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Non-Fluorescence label protein sensing with track-etched nanopore decorated by avidin/biotin system

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ABSTRACT

Single nanopore technology permits to design a biosensor, able to detect proteins without the use of specific probe. In this work, we have fashioned cylindrical nanopore by track-etched technique and atomic layer deposition. The nanopore was then decorated by the asymmetric tethering of biotin. From the rectification of the I–V response induced by the asymmetry of the nanopore, we demonstrate the potentiality of this strategy to detect and to discriminate avidin and streptavidin with respect to their global charge. Based on the nanopore decorated with avidin and streptavidin, we show the possible detection layer by layer of biotinylated protein (IgG and BSA) as well as antibody.

1. Introduction

The development of nanopore technologies have become an important research area over the last two decades as a new way for DNA sequencing [1–4] as well as for biosensing [5,6]. Among the various approaches investigated so far, solid state nanopores are an alternative to the fragile biological ones. Indeed, they present obvious advantages like their stability, easy handling, and up-scaled production [7]. Solid state nanopores can be made of different types of materials such as: polymer [8], silicon based materials [9,10], glass [11], graphene [12,13], and MoS_2 [14]. They are defined by their compositions, aspect ratio, and surface state (typically chemical function). The polymeric nanopores obtained by track-etched methods, offer a large scale of applications regarding their shapes (*i.e.* cylindrical, conical) and the ability to decorate them with suitable functions to design for instance nanovalve pH-dependent or ionic diode [15,16].

Polymeric single nanopores have also been investigated to fashion biosensor for detection using mainly two methods. The first one is the resistive pulse method which permits the detection of a single molecule [17–21]. Basically this method consists to apply a voltage through a nanopore and record the current fluctuations induced by the translocation of macromolecules or nanoparticles. These fluctuations are linked to the macromolecule properties such as, its size, charge, and shape. The second one is based on the modification of the current rectification induced by a charge modification at the entrance of an asymmetric nanopore. It is the case for both, conical nanopores after a modification of their surface charge [22], as well as an asymmetric functionalization of cylindrical nanopores with protein [23]. Based on this concept, biomolecular conjugations inside single synthetic nanopores have been studied extensively due to their interest to fashion a large range of biosensors. Those biomolecular conjugations can be achieved by the immobilization, on the inner wall of the nanopore, of recognition sites for biomolecules such as, aptamer [23], biotin [24–26], Nitrilotriacetic acid (NTA) [27] or antibodies[28]. Multi-step functionalization using proteins have been performed with multipore membranes in order to detect, T4 Polynucleotide Kinase [29] or glucose [30] as well as to evaluate enzymatic reaction under confinement [31]. Beside these two detection methods for sensor applications, chronopotentiometry [32] and impedance [33] have also been reported.

In most previous studies, the functionalization of nanopores is performed directly after the etching process. This way seems the most suitable but it has two limitations. The first one is the difficulty to precisely predict the diameter of the nanopore before chemical etching. The second one is the location of chemically active groups (typically COOH groups for Polyethylene terephthalate (PET)), which are only inside the nanopore, on its surface walls. This permits a selective functionalization inside the nanopore. However if we consider the decoration of a nanopore with protein

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or for the detection of proteins, the unspecific adsorption close to the nanopore entrance could induce interferences. In order to tackle these problems, the nanopore can be modified by atomic layer deposition (ALD) [34]. This method is particularly adapted to fashion nanopore since it permits (i) to coat identically and homogeneously the inside and the outside of the nanopore [35], (ii) to functionalize the surface with chemically active groups such as amine and (iii) to control precisely the nanopore diameter up to 1 nm [36].

In a previous work [37], we proved that this approach is suitable to functionalize nanopore with streptavidin using a long Polyethylene glycol (PEG)-biotin spacer. This nanopore exhibits both, pH-gating and potential sensing properties. In this paper, we have used this approach and investigated the sensing aspects more in details. Different parameters such as (i) the size of the PEG spacer, and (ii) the protein used (avidin and streptavidin) were investigated as a proof of concept for protein detection. In order to work with a well-known system we have selected biotinilated protein (bovine serum albumin (BSA) and immunoglobulin G (IgG)) and antibody (AntiBSA).

