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Tunable Thermoresponsive Polymeric Platforms on Gold by "Photoiniferter"-Based Surface Grafting**

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The surface tethering of "smart" polymers has so far been explored with a variety of methods, including the "grafting-to" approach,[1] physical adsorption,[2,3] and the "grafting-from" technique.[4–8] The last of these methods is recognized as being very versatile, and enables the formation of patterned polymeric brushes. It exploits such techniques as microcontact printing (μCP)[9] and tip-assisted nanolithography[7] in order to deposit the initiators from which the polymer chains may eventually be grown. There has been great interest in using poly(*N*-isopropylacrylamide) (PNIPAM) as a prototype "smart" polymer in biological applications, as it exhibits a lower critical solution temperature (LCST) in aqueous environments. Upon passing the LCST by heating, PNIPAM precipitates at physiologically relevant temperatures, which form the basis for responsive applications. PNIPAM is also a prime target polymer for surface- and interface-related use, as many biological, biomedical, and sensing applications, such as protein adsorption, cell adhesion, and biosensors, benefit from thermal control of the conformation of surface-grafted chains.[10–14] The possibility of controlling the chemistry and, at the same time, the topography of a surface-bound polymeric platform represents a fundamental issue in materials science and biotechnology.[15–19]

In the present study we have focused on the synthesis of thermosensitive surfaces, including controlled grafting of PNIPAM brushes using a newly developed disulfide-containing photoinitiator immobilized on Au, and on in situ atomic force microscopy (AFM) monitoring of reversible changes of the volume and adherence of grafted patterns. Our approach allows tuning of the composition of the polymer-chain terminal groups exposed to the brush/liquid interface. The system proposed in this study is, furthermore, fully compatible with a successive single-molecule-level fabrication exploiting AFM tip-assisted nanomodification techniques.[20]

PNIPAM brushes were grafted in a controlled manner from mixed self-assembled monolayers (SAMs) (consisting of an alkane thiol and the disulfide-based initiator) on Au substrates. The "grafting-from" polymerization was carried out by employing a new initiator–transfer–terminator agent (iniferter) as the initiator [21] for photopolymerization. The disulfide derivative of the photoinitiator was designed for easy deposition on Au surfaces by usual soft- and nanolithographic methods. The choice of Au as a substrate has several advantages over the already-reported photoiniferter-based grafting from silicon surfaces:[5,22] SAMs of thiols or disulfides on Au surfaces are well ordered, their compositions may be easily varied, the deposition procedure is compatible with the lithographic techniques mentioned above, and the chemistry is not sensitive to humidity variations.[23–26]

The disulfide-containing photoiniferter dithiodiundecane-11,1-diylbis[4({[(diethylamino)carbonothioyl]thioethyl)phenyl]carbamate} (DTCA; see Fig. 1) developed in this study can be easily deposited on Au and will initiate a controlled radical polymerization from aqueous *N*-isopropylacrylamide

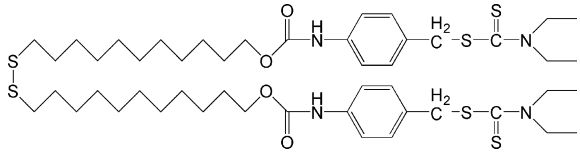

Figure 1. The disulfide-based photoiniferter (DTCA).

(NIPAM) solutions, which can be triggered by UV light and performed at room temperature. The diethyldithiocarbamil group remains at the end of the growing tethered chain,[21] which allows one to tune the chain length by variation of the irradiation time. In addition, the use of this polymerization reaction gives control over the end groups, which can be easily exchanged or chemically modified.[19] The biocompatibility of this technique should also be underlined: no organic solvents are used and no toxic metal/compounds are involved in the polymerization process.

The UV-initiated polymerization from SAMs on Au was considered an impossible (or notoriously difficult) task because of the instability of the thiol-Au bond upon UV irradiation.[22] However, we demonstrate here that by using lamps emitting at 300 nm coupled with a 280 nm cut-off filter as a precaution, it

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is possible to perform a controlled photopolymerization and avoid any degradation of the starting monolayer.

