Towards Self-regenerating Antimicrobial Polymer Surfaces.

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Supporting Information

Experimental

General.

All chemicals used were reagent grade and obtained from Acros, Carl Roth, Sigma-Aldrich or TCI Europe. They were used as obtained. Solvents for gel-permeation chromatography (GPC) were HPLC quality and obtained from Carl Roth.

The silicon wafer substrates used were either single side polished or double side polished (for FTIR measurements), with [100] orientation, 100 mm diamenter and about 700 µm thickness, from Si-Mat, Kaufering, Germany.

The spin-coater used was a SPIN150-NPP (SPS-Europe, Netherlands). The UV irradiation unit was a BIO-LINK-Box (Vilber Lourmat GmbH, Germany), with 254 nm light bulbs.

NMR spectra were recorded on a Bruker 250 MHz specrometer (Bruker, Madison/WI, USA).

The auto-nulling imaging ellipsometer Nanofilm EP^3 (Nanofilm technology GmbH, Göttingen, Germany) with a 532 nm laser was used to measure film thickness and refractive index. The angle of incidence was varied. Each sample was measured at 4-6 different

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locations. The EP4 model was used to fit the data.

FTIR spectra were measured on a Bio-Rad Excalibur spectrometer (Bio-Rad, Munich, Germany). Samples were measured under nitrogen atmosphere. A non-coated Silicon wafer was used as background sample. 64-128 scans were taken for each measurement.

Fluorescence microscopy images were taken on a Nikon Eclipse T*i*-S inverted microscope (Nikon GmbH, Düsseldorf, Germany) using a green-fluorescent-protein filter and 20x magnification. The imagining time was varied between 80 ms and 1 s. The fluorescence intensity was determined using a Perl Data Language script, with p dims($im) being the size of the structure, and p sum($im) the unweighted sum of all pixel intensities.

Antimicrobial activity tests with *E. coli* (ATCC 25922) were carried out using previously reported protocols.[1] In short, bacteria were sprayed onto the test surfaces under defined conditions. After 2 hours incubation, they were removed from the surface, plated out on agar plates, incubated to allow colony growth, and counted.

Feldfunktion geändert

Polymer Synthesis.

Polymer for SMAMP bottom layer.

Propyl Monomer: The propyl monomer (Figure S1) was synthesized as described previously.[2] (1) In short, 10.0 g (102 mmol) maleic acid anhydride was dissolved in 100 mL toluene. 15 mL (14.0 g, 205 mmol) furane were added. The reaction mixture was stirred for three days at room temperature. The product, oxonorbornene anhydride, precipitated as colorless solid from that mixture and was removed by filtration, washed with toluene, and dried in dynamic vacuum. (2) The thus obtained oxonorbornene anhydride (5.00 g, 30 mmol) was dissolved in 60 mL dry THF under nitrogen. Dry 1-propanol (5.60 g, 60 mmol) and 10 mol% (3.0 mmol, 1.67 g) 4-(dimethylamino)pyridine (DMAP) were added. The mixture was stirred overnight. To avoid a retro-Diels-Alder reaction during work-up, the solvent was removed quickly at room temperature. The product was dissolved in dichloromethane and added dropwise into ice-cooled n-hexane. It precipitated as a colorless solid and was dried in dynamic vacuum (3) The product of the second step (2.00 g, 9.0 mmol) was dissolved in 25 mL dry dichloromethane under nitrogen. 1.42 g (9.0 mmol) N-Boc-ethanolamine and 10 mol% (0.53 g) DMAP were added. The solution was cooled to 0°C, and 1.86 g (9.0 mmol) dicyclohexyl carbodiimid (DCC) were added. The suspension was stirred overnight. It was filtered over a short alumina column until a clear solution was obtained. It was then cooled to -20°C, and further precipitate was removed by filtration over alumina. The solvent was then removed and the product was re-dissolved in a small amount of dichloromethane. It was added dropwise into ice-cooled n-hexane. The colorless precipitate was removed by filtration, and dried in dynamic vacuum.

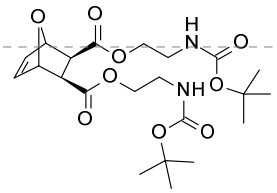


Figure S1: Propyl monomer

Feldfunktion geändert

^{1}H-NMR (250 MHz, $CDCl_3$): δ / ppm = 0.96 (t, 3H, CH_2-CH_3); 1.45 (s, 9H, BOC-CH_3); 1.67 (m, 2H, -CH_2-CH_3); 2.83 (q, 2H, C-CH-); 3.40 (2H, N-CH_2); 3,99 – 4.30 (m, 4H,O-CH_2-); 5.07 (s, breit, 1H, NH); 5.28 (m, 2H, O-CH); 6.47 (m, 2H, C=CH).

^{13}C-NMR (62.9 MHz, $CDCl_3$): δ / ppm =9.9 (CH_3); 22.2 (-CH_2-CH_3); 28.7 (BOC-C-CH_3); 39.4 (NCH_2); 47.2 und 47.6 (C-CH); 65.2 und 67.4 (O-CH_2); 79.7 (BOC-C-CH_3); 80.8 und 81.1 (O-CH-); 137.0 und 137.1 (C=CH); 156.2 (N-C=O(O)); 172.0 und 172.1 (CH-C=O(O)).

