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Supporting Information

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Molecular Printboards as a General Platform for Protein Immobilization: a Supramolecular Solution to Nonspecific Adsorption**

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Calorimetry results

Isothermal titration calorimetry (ITC) experiments were performed to investigate the possible aggregation of **3**, and to determine the binding constant of **3** and **5** to βCD in solution. When 5 mM of **3** was titrated to PBS buffer (Figure 1a), only very small heat

effects were observed which are attributed to dilution. Thus aggregation of **3** is not observed and this indicates that **3** can be used at least up to 1 mM in order to suppress nonspecific protein adsorption at βCD SAMs. An ITC titration of 10 mM βCD (**1**) to 1 mM **3** showed a typical 1 : 1 binding event (Figure 1b). Fitting to a 1 : 1 binding model yielded K_a = (6.6 ± 0.3) × 10^4 M^{-1}, and $\Delta H°$ = -5.2 ± 0.4 kcal mol^{-1}. An ITC titration of 10 mM βCD to 1 mM **5** (Figure 1c) similarly led to K_a = (5.5 ± 1.3) × 10^4 M^{-1}, and $\Delta H°$ = -6.5 ± 0.4 kcal mol^{-1}. In both cases these values are typical for βCD-adamantyl interactions.[15] As a test protein, we used 0.1 mM BSA to investigate a possible interaction with **3** (Figure 1d). Notably, an interaction of **3** with BSA is absent.

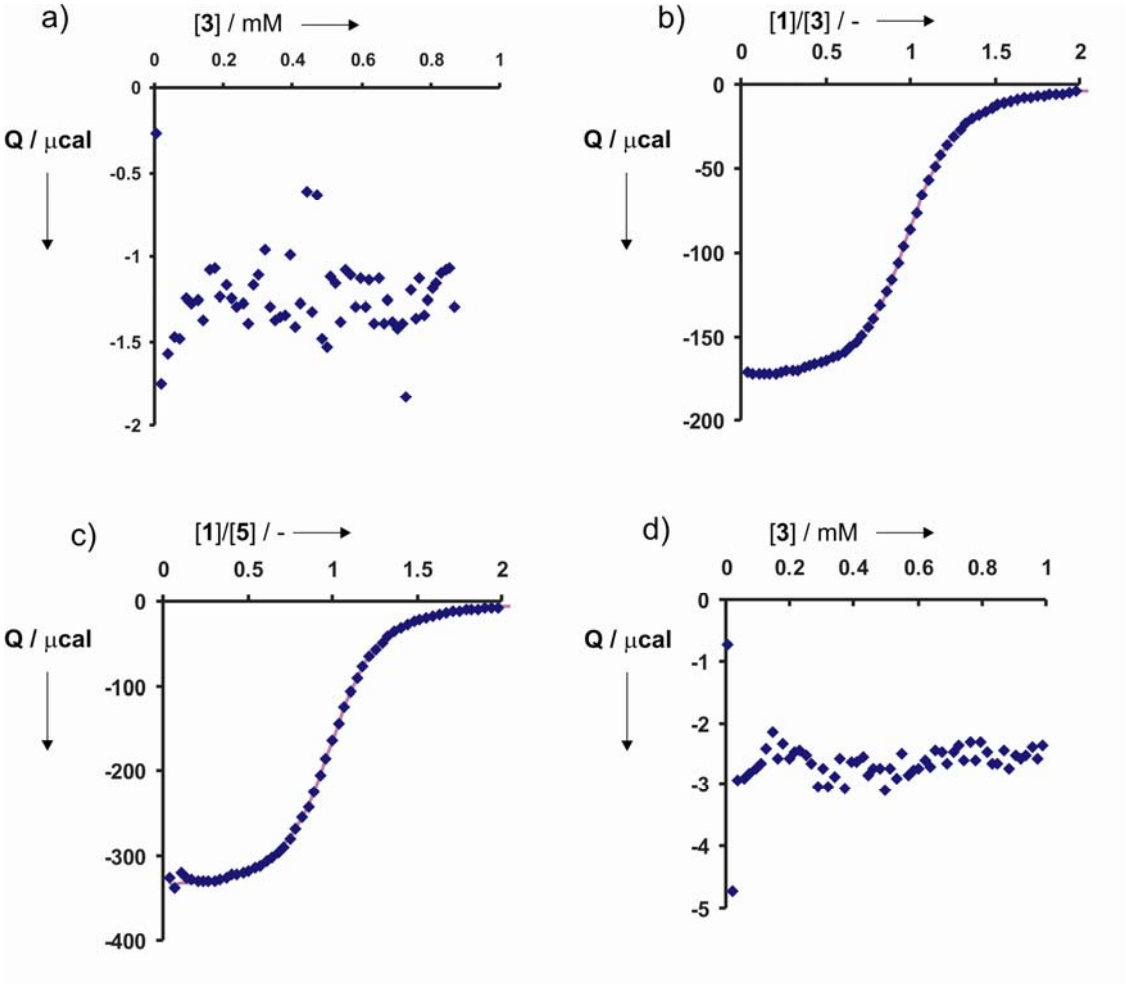

Figure 1. Heat evolved per injection (markers) and fits to a 1:1 model (lines) for the isothermal calorimetric titrations of 5 mM **3** in PBS (a), of 10 mM βCD to **3** (b), 10 mM βCD to **5** (c), and 5 mM **3** in PBS to 0.1 mM BSA (d).

