Biophysical Journal, Volume *112*

Supplemental Information

Refinement of OprH-LPS Interactions by Molecular Simulations

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Table S1. System information.

Systems		Lipid composition		# Lipids		System Size	# Atom	# Water
		Top	Bottom	Top	Bottom			
OM-Only	Ec.Kdo	*E. coli* lipid A + two Kdo sugars	PPPE/PVPG/PVCL2	36	75/20/5	80×80×95	~61,000	~12,000
	Ec.K12	*E. coli* Lipid A + K12 core	PPPE/PVPG/PVCL2	36	75/20/5	78×78×116	~73,000	~13,000
	Pa.Kdo	*P. aeruginosa* Lipid A + two Kdo sugars	PPPE/PVPG/PVCL2	36	75/20/5	79×79×93	~60,000	~12,000
	Pa.G2	*P. aeruginosa* Lipid A + G2 core	PPPE/PVPG/PVCL2	36	75/20/5	77×77115	~71,000	~13,000
	Pa.G2.O10	*P. aeruginosa* Lipid A + G2 core + two O10 O-antigen	PPPE/PVPG/PVCL2	36	75/20/5	77×77×139	~85,000	~16,000
OM-OprH	Ec.Kdo	*E. coli* lipid A + two Kdo sugars	PPPE/PVPG/PVCL2	35	75/20/5	83×83×115	~82,000	~18,000
	Ec.K12	*E. coli* Lipid A + K12 core	PPPE/PVPG/PVCL2	35	75/20/5	83×83×118	~84,000	~16,000
	Pa.Kdo	*P. aeruginosa* Lipid A + two Kdo sugars	PPPE/PVPG/PVCL2	35	75/20/5	83×83×113	~80,000	~18,000
	Pa.G2	*P. aeruginosa* Lipid A + G2 core	PPPE/PVPG/PVCL2	35	75/20/5	83×83×116	~81,000	~16,000
	Pa.G2.O10	*P. aeruginosa* Lipid A + G2 core + two O10 O-antigen	PPPE/PVPG/PVCL2	35	75/20/5	83×83×138	~98,000	~19,000

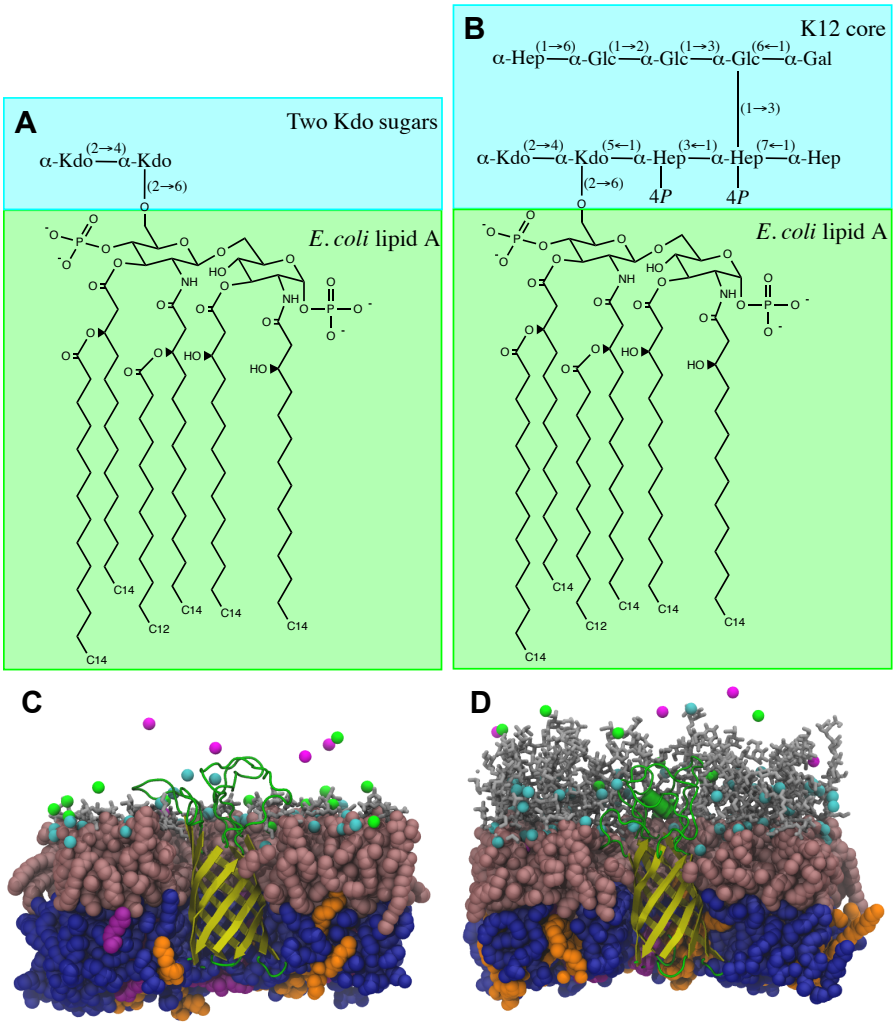

Figure S1. Chemical structures of lipid A and sequences of LPS core considered in this study: (A) Ec.Kdo and (B) Ec.K12 (1, 2) and representative snapshots of OprH embedded in (C) Ec.Kdo and (D) Ec.K12 OMs. The *E. coli* K12 core has two Kdo residues and three Hep residues, two of which have a monophosphoester group at O4 positions in the inner core. The outer core consists of three Glc residues, one Gal residue, and one Hep residue, which are α-linked. Lipid A is represented as pink spheres, core sugars as gray sticks, O10-antigen polysaccharides as orange sticks, PPPE as blue spheres, PVPG as orange spheres, PVCL2 as magenta spheres, Ca^{2+} ions as small cyan spheres, K^{+} ions as small magenta spheres, and Cl^{-} ions as small green spheres. For clarity, some portion of each system is truncated and water molecules are not shown.

