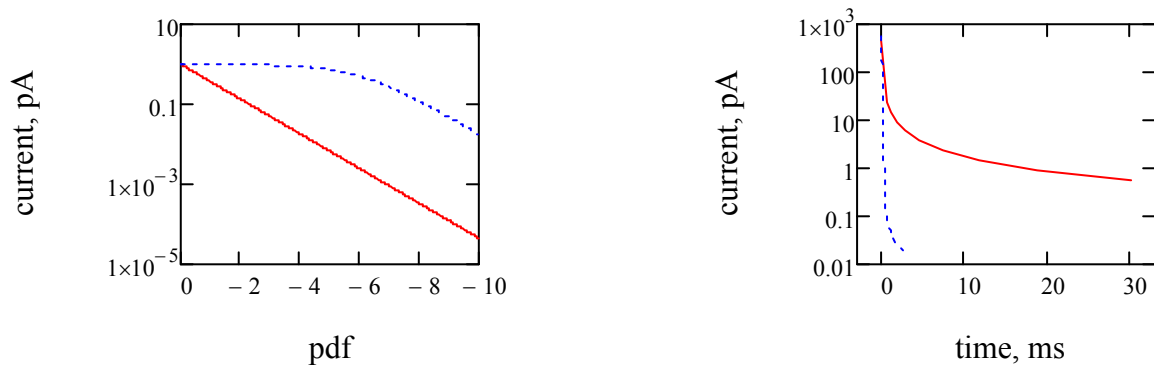Fig. S1. Dependence of the electric current $j_{pump} = e_0 J_{pump}$ on the dimensionless proton motive force $pdf = \Delta\tilde{\mu}_{H^+}/RT$ (left) and time (right). Full lines, Eq. (3). Doted lines, Eq. (6).
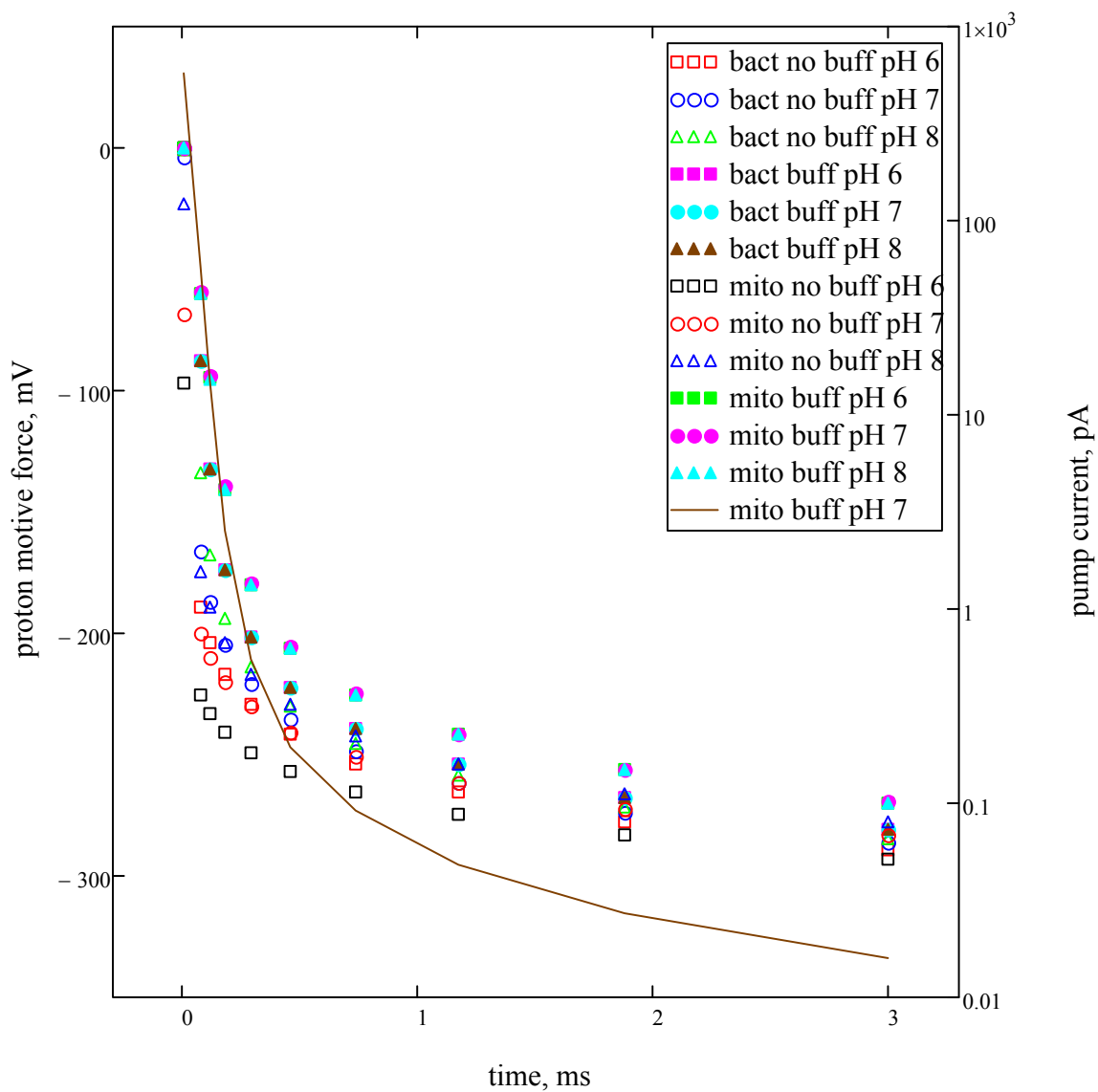

Fig. S2. Kinetics of generation of pdf (points, left ordinate, mV) and electric current j_{pump} (line, right ordinate, pA) calculated by Eq. (6)

1

Calculations of the rate constants for proton removal from region a

The rate constants in Eq. (9) are

$$k_{a,\text{off,sp}} = \tau_c^{-1} e^{-U/RT} \equiv \tau_c^{-1} 10^{-\text{pK}_a} , \qquad (S1)$$

$$k_{a,\text{off,B}^-} = \kappa_{a,\text{off,B}^-} c_{\text{B}^-} , \qquad (S2)$$

where $\tau_c = 10$ ps [1], $\kappa_{a,\text{off,B}^-}$ is the bimolecular rate constant for proton transfer from the surface to the bulk and c_{B^-} is the B^- concentration. In order to estimate this constant, we will write down the equilibrium condition for the proton exchange reaction between the surface and the mobile buffer [2],

$$\kappa_{a,\text{off,B}^-} = \kappa_{a,\text{on,BH}} 10^{\text{pK}_\text{B} - \text{pK}_a} , \qquad (S3)$$

where

$$\text{pK}_a = U/(RT \ln 10) \qquad (S4)$$

is an apparent pK of the surface equal to 2.2-6.9 at $U = (5 \div 16) RT$ [1]. Assuming

