Supporting Information for

Site Specific Protein Immobilization Into Structured Polymer Brushes Prepared by AFM Lithography

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General

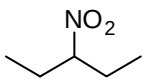
All reactions were performed under an argon atmosphere using standard Schlenk technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DPX-300 (300 MHz), a Bruker AV 400 (400 MHz) or a Varian Inova 500 (500 MHz). Chemical shifts δ in ppm are referenced to $SiMe_4$ as an internal standard or to the solvent residual peak. TLC was carried out on Merck silica gel 60 F_{254} plates; detection by UV or dipping into a solution of $KMnO_4$ (1.5 g), $NaHCO_3$ (5.0 g) and H_2O (400 mL) followed by heating. Flash chromatography (FC) was carried out on Merck silica gel 60 (40 – 63 μm) at about 0.4 bar. Melting points were determined on a SMP 10 apparatus (Stuart Scientific) and are uncorrected. IR spectra were recorded on a Digilab FTS 4000 equipped with a MKII Golden Gate Single Reflection ATR System or on a Bruker IFS 28 or on a Shimadzu FTIR 8400S. ESI-MS and HRMS were

performed using a Bruker MicroTof and a Waters-Micromass Quattro LCZ (only ESI-MS). Size exclusion chromatography (SEC) was carried out with degassed THF as eluent at a flow rate of 1.0 mL/min at rt on a system consisting of a L6200A Intelligent Pump (Merck Hitachi), a set of two PLgel 5 µm MIXED-C columns (300 × 7.5 mm, Polymer Laboratories) and a Knauer RI Differential-Refraktometer detector. Data were analyzed with PSS WinGPC Compact V.7.20 software (Polymer Standards Service) based upon calibration curves built upon polystyrene and poly(methyl-methacrylate) standards (Polymer Laboratories Polystyrene Medium MW Calibration Kit S-M-10 to determine the molecular weight of styrene and poly(methyl-methacrylate) Medium MW Calibration Kit M-M-10 to determine the molecular weight of *n*-butyl acrylate) with peak molecular weights ranging from 1660 to 1000000 g/mol. Elemental analyses were performed on a Vario EL III (Elementar-Analysensysteme GmbH) at the University of Münster. All AFM measurements were done on a commercial AFM (Digital Instruments, Dimension 3000 with a Nanoscope IIIa controler) running in tapping mode under ambient conditions. Si cantilevers (Nanosensors) with eigenfrequencies of 250–350 kHz were used. AFM lithography was performed on the same instrument in contact mode. Fluorescence microscopy was conducted on an Olympus BX41 (Japan) Fluorescence microscope.

Materials and Synthesis

Styrene and *n*-butyl acrylate were both distilled under reduced pressure from CaH_2 to remove stabilizer. Et_2O was distilled over K/Na, benzene was distilled from Na, THF was distilled from K and CH_2Cl_2 was distilled from P_2O_5. All other chemicals were used as received. All proteins used in this study are commercially available. Streptavidin was received from Luminartis, concanavalin A from Vector Laboratories and bovine serum albumin from Invitrogen. The silicon wafer with 300nm oxide layer was purchased from Si-Mat GmbH, Germany.

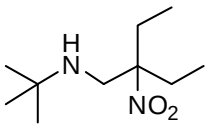
3-Nitropentane (4)



3-Brompentane (95%, 42.4 mL, 330 mmol, 1.00 eq) was dissolved in DMSO (260 mL) and $NaNO_2$ (18.4 g, 415 mmol, 1.26 eq) was added. The reaction mixture was stirred for 21 h at 33-35 °C. The reaction was stopped by adding a water/ice mixture (310 mL) and the resulting solution was extracted with pentane (3 × 100 mL). The combined organic layers were washed with water and dried over $MgSO_4$. After filtration and removal of the solvents *in vacuo*, the crude product **4** was obtained as a yellow liquid and was

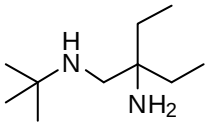
used without further purification (18.3 g, 156 mmol, 47%). The analytical data were in accordance to those described in the literature.[1] ^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ = 4.40-4.27 (*m*, 1H, CH), 2.01-1.81 (*m,* 2H, CH_2), 1.81-1.63 (*m*, 2H, CH_2), 0.89 (*t*, *J* = 7.4 Hz, 6H, 2×CH_3).

N-tert-Butyl-(2-ethyl-2-nitrobutyl)-amine (5)



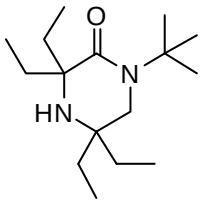
tert-Butylamine (165 mL, 156 mmol, 1.00 eq) was added to 3-nitropentane (18.3 g, 156 mmol, 1.00 eq). Formaldehyde (37% *aq.*, 11.7 mL, 156 mmol, 1.00 eq) was added over a period of 10 min at 20-30 °C. The reaction mixture was stirred for 18 h at 50 °C. Pentane (70 mL) was added at rt and the phases were separated. The organic phase was washed with water (50 mL) and dried over $MgSO_4$. After filtration and removal of the solvents *in vacuo*, the crude product was purified by distillation (95 °C, 6 mbar). Amine **5** was isolated as a colorless oil (18.0 g, 89.0 mmol, 57%). The analytical data were in accordance to those described in the literature.[2] ^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ = 2.91 (*s*, 2H, CH_2), 1.94 (*q, J* = 7.5 Hz, 4H, 2×CH_2), 1.03 (*s*, 9H, 3×CH_3), 0.83 (*t*, *J* = 7.5 Hz, 6H, 2×CH_3).

N-tert-Butyl-(2-ethyl-butane)-1,2-diamine (6)



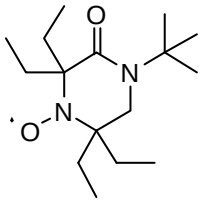
Amine **5** (18.0 g, 88.7 mmol, 1.00 eq) was dissolved in a mixture of AcOH and H_2O (1:1.5, 390 mL). Zinc powder (46.5 g, 710 mmol, 8.00 eq) was added and the resulting suspension was stirred at 80 °C for 2 h. The reaction mixture was stirred at 80 °C for 2 h. It was filtrated directly and the filtrate was evaporated to dryness and the precipitate was dissolved in H_2O (20 mL), treated with NH_3 (*aq. conc.*, 2 mL → pH = 9) and extracted with Et_2O (3 × 40 mL). The combined organic layers were dried over K_2CO_3, filtrated, and the solvent was evaporated *in vacuo*. Diamine **6** was obtained as a colorless oil (12.4 g, 72.0 mmol, 81%) without further purification. The analytical data were in accordance to those described in the literature.[2] ^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ = 2.25 (*s*, 2H, CH_2), 1.35-1.08 (*m*, 4H, 2×CH_2), 0.96 (*s*, 9H, 3×CH_3), 0.72 (*t*, *J* = 7.5 Hz, 6H, 2×CH_3).

