Electronic Supplementary Information (ESI†)

PANI supported lipid bilayer that contains NhaA transporter proteins provides a basis for a biomimetic biocapacitor

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Chemicals and materials

Aniline monomer (99%) was purchased from Sigma Aldrich (USA). DOPC phospholipid (dissolved in chloroform) were purchased from Avanti Polar Lipids (Alabaster, AL). (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) buffer (*p*H 7.4) consisting of 10 mM HEPES (assay 99%) from Fisher Scientific and 150 mM NaCl (assay ≥99.5%) from Sigma-Aldrich, Diegem, Belgium, was used for hydration of the dried DOPC lipid film. Absolute ethanol and isopropanol (≥99.7%) were purchased from VWR Prolab Chemicals.

Phosphate buffered saline (PBS) (containing 137 mM sodium chloride, 10 mM sodium phosphate, 2.7 mM potassium chloride, 2.0 mM potassium phosphate, pH 7.4) was prepared from tablets (Sigma).

NhaA bacterial production in detergent form:

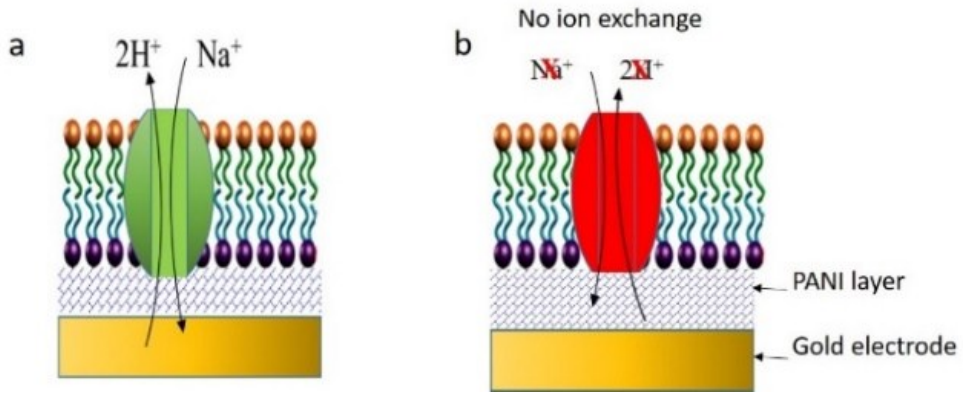

Fig. S1. *Principle of Ion-co-transport for the NhaA membrane protein incorporated in the SLB.* ***(a)*** *For the* ***active*** *protein, there is an electrogenic ion current established across the lipid bilayer membrane due to the exchange of 2 H^+ ions for 1 Na^+ ion.* ***(b)*** *For the* ***inactive*** *mutated protein (see ESI), there is no ion transport.*

(a) Subcloning in pET15b of active and inactive form of NhaA:

pET15b::NhaA was constructed from previously constructed pIVEX2.4b::NhaA and pET15b expression vector (Novagen). Plasmids were transformed in DH5a Escherichia coli competent cells, and recombinant clones were identified by direct PCR screening and then sequenced. The inactive double mutant NhaA* has been extensively described in Galili *et al.*[1]

The cloning was performed by using a Quickchange site-directed mutagenesis strategy.[2] The protocol was adapted from Weiner et al (1994). The high fidelity polymerase was purchase from Jena Bioscience. 3 cycles (95°C/30s-68°C/6min) were performed with 100 ng of plasmid. 1 µL of DnpI (from Roche) was incubated in the PCR reaction at 37°C during 1 h before transformation of DH5α competent cells.

We first constructed the T132C mutant from the pET15b::NhaA subclone with the following primer pairs: 5'- GCG ATC CCG GCG GCT **TGT** GAC ATT GCT TTT GCA -3' and 5'- TGC AAA AGC AAT GTC **ACA** AGC CGC CGG GAT CGC -3'. The pET15b::T132C mutant was sequenced and used for the second round with the primers : 5'-ATC CTG TGC GGT ATC **TGT** TTT ACT ATG TCT ATC -3' and 5'- GAT AGA CAT AGT AAA **ACA** GAT ACC GCA CAG GAT -3' to produce the inactive double mutant NhaA* containing T132C and Q338C.

(b) Recombinant expression of NhaA and NhaA*

pET15b::NhaA and pET15b::NhaA* plasmids were transformed in the expression host (E. coli C43(DE3) cells, NEB) and overexpressed by developing an optimised protocol based on the work of Kubicek *et al.*[3] C43 transformed cells were cultivated in LB medium supplemented with ampicillin (100 µg/ml), at 37°C until OD_{600} = 0,4. Induction was done by adding 200 µM IPTG during 5 hours. Cells were harvested by centrifugation in falcon tubes at 8000 rpm during 5 min. Pellets of 1.8 g of wet weight were stored at -80°C.

