ADVANCED MATERIALS

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Amphiphilic Copolymers for Versatile, Facile, and In Situ Tunable Surface Biofunctionalization

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Keywords: adsorptive surface functionalization, styrene-maleic anhydride copolymers, cell-instructive properties, antimicrobial surface properties

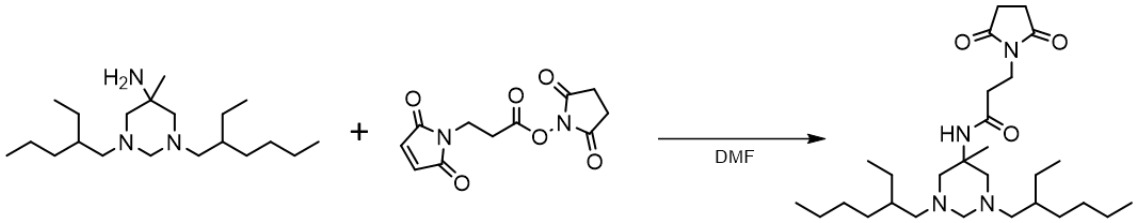
Experimental section

Materials

All chemicals were purchased from Merck KGaA (Darmstadt, Germany)–if not indicated otherwise–and used as purchased without further purification unless stated otherwise. Water is used ultrapure (Millipore Direct-Q, 18 MΩ cm resistivity). The following peptides were purchased from CASLO ApS (Kongens Lyngby, Denmark): Ac-GCWGGRGDSP-NH_2; Ac-GCWGGSIRT-NH_2; Ac-GCWGGEIDGIELT-NH_2; Ac-GCWGGIKVAV-NH_2; cyclo[RGDfC]; Ac-CWGGPQVTRGDVFTMP-NH_2; Ac-GCWGRKKGRKKGRKKGRKK-NH_2

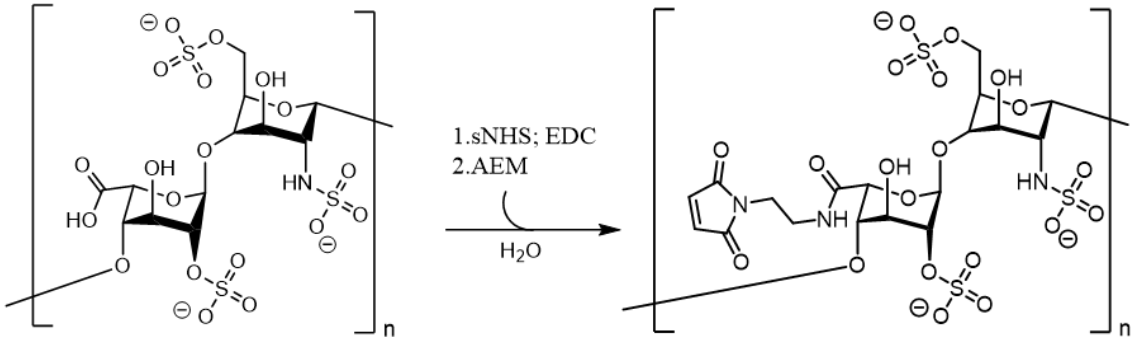
Syntheses of modified bioactives

Synthesis of Hexetidine-Maleimide



A solution of N-(3-Maleimidopropionyloxy)-succinimide (0.314 g, 1.18 mmol) in 5 mL DMF was added to a solution of hexetidine (0.2 g, 0.589 mmol) in 5 mL *N, N*-dimethylformamide (DMF). The reaction mixture was stirred for 24 h at RT and directly used for further analysis and purification via HPLC/HPLC-MS. Structure and purity were determined by LC-ESI-ToF MS (yield: 65 %).

Synthesis of Heparin-Maleimide



The synthesis of heparin conjugates with one group of maleimide (Hep-Mal1) was performed according to a previously reported procedures.[1] Briefly, heparin (0.5 g, 0.033 mmol) was dissolved in 3 ml ice-cold MilliQ followed by the addition of (0.217, 0.06 mmol) N-hydroxysulfosuccinimide (s-NHS) in 0.4 mL ice-cold MilliQ and (0.192 g, 0.12 mmol) 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in 0.2 mL ice-cold MilliQ and placed in an ice bath. The reaction was allowed to proceed before adding of (0.254 g, 0.04 mmol) N-(2-aminoethyl)-maleimide (AEM). The mixture was allowed to react overnight with continuous stirring at room temperature and purified via dialysis (MWCO = 8 kDa) against 1 M NaCl for

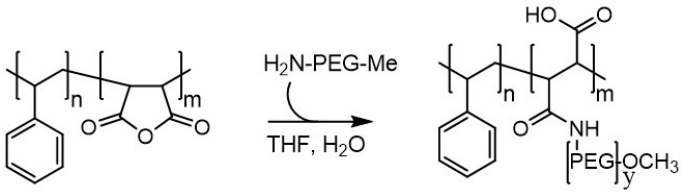
three times and for three times against MilliQ over the course of multiple days, followed by lyophilization (yield: 83 %). The structure was determined by ^{1}H NMR using D_2O as solvent.

The degree of maleimidation was determined via comparing the intensities of signals of the maleimide group at δ= 7.3 (2H) to the heparin's N-acetyl peak at δ= 2.4 (3H).

Synthesis of modified SMA copolymers

PEGylation

α-Methoxy-ω-amino PEG



Styrene-maleic anhydride (SMAnh) copolymers XIRAN® SZ 10010, 30010, 26080 and 26120 were kindly provided by Polyscope Polymers B.V. (Geleen, The Netherlands). Scirpset 520™ was provided by Solenis (Delaware, USA). Polyethylene glycols (α-Methoxy-ω-amino PEG, 122000-2, 125000-2, 1210000-2, 1220000-2) were purchased from Rapp Polymere (Tübingen, Germany). Prior to use, SMAnh copolymer granules were dissolved in anhydrous tetrahydrofuran (THF) and precipitated in anhydrous diethyl ether, collected by vacuum filtration and dried under nitrogen flow to gain a fine, white powder. In brief, PEGylation was performed by dissolving the SMAnh copolymers in THF and PEG in a THF /MilliQ (10:1, v/v) mixture before combining both solutions.[2] The reaction mixture was stirred for 5 days at RT and purified by dialysis (MWCO = 10, 25 and 50 kDa, depending on MW of used PEG) against 1 M NaCl for three times and against MilliQ for three times over the course of multiple days, which was followed by lyophilization. The molar excess of PEG used for this synthesis was calculated from the degree of polymerization DP_{MAnh} for each of the SMAnh copolymers and for different, anticipated PEGylation degrees using equation (1).

$$DP_{MAnh} = \frac{M_{(SMAnh)}}{\sum(M_i \times x_i)} \times x_{MAnh} \qquad (1),$$

$M_{(SMAnh)}$ corresponds to the molar mass of the copolymer, M_i to the molar mass of the monomers and x_i to the percentage of each monomer, while x_{MAnh} corresponds to the percentage of MAnh, respectively. The structure and PEGylation degree were determined by ^{1}H NMR and FT-IR.

Table S1. Synthesized AP derivatives Synthesized anchor polymer (AP) derivatives and corresponding yields are listed in the following table.

SMAnh	PEGylation (100 %)	AP	PEGylation (50 %)	AP	yield
XIRAN® SZ **10010** (0.1 g, 0.02 mmol)	2k (0.155 g, 0.11 mmol)	10-10-6-2	2k (0.078 g, 0.05 mmol)	10-10-3-2	87 % (PEG = 1) 92 % (PEG = 0.5)
XIRAN® SZ **10010** (0.1 g, 0.02 mmol)	5k (0.483 g, 0.11 mmol)	10-10-14-5	5k (0.241 g, 0.05 mmol)	10-10-7-5	93 % (PEG = 1) 94 % (PEG = 0.5)
XIRAN® SZ **10010** (0.5 g, 0.01 mmol)	20k (0.908 g, 0.05 mmol)	10-10-58-20	20k -	-	82 % (PEG = 1) -
XIRAN® SZ **30010** (0.1 g, 0.01 mmol)	2k (0.371 g, 0.25 mmol)	10-30-16-2	2k (0.185 g, 0.13 mmol)	10-30-8-2	82 % (PEG = 1) 79 % (PEG = 0.5)
XIRAN® SZ **30010** (0.025 g, 0.004 mmol)	5k (0.288 g, 0.06 mmol)	10-30-40-5	5k (0.144 g, 0.03 mmol)	10-30-20-2	93 % (PEG = 1) 95 % (PEG = 0.5)
XIRAN® SZ **30010** (0.025 g, 0.004 mmol)	10k (0.645 g, 0.06 mmol)	10-30-84-10	10k -	-	97 % (PEG = 1) -
XIRAN® SZ **30010** (0.025 g, 0.004 mmol)	20k (1.107 g, 0.06 mmol)	10-30-165-20	20k -	-	91 % (PEG = 1) -
XIRAN® SZ 26080 (0.1 g, 0.001 mmol)	2k (0.313 g, 0.214 mmol)	80-30-13-2	2k (0.156 g, 0.107 mmol)	80-30-6-2	95 % (PEG = 1) 88 % (PEG = 0.5)
XIRAN® SZ 26080 (0.1 g, 0.001 mmol)	5k (0.974 g, 0.214 mmol)	80-30-32-5	5k (0.487 g, 0.107 mmol)	80-30-16-5	96 % (PEG = 1) 98 % (PEG = 0.5)
XIRAN® SZ 26120 (0.1 g, 0.9 µmol)	2k (0.313 g, 0.214 mmol)	120-30-13-2	2k (0.156 g, 0.107 mmol)	120-30-6-2	78 % (PEG = 1) 98 % (PEG = 0.5)
XIRAN® SZ 26120 (0.025 g, 0.23 µmol)	5k (0.243 g, 0.05 mmol)	120-30-33-5	5k (0.121 g, 0.025 mmol)	120-30-16-5	95 % (PEG = 1) 91 % (PEG = 0.5)

Scirpset 520™ (0.05 g, 0.1 µmol)	2k (0.362 g, 0.247 mmol)	350-50-45-2	2k (0.181 g, 0.123 mmol)	350-50-22-2	80 % (PEG = 1) 73 % (PEG = 0.5)
Scirpset 520™ (0.02 g, 0.06 µmol)	5k (0.450 g, 0.1 mmol)	350-50-115-5	5k -	-	91 % (PEG = 1) -
Scirpset 520™ (0.02 g, 0.06 µmol)	10k (1.01 g, 0.1 mmol	350-50-238-10	10k -	-	92% (PEG = 1) -
Scirpset 520™ (0.01 g, 0.03 µmol)	20k (0.845 g, 0.05 mmol)	350-50-470-20	20k -	-	94 % (PEG = 1) -

P(PEG)/S(Styrene) ratio

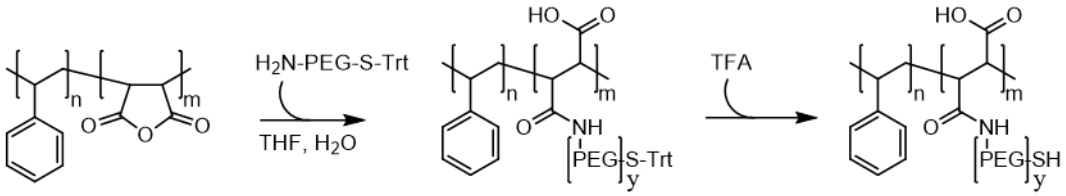
P_{EG}/S ratios of APs were calculated using the equations (2) and (3).

$$\left(\frac{DP_{PEG} \times DP_{MAnh}}{(DP_{MAnh} + DP_{STY})}\right) \times degPEG = P_{EG} \quad (2),$$

$$\frac{DP_{STY}}{(DP_{MAnh} + DP_{STY})} = S \quad (3),$$

With DP_{PEG} the amount of PEG repeating units per PEG chain, DP_{MAnh} the number of repeating units of MAnh monomer per AP = PEG chains per AP, degPEG, the degree of PEGylation and DP_{STY} the number of repeating units of styrene monomers per AP.

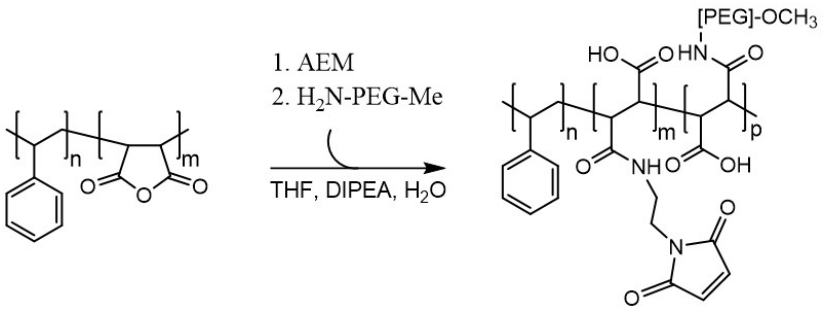
α-Amino-ω-tritylthio PEG



PEGylation was performed by dissolving SMAnh copolymers in THF and PEG (133000-20-41) (Rapp Polymere, Tübingen, Germany) in a THF/MilliQ (10:1, v/v) mixture before combining both solutions. The reaction mixture was stirred for 5 days at RT and purified via dialysis (MWCO = 10 kDa) against 1 M NaCl for three times and for three times against MilliQ over the course of multiple days, followed by lyophilization. The molar excess of PEG used for this synthesis was calculated as previously described. Deprotection of the trityl protection group was accomplished by dissolving the anchor polymer (AP)-Triphyenylmethyl(Trt) in a mixture of trifluoroacetic acid (TFA), phenol, tri-isopropyl silane and MilliQ-water (35:2:1:2, v/v). The reaction mixture was stirred for 3h at RT. Afterwards, the mixture was subsequently diluted using MilliQ and quickly transferred to a 10 kDa dialysis membrane. Dialysis was carried out as described above. To prevent thiol group oxidation, dialysis solution was vigorously saturated by bubbled nitrogen. After purification the product was immediately freeze-dried. The structure and PEGylation degree were determined by ^{1}H NMR and Ellman's assay (yield: 78 %).

