Supplementary material

Functionalization of hydrophobic surfaces with antimicrobial peptides immobilized on a bio-interfactant layer

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Laccase purification

Bio-Spin 30 column (Bio-Rad, Germany) was used to purify the enzyme protein using size exclusion chromatography. The column was filled with Bio-Gel P-30 gels and fully hydrated in Tris buffer. The column was used to remove salts and low molecular weight compounds. (lower than 40000 g/mol such as the maltodextrin according to the supplier's specifications) from aqueous protein samples. The buffer was exchanged for a sodium acetate buffer 0.2 M (SAB) and the protein was purified according to the supplier's protocol.

Table S1. XPS-based elemental atomic surface concentration (in at%) for PS substrates modified with laccase mixture suspension and laccase suspension, respectively, using 30 minutes of time in contact .

Sample	**[O]**	**[N]**	**[N]/[O]**
30 min laccase mixture suspension	9.8	3.9	0.40
30 min laccase purified suspension	11.4	5.4	0.47

Table S1 shows higher [N]/[O] ratio for the PS surface when modified with the purified laccase than after modification with the laccase/maltodextrin suspension (LMS). These results suggest co-adsorption of the laccase and maltodextrin.

Activity of laccase immobilized on PS

Laccase enzymatic oxidation activity on the PS modified surface was assayed by adding 50 µL/well of 500 µg/mL solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (purity 98%, Sigma Aldrich, Germany) in 0.2 M sodium acetate (pH 4.5) to wells previously modified with laccase/maltodextrin suspension at 25°C. The absorbance at 420 nm was monitored for 30 minutes. Fresh LMS in the same volume was added to the well plate to determine the maximum catalytic activity expected.

TableS2. Laccase enzyme surface activity.

	Absorption at 420 nm	
Enzymatic activity	**1 min**	**30 min**
Aqueous suspension of 50 µl ABTS 500 µg/mL + 50 µl LMS 0.1 mg/mL (freshly prepared) acetate buffer	0.33	0.41
PS surface modified with LMS and next in contact with 50 µL ABTS + 50 µL acetate buffer	0.05	0.29
Aqueous LMS removed from the PS surface, adding 50 µL ABTS afterwards without acetate buffer	0.31	0.39

The increase in absorption values between 1 min and 30 min which is observed in a pronounced manner is correlated to the oxidation of ABTS and suggests that the enzyme is active after adsorption on the surface.

Isothermal titration calorimetry (ITC).

ITC studies were performed using a TA Nano ITC Instrument. 0.0899 mmol/L peptide solutions were prepared in sodium 100 mM acetate buffer, at a pH value of 4.5. Tet 124-G-BrPh-DOPA-G or Tet-124 peptide solutions were titrated into 40 µg/mL laccase/maltodextrin suspension. The titration was carried out by 25 step-by-step injections for aliquots of 10 µL peptide solution at intervals of 300 s into the cell containing 900 µL of laccase/maltodextrin while stirring at 300 rpm.

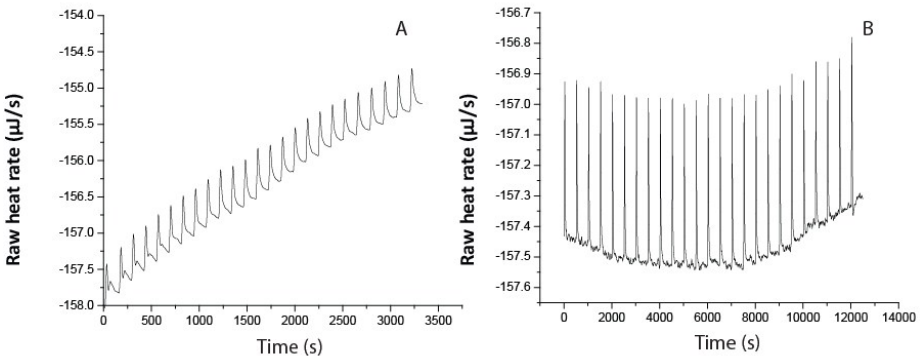

Figure S1. Representative ITC data for A) Tet 124-G-BrPh-DOPA-G B) Tet-124-G-Br-G titrated into laccase/maltodextrin suspension. The reaction of the laccase with the Tet-124-G-BrPh-DOPA-G is correlated to an increase in the instrumental thermal power that is observed by the shift of the baseline when plotting the differential heat (d/dt).

