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Redox-Driven Proton Pumps of the Respiratory Chain

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ABSTRACT In aerobic cells, the proton gradient that drives ATP synthesis is created by three different proton pumps—membrane enzymes of the respiratory electron transport chain known as complex I, III, and IV. Despite the striking dissimilarity of structures and apparent differences in molecular mechanisms of proton pumping, all three enzymes have much in common and employ the same universal physical principles of converting redox energy to proton pumping. In this study, we describe a simple mathematical model that illustrates the general principles of redox-driven proton pumps and discuss their implementation in complex I, III, and IV of the respiratory chain.

INTRODUCTION

A proton pump is a membrane enzyme, which functions as a molecular device that "catches" protons on one side of the membrane and expels them on the other. The transfer of protons requires energy because the membrane is electrically charged and protons need to overcome a potential difference; in addition, there is a proton concentration difference on both sides of the membrane so that the transfer of protons occurs against both the electric and the concentration ("electrochemical") gradient. In fact, the proton pumping itself gives rise to an electrochemical gradient on the membrane, as the expulsion of protons occurs either from or to a closed organelle such as a mitochondrion, bacterial cell, or chloroplast. According to Mitchell's basic principles of bioenergetics, it is this electrochemical proton gradient, also known as the Proton Motive Force (PMF) that drives the synthesis of ATP in the cell (1,2).

In a sense, a proton pump is a molecular version of Maxwell's demon (3,4) that operates in the membrane but does require energy for its operation. There are different types of proton pumps: some are powered by ATP; others by a concentration gradient of an ion that exchanges with the proton or vice versa, the proton gradient drives pumping of other ions (2). The pumps that create PMF for ATP synthesis are powered by the redox energy of oxygen reduction of cell respiration. In this process, electrons are transferred from an initial substrate NADH through a chain of enzymes—complexes I–IV and their intermediates quinone and cytochrome c—to oxygen, which is converted, with the addition of four electrons and four protons, to two molecules of water. Three of the four enzymes of the electron transport chain—NADH dehydrogenase, quinol:cytochrome c oxido-reductase, and cytochrome c oxidase, also known as complex I, III, and IV, respectively—are proton pumps (5). Sometimes complex III is said to be not a true pump but rather a Mitchellian proton-loop machine (because protons are not directly translocated through the enzyme but instead carried by the diffusing quinone molecules in the membrane); however, we will show that all thee follow the same basic physical principle of pumping despite their remarkably different molecular mechanisms. These enzymes, therefore, are molecular machines that convert redox energy into proton pumping force (PPF), i.e., the ability to pump protons against an electrochemical gradient. Quantitatively, PPF can be defined as a maximal PMF against which a pump can pump protons. (Both PMF and PPF are of course just a difference of chemical potential of the protons on the opposite sides of the membrane).

The structures of all proton pumps of the respiratory chain have been solved in the past two decades, complex I's complete structure being the most recent achievement of the field (6). The revealed structures suggested molecular mechanisms, and although important details are still missing, a rough picture has already emerged (5).

The purpose of this work is to introduce a mathematical model that describes the simplest and universal scheme of pumping and to discuss the general pumping principles that underlie the function of the respiratory chain; earlier

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discussions of this subject include (7–12). Within the framework of our model, we compare the molecular mechanisms of three different pumps, placing special emphasis on the question of how redox energy of electron transfer reactions is converted to proton pumping power of the enzymes. We show that all three enzymes employ the same universal physical principle of generation of PPF, although their molecular mechanisms are strikingly different.

METHODS

Pump equation

The schematic of the model is shown in Fig. 1. The protons on the opposite sides of the membrane are described by two proton reservoirs, R1 and R2, with concentrations p_1 and p_2 ($p_2 > p_1$), and energies, including their electric potentials, μ_{01} and μ_{02}. For convenience, it will be assumed that both reservoirs have unit volumes so that concentrations p_i are proportional to total number of protons in the reservoirs. The electrochemical potential of the protons can then be written as

$$\mu_i = \mu_{0i} + kT \ln p_i, \quad \Delta\mu_{0i} = e\Delta\varphi, \tag{1}$$

where $\Delta\varphi$ is the membrane electric potential.

The two reservoirs are connected by the enzyme, E, which is represented by a single site called the proton loading site (PLS) located in the enzyme. (The terminology of the PLS is from the original discussion of pumping in cytochrome c oxidase, complex IV; see 13,14). There is an access of protons from both sides of the membrane to this site. The rate constants of on- and off- reactions are $k_{i+} = k_i p_i$ and k_{i-}. The occupancy of the PLS is p_0. A single occupancy (one proton maximum) of the PLS will be assumed. The kinetics of the model is illustrated in Fig. 2.

If the enzyme is in a resting state, i.e., does not do anything, the parameters of the model—rate constants, the energy of the PLS, and its population—are fixed to certain values and do not change. Obviously, no matter what the parameters of the model are, the stationary flux of protons will be directed along the electrochemical gradient from high-potential R2 to low-potential R1.

We now ask the question of whether it is possible to change the parameters of the model—periodically, to reflect the cycles of the enzyme turnovers—in such a way so as to reverse the direction of the net flux. In other words, we ask what the enzyme "should do" to generate PPF.

The equations that describe the kinetics of the system are as follows:

$$\begin{aligned} \dot{p}_1 &= -p_1(1-p_0)k_1 + p_0 k_{-1}, \\ \dot{p}_0 &= +p_1(1-p_0)k_1 + p_2(1-p_0)k_2 - p_0(k_{-1}+k_{-2}), \text{ and} \\ \dot{p}_2 &= -p_2(1-p_0)k_2 + p_0 k_{-2}. \end{aligned} \tag{2}$$

The flux of protons between R1 and R2, J_{12}, is defined as

$$J_{12} = (\dot{p}_2 - \dot{p}_1)/2. \tag{3}$$

FIGURE 1 Schematics of the model. Two reservoirs (R1 and R2) are exchanging particles (protons) through enzyme E.

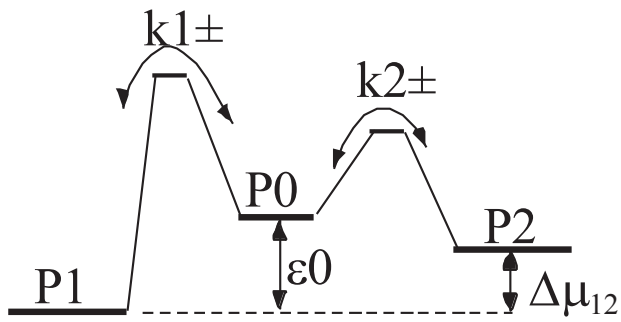


FIGURE 2 Schematics of the kinetic model. Two reservoirs (R1 and R2) are described by concentrations/populations P1 and P2 and have different chemical potentials: R1 is a low-potential and R2 is a high-potential reservoir. The enzyme is described by a single proton loading site (PLS) with population P0. The energy of PLS (which defines its pKa) is ε_0. The rates k_{12} describe the on- ($\mathbf{k}_+$) and off- ($\mathbf{k}_-$) proton transfers to/from the PLS from R1 and R2.

