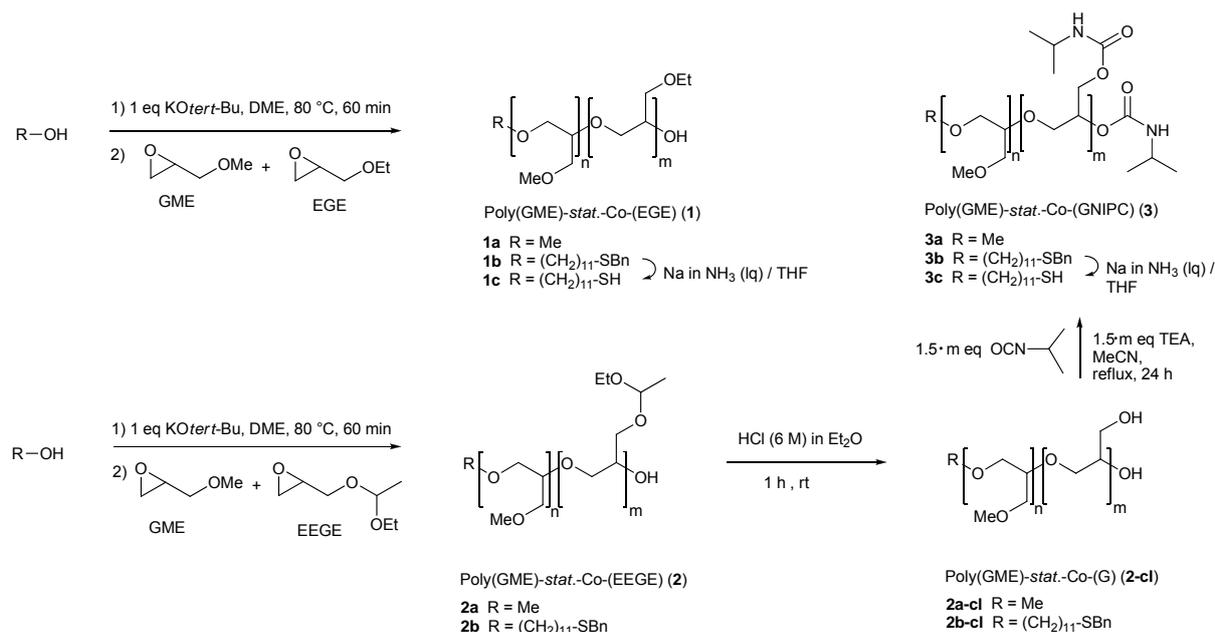


Supporting Information

Switchable, Biocompatible Surfaces Based on Glycerol Copolymers

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SI1. Synthesis



Scheme S1. Synthesis of glycerol based, thermoresponsive copolymers via anionic polymerization.

Materials

All chemicals used were reagent grade, used as received and purchased from Acros Organics (Geel, Belgium) or Sigma-Aldrich (Schnelldorf, Germany) unless stated otherwise. Glycidyl methyl ether (GME) (85%) and ethyl glycidyl ether (EGE) (>98%) were purchased from TCI (Eschborn, Germany) and further purified via fractional distillation before use. Dimethoxyethane (DME) as a solvent for the anionic polymerization was dried over sodium/benzophenone and freshly distilled before use. Fibrinogen (from bovine plasma, F8630), lysozyme (chicken egg white, E.C. 3.2.1.17, L6876), pepsin (porcine gastric mucosa, E.C. 3.4.23.1, P7012) and albumin (bovine serum, Cohn Fraction V, A7030) were purchased from Sigma-Aldrich (Steinheim, Germany) and used as received. Trypsin-EDTA was purchased from Invitrogen (Darmstadt, Germany) and cell culture medium PAA® DMEM (with 10% FCS and 1% penicillin/streptomycin) was supplied by PAA Laboratories GmbH (Pasching, Austria). Phosphate buffered saline (PBS; 10 x concentrated; 90 gL⁻¹ NaCl, 7.95 gL⁻¹ Na₂HPO₄ and 1.44 gL⁻¹ KH₂PO₄; pH 7.4) was purchased from Lonza (Köln, Germany). PBS (1 x concentrated) was freshly prepared by dilution in deionized water and degassed by filtration through 0.22 µm filters prior to use. Degassed (ultrasonification) protein solutions (1 mg mL⁻¹ in PBS) and SDS solution (1 wt % and 4 wt % in PBS) were freshly prepared and filtered through 0.22 µm syringe filters. The deionized water used was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩcm. For SPR measurements and cell adhesion-detachment tests gold coated (48 nm gold, 2 nm adhesive chromium layer on glass) sensor chips (SIA Kit Au) purchased from Biacore (Uppsala, Sweden) and for IRRAS measurements gold coated (200 nm, 2.5 nm adhesive chromium layer, 11x11 mm) glass slides supplied by Arrandee (Berlin, Germany) were used. Both kind of gold surfaces were pre-

cleaned by immersion into freshly prepared piranha solution (3:1, v/v, H₂SO₄:H₂O₂) for 25 s and subsequent exhaustive washing with deionized H₂O (Milli-Q) and EtOH (p.a.) and drying in a stream of N₂ prior to monolayer formation. For the cell culture experiments NIH-3T3 adherent cells (mouse fibroblasts) were used.

