Electronic Supplementary Information for

Bacterial lipids drive compartmentalization on the nanoscale

Antonio De Nicola,[a,§] Costanza Montis,[b,*] Greta Donati,[c, †] Antonio Molinaro,[d,*] Alba Silipo,[d] Arianna Balestri,[b] Debora Berti,[b] Flaviana Di Lorenzo [d] You-Liang Zhu,[e] Giuseppe Milano[f,*]

[a.] Scuola Superiore Meridionale, Via Largo San Marcellino 10, 80132 Napoli, Italy.
[b.] Dipartimento di Chimica "Ugo Schiff", Università degli Studi di Firenze, 50019 Firenze, Italy
[d.] Department of Chemical Sciences, University of Napoli Federico II, Complesso Universitario Monte Santangelo, Via Cintia 4, I-80126 Napoli, Italy.
[c.] Department of Chemistry and Biology, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy.
[e.] State Key Laboratory of Theoretical and Computational Chemistry, Institute of Theoretical Chemistry, Jilin Univeristy, Changchun, China.
[f.] University of Naples Federico II, Department of Chemical, Materials and Production Engineering, Piazzale V. Tecchio, 80, 80125 Napoli, Italy.
[§]Graduate School of Organic Materials Science, Yamagata University, Jonan 4-3-16, Yonezawa, Yamagata, 992-8510, Japan.
[†]Current Address: Department of Pharmacy, University of Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli, Italy.

Email: giuseppe.milano@unina.it, costanza.montis@unifi.it, antonio.molinaro@unina.it

Purification of LPS

LPS from *E. coli* and *P. aeruginosa* have been extracted by the hot phenol/water procedure.[1] The crude extracts have been enzymatically digested with RNase (Roth), DNase (Roth), and Proteinase K (Roth), followed by dialysis, ultracentrifugation, and size-exclusion chromatography. An aliquot of each purified LPS (30 mg) was treated with acetate buffer (pH 4.4, 2h, 373 K) in order to cleave the linkage between the lipid A portion and the saccharide part of the LPS. A mixture of $CHCl_3$ and CH_3OH was added to the hydrolysis product to obtain a $CHCl_3$/CH_3OH/hydrolysate 2:2:1.8 (v/v/v) ratio. The mixture was then shaken and centrifuged for several minutes. The chloroform phase, containing the lipid A, was collected, and washed with the water phase of a freshly prepared Bligh/Dyer mixture ($CHCl_3$/CH_3OH/H_2O, 2:2:1.8).[2] The organic phases were pooled, dried, and double checked by MALDI-TOF MS (SCIEX, Concord, ON, Canada).[3]

Small Angle X-Ray Scattering

SAXS measurements have been performed by using a Xenocs Xeuss 3.0HR instrument equipped with a Genix3D (Cu) X-Ray source (λ = 1.34 $Å^{-1}$) and a Dectris 1 M Eiger detector. Data were acquired at Sample-Detector (S-D) distances of 450 mm for 1800 seconds in ultrahigh-resolution mode. The wave vector range (q-range) accessed in experiments was $1.3 \cdot 10^{-2}$ $Å^{-1} \leq q \leq 3 \cdot 10^{-1}$ $Å^{-1}$. The intensities were normalized to transmission and sample thickness and azimuthally averaged using the XSact software.[4] The SAXS measurements on the Lipid A/water system were performed on gel-holders at r.t.. Supramolecular aggregates of Lipid A were prepared by adding directly MilliQ water to the system in the following ratio (~65/35 w/w %), stored at 273.15 K over 48h before the measurements.

hPF Coarse-Grained (CG) Lipid-A Models

For this study we selected two hexa-acylated structures of Lipid A originated by Escherichia Coli and Pseudomonas bacteria. The chemical skeletons of both Lipids A and the adopted mapping scheme are reported in Figure S1. The CG model of hexa-acylated Lipid A from Pseudomonas has been originally developed in our previous publication at reference.[5] The mapping scheme for the

hexa-acylated Lipid A from Escherichia Coli has been adapted to its molecular skeleton. The main difference between the two structures arises in the different distribution of alkyl side chains linked to the sugar fragments (belonging the head of the lipid). Force Field parameters of the CG model have been took from reference [5]. In the following, the functional form of bonded and non-bonded interaction potentials and parameter set are reported.[6]

