Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information for:

Combining the incompatible: Block copolymers consecutively displaying activated esters and amines and their use as protein-repellent surface modifiers with multivalent biorecognition

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Content:

1. Materials

2,2'-Azobis(2-methylpropionitrile) (AIBN) (98%, Aldrich) was purified by recrystallization twice in methanol before use. All further reagents were used as received without any purification. 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) (98%), acryloyl chloride (≥98%), ammonium hydroxide solution (25%), biotin (≥99%), dioxane (anhydrous, 99.8%), *N,N*-diisopropylethylamine (DIPEA) (≥99%), *N,N*-dimethylformamide (DMF) (anhydrous, 99.8%), *N*-(*tert*-butoxycarbonyl)ethanolamine (98%), pentafluorophenol (≥99%), rhodamine B isothiocyanate, sodium azide (≥99.5%), sodium bicarbonate, triethylamine (≥99%), triethylene glycol monomethyl ether (TEG-OH) (≥99%) and triphenylphosphine (≥98.5%) were purchased from Aldrich. Methanol (≥99%), n-hexane (98%), tetrahydrofuran (THF) (≥99.5%) and toluene (≥99%) were purchased from VWR. Diethyl ether (≥99%) and p-toluenesulfonyl chloride (≥99%) were purchased from Fluka. Silica gel 60 was purchased from AppliChem, dichloromethane (DCM) (≥99%) was purchased from Th. Geyer and magnesium sulfate anhydrous (≥99%) and dimethylsulfoxide-d6 was purchased from Merck. *O*-(1*H*-Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) (98%) and trifluoroacetic acid (99%) were purchased from ABCR. NeutrAvidin Oregon Green 488 conjugate (NAv) and 4.9 μm (SD = 0.23 μm), 4.0% (w/v) surfactant-free, aldehyde/sulfate latex polystyrene (PS) beads were purchased from Invitrogen. Chloroform-d1 was purchased from Deutero GmbH and 1-(Boc-amino)-4,7,10-trioxa-13-tridecaneamine was purchased from Iris Biotech GmbH.

2. Instrumentation

NMR Spectroscopy. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{19}F NMR (376 MHz) spectra were recorded using a Bruker DPX-400. All spectra were referenced to the residual proton signals of the deuterated solvent.

Size exclusion chromatography. SEC in THF (250 g/L 3,5-di-*tert*-4-butylhydroxytoluene as internal standard) was performed at 1.0 mL/min (20 °C) using a pre-column (8x50 mm) and four MZ-DVB gel columns (8x300 mm, SDplus, MZ Analysentechnik, nominal pore widths were 50, 10^2, 10^3 and 10^4 Å) with a refractive index detector (RI-2031plus, Jasco). SEC was calibrated using narrowly distributed poly(methyl methacrylate) standards (PSS Mainz).

SEC in DMF was performed using an Agilent 1100 system equipped with a dual RI-/Visco detector (ETA-2020, WGE). The eluent contained 1 g/L LiBr as internal standard. The sample solvent contained traces of distilled water as internal standard. One pre-column (8x50 mm) and four PSS GRAM gel columns (8x300 mm) were used at a flow rate of 1.0 mL/min at 20 °C. The diameter of the gel particles measured 10 μm, the nominal pore widths were 30, 10^2, 10^3 and 3000 Å. SEC was calibrated using narrowly distributed poly(methyl methacrylate) standards (PSS Mainz).

SEC in dimethylacetamide (DMAc) was performed using a Varian PL50 system, comprising an auto injector and a differential refractive index detector. The eluent contained 0.32 g/L LiBr. A Polymer Laboratories 5.0 μm bead-size guard column, followed by three linear PL columns (PLgel 5 μm

MIXED-C) were used at a flow rate of 1.0 mL/min at 50 °C. SEC was calibrated using narrowly distributed polystyrene standards (PSS Mainz).

