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Reversible Switching of Microtubule Motility Using Thermoresponsive Polymer Surfaces

Leonid Ionov,[†,‡] Manfred Stamm,[‡] and Stefan Diez*[,†]

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

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ABSTRACT

We report a novel approach for the dynamic control of gliding microtubule motility by external stimuli. Our approach is based on the fabrication of a composite surface where functional kinesin motor-molecules are adsorbed onto a silicon substrate between surface-grafted polymer chains of thermoresponsive poly(*N*-isopropylacrylamide). By external temperature control between 27 and 35 °C, we demonstrate the reversible landing, gliding, and releasing of motor-driven microtubules in response to conformational changes of the polymer chains. Our method represents a versatile means to control the activity of biomolecular motors, and other surface-coupled enzyme systems, in bionanotechnological applications.

The development of methods to transport and manipulate nano-objects in engineered environments is a challenging task toward the design of miniaturized bionanodevices. While atomic force microscopy and optical tweezers have recently been applied for the positioning of nanoparticles, polymer molecules, and proteins[1−5] their applicability is limited to single molecules at a time. An alternative method of nanomanipulation, which holds the promise to be applicable in a highly parallel manner, is the in vitro use of biomolecular motors.

One well-studied motor protein is kinesin,[6,7] an ATP-hydrolyzing enzyme that moves in a cellular environment vesicles and organelles along microtubules.[8] Microtubules are hollow, cylindrical, protein filaments that can be formed in vitro by self-assembly of tubulin heterodimers. Microtubules have an outer diameter of 24 nm and can be as long as several micrometers. In vitro gliding motility assays, where microtubules are propelled over a substrate by surface-bound kinesin molecules in the presence of ATP, have been widely used to study the interaction between motor proteins and microtubules.[7]

Recently, the kinesin−microtubule system has been successfully implemented into synthetic environments in order to facilitate technological tasks such as nanotransport and nanostructuring.[9−12] In such applications, a highly specific, spatiotemporal control over the transport activity is desired. Static spatial control over the motility has been achieved by topographical or/and chemical surface modifications of substrate.[13−15] Moreover, electrical fields[16−18] and hydrodynamic flow[19,20] have been used to dynamically influence the motion of gliding filaments. Temporal control has also been achieved by manipulating the ATP concentration either by buffer exchange[20,21] or by ATP uncaging.[9]

In this paper, we report an approach to control the motility of gliding microtubules using stimuli-responsive polymer surfaces. In particular, we fabricated composite surfaces where functional kinesin motor molecules were adsorbed onto a silicon substrate between surface-grafted polymer chains of thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM).[22,23] In aqueous solution the PNIPAM polymer chains hydrate to form extended structures when the temperature is below the lower critical solution temperature (LCST, 32−33 °C for PNIPAM[24]) but become compact structures by dehydration when heated above the LCST. We used this effect to repel gliding microtubules from the surface at 27 °C while they were able to land and glide at 35 °C (Figure 1).

Fabrication of PNIPAM Gradient Surfaces. To determine the optimal polymer surface density for the temperature-controlled switching of microtubule motility, we performed our experiments on a PNIPAM layer with a lateral gradient of the grafting density. Gradient PNIPAM layers were prepared via a two-step procedure similar to the methods described elsewhere.[12,25] In brief, highly polished single-crystal silicon wafers of {100} orientation with a native silicon oxide layer (1.6 nm) (Semiconductor Processing Co.) were used as substrates. Silicon chips, which were

* Corresponding author. E-mail: diez@mpi-cbg.de.
[†] Max-Planck-Institute of Molecular Cell Biology and Genetics.
[‡] Leibniz Institute of Polymer Research Dresden.

