Supporting information

MATERIAL AND METHODS

Material

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) ($Zn(CH_2CH_3)_2$, 95% purity, CAS: 557-20-0), Trimethylaluminum (TMA) ($Al(CH_3)_3$, 97% purity, CAS: 75-24-1), sodium chloride (S9888), Phosphate buffered saline (P4417), MES hemisodium 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 Mn = 3800 g mol^{-1} (757799), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (03449), Poly(ethylene glycol) 2-aminoethyl ether biotin Mn = 5300 g mol^{-1} (757772), γ-Globulins from bovine blood (G7516), and N-[3-(Trimethoxysilyl)propyl]ethylenediamine **(104884)** were purchased from Sigma Aldrich. Potassium chloride was purchased from VWR (26764.298).

Buffer preparation

The solutions for the pH response measurements were made as follow: pH10 and 9 (NaCl 100 mM, CAPS 1 mM); pH8 and pH7 (NaCl 100 mM, HEPES 1 mM); pH6 and pH 5 (NaCl 100 mM, MES 1 mM); pH4 (NaCl 100 mM, Citrate 1 mM). The final pH of the solutions was

adjusted with HCl and NaOH solutions using pH meter (Hanna HI 221 pH meter, pH electrode HI 1131).

Current-voltage measurement

I-V curves were performed using a patch-clamp amplifier (EPC10 HEKA electronics, Germany). Typically trace currents were recorded as a function of time under applied voltages from 1V to -1V by 200 mV step. Each measurement was repeated three times. The single nanopore is sandwiched between two Teflon chambers containing the same electrolyte solution. The current is measured by Ag/AgCl, 1M KCl electrodes connected to the cell chamber by agar-agar bridges. Data has been recorded at 2 kHz using Patchmaster software (Heka Elektronik, Germany). Recorded currents were analyzed by Fitmaster (Heka Elektronik, Germany). One electrode was plugged to the positive end of the amplifier (trans chamber) and the other electrode connected to the ground (cis chamber).

Confocal fluorescence spectroscopy

Avidin localization in nanopore membranes was investigated on multiporous membranes (10^5 pore cm^{-2}) and flat PET surface by confocal fluorescence spectroscopy using the set-up described elsewhere.[1] In order to detect avidin, the latter was previously labeled using alexa fluor 594 succinimidyl ester kit (InvitroGen, A30008). Typically, 500 µL of protein solution was added to dry fluorophore Alexa-594 in molar ratio 1:1 and allowed to react for 0.5 h at 20 °C. Then the unreacted Alexa and avidin was separated by centrifugation (16 000 g, 1 min) using a filter (Biospin P6) according to the supplier of the kit. The labeling ratio [alexa]/[protein] (0.3) was determined from the UV-Visible spectrometry.

Track-etching nanopore

Single nanopores were tailored by track-etched method described elsewhere.[2] 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 nanopore was performed under NaOH solution (3 M, ~4 min, 55°C). Then the nanopore was immerged 24h hours under ultrapure water. The etching of conical nanopore was performed under dissymmetric condition. The PET foil is mounted in a chemical cell in Teflon. On one side of the cell, there is the etchant solution: 9 M NaOH, and on the other side the stopping solution: 1 M KCl and 1M of acetic acid. The time is recorded as soon as the etching solution is inserted. A 1V potential is applied across the membrane, the reference electrode is in the stopping solution and the active electrode is in the etchant. When the current rises to a hundred of pA, there is a breakthrough. To stop the etching process, the NaOH solution is replaced by the stopping one.