MALDI-TOF MS was performed on a Bruker ultrafleXtreme in linear mode. Samples were prepared by dissolving the matrix (trans-2-[3-(4-tert-butylophenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) in DCM (30 g/L) and mixing with the polymer in DCM (1 g/L) in the ratio 1:50 (v/v). Then, 2 µL were spotted onto a thin layer of sodium trifluoroacetate (NaTFAc), which was previously prepared from a solution in acetone (19 g/L). For spectra in the linear mode 6000 laser shots with 33% laser power were collected. The laser repetition rate was 1000 Hz and the pulsed ion extraction time 360 ns.

Confocal (fluorescence) microscopy was performed on a Leica TCS SP8 confocal laser scanning microscope based on a Leica DMI6000 optical microscope with 63x immersion objective. Fluorescence excitation was conducted using a 488 nm Argon laser, fluorescence emission was detected by a Leica HyD(TM) hybrid detector. Bright field images were recorded with a photomultiplier detector. Image processing was performed using ImageJ version 1.6.0_20.

Fluorescence spectra were measured by a Perkin Elmer LS-50 photoluminescence spectrometer.

Zeta potential measurements were carried out at 25 °C using a Zetasizer Nano ZS from Malvern Instruments. The samples of non-modified or polymer coated PS particle dispersions (0.4% w/v in water) were washed twice with ethanol, twice with NaCl solution (500 mM in water) to break interpolyelectrolyte complexes and three times with water before the measurement. Every data point represents an average value of three measurements. Each measurement consists of a set of runs, which is performed until convergence (max. 100 runs). The Malvern software calculates the zeta potential from the electrophoretic mobility based on the Helmholtz-Smoluchowski relationship.

3. Monomer Syntheses

3.1 Synthesis of pentafluorophenyl acrylate (PFPA)

10.06 g (54.67 mmol) pentafluorophenol was dissolved in 50 mL DCM and 5.65 g (55.84 mmol; 1.02 eq) triethylamine was added dropwise at 0 °C. 5.41 g (59.75 mmol; 1.09 eq) acryloyl chloride was subsequently added dropwise and the reaction vessel was kept under cooling for 10 min before it was allowed to proceed at room temperature. Upon complete conversion (monitored by thin-layer chromatography (TLC)), precipitated triethylammonium chloride was removed by filtration and the filtrate was washed twice with acidic water (HCl; pH = 2.0) and twice with distilled water. The organic phase was dried over anhydrous magnesium sulfate ($MgSO_4$) and remaining solvent was removed under reduced pressure to yield a yellow liquid. The residue was purified by flash chromatography (Silica gel, petroleum ether) to obtain 9.42 g (39.51 mmol; 70%) of colorless pentafluorophenyl acrylate.

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 6.71 (d, -CH–$CH_a\mathbf{H_b}$), 6.37 (dd, -C(O)-C**H**–CH_aH_b), 6.17(d, -CH–C$\mathbf{H_a}H_b$). **^{19}F NMR ($CDCl_3$, 376 MHz):** δ/ppm: -162.6, -158.4, -158.9.

