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Multivalent Host–Guest Interactions between β-Cyclodextrin Self-Assembled Monolayers and Poly(isobutene-*alt*-maleic acid)s Modified with Hydrophobic Guest Moieties

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Abstract: Poly(isobutene-*alt*-maleic acid)s modified with *p*-*tert*-butylphenyl or adamantyl groups interact with β-cyclodextrin self-assembled monolayers (β-CD SAMs) by inclusion of the hydrophobic substituents in the β-cyclodextrin cavities. The adsorption was shown to be strong, specific, and irreversible. Even with a monovalent competitor in solution, adsorption to the β-CD SAMs was observed, and desorption proved impossible. The adsorbed polymer layer was very thin as evidenced by surface plasmon resonance spectroscopy and AFM. Apparently, all or most hydrophobic groups of the polymers were employed efficiently in multivalent binding, as was further supported by the absence of specific binding of β-CD-modified gold nanoparticles to the polymer surface assemblies. Supramolecular microcontact printing of the polymers onto the β-CD SAMs led to assembly formation in the targeted areas of the substrates.

Keywords: cyclodextrins · host–guest systems · polymers · self-assembled monolayers · surface plasmon resonance

Introduction

Multivalent interactions involve the simultaneous binding of multiple ligand sites on one entity to multiple receptor sites on another,[1] and can result in the formation of numerous simultaneous complexation events that afford a high functional affinity. These interactions occur throughout biology,[2] for example, processes such as cell–cell recognition often depend on the formation of multiple receptor–ligand complexes at the cell surface.[3] Multivalent ligands, in contrast to monovalent ligands, can interact with receptors by different mechanisms.[4] Therefore, an understanding of these mechanisms in well-defined synthetic systems will help to understand how natural systems function. The nature of the binding elements, structure of the scaffold,[5] number of binding groups, and density of binding elements[6] are some of the parameters that influence the mechanisms by which a multivalent synthetic ligand acts.[7]

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Polymer systems are currently the most extensively studied[8] of all multivalent ligands, and serve as the prototypical system for the design of reagents for biochemistry and biology. Polymers tethered onto surfaces have been a subject of attention owing to their potential use in many surface-based devices phenomena and technologies such as switchable membranes, sensors, cell growth control, and biomimetic materials.[9] For example, Ravoo and co-workers studied the interaction between polymers modified with hydrophobic groups and β-cyclodextrin-modified bilayer vesicles[10] by means of capillary electrophoresis.[11]

In our group we have prepared self-assembled monolayers (SAMs) of a β-cyclodextrin (β-CD) heptathioether adsorbate on gold substrates[12] for the formation of densely packed, well-ordered SAMs,[13] the hexagonal packing of which has been visualized by atomic force microscopy (AFM).[14] We have recently achieved the stable positioning and patterning of molecules on these SAMs by means of multiple hydrophobic interactions. Thus, these SAMs constitute molecular printboards for the binding, organization, and local functionalization of polyvalent systems.[15,16] Moreover, the thermodynamic and kinetic stabilities of the resulting patterns can be tuned, and has led to, for example, electrochemically induced desorption.[16]

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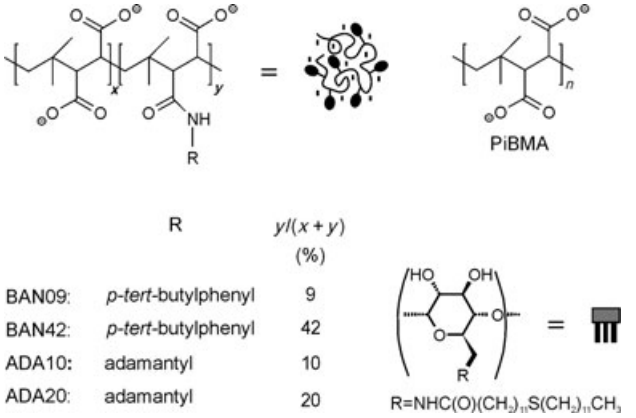
Here, we describe the molecular recognition by β-CD SAMs of poly(isobutene-*alt*-maleic acid)s modified with hydrophobic *p-tert*-butylphenyl or adamantyl groups (guest polymers). The multivalent noncovalent interactions of the guest polymers with the β-CD SAMs were investigated as a function of the nature and number of hydrophobic groups that interact with the β-CD surface and the intramolecular interactions within the polymer.