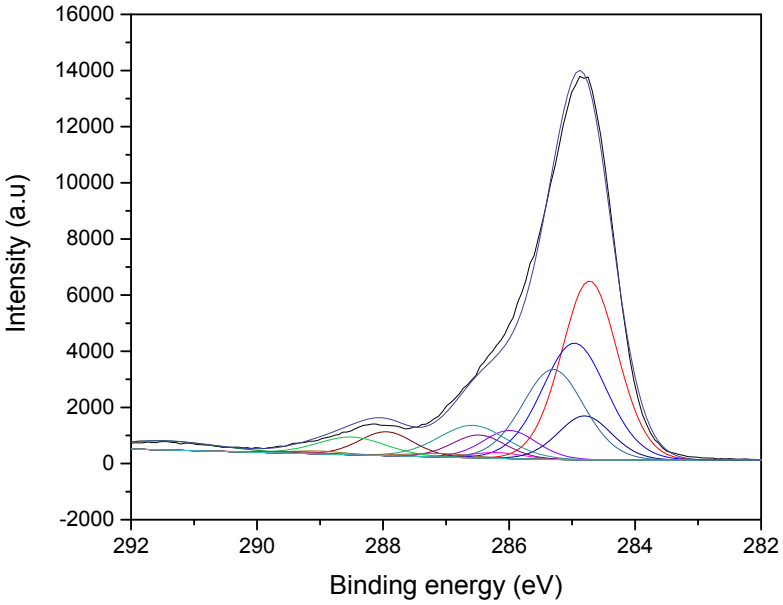

Figure S2. C1s XPS findings for PS modified with LM/Tet-124-G-BrPh-DOPA-G after derivatization reaction showing similar hydrocarbonaceous contributions after the reaction.

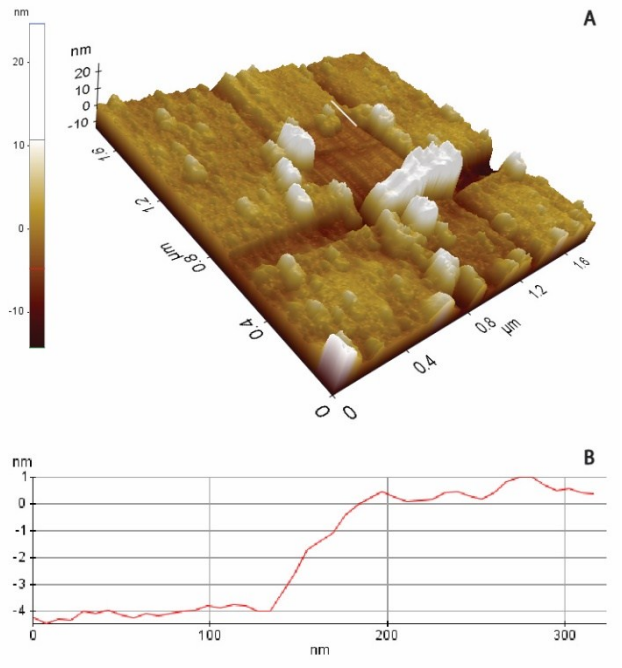

Figure S3. A) AFM height image of LM/Tet-G-Br-DOPA-G adsorbed on HOPG. B) Height profile of line highlighted on image A. Similar procedure applied for the PS surfaces showed in Fig.4 was applied.