The diameter of cylindrical nanopores *d* was obtained from the dependence of the conductance *G* with NaCl concentration, assuming bulk-like ionic conductivity inside the nanopores. In order to correct the conductivity at high salt concentration, the diameter is calculated from equation 1 using the ionic conductivity of solution κ. The latter have been measured using a conductimeter (Hanna HI 255 combined meter with conductivity and electrode HI 76310) after preparation.

$$G = \kappa \pi d^2/4L \quad (1)$$

Where *L* is the nanopore length

The tip diameter d_t of conical nanopore is also estimate from conductance using equation 2

$$G = \kappa \pi d_b d_t / L \quad (2)$$

Where d_b is calculate from total etch time t using relationship $d_b = 2.5t$. The factor 2.5 was determinate at lab using mutlipore membrane track. This factor is close to 2.13 usually find in literrature

Nanopores functionalization

PEG-biotin-NH_2 linkage in native nanopore (conical and cylindrical): Poly(ethylene glycol) 2-aminoethyl ether biotin (PEG) was attached using a one-step EDC modification process. A solution of 10 mg mL^{-1} PEG, 0.1M of KCl, 1mM HEPES at pH 7.1 and 3.8 mg mL^{-1} EDC is placed in a reservoir of the cis side of the electrochemical cell and is allowed to sit for 24 hours. After the 24 hour period the chemical cell is rinsed with water at least three times and time average of the current is taken from -1V to +1V.

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 (H_2O) with the following cycle times: 0.2 s pulse (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 (H_2O) with the following cycle times: 0.2 s pulse (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 purge times were chosen conservatively to ensure completion of the ALD surface reactions and to prevent mixing of the reactive gases. The growth per cycle was about 2 Å/cycle for

Al_2O_3 and 2.1 Å/cycle for ZnO. A sequence of five cycles of Al_2O_3 preceding five cycles of ZnO was used to reduce the pore diameter. After ALD deposition, a 48 h N-[3-(Trimethoxysilyl)propyl]ethylenediamine vapour exposure treatment was performed under vacuum to create a pore surface with a $–NH_2$ terminal. The nanopores modification was approved by XPS measurement (ESCALAB 250 Thermo Electron).

PEG-biotin-NSH linkage in amine modified nanopore : A 0.5mg of Poly(ethylene glycol) (N-hydroxysuccinimide 5-pentanoate) ether 2-(biotinylamino)ethane was added in 1mL of a solution H_2O/MeOH 95/5. 50µL of that solution were added in the cis side of the cell in a 1.8 mL PBS solution (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C.), for 1h.

Avidin or Streptavidin insertion : 100µL of StreptAvidin (concentration) were added, in the cis side of the cell, in 1.8mL of NaCl (100mM) and HEPES (1mM) at pH7, under an applied voltage of 100mV, for 30min. This modus operandi was applied for all the pores.

Biotinylation of γ globulin : 1 mL of protein in PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 C.) was added to Poly(ethylene glycol) (N-hydroxysuccinimide 5-pentanoate) ether 2-(biotinylamino)ethane dry fluorophore Alexa-594 in molar ratio 1:1 and allowed to react for 0.5 h at 20 °C. Then the unreacted biotin-PEG-NHS was separated by centrifugation (16 000 g, 1 min) using a filter (Biospin P6)

Biotin-PEG-γ -globulin insertion : 100µL of **Biotin-PEG-** γ -globulin were added, in the cis side of the cell, in 1.8mL of NaCl (100mM) and HEPES (1mM) at pH7, under an applied voltage of 100mV, for 1h.