A diluted solution of octadecylthiol (ODT) (40 mol %) mixed with DTCA (60 mol %, with respect to the iniferter groups) in ethanol was placed in contact with Au using a polydimethylsiloxane (PDMS) elastomeric stamp, thus forming regular patterns consisting of mixed SAMs. Assuming that the deposition of the two components on the Au surface followed the same adsorption kinetics in the mixture, the SAM formed should exhibit a component ratio equal to the feed composition. This allowed us to decrease the surface concentration of DTCA. Control of "surface dilution" played an important role in the subsequent "grafting-from" photopolymerization, as it allowed us to essentially eliminate the recombination of the radicals that were formed on the surface. Obviously, radical recombination on the surface would yield bridging and thus inhibit the growth of the tethered chains. In addition, a high concentration of radicals may induce the formation of a bulk polymer in the surroundings.[5,27–29]

The Au substrate covered with a printed pattern of mixed SAMs, consisting of parallel stripes with a 15 μm width separated by 5 μm Au, was immersed in a deoxygenated aqueous solution of the monomer NIPAM and subsequently irradiated with UV light. Figure 2 presents height images from tapping-mode AFM measurements of the patterned surface with the grafted PNIPAM chains in the dry state. After only 5 min of irradiation of the sample immersed in 5 % monomer solution, features of (10 ± 2) nm height were obtained (Fig. 2a) on the initiator-covered stripes. We performed a chain-extension test by placing the sample, following the interruption of polymerization, back in the monomer solution and irradiating for another 5 min. As can be seen in Figure 2b, the height of the brushes doubled after the second irradiation step, reaching values of (20 ± 3) nm.

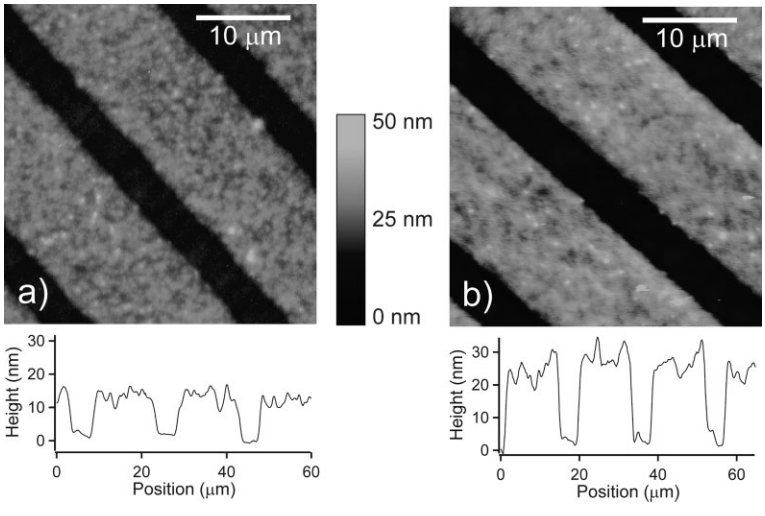

Figure 2. Height images from AFM measurements in tapping mode of the patterned surface with the grafted PNIPAM chains (in the dry state) after a) 5 min and b) an additional 5 min of irradiation. The respective cross sections are displayed below each image.

The sample obtained was subsequently imaged in an aqueous medium to monitor the morphological changes of the PNIPAM brushes at temperatures around the LCST. Whereas in recent years the works of Kidoaki et al.[4] and of Jones et al.[9] focused on AFM force measurements below and above the LCST, to our knowledge this is the first study providing AFM topography images and force–volume measurements recorded in situ at several temperatures in the range 30–36 °C. In Figure 3 height images of the PNIPAM brushes at 31.0 and 36.0 °C can be seen.

A representative height image at 31.0 °C shows how the polymeric features that exhibited an average height of 20 nm in the dry state swelled profusely and gave rise to height values of (109 ± 6) nm when immersed in water (Fig. 3a). Assuming that in the presence of a good solvent the height of the stretched brushes h is comparable to the length of the polymer chains L,[30] we can estimate the number-average molar mass M_n knowing the monomer length l. Assuming l to be 3 Å for acrylic monomers,[31] we estimated an M_n value for the grafted chains of 40 000 g mol^{-1}. The number-average molar mass had the same order of magnitude as that obtained by gel permeation chromatography (GPC) measurements (data not shown) on bulk systems with a similar iniferter-based initiator.. The grafting density can thus be estimated to be 0.33 chain nm^{-2}, confirming the formation of a tightly packed brush.[32]

When the temperature was raised to above the LCST value (32.0 °C, as measured in the bulk for this polymer), the chains collapsed, aggregated, and formed globular features (Fig. 3b, image captured at 36.0 °C). The calculated average height of the polymeric features dropped to 34 nm but the height profile in Figure 3b clearly shows that the chains not only shrank but, in many cases, aggregated without any significant difference in peak height. The aggregation of the polymer brushes was accompanied by the exclusion of water from the polymeric structure. From a bearing analysis on the AFM images, we concluded that the volume occupied by the polymer decreased by more than twice, from (0.046 ± 0.002) μm^3 μm^{-2} at 31 °C to (0.022 ± 0.002) μm^3 μm^{-2} at 36 °C. The formation of hydrophobic polymeric aggregates caused an abrupt change in the morphology leading to a dramatic increase in the values of root-mean-squared (RMS) roughness of the surface from (4.9 ± 0.8) nm, at 31 °C, to (37 ± 2) nm at 36 °C (values calculated over a 10 μm × 10 μm area).

