Scaling behavior of ionic transport in membrane nanochannels

María Queralt-Martín[1#], Maria Lidón López[2#], Marcel Aguilella-Arzo[2], Vicente M. Aguilella[2] and Antonio Alcaraz[2*]

[1] *Section on Molecular Transport, Eunice Kennedy Shriver NICHD, National Institutes of Health, Bethesda, Maryland 20892, USA.*

[2] *Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Av. Vicent Sos Baynat s/n 12071 Castellón, Spain.*

*Author to whom correspondence should be addressed. Phone: +34 964 72 8044. Electronic mail alcaraza@uji.es

[#] Equal contribution

SUPPORTING INFORMATION

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S1. Representative current traces

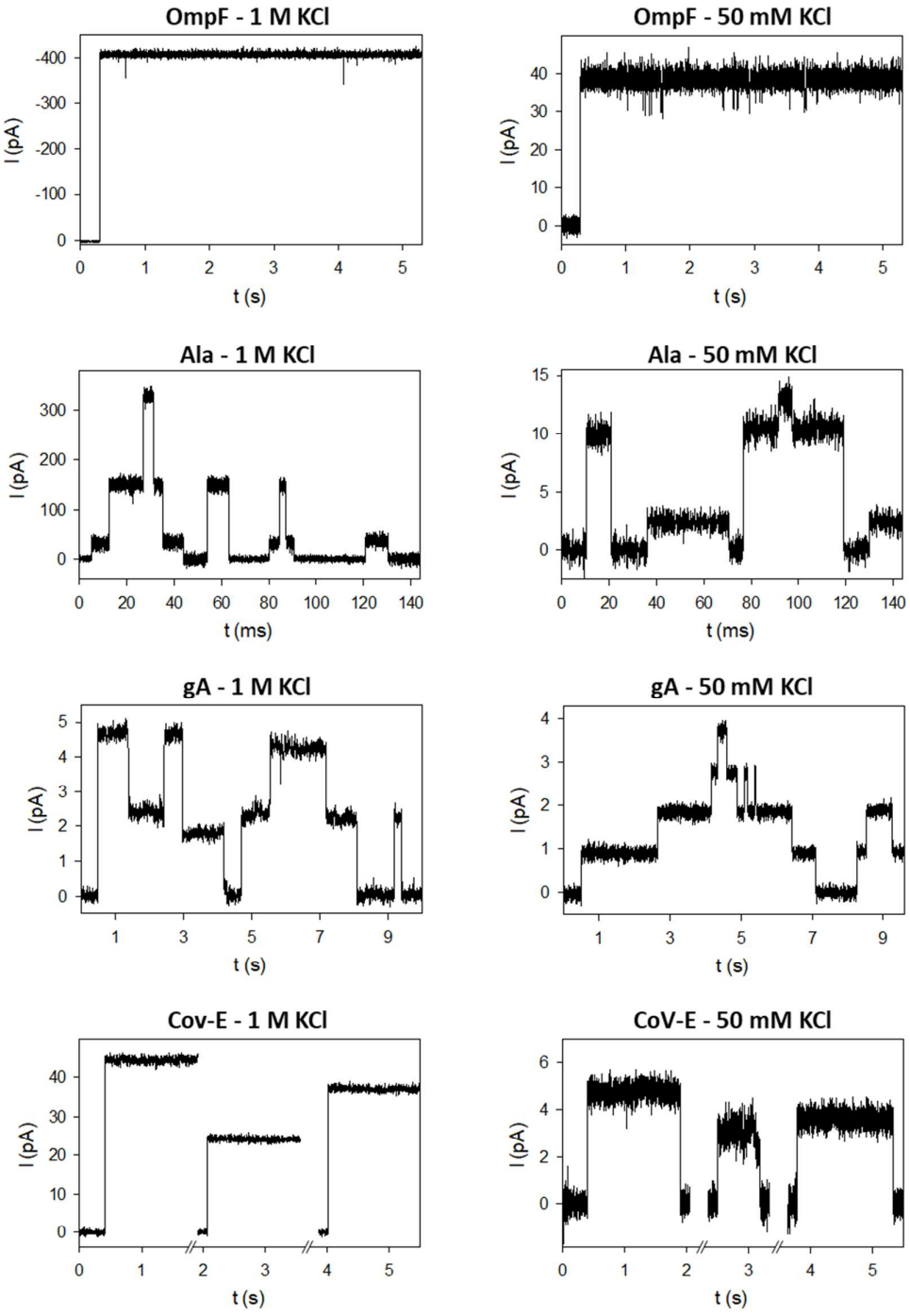

Figure S1. Representative current traces of the different protein channels used in our study, at high (1 M) and low (50 mM) KCl concentrations at pH 6 when reconstituted in neutral lipid (DPhPC). Current records were digitally filtered at different sampling rates for each channel using a low pass Bessel (8-pole) filter: 5 kHz for OmpF, 2 kHz for alamethicin (Ala)

only at 50 mM KCl, 300 Hz for SARS CoV E protein (CoV-E), and 100 Hz for gramicidin A (gA).

