

Supplementary Information

Chemicals and Reagents. Reaction and washing solvents employed in this experiment were HPLC grade from Fisher Scientific. Anhydrous ethanol was further purified with molecular sieves (4A, Aldrich) and anhydrous CH_2Cl_2 for the dendrimer synthesis was purchased from Aldrich in Sure/Seal bottles. Deionized water (18 $\text{M}\Omega\cdot\text{cm}$) was obtained by passing distilled water through a Barnstead E-pure 3-Module system. (11-Mercaptoundecyl)ammonium chloride (MA) was synthesized by following a literature method.¹ *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS) was purchased from Fluka, and sodium azide from Janssen Chemica. Succinimidyl D-biotin (NHS-biotin) and (+)-biotinyl-3,6,9-trioxaundecanediamine (biotin-LC-PEO-amine) were purchased from Molecular Probes. Immunopure streptavidin was obtained from Sigma. All other reagents purchased were of the highest quality available. For the buffer solutions, a phosphate buffered saline (PBS) solution containing 10 mM phosphate, 2.7 mM KCl, and 137 mM NaCl and a triethanolamine (TEA) buffer solution (50 mM, pH 8.0, 0.25 M NaCl) were used.

General Procedure for Preparing the Substrates for SPR. Substrates were prepared by sequential thermal deposition of chromium (1.5 nm) and gold (50 nm) onto glass cover slips (thickness 0.20 mm, Corning No. 2) and all modifications of substrates were carried out in teflon reaction vessels at ambient condition.

For the biotinylated dendrimer monolayer, gold coated substrates were cut into squares (1.0 cm x 1.0 cm), immersed in an ethanolic solution of MA (2.0 mM) for 24 h, rinsed with ethanol, and dried with a stream of dry nitrogen. The carboxylic group of the dendrimer

(5.0 mM) was activated with an aqueous solution containing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC, 0.10 M) and sulfo-NHS (0.10 M) for 1 h leading to the amine-reactive ester group. The above-prepared MA self-assembled substrate was placed in this solution at room temperature for 2 h, and the substrate was then placed in bicarbonate buffer solution (0.10 M, pH 9.5) for 30 min in order to hydrolyze the remaining ester group. A few residual amines of MA were blocked through a "capping process". In this process, several pieces of the above dendrimer-modified substrate were placed in a dichloromethane solution (20 mL) dissolving 4-(dimethylamino)pyridine (1.0 mg, 8.2 μ mol) and acetic anhydride (1.0 mL, 11 mmol) at room temperature for 12 h. After the reaction, plates were taken out of the flask, washed with CH_2Cl_2 and methanol, and were evacuated to dryness. For Fmoc (or 9-fluorenylmethoxycarbonyl) group deprotection, the substrate was dipped in 5 % (v/v) piperidine in DMF for 20 min, washed with DMF, and dried with a flow of nitrogen. The substrate was then biotinylated with the appropriate amount of NHS-biotin in a DMSO/bicarbonate buffer (55 mM, pH 8.5) solution (4:6 (v/v)) for 2 h, and washed with DMSO and deionized water.

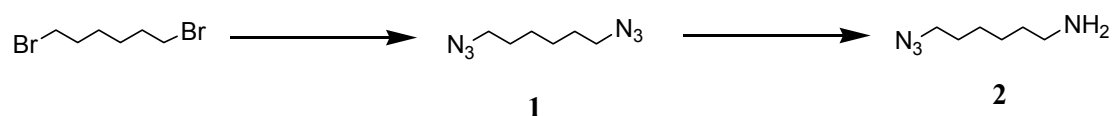
For the mixed monolayers, gold coated substrates were treated with an ethanolic solution (final concentration, 2.0 mM) dissolving 16-MHA/11-MUOH in a 1:12 or 1:100 ratio. The resulting surface was activated with EDC (0.40 M) and sulfo-NHS (0.10 M) to give a reactive ester group. Subsequently, the mixed SAM was derivatized with biotin-LC-PEO-amine in a TEA buffer solution (1.2 mg/mL) for 2 h. Finally, the substrate was placed in a bicarbonate buffer solution (0.10 M, pH 9.5) for 30 min in order to hydrolyze the remaining reactive ester group.