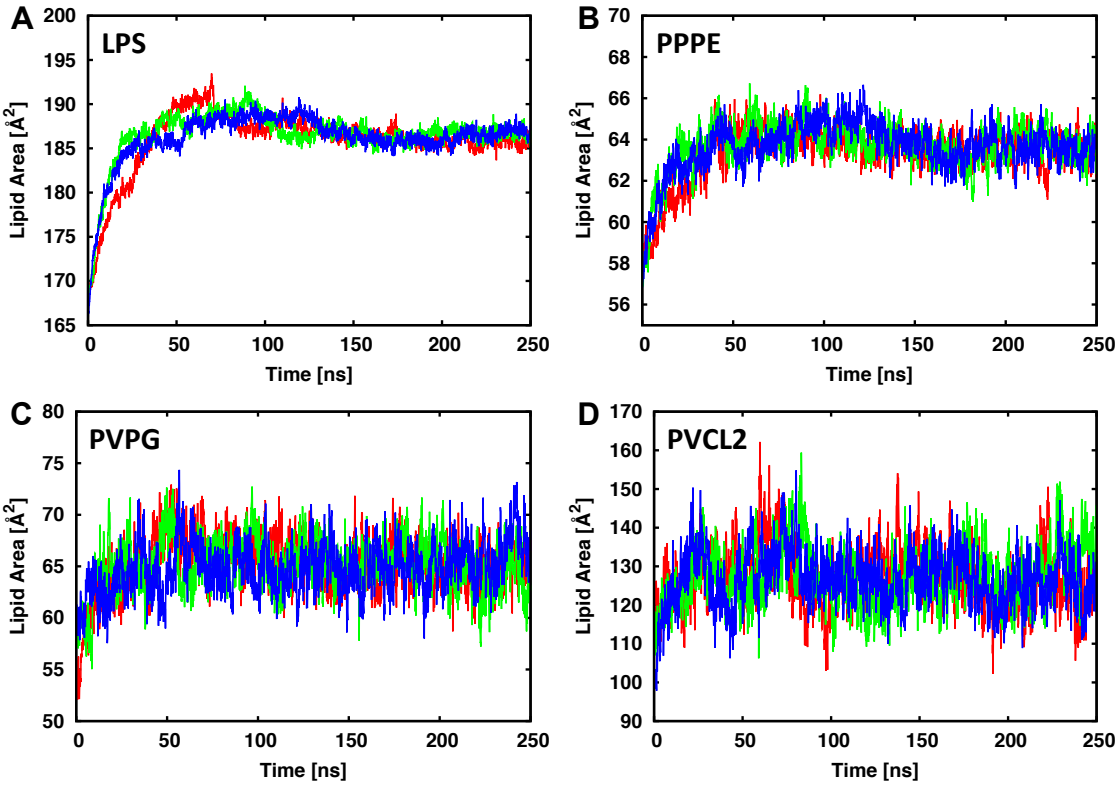

Figure S2. Time-series of the area per lipid (APL) of (A) LPS, (B) PPPE, (C) PVPG, and (D) PVCL2 in OM-only Pa.G2.O10 with three independent systems (replica 1 with red line, replica 2 with green line, and replica 3 with blue line).

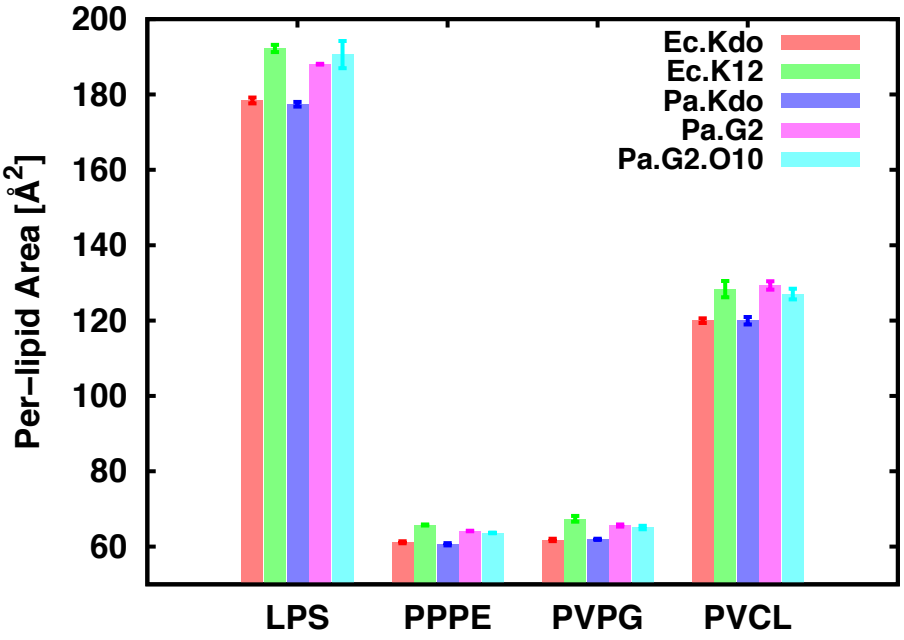

Figure S3. Average area per lipid (APL) of each lipid in OM-only system with the standard errors over three replicas. The APL of each lipid type was calculated using a Voronoi tessellation approach with the following atom selections: carbonyl carbon atoms (i.e., C11, C21, C31, C41, C51, and C61) to define acyl chains of lipid A, two carbon atoms (C21 and C31) for PPPE and PVPG, and four carbon atoms (CA1, CB1, CC1, and CD1) for PVCL2 lipid chains.

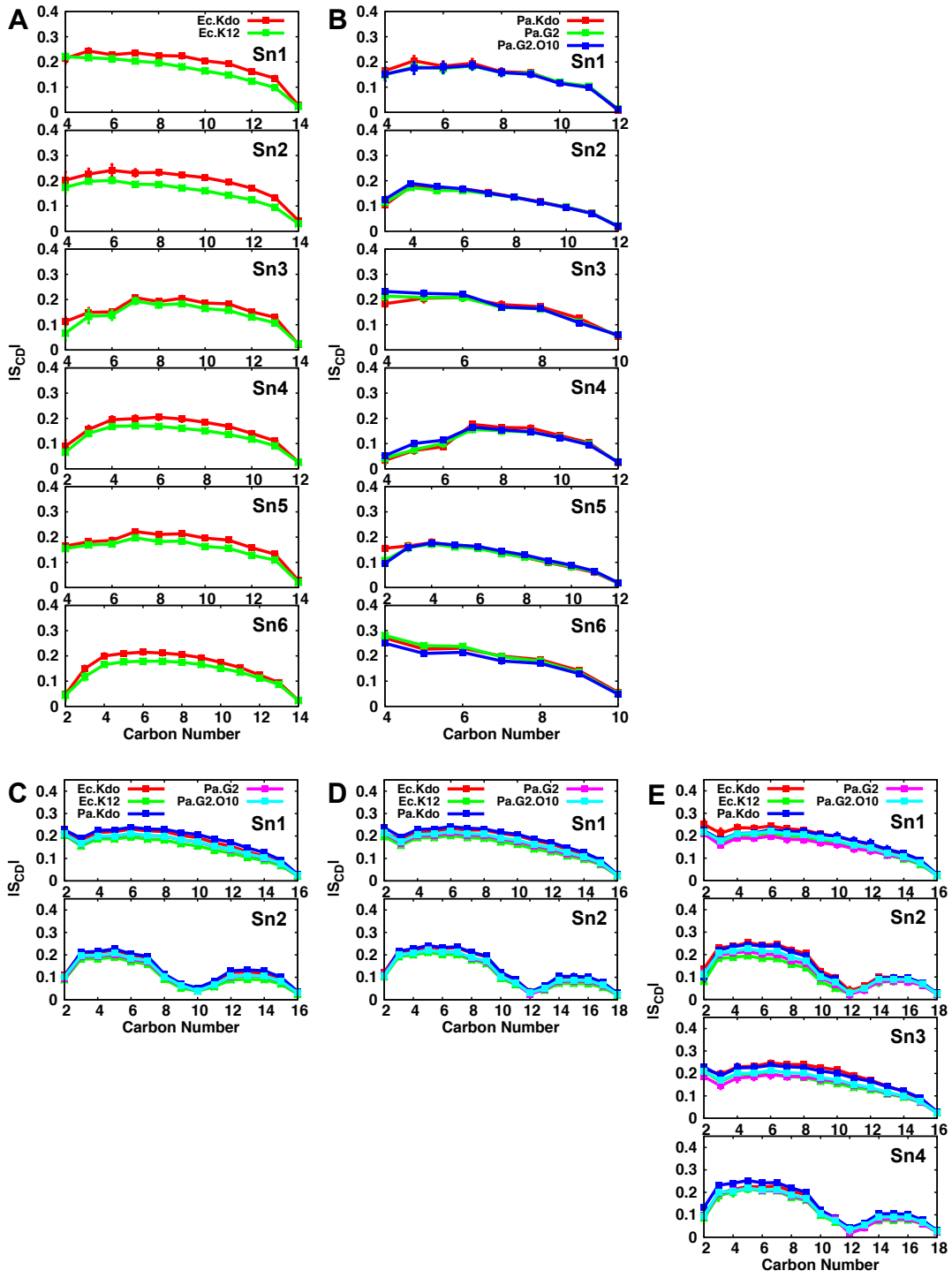

Figure S4. Deuterium order parameters for (A) *E. coli* lipid A, (B) *P. aeruginosa* lipid A, (C) PPPE, (D) PVPG, and (E) PVCL2 of OM-only systems.

