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Ultrathin Microstructured Polypeptide Layers by Surface-initiated Polymerization on Microprinted Surfaces**

By *Thomas Kratzmüller*, *Dietmar Appelhans*, and *Hans-Georg Braun**

Immobilization of thin biopolymer layers on solid substrates is of particular interest for the development of biosensors,[1] microanalytical devices,[2] or microbioreactors.[3] Furthermore, polypeptides or proteins located on surfaces are regarded as key compounds for the control of biomimetic steps in biomineralization processes.[4–6] Finally, some synthetic polypeptides, such as poly-γ-benzyl-glutamate, reveal interesting material properties, such as piezoelectric[7] or nonlinear optic[8] behavior, when are attached to solid substrates.

The microstructural arrangement in biopolymers is of great interest for the integration of biomolecules into microanalytical or microreaction devices. Patterning of biopolymer layers is commonly achieved using techniques such as photolithography, which was employed for the preparation of structured surfaces. The use of appropriate photolabile protecting groups enables the patterning of a surface with reactive and unreactive functionalities, which can be reacted with biopolymers such as proteins[9,10] or oligo-DNA strands.[11] It has also been demonstrated by different authors[12,13] that capillary extrusion could be another convenient method for the microstructured deposition of proteins on surfaces. Recently microprinting techniques were used to pattern surfaces by direct transfer of protein molecules onto solid substrates.[14]

In this communication we describe a new approach to preparing microstructured polypeptides that are covalently attached to a solid surface. This approach is based on a procedure called microcontact printing (μCP) developed by Whitesides et al..[15] This is a method for chemical surface

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heterogenization resulting in well-defined microstructured areas. Using this method we have been able to pattern gold surfaces with monolayers of appropriate initiator molecules, which can start the polymerization of N-carboxy anhydrides (NCA).[16] The surface-initiated polymerization results in the growth of thin surface-bound polypeptide layers within well-defined areas. The different steps, which involve surface modification and polymerization, are shown in Figure 1.
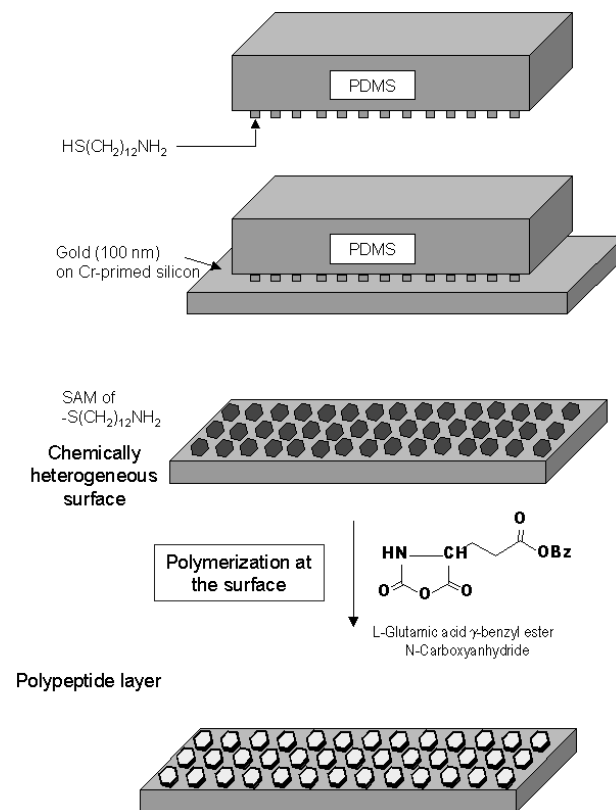

Fig. 1. Schematic representation of the preparation of thin patterned polypeptide layers by microprinting and surface-initiated polymerization.

The surface of a PDMS (polydimethylsiloxane) stamp was treated with a chemical compound necessary for local surface modification, in the following called ink. For chemisorption on a gold surface, the ink molecules are terminated with a thiol group. The initiation of the polymerization requires primary amino groups at the opposite end of the molecule. The molecular self-assembly can be achieved by a linear aliphatic chain segment in between the two functional groups. The compound we synthesized (see Experimental) and used as the initiator system was 12-mercaptododecylamine.

