Electronic Supplementary Information

Reactive protein-repellent surfaces for the straightforward attachment of small molecules up to whole cells

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1. General Information

Unless otherwise stated, all chemicals were obtained from commercial sources and used without further purification. Dichloromethane (CH_2Cl_2) was distilled under nitrogen prior to use. Dry solvents were purchased as dry solvents from commercial suppliers and stored sealed under N_2. Cyclic Arg(Pmc)-Gly-Asp(OtBu)-D-Phe-Lys(Boc) was a kind gift from Dr. Dennis Löwik, Radboud University Nijmegen. BCN-*p*-nitrophenyl carbonate was obtained from SynAffix B. V., Nijmegen, The Netherlands. Poly(ethylene glycol)s were obtained from Rapp polymere, Tübingen, Germany. PLL•HBr (MW 20 kDa) was obtained from Sigma Aldrich.

^{1}H- and ^{13}C-NMR spectra were recorded at 20 °C on a Bruker AM 250 SY spectrometer (250 MHz and 50 MHz for ^{1}H and ^{13}C, respectively) or on a Bruker Topspin spectrometer (300 MHz and 75 MHz for ^{1}H and ^{13}C, respectively). ^{1}H-NMR chemical shifts (δ) are reported in parts per million (ppm) relative to a residual proton peak of the solvent, δ = 1.94 for CD_3CN, δ = 7.26 for $CDCl_3$, δ = 2.05 for acetone-d_6 and δ = 4.79 for D_2O. Multiplicities are reported as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets) or m (multiplet). Broad peaks are indicated by br. Coupling constants are reported as a *J* value in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. ^{13}C NMR chemical shifts (δ) are reported in ppm relative to CD_3CN (δ = 118.53). Mass spectra (low and high resolutions) were performed by the Laboratoire de Spectrométrie de Masse de l'ENSCP (Paris) and the Laboratoire de Spectrométrie de Masse de l'ICSN (Gif sur Yvette). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 pre-coated plates (UV detection at 254 nm) and Column chromatography was conducted using Merck silica gel 60 (0.040-0.063 nm). MilliQ water was purified using a Direct-Q 5 instrument (Millipore, Billerica, MA). Imaging and scanning ellipsometry were performed at 20 °C with an EP^3-SE ellipsometer (Nanofilm technologies, Göttingen) equipped with a laser (λ = 658 nm); we thank Cecile Monteux (ESPCI, Paris) for providing access to this Ellipsometer. Infrared spectra were measured on a Spectrum 100 FTIR spectrometer (PerkinElmer, Waltham, MA) fitted with an ATR cell. The HPLC analyses and purifications were run on a Waters HPLC (Millford, MA) equipped with either a semi-preparative or an analytical C_{18} column. Purifications were performed using a linear gradient of CH_3CN in an aqueous solution containing 0.1 % (v/v) TFA. Enzyme-linked assays were measured using a spectra max 250 plate reader (Molecular Devices, Sunnyvale, CA.)Fluorescence microscopy was done using an eclipse TS100 microscope (Nikon) equipped with a DS-Fi1 camera (Nikon).

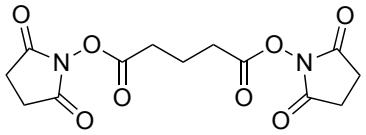
Polydimethoxysilane (PDMS) microchannels were fabricated as previously described.[1]

HeLa cells were cultured in DMEM/Glutamax (Gibco) with 10% fetal calf serum (FCS) and antibiotics (penicillin/streptomycin; Gibco). During live cell imaging experiments, HeLa cells were cultured in Leibovitz's CO_2-independent medium without phenolsulfonphthalein (phenol red). Cell recordings were done at 37 °C under a 5% CO_2 atmosphere using an Axio Observer inverted microscope (Zeiss) equipped with a Coolsnap HQ2 camera (Roper Scientific) and were controlled by Axio vision software (Zeiss).

A note on reaction times: throughout this report, reactions with BCN-linked compounds are consistently allowed to proceed for exactly one hour. This is mainly to present a standardised protocol across all different applications, and not out of experimental necessity. In fact, empirical evidence has shown that conjugations that ran for only 40 minutes gave indistinguishable results in the case of nanoparticle-capture. If reaction times are of the essence to an assay, we recommend that durations prescribed here merely be taken as a starting point for further optimisation. For reference, literature values[2] for SPAAC with BCN give a reaction rate constant of 0.29 M^{-1} s^{-1} in CD_3CN/D_2O (1:2).

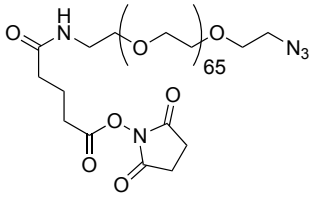
2. Synthesis

a. di-NHS-glutaric acid ester (S1)



