Supporting Information

Nylon-3 co-polymers that generate cell-adhesive surfaces identified by library screening

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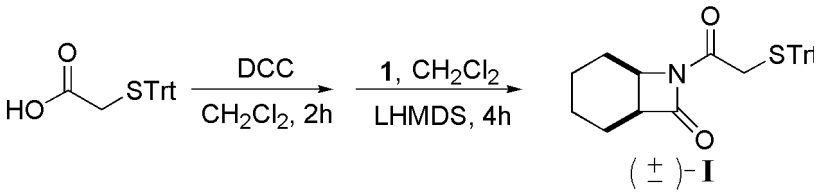
General Methods.

Chemicals, including polyethylene glycol (Mn 3400, 202444), *O*-(2-aminoethyl)polyethylene glycol 3000 (07969), *O,O`*-bis(2-aminoethyl)polyethylene glycol (Mw 3000, 14502), and chambered coverslips (C7735) were purchased from Sigma-Aldrich, amine-functionalized glass (SMM2) from ArrayIt, collagen (PureCol^{TM}) from Inamed Biomaterials, Irgacure 2959 from Ciba, Dulbecco's Modified Eagle's Media (DMEM), LIVE/DEAD viability/cytotoxicity kit (L3224), Quant-iT^{TM} picogreen dsDNA Assay kit (P7589) and NanoOrgane Protein Quantitation Kit (N666) from Invitrogen, Syringe Prefilter plus GF Prefilter with 0.2 C.A. membrane (sterile filter, 192-2520) from Nalgene, round cover glasses (12-545-80 12CIR.-1) from Fisher Scientific.

After they had been seeded on a surface and allowed to grow for a designated time, NIH 3T3 cells were stained with the LIVE/DEAD kit and scanned using a GeneTAC UC 4x4 scanner (Genomic Solution). All scanned data were analyzed using the GeneTAC quantitation program or the GenePix Pro 6.0 demo version.

β-Lactams used for polymer synthesis, **CH**[1], **CO**[2], **MM**[3], **DM**[3b] and **DH**[4], were prepared according to reported procedures.

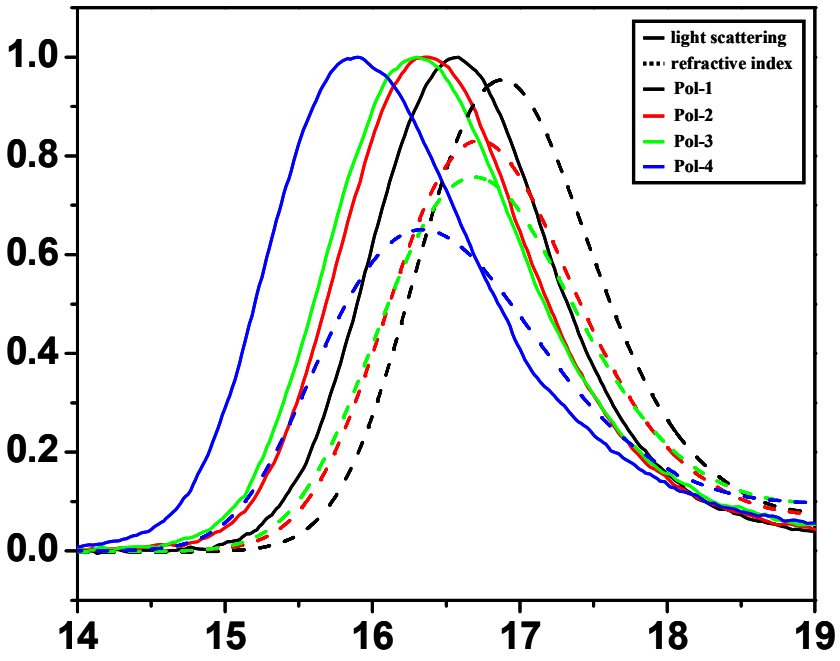


Co-initiator I. To a stirred solution of tritylthioacetic acid (8.9 g, 26.8 mmol) in dry CH_2Cl_2 (190 mL) was added DCC (2.8 g, 13.4 mmol). After 2 h, the reaction mixture was filtered through celite to remove the urea by-product and rinsed with Et_2O. The filtrate was concentrated *in vacuo*. To a stirred solution of the resulting residue and **CH** (1.5 g, 12.1 mmol) in dry CH_2Cl_2 (40 mL) was added 1 N lithium bis-