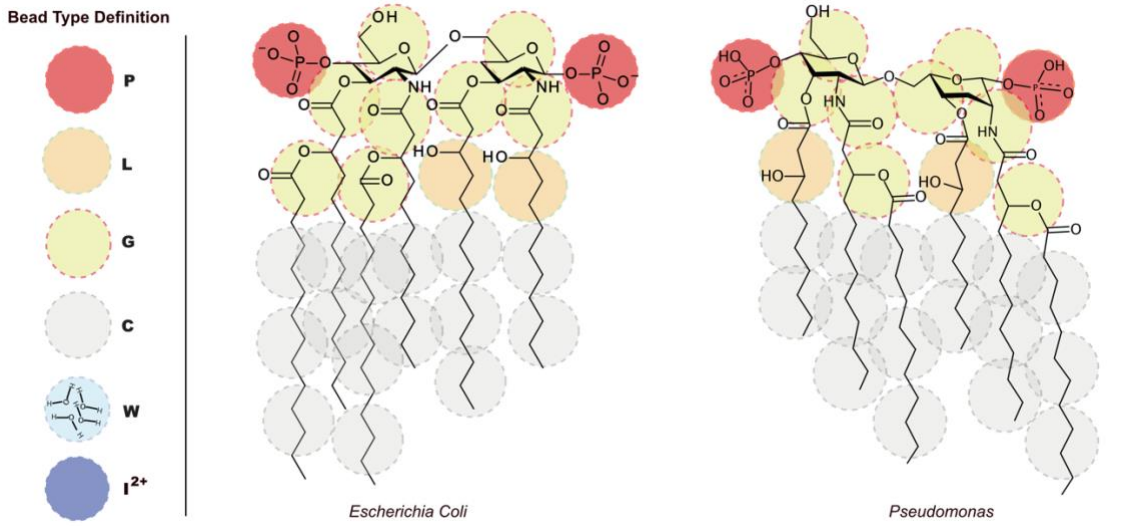

Figure S1. Chemical structures of Lipid A from Escherichia Coli and Pseudomonas. Coarse-Grained Bead type definition are reported on the left of the figure. The Mapping scheme adopted for the used Coarse-Grained model is overlayered on the chemical structures. The colour code is used to distinguish for different bead types. The bead type I^{2+} indicates a bivalent positive counter ion, while the label W indicates a bead of water solvent. Each W bead corresponds approximatively to 4 atomistic H_2O molecules.

Bond Stretching Potential:

$V_{bond} = \frac{K_{bond}}{2}(r_{ij} - r_0)^2$ (S12)

Table S1: Interaction bond terms

Bond Type	K_{bond} (kcal/mol/Ang.^{2})	r_0 (Ang.)
L-G	1250	4.7
L-C	1250	4.7
G-P	1250	4.7
G-G	1000	4.7
G-C	1250	4.7
C-C	1250	4.7

Angle Bending Potential:

$$V_{angle} = \frac{K_{angle}}{2}(\theta_{ij} - \theta_0)^2 \qquad \text{(S13)}$$

Table S2: Interaction Angle Terms

Angle Type	$\boldsymbol{K_{angle}}$ (kcal/mol/rad^2)	$\boldsymbol{\theta_0}$ (deg.)
G-G-G	850	120
G-G-L	25	120
L-C-C	25	180
C-C-C	25	180

Non-Bonding hPF interaction parameters:

Table S3. Interaction matrix $\chi_{KK'} \times RT\ (kJ\ mol^{-1})$.

$\chi_{KK'}$	**L**	**P**	**G**	**C**	**I^{2+}**	**W**
L	0	0	4.5	13.25	0	0
P	0	0	4.5	20	-7.2	-3.6
G	4.5	4.5	0	8.3	0	4.5
C	13.25	20	8.3	0	13.25	33.75
I^{2+}	0	-7.2	0	13.25	0	0
W	0	-3.6	4.5	33.75	0	0

System Building

Small Systems (E1, P1). In this work we simulate cubic box systems at two different sizes, small (E1, P1 in Table S4) and large (E2, P2 in Table S4). The small system of Lipid A from Pseudomonas (P1) was obtained in our previous manuscript at reference,[5] while the system E1 has been simulated for the first time in the present work. Initial configuration for the system E1 (Escherichia Coli) is get by randomly placing Lipid A, water and counterions molecules in the simulation box. The initial packing of molecules has been done using Packmol package.[7]

Large Systems (E2, P2). Large size systems have been built by duplicating the unitary small system (E1 and P1) in each *x,y,z* direction. At the end, a double size cubic box is obtained. Equilibrium configurations of systems E1 and P1 are used for the duplication procedure.

