Supplementary Information

Nanoengineering Multifunctional Hybrid Interfaces Using Adhesive Glycogen Nanoparticles

Pietro Pacchin Tomanin, Jiajing Zhou, Alessia Amodio, Rita Cimino, Agata Glab, Francesca Cavalieri, Frank Caruso

Table of Contents

1 Experimental section

1.1 Synthesis

1.1.1 Preparation of fluorescent labeled L-PG

L-PG (2 mg) was dispersed in carbonate buffer (1 mL, 10 mM, pH 9.2). To this dispersion, AF488 solution (30 µL, 1 mg mL^{-1} in dimethyl sulfoxide (DMSO)) was added and the resulting solution was mixed overnight. Excess fluorophore was removed using an illustra Nap 10 Sephadex column (GE Healthcare Life Sciences), and the resulting product was freeze-dried to generate L-PG@AF488.

1.1.2 Synthesis of dodecane-conjugated phytoglycogen (DD-PG)

DD-PG was prepared using the same synthesis procedure as that used for preparing L-PG, with the exception that dodecanoic acid (0.06 eq.) was used instead of lipoic acid. The degree of functionalization of modified glycogen, as determined by NMR spectroscopy, was 1% (dodecanoic moieties/glucose mol/mol).

1.1.3 Synthesis of gold nanoparticles (AuNPs)

AuNPs of 14 nm in diameter were prepared by citrate reduction of $HAuCl_4$ in aqueous phase. Typically, a sodium citrate (102 mg) water solution (2 mL) was rapidly injected into a boiling aqueous $HAuCl_4$ solution (30 mg in 200 mL water) under vigorous stirring. After boiling for 15 min, the solution was cooled to room temperature. The colloidal dispersion was stored at 4 °C in the dark until further use.

1.2 Characterization

1.2.1 Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectra were obtained on a TENSOR II ATR−FTIR spectrometer (Bruker) and analyzed using the software OPUS 7.8. The number of scans was 64 with a minimum resolution of 4 cm^{-1}.

1.2.2 UV–Vis spectroscopy

UV–Vis absorbance spectra were recorded on a SPECORD 250 PLUS spectrophotometer (Analytik Jena, Germany) and analyzed using the software Aspect UV. Solid and liquid samples spectra were recorded using, respectively, a solid sample holder and a reduced volume quartz cuvette with a path length of 1 cm mounted on a standard cell holder. Spectra were acquired with a scan speed of 20 nm s^{-1}. The spectra of PG and L-PG were recorded using a 1 mg mL^{-1} water dispersion.

1.2.3 Dynamic light scattering (DLS): hydrodynamic diameter and ζ-potential

The hydrodynamic diameter and ζ-potential of the nanoparticles were measured using a Zetasizer Nano-ZS instrument (Malvern Instruments, Malvern, UK) equipped with a He–Ne ion laser (λ = 633 nm). To evaluate the hydrodynamic diameter based on the scattered intensity, an L-PG or PG dispersion (0.1 mL, 1 mg mL^{-1} in water) was analyzed in a micro cuvette (ZEN0040, Malvern Instruments). To evaluate the ζ-potential, a nanoparticle dispersion (0.8 mL, 3 mg mL^{-1}) in PBS (0.1×, pH 7.4) was analyzed in a capillary cell (DTS1070, Malvern Instruments). The analysis was performed using a standard operation procedure with automatic attenuation and measurement position, executing 5 runs consisting of at least 10 acquisitions for each sample.

1.2.4 Atomic force microscopy (AFM)

AFM experiments were performed on a JPK NanoWizard II BioAFM instrument. Images were obtained in tapping mode using Tap300-G (BudgetSensors, Bulgaria) monolithic silicon cantilevers with a spring constant and

resonance frequency of about 40 N m^{-1} and 300 kHz, respectively. For the analysis of single nanoparticles, an L-PG aqueous dispersion (40 µL, 1 µg mL^{-1}) was incubated on a freshly gold-sputtered mica substrate (20 nm gold layer) for 60 min, extensively washed with water, and dried with a N_2 gun. Height and thickness values were averaged over 250 single nanoparticles. For the thickness and roughness analysis of the films, glass substrates were first cleaned with EtOH and water and then completely immersed in a 0.1 mg mL^{-1} PG, DD-PG or L-PG solution for 10 min. The glass substrates were then rinsed with water several times to remove all excess and unbound polymer, and dried with a N_2 gun to obtain PG-, DD-PG- or L-PG-functionalized glass substrates. Scratches were made using a metallic doctor blade. Films thicknesses and roughness were analyzed (at least 20 different profiles for each sample) using the JPK SPM image processing software.

