Supporting Information

Immobilization of antimicrobial peptide IG-25 onto fluoropolymers via fluorous interactions and click chemistry

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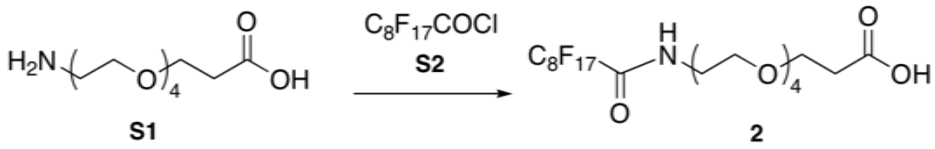
Synthesis.

Compounds **1** and **3** were synthesized according to our published method.[1]

*18,18,19,19,20,20,21,21,22,22,23,23,24,24,25,25,25-heptadecafluoro-17-oxo-4,7,10,13-tetraoxa-16-azapentacosan-1-oic acid (**2**)*



A solution of $C_8F_{17}COCl$ (**S2**, 385 mg, 0.80 mmol) in dry THF (0.5 mL) was dropwise added to a solution of the amine **S1** (200 mg, 0.75 mmol) and triethylamine (0.14 mL, 1.0 mmol) in dry THF (1 mL) at 0 °C under nitrogen. After being stirred for 1.5 h at 0 °C, the reaction mixture was stirred overnight at room temperature. Saturated aqueous NH_4Cl (5 mL) was added, and the mixture was extracted three times with Et_2O. The combined organic layers were washed with water, brine, and dried over Na_2SO_4. Flash chromatography (EtOAc:MeOH 10:1) gave the acid **2** (320 mg, 0.45 mmol, 60%) as a yellow oil. ^{1}H NMR (300 MHz, CD_3OH, δ 3.71-3.34 (m, 14H), 2.58 (t, J = 4.5 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OH): δ 77, 164.1, 108.4-125.5, 71.9, 71.8, 71.2, 68.4, 41.5, 36.0. MS (ESI) m/z calcd for $C_{20}H_{22}F_{17}NO_7$: 711.3; found: 734.4 ($[M + Na]^+$).

XPS measurements. A PHI 5700 X-ray photoelectron spectrometer was equipped with a monochromatic Al Kα X-ray source ($h\nu$=1486.7 eV) incident at 90° relative to the axis of a hemispherical energy analyzer. The spectrometer was operated both at high and low resolutions with pass energies of 23.5 eV and 187.85 eV, a photoelectron take off angle of 45° from the surface, and an analyzer spot diameter of 1.1 mm. The survey spectra were collected from 0 to 1400 eV, and the high-resolution spectrum was obtained for photoelectrons emitted from C1s,

O1s, Si 2p, N1s, and F1s. All spectra were collected at room temperature with a base pressure of 1 x10^{-8}torr. Electron binding energies were calibrated with respect to the alkyl C1s line at 284.5 eV. A PHI Multipak software (version 5.0A) was used for all data processing. The high-resolution data were analyzed first by background subtraction using the Shirley routine and a subsequent non-linear fitting to mixed Gaussian-Lorentzian functions. Atomic compositions were derived from the high-resolution scans. Peak areas were obtained after subtraction of the integrated baseline and corrected for sensitivity factors.

Estimate of surface density of IG-25 (ρ_p). Ignoring the attenuation of C1s and N1s signals, the density of IG-25 (ρ_p) on the film **B** or **E** can be estimated from XPS measurement using the equation [1] and is summarized in table S1.

