Letter to the Editor

Forces Determining Ion Permeation

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I would like to address two key issues related to the *Perspectives on Ion Permeation*. The first is the estimate of the size of the physical forces relevant for ion transport. Any good physical model of ion permeation requires the identification of the dominant forces. The second subject concerns a careful definition of the terms "mean field" and "mean field approximation." Several articles in the field of ion permeation show considerable confusion about the mean field concept.

The Size of the Forces

What is the relative size of the forces that ions experience while passing through a channel? To enter the channel ions have to become partly, if not completely dehydrated. Highly polar and even charged functional groups forming the channel walls compensate for this loss of hydration energy. In addition, there are two kinds of strong ion–ion forces: long range electrostatic forces and short range hard core interactions leading to volume exclusion effects.

Considerable simplifications are necessary to compute current–voltage curves of physical model channels. At present only two theories of ion transport are sufficiently simple for direct comparison between theory and experiment. These two theories consider different parts of the relevant forces as the most strong ones. In reaction-rate theories, also designated barrier models, the interaction of the ion with its environment and volume exclusion effects among ions are considered to dominate. Neglecting electrostatic ion–ion interactions, the rates of barrier crossing can be computed from first principles (Laio and Torre, 1999). In contrast, Poisson-Nernst-Planck (PNP) theory assumes that electrostatic forces between the ions determine ion transport.

Reaction-rate theories apply only if environmental forces surpass the electrostatic forces between ions. Otherwise, environmental interactions would not determine ion transport. Consequently, typical energies of electrostatic ion–ion interactions inside the channel

represent lower limits for the energy differences between barriers and wells. Using a simple Coulomb law with a dielectric constant of 10, the energy required to bring two positively charged monovalent ions as close as 0.58 nm requires at least 250 milli electron volts (10 kT). Thus, environmental forces only dominate if the barrier energies are much larger.

Reaction-rate theories explain the saturation of the channel conductance with increasing external concentrations by hard core ion–ion interactions. To experience the short range volume exclusion interactions, the ions must come rather close to each other. The distances of closest approach between ions including a single intermediate water molecule are in the order of 0.3–0.5 nm. Consequently, reaction-rate theories predicting conductance saturation automatically involve strong electrostatic ion–ion interactions. Thus, even single ion channels require large barriers to dominate these electrostatic forces between ions. A more general discussion of the forces important for ion transport was published recently (Syganow and von Kitzing, 1999a).

The identification of the dominant forces is crucial for understanding ion transport. Only those models of ion permeation that include the strongest interactions can provide a reasonable picture of what is going on inside biological ion channels.

Mean Fields and Mean Field Approximation

In his editorial, Andersen (1999) questions the applicability of the mean field approximation for the situation of ions in the narrow pore of the channel: "Finally, notwithstanding the utility of the mean field approximation, is it appropriate for narrow channels that are occupied by only a few ions?" Similar doubts are mentioned in other *Perspectives*. Therefore, a critical inspection of the concepts of mean fields and mean field approximation in statistical physics applied to biological ion channels is timely. Whereas the introduction of mean fields generally does not involve any approximation, the mean field approximation is necessary to account for the nonlinear long-range electrostatic interaction between permeant ions.

During a 10-pA, 1-s channel opening, $6.3 \cdot 10^7$ ions pass the channel. With a time resolution in the millisec-

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ond range, the experimental mean current samples >60,000 ions. Passing the bottleneck of the channel each of those ions will see different forces. Side chains at the channel wall may change their orientations between the passage of two ions, and sometimes they even block the path. Also, the position of other ions differs at different ion passages, resulting in different electrostatic forces. For instance, the forces seen by an ion entering a channel differ considerably whether the channel is occupied by another ion or not. However, these very different forces add up linearly. If the channel is 50% occupied by other ions, on the average the incoming ion sees a half-occupied channel. The average of 10^5 different configurations results in a mean force. The absolute value of the force seen at each passage is generally large compared with that of the mean force. What is important for the measured mean current is the mean force, the linear average over all those very different contributions.

We do not measure mean forces, but their integrals over the paths of the ions. Current–voltage relations represent integral properties of the channel (Syganow and von Kitzing, 1999b). Many particularities of the channel structure are averaged out. This explains why extremely simple theories, such as reaction-rate theories with few barriers or PNP equations without particular structural elements, often can reproduce experimental data. Since we are measuring integral channel properties averaged over many configurations, mean fields are the appropriate physical tools to mathematically describe ion transport through biological ion channels.