$$\kappa_{a,\text{on,BH}} = 2\pi d N_\text{A} D_\text{BH} \qquad (S5)$$

(we introduced Avogadro number N_A in order to express the constant in units of $\text{M}^{-1}\text{s}^{-1}$), $d = 0.6$ nm (two H bonds), and taking the mobile buffer parameters as follows: the diffusion coefficient $D_\text{BH} = 10^{-7} \text{cm}^2\text{s}^{-1}$ [2] and the concentration[1] $c_{\text{B}^-} = 0.5 c_\text{B} = 0.5$ mM, we obtain from (S5) and (S2)

$$\kappa_{a,\text{on,BH}} = 2.3 \times 10^7 \text{M}^{-1}\text{s}^{-1} \qquad (S6)$$

and

$$k_{a,\text{off,B}^-} = 10^4 \text{s}^{-1} \times 10^{\text{pK}_\text{B} - \text{pK}_a} , \qquad (S7)$$

which is of the same order of magnitude as (S1). For instance, at an intermediate value of the barrier, $U = 10$ *RT*, they are both $5 \times 10^6 \text{s}^{-1}$. The total rate constant for proton depopulation of the surface, Eq. (9), can be less than (or on the order of) the rate of the initial pmf increase, in which case appreciable local pH changes should be expected at low pmf.

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[1] Mobile buffer concentration is assumed to be much less than the total buffer concentration, the latter being usually about 10 to 100 mM.

The membrane is assumed to attract protons. The potential well at the membrane surface (region a) has width d and depth U. The bulk space outside it is denoted region b. The free and bound proton concentrations in these regions as functions of time and the space coordinate normal to the surface are represented in the form

$$\sigma_{a,H^+}(t) = \sigma_{a,eq,H^+} + S(t), \tag{S8}$$

$$c_{b,H^+}(z,t) = c_{b,eq,H^+} + N(z,t), \tag{S9}$$

$$c_{b,BH}(z,t) = c_{b,eq,BH} + N(z,t), \tag{S10}$$

where[2]

$$\sigma_{a,H^+} = c_{a,H^+} d \tag{S11}$$

is the surface concentration, c_{a,H^+} and c_{b,H^+} stand for the volume concentrations (in M), S and N are the non-equilibrium proton concentrations created by the proton pumping. The medium in general contains both non-equilibrium free protons H^+ and non-equilibrium bound protons BH, where B stands for the buffer. We will not consider these two non-equilibrium[3] proton pools simultaneously since it would be a significant yet unnecessary complication. Instead, we assume that H^+ can be ignored when BH is present in physiological concentrations of 1-100 mM. Thus, the unknown functions in the problem are (S8) and either (S9) or (S10) plus dimensionless $\Delta\psi$.

The equilibrium concentrations obey the following relations similar to (S11) and (8):

$$\sigma_{a,eq,H^+} = c_{a,eq,H^+} d, \tag{S12}$$

$$c_{a,eq,H^+} = c_{b,eq,H^+} e^{U/RT}, \tag{S13}$$

$$\sigma_{a,eq,H^+} = L_{0,H^+} c_{b,eq,H^+} = L_{0,B} c_{b,eq,BH}. \tag{S14}$$

Equation (S12) is merely the definition of the surface concentration. Equation (S13) reflects the increase of the surface concentration due to the attraction. Equations (S14) state that the free and bound proton concentrations in the bulk are proportional to each other and to the surface concentration of the free protons (BH is assumed to be unable to penetrate into region a), L_{0,H^+} and $L_{0,B}$ being the equilibrium constants for the free and bound protons with dimension of length. Combining these equations, we obtain

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[2] The same notation $N(z,t)$ is used in Eqs. (S9) and (S10), which does not lead to confusion since either free or bound protons are accounted for in the bulk, see below.

[3] The equilibrium protons, neither free nor bound, cannot be ignored since they both are responsible for the pH of the solution.

$$L_{0,\mathrm{H}^+} = d \exp(U/RT), \tag{S15}$$

$$L_{0,\mathrm{B}} = L_{0,\mathrm{H}^+} \frac{c_{b,\mathrm{eq},\mathrm{H}^+} + K_\mathrm{B}}{c_\mathrm{B}}, \tag{S16}$$

where

$$c_\mathrm{B} \equiv c_{\mathrm{BH}}(z,t) + c_{\mathrm{B}^-}(z,t) \tag{S17}$$

is the total concentration of the buffer,

$$K_\mathrm{B} = 10^{-\mathrm{pK_B}}\,\mathrm{M} \tag{S18}$$

is the equilibrium constant of the buffer, and

$$c_{b,\mathrm{eq},\mathrm{H}^+} = 10^{-\mathrm{pH}_{b,\mathrm{eq}}}\,\mathrm{M} \tag{S19}$$

is the equilibrium concentration of the free protons in the bulk. The length scale of (S15) or (S16) (shortly L_0) combined with the respective diffusion coefficients (shortly D_b) provides for a time scale of

$$t_{0,\mathrm{H}^+} = \left(L_{0,\mathrm{H}^+}\right)^2 \big/ D_{b,\mathrm{H}^+} \tag{S20}$$

or

$$t_{0,\mathrm{B}} = \left(L_{0,\mathrm{B}}\right)^2 \big/ D_{b,\mathrm{BH}} \tag{S21}$$

(shortly t_0) for dissipation of the non-equilibrium free or bound protons in the bulk [1-3], which should be compared with the proton dwell time at the surface,

$$\tau_{\mathrm{dw}} = k_{a,\mathrm{off}}^{-1}. \tag{S22}$$

Here,

$$k_{a,\mathrm{off}} = k_{a,\mathrm{off,sp}} + k_{a,\mathrm{off,B}^-} \tag{S23}$$

is the rate with which the protons leave region a either spontaneously or due to collisions of B^- with the surface. The dwell time is the time required for a pumped proton at the P side to leave the surface for the first time. If the diffusion is fast, so that $t_0 \ll \tau_{\mathrm{dw}}$, the proton released will rapidly escape to the bulk with no chance to return back to the surface. However, typically, the situation is just opposite: after leaving the surface, the proton stays long next to it and moves back and forth[4] making

$$\nu = k_{a,\mathrm{off}} t_0 \gg 1 \tag{S24}$$

jumps before the full equilibration with the bulk is reached. In other words, due to slow equilibration with the bulk, the non-equilibrium protons in the bulk create back flow to the surface,

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[4] We called this the fast-exchange limit, as opposed to the slow-exchange one, $\nu < 1$ [2].

thereby retarding the decay of the surface population. The back flow largely compensates the forth one, so that quasi-equilibrium is established between the surface and bulk non-equilibrium proton concentrations at every moment of time similar to (S14),

$$S(t) = L_0 N(0,t). \tag{S25}$$

Similar considerations apply to the N side as well[5] where the proton population depleted due to the pumping is slowly restored because of slow diffusion from the bulk towards the surface.