1-*tert*-Butyl-3,3,5,5-tetraethyl-2-piperazinon (7)



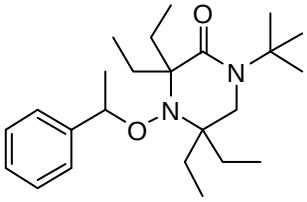
Diamine **6** (12.4 g, 71.7 mmol, 1.00 eq) was dissolved in $CHCl_3$ (8.68 mL, 108 mmol, 1.50 eq). 3-Pentanone (91.0 mL, 861 mmol, 12.0 eq) and powdered KOH (20.0 g, 359 mmol, 5.00 eq) was added at 10 °C. The reaction was stirred at rt for 18 h and stopped by adding H_2O (60 mL). The phases were seperated, the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$. After filtration and removal of the solvents *in vacuo*, the crude product was purified by FC (pentane:MTBE, 2:1) and piperazinone **7** was obtained as a yellow oil (11.1 g, 41.4 mmol, 58%). The analytical data were in accordance to those described in the literature.[2] ^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ = 3.12 (*s*, 2H, CH_2), 1.55 (*q*, *J* = 7.5 Hz, 4H, 2×CH_2), 1.44–1.29 (*m*, 4H, 2×CH_2), 1.38 (*s*, 9H, 3×CH_3), 0.83 (*t*, *J* = 7.5 Hz, 6H, 2×CH_3), 0.80 (*t*, *J* = 7.5 Hz, 6H, 2×CH_3).

1-*tert*-Butyl-3,3,5,5-tetraethyl-2-piperazinone-4-oxyl radical (8)



Peroxyacetic acid (39% in AcOH, 11.3 mL, 58.8 mmol, 1.50 eq) was added dropwise over a period of 20 min to a solution of piperazinone **7** (11.1 g, 39.2 mmol, 1.00 eq) in EtOAc at 0 °C. After stirring for 2.5 h the reaction mixture was hydrolyzed with water, the phases were separated, and the aqueous layer was extracted with pentane (3 × 40 mL). The combined organic layers were washed with $NaHCO_3$ (*aq. sat.*, → pH = 7) and was dried over $MgSO_4$. After filtration solvents were evaporated *in vacuo*. The crude product was purified by FC (pentane:MTBE, 10:1) and nitroxide **8** was obtained as an orange solid (10.0 g, 35.3 mmol, 90%). The analytical data were in accordance to those described in the literature.[2] ESI-MS: m/z: 306 $[M+Na]^+$, 589 $[2M+Na]^+$. HRMS (ESI): m/z calcd for $[M+Na]^+$: 306.2278; found: 306.2277.

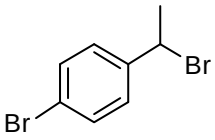
1-*tert*-Butyl-3,3,5,5-tetraethyl-4-(1-phenylethoxy)-piperazin-2-on (2)



(1-Bromoethyl)benzene (550 µL, 3.50 mmol, 1.00 eq), nitroxide **8** (1.09 g, 3.85 mmol, 1.10 eq), copper powder (234 mg, 3.68 mmol, 1.05 eq), $Cu(OTf)_2$ (13 mg, 35 µmol, 1 mol%), and 4,4′-di-*tert*-butyl-2,2′-bipyridyl (38 mg, 140 µmol, 4 mol%) were suspended in benzene (5.0 mL) in a sealed tube. The reaction mixture was stirred at 75 °C for 18 h. Solids were removed by filtration over silica gel, followed by evaporation of the solvents *in vacuo*. Purification by FC (pentane:MTBE, 2:1) afforded the alkoxyamine **2** as a colorless solid (1.20 g, 3.09 mmol, 92%). The analytical data were in accordance to those described in the

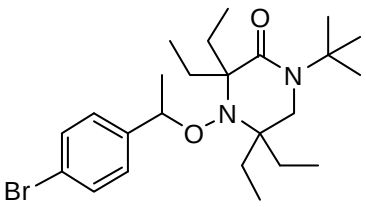
literature.[2] ^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ = 7.38-7.16 (*m*, 5H, Ar-H), 4.78-4.57 (*m,* 1H, C*H*CH_3), 3.23-2.88 (*m*, 2H, CH_2N), 2.16-1.27, 1.67-1.18, 1.12-0.52 (each *m*, 32 H, 4×CH_2, 8×CH_3).

1-Bromo-4-(bromoethyl)benzene (9)



1-(4-Bromophenyl)ethanol (7.04 g, 35.0 mmol, 1.00 eq) was dissolved in CH_2Cl_2 (40 mL) and HBr (33% in AcOH, 7.77 mL, 45.5 mmol, 1.30 eq) was added dropwise at 0 °C. The reaction mixture was stirred for 12 h at rt and $NaHCO_3$ (*aq. sat.*, 20 mL) was added carefully. The phase was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and after filtration solvents were evaporated *in vacuo*. The crude product was purified by FC (pentane) and bromide **10** was obtained as a colorless liquid (8.07 g, 30.6 mmol, 87%). The analytical data were in accordance to those described in the literature.[3] ^{1}H NMR (300 MHz, $CDCl_3$, 295 K): *δ* = 7.47 (*d*, *J* = 8.5 Hz, 2H, Ar-H), 7.31 (*d*, *J* = 8.4 Hz, 2H, Ar-H), 5.15 (*q*, *J* = 6.9 Hz, 1H, BrC*H*CH_3), 2.02 (*d*, *J* = 6.9 Hz, 3H, BrCHCH_3).

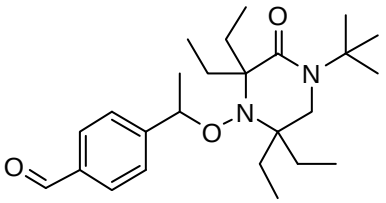
4-(1-(4-Bromophenyl)-ethoxy)-1-*tert*-butyl-3,3,5,5-tetraethylpiperazine-2-on (10)



Nitroxide **8** (2.26 g, 8.00 mmol, 1.05 eq), 1-bromo-4-(1-bromoethyl)benzene (2.22 g, 7.62 mmol, 1.00 eq), $Cu(OTf)_2$ (28 mg, 76 µmol, 1 mol%) and 4,4'-*tert*-butyl-2,2'-bipyridyl (82 mg, 0.31 mmol, 4 mol%) and copper powder (0.51 g, 8.0 mmol, 1.1 eq) were suspended in benzene (20 mL) in a sealed tube. The reaction mixture was stirred at 70 °C for 18 h. Solids were removed by filtration over silica gel, followed by evaporation of the solvents *in vacuo*. Purification by FC (pentane:MTBE, 20:1) afforded the alkoxyamine **10** as a colorless solid (2.23 g, 4.77 mmol, 63%). Mp: 92 °C. IR (neat): 1643*s*, 1483*w*, 1450*w*, 1414*w*, 1303*w*, 1205*m*, 1057*m*, 994*m*, 941*w*, 916*w*, 824*m*, 792*w* cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 295 K): *δ* = 7.42 (*d*, *J* = 8.3 Hz, 2H, Ar-H), 7.15 (*d*, *J* = 7.5 Hz, 2H, Ar-H), 4.71-4.61 (*m*, 1H, OC*H*CH_3), 3.21-3.00 (*m*, 2H, NCH_2), 2.19-1.67, 1.65-1.49, 1.46-1.26, 1.20-0.55 (*m*, 32H, 4×CH_2, 8×CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K)*: δ = 173.0 (C), 143.5 (C), 143.3 (C), 131.5 (CH), 131.4 (C), 128.8 (C), 128.6 (CH), 121.5 (C), 121.1 (C), 82.3 (CH), 73.7 (C), 73.3 (C), 62.8 (C), 62.5 (C), 57.3 (C), 47.1 (CH_2), 46.2 (CH_2), 34.9 (CH_2), 33.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.4 (CH_3), 26.9 (CH_2), 26.7 (CH_2), 24.7 (CH_2), 23.2 (CH_3), 22.2 (CH_3), 11.8 (CH_3), 11.4 (CH_3), 9.5 (CH_3), 8.4 (CH_3), 7.8 (CH_3). ESI-MS: m/z: 469 $[M+H]^+$, 491 $[M+Na]^+$, 959 $[2M+Na]^+$. HRMS (ESI): m/z calcd for