In order to obtain a thin layer of initiator for the surface polymerization on a gold surface we used a solution of 12-mercaptododecylamine in ethanol (1 mM) as ink. After the solvent had been evaporated with a gentle stream of nitrogen, the stamp was brought into contact with the gold surface (100 nm on silicon primed with 2.5 nm of Cr). The thiol was transferred from the stamp to the gold and there-

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by a patterned self-assembled monolayer (SAM) of the aminothiol compound formed. After the stamp had been removed the surface was washed with copious amounts of ethanol. A microstructured amino functionalized surface was produced using this procedure.

Atomic force microscopy (AFM) operated in the lateral force mode is an appropriate method for the analysis of microprinted molecular layers.[17] A typical AFM micrograph (Fig. 2) of a printed aminothiol layer obtained under these experimental conditions demonstrates that the stamped layers are essentially homogeneous and of about one monolayer in thickness.
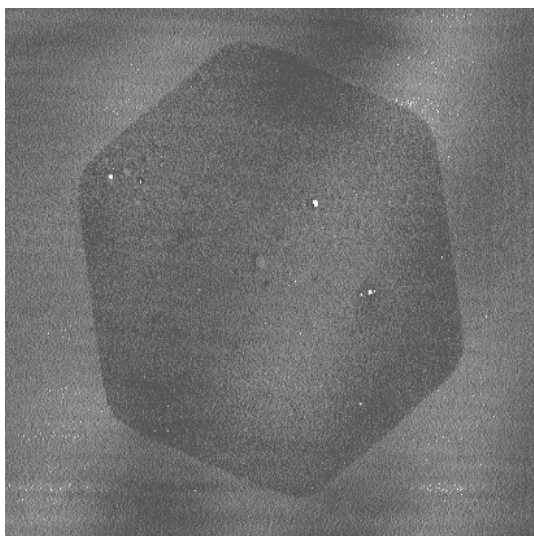

Fig. 2. Lateral force microscopy image of a transferred aminothiol layer.

In order to obtain a surface-bound polypeptide layer we used the structured amine surface as a layer of initiator for peptide polymerization. We used the NCA of γ-benzyl-L-glutamate for the polymerization. The polymerization step was carried out in THF at room temperature under argon. The monomer was readily dissolved in THF without any insoluble residue. The flask was purged with argon and closed for 24 h. After this period of time the gold substrates were removed from the solution and washed several times with a mixture of dichloroacetic acid and chloroform (8:2, v:v). Details of the procedure will be published separately.

To examine the polymerization process with regard to the thickness, orientation, and secondary structure, we also used a gold substrate, which was totally covered with the aminothiol. After 16 h in a 10 mM solution of the thiol in ethanol a self-assembled monolayer had formed on the surface. Next the substrates were removed from the solution, rinsed several times with ethanol and blown dry with a stream of nitrogen. Finally, the unstructured substrates were subjected to the same conditions as the structured ones. The substrates, which were completely covered with the polymer, were then examined with ellipsometry and external reflectance Fourier transform infrared (FTIR) spectroscopy. The thickness of the peptide layer formed during the polymerization was about 30 nm and no other conformation than α-helical could be detected by FTIR spectroscopy.

The structured peptide layers were analyzed using conventional light microscopy with difference interference contrast, low voltage scanning electron microscopy (SEM), and AFM (Fig. 3). We used low voltage SEM because of the low penetration depth of the electrons. This enables the examination of thin organic films on the surface of gold. The images reveal that the polymerization process acts only in the regions where the initiator had been transferred. It is remarkable that the geometrical features introduced by microprinting are retained accurately during the polymerization process. We prepared micropatterned peptide layers with individual repeating motifs with dimensions between 3.8 μm and 100 μm.

From the structured surface it is possible to determine the thickness using AFM. The section analysis of the AFM images shows that the peptide layers are about 32 nm in height. This is in good agreement with the ellipsometric data obtained with the totally covered sample. Deviations in height between different polypeptide patches are less than 2 nm. With the assumption of a perpendicular orientation of the helices, the observed layer thickness corresponds to a degree of polymerization of around 200. We conclude from the analysis of AFM measurements in lateral force mode that the gold surface between the polymerized peptide structure remains free of any peptide adsorbed from the solution.