Propyl SMAMP homopolymer: The SMAMP homopolymer with a molecular weight of 100 000 g mol^{-1} was synthesized after literature procedures (500 mg propyl monomer, 10 mL dry dichloromethane, 3.63 mL of a stock solution containing Grubbs third generation catalyst (1 mg mL^{-1} in dichloromethane)).[2] For removal of the catalyst, the polymer was dissolved in dichloromethane and stirred for 30 min over silica gel. The resulting suspension was filtered using sand as filtration aid. The solution was precipitated into ice-cold n-hexanes, yielding the pure polymer.

Feldfunktion geändert

^{1}H-NMR (250 MHz, $CDCl_3$): δ / ppm = 5.91 (s, 1H, C=CH, trans); 5.91 (s, 1H, C=CH, trans); 5.62 (s, 1H, C=CH, cis); 5.39 (br s, 1H, NH); 5.14 (s, 1H, O-CH, cis); 4.74 (s, 1H, O-CH, trans); 4.02-4.26 (m, 4H, O-CH_2); 3.39 (s, 2H, N-CH_2); 3.14 (s, 2H, C-CH); 1.65 (m, 2H, CH_2-CH_3); 1.46 (s, 9H, BOC-CH_3); 0.95 (t, 3H, -CH_3).

GPC ($CHCl_3$, SDV column (PSS, Mainz, Germany) flow rate 1 mL min^{-1}, PMMA standards): M_n 220 000 gmol^{-1}, PDI = 1.10.

The N-Boc protected propyl SMAMP (500 mg) was dissolved in 20 mL of dry chloroform under nitrogen. To this solution, 20 mL of 4 M HCl in dioxane was added. After a few minutes, 5-10 vol% methanol were added to maintain solubility of the hydrolyzing polymer. The mixture was stirred for 18 hours at room temperature. The solvent was removed and the precipitate was re-dissolved in methanol. It was purified by precipitation into ice-cooled diethyl ether. Up to 10 vol% n-hexanes were added in case the polymer did not precipitate.

^{1}H-NMR (250 MHz, $CDCl_3$): δ / ppm = 5.99 (br s, 1H, C=CH, trans); 5.75 (br s, 1H, C=CH, cis), 5.14 (s, 1H, O-CH, cis); 4.73 (s, 1H, O-CH, trans); 4.11-4.26 (m, 4H, O-C**H**$_2$-CH_2); 3.74 (m, 2H, N-C**H**$_2$); 3.36 (s, 2H, C-CH + solvent); 1.69 (m, 2H, CH_2-CH_3); 0.99 (m, 3H, -CH_3).

Poly(sebacid anhydride) (PSA) for the degradable interlayer:

The structure of the PSA **2** copolymer is shown in Figure 2, the reaction is illustrated in Scheme S1. PSA was synthesized after literature procedures:[3] (1) The reaction was performed in dry glassware under nitrogen. 9.0 g (45 mmol) sebacid acid was mixed with a large excess (90 mL, 97.2 g, 0.95 mol) acetic anhydride and refluxed for one hour. The solvent was then removed in dynamic vacuum at 50°C. A gel-like residue was obtained. It was mixed with 10 mL dry toluene and stirred. 20 mL of dry diethyl ether and 20 mL dry petrol ether were added. The precipitate was removed by vacuum filtration and washed with a 1:1 v/v mixture of dry petrol ether and ethyl ether. After drying in dynamic vacuum, 1.88 g of a colorless material were obtained (18% yield).

Feldfunktion geändert

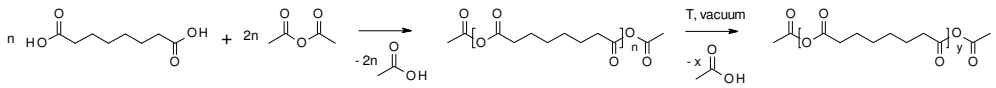
^{1}H-NMR (250 MHz, $CDCl_3$): δ / ppm = 2.47 (t, 4H, C(=O)-CH_2); 2.24 (s, 0.5H, -CH_3, end group) 1.60-1.75 (m, 4H, C(=O)-CH_2-CH_2); 1.25-1.45 (m, 8H, -CH_2).

(2) The pre-polymer obtained in step (1) (1.80 g) was placed in a dry Schlenk flask, which was evacuated (10^{-3} mbar) and placed into a pre-heated oil bath at 180°C. The polycondensation that yielded the PSA polymer was quenched after 90 min by cooling with an ice bath and flushing the flask with nitrogen. The raw product was dissolved in 20 mL dry

chloroform and added dropwise into 50 mL ice-cooled dry diethyl ether. A colorless, fluffy precipitate was obtained. The product was recovered by vacuum filtration, dried in dynamic vacuum, and stored at -18 °C. The yield was 1.30 g (69%).

