Enhancing molecular flux through nanopores by means of attractive interactions

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The classic experiments by Galvani and colleagues (as discussed by Piccolino in ref. 1) in the 1790s led him to suggest that neural conduction and muscle contraction are governed by a form of electricity. Nearly 100 years later, Santiago Ramon y Cajal's (2) use of Golgi stains provided stunning images of the complex neural architecture, and hinted at how signals might be propagated along relatively vast distances within the body. In the mid-20th century, electrophysiological experiments on giant squid axons by Hodgkin and Huxley (3) confirmed Galvani's conjecture; the rapid transmission of information along nerve fibers is indeed electrical. Specifically, the propagation of the action potential in nerve is controlled by the spatial-temporal opening and closing of separate pathways for Na^+ and K^+ ions in nerve cell membranes (3–6). By computing the energy for transporting ions across an ultrathin cell membrane (≈4 nm thick) that has a low dielectric constant ($\varepsilon \sim 2$), it was shown that ion-selective transporters, now known as protein ion channels, are water-filled pores (7). The ability to observe single molecules of excitatory material in artificial cell membranes (8–10) and in frog muscle fibers (11, 12) provided keen insight into the structure–function relationship of ion channels. Channels, and channel-like entities, also facilitate the transport of macromolecules in a wide variety of processes including protein translocation across membranes (13), gene transduction between bacteria, and the transfer of genetic information from some viruses and bacteriophages to cells (14). The theoretical work by Bauer and Nadler (15) in this issue of PNAS brings us one step closer to understanding the mechanisms and advantages of molecular selectivity.

With few exceptions (16), the channel pore diameter and simple chemical binding site stoichiometry (17) are not a sufficient basis for enhanced selective transport. Indeed, potassium channels admit $\approx 10^4$ K^+ ions for every Na^+ ion. This remarkable selectivity requires sophisticated physical and chemical machinery with minimal molecular mass; as illustrated in Fig. 1 *Left* (18). The K^+ channel barely spans a cell membrane and is much smaller than the channels formed by the bacterial toxins *Staphylococcus aureus* α-hemolysin (19) (Fig. 1 *Center*) and *Bacillus anthracis* protective antigen 63 (PA_{63}; Fig. 1 *Right*) (20). The various shapes and sizes of ion channels reflect the scope of nanopores used in nature.