Synthesis and characterization

General. ¹H NMR and ¹³C NMR spectra were recorded at 25 °C at concentrations of 100 g·L⁻¹ on a Joel ECX 400 spectrometer, operating at 400 MHz and 100 MHz respectively. NMR chemical shifts were reported as δ values in ppm, coupling constants *J* are given in Hz, and the deuterated solvent peak was used for calibration. Mass spectral data were obtained on an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA) spectrometer at flow rates of 4 μl/min and spray voltage of 4 kV or a Bruker Ultraflex II (MALDI-TOF) instrument using α-hydroxycinnamic acid (HCCA) as matrix material. Analytical GPC measurements were performed on an Agilent 1100 Series instrument including a UV detector (254 nm) as well as a refractive index detector. PS standards have been used for calibration and calculation was performed with PSS Win-GPC software. The measurements were run in THF as the eluent (1 mL min⁻¹, 20 °C), using an array of Suprema Lux 100, Suprema 1000 and Suprema Lux 3000 columns (dimensions: 8 x 300 mm, particle size: 10 μm, PSS, Mainz, Germany). FT-IR measurements were recorded on a Nicolet Avator 320 FT-IR instrument operating from 4000–600 nm and analyzed with the software program EZ OMNIC ESP 5.2. Reactions under exclusion of moisture and air were performed in flame-dried glass ware under Ar atmosphere.

Synthesis of linear statistical copolymers. 11-benzylthio-1-undecanol¹ as initiator of the anionic polymerization, as well as ethoxyethyl glycidyl ether (EEGE)² as a monomer were synthesized according to literature. Anionic statistical copolymerizations of GME, EGE or EEGE were performed after a slightly modified procedure of Hans et al.³ using 11-benzylthio-1-undecanol or methanol as a starter of the polymerization and both GME and EGE, or GME and EEGE as the oxirane comonomers, respectively. In general the anionic ring-opening polymerizations were performed on a scale of a 2–10 g, while the degree of polymerization *x_n* was adjusted via the monomer to initiator ratio (*x_n* = [monomer]/[initiator]) and the statistical copolymer composition was controlled via the ratio of the respective comonomers. In brief, the respective alcohol initiator (1 eq) and KO^{*tert*}-Bu (1 eq) were dissolved in dry DME (minimum quantity) under Ar atmosphere and heated to 80 °C for at least 30 min until the alcohol initiator was fully deprotonated. The generated *tert*-BuOH was removed from the system by distillation under reduced pressure together with DME. The remaining solid alcoholate initiator was dissolved in dry DME (equals the volume of monomer) and heated to 110 °C under Ar atmosphere. The respective, freshly distilled monomers GME and EGE or GME and EEGE (equivalents dependent on the desired *x_n* and the copolymer composition) was added via syringe and septum to the alcoholate and polymerized for 24 h under Ar atmosphere. The reaction was quenched by the addition of H₂O (2–5 mL), concentrated under reduced pressure, and subsequently dried in high vacuum. For further purification the obtained cloudy, yellow oil was dissolved in Et₂O and centrifuged in order to separate from inorganic salts. After concentration of the decanted top layer and drying under reduced pressure the product was observed as a bright, yellow oil in about 90% yield.

Methoxide initiated Poly(GME)-*stat.*-Co-(EGE) (1a)

¹H NMR (400 MHz; CDCl₃): δ 1.14 (42 H, t, *J* = 6.9, CH₃CH₂O); 3.31 (12 H, s, OMe); 3.32 (3 H, s, OMe-Initiator); 3.35–3.75 (118 H, m, polymer backbone + CH₃CH₂O); 3.81–3.95 (1 H, m, terminal CH-OH) ppm. ¹³C NMR (100 MHz; CDCl₃): δ 15.1 (CH₃CH₂O); 59.0 (OMe); 59.1 (OMe-Initiator); 66.6; 69.8; 70.0; 72.6; 78.7 (polymer backbone + CH₃CH₂O) ppm. IR (KBr) *v*_{max}/cm⁻¹: 3497 (w); 2974 (m); 2867 (m); 2814 (w); 1456 (m); 1379 (m); 1352 (w); 1301 (w); 1259 (w); 1197 (m); 1103 (vs); 961 (m); 858 (m). MALDI-TOF: *M_n* = 1836 m/z (4 x GME-unit, 14 x EGE-unit with MeO⁻ initiator + Na⁺). GPC (PS-Standard): PDI = 1.1; *M_n* = 2100 g/mol.

Benzylthioundecanolate initiated Poly(GME)-*stat.*-Co-(EGE) (1b)

¹H NMR (400 MHz; CDCl₃): δ 1.14 (42 H, t, *J* = 6.9, CH₃CH₂O); 1.18–1.35 (14 H, m, CH₂-alkyl); 1.44–1.56 (4 H, m, CH₂CH₂O and CH₂CH₂S); 2.37 (2 H, t, *J* = 7.4, CH₂-SBn), 3.31 (15 H, s, OMe); 3.34–3.77 (130 H, m, polymer backbone + CH₃CH₂O + CH₂-benzyl + alkyl-CH₂O); 3.81–3.94 (1 H, m, terminal CH-OH); 7.16–7.29 (5 H, m, CH_{arom.}) ppm. ¹³C NMR (100 MHz; CDCl₃): δ 15.1 (CH₃CH₂O); 26.0, 28.7, 29.1, 29.2, 29.3, 29.4, 29.4, 29.5, 29.6 (CH₂-alkyl); 31.3 (alkyl-CH₂-S); 36.2 (CH₂-benzyl); 59.0 (OMe); 66.6; 69.8; 70.0; 70.5; 71.5, 72.6, 78.7 (polymer backbone + CH₃CH₂O + alkyl-CH₂O); 126.7; 128.3; 128.7; 138.6 (CH_{arom.}) ppm. IR (KBr) *v*_{max}/cm⁻¹: 2974 (m); 2926 (m); 2865 (m); 2810 (w); 1455 (m); 1379 (m); 1353 (w); 1326 (w); 1300 (w); 1274 (w); 1197 (m); 1106 (vs); 963 (m); 928 (m);

883 (w). MALDI-TOF: $M_n = 2187$ m/z (5 x GME-unit, 14 x EGE-unit with benzylthioundecanolate starter + Na^+). GPC (PS-Standard): PDI = 1.2; $M_n = 2700$ g/mol.

