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Stochastic pumping of ions based on colored noise in bacterial channels under acidic stress

M. Lidón López, María Queralt-Martín and Antonio Alcaraz*

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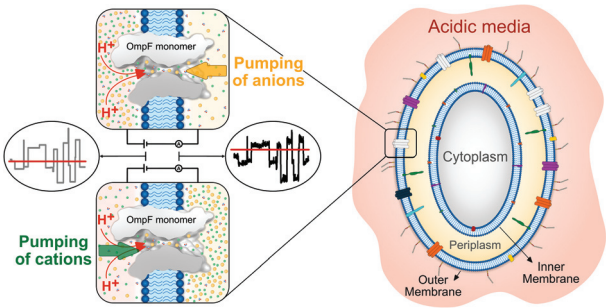

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PAPER

Stochastic pumping of ions based on colored noise in bacterial channels under acidic stress†

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Fluctuation-driven ion transport can be obtained in bacterial channels with the aid of different types of colored noise including the biologically relevant Lorentzian one. Using the electrochemical rectification of the channel current as a ratchet mechanism we observe transport of ions up to their concentration gradient under conditions similar to that met *in vivo*, namely moderate pH gradients and asymmetrically charged lipid membranes. We find that depending on the direction of the concentration gradient the channel can pump either cations or anions from the diluted side to the concentrated one. We discuss the possible relevance of this phenomenon for the pH homeostasis of bacterial cells.

Introduction

Thermodynamic arguments assure that free energy transduction cannot be obtained from equilibrium fluctuations.[1] However, a net flux of energy could appear when external oscillating fields couple to internal conformational fluctuations contributing to the maintenance of a non-equilibrium state.[1–4] This constitutes a general design principle for implementing engineering applications[5] like separation processes and energy conversion.[6–8] Living cells provide an ideal environment for fluctuation-driven energy transfer.[2] For instance, significant transient changes in voltage (~100 mV) are found in the vicinity of protein ion channels undergoing transitions between different states.[9] Specifically, ratchet mechanisms observed in biochannels are based on the fact that for one voltage polarity the force required to move the ions through the pore is smaller than that under the opposite polarity. Thus, when the electric voltage across the membrane fluctuates with a zero mean, a net flow of ions (the so-called stochastic pumping) can be observed.[6,10–14]

Previous studies showed that OmpF porin, a non-specific wide channel found in the outer membrane of *Escherichia coli* (*E. coli*),[15,16] may perform as a molecular ratchet using diverse mechanisms. For instance, the addition of small amounts of multivalent cations (*i.e.* lanthanum) changes the internal electric potential of the channel yielding reversible current rectification.[14,17] In contrast, non-reversible current asymmetry was obtained using site-directed mutations in the selectivity filter of the protein.[18] In this case, no change in the external solutions is needed but the large number of mutations required jeopardizes the appropriate folding of the protein into a functional channel. Alternatively, solution acidity can act as an external modulator so that by the selective titration of the protein residues[19] the pore conductive properties can be tuned at will, from almost ohmic conduction to a bipolar diode resembling the solid state p–n junctions.[19–21] Unfortunately, the extreme pH conditions (for instance $pH_{cis} = 3/pH_{trans} = 12$) required to obtain substantial rectification put into question not only its physiological relevance[20] but also the potential use of such a nanofluidic diode in technological applications.[19]

In the present study we investigate how rectifying conductive channels can be achieved under conditions more similar to that met *in vivo*. To this end, only moderate pH gradients are considered and asymmetrically charged lipid bilayers are used to mimic the effects of acidic stress on the membrane properties. Former approaches stressed that enzymes can capture and transmit free energy from oscillating electric fields, as in the case of active transport of Rb^+ by using the Na^+, K^+-ATPase.[2] We show that zero-average electric potentials similar to those actually measured in *E. coli*[22] can be used to obtain electrical pumping of ions against an external concentration gradient without the need for countertransport of other charged species, as depicted in Fig. 1.

Although this possibility was already explored theoretically in a series of seminal papers by Astumian and coworkers,[1,2] we present here the experimental realization of the concept in diverse experiments performed at the single protein level as opposed to studies concerning assemblies of many biomolecules in solution.[23] The novelty of the present approach lies in the nature of both the voltage oscillations and the mechanism of pumping. We consider different kinds of colored noise (noise signal whose power spectrum is not flat) including the biologically relevant[1,24] Lorentzian one (random