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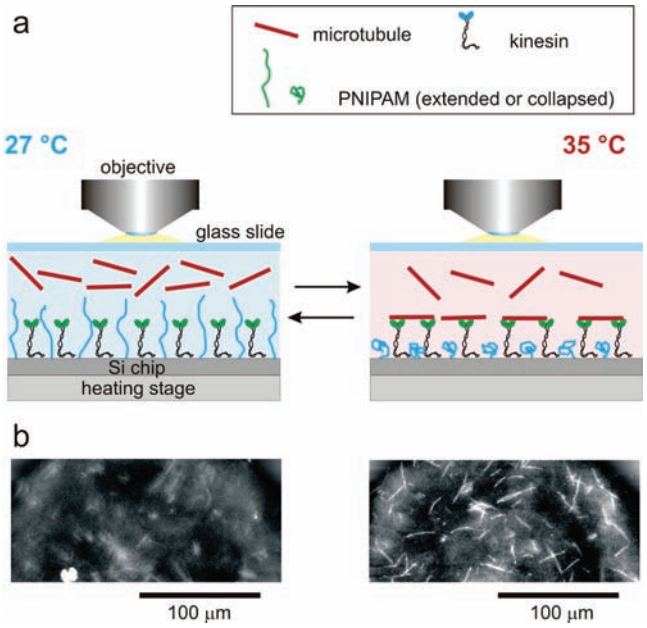

Figure 1. Gliding motility of microtubules on a thermoresponsive PNIPAM surface with adsorbed kinesin. (a) Schematics of the experimental setup. Repeated changes in temperature resulted in the reversible switching of PNIPAM chains between the extended conformation (where microtubules are repelled from the surface and cannot bind to the kinesin heads) and the collapsed conformation (where microtubules can glide unhindered on the kinesin molecules). (b) Fluorescence micrographs of rhodamine-labeled microtubules on a substrate surface with grafted PNIPAM chains ($\Gamma_{PNIPAM} \approx 6$ mg/m^2) and adsorbed kinesin. No gliding of microtubules is observed at 27 °C (left image) while the microtubules glide at 35 °C (right image).

cut from the wafers in different sizes, were first ultrasonically cleaned in chloroform for 30 min, placed in hot piranha solution (3:1 concentrated sulfuric acid and 30% hydrogen peroxide) for 1 h, and finally rinsed several times with high-purity water. A thin layer of polyglycidyl methacrylate (PGMA, $M_n = 84\,000$ g/mol, synthesized by free radical polymerization) was deposited on top of the chips by spin-coating of a 0.01% PGMA solution in chloroform and annealed at 110 °C for 5 min. Afterward, a thick film (200 nm) of carboxy terminated poly(*N*-isopropylacrylamide) (PNIPAM-COOH, $M_n = 49\,900$ g/mol, PDI = 1.46, synthesized by anionic polymerization, purchased from Polymer Source, Inc.) was spin-coated on top of the PGMA layer from a 2% solution in chloroform and annealed for 1 h on a stage exhibiting a temperature gradient. The temperature changed gradually from 130 to 180 °C along the 50-mm length of the sample. Upon heating, the chemical reaction between the terminating carboxyl groups of the PNIPAM and the epoxy groups of the PGMA resulted in the formation of the grafted PNIPAM layer with a gradient in the grafting density caused by the temperature dependence of the grafting kinetics. Nongrafted polymer was removed using Soxhlet extraction in chloroform for 3 h.

