Supporting Information

Outer Membranes of Polymyxin-Resistant *Acinetobacter baumannii* with Phosphoethanolamine-modified Lipid A and Lipopolysaccharide Loss Display Different Atomic-Scale Interactions with Polymyxins

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Table S1. Number of Ca^{2+} bound to the outer leaflet of the outer membrane (OM).

System	Total number of bound Ca^{2+}	Number of bound Ca^{2+} per lipid
Lipid A OM	60	1.82
Lipid A-pEtN OM	48	1.45
LPS-deficient OM	19	0.24

Table S2. Components of the outer membrane models.

Outer membrane	Membrane composition	Lipid number	Membrane size
Lipid A	Outer leaflet: 75% lipid A, 18% POPE, 4% POPG, 3% PVCL2 Inner leaflet: 72% POPE, 16% POPG, 12% PVCL2	Lipid A: 25 POPE: 60 POPG: 14 PVCL2: 11	7.6 nm × 7.6 nm (x-y plane)
Lipid A-pEtN	Outer leaflet: 75% lipid A-pEtN, 14.5% POPE, 6.5% POPG, 4% PVCL2 Inner leaflet: 58% POPE, 26% POPG, 16% PVCL2	Lipid A-pEtN: 25 POPE: 48 POPG: 22 PVCL2: 14	7.7 nm × 7.7 nm (x-y plane)
LPS-deficient	Outer and inner leaflets: 64% POPE, 26% POPG, 10% PVCL2	POPE: 100 POPG: 40 PVCL2: 16	7.6 nm × 7.6 nm (x-y plane)

POPE: phosphoethanolamine (16:0-18:1); POPG: phosphoglycerol (16:0-18:1); PVCL2: cardiolipin (16:0, 18:1-16:0, 18:1).

Table S3. Details of molecular dynamics simulation systems.

Outer membrane	System	Colistin number	Simulation method	Simulation length
Lipid A	OM	0	Unconstrained MD	100 ns
	OM + colistin	1	Steered MD	50 ns (n = 3)
	OM + colistin	1	Umbrella sampling MD	50 ns (×24 windows)
Lipid A-pEtN	OM	0	Unconstrained MD	100 ns
	OM + colistin	1	Steered MD	50 ns (n = 3)
	OM + colistin	1	Umbrella sampling MD	50 ns (×24 windows)
LPS-deficient	OM	0	Unconstrained MD	100 ns
	OM + colistin	1	Steered MD	50 ns (n = 3)
	OM + colistin	1	Umbrella sampling MD	50 ns (×28 windows)

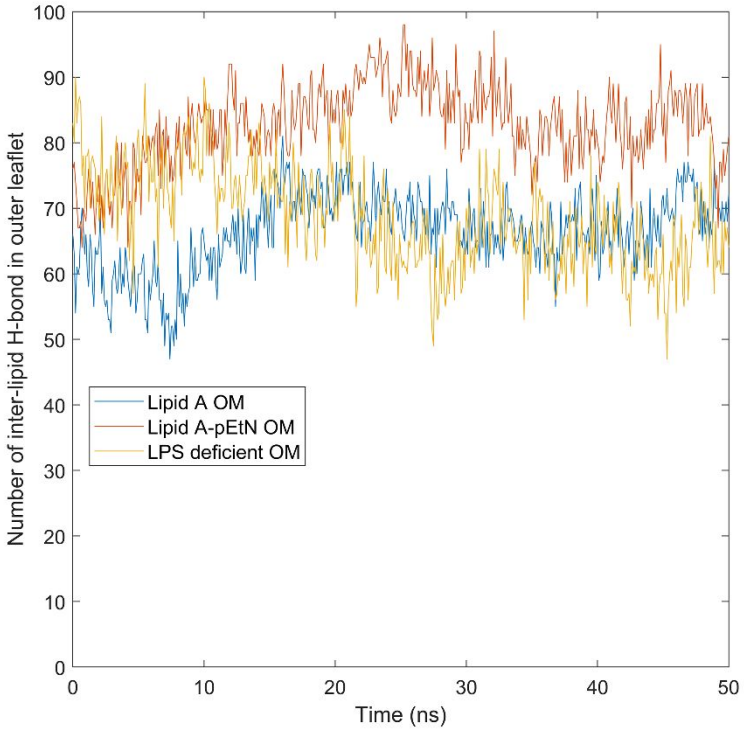

Figure S1. Changes in the number of the inter-lipid hydrogen bonds in different systems calculated based on the steered simulation trajectories. The colistin penetration decreased the intramolecular hydrogen bonds after approximately 18-ns, 10-ns and 25-ns simulations in the lipid A, lipid A-pEtN and LPS-deficient OM systems, repectively.