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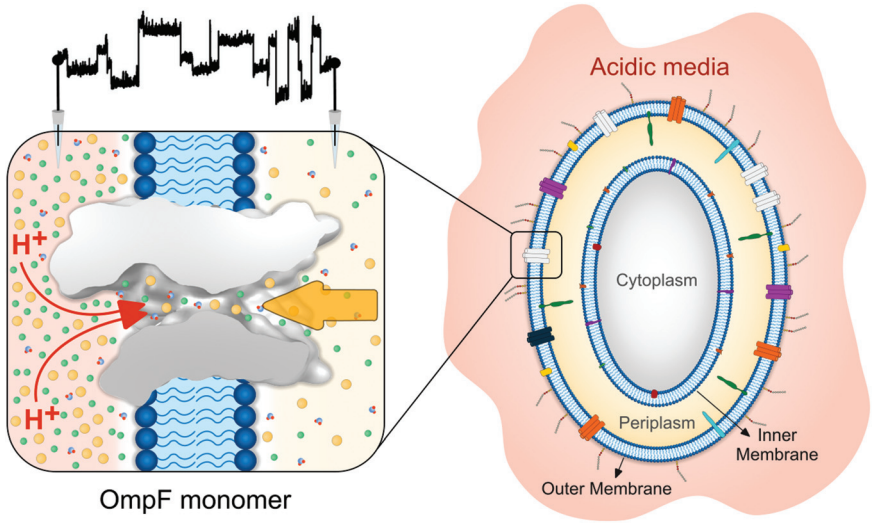

Fig. 1 Acidic stress has potential to trigger electrical pumping of ions (represented as a yellow arrow) through wide channels in the presence of zero-average colored noise.

telegraph noise) as opposed to square or sinusoidal waves used in previous studies.[11,14] Additionally, we show that the uphill transport of ions obtained in an OmpF channel under acidic stress is directional. Depending on which side of the membrane is placed the diluted solution, the system is able to pump either cations or anions against their concentration gradient. We discuss the potential implications of this phenomenon in the regulatory mechanisms of bacterial cells against acidic stress.

Experimental

Wild-type OmpF, kindly provided by Dr S. Bezrukov (NIH, Bethesda, USA), was isolated and purified from an *E. coli* culture. Planar membranes were formed by the apposition of monolayers across orifices with diameters of 70–100 μm on a 15 μm-thick Teflon partition using diphytanoyl phosphatidylcholine (DPhPC) or diphytanoyl phosphatidylserine (DPhPS). The orifices were pre-treated with a 1% solution of hexadecane in pentane. An 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) in the voltage-clamp mode was used to measure the current and applied potential. Except otherwise noticed, 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). The pH was adjusted by adding HCl or KOH and controlled during the experiments with a GLP22 pH meter (Crison). Except where noted, measurements were done at $T = (23 \pm 1.5)$ °C. The reversal potential was measured as the potential needed to achieve zero current when several channels were inserted into the bilayer. It was corrected with the liquid junction potential calculated from Henderson's equation, as described in detail elsewhere.[25]

Results and discussion

E. coli can survive inside the stomach under considerable external acidic stress (pH 2–3),[26–28] while the cytoplasmic pH can remain close to pH = 7 by means of diverse internal regulatory mechanisms.[29,30] We explore this issue by considering different pH arrangements that will be henceforth represented as $pH_{cis}||pH_{trans}$. To mimic the effects of acidic stress on the membrane surface charge, we consider that negatively charged lipids are present in the *trans* monolayer kept at pH = 7 whereas the *cis* monolayer facing an acidic solution is made of neutral lipids (note that at low pH the lipid polar heads should be protonated).[31] We focus exclusively on the effect of charge aiming to reduce a particularly complex system – the outer membrane of *E. coli* is a heterogeneous mixture of lipopolysaccharides and phospholipids[32] – into a simple one that could be understood in terms of electrostatic interactions.

Fig. 2a shows the current–voltage (I–V) curves of the bacterial porin OmpF in the 3||7 configuration for different lipid mixtures. Rectification ratios ($r = |I_{-V}/I_{+V}|$) of the corresponding curves are shown in Fig. 2b. When both monolayers are made of DPhPC only slight rectification appears ($r \sim 2$ for $V = 150$ mV). In contrast, when the monolayer facing the side of pH = 7 is prepared from DPhPS, the rectification ratio increases up to 14 for $V = 150$ mV. This suggests that negative charges in the lipid monolayer facing the *trans* side help to create a diode-like structure responsible for the observed current rectification,[19] similarly to the case of syringomycin E

