Biophysical Journal, Volume *110*

Supplemental Information

Dynamics and Interactions of OmpF and LPS: Influence on Pore Accessibility and Ion Permeability

Dhilon S. Patel, Suyong Re, Emilia L. Wu, Yifei Qi, Phillip E. Klebba, Göran Widmalm, Min Sun Yeom, Yuji Sugita, and Wonpil Im

Biophysical Journal

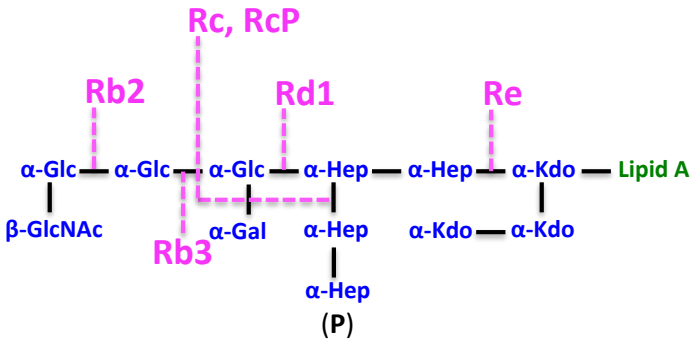
Supporting Material

Dynamics and Interactions of OmpF and LPS: Influence on Pore Accessibility and Ion Permeability

Dhilon S. Patel,[1] Suyong Re,[2,3,4] Emilia L. Wu,[1] Yifei Qi,[1] Phillip E. Klebba,[5] Göran Widmalm,[6] Min Sun Yeom,[7] Yuji Sugita,[2,3,4] and Wonpil Im[1,*]

[1]Department of Molecular Biosciences and Center for Computational Biology, The University of Kansas, Lawrence, Kansas; [2]RIKEN Theoretical Molecular Science Laboratory, Hirosawa, Wako, Saitama, Japan; [3]RIKEN Quantitative Biology Center (QBiC), IMDA 6F, and [4]RIKEN Advanced Institute for Computational Science, Chuo-ku, Kobe, Hyogo Prefecture, Japan; [5]Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, Kansas; [6]Department of Organic Chemistry and Stockholm Center for Biomembrane Research, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden; and [7]Korean Institute of Science and Technology Information, Yuseong-gu, Daejeon, Korea

*Correspondence: wonpil@ku.edu



mAb	Wtb	Wtc	Rb2	Rb3	Rc	RcP	Rd1	Re
2	✔	✔	✔	✔	✔	✔	✔	✔
10	✔	✔	✔	✔	✔	✔	✔	✔
12	✔	✔	✔	✔	✔	✔	✔	✔
14	✔	✔	✔	✔	✔	✔	✔	✔
15	✔	✔	✔	✔	✔	✔	✔	✔
6	⊙	✔	✔	✔	✔	✔	✔	✔
8	☒	✔	✔	✔	✔	✔	✔	✔
11	☒	☒	⊙	⊙	✔	✔	✔	✔
16	☒	☒	⊙	⊙	✔	✔	⊙	✔
4	☒	☒	⊙	⊙	✔	✔	✔	✔
7	☒	☒	☒	✔	✔	✔	✔	✔
60	☒	☒	☒	⊙	⊙	⊙	⊙	✔
5	☒	☒	☒	☒	✔	✔	✔	✔
9	☒	☒	☒	☒	✔	✔	⊙	✔
1	☒	☒	☒	☒	✔	✔	✔	✔
13	☒	☒	☒	⊙	☒	✔	☒	☒
19	☒	☒	☒	☒	✔	✔	☒	✔
69	☒	☒	☒	☒	☒	☒	☒	⊙
44	☒	☒	☒	☒	☒	☒	☒	☒
49	☒	☒	☒	☒	☒	☒	☒	☒
20	☒	☒	☒	☒	☒	☒	☒	☒

FIGURE S1. LPS core structure of *E. coli* K12 with chemotypes (Ra to Re). Experimental data for accessibility of mAbs with truncation of *E. coli* K12 core. ☒ represents no activity for mAb, ⊙ represents low activity for mAb, and ✔ represents high activity for mAb. mAbs are grouped to S1 (green) binding to loop L4, S2 (magenta) binding to loop L5, and S3c (red) binding to loop L1. Abbreviations: GlcNAc, *N*-acetyl-D-glucosamine; Glc, D-glucose; Gal, D-galactose; Hep, L-*glycero*-D-*manno*-heptose; P, phosphate, Kdo, 2-keto-3-deoxyoctulosonic acid. (see Ref. 14, 15 for more details).

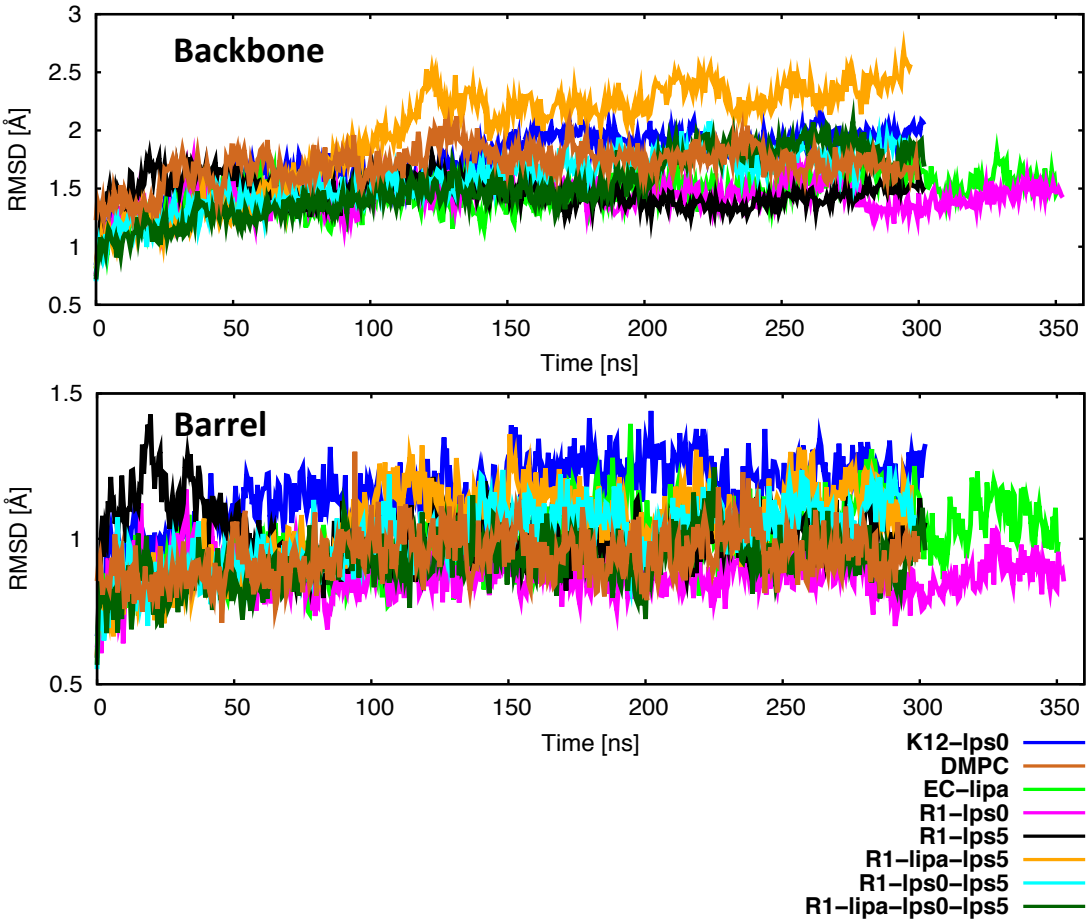


FIGURE S2. RMSD time-series of backbone and barrel atoms.

