Supplementary information

Antimicrobial Peptide LL-37 on Surfaces Presenting Carboxylate anions

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Surface Density Estimated from Ellipsometry Thickness and XPS Data

In the first method based on ellipsometry data, the densities of LL-37 on SAMs **1a–4a** were estimated using the following formula. The results are summarized in Table S1.

$$N_{LL-37} = \frac{\Delta t \times \rho \times N_A \times 10^{-8}}{Mw_{LL-37}} \qquad (1)$$

where Δt is the thickness increase in Å, N_A is Avogadro's number (6.02×10^{23} molecules/mol), Mw_{LL-37} is the molecular weight of LL-37 (4493 g/mol), and ρ is the density of LL-37, estimated to be 1.55 g/mL using the following formula proposed by Fischer *et al*.[2]

$$\rho = \left[1.41 + 0.145\exp\left(-\frac{Mw(kDa)}{13}\right)\right] = 1.55\ \text{g/cm}^3 \qquad (2)$$

Table S1. Ellipsometric thickness of SAMs prepared from **HS-COOH** and **HS-OH** of various molar ratios, and the derived surface density of the adsorbed LL-37 (N_{LL-37}/cm^2)

SAM	Thickness (Å)[a)]		Thickness (Å)[a)]		Thickness increase (Å)	N_{LL-37}/cm^{2} [b)]
1a (100% COOH)	**1a**	33.1±0.2	**1b**	45.6±0.9	12.5±0.9	2.6×10^{13}
1b (~80% COOH)	**2a**	33.0±0.6	**2b**	43.7±2.6	10.7±2.6	2.2×10^{13}
1c (~2% COOH)	**3a**	32.3±0.2	**3b**	33.1±0.5	0.8±1.5	1.7×10^{12}
1d (0% COOH)	**4a**	34.1±0.3	**4b**	32.2±0.6	-1.9±0.6	0

a) Average thickness values obtained on three random locations with the standard deviation.

b) The sources of error include the ellipsometric measurement and the estimation of the peptide weight density (formula 2 was proposed for crystals of proteins with a molecular weight higher than 7 kDa).

To directly derive the densities of the absorbed LL-37, the density of **HS-OH** containing a nitrogen atom was first estimated using the following formula:

$$(C/N)_{XPS} = \frac{C_{HS-OH}\rho_{HS-OH} + C_{HS-COOH}\rho_{HS-COOH}}{N_{HS-OH}\rho_{HS-OH}} \quad (3)$$

where $(C/N)_{XPS}$ is the measured atomic C/N ratio, C_{HS-OH} and $C_{HS-COOH}$ are the number of C atoms per **HS-OH** and **HS-COOH** molecule, which are 28 and 25, respectively. N_{HS-OH} is the number of N atoms per **HS-OH** molecule, which is 1. ρ_{HS-OH} and $\rho_{HS-COOH}$ are the surface densities of **HS-OH** and **HS-COOH** molecules. Using the idealized total density of (ρ_{HS-OH} + $\rho_{HS-COOH}$) = 21 Å^2/molecule (4.67×10^{14}/cm^2) for alkanethiolate SAMs,[3] the densities of **HS-COOH** ($\rho_{HS-COOH}$) in SAMs **2a** and **3a** can then be obtained from the above formula and listed in Table S2.

Knowing the composition of the mixed SAMs, the densities of LL-37 on SAMs **1a–4a** can be derived using the following formula:

$$(C/N)_{XPS} = \frac{C_{HS-OH}\rho_{HS-OH} + C_{HS-COOH}\rho_{HS-COOH} + C_{LL-37}\rho_{LL-37}}{N_{HS-OH}\rho_{HS-OH} + N_{LL-37}\rho_{LL-37}} \quad (4)$$

where C_{LL37} and N_{LL37} are the numbers of C and N atoms per LL-37 molecule, which are 205 and 60, respectively. The results are listed in Table S2. Note that the attenuations of N1*s* and C1s are not considered, which leads to an overestimate of the N/C ratio and accordingly the peptide density. However, this systematic error does not affect the trend for the antibacterial efficiency vs surface density of LL-37. Indeed, using the LL-37 density data derived from the XPS measurement as compared to ellipsometric measurement, the plot of PA viability vs density of LL-37 gives a similar trend (Figure S2 vs Figure 5).

Table S2. XPS derived densities of COOH groups (N_{COOH}/cm^2) of the SAMs prepared from **HS-COOH** and **HS-OH** of various molar ratios, and the densities of LL-37 (N_{LL-37}/cm^2) adsorbed on these SAMs

HS-COOH/ HS-OH	N_{COOH}/cm^2	C/N ratio				N_{LL-37}/cm^2*
1:0	4.7×10^{14}	**1a**	\	**1b**	9.1	3.3×10^{13}
1:1	3.8×10^{14}	**2a**	127.1	**2b**	9.5	3.1×10^{13}
1:9	7.4×10^{12}	**3a**	28.4	**3b**	16.9	6.6×10^{12}
0:1	0	**4a**	21.0	**4b**	21.1	0

* Derived from the measured C/N ratios before and after attachment of LL-37 using equations 3 & 4. The density of the mixed monolayer of **HS-COOH/HS-OH** was assumed to be 21.4 Å^2/molecule.[3] Assuming the peptide is adapting a bilayer structure as illustrated in Figure 2, *on average* there are about 28, 24, and 2 COOH groups under each peptide molecule in the bottom of the peptide bilayer in **1b**, **2b**, and **3b**, respectively.

