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Environment-Friendly Photolithography Using Poly(*N*-isopropylacrylamide)-Based Thermoresponsive Photoresists

Leonid Ionov*,†,‡ and Stefan Diez†

Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauer Str. 108, 01307 Dresden, Germany, and Leibniz Institute of Polymer Research Dresden, Hohe Str. 6, 01069 Dresden, Germany

Received April 3, 2009; E-mail: ionov@ipfdd.de; ionov@mpi-cbg.de

Abstract: We report a novel approach for the temperature-triggered development of water-soluble photoresists based on photocleavable poly(N-isopropylacrylamide) copolymers. These copolymers are soluble in an aqueous environment below their Lower Critical Solution Temperature (LCST). Upon UV irradiation, the photocleavable groups are deprotected resulting in an increased LCST. Thus, the illuminated parts of spin-coated copolymer layers dissolve at higher temperatures than the surrounding areas, leading to pattern development. The photoresist can finally be completely removed at low temperature. We demonstrate the applicability of this novel photolithographic approach by the patterning of fluorescent proteins.

Development of photopatterning technology is of importance for microelectronics,[1] sensor design,[2,3] microfluidics,[4,5] biotechnology,[6−8] bioanalytics,[9,10] and tissue engineering.[11] Thereby, different compounds undergoing photoinduced degradation[12] or cross-linking[13] are implemented as photoresists for fabrication of structured surfaces. In fact, the design of photosensitive compounds determines the applicability of any particular sort of photolithography. For example, water-soluble photoresists[12,14] are of particular interest for the *in situ* patterning of proteins and cells[15−17] under biologically relevant conditions. Recently, various photoresists that are water-soluble and can be developed in an aqueous environment have been designed. However, most of them undergo immediate development upon illumination. This causes an undesired contamination of the surrounding due to the uncontrolled release of dissolved photoresists.

Here, we demonstrate a novel approach for the design of photoresists which can be fully processed in aqueous environment but which development is triggered by temperature. Our design is based on random copolymers of poly(*N*-isopropylacrylamide) (PNIPAM) with photocleavable groups. In an aqueous environment, PNIPAM (homopolymer) reversibly changes its solubility at the Low Critical Solution Temperature (LCST = 33 °C). Due to its thermoresponsive properties, PNIPAM has been already applied for cell culturing,[18] directed protein adsorption,[19] control of biomolecular motors,[20] protein purification,[21] and drug delivery.[22] In our approach, we incorporated hydrophobic 2-nitrobenzyl photocleavable groups into the PNIPAM chains and prepared random poly(2-nitrobenzyl acrylate-co-*N*-isopropylacrylamide) copolymers (poly(NIPAM-NBA)) resulting in a lowered LCST. Upon UV irradiation, the LCST of the copolymers increases due to the formation of poly(acrylic acid-co-*N*-isopropylacrylamide) (poly(NIPAM-AA)). Consequently, when photostructured copolymer layers

† Max-Planck-Institute of Molecular Cell Biology and Genetics.
‡ Leibniz Institute of Polymer Research Dresden.

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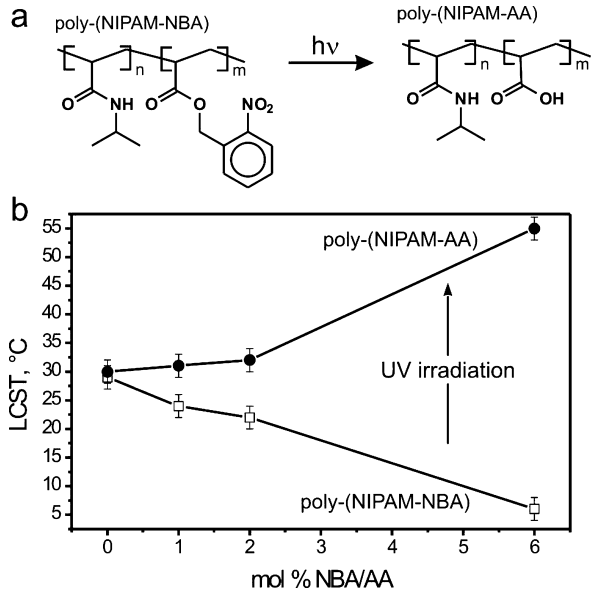

