CONTRIBUTIONS to SCIENCE
OPEN ACCESS

CONTRIB SCI 11:181-188 (2015)
doi:10.2436/20.7010.01.229

SOFT MATTER
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Ion transport through biological channels

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Summary. The transport of ions across single-molecule protein nanochannels is important both in the biological context and in proposed nanotechnological applications. Here we discuss these systems from the perspective of non-equilibrium physics, and in particular, whether the concepts underlying the physics of diffusive and electrokinetic transport can be employed to predict and understand these systems. [**Contrib Sci** 11(2): 181-188 (2015)]

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A brief introduction to biological ion channels

All living cells are immersed in a solution of salts and separated from the external environment by the cell membrane. To precisely regulate the entry and exit of ions and other molecules, cells are equipped with structures that control ion passage bidirectionally. These remarkable nanostructures are made up of proteins that make subtle use of the principles of non-equilibrium physics to achieve their essential functions. There are two different types of ion transport across the cell membrane: active transport by ion pumps or ion transporters requires energy input from the cell, whereas passive transport across selective ion channels occurs without the direct consumption of energy.

Active transport acts against the natural flow of ions. A classical example is the sodium-potassium pump, discovered in 1957 by Jens Christian Skou (Nobel Prize in Chemistry in 1997). This pump is responsible for maintaining a high concentration of K^+ ions and a relatively low concentration of Na^+

Keywords: ionic transport · protein channels · non-equilibrium physics · Poisson-Nernst-Planck equation · molecular dynamics

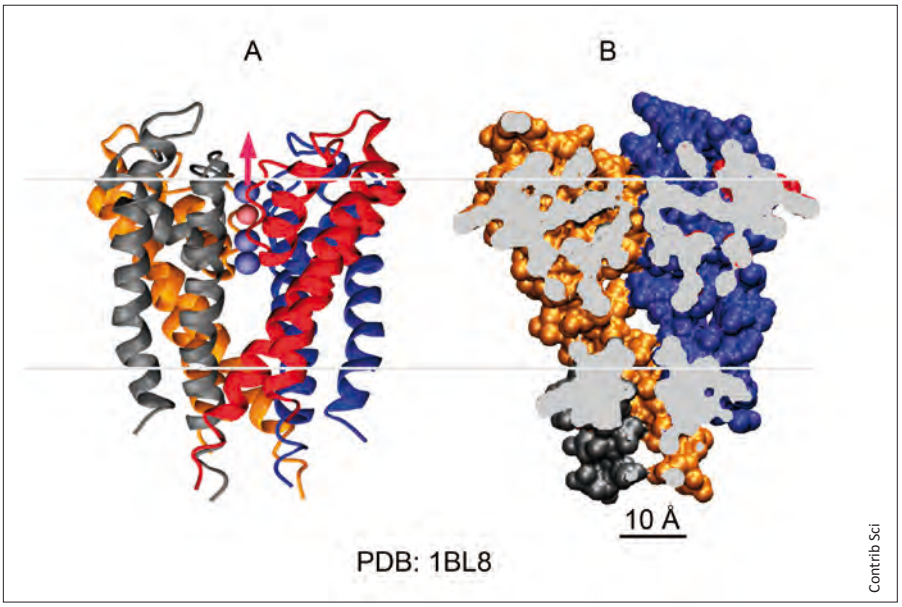

Fig. 1. Lateral view of the central region (the so-called selectivity filter) of the potassium channel (KSCA) of Streptomyces lividans (Protein Data Bank accession code: 1BL8). (**A**) Cartoon view of the channel inserted in the cell membrane (indicated by horizontal lines). There are three potassium ions (blue spheres) in the channel, together with an oxygen atom from a hydration water molecule (red sphere), as found in the structure obtained by X-ray. The red arrow indicates the transmembrane direction followed by the ions. (**B**) Surface representation of the potassium channel using a 0.7Å probe radius. The orientation and color code are the same as in the left panel. To visualize the narrow permeation pathway available to ions, a cut has been made near the permeation pathway, in a plane perpendicular to the observer's view.

ions inside the cell. In each cycle, the pump transports two K^+ from the exterior into the cell and releases three intracellular Na^+ to the exterior. During this exchange, energy is supplied by the hydrolysis of one ATP molecule. The pump is responsible for maintaining a concentration gradient of sodium and potassium ions across the cell membrane. This gradient is essential for many biological functions and for establishing the resting electrical potential of the cell membrane. Active transport accounts for a substantial part of the energy budget of animal cells, but a full understanding of the energetic requirements of active transport remains elusive [18,19].