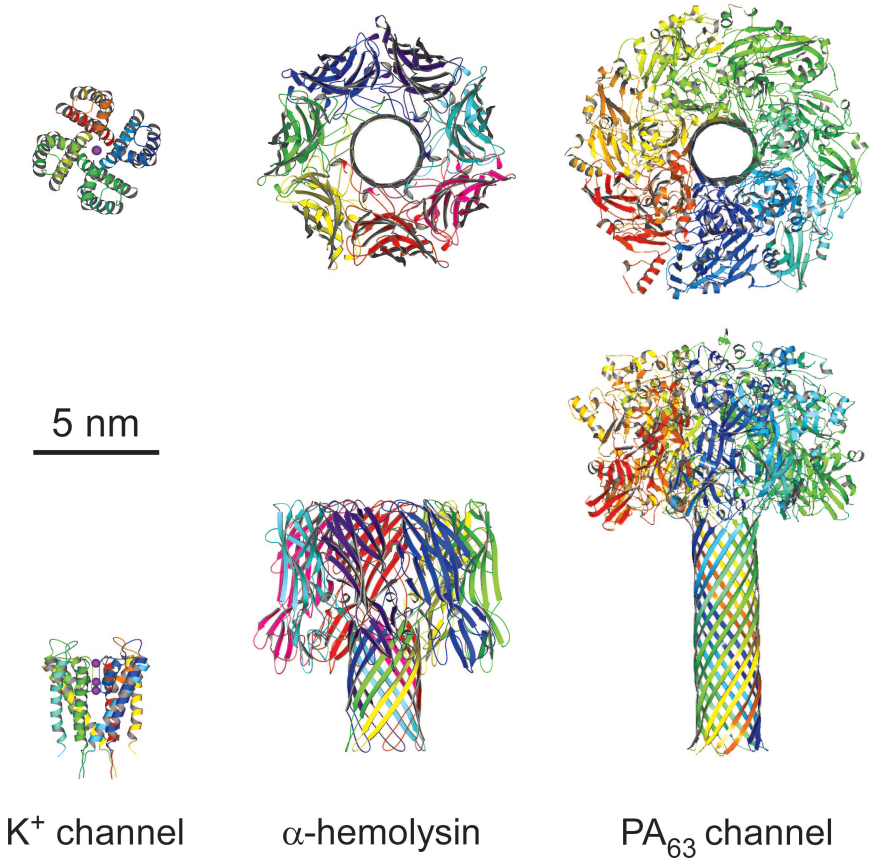

Fig. 1. Representations of three archetypal protein ion channels. (*Left* and *Center*) Crystal structures of a K^+ selective ion channel (18) (*Left*) and the channel formed by *S. aureus* α-hemolysin (19) (*Center*). (*Right*) A model for the channel formed by *B. anthracis* protective antigen 63 (PA_{63}) (20). Each color denotes a single polypeptide monomer. The bacterial toxin channels are far less selective than the K^+ channel. In addition, they only discriminate significantly between cations and anions, e.g., Na^+ and Cl^- (e.g., ref. 21).

Ignoring molecular-sieving mechanisms, selective transport requires that the diffusing particle bind to the channel. A greater attractive interaction implies a greater mean residence time of the particle inside the pore and seemingly a decrease in particle throughput. In fact, that is the case for an infinitely thin channel. However, Berezhkovskii and Bezrukov (17, 22) demonstrated theoretically that for finite length channels, with potential wells that span the length of the pore, and for diffusive motion of particles, the flux is nonmonotonic in binding energy and can exceed that through a nonbinding channel. Moreover, there is an optimum interaction strength that maximizes the flux. In this analysis, it was assumed that the channel-permeated species are point

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particles that do not interact with each other. Their findings are in good agreement with experimental studies of a variety of channels that transport specific nucleotides, sugars, and antibiotics (23–26).

Bauer and Nadler (15) developed an analytical theory that also addressed the question of how the binding of particles to a channel could affect their diffusive flow through ion-selective pores. In this model, the idealized particles have finite extent and can interact with each other in blocking the channel. In addition, the attractive potential well spans part or all of the channel length plus the length of a particle and need not be centered at the middle of the channel. As with Berezhkovskii and Bezrukov (17, 22), Bauer and Nadler (15) found that a binding site can increase the flow compared with that in the absence of particle–channel interactions over a range of bulk particle concentrations and well depths. The increase in particle density in the pore caused by the binding site sufficiently offsets the increase in the mean residence time of the particle in the pore because of the attractive potential.

For a binding site that spans part of the pore, Bauer and Nadler (15) discovered that the site's position qualitatively affects the net flux of particles. Surprisingly, the flow decreases when the binding site is closest to the side of greater particle concentration. Conversely, the flow is enhanced when the site is opposite the entrance with the greater concentration. According to the model, once the particle binds to the pore, it is more likely to exit the pore on that side than to back-diffuse and exit the channel whence it came.

The method of Bauer and Nadler (15) may lead to an even better understanding of other complex transport problems. For example, it was demonstrated that single-stranded DNA can be driven through the α-hemolysin channel (Fig. 1 *Center*) by an applied electric field (27). For a given length of polynucleotide, the DNA-induced ion current blockade patterns and lifetimes inside the channel depended on the base composition (28), which suggests that intraparticle interactions may also play a role in the dynamics of molecular transport through highly confined spaces.

The key question addressed by Berezhkovskii and Bezrukov (17, 22) and by Bauer and Nadler (15) was whether an attractive site in the pore can enhance particle flow through a selective ion channel. They found that as the strength of binding increases past an optimum value, the enhanced flow decreases. However, maximizing flow may not be the desired outcome for all channel-based transport systems. For example, *B. anthracis* exerts its lethal action on cells by means of three anthrax toxins [lethal factor (LF), edema factor (EF), and protective antigen 83 (PA_{83})] that are secreted by the bacterium (29, 30). PA_{83} binds to cell membranes and is cleaved into two fragments. One of these, PA_{63}, remains bound to the membrane and self-assembles into an ion channel (Fig. 1 *Right*). LF and EF bind tightly to the PA_{63} pore (the binding constants are both $\approx$40 pM) and block the channel conductance (31, 32). Recent experimental evidence suggests that LF might thread through the PA_{63} channel (refs. 33 and 34, but see also ref. 31). Unlike metabolites that are consumed and that need to be replenished on a moment-to-moment basis, LF and EF are enzymes that are recycled inside the cell. Maximizing their concentration in the cell may not be essential to cause cell death by anthrax infection.

Nanometer-scale pores play many roles in cells and organelles. In some instances, the selective transport of ions or macromolecules is not only important but is also critical to the survival of an organism. For example, a defect in a chloride-selective ion channel is the molecular basis for cystic fibrosis (35). Thus, theories that advance our understanding of the mechanism of ion channel selectivity are and will continue to be of great import.

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