(c) Purification of recombinant NhaA and NhaA*

The purification comprises two steps. In the all process, NaCl was replaced by KCl to avoid presence of Na^+.

Membrane fraction preparation and protein solubilization: The cells were placed in 30 ml of binding buffer (20 mM Tris, 500 mM KCl, 10 mM imidazole, and 12.6 % (w/v) glycerol) containing 28 mg lysozyme and 525U of Benzonase Nuclease (both purchase from Sigma Aldrich). After an incubation time of 30 min. at 37°C, cells were disrupted in a French pressure cell, and the resulting crude extract was clarified by centrifugation at 14,000 rpm for 20 min. Membrane fraction was obtained after an ultracentrifugation of the cell lysate at 36 000 rpm (160 000g) in a SW41 rotor (Beckman) during 2 hours. The membrane faction was resuspended in 7 ml of binding buffer containing 20 mM DDM and the tube was incubated overnight on an end-over-end rotator at 4°C.

IMAC purification: The His-Tagged NhaA protein was purified on an immobilized metal affinity chromatography (IMAC) column (Ni-NTA agarose, Qiagen). The column (700 to 800 µL) was equilibrated with the binding buffer prior to purification. The solubilized membrane fraction was then

incubated with the column during 2 hours under smooth agitation at RT. It was then drain by gravity flow and washed with 20 volume of washing buffer (20 mM Tris, 500 mM KCl, 40 mM imidazole, 12.6 % (w/v) glycerol and 0,225 mM DDM). The purified protein was recovered in 1.5 column volume of elution buffer (20 mM Tris, 500 mM KCl, 300 mM imidazole, 12.6 % (w/v) glycerol and 0,225 mM DDM). The purified protein concentration was adjusted to 0.75 µg/µL.

Vesicle preparation

DOPC were dissolved in spectroscopic grade chloroform and the solvent was then evaporated under a mild flow of nitrogen in a round bottomed flask. The resulting lipid film was kept under vacuum for 2 hours to remove residual solvent. Then, the lipid mixture was hydrated with Tris buffer. Hydration to 0.5 mg/ml was carried out under room temperature for DOPC (well above its melting temperature T_m ~ –17 °C), followed by six twenty-second cycles of ultrasonic treatment using a probe. Large unilamellar vesicles were formed by extrusion through a filter support (Avanti Polar Lipids) with a pore size of 100 nm and 50nm for 21 times. The vesicle dispersions were stored at 4 °C and used within 2 days.

