Supporting Information

Bacterial Adhesion to Graphene Oxide (GO)-Functionalized Interfaces is Determined by Hydrophobicity and GO Sheet Spatial Orientation

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Environ. Sci. Technol. Lett.

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Contents: 17 pages and 12 figures

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Supporting Materials and Methods

Synthesis of GO

Graphene oxide (GO) was prepared *via* chemical exfoliation of graphite (Bay Carbon, SP-1, 325 mesh) using a modified Hummers method.[1] First, 2.0 g of graphite was placed in 5 mL of concentrated sulfuric acid at 80 °C. Next, 2.0 g each of $K_2S_2O_8$ and P_2O_5 were added and the suspension was allowed to react at 80 °C for 4.5 hours. After reaction, the mixture was transferred into 320 mL of ultrapure water (18.2 MΩ cm, Barnstead, Thermo Fisher) and allowed to settle overnight. The mixture was subsequently vacuum filtered using PTFE membranes (0.45 μm, Whatman TE 36) and dried overnight at room temperature. Next, the obtained black solid was mixed with 80 mL of concentrated sulfuric acid over an ice bath, and 10.0 g of $KMnO_4$ was slowly added so that the temperature of the mixture did not exceed 10 °C. The mixture was then slowly heated to 35 °C over a period of 2.5 hours. Next, 154 mL of ultrapure water was slowly added, preventing the suspension temperature from exceeding 50 °C, and reacted for 2 hours at room temperature. Lastly, the mixture was transferred to 480 mL of ultrapure water, and 8.4 mL of 30% H_2O_2 was added, causing the mixture to acquire a yellowish-brown color. The suspension was allowed to settle for 2 days, and the precipitate was subsequently recovered by multiple centrifugation steps (12,000 × g, 30 min), initially re-suspending the product in 10% HCl to remove chemical residues and finishing with resuspension in water until the supernatant reached a pH of about 3.5. Finally, the suspended product was purified *via* dialysis (3.5 kDa membranes, Spectrum Labs) for 4 days and lyophilized for 4 days.

Preparation of PLL-GO Surfaces

GO sheets were covalently tethered to poly-L-lysine (PLL, MW = 150-300 kDa) immobilized on glass surfaces (Poly-Prep slides, Sigma Aldrich) *via* amine coupling[2] mediated by EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) and NHS (*N*-hydroxysuccinimide), following established protocols.[2,3] MES buffer (2-(*N*-morpholino)ethanesulfonic acid, 100 mM, pH 6.0) was mixed with GO aqueous dispersion (250 μg L^{-1}) at a 1:5 volume ratio. Next, EDC (20 mM) and NHS (50 mM) solutions prepared in 10 mM MES buffer were sequentially mixed with the GO suspension. During this step, carboxylic acid groups in GO are converted to amine-reactive esters. The reaction proceeded for 15 min at pH ~5.5. The pH of the suspension was subsequently adjusted to ~7.2 before immersing in the suspension a PLL-coated glass coupon. The suspension was placed on a shaker table (~30 rpm) for 1 h, after which the coupon was rinsed with ultrapure water, and bath-sonicated for ~10 min to remove unbound GO sheets. Prepared PLL-GO coupons were stored in ultrapure water at 4 °C until use.

Preparation of Si-GO Surfaces

Si-GO substrates were prepared *via* dip coating of Langmuir-Blodgett (LB) GO films on P-type silicon wafers (100 orientation, single side polished, test grade, 500-µm thickness). The bare silicon substrate was first soaked in acetone for 15 min, rinsed with copious amounts of ultrapure water, and washed with isopropyl alcohol to eliminate water residues. After air drying, the wafer was placed in a UV/O_3 cleaner for 20 min to eliminate organic residues (ProCleaner™ Plus, Bioforce Nanosciences). The cleaned wafer was stored in a nitrogen-purged desiccator before use.

The LB trough (effective area = 172 cm^2) was cleaned with Alconox solution followed by thorough rinsing with ultrapure water. Thereafter the trough was filled with a sublayer consisting of HCl solution, pH 1.0. The Si wafer was then dipped vertically into the trough well with the upper end clamped on the dipper. Surface pressure was monitored using a Wilhelmy plate positioned parallel to the Si substrate. A mixture of GO dispersion (2 mg mL^{-1}) and methanol (v/v = 1/5) was added to the acidic water sublayer dropwise in 0.5-mL aliquots to a total of 2.5 mL. Five min was allowed between aliquot additions. The setup was left overnight for methanol to completely evaporate. Finally, the Si substrate was pulled up at a constant speed of 0.03 mm/s with a surface pressure of 5 mN/m. More information on GO LB film preparation is documented elsewhere.[4–6]

Confocal Raman Microscopy

Confocal Raman Microscopy (Witec Alpha300R) was performed in the study to confirm the two characteristic bands of GO materials and GO coverage on the model surfaces. Sample surfaces were scanned using a Nikon 100× objective, 532-nm laser excitation and 1800 grooves/mm grating.

The optimal depth for mapping was determined by performing an x-z scan over a 20 × 8 μm^2 (length × depth) cross-section. Next, the x-y Raman map was generated over a 20 × 20 μm^2 scan area at the determined depth, with a resolution of 1 µm. Two spectra were measured per micron. The sum of the area under the D and G peaks of GO (found at ~1350 cm^{-1} and ~1590 cm^{-1}, respectively[7]) was used to generate the signal intensity maps.

