Appendix 2

Relaxation normal mode (2). The rate laws for the constituting steps in scheme (9) are, respectively,

\[
\frac{d[C]}{dt} = -k'_{1}[C] + k'_{1}[C_1] , \quad \frac{d[P_2]}{dt} = -k'_{2}[P_2] + k'_{2}[C_1] ,
\]

(A2.1)

In terms of temporal deviations \( \delta c_i = c_i(t) - \bar{c} \) from the resulting equilibrium values \( \bar{c} \), we obtain, respectively,

\[
\frac{d\delta[C]}{dt} = -k'_{1i}[C] + k'_{1i}[C_1] , \quad \frac{d\delta[P_2]}{dt} = -k'_{2i}[P_2] + k'_{2i}[C_1] .
\]

(A2.2)

Mass conservation implies that \( \delta[C] + \delta[C_1] + \delta[P_2] = 0 \), hence \( \delta[C_1] = -\delta[C] - \delta[P_2] \).

When during the slower transition \( C \ll C_1 \), the assumed equilibration quality \( d[P_2] / dt = 0 \) holds, the relation \( \delta[P_2] = K'_{2i} \cdot \delta[C_1] \) is applicable.

Substitution of \( \delta[C_1] = \delta[P_2] / K'_{2i} \) into Eqs. (A2.2) yields
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\[
\frac{d\delta[C]}{dt} = - \left( k'_1 + k'_{-1} \cdot \frac{1}{1 + K'} \right) \delta[C] = - \frac{1}{\tau_2} \delta[C] , \quad \text{(A2.3)}
\]

where \(1/\tau_2\) is given by Eq.(14) of the text. Since formally

\[
\delta[C] = - \delta([P_2] + [C_1]) = - \frac{\delta([P_2] + K'_2)}{K'_2} \text{ substitution in Eq. (A2.3) leads to}
\]

\[
\frac{d\delta([P_2])}{dt} = - \frac{1}{\tau_2} \cdot \delta[P_2] \quad \text{(A2.4)}
\]

Integration, within the boundaries \(t = 0\) and \(t\), yields

\[
\delta[P_2(t)] = \delta[P_2^0] \cdot \exp\left[\frac{-t}{\tau_2}\right] , \quad \text{(A2.5)}
\]

where \(\delta[P_2(t)] = [P_2(t)] - [\bar{P}]\) and the initial maximum deviation is \(\delta[P_2^0] = [P_2^0] - [\bar{P}]\). The superscript zero refers to \(t = 0\) and the bar in \([\bar{P}]\) to \(t \to \infty\) (equilibrium). Substitution yields

\[
[P_2(t)] - [\bar{P}] = ([P_2^0] - [\bar{P}]) \cdot \exp\left[\frac{-t}{\tau_2}\right] . \quad \text{Since by Eqs. (11), } f_2 = [P_2]/c_0 , \text{ rearrangement leads to Eq. (12) of the text.}
\]

Relaxation normal mode (3). For the scheme (16), the rate law of the slow mode (3) is given by:

\[
\frac{d[P_3]}{dt} = - k'_{-3} [P_3] + k'_3 [P_2] = - k'_{-3} \delta[P_3] + k'_3 \delta[P_2] . \quad \text{(A2.6)}
\]

The rapid equilibrations during this slow mode refer to the rapid mode (2), where \(\delta[P_2]\) is given by \(\delta[P_2] = K_2 (\delta[C] + \delta[C_1]) = - K_2 (\delta[P_2] + \delta[P_3])\). Obviously, the total mass conservation applied to mode (3) reads \(\delta[C] + \delta[C_1] + \delta[P_2] + \delta[P_3] = 0\). Since now \(\delta[P_2] = - \delta[P_3] \cdot K_2 / (1 + K_2)\), substitution in Eq. (A2.6) yields:

\[
\frac{d\delta[P_3]}{dt} = - \left( k'_{-3} + k'_3 \cdot \frac{K_2}{1 + K_2} \right) \delta[P_3] = - \frac{1}{\tau_3} \delta[P_3] , \quad \text{(A2.7)}
\]

where \(1/\tau_3\) is given by Eq. (20) of the text. With Eqs. (5) and (18) integration within \(t = 0\) and \(t\) yields:

\[
\frac{\delta[P_3]}{\delta[P_3^0]} = \frac{Y_3 - Y_3(t)}{Y_3} = 1 - \frac{t - \tau_2 (1 - \exp[\frac{-t}{\tau_2}])}{\tau_3} . \quad \text{(A2.8)}
\]
Analogous to the derivation of Eq. (A2.5), we apply $f_3 = [P_3]/c_0$ and obtain the Eq. (18) of the text. Because the amplitude $Y_3 \gg Y_3(t_f)$, thus $f_3 \gg f_2$, the inequality $K_3' = [P_3]/[P_2] = f_3^1/f_2 \gg 1$ applies, suggesting that the $P_3$-pores are formed at the expense of $P_2$-pores. Hence $k_3' \gg k_2'$ such that $k_3'K_2/(1 + K_2) > k_3'$, accounting for the increase of $1/\tau_3$ with increasing $E$.