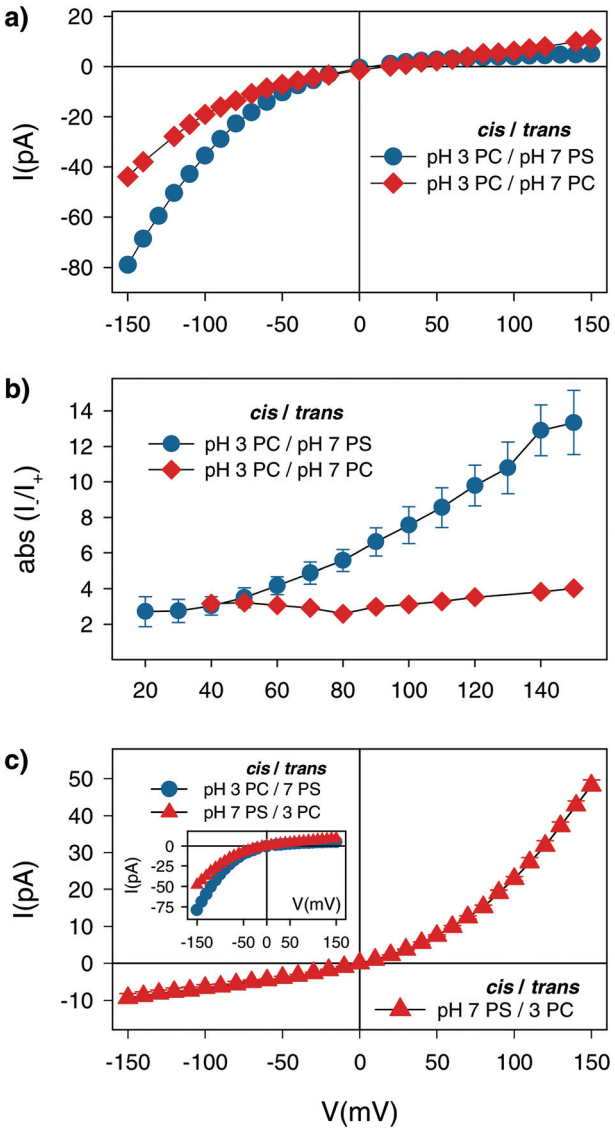

Fig. 2 (a) Current–voltage (*I–V*) curves of the OmpF channel in the 3||7 configuration for two different lipid mixtures at 25 mM KCl. (b) Rectification ratio ($r = |I_{-V}/I_{+V}|$) dependence on the applied voltage for the same conditions. (c) *I–V* curve in the reversed configuration pH 7 DPhPS||pH 3 DPhPC at 25 mM KCl. The inset compares this *I–V* curve (with inverted ground) with that of pH 3 DPhPC||pH 7 DPhPS.

channels.[33] In fact, the current–voltage curves shown in Fig. 2a can be compared with the characteristic equations of semiconductor p/n diodes.[34] The diode current I_d as a function of applied voltage V can be written as follows:[35]

$$I_d \propto \left[\exp\left(\frac{-eV}{nk_BT}\right) - 1\right] \quad (1)$$

where e, k_B and T have their standard meaning and n is an ideality factor accounting for the recombination enhancement by defects that typically ranges between 1 and 2. Fitting eqn (1) to data in Fig. 2a yields $n \sim 2.4$ for the whole curve and $n \sim 1.9$ for $|V| < 100$ mV (ESI†). Such high values of n probably arise from non-uniformities in the distribution of fixed charges[34] showing in any case that our protein diode is far from being an ideal junction ($n = 1$).

One could wonder whether the rectification mechanism depicted above requires a specific channel orientation incompatible with that found *in vivo*, given the fact that the actual direction of insertion of OmpF when reconstituted *in vitro* into planar bilayers is still unclear.[36] Control experiments indicate that in the protocol employed here the protein inserts almost always (>95%) in a particular orientation,[17,37] whichever it is. Fig. 2c shows the *I–V* curves of OmpF in the reverse configuration to that shown in Fig. 2a, this is to say 7||3 for DPhPS||DPhPC membranes. Rectification appears just in the opposite direction to the 3||7, now the current being higher under positive applied voltages. The inset shows the superposition of the absolute values of the current in both configurations (7||3 and 3||7), which are not identical but pretty similar suggesting that orientation of the channel is not a critical issue.

Similarly to most ion channels and nanopores,[12,38–42] an OmpF channel is not ideally selective,[36,37] so that both cations and anions contribute to the overall current.[21,43] To probe how the ionic selectivity depends on solution acidity and the electrolyte concentration we calculated the permeability ratio (P_-/P_+)[44] from the measured reversal potential (potential needed to achieve zero current) in experiments with gradients of both pH and salt concentration, as shown in Fig. 3.

Customarily, ionic selectivity is not reversed when inverting the direction of the concentration gradient.[36] This is the situation that we find in the 2||7 configuration. However, for values of pH_{cis} between 2.4 and 3 we find a different scenario. The influx of cations is preferred when $c_{cis} > c_{trans}$ but this is overturned when $c_{cis} < c_{trans}$, the outflux of anions being favored. The fact that $c_{cis} \neq c_{trans}$ yields different screening on each channel mouth changes the conductive properties of the channel. Fig. 4 shows the *I–V* curves and rectification ratios for the 2.7||7 configuration under the conditions as shown in Fig. 3. The shape of the *I–V* curves is similar in both salt gradi-

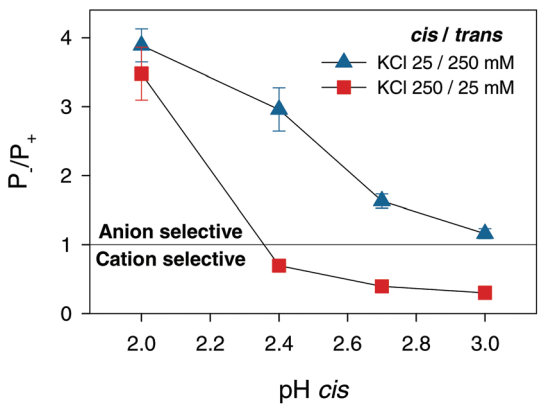

Fig. 3 Dependence of OmpF selectivity, presented as the permeability ratio (P_-/P_+), with pH of the *cis* side. The lipids used were DPhPC at the *cis* side and DPhPS at the *trans* side. The pH at the *trans* side was always 7 and the pH at the *cis* side was varied between 3 and 2.