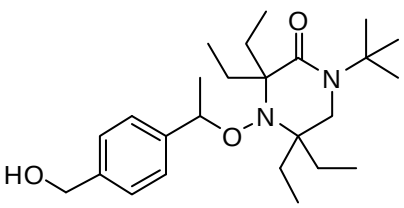
$[M+H]^+$: 469.2248; found: 469.2267. Anal. calcd for $C_{24}H_{39}BrN_2O_2$: C: 61.66, H: 8.41, N: 5.99; found: C: 61.40, H: 8.52, N: 5.86.

4-[1-(4-*tert*-Butyl-2,2,6,6-tetraethyl-3-oxopiperazine-1-yloxy)-ethyl]-benzaldehyde (11)



Alkoxyamine **10** (2.23 g, 4.77 mmol, 1.00 eq) was dissolved in THF (50 mL) and the resulting solution was cooled to -78 °C. *tert*-Butyllithium (1.6M in hexane, 6.26 mL, 10.0 mmol, 2.10 eq) was added slowly and the resulting slurry was stirred at -78 °C for 2 h. DMF (2.10 mL, 27.2 mmol, 5.70 eq) was added slowly and the reaction mixture was warmed to rt over a period of 2 h. NH_4Cl (*aq. sat.*, 4 mL) was added subsequently and the phases were separated. The aqueous phase was extracted with Et_2O (3 × 20 mL) and the combined organic layers were dried over $MgSO_4$. Volatiles were evaporated *in vacuo* after filtration. Aldehyde **11** was obtained as a yellow solid (1.83 g, 4.39 mmol, 92%) and used without further purification. Mp: 101-102 °C. IR (neat): 2961*m*, 2930*w*, 2876*w*, 1695*m*, 1633*s*, 1610*w*, 1456*w*, 1420*w*, 1391*w*, 1344*w*, 1304*w*, 1262*w*, 1206*m*, 1151*w*, 1135*w*, 1059*m*, 990*m*, 936*w*, 911*w*, 889*w*, 834*m*, 803*m*, 760*m*, 701*w*, 617*w*, 546*m* cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 295 K): δ = 9.99 (*s*, 1H, CHO), 7.83 (*d*, *J* = 7.7 Hz, 2H, Ar-H), 7.45-7.43 (*m*, 2H, Ar-H), 4.79-4.74 (*m*, 1H, OC*H*CH_3), 3.21-2.95 (*m*, 2H, NCH_2), 2.12-1.69, 1.67-1.16, 1.11-0.51 (each *m*, 32H, 4×CH_2, 8×CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, 300 K)*; δ = 192.2 (CH), 172.8 (C), 172.7 (C), 151.5 (C), 151.2 (C), 135.9 (C), 135.6 (C), 129.9 (CH), 127.6 (CH), 127.3 (CH), 82.7 (CH), 82.7 (CH), 73.7(C), 73.2 (C), 62.8 (C), 62.6 (C), 57.3 (C), 47.1 (CH_2), 46.1 (CH_2), 34.3 (CH_2), 33.3 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 28.3 (CH_3), 26.9 (CH_2), 26.7 (CH_2), 24.7 (CH_3), 23.3 (CH_3), 22.3 (CH_3), 11.8 (CH_3), 11.3 (CH_3), 9.7 (CH_3), 9.5 (CH_3), 9.2 (CH_3), 8.4 (CH_3), 7.8 (CH_3). ESI-MS: m/z: 417 $[M+H]^+$, 439 $[M+Na]^+$, 856 $[2M+Na]^+$. HRMS (ESI): m/z calcd for $[M+H]^+$: 417.3112; found: 417.3123.

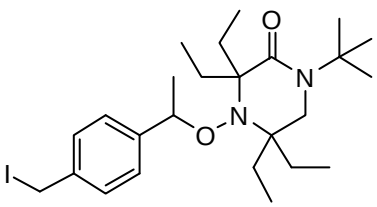
1-*tert*-Butyl-3,3,5,5-tetraethyl-4-[1-(4-hydroxymethylphenyl)-ethoxy]-piperazin-2-on (12)



Aldehyde **11** (2.21 g, 5.30 mmol, 1.00 eq) was dissolved in a mixture of MeOH/AcOH (3:1, 40 mL) at 0 °C and $NaCNBH_3$ (2.21 g, 58.3 mmol, 11.0 eq) was added. The reaction mixture was stirred for 1 h at rt. The solution was carefully neutralized with K_2CO_3 (s) and subsequently extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with NaCl (*aq. sat.*, 30 mL) and dried over

$MgSO_4$. After filtration solvents were evaporated *in vacuo*. The crude product was purified by FC (pentane:Et_2O, 15:1) and alcohol **12** was obtained as a colorless oil (2.19 g, 5.23 mmol, 98%). IR (neat): 2974*w*, 2937*w*, 1634*s*, 1456*w*, 1419*w*, 1205*m*, 1151*w*, 1060*m*, 1017*w*, 992*w*, 910*s*, 818*w*, 730*vs*, 646*w* cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 300 K): δ = 7.35-7.15 (*m*, 4H, Ar-H), 4.74-4.54 (*m*, 3H, OC*H*CH_3, CH_2OH), 3.23-2.89 (*m*, 2H, NCH_2), 2.45 (*bs*, 1H, OH), 2.20-1.50, 1.47-1.28, 1.11-0.53 (each *m*, 32H, 4×CH_2, 8×CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K)*: δ = 173.2 (C), 173.0 (C), 143.7 (C), 143.5 (C), 140.6 (C), 140.2 (C), 127.2 (CH), 126.9 (CH), 82.6 (CH), 73.6 (C), 73.3 (C), 65.1 (CH_2), 62.7 (C), 62.5 (C), 57.3 (C), 47.2 (CH_2), 46.2 (CH_2), 34.8 (CH_2), 33.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 28.3 (CH_3), 26.9 (CH_2), 26.7 (CH_2), 24.7 (CH_2), 24.6 (CH_2), 23.3 (CH_3), 22.2 (CH_3), 11.8 (CH_3), 11.4 (CH_3), 9.7 (CH_3), 9.4 (CH_3), 9.2 (CH_3). 8.4 (CH_3), 7.8 (CH_3). ESI-MS: m/z: 419 $[M+H]^+$, 441 $[M+Na]^+$, 860 $[2M+Na]^+$. HRMS (ESI): m/z calcd for $[M+H]^+$: 419.3268; found: 419.3271. Anal. calcd for $C_{25}H_{42}N_2O_3$: C: 71.73, H: 10.11, N: 6.69; found: C: 71.45, H: 9.83, N: 6.43.

