Letter to the Editor

Theories of Ion Permeation: A Chaser

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The *Perspectives on Ion Permeation* in the June issue of *The Journal of General Physiology* provided a unique exposition of contrasting views regarding kinetic rate models, electrodiffusions, Brownian dynamics, and Poisson-Nernst-Planck (PNP). The debate is timely, healthy, and desirable, because recent progress in the determination of the three-dimensional structure of biological ion channels at atomic resolution gives a fresh impetus to efforts directed at understanding the fundamental principles governing ion permeation (Cowan et al., 1992; Chang et al., 1998; Doyle et al., 1998). Theory is expected to play an important role in those efforts, though we are hard pressed to develop a consensus toward specific approaches. A necessary prerequisite for making any global judgement, however, is to understand the significance of the various treatments and how they relate to one another. In my view, there remains significant confusion about these issues that I hope the present contribution will help clarify.

Misconception About Molecular Dynamics Simulations

Several statements about molecular dynamics (MD) indicate that the approach is misunderstood. The approach consists of constructing detailed atomic models of the macromolecular system of interest and, having described the microscopic forces with a potential function, use Newton's classical equation, F = MA, to literally "simulate" the dynamical motions of all the atoms as a function of time (Brooks et al., 1988). The calculated trajectory, though an approximation to the real world, provides detailed information about the time course of the atomic motions, which is difficult to access experimentally. Despite its limitations, MD provides arguably the best available representation of biomolecular systems. Even though the trajectories are typically on the order of nanoseconds, MD simulations are not limited to rapid processes occurring within that time scale. If a well-defined slow process can be identified (e.g., an allosteric gating transition, or the ion movement across a free energy barrier), one can fully characterize such processes using special computational techniques. These well-developed techniques are routinely used by computational chemists and physicists (Chandler, 1978). For example, such methods were used to compute kinetic gating transition rates for dioxolane-linked gramicidin that were on the order of a millisecond (Crouzy et al., 1994).

It is wrong to state that numerical integration of Newton's equation of motions is not reliable for times longer than several picoseconds because the calculated trajectories are very sensitive to initial conditions and round-off errors, and therefore diverge exponentially. This question was debated among theoretical chemists and physicists in the late 1960's or so, and it was shown that these concerns are perfectly solvable (Brooks et al., 1988). The resolution is based on the fact that imperfections in any single numerical trajectory will cancel when considering average value. This is because molecular memory is very short term, meaning that the system forgets rapidly its past. The decay time of the relevant correlations (i.e., the time after which randomization sets in) are therefore very short, which is also one of the reasons why the response of a molecular system to a small time-dependent perturbation is linear (Brooks et al., 1988).

Misconception About Kinetic Rate Models

Kinetic models are constructed on the basis of two assumptions: first, it is assumed that the total configurational space of the whole system comprises a complete collection of distinct subspaces (the states), and, second, it is assumed that the system possesses no dynamical memory when it leaves one state to enter another (the Markov assumption). While it is always possible to define a complete collection of discrete states for any system, the Markov assumption is not necessarily valid (e.g., if there are no free energy barriers between different regions and the movement is purely diffusive). A kinetic rate model is valid as long as the long-time behavior of a molecular system can be described by a finite number of states whose lifetimes are exponentially distributed. Equilibrium properties do not rely on the

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Markov assumption, as it can be shown rigorously that the equilibrium probabilities for occupancy of multiply occupied channels have the familiar algebraic form for saturation behavior that is obtained from kinetic models with discrete states corresponding to a specific number of ions inside the pore (Roux, 1999).

The concept of a rate constant has been used and discussed for a long time (Arrhenius, 1887; Kramers, 1940; Zwolinski et al., 1949; Läuger, 1972), but it was only in the 1970's that its fundamental microscopic basis was clarified (Chandler, 1978). Perhaps for this reason, there remain many misconceptions about the physical significance of kinetic rate models. First, rate models do not assume that ions literally jump over tens of angstroms. Second, rate models do not neglect ion–ion electrostatic interactions. Third, rate models do satisfy Coulomb's law (or Poisson's equation)—if properly constructed. Fourth, rate models do not assume that proteins are rigid. Fifth, the validity of rate models do not hinge on assumptions about the prefactor $k_B T/h$, where k_B, T, and h are Boltzmann's constant, the temperature in Kelvin, and Plank's constant, respectively. This prefactor should never be used for dense systems such as aqueous solutions of membrane-spanning pores. Even if the Markov assumption holds, an experimentally determined rate constant is a single number; without further information, there is no way to determine a unique dynamical prefactor and a unique activation free energy.

The best aspect of kinetic rate models is their flexibility; one can adjust the rate constants constituting the model to fit most complex observed behaviors. The worst aspect of kinetic rate models is also their flexibility. The lack of internal constraints between the various rate constants makes it virtually impossible to guarantee a unique interpretation of what is going on at the microscopic level. The most sensible choice is thus to keep the models as simple as possible, even though a complex reality is not necessarily best described by a simple model.