Table S4. Composition of the simulated systems.

System	Lipid A (Escherichia Coli)	Lipid A (Pseudomonas)	Counter ions	Water	Particles	Cubic Box Side Length [nm]	Time [μs]
E1	1400	-	1400	15000	54200	17.0	5
P1	-	1400	1400	15000	54200	17.0	5
E2	11200	-	11200	120000	433600	34.0	20
P2	-	11200	11200	120000	433600	34.0	20

Additional Results

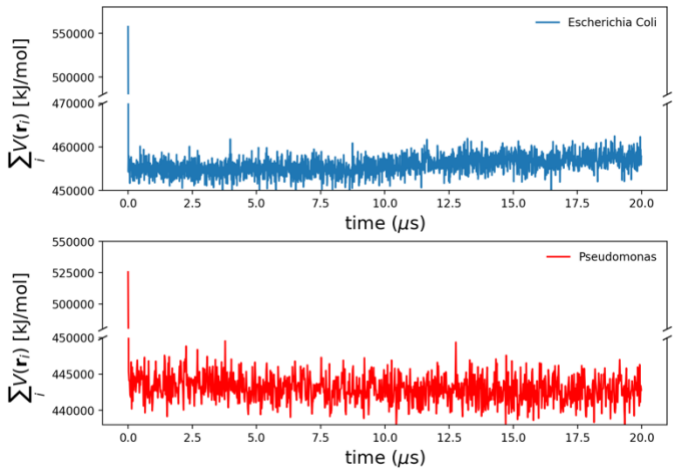

Figure S2. Time evolution of $\sum_i V(\boldsymbol{r}_i)$ hPF potentials for large systems (E2 and P2, Table S4) of Escherichia Coli and Pseudomonas. $\boldsymbol{r}_i$ is the position of i-th particle in the system.

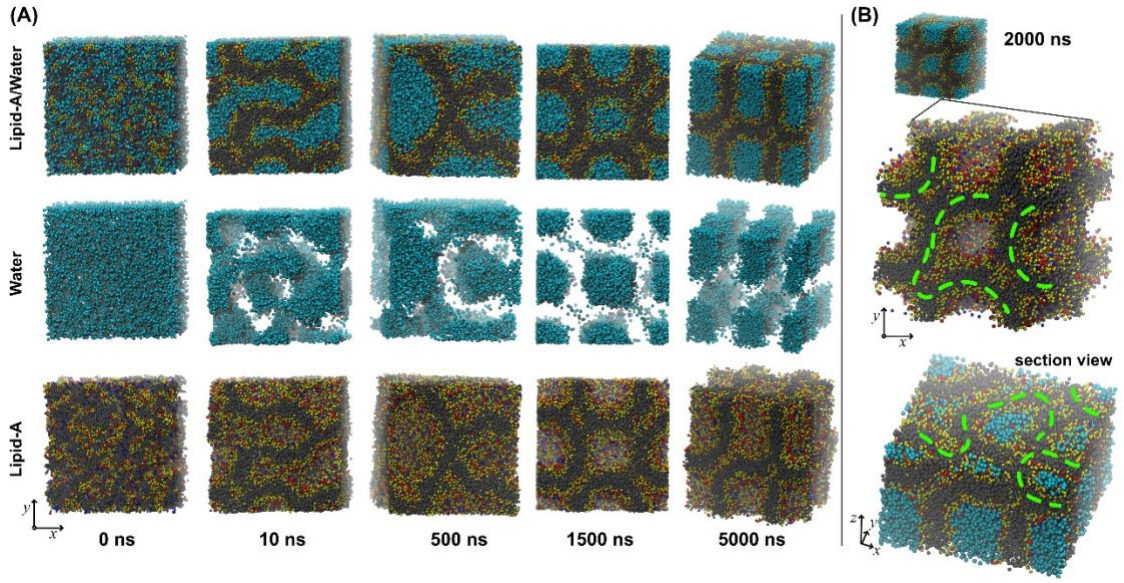

Figure S3. (A) Snapshot sequence to show morphology time evolution of Lipid-A mixture from Escherichia Coli. In the upper row, snapshots of the whole system (Lipid A/water/ions) are shown, while in medium and lower rows are selectively shown water and Lipid A components. (B) Snapshot corresponding at 2 μs is zoomed in two views: once without water, and a second in a section (cut along z direction). The green lines drawn on the snapshot are the meaning of eye guide to highlight the Lipid A junction sections.