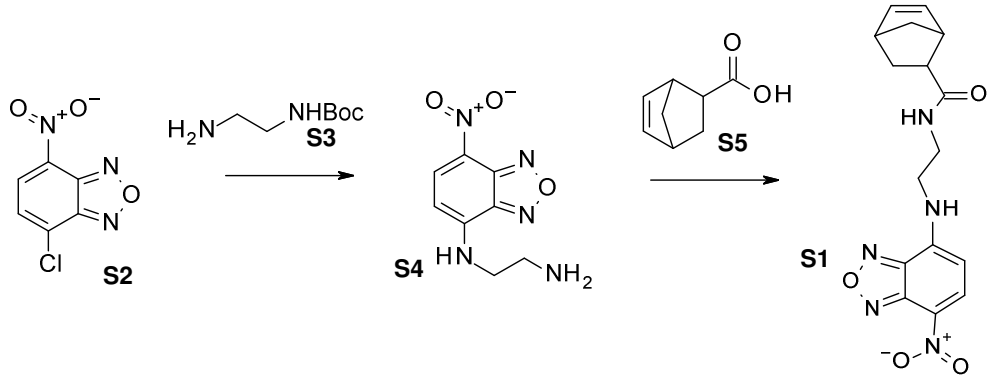
^{1}H-NMR (250 MHz, $CDCl_3$): δ / ppm = 2.47 (t, 4H, C(=O)-CH_2); 2.24 (s, 0.07 H, -CH_3, end group) 1.60-1.75 (m, 4H, C(=O)-CH_2-CH_2); 1.25-1.45 (m, 8H, -CH_2).

GPC ($CHCl_3$, SDV column (PSS, Mainz, Germany), flow rate 1 mL min^{-1}, PMMA standards): M_n = 35 000 g mol^{-1}, PDI = 2.66



Scheme S1: Synthesis of poly(sebacic anhydride) in two steps.
Polymer for Fluorescent SMAMP top layer.

2-(4-nitro-2,1,3-benzoxadiazol-7-yl)norbornen (NBD) Monomer: The synthesis of the NBD-Monomer **S1** is shown in Scheme S2. The chloro derivative of the NBD dye (**S2**) was reacted with the partially N-Boc-protected ethylene diamine **S3** as described in the literature.[4]



Scheme S2: Synthesis of the 2-(4-nitro-2,1,3-benzoxadiazol-7-yl)norbornen (NBD) Monomer

Step 1: Synthesis of N-Boc-ethylene diamine (**S3**): **S3** was synthesized as described in literature[5],[6]. Di-*tert*-butyl dicarbonate (16.34 g, 74.68 mmol) was dissolved in dichloromethane (400 mL) and added dropwise to a solution of ethylene diamine (22.50 g, 374.3 mmol, 5.0 eq) in dichloromethane (50 mL) over 6 h, while stirring at room temperature. The reaction mixture was further stirred at room temperature for 24 h. Aqueous sodium bicarbonate (saturated, 300 mL) was added to the reaction mixture and the organic phase was separated. The water phase was extracted with dichloromethane (3x 100 mL). The organic phases were combined and dried over sodium sulphate. The solvent was evaporated under reduced pressure. The product **S3** was received as a colorless oil. Yield: 11.7 g, 98 %.

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^{1}H NMR (250 MHz, $CDCl_3$): δ / ppm) = 4.92 (br. s., 1H), 3.19 (td, *J* = 5.80 Hz, 2H), 2.81 (t, *J* = 5.92 Hz, 2H), 1.46 (s, 9 H), 1.33 (s, 2H).

Step 2: Synthesis of the 4-diaminoethyl-7-nitrobenzofurazan (**S4**): **S4** was synthesized as described in the literature[4]. N-Boc-ethylenediamine (0.47 g, 2.7 mmol, 1.1 eq) and N,N-diisopropyl ethylamine (0.69 g, 0.90 mL, 5.0 mmol, 2.0 eq) were dissolved in dry dichloromethane (20 ml). 4-Chloro-7-nitrobenzofurazan (NBD-chloride, 0.50 g, 2.5 mmol) was added to the solution at room temperature under nitrogen. The reaction flask was wrapped in aluminum foil, and the dark brown solution was stirred overnight. The solution was diluted with dichloromethane (200 mL) and washed with aqueous sodium bisulphate (10 w% in water, 50 mL), aqueous sodium bicarbonate (saturated, 50 mL) and water (50 mL). The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure. The product was dissolved in methanol (300 mL) and washed with hexane (2x 100 mL). The solvent was evaporated under reduced pressure. The product **S4** was purified by column chromatography on silica gel (hexane/ethyl acetate: 1/3). The resulting intermediate product was dissolved in dichloromethane (10 mL). Trifluoroacetic acid (10 mL) was slowly added. After 1.5 h, the reaction mixture was slowly dropped into diethyl ether (250 mL). The precipitate was filtrated, dissolved in acetone (5 mL) and again precipitated in diethyl ether (200 mL). The product was collected by vacuum filtration. Yield: 0.18 g, 33 %.

Feldfunktion geändert

Feldfunktion geändert

^{1}H NMR (250 MHz, methanol-d_4): δ / ppm = 8.59 (d, *J* = 8.69 Hz, 1H, H ortho to NO_2), 6.47 (d, *J* = 8.69 Hz, 1H, H ortho to NH), 3.89 (t, *J* = 6.24 Hz, 2H, CH_2), 2.16-2.18 (m, 2H, CH_2).

