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Severed Channels Probe Regulation of Gating of CFTR by its Cytoplasmic Domains

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Applying the Schwarz Criterion to the Distribution of Open Times to Distinguish Between One or More Open States, in the Case of Only One Closed State

Consider a gating scheme with 1 closed state and any number of open states. Let the data consist of M openings (without restricting generality, assume that the series starts and ends with closed events). The events list consists of the series of dwell times $t_{c,1}$, $t_{o,1}$, $t_{c,2}$, $t_{o,2}$,...$t_{o,M}$, $t_{c,M+1}$. The likelihood of such a series of dwell times (Fredkin, D.R., M. Montal, and J.A. Rice. 1985. Identification of aggregated Markovian models: application to the nicotinic acetylcholine receptor. In Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack Kiefer. L.M. Le Cam and R.A. Ohlsen, editors. Wadsworth Publishing Co., Belmont, CA. 269–289) is:

$$L = \mathbf{p}_c^T(0)\prod_{i=1}^{M}(e^{\mathbf{Q}_{cc}t_{c,i}}\mathbf{Q}_{co}e^{\mathbf{Q}_{oo}t_{o,i}}\mathbf{Q}_{oc})e^{\mathbf{Q}_{cc}t_{c,M+1}}\mathbf{Q}_{co}\mathbf{1}_o, \text{ where } \mathbf{p}_c^T(0) = \frac{\mathbf{p}_o^T(\infty)\mathbf{Q}_{oc}}{\mathbf{p}_o^T(\infty)\mathbf{Q}_{oc}\mathbf{1}_c}, \tag{S1}$$

where $\mathbf{Q}_{cc}$, $\mathbf{Q}_{oo}$, $\mathbf{Q}_{co}$, and $\mathbf{Q}_{oc}$ are submatrices of the state matrix $\mathbf{Q}$, and $\mathbf{p}_o^T(\infty)$ and $\mathbf{p}_c^T(\infty)$ (see below) are row vectors of steady state occupancy probabilities, and $\mathbf{1}_o$ and $\mathbf{1}_c$ summation vectors, for the sets of closed and open states, respectively.

Because there is only one closed state, the following identities apply:

$$\mathbf{Q}_{co} = \mathbf{Q}_{co}\mathbf{1}_o\mathbf{p}_o^T(0), \text{ where } \mathbf{p}_o^T(0) = \frac{\mathbf{p}_c^T(\infty)\mathbf{Q}_{co}}{\mathbf{p}_c^T(\infty)\mathbf{Q}_{co}\mathbf{1}_o}. \tag{a}$$

This is because $\mathbf{p}_c^T(\infty)$ is simply a scalar number, and so:

$$\mathbf{p}_o^T(0) = \frac{\mathbf{Q}_{co}}{\mathbf{Q}_{co}\mathbf{1}_o} \text{ and } \mathbf{Q}_{co}\mathbf{1}_o\mathbf{p}_o^T(0) = \frac{(\mathbf{Q}_{co}\mathbf{1}_o)\mathbf{Q}_{co}}{\mathbf{Q}_{co}\mathbf{1}_o} = \mathbf{Q}_{co}.$$

$$\mathbf{Q}_{oc} = \mathbf{Q}_{oc}\mathbf{1}_c\mathbf{p}_c^T(0), \text{ [since } \mathbf{1}_c\mathbf{p}_c^T(0) \text{ is simply the (scalar) number 1].} \tag{b}$$

Substituting from Eqs. a and b into Eq. S1, the likelihood of the whole time series becomes

$$\begin{aligned} L &= \mathbf{p}_c^T(0)\prod_{i=1}^{M}[e^{\mathbf{Q}_{cc}t_{c,i}}\mathbf{Q}_{co}\mathbf{1}_o\mathbf{p}_o^T(0)e^{\mathbf{Q}_{oo}t_{o,i}}\mathbf{Q}_{oc}\mathbf{1}_c\mathbf{p}_c^T(0)]e^{\mathbf{Q}_{cc}t_{c,M+1}}\mathbf{Q}_{co}\mathbf{1}_o \\ &= \prod_{i=1}^{M}\{[\mathbf{p}_c^T(0)e^{\mathbf{Q}_{cc}t_{c,i}}\mathbf{Q}_{co}\mathbf{1}_o][\mathbf{p}_o^T(0)e^{\mathbf{Q}_{oo}t_{o,i}}\mathbf{Q}_{oc}\mathbf{1}_c]\}[\mathbf{p}_c^T(0)e^{\mathbf{Q}_{cc}t_{c,M+1}}\mathbf{Q}_{co}\mathbf{1}_o] \\ &= \left[\prod_{i=1}^{M+1} pdf_c(t_{c,i})\right]\cdot\left[\prod_{i=1}^{M} pdf_o(t_{o,i})\right]. \end{aligned} \tag{S2}$$

