Proton Transport via the Membrane Surface

Yuri Georgievskii,* Emile S. Medvedev,† and Alexei A. Stuchebrukhov*
*Department of Chemistry, University of California, Davis, California 95616 USA and †Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow, Russia

ABSTRACT Some proton pumps, such as cytochrome *c* oxidase (C_cO), translocate protons across biological membranes at a rate that considerably exceeds the rate of proton transport to the entrance of the proton-conducting channel via bulk diffusion. This effect is usually ascribed to a proton-collecting antenna surrounding the channel entrance. In this paper, we consider a realistic phenomenological model of such an antenna. In our model, a homogeneous membrane surface, which can mediate proton diffusion toward the channel entrance, is populated with protolytic groups that are in dynamic equilibrium with the solution. Equations that describe coupled surface-bulk proton diffusion are derived and analyzed. A general expression for the rate constant of proton transport via such a coupled surface-bulk diffusion mechanism is obtained. A rigorous criterion is formulated of when proton diffusion along the surface enhances the transport. The enhancement factor is found to depend on the ratio of the surface and bulk diffusional constants, pK_a values of surface protolytic groups, and their concentration. A capture radius for a proton on the surface and an effective size of the antenna are found. The theory also predicts the effective distance that a proton can migrate on the membrane surface between a source (such as CcO) and a sink (such as ATP synthase) without fully equilibrating with the bulk. In pure aqueous solutions, protons can travel over long distances (microns). In buffered solutions, the travel distance is much shorter (nanometers); still the enhancement effect of the surface diffusion on the proton flow to a target on the surface can be tens to hundreds at physiological buffer concentrations. These results are discussed in a general context of chemiosmotic theory.

INTRODUCTION

Proton translocation across biomembranes is a key step in biological energy conversion in mitochondria and chloroplasts. The translocation is carried out by proton pumps, membrane enzymes that utilize redox energy (or light energy in photosynthetic systems) to move protons from one side of the membrane to the other, thereby creating electrochemical gradients. Later, the free energy stored in proton gradients is utilized in synthesis of ATP or other energy-requiring processes (Skulachev, 1988; Cramer and Knaff, 1990)

Proton pumps, such as cytochrome oxidase (CcO) (Wikström, 1998), are very efficient—they are capable of pumping more than 10^3 H^+ per second (Babcock and Wikström, 1992). The corresponding time scale for overall processing of a single proton is as short as 1 ms. Moreover, some proton-transfer reactions during the catalytic cycle of CcO occur on even a shorter time scale of 0.1 ms, and the reprotonation of some protolytic sites of the enzyme from the bulk is believed to occur even faster (Karpefors et al., 1999, 2000; Kotelnikov et al., 2001). Similar time scales of proton transfer reactions have been reported for bacteriorhodopsin (Heberle, 2000).

The high rate of proton pumping raises the question about the maximum possible rate of supply of protons by the organelle's medium to the pump, given the fact that the concentration of available protons is limited and the diffusion occurs with only a finite speed (e.g., in the bulk free protons diffuse with coefficient $D_b \sim 10^{-4}$ cm^2s^{-1}, see review in Gutman and Nachliel, 1997). Although the diffusion control has not been directly implicated in proton pumps, such a diffusion-limited kinetics does seem to occur in a number of proton-conducting channels (DeCoursey and Cherny, 1999). The absence of the diffusion bottleneck in CcO is ascribed to a proton-collecting antenna surrounding the entrance of the proton-conducting channel, which enhances the proton influx from the solution to the channel entrance (Gutman and Nachliel, 1997; Brandsburg-Zabary et al., 2000). Hence, the rate of proton supply is modified by the properties of the surface of the protein, or that of the surrounding membrane. The lateral membrane diffusion has been also suggested to occur in other proton-conducting systems (DeCoursey and Cherny, 1999). The relevant question then is how to correctly estimate such a diffusion-limited and surface-enhanced rate of proton supply from the medium to the entrance of the proton-conducting channel.

The above question is complicated by the fact that the nature of the protons participating in the process is not always known with certainty. For instance, under physiological conditions, the concentration of free protons in the organelles is of an order of 10^{-7} to 10^{-8}M. Hence, in a typical organelle with dimension of 1 μm, there will be only an order of one free proton or less(!) on average. The splitting of water is energetically unfavorable, hence the involved protons must belong to buffer molecules that contain acidic or basic groups. These buffer molecules can be

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Dr. Georgievskii's present address is Combustion Research Facility, MS9055, Sandia National Laboratories, P.O. Box 969, Livermore, CA 94551.

Address reprint requests to Alexei A. Stuchebrukhov, Univ. of California, Dept. of Chemistry, One Shields Ave., Davis, CA 95616. Tel.: 530-752-7778; Fax: 530-752-8995; E-mail: stuchebr@chem.ucdavis.edu.

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both mobile and immobile, i.e., fixed on the surface of membrane proteins and on the membrane itself (Gutman and Nachliel, 1997; Brandsburg-Zabary et al., 2000). The presence of fixed and mobile buffers changes the effective concentration and mobility of the protons (Junge and McLaughlin, 1987).

The question of how the membrane surface can modify the proton transport is also of interest in a more general context of chemiosmotic coupling (Nicholls and Ferguson, 1992; Ferguson, 1995). Previously, it has been suggested that the diffusion of protons along the membrane may play a major role in translocation of protons between the generators, such as CcO or bacteriorhodopsin, and consumers, such as ATP synthase (Scherrer, 1995; Teissié, 1996; Gabriel and Teissié, 1996; Nachliel et al., 1996; Antonenko and Pohl, 1998; Krasinskaya et al., 1998). Long-distance migration of protons along membranes has been observed in purple membranes and reconstituted bacteriorhodopsin (Heberle and Dencher, 1992; Alexiev et al., 1994, 1995; Heberle et al., 1994; Scherrer et al., 1994; Nachliel et al., 1996; Riesle et al., 1996). Long-range proton diffusion has also been observed along lipid (Gabriel et al., 1994) and stearic acid monolayers (Slevin and Unwin, 2000; Slevin and Unwin proposed a similar model to what is discussed in this paper, and analyzed it numerically. They found that, for stearic acid monolayers, the surface diffusion coefficient is 1.2×10^{-5} cm^2s^{-1}). The effect of the surface on the proton transport depends on its properties—diffusion coefficient, nature of the lipid head groups, their pK_a, concentration, etc. (Scherrer, 1995; Teissié, 1996). Because these properties are not easily defined, since the proposal of the chemiosmotic theory, there has been a continuing debate about whether the surface participates in the proton transport.

To formulate an exact criterion of when and how the surface can affect the proton transport is also a nontrivial theoretical problem. Various aspects of the effect of reduced dimensionality have been discussed by many authors in a general context of diffusion-controlled ligand–receptor binding (Adam and Delbrück, 1968; Berg and Purcell, 1977; Berg and Blomberg, 1976; Hardt, 1979, 1981; Schranner and Richter, 1978; Berg, 1985). It has been recognized that, when diffusion is limited to one or two dimensions, for example, DNA molecule or a membrane, the rate of finding the target can be significantly increased.

In our previous paper (Georgievskii et al., 2002), a phenomenological model was developed that describes diffusion of protons near the entrance of a proton-conducting channel. The transport of protons occurs both through the bulk and along the membrane surface, which can exchange protons with the bulk. There are two regimes of the proton transport. In the first regime, the exchange between the bulk solution and the membrane is so fast that a local equilibrium is always established between the surface and bulk concentrations at neighboring points. This case is most likely to occur in a typical biological environment. In the second regime, the exchange is slow, so that the local equilibrium is not established. A rigorous solution of the model was obtained with no restrictions on the exchange kinetics.

In this paper, we present a detailed analysis and application of the fast exchange regime to the relevant experimental studies of proton migration along biological membranes. The plan of the paper is as follows. In the next section the model is presented and the fast exchange approximation is introduced. In the following section, the solution of the model is described and simplified derivation for the fast exchange regime is given in Appendix A. The concept of the capture radius is introduced in the next to last section. In the last section, we discuss the results and parameters of our model, and give numerical estimates for pure unbuffered and buffered aqueous solutions and the rate expression derived in this paper. The concept of proton lifetime on the surface, for which an explicit formula is derived in Appendix B, the extension of the theory to buffered solutions, and the capture radius are also discussed in the last section.