Thiol functionalized Poly(GME)-*stat.*-Co-(EGE) (**1c**)

Deprotection of the thiol-group of **1b** was performed as described earlier.⁴ In brief, **1b** (4 g) was dissolved in a mixture of liquid ammonia/THF = 3:1 under careful exclusion of oxygen. Small amounts of elemental sodium were added until the mixture turned blue. After 1 h stirring at rt, the reaction was quenched by addition of degassed sat. aqueous NH_4Cl . The product was extracted with previously degassed Et_2O under exclusion of oxygen, concentrated under reduced pressure and dried in high vacuum to yield the thiolated polymer **1c** (3.2 g, 80%) as colourless, viscous oil.

^1H NMR (400 MHz; CDCl_3): δ 1.16 (42 H, t, $J = 7.0$, $\text{CH}_3\text{CH}_2\text{O}$); 1.21-1.30 (14 H, m, CH_2 -alkyl); 1.47-1.63 (4 H, m, $\text{CH}_2\text{CH}_2\text{O}$ and $\text{CH}_2\text{CH}_2\text{S}$); 2.50 (2 H, q, $J = 7.6$, CH_2 -SH), 3.31 (15 H, s, *OMe*); 3.36-3.75 (127 H, m, polymer backbone + $\text{CH}_3\text{CH}_2\text{O}$ + alkyl- CH_2O); 3.85-3.97 (1 H, m, terminal CH-OH) ppm. ^{13}C NMR (100 MHz; CDCl_3): δ 15.1 ($\text{CH}_3\text{CH}_2\text{O}$); 26.0, 28.3, 28.9, 29.4, 29.4, 29.4, 29.5, 29.6, 29.6 (CH_2 -alkyl); 33.9 (alkyl- CH_2 -S); 59.1 (*OMe*); 66.6; 69.8; 70.0; 70.4; 71.5, 72.6, 78.7 (polymer backbone + $\text{CH}_3\text{CH}_2\text{O}$ + alkyl- CH_2O) ppm. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2974 (m); 2926 (m); 2865 (m); 2810 (w); 1456 (m); 1379 (m); 1353 (w); 1326 (w); 1301 (w); 1259 (w); 1197 (w); 1106 (vs); 962 (m); 923 (m); 884 (m); 806 (m).

Methoxide initiated Poly(GME)-*stat.*-Co-(EEGE) (**2a**)

^1H NMR (400 MHz; CDCl_3): δ 1.19 (30 H, t, $J = 7.0$, $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 1.23 (30 H, d, $J = 7.0$, $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 3.28 (36 H, s, *OMe*); 3.32-3.75 (125 H, m, polymer backbone + $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 3.77-3.90 (1 H, m, terminal CH-OH); 4.63 (10 H, m, $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$) ppm. ^{13}C NMR (100 MHz; CDCl_3): δ 15.2 ($\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 19.6 ($\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 58.9 (*OMe*); 59.0 (*OMe*-Initiator); 60.6; 69.6; 69.8; 72.5; 78.5 (polymer backbone + $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 99.5 ($\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$) ppm. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 2977 (m); 2875 (m); 2812 (w); 1456 (m); 1380 (m); 1339 (m); 1196 (m); 1082 (vs); 1056 (s); 947(m); 930 (m); 875 (w). MALDI-TOF: $M_n = 2485$ m/z [11 x GME-unit, 10 x EEGE-unit with MeO^- starter + Na^+]; GPC (PS-Standard): PDI = 1.1; $M_n = 3000$.

Methoxide initiated Poly(GME)-*stat.*-Co-(G) (**2a-cl**)

Acidic cleavage of the acetal groups of copolymer **2a** was performed by dissolving **2a** (10 g) in Et_2O (50 mL) and subsequent addition of a 6 M HCl (1 mL) at room temperature. The mixture was stirred for 1 h while the side chain deprotected copolymer precipitated. The acidic solution was decanted and the remaining crude product was purified by repeated precipitation in Et_2O to yield 6.2 g (87%) of the title compound as a colourless oil after drying in high vacuum.

^1H NMR (400 MHz; MeOH-d_4): δ 3.36 (36 H, s, *OMe*); 3.39-3.76 (105 H, m, polymer backbone) ppm. ^{13}C NMR (100 MHz; MeOH-d_4): δ 59.5 (*OMe*); 62.7, 70.9, 73.6, 79.9, 81.5 (polymer backbone) ppm. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3428 (m); 2921 (s); 2875 (s); 2815 (m); 1456 (s); 1397 (m); 1347 (m); 1305 (m); 1258 (m); 1197 (s); 1077 (vs); 962 (s); 922 (s); 857 (m).

Methoxide initiated Poly(GME)-*stat.*-Co-(GNIPC) (**3a**)

Copolymer **2a-cl** (5 g, 2.87 mmol) was dissolved in dry acetonitrile (100 mL) and triethylamine (TEA, 0.086 mol, 6 mL, 15 eq) as well as N-isopropyl isocyanate (0.086 mol, 8.5 mL, 15 eq) were added. The reaction mixture was refluxed for 24 h and then concentrated under reduced pressure. The crude product was dialysed against methanol for 24 h while the solvent was replaced 4 times. The title compound was obtained as a colourless oil (7.4 g, 99%).