Results and Discussion

Interactions of guest polymers with β-CD SAMs: The guest polymers with hydrophobic *p-tert*-butylphenyl groups, BAN09 and BAN42, and adamantyl groups, ADA10 and ADA20 are shown in Scheme 1, as well as the reference



	R	$y/(x+y)$ (%)
BAN09:	*p-tert*-butylphenyl	9
BAN42:	*p-tert*-butylphenyl	42
ADA10:	adamantyl	10
ADA20:	adamantyl	20

Scheme 1. Chemical structures of guest polymers and host adsorbate used in this study.

compound, poly(isobutene-*alt*-maleic acid) (PiBMA), which lacks such hydrophobic groups. Note that throughout the work described herein, the concentration of guest polymers is expressed as the concentration of hydrophobic substituents.[10a] β-CD SAMs of a β-CD heptathioether adsorbate (Scheme 1) were prepared as described before.[12a]

Binding of the guest polymers to β-CD SAMs was studied by surface plasmon resonance (SPR) spectroscopy.[17] SPR titrations were performed in the presence of 10 mM phosphate buffer. As illustrated in Figure 1, a change in the SPR angle was observed after injection of an aqueous solution of BAN09 (A), indicative of adsorption. The adsorption was monitored for 30 min showing an increase of 0.11°. Rinsing with a 10 mM phosphate buffer solution (B) reduced the change in angle to about 0.05°. Extensive rinsing of the cell with buffer (B) and with 8 mM β-CD (C) did not completely restore the signal to the baseline. Subsequent polymer additions showed smaller changes in the angle, and extensive rinsing always led back to the change in angle of 0.05° obtained during the first addition.

Our interpretation is that the change in angle of 0.05° reflects the strong, irreversible adsorption of polymer through

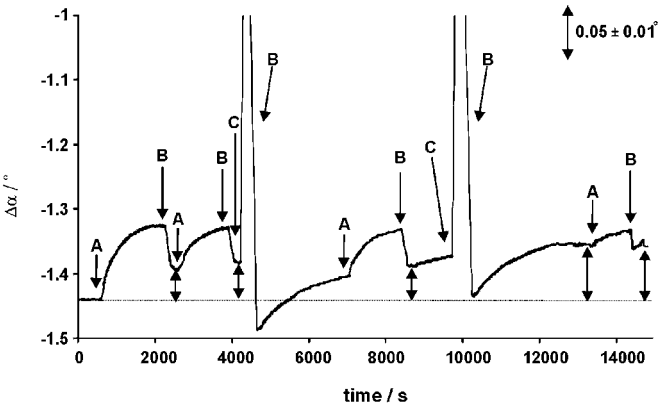


Figure 1. Surface plasmon resonance (SPR) spectroscopy time traces of the adsorption and attempted desorption of BAN09 (0.025 mM in hydrophobic moieties) onto a β-CD SAM; solutions (all in phosphate buffer 10 mM, pH 7): A: BAN09; B: buffer; C: 8 mM β-CD.

specific, multivalent interactions, whereas the remainder of the angle change of the first addition and the entire angle change of subsequent additions is due to nonspecific adsorption. When compared to a maximal angle change of 0.09° observed for small guests such as acetamidoadamantane,[12a] the angle change of 0.05° observed here suggests that a thin layer of polymer is adsorbed with efficient use of all or most of the hydrophobic groups (upper right sketch in Figure 2).

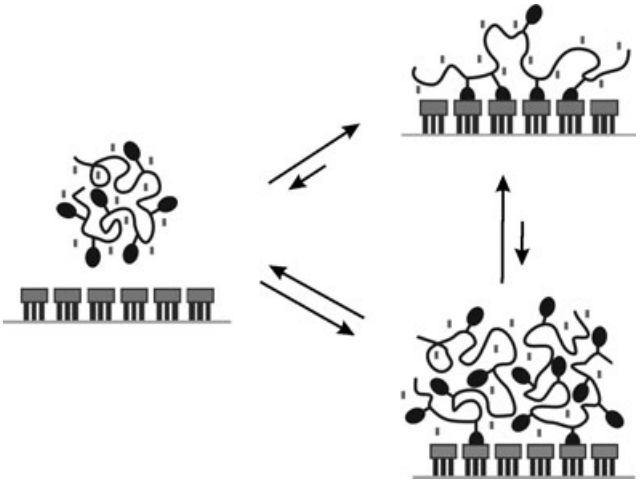


Figure 2. Schematic representation of possible binding modes of guest polymers onto β-CD SAMs.