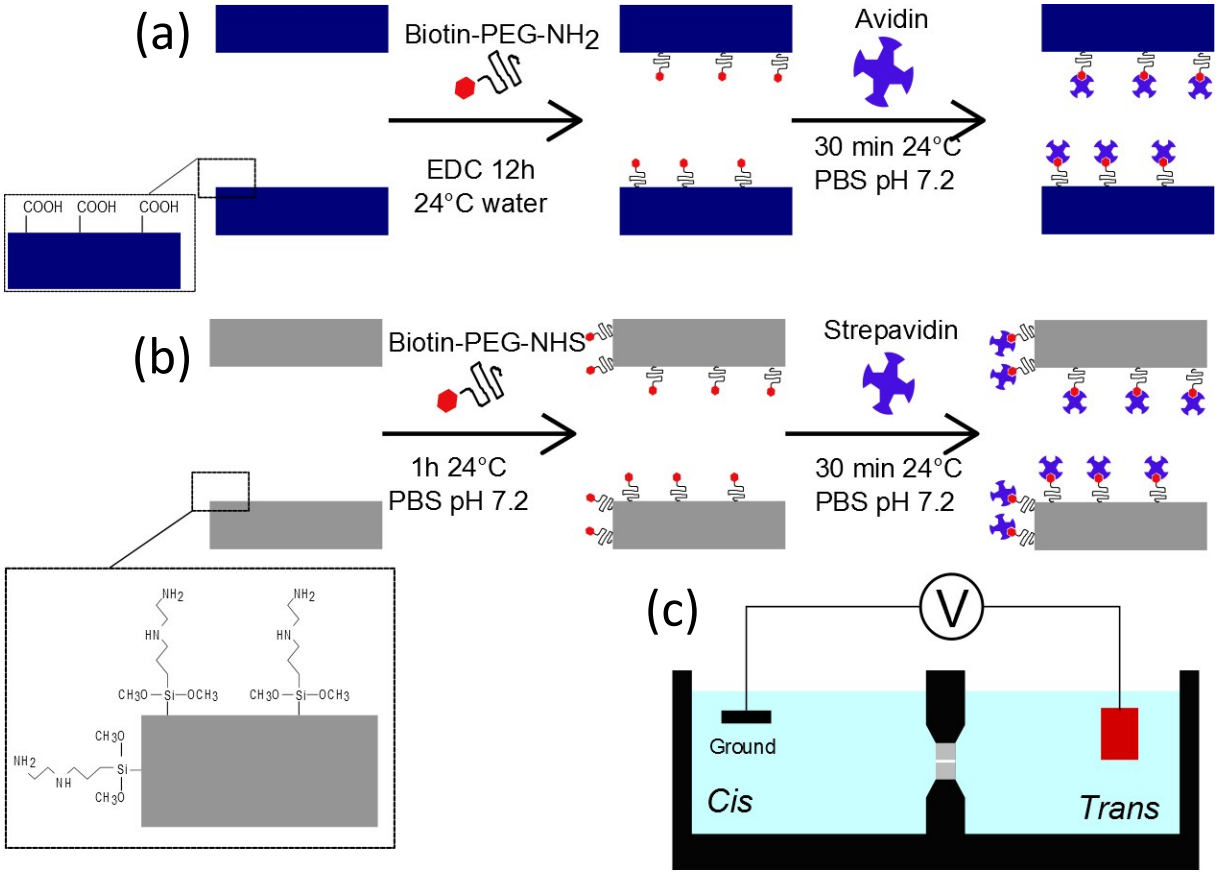

Figure SI-1. *Schematic representation of a nanopore functionalized directly after chemical etching (a) and the nanopore functionalized after the diameter was adjusted by ALD and the surface was functionalized with amine groups (b); Schematic representation of the experimental setup used to apply a voltage across the nanopore (c).*