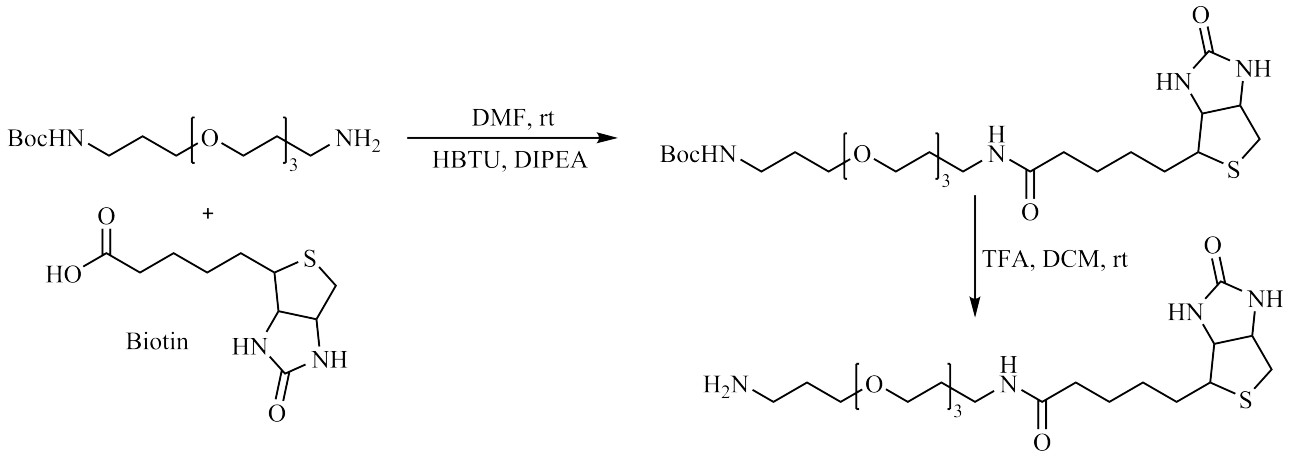
3.2 Synthesis of N-(tert-butoxycarbonyl)aminoethyl acrylate (BocAEA)

8.08 g *tert*-butyl *N*-(2-hydroxyethyl) carbamate (49.62 mmol) were mixed with 6.06 g (59.88 mmol; 1.19 eq) triethylamine in 100 mL DCM. Afterwards, 4.79 g (52.92 mmol; 1.06 eq) acryloyl chloride was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The solution was washed twice with citric acid solution (10 wt%) and twice with distilled water. The organic phase was dried over anhydrous $MgSO_4$ and remaining solvent was removed under reduced pressure to yield a yellowish solid. The crude product was purified by recrystallization from DCM/hexane to obtain 8.60 g (39.95 mmol; 80%) of white BocAEA.

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 6.34 (d, -CH–CH_a**H_b**), 6.04 (dd, -C(O)-C**H**–$CH_a H_b$), 5.76 (d, -CH–C**H_a**H_b), 5.01 (s, –CH_2-N**H**-Boc), 4.13 (t, -O-C**H_2**-CH_2-), 3.34 (br, -CH_2-C**H_2**-NH-), 1.35 (s, -NH-**Boc**)). **^{13}C NMR ($CDCl_3$, 100 MHz):** δ/ppm: 166.0, 155.8, 131.2, 128.1, 79.4, 63.7, 53.4, 39.6, 28.3.

4. Synthesis of TEG-NH_2 and Biotin-TEG-NH_2

4.1 Synthesis of Biotin-TEG-NH_2



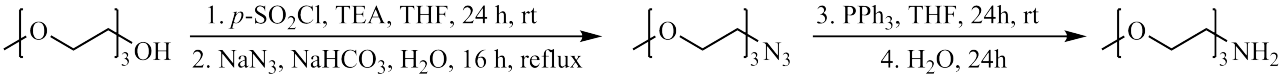
N,N-diisopropylethylamine (DIPEA) (0.21 g, 1.62 mmol, 1.49 eq) was added to a solution of biotin (0.35 g, 1.42 mmol, 1.30 eq) and *O*-(1*H*-Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) (0.51 g, 1.33 mmol, 1.22 eq) in 4 mL dry DMF. The mixture was stirred at room temperature for 10 min before it was added dropwise to a solution of *N*-Boc-4,7,10-trioxa-1,13-tridecanediamine (BocNH-TEG-amine) ((0.35 g, 1.09 mmol) in 3.5 mL DMF. Upon complete conversion after 22 h (monitored by TLC), the solvent was removed *in vacuo* and the crude product was purified by flash chromatography (Silica gel, DCM/MeOH 1:0.1).

The obtained BocNH-TEG-Biotin was deprotected as follows. BocNH-TEG-Biotin (0.15 g, 0.27 mmol) was dissolved in 2.5 mL DCM under nitrogen atmosphere and the solution was cooled to 0 °C before 2 mL TFA was added. After 40 min full conversion was identified by TLC and the TFA was evaporated under reduced pressure to give a yellow oil. The residue was purified by flash chromatography (Silica gel, $CHCl_3$/MeOH/NH_3 (25 wt%) 1:0.1:0.01) to afford 100 mg (84 %) slightly yellow, waxy product.