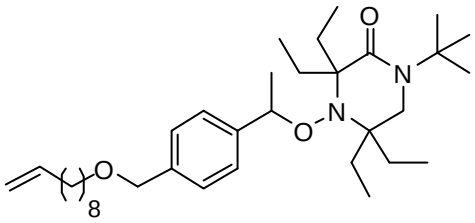
1-*tert*-Butyl-3,3,5,5-tetraethyl-4-[1-(4-iodomethylphenyl)-ethoxy]-piperazine-2-on (13)



Alcohol **12** (1.58 g, 3.70 mmol, 1.00 eq) was dissolved in acetonitrile (20 mL). NaI (1.41 g, 9.40 mmol, 2.50 eq) and TMSCl (0.81 mL, 6.3 mmol, 1.7 eq) was added at 0 °C and the reaction mixture was stirred for 3 h at rt. The reaction was stopped by adding H_2O (20 mL) and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (30 mL), the combined organic layers were washed with $Na_2S_2O_3$ (*aq. sat.*, 2 × 50 mL) and dried over $MgSO_4$. After filtration solvents were evaporated *in vacuo*. Alkoxyamine **13** was obtained as a yellow oil (1.75 g, 3.31 mmol 88%) and used without further purification. IR (neat): 2972*m*, 2938*w*, 1647*vs*, 1457*m*, 1415*w*, 1363*m*, 1327*w*, 1206*s*, 1155*m*, 1061*m*, 992*w*, 913*m*, 842*m*, 731*s*, 583*m* cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 295 K): δ = 7.41-7.28 (*m*, 2H, Ar-H), 7.25-7.16 (*m*, 2H, Ar-H), 4.44 (*s*, 2H, ICH_2), 3.46 (*q*, *J* = 7.0 Hz, 1H, OC*H*CH_3), 3.24-2.93 (m, 2H, NCH_2), 2.22-1.49, 1.48-1.27, 1.24-1.14, 1.10-0.52 (each *m*, 32H, 4×CH_2, 8×CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, 295 K)*: δ = 173.0 (C), 172.8 (C) 144.0 (C), 143.8 (C), 138.7 (CH), 138.3 (CH), 128.8 (CH), 127.6 (CH), 127.4 (CH), 82.2 (CH), 73.6 (C), 73.2 (C), 62.7 (C), 62.4 (C), 57.2 (C), 47.1 (CH_2), 46.2 (CH_2), 34.9 (CH_2), 33.3 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.3 (CH_3), 26.8 (CH_2), 26.6 (CH_2), 24.6 (CH_2), 22.8 (CH_3), 21.9 (CH_3), 11.8 (CH_3), 11.4 (CH_3), 9.7 (CH_3), 9.5 (CH_3), 9.3 (CH_3),

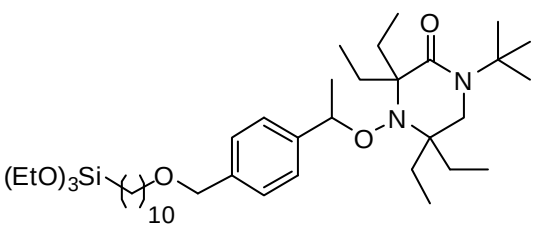
8.4 (CH_3), 7.7 (CH_3), 5.9 (CH_3), 5.8 (CH_3). ESI-MS: m/z: 529 $[M+H]^+$, 551 $[M+Na]^+$, 1079 $[2M+Na]^+$. HRMS (ESI): m/z calcd for $[M+Na]^+$: 551.2105; found: 551.2107.

1-*tert*-Butyl-4-{1-[4-(dec-9-eneyloxymethyl)-phenyl]-ethoxy}-3,3,5,5-tetraethyl-piperazine-2-on (14)



9-Decenol (1.0 mL, 3.0 mmol, 2.0 eq) was dissolved in THF (90 mL) and NaH (60% in mineral oil, 0.24 g, 6.0 mmol, 2.0 eq) was added slowly at rt. The reaction mixture was refluxed for 1.5 h and a solution of alkoxyamine **13** (1.59 g, 3.01 mmol, 1.00 eq) in THF (30 mL) was added at rt. The resulting suspension was refluxed for 24 h and hydrolyzed with NH_4Cl (*aq. sat.*, 30 mL). It was added HCl (*1M aq.*, → pH = 4) and phases were separated. The aqueous layer was extracted with Et_2O (3 × 30 mL) and the combined organic layers were dried over $MgSO_4$. It was filtrated and the crude product was purified by FC (pentane:Et_2O, 70:1). Olefin **14** was obtained as a colorless oil (1.15 g, 2.07 mmol, 68%). IR (neat): 2976*w*, 2961*m*, 1643*vs*, 1483*w*, 1450*m,* 1414*w*, 1341*m*, 1303*w*, 1204*m*, 1131*w*, 1057*m*, 1012*m*, 994*m*, 915*w*, 834*m*, 824*s* cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 295 K): δ = 7.35-7.14 (*m*, 4H, Ar-H), 5.89-5.70 (*m*, 1H, CH_2=C*H*), 5.03-4.85 (*m*, 2H, CH_2=CH), 4.73-4.60 (*m*, 1H, OC*H*CH_3), 4.47 (*s*, 2H, OCH_2Ar), 3.49-3.35 (*m*, 2H, OCH_2CH_2), 3.22-2.91 (*m*, 2H, NCH_2), 2.21-1.96, 1.65-1.52, 1.46-1.19, 1.09-0.54 (each *m*, 46H, 11×CH_2, 8×CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, 295 K)*: δ = 173.2 (C), 173.0 (C) 143.7 (C), 143.4 (C), 139.4 (CH), 138.2 (C), 137.8 (CH), 127.6 (CH), 127.2 (CH), 126.9 (CH), 114.3 (CH_2), 82.6 (CH), 73.6 (C), 73.3 (C), 72.9 (CH_2), 72.8 (CH_2), 70.7 (CH_2), 70.6 (CH_2), 62.8 (C), 62.5 (C), 57.2 (C), 47.1 (CH_2), 46.2 (CH_2), 34.9 (CH_2), 34.0 (CH_2), 33.4 (CH_2), 30.0 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.4 (CH_3), 26.9 (CH_2), 26.6 (CH_2), 26.4 (CH_2), 24.7 (CH_2), 23.3 (CH_2), 22.2 (CH_3), 11.9 (CH_3), 11.4 (CH_3), 9.8 (CH_3), 9.5 (CH_3), 9.3 (CH_3), 8.5 (CH_3), 7.8 (CH_3). ESI-MS: m/z: 557 $[M+H]^+$, 579 $[M+Na]^+$, 1114 $[2M+H]^+$, 1136 $[2M+Na]^+$. HRMS (ESI): m/z calcd for $[M+Na]^+$: 579.4496; found: 579.4495.