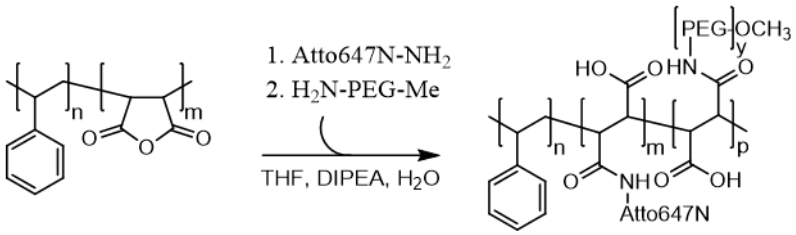
Backbone modifications

Maleimidation



Maleimidation was performed by dissolving the SMAnh (XIRAN® SZ, 30010; Scirpset 520™) copolymer (0.3 g, 0.043 mmol; 0.1 g, 0.29 µmol) in 40 mL THF, α-Methoxy-ω-amino PEG 122000-2 (0.56 g, 0.38 mmol; 0.362 g, 0.25 mmol) in 20 mL of a mixture of THF and MilliQ (3:1, v/v) and AEM (0.096 g, 0.38 mmol; 0.063 g, 0.25 mmol) in 5 mL MilliQ. The AEM solution was combined first with the SMAnh solution and after 1 h of stirring at RT the PEG solution was added to the reaction mixture. The reaction mixture was subsequently stirred for 3 days at RT. AP of the 40-50-19-0.5 type (AP with the composition 40-50-19-0.5 was kindly received as a gift from BASF; BASF SE, (Ludwigshafen, Germany)) were modified with maleimide moieties using EDC/sNHS chemistry. Briefly, PEGylated copolymers (1 g, 16 µmol) were dissolved in 100 mL PBS, EDC (0.061 g, 0.32 mmol) and sNHS (0.034 g, 0.16 mmol) were added and the mixture was stirred for 1 h at 4 °C before AEM (0.02 g, 0.08 mmol) was additionally added. The reaction mixture was stirred over night at RT. Purification was performed via dialysis as well as freeze-drying via lyophilization as described above. The structure, PEGylation and maleimidation degree were determined by ^{1}H NMR and Ellman's assay (yield: 68 %; 51 %; 83 %).

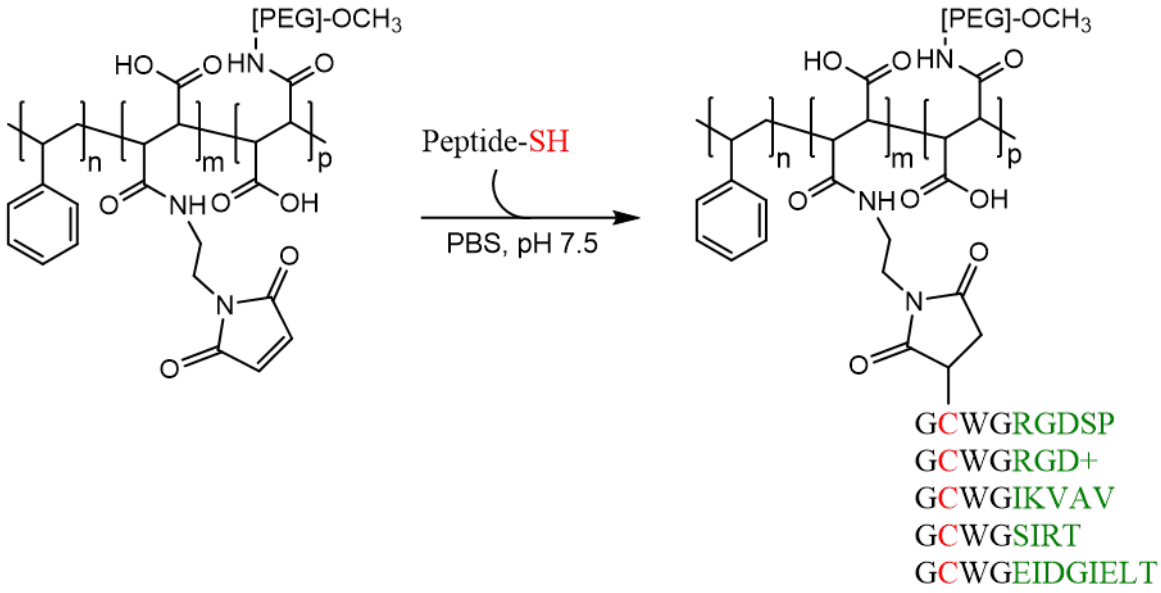
Fluorescent dyes



Fluorescent dye Atto 647N-NH_2 (Atto-TEC GmbH, Siegen, Germany) was covalently attached to the backbone of SMAnh (XIRAN® SZ, 30010 and 26120) copolymers (0.1 g, 0.014 mmol; 0.1 g, 0.9 µmol) by dissolving the SMAnh copolymers in 40 mL THF, PEG (0.369 g, 0.252 mmol; 0.312 g, 0.213 mmol) in in a mixture of 20 mL of THF and MilliQ (3:1, v/v) and Atto 647N-NH_2 (1.3 mg, 1.4 µmol; 0.09 mg, 0.09 µmol in 1 mL THF. The Atto 647N-NH_2 solution was combined first with the SMAnh solution, *N,N*-Diisopropylethylamine (DIPEA) (5.3 mg, 42.9 µmol; 0.36 mg, 0.093 µmol) was subsequently added and after 10 min stirring at RT, the PEG solution was finally added to the reaction mixture. The reaction mixture was stirred for 5 days at RT and purified by dialysis. The final product was freeze-dried by lyophilization as described above (yield: 92 %; 89 %).

Sidechain modifications (bioactives)

Peptides

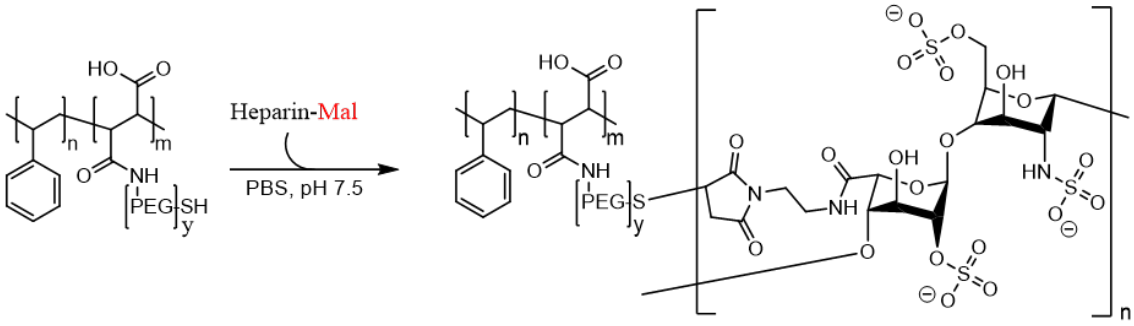


Maleimide functionalized 10-30-8-2-Mal; 350-50-22-2-Mal, 40-50-19-0.5-Mal (AP-Mal) copolymers and peptides containing a free thiol moiety were dissolved in PBS (pH 7.4). The molar excess of peptides used for this synthesis was calculated according to the amount of free maleimide moieties as determined by Ellman's test. Both solutions were combined, the pH was adjusted to 7.5, and the reaction mixture was stirred for 4 h at RT. Purification by dialysis (MWCO: 10 kDa) followed by lyophilization was performed as described above (see table 2).

Table S2. AP-peptide derivatives

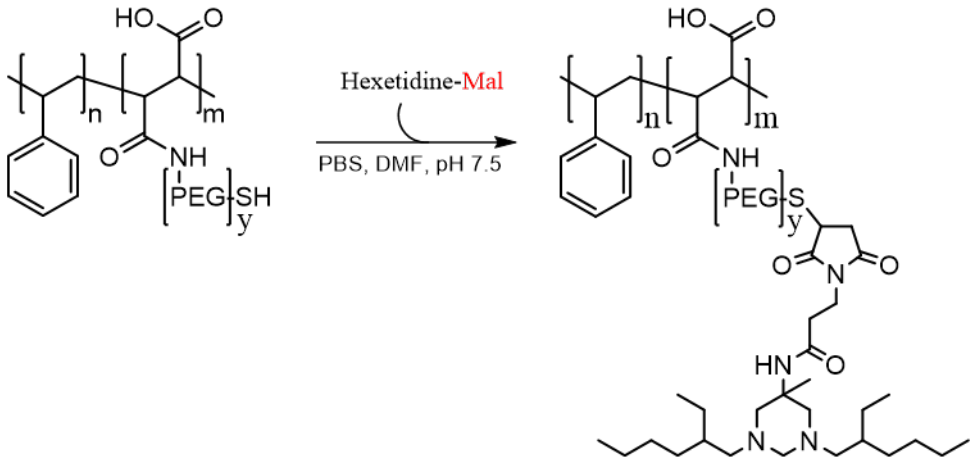
AP-Mal	Peptide	yield
(60 mg, 3 µmol)	Ac-GCWGGRGDSP-NH_2 (23.7 mg, 24 µmol)	86 %
(200 mg, 3.3 µmol)	RGD+: cyclo[RGDfC] Ac-CWGGPQVTRGDVFTMP-NH_2 Ac-GCWGRKKGRKKGRKKGRKK-NH_2 (112.8 µmol)	92 %
(20 mg, 0.9 µmol)	Ac-GCWGGSIRT-NH_2 (16 mg, 8 µmol)	72 %
(20 mg, 0.9 µmol)	Ac-GCWGGEIDGIELT-NH_2 (19.3 mg, 8 µmol)	64 %
(20 mg, 0.9 µmol)	Ac-GCWGGIKVAV-NH_2 (16.5 mg, 8 µmol)	69 %

Heparin



Heparin-maleimide1 (0.376 g, 0.025 mmol) and AP-SH (0.1 g, 0.0022 mmol) were dissolved in PBS. Both solutions were combined, the pH was adjusted to 7.5 and the reaction mixture was stirred for 4 h at RT. Purification by dialysis (MWCO = 50 kDa) as well as lyophilization was performed as described above (yield: 95 %).

Hexetidine



Hexetidine-Maleimide (0.024 g, 0.048 mmol) was dissolved in 5 mL DMF and AP-SH (0.1 g, 0.0018 mmol) was dissolved in 50 mL PBS. Both solutions were combined, the pH was adjusted to 7.5 and the reaction mixture was stirred for 4 h at RT. Purification by dialysis (MWCO = 10 kDa) as well as lyophilization was performed as described above (yield: 61 %).

Methods

High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)

HPLC-MS was carried out on a 1260 Infinity LC System coupled to an Agilent 6230 time-of-flight (TOF) LC/MS device from Agilent Technologies (Santa Barbara, USA). The analytical experiments were performed using a Luna® C-18 column (5 µm particle size, 100 Å pore size, 250 × 3 mm from Phenomenex Ltd. (Torrance, USA) over 45 min with a flow rate of 0.5 mL/min. A linear gradient of MilliQ-water/acetonitrile (AcN) and formic acid (φ_z = 0.1 %) was used as mobile phase. A UV/VIS diode array detector was used to monitor wavelengths at 210 and 278 nm. MS measurements were performed simultaneously while HPLC experiments and ionization of the analytes was achieved using electrospray ionization (ESI-MS). The ESI-MS was equipped with a dual nebulizer ion source to run an internal reference mass standard (121-922 Da) in parallel to the sample flow. The following settings were used for ESI and MS-ToF (gas temperature 325 °C; capillary, nozzle, and fragmentor voltages was set to 3500, 500, and 100 V, respectively; skimmer was set to 65 V and octopol to 750 V). The instrument state was set to high resolution mode (4 GHz). Tuning and calibration were performed before sample runs. Data collection and integration were performed using MassHunter workstation software (version B.08.00). The data was collected in the range of 100 and 2000 m/z.

Gel permeation chromatography (GPC)

GPC was performed with a PL-GPC 120 using *N,N*-dimethylacetamide (DMAc) as eluent containing 5 g/L LiBr and 1% MilliQ with a flow rate of 1 mL/min at 70 °C. For protection, a precolumn (GRAM, 8mm × 50mm, 10µm) was applied onto two GRAM 1000 columns (8mm × 300mm, 10µm)) (PSS, Mainz, Germany) for sample separation. The injection volume was 100 µL and a refractive index (RI) detector was used for detection. The system was calibrated against PMMA standards PSS (Mainz, Germany) in the range of 500 to 500,000 g/mol.

Transmission measurements

Turbidity measurements were recorded on a Spark® multimode microplate reader Tecan Group AG (Männedorf, Switzerland). The optical transmittance of the light beam was recorded at 450 nm. AP coatings with various PEGylation degrees, maleic anhydride to styrene ratios and molecular weights were analyzed using a range of AP concentrations (0.005; 0.05; 0.5; 1, 10 and 20 mg/mL) in PBS at RT. Data collection and integration were performed using the SparkControl™ software (Männedorf, Switzerland).