AFM Topography and Surface Roughness

An MFP-3D-Bio atomic force microscope (Asylum Research) was used to image the surfaces and measure their nanoscale roughness using tapping (AC) mode AFM in phosphate buffered saline (PBS, pH 7.4). Bruker SNL probes were used (cantilever C, nominal k = 0.24 N/m). Surface images were collected at 0.5 Hz over areas of 20 × 20 μm^2, 5 × 5 μm^2, and 2 × 2 μm^2, on each surface type. Root-mean-square (RMS) roughness was calculated from three randomly selected spots (0.5 µm × 0.5 µm) on each surface type.

Contact Angle Goniometry

Contact angle measurements were performed with a Model 200 contact angle goniometer (Ramé-Hart) equipped with a fluid cell for captive bubble measurements. *n*-Decane drops (2 μL) were injected into the fluid cell (filled with PBS, pH 7.4) and deposited on the surface using a syringe fitted with a J-shaped needle (Type 304 stainless steel, 22 gauge). The left and right contact angles were recorded after 60 s from digital images using the DROPImage Standard software (Ramé-Hart). For each surface, 4 replicate specimens were measured for a total of ~10 deposited droplets.

Surface Charge (ζ-Potential)

The ζ-potential of the surfaces was determined from streaming current measurements using an electrokinetic analyzer (SurPASS, Anton Paar). Two 10 × 20 mm^2 specimen coupons were attached to sample holders using double-sided tape; sample holders were subsequently mounted in an adjustable gap cell, setting the gap size to ≈ 110 μm. The streaming current was measured by flowing the electrolyte solution (1 mM KCl) through the gap (i.e., parallel to the specimen coupons) as the pressure difference was increased to 400 mbar. A linear dependence of the streaming current with the pressure difference was observed, in accordance with the Helmholtz-Smoluchowski equation,[8] and the ζ-potential was determined from the slope. Streaming potential measurements were performed at pH ~5.5 – 10 adding aliquots of 0.05 M NaOH. Two independently prepared samples of each surface type were characterized.

Bacterial Culture Conditions

The as-received freeze-dried bacterial culture powder (*P. fluorescens* ATCC 13525) was used to inoculate 6 mL of LB broth (Miller, Sigma-Aldrich). Following incubation for 2 hours at 30 °C, agar plates were streaked and incubated at 30 °C overnight to grow bacterial colonies. Bacterial suspensions were prepared by transferring a colony with a pipette tip to 50 mL of LB medium. The suspension was incubated overnight at 30 °C and 125 RPM shaking speed, and diluted (1:25) with fresh LB broth. After further incubation for ~3 hours at 30 °C and 175 RPM, cells were harvested in mid-exponential phase ($OD_{600\ nm}$ ≈ 0.4 – 0.6), centrifuged at 5000 × g for 1 min, and re-suspended in PBS, pH 7.4. This step was repeated thrice. All materials and reagents used in cell culture were autoclaved before use.

Sample Preparation and Cantilever Functionalization

A specimen of sample surface with a dimension of ~1 × 0.5 cm^2 was adhered using epoxy (3M Quick Set Epoxy Adhesive) to a piranha- and UV/O_3-cleaned 35-mm circular glass disc (Asylum Research). After a 15-min epoxy curing step, a 20-μL droplet of bacterial suspension was placed on the glass disc beside the specimen. The droplet was let to stand for 30 min to permit bacterial deposition on the glass surface. Afterward, 4 mL of PBS was used to rinse off excess unattached cells, avoiding contact between the specimen surface and the bacterial suspension. The glass disc

was mounted in the AFM fluid cell (Fluid Cell Lite, Asylum Research), which was then filled with 2 mL of PBS buffer (pH 7.4).

Tipless silicon nitride cantilevers with nominal $k = 0.01$ N/m (Bruker MLCT-O10 probe "C") were used in force spectroscopy experiments. Cantilevers were cleaned in a UV/O_3 chamber for 25 min before use. A self-adherent polydopamine (PDA) coating[9,10] was deposited on the AFM probe to enable attachment of a bacterial cell to the end of the cantilever. PDA deposition was conducted for 15 min (65 RPM shaking speed) from a solution containing 4 mg of dopamine hydrochloride (Sigma-Aldrich) per milliliter of Trizma buffer (10 mM, BioReagent, Sigma-Aldrich) buffered to pH 8.5. Following deposition, the probe was rinsed with ultrapure water and dried in a nitrogen-purged desiccator for 5 min. Prior to bacterial attachment, the cantilever optical lever sensitivity was measured over the bare glass surface, and the spring constant (k) was calibrated using the thermal noise method[11] (the values of k were within the range specified by the manufacturer). The AFM probe was mounted onto the AFM probe holder, and the AFM head was thereafter lowered into the fluid cell, allowing ~40 min for the cantilever deflection signal to reach a stable value. To prepare a single-cell AFM probe, the PDA-coated cantilever was engaged at a 1 nN loading force on a single bacterial cell identified using the 63× objective of the inverted optical microscope (Zeiss Axio Observer A.1). After 5 min, the cantilever (functionalized with the bacterial cell) was withdrawn.