Step 3: Synthesis of the 2-(4-nitro-2,1,3-benzoxadiazol-7-yl)norbornen (NBD) Monomer (**S1**): 5-Norbornene-2-carboxylic acid **S5** (0.044 g, 0.32 mmol), 4-diaminoethyl-7-nitrobenzofurazan **S4** (0.071 g, 0.32 mmol, 1.0 eq) and 4-(dimethylamino)-pyridin (0.037 g, 0.31 mmol, 1.0 eq) were dissolved in anhydrous tetrahydrofuran (45 ml) under nitrogen and stirred at 0°C. A solution of *N,N'*-dicyclohexylcarbodiimide (0.072 g, 0.35 mmol, 1.1 eq) in tetrahydrofuran was added dropwise. The reaction mixture was stirred at room temperature for 24 h. The product mixture was filtered, washed with aqueous potassium bisulphate (10 w% in water, 2x 50 mL) and with aqueous sodium bicarbonate (saturated, 1x 50 mL). The organic phase was dried over sodium sulphate and the solvent was evaporated under reduced pressure. The product **S1** was purified by column chromatography on silica gel (hexane/acetone: 3/2). Yield: 0.035 mg, 33%.

^{1}H NMR (250 MHz, DMSO-d_6) δ / ppm = 9.46 (br. s., 1H), 8.54 (d, *J* = 8.85 Hz, 1H), 8.10 (t, 1H), 6.46 (d, *J* = 9.16 Hz, 1H), 6.01 - 6.23 (m, 2H) 3.56 (br. s., 2H) 3.38 - 3.47 (m, 2H) 2.75 - 2.85 (m, 2H) 1.92 - 2.05 (m, 1H) 1.70 - 1.82 (m, 1H) 1.52 - 1.62 (m, 1H) 1.01 - 1.22 (m, 2H).
^{13}C NMR (63 MHz, DMSO-d_6) δ = 176.17, 146.33, 145.32, 144.97, 138.69, 136.98, 121.64, 100.11, 47.57, 46.49, 43.96, 41.80, 38.26, 30.58.

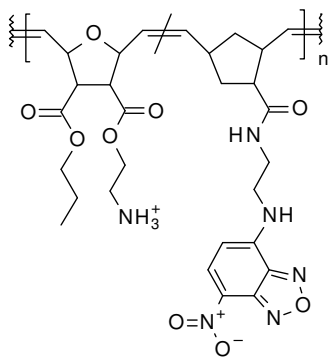


Figure S2: structure of Propyl-SMAMP-NBD-copolymer

Propyl-SMAMP-NBD copolymer: The structure of the Propyl-SMAMP-NBD **5** copolymer is shown in Figures 2 and S2. To synthesize this polymer, Propyl-Monomer

(Figure S1, 500.3 mg, 1.36 mmol) and NBD-Monomer **S1** (30 mg, 0.087 mmol) were dissolved in 2 mL dry DCM. Grubbs 3rd generation catalyst (7.27 mg, 0.01 mmol) was dissolved in 0.5 mL DCM. Both solutions were stirred rigorously. Three freeze-pump-thaw cycles were conducted with each of the solutions. The catalyst solution was then added in one shot to the monomer solution. After 25 min stirring, 0.8 mL ethylvinyl ether were added to quench the polymerization. After another 45 min stirring, the viscous mixture was dropwise added to stirred, ice-cooled n-hexane (500 mL). The polymer **5** precipitated as a brownish solid and was dried in high vacuum overnight.

^{1}H NMR (250 MHz, d-methanol) δ / ppm = 5.91 (s, 1H, C=CH, trans); 5.63 (s, 1H, C=CH, cis); 5.43 (br s, 1H, NH); 5.14 (s, 1H, O-CH, cis); 4.72 (s, 1H, O-CH, trans); 4.02-4.28 (m, 4H, O-CH_2); 3,38 (s, 2H, N-CH_2); 3.14 (s, 2H, C-CH); 1.66 (m, 2H, CH_2-CH_3); 1.47 (s, 9H, BOC-CH_3); 0,96 (t, 3H,-CH_3).

GPC ($CHCl_3$, SDV column (PSS, Mainz, Germany), flow rate 1 mL min^{-1}, PMMA standards): M_n = 180 000 g mol^{-1}, PDI = 1.1.

Multi-stack assembly.

Functionalized silicon wafers.

The anchor group benzophenone-triethoxysilane (3EBP) shown in Figure S3 was synthesized after literature procedures.[7] 3EBP was dissolved in dry toluene (10 mg mL^{-1}). This stock solution was stored at room temperature in an aluminum-foil wrapped Schlenk flask in the dark under nitrogen. A silicon wafer was washed with isopropanol, methanol and toluene on the spin coater. The 3EBP solution was applied to the thus cleaned substrate by spin-coating at 500 rpm for 120 s. The wafer was then placed for 45 min onto a pre-heated hot plate (110°C) and covered with an aluminum-foil wrapped Petri dish. The wafer was then washed with toluene and dried under nitrogen flow. It was then cut into the desired size for further experiments.