The log of the likelihood, $LL = \ln(L)$ then becomes:

$$LL = \left\{\sum_{i=1}^{M+1}\ln[pdf_c(t_{c,i})]\right\} + \left\{\sum_{i=1}^{M}\ln[pdf_o(t_{o,i})]\right\} = LL_c(\mathbf{t}_c) + LL_o(\mathbf{t}_o), \tag{S3}$$

where $\mathbf{t}_c$ and $\mathbf{t}_o$ represent the separated vectors of closed and open dwell times, respectively. Hence, the log likelihood of the whole time series separates into the sum of the log likelihoods of the closed and open times. Moreover, the latter two log likelihoods depend on disjoint subsets of parameters: let r_{co} denote the sum of all rates leading from the unique closed state to all adjacent open states. LL_o does not depend on the size of r_{co}, while LL_c depends solely on r_{co} through:

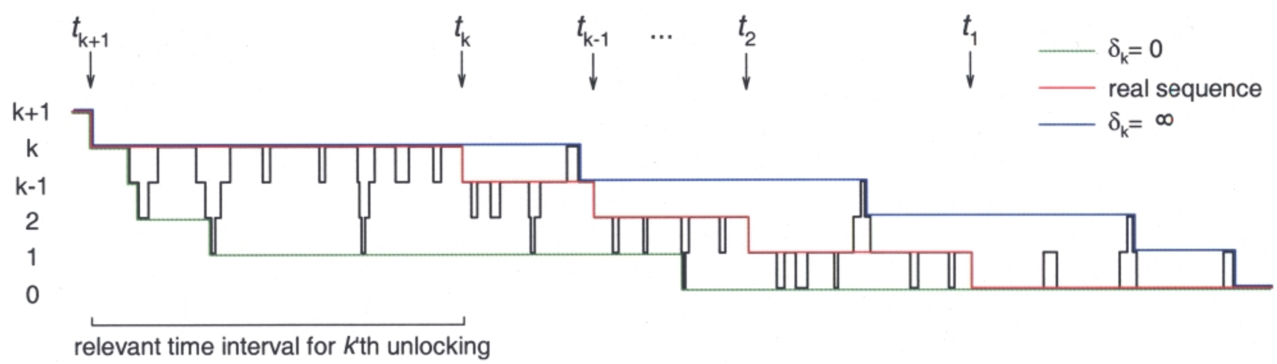
$$LL_c = \sum_{i=1}^{M+1} \ln(r_{co} e^{-r_{co} t_{c,i}}).$$

If Θ denotes the gating scheme with its rate constants, then optimization of LL with respect to Θ can be achieved by separately optimizing LL_c with respect to r_{co}, and LL_o with respect to $\Theta \cap \overline{\{r_{co}\}}$. Moreover, LL_c at the peak is the same for any two schemes with one closed state: $LL_c(\mathbf{t}_c|\Theta_2) = LL_c(\mathbf{t}_c|\Theta_1)$, from which:

$$\Delta LL = LL(\mathbf{t}|\Theta_2) - LL(\mathbf{t}|\Theta_1) = LL_o(\mathbf{t}_o|\Theta_2) - LL_o(\mathbf{t}_o|\Theta_1) = \Delta LL_o. \tag{S4}$$

Thus, ΔLL_o, the log of the likelihood ratio for M open times, corresponds to ΔLL for a time series of $2M(+1)$ events for any pair of schemes that both have one closed state.

Reconstruction of the Time Sequence of Unlocking Events



Task. N channels, N_o locked initially, reconstruct time sequence $t_{N_o} < t_{N_o - 1} < \ldots < t_k < \ldots t_1$ of unlocking events.

Strategy. The kth channel is considered to become unlocked at the start of a gap to lower conductance levels of duration exceeding a defined cutoff δ_k.

