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Flux, Coupling, and Selectivity in Ionic Channels of One Conformation

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ABSTRACT Ions crossing biological membranes are described as a concentration of charge flowing through a selective open channel of one conformation and analyzed by a combination of Poisson and Nernst-Planck equations and boundary conditions, called the PNP theory for short. The ion fluxes in this theory interact much as ion fluxes interact in biological channels and mediated transporters, provided the theoretical channel contains permanent charge and has selectivity created by (electrochemical) resistance at its ends. Interaction occurs because the flux of different ionic species depends on the same electric field. That electric field is a variable, changing with experimental conditions because the screening (i.e., shielding) of the permanent charge within the channel changes with experimental conditions. For example, the screening of charge and the shape of the electric field depend on the concentration of all ionic species on both sides of the channel. As experimental interventions vary the screening, the electric field varies, and thus the flux of each ionic species varies conjointly, and is, in that sense, coupled. Interdependence and interaction are the rule, independence is the exception, in this channel.

INTRODUCTION

Measurements of unidirectional fluxes of tracers have been widely used to characterize and distinguish (if not define) mediated (1) and channel transport in biological membranes (2, 3), ever since the introduction of radioactive isotopes allowed their measurement. The ratio of unidirectional influx and efflux (of one species) is called the flux ratio (4–7). In biological systems the flux ratio nearly always deviates from its ideal value, that found in ionic solutions and classical Nernst-Planck theories. The deviations are usually explained as interactions of individual ions and the membrane protein through which they flow, the interaction depending on the type of deviation. If the flux ratio is less than ideal, the interaction is attributed to single-filing effects, ions flowing in one direction interfering with ions flowing in the other (8). If the flux ratio is larger than ideal, the interaction is usually attributed to an intrinsic property of the transporting protein, which is supposed to arise, somewhat mysteriously, from correlated changes in the conformation of the protein, either its binding sites or its pore (9–11).

Interacting fluxes are usually shown as arrows, linked by a circle, extending from one side of a membrane to the other (Fig. 1). The linked arrows are widely used to describe the motion of one atom (across the membrane) obligatorily linked to the motion of another atom. But the measurements represented by such arrows are not of individual atoms. Rather, the measurements are of large numbers of atoms (10^9-10^{12}) moving through substantial, perhaps variable, numbers of transporters (10^6-10^7) under varying experimental conditions. A typical experiment might measure the fluxes of two types of ion, as the concentration of one of those ions is changed on one side of the channel. If those fluxes vary conjointly, they are usually said to be coupled and are thought to arise from the motions of individual atoms linked together by conformation changes of the transporter protein.

Covariation can arise in many ways. One way is the obligatory coupling of the movement of one atom with another assumed in traditional treatments. Another way is presented in this paper. Here covariation of flux is produced by common dependence on the electric field within a channel, the electric field in turn being changed by the concentration and flux of each ion, because the system is thoroughly coupled by Poisson's equation and the selectivity filters at the ends of the channel. Fluxes and concentrations interact in this theory through the electric field they (and other charges) create. Experimental interventions change the electric field, directly or indirectly through Poisson's equation and boundary conditions. The electric field in turn changes the flux of all species of ions. The flux of every species varies conjointly and thus they are linked. In particular, variation of the concentration of ions on one side of the channel changes the electric field acting on all ions, thus changing the flux of every ion, with the ratio of the fluxes depending chiefly on the asymmetrical selectivity ratio of the ions. Interdependence and interaction are the rule, independence is the exception, in this channel.

This paper examines a simple macroscopic system, in which a concentration of ions flows through channels of one conformation. The theory describes the ionic flux driven by electrodiffusion using a combination of Poisson and Nernst-Planck equations (which we call the PNP theory), building on earlier work (12–15). In this theory, the atoms of the protein surrounding the pore do not move, and so motions of individual ions through a channel are not linked by conformational changes; nor are they tied together by collisions in the channel's pore. Nonetheless, the fluxes of different ionic species co-vary and are (in that sense) coupled. Similarly, the ratio of the (unidirectional) influx and efflux of one ionic species may or may not have its ideal value, depending on the behavior of ions at the ends of the channel. If the ends or antechambers of the channel are at equilibrium, the flux

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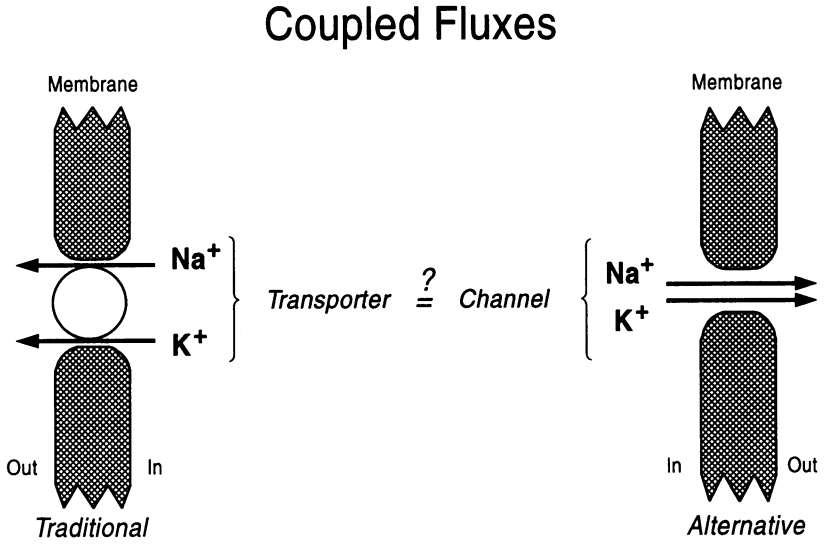

FIGURE 1 Schematic drawing of the traditional description of coupled fluxes in mediated transport (*left-hand side*) and the alternative view of the PNP theory used here.

ratio does have the same value as in free solution, its ideal value, as in many theories (5–7), including an earlier version of the PNP theory (12). If the ends or antechambers are not at equilibrium, because selectivity arises there, the flux ratio can have many values, those classically classified as single filing or exchange diffusion, i.e., as channel or mediated transport, depending on the asymmetry of the channel. In this way a single simple structure, a protein pipe, can produce most of the phenomena of membrane transport.

The idea that "all translocator proteins must . . . be built as channels" (Nikaido and Saier, Ref. 16) is certainly not new (e.g., Wilson, Ref. 17). It has been implicit in the work of Läuger (11) for years and has been discussed informally in many places, including our laboratory, ever since we learned of ping-pong models (18) and then occluded states (19–21). It was immediately apparent that a transporter in an occluded state closely resembles an inactivated ($h = 0$) and deactivated ($m = 0$) sodium channel (22) if inactivation and activation gates are on opposite ends of the pore (2).

What may be new in this paper is the realization of those ideas in a specific theory, incorporating screening and selectivity, in a transporter of one conformation, a theory that explains and predicts a range of experimental results and shows how the spatial distribution of potential, concentration, and charge inside a pore can vary with experimental conditions to produce the covariation of fluxes called flux coupling.

DESCRIPTION OF THE CHANNEL

Consider an open channel with a distribution of permanent charge $P(z)$ extending from $z = 0^-$ to $z = 1^+$ (Fig. 2)

$$P(z) = \frac{\hat{P}(z)}{I_c} \tag{1}$$

where $\hat{P}(z)$ is the dimension*al* concentration of permanent charge (units: moles·m^{-3}) and $P(z)$ is the dimension*less* concentration. I_c is the scaling factor for all concentrations, taken as the largest value of the concentration of the ions in the left bath $\hat{C}_j(L)$ and right bath $\hat{C}_j(R)$ (units: moles·m^{-3})

$$I_c \equiv \max\{\hat{C}_j(L), \hat{C}_j(R)\}; \qquad j = 1, 2, \ldots \quad \text{e.g.,} \quad j = \mathrm{K}^+, \mathrm{Na}^+, \mathrm{Cl}^- \tag{2}$$

The distance is described by the dimensionless variable $z = x/d$, where d is the length of the channel (units: meters) and x is the dimensional spatial variable in the same units. The permanent charge $P(0^-)$ and $P(1^+)$ just outside the pore is accessible to the bathing solutions. An offset in electrochemical potential, the phase-boundary potential $\delta(0)$ or $\delta(1)$ (dimensionless, defined below) of the selectivity filter, is found just inside the pore, where it is not accessible to the bathing solution.

Phase-boundary potentials characterize most interfaces (Bockris and Reddy, Ref. 23, chapter 7; Aveyard and Haydon, Ref. 24, chapter 2) and arise in a variety of ways, perhaps most generally (in our context) as an approximation to the selectivity process in which ions move through an antechamber (25, 26) or selectivity filter (2), as a result of the difference in the standard state of an ion in the bath and in the channel, caused by the difference in free energy of solvation (i.e., hydration) of an ion by water (in the bath) and "hydration" of that ion in the pore by the channel protein (and water). Because both solvation energies are large compared to the energy barriers to ion permeation, small mismatches in solvation between pure water and water/channel protein can be expected to have large effects on selectivity, permeation, and perhaps coupling (see Appendix: Entropy Production and Thermodynamic Constraints). The phase-boundary potential might also arise from the distribution of (permanent) charge in the channel protein, in accessory proteins, or even in lipid in the "far field," away from the channel (12). Phase-boundary potentials are likely to be functions depending on many variables (e.g., diffusion coefficients, geometry of the mouth of the channel, flux through the channel, electrochemical potential) and so they are written as

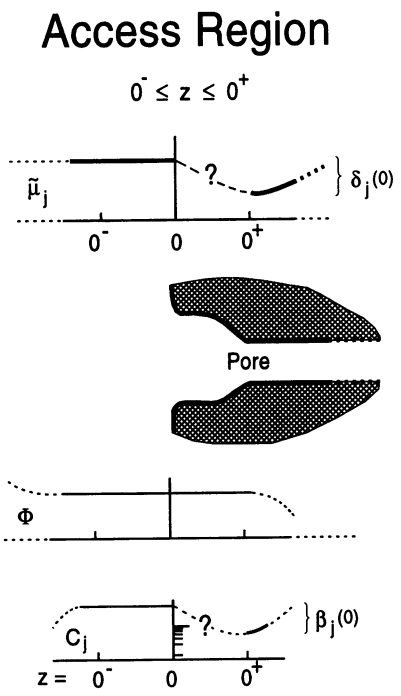

FIGURE 2 Our view of the access region. Only the region on the left $0^- \le z \le 0^+$ is shown, which can also be written as $z = [0^-, 0^+]$. The other side of the channel is similar. The electrochemical potential $\tilde{\mu}_j$ of ion j is shown in the upper panel. Note that the electrochemical potential $\tilde{\mu}_j(z)$ is constant in the interval $z = [0^-, 0]$, but it jumps by an amount $\delta_j(0)$, different for each ion, in the adjoining region, deeper within the channel, namely in the region of the selectivity filter $z = [0, 0^+]$. The jumps $\delta_j(0)$ have some of the properties of interfacial resistances and phase-boundary potentials: they depend on the flux J_j, and overall driving force $V - V_j$ being zero when that flux or driving force is zero.The electrical potential $\Phi(z)$, shown in the panel just beneath the pore, is assumed constant in the interval $z = [0^{-p}, 0^+]$ because we imagine that ionic charge flows quickly enough to neutralize any preexisting dipoles by the time physiological measurements are made (after, say, 10 μs).The concentration $C_j(z)$ is shown in the bottom panel. The concentration of each ion is constant in the region $z = [0^-, 0]$. It jumps in the region $z = [0, 0^+]$, thereby creating a jump in the electrochemical potential. Thevariables $\tilde{\mu}_j(z)$, δ_j, $V - V_j$, and β_j are defined precisely in the text.

$\delta_j(0; \cdot)$ or $\delta_j(1; \cdot)$, where the subscript j shows that the phase-boundary potential in this theory is selective, and different for different ionic species. If the phase-boundary potential δ_j depends on flux (or driving force), as assumed later in this paper, it is a nonequilibrium process, an interfacial "resistance," like the potential drop across a resistor or the overpotential accompanying flux across an interface (Bockris and Reddy, Ref. 23, p. 879). The phase-boundary potential has some of the properties of a diffusion potential (like the Henderson liquid junction potential); for example, it is not restricted to a value smaller than its own equilibrium potential (see Eq. 37 below), even in the absence of net flux or current. Ward and his co-workers (27–31) have developed a statistical rate theory of interfacial transport in several systems, deriving an interfacial resistance similar to that defined in Eq. 33 below.

The jump $\delta_j(0)$ and $\delta_j(1)$ in electrochemical potential at the end of the channel is just the difference in electrochemical potential $\tilde{\mu}_j(\cdot)$ inside and outside the channel, defined (using the usual physiological sign conventions) as

$$\delta_j(0) \equiv \tilde{\mu}_j(0) - \tilde{\mu}_j(0^+) \tag{3}$$

$$\delta_j(1) \equiv \tilde{\mu}_j(1^-) - \tilde{\mu}_j(1) \tag{4}$$

where the (dimensionless) electrochemical potential $\tilde{\mu}_j(\cdot)$ for an ion is defined in terms of the (dimensionless) electrical potential Φ and the (dimensionless) concentration C_j as

$$\tilde{\mu}_j \equiv z_j\Phi + \ln C_j. \tag{5}$$

We use dimension*less* variables Φ and $\tilde{\mu}_j$, related to the dimension*al* variables of the laboratory, the electrical potential φ (units: volts), and the electrochemical potential $M_u(j)$ (units: joules·mole^{-1}) of species

$$\Phi \equiv \frac{\varphi F}{RT}; \qquad \tilde{\mu}_j \equiv \frac{M_u(j)}{RT} \tag{6}$$

where F is Faraday's constant (units: coulombs·mole^{-1}): $F = eN_A$, where e is the charge on a proton (units: coulombs) and N_A is Avogadro's number (units: mole^{-1}). RT is the thermal energy per mole (units: joules·mole^{-1}), where R is the (molar) gas constant (units: joules·mole^{-1}·°K^{-1}), and T is the absolute temperature (units: °K).

