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A CHANNEL MECHANISM FOR ELECTROGENIC ION PUMPS

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Summary

A model of active ion transport is analyzed in which an essential part of the pump molecule is an ion channel. Ion translocation in the channel is described as a series of jumps between binding sites which are separated by energy barriers. Pumping action results from a transient energy-dependent modification of the barrier structure of the channel and requires only minor conformational changes of the pump molecule. This model is applied to the light-driven proton pump of *Halobacterium* and to redox-coupled proton pumps in the mitochondrial respiratory chain. Similar considerations may be used to describe ATP-dependent ion transport.

Introduction

Transmembrane ion transport against an electrochemical potential gradient may be driven by a number of different energy sources, such as ATP hydrolysis, redox energy, or light. Well-known examples of ATP-dependent active transport systems are the Na^+/K^+ pump [1], the Ca^{2+} transport system in the sarcoplasmic reticulum [2] and the H^+ transport system in *Neurospora* [3]. In chloroplasts of green plants and in mitochondria, electron flow driven by redox-potential differences is coupled to active H^+ transport [4,5]. Light, besides being the primary energy source of the electron transport chain in chloroplasts, may also drive proton transport directly (without intermediate redox reactions) in the purple membrane of halophilic bacteria [6—8]. In most (or all) of these systems the transport is electrogenic, i.e. the translocation of the ion is accompanied by a net transfer of electrical charge across the membrane. So far, the molecular mechanism of coupling between energy input and ion translocation is poorly understood. One possibility which has been

frequently discussed in the past [9,10] is a mobile carrier which is chemically modified (for instance, by phosphorylation) on one side of the membrane. In this way, the carrier acts as a pump if the chemical modification leads to an asymmetric change of binding strength of the transported ion. In recent years, however, evidence has been accumulated that many transport systems are relatively large proteins spanning the entire thickness of the membrane. Such a protein is unlikely to act as a mobile carrier and it is more probable that pumping is brought about by some conformational change of the protein while the molecule as a whole remains more or less fixed within the membrane [45, 10,11]. For instance, bacteriorhodopsin which acts as a light-driven proton pump is organized in a two-dimensional crystalline array in the purple membrane [7,8]. Bacteriorhodopsin exerts its pumping function despite the fact that the rigid lattice virtually prevents any translational or rotational motion of the protein [12].

In this paper, a model is analyzed in which active ion translocation requires only minor conformational changes of the transport systems. The model is based on the assumption that the transport system has a number of ion-binding sites which are arranged in a sequence bridging the thickness of the membrane. Ion translocation may then be visualized as a series of jumps between energy minima (the binding sites), which are separated by activation-energy barriers. This concept has been widely used to describe passive ion transport across transmembrane channels [13—17]. Such an ion channel functions as a pump if the barrier structure of the channel is transiently modified by an energy-supplying reaction. For instance, absorption of a light quatum or transition to another redox state may alter the pK of a proton-binding site in the channel and, at the same time, change the height of the adjacent barriers. In this way a proton is preferentially released to one side of the membrane; during the transition back to the original state of the channel, another proton is taken up from the opposite side. In the following, such a barrier model is applied to light-driven proton transport; in a later section application to redox pumps is discussed.

Basic properties of the barrier model: application to light-driven proton transport

We consider a transmembrane proton channel consisting of a series of binding sites separated by energy barriers (Fig. 1). In the ground state a proton occupying the channel is assumed to be located most of the time in the deepest energy minimum (the main binding site). This binding site is easily accessible from the left (cytoplasmic) phase but separated from the right (external) phase by a high-energy barrier (Fig. 1). Absorption of a light quantum shifts the energy level of the binding site upward (corresponding to a decrease in binding strength) and changes the height of the neighbouring barriers in such a way, that the protein is released preferentially to the right (external) medium. In the case of bacteriorhodopsin there is evidence that excitation of the retinylidene chromophore leads to dissociation of a proton from the Schiff base [18—22]. A modification in the height of an activation-energy barrier may result from a small conformation change in the ligand system of the proton channel. The idea that bacteriorhodopsin contains a proton channel consisting of a sequence

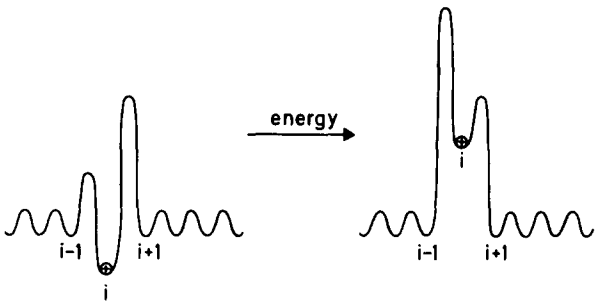

Fig. 1. Transmembrane ion channel consisting of a series of ion-binding sites separated by energy barriers. The barrier structure of the channel is modified by an energy-supplying reaction.

of binding sites has already been discussed in the literature [20,23,25].

A reaction scheme for the whole pumping cycle is depicted in Fig. 2. Absorption of a photon in the ground state (HP) leads to a short-lived excited state HP** which relaxes to the activated state HP* from which the proton is released to the external medium (phase''). The state HP** (which may represent a higher vibrational level of the chromophore) is introduced here in order to account for the limited reversibility of the pump (see below). For generality we assume that there is a finite probability for thermal deactivation of state HP* directly back to the ground state HP (rate constant k''). After release of the proton from state HP*, a dark transition back to the conformation with a low barrier on the left (cytoplasmic) side may take place (P* → P). The original state is restored by uptake of H^+ from the cytoplasmic side (P → HP). The whole cycle is equivalent to the net transfer of one proton from the cytoplasm to the external medium. State HP* may be tentatively assigned to the L_{550} intermediate, state P* to the deprotonized M_{412} product and state P to the

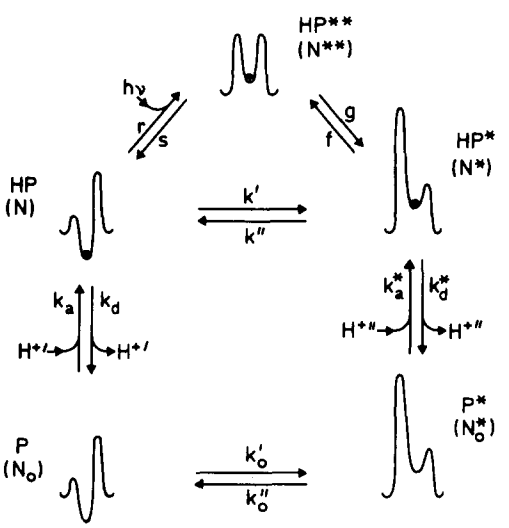

Fig. 2. Transitions between the different states of the proton channel during the pumping cycle. Only the main proton-binding site and the two neighbouring energy barriers are shown. N, N^*, N^{**}, N_0 and N_0^* are the number of channels in states HP, HP*, HP**, P and P*, respectively.