$$C/N = \frac{a + (\rho_p / \rho_f)m}{b + (\rho_p / \rho_f)n} \qquad [1]$$

where the C/N atomic ratio is measured by XPS using C1s peak position within 284–288 eV (excluding the signal from CF_2 at 290 eV) and N1s at ~400 eV, contributed from the alkyne **1** or carboxylic acid **2**, represented as C_aN_b, *a* and *b* being the number of C atoms (other than CF_2) and N atoms, on the precursor films, and the peptide **N_3-EG_{12}-IG-25** on the film **B** or **IG-25** on the film **E**, represented as C_mN_n, *m* and *n* being the number of C atoms and N atoms, and ρ_p / ρ_f is the density ratio of the peptide over the fluorous chains **1** or **2**. Thus, for the film **B**: *a* = 11, *b*= 1, *m*= 137 + 27 = 164, *n* = 42 + 3 = 45, and for the film **E**: *a* = 9, *b* = 1, *m* = 137, *n* = 42. The density (ρ_f) of the fluorous chains **1** or **2** is estimated by the following formula:[2,3]

$$\rho_f = \frac{\rho d N_a}{M_w} \qquad [2]$$

where ρ is the density of **1** or **2** on film **A** or **C**, assuming to be 1 gm/cm^3, N_A is the Avogadro's number, M_w is the molecular weight of **1** (677 gm/mol) or **2** (726 gm/mol), and d is the thickness of **1** or **2** on the film **A** or **C**, estimated to be 22.8 Å for film **A** and 23.0 Å for film **C** based on the angle-resolved XPS data shown in Tables S2 and S3 and Figure S1, using the method described in the literature.[4] Table S1 shows data for the estimation of the surface density.

Table S1. Data for estimation of the density of proteins deposited from solutions of various concentrationon surface B (20mg/mL) and surface E (200 mg/mL and 20 mg/mL). The data are expressed in molecules of IG-25/nm^2 and are calculated using equation [1].

Analyzed surface	(C/N) ratio	(a b)	(m n)	*ρ_f (molecules/nm^2)	ρ_p (molecules/nm^2)
Surface B	5.5	(11 1)	(164 45)	2.0 x 10^{14}	1.4 x 10^{13}
Surface E (200mg/L)	3.3	(9 1)	(137 45)	1.9 x 10^{14}	1.2 x 10^{13}
Surface E (20mg/L)	3.5	(9 1)	(137 45)	1.9 x 10^{14}	5.2 x 10^{13}

* Calculated using equation [2].

Table S2. Data of C 1s Signals Intensities of the COOH-modified ($I_{C1s}^{COOH\text{-}modified}$) and the Unmodified ($I_{C1s}^{unmodified}$) Fluorous Substrates at Different Take-off Angles (θ)

θ	1/sin θ	$I_{C1s}^{COOH\text{-}modified}$	$I_{C1s}^{unmodified}$	-ln A
30	2.00	20	93	1.54
40	1.41	30	96	1.16
60	1.16	35	98	1.03
75	1.04	37	99	0.98

Table S3. Data of C 1s Signals Intensities of the Alkynyl-modified ($I_{C1s}^{Alkyne\text{-}modified}$) and the Unmodified ($I_{C1s}^{unmodified}$) Fluorous Substrates at Different Take-off Angles (θ)

θ	1/sin θ	$S_{C1s}^{alkyne\text{-}modified}$	$S_{C1s}^{unmodified}$	-ln A
30	2.00	21	93	1.49
40	1.41	32	96	1.10
60	1.16	34	98	1.06
75	1.04	37	99	0.98