The benzylester group of poly(γ-benzyl-L-glutamate) (PBLG) is sensitive to hydrolysis under acidic conditions. The polymer-analogous reaction of surface-bound PBLG leads to surface-bound poly(L-glutamic acid). This is a convenient route for the preparation of ultrathin layers of surface-bound polyelectrolytes. HBr in acetic acid was used for the hydrolysis of the benzylester group of poly-γ-benzyl-glutamate. The hydrolysis of the benzylester group could also be monitored by FTIR spectroscopy until no ester group was detectable. The thin layer of microstructured surface-bound poly(L-glutamic acid) shows varying degrees of ionization, depending on the pH, leading to a layer with a different content of carboxylate groups.

In this communication the polymerization of poly(γ-benzyl-L-glutamate) on amine-modified gold surfaces is described for the first time. The patterning of self-assembling initiator molecules allowed the surface growth of well-defined poly(γ-benzyl-L-glutamate) domains on the gold surfaces. By subsequent hydrolysis of the benzylester groups of surface-immobilized PBLG, microstructured poly(L-glutamic acid) layers were available for the first time.

The preparation of microstructured surface-bound polyelectrolytes could have a great impact on future material design through biomimetic reactions at well-defined biopolymer interfaces. Possible conformational changes within these defined polypeptide layers in combination with ion complexation capability would be of great interest for the

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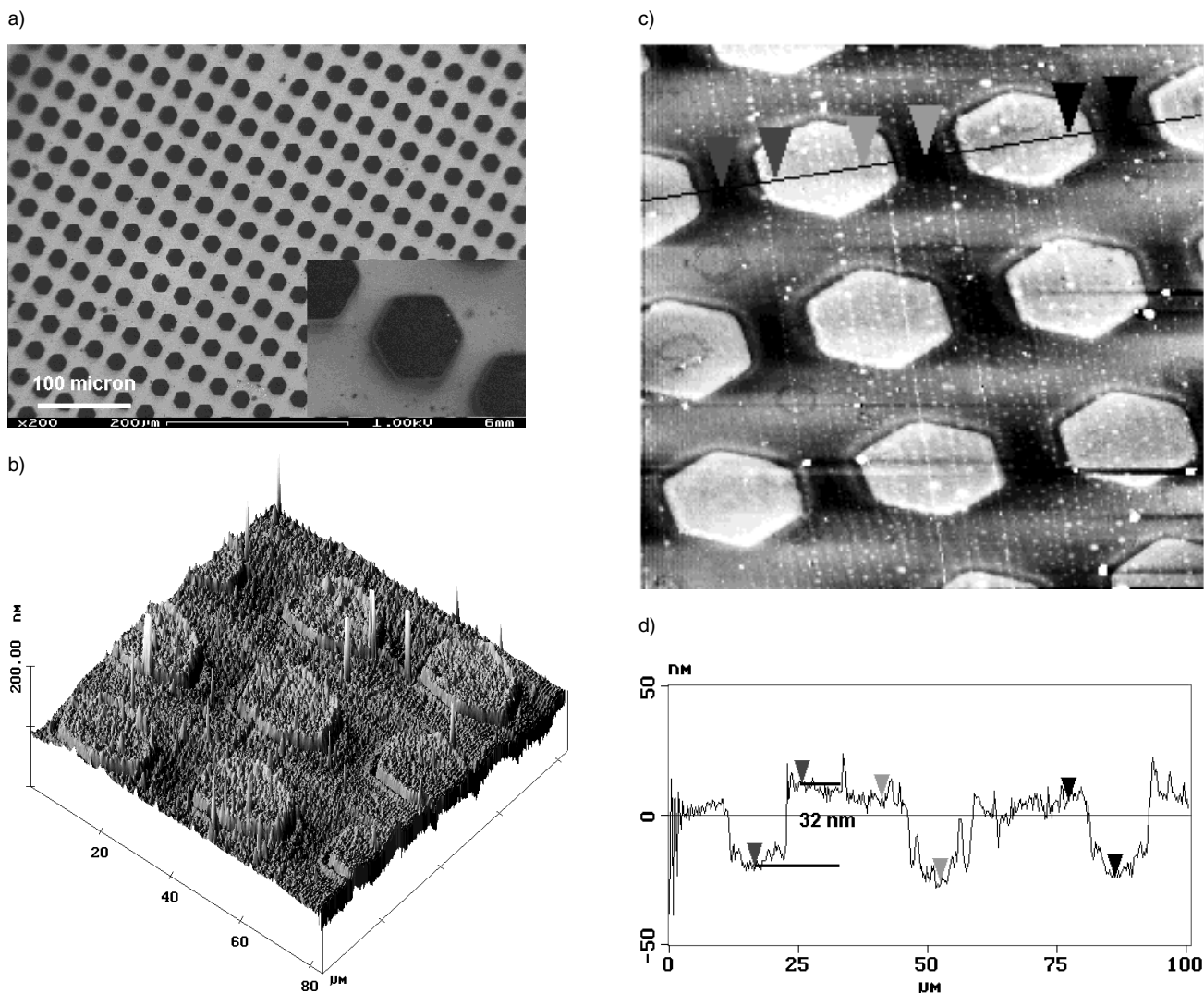
a)