We introduce the following notation

$$\begin{aligned} \kappa_\pm &= \frac{k_1 p_1}{\overline{p}_{01}} \pm \frac{k_2 p_2}{\overline{p}_{02}} \\ \overline{p}_{0i} &= \frac{p_i}{p_i + K_i}, \quad K_i = k_{-i}/k_i \end{aligned} \tag{4}$$

and define the stationary adiabatic PLS population and the flux (i.e., those stationary values that would be in case of time-independent parameters of the system):

$$J_{12} = \overline{J}_{12} + \delta J_{12}. \tag{5}$$

The stationary flux—assumed to be negative (backflow), from the high-potential reservoir R2 to the low-potential R1—is

$$\overline{J}_{12} = \frac{k_1 p_1 k_2 p_2}{\kappa_+}\left(e^{\frac{-\mu_2}{kT}} - e^{\frac{-\mu_1}{kT}}\right)e^{\frac{\varepsilon_0}{kT}} \tag{6}$$

(for $\mu_2 > \mu_1$, $\overline{J}_{12} < 0$). Whereas for additional nonstationary flux δJ_{12}, we have

$$\delta J_{12} = \frac{1}{2}\frac{\kappa_-}{\kappa_+}\dot{p}_0. \tag{7}$$

It can be either positive or negative (because both κ_- and $\dot{p}_0$ can have either sign). This contribution to the flux will be called the pumped flux.

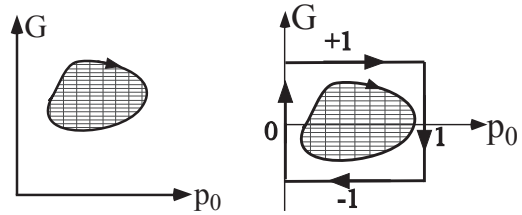
The total nonstationary pumped charge crossing the membrane over a time interval T (e.g., during one cycle of the reaction) is

$$\delta Q_{12} = \int_0^T \delta J_{12} dt = \frac{1}{2}\int_0^T \frac{\kappa_-}{\kappa_+}\dot{p}_0 dt. \tag{8}$$

If the PLS population p_0 is treated as an independent variable and a periodic cycling of the enzyme is assumed, for one cycle we have

$$\delta Q_{12} = \frac{1}{2}\oint G dp_0, \tag{9}$$

where we introduce the gate function G,



(3A) (3B)

FIGURE 3 The pumping cycle is described by a curve in the plane G vs. p_0, where G is the gate value and p_0 is the PLS population, left (*A*). The number of particles pumped in one cycle is the area of the enclosed curve (*filled*). By definition, $|G| < 1$ and $0 < p_0 < 1$. The maximal pumping efficiency is achieved for the cycle of maximal area shown on the right (*B*). Here, for comparison, we also show the cycle of (*A*) to emphasize that an arbitrary cycle (*A*) has lower than the maximal enclosed area and hence lower efficiency.

$$G = \frac{\kappa_-}{\kappa_+} = \frac{\frac{k_1 p_1}{\overline{p}_{01}} - \frac{k_2 p_2}{\overline{p}_{02}}}{\frac{k_1 p_1}{\overline{p}_{01}} + \frac{k_2 p_2}{\overline{p}_{02}}}, \tag{10}$$

which varies in the range from -1 to $+1$. When the gate is open on the R1 side and closed on R2, then $G = +1$ (for $dp_0 > 0$, this corresponds to loading PLS from R1 only); in the opposite case, of a closed gate on the R1 side/open on R2, $G = -1$ (for $dp_0 < 0$, this corresponds to unloading PLS to R2 only). The factor of 1/2 in Eq. 9 is simply due to the fact that getting a full proton from R1 to the PLS is equivalent only to a "half"-transferred proton; the subsequent expulsion of the proton from the PLS to R2 would complete the full transfer. The obtained Eqs. 9 and 10 will be referred to as the "pump equation."

The final expression Eq. 9 has a clear physical meaning. Namely, the net pumped charge over a small time interval dt is a product of variation of PLS population $dp_0(t)$ times the value of the gate $G(t)$, which regulates to/from which side of the membrane population dp_0 is transferred. The product Gdp_0 is similar (this similarity is formal but useful nevertheless conceptually) to a familiar expression for a mechanical work, Fdx, or PdV, where dp_0 is equivalent to the displacement, or volume change, and the gate function G is equivalent (formally) to force, or pressure. Similar to the mechanical work, if a periodic process is involved, the total work or transferred charge is given by a cyclic integral (Eq. 9). This formal analogy goes further: although population p_0 is a unique function of time-dependent parameters of the system and hence of the gate G, the reverse is not true, i.e., population $p_0(t)$ does not uniquely define the parameters of the gate; we have the same relation for coordinate $x(t)$ and the force acting on the body $F(t)$ (or V vs. P). This is, in fact, the formal reason why the cyclic integral Eq. 9 is nonzero. Other examples of physical systems involving a cyclic integral similar to Eq. 9 are discussed in the Appendix.

From the above discussion, it is clear that if there is a periodic variation of the PLS population in such a way that when $dp_0(t) > 0$, $G(t) > 0$, and when $dp_0(t) < 0$, $G(t) < 0$, there will be nonzero nonstationary flux from the low-potential reservoir R1 to the high-potential reservoir R2. Our goal is now to see how to make this nonstationary flux the maximum possible to successfully compete with a stationary flux in the opposite direction, i.e., the leak of protons through the pump.

The integral defining the number of pumped particles per cycle (Eq. 9) is convenient to describe graphically in the plane of variable G and p_0. The number of pumped particles is the area enclosed by a closed curve in the (p_0,G) plane describing the cycle, as shown in Fig. 3 *A*. Obviously, the maximal pumping efficiency is achieved for the cycle of maximal area shown in Fig. 3 *B*. The maximal efficiency, achieved for a special cycle, brings up the analogy with the Carnot engine (15). In this case, maximal efficiency is known to be achieved for a special (Carnot) cycle consisting of two isotherms and two adiabats in the (P,V) plane. But in the plane of entropy S and temperature T, (S,T), the Carnot cycle is exactly equivalent to what we described for the pump and have shown in Fig. 3 *B*. The analogy with the Carnot engine goes further and is discussed in more detail later in the study.

