Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

SUPPLEMENTARY INFORMATION

Self-Standing, Conducting and Capacitive Biomimetic Hybrid Nanomembranes for Selective Molecular Ions Separation

Anna Puiggalí-Jou,[1,2] Brenda G. Molina,[1,2] Maximilien Lopes-Rodrigues,[1,2,3] Catherine Michaux,[3] Eric A. Perpète,[3] David Zanuy[1] and Carlos Alemán [1,2,4,*]

[1] Departament d'Enginyeria Química, EEBE, Universitat Politècnica de Catalunya, C/Eduard Maristany 10-14, Edif. I2, 08019, Barcelona, Spain.

[2] Barcelona Research Center for Multiscale Science and Engineering, EEBE, Universitat Politècnica de Catalunya, C/Eduard Maristany 10-14, Edif. C, 08019, Barcelona, Spain.

[3] Laboratoire de Chimie Physique des Biomolécules, Unité de Chimie Physique Théorique et Structurale (UCPTS), University of Namur, Rue de Bruxelles, 61, 5000 Namur, Belgium.

[4] Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Baldiri Reixac 10-12, 08028 Barcelona Spain

METHODS

Materials

Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) 1.3 wt. % dispersion in H_2O, 3,4-ethylenedioxythiophene (EDOT), PVA 87-89% hydrolysed and lithium perchlorate ($LiClO_4$) were purchased from Sigma-Aldrich (USA). PLA 2002D, a product of Natureworks, was kindly supplied by Nupik International (Polinyà, Spain). According to the manufacturer, this PLA has a D content of 4.25%, a residual monomer content of 0.3%, density of 1.24 g/cm^3, glass transition temperature (T_g) of 58 ^oC, and melting temperature (T_m) of 153 ^oC. Acetonitrile and hexafluoroisopropanol (HFIP) were purchased from Panreac Quimica S.A.U. (Spain).

Spin-coating

Spin-coating was performed using the conditions described in the main text with a spin-coater (WS-400BZ-6NPP/A1/AR1 Laurell Technologies Corporation).

The PEDOT:PSS sacrificial layer was obtained by spin-coating a commercial water dispersion (1.3 wt. %) at a speed of 800 rpm for 1 min. Nanoperforated PLA (npPLA) layers were obtained by blending PLA and PVA with a ratio of 90:10 v/v (PLA:PVA), prepared by mixing PLA (10 mg/mL) and PVA (10 mg/mL) HFIP solutions. The spin-coating of the PLA:PVA mixture was conducted at 1200 rpm for 1 min. The resulting PLA:PVA nanomembrane was transformed into npPLA by eliminating the PVA phase. For this purpose, selective solvent etching was carried out by dropping milli-Q water on the surface for 1 h without agitation at 20 ºC.

Anodic polymerization

Anodic polymerizations were performed using a VersaStat II potentiostat-galvanostat connected to a computer controlled through a Metrohm Autolab Nova program. Electrochemical experiments were conducted in a three-electrode two-compartment cell under nitrogen atmosphere (99.995% in purity) at 20 ºC. The anodic compartment was filled with 40 mL of a 10 mM EDOT solution in acetonitrile containing 0.1 M $LiClO_4$ as supporting electrolyte, while the cathodic compartment was filled with 10 mL of the same electrolyte solution. Steel AISI 316L sheets (1 cm^2), previously washed with acetone and ethanol, were employed as working (coated with a PEDOT:PSS layer) and counter (bare) electrodes. The reference electrode was an Ag|AgCl electrode containing a KCl saturated aqueous solution (E^o = 0.222 V at 25 ºC), which was connected to the working compartment through a salt bridge containing the electrolyte solution. All anodic polymerizations were conducted by chronoamperometry under a constant potential of +1.40 V and adjusting the polymerization charge to 90 mC/cm^2.

Expression, purification and immobilization of the VDAC36 protein

Escherichia coli BL21 (DE3) bacteria were transformed to produce 6-His-tagged VDAC36 proteins in inclusion bodies. Bacteria were then lysed and centrifuged to obtain the final pellet corresponding to the non-purified proteins. VDAC36 was then purified by Ni^{2+} affinity column. VDAC36 was eluted and the buffer was exchanged to 20 mM phosphate (pH 7.4), 60 mM sodium dodecyl sulphate (SDS) and 1.5 M 2-methyl-2,4-pentanediol (MPD) with a PD10 desalting column.