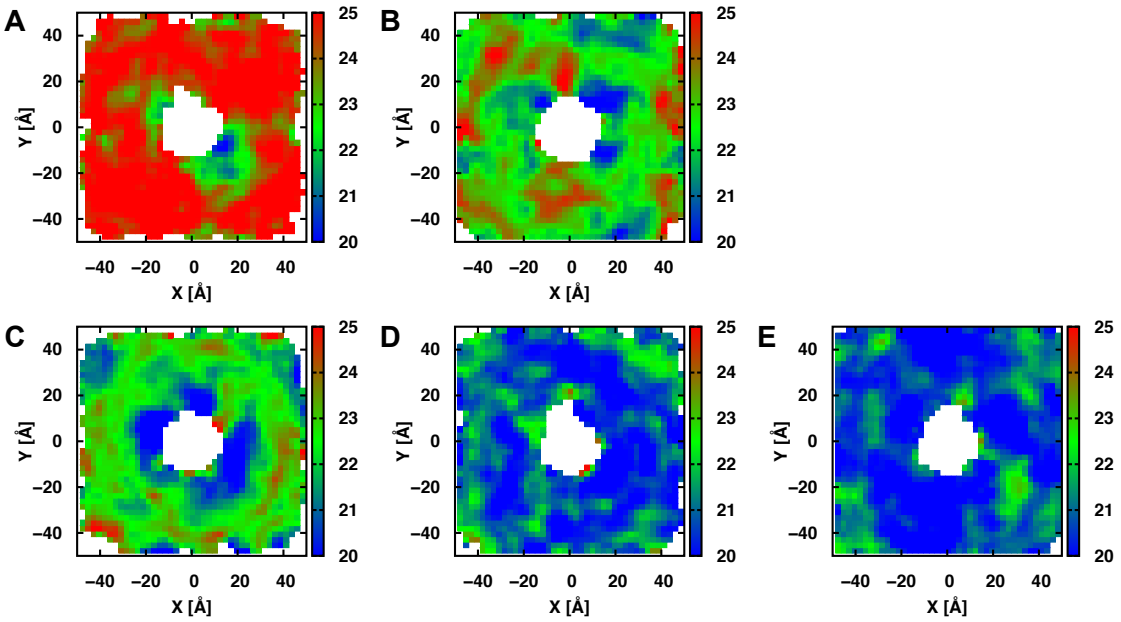

Figure S5. Two-dimensional thickness distributions of the OMs with OprH in (A) Ec.Kdo, (B) Ec.K12, (C) Pa.Kdo, (D) Pa.G2, and (E) Pa.G2.O10.

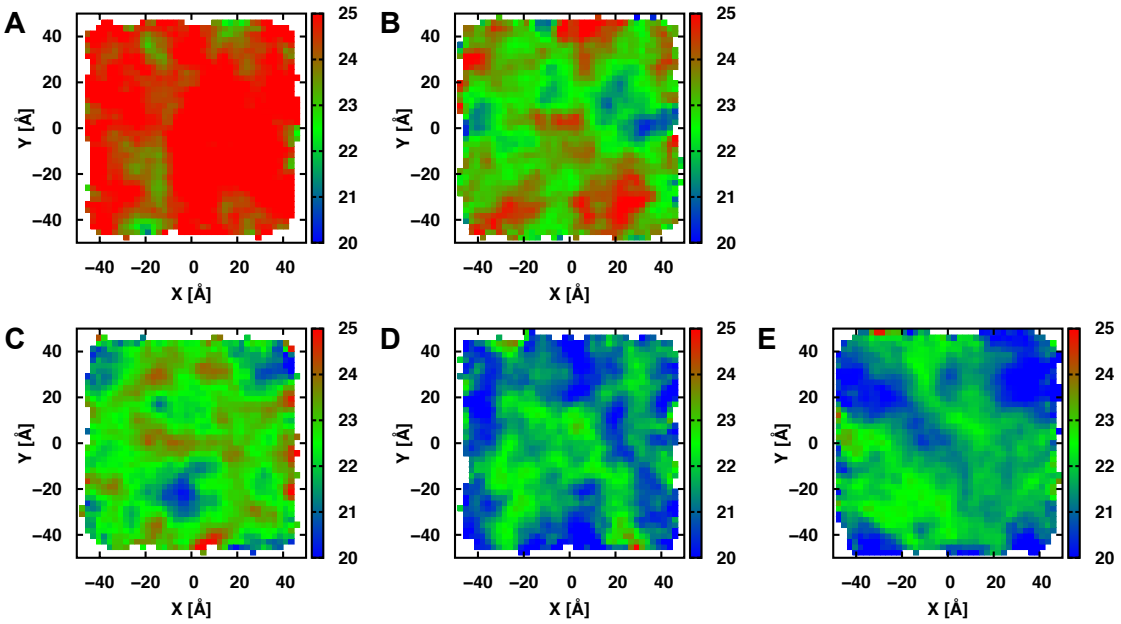

Figure S6. Two-dimensional thickness distributions of the OMs with (A) Ec.Kdo, (B) Ec.K12, (C) Pa.Kdo, (D) Pa.G2, and (E) Pa.G2.O10.

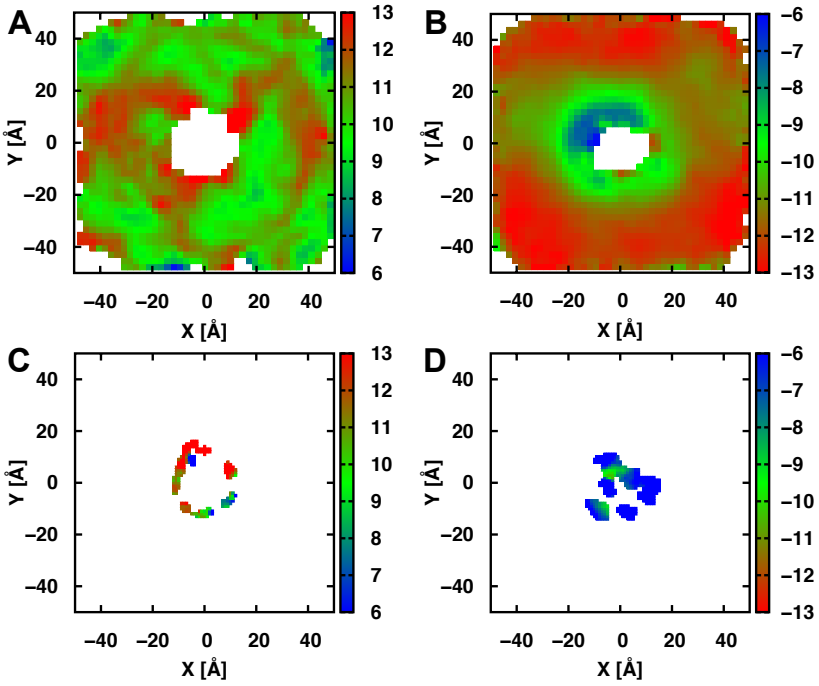

Figure S7. Two-dimensional z-position distributions of (A) the C2 and C4 atoms of lipid A and (B) the acyl chain C2 atoms of phospholipids in Pa.Kdo, as well as two-dimensional z-position distributions of the center of mass of the side chain of the hydrophobic residues on the rim of each β-strand for (C) upper leaflet and (D) lower leaflet in OprH-Pa.Kdo.