Fluorescent Imaging of PAO1-GFP on LL-37 Surfaces for Evaluation of the Antibacterial Efficiency of the Surfaces

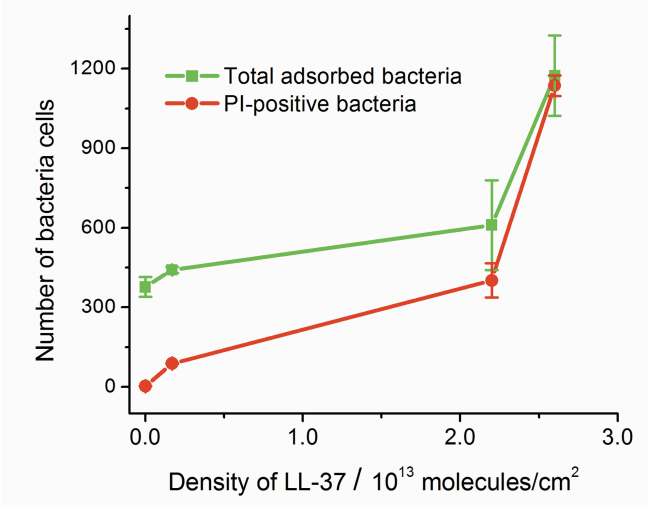

Figure S1. Plot of the total number of bacteria (green) and among them the number of PI-positive bacteria (red), which were adsorbed on an area of 100×100 μm^2 presenting LL-37 of various densities. The densities of LL-37 were derived from ellipsometric data (Table S1). Data are expressed as mean number of bacteria ± standard deviation of three experiments. For the plot based on densities of LL-37 derived from the XPS data, see Figure 6.

Cytotoxicity of the Surfaces Presenting LL-37 towards SV40-transformed HCECs

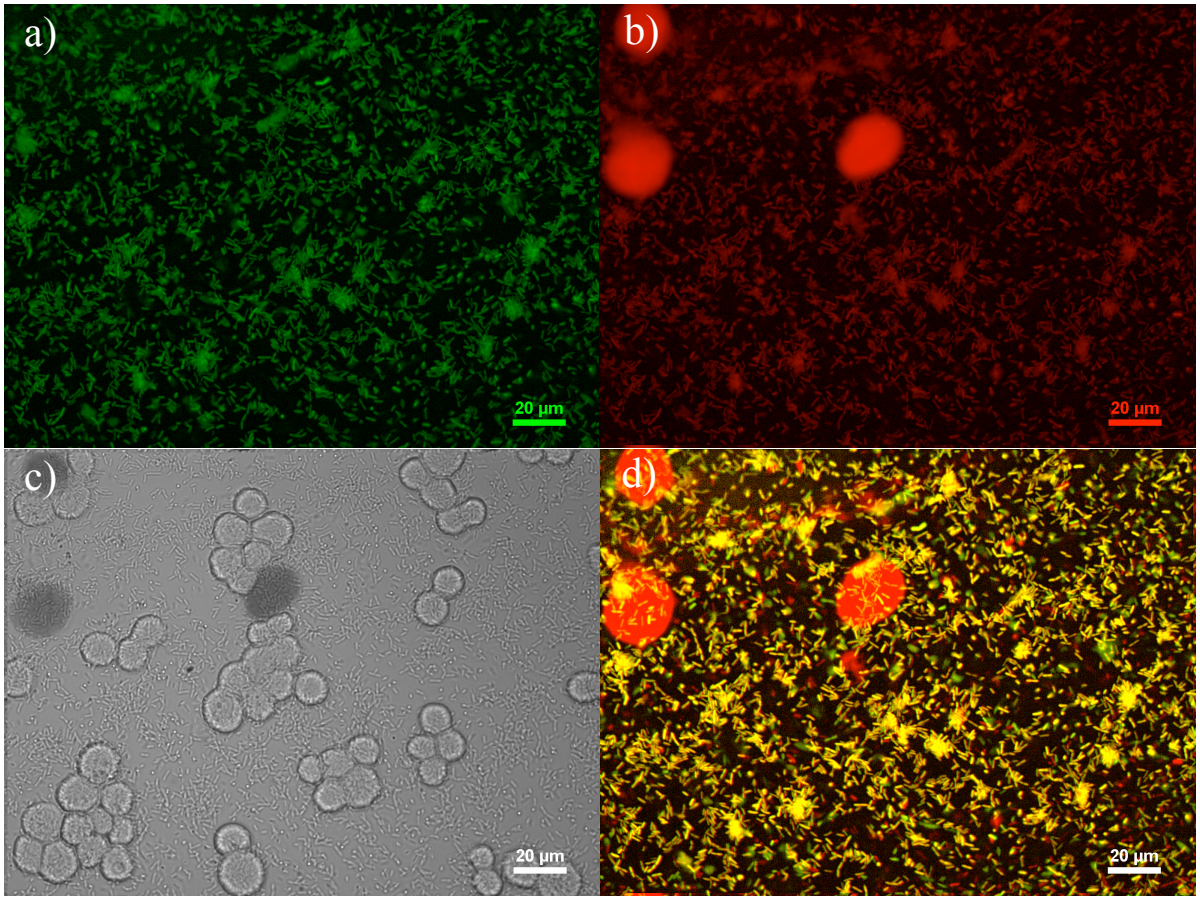

Figure S2. Fluorescence (a, b) and bright-field (c) images of the same field of view of PAO1-GFP and HCECs after incubation for 90 minutes on LL-37 immobilized on 100% COOH surface **1a**. The green fluorescence image (a) was obtained with a FITC filter showing all adsorbed PA, the red fluorescence image (b) with a TRITC filter, and their overlay is shown in (d). The overlay is also provided in Figure 6. The large red spots (PI positive) indicate dead HCEC that were also found on films without LL-37.

References

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(3) H. Harder, M. Grunze, R. Dahint, G. M. Whitesides, P. E. Laibinis, *J. Phys. Chem. B* **1998**, *102*, 426.