In passive transport, ions flow across *ion channels* by following the spontaneous flow dictated by gradients of concentration and electrostatic potential [17]. Ion channels are proteins embedded in the cell membrane; they form hydrophilic channels connecting the extracellular and intracellular environment. The importance of ion channels can be appreciated by considering that a significant fraction of DNA-encoded proteins are ion channels, which is the reason for the many diseases linked to their abnormal functioning (known as *channelopathies* [14]). An important property of ion channels is their selectivity: only certain ions are transported across the channel. For example, only K^+ ions can significantly cross potassium channels, a family of channels widely found among organisms [16].

Figure 1 shows an example of the potassium channel KSCA from *Streptomyces lividans*. This extremely narrow protein channel allows the passage of ions in single file. Other ion channels are less specific as they are selective for particular kinds of ions, for example, cations or anions. This class of channels includes outer membrane bacterial porin F (OmpF) from *Escherichia coli* (Fig. 2). OmpF is a relatively wide channel in that it allows the simultaneous permeation of both cations and anions (in hydrated form) as well as the entry of relatively large molecules, such as antibiotics and polymers. In the case of monovalent electrolyte solutions (KCl, NaCl, LiCl, CsCl) the channel has slight cationic selectivity, i.e., the current from cations is larger than that from anions [1,2]. Interestingly, the channel blocks the passage of multivalent cations (such as Mg^{2+} or La^{3+}), in which case the current is due only to anions [3]. These two channels are representative of the narrow and wide ionic channels that have been extensively studied, both experimentally and theoretically. The basic transport concepts emerging from these studies are the subject of this article.

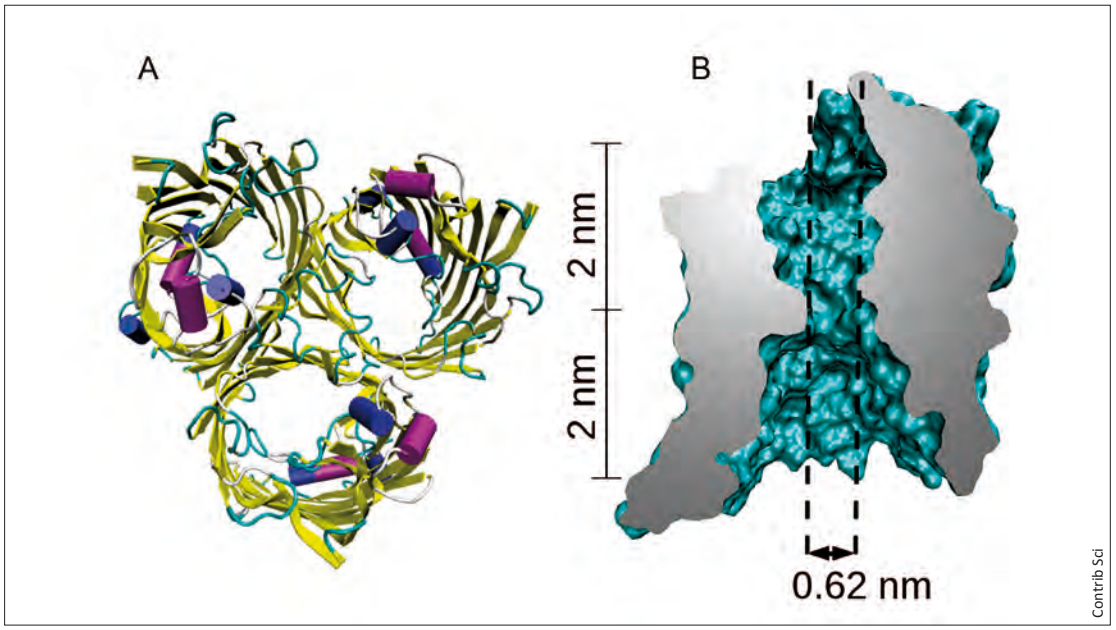

Fig. 2. Trimeric OmpF protein channel from *Escherichia coli* (Protein Data Bank accession code: 2OMF). (**A**) Top view of a cartoon representation of the trimeric protein channel. (**B**) Surface representation of one of the channels of the trimeric protein. The sizes of the protein and the narrow constriction zone are shown.