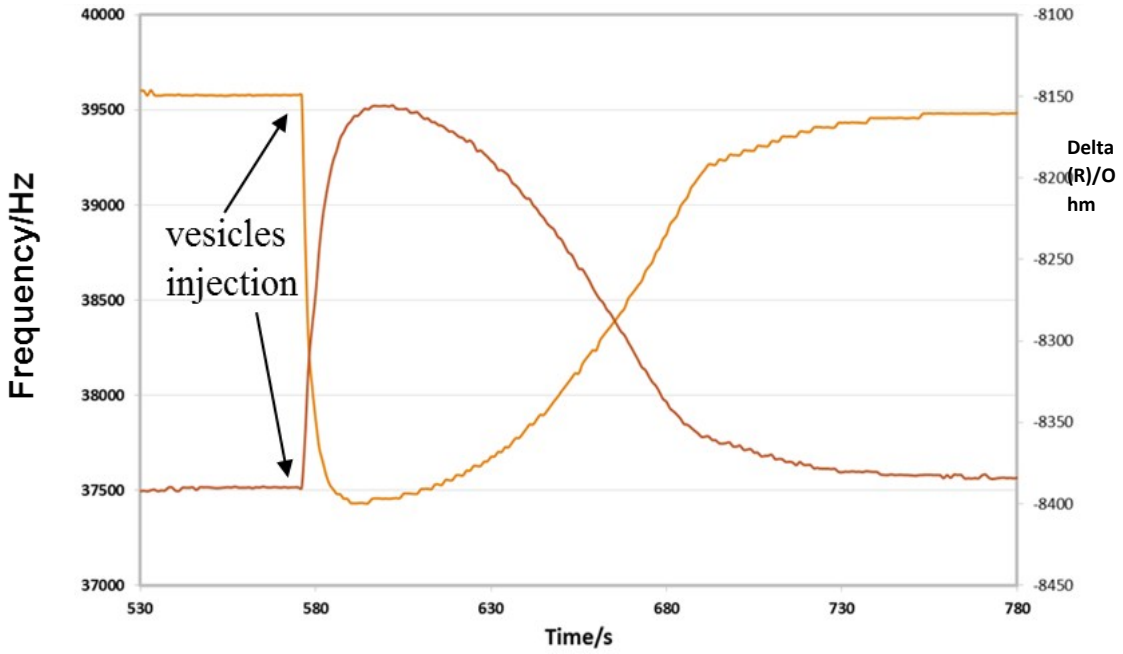

Fig. S2. *QCM-D responses for PANI modified gold electrode before and after vesicle injection. The orange line shows the resonance frequency and the red line shows the change in dissipation. After the vesicles were injected (580 s) there was a diminution in the resonant frequency and a concomitant increase in the dissipation. After approximately 200 s both the resonant frequency and dissipation reached steady-state levels which were close to the initial values.*

Electrochemical measurements

A BIOLOGIC Instruments 650 potentiostat interfaced to a PC was used for all experiments along with a saturated calomel electrode (SCE) as reference and a platinum mesh as the counter electrode, unless otherwise stated. The gold electrode modified quartz were employed as the working electrodes.

Electrochemical polymerization of aniline was carried by cyclic voltammetry (CV) in PBS buffer containing 2 mM of aniline solutions was done at a gold disc electrochemical cell. All experiments were carried out at room temperature (20°C). CV measurement were performed in the potential range -0.5 to +1 V (vs SCE) at 10mV/s. PANI growth was controlled by the number of deposition cycles. After electropolymerization, the electrodes were thoroughly rinsed with ethanol and buffer to remove any remaining monomeric. Electrochemical impedance measurements were performed in the range from 1 Mhz to 0.5 Hz to 1 MHz carried at open circuit potential (OCP) in a P.BS (pH7).

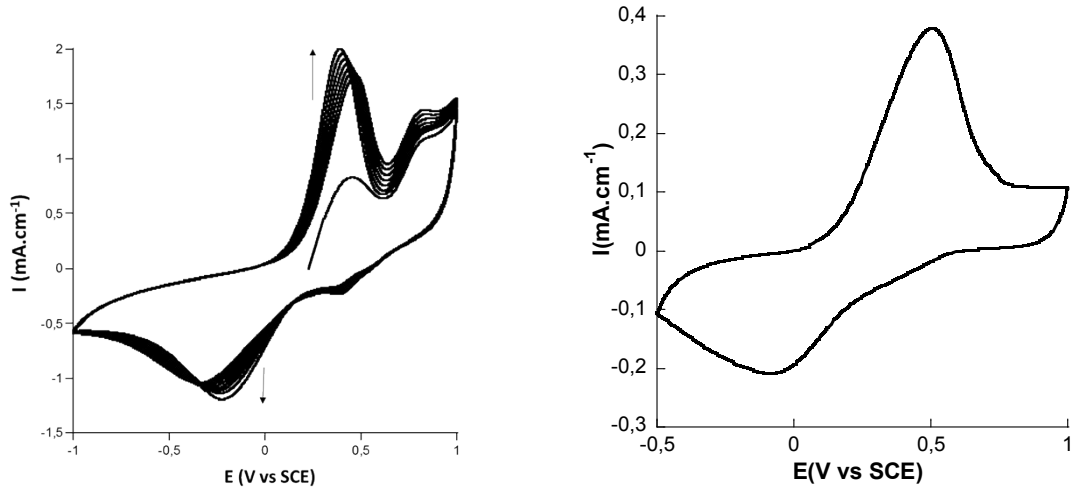

***Fig. S3. Left**, Consecutive cyclic voltammograms for electro polymerization of aniline obtained on gold electrode in PBS (pH 7) containing 2 mM of aniline, scan rate 10 mV/s. **Right**, cyclic voltammetry of PANI modified gold electrode in PBS (pH7), scan rate 10mV/s.*

Cyclic voltammograms *(Fig S3. left*) of PANI deposited on gold electrode is characterized by two pairs of current peaks (two oxidation peaks and two reduction peaks). The oxidation peak around +500 mV is attributed to the conversion from leucoemeraldine into emeraldine form, the second peak appearing around +800 mV is due to emeraldine/pernigraniline transformation. On the way back there are two small reduction peaks appeared at about 0.4 V and -0.2 V corresponding to the reduction of fully oxidized PANI pernagriniline base to emeraldine salt (ES) and the reduction of subsequent emeraldine salt to leucomeraldine base.