Supporting Results

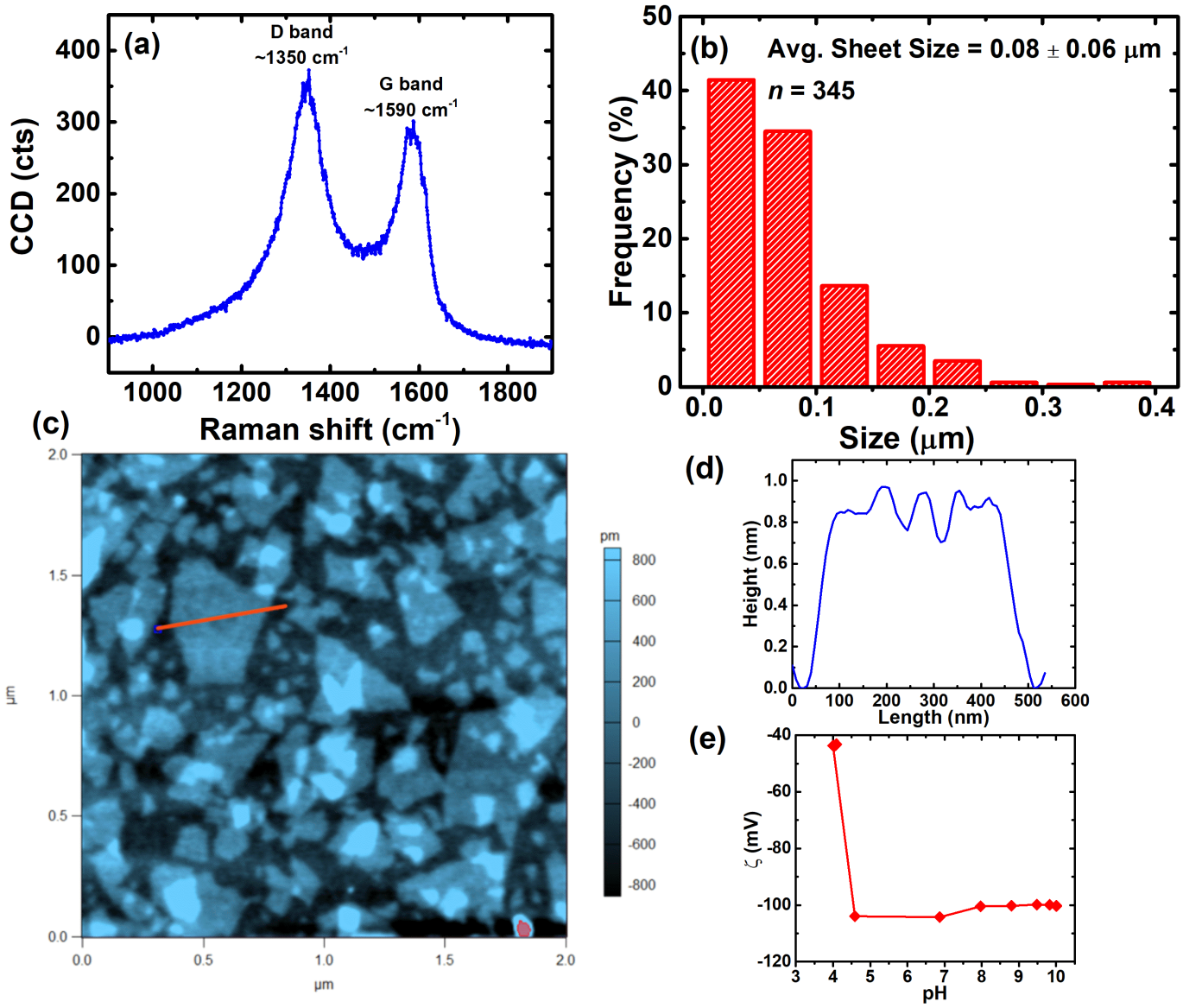

Figure S1: Characterization of graphene oxide (GO): (a) Raman spectrum; (b) sheet size (equivalent radius) distribution; (c) tapping mode AFM image of GO sheets deposited on silicon; (d) GO sheet height profile (determined along the red line in panel c); (e) zeta potential of GO in aqueous dispersion at a concentration of 250 μg mL^{-1}.

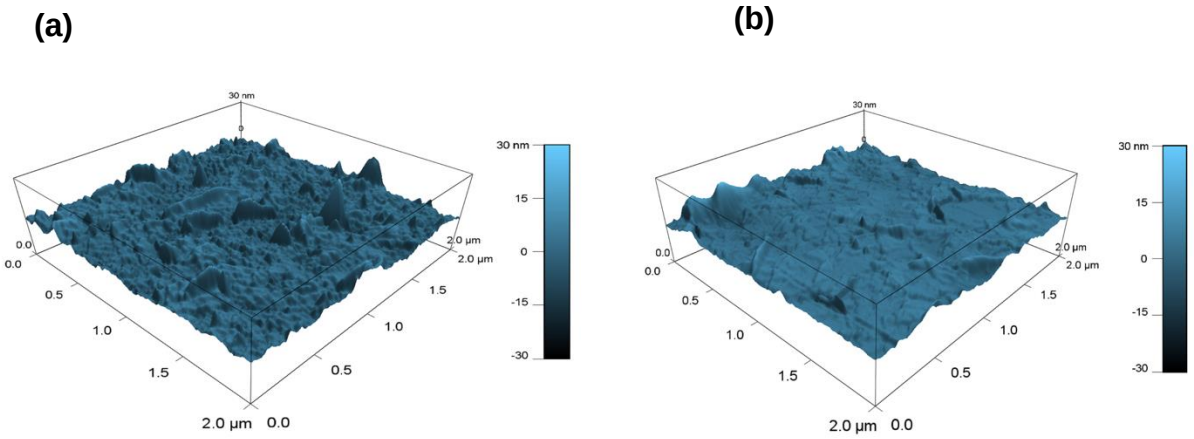

Figure S2: Surface topography visualized by tapping mode AFM: (a) PLL-GO and (b) Si-GO.