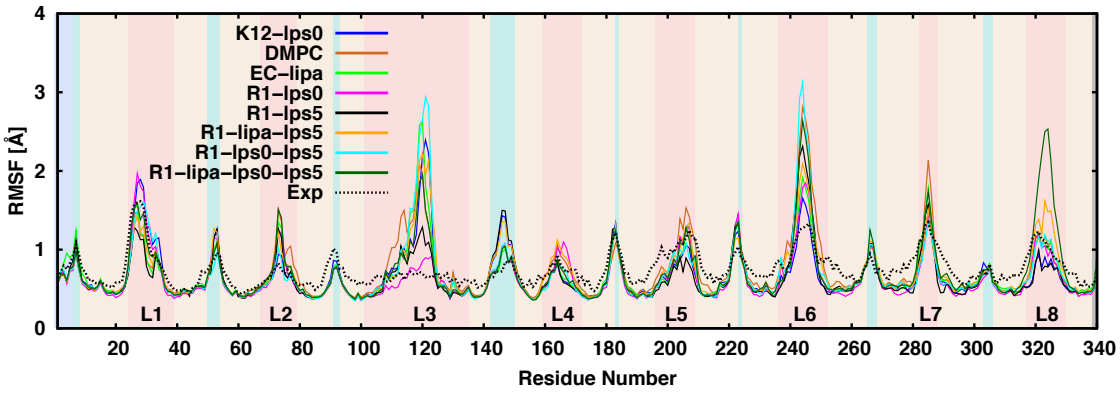


FIGURE S3. Root mean-square fluctuations (RMSFs) of the OmpF backbone atoms compared with experimental (Exp). Protein secondary structure is indicated by the background color: β-barrel (beige), loop (coral), turn (turquoise), N terminus (light blue), and C terminus (gray).

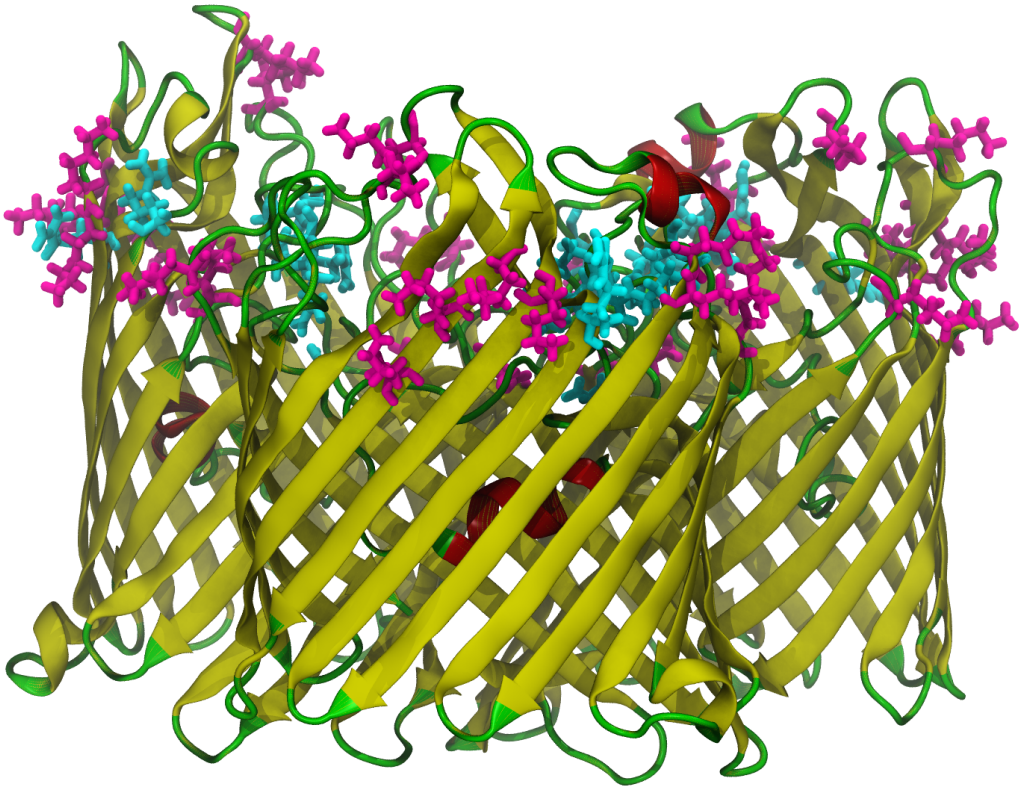


FIGURE S4. Lys (magenta) and Arg (cyan) residues (z > 10) that are prominently located on the outer surface.

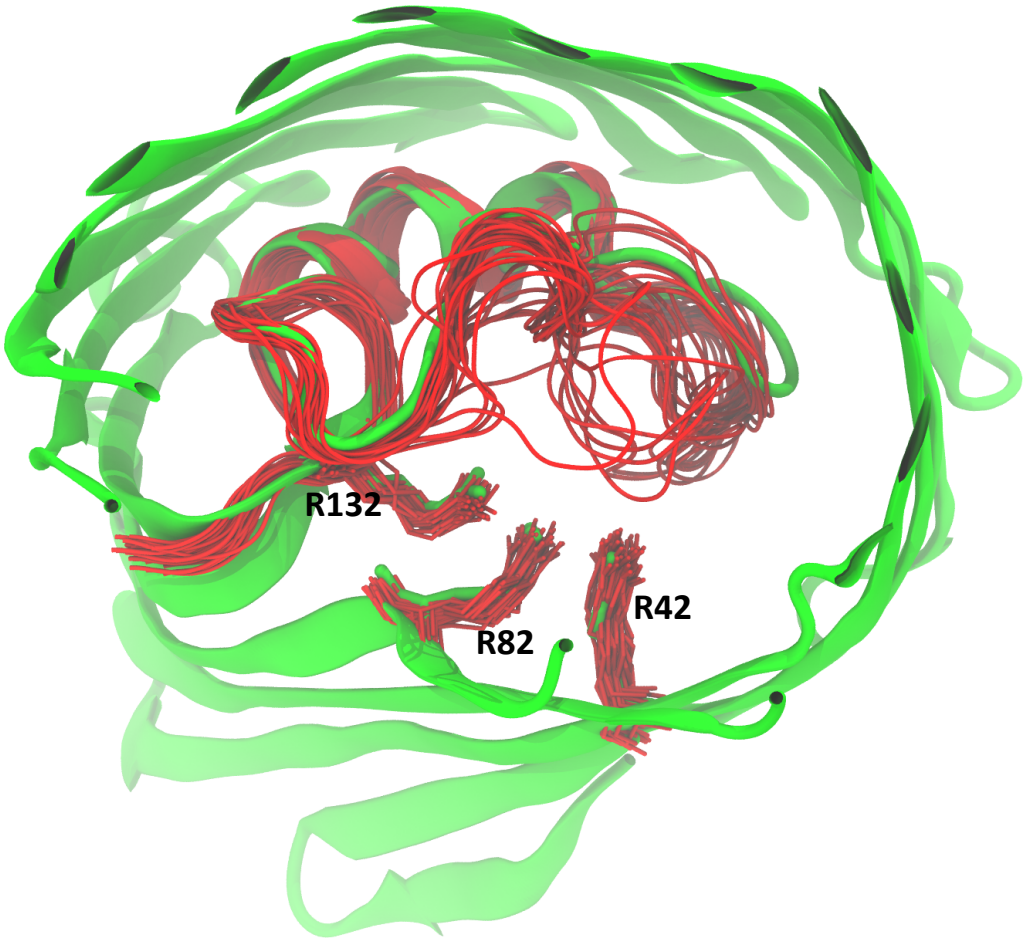


FIGURE S5. Fluctuations (Red) of the loop L3 and three cationic residues R42, R82, and R132 in one of the monomers superimposed with X-ray structure (Green).

