From Solutions to Surfaces: A Novel Molecular Imprinting Method Based on the Conformational Changes of Boronic-Acid-Appended Poly(L-lysine)

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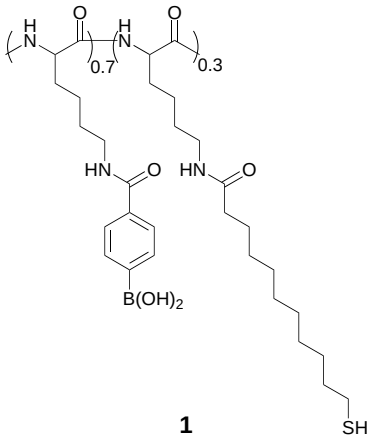
Molecular imprinting is usually defined as a process consisting of the copolymerization of functional and cross-linking monomers in the presence of a template (guest) molecule.[1] Subsequently, the template molecule is removed, revealing well-defined binding sites in which the arrangement of functional groups is particularly suited for binding the template molecule or other structurally related molecules. In the first approach to molecular imprinting, Wulff and co-workers covalently coupled the monomers to the template molecule.[2] Shortly thereafter, Mosbach and co-workers presented an imprinting method relying on noncovalent linkages that allows a wider choice of functional monomers and template molecules.[3] Recently, an imprinting method based on the immobilization of the template on a solid support has been reported.[4] Hitherto, molecular imprinting has usually been carried out in solution,[5] and only a few examples of imprinting at surfaces are known.[6] In this paper we present a novel, solution-to-surface imprinting method based on the different higher-order conformations adopted by boronic-acid-appended poly(L-lysine) **1**, in the presence of



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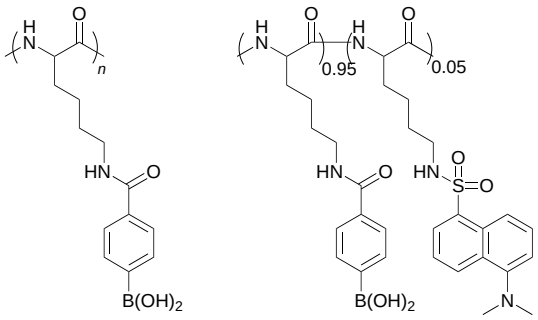
sugars. In a three-step procedure the polymer–template complex initially formed in solution is subsequently anchored to a metal surface, which is then rinsed to remove the template molecules, resulting in a glucose-selective, molecularly imprinted interface.

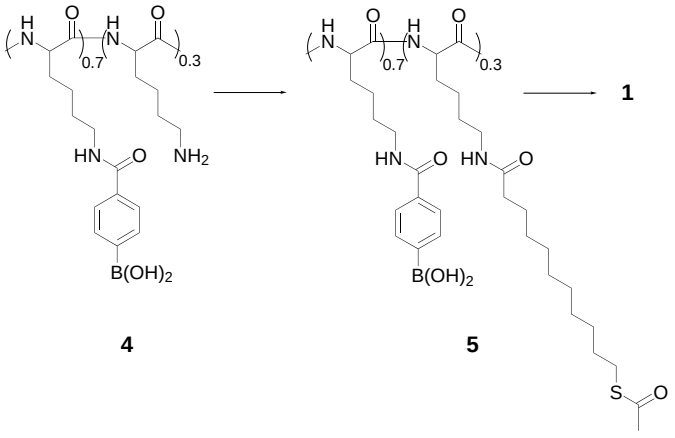
The conformational transitions of poly(L-lysine) are related to very subtle changes in secondary forces such as hydrogen-bonding interactions, electrostatic effects, and hydrophobicity.[7] At neutral pH, poly(L-lysine) assumes a β-sheet structure, in the medium to high pH region, an α-helix, and at even higher pH values, a random coil.[7] In our previous work we found that the addition of monosaccharides to boronic-acid-appended poly(L-lysine) **2** increases the α-helix content of the



2 **3**

polypeptide chain,[8] and the pH at which the maximum α-helix content is usually observed is shifted to a lower pH region in the presence of sugars. Recently, Kobayashi et al.[9] found that at pH > 10 and in the presence of D-glucose, polymer **3** has mainly a β-turn structure. The stabilization of this β-turn is attributed to the capability of D-glucose to bind concomitantly to two boronic acid moieties of the polypeptide chain, thus forming intrapolymeric cross-links.