^{1}H NMR (CD_3OD, 400 MHz): δ/ppm: 4.49 (dd, J = 7.8, 4.4 Hz, -NH-C**H**(CH)-CH_2-), 4.30 (dd, J = 7.8, 4.4 Hz, -NH-C**H**(CH)-CH-), 3.65-3.55 (m, **TEG**), 3.52 (t, J = 6.2 Hz, TEG-C**H_2**-CH_2-NH-

C(O)-), 3.26 (t, J = 6.8 Hz, NH_2-C**H$_2$**-TEG), 3.24-3.18 (m, -C**H**-S-), 2.93 (dd, J = 12.7, 5.0 Hz, S-CH_a(**H$_b$**)-CH-), 2.78-2.68 (m, S-C**H$_a$**(H_b)-CH- and CH_2-C**H$_2$**-NH-C(O)-), 2.20 (t, J = 7.4 Hz, NH-C(O)-C**H$_2$**-CH_2-), 1.81-1.55 (m, **TEG** and -C**H$_2$**-CH_2-CH_2-CH(S)-), 1.45 (dd, J = 15.2, 7.4 Hz, -CH_2-C**H$_2$**-CH_2-CH(S)-). **^{13}C NMR (CD_3OD, 100 MHz):** δ/ppm: 176.2, 166.3, 71.8, 71.5, 71.4, 70.7, 70.1, 63.6, 61.8, 57.2, 41.3, 40.3, 38.0, 37.1, 33.5, 30.7, 29.7, 27.1.

4.2 Synthesis of TEG-NH_2



Triethylene glycol monomethyl ether (TEG-OH) (41 g, 0.25 mol) and *p*-toluenesulfonyl chloride (74.5 g, 0.39 mol, 1.56 eq) were dissolved in dry THF (450 mL) and the mixture was cooled to 0 °C under N_2 atmosphere. Triethylamine (44.5 g, 0.44 mol, 1.76 eq) was added dropwise to the solution and stirring was continued at room temperature for 24 h. To this mixture, an aqueous solution (300 mL) of $NaHCO_3$ (30 g, 0.36 mol, 1.43 eq) was added, followed by sodium azide (28.5 g, 0.44 mol, 1.75 eq) in one portion while stirring. THF was distilled off by heating the suspension to 75 °C and the solution was refluxed for 16 h. The cooled reaction mixture was extracted with ethyl acetate (3 x 150 mL) and the organic layer was washed twice with brine. After drying over anhydrous $MgSO_4$, the solvent was removed *in vacuo* to yield a slightly yellow oil.

Afterwards, the azide was reduced to an amine by reaction of the azide with triphenylphosphine (80 g, 0.31 mol) in dry THF (300 mL). The mixture was stirred at room temperature for 24 h until water (100 mL) was added to hydrolyze the phosphorous intermediate during additional stirring for 24 h. The THF was evaporated and the aqueous phase was washed with toluene (3 x 50 mL). The water phase was evaporated to obtain a pale yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 3.47-3.21 (m, **TEG**), 3.13 (s, -O-C**H$_3$**), 2.61 (t, J = 5.2 Hz, -CH_2-C**H$_2$**-NH_2), 1.15 (br s, -CH_2-N**H$_2$**). **^{13}C NMR ($CDCl_3$, 100 MHz):** δ/ppm: 73.1, 71.5, 70.2, 70.1, 69.8, 58.5, 41.4.