The flow through the channel is governed by the same combination of Poisson and Nernst-Planck equations and boundary conditions previously analyzed (12), except that we place nonequilibrium boundary conditions at the ends of the channel. We remind the reader that the PNP system is thoroughly coupled; neither the Poisson equation nor the Nernst-Planck equations can be solved independently of each other or of boundary conditions, even approximately (over a range of experimental conditions).

Built-in potential (in the baths)

A built-in (i.e., Donnan) electrical potential Φ_{bi} extends into the bath in our model, created by the permanent charge just outside the channel proper, just in the bath. More precisely, the permanent charge $P(0^-)$ and $P(1^+)$ just outside the channel is "neutralized" by (all the) ions present in the baths

$$P(0^-) + \sum_j z_j C_j(0^-) = 0 \tag{7}$$

$$P(1^+) + \sum_j z_j C_j(1^+) = 0 \tag{8}$$

The charges $P(0^-)$ and $P(1^+)$ create potentials $\Phi(0^-)$ and $\Phi(1^+)$ in the baths (for $z \le 0^-$ or $z \ge 1^+$), related to the potential $\Phi(0)$ and $\Phi(1)$ just inside the channel

$$\Phi(0^-) = \Phi(0) = \Phi(L) - \Phi_{bi}(0^-) \tag{9}$$

$$\Phi(1^+) = \Phi(1) = \Phi_{bi}(1^+) + \Phi(R) \tag{10}$$

where $\Phi(L)$ and $\Phi(R)$ are the (electrical) potentials measured

and controlled in experiments, the potentials far away in the baths, at $z = -\infty \equiv L$ and $z = +\infty \equiv R$. The built-in potentials Φ_{bi} are the offsets between the edge of the channel and the distant bath

$$\Phi_{bi}(0^-) \equiv \Phi(L) - \Phi(0^-) \tag{11}$$

$$\Phi_{bi}(1^+) \equiv \Phi(1^+) - \Phi(R) \tag{12}$$

The sign convention chosen for potential differences is (potential at left) − (potential at right), following the usual convention of electrostatics and physiology. Ohm's law has a positive sign in that case. Note that Ohm's law (in electrostatics and in physiology in the absence of concentration gradients) has a minus sign if its potential difference is computed as a forward difference (i.e., [potential at right] − [potential at left]) as in mathematical operators like the derivative and gradient.

The built-in potentials are created by the permanent charge at the ends of the channel. Both the electrical potentials Φ and the concentrations C_j are continuous at the ends of the channel and so are the electrochemical potentials $\tilde{\mu}_j$. There are no jumps in electrical potential, concentration, or electrochemical potential between $z = 0^-$ and $z = 0$ or between $z = 1$ and $z = 1^+$. Jumps in electrochemical potential, namely $\delta_j(0)$ and $\delta_j(1)$, occur between $z = 0$ and $z = 0^+$ or between $z = 1^-$ and $z = 1$, as we shall see later in this paper. That is to say,

$$\tilde{\mu}(L) = \tilde{\mu}(0^-) = \tilde{\mu}(0) \neq \tilde{\mu}(0^+) \tag{13}$$

$$\tilde{\mu}(R) = \tilde{\mu}(1^+) = \tilde{\mu}(1) \neq \tilde{\mu}(1^-) \tag{14}$$

Concentration and potential in the baths are described by Nernst-Planck equations integrated for the case of zero flux $J_j = 0$, giving the concentrations $C_j(0^-)$ and $C_j(1^+)$ at the ends of the channel as a function of the experimentally measured and controlled concentrations $C_j(L)$ and $C_j(R)$ far away in the bath.

$$C_j(0) = C_j(0^-) = C_j(L)\exp[z_j\Phi_{bi}(0^-)] \tag{15}$$

$$C_j(1) = C_j(1^+) = C_j(R)\exp[-z_j\Phi_{bi}(1^+)] \tag{16}$$

or equivalently,

$$\Phi_{bi}(0^-) = \frac{1}{z_j}\ln\frac{C_j(0^-)}{C_j(L)} \tag{17}$$

$$\Phi_{bi}(1^+) = \frac{1}{z_j}\ln\frac{C_j(R)}{C_j(1^+)} \tag{18}$$

where z_j describes the sign and number of charges on the ion j.

The built-in potential can be determined from Eqs. 7–18 once the composition of the bathing solutions is specified, thus determining a relation between the C_j's far from the channel,

$$\sum_j z_j C_j(L) = 0 \tag{19}$$

$$\sum_j z_j C_j(R) = 0 \tag{20}$$

The resulting system of equations determines the built-in potentials, for example, $\Phi_{bi}(0^-)$, as a function of the concentrations and permanent charge at the mouth of the channel,

$$\Phi_{bi}(0^-) = \mathcal{F}\{C_j(L); P(0^-); z_j\} \tag{21}$$

The form of the relation $\mathcal{F}(\cdot)$ depends on the composition of the solutions but it does not depend on the properties of the channel (i.e., parameters or variables in the region $0^+ \leq z \leq 1^-$), or the flux through it, or the potential. Explicit formulae for $\mathcal{F}\{\cdot\}$ are found in Chen and Eisenberg (12) for solutions containing only univalent ions and have been derived for solutions containing divalents as well.

This treatment of the built-in potentials follows the traditional equilibrium assumption of semiconductor physics, closely related to the Gouy-Chapman theory (23, 32) of electrochemistry: the volume, concentration, and total contents of the baths are assumed to be so large that flux through the channel does not disturb the (equilibrium distribution of) concentrations $C_j(z)$, for $z \leq 0^-$ or $z \geq 1^+$.

FLUX THROUGH THE CHANNEL

The net flux of an ion J_j(net), or J_j for short, is estimated in single channels from measurements of the open channel current. That current is (nearly; see Eq. 25 below) the difference of the unidirectional fluxes $J_j(L \rightarrow R)$ and $J_j(R \rightarrow L)$ measured in tracer experiments in which isotope is only on one side of a membrane (see Appendix):

$$J_j \equiv J_j(\text{net}) \equiv \overbrace{J_j(L \rightarrow R)}^{\text{efflux}} - \overbrace{J_j(R \rightarrow L)}^{\text{influx}} \tag{22}$$

The unidirectional fluxes measured in tracer experiments flow through many ($\sim 10^6$) transporters and are usually assumed to move through a constant number of transporters of just one type. When comparing the predictions of our theory with experimental results, it is important to remember that the theory describes just one channel, permanently open, with no gating. Thus, the present version of the PNP theory cannot describe phenomena that depend on gating, e.g., that depend on changes in open probability with experimental conditions.

Interestingly, unidirectional fluxes also appear naturally in models of stochastic movement over potential barriers (33) even though these theories contain no mention of tracer or isotope flux. In the stochastic theory, the unidirectional fluxes appear as components of the total flux, describing the random motions of a single particle moving

across a channel from a source to an "absorbing" boundary, an artificial (i.e., not necessarily physical) boundary condition introduced to define the first passage time of a random walker.

The dimension*less* net flux J_j(net) can be determined directly by integration (12) of the Nernst-Planck equations (see Appendix, and Ref. 2, p. 345, eq. 13-13) in the domain $0^+ \le z \le 1^-$:

$$J_j(\text{net}) = \frac{C_j(0^+)\exp\{z_j\Phi(0^+)\} - C_j(1^-)\exp\{z_j\Phi(1^-)\}}{\int_{0^+}^{1^-} \exp\{z_j\Phi(\varsigma)\}\, d\varsigma} = \frac{e^{\tilde{\mu}_j(0^+)} - e^{\tilde{\mu}_j(1^-)}}{\int_{0^+}^{1^-} \exp[z_j\Phi(\varsigma)]\, d\varsigma}. \tag{23}$$

The dimension*al* flux $\mathscr{J}_j$(net) (units: moles·m^{-2}·s^{-1}) is

$$\mathscr{J}_j(\text{net}) = J_j(\text{net})\frac{D_j I_c}{\text{d}}. \tag{24}$$

D_j is the diffusion coefficient (units: m^2·s^{-1}). Note that the flux through a single channel is $\mathscr{A} \cdot \mathscr{J}_j$ where $\mathscr{A}$ is the cross-sectional area of the channel's pore. The dimension*al* electric current $\mathscr{I}$(units: amperes) through one channel is the sum of the current $\mathscr{J}_j$(net) carried by each species through that channel:

$$I = \sum_j \mathscr{J}_j(\text{net}) = \mathscr{A}F\sum_j z_j \mathscr{J}_j(\text{net}) = \mathscr{A}\frac{I_c F}{d}\sum_j z_j D_j J_j(\text{net}). \tag{25}$$

Although Poisson's equation is not needed for the integration giving Eq. 23, it is needed to determine the potential profile $\Phi(\varsigma)$. Because Poisson's equation depends on $\sum z_j C_j$, the system is thoroughly coupled. The potential profile $\Phi(\varsigma)$, and its derivative the electric field, is not a constant, but rather a function of experimental conditions.

The potential profile $\Phi(\varsigma)$ is the solution of Poisson's equation and boundary conditions, the consequence of an unchanging distribution of permanent charge, interacting with mobile charge carried by ions in and out of the pore. The total charge in the pore is not at all constant in the PNP theory, because the mean number of ions in the pore is comparable to the amount of permanent charge; the number of ions in the pore varies with experimental conditions, thus changing the shielding of the permanent charge within the channel. The potential profile cannot be constant (under varying experimental conditions) if the total charge varies, i.e., if the screening varies, as a consequence of Poisson's equation (Ref. 34). Indeed, the dramatic variation of the potential profile, and underlying charge distributions, with experimental conditions (Ref. 12) gives the channel most of its interesting behavior.

The unidirectional fluxes can now be written in terms of the experimentally controlled concentrations $C_j(L)$ and $C_j(R)$, and transmembrane potential, by substituting the boundary conditions defining the built-in potential (Eqs. 11–12) and phase-boundary potential (Eqs. 3–4) into the integrated Nernst-Planck equations (Eq. 23)

$$J_j(\text{net}) = \frac{C_j(L)\exp\{z_j V - \delta_j(0;\cdot)\} - C_j(R)\exp\{z_j\delta_j(1;\cdot)\}}{\int_{0^+}^{1^-} \exp\{z_j\Phi\varsigma\}\, d\varsigma}. \tag{26}$$

In these equations, the potential far from the channel on the left is defined as the transmembrane potential V

$$\Phi(L) \equiv V \tag{27}$$

because the outside potential $\Phi(R)$ far from the channel on the right is set to zero, universally since Hodgkin's Croonian lecture (35) (if not earlier (36)):

$$\Phi(R) \equiv 0. \tag{28}$$

The built-in potentials have "canceled out" of the (numerator of the) final expression for the fluxes (Eqs. 23 and 26), as they must, because the bath ($L \le z < 0$; $1 < z \le R$) is assumed to be at equilibrium, in contrast to the channel itself. The built-in potentials Φ_{bi} have an important effect on the denominator of the flux expression, Eq. 26. The built-in potentials Φ_{bi} directly change the potential $\Phi(z)$ in the channel (Eqs. 9 and 10). They also indirectly change the concentration of ions $C_j(0^-)$ and $C_j(1^+)$ at the ends of the channel (Eqs. 15 and 16), which in turn modify the fluxes (through Eq. 23, etc.). The built-in potentials Φ_{bi} and potential profile $\Phi(z)$ have profound effects on the unidirectional and net flux but not on the ratio of the unidirectional fluxes. Similarly, the physical origin of δ_j has an effect on the unidirectional and net flux but not on the ratio of the unidirectional fluxes. The flux ratio does not depend on whether the phase-boundary potential comes from a jump in electrical potential, a jump in concentration, or a mixture of both.

If a theory (12) does not include (nonequilibrium) phase-boundary potentials, flux ratios like $J_j(R \to L)/J_j(L \to R)$ are independent of the entry process and the properties of the ends of the channel (5–7). In particular, they do not depend on the built-in potentials Φ_{bi}, because the numerator of the expression for the unidirectional flux (like our Eq. 26) is independent of the built-in potentials Φ_{bi}. The (lowest common) denominator of flux equations does depend on the built-in potentials Φ_{bi}, but the flux ratio does not, because the denominator "cancels out" of the resulting fraction.

Phase-boundary potentials $\delta_j(\cdot)$ alter flux ratios in contrast to built-in potentials Φ_{bi}, which do not. Physically, the flux ratios vary because of the nonequilibrium character of $\delta_j(0;\cdot)$ and $\delta_j(1;\cdot)$. Mathematically, the flux ratios vary because $\delta_j(0;\cdot)$ and $\delta_j(1;\cdot)$ appear in the numerators of Eqs. 23 and 26. Of course, the phase-boundary potentials also change the denominator of those expressions, rather like the effect of

Φ_{bi}, because they too modify the potential profiles $\Phi(z)$ and the concentration profiles $C_j(s)$.