O_{640} product which is reprotonated to give the original R_{570} protein [23,24, 26–28]. It should be emphasized that this kinetic scheme does not account for all photo-chemical intermediates observed so far with bacteriorhodopsin, but has to be regarded as a minimal model of a light-driven proton pump. Such a model may be useful for an approximate description, since the kinetics of the overall process are mainly determined by a limited number of relatively long-lived intermediates.

For the formal analysis of the model, we introduce the following assumptions:

(1) The bandwidth of the incident radiation is sufficiently narrow so that light is absorbed by species HP only (Fig. 2).

(2) The rate-limiting barriers for proton transport are the two barriers on either side of the main binding site (i). The outer barriers are low enough so that binding sites (i − 1) and (i + 1) are always in equilibrium with the respective aqueous phases (Fig. 1). Furthermore, the binding strength in all energy minima besides the ith minimum is so low that these minima are rarely occupied.

(3) In the ground state (HP) the barrier to the right of the main binding site is of virtually infinite height and in the activated state (HP*) the barrier to the left. This means that, in the ground state, the binding site may exchange a proton only with the left-hand solution and in the activated state only with the right-hand solution.

(4) In the primary excited state (HP**), proton dissociation from the binding site may be neglected, either because of the height of the barriers or because of the low concentration of species HP** (or both).

(5) Transition between HP** and HP* are fast compared with the other reactions so that species HP** and HP* are always in equilibrium with each other.

These assumptions have been introduced here mainly for mathematical convenience and may be omitted in the framework of a more general treatment.

If N_p is the total number of light-activated proton channels in the membrane, the number N_{i-1} of channels with a proton in the (i − 1)th binding site is given by (according to assumption 2):

$$N_{i-1} = N_p \theta' a' \tag{1}$$

a' is the proton activity in the left-hand aqueous phase (phase′) and θ' a constant. Denoting the rate constant for jumps from the (i − 1)th minimum into the binding site by k'_{i-1} and the number of channels with unoccupied binding site by N_0, the rate of association between protons from phase′ and the binding site may be written as

$$k'_{i-1} N_{i-1} \frac{N_0}{N_p} = k'_{i-1} \theta' a' N_0 = k_a a' N_0 \tag{2}$$

N_0/N_p is the probability that the binding site is empty and $k_a = \theta' k'_{i-1}$ is the association rate constant. If N is the number of channels with occupied binding site and k_d the dissociation rate constant, the dissociation rate is given by $k_d N$. In the equilibrium state both rates are equal, so that

$$\frac{a' \overline{N_0}}{\overline{N}} = \frac{k_d}{k_a} = K \tag{3}$$

$\bar{N}$ and $\bar{N}_0$ are the equilibrium values of N and N_0 and K is the equilibrium constant of dissociation. Introducing the number N_0^* and N^* of activated channels with empty and occupied binding site (states P* and HP* in Fig. 2) and using an analogous argument as above, the equilibrium constant of dissociation in the activated state, K^*, is obtained as

$$\frac{a''\bar{N}_0^*}{\bar{N}^*} = \frac{k_d^*}{k_a^*} = K^* \tag{4}$$

a'' is the proton activity in the right-hand solution and k_d^* and k_a^* are the rate constants of dissociation and association in the activated state.

If the system is irradiated with light of intensity J the rate of transition from the ground state HP to the excited state HP** is equal to rN, the rate constant r being given by

$$r = r_0 + \gamma J \tag{5}$$

where r_0 is the rate constant for spontaneous excitation in the dark ($J = 0$) and γ is the absorption cross-section. γ is related to the molar extinction coefficient ϵ by the relation $\gamma = 1000(\epsilon/L)\ln 10$, where L is Avogadro's constant. The rate constant, s, for the reverse transition (HP** → HP) may be written as

$$s = s_0 + \gamma J \tag{6}$$

The term γJ accounts for the possibility of induced emission which has, at a given light intensity, the same probability as absorption [29]. (Although the actual rate $\gamma J N^{**}$ of induced emission is usually very low, the term γJ has to be included in order to make the model internally consistent.) For the representation of the final results, it is useful to introduce the following equilibrium constants:

$$\frac{\bar{N}^{**}}{\bar{N}} = \frac{r_0}{s_0} = A \tag{7}$$

$$\frac{\bar{N}^{**}}{\bar{N}^*} = \frac{f}{g} = B \tag{8}$$

$$\frac{\bar{N}^*}{\bar{N}} = \frac{k'}{k''} = S \tag{9}$$

$$\frac{\bar{N}_0^*}{\bar{N}_0} = \frac{k_0'}{k_0''} = S_0 \tag{10}$$

The equilibrium values of N, N^*, etc. are denoted by a bar. In general, the rate constants r_0, s_0, f, g, k', k'', k_0' and k_0'' (Fig. 2), as well as the equilibrium constants A, B, S and S_0, are functions of voltage. It is seen from Eqns. 7—9 that the following relationship holds:

$$A = BS \tag{11}$$

Furthermore, Eqns. 3, 4, 9 and 10 together yield $SK^*/S_0K = a''/a'$, where the ratio a''/a' refers to the equililibrium state (vanishing proton flux and vanishing light intensity). An equilibrium state for $J = 0$ and $a' \neq a''$ is only possible if a voltage $V_m = V_m^e$ (the equilibrium potential for H^+) is present across the mem-

brane, which is given by the Nernst equation (the index e denotes the equilibrium state):

$$\exp(u_e) = \frac{a''}{a'} \tag{12}$$

$$u_e = \frac{V_m^e}{kT/e_0} = \frac{(\psi' - \psi'')_e}{kT/e_0} \tag{13}$$

k is the Boltzmann constant, T the absolute temperature, e_0 the elementary charge and ψ' and ψ'' are the electrical potentials in the aqueous phases ′ and ″, respectively. This yields the relation $SK^*/S_0K = \exp(u_e)$. However, as any value of u_e may be obtained by a suitable choice of the ratio a''/a' and as S, S_0, K and K^* are independent of a' and a'', we have, at arbitrary voltages $u = V_mF/RT$,:

$$\frac{SK^*}{S_0K} = \exp(u) \tag{14}$$

In the following we consider the stationary state of the transport system in the presence of a constant light intensity J. Absorption of photons raises the number of activated pump molecules (state HP*) above the equilibrium value (which may be close to zero) and thus creates a driving force for the reaction HP* → P* → P → HP during which a proton is released to phase″ and another proton is taken up from phase′ (Fig. 2). The stationary proton flux Φ from phase′ to phase″ is equal to the rate of transition from HP* to P* minus the rate of reverse transition (P* → HP*):

$$\Phi = k_d^* N^* - k_a^* a'' N_0^* \tag{15}$$

By determining the stationary values of N^* and N_0^*, the following result is obtained (Appendix A):

$$\Phi = \frac{N_p}{\beta}[(r_0 + k')(1 - w) + \gamma J(1 - Aw)] \tag{16}$$

$$w \equiv \frac{a''}{a'} \exp(-u) = \exp(-\Delta\tilde{\mu}_H/RT) \tag{17}$$

$$\begin{aligned}\beta \equiv\ & 1 + wS(1 + B) + \frac{1 + S_0}{a'/K} + (r_0 + k')\left[\frac{1 + S_0}{a'k_a} + \frac{1}{k_0''} + \left(1 + B + \frac{1}{S}\right)\cdot\right. \\ & \left.\cdot\left(\frac{wS}{k_d} + \frac{a''/K^*}{k_0''} + \frac{1}{k_d^*}\right) + w\left(\frac{1 + 1/S_0}{a''k_a^*} + \frac{1}{k_0'}\right)\right] \\ & + \gamma J\left[(1 + S_0)\left(\frac{1}{a'k_a} + \frac{B}{k_d^* a'/K}\right) + \frac{1}{k_0''} + \frac{wBS}{k_0'}\right. \\ & \left. + (1 + 2B)\left(\frac{wS}{k_d} + \frac{a''/K^*}{k_0''} + \frac{1}{k_d^*}\right)\right]\end{aligned} \tag{18}$$

R is the gas constant and $\Delta\tilde{\mu}_H = \tilde{\mu}_H' - \tilde{\mu}_H''$ is the difference of the electrochemical potentials for H^+ between phase′ and phase″. Eqns. 16–18 describe the proton flux Φ as a function of the external variables a', a'', u and J (the mem-

brane voltage u is also implicitly contained in rate constants and the equilibrium constants). It is seen that, in the absence of an electrochemical potential gradient ($w = 1$), the proton flux Φ is a linear function of light intensity J at small values of J. On the other hand, Φ becomes independent of light intensity in the limit $J \to \infty$ since β is a linear function of J. In experiments with purple bacteria, saturation of proton flux is observed at light intensities of the order of 10 mW/cm^2 (at a wavelength of 575 nm) or $3 \cdot 10^{16}$ quanta/cm$^2 \cdot$ s [30]. With a peak extinction coefficient of $\epsilon \approx 5 \cdot 10^4$ M$^{-1} \cdot$ cm^{-1} [7], or an absorption cross-section of $\gamma \approx 2 \cdot 10^{-16}$ cm^2, this corresponds to an absorption rate of $\gamma J \approx 6$ s^{-1}.

Short-circuit current, turnover rate and reversal potential

A simple situation arises when the proton activities on both sides of the membrane are the same ($a' = a''$) and when the membrane voltage vanishes (short-circuit conditions). In this case, $w = 1$ holds. Eqn. 16 therefore yields:

$$\Phi_{sc} = \frac{N_p}{\beta}(1 - A)\,\gamma J \tag{19}$$

The corresponding electrical short-circuit current I_{sc} is given by $I_{sc} = e_0 \Phi_{sc}$ (e_0 is the elementary charge).

In photochemical experiments with suspensions of isolated purple membranes, the so-called turnover rate ρ is determined under the condition $a' = a'' = a$, $u = 0$, $J \to \infty$. ρ is the number of photocycles performed by a single pump molecule in 1 s at saturating light intensity. From Eqns. 14, 18 and 19, the turnover rate is obtained as

$$\rho = \left(\frac{\Phi_{sc}}{N_p}\right)_{J \to \infty} = \frac{1 - A}{(1 + S_0)\left(\frac{1}{ak_a} + \frac{B}{k_d^* a/K}\right) + \frac{1}{k_0''} + \frac{A}{k_0'} + (1 + 2B)\left(\frac{S}{k_d} + \frac{a/K^*}{k_0''} + \frac{1}{k_d^*}\right)} \tag{20}$$

It is seen from Eqn. 20 that the pH dependence of ρ may be rather complicated. Eqn. 20 assumes a much simpler form if the following two additional assumptions are introduced: (a) The energy of the excited state is so high that $\bar{N}^{**} \ll \bar{N}$ and $\bar{N}^{**} \ll \bar{N}^*$; this means that $A \ll 1$ and $B \ll 1$ (Eqns. 7 and 8). (b) The rate constants of protonation ($a'k_a$, $a''k_a^*$) are much larger than the other rate constants. Under these condition, Eqn. 20 reduces to

$$\rho = \left(\frac{S}{k_d} + \frac{1}{k_d^*} + \frac{1 + a/K^*}{k_0''}\right)^{-1} \tag{21}$$

In experiments with isolated purple membrane fragments the turnover rate was found to be $\rho \approx 100$ s^{-1} [8]. On the basis of Eqn. 21 this would mean that at least one of the quantities S/k_d, k_d^* and k_0'' is of the order of 100 s^{-1} (while the other two may be much larger).

The reversal potential u_0 of the pump is the membrane voltage for which, at a given light intensity J, the proton flux through the pump vanishes. From

Eqns. 16 and 17 one finds

$$u_0 = \ln \frac{a''}{a'} + \ln \frac{r_0 + k' + A\gamma J}{r_0 + k' + \gamma J} \tag{22}$$

At $u = u_0$ electrochemical potential gradient for protons across the membrane exactly compensates the driving force of the pump. According to Eqn. 17, Eqn. 22 may be written in the equivalent form

$$(\Delta\tilde{\mu}_H)_0 = RT \ln \frac{r_0 + k' + A\gamma J}{r_0 + k' + \gamma J} \tag{23}$$

where $(\Delta\tilde{\mu}_H)_0$ is the electrochemical potential difference for which the proton flux through the pump vanishes. If the pump starts to work under the initial condition $u = 0$, $a' = a''$ ($\Delta\tilde{\mu}_H = 0$), an electrochemical potential difference gradually builds up, which consists partly of a voltage u and partly of a pH difference. With increasing absolute value of $\Delta\tilde{\mu}_H$, the rate of the reverse process ($P^* + H^+ \rightarrow HP^*$) is enhanced. In this condition and in the absence of leakage pathways, the electrochemical potential difference reaches the limit $(\Delta\tilde{\mu}_H)_0$ at which the forward and backward rates become equal ($\Phi = 0$). Thus, $(\Delta\tilde{\mu}_H)_0$ is the maximum electrochemical potential difference which may be built up by the pump at a given light intensity J. The quantity $(\Delta\tilde{\mu}_H)_0/F = (RT/F)(u_0 + \ln a'/a'')$ is sometimes called the protonmotive force of the pump [38].