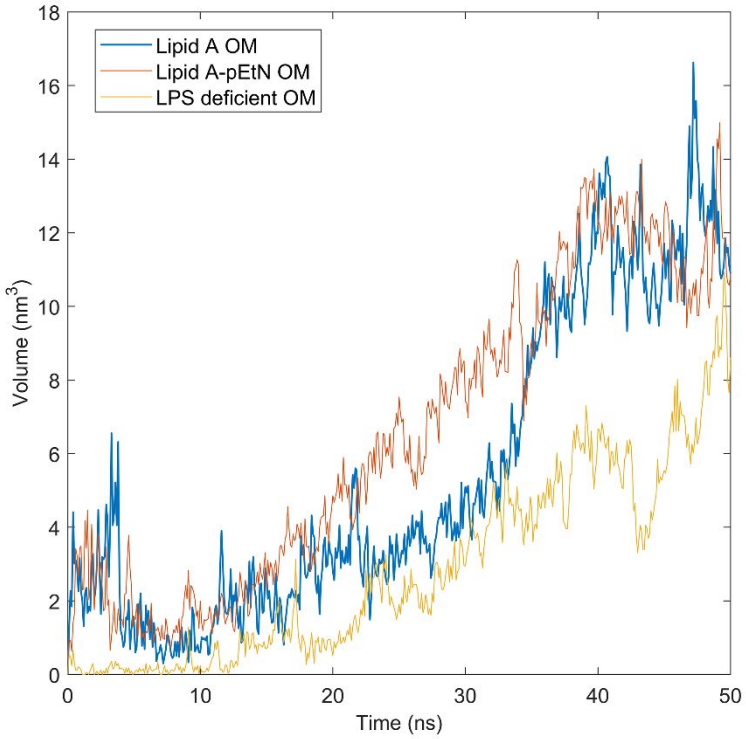

Figure S2. Changes in the volume of the water pores in different outer membrane systems calculated based on the steered simulation trajectories.