The supported npPLA/PEDOT/npPLA 3-layered film was used to immobilize the protein. For this purpose, samples were placed on a 24-well plate and incubated for 12 h at 20 ºC with 1 mL of a protein solution containing 0.85 mg/mL VDAC36, 60 mM SDS and 1.5 M MPD. The resulting npPLA/PEDOT/PLA/npVDAC samples

were rinsed three times with milliQ water to remove residues. Blank film was obtained using the same procedure (*i.e.* 60 mM SDS and 1.5 M MPD) but without VDAC36.

For EIS experiments using films functionalized with the unfolded VDAC36, the protein was immobilized before the refolding step. Particularly, the membranes were incubated with a solution of 0.85 mg/mL VDAC36 in 20 mM phosphate pH 8, 100 mM NaCl, 0.1% [wt/vol] SDS for 12 h at 20 ºC. The resulting npPLA/PEDOT/PLA/VDAC(u) samples were rinsed three times with milliQ water to remove residues.

Characterization

Film thickness measurements were carried out using a Dektak 150 stylus profilometer (Veeco, Plainview, NY). Different scratches were intentionally caused on the films and measured to allow statistical analysis of data. At least eighteen independent measurements were performed for three samples of each examined condition. Imaging of the films was conducted using the following optimized settings: tip radius = 2.5 μm; stylus force = 3.0 mg; scan length = 1 mm; and speed = 50 μm/s.

Detailed inspection of films and layers was conducted by scanning electron microscopy (SEM). A Focus Ion Beam Zeiss Neon 40 instrument (Carl Zeiss, Germany) equipped with an energy dispersive X-ray (EDX) spectroscopy system and operating at 1 kV was used. Films supported onto steel sheets were mounted on a double-sided adhesive carbon disc and sputter-coated with an ultra-thin carbon layer (6-10 nm) to prevent sample charging problems. The diameter of the perforations was measured with the SmartTiff software from Carl Zeiss SMT Ltd.

Imaging was performed using an Axio Observer Z1 fluorescence microscope (Carl Zeiss) confocal laser scanning microscope with a 60x oil objective.

Atomic force microscopy (AFM) images of PLA nanomembranes before and after application of selective solvent etching were obtained with a Molecular Imaging PicoSPM using a NanoScope IV controller under ambient conditions. The tapping mode AFM was operated at constant deflection (*i.e.* vertical constant force with triangular shaped gold-coated silicon nitride). The row scanning frequency was set to 0.87 or 0.68 Hz, depending on the sample response, and the physical tip sample motion speed was 10 mm/s.

Samples were characterized by Raman spectroscopy using a commercial Renishaw inVia Qontor confocal Raman microscope. The Raman setup consisted of a laser (at 785 nm with a nominal 300 mW output power) directed through a microscope (specially adapted Leica DM2700 M microscope) to the sample, after which the scattered light is collected and directed to a spectrometer with a 1200 lines·mm^{-1} grating. The exposure time was 1 s, the laser power was adjusted to 0.1% of its nominal output power depending on the sample, and each spectrum was collected with three accumulations.

FTIR spectra were recorded on a FTIR Jasco 4100 spectrophotometer. The powder and films were deposited on an attenuated total reflection accessory (Top-plate) with a diamond crystal (Specac model MKII Golden Gate Heated Single Reflection Diamond ATR). Samples were evaluated using the spectra manager software and, for each sample, 32 scans were performed between 4000 and 600 cm^{-1} with a resolution of 4 cm^{-1}.

X-ray photoelectron spectroscopy (XPS) analyses were performed in a SPECS system equipped with a high-intensity twin-anode X-ray source XR50 of Mg/Al (1253 eV/1487 eV) operating at 150 W, placed perpendicular to the analyser axis, and using a

Phoibos 150 MCD-9 XP detector. The X-ray spot size was 650 mm. The pass energy was set to 25 and 0.1 eV for the survey and the narrow scans, respectively. Charge compensation was achieved with a combination of electron and argon ion flood guns. The energy and emission currents of the electrons were 4 eV and 0.35 mA, respectively. For the argon gun, the energy and the emission currents were 0 eV and 0.1 mA, respectively. The spectra were recorded with a pass energy of 25 eV in 0.1 eV steps at a pressure below $6 \cdot 10^{-9}$ mbar. These standard conditions of charge compensation resulted in a negative but perfectly uniform static charge. The C1s peak was used as an internal reference with a binding energy of 284.8 eV. High-resolution XPS spectra were acquired by Gaussian/Lorentzian curve fitting after S-shape background subtraction. The surface composition was determined using the manufacturer's sensitivity factors.

