Electronic Supplementary Material (ESI) for Chemical Communications

Tuning nanopore surface polarity and rectification properties through enzymatic hydrolysis inside nanoconfined geometries

Mubarak Ali,[a,b]* Saima Nasir,[a,b] Ishtiaq Ahmed,[c] Ljiljana Fruk,[c] and Wolfgang Ensinger[b]

[a] *Technische Universität Darmstadt, Fachgebiet Materialanalytik, Petersenstr. 23, D-64287 Darmstadt, Germany.*

[b] *Matrialforschung, GSI Helmholtzzentrum für Schwerionenforschung, Planckstr. 1, D-64291 Darmstadt, Germany.*

[c] *Karlsruher Institute of Technology, DFG-Center for Functional Nanostructures, Wolfgang-Gaede-Str. 1, D-76131 Karlsruhe, Germany.*

Address correspondence to: m.ali@gsi.de

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 Au ions (energy: 11 MeV/u; ion fluence is either single or 5×10^7 ions cm^{-2}). Subsequently, ion tracked PET membrane were further treated with UV light (50 W m^2) from each side for 15 minutes in order to sensitize the latent tracks for etching process. The UV lamp used for irradiation of polymer membranes is homemade and equipped with T-30M UV-b fluorescent tubes which purchased from Vilber-Lourmat, Germany. The power consumption of the tube is 30W and gives light of wavelength 312 nm (280-380nm) as shown in the spectrum curve of UV tube.[1]

N-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 98%,), pentafluorophenol (PFP, 99+ %,), *N*-*t*-Butoxycarbonyl-6-aminohexanoic acid (99%), 2-chloro-*N*,*N*-dimethylethanamine (99%), ethyl acetate (99.8%), iodomethane (≥99.0%), acetone (99.9%) and glycine p-nitroanilide, acetylcholinesterase from Electrophorus electricus and protease from Bacillus licheniformis were purchased from Sigma-Aldrich, Taufkirchen, Germany, and used without further purification. The solution ^{1}H-NMR spectra were measured on a Bruker Spectrospin 300 (300 MHz).

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.[2] A custom-made conductivity cell with three chambers is used for the fabrication of single conical nanopore and arrays of conical nanopores at the same time. A single-shot membrane and a membrane irradiated with 10^7 ions/cm^2 are placed on both sides of the middle chamber of the conductivity cell and clamped tight. The middle chamber, having holes on both sides, is filled with the aqueous chemical etchant (9 M NaOH) while the other two chambers are filled with a stopping solution (1 M KCl + 1 M HCOOH). The etching process is carried out at room temperature. During the etching process, a voltage of -1 V is 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 has reached a certain value. The pore is then 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

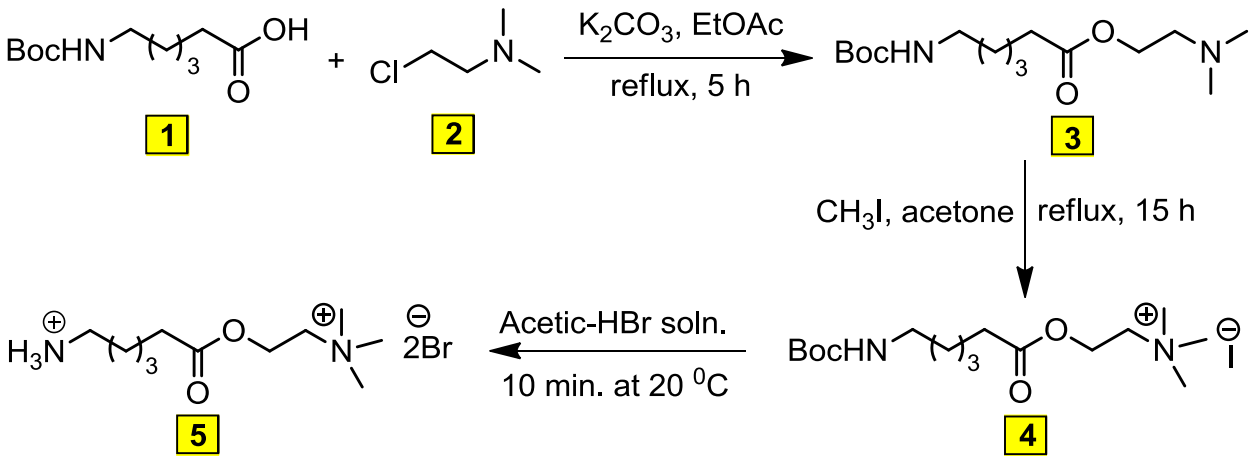
After etching, the diameter of the large opening (*D*) of the channel was determined by field emission scanning electron microscopy (FESEM). For this purpose a multipore membrane is etched simultaneously with the single channel under the same conditions. The diameter of the small opening (*d*) was estimated by assuming the conical geometry of the channel from its conductivity using the following relation[1]