The above arguments imply that transient non-equilibrium proton populations can be created by proton pumping and maintained for a while at both sides of the membrane. The obvious conditions for this to occur are that the pumping of local pH changes in regions a, first, be faster than the buildup of $\Delta\psi$ because otherwise the pumps will stop working before any significant ΔpH is created, and second, be faster than the proton dissipation along the two above-mentioned pathways, i.e., the removal of the non-equilibrium protons from region a with the rate $k_{a,\text{off}}$ and their ultimate escape to the bulk with the rate t_0^{-1} preventing their return to the surface. In order to put quantitative criteria, we introduce characteristic rates $\tau_{a,\text{pH}}^{-1}$ and τ_ψ^{-1} [to be specified later, see Eqs. (S66) and (S69)] that are required for the pumps to change ΔpH and $\Delta\psi$ by unity. The pumping will be efficient in producing local pH changes when, first, ΔpH increases faster than $\Delta\psi$,

$$\tau_{a,\text{pH}}^{-1} \geq \tau_\psi^{-1}, \tag{S26}$$

and second, faster than at least one of the above two dissipation rates, $k_{a,\text{off}}$ and t_0^{-1}. In the fast-exchange limit (S24), two scenarios are conceivable,

$$\tau_{a,\text{pH}}^{-1} \gg k_{a,\text{off}} \gg t_0^{-1} \tag{S27}$$

and

$$k_{a,\text{off}} \gg \tau_{a,\text{pH}}^{-1} \gg t_0^{-1}, \tag{S28}$$

which correspond to mechanisms 1 and 2 mentioned in the main text. Mechanism 1 is the most efficient due to higher proton pumping rate. However, as will be seen later, based on estimates of the parameters, it is expected to operate only in strongly basic media whereas mechanism 2 is not subject to such a restriction.

After these preliminary considerations we turn to the derivation and solution of the basic equations. The proton diffusion in the bulk is described by the equation

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[5] We tacitly assume that parameters L_0, D_b, $k_{a,\text{off}}$, and hence t_0 and ν, are identical at both sides of the membrane.

$$\frac{\partial N(z,t)}{\partial t} = D_b \frac{\partial^2 N(z,t)}{\partial z^2}, \tag{S29}$$

where either free or bound protons are implied depending on the solution. The boundary condition at infinity is

$$N(z \to +\infty, t) \to 0. \tag{S30}$$

At the membrane surface, the proton pumping and the proton flow between the surface and the bulk (protons per second) result in changes of the total number of protons in region a,

$$S_{memb} N_A D_b \frac{\partial N(z,t)}{\partial z}\bigg|_{z=0} + \varepsilon J_{pump}(t) = S_{memb} N_A \dot{S}(t), \tag{S31}$$

where ε and the meaning of the terms are explained below. Since the pumps transfer proton numbers,

$$n_{a,H^+} = V_a N_A c_{a,H^+} = S_{memb} N_A \sigma_{a,H^+}, \tag{S32}$$

rather than concentrations c_{a,H^+} in volume $V_a = S_{memb} d$, it was convenient for us to write (S31) in terms of proton numbers. Now, dividing it by $S_{memb} N_A$, we obtain a more compact equation

$$D_b \frac{\partial N(z,t)}{\partial z}\bigg|_{z=0} + \frac{\varepsilon J_{pump}(t)}{S_{memb} N_A} = \dot{S}(t). \tag{S33}$$

The first term is the proton flow due to gradient of the proton concentration in the bulk. The second term is the proton pumping due to pump current J_{pump} defined in the main text as a function of pmf [see Eq. (6)], which in turn depends on t. For the sake of argument, we consider cytochrome-c-oxidase as a model proton pump. Then, $\varepsilon = \frac{1}{2}$ for the P side and -1 for the N side because only one half of the protons uptaken at the N side reach the P side; S_{memb} is the surface of the membrane. In the right-hand side, we neglected the term due to recombination of H^+ with OH^- or dissociation of water because the assumed attraction of protons implies the reduced concentration of hydroxyl, so that the hydroxyl contribution is small in neutral and acidic media[6].

It is useful to define the total number of protons in the bulk region,

$$N_{tot}(t) = \int_0^\infty N(z,t)dz. \tag{S34}$$

The conservation of the total number of protons is easy to derive from Eqs. (S29), (S30), and (S33),

$$\frac{d}{dt}\big[N_{tot}(t) + S(t)\big] = \frac{\varepsilon J_{pump}(t)}{S_{memb} N_A}. \tag{S35}$$

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[6] However, this is not true in alkaline media.

This relation will be used later.

Following our previous approach [2, 4], we approximate the first term in Eq. (S33) by the so-called Langmuir kinetics,

$$D_b \left. \frac{\partial N(z,t)}{\partial z} \right|_{z=0} = k_{a,\text{off}} \left[L_0 c_b (0,t) - \sigma_{a,\text{H}^+} (t) \right], \tag{S36}$$

which represents the net proton flow from the bulk to the surface as a difference between the incoming and outgoing proton fluxes, both being proportional to the respective proton concentrations (c_b is concentration of either free or bound protons in the bulk). In equilibrium, the flow vanishes according to (S14). Inserting this into (S33) and using (S8)-(S10), we obtain

$$k_{a,\text{off}} \left[L_0 N(0,t) - S(t) \right] + \frac{\varepsilon J_{\text{pump}}(t)}{S_{\text{memb}} N_A} = \dot{S}(t). \tag{S37}$$

At this junction, it is worthwhile to comment on the application of the fast-exchange limit (S24). In this limit, both incoming and outgoing fluxes in the right-hand side of (S36) are large whereas their difference is small, so that relation (S25) is approximately fulfilled. However, their small difference must be retained in Eq. (S33) because it is the slow diffusion in the bulk that is responsible for the slow decay of the surface population. The diffusion equation (S29) with boundary conditions (S30), (S33) without the pump term but with the initial condition $S(0) = 1$, was solved in our BJ publication [2]; we will denote it $S^{\text{BJ}}(t)$. A more general problem, i.e., without invoking the fast-exchange approximation, but in the steady-state case, was also solved in our JCP publication [4]. Here, we are interested to find a solution of the time-dependent problem in the general case because we need to consider short times after the proton pumping starts, at which no back flow is yet developed. We will show how our present solution denoted as $S^{\text{JCP}}(t)$ is related to $S^{\text{BJ}}(t)$.

