Appendix on the Inverse Problem of Determining the Rate Constants of a Channel from Patch-Clamp Data

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Consider a channel model with the following states and transitions

$$\begin{tikzcd} C \arrow[r, shift left, "\alpha"] \arrow[dr, shift left, "\delta"] & O \arrow[l, shift left, "\beta"] \arrow[d, shift left, "\gamma"] \\ & I \arrow[u, shift left, "\mu"] \arrow[ul, shift left, "\nu"] \end{tikzcd}$$

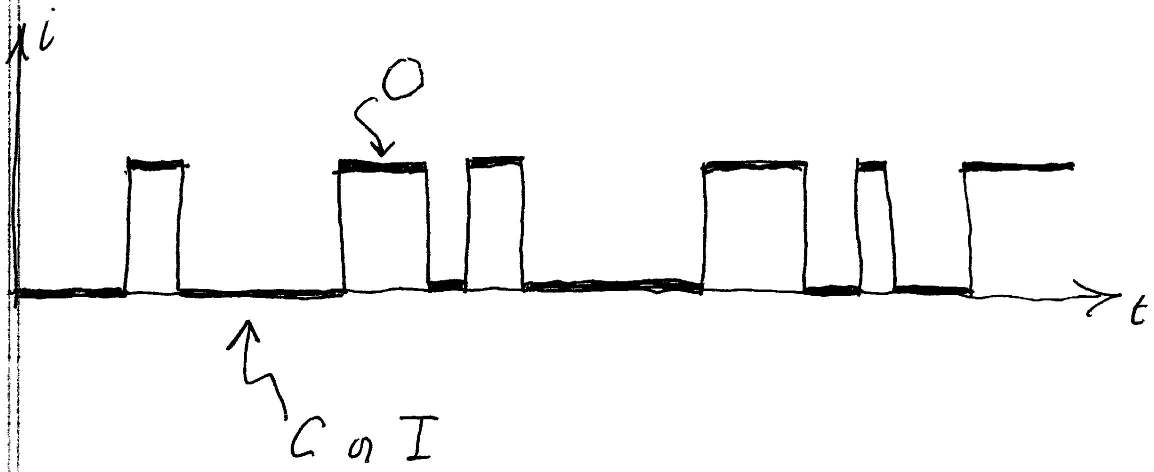
where

C = <u>closed</u> state
O = <u>open</u> state
I = <u>inactivated</u> state (which, like C, is also closed).

The rate constants $\alpha, \beta, \gamma, \delta, \mu, \nu$ are all functions of voltage, but we assume that the voltage is held constant unless otherwise noted.

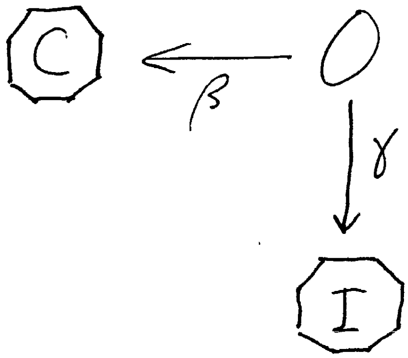
We consider the inverse problem of determining the rate constants from patch clamp data in which one can distinguish the state O from the other two states but cannot distinguish C from I.

At fixed voltage, patch clamp data showing single-channel current as a function of time will look something like this



Note that the open state is directly observable in such a record, since the channel then conducts a nonzero current (assuming that the voltage is not equal to the reversal potential). When the channel is not conducting current, however, it may be in state C or I, and these cannot be distinguished. Moreover, transitions of the type C→I or I→C are invisible, and one cannot tell whether the transitions to (or from) the state O are from (or to) the state C or I.

When the system (i.e., the channel) is in state O, it has the following possible transitions



Here, C and I are absorbing states (indicated by the stop signs), since we are interested in the sub-process that stops when the channel leaves the state O.

It follows that the dwell time in state O is exponential with mean $1/(\beta+\gamma)$.