MODEL

We consider the following idealized model. A half-space filled with a solution of protons (free or attached to buffer molecules) is limited by a membrane. The membrane is populated with protolytic groups and can exchange protons with the bulk. The protons can diffuse both in the bulk and on the surface of the membrane. The diffusion coefficients on the surface and in the bulk are different. There is a specific finite lifetime of the protons on the surface, during which protons can migrate along the surface. The protons are dynamically exchanged between the surface and the bulk. On the surface of the membrane, there is a sink (or a source) of the protons of molecular size through which protons are removed (supplied) to the system. (For simplicity we will talk about the removal of the protons from the system. The results for the reversed process are identical in the approximation considered in this paper.) We model this sink of the protons as an absorbing spot on the surface, such that, after a proton gets to this spot, it is immediately removed from the system. Thus, the absorbing conditions are ideal, and there is no reverse reaction with the sink. The absorbing spot models the entrance of the proton channel, through which protons are pumped out of the system. The questions in which we are interested are: 1) what is the maximum (diffusion-limited) rate at which protons can be collected (pumped out) from such a system, and 2) at what distance from the absorbing spot the equilibrium concentration both in the bulk and on the surface will be established. The former question is related to the efficiency of proton pumps and the latter to the discussion of the nature of chemiosmotic coupling between the source and the sink of the protons on the membrane. The effects of the finite size of the organelle on the results can be easily incorporated and will be discussed in the paper.

TABLE 1 Symbols used in the equations

Symbol	Meaning	Equation(s) Defining
x, y	Cartesian coordinates on the surface	1
r	Polar radius on the surface	9
z	Cartesian coordinate orthogonal to the surface	1
σ	Surface proton density at x, y	3
σ_{eq}	Equilibrium surface proton density at infinity	14
n	Bulk proton density at x, y, z	1
n_0	Equilibrium bulk proton density at infinity	12
k_b	Bulk proton flux through the channel entrance area	10, 12
k_s	Surface proton flux through the borderline of the channel entrance	11, 15, 23
L_{sb}	First characteristic length parameter of the theory	6, 9
L_s	Second characteristic length parameter of the theory	7
T_{dw}	Proton dwell time at a single surface protolytic group	8
R_0	Effective radius of the channel for unrestricted surface area available for proton diffusion	17
R^{eff}	Effective radius of the channel when the surface area available for proton diffusion is restricted by L_{max}	The Rate
L_{max}	Dimension of the cluster of protolytic groups around the channel entrance	The Rate
R_c	Capture radius	20–22
P_c	Capture probability	19, 22
C_0	Numerical constant	13, A13
T_1	Depletion time	24
Parameters for buffered solutions		Buffers
Other symbols		Table 2

In a system of a finite size, after some transient period, a quasi-stationary flow and a distribution of the concentration of protons are established. In our idealized infinite model, these conditions can be described as completely stationary by assuming that there is a compensating source of the protons at infinity such that the equilibrium is maintained at an infinitely large distance from the sink.

The stationary proton concentration in the bulk, $n(x, y, z)$, obeys the three-dimensional diffusion equation

$$\frac{\partial^2 n}{\partial x^2} + \frac{\partial^2 n}{\partial y^2} + \frac{\partial^2 n}{\partial z^2} = 0. \quad (1)$$

(The symbols used in the equations and their meanings are listed in Table 1.) The boundary condition on the surface depends on the properties of the surface. We assume that the coupling between the bulk and surface protons is described by the Langmuir kinetics of adsorption/desorption. The surface groups can be protonated from the bulk with a bimolecular rate constant k_{on} (in units of $M^{-1}s^{-1}$) and deprotonated with a monomolecular rate of desorption k_{off} (in units of s^{-1}). The rate of protonation is proportional to $\sigma_0 - \sigma$, where $\sigma(x, y)$ is the stationary surface density of protons captured at a given point (x, y) at the surface, and σ_0 is the concentration of the protolytic groups on the surface. We will consider the case when the surface protolytic groups are far from saturation, $\sigma \ll \sigma_0$, a regime that is practically the most important one. Then the proton flux from the bulk to the surface is given by the equation

$$D_b \left.\frac{\partial n}{\partial z}\right|_{z=0} = \kappa_{on} n \sigma_0 - k_{off}\sigma, \quad (2)$$

where D_b is the bulk diffusion coefficient. This equation is the boundary condition to Eq. 1. It relates the bulk concentration of protons and its normal derivative at $z = 0$ to the surface concentration.

The second assumption of the model is that there exists a long-range connectivity between the protonatable groups on the surface (as described, e.g., in the percolation model [Rupley and Careri, 1991; Gutman and Nachliel, 1995, 1997]), resulting in a Brownian proton migration along the surface. The stationary concentration of the protons on the surface satisfies the two-dimensional diffusion equation,

$$D_s\left(\frac{\partial^2 \sigma}{\partial x^2} + \frac{\partial^2 \sigma}{\partial y^2}\right) = -D_b \left.\frac{\partial n}{\partial z}\right|_{z=0}, \quad (3)$$

where D_s is the surface diffusion coefficient. The bulk diffusion flux $D_b \partial n/\partial z$ at $z = 0$ is a source of protons on the surface.

The proton channel entrance, which serves as a sink for both the bulk and surface protons, is modeled by a circle of radius r_0 on the membrane surface. Eqs. 1–3 supplemented by the absorbing boundary condition inside the channel entrance and the equilibrium condition at infinity were solved in our previous paper (Georgievskii et al., 2002).

Qualitatively, there are two regimes of proton transport, depending on the rate with which protons are exchanged between the surface and the bulk. In the fast-exchange regime, each of the two Langmuir terms in the right-hand side of Eq. 2 is much larger than the total diffusional flux on the surface, the term on the left-hand side. Then the surface density and the local bulk density of the protons in every

point on the surface are essentially in equilibrium and are related as

$$\sigma = L_0 n. \tag{4}$$

The equilibrium association constant L_0 in Eq. 4 is expressed in terms of the parameters of the Langmuir kinetics,

$$L_0 = \frac{\kappa_{on}}{k_{off}}\sigma_0 = 10^{pK_a^{(s)}}\sigma_0, \tag{5}$$

where $pK_a^{(s)}$ refers to the protolytic surface groups. Note that, in this definition, $10^{pK_a^{(s)}}$ has a dimension of inverse concentration, M^{-1}, where M = mole/liter = $N_A \times 10^{-3}$ cm^{-3} and N_A is the Avogadro number. Because σ_0 is the number of groups per unit surface, L_0 has a dimension of length. The physical meaning of L_0 is the height of a column in the bulk equilibrium solution that contains the same number of protons as a spot on the surface with the same area as the column's cross-section. Eq. 5 is written for a pure (unbuffered) solution. In the presence of a buffer, it is modified as discussed in the Discussion, Buffers.

Eq. 4 is a modified boundary condition to Eq. 1 (replacing Eq. 2) and it provides a direct coupling between the surface and bulk diffusion. The analysis shows (Georgievskii et al., 2002) that the formal condition for the local equilibrium described by Eq. 4 is expressed in terms of two length parameters characterizing the system. The first parameter,

$$L_{sb} = \frac{D_s}{D_b}L_0, \tag{6}$$

is of the order of an effective size of the collecting antenna, or a capture radius (see the Capture Radius). Another parameter,

$$L_s = \sqrt{D_s/k_{off}}, \tag{7}$$

is the average distance that a proton migrates along the surface during its surface dwell time,

$$\tau_{dw} = k_{off}^{-1}. \tag{8}$$

The formal condition for the fast exchange reads $L_{sb} \gg L_s$, whereas the opposite case, $L_{sb} \ll L_s$, corresponds to the slow exchange regime. In this paper, we will consider in more detail the case of the fast exchange because it is most relevant to the proton transport in biological systems.

Using the cylindrical symmetry of the problem and the coupling condition 4, we rewrite Eq. 3 as

$$\frac{d^2n}{dr^2} + \frac{1}{r}\frac{dn}{dr} + L_{sb}^{-1}n_z' = 0, \quad r > r_0, \quad z = 0, \tag{9}$$

where r is the distance on the surface, measured from the center of the proton channel, $n \equiv n(r, z)$, $n_z' = \partial n/\partial z$ at $z = 0$, and L_{sb} is given by Eq. 6. A simplified method to solve the coupled Eq. 1 and 9 with appropriate boundary conditions is presented in Appendix A. The solution to these equations is the function $n(r, z)$ given by Eqs. A1, A6, A8, and A14. The function $n(r) \equiv n(r, z = 0)$ necessary to calculate the surface proton flux in the next section is given by Eq. A15.