Absorption measurements for determination of thiol group reactivity

UV/VIS measurements were performed on a Spark® multimode microplate reader at room temperature measuring the absorption of Ellman's reagent at 412 nm for determining the thiol group reactivity of AP-SH coatings following a protocol adapted from Thermo Fisher Scientific (Waltham, USA) in a 96-well plate format.[3]

Absorption measurements for determination of maleimide group reactivity

UV/VIS measurements were performed on a Spark® multimode microplate reader at RT measuring the absorption of Ellman's reagent at 412 nm in a 96-well plate format. First, a 1 mM cysteine solution, a 0.1 mM AP-Mal and a 2 mM Ellman's reagent solution were prepared in PBS. The AP-Mal and cysteine solution were combined at different ratios (excess of added cysteine solution depends on the theoretical amount of free maleimide groups to be expected for the analyzed AP-Mal coatings) and topped with PBS for a total volume of 200 µL for each combination. The combinations and a cysteine solution without AP-Mal were mixed and incubated for 15 min at RT. A volume of 100 µL of Ellman's reagent solution and 100 µL of PBS solution were added to those solution combinations. The solutions were well mixed and incubated for another 15 min, before measuring the absorbance at 412 nm. For blank subtraction a volume of 100 µL Ellman's reagent solution was combined with 100 µL PBS to receive a total volume of 200 µL and measured. All combinations were measured as triplicates and data collection and integration were performed using the SparkControl™ software. The amount of maleimide was calculated using equation (4)

$$n_{Maleimide} = excess - (excess \times \frac{A_{polymer}}{A_{no\ polymer}}) \qquad (4),$$

with $n_{Maleimide}$ the amount of free maleimide groups coupled to the backbone of the AP, *excess* the excess amount of cysteine (in mM) in contrast to the amount of AP-Mal (in mM). The parameters $A_{polymer}$ and $A_{no\ polymer}$ correspond to the measured absorbance of the AP-Mal cysteine combinations ($A_{polymer}$) and the absorbance of the pure cysteine solution ($A_{no\ polymer}$).

Nuclear magnetic resonance spectroscopy (NMR)

^{1}H NMR spectroscopy was performed on a Bruker DRX 500 P from Bruker (BioSpin GmbH, Rheinstetten, Germany) at 500 MHz and 298 K, using D_2O and DMSO-d6 as solvents. ^{1}H NMR data are reported in ppm relative to tetramethylsilane (TMS) or to DMSO-d6 at 2.5 ppm relative to TMS. First, all PEG derivatives used for the AP synthesis were analyzed to determine the number of protons for each PEG derivative. The integral for the methoxy singlet (3.33 ppm) was normalized to 3 protons and compared to the integral for the polymer backbone (3.51 ppm). This ratio is equal to the number of protons in the backbone. To analyze the degree of PEGylation for the different AP coatings, the integral for the PEG backbone (3.51 ppm) was normalized to the determined number of protons for the different PEG derivatives and compared to the integral for the styrene signal (5.9-7.8 ppm) in a first step. Next, the number of styrene repeating units in the AP copolymers was calculated using equation (5).

$$N_{styrene} = \frac{I_{styrene}}{nH_{styrene}} \qquad (5),$$

with $N_{styrene}$ the number of styrene repeating units, $I_{styrene}$ the integral of the styrene signal and $nH_{styrene}$ the theoretical number of protons per styrene monomer (5H). Next, equation (6) was used to calculate the number of maleic acid (MA) units per PEG chain.

$$N_{MA_{PEG}} = \frac{\chi_{MA}}{\chi_{styrene}} \times N_{styrene} \quad (6),$$

with $N_{MA_{PEG}}$ the number of MA units per PEG chain, χ_{MA} the ratio of MA in the SMAnh copolymers, $\chi_{styrene}$ the ratio of styrene in the SMAnh copolymers and $N_{styrene}$ the number of styrene repeating units. The degree of PEGylation for the different AP coatings is equal to the reciprocal of $N_{MA_{PEG}}$. Data integration and analysis was performed using the ACD Labs software (version 12.0, Toronto, Canada).

FT-IR

IR measurements were performed on a FT-IR spectrometer (Vertex 80v) from Bruker Optik (Ettlingen, Germany) in attenuated total reflection (ATR) mode. The FT-IR spectra were acquired in the range from 600 to 4,000 cm^{-1}. The spectral resolution was 4 cm^{-1} and the number of scans per spectrum was 100.

Thin film preparation

QCM crystals (QSX 303 SiO_2) from Biolin Scientific (Västra Frölunda, Sweden), silicon thermal oxide wafers (thermal oxidation done by TUD, Institute of Semiconductors and Microsystems) and coverslips (13 mm diameter) from Thermo Fisher Scientific (Waltham, USA) were cleaned prior to thin film coating, using a standard Radio Corporation of America (RCA) cleaning-protocol.[4] Coverslips and QCM crystals were silanized using Hexamethylsilazane and thin film coatings were prepared using the following polymers (polystyrene (PS), 182427, Mw 280,000 g/mol; polyethersulfon (PES) Ultrason ® E 6020 P, Mw 75,000 g/mol, BASF SE, (Ludwigshafen, Germany); aliphatic thermoplastic polyurethane (TPU), Tecoflex™ SG-80A, Lubrizol, (Wickliffe, USA); polyamide (PA), VESTAMID® L – polyamide 12, (Essen, Germany). PS (1 wt %) was dissolved in toluene, PES (0.5 wt %) was dissolved in dichloromethane, TPU (0.5 wt %) was dissolved in THF, and PA (0.2 wt %) was dissolved in hexafluoro-2-propanol. Spin-coating of PS, PES, TPU and PA was performed on a Labspin6 semi-automatic spin-coater from Süss MicroTec (München, Germany)with the following settings (PS: time = 30 s, speed = 2,000 rpm, acceleration = 1,500 rpm/s, drying = 2 h at 90 °C; PES: time = 30 s, speed = 4,000 rpm, acceleration = 1,500 rpm/s, drying = 1 h at 120 °C; TPU: time = 30 s, speed = 4,000 rpm, acceleration = 1,500 rpm/s, drying = 2 h at 60 °C and PA: time = 30 s, speed = 4,000 rpm, acceleration = 1,500 rpm/s, drying = 2 h at 60 °C). Low density polyethylene (LDPE), ET 311301, Goodfellow (Coraopolis, USA) was dissolved in toluene (1 wt %) at 72 °C and dip coated at 72°C at a velocity of 100 mm/min using a dip coater approach.

Quartz Crystal Microbalance with dissipation monitoring (QCM-D)

Measurements were carried out on a QSense E4 (Biolin Scientific, Sweden) using precoated (PS, PES, TPU, LDPE, PA) QCM crystals. The crystals are characterized by a fundamental resonance frequency of f_0 = 5 MHz and a sensitivity constant of C = 0.177 mg $(m^2\ Hz)^{-1}$. Measurements were performed for all coatings using a mass concentration of 1 mg/mL and additional, measurements were repeated for PS precoated wafers using constant molar concentrations for various concentration ranges. The polymer-free solution (PBS) was first flushed through a flow-cell to establish a stable baseline; then the PBS was switched to the polymer solution, which was flown (flow rate = 0.1 mL/min) until a stable baseline was established. The polymer solution was exchanged by PBS in order to displace loosely bound polymer chains. For protein adsorption measurements BSA solution (4 wt.%) in PBS was flown (flow rate = 0.1 mL/min) until a stable baseline was established. The BSA solution was exchanged afterwards by PBS in order to displace loosely bound proteins. We measured the polymer and protein adsorption for the 3rd to 11th overtone of the fundamental frequency at 20 °C. Data collection and analysis were performed using QSense Dfind software to calculate film thickness and mass with the Sauerbrey model[5] (Biolin Scientific, Sweden). Additionally to consider viscoelastic properties, measured frequency and dissipations shifts were fitted to the Johannsmann model using the QTE software and corresponding layer thicknesses were calculated.[6, 7] Each AP (various PEGylation degrees, maleic anhydride to styrene ratios, molecular weights and bioactive modifications) was measured at least eight times.

Contact Angle

Contact angle measurements were performed on an OCA30 (Data Physics Instruments GmbH, Filderstadt, Germany). Static and dynamic angles were measured on PS, PES, TPU coated silicon thermal oxide wafers using a droplet of degassed deionized water with volume of 20 µL. All measurements were conducted as duplicates for each surface.

Atomic force microscopy (AFM)

AFM-based force spectroscopy measurements were used to determine the repulsive forces exerted by different APs immobilised on PS substrates on an approaching object. Prior to the measurements, the substrates were equilibrated in MilliQ water for 10 min at 22 °C and then analyzed using a NanoWizard IV AFM (JPK Instruments, Berlin, Germany) mounted on an inverted light microscope (Axio Observer D.1, Zeiss, Jena, Germany). For the measurements, tipless triangular cantilevers (PNP-TR-TL-Au, Nanoworld) were modified with a 10 µm silica bead (Kisker Biotec GmbH, Steinfurt, Germany) as described. First, cantilevers were calibrated before measurements using the equipartition theorem. Second, force spectroscopy was performed with a set point of 4 nN and approach/retraction velocity of 500 nm/s. During measurements, the temperature was controlled using a PetriDishHeater (JPK Instruments) sample chamber. For each sample at least nine different locations on the sample surface were probed. The repulsion distance is defined as the distance from the point at which the approaching AFM cantilever is first deflected from its neutral position to the point at which the

colloid sample at the cantilever comes into hard contact with the substrate (S8). The repulsion distance was extracted from the approach force−distance curves using routines implemented in the data analysis software provided by the AFM manufacturer (JPK Instruments).

Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

A ToF-SIMS 5-100 instrument from IONTOF (Münster, Germany) equipped with a 30 kV Bi liquid metal ion gun was used to provide a semi-quantitative proof for the immobilization of anchor molecules on polystyrene surfaces. Spectra were acquired in the Bi3++ mode in the mass range m/z 0-913. The area of analysis was 250 × 250 μm^2 which was scanned randomly in a pattern of 128 × 128 pixels. 50 scans with positive polarity were accumulated per spectrum. The mass scale of the obtained spectra was calibrated against a list of reference peaks (SurfaceLab7, IONTOF). The signal of $C_2H_5O^+$ at m/z = 45.034 was considered as an indicator for successful immobilization. It is highly characteristic for the poly(ethylene glycol) chain in the anchor molecules (SurfaceLab7 database) and not expected for clean polystyrene.

Fluorescence measurements

Coating stability

To measure the stability of AP coatings on different model surfaces, in different media and under realistic conditions for up to 2 weeks, precoated (PS, PES, TPU, LDPE, PA) coverslips (13mm diameter) were used. Precoated coverslips were incubated with AP-Atto 647N copolymers by adding droplets (V = 300 µL) from an AP-Atto 647N solution (c = 1 gL^{-1}), dissolved in PBS, for 1 h on top of the precoated coverslips. After 1h incubation the AP-Atto647N solution was removed from the coverslips, coverslips were rinsed twice with PBS and MilliQ, dried under a flow of nitrogen and transferred to a 24 well plate (TPP tissue culture plates, Merck KGaA, Darmstadt, Germany). A volume of 1 mL PBS including ProClin™ 950 (0.1%, v/v), was added to every well on top of the coverslips to remove unbound AP. The well plate was incubated for 24 h at 37 °C on a plate shaker. The PBS solution was removed and the wells were washed twice with PBS and MilliQ, before measuring the fluorescence intensity using a FLA 5100 fluorescence scanner from Fujifilm (Minato, Japan) at t = 0 h. After performing the initial fluorescence measurement, the polymer samples were incubated in one of the following solutions (1mL total volume): PBS, Tris-HCL (50 mM) + 4 % Bovine Serum Albumin (BSA), citrated plasma (sodium citrate dihydrate 0.14 M; Haema AG; Germany) and Tween20 (4 mM). Additionally, ProClin™ 950 (0.1% v/v) was added to every solution to avoid bacterial contaminations. Fluorescence measurements were performed as triplicates for all model surfaces and repeated at 24 h, 48 h, 72 h and 168 h. For the time of the measurements all well plates were incubated at 37 °C on a plate shaker. Data collection and integration were performed using the multi gauge software (version 3.1 from Fujifilm (Minato, Japan). Measured intensities were normalized to values between 0 and 100 %. Hereby, the measured fluorescence values after 24 h (t = 0 h) washing in PBS were set to 100 %. Further, intensities measured after t = 24 h, t = 48 h, t = 72 h, t = 168 h and t = 336 h were transformed to relative values using equation (7).

$$rF(t) = \frac{F_{t=i}}{F_{t=0}} \times 100\,\% \qquad (7),$$

with rF the relative fluorescence intensity in %, $F_{t=i}$ the fluorescence intensity at different timepoints i and $F_{t=0}$ the initial fluorescence intensity measured directly after 24 h washing in PBS. Each AP (various PEGylation degrees, maleic anhydride to styrene ratios, molecular weights) was measured at least five times.

Protein adsorption

For fluorescence protein adsorption experiments, bovine serum albumin (BSA) was conjugated with ATTO 565 NHS ester according to the manufacturer instructions. The average labelling degree for BSA ATTO 565 was 0.7 mol of dye per mol of protein. For protein adsorption, the anchor polymer surfaces were immersed in solutions of BSA ATTO 565 at a concentration of 100µg/mL in PBS. ATTO 565 were excited with laser lines at 561nm. After 1 h adsorption, the anchor polymer surfaces fluorescence intensities were measured using a Leica confocal laser scanning microscope SP5 (Leica Microsystems, Germany) equipped with a 40×, NA1.25 oil immersion objective (Leica) and image frequency of 400 Hz and 512×512 pixels with a six frames average per image. The adsorption behavior (fluorescence intensity measurements) was analyzed at time points 0, 1 and 4 hours for all surfaces, each data set is from two independent samples and at least 6 random spots for each surface. The image analysis was done using the FIJI plugin Measure stack.[8] The fluorescence intensities were normalized to the control surfaces at each time point.

