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An ON/OFF biosensor based on blockade of ionic current passing through a solid-state nanopore

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ABSTRACT

Single nanopores have attracted interest for their use as biosensing devices. In general, methods involve measuring ionic current blockades associated with translocation of analytes through the nanopore, but the detection of such short time lasting events requires complex equipment and setup that are critical for convenient routine biosensing. Here we present a novel biosensing concept based on a single nanopore in a silicon nitride membrane and two anchor-linked DNA species that forms trans-pore hybrids, realizing a stable blockade of ionic current through the pore. Molecular recognition events affecting the DNA hybrids cause a pore opening and the consequent establishment of an ionic current. In the present implementation of the device, we constructed a magnetic bead/streptavidin/biotin-DNA1/DNA2-biotin/streptavidin/Quantumdot-cluster complex (where DNA1 is a mismatched reverse complement of DNA2) through a sub-micrometric pore and monitored DNA strand displacement events occurring after addition of an oligonucleotide complementary to DNA2. The electric and mechanical aspects of the novel device, as well as its potential in biosensing are discussed.

1. Introduction

Current trends in clinical diagnostic and environmental monitoring sustain an increasing demand for a portable, rapid and highly sensitive, yet user-friendly and inexpensive biosensing technology. As far as DNA hybridization based approaches are concerned, methods that perform electrical signal detection cut down consistently equipment costs and are amenable to integration in portable instruments. However, approaches that conjugate low cost and high sensitivity are, to date, still technically challenging (Song et al., 2006). Considerable interest toward the miniaturization of devices has stimulated the integration of different sciences such as materials engineering, physics and biology for the development of novel biotechnology applications. A major exercise of integrating technology directed towards miniaturized laboratory and high sensitivity has been, in the last decade, molecular characterization through artificial nanopores.

In recent years the use of the alpha-haemolysin pore for the characterization of nucleic acids has addressed rewarding promises. The technique is usually applied to study properties of nucleic acids passing through the lumen or with sequencing purpose (Kasianowicz et al., 1996; Li et al., 2003; Meller et al., 2000). Converting these promises into reality for the field of molecular diagnostics, however, remains an ambitious goal. The analysis of ionic current signatures as DNA translocates through a nanopore, is a very difficult task given the translocation rate (1–30 nt/μs at 100 mV depending on estimates) and the noise in the ionic current signal. Detection of DNA features requires an electronic system with a bandwidth which is currently not available when designing a bench-top diagnostic system. Moreover, the use of a lipid bilayer as a supporting membrane severely limits the applications of nanopore-based sensors for the detection of target molecules, as geometric disposition of pores, their number and size are constrained by the biological intrinsic properties of the system; the protein pore is not reusable and the entire reconstruction process has to be done before starting a new experiment.

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Therefore recent research was addressed to solid-state nanopores. The most widely used method to produce artificial pores is based on the electron or ion beam sculpting in silicon nitride or silicon oxide membranes (Li et al., 2001), although several other methods are available (Saleh and Sohn, 2003; Siwy and Fulinski, 2002; Sun and Crooks, 2000). Synthetic nanopores are widely used for force measurements on proteins and nucleic acids (Fologea et al., 2007a; Keyser et al., 2006) or transient current-blockade detection (Choi et al., 2006), but to date there are no reports on their use in diagnostic assays.

In this paper we present a novel biosensing principle that exploits the ability to easily measure ionic currents passing through small pores but do not require the sophisticated setup and equipment needed for real time monitoring of rapid events. Our sensor differs from previous pore-based approaches in that the recorded ionic current variation events are not determined by the transient blockade caused by the analyte crossing through the synthetic pore, but by stable, yet analyte-dependent pore clogging caused by buoys associated with probe molecules. Thus the molecular events concerning a small number of molecules can trigger a physical and electric reversible switch that is easily recorded, paving the way to the development of portable and highly sensitive devices.

2. Materials and methods

2.1. Reagents

Several different beads were used as molecular anchor and linked to ligand molecules (nucleic acids in most experiments) exploiting the streptavidin/biotin link. Particles with superparamagnetic core were Dynal MyOne Streptavidin C1, 1.0 ± 0.1 μm diameter; fluorescent particles were either quantum dots, i.e. Qdot® Streptavidin Conjugates 605 nm emitters 20 nm in diameter, or Fluosphere 1.0 ± 0.1 μm diameter, biotin coated, emitting at 420 nm. All particles were purchased from Invitrogen (Carlsbad, USA).