1.2.5 Contact angle measurements

Contact angle experiments were performed using H_2O (3 µL) at 21 °C on an optical contact angle tensiometer (DataPhysics) using the sessile drop method. The substrates were placed in the contact angle instrument window analysis. The drop shape was fitted using the elliptic method. The contact angle values and standard deviations were calculated based on the left and right contact angles of at least 15 different drops per sample.

1.2.6 Cytotoxicity assay

Cell viability was evaluated via a standard thiazolyl blue tetrazolium bromide (MTT) assay protocol. Cells (MDA-MB-231) were plated on a 96-well plate (Costar 3596, Corning, USA) with a seeding density of 10^4 cells per well in Dulbecco's modified Eagle medium (100 µL) with 10% fetal bovine serum. After 24 h, L-PG nanoparticles were added to the culture media (final concentrations as reported) and the systems were incubated for 24 h. Then, MTT reagent was added and after 4 h, the resulting formazan crystals were dissolved in DMSO. The cell viability was determined by absorbance measurements at 554 and 670 nm (as reference) with an Infinite M200 microplate reader (Tecan, Switzerland).

1.2.7 Degradation of PG and L-PG nanoparticles by β-amylase

The rate of glycogen nanoparticle hydrolysis by β-amylase was determined using the Somogyi–Nelson assay adjusted for a microtiter plate.[1] For the assay, a PG or L-PG solution (25 µL, 2 mg mL^{-1}) in 16 mM sodium acetate buffer (pH 4.8) was treated with β-amylase solution in H_2O (25 µL, 1 U mL^{-1}) for 20 min, 1 h, 2 h, or 24 h on a 96-well plate (Costar 3596, Corning, MA, USA); the experiments were performed in triplicates. After the incubation, the working solution was added (50 µL), the plate was covered and heated in a water bath at 95 °C for 20 min. Then, the plate was cooled to room temperature, and the arsenomolybdate color reagent was added (50 µL) into each well, followed by incubation for 1.5 h for complete color development. The absorbance was measured at 750 nm with an Infinite M200 microplate reader. To prepare the working solution, sodium potassium tartrate tetrahydrate (1.2 g), sodium carbonate (2.4 g), sodium bicarbonate (1.6 g), and sodium sulfate (14.4 g) were dissolved in water, and the resulting solution was diluted to 80 mL to obtain stock Solution I. Copper sulfate pentahydrate (0.4 g) and sodium sulfate (3.6 g) were dissolved in water, and the resulting solution was diluted to 20 mL to obtain stock Solution II. The solutions were stored separately to prevent copper oxidation.[2] Four parts of Solution I and one part of Solution II were freshly mixed to prepare the working solution before analysis. To prepare the color reagent, ammonium molybdate (2.5 g) was dissolved in water (45 mL) and concentrated sulfuric acid (2.1 mL). Sodium arsenate dibasic pentahydrate (0.3 g) was dissolved in water (2.5 mL) and mixed with the ammonium molybdate solution. The reagent was incubated at 37 °C for 24–48 h and stored in a brown bottle.[3]

1.2.8 Stability of L-PG coatings on glass substrates

The chemical stability of the L-PG coatings was studied via fluorescence analysis. First, square-shaped glass substrates were cleaned with ethanol (EtOH) and water and then completely immersed in a 0.1 mg mL^{-1} AF488-labeled L-PG solution for 10 min. Then, the functionalized glass substrates were rinsed with water several times to remove all excess and unbound polymer. The prepared glass substrates were then separately incubated for 60 min in several solutions: H_2O, 10 mM phosphate-buffered saline (PBS; pH 7.4), 1 M HCl, 1 M HNO_3, 1 M H_2O_2, 10 mM glutathione (GSH), 17 mM sodium dodecyl sulfate (SDS), EtOH, and 0.5 U mL^{-1} β-amylase (in 8 mM acetate buffer, pH 4.8). Following incubation, each glass substrate was rinsed with water and dried with a N_2 gun. The fluorescence emission intensities were recorded by mounting the samples on a solid-state sample holder in a Fluorolog instrument (Jobin Yvon Horiba). Emissions intensities were recorded at a wavelength of 515 nm using an excitation wavelength of 488 nm (slit aperture 5). The data were recorded using the software FluorEssence.

