

Electronic Supplementary Information

Bio-interfactants as double-sided tapes for Graphene Oxide

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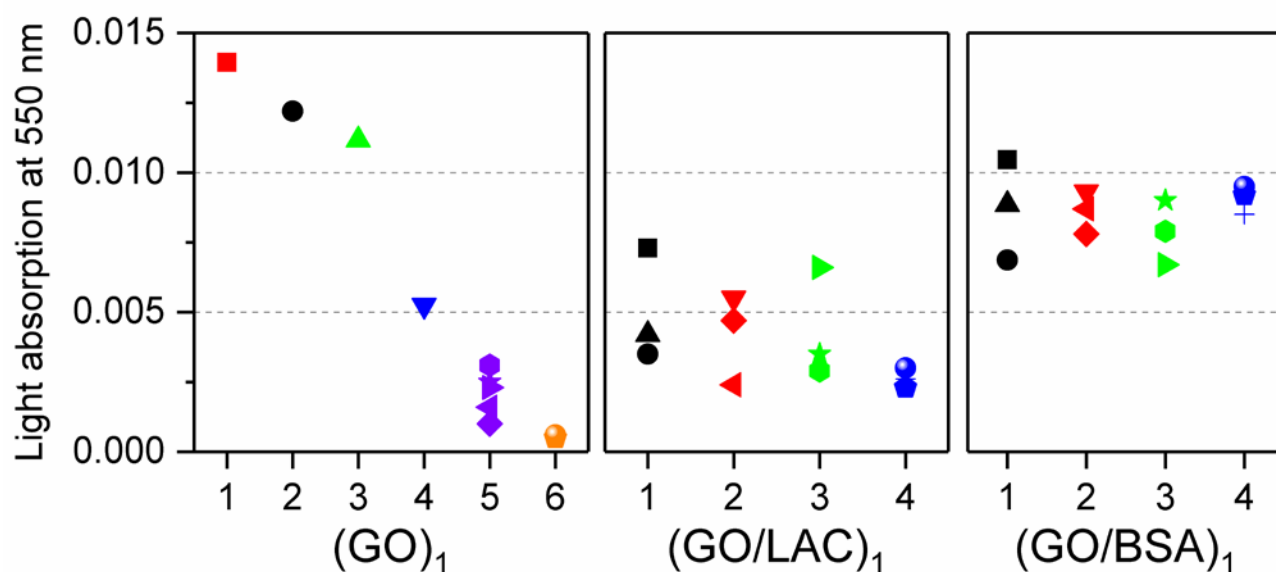


Figure S1. Reproducibility of LBL systems can be evaluated using light absorbance on several samples (coatings deposited on fused quartz). The (GO)₁ system showed low reproducibility among 6 samples but relative similar values within same samples (for instance in sample 5 and 6). As a consequence of low reproducibility, some samples after the thermal reduction failed to have a measurable electrical conductivity, probably for being below the percolation threshold, provided by overlapping GO flakes. (GO/LAC)₁ displays absorbance values on a narrower window and (GO/BSA)₁ shows the lowest spread of values within samples and among samples. The latter coating also shows the highest light absorption in average.

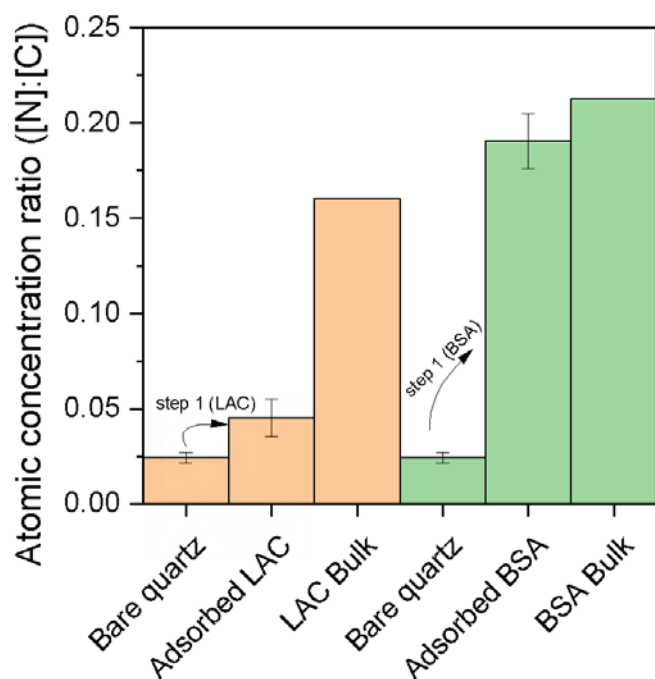


Figure S2. Evolution of $[N]_{at}: [C]_{at}$ before and after step 1 for adsorption of LAC and BSA on quartz. Natively the quartz contains a low fraction of N:C ratio (0.025), which is substantially increased after step 1, indicating adsorption of the bio-interfactants LAC and BSA. The diminished increase of $[N]$ for LAC adsorption is related to high adsorption of maltodextrine (free of nitrogen) that is present in the comercial mixture with Laccase from *Trametes versicolor*. The ratio of the bio-interfactants (LAC and BSA) in bulk powder are also plotted as a reference (third and sixth columns).