Similar SPR titrations were performed with the other guest polymers, ADA10, ADA20, and BAN42, and the same behavior was observed for all polymers (data not shown) suggesting a strong affinity for the β-cyclodextrin SAMs.

Titrations performed with ADA10 on 11-mercapto-1-undecanol reference SAMs (lacking the host sites) and with PiBMA (lacking the guest sites) on β-CD SAMs only exhibited a small refractive index effect on the SPR signal, which could be instantaneously restored by rinsing the SAMs with

the solutions indicated above. No clear adsorption or desorption traces could be recorded, thus indicating the need for specific interactions between guest polymers and β-CD SAMs to form stable assemblies.

From these results, it was concluded that the binding of guest polymers to β-CD-coated gold surfaces was due to the formation of inclusion complexes between adamantyl or *p-tert*-butylphenyl groups of the guest polymers and β-CD sites immobilized on the SAMs, and that the binding between polymer and surface was irreversible.

The adsorption of ADA10 on β-CD SAMs was also studied by electrochemical impedance spectroscopy. The initial value of the charge-transfer resistance (R_{CT}) of the β-CD SAM using $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ as a redox couple was $110 \pm 10\,k\Omega$, indicating a highly ordered monolayer that blocks the redox current effectively.[18] Adsorption of ADA10 from solution (0.1 mM in adamantyl moieties) resulted in an increase of R_{CT} up to $300 \pm 50\,k\Omega$ due to the electrostatic repulsion between the carboxylate anions of the polymer and the redox couple. When using the positively charged $[Ru(NH_3)_6]^{2+}/[Ru(NH_3)_6]^{3+}$ as the reporter redox couple, EIS showed a decrease of the charge-transfer resistance upon adsorption of ADA10 from 47 to $24(\pm 15)k\Omega$ resulting from the electrostatic attraction between the negatively charged polymer and the positively charged redox pair. Thus, EIS confirmed the adsorption of ADA10 on the β-CD SAMs.

AFM was used for a direct determination of the thickness of the guest polymer film.[19,20] Adsorption of the polymer was achieved by immersion of a β-CD SAM in an ADA10 solution (1 mM in adamantyl functionalities), followed by rinsing with a 10 mM phosphate buffer solution. The AFM tip was used to create a scratch down to the gold, and the thickness was determined by scanning across the scratch with the AFM tip. The thickness (1.77 ± 0.03 nm) was compared to the thickness of a bare β-CD SAM (1.34 ± 0.03 nm). Thus, an estimate of the polymer thickness of 0.44 ± 0.06 nm was obtained. In addition, the thickness of the absorbed guest polymer layer was also estimated from microcontact printed substrates (see below) to be about 0.50 nm, corroborating the scratching experiments.

From the diffusion coefficients of the polymers as determined before,[10a] the hydrodynamic radii of the polymers in solution were estimated to be about 10 nm by using the Stokes–Einstein equation. It should be emphasized that this equation assumes a spherical conformation of the polymer chains. Nevertheless, the comparison between these radii and the values for the thickness of adsorbed polymers clearly indicates that the strong binding observed in the latter case, using efficiently all or most hydrophobic groups, leads to strong stretching and flattening of the polymers when adsorbing to the β-CD SAMs (Figure 2, top right).

To evaluate the effect of the polymer concentration on the adsorption process onto the β-CD surface, the interaction of ADA10 with β-CD SAMs was studied at 1 μM, 0.1 mM, and 1 mM in adamantyl moieties. SPR titrations were performed under the same conditions as described above. For the titration at 1 μM, adsorption appeared to be very slow probably due to severe diffusion limitation. As a consequence, an exact value for the SPR angle change was difficult to determine. In contrast, titration of 0.1 mM ADA10 showed a maximum SPR angle change of 0.05°. After thorough rinsing with 10 mM phosphate buffer and 8 mM β-CD solution, 0.03° remained. Similarly, titration of 1 mM ADA10 showed a maximum SPR angle change of 0.08°, indicating more nonspecific adsorption at this concentration. After the sample had been rinsed, a residual SPR angle change of 0.03° was observed. These experiments led us to conclude that the mode in which guest polymers bind to the molecular printboard is not concentration dependent in this concentration range. Combined with the thickness measurements discussed above, it is concluded that the polymers bind under all conditions employed here as a thin layer, making efficient use of the hydrophobic groups, (Figure 2, top right), and that a more spherical adsorption (Figure 2, bottom right) is not observed, although it can not be excluded that this is a rapidly progressing intermediate state.