1-*tert*-Butyl-3,3,5,5-tetraethyl-4-{1-[4-(-(10-triethoxysilyl)-decyloxymethyl)phenyl]-ethoxy}piperazine-2-on (1)



Olefin **14** (0.24 g, 0.43 mmol, 1.0 eq) and triethoxysilane (71 µL, 0.39 mmol, 0.90 eq) was heated to 40 °C under an argon atmosphere. *Karstedt*-catalyst (0.30 mL, 10 µmol, 1 mol%) was added and the reaction mixture was stirred for 2 h at 40 °C in a sealed tube. Cyclohexane and propylencarbonat was added and the phases were separated. The cyclohexane phase was concentrated and the crude material was purified by FC (pentane:Et_2O, 40:1→4:1). Alkoxyamine **1** was isolated as a colorless oil (0.15 g, 0.21 mmol, 58%) IR (neat): 2973*w*, 2927*m*, 1651*m*, 1208*w*, 1166*w*, 1102*vs*, 1077*vs*, 956*m*, 778*m* cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 300 K): δ = 7.34-7.16 (*m*, 4H, Ar-H), 4.74-4.59 (*m*, 1H, OC*H*CH_3), 4.47 (*s*, 2H, OCH_2Ar), 3.79 (*q*, *J* = 7.0 Hz, 6H, $SiOCH_2$), 3.52-3.35 (*m*, 2H, OC*H*$_2CH_2$), 3.23-2.91 (*m*, 2H, NCH_2), 2.18-1.50, 1.47-1.13, 1.08-0.52 (each *m*, 59H, 13×CH_2, 11×CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K)*: δ = 173.2 (C), 173.0 (C), 143.7 (C), 143.5 (C), 138.2 (C), 137.8 (C), 127.6 (CH), 127.2 (CH), 126.9 (CH), 82.6 (CH), 73.7 (C), 73.3 (C), 72.8 (CH_2), 70.7 (CH_2), 70.6 (CH), 62.8 (C), 62.6 (C), 58.5 (CH_2), 57.2 (C), 47.2 (CH_2), 46.3 (CH_2), 34.8 (CH_2), 33.4 (CH_2), 30.0 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 28.4 (CH_3), 26.9 (CH_2), 26.7 (CH_2), 26.4 (CH_2), 24.7 (CH_2), 23.3 (CH_3), 23.0 (CH_2), 22.2 (CH_3), 18.5 (CH_3), 11.8 (CH_3), 11.4 (CH_3), 10.6 (CH_2), 9.8 (CH_3), 9.5 (CH_3), 9.3 (CH_3), 8.4 (CH_3), 7.8 (CH_3). ESI-MS: m/z: 722 $[M+H]^+$, 744 $[M+Na]^+$. HRMS (ESI): m/z calcd for $[M+H]^+$: 721.5545; found.: 721.5552.

* double set of resonance obtained for specific carbon atoms

Sample Preparation, SIP (NMP) and Protein Immobilization at Surfaces

Silicon wafers with an 300 nm oxide layer were cleaned by ultrasonication in solvents of increasing polarity (pentane, CH_2Cl_2, acetone, methanol, ultrapure water) for 5 min each. The clean surfaces were oxidized with freshly prepared piranha solution (conc. H_2SO_4/H_2O_2 (30%) = 7:3) for 45 min. The surfaces were rinsed again with ultrapure water and dried in an argon flow. The oxidized wafers were placed into a sealed tube and a solution of **1** was added (1.5 mL, 10 mmol/L in dry toluene). The mixture was allowed to stand at rt for 3 d. The surfaces were rinsed with CH_2Cl_2 followed by ultrasonication (5 ×) in CH_2Cl_2 for 5 min each.

Typical Procedure for the Surface Initiated Polymerization of Styrene

A Schlenk tube was charged with alkoxyamine **2** and styrene. The tube was subjected to three freeze-thaw cycles, a silicon wafer (type **A**) containing immobilized alkoxyamine initiator **1** was added and the tube was sealed off under argon. The polymerization was carried out under argon at 105 °C for 24 h. The resulting mixture was cooled to rt and dissolved in CH_2Cl_2. The wafer was taken out of the solution, rinsed with CH_2Cl_2 followed by ultrasonication (5 ×) in CH_2Cl_2 for 5 min each and AFM experiments were carried out. CH_2Cl_2 was removed from the styrene/polystyrene (PS) solution under reduced pressure and residual monomer was removed in a vacuum-drying cabinet at 60 °C for 12 h. Conversion was evaluated gravimetrically; molecular weight and polydispersity index (PDI) were determined by size exclusion chromatography.

1. Styrene (3 mL, 29.1 mmol), external initiator **2** (7.1 mg, 0.063 mol%).

2. Styrene (2 mL, 19.4 mmol), external initiator **2** (8.0 mg, 0.10 mol%).

3. Styrene (1.5 mL, 14.6 mmol), external initiator **2** (11 mg, 0.20 mol%).

4. Styrene (1.5 mL, 14.6 mmol), external initiator **2** (23 mg, 0.40 mol%).

Table 1. PS brushes prepared by NMP.

entry	conversion in %	$M_{n,theo}$ [g/mol]	$M_{n,exp}$ [g/mol]	PDI	thickness [nm]	graft density [chains/nm^2]
1	46%	55410	59360	1.20	50	0.53
2	47%	48505	48201	1.16	22	0.29
3	75%	38866	38454	1.18	10	0.16
4	56%	14674	9713	1.30	5	0.33

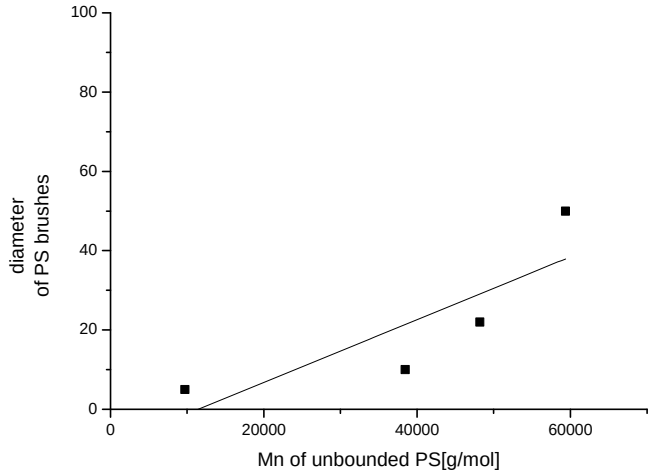


Figure 1. Variation of PS brush thickness in relation to the molecular weight of unbounded PS.