1.2.9 Quartz crystal microbalance with dissipation (QCM-D)

QCM-D experiments were performed using a QSense E4 (Biolin Scientific, Sweden) equipped with a peristaltic pump. Before the measurements, gold sensor chips (QSX 301 Gold, Biolin Scientific) were cleaned with piranha solution, rinsed with Milli-Q water, dried with a N_2 gun and sterilized with a UV/ozone treatment (Bioforce Nanoscience, UV.TC.EU.003, USA) for 20 min.

1.2.10 Differential interference contrast (DIC) microscopy

DIC microscopy images were recorded using an inverted Olympus IX71 microscope.

1.2.11 Transmission electron microscopy (TEM)

For TEM analysis of the bare, L-PG- and L-PG/AuNP-coated PS microparticles, the corresponding water dispersion was dropped onto an ozone-cleaned copper grid and allowed to dry in air overnight. TEM images were acquired using a Tecnai Spirit (FEI, USA) instrument with an operation voltage of 120 kV.

1.2.12 Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy

For SEM and EDX analysis, the sample-containing copper grid prepared for TEM analysis was attached onto a carbon tape and analyzed as is. SEM images and EDX spectra and mapping were acquired with a FlexSEM 1000 benchtop scanning electron microscope (Hitachi, Japan) equipped with a Quantax 80 EDX system (Bruker, USA). Typically, images and spectra of samples were recorded using a beam voltage of 15 kV, operating in SEM pressure mode and with an acquisition time of 5 min.

1.2.13 Scanning helium ion microscopy (HIM)

HIM was performed on an ORION NanoFab (Zeiss, Peabody, USA) using a 25 keV He^+ probe with a current of 0.3 pA, and the image was obtained using an Everhart–Thornley-type secondary electron detector. The sample was mounted on a 45° holder and the stage tilted by a further 30° to give a final viewing angle of 75°.

1.2.14 Stability of L-PG/AuNP hybrid coatings on glass substrates

To assess the chemical stability of the L-PG/AuNP hybrid coatings, square-shaped glass substrates were first cleaned with EtOH and water and then completely immersed in a 0.1 mg mL^{-1} L-PG solution for 10 min. The functionalized glass substrates were then rinsed with water several times to remove all excess and unbound polymer. The L-PG-coated substrates were then completely immersed in a AuNP colloidal dispersion for 10 min and subsequently rinsed with water to obtain 1 bilayer coating of L-PG/AuNP on each side. The prepared glass

substrates were then separately incubated for 60 min in several solutions: H_2O, 10 mM PBS (pH 7.4), 1 M HCl, 1 M HNO_3, 10 mM $NaBH_4$, 1 M H_2O_2, *n*-hexane, EtOH, 17 mM SDS, and 10 mM GSH. After that, each glass substrate was washed with water and dried with a N_2 gun. The UV–vis spectrum of each glass substrate was recorded from 350 to 800 nm.

1.2.15 Catalytic evaluation of AuNPs in L-PG/AuNP-coated 15 mL polypropylene (PP) tubes

PP tubes functionalized with an inner coating of L-PG/AuNPs were used to perform the decoloration of methylene blue (MB; blue solution) to leucomethylene blue (LMB; colorless solution) via $NaBH_4$ in the presence of catalyst AuNPs. In a typical reaction, 5 mL of a 0.025 mM MB and 10 mM $NaBH_4$ solution were introduced into the tube and vortexed for 45 min. At intervals of 5 min, aliquots (0.3 mL) were withdrawn. The UV–vis spectra of the aliquots were measured using a plate reader in the range of 450 to 750 nm, with a recording interval of 2 nm. To obtain the kinetics graph, the absorbance intensity at 664 nm was recorded for each sample and plotted against the reaction time.