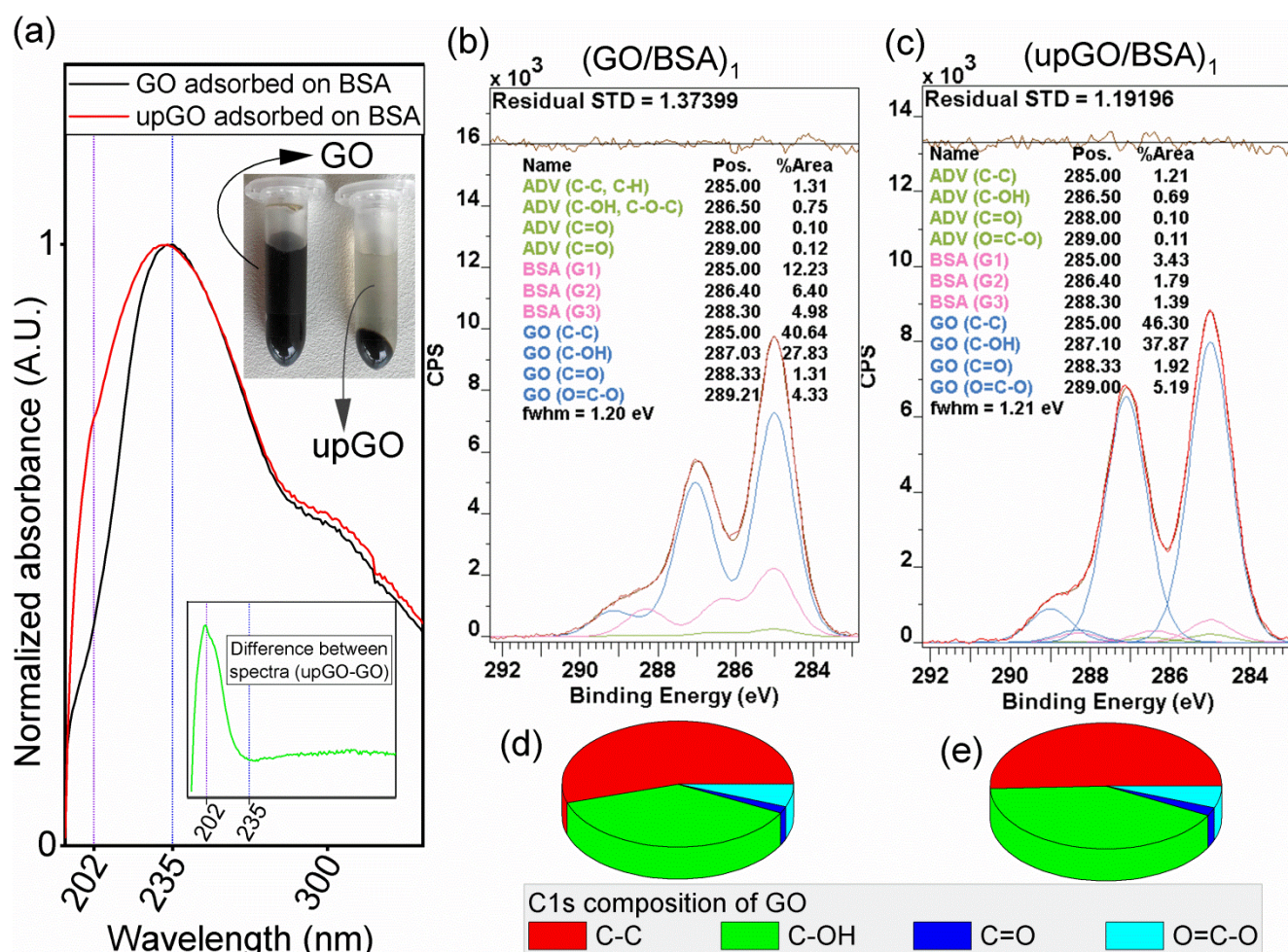


Figure S3. (a) Normalized UV-Vis contribution of the GO type on BSA when using different fractions of the GO mixture. Inset picture in (a) shows the difference between these two spectra, which is characterized by a sharp absorption peak at 202 nm when the fraction dGO is used. (b) and (c) XPS spectra of C1s spectra of (GO/BSA)₁ and (upGO/BSA)₁, respectively, and the their proportion of components attributed to GO explicit in (d) and (e) respectively. The spectra was fitted with the adventitious carbon from the substrate (corrected by the substrate attenuation). The BSA contribution in the spectra respected the ratio expected for it¹. Components from GO were plotted using four components within a range broadly accepted in the literature²⁻¹⁰. Sample (upGO/BSA)₁ showed a smaller proportion of C-C species, which is arguably related to smaller GO flakes with different defects, such have different electronic states¹¹ and therefore a different UV-Vis spectra.

