Electronic Supplementary Material (ESI) for ChemComm.

Bioconjugation-induced ionic current rectification in aptamer-modified single cylindrical nanopores

Mubarak Ali,* Saima Nasir and Wolfgang Ensinger

Technische Universität Darmstadt, Fachgebiet Materialanalytik, Alarich-Weiss-Str. 2, D-64287 Darmstadt, Germany.

Materialforschung, GSI Helmholtzzentrum für Schwerionenforschung, Planckstr. 1, D-64291 Darmstadt, Germany.

Materials and Methods:

Polyethyleneterephthalate (PET) membranes (Hostaphan RN 12, Hoechst) of 12 μm thickness were irradiated at the Helmholtz Centre for Heavy Ion Research (GSI, Darmstadt) with single Au ions (energy: 11 MeV/u). Subsequently, ion tracked PET membranes were further irradiated with soft UV light from each side for 15 minutes in order to sensitize the latent tracks for etching process.

Lysozyme from chicken egg (L6876), cytochrome C from equine heart (C2560), avidin from egg white (A9275), hemoglobin from bovine blood (H2500), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC, 98%,), *N*-hydroxysulfosuccinimide (sulfo-NHS, 98.5+ %) and DNA aptamer 5′-NH_2-$(CH_2)_{12}$-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′ (LyzAp–NH_2) were purchased from Sigma-Aldrich, Taufkirchen, Germany, and used without further purification.

Fabrication of single cylindrical nanopores

The fabrication of cylindrical nanopores in single heavy ion irradiated polymer membranes was achieved via symmetric track-etching technique.[1] For this purpose, the track-etching process was carried out in a double walled isothermal bath, which was half-filled with 2M sodium hydroxide (NaOH) solution. The temperature of etching solution was maintained at 50˚C by a circuit of heated and cooling water flowing through the double walls of the beaker. The ion tracked polymer membranes were first fixed in the sample holders. Then the sample holder with membranes was immersed in the preheated etching bath. The concentration of the solution and the temperature in the vicinity of the ion tracked polymer membranes were kept approximately constant throughout the whole etching process due to constant stirring of the

etchant. The etching of the samples was carried out for 5 minutes. After etching, the track-etched membranes were thoroughly washed with deionized water and then immersed in deionized water overnight for the further removal of the residual salts.

For the case of single cylindrical nanopore the conductance was measured from their linear *I*–*V* curves and the pore diameter was estimated by the following relation.

$$d = \sqrt{\frac{4LI}{\pi\kappa U}}$$

where L is the length of the pore which is almost equal to the membrane thickness (~12 μm), d is the diameter of the pore; κ is the specific conductivity of the electrolyte (11.377 S/m for 1 M KCl at 26 °C), U is the voltage applied across the membrane, and I is value of ionic current obtained from the *I*–*V* curve (Fig. S1).

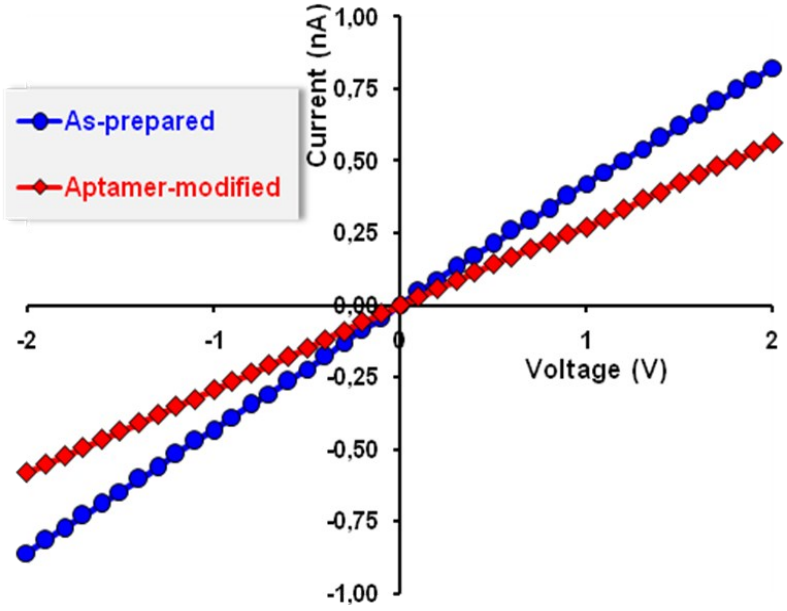

Fig. S1. *I*–*V* curves of a single cylindrical nanopore in 1 M KCl solution obtained before and after the immobilization of DNA aptamer.

Functionalization of LyzAp-amine on nanopore surface:

The solutions used for chemical modification of the channel surface were prepared in 0.1 M MES buffer [2-(*N*-morpholino) ethanesulfonic acid], pH = 5.5. To maintain the structural robustness of the aptamer, the aptamer solution was first heated at 70 °C for ~4–5 minutes and then allowed to cool down at room temperature.[2] This pre-heated aptamer solution was used for the functionalization of the nanopore surface.

The heavy ion irradiation and subsequent chemical etching of ion tracks in polymer membranes resulted in the generation of carboxyl groups on the surface and inner wall of the nanopore. These native carboxylic acid groups were exploited for the immobilization of

aptamer molecules on the pore surface. The aptamer immobilization was carried out in a two-step reaction process: Firstly, the carboxylic acid groups on the pore surface were activated into amine-reactive ester molecules by immersing the track-etched single pore membrane in a solution of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, 100 mM) and *N*-hydroxysulfosuccinimide (sulfo-NHS, 100 mM) prepared in MES buffer: The activation process was carried out for 45 minutes. After activation, the membrane was washed with the same buffer solution. Subsequently, the sulfo-NHS-ester molecules were covalently coupled with the amine group present at the 5′-terminus of the DNA aptamer (LyzAp-NH_2 ; 5 μM) for 30 hours. Finally, the aptamer-modified channel was washed thoroughly with the same buffer solution followed by deionized water.

Current–voltage measurements

The single-pore membrane (as-prepared and aptamer-modified) were characterised by measuring the current–voltage (*I–V*) curves. To this end, the single-pore membrane was clamped between the two halves of the conductivity cell. An electrolyte (0.1M KCl, pH 7.2) prepared in phosphate buffer (10 mM) solution was filled on both sides of the membrane. An Ag/AgCl electrode was placed into each half-cell solution and the ionic current flowing through the single channel membrane was measured with a picoammeter/voltage source (Keithley 6487, Keithley Instruments, Cleveland, OH). *I–V* curves were recorded by applying a scanning triangle voltage signal from -2 to +2 V across the membrane.

Biomolecular recognition and conjugation

The target protein (lysozyme) solutions of different concentrations were prepared in phosphate buffer and were introduced on the right side of the single-pore membrane (separately). The protein was allowed to react with the DNA aptamer for 30 minutes. Then the pore was washed with deionized water to get rid of unbound protein. The selective immobilization of protein on the pore surface was confirmed from the changes *I–V* measurement. For the control experiment, aptamer-modified single pore membrane was first treated with control protein cytochrome C, avidin, bovine hemoglobin followed by lysozyme solution prepared in the same buffer.

References

1. Q. H. Nguyen, M. Ali, V. Bayer, R. Neumann and W. Ensinger, *Nanotechnology*, 2010, **21**, 365701.
2. R. Kirby, E. J. Cho, B. Gehrke, T. Bayer, Y. S. Park, D. P. Neikirk, J. T. McDevitt and A. D. Ellington, *Anal. Chem*., 2004, **76**, 4066-4075.