

Supporting Information

Modulation of surface biofunctionality by using gold nanostructures on protein repellent surfaces.

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PEO-like coating Characterization

X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed with an Axis Ultra spectrometer (Kratos, UK), using the $K\alpha$ Al monochromatic radiation ($h\nu=1487$ eV) and X-ray spot size of $100\ \mu\text{m}^2$ operating in hybrid mode. For each sample, a survey spectrum (0-1150 eV), from which the surface chemical composition (at %) was determined, was recorded at pass energy of 160 eV. Moreover, two sets of high-resolution spectra (PE = 20 eV) was recorded to obtain information about the chemical bonds involved. Surface charge was compensated by a filament ($V=3.6\text{V}$, $I=1.9\text{A}$) placed under the magnetic lens and all spectra were corrected by setting the C1s hydrocarbon peak to 285 eV.¹ The take-off angle for the acquisitions was 90° respect to the sample surface. The data were processed using Vision2 software (Kratos Analytical, UK) and the analysis of the XPS peaks was carried out using a commercial software package (Casa XPS v2.3.10, Casa Software Ltd, UK). The peak fitting was performed.

XPS elemental composition analysis was employed to investigate the quality of plasma-deposited PEO-like coatings produced (**Fig. S1 A and B**). The high-resolution C1s core level spectra of PEO-like coating were resolved by a combination of four distinct components (**Fig. S1 B**). A C0 contribution at 285.0 eV corresponding to C-C and C-H moieties, a C1 contribution at 286.5 eV corresponding to C-OH and/or C-O-C moieties, a C2 contribution at 288.0 eV (C=O moieties) and a last component C3 at 289.2 eV (O-C=O moieties). The quality of PEO-like coatings associated to its non-adhesive properties are commonly associated to the relative contribution of the ether peak component of the C1s XPS signal ($> 70\%$) and the O/C ratio.^{2,3,4} The results revealed that the coatings were mainly composed of carbon (62.6%) and oxygen

(36.5%) with an O/C ratio of 0.58 which is close to the value of pure DEGDM (0.5) indicating a good retention of the monomer precursor. Furthermore, the samples produced experimentally showed a very good PEO character (C-O) over 70%.