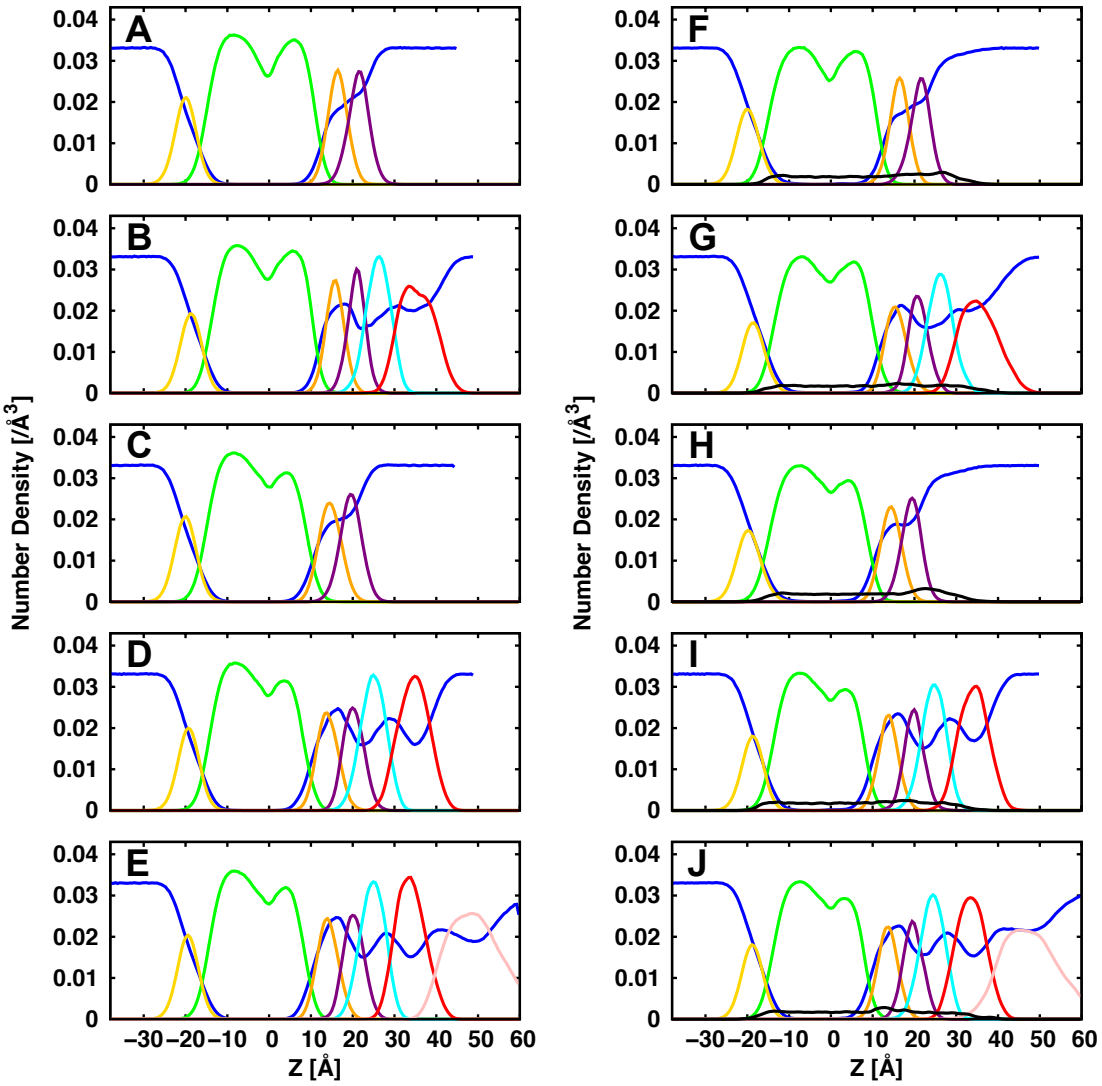

Figure S8. Density profiles of water (blue), phospholipid head groups (yellow), lipid carbon tail (green), LPS head groups (orange), LPS Kdo sugar (purple), LPS inner core (cyan), LPS outer core (red), LPS O-antigen (pink), and protein backbone atoms (black) along the membrane normal (i.e., the z axis) in (A) Ec.Kdo, (B) Ec.K12, (C) Pa.Kdo, (D) Pa.G2, and (E) Pa.G2.O10 of OM-only systems, and (F) Ec.Kdo, (G) Ec.K12, (H) Pa.Kdo, (I) Pa.G2, and (J) Pa.G2.O10 of OprH-OM systems.

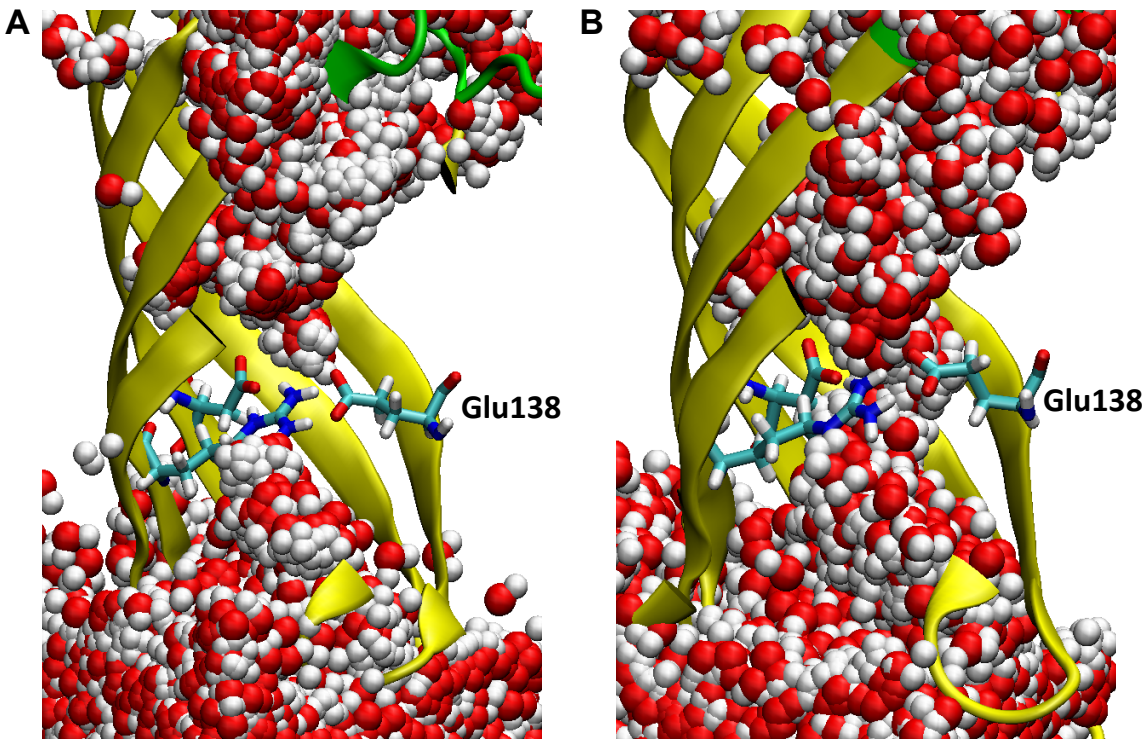

Figure S9. Overlaid views with 100 snapshots of the water molecules in OprH pore in (A) Pa.G2.O10 and (B) Pa.Kdo. Each snapshot was extracted every 2 ns from the last 200 ns. Arg54, Asp81, and Glu138 are shown with sticks.