5. Synthesis of P(AEA-*co*-TEGA) and P(AEA-*co*-TEGA-*co*-BiotinTEGA)

5.1 Synthesis of P(BocAEA-*co*-PFPA)

t-Boc-aminoethyl acrylate (BocAEA) (1.08 g, 5 mmol, 40 eq) and 1.19 g pentafluorophenyl acrylate (PFPA) (5 mmol, 40 eq) were mixed in 2 mL anhydrous dioxane and the mixture was degassed by three freeze-thaw evacuation cycles. The reaction vessel was placed in a preheated oil bath at 70 °C and 0.048 mL of a degassed stock solution of AIBN (0.03 mmol in 2 mL dioxane) was subsequently injected in a ratio of [M]:[CTA]:[AIBN] = 80/1/0.05. The polymerization was conducted under nitrogen atmosphere and samples for NMR analysis were withdrawn to monitor conversion and to obtain P(BocAEA-*co*-PFPA) random copolymer of desired compositions. After certain reaction time, the reaction was stopped by freezing the vessel in liquid nitrogen. Precipitation from dioxane into hexane/diethyl ether mixture (3:1) was repeated twice before the polymer was freeze-dried from dioxane yielding a yellow solid polymer.

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 5.29 (br, –CH_2-N**H**-Boc), 4.14 (br, -O-C**H_2**-CH_2-), 3.34 (br, -CH_2-C**H_2**-NH-), 3.12-1.59 (br, backbone -C**H_2**-C**H**-), 1.39 (br, -NH-**Boc**), 1.30-1.20 (br, -SC(S)S-CH_2-(C**H_2**)$_{10}$-CH_3), 0.86 (t, -SC(S)S-CH_2-$(CH_2)_{10}$-C**H_3**). **^{13}C NMR ($CDCl_3$, 100 MHz):** δ/ppm: 174.4, 170.8, 156.1, 142.3, 140.9, 139.9, 139.3, 138.6, 136.7, 124.7, 79.5, 64,4, 41.0, 39.5, 35.0, 31.8, 29.5, 29.4, 29.7, 29.4, 29.0, 28.5, 22.7, 14.2. **^{19}F NMR ($CDCl_3$, 376 MHz):** δ/ppm: -161.9, -156.9, -152.9.

5.2 Post-polymerization modification of P(BocAEA-*co*-PFPA) and deprotection

P(BocAEA20-*co*-PFPA20) ($M_{n,calc}$ = 9500 g/mol, 0.3 g, 0.64 mmol in PFPA moieties) was dissolved in anhydrous DMF (3 mL) and the solution was purged with nitrogen. BiotinTEG-NH_2 (14 mg, 0.03 mmol, 0.05 eq) in 2 mL DMF and triethylamine (TEA) (3 mg, 0.03 mmol) was added to this solution. The mixture was stirred for 24 h under a nitrogen atmosphere at room temperature. Afterward, an excess of TEG-NH_2 (0.16 g, 0.96 mmol, 1.50 eq) was added together with TEA (0.09 g, 0.96 mmol). The mixture was stirred for additional 24 h at room temperature to ensure complete conversion of all active ester groups (monitored by ^{19}F NMR). The solution was concentrated in vacuo, precipitated three times in a hexane/diethyl ether mixture (7:3) and freeze-dried from dioxane yielding a yellow highly viscous polymer. The corresponding random copolymer without biotin function was prepared in an analogous way by post-functionalization of P(BocAEA-*co*-PFPA) with a slight excess of TEG-NH_2 and omitting BiotinTEG-NH_2.

Subsequently, the random copolymer P(BocAEA-*co*-TEGA-*co*-BiotinTEGA) or P(BocAEA-*co*-TEGA) was dissolved in dichloromethane (DCM) (2.5 mL) and the solution was cooled to 0 °C while it was purged with nitrogen. Trifluoroacetic acid (TFA) (2.5 mL) was added dropwise to the cooled solution and the reaction mixture was stirred for 4 h at room temperature. After DCM and TFA were removed by evaporation, the residue was dissolved in dioxane and precipitated three times in diethyl ether. The resultant precipitate was collected by centrifugation and lyophilized to give a highly viscous polymeric liquid.