To verify the absence of free, uncomplexed guest moieties in adsorbed guest polymers, the binding of β-CD-covered gold nanoparticles was attempted. A β-CD SAM was saturated with a 0.1 mM solution of ADA10. After thoroughly rinsing with phosphate buffer, the surface was exposed to a solution of 0.1 mM β-CD-modified gold nanoparticles.[21] An SPR angle change of 0.25° was observed, but after copious rinsing with water, the SPR angle change was restored to the baseline (data not shown). It was shown before by us that a divalent adamantyl-β-CD interaction is already strong enough to prohibit dissociation by rinsing with water;[16,22,23] only upon rinsing with competing β-CD in solution, significant dissociation can occur. Therefore, the results shown here demonstrate that the binding of the gold nanoparticles has occurred by physisorption and/or maximally one host–guest interaction per particle.[24] In conclusion, the surface concentration of free guest sites for a substrate with ADA10 adsorbed is significantly lower than the surface concentration of adamantyl-β-CD complexes between the β-CD SAM and the polymer, which confirms that the binding of the guest polymer to the molecular printboard is efficient using most or all hydrophobic groups.

Competition experiments with monovalent hosts and guests: ADA10 and BAN42 were dissolved in a 10 mM phosphate buffer solution containing a high concentration of competing monovalent host (8 mM β-CD). In SPR titrations, SPR angle changes of 0.08° and 0.20° for ADA10 and BAN42, respectively, were observed after injection of the aqueous solution of the respective guest polymer (0.025 mM in hydrophobic moieties; Figure 3). The adsorptions of ADA10 and BAN42 showed rapid kinetics (about 80% of binding after 5 min). After thorough rinsing with 8 mM β-CD in 10 mM phosphate buffer, approximately 0.06° and 0.12° remained, in agreement with the experiments described above. Consecutive additions of polymer did not lead to specific adsorption. These

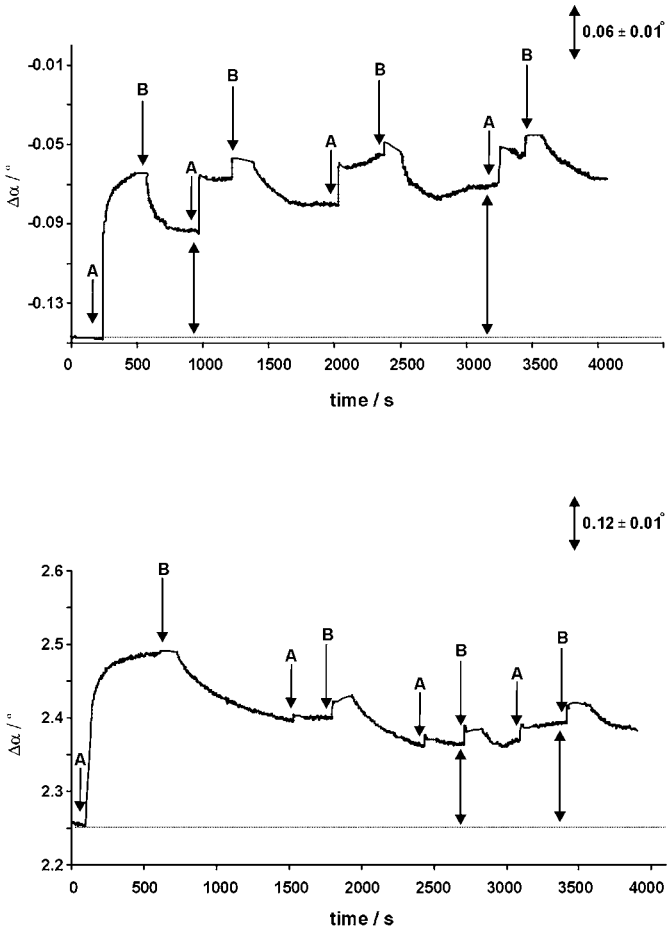


Figure 3. Surface plasmon resonance (SPR) spectroscopy time traces of the adsorption and attempted desorption of ADA10 (top) and BAN42 (bottom) (0.025 mM in hydrophobic moieties) onto a β-CD SAM in competition with monovalent host in solution; solutions (all in 10 mM phosphate buffer and 8 mM β-CD): A: guest polymer; B: buffer with 8 mM β-CD.