For the pump, the maximal efficiency cycle (Fig. 3 *B*, *solid square curve* with *arrows*) is such that the PLS is first loaded from R1 (i.e., p_0 is changing from 0 to 1 while $G = +1$) and then unloaded to R2 (i.e., p_0 is changing from 1 to 0 while $G = -1$). Such a process is shown schematically in Fig. 4. Upon loading (Fig. 4, *left*), the energy of PLS ε_0 is made lower than the chemical potential of R1, or equivalently, pKa of the PLS is higher than the pH of R1, whereas a high barrier blocks access from R2. In the unloading stage (Fig. 4, *right*), the access from R1 is now blocked by a high barrier, whereas the access to R2 is open; at the same time, the energy of the loaded PLS is made higher than the potential of R2, or equivalently, pKa of the PLS is now lower than the pH of R2, providing unloading of the PLS proton to the R2 side. In such a cycle, obviously, a proton is pumped from the low-potential reservoir R1 to high-potential R2.

The efficiency of the cycle (i.e., the number of protons per cycle pumped), as it follows from the above discussion, depends on the tight correlation/coupling between the gate opening/closing and the kinetics of the PLS protonation/deprotonation. However, the overall efficiency of the pump depends also on the control of the backflow (leakage) of protons through the pump. This will be discussed in the next subsection. To operate the pump, one needs an input of energy, and there is also a question of the efficiency of energy usage for the pumping; this will be still further discussed in the following (Adiabatic Pumping: Relation to Thermodynamics and in Relation to Carnot Heat Engine on the connection to the Carnot machine).

Net flux

The net flux is the sum of the stationary backflow in the direction from high-potential R2 to low-potential R1 and the nonstationary pumped flux from R1 to R2 (Eq. 5). The backflow, in principle, can be made arbitrary small (by always keeping at least one of the barriers infinitely high), and thus the net flux can be made to flow from the low-potential to the high-potential reservoir.

To maintain the net flux positive (from R1 to R2), however, the changes in the system cannot be arbitrary slow because during one cycle, a maximum of one particle is transferred from R1 to R2, whereas the number of transferred particles from the stationary backflow flux is proportional to the cycle duration time. Hence, the changes should be fast enough so that the negative stationary flux would not exceed the pumped one. On the other hand, for efficient pumping, i.e., to pump the maximal possible number of particles per cycle (in our case, one), the changes cannot be too fast, as it follows from the previous discussion. The quantitative estimates can be made as follows.

Consider the diffusion-limiting (maximal) rate of particles supply $k_{on} = \kappa^{BM}[H^+]$, where κ^{BM} is a bimolecular rate constant (for protons, on the order of 10^{11} M^{-1} s^{-1}) and $[H^+]$ concentration. (This rate is represented by $k_{i+} = k_i p_i$ terms in the kinetic equations.) The corresponding timescale of protonation is $\tau_0 = 1/k_{on}$.

For efficient pumping, the cycle should be obviously slower than the time needed for one protonation of PLS to occur, i.e., τ_0. On the other hand, the stationary backflow flux is

$$\overline{J}_{12} \sim k_{on} e^{-\frac{V_{\max}}{k_B T}}, \tag{11}$$

where $V_{\max}$ is the maximal barrier that blocks the access to PLS from the "wrong" side (i.e., from R2 upon loading and to R1 upon unloading).

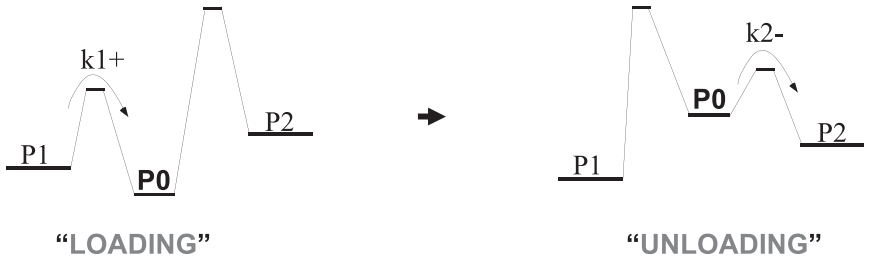


FIGURE 4 Schematics of the maximal efficiency pumping cycle. Upon loading (*left*), pKa of the PLS is higher than the pH of R1, while a high barrier blocks access from R2. In the unloading stage (*right*), the access from R1 is blocked by a high barrier, and pKa of the PLS is lower than the pH of R2.

Then the exact formal condition for the cycle duration T_{cyc} can be written as follows:

$$\tau_0 < T_{cyc} < \tau_0 e^{\frac{V_{\max}}{k_B T}}. \tag{12}$$

Adiabatic pumping: Relation to thermodynamics

The efficiency of energy usage to pump protons against the electrochemical gradient also depends on the timescale of the cycle; as in general thermodynamics, the maximal efficiency is achieved in an adiabatic process, which is discussed next.

The gate G is an explicit function of various rate constants of the system Eq. 10, which themselves depend on several kinetic parameters of the system such as barrier heights separating the PLS from the two sides of the membrane (V_1 and V_2), energy of the PLS (ε_0), and others. If one or more of such parameters varies in time, so does the gate function $G(t)$. This dependence on time is parametric in the sense that G at time t is defined by the values of the parameters of the system at that moment of time.

In contrast to $G(t)$, the PLS population $p_0(t)$, the second variable in the cyclic integral Eq. 9, is not a function, but a *functional* of time-dependent parameters of the system. Unlike $G(t)$, the external parameters at a given time t define the rate of change of $p_0(t)$ (Eq. 2), not $p_0(t)$ itself; naturally, then, $p_0(t)$ depends not only on those parameters at time t but also on the previous moments of time—the system has memory. This is typical for a dynamic variable that is driven by an external source. Explicitly, the expression for $p_0(t)$ in the integral form is given in the Appendix.

In general, therefore, in the contour integral Eq. 9, G is not a function of p_0 but rather a specific value of $G(t)$, whereas the PLS population has its own value $p_0(t)$ (and an increment $dp_0(t)$) so that the integral is the sum of $G(t_i)dp_0(t_i)$ over instances of time t_i values. Thus, in general, there is no correlation between $G(t)$ and $p_0(t)$.