THE RATE CONSTANT

The total rate of proton transport to the channel consists of two proton fluxes, $k = k_b + k_s$. The bulk proton flux k_b through the channel is given by

$$k_b = 2\pi D_b \int_0^{r_0} n_z' r\, dr. \tag{10}$$

The surface proton flux k_s through the border of the channel entrance is

$$k_s = 2\pi r_0 D_s \left.\frac{d\sigma}{dr}\right|_{r=r_0}. \tag{11}$$

The expressions for the stationary distributions of both the surface and bulk protons are given in Appendix A and Eq. 13.

The mechanism of proton transport to the proton channel is different for different values of L_{sb}. If $L_{sb} \lesssim r_0$, the proton transport occurs mainly via absorption of protons from the bulk solution by the channel entrance, i.e., $k_b \gg k_s$. In this situation, one can neglect the surface diffusion and replace Eq. 3 with $n_z' = 0$, $r > r_0$. This is the standard model for proton transfer. The rate constant of such a process is given by (Crank, 1990)

$$k_b = 4r_0 D_b n_0. \tag{12}$$

More interesting is the opposite case, $L_{sb} \gg r_0$. The distribution of the surface density is given by the equation

$$\sigma(r) = \sigma_{eq}\frac{\ln(r/r_0)}{\ln(C_0 L_{sb}/r_0)}, \quad r_0 < r \lesssim L_{sb}, \tag{13}$$

which is obtained by substituting Eq. A15 into Eq. 4. Here, $C_0 \approx 1.1229$ (see Eq. A13), and σ_{eq} is the equilibrium concentration of protons on the surface,

$$\sigma_{eq} = L_0 n_0 = \sigma_0 10^{pK_a^{(s)} - pH}, \quad pK_a^{(s)} \lesssim pH. \tag{14}$$

Inserting Eq. 13 into Eq. 11, we obtain the rate constant due to the surface diffusion mechanism,

$$k_s = \frac{2\pi D_s \sigma_{eq}}{\ln(C_0 L_{sb}/r_0)}. \tag{15}$$

The rate constant k_s depends on the size of the proton channel entrance in a much weaker, logarithmic fashion than the rate constant k_b for the bulk diffusion mechanism, Eq. 12. As shown by Georgievskii et al. (2002), in the

slow-exchange regime, when $L_s \gg L_{sb} \gg r_0$, the expression for the rate has the same form as Eq. 15, but, in the logarithmic factor, the length L_{sb} is replaced with L_s. In a different context, Berg (1985) obtained an expression for surface-enhanced diffusion flux similar to our Eq. 15.

For the purpose of comparison, the above equation is rewritten in the form equivalent to the bulk rate, Eq. 12,

$$k_s = 4R_0 D_b n_0, \tag{16}$$

where R_0 is the effective radius of the channel, a formal parameter that tells what the radius of the channel should be, in the absence of the surface diffusion, to provide the same absorption rate as in the presence of the surface diffusion. The expression for R_0 thus obtained is

$$R_0 = \frac{\pi}{2}\frac{L_{sb}}{\ln(R_c/r_0)}, \tag{17}$$

where R_c is the capture radius (see Eq. 20). This expression is valid for $R_c \gg r_0$. The effect of the surface diffusion can now be measured directly by comparing the effective radius of the channel R_0 with the actual radius r_0.

THE CAPTURE RADIUS

The above-introduced effective radius of the channel R_0 is a more or less formal parameter, which is useful for comparison with the usual bulk diffusion. It should not be confused with the actual size of the area on the membrane surface from which protons are collected to the channel in the process of the coupled surface-bulk diffusion. The size of such an area is described in terms of the capture radius, R_c. The latter is defined as the distance from which a proton once on the surface will be absorbed by the channel.

The capture radius R_c can be also understood as a distance between a generator and a consumer of protons on the surface of the membrane at which the exchange between the two occurs mainly via the surface, before equilibrating with the bulk. The coupling between the source and the sink in this case is said to be local. The opposite case of delocalized coupling is realized when the source and the sink are each in equilibrium with the bulk. The rate of absorption by the consumer in this case will neither depend on the rate of proton generation (assuming infinite volume), nor on the distance between the generator and the consumer (Ferguson, 1995).

The probability of capture by the channel via the surface diffusion is described by the function,

$$P_c(r) = 1 - \frac{\sigma(r)}{\sigma_{eq}} = 1 - \frac{n(r)}{n_0}. \tag{18}$$

The deviation of $\sigma(r)$ from the equilibrium value σ_{eq} is due to absorption by the channel, and is a measure of the probability that the proton will be absorbed by the channel from a specified position. At the boundary of the absorbing channel, $\sigma = 0$ and the probability is exactly one. At an infinitely large distance from the channel, $\sigma = \sigma_{eq}$ and the probability of absorption is zero.

From Eq. A15, we find that

$$P_c(r) = \frac{\ln(C_0 L_{sb}/r)}{\ln(C_0 L_{sb}/r_0)}, \quad r_0 < r \lesssim L_{sb}. \tag{19}$$

The capture radius defined formally as the distance at which the probability of capture decreases to zero is

$$R_c = C_0 L_{sb}. \tag{20}$$

One should notice that, because the dependence of the probability of capture on the distance is logarithmic, the capture radius should be understood only as defining an order of magnitude of the relevant characteristic length (say, increasing R_c by a factor of e will change the probability only by $1/\ln(L_{sb}/r_0) \ll 1$, because we assume $L_{sb} \gg r_0$). Thus, the constant $C_0 \sim 1$, Eq. A13, is essentially irrelevant in the above expression for R_c. It is recalled that, here, we treated the case $L_{sb} \gg L_s$. In a general case, as shown by Georgievskii et al. (2002), the capture radius is

$$R_c \simeq \max(L_{sb}, L_s), \tag{21}$$

and the capture probability is given by

$$P_c(r) = \frac{\ln(R_c/r)}{\ln(R_c/r_0)}. \tag{22}$$

This expression is valid for $R_c \gg r_0$ and $r_0 < r \lesssim R_c$.

DISCUSSION AND CONCLUSIONS

Summary

The surface can enhance the flow of protons to a target, such as the entrance of a proton channel, via its low-pK protonatable groups capable of catching protons from the bulk and then shuttling them along the surface. If there are only a few such groups, then, quantitatively, the enhancement can be expressed in terms of so-called virtual bimolecular rate constants introduced by Gutman and coworkers (Yam et al., 1988). When there are many such groups, and the connectivity between them is established, the transport to the target on the surface is a combined surface–bulk diffusion. The model discussed in this paper refers to the latter type of proton transfer.

The theory developed allows one to evaluate the maximum (diffusion-limited) rate at which a proton pump can translocate protons across a membrane. The model is phenomenological. It assumes that the protons are collected by a proton channel in the membrane, and that they can diffuse both along the surface and from the bulk toward the entrance of the channel. There is a dynamic proton exchange between the surface and the bulk, which is described by the Langmuir adsorption/desorption kinetics. Such a coupling

TABLE 2 The parameters of the model

Characteristics of the surface	
$pK_a^{(s)} = 5$	pK_a of the protolytic groups
$r_0 = 1$ Å	Radius of the channel entrance
$\sigma_0 = 10^{-2}$ Å^{-2}	Density of the protolytic groups
$d = 6$ Å	The reaction radius of the protolytic groups
$D_s = (10^{-7} - 10^{-4})$ cm^{2}s^{-1}	Diffusion coefficient of protons on the surface
Pure aqueous solutions, pH = 7	
$D_b = 10^{-4}$ cm^{2}s^{-1}	Diffusion coefficient of protons in bulk water
$K_{on} = 2\pi dD_b = 2.3 \times 10^{10}$ M^{-1}s^{-1}	Rate constant for proton association on the surface
$k_{off} = 2.3 \times 10^5$s^{-1}	Rate constant for proton dissociation from the surface
$L_0 = \sigma_0 10^{pK_a^{(s)}} = 170$ μm	Equilibrium constant for proton association on the surface*
$\sigma_{eq}/\sigma_0 = 10^{pK_a^{(s)}} - pH = 0.01$	The equilibrium degree of saturation of the surface†
Buffered aqueous solutions, pH = 7	
$pK_a^{(B)} = 6$	pK_a of buffer molecules
$D_b' = 5 \times 10^{-6}$ cm^{2}s^{-1}	Diffusion coefficient of buffer molecules in bulk water
$K_b' = 3 \times 10^{10}$ M^{-1}s^{-1}	Rate constant for proton binding to a buffer molecule
$k_d' = 3 \times 10^4$ s^{-1}	Rate constant for proton dissociation from a buffer molecule
$K_{on}' = 10^8$ M^{-1}s^{-1}	Rate constant for collisional proton transfer from buffer to surface
$K_{off}' = 10^9$ M^{-1}s^{-1}	Rate constant for collisional proton transfer from surface to buffer