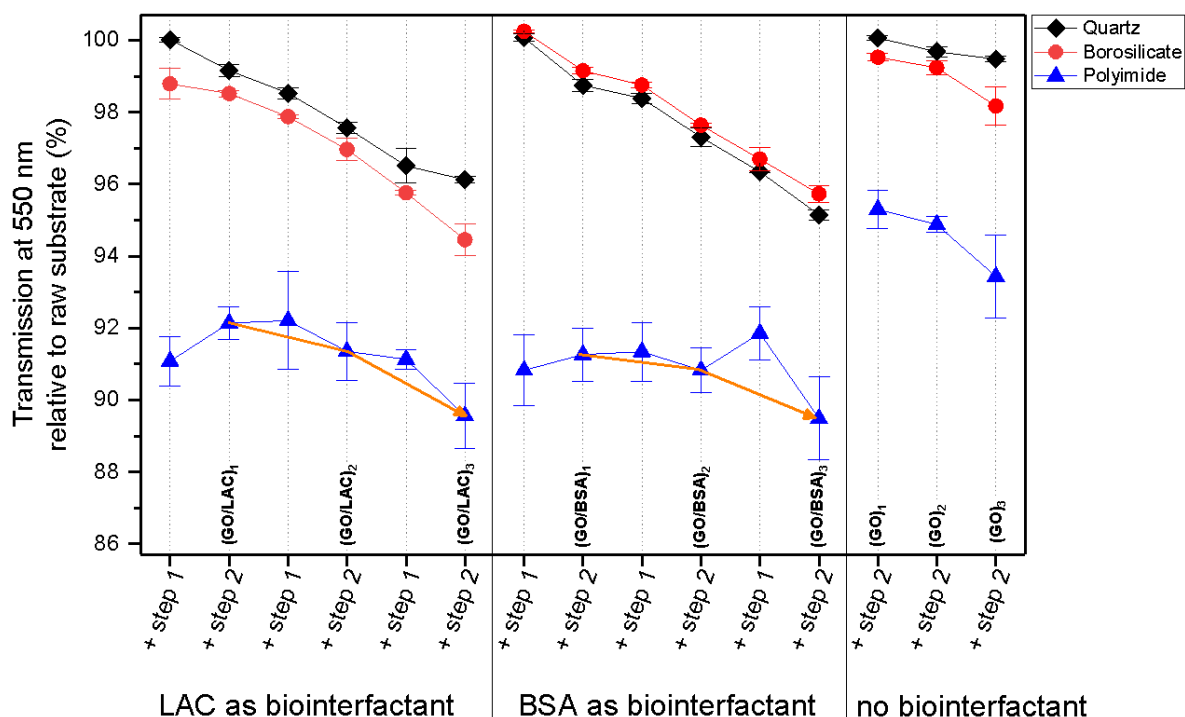


Figure S4. 550 nm light transmission through substrates (quartz, borosilicate and polyimide) for the three building strategies; using LAC as bio-interfactant (left part of the chart), BSA as bio-interfactant (central part of the chart) and without the use of bio-interfactants (right part of the chart). Each following step to the right on the x-axis represents a further process from the original raw substrate. The orange lines indicate decrease of transmittance from 1 to 3 intercalations of bio-interfactant and GO.

Absorbance @ 280 nm (1 cm path length)	Mixture concentration (mg/ml)	Molar absorption coefficient (for molecule 1GYC) $\varepsilon = n_w \cdot 5500 + n_v \cdot 1490 + n_c \cdot 125 = 61475$		
		n_w (# tryptophan)	n_v (#tyrosine)	n_c (#cysteine)
0.1434	2.1	7	15	5

Figure S5. Considering the theory and methods for estimating a polypeptide concentrations¹²⁻¹⁵, it was determined that the commercial mixture with LAC contains ~6.2% of enzymes and the remaining is assumed to be polysaccharides that have the function of stabilizing the enzyme. The equation that calculates the concentration is $A_\lambda = \varepsilon \cdot c \cdot L$, where A_λ is the light absorbance, ε is the molar absorption coefficient, c the concentration and L the path length.

