# Hv1 Proton Channel Opening Is Preceded by a Voltage-independent Transition

Carlos A. Villalba-Galea[1,*]

[1]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, Virginia

## SUPPLEMENTARY INFORMATION

### *Kinetic analysis*

Current recordings were fitted to a first-order ordinary differential equation system representing a four-state sequential model (Fig. 2A) using MatLab. The transition rates were considered to exponentially depend on the membrane potential. The forward (α) and backward (β) rates were defined as:

$$\alpha_i = \alpha_{0i} e^{z_{\alpha i} V/kT} \qquad (1)$$

$$\beta_i = \beta_{0i} e^{-z_{\beta i} V/kT} \qquad (2)$$

where, $\alpha_{0i}$ and $\beta_{0i}$ are the rate constants at 0 mV of the *i*-th transition; $z_\alpha$ and $z_\beta$ are the charge (valence) associated with the *i*-th transition; *V* is the membrane potential; *k* and *T* are the Boltzmann constant and the absolute temperature. In terms of differential equations, a sequential 4-state model (below) can be described as follows:

$$C_1 \underset{\beta_1}{\overset{\alpha_1}{\rightleftharpoons}} C_2 \underset{\beta_2}{\overset{\alpha_2}{\rightleftharpoons}} C_3 \underset{\beta_3}{\overset{\alpha_3}{\rightleftharpoons}} O$$

$$\frac{dC_1}{dt} = -\alpha_1 C_1 + \beta_1 C_2$$

$$\frac{dC_2}{dt} = \alpha_1 C_1 - (\beta_1 + \alpha_2) C_2 + \beta_2 C_3$$

$$\frac{dC_3}{dt} = \alpha_2 C_2 - (\beta_2 + \alpha_3) C_3 + \beta_3 O$$

$$\frac{dO}{dt} = \alpha_3 C_3 - \beta_3 O$$

Rearranging these differential equation into matrix form yields

$$\frac{\partial}{\partial t}\begin{pmatrix} C_1 \\ C_2 \\ C_3 \\ O \end{pmatrix} = \begin{pmatrix} -\alpha_1 & \beta_1 & 0 & 0 \\ \alpha_1 & -(\beta_1+\alpha_2) & \beta_2 & 0 \\ 0 & \alpha_2 & -(\beta_2+\alpha_3) & \beta_3 \\ 0 & 0 & \alpha_3 & -\beta_3 \end{pmatrix}\begin{pmatrix} C_1 \\ C_2 \\ C_3 \\ O \end{pmatrix}$$

In general, these kinds of systems can be expressed as

$$\frac{\partial}{\partial t}\vec{S} = \boldsymbol{A}\vec{S} \qquad (3)$$

$$\vec{S} = \begin{pmatrix} C_1 \\ C_2 \\ C_3 \\ O \end{pmatrix};\ \boldsymbol{A} = \begin{pmatrix} -\alpha_1 & \beta_1 & 0 & 0 \\ \alpha_1 & -(\beta_1+\alpha_2) & \beta_2 & 0 \\ 0 & \alpha_2 & -(\beta_2+\alpha_3) & \beta_3 \\ 0 & 0 & \alpha_3 & -\beta_3 \end{pmatrix}$$

where, $\vec{S}$ is a column vector (state vector) the elements of which correspond to the population fraction of each state and $\boldsymbol{A}$ is the matrix (state matrix) containing the rate constants governing the reaction. The general solution of this system has the form

$$\vec{S}(t) = \vec{\eta}e^{\lambda t} \quad (4)$$

From this proposed solution, the derivative of the state vector is

$$\frac{\partial}{\partial t}\vec{S}(t) = \lambda\vec{\eta}e^{\lambda t} \qquad (5)$$