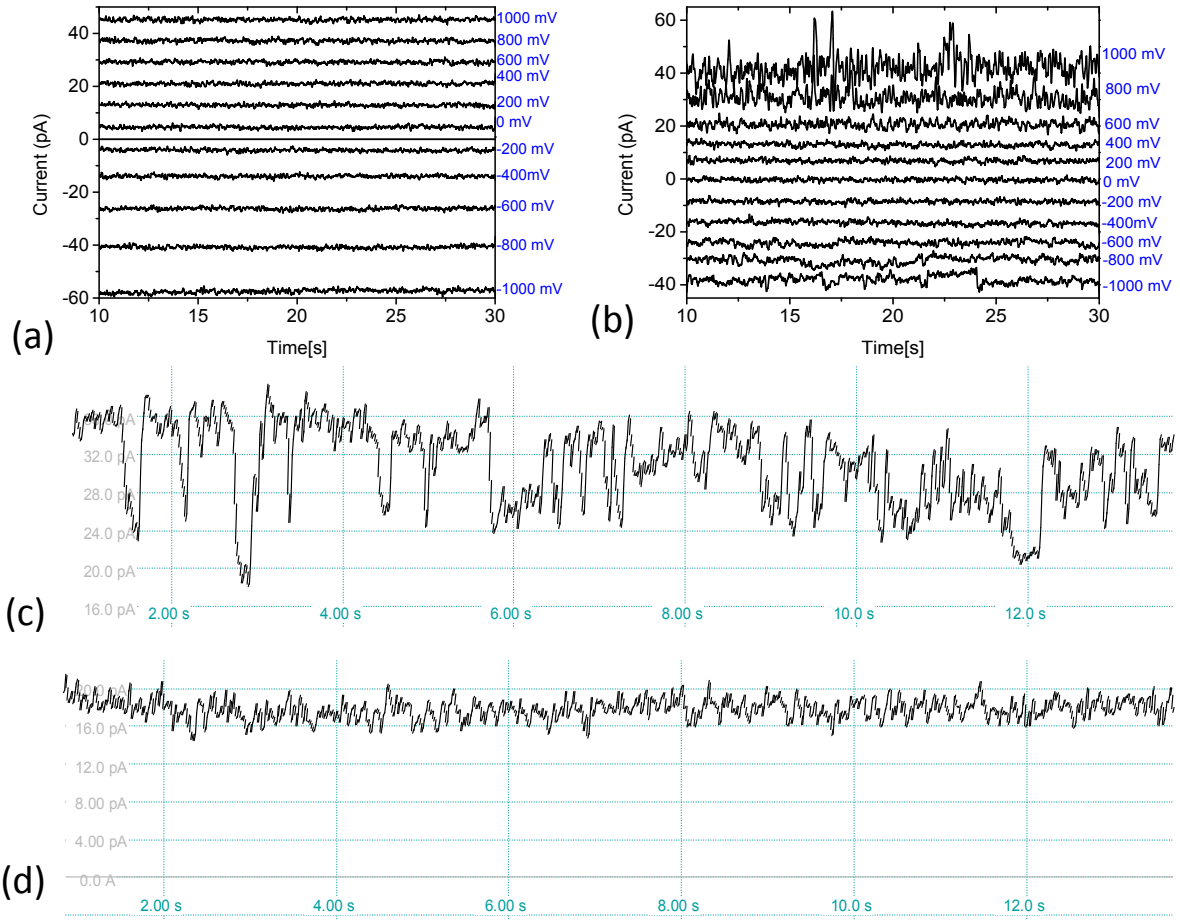

Figure SI-2. *Current trace of cylindrical nanopore (diameter 18 nm, length 13 micron) after chemical etching (a) and after functionalization by PEG-avidin (b). (c) and (d) current trace record after avidin linkage under 950 mV and 700 mV respectively*

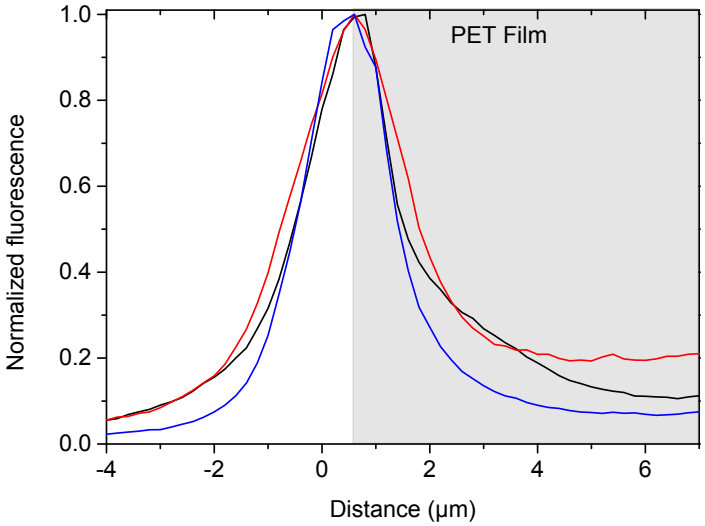

Figure SI-3. *Characterization of the labeled avidin by confocal fluorescence spectroscopy inside multi pore membrane previously functionalized by PEG-biotin (red line), unmodified (black line) and on PET film without pore (blue line).*