2. Materials and method

2.1. Materials

NaCl was purchased from ACROS Organics (99,5%, 207790010). Ultra-pure water was produced from a Q-grad®-1 MilliQ system (Millipore). Poly(Ethylene Terephthalate) (PET) film (thickness 13 μm, biaxial orientation) was purchased from Goodfellow (ES301061). Diethyl zinc (DEZ) (Zn (CH2CH3)2, 95% purity, 557-20-0), Trimethylaluminum (TMA) (Al(CH3)3, 97% purity, 75-24-1), *N*-[3-(Trimethoxysilyl)propyl]ethylenediamine (104884), sodium chloride (S9888), phosphate buffered saline (P4417), MES hemi-sodium salt (M0164), HEPES (H4034), CAPS (C2632) Avidin from egg white (A9275), Streptavidin from Streptomyces avidinii (S0677), Biotinamidohexanoyl-6-aminohexanoic acid N-hydroxysuccinimide ester (B3295) Poly(ethylene glycol) (*N*-hydroxysuccinimide 5-pentanoate) ether 2-(biotinylamino)ethane M_n 3800 (757799), γ-Globulins from bovine blood (G7516), Bovine Serum Albumin (A215), monoclonal Anti-BSA antibody produced in mouse (SAB5300158) were purchased from Sigma Aldrich. Potassium chloride was purchased from VWR (26764.298).

2.2. Buffer preparation

The buffer solutions were made as follow: pH 10 and pH 9 (NaCl 100 mM, CAPS 1 mM); pH 8 and pH 7 (NaCl 100 mM, HEPES 1 mM); pH 6 and pH 5 (NaCl 100 mM, MES 1 mM); pH 4 (NaCl 100 mM, Citrate 1 mM). The final pH of the solutions was adjusted with HCl and NaOH solutions using a pH meter (Hanna HI 221 pH meter, pH electrode HI 1131).

2.3. Current-voltage measurements

I-V curves were recorded using a patch-clamp amplifier (EPC10 HEKA electronics, Germany). Typically current traces were recorded as a function of time under applied voltages from 1 V to −1 V by 100 mV steps during 20 s (sampling rate 2 kHz). Each measurement was repeated three times. The single nanopore is placed between two Teflon chambers containing the same electrolyte solution. The current is measured by Ag/AgCl, 1 M KCl electrodes connected to the cell chamber by agar-agar bridges. One electrode was plugged to the positive end of the amplifier (trans chamber) and the other electrode connected to the ground (cis chamber). Recorded currents were analyzed by Fitmaster (Heka Elektronik, Germany).

2.4. Track-etching nanopore

Single nanopores were tailored by track-etched methods. The single tracks were produced by Xe irradiation (8,98 MeV u^{-1}) of PET film (13 μm) (GANIL, SME line, Caen, France). The activation of track was performed by UV exposition 24 h per side, (Fisher bioblock; VL215.MC, λ = 312 nm) before chemical etching process. The chemical etching of cylindrical nanopores was performed under NaOH solution (3 M, 4–5 min, 55 °C, see Table 1). Then the nanopore was immerged 24 h hours in ultrapure water. The diameter of cylindrical nanopores d was obtained from the dependence of the conductance G with NaCl concentration from 10 mM to 750 mM, assuming bulk-like ionic conductivity inside the nanopores. In order to correct the conductivity at high salt concentration, the diameter is calculated from Eq. (1) using the ionic conductivity of bulk solution κ. The latter have been measured using a conductimeter (Hanna HI 255 combined meter with conductivity and electrode HI 76310) after preparation.

$$G = \frac{\kappa \pi d^2}{4L} \quad (1)$$

where L and d are the length and diameter of the nanopore.

2.5. Functionalization of the nanopores

Atomic layer deposition: The nanopore diameter was reduced by depositing thin Al_2O_3/ZnO films using a custom-made ALD setup. The Al_2O_3 films were deposited by alternating exposures of TMA and deionized water (H2O) with the following cycle times: 0.2 s pulse of TMA, 30 s exposure, and 40 s purge with 100 sccm dry Argon. A 2 s pulse, 30 s exposure, and 60 s purge with dry argon were used for H_2O. The ZnO films were deposited by alternating exposures of DEZ and deionized water with the following cycle times: 0.2 s pulse of DEZ, 30 s exposure, and 40 s purge. Again a 2 s pulse, 30 s exposure, and 60 s purge with dry argon were used for H_2O. ALD was carried out at 60 °C. These pulses, exposure, and

Table 1
Details of studied nanopore.

Pore name	Etching time, at 55 °C (s)	N° of Al_2O_3/ZnO bilayers	Diameter after ALD (nm)	Biotin	1st protein	2nd protein	3rd protein
NP_{24Biot}	304	15	24	– biotin	StreptAvidin	IgG-biotin	–
NP_{11Biot}	276	12	11	– biotin	Avidin	IgG-biotin	–
NP_{22Biot}	324	15	22	– biotin	StreptAvidin	BSA-biotin	–
NP_{18Biot}	300	9	18	– biotin	Avidin	BSA-biotin	Anti-BSA
NP_{16} PEG_{biot}	263	10	16	– PEG – biotin	StreptAvidin	BSA-biotin	Anti-BSA
NP_{22} PEG_{biot}	251	7	22	– PEG – biotin	StreptAvidin	IgG-biotin	–
NP_{26} PEG_{biot}	244	5	26	– PEG – biotin	Avidin	BSA-biotin	Anti-BSA