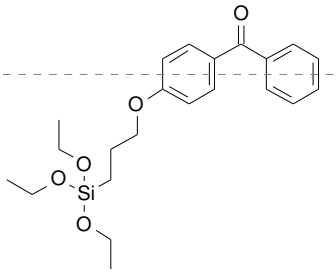


Figure S3: Benzophenone-triethoxysilane

Feldfunktion geändert

SMAMP bottom layer.

The protected SMAMP polymer was dissolved in dry chloroform (30 mg mL^{-1}). The cross-linker pentaerythritol tetrakis(3-mercaptopropionat) (**4** in Figure 3) was also dissolved in dry chloroform (4.9 mg mL^{-1}). After 30 min stirring, equal amounts of each solution were mixed, yielding a solution with 15 mg mL^{-1} polymer and 2.5 mg mL^{-1} cross-linker (0.5 SH groups per double bond). The thus obtained solution was stirred for 30 min. It was filtered through a syringe filter (hydrophilic PTFE, pore size 0.2 µm, Millipore) onto a static 3EBP wafer. The wafer was spin-coated with an acceleration of 1000 rmp s^{-1} and final speed of 3000 rpm for 30 s. It was then irradiated at 254 nm for about 15 min, until an energy dose of 3 J cm^{-1} was reached. The not covalently bound part of the thus obtained polymer network was extracted

by immersion into dry dichloromethane. It was then immersed for three hours in 4 M HCl in dioxane under nitrogen, rinsed thoroughly with ethanol, and dried under nitrogen flow.

PSA Interlayer.

The PSA polymer (**2**) was dissolved in dry chloroform at 15 mg mL^{-1} and stirred for 30 min. It was filtered through a syringe filter (hydrophilic PTFE, pore size 0.2 µm, Millipore) and added dropwise onto the non-rotating 3EBP wafer piece that carried the SMAMP bottom layer. The wafer was spin-coated with an acceleration of 1000 rmp s^{-1} and a final speed of 3000 rpm for 30 s. It was then dried under nitrogen flow.

Fluorescent SMAMP top layer.

The deprotected SMAMP-NBD copolymer with a NBD content of 2 mol% was dissolved in a mixture of methanol and ethyl acetate (28 mg mL^{-1}). The cross-linker pentaerythritol tetrakis(3-mercaptopropionat) (**4** in Figure 3) was dissolved in the same solvent mixture at 1 mg mL^{-1}. After 30 min stirring, equal amounts of each solution were mixed, yielding a solution with 14 mg mL^{-1} polymer and 0.5 mg mL^{-1} cross-linker (0.1 SH group per double bond). The thus obtained solution was stirred for 30 min. It was filtered through a syringe filter (hydrophilic PTFE, pore size 0.2 µm, Millipore) onto a non-rotating 3EBP wafer that carried the SMAMP bottom layer and the PSA layer. The wafer was spin-coated with an acceleration of 1000 rmp s^{-1} and final speed of 3000 rpm for 30 s. It was then irradiated at 254 nm for about 15 min, until an energy dose of 3 J cm^{-1} was reached. The system was washed with ethanol and dried under nitrogen flow.

Multi-stack Characterization

Thickness determination by ellipsometry:

SMAMP: d = 108 ± 4 nm; SMAMP+PSA: d = 228 ± 4 nm; SMAMP+PSA+SMAMP-co-NBD: d = 260±4 nm.
As expected, the stack thickness increases with each added layer.

FTIR spectra:

As shown in Fig. S4, the carbonyl stretching vibration at 1730 cm^{-1} is the most prominent feature of the SMAMP layer spectrum (black). When the PSA layer is added (red), an additional peak at 1810 cm^{-1} appears, corresponding to the asymmetric stretching vibration of the anhydride in PSA. The spectrum of the three layer system (blue) features a relative increase of the ester peak height compared to the anhydride peak, which confirms the addition of another SMAMP layer.

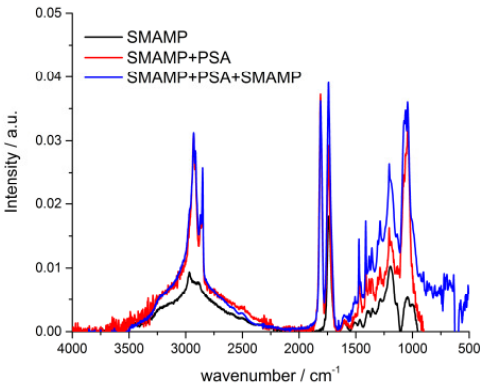


Figure S4: FTIR spectra of three-layer system build-up. Black: SMAMP bottom layer, Red: PSA layer on top of

SMAMP bottom layer (characteristic anhydride peak at 1810 cm^{-1}), Blue: NBD-SMAMP-layer on top of PSA and bottom SMAMP layer.

Flourescence microscopy images:

The fluoresecence images of the SMAMP layer, the SMAMP layer+PSA, and the SMAMP layer+PSA+NBD-SMAMP are shown in Figure S5. These images indicate that both the bottom SMAMP layer (A) and the additional PSA layer (B) do not have a fluorescent chromophore. In contrast, the three-layer-system with the dye-labelled SMAMP polymer as the top layer features the expected high fluorescence intensity. These control images confirm that we can attribute all the fluorescence intensity of the sample to the presence of the NBD chromophore.

