Supporting Information

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SI Text

SI Materials and Methods. ***z-resolution of the X-ray fluorescence experiments.*** As shown in Fig. 3*A*, the normalized K^+ fluorescence signal from purified rough mutant lipopolysaccharides (LPS Re) monolayer on Ca^{2+}-free buffer can be well modeled with $z_{max} = 5$ Å. If we assume $z_{max} = 20$ Å, coinciding with a K^+ concentration peak well outside the saccharide head group slab, the fit is significantly worse [see also mean square deviation (MSD) vs. z_{max} plot in Fig. 3*A*]. This is illustrated in Fig. S1, where both model curves are plotted together with the experimental data points.

LPS Re monolayer slab model parameters and fits from grazing-incidence X-ray scattering out of the specular plane (GIXOS) measurements. Table S1 contains the best matching model parameters of LPS Re monolayers at a lateral pressure of $\pi = 20$ mN/m on Ca^{2+}-free and Ca^{2+}-loaded buffer as obtained from least-square fits to the measured GIXOS signals. ρ and d denote the electron density and the thickness of each medium, whereas σ denotes the RMS roughness of each interface between two neighboring media. Fig. S2 shows the measured GIXOS signals and the modeled curves according to the best matching model parameters.

Surface pH and surface pK_a of carboxyl groups at LPS Re monolayers. Ref. 1 provides a relation between the surface pH value (pH_s), the bulk pH value, and the electric surface potential ψ:

$$pH_s = pH + \frac{e\psi}{\ln 10 \cdot k_B T}. \qquad [S1]$$

In our experimental system, $\psi = 128$ mV was roughly approximated in a Poisson–Boltzmann approach from the measured average charge per LPS Re molecule ($Q_{tot} = -2.3$ e), the average area per molecule ($A = 166$ Å^2), and the bulk ion concentrations in Ca^{2+}-free buffer. With these values, Eq. **S1** yields $pH_s = 5.25$.

Ref. 1 also provides a relation between the surface pK_a value, the electric surface potential ψ and the degree of dissociation f of surface-confined acids:

$$\log_{10}\frac{f}{1-f} = pH - pK_a + \frac{e\psi}{\ln 10 \cdot k_B T} = pH_s - pK_a. \qquad [S2]$$

We applied Eq. **S2** to the two carboxyl groups in LPS Re monolayers. Because the pK_a values of phosphate and amino groups are far from the experimental pH values (2), it is plausible that they are fully ionized. Thus, f was determined from Q_{tot}, from the charge of the two carboxyl groups at full dissociation ($Q_c^{max} = -2$ e), and from the average charge per molecule at full dissociation ($Q_{tot}^{max} = -3.6$ e, ref. 3). This leads to the result $f = (Q_{tot}^{max} - Q_c^{max} - Q_{tot})/2 = 0.35$, and Eq. **S2** yields a surface $pK_a = 5.5$.

1. Hu K, Bard AJ (1997) Use of atomic force microscopy for the study of surface acid-base properties of carboxylic acid-terminated self-assembled monolayers. *Langmuir* 13:5114–5119.
2. Lide DR (2009) *CRC Handbook of Chemistry and Physics* (CRC, Boca Raton), 90th Ed.
3. Hagge SO, Hammer MU, Wiese A, Seydel U, Gutsmann T (2006) Calcium adsorption and displacement: Characterization of lipid monolayers and their interaction with membrane-active peptides/proteins. *BMC Biochem* 7(15):1–13.

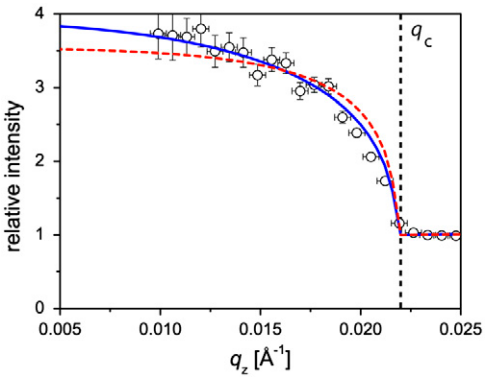

Fig. S1. Fit quality subject to different values of z_{max}. Solid blue line: $z_{max} = 5$ Å; dashed red line: $z_{max} = 20$ Å. Data: K^+ fluorescence signal from LPS Re monolayer of Ca^{2+}-free buffer.

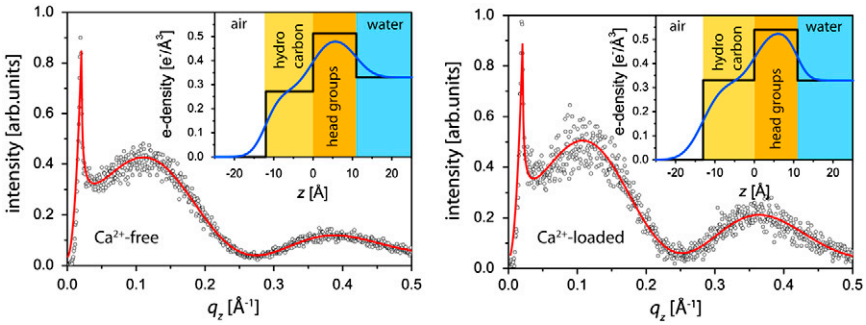

Fig. S2. Measured GIXOS signals (open circles) and best fits (solid lines) from LPS Re monolayers on Ca^{2+}-free (*Left*) and Ca^{2+}-loaded (*Right*) subphase at a lateral pressure of $\pi = 20$ mN/m. (*Inset*): Electron density profiles (blue) and box models (black) corresponding to the best fitting parameters.

Table S1. Electron density model parameters

Ca^{2+}-loaded, 140 Å^2	ρ [e/Å^3]	d [Å]	σ [Å]
Air	0.00	infinity	—
Hydrocarbon chains	0.33	13	3.9
Head group	0.54	11	3.2
Water	0.33	infinity	2.4
Ca^{2+}-free, 166 Å^2	ρ [e/Å^3]	d [Å]	σ [Å]
Air	0.00	infinity	—
Hydrocarbon chains	0.27	12	2.9
Head group	0.51	11	3.8
Water	0.33	infinity	3.6