$$d = \frac{4LI}{\pi\kappa UD}$$

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, κ is the specific conductivity of the electrolyte (1.313 S/m for 0.1 M KCl at 26 ^{o}C), *U* is the voltage applied across the membrane and *I* is the measured current.

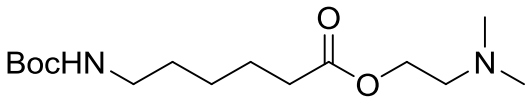
Synthesis of *O*-(6-aminohexanoyl)-choline bromide hydrobromide (5)

The chemical compound *O*-(6-aminohexanoyl)-choline bromide hydrobromide (**5**) which acted as possible AChE substrate, was synthesized according to the procedure reported by Frank *et al.* (scheme 1).[3]



Scheme S1. Chemical reaction scheme for the synthesis of *O*-(6-aminohexanoyl)-choline bromide hydrobromide (**5**)

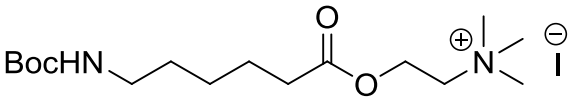
Synthesis of *N*-Dimethyl-*O*-*(N*-*t*-butoxycarbonyl-6-aminohexanoyl*)*-colamine (3)



3

To a solution of *N*-*t*-Butoxycarbonyl-6-aminohexanoic acid (**1**) (1 g, 4.32 mmol) and 2-chloro-*N*,*N*-dimethylethanamine (**2**) (freshly recrystallized from ethanol) (2.49 g (23.27 mmol) in 50 ml ethyl acetate were added finely ground, anhydrous K_2CO_3 (1.20 g, 8.69 mmol). The reaction mixture was refluxed for 6 h. After cooling to room temperature, the insoluble material was removed by filtration and the filtrate was diluted with ethyl acetate and extracted with $NaHCO_3$ and saturated aq. NaC1-solution. The organic layer was concentrated under reduced pressure to yield *N*-dimethyl-*O*-*(N*-*t*-butoxycarbonyl-6-aminohexanoyl*)*-colamine (**3**) **(**1.20 g, 3.97 mmol, 92%**).** TLC analysis showed that the product was pure enough to be used for next step without further purification. It was not further identified because the ester linkage was quite labile upon storage and was used directly in the next step. TLC: 0.45 ($CHC1_3$/MeOH 4: 1).

Synthesis of *O*-*(N*-*t*-Butoxycarbonyl-6-aminohexanoyl)-choline iodide (4)



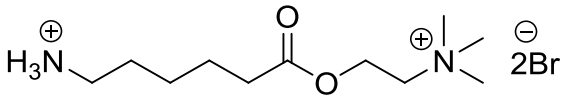
4

To a solution of crude *N*-dimethyl-*O*-*(N*-*t*-butoxycarbonyl-6-aminohexanoyl*)*-colamine (**3**) (1 g, 3.31 mmol) in acetone (30 ml) was added CH_3I (515 mg, 3.46 mmol). The reaction mixture was refluxed for 15 h. After cooling to room temperature, the slight amount of insoluble material was removed by filtration and the clear filtrate was concentrated under reduced pressure to yield yellow, oily residue. The yellow residue was dissolved in water and extracted with ethyl acetate

to remove the impurities. The extraction process was repeated until the aqueous phase became colourless. The colourless aqueous phase was lyophilized and the residue was recrystallized from 2-propanol/diisopropy1 ether to yield colourless crystals of *O*-(*N*-*t*-butoxycarbonyl-6-aminohexanoyl)-choline iodide (**4**) (1.2 g, 2.71 mmol, 82%), m.p. 96 ^{0}C. TLC.: 0.21 ($CHC1_3$/MeOH 4: 1).