However, if parameters change slowly (the exact condition can be deduced from the equation given in the Appendix), the population of the PLS becomes a function of time-dependent parameters:

$$\overline{p}_0(t) = \frac{k_1(t)p_1 + k_2(t)p_2}{\kappa_+(t)}. \tag{13}$$

Similar to $G(t)$, $\overline{p}_0$ now depends explicitly on a number of kinetic parameters—such as barrier heights, pKa value of the PLS, etc.—that change in time, and so does $\overline{p}_0(t)$. This is an "adiabatic" approximation of $p_0(t)$. Now both G and p_0 are functions of the same kinetic parameters, and therefore, they are uniquely related to each other at a given instance of time. Both G and p_0 are now "state functions" and do not depend on the manner of how state parameters change in time, i.e., $G = G(\lambda_1, \lambda_2, \ldots)$, $p_0 = p_0(\lambda_1, \lambda_2, \ldots)$, and their time dependence is parametric: $G(t) = G(\lambda_1(t), \lambda_2(t), \ldots)$, $p_0(t) = p_0(\lambda_1(t), \lambda_2(t), \ldots)$.

This is similar to "reversible" and "irreversible" or quasi-equilibrium changes in thermodynamics, which are key for the efficiency of energy usage. For reversibility, the change in the system parameters should be made slowly so that the entropy of the system would always be close to its equilibrium/maximal value for a given state of parameters of the system. The rate of change obviously should be slower than the relaxation rate with which the system finds its new state of equilibrium for changed conditions.

In fact, our system depends most strongly only on two parameters: the energy ε_0 of the PLS (or its equivalent pKa), and the difference in the heights of two barriers separating the PLS from the two reservoirs (Fig. 2), defined as $V = V_1 - V_2$. The explicit expressions for $G(\varepsilon,V)$ and $p(\varepsilon,V)$ (for short, here we write $\varepsilon \equiv \varepsilon_0$ and $p \equiv p_0$) are given in the Appendix. Given these explicit functions, the charge transferred in one adiabatic cycle can be expressed as follows:

$$\begin{aligned}\delta Q_{12} &= \frac{1}{2}\oint G dp = \frac{1}{2}\iint dG dp \\ &= \frac{1}{2}\iint \left(\frac{\partial G}{\partial V}\frac{\partial p}{\partial \varepsilon} - \frac{\partial G}{\partial \varepsilon}\frac{\partial p}{\partial V}\right) d\varepsilon dV.\end{aligned} \tag{14}$$

The total work needed per cycle is

$$W = \oint p d\varepsilon = \iint dp d\varepsilon = \iint \left(\frac{\partial p}{\partial V}\right)_{\varepsilon} d\varepsilon dV, \tag{15}$$

where G and p are treated as functions of "state variables" ε and V.

In an adiabatic cycle, the work of pumping is the minimum possible, and its expression is given above. If pumping is not adiabatic (i.e., not reversible), the work is greater. This is similar to ordinary thermodynamic gas expansion/contraction: minimal work to achieve a given volume change is done in an adiabatic process (in which entropy is not changed; otherwise, additional work is lost to increased entropy, i.e., heat). Here, too, the minimal work for a maximal number of protons per cycle is achieved in the adiabatic process shown in Fig. 3 *B*; this work is

$$W_{\min} = \oint p d\varepsilon = (p = 1) \times \Delta\varepsilon = kT \ln 10 \Delta pKa. \tag{16}$$

On the other hand, the minimal work is just a difference in chemical potentials of the reservoirs, $\Delta\mu_{12} = (\mu_2 - \mu_1)$. Hence, we naturally find that in general, the change of pKa of the PLS must be greater than $\Delta\mu_{12}$, as expected, and the work spent is that needed to change pKa of the PLS during the cycle. Additional thermodynamic energy and work relations are given in the Appendix.

Relation to Carnot heat engine

First of all, it is noticed that the Carnot heat engine can be thought of as an entropy pump. It takes entropy from a heated body and releases it to a cold body; the difference in heat taken and returned is the work done by the Carnot engine. In the reverse process, it pumps heat and entropy from a cold body to a hot body, in which case the external work is needed. In an ideal machine, the pumped entropy in the cycle is conserved and is analogous to the pumped protons. This analogy can be extended further. The temperatures of two heat reservoirs T_1 and T_2 in the Carnot machine play the role

of the chemical potentials μ_1 and μ_2 in the proton pump model. This analogy is already seen in the expression

$$TdS = dE + PdV - \mu dN, \tag{17}$$

namely, for energy E at constant V, temperature T plays the same role (conjugate) with respect to S as μ with respect to N. The work to pump entropy from a cold body to a heated body is

$$W = -\oint PdV. \tag{18}$$

Changing variables from (P,V) to (S,T) in the above expression (see Appendix), the work is

$$W = -\oint TdS = (T_2 - T_1)\Delta S, \tag{19}$$

where the second expression gives the minimal work required to pump ΔS from low temperature T_1 to high temperature T_2. This is analogous to our expression for the work of the pump:

$$W = -\oint \varepsilon dp = (\mu_2 - \mu_1)\Delta p \tag{20}$$

(see Eq. A34 in the Appendix), where Δp is the average number of protons pumped per cycle. In our case, $\Delta p \leq 1$, but this limitation is not important; one can imagine that the pump has several PLSs and pumps several protons in the cycle—e.g., as in complex I, for which Δp is 3 or 4, or anything less than that depending on the efficiency of pumping.

In variables (P,V), the maximal work of the Carnot engine and corresponding minimal work to run the Carnot engine in reverse is achieved for a cycle (Eq. 18) consisting of two isotherms and two adiabats. In variables (S,T), the maximal efficiency cycle of the Carnot machine given by Eq. 19 is represented now by a rectangle, with two sides representing the change of entropy at constant temperature and two sides corresponding to changing temperature at constant entropy. This is exactly analogous to a cycle shown in Fig. 3 *B*. This analogy is further elaborated below.

The cycle for the working body of the Carnot engine (equivalent to the PLS) consists of the following: 1) making a contact with the low-temperature reservoir R1, i.e., opening the gate G to R1, and isothermal expansion at low temperature T_1, i.e., taking entropy (and heat) from a cold body (equivalent to loading); 2) closing the gate G to R1 and adiabatic compression to high temperature T_2 (this is equivalent to raising the energy of the PLS, ε_0, from a low value μ_1 to a high value μ_2, or changing pKa of the PLS from a high-value pH_1 to a low-value pH_2); 3) making contact with R2, i.e., opening the gate G to R2, and isothermal compression at high temperature T_2, releasing entropy ΔS and heat to high-temperature reservoir R2; and finally, 4) closing the gate G to R2 and adiabatic expansion in which temperature is lowered from high T_2 to a low-value T_1.