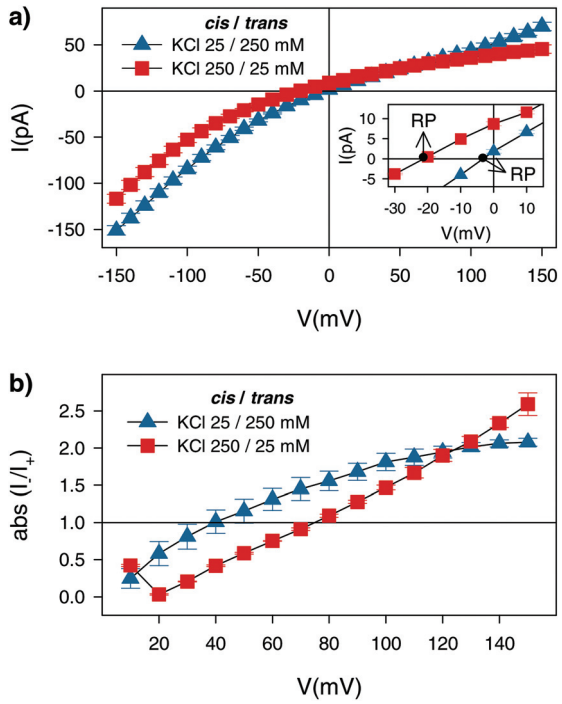

Fig. 4 (a) OmpF single-channel *I*–*V* curves in the pH 2.7 DPhPC||pH 7 DPhPS configuration for two reversed salt concentration ratios. Inset: Zoom highlighting the negative reversal potentials (RP) obtained. (b) Rectification ratio ($r = |I_{-V}/I_{+V}|$) dependence on the applied voltage for the same conditions.

ents (Fig. 4a), with slightly higher currents for negative applied voltages. This means that the current rectification (Fig. 4b) is modulated by the concentration gradient employed so that the rectification ratios are considerably lower than those in the case of symmetrical 25 mM solutions (Fig. 2b).

To probe the transport of ions using colored noise, we apply different kinds of zero mean fluctuating potentials to the OmpF channel for the pH 2.7||7 configuration and concentration gradient 25||250 mM. Random telegraph noise (RTN) is used to simulate biologically-relevant signals as those generated by the time-dependent oscillation of chemical reactions[45] or the current and voltage fluctuations in cells due to the stochastic opening and closing of channels in the membrane.[46] Fig. 5 shows single-channel current traces showing the response to a RTN signal (red lines) with Gaussian-distributed voltages and different characteristic lifetimes. Interestingly, the output signal is slave of the input one. This indicates that the characteristic response time of the system is much shorter than the period of the signals considered here (typically in the range of milliseconds)[21] so that the average current obtained for the whole signal does not depend on the pulse lifetime.

In addition to RTN with Gaussian-distributed amplitudes, we probed the response of the system to a RTN with constant amplitude, and a random noise (RN) (Fig. 6). RN simulates thermal noise, also known as Johnson or Nyquist noise, originated from dissipative sources of fluctuations and expected from any conductor.[24]