The thickness profile of the one-dimensional PNIPAM-gradient was determined by ellipsometric mapping using a SENTECH SE-402 scanning microfocus ellipsometer at $\lambda = 633$ nm at an angle of incidence of 70°. The thicknesses of the polymer layers obtained from ellipsometry corresponded to a four-layer model Si/SiO_2/PGMA/PNIPAM where it was assumed that the polymer films (PGMA and PNIPAM) have the same refractive index as the corresponding bulk polymers. Because the thickness of a PNIPAM layer strongly depends on the degree of swelling, we will further on use the grafting density Γ_{PNIPAM} rather than the thickness h_{PNIPAM} of the polymer layer. With a polymer mass density ρ of about 1000 kg/m^3, the grafting density is given by $\Gamma_{PNIPAM} = h_{PNIPAM,dry}\rho$, where $h_{PNIPAM,dry}$ is the measured ellipsometric thickness of the PNIPAM layer in the dry state. The number of individual polymer chains per surface area S_{PNIPAM} is related to the grafting density by $S_{PNIPAM} = \Gamma_{PNIPAM}N_A/M_n$, where N_A is the Avogadro number and M_n is the averaged molecular weight of PNIPAM. The averaged distance between individual polymer chains can be estimated by $D_{PNIPAM} = S_{PNIPAM}^{1/2}$. Concerning the behavior of a PNIPAM layer in water, it is known that the thickness at 35 °C is approximately the same as that in the dry state, whereas the thickness increases about 8−10 times upon swelling at 27 °C.[26] A summary of the PNIPAM characteristics for three different grafting densities is given in Table 1. Figure 2a shows that the PNIPAM grafting density gradually increased with lateral position x on our sample, where $x = 0$ is assigned to the position with the lowest grafting density.

Table 1. Correlation of the Measured Thickness of the PNIPAM Layer in the Dry State with the Grafting Density, the Number of Individual Polymer Chains per Surface Area, the Average Distance between Individual Polymer Chains, and the Expected Thickness of the Polymer Layer in Water at 27 °C

$h_{PNIPAM,dry}$ (nm)	Γ_{PNIPAM} (mg/m^2)	S_{PNIPAM} (μm^{-2})	D_{PNIPAM} (nm)	$h_{PNIPAM,water,27°C}$ (nm)
1.6	1.6	19500	7.1	13−16
5.1	5.1	62500	4.0	41−51
6.0	6.0	73500	3.7	48−60

Preparation of Microtubule Motility Assays. Motility experiments were performed in a 5-mm-wide flow cell constructed from a silicon chip containing the PNIPAM gradient, a cover slip (Corning, 50 × 24 mm^2), and two pieces of double-sided sticky tape (Scotch 3M, thickness 0.1 mm). A casein-containing solution (0.5 mg/mL in BRB8 (80 mM PIPES)/KOH pH 6.9, 1 mM EGTA, 1 mM $MgCl_2$) was perfused into the flow cell and allowed to adsorb to the surfaces for 5 min. Next, 50 μL of a motor solution containing 2 μg/mL wild-type kinesin in BRB80 (full length drosophila conventional kinesin expressed in bacteria and purified as described in ref 27) was perfused into the flow cell and allowed to adsorb for 5 min. Depending on the conditions of the experiments, the casein and kinesin solutions were adsorbed at 27 or 35 °C. Thereafter, a motility solution containing rhodamine-labeled taxol-stabilized microtubules[12] was applied. To perform the motility experiments under conditions where the binding and unbinding of microtubules to/from the surface is in equilibrium, unbound microtubules were not washed out.

Temperature Control and Optical Imaging. For the data in Figures 2, 3, and 5, the temperature in the flow cell was

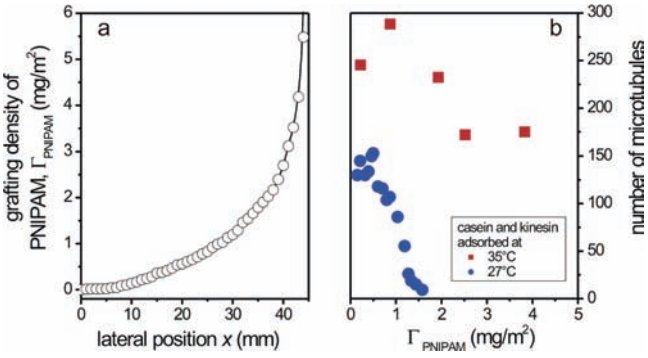

Figure 2. Characterization of the gradient PNIPAM layer. (a) Ellipsometric mapping of the gradient PNIPAM layer in the dry state. (b) Number of gliding microtubules per field of view vs PNIPAM grafting density. Casein and kinesin solutions were perfused at either 27 °C (blue circles) or 35 °C (red squares). Perfusion of motility solution and microscopic imaging were performed at 27 °C.