S2. Representative I-V curves

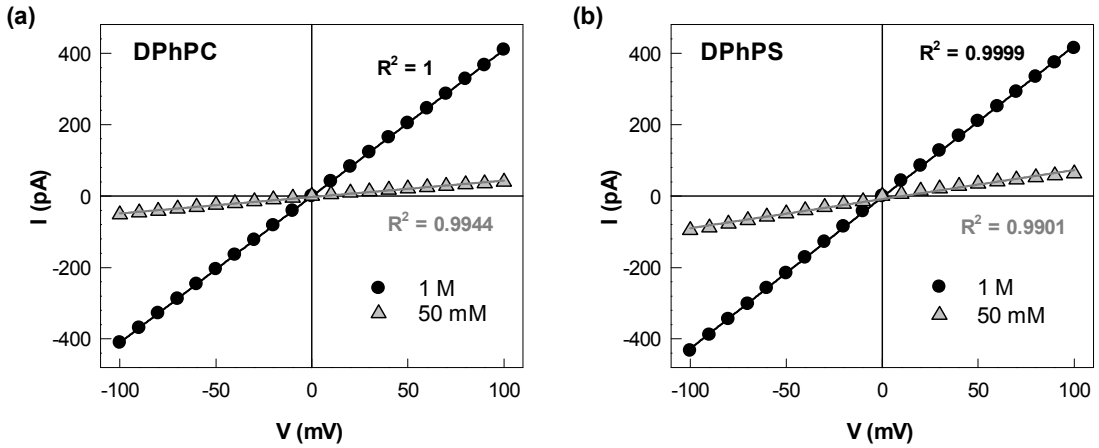

Figure S2. Representative IV curves of OmpF single channel-currents showing the absence of current rectification at high (1 M) and low (50 mM) KCl concentrations at pH 6 when reconstituted in neutral ((**a**), DPhPC) or charged ((**b**), DPhPS) lipids. Solid lines correspond to linear fits. Value of squared R is shown for each fitting.

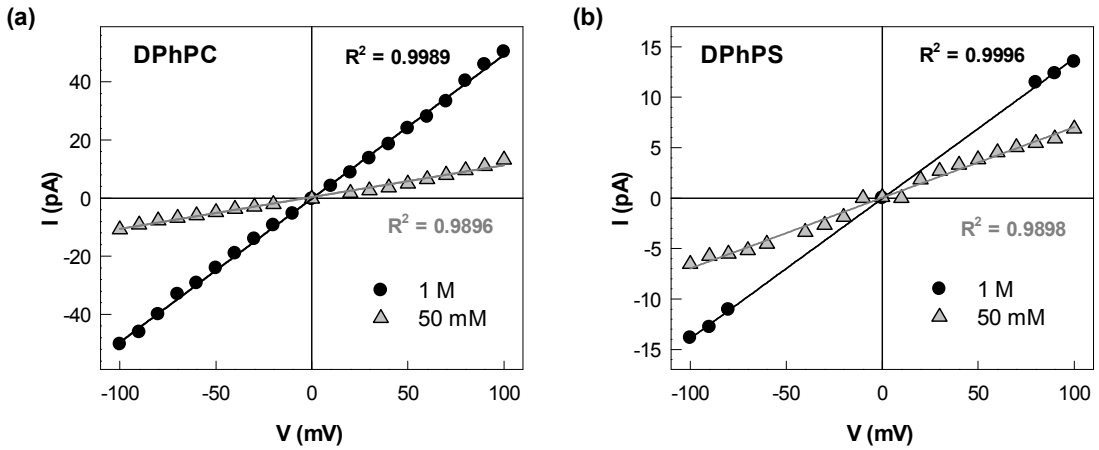

Figure S3. Representative IV curves of SARS CoV E single-channel currents at high (1 M) and low (50 mM) KCl concentrations at pH 6 when reconstituted in neutral ((**a**), DPhPC) or charged ((**b**), DPhPS) lipids. Solid lines correspond to linear fits. Value of squared R is shown for each fitting.

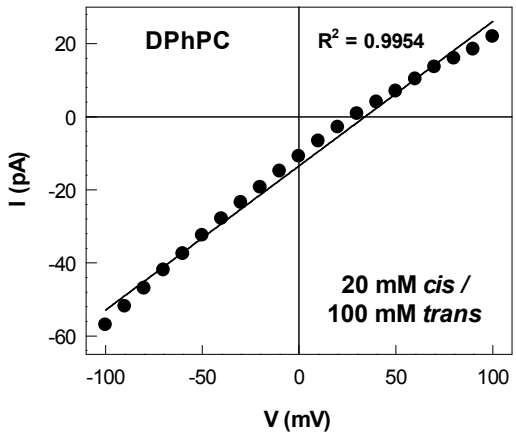

Figure S4. Representative IV curve of OmpF single channel-current showing a slight current rectification in a KCl concentration gradient of 20 mM *cis* / 100 mM *trans*. at pH 6 in neutral lipid. Solid line corresponds to a linear fit.