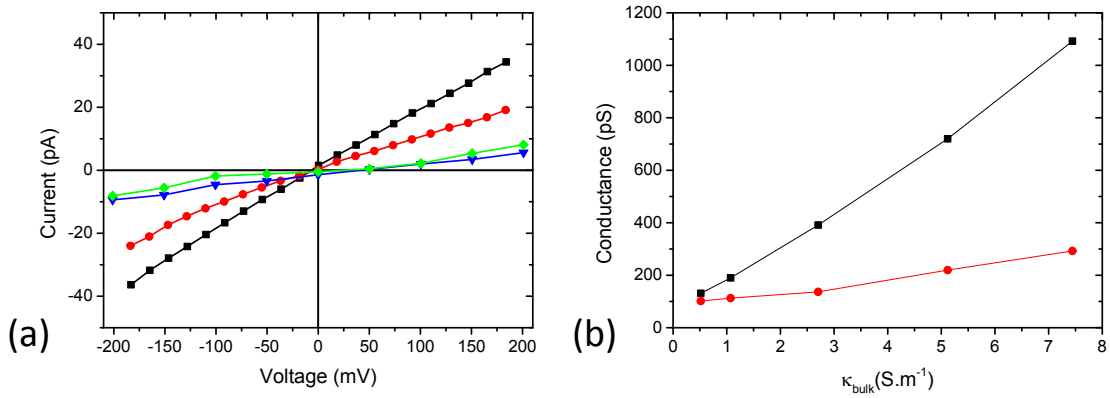

Figure SI-4. *(a) Example of I-V curve (here under 100 mM NaCl pH 7) of nanopore at each step, directly after chemical etching (black), after ALD (red), and after PEG-streptavidin grafting (blue), and after γ -globulin insertion (green) (b) conductance/salt conductivity dependence of cylindrical nanopore, directly after chemical etching (black), and after ALD (red)*

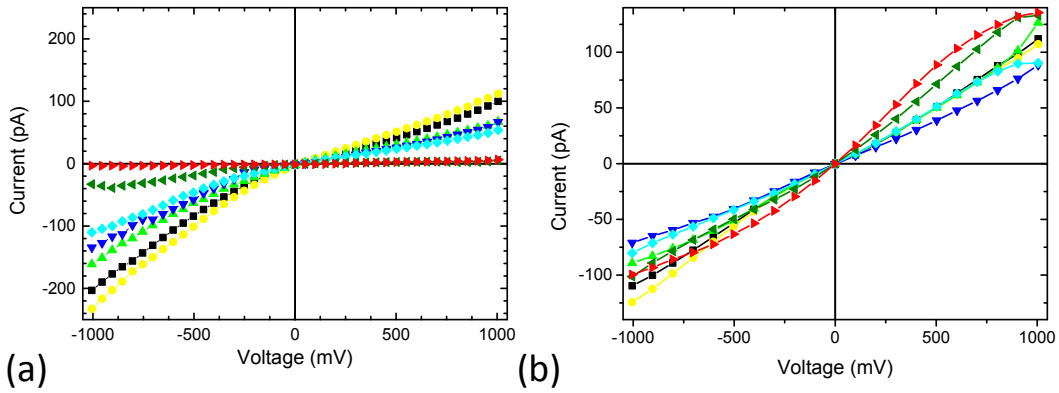

Figure SI-5. *(a) Example of I-V curve (here under 100 mM NaCl) of nanopore after PEG-biotin-streptavidin grafting at different pH, pH10 (black), pH9 (yellow), pH8 (green), pH7 (blue), pH6 (cyan), pH5 (olive) and pH4 (red) (b) Example of I-V curve (here under 100mM NaCl) of nanopore after biotin-streptavidin grafting at different pH, pH10 (black), pH9 (yellow), pH8 (green), pH7 (blue), pH6 (cyan), pH5 (olive) and pH4 (red)*

