Synthesis of Functionalized Polymer Monolayers from Active Ester Brushes

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ABSTRACT: The synthesis of densely grafted monolayers of polymers carrying a wide spectrum of functional groups is described. To this surface-attached chains with active ester groups were generated through surface initiated polymerization of *N*-methacryloyl-*β*-alanine *N*′-oxysuccinimide ester (MAC_2AE) using a self-assembled monolayer of an azo initiator on the surfaces of silicon oxide substrates. The layer thickness of the polymer monolayers can be easily controlled through the adjustment of the monomer concentration during polymerization. The aminolysis of the active ester groups within the brush with various *n*-alkylamines (C_3−C_{18}) was studied by infrared and surface plasmon spectroscopy. It is also shown that amines that carry additional functional groups such as amino acids, sugars, dyes, crown ethers, and aminoterminated polymers such as poly(ethylene glycol) derivatives can be used to incorporate many different functional moieties into the polymers.

Introduction

The decoration of surfaces with specific functional groups is of interest in many fields of research, especially in the life sciences. For example, systems are currently being developed for biomedical implants where short sequences of cell recognition proteins are immobilized on surfaces to enhance cell adhesion.[1] In other systems, antibodies are attached to particle surfaces and then used for bioanalytical purposes. Functionalized (self-assembled) monolayers are also the basis for sensors where probe molecules are attached to the surface of a chip, which create specific binding sites for biomolecules such as DNA or proteins contained in an analyte ("biochips").[2] These and other applications have led to a vast effort on the generation of self-assembled monolayers carrying functional groups. Although, the self-assembly strategy is from a principal point of view simple and straight forward, one drawback of the monolayer approaches is the inherently small number of probe molecules provided through this approach. The low probe surface density frequently leads to sensitivity problems of the thus obtained analytical devices. Furthermore, the determination of the binding efficiency of such surface functionalized chips is not straightforward. Simple measurements of an increase of the mass of the surface layer due to incorporation of additional material from the analyte, e.g., by SPR, is often difficult mainly for two reasons: (a) the mass increase of the monolayer is rather low and (b) nonspecific adsorption/binding of competing molecules from the analyte might contribute significantly to the measured signal.[3] To circumvent this problem one needs either to introduce labeling techniques, which improve the sensitivity, or to establish fabrication techniques that increase the density of the probe groups on the surface. One promising strategy is to follow a "skyscraper approach" and to incorporate these groups into a polymer matrix on top of the sensor chip.

In recent years, we have developed a technique to grow dense monolayers of terminally grafted polymer chains on various solid substrates using surface-initiated polymerization.[4−8] This technique makes use of self-assembled azo-initiator monolayers for the direct polymerization of suitable monomers on the surface of the substrate. Using this approach we were able to synthesize polymer brushes in a well controlled way from monomers like styrene or methyl methacrylate with a thickness of up to 2200 nm and graft densities that correspond to averaged anchor distances down to 2 nm.[7,8]

In order to create functionalized polymer brushes on a surface following this approach one can of course use monomers that carry the desired groups and grow the layer directly using these monomers. However, in order to generate thick brushes at a given graft density, a high molecular weight of the surface-attached chains is needed. The molecular weight of the generated chains scales linearly with the monomer concentration used in the polymerization reaction. Thus, in typical experimental procedures, even for small substrates, milligram to gram amounts of the monomer are needed. Such requirements would render the process prohibitively expensive, in particular, if complicated biomolecules are used, as such large amounts of monomer will not be available for all systems at a reasonable price. Additionally, each variation of the functional monomer requires that the entire polymerization behavior of this monomer is studied anew. Finally, the brush growth process does not tolerate the presence of all possible functionalities that might be of interest. As in any other chain growth process, groups which act as terminating or strong transfer agents prevent the formation of high-molecular weight surface-attached chains and thus limit the number of functional groups contained in the film. Thiol or amine groups, for example, contained in the monomer will limit the molecular weight of the growing chains very strongly and render only unacceptably thin films.

On the basis of these considerations, we choose an alternative route and used a monomer with a reactive group which is sufficiently stable under polymerization conditions and can then be transformed into the desired function after the preparation of the polymer monolayer (Figure 1a).

Experimental Section

Materials. Methacryloyl chloride, *β*-alanine, *N*-hydroxysuccinimide, *n*-alkylamines (C_3∼C_{18}), *O*-(2-aminoethyl)-*O*′-methyl-poly(ethylene glycol) (monoaminoPEG) 5000, 2000, and 750, 2-aminomethyl-15-crown-5, allyl alcohol and 4,4′-azobis(4-cyanopentanoic acid) were purchased from Fluka. D-Glucosamine hydrochloride, L-alanine methylester hydrochloride, and amino-