*$10^{pK_a^{(s)}}$ has the dimension of inverse concentration, see Eq. 5.
†See the definition of σ_{eq} in Eq. 14.

results in a nontrivial coupled surface and bulk proton diffusion in the system. The surface was assumed to be infinite, homogeneous, and characterized by a surface proton diffusion coefficient, D_s, pK_a of the surface protolytic groups, $pK_a^{(s)}$, their density σ_0 (the number of groups per unit surface), and the parameters of the Langmuir kinetics—the first-order rate constant of desorption, k_{off}, and the second-order rate constant of adsorption, k_{on}. The radius of the proton sink on the surface is r_0. The bulk protons are characterized by the bulk diffusion coefficient D_b and the equilibrium bulk density n_0.

In this phenomenological description, we do not specify the precise nature of the protonatable groups on the surface, but rather characterize them by their pK_a, their density σ_0, and the proton diffusion coefficient D_s. The protonatable groups on the surface of the protein (Gutman and Nachliel, 1997), and the protonatable lipid head groups of the membrane both can play the role of the proton-transmitting elements on the surface (Scherrer, 1995; Teissié, 1996). In this section, quantitative estimates will be made with the use of parameters from Table 2, where the values of pK_a and σ_0 are characteristic of carboxylates on the surface of proteins such as cytochrome *c* oxidase and bacteriorhodopsin, and are in the range of values for protonatable lipid head groups studied by Scherrer (1995).

Modeling the channel entrance as a plain sink of a given radius r_0 with free proton diffusion in its vicinity may look, at a first glance, as an oversimplification. Indeed it has been suggested that the entrance of protein proton channels may be surrounded by negative charges that provide a guiding electrostatic potential, which extends to some distances, leading to both directed and enhanced bulk diffusion toward the channel entrance. The range of action of the attracting potential, however, may be expected to be only of the order of the Coulomb cage radius, say, 7–14 Å. Because the phenomena described by the present model typically occur on a much larger scale, as we demonstrate below, this external "short-range" potential can be described in our phenomenological model by simply increasing the effective radius of the channel from a few angstroms to 10–15. The rate of surface transport, as we saw, only weakly, logarithmically, depends on the channel radius. Thus, the increase of the effective size of the channel may be insignificant. However, the relative contribution of the bulk and the surface transport may be indeed affected, because the bulk contribution is proportional to r_0. This uncertainty in r_0 should be kept in mind when estimates are made using this theory.

Within the model developed, the theory also allowed us to formulate a criterion of when the coupling between a source and a sink on the membrane surface is localized, i.e., the transport between the two occurs before the protons generated by the source are fully equilibrated with the bulk.

Both the issue of diffusion-limited transport and the range of delocalization of the surface protons are of interest in a general context of chemiosmotic theory of energy transduction.

The rate

In this paper, we treated the case of the fast exchange between the surface and the bulk, a regime in which the surface and bulk protons are in local equilibrium. Formally, the condition for the fast exchange is $L_{sb} \gg L_s$. The study of the opposite case of the slow exchange (Georgievskii et al., 2002), $L_{sb} \leq L_s$, yields the expression for the rate similar to the one obtained here.

In the regime where the surface diffusion dominates the transport, in both cases of the fast and slow exchange between the surface and the bulk, the rate can be written in the form

$$k_s = \frac{2\pi D_s \sigma_{eq}}{\ln(R_c/r_0)}, \quad (23)$$

where R_c is essentially the maximum of two lengths, L_s and L_{sb} (the factor C_0 in the exact definition is close to unity and can be neglected). The logarithmic factor is expected to be roughly in the range of one to ten. Due to the logarithmic dependence, the radius of the channel r_0 has a minor effect on the rate.

The effect of the surface on the maximum rate of absorption by the channel is described in terms of the effective radius of the channel, R_0, as defined in Eq. 17. When R_0 is much larger than the actual radius of the channel, r_0, the surface-modified diffusion enhances the rate by a factor of R_0/r_0. Analytical results could be obtained in the regime where the surface dominates proton transport, $R_0/r_0 \gg 1$, and the protonation of the surface is far from saturation, $\sigma_{eq} \ll \sigma_0$. The latter condition makes the coupling between the bulk and the surface linear.

Because the surface diffusion coefficient typically is not expected to be much different from that of the bulk (the reported values for D_s/D_b are in the range of 10^{-3} to 1 [Heberle et al., 1994; Scherrer et al., 1994; Slevin and Unwin, 2000]), but the surface density can be much higher than the equivalent bulk density (the measure is given by the equilibrium constant L_0, see Eqs. 4 and 5), the surface can provide an extremely high output of protons. For example, using $D_s = 10^{-5}$ cm^{2}s^{-1} and other parameters from Table 2, we obtain $\tau_{dw} = 4$ μs by Eq. 8, $L_0 = 170$ μm by Eq. 5, $L_{sb} = 17$ μm by Eq. 6, $L_s = 660$ Å by Eq. 7, $R_c = 17$ μm by Eq. 20, and $R_0 = 2$ μm by Eq. 17. Thus, the enhancement effect would be $R_0/r_0 \sim 10^4$, provided that the surface available for the proton diffusion was infinite.

If the proton diffusion on the surface around the channel entrance is restricted by a finite area, such as a cluster of protonatable groups of dimension L_{max} and $L_{max} < R_c$, then the enhancement factor is less than R_0/r_0. In this case, a proton, once adsorbed by the cluster with a high probability, will be dragged into the channel before returning to the bulk. The cluster, therefore, will act as an absolutely absorbing disk for which the rate of absorption is given by Eq. 12 where r_0 should be replaced with L_{max}. The effective radius of the channel, R^{eff}, will be equal to L_{max}, and the enhancement effect will be L_{max}/r_0.

In the opposite limit of $L_{max} > R_c$ the surface proton flow is independent of L_{max}, and R^{eff} is gradually approaching R_0 somewhere between R_0 and R_c. This behavior is schematically depicted in Fig. 1. One can see, therefore, that the length parameter R_c is a critical parameter that determines the rate, although formally the rate depends only logarithmically on it.

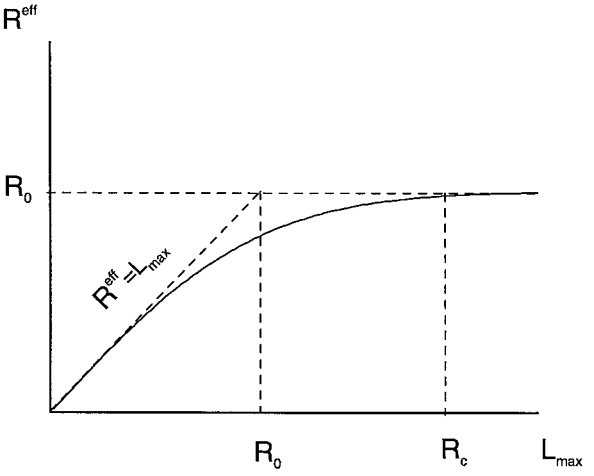

FIGURE 1 Schematic dependence of the effective channel radius R^{eff} on L_{max}, the maximum dimension of the surface area available for the proton diffusion. The effective radius is equal to L_{max} when L_{max} is smaller than the capture radius R_c, and reaches its limiting value R_0 at $L_{max} > R_c$.

Above, we gave estimates of the effective radius of the channel and the surface enhancement effect for a pure unbuffered solvent. Buffers can change these estimates dramatically. However, the surface is still predicted to dominate the transport up to a high buffer concentration (see Buffers subsection).