Molecular dynamics simulations of ion channels

Molecular dynamics (MD) simulations consist of solving numerically (using a computer) the Newtonian equations of motion in a system made up of a large number of atoms, taking into account their mutual interactions and external thermodynamic constraints (such as the presence of a barostat or a thermostat) and/or external fields (such as an applied electric field). In the case of ion channels, this technique is relevant as long as the 3D structure of the channel and the atomic details thereof are available. Due to the impressive advances in protein crystallography, atomistically resolved structures are available for a large number of channels (at the time of this writing, the Protein Data Bank database has 3882 entries for ion channels). These can be investigated in MD simulations, as a kind of computational microscope, to obtain dynamic images of the respective systems and their transport processes at the atomic level. Typical simulation scales are of the order of 10^5 atoms, tens of nm, and hundreds of ns [10]. These scales allow studies of ion-channel interactions, ion kinetics, and calculations of the conductivity properties of the channels. However, because these simulations require huge amounts of computer power, they cannot be employed to exhaustively analyze the behavior of a protein channel under different conditions (for example, different ion concentrations). Rather, these MD simulations are, at least thus far, limited to investigating specific, fundamental questions. More extensive calculations can then be performed by combining the results of MD simulations with theoretical approaches or by employing other simulations that use less precise but also less computationally demanding techniques, such as Brownian dynamic simulations [8]. The results of these MD simulations provide insights into the physical mechanisms of ion transport across ion channels.

A minimal model of an ion channel

To gain a better understanding of the physical properties of ion channels, it is instructive to consider a minimalistic model (Fig. 3), in which the ion channel is modeled as a cylindrical pore of known radius *a* through a low dielectric medium of width *L* (the membrane). The low dielectric constant of the membrane ($\varepsilon_r \sim 2$–3) presents a strong barrier to the passage of electrical charges, so that ions are forced to cross through the pores. In our simplified channel model, selectivity is achieved primarily by the fixed charges anchored to the channel. These electric charges exert repulsive forces on ions of the same sign, reducing their relative numbers inside the channel, a property known as *electrostatic exclusion*. The opposite occurs with charges of opposite sign, and their concentration in-