To take advantage of such sugar-induced conformational changes for the realization of a molecularly imprinted interface, we synthesized poly(L-lysine) derivative **1** according to Equation (1). This polymeric adsorbate contains boronic acid moieties, which can bind to saccharides, and also 10-sulfanyldecyl moieties, which can anchor the polypeptide to a gold surface without hindering its conformational flexibility.



4 **5** **1** (1)

Molecular imprinting of adsorbate **1** was carried out at pH 11 in the presence of a large excess of either D-fructose or D-glucose, since these experimental conditions had previously shown the largest differences in conformation adopted by

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poly(L-lysine) derivative **3**.[8b, 9, 10] Circular dichroism (CD) spectroscopy in homogeneous solution (Figure 1) confirmed that in the absence of saccharides, **1** adopts a random coil conformation with a small percentage of α-helix structure.[7, 11] The percentage of α-helix content can be estimated by using

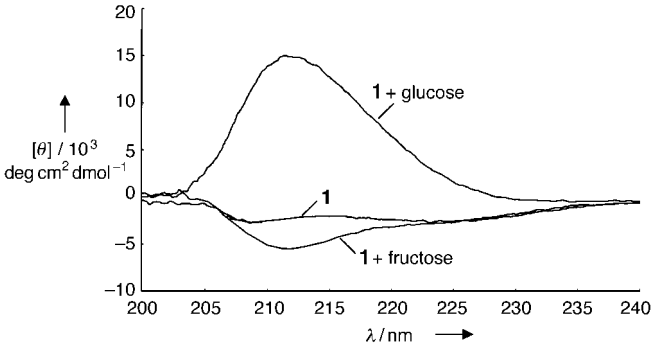


Figure 1. CD spectra of **1**, **1** + D-fructose, and **1** + D-glucose, at pH 11.

the relationship established by Greenfield and Fasman for poly(L-lysine) [Eq. (2)].[11a, 12] CD spectroscopy shows that in the polymer chain of **1** about 13% α-helix is present. The CD spectrum of **1** and D-fructose indicates that the major part of the polypeptide adopts the random coil conformation. However, in the case of **1** and D-glucose, the CD sign is inverted to positive values indicating the expected formation of the β-turn structure along the polypeptide chain, as was previously observed for compound **3**.[9]

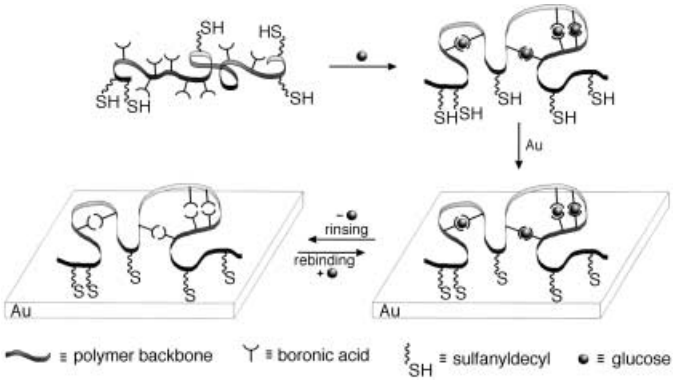
$$\%\ \alpha\text{-helix} = -([\theta]_{208} + 4000)/29000 \qquad (2)$$

An aqueous solution of **1** containing either D-fructose or D-glucose was equilibrated for approximately 20 min, and subsequently a gold-covered quartz crystal microbalance (QCM) resonator was immersed in this solution for 5 h. This treatment allows the polymer, either as a random coil or as a β-turn, depending on the saccharide, to be fixed onto the gold surface by means of the sulfanyl moieties present in **1**.[13] To remove the templating saccharide from the surface-bound polypeptide, the QCM resonator was rinsed thoroughly with acetate-buffered aqueous solution (pH 5). Subsequently, the rebinding of D-glucose and D-fructose by this poly(L-lysine) derivative **1** was monitored by following the changes in frequency (ΔF) of the resonator upon addition of the saccharide guests (Table 1). In the case of a QCM resonator covered with (nonimprinted) polypeptide **1**, addition of 1 mmol of D-fructose caused a significantly larger *decrease* in frequency ($\Delta F = 72 \pm 11$) than the same amount of D-glucose ($\Delta F = 42 \pm 7$).[14] These responses are in accordance with the fact that the interaction between boronic acid and D-fructose ($\lg K_a = 3.64$) is stronger than that between boronic acid and D-glucose ($\lg K_a = 2.04$).[10] However, the opposite was observed when a D-glucose-imprinted QCM resonator was exposed to the same two sugars (see Table 1). These results are indicative of a "molecular memory" effect induced by the imprinting process. Moreover, the QCM resonator covered with D-glucose-imprinted polypeptide **1** could be reused at least three times for the detection of D-glucose, with deviations $\leq 10\%$ of the initial value. The smaller response observed for D-fructose on the D-glucose-imprinted surface, compared to the responses on the nonimprinted polymer **1** surface, might be due to the conformation adopted by the D-glucose-imprinted polymer. D-Glucose can bind to two boronic acid groups and this brings the two boronic acid units in close proximity, giving rise to β-turn structures (Scheme 1).