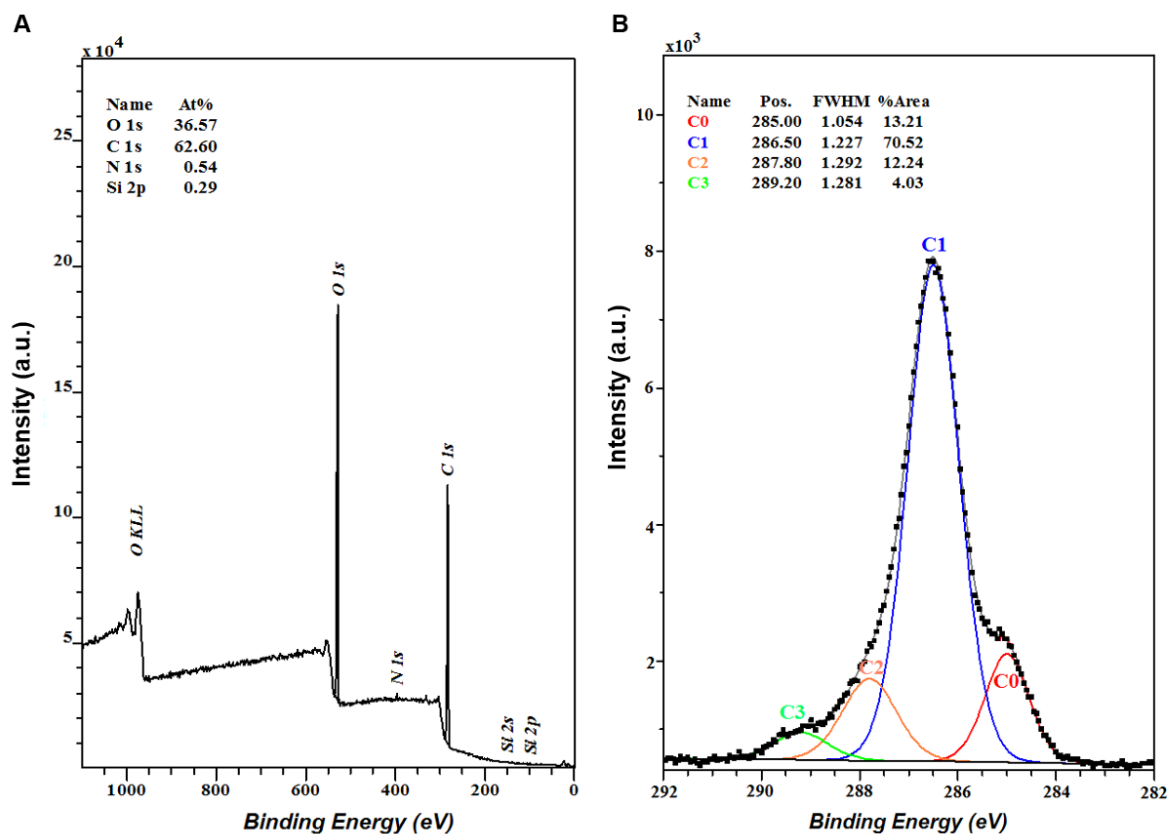


Fig. S1. XPS analysis of PEO-like coatings. **A)** Survey of X-ray photoelectron spectrum of PEO-like coatings. **B)** Deconvoluted high resolution C1s spectrum of PEO-like coatings.

Moreover, the stability of such coatings in solution is an essential parameter for *in vitro* cell culture and biosensing applications. Prior studies showed that such PEO-like coatings keep their antifouling and cell repellent properties for at least a week in biological solution. However, in most cases, a decrease in the refractive index is observable in real-time (by ellipsometry and

QCM (data not shown)) which stabilizes in general after one or two hours. This phenomenon was thoroughly discussed in a previous paper ⁵ which demonstrated that such decrease is mainly due to a loss of density of the coating attributed either to the creation of new terminal bonds and/or to the diminution of the crosslinking of the coatings. In addition, it was also shown that the chemical composition of PEO-like coatings (obtained by XPS analysis) remain quite constant (variation < 5%) after its immersion water for 5 days. Fortunately, such microstructural rearrangements in solution do not affect the coating's primary functions concerning protein and cell resistance.

Ellipsometry

The thickness and refractive index of PEO-like coating were analysed by ellipsometric measurements. Data were acquired with a variable angle single-wavelength imaging ellipsometer (JA. Woollam® VUV VASE™ Ellipsometer). All measurements were performed in air at room temperature for different angles of incidence (between 40° and 70°) with a step width of 0.5° and a low-capacity laser with the wavelength $\lambda=554.3$ nm was used as a light source. Conventional Polarizer-Compensator-Sample-Analyzer (PCSA) null-ellipsometric procedure is used to obtain maps of the Δ and ψ angles. The thickness and the complex refractive index are calculated from these two angles maps by point-by point modelling using the software EP3 View provided with the ellipsometer, using a two-layer model with the silicon wafer as first layer and PEO as the second. The thickness of PEO coatings was $30 \text{ nm} \pm 3 \text{ nm}$ and the refractive index was 1.51.

Surface Zeta Potential analysis

The surface zeta potential of PEO-like coatings was measured to comprehend if electrostatic interactions are the main driving forces regarding the mechanism of nanoparticle adsorption on PEO-like coatings (**Fig. S2**). The results showed a point of zero charge (PZC) of approximately at pH 4, which is typical for polymeric surfaces without dissociable functional groups.^{6, 7} The zeta potential values obtained in the pH region between 6.5 and 7.2, in which the nanoparticles adsorption and functionalization experiments were performed, showed that the surface is slightly negatively charged (about -30 mV).