This follows the cycle of the proton pump almost exactly; the only difference is that we assume no work is done upon transfer of particles from/to the PLS. Instead, in proton pumps, there is heat exchange upon binding and releasing protons, as described in the Appendix. This heat is significant, on the order of 0.5 V or some 12 kcal/mol. Upon binding, this heat is released (due to decrease of entropy of a proton) locally to solvent water molecules, i.e., to the environment. In the reverse process of proton dissociation, the same energy is needed to break the bond between the proton and the PLS; this energy obviously comes from the environment.

In the pump, the work is done only when we change the energy of the PLS as given by 20; in the Carnot engine, the work is given by Eq. Eq. 19. The analogy between T and μ in Eqs. 19 and 20 is evident. The efficiency of the Carnot engine generating work by transferring heat from the high-temperature (T_2) reservoir to the lower one (T_1) is defined as a fraction of total energy (transferred) converted to work,

$$\eta_C = 1 - \frac{T_1}{T_2}. \tag{21}$$

The same can be done for the pump when it works in reverse (i.e., generating work by the natural flux of protons); the efficiency of conversion to work of the high potential protons μ_2 (low pH, assuming for simplicity no membrane potential ($\Delta\varphi = 0$)) to low potential protons μ_1 (high pH) is

$$\eta_P = 1 - \frac{\mu_1}{\mu_2} = 1 - \frac{pH_2}{pH_1}. \tag{22}$$

The formal efficiency of an ideal pump defined in this way, however, is only of limited practical use, as chemical potential can be negative or can take arbitrary high or low values depending on the concentration of ions. Also, unlike temperature, chemical potential difference $\Delta\mu$ is directly related to work w produced (for an ideal machine) or needs to be produced to go from a low potential to a high potential. The potential difference in the pump $\mu_2 - \mu_1 = w$ is in fact physically equivalent to $w = Q_2 - Q_1$ of the Carnot machine, and Eq. 22 is equivalent to $\eta_C = 1 - Q_2/Q_1$. Formal efficiency is further discussed in the Appendix (Thermodynamic Energy and Work by the Pump).

More practically important, when discussing the pumps, is how far or close they are to ideal pumps. In fact, it is this aspect that is usually implied when "efficiency" of the pumps is discussed. An ideal pump (100%) can convert all the work/energy input (redox energy in case of redox-driven proton pumps) to proton-motive force, i.e., into $\Delta\mu$ of the pumped protons. Real systems are surprisingly close to this ideal, being in the range of 60–80% in the best-known cases. Such efficiency achieved in molecular realization of the enzymes is truly remarkable. Three major proton pumping enzymes of the respiratory chain are discussed next.

RESULTS AND DISCUSSION

As evident from their structure, the three proton pumps of the respiratory chain—complexes I, III, and IV—operate by drastically different molecular mechanisms, yet they all appear to follow the same fundamental principles described by our model. Namely, the mechanism, whatever its molecular implementation, involves a PLS of variable pKa value and two gates that control the access to the PLS from each sides of the membrane. The pump works as a two-stroke device, involving a "loading" stage, in which pKa of the PLS is high (higher than the pH of the N-side of the membrane) and the input gate is open from the N-side of the membrane while the output gate from the P-side is closed; and the unloading or "firing" stage, in which pKa of the PLS is lowered (below the pH of the P-side of the membrane), the input gate shuts the access to the N-side of the membrane, and the output gate opens access to the P-side. In the loading stroke, a proton from the N-side of the membrane is transferred to the PLS, whereas in the firing stroke, the same proton is expelled from the PLS to the P-side of the membrane, Fig. 4. Several such pump channels can operate synchronously, as in complex I.

The principles of the pumping scheme, involving a PLS of variable energy and the alternating access of the PLS from both sides of the membrane, of course are not new and were recognized as such long ago. What the mathematical treatment presented here adds is a precise formulation that allows a formal analysis of various aspect of the pumping, such as adiabatic limit, efficiency, relation to thermodynamics and Carnot engine, etc. Although the model is too

general to be applied to specific enzymes, it is of interest to discuss how these general principles are realized (i.e., their molecular implementation) in enzymes of such different structures and natures.

Complex IV

These principles are realized most clearly in cytochrome c oxidase (complex IV); in fact, the model was developed with complex IV in mind, as can be seen in our earlier modeling (13,14). The role of the PLS here is played by a group of protonatable sites that include two propionates of heme a3 (5,16–18) and/or a His ligand to CuB (13) and/or nearby water molecules (19); the exact identity of the PLS is still difficult to pinpoint on the basis of current experimental data (20). The two-stroke pumping cycle is driven by the reduction and the following protonation of Fe-Cu binuclear center, BNC. Upon reduction, pKa of the PLS increases, and it gets protonated by a proton from the N-side of the membrane; upon the following BNC protonation, pKa of the PLS drops down, and the pump proton is expelled from the PLS to the P-side of the membrane. The access to the PLS is controlled by two gates. The input gate has been suggested to be played by a conformational motion of Glu286 (21), perhaps in conjunction with water chains that connect Glu286 alternatively either to the PLS or to the BNC (22–24) (cf. (25); other possibilities were also discussed in the literature (24,26,27)).The output gate was suggested to be operated by the correlated motion of protons in the exit channel that connects the PLS (via Prop A of heme a3) to the P-side of the membrane (14). The energy profile of Fig. 4 was already envisioned in the early studies (see Fig. 7 of (14)) as the most obvious mechanism of pumping.

Another possibility to prevent the backflow is based on a remarkable fact that the PLS group is located "above" the BNC along a one-dimensional proton transfer pathway that connects the N- and P-side of the membrane. This arrangement provides another possible mechanism by which the chemical proton that arrives to the BNC, after the pumped proton has been loaded to the PLS, naturally pushes the pump proton along the path in the direction of P-side and naturally blocks the transfer in the opposite direction. This is a purely Coulomb-based mechanism that ensures kinetic gating (13,28).

The model discussed here shows that a minimum of two independent parameters should control the pump: the energy of the PLS (or its pKa) and the difference in the barrier heights of the input and the output gates. In other words, only one gate can be operated, but another can remain the same, provided it is high enough to slow down the stationary backflow of protons.

This appears to be exactly the arrangement in complex IV, in which the input gate undergoes periodic changes while the output gate remains the same, providing only slow back transfer of protons that needs to be compensated by the faster rate of pumping (14) (Net Flux).

Complex III

In complex III, or bc1 complex (29), the mechanism is usually referred to as Mitchellian loop (5) rather than proper proton pumping, yet the described mechanism can be recognized here as well. The enzyme (which is a dimer (30,31), but we discuss only one monomer) contains two binding sites, Qo and Qi, for electron and proton carrier molecule ubiquinone, Q. The ubiquinone molecule plays the role of the "PLS" here. The unique aspect of the system is that the proton loading site—the Q molecule—is physically moving between two sites, Qi and Qo, where loading and unloading occur, respectively.