In a real membrane with finite leakage conductance, the stationary state is determined by the condition that the fluxes through the pump and through the leakage pathways cancel each other. This condition is already reached at $|\Delta\tilde{\mu}_H| < |(\Delta\tilde{\mu}_H)_0|$.

Under most experimental conditions the term $A\gamma J$ in Eqns. 22 and 23 is negligibly small. With a molar extinction coefficient of $\epsilon \approx 5 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ at 560 nm [7], the absorption cross-section of bacteriorhodopsin at 560 nm is $\gamma = 1000(\epsilon/L)\ln 10 \approx 2 \cdot 10^{-16}\ cm^2$. In an experiment with steady illumination, the light intensity is usually less than $1\ W \cdot cm^{-2}$, corresponding (at 560 nm) to a quantum flux density of $J \approx 3 \cdot 10^{18}\ cm^{-2} \cdot s^{-1}$, so that the rate of induced emission, γJ, is of the order of $10^3\ s^{-1}$ or smaller. On the other hand, the rate $s_0 = r_0/A$ of sponteneous deactivation of an excited singlet state is many orders of magnitude larger. This means $\gamma J << s_0$, or $A\gamma J << r_0$, so that Eqn. 23 reduces to

$$(\Delta\tilde{\mu}_H)_0 \approx RT \ln \frac{r_0 + k'}{r_0 + k' + \gamma J} \tag{24}$$

On the other hand, it is seen from Eqn. 23 that $(\Delta\tilde{\mu}_H)_0$ never exceeds the value $RT \ln A$. The equilibrium constant A between the excited state and the ground state is related to the energy $h\nu$ of the incident light quanta:

$$A = \exp(-h\nu/kT) \tag{25}$$

Thus, $(\Delta\tilde{\mu}_H)_0$ obeys the relation

$$|(\Delta\tilde{\mu}_H)_0| \leqslant Lh\nu \tag{26}$$

where $L = R/k$ is Avogadro's constant. Eqn. 26, of course, has to be expected a priori from energy considerations and from the stoichiometry of the model.

It is interesting to note that the expression for $(\Delta\tilde{\mu}_H)_0$ (Eqn. 23) does not contain the proton dissociation constants K and K^*. This means that a pK difference between the ground state and the excited state is not critical for the thermodynamic efficiency of the pump. The essential feature of this pumping mechanism is the light-induced change in the barrier structure of the channel which switches the proton-binding site from a left exposed to a right-exposed state. On the other hand, it is seen from Eqn. 21 that a low pK of the excited state ($K^* >> a$) is favourable for a high turnover rate of the pump.

Current-voltage characteristic

Further information on the kinetic parameters of the pump may be obtained by measuring the proton flux Φ (or the corresponding electrical current $I = e_0\Phi$) as a function of voltage u. Although, in general, all rate constants may depend on voltage, it is reasonable to assume that the main voltage dependence results from field effects on the translocation rate constants of the proton in the channel. Accordingly, we regard all other rate constants as voltage independent. If $\alpha_1 u$ is the voltage dropping between the left-hand solution and the (i -- 1)th energy minimum (Fig. 1) and $\alpha_{i-1}u$ the voltage between the (i — 1)th and the ith minimum, then, according to the theory of absolute reaction rates [13], the quantities θ' (Eqn. 1) and k'_{i-1} (Eqn. 2) are given by $\theta' = \tilde{\theta}' \exp(\alpha_1 u)$, $k'_{i-1} = \tilde{k}'_{i-1} \exp(\alpha_{i-1}u/2)$, where $\tilde{\theta}'$ and $\tilde{k}'_{i-1}$ are the values of θ' and k'_{i-1} at $u = 0$. Therefore,

$$k_a = \tilde{k}_a \exp[(\alpha_1 + \alpha_{i-1}/2)u] \tag{27}$$

$$k_d = \tilde{k}_d \exp[-(\alpha_{i-1}/2)u] \tag{28}$$

$$K = \tilde{K} \exp[-(\alpha u)] \tag{29}$$

(compare Eqns. 1—3). $\alpha u = (\alpha_1 + \alpha_{i-1})u$ is the voltage between the left-hand solution and the main binding site in the ground state. According to Eqn. 27 the pK value of the binding site is a function of external voltage. Similarly, one obtains for the activated state:

$$k_a^* = \tilde{k}_a^* \exp[-(\alpha_r^* + \alpha_i^*/2)u] \tag{30}$$

$$k_d^* = \tilde{k}_d^* \exp(\alpha_i^* u/2) \tag{31}$$

$$K^* = \tilde{K}^* \exp(\alpha^* u) \tag{32}$$

$\alpha_r^* u$ is the voltage between the (i + 1)th minimum and the right-hand solution, $\alpha_i^* u$ the voltage between the ith and the (i + 1)th minimum and $\alpha^* u = (\alpha_r^* + \alpha_i^*)u$ the voltage between the main binding site and the right-hand solution. As $S = k'/k''$ and $S_0 = k'_0/k''$ have been assumed to be voltage independent, we see from Eqn. 14 that the relation $\alpha + \alpha^* =$ holds.

The current-voltage behaviour of the pump is described by Eqns. 16 and 18 together with Eqns. 27—32. It is seen from the form of these equations that $I(u)$ approaches voltage-independent limiting values both for large positive and large negative voltages:

$$I(+\infty) = e_0 N_p k_0'' \frac{r_0 + k' + \gamma J}{r_0 + k' + k_0'' + \gamma J} \tag{33}$$

$$I(-\infty) = -e_0 N_p k_0' \frac{s_0 B + k'' + B\gamma J}{s_0 B + k'' + k_0'(1 + B) + B\gamma J} \tag{34}$$

The current-voltage characteristic is represented schematically in Fig. 3.