adjusted by using a Tempcontrol 37-2 digital unit (PeCon GmbH, Germany) which simultaneously controlled the temperature of the objective and the sample holder. Fluorescent images were obtained using an Axiovert 200M inverted microscope with a 40× oil immersion objective (Zeiss, Oberkochen, Germany) in conjunction with a CoolSnap HQ camera (Photometrics, Tucson, AZ) in 2 × 2 binning mode yielding a field of view of 226 × 169 μm^2. Because the time scale in these experiments was slow and the flow cell was thermally well coupled to the objective by immersion oil as well as to the sample holder by heat conducting paste, the temperature values indicated by the Tempcontrol unit were used for data evaluation. To obtain the data in Figure 4 the silicon chip was directly attached to a Peltier element (50 mm × 50 mm, Conrad Electronic GmbH, Hirschau, Germany) by heat conducting paste. Heating and cooling were then performed by changing the electrical polarity on the Peltier element by a conventional power supply. In this experiment the temperature was recorded by a BAT-10 multipurpose thermometer (Physitemp Instruments, Clifton, NJ) and a 20× air objective (Zeiss, Oberkochen, Germany) in conjunction with a MicroMax 512 BFT back-illuminated CCD camera (Photometrics, Tucson, AZ) was used for image acquisition. Image processing was performed using MetaMorph software (Universal Imaging, Downingtown, PA).

In the following, we report on the characterization of microtubule motility on the gradient PNIPAM surfaces at constant temperature (experiments 1 and 2) and the reversible switching of motility on selected locations along the gradient (experiments 3 and 4).

Adsorption of Casein and Kinesin on Extended PNIPAM Chains (Experiment I). We started with the investigation of microtubule motility after adsorbing casein and kinesin onto the PNIPAM gradient layer at 27 °C; i.e., when the PNIPAM chains were in the extended state. When investigating microtubule motility at 27 °C, we found that the number of gliding microtubules decreased strongly with increasing PNIPAM grafting density (Figure 2b, blue circles) and no microtubules were observed at $\Gamma_{PNIPAM} > 1.6$ mg/m^2. This behavior is in accordance with earlier experiments[12] where casein and kinesin were adsorbed onto a gradient layer of poly(ethylene glycol), a polymer with similar protein repelling properties as PNIPAM at 27 °C. However, in the range where motility occurred, the gliding velocity of microtubules was independent of the amount of grafted PNIPAM, $v = 0.84 \pm 0.08$ μm/s (mean ± standard deviation, averaged for all polymer densities, data obtained from at least 20 gliding microtubules per polymer density).

When the temperature of the flow cell was raised from 27 to 35 °C, the gliding velocity increased to $v = 1.22 \pm 0.07$ μm/s, as expected from the faster rate of ATP hydrolysis.[21] However, the number of gliding microtubules—observed at $\Gamma_{PNIPAM} = 1.6$ mg/m^2—increased only marginally and remained constant upon further cyclic changes of the temperature. We conclude that in the range of grafting densities where motility was observed the number of PNIPAM chains was too small to significantly influence the microtubule−kinesin interactions.

Adsorption of Casein and Kinesin on Collapsed PNIPAM Chains (Experiment II). In a second experiment, we incubated a PNIPAM gradient layer of the same kind as used in experiment I with casein and kinesin at 35 °C. At this temperature, the PNIPAM chains were collapsed and the perfused proteins were able to bind on all positions along the polymer gradient (information obtained from the perfusion of fluorescent casein, data not shown). The flow cell was then cooled to 27 °C. This particular cooling step was performed in order to release the kinesin molecules, which might have initially adsorbed onto the PNIPAM chains themselves at 35 °C.[23] The nonadsorbed kinesin was then removed from the flow cell by perfusion of the microtubule-containing motility solution at 27 °C. We assume that after this procedure, the majority of bound kinesin molecules are attached to the substrate between the PNIPAM chains and there is no kinesin in solution. Further switching of the PNIPAM layer between collapsed and extended states should not influence the total concentration of kinesin molecules on the surface. When investigating microtubule motility at 27 °C (similar as in experiment I), we observed that—at any given PNIPAM grafting density—the number of gliding microtubules was higher than that in experiment I and motility was observed up to grafting densities of 4 mg/m^2 and higher (Figure 2b, red squares). However, similar to experiment I, the number of gliding microtubules gradually decreased with increasing Γ_{PNIPAM}.