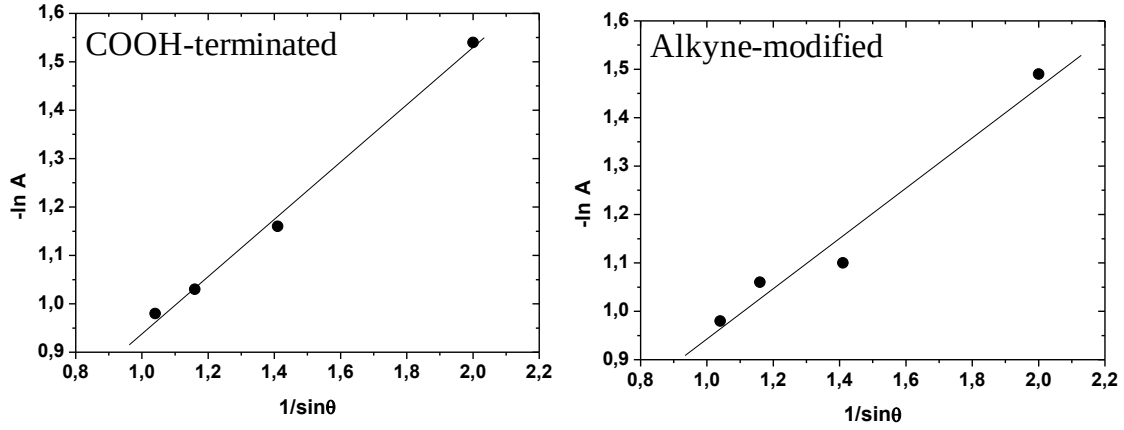


Figure S1. Plot of –Ln *A* as a function of 1/sinθ for the COOH-terminated and for the alkyne-modified fluorous surfaces **C** and **A**, respectively.

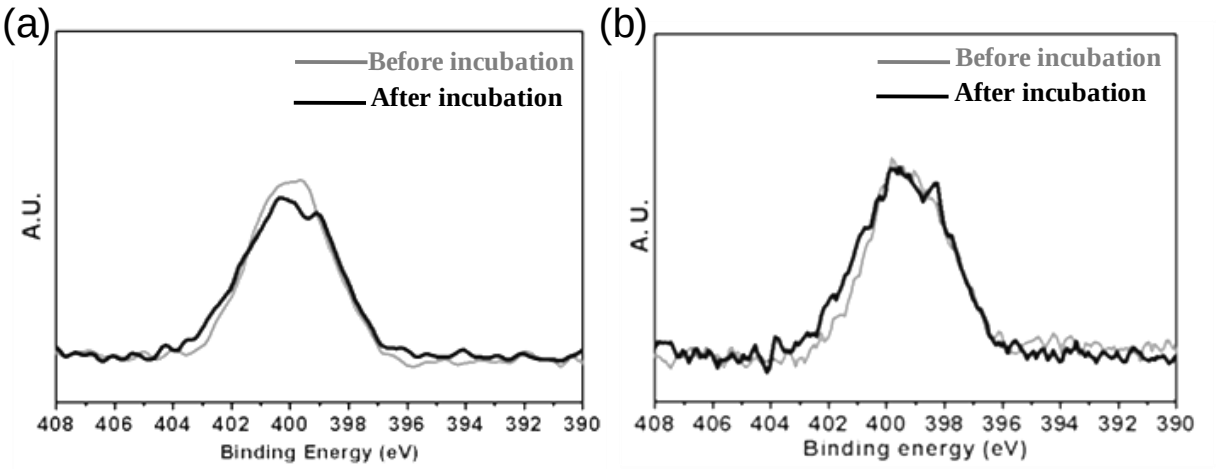


Figure S2. XPS N 1s spectra of the IG-25-presenting surfaces prepared by (a) the carbodiimide- and (b) the click-modified IG-25 fluorous surfaces E and B before and after incubation in PBS for 24 h.

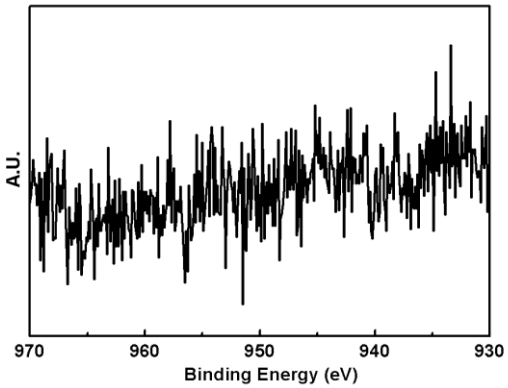


Figure S3. XPS Cu 2p spectra of the IG-25-modified contact lens after click functionalization.