THERMOSTATIC CONSTRAINTS

The PNP theory is kinetic; it does not include thermostatic[1] constraints and so we must check to see that these are satisfied. In particular, in a passive system thermostatics requires that

$$\{\text{no driving force}\} \Rightarrow \{\text{no net flux}\}. \tag{29}$$

That is to say, when $C_j(L) = C_j(R)$ and $V = 0$, the net flux must be zero in a passive system. (Obviously, this restriction would not have to apply in active transport systems like the sodium pump that derive energy from ATP.) Thus, Eqs. 26 and 29 require the net phase-boundary potential to be zero when there is no gradient of concentration or electrical potential, when the driving force is zero:

Thermostatic Constraint

$$\delta_j(0; J_j) = \delta_j(1; J_j) = 0 \tag{30}$$

$$\text{when} \left\{ \begin{array}{c} C_j(L) = C_j(R) \\ \text{and} \\ V = 0 \end{array} \right\} \Rightarrow J_j(\text{net}) = 0.$$

Note that the constraint is strictly thermostatic; the equalities need not apply away from equilibrium, when there are finite fluxes, gradients of potential, or concentration. A potential proportional to current (or flux) would, for example, be a nonequilibrium process that would automatically satisfy Eq. 30 at equilibrium, when current and flux are zero, but not otherwise.

SELECTIVITY IN AN ACCESS RESISTANCE

A selectivity filter will naturally produce the balance in concentration and/or charge required (at equilibrium) by the thermostatic constraint (Eq. 30). An (electrochemical) access "resistance" r_j at the end of the channel, producing a (nonequilibrium) drop in electrochemical potential δ_j in the selectivity process, across the antechamber or entry region, or in the filter, produces potentials $\delta_j(0; J_j)$ and $\delta_j(1; J_j)$ that automatically satisfy the thermostatic constraint 30, as would *IR* potential drops in a voltage divider or an antechamber described by the PNP model (25, 26). In that way, the constraint is satisfied even if one side of the channel does not interact with the other. In our nonequilibrium system, each phase-boundary potential is separately equal to zero when $J_j = 0$, and they are equal, for that reason, but no other, when the system is at equilibrium, and the net flux $J_j(\text{net})$ is zero.

More precisely, we require either

$$\delta_j(0; J_j) = z_j\alpha_j(0) \cdot [V - V_j] \tag{31}$$

$$\delta_j(1; J_j) = z_j\alpha_j(1) \cdot [V - V_j] \tag{32}$$

or, (nearly) equivalently,

$$\delta_j(0; J_j) = r_j(0; \cdot) \cdot z_jJ_j\,(\text{net}) \tag{33}$$

$$\delta_j(1; J_j) = r_j(1; \cdot) \cdot z_jJ_j\,(\text{net}) \tag{34}$$

implying the formal relations

$$r_j(0; \cdot) = \frac{\alpha_j(0) \cdot J_j\,(\text{net})}{V - V_j} \tag{35}$$

$$r_j(1; \cdot) = \frac{\alpha_j(1) \cdot J_j\,(\text{net})}{V - V_j}, \tag{36}$$

where we introduce a number of new variables and names. The access resistance on the left is $r_j(0; \cdot)$ and on the right $r_j(1; \cdot)$ and the selectivity on the left is $\alpha_j(0)$ and on the right $\alpha_j(1)$. The unusual listing of arguments indicates that the functions might depend on many variables, e.g., flux, concentration, potential, and on each other, although in the calculations reported here the α_j's are held strictly constant for the sake of simplicity.

The (dimension*less*) equilibrium or reversal potential V_j is defined as the transmembrane potential (i.e., value of $\Phi(L)$) at which net flux J_j is zero (i.e., reverses direction) in conventional uncoupled Nernst-Planck systems (2), using the usual physiological sign conventions (positive equilibrium potential for an inward, i.e., right to left, diffusive gradient, in which the outside concentration of a positive ion is larger than the inside concentration):

$$V_j \equiv \frac{1}{z_j} \ln \frac{C_j(R)}{C_j(L)} \tag{37}$$

or, equivalently,

$$\frac{C_j(R)}{C_j(L)} = \exp\{z_jV_j\}. \tag{38}$$

The selectivities α_j of Eqs. 31 and 33 are assumed here to be constants, independent of the driving force $V - V_j$, in the calculations shown in Results, for the sake of simplicity (e.g., in writing analytical expressions for fluxes 48–58), and for little other reason. The access resistances $r_j(\cdot)$ can also be constants, if the flux is a linear function of electrochemical potential $V - V_j$. In general, however, the access resistances will depend on many variables even if the α_j are constant, because (in general) the flux J_j is a nonlinear function(al) of (for example) the driving force $V - V_j$, concentrations C_j, and potential $\Phi(z)$; see, for example, Eq. 23.

[1] We think it might be seriously misleading to use the word "thermodynamic" to describe a constraint true *only* at equilibrium. "Thermodynamic" is used here to describe constraints, and situations away from equilibrium, where J_j need not be zero (see Appendix: Entropy Production, and Thermodynamic Constraint, particularly Eq. 71).

We are aware that a complete analysis of the (time-dependent) selectivity and entry process using mesoscopic or stochastic analysis, or molecular dynamics simulations, is likely to uncover currently unknown dependence on many parameters, and thus is needed, for this, among other reasons.

Nonequilibrium jumps at the boundaries

The ratios of unidirectional fluxes are determined by the phase-boundary potentials δ_j, and not by the physical nature of those potentials (as can be seen from Eq. 23). In particular, the flux ratios are the same, whether δ_j represents a jump in concentration, a jump in electrical potential, or a combination of both. Thus, the nature of the phase-boundary potential does not affect the main conclusion of this paper, that flux coupling of many types can occur in a channel of one conformation, if the channel has a nonequilibrium selectivity filter at its end.

To write an explicit expression for an individual flux (as opposed to the ratio of fluxes), we must be more specific in describing the selectivity process, that is to say, in describing the nonequilibrium jumps at the boundaries.

In our view of the entry and selectivity process, charges flow (before biological functions start or are measured) to make the electrical potential Φ continuous at the ends of the channel, i.e., the potential has one value at $z = 0^-$, $z = 0$, and $z = 0^+$ and another at $z = 1^-$, $z = 1$, and $z = 1^+$,

$$\Phi(0) = \Phi(0^+) = \Phi(0^-) \tag{39}$$

$$\Phi(1^-) = \Phi(1) = \Phi(1^+), \tag{40}$$

even where there is a discontinuity in electrochemical potential $\delta_j(\cdot)$, namely between $z = 0$ and $z = 0^+$ or between $z = 1^-$ and $z = 1$. There, the concentration is discontinuous, as traditionally described by the partition coefficients $\beta_j(\cdot)$, namely,

$$\frac{C_j(0^+)}{C_j(0)} = \beta_j(0; J_j) \equiv \exp\{-\delta_j(0; J_j)\} \tag{41}$$

and

$$\frac{C_j(1^-)}{C_j(1)} = \beta_j(1; J_j) \equiv \exp\{\delta_j(1; J_j)\}, \tag{42}$$

or equivalently one can say that the (nonequilibrium) concentration ratio determines the offsets in equilibrium potential:

$$-\delta_j(0; J_j) = \ln \frac{C_j(0^+)}{C_j(0)} = \ln \beta_j(0; J_j) \tag{43}$$

and

$$\delta_j(1; J_j) = \ln \frac{C_j(1^-)}{C_j(1)} = \ln \beta_j(1; J_j). \tag{44}$$

Here the δ_j's are determined by Eqs. 31 and 33, and we use the partition coefficient β_j of Hodgkin and Katz (37) (following others, no doubt), and Hodgkin and Frankenhaeuser (38), but now generalized to be functions (not constants), dependent on many things, including concentration, potential, and flux (see Historical Note).

In this model, we attribute all discontinuities to the phase-boundary potentials located between $z = 0$ and $z = 0^+$ and between $z = 1^-$ and $z = 1$, while the concentrations and electrical potentials are continuous between $z = 0^-$ and $z = 0$ and between $z = 1$ and $z = 1^+$; that is to say, the potential and concentrations are the same at the end of the channel and in the immediately adjacent bath:

$$C_j(0^-) = C_j(0); \qquad \Phi(0^-) = \Phi(0) \tag{45}$$

$$C_j(1) = C_j(1^+); \qquad \Phi(1) = \Phi(1^+). \tag{46}$$

This model (in particular, Eqs. 39–42) attributes the jump in electrochemical potential, dependent on flux, to a jump in concentration at the ends of the channel, not a jump in electrical potential; we suppose that no (infinitely thin) layers of electrical dipoles, dependent on flux, exist in the ionic solution at the ends of the channel by the time the physiological time scale (tens of microseconds) is reached, after the rapid (nanosecond) charging and coupling process described by the (underlying) time-dependent PNP equations. We assume (pending further analysis) that only a jump in concentration exists by that time because induced and mobile charges have moved previously to neutralize any preexisting discontinuities in electrical potential, leaving the channel in the steady state satisfying Eqs. 39–46.

In this model, the end of the channel is supposed to be a solution of ions in water with no permanent structure, so that an (infinitely thin) layer of electrical dipoles is not permitted there. If the water or the ions did create a dipole layer, and thus a jump in electrical potential dependent on flux, our theory would have to be modified. However, the expressions for the flux ratio remain the same, because they depend on the jumps $\delta_j(\cdot)$ in electrochemical potential, the phase-boundary potential, as can be seen explicitly in Eq. 26. The flux ratio is independent of the source of the phase-boundary potentials, independent of their origin as a jump in concentration or as a jump in electrical potential, provided the $\delta_j(\cdot)$ themselves do not change. We have in fact constructed a model in which the $\delta_j(\cdot)$ arise from an arbitrary combination of jumps in concentration and electrical potential, but we think it unnecessary to present the details here.

The model of the entry process and selectivity filter presented here is consistent with the general theory of interfacial phenomena developed by Ward and his colleagues

TABLE 1 Direction of net transport

Asymmetry	Transport
$\gamma_j > 0$	Downhill
$\gamma_j = 0$	Futile ("exchange diffusion")
$\gamma_j < 0$	Uphill

Uphill means J_j and $V - V_j$ in the opposite direction. Futile means no net transport; unidirectional fluxes may be large. Downhill means J_j and $V - V_j$ in the same direction.

(27–31); our definition (Eq. 33) of interfacial resistance is similar to equation 53 of Ward, Findlay, and Rizk (30). It can also be viewed as a lumped approximation to a (spatially distributed) PNP model of the antechambers at the ends of channels (25, 26).

Selectivity ratios arise naturally in our PNP model in several ways, for example, as the ratio of (nonequilibrium) partition coefficients β_j/β_k used to describe (but not understand) the selectivity filter and process. The selectivity produces a drop in electrochemical potential dependent on flux (and other variables) described by Eqs. 41 and 42 and 31 and 33. As we shall see (in Table 1 and Eqs. 53 and 51), it is precisely the asymmetry in the channel, the difference in its selectivity at each end, that determines the driving force for electrodiffusion and thus the linkage of fluxes moving through the channel, whether these are unidirectional fluxes of the same species or net fluxes of different species.

In this PNP model, coupling of fluxes is a direct result of asymmetrical selectivity. Selectivity, asymmetry, and coupling—each so characteristic of biological channels and transporters—are unavoidably linked in our model, and perhaps in real channels as well.

FLUX THROUGH THE SELECTIVE CHANNEL

It is now possible to write a general expression for the net flux $J_j(\text{net}) \equiv J_j \equiv J_j(L \to R) - J_j(R \to L)$ in terms of the partition coefficients (defined in Eq. 41, starting from Eq. 26):

$$J_j\,(\text{net}) = \frac{\beta_j(0;\cdot)C_j(L)\exp\{z_jV\} - \beta_j(1;\cdot)C_j(R)}{\int_{0^+}^{1^-}\exp\{z_j\Phi(\varsigma)\}\,d\varsigma}. \qquad (47)$$

However, this expression does not automatically satisfy the thermostatic constraint, Eq. 30.

If we introduce the access resistance and selectivities, according to Eqs. 31 and 33, it is possible to write an explicit expression that automatically satisfies the thermostatic constraint and permits the evaluation of flux ratios, either the ratios of unidirectional fluxes of the same ionic species (*homo-flux* ratios) or ratios of the fluxes of different species (*hetero-flux* ratios).

The generally valid expression for the net flux $J_j(\text{net})$ is then the difference of the influx (i.e., $R \to L$) and the efflux (i.e., $L \to R$)

$$J_j = \frac{C_j(R)\exp\{\alpha_j(1)z_j[V - V_j]\}}{\int_{0^+}^{1^-}\exp\{z_j\Phi(\varsigma)\}\,d\varsigma} \times \left[\underbrace{-1}_{\text{influx}} + \underbrace{\exp\{z_j\gamma_j[V - V_j]\}}_{\text{efflux}}\right] \qquad (48)$$

where

$$\gamma_j = 1 - \alpha_j(0) - \alpha_j(1). \qquad (49)$$

The direction of the flux of each ion (i.e., the sign of J_j) is determined by the sign of the second factor on the right-hand side of Eq. 48, because the preceding fraction is never negative. In other words, the direction of transport is determined entirely by the asymmetrical selectivity factor γ_j, that is, by the boundary conditions Eqs. 31 and 33 and not by the distribution of potential $\Phi(\varsigma)$ or permanent charge (12) $P(\varsigma)$.

The asymmetry constants γ_j are determined by the asymmetry in the entrance process, selectivity filter, or antechambers of the channel. If the physical processes at the two phase boundaries are identical, then $\alpha_j(0) = -\alpha_j(1)$ (because of the usual physiological sign conventions). The asymmetry constant γ_j is then equal to 1, and the ratio of unidirectional fluxes assumes its ideal value, as in free solutions.