(trimethylsilyl)amide (12 mL) in THF. After 4 h, the reaction mixture was diluted with EtOAc and washed with 1 N HCl, sat. aq. $NaHCO_3$ and then brine. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by SiO_2 column chromatography (6:1 to 3:1 hexane/EtOAc containing 1% Et_3N) to give **I** in 34% yield as a solid.: mp 156-159 ^oC; ^1H NMR (300 MHz, CD_3OD) δ 7.48-7.38 (m, 6 H), 7.34-7.16 (m, 9 H), 4.09-4.01 (m, 1 H), 3.49-3.36 (m, 2 H), 3.24-3.14 (m, 1 H), 2.06-1.93 (m, 1 H), 1.91-1.62 (m, 3 H), 1.59-1.38 (m, 4 H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.7, 166.6, 144.2, 129.7, 128.0, 126.9, 67.2, 49.8, 46.5, 36.8, 23.1, 19.4, 18.9, 16.9; HRMS (m/z, ESI) calcd for $C_{14}H_{19}NO_4$ $(M+Na)^+$ 464.1655, found 464.1676.

PEG-Diacrylate. To a stirred solution of polyethylene glycol (2 g, 0.59 mmol) in dry CH_2Cl_2 was added Et_3N (0.18 g, 1.77 mmol) and acryloyl chloride (0.16 g, 1.77 mmol) at 0^oC. After 24 h at room temperature, the reaction mixture was poured into Et_2O (400 mL). The precipitated solid was isolated by filtration and dried. After drying, the crude product was dissolved in deionized water (20 mL) and dialyzed to remove $Et_3N{\cdot}HCl$ and minor impurities using 1 L deionized water (2 x 8 h). After dialysis, the solution was lyophilized to give PEG-diacrylate in 85% yield as a solid.: ^1H NMR (300 MHz, $CDCl_3$) δ 6.42 (dd, 1 H, J = 17.4, 1.5 Hz), 6.15 (dd, 1 H, J = 17.4, 10.5 Hz), 5.83 (dd, 1 H, J = 10.8, 1.8 Hz), 4.34-4.27 (m, 2 H), 3.91-3.37 (m, 219 H).

Nylon-3 polymer synthesis and characterization. Polymers Pol-1 to Pol-20 were prepared using reported procedures[3]. The side chain-protected forms of these polymers were analyzed by GPC using a Wyatt MALS and RI detection system (Figure S1). Polymer molecular weights were calculated using ASTRA 5.3.2.15 software. In order to calculate the number-averaged molecular weight (Mn) and polydispersity index (PDI), a dn/dc value 0.1 ml/g was used for all polymers. This value was chosen based on dn/dc measurements made with six selected copolymers (protected members from the CH+MM and DH+MM sets) (Table S1).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refractive index
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Pol-2
Pol-3
Pol-4
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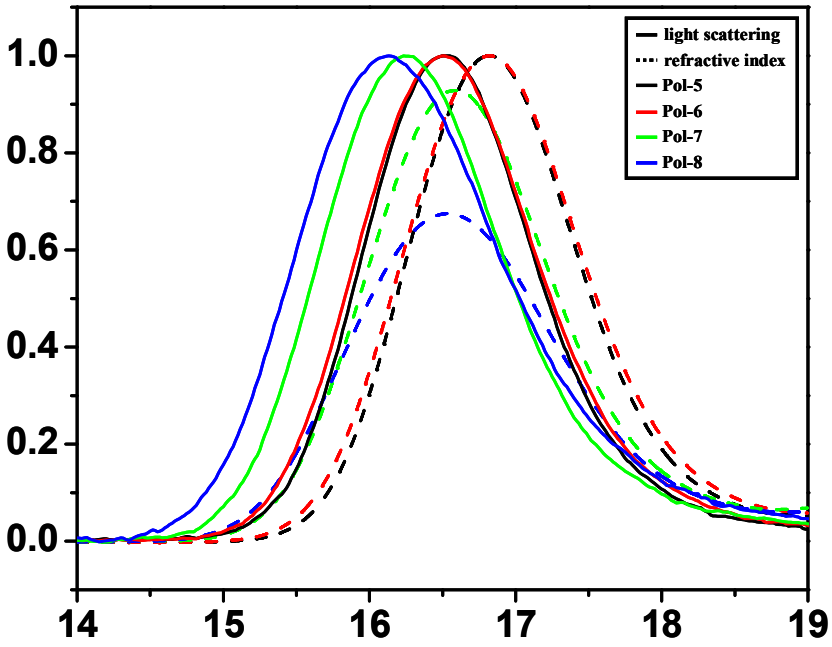
0.6
0.4
0.2
0.0
14
15
16
17
18
19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Pol-6
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17
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19