^1H NMR (400 MHz; CDCl_3): δ 1.09 (60 H, d, $J = 5.5$, CH_3 -isopropyl); 3.29 (36 H, s, *OMe*); 3.33-3.81 (95 H, m, polymer backbone); 3.83-4.33 (20 H, m, $\text{CH}_2\text{C}(\text{O})\text{NH}$); 4.74-5.64 (br s, 10 H, NH) ppm. ^{13}C NMR (100 MHz; CDCl_3): δ 22.6 (CH_3 -isopropyl); 42.8 (CH -isopropyl); 58.9 (*OMe*); 61.7, 63.5, 69.7, 72.3, 78.4, 79.5 (polymer backbone); 155.4 ($\text{C}=\text{O}$) ppm. IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3445 (m); 3327 (m); 2972 (s); 2923 (s); 2878 (s); 2812 (w); 1722 (s); 1714 (s); 1696 (s); 1537 (s); 1531 (s); 1459 (s); 1387 (m); 1367 (m); 1324 (m); 1248 (s); 1197 (m); 1095 (vs); 960 (w); 851 (w). GPC (PS-Standard): PDI = 1.2; $M_n = 3000$ g/mol.

Benzylthioundecanolate initiated Poly(GME)-*stat.*-Co-(EEGE) (**2b**)

^1H NMR (400 MHz; CDCl_3): δ 1.13 (30 H, t, $J = 7.0$, $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 1.16-1.31 (44 H, m, CH_2 -alkyl + $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 1.41-1.53 (4 H, m, $\text{CH}_2\text{CH}_2\text{O}$ and $\text{CH}_2\text{CH}_2\text{S}$); 2.33 (2 H, t, $J = 7.4$, CH_2 -SBn), 3.31 (33 H, s, *OMe*); 3.32-3.74 (130 H, m, polymer-backbone + $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$ + CH_2 -benzyl + alkyl- CH_2O); 3.79-3.88 (1 H, m, terminal CH-OH); 4.58-4.69 (10 H, m, $\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 7.14-7.25 (5 H, m, $\text{CH}_{\text{arom.}}$) ppm. ^{13}C NMR (100 MHz; CDCl_3): δ 15.1 ($\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$); 19.6 ($\text{CH}(\text{Me})\text{OCH}_2\text{CH}_3$)

25.9, 27.3, 28.7, 29.0, 29.3, 29.3, 29.4, 29.4, 29.5 (CH₂-alkyl); 31.2 (alkyl-CH₂-S); 36.1 (CH₂-benzyl); 59.0 (OMe); 60.6 64.6, 69.8, 71.4; 72.6, 78.5 (polymer backbone + CH(Me)OCH₂CH₃ + alkyl-CH₂O); 99.5 (CH(Me)OCH₂CH₃); 126.7; 128.2; 128.6; 138.5 (C_{arom.}) ppm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3469 (w); 2976 (m); 2925 (m); 2875 (m); 2813 (w); 1456 (m); 1380 (m); 1340 (m); 1303 (w); 1258 (w); 1197 (m); 1082 (vs); 1056 (vs); 948 (m); 930 (m); 873 (m). MALDI-TOF: $M_n = 2748$ m/z [11 x GME-unit, 10 x EEGE-unit with benzylthioundecanolate starter + Na⁺]; GPC (PS-Standard): PDI = 1.2; $M_n = 3000$ g/mol.

Benzylthioundecanolate initiated Poly(GME)-*stat.*-Co-(G) (**2b-cl**)

Acidic cleavage of the acetal side chains of copolymer **2b** (5 g) was performed as described for **2a-cl** and yielded 3.5 g (95%) of the title compound as a colourless oil.

¹H NMR (400 MHz; CDCl₃): δ 1.16-1.34 (14 H, m, CH₂-alkyl); 1.40-1.58 (4 H, m, CH₂CH₂O and CH₂CH₂S); 2.36 (2 H, t, $J = 7.4$, CH₂-SBn), 3.31 (33 H, s, OMe); 3.32-3.74 (110 H, m, polymer backbone + CH₂-benzyl + alkyl-CH₂O); 4.11 (10 H, br s, OH); 7.15-7.28 (5 H, m, CH_{arom.}) ppm. ¹³C NMR (100 MHz; CDCl₃): δ 25.9, 27.2, 28.7, 29.0, 29.0, 29.3, 29.4, 29.4, 29.4 (CH₂-alkyl); 31.2 (alkyl-CH₂-S); 36.1 (CH₂-benzyl); 59.0 (OCH₃); 61.3, 69.6, 71.5, 72.1, 78.4, 79.7 (polymer backbone + alkyl-CH₂O); 126.7; 128.3; 128.6; 138.5 (CH_{arom.}) ppm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3428 (m); 2976 (m); 2925 (m); 2875 (m); 2813 (w); 1456 (m); 1380 (m); 1339 (m); 1304 (w); 1258 (w); 1197 (m); 1080 (vs); 1056 (vs); 948 (m); 930 (m); 873 (m).

Benzylthioundecyl functionalized Poly(GME)-*stat.*-Co-(GNIPC) (**3b**)

Reaction of **2b-cl** (3 g, 1.5 mmol) with N-isopropyl isocyanate was performed as described above for **3a** and yielded the title compound in 96% (4.1 g) as a colourless oil after drying in high vacuum.