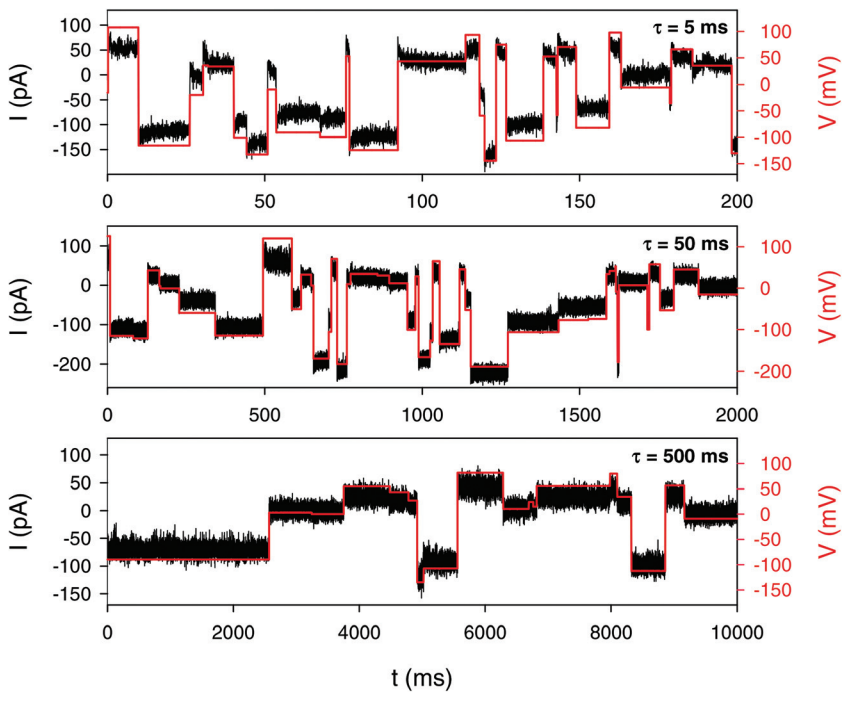

Fig. 5 Single-channel current traces (black lines) showing the response to a RTN signal (red lines) with Gaussian-distributed voltages (σ = 100 mV) and different characteristic lifetimes (5 ms, top panel; 50 ms, middle panel; 500 ms, bottom panel). Traces are shown at the originally measured sampling frequency (50 kHz) except for the trace at the bottom panel, which is displayed at a 5 kHz sampling frequency. Experimental conditions: KCl 25 mM pH 2.7 DPhPC *cis*||250 mM pH 7 DPhPS *trans*.

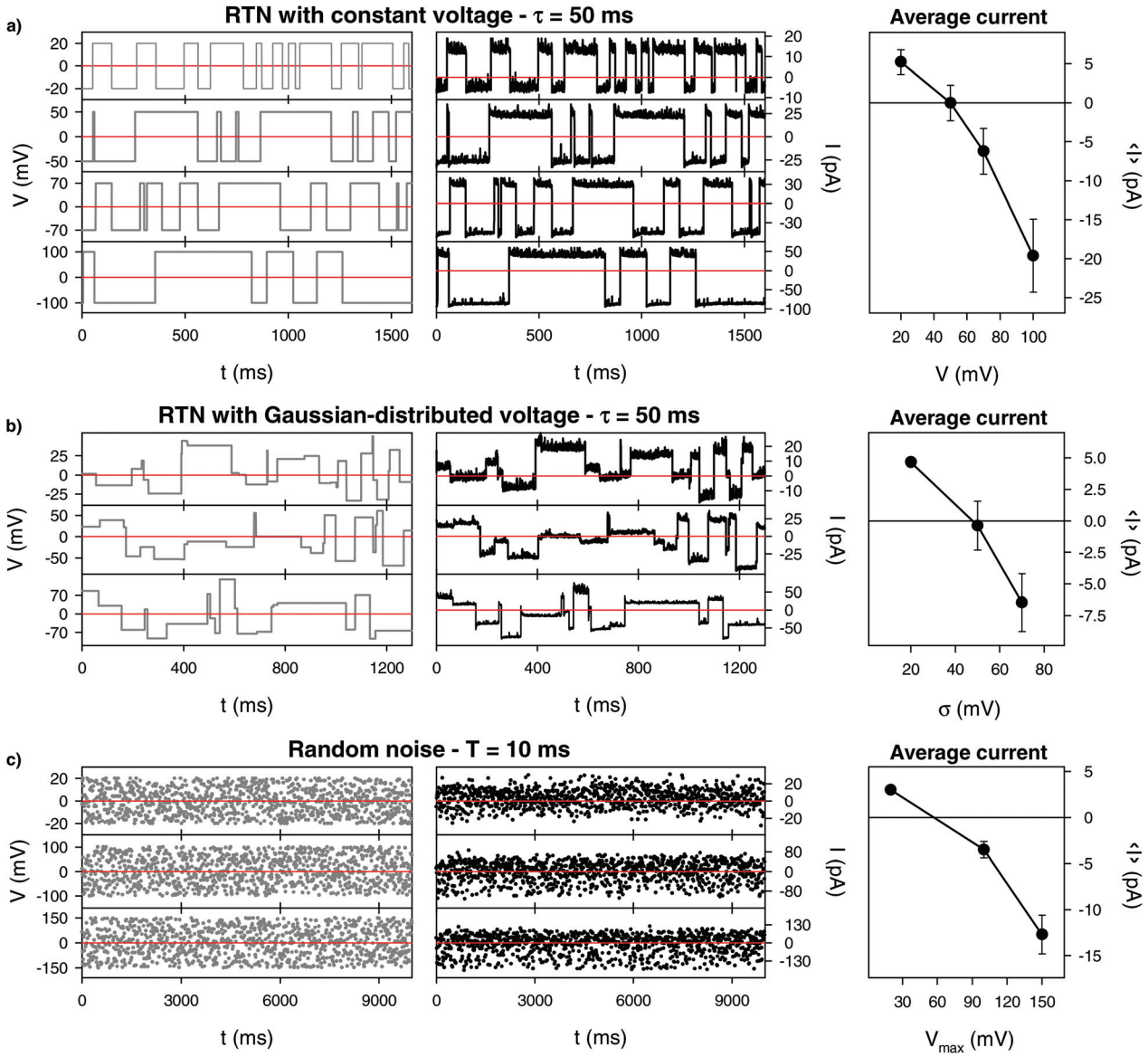

Fig. 6 Single-channel current traces (black lines/dots) showing the response to different types of input voltage signals (grey lines/dots), together with the corresponding calculated average current $\langle I \rangle$. (a) RTN with constant voltage (V = 20, 50, 70, and 100 mV) and 50 ms lifetime. (b) RTN with Gaussian-distributed voltages (σ = 20, 50, and 70 mV) and 50 ms lifetime. (c) RN with different maximum voltages (V_{max} = 20, 100, and 150 mV) and 10 ms period. Traces have been filtered at 1 kHz using a digital 8-pole Bessel filter, except for the trace in (c), which is displayed at the recorded 100 Hz sampling frequency without further filtering. Experimental conditions: KCl 25 mM pH 2.7 DPhPC *cis*||250 mM pH 7 DPhPS *trans*.