The surface properties of the grafted polymer were monitored by AFM in force–volume mode. As shown in Figure 4, the adherence can be easily tuned by a repetitive heating–cooling sequence, which causes the polymer features to alternate between swollen and collapsed states (compare the heights in Fig. 3).

The tip–sample interaction at 31.0 °C was dominated by repulsive forces (brighter horizontal areas, and force curve A in Fig. 4) whereas adhe-

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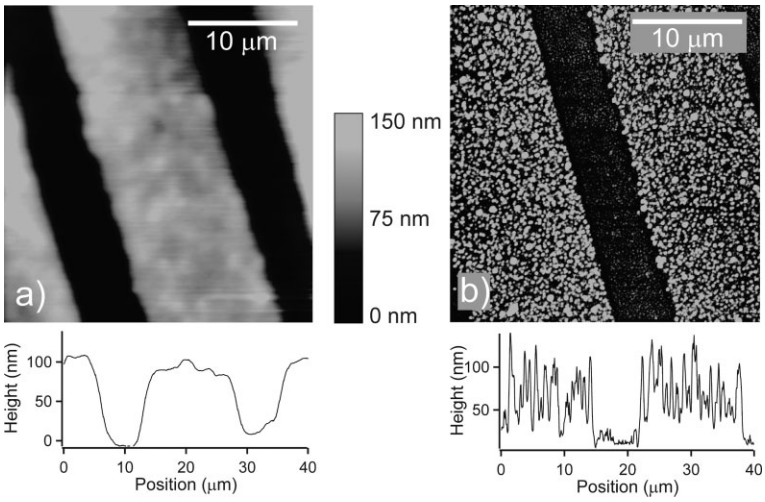

Figure 3. Height images from tapping-mode AFM measurements of the patterned surface with the grafted PNIPAM chains in water at a) 31.0 and b) 36.0 °C. The respective cross sections are displayed below each image.

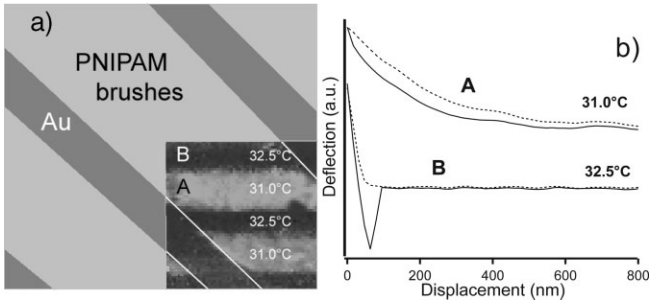

Figure 4. a) The area captured shows a 40 μm × 40 μm section exhibiting a schematic of the structure of the surface-grafted polymer platform. The bottom right is an experimental AFM force–volume image (20 μm × 20 μm) of the grafted PNIPAM chains in water. The grayscale in the AFM image corresponds to areas of high (dark) and low (bright) adherence. The temperature was cycled between 31.0 and 32.5 °C. b) Force–displacement curves for the two regimes, captured on the polymeric features.

sive interactions occurred with the nonprinted Au surface. At 32.5 °C the contrast between Au and the polymer disappeared as a result of the transition of the brushes to collapsed aggregates above the LCST. This resulted in attractive interactions and "conventional" adhesive pull-off forces upon tip withdrawal (see force curve B in Fig. 4). The typical force–distance curves at the two temperatures (Fig. 4b) confirm that the repulsive forces upon compression of the swollen chains at 31.0 °C, which are typical for packed brushes,[32–34] disappeared at 32.5 °C, above the LCST.[9]

As mentioned earlier, the grafting procedure should permit the control of the chain ends of the tethered polymers. In order to test this notion, diethyldithiocarbamyl groups were exchanged with stable 2,2,6,6-tetramethylpiperidyl-1-oxyl (TEMPO) radicals.[35]

The tethered polymer chains were irradiated in the presence of 4-amino-TEMPO radicals that irreversibly couple at room temperature with the macroradicals formed in situ. Figure 5a displays a schematic representation of this exchange procedure. In the next step a fluorophore (fluorescamine) was reacted with the primary amine moieties of the exchanged groups. The contrast shown in the fluorescence microscope image in Figure 5b comes from the fluorescamine adducts formed at the chain ends of the polymers grafted from the μCP patterns. Thus, with chain-terminal-group-exchange strategies, such as the approach we used, it is possible to easily introduce the desired functionalities at the end of the tethered polymer chains.