Each site has exclusive proton access to only one side of the membrane: the Qo site is connected to the outer (P-) side, and Qi is connected to the inner (N-) side of the membrane. There is no proton connectivity between the sites. The redox process is organized in the form of a Q-cycle (32,33). Upon reduction at the Qi site, ubiquinone molecule Q receives two protons from the N-side of the membrane, which obviously corresponds to loading of the PLS.

Because protonation at Qi occurs exclusively from the N-side, it corresponds to a low proton transfer barrier on the N-side and a high barrier on the P-side of the membrane (because there is no proton connectivity between the sites), as shown in Fig. 4 (*left*). Upon reduction and proton loading of Q, subsequent unbinding of QH2, and the following migration across the membrane and the following binding to Qo site, the protonated PLS (in the form of QH2) gets access to the P-side of the membrane, which corresponds to a configuration shown in Fig. 4 (*right*), with a low barrier for proton transfer on the P-side and a high barrier on the N-side.

The change of pKa of the PLS, in the form of a QH2/Q couple, occurs as QH2 physically moves between the Qi and Qo sites, where QH2/Q has different redox potentials and pKa values. At the Qo site, QH2 is oxidized in a bifurcated ET reaction in which one electron is taken up by a high-potential Rieske FeS cluster and the second by the low-potential heme H*bL*; the protons are then released on the P-side, which is clearly recognized as the "unloading" or firing stage of the pump cycle. At the Qi site, ubiquinone Q is reduced by the electrons of the low potential chain, and receives protons from the N-side, which is recognized as a PLS loading stage, as already mentioned.

To pump protons in this scheme, the only additional requirement that needs to be satisfied is that the redox potentials of Q/QH and QH/QH2 couple at the Qi site have to be higher, by the magnitude of the membrane potential $\Delta\mu$, than the redox potential of QH/Q couple at the Qo site. Otherwise, the analogy between the fundamental pumping

scheme discussed in the study and redox-driven proton translocation in bc1 complex is obvious.

It is of interest to mention one additional aspect of bc1 that might be also related to pumping—in this case, electron pumping. It concerns the passage of electrons coming from QH2 at the Qo site along the so-called high-potential chain. The key fact here is that the first electron of QH2 oxidation is of very high potential (high affinity of an electron to semiquinone QH)—perhaps in excess of some 0.5 V (data collected in (34), whereas the final acceptor in the chain—cytochrome c, which shuttles electrons between bc1 and cytochrome c oxidase—has a potential only in the range of 0.25 V. Thus, the transfer is 0.25 V uphill in energy. This obviously requires a special arrangement (electron pumping), as the energy of a quarter of a volt is needed to push the electron against the energy gradient along the chain. The energy can only come from the second electron, which in contrast has very low potential, some −0.3 V, so that the total average potential of QH2 is around typical +0.1 V, i.e., sufficient to transfer two electrons. Exactly how the exchange of energy between the two electrons occurs is still not clear (35,36). One theoretical possibility is suggested by our model.

Namely, to get the first electron from QH2, the Rieske FeS cluster, which plays the role of the electron loading site of the pump here, in the proximal position to Qo, should have a potential higher than that of QH2, i.e., in excess of 0.5 V. Upon reduction, the Rieske group containing the FeS cluster (37) unbinds from the proximal docking site and rebinds to a distal site, from which it passes the electron to cytochrome c1, which has a potential of the same order as that of cytochrome c, i.e., around 0.25 V (34). After oxidation, the Rieske cluster returns to the proximal position near the Qo site. If so, the redox potential of the FeS cluster at the distal site should have redox potential lower than that at the proximal site by ∼0.25 V. Hence, the supply of energy is needed to lower the potential at the distal site in exact analogy to lifting the PLS energy level (or shifting its pKa) to a higher energy (shown in Fig. 4). This energy can come from the second electron; quite likely the energy transfer then occurs via a conformational change generated by the transfer of the second electron to the low potential chain, which is transmitted (somehow) to the Rieske cluster (31). The mechanism of this electron pumping then clearly follows the principles of our pumping model.

The electron pumping along the high-potential chain in bc1 complex is still debatable; what we described is only one possibility. The other two possibilities for the mechanism at the Qo site are discussed in (35,36).

Complex I

It seems that the described two-stroke proton pumping mechanism is most directly realized in complex I. The mechanism is suggestive by the solved structure of the entire enzyme (6,38,39) and in particular by its membrane part (40), in which several proton pumping conduits (three or four) are well recognized (41). Each pumping unit consists of two half-channels that connect a protonatable group(s) PLS in the middle of the membrane to its N- and P-sides. The exact identity of the PLS is still unknown, but the only candidates are the Lys and Glu residues located in the middle of the mne, which stand out as the most obvious candidates. The change of pKa of the groups is likely regulated by the conformational change (41) generated by either the alternating redox state of the terminal FeS cluster N2 and/or by the reduction reaction of a quinone acceptor (39). Evidently, the redox reaction at the N2 center, not resolved in detail yet, drives the cycling of the conformation of the enzyme between two states, in which the two half channels in the membrane part open and close, alternatively providing alternating access to the PLS from the opposite sides of the membrane; this occurs in concert with the corresponding changes of pKa of the PLS groups, exactly as prescribed by the model discussed. There is little doubt that the fundamental principles of the pumping mechanism in complex I are the same as those of other enzymes of the respiratory chain and the same as in the model presented here; however, the molecular implementation of these pumping principles is strikingly different from two other enzymes. The variability of molecular implementation of the same principles in different enzymes is absolutely remarkable.

In conclusion, all three redox-driven proton pumps of the respiratory chain follow the same fundamental pumping principles for which a mathematical description was outlined in this work. Whenever a transfer of a particle from a low chemical potential to a high potential is needed, it takes energy and a special arrangement, pumping, by which the energy is spent to overcome the chemical potential difference. The model we described provides the simplest and universal scheme for such a process. However, most remarkable is the molecular realization of these general principles found in specific enzymes; in particular, the variability of implementation of the same idea in different respiratory complexes of the electron transport chain is simply astonishing.

APPENDIX

Adiabatic pumping: relation to thermodynamics

The gate G is an explicit function of various rate constants of the system Eq. 10, which themselves depend on several kinetic parameters of the system such as barrier heights separating the PLS from the two sides of the membrane (V_1 and V_2), energy of the PLS (ε_0), and others. If one or more of these parameters varies in time, so does the gate function G. This dependence on time is parametric in the sense that G at time t is defined by the values of kinetic parameters of the system at that moment of time t.