¹H NMR (400 MHz; CDCl₃): δ 1.12 (60 H, d, $J = 6.4$, CH₃-isopropyl); 1.19-1.34 (14 H, m, CH₂-alkyl), 1.46-1.56 (4 H, m, CH₂-CH₂-O + CH₂CH₂-S); 2.37 (2 H, t, $J = 7.4$, CH₂CH₂-S); 3.32 (33 H, s, OMe); 3.34-4.38 (120 H, m, polymer-backbone + NHCH(Me)₂ + CH₂-CH₂-O + CH₂-benzyl); 4.79-5.71 (10 H, br s, NH); 7.16-7.29 (5 H, m, CH_{arom.}) ppm. ¹³C NMR (100 MHz; CDCl₃): δ 22.9 (CH₃-isopropyl); 26.0, 27.4, 27.8, 28.8, 29.3, 29.4, 29.5, 29.5, 29.6 (CH₂-alkyl); 31.3 (CH₂-S); 36.2 (CH₂-benzyl); 42.9 (CH-isopropyl); 59.1 (OCH₃); 64.0, 69.9, 71.5, 72.5, 77.6, 78.5 (polymer backbone + CH₂-CH₂-O); 126.8, 128.4, 128.7, 138.6 (CH_{arom.}); 155.7 (C=O) ppm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3326 (m); 2971 (m); 2926 (m); 2875 (m); 2813 (w); 1694 (s); 1530 (s); 1457 (m); 1387 (m); 1367 (m), 1346 (m); 1323 (m); 1243 (s); 1198 (m); 1080 (vs); 957 (m); 849 (m). GPC (PS-Standard): PDI = 1.2 $M_n = 3550$ g/mol.

Thiol functionalized Poly(GME)-*stat.*-Co-(GNIPC) (**3c**)

Deprotection of the thiol-group of **3b** (2.5 g) was performed in analogy to **1c** and yielded compound **3c** in 82% (1.98 g) yield.

¹H NMR (400 MHz; CDCl₃): δ 1.14 (60 H, d, $J = 6.4$, CH₃-isopropyl); 1.23-1.35 (14 H, m, CH₂-alkyl), 1.51-1.63 (4 H, m, CH₂-CH₂-O + CH₂CH₂-S); 2.51 (2 H, q, $J = 7.2$, CH₂CH₂-SH); 3.33 (33 H, s, OMe); 3.37-3.84 (118 H, m, polymer backbone + NHCH(Me)₂ + CH₂-CH₂-O); 4.78-5.66 (10 H, br s, NH) ppm. ¹³C NMR (100 MHz; CDCl₃): δ 22.9 (CH₃-isopropyl); 26.0, 28.3, 29.0, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6 (CH₂-alkyl); 33.9 (CH₂-S); 43.0 (CH-isopropyl); 59.1 (OCH₃); 64.0, 69.8, 71.6, 72.6, 78.5 (polymer backbone + CH₂-CH₂-O); 155.7 (C=O) ppm. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3330 (m); 2971 (m); 2927 (m); 2876 (m); 2813 (w); 1695 (s); 1530 (s); 1457 (m); 1387 (w); 1367 (w); 1323 (w); 1243 (s); 1197 (m); 1080 (vs); 958 (m); 850 (m).

SI 2. Methods

UV transmittance measurements for LCST (cloud point T_{cp}) determination

UV measurements were performed on a Perkin Elmer Lambda 950 UV/vis/NIR spectrometer equipped with a Perkin Elmer PTP-6 Peltier System and the Perkin Elmer software program UV-WinLab DVP Vesion 1.00.00.0010 and TempLab version 2.13. Temperature dependent transmittance measurements of all samples were performed at $\lambda = 500$ nm with a heating rate of 0.5 °C·min⁻¹ and measuring interval of 0.2 °C. The samples were dissolved in PBS to final concentrations of 0.5 mM and 2.5 mM, respectively, and passed through a 0.22 μm pore size syringe filter prior to the measurement. All measurements were performed with five heating and cooling cycles, respectively and the LCSTs were determined from the inflexion points of the obtained curves. The stated values are the mean from five curves. In order to evaluate the effect of the presence of protein on the LCST temperature dependent transmittance of both copolymers was also performed in a solution of 1 mg·mL⁻¹ BSA in PBS.

SAM formation on gold

SAMs from thiol-endfunctionalized polymers **1c** and **3c** were prepared by immersing a pre-cleaned Au-surface into a 1 mM solution of the respective polymer in ethanol for 1 h. The hydrophobic reference SAM of hexadecanethiol (HDT) was prepared by immersing the pre-cleaned gold slide into a 2 mM solution in ethanol for 24 h. Afterwards the SAM modified surfaces were washed thoroughly with ethanol (p.a.) and dried in a stream of N₂.

Surface IR Spectroscopy (IRRAS)

IR-reflection-absorption spectroscopy (IRRAS) of SAM modified Au surfaces from **1c** and **3c** was performed with a nitrogen purged NICOLET 8700 FT-IR spectrometer equipped with a liquid nitrogen cooled MCT detector at 4 cm⁻¹ resolution with 2048 scans at an incident angle of 85° relative to the surface normal from 4000 to 700 cm⁻¹. The spectra are reported in extinction after linear baseline correction. For background measurement a freshly piranha cleaned gold chip was used.