Efficiency and quantum yield

The quantum yield Q of he pump may be generally defined by

$$Q = \frac{\Phi - \Phi_d}{j} \tag{35}$$

where Φ_d is the proton flux in the dark and j is the rate of net uptake of light quanta. In the following, we apply Eqn. 35 to the case of vanishing electrochemical potential gradient ($\Delta\tilde{\mu}_H = 0$) where Φ_d is equal to zero. For the representation of the result we first introduce the relation

$$s_0 = s_0^0 + s_0^* \tag{36}$$

which expressess the fact that the rate s_0 of transition from state HP** to state HP is the sum of the rate of radiationless transition, s_0^0, and the rate of spontaneous emission, s_0^*. The quantum yield is then obtained in the form (see Appendix B)

$$Q = \frac{1}{1 + (k'' + s_0^0 B)\left(\frac{S}{k_d} + \frac{1}{k_d^*} + \frac{a''/K^*}{k_0''}\right)} \tag{37}$$

It is seen from Eqn. 37 that Q approaches unity when the rate constants k'' and s_0^0 became small. k'' and s_0^0 determine the rate of thermal desactivation of state HP* which may occur either directly (rate constant k'') or via state HP** (rate constant s_0^0); as we have assumed that HP* and HP** are always in equilibrium with each other, the rate constant for the transition HP* → HP** does not enter explicitly into the expression for Q. Experimental quantum yields range between 0.5 and 0.8 translocated protons/absorbed photon for the purple membrane [8].

It is well known that the Na^+/K^+ pump, as well as the Ca^{2+} pump, in the sarcoplasmic reticulum may be operated in the reverse direction, i.e. an ion concentration difference of sufficient magnitude may drive the pump backwards, resulting in net synthesis of ATP from ADP [32,33]. In the case of a light-driven ion pump, backward operation of the pump should lead to

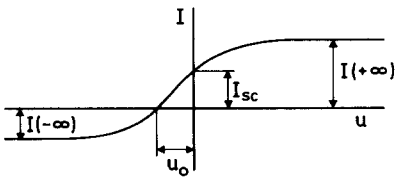

Fig. 3. Current-voltage characterisitic of the pump. I_{sc} is the short-circuit current and u_0 the reversal potential.

emission of light, at least in principle. Such a light emission has not been observed from purple membranes so far. Nevertheless it is interesting to calculate the quantum yield Q_e of the emission process for the transport model considered here. Q_e is defined as the ratio of the photon emission rate, divided by the proton flux under the condition $J = 0$. As shown in Appendix B, Q_e is given by

$$Q_e = \left(\frac{j}{\Phi}\right)_{J=0} = \frac{s_0^*}{s_0 + k''/B} \tag{38}$$

The primary excited state HP** probably has a much higher energy than the activated state HP* so that the equilibrium constant B is much less than unity. This means that Q_e is likely to be very small.

The efficiency η is defined as the ratio of the electrochemical power $-(\Phi - \Phi_d)\Delta\tilde{\mu}_H$ which is generated by the pump, divided by the net rate $jh\nu L$ of light-energy absorption:

$$\eta = -\frac{(\Phi - \Phi_d)\Delta\tilde{\mu}_H}{jh\nu L} = -\frac{\Delta\tilde{\mu}_H}{h\nu L} Q \tag{39}$$

(It should be noted that $(\Phi - \Phi_d)\Delta\tilde{\mu}_H$ is negative when $\Delta\tilde{\mu}_H$ is generated by a light-driven proton flux.) An explicit expression for η (not given here) may be obtained from Eqns. 16, 39, and B4. Theoretically, the efficiency may approach unity in the limit of small values of k', k'', r_0, and s_0, but experimentally observed efficiencies with *Halobacterium* cells are only of the order of 5–10% [8].

The pump in the vicinity of equilibrium

We now consider the case that both the light intensity and the electrochemical potential difference of H^+ are small, so that the pump operates close to equilibrium. This means that

$$u \approx u_e = \ln \frac{a''}{a'} \tag{40}$$

$$\left|\frac{\Delta\tilde{\mu}_H}{RT}\right| = |u - u_e| << 1 \tag{41}$$

and $w \approx 1 - \Delta\tilde{\mu}_H/RT$ (compare Eqns. 12 and 17). Eqn. 16 then reduces to:

$$\Phi = \frac{N_p}{\beta_0}\left[(r_0 + k')\frac{\Delta\tilde{\mu}_H}{RT} + (1 - A)\gamma J\right] \tag{42}$$

where β_0 is the value of β for $w = 1$ and $J = 0$. In Eqn. 42 the proton flux Φ is represented by a linear function of the 'driving forces' $\Delta\tilde{\mu}_H$ and J. Thus, Eqn. 42 has the form of the phenomenological equations used in the thermodynamics of irreversible processes [34,35].

It can be seen from Eqn. 42 that the proton pump, in general, acts as a passive proton channel in the dark ($J = 0$) unless the rate constants r_0 and k' are small. The dark conductance Λ_d of the single channel is given by

$$\Lambda_d = \frac{1}{N_p}\frac{e_0\Phi}{V_m - V_m^e} = \frac{e_0^2}{kT}\frac{r_0 + k'}{\beta_0} \tag{43}$$

Eqn. 42 further shows that differences in pH and electrical potential always enter into the relationship for Φ in the combination

$$\frac{\Delta\tilde{\mu}_H}{RT} = \ln\frac{a'}{a''} + u = -2.30\Delta(\mathrm{pH}) + (F/RT)V_m \qquad (44)$$

In this sense, a pH difference and a voltage may be considered to be kinetically equivalent. This, however, is only true for small values of Δ(pH) and V_m. In the general case, where Eqn. 42 has to be replaced by Eqn. 16, a pH difference Δ(pH) and a voltage V_m of magnitude $-2.30(RT/F)\Delta$(pH) may have different effects on Φ although they are thermodynamically equivalent (in terms of Eqn. 23). Another case, where an ion-concentration difference and a voltage have different kinetic effects, has recently been analyzed for an electrogenic cotransport system [36].

Redox-coupled proton pumps

In the respiratory chain of mitochondria and bacteria, redox energy is converted into an electrochemical potential difference of protons [37]. An early proposal for the mechanism by which $\Delta\tilde{\mu}_H$ is generated was based on the assumption that the transfer of an electron across the membrane in one direction is coupled to the transfer of neutral hydrogen in the opposite direction, resulting in proton uptake on one side and proton release on the other [38]. An alternative possibility is a proton pump which is driven by the redox reaction [39,40,46]. This second mechanism, which has been termed 'vectorial Bohr effect' [39], has recently gained support from experiments with cytochrome *c* oxidase [40—42] and with transhydrogenase [46]. From the results of these experiments, it has been proposed that the oxidized and the reduced state correspond to different conformation states of the enzyme and that the conformational changes associated with the redox cycle are coupled to the translocation of H^+ across the membrane.