Figure 1. Properties of photocleavable PNIPAM copolymers. (a) Schematic conversion of poly(NIPAM-NBA) into poly(NIPAM-AA) upon UV irradiation. (b) Thermoresponsive behavior (Low Critical Solution Temperature, LCST) of poly(NIPAM-NBA) (□) and poly(NIPAM-AA) (●) in PBS 100 mM buffer (pH = 7) as a function of copolymer composition.

are cooled down from a high temperature (where they are insoluble), poly(NIPAM-AA) and poly(NIPAM-NBA) dissolve in a sequential manner.

We have synthesized poly(NIPAM-NBA) with different fractions of photocleavable comonomer as described elsewhere[7] and investigated its thermoresponsive behavior in an aqueous environment (Figure 1). We found that the incorporation of nitrobenzyl acrylate reduced the LCST[23] by more than 20 °C. The conversion of hydrophobic nitrobenzyl acrylate groups into carboxylic acid occurred upon irradiation with UV light (Figure 1a). The LCST of the polymer thereby significantly increased due to the formation of hydrophilic groups. This remarkable change in the thermoresponsive behavior of PNIPAM-based copolymers after UV irradiation, in combination with the possibility to deposit it onto substrates from aqueous solutions, allows their use as photoresists with thermotriggered development.

The general concept of this novel type of photolithography using PNIPAM-based photoresists is illustrated in Figure 2. A thin film of poly(NIPAM-NBA) (Figure 2a) is deposited on a substrate and irradiated with UV light through a mask at elevated temperature (Figure 2b). Reducing the temperature slightly below the LCST of the poly(NIPAM-AA) leads to pattern development in the biological buffer (Figure 2c). While proteins can bind to the patterned surface everywhere (Figure 2d), proteins adsorbed to the top of the photoresist are removed by lowering the temperature below the LCST of the poly(NIPAM-NBA) (Figure 2e).

To experimentally demonstrate the thermotriggered development of the PNIPAM-based photoresist, we spin-coated 100−150 nm thick films of poly(NIPAM-NBA) (6 mol % of NBA, M_w = 114 000; PDI = 1.7, LCST = 6 °C) with an admixed small amount of fluorescent nanoparticles[24] on a glass substrate. Illumination with the UV light through a mask caused the photocleavage of nitrobenzyl groups and changed the optical properties of the photoresist. As a result, the contrast between illuminated and nonilluminated areas could be detected using epi-fluorescence microscopy (Figure 3a). The illuminated polymer was removed by rinsing in PBS buffer at 25 °C (below LCST of poly(NIPAM-AA) which is T = 55 °C) leading to pattern development (Figure 3b). The photoresist pattern was completely removed by rinsing in cold PBS buffer at 4 °C (below LCST of poly(NIPAM-NBA) which is 6 °C) (Figure 3c).

We quantified the thickness of the photoresist layer on each step of processing using null-ellipsometry. For this, we spin-coated a thin layer of the photoresist (87 nm) onto a silicon wafer and illuminated half of the film with UV light for 10 min. After 20 min of development in PBS buffer at room temperature (corresponds to Figure 2c and 3b), we found that the thickness of the photoresist in illuminated areas was reduced down to 0.1 nm, while the thickness in nonilluminated areas was reduced only slightly (down to 72 nm). After additional rinsing in PBS buffer at T = 4 °C for 20 min, the thickness of the photoresist in nonilluminated areas was completely removed; the thickness was reduced down to 0.1 nm. In agreement with the results of null-ellipsometry, fluorescence microscopy shows that the amount of photoresist in nonilluminated areas is slightly reduced after development at room temperature (reduce of fluorescence after development, Figure 3a and b). Moreover, fluorescence intensity in developed areas is almost equal to that after complete removal of the photoresist (Figure 3b and c). Thus, we can argue that the photoresist film can be completely removed both in the illuminated areas after development at room temperature and in the nonilluminated areas at reduced temperature.