S3. Experimental methods

Planar membranes were formed by the apposition of monolayers[1] across orifices with diameters of 70–100 μm on a 15-μm-thick Teflon partition using diphytanoyl phosphatidylcholine (DPhPC) or diphytanoyl phosphatidylserine (DPhPS). All lipids were purchased from Avanti Polar Lipids (Alabaster, AL). Lipids were dissolved in pentane at 5 mg/mL and amounts of 10-20 μL were added to each chamber. The orifices were pre-treated with a 1% solution of hexadecane in pentane. Membranes were formed at least 10 minutes after hexadecane and lipid addition to ensure pentane evaporation. Proteins were obtained from different sources: OmpF was a generous gift of Sergey M. Bezrukov, NIH, Bethesda (MD), USA; Alamethicin and Gramicidin were purchased at Sigma-Aldrich (St. Louis, MO; catalog numbers A4665 and 50845, respectively); SARS Coronavirus Envelope Protein (CoV-E) was kindly provided by Dr. Jaume Torres, School of Biological Sciences, Nanyang Technological University, Singapore. Depending on the protein, a different amount was added to the 2 mL aqueous phase at the *cis* side of the membrane chamber to achieve channel

insertions: ~0.1 μL of OmpF at 1 ng/μL in 0.1 M KCl and 1% (v/v) OctylPOE (Alexis, Switzerland); ~1 μL of Alamethicin at 10^{-5} M in ethanol; ~1-2 μL of Gramicidin A at 10^{-10} M in ethanol; 0.5-1 μL of CoV-E protein at 300 ng/μL in acetonitrile:isopropanol (40:60). After protein addition, channel insertion was monitored by current steps at an applied potential of 100-140 mV. The electric potential was applied using Ag/AgCl electrodes in 2 M KCl, 1.5% agarose bridges assembled within standard 250 ml pipette tips. The potential was defined as positive when it was higher on the side of the protein addition (the *cis* side of the membrane chamber), whereas the *trans* side was set to ground. An Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA) in the voltage-clamp mode was used to measure the current and applied potential. The signal was digitalized at 50 kHz sampling frequency after 10 kHz 8-pole in-line Bessel filtering. The chamber and the head stage were isolated from external noise sources with a double metal screen (Amuneal Manufacturing Corp., Philadelphia, PA, USA). Solutions were buffered with 5 mM of either HEPES or MES and pH was adjusted by adding HCl or KOH and controlled during the experiments with a GLP22 pH meter (Crison, Barcelona, Spain). Measurements were obtained at room temperature. For the case of gramicidin A, 1 mM EDTA was also added to assure removal of free divalent cations from solution. Channel conductance was calculated as the ratio between measured current and applied potential. The applied voltage and method for measuring single channel current was different for each channel: in OmpF, single-channel insertions were monitored and current was recorded under a +100 mV applied voltage; with gramicidin A and SARS CoV E proteins, conductance was calculated from current steps recorded while applying +100 mV; for alamethicin, conductance was also obtained from insertion steps, but in this case when a voltage of +140 mV was applied.

S4. Theoretical calculations

The three-dimensional Poisson–Nernst–Planck model was implemented as described in detail elsewhere.[2–4] Briefly, after calculating the pKa and channel fixed charge for each condition using the University of Houston Brownian Dynamics (UHBD) code, the Poisson-Nernst-Planck equations were solved numerically using FiPy, a finite volume solver of partial differential equations written in Python.[5] The three-dimensional structures of each protein were obtained from the Protein Data Bank (PDB codes 2OMF for OmpF and 1JNO for gramicidin A). Ion diffusion coefficients were introduced as free parameters, using values slightly lower than the tabulated ones for bulk solution (0.8 D_{bulk} for OmpF and 0.5 D_{bulk} for gramicidin A).

S5. Supplemental references

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(2) Kurnikova, M. G.; Coalson, R. D.; Graf, P.; Nitzan, A. A Lattice Relaxation Algorithm for Three-Dimensional Poisson-Nernst-Planck Theory with Application to Ion Transport through the Gramicidin A Channel. *Biophys. J.* **1999**, *76* (2), 642–656.

(3) Aguilella-Arzo, M.; Queralt-Martín, M.; Lopez, M.-L. M.-L.; Alcaraz, A. Fluctuation-Driven Transport in Biological Nanopores. A 3D Poisson–Nernst–Planck Study. *Entropy* **2017**, *19* (3), 116.

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(5) Guyer, J. E.; Wheeler, D.; Warren, J. A. FiPy: Partial Differential Equations with Python. *Comput. Sci. Eng.* **2009**, *11* (3), 6–15.