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Ultrafast molecular motor driven nanoseparation and biosensing

Mercy Lard [a,1], Lasse ten Siethoff [b,1], Saroj Kumar [b], Malin Persson [b], Geertruy te Kronnie [c], Heiner Linke [a], Alf Månsson [b,*]

[a] The Nanometer Structure Consortium (nmC@LU), Division of Solid State Physics, Lund University, SE-221 00 Lund, Sweden
[b] Department of Chemistry and Biomedical Sciences, Linnaeus University, SE-391 82 Kalmar, Sweden
[c] Department of Women's and Children's Health, University of Padova, 35131 Padova, Italy

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ABSTRACT

Portable biosensor systems would benefit from reduced dependency on external power supplies as well as from further miniaturization and increased detection rate. Systems built around self-propelled biological molecular motors and cytoskeletal filaments hold significant promise in these regards as they are built from nanoscale components that enable nanoseparation independent of fluidic pumping. Previously reported microtubule-kinesin based devices are slow, however, compared to several existing biosensor systems. Here we demonstrate that this speed limitation can be overcome by using the faster actomyosin motor system. Moreover, due to lower flexural rigidity of the actin filaments, smaller features can be achieved compared to microtubule-based systems, enabling further miniaturization. Using a device designed through optimization by Monte Carlo simulations, we demonstrate extensive myosin driven enrichment of actin filaments on a detector area of less than 10 μm^2, with a concentration half-time of approximately 40 s. We also show accumulation of model analyte (streptavidin at nanomolar concentration in nanoliter effective volume) detecting increased fluorescence intensity within seconds after initiation of motor-driven transportation from capture regions. We discuss further optimizations of the system and incorporation into a complete biosensing workflow.

1. Introduction

Portable biosensors with increased sensitivity, rate of detection and capacity for multiplexing (Giljohann and Mirkin, 2009; Jokerst et al., 2010; Ng et al., 2010) are of appreciable interest for improved point-of-care medical diagnostics and related applications. Realizations of high-performance devices have been proposed using nanostructures (Giljohann and Mirkin, 2009; Lee et al., 2004; Nam et al., 2003; Zhang et al., 2005a, 2005b) combined with microfluidics/nanofluidics for specific concentration of analytes from a complex sample mixture (Jokerst et al., 2010; Ng et al., 2010; Whitesides, 2006). However, particularly nanofluidics requires strong driving forces for liquid transport (Månsson et al., 2005), and depends on bulky accessory equipment such as pumps and various control devices (Jokerst et al., 2010; Whitesides, 2006). One way to circumvent these problems would be to use Adenosine-5'-triphosphate (ATP)-driven biological molecular motors for separation and concentration of analyte molecules on detector sites (Fischer et al., 2009; Korten et al., 2010; Lin et al., 2008). In this process, the analyte molecules are linked to motor-propelled cytoskeletal filament shuttles (Bachand et al., 2006; Brunner et al., 2007; Hess et al., 2001; Månsson et al., 2004; Ramachandran et al., 2006) and guided on nanopatterned surfaces (Ashikari et al., 2012; Bunk et al., 2005b; Byun et al., 2007; Hess et al., 2001; Nicolau et al., 1999; Sundberg et al., 2006b; Suzuki et al., 1997) to the desired sites. In addition to advantages over microfluidics driven separation, the actual detection of analytes may be achieved in unique ways using motor driven devices e.g. by the actual observation of cotransportation of filaments and analytes (e.g. Korten et al., 2013). One automated approach that combines this unique biosensing principle with separation is transportation of analytes to a pre-determined detection site that can be readily reached only by motor driven transportation. Whereas proof-of-principle devices of this type have been reported using the microtubule-kinesin motor system (Fischer et al., 2009; Lin et al., 2008) these devices have shown orders of magnitude, lower rates of detection than other high-sensitivity methods (Georganopoulou et al., 2005; Mulvaney et al., 2009; Nam et al., 2003; Rissin et al., 2010).