As we shall see, γ_j determines the qualitative properties of the coupling between fluxes mathematically because of their location in Eqs. 48 (see also Table 1) and physically because they determine the driving force across the channel (see Eq. 51 below). In using these expressions, it is prudent to remember that α_j, and thus γ_j, has been assumed to be constant, independent of $V - V_j$, for the sake of convenience (e.g., in writing analytical expressions for fluxes 48–58), but not much else.

Uphill and downhill transport: sign of net flux of an ion

Traditionally, the direction of the current I_j carried by each type of ion j is supposed to be downhill in any Nernst-Planck model; the (dimensionless) current carried by an ion

$$I_j = z_j \cdot J_j(\text{net}) = z_j\{J_j(L \to R) - J_j(R \to L)\} \qquad (50)$$

and its driving force $z_j(V - V_j)$ are supposed to necessarily have the same sign. Uncoupled Nernst-Planck models, in which the potential distribution is independent of experimental conditions, have that property and so do coupled models (12) without a selectivity filter or nonequilibrium entry process. But the model considered here is not so restricted. It includes a nonequilibrium selectivity filter, involving many ions, that allows the asymmetrical coupling constant, $\gamma_j = 1 - \alpha_j(0) - \alpha_j(1)$ to have either sign, along with the ratio of (net) flux to driving force. For example, if $\alpha_j(0) = 0.5$, and $\alpha_j(1) = 0.6$, perfectly respectable values, net flux of one ionic species j would be uphill.

In particular, three combinations of the signs of γ_j can occur in Eq. 48. Those with $\gamma_j > 0$ give downhill transport, currents in the same direction as the driving force. Those with $\gamma_j < 0$ give uphill transport, with current in the direction opposite from that of the driving force (Table 1). Interestingly, if the selectivity filter, entry process, and properties of the antechambers are so balanced that $\gamma_j = 0$, i.e., $\alpha_j(1) + \alpha_j(0) = 1$, the current is zero, but ions (e.g., radioactive isotopes acting as tracers of each species on both sides of the channel) can still exchange their positions through unidirec-

tional fluxes. The unidirectional fluxes need not be individually zero when $\gamma_j = 0$. Such "exchange diffusion" has been found in many biological transport systems.

We must conclude that in the PNP model uphill net transport of one species can occur in a channel of one conformation if it has a selectivity filter, an asymmetrical nonequilibrium entry step, with $\gamma_j < 0$, no matter what the potential distribution Φ (ς) is inside the channel, i.e., in the region, $0^+ < \varsigma < 1^-$.

Driving force within the channel

The physical reason for the central role of the asymmetry constant γ_j in determining the flux ratio was not clear until we examined the driving force within the channel, in the region $0^+ < z < 1^-$. The difference in electrochemical potential across this region $\tilde{\mu}_j(0^+) - \tilde{\mu}_j(1^-)$ is the driving force for electrodiffusion, the source term in the (Poisson) Nernst-Planck system within the channel (see Eq. 23). This term can be evaluated by combining several earlier expressions, Eqs. 3, 4 and 31, 32. Then,

$$\begin{aligned}\tilde{\mu}_j(0^+) - \tilde{\mu}_j(1^-) &= [1 - \alpha_j(0) - \alpha_j(1)] \cdot [V - V_j] \\ &= \gamma_j[V - V_j].\end{aligned} \tag{51}$$

We see that the asymmetry constant γ_j determines the driving force for electrodiffusion within the channel, in $0 < z < 1$. The driving force across the channel $\tilde{\mu}_j(0^+) - \tilde{\mu}_j(1^-)$ is independent of the potential distribution Φ (z) inside the channel, i.e., in the region, $0^+ < z < 1^-$.

The parameter γ_j has three distinct roles. It is a selectivity constant reflecting the asymmetry in phase-boundary potentials on the two sides of the channel; it is an asymmetry constant determining the direction of net flux; and it is a coupling constant determining the size and sign of flux ratios (Table 1). In this way phase-boundary potentials, asymmetry, and coupling arise together in the selectivity filters of the PNP model.

Asymmetry constants γ_j and diffusion potentials

The phase-boundary potentials δ_j that determine γ_j are closely related to the diffusion potentials across interfaces discussed in electrochemistry, the difference being the presence of the protein and its selectivity filter and antechamber. Few (simple, general) rules are known that constrain diffusion potentials ϕ_j across phase boundaries of solutions in the nonequilibrium case, when many ionic species are present, intermixing and flowing in complex combinations of fluxes and currents (see Appendix: Entropy Production and Thermodynamic Constraints). Diffusion potentials at the boundary of solutions can have any sign, depending on the relative diffusion constant of the ions, even when current flow is negligible. The diffusion potential ϕ_j and the flux J_j of each ion j depend on all the other ions $k \neq j$ and on those ions' diffusion constants D_k (as well as other parameters), thus allowing a range of values of the potential ϕ_j and many forms of coupling between the movements of ions J_j and J_k, often described by the so-called Onsager reciprocal relations, which predict how the flux of one species (say, j) is influenced by the driving force for another, say k.

Similar richness of behavior should be expected in the phase-boundary potentials δ_j, selectivities, α_j, and asymmetrical coupling constants γ_j, and that richness can be controlled and exploited by the channel protein for its own purposes. The selectivity of the channel is determined by the protein's structure and flexibility and the ions, solvation. In the PNP model, the structure is described by the spatial distribution of permanent charge and the flexibility by the spatial distribution of induced charge, that is, the spatial distribution of the local dielectric constant. Together with solvation, these determine its selectivities $\alpha_j(\cdot)$. The $\alpha_j(\cdot)$ have presumably been selected by evolution to confer the flux coupling and ionic selectivity needed for the biological function of the channel.

Ratio of unidirectional fluxes of one species

The ratio of unidirectional fluxes carried by the same ion (the so-called homo-flux ratio) can easily be predicted if the potential profile $\Phi(x)$ does not depend on the direction of current flow. Then, Eqs. 26 and 41–47 predict a flux ratio, but one that does not automatically satisfy the thermostatic constraint and thus is not generally valid:

$$\begin{aligned}\frac{\text{efflux}}{\text{influx}} &= \frac{J_j(L \to R)}{J_j(R \to L)} = \frac{\beta_j(0;\cdot)}{\beta_j(1;\cdot)} \frac{C_j(L)}{C_j(R)} \exp(z_j V) \\ &= \frac{C_j(L)}{C_j(R)} \exp\{z_j V - \delta_j(0;\cdot) - \delta_j(1;\cdot)\} \\ &= \exp\{z_j[V - V_j] - \delta_j(0;\cdot) - \delta_j(1;\cdot)\}.\end{aligned} \tag{52}$$

When the access resistance is included in the model (as in Eqs. 31 and 48), the phase-boundary potentials are constrained, and the flux ratio automatically satisfies the thermostatic condition (Eq. 30), but it does not necessarily have its ideal value, found in free solution. The resulting generally valid expression is then

$$\ln \frac{J_j(L \to R)}{J_j(R \to L)} = z_j \gamma_j [V - V_j]. \tag{53}$$

In free solution $\gamma_j = 1$, its ideal value. But in the PNP theory, the asymmetrical coupling constant γ_j can have many values and so can the flux ratio. Values of γ_j greater than 1 are traditionally taken as a sign of single filing or other long pore effects. Values less than 1 are called exchange diffusion and are traditionally taken as signs of intrinsic coupling in the transport mechanism, the protein molecule itself.

Ratio of fluxes of different species

The ratio of fluxes of different species is also significant. In the derivation of our model, and in the calculations reported

in Results, the potential profiles for different ions (and for the same ion moving in different directions) are the same. We know we risk inconsistency if each ion interacts with a different potential distribution $\Phi_j(\varsigma)$ just in the output of the theory (namely, the flux formulae, Eqs. 54–58 below) and not in the original differential equations and boundary conditions.[2] Nonetheless, we think it would be even worse to present formulae in which much of the physics is absent, because many terms have canceled, all because the $\Phi_j(\varsigma)$ were set equal, for no good physical reason.

After all, potential profiles $\Phi(\varsigma)$ for different ionic species are likely to be different. Different ions have quite different sizes and physical characteristics (e.g., different charge densities) and chemical properties (e.g., interactions with water), and so the parameters of any macroscopic theory must be viewed as approximate integral representations of the microscopic reality, as effective but not exact parameters, that will depend, perhaps subtly, on the ionic species occupying the channel. In particular, the permanent charge density (with which the ion interacts in the PNP theory) (12), as well perhaps as the dielectric constant, pore diameter, and even pore length must be seen as what they are, namely effective parameters that may depend on the ionic contents and occupancy of the channel.

Some of this subtlety can be described by using different potential distributions $\Phi_j(\varsigma)$ for each species j of ion. Each potential distribution $\Phi_j(\varsigma)$ is determined from the permanent charge density $P_j(\varsigma)$ appropriate for that ion, using the coupled system of Poisson, Nernst-Planck, and boundary equations of the PNP theory.

Ratio of net fluxes of different species

We can then write the net flux ratio of ions of different types (hetero-flux ratios):

$$\frac{J_j(\text{net})}{J_k(\text{net})} = \frac{\int_{0^+}^{1^-} \exp\{z_k\Phi_k(\varsigma)\}\,d\varsigma}{\int_{0^+}^{1^-} \exp\{z_j\Phi_j(\varsigma)\}\,d\varsigma} \times \frac{C_j(R)\exp\{\alpha_j(1)z_j[V - V_j]\}}{C_k(R)\exp\{\alpha_k(1)z_k[V - V_k]\}} \times \frac{-1 + \exp\{z_j\gamma_j[V - V_j]\}}{-1 + \exp\{z_k\gamma_k[V - V_k]\}}. \quad (54)$$

The sign of the heteroflux ratio can be further analyzed, because it is determined by only the last factor in Eq. 54, the other factors being strictly non-negative. If the flux of two ions is in the same direction, so that J_j and J_k have the same sign, the transport system is often called a cotransporter. If the flux is in opposite directions, so that J_j and J_k have opposite signs, the transport system is often called a countertransporter. The direction of each flux depends on the sign of γ_j or γ_k, as shown in Table 1.

Futile transport occurs in several forms. For example, if γ_j is zero, the net flux of that ion $J_j(\text{net})$ will be zero, even if the driving force $V - V_j$ is quite substantial (see Eq. 48). But unidirectional fluxes of that species (e.g., $J_j(L \to R)$) will not necessarily be zero, nor will the net or unidirectional fluxes of the other species, $J_k(\cdot)$. If both $\gamma_j = 0$ and $\gamma_k = 0$, then no net transport of either species will occur, but unidirectional fluxes of each species can still flow and be coupled. Such "exchange diffusion" systems have been widely studied.

Ratio of unidirectional fluxes of different species

The ratios of unidirectional fluxes come in four flavors, all strictly non-negative, illustrated below by one *trans* ratio, the unidirectional efflux of ion j divided by the influx of ion k,

$$\frac{J_j(L \to R)}{J_k(R \to L)} = -\exp\{z_j\gamma_j[V - V_j]\} \cdot \frac{\int_{0^+}^{1^-} \exp\{z_k\Phi_k(\varsigma)\}\,d\varsigma}{\int_{0^+}^{1^-} \exp\{z_j\Phi_j(\sigma)\}\,d\varsigma} \times \frac{C_j(R)\exp\{\alpha_j(1)z_j[V - V_j]\}}{C_k(R)\exp\{\alpha_k(1)z_k[V - V_k]\}} \quad (55)$$

$$= -\frac{\int_{0^+}^{1^-} \exp\{z_k\Phi_k(\varsigma)\}\,d\varsigma}{\int_{0^+}^{1^-} \exp\{z_j\Phi_j(\varsigma)\}\,d\varsigma} \times \frac{C_j(R)\exp\{z_j[(1 - \alpha_j(0))(V - V_j)]\}}{C_k(R)\exp\{z_k[\alpha_k(1)(V - V_k)]\}} \quad (56)$$

and one of the *cis* ratios, unidirectional efflux of ion j over efflux of ion k:

$$\frac{J_j(L \to R)}{J_k(L \to R)} = \frac{\int_{0^+}^{1^-} \exp\{z_k\Phi_k(\varsigma)\}\,d\varsigma}{\int_{0^+}^{1^-} \exp\{z_j\Phi_j(\varsigma)\}\,d\varsigma} \times \frac{\exp\{z_j\gamma_j[V - V_j]\}}{\exp\{z_k\gamma_k[V - V_k]\}} \times \frac{C_j(R)\exp\{\alpha_j(1)z_j[V - V_j]\}}{C_k(R)\exp\{\alpha_k(1)z_k[V - V_k]\}} \quad (57)$$

$$= \frac{\int_{0^+}^{1^-} \{z_k\Phi_k(\varsigma)\}\,d\varsigma}{\int_{0^+}^{1^-} \exp\{z_j\Phi_j(\varsigma)\}\,d\varsigma} \times \frac{C_j(R)\exp\{z_j[(1 - \alpha_j(0))(V - V_j)]\}}{C_k(R)\exp\{z_k[(1 - \alpha_k(0))(V - V_k)]\}}. \quad (58)$$

Note that the ratio only depends on the selectivity (i.e., phase-boundary potentials) on the left-hand side of the channel, at $z = 0$.

[2] And a full derivation of this case requires a stochastic model that we are struggling to formulate, and solve!