Standard oligonucleotides were M13RC: 5′-ATGGTCATAGCTGTTTCC-3′, its perfect reverse complement M13R: 5′-GGAAACAGCTATGACCAT-3′, its not-perfect reverse complement M13RA: 5′-GGAAACAGCATTTAAATA-3′ containing 9 bases mismatch (underlined) and the non-complementary oligonucleotide M13F: 5′-TTGTAAAACGACGGCCAG-3′. The oligonucleotides were synthesized with either a 5′ biotin (M13Rbio, M13RCbio, M13RAbio, M13Fbio) or a 5′ fluorescein (M13Rfluo, M13RCfluo).

Used buffers were: 1× SSC (0.15 M NaCl, 0.015 M Na–Citrate, pH 7.0), 1× WBB (Tris–HCl 10 mM, 1 M NaCl, pH 7.5), Qbuffer (Tris–HCl 10 mM, 0.1 M NaCl, pH 7). Gel electrophoresis was carried out in 4% agarose under standard conditions.

2.2. Pore fabrication

Silicon (1 0 0) wafers (Jocam srl, Milano, Italy) 100 nm Si_3N_4 coated on both sides were used to fabricate silicon nitride membranes 56 μm wide into frames 15 mm sided. An optical mask was made starting from a squared glass sheet (75 mm × 3 mm) coated with 300 nm chrome. Polimethylmetacrylate (PMMA 950 kDa) positive resist (Shipley) was spun on the sheet followed by electron beam exposure of the pattern. PMMA was developed in MIBK: IPA = 1:1 solution (Shipley) and chrome layer was chemically etched. Resist was removed in hot acetone solution. The optical mask was used to expose silicon nitride wafer coated with S1828 optical resist (Shipley) under UV light into optical stepper for MUV lithography. The wafer was further developed in MF322 solution (Shipley). Silicon nitride exposed after developing was etched with O_2/CF_4 = 1.5/28.5, 150 W power and 250 V Bias in Systec RIE 600 System. After total removing of resist with acetone (5 min at 50 °C), exposed silicon was wet etched in 5 M KOH solution at 80 °C for 10 h. Single pores were milled without further processing of the wafer in a NOVA 600i system (FEI Company, Hillsboro, USA) employing a focused ion beam (FIB) of gallium ions at 10 keV energy high tension and 50 pA current. Pores wide from 450 to 800 nm were obtained with time exposures from 10 to 60 s, respectively. In the experiments involving current measurement reported in this paper, a membrane with a single 804 nm pore was used.

2.3. The electromagnet

An electromagnetic device was home made in order to have a localised tip magnetic force acting on the superparamagnetic particle positioned on the pore. A 0.5 mm copper wire was coiled twofold on a paramagnetic needle, then the magnetic tip was enveloped into a paraffin film (Parafilm M, Sigma–Aldrich). Connection to a power supply typically working at 1.5 V allowed a 0.25 A current through the wire.

2.4. Free standing membrane

In some experiments the membrane frame was leaned with back side (the side etched by KOH) up on a plastic *o*-ring over a microscope glass. Two microliters of Qbuffer were loaded on the front side and 10 μl containing Dynal functionalized particles (1 mg/ml) on the back side. The closing of the pore by particles was observed under an optical microscope. When the pore appeared to be perfectly closed, 1 μl of solution containing functionalized Fluosphere (1 mg/ml) or functionalized Qdots (1 μM) was loaded on the front side, then the frame was turned upside down and incubated for 1 h or overnight with gentle orbital shaking inside a humidified chamber to prevent evaporation. After incubation, unreacted particles were washed out with Qbuffer.

2.5. Detector setup

For experiment involving current measures, a dedicated cell was built (Fig. 1). The silicon membrane frame as described above was placed between two sealing silicone rubber *o*-rings generating two chambers separated by the pored membrane. The two compartments were maintained vertical. The electromagnet is positioned nearby the pore inside the top chamber. Electrophoretic solution (Qbuffer) was loaded into the two compartments. Platinum wire mobile electrodes were dipped in each chamber solution and connected to a current/voltage converter and a power supply set at 5 V. Data acquisition and processing was made with LabJack (LabJack Co., Lakewood, CO) and DAQFactory (Azeotech Inc., Boulder, CO).

2.6. Particles biofunctionalization

Oligonucleotides were reacted with their reverse-complements in equimolar amount in a water solution containing 2× SSC. The mix solution was incubated briefly in a water bath at 60 °C, and then allowed to cool at room temperature for 2 h. Single or double stranded oligonucleotides with biotin at 5′ end were reacted in 10:1 proportion with Dynal in buffer 1× WBB according to the manufacturer instructions. Reactions that included different particles were carried out in Qbuffer.