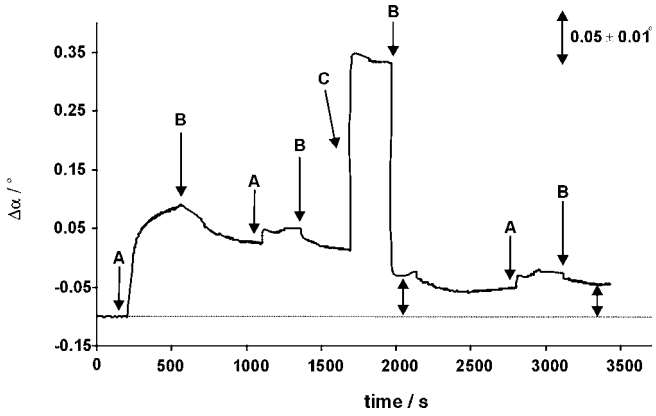


Figure 4. Surface plasmon resonance (SPR) spectroscopy time traces of the adsorption and attempted desorption of BAN09 (0.025 mM in hydrophobic moieties) onto a β-CD SAM in competition with monovalent guest in solution; solutions (all in 10 mM phosphate buffer): A: BAN09; B: buffer; C: 5 mM 1-adamantylamine.

experiments confirmed our earlier statement that guest polymers are bound to β-CD SAMs in a strong and irreversible fashion, probably using nearly all hydrophobic groups available. The resulting assemblies even formed and remained stable at high concentrations of competing monovalent β-CD in solution.

BAN09 was chosen to carry out SPR titrations in competition with monovalent guest in solution due its lowest number of hydrophobic groups and weakest type of interaction.[25] After adsorption of BAN09 (0.1 mM in hydrophobic moieties) to the β-CD SAM and rinsing with 5 mM 1-adamantylamine, an SPR angle change of 0.06° was observed (Figure 4). This value is comparable to the values obtained for ADA10 and BAN42 in competition with monovalent host in solution. Again, these results confirm that competition with a monovalent competitor only leads to partial desorption of material from the CD surface, but that specifically and strongly bound guest polymer remains. The material that is removed is most likely physisorbed material, but the removal of a small fraction of specifically, but weakly, bound polymer cannot be excluded.

To estimate the binding strength of the polymers to the surface quantitatively, a recently developed model for multivalent interactions at interfaces was applied.[22,23] This model employs an effective concentration parameter, C_{eff}, which represents the concentration of free, uncomplexed surface host sites experienced by a noncomplexed guest site connected to a surface-bound guest site by a linker. Thus, C_{eff} is surface-coverage-dependent, and is assumed to be independent of the number of binding sites of the guest but only dependent on its molecular geometry (linker length, stiffness, etc.) and the number of host sites that a nonattached guest site can reach at the surface.

Table 1 gives estimates of the maximal C_{eff} values (reached at low surface coverages) as determined from the linker lengths,[22] which were assumed equal to the average distances between hydrophobic groups in the guest polymers based on the extended conformation of the polymer backbone. In our case the $C_{eff,max}$ for the different polymers was calculated to be 0.15–0.35 M. Thus it can be clearly seen that this value is always higher than can be reached by a monovalent competitor in solution (ca. 15 mM for β-CD and ca. 50 mM for hydrophobic guest), thus the adsorption is always

Table 1. Degree of substitution, average spacing of substituents, and effective concentrations of the hydrophobic group-modified guest polymers.

Polymer	Average number of groups per polymer chain	Average distance between groups [nm]	$C_{eff,max}$[a] [M]
BAN09	35	5.4	0.15
BAN42	164	1.6	0.34
ADA20	78	2.6	0.28
ADA10	39	5.4	0.15

[a] effective concentration employed in the multivalency model (see text and reference [22]).

favored. The absolute stability constant K of the polymer on the β-CD SAMs, can be estimated by using Equation (1).[22]

$$K = K_{i,s}{}^{n}\ C_{eff,\,max}{}^{n-1} \quad (1)$$

Assuming that the known intrinsic binding constants of the guest polymers in solution[10a] are equal to the intrinsic binding constants at the surface, $K_{i,s}$, it can be estimated that for all polymers $K > 5\times10^{8}\,\text{M}^{-1}$.[26] This supports the observed stabilities and irreversibility of the polymer assembly formation.