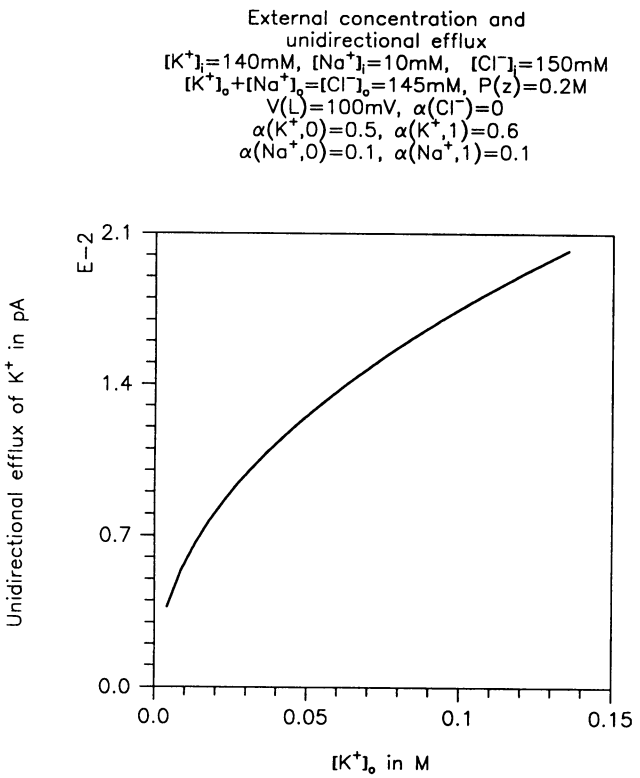

FIGURE 3 The variation in unidirectional efflux with external $[K^+]$. The conditions are specified in the figure itself, the text, and Table 3. Unidirectional efflux is defined in the text and in Appendix 2.

METHODS

The concentration and potential within a channel, and the flux and current through it, have been calculated from the PNP theory (12) modified to include the selectivities α_j and their asymmetries γ_j as defined in Eqs. 31 *et seq.* and Eq. 49. The selectivities and their asymmetries are constants in these calculations. The differential equations have been numerically integrated (12) and the results graphed. The summary formulae presented in this paper are used only for purposes of comparison and understanding. All the graphs shown are the outputs of the full numerical calculations. The permanent charge density $P(z)$ is taken as a constant, independent of z, in the calculations illustrated in Results, for the sake of simplicity and nothing else. The differential equations, numerical analysis, and summary formulae all allow a general spatially dependent $P(z)$. Spatially dependent diffusion constants $D(z)$ can be incorporated into the PNP theory with little trouble (see Appendix and Ref. 12), when warranted by experimental data or forthcoming simulations of molecular dynamics.

RESULTS

The unidirectional efflux through a classical Nernst-Planck system is independent of the *trans* concentration because its ionic concentrations move independently. Fig. 3 shows that the unidirectional efflux of ionic concentration in a PNP system depends on the *trans* concentration even though the concentrations of this system can move through each other. The dependence on *trans* concentration occurs because the electric field within the channel depends on the *trans* concentration. Fig. 4 shows the spatial distribution of the electrical potential $\Phi(z)$ and the energy density $W(z)$ for two different *trans* concentrations, where

$$W(z) \equiv \sum_j z_j C_j(z)[\Phi(z) - \Phi_{bi}(1^+)]. \qquad (59)$$

The electrical potential measures the potential energy of a single (positive) charge. The energy density measures the potential energy of the concentration of ions actually in the channel at that point. Fig. 5 shows those concentrations and the electrochemical potential at each location z.

This channel was 2.5 nm long and 0.4 nm in diameter and had a uniform permanent charge density of 0.2 M, corresponding to $1/15 \simeq 0.04$ positive charges/channel. The diffusion constants were $D_K = 1.0 \times 10^{-6}$; $D_{Na} = 10^{-5}$; $D_{Cl} = 10^{-7}$ cm^{-2}·s^{-1}. Concentrations and selectivities are given in Table 2.

The expected fluxes are shown in Fig. 6. The homoflux ratio (Fig. 6 *A*) has the slope predicted from our Eq. 53; the *trans* and *cis* flux ratios are shown in Fig. 6 (*B* and *C*). The current-voltage relation is in Fig. 6 *D*.

When comparing these results with experimental data, it is important to remember that the PNP theory contains no gating phenomena. It describes (a constant number of) permanently "open" transporters, whereas the macroscopic fluxes observed experimentally come from a variable number of transporters: the number of open transporters is likely to vary with, for example, $[K^+]_{out}$.

The strange current-voltage relation of Fig. 6 *D* is a reflection of the value of α_j chosen in this calculation, namely $\alpha(K^+, 0) = 0.5$ and $\alpha(K^+, 1) = 0.6$. If an unselective channel is used in the calculation, the current voltage relation reverses as anticipated.

Fig. 7 *A* shows the counter-flux J_k predicted by Table 1 for these conditions. The concomitant current-voltage relation is shown in Fig. 7 *B*.

DISCUSSION

The finding of uphill transport (counter-transport) in a channel of one conformation surprised us. At first we thought it forbidden by the second law of thermostatics. But that law does not apply to open systems, to which matter and energy are supplied or withdrawn, unless the systems are made closed by explicit analysis of the sources of matter and energy. The model system considered here maintains constant concentrations and electrical potentials in both baths (as do real physiological systems in the laboratory and in life), even though flux and current flow through the channel. Thus, the boundary conditions are sources that supply charge, matter, and energy to the system and must be included in any

thermostatic analysis. Thermostatic analysis, including boundary sources, is rarely performed in kinetic models, field theory, or boundary value problems, in general, and is not attempted here. Of course, any thermostatic analysis, even including boundary sources, would not be of much use in the present situation. It would apply only at equilibrium, when all fluxes are zero, in which case our results are clearly consistent with the second law, if only because of the thermostatic constraint we impose, namely Eq. 30.

Although thermostatics does not provide a useful check on our analysis, thermodynamics does—particularly the nonequilibrium thermodynamics (39, 40) derived (41) to describe diffusion processes in open systems like the PNP channel (see Appendix: Entropy Production and Thermodynamic Constraints). Nonequilibrium thermodynamics defines the local entropy production in a diffusion process as a generalization of the familiar IV heat production in a resistor. If the local entropy production is positive, the process is dissipative at that place in an open system. Entropy production can be negative in one location and positive in another in the general theory, if spatial coupling or spontaneous symmetry breaking occurs, but we do not allow such complexities here; the PNP model is required to be dissipative everywhere, including in the selectivity filters themselves.

The PNP channel considered here is necessarily dissipative in the interior of the channel $0^+ < z < 1^-$, no matter what the parameter values. The selectivity filters $\alpha_j(0)$ and $\alpha_j(1)$ of the PNP channel are forced to be dissipative by the thermodynamic constraint (Eq. 71 of Appendix: Entropy Production and Thermodynamic Constraints). More general theories, in which entropy is decreased in one selectivity filter by entropy production elsewhere, might be valid descriptions of ATP-driven active transport, although they would be too remote and abstract for our taste.

The selectivity parameters α_j are mathematical idealizations of the selectivity filter of the PNP channel. Physically, selectivity must depend on the (small $\simeq kT$) energy difference between an ion in the bath and an ion in the channel, chiefly reflecting the difference in the energy of solvation of the ion in the bath (i.e., the hydration energy of the ion $\gg kT$) and the energy of solvation in the channel, also much larger than the thermal energy. In the bath, all the nearest neighbors of the ion are waters; in the channel most are atoms of the protein (e.g., carbonyl oxygens in gramicidin) and some are oxygens of water. (In gramicidin two of the nearest neighbors are water oxygens.) The small difference of the hydration and channel solvation energies can be either positive or negative, depending on the details of the atomic interactions, for example, the structure and dynamics of the protein. Because the entry step depends on such small differences in large energies, it is an easy place for the protein to exert control over ionic movement, producing selectivity,

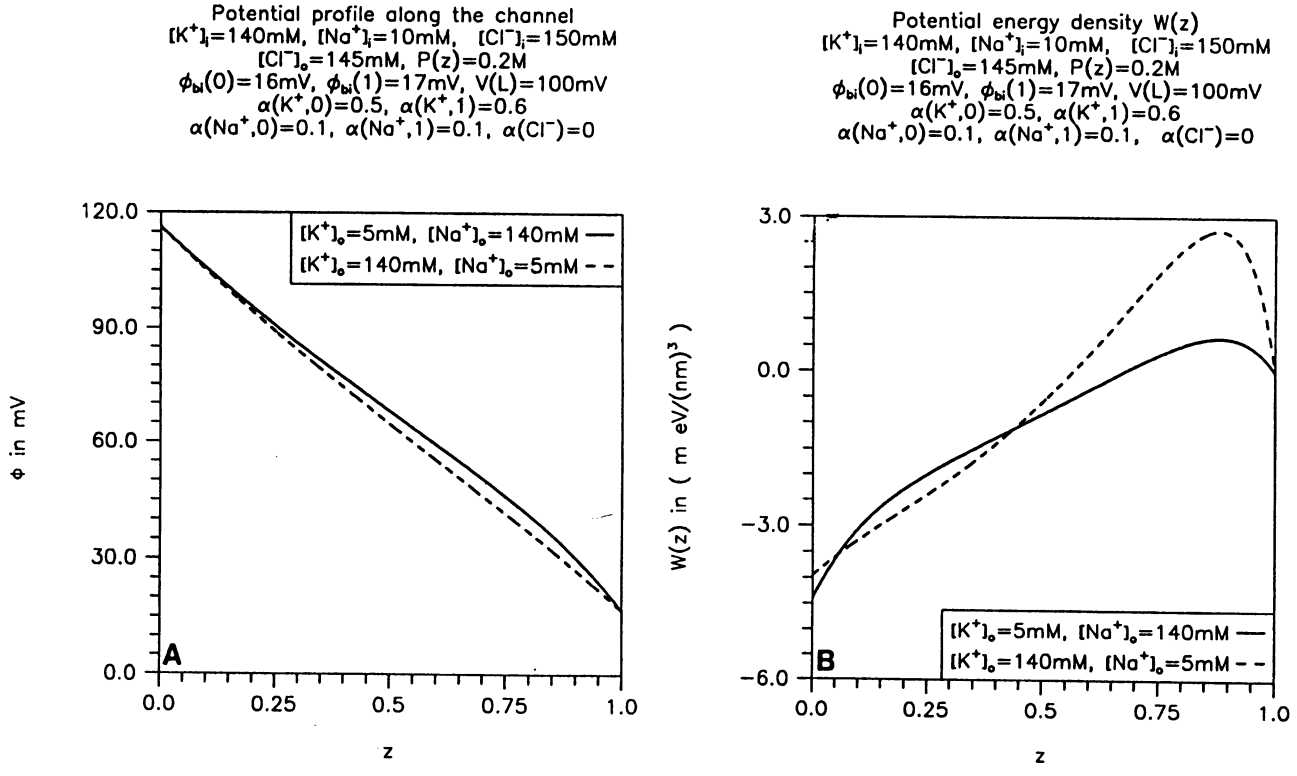

FIGURE 4 (A) The electrical potential $\Phi(z)$ under two different ionic conditions. The conditions are specified in the figure itself, the text, and Table 3. The difference in potential seems small to the eye, but it appears in integrals of exponential functions of the potential and so it has a significant effect on the concentration $C_{(z)}$ and the flux, particularly its qualitative properties shown in Fig. 3. Coupling occurs in the PNP model because the change in bathing concentrations changes the distribution of potential Φmi(z). (B) The energy density $W(z)$ under two different ionic conditions. $W(z)$ measures the potential energy of the concentration of all ions $z_jC_j(z)$ at that location; it determines the macroscopic flux J_j more directly than the electrical potential $\Phi(z)$ and so, not surprisingly, changes more dramatically than $\Phi(z)$ when ionic concentrations are changed (as specified in the figure itself, the text, and Table 3).

resistance, and perhaps even flux coupling (see Appendix: Entropy Production and Thermodynamic Constraints).

In the PNP theory presented here, the selectivity parameters are chosen so that the selectivity filter is a dissipative structure, in which the total entropy production is always positive, as just described. In some ionic conditions (e.g., when only one ionic species permeates and it is close to equilibrium) α_j may be restricted to the unit interval $0 \leq \alpha_j \leq 1$, but in general it is not, neither by the thermodynamic requirement that the selectivity filter be dissipative, nor by estimates of the energies of hydration and solvation.

