APPENDIX A

EXPERIMENTAL PROTOCOLS

A.1 Bacterial growth in M9 minimum

First, Petri dishes are inoculated with *E. coli* MG1655 strain and placed during one night at $37°C$. Then, some colonies are taken out from the Petri dish and seeded in 20 ml of LB Broth medium which is then placed at $37°C$, 180 rpm during one night. This is the overnight culture. The next morning, 5 ml are taken from the overnight culture and diluted with 20 ml of fresh LB broth medium. This solution is again placed at $37°C$, 180 rpm for 2 h. Then, 6 eppendorfs of this culture are retrieved and the bacteria are harvested by centrifugation (3 min, 10000 rpm). The bacteria are then suspended in M9 minimum medium. This procedure is repeated three times. These 6 eppendorfs are then brought together in a tube forming the bacterial suspension . The obtained solution is diluted with M9 minimum medium to reach an optical density (OD) between 0.22 and 0.24 at 600 nm, corresponding to about $2.5 * 10^8$ cells/mL. This bacterial suspension was distributed in different eppendorf tubes and placed at 37°C. Optical density was evaluated after different times. The results are presented in figure A.1.

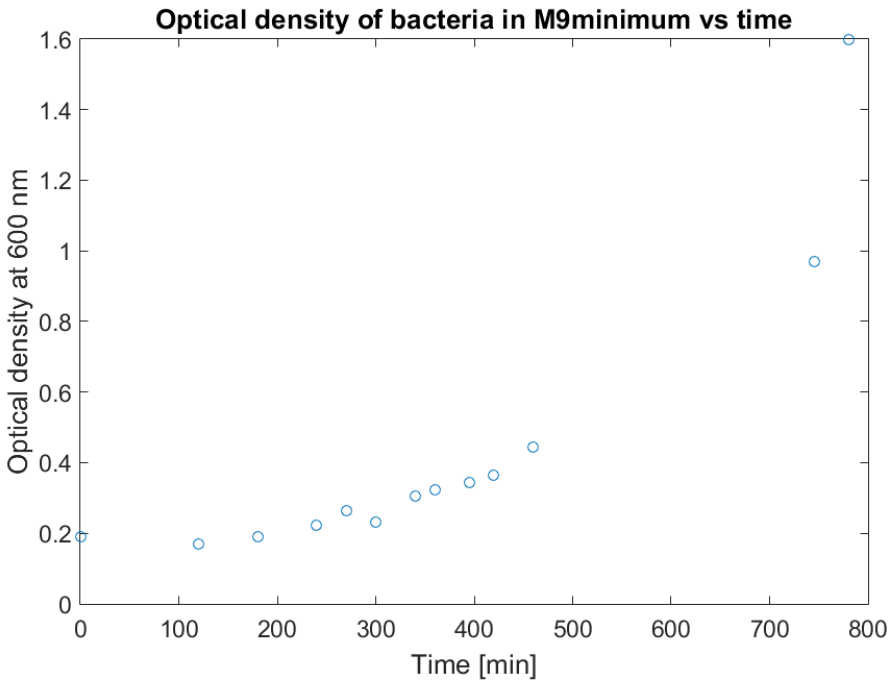

Figure A.1 – Bacterial growth in M9 minimum medium

A.2 Surfaces preparation

A.2.1 Nanoimprint lithography

The nanoimprint lithography process was entirely performed in the WinFab laboratories at Université catholique de Louvain (UCL). Two silicon mold (400 mold and 250 mold as described in section 3.2) with an area of 1×1 cm^2 (purchased from AMO, GmbH) were used. Silicon substrates were first cleaned in a piranha solution made up of a 3:1 mix of H_2O_2 and H_2SO_4 to remove organic residues. The clean substrates were then subjected to a nanoimprint lithography procedure. A 4% (w/w) poly(methyl methacrylate) (PMMA) solution was prepared in toluene and filtered through a 0.45 µm membrane. PMMA was spin-coated onto the silicon (Si) samples using a Laurell WS-650MZ-23NPP/LITE spin-coater at a speed of 6400 rpm with an acceleration of 4000 rpm/s for 60 sec for the 400 mold and a speed of 2800 rpm with an acceleration of 2500 rpm/s for 60 sec for the 250 mold. The thickness of the mask was measured by ellipsometry to be approximately 115-120 nm for the 400 mold and 150-160 nm for he 250 mold. To perform the imprint, the mold was pressed onto the polymer mask using an Obducat nanoimprinter. The sample was pre-heated at 170°C for 1 min, then the pressure was increased from 5 bars to 60 bars and left for 3 min to perform the imprint. The system was then cooled down to 60 °C and the mold was detached from the sample. Samples were subjected to a descum process for 3 min 40 sec using an Electrotech Plasmafab 310/340 reactive ion etcher, in oxygen plasma, to remove the residual polymer mask left on the patterned regions of the sample, leaving bare silicon with exposed silanol groups for the subsequent functionalization steps. Samples were immediately subjected to the next step to avoid surface contamination.