The results from experiments I and II show that the use of a PNIPAM gradient layer allows the fabrication of surface gradients of active kinesin. Our finding that the gliding velocity did not change along the gradient indicates that the bioactivity of individual kinesin molecules is independent of the PNIPAM grafting density. Moreover, we observed only very few stuck microtubules. This indicates a high percentage of active motor molecules on the polymer surface, since it is known that inactive motors tend to stick microtubules to the surface.

When casein and kinesin were adsorbed to the surface at 27 °C, we found that the grafting density of PNIPAM—at

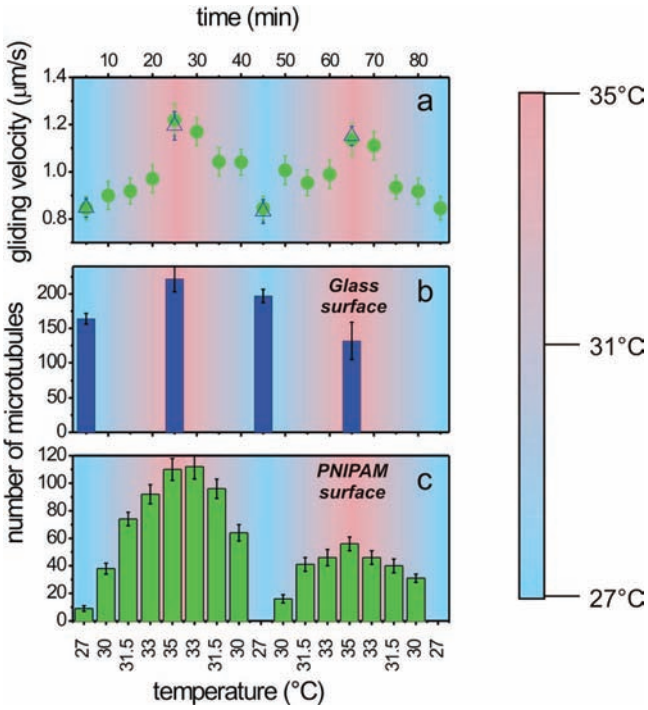

Figure 3. Temperature-induced changes of gliding microtubule motility. (a) Dependence of the averaged gliding velocity with the temperature on the PNIPAM surface (green circles) and glass (blue triangles). (b) Number of gliding microtubules on the glass surface. The measurements were performed at 27 and 35 °C only, but motility also occurred at intermediate temperatures. (c) Number of gliding microtubules during two sequential cycles of heating and cooling at $\Gamma_{PNIPAM} \approx 6$ mg/m^2 (see also Supporting Information). Casein and kinesin were adsorbed at 35 °C. The underlain color code is used for qualitative comparison.