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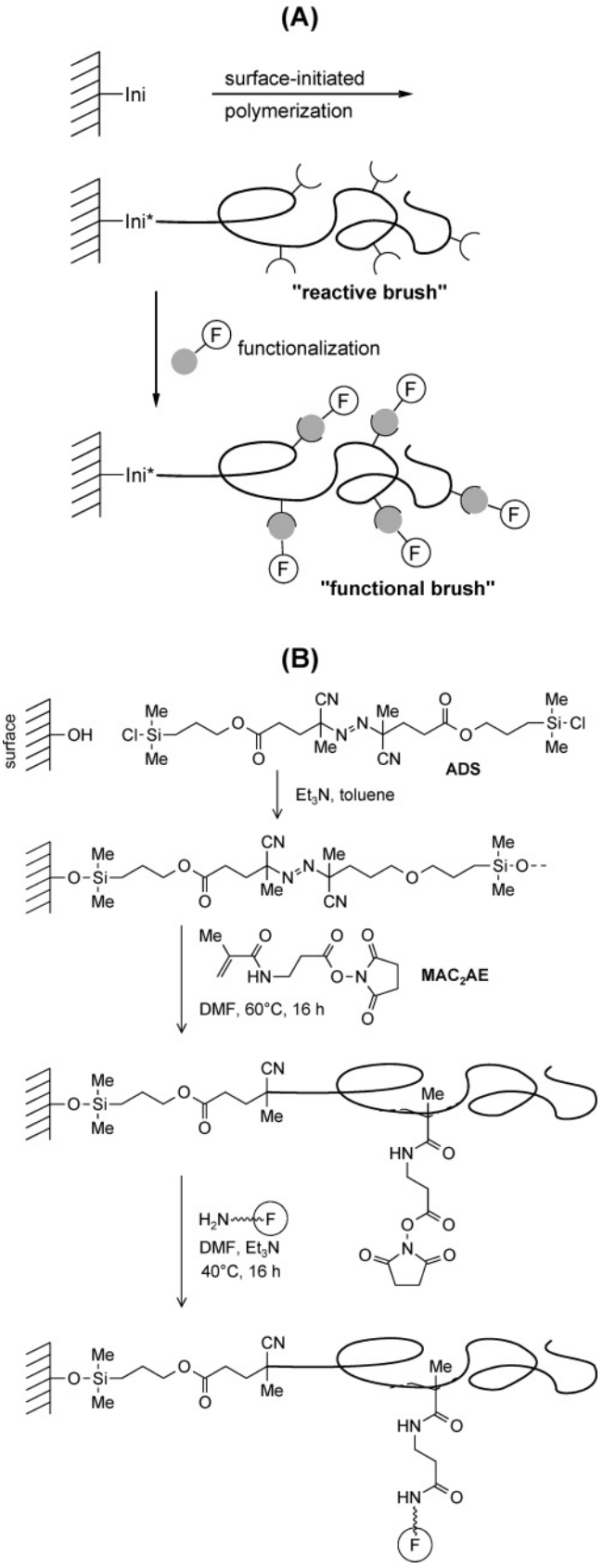

Figure 1. (A) Schematic illustration for the two-step process used for the generation of functional polymer brushes. First, a brush containing reactive sites is generated through surface initiated copolymerization which is then transformed to the desired functional brush following a polymer analogous reaction. (B) The reactive brushes are generated from a monolayer of an azo initiator using a active ester bearing methacrylate as reactive monomer. These reactive sites are then aminolyzed using appropriate functional amines.

methylpyrene hydrochloride were purchased from Aldrich. The substrates for suface plasmon spectroscopy (SPS) were prepared by evaporating Ag (50 nm) and then SiO_x ($1 \leq \times \leq 2$, 30 nm) layers onto glass slides (BK7 and LaSFN9). The substrates for transmission FTIR spectroscopy were silicon wafers polished on both sides (Aurel) with a natural SiO_{-2} layer of approximately 2.5 nm. *N*,*N*-Dimethylformamide (DMF) was distilled from activated molecular sieves (4 Å, 300 °C for 16 h) after drying with calcium hydride. Toluene was distilled from molten sodium using benzophenone as an indicator. Triethylamine was distilled from calcium hydride. All drying procedures were performed in an atmosphere of dry argon and the resulting dry chemicals and solvents were also stored under such an atmosphere.

Characterization. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 250 spectrometer in $CDCl_3$. Routine FT-IR spectra were obtained with a Perkin-Elmer Paragon 1000. Transmission FT-IR spectra of the attached polymer layers were recorded using a Nicolet 730 FTIR spectrometer. Melting points (mp) were measured on a Laboratory Devices Mel-Temp II. The layer thickness of the grafted polymers were obtained by SPS[9] using a set up in Kretschmann configuration[10] with a He−Ne laser (λ = 632.8 nm), 90° prism (BK7, $n_D = 1.5151$, Spindler & Hoyer, and LaSF9N, $n_D = 1.8449$, Berliner Glas) and index match liquid ($n_D = 1.5160$ and 1.700, Cargill). The refractive index of the polymer brushes on the substrates were assumed to be $n = 1.50$.

Synthesis of the Monomer. *N*-Methacryloyl-*β*-alanine. To a solution of *β*-alanine (8.9 g, 100 mmol) and sodium hydrogen carbonate (18.5 g, 220 mmol) in H_2O (100 mL) and 1,4-dioxane (50 mL) was added 10.3 mL of methacryloyl chloride (105 mmol) in 1,4-dioxane (50 mL) slowly at 0 °C, and the resulting mixture was stirred at room temperature overnight. The mixture was washed twice with ethyl acetate (100 mL) after adding H_2O (100 mL). The water layer was acidified with 6 M HCl to pH 2 at 0 °C and then extracted five times with ethyl acetate (100 mL). The organic layers were dried over anhydrous $MgSO_4$, filtered, and the solvent was evaporated. The resulting residue was purified by recrystallization from ethyl acetate/*n*-hexane (1/9, v/v); yield 12.3 g (78%), mp 74−76 °C (lit.[11] 70−73 °C). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.08 (s, 3 H, CH_3 (allyl)), 2.77 (t, 2 H, $J = 5.96$ Hz, $NHCH_2CH_2CO$), 3.74 (t, $J = 5.96$ Hz, $NHCH_2CH_2CO$), 5.49 (s, 1 H, CH (olefin)), 5.86 (s, 1 H, CH (olefin)), 6.81 (broad s, 1 H, amide), 10.39 (broad s, 1 H, carboxylic acid). IR (KBr): 3354, 2957, 2598, 1715, 1651, 1601, 1546, 1453, 1223, 1196, 1095, 1041, 875, and 800 cm^{-1}.