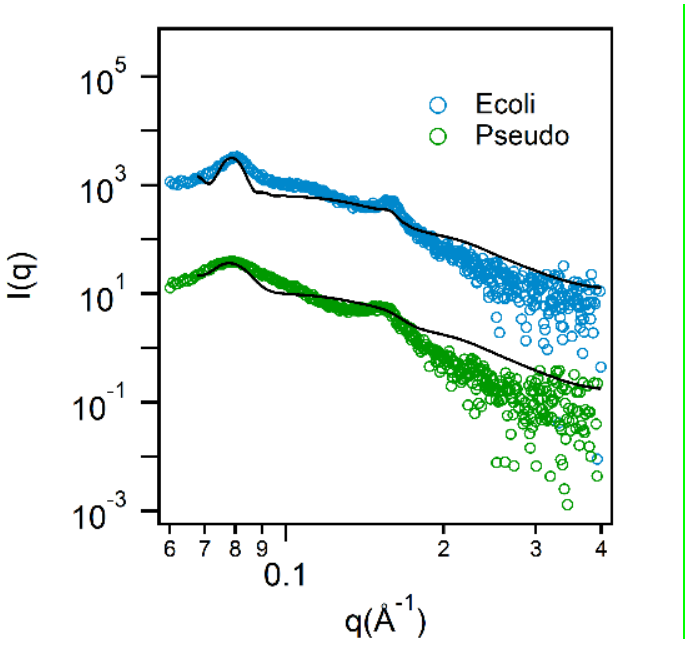

Figure S4. Experimental X-Ray profile of Escherichia Coli (blue circles) and Pseudomonas (green circles) together with lamellar_hg_stack_caille fitting (black lines). The scattering intensity is calculated by using random lamellar sheet model characterized by 10 layers of 7 nm thickness (head thickness= 1.5 nm and tail thickness = 2.0 nm) with an interlamellar distance d = 8.0 nm.

Experimental scattering intensity of Escherichia Coli and Pseudomonas was analysed by lamellar_hg_stack_caille fitting of the SASVIEW program. The experimental profiles at high q values, $q > 10^{-2}$ A^{-1}, have been fitted considering a lamella repetition distance d =8.0 nm and keeping the thickness constant at a value of 7 nm (head thickness= 1.5 nm and tail thickness = 2.0 nm).

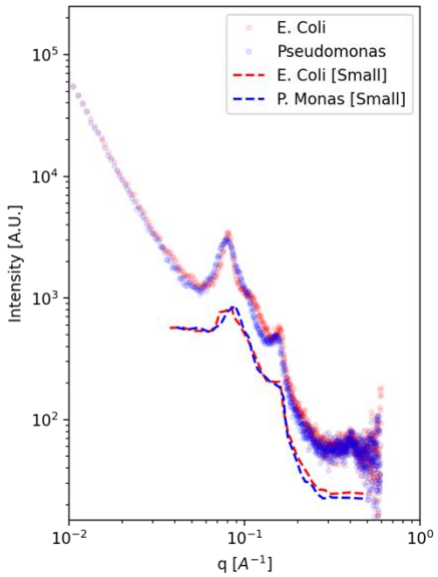

Figure S5. Comparison between measured (points) and calculated SAXS profiles for Escherichia Coli and Pseudomonas Lipid A for smaller size (systems E1, P1 in Table S4. segmented lines).

The exponent α of the power law $I(q) = q^{\alpha}$ is calculated from the fitting by using the equation $\log[I(q)] = \alpha \log(q)$. In Table S5, α from the best fitting is reported for experimental and calculated SAXS profiles. The log-log plot is reported in Figure 3A of the main text.

Table S5. α values. The values in the brackets have the meaning of standard error.