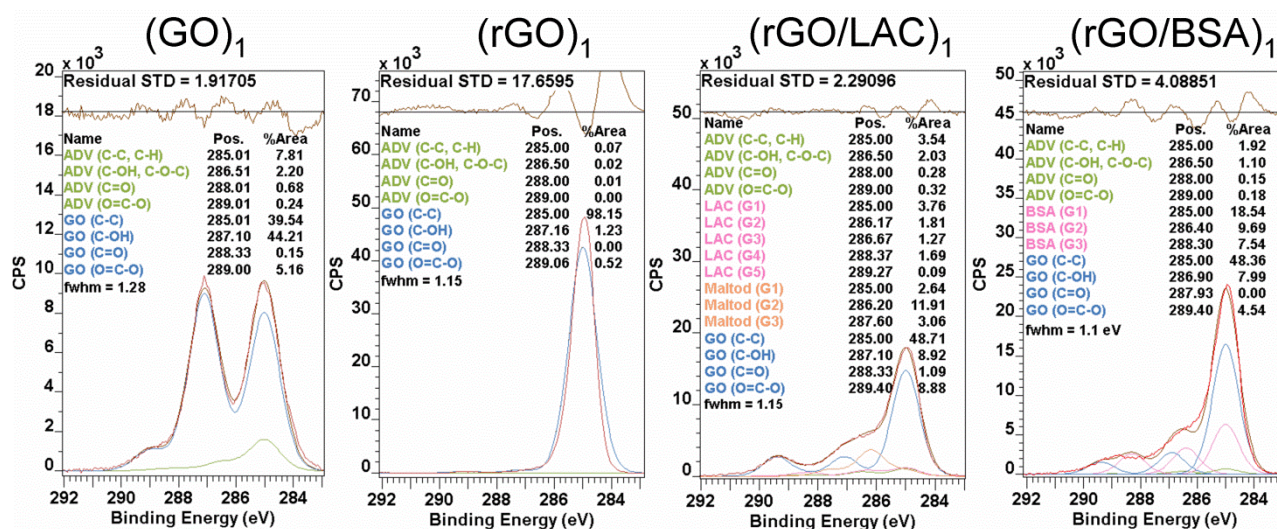


Figure S6. C1s high resolution XPS spectra of (from left to right) (GO)₁, (rGO)₁, (rGO/LAC)₁ and (rGO/BSA)₁. The first spectra is the only one with high peak ~2 eV higher than the reference peak at 285 eV, which is feature of highly oxidized GO¹⁶. The reduced forms of the three coatings; (rGO)₁, (rGO/LAC)₁ and (rGO/BSA)₁ show the peak at ~287 eV with substantially low contribution to the spectra. Green area is attributed to adventitious carbon from the quartz substrate, which was corrected by Si 2p attenuation. Spectra from (rGO)₁ is a region particularly thick, which stems from the low reproducibility of process when unassisted by bio-interfactants.

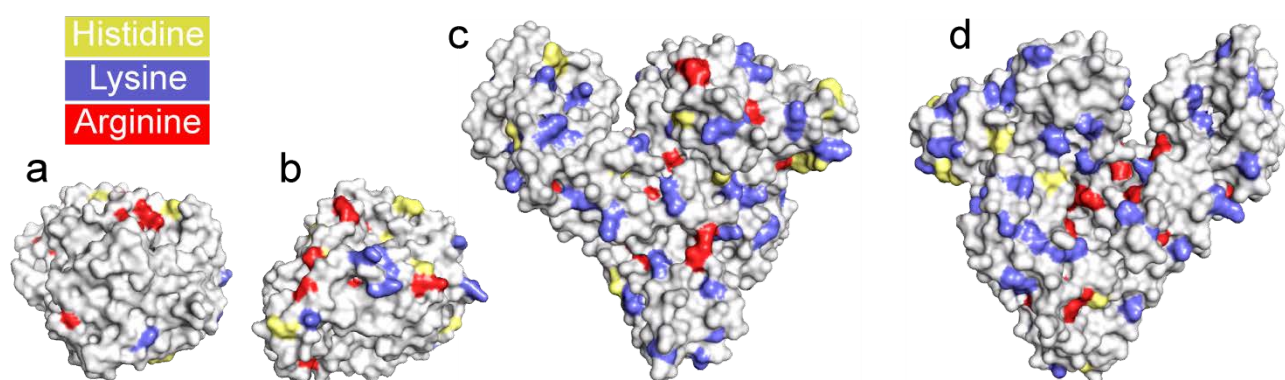


Figure S7. Representation of polypeptide models; (a) and (b) are opposite sides of Laccase (PDB code: 1GYC). (c) and (d) BSA are two sides of BSA molecule (PDB code: 3V03). Key amino acids are highlighted with different colours. BSA displays more homogeneous distribution of these sites comparing to Laccase.