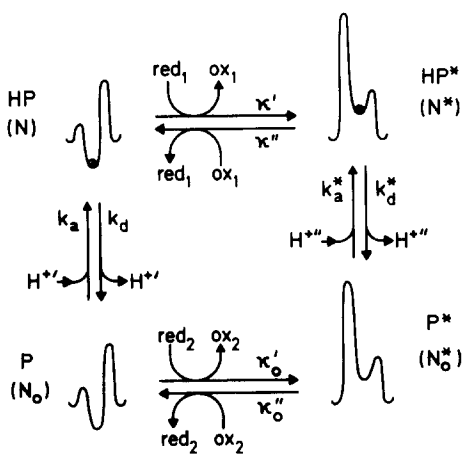

Fig. 4. Coupling between changes in the redox state of the pump and translocation of H^+ across the membrane. HP and P are oxidized states of the pump, HP* and P* are reduced states. The pump operates between two redox systems red_1/ox_1 and red_2/ox_2.

Such a redox-coupled proton pump may operate by a similar mechanism to that proposed above for bacteriorhodopsin. The redox enzyme (e.g. cytochrome *c* oxidase) itself may have the property of a proton channel or it may be closely associated with a proton channel in the membrane. Donation of an electron to one of the heme groups of cytochrome *c* oxidase may result in a change of barrier structure of the proton channel leading to vectorial proton release [41]. In the reaction scheme depicted in Fig. 4, it is assumed that, in the oxidized state (HP/P) of the redox pump, the proton binding site is exposed to the left, whereas in the reduced state (HP^*/P^*) the binding site is exposed to the right. During the pumping cycle, the pump accepts an electron from redox system 1 and donates an electron to redox system 2; at the same time a proton is translocated from left to right. We again assume that the outer barriers are not rate limiting and that, in state HP/P, the main binding site can exchange a proton only with the left-hand solution and in state HP^*/P^* only with the right-hand solution (assumptions 2 and 3 of the preceding section). Furthermore, we assume that the protonated form of the channel (HP/HP^*) reacts only with redox system 1 and the unprotonated form only with system 2. This means that the ratio of proton flow to electron flow is unity (complete coupling).

Redox systems 1 and 2 may be present in the external aqueous phases or they may be components of the electron transport chain in the membrane. In the later case, they are connected either directly or via other components of the chain to external redox pools. If the average concentrations of the oxidized and the reduced form of system i ($i = 1, 2$) are denoted by $[ox_i]$ and $[red_i]$, the redox potential E_i of system i is given by

$$E_i = E_{i0} + \frac{RT}{F} \ln \frac{[ox_i]}{[red_i]} = E_{i0} + \frac{RT}{F} \ln \frac{1 - p_i}{p_i} \tag{45}$$

E_{i0} is the standard redox potential and $p_i = [red_i]/([ox_i] + [red_i])$ is the probability of finding system i in the reduced form. The difference $E_2 - E_1$ is related to the difference $\Delta\mu_e$ in the chemical potentials of the electron in system 1 and 2:

$$\Delta\mu_e \equiv \mu_e(1) - \mu_e(2) = F(E_2 - E_1) \tag{46}$$

In order to calculate the difference $\Delta\tilde{\mu}_e$ in the electrochemical potentials of the electron, one has to take into account that the transition of an electron from system 1 to system 2 involves, in general, a transfer across part of the membrane dielectric. Denoting by $\alpha_{12}u$ the fraction of the external membrane voltage u which drops between systems 1 and 2, one obtains:

$$\Delta\tilde{\mu}_e = F(E_2 - E_1) - RT\alpha_{12}u \tag{47}$$

$$\alpha_{12} \equiv \frac{1}{u}(\varphi_1 - \varphi_2)$$

φ_1 and φ_2 are the (dimensionless) electrical potentials which are created by the external voltage at the sites of systems 1 and 2. Depending on the relative position of systems 1 and 2, α_{12} may be positive or negative ($-1 \leqslant \alpha \leqslant 1$). It should be noted that u is the externally applied voltage, so that any contribution of an

intrinsic electrical field strength in the membrane (which may result from a difference in the surface potentials) of the membrane is contained in $(E_2 - E_1)$. The redox reactions between the pump and systems 1 and 2 may be described by the rate constants κ', κ'', κ_0' and κ_0'' (Fig. 4). Denoting again the number of pump molecules in states HP, HP*, P, and P* by N, N^*, N_0 and N_0^*, respectively, the rates of the transitions HP → HP* and HP* → HP may be written as $\kappa' p_1 N$ and $\kappa''(1 - p_1)N^*$. In the equilibrium state (i.e. at vanishing proton flow) the following relations hold:

$$\frac{(1-p_1)\bar{N}^*}{p_1\bar{N}} = \frac{\kappa'}{\kappa''} = H \tag{48}$$

$$\frac{(1-p_2)\bar{N}_0^*}{p_2\bar{N}_0} = \frac{\kappa_0'}{\kappa_0''} = H_0 \tag{49}$$

As shown in Appendix C, the equilibrium constants H, H_0, K and K^* are connected by the following relation:

$$\frac{HK^*}{H_0K} = \exp\left[\frac{E_{20} - E_{10}}{RT/F} + (1 - \alpha_{12})u\right] \tag{50}$$

The stationary proton flux Φ is again obtained from Eqn. 15 by calculating the steady-state values of N^* and N_0^* (Appendix C); this yields

$$\Phi = \frac{N_p}{\beta}\kappa' p_1(1 - v) \tag{51}$$

$$v \equiv \frac{a''}{a'}\exp\left[\frac{E_1 - E_2}{RT/F} - (1 - \alpha_{12})u\right] = \exp\left(-\frac{\Delta\tilde{\mu}_H + \Delta\tilde{\mu}_e}{RT}\right) \tag{52}$$

$$\begin{aligned}\beta \equiv 1 &+ \frac{vHp_1}{1-p_1} + \frac{K}{a'}\left(1 + \frac{H_0p_2}{1-p_2}\right) + \kappa' p_1\left\{\frac{1}{a'k_a}\left(1 + \frac{H_0p_2}{1-p_2}\right)\right. \\ &+ \frac{1}{\kappa_0''(1-p_2)} + \left(1 + \frac{1-p_1}{p_1H}\right)\left[\frac{vHp_1}{k_d(1-p_1)} + \frac{a''/K^*}{\kappa_0''(1-p_2)} + \frac{1}{k_d^*}\right] \\ &\left. + v\left[\frac{1}{a''k_a^*}\left(1 + \frac{1-p_2}{H_0p_2}\right) + \frac{1}{\kappa_0'p_2}\right]\right\}\end{aligned} \tag{53}$$

Implicit in the derivation of Eqn. 51 is the assumption that the quantities p_1 and p_2 which describe the redox state of systems 1 and 2 are maintained at constant values, irrespective of the magnitude of the proton flux Φ. This condition is met if systems 1 and 2 are buffered by redox pools in the external phases (for a discussion of the steady-state kinetics of the respiratory chain, see Ref. 44).