We tested the potential of our method for the *in situ* photoresist development in microfluidic devices. For this, fluorescent neutravidin-coated 200 nm large beads were adsorbed on poly(NIPAM-NBA) (with 1 mol % of NBA) from an aqueous environment at temperature T = 35 °C (above the LCST of the deprotected poly(NIPAM-AA), see Figure 1b). The polymer layer was then illuminated through a 100× oil objective (1.4 numerical aperture) by UV light. We observed that the particles stayed attached to the illuminated polymer if the surrounding temperature was higher than the LCST of poly(NIPAM-AA) (Figure 4a; note that due to photobleaching the fluorescent beads in the illuminated area appear slightly dimmer than those in the surrounding area.). The illuminated polymer dissolved, and the fluorescent beads were released after the temperature was reduced below the LCST of the photodeprotected polymer (Figure 4b). Further cooling down to T = 8 °C removed the photoresist completely (Figure 4c).[25] This experiment demonstrated the possibility for *in situ* patterning using poly(NIPAM-NBA) photoresists.

Finally, we demonstrated the applicability of the developed method for protein patterning. Poly(NIPAM-NBA) (with 6 mol % of NBA and admixed red-fluorescent CdSeS quantum dots, as tested in the experiment illustrated in Figure 3) was used as a photoresist. After illumination through a mask and development at moderate temperature (T = 30 °C), fluorescent casein

(23) The LCST of all polymers were measured in PBS 100 mM buffer, pH = 7.

(24) Nanoparticles were used to make the polymer fluorescent and detectable using fluorescence microscopy. The CdSeS nanocrystals were kindly provided by Dr. A. Rogach (LMU).

(25) We note that the efficient development of photoresist inside a narrow channel occured at lower temperatures than the LCST of poly(NIPAM-AA). This finding can be attributed to slow polymer dissolution because no stirring was applied and the process was limited by diffusion.