SPR Analysis of Streptavidin-Biotin Interaction. The SPR instrument used in this study was a Biacore-X (BIAcore) and sensor chips were prepared according to Whitesides's method (G. B. Sigal, M. Mrksich and G. M. Whitesides, *J. Am. Chem. Soc.*, 1998, **120**, 3464.). To stabilize the baseline, the biotinylated surfaces were pre-rinsed with PBS for 10 min, and the streptavidin solution (0.50 μM , in PBS) was injected with a flow rate of 1.0 $\mu\text{L}/\text{min}$ for 30 min. After washing with PBS for 30 min, the increase in response (ΔRU) of each layer for streptavidin association was estimated.

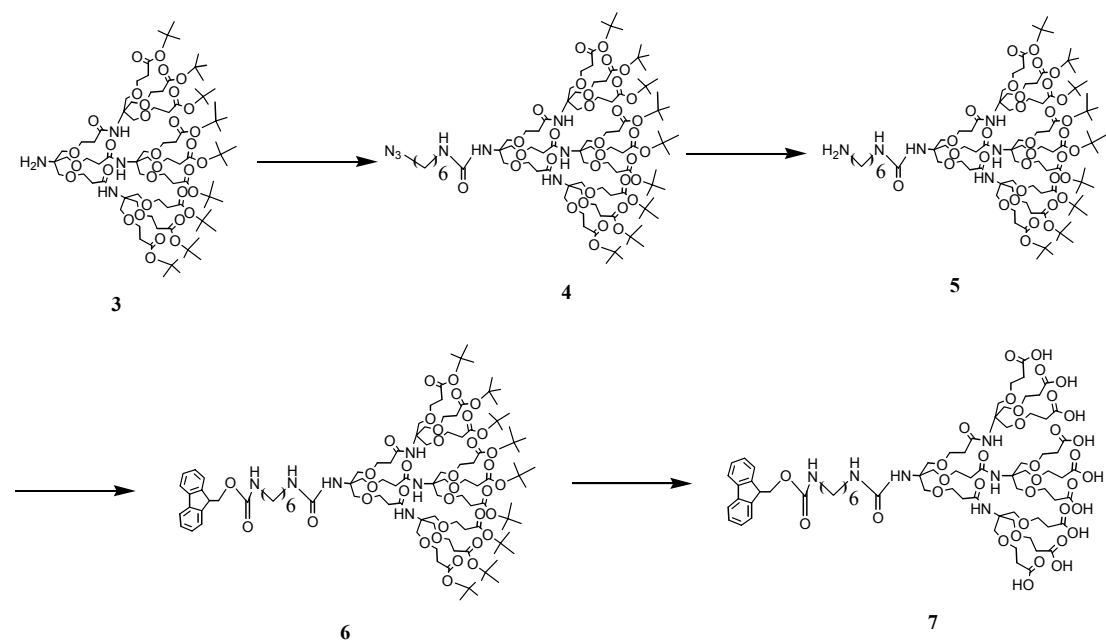
Surface Amine Density of the Dendrimer-Modified Monolayer. As established in this laboratory (B. J. Hong, J. Y. Shim, S. J. Oh and J. W. Park, *Langmuir*, 2003, **19**, 2357.), the strong fluorescence nature of 9-anthraldehyde was used for the measurement. Immediately after Fmoc group deprotection, the substrate was immersed into anhydrous ethanol containing 9-anthraldehyde (1.0 mg/ml) at 50 °C for 6 h under nitrogen atmosphere. After the imine formation, the substrate was washed and sonicated in methanol for 1 min., and then dried with a flow of nitrogen gas. The imine-coupled 9-anthraldehyde was hydrolyzed by placing the substrate in a known amount of water and heating the aqueous solution at 50 °C for 2 h. The resulting solution was sampled, and the fluorescence of 9-anthraldehyde was measured at 523 nm.

Synthesis of the Dendrimer.

1,6-Diazidohexane (**1**) and 6-azidohexylamine (**2**) were prepared from 1,6-dibromohexane according to Kim's method.²



Scheme 1 Synthetic scheme for the dendrimer



N-Tris[2-[[[tris{2-(*tert*-butoxycarbonyl)ethoxy}methyl)methyl]amino]carbonyl]-ethoxy)methyl]methylamine (**3**). This molecule was previously synthesized and characterized by Gawley and a co-worker.³ Gawley's scheme was followed to prepare the nona-*tert*-butyl ester (**3**).