Appendix 3

Calculation of the pore conductivity $\lambda_{p,i}$ (the barrier–model). The electrostatic interactions of ions with the low-dielectrics of the wall of an aqueous pore cause an energy barrier for ions entering and passing through the narrow pores. An externally applied field can reduce the barrier and increase the conductivity $\lambda_{p,i}$ of the pore of type $i$ relative to the zero-field value $\lambda_{p,i}^0$. For large induced potential differences, $|\Delta \phi_{ind}| \gg 25 \text{ mV}$, the pore conductivity is given by [26]:

$$\lambda_{p,i} = \frac{\lambda_{ex} + \lambda_{in}}{2} \exp\left[\left(\alpha_{p,i} \cdot n_i \cdot |\Delta \phi_{ind}| - \phi_{im,i}^0\right) \frac{F}{RT}\right], \quad (A3.1)$$

where $\alpha_{p,i} = 1 - RT/(F \cdot \phi_{im,i}^0)$ and $F$ is the Faraday constant. Here, $\lambda_{ex} = 0.17 \text{ mS/cm}$ and $\lambda_{in} = 5 \text{ mS/cm}$ are the external and internal conductivities, respectively. The intrinsic pore barrier potential $\phi_{im,i}^0$ (by image forces) is approximated by $\phi_{im,i}^0 \approx RT \cdot d_{im}/(F \cdot \tau_i)$, [34]. The mean pore radius $\bar{\tau}_i$ is determined from $b_i^0$ in $\left(f_i^0/f_i^0\right) = \left(K_i/K_i^0\right) = \exp\left(b_i^0 \cdot \bar{a}_i^0 \cdot E^2\right)$. Here, the geometrical parameter $n = h/d_{im}$ of the trapezium model for the energy barrier (of both $P_2$ and $P_3$ pores) has been previously given as $n = 0.12$ (lipid vesicle approximation) [26].

See Fig. A1. Substitution of the pole cap approximation $\Delta \phi_{ind} = -\bar{a}_c \cdot f_{x,2,3} \cdot E$, Eq. (4), into Eq. (A3.1) leads to

$$\lambda_{p,i} = \lambda_{p,i}^0 \cdot \exp[\alpha_{p,i} \cdot n \cdot \bar{a}_c \cdot f_{x,i} \cdot E \cdot F/(RT)] \quad , \quad (A3.2)$$
At $T = 293$ K for $\tau_2 = 1.0 \text{ nm}$ we obtain $\phi_{im,2} = 0.13 \text{ V}$ yielding $\lambda_{p,2}^0 = 0.017 \text{ mS/cm}$; for $\tau_3 = 1.5 \text{ nm}$ we obtain $\phi_{im,3}^0 = 0.084 \text{ V}$ yielding $\lambda_{p,3}^0 = 0.1 \text{ mS/cm}$. Using $c_i^0 = c_i / f_i = \alpha_{p,i} \cdot n \cdot \bar{a}_i \cdot F / RT$ for $\tau_2$ and $\alpha_{p,2} = 0.80$ we calculate $c_{2} = 2.8 \text{ cm/kV}$ (Fig. A1). For $\tau_3$ and $\alpha_{p,3} = 0.69$ we obtain $c_{3} = 2.4 \text{ cm/kV}$. In Fig. A1 (d) it is seen that both $\lambda_{p,2}$ and $\lambda_{p,3}$, first steeply increase up to $E \approx 0.8 \text{ kV/cm}$ and then level-off, consistent with the levelling-off of the membrane voltage $U_m$.