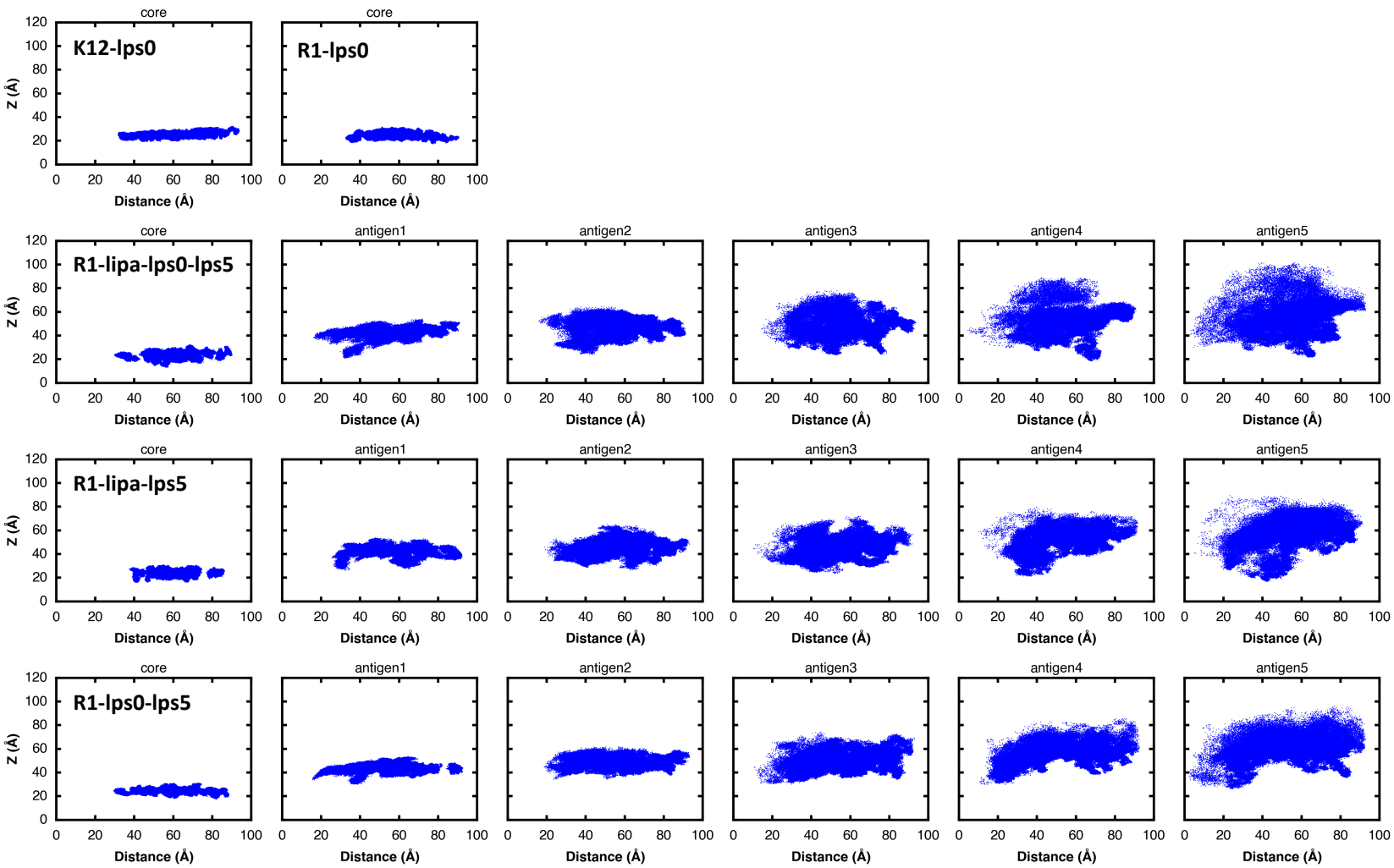


FIGURE S6. Flexibility of the core and O-antigens. 2D-scatter plots of the center of mass of core and O-antigen repeating units along the Z-axis and the distance from the center of mass of OmpF trimer for all systems. The bilayer is re-centered to z = 0.

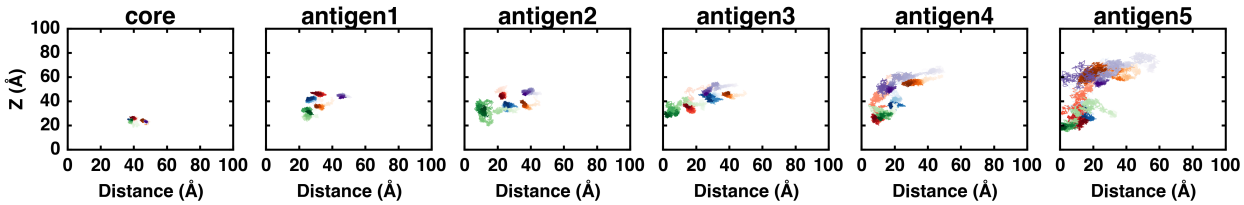


FIGURE S7. Representative examples of O-antigen flexibility during the simulation time. The positions of core and O-antigens of five selected LPS molecules are shown with lightest to darkest shade depicting transition from initial position to final position at end of simulation. Each color scheme in all figures represents positions of individual LPS molecule.

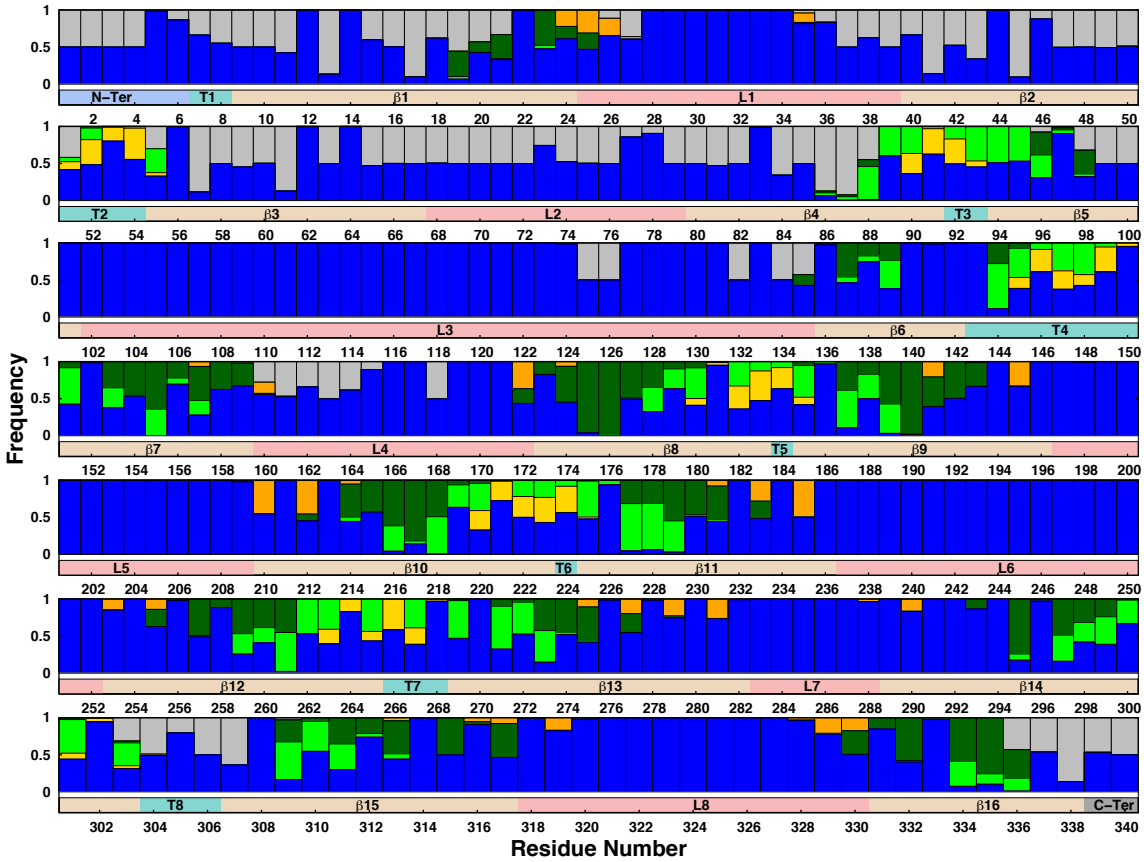


FIGURE S8A. Interaction patterns of OmpF residues with their surrounding environments in EC-lipa. For color coding please check main text Fig. 5.