Contact angle measurements were carried out using the water sessile drop method. Images of 0.5 μL distillated water drops were recorded after stabilization with the equipment OCA 15EC (Data-Physics Instruments GmbH, Filderstadt). SCA20 software was used to analyze the images and determine the contact angle value, which was obtained as the average of at least fifteen independent measures for each sample.

Circular dichroism (CD) spectra were recorded between 190 and 260 nm at room temperature using a Jasco J-815 equipment with a protein concentration of approximately 110 μg/mL and a 0.1 cm cell path. Spectra were acquired at a scan speed of 50 nm min^{-1} with a 0.2 nm data pitch using a 2 nm bandwidth and a 4 second digital integration time. Spectra were averaged after two accumulations and corrected by subtraction of the background spectrum.

EIS measurements were performed using a conventional three-electrode cell and an AUTOLAB-302N potentiostat/galvanostat operating between the frequency range of 10^5 Hz and 10^{-2} Hz and 10 mV of amplitude for the sinusoidal voltage. All experiments

were performed at room temperature with 3-layered membranes deposited onto steel and using a NaCl, ATP and L-lysine electrolyte solutions. Steel was used as working-electrode and platinum as counter-electrode, whereas Ag|AgCl saturated (KCl 3M) was employed as reference electrode. After data collection, EIS results were then processed and fitted to an electrical equivalent circuit (EEC).

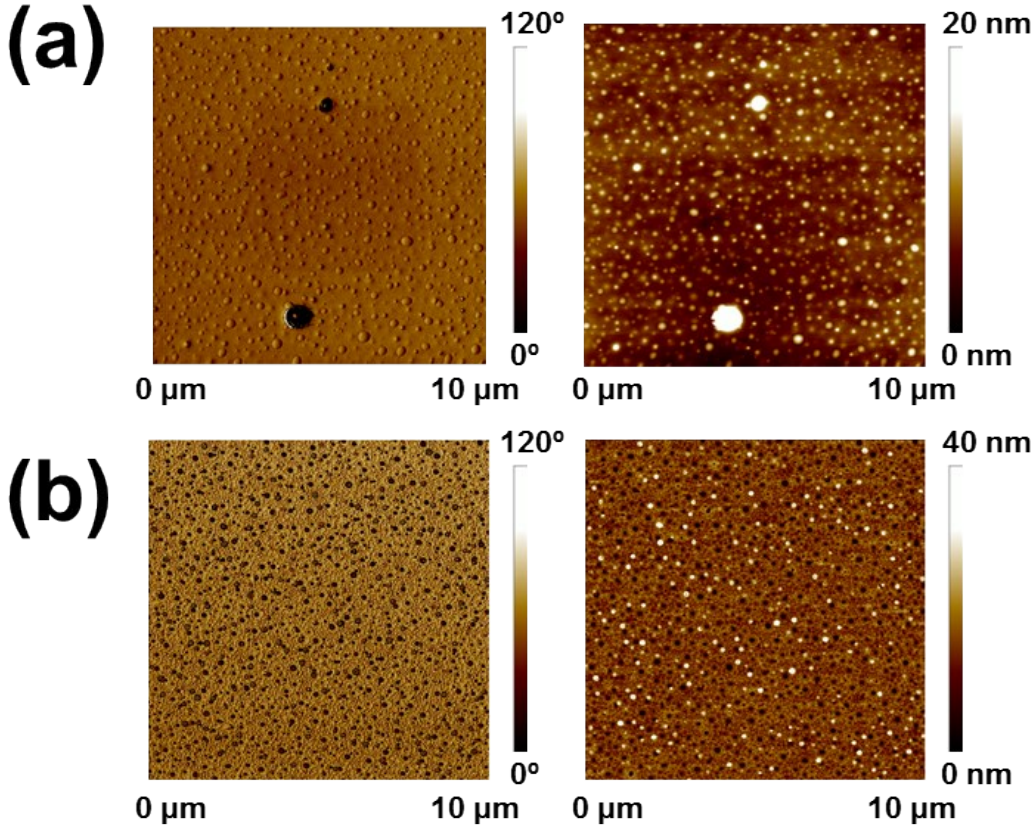

Figure S1. AFM phase (left) and topographic (right) images of (a) PLA:PVA and (b) npPLA films.