In Fig. 2, the proton flux into the channel due to the surface-diffusion mechanism is shown as a function of the surface-diffusion constant. Within the assumed range of D_s, the rate of proton supply to a channel may vary from 10^5 up to 4×10^7 s^{-1}. In cytochrome *c* oxidase, the rates of internal proton transfer in different redox states of the enzyme were estimated in the previous work from this

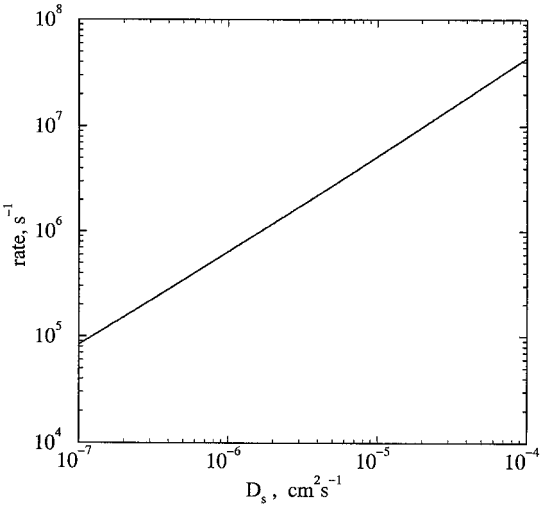

FIGURE 2 The dependence of the rate of the surface-mediated proton supply to the channel entrance, k_s, on the surface diffusion coefficient, D_s, calculated by Eq. 15 with parameters from Table 2 for pure solution.

group (Kotelnikov et al., 2001) to be $(10^2–10^4)$ s^{-1}, so that, even at the lowest diffusional mobility on the surface, the calculated rate of proton delivery is still sufficiently high to prevent a bottleneck in CcO turnover. Further, in the work cited, the proton channel was represented by a single protonatable group that was in the fast equilibrium with the bulk at the apparent protonation rate of 5×10^{11} $M^{-1}s^{-1}$, which is an order of magnitude higher than that expected for a diffusion-controlled protonation from the bulk. From Fig. 2, it is seen that, at the lowest diffusion constant, the flux is 10^5 s^{-1}, which corresponds to the apparent bimolecular protonation rate from the bulk of 10^{12} $M^{-1}s^{-1}$ at pH = 7. Thus, the results shown in Fig. 2 are consistent with our previous findings.

The high surface rate is due to a relatively high concentration of protons on the surface, their high mobility, and, most importantly, to the reduced dimensionality of diffusion. The latter results in practical independence of the rate of absorption on the actual radius of the channel. In contrast, the bulk diffusion to the channel is limited by the small size of the channel and a relatively low bulk density of protons.

An increased effective radius of the channel, R_0, is equivalent to the effect of a proton-collecting antenna (Gutman and Nachliel, 1997). In the antenna model, special properties of the groups surrounding the entrance to the channel are required (high mobility, overlap of their Coulomb wells, etc.). We find that, in fact, any surface area surrounding the channel that conducts protons can already significantly increase the output of protons through the channel. That is, if the enhancement of the infinite surface is significant, $R_0/r_0 \gg 1$, a patch of a similar surface with dimensions of $L_{max} < R_c$ can also serve as an enhancement factor of the output of the channel. In this case, the proton conducting patch of the surface is exactly equivalent to the antenna model of Gutman and Nachliel (1997; see also the Capture Radius subsection). In contrast, when $L_{max} > R_c$, only a part of the proton-collecting collecting cluster will work as a collecting antenna, see Fig. 1. The main point here is that no special properties, such as an extremely fast exchange between the groups of the antenna, are in fact required. The two-dimensional nature of the antenna can significantly relax the requirements on the transport and the collecting properties of the groups making up the antenna.

It is well recognized that the effect of the surface can be significant only if the dwell time on the surface is relatively large. In this case, the proton can migrate a long distance on the surface during its dwell time, $L_s \gg r_0$, and L_s is often tacitly assumed to be the natural length that determines the size of the region on the surface from which protons can migrate to a target. We find, however, that such a region, in fact, can be much larger than L_s, and is equal to L_{sb} instead when $L_{sb} \gg L_s$. Moreover, we find that this is a typical situation. When the exchange between the surface and the bulk is fast (in the sense discussed in Model section and below in the Parameters and Assumptions subsection), the proton can many times desorb and be readsorbed by the surface, staying, on average, close to the surface, and hence make use of the reduced dimensionality of the space in which its migration occurs.

The surface depletion rate and the dwell time

The above result can be qualitatively understood if it is recognized that the actual average time that the proton spends at or near the surface, τ_l, is not the same as the inverse of the desorption rate, k_{off}^{-1}. Indeed, the proton that desorbs from the surface can be recaptured again by the surface groups. Therefore, the actual time during which a proton will be localized in the vicinity of the surface will be defined by how quickly the proton migrates into the bulk from the surface (we assume that the desorption rate k_{off} is high, see Eq. 29). A detailed description of such a surface-depletion kinetics is given in Appendix B. The probability of finding a proton on the surface decays with time as $t^{-1/2}$, and the depletion time τ_l, apart from the numerical factor of the order of one, is

$$\tau_l \sim \frac{L_0^2}{D_b}, \tag{24}$$

where L_0 is given by Eq. 5. This time is directly related to the coupled surface–bulk diffusion of the proton, and is typically much longer than k_{off}^{-1}. For pure unbuffered solutions and the parameters from Table 2, we obtain $\tau_l \sim 1$ s.

The capture radius

The capture radius R_c describes the maximum distance at which a source and a sink on the surface can exchange protons without full equilibration with the bulk. Thus, if the distance between the donor and acceptor is less than R_c, the coupling is localized.

Eq. 19 and a more general Eq. 22 provide expressions for the probability of capture of a proton that was initially localized on the surface at a distance r from the channel. We find that the capture radius R_c is the larger of two lengths, L_{sb} and L_s.

Recently, the question of the nature of communication between a source and a sink on the membrane surface has been directly addressed experimentally by studying two connected ion channels (Antonenko and Pohl, 1998). The reported distance of "direct" coupling between the donor and acceptor is of the order of 100 μm, which is in a remarkably good agreement with our estimate of $R_c = 17$ μm in the Rate subsection above. Similar distances in the micrometer range are measured by other authors (Heberle and Dencher, 1992; Heberle et al., 1994; Alexiev et al., 1994, 1995; Scherrer et al., 1994). Lateral proton movement on a macroscopic scale has been also observed both in pure aqueous solutions and buffered solutions (Gabriel et al.,

1994, and references therein). We note that, according to the present theory, R_c is a function not only of the properties of the surface but also those of the buffers (see the Buffers subsection below).

It should be noticed that a distance as long as $L_{max} = 100$ μm between the source and the sink requires a very long lifetime even if proton diffusion on the surface is as fast as in the bulk. For $D_s = 10^{-4}$ cm^{2}s^{-1}, one obtains $L_{max}^2/4D_s = 0.25$ s. This long time clearly can not be a simple desorption time τ_{dw} (= 4 μs by the estimate given in the Rate subsection). However, it is very close to our estimate of the surface depletion time, $\tau_l \sim 1$ s, given in the previous subsection. Thus, the long distance of direct and local coupling can be explained by the coupled surface–bulk diffusion considered in this paper.

In contrast to our calculations and the above-cited experimental data, Gutman and coworkers derived from their experimental results that the proton-collecting antenna consisted of a few (typically 2–3) surface groups in a close vicinity of the fluorescein probe (Flu) and its dimension did not exceed 30 Å (Gutman and Nachliel, 1997). This disagreement might be a consequence of the way the experimental data were described. A notable example is the work on cytochrome *c* oxidase (Marantz et al., 1998). The Flu protonation on the enzyme surface was found to be mediated by one carboxylate (COO^-_{near}) and one histidine (His_{near}) located within 10 Å from the probe. In addition, two moieties of 15 distant carboxylates and 10 distant histidines were found to affect the dynamics of Flu protonation indirectly, via proton exchange with COO^-_{near} and His_{near}. Obviously, in this case, the antenna extends far beyond the 10 Å distance and involves many surface groups. Thus, what we describe in terms of the surface diffusion, Gutman and Nachliel describe in terms of shuttling protons between distant and nearby groups. In the work on bacteriorhodopsin (Checover et al., 1997), it is also obtained that the size of the antenna is comparable to the dimension of the molecule. In other experiments by Gutman and Nachliel (1997), where the antenna dimension was found to be small, the contributions of distant groups might not be properly resolved because the kinetic constants for all numerous proton-transfer reactions were extracted from a single kinetic curve, the protonation dynamics of the probe.

