Supporting Information

ATP-Modulated Ionic Transport through Synthetic Nanochannels

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MATERIALS AND METHODS

Polymer foils of polyethylene terephthalate (PET) (Hostaphan RN 12, Hoechst) of 12 μm thickness were irradiated at the linear accelerator UNILAC (GSI, Darmstadt) with swift heavy ions (Pb, U and Au) having an energy of 11.4 MeV per nucleon. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 98%, Fluka), *N*-hydroxysulfosuccinimide (sulfo-NHS, 98.5+ %, Aldrich) and polyethyleneimine (PEI; Mn ~1200, 50 wt. % in H_2O, Aldrich) were used as received for the chemical modification. Adenosine 5´-triphosphate (ATP, 99+ %, Aldrich) and 1,5-naphthalene disulfonate di-sodium salt (NDS^{2-} ; Aldrich) were purchased from Sigma-Aldrich.

FABRICATION OF CONICAL NANOCHANNELS:

The etching method for the fabrication of conical nanochannels in PET membranes was developed by Apel *et al.*[1] Briefly, the heavy ion-irradiated membrane was placed between the two halves of a conductivity cell in which it served as a dividing wall between the two compartments. An etching solution (9 M NaOH) was added on one side and the other side of the cell was filled with stopping solution (IM HCOOH + 1M KCl). The etching process was carried out at room temperature. During the etching process, a potential of -1 V was applied across the membrane in order to observe the current flowing through it. The current remains zero as long as the channel is not yet etched through. After the break through, the stopping solution on the other side of the membranes neutralizes the etchant. The etching process was stopped when the current was reached at a certain value and the channel was washed first with stopping solution in order to quench the etchant, followed with deionised water. The etched membrane was immersed in deionised water in order to remove the residual salts. After etching, the diameter of the large opening (*D*) of the channel was determined from the bulk etch rate which was 2.13 nm/min for PET in 9M NaOH at room temperature.[1] The diameter of the small opening (*d*) was estimated from its conductivity by the following relation[1],

$$d = 4LI/\pi D\kappa V$$

where L is the length of the channel which could be approximated to the thickness of the membrane, d and D are the small and large opening diameter of the channel respectively, κ is the specific conductivity of the electrolyte (1 M KCl), V is the voltage applied across the membrane and I is the measured current. The conical nanochannels used in our study have a small opening (tip) diameter < 10 nm and the large opening (base) diameter of several hundreds of nanometer.

FABRICATION OF CYLINDRICAL NANOCHANNELS:

Before etching, heavy ion tracked polymer membranes were sensitized with UV light (wavelength 320 nm) for one hour on each side. In order to obtain cylindrical nanochannels, the membranes were etched by suspending them in a double-walled beaker filled with etchant (2 M NaOH) for a preset of time. The temperature of the etching solution was adjusted at 50°C by a circuit of heated water flowing through the double walls of the beaker. After the etching process, the membranes were thoroughly washed with distilled water. For further removal of the residual salts, the membranes were additionally immersed in deionized water overnight.

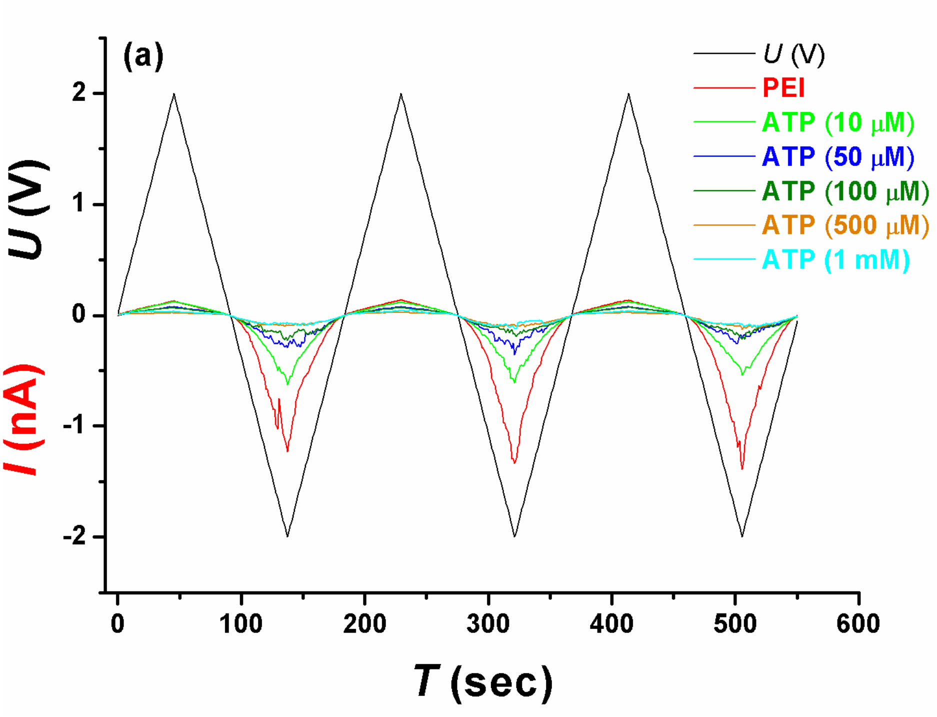
FUNCTIONALIZATION WITH POLYETHYLENEIMINE:

The solutions used for chemical modification of the channel surface were prepared in 0.1M MES buffer [2-(*N*-morpholino) ethanesulfonic acid], pH = 5.5. Functionalization of nanochannel walls with PEI was carried out in the same conductivity cell used for etching of tracked polymer foils. The carboxylic (-COOH) groups generated on the channel surface during the track-etching process were functionalized with PEI by the following procedure. The carboxyl groups were first activated into sulfo-NHS-esters by using a solution of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC, 10 mM) and *N*-hydroxysulfosuccinimide (sulfo-NHS, 20 mM) for 30 minutes. After activation, foil was washed with the same buffer solution. Subsequently, the amine-reactive sulfo-NHS-esters were covalently coupled with the amino group present on the backbone of PEI (2mg / ml) for overnight. Finally, the modified channel was washed with buffer solution thoroughly. Same procedure was used for the chemical modification of multi-channel membranes.

CURRENT-VOLTAGE MEASUREMENTS:

The membrane containing the single conically nanochannel was mounted between the two halves of the conductivity cell, and both half of the cell were filled with 10mM phosphate buffer (pH = 6.5) prepared in 0.1M KCl solution. A Ag/AgCl electrode was placed into each half-cell solution, and the Keithley 6487 picoammeter/voltage source (Keithley Instruments, Cleveland, OH) was used to apply the desired transmembrane potential in order to measure the resulting ion current flowing through the nanochannel by applying a scanning triangle voltage from -1V to +1V on the tip side while the base side of the channel remain connected to the ground electrode.

The various concentrations of Adenosine 5′-triphosphate prepared in the same electrolyte solution were used for the measurement of respective *I*-*V* curve.