6. Comparison of protein fouling after coating PS colloids with homopolymer PAEA and with random copolymers P(AEA-*co*-TEGA) and P(AEA-*co*-TEGA-*co*-BiotinTEGA)

To allow a better understanding and comparability of the protein-fouling results of the block copolymers discussed within the manuscript, we here make a further comparison to compositionally similar random copolymers (P(AEA-*co*-TEGA) and P(AEA-*co*-TEGA-*co*-BiotinTEGA)) and to the pristine homopolymer (PAEA).

The copolymers were synthesized by RAFT co-polymerization of BocAEA and PFPA as detailed above in Chapter 5.1. After polymerization the PFPA segments were substituted by TEG-NH_2 and Biotin-TEG-NH_2 in similar ratios as compared to PAEA25-*b*-P(TEGA-*co*-BiotinTEGA)33

In detail:

- P(BocAEA-*co*-PFPA) contains 20 units of AEA and 20 units of PFPA, $M_{n,calc,NMR}$ = 9,500 g/mol, $M_{n,SEC}$ = 9,900); $Đ$ = 1.13
- P(BocAEA-*co*-TEGA) contains 20 units of AEA and 20 units of TEG-NH_2, $M_{n,calc,NMR}$ = 7,900, $M_{n,SEC}$ = 7,500); $Đ$ = 1.20
- P(BocAEA-*co*-TEGA-*co*-BiotinTEGA) contains 20 units of AEA and molar ratio of TEG-NH_2/biotin-TEG-NH_2 20:1, $M_{n,calc,NMR}$ = 8,100, $M_{n,SEC}$ = 8,700); $Đ$ = 1.27
- PBocAEA has an $M_{n,calc,NMR}$ = 5,700 g/mol ; $M_{n,SEC}$ = 7,100; $Đ$ = 1.16.

All polymers were used after deprotection.

Figure SI1 summarizes the results.