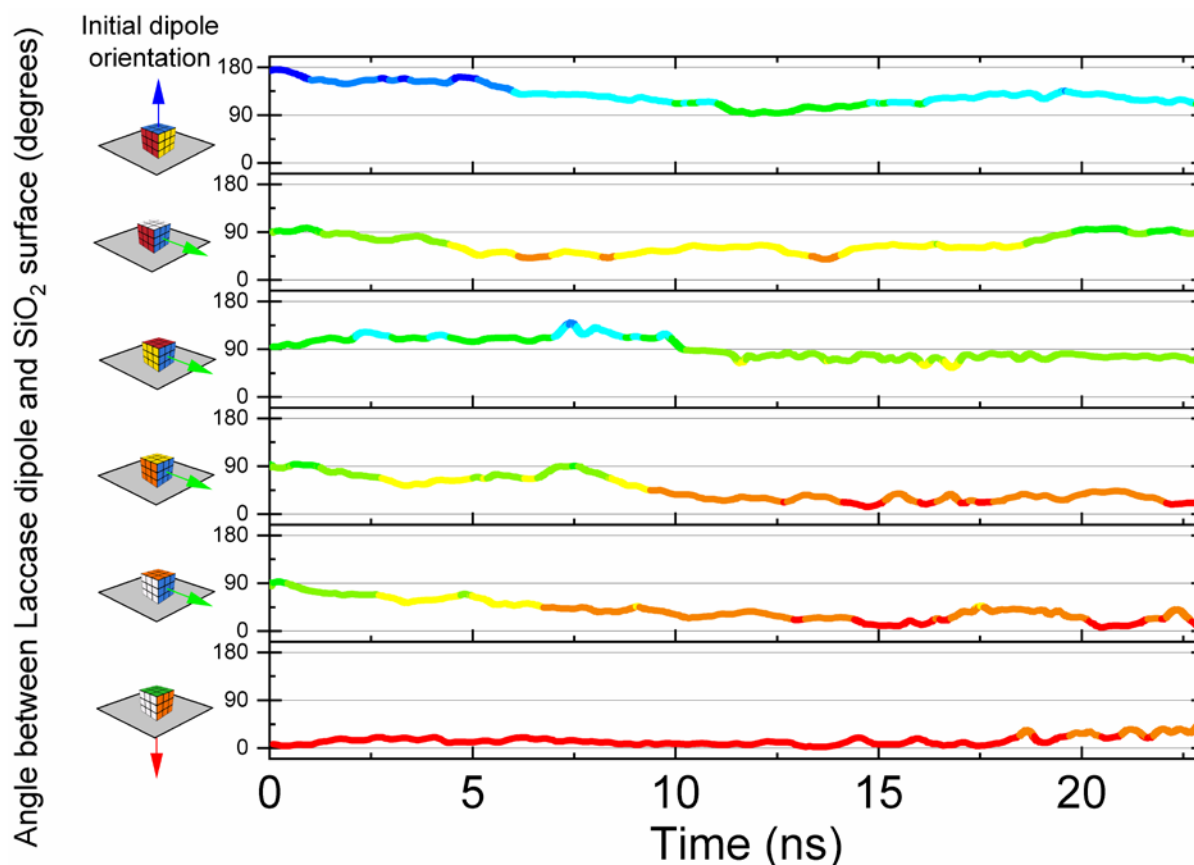


Figure S8. The Laccase molecule was approached towards SiO₂ surface in six distinct initial orientations (represented by the Rubik's cube). In the image, the arrow represents the dipole direction, the color scale in the arrows and on the chart (from blue to red) represents the angle at a given time of the experiment. The first orientation (dipole facing out the surface) shifted towards a parallel orientation on the experiment time frame. Positions 2 and 3 began with the dipole parallel to the surface and across experiment the dipole remained parallel. Positions 4 and 5 also have parallel orientations in relationship to the surface but with the time experiment the dipole sat towards the surface. Finally position 6 began with the dipole facing the silica surface, and in the experiment time remained in angles pointing to the surface. In conclusion, the initial angle of the dipole tends to either remain in the initial position or point towards the silica surface throughout this experiment time, as expected since the silica surface has a negatively charged surface.

The Laccase structure 1GYC with a resolution of 1.9 Angström at 298.15 K was taken from the Protein Data Bank. All sugar molecules which were included in the file were manually removed. To account for the protonation state of ASP, GLU, HIS, LYS, CYS and TYR at a pH of 4.75 constant-pH calculations within the AMBER simulation package¹⁷ were employed. This resulted for the laccase in a Nettocharge of +15 e, whereby +7 e can be attributed to the protonation state of the amino acids and +8 e to the Copper(II) centers within the protein. Thereafter, the protein structure was equilibrated with consecutive NVT, NPT and NVE ensembles and with the AMBER-14SB forcefield in explicit TIP3P water. Due to periodic boundary conditions the charge of the system had to be compensated with Cl⁻ Anions.¹⁸

The amorphous SiO₂ was adopted from Cole et al.¹⁹ In addition, the forcefield from Butenuth et al.²⁰ was applied to contribute for the SiO₂ and water interactions. Nettocharges of the surface were taken from experimental data^{21,22}, which lead in the end to a charge of -5e and a surface area of 81 nm².

The molecular dynamics simulations for the adsorption of Laccase on the SiO_2 Surface was carried out using the AMBER-14SB force field in GROMACS²³ under periodic boundary conditions and with TIP3P water. In order to maintain electrical neutrality 10 Cl^- Anions were inserted into the system. Applying a NPT simulation beforehand, the density of TIP3P water in the system at 300K was regulated to $0.983 \pm 1 \text{ g/cm}^3$ ¹⁷. During the the simulation in a NVT ensemble, all surface atoms except for the H-Atoms were kept in their initial position²⁴. The system is weakly coupled to a modified Berendsen thermostat (Coupling constant = 100ps), allowing for changes of maximal 5K²⁵. Consequently, the total energy of the system remains approximately constant, resulting in NVE-like conditions. The cutoff was set to 12 Angström, furthermore, utilizing a timestep of 2fs and the Particle Mesh Ewald Method to describe electrostatic interactions. With the use of VMD²⁶ the trajectories have been analyzed.

