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Control and gating of kinesin-microtubule motility on electrically heated thermo-chips

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Abstract First lab-on-chip devices based on active transport by biomolecular motors have been demonstrated for basic detection and sorting applications. However, to fully employ the advantages of such hybrid nanotechnology, versatile spatial and temporal control mechanisms are required. Using a thermo-responsive polymer, we demonstrated a temperature controlled gate that either allows or disallows the passing of microtubules through a topographically defined channel. The gate is addressed by a narrow gold wire, which acts as a local heating element. It is shown that the electrical current flowing through a narrow gold channel can control the local temperature and as a result the conformation of the polymer. This is the first demonstration of a spatially addressable gate for microtubule motility which is a key element of nanodevices based on biomolecular motors.

Keywords Nanodevices · Molecular motors · Thermo-responsive polymer · Poly(N-isopropylacrylamide) · Microtubules · Kinesin

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1 Introduction

The control of the location of biomolecules with nanometer precision is a key performance criterion for advanced nanodevices, such as spatially-addressable positioning of nanoarrays (Lee et al. 2002; Christman et al. 2006); and nanodevices based on protein molecular motors (Diez and Howard 2009; Fulga and Nicolau 2009; Korten et al. 2010). Linear motor proteins, including the ATP-hydrolyzing enzyme kinesin, are responsible for numerous critical biological functions *in vivo* such as the transport of cellular cargo (Howard 2001). The development of the *in vitro* motility assays, where purified motor proteins work in an artificial environment, has allowed better access to study the fundamentals of transport by molecular motors (Lee et al. 2002; Diez and Howard 2009; Fulga and Nicolau 2009; Korten et al. 2010). As a result they have been studied in detail for their potential implementation in bio-devices as they provide a very efficient system for the transportation of cargo at the nanometer scale (Howard et al. 1989; Hunt et al. 1994; Korten et al. 2010; Hess et al. 2011). Most promising for nanotechnological applications is the *gliding motility assay*, in which the cytoskeletal filaments move across a surface functionalized with motors proteins (Kron and Spudich 1986; Cohn et al.

1989; Howard 2001). Because this assay is robust as well as easy to implement and operate, gliding motility assays have been used as templates for numerous prototypes of dynamic devices based on the use of protein linear molecular motors (Korten and Diez 2008; Fischer et al. 2009).

The use of protein molecular motors in effective nanodevices requires the precise control of the direction and speed of movement of the cytoskeletal filaments. This has been achieved in various ways, ranging from chemical modification (Nicolau et al. 1999, 2007) to creating physical barriers such as topographical features to guide the movement of cytoskeletal filaments in the desired direction (Clemmens et al. 2003; Bunk et al. 2005; van den Heuvel et al. 2005). Despite their success, a disadvantage of these methods is that they control motility in a static fashion, as the surface or the features cannot be modified during the operation of the nanodevice. Temporal control has been achieved using electric fields and thermo-responsive polymers to dynamically guide and switch the motility of cytoskeletal filaments (Riveline et al. 1998; Hanson et al. 2005; Ionov et al. 2006; van den Heuvel et al. 2006; Korten et al. 2012; Schroeder et al. 2013). Electric guiding allows for the change of directionality during a motility assay and the on/off switching of the directionality control. However, the electric fields required are relatively large, thus creating the potential of introducing gasses and radicals in the motility assay chamber, which are harmful to the activity of protein molecular motors. The thermo-responsive polymer poly(N-isopropylacrylamide) (PNIPAM), on the other hand has been used (i) to control the overall motility of microtubules on a kinesin-coated surface (Ionov et al. 2006), (ii) to selectively control a specific population of microtubules (Korten et al. 2012), and (iii) to dynamically guide microtubules at a planar junction (Schroeder et al. 2013). However, so far this polymer has not been integrated with static topographical guiding structures, where the topographical confinement leads the microtubules towards a gated area containing the PNIPAM molecules. There, an essential operational element is a 'traffic light' system that acts as a gate to the transport of the cytoskeletal filaments, i.e. allowing the passage through a geometric local bottleneck only when the gate is open. Here, we demonstrate the implementation of such a PNIPAM gate in topographical channels in combination with localized heating to gate the motility of kinesin-powered microtubules.