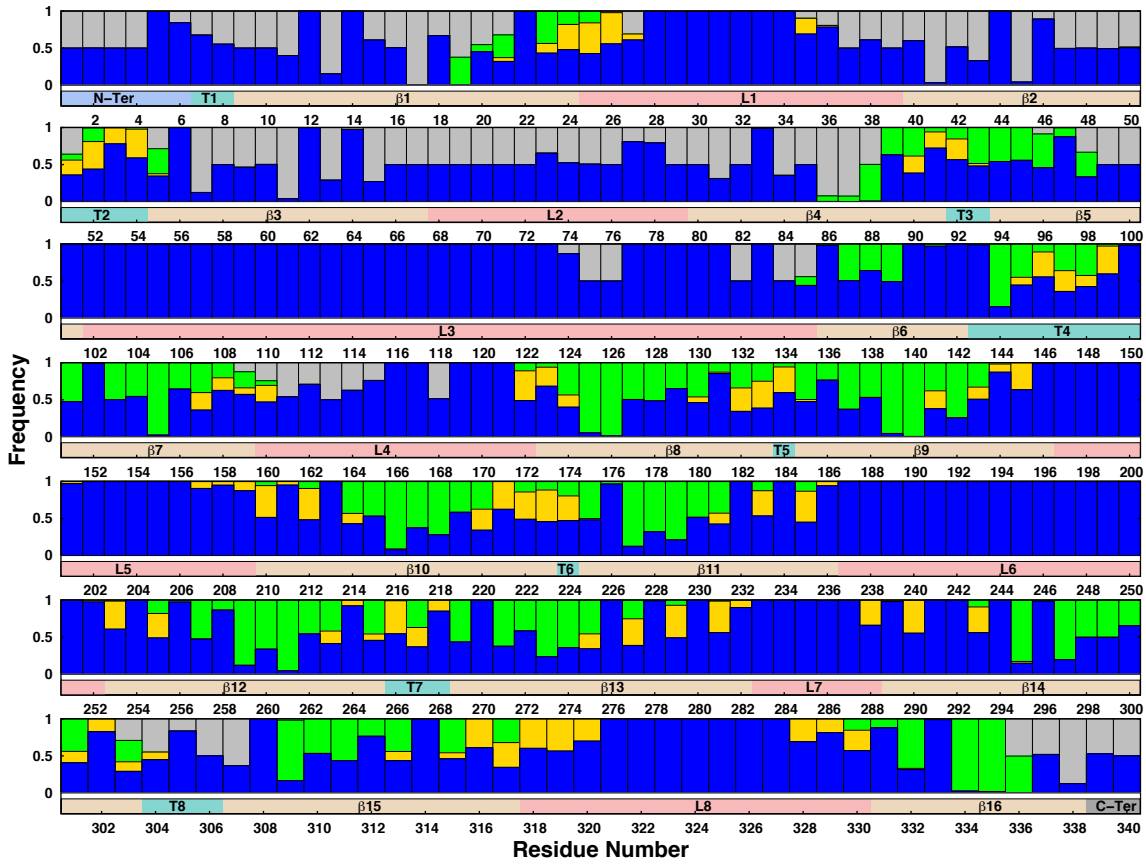


FIGURE S8B. Interaction patterns of OmpF residues with their surrounding environments in DMPC. For color coding please check main text Fig. 5.

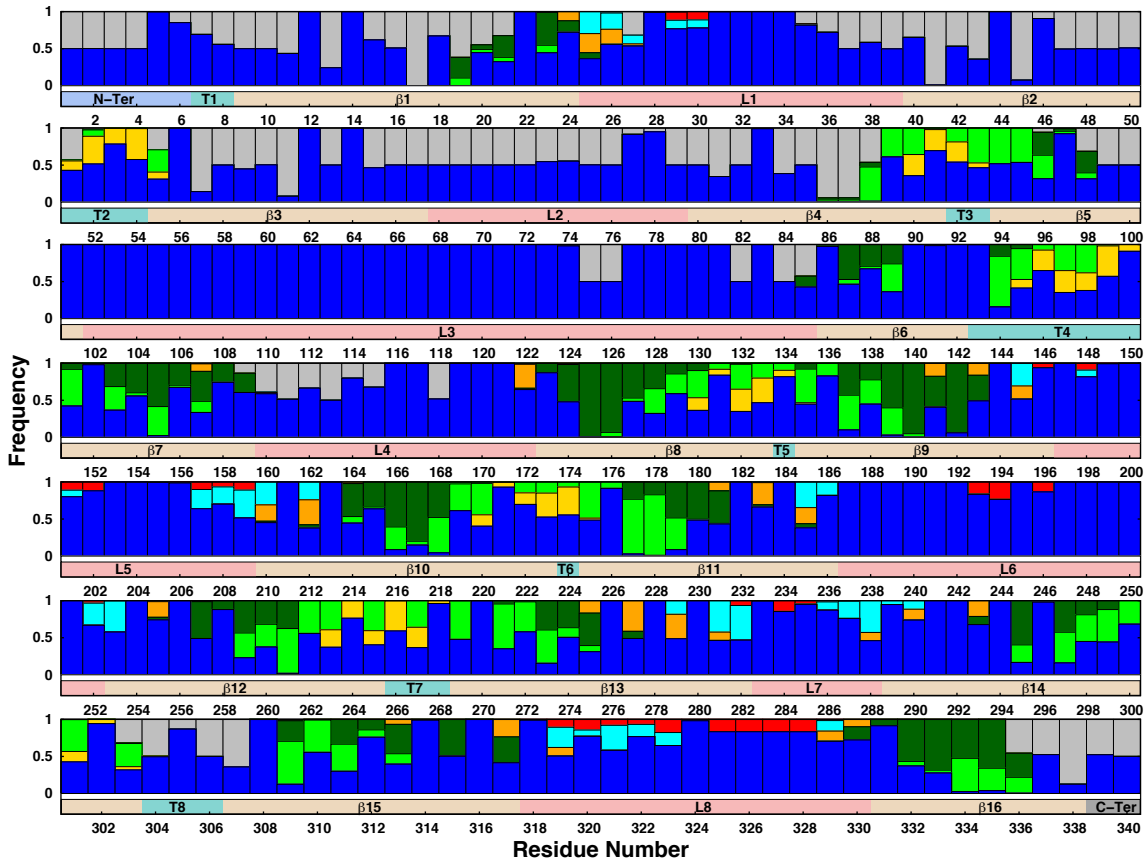


FIGURE S8C. Interaction patterns of OmpF residues with their surrounding environments in R1-lps0. For color coding please check main text Fig. 5.