***N*-Methacryloyl-*β*-alanine Succinimide Ester (MAC_2AE).** To a solution of *N*-methacryloyl-*β*-alanine (8 g, 51 mmol) and *N*-hydroxysuccinimide (5.9 g, 51 mmol) in dichloromethane (100 mL) was added dicyclohexylcarbodiimide (DCC, 10.5 g, 51 mmol) at 0 °C, and the resulting mixture was kept in a refrigerator overnight. The precipitated urea was filtered off and the filtrate was concentrated to 30 mL, causing the separation of the product. The material was purified by recrystallization from 2-propanol (150 mL): yield 9 g (70%), mp 143−144 °C (lit.[11] 125−126 °C). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.82 (s, 3 H, CH_3 (allyl)), 2.72−2.76 (m, 6 H, CH_2 (succinimide) and $NHCH_2CH_2CO$), 3.54−3.61 (t, 2 H, $J = 5.96$ Hz $NHCH_2CH_2CO$), 5.21 (s, 1 H, olefin), 5.61 (s, 1 H, olefin), 6.51 (broad s, 1 H, NH (amide)). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 18.9 ($CH2{=}C(CH_3)$), 26.0 ($COCH_2CH_2CO$ in succinimide), 32.3 ($NHCH_2CH_2CO$), 35.7 ($NHCH_2CH_2CO$), 120.7 ($CH_2{=}C$), 139.8 ($CH_2{=}C$), 167.9, 168.9, and 169.6 (C=O), IR (KBr): 3411 and 3322 (N−H), 2996 and 2931 (C−H), 1809, 1781, and 1725 (C=O (succinimide ester)), 1657 (C=O (amide)), 1619 (C=C), 1540 (N−H), 1226, 1210, 1196, 1081, 1064, 879, and 649 cm^{-1}.

Synthesis of the Azo Initiator 4,4′-Azobis(4-cyanopentanoic acid-(3′-chlorodimethylsilyl)propyl ester) (ADS). The initiator ADS was obtained by esterification of the acid chloride of 4,4′-azobis(4-cyanopentanoic acid) with allyl alcohol followed by hydrosilylation with dimethylchlorosilane. Details of the synthesis of this and related compounds have been reported.[4−6,12]

Immobilization of ADS on SiO_x Substrates. Substrates for SPS and FTIR mesurements were dried in vacuo. Then, toluene (20 mL), triethylamine (1 mL), and 2 mL of ADS solution (ADS 200 mg/

toluene 20 mL) were added under a dry argon flow and kept in the dark at room temperature overnight. The modified substrates were then rinsed with toluene, methanol, and acetone.

Polymerizations. Initiator modified substrates were placed in a Schlenk tube and covered with monomer and DMF in the desired ratio. The monomer solutions were degassed by five freeze−pump−thaw cycles and then heated to 60 °C for 16 h. The samples were then extracted with DMF for at least 6 h to remove free polymer from the layer and finally rinsed with acetone to remove DMF. The samples were dried in vacuo prior to SPS and FTIR measurements.

Aminolysis of the Active Ester of the Polymer Brushes. To the substrates carrying active ester polymer brushes in DMF (3 mL), the desired amine (50 mg or 50 μL) and triethylamine (50 μL) were added and the reactor was kept at 40 °C for 16 h. The substrates were then rinsed with DMF and acetone and finally dried in vacuum.

Results and Discussions

General Description of the System. The system used in this study is depicted in Figure 1b. Many molecules that are interesting for sensor applications contain amino groups and for this reason we decided to first use a monomer that carries a group that reacts specifically with amines. *N*-hydroxysuccinimide esters are suitable candidates for this purpose as they are well-established reagents for the attachment of amino group containing molecules under mild conditions with excellent yields.[11,13−20]

It has been shown previously that such succinimide groups can be incorporated into polymers and copolymers via free radical polymerization, resulting in well-defined polymers.[11,17−20]

In this paper, we describe the synthesis of "active ester polymer brushes" on planar substrates using *N*-methacryloyl-β-alanine *N*′-oxysuccinimide ester (MAC_2AE) as a monomer and monolayers of 4,4′-azobis(4-cyanopentanoic acid-(3′-chlorodimethylsilyl)propyl ester) (ADS) to start the surface-initiated polymerizations. We have chosen this specific monomer as it has a short ethylene spacer between the polymer backbone and the active ester unit which should avoid possible steric problems during reaction of the amine with the succinimide ester moiety.

We have first studied the aminolysis reaction of the active ester polymer brushes with simple *n*-alkylamines as model compounds. Second, functional amines were used to explore the versatility of the approach: L-alanine methylester and D-glucosamine as examples for biomolecules; aminomethylpyrene as a simple dye molecule; 2-aminomethyl-15-crown-5 as an example for a moiety with complexation properties; *O*-(2-aminoethyl)-*O*′-methylpoly(ethylene glycol) (monoaminoPEG) as a typical compound for the improvement of the biocompatibility.

The latter polymeric amines are also interesting model compounds for the investigation of the incorporation of larger molecules into brush monolayers. The structures of all compounds used in this study are shown in Figure 2.