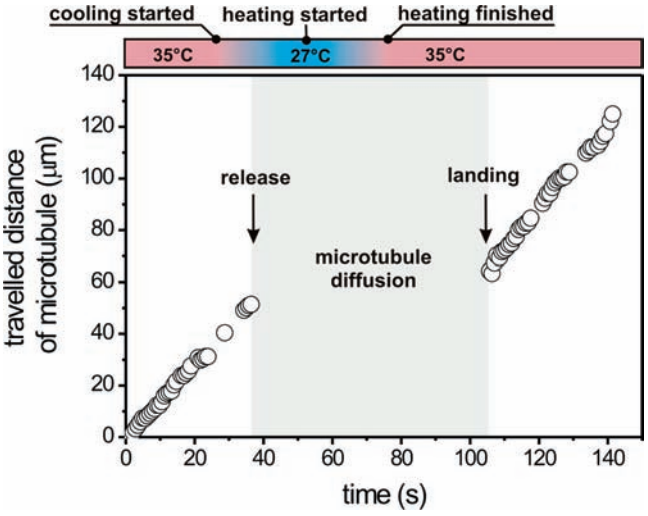

Figure 4. Behavior of a single microtubule during rapid cooling and heating of the PNIPAM−kinesin surface. Plotted is the traveled distance of one microtubule (as determined by manual tracking of the trailing end of the microtubule) as a function of time. The corresponding temperature conditions are indicated in the upper panel. The microtubule first glided at 35 °C with constant velocity, released when the temperature was lowered to 27 °C, and diffused in the solution close to the surface. During the diffusive period, tracking of the microtubule was not possible. After the surface was heated to 35 °C again the microtubule rebound after about 20 s and was gliding again at constant velocity.

which motility was observed—was insufficient to influence microtubule motility in a temperature-induced way. Therefore, we concluded that the adsorption of casein and kinesin at 35 °C (when the PNIPAM chains are in the collapsed state and higher kinesin densities can be bound to the surface) will be advantageous when aiming for the temperature-induced switching of motility.

Reversible Switching of Microtubule Motility (Experiment III). To demonstrate the temperature-induced switching of microtubule motility, we incubated a PNIPAM gradient surface with casein and kinesin at 35 °C. We then gradually raised and lowered the temperature between 27 and 35 °C and investigated the gliding motion of microtubules at $\Gamma_{PNIPAM} \approx 6$ mg/m^2. Again, the gliding velocity was found to change in phase with the temperature both on the polymer and on the glass surface of the flow cell (Figure 3a). While the number of gliding microtubules did not change significantly on the glass side (Figure 3b), a pronounced temperature dependence became apparent on the polymer surface (Figure 3c and Supporting Information).[28] Upon the gradual increase of the temperature, the number of gliding microtubules significantly increased. Upon cooling, this number lowered until eventually no gliding microtubules were observed at 27 °C. The same behavior was observed during a second, consecutive cycle of heating and cooling. The slightly lower number of gliding microtubules during the second cycle was most likely due to partial denaturation of the adsorbed kinesin. Note, that a similar effect was observed on the glass surface, where the number of gliding microtubules decreased to about 60% of the initial value during the second temperature cycle.

To obtain the data in Figure 3, heating and cooling were performed slowly and emphasis was on performing the measurements under conditions where the gliding microtubules were in equilibrium with the ones in solution. To perform measurements on a faster time scale, we extended our experimental setup by a Peltier element. This way we were able to switch the temperature in the flow cell between 27 and 35 °C within about 25 s. We observed that the release of microtubules occurred almost concurrent with the temperature decrease from 35 to 27 °C. In contrast, landing started only about 20 s after the temperature had risen to 35 °C again. This time lag was presumably caused by the limited diffusion of the released microtubules back to the kinesins on the surface. Figure 4 shows a typical example of the behavior of a single microtubule during the rapid cooling and heating of the PNIPAM−kinesin surface ($\Gamma_{PNIPAM} \approx 6$ mg/m^2).

Temperature-Controlled Sorting of Microtubules (Experiment IV). Similar to experiment III, we incubated a PNIPAM gradient surface with casein and kinesin at 35 °C but investigated the gliding motility at a location with a less dense PNIPAM layer ($\Gamma_{PNIPAM} \approx 5$ mg/m^2). We again observed a periodic increase and decrease of the number of gliding microtubules upon heating and cooling (Figure 5a). However, cooling did not lead to a complete release of microtubules at 27 °C. We found that the cyclic change of the temperature was accompanied by a cyclic change of the