Replacing 4 and 5 in 3 yields

$$\lambda\vec{\eta}e^{\lambda t} = \boldsymbol{A}\vec{\eta}e^{\lambda t}$$

Rearranging this equation yields

$$\boldsymbol{A}\vec{\eta}e^{\lambda t} - \lambda\vec{\eta}e^{\lambda t} = \vec{0} \quad \Longrightarrow \quad (\boldsymbol{A} - \lambda\boldsymbol{I})\vec{\eta}e^{\lambda t} = \vec{0} \qquad (6)$$

where, $\boldsymbol{I}$ is an identity matrix of the same dimension than $\boldsymbol{A}$. Because $e^{\lambda t} > 0$, equation 7 becomes

$$(\boldsymbol{A} - \lambda\boldsymbol{I})\vec{\eta} = \vec{0} \qquad (7)$$

In this latter equation, the scalar $\lambda$ and the vector $\vec{\eta}$ are known as the eigenvalue and eigenvector of the matrix $\boldsymbol{A}$. In general, there are as many eigenvalue-eigenvectors pairs as elements in the state vector. For this particular case, there are 4 eigenvalues and eigenvectors. Finally, the general solution of this kind of system will be:

$$\vec{S}(t) = \sum_{i=1}^{n} c_i \vec{\eta}_i e^{\lambda_i t} \quad (8)$$

where, $n$ is the number of states and $c_i$ are integration constants. To calculate these latter values, Equation 8 was evaluated at time zero, making the exponential equal to 1. Thus,

$$\vec{S}(0) = \sum_{i=1}^{n} c_i \vec{\eta}_i \qquad (9)$$

At time 0, the values for $\vec{S}(t)$ correspond to those of the initial condition. Therefore, the values of $c_i$ can be readily calculated, since Equation 9 is an algebraic system of $n$ equation with $n$ unknown ($c_i$). Replacing the calculated $c_i$ in equation 8 gives an expression that yields by fractional population of each state of the model in time.

Finally, having found $\vec{S}(t)$ and the fraction of open channels ($O(t)$) which is the last element of the vector $\vec{S}(t)$, current can be calculated using the following expression:

$$I(t) = O(t) G_{MAX} (V - V_{H^+})$$

where, $G_{MAX}$ is the maximum conductance for protons, $V$ is the membrane potential, and $V_{H^+}$ is the reversal potential for protons.

***Table S1***

| | $pH_I$ 4.5 (*n*=5) | | $pH_I$ 5.5 (*n*=5) | | $pH_I$ 6.5 (*n*=8) | |
|---|---|---|---|---|---|---|
| | mean | S.D. | mean | S.D. | mean | S.D. |
| $\alpha_{0,1}$ | $4.5940x10^{-4}$ | $2.9x10^{-4}$ | $2.1092x10^{-4}$ | $8.0x10^{-5}$ | $3.6745x10^{-4}$ | $2.8x10^{-4}$ |
| $\beta_{0,1}$ | $3.7759x10^{-7}$ | $1.0x10^{-7}$ | $8.8539x10^{-7}$ | $2.8x10^{-7}$ | $3.6000x10^{-6}$ | $1.6x10^{-6}$ |
| $z_{\alpha 1}$ | $7.4982x10^{-1}$ | $2.6x10^{-1}$ | $4.8815x10^{-1}$ | $7.3x10^{-2}$ | $1.6265x10^{-1}$ | $7.4x10^{-2}$ |
| $z_{\beta 1}$ | 2.0726 | $5.1x10^{-1}$ | 2.0954 | $5.9x10^{-1}$ | 2.4175 | $1.6x10^{-1}$ |
| $\alpha_{0,2}$ | $1.3196x10^{-3}$ | $6.4x10^{-4}$ | $2.4286x10^{-4}$ | $1.6x10^{-4}$ | $3.943x10^{-5}$ | $2.8x10^{-5}$ |
| $\beta_{0,2}$ | $4.4267x10^{-3}$ | $2.6x10^{-3}$ | $2.0368x10^{-2}$ | $1.1x10^{-2}$ | $1.1001x10^{-1}$ | $5.0x10^{-2}$ |
| $z_{\alpha 2}$ | 1.0120 | $1.3x10^{-1}$ | $9.8428x10^{-1}$ | $1.5x10^{-1}$ | $9.2826x10^{-1}$ | $8.4x10^{-2}$ |
| $z_{\beta 2}$ | 2.0705 | $2.8x10^{-1}$ | 1.8043 | $9.9x10^{-1}$ | 1.5173 | $2.0x10^{-1}$ |
| $\alpha_{0,3}$ | $1.5538x10^{-2}$ | $3.8x10^{-3}$ | $1.1932x10^{-2}$ | $6.0x10^{-3}$ | $6.6033x10^{-3}$ | $1.5x10^{-3}$ |
| $\beta_{0,3}$ | $8.6658x10^{-4}$ | $6.3x10^{-4}$ | $1.5240x10^{-3}$ | $3.8x10^{-4}$ | $1.654x10^{-3}$ | $6.7x10^{-4}$ |
| $z_{\alpha,3}$ | $1.3479x10^{-5}$ | $1.0x10^{-5}$ | $1.4769x10^{-5}$ | $6.1x10^{-6}$ | $2.965x10^{-5}$ | $1.2x10^{-5}$ |
| $z_{\beta,3}$ | $1.6309x10^{-7}$ | $1.1x10^{-7}$ | $5.1742x10^{-7}$ | $2.2x10^{-7}$ | $2.7365x10^{-7}$ | $1.2x10^{-7}$ |