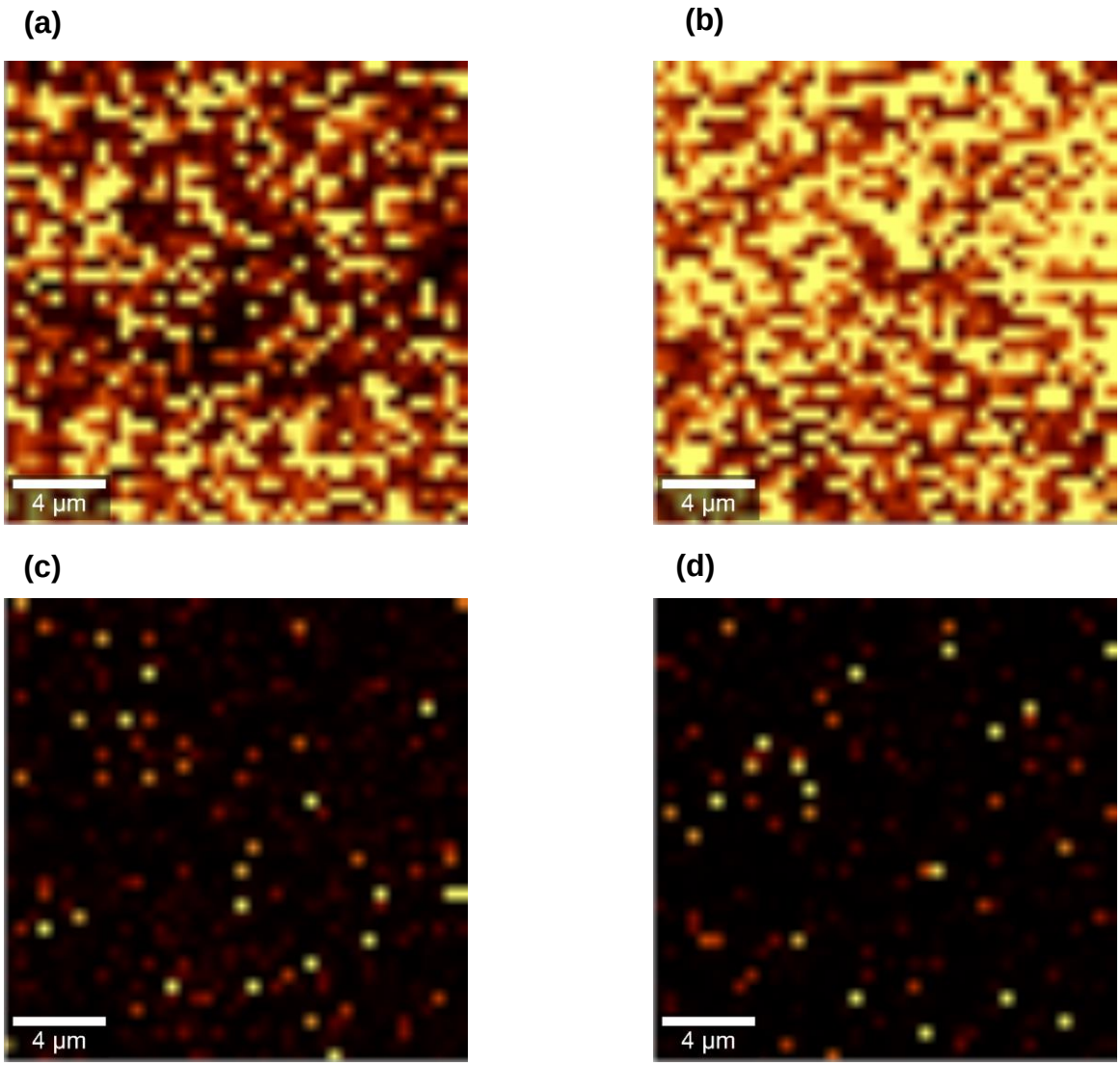

Figure S3: Raman spectroscopy images of (a) PLL-GO, (b) Si-GO, (c) PLL, and (d) Si surfaces.

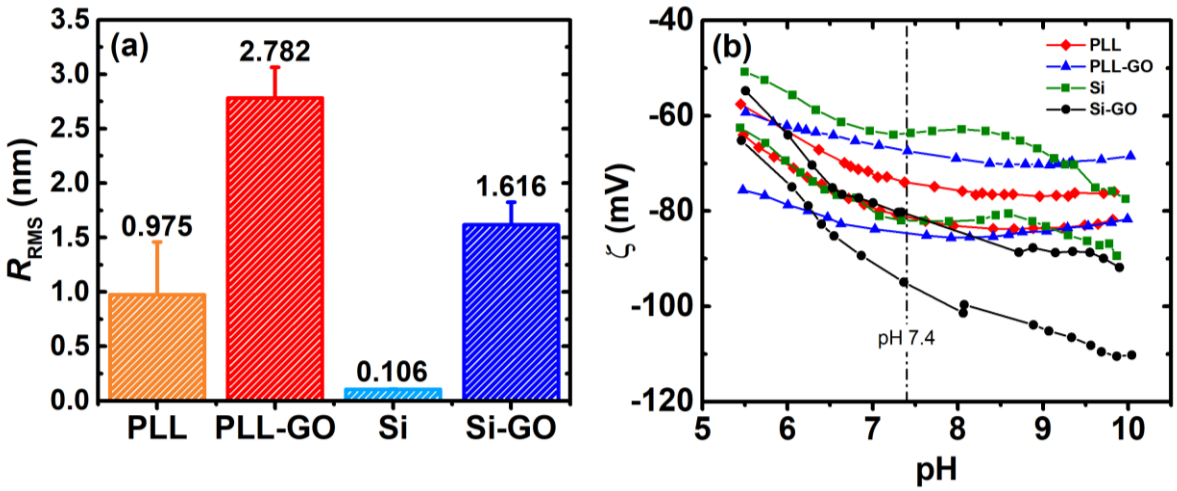

Figure S4: RMS roughness values (a) and ζ-potential as a function of pH (b) for the different surfaces.