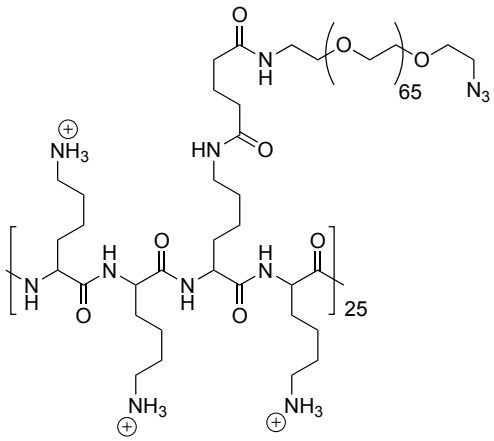
1-({5-[(2,5-dioxo-1-pyrrolidinyl)oxy]-5-oxopentanoyl}oxy)-2,5-pyrrolidinedione was synthesised using a modified procedure from Roelfes et al.[3] To a cooled (0 °C) solution of *N*-hydroxysuccinimide (NHS, 3.42 g; 27.7 mmol; 1.1 equiv.) in dry THF (75 mL) was added triethyl amine (Et_3N, 3.83 mL; 27.7 mmol; 1.1 equiv.) followed by the dropwise addition of glutaryl dichloride (1.61 mL; 12.6 mmol; 1 equiv.). The resulting white suspension was stirred for 2 hours at room temperature. The solvent was evaporated and the residue was taken up in CH_2Cl_2 (100 mL), washed with water (3 × 50 mL) and dried over $MgSO_4$. Filtration and evaporation of the solvent yielded a white solid (**S1**; 3.95 g, 96%), which was recrystallised from isopropyl alcohol (Y = 85%).
^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.85 (s, 8H), 2.80 (t, *J* = 7.2 Hz, 4H), 2.20 (quintet, *J* = 7.2 Hz, 2H) ppm.

b. NHS-PEG$_{3000}$-azide (S2)



To a solution of α-amino-ω-azido-poly(ethylene glycol)$_{67}$ (102 mg; 0.034 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (5.7 μL; 0.07 mmol; 2 equiv.), after which the solution was stirred for 15 minutes. Subsequently, **S1** (111 mg; 0.34 mmol; 10 equiv.) was added, and the reaction was stirred for an additional 2 hours at room temperature. The solution was washed with water (2 × 10 mL) and brine (2 × 10 mL), after which the solvent (CH_2Cl_2) was evaporated. The residue was taken up in a minimal amount of CH_2Cl_2 and loaded on a silica column. Elution with 5% methanol in CH_2Cl_2 removed excess **S1** and NHS, after which the product was eluted using 15% methanol in CH_2Cl_2. The product-containing fractions were combined, evaporated to dryness, and subsequently lyophilised from MilliQ yielding a white fluffy solid (**S2**; 72 mg, 71%).
^{1}H NMR (250 MHz, acetone-d_6): δ = 3.66 (broad, ~260H), 3.42 (t, *J* = 4.0 Hz, 2H), 2.78 (s, 4H), 2.62 (t, *J* = 5.8 Hz, 2H) 2.26 (quintet, *J* = 6.0 Hz, 2H), 2.03 (m, 4H) ppm. FTIR-ATR: 2879, 2100 (ν N_3), 1952, 1715, 1652, 1466, 1341, 1279, 1240, 1124, 1096, 960, 841, 658 cm^{-1}.

c. APP (100% azide content) (2)



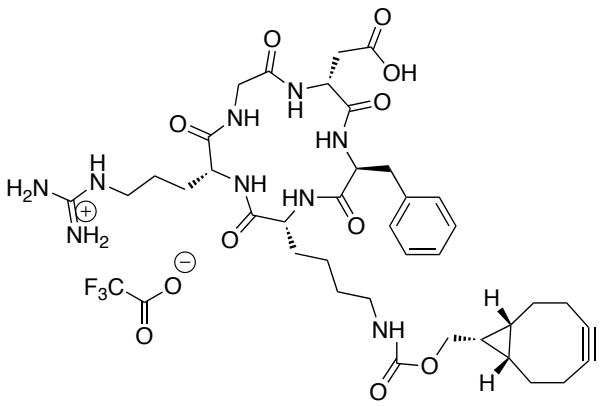
General strategy for the synthesis of APP or PLL-g-PEG (if methoxy-PEG is used): PLL•HBr with an average molecular weight of 20 kDa is dissolved in a sodium tetraborate buffer (STBB, 50 mM in D_2O, 1 mg PLL•HBr per 50 μL STBB). After dissolution, the pH of the solution is set to 8.5. Subsequently, the solution is added to the desired amount of NHS-activated PEG (based on the desired grafting density - assuming a quantitative reaction), and the suspension is shaken vigorously to promote dissolution of the NHS-activated PEG. The reaction progress is monitored by ^{1}H-NMR. Upon achieving the desired grafting ratio (which generally takes only several minutes), the solution is transferred to a dialysis tube (MW cut-off 8 kDa) and dialysed against MilliQ for 16 hours, with at least three changes of the dialysis bath. The solution is lyophilised producing the desired APP as a white fluffy powder (near quantitative yield). APP used in this research had a grafting density of 1 PEG chain per 3 lysine residues. See section 5a for NMR analysis. FTIR-ATR: 3297, 2882, 2103 (ν N_3), 1982, 1649, 1544, 1467, 1342, 1280, 1241, 1098, 962, 841 cm^{-1}.

d. APP (28% azide content)

To obtain APP with a lower azide density, a variation on the protocol described in section 2c is followed, where the total amount of NHS-PEG used is partitioned between NHS-PEG-azide (**S2**) and NHS-PEG_{2000}-OMe. For example, the following protocol produces APP with 28% azide density:

PLL•HBr (7.2 mg; 0.36 μmol; 36 μmol lysine residues) was dissolved in STBB (360 μL) and the pH of the solution was set to 8.5. This solution was subsequently added to **S2** (8.6 mg; 2.8 μmol) and the reaction progress was monitored by ^{1}H-NMR. Next, the solution was added to NHS-PEG_{2000}-OMe (19.4 mg; 9.7 μmol) and after two minutes it was again analysed by ^{1}H-NMR. The solution was transferred to a dialysis tube (MW cut-off 8 kDa) and dialysed against MilliQ water for 16 hours. Lyophilisation yielded the final product as a white fluffy powder. See section 5a for NMR analysis. FTIR-ATR: 3297, 2881, 2103 (ν N_3), 1982, 1649, 1544, 1467, 1342, 1279, 1241, 1097, 960, 841 cm^{-1}.

e. BCN-RGD (5)



Deprotection of the cyclic peptide: Cyclic Arg(Pmc)-Gly-Asp(OtBu)-D-Phe-Lys(Boc) (150 mg) was deprotected using a solution containing 95% trifluoroacetic acid (TFA), 2.5% 2-mercaptoethanol and 2.5% H_2O (5 mL). After stirring for 3 hours, the solution was injected in diethyl ether (Et_2O, 45 mL) and centrifuged. The liquid was decanted, and the solid pellet was taken up in methanol, after which it was precipitated in Et_2O (48 mL) and isolated. c[RGDfK]•(2 TFA) was obtained with a purity > 95 %, according to analytical HPLC. (103 mg; 85%)
[M+H] calc.: 604.3; found: 604.7.

BCN-conjugation of c[RGDfK]: A solution of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate (BCN-*p*-nitrophenyl carbonate; 5.13 mg; 16.3 μmol; 1 equiv.) in dimethylformamide (DMF, 1 mL) was added to a solution of c[RGDfK]•(2 TFA) (14.87 mg; 17.9 μmol; 1.1 equiv.) and *N,N*-Diisopropylethylamine (DIPEA; 9 μL; 52 μmol; 3.2 equiv.) The reaction was stirred for 16 hours, after which the solvents were evaporated. The residue was taken up in 2 mL H_2O and then purified by HPLC. Lyophilisation of the collected fractions yielded the TFA salt of the title compound as a white powder (**5**; 12 mg; 82%).
HRMS [M+H] calc.: 780.4044; found: 780.4055
^{1}H NMR (300 MHz, D_2O/CD_3CN): δ = 7.49 - 7.34 (m, 5H), 4.85 (dd, *J* = 6.3, 7.5 Hz, 1H), 4.42 (dd, *J* = 5.4, 8.7 Hz, 1H), 4.28 (br. m, 3H), 3.96 (dd, *J* = 3.9, 10.2 Hz, 1H), 3.57 (d, *J* = 15.0 Hz, 1H), 3.26 (m, 2H), 3.11 (br. m, 4H), 2.88 (m, 3H), 2.14 (quintet, *J* = 2.4 Hz, 4H), 1.95 (b, 2H), 1.8 - 1.4 (br. m, 13H), 1.08 (br. t, *J* = 9.0 Hz, 3H) ppm. ^{13}C NMR (75 MHz, D_2O/CD_3CN): δ = 174.1, 173.4, 172.1, 170.6, 166.8, 164.7, 162.1, 156.2, 149.7, 148.3, 147.6, 135.7, 128.7, 128.3, 126.7, 125.8, 99.4, 87.0, 78.1, 68.2, 49.1, 40.1, 36.6, 33.9, 29.8, 28.2, 24.0, 20.3, 19.3, 16.9, 16.1 ppm.

3. Surface Coating

a. Coating of quartz and photopatterning

Quartz wafers were exposed to O_2 plasma for 45 seconds. Cleaned wafers were then laid on top of a 200 μL drop of 0.1 mg/mL PLL-g-PEG (**1**, Surface Solutions, Switzerland) or APP (**2**) in 10 mM HEPES, pH 7.4 (200 μL) at room temperature for 1 hour (this volume has to be adapted to the wafer size to allow complete coverage of the wafer by the solution, but use a minimal amount). The wafers were rinsed once with PBS, twice with MilliQ and then dried.

For photopatterning, a chromium synthetic quartz photomask (Delta mask, Enschede, The Netherlands) was exposed to deep UV light for four minutes. Then, an APP-coated quartz wafer was placed on the mask with 4.6 μL of water (this volume has to be adapted to the wafer size to allow complete coverage of the wafer by water, but use a minimal amount). The masked substrate was then exposed to deep UV light for 7 min, after which the wafer was dried and measured by ellipsometry (see section 5b). Glass cover slips (25 mm diameter) were patterned in a similar fashion, using 2.3 μL of water. The irradiated areas were coated with fibronectin after patterning.

b. Coating of glass

Fluorodishes (Glass bottom petri dishes from World Precision Instruments, FL) with a diameter of 35 mm were exposed to O_2 plasma for 45 seconds. The glass insets of cleaned dishes were incubated with 0.1 mg/mL PLL-g-PEG (**1**, Surface Solutions, Switzerland) or APP (**2**) in 10 mM HEPES, pH 7.4 (200 μL) at room temperature for 1 hour. The treated glass surfaces were then rinsed once with PBS, twice with MilliQ water, dried, and stored at 4 °C.