Preparation of Active Ester Brushes on the Substrates. The first step in the preparation of polymer brushes via radical "grafting from" polymerization is the self-assembly of the initiator species on the surface of the substrates. In this study, we have used an azo-initiator that carries two dimethylchlorosilane end groups that can both potentially react with silanol groups on SiO_x surfaces and result in either mono- or bidentate attachment.[4−6,12] While it is generally more straight forward to use an initiator species that can only react with one reactive site, it has been shown before,[12] that the compound ADS is very easily synthesized and gives well reproducible initiator layers if appropriate dry conditions are chosen.

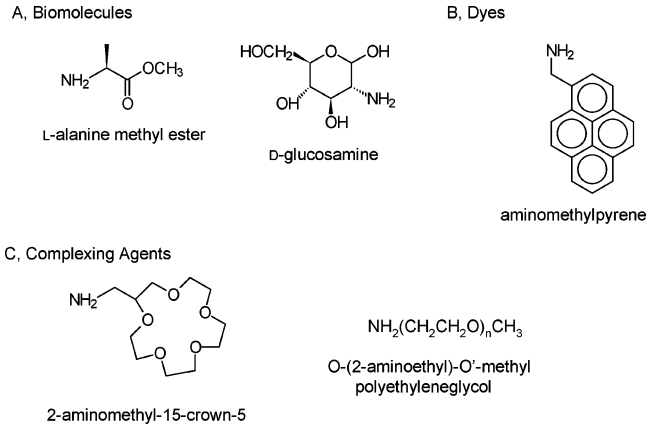

Figure 2. Chemical structures of the amines used for the generation of functional polymer brushes from active ester brushes.

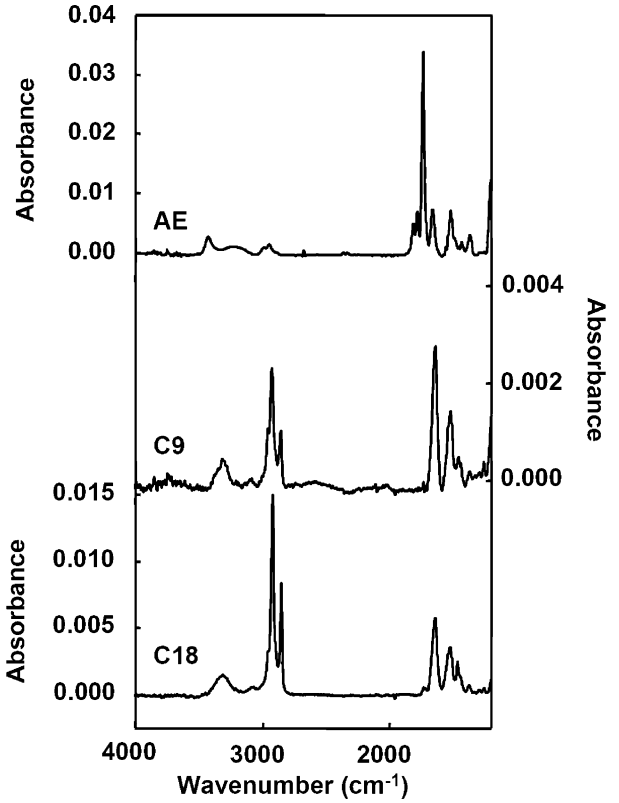

Figure 3. Transmission FTIR spectra of polymer brushes on SiO_x substrates: (AE) active ester polymer brushes; (C9) brush reacted with 1-nonylamine; (C18) brush reacted with 1-octadecylamine.

The surface initiated polymerizations using MAC_2AE as an active ester monomer were carried out at 60 °C for 16 h in DMF. After completion of the polymerization reaction the active ester polymer brushes were rinsed and extensively extracted with DMF for at least 6 h to remove nonattached polymer chains.

As quite thick brushes were obtained, it was possible to characterize the structures of the active ester polymer brushes directly by transmission FTIR spectroscopy, as shown in Figure 3 (for AE). Apart from absorption bands around 3400 (N−H) and 3000 cm^{-1} which can be attributed to C−H stretching vibrations, typical signals from the *N*-oxysuccinimide ester groups are found at 1815, 1785, and 1737 cm^{-1}. Absorptions at 1661 and 1520 cm^{-1} can be assigned to the amide I and II bands, respectively. The area below ca. 1200 cm^{-1} is dominated by absorption bands caused by solid-state vibrations of the silicon oxide layer.