A.2.2 Growth of polymer brush

Gas-phase silanization

Materials 5'-dimethyl dichloro silyl propyl) 2-bromo-2-methylpropionate molecules were synthesized by Catherine Phillipart in the BSMA laboratories.

Method A Teflon sample holder, a Schlenk reactor and 2 silicon wafers used as control were cleaned by standard piranha procedure, rinsed with milli-Q water and dried in a heat chamber. The septum, valve and micro-syringe were rinsed with methanol and dried with nitrogen. Control surfaces were also cleaned 10 min by UV ozone. Control and descummed samples were placed on a Teflon sample holder in a Schlenk reactor heated in a silicone oil bath at 80 °C. The reactor was filled with argon for 5 min, then vacuumpumped for 10 min, filled again with argon for 15 min and finally vacuumpumped for 90 min before being isolated. $20\mu l$ of ATRP-silane was withdrawn in a glove box with a micro-syringe, which was previously purged 3 times. It was then injected into the reactor. The reaction was run 2 h at 80 °C. To stop the reaction, the samples were removed from the Schlenk reactor and immediately rinsed with methanol.

Liquid-phase silanization

Materials 2-[Methoxy(polyethyleneoxy)propyl]trichlorosilane (95%) was purchased from FluoroChem. Extra dry Toluene (99.85%) was purchased from Fisher Scientific. Triethylamine (99%) was purchased from Acros.

Method Two crystallizers were cleaned by standard piranha procedure, rinsed with milli-Q water and dried in a heat chamber. The samples were first placed in heated acetone (55°C) for 1 min in order to remove the PMMA mask. They were then placed for 10 sec in acetone in a ultrasound bath, rinsed with methanol and dried with a stream of nitrogen. The 8 samples were placed in 2 crystallizers. Then, 8 ml of toluene, 6 drops of PEG silane and 3 drops of triethylamine were added in each crystallizer. The reaction was run overnight in a glove box with a controlled argon atmosphere to ensure the reaction environment was completely free of water and oxygen. Samples were then rinsed with toluene, placed 20 sec in methanol in an ultrasound bath, rinsed again with methanol and dried with a stream of nitrogen.

P(HOEGMA) polymer brush

Materials Hydroxyl-terminated oligo(ethylene glycol) ether methacrylate (HOEGMA) monomers of molar mass 360 g/mol were obtained from Aldrich and were used without purification. Copper(I) chloride (99.995+%) (Cu^ICl), copper(II) chloride (99.999+%)($Cu^{II}Cl_2^I$), and 2,2'-dipyridyl (99+%) (bipy) were from Aldrich. Milli-Q water (resistivity higher than $18.2M\Omega.cm$) was obtained from a Millipore Simplicity 185 system.