c. Coating of polystyrene

Cell culture treated polystyrene petri dishes (34 mm) or flat-bottom 96-well plates (Techno Plastic Products, Trasadingen, Switzerland) were exposed to O_2 plasma for 45 seconds. Cleaned surfaces were incubated with 0.1 mg/mL PLL-g-PEG (**1**, Surface Solutions, Switzerland) or APP (**2**) or a mixture thereof in 10 mM HEPES, pH 7.4 (400 μL per dish, or 100 μL per well) at room temperature for 1 hour. The treated polystyrene surfaces were then rinsed once with PBS, twice with MilliQ, dried and stored at 4 °C.

d. Coating of PDMS

Based on a published protocol,[1] PDMS microchannels were exposed to O_2 plasma for 45 seconds, after which they were filled with 0.1 mg/mL PLL-g-PEG (**1**, Surface Solutions, Switzerland) or APP (**2**) in 10 mM HEPES, pH 7.4 (5 μL) and left to incubate at room temperature for 1 hour. After removal of as much liquid as possible using a pipette, the microchannels were rinsed with MilliQ and emptied again, for at least three times. The microchannels were then dried under vacuum for at least three hours.

e. Coating of patterned glass slides with fibronectin

UV-exposed regions of glass slides were made adhesive to cells by incubation with fibronectin (100 μL, 25 μg ml^{-1} in 100 mM $NaHCO_3$, pH 8.6) for 1 h at room temperature. The slides were rinsed once with PBS, twice with MilliQ water, and used immediately.

4. Surface Functionalisation

a. Conjugation of BCN-rhodamine (BCN-ϱ)

*A stock solution of **3**, (BCN-rhodamine, SX-A1027 from SynAffix, 100 μM in milliQ) was prepared and stored in small aliquots at -20 °C.*

PDMS microchannels (coated with APP or PLL-g-PEG, or uncoated) were filled with 5 μL BCN-rhodamine solution and reacted at room temperature for 12 hours. Afterwards, as much liquid as possible was removed using a pipette. Channels were then repetitively submerged in MilliQ water to fill the channels and subsequently emptied again (3 x) to thoroughly wash the channels. They were then placed under vacuum for three hours, after which they were washed as described above for two more times. Finally, the microchannels were dried in a vacuum for 1 hour.

b. Conjugation of BCN-biotin

*A stock solution of **4**, (BCN-biotin, SX-A1029 from SynAffix, 100 μM in PBS pH 7.5 containing 0.1% DMF) was prepared and stored in small aliquots at -20 °C.*

The glass insets of Fluorodishes, or wells in a 96-well plate (coated with APP, with PLL-g-PEG, or with a mixture thereof) were filled with BCN-biotin solution (200 μL for dishes, 100 μL for wells) and left to react at room temperature for 1 hour. Afterwards, the surfaces were washed with PBS, and rinsed with MilliQ for at least three times. The surfaces were either used immediately after preparation, or dried under an air stream and stored at 4 °C.

c. Capture of polystyrene beads

Biotinylated Fluorodishes (or just PLL-g-PEG or APP-coated ones in control experiments) were filled with a FluoSphere suspension (FluoSpheres F-8776 from Invitrogen) containing 0.01% solids in PBS (200 μL) for 45 minutes at room temperature. The dishes were then filled with PBS and gently shaken on an orbital shaker for five minutes, after which they were emptied and carefully refilled with fresh PBS (we found that direct jets of PBS from squirt bottles can tear the microspheres from the surface, due to their relatively large size). After three such washing steps, the dishes were filled with PBS and observed by fluorescence microscopy.

d. Enzyme-linked assay

An HRP-based enzyme-linked assay was performed using an OptEIA kit (BD Biosciences) according to the included protocol. In brief, each well was filled with a streptavidin-HRP solution (10.000-fold dilution from the kit stock) and incubated at room temperature for 1 hour. The wells were then washed with PBS (5 x) by filling and emptying them, successively. Then, each well was filled with 50 μL of "solution A" and 50 μL of "solution B" (the TMB stock solution, and the H_2O_2 stock solution provided by the kit) and the plate was immediately placed in a plate reader for analysis.

e. Conjugation of BCN-RGD

*A stock solution of **5**, (BCN-RGD, 100 μM in PBS pH 7.5) was prepared and stored in small aliquots at -20 °C.*

The glass insets of Fluorodishes (coated with either APP or PLL-g-PEG) were filled with BCN-RGD solution (200 μL) and left to react at room temperature for 1 hour. Afterwards, the surfaces were washed with PBS, and repetitively rinsed (3 x) with MilliQ. The conjugated surfaces were subsequently dried with an air stream, and rinsed with cell culture medium prior to use.

f. Cell seeding for time lapse movies

Fluorodishes were washed with culture medium before use. Cells were detached using Versene (Invitrogen) and pelleted for 3 minutes at 1300 rpm. The cell pellet was resuspended in Leibovitz's CO_2-independent medium without phenol red at a concentration of 150.000 cells per mL. Each dish was then seeded with 1 mL of medium, after which live imaging was started immediately.