In general, in contrast to $G(t)$, PLS population $p_0(t)$ is not a function but a functional of time-dependent parameters of the system. Explicitly, one can find from Eq. 2:

$$p_0(t) = p_0(0)\exp\left(-\int_0^t \kappa_+(t')dt'\right) + \int_0^t k_+(t-t')\exp \times \left(-\int_{t'}^t \kappa_+(t'')dt''\right)dt', \tag{A1}$$

where κ_+ and k_+ are given by the following:

$$\kappa_+ = \frac{k_1 p_1}{\overline{p}_{01}} + \frac{k_2 p_2}{\overline{p}_{02}}$$
$$\overline{p}_{0i} = \frac{p_i}{p_i + K_i},\ K_i = k_{-i}/k_i \tag{A2}$$

and

$$k_+ = k_1 p_1 + k_2 p_2. \tag{A3}$$

If the parameters of the systems change sufficiently slowly (adiabatic pumping), Eq. A1 simplifies to the following:

$$p_0(t) \approx \frac{k_+(t)}{\kappa_+(t)} = \frac{k_1(t)p_1 + k_2(t)p_2}{\kappa_+(t)}. \tag{A4}$$

That is, the PLS population is now a simple function of time-dependent parameters (not a functional) of the systems, as was stated in the main text (Eq. 13). The parameters themselves depend on time, and so does the PLS population. Both $G(t)$ and $p_0(t)$ are now functions of the same set of (time-dependent) parameters of the system. This is the key for adiabatic approximation, which is equivalent to quasi-equilibrium processes in thermodynamics; see additional comments in the main text.

Our system depends most strongly only on two parameters: the energy ε_0 of the PLS (or its equivalent pKa), and the difference in the heights of two barriers separating the PLS from the two reservoirs (Fig. 2), defined as $V = V_1 - V_2$. The explicit dependence of $G(\varepsilon,V)$ and $p(\varepsilon,V)$ (for short, here we write $\varepsilon \equiv \varepsilon_0$ and $p \equiv p_0$) is as follows:

$$G = \frac{1-\eta}{1+\eta}, \tag{A5}$$

$$\eta = e^{-V/kT}\frac{p_2 + e^{\varepsilon_0/kT}}{p_1 + e^{\varepsilon_0/kT}}, \tag{A6}$$

and

$$p_0 = \frac{1}{1+\theta}, \tag{A7}$$

$$\theta = e^{\varepsilon_0/kT}\frac{1 + e^{-V/kT}}{p_1 + p_2 e^{-V/kT}}. \tag{A8}$$

The last two equations, with time-dependent $\varepsilon_0(t)$ and $V(t)$, are equivalent to Eq. 13 of the main text.

Heat transfer in the pump process

The chemical potentials of protons are

$$\mu_i = \varepsilon_i - Ts_i. \tag{A9}$$

The work to pump a single proton is

$$W = \Delta E + \Delta Q = \Delta\varepsilon - T\Delta s. \tag{A10}$$

Heat released to the environment is

$$\Delta Q = T(s_1 - s_2) = k_B T \ln 10 \Delta pH. \tag{A11}$$

In mitochondria, ΔpH is only around one pH unit and ΔQ is rather small. More interesting is heat transfer upon loading and unloading; in this case, the protonation brings a free proton to a bound state on the PLS (and releases a proton in deprotonation). We assume that PLS entropy is zero (this simply means that the entropy of a bound proton is much less than that of a free solvated proton), which should be compared with that of a free proton. For chemical potentials of the initial and final states, we have

$$\varepsilon_0 = \varepsilon_i - Ts_i. \tag{A12}$$

Hence, the heat released (upon protonation) is

$$\Delta Q_i = k_B T \Delta s_i = k_B T \ln\frac{[H_2 0]}{[H^+]} \approx 60mV(pH_i + 1.7) \approx 0.5V. \tag{A13}$$

This is quite a significant heat amount. It means that upon protonation, this energy is released locally. A similar conclusion is reached when recalling that pKa of a protonated water (hydronium ion) is about -2 and pKa of the PLS is some 7 or 8; thus, the difference of energy between the nearest H+ on water and on the PLS must be released as heat. Upon unbinding or deprotonation, however, about the same energy is taken up from the environment. This would appear to be a much slower process, yet it is all nicely accounted for in the relations for the "on" and "off" protonation reaction rates:

$$k_{on} \approx 10^{11-pH}$$
$$k_{off} \approx 10^{11-pKa}. \tag{A14}$$

In an ideal pump, the total entropy in the system plus environment is conserved because of heat exchange with the environment.

Analogy with the Carnot engine

A rigorous result of our pumping model is that the charge transferred through the membrane is expressed by the cyclic integral (the pump equation):

$$Q = 1/2\oint G dp_0. \tag{A15}$$

There is an analogy with the Carnot engine, or the more general thermodynamic expression for the work done by the working body:

$$w = \oint P dV. \tag{A16}$$

As in the pump equation, the cyclic integral is nonzero because the integrand is a function of not only V but also of some other parameter, like T;

thus, it can be different for two halves of the cycle. (This additional parameter returns back to its initial value but varies independently of V, and this is the key for nonzero value of the integral. It is interesting that a similar mechanism is at work in many so-called geometric phase quantum systems ((35)), such as the Berry phase, or even in the action integral in classical mechanics.)

To see the analogy clearly, we change variables and go from (V,T) to (S,T):

$$w = -\oint TdS = -\oint dQ. \tag{A17}$$

The change of variables is done as follows: we integrate the area

$$dPdV = \frac{\partial(P,V)}{\partial(S,V)}\frac{\partial(S,V)}{\partial(S,T)}dSdT = \left(\frac{\partial P}{\partial S}\right)_V\left(\frac{\partial V}{\partial T}\right)_S dSdT, \tag{A18}$$

but from

$$TdS = dE + PdV, \tag{A19}$$

we have

$$\begin{gathered}\left(\frac{\partial P}{\partial S}\right)_V = -\left(\frac{\partial T}{\partial V}\right)_S, \text{ hence} \\ \left(\frac{\partial P}{\partial S}\right)_V\left(\frac{\partial V}{\partial T}\right)_S = -1.\end{gathered} \tag{A20}$$

Thus, we have

$$dPdV = -dSdT. \tag{A21}$$

And thus, the integral

$$w = \oint PdV = \iint dPdV = -\iint dTdS = -\oint TdS. \tag{A22}$$

This relation just reminds us that the work is related to the flux of heat through the working body, similar to the flux through the pump site. In the Carnot case, the overall integral is heat in minus heat out in one cycle; in the pump site, it is flux in minus flux out, but because the total flux is conserved, flux in is the same as flux out, and thus the factor 1/2 is needed.