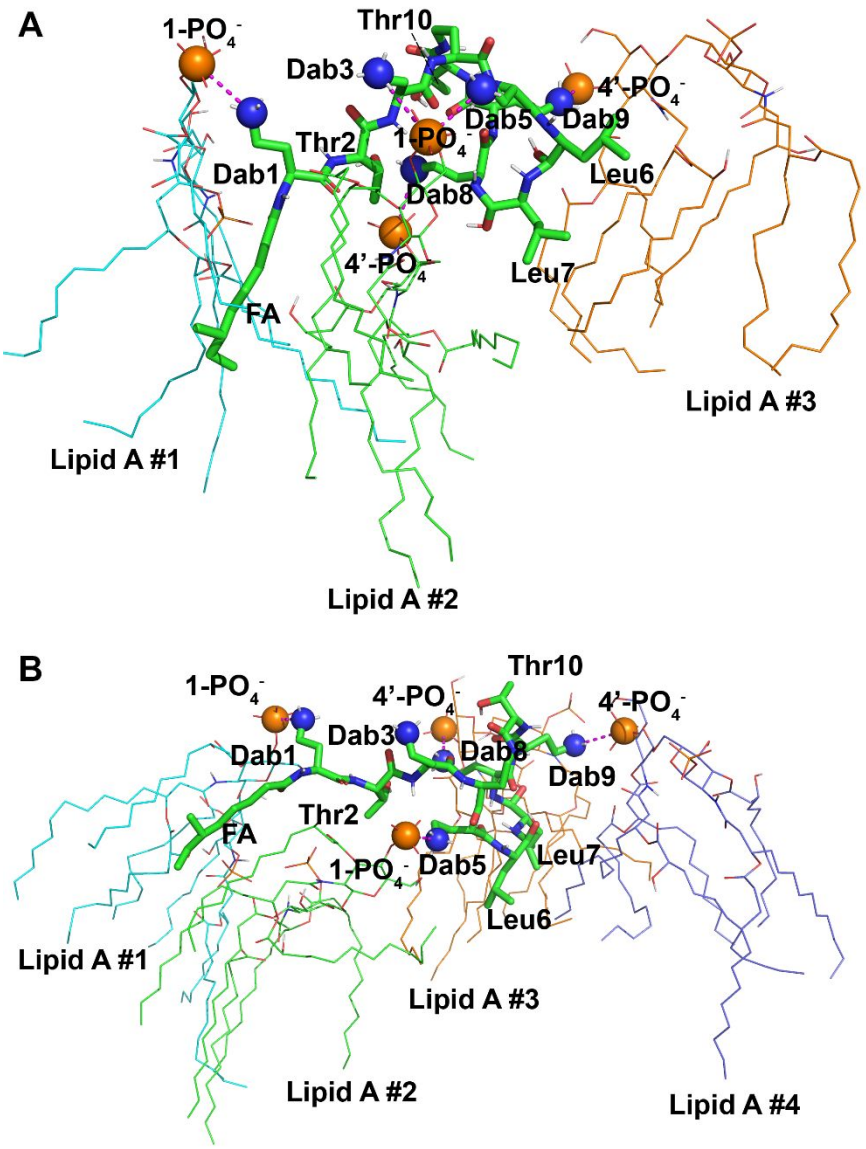

Figure S3. Interaction of colistin A with the lipid A OM. The results were calculated from the (A) first and (B) second steered simulation replicates in the lipid A OM system. The electrostatic interactions between the Dab residues and the lipid A phosphate groups are shown in purple dashed lines.

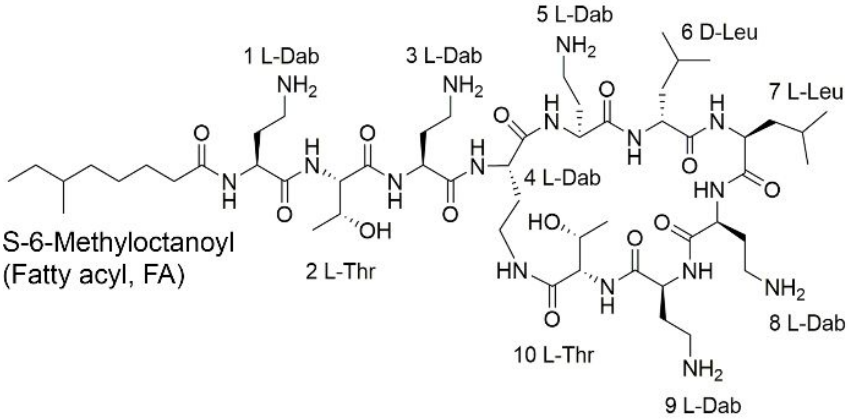

Figure S4. Chemical structure of colistin A. The colistin molecule consists of a cyclic heptapeptide, a linking tripeptide and an *N*-terminal fatty acyl group. The hydrophobic fatty acyl tail, Leu6 and Leu7, and the positively charged Dab residues (at positions 1, 3, 5, 8 and 9) confer the amphipathic nature.