Coating displacement

Displacement of AP coatings on different model surfaces was performed in PBS and up to 2 weeks on precoated (PS, PES, TPU, LDPE) coverslips (13mm diameter). The coverslips were incubated with AP-Atto 647N copolymers by applying droplets (V = 300 µL) of an AP-Atto647N (10-30-16-2- Atto647N; 120-30-13-2- Atto647N) solution in PBS for 1 h on top of each precoated polymer surface. Four different displacement experiments (homo and hetero exchange) were performed using 2 different conditions (constant molar concentration and constant mass concentration) for each experiment. 10-30-16-2-Atto647N and 120-30-13-2-Atto647N were used to coat the model surfaces and as displacement solutions we used solutions containing AP 10-30-16-2 and 120-30-13-2. As described above measured fluorescence intensities were normalized to values between 0 and 100 %. Hereby, the measured fluorescence values after 24 h (t = 0 h) washing in PBS at 37 °C were set to 100 %. After performing the initial fluorescence measurement, a volume of 1 mL of displacement solution was added on top of each coverslip. For the time of the measurements all well plates were incubated at RT. Further, intensities measured after t = 24 h, t = 48 h, t = 72 h, t = 168 h and t = 336 h were transformed to relative values using equation (7). Each AP displacement combination was measured at least nine times.

Cell culture experiments

Human umbilical vein endothelial cells (HUVECs) were purchased from Promocell, (Heidelberg, Germany) and maintained on fibronectin-coated 75 cm^2 culture flasks in Endothelial Cell Growth Medium (Promocell, Heidelberg, Germany) containing a supplement mix with 2% FCS and 1 % penicillin-streptomycin at 5% CO_2 and 37°C in a humidified incubator.[9] After reaching 80% of confluency, the cells were detached (using 0.5% trypsin-EDTA solution), collected, centrifuged at 150 g, and reseeded at appropriate cell densities until further use. Cells from passage 2-6 were used for all experiments on AP coated non-treated PS Falcon® 24-well plates (Thermo Fisher Scientific Inc., Watham, USA).

The human induced pluripotent stem cell line CRTD3 was derived at the stem cell and engineering facility at the Center for Regenerative Therapies, Dresden (CRTD) at the TU Dresden and generously provided for this work. The cells were routinely maintained on hESC-qualified Matrigel™ (Corning Inc., Corning, USA)-coated 6 well plates (TPP, Trasadingen, Switzerland) in mTeSR™1 medium (Stem Cell Technologies Inc., Seattle, USA) at 5% CO_2 and 37°C in a humidified incubator. The medium was changed daily. After reaching 80-90% confluency, the cells were detached as aggregates using the non-enzymatic reagent ReLeSR™ (Stem Cell Technologies Inc., Seattle, USA), and reseeded at appropriate cell densities until further use.
For cytometry analyses, the cells were passaged as single cells using TrypLE for 3 min at 37°C. The cells were resuspended in DMEM/F12 (Gibco), centrifuged (1000 rpm, 3min), counted and resuspended in PBS+5% FBS at a cell concentration of $3x10^6$ cells /ml.

Preparation of AP coatings for cell culture

To prepare AP coated tissue culture cell plates for HUVECs, a volume of 0.5 mL (0.001-0.1 wt. %) of AP coatings (adhesive and non-adhesive coatings, bioactive modified) dissolved in PBS containing 1 % penicillin-streptomycin was added into each well. The well plates were incubated for 1 h at RT under a laminar flow hood. The polymer solution was removed and the wells were rinsed three times with sterile PBS to remove any unbound AP. Subsequently, 1 mL suspension of HUVECs (5×10^4 cells per mL) in cell culture medium (Endothelial Cell Basal Medium, PromoCell, Germany) containing 1 % penicillin-streptomycin and 2 % FCS was added to the AP coated wells. Cells were cultured at 5 % CO_2 and 37 °C.

To prepare AP coated plates to culture undifferentiated hiPSCs, a volume of 1ml (0.1 wt. %) of AP-RGD+-containing peptide-conjugates dissolved in PBS was added into each well of a non-treated PS 6 well plate (Thermo Fisher Scientific Inc., Watham, USA). The well plates were incubated for 1 h at RT in a laminar flow hood. The polymer solution was removed and the wells were rinsed three times with sterile PBS to remove unbound AP. Subsequently, 1ml mTeSR™1 medium was added and the plates were kept in the cell incubator for immediate use or in PBS at 4°C for later use. The cells were added after passaging and cultured as described above.

Live/Dead cell staining and viability assay

After removal of the medium and washing with sterile HBSS (Hank's balanced salt solution, Thermo Fisher Scientific, Waltham, USA), the cells were stained with 3 µM Calcein-AM and 2.5 µM propidium iodide (PI, both Thermo Fisher Scientific, Waltham, USA) in HBBS for 30 min at 37°C before analysis by light microscopy on a Zeiss Axio Observer microscope (Carl Zeiss AG, Oberkochen, Germany). The number of dead, PI^+ cells was determined using ImageJ.[8]
To assess cell viability Prestoblue cell viability reagent (Thermo Fisher Scientific, Waltham, USA) according to manufacturer's instructions. In brief, cells were incubated for 24 h before adding 10% Prestoblue reagent to the medium. After 1h incubation at 37°C, the fluorescence intensity (excitation 535 nm and emission 590 nm) was measured on a Tecan Spark plate reader (Tecan, Männedorf, Switzerland). Results were obtained from two independent experiments.

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 20 min at RT, followed by quenching (1xPBS, 100 mM Glycine, 0.3% Triton X-100) and blocking (1x PBS, 0,5% BSA, 0.3% Triton X-100). Immunostaining was performed in blocking buffer and each antibody reaction was done overnight at 4°C. The primary antibodies and their working dilutions were as follows: mouse anti OCT3/4 (1:200, BD Biosciences, Franklin Lakes, USA); mouse anti SOX2 (1:200, R&D Systems, Minneapolis, USA). After washing with PBS + 0.3% Triton X-100 the cells were incubated with the secondary antibodies (each 1:200; donkey anti mouse Alexa Fluor 488 and donkey anti mouse Alexa Fluor 546) as well as Alexa Fluor™ Plus Phalloidin 405 (1:40) and Draq5 (1:200; all reagents from Invitrogen, Thermo Fisher Scientific Inc, Watham, USA) in blocking solution overnight at 4°C. After washing with PBS the cells were imaged at an Andor Dragonfly spinning disk confocal microscope (Oxford Instruments, Abindon, UK). Results were obtained from two independent experiments.

F-Actin cell staining

After removal of the medium and washing with sterile PBS with Mg^{2+} and Ca^{2+}, the cells were fixed with 2% paraformaldehyde in PBS for 15 min and permeabilized with 0.1% Triton-X100 for 10 min. Staining was performed with 50 nM Phalloidin- Atto55 (AttoTec, Siegen, Germany) and 1 µg/ml Hoechst 33342 (Thermo Fisher Scientific, Waltham, USA) for 30 min at RT before analysis by light microscopy on a Zeiss Axio Observer microscope. Results were obtained from three independent experiments.

Cytometry analysis

For cell surface stainings, the cells were stained with TRA-1-60-FITC and SSEA4-PE (human iPS selection kit, SCR502) from Merck KGaA (Darmstadt, Germany), at room temperature in

the dark, washed with PBS + 5% FBS + 0.03% NaN_3 and analyzed at the Fortessa (Becton Dickinson). As controls cells were incubated with the corresponding isotype controls.
For intranuclear stainings, the cells were fixed and permeabilized using the Fixation/Permeabilization Solution Kit (BD Biosciences) according to the manufacturer's protocol and were stained with OCT3/4-488 (BD Biosciences) and NANOG-647 (BD Biosciences). As controls cells were incubated with the corresponding isotype controls.
All samples were analyzed using a BD Fortessa Flow Cytometer. Results were obtained from two independent experiments.

Displacement of HUVECs

To displace adherent cells from the AP surface, 1 mL medium with varying concentrations of AP (up to 5g/L) was added to the wells. The cell number of adherent cells was determined using light microscopy after 24 h and 72 h of incubation. At every time point, the medium was removed, the wells were rinsed three times with sterile PBS before adding fresh displacement medium. AP coatings were tested with three independent samples per experiment. Each experiment was performed at least three times.

Cell morphology of HUVECs

To determine cell spreading on the different AP coating, HUVECS were cultured in heparin-free cell culture medium (MV2, PromoCell, Germany) containing 1 % penicillin-streptomycin and 5 % FCS at 5 % CO_2 and 37 °C for 72 h. Cell spreading was analyzed using cell profiler by threshold-based identification of nuclei and propagation of cell body from these seed points.[10] Subsequently, the cell shape of cell bodies with less than three neighbor cells was determined. AP coatings were tested with three independent samples per experiment. Each experiment was performed at least three times.

Ligand densities (bioactives)

Ligand densities of different bioactives were calculated according to equation (8).

$$n_{bioactive} = \frac{m_{AP}}{Mw_{AP}} \times \chi_{MA} \qquad (8),$$

with $n_{bioactive}$ the surface densities of various bioactives (expressed as amount of substance of bioactive per unit area), m_{AP} mass of AP adsorbed per unit area, Mw_{AP} molecular weight of APs and χ_{MA} the ratio of MA in the AP copolymers. Hereby, m_{AP} was calculated from QCM-D measurements (using the QSense Dfind software to calculate film thickness and mass with the Sauerbrey model). To calculate the bioactive density of RGDSP peptides per unit area equation (8) was converted into equation (9).

$$n_{RGDSP} = \frac{m_{AP}}{Mw_{AP}} \times 0.5\chi_{MA} \quad (9),$$

with n_{RGDSP} the surface density of RGDSP peptides (expressed as pmol of RGDSP per cm^2), m_{AP} mass of AP 10-30-8-2-RGDSP (3.2×10^5 pg/cm^2) adsorbed per unit area, Mw_{AP} molecular weight of AP 10-30-8-2-RGDSP (31169 g/mol) and $0.5\chi_{MA}$ (8.7) the ratio of MA in the AP 10-30-8-2-RGDSP multiplied by 0.5 (50 % PEGylation; 50% Peptide). Hereby, mAP was calculated from QCM-D measurements (using the QSense Dfind software to calculate film thickness and mass with the Sauerbrey model).

Bacteria experiments

Solution-based assay

We prepared overnight bacterial cultures of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* W3110 from a single colony picked from a Lysogeny broth (LB) agar plate in LB at 30°C and 220 rpm. The next morning, we diluted the overnight culture 1:1,000 in 100 mL fresh LB. The culture was grown at 37°C and 220 rpm to an OD~0.1. At OD~0.1, we added 2mL of the bacteria culture on glass slides (containing AP coatings equipped with anti-adhesive or antiseptic function) in 6-well plates. The samples were incubated for 1h at 37°C without shaking. The LB medium was removed and the glass slides were gently washed in PBS (Sigma-Aldrich). The glass slides with pre-adsorbed bacteria were placed into 6-well plates containing fresh medium (M9 depleted medium, consisting of modified and supplemented M9 with 2% casein hydrolysate broth and 0.4% glucose). The samples were imaged at time points 0 h, 1 h, 4 h and 24 h using phase contrast microscopy with a Zeiss Axiovert 200 inverted microscope (Zeiss, Jena, Germany) equipped with an Axiocam 503 mono CCD (Zeiss, Jena, Germany) and a Zeiss EC Plan-Neofluar 40×/0.75NA objective (Zeiss, Jena, Germany). In between measurements, the samples were incubated at 37°C without shaking. Before each measurement, the M9 depleted medium was removed and the sample surfaces gently washed in PBS. The raw data images were processed with a bandpass filter, thresholded and analysed using the ImageJ 'analyse particle' function to extract total bacterial coverage (area) with a minimum feature size of a single *S. aureus* cell (approximately 0.75 μm^2) or *E. coli* cell (approximately 1 μm^2) of at least 10 images per sample condition with two independent samples per experiment.[8] Each experiment was performed at least three times.

Bacteria Direct Contact Experiments

We casted 1.5% LB agar plates (Falcon). We adapted a previously described protocol.[11] We prepared overnight bacterial cultures of *Staphylococcus aureus* ATCC 25923 from a single colony in LB at 30°C and 220 rpm. The next morning, we diluted the overnight culture 1:1,000 dilution in 1 mL fresh LB. The culture was grown at 37°C and 220 rpm to early or mid-exponential growth phase. At OD~0.2-0.3, we diluted the culture to OD~0.02 (~10^5 CFU/mL). 5 µL of this solution were applied on 2 cm sample diameter glass slide (control PS and equipped

with AP containing a hexetidine functionality) and spread homogeneously over the surface using a cell scraper (Sarstedt). The bacteria solution was dried for 10 min at RT to remove excess liquid and incubated for t = 0, 1, 4, and 24 hours at room temperature. At the indicated time points, the glass slides were flipped and gently pressed on 1.5% LB agar plates for 10 sec and then removed. The LB agar plates were grown at 37°C overnight and images of the colonies were taken with a smartphone camera. The CFUs were counted using the ImageJ plugin cell counter.[8]

Statistical Analysis

Statistical analysis was performed in Origin 2020. One-way analysis of variance (ANOVA) followed by a post hoc Tukey's multiple comparisons test was carried out for group differences. Significance is indicated by $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). All results are displayed as mean ± standard deviation.