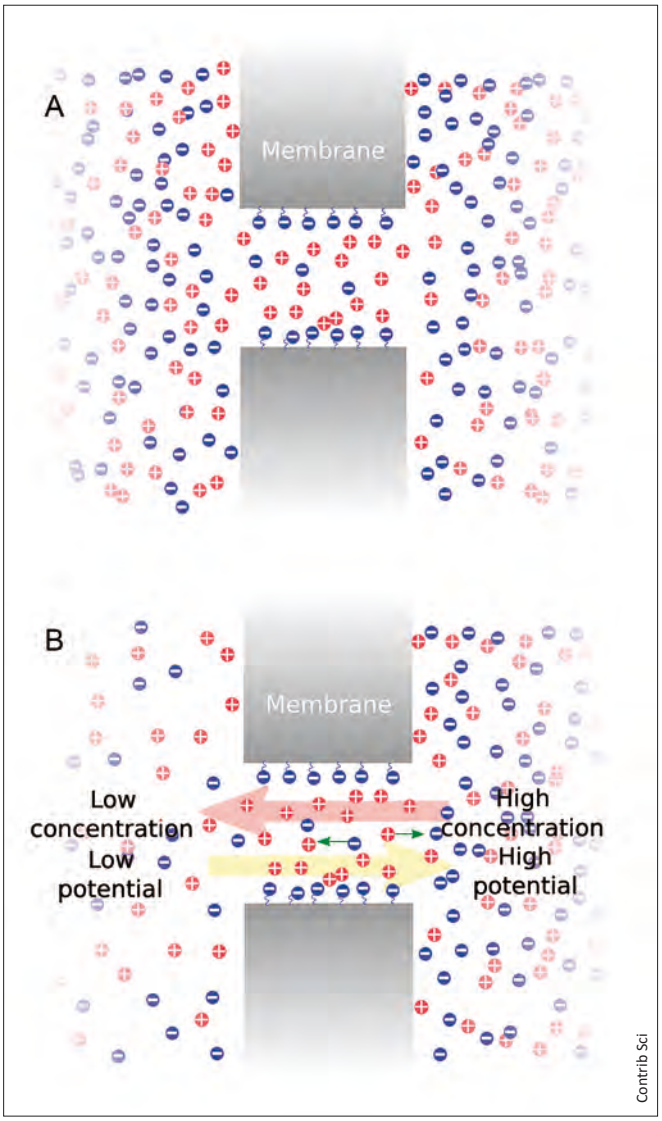

Fig. 3. A simple model of an ionic channel in equilibrium and in the presence of concentration and electric potential gradients. (**A**) A low dielectric region representing the cell membrane (gray area) is traversed by an aqueous pore channel in equilibrium with an ionic solution. The ions in the solution region are represented by blue (negative charge) and red (positive charge) spheres with a charge sign. Some of the negative charges are anchored to the pore wall (blue anchored spheres), causing ion selection inside the pore channel and thus exclusion (co-ions) or favoring (counter-ions) of ions from the ionic solution, according to their electric charge. This leads to a passive charge selectivity of the channel. (**B**) Same as (A) but now in the presence of concentration and electrostatic potential gradients. A concentration gradient through the system creates a net flow of ions from the side with a higher concentration to the side with a lower concentration (the light red arrow in the pore region). An electric field that is axially applied through the system (light yellow arrow) causes the positive and negative ions to move in opposite directions (green arrows).

side the channel increases (Fig. 3A). If this channel is placed in contact with an electrolyte-containing aqueous solution (e.g., KCl), then in the equilibrium state the concentrations of anions and cations will be different (Fig. 3A). Due to the negative charge of the channel, there will be an excess of K^+ ions over Cl^- ions in order to compensate for the fixed charge present in the channel walls. Thus, a positively charged channel becomes an *anion-selective* channel, and a negatively charged channel a *cation-selective* channel. However, the effect of the wall charges is only relevant at distances sufficiently small from the charged wall and declines rapidly with distance.

The passive transport of ions can be induced by a concentration gradient, an electrostatic potential difference, or both (Fig. 3B). Cells are characterized by substantial concentration gradients of ions, such as K^+, Na^+, and Cl^-, and membrane potentials of the order of a few hundreds of mV are common. In the laboratory, larger concentration and/or potential gradients can be easily induced by the experimenter. From a physical standpoint, passive transport through ion channels can be described, in the continuum approach, as the movement of ion species in response to the electrochemical potential gradient. The concentration gradient induces an ion flux in the direction of the low concentration, proportional to the concentration gradient and to the diffusion coefficient D, as dictated by Fick's law of diffusive transport. The presence of an electrostatic potential difference gives the ions a drift velocity in the same or in the opposite direction of the electric field, depending on whether the ion is positively or negatively charged and proportional to the gradient of the electrostatic potential. Mathematically, the electrodiffusion of an ion species is described by the Nernst-Planck equation
(Eq. 1):

$$\frac{\partial c}{\partial t} = -\nabla(D\nabla c + \frac{Dze}{k_B T} c\nabla\varphi) \qquad (1)$$

where c is the local concentration of electrolyte, D is the diffusion coefficient, e is the elementary charge, k_B is the Boltzman constant, T is the absolute temperature, and z is the valence of the ion species. The Nernst-Planck equation contains two terms: the first corresponds to diffusion and is also known as the Fick equation, while the second describes the motion of charged ions under the influence of electric fields. Eq. (1), together with the Poisson equation (Eq. 2)