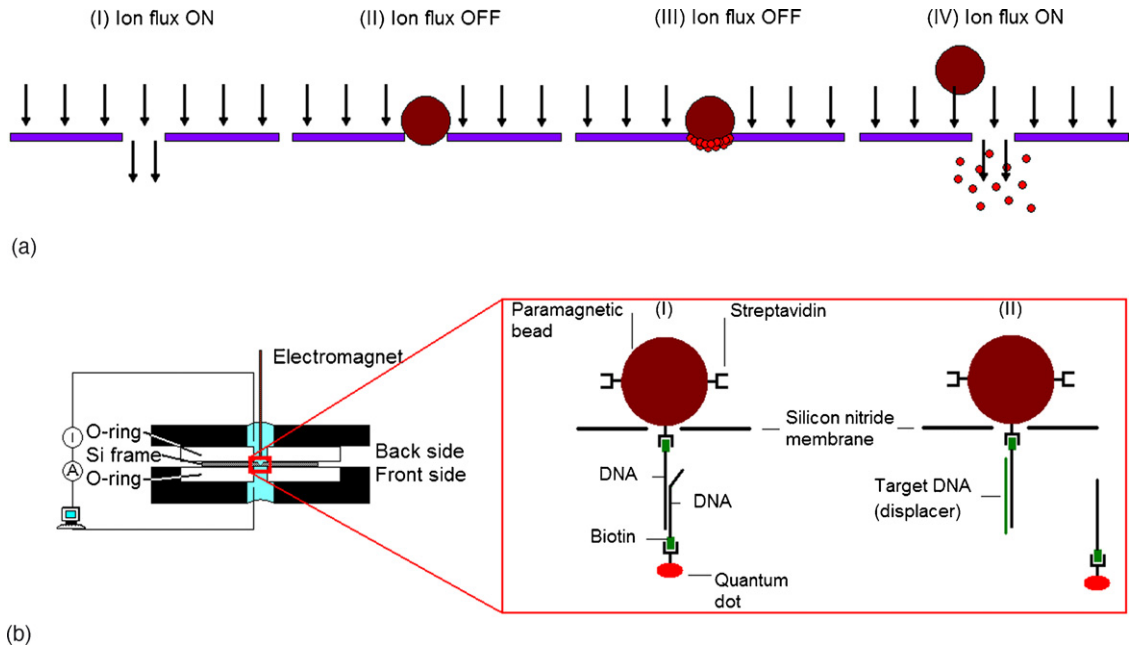

Fig. 1. Diagrams of the detection principle and its implementation. (a) Principle: (I) ON: no particles in solution, ionic current through the pore. (II) OFF: magnetic particle settling on the pore, ion flux greatly diminishes. (III) OFF: trans-membrane interaction is perceived as unceasing OFF signal. (IV) ON: strand-displacement due to interaction between nucleic acids restores ion flux after activation of the electromagnet. (b) Drawing of the device setup, at macro- and micro-scale. A silicon nitride membrane is sealed between two *o*-rings (white) pressed with four screws between two rigid plastic rings (black). The nanopore membrane inside the red square shows in its cross section enlarged in the drawing the paramagnetic bead/streptavidin/biotinylated DNA1/biotinylated DNA2/streptavidin/Quantum dot complex before (I) and after (II) displacement (for clarity only three of the about 60,000 streptavidins available per paramagnetic bead are drawn). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.7. Strand displacement

Five microliters Dynal streptavidin (1 mg/ml) were reacted with 500 pmol of M13RCbio/M13RAbio in 1× WBB for 1 h with gentle shaking. The modified beads were washed in 1× WBB containing biotin at 0.02 g/l concentration and resuspended in 500 μl Qbuffer. One microliter of this solution was then reacted with 10 μl Qdots (1 μM) for 1 h, then washed with Qbuffer and observed by epifluorescence microscopy (480×). After checking the hybridization, an excess of M13Rfluo in Qbuffer was added and incubated 2 h at room temperature with gentle shaking to displace M13RAbio (Collins et al., 1988).