Fig S3 (right) shows the cyclic voltammetry of PANI modified gold electrode and we can see the presence of two peaks. There is an oxidation peak at 0.5 V corresponding to the PANI electro-oxidation and a reduction peak corresponding to the PANI reduction.

Enlarged representations of the Cole-Cole plots from Figures 4 and 5

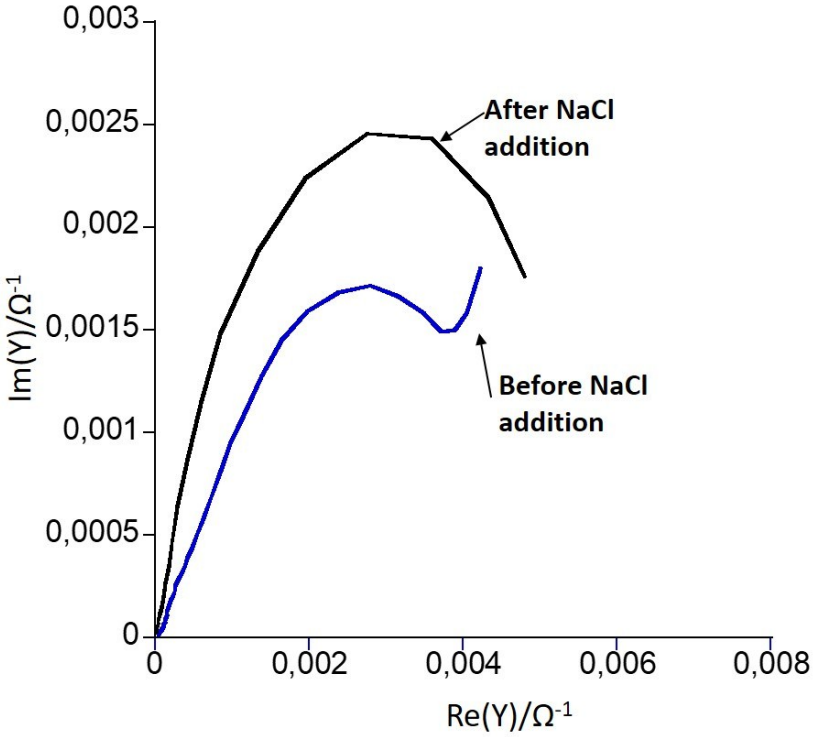

Fig. S4. *The same data from Fig. 4 for the electrochemical impedance spectroscopy response (Cole-Cole diagram) of SLB-**inactive**-NhaA-PANI modified gold electrode before and the after the addition of NaCl 10 mM. The scale is reduced for better visibility of the differences before and after the addition of NaCl. Although the difference appears to be large at this scale, it is important to note that the inactive NhaA protein did not respond to the addition of NaCl.*

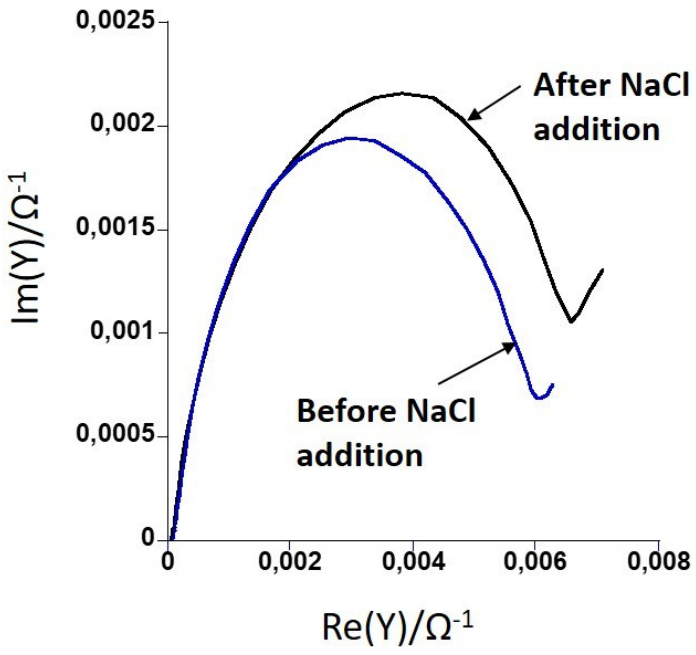

Fig. S5. *The same data from Fig. 5 for the electrochemical impedance spectroscopy response (Cole-Cole diagram) of PANI modified gold electrode before and the after the addition of NaCl 10 mM. Note that NhaA was not incorporated into this system. The scale is reduced for better visibility of the differences before and after the addition of NaCl. Although the difference appears to be large at this scale, it is important to note that the PANI-modified electrode without any added NhaA protein did not respond to the addition of NaCl.*