Method The different schlenks and laboratory glasswork used were cleaned by a standard piranha procedure, rinsed with milli-Q water and dried in a heat chamber. Septums

and valves were rinsed with methanol and dried. For ten substrates, 2 homogeneous surfaces were used as control. The monomer (21ml) was dissolved in a mixture of water (14.7 ml) and methanol (14.7 ml) in a round-bottom flask sealed with a rubber septum. Bipy (521.3 mg) and $CuCl_2$ (14.1 mg) were added to this solution, which was stirred and degassed with a stream of argon for 45 min. $CuCl$ (105.8 mg) was then added quickly to the solution. The solution was stirred and further degassed for 45 min.
Meanwhile, the initiator-grafted Si wafers were sealed into Schlenk tubes (polished face downward) and were degassed (the reactor was vacuumpumped for 5 min and then filled with argon for 15 min, this process was repeated 3 times). Then, 3 ml of polymerization solution was syringed and quickly transferred to each Schlenk tube. The Schlenk tubes containing the samples were kept at room temperature under an overpressure of argon in the absence of stirring, for 2 h. After 2 h, 1 homogeneous sample was removed, washed with water then methanol, dried with a stream of nitrogen, and measured by ellipsometry to assess the remaining polymerization time in order to reach approximatively 30 nm. Finally, the remaining samples were removed, washed with water then methanol and dried with a stream of nitrogen. The last homogeneous surface was measured by ellipsometry to obtain the final brush tickness. All samples were then stored under argon before use. The schlenks were finally cleaned with soap and water.

A.2.3 Biofunctionalization of the polymer brushes

Linker grafting

Materials P-Maleimidophenyl isocyanate (PMPI) was purchased from Apolo Scientific. Tetrahydrofuran (THF) was purchased from Acros.

Methods Glassworks used were cleaned by a standard piranha procedure, rinsed with milli-Q water and dried in a heat chamber. The samples were first rinsed with milli-Q water, methanol and dried with a stream of nitrogen before being placed in schlenk tubes. The reactors were vacuumpumped for 5 min and then filled with argon for 15 min and this process was repeated 3 times. A 6mM PMPI solution prepared in distilled dry THF was placed at 30°C and 700 rpm for 30 min Then, 2 ml of this solution were added to each Schlenk tube. After a reaction time of 6 h at room temperature, the samples were sonicated in distilled THF for 5 min, and thoroughly washed 3 times with distilled THF before finally being dried in a stream of nitrogen.

Peptides grafting

Materials KRGDSPC (95+%), LL-37 with an additional C-terminal cysteine residue (LL37-Cys) (95+%), Magainin I derivatives with an additional C-terminal cysteine residue (MAG-I-Cys) (95+%) and tris(hydroxymethyl)aminomethane (tris) (99.8+%) were purchased from Sigma Aldrich.

Method Glassworks used were cleaned by a standard piranha procedure, rinsed with milli-Q water and dried in a heat chamber. A 100 mM tris buffer solution was prepared and the pH was ajdusted to 7.05. The substrates were then rinsed with filtered trisbuffer and dried with a nitrogen steam. Peptides were withdrawn in glove box and a $280\mu M$ peptide solution was prepared in trisbuffer. This solution was sonicated for 15 min.

Each sample was immersed in 1 ml of this solution for 2 h 30 min at room temperature. The substrates were then sonicated 5 min in trisbuffer, thoroughly washed 3 times with Milli-Q water, and finally dried in a stream of nitrogen before being stored under argon.

A.3 Surfaces characterization

A.3.1 Ellipsometry

As previously mentioned, the three first measures were taken in the UCL cleanroom facilities with a Sentech SE 850 Ellipsometer using SpectraRay software. The data were fit using a model composed of three layers. The first layer corresponded to the silicon layer characterized by a refractive index of n=3.6180 and k = 0.0015 (tabulated values). The next layer consisted in a 1.5 nm-thick layer of silicon oxide whose index of refraction was modelized by a Cauchy model with parameters $n_0 = 1.452$ and $n_1 = 36$. The last layer was the PMMA layer also modelized by a Cauchy model with parameters $n_0 = 1.474$ and $n_1 = 47$. Five measurements were taken and averaged for each sample. The next ellipsometric measurements were carried out with a spectroscopic ellipsometer (RSE from J. A. Woollam Co., Inc.). The data were fit using a Cauchy model for the index of refraction of the organic layer, using tabulated values for the index of refraction of silicon. Five measurements were taken and averaged for each sample.