3. Results

3.1. A new sensor concept

The sensing strategy used in this work is based on the ionic current passing through a pore, whose value defines either an "ON" or an "OFF" state of a switch triggered by molecular interaction. The general concept (Fig. 1) is that the reciprocal recognition of two molecular species, reacting through a membrane pore, may produce a distinct signal by obstruction of the pore lumen. Pore closure is recorded by measuring current passing through the pore. In order to produce a stable pore blockade, the two reacting molecular species are linked to relatively large buoys (or to a cluster of buoys, in the case of Quantum dots) that cannot cross the pore. Sensor operation is obtained introducing a third molecular species (target) having high affinity to one of the reacted molecular species that removes the block in the pore by displacing the other molecule. In the setup presented here, the sensor is interrogated by applying an electromagnet that can remove the paramagnetic bead settled in the pore only if DNA strand displacement has occurred and therefore the paramagnetic bead is not constrained in the pore by molecular buoys on the other side.

3.2. Experimental setup

Several biomolecular constrains of the experimental setup were dictated by the physical and mechanical characteristics that were implemented in the final device. Silicon nitride membranes 100 nm thick or 500 μm wide were found to be particularly fragile under the pressure of electrophoretic solution and during routine manipulation. In order to provide sufficient mechanical resistance, a membrane 100 nm thick and 56 μm wide was routinely used. Transmembrane capture through a 100 nm channel is however particularly challenging. The recession in the pore of the capturing molecule and the size of the molecule being captured both greatly influence the efficiency of the capture process. Therefore, pore dimension was essentially chosen on the assumption that the particle closing the pore has to expose itself through the pore to allow particles loaded in the front side to react. Pores with diameter of 900 nm or wider did not permit easy removing of the 1000 nm particle blocked and remain permanently clogged. Advantages of pores of diameter of about 800 nm include the allowance of ionic currents of the order of hundreds of nA that can be easily detected with low cost equipment and very good signal to noise ratio. The relevant current drop due to pore blockade made thermal noise negligible and no temperature control was necessary in the experiments reported below.

3.3. Free solution assays

All the beads and oligonucleotides combinations were tested in free solution before their use with Si_3N_4 pore membranes. The bead/DNA/bead complex was built in free solution with four steps:

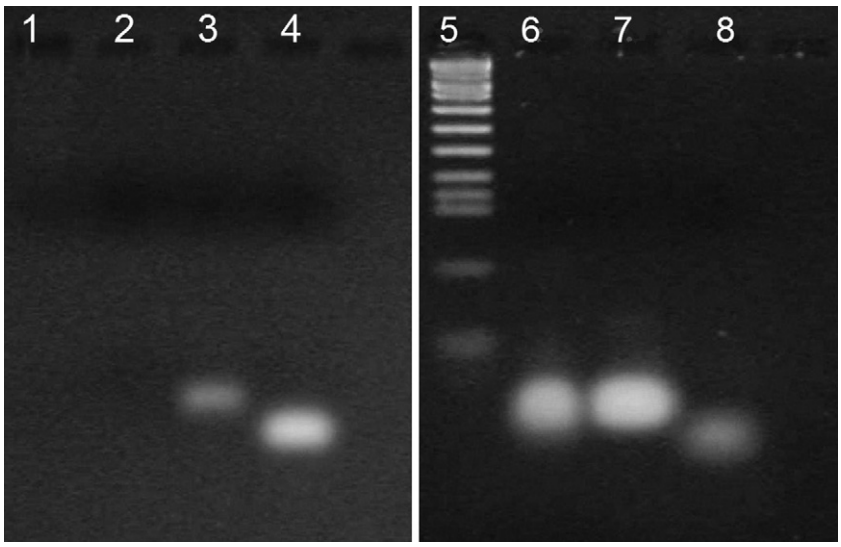

Fig. 2. Gel electrophoresis showing formation of double stranded oligonucleotides and strand displacement before (lanes 1–4) and after (lanes 5–8) ethidium bromide staining. 1,5: DNA Marker VIII (Roche). 2,6: M13RCbio + M13RAbio hybrid. 3,7: As in lane 2 after the addition of the displacing oligonucleotide M13Rfluo. 4,8: M13Rfluo.

(i) M13Rbio was hybridized to its reverse complement M13RCbio; (ii) the hybrid was reacted with streptavidin coated paramagnetic beads, then washed; (iii) unreacted streptavidins were saturated with an excess biotin; (iv) the dsDNA/paramagnetic bead complex was reacted with either biotin labelled Qdots or biotin labelled Fluospheres, magnetically purified and observed by epifluorescence microscopy. Qdots promptly reacted with Dynal/dsDNA and all magnetic beads were fluorescently labelled even with short incubation, while Fluospheres linked about 70% of Dynal/dsDNA particles. As a control to check for unwanted background interactions, the procedure was repeated using a biotinylated, non-complementary oligonucleotide to M13Rbio in step (i), resulting in a dark image (not shown). The efficiency of strand displacement was tested by gel electrophoresis. As shown in Fig. 2, the fluorescent oligonucleotide M13Rfluo when mixed with the non-perfect hybrid M13RCbio/M13RAbio (lane 2,6) displaces M13RAbio forming the green fluorescent, perfect hybrid M13RCbio/M13Rfluo (lane 3,7) which migrates slower than M13Rfluo alone (lane 4,8).