2 Experimental methods

2.1 Surface engineering

The method for grafting PNIPAM onto surfaces was adapted from (Ionov et al. 2006). Briefly, 200 nm thick gold-on-glass chips (20×14×1 mm, Ssens, Enschede, The Netherlands) were cleaned with Piranha solution (*3:1 concentrated H_2SO_4 and H_2O_2; danger, extremely corrosive and explosive when mixed with organic solvents!*). The clean substrates were then spin-coated (ramped at 500 rpm/s to 2,000 rpm for 30 s) with a 0.01 % Poly(glycidyl methacrylate), (PGMA, M_n= 65,000 g mol^{-1}, Polymersource Inc., Dorval, (Montreal), Canada) solution in chloroform. The PGMA was annealed at 130 °C for 20 min in a vacuum oven. After annealing, the substrates were placed in chloroform (70 °C) in order to remove unbound PGMA. After the deposition of the PGMA, the topographical structure and the electrodes were fabricated by laser microablation of the chip surface (QuikLase-50ST from ESI/NewWave Research). Poly(N-isopropylacrylamide) (PNIPAM, Mn=45,000 g/mol, Polymersource Inc., Dorval (Montreal), Canada) was dissolved in chloroform (1 % solution). The surface of the substrates was then completely covered with a droplet of the PNIPAM solution. After the chloroform evaporated, the substrates were placed in the vacuum oven at 160 °C for 60 min to anneal the PNIPAM. Unbound PNIPAM was removed by washing the substrates in hot chloroform (70 °C).

2.2 Microtubules gliding assay

Microtubules were polymerized from rhodamine-labeled porcine brain tubulin in BRB80 (80 mM PIPES, adjusted to pH 6.9 with KOH, 1 mM EGTA, 1 mM $MgCl_2$) with 5 mM $MgCl_2$, 1 mM GTP, 5 % DMSO at 37 °C for 30 min. The microtubules were stabilized and diluted 200-fold in BRB80 containing 10 μM taxol. Flow cells for motility experiments were assembled with the structured chips, two stripes of parafilm across the surface and closed with a glass cover slip. The chips were then mounted onto a microscope stage that could be temperature-controlled using a Peltier element, see also Fig. 2a (Ionov et al. 2006). A casein containing solution (BRB80 with 0.5 mg ml^{-1} casein) was perfused into the flow cell and allowed to adsorb for 5 min. This solution was exchanged for a kinesin solution (BRB80 with 10 μg ml^{-1} Kinesin-1, full length, from Drosophila melanogaster, expressed in bacteria (Coy et al. 1999); 0.2 mg/ml casein, 1 mM ATP, 10 mM dithiothreitol), which was allowed to adsorb for 5 min. Finally, a microtubule containing solution (motility solution: BRB80 with 10 mM taxol, microtubules (equivalent of 32 nM tubulin), 1 mM ATP, 40 mM D-glucose, 55 μg ml^{-1} glucose oxidase, 11 mg ml^{-1} catalase, 10 mM dithiothreitol) was inserted and imaging was started. The imaging was performed using an Axiovert 200 M inverted optical microscope (Zeiss) equipped with a back-illuminated CCD camera (MicroMax 512 BFT, Roper Scientific) in conjunction with a Metamorph imaging system (Universal Imaging Corp.).

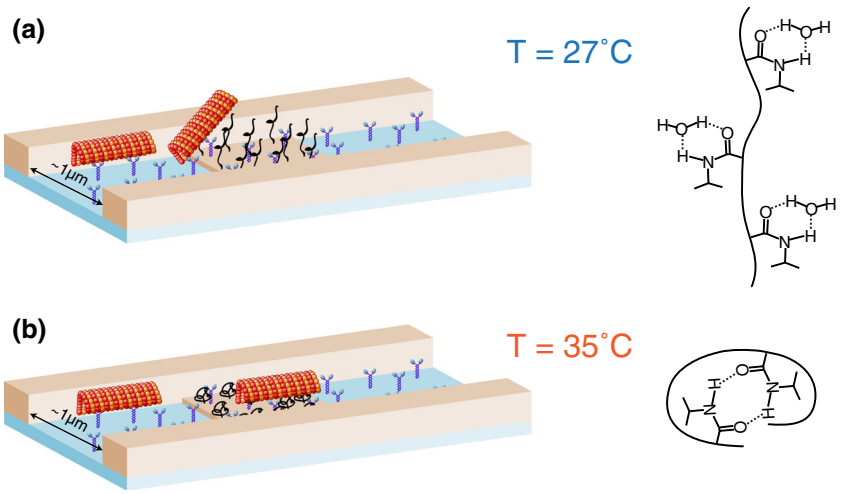

Fig. 1 Schematic showing the operation of a PNIPAM gate. On the right are schematics of the two conformations of the PNIPAM molecule. In both situations the microtubules are travelling from the left to the right inside a topographically defined channel. **a** At 27 °C the PNIPAM will be in its fully extended state and will not allow the binding of the microtubule to the kinesin motors and no microtubules are able to traverse the gate area. **b** Once heated to 35 °C, the PNIPAM chains will collapse, thus allowing the motility to proceed uninhibited and the gate to be open