Discussion on Surface coverage

Earlier work by Whitesides,[1] showed that mixed SAMs with OEG thiols resist the nonspecific adsorption of proteins when the fraction of the OEG thiol is at least 0.4-0.6, corresponding to an absolute coverage of about 4×10^{-10} mol/cm^{2}.[1] When this value is compared to the surface coverages of **3** achieved here, it appears that much lower

densities of OEG moieties can be effective in our case (max. about 5×10^{-11} mol/cm^2). This may be in part attributed to the dynamic nature of the supramolecular approach presented here. Moreover, although no surfactant behavior of **3** has been detected (see described ITC results), interactions of **3** with proteins in solution cannot be ruled out completely.

Experimental

Synthesis

1-Biotin-3-(3,5-di(tetraethylene glycol adamantyl ether) benzylamide **4** was synthesized as described before.[2]

Hexa(ethylene glycol) mono(adamantyl ether) (**3**) was synthesized by the reaction of hexa(ethylene glycol) (12.5 ml, 49.9 mmol) with 1-bromoadamantane (1.1 g, 5.0 mmol) at 180 °C in the presence of Et_3N (2.0 ml, 15.0 mmol). After cooling to room temperature, dichloromethane (50 ml) was added. The solution was washed with 2 M hydrochloric acid (4 × 50 ml) and once with brine (50 ml). the organic layer was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to give **3** as a yellow-brown oil (1.9 g, 4.5 mmol; 90 %). ^{1}H NMR ($CDCl_3$): δ 3.70 (t, 2H, AdO*CH$_2$*CH$_2$), 3.66-3.62 (m, 16H, HEG CH$_2$), 3.61-3.54 (m, 6H, AdOCH$_2$*CH$_2$* + *CH$_2$CH$_2$*OH), 3.02 (s, 1 H, CH$_2$*OH*), 2.10 (m, 3H, CH$_2$*CH*CH$_2$Ad), 1.75-1.70 (m, 6H, CH*CH$_2$*CAd, 1.65-1.53 (m, 6H, CH*CH$_2$*CHAd); ^{13}C NMR ($CDCl_3$): δ 72.8, 72.5, 71.5, 70.8, 70.6, 70.4, 61.9, 59.5, 41.7, 36.7, 30.7. MS (FAB): *m/z* calcd for $[M+H]^+$ 417.3; found 417.1.

Mono(adamantyl) L-lysine-nitrilo-tri(acetic acid)-(O*t*Bu)$_3$ (**5**)-(O*t*Bu)$_3$ was synthesized as follows. L-Lys-NTA(O*t*Bu)$_3$[2] (1.0 gram, 2.3 mmol) was dissolved in dichloromethane (50 mL) and DIPEA (0.5 mL, 2.4 mmol) and adamantane acid chloride (0.4 gram, 2.2 mol) were added subsequently. The solution was stirred at room temperature for 2 h., washed with 1N NaOH (2 × 75 mL), 1N HCL (2 × 75 mL), and brine (75 mL), dried over $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography (SiO_2, cyclohexane/ethylacetate [3:1]) to give the desired product in 99% yield as a colorless oil (1.3 gram, 2.2 mmol).

^{1}H NMR (250 MHz, $CDCl_3$): δ 5.82 (t, 1H, NH), 3.50 (m, 4H, CH_2COO), 3.25-3.36 (m, 3H, NCH + CONHC$\underline{H}_2$), 2.09 (m, 3H, AdCH), 1.87 (m, 6H, AdCH_2), 1.63-1.79 (m, 8H, AdCH_2 + C$\underline{H}_2$CH), 1.40-1.58 (m, CH_2C$\underline{H}_2$C$\underline{H}_2$$CH_2$ + CH_3); ^{13}C NMR (63 MHz, $CDCl_3$): δ 178.0, 172.3, 170.6, 81.1, 80.7, 65.1, 53.8, 40.5, 39.2, 39.0, 36.6, 30.2, 29.0, 28.2, 28.1, 26.9, 23.2; MS (MALDITOF): *m*/*z* calcd for [M] 592.8, found $[M+H]^+$ 594.0.