These conclusions are in marked contrast to the observations made for the inclusion of the guest polymers with vesicles composed of modified β-cyclodextrin.[10b] The guest polymers bind to these vesicles in a brush- or mushroom-type conformation (Figure 2, bottom right) with an affinity of $2\times10^{6}\,\text{M}^{-1}$ at most. It is likely that the oligo(ethylene glycol) residues protruding from the surface of the vesicles prevent optimal multivalent interaction with the guest polymers. This type of steric repulsion is well known for colloids and surfaces decorated with poly(ethylene glycol).[27]

Supramolecular microcontact printing: We used supramolecular microcontact printing[16,28b,29] to transfer the guest polymers onto the β-CD SAMs. Owing to the hydrophilicity of the ink, oxidation of the PDMS stamp by mild oxidation in an ozone plasma for 30 min was required to ink the stamp.[30] After immersing the hydrophilic stamps in an ADA10 solution (1 mM in adamantyl moieties), they were applied by hand onto the molecular printboard for 60 s. As seen from Figure 5 (top), a pattern was observed in height (left), but more clearly in friction (right), confirming the transfer of polymer onto the substrate. The darker lines in the latter image represent the β-CD SAM areas, whereas the brighter ones are the areas printed with ADA10.

As described before for small guest molecules,[16] the printed substrates were rinsed with copious amounts of 8 mM β-CD in 10 mM phosphate buffer. AFM friction images (Figure 5 A) confirmed the SPR results, as it can be clearly seen that the transferred pattern is still present even after competitive rinsing.

A similar printing experiment was applied on a 11-mercapto-1-undecanol SAM. These layers have a polarity comparable to the β-CD layers, but lack the possibility to form specific host-guest complexes. Patterns after printing were observed similar to the patterns on the β-CD SAMs. However, exposing the printed pattern to the β-CD rinsing procedure led to the complete removal of the pattern, proving physisorption in this case (Figure 5B).

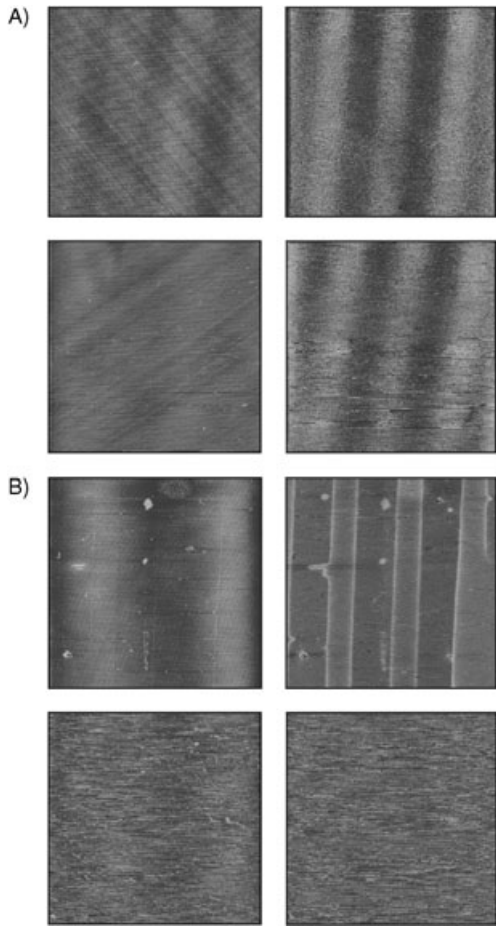


Figure 5. CM-AFM height (left, z range 5 μm) and friction (right, z range 0.1–0.2 V) images (50x50 μm^2) in air of β-CD (A) and OH SAMs (B) after CPμ of ADA10 (0.1 mM in adamantyl moieties) before (top) and after (bottom) rinsing with 10 mM phosphate buffer containing 8 mM β-CD.