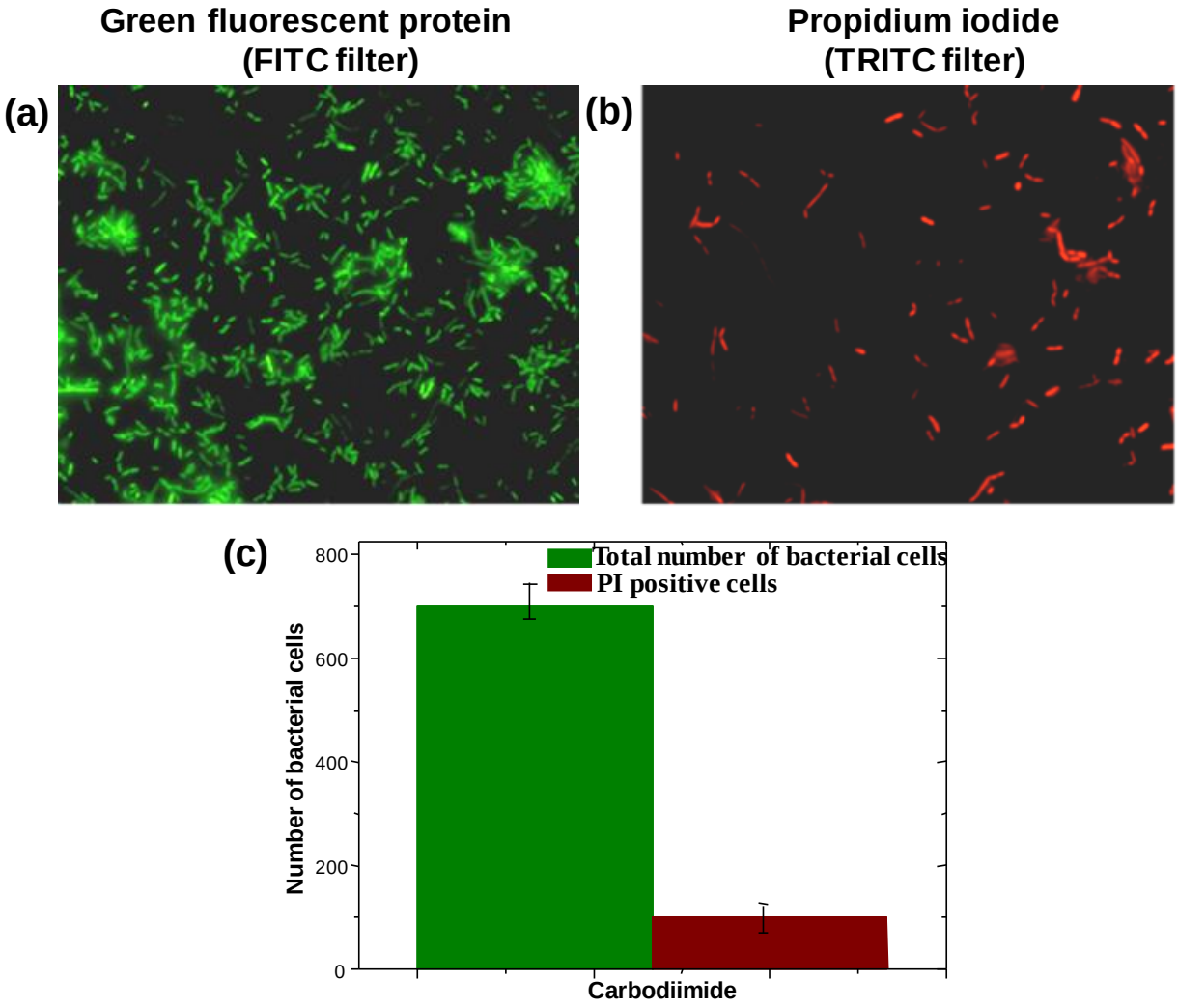


Figure S4. Fluorescence images (a) and (b) (Area: 149 x 112 μm^2) of the GFP-transformed *Pseudomonas aeruginosa* on a lower density carbodiimide-modified IG-25 surface E (ρ_p=5.2 x 10^{13} molecules/cm^2). (c) Plot of the total number of bacteria (green) and among them the number of PI-positive bacteria (red) on the field of view (149×112 μm^2), which were adsorbed on the surface E where lower density of IG-25 was attached via carbodiimide chemistry. The unmodified fluorous slide serves as a control.

References

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