Thus, following our method [2, 5], we apply the Laplace transform[7],

$$f_\lambda = \int_0^\infty f(t) e^{-\lambda t} dt, \tag{S38}$$

to Eqs. (S29), (S34), (S35), and (S37):

$$D_b \frac{d^2}{dz^2} N_\lambda(z) = \lambda N_\lambda(z), \tag{S39}$$

$$N_{\text{tot},\lambda} = \int_0^\infty N_\lambda(z) dz, \tag{S40}$$

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[7] The functions f_λ and $f(t)$ are called "the Laplace transform" and "the original", respectively.

$$\lambda\left(N_{\text{tot},\lambda}+S_\lambda\right)=\frac{\varepsilon J_{\text{pump},\lambda}}{S_{\text{memb}}N_A}\,,\tag{S41}$$

$$\lambda S_\lambda=\frac{\varepsilon J_{\text{pump},\lambda}}{S_{\text{memb}}N_A}+k_{a,\text{off}}\left[L_{0,B}N_\lambda(0)-S_\lambda\right].\tag{S42}$$

The general solution to Eq. (S39) obeying condition (S30) is

$$N_\lambda(z)=N_\lambda(0)\exp\left(-z\sqrt{\lambda/D_b}\right).\tag{S43}$$

Inserting this into Eq. (S40), we obtain

$$N_{\text{tot},\lambda}=\frac{N_\lambda(0)}{\sqrt{\lambda/D_b}}=\frac{L_0 N_\lambda(0)}{\sqrt{\lambda t_0}}\,.\tag{S44}$$

Thus, we have three equations (S41), (S42), and (S44) for three unknowns, from which we find

$$S_\lambda=\frac{\varepsilon J_{\text{pump},\lambda}}{S_{\text{memb}}N_A}\left(\lambda+\frac{\nu\sqrt{\lambda/t_0}}{\nu+\sqrt{\lambda t_0}}\right)^{-1}\equiv\frac{\varepsilon J_{\text{pump},\lambda}}{S_{\text{memb}}N_A}S_\lambda^{\text{JCP}}\,,\tag{S45}$$

where the right-hand identity is the definition of S_λ^{JCP}. In the fast-exchange limit (S24) it turns into

$$S_\lambda^{\text{BJ}}=\left(\lambda+\sqrt{\lambda/t_0}\right)^{-1}\,,\tag{S46}$$

which was obtained in [2]. Finally, we have to perform the inverse Laplace transform [6],

$$f(t)=\frac{1}{2\pi i}\int_{a-i\infty}^{a+i\infty}f_\lambda e^{\lambda t}d\lambda\,,\tag{S47}$$

where a is an arbitrary positive number. The product of the Laplace transforms in (S45) turns into the convolution of the originals [6],

$$S(t)=\frac{\varepsilon}{S_{\text{memb}}N_A\tau_{\text{pump}}}\int_0^t S^{\text{JCP}}(t-t_1)\tilde{J}_{\text{pump}}(t_1)dt_1\,,\tag{S48}$$

where the dimensionless current has been introduced,

$$\tilde{J}_{\text{pump}}(t)=\tau_{\text{pump}}J_{\text{pump}}(t).\tag{S49}$$

The other function in the integrand is

$$S^{\text{JCP}}(t)=\frac{1}{2\pi i}\int_{a-i\infty}^{a+i\infty}S_\lambda^{\text{JCP}}e^{\lambda t}d\lambda\,.\tag{S50}$$

This function represents the solution to Eq. (S37) with no pump but with the initial condition

$$S^{\text{JCP}}(0)=1.\tag{S51}$$

It is easy to show that

$$S_\lambda^{\text{JCP}} = \left(\lambda + \frac{v\sqrt{\lambda/t_0}}{v + \sqrt{\lambda t_0}} \right)^{-1} \tag{S52}$$

[see Eq. (S45)] is regular everywhere in the complex plane of λ except for a cut along the negative real half-axis. To be more specific, we make the replacements

$$\lambda = \xi k_{a,\text{off}}, \quad t = \tilde{\tau} k_{a,\text{off}}^{-1}, \tag{S53}$$

so that

$$S^{\text{JCP}}(t) = \frac{1}{2\pi i} \int\limits_{a-i\infty}^{a+i\infty} \left(\xi + \frac{\sqrt{\xi v}}{v + \sqrt{\xi v}} \right)^{-1} e^{\xi\tilde{\tau}} d\xi. \tag{S54}$$

We draw the cut along the negative real half-axis and put

$$\xi = se^{i\varphi}, \quad -\pi \le \varphi \le \pi. \tag{S55}$$

The denominator in the integrand of (S54) vanishes only at $\text{Re}\sqrt{\xi} < 0$, which is obtained by solving a quadratic equation. However, according to (S55),

$$\text{Re}\sqrt{\xi} = \sqrt{s}\cos\left(\tfrac{1}{2}\varphi\right) \ge 0, \tag{S56}$$

so that no other singularities are present in addition to the cut. Then, the integration contour can be displaced to the left to circumvent the cut,

$$S^{\text{JCP}}(t) = \frac{1}{2\pi i} \int\limits_{\infty}^{0} \left(-s + \frac{-i\sqrt{sv}}{v - i\sqrt{sv}} \right)^{-1} e^{-s\tilde{\tau}}(-ds) + \frac{1}{2\pi i} \int\limits_{0}^{\infty} \left(-s + \frac{i\sqrt{sv}}{v + i\sqrt{sv}} \right)^{-1} e^{-s\tilde{\tau}}(-ds). \tag{S57}$$

A simple calculation, after replacement $s = x^2$, gives

$$S^{\text{JCP}}(t) = \frac{2}{\pi} \int\limits_{0}^{\infty} \frac{\sqrt{v}dx}{vx^2 + \left(1 - x^2\right)^2} e^{-x^2\tilde{\tau}}. \tag{S58}$$

This expression is suitable when $v \le 1$ and $\tilde{\tau} \le 1$, i.e., when mechanism 1 is operating. In particular, when $v \ll 1$, the integral comes from a vicinity of $x = 1$. Replacing the integration variable, $x = 1 + \left(\sqrt{v}/2\right)y$, and extending integration over y from $-\infty$ to $+\infty$, results in

$$S^{\text{JCP}}(t) \approx \frac{1}{\pi} \int\limits_{-\infty}^{+\infty} \frac{dy}{1 + y^2} e^{-\tilde{\tau}} = e^{-\tilde{\tau}}. \tag{S59}$$

This result is easy to understand. In the original Eq. (S37), the incoming flux is absent because $v \ll 1$ means rapid dissipation (due to small t_0) of the non-equilibrium protons in the bulk, and no pump is working since initial condition (S51) is assumed instead. Then, the exponential decay (S59) is immediately obtained.

In the opposite case, $v > 1$, we rewrite Eq. (S58) by replacing x with $x/\sqrt{v}$ and $\tilde{\tau}$ with $\tau = \tilde{\tau}/v = t/t_0$, which gives

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$$S^{\mathrm{JCP}}(t) = \frac{2}{\pi} \int_0^\infty \frac{dx}{x^2 + \left(1 - v^{-1}x^2\right)^2} e^{-x^2\tau}, \quad \tau = \frac{t}{t_0}.$$ (S60)

In particular, in the fast-exchange limit of Eq. (S24), this turns into the expression

$$S^{\mathrm{BJ}}(t) = \frac{2}{\pi} \int_0^\infty \frac{dx}{x^2 + 1} e^{-x^2\tau}$$ (S61)

obtained in [2], which can be also derived by taking inverse Laplace transform of Eq. (S46). It is suitable for treating mechanism 2.