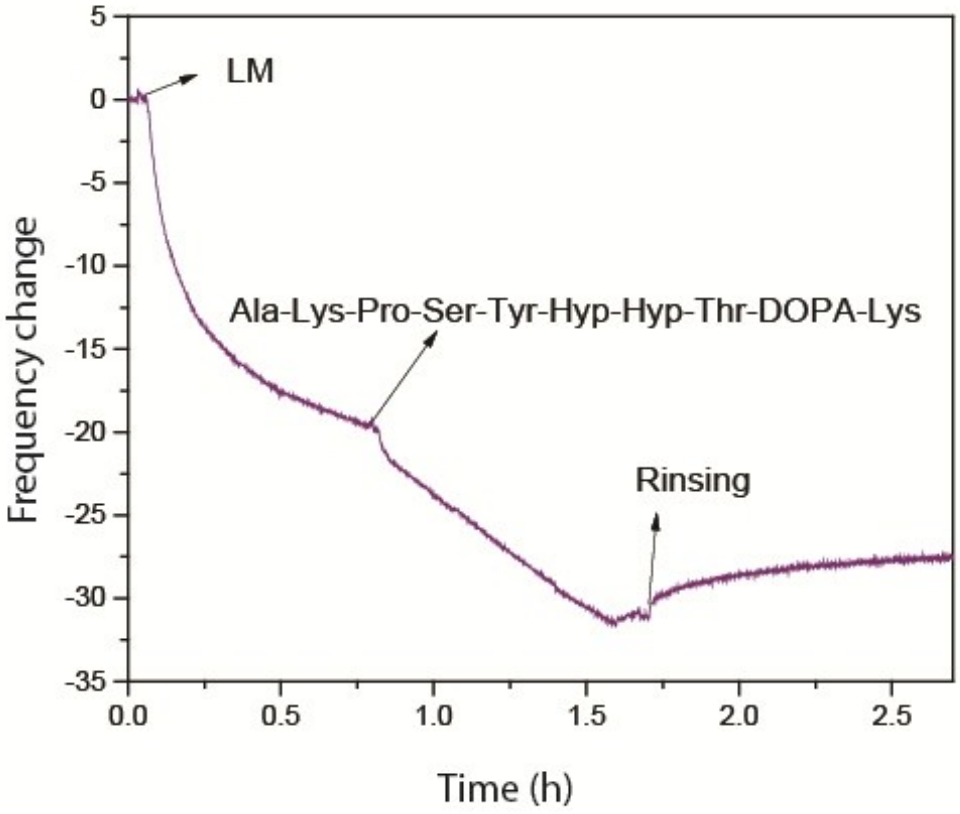

Figure S4. A) Resonance frequency shift Δf as a function of time for a crystal surface initially exposed to 0.08 mg/mL LMS and later to 0.1 mg/mL Ala-Lys-Pro-Ser-Tyr-Hyp-Hyp-Thr-DOPA-Lys in sodium acetate buffer.

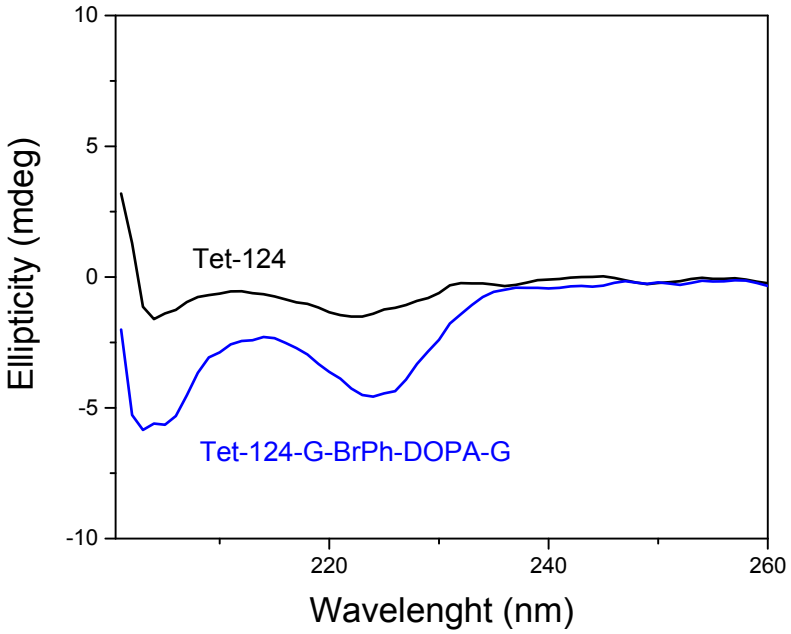

Figure S5. CD analysis of the peptide Tet-124 and Tet-124-G-BrPh-DOPA-G; concentration 0.05 mg/ml. The measurements were obtained using a circular dichroism instrument a Jasco J-715 spectropolarimeter.