A.3.2 XPS measurements

Measures parameters

The analysis were performed on a SSX 100/206 photoelectron spectrometer from Surface Science Instruments (USA) equipped with a monochromatized micro focused Al Xray source (powered at 20 mA and 10 kV). Samples were stuck onto the aluminium conductive carousel with double sided adhesive tape. The pressure in the analysis chamber was around 10 Pa. The angle between the surface normal and the axis of the analyser lens was 55°. The analysed area was approximately 1.4 mm2 and the pass energy was set at 150 eV. In these conditions, the full width at half maximum (FWHM) of the Au 4f peak of a clean gold standard sample was about 1.6 eV. A flood gun set à 8 eV 7/2 and a Ni grid placed 3 mm above the sample surface were used for charge stabilisation. The C(C,H) component of the C1s peak of carbon has been fixed to 284.8 eV to set the binding energy scale. Data treatment was performed with the CasaXPS program (Casa Software Ltd,UK), some spectra were decomposed with the least squares fitting routine provided by the software with a Gaussian/Lorentzian (85/15) product function and after subtraction of a nonlinear baseline. Molar fractions were calculated using peak areas normalized on the basis of acquisition parameters and sensitivity factors provided by the manufacturer.

Percentage of grafted monomers determination

Here, the developments leading to equations 3.1 and 3.2 page 38, allowing to compute the percentage of monomer units grafted by the different peptides, are detailed. By defining

- C_{HOEGMA}, the number of carbon atoms in one HOEGMA monomer,

- $C_{HOEGMA_{peptide}}$ the number of carbon atoms in one peptide-grafted HOEGMA monomer,
- $N_{HOEGMA_{peptide}}$ the number of nitrogen atoms in one peptide-grafted HOEGMA monomer,
- m_{HOEGMA} the number of HOEGMA monomers,
- $m_{HOEGMA_{peptide}}$ the number of peptide-grafted monomers.

From these definitions, it comes :

$$\frac{\%N_{tot}}{\%C_{tot}} = \frac{N_{HOEGMA_{peptide}} * m_{HOEGMA_{peptide}}}{C_{HOEGMA} * m_{HOEGMA} + C_{HOEGMA_{peptide}}} * m_{HOEGMA_{peptide}} \tag{A.1}$$

In addition, by defining x the percentage of grafted monomers and n_{tot} the total number of monomers, it comes:

- $m_{HOEGMA} = n_{tot} * (1 - x)$
- $m_{HOEGMA_{peptide}} = n_{tot} * x$

We can thus rewrite equation A.1 :

$$\frac{\%N_{tot}}{\%C_{tot}} = \frac{N_{HOEGMA_{peptide}} * x * n_{tot}}{C_{HOEGMA} * (1 - x) * n_{tot} + C_{HOEGMA_{peptide}} * x * n_{tot}} \tag{A.2}$$

$$\frac{\%N_{tot}}{\%C_{tot}} = \frac{N_{HOEGMA_{peptide}} * x}{C_{HOEGMA} * (1 - x) + C_{HOEGMA_{peptide}} * x} \tag{A.3}$$

$$\frac{\%N_{tot}}{\%C_{tot}} = \frac{N_{HOEGMA_{peptide}} * x}{C_{HOEGMA} + (C_{HOEGMA_{peptide}} - C_{HOEGMA}) * x} \tag{A.4}$$

By rearranging equation A.4 to isolate x, equation 3.1 is obtained:

$$\frac{\%N_{tot}}{\%C_{tot}} * C_{HOEGMA} + \frac{\%N_{tot}}{\%C_{tot}} * (C_{HOEGMA_{peptide}} - C_{HOEGMA}) * x = N_{HOEGMA_{peptide}} * x \tag{A.5}$$

$$x = \frac{\frac{\%N_{tot}}{\%C_{tot}} * C_{HOEGMA}}{\frac{\%N_{tot}}{\%C_{tot}}(C_{HOEGMA} - C_{HOEGMA_{peptide}}) + N_{HOEGMA_{peptide}}} \tag{A.6}$$