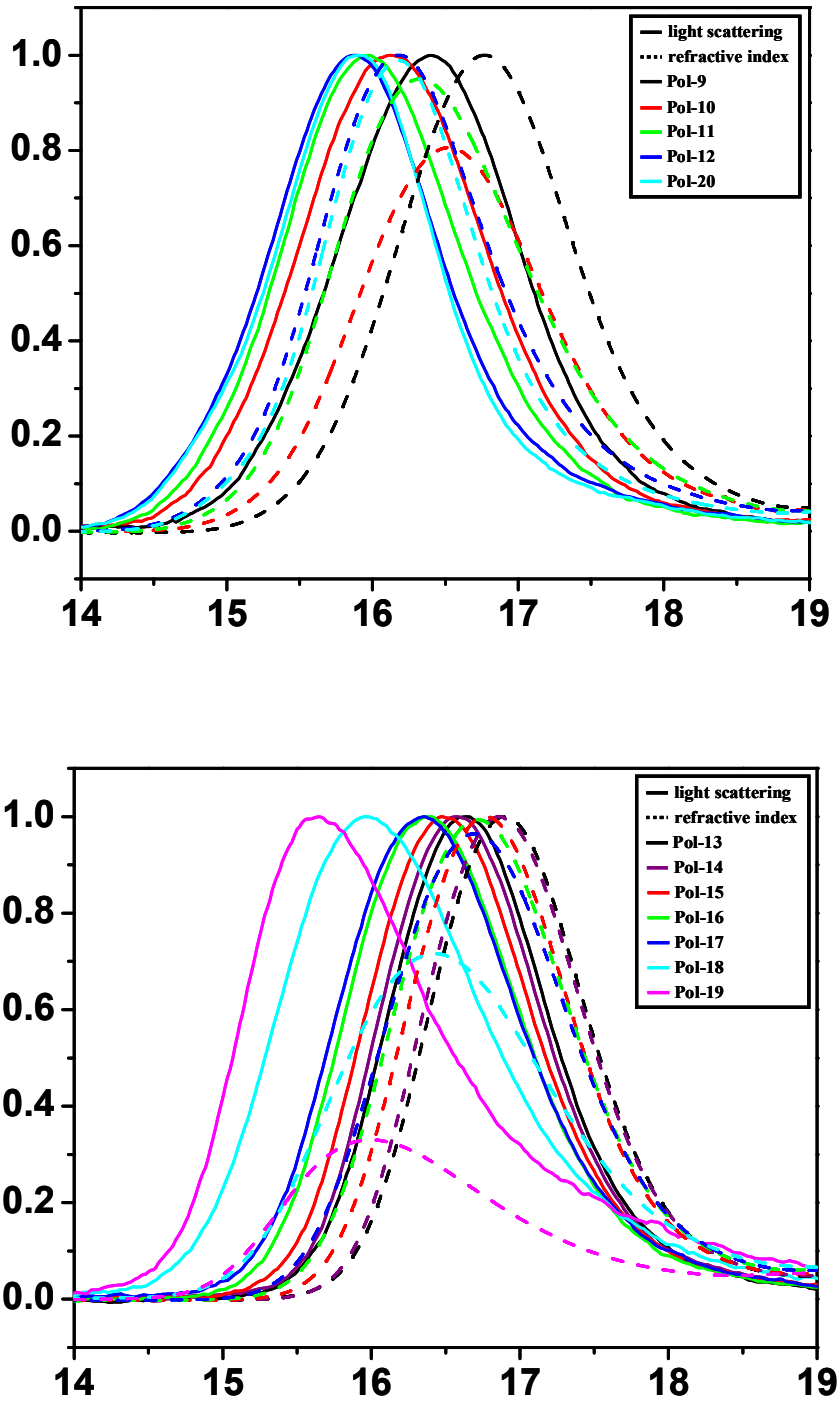


Figure S1. GPC data of polymers Pol-1 to Pol-20

polymer	exp residue	cal residue	Mn	Mw	PDI	Mn(TFA salt)
Pol-1	21	25	4675	5671	1.21	4630
Pol-2	21	26	5038	6114	1.21	5034
Pol-3	21	24	4852	5942	1.23	4860
Pol-4	21	26	5634	7013	1.25	5708
Pol-5	21	26	5125	6318	1.23	5089
Pol-6	21	24	4921	6005	1.22	4901
Pol-7	21	27	5604	6747	1.20	5646
Pol-8	21	26	5658	6806	1.20	5729
Pol-9	21	22	4271	5962	1.40	4199
Pol-10	21	23	4760	6577	1.38	4731
Pol-11	21	23	5103	7039	1.38	5110
Pol-12	21	28	6253	7945	1.27	6344
Pol-13	21	25	5321	6422	1.21	4639
Pol-14	21	24	5117	5372	1.25	4579
Pol-15	21	24	5253	6221	1.18	4833
Pol-16	21	23	4986	6251	1.25	4694
Pol-17	21	22	4962	6172	1.25	4786
Pol-18	21	26	5848	7216	1.23	5822
Pol-19	21	39	8597	9918	1.15	8884
Pol-20	21	26	6250	7894	1.26	6361

Table S1. Molecular weight (Mn, Mw) and PDI of the side chain-protected forms of polymers Pol-1 to Pol-20. The expected number of subunits ("exp residue") in each case is 21 because 5 mol % of the co-initiator was used in each case, and the co-initiator itself contains one residue. The average number of subunits ("cal residue") was calculated based on Mn measured by GPC and the β-lactam proportion used to prepare the polymer.