Appendix 4

Increase in conductivity due to efflux of electrolyte from the cells. If the in-field conductivity increase would be caused by the efflux of electrolyte through pores, the relative decrease in the cell volume $\Delta V/V_0$ can be calculated from the relative increase in the pellet conductivity $Y(t) = \Delta \lambda/\lambda_0$ (in-field), or from the remaining relative conductivity $Y_{off}(t \to 0)$ immediately after the electric pulse (Fig. 7). Applying Hagen-Poiseuille’s equation [17], for instance to the data at $E = 1.5 \text{ kV/cm}$, where $f_2 = 8.3 \cdot 10^{-4}$, $\tau_2 = 1.0 \text{ nm}$, $f_3 = 3.0 \cdot 10^{-5}$, $\tau_3 = 1.5 \text{ nm}$, $\Delta t = t_E = 1 \text{ ms}$ and $V_0 = 4/3 \cdot \pi \cdot \bar{a}_i^3$, it is found that

$$\frac{\Delta V}{V_0} = -\frac{9}{160} \cdot \varepsilon_0 \cdot \varepsilon_r \cdot E^2 \cdot (f_2 \cdot \bar{E}_2^2 + f_3 \cdot \bar{E}_3^2) \cdot \Delta t = -2.1 \cdot 10^{-8},$$

(A4.1)

where $\eta = 1.002 \cdot 10^{-3} \text{ kg m}^{-1} \text{s}^{-1}$ is the viscosity of water at $T = 293$ K (20°C). The value (without sign) $\Delta V/V_0 = -((\Delta \lambda_2 + \Delta \lambda_3(t_E))/\lambda_0)/\zeta = -0.015$ calculated with $Y_2$ and $Y_3$ is much larger than the “theoretical” value $\Delta V/V_0 = -2.1 \cdot 10^{-8}$ (Eq. A4.1); here $\zeta = \lambda_{max} / \lambda_0 = 55$ is the factor for maximum release of electrolyte from cells and $\lambda_0 = 0.09 \text{ mS/cm}$. The value of the remaining relative conductivity $Y_{off}(t \to 0) \approx \Delta \lambda_3(t_E)/\lambda_0 = 0.2$, however, would require
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\[
\Delta V / V_0 = -\Delta \lambda_3(t_E) / (\lambda_0 \cdot \varsigma) = -3.6 \cdot 10^{-3}, \text{ which is much larger than } -2.1 \cdot 10^{-8} (\text{Eq. A4.1}).
\]

Hence, finite release of the electrolyte can cause neither phases \( Y_2 \) and \( Y_3 \), respectively, nor \( Y_{\text{off}}(t \to 0) \).

Appendix 5

Conductivity Increase due to Cell Electroelongation. The pellet conductance \( \Lambda = \lambda \cdot A / l \) is proportional to the geometrical cross section \( A \) for the ion current. If the relative increase \( (\Delta \lambda_2 + \Delta \lambda_3) / \lambda_0 = \Delta \Lambda / \Lambda_0 = (\Lambda - \Lambda_0) / \Lambda_0 \) in the pellet conductivity were due to cell electroelongation, e.g., under Maxwell stress, it would be proportional to the relative increase in the cross section \( \Delta A / A_0 = (A - A_0) / A_0 \) in the direction of cell elongation, where \( \Lambda \) and \( \Lambda_0 \) are the conductances in the presence of \( E \) and at \( E = 0 \), respectively (Fig. A2). Explicitly,

\[
\frac{\Delta \lambda}{\lambda_0} = \frac{\Delta \lambda_3 + \Delta \lambda_2}{\lambda_0} = \frac{\Delta A}{A_0}, \quad (A5.1)
\]

where \( A_0 = L^2 - \pi a_c^2 \) is the original cross section and \( A = L^2 - \pi b^2 \) is the cross section increased by the electroelongation (Fig. A2). Note that at a volume fraction \( f_c = 0.6 (< 0.74) \) the cell envelopes are on average not in tight contact such that there is a finite value of the ion current through the cross section \( A_0 \). Here \( b \) and \( c \) are the half axes of the ellipsoid. The mean distance \( L \) between the cell centres is calculated from the cell density \( \rho = N / V \) according to \( L = 1 / \rho^{1/3} \). For \( f_c = 0.6, \rho = 2.4 \cdot 10^8 \text{ cm}^{-1} \), hence \( L = 16 \mu\text{m} \). The value of \( b \) required to rationalize the relative increase \( \Delta \lambda / \lambda_0 = (\Delta \lambda_3(t_E) + \Delta \lambda_2) / \lambda_0 = (0.2 + 0.6) = 0.8 \) at \( E = 1.5 \text{ kV/cm} \) and \( t = t_E \) is given by

\[
b = \sqrt{a_c^2 - \Delta \lambda / \lambda_0 \cdot (L^2 - \pi \cdot a_c^2) / \pi} = 5.9 \mu\text{m} \quad (A5.2)
\]