Typical Procedure for the Surface Initiated Polymerization of *n*-Butyl Acrylate

A Schlenk tube was charged with alkoxyamine **2** and *n*-butyl acrylate. The tube was subjected to three freeze-thaw cycles, a silicon wafer containing immobilized alkoxyamine initiator **1** was added and sealed off under argon. The polymerization was carried out under argon at 125 °C for 24 h. The resulting mixture was cooled to rt and dissolved in CH_2Cl_2. The wafer was taken out of the solution, rinsed with CH_2Cl_2 followed by ultrasonication (5 ×) in CH_2Cl_2 for 5 min each and AFM experiments were carried out. CH_2Cl_2 was removed from the *n*-butyl acrylate/poly-*n*-butyl acrylate (PNBA) solution under reduced pressure and residual monomer was removed in a vacuum-drying cabinet at 60 °C for 12 h. Conversion was evaluated gravimetrically; molecular weight and polydispersity index (PDI) were determined by size exclusion chromatography.

1. *n*-Butyl acrylate (3 mL, 20.7 mmol), external initiator **2** (8.0 mg, 0.10 mol%).

Table 2. PNBA brushes prepared by NMP.

entry	conversion in %	$M_{n,theo}$ [g/mol]	$M_{n,exp}$ [g/mol]	PDI	thickness [nm]	graft density [chains/nm^2]
1	89%	114058	135730	1.31	100	0.47

IR analysis of typical polymer brushes

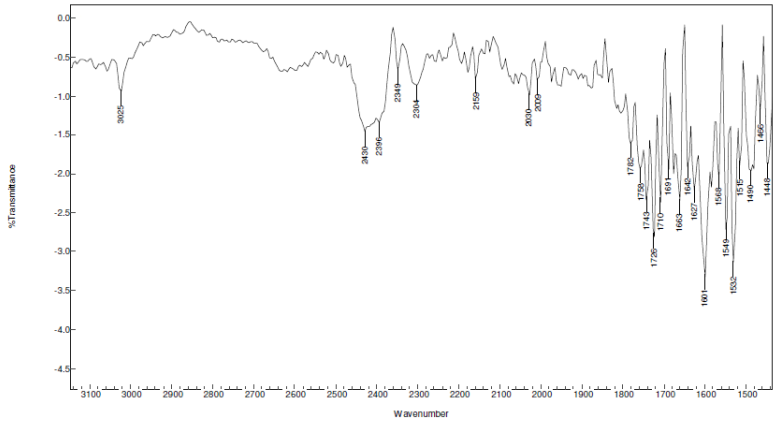


Figure 2. Typical surface IR spectra of a polystyrene based polymer brushes (thickness: 40 nm).

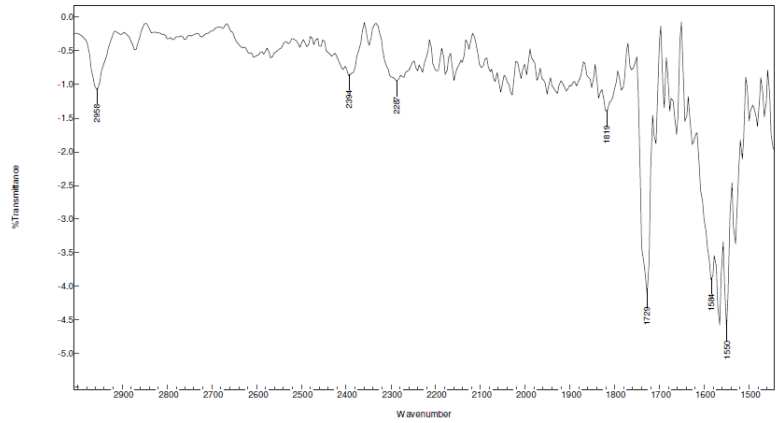


Figure 3. Typical surface IR spectra of a poly *n*-butyl acrylate based polymer brushes (thickness: 40 nm).

Contact Angle measurements of PS and PNBA brushes in correlation of brush thickness

Table 3. Water contact angles of PS and PNBA brushes prepared by NMP.

entry	monomer	thickness [nm]	CA(adv) in degree	CA(rec) in degree
1	styrene	43	87.7±1.4	77.4±0.9
2	styrene	13	86.5±1.4	75.7±1.5
3	*n*-butyl acrylate	32	103.0±1.3	55.1±3.7
4	*n*-butyl acrylate	20	95.9±1.7	57.1±3.1
5	SAM based on **1**	-	73.3±1.2	38.6±1.0

Determination of Lithography Threshold Force

According to our previously reported results AFM lithography experiments with single tips (silicon tapping mode cantilevers, k≈42 N m-1) were performed on a *Dimension 3000 AFM*, operating in contact mode and at a set point of 5V yielding a loading force of about 22 µN, which allowed to engender the reproducible and reliable lithography process.[4]

For achieving large area nanolithography, we utilized multiple tips of AFM system (*DPN 5000 system, NanoInk, Skokie*, USA) to perform parallel writing. Since the cantilever arrays were designed for contact mode application with small k constant (0.5 N/m), we applied a sufficient pressure to the tips to provide reasonable force and therefore achieved an effective removal of the polymer brushes, relying on visual inspection of the cantilever deflection.

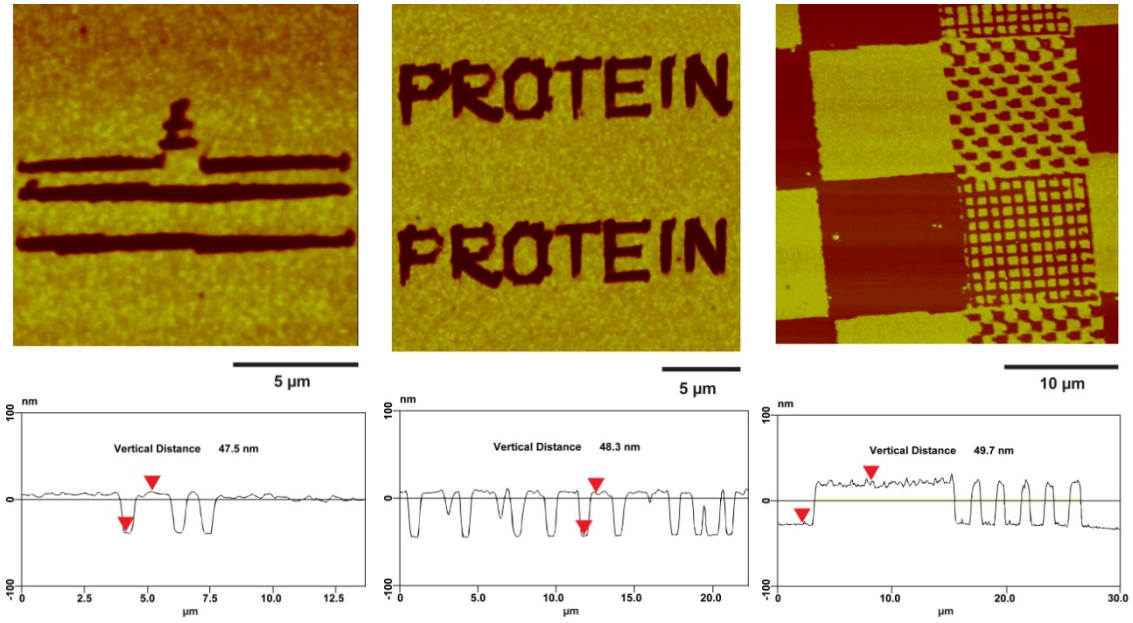


Figure 4. AFM images and elevation profile of PS brush patterns by multi tip based AFM lithography.