O 1s	Si 2p	C 1s	N 1s
59.27 ± 1.11	31.24 ± 0.71	9.26 ± 1.55	0.23 ± 0.06

Figure S9. XPS evaluation of 14 positions on the total of 5 quartz samples treated with piranha solution protocol, showing the homogeneity of atomic concentrations inter and intra samples. The carbon and nitrogen were considered impurities and subtracted from calculations of of the coating atomic contents proportionally.

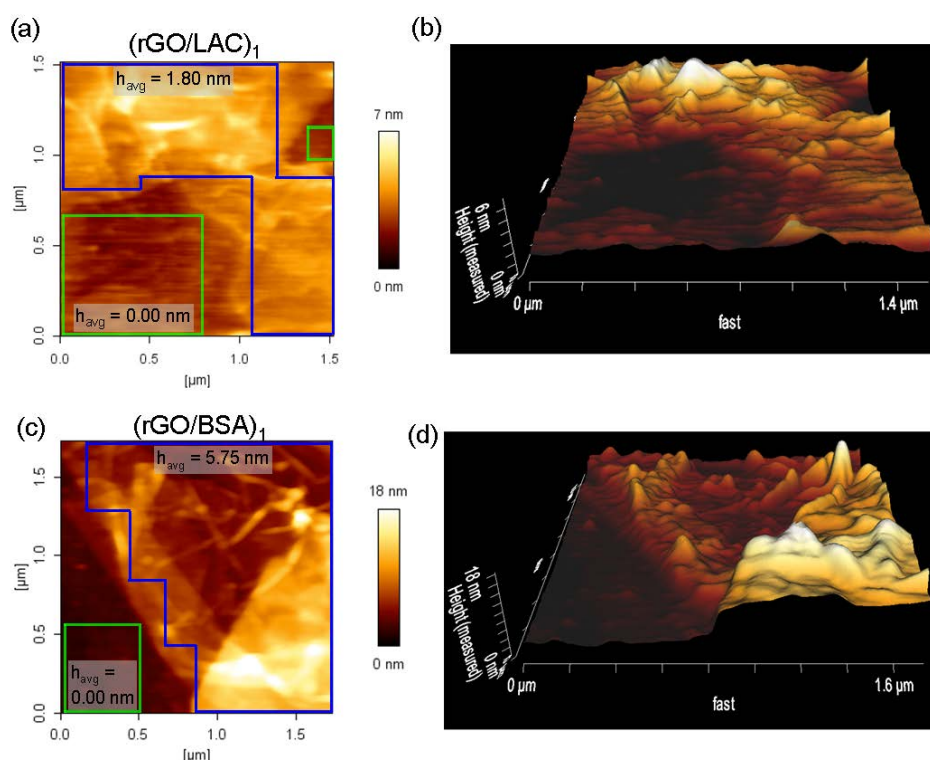


Figure S10. AFM of coatings (rGO/LAC)₁ (a-b) and (rGO/LAC)₁ (c-d), images (b) and (d) being the 3D projection of the left images. The height of the coating (average of areas in blue perimeter) was compared to the height of the quartz glass substrate (highlighted with green perimeter).