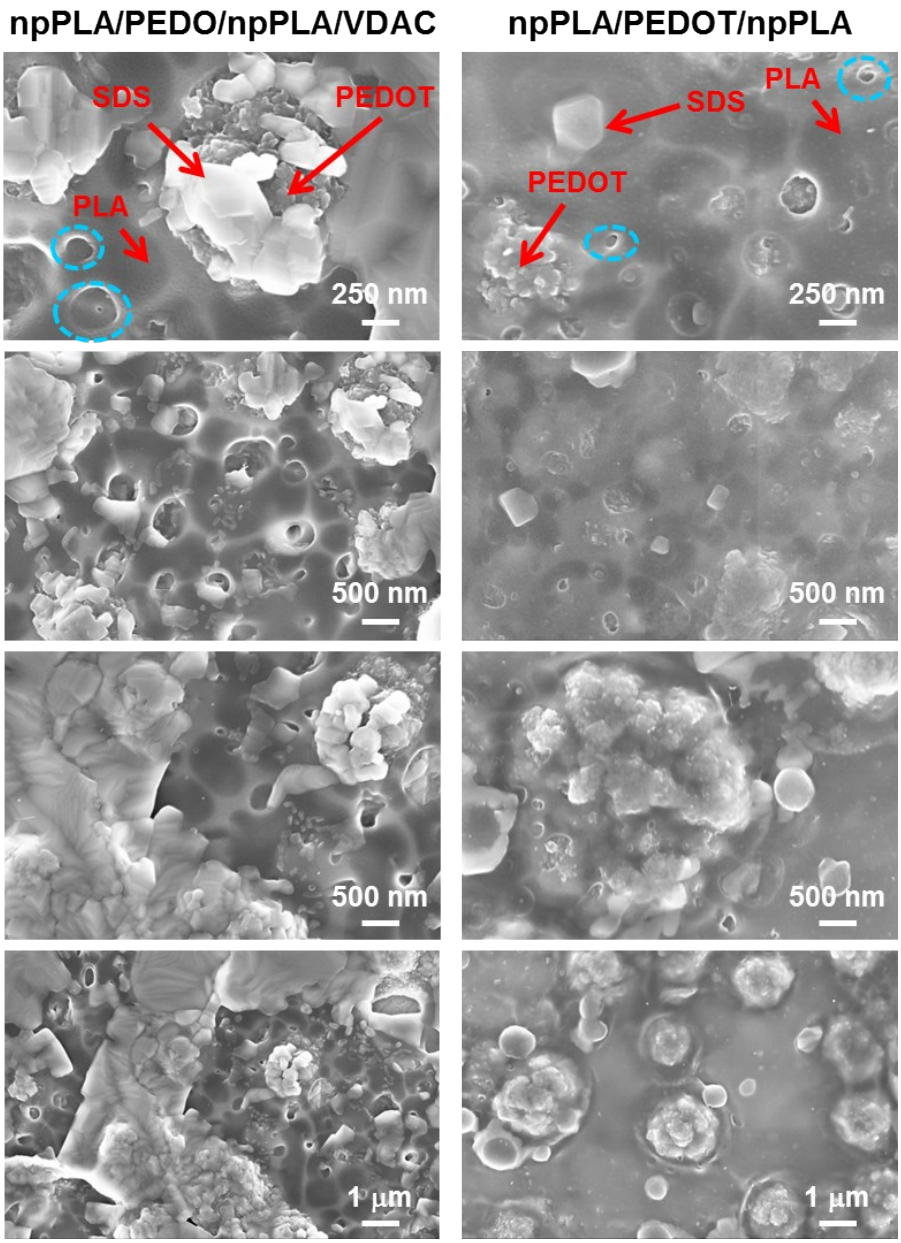

Figure S2. SEM micrographs with decreasing magnification (bottom to top) of npPLA/PEDOT/npPLA/VDAC (left column) and npPLA/PEDOT/npPLA (right column) 3-layered films. npPLA/PEDOT/npPLA/VDAC was obtained after incubating the film into a protein solution with ~0.85 mg/mL VDAC36, 60 mM SDS and 1.5 M MPD for 12 h, while the npPLA/PEDOT/npPLA was incubated in a blank solution without protein. The different elements of the film (*i.e.* npPLA of the upper layer, PEDOT clusters emerging from the intermediate layer and SDS crystals) are marked in red, while representative nanoperforations of the upper npPLA layer are marked in blue.