$$\nabla(\varepsilon\nabla\varphi) = -\rho \qquad (2)$$

describing the connection between the electrostatic potential (φ) and its sources, i.e., the electric charge density (ρ), forms a closed system of equations known as the Poisson-Nernst-Planck equations (PNP). The surface charge density σ of the channel walls also enters into the PNP equations as a boundary condition. Before the advent of computers, the system de-

scribed by the PNP equations was solved by applying various simplifying approximations, such as the existence of a constant electric field across the membrane (sometimes referred to as the Goldman hypothesis); the important theoretical results obtained in this way are still widely used today. One of the most famous equations is that of Goldman-Hodgkin-Katz (GHK), for the resting potential across a membrane [11]. The advent of computers allowed the numerical resolution of the differential equations comprising the PNP equations. Today, it is possible to numerically solve the equations for a system such as the one described in Fig. 3B using a standard personal computer.

Real channels are more complex than the simple illustration provided in Fig. 3 (compare, for example, Fig. 3 with Figs. 1 and 2). Nonetheless, Eqs. (1) and (2) can be solved such that they include all the structural information of the protein in its full three dimensions, without a substantial increase in the computational cost. However, there are other simplifications present in the PNP equations that have prompted the search for fixes for some of the resulting shortcomings. Among these fixes are the inclusion of the finite size of the ions [15], as steric effects (although there already are generalizations to incorporate correlations between ions), and improved numerical algorithms based on finite element, finite volume, etc. [6]. The continuum approach, implicit in Eqs. (1) and (2), assumes that the system under study is much larger than the typical distance between its elementary constituents, that is, the atoms, ions, and molecules forming the system, and thus can be described mathematically by fields. This approach, however, may be a bit harsh, given that ion channels have dimensions in the nanometer scale, which is only an order of magnitude greater than the size of many atomic species.

The question is how this complexity affects the validity of the basic physical principles and to what extent it is relevant for the prediction of transport. This is discussed in the following sections, using results from the PNP equations and from MD simulations.

The physics of transport in wide ion channels

OmpF provides a useful model of a wide ion channel (Fig. 2). Like other wide channels it has a net charge (at pH 7, the charge of an OmpF pore is about –11e), due to the complex arrangement of the local positive and negative charges from amino acids. Some of these charges are located at the surface while others are buried inside the protein but still influence the ions. In these channels, the *electrostatic exclusion* mechanism described schematically in Fig. 3A is operative. For example, computer simulations [10,13] have shown that in the presence of 1 M KCl the inner aspect of the OmpF channel has an average of 7 Cl^- anions and 11.8 K^+ cations (Table 1). In other words, outside the channel, there is one K^+ cation for each Cl^- anion but inside the channel there is an average of 1.68 cations (K^+) for each Cl^- ion. Under an applied voltage, the contribution of K^+ to the current is larger than that of Cl^-, but the ratio is 1.4, which is smaller than expected based on the channel population. This is due to a greater reduction of the K^+ mobility inside the channel.

The concept that the excess of cations inside the channel is due to electrostatic compensation of the protein charge (charge neutralization) can be further tested by considering mutants of this protein channel. In the OmpF-CC mutant, two negatively charged amino acids (one aspartic and one glutamic acid) present inside the channel are replaced by two neutral cysteine amino acids. In the OmpF-RR mutant, these two negatively charged amino acids are replaced by positively charged arginines. Therefore, the protein charge changes by +2e in OmpF-CC and by +4e in OmpF-RR (vs. the wild type OmpF channel). Our simulations [10] indicate that the charge due to ions inside the mutant channels changes to compensate for these alterations and maintain electroneutrality (Table 1). Inside the OmpF-CC channel, there is an increase of Cl^- and a reduction of K^+ ions to compensate for the increase of +2e of the channel. In OmpF-RR, the increase of +4e is compensated by a concomitant increase in the Cl^- population inside the channel (Table 1). Interestingly, for both mutants the current attributable to K^+ is extremely low and almost all ion transport is due to Cl^- (with a flux of Cl^- similar to that observed for the wild type). In addition to their effect on the total charge (which determines the population of ions), the small changes in the channel wall that are caused by these mutations also strongly impact the mobility of the K^+ ions inside the channel, which is severely reduced. This effect illustrates that the simple relation between the static and dynamic case (compare Fig. 3a and Fig. 3b) is lost in a protein channel. A further illustration of these complexities of ionic channels is seen in the behavior of the channels in response to multivalent cations. In the presence of 1 M $MgCl_2$, there is again an excess of cations over anions inside the channel. In bulk solution, there is one Mg^{2+} cation for each two Cl^- anions, whereas inside the channel there are 1.44 Mg^{2+} cations for each two Cl^- anions. However, because these Mg^{2+} cations are tightly bound to certain anionic amino acids, their mobility is extremely low and almost all the current is due to anions [3].