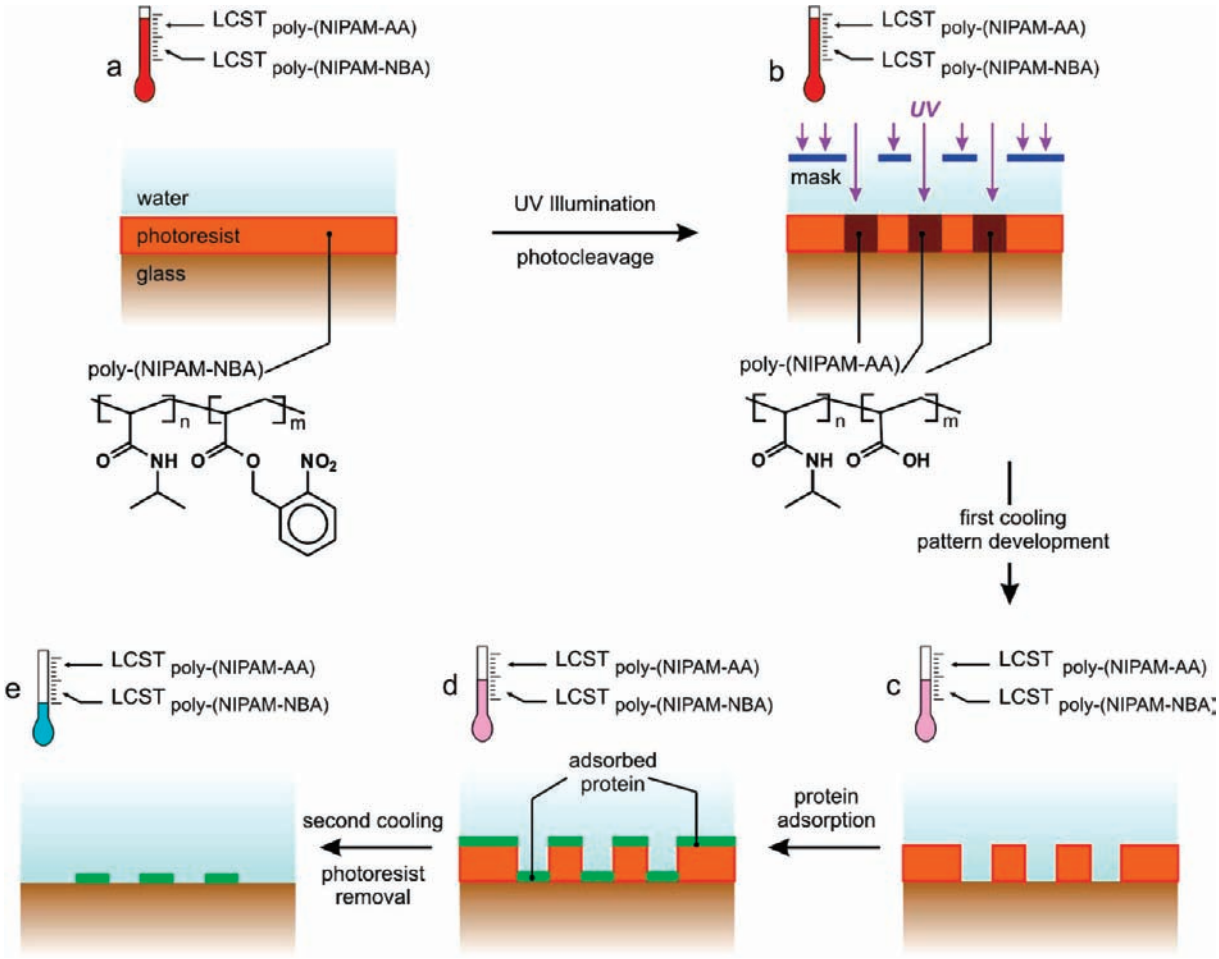

Figure 2. Scheme of photopatterning using PNIPAM-based photoresists with temperature-triggered development. Thermoresponsive poly(2-nitrobenzyl acrylate-co-*N*-isopropylacrylamide) is deposited as photoresist onto a substrate (a). Illumination of the photoresist (b) results in photocleavage of nitrobenzyl acrylate groups, increasing the LCST. After the photoresist pattern is developed at slightly lower temperature (c), proteins are adsorbed (d). The photoresist (together with the proteins on top) can be completely removed in aqueous environment at low temperatures (e).

(10 mg/mL) was adsorbed to the patterned surface. The unbound casein was then washed out by rinsing in warm PBS buffer (T = 30 °C),[26] and the residual photoresist was removed by rinsing in cold PBS buffer (T = 4 °C). The thickness of the casein layer as measured by null-ellipsometry is 2.4 nm. We observed a clear pattern of fluorescent casein (green) on the surface (Figure 5a) and a minor residual red signal in the areas which were covered by the photoresist (Figure 5b). On the other hand, we did not observe the red signal if the experiment was performed using the polymer without added fluorescent nanocrystals (images are not shown). Therefore, we attribute the red signal in the areas previously occupied by the nonilluminated photoresist to precipitation of water-insoluble fluorescent nanoparticles, which were added to photoresist.[24]

Because carboxylic groups are formed upon illumination of poly(NIPAM-NBA), we expect that our thermoresponsive photoresist can demonstrate pH-dependent development similarly to the pH-sensitive photoresist developed by Irvine.[15] Therefore, our photoresist can be used in the same way as it was suggested by Irvine: it can be deposited onto a positively charged substrate and form a bilayer after patterning.[15] On the other hand, since negatively charged glass was used as the substrate, the photoresist could be completely removed in illuminated areas after pattern development at moderate temperature and in nonilluminated areas after additional rinsing at low temperature. In contrast to the photoresist developed by Irvine, our photoresist can be developed by applying different temperatures.