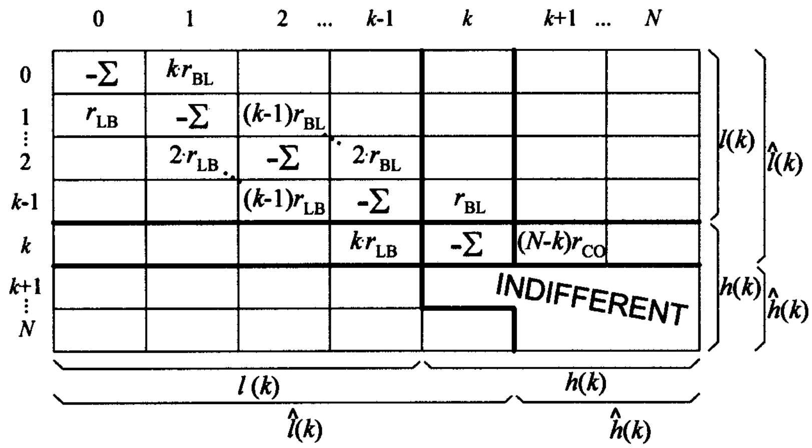
Problem. How to choose a cutoff δ_k separately for each conductance level, such that the probabilities of assigning a particular unlocking event too early or too late are equal?

Single-channel model. Consider a patch with several (5–20) channels, each of which can transit from a closed state (C) to either a relatively short-lived open state (O), or a very long-lived conducting state (L, for "locked open"). State L also communicates with a brief blocked (B, nonconducting) state. At steady state, the rates are suddenly changed such that rate $r_{CL} = 0$, and r_{CO} becomes extremely small. The system can now be described by the scheme:

$$[B \underset{r_{LB}}{\overset{r_{BL}}{\leftrightarrow}} L \overset{r_{LC}}{\rightarrow} C \underset{r_{OC}}{\overset{r_{CO}}{\leftrightarrow}} O] \times N,$$

where, initially, N_o channels are in L, and N–N_o in C. First, obtain estimates of r_{LB} and r_{BL} from sections of record with just one channel locked ($r_{LB} \approx 3\ s^{-1}$, $r_{BL} \approx 13\ s^{-1}$). Second, obtain estimate of apparent reopening rate r_{CO} after all channels are unlocked ($r_{CO} < 0.005\ s^{-1}$ typically).

Macroscopic model. Consider time interval after $(k+1)$th channel has unlocked, but kth channel has not unlocked yet. During this time interval, describe macroscopic system by simplified two-dimensional macroscopic state vector (n_S, n_A), corresponding to the number of shut (closed or blocked) and active (locked or open) channels, respectively ($n_S + n_A = N$). Hence, the number of macroscopic states is $N + 1$. Index macroscopic states in increasing order of n_A, build state-matrix $\mathbf{Q}(k)$. Neglecting the effect of channel reopening within flickery gaps ($r_{CO} << r_{BL}$), $\mathbf{Q}$(k) simplifies to:



Define $l(k)$ as the set of macroscopic states indexed from 0 to $k-1$ (i.e., $n_A = 0, 1,\ldots$or $k-1$). Similarly, define $h(k) = \{k, k+1,\ldots N\}$, $\hat{l}(k) = \{0, 1,\ldots k\}$, $\hat{h}(k) = \{k+1,\ldots N\}$, and partition $\mathbf{Q}$ correspondingly.

Errors. $P_1(\mathrm{k}) = P$ (unlocking of kth channel assigned too late) $= P$ [leaving from $\hat{l}(k-1)$ to $\hat{h}(k-1)$ occurs between t_k and $t_k + \delta_k$] =

$$1-[0, \ldots, 0, 1]e^{\mathbf{Q}_{\hat{l}\hat{l}}(k-1)\delta_k}\mathbf{1}_{\hat{l}(k-1)}.$$

$P_2(\mathrm{k}) = P$ (unlocking of k'th channel assigned too early) $= 1 - P$ (unlocking of k'th channel is not assigned too early) $= 1 - P$ (all leavings from $h(k)$ to $l(k)$ between time t_{k+1} and t_k are shorter than δ_k] $= 1 - \{P$ [a leaving from $h(k)$ to $l(k)$ between time t_{k+1} and t_k is shorter than δ_k]$\}^{\upsilon}$ =

$$1-\{1-[0, \ldots, 0, 1]e^{\mathbf{Q}_{ll}(k)\delta_k}\mathbf{1}_{l(k)}\}^{\upsilon},$$

where υ is the expected number of leavings from $h(k)$ to $l(k)$ between time t_{k+1} and t_k. Both $P_1(\mathrm{k})$ and $P_2(\mathrm{k})$ are functions of the cutoff time δ_k. Search for δ_k such that calculated $P_1(\mathrm{k})$ equals $P_2(\mathrm{k})$. The total probability of misassigning t_k is $P(\mathrm{k}) = P_1(\mathrm{k})+P_2(\mathrm{k})$.