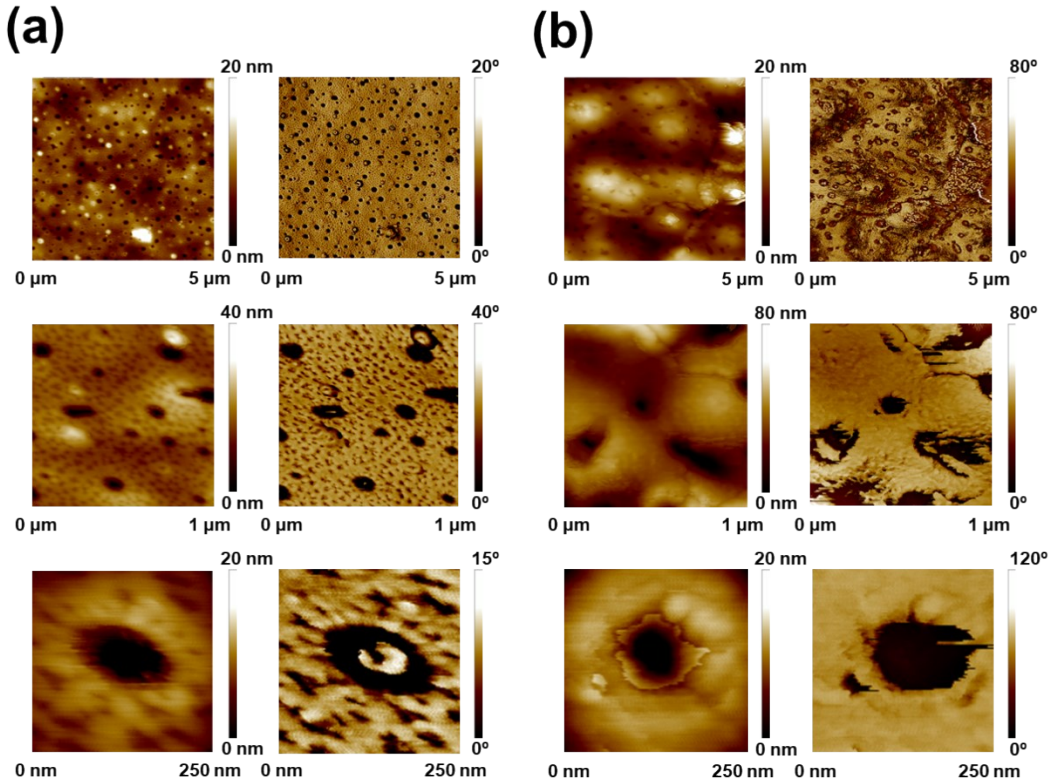

Figure S3. 2D topographic and phase contrast AFM images (left and right, respectively) for (a) npPLA/PEDOT/npPLA and (b) npPLA/PEDOT/npPLA/VDAC: 5 × 5 µm^2 (top), 1 × 1 µm^2 (middle) and 0.25 × 0.25 µm^2 (bottom) windows. Comparison of the images recorded for the 3-layered film reflects the apparition of small protuberances after incubation in the protein solution. These protuberances, which have been attributed to small protein aggregates, are located but onto the film surface and inside and around the nanopores of the npPLA layer.