3 Experimental results

PNIPAM is a thermo-responsive polymer that changes conformation as a function of the applied temperature (Nath and Chilkoti 2002; Huber et al. 2003). This structural change occurs in the polymer at a very specific temperature, designated as the Lower Critical Solution Temperature (LCST), which for PNIPAM is 32–33 °C (Sun et al. 2004). Below the LCST the polymer exists in a hydrated state with an extended structure (Fig. 1a). Upon heating the polymer is dehydrated and the chains collapse, as presented in Fig. 1b. Because kinesin-1 holds microtubules approximately 20 nm above the surface in a gliding assay (Kerssemakers et al. 2006), it has been shown that the length of the PNIPAM polymer can be chosen such that it sterically prevents the attachment of the microtubules to the kinesin when the PNIPAM is in its extended state (Ionov et al. 2006). In contrast, when the PNIPAM is in its collapsed state, the kinesin molecules become accessible to the microtubules leading to their propulsion. With an appropriate design of the surface, we aimed to localize the effect on a micron-sized area within a channel, thus creating a gate.

The chip was designed to possess topographical features that guide the microtubules towards a PNIPAM-functionalized area, which can be heated locally by an electrical current that can be switched on and off during the motility assay. The micro-structured surface was created by laser ablation of a gold layer on a glass slide. The gold layer was pre-coated with PGMA to allow specific attachment of PNIPAM on top of the gold and not inside the channels. The structure consisted of a microtubule collecting area where microtubules could land and move towards a narrow channel (Fig. 2). A gap in this channel was left, creating a small strip of gold which was narrower than the surrounding gold connectors (Fig. 2b). Thus the resistivity in the small strip of gold was higher and an applied current could generate heat localized to the strip of gold allowing this PNIPAM-coated gold strip to act as a gate for microtubule motility.

Before demonstrating local effects, the thermo-responsive properties of the PNIPAM were tested on the whole surface of the flow cell using a Peltier element attached to the flow cell for temperature control. Maximum intensity projections are shown at temperatures below (Fig. 3a) and above (Fig. 3b) the LCST. Below the LCST, when the polymer was extended,

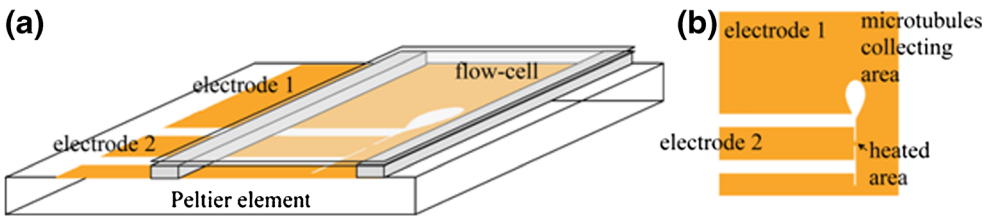

Fig. 2 Schematic showing the experimental setup. **a** The flow cell in which the motility occurs. The two electrodes needed to generate to localized heating as well as the Peltier element at the back of the chip are labeled. **b** Design of the chip. The *white areas* are coated with kinesin only and the *yellow areas* are coated with both kinesin and PNIPAM