Parameters and assumptions

The phenomenological parameters of this theory listed in Table 2 are actually not all independent. For instance, the ratio of κ_{on} and k_{off} for the unbuffered solution is expressed in terms of $pK_a^{(s)}$, Eq. 5, which runs typically from 4 to 6.5 (see, e.g., data for cytochrome *c* [Marantz and Nachliel, 1999], bacteriorhodopsin [Nachliel and Gutman, 1996], and cytochrome *c* oxidase [Marantz et al., 1998]). The absolute value of k_{off} is expected to strongly depend on $pK_a^{(s)}$ (Gutman and Nachliel, 1995), whereas that of κ_{on} on the bulk diffusion coefficient D_b, see Eq. 25.

It is recognized that the surface diffusion coefficient can depend on both $pK_a^{(s)}$ and k_{off}. The potential field around a binding site on the surface is characterized by two energy barriers. The first one is for the proton displacement in the direction perpendicular to the surface (Teissié, 1996), and the second is for the lateral movement. The first barrier is always associated only with $pK_a^{(s)}$, but the second will also depend on the distance between the surface sites and on how much their Coulomb cages overlap (Matthew and Richards, 1982; Gutman and Nachliel, 1990, 1997; Peitzsch et al., 1995). If the sites are close enough, the lateral barrier will be small (due to overlaps of the neighboring wells), and, for some range of values of $pK_a^{(s)}$, the surface diffusion coefficient should be independent of $pK_a^{(s)}$. At some high $pK_a^{(s)}$, however, D_s should be expected to decrease exponentially with the increase of $pK_a^{(s)}$, because the barrier for the lateral translation will increase. The understanding of all intricate relations between $pK_a^{(s)}$, D_s, k_{off}, and κ_{on} requires a detailed microscopic model of the surface and solving the diffusion equation in the Coulomb potential, similar to the approach developed by Agmon (1988) for bulk reactions, which is beyond the scope of the present paper.

The parameters L_{sb} and L_s are also related. The rate constant of the diffusion-controlled protonation of a single surface group from the bulk can be written as (Crank, 1990)

$$\kappa_{on} = 2\pi d D_b, \tag{25}$$

where d is the reaction radius. We disregard other factors due to electrostatic interactions (Gutman and Nachliel, 1995) and adopt the rate expression for the reaction on a half-sphere. Inserting Eqs. 25, 5, and 7 into 6, we obtain

$$\frac{L_{sb}}{L_s} = (2\pi d r_0 \sigma_0)\left(\frac{L_s}{r_0}\right), \tag{26}$$

$$\frac{L_{sb}}{L_s} = [(2\pi d 10^{pK_a^{(s)}})^{1/2}\sigma_0]\left(\frac{D_s}{D_b}\right)^{1/2}. \tag{27}$$

Thus, L_{sb} depends quadratically on L_s, and the ratio L_{sb}/L_s increases linearly with L_s.

It can be seen from the above relations that, to have an enhancement effect in the transport rate k_s, the dwell time on the surface should be sufficiently large, $\tau_{dw} \gg r_0^2/D_s$, so that $L_s \gg r_0$ (see Eqs. 7 and 8). Indeed, the first factor in Eq. 26 is ≤ 1 because both the reaction radius and the target size are of the same order or smaller than the separation between the surface groups. Therefore, if $L_s \leq r_0$, we obtain $L_{sb} \leq r_0$ as well, and, according to the results of the Rate Constant section, no enhancement effect would be obtained in this case. However, typically, L_s is expected to be large and the condition $L_s \gg r_0$ to be satisfied.

In Eq. 27, the first factor is $\sim 10^3$ for the unbuffered solution. For the reported values of $D_s/D_b = 10^{-3} - 10^0$

(Heberle et al., 1994; Scherrer et al., 1994) one has $L_{sb} \gg L_s$, i.e., only the fast exchange regime is realized (see the Introduction). Thus, for the unbuffered solutions, we obtain the estimate for L_s using $d = 6$ Å,

$$L_s = 0.2\ \mu\text{m} \times \sqrt{\frac{D_s}{D_b}} = 60 - 2000\ \text{Å}. \qquad (28)$$

Under the same conditions $L_{sb} = 0.17 - 170\ \mu$m, and therefore typically we have $L_{sb} \gg L_s$.

Our final remark is on the conditions under which the surface and bulk protons are in local equilibrium, resulting in Eq. 4. It is qualitatively clear that it should be the case when the exchange rate between the surface and the bulk is sufficiently high. It is not immediately obvious, however, what this rate should be compared with (to determine if it is indeed sufficiently high). The relevant estimate can be obtained as follows. The formal condition could be directly derived from Eqs. 2 and 3, where the terms describing the surface–bulk exchange kinetics must dominate over the surface diffusion term. The analysis shows that k_{off}^{-1} should be short compared with the characteristic diffusion time,

$$k_{off}^{-1} \ll \tau_c \sim \frac{R_c^2}{D_s}. \qquad (29)$$

Thus, the condition for the fast exchange can be stated as $\tau_{dw} \ll \tau_c$. Using the definitions in Eqs. 7, 8, and 21, we obtain $L_{sb} \gg L_s$.

It is interesting to note that, because L_{sb} is increasing quadratically with L_s, see Eq. 26, the greater the length L_s is, the better condition $L_{sb} \gg L_s$ is satisfied. The latter is a condition for the local equilibrium between the surface and the bulk protons, which requires a fast exchange with the bulk, i.e., a "short" dwell time. However, with increasing L_s the dwell time of the proton on the surface is increasing instead(!). The seeming contradiction is resolved when it is recognized that, although with increasing L_s the dwell time τ_{dw} does increase, the corresponding time scale of the diffusion, τ_c, with which τ_{dw} should be compared, increases even more rapidly due to its quartic dependence on L_s.

Buffers

The present theory is easily generalized to the case when an unsaturated buffer is present in the solution,

$$HB \rightleftharpoons B^- + H^+, \quad pK_a^{(B)} \lesssim pH, \qquad (30)$$

and when the collisional proton exchange between HB and the surface groups is the dominant pathway of the proton movement.

Reaction 30 introduces proton generation/consumption in the bulk (Nunogaki and Kasai, 1988) with a characteristic time,

$$\tau' = (k'_b[H^+]_{eq} + k'_d)^{-1}, \qquad (31)$$

where k'_b and k'_d are the proton-binding and dissociation rate constants, respectively. In deriving Eq. 31, we assumed that $[H^+] \simeq$ const. Here and below, the primed parameters refer to the buffered solutions. Using the parameters from Table 2, we calculate the characteristic diffusion length

$$\sqrt{6D'_b\tau'} = 3000\ \text{Å}.$$

We will see in this section that this length is much longer than the capture radius in our problem, hence the exchange reaction 30 is very slow on a relevant time scale and can be neglected. The above estimate for τ', Eq. 31, was obtained under the assumption that the proton diffusion is sufficiently fast in comparison with that of the buffer molecules and, therefore, $[H^+] \simeq$ const. In the oposite limit of slow proton diffusion, the supply of protons to the reaction region will be a limiting factor, and reaction 30 can be neglected as well.

In this situation, the concentration of the protonated buffer molecules, [HB], plays the role of the concentration of free protons in the solution. The proton flux F from the bulk to the surface can be written in a way identical to Eq. 2,

$$F = \kappa'_{on} n\sigma_0 - k'_{off}\sigma, \quad k'_{off} = \kappa'_{off}[B^-], \qquad (32)$$

where n now stands for [HB] and the primed constants related to the collisional proton exchange between the buffer and the membrane groups, which we assume to be the main mechanism of proton supply to the surface. Then the rate constants obey the relation

$$\frac{\kappa'_{off}}{\kappa'_{on}} = 10^{pK_a^{(B)} - pK_a^{(s)}}. \qquad (33)$$

With the above substitutions, one can then use all the derived formulas in this paper.

The diffusion coefficient D'_b now describes the diffusion of the buffer molecules in the solution. The parameter L'_0 for the buffered solution, which comes into the expression $L'_{sb} = L'_0(D_s/D'_b)$ (cf. Eq. 6) can be written as

$$L'_0 = \frac{\sigma_{eq}}{[HB]_{eq}}, \qquad (34)$$

where σ_{eq} is independent of the buffer. Substituting Eq. 14 into Eq. 34, one finds

$$L'_0 = L_0 \frac{[H^+]_{eq}}{[HB]_{eq}} \equiv L_0 \frac{10^{-pK_a^{(B)}}}{[B^-]_{eq}}, \qquad (35)$$

where L_0, as before, relates to the unbuffered solution, Eq. 5.