The displacement was then tested on the particle/dsDNA/particle complex. After reacting the red fluorescent Dynal/M13RCbio/M13RAbio/Qdots with the green fluorescent M13Rfluo at 25 °C for 3 h, the subsequent magnetic capture of paramagnetic beads recovered a complex containing about 70% of particles that were green fluorescent (Dynal/M13RCbio/M13Rfluo) due to the displacement of the M13RAbio/Qdots component (not shown).

3.4. Trans-pore reaction (free standing membranes)

The dynamic of molecular recognition through pores was first monitored by fluorescence microscopy. The bead/dsDNA/bead complex was built through a single nanopore using a modification of the procedure detailed above for free solution experiments. After step (iii) the Dynal/M13RCbio/M13Rbio complex was loaded on the back side of the membrane and incubated for 5 min. As detected by optical microscopy, the bead/DNA complex settled into the pore driven by the liquid flow. The membrane was then turned upside-down and Qdots were loaded on the front side of the membrane (step iv); following incubation for 1 h at room temperature, mild washes were carried out to remove all unreacted Dynal beads. As shown in Fig. 3, when trans-pore molecular recognition occurred, the fluorescent beads could not be removed and remained localized on the pore. Similar experiments were also carried out with Fluospheres, but the larger particles resulted much less efficient than Qdots in transmembrane reactions and a Dynal/dsDNA/Fluosphere complex formed only rarely.

Therefore only Qdots were used in strand displacement experiments inside the electrophoretic cell. The transmembrane complex was constructed as above but with Dynal/M13RCbio/M13RAbio/Qdots. After addition of the oligonucleotide M13R, perfectly matching M13RCbio, incubation for 4 h at 25 °C and mild washes, epifluorescence microscopy revealed that neither Qdots nor Dynal beads remained in the pore since a strand displacement event has occurred.

3.5. Detection of trans-membrane events by current blockade

A single pore holding membrane (Fig. 4) was settled inside a vertical electrophoretic cell, with a needle shaped electromagnet dipped in the upper chamber. With an 804 nm diameter pore, an

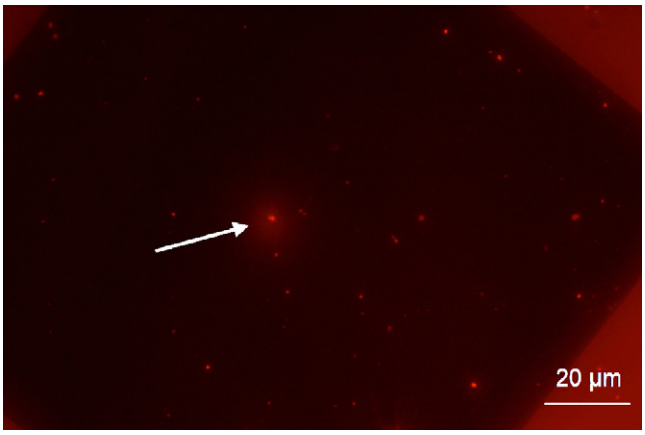

Fig. 3. Red fluorescent Qdots-streptavidin interaction with Dynal/dsDNA-biotin through the single pore in the silicon nitride membrane. The picture shows part of the silicon frame (bright background) of the silicon nitride membrane (dark background), with a single nanopore in its middle (white arrow), closed by the Dynal bead on the opposite side. The red fluorescent Qdots-streptavidin accumulated and formed a bright cluster on the nanopore following transmembrane reaction with the Dynal/dsDNA-biotin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