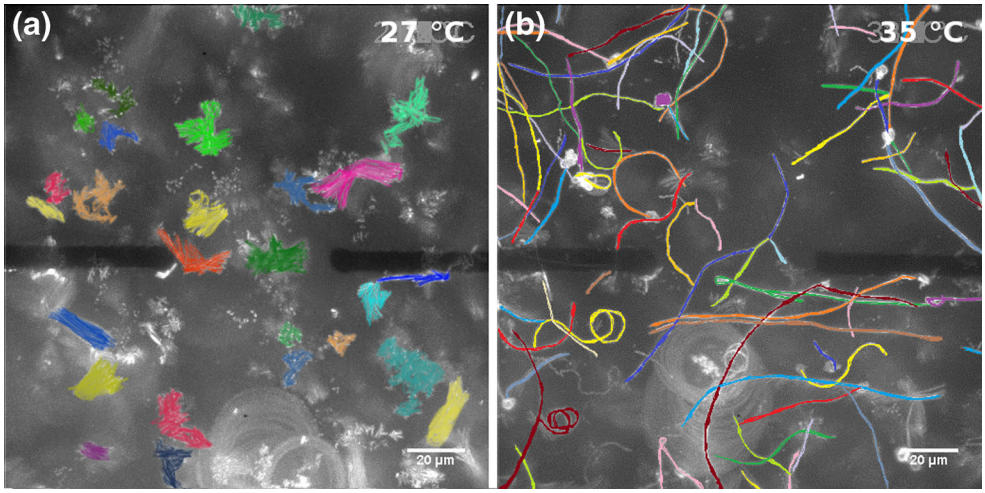

Fig. 3 Visualization of the control of motility by heating of the PNIPAM surface in these experiments the whole sample has been heated. **a** When the temperature of the surface was held at 27 °C, the fully extended PNIPAM chains prevented the microtubules from reaching the kinesin molecules. **b** When the temperature of the surface was held at 35 °C, the collapsed PNIPAM chains allowed the normal motility function. Both images show maximum projections of the pixel intensities over the complete video (100 ms time lapse, 100 s)

microtubules showed only random diffusion and no directed motion resulting in a blurry appearance in the maximum projection. In contrast, when the temperature of the Peltier element was raised, the PNIPAM molecules collapsed and microtubules started to move in clearly visible lines indicating directed motion. The difference in fluorescence intensity on the ablated channel and the non-ablated gold surface was a result of fluorescence interference contrast on the reflective gold surface (Kerssemakers et al. 2006).

In order to study the effect of locally heating the small strip of gold, the temperature of the Peltier element was fixed at 23 °C to limit the distribution of the heat generated by the resistive element. Without the presence of the Peltier element the size of the area affected by the heating in the gate area was far more difficult to control. An electrical potential was then applied over the gold bridge (0.3 V at 52 mA). This resulted in 15.6 mW of heat generated at the gold junction. When looking at Fig. 4 the potential is applied between the top and the bottom of the image and the constriction, which is the resistive element is located between the two dark channels. Although the heat is generated in this relatively small area the thermal transport properties of the materials used result in a slight larger heated area.

As the temperature on the junction was raised above the LCST the kinesin was able to propel microtubules over the gold gate. Figure 4 shows an example microtubule that moved along the channel (Fig. 4a), crossed the gate boundary and started to move orthogonally to the channel (Fig. 4b). When the microtubule moved further away from the junction, it entered a region where the temperature had not risen sufficiently to reach the LCST. Approximately 40 µm away from the junction, the microtubule started to detach and appeared blurry (Fig. 4c). This result shows that it is possible to gate the movement of a microtubule. Further optimization of the topography of the motility channel and the heating element will provide a better confinement and directionality of the

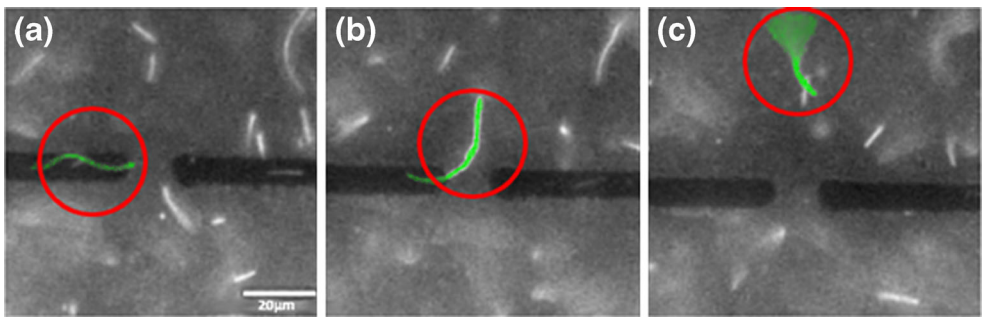

Fig. 4 Demonstration of the gating of motility. The resistive element is the area between the two dark channels, a potential was applied between the *top* and *bottom* of the images causing heat generation in the resistive element. The Figures A to C have subsequently. **a** Highlighted area (*red circle*) presents a microtubule gliding inside a channel. **b** The microtubule was allowed to glide in the locally heated area of the gate where the PNIPAM was in its collapsed state. **c** When the microtubule reached the edge of the heated gate, the extended PNIPAM no longer allowed the contact with the kinesin motors and the microtubule detached from the surface. The *circle* indicates the same microtubule moving throughout the video (52 s)

microtubule. This can be assisted by the fine tuning of the applied potential on the heating element, which will limit the affected area.

4 Conclusion

In conclusion, the thermo-chip designed and demonstrated here was able to effectively localize the heating on the surface and thus enabled the PNIPAM-facilitated gating of microtubule motility. The localized heating at the microfabricated gate was tuned such that the PNIPAM was successfully dehydrated while not destroying the immobilized kinesin motor proteins. The combination of the PNIPAM conformation with the localized heating enabled the gating of biomolecular transport, which is potentially a critical part of any future dynamic nanodevice based on the microtubule-kinesin system.

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