Conclusion

The binding of hydrophobic guest-functionalized poly(isobutene-*alt*-maleic acid)s and β-CD SAMs through multiple inclusion of the guest substituents of the polymers into the cavities of the β-CDs was shown to be very strong and irreversible. The polymer adsorption led to very thin polymer films on the surface, apparently using all or many of the hydrophobic groups, even though the polymers in solution are known to be spherical and to have strong intramolecular hydrophobic interactions leading to reduced affinity for β-CD in solution.[10a] Variations of the nature and number of hydrophobic groups in the polymer, and the polymer concentration in solution did not lead to significant differences in adsorption behavior. Even competition with a monovalent host and guest in solution did not lead to measurable polymer desorption, even though competition is known to enhance multivalent dissociation kinetics.[31] This behavior is attributed to the large number of hydrophobic groups present in the polymer and to the close-to-optimal linker lengths (1.6–5.4 nm) between the hydrophobic groups relative to the periodicity of the β-CD lattice (ca. 2 nm)[14,22] leading to high effective concentrations at the β-CD SAMs. These aspects cause the guest polymers–β-CD SAM assemblies to reach huge stability constants and concomitantly immeasurably long lifetimes, even under competitive conditions. Thus we have proven that multivalent polymer assemblies can be

thermodynamically and kinetically stable, even though intrinsically weak and rapidly reversible supramolecular interactions are employed. This paradigm can be of value for nanofabrication.

Experimental Section

Materials: Guest polymers BAN09, BAN42, ADA10 and ADA20, were kindly donated by Professor Gerhard Wenz (Saarland University, Germany) or prepared as described[32] by amidation of poly(isobutene-*alt*-maleic anhydride) (PiBM) of MW = 60 kD with varying amounts of *p*-*tert*-butylaniline or adamantylamine, followed by hydrolysis of the remaining anhydride groups. Throughout this paper, the concentration of guest polymers is expressed as the concentration of hydrophobic substituents.[10a] Poly(isobutene-*alt*-maleic acid) (PiBMA) was obtained by hydrolysis of PiBM using aqueous NaOH. *p*-*tert*-Butylbenzoic acid was obtained from Aldrich and converted to the sodium salt by addition of one equivalent of aqueous NaOH. Synthesis of the β-cyclodextrin heptathioether adsorbate was reported previously.[12a]

β-CD-coated gold nanoparticles were synthesized according to reference [21] by reduction of $AuCl_4^-$ in DMSO solution containing perthiolated β-CD[33] in a ratio [β-CD]/[$AuCl_4^-$] = 0.30:1. The reaction mixture became deep brown immediately upon the addition of the reducing agent $NaBH_4$. The β-CD-modified gold particles were isolated by precipitation from CH_3CN and characterized by UV/Vis spectroscopy, ^{1}H NMR, and transmission electron microscopy (TEM). Using TEM, a mean particle size of 2.5 ± 0.6 nm was found.

Substrate and monolayer preparation: All glassware used to prepare monolayers was immersed in piraña (conc. H_2SO_4 and 33 % H_2O_2 in a 3:1 ratio). (**Warning**! *piraña should be handled with caution; it has detonated unexpectedly*). The glassware was rinsed with large amounts of high purity water (Millipore). All adsorbate solutions were prepared freshly prior to use. Round glass supported gold substrates for SPR (2.54 cm diameter; 47.5 nm Au) and gold substrates for μCP (20 nm of gold on a 7.5-cm silicon wafer with a 2 nm titanium adhesion layer) were obtained from Ssens BV (Hengelo, The Netherlands). Prior to use the substrates were cut to the preferred shape and size. Substrates were cleaned by immersing the substrates in piraña for 5 s and leaving the substrates for 5 min in absolute EtOH.[34] The substrates were subsequently immersed into a 0.1 mM β-CD heptathioether adsorbate solution in EtOH and $CHCl_3$ (1:2 v/v) for 16 h at 60 °C. The samples were removed from the solution and rinsed with substantial amounts of chloroform, ethanol, and Milli-Q water. 11-Mercapto-1-undecanol was purchased from Aldrich, and cleaned gold substrates were immersed with minimal delay into a 0.1 mM adsorbate solution in EtOH for 24 h. Subsequently, the substrates were removed from the solution and rinsed repeatedly with chloroform or dichloromethane, ethanol, and water to remove any physisorbed material. Gold substrates for the direct determination of the thickness of the guest polymer films were flame-annealed in a H_2 flame. After the annealing procedure, the substrates were immersed into a 0.1 mM β-CD heptathioether adsorbate solution in EtOH and $CHCl_3$ (1:2 v/v) for 16 h at 60 °C. The same rinsing procedures were applied as described above. All solvents used in monolayer preparation were of p.a. grade.