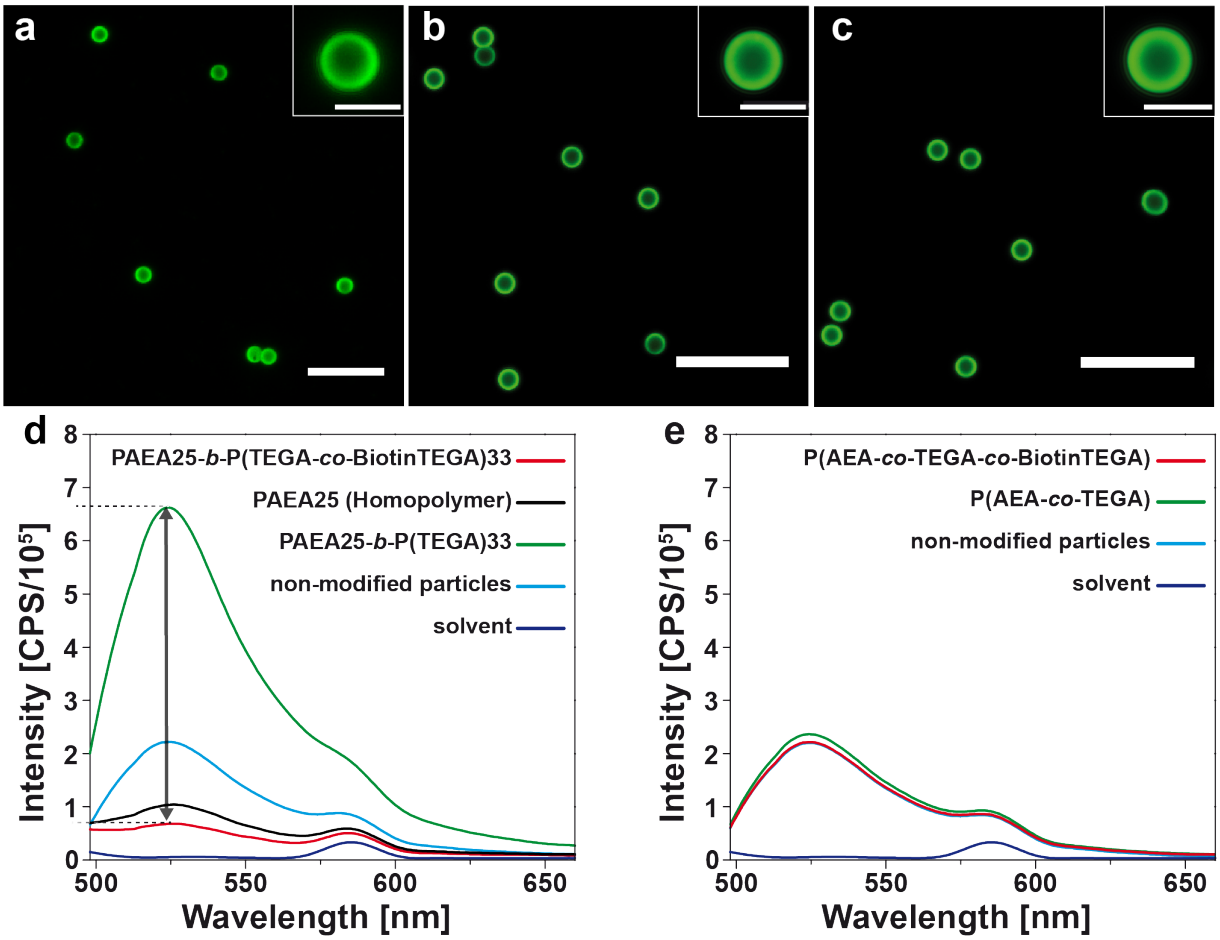


Figure SI 1. Comparison of protein fouling after coating PS colloids with homopolymer PAEA and with random copolymers P(AEA-*co*-TEGA) and P(AEA-*co*-TEGA-*co*-BiotinTEGA). (a-c) Confocal fluorescence micrographs of PS colloids after polymer immobilization and subsequent treatment with fluorescent NAv. Preparation and imaging conditions are equal for all samples. Fluorescence image (a) shows strong protein adsorption after coating with PAEA homopolymer. Fluorescence images (b, c) show strong protein adsorption on PS colloids coated with random copolymers without (a) and with biotin (b). Please see text above for details on polymer composition. All scale bars are 25 µm (insets 5 µm). (d, e) Fluorescence emission spectra (λ_{ex} = 488 nm) of the supernatant to monitor non-adsorbed fluorescent NAv in solution after exposure to differently coated PS colloids. (d) Comparison of homopolymer-coated PS colloids to block copolymer-coated ones and pristine particles. The PAEA homopolymer-coated PS colloids show even stronger non-specific protein adsorption (protein fouling) as the initial non-modified PS particles. A related block copolymer is shown for comparison. The black arrow and dashed lines highlight the differences when incorporating the biotin units into the block copolymers, for which they undergo a change in properties from protein repellent to selective adsorption via biotin/NAv recognition. (e) Comparison of random copolymers with and without biotin segments. Both PS colloids coated with the random copolymers show a similar level of protein adsorption (red and green curve), in fact comparable to untreated PS particles (blue curve). We relate the lack of enhanced protein adsorption after incorporation of biotin segments to possible back-folding of the hydrophobic biotin tags onto the hydrophobic PS particles.

We find that PAEA-coated PS colloids lead to an even higher extent of protein fouling compared to pristine PS colloids (Figure SI1d, compare black and blue curve). This may relate to the changes in ζ-potential from negative to positive values upon coating of the PS colloids with PAEA (see main manuscript). Furthermore, the results clearly show that random copolymers are unsuitable to achieve protein repellency and selective biorecognition. PS colloids coated with the random polymers with and without biotin show a similar level of protein adsorption, in fact comparable to untreated PS particles. We relate the lack of enhanced protein adsorption after incorporation of biotin segments to possible back-folding of the hydrophobic biotin tags onto the hydrophobic PS particles. Obviously the protein fouling is dominated by PAEA segments, which are exposed to the environment for random copolymers, while they are buried as anchor layers in block copolymers.