Table 1. Charge balance inside the OmpF ion channel as computed by molecular dynamics simulations (data from [10]). Qc is the total charge of a (monomer) channel at neutral pH. The charge balance is the difference in charge between the mutant proteins and the wild type (ΔQc) and their corresponding difference in ionic charge inside the channel (ΔQions). All charges are given in units of the elementary charge *e*

	Number of ions inside the channel			Charge balance	
	Qc	Cl^-	K^+	ΔQc	ΔQions
OmpF (wild type)	–11	7.0 ± 0.1	11.8 ± 0.1	–	–
CC mutant	–9	7.73 ± 0.03	11.0 ± 0.5	+2	–1.5 ± 0.7
RR mutant	–7	10.13 ± 0.03	11.5 ± 0.5	+4	–3.4 ± 0.7

Another complexity of ion channels, which is missing in the simple picture provided in Fig. 3, is that not only the total charge of the channel matters for electrostatic exclusion but also the fact that the walls of the channel have patches or regions of opposite charge. This charge distribution creates well-defined regions in which cations or anions are excluded. For example, in computer simulations of OmpF, [10,13], K^+ and Cl^- ions are distributed inside the channel, occupying well-defined regions guided by the charge distribution: cations are located near walls with anionic amino acids and anions near walls with cationic amino acids (Fig. 4). Recently, these highly local electrostatic exclusion effects have been observed experimentally using anomalous X-ray diffraction [9].

Another interesting aspect that can be explored by computer simulations is the nature of the motion of ions inside the protein channel. As noted above, the basic electrodiffusion theory described in the previous section assumes that ion transport can be described by the superposition of a diffusive motion (characterized by the diffusion coefficient D) and an electrostatic drift resulting from the applied voltage difference. However, given the complex interactions between ions and channel walls, the motion of atoms is likely to be more complicated. For example, how do we know that the diffusion coefficient D of a given ion inside the channel is equal to the diffusion coefficient measured for this same ion in a simple electrolyte solution? This question has been addressed in many studies, whose results suggest that the diffusion coefficient inside the channel is lower than the diffusion coefficient of the same ion in bulk electrolyte solutions. Our own simulations indicate that, inside the OmpF channel, the diffusion coefficients of K^+ and Cl^- ions are substantially lower than their