Now we are in position to calculate the local time-dependent variations of pH within region a. According to Eqs. (19), (S8), and (S11) they are given by

$$\delta \mathrm{pH}_a(t) = -\frac{q_{a,\mathrm{H}^+}(t)}{\ln 10},$$ (S62)

where

$$q_{a,\mathrm{H}^+}(t) = \frac{\delta \mu_{a,\mathrm{H}^+}(t)}{RT} = \ln\left(1 + \frac{S(t)}{\sigma_{a,\mathrm{eq,H}^+}}\right)$$ (S63)

is the change of the local dimensionless chemical potential with respect to its equilibrium value. Substituting (S48) for S, we obtain

$$q'_{a,\mathrm{H}^+}(t) = \ln\left(1 - \frac{1}{\tau'_{a,\mathrm{pH}}} \int_0^t S^{\mathrm{JCP}'}(t - t_1) \tilde{J}_{\mathrm{pump}}(t_1) dt_1\right)$$ (S64)

for the N side and

$$q''_{a,\mathrm{H}^+}(t) = \ln\left(1 + \frac{1}{2\tau''_{a,\mathrm{pH}}} \int_0^t S^{\mathrm{JCP}''}(t - t_1) \tilde{J}_{\mathrm{pump}}(t_1) dt_1\right)$$ (S65)

for the P-side, where

$$\tau'_{a,\mathrm{pH}} = n'_{a,\mathrm{eq,H}^+} \tau_{\mathrm{pump}}, \quad \tau''_{a,\mathrm{pH}} = n''_{a,\mathrm{eq,H}^+} \tau_{\mathrm{pump}}$$ (S66)

are the timescale for proton pumping at two sides of the membrane mentioned earlier and $S^{\mathrm{JCP}'}$ or $S^{\mathrm{JCP}''}$ are S^{JCP} with $t_0, v = t'_0, v'$ or t''_0, v''.

The trans-membrane difference of the electric potential obeys the equation

$$\Delta\dot{\varphi} = \varphi_e J_{\mathrm{pump}}, \quad \varphi_e = \frac{e_0}{C_{\mathrm{memb}} S_{\mathrm{memb}}}.$$ (S67)

Introducing the dimensionless potential,

$$\psi = \frac{F\varphi}{RT}, \quad \psi_e = \frac{F\varphi_e}{RT},$$ (S68)

and the time-scale for ψ generation,

$$\tau_\psi = \psi_e^{-1}\tau_{pump}, \tag{S69}$$

we obtain the following equation describing the kinetics of generation of the trans-membrane potential difference:

$$\Delta\dot{\psi}(t) = -\tau_\psi^{-1}\tilde{J}_{pump}(t) \tag{S70}$$

or, in the integral form,

$$\Delta\psi(t) = \Delta\psi(0) - \tau_\psi^{-1}\int_0^t \tilde{J}_{pump}(t_1)\,dt_1. \tag{S71}$$

Using Eqs. (1), (2), (7), and (S68), the dimensionless current (S49) as function of the pmf components is rewritten as

$$\tilde{J}_{pump}(t) = \frac{1}{1+\exp\left[-\Delta\psi(t)-6-q'_{a,\mathrm{H}^+}(t)+q''_{a,\mathrm{H}^+}(t)\right]}. \tag{S72}$$

Thus, we have three non-linear integral equations (S64), (S65), and (S71) to find three unknown functions $q'_{a,\mathrm{H}^+}(t)$, $q''_{a,\mathrm{H}^+}(t)$, and $\Delta\psi(t)$. If the pump current $\tilde{J}_{pump}(t)$ was a known function of time, the above equations would provide these functions. This implies that the problem could be solved by iterations. Namely, assuming some initial (zero-order) iteration, say,

$$q'_{a,\mathrm{H}^+}(t) \equiv 0, \quad q''_{a,\mathrm{H}^+}(t) \equiv 0, \quad \Delta\psi(t) = -\ln\left(1+\tau_\psi^{-1}t\right), \tag{S73}$$

we calculate step-by-step the 1st, the 2nd, etc. iterations by inserting the current found from Eq. (S72) at the previous step into Eqs. (S64), (S65), and (S71). Convergence of the iterations is shown in Figs. S3 and S4. A significant problem with this method is that $\sigma'_{a,\mathrm{H}^+} < 0$ can occur at the N side at an intermediate iteration because of a bad selection of the initial iteration, making q'_{a,H^+} complex, which prevents developing subsequent iterations. The natural idea to introduce buffering function due to hydroxyl does not work, unfortunately, because our method is not applicable. We overcame this difficulty by dividing $S/\sigma_{a,eq,\mathrm{H}^+}$ (which is negative at the N side) by its maximum value (and subtracting additional 0.1 to avoid occasional zero). However, often this method did not work, e.g., at pH > 7.2 or $c_\mathrm{B} < 1\,\mathrm{mM}$. Therefore we also employed a second method where the unknown functions are propagated in small time steps starting at $t = 0$.

Equation (S71) can be rewritten alternatively in the form

$$\Delta\psi(t) = \Delta\psi(0) - \ln\left[1+\tau_\psi^{-1}\int_0^t e^{\Delta\psi(0)-\Delta\psi(t_1)}\tilde{J}_{pump}(t_1)\,dt_1\right], \tag{S74}$$

which can be used to control the accuracy of the calculations.

Calculation of the global ΔpH

In the absence of the surface effect, and assuming fast equilibration of the protons pumped with the bulk solution, we can calculate the kinetics of generation of the global ΔpH. The concentrations of free and bound protons are changing with time according to the equations

$$\dot{c}'_{\mathrm{H}^+} + \dot{c}'_{\mathrm{BH}} - \dot{c}'_{\mathrm{OH}^-} = -\frac{\tilde{J}_{\mathrm{pump}}}{V' N_{\mathrm{A}} \tau_{\mathrm{pump}}}, \tag{S75}$$

$$\dot{c}''_{\mathrm{H}^+} + \dot{c}''_{\mathrm{BH}} - \dot{c}''_{\mathrm{OH}^-} = \frac{\tilde{J}_{\mathrm{pump}}}{2V'' N_{\mathrm{A}} \tau_{\mathrm{pump}}}, \tag{S76}$$

$$\Delta\dot{\psi} = -\tau_{\mathrm{pump},\psi}^{-1} \tilde{J}_{\mathrm{pump}}, \tag{S77}$$

$$\tilde{J}_{\mathrm{pump}} = \frac{1}{1 + \exp\left[-\Delta\psi - 6 - q'_{\mathrm{H}^+} + q''_{\mathrm{H}^+}\right]}, \tag{S78}$$

where $V' = V'' = 1\ \mu\mathrm{m}^3$ for membrane M and $V' = 1\ \mu\mathrm{m}^3, V'' = \infty$ for membrane B. At every moment of time, the concentrations of bound protons and hydroxyl obey the conditions of thermodynamic equilibrium,

$$c_{\mathrm{BH}} = c_{\mathrm{B}}\left(1 + \frac{K_{\mathrm{B}}}{c_{\mathrm{H}^+}}\right)^{-1}, \quad c_{\mathrm{OH}^-} = \frac{(0.1\ \mu\mathrm{M})^2}{c_{\mathrm{H}^+}}. \tag{S79}$$