Adsorption Studies and Immobilization of Proteins at Polymer brushes

A silicon wafer with grafted polystyrene or poly(*n*-butyl acrylate) brushes was placed into a sealed tube and a buffered solution of Concanavalin A (Rhodamine labeled, 5 mg/mL; buffer: 10 mM HEPES, 0.15 M NaCl, pH=7.5, 0.1 mM Ca^{2+}, 0.08% NaN_3, 0.01 mM Mn^{2+}), streptavidin (Oyster®-488 conjugate in PBS buffer, pH=7.4, contains 1% BSA as stabilizer) or BSA (Rhodamine labeled, 1 mg/mL in NH_4HCO_2 buffer, pH=7.4) was added. The sample was allowed to stand at rt for 1 d. The wafer was ultrasonicated in ultrapure water 3 times for 5 min or washed by simple rinsing the samples with water, without detectable differences. The surface was dried in an argon stream prior to analysis by fluorescent microscopy. We

used PS brushes of 5 nm, 10 nm and thicknesses greater 40 nm. In case of PNBA brushes we used brushes with thicknesses greater 40 nm (e.g. 59 nm or 100 nm).

We applied the same protocol for adsorption of Concanavalin A and streptavidin (rhodamine labeled) towards 22 nm PS brushes and observed adhesion of proteins at the polymer film via fluorescence microscopy.

For site selective protein immobilization the described protocol was applied to silicon wafer with patterns generated by AFM lithography as previously reported.[4]

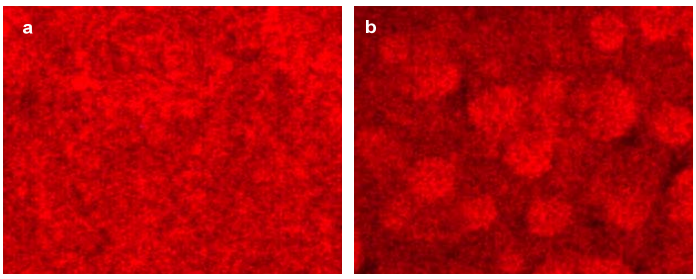


Figure 5. Protein adsorption at polymer brushes with a thickness of 22 nm. Fluorescence images of rhodamine labeled Con A (**a**) and streptavidin (**b**).

Protein Adsorption at PS thin films at SiO_2 substrates prepared by spin coating

Preparation of PS for the spin coating process was achieved as following: a *Schlenk* tube was charged with alkoxyamine **2** (22 mg, 58.2 µmol, 0.2 mol%) and styrene (3.0 mL, 29.1 mmol, 1.0eq). The tube was subjected to three freeze-thaw cycles and the tube was sealed off under argon. The polymerization was carried out under argon at 105 °C for 24 h. The resulting mixture was cooled to rt, dissolved in CH_2Cl_2 and transferred to round bottom flask. CH_2Cl_2 was removed from the styrene/polystyrene solution under reduced pressure and residual monomer was removed in a vacuum-drying cabinet at 60 °C for 12 h and polystyrene I was obtained as colorless solid. Conversion was evaluated gravimetrically; molecular weight and polydispersity index (PDI) were determined by size exclusion chromatography. Conversion: 42%; $M_{n,theo}$: 21952 g/mol; $M_{n,exp}$: 25399 g/mol; PDI: 1.04.

Polystyrene I was transferred to the silicon substrate (silicon wafer bearing a 300 nm oxide layer) via spin coating.

110 nm PS at silicon surface: spin coating with PS in toluene (20 mg/mL) using 1000 rpm.

CA(adv) = 88.2±1.4 °; CA (rec) = 62.6±1.9 °.

300 nm PS at silicon surface: spin coating with PS in toluene (20 mg/mL) using 1500 rpm.

CA(adv) = 104.6±1.9 °; CA (rec) = 82.5±0.79 °.

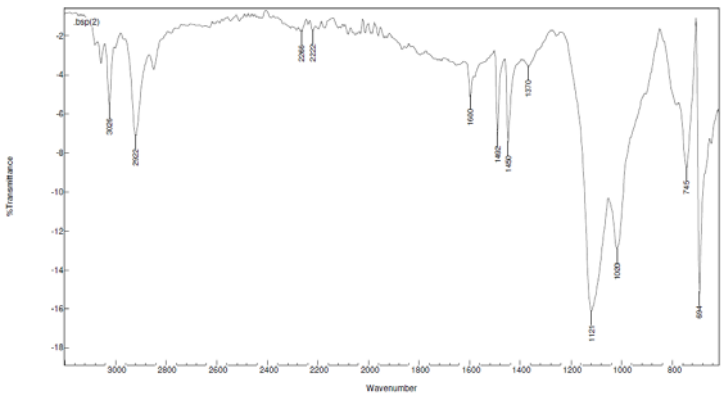


Figure 6. Typical surface IR spectra of a silicon wafer with a polystyrene thin layer, prepared by spin coating (thickness: 300 nm).

A silicon wafer with spin coated polystyrene was placed into a sealed tube and a buffered solution of Concanavalin A (Rhodamine labeled, 5 mg/mL; buffer: 10 mM HEPES, 0.15 M NaCl, pH=7.5, 0.1 mM Ca^{2+}, 0.08% NaN_3, 0.01 mM Mn^{2+}), streptavidin (Oyster®-488 conjugate in PBS buffer, pH=7.4, contains 1% BSA as stabilizer) or BSA (Rhodamine labeled, 1 mg/mL in NH_4HCO_2 buffer, pH=7.4) was added. The sample was allowed to stand at rt for 1 d. The wafer was washed carefully and briefly (due to low stability of spin coated PS at

surfaces) by rinsing the samples with water. The surface was dried in an argon stream prior to analysis by fluorescent microscopy. Spin coated PS of 110 nm and 300 nm was used for the adsorption study. In this case all investigated proteins adsorb to the PS layer (110 nm and 300 nm, Figure 7) and there is no difference in recognition behavior of the corresponding layer thickness by the proteins.

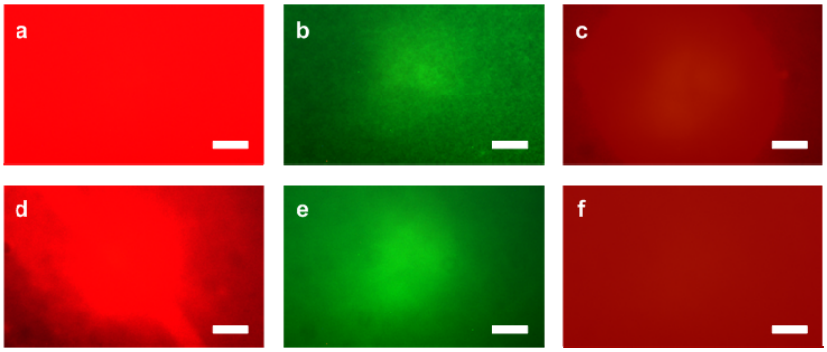


Figure 7. Adsorption studies: adsorption of fluorescent dye labeled Con A (a), streptavidin (b) and BSA (c) on spin coated PS (110 nm). Adsorption of fluorescent dye labeled Con A (a), streptavidin (b) and BSA (c) on spin coated PS (300 nm) as control experiments. Scale bars for a-f: 10 µm.