This value requires that the degree of elongation is \( p_e = c / b = 1.87 \), calculated from:

\[
b = \bar{a}_c \cdot \sqrt{\frac{1 + \Delta V(p_e) / V_0}{p_e}} \quad (A5.3)
\]
where $\Delta V/V_0$, is given by [8,9]:

$$\frac{\Delta V}{V_0} = \left( \frac{\arcsin \sqrt{1-p_s^2} + p_s^2}{2 \sqrt{p_s^2 - 1}} \right)^{-2/3} \cdot p_s^2 - 1 = -0.088 \quad (A5.4)$$

This value is a factor of about 6 larger than the “experimentally” justifiable value of $\Delta V/V_0 = -((\Delta \lambda_2 + \Delta \lambda_3(t_E))/\lambda_0)/\zeta = -0.015$ (see the appendix 4). Therefore, elongation of cells due to efflux is not suited to rationalize the conductivity phases 2 and 3. Additionally, applying $p_s = 1.87$, we obtain for the main half axis:

$$c_s = p_s \cdot b = \bar{a}_c \sqrt{2/\left( p_s^{-2} + \arcsin(\sqrt{1-p_s^2})/\sqrt{p_s^2 - 1} \right)} = 11\, \mu m. \quad (A5.5)$$

The distance $L = 16\, \mu m$ would be not sufficient to permit an increase of the main axis by $2c_s = 22\, \mu m > L$. Therefore, also cell elongation at constant surface area can not rationalize the experimental conductivity increase. Alternatively, if the cell volume would remain constant, the value $\Delta \lambda/\lambda_0 = 0.8$ requires the relative increase $\Delta S/S_0$ in the cell surface area at constant volume (subscript v) by [9]:

$$\frac{\Delta S}{S_0} = \frac{p_v^{2/3}}{2} + \frac{p_v^{1/3} \cdot \arcsin(\sqrt{1-p_v^2})}{2 \cdot \sqrt{1-p_v^2}} - 1 = 0.082, \quad (A5.6)$$

where $p_v = c_v/b = 2.05$, $c_v = \bar{a}_c^3/b^2 = 12.1\, \mu m$ and $b = 5.9\, \mu m$ (see Eq. A5.2). The available relative increase in the membrane surface by electropores at $E = 1.5\, kV/cm$ and $t = t_E$ is, however, $f_2 + f_3 = 8.6 \cdot 10^{-4} << 0.082$ (see the appendix 4). Additionally, the main cell axis $2 \cdot c_v = 24.2\, \mu m$ is much larger than the available distance $L = 16\, \mu m$. Hence, cell electrolengation at constant volume can not be the origin of the conductivity phases (2) and (3). Alternatively, if we assume that solely the conductivity phase (3) refers to cell elongation at the expense of the increase in surface area due to the electropores of the phase (2), the small half-axis $b_3$ would be:

$$b_3 = \sqrt{\bar{a}_c^2 - \Delta \lambda_3/\lambda_0 \cdot (L^2 - \pi \cdot \bar{a}_c^2)}/\pi = 7.1\, \mu m \quad . \quad (A5.7)$$
Because $c_v = \frac{a^3}{b^2} = 8.3 \, \mu m$, we obtain $p_v = c_v / b_1 = 1.16$ and $\Delta S / S_0 = 3.7 \cdot 10^{-3}$. Again,

$$\Delta S / S_0 = 3.7 \cdot 10^{-3} > f_2 = 8.3 \cdot 10^{-4} \, \text{ (at } E = 1.5 \, \text{kV/cm and } t = t_E).$$

Although $2c_v = 16.6 \, \mu m \geq L = 16 \, \mu m$, the inequality $\Delta S / S_0 > f_2$ by a factor about 5 indicates that the conductivity phase (3) is not related to cell elongation.

**References**

Fig. A1. Theoretical models for the pore states $P_2$ (a) and $P_3$ (b), respectively. The entrance parameter is $n = h/d_m = 0.12$, the membrane thickness $d_m = 5$ nm and the different pore radii are $\bar{r}_2 = 1.0$ nm ($P_2$ - pores) and $\bar{r}_3 = 1.5$ nm ($P_3$ - pores), respectively.
Fig. A2. Model for the relative increase $\Delta A/A_0$ in the cross section $A$ for the ion current along the field lines $E$, due to electroporative electro-elongation of the cells in the pellet; (a) cells ($\bar{a}_c = 7.5 \, \mu m$) in the absence of the electric field, and (b) elongated cells in the presence of the electric field $E$, causing an increase $\Delta A = A - A_0$ of the cross section of electric current from $A_0$ at $E = 0$ to $A$ at $E > 0$. For $RCF = 90 \, g$ the mean distance between the cell centers is $L = 16 \, \mu m$; see also the legend to Fig. 1 of the main text.