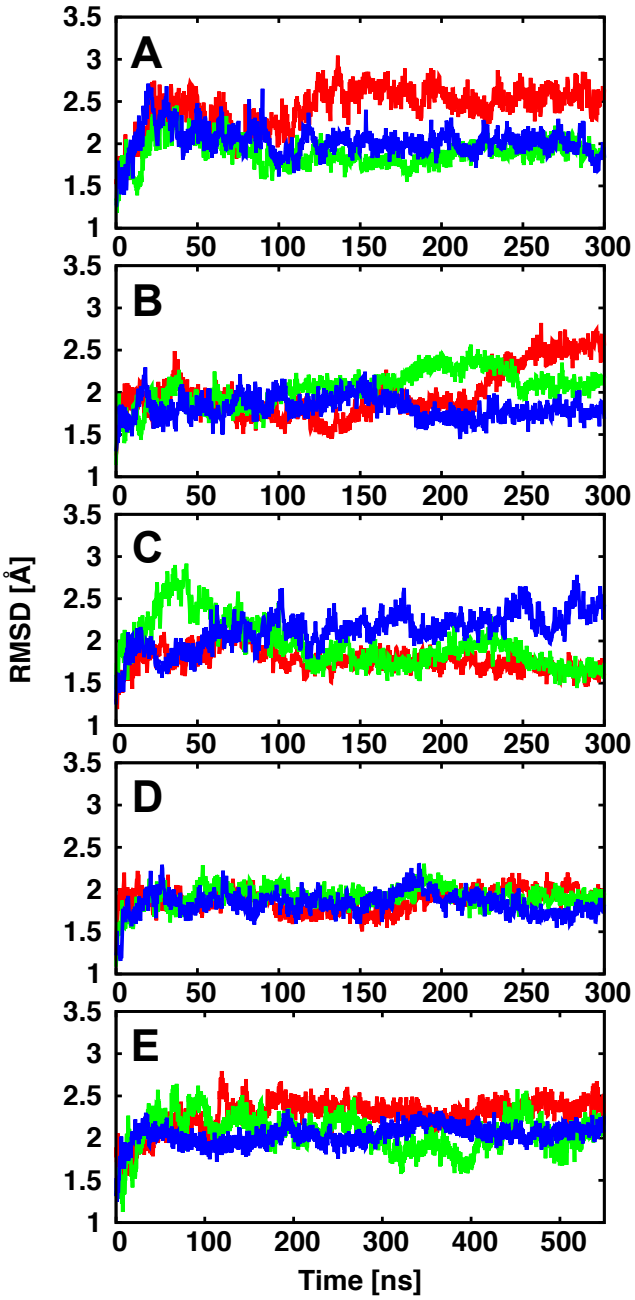

Figure S10. Time-series of the root-mean-square deviation (RMSD) of OprH β-barrel backbone atoms from the starting NMR structure in three independent systems (replica 1 with red line, replica 2 with green line, and replica 3 with blue line) in (A) Ec.Kdo, (B) Ec.K12, (C) Pa.Kdo, (D) Pa.G2, and (E) Pa.G2.O10.

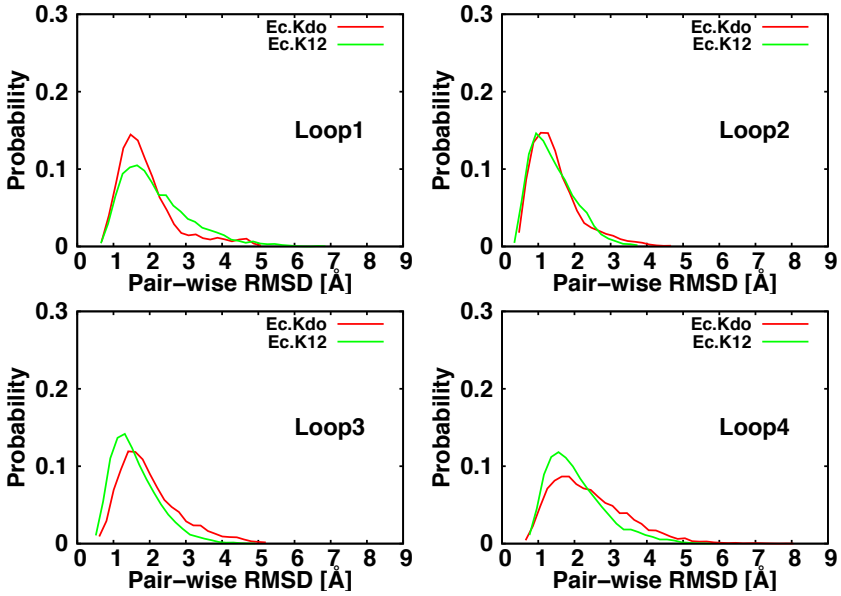

Figure S11. Pairwise RMSD distributions of the OprH loops for Ec systems, calculated by aligning each of last 100-ns snapshot structures to the β-barrel CA atoms of the initial structure and then measuring the RMSD of each pair using the loop Cα atoms.

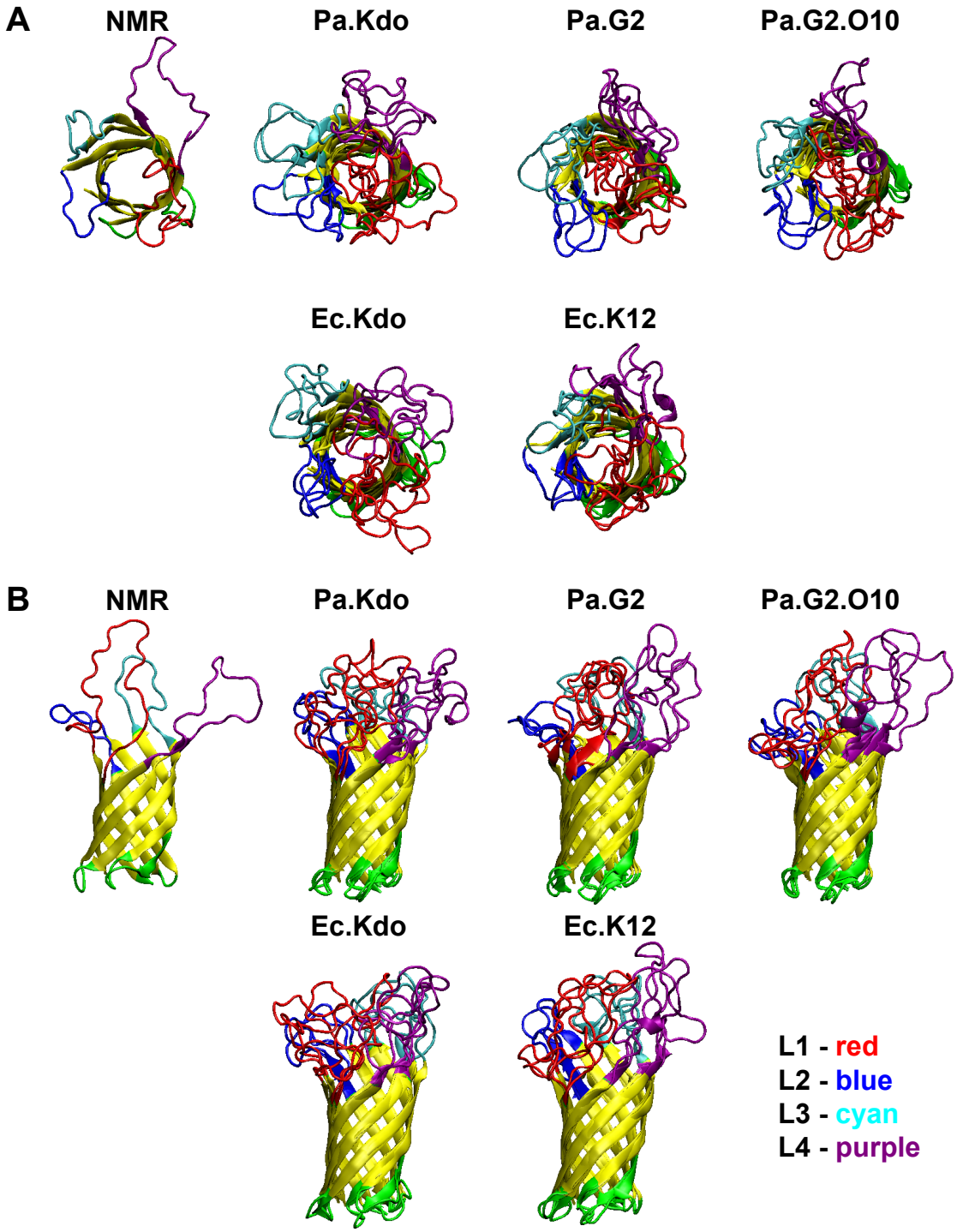

Figure S12. Comparison of the starting NMR structure and MD-averaged structures (three replicas in each system): (A) top and (B) side view.