Mathematically, the thermodynamic constraints of the PNP theory (Eq. 71) imply that the selectivities $\alpha_j(\cdot)$ are in some (ill-defined, multivalued) sense functions of the electrochemical potentials and diffusion constants of all ions. This dependence resembles the dependence of phase-boundary (i.e., diffusion) potentials on the properties of all ions, even when these diffusion potentials arise in free solutions, without organizing structure or proteins. Nonetheless, in the calculations shown in Results, the selectivities $\alpha_j(\cdot)$ are strictly constant; they are one set of numbers, chosen so that the thermodynamic constraint (Eq. 71) is

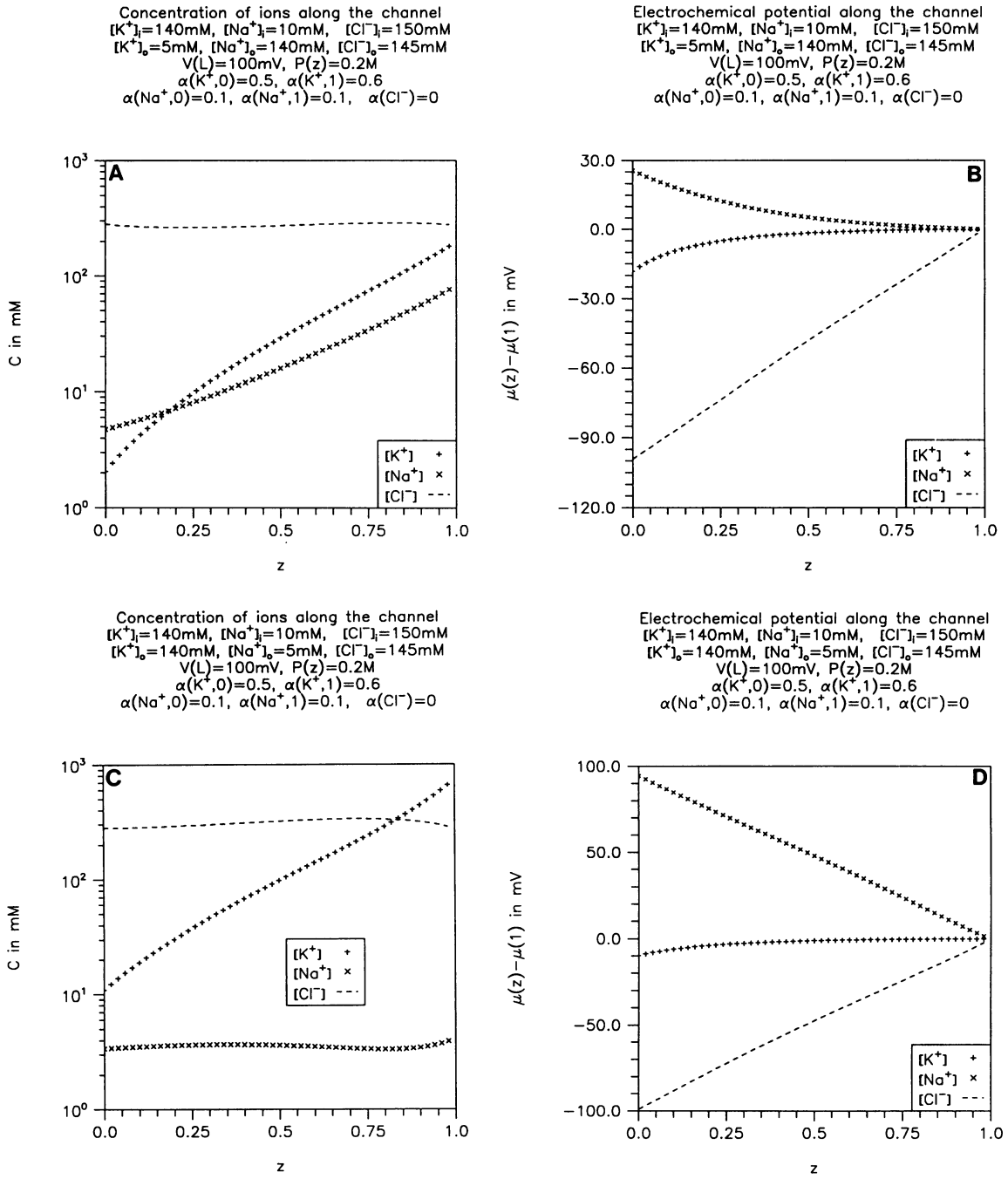

FIGURE 5 The concentration of ions (*A* and *C*) and the electrochemical potential (*B* and *D*) under the two ionic conditions (*A* and *B* and *C* and *D*). The conditions are specified in the figure itself, the text, and Table 3.

TABLE 2 Coupling of net transport

Asymmetry		$\gamma_k > 0$: downhill	$\gamma_k = 0$: futile	$\gamma_k < 0$: uphill
$\gamma_j > 0$	downhill	Cotransport	Net transport is uncoupled	Counter-transport
$\gamma_j = 0$	futile	Net transport is uncoupled	Futile transport "exchange diffusion"	—
$\gamma_j < 0$	uphill	Counter-transport	—	—

Cotransport means both fluxes are in the same direction. Counter-transport means the fluxes are in the opposite direction. Uncoupled means the *net* flux of one species is zero: the net flux of the other species and unidirectional fluxes of both species may be large. Futile transport means no *net* transport; unidirectional fluxes of both species may be large. Uphill flux of one species can occur only when the flux of the other species is downhill.

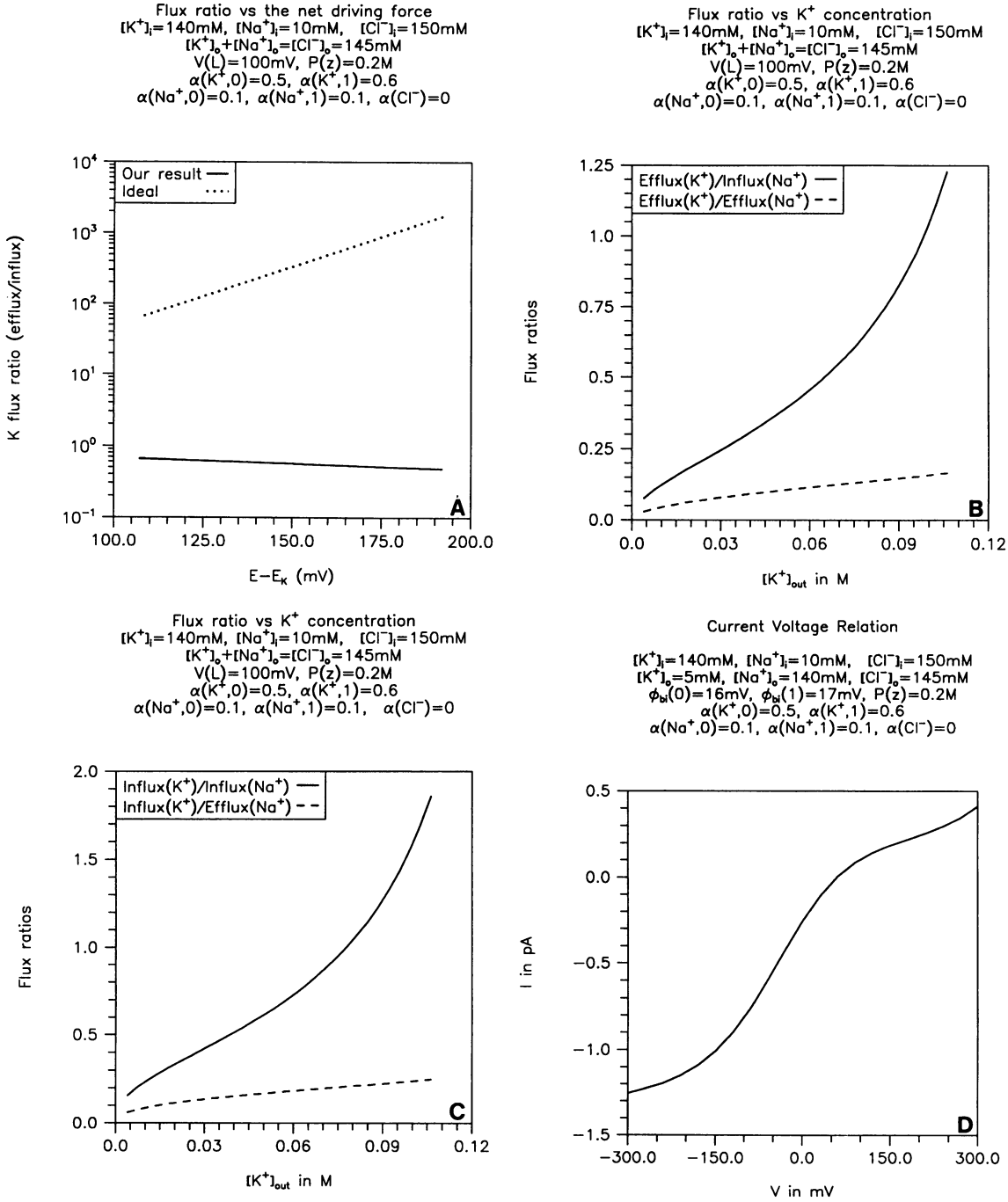

FIGURE 6 The flux ratios as a function of driving force (*A* and *B*) and concentration (*C*), and the concomitant current voltage relation (*D*). The conditions are specified in the figure itself, the text, and Table 3. $E - E_K$ is the dimension*al* version of the dimension*less* driving force $V - V_K$ of the text:

$$E - E_K \equiv RT[V - V_K]$$

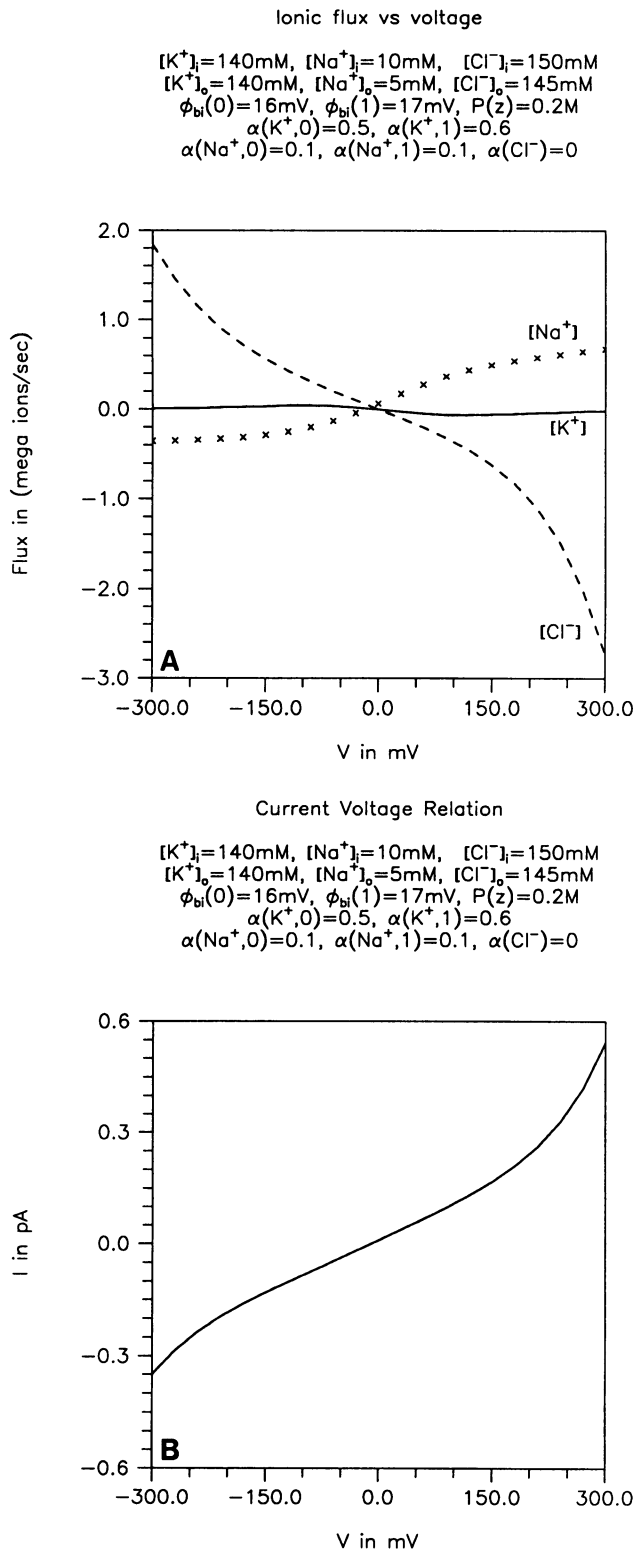

FIGURE 7 The ionic flux and current as a function of potential. The conditions are specified in the figure itself, the text, and Table 3.

satisfied for all the concentrations and transmembrane potentials investigated, with just that one set of values $\alpha_j(\cdot)$. The coupling phenomena reported here do not arise from

TABLE 3 Uniform channel

Concentration	Inside = $C_j(L)$	Outside = $C_j(R)$
C_1 = [K^+] (mM)	140	5
C_2 = [Na^+] (mM)	10	140
C_3 = [Cl^-] (mM)	150	145
Selectivity = $\alpha_j(\cdot)$	$\alpha_1(L)=\alpha_K(L)=0.5$	$\alpha_1(R)=\alpha_K(R)=0.6$
	$\alpha_2(L)=\alpha_{Na}(L)=0.1$	$\alpha_2(R)=\alpha_{Na}(R)=0.1$
	$\alpha_3(L)=\alpha_{Cl}(L)=0$	$\alpha_3(R)=\alpha_{Cl}(R)=0$

an implicit dependence of the selectivity $\alpha_j(\cdot)$ of one species on the selectivity $\alpha_k(\cdot)$ of another species.

The model presented here is most vague, and thus weakest, in its treatment of selectivity. In particular, we assume that selectivity arises entirely as the ion enters and diffuses through the pore, as described by the parameters α_j and D_j, with whatever values are necessary to fit classical permeability (2) and flux ratios. Thus, the values of the selectivity α_j and diffusion constants D_j are surmised, but not derived or justified, as they should be. That derivation requires a specific physical model of channel structure, permeation, selectivity, and entry, dealing with many of the properties we idealize away in this paper, like the finite size of the ions. We suspect that such a complete theory would yield values of α_j and γ_j (at all concentrations and potentials, etc.) falling only in the range allowed by the thermodynamic constraint, Eq. 71. In the absence of such a theory, the selectivities must be chosen with care, so that they fall in that range under all experimental conditions.