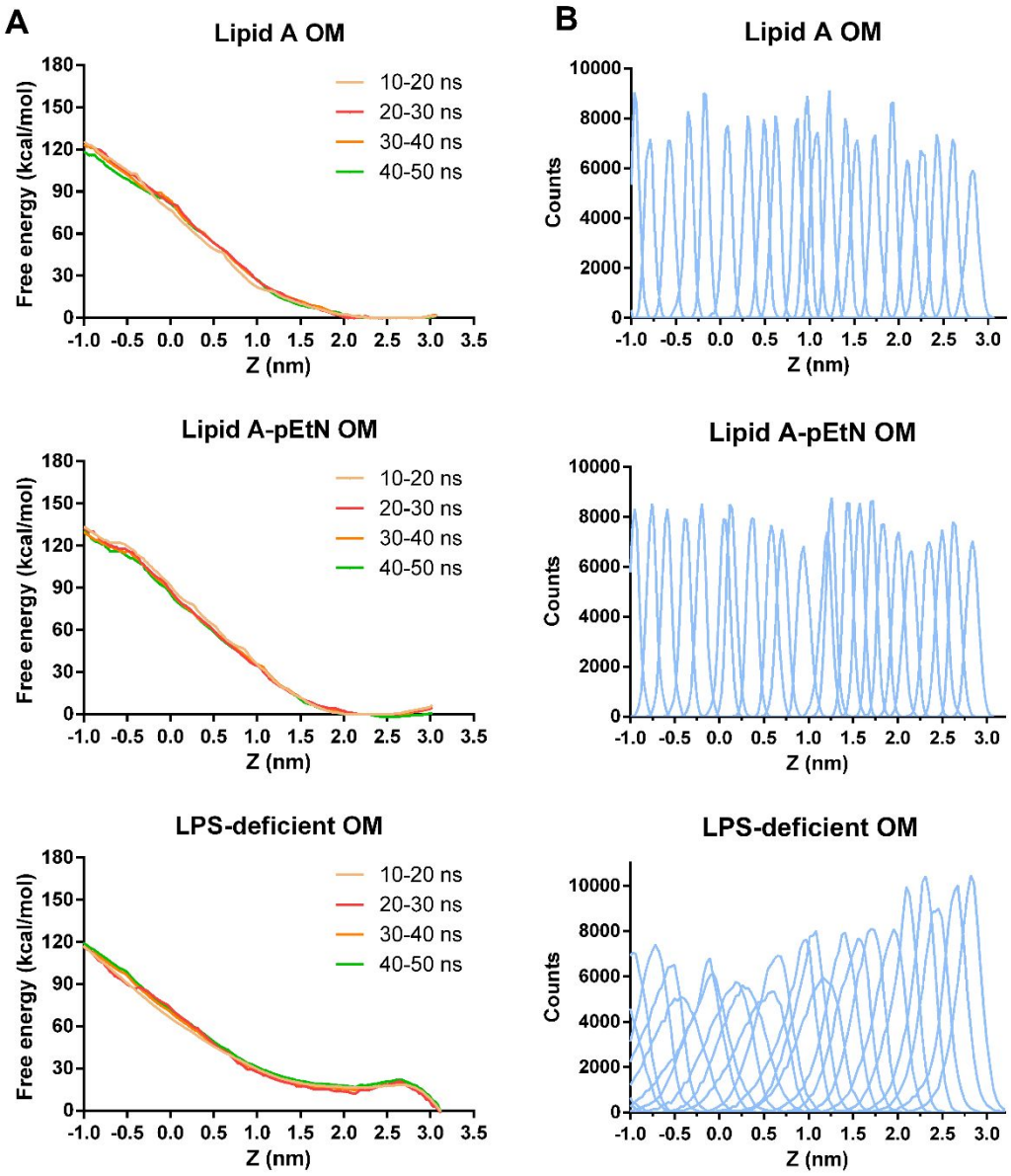

Figure S5. Free energy profiles (PMFs) calculated from 10-ns time blocks (A) and position distribution of colistin A in each umbrella sampling window (B). The minor variations between the PMFs from four time blocks and the overlap between adjacent windows indicate the convergence of free energy calculations.

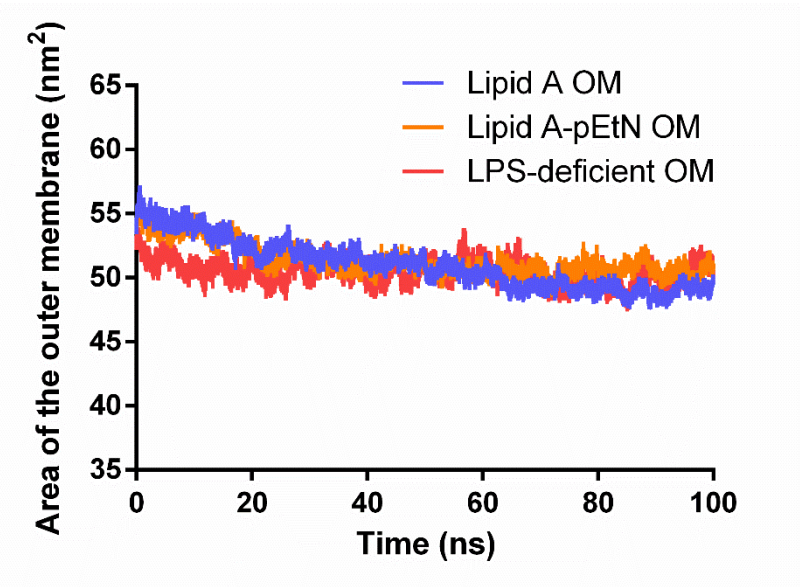

Figure S6. Surface area of the outer membrane (x-y plane) in the unrestrained molecular dynamics simulations.

Movie S1. Structural recovery of the lipid A outer membrane. The movie was prepared based on the simulation trajectory from the 24th umbrella sampling window.

Movie S2. Structural recovery of the lipid A-pEtN outer membrane. The movie was prepared based on the simulation trajectory from the 24th umbrella sampling window.

Movie S3. Structural recovery of the LPS-deficient outer membrane. The movie was prepared based on the simulation trajectory from the 28th umbrella sampling window.