1.2.16 AuNP stability in modified 15 mL PP tubes

A 3-bilayer L-PG/AuNP-functionalized PP tube was prepared as described before, with AuNPs as the last layer. After each addition of AuNP solution, the supernatant was withdrawn and the UV–vis spectra were acquired. A 10 mM $NaBH_4$ solution (2 mL) was incubated by vortexing in the prepared tube for 45 min. At intervals of 5 min, aliquots (0.3 mL) were withdrawn. The aliquots were analyzed via UV–vis spectroscopy. To calculate the concentration (mM) of AuNPs in the supernatant, the following formula was used: OD@400nm:1.2 = 0.5 mM:*x* mM (where OD is the optical density).

1.2.17 Surface-enhanced Raman scattering (SERS) sensing using L-PG/AgNP hybrid coatings

A 3-bilayer L-PG/AgNP glass substrate was prepared as described before, with AgNPs as the last layer. Liquid samples at different concentrations of crystal violet were loaded onto the functionalized substrate and the SERS intensity was then measured with a RENISHAW Raman microscope equipped with a He−Ne laser operating at 532 nm. The laser spot size was 5 μm, focused by a 50× lens. Single scans were run with an integration time of 15 s using a grating of 1800 lines mm^{-1} from 300 to 1800 cm^{-1}. The acquired data were processed with the WIRE 2.0 software.

2 Figures

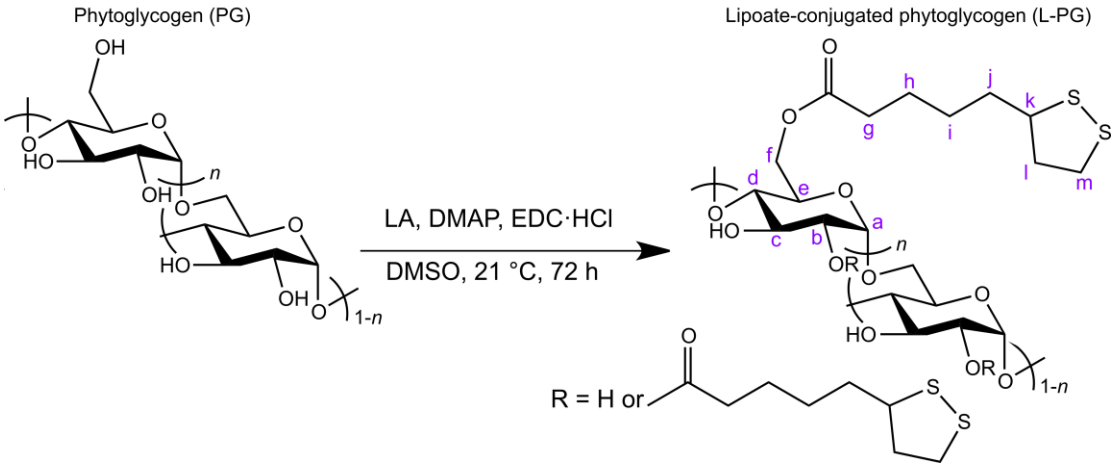

Figure S1: PG functionalization reaction with lipoic acid to obtain L-PG via Steglich esterification.

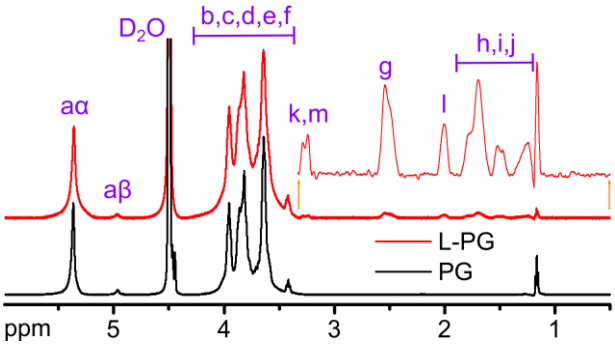

Figure S2: H-NMR spectra of L-PG and PG. The thin red line is a magnification of the lipoate peaks from 3.4 to 0.5 ppm.