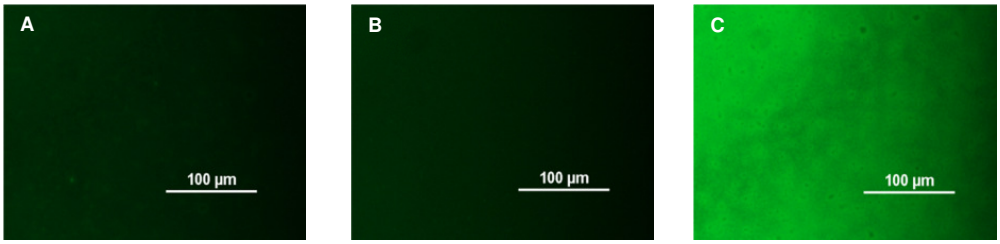


Figure S5: Flourescence microscopy images of three-layer system build-up. (A) SMAMP bottom layer, low fluorescence; (B) PSA layer on top of SMAMP bottom layer, low fluorescence; (C): NBD-SMAMP-layer on top PSA and bottom SMAMP layer, significantly higher fluorescence intensity (see below).

Layer Shedding

The kinetics of layer shedding was investigated in aqueous 0.1 M HCl and 3 M HCl. In each case, duplicates of the three-layer stack were immersed into the degradation solution. At defined time points, one duplicate was removed and quickly dried under nitrogen flow. It was then immediately analyzed, either by imaging with the fluorescence microscope, or the FTIR spectra were taken, or the layer thickness was measured using ellipsometry.

Degradation in 0.1 M HCl:

a) Fluorescence microscopy:

Fluorescence images at an exposure time of 80 ms are shown in Figure S6, the corresponding images at an exposure time of 1 s are shown in Figure S7. A green fluorescent protein filter at 20x magnification was used for imaging. The fluorescence intensity was quantified using a Perl Data Language script as described in the General experimental part. The results are summarized in Figure S8.

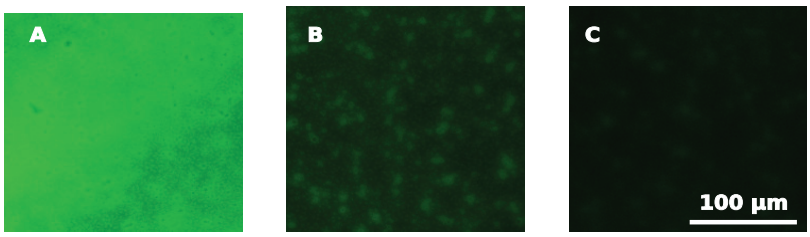


Figure S6: Fluorescence images of the three layer system (exposure time t = 80 ms): before degradation (A), after 10 min degradation (B) and after 30 min degradation (C) in 0.1 M HCl at 60 °C.

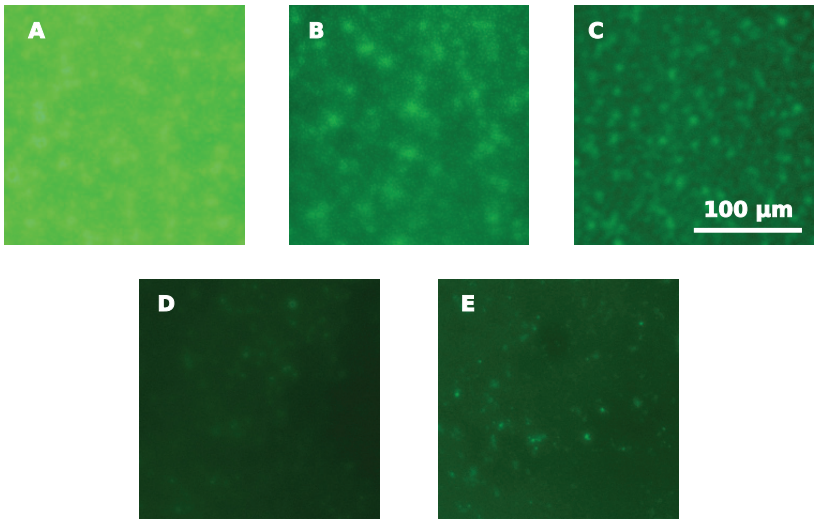


Figure S7: Fluorescence images of the three layer system (exposure time t = 1 s) during degradation: (A) after 10 min, (B) after 20 min, (C) after 30 min, (D) after 90 min, (E) after 120 min degradation in 0.1 M HCl at 60 °C.