Table S3. Basic compositional parameters of the developed AP platform and respective parameter ranges

back-bone	MW_{bb}[a] [kDa]	MA[b] [%]	P_{EG}/S[c]	MW_{PEG}[d] [kDa]	AM[e]	CI[f]	CI[g]	Fl.-dye[h]
1	10	10	3;6	2	-	-	-	-
	10	10	14	5	-	-	-	-
	10	10	58	20	-	-	-	-
2	10	30	8; 16	2	***HEX***	***RGDSP, EIDGIELT; SIRT; IKVAV***	***HEP***	***647N***
	10	30	20; 40	5	-	-	-	-
	10	30	84	10	-	-	-	-
	10	30	165	20	-	-	-	-
3	80	30	6	2	-	-	-	-
	80	30	32	5	-	-	-	-
4	120	30	6	2	-	-	-	***647N***
	120	30	33	5	-	-	-	-
5	40	50	19	0.5	-	***RGD+***	-	-
	350	50	22; 45	2	***HEX***	***RGDSP***	-	-
	350	50	58; 115	5	-	-	-	-
	350	50	238	10	-	-	-	-
	350	50	470	20	-	-	-	-

[a] molecular weight of polymer backbone; [b]maleic acid content of polymer backbone, styrene content (S): 100 – MA.; [c] ratio PEG to styrene units; [d]molecular weight of the PEG chain; [e]antimicrobial bioactive: hexetidine (***HEX***); [f]cell-instructive bioactives: peptides (***RGDSP, RGD+; EIDGIELT; SIRT; IKVAV***); [g]cell-instructive bioactives; heparin (***HEP***); [h]fluorescence dye (Atto-647N)

Table S4. Calculated brush vs. mushroom regime and desorption threshold values for the developed AP platform after Sartori et al.

MW_{bb}[a] [kDa]	MA[b] [%]	P_{EG}/S[c]	MW_{PEG}[d] [kDa]	N_B[e]	N_{MA}[f]	N_{PEG}[g]	$N_B^{\frac{5}{6}}$[h]	$N_B^{\frac{11}{6}}$[j]
10	10	6	2	38.7	4.8	215	21	814
10	10	14	5	38.7	4.8	541	21	814
10	10	58	20	38.7	4.8	2172	21	814
10	30	16	2	50.8	17.4	783	26	1342
10	30	165	20	50.8	17.4	7899	26	1342
40	50	19	0.5	198	198	2257	82	16239
80	30	6	2	546.2	154.1	6933	191	104364
80	30	32	5	546.2	154.1	17410	191	104364
120	30	6	2	815.6	230	10352	267	217615
120	30	33	5	815.6	230	25944	267	217615
350	50	45	2	1730.9	1730.9	77889	500	864609
350	50	115	5	1730.9	1730.9	195589	500	864609
350	50	238	10	1730.9	1730.9	392908	500	864609
350	50	470	20	1730.9	1730.9	785817	500	864609

[a)] molecular weight of polymer backbone; [b)]maleic acid content of polymer backbone, styrene content (S): 100 – MA.; [c)] ratio PEG to styrene units; [d)]molecular weight of the PEG chain; [e)] number of styrene repeating units per AP; [f)] number of maleic acid repeating units per AP; [g)] number of PEG repeating units per AP. [h)] brush vs. mushroom regime; [j)] desorption threshold

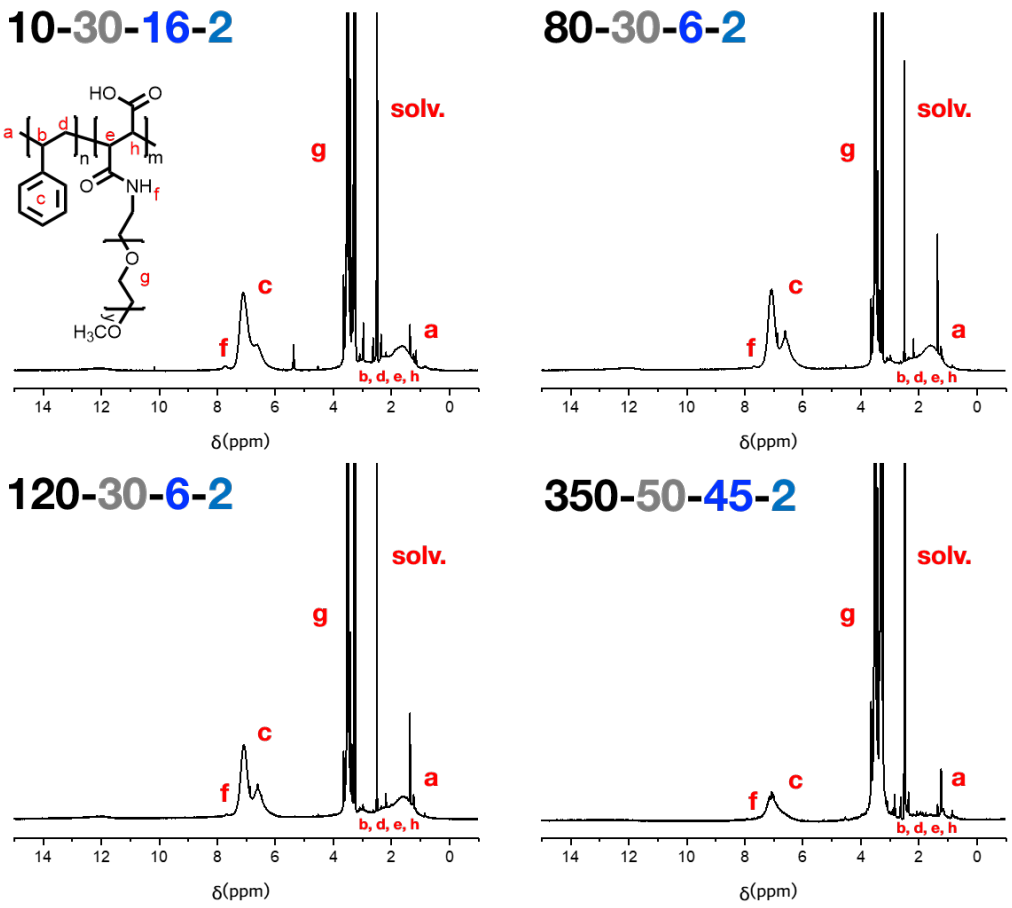


Figure S1. Representative ^{1}H NMR spectra of 4 different APs (10-30-16-2; 80-30-6-2; 120-30-6-2 and 350-50-45-2).

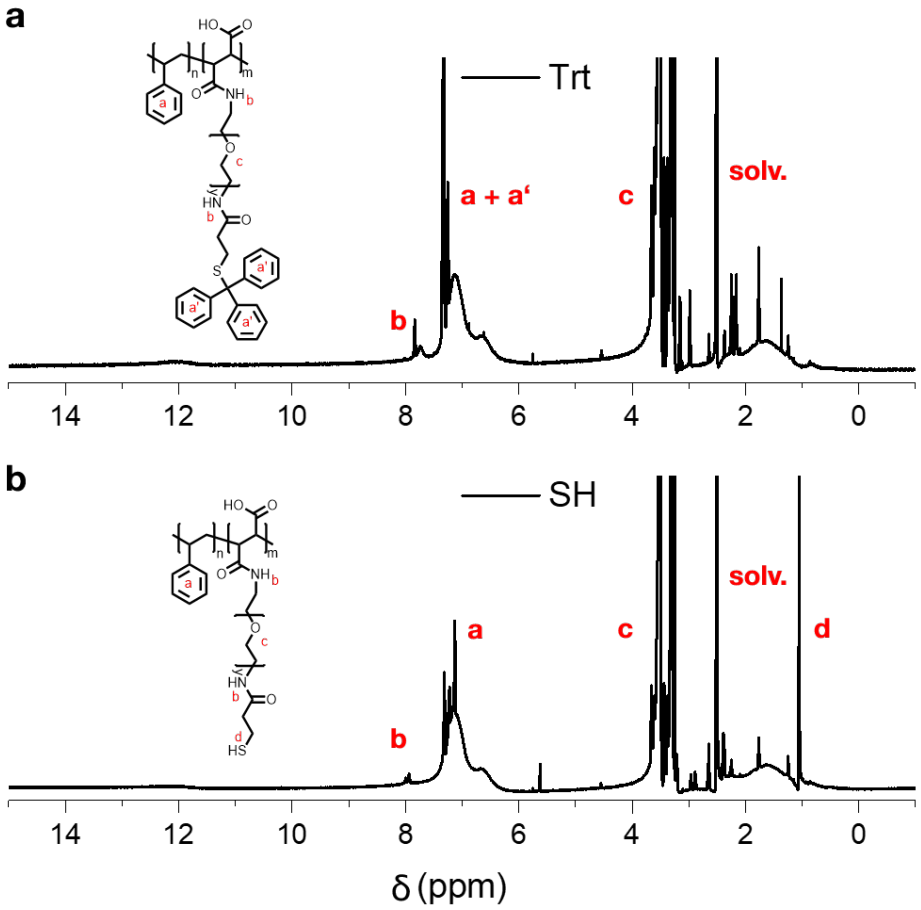


Figure S2. ^{1}H NMR spectra showing AP-SH before (a) deprotection and after (b) deprotection of Trt protection group.

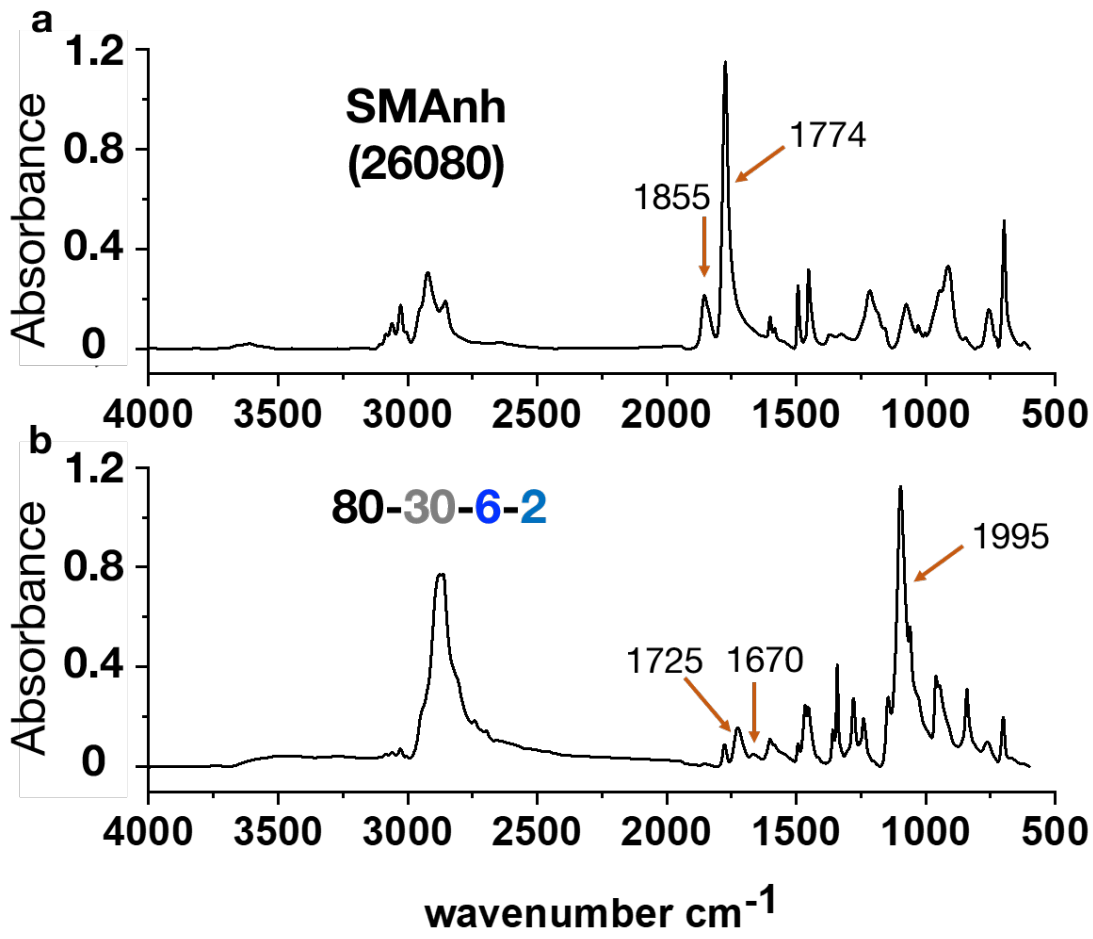


Figure S3. Representative FT-IR spectra showing successful PEGylation of SMAnh copolymers. (a) SMAnh before PEGylation. The symmetric C=O stretching absorption band (1774 cm^{-1}) and the asymmetric one (1855 cm^{-1}) of the anhydride are indicated. (b) SMAnh after PEGylation (80-30-6-2). The Amide II absorption band (1670 cm^{-1}), the C=O stretching band of the protonated carboxyl group (1725 cm^{-1}) and absorption band corresponding to the coupled PEG (1995 cm^{-1}) are indicated.