Table S1: Chemical modification reactions of PG into L-PG

Type of glycogen	Targeted degree of functionalization (LA/glucose mol/mol)	Actual degree of functionalization (LA/glucose mol/mol)	Solubility
PG	30	Not measured	Non-soluble in water, EtOH, DMSO, or *n*-hexane
PG	20	Not measured	Non-soluble in water, EtOH/water (50:50), or DMSO
PG	10	2% on total glucose, 10.6% on H4 terminal	Water soluble, up to 20 mg mL^{-1}
PG	3	Below H-NMR limit of detection	Water
PG	1	Below H-NMR limit of detection	Water

PG, phytoglycogen; EtOH, ethanol; DMSO, dimethyl sulfoxide.

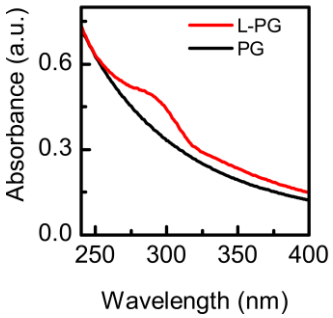

Figure S3: UV–vis spectra of L-PG and PG.

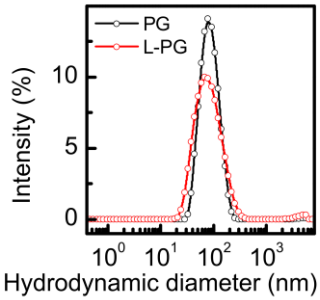

Figure S4: DLS hydrodynamic diameter populations of L-PG and PG.

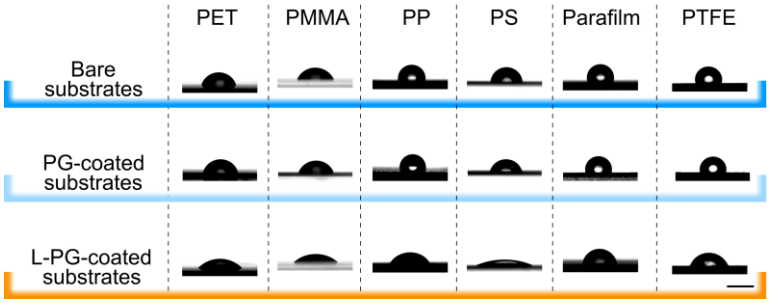

Figure S5: Photographs of water droplets on bare, PG-, and L-PG-coated substrates; the contact angle values are given in Figure 2c. Scale bar: 2 µm.

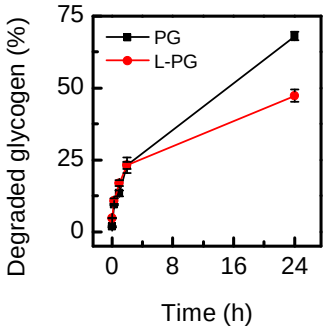

Figure S6: Enzymatic degradation of PG and L-PG.

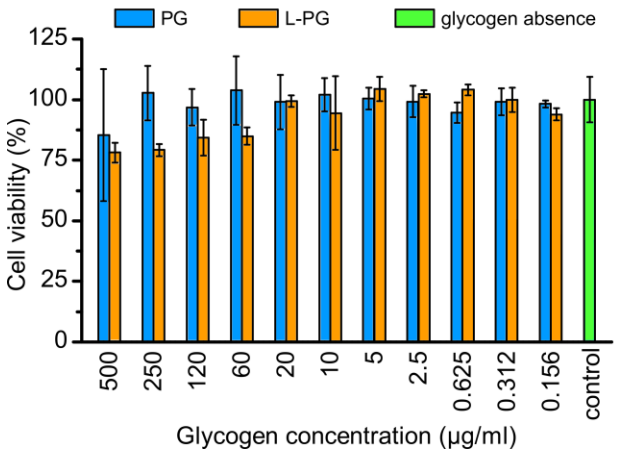

Figure S7: Cytotoxicity studies of PG and L-PG.