In conclusion, we developed a novel approach for the design of environment-friendly (water-soluble/water-developing) photoresists with stimuli-triggered development based on photocleavable copolymers of poly(*N*-isopropylacrylamide). The proposed photoresists possess a unique combination of advantages: (i) they are soluble in biological buffers, (ii) their photocleaved products are soluble in an aqueous environment as well, and (iii) their development is triggered by temperature in physiological buffer in a controllable way and no change of pH is required. We demonstrated the applicability of the presented method for *in situ* patterning inside microfluidic channels and for protein patterning on surfaces. We foresee a strong potential of our method for patterning and harvesting proteins, particles, and cells in microfluidic devices, where all procedures have to be performed in biological buffers.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAM, Aldrich), acetone (Aldrich), anhydrous dichloromethane (Aldrich), triethylamine (Fluka), 2-nitrobenzyl alcohol (Fluka), acryloyl chloride (Fluka) *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDTA, Aldrich),

(26) Warm buffer was used to prevent further pattern development.

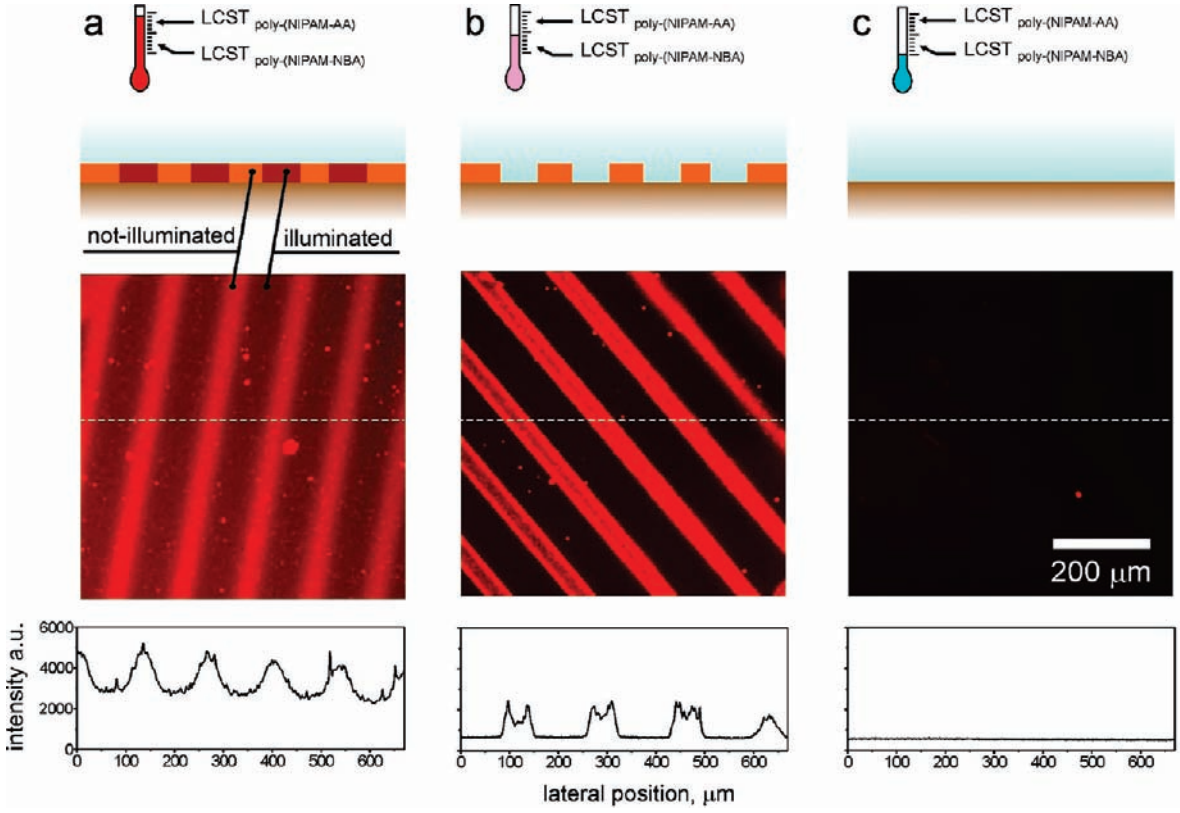

Figure 3. Fluorescence micrographs of poly(NIPAM-NBA) photoresist (% NBA = 6 mol %; M_w = 114 000; PDI = 1.7; $LCST_{poly(NIPAM-NBA)}$ = 6 °C; $LCST_{poly(NIPAM-AA)}$ = 55 °C) with admixed fluorescent CdSeS nanocrystals[16] at different stages of processing: (a) after illumination; (b) after development at 25 °C; and (c) after cooling to 4 °C.