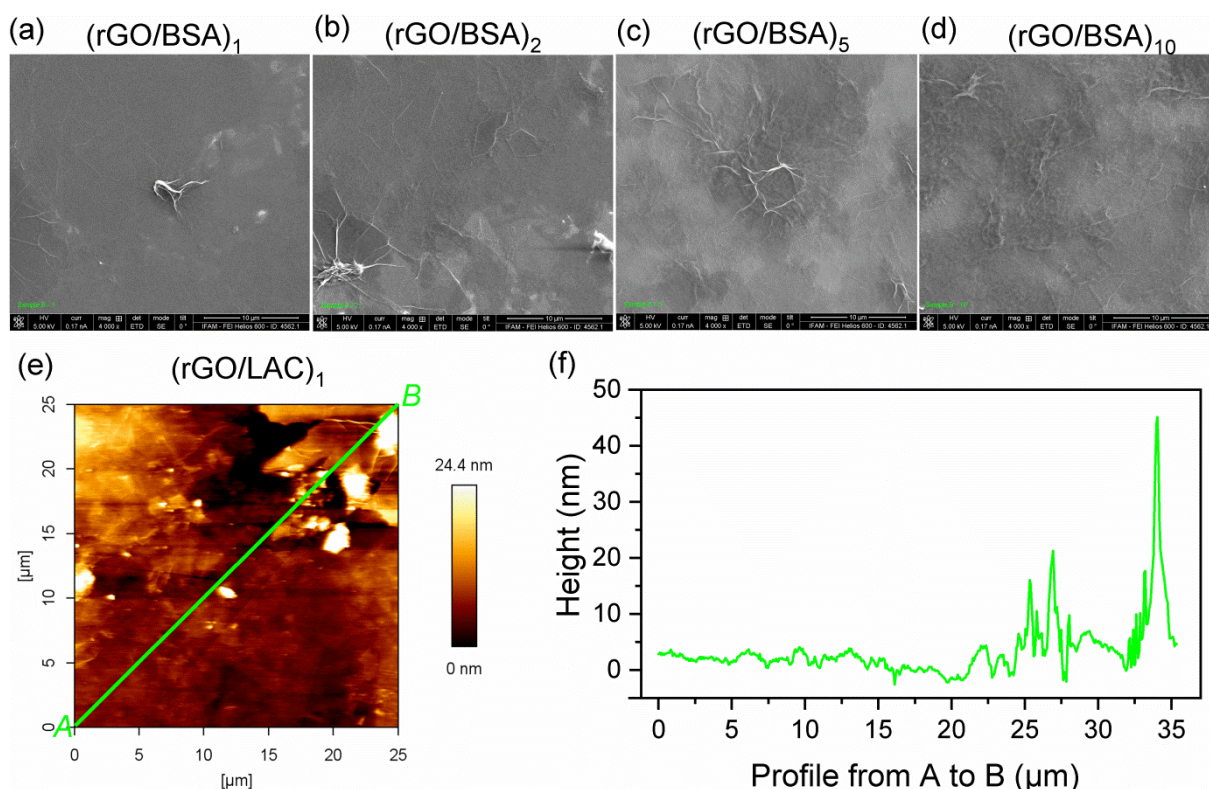


Figure S11. (a to d) SEM images of coatings with BSA with 1, 2, 5 and 10 layers of deposition, respectively. Lateral homogeneity is observed in a broad area of the coatings. (e) AFM of large area of film with LAC. Similar to the coatings with BSA, (rGO/LAC)₁ also shows broad flat region with some local areas of corrugations. (f) AFM height profile of line AB highlighted in figure (e), detailing the topographic profile in micrometer scale.

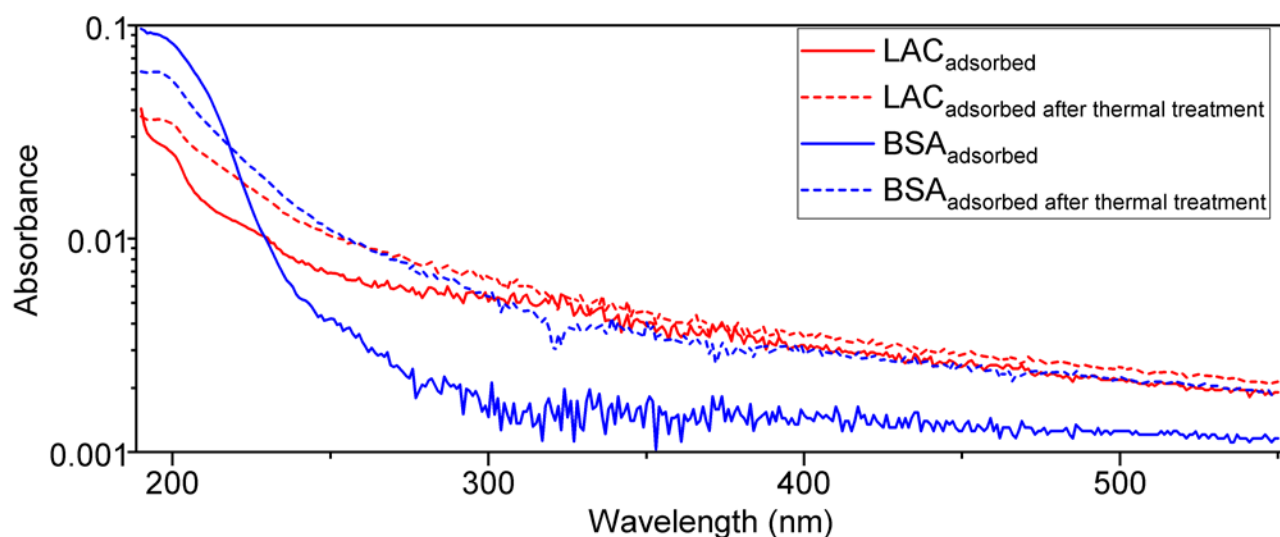


Figure S12. Evaluation of thermal treatment effect on bio-interfactants using UV-Vis absorbance of bio-interfactants adsorbed on quartz substrate before and after thermal reduction (up to 250°C in N₂ atmosphere and kept for 15 minutes). The light absorbance in this range in general is increased as opposed to decreased to zero (which would mean cleansing the substrate towards original reference value). At 550 nm, the equivalent opacity of these coatings after thermal treatment are approximately 0.4%.