Iteration. $\upsilon \approx r_{LB}/r_{LC}$, but r_{LC} is not known a priori. Solve by iteration: in the first round, set $\upsilon^{(1)} = 1$, calculate $\delta_k^{(1)}$ (as above), and then find $t_k^{(1)}$ for each $k = N_o,\ldots 1$. Estimate r_{LC} by

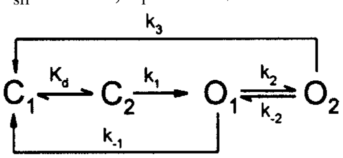
$$r_{LC}^{(1)} = N_o/\sum_{k=1}^{N_o} t_k^{(1)}.$$

Repeat by setting $\upsilon^{(i)} = (r_{LB}/r_{LC}^{(i-1)})$. Stop iteration if $t_k^{(i)} = t_k^{(i-1)}$ for all $k = N_o,\ldots 1$.

Results. Time sequence $t_{N_o} < t_{N_o-1} < \ldots < t_k < \ldots t_1$ of unlocking events, error estimate $P(\mathrm{k})$ for each conductance level, estimate for r_{LC} (unlocking rate, estimated from the average wait time for unlocking).

How Applicable Is a Closed-Open-Blocked Fit to Scheme I?

A valid concern is that Scheme I (itself a four-state scheme), extended by short-lived ("flickery") closed states, is not readily seen to reduce to a simple three-state, Closed-Open-Blocked (C-O-B) scheme. How do the burst durations determined from the multichannel C-O-B fit compare to the fits to the distributions of single-channel burst durations (particularly for constructs 835+837, cut-ΔR, and Flag-cut-ΔR, which showed two components in the distributions of burst durations)? This is justified as follows. The C-O-B fit merely separates out short flickery closures (i.e., it is the multichannel implementation of single-channel burst analysis), and it works because the mean duration of flickery closures (~10 ms) is >100-fold shorter than that of interburst closures, while the length of the mean burst durations varied only less than fourfold. So, for the purpose of separating flickery from interburst closures, pooling the open states is certainly justifiable. This assertion is supported by the good agreement between τ_b extracted from multichannel fits (see Table I in text), and the means of the distributions of bursts obtained from isolated openings (Fig. 8). But since we were also concerned about the validity of this approach, we tested it extensively on simulated traces (as briefly mentioned in the DISCUSSION). As an example, we took Scheme I with rates (s^{-1}) $k_1 = 0.7$, $k_{-1} = 3.5$, $k_2 = 0.3$, $k_{-2} = 0.2$, $k_3 = 0.9$, a typical set for cut-ΔR channels (rates between C_1 and C_2 were set to simulate saturating [ATP], $r_{C1,C2} = 10\ s^{-1}$, $r_{C2,C1} = 0.1\ s^{-1}$). We next extended this scheme with blocked states B_1 and B_2, communicating with O_1 and O_2, respectively, and set $r_{O1,B1} = r_{O2,B2} = 3\ s^{-1}$, $r_{B1,O1} = r_{B2,O2} = 100\ s^{-1}$, to elicit flickery closures with characteristics typical of those seen in our recordings. We then simulated a 3-min segment with four channels obeying this extended six-state scheme, and repeated the simulation five times with the same rate constants, but a different random seed value. The five traces were then idealized, and fitted separately using the C-O-B scheme, as described in METHODS (compare Csanády, 2000), with $t_d = 4$ ms. The mean (±SEM) estimates from the five fits were $r_{CO} = 0.68 \pm 0.02\ s^{-1}$, $r_{OC} = 3.0 \pm 0.2\ s^{-1}$, $r_{OB} = 2.7 \pm 0.1\ s^{-1}$, $r_{BO} = 94 \pm 2\ s^{-1}$, predicting mean burst and interburst durations of $\tau_b = 353 \pm 26$ ms, and $\tau_{ib} = 1{,}485 \pm 41$ ms, respectively; the latter values being closely similar to the $\tau_b = 340$ ms and $\tau_{ib} = 1{,}543$ ms predicted by the model (τ_b is given by Eq. 1 of text, $\tau_{ib} \approx 1/k_1$). This is despite the fact that the burst duration distribution for this scheme is characterized by two clearly discernible components with parameters $\tau_{sh} = 262$ ms, $\tau_l = 928$ ms, $a_{sh} = 0.88$, $a_l = 0.12$.