Unfortunately, the introduction of mean forces is not sufficient to handle the strong, long-range electrostatic ion–ion interactions. To describe the behavior of plasmas, Vlasov (1938) approximated the mean conditional force seen by a single ion by the mean force, the electric force of the mean charge density. This approximation generalizes the Gouy-Chapman theory (Gouy, 1910; Chapman, 1913) to nonequilibrium systems. What is the difference between the mean force and the conditional mean force? One of the oldest problems in physical chemistry is the nonlinear concentration dependence of the conductivity of electrolytes. In 1926, Onsager (1926, 1927) explained this effect by the difference between the mean force (due to the electric field across the electrolyte) and the conditional mean force (the mean force seen by a single ion). The nonlinear deviation originates from the fact that the motion and distribution of ions in solution is correlated. This correlation leads to two mechanisms: electrophoresis and electropolarization. Each moving ion pushes a part of the solvent molecules, and thus induces hydrodynamic ion–ion forces. This electrophoresis results in a modified effective mobility of the ions in the solvent (Hubbard, 1987). The electric field de-

forms the counter ion cloud around each ion. This polarized counter ion cloud produces a local electric field that shields its host ion from the external electric field. Recently, Lehmani et al. (1997) included ion–ion correlations in the conductivity of ion-exchange membranes with large pores.

Also within biological ion channels, one should expect effects because of ion–ion correlations. Solvent-mediated ion–ion interactions lead to single filing. This "electrophoretic" effect may be strong in channels such as the potassium channel. Consequently, PNP theories need to implement this mechanism, as for instance suggested by Conti and Eisenman (1966). Also, electropolarization may influence ion transport. Because of long-range strong electrostatic interactions, the occupancy of the channel depends on whether an ion is placed at the channel entrance or not. Therefore, the electric field due to the other mobile ions seen by the ion ready to enter differs from the field of the mean ion distribution at the same position.

How reliable are these mean field approximations? In physics, such mean field approximations are frequently employed with different success. Unfortunately, the comparison (Cooper et al., 1985) between the ion concentrations obtained from PNP and Brownian dynamics cannot be used to evaluate the PNP solution because the two ion channel models employed different forms of electrostatic interactions (Syganow and von Kitzing, 1999a). However, excellent tools to test the quality of mean field approximations are the so-called sum rules (Henderson, 1992). These are exact relations generally derived for equilibrium systems; any exact theory strictly obeys these rules. There are two rules that are particularly relevant for homogeneous and inhomogeneous electrolytes (Blum and Henderson, 1992). The screening sum rule state that all charges, dipoles, and higher multipoles within an electrolyte system are screened by respective counter charges, dipoles, or higher multipoles. It has been shown (Blum and Henderson, 1992) that the PNP theory fulfills this rule exactly. The other important sum rule is the contact theorem. It relates the electric field at a certain boundary to the respective ion concentrations. PNP follows this rule only approximately (Henderson et al., 1979; Blum and Henderson, 1992). The agreement becomes almost exact in the case of strong electric fields. Because both sum rules apply to equilibrium, they justify to some extent the neglect of the counter ion cloud polarization. However, they say nothing about the importance of single filing.

Thus, in accord with the estimates of the size of the forces inside the channel, the PNP theory, using the mean field approximation for the electrostatic interactions, is the method of choice for modeling ion channels dominated by strong electrostatic fields. This the-

ory obeys two important sum rules derived in statistical physics. The fact that the current–voltage relations used to compare theory with experiment are integral properties of the channel renders the judgment of the sum rules particularly valid. In contrast, commonly used reaction-rate theories obey none of these rules. Therefore, they cannot account correctly for strong, long range electrostatic interactions.

Perspectives and Outlook

McCleskey (1999) and Miller (1999) discuss the value of reaction-rate and PNP2 theories according to their ability to reproduce particular experimental current–voltage curves. They neglect the question of whether the basic physical assumptions of those theories are satisfied or not. As shown above, the applicability of any of the two theories depends critically on the size of electrostatic interactions compared with environmental forces.

To study the fundamentals of ion permeation, simpler channels than the potassium or calcium channel should also be considered. For instance, the kind and position of mutations in the acetylcholine receptor channel (Konno et al., 1991) has no correlation with the obtained barrier energy profile. Such correlation, however, would be expected if this profile is assumed to represent the interactions of the ions with their environment. Random changes of the parameters of reaction-rate theories generally lead to nonlinear current–voltage curves (Levitt, 1986). If reaction-rate theories represented the physics of ion channels such as the acetylcholine receptor channel, most single point mutations should result in nonlinear current–voltage curves. In contrast, nearly all mutations in this channel lead to fairly linear current–voltage curves. Such behavior is characteristic for ion channels dominated by electrostatic interactions (Syganow and von Kitzing, 1999b).

Today, the formulation of the Gouy–Chapman theory (Gouy, 1910; Chapman, 1913) is considered as an important first step in understanding the behavior of strong, inhomogeneous electrolytes. Since then, electrolyte theories have considerably improved (Blum and Henderson, 1992). In the same sense, PNP theory should be considered as a first step to describe strong, long range electrostatic interactions in ion channels. The inclusion of ion size effects such as single filing must be one of the next steps.

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