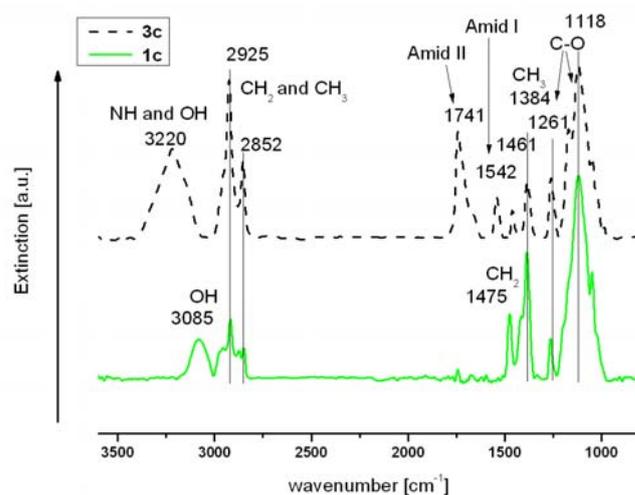


Fig. S1. IRRAS spectra of **1c** and **3c** SAMs on gold.

Contact angle measurements

Static contact angles were measured at the liquid-liquid-solid interface using a Dataphysics OCA-20 system equipped with a CCA camera and evaluated with the software SCA202 applying Young-Laplace fitting. The liquid-liquid-solid interface set up was chosen since the phase transition of the polymers requires the presence of water or in this case PBS which resembles the media of the SPR experiments. Therefore the coated gold slide was placed into PBS for 30 minutes at 25 °C and 40 °C (below and above the LCST of polymer **1c** and **3c** on the surface), respectively for equilibration. Then a drop of diiodomethane (2 μL) was placed on the surface. The drop was allowed to equilibrate for 30 s before imaging. Thereby the SAM surface of **1c** revealed a contact angle of 84.3 ± 2.4 at 25 °C and 78.1 ± 3.1 at 40 °C, while the SAM surface of **3c** showed a contact angle of 89.3 ± 1.6 at 25 °C and 76.7 ± 0.4 at 40 °C. In contrast the contact angle on a bare gold reference did not change within the range of error upon the increase of temperature from 25 °C to 40 °C. Contact angles and standard deviation are the mean from at least three measurements on different locations on the surface. These data further support the presence of a phase transition on the surface by a slight change in hydrophilicity of the surface. At ambient temperature the hydrated polymer yields a more hydrophilic surface compared to the dehydrated polymer above the LCST at 40 °C, therefore the diiodomethane contact angle at ambient temperature is higher than at 40 °C for both polymers, though the difference in contact angle between both states is rather small. This small change in contact angle for these SAM surfaces could be due to the short polymer chains which were applied and might be more pronounced upon increasing the film thickness on the surface or are the result of still a reasonable amount of water fraction within the polymer chains in the collapsed state as it was recently demonstrated for a PEG-based thermoresponsive polymer tethered on surfaces.⁵

SPR experiments

The adsorption of the test proteins to the prepared SAMs of compound **1c** and **3c** was measured following Whitesides's protocol⁶ at different temperatures on a BiacoreTM 3000 Instrument equipped with the BiacoreTM 3000 control software and an integrated thermostat. As the response signal from the measurement given in response units (RU) is highly temperature dependant, temperature is fixed for each measurement and precisely controlled by the integrated thermostat (variation from the set temperature < 0.01 °C). Therefore, temperature cannot be changed during one run.

In brief, the measuring protocol is as follows: (i) passing SDS (0.5 wt% in PBS) over the SAM surface for 3 min and then rinsing the surface with PBS buffer for 10 min; (ii) passing the adequate protein (1 mg mL⁻¹ in PBS) for 30 min over the surface; and finally, (iii) allowing PBS buffer to flow over the surface for an additional 10 min. The flow rate used for all experiments was 10 µL min⁻¹. The baseline difference given in response units ($\Delta RU_{\text{sample}}$) before and after exposure of the sample surface to a protein solution was evaluated relative to the baseline difference (ΔRU_{HDT}) of a hydrophobic HDT reference surface according to the equation given below in % protein adsorption (% PA). The adsorbed amount of proteins on HDT is assumed to be a monolayer of proteins' and therefore the resulting difference in response units was set to 100% protein adsorption.

$$\text{Protein Adsorption [\%]} = \frac{\Delta RU_{\text{sample}}}{\Delta RU_{\text{HDT}}} \cdot 100\%$$

The measurements were performed the way that first the general protein adsorption screening at 25 °C with all test proteins (fibrinogen, pepsin, BSA and lysozyme) was performed. For the temperature dependant adsorption tests only fibrinogen was used as the most "sticky" one from these model proteins. As with elevated temperature issues due to generated air bubbles within the microfluidic device arise, it is suggested to perform temperature dependant measurements starting with the highest temperature and further decrease it, in order to prevent air bubbles which disturb the measurement and generate huge spikes in the sensorgram. Therefore, after the protein adsorption screening at 25 °C temperature was set to 40 °C while PBS was constantly flushing the surface at decreased flow rate. The chip was equilibrated for further 15 minutes after the set temperature was reached. After the measurement temperature was successively decreased to 37 °, 34 °, 31 °, 28 °, and again 25 °C with 15 minutes equilibration at each temperature prior to the measurement. The repeated use of one SAM modified gold slide for all measurements was possible as the SAM surface could always be regenerated after each measurement (performed in duplicate) by an additional SDS (4 wt% in PBS) wash cycle which removed adsorbed proteins indicated by the recovery of the initial response signal before protein injection.