***N*-(6-Azidoheptyl)-*N'*-tris[(2-{{(tris{[2-(*tert*-butoxycarbonyl)ethoxy]methyl)methyl-amino]carbonyl}ethoxy)methyl]methylurea (4).** Triphosgene (58 mg, 0.20 mmol) was dissolved in anhydrous CH₂Cl₂ (2.0 mL). A mixture of 6-azidoheptylamine (76 mg, 0.53 mmol) and *N,N*-diisopropylethylamine (DIEA, 0.11 mL, 0.63 mmol) in anhydrous CH₂Cl₂ (2.0 mL) was added dropwise to the stirred solution of triphosgene over a period of 30 min using a syringe pump. After further stirring for 6 min, a solution of **3** (0.96 g, 0.53 mmol) and DIEA (0.11 mL, 0.63 mmol) in anhydrous CH₂Cl₂ (2.0 mL) was added. The reaction mixture was stirred for 12 h at room temperature under nitrogen, and washed with 0.5 M HCl and brine. The organic layer was then dried over anhydrous MgSO₄, and the solvent was removed by evacuation. Purification with column chromatography (silica, 2:1 EtOAc/hexane) yielded colorless oil (0.60 g, 58 %). ¹H NMR (CDCl₃, 300 MHz): δ 1.45 (s, (CH₃)₃C, 81H); 1.36-1.58 (m, CH₂CH₂CH₂CH₂, 8H); 2.40-2.47 (m, CH₂CH₂O gen. 1 & 2, 24H), 3.13 (m, CONHCH₂, 2H), 3.26 (t, N₃CH₂, 6.9 Hz, 2H), 3.62-3.69 (m, CCH₂O gen. 1 & 2, CH₂CH₂O gen. 1 & 2, 48H); 5.36 (t, CH₂NHCO, J=6.7 Hz, 1H), 5.68 (br, CONHC, 1H), 6.28 (br, amide NH, 3H). ¹³C NMR (CDCl₃, 75 MHz): δ 26.59, 26.69, 28.91, 30.54 (CH₂CH₂CH₂CH₂); 28.22 ((CH₃)₃C); 36.20 (CH₂CH₂O gen. 2); 37.43 (CH₂CH₂O gen. 1); 39.81 (CONHCH₂); 51.47 (N₃CH₂); 58.93 (CCH₂O gen. 1); 59.89 (CCH₂O gen. 2); 67.15 (CH₂CH₂O gen. 2); 67.68 (CH₂CH₂O gen. 1); 69.23 (CCH₂O gen. 2); 70.12 (CCH₂O gen. 1); 80.57 ((CH₃)₃C); 158.25 (NHCONH); 171.01 (COO*t*-Bu) 171.41 (CONH amides). MALDI-MS: 1989.8 (MNa⁺), 2005.8 (MK⁺).

***N*-(6-Aminoheptyl)-*N'*-tris[(2-{{(tris{[2-(*tert*-butoxycarbonyl)ethoxy]methyl)methyl-amino]carbonyl}ethoxy)methyl]methylurea (5).** Nona-*tert*-butyl ester **4** (0.37 g, 0.20

mmol) was stirred with 10 % Pd/C (37.0 mg) in ethanol (20.0 mL) under H₂ at room temperature for 12 h. After checking completion of the reaction with TLC, the mixture was filtered with a 0.2 μm Millipore filter. After the filter paper was rinsed with CH₂Cl₂, the combined solvent was removed in vacuo, and colorless oil was recovered.

***N*-{6-(9-fluorenylmethoxycarbonyl)aminohexyl}-*N'*-tris[(2-{{(tris{[2-(*tert*-butoxycarbonyl)ethoxy]methyl)methyl}amino]carbonyl}ethoxy)methyl]methylurea (6).**