Preparation of functionalized glass surfaces. Amine-covered glass slides were placed in a staining jar. A 3% solution of succinic anhydride and diisopropylethylamine in DMF (30 mL) was poured into the staining jar, which was then shaken gently for 3 h. After thorough rinsing of the slides with DMF, a 3% solution of DIC and N-hydroxysuccinimide (NHS) in DMF (30 mL) was poured into a staining jar. After gentle shaking for 3 h, the slides were washed with DMF and then dried with N_2 gas. Each dried NHS-activated glass slide was covered with coverslip containing 50 wells. An 8 μL aliquot of control solution 1 (5% (v/v) 2-aminoethanol in 100 mM $NaHCO_3$ containing 15% glycerol), control solution 2 (5% (v/v) ethylenediamine in 100

mM $NaHCO_3$ containing 15% glycerol), control solution 3 (100 mM $NaHCO_3$ containing 15% glycerol), control solution 4 (5 %(w/v) H_2N-PEG-OH (Mw = 3000) in 100 mM $NaHCO_3$ containing 15% glycerol), control solution 5 (5% (w/v) H_2N-PEG-NH_2 (Mw = 3000) in 100 mM $NaHCO_3$ containing 15% glycerol) or of a solution containing one among polymers Pol-1 to Pol-20 (1 mM in 100 mM $NaHCO_3$ containing 15% glycerol) was added into each well (in a predetermined pattern). The glass slide was placed in a water-loaded petri dish humidifying chamber. After 12 h at room temperature, the glass slides were thoroughly washed using a squeeze bottle containing deionized water and then dried with N_2 gas (Figure S2).