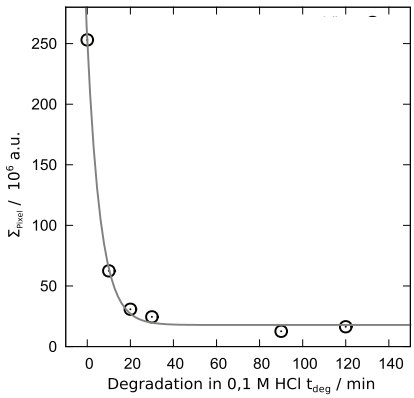


Figure S8: Fluorescence intensity of the three layer system during degradation (exposure time t = 80 s). The intensity of the fluorescence was obtained from the 450 x 450 pixel images shown in Figure S7. The data was fitted exponentially, with $\sum pixel = 17 \cdot 10^6 + 235 \cdot 10^6 \cdot e^{-0.16 \cdot t_{deg}}$. The intensity at t = 0 min was calculated from that formula.

The fluorescence intensity was fitted exponentially, with $\sum pixel = 17 \cdot 10^6 + 235 \cdot 10^6 \cdot e^{-0.16 \cdot t_{deg}}$. This yielded a degradation rate constant of 0.16 s^{-1} and a base fluorescence level of $17 \cdot 10^6$ a.u.. The intensity at t = 0 min for an exposure time of 1 s was calculated from the formula above because it was out of the measurement range of the microscope.

b) FTIR spectroscopy and ellipsometry measurements

The removal of the PSA layer was monitored by quantifying the ratio of the ester and anhydride peaks of the FTIR spectrum, which are also discussed in the multi-stack buildup section and the main text. The results are shown in Figure S9. While the asymmetric stretching vibration peak of the anhydride continuously shrinks, it does not fully vanish. This indicates that some fragments of the PSA layer, presumably low molecular weight molecules that diffused into the bottom SMAMP layer, remained on the surface. This was confirmed by matching ellipsometry results (Fig. S10). Here, the relative layer thickness, i.e. the thickness of all material on top of the bottom SMAMP layer, did not fall back to 0 nm, but remained at about 25 nm. This could be incomplete layer shedding, or low molecular weight fragments that migrated into the bottom SMAMP network, and thus made it thicker.

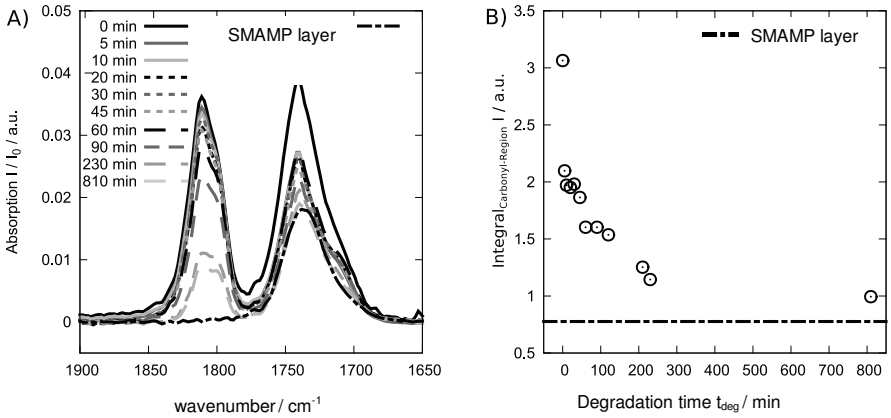
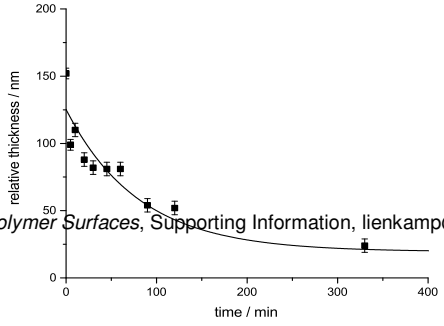


Figure S9: FTIR sprectra of the three layer system (A), with the SMAMP bottom layer as reference. The relative intensity of the carbonyl peaks versus time was obtained by integration, and plotted versus degradation time (B). The data, compared to the integral of the original SMAMP bottom layer, indicates that some anhydride groups remain in the material, presumably due to low molecular



weight fragments in the pores of the bottom SMAMP network.

Figure S10: The relative layer thickness layer of the three-layer system (= measured thickness minus SMAMP bottom layer thickness) was followed over time with ellipsometry measurements. The data indicated a remaining layer of about 25 nm thickness.

Since the fluorescence measurements monitor only the shedding of the top layer of the system, while the ellipsometry data looks at all three layers simultaneously, there is a certain discrepancy of the degradation time and rates observed by these methods. The fluorescence essentially decayed to baseline level after 30 minutes, while it took more than 300 min for the thickness measured by ellipsometry to reach baseline. Thus, the top layer floats off once a substantial amount of the inter-layer is degraded, but it takes much longer for that inter-layer to be removed.

In summary, the data shows that material of the top two layers remained on the bottom layer after 120 min degradation time in 0.1 M HCl. The experimental series was therefore repeated in 3 HCl to obtain faster degradation.