According to Eqns. 51 and 52 the proton flux vanishes for $\Delta\tilde{\mu}_H + \Delta\tilde{\mu}_e = 0$. The reversal potential u_0 and the proton-motive force $(\Delta\tilde{\mu}_H)_0/F$ of the pump are then obtained from Eqns. 44 and 47 as

$$u_0 = \frac{V_m^0 F}{RT} = \frac{\ln(a''/a') + F(E_1 - E_2)/RT}{1 - \alpha_{12}} \tag{54}$$

$$(\Delta\tilde{\mu}_H)_0/F = E_1 - E_2 + \alpha_{12}V_m \tag{55}$$

In the simplest case, where an electron transfer from system 1 to system 2 is voltage independent ($\alpha_{12} = 0$), the protonmotive force is equal to the difference $E_1 - E_2$ of the redox potentials. This situation arises, for instance, when both redox systems are present in the same external solution. On the other hand, when systems 1 and 2 are located on opposite sides of the membrane, an electron transition from systems 1 and 2 involves a transfer of the electron across the entire membrane dielectric so that either $\alpha_{12} = 1$ or $\alpha_{12} = -1$. Under these circumstances, the protonmotive force becomes voltage-dependent (Eqn. 55). When system 2 is located on the side of the solution into which protons are pumped, the charge transport by H^+ is cancelled by a simultaneous transfer of e^- in the same direction ($\alpha_{12} = 1$); according to Eqn. 54, this means that in this case the pump can operate against arbitrarily high membrane voltages ($|u_0| \to \infty$). In the opposite limiting case ($\alpha_{12} = -1$), the reversal potential u_0 has half the value as under the condition $\alpha_{12} = 0$ [40,41].

The pump is driven by the difference $E_1 - E_2$ in the redox potentials of systems 1 and 2. If this driving force becomes large, i.e. if system 1 is near the fully reduced state ($p_1 \approx 1$) and system 2 near the fully oxidized state ($p_2 \approx 0$), the pump approaches a constant limiting rate. The maximum turnover rate may be obtained by inserting $p_1 \approx 1$, $p_2 \approx 0$ and $v \approx 0$ ($E_1 - E_2 << -RT/F$) into Eqns. 51—53:

$$\rho \equiv \left(\frac{\Phi}{N_p}\right)_{\substack{p_1 \approx 1 \\ p_2 \approx 0}} = \left[\frac{1}{\kappa'}\left(1 + \frac{K}{a'}\right) + \frac{1}{\kappa_0''}\left(1 + \frac{a''}{K^*}\right) + \frac{1}{a' k_a} + \frac{1}{k_d^*}\right]^{-1} \tag{56}$$

According to Eqn. 56, a high pK value in the oxidized state of the pump ($K << a'$) and a low pK value in the reduced state ($K^* >> a''$) are favourable for a high transport rate. On the other hand, the proton dissociation constants K and K^* do not enter into the expression for the protonmotive force (Eqn. 55).

If the pump operates close to equilibrium ($\Delta\tilde{\mu}_e \approx 0$, $\Delta\tilde{\mu}_H \approx 0$), the proton flux becomes a linear function of $\Delta\tilde{\mu}_e$ and $\Delta\tilde{\mu}_H$ (Eqn. 51):

$$\Phi \approx \frac{N_p \kappa' p_1}{\beta RT}(\Delta\tilde{\mu}_H + \Delta\tilde{\mu}_e) \tag{57}$$

Thus $\Delta\tilde{\mu}_H$ and $\Delta\tilde{\mu}_e$ have the same effect on the pump rate Φ. This is, of course, a consequence of the assumption that the flows of proton and of electrons are completely coupled.

Conclusion

A mechanism of active ion transport has been described in which an essential part of the pump molecule is an ion channel. Pumping action results from an energy-dependent change in the barrier structure of the channel. This model of an 'activated channel' has been explicitly applied to light-driven proton transport and to redox-coupled proton transport. Similar considerations could be

used to describe ATP-dependent ion transport. Modifications of the potential energy profile of an ion in the channel (as required by the model) may result from minor conformational changes, or even from changes in the dipolar moment of a functional group close to the pathway of the ion [43]. In the treatment of the model, a number of assumptions have been introduced (such as the strict coupling between electron and proton transport in the case of the redox pump) in order to simplify the formal analysis. These assumptions are not essential for the model and may be easily omitted, if necessary. A direct comparison of the prediction of the model with experimental data is still difficult, but should become possible as soon as detailed information on kinetic parameters from reconstituted systems become available. It is interesting to note that this mechanism is intermediate between a 'pure' carrier and a 'pure' channel mechanism. A carrier may be generally defined as a transport system in which the binding site is alternately exposed to the left and to the right external phase. This requirement is fulfilled by the proposed model in the limiting case, where, in the ground state, the barrier to the right of the main binding site is impenetrable and, in the activated state, the barrier to the left. On the other hand, in the more general case where all barriers are of finite height, the model approaches a channel mechanism sensu stricto.

Appendix

(A) Derivation of Eqns. 16—18

If N, N^*, N^{**}, N_0 and N_0^* are the numbers of pump molecules in states HP, HP*, HP**, P and P*, respectively, then the total number N_p is given by

$$N_p = N + N^* + N^{**} + N_0 + N_0^* \tag{A1}$$

According to assumption 5, the reaction between species HP* and HP** is always in equilibrium so that

$$N^{**} = BN^* \tag{A2}$$

(compare Eqn. 8). In the stationary state the time derivatives of N, N^*, etc. vanish:

$$\frac{dN}{dt} = 0 = -(r + k' + k_d)N + sN^{**} + k''N^* + k_a a' N_0 \tag{A3}$$

$$\frac{dN_0}{dt} = 0 = -(k_0' + k_a a')N_0 + k_0'' N_0^* + k_d N \tag{A4}$$

$$\frac{dN^*}{dt} = 0 = -(f + k'' + k_d^*)N^* + gN^{**} + k'N + k_a^* a'' N_0^* \tag{A5}$$

$$\frac{dN^{**}}{dt} = 0 = -(g + s)N^{**} + fN^* + rN \tag{A6}$$

Addition of Eqns. A5 and A6 and introduction of Eqn. A2 yields

$$0 = (k' + r)N - (k'' + k_d^* + sB)N^* + k_a^* a'' N_0^* \tag{A7}$$

From the five equations A1—A4 and A7 the five unknown quantities N, N^*, N^{**}, N_0 and N_0^* may be obtained.