The probability that the channel exits state O via the transition $O \to C$ is $\beta/(\beta+\gamma)$, and the probability that it exits via $O \to I$ is $\gamma/(\beta+\gamma)$.

The exit path is independent of the dwell time.

When the channel is known to be in the state C or I, but we don't know which, the situation is more complicated:

$$
\begin{tikzcd}
C \arrow[rr, "\alpha"] \arrow[drr, shift left, "\delta"] & & O \\
& & I \arrow[u, "\mu"'] \arrow[ull, shift left, "\nu"]
\end{tikzcd}
$$

Here O is an absorbing state, since we are interested in the dwell time in the composite state C or I

Let

$[C](t)$ = probability of being in state C at time t

$[I](t)$ = probability of being in state I at time t

Then

$$\frac{d}{dt}\begin{pmatrix}[C]\\ [I]\end{pmatrix} = \begin{pmatrix} -(\alpha+\delta) & \nu \\ \delta & -(\mu+\nu)\end{pmatrix}\begin{pmatrix}[C]\\ [I]\end{pmatrix}$$

At time $t=0$, we assume that the system is in state C with probability $[C]_0$ and in state I

with probability $[I]_0$, such that

$$[C]_0 + [I]_0 = 1$$

Then the probability density function $\rho(t)$ for the dwell time in the composite state C or I will be given by

$$\rho(t) = (\alpha \quad \mu)\begin{pmatrix}[C](t)\\ [I](t)\end{pmatrix} = \alpha[C](t) + \mu[I](t)$$

As a check, note that

$$\rho(t) = -\frac{d}{dt}\Big([C](t) + [I](t)\Big)$$

We now proceed to solve the above system of differential equations for $[C](t), [I(t)]$. First, look for solutions of the form

$$\begin{pmatrix}[C](t)\\ [I](t)\end{pmatrix} = \begin{pmatrix}[C]_\lambda\\ [I]_\lambda\end{pmatrix} e^{\lambda t}$$

This leads to

$$\begin{pmatrix} \lambda + \alpha + \delta & -\nu \\ -\delta & \lambda + \mu + \nu \end{pmatrix} \begin{pmatrix} [C]_\lambda \\ [I]_\lambda \end{pmatrix} = 0$$

which has nontrivial solutions if and only if

$$0 = (\lambda + \alpha + \delta)(\lambda + \mu + \nu) - \delta\nu$$

$$= \lambda^2 + (\alpha + \delta + \mu + \nu)\lambda + (\alpha+\delta)(\mu+\nu) - \delta\nu$$

The roots are real, since

$$(\alpha + \delta + \mu + \nu)^2 - 4\left[(\alpha+\delta)(\mu+\nu) - \delta\nu\right]$$

$$= \left((\alpha+\delta) - (\mu+\nu)\right)^2 + 4\delta\nu > 0$$

(We assume that all of the rate constants are positive.)

We may write the roots in the following way

$$\lambda_\pm = \frac{-(\alpha+\delta+\mu+\nu) \pm \sqrt{\left((\alpha+\delta)-(\mu+\nu)\right)^2 + 4\nu\delta}}{2}$$

From the characteristic equation itself, we have

$$\lambda_+ \lambda_- = (\alpha+\delta)(\mu+\nu) - \delta\nu > 0$$

$$\lambda_+ + \lambda_- = -(\alpha+\delta+\mu+\nu) < 0$$

It follows that both roots are negative, and

$$\lambda_- < \lambda_+ < 0$$

For $[C]_\lambda$, $[I]_\lambda$ we may take

$$\begin{pmatrix} [C]_\lambda \\ [I]_\lambda \end{pmatrix} = \begin{pmatrix} \nu \\ \lambda+\alpha+\delta \end{pmatrix}$$

and we write $[C]_\pm$, $[I]_\pm$ as shorthand for $[C]_{\lambda_\pm}$, $[I]_{\lambda_\pm}$. The general solution may now be written