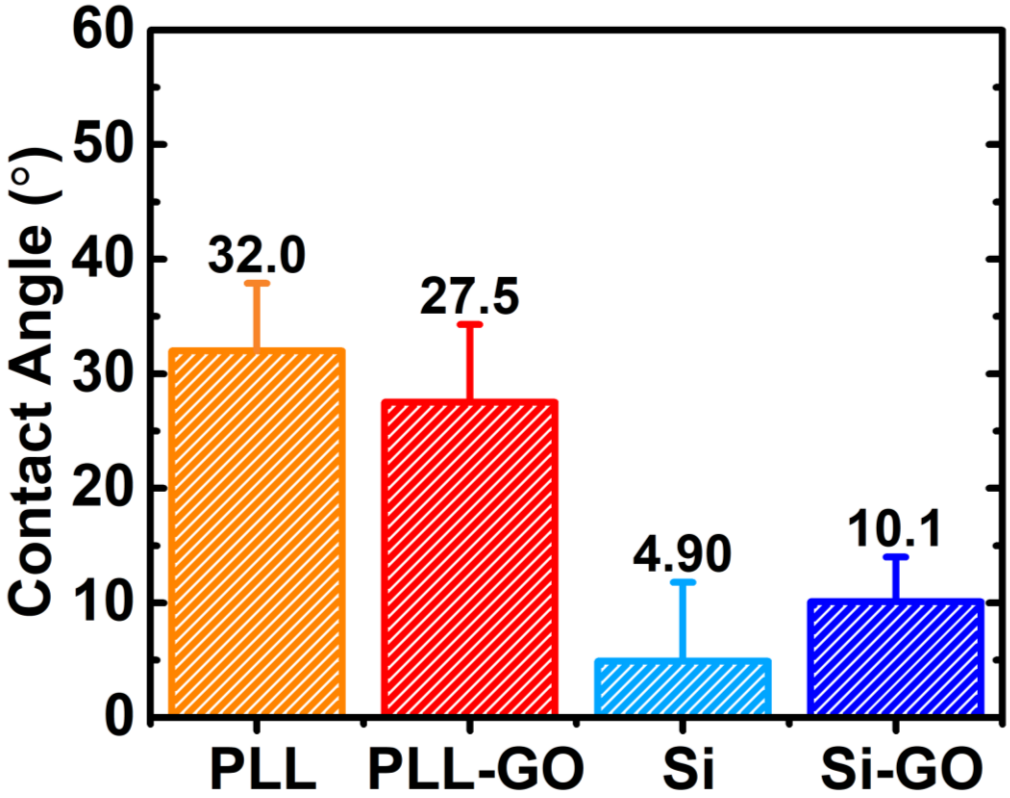

Figure S5: Contact angles obtained through the captive bubble method using *n*-decane droplets in PBS buffer (pH 7.4).

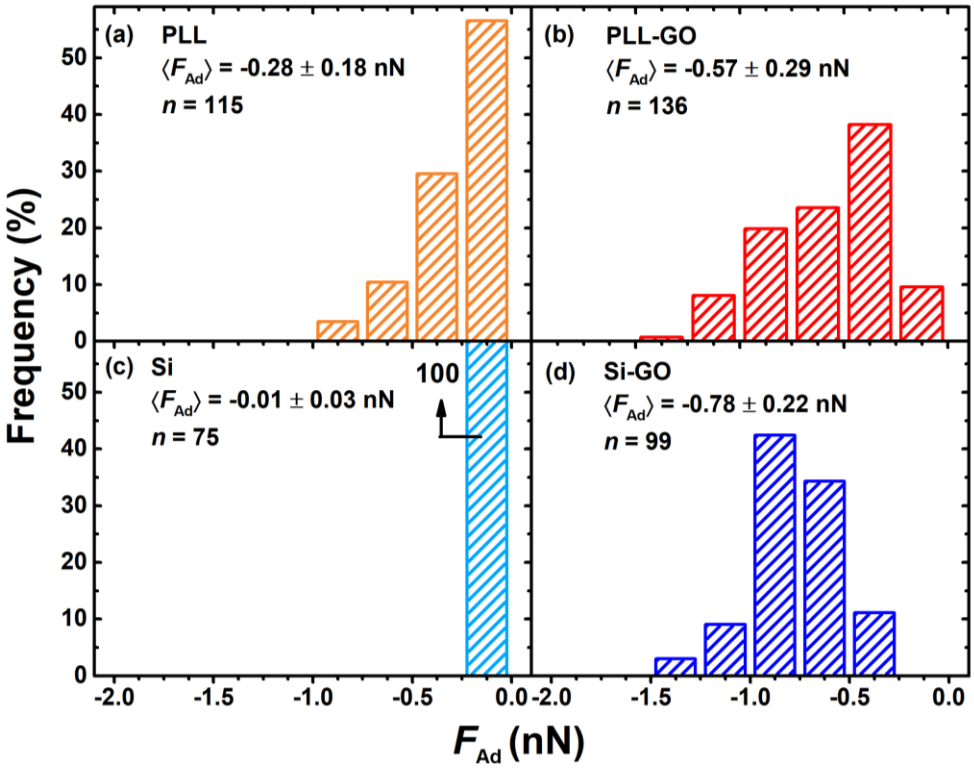

Figure S6: Distribution of maximum adhesion forces (F_{Ad}) of single *P. fluorescens* cells on: (a) poly-L-lysine-coated glass (PLL); (b) graphene oxide (GO)-functionalized PLL surfaces (PLL-GO); (c) Si wafers; (d) Langmuir-Blodgett GO films deposited on Si wafers by dip-coating (Si-GO). The inset shows the histogram average ($\langle F_{Ad} \rangle$), standard deviation, and number of measurements (n). Measurements were performed in PBS buffer (pH 7.4).