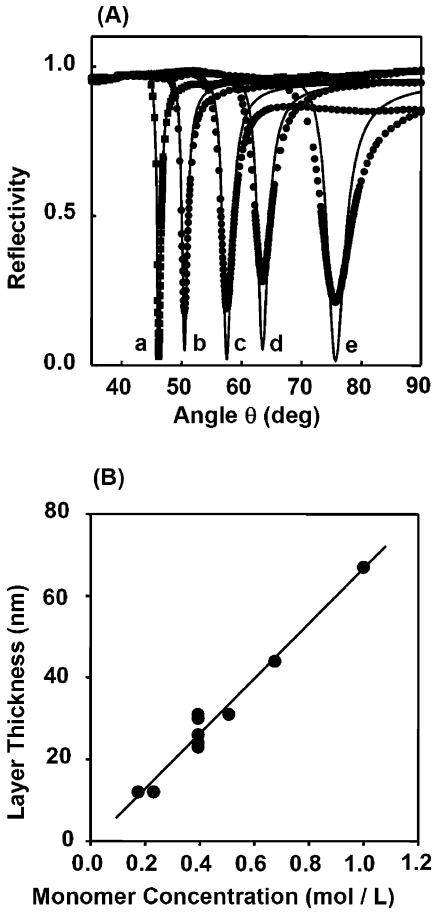

Figure 4. (A) SPR reflectivity curves of active ester polymer brushes obtained on the surface of SiO_x-Ag-glass substrates by surface-initiated polymerization in DMF at 60 °C for 16 h. Key: (a) blank (SiO_x; 30 nm, Ag; 50 nm, and BK7); (b) monomer concentration [M] = 0.18 mol/L (12 nm), (c) [M] = 0.51 mol/L (31 nm), (d) [M] = 0.68 mol/L (44 nm), or (e) [M] = 1.00 mol/L (67 nm). Solid lines represent results of Fresnel calculations, respectively. (B) Layer thickness of active ester polymer brushes as a function of the monomer concentration used during "grafting from" polymerization with SAIMs in DMF at 60 °C for 16 h.

For quantitative analysis, the thicknesses of the grafted active ester polymer brushes were determined by surface plasmon resonance (SPR). The reflectivity curves obtained from these samples (see Figure 4a) were compared to those obtained by Fresnel model calculations (results shown as solid lines in Figure 4a).[9,10] To obtain the thickness values of the samples a simple box model was employed and a refractive index of $n = 1.50$ for the polymer monolayers was assumed. In Figure 4a the reflectivity curves of a blank substrate (a) and of samples with surface-attached polymer monolayers obtained from polymerizations at different monomer concentrations (b−e) are shown. It can be easily seen that the resonance angles are strongly shifted to higher values for samples obtained at higher monomer concentrations. The thickness values derived from the Fresnel analysis of these curves are plotted in Figure 4b as a function of the monomer concentration. The layer thicknesses obtained under the given reaction conditions range from 12 nm (0.18 mol/L) to 67 nm (1.0 mol/L). As expected for a free radical polymerization process, a linear increase of the sample thickness with increasing monomer concentration was found, which shows that the chosen parameters and reaction conditions offer good control over the brush growth.

Reaction of the Active Ester Polymer Brushes with *n*-Alkylamines. The active ester groups in the brushes can be used to introduce functional groups via aminolysis. This reaction of *N*-oxysuccinimide esters in solution has been well-studied.[12−15] However, the constrained geometry inside a brush might influence the accessibility of the active ester groups which in turn might lower the degree of functionalization obtained in this reaction. To study this, we first investigated the polymer analogous reaction of the active ester brushes with simple alkyl amines of variable molecular weight (C_3−C_{18}).

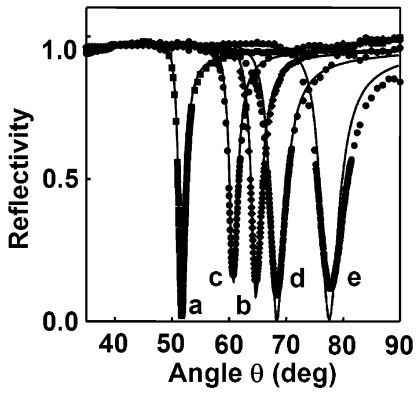

Figure 5. SPR reflectivity curves of polymer brushes before and after reaction with *n*-alkylamines in DMF at 40 °C for 16 h. Key: (a) blank (SiO_x; 50 nm, Ag; 50 nm, and BK7); (b) active ester polymer brush before reaction with amine (30 nm); (c) sample reacted with 1-propyl (18 nm), (d) 1-dodecyl (39 nm), or (e) 1-octadecylamine (52 nm). Solid lines represent results of Fresnel calculations.

The reactions were carried out at 40 °C for 16 h in DMF as a solvent. After completion of the aminolysis reaction, the substrates were extracted with DMF and acetone. For solubility reasons all samples where 1-octadecylamine was employed were extracted with chloroform. All substrates were dried in vacuum prior to characterization.

The chemical structures of the polymer brushes obtained after aminolysis with C_9 and C_{13} amines were at first studied qualitatively by transmission FTIR spectroscopy. The spectra obtained after aminolysis of the active ester brushes with C_9 and C_{18} amines are shown in Figure 3. All spectra of the amine modified brushes are dominated by absorption bands of the C−H stretching vibration originating from the alkyl groups of the amines. Furthermore, in the region of the C═O stretching vibration the bands due to the *N*-oxysuccinimide moiety of the active ester brushes between 1815, 1785, and 1737 cm^{-1} are no longer visible. This is a first evidence that the aminolysis of the active ester groups within the polymer brushes proceeds with high yield.

All samples obtained after reaction with the various alkylamines were further investigated by SPR. In Figure 5, the SPR reflectivity curves of (a) a blank substrate, (b) of an active ester polymer brush prior to aminolysis, and (c−e) of various polymer brushes after reaction with selected amines (1-propylamine, 1-dodecylamine, and 1-octadecylamine) are shown. The resonance angle of the sample reacted to 1-propylamine is shifted to a lower value compared to that of the active ester polymer brush prior to reaction, while the resonance angles of the samples after reaction with 1-dodecyl and 1-octadecylamine are shifted to significantly higher values.

For further analysis, the SPR curves were used to obtain layer thicknesses of the polymer brushes after reaction with the alkylamines. Again, a refractive index of $n = 1.50$ was assumed for all polymer monolayers. In all cases an active ester brush with an identical thickness of approximately 30 nm was used as starting material. Upon aminolysis, the thickness of the layer changes depending on the chain length of the alkylamine used.