System	***q*** range [Å- 1]	$\boldsymbol{\alpha}$ (from simulations)	$\boldsymbol{\alpha}$ (from experiments)
E2 (E. coli)	0.001-0.005	-1.8(0.3)	-2.1
	0.006-0.15	-3.3 (0.4)	-3.4
	0.15-0.22	-3.8 (0.21)	-4
P2 (Pseudomonas)	0.001-0.005	-1.9(0.14)	-2.13
	0.006-0.15	-3.2 (0.37)	-3.4
	0.15-0.22	-3.9 (0.18)	-4

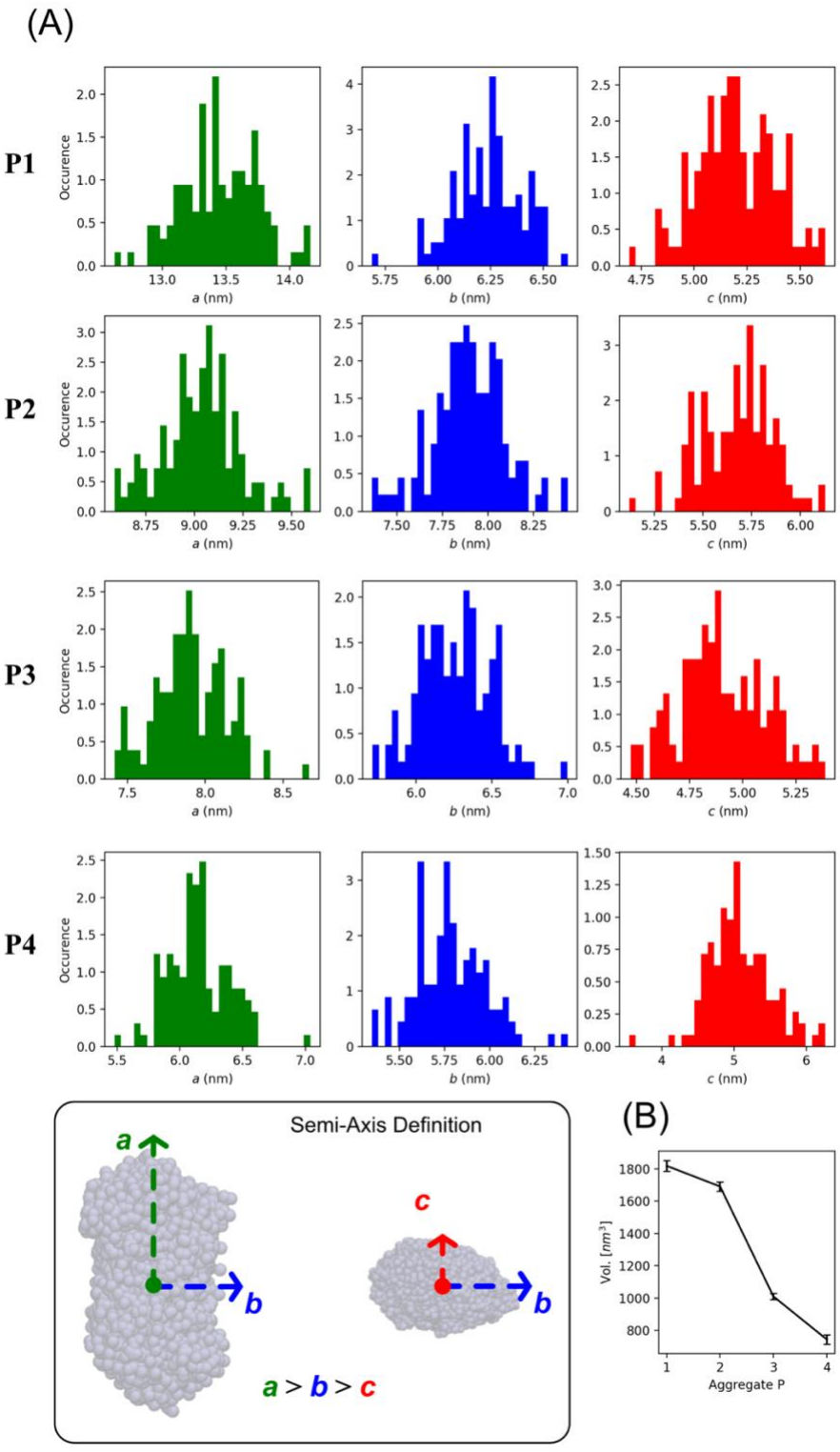

Figure S6. (A) Distributions of semi-axes lengths (*a,b,c*) calculated by using the PCA. Definition of semi-axis is reported in the bottom-right side of the panel. (B) Estimated volume of water aggregates for each P-type aggregate. the distributions of (*a, b, c*) are calculated considering 150 configurations from MD trajectories.

References:

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