To prepare a well containing collagen, an untreated amine-covered glass slide was covered with a coverslip and placed in a Petri dish. An 8 μL aliquot of collagen (1.75 μg/mL in bicarbonate coating buffer) was added into each well. The petri dish was placed in a cold room and incubated 20 h. After incubation, the slide was washed with deionized water (10 uL in each well x 2) and then dried with N_2 gas.

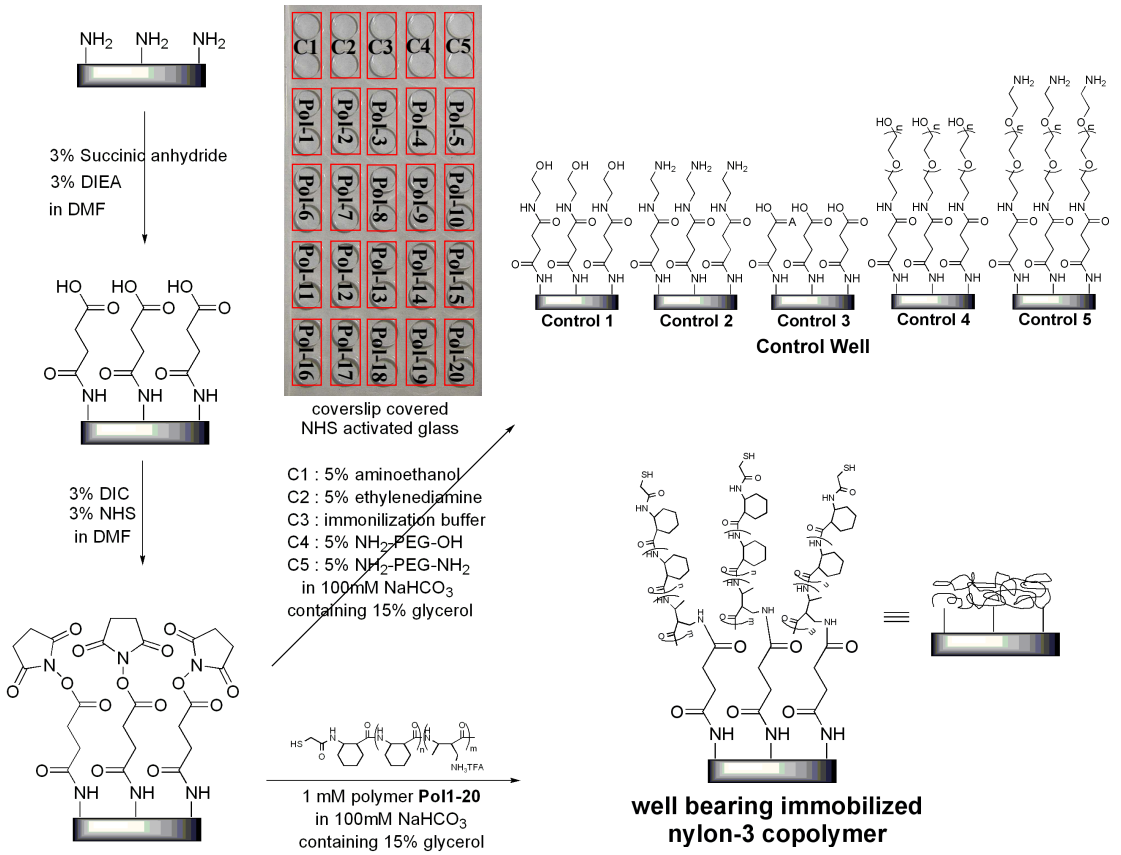


Figure S2. Preparation of functionalized glass surfaces

Screening of NIH 3T3 cell adhesion. 3T3 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine at 37 ^{o}C for 4–5 days. Cells were detached from the TCPS surface using 1% trypsin and collected using centrifugation. The collected cells were suspended in cell culture medium and diluted to the desired density (125000 cells/mL). For experiments conducted without