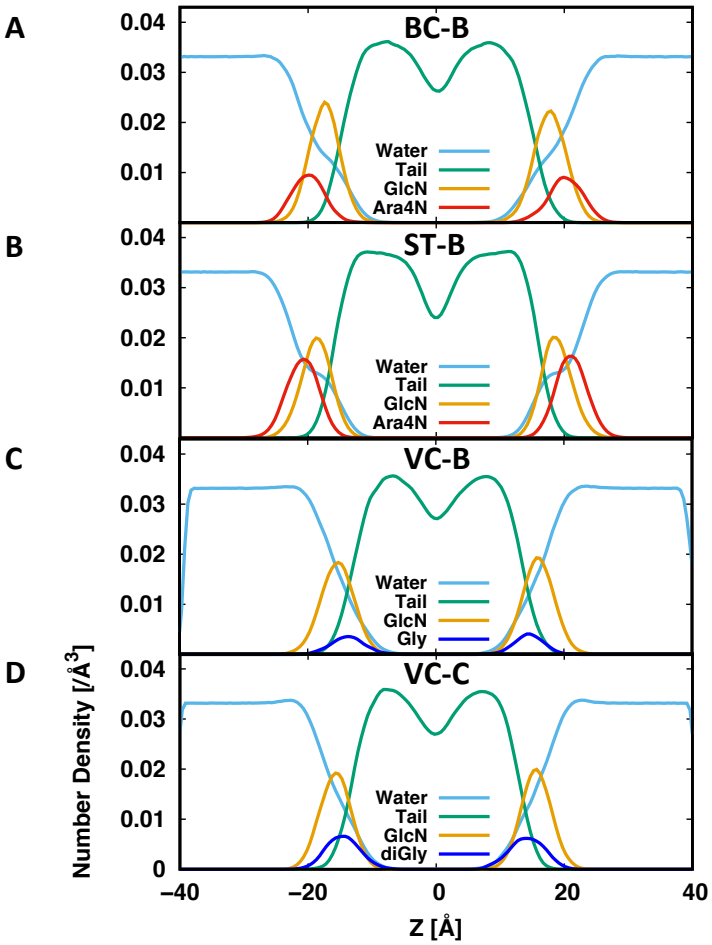


Figure S3 Density profiles along the *z*-axis (i.e., the membrane normal with the membrane center at $z = 0$) for (A) BC-B, (B) ST-B, (C) VC-B, and (D) VC-C systems with skyblue for water; green for acyl tail, orange for disaccharide, and red for L-Ara4N. (E) In VC-B system, Gly interacts with inter or intra phosphate groups. (F) In VC-C system, diGly interacts with intra phosphate group.



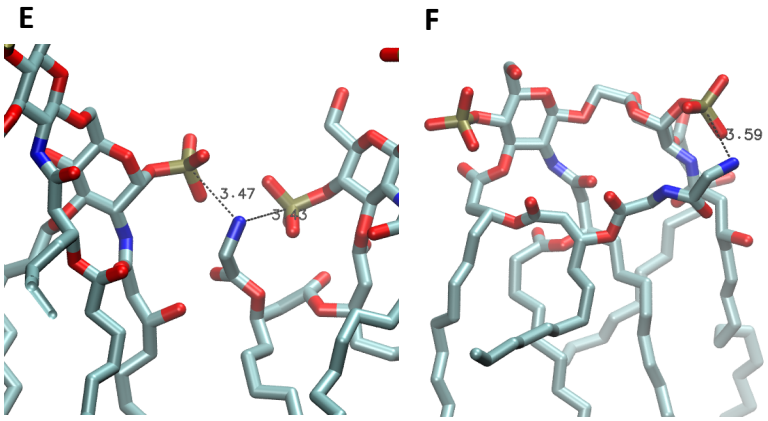


Figure S4 ϕ-ψ and ψ-ω distributions of the disaccharide's β(1→6) glycosidic linkages in (A) AB-A, (B) AB-B, and (C) EC-A system. The twenty-seven black dots represent x-ray structure of lipid A from eighteen PDBs (1QFG, 2GRX, 1FI1, 1QFF, 1QJQ, 1QKC, 3FXI, 3VQ2, 4G8A, 4LKV, 3ULA, 2Z65, 4CU4, 2E59, 3VQ1, 1FCP, 2FCP, and 3MU3) that contain the intact disaccharide with β(1→6) linkage preserved. The glycosidic torsion angle definitions are: O5′−C1′−O6−C6 (ϕ), C1′−O6−C6−C5 (ψ), and O6−C6−C5−O5 (ω).