Microcontact-printed substrates: Microcontact-printed substrates were prepared according to reference [28]. Stamps were prepared by casting a 10:1 (v/v) mixture of poly(dimethylsiloxane) and curing agent (Sylgard 184, Dow Corning) against a patterned silicon master. After curing, the stamps were mildly oxidized in an ozone plasma reactor for 30 min and then inked by soaking them in the polymer solution (1 mM in hydrophobic groups) for 30–45 min. The master employed to prepare the PDMS stamp had 10 μm line features with 5 μm gaps, but the ozone treatment of the stamp decreased the features to about 8 ± 1 μm. Before printing, the stamps were blown dry in a stream of N_2. The stamps were applied manually (without pressure control) for 60 s on preformed SAMs (β-CD or 11-mercapto-1-undecanol) on gold and then carefully removed. After each printing step the inking procedure was repeated. Microcontact-printed substrates were thoroughly rinsed with 200 mL of aqueous solutions of either β-CD (8 mM in 10 mM phosphate buffer pH 7) or phosphate buffer (10 mM pH 7).

Monolayer characterization: Advancing and receding contact angles were measured on a Krüss G10 Contact Angle Measuring Instrument equipped with a CCD camera during the growth and shrinkage of a water droplet, respectively. Electrochemical measurements (cyclic voltammetry and impedance spectroscopy) were performed by using an Autolab PGSTAT10 (ECOCHEMIE, Utrecht, The Netherlands) in a three-electrode configuration consisting of a gold working electrode (clamped to the bottom of the cell, exposing a geometric area of 0.44 cm^2 to the electrolyte solution), a platinum counter electrode, and a mercury/mercurous sulfate reference electrode (+0.61 V_{NHE}). Cyclic voltammetric capacitance measurements were conducted in 0.1 M K_2SO_4 between −0.35 V_{MSE} and −0.25 V_{MSE} at scan rates ranging from 0.1 to 2.0 Vs^{-1}. Impedance spectroscopy measurements were performed in aqueous $K_3Fe(CN)_6/K_4Fe(CN)_6$ (both 1 mM) containing 0.1 M K_2SO_4 at −0.2 V_{MSE} with an amplitude of 5 mV using a frequency range from 50 kHz to 0.1 Hz. The charge-transfer resistance of the monolayer was obtained by fitting the experimental data to an equivalent circuit consisting of the monolayer resistance parallel to the monolayer capacitance, in series with the solution resistance.[35]

AFM: AFM experiments were carried out with a NanoScope IIIa Multimode AFM (Digital Instruments, Veeco Metrology Group, USA) in contact mode using V-shaped Si_3N_4 cantilevers (Nanoprobes, Veeco) with a nominal spring constant of 0.32 Nm^{-1}. The AFM was equipped with a J scanner. Before thickness determination the scanner was calibrated in the *z* direction. The error was about 2 %. Gold-coated AFM tips were functionalized with 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol (purchased from Fluorochem) to avoid the adhesion of polymer chains to the AFM tip during imaging. The fluorinated AFM tips were immersed into a 0.1 mM 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol solution in CH_2Cl_2 overnight. The AFM tips were removed from the solution and rinsed with substantial amounts of dichloromethane, ethanol, and Milli-Q water. Images were captured in ambient atmosphere (25 °C).

Surface plasmon resonance spectroscopy: SPR measurements were performed in a two-channel vibrating mirror angle scan set-up based on the Kretschmann configuration, described by Kooyman et al.[36] Light from a 2-mW HeNe laser was directed onto a prism surface by means of a vibrating mirror. The intensity of the light was measured by means of a large-area photodiode. This set-up allows the determination of changes in plasmon angle with an accuracy of 0.002°. The gold substrates with the monolayer were optically matched to the prism using an index matching oil. A Teflon cell was placed on a monolayer through an O-ring, to avoid leakage, and filled with 800 μL of 10 mM phosphate buffer solution. After stabilization of the SPR signal, titrations were performed by removing an amount of the buffer solution from the cell and adding the same amount of stock solution of guest polymers in phosphate buffer at different hydrophobic group concentrations (1 μM, 0.1 mM, or 1 mM). After each addition, the cell was thoroughly washed with 10 mM phosphate buffer pH 7 (five times 700 μL) or 8 mM β-CD in 10 mM phosphate buffer pH 7. SPR time traces shown in the figures are corrected for baseline drifts by subtraction of the reference channel intensities.

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