

## Silicatein conjugation inside nanoconfined geometries through immobilized NTA–Ni(II) chelates

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### Materials and Methods:

Polyethyleneterephthalate (PET) membranes (Hostaphan RN 12, Hoechst) of 12  $\mu\text{m}$  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 membrane were further irradiated with soft UV light from each side for 15 minutes in order to sensitize the latent tracks for etching process. The UV lamp used for irradiation of the polymer membranes is homemade and equipped with T-30M UV-b fluorescent tubes which were purchased from Vilber-Lourmat, Germany. The power consumption of the tube is 30 W and gives light of wavelength 312 nm (280-380 nm) as shown in the spectrum curve of UV tube.<sup>1</sup>

*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 98%), *N*-hydroxysulfosuccinimide (sulfo-NHS, 98.5+ %) and *N*<sub>ω</sub>*N*<sub>α</sub>-Bis(carboxymethyl)-L-Lysine hydrate (NTA-NH<sub>2</sub>, 97%) were purchased from Sigma-Aldrich, Taufkirchen, Germany, and used without further purification.

### **Fabrication of single conical nanopores**

An asymmetric track-etching technique developed by Apel *et al* is used to fabricate the conical nanopores in heavy ion tracked PET membranes.<sup>2</sup> A custom-made conductivity cell having three chambers is used for the fabrication of single conical and arrays of conical nanopores at the same time. A single-shot membrane is placed in between the two chamber of the conductivity cell and clamped tight. The one chamber was filled with aqueous chemical etchant (9 M NaOH), while the other chamber filled with a stopping solution (1 M KCl + 1 M HCOOH). The etching process was carried out at room temperature. During the etching process, a voltage of -1 V was applied across the membrane in order to observe the current flowing through the nascent nanopore. The current remains zero as long as the pore is not yet etched through, and after the breakthrough an increase in ionic current is observed. The etching process is stopped when the current was reached at a certain value and the pore is washed first with stopping solution in order to neutralize the etchant, followed by with deionized water. The etched membranes are further immersed in deionized water for overnight in order to remove the residual salts.

After etching, the diameter of the large opening ( $D$ ) of the channel was determined by field emission scanning electron microscopy (FESEM). For this purpose a PET sample containing  $10^7$  channels  $\text{cm}^{-2}$  was etched simultaneously with the single channel under the same conditions. The diameter of the small opening ( $d$ ) was estimated by assuming the conical geometry of the channel from its conductivity using the following relation<sup>1</sup>

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

where  $L$  is the length of the pore which could be approximated to the thickness of the membrane,  $d$  and  $D$  are the small and large opening diameter of the channel respectively,  $\kappa$  is

the specific conductivity of the electrolyte (1.313 S/m for 0.1 M KCl at 26 °C),  $U$  is the voltage applied across the membrane and  $I$  is the measured current.

### **Functionalization of NTA-amine on nanopore surface:**

The above-described etching procedure results in the generation of carboxyl groups on the surface and inner wall of the nanopore. 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. Functionalization of the nanopore surface and inner walls with NTA molecules was carried out in the same conductivity cell used for the chemical etching of tracked polymer membranes. The carboxyl groups were first activated into amine-reactive ester molecules 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, the foil was washed with the same buffer solution. Subsequently, the sulfo-NHS-ester molecules were covalently coupled with the amine group present at the N-terminus of the NTA (1 mg/ml prepared in 5 mM NaHCO<sub>3</sub> aqueous solution) for overnight. Finally, the NTA-modified channel was washed thoroughly with NaHCO<sub>3</sub> solution.

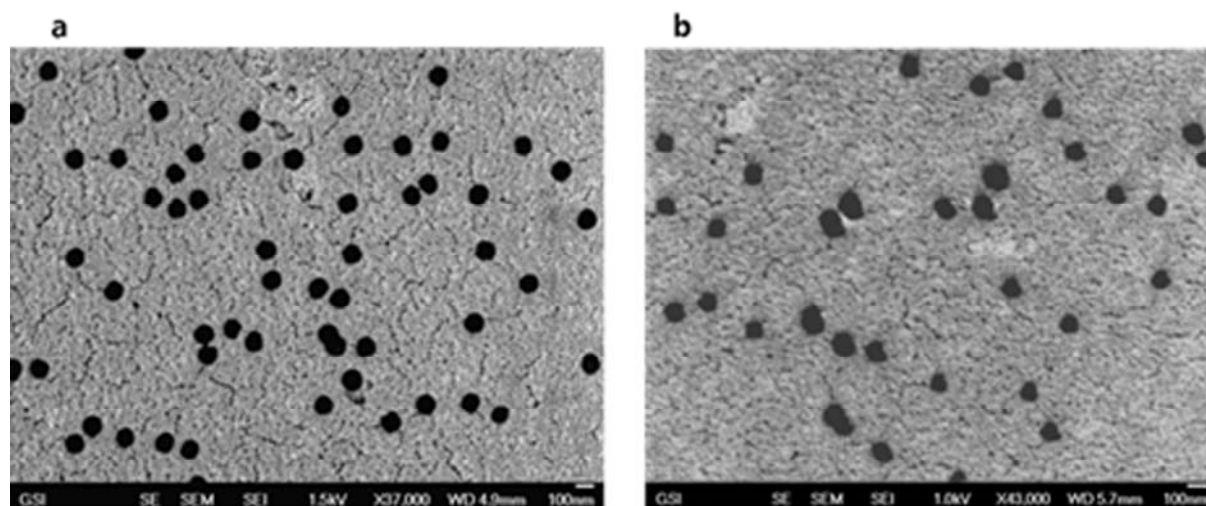
### **Current–voltage measurements**

The single-pore membrane was characterised by measuring the  $I$ - $V$  curves. To this end, the membrane was clamped between the two halves of the conductivity cell. An electrolyte (0.1M KCl,) 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). The

ground electrode was placed on the base opening side of the conical nanochannel and the  $I-V$  curves were recorded by applying a scanning triangle voltage signal from -1 to +1 V across the membrane.

### NTA-Ni(II) complexation and Silicatein immobilization

A solution of  $\text{NiCl}_2$  (50 mM) was prepared in deionised water. The pH of the  $\text{NiCl}_2$  solution was adjusted to 10 with dilute NaOH. A polymer membrane containing a single NTA-modified pore was mounted in the conductivity cell. In order to achieve NTA-Ni(II) chelates, the modified pore was exposed to  $\text{NiCl}_2$  solution for four hours at room temperature. After washing with NaCl solution, the  $I-V$  curves were measured using 0.1 M KCl prepared in PBS (pH = 7.6) as an electrolyte for the examination of successful NTA-Ni(II) complexation. Then silicatein solution (30 nmol) was introduced on both sides of the single-pore membrane and allowed to react with the NTA-Ni(II) chelates for two hour. Then the pore was washed with deionized water to get rid of unbound protein. The immobilization of silicatein on the pore surface was confirmed from the  $I-V$  measurement.



**Fig. S1.** FESEM micrographs of track-etched polymer membranes containing nanopore arrays with (a) and without (b) UV treatment before the etching process.

## References

1. [http://www.vilber.com/products/download/uv\\_instruments.pdf](http://www.vilber.com/products/download/uv_instruments.pdf)
2. P. Y. Apel, Y. E. Korchev, Z. Siwy, R. Spohr and M. Yoshida, *Nucl. Instrum. Methods Phys. Res., Sect. B*, 2001, **184**, 337-346.