Table 1. Changes in resonant frequency (ΔF) of QCM resonators covered with nonimprinted and imprinted surfaces of adsorbate **1**, upon addition of D-fructose or D-glucose (1 mmol, pH 8).[a]

Guest	ΔF		
	Nonimprinted	D-Glucose-imprinted	D-Fructose-imprinted
D-fructose	72 ± 11	41 ± 10	69 ± 9
D-glucose	42 ± 7	64 ± 5	33 ± 8

[a] All values correspond to decreases in frequency and are the average of at least three measurements.[14]



Scheme 1. Schematic representation of the solution-to-surface imprinting process based on the conformational changes of **1** in the presence of D-glucose.

If the conformation of the polypeptide is fixed through its adsorption to the gold surface, the boronic acid units will remain in close proximity even after the removal of the D-glucose template molecules (Scheme 1). D-Fructose, however, binds to boronic acid in a 1:1 fashion. The close proximity of two boronic acid moieties in the D-glucose-imprinted polymer might sterically hinder the complexation of two D-fructose molecules and thus lead to a lower ΔF value.

When a QCM resonator covered with D-fructose-imprinted polypeptide **1** was exposed to a D-fructose solution, the signal observed ($\Delta F = 69 \pm 9$) was similar to that obtained when the nonimprinted polymer surface was exposed to the same amount of D-fructose ($\Delta F = 72 \pm 11$; Table 1). Moreover, the addition of D-glucose to the D-fructose-imprinted interface also caused a response in the same range as that of D-glucose on the nonimprinted polymer surface (Table 1). Therefore, the D-fructose-imprinted polymer **1** interface does not show a better selectivity towards D-fructose than the nonimprinted polypeptide interface does. If the interaction of D-fructose with poly(L-lysine) derivative **1** does not cause significant conformational changes to the polypeptide, as indicated by circular dichroism, then it seems likely that the imprinting process will not give rise to a significant "molecular memory" effect. Interestingly, this observation implies that CD spec-

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troscopy could be used to predict whether a polymer–template system in solution would result in a successfully imprinted polymer on a surface.

The sensitivity of the D-glucose-imprinted polypeptide **1** layer towards successive additions of D-glucose to the measurement cell was studied as a function of the guest concentration (Figure 2). Saturation behavior was observed,

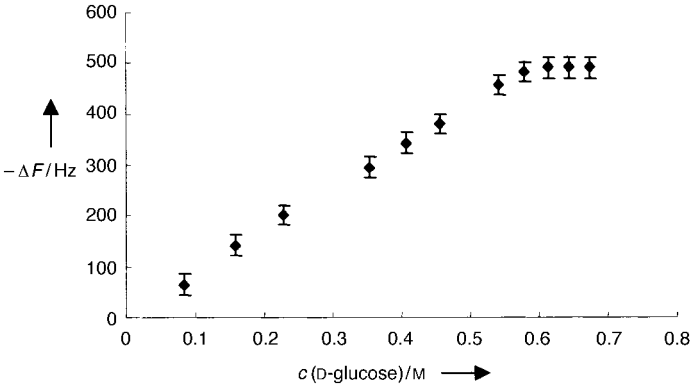

Figure 2. Frequency changes of a QCM with a D-glucose-imprinted polypeptide **1** interface upon successive additions of a D-glucose solution (pH 8).

with a maximum change in frequency corresponding to a 0.67 M concentration of D-glucose in the cell. Further additions of the guest no longer resulted in a decrease of the frequency of the resonator.[15] Moreover, the number of boronic acid recognition sites (3.0×10^{-4}) calculated from the saturation value ($\Delta F = -500$ Hz) of the curve in Figure 2 compares very well to the number of boronic acid units estimated to be present on the polymer adsorbed to the gold surface (between 1.7×10^{-4} and 4.1×10^{-4}).[16]

In conclusion, we have presented a new molecular imprinting method based on the conformational changes of poly(L-lysine) derivative **1**, arising from the interaction between the polymer and the template molecules. Subsequent adsorption of the imprinted polymer onto a gold surface fixes the conformation of the polypeptide, thereby creating a novel molecularly imprinted interface. Furthermore, the application of a D-glucose-imprinted polymer interface for the selective detection of D-glucose over D-fructose has been demonstrated successfully. We hope to extend this novel imprinting concept to other polymer/metal surface systems to create new, selective sensor interfaces for small molecules.