serum, DMEM supplemented with only 1% penicillin/streptomycin and 1% L-glutamine was used. An 8 μL aliquot of cell suspension was added into each well on the glass slide, which had been placed in a petri dish. After incubation for 2 h, the petri dish was filled with culture medium (25-30 mL). After 1 day, excess culture medium was removed by vacuum suction without removing the medium in the wells. A solution of LIVE/DEAD staining fluorescence dye (Ethidium Homodimer-1 (2 μL, final 4 mM) and Calcein AM (0.5 μL, final 2mM) in PBS (650 μL)) was added to the coverslip. After 20 min, the adhered cells in the well were quantitatively analyzed using a GeneTAC UC 4x4 scanner, and then photos were obtained using a fluorescence microscope (Figure S3).

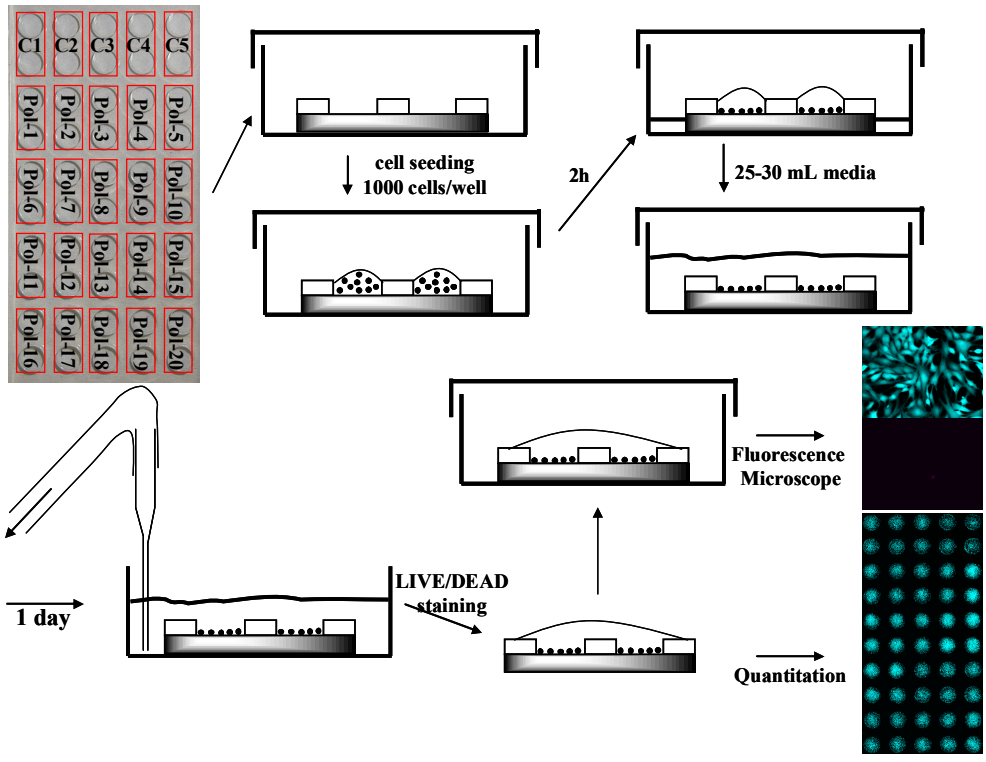


Figure S3. Screening of functionalized glass surfaces for NIH 3T3 cell adhesion.