This situation is different as compared to the pure unbuffered solution, because the ratio $[H^+]_{eq}/[HB]_{eq}$ can be several orders of magnitude smaller than one. Hence, L'_{sb}, the capture radius R'_c (Eq. 21 with primed parameters), and the effective radius of the channel,

$$R'_0 = \frac{\pi}{2}\frac{L'_{sb}}{\ln(R'_c/r_0)}, \tag{36}$$

will be significantly decreased as compared to the pure unbuffered solution. Thus, the buffer can be a major factor that determines whether the proton transfer occurs via the buffer in the bulk solution or through the surface-diffusion mechanism.

To discuss this issue quantitatively, we will write the expressions for L'_{sb} and L'_s in the form

$$\frac{L'_{sb}}{r_0} = \frac{[B]_0}{[B]}, \tag{37}$$

$$\frac{L'_s}{r_0} = \sqrt{Q\frac{[B]_0}{[B]}}, \tag{38}$$

where $[B] = [BH] + [B^-] \simeq [B^-]$ is the total concentration of the buffer, and

$$[B]_0 = \frac{D_s\sigma_0}{D'_b r_0}\, 10^{pK_a^{(s)} - pK_a^{(B)}}, \tag{39}$$

$$Q = \frac{D'_b}{r_0\sigma_0\kappa'_{on}}. \tag{40}$$

The parameters $[B]_0$ and Q are useful measures of enhancement of the proton transport. According to the present theory, the enhancement effect in the buffered solutions occurs when L'_{sb} is large, $L'_{sb} \gg r_0$. However, L'_{sb} is rapidly decreasing with increasing buffer concentration, and $[B]_0$ represents the maximum concentration of the buffer under which the effect of the surface enhancement can still be observed. For $D_s = 10^{-5}$ cm^{2}s^{-1} and other parameters from Table 2, we obtain $[B]_0 = 3.3$ M. With regard to the latter estimate, we notice that Gabriel et al. (1994) measured the influence of both inorganic (sulphate) and physiological (TRIS) buffers on the lateral diffusion and found that the buffers do not destroy it up to [B] = 200 mM.

The meaning of Q will be clear if we insert $\kappa'_{on} = 2\pi w' d' D'_b$ similar to Eq. 25, supplemented with a factor $w' < 1$, taking into account that the protonation reactions via buffers are not generally diffusion-controlled. Then we obtain

$$Q = (2\pi w' d' r_0 \sigma_0)^{-1} > 1. \tag{41}$$

Thus, Q is a numerical factor greater than unity because both the reaction radius and the radius of the channel are smaller than the distance between the groups on the surface, $\sigma_0^{-1/2}$. For $\kappa'_{on} = 10^8$ M^{-1}s^{-1}, which corresponds to $w'd' = 0.5$ Å, we obtain $Q = 30$.

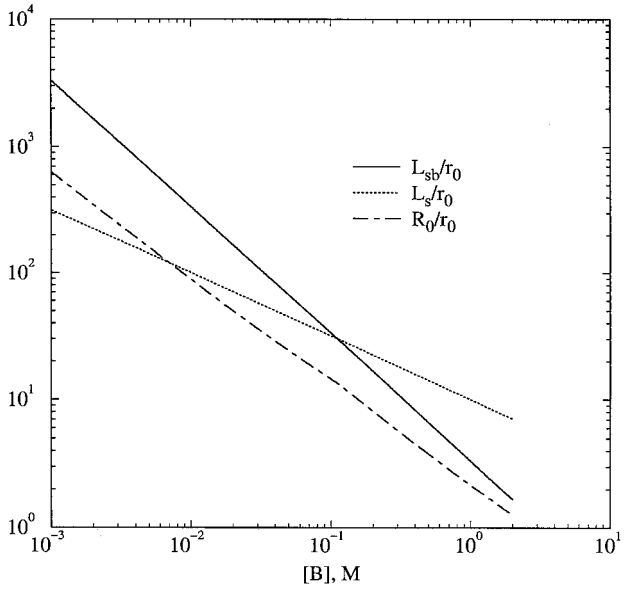

FIGURE 3 The dependence of L'_{sb} (*solid line*), L'_s (*dotted*), and R'_0 (*dot-dashed*) in units of r_0 on the buffer concentration $[B]$ calculated by Eqs. 37, 38, and 36, respectively, with the parameters from Table 1. $[B]_0 = 3.3$ M by Eq. 39, $Q = 30$ by Eq. 40. R'_0 is the effective channel radius in the presence of a buffer, L'_{sb} and L'_s are two length parameters for buffered solutoins that define the regime of proton diffusion, $[B_0]$ is the maximal concentration of the buffer beyond which the effect of the surface disappears, and Q is a dimensionless parameter (see Eqs. 40 and 41). In a vicinity of $[B] = 0.1$ M, a change of the regime of the surface diffusion occurs from the doupled (fast exchange) regime, $L'_{sb} \gg L'_s$, to the uncoupled (slow-exchange) one, $L'_{sb} \ll L'_s$. Because we did not treat the case $L'_{sb} \simeq L'_s$, the behavior of R'_0 near the intersection of the two curves is shown only qualitatively.

Figure 3 shows L'_{sb}, L'_s, and R'_0 as functions of the buffer concentration. This figure demonstrates that, even though both the two characteristic lengths and the enhancement factor R'_0/r_0 are very rapidly decreasing with increasing buffer concentration, a large enhancement effect of 10–600 still can be obtained at reasonable buffer concentrations from 1–100 mM. The highest concentration where the effect disappears is $[B]_0 = 3.3$ M.

The L'_{sb} and L'_s curves cross at $[B] \simeq 0.1$ M, which means that a change in the regime of proton transfer occurs from the coupled (fast-exchange) regime at smaller concentrations to the uncoupled (slow-exchange) one at higher concentrations. Yet, both regimes are similar with respect to the ability of the surface to accelerate the proton delivery to the target, the difference in the rate being only in the logarithmic factor. The capture radius R_c (the larger of L'_{sb} and L'_s) and the enhancement effect of antenna, R'_0/r_0, both decrease with the buffer concentration.

Figure 4 shows the calculated proton fluxes along the surface and from the bulk. The supply of protons from the bulk due to the collisional proton transfer from the buffer

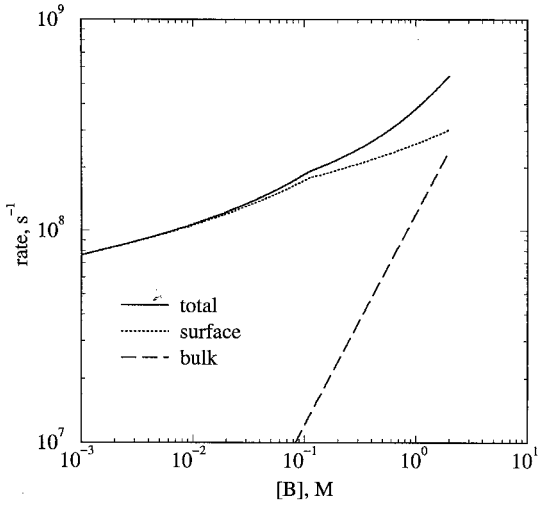

FIGURE 4 The calculated dependence of the rate of the surface-mediated proton supply to the channel entrance on the buffer cocentration [B]. *Solid line*, the total rate $k = k_s + k_b$; *dotted line*, the surface rate k_s; *dashed line*, the bulk rate k_b. The parameters are from Table 2. A spike at [B] $\simeq$ 0.1 M is due to our neglect of regorously solving the equations for the surface–bulk diffusion in the intermediate case between the two limiting cases.

molecules dramatically decreases at low buffer concentrations. On the contrary, the surface flux decreases much slower, only logarithmically, due to the reduced dimensionality of the diffusion space.

Gutman and Nachliel (1990, 1995, 1997) argued that buffers change the proton dynamics significantly. The above results are consistent with this expectation and give quantitative estimates of the buffer effect on the coupled surface–bulk proton diffusion.

APPENDIX A: THE SOLUTION OF THE MODEL IN THE FAST-EXCHANGE REGIME

Here, a simplified derivation of the solution of the coupled surface–bulk Eqs. 1 and 9 is presented. A more regorous and complete solution of the problem is given in Georgievskii et al. (2002).