Degradation in 3 M HCl at 60 °C

FTIR spectra after degradation in 3 M HCl are shown in Figure S11. The peak of the asymmetric anhydride stretching vibration completely vanished, indicating quantitative removal of the PSA layer. The ester peak also fell back to the level of the initial SMAMP single layer (dashed line in Figure S11).

a) FTIR-spectroscopy

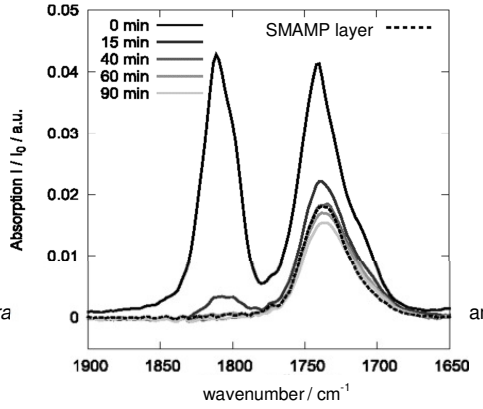


Figure S11: FTIR sprectra of the three layer system, with the SMAMP bottom layer as reference. The data, compared to the original SMAMP bottom layer, indicates that the anhydride layer was quantitatively removed.

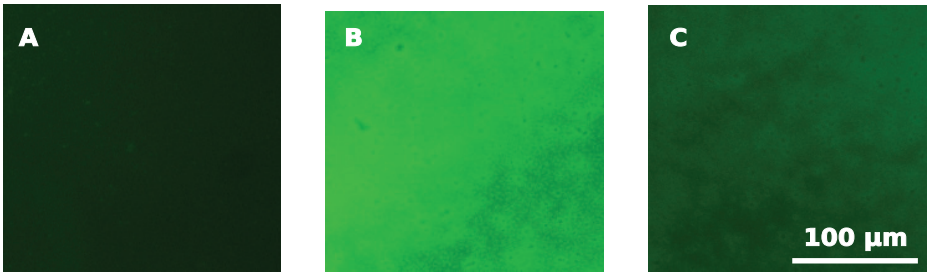


Figure S12: Fluorescence images of the three layer system during build-up and degradation: (A) bottom layer, exposure time t = 1 s, fluorescence intensity I = $9.7 \cdot 10^6$ a.u.; (B) three-layer system, t = 80 ms, I = $42.9 \cdot 10^6$ a.u.; (C) degraded system, t = 1 s, fluorescence intensity I = $18.3 \cdot 10^6$ a.u.

Quantitative fluorescence microscopy images are shown in Figure S12. The degraded system had about twice the intensity of initial SMAMP layer ($18.3 \cdot 10^6$ a.u. vs. $9.7 \cdot 10^6$ a.u.). The intensity for the three-layer system was $42.9 \cdot 10^6$ a.u. at t = 80 ms, and out of the measurement range for t = 1 s. We can calculate the corresponding 'measured' intensity at t = 1 sec by taking $42.9 \cdot 10^6 \text{ a.u.} \cdot \frac{1\ s}{80\ ms}$, which yields $536 \cdot 10^6$ a.u.. Thus, the residual fluorescence precentage in Figure S12C, can be estimated by taking $\frac{18.3-9.7}{536-9.7} \cdot 100$. This yielded 1.6% more fluorescence for the degraded system than for a single layer. This is not a significant increase, considering the crudeness of the method.

The antimicrobial activity was determined as discussed above. The data is shown in Table S1 and Figure S13. The number of colony forming units (CFUs, i.e. surviving bacteria that were not killed by the coating) was zero for the positive control, the initial three-layer system ('Before degradation'), and the degraded system ('after degradation' in Table S1), while the negative control had a colony count of 1059 ± 14. This data demonstrates that the degraded system was as active as the pristine three-layer system.

Table S1: Results of the antimicrobial activity assay. The number of surviving colony forming units (CFUs) on the negative control (blank silicon wafer), the positive control (disinfectant chlorhexidine digluconate), and the two test surfaces are shown. 'Before degradation' designates the pristine three-layer system after build-up. 'After degradation' is the three-layer system after exposure to 3 M HCl and shedding of the top layers.

	Positive control	Negative control	Before Degradation	After Degradation
Data of individual samples	0	1245	0	0
		1086	0	0
		1018	0	0
		988	0	0
		956	0	0
Average	0 ± 0	1059 ± 14	0 ± 0	0 ± 0

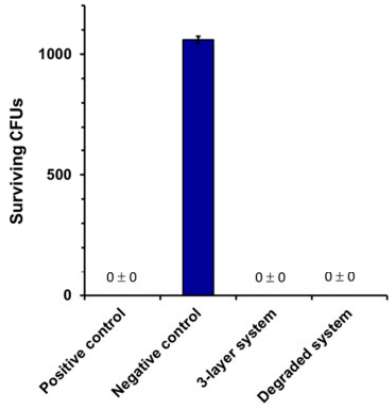


Figure S13. Bar diagram of the data shown in Table S1. The data indicates that the degraded system and the three-layer system had an equally high antimicrobial activity.

Author contributions:

K.L. designed and directed the project, evaluated the data for this manuscript and her habilitation thesis, made the figures in the main text and some in the supporting information, and wrote the main text manuscript and most of the text in the supporting information. F.D. compiled the data and made most of the figures for the Supporting Information, and independently evaluated the data for her doctoral thesis. D. B., A. S. and W. H. developed the synthesis of the NBD-labeled polymer. A. A.-A. directed the antimicrobial experiments.

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