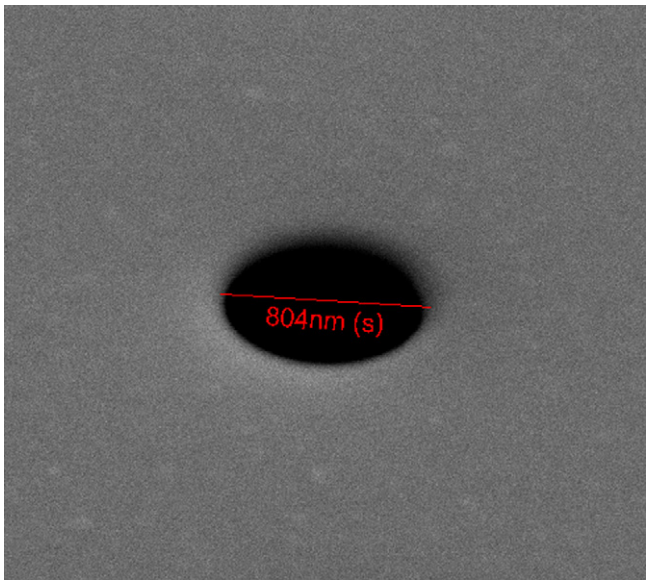

Fig. 4. SEM image of single pore (804 nm) after FIB milling.

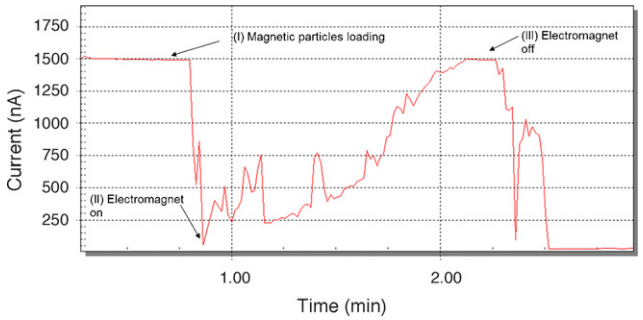

Fig. 5. Current variation during electromagnet operation. ON state (1500 nA) is turned into OFF state (100 nA) when particles reach the pore. Electromagnet activation restores ionic current to 1500 nA. When the electromagnet is turned off the particles close the pore and the current diminishes again.

applied tension of 5 V, a buffer ionic strength determined by 0.1 M NaCl, current measured passing through the pore was typically around 2000 nA (ON state). A typical background signal of 30 nA was determined using a membrane without pores. In trans-pore molecular recognition experiments, a Dynal/dsDNA complex was loaded on the back (upper) side on an electrophoretic cell powered with 5 V. Within seconds, a current drop from 1500 to 500 nA (OFF state) was detected upon occurrence of particle encountering the pore. Then the electromagnet was turned on briefly, and an increase in ionic current passing through the pore was measured, a behaviour that was interpreted as a return to the ON state by pore opening. When the electromagnet was turned off, current measure dropped again to a value corresponding to the OFF state (Fig. 5). This behaviour was interpreted as a pore closing. When the same experiment was repeated without loading magnetic particles no current changes were detected.

With the electromagnet turned off and the pore closed (OFF state), Qdots were injected in the lower chamber. Electrophoresis was stopped and the Qdots allowed to react with the exposed biotin of the trans-membrane magnetic particle/M13RCbio/M13Rbio complex for 15 min. When electrophoresis was reactivated, the measured current was on the OFF state. The electromagnet was turned on and no increase in the current measured was detected, while in control experiment, carried out with non-complementary oligonucleotides, the current could be returned to the higher values corresponding to the ON state. It was argued that Qdots reacting with Dynal/dsDNA blocked the particles in the pore, thus preventing the pore to be opened by the electromagnet and the ionic current to be re-established. Observation of the sample in the epi-fluorescence microscope confirmed that Dynal/dsDNA were settled on the pore and that Qdots reacted with them through the aperture, clustering on the opposite side of the membrane.

For trans-membrane strand displacement assay in the electrophoretic cell, the construct Dynal/M13RCbio/M13RAbio/Qdots was used to reach the OFF state of the device. The cell was powered off, and M13R was loaded on the lower chamber and incubated at 25 °C for 6 h. When the cell and the electromagnet were powered on again the ON state could be re-established. Fig. 6 reports the current readings obtained in a typical experiment: the initial ON state (a), the OFF state signal due to Dynal encountering the pore opening (b), the stable pore occlusion following the introduction of Qdots (c), characterized by an OFF state that cannot be reverted by electromagnet operation (d), and finally restoration of ionic current signal (ON state) after oligonucleotide displacement and electromagnet activation (e). Introduction of Qdots did not usually modify OFF cur-