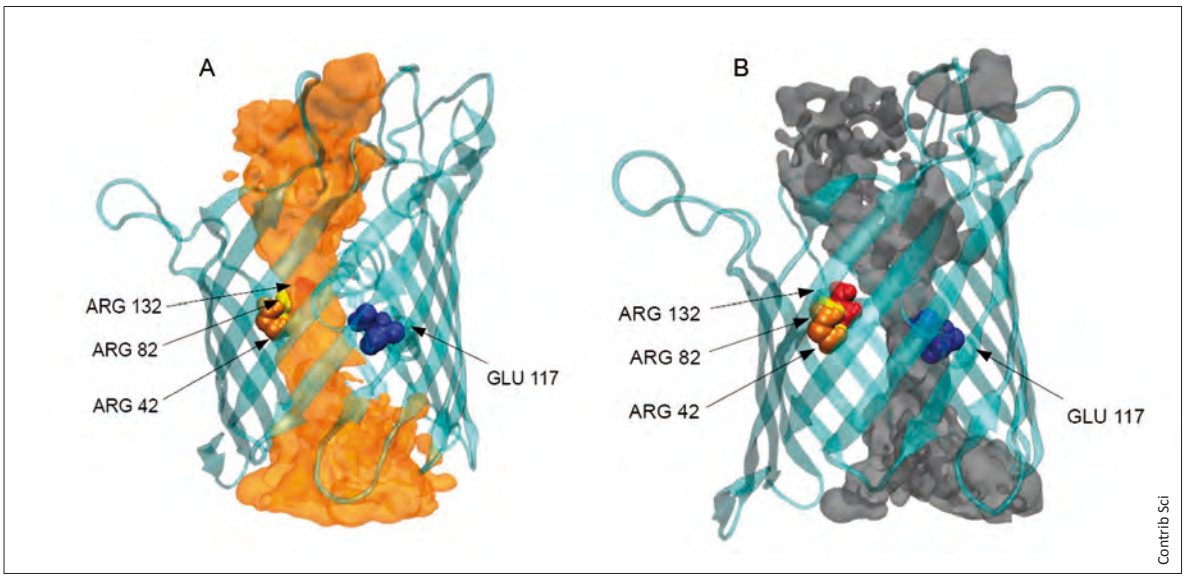

Fig. 4. Different regions occupied by ions inside the OmpF channel (shown as a light blue cage) according to molecular dynamics simulations [10]. (**A**) region occupied by Cl^- ions (shown in orange); (**B**) region occupied by K^+ cations (shown in gray). The charged amino acids (positive Arg and negative Glu) responsible for the ionic distribution (shown in van der Waals representation) are indicated by arrows.

bulk values [5] (by a factor of about 4 in the wider regions of the channel and by a factor of about 10 in the constriction located in the center of the channel). In addition, we were able to show that the drift of the ions in the direction of the applied voltage is strongly influenced by an additional force, due to changes in the geometry of the channel. This is an entropic effect, predicted by non-equilibrium thermodynamics [20], in which ions are dragged toward regions with larger cross-sectional areas.

These atomic-level studies can be complemented by examining several macroscopic aspects. For example, the transport of ions under an applied voltage is usually ohmic in ionic channels, which means that the current intensity (I) and the applied voltage difference (ΔV) follow Ohm's law: $\Delta V = I \times R$, where R is the resistance. In general, it is customary in this type of application to report the conductance, $G = 1/R$. The conductance of a channel for a given concentration of electrolyte solution can be calculated. For example, using MD simulations, after the application of an external voltage the flow of ions can be counted to determine both the stationary current I and the conductance G [10]. These simulations yield reasonably accurate results. For example, in the presence of 1 M KCl, simulations predict [10] a conductance of 2.7 nanoSiemens (nS), in agreement with experimental results.

Other quantities of interest for these channels are more difficult to predict from MD simulations, but they can be predicted with the help of PNP equations. A particularly important example is the reversal potential (RP), which is defined experimentally as follows. In a channel under a concentration gradient, the flow of ions will behave according to Fick's law. The RP is defined as the (external) electrostatic potential difference that has to be applied to counteract the effect of this concentration gradient and to obtain a net current of zero. This is difficult to determine directly from simulations, because it requires the maintenance of a concentration gradient (which is difficult in simulations) and the testing of different voltages to find the RP. However, this is an example of a quantity that can be obtained as a combination of results from MD simulations and a 3D version of the PNP equations that accounts for the detailed channel geometry. From the MD simulations, the binding sites of ions can be determined and suitable values for the diffusion coefficients of ions inside the channel proposed. This information can be entered into a PNP calculation. Figure 5 provides an example of this type of calculation, made for the OmpF channel in the presence of KCl. There is excellent agreement with the experimental results.