Table S1.- Fitted average parameters for forward and backward transitions. Values for $\alpha_{0,1}$ and $\beta_{0,1}$ are in $ms^{-1}$. Values for z are in $e^-$.

***Figure S1***

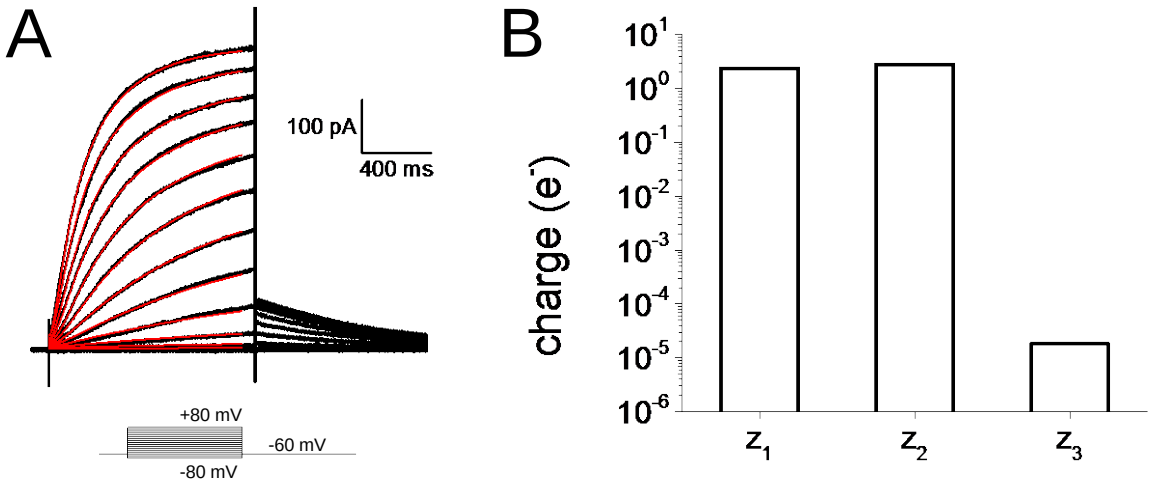


Figure S1- Simultaneous fitting of activating currents to a multi-state model. (*A*) A four-state sequential model was used to fit the activation kinetics of Hv1, where the first 3 states of the model are non-conductive (closed, $C_1$-$C_3$). All forward ($\alpha_i$) and backward ($\beta_i$) transition rates are considered essentially voltage-sensitive (Eqs. 1 and 2 in Materials and Methods). (*B*) Simultaneous fitting of currents traces recorded at potentials ranging from -40 to +80 mV at $pH_I$ 4.5 and $pH_O$ 6.5. Traces between -80 and -50 were not included in the fitting process. (*C*) There were two parameters for each transition rate, one is the rate at 0 mV ($\alpha_0$ or $\beta_0$, accordingly) and the other is the apparent charge associated with the rate ($z_{\alpha 0}$ and $z_{\beta 0}$, accordingly). The total charge of the transitions between two states was obtained by the sum of the individuals charges. As shown in the graph, the gating charge associated with the first and second transitions, $z_1$ and $z_2$, respectively, displayed values of ~2.5 $e^-$ each, while the last transition showed about $2x10^{-5}$ $e^-$.