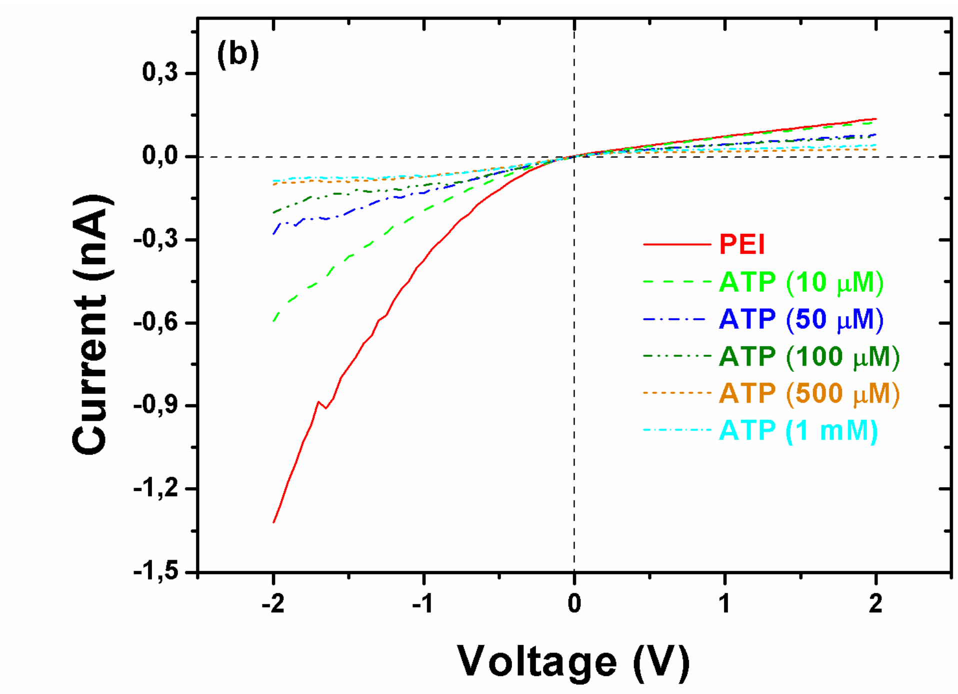


Figure S1: *(a) Applied voltage and measured current signals with respect to time and (b) the corresponding current-voltage (I-V) characteristics of a single conical nanochannel functionalized with PEI, prior to (red) and after the co-addition of various concentrations of hydrogen peroxide in electrolyte solution.*

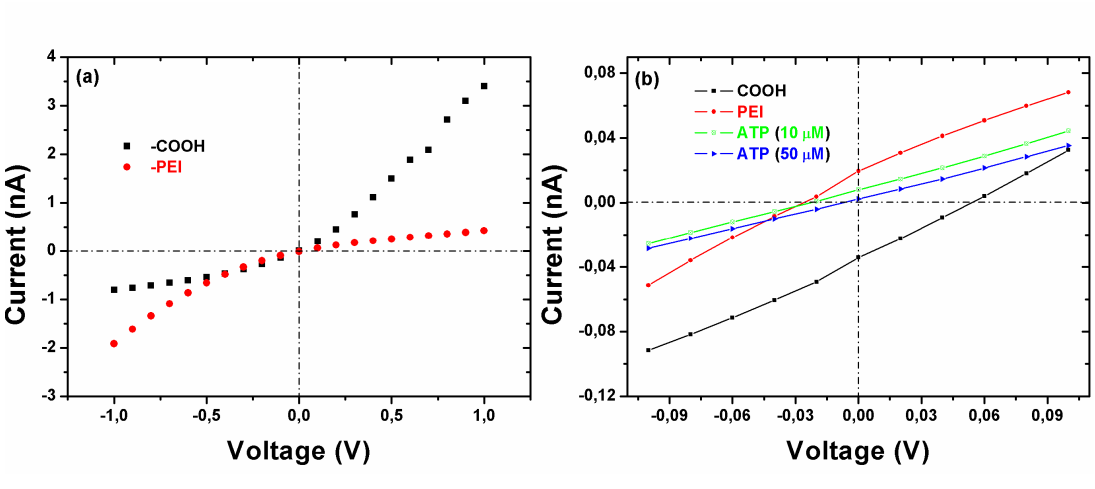


Figure S2: (a) *Current–voltage (I–V) curves corresponding to a single conical nanochannel prioe to and after modification with PEI using 0.1 M KCl as an electrolyte solution. The dimensions of the channel were tip d ~28 nm and base opening D ~270 nm* ***(b)*** *The sections of I–V curves recorded under a tenfold concentration gradient with 0.1 M KCl on the base and 0.01 M KCl on the tip opening, prior to (black line with squares) and after modification (red line with circles). Further, the I–V curves recorded under the same electrolyte condition (0.1 M KCl on base and 0.01 M KCl on tip side) with the addition of 10 µM (green line with crossed circles) and 50 µM ATP concentrations.*

MASS-TRANSPORT EXPERIMENT:

The modified membranes were used for the selective diffusion of doubly charged organic dye molecules 1,5-disulfonate naphthalene (NDS^{2-}). The analyte molecules were prepared in solutions buffered at pH = 6.5 with phosphate buffer saline. For the transport experiments, PEI-modified multi-channel membranes were mounted between the two halves of the conductivity cell. Each cell volume was 3.4 ml with an effective permeation area of the membrane of 1.15 cm^2. The feed half-cell contained a known concentration of an analyte in the buffer solution, whereas the permeate half-cell was filled with pure buffer solution. The concentration of NDS^{2-} in the feed half-cell was 10 mM. Both solutions were continuously stirred during the whole process. After a preset time, the concentration of analyte in the permeate half-cell was determined by measuring the UV absorbance with a UNICAM UV/VIS spectrometer.

References

1. P. Y. Apel, Y. E. Korchev, Z. Siwy, R. Spohr and M. Yoshida, *Nucl. Instrum. Methods Phys. Res. B*, 2001, **184**, 337-346.