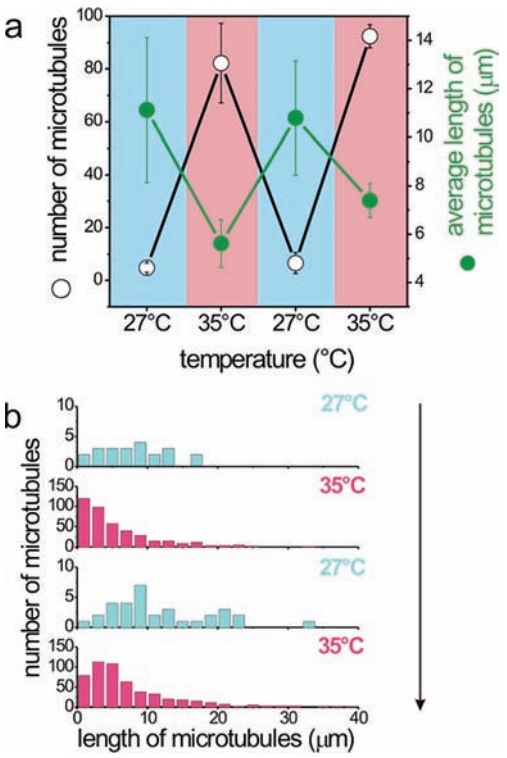

Figure 5. Dependence of the number and the length of motile microtubules on temperature at $\Gamma_{PNIPAM} \approx 5$ mg/m^2. (a) Average microtubule length (green, filled circles) and number of microtubules (black, open circles) per field of view as function of temperature. (b) Histograms of microtubule lengths at different temperatures (data represent total length distributions of microtubules in four fields of view). The cyclic change of temperature was accompanied by a cyclic change of the average microtubule length. The arrow shows the temporal order of the experiments.

average microtubule length (Figure 5a). Analysis of the length distributions of gliding microtubules revealed that cooling predominantly caused the release of the shorter microtubules (Figure 5b). This finding can be explained as follows: Swelling of the PNIPAM chains leads to the screening of part of the kinesin motors. Consequently, the average distance between neighboring kinesin molecules that can be reached by the microtubules significantly increases. Because for continuous gliding, microtubules always need to be in contact with at least two kinesin motors at one time, the variable motor density leads to a sorting effect of the microtubules according to their length similar to the static phenomenon described in ref 12.

The release of the gliding microtubules in experiments III and IV can be understood by the dimension of the swollen PNIPAM layer at 27 °C (see Table 1). At high grafting densities the length of the extended PNIPAM chains exceeds the height of about 15−20 nm at which kinesin-driven microtubules are assumed to hover above the surface.[29] Thus, the kinesin heads are likely be hidden within the surrounding PNIPAM chains. Heating leads to a collapse of the PNIPAM chains and makes the kinesin molecules accessible for the microtubules.

In summary, we developed a novel approach for the in vitro control of microtubule motility on kinesin-coated substrates using stimuli-responsive polymers. Reversible switching of motility was achieved by the covalent attachment of a thermoresponsive PNIPAM onto Si chips where kinesin motors were subsequently adsorbed. We observed that microtubules were able to land and glide on the composite PNIPAM−kinesin surface at 35 °C but were repelled from the surface at 27 °C. Moreover, we demonstrated the applicability of the composite PNIPAM−kinesin layer for the stimuli-controlled sorting of microtubules according to their length.

We believe that the reported method can be implemented for the control of microtubule guiding in various nanotechnological applications. For example, patches of PNIPAM polymer grafted onto the bottoms of narrow grooves could be used for the externally controlled gating of nanotransport: microtubules could pass the gate if the PNIPAM patch is locally heated but would be expelled at room temperature. Such a configuration could also be applied for the thermocontrolled sorting of microtubules according to their length when microtubules of length shorter than a threshold value cannot pass such a gate. Because the presented method operates in a temperature range that does not impact the functioning of most biological systems, we foresee a strong potential of the system for the external control over a wide range of surface-coupled enzyme systems.

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Supporting Information Available: A sequence of fluorescent images of microtubules gliding over a PNIPAM−kinesin surface (field of view 226 × 169 μm, PNIPAM grafting density of about 6 mg/m^2) at different temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

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