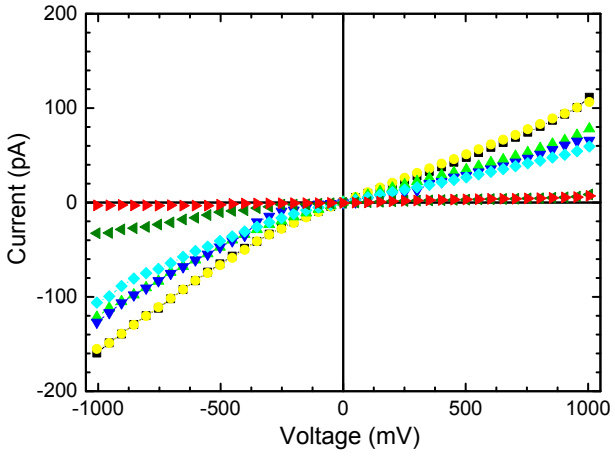

Figure SI-6. *Example of I-V curve (here under 100 mM NaCl) of nanopore after γ -globulin insertion on a PEG-streptavidin at different pH, pH10 (black), pH9 (yellow), pH8 (green), pH7 (blue), pH6 (cyan), pH5 (olive) and pH4 (red)*

XPS of atomic layer deposition (ALD) nanopore functionalized by amine groups

The success of the grafting has been attested by XPS measurement (Table SI6). XPS measurement on PET membrane with 3 bilayers of 5 cycle Al_2O_3 / 5 cycle ZnO has been performed before (Table SI6a) and after (Table SI6b) N-[3-(Trimethoxysilyl)propyl]ethylenediamine treatment in order to analyze the effect of the amine groups treatment on the surface properties. In addition to the Al, Zn, C and O element detected in the PET membrane modified with 3 bilayers of 5 cycle Al_2O_3 / 5 cycle ZnO (Table SI-6a) before functionalization, the Si and N element were detected in the modified samples (Table SI-6b) attested the success of the functionalization step.

Table SI-7a. XPS measurement on PET membrane covered with 3 bilayers of 5 cycles Al_2O_3/ 5 cycles ZnO

Name	***Peak BE***	***Height Counts***	***FWHM eV***	***Area (P) CPS.eV***	***Area (N)***	***At. %***	***Q***
Al2p	73.83	2921.12	1.43	4446.39	0.19	11.96	1
C1s	284.82	9118.87	1.13	14606.54	0.34	21.40	1
O1s	531.32	37845.89	2.33	90833.00	0.74	46.76	1
Zn2p3	1021.56	117034.65	1.61	213266.32	0.31	19.88	1

Table SI-7b. XPS measurement on PET membrane covered with 3 bilayers of 5 cycles Al_2O_3 and 5 cycles ZnO treated with amine groups

Name	*Peak BE*	*Height Counts*	*FWHM eV*	*Area (P) CPS.eV*	*Area (N)*	*At. %*	*Q*
Al2p3	74.08	1259.59	1.30	1773.75	0.11	10.32	1
Al2p1	74.62	629.80	1.30	886.87	0.00	0.00	0
Si2p3	101.90	886.32	1.45	1394.43	0.06	5.38	1
Si2p1	102.43	452.17	1.45	711.39	0.00	0.00	0
C1s	284.81	11435.45	1.23	15236.65	0.35	32.18	1
C1s	286.28	638.92	1.26	872.56	0.02	1.84	1
C1s	289.19	664.13	1.49	1069.10	0.02	2.26	1
C1s	291.80	943.29	0.89	904.26	0.02	1.91	1
C1s	293.71	429.61	0.97	452.12	0.01	0.96	1
N1s	400.16	277.60	2.02	606.87	0.01	0.72	1
O1s	531.70	19504.98	2.36	47239.38	0.38	35.06	1
Zn2p3	1021.90	36960.13	1.74	69764.73	0.10	9.37	1

1. Balme, S.; Janot, J. M.; Dejardin, P.; Vasina, E. N.; Seta, P., *J Membrane Sci*, 2006, **284**, 198-204.
2. Cabello-Aguillar, S.; Balme, S.; Abou-Chaaya, A.; Bechelany, M.; Balanzat, E.; Janot, J. M.; Pochat-Bohatier, C.; Miele, P.; Dejardin, P., *Nanoscale*, 2013, **5**, 9582-9586.