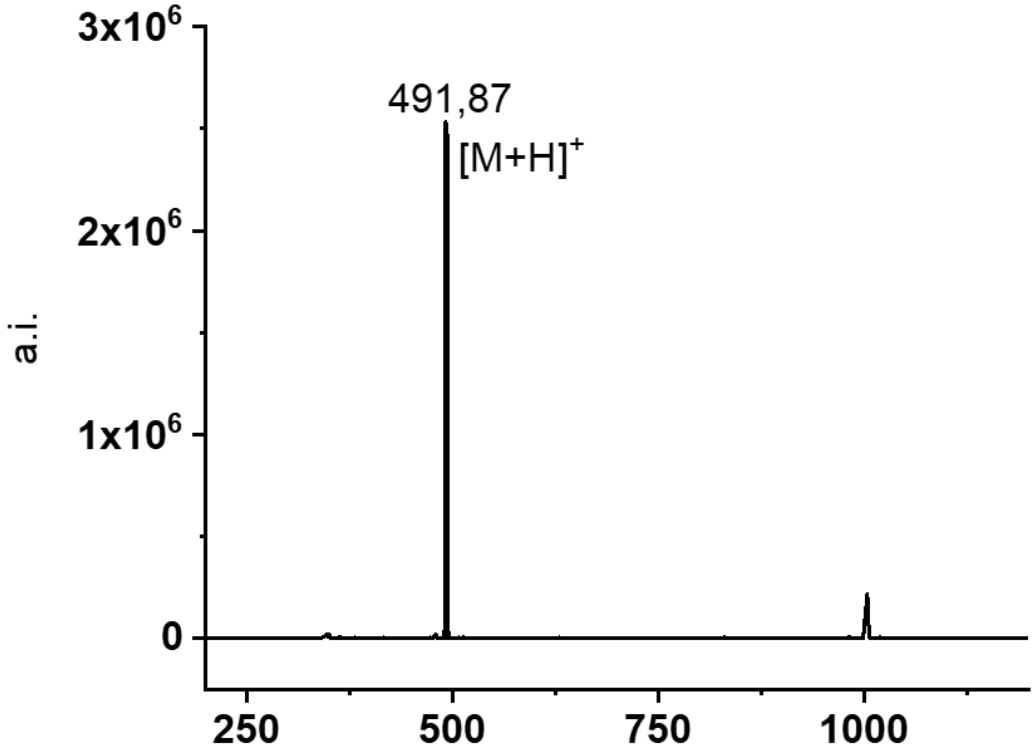


Figure S4. ESI-ToF MS spectrum of Hexetidine-Maleimide

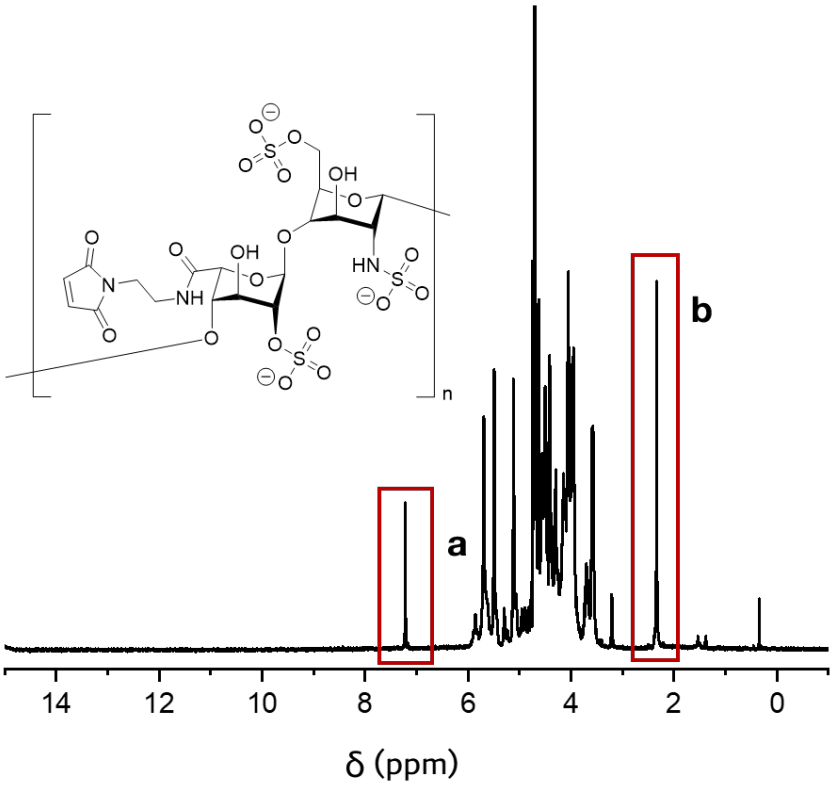


Figure S5. ^{1}H NMR spectra of Heparin-Maleimide1. The degree of maleimidation was determined via comparing the intensities of signals of the maleimide group (a) at δ= 7.3 (2H) to the heparin's N-acetyl (b) peak at δ= 2.4 (3H).

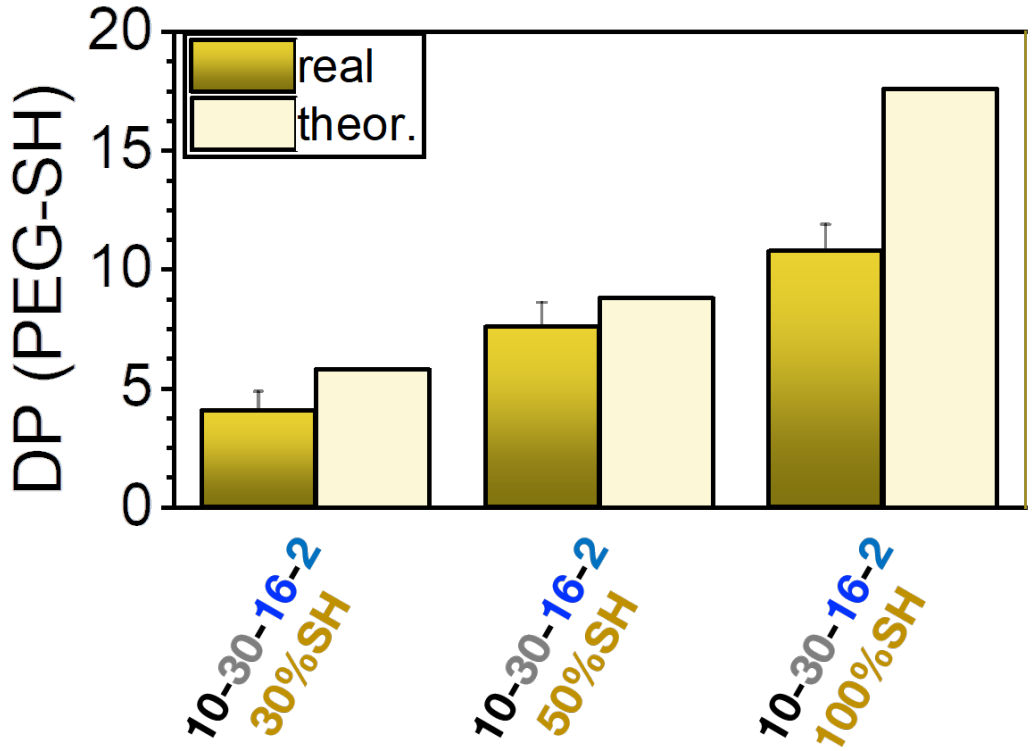


Figure S6. AP 10-30-16-2 equipped with different amounts of accessible SH moieties shown as degree of polymerization (DP) of PEG-SH units verified via Ellman's reagent.

surface	advancing water contact angle (°)	receding water contact angle (°)	Average surface roughness (nm)
PS	89.8 ± 0.7	75 ± 0.9	0.3 ± 0.03
PS + 10-30-16-2	80.3 ± 1.8	31.9 ± 2.1	0.5 ± 0.06
PS + 10-30-165-20	77.9 ± 1.6	36 ± 4.6	-
PES	78.3 ± 0.4	38.4± 5.2	2.1 ± 0.7
PES + 10-30-16-2	67.2 ± 1.9	26.2± 2.2	5.5 ± 0.8
PES + 10-30-165-20	59.9 ± 2.3	22.9 ± 3.0	-
TPU	82.9 ± 2.0	51.8 ± 0.8	2.1 ± 0.1
TPU + 10-30-16-2	72.7 ± 2.2	43.3 ± 2.6	2.9 ± 0.7
TPU + 10-30-165-20	70.9 ± 2.4	45.5 ± 1.8	-

Figure S7. Dynamic contact angles of droplets of water on AP (10-30-16-2; 10-30-165-20) coated and uncoated polymeric surfaces (PS; PES and TPU) + average surface roughness measured via AFM.

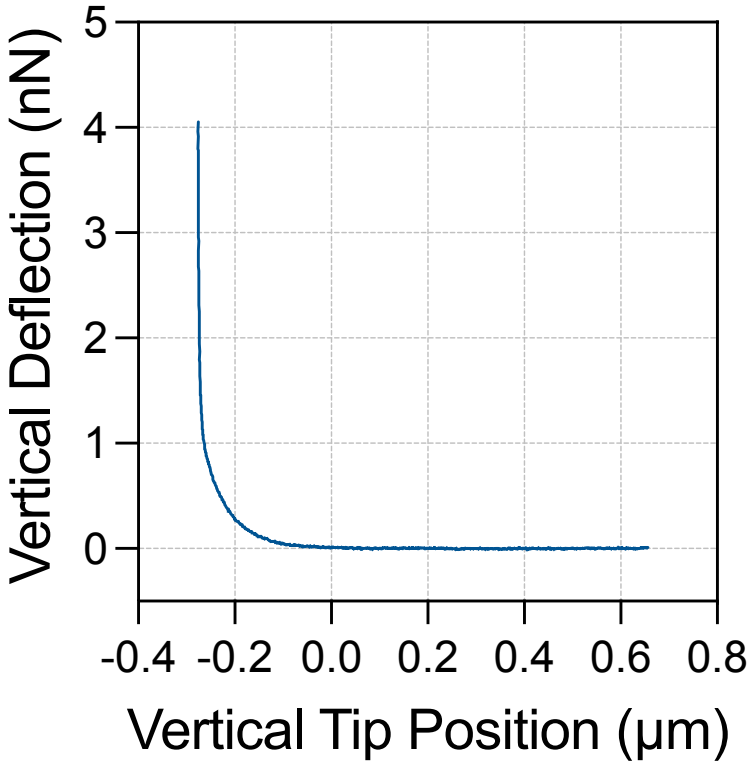


Figure S8. Example force curve of AFM-based force spectroscopy measurements

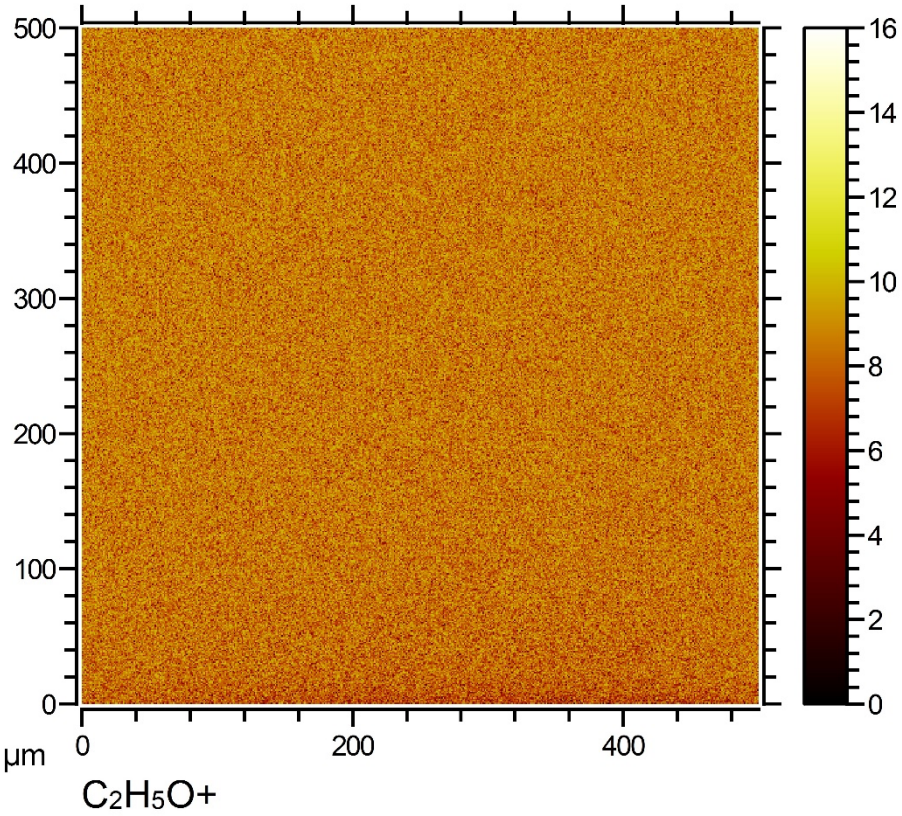


Figure S9. ToF SIMS produced heightmap based on the ion intensity signal of $C_2H_5O^+$ at m/z = 45.034 from 10-30-16-2 coated PS surfaces.