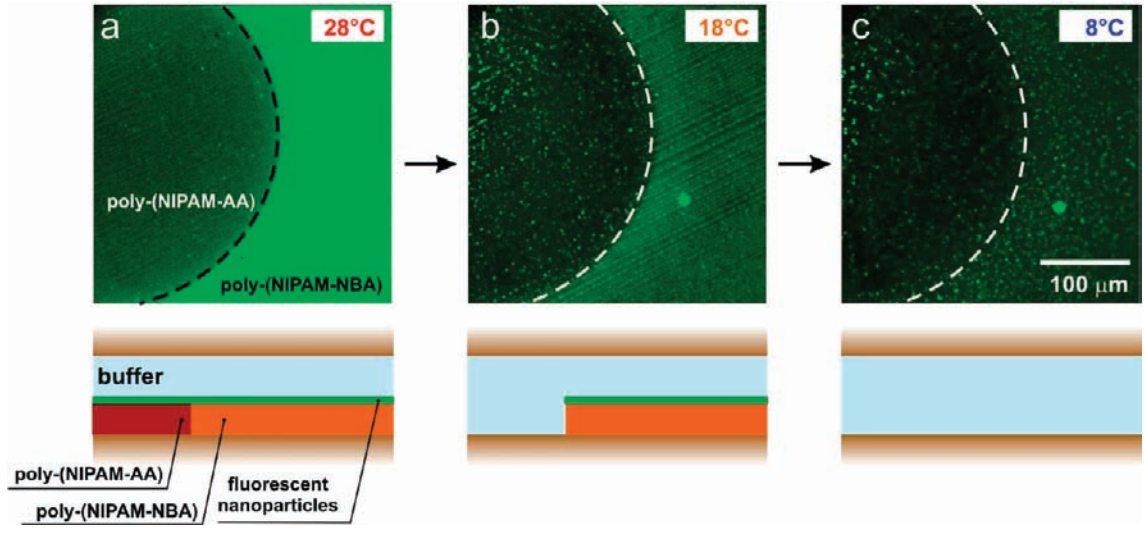

Figure 4. Fluorescence micrographs of in situ temperature-triggered development of poly(NIPAM-NBA) photoresist (% NBA = 1 mol %; $LCST_{poly(NIPAM-NBA)}$ = 25 °C; $LCST_{poly(NIPAM-AA)}$ = 29 °C; M_w = 25 000; PDI = 1.5) at different temperatures.

ethyl-2-bromoisobutyrate (EBiB), ethylenediamine (ED, Fluka), anhydrous dichloromethane (Aldrich), 2-bromo-2-methylpropanoyl bromide (BMPB, Aldrich), triethylamine (Fluka), L-ascorbic acid (Sigma), and copper bromide (Aldrich) were used as received.

Synthesis of 2-Nitrobenzyl Acrylate (NBA). 3 g (1.9×10^{-2} mol) of 2-nitrobenzyl alcohol and 2.1 g (2×10^{-2} mol) of triethylamine were dissolved in 20 mL of anhydrous dichloromethane, and 1.7 mL (2×10^{-2} mol) of acryloyl chloride were added dropwise to the resulting solution. Stirring continued for 1 h. After filtering out the resulting salt, the filtrate was concentrated and purified by column chromatography (packed material: silica gel; eluent: hexane/ethyl acetate = 10/1). Thus, 2.7 g of an oily liquid were recovered and confirmed to be 2-nitrobenzyl acrylate by NMR spectroscopy. δ = 5.6 (s, 2H), δ = 5.8−6.5 (m, 3H), δ = 7.1−8.1 (m, 4H).