5. Supporting Experimental Data

a. Determination of PEG grafting ratio

All APP used in this report was characterised by ^{1}H-NMR spectroscopy, to establish the grafting ratio of PEG to the PLL backbone. Supplementary Figure S1 shows a typical NMR spectrum of APP with a grafting density of 1 PEG chain per 3 lysine residues. Based on the spectra of PLL and PEG (data not shown), all peaks in the spectrum can be unambiguously assigned. The grafting ratio is determined by comparison of the signals for the PLL backbone's chiral proton (one per residue, highlighted in red) and the α-amide protons in the grafted side chains (two per residue, highlighted in blue).

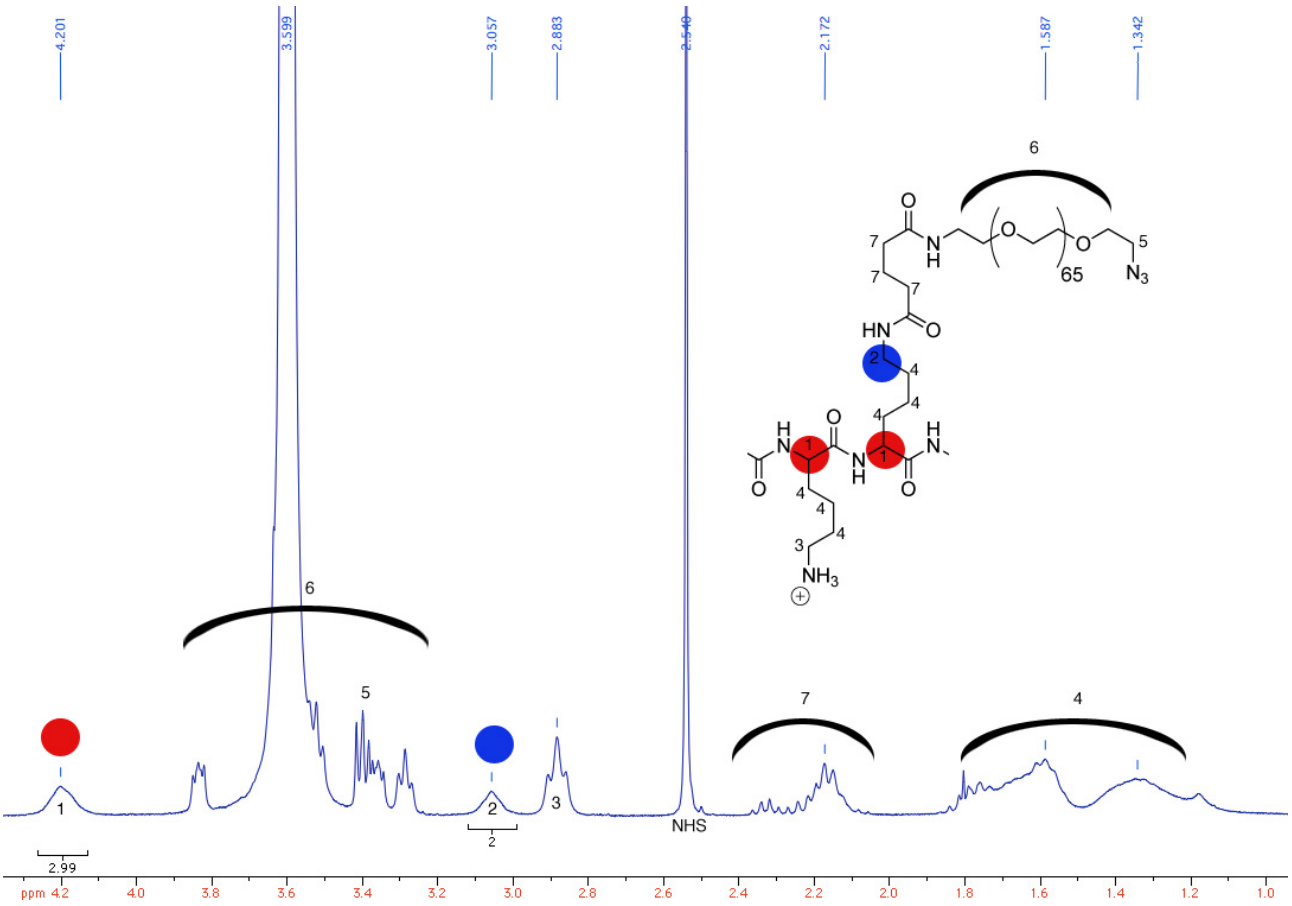


Figure S1. *^{1}H-NMR spectrum of an APP polymer with a grafting ratio of 1 PEG chain per 3 lysine residues, and assignment of the peaks according to the PLL-g-(PEG-N_3) structural formula.*

For APP with a reduced azide content (e.g. 28%, as described in section 2d), two ^{1}H-NMR spectra are required to determine both the grafting ratio of the azide chains, and the total grafting ratio after NHS-PEG-OMe grafting. Supplementary Figure S2 shows the spectra of APP (28%) when only the azido-PEG has been grafted (Figure S2a) and after additional methoxy-PEG grafting (Figure S2b). For both steps, comparison of the highlighted integrals reveals the grafting ratios. In turn, the ratio between these values provides the amount of PEG-chains that has an azide moiety. In the case of Figure S2, which shows spectra recorded for the APP synthesised in section 2d, this ratio was found to be 28% (i.e. 3.01 divided by 10.7).