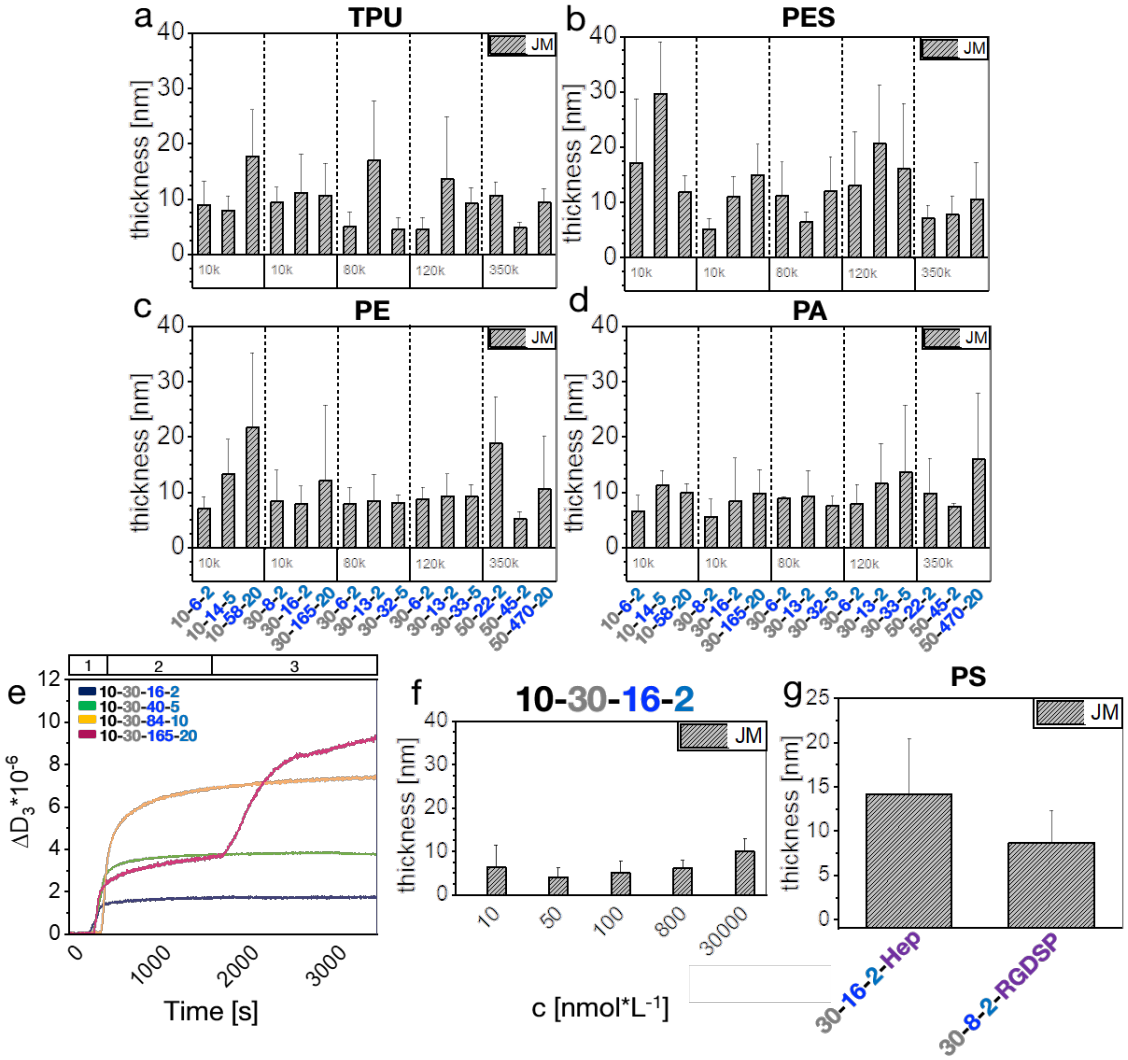


Figure S10. QCM-D-based adsorption measurements. Layer thicknesses are calculated from Johannsmann (JM) based viscoelastic model. (a, b, c, d) Adsorption measurements of APs (c = 1 gL-1) dissolved in PBS on PS, PES, TPU, PE and PA substrates plotted in regard to increasing P/S ratios. APs with the identical backbone molecular weights are grouped. (e) Dissipation shifts of four different APs plotted as a function of time. Corresponding experiments were performed in three consecutive steps: the polymer-free solution (PBS) was first flushed through the flow-cell to establish a stable baseline (1); then the PBS was exchanged to the polymer solution (2), which was flowed until it was replaced again by the PBS (3) in order to wash away the loosely adsorbed polymer chains. (f) Adsorption measurements of AP 10-30-16-2 on PS dissolved in PBS using a broad concentration range (c = 10 – 30000 nmol L^{-1}). (g) Adsorption measurements of Heparin (10-30-16-2-Hep) and RGDSP (10-30-8-2-RGDSP) modified AP on PS.

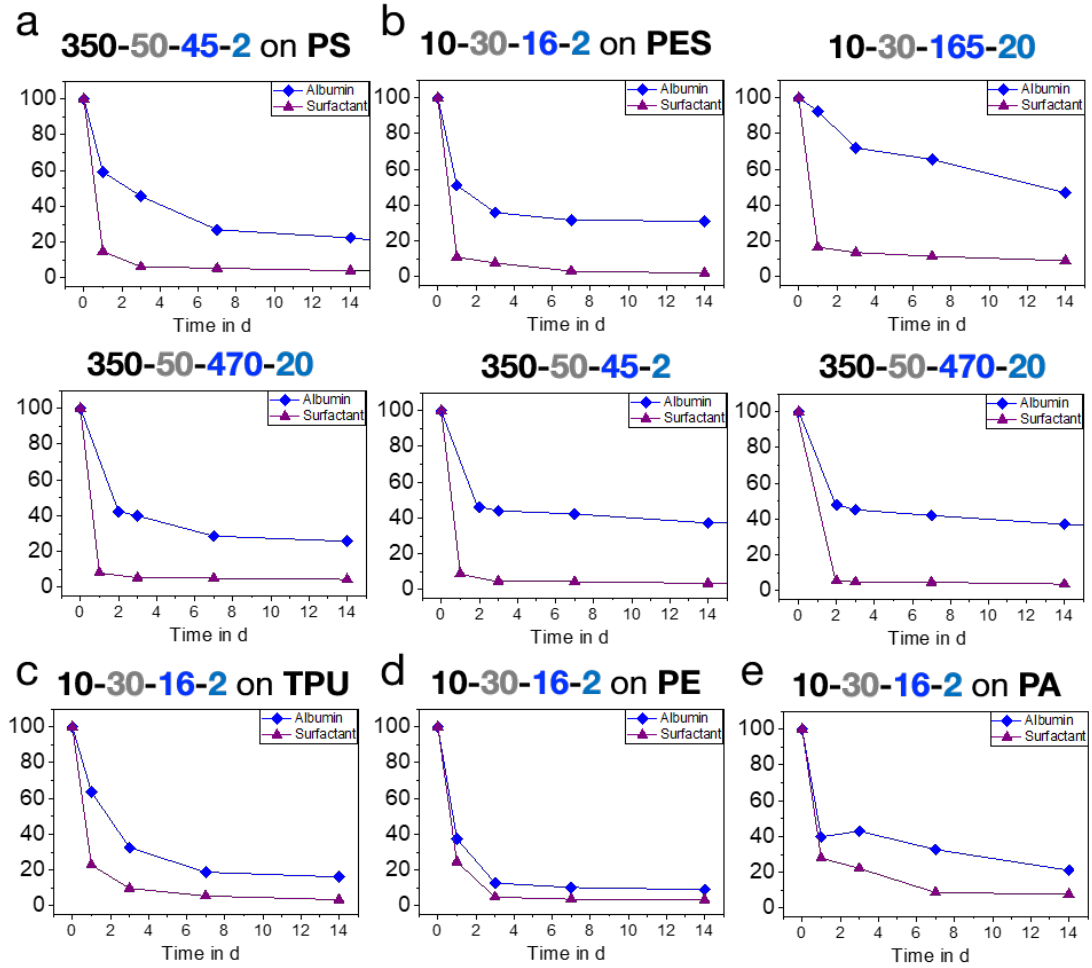


Figure S11. Kinetics of fluorescently labelled AP coatings on PS (a) 350-50-45-2, 350-50-470-20), PES (b) 10-30-16-2, 10-30-165-20, 350-50-45-2, 350-50-470-20; TPU (c) 10-30-16-2; PE (d) 10-30-16-2 and PA (e) 10-30-16-2 substrates incubated under application relevant conditions (37°C; plate shaker) for 14 d shown as % in relative fluorescence intensity.

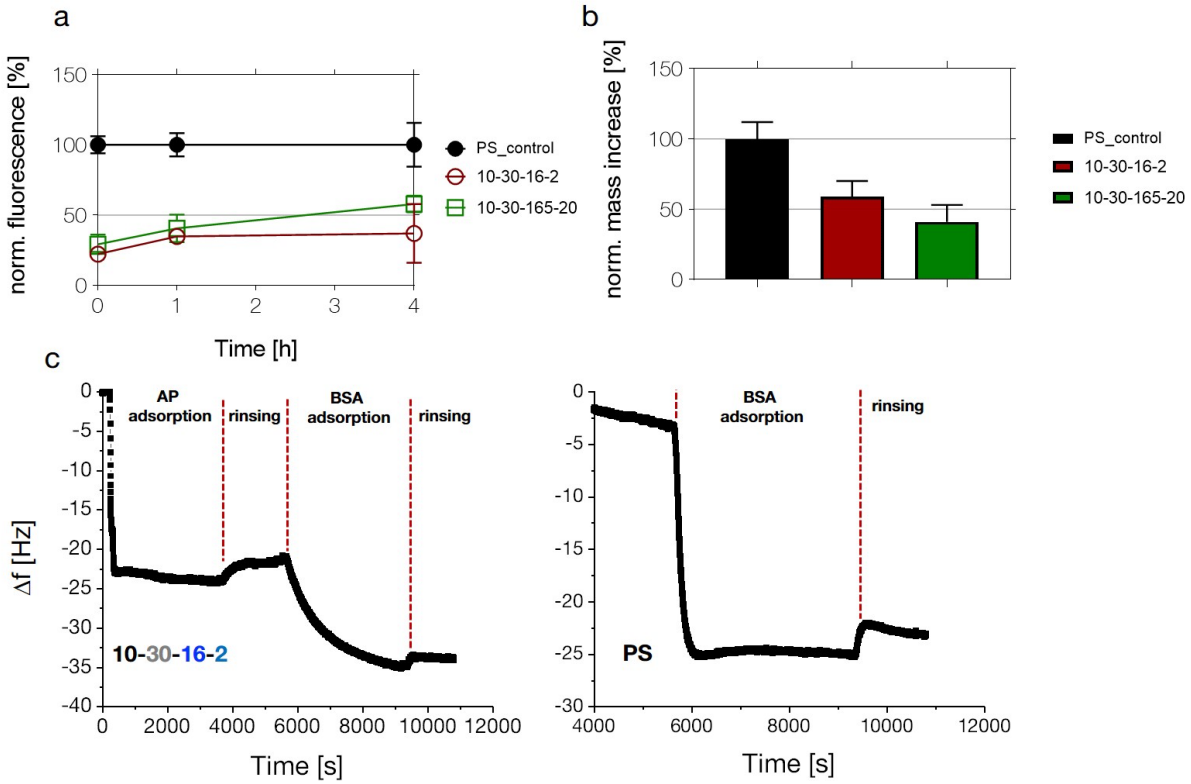


Figure S12 Protein adsorption to AP-prefunctionalized surfaces. (a) Adsorption of Atto-565 labeled BSA (c = 0.1 mg/mL in PBS) on AP 10-30-16-2 and PS after incubation for t = 4 h displayed as a percentage of normalized fluorescence intensity (n = 3). (b) QCM-D measured amount of adsorbed BSA (c = 40 mg/mL in PBS) on AP 10-30-16-2 and pure PS displayed as a percentage of normalized mass increase. (c) QCM-D measured adsorption kinetics during adsorption of BSA (c = 40 mg/mL in PBS) on AP 10-30-16-2 and pure PS plotted as frequency (5th overtone, black curves) shift Δf over time. n = 6.

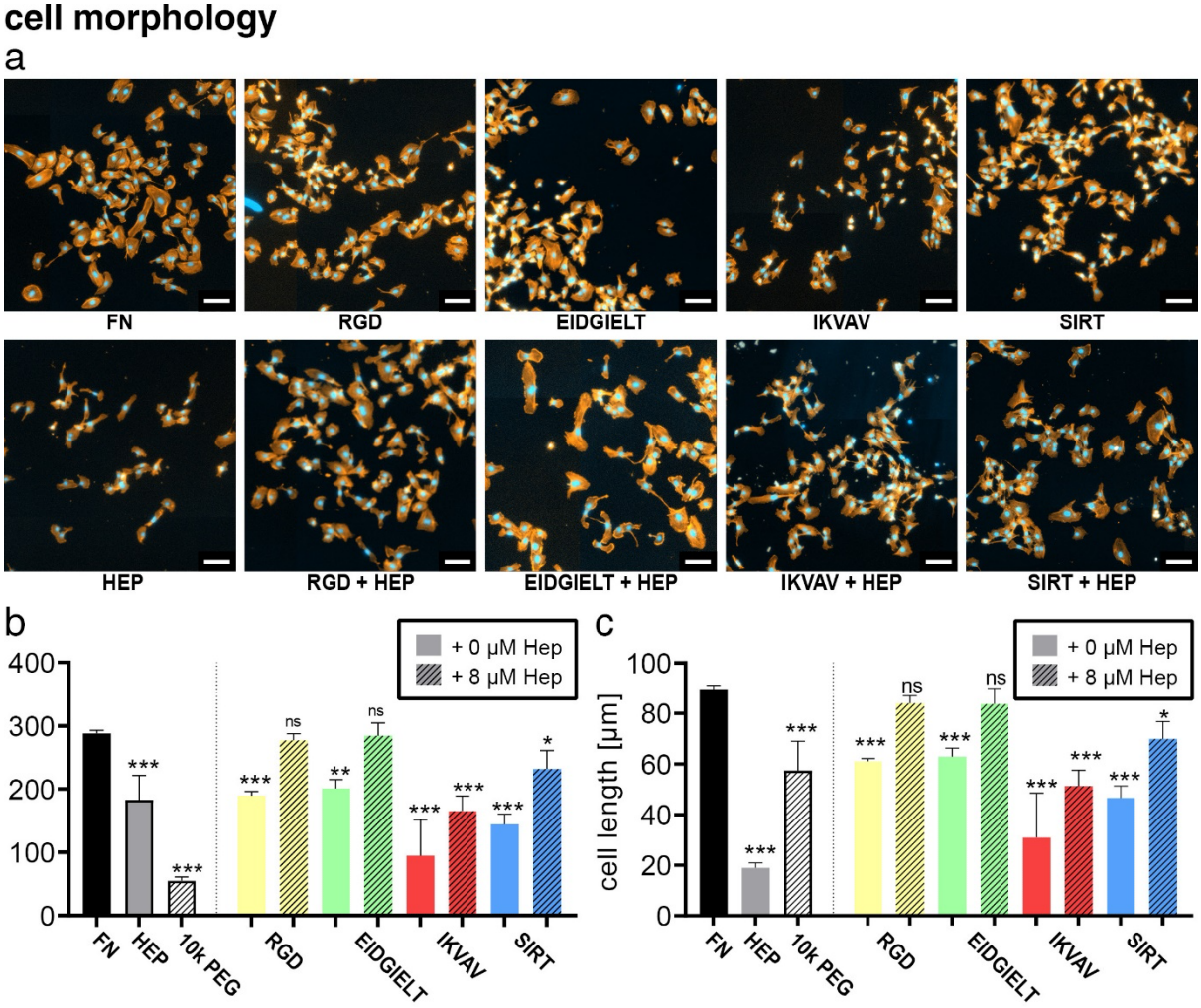


Figure S13. Cell-instructive properties of APs with different functionality. (a) Programming cell instructive signals of HUVECs by variation of ligand type 10-30-8-2-RGDSP, 10-30-8-2-EIDGIELT, 10-30-8-2-IKVAV, 10-30-8-2-SIRT and by combinations of (RGDSP; EIDGIELT; IKVAV; SIRT) with 10-30-16-2-Hep. Quantification of cell area (b) and cell elongation (c) of HUVECs comparing different cell ligand types alone and in combination with 10-30-16-2-Hep (staining: nucleus: DAPI (blue), Actin: Phalloidin-Atto555 (orange), scale bar: 100 µm).