Quantification of adsorbed serum protein. A BSA standard curve was generated as follows. A stock solution of 2 mg/mL BSA was diluted 200-fold with 1 X

NanoOrange working solution to generate a 10 μg/mL BSA solution, which was further diluted with 1 X NanoOrange working solution to generate 1, 2.5, 5.0, and 7.5 μg/mL standard solutions. An untreated amine-covered glass slide was covered with a coverslip, and then 10 μL each standard solution (and of a solution containing no BSA) was added into predetermined wells. After 1 h incubation in a water-loaded Petri dish humidifying chamber at room temperature, the slide was scanned using a GeneTAC UC 4x4 scanner (Figure S4).

The amount of protein on glass surfaces exposed to culture medium was then determined as follows. Glass slides with wells having a control modified surface or a surface modified with a polymer (Pol-1 to Pol-20) were placed in a water-loaded Petri dish humidified chamber. An 8 μL aliquot of DMEM supplemented with 10 % fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine was added into each well. After 30 min, one slide was washed with deionized water (10 uL in each well x 2) and then dried with N_2 gas; the other slide was washed and dried similarly after 2 hr. A 10 μL aliquot of 1 X NanoOrange working solution was added to each well. After a 1 h incubation in a water-loaded Petri dish humidifying chamber at room temperature, the slide was scanned using a GeneTAC UC 4x4 scanner (Figure S5).

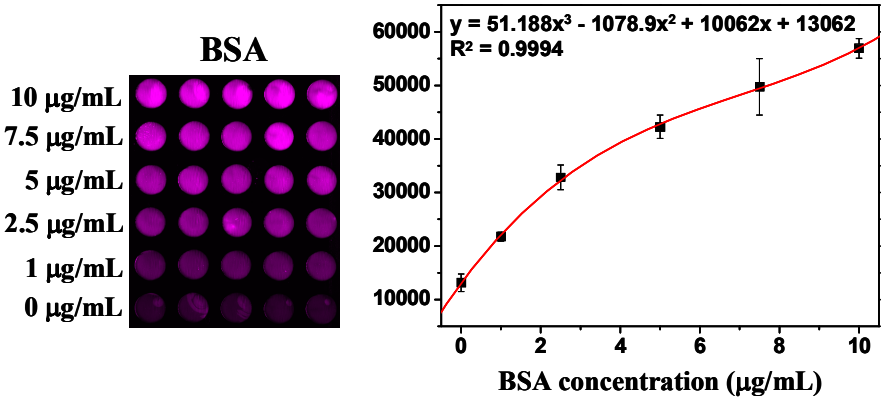
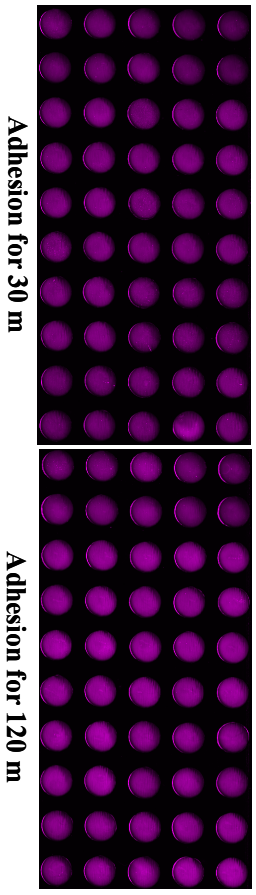


Figure S4. Calibration curve for protein adsorption on glass generated via BSA treatment.