Experimental Section

Synthesis of **1** [Eq. (1)]: **5**: To a solution of 11-acetylsulfanylundecanoic acid (0.025 g, 0.096 mmol) in MeOH/THF (1:3, 15 mL) cooled to 0 °C, were added 1-hydroxybenzotriazole (HOBT; 0.013 g, 0.096 mmol) and 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (EDC, 0.018 g, 0.096 mmol). The solution was stirred at 0 °C for 1 h and subsequently at room temperature for 1 h. It was then added dropwise to a solution of **4** (0.085 g, 0.477 unit mmol) and *N*-ethylmorpholine (0.1 mL) in MeOH (20 mL), and the reaction mixture was stirred at room temperature for 24 h. The solvent was subsequently removed under reduced pressure and the residue dissolved in a minimum amount of MeOH. Upon addition of acetone (15–20 mL) a white precipitate formed which was filtered off and dried to afford **5** in 50% yield. The content of the boronic acid moieties and the acetylsulfanylundecanoic amide moieties as determined by ^{1}H NMR spectroscopy was found to be 70 and 30 mol%, respectively. ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD = 4:1, 25 °C): δ = 8.20–7.90 (brs, 4H; ArH), 3.58 (brs, 1H; CH), 3.24 (brs, 2.9H; CH_2N), 2.71 (brs, 0.9H; CH_2S), 2.20 (brs, 0.9H; $C(O)CH_2$ and brs, 1.3H; CH_3), 1.80–1.40 (m, 6H; CH_2 in poly(L-lysine)), 1.13 (m, 6.9H; CH_2 in acetylsulfanylundecanoic amide side chain).

1: To a nitrogen-saturated solution of **5** (0.063 g, 0.230 mmol) in MeOH (20 mL) was added an aqueous solution of K_2CO_3 (0.5 g in 20 mL), and the solution was heated at reflux for 30 min. After the solution had cooled to room temperature, 2N HCl was added (2 mL) and the organic solvent was subsequently removed under reduced pressure. The residue was dissolved in a minimum amount of MeOH, and upon addition of acetone (15–20 mL) a pale yellow precipitate formed, which was then filtered off and dried to afford **1** in 95% yield. ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 8.20–7.90 (brs, 4H; ArH), 3.98 (brs, 1H; CH), 3.25 (brs, 2.9H; CH_2N), 2.73 (brs, 0.9H; CH_2S), 2.22 (brs, 0.9H; $C(O)CH_2$), 2.00–1.20 (m, 6H; CH_2 in poly(L-lysine)), 1.13 (m, 6.9H; CH_2 in acetylsulfanylundecanoic amide side chain).

QCM measurements were carried out as described in the literature.[17] For the preparation of the QCM resonators see the Supporting Information.

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[12] $[\theta]_{208}$ is the mean residue ellipticity, in deg cm^2 dmol^{-1}, at an extremum of the curve for the α-helix form of poly(L-lysine). See ref. [11].

[13] Considering that the molecular weight of the poly(L-lysine) starting material employed in these experiments is 5000–15000 and that the molecular weight per poly(L-lysine) unit is 208, every polymer chain consists of 24–72 units of which 30% (7–22 units) are surface-anchoring units (i.e. 10-sulfanyldecyl units).

[14] All ΔF values measured are uncorrected for the effect of surface roughness. a) A. Janshoff, H.-J. Galla, C. Steinem, *Angew. Chem.* **2000**, *112*, 4164; *Angew. Chem. Int. Ed.* **2000**, *39*, 4004; b) C. Malitesta, I. Losito, P. G. Zambonin, *Anal. Chem.* **1999**, *71*, 1366.

[15] Although the data in Figure 2 are reminiscent of a Langmuir adsorption isotherm, adsorption of a guest molecule at a given site cannot be considered independent of adsorption occurring at neighboring sites since many of the binding sites (i.e. boronic acid moieties) are located on the same polymer chain, which changes conformation upon binding of the D-glucose guest molecules.

[16] See Supporting Information for details.

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