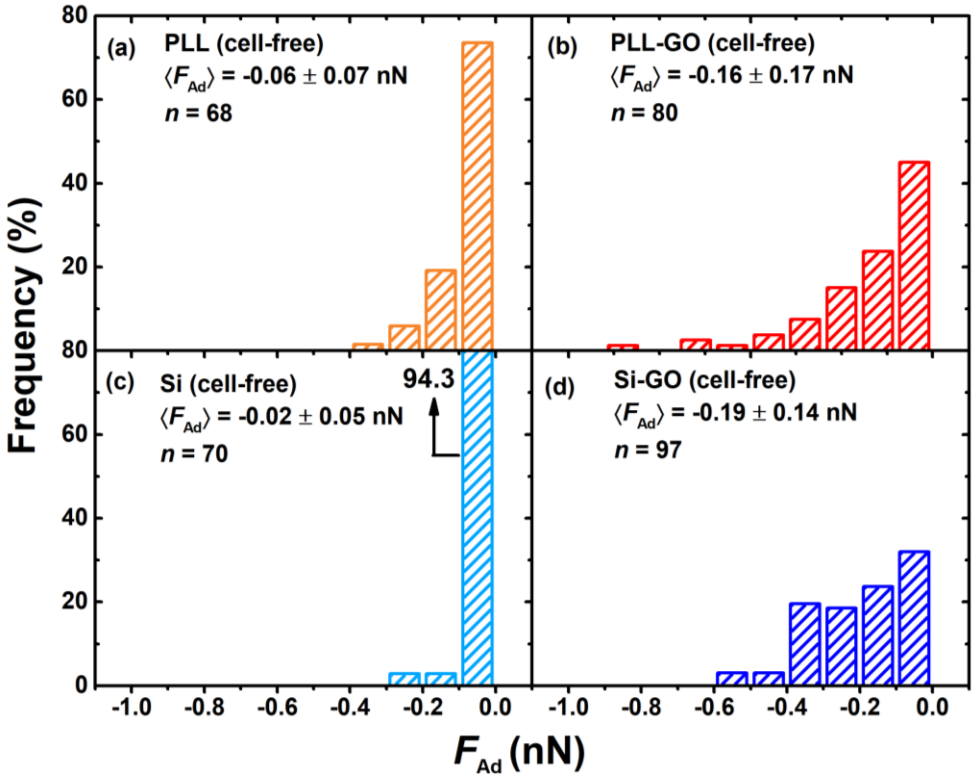

Figure S7: Distribution of maximum adhesion forces (F_{Ad}) of cell-free polydopamine-coated cantilevers on: (a) poly-L-lysine-coated glass (PLL); (b) graphene oxide (GO)-functionalized PLL surfaces (PLL-GO); (c) Si wafers; (d) Langmuir-Blodgett GO films deposited on Si wafers by dip-coating (Si-GO). The inset shows the histogram average ($\langle F_{Ad} \rangle$), standard deviation, and number of measurements (n). Experiments were performed in PBS buffer (pH 7.4).