By applying the same reasoning to nitrogen and oxygen atoms, equation 3.2 is obtained:

$$x = \frac{\frac{\%N_{tot}}{\%O_{tot}} * O_{HOEGMA}}{\frac{\%N_{tot}}{\%O_{tot}}(O_{HOEGMA} - O_{HOEGMA_{peptide}}) + N_{HOEGMA_{peptide}}} \tag{A.7}$$

A.4 Bacteria adhesion and growth on biofunctionalized surfaces

A.4.1 Materials

LB Broth with agar (Lennox), LB Broth (Lennox), Ampicillin sodium salt, sodium chloride (99%+) (NaCl), D-(+)-Glucose (≥99.5%), ammonium chloride (NH_4Cl) (>99.5%), sodium phosphate monobasic (99%) (KH_2PO_4), sodium phosphate dibasic (≥99.5%) (Na_2HPO_4), magnesium sulfate heptahydrate (>99%) ($MgSO_4 \cdot 7H_2O$), calcium chloride dehydrate (>99%) ($CaCl_2 \cdot 2H_2O$) were purchased from Sigma-Aldrich. Pure casein was purchased from Acros. The water used was of Milli-Q grade.LIVE/DEAD® BacLight™Bacterial Viability Kit L7007 containing SYTO 9 and propidium iodide dyes was purchased from Molecular Probes.

A.4.2 Medium preparation

- Ampicilline solution of 100 µg/ml. Add 10 mg of amipicilline in 10 ml autoclaved water. Dilute 10 times, filter and store in the freezer. Unfreeze before use.
- Petri dishes. Dissolve 7 g of LB Broth with agar in 200 ml of water. Autoclave during 10 min and let it cool then add 200 µl of ampicilline solution. Pour the petri dishes.
- LB Broth medium. Dissolve 4 g of LB Broth in 200 ml water. Autoclave during 10 min and let it cool then add 200 µl of ampicilline solution.
- 0.15M NaCl solution. Dissolve 2.19 g of NaCl in 250 ml of water. Autoclave during 10 min. Adjust pH to 7.25 right before use.
- 20% glucose solution. Dissolve 4 g of glucose in 20 ml of water. Autoclave during 10 min.
- M9 saline solution. Add 0.1 g of NaCl, 0.2 g NH_4Cl, 0.6 g of KH_2PO_4, 1.1565 g of Na_2HPO_4 in 200 ml of water. Adjust pH to 7.2 and autoclave during 10 min.
- 1M $MgSO_4 \cdot 7H_2O$ solution. Dissolve 246 mg of $MgSO_4 \cdot 7H_2O$ in 1 ml of autoclaved water. Filter and store in the fridge.
- 10mM $CaCl_2 \cdot 2H_2O$ solution. Dissolve 14.7 mg of $CaCl_2 \cdot 2H_2O$ in 10 ml of autoclaved water. Filter and store in the fridge.
- Casein solution. Add 1 g of casein in 5 ml autoclaved water. Prepare this solution just before use.
- M9 minimum solution. Add 75 ml of autoclaved water, 25 ml of filtered M9 saline solution, 0.2 ml of $MgSO_4 \cdot 7H_2O$ solution, 1 ml of $CaCl_2 \cdot 2H_2O$ solution. Adjust the pH at 7.25. Add 1 ml of glucose solution and 1 ml of casein solution. Agitate the solution until use. Prepare this solution just before use.

A.4.3 Epifluorescence microscopy

Fluorescence images were taken at room temperature with a Olympus IX71 microscope equipped with a FITC filter set and an Epi-Fluorescence module. The samples are placed in filtered 0.15 M NaCl solution (pH$\simeq$ 7.25) in Lab-Tek chambered covered glass. The bacteria were stained with LIVE/DEAD® BacLight™Bacterial Viability Kit: 30 µl of solution (SYTO 9 dye, 1.67 mM / Propidium iodide, 18.3 mM) was added to 20ml of filtered 0.15 M NaCl solution at neutral pH. Each samples was immersed in 0.8 ml of this solution for 15 min in a container protected from light. After staining, the samples were rinsed in one tube filled with M9 minimum solution and placed in filtered NaCl (pH 7.25) for observation.