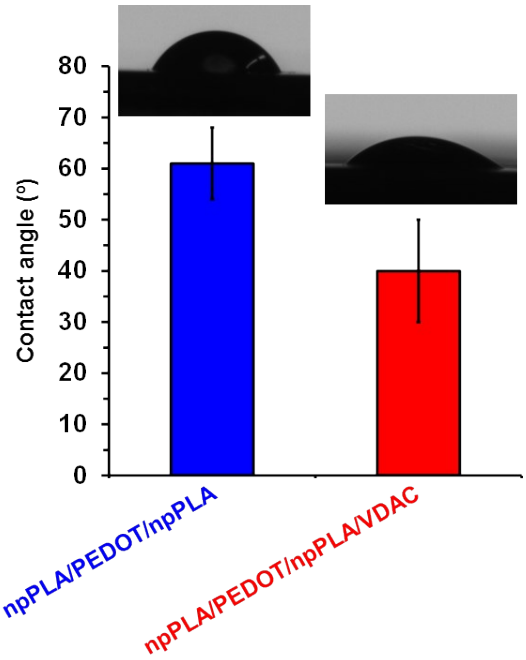

Figure S4. Contact angle of the studied 3-layered films.

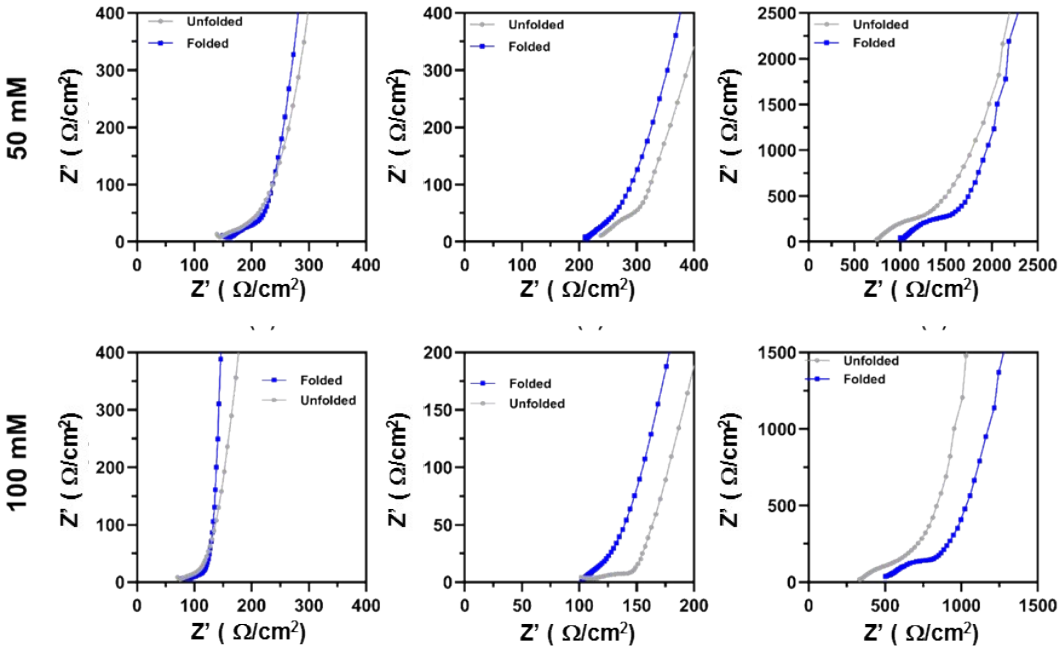

Figure S5. Nyquist plots in a 50 mM (top) and 100 mM (down) NaCl, ATP and L-lysine aqueous solution for npPLA/PEDOT/npPLA/VDAC(f) (folded) and npPLA/PEDOT/npPLA/VDAC(u) (unfolded).

Table S1. R_p values measured for the npPLA/PEDOT/npPLA/VDAC(u) and npPLA/PEDOT/npPLA/VDAC(f) films in 10, 50, 100 and 500 mM NaCl, ATP and L-lysine aqueous solution solutions.

Concentration (mM)	**NaCl**	**ATP**	**L-Lysine**
npPLA/PEDOT/npPLA/VDAC(u)			
10	330	481	6711
50	206	269	1475
100	89	100	595
500	21	93	218
npPLA/PEDOT/npPLA/VDAC(f)			
10	464	255	11877
50	275	256	1640
100	114	200	711
500	24	115	307