Derivation of Observable Parameters for Scheme I

Mean open time (burst duration). The mean open time (see τ_b in text) is the weighted average of the durations of various types of openings, each weighted by its fractional occurrence. Openings either close from O_1, after venturing m times to O_2, $m = 0,\ldots\infty$, "type 1_m;" or from O_2, upon entering O_2 the m^{th} time, $m = 1,\ldots\infty$, "type 2_m." The mean durations of individual dwells at O_1 or O_2 are $\tau_{O1} = 1/(k_{-1} + k_2)$ and $\tau_{O2} = 1/(k_{-2} + k_3)$, respectively. Let π_{-1} be the probability that a channel in O_1 will next exit to C_1, and π_2 the probability that it will next exit to O_2. Similarly, π_{-2} and π_3 denote probabilities that a channel in O_2 next exits to O_1 or C_1, respectively. Hence, $\pi_{-1} = k_{-1}/(k_{-1} + k_2)$, $\pi_{-2} = k_{-2}/(k_{-2} + k_3)$, and $\pi_3 = k_3/(k_{-2} + k_3)$. The mean duration of a type 1_m opening is $(m + 1) \cdot \tau_{O1} + m \cdot \tau_{O2}$, with fractional occurrence $\pi_2^m \pi_{-2}^m \pi_{-1}$, while type 2_m openings last for $m \cdot (\tau_{O1} + \tau_{O2})$, and occur with $\pi_2^m \pi_{-2}^{m-1} \pi_3$ probability. Hence,

$$\tau_b = \sum_{m=0}^{\infty} \pi_2^m \pi_{-2}^m \pi_{-1}[(m+1) \cdot \tau_{O1} + m \cdot \tau_{O2}] + \sum_{m=1}^{\infty} \pi_2^m \pi_{-2}^{m-1} \pi_3 [m \cdot (\tau_{O1} + \tau_{O2})]$$

$$= (\pi_{-1} + \pi_3/\pi_{-2}) \cdot (\tau_{O1} + \tau_{O2}) \cdot \sum_{m=0}^{\infty} m \cdot (\pi_2\pi_{-2})^m + \pi_{-1} \cdot \tau_{O1} \cdot \sum_{m=0}^{\infty} (\pi_2\pi_{-2})^m,$$

from which, using identities

$$\sum_{m=0}^{\infty} m \cdot q^m = q/(1-q)^2 \text{ and } \sum_{m=0}^{\infty} q^m = q/(1-q) \text{ for } q < 1:$$

$$\tau_b = (\pi_{-1} + \pi_3/\pi_{-2}) \cdot (\tau_{O1} + \tau_{O2}) \cdot \pi_2\pi_{-2}/(1 - \pi_2\pi_{-2})^2 + \pi_{-1} \cdot \tau_{O1}/(1 - \pi_2\pi_{-2}). \tag{S5}$$

Substituting for π_{-1}, π_2, π_{-2}, π_3, τ_{O1}, and τ_{O2} from the rates, Eq. S5 reduces to Eq. 1 in the text.

Survivor function of open times (burst durations). Numbering the states of Scheme I in the order C_1, C_2, O_1, O_2, the **Q** matrix of the system is:

$$\left|\begin{array}{cc|cc} -k'_{on} & k'_{on} & 0 & 0 \\ k_{off} & -(k_{off} + k_1) & k_1 & 0 \\ \hline k_{-1} & 0 & -(k_{-1} + k_2) & k_2 \\ k_3 & 0 & k_{-2} & -(k_3 + k_{-2}) \end{array}\right|,$$

in particular, the submatrix of the set of states $\{O_1, O_2\}$ is:

$$\mathbf{Q}_{\{O_1, O_2\}\{O_1, O_2\}} = \begin{vmatrix} -(k_{-1} + k_2) & k_2 \\ k_{-2} & -(k_3 + k_{-2}) \end{vmatrix}.$$