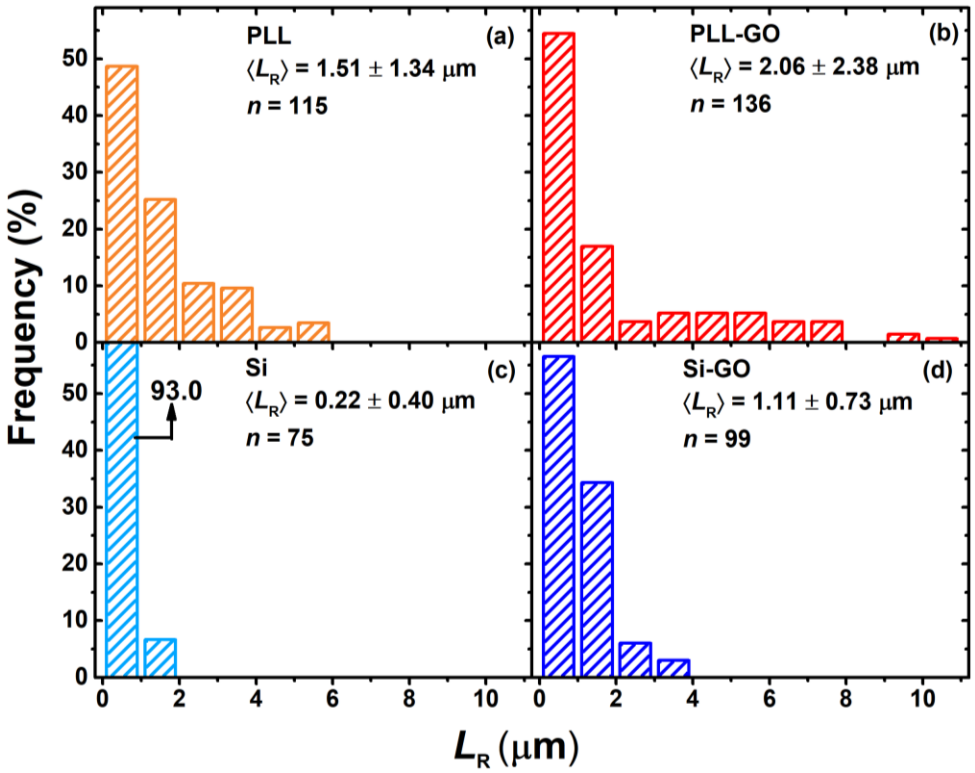

Figure S8: Distribution of rupture separation (L_R: distance from the surface at which adhesion forces vanish) for single *P. fluorescens* cells on: (a) poly-L-lysine-coated glass (PLL); (b) graphene oxide (GO)-functionalized PLL surfaces (PLL-GO); (c) Si wafers; (d) Langmuir-Blodgett GO films deposited on Si wafers by dip-coating (Si-GO). The inset shows the histogram average (⟨L_R⟩), standard deviation, and number of measurements (n). Experiments were performed in PBS buffer (pH 7.4).

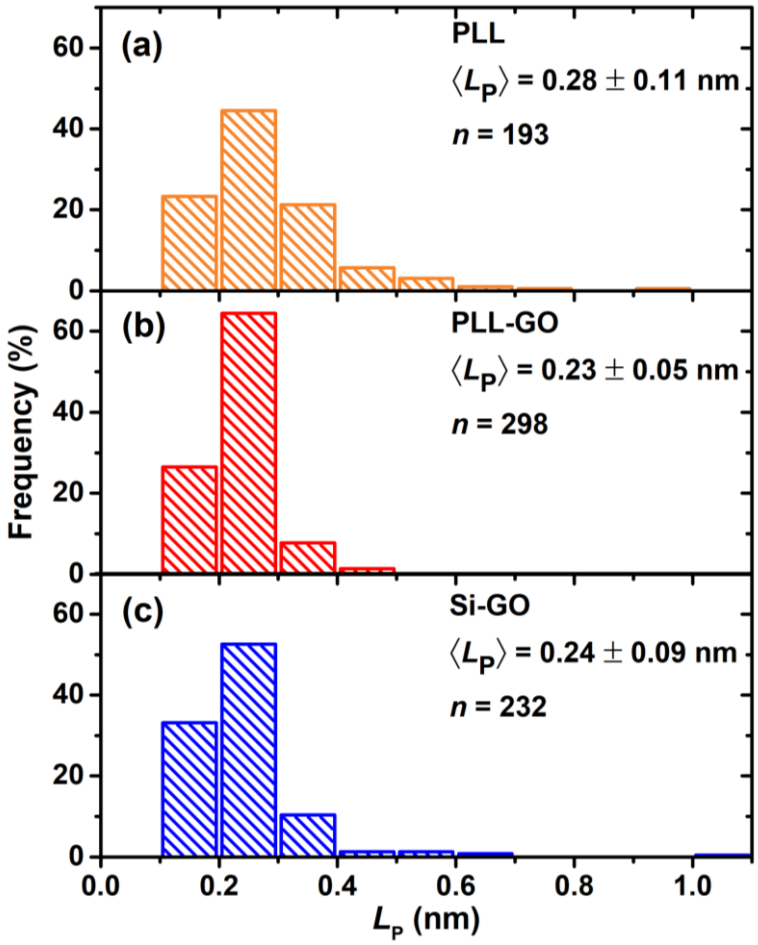

Figure S9: Distribution of best-fit persistence length values (L_P), obtained from WLC model fits to the pull-off force curve of single *P. fluorescens* cells on: (a) poly-L-lysine-coated glass (PLL); (b) graphene oxide (GO)-functionalized PLL surfaces (PLL-GO); (c) Langmuir-Blodgett GO films deposited on Si wafers by dip-coating (Si-GO). The inset shows the histogram average (⟨L_P⟩), standard deviation, and number of measurements (n). Experiments were performed in PBS buffer (pH 7.4).

