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Binding Control and Stoichiometry of Ferrocenyl Dendrimers at a Molecular Printboard

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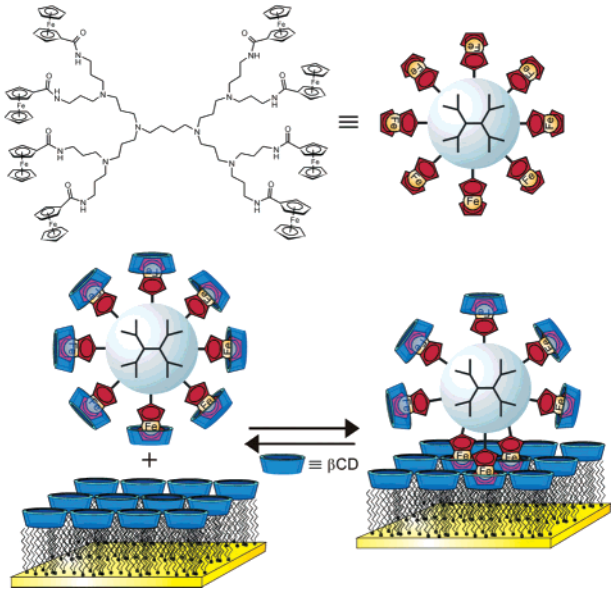
Positioning of (bio)molecules at surfaces with high accuracy is a key issue for various applications, e.g., molecular electronics[1] and biochips,[2] and for conducting single molecule experiments. In general, molecules are either covalently attached or physisorbed at surfaces. A disadvantage of chemical modification of surfaces is that neither self-correction nor intentional desorption is possible. In contrast, physisorption allows self-correction, but the thermodynamic and kinetic parameters are difficult to control.[3] Supramolecular interactions are directional, specific, and reversible, and a wealth of information is commonly available about their binding strength and kinetics, and they are therefore of special interest for placing molecules at surfaces.[4]

For this reason, we have developed molecular printboards, which consist of self-assembled monolayers (SAMs) of heptathioether-functionalized β-cyclodextrin (βCD) on gold.[5] These form well-ordered and densely packed monolayers with a hexagonal lattice onto which a variety of univalent or multivalent guest molecules can be positioned by either adsorption from solution, microcontact printing, or dip-pen nanolithography with submicron resolution.[6] Single supramolecular interactions are commonly relatively weak, but *multivalent* interactions,[7] that is, the simultaneous binding of multiple binding sites on one (bio)molecule to a system or surface with multiple receptors, can overcome this problem. The formation of stable assemblies at surfaces, but still having control over the binding by external stimuli, is essential for applications. However, quantitative information about the specific number of interactions and the resulting binding strength is often hard to obtain, especially for multivalent systems linked to surfaces.

Dendrimers constitute a particularly interesting class of polyfunctional guest molecules that can be placed onto these molecular printboards. Recently, we reported the adsorption of βCD-complexed adamantyl- and ferrocenyl-functionalized poly(propylene imine) (PPI) dendrimers from aqueous solution to βCD SAMs.[6,8] These molecules form stable assemblies at βCD SAMs due to the formation of multiple specific host−guest interactions with the host surface. Here we report the determination of the numbers of interactions of ferrocenyl-terminated PPI dendrimers at βCD SAMs by electrochemistry, quantitatively confirmed by surface plasmon resonance (SPR) spectroscopy, and the effective electrochemically induced desorption of the dendrimers, revealing the advantage of the molecular printboard for intentional desorption.