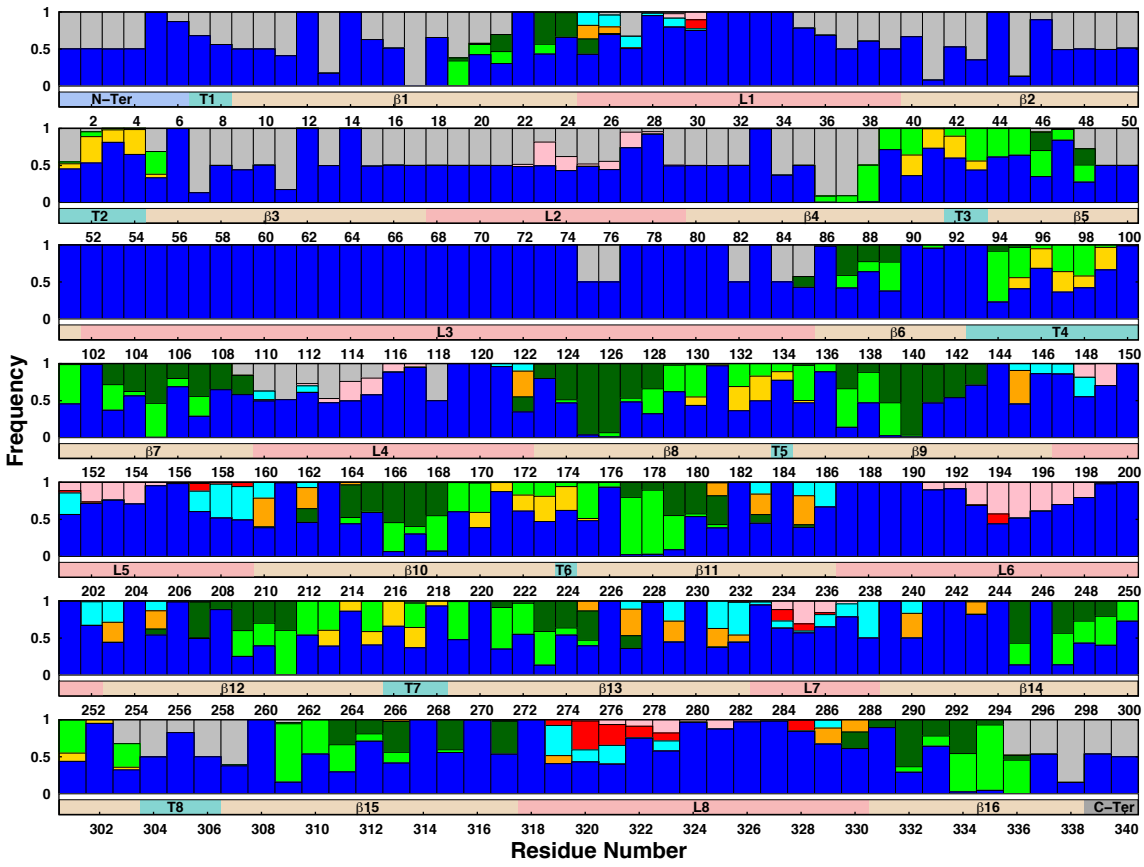


FIGURE S8D. Interaction patterns of OmpF residues with their surrounding environments in R1-lps5. For color coding please check main text Fig. 5. In addition the graph shows, for each residue, the frequency of occurrence within 5 Å of an O-antigen (pink).

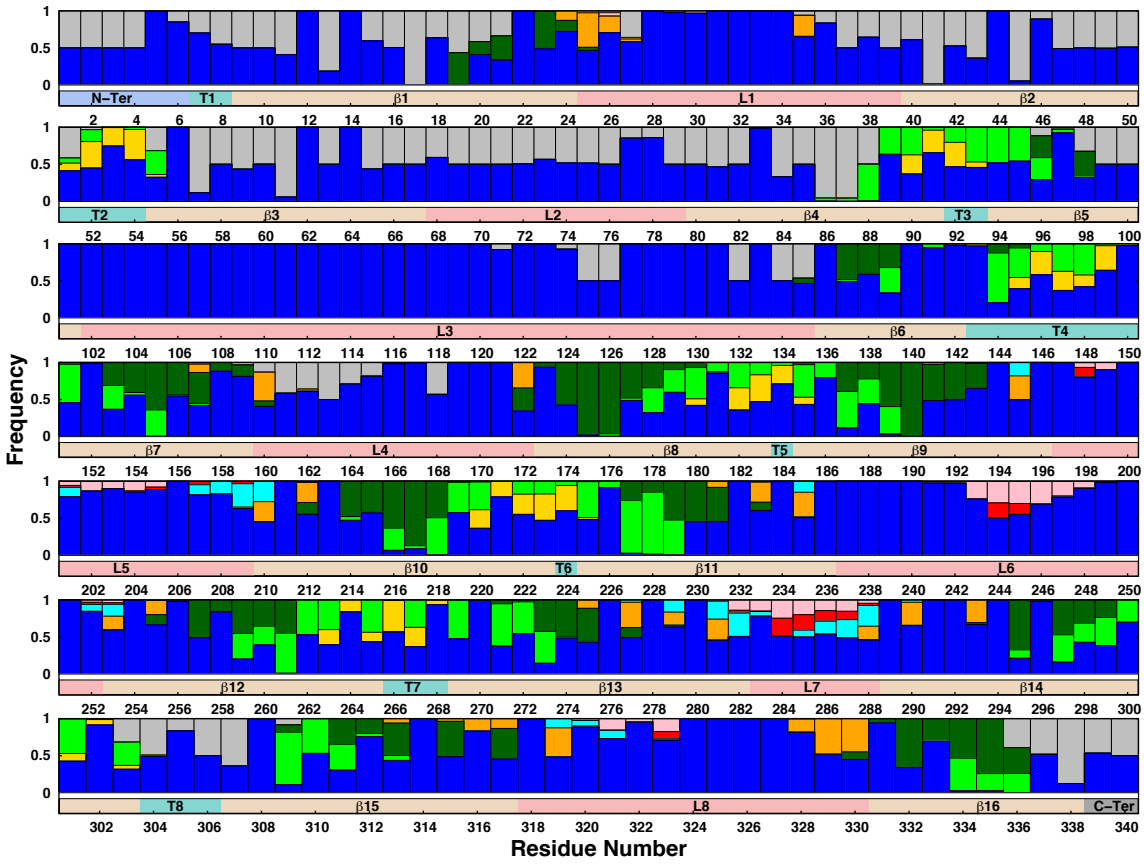


FIGURE S8E. Interaction patterns of OmpF residues with their surrounding environments in R1-lipa-lps5. For color coding please check main text Fig. 5. In addition the graph shows, for each residue, the frequency of occurrence within 5 Å of an O-antigen (pink).