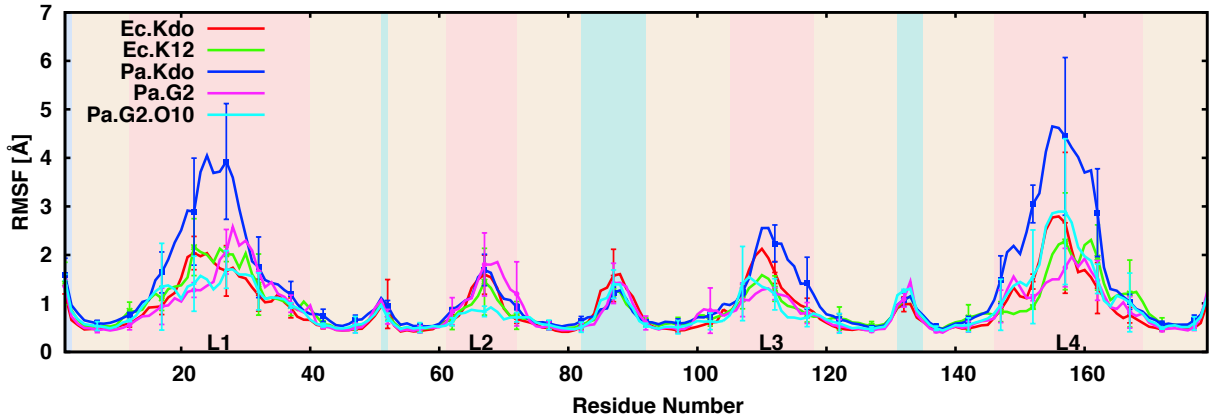

Figure S13. Root mean-square fluctuations (RMSF) of the OprH backbone atoms with the standard errors over three replicas for each system, which were calculated by aligning each structure to the β-barrel CA atoms of the initial structure using last 100-ns trajectories. Protein secondary structure is indicated by the background color: β-barrel (beige), loop (coral), and turn (turquoise).

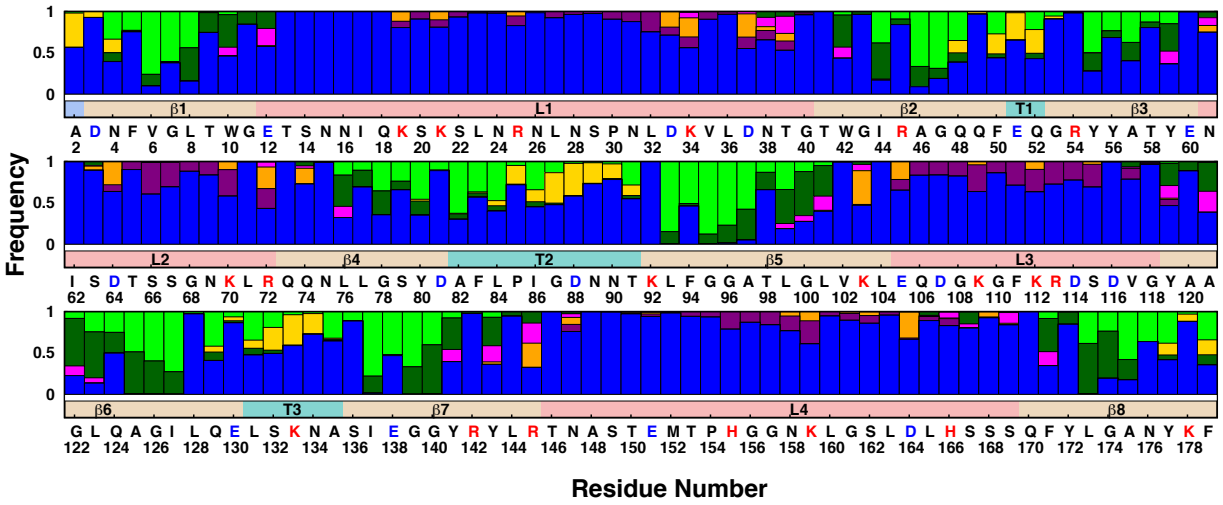

Figure S14. Interaction patterns of protein residues with their surrounding environments in Pa.Kdo. The frequencies of various environmental entities coming within 4 Å proximity of each residue of OprH are shown for water (blue), phospholipid head groups (yellow), phospholipid carbon tails (green), lipid A tails (dark green), lipid A head groups (orange), the region between lipid A head groups and lipid A tails (magenta), Kdo sugars in inner core (purple), Hep sugars in inner core (cyan), outer core (red), and O-antigen (pink). The bar below each set of patterns indicates the protein secondary structure: β-barrel (beige), loop (coral), turn (turquoise), and N terminus (light blue). The red and blue colored characters indicate basic and polar residues, respectively.

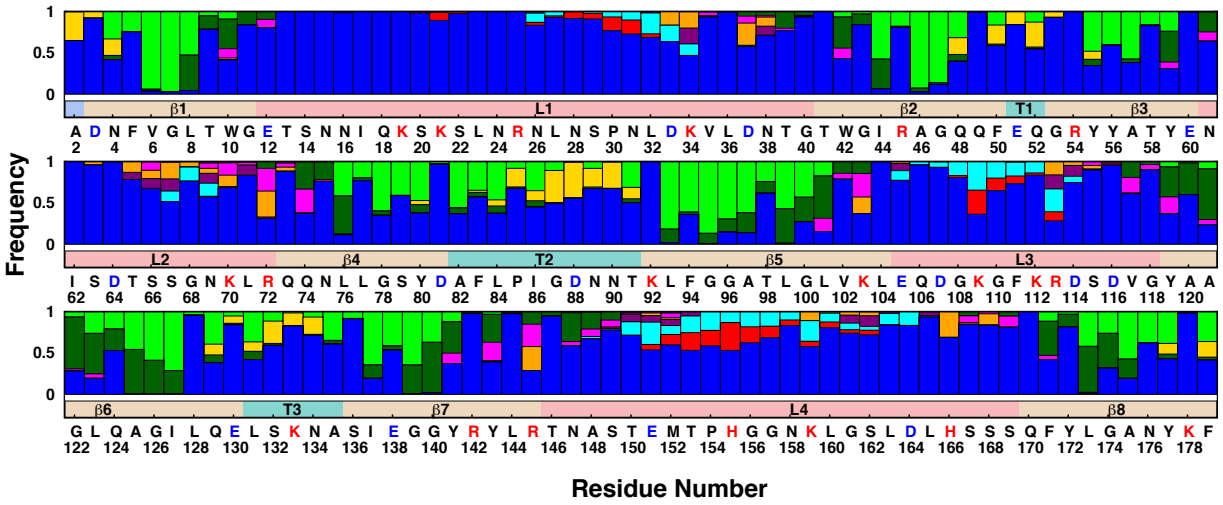

Figure S15. Interaction patterns of protein residues with their surrounding environments in Pa.G2. The frequencies of various environmental entities coming within 4 Å proximity of each residue of OprH are shown for water (blue), phospholipid head groups (yellow), phospholipid carbon tails (green), lipid A tails (dark green), lipid A head groups (orange), the region between lipid A head groups and lipid A tails (magenta), Kdo sugars in inner core (purple), Hep sugars in inner core (cyan), outer core (red), and O-antigen (pink). The bar below each set of patterns indicates the protein secondary structure: β-barrel (beige), loop (coral), turn (turquoise), and N terminus (light blue). The red and blue colored characters indicate basic and polar residues, respectively.