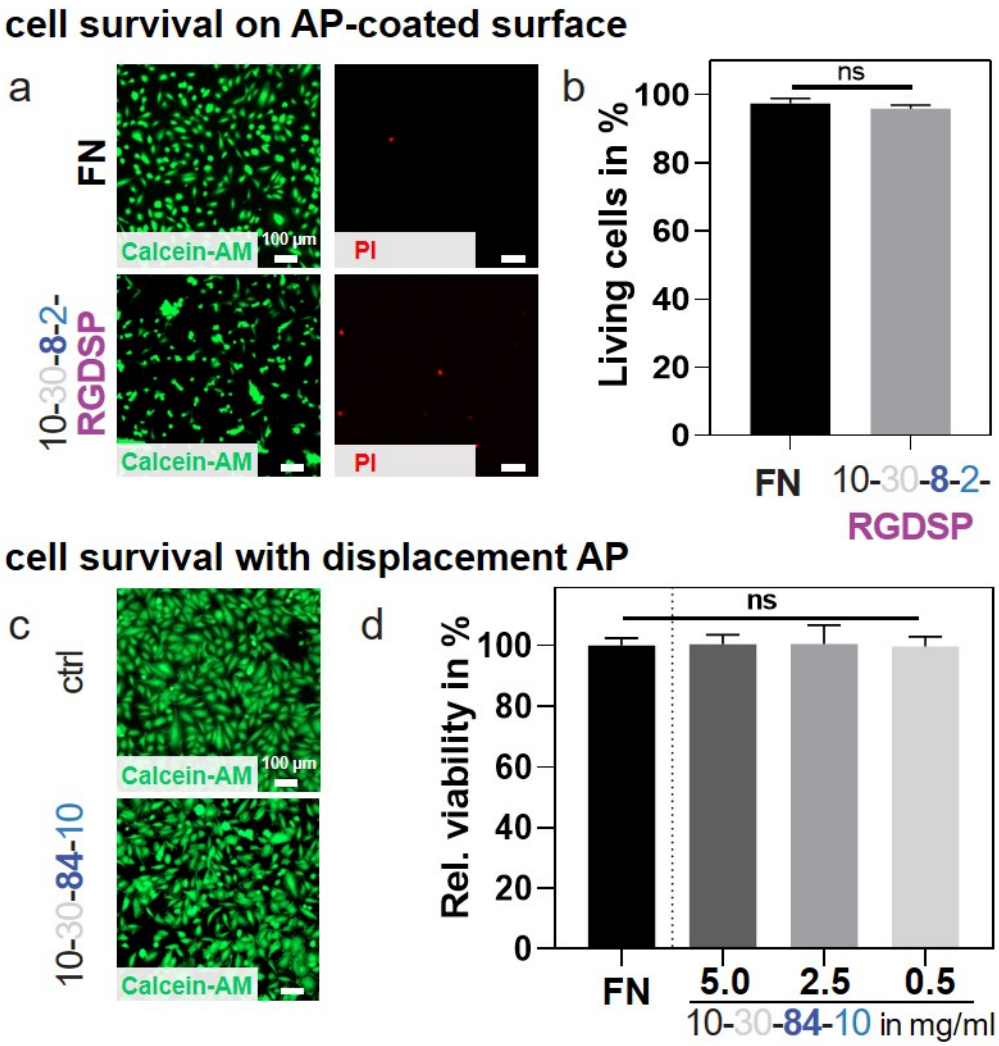


Figure S14. Cell survival and viability with AP. (a) HUVECs cultured for 24h on AP-coated surface show similar survival to FN-coated surface. (b) Quantification of living cells on AP-coated surface. (c) HUVECs cultured on FN-coated surface over 24h with varying concentrations of 10-30-84-10 in the culturing medium. (d) Quantification of cell viability with 10-30-84-10 in the culturing medium. (staining: live cells: Calcein-AM (green), dead cells: PI (red), scale bar: 100 µm)**.**

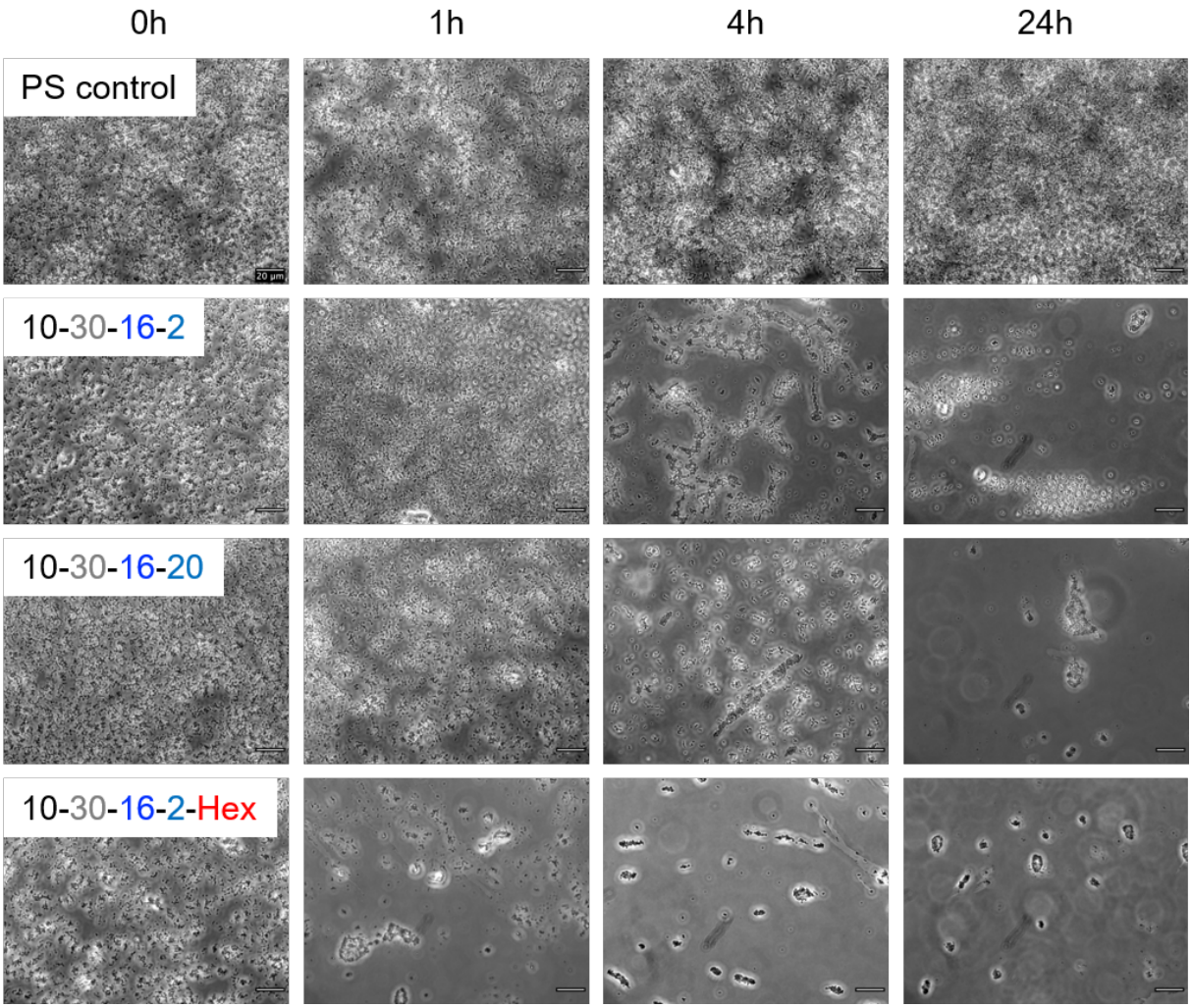


Figure S15. Phase contrast microscopy images taken at four different time points of *S.aureus* growing on AP coated PS surfaces. Scale bar: 20µm.

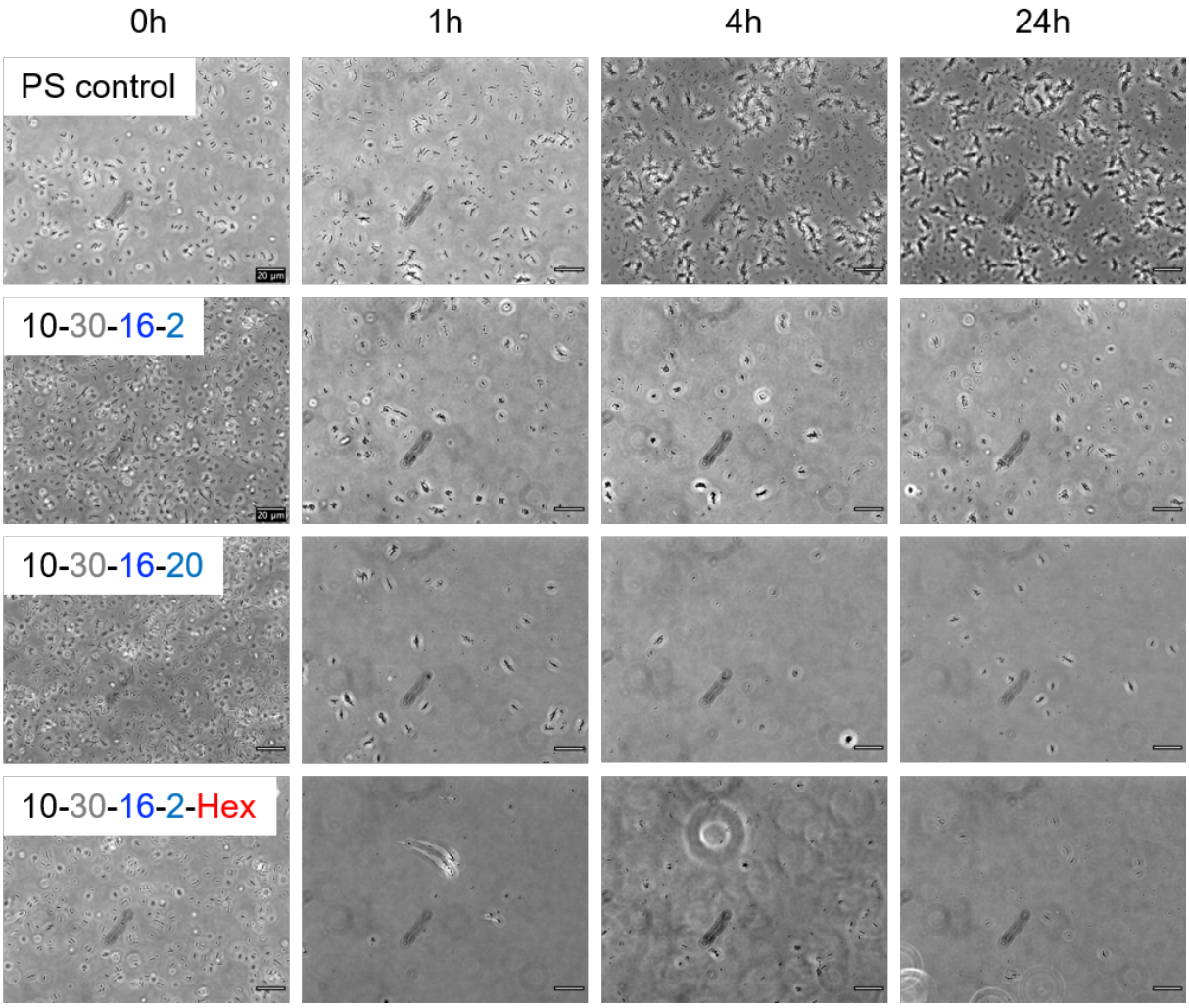


Figure S16. Phase contrast microscopy images taken at four different time points of *E.coli* W3110 growing on AP coated PS surfaces. Scale bar: 20µm.

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