Fig. 6a and b show RTN (exponentially-distributed lifetime) with constant and Gaussian-distributed amplitudes, respectively, and Fig. 6c shows RN. The current traces are presented together with the corresponding calculated average current $\langle I \rangle$. In contrast to its non-dependence on the pulse lifetime, the average current is absolutely dictated by the potential amplitude distribution. Smallest values correspond to RN whereas largest ones correspond to RTN with constant voltage. Hence, when using RN, the average current changes from positive to negative (a necessary condition to obtain ion pumping)[21] at $V_{max} \sim 70$ mV, while a 50 mV amplitude is enough to reverse the sign when a RTN with constant voltage is applied.

In the case of RTN with Gaussian-distributed amplitudes, significant negative average currents are obtained when $\sigma >$ 50 mV. Because approximately 30% of the voltage values in this distribution are higher than σ, the actual average potential applied in this case to obtain negative $\langle I \rangle$ is much higher than for RTN with constant amplitude.

Next, we wish to know if oscillating signals could lead to directional uphill transport. To this end, we analyze the response of the system to a RTN with constant amplitude under two opposite KCl concentration ratios in the pH 2.7||7 configuration, as shown in Fig. 7. In both cases the average current changes from positive to negative values with

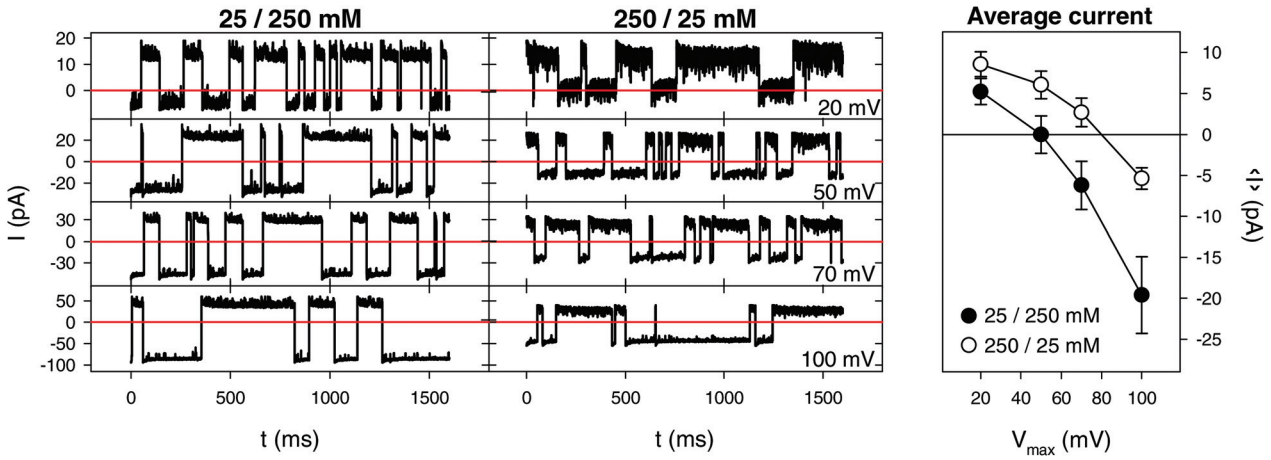

Fig. 7 Single-channel current traces showing the response to a RTN signal (τ = 50 ms) with constant voltage together with the corresponding calculated average current $\langle I \rangle$, for two concentration ratios: r = 0.1 (KCl 25/250 mM) and r = 10 (KCl 250/25 mM). Traces were filtered at 1 kHz using a digital 8-pole Bessel filter. Experimental conditions: pH 2.7 DPhPC *cis*||pH 7 DPhPS *trans*.

increasing V_{max}, meaning different things in each case. Thus, when the diluted solution is in the *trans* side the channel is selective to cations (see Fig. 3) so that $\langle I \rangle < 0$ means that cations could be transported from *trans* to *cis* against their external concentration gradient. In contrast, when the diluted solution is in the *cis* side the channel is selective to anions (see Fig. 3) and then $\langle I \rangle < 0$ could imply the uphill transport of anions (see Fig. 3) from *cis* to *trans* against their external concentration gradient (see ref. 21 for extended discussion).