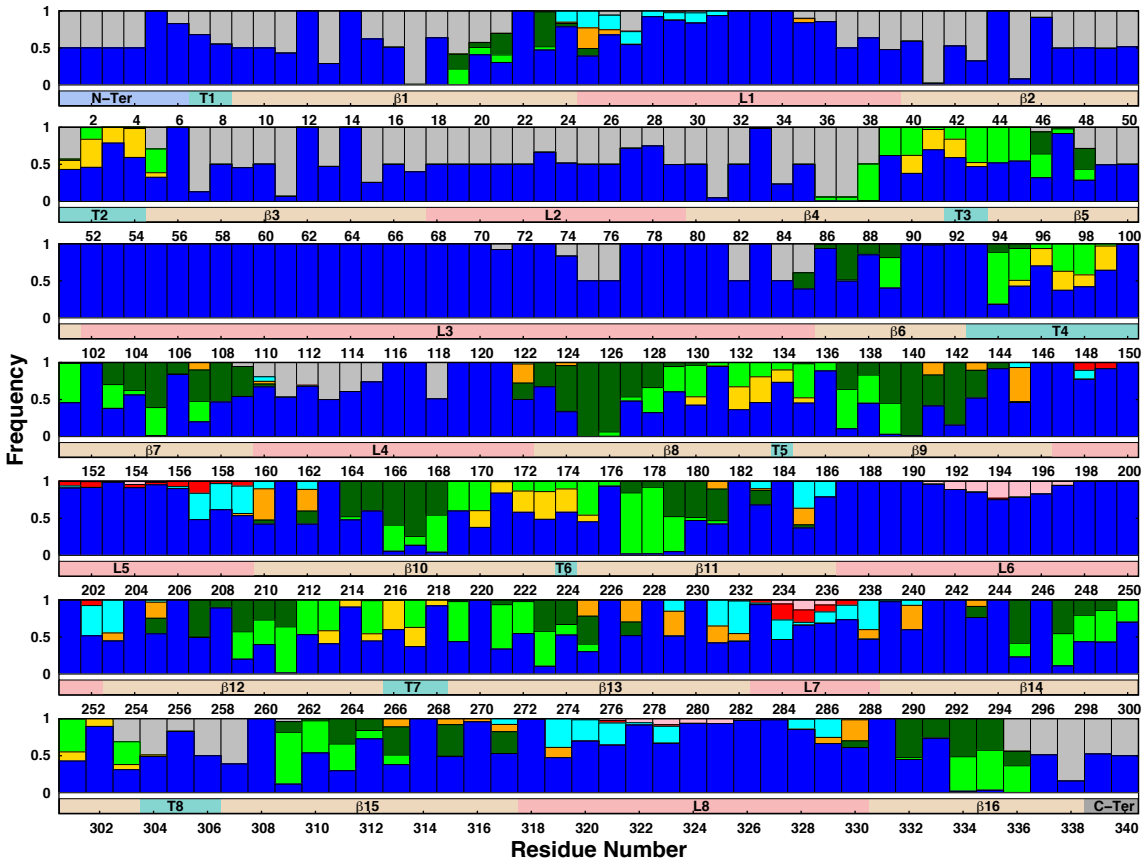


FIGURE S8F. Interaction patterns of OmpF residues with their surrounding environments in R1-lps0-lps5. For color coding please check main text Fig. 5. In addition the graph shows, for each residue, the frequency of occurrence within 5 Å of an O-antigen (pink).

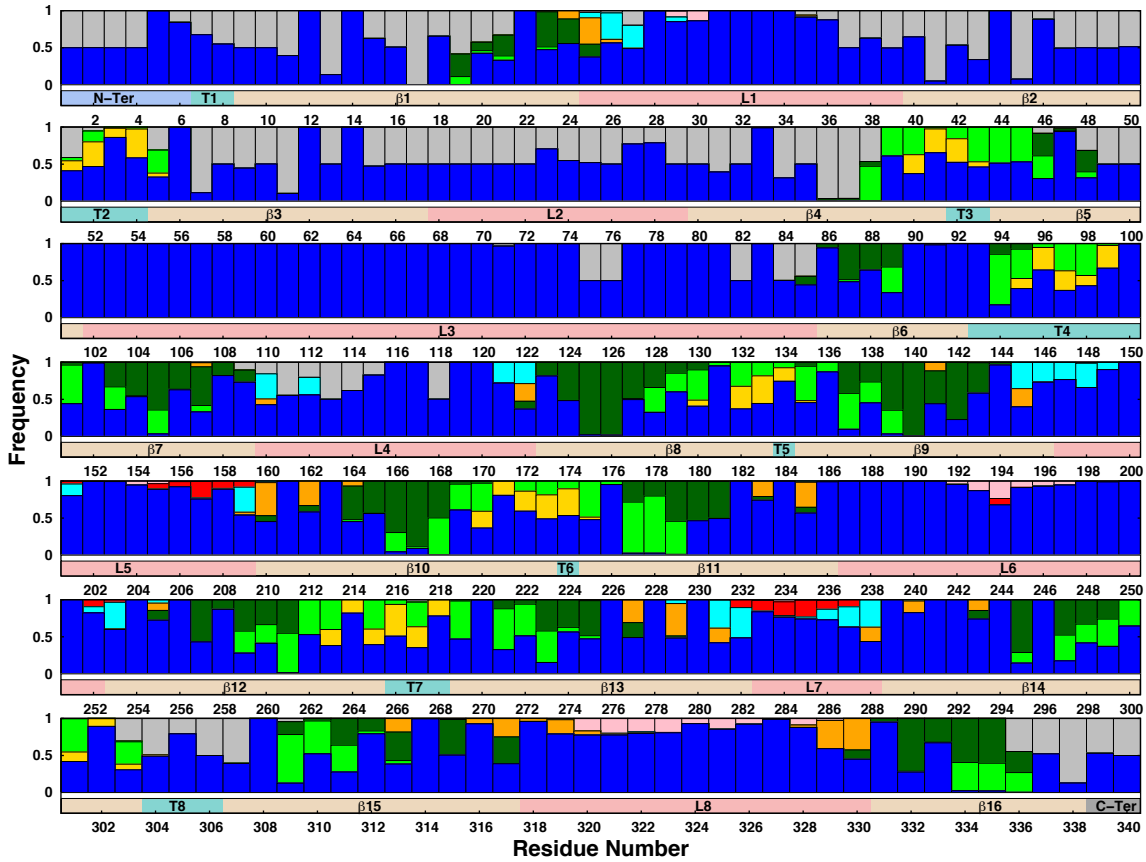


FIGURE S8G. Interaction patterns of OmpF residues with their surrounding environments in R1-lipa-lps0-lps5. For color coding please check main text Fig. 5. In addition the graph shows, for each residue, the frequency of occurrence within 5 Å of an O-antigen (pink).