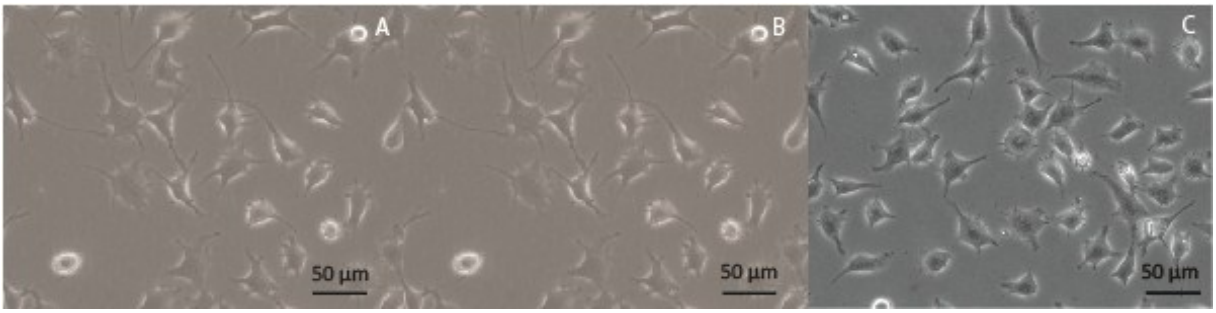

Figure S6. Fibroblast L929 cell growth on A) PS surface, B) PS/LM, or C) PS/LM/Tet-124-G-BrPh-DOPA-G surface. Cells were imaged using a bright field microscope.

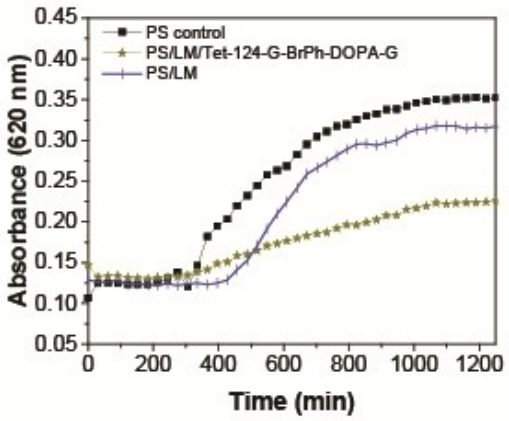

Figure S7. Time-dependent growth of E.coli bacteria after detachment procedure (Three different substrates, namely PS, PS modified with laccase mixture suspension (LMS), and PS modified with LM/tet-124-G-BrPh-DOPA-G, were investigated during four hours during contact of the substrate surface with 10^6 CFU/mL E.coli suspension. We did not detect any increase in bacteria adhesion as can be seen from the delay in the bacteria growth from solutions that were in contact with the functionalized surfaces.

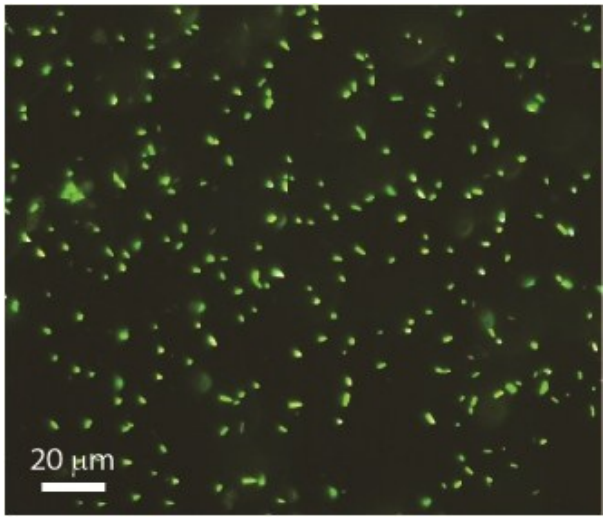

Figure S8. Fluorescence image of live (green) and dead (red) E.coli bacteria (LIIVE/DEAD staining) on PS neat surface; control sample from experimental results shown in Figure 7 .