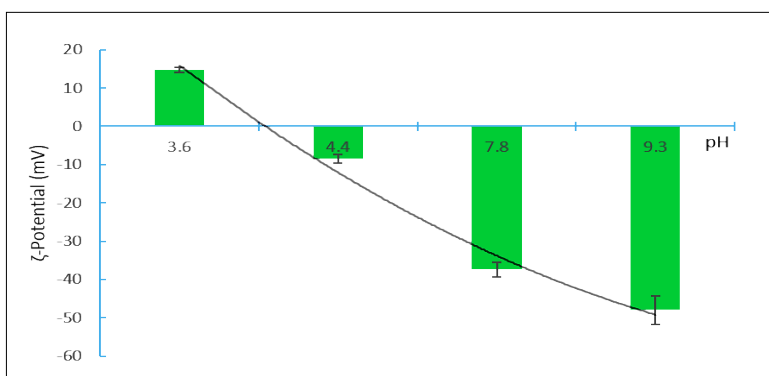


Fig. S2. Zeta Potential analysis of PEO-like coatings. The black line represent polynomial best fit curve with an approximate point of zero charge at pH 4.

The zeta potential of the ultrathin PEO-like films were determined using an ElectroKinetic Analyzer (Anton Paar, Austria) with a rectangular clamping cell suitable for small flat substrates, based on the streaming potential method. Inside the cell, the sample was pressed against a PMMA spacer with seven rectangular channels. Therefore, the measured zeta-potential includes a contribution from the PMMA spacer, which can be eliminated by measuring a reference PMMA surface under the same experimental conditions. The starting pH was adjusted at 10 by adding 0.1 M HCl or 0.1 M NaOH. The raw zeta-potential values for both samples were

measured in a solution of 1 mM KCL and in steps of approximately 2 pH units by automatic titration with 0.1 M HCl. For each stabilized pHs, four single measurements were taken, with alternating flow direction in order to ensure good statistics. The zeta potential was calculated based on the Helmholtz–Smoluchowsky equation: $\zeta = (dU / dp) \times (\eta / \epsilon \epsilon_0) \times K$, where ζ is the zeta potential, dU is the streaming potential, dp is the pressure differential across the sample, η is the viscosity of the electrolyte solution, ϵ is the relative dielectric constant of the fluid, ϵ_0 is the vacuum permittivity and K is the specific electrical conductivity of the electrolyte solution. The corrected zeta-potential (ζ_c) values for the PEO-coated sample was obtained by using the equation $\zeta_c = 2 \times \zeta_{PEO} - \zeta_{PMMA}$ for each concerned pH.

Contact angle analysis

The contact angle measurements on 'PEO-like' coatings were obtained in ambient air at room temperature using a goniometer (MSE Digidrop BBX, GBX Scientific Instrumentation, France) controlled remotely by a computer. The contact angles were measured from a sessile drop on the surface by lowering a drop of 1 μ l of ultrapure water (Millipore System Q), Glycerol and Bromonaphtalene suspended on the needle of a syringe, using an optical system for capture the profile of the drop on the solid surface. The choice of the three liquids has been made according to the work of Della Volpe *et al.*⁸ in order to measure the surface energy components following the modified Van Oss law. Both sides of the drop were measured to obtain the angle of repose of the contact using the drop Windrop software. This process was repeated three times for each drop to obtain an average contact angle. The accuracy is $\pm 1^\circ$. The measurements has been made in the same way on the PEO-like coating and the e-beam modified PEO-like coating. The results are shown in Table S1 and S2.

Table S1: Contact angle value with three liquids for PEO unmodified surface and FIB modified surface.

Liquid	Contact angle e-beam treated PEO-like(°)	Contact angle as deposited PEO-like (°)
Water	62.3	64.1
Glycerol	67.3	67.7
α -bromonaphthalene	25.0	24.0

The acid-base components of the solid calculated with the programme developed by Della Volpe *et al.* are reported in Table S2.

Table S2: Values of the surface energy components for the PEO modified surface, inside and outside.