A way to overcome the limitations in speed may be to use myosin-propelled actin filaments that are ten-fold faster than kinesin-propelled microtubules. This idea is supported by the recent demonstration of consistent heavy meromyosin (HMM)

* Corresponding author. Tel.: +46 480 446 243; fax: +46 480 446 262.
E-mail address: alf.mansson@lnu.se (A. Månsson).
[1] These authors contributed equally to this work.

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driven transportation of a large number of antibody–antigen complexes (Kumar et al., 2012). Moreover, the low flexural rigidity (Vikhorev et al., 2008a) of actin filaments compared to microtubules would enable more extensive miniaturization that may also contribute to further increased detection rate. We thus hypothesize that actin filaments can be concentrated by myosin driven transport, orders of magnitude faster than in previous motor driven devices (Fischer et al., 2009; Lin et al., 2008). Moreover, we expect that the use of actin filaments allows further miniaturization, e.g. of the detector site, with favorable effects on the signal/noise ratio (Katira and Hess, 2010b). With the aim to test these ideas, we first present experiments using a generic concentrator device to validate a Monte-Carlo simulation approach (Nitta et al., 2006, 2008) that is then used for rational design of an optimized device. Experiments employing the latter device showed rates of concentration that were nearly two orders of magnitude faster than in earlier motor-driven devices but also faster than key amplification steps in non-motor based diagnostic tests (Georganopoulou et al., 2005; Nam et al., 2003). Moreover, further miniaturization compared to microtubule-kinesin devices, allowed actin filament capture and subsequent guidance to detector site of less than 10 μm^2 total area. We discuss further optimizations and the most effective incorporation of the tested device into complete workflows for high-sensitivity detection of analytes in, for example, clinical diagnostics and environmental monitoring.

2. Materials and methods

2.1. Nanostructuring and surface preparation

An SiO_2 layer of 825 nm thickness was grown by wet thermal oxidation on a Si wafer followed by covering with lift-off resist (LOR 0.7A; Microchem Corporation, Newton, MA, USA) using spin coating at 1500 RPM for 30 s and subsequent baking at 180 °C for 15 min on a hot plate. Next, polymethylmethacrylate, (PMMA 950A5; Microchem Corporation, Newton, MA, USA) was spin-coated on top at 6000 RPM for 60 s, followed by baking at 160 °C for 15 min. This PMMA resist layer was exposed by electron-beam lithography (EBL; Raith 150, Dortmund, Germany) giving top line widths of approximately 185 nm in the PMMA. The PMMA was then developed with methyl isobutyl ketone and isopropanol (MIBK:IPA; Merck KGaA, Darmstadt, Germany) at a ratio of 1:3 for 1 min, followed by rinsing with IPA for 30 s. Next, the sample was subjected to O_2 plasma ashing in a Plasma Preen at 5 mBar for 15 s. Then, the LOR layer was etched with MF-319:H_2O (Microposit MF-319 Developer, Rohm and Haas Electronic Materials, Coventry, UK) in the ratio 1:1 for 4 min and rinsed with H_2O for 30 s to halt etching. The resulting width of the bottom channel was 260 nm, with an under-cut of approximately 40 nm from the opening of the top channel (Fig. 1b). The sample was then treated with another plasma ashing step at 5 mBar for 15 s in order to remove possible LOR residue on the bottom of the channel and making the PMMA hydrophilic and thereby incapable of supporting motility. Finally, the sample was silanized, in a chemical vapor phase deposition (CVD) process (Bunk et al., 2005a; Sundberg et al., 2003), with trimethylchlorosilane (TMCS, > 97%, GC, Sigma-Aldrich, Sweden AB, Stockholm, Sweden) in a closed glass container inside a glove box, with sub-ppm levels of O_2 and H_2O. As a final step before the in vitro motility assay procedure, the sample was steamed in a deionized water bath for 30 s to wet the channels and remove possible air bubbles. The surfaces were rinsed in distilled water for 10 min prior to use. The above account describes the key steps in producing the first generation concentration device. The second-generation concentration device was fabricated in a similar way but without any LOR layer (Fig. 4a).