Inserting this into Eqs. (S75) and (S76) gives

$$\dot{c}'_{\mathrm{H}^+} = -\frac{\tilde{J}_{\mathrm{pump}}}{V' N_{\mathrm{A}} \chi(c'_{\mathrm{H}^+}) \tau_{\mathrm{pump}}}, \quad \dot{c}''_{\mathrm{H}^+} = \frac{\tilde{J}_{\mathrm{pump}}}{2V'' N_{\mathrm{A}} \chi(c''_{\mathrm{H}^+}) \tau_{\mathrm{pump}}}, \tag{S80}$$

where the buffering function is

$$\chi(c_{\mathrm{H}^+}) = 1 + \left(\frac{0.1\ \mu\mathrm{M}}{c_{\mathrm{H}^+}}\right)^2 + \frac{c_{\mathrm{B}} K_{\mathrm{B}}}{(c_{\mathrm{H}^+} + K_{\mathrm{B}})^2}. \tag{S81}$$

We divide Eqs. (S80) by equilibrium concentrations $c_{\mathrm{eq,H}^+}$ and introduce the following definitions:

$$q_{\mathrm{H}^+} = \ln\left(\frac{c_{\mathrm{H}^+}}{c_{\mathrm{eq,H}^+}}\right) = -(\ln 10)\delta\mathrm{pH}, \tag{S82}$$

$$\tau_{\mathrm{pH}} = V c_{\mathrm{eq,H}^+} N_{\mathrm{A}} \tau_{\mathrm{pump}}. \tag{S83}$$

Then,

$$\dot{q}'_{\mathrm{H}^+} = -\frac{\tilde{J}_{\mathrm{pump}}}{\chi' \tau'_{\mathrm{pH}}}, \quad \dot{q}''_{\mathrm{H}^+} = \frac{\tilde{J}_{\mathrm{pump}}}{2\chi'' \tau''_{\mathrm{pH}}}. \tag{S84}$$

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Thus, we have to solve three equations (S77) and (S84) with the initial conditions

$$\Delta\psi(0) = q'_{H^+}(0) = q''_{H^+}(0) = 0 .$$ (S85)
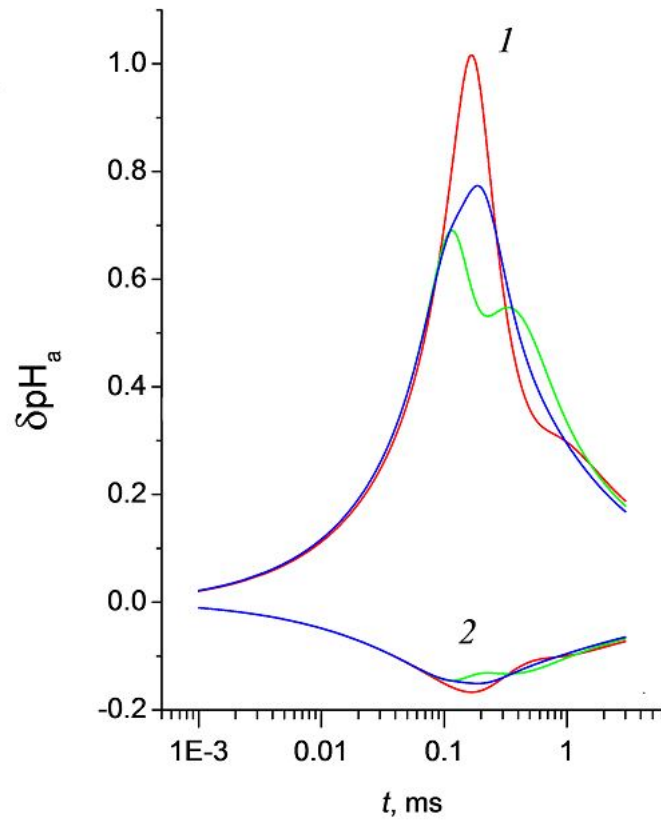
The results are shown in Figs. S7-S9.

Fig. S3. Convergence of the iterations for membrane M. Shown are the 3rd (red), 4th (green), and 5th (blue) iterations in $\delta pH'_a$ (*1*) and $\delta pH''_a$ (*2*). The final iteration is shown in Fig. 2.
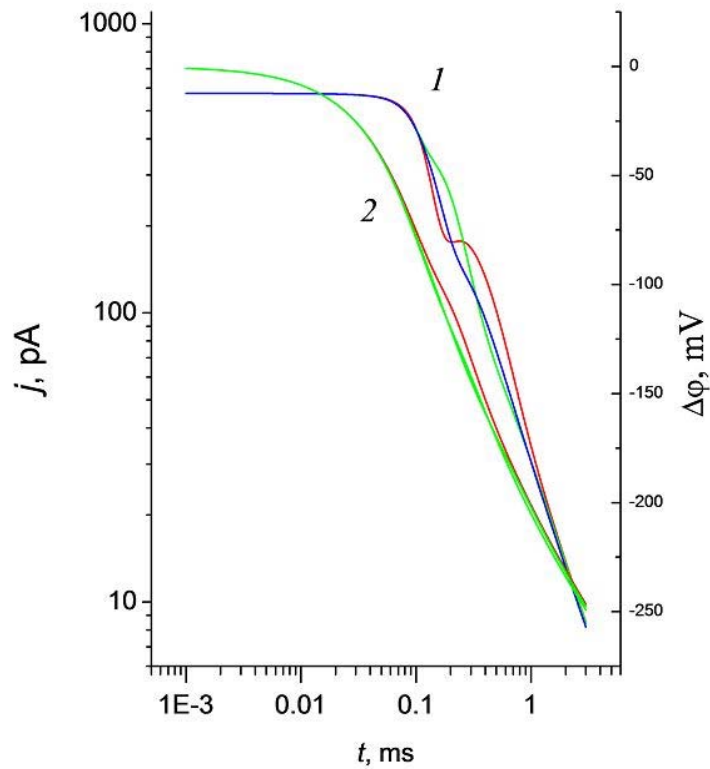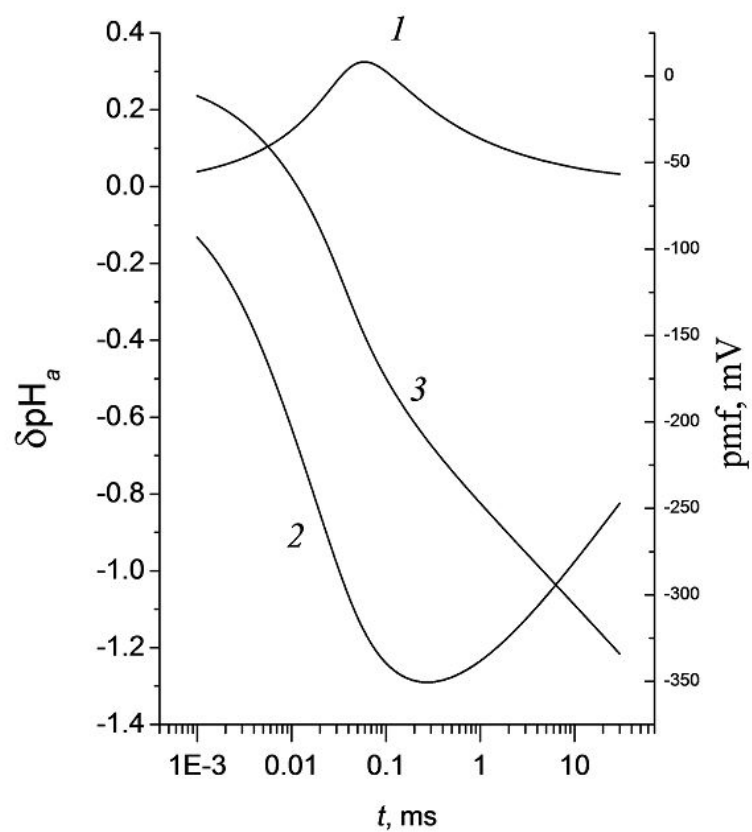