Branched channels as active transporters

It is important to remember that some channels may have complex structure with important consequences for flux and flux coupling. In particular, some channels are branched pipes, shaped something like the letter Y, as inferred originally by Miller (42, 43) for a chloride channel, by Matsuda (44) for a potassium channel, and then shown directly by Schulz's group (45, 46) for a porin channel. If the short branches of the channel (i.e., the diagonal strokes, the upper branches of the letter Y) had different selectivities, it seems quite likely that the concentration and electrical potential at the node, and thus electrochemical potential drop across those branches, would depend on the properties (e.g., diffusion constants) of each branch. The effective selectivity $\alpha_j(0)$ would then describe the combined properties of both branches and would be defined by generalizing Eq. 31 to the branched geometry, using Kirchoff's laws (23). If ATP hydrolysis occurred at the end of the channel and anion (e.g., phosphate) current were confined to one or two branches, the electrochemical potential at the node of the Y-shaped channel would be determined by the ATP hydrolysis. Such a branched channel would have the energy coupling needed in a model of ATP-driven active transport. It could also move nonelectrolytes (as could an unbranched channel, for that matter) as a nonuniform electric field moves nonelectrolytes during dielectrophoresis (47). Explicit treatment of the occlusion

found in biological active transport systems requires a generalization of the PNP theory to time-dependent phenomena analogous to gating (48).

Direct visualization of the structure of an active transporter, as well as a more realistic analysis of entry and selectivity, is clearly needed to discipline and motivate our further speculation.

Other models of selectivity and entry

Other physical models of selectivity, ion entry (e.g., through antechambers) (25, 26) or activated movement across large barriers (49–51), and phase-boundary potentials could no doubt replace Eqs. 31–42. Alternative models might include a discontinuity in electrical potential at either end of the channel, as well as a discontinuity of concentration. We have, in fact, constructed, computed, and rejected models in which the discontinuity at the ends of the channel was entirely in the electrical potential, because the predictions of the fluxes at different concentrations and potentials seemed strange, at least to us.

The flux ratio predicted by any of these models is the same, however, because the flux ratio depends on the jump δ_j in electrochemical potential (see Eq. 26); the flux ratio does not depend on how much of the phase-boundary potentials δ_j arise from jumps in electrical potential or jumps in concentration.

Measurement of unidirectional tracer fluxes at various concentrations and potentials in channels (8, 52–56) and transporters (Hille, Läuger, and Stein supply gateways to this huge literature) (1, 11, 57) could limit the choice of models of δ_j, if such macroscopic measurements accurately reflect the behavior of a fixed number of a single type of channel. More likely, the macroscopic measurements will depend to some extent on "gating" phenomena (2) and so will not always involve a fixed number of channels and thus not be directly comparable to our predictions.

Theoretical analysis will also be helpful in determining the phase-boundary potentials, their physical meaning, functional dependence, and physiological consequences. An hierarchy of models may allow a better analysis of the selectivity filter and access process, including, for example, macroscopic analysis of the transient problem; macroscopic and stochastic analysis of the entry and selectivity process and filter; and simulations of the molecular dynamics of channel protein, water, and ions, with reasonable properties and finite size, as they enter and pass through the channel, producing, we suspect, the coupling implied by our boundary conditions (see Appendix: Entropy Production and Thermodynamic Constraints).

Meanwhile, the crude model of access resistance presented here is enough to demonstrate the main point: flux ratios can have many values, even in a simple Nernst-Planck system if selectivity is included as a purely dissipative nonequilibrium access resistance, even though ions are described, somewhat ridiculously, as concentrations that can flow through each other in a channel of one conformation. Such a system can have properties usually characterized as "single filing in narrow channels" or "exchange diffusion in a mediated transporter," even though it does not include explicit collisions and interatomic interactions, or multiple states and intrinsic coupling (although its ionic concentrations do interact through Poisson's equation), even though it is dissipative in every location, including its boundaries.

The coupling of macroscopic fluxes in this model channel is a direct consequence of its selectivity.

CONCLUSION

The PNP theory of a channel containing permanent charge and a nonequilibrium selectivity process predicts the range of flux ratios observed in biological transport, whether mediated or through channels, suggesting that more microscopic and elaborate descriptions of interactions are not necessary, calling into question the usual description of flux coupling as a necessary, intrinsic, but unexplained property of a membrane transport protein.

APPENDIX: HISTORICAL NOTE

The thermostatic constraint on phase-boundary potentials (Eq. 30) implies a coupling of the partition coefficients $\beta_j(0; J_j)$ and $\beta_j(1; J_j)$ through Eqs. 41 and 42, namely,

Thermostatic Constraint

$$\beta_j(0; J_j) = \beta_j(1; J_j) \quad \text{when} \quad \left\{ \begin{array}{c} C_j(L) = C_j(R) \\ \text{and} \\ V = 0 \end{array} \right\} \Rightarrow J_j(\text{net}) = 0 \tag{60}$$

Note that the constraint need not apply away from equilibrium. The partition coefficients need not be equal in the presence of concentration gradients or transmembrane potentials.

Traditional treatments (e.g., Hodgkin and Katz, Ref. 37, following others, no doubt) used only one partition coefficient β_j (under all conditions) to describe concentration ratios on two sides of an (asymmetrical) channel, sides that were already known to have quite different properties, and presumably different composition, chemical properties, and permanent charge. The partition coefficient was assigned its equilibrium value even far from equilibrium, and the likely existence of different partition coefficients on both sides of the membrane was ignored, at least in the printed work, possibly because the existence of different partition coefficients at two sides of the membrane (with different values even at zero flux) was known to imply a violation of thermodynamics, namely a flux when the driving force was zero. Perhaps that is why the traditional treatments of β_j were not pursued further, for example, extended, as in this paper, to nonequilibrium conditions to calculate flux ratios. It is the treatment of the partition coefficient as a nonequilibrium process that allows flux coupling in the PNP theory while satisfying the thermostatic constraint (Eq. 60), just at $J_j = 0$.

In the PNP theory of this paper, the equality of $\beta_j(0; J_j)$ and $\beta_j(1; J_j)$ seems less arbitrary, at least to us (and perhaps others; see Refs. 31, 48–50), because it need be true only under equilibrium conditions. In general, our β_j's are unequal—the variable consequences of variable offsets in electrochemical potential, the steady-state outcome of a transient charging and coupling process. The $\beta_j(\cdot)$'s are in essence phase-boundary potentials $\delta_j(\cdot)$ written on an expanding scale, in exponential units, unconstrained, except at equilibrium. The $\delta_j(\cdot)$'s are rather like IR potential drops in resistors, being equal and zero, only when flux is zero, at equilibrium. In fact, the channel system in some ways resembles a potentiom-

eter, with access "resistances" $r_j(0)$ and $r_j(1)$, flanking a pore $0^+ \le z \le 1^-$, the effective resistance of which (i.e., potential $V = \Phi(L)$ divided by flux J_j) is described by Eq. 23, etc.

APPENDIX: FLUX EXPRESSION

We present the derivation of the integrated flux formula 23 responding to requests to make our presentation reasonably self-contained, although, no doubt, the derivation has already appeared many times in the literature.

The dimensional steady-state Nernst-Planck equations are

$$\mathscr{J}_j = -D_j(x)\left[\frac{d\hat{C}_j}{dx} + \frac{z_jF}{RT}\hat{C}_j\frac{d\varphi}{dx}\right]. \tag{61}$$

Divide both sides of the equation by $D_j(x)$, and then multiply by the integrating factor $\exp[(z_jF/RT)\varphi(x)]$, and write the "total differential"

$$\begin{aligned}\frac{\mathscr{J}_j}{D_j(x)}\exp\left[\frac{z_jF}{RT}\varphi(x)\right] &= -\left\{\frac{d\hat{C}_j}{dx}\exp\left[\frac{z_jF}{RT}\varphi(x)\right] + \hat{C}_j(x)\frac{z_jF}{RT}\frac{d\varphi}{dx}\exp\left[\frac{z_jF}{RT}\varphi(x)\right]\right\} \\ &= -\frac{d}{dx}\left\{\hat{C}_j(x)\exp\left[\frac{z_jF}{RT}\varphi(x)\right]\right\}.\end{aligned} \tag{62}$$

Integration from one position x_1 inside the channel (where the Nernst-Planck equations are valid) to any other position inside the channel x_2, followed by a division, gives the flux expressions we call the integrated Nernst-Planck equations:

$$\mathscr{J}_j = \frac{\hat{C}_j(x_1)\exp\left[\frac{z_jF}{RT}\varphi(x_1)\right] - \hat{C}_j(x_2)\exp\left[\frac{z_jF}{RT}\varphi(x_2)\right]}{\int_{x_1}^{x_2}\frac{\exp[(z_jF/RT)\varphi(\varsigma)]}{D_j(\varsigma)}d\varsigma}. \tag{63}$$

Note that in this one-dimensional steady-state problem $\mathscr{J}_j$ is independent of x.

Using the nondimensional variables defined in the text, and assuming D_j to be independent of x, the now nondimensional flux can be written in terms of parameters just inside the boundaries, namely at $x = 0^+$ and $x = 1^-$, that is in terms of the boundary concentrations $C_j(0^+)$ and $C_j(1^-)$, the boundary electrical potentials $\varphi_j(0^+)$ and $\varphi(1^-)$ or the boundary electrochemical potentials $\bar{\mu}_j(0^+)$ and $\bar{\mu}_j(1^-)$. Note that the denominator depends on the potential everywhere $\Phi(\zeta)$, $0^+ \le \zeta \le 1^-$, but the numerator depends only on the (electrical) potentials, concentrations, or electrochemical potentials just inside the boundary:

Integrated Nernst-Planck Equation

$$\begin{aligned}J_j &= \frac{C_j(0^+)\exp[z_j\Phi(0^+)] - C_j(1^-)\exp[z_j\Phi(1^-)]}{\int_{0^+}^{1^-}\exp[z_j\Phi(\zeta)]\,d\zeta} \\ &= \frac{e^{\bar{\mu}_j(0^+)} - e^{\bar{\mu}_j(1^-)}}{\int_{0^+}^{1^-}\exp[z_j\Phi(\zeta)]\,d\zeta}.\end{aligned} \tag{64}$$

The potential everywhere $\Phi(\zeta)$ can be determined only by solving Poisson's equation, and boundary conditions, which in turn depends on the concentrations of each ion everywhere $C_j(\zeta)$, and the density of permanent charge, etc. Thus, complete knowledge of the flux requires solution of the coupled PNP model.

Expressions for the flux ratio, however, do not require knowledge of potential and concentration everywhere. Rather, they depend only on the concentration, electrical potential, and/or electrochemical potential just inside the boundary. The denominator cancels out of expressions for flux ratios; and the denominator is the only place the potential profile $\Phi(\zeta)$ appears in the integrated flux expression, Eq. 64.

APPENDIX: ENTROPY PRODUCTION AND THERMODYNAMIC CONSTRAINTS

The PNP theory is a macroscopic description of atoms diffusing in random walks with probability densities described by a local Gaussian distribution (58) or (equivalently) by a global Fokker-Planck equation (see Ma, Ref. 59, pp. 214–219, 376–379). Thermostatics has been extended to describe flux in such diffusion systems (also called Gaussian-Markov in De Groot and Mazur, Ref. 41, chapter 7, particularly pp. 111–119; Callen, Ref. 39, chapters 15 and 16).

Although the extension of thermostatics to general systems has not been successful (as emphasized by Truesdell, Ref. 60, pp. 365–404; and Jaynes, Ref. 61; see also Refs. 62 and 63), it can be extended to systems of electrodiffusion, like those of interest here, by introducing the idea of entropy production $\sigma(x)$ (units: joules·m^{-3}·s^{-1}) at location x. We found the treatment of Katchalsky and Curran (Ref. 40, chapter 7, particularly p. 82, equation 7-29, and p. 90, equation 8-8) most helpful.

$$\sigma(x) \equiv \sum_j \mathscr{J}_jX_j \equiv -\sum_j \frac{\mathscr{J}_j}{T}\frac{\partial M_u(j)}{\partial x}, \tag{65}$$

where we use the absolute temperature T, dimensional fluxes $\mathscr{J}_j$, electrochemical potentials $M_u(j)$, and corresponding generalized forces $X_j(x)$. For purely electrical systems (i.e., systems without diffusion because concentrations are everywhere constant, $\partial C_j/\partial x \equiv 0$) the entropy production is analogous to the familiar irreversible IR heat production in a resistor, given by $IV(x)$ in that special case.

In general, the fundamental Nernst-Planck Eq. 61 can be written

$$\mathscr{J}_j = -\frac{D_j(x)C_j(x)}{RT}\frac{\partial M_u(j)}{\partial x}. \tag{66}$$

Remember that $\mathscr{J}_j$ is independent of x in this stationary problem, although it depends on all the other parameters of the problem, implicitly and explicitly as shown in Eq. 64, for example.

In the interior of the channel $0^+ < x < d^-$, the system is dissipative: the total entropy production $\sigma(x)$ is positive. The flux of each ion is also dissipative because the entropy production for each species is positive $\sigma_j(x) > 0$:

$$\sigma(x) \equiv \sum_j \sigma_j(x) = \frac{1}{RT^2}\sum_j D_j(x)C_j(x)\left[\frac{\partial M_u(j)}{\partial x}\right]^2 > 0; \qquad 0^+ < x < d^-. \tag{67}$$

The system is entirely dissipative in this region, no matter what the direction of flow, or type of transport, or coupling of fluxes, or values of parameters.