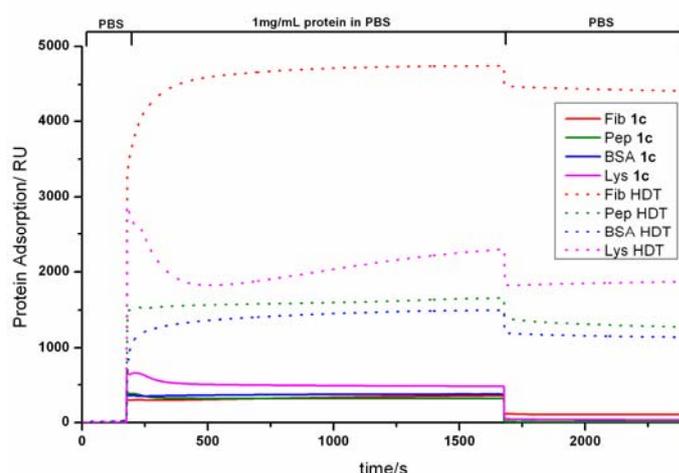


Fig. S2. Typical SPR sensorgram showing the adsorption of fibrinogen, pepsin, BSA and lysozyme on a SAM of **1c** (straight lines) at 25 °C in comparison to the adsorption on a HDT SAM surface (dotted lines).

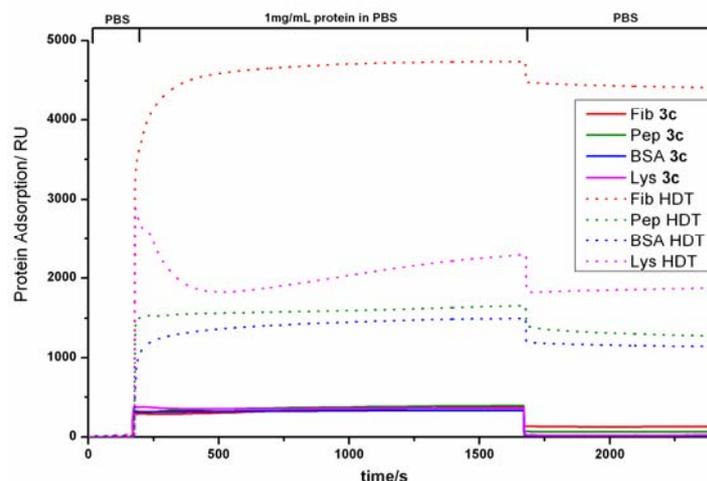


Fig. S3. Typical SPR sensorgram showing the adsorption of fibrinogen, pepsin, BSA and lysozyme on a SAM of **3c** (straight lines) at 25 °C in comparison to the adsorption on a HDT SAM surface (dotted lines).

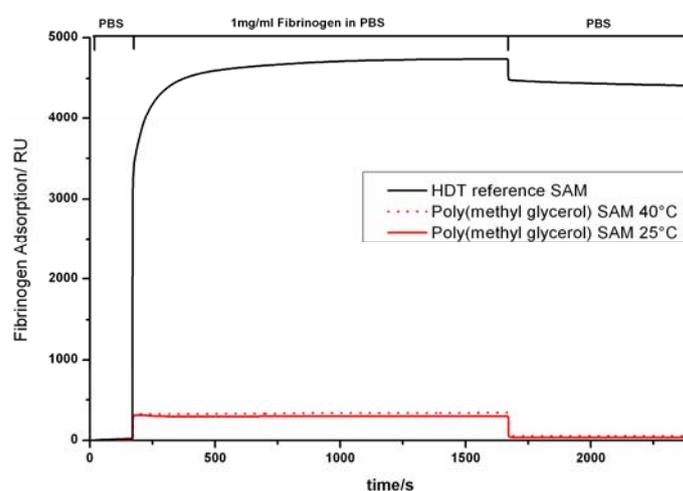


Fig. S4. SPR sensorgram of fibrinogen adsorption on a poly(methyl glycerol) homopolymer SAM surface at 25 °C and 40 °C.

Cell experiments

NIH 3T3 mouse fibroblasts were cultivated at 37 °C and 5% CO₂ in DMEM (PAA Laboratories GmbH, Pasching, Austria) supplemented with 10% FBS, 100 u/mL penicillin, and 100 µg/mL streptomycin. Fibroblasts were harvested from petri dishes by incubation in trypsin (25% Trypsin-EDTA, Gibco Invitrogen) for 5 minutes. The cell suspension was washed from trypsin by centrifugation, the top layer was removed and the remaining cells were resuspended in fresh DMEM. Cell adhesion and detachment studies with the fibroblasts were performed with SAMs of **1c** and **3c** on gold, respectively. A bare gold slide and a tissue culture polystyrene (TCPS) petri dish were used as a reference control. The slides were placed into sterile 12-well TCPS-plates (BD Falcon™). 1 x 10⁶ cells (determined via Neubauer chamber) per well were seeded onto the coated or uncoated gold substrates in 4 mL fresh DMEM. After cell cultivation for 48 h at 37 °C and 5% CO₂ all slides as well as the TCPS control surface were covered with adherent cells at a low degree of confluence. The slides were immediately transferred to a microscope (Zeiss Axio observer Z1 equipped with a Axio Cam MRm camera and a 10 x and 40 x objective) and images of the slides at 37 °C were taken. After 20 minutes at room temperature in cell medium and cell detachment from the SAM coated slides, all surfaces were gently rinsed with fresh medium and again imaged. Representative images are shown. SAM coated gold slides have been used twice in this adhesion and detachment test and did not show any difference in their performance upon reusing them. It should be noted that in case of a high degree of confluence of the adherent cells, whole cell sheets were released from the SAM coated surfaces which occurred much faster than the release of single cells under low confluence conditions.