Instead of $n(r, z)$ and $n(r)$, we will consider the new functions,

$$u(r, z) = 1 - \frac{n(r, z)}{n_0}, \tag{A1}$$

and $u(r) = u(r, z = 0)$, where n_0 is the equilibrium bulk concentration of protons. Inserting them into Eq. 9 gives

$$\frac{d^2u}{dr^2} + \frac{1}{r}\frac{du}{dr} + L_{sb}^{-1}u'_z = 0, \quad r > r_0. \tag{A2}$$

The boundary conditions to Eq. A2 are

$$u(r) = 1, \quad r < r_0, \qquad u(r) \to 0, \quad r \to \infty. \tag{A3}$$

The function $u(r)$ has an important meaning of the probability of capture of a proton that was initially on the surface at a distance r from the channel entrance. Similarly, $u(r, z)$ is the probability of capture of a proton that was initially at the point (r, z) in the bulk solution.

If $L_{sb} \ll r_0$, one can neglect the surface diffusion in Eq. A2, which then reduces to $u'_z = 0$ for $r > r_0$. This is a standard problem of an absorbing spot on a reflecting surface (Crank, 1990); the proton transport occurs via the bulk, and the rate is given by Eq. 12.

Below, the solution to Eqs. 1 and A2 is obtained in the regime when $L_{sb} \gg r_0$. As we will see, in this case, the proton flux incoming from the bulk is collected from a large surface area. Therefore, at small r, one can neglect the term $L_{sb}^{-1}u'_z$ in Eq. A2 and write

$$\frac{d^2u}{dr^2} + \frac{1}{r}\frac{du}{dr} = 0, \quad r \gtrsim r_0. \tag{A4}$$

The general solution of Eq. A4 can be written as

$$u(r) = \ln(R_c/r)/\ln(R_c/r_0), \quad r \gtrsim r_0, \tag{A5}$$

where we have used the fact that $u = 1$ at $r = r_0$. To find the unknown length parameter R_c, we will make a Fourier–Bessel transform of Eq. A2 over r. Because its right-hand side is different from 0 only at $r < r_0$, without restriction of generality, the Fourier transform of Eq. A2 can be written as

$$\mu(q)(q^2 + L_{sb}^{-1}q) = C, \quad qr_0 \lesssim 1, \tag{A6}$$

where C is constant, $\mu(q)$ is the Fourier transform of $u(r)$,

$$\mu(q) = \int_0^\infty J_0(qr)u(r)r\,dr, \tag{A7}$$

and $J_0(x)$ is the Bessel function. In Eq. A6, we have used the fact that a general, cylindrically symmetric solution of the diffusion equation, Eq. 1, can be written as

$$u(r, z) = \int_0^\infty e^{-qz}J_0(qr)\mu(q)q\,dq. \tag{A8}$$

As a result, the Fourier transform of $u_z(r)$ is given by

$$-\int_0^\infty J_0(qr)u_z(r)r\,dr = q\mu(q). \tag{A9}$$

Making the inverse Fourier transform of Eq. A6, one obtains, for $u(r)$,

$$u(r) = C\int_0^\infty dx J_0(x)\frac{1}{x + r/L_{sb}}, \quad r \gtrsim r_0. \tag{A10}$$

Only $x \lesssim 1$ contribute to this integral. At $r \gg L_{sb}$, the integral can be estimated as

$$u(r) \simeq CL_{sb}r^{-1}\int_0^\infty dx J_0(x) = C\frac{L_{sb}}{r}, \quad r \gtrsim L_{sb}. \tag{A11}$$

Thus, one can see that $u(r)$ decreases as $1/r$.

To estimate $u(r)$ at $r_0 \lesssim r \lesssim L_{sb}$, the integral in Eq. A10 can be taken by parts,

$$u(r) \simeq C \ln(C_0 L_{sb}/r), \quad r_0 \lesssim r \lesssim L_{sb}. \quad \text{(A12)}$$

The constant C_0 is given by (Gradshteyn and Ryzhik, 1994)

$$C_0 = \exp\left(\int_0^\infty dx \ln x J_1(x)\right) = 2e^{-\gamma} \simeq 1.1229, \quad \text{(A13)}$$

where $J_1(x)$ is the Bessel function, and $\gamma \simeq 0.5772$ is Euler's constant.

Comparing Eqs. A5 and A12, one finds Eq. 20 and

$$C = \frac{1}{\ln(C_0 L_{sb}/r_0)}. \quad \text{(A14)}$$

Having the above solution for $u(r)$, one can now find $\sigma(r)$, $n(r)$, $u(r, z)$, and $n(r, z)$. Thus, using Eqs. A12–A14 and Eq. A1 at $z = 0$, we obtain

$$n(r) = n_0 \frac{\ln(r/r_0)}{\ln(C_0 L_{sb}/r_0)}. \quad \text{(A15)}$$

Then, Eq. 13 is obtained by substituting Eq. A15 into Eq. 4.

APPENDIX B: THE LIFETIME OF THE PROTON ON THE SURFACE

In this section, we consider the following problem. Let a proton be initially localized on the membrane surface, and the rate of proton exchange between the surface and the bulk be high. Then, the rate-limiting step in the proton dissociation process will be the bulk diffusion, and the question is: how long will it take for the probability of finding the proton on the surface to decrease to a given value, say 0.5? We will denote this time τ_1.

Because the r coordinate on the surface plane is not essential to the problem, it can be integrated out,

$$S = \int \sigma(r) r \, dr, \quad \text{(B1)}$$

$$N(z) = \int n(r, z) r \, dr.$$

Then $N(z)$ satisfies the one-dimensional diffusion equation,

$$\frac{\partial N}{\partial t} = D_b \frac{\partial^2 N}{\partial z^2}, \quad \text{(B2)}$$

with the boundary conditions, cf. Eqs. 3 and 4,

$$D_b \frac{\partial N}{\partial z} = \frac{dS}{dt}, \quad \text{(B3)}$$

$$S(t) = L_0 N(0, t). \quad \text{(B4)}$$

The initial conditions to Eqs. B2 and B3 are

$$S(0) = 1, \quad N(z, 0) = 0. \quad \text{(B5)}$$

To solve Eqs. B2–B5, we will use the Laplace transform method,

$$N_\lambda(z) = \int_0^\infty e^{-\lambda t} N(z, t) \, dt, \quad \text{(B6)}$$

$$S_\lambda = \int_0^\infty e^{-\lambda t} S(t) \, dt.$$

The Laplace transforms of Eqs. B2–B4 are

$$\lambda N_\lambda(z) = D_b \frac{d^2 N_\lambda}{dz^2}, \quad \text{(B7)}$$

$$D_b \frac{dN_\lambda}{dz} = -1 + \lambda S_\lambda, \quad \text{(B8)}$$

$$S_\lambda = L_0 N_\lambda(0). \quad \text{(B9)}$$

In deriving Eqs B7 and B8, we have used the following property of the Laplace transform:

$$f'_\lambda = -f(0) + \lambda f_\lambda, \quad \text{(B10)}$$

where f'_λ is the Laplace transform of the derivative $f'(t) \equiv df/dt$. The solution of Eq. B7, which does not increase at infinity, can be written as

$$N_\lambda(z) = N_\lambda(0) \exp(-\sqrt{\lambda/D_b}\, z). \quad \text{(B11)}$$

Substituting this into Eq. B8 gives

$$\sqrt{D_b \lambda} N_\lambda(0) + \lambda S_\lambda = 1. \quad \text{(B12)}$$

From Eqs. B9 and B12, on finds

$$S_\lambda = \frac{1}{\lambda + L_0^{-1}\sqrt{D_b \lambda}}. \quad \text{(B13)}$$

Making the inverse Laplace transform of Eq. B13, one arrives at the following expression for $S(t)$:

$$S(t) = \frac{\sqrt{D_b}}{\pi L_0} \int_0^\infty d\lambda \frac{e^{-\lambda t}}{\sqrt{\lambda}(\lambda + D_b/L_0^2)} = \frac{1}{\pi} \int_{-\infty}^{+\infty} dp \frac{e^{-p^2 t D_b/L_0^2}}{p^2 + 1}. \quad \text{(B14)}$$

For $t \ll L_0^2/D_b$, one has $S(t) \simeq 1$. For $t \gg L_0^2/D_b$, the probability decreases very slowly, $S(t) \simeq 1/\sqrt{\pi t D_b/L_0^2}$. Numerical integration gives that $S(t) = 0.5$ at $t = 0.59 L_0^2/D_b \equiv \tau_1$.

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