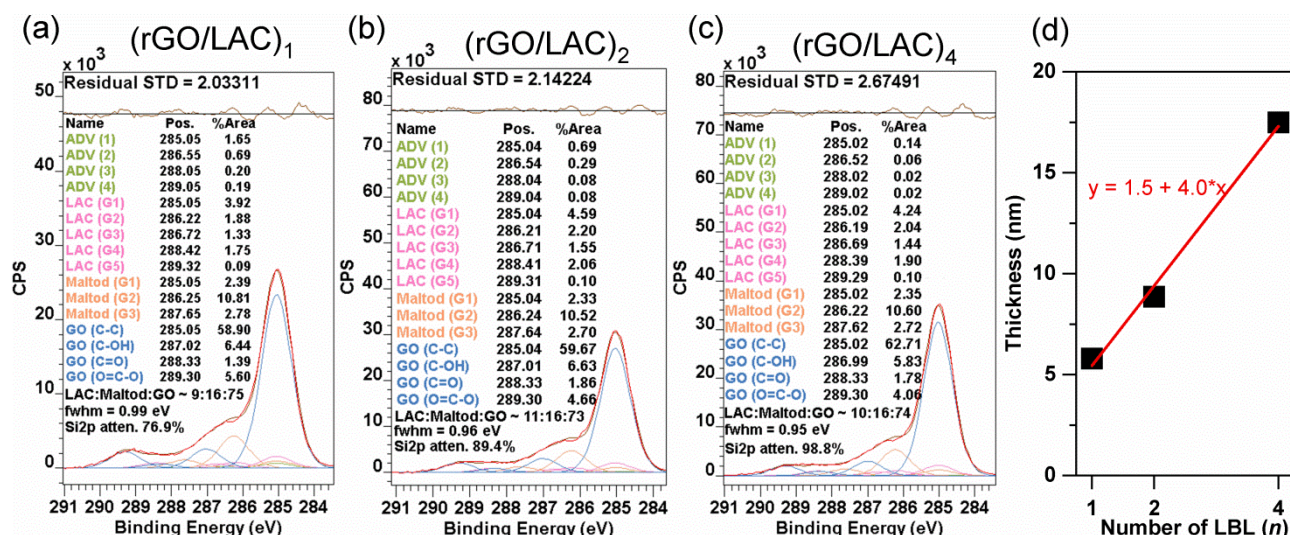


Figure S13. C1s high resolution XPS spectra of 1, 2 and 4 LBL of process using LAC as bio-interfactant (images a to c). The deconvolution of the spectra were corrected by adventitious species from the substrate and plotted using components from the substances used on the process (LAC²⁷, Maltodextrine²⁷ and BSA²⁸), the best fitting indicated that the ratios among these substances do not change substantially. From the substrate attenuation it was also possible to plot the layer thickness in (d). (d) indicates that the thickness is incremented by similar block every process repetition, adding approximately 4 nm of material every process repetition.

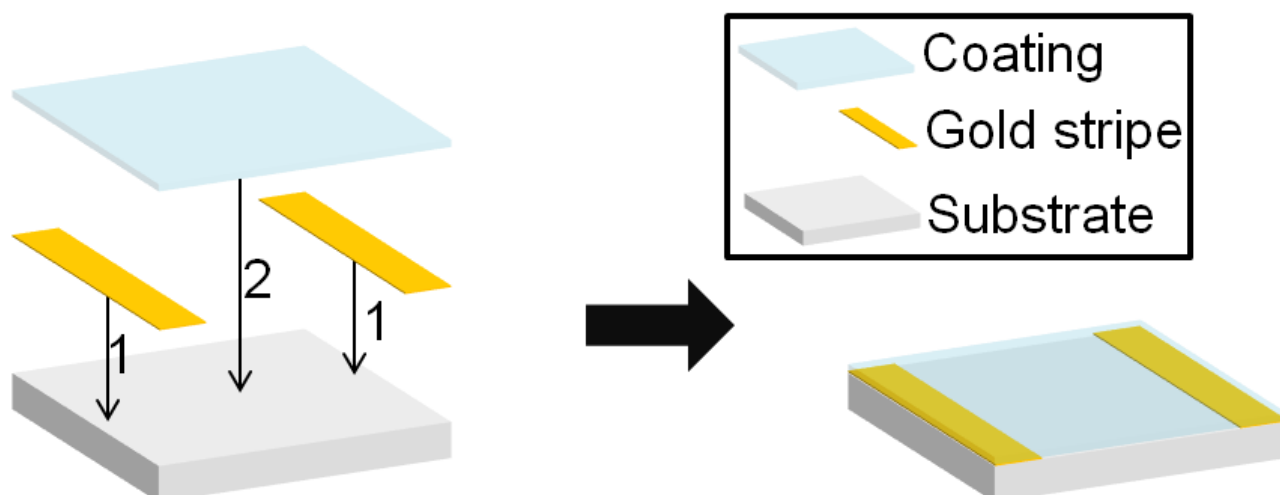


Figure S14. Sample preparation: Gold is sputtered on a substrate (1) before the coating process (2) for continuous electrical contact with the bottom part of the coating.

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