Quartz crystal microbalance with dissipation (QCM-D) measurements

Liposome adsorption and the supported lipid bilayer formation processes were monitored by QCM-922A (SEIKO EG&G, Japan) and repeated several times. Just prior to the measurements, the sensor crystals (silica-coated QCM-D sensors with a fundamental frequency of 5 MHz obtained from Q-Sense AB) were rinsed with ethanol. The frequency and dissipation changes were plotted and analyzed using Biologic EC-Lab software. A typical experiment was performed at room temperature as follows: 2 mL of PBS buffer solution was injected in the QCM cell. Then, 100μL of lipid solution was added. Finally, liposomes with a selected size distribution were adsorbed onto a polyaniline coated silica QCM-D sensor.

Contact angle measurement: surface wettability

The hydrophobicity and the surface wettability condition of the PANI coated gold were observed by measuring contact angle (CA), to understand the effect of electropolymerization synthesis of the PANI on the surface of gold. Contact angle is an important parameter to evaluate the wetting ability of polymer membrane surfaces.[4] For the measurements we performed with our system, the material for testing was placed under a needle tip of a syringe which deposited 2μL water droplets onto the surface of the material. A goniometer provided the measure of the initial contact angle of the 2 μl drop of water. The advancing contact angle of the water droplets was measured with the optical contact measuring system (OCA 5, Dataphysics Instruments GmBH, Filderstadt, Germany), which provides a ± 0.2° reading accuracy and data acquisition rate of eight images per second.[5] The contact angle was measured from profile images of the water drops that were extracted from the movie (Fig. S6).

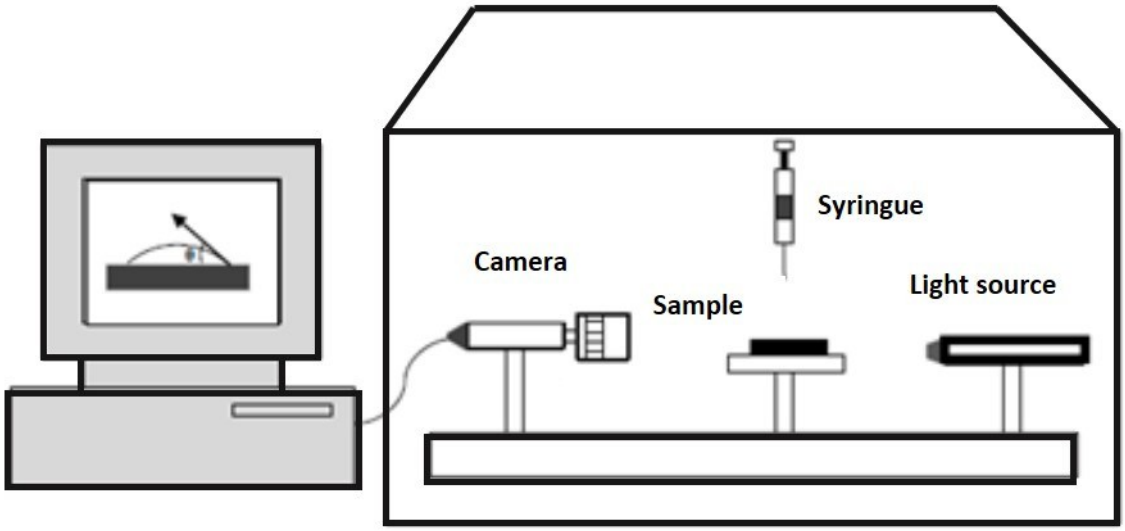

Fig. S6:*Schematic illustration of the contact angle measuring device*

The measured contact angles were 84 ± 2° for the bare gold surface and 44 ± 1° for the PANI surface. The PANI surfaces has a more hydrophilic surface compared to that of the bare gold electrode. This increased wettability of the PANI surface is advantageous for the adsorption of lipids.

References

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2. M.P. Weiner, G.L. Costa, W. Schoettlin, J. Cline, E. Mathur and J.C. Bauer, *Gene*, 1994, **151**, 119-123.
3. K. Kubicek, H. Block, B. Maertens, A. Spriestersbach and J. Labahn, *Meth. Enzymol*., 2014, **541**, 117-140.
4. A. V. Prydatko, L. A. Belyaeva, L. Jiang, L. M. C. Lima and G. F. Schneider, *Nat. Commun.*, 2018, **9**, 4185, 2018.
5. D. Y. Kwok, T. Gietzelt, K. Grundke, H.-J. Jacobasch and A. W. Neumann, *Langmuir*, 1997, **13**, 2880-2894.