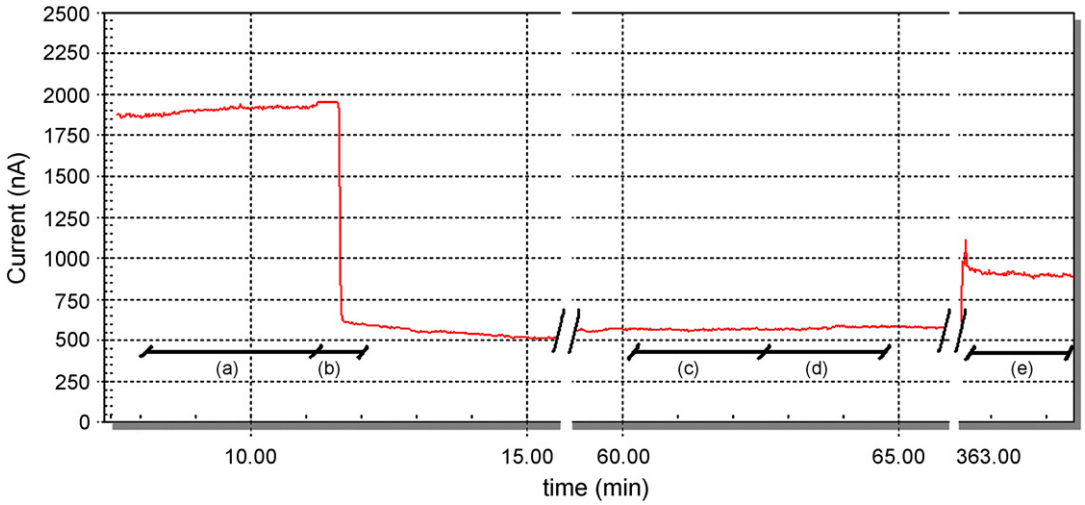

Fig. 6. Current reading during biosensor operation: initial ON state (a), OFF state signal due to pore closure by Dynal (b), introduction of Qdots (c), electromagnet operation (d), strand displacement and electromagnet operation (e).

rent signal, but occasionally an up to 100 nA decrement has been observed. After strand displacement, the switch to the ON position following electromagnet operation did not always fully restored the current to the exact initial value; this behaviour was possibly due to partial occlusion of the pore due to nonspecific adsorption of Qdots on pore margins. This unwanted effect could be avoided with shorter incubation times, but this provided unreliable results on strand displacement. With proper incubation, ON and OFF levels were always unambiguously recorded, although variations of the absolute current values varied between experiments.

4. Discussion

The advent of nanoscience has revived the old and widely applied principle of Coulter counter, introduced by Kubitschek (1958), stating that particle detection can be obtained by recording the change in conductance within a small electrolyte channel. Bezrukov et al. (1994) reported that the alpha haemolysin pore protein in a lipid bilayer could mimic the action of the Coulter-counter at the nano scale, thus opening a prolific research for the following decade. However, the exploitation in the field of biosensing and diagnostics of this revisitation of the Coulter counter at the nano scale has been hampered by the sensitivity and speed needed for the detection, as well as by the critical experimental setups. Promising modifications of the initial approach for the diagnostic field were obtained by engineering the alpha-haemolysin pore (Capone et al., 2007; Cheley et al., 2002; Gu and Bayley, 2000; Movileanu et al., 2000), while significant progress in improving the stability of the system has been achieved with the use of synthetic rather than biological pores. Despite the progress in the manufacturing technology, that permits the production of 1 nm synthetic pores and the incorporation of 2 nm proteic pores in teflon (Howorka et al., 2001), glass (Sandison et al., 2007) and silicon supports (Peterman et al., 2002) the use of these bio-inorganic hybrid devices in diagnostics is still in its early infancy.

With the few exceptions (discussed below), the methods proposed to date rely on the transient capture or detection of molecules (Deamer and Branton, 2002; Fologea et al., 2007b; Lee et al., 2004; Meller et al., 2000). Here, we exploited the recent progress in pore manufacturing to demonstrate the principle of operation of a novel biosensing method, based on a DNA driven valve in the sub-micrometric scale. Although the device is at its first, rough implementation, there are at least three unique prerogatives of this innovative method that are worth considering.

First, the system is based on two loadable, membrane separated compartments, with no interacting molecules being linked to the pore or the silicon nitride membrane or other structural parts. This fact implies that on the same device, different targets and different probes can be tested sequentially and independently. Once a reaction has occurred either one or both compartments may be freed using fluidic and magnetic tools, and then reloaded for a new, different assay. In addition, once a reaction has occurred in the testing compartment (e.g. lower chamber, loaded with the target), the latter is not disturbed by the events in the detection compartment (e.g. the upper chamber loaded with the probe). Thus the analyte molecules, that have been captured, may be further analyzed or manipulated.