Fig. S4. Convergence of the iterations for membrane M. Shown are the 3rd (red), 4th (green), and 5th (blue) iterations in j (*1*) and $\Delta\varphi$ (*2*).

Fig. S5. Kinetics of $\delta\text{pH}_a'$ (line *1*), $\delta\text{pH}_a''$ (line *2*), and pmf (line *3*) in membrane B at $U = 8RT$.
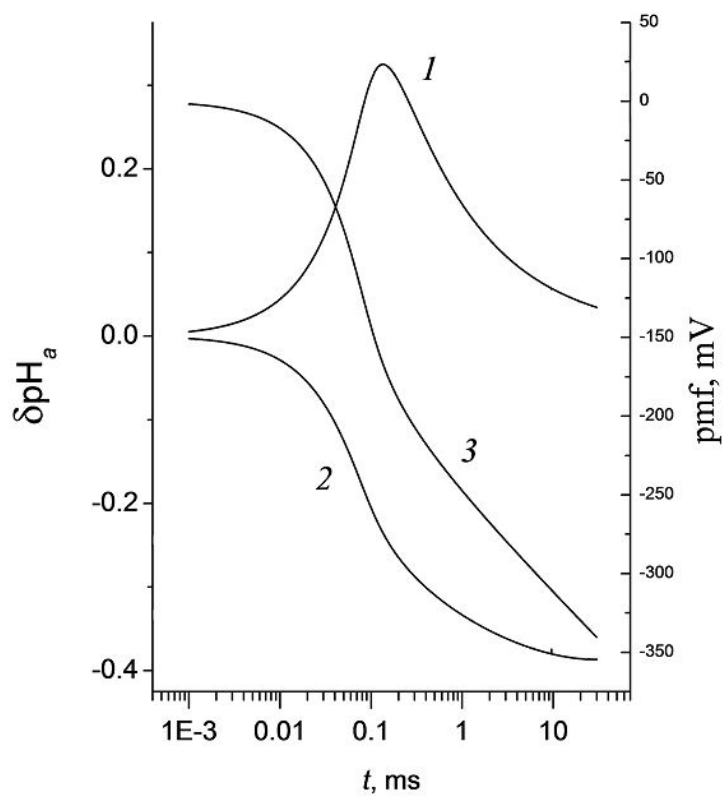
Fig. S6. Kinetics of $\delta\text{pH}_a'$ (line *1*), $\delta\text{pH}_a''$ (line *2*), and pmf (line *3*) in membrane B at $U = 12RT$.
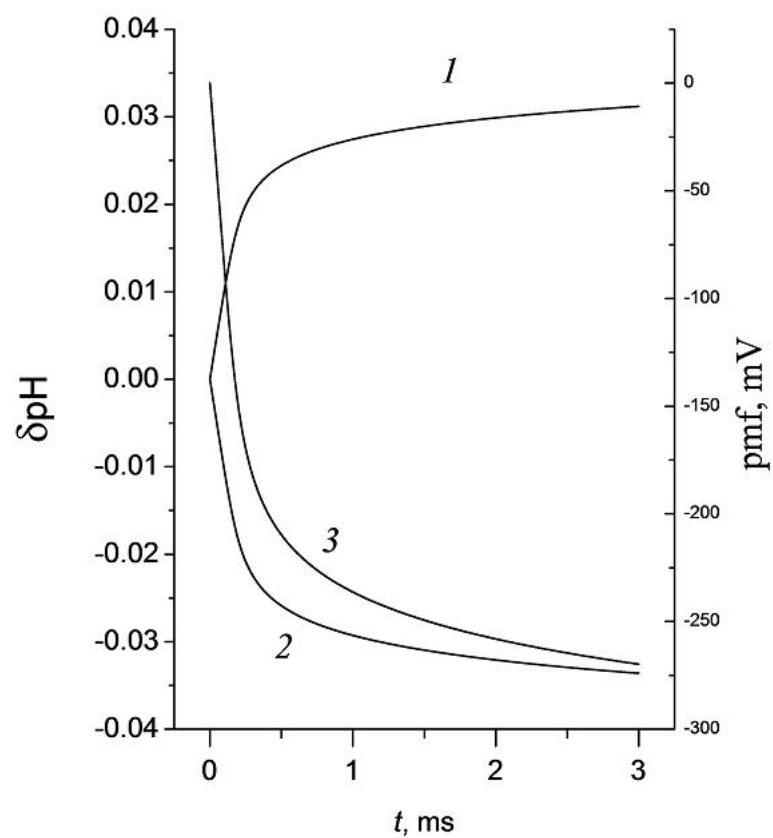
Fig. S7. Kinetics of generation of global $\delta pH'$ (line *1*), $\delta pH''$ (line *2*), and pmf (line *3*) in membrane M. Buffer concentration is 100 mM.
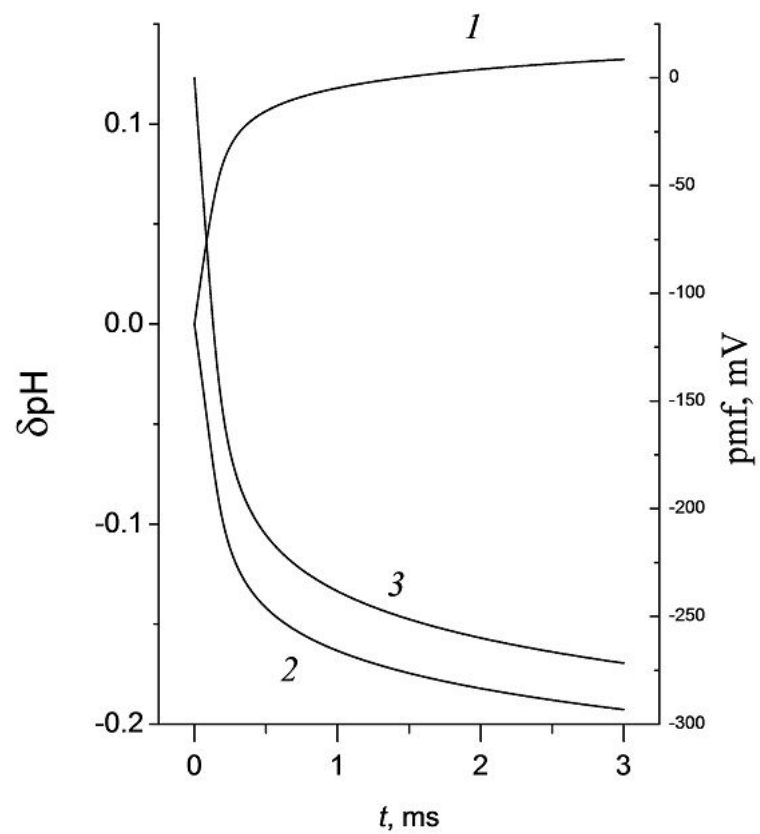
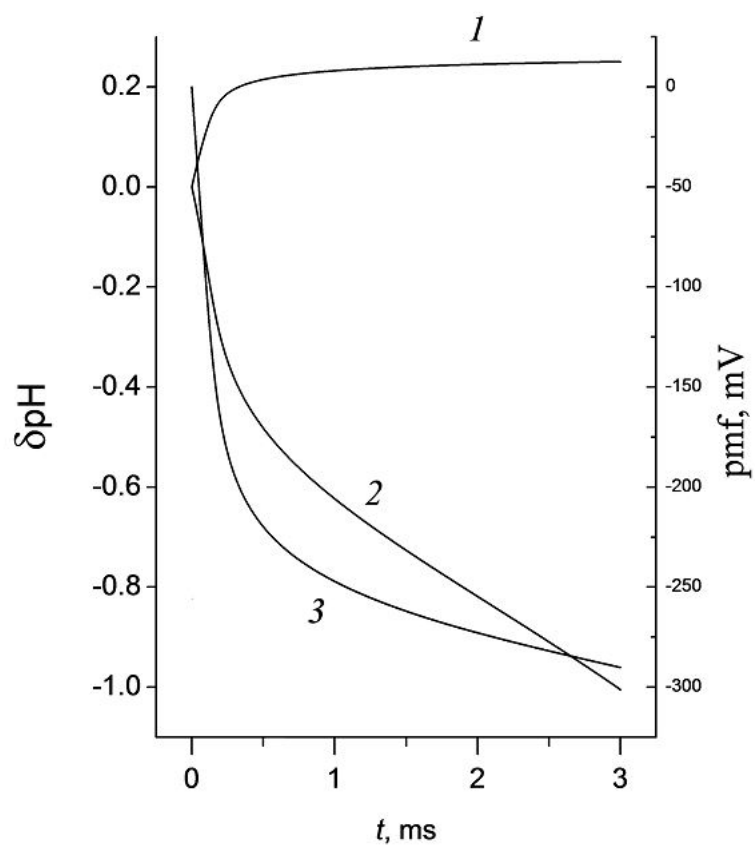**Fig. S8**. Same as **Fig. S7**, buffer concentration is 20 mM.