b)

c)

d)

Fig. 3. a) Scanning electron microscopy image of peptide layer. b) AFM image of the structured polypeptide layers. c) Section analysis (image). d) Section analysis.

spatially controlled adsorption of oppositely charged poly-electrolytes from solution,[18] or for the conversion of inorganic ions to inorganic materials with interesting material properties.[19]

Experimental

12-mercaptododecylamine was prepared according to the following procedure: Reaction of 11-bromoundec-1-ene (Lancaster) with thioacetic acid in the presence of AIBN in refluxing $CHCl_3$ yields 11-bromoundecane-1-thioacetate.

NaCN (2.0 g, 45 mmol) was dissolved in anhydrous DMSO under argon at 100 °C and 11-bromoundecane-1-thioacetate (11.5 g, 38.5 mmol) was added to this solution. The reaction was stirred at 90 °C for 6 h. The mixture was cooled to room temperature, diluted with water, and extracted three times with petroleum ether. The combined organic layers were washed twice with saturated aqueous NaCl and dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was distilled under vacuum. 11-cyanoundecane-1-thioacetate (7.65 g, 81 %) was obtained as a clear liquid.

Under argon, sodium (0.52 g, 22.7 mmol) was dissolved in absolute methanol. 11-cyanoundecane-1-thioacetate (4.16 g, 16.3 mmol) was added and stirred overnight at room temperature. The reaction solution was cooled

to 0 °C and quenched with aqueous NH_4Cl and diethyl ether. The aqueous layer was extracted three times with petroleum ether and the combined organic layer was washed twice with deionized water and dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was distilled under vacuum. 11-cyanoundecanethiol (3.15 g, 80 %) was obtained as a clear liquid.

To a stirred solution of 11-cyanoundecanethiol (0.56 g, 2.6 mmol) in anhydrous dichloromethane was added borane-dimethylsulfide complex in THF (1 M; 10 mL) under argon at room temperature. The reaction was stirred for 1 h at 40 °C. After cooling to room temperature methanol (20 mL) was slowly added to the reaction solution. The solvent was removed under reduced pressure to leave 12-mercaptoundecylamine (0.55 g, 97 %).

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Increasing the Coercivity in Layered Molecular-based Magnets A[M^II M^III(ox)3] (M^II = Mn, Fe, Co, Ni, Cu; M^III = Cr, Fe; ox = oxalate; A = organic or organometallic cation)**

By *Eugenio Coronado,* José R. Galán-Mascarós, Carlos J. Gómez-García*, and *José M. Martínez-Agudo*

In the search for new molecular-based magnets, the extended networks based on transition metal oxalate complexes have been attracting considerable attention since the discovery at the beginning of the nineties of the series of bimetallic magnets $A[M^{II}M^{III}(ox)_3]$ (M^{II} = Mn, Fe, Co, Ni, Cu; M^{III} = Cr, Fe), where A is a bulky organic cation of the type $[XR_4]^+$ (X = N, P; R = phenyl, *n*-propyl, *n*-butyl, etc.). All these compounds behave as magnets with coercive fields, H_{coer}, ranging from < 1 mT to about 100 mT at 2 K, and critical temperatures, T_c, ranging from 5 to 44 K.[1–4] Their structures consist of honeycomb layers of the two metal atoms connected through ambidentate oxalate ligands. These inorganic layers are interleaved by cations, A, which control the interlayer separation, as well as the stacking of the layers.[5] By varying M^{II}, M^{III}, and A it has been possible to obtain a large number of bimetallic compounds exhibiting a wide variety of magnetic structures including ferro, ferri, and canted antiferromagnets.