Synthesis of Poly(NIPAM-NBA) Copolymers. The poly(NIPAM-NBA) copolymer with 6 mol % of NBA was prepared according to the following procedure. *N*-Isopropylacrylamide (4 g, 3.5×10^{-2} mol), 2-nitrobenzyl acrylate (465 mg, 2.25×10^{-3} mol), and EBIB (8 mg, 4.1×10^{-5} mol) were dissolved were dissolved in 6 mL of acetone solution of $CuBr_2$ (0.4 mg, 1.8×10^{-6} mol) and PMDTA (0.31 mg, 1.8×10^{-6} mol). The reaction solution was added to the

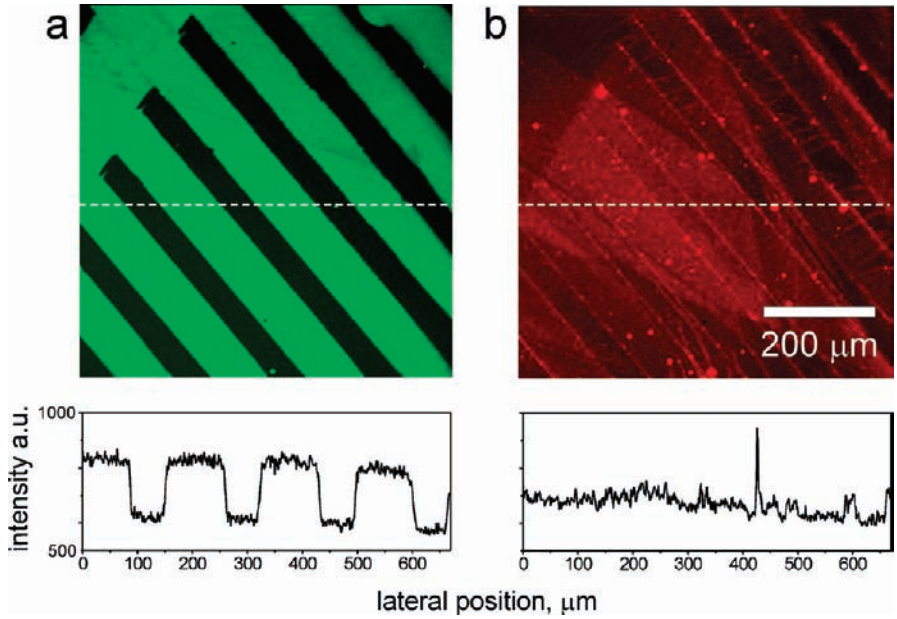

Figure 5. Fluorescence micrographs of a FITC-casein pattern obtained by photolithography using poly(NIPAM-NBA) (% NBA = 6 mol %; M_w = 114 000; PDI = 1.7; $LCST_{poly(NIPAM-NBA)}$ = 6 °C; $LCST_{poly(NIPAM-AA)}$ = 55 °C) photoresist with added CdSeS fluorescent nanocrystals. (a) Signal of FITC-casein (green) and (b) signal of poly(NIPAM-NBA) with admixed CdSeS quantum dots with partial cross-talk from the FITC-casein and from precipitated fluorescent nanocrystals (red).

tube and sealed with a rubber septum; a solution of L-ascorbic acid (18 mg, 1×10^{-4} mol) in water (0.5 mL) was injected. The vial was placed in a 70 °C oil bath. The polymerization was carried out under stirring and was stopped after 4 h. The polymer was precipitated in diethylether. The copolymers with different composition were prepared using a similar route. The LCST of polymers was measured in PBS 100 mM buffer, pH = 7.

Fluorescent Microscopy. Fluorescence images were obtained using an Axiovert 200 M inverted microscope with a 10× objective (Zeiss, Oberkochen, Germany) equipped with a FluoArc lamp. For data acquisition a standard TRITC filterset (excitation: HQ 535/50; dichroic: Q 565 LP; emission: HQ 610/75, Chroma Technology) and FITC filterset (excitation: HQ 480/40, dichroic: Q 505 LP, emission: HQ 535/75, Chroma Technology, Rockingham, VT) in conjunction with a Micromax 512 BFT camera (Photometrics, Tucson, AZ) and a MetaMorph imaging system (Universal Imaging, Downingtown, PA) were used.

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