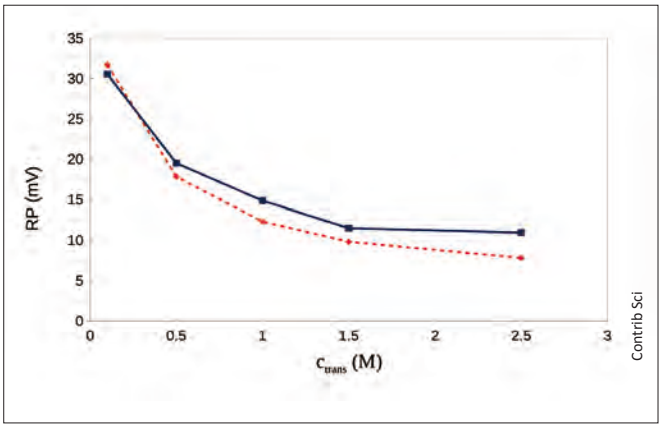

Fig. 5. Reversal potential of the OmpF channel at neutral pH (pH = 7) at different "trans" (extracellular) side KCl concentrations. The concentration on the "cis" (intracellular; negative z coordinates in the Protein Data Bank coordinates) side is maintained to yield a ccis/ctrans ratio of 0.2. The blue line shows the experimental data and the orange points the theoretical data obtained through numerical solutions of the 3D PNP equations [Eqs. (1) and (2) in the text]. The full 3D structure, obtained from the Protein Data Bank (code: 2OMF) was used as input.

The physics of transport in narrow channels

As in the case of wide channels, the basic concepts describing the mechanism of ion transport through narrow channels, such as the potassium channel shown in Fig. 2, need further refinement. Ions cross the channel of very narrow pores in single file, with fewer ions occupying the narrower part of the channel, which acts as a selectivity filter. The transport mechanism in these narrow channels combines the presence of binding sites (due to strong, attractive, and highly specific ion-channel interactions) and ion-ion repulsion. For example, in the case of the KcsA K^+ channel of Fig. 1, conduction proceeds as follows [4]: Inside the channel, there are five specific sites for K^+ ions. The channel has two states, one with two K^+ ions inside the selectivity filter of the channel and another with three K^+ ions (this latter state is shown in Fig. 1). In the state with two K^+ ions, the outer K^+ ion is adsorbed in a deep free energy well near the exit, where it is trapped. As a new ion enters the channel, the three ions became located as shown in Fig. 1 and the strong repulsion of the two other K^+ ions forces the outer K^+ ion to leave the channel. Interestingly, without the two other K^+ ions, this outer K^+ can remain adsorbed indefinitely. Inside the selectivity filter, the K^+ ions are hydrated by a single water molecule (Fig. 1) and full hydration is recovered at the more exterior site. During this very rapid (about 10^8 ions/s) *vacancy transport*, the flexibility

of the protein plays a substantial role, as a rigid protein is unable to correctly accommodate the unsolvated K^+ ions and their transitions between adjacent states.

Mechanisms similar to that of the potassium channel have been determined for other channels, where transport proceeds single file and involves strong ion-channel affinity and ion-ion repulsion. For example, in the transport of Cl^- ions through the CmCLC transporter, a chloride ion channel [7], a single Cl^- ion has a strong affinity for a binding site at the central region of the channel and thus remains adsorbed. The entrance of a second Cl^- induces both a strong repulsion to the previously adsorbed Cl^- ion and the deprotonation of a particular amino acid. The released proton is transported to the exterior of the cell and the two Cl^- ions are transported toward the interior.

Conclusions

Biophysical studies of transport in ion channels have pushed the concepts of nonequilibrium physics to their limits in describing transport processes. In these systems, gradients are extremely large (a 100-mV drop along a 4-nm-thick membrane results in an electric field of 2.5×10^7 V/m) and transport processes occur in extremely narrow regions (such as the narrow constriction zone of OmpF, which is < 1 nm across). Nonetheless, approaches based on diffusion coefficients and classical electrodiffusion theory are still useful. They are complemented by novel techniques such as MD simulations with atomistic resolution, which have also revealed the limits of applicability of non-equilibrium physics. Using these physical techniques will aid biologists in elucidating the relation between the structural details of the proteins and their biophysical mechanisms of operation, the *structure-function relationship.*

Acknowledgements. This work was supported by the Spanish Government (grant FIS2011-1305 1-E) and of University Jaume I grant P1·1B2012-16. All images of protein structures have been made using the free software Visual Molecular Dynamics software [12].

Competing interests. None declared.

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