The solution reads

$$N = N_p \frac{\beta_1}{\beta}\ ; \quad N^* = N_p \frac{\beta_2}{\beta}\ ; \quad N_0 = N_p \frac{\beta_3}{\beta} \tag{A8}$$

$$\beta_1 = 1 + \left(\frac{r_0 + k'}{S} + B\gamma J\right)\left(\frac{wS}{k_d} + \frac{a''/K^*}{k_0''} + \frac{1}{k_d^*}\right) \tag{A9}$$

$$\beta_2 = wS + (r_0 + k' + \gamma J)\left(\frac{wS}{k_d} + \frac{a''/K^*}{k_0''} + \frac{1}{k_d^*}\right) \tag{A10}$$

$$\beta_3 = \frac{K}{a'} + (r_0 + k')\left(\frac{1}{a'k_a} + \frac{1}{Sk_d^* a'/K} + \frac{w}{k_0'}\right) + \gamma J\left(\frac{1}{a'k_a} + \frac{B}{k_d^* a'/K} + \frac{wBS}{k_0'}\right) \tag{A11}$$

$$N^{**} = BN^*\ ; \quad N_0^* = N_p - N - (1 + B)N^* - N_0 \tag{A12}$$

The quantities w and β are given by Eqns. 17 and 18.

(B) Derivation of Eqns. 37 and 38

The rates r_0 and s_0 of transitions between states HP and HP** (Eqns. 5 and 6) may be written as

$$r_0 = r_0^0 + r_0^*\ ; \quad s_0 = s_0^0 + s_0^* \tag{B1}$$

r_0^0 and s_0^0 account for radiationless transitions (thermal activations and desactivations) and r_0^* and s_0^* for transitions involving the exchange of a photon with the surroundings at equilibrium ($J = 0$). According to the principle of microscopic reversibility, the relations $r_0^0\overline{N} = s_0^0\overline{N}^{**}$, $r_0^*\overline{N} = s_0^*\overline{N}^{**}$, hold at equilibrium. This gives, together with Eqn. 7:

$$\frac{r_0^0}{s_0^0} = \frac{r_0^*}{s_0^*} = \frac{r_0}{s_0} = A \tag{B2}$$

The rate j of net photon uptake may then be written as

$$j = (r_0^* + \gamma J)N - (s_0^* + \gamma J)N^{**} \tag{B3}$$

The term γJN^{**} accounts for induced emission. Using Eqns. 11, A2, A8—A10 and B1—B3 yields

$$j = \frac{N_p}{\beta}\left\{\gamma J\left[1 - Aw + (1 - A)(k'' + s_0^0 B)\left(\frac{wS}{k_d} + \frac{1}{k_d^*} + \frac{a''/K^*}{k_0''}\right)\right] + r_0^*(1 - w)\right\} \tag{B4}$$

By combining Eqns. 16 and B4 under the condition $\Delta\tilde{\mu}_{H^+} = 0$ ($w = 1$), Eqn. 37 is obtained. In a similar way Eqn. 38 is derived from Eqns. 16 and B4 under the condition $J = 0$.

(C) Derivation of Eqns. 50—53

Combining Eqns. 3, 4, 48 and 49 yields

$$\frac{HK^*}{H_0K} = \left(\frac{a''}{a'} \cdot \frac{1-p_1}{p_1} \cdot \frac{p_2}{1-p_2}\right)_{eq} \tag{C1}$$

whereas the subscript 'eq' denotes the equilibrium state. From the equilibrium condition

$$\Delta\tilde{\mu}_H + \Delta\tilde{\mu}_e = 0 \tag{C2}$$

together with Eqns. 17, 45 and 47, one obtains

$$\left(\frac{a''}{a'} \cdot \frac{1-p_1}{p_1} \cdot \frac{p_2}{1-p_2}\right)_{eq} = \exp\left[\frac{E_{20}-E_{10}}{RT/F} + (1-\alpha_{12})u_{eq}\right] . \tag{C3}$$

Introducing Eqn. C3 into Eqn. C1 yields

$$\frac{HK^*}{H_0K} = \exp\left[\frac{E_{20}-E_{10}}{RT/F} + (1-\alpha_{12})u_{eq}\right] . \tag{C4}$$

As any value of the equilibrium voltage u_{eq} may be obtained by a suitable choice of a''/a', p_1 and p_2, and as H, H_0, K and K^* do not explicitly depend on a', a'', p_1 and p_2, Eqn. C4 holds for arbitrary voltages u. This proves Eqn. 50.

For the calculation of the stationary proton flux, we use the condition that the time derivatives of N, N^* and N_0 vanish in the steady state:

$$\frac{dN}{dt} = 0 = -(\kappa' p_1 + k_d)N + \kappa''(1-p_1)N^* + k_a a' N_0 \tag{C5}$$

$$\frac{dN^*}{dt} = 0 = -[\kappa''(1-p_1) + k_d^*]N^* + \kappa' p_1 N + k_a^* a'' N_0^* \tag{C6}$$

$$\frac{dN_0}{dt} = 0 = -(\kappa_0' p_2 + k_a a')N_0 + \kappa_0''(1-p_2)N_0^* + k_d N \tag{C7}$$

These equations require that the probabilities p_1 and p_2 are constant, irrespective of the state (HP, HP*, P, P*) of the pump molecule. This assumption is reasonable as long as systems 1 and 2 are buffered by external redox pools. Eqns. C5—C7 together with

$$N_p = N + N^* + N_0 + N_0^* \tag{C8}$$

represent a system of four equations for the four unknown quantities N, N^*, N_0 and N_0^*. The solution reads:

$$N = N_p \frac{\beta_1}{\beta} ; \quad N^* = N_p \frac{\beta_2}{\beta} ; \quad N_0 = N_p \frac{\beta_3}{\beta} \tag{C9}$$

$$\beta_1 = 1 + \kappa''(1-p_1)\left[\frac{vHp_1}{k_d(1-p_1)} + \frac{a''/K^*}{\kappa_0''(1-p_2)} + \frac{1}{k_d^*}\right] \tag{C10}$$

$$\beta_2 = \frac{vHp_1}{1-p_1} + \kappa' p_1\left[\frac{vHp_1}{k_d(1-p_1)} + \frac{a''/K^*}{\kappa_0''(1-p_2)} + \frac{1}{k_d^*}\right] \tag{C11}$$

$$\beta_3 = \frac{K}{a'} + \kappa' p_1\left[\frac{1}{a'k_a} + \frac{K(1-p_1)}{a'Hp_1k_d^*} + \frac{v}{\kappa_0' p_2}\right] \tag{C12}$$

The quantities v and β are given by Eqns. 52 and 53.

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