Surface energy components	Value e-beam treated PEO like (mJ m ⁻²)	Value PEO-like (mJ m ⁻²)
γ_s^{LW}	40.33751	40.64439
γ_s^+	0.12309	0.11119
γ_l^-	25.37612	22.99839

Au NPs characterization

In order to assess the particle size distributions of the nanoparticles Centrifugal Liquid Sedimentation (CLS) measurements were performed on the as synthesized nanoparticle dispersions. Measurements were made in a CLS instrument model DC24000UHR by CPS Instruments Measurements were performed in an 8wt%-24wt% sucrose density gradient with a disc speed of 22000 rpm. Each sample injection of 100 μ l was preceded by a calibration step performed using certified PVC particle size standards with weight means size of 380 nm.

The particle size distribution was also determined Dynamic Light Scattering (DLS) by Malvern Zetasizer Nano-ZS instrument with temperature control. Prior use, Au NPs were centrifuged for three hours at 3000 rfc and the supernatant were removed to reach 10X concentration of gold. Then, the Au NPs were suspended in citrate solution to final concentration of 0.5 mM of gold in all Au NPs.

The characterization of the size and size distribution of Au NPs were performed by CLS and DLS and the data are summarised in **Table S1**. Scanning Electron Microscopy (SEM) images were collected to verify the spherical shape of Au NPs as shown in **Fig. S3**.

sample	15 nm	40 nm	75 nm
d_{CLS} (nm)	11	47	70
σ_{CLS} (nm)	1.5	7.9	11.9
DI_{CLS} (nm)	1.08	1.1	1.09
d_{DLS} (nm)	14	51	71
σ_{DLS} (nm)	2.3	17	26
PDI_{DLS} (nm)	0.029	0.118	0.142

Table S3. d_{CLS} (nm) represents the average size of the Au NPs calculated by CLS, σ_{CLS} (nm) is the size distribution by CLS calculated at Half Height Width (HHW) of the AuNPs peak. DI_{CLS} (nm) is the CLS Polydispersity Index (expressed as the ratio D_w/D_n where the D_w is the average particle size calculated from the particle weight-size distribution while D_n is the average particle size calculated from the equivalent number-size distribution). d_{DLS} (nm) is the average size

calculated by DLS, σ_{DLS} is defined as $\sigma_{DLS} = d_{DLS} (\text{PDI})^{1/2}$. PDI_{DLS} is the dimensionless polydispersity index.

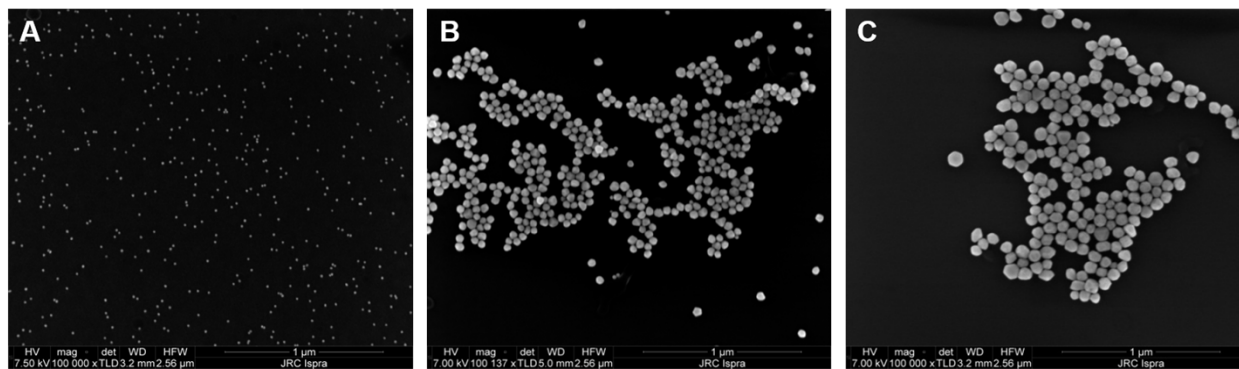


Fig. S3. SEM images of Au NPs. A) 15 nm, B) 40 nm, C) 75 nm

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