Ferrocenyl (Fc)-terminated PPI dendrimers[9] of generations 1 (**G1**, 4 Fc groups), 2 (**G2**, 8 Fc groups), 3 (**G3**, 16 Fc groups), 4 (**G4**, 32 Fc groups), and 5 (**G5**, 64 Fc groups) were solubilized in water by complexation of the Fc endgroups with native βCD (Scheme 1).[10] The full degree of complexation was confirmed by cyclic voltammetry (CV) at different scan rates showing a single reversible wave for all generations of dendrimers, except for **G5** (See Supporting Information). Thus, the stoichiometries of the dendrimer-βCD assemblies in solution are **G1**·(βCD)$_4$, **G2**·(βCD)$_8$, **G3**·(βCD)$_{16}$, and **G4**·(βCD)$_{32}$, the last having a molecular weight of more than 46 kDa.

Scheme 1. **G2** Ferrocene Dendrimer (top) and Adsorption of the Dendrimer−βCD Assembly at the βCD SAM (bottom)



The dendrimers were adsorbed at the βCD SAMs on gold (Scheme 1) by immersion of the substrates into aqueous solutions of the dendrimer-βCD assemblies for at least 1 h, washing with water, and briefly drying in a stream of N_2. A typical CV for adsorbed **G1** is shown in Figure 1. During every scan, all Fc groups are oxidized, leading to desorption of the dendrimers from the surface. Upon reduction, only the dendrimers which remained close to the surface can rebind, leading to a concomitant decrease of the intensity for subsequent scans (see Supporting Information). It is well-known that ferrocene is able to form inclusion complexes with βCD in aqueous media and that the complexation is strongly diminished upon oxidation of the Fc groups to ferrocenium cations.[11]

In the case of **G1**, two signals were recorded at low scan rates (Figure 1). This is interpreted by assuming that two of the four Fc endgroups are bound to βCD at the surface (at 91 mV) and the other two unbound (at 121 mV).[12] Similar CVs were recorded for the other dendrimers (see Supporting Information). The signal attributed to bound ferrocene moieties was observed as a shoulder in these cases, because the ratio between bound and unbound endgroups decreases for higher generations (see below).

Integration of the (*i*,*E*)-curves gives the total charge (*Q*), which is, for a surface-confined process, independent of the scan rate.[13] For all dendrimers adsorbed at the βCD SAMs, the first scan of the CVs was integrated and *Q* was found to be constant for scan rates of 10−200 mV/s. From the total charge *Q*, the surface

10.1021/ja048271n CCC: $27.50

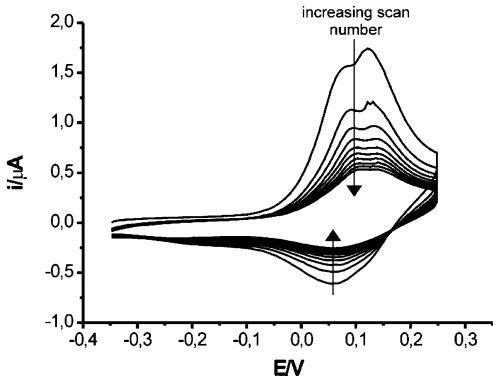


Figure 1. Electrochemical desorption of **G1** from a βCD SAM induced and observed by CV, scan rate = 25 mV/s, 0.1 M K_2SO_4, 10 scans.

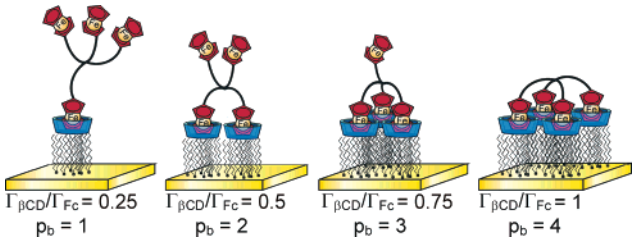


Figure 2. Schematic representation of the four possible binding modes of G1 with the numbers, p_b, of bound sites and the predicted $\Gamma_{\beta CD}/\Gamma_{Fc}$ ratios depicted below.

coverage of the Fc moieties, Γ_{Fc} (mol/cm^2), was calculated using the following formula: $\Gamma_{Fc} = Q/nFA$, in which n = number of electrons per mole of reaction, F = Faraday constant, and A = surface area of the electrode.[13]