These results may have interesting implications for pH homeostasis in bacterial cells. It is known that *E. coli* cannot maintain internal pH two units over the external one.[22] So, when bacterial cells are subjected to extreme acid stress there should be an influx of protons which decreases the periplasmic pH inhibiting the metabolic activity and triggering the acid resistance mechanisms like antiport exchange associated with decarboxylase activity.[22,29,47] Interestingly, it has been reported that *E. coli* outer membrane porins (OMPs) like OmpF contribute to the acid stress response by controlling the proton influx in several ways, either decreasing gradually the pore conductance by the selective titration of certain residues[48] or by promoting the binding of polyphosphate or cadaverine causing the channel block.[49–51] However, it is unclear whether protons use membrane channels as the principal permeation pathway or they just cross damaged lipid bilayers.[22] Given that the maintenance of proton gradients is essential to perform work such as rotating flagella or generating ATP[22,52,53] it seems unlikely that such a delicate function could be performed exclusively by using passive filters like defects or holes in membranes. It is more plausible that proton transport requires that membrane pores could act as externally activated valves allowing fine-tune permeation mechanisms. In this sense, it is tempting to speculate that the above reported directional selectivity of OmpF (or other similar mechanisms) may play a role promoting or impeding the transport of particular charged species, either passively downhill or uphill taking advantage of the fluctuating electric potentials characteristic of living cells. Appealingly, the fact that non-equilibrium fluctuations can cause a protein to cycle through several conformations would explain the high efficiencies observed for some biological energy-transduction processes.[54]

Conclusions

We use electrochemical rectification of the OmpF channel current as a ratchet mechanism to obtain uphill transport of ions with the only aid of fluctuating electric potentials similar to those found in living cells. We find that depending on the direction of the concentration gradient the channel can pump either cations or anions from the diluted side to the concentrated one. Our results provide new insights into the pH homeostasis in bacterial cells and also offer interesting clues for the understanding of active transport. Acid resistance mechanisms occurring in the inner membrane involve remarkable fluctuations of the transmembrane potential and require the influx and outflux of diverse substances catalyzed by amino acid antiporters and chloride transports. We hypothesize that OMPs could participate in the equilibration process of ion imbalances occurring in the periplasm and the extracellular media by allowing a variety of permeation mechanisms that could not be accomplished by membrane holes or defects. OMPs could not only discriminate the passive diffusive transport but also benefit from transmembrane potential fluctuations to operate like directional ion pumps.

Acknowledgements

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References

1 R. D. Astumian, P. B. Chock, T. Y. Tsong, Y. D. Chen and H. V. Westerhoff, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 434–438.

2 H. V. Westerhoff, T. Y. Tsong, P. B. Chock, Y. D. Chen and R. D. Astumian, *Proc. Natl. Acad. Sci. U. S. A.*, 1986, **83**, 4734–4738.

3 R. D. Astumian, *Phys. Chem. Chem. Phys.*, 2009, **11**, 9592–9594.

4 Z. S. Siwy, M. R. Powell, E. Kalman, R. D. Astumian and R. S. Eisenberg, *Nano Lett.*, 2006, **6**, 473–477.

5 T. Reichenbach and A. J. Hudspeth, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 4973–4978.

6 J. Cervera, P. Ramirez, S. Mafe and P. Stroeve, *Electrochim. Acta*, 2011, **56**, 4504–4511.

7 G. A. Griess, E. Rogers and P. Serwer, *Electrophoresis*, 2001, **22**, 981–989.

8 J. C. T. Eijkel and A. Van Den Berg, *Microfluid. Nanofluid.*, 2005, **1**, 249–267.

9 L. D. Mosgaard, K. A. Zecchi, T. Heimburg and R. Budvytyte, *Membranes*, 2015, **5**, 495–512.

10 Z. S. Siwy and A. Fuliński, *Phys. Rev. Lett.*, 2002, **89**, 198103.

11 Z. S. Siwy and A. Fuliński, *Am. J. Physiol.*, 2004, **72**, 567.

12 J. Cervera, B. Schiedt, R. Neumann, S. Mafé and P. Ramírez, *J. Chem. Phys.*, 2006, **124**, 104706.

13 J. Cervera, A. Alcaraz, B. Schiedt, R. Neumann and P. Ramirez, *J. Phys. Chem. C*, 2007, **111**, 12265–12273.

14 C. Verdiá-Báguena, M. Queralt-Martín, V. M. Aguilella and A. Alcaraz, *J. Phys. Chem. C*, 2012, **116**, 6537–6542.

15 A. H. Delcour, *Front. Biosci.*, 2003, **8**, D1055–D1071.

16 H. Nikaido, *Microbiol. Mol. Biol. Rev.*, 2003, **67**, 593–656.

17 M. Queralt-Martín, C. Verdiá-Báguena, V. M. Aguilella and A. Alcaraz, *Langmuir*, 2013, **29**, 15320–15327.

18 H. Miedema, M. Vrouenraets, J. Wierenga, W. Meijberg, G. Robillard and B. Eisenberg, *Nano Lett.*, 2007, **7**, 2886–2891.

19 A. Alcaraz, P. Ramírez, E. García-Giménez, M. L. López, A. Andrio and V. M. Aguilella, *J. Phys. Chem. B*, 2006, **110**, 21205–21209.