It is of interest also to mention the formal (purely mathematical) relation of the work integral of the Carnot machine to quantum phase integrals and magnetic fluxes.

Consider the work integral

$$w = \oint PdV$$

and introduce new variables (T,S). Then

$$\begin{aligned} w = \oint PdV &= \oint P(T,S)\left(\frac{\partial V}{\partial S}dS + \frac{\partial V}{\partial T}dT\right) \\ &= \oint A_S dS + A_T dT = \oint\left(\vec{A}\cdot d\vec{l}\right). \end{aligned} \tag{A23}$$

Here, we introduced the "vector potential" $\vec{A}$, whose components are $P\frac{\partial V}{\partial S}$ and $P\frac{\partial V}{\partial T}$ in the line integral in the plane of (S,T). Applying Stocks' theorem, the line integral becomes an area integral of the flux of the field, which is the curl of $\vec{A}$. The two-dimensional "curl of $\vec{A}$" is

$$\left(\frac{\partial P}{\partial S}\right)_T\left(\frac{\partial V}{\partial T}\right)_S - \left(\frac{\partial P}{\partial T}\right)_S\left(\frac{\partial V}{\partial S}\right)_T = \frac{\partial(P,V)}{\partial(S,T)} = -1. \tag{A24}$$

Thus, again, we conclude that the work integral is

$$w = \oint PdV = -\iint dTdS = -\oint TdS. \tag{A25}$$

Thermodynamic energy and work by the pump

Additional thermodynamic energy and work relations of interest for the pumping theory are as follows.

The total energy of the system is (here, to simplify notation, we assume $\varepsilon \equiv \varepsilon_0$ and $p \equiv p_0$)

$$E = \varepsilon p + \mu_1 N_1 + \mu_2 N_2. \tag{A26}$$

The change of total energy of the system is due to work, which is due to change of energy of the PLS, $dw = pd\varepsilon$, and due to exchange of heat with environment (heat reservoir)

$$dE = pd\varepsilon + dQ. \tag{A27}$$

Substitution to Eq. A26 gives the following:

$$dQ = \varepsilon dp + (\mu_1 dN_1 + \mu_2 dN_2). \tag{A28}$$

Given conservation of total number of particles, $p + N_1 + N_2 = const$ and the gate Eq. 9, we have

$$\begin{aligned} -dp &= dN_1 + dN_2 \\ -Gdp &= dN_1 - dN_2 \end{aligned}. \tag{A29}$$

Hence,

$$\begin{aligned} dN_1 &= -\frac{1}{2}(1+G)dp \\ dN_2 &= -\frac{1}{2}(1-G)dp \end{aligned}, \tag{A30}$$

and Eq. A28 takes the following form:

$$\begin{aligned} dQ &= \varepsilon dp - \frac{\mu_1}{2}(1+G)dp - \frac{\mu_2}{2}(1-G)dp \\ &= \left(\varepsilon - \frac{\mu_1+\mu_2}{2}\right)dp - \frac{1}{2}(\mu_1-\mu_2)Gdp. \end{aligned} \tag{A31}$$

In one cycle,

$$\begin{aligned} \oint dQ &= \oint\left(\varepsilon - \frac{\mu_1+\mu_2}{2}\right)dp - \frac{1}{2}(\mu_1-\mu_2)Gdp \\ &= \oint \varepsilon dp + (\mu_2-\mu_1)\frac{1}{2}\oint Gdp. \end{aligned} \tag{A32}$$

On the other hand, in one cycle, the change of the total energy is

$$\Delta E = (\mu_2 - \mu_1)\frac{1}{2}\oint G dp, \tag{A33}$$

i.e., $(\mu_2 - \mu_1)$ times number of pumped particles, $\delta N = 1/2\oint G dp$. Hence, overall in one cycle, we have

$$-\oint \varepsilon dp = \oint dw. \tag{A34}$$

If we assume a cycle in which loading occurs in the condition that $\varepsilon < \mu_1$ and unloading $\varepsilon > \mu_2$, and in loading $\Delta p = 1$ and in unloading $\Delta p = -1$, we have total work during the cycle

$$W = \oint dw \geq (\mu_2 - \mu_1), \tag{A35}$$

as expected.

The above expression for work Eq. A34 could be derived more directly from

$$\oint d(\varepsilon p) = 0, \tag{A36}$$

and because work $dw = pd\varepsilon$, we immediately have expression Eq. A34.

On the general form of the pump equation

As shown in this work, the general theory of pumping can be described in the framework of a simple three-level system with periodically changing kinetic parameters. Its key result—the pump equation—is formally related, in its mathematical formulation, to a variety of other concepts such as the Berry phase (and related subjects (42), such as electronic adiabatic pumping (43)), the action variable, the Carnot cycle, and a general expression for thermodynamic work, to name a few. All these seemingly unrelated subjects involve a cyclic integral of the form

$$\oint_{closed\ path} A dB. \tag{A37}$$

The key is that in all such integrals, the integrand is not a single valued function of the integration variable: for example, the momentum in the action integral $p(q)$ has two values different in sign; in thermodynamic work, pressure P depends not only on V but also on T along the path; the phase increment in the Berry phase integral (42) depends on the sign of the coupling matrix element along the path, etc. In other words, in all of these systems, the integrand depends on an additional "hidden" variable, which makes the integrand a double-valued function of the integration variable, which gives rise to a nonzero cyclic integral. In the pump equation, the gate G is double valued, i.e., can be open either to R1 or to R2, for the same value of the PLS population p_0 along which the cyclic integration is performed.

Finally, it is also worth mentioning a connection to Brownian ratchet motors (44,45). The Brownian ratchet involves a particle in a periodic potential $U(x)$. Suppose the potential is also changing in time; if the timescale of potential changes is much greater than the relaxation time τ_0, the particle is always located at the bottom of the local minimum of $U(x)$. If periodic change is such that this local minimum is moving left or right, the particle moves together with the minimum; i.e., if potential has the form $U(x - vt)$, the particle moves with velocity v (43). This is adiabatic pumping in the Brownian ratchet. In a nonadiabatic ratchet, the potential can be thought to change in sudden jumps between two periodic structures that are obtained by repetition in the x-direction of the potential profiles shown in Fig. 4 for two reservoirs of the same chemical potential, $\mu_1 = \mu_2$. In this case, the left-right symmetry of two identical reservoirs is broken and a unique direction of the flux is generated, which is equivalent to pumping ions in one specific direction (rectified diffusion) in a totally symmetric system.

AUTHOR CONTRIBUTIONS

The author (A.A.S.) designed the project, did all the analysis, and wrote the manuscript.

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