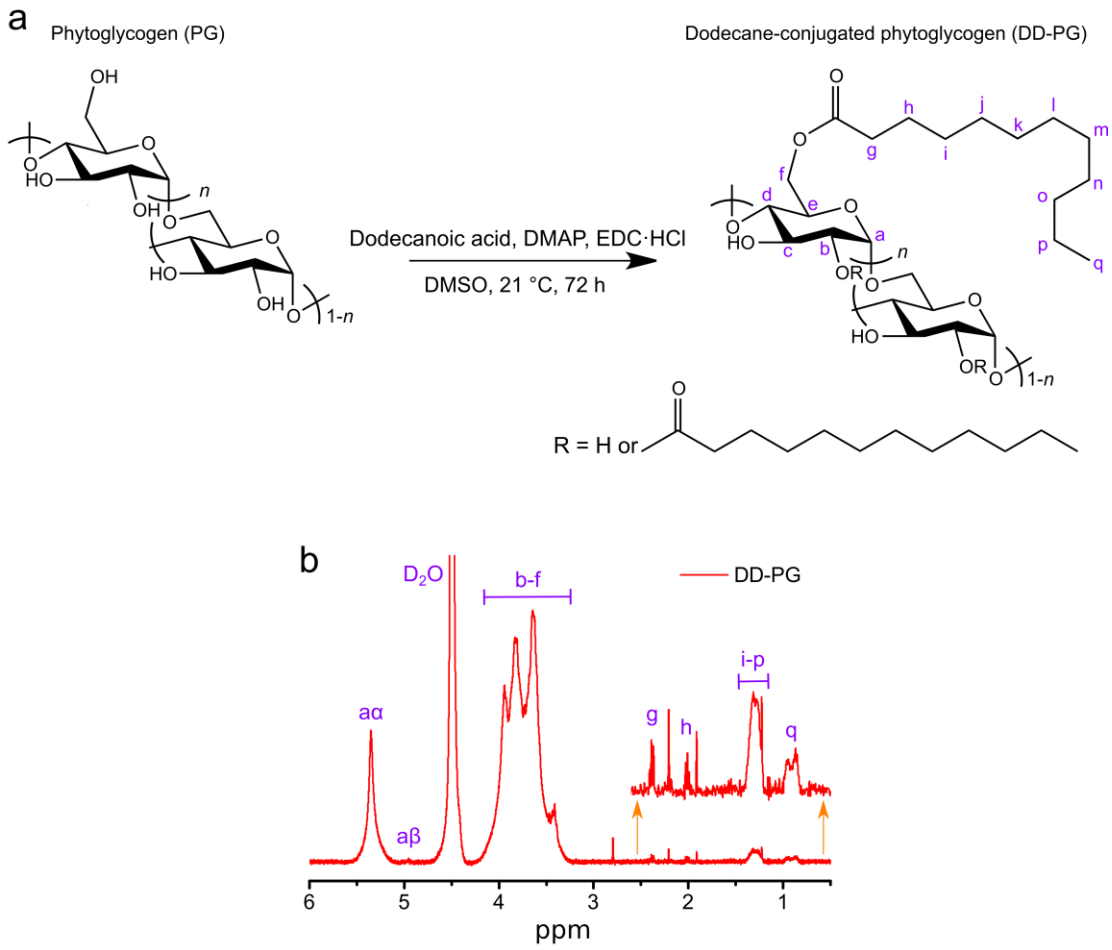

Figure S8: (a) PG functionalization reaction with dodecanoic acid to obtain DD-PG via Steglich esterification and (b) H-NMR spectra of DD-PG with magnification of the peaks corresponding to the dodecane moiety from 2.5 to 0.5 ppm.

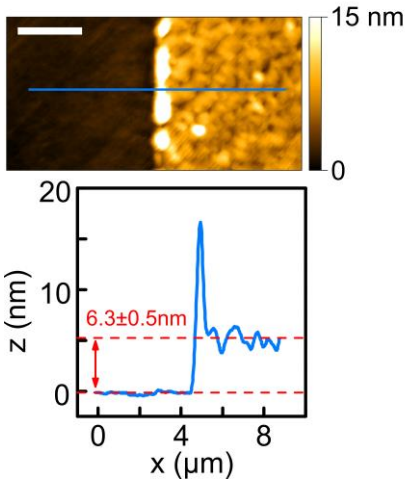

Figure S9: Dry-state AFM image and corresponding height profile of DD-PG-coated glass substrates (scale bar = 2 μm).