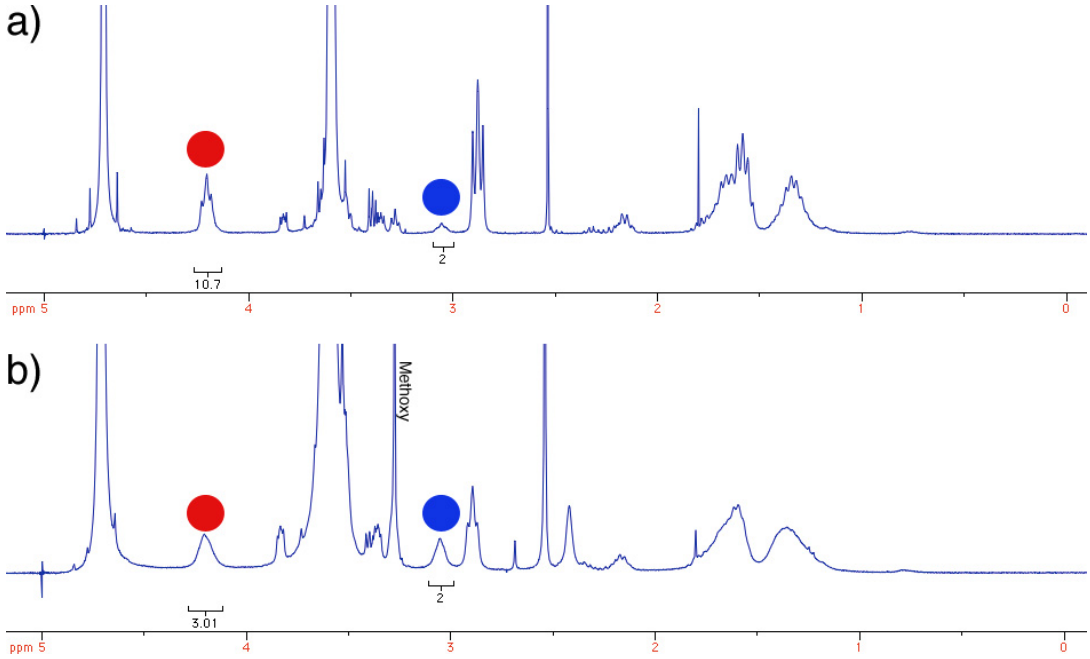


Figure S2. *a) ^{1}H-NMR spectrum of APP with a grafting ratio of 1 azido-PEG chain per 10.7 lysine residues; b) ^{1}H-NMR spectrum of the same APP polymer, after additional NHS-PEG-OMe grafting. The final grafting ratio is 1 PEG chain per 3 lysine residues, with an azide content of 28%*

b. Chain density determination

An imaging ellipsometer EP^3-SE (Nanofilm technologies, Göttingen, Germany) equipped with a laser (λ = 658 nm) was used to determine thickness maps with ca. one micrometer lateral resolution. As for conventional ellipsometry, the change of polarisation of light upon reflection on the wafer (in air) was quantified (at each pixel) by the complex reflectance ratio Rp/Rs = $\tan(\psi).e^{i\Delta}$, where Rp and Rs are the reflectivity coefficients parallel and perpendicular to the plane of incidence. Δ and Ψ maps were transformed into a thickness map by analysing data in the framework of a three layer model consisting of an upper thin organic layer, an intermediate thin layer of silicon oxide, and a bottom thick layer of silicon. Cleaned quartz wafers were measured under an angle of 75° to determine the thickness of the oxide layer (1.8 nm). They were then coated with APP and subsequently photopatterned as described in section 3a using a mask with a 9 µm stripe pattern. The wafers were then measured by ellipsometry under an angle of 75°, taking Ψ and Δ values for both the patterned and the intact areas. Mean values of five such experiments are given in Supplementary Table S1. Using EP4 model software 1.0.0 and the values measured for the oxide layer, this data was fitted to a layer thickness simulation using a refractive index of 1.459 for APP (the literature value for the refractive index of PEG_{3000}). A plot of Ψ against material thickness is shown in Supplementary Figure S3. Resulting layer thickness values are reported in Supplementary Table S1.

	Δ	Ψ	nm
APP coating	124	1.8	1.1
Patterned area	130	1.3	0.0

Table S1. *Ellipsometry values and modelled layer thickness for an APP-coating on quartz.*

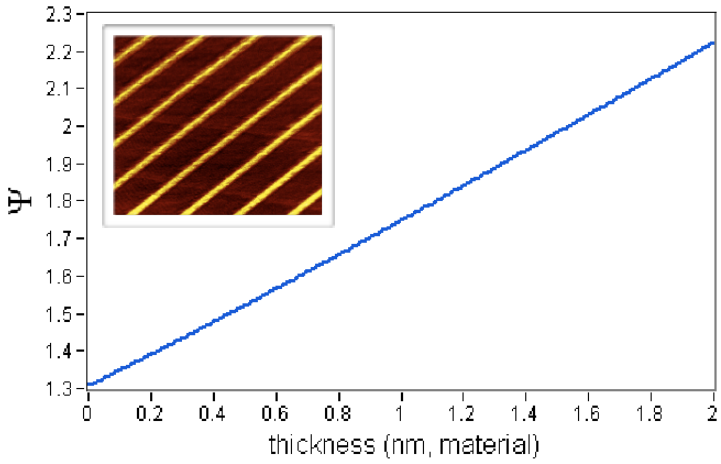


Figure S3. *Layer thickness simulation. Inset: area scan of a patterned APP surface.*