^{1}H-NMR (300 MHz, DMSO-d_6): δ ppm = 1.39 (15 H, Boc$(CH_3)_3$, $(CH_2)_3$), 2.34 (2 H, CH_2COO), 2.92 (2 H, CH_2N), 3.19 (9 H, $(NCH_3)_3{}^{+}I^{-}$), 3.74 (2 H, $NHCH_2$), 4.49 (2 H, $COOCH_2$), 6.71(1 H, NH).

Synthesis of *O*-(6-aminohexanoyl)-choline bromide hydrobromide (5)



5

O-(*N*-*t*-Butoxycarbonyl-6-aminohexanoyl)-choline iodide (**4**) (1 g, 2.25 mmol) was dissolved in dilute acetic HBr-solution. The reaction mixture was stirred for 15 min at 20 ^{0}C. Ether was added to precipitate the crude product **5** as an oily residue. This was dissolved in water, extracted with $CHC1_3$ and ethyl acetate to remove impurities. The aqueous phase was lyophilized to give slight yellow rresidue. The residue was dissolved in 2-propanol/MeOH/ 5:1, and treated with a few drops of diisopropyl ether. After 24 h the colourless crystals of crude *O*-(6-aminohexanoyl)-choline bromide hydrobromide (**5**) were isolated (766 mg, 2.02 mmol, 93%), m.p. 129-131 ^{0}C. TLC.: 0.16 ($BuOH/AcOH/H_20$ 5:2:3).

^{1}H-NMR (300 MHz, DMSO-d_6): δ ppm = 1.47 (6 H, $(CH_2)_3$), 2.33 (2 H, CH_2COO), 2.72 (2 H, $NH_3{}^{+}CH_2$), 3.21 (9 H, $(NCH_3)_3{}^{+}2Br^{-}$), 3.76 (2 H, CH_2N), 4.47 (2 H, $COOCH_2$), 7.04(3 H, $^{+}$NH3).

Functionalization of nanopore surface

The carboxylic acid groups generated during chemical etching process are first converted into amine-reactive pentafluorophenyl esters via coupling chemistry by using pentafluorophenol (PFP) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC). For activation, the track-etched membranes are immersed in an ethanolic solution containing a mixture of EDC (100 mM) and PFP (200 mM) at room temperature for one hour. The activated membrane is washed with ethanol several times. Then the single-pore membrane is further dipped in a solution of *O*-(6-aminohexanoyl)-choline bromide hydrobromide (50 mM) prepared in anhydrous ethanol and left for overnight. During this reaction period, reactive PFP-esters are covalently coupled with terminal amine group of *O*-(6-aminohexanoyl)-choline bromide hydrobromide. Then, the modified membranes are washed thoroughly first with ethanol followed by careful rinsing with deionized water.

For the immobilization of protease substrate glycine p-nitroanilide (50 mM) is dissolved in a mixture of ethanol and acetone (C_2H_5OH/CH_3COCH_3, 6:4 by volume). Then the PFP-activated single-pore membrane was immersed in the glycine p-nitroanilide solution for overnight.

Current–voltage measurements

The single-pore membrane is characterized by measuring the *I–V* curves. To this end, the membrane is clamped between the two halves of the conductivity cell. An electrolyte (0.1M KCl,) prepared in a phosphate buffer (10 mM, pH = 7.2) solution is filled on both sides of the membrane. An Ag/AgCl electrode is placed into each half-cell solution and the ionic current flowing through the single pore membrane is measured with a picoammeter/voltage source (Keithley 6487, Keithley Instruments, Cleveland, OH). The ground electrode is placed on the

base opening side of the conical pore and the *I–V* curves are recorded by applying a scanning triangle voltage signal from -2 to +2 V across the membrane.

References

1. Lab & UV Instruments at http://www.vilber.com

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.

3. J. Frank, V. M. Kriwaczek, C. Marchand and R. Schwyzer, *Helv. Chim. Acta*, 1977, **60**, 2550.