***Figure S2***

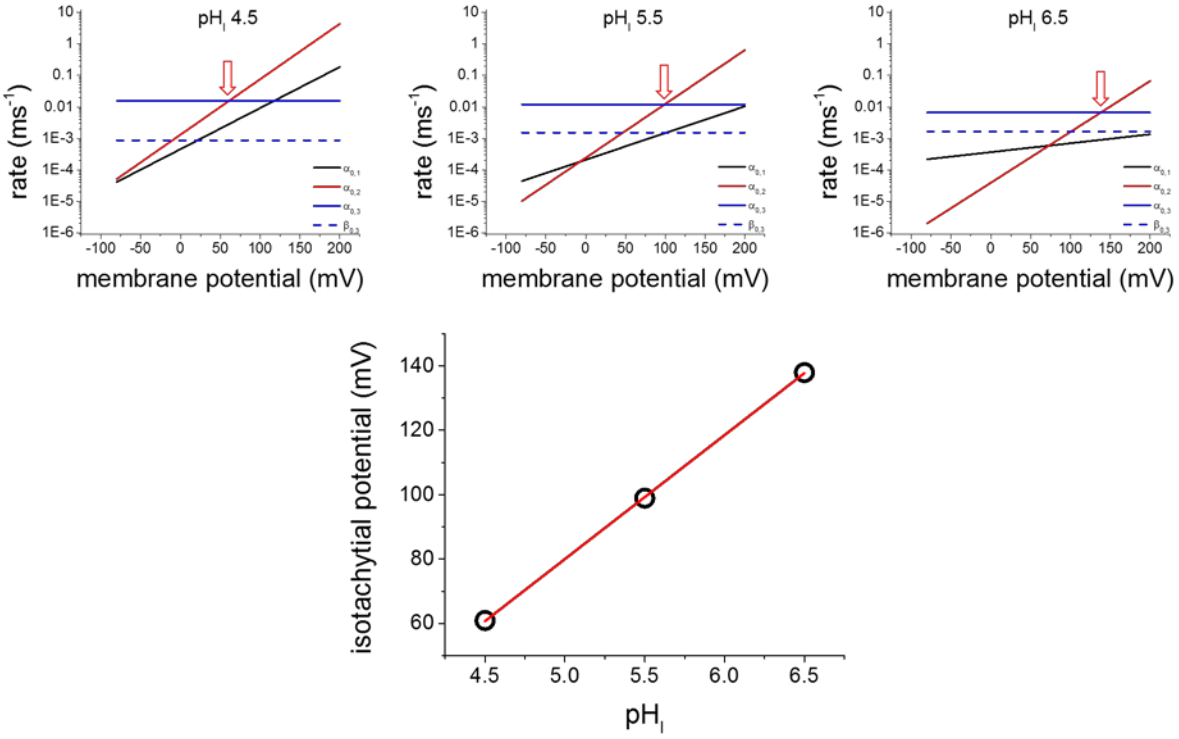


Figure S2.- *Top*) Calculated values of $\alpha_1$ (black), $\alpha_2$ (red) and $\alpha_3$ (blue) as a function of the membrane potential at $pH_I$ 4.5 (left), 5.5 (center) and 6.5 (right). *Bottom*) Plot of the membrane potential at which $\alpha_2$ equals $\alpha_3$ as a function of $pH_I$. At voltages above these potentials, or isotachytial potentials ($V_{IT}$; from the Greek words ίσος (isos) = equal and ταχύτητα (tachýtita) = speed), the last transition can be considered to be rate-limiting. Noteworthy, a linear fit of $V_{IT}$ versus pHI plot yielded a slope of 38.5±0.3 mV/Δ$pH_I$, which matches the $pH_I$ sensitive of the overall voltage-dependence of Hv1. The $V_{IT}$ was calculated using the equation $V_{IT} = \frac{kT \ln\left(\alpha_{0,2}/\alpha_{0,3}\right)}{z_{\alpha,3} - z_{\alpha,2}}$.