The surface coverage of the βCD adsorbate molecules, $\Gamma_{\beta CD}$ (8×10^{-11} mol/cm^2), was estimated from molecular size considerations and from AFM studies.[5] The relative coverages $\Gamma_{\beta CD}/\Gamma_{Fc}$ provide the number p_b of bound interactions per dendrimer molecule with the host surface, using $p_b = p_{tot}\Gamma_{\beta CD}/\Gamma_{Fc}$, where p_{tot} is the total number of endgroups. All possible binding modes of **G1** with the surface and the theoretical values of the $\Gamma_{\beta CD}/\Gamma_{Fc}$ ratios are depicted in Figure 2. For **G1**, the experimental $\Gamma_{\beta CD}/\Gamma_{Fc}$ ratio is 0.56, implying two Fc moieties per βCD adsorbate molecule, when assuming full surface coverage. This confirms the 1:1 peak splitting observed by CV as described above. For **G2**, **G3**, **G4**, and **G5**, the $\Gamma_{\beta CD}/\Gamma_{Fc}$ ratios were 0.38, 0.26, 0.22, and 0.11, respectively. These values suggest that **G2** has 3 (out of 8) bound Fc units and 5 unbound, **G3** has 4 (out of 16) bound and 12 unbound Fc groups, while **G4** has 7 (out of 32) bound and 25 unbound, and **G5** has 7 (or 8) (out of 64) bound. For **G1**, the ratio of 0.56 is significantly higher than the theoretical value of 0.5 for full surface coverage. This indicates that the molecule may slowly desorb from the surface upon rinsing with water. For the other dendrimers, the observed coverages are very close to the theoretical values of 0.38, 0.25, 0.22, and 0.11 for full coverage, probably due to the stronger interactions of the higher generation dendrimers with the surface.

The number of interactions p_b of **G1** and **G2** to βCD SAMs was also determined by SPR spectroscopy,[14] and the data were fitted to a surface binding model for multivalent host−guest interactions.[15] Using an intrinsic binding constant (K_i) for an individual ferrocene−βCD interaction of 1.2×10^3 M^{-1}, as reported by Kaifer,[16] the best fit was obtained with two interactions per molecule to the surface. This gave an intrinsic binding constant at the surface K_{surf} of 5.5×10^2 M^{-1} per interaction.[1] The binding data of **G2** gave a best fit when assuming three interactions and a K_{surf} of 1.1×10^3 M^{-1} per ferrocene−βCD interaction. Both values are, within error, equal to the intrinsic interaction constant K_i, and thus the observation made earlier,[1] that the binding to βCD SAMs is comparable to solution, also holds for multivalent systems. For the larger dendrimers, spontaneous desorption is too slow for reliable SPR titration data to be obtained.

In summary, we have been able to determine the number of interactions of ferrocenyl-functionalized dendrimers at βCD SAMs. The numbers of interactions increased with increasing dendrimer size and were quantified by CV and SPR spectroscopy. These are in good agreement with the molecular sizes of the dendrimers compared to the spacing between the βCD cavities at the surface (see Supporting Information). It can be shown from CPK models of the maximally extended configuration that **G1** can interact only with two βCD cavities at the surface. For similar reasons, **G2** can interact with three βCD sites and **G3** with four, and **G4** and **G5** can access seven sites.

A good fundamental understanding of multivalent interactions at interfaces is a prerequisite for the understanding of recognition at, e.g., cell membranes, and for a wide range of applications. The concept of multivalent interactions, and the electrochemical binding control of such molecules at interfaces, can be of particular interest for nanoconstruction.

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Supporting Information Available: Detailed descriptions of sample preparations, SPR and CV measurements, CVs of **G2**−**5**, mechanism of desorption and rebinding, and size considerations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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