To extend the range of examples, our first approach was to replace the organic cation A with the organometallic cations decamethylferrocenium, $[FeCp*_2]^+$, and decamethyl-

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cobaltocenium, $[CoCp*_2]^+$. The insertion of these complexes between the bimetallic magnetic layers led to a novel series of molecular magnets whose cooperative magnetic properties were very close to those reported for the XR_4^+ salts, which is consistent with the lack of short contacts between the organometallic cations and the inorganic layers.[6] The only noticeable difference was observed in the $CoCp*_2$ derivatives, which showed important variations in the coercive fields.[7] In particular, the coercive field for the $[Fe^{II}Cr^{III}]$ derivative was twice that found in the XR_4^+ or $[FeCp*_2]^+$ analogues (203 mT compared with 110 mT).

A much more profound effect on the magnetic properties is expected to occur when using mixtures of Cr^{III} and Fe^{III} in the construction of the anionic network, because competitive ferromagnetic M^{II}–Cr^{III} and antiferromagnetic M^{II}–Fe^{III} interactions will coexist within the magnetic layer. Such a possibility was first investigated by Bhattacharjee and Ijima[8] who examined the static (d.c.) magnetic susceptibility behavior of the mixed-metal compounds $[NBu_4][Mn^{II}Fe^{III}_{0.5}Cr^{III}_{0.5}(ox)_3]$ and $[NBu_4][Fe^{II}Fe^{III}_{0.5}Cr^{III}_{0.5}(ox)_3]$. We have undertaken a detailed magnetic study of the series of trimetallic compounds of general formula $A[M^{II}Fe^{III}_x Cr^{III}_{(1-x)}(ox)_3]$ (M^{II} = Mn, Fe, Co, and Ni; A = $[NBu_4]$, $[FeCp*_2]$, and $[CoCp*_2]$), paying particular attention to the magnetic susceptibility in the presence of an oscillating field (a.c. susceptibility) and the hysteretic behavior, the final aim being to understand the relationship of these properties to the chemical composition. The first results of this study are reported here.

The compounds were prepared as microcrystalline powders by the one-pot reaction of the appropriate mixture of the tris(oxalate)complexes $[M^{III}(ox)_3]^{3-}$ of Cr^{III} and Fe^{III} with the divalent metal ion M^{II} and A^+ cations in water. The X-ray diffraction patterns of these samples were indexed with the same unit cells as the pure Cr or Fe salts. This shows that the purity and crystallinity of the materials, as well as the 2-D layer structure, are maintained.

Looking at the magnetic properties we observed that the magnetic susceptibilities in the high temperature region (i.e., above 50 K) closely obey the Curie–Weiss law, with Weiss constants (Θ) passing, as expected, from positive to negative values when the ratio x is increased. For example, in the iron (II) series $[NBu_4][Fe^{II}Fe^{III}_x Cr^{III}_{(1-x)}(ox)_3]$, Θ varies monotonically between 19 and –90 K when passing from the Cr^{III} (x = 0) to the Fe^{III} (x = 1) derivatives, concomitant with the dominance of ferromagnetic or antiferromagnetic interactions, respectively, between the metal centers (see Table 1). In the intermediate region the two near-neighbor exchange pathways compensate, so that when $x \approx 0.33$, Θ is close to zero. Similarly, the transition temperatures of these Cr/Fe metal alloys are also between the values of the two limit compositions in such a way that T_c can be tuned continuously by variation of x (Fig. 1 and Table 1). The values of T_c are derived from a.c. susceptibility measurements, which exhibit a sharp peak in the in-phase susceptibility as well as an out-of-phase signal at T_c. Inter-