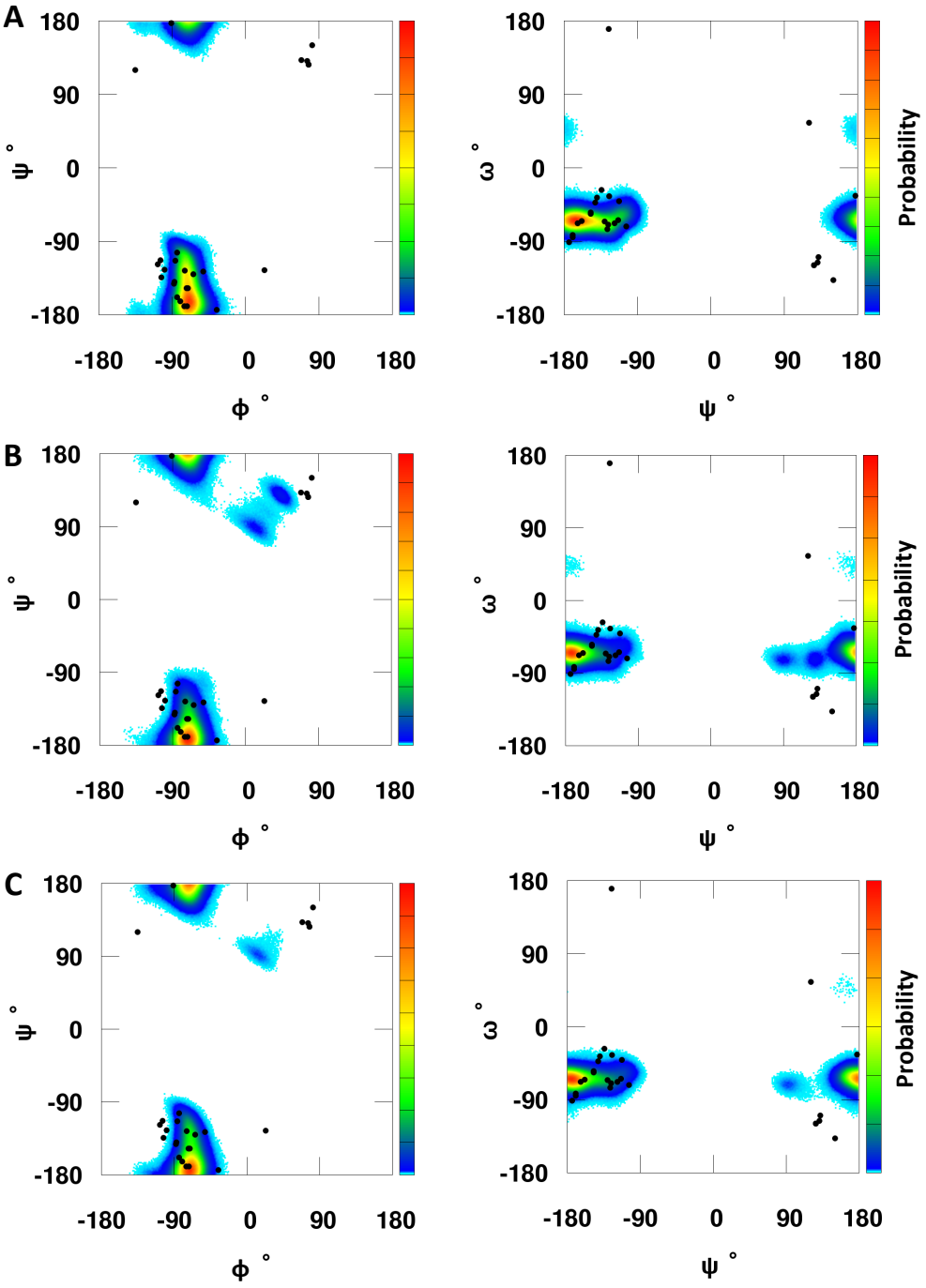
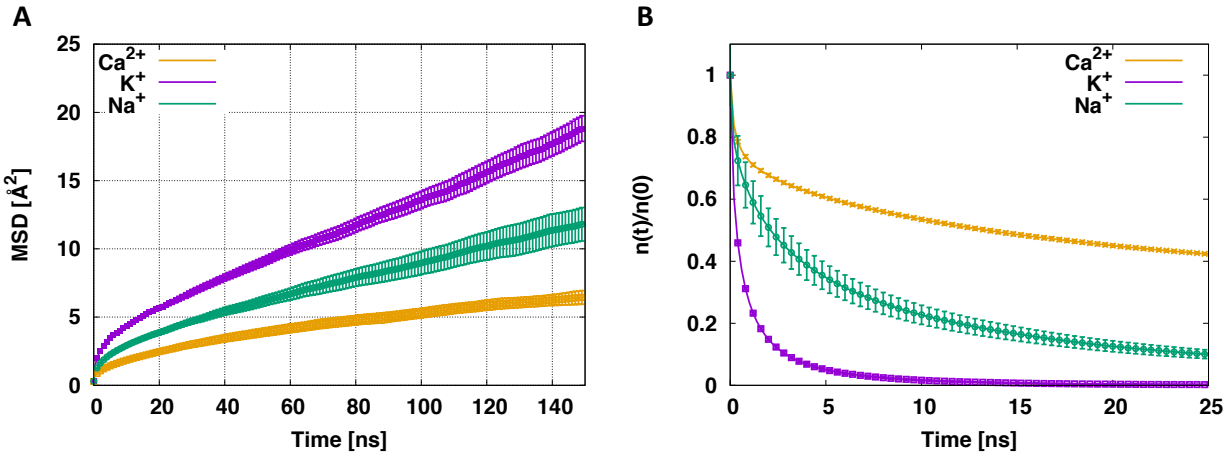


FIGURE S5 (A) Mean square displacement (MSD) and (B) residence time plot in AB-A systems with the different neutralizing ion types. The error bars indicate the standard errors from the 3 replicas.



Reference

1. Rawicz, W., K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans. 2000. Effect of chain length and unsaturation on elasticity of lipid bilayers. Biophys. J. 79:328-339.