In conclusion, we developed a disulfide-containing photointiator for surface-tethered polymerization (grafting from Au) from mixed SAMs. This process allowed us to efficiently produce tunable temperature-responsive PNIPAM brushes. The length of tethered chains could be controlled by the irradiation time, whereas the grafting density could be adjusted with SAM mixing ratios. We monitored, by using in situ AFM measurements, the temperature-induced morphology and adherence changes of microscale-patterned surfaces of the brush around its LCST. The initiator developed could be delivered on Au surfaces exploiting nanolithographic approaches. The method enables control of chain ends, as was proven by a radical-exchange experiment. All these characteristics make this grafting technique a promising tool for producing "smart", temperature-responsive platforms, with the potential for chemical control of the chain-terminating groups.

Experimental

Synthesis of the Disulfide Based Photoinitiator (DTCA): 11-Mercaptoundecan-1-ol was oxidized to the corresponding disulfide, 11,11′-dithiodiundecan-1-ol, using an equimolar aqueous solution of iodine

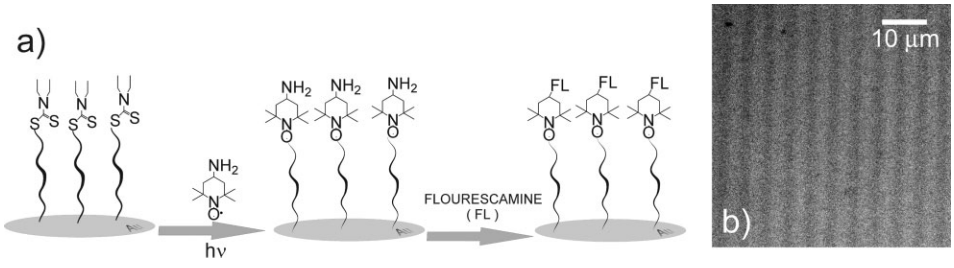

Figure 5. a) The scheme of the exchange procedure of the PNIPAM-polymer-brush end groups with the stable 4-amino-TEMPO radicals and the subsequent fluorescent labeling of the amino groups with fluorescamine. b) A fluorescence microscope image of the patterned PNIPAM brushes modified according to the mentioned procedure.

and potassium iodide. The disulfide was reacted with 4-(chloromethyl)phenyl isocyanate in chloroform with dibutyltin dilaurate as a catalyst, giving dithiodiundecane-11,1-diylbis{[4-(chloromethyl)phenyl]carbamate} as a product. The latter compound was reacted with the diethylammonium salt of diethyldithiocarbamic acid in tetrahydrofuran (THF) at 40 °C giving the final compound dithiodiundecane-11,1-diylbis[4({[(diethylamino)carbonothioyl]thioethyl)phenyl]carbamate} (DTCA).

Grafting of PNIPAM: A mixture of the initiator DTCA and ODT was deposited on Au samples (cleaned with a "piranha" solution) from ethanolic solutions with a PDMS stamp. The formation of the mixed SAM was confirmed by grazing-angle Fourier-transform infrared spectroscopy (FTIR) (Biorad model FTS575C, data not shown). The samples were then placed in quartz flasks equipped with a 280 nm cut-off filter and containing 5 % aqueous solution of NIPAM. They were purged with nitrogen and subsequently irradiated for the necessary time by six UV-B lamps (15 W G15T8E, Ushio Japan) at a distance of 20 cm. After the photopolymerization, the substrates were extensively rinsed with water and methanol.

Chain-End Exchange Experiment: After the grafting of PNIPAM, the substrates were covered with a drop of 0.01 M aqueous solution of 4-amino-TEMPO free radicals and irradiated for 5 min with UV light in the above-described system. The samples were subsequently rinsed with water and placed in an acetone solution of fluorescamine (0.5 mg mL^{-1}) for a further 5 min. After extensive rinsing with acetone the substrates were imaged with a fluorescence microscope (Olympus IX71).

AFM Measurements: The AFM measurements in tapping mode were carried out on a NanoScope III multimode AFM (Digital Instruments/Veeco, Santa Barbara, CA) equipped with a heating stage, a liquid cell, and an external thermocouple, which allowed control of the actual temperature of the medium.

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