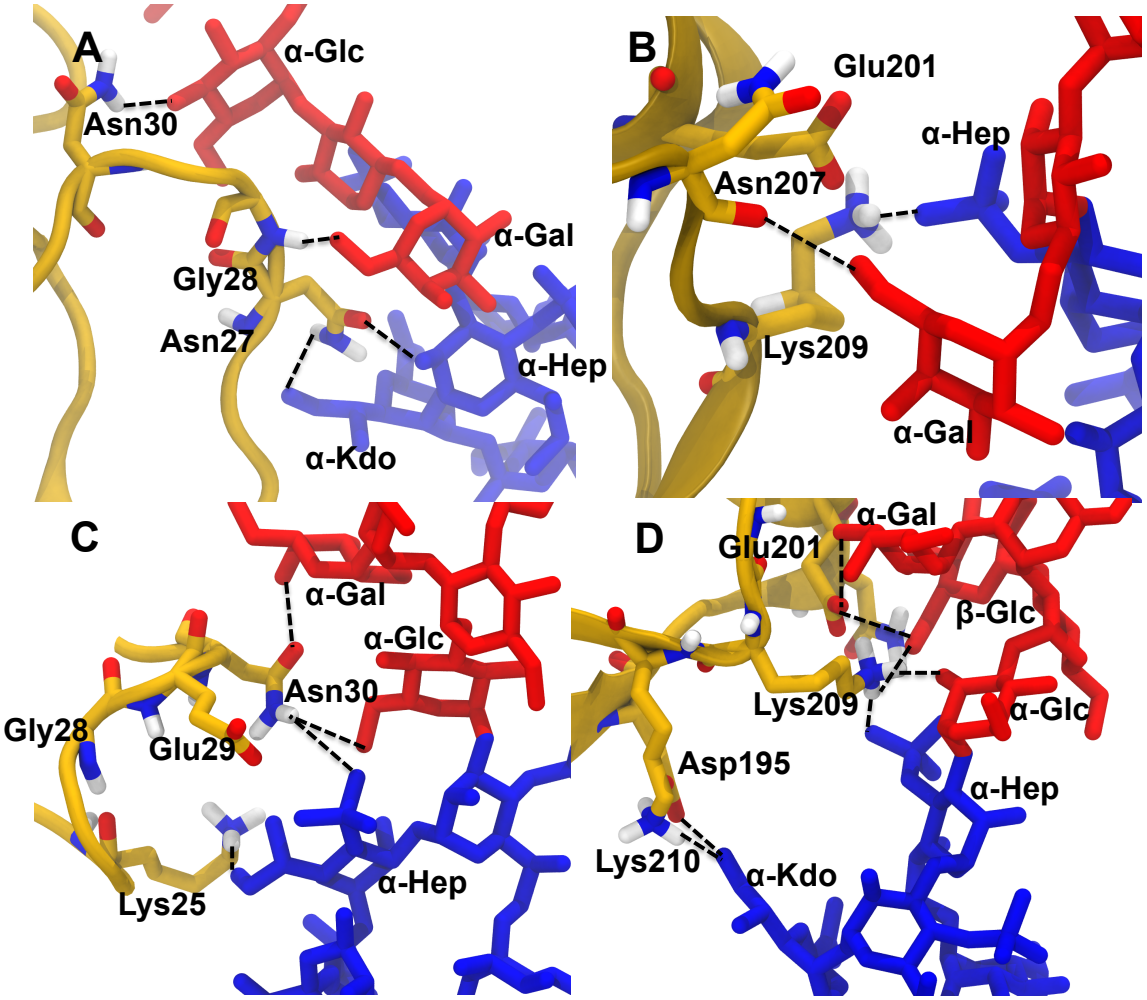


FIGURE S9. Snapshots of interactions of L1 (epitope site S3c) and L5 (epitope site S2) loop residues with LPS in K12-lps0 (A and B) and in R1-lps0 (C and D) systems. Protein residues are colored yellow, whereas LPS inner core and outer core sugars are colored blue and red respectively. Exocyclic hydroxylmethyl (CH_2OH) group of α-Gal (outer core sugar) of K12 core makes H-bonding with backbone NH of Gly28 and C=O of Asn207 in loop L1 and L5, respectively (A and B). In addition, K12-lps0 system showed H-bonding and electrostatic interactions between α-Hep (inner core sugars) and protein residues (especially Asn27 in L1 and Lys209/Glu201 in L5). Variable α-Hep of inner core and terminal α-Gal and α-Glc/β-Glc of outer core sugars in R1-lps0 system are interacting with loop L1 and L5 residues (C and D).

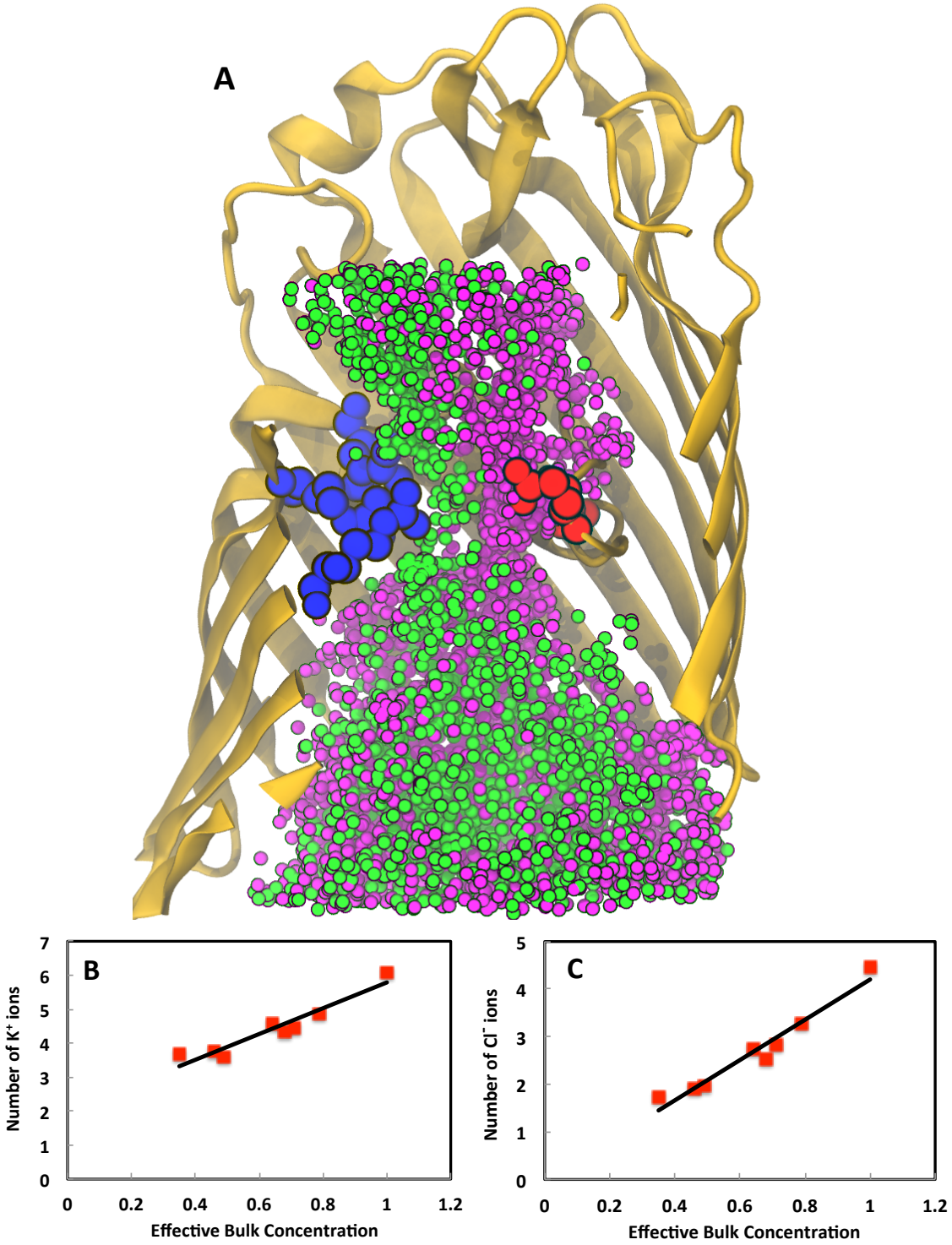


FIGURE S10. Number of K^+ and Cl^- as a function of effective KCl bulk concentration. (A) Superposition of ion positions extracted every 2 ns along a 300-ns MD trajectory from all three monomers in K12-lps0 system (magenta for K^+ and green for Cl^-) and with constriction zone defined by positively charged residues Arg42, Arg82, and Arg132 (blue) and negatively charged residues Asp113 and Glu117 (red). Number of (B) K^+ and (C) Cl^- as a function of effective KCl bulk concentration.