20 E. García-Giménez, A. Alcaraz, V. M. Aguilella and P. Ramírez, *J. Membr. Sci.*, 2009, **331**, 137–142.

21 M. Queralt-Martín, E. García-Giménez, V. M. Aguilella, P. Ramirez, S. Mafe and A. Alcaraz, *Appl. Phys. Lett.*, 2013, **103**, 043707.

22 J. W. Foster, *Nat. Rev. Microbiol.*, 2004, **2**, 898–907.

23 D. S. Liu, R. D. Astumian and T. Y. Tsong, *J. Biol. Chem.*, 1990, **265**, 7260–7267.

24 L. J. DeFelice, *Introduction to Membrane Noise*, Springer US, Boston, MA, 1981.

25 A. Alcaraz, E. M. Nestorovich, M. L. López, E. García-Giménez, S. M. Bezrukov and V. M. Aguilella, *Biophys. J.*, 2009, **96**, 56–66.

26 J. Gorden and P. L. Small, *Infect. Immun.*, 1993, **61**, 364–367.

27 M. P. Castanie-Cornet, T. A. Penfound, D. Smith, J. F. Elliott and J. W. Foster, *J. Bacteriol.*, 1999, **181**, 3525–3535.

28 J. Lin, I. S. Lee, J. Frey, J. L. Slonczewski and J. W. Foster, *J. Bacteriol.*, 1995, **177**, 4097–4104.

29 F. Diez-Gonzalez and J. B. Russell, *Microbiology*, 1997, **143** (Pt 4), 1175–1180.

30 K. N. Jordan, L. Oxford and C. P. O'Byrne, *Appl. Environ. Microbiol.*, 1999, **65**, 3048–3055.

31 H. Tyäuble, M. Teubner, P. Woolley and H. Eibl, *Biophys. Chem.*, 1976, **4**, 319–342.

32 H. Nikaido and M. Vaara, *Microbiol. Rev.*, 1985, **49**, 1–32.

33 A. Bessonov, J. Y. Takemoto and F. C. Simmel, *ACS Nano*, 2012, **6**, 3356–3363.

34 A. Jain and A. Kapoor, *Sol. Energy Mater. Sol. Cells*, 2005, **85**, 391–396.

35 P. A. Gurnev and S. M. Bezrukov, *Langmuir*, 2012, **28**, 15824–15830.

36 A. Alcaraz, E. M. Nestorovich, M. Aguilella-Arzo, V. M. Aguilella and S. M. Bezrukov, *Biophys. J.*, 2004, **87**, 943–957.

37 E. M. Nestorovich, T. K. Rostovtseva and S. M. Bezrukov, *Biophys. J.*, 2003, **85**, 3718–3729.

38 J. E. Hall, I. Vodyanoy, T. M. Balasubramanian and G. R. Marshall, *Biophys. J.*, 1984, **45**, 233–247.

39 E. Gouaux, *J. Struct. Biol.*, 1998, **121**, 110–122.

40 Z. S. Siwy and S. Howorka, *Chem. Soc. Rev.*, 2010, **39**, 1115–1132.

41 X. Hou, W. Guo and L. Jiang, *Chem. Soc. Rev.*, 2011, **40**, 2385–2401.

42 M. Colombini, *Biochim. Biophys. Acta*, 2012, **1818**, 1457–1465.

43 V. M. Aguilella, M. Queralt-Martín, M. Aguilella-Arzo and A. Alcaraz, *Integr. Biol.*, 2011, **3**, 159–172.

44 N. Lakshminarayanaiah, *Equations of Membrane Biophysics*, Academic Press, New York, 1984.

45 T. D. Xie, P. Marszalek, Y. D. Chen and T. Y. Tsong, *Biophys. J.*, 1994, **67**, 1247–1251.

46 A. Fuliński, Z. Grzywna, I. Mellor, Z. Siwy and P. Usherwood, *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.*, 1998, **58**, 919–924.

47 D. De Biase and P. A. Lund, *Adv. Appl. Microbiol.*, 2015, **92**, 49–88.

48 A. Alcaraz, M. Queralt-Martín, E. García-Giménez and V. M. Aguilella, *Biochim. Biophys. Acta, Biomembr.*, 2012, **1818**, 2777–2782.

49 H. Samartzidou and A. H. Delcour, *J. Bacteriol.*, 1999, **181**, 791–798.

50 H. Samartzidou, M. Mehrazin, Z. Xu, M. J. Benedik and A. H. Delcour, *J. Bacteriol.*, 2003, **185**, 13–19.

51 U. Kanjee and W. A. Houry, *Annu. Rev. Microbiol.*, 2013, **67**, 65–81.

52 R. M. Macnab and S. I. Aizawa, *Annu. Rev. Biophys. Bioeng.*, 1984, **13**, 51–83.

53 M. Yoshida, E. Muneyuki and T. Hisabori, *Nat. Rev. Mol. Cell Biol.*, 2001, **2**, 669–677.

54 R. D. D. Astumian, *Appl. Phys. A: Mater. Sci. Process.*, 2002, **75**, 193–206.