If, for example, 1-propylamine is used, a reduction of the layer thickness to 18 nm is obtained, whereas reaction with dodecyl- and octadecyl amine leads to an increase of the layer thickness to 39 and 52 nm, respectively.

In order to understand this behavior, we have to consider that the aminolysis reaction changes the molecular mass of the attached polymer molecules and, therefore, the total mass of the monolayer. This translates directly to changes in the thickness of the layer before and after aminolysis.

The thickness L of a polymer monolayer is given by

$$L = \frac{\Gamma \bar{M}_n}{\rho} \tag{1}$$

In this equation Γ is the graft density of the brush and $\bar{M}_n$ the number-average molecular weight of the attached polymer chains. ρ is the materials density of the monolayer. Now, the ratio (L_2/L_1) of the layer thickness before (1) and after (2) reaction with an amine can be expressed as

$$\frac{L_2}{L_1} = \frac{\Gamma_2 \cdot \bar{M}_{n2} \cdot \rho_1}{\Gamma_1 \cdot \bar{M}_{n1} \cdot \rho_2} \tag{2}$$

If it is assumed, that (a) the chain length of the surface-attached molecules does not change during the reaction, (b) the aminolysis proceeds quantitatively, and (c) the graft density of the layers is unaffected by the reaction (no chains lost during the reaction, $\Gamma_1 = \Gamma_2$), we can rewrite eq 2 as follows:

$$\frac{L_2}{L_1} = \frac{M_2 \rho_1}{M_1 \rho_2} \tag{3}$$

Here M_1 and M_2 are the molar masses of the repeat units before (1) and after aminolysis (2). As a first approximation a constant material density of the brushes ($\rho_1 = \rho_2$) can be assumed. Then, L_2/L_1 should be equal to the ratio of the molar masses of the repeat units before and after the reaction (M_2/M_1):

$$\frac{L_2}{L_1} = \frac{M_2}{M_1} \tag{4}$$

Reactions with short alkylamines ($n \leq 8$) reduce the molar mass of the repeat units because the mass of the corresponding alkyl chain is smaller than that of the *N*-hydroxy succinimide leaving group. Hence, a lower film thickness of the brush after aminolysis is expected and also observed experimentally. For the longer alkyl amines we also find the expected increase in film thickness after aminolysis.

In Figure 6a, the two ratios M_1/M_2 (open square) and L_1/L_2 (open circles) are shown as a function of the chain length of the amines. The data confirm the expected behavior fairly well. The slight difference between the slopes of the regression curve of the experimental data and the values calculated according to eq 4, can be attributed to the different material densities of the respective layers. This is indeed expected as the density of alkyl chains is smaller than that of the backbone polymer and the relative contribution of the alkyl substituents to the total mass of the polymer increases with increasing chain length of the alkyl groups. Although the exact materials densities of the polymers before and after aminolysis are unknown, a rough estimation of these values can be made if the density increments based on van der Waals radii of suitable molecular fragments in the amorphous state are used.[21,22] Details on these calculations are available in the Supporting Information. If the obtained density values are used to refine the calculation of the L_2/L_1 ratios according to eq 3, a good agreement between theory and experiment is observed (Figure 6b).

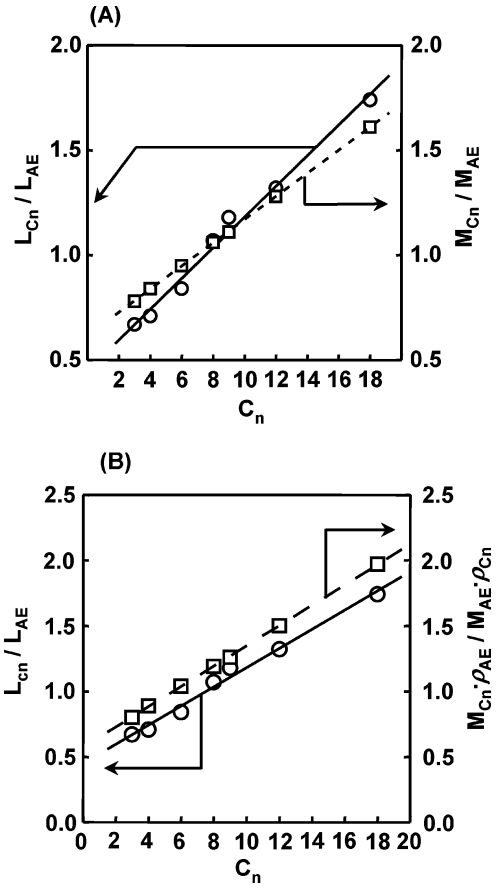

Figure 6. (A) Layer thickness ratio (L_{Cn}/L_{AE}; ○) and repeat unit mass ratio (M_{Cn}/M_{AE}; □) as well as (B) layer thickness ratio (L_{Cn}/L_{AE}; ○) and density corrected mass ratio of polymer brushes as a function of the molecular mass of the alkylamines used for transformation of active ester brushes. The subscript Cn denotes the situation after aminolysis; the subscript AE denotes the active ester brush prior to aminolysis.

Preparation of Functional Polymer Brushes. On the basis of the above-mentioned findings, we wanted to explore the spectrum of functionalities that might be introduced following this approach. For that, we picked a number of functionalized amines and reacted them with the active ester brushes. The structures of these molecules are given in Figure 2. The reaction conditions were rather similar to those described above for alkyl amines (triethylamine; $T = 40$ °C; $t = 16$ h; DMF). After the reactions the samples were extracted in DMF and acetone, and dried in vacuum. Water was used in the cases of D-glucosamine and the monoamino PEGs.