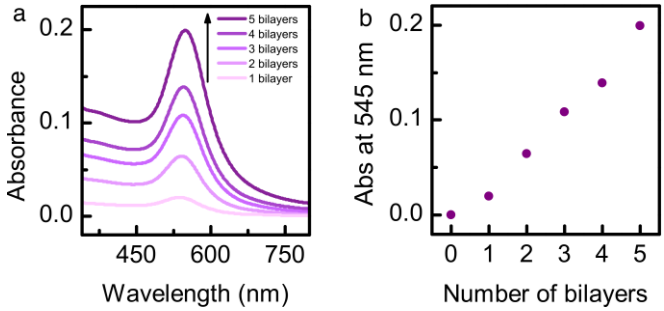

Figure S10: (a) UV–Vis spectra of glass substrates coated with increasing number of L-PG/AuNP bilayers. (b) Localized surface plasmon resonance absorbance profile at 545 nm as a function of the bilayer number.

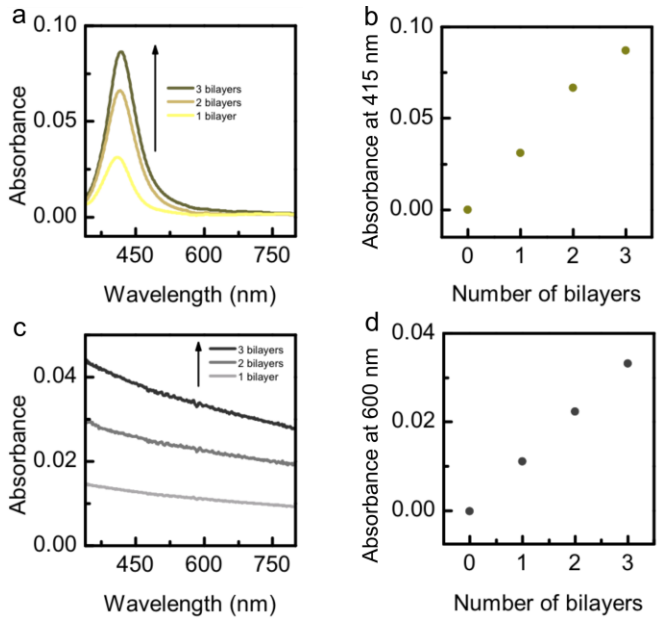

Figure S11: UV–Vis spectra of glasses coated with increasing number of (a) L-PG/AgNP and (c) L-PG/PtNP bilayers with corresponding absorbances at (b) 415 nm and (d) 600 nm as a function of the bilayer number.

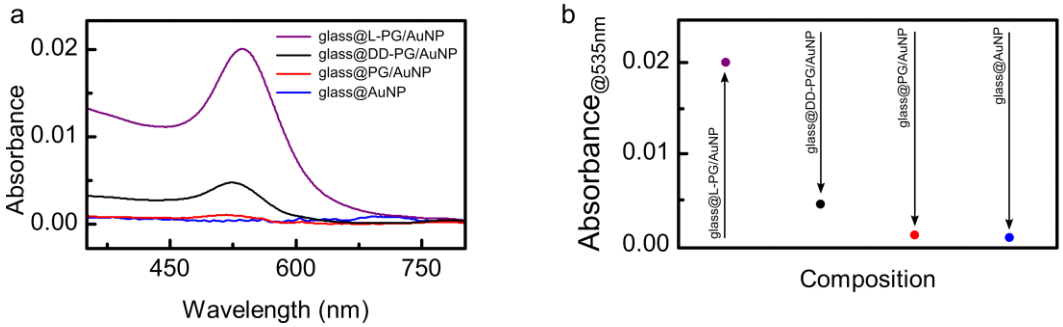

Figure S12: (a) UV–Vis spectra of glass substrates coated with L-PG/AuNP, DD-PG/AuNP, PG/AuNP assemblies or AuNPs alone and (b) corresponding absorbances at 545 nm.

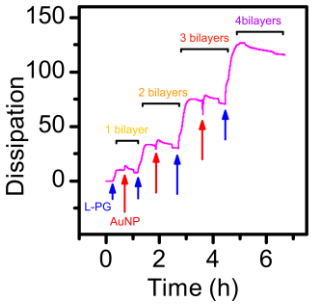

Figure S13: QCM-D dissipation profile of an L-PG/AuNP LbL assembly on gold chip obtained upon sequential deposition of L-PG (blue arrow) and AuNPs (red arrow); the corresponding QCM-D frequency profile is shown in Figure 3b.