Based on a layer thickness value of 1.1 nm and the density of PEG_{3000} as 1.2 g/cm^3, it follows that an APP coating has a mass of $1.32 \cdot 10^{-7}$ g/cm^2. The molecular weight of PEG in APP is 3 kDa per PEG chain, leading to a chain density of $4.4 \cdot 10^{-11}$ mol/cm^2. This corresponds to $4.4 \cdot 10^{-25}$ chains per nm^2, and division by the Avogadro constant gives an individual chain density of 0.265 chains per square nanometer for APP with a grafting density of 1 PEG chain per 3 lysine residues.

c. APP with diluted azide content

APP coatings with a lowered azide density were created as described in section 3b using APP with a 28% azide content as described in section 2d. These coatings were biotinylated according to the protocol given in section 4b, followed by immobilisation of FluoSpheres as described in section 4c. Supplementary Figure S4 shows representative fluorescence microscopy images of captured FluoSpheres using APP(28%) and APP(100%). The amounts of FluoSpheres shown are 80 and 303, respectively, indicating an effective ratio of 26.4%, which is in good correspondence with the ratio of available azides.

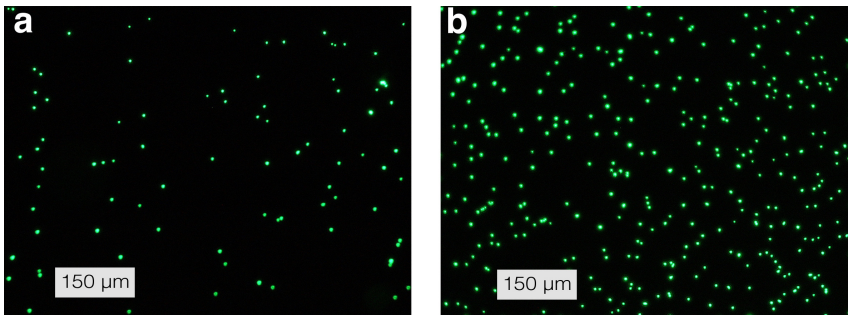


Figure S4. *Immobilised FluoSpheres on biotinylated APP a) with a 28% azide density (80 spheres). b) with a 100% azide density (303 spheres). The ratio of captured spheres is 26.4%.*

d. A measure for the protein-repellent character of APP

To assess the protein-repellent character of APP as compared to PLL-g-PEG, we performed an enzyme-linked assay as described in section 4d. In a comparative experiment, we prepared wells coated with 100% APP and with 100% PLL-g-PEG. We also left a set of wells without a coating. Subsequently, the PLL-g-PEG and the bare wells were incubated with BCN-biotin, but not the wells coated with APP. After 1 hour of incubation, the wells were washed with PBS (3x) and with MilliQ (3x), followed by the addition of streptavidin-HRP to each well. After half an hour incubation, the wells were again washed with PBS and MilliQ. HRP activity was then detected using the TMB and H_2O_2 stock solutions (50 μL each). Figure S5 shows representative progress curves for the specified three experiments. It is apparent that the uncoated wells retained much HRP activity, which must be attributed to non-specifically adsorbed protein. To prevent this known problem, most standard protocols for enzyme-linked assays habitually include washing steps employing detergents such as Triton X-100 or Tween. Our results show that the use of detergents is not required when sample wells are PEGylated with either APP or PLL-g-PEG. A direct comparison between these two PEGylating agents reveals that their effects are nearly similar. The inset in Figure S5 shows regression analysis of the first 75 data points of the two relevant data sets, which excludes the plateau of identical values, and an R^2 value of 0.96613 was calculated.