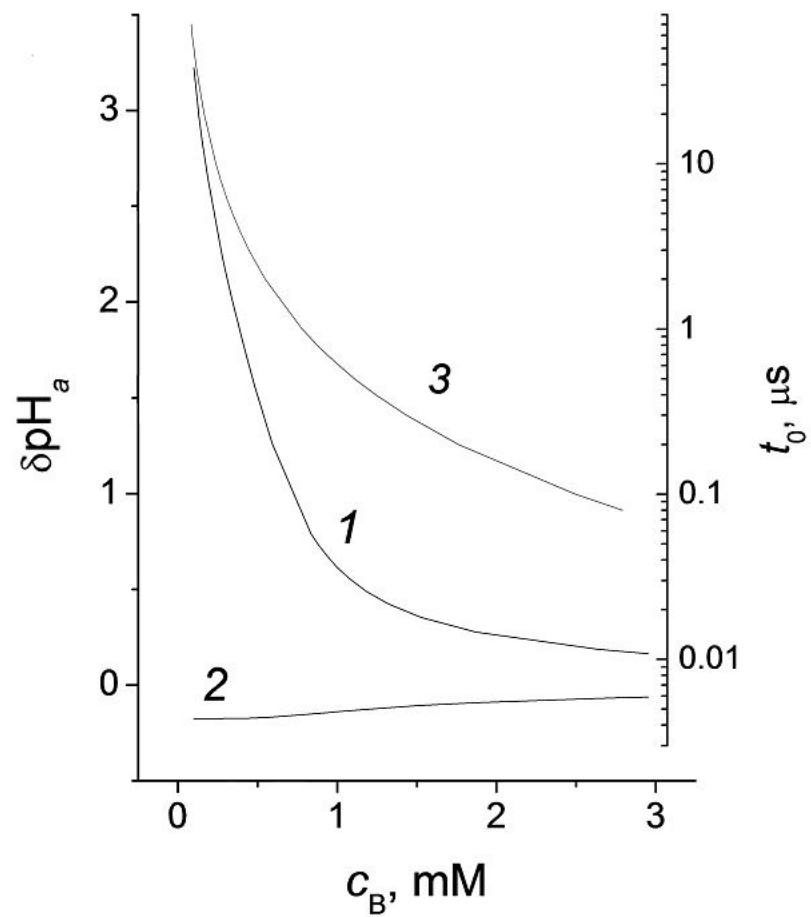**Fig. S9**. Same as **Fig. S7**, buffer concentration is 8 mM.

Fig. S 10. Dependence of maximum $\delta pH_a'$ (*1*), minimum $\delta pH_a''$ (*2*), relaxation time t_0 (*3*) upon free-buffer concentration (*3*) for mitochondrial membrane.

Table S1. Calculated time parameters (mks) for membrane B with various barriers

U/RT	8	10	12
$k_{a,\text{off}}^{-1}$	0.02	0.1	1
t_0'	0.04	0.7	40
t_0''	300	10^4	10^6
τ_{ψ}	20	20	20
$\tau_{a,\text{pH}}$	1	10	70

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