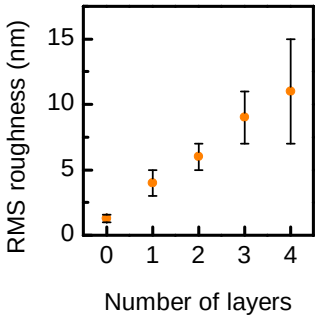

Figure S14: Root mean square (RMS) roughness of dry films as a function of the L-PG/AuNP bilayer number.

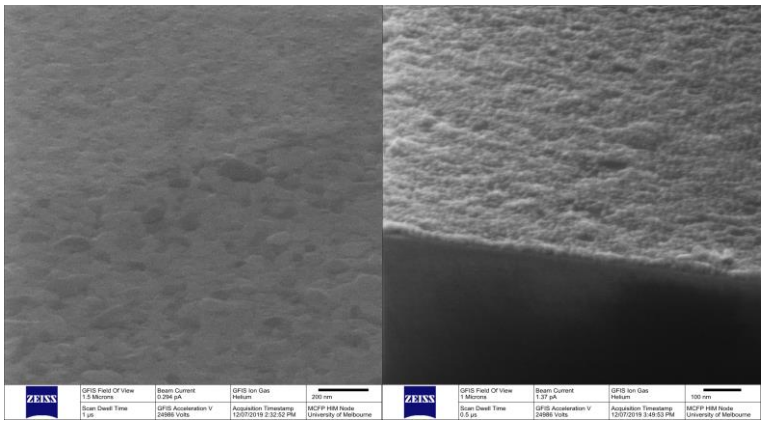

Figure S15: HIM images of L-PG/AuNP LbL assembly on glass with the final layer as L-PG (left) and AuNP (right).

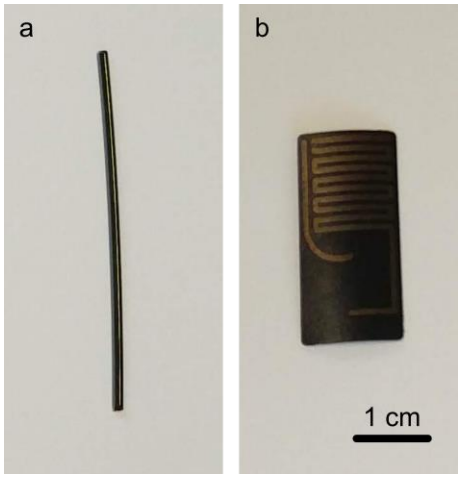

Figure S16: Photographs of L-PG/AuNP assembly on (a) polyether ether ketone and (b) Viton.

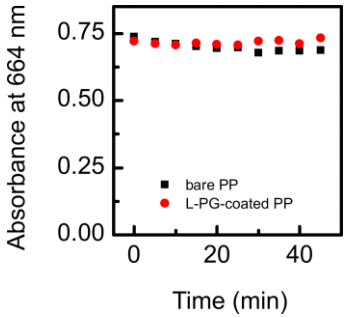

Figure S17: Degradation kinetics of MB via $NaBH_4$ on a bare PP tube and L-PG-coated PP tube. Both reaction systems do not contain AuNPs.

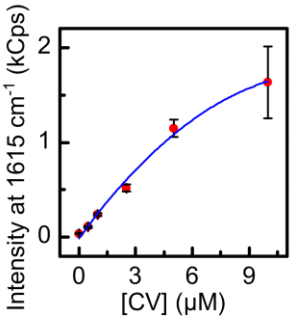

Figure S18: SERS signal intensity at 1615 cm^{-1} as a function of the concentration of the crystal violet (CV) solution; the corresponding SERS intensity profiles are shown in Figure 5d.

3 References

(1) Y. Shao and A. H.-M. Lin, *Food Chem.,* 2018, **240**, 898.
(2) M. Somogyi, *J. Biol. Chem.,* 1952, **195**, 19.
(3) N. Nelson, *J. biol. Chem.,* 1944, **153**, 375.