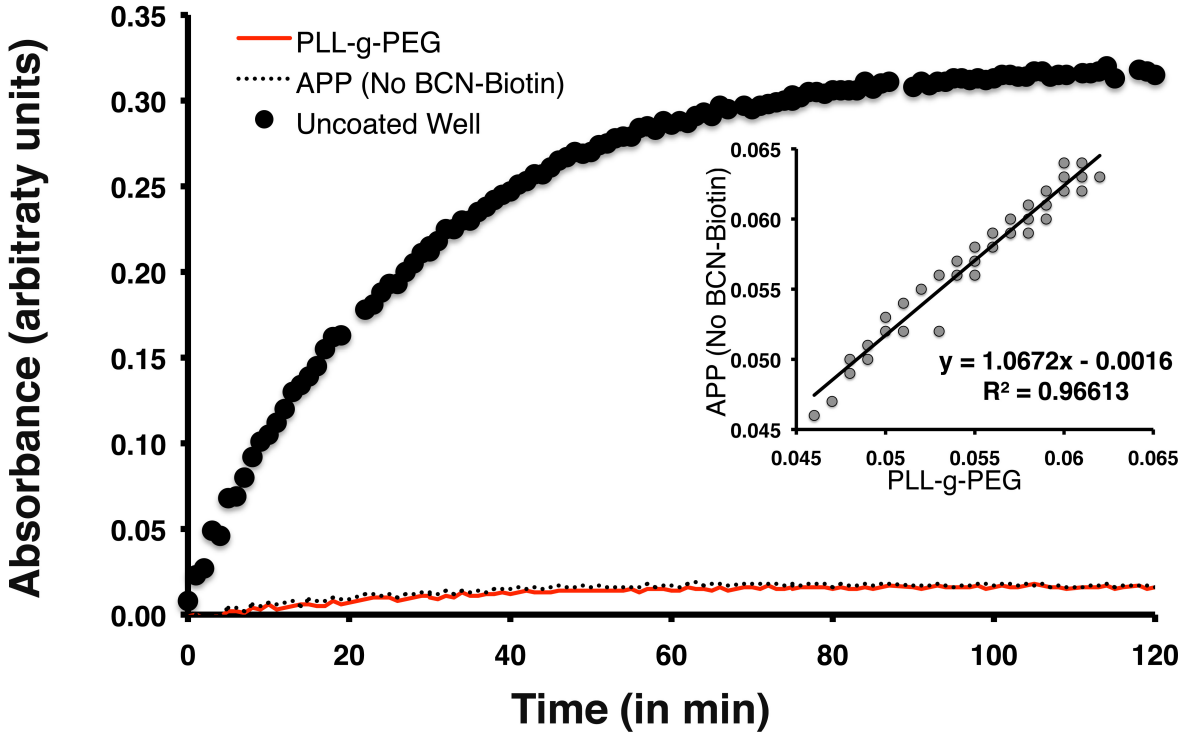


Figure S5. *Representative progress curves of the oxidation of TMB by HRP for wells with different contents. Polystyrene wells were coated with APP, with PLL-g-PEG, or they were left without coating. The well coated with APP was not treated with BCN-biotin. Inset: regression analysis of the first 75 data points for PLL-g-PEG and for APP (No BCN-Biotin).*

e. Time lapse movies of cell attachment

Fluorodishes were coated with APP or PLL-g-PEG (section 3b), and were then either left untreated, or reacted with BCN-RGD (section 4e). Subsequently, HeLa cells were seeded as described in section 4f The cells were observed using live cell video microscopy (1 image per 5 minutes), and the resulting movie files (7 fps) can be found in the electronic supplementary information, available via the internet. The surfaces used in the movie files are given in table S2.

Movie	Name	Glass coating	Additional treatment
1	OMe	PLL-g-PEG	BCN-RGD
2	RGD	APP	BCN-RGD
3	N_3	APP	none
4	Glass	none	none

Table S2. *Systems used in the supplied movie files.*

f. Cell release from confinement

Different 200 μm disk patterns of fibronectin in APP or of fibronectin in PLL-g-PEG were prepared as described in section 3. HeLa cells were then seeded as described in section 4f. After 20 minutes, unattached cells were gently washed away. Remaining cells were allowed to proliferate to fill the available space in a cell culture incubator. Then, the medium was replaced by 1 mL of a BCN-RGD solution (1 mM in PBS) and the cells were placed in the incubator. In a control experiment, unmodified RGD (that is, c[RGDfK]) was used at the same concentration. After one hour, the PBS was replaced by fresh medium and the cells were incubated for 18 or 30 hours, after which they were observed by light microscopy. Figure S6 shows control experiments that demonstrate how the use of PLL-g-PEG instead of APP does not result in controlled migration of cells, and how BCN-RGD cannot be replaced by unmodified RGD.

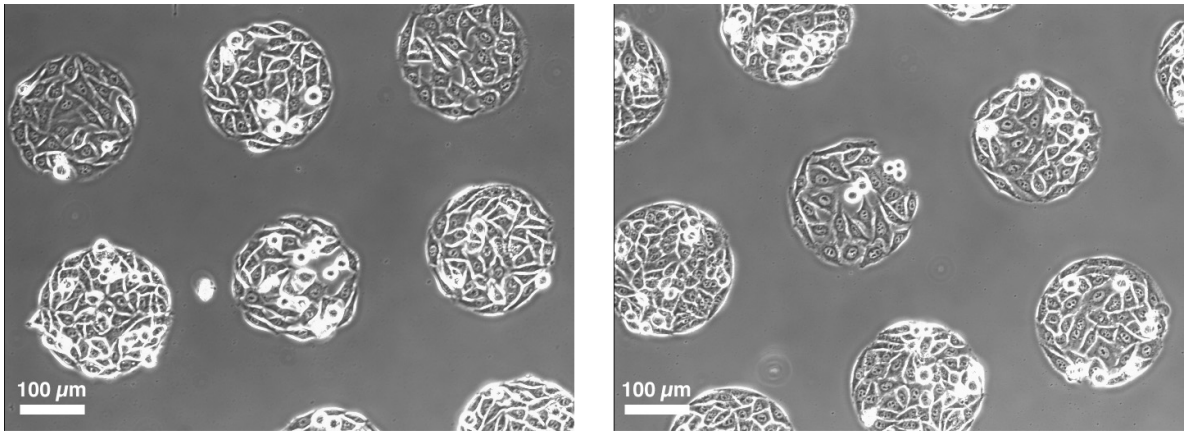


Figure S6. *Phase contrast micrographs of HeLa cells 18 h after seeding on 200 µm fibronectin disks in an APP or PLL-g-PEG matrix, as indicated. At T = 0 h, the wells were treated with RGD or BCN-RGD as indicated. In both cases, the cells remain confined within their patterns.*

6. References

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