Now, consider the selectivity filters, the boundary regions $0 \le x \le 0^+$ and $d^- \le x \le d$ across which there are (nonequilibrium) drops $\delta_j(0)$ and $\delta_j(d)$ in the (nondimensional) electrochemical potential (see text, Eqs. 3, 4, and 39–46), defined with the usual physiological sign conventions. The total entropy production in the selectivity filters, the end regions of length h, can be written from Eq. 65, for example,

$$\begin{aligned}\sigma(\xi_0)\cdot h &= -\sum_j \frac{\mathscr{J}_j}{T}[M_u(j; x = 0^+) - M_u(j; x = 0)] \\ &= R\sum_j \mathscr{J}_j\cdot\delta_j(0),\end{aligned} \tag{68}$$

where ξ_0 is some location within the left-hand selectivity filter, $0 < \xi_0 < 0^+$.

If the selectivity filters are described as electrochemical resistors (as in text, Eqs. 31 and 32), using the dimensionless spatial variable $z = x/d$ as the argument of the function,

$$\delta_j(0) = z_j\alpha_j(0)[V - V_j]; \qquad \delta_j(1) = z_j\alpha_j(1)[V - V_j], \tag{69}$$

their entropy production, accompanying selectivity, is

$$\sigma(\xi_0) \cdot h = R \sum_j \mathscr{J}_j z_j \alpha_j(0)[V - V_j]$$

$$\sigma(\xi_1) \cdot h = R \sum_j \mathscr{J}_j z_j \alpha_j(1)[V - V_j]. \quad (70)$$

Clearly, if transport is downhill (i.e., $V - V_j$ and J_j have the same sign; Tables 1 and 2), and the selectivities $\alpha_j(\cdot)$ are positive, the entropy production in the selectivity filters is positive, as it must be, if the selectivity filters are to be strictly dissipative and local, as we suppose in this theory.

The situation is somewhat more complex if several species are present and one species, say k, moves uphill through the selectivity filter (say, the one at $x = 0$) while another species, say m, moves downhill through it. Then (see Tables 1 and 2), $V - V_k$ and J_k have opposite signs. The uphill flux of k is accompanied by, of course, negative entropy production $\sigma_k(0) < 0$ for that component in that selectivity filter. In a locally dissipative system like the selectivity filters envisioned here, negative entropy production by one ion k can occur only when it is balanced (at that location) by the positive entropy $\sigma_m(0) > 0$ generated by another ion m, moving downhill, at that location. Thus, uphill transport can occur in a dissipative selectivity filter, if, but only if, the selectivities $\alpha_j(0)$ (describing the selectivity filter at $x = 0$) are constrained so that the total entropy production there $\sigma(0)$ is positive. The same discussion applies separately to the entropy production $\sigma(1)$ (and its components $\sigma_k(1)$ and $\sigma_m(1)$), at the other selectivity filter, at $x = 1$; in a locally dissipative system, like that considered in this paper, a decrease in entropy at one location cannot be balanced by an increase at another location.

In the PNP channel, flux coupling occurs because of dissipative processes in each of the selectivity filters. In these filters the selectivity parameters of different ions (say, $\alpha_m(1)$ and $\alpha_k(1)$ or $\alpha_m(0)$ and $\alpha_k(0)$) are constants, not varying with experimental conditions, not coupled to one another in any way. This description of selectivity is compatible with thermodynamics provided the selectivities $\alpha_j(0)$ at one end of the channel and the set $\alpha_j(1)$ at the other both satisfy thermodynamic constraints:

Thermodynamic Constraint

$$\sum_j \mathscr{J}_j z_j \alpha_j(0)[V - V_j] \geq 0$$

$$\sum_j \mathscr{J}_j z_j \alpha_j(1)[V - V_j] \geq 0. \quad (71)$$

These constraints are required in addition to the thermostatic constraints on the phase-boundary potentials $\delta_j(0)$ and $\delta_j(1)$, described in text (Eq. 30). It is not difficult to choose selectivities that satisfy both constraints, as do the selectivities used in the calculations reported in Results. The need for these constraints, in addition to the differential equations and boundary conditions of the PNP model itself, is not a weakness of the model. In the analysis of many nonequilibrium systems, thermodynamics is needed to impose "constraints on the transport coefficients by predicting that they must obey a certain number of inequalities" (Balian, Ref. 63, p. 267).

The PNP model does not specify the physical mechanism by which the entropy consumed by uphill transport of one ion in one selectivity filter is coupled to the entropy production produced there by the downhill movement of other ions. Indeed, it seems clear that the physical processes described by the PNP differential equations cannot account for that coupling of entropy consumption and production by themselves, without boundary conditions like Eq. 31. On the other hand, the underlying processes are probably closely related to interactions found in free solution or gases in which the flux of one species is driven by the driving force of another, usually described by Onsager reciprocal relations or generalized diffusion coefficients D_{jk} (see pp. 540, 715–717 of Hirschfelder et al., Ref. 64, for the analogous treatment of gases). Such interactions can be expected at the ends of channels, where massive dehydration must be balanced by massive re-solvation, with small imbalances having large effects on permeation, selectivity, and coupling.

APPENDIX: UNIDIRECTIONAL AND TRACER FLUXES

The unidirectional fluxes defined in this paper (e.g., Eq. 22) were theoretical constructs until radioactive tracers became available. Since then, however, hundreds if not thousands of papers have used the net flux of tracer (measured when tracer is present in significant amounts on only one side of a membrane) as an estimate of the unidirectional flux of the main species through channels (2, 3) or transporters (1, 11, 65). In the present context, channel transport would be expected in systems with $\gamma_j > 1$ and mediated transport in systems with $\gamma_j \leq 1$.

Tracer fluxes reported in the literature flow, of course, through macroscopic numbers ($\sim 10^5$) of channels or transporters because the flux of ions through a single channel or transporter protein is much too small to be measured directly, by chemical or isotope counting techniques. The electrical charge carried by ions through a single channel is, however, large enough to measure—indeed that current is now measured routinely in hundreds of laboratories every day with the patch clamp method—but the charge moving through other transport proteins is expected to be invisible in the background noise of presently available patch-clamp amplifiers (see Wang, Tang, and Eisenberg, Ref. 66, for a possible exception).

Unidirectional flux through gated channels or transporters

A macroscopic tracer flux can be interpreted as a unidirectional flux through a single channel or transport molecule only if the number of open channels through which the flux flows does not change as experimental and biological conditions are manipulated. That is to say, the area through which the flux flows must remain constant; experimental interventions used to manipulate the system and fluxes must not change gating.

Measurements of currents through single open channels have shown that many experimental manipulations modify the gating of channels, without changing the current flowing through a single open channel. Measurements of current through single transport proteins (other than channels) are rare, and measurements of tracer flux nonexistent, but their scarcity represents a technological and historical constraint rather than a biological phenomenon. Our present inability to observe gating in most transport proteins does not mean gating is absent in these molecules.

Indeed, one must suspect that transport proteins are able to transport ions only a certain fraction of the time, just like channels; one must suspect that they are sometimes active, sometimes inactive, just like channels. If so, the current through them, when they are open (i.e., active), may be much larger than commonly supposed. In fact, the usual estimates of flux through the transport protein would have to be divided by the fraction of time the protein is active.[3] In that case, experimental interventions might change macroscopic unidirectional flux by changing the fraction of time the transport protein is transporting, as well as by changing the amount of transport when the protein is active. Without a description of such "gating" phenomena, no theory, certainly not that presented here, will be able to describe isotope fluxes through macroscopic numbers of channels observed experimentally and their variation with ionic and electrical conditions.

Unidirectional flux estimated by the net flux of tracer

It is perhaps worthwhile presenting a careful analysis of the usual physiological method of estimating unidirectional fluxes, because our colleagues in the physical and mathematical sciences seem unaware of the method and

[3] The fraction of time a single protein molecule is active equals the probability of any one molecule being open in a patch of membrane (about 0.1 in many cases) divided by the number of those proteins in that patch (say 20,000 in one case of interest) (66).

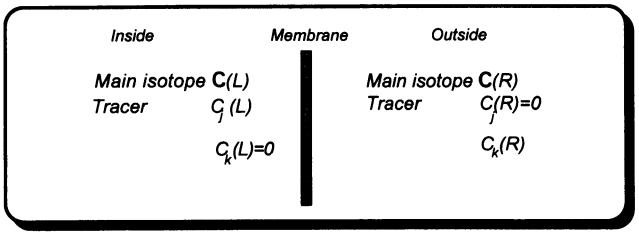

FIGURE 8 Typical set-up for measuring unidirectional isotope flux. Note the concentration of main isotope (nonradioactive) is $\mathbf{C}(L)$ written without a subscript. The concentration of the j isotope, confined to the left, is $C_j(L)$; the concentration of the k isotope, confined to the right, is $C_k(R)$.

are unconvinced of its power by the derivations and arguments we have located for them in the physiological literature.

Consider a set-up for measuring tracer flux (Fig. 8) in which three different isotopes of the same ion species are present. The concentration of the main isotope on the left is $\mathbf{C}(L)$, written without a subscript in bold face, and the concentration of one tracer (also on the left) is denoted by a subscript j, namely $C_j(L)$. The concentration of the main isotope on the right is $\mathbf{C}(R)$, written without a subscript, and the concentration of a different tracer (on the right), is denoted by a subscript k, namely $C_k(R)$. Each tracer is confined to one side of the channel: the concentration of one tracer $C_j(R)$ on the right is kept insignificant, as is the concentration of the other tracer $C_k(L)$ on the left. That is to say, the *trans* concentrations of tracer are negligible compared to the concentrations of tracer on the *cis* side:

$$C_j(L) \ll \mathbf{C}(L); \qquad C_k(R) \ll \mathbf{C}(R). \tag{72}$$

Because the isotopes are indistinguishable except for their radioactivity, all functions, variables, and parameters except concentration and flux are the same for all species. In particular, one set of functions $\Phi(x)$, $\beta(0)$, $\beta(1)$, etc. describe all the isotopes.

The unidirectional fluxes of the main species are written in this Appendix without subscript and in bold face, using Eq. (47):

$$\text{Unidirectional efflux} = \mathbf{J}(L \to R) = \mathbf{C}(L) \cdot \frac{\beta(0; \cdot)\exp\{zV\}}{\int_{0^+}^{1^-} \exp\{z\Phi(\varsigma)\}d\varsigma} \tag{73}$$

$$\text{Unidirectional influx} = \mathbf{J}(R \to L) = \mathbf{C}(R) \cdot \frac{\beta(1; \cdot)}{\int_{0^+}^{1^-} \exp\{z\Phi(\varsigma)\}d\varsigma}. \tag{74}$$

The unidirectional and net flux $J_j(\cdot)$ of the tracer species j are written with subscripts. Then,

$$\begin{gathered}\text{Tracer influx} = J_j(R \to L) = 0,\\ \text{because } \textit{trans} \text{ concentration } C_j(R) = 0.\end{gathered} \tag{75}$$

$$\text{Tracer efflux} = J_j(L \to R) = C_j(L) \cdot \frac{\beta(0; \cdot)\exp\{zV\}}{\int_{0^+}^{1^-} \exp\{z\Phi(\varsigma)\}\, d\varsigma} = J_j\,(\text{net}). \tag{76}$$

The unidirectional and net flux $J_k(\cdot)$ of the other tracer species k are also written with subscripts. Then,

$$\text{Tracer influx} = J_k(R \to L) = C_k(R) \cdot \frac{\beta(1; \cdot)}{\int_{0^+}^{1^-} \exp\{z\mathbf{C}(L)\Phi(\varsigma)\}\, d\varsigma} = J_k\,(\text{net})$$

$$\text{Tracer efflux} = J_k(R \to L) = 0$$

$$\text{because } \textit{trans} \text{ concentration } C_k(L) = 0.$$

The result is that each unidirectional flux $\mathbf{J}$ of the main species is proportional to the unidirectional flux of one of the tracer species, which is also the net flux J_j of that tracer isotope.

$$\mathbf{J}(L \to R) = \left\{\frac{\mathbf{C}(L)}{C_j(L)}\right\} \cdot J_j(L \to R) = \left\{\frac{\mathbf{C}(L)}{C_j(L)}\right\} \cdot J_j\,(\text{net})$$

$$\mathbf{J}(R \to L) = \left\{\frac{\mathbf{C}(R)}{C_k(R)}\right\} \cdot J_k(R \to L) = \left\{\frac{\mathbf{C}(R)}{C_k(R)}\right\} \cdot J_k\,(\text{net})$$

The proportionality constants, shown in the big braces, are the reciprocal of the specific activity of each isotope on the *cis* side and can easily be estimated experimentally as the ratio of the concentration of the main species to the number of radioactive disintegrations (of a particular isotope) observed in a unit time on the *cis* side. (On the *trans* side the specific activity is negligible, that is, "zero," because the concentration of isotope is kept negligible, compared to that on the *cis* side, by the experimental apparatus and protocol.) In this way, measurement of the net flux of isotope allows easy estimation of the unidirectional fluxes of the main species.

The derivation presented here uses the PNP theory for the sake of clarity and specificity. It can easily be generalized to any theory of ionic motion, provided

(a) the concentration (in moles/liter) of all tracers is negligible compared to the main species;
(b) the tracer species is chemically and physically indistinguishable from the main species (except for its radioactive disintegrations);
(c) the concentration of isotope on the *trans* side of the channel is negligible; and
(d) the functionals describing unidirectional influx and efflux (analogous to the right-hand sides of Eqs. 73 and 74) are zero when the *cis* concentrations are zero, i.e., when $\mathbf{C}(L) = 0$ and $\mathbf{C}(R) = 0$, respectively.

Then, the functionals (analogous to those on the right-hand sides of Eqs. 74 and 77) are equal and so the result, namely Eqs. 79 and 80, is established.

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