Misconception About the Transmembrane Potential

There are many misconceptions about the transmembrane potential and its role in ion permeation. For example, in rate models, the transmembrane potential is usually treated as an external (constant?) field independent of the free energy profile (Läuger, 1972; Zwolinski et al., 1949). The statistical mechanical basis for such a separation has been clarified recently (Roux, 1999). The total electrochemical free energy profile of an ion along the channel axis can be rigorously expressed as an intrinsic ion-pore free energy profile (independent of the applied voltage) and other contributions that arise from the transmembrane potential. According to this analysis, the transmembrane potential arises from the electrostatic potential of the ions that are in the bulk solution, but not in the immediate vicinity of the pore. Detailed calculations, based on an atomic structure of the gramicidin channel in a DMPC bilayer, show that the transmembrane potential is in fact quite linear over the length of the channel, thus providing validity to the concept of electric distance (Roux, 1999).

Misconception About Poisson-Nernst-Planck Electrodiffusion

PNP electrodiffusion is an approximate theory combining the diffusion equation under the influence of an electric field, which itself is evaluated based on a continuum electrostatic approximation using the average (mean) charge density of the diffusing ions. Hence its "mean-field" characteristic. Although the earlier form probably dates back to Planck, a more complete description was developed by L. Onsager in the 1940's (Berne and Pecora, 1976). Initially, a one-dimensional reduced model was used to describe ion permeation (1D-PNP; Chen et al., 1997), but full three-dimensional (3D-PNP) theories are now available (Kurnikova et al., 1998). In the absence of ion flux, the 3D-PNP theory reduces to the standard nonlinear equilibrium Poisson-Boltzmann equation. Such an equivalence cannot be made for the 1D-PNP theory. For example, the significance of the 1D charge density profile used to fit experimental data is not known (Nonner et al., 1999). Thus, the 1D-PNP theory involves even further approximations that are difficult to assess and the 3D-PNP theory is preferable. I will limit my comments to 3D-PNP.

The best aspect of PNP is that it aims at doing everything at once: ion–ion, ion–channel, ion–water interactions, and the transmembrane potential are all treated in a consistent way. The worst aspect of PNP is that, while it aims at doing everything at once, it leaves out much of the atomic reality that we know is important at the microscopic level (e.g., van der Waals interactions, core repulsion, induction, hydrogen bonding, solvation structure, and protein flexibility). In practice, PNP is based on several simplifications: rigid channel structure, structureless dielectric solvent, and mean-field ion–ion interactions. If one is to adopt a continuum electrodiffusion approach, such simplifications are necessary to have partial differential equations that can be solved numerically. Interestingly, the debate about PNP often hinges on the use of the mean-field approximation to represent ion–ion interactions. While this is a nontrivial approximation (see below), the most fundamental problems with PNP are related to the approximations about channel rigidity and the representation of the solvent in terms of continuum electrostatics. Let us examine the physical significance of those approximations.

The flexibility of ion channels, as any proteins, plays an important role in its function (Brooks et al., 1988); atomic fluctuations are usually on the order of 0.5–1.0 Å root-mean squared. Ion–protein interactions are very

large (Roux and Karplus, 1995). Although continuum electrostatics is successful in treating processes taking place in bulk solution; i.e., Born model of solvation (Born, 1920), Debye-Hückel theory of electrolytes (Debye and Hückel, 1923), finite-difference Poisson-Boltzmann calculations (Honig and Nicholls, 1995), there are significant effects arising from the granularity of water molecules and their ability to form hydrogen bonds. Continuum electrostatic models depend on empirical parameters (e.g., Born radii) that must be fitted to yield quantitatively accurate results (Born, 1920; Roux et al., 1990). In bulk solution, continuum dielectric behavior is observed only at distances larger than a few water diameters (5–6 Å; Pettitt and Rossky, 1986), the effective ion–ion interaction energy has some microscopic structure (wells, barriers, bumps, and crevasses) and deviates from the smooth and simple Coulomb's law $q_1 q_2 / \epsilon r_{12}$; the interaction energy between two anions or two cations in bulk water are different, while continuum electrostatics is unable to make that distinction. In single file channels, the deviations from the continuum behavior are expected to be even more significant; e.g., ion–ion interactions in the gramicidin channel are species dependent even at a distance of 20 Å (Becker et al., 1992; Roux et al., 1995). From that point of view, the mean-field ion–ion approximation is not the main problem of PNP. This approximation may or may not hold depending on the situation. The Poisson-Boltzmann equation (the equilibrium equivalent of PNP) works well at a low ion concentration. In a channel with a high probability of occupancy, the situation essentially corresponds to that of an effective high concentration, where the Poisson-Boltzmann equation could have problems.