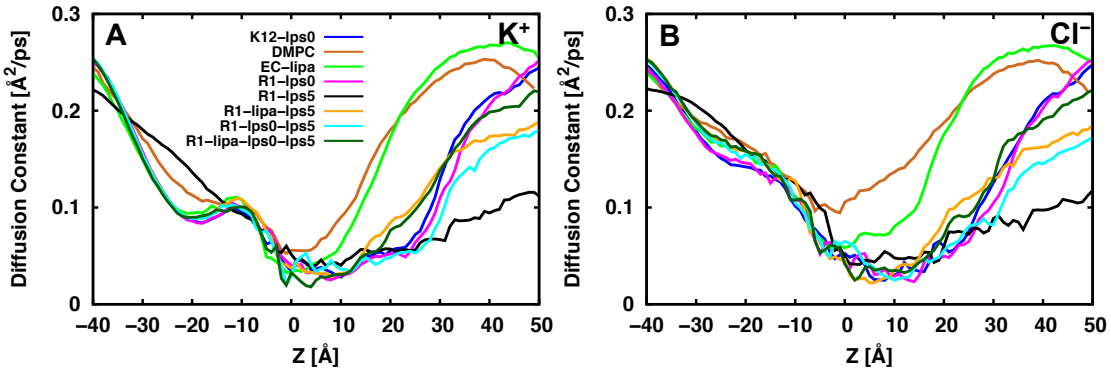


FIGURE S11. The diffusion constant profiles of (A) K^+ and (B) Cl^- ions along the channel axis. The OmpF's center of mass in each system is re-centered to z = 0. PLs reside on the side of z < 0 and LPS on the side of z > 0.

Table S1. Details of size and composition of all systems.

Segment\System	K12-lps0	EC-lipa	R1-lps0	R1-lps5	R1-lipa-lps5	R1-lps0-lps5	R1-lipa-lps0-lps5	DMPC
Protein	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)	340 x 3 (PROA/B/C)
Membrane	200 (PPPE, PVPG, PVCL2)	200 (PPPE, PVPG, PVCL2)	200 (PPPE, PVPG, PVCL2)	200 (PPPE, PVPG, PVCL2)	200 (PPPE, PVPG, PVCL2)	200 (PPPE, PVPG, PVCL2)	200 (PPPE, PVPG, PVCL2)	312 (DMPC)
Lipa	-	77	-	-	38	-	25	-
Lps0	73	-	73	-	-	36	24	-
Lps5	-	-	-	69	37	35	24	-
P-bound Water	618	618	618	618	618	618	618	-
Water	34,414	33,128	31,249	43,129	50,563	47,385	51,611	23,382
$Ca^{2+}/K^{+}/Cl^{-}$	365/470/380	154/470/379	365/470/380	345/703/613	261/474/384	355/470/380	290/470/380	342/312
No. Atoms	188,178	165,658	176,420	250,731	247,937	244,223	245,521	122,835
Box size($Å^3$)	132.8 x 132.8 x 116.6	132.7 x 132.7 x 102.2	132.8 x 132.8 x 109.7	132.7 x 132.7 x 173.1	133.4 x 133.4 x 150.6	132.7 x 132.7 x 150.8	132.7 x 132.7 x 150.7	119.1 x 119.1 x 88.2
Simulation Length in ns	300	350	350	300	300	300	300	300

Table S2. The RMSDs of main structural elements: backbone (BB), side chain (SC), barrel, loop, and turn of three monomers of OmpF in all systems. The standard errors for three monomers are also given.

System	Monomer	All atoms	BB	SC	Barrel	Loop	Turn
	ProA	2.29	1.95	2.61	1.01	2.84	1.35
DMPC	ProB	2.08	1.67	2.44	0.90	2.40	1.27
	ProC	1.93	1.46	2.33	0.90	1.98	1.37
Std. err.		*0.09*	*0.12*	*0.07*	*0.03*	*0.20*	*0.03*
	ProA	2.02	1.58	2.40	1.08	2.06	1.58
EC-lipa	ProB	1.83	1.46	2.16	0.93	1.95	1.46
	ProC	1.66	1.28	2.00	0.95	1.60	1.34
Std. err.		*0.08*	*0.07*	*0.10*	*0.04*	*0.11*	*0.06*
	ProA	2.06	1.69	2.40	1.30	2.11	1.69
K12-lps0	ProB	2.38	2.01	2.73	1.09	2.86	1.67
	ProC	1.83	1.43	2.17	1.08	1.78	1.52
Std. err.		*0.13*	*0.14*	*0.14*	*0.06*	*0.26*	*0.04*
	ProA	2.18	1.80	2.52	0.94	2.60	1.40
R1-lps0	ProB	1.72	1.24	2.12	0.79	1.65	1.25
	ProC	1.55	1.13	1.91	0.89	1.32	1.41
Std. err.		*0.15*	*0.17*	*0.15*	*0.04*	*0.31*	*0.04*
	ProA	2.15	1.77	2.50	1.11	2.41	1.63
R1-lps5	ProB	1.80	1.40	2.15	1.02	1.73	1.60
	ProC	1.66	1.17	2.05	0.90	1.38	1.47
Std. err.		*0.12*	*0.14*	*0.11*	*0.05*	*0.24*	*0.04*
	ProA	2.69	2.36	3.00	1.20	3.44	1.67
R1-lipa-lps5	ProB	1.92	1.55	2.26	0.88	2.17	1.30
	ProC	2.28	1.92	2.61	1.01	2.74	1.59
Std. err.		*0.18*	*0.19*	*0.18*	*0.08*	*0.30*	*0.09*
	ProA	1.94	1.48	2.32	0.83	2.09	1.23
R1-lps0-lps5	ProB	1.88	1.51	2.21	1.03	1.95	1.61
	ProC	2.05	1.70	2.37	1.12	2.29	1.55
Std. err.		*0.04*	*0.06*	*0.04*	*0.07*	*0.08*	*0.09*
	ProA	1.73	1.30	2.10	0.88	1.66	1.45
R1-lipa-lps0-lps5	ProB	1.66	1.25	2.01	0.94	1.55	1.34
	ProC	2.35	1.92	2.74	0.93	2.81	1.30
Std. err.		*0.18*	*0.18*	*0.19*	*0.02*	*0.33*	*0.04*

Table S3. Numbers of K^+ and Cl^- for all the systems in each pore along with the occupancy ratio of ions (N_K/N_{Cl}). The standard errors for all three pores are presented in parentheses.

System	K^+			Avg. K^+ (N_K)	Cl^-			Avg. Cl^- (N_{Cl})	Avg. N_K/N_{Cl}
	ProA	**ProB**	**ProC**		**ProA**	**ProB**	**ProC**		
DMPC	4.7	5.1	4.8	4.9 (0.08)	3.1	3.3	3.4	3.3 (0.08)	1.49
EC-lipa	4.2	4.7	4.8	4.6 (0.15)	2.5	3.0	2.7	2.7 (0.12)	1.70
K12-lps0	4.6	4.0	4.4	4.3 (0.14)	2.9	1.9	2.8	2.5 (0.27)	1.72
R1-lps0	4.4	4.9	4.1	4.5 (0.20)	3.0	2.9	2.6	2.8 (0.10)	1.61
R1-lps5	6.0	6.5	5.8	6.1 (0.18)	4.5	4.6	4.3	4.5 (0.07)	1.36
R1-lipa-lps5	3.3	4.0	4.0	3.8 (0.19)	1.6	2.4	1.7	1.9 (0.22)	2.00
R1-lps0-lps5	3.6	3.8	3.4	3.6 (0.09)	1.8	1.9	2.2	2.0 (0.12)	1.80
R1-lips-lps0-lps5	4.0	3.6	3.4	3.7 (0.16)	2.1	1.9	1.2	1.7 (0.24)	2.18

Movie S1: Movie (top view) of O-antigen polysaccharides in R1-lps5 system with inner core (gray), antigen1 (orange), antigen2 (red), antigen3 (ochre), antigen4 (cyan), and antigen5 (blue) stick model representation. For clarity, ions and water molecules are not shown. The movie represents flexibility of O-anitgens above the OmpF vestibule from the beginning to the end of simulations (~300 ns).