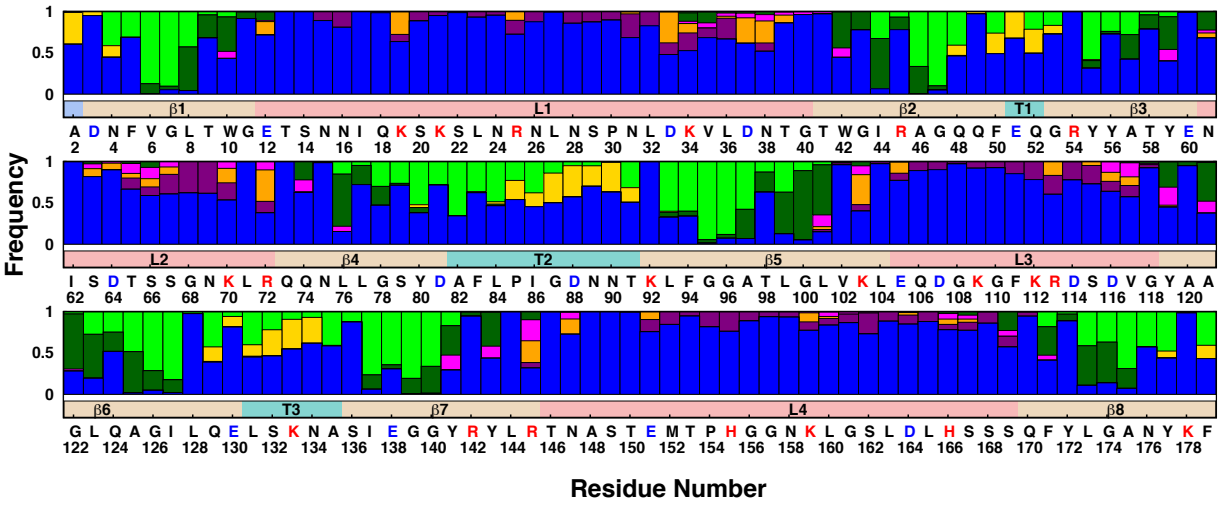

Figure S16. Interaction patterns of protein residues with their surrounding environments in Ec.Kdo. The frequencies of various environmental entities coming within 4 Å proximity of each residue of OprH are shown for water (blue), phospholipid head groups (yellow), phospholipid carbon tails (green), lipid A tails (dark green), lipid A head groups (orange), the region between lipid A head groups and lipid A tails (magenta), Kdo sugars in inner core (purple), Hep sugars in inner core (cyan), outer core (red), and O-antigen (pink). The bar below each set of patterns indicates the protein secondary structure: β-barrel (beige), loop (coral), turn (turquoise), and N terminus (light blue). The red and blue colored characters indicate basic and polar residues, respectively.

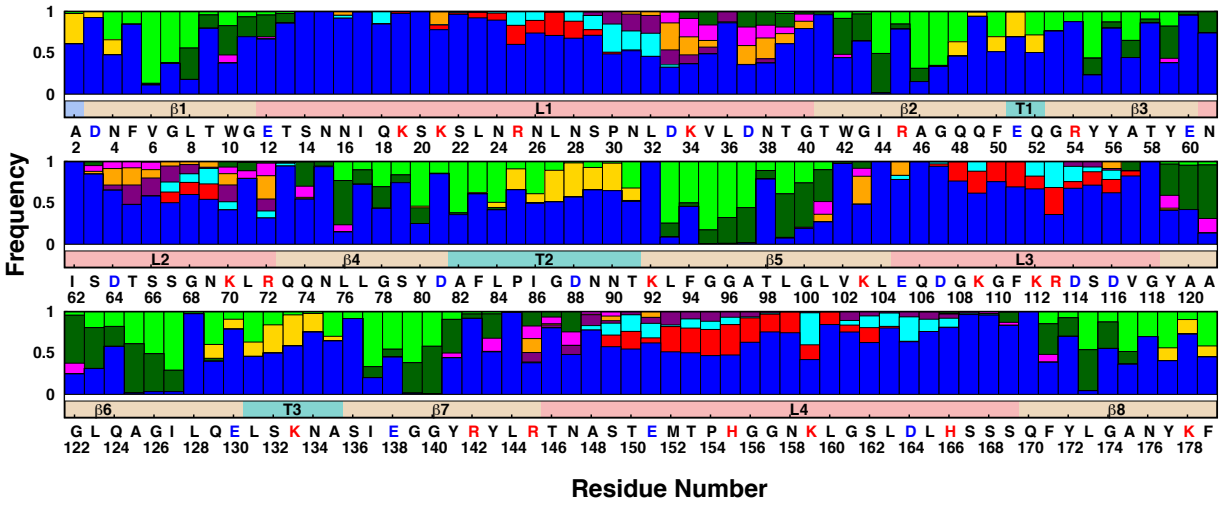

Figure S17. Interaction patterns of protein residues with their surrounding environments in Ec.K12. The frequencies of various environmental entities coming within 4 Å proximity of each residue of OprH are shown for water (blue), phospholipid head groups (yellow), phospholipid carbon tails (green), lipid A tails (dark green), lipid A head groups (orange), the region between lipid A head groups and lipid A tails (magenta), Kdo sugars in inner core (purple), Hep sugars in inner core (cyan), outer core (red), and O-antigen (pink). The bar below each set of patterns indicates the protein secondary structure: β-barrel (beige), loop (coral), turn (turquoise), and N terminus (light blue). The red and blue colored characters indicate basic and polar residues, respectively.