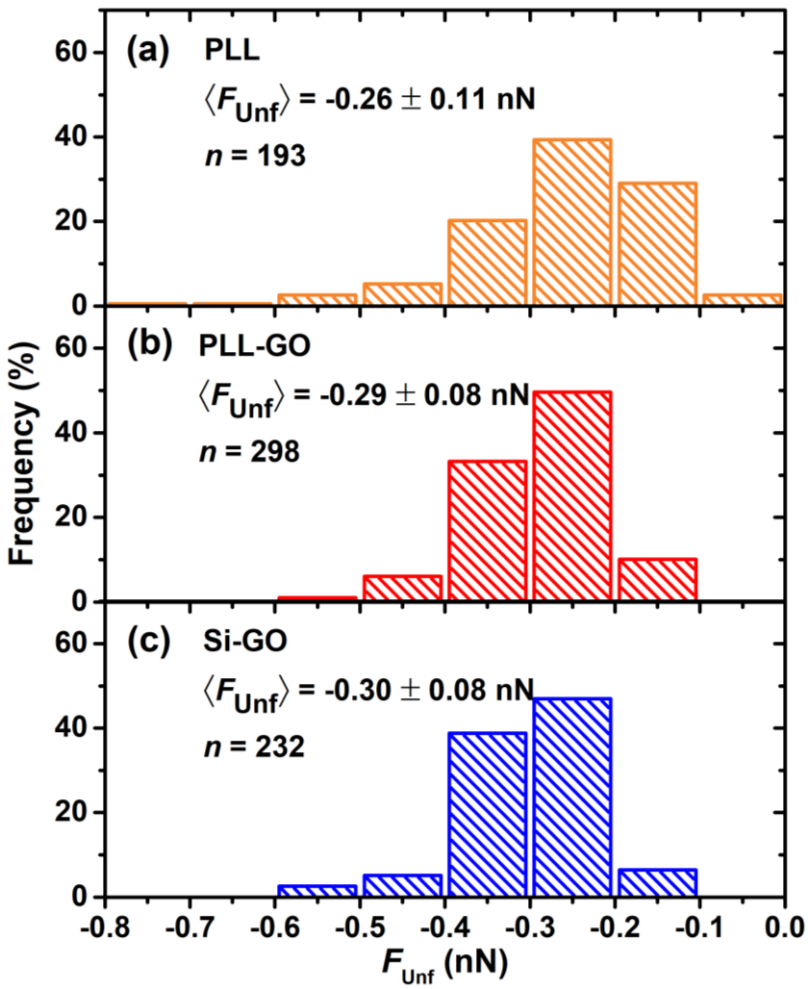

Figure S10: Distribution of the unfolding forces (F_{Unf}, the force measured at the sawtooth peak, cf. Figure 3(a)), obtained from the pull-off force curve of single *P. fluorescens* cells on: (a) poly-L-lysine-coated glass (PLL); (b) graphene oxide (GO)-functionalized PLL surfaces (PLL-GO); (c) Langmuir-Blodgett GO films deposited on Si wafers by dip-coating (Si-GO). The inset shows the histogram average ($\langle F_{\mathrm{Unf}} \rangle$), standard deviation, and number of measurements (n). Experiments were performed in PBS buffer (pH 7.4).

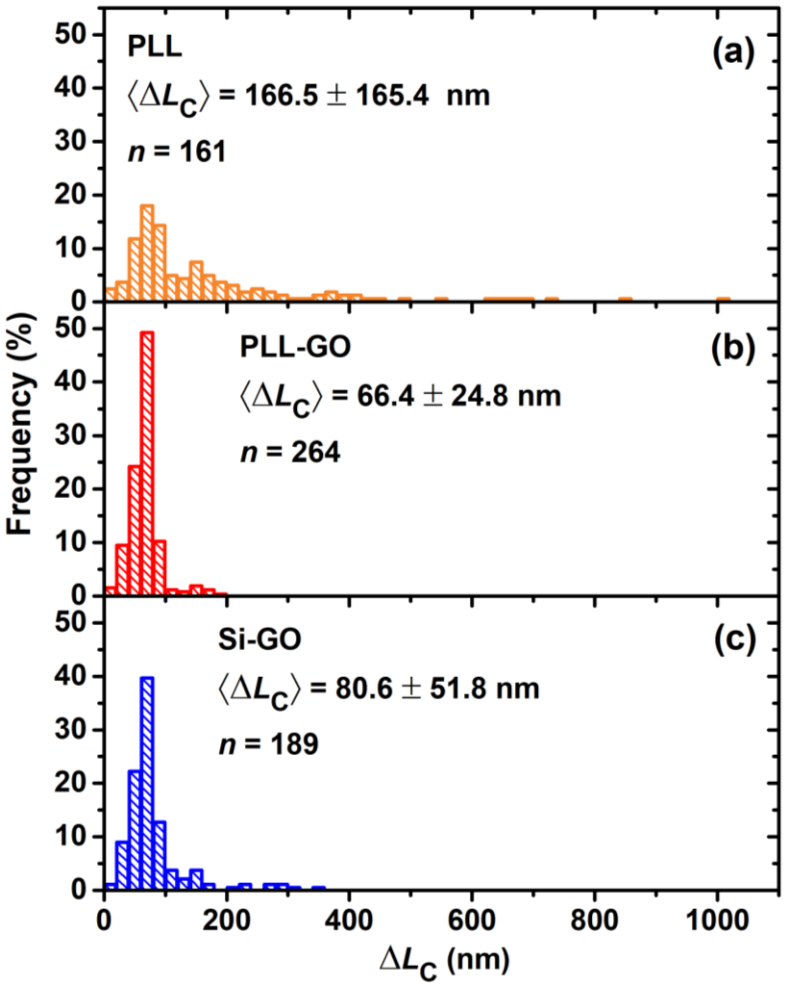

Figure S11: Distribution of ΔL_C (the difference in contour length between two consecutive sawtooth peaks), obtained from the pull-off force curve of single *P. fluorescens* cells on: (a) poly-L-lysine-coated glass (PLL); (b) graphene oxide (GO)-functionalized PLL surfaces (PLL-GO); (c) Langmuir-Blodgett GO films deposited on Si wafers by dip-coating (Si-GO). The inset shows the histogram average ($\langle \Delta L_C \rangle$), standard deviation, and number of measurements (n). Experiments were performed in PBS buffer (pH 7.4).

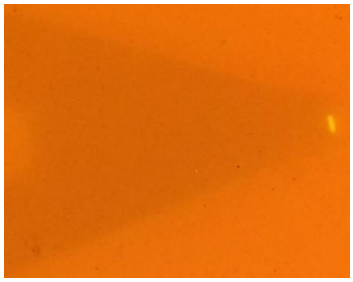

Figure S12: Bacterial cell probe imaged after force measurements. The bacterial cell (*P. fluorescens*) was attached to the front edge of a tipless AFM cantilever using polydopamine wet adhesive. The observed green fluorescence indicates that the cell remained viable throughout the experiment.

Supporting References

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