2.2. Protein preparations and in vitro motility assays

Myosin II was purified from rabbit fast skeletal muscle (Sata et al., 1993) and then digested by α-chymotrypsin to yield HMM (Kron et al., 1991). Actin filaments were prepared from rabbit skeletal muscle (Pardee and Spudich, 1982) and fluorescently labeled with Alexa Fluor 488® phalloidin (APh) or tetramethylrhodamineisothiocyanate (TRITC)–phalloidin (RhPh; Molecular Probes Invitrogen, Eugene, OR) (Balaz and Mansson, 2005).

Flow cells were constructed from one cover-slip and one nanostructured chip on top with double-sided sticky tape as spacers. All solutions that were added to the flow cell were based on buffer A (1 mM $MgCl_2$, 10 mM 3-(N-morpholino)propanesulfonic acid MOPS, 0.1 mM K_2-ethylene glycol tetraacetic acid (EGTA), pH 7.4) and all proteins were diluted in buffer B (buffer A with 1 mM dithiothreitol (DTT) and 50 mM KCl). The flow cell was pre-incubated essentially as described previously (Kron et al., 1991; Sundberg et al., 2006b): (i) HMM (120 μg mL^{-1}) for 4 min, (ii) bovine serum albumin (BSA; 1 mg mL^{-1}) for 1 min, (iii) blocking actin (1 μM unlabeled sheared actin filaments with 1 mM MgATP) for 2 min. These pre-incubation steps were followed by (iv) wash with a50 assay solution (buffer A with 10 mM DTT, 1 mM MgATP, 35 mM KCl, ionic strength 50 mM) and (v) addition of actin filaments at 10 nM (second generation) or 100 nM (first generation). After an incubation period of 1 min, flow cells were washed with buffer B and (vi) incubated with rigor solution (r60) for initial observation in the microscope. The latter solution had the same composition as the a50 solution but without MgATP and with 45 mM instead of 35 mM KCl, giving an ionic strength 60 mM. An anti bleach mixture of 3 mg mL^{-1} glucose, 100 μg mL^{-1} glucose oxidase and 870 U mL^{-1} catalase was added. Soon after the recording was started, flow cells were (vii) incubated with a60 solution (r60 with 1 mM MgATP and an ATP re-generating system: 2.5 mM creatine phosphate and 3.5 U mL^{-1} creatine phosphokinase). For the second generation concentrator device the procedure was similar as described above, but the incubation step with block actin and subsequent washing steps (steps iii and iv) were omitted.

To demonstrate analyte concentration we rinsed the second generation device after previous use with (i) a130 solution (similar to a60 but with an ionic strength of 130 mM) and (ii) buffer B before incubation with (iii) 10 nM biotinylated APh labeled actin filaments for one minute. The flow cell was then rinsed with (iv) buffer B and, incubated with (v) TRITC–streptavidin (2 nM) for one minute. To block remaining sites on streptavidin and avoid cross-linking of filaments via streptavidin–biotin links, the flow cell was then rinsed with (vi) biotin (30 μM). Subsequently, (vii) buffer B was infused. Flow cells were then incubated with (viii) r60 solution and observed in the microscope before motility was induced by (ix) addition of a60 solution.

2.3. Data collection and analysis of in vitro motility assays

Fluorescently labeled actin filaments were observed using a Nikon Eclipse TE300 inverted fluorescence microscope (Nikon Corporation, Tokyo, Japan) equipped with a temperature-regulated Nikon (100 × 1.4 NA) oil immersion objective and TRITC (Ex. 540/25, DM 565, and BA 605/25) and FITC (Ex. 465–495, DM 505, and BA 515–555) filter sets. A cooled Hammamtsu EMCCD camera (C9100-12) was used to record image sequences (Persson et al., 2010) which were then analyzed using algorithms developed in the MATLAB (Mathworks Inc., Natick, MA; Mansson and Tagerud, 2003). Image sequences were further analyzed using Image J (Rasband, W.S., ImageJ, U S National Institutes of Health, Bethesda,