PNP is a consistent but approximate theory. It may, or may not, provide a useful picture of ion permeation because it relies on several physical approximations (rigid channel, continuum electrostatics, and mean-field ion–ion interactions) that are of unknown validity in the context in which they are used. Ultimately, the significance of that picture should not be expected to exceed that of the physical approximations upon which it is built, as is the case of the Born model of solvation, Debye-Hückel, or Poisson-Boltzmann theories. It would be useful to determine the validity of those physical approximations, but that would require more than algebraic mathematical considerations. It requires a comparison between the results from atomic models with explicit molecules and experimental data. Prediction of experimental results alone cannot reveal the limitations of PNP at the microscopic level.

What Is the Role of Theory in Biology?

Rather than focussing on the narrow question, "Which is the best: kinetic rate models or electrodiffusion?," one should ask the deeper question, "Where do these theoretical models stand within modern biology and ion channel science?" It is necessary to step back and try to address more fundamental questions about the role of theory in biology.

Understanding the function of biological systems is one the greatest scientific challenges of our times. To this day, biology remains primarily an experimental science, as it should. Complex biological systems are broken down in isolated elementary constituents, which are then analyzed for their structural and functional properties. These efforts represent more or less what one might (rather pompously) call the enterprise of modern scientific reductionism (Bock and Goode, 1998) that has been so successful in deciphering the laws of physics during the last two centuries. Historically, theory played a huge role in the development of physics. Should one expect that theory will contribute to biology in an equivalent way? What is the role of theory and theoretical models in biology?

When there is no three-dimensional atomic structure available, the goal of theory in biology is to help formulate plausible and reasonable models to help organize the information from experimental data (e.g., current–voltage-concentration relation). Simple models with a limited number of adjustable parameters are most desirable. There is no reason to complicate theoretical models when the atomic structure is not known.

When a three-dimensional atomic-resolution structure is available, the goal of theory is to analyze all the details that play an important role (van der Waals, electrostatic and hydrogen bonding interactions, protein flexibility, and hydration/dehydration processes) on the observed properties (permeation, selectivity, and gating). Can one predict observed properties such as a channel conductance from the atomic structure using only the fundamental laws of physics? At the present time, this is not really possible. The complexity of biological systems requires a hierarchy of inter-related levels of descriptions, the laws governing each level emerging from the fundamental behavior of the lower level: electrons and atoms obey, more or less, the laws of quantum mechanics as described by the Schrödinger equation, the forces acting on atoms and molecules are, more or less, described by the Born-Oppenheimer approximation, the dynamical trajectory of molecules follows, more or less, the laws of classical mechanics, the complexity of dense molecular systems leads, more or less, to chaotic diffusive motions guided by some sort of free energy potential surface, variations in the local composition may (or may not) follow a Markovian kinetic rate process, and so on. One should be able to continue like this all the way to the macroscopic physiological level (Hand et al., 1996). These multiple levels become an absolute necessity to describe biological sys-

tems because of their complexity. Such a construct is not as necessary in physical sciences, and this is a fundamental difference between theoretical physics and theoretical biology.

Useful contributions in theoretical biology should clearly acknowledge their relationship to the other levels of description (above and below). One level below connects with a more fundamental basis, one level above connects to the macroscopic laws. The need to consider and incorporate several levels of description is an important methodological aspect of theoretical biology. Good theory teaches us all (experimentalists and theoreticians) how to better understand the behavior of biological systems by providing an integration of the information from different levels of description. For example: "How does an atomic model of an ion channel relate to a continuous electrodiffusion or to the kinetic rate model?" or "Can one make such a relation?" Detailed computations based on atomic models can contribute by helping in assessing quantitatively the relative importance of microscopic factors.

Theoretical models, at any level, are approximations. Excessive criticism of a theoretical model therefore becomes irrelevant when it is taken out of context. For example, kinetic rate models have been criticized extensively by the proponents of continuum electrodiffusion. But many of the criticisms of kinetic rate theory are largely unjustified and reflect misunderstandings and misconceptions about fundamental molecular statistical physics. The danger becomes that such criticism deters experimentalists from doing quantitative analysis of experimental data because they fear that they cannot attain the required level of theoretical rigor. Such an outcome would be very unfortunate because a quantitative characterization of biological systems (a painstaking task that is not always glamorous) is very important.

The ultimate goal should be to understand mechanisms better, not to develop a black-box that spits out numbers. A useful calculation can reveal important aspects of the function of an ion channel while it fails to reproduce exactly the conductance of the channel. In that sense, accurate reproduction of experimental data is desirable, but not necessarily the absolute criteria because no one at this point can reproduce the observed macroscopic behavior of a biological system starting from the most fundamental level. One must be patient and not ask too much too soon.

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