	30m	120m
C1	1.0 μg/mL	1.3 μg/mL
C2	1.2 μg/mL	1.5 μg/mL
C3	1.2 μg/mL	1.9 μg/mL
C4	0.7 μg/mL	1.2 μg/mL
C5	0.4 μg/mL	0.8 μg/mL
Pol-1	1.6 μg/mL	2.3 μg/mL
Pol-2	1.9 μg/mL	2.7 μg/mL
Pol-3	1.6 μg/mL	2.7 μg/mL
Pol-4	1.7 μg/mL	2.6 μg/mL
Pol-5	1.7 μg/mL	2.7 μg/mL
Pol-6	1.7 μg/mL	2.8 μg/mL
Pol-7	2.0 μg/mL	3.1 μg/mL
Pol-8	1.7 μg/mL	2.8 μg/mL
Pol-9	1.7 μg/mL	2.5 μg/mL
Pol-10	1.6 μg/mL	2.7 μg/mL
Pol-11	1.6 μg/mL	2.5 μg/mL
Pol-12	1.9 μg/mL	3.1 μg/mL
Pol-13	1.4 μg/mL	2.0 μg/mL
Pol-14	1.3 μg/mL	2.2 μg/mL
Pol-15	1.1 μg/mL	1.9 μg/mL
Pol-16	1.0 μg/mL	1.7 μg/mL
Pol-17	1.2 μg/mL	2.1 μg/mL
Pol-18	1.3 μg/mL	2.3 μg/mL
Pol-19	1.9 μg/mL	2.6 μg/mL
Pol-20	1.6 μg/mL	2.8 μg/mL

Figure S5. Quantification of adsorbed serum protein on modified glass surfaces.

Cell adhesion on modified PEG hydrogels. PEG-diacrylate (20% (w/v)) in PBS buffer was sterilized using a sterile filter (Syringe Prefilter plus GF Prefilter with 0.2 C.A. membrane). The PEG hydrogels were prepared as follows. Each hydrogel sample was prepared from a solution containing 280 μL 15% (w/v) sterile PEG-diacrylate and 0.05% photoinitiator. The negative control sample contained no other additive. The positive control sample contained 1 mM CGRGDS. The nylon-3 copolymer samples contained 1 mM of Pol-5, Pol-9 or Pol-15. Each of these solutions (60 μL x 4 for each condition) was loaded into a round cover glass, and then another round cover glass was placed on top. The PEG solutions sandwiched between round cover glasses were placed in a UV reactor and irradiated for 5 min. After polymerization, the upper cover glass was removed, and the cover glasses bearing the hydrogels were placed into a 24-well plate. The PEG hydrogels were preincubated with 500 μL cell culture medium, which was exchanged three times (after 8 h in each case) to remove unreacted solutes. Meanwhile, NIH 3T3 cells were suspended in medium and diluted

to the desired density (200,000 cells/mL). A 500 μL aliquot of cell suspension was added into each well. After incubation for 1 day, the culture medium was removed. A 250 μL aliquot of LIVE/DEAD staining fluorescence dye solution (Ethidium Homodimer-1 (11 μL, final 4 mM) and Calcein AM (2.75 μL, final 2 mM) in PBS (5.5 mL)) was added to each well. After 5 min, photographs were obtained with a fluorescence microscope. Then adhered cells are quantified with a picogreen assay. PEG hydrogels were then moved carefully to new wells, and 250 μL M-Per (cell lysis solution) was added to each well. After 30-60 min, cell lysates were collected, and 20 μL of each lysate was diluted 10-fold with 1 X TE buffer. Meanwhile, DNA standard solutions were prepared by diluting a 100 μg/mL DNA stock solution with 1 X TE butter to make 1, 5, 10, 100, 500 and 1000 ng/mL solutions (minimum 200 μL each). A 150 μL aliquot of each DNA standard solution, and a 150 μL aliquot of each 10-fold diluted cell lysate, along with 150 μL 1 X TE buffer (blank) were placed into a 96-well plate. Each solution was mixed with 150 μL 1 X picogreen working solution. After incubation 5 min, the fluorescence of each solution was measured using a fluorescence microplate reader.

References

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3. (a) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 15474-15476. (b) Zhang, J.; Kissounko, D. A.; Lee, S. E.; Gellman, S. H.; Stahl, S. S. *J. Am. Chem. Soc.* **2009**, *131*, 1589-1597.
4. Lee, M.; Stahl, S. S.; Gellman, S. H. *Org. Lett.* **2008**, *10*, 5317-5319.