The transmission FTIR spectra of the obtained functional brushes are given in Figure 7. These spectra show that the aminolysis proceeds with high yields as the adsorption bands of the *N*-oxysuccinimide ester moiety are completely missing in all product spectra. In addition, the spectra of the various functionalized brushes clearly show adsorption bands that are indicative for the incorporated moieties. The details of the absorption spectra are summarized in Table 1.

The layer thicknesses of the polymer brushes after reaction with L-alanine methyl ester or D-glucosamine were almost identical to that of active ester polymer brushes. This behavior is due to the fact that the groups attached to brush have roughly the same molar mass as the leaving group *N*-hydroxysuccimide. Figures 8 and 9 show SPS curves of the polymer brushes before (b) and after reaction with aminomethylpyrene (c, Figure 8) and

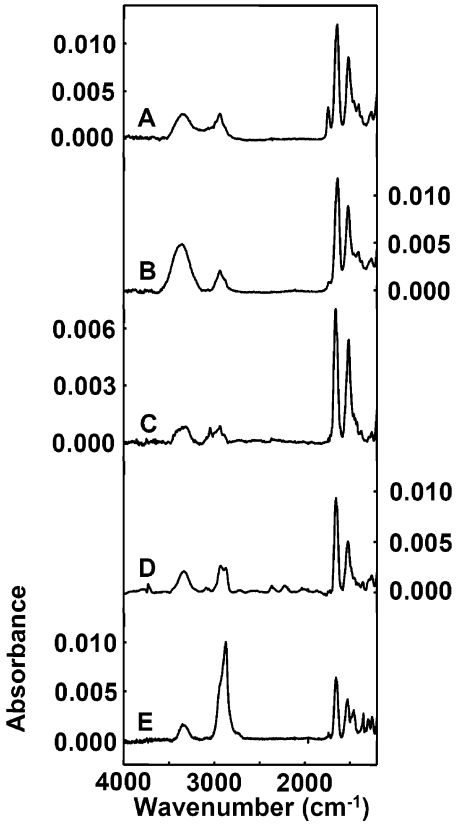

Figure 7. Transmission FTIR spectra of aminolyzed active ester polymer brushes on SiO_x substrate. Key: (A) reacted with L-alanine methyl ester, (B) D-glucosamine, (C) aminomethylpyrene, (D) aminomethyl-15crown-5, or (E) monoaminoPEG ($\bar{M}_n$ = 750).

Table 1. IR Absorption Bands of "Functional" Polymer Brushes

run	amine	absorption (cm^{-1})	peak assignment
A	L-alanine methyl ester	3353	N−H; amide
		2936	C−H
		1740	C═O; ester
		1640	C═O; amide
		1541	N−H; amide
B	D-glucosamine	3356	O−H; hydroxy, N−H; amide
		2933	C−H
		1636	C═O; amide
		1518	N−H; amide
C	aminomethylpyrene	3313	N−H; amide
		3044	C−H; pyrene ring
		2934	C−H
		1655	C═O; amide
		1512	N−H; amide
D	aminomethyl-15-crown-5	3328	N−H; amide
		2927	C−H
		2896	CH_2−O; crown unit
		1652	C═O; amide
		1522	N−H; amide
E	monoaminoPEG$_{750}$	3351	N−H; amide
		2870	C−H
		1653	C═O; amide
		1522	N−H; amide

with aminomethyl-15-crown-5 (c, Figure 9). For both amines the SPS resonance angles are shifted to higher values after reaction. This suggests that the layer thickness is increased due to the increasing molar mass of the monomer unit after reaction with the amines.

Grafting of Polymers onto Polymer Brushes. Interesting questions arise if instead of low molecular weight reagents oligomers or polymers are to be attached to the brushes. In particular, in the latter case, it is expected that the penetration

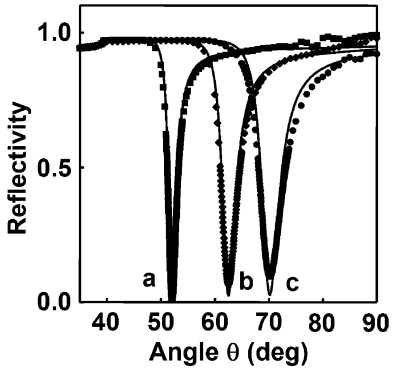

Figure 8. SPR reflectivity curves of polymer brushes before and after reaction with aminomethylpyrene in DMF 40 °C for 16 h. Key: (a) blank (SiO_x; 50 nm, Ag; 50 nm, and BK7); (b) active ester polymer brushes before reaction (24 nm); (c) sample after reaction with aminomethylpyrene (38 nm). Solid lines represent results of Fresnel calculations.

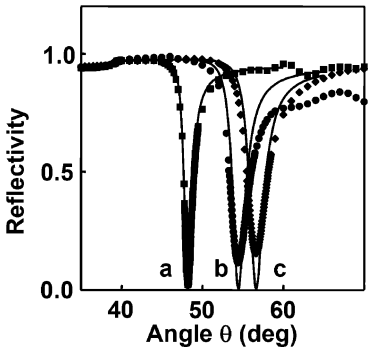

Figure 9. SPR reflectivity curves of polymer brushes before and after reaction with 2-aminomethyl-15-crown-5 in DMF 40 °C for 16 h. Key: (a) blank (SiO_x; 30 nm, Ag; 50 nm, and BK7); (b) active ester polymer brushes before reaction (18 nm); (c) sample after reaction with aminomethyl-15-crown-5 (23 nm). Solid lines represent results of Fresnel calculations.

of polymer molecules from the environment into a densely grafted, swollen brush is difficult and that not all reactive (active ester) moieties may find an appropriate reaction partner from solution. Obtaining high degrees of conversion might be difficult both for kinetic and thermodynamic reasons.