The Eigenvalues of the submatrix are:

$$\lambda_{\pm} = \frac{-(k_{-1} + k_2 + k_{-2} + k_3) \pm \sqrt{D}}{2},$$

where $D = (k_{-1} + k_2 + k_{-2} + k_3)^2 - 4(k_{-1}k_3 + k_{-1}k_{-2} + k_2k_3)$. The exponential of the submatrix is:

$$e^{\mathbf{Q}_{\{O_1,O_2\}\{O_1,O_2\}}t}$$

$$= \begin{vmatrix} \frac{(e^{\lambda_+ t} + e^{\lambda_- t})}{2} - \frac{k_{-1} + k_2 - k_{-2} - k_3}{2\sqrt{D}} \cdot (e^{\lambda_+ t} - e^{\lambda_- t}) & \frac{k_2}{\sqrt{D}} \cdot (e^{\lambda_+ t} - e^{\lambda_- t}) \\ \frac{k_{-2}}{\sqrt{D}} \cdot (e^{\lambda_+ t} - e^{\lambda_- t}) & \frac{(e^{\lambda_+ t} + e^{\lambda_- t})}{2} + \frac{k_{-1} + k_2 - k_{-2} - k_3}{2\sqrt{D}} \cdot (e^{\lambda_+ t} - e^{\lambda_- t}) \end{vmatrix}.$$

The survivor function of $\{O_1,O_2\}$ is:

$$surv_{\{O_1,O_2\}}(t) = \mathbf{p}_{\{O_1,O_2\}}(0)^{\mathrm{T}} e^{\mathbf{Q}_{\{O_1,O_2\}\{O_1,O_2\}}t} \mathbf{1}_{\{O_1,O_2\}}$$

(see Csanády, 2000), where $\mathbf{p}_{\{O_1,O_2\}}(0)^{\mathrm{T}} = [1, 0]$ (since all openings start in O_1); i.e., the survivor function simplifies to the sum of the first row of the submatrix:

$$surv_{\{O_1,O_2\}}(t) = \frac{\sqrt{D} - k_2 - k_{-2} - k_3 + k_{-1}}{2\sqrt{D}} \cdot e^{\lambda_- t} + \frac{\sqrt{D} + k_2 + k_{-2} + k_3 - k_{-1}}{2\sqrt{D}} \cdot e^{\lambda_+ t}. \tag{S6}$$

The coefficient of the first term and $-\lambda_-^{-1}$ give a_{sh} and τ_{sh}, respectively (Eqs. 2 and 3 in text), while the second coefficient and $-\lambda_+^{-1}$ yield a_l and τ_l (Eq. 4 in text). As a check, the mean of the above distribution, $\langle t \rangle = a_{sh}\tau_{sh} + a_l\tau_l$, is identical to τ_b in Eq. S5 (Eq. 1 in text).

Time course of unlocking from AMPPNP-mediated lock. The predicted time course of unlocking is obtained by solving the differential equation describing scheme

$$O_2 \xrightarrow{k_{-2}} O_1 \xrightarrow{k_{-1}} C_1,$$

with initial condition $o_1(0) = k_{-2}/(k_2 + k_{-2})$, $o_2(0) = k_2/(k_2 + k_{-2})$. The vector solution of equation:

$$\frac{d}{dt}\begin{bmatrix} o_1 \\ o_2 \end{bmatrix}(t) = \begin{bmatrix} -k_{-1} & k_{-2} \\ 0 & -k_{-2} \end{bmatrix}\begin{bmatrix} o_1 \\ o_2 \end{bmatrix}(t),$$

with the above initial condition is:

$$\begin{bmatrix} o_1 \\ o_2 \end{bmatrix}(t) = \frac{k_{-2}}{k_2 + k_{-2}} \cdot \begin{bmatrix} 1 - \frac{k_2}{k_{-1} - k_{-2}} \\ 0 \end{bmatrix} \cdot e^{-k_{-1}t} + \frac{k_2}{k_2 + k_{-2}} \cdot \begin{bmatrix} \frac{k_{-2}}{k_{-1} - k_{-2}} \\ 1 \end{bmatrix} \cdot e^{-k_{-2}t}, \tag{S7}$$

from which the time course, obtained as $o(t) = o_1(t) + o_2(t)$, contains a slow component with time constant $1/k_{-2}$ (see Eq. 5 in text) and fractional amplitude $[k_2/(k_2 + k_{-2})] \cdot [k_{-1}/(k_{-1} - k_{-2})]$ (see Eq. 6 in text); as well as a fast component with time constant $1/k_{-1}$ and complementary fractional amplitude.