Protein Adsorption at SiO_2 substrates and Wafer of Type A

A silicon wafer with 300 nm SiO_2 layer or a wafer of type **A** was placed into a sealed tube and buffered solutions of Concanavalin A (Rhodamine labeled, 5 mg/mL; buffer: 10 mM HEPES, 0.15 M NaCl, pH=7.5, 0.1 mM Ca^{2+}, 0.08% NaN_3, 0.01 mM Mn^{2+}), streptavidin (Oyster®-488 conjugate in PBS buffer, pH=7.4, contains 1% BSA as stabilizer) or BSA (Rhodamine labeled, 1 mg/mL in NH_4HCO_2 buffer, pH=7.4) were added. The samples were allowed to stand at rt for 1 d. The wafers were washed carefully by rinsing the samples with water. The surface was dried in an argon stream prior to analysis by fluorescent microscopy. In these cases we observed protein adsorption at least for rinsing conditions.

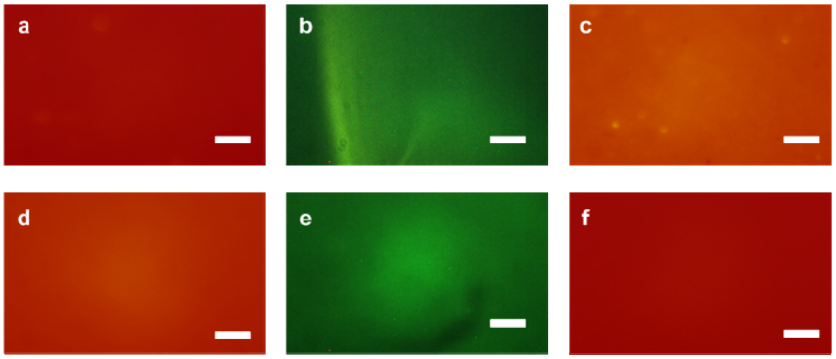


Figure 7. Adsorption studies: adsorption of fluorescent dye labeled Con A (a), streptavidin (b) and BSA (c) on SiO_2 layer at silicon wafer. Adsorption of fluorescent dye labeled Con A (a), streptavidin (b) and BSA (c) on wafer of type **A** as control experiments. Scale bars for a-f: 10 µm.

Protein Adsorption using different concentrations of Con A at PS brushes

A silicon wafer with grafted polystyrene or poly(n-butyl acrylate) brushes was placed into a sealed tube and a buffered solution of Concanavalin A (Rhodamine labeled, 5 mg/mL, 0.05 mg/mL, 0.02 mg/mL and 0.01 mg/mL; buffer: 10 mM HEPES, 0.15 M NaCl, pH=7.5). The sample was allowed to stand at rt for 1 d and washed by simple rinsing the sample with water. The surface was dried in an argon stream prior to analysis by fluorescent microscopy.

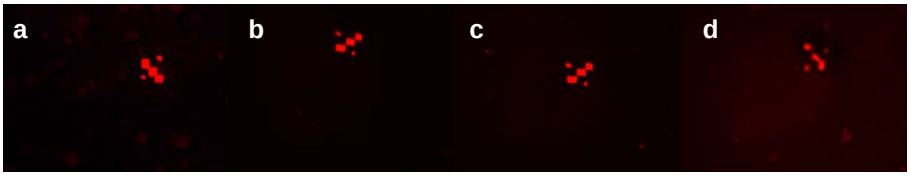


Figure 7. Protein immobilization using different concentrations of Con A at PS brushes 5 mg/mL (**a**), 0.05 mg/mL (**b**), 0.02 mg/mL (**c**) and 0.01mg/mL (**d**) of Con A in HEPES buffer.

BSA-antibody (anti-BSA) interaction at surface

A silicon wafer with grafted poly(*n*-butyl acrylate) brushes (57 nm) was placed into a sealed tube and a buffered solution of BSA (1 mg/mL in NH_4HCO_2 buffer, pH=7.4). The sample was allowed to stand at rt for 1 d. The wafer was ultrasonicated in ultrapure water 3 times for 5 min. The surface was dried in an argon stream. The sample was treated with FITC conjugated sheep anti-BSA (1 mg/mL in PBS buffer, pH=7.2;) at 4°C for 2 h and washed under mild rinsing conditions prior to analysis by fluorescence microscopy (Figure 4a). As a control experiment a silicon wafer with grafted poly(*n*-butyl acrylate) brushes (59 nm) was placed

into a sealed tube. The sample was treated with a buffered solution of FITC conjugated sheep anti-BSA (1 mg/mL in PBS buffer, pH=7.2;) at 4°C for 2 h and washed by mild rinsing conditions prior to analysis *via* fluorescence microscopy (Figure 4b).

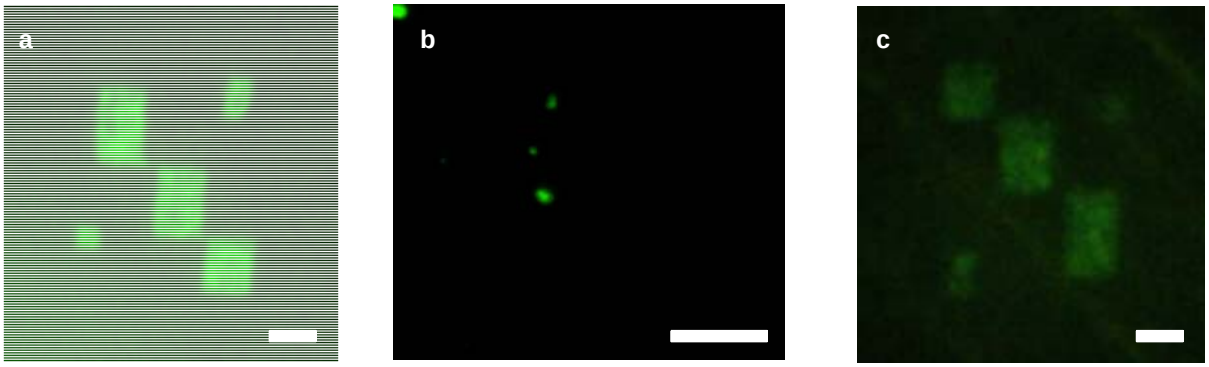


Figure 8. BSA adsorption at patterned poly(*n*-butyl acrylate) brushes and subsequent treatment with a dye tagged sheep anti-BSA (**a**) and treatment of patterned poly(*n*-butyl acrylate) brushes with a dye tagged sheep anti-BSA as a control experiment for 2 h at 4 °C (**b**) and for 24 h at rt (**c**).

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