Second, the system gives easily detectable, low noise signals that are dependent on a relatively small threshold number of interacting molecules. According to the binding capacity declared by the manufacturer, a single magnetic bead used in this work (Dynal) binds less than 100 zeptomoles DNA, corresponding to 6×10^4 molecules; given the geometry of the device, we estimate that about 10^3 to 10^4 molecules govern the ability of the pore to remain closed when the electromagnet is operated, i.e. switching between its ON and OFF positions. These characteristics made this system an interesting technology for the implementation of DNA-based logics and hybrid computing.

Finally, the small size and potential sensitivity of this biosensor make it a candidate for use in intracellular probes. The sensing pore may be conveniently placed on the tip of a needle to puncture a cell, using the cell cytoplasm as the lower chamber.

Before the new technology may find the practical exploitations, several aspects of the device need to be optimized. This involves aspects of nanotechnology, molecular biology and fluidics.

As far as the construction of the pore is concerned, several methods are available, such as track-etching in PET foils (Siwy and Fulinski, 2002), micromolding techniques with PDMS (Saleh and Sohn, 2003) or embedding carbon nanotubes into epoxy membranes (Sun and Crooks, 2000). In this work, the simple approach of membrane ion milling was found very reliable and repeatable. Silicon nitride was the material of choice for its insulating properties and low background signal in electrophoresis. Repeated polishing with acid solution oxidized deeply the surface, affecting its charge, and slightly enlarged the pore diameter, thus limiting the durability of a single pore membrane to 20–25 reuses. The effectiveness of the experiment was easily achieved with pore diameters in the order of hundreds nanometres, although the diameter of the pore can be reduced down to 20 nm according to the FIB milling limit capacity tested in our preliminary experiments. In case of pore diameter reduction, the thickness of the membrane should be reduced accordingly, given the spherical shape of the buoy settled. Indeed the thickness of the membrane and its relation with the size of the active molecules and particles was a challenge more serious than pore milling.

The sensor strategy presented in this paper includes transmembrane detection through a sub-micrometric pore, a process that have been successfully attempted to date with an alpha haemolysin proteic pore in a lipid membrane by Nakane et al. (2004); those authors used a molecular anchor-linked DNA that crossed the pore, reacted trans-membrane with another DNA complementary to its 3′ end forming an hybrid that acted as a block in the pore. We adopted a similar approach with a synthetic membrane, but having the focus of the diagnostic in a strand displacement event, that we found more reliable than the capture event. The device is therefore not dependent on the critical and stochastic bead positioning and transmembrane capture, but by the physically well defined process of displacement and can be interrogated at user will by action on the electromagnet.

In the setup used in this work, the probe-carrying beads exposed a considerable part of their surface on the trans side of the Si_3N_4 membrane in order to interact with particles loaded in the other side, as the nucleic acids linked to the surface were shorter than the thickness of the membrane. Using longer nucleic acids may give limited benefits, as the conditions for maintaining nucleic acid longer than 100 nm in an extended configuration could compromise molecular recognition and interaction. Mixed configurations (extended within the pore, supercoiled outside) have however been recently obtained (Keyser et al., 2006) and could find application in this system. It should be noted, however, that they require very small pores and very long DNA molecules, characteristics that may dramatically affect the reliability and robustness of the device. Moreover, a relatively large pore sustain substantial ionic current even in low salt buffer, thus allowing to tune the salt concentration to the needs dictated by the hybridization or displacement reaction that are critical in governing specificity. High salt concentration may also determine precipitation and clogging of particles.

The use of relatively larger pores is therefore advantageous for the mechanical and electrical properties of the device, although it

introduced difficulties related to the molecular recognition events. In facts, our preliminary approach consisting in the reaction of beads of similar size, larger than the pore, on both membrane sides (i.e. Dynal and Fluosphere) failed for the scarcity of the encounters between the large bodies, due to the adverse influence of the low diffusion coefficients of large particles.

5. Conclusion

While recent research exploited small pores for characterization of molecules during their transit, here we present a new strategy that relies on the stable localization and manipulation of molecules within the sensing pore. Here, the pore is intended as a mechanical amplifier of signals associated to molecular interactions. We made and tested a device that reliably restores high ionic current between two pore-separated compartments when it senses a target DNA, although the optimization of the setup including pore size/bead size/membrane thickness still needs further investigations and trials. By definition, it is an ON/OFF device and, as such, it may find its optimal use as a part of hybrid bio-electronic circuits as a biological/electronic signal converter.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.03.047.

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