Mono(adamantyl) L-lysine-nitrilo-tri(acetic acid) (**5**) was synthesized as follows. **5**-(O*t*Bu)$_3$ (0.7 gram, 1.1 mmol) and triethylsilane (1.4 mL, 8.4 mmol) were dissolved in trifluoroacetic acid (20 mL). The solution was stirred at room temperature for 4 h and diethylether (20 mL) was added to give a white precipitate. The precipitate was isolated by filtration over a glass filter (por 4) and rinsed thoroughly with diethylether to give the desired product in 71 % yield as a white powder.

^{1}H NMR (250 MHz, DMSO): δ 11.70 (bs, 3H, COOH), 7.29 (t, 3H, 1H, NHCO), 3.51 (m, 4H, CH2COO), 3.36 (t, 1H, NCH), 3.01 (m, 2H, NHC$\underline{H}_2$), 1.95 (m, 3H, AdCH), 1.55-1.77 (m, 14H, AdCH_2 + AdCH_2 + C$\underline{H}_2$CH), 1.25-1.42 (m, 4H, CH_2C$\underline{H}_2$C$\underline{H}_2$$CH_2$);

^{13}C NMR (63 MHz, DMSO): δ 175.0, 174.8, 66.4, 55.7, 41.3, 39.8, 39.6, 37.2, 30.6, 29.1, 24.4; MS (MALDITOF): *m/z* calcd for [M] 424.6, found $[M+H]^+$ 425.8.

Streptavidin was bought from Aldrich and used as received. Maltose binding protein (MBP) with a C-terminal hexahistidine tag was expressed and purified as previously described.[3] For all experiments 10 mM phosphate buffer, pH 7.5 with 100 mM NaCl, phosphate buffered saline (PBS), was used.

Monolayer preparation

Gold substrates for SPR (BK7 glass/2-4 nm Ti/50 nm Au) were obtained from Ssens B.V., Hengelo, The Netherlands. Gold substrates were cleaned by dipping them into piranha (1:3 mixture of concentrated H_2SO_4 and 30% H_2O_2) for 5 s. (*Warning:* piranha should be handled with caution; it can detonate unexpectedly.) After thorough rinsing with Millipore water, they were placed for 10 min in absolute EtOH in order to remove the oxide layer. Subsequently SAMs were prepared as described before.[4] All solvents used in the monolayer preparation were of p.a. grade.

Calorimetric titrations

Calorimetric titrations were performed at 20 °C using a Microcal VP-ITC titration microcalorimeter. Aggregation studies were performed by adding 5 μl aliquots of a 5 mM solution of **3** in PBS to PBS or 0.1 mM BSA. Titrations were performed by adding 5 μl aliquots of a 10 mM βCD solution to a 1 mM solution of **3**. The titrations were analyzed

with a least-squares curve fitting procedure. Each ITC experiment was repeated at least two times.

SPR

SPR measurements were performed on a Resonant Probes Gmbh SPR instrument. The instrument consists of a HeNe laser (JDS Uniphase, 10 mW, λ = 632.8 nm) of which the laser light passes through a chopper that is connected to a lock-in amplifier (EG&G 7256). The modulated beam, is directed through two polarizers (OWIS) to control the intensity and the plane of polarization of the light. The light is coupled via a high index prism (Scott, LaSFN9) in the Kretschmann configuration to the backside of the gold-coated substrate which is optically matched through a refractive index matching oil (Cargille; series B; $n_D^{25°C} = 1.700 \pm 0.0002$) at the prism, mounted on a θ-2θ goniometer, in contact with a Teflon cell with a volume of 39 μl and a diameter of 5 mm. The light that leaves the prism passes through a beam splitter, subsequently, the s-polarized light is directed to a reference detector, and the p-polarized light passes through a lens which focuses the light onto a photodiode detector. Laser fluctuations are filtered out by dividing the intensity of the p-polarized light (I_p) by the intensity of the s-polarized light (I_s). All measurements were performed at a constant angle by reflectivity tracking.

A Reglo digital MS-4/8 Flow pump from Ismatec with four channels was used. In this flow pump, Tygon R3607 tubings with a diameter of 0.76 mm were used, obtained from Ismatec.

The SPR experiments were performed in a flow cell with a volume of 3.9×10^{-2} ml, under flow. A continuous flow of 0.5 ml/min was used. Before a new experiment was

started, the gold substrates were rinsed thoroughly with 10 mM βCD in 10 mM PBS containing 150 mM NaCl, and 10 mM PBS containing 150 mM NaCl. Experiments were started after the baseline was stable. When the solution had to be changed, the pump was stopped, and immediately after changing the solution the pump was switched on again.

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