The amine **5** (0.33 g, 0.17 mmol) and DIEA (33 μL, 0.19 mmol) were dissolved in 5.0 mL of CH₂Cl₂, and stirred for 30 min under nitrogen atmosphere. 9-Fluorenylmethyl chloroformate (48 mg, 0.19 mmol) in 2.0 mL of CH₂Cl₂ was added, and the reaction mixture was stirred for 3 h at room temperature. The solvent was removed under reduced pressure and washed with 0.5 M HCl and brine. The residue was purified with column chromatography (silica, EtOAc) to yield colorless oil (0.18 g, 64 %). ¹H NMR (CDCl₃, 300 MHz): δ 1.45(s, (CH₃)₃C, 81H); 1.23-1.58 (m, CH₂CH₂CH₂CH₂, 8H); 2.37-2.47 (m, CH₂CH₂O gen. 1 & 2, 24H); 3.10-3.22 (m, CONHCH₂, 4H); 3.62- 3.70 (m, CCH₂O gen. 1 & 2, CH₂CH₂O gen. 1 & 2, 48H); 4.22 (t, CH(fluorenyl)-CH₂, *J*=7.1 Hz, 1H); 4.36 (d, fluorenyl-CH₂, *J*=7.1 Hz, 2H); 5.27-5.35 (m, CH₂NHCO, 2H); 5.67 (br, CONHC, 1H); 6.25 (br, amide, 3H); 7.28 -7.77 (fluorenyl, 8H). ¹³C NMR (CDCl₃, 75 MHz): δ 26.85, 27.02, 30.27, 30.88 (CH₂CH₂CH₂CH₂); 28.49 ((CH₃)₃C); 36.48 (CH₂CH₂O gen. 2); 37.73 (CH₂CH₂O gen. 1); 40.03, 41.34 (CONHCH₂); 47.68 (CH(fluorenyl)-CH₂); 59.22 (CCH₂O gen. 1); 60.16 (CCH₂O gen. 2); 66.87 (fluorenyl-CH₂); 67.43 (CH₂CH₂O gen. 2); 67.98 (CH₂CH₂O gen. 1); 69.52 (CCH₂O gen. 2); 70.42 (CCH₂O gen.1); 80.84 ((CH₃)₃C); 120.28,

125.52, 127.38, 127.98, 141.65, 144.48 (fluorenyl); 156.88 (OCONH); 158.52 (NHCONH);
171.27 (COO*t*-Bu) 171.65 (amide CONH). MALDI-MS : 2186.8 (MNa⁺), 2002.8 (MK⁺).

***N*-{6-(9-fluorenylmethoxycarbonyl)aminoethyl}-*N'*-tris[(2-[(tris{2-carboxy-ethoxy}methyl)methyl]amino)carbonyl]ethoxy)-methyl]methylurea (7).** Nona-*tert*-butyl ester having a protecting group **6** (0.12 g, 72 μmol) was stirred in 10 mL of 96 % formic acid for 18 h. The formic acid was then removed at reduced pressure at 50 °C to produce colorless oil in a quantitative yield. ¹H NMR (CD₃COCD₃, 300 MHz): δ 1.23-1.51 (m, CH₂CH₂CH₂CH₂, 8H); 2.44-2.58 (m, CH₂CH₂O gen. 1 & 2, 24H); 3.15-3.18 (m, CONHCH₂, 4H); 3.61-3.75 (m, CCH₂O gen. 1 & 2, CH₂CH₂O gen. 1 & 2, 48H); 4.23 (t, CH(fluorenyl)-CH₂, *J*=7.0 Hz, 1H); 4.35 (d, fluorenyl-CH₂, *J*=7.0 Hz, 2H); 5.85, 6.09 (br, CH₂NHCO, 2H); 6.57 (br, CONHC, 1H); 6.88 (br, amide NH, 3H); 7.31-7.88 (fluorenyl, 8H). ¹³C NMR (CD₃COCD₃, 75 MHz): δ 27.21, 27.33, 30.69, 30.98 (CH₂CH₂CH₂CH₂); 35.31 (CH₂CH₂O gen. 2); 37.83 (CH₂CH₂O gen. 1); 40.56, 41.54 (CONHCH₂); 48.10 (CH(fluorenyl)-CH₂); 59.93 (CCH₂O gen. 1); 61.10 (CCH₂O gen. 2); 66.86 (fluorenyl-CH₂); 67.81 (CH₂CH₂O gen. 2); 68.37 (CH₂CH₂O gen. 1); 69.80 (CCH₂O gen. 2); 70.83 (CCH₂O gen.1); 120.84, 126.13, 127.98, 128.56, 142.10, 145.16 (fluorenyl); 157.50 (OCONH); 159.82 (NHCONH); 173.20 (amide CONH); 173.93 (COOH).

Reference

- 1 J. Tien, A. Terfort and G. M. Whitesides, *Langmuir*, 1997, **13**, 5349.
- 2 J. W. Lee, S. I. Jun and K. Kim, *Tetrahedron Lett.*, 2001, **42**, 2709.
- 3 C. M. Cardona and R. E. Gawley, *J. Org. Chem.*, 2002, **67**, 1411.