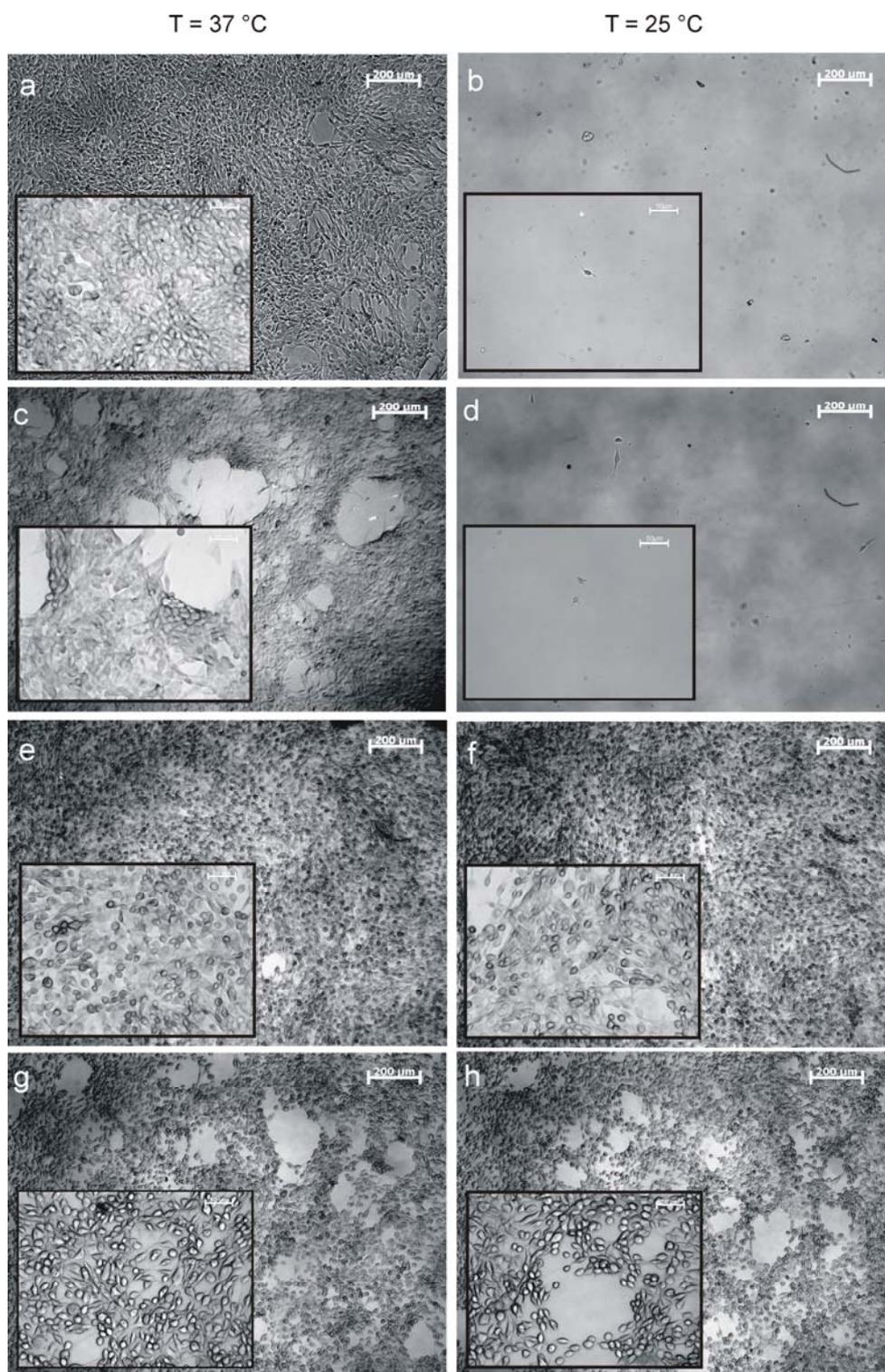


Fig. S5. Representative images of NIH 3T3 fibroblast cells on a **1c** (a,b) and a **3c** (c,d) SAM on a gold as well as on a bare gold (e,f) and a cell culture polystyrene (g, h) reference surface at 37 °C and 25 °C, respectively, with the inserts showing an amplification.

SI3. References

- 1 J. Zhang, J. Kirkham, C. Robinson, M. L. Wallwork, D. A. Smith, A. Marsh and M. Wong, *Anal. Chem.*, 2000, **72**, 1973.
- 2 A. O. Fitton, J. Hill, D. E. Jane and R. Millar, *Synthesis*, 1987, **1987**, 1140.
- 3 M. Hans, H. Keul and M. Moeller, *Biomacromolecules*, 2008, **9**, 2954.
- 4 M. Weinhart, I. Grunwald, M. Wyszogrodzka, L. Gaetjen, A. Hartwig and R. Haag, *Chem. - Asian J.*, 2010, **5**, 1992.
- 5 X. Gao, N. Kucerka, M.-P. Nieh, J. Katsaras, S. Zhu, L. Brash John and H. Sheardown, *Langmuir*, 2009, **25**, 10271.
- 6 R. G. Chapman, E. Ostuni, S. Takayama, E. Holmlin, L. Yan and G. M. Whitesides, *J. Am. Chem. Soc.*, 2000, **122**, 8303.
- 7 E. Ostuni, R. G. Chapman, R. E. Holmlin, S. Takayama and G. M. Whitesides, *Langmuir*, 2001, **17**, 5605.