On the one hand, the polymer molecules have to diffuse against the steep concentration gradient built up by the segment density profile of the surface-attached polymer chains in order to react with active ester moieties in the inner parts of the brush. The diffusion barrier will slow down the diffusion into the brush very strongly and will push the time required for complete conversion beyond experimentally accessible time frames. With increasing conversion, the barrier created by such an "uphill" diffusion process will become even higher and higher. The argument here is very similar to that used in the case for the chemisorption of polymer chains onto a solid substrate ("grafting to" process for the formation of polymer monolayers) which also shows such a self-limiting behavior.[23−25]

On the other hand the polymer−polymer interactions will not just lead to chain stretching and a corresponding reduction of the entropy in the polymer backbone. The same is true for the PEGs that enter the brush. This loss of entropy of all participating polymer chains is compensated only by the formation of one single chemical bond, which renders very high conversions also for thermodynamic reasons unfavorable.

Amino-terminated PEGs were chosen as a model polymers for this study for availability reasons and because monolayers of PEG are known to prevent protein adsorption to the coated

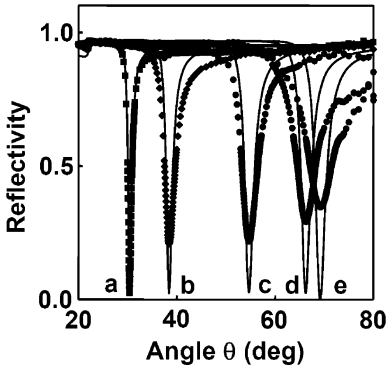

Figure 10. SPR reflectivity curves of polymer brushes before and after reaction with PEGs in DMF at 40 °C for 16 h. Key: (a) blank (SiO_x; 30 nm, Ag; 50 nm, and LaSFN9); (b) active ester polymer brushes before reaction (24 nm); sample (c) reacted with monoaminoPEG, $\bar{M}_n$ = 750 (78 nm), (d) $\bar{M}_n$ = 2000 (147 nm), or (e) $\bar{M}_n$ = 5000 (181 nm). Solid lines represent the results of the Fresnel calculations.

surfaces.[26] The SPS curves obtained from samples before and after reaction with monoamino PEGs of different molecular weights ($\bar{M}_n$ = 750, 2000, and 5000) are shown in Figure 10. The resonance angles after the reactions shift to significantly higher values corresponding to film thickness of 78, 147, and 181 nm. This indicates an increase of the thickness by a factor of 4 (PEG 500), 8 (PEG 750), and 10 (PEG 5000), respectively.

To determine the degree of conversion f of the active ester groups, we again assume that the brush's material density remains unaltered by the aminolysis reaction. Equation 3 can then be rewritten as

$$\frac{L_{PEG}}{L_{AE}} = \frac{M_{PEG}f + M_{AE}(1-f)}{M_{AE}} \tag{5}$$

According to this equation the conversion of the reaction with the monoamino PEGs can be roughly estimated to 89% ($\bar{M}_n$ = 750), 65% ($\bar{M}_n$ = 2000), and 34% ($\bar{M}_n$ = 5000). In agreement with the theoretical considerations described above the degree of conversion decreases once the side chains start to contribute significantly to the mass of the brush chains for higher molecular weight amines. However, it is interesting to note that even for a brush with a high grafting density a polymer with roughly 125 repeat units can be attached to every third repeat unit of the brush. The resulting polymeric layer then consists of roughly of 90 wt % of poly(ethylene oxide) chains, and only 10% of the total film weight is due to the contribution of the brush backbone.

Conclusions

In this paper, the synthesis of polymer brushes containing a variety of low and high molecular weight functional groups was described. These surface architectures were built through growth of polymer chains containing succinimide ester groups in a surface-initiated polymerization reaction. Succinimide moieties embedded into the surface-attached monolayers were used for the attachment of various functional compounds containing alkyl amino groups. We find that the aminolysis with low molecular weight amines proceeds quantitatively. If polymeric reagents are used, the conversion decreases with the molecular weight of the chains that are attached. However, under the conditions described in this contribution still a very significant amount of polymer can be covalently attached to the brush. In one example, a 5000 g/mol molecular weight poly(ethylene glycol) was attached to every third repeat unit of the surface-attached chains. It will be interesting to study the influence of the graft density of the polymer and the molecular weight of the attaching polymer upon the conversion of the reactive sites. Such studies are currently underway.

We expect that functional polymer monolayers such as the ones described here will prove to be useful for a wide range of different applications. We see a strong potential especially in the field of biosensors because they offer the unique opportunity to provide a high number of sensor units per surface area which in turn allows the simplification of the read-out techniques needed to quantify the sensor signals (label-free detection). Experiments along these lines are currently performed in our laboratories.

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Supporting Information Available: Text describing the estimation of polymer densities before and after aminolysis and tables giving data for the calculation of molar volumes and the molar mass, molar volume, and density values and denisty corrected ratio of the molar masses of the polymer brush repeat units before and after aminolysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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