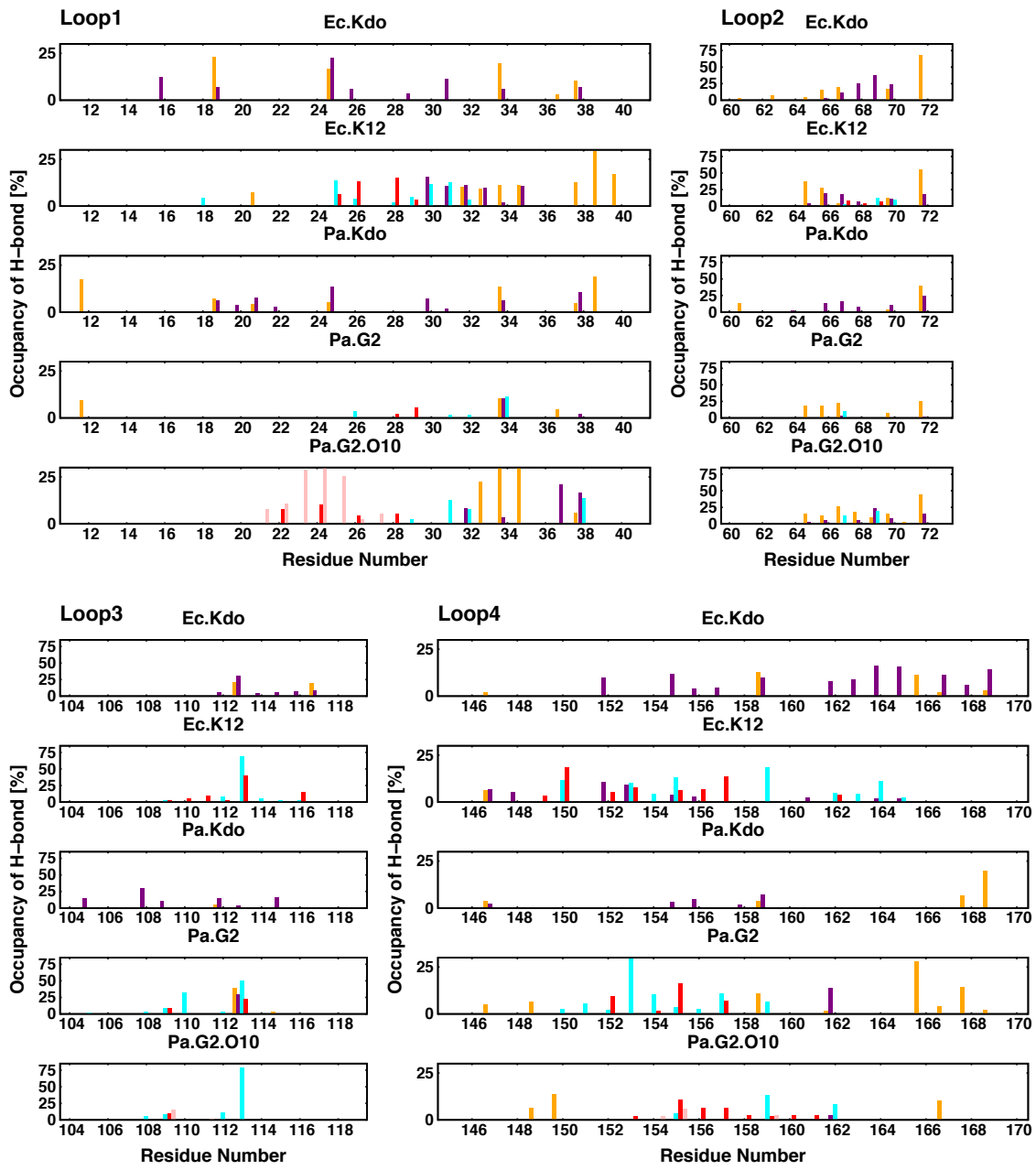

Figure S18. The occupancy of hydrogen bonding between protein loops and each component in LPS molecules (lipid A with orange, Kdo sugars in inner core with purple, Hep sugars in inner core with cyan, outer core with red, and O-antigen with pink). A hydrogen bond is assigned when donor and acceptor atoms are closer than 2.4 Å, and the occupancy indicates an average lifetime of hydrogen bonds over three replicas during last 100-ns trajectories.

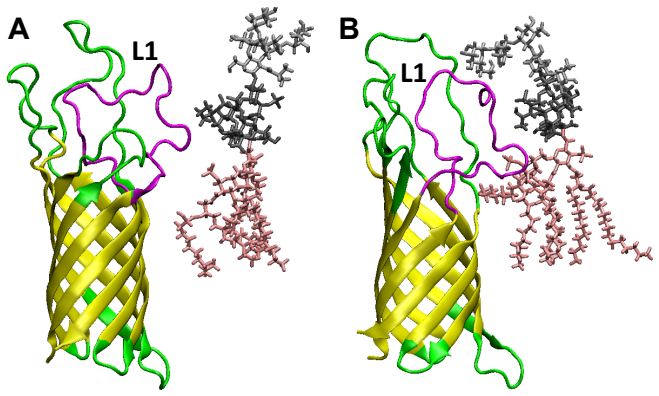

Figure S19. Representative snapshots showing interactions of L1 with LPS: (A) L1 of Pa.G2 and (B) L1 of Ec.K12. L1 is colored as magenta, whereas lipid A is represented as pink sticks, inner core sugars as dark gray sticks, outer core sugars as gray sticks.

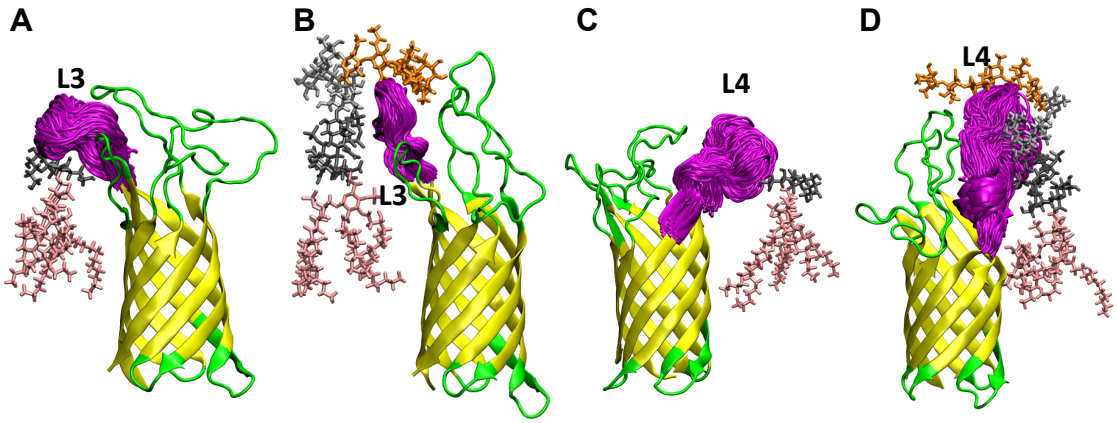

Figure S20. Fluctuations of the L3 and L4 (magenta) and the nearest LPS molecule: (A) L3 in Pa.Kdo, (B) L3 in Pa.G2.O10, (C) L4 in Pa.Kdo, and (D) L4 in Pa.G2.O10 from representative replica of the systems. Lipid A is represented as pink sticks, inner core sugars as dark gray sticks, outer core sugars as gray sticks, and O-antigen polysaccharide as orange sticks. Fluctuations of L3 and L4 were recoded from last 200ns of trajectories and snapshots were saved at every 1ns of time interval. Similar fluctuations for L3 and L4 were observed for other replicas of the representative systems.

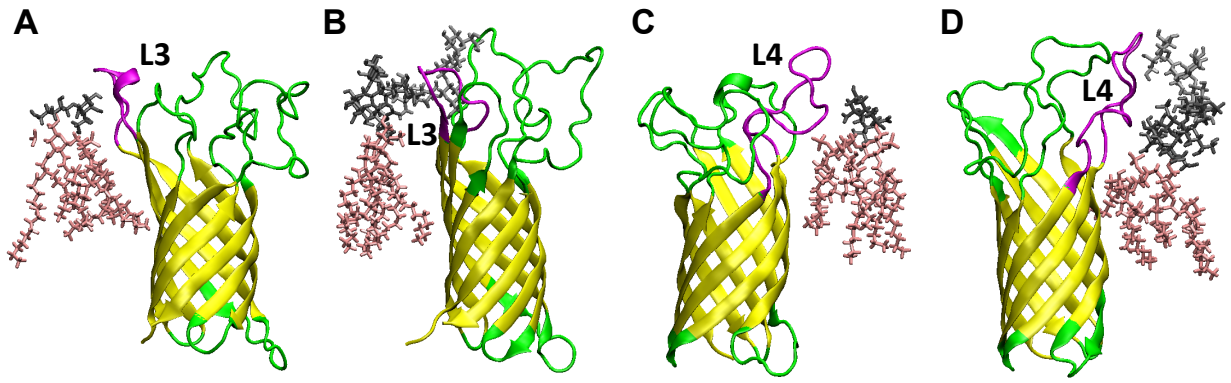

Figure S21. Representative snapshots showing interactions of L3 and L4 with LPS molecule: (A) L3 of Ec.Kdo, (B) L3 of Ec.K12, (C) L4 of Ec.Kdo, and (D) L4 of Ec.K12. Each loop that interacts with LPS molecule is colored as magenta, whereas lipid A is represented as pink sticks, inner core sugars as dark gray sticks, outer core sugars as gray sticks, and O-antigen polysaccharide as orange sticks.

Reference

1. Knirel, Y. A., and M. A. Valvano. 2011. Bacterial Lipopolysaccharides. SpringerWienNewYork.
2. Raetz, C. R., and C. Whitfield. 2002. Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635-700.