$$\begin{pmatrix} [C](t) \\ [I](t) \end{pmatrix} = a_+ e^{\lambda_+ t} \begin{pmatrix} \nu \\ \lambda_+ + \alpha + \delta \end{pmatrix} + a_- e^{\lambda_- t} \begin{pmatrix} \nu \\ \lambda_- + \alpha + \delta \end{pmatrix}$$

where a_+ and a_- are determined by imposing the initial conditions

$$\begin{pmatrix} [C]_0 \\ [I]_0 \end{pmatrix} = \begin{pmatrix} \nu & \nu \\ \lambda_+ + \alpha + \delta & \lambda_- + \alpha + \delta \end{pmatrix} \begin{pmatrix} a_+ \\ a_- \end{pmatrix}$$

These equations have the solution

$$a_+ = \frac{(\lambda_- + \alpha + \delta)[C]_0 - \nu [I]_0}{\nu(\lambda_- - \lambda_+)}$$

$$a_- = \frac{\nu [I]_0 - (\lambda_+ + \alpha + \delta)[C]_0}{\nu(\lambda_- - \lambda_+)}$$

Then

$$\rho(t) = \begin{pmatrix}\alpha & \mu\end{pmatrix} \begin{pmatrix}[C](t) \\ [I](t)\end{pmatrix}$$

$$= a_+\left(\alpha\nu + \mu(\lambda_+ + \alpha + \delta)\right) e^{\lambda_+ t}$$

$$+ a_-\left(\alpha\nu + \mu(\lambda_- + \alpha + \delta)\right) e^{\lambda_- t}$$

$$= \frac{[C]_0}{\nu(\lambda_- - \lambda_+)} \Big[(\lambda_- + \alpha + \delta)\left(\alpha\nu + \mu(\lambda_+ + \alpha + \delta)\right) e^{\lambda_+ t} - (\lambda_+ + \alpha + \delta)\left(\alpha\nu + \mu(\lambda_- + \alpha + \delta)\right) e^{\lambda_- t} \Big]$$

$$+ \frac{[I]_0}{\lambda_- - \lambda_+} \Big[-\left(\alpha\nu + \mu(\lambda_+ + \alpha + \delta)\right) e^{\lambda_+ t} + \left(\alpha\nu + \mu(\lambda_- + \alpha + \delta)\right) e^{\lambda_- t} \Big]$$

But $$\left(\lambda_\pm + \alpha + \delta\right) = \frac{(\alpha+\delta)-(\mu+\nu) \pm \sqrt{\left((\alpha+\delta)-(\mu+\nu)\right)^2 + 4\nu\delta}}{2}$$

So

$$\left(\lambda_+ + \alpha + \delta\right)\left(\lambda_- + \alpha + \delta\right) = -\frac{4\nu\delta}{4} = -\nu\delta$$

Therefore

$$\rho(t) = \frac{[C]_0}{\lambda_- - \lambda_+}\Big[\left(-\mu\delta + \alpha(\lambda_- + \alpha + \delta)\right)e^{\lambda_+ t} + \left(\mu\delta - \alpha(\lambda_+ + \alpha + \delta)\right)e^{\lambda_- t}\Big]$$

$$+ \frac{I_0}{\lambda_- - \lambda_+}\Big[\left(-\alpha\nu - \mu(\lambda_+ + \alpha + \delta)\right)e^{\lambda_+ t} + \left(\alpha\nu + \mu(\lambda_- + \alpha + \delta)\right)e^{\lambda_- t}\Big]$$

$$= \frac{[C]_0}{\lambda_+ - \lambda_-}\Big[\left(\mu\delta - \alpha(\lambda_- + \alpha + \delta)\right)e^{\lambda_+ t} + \left(-\mu\delta + \alpha(\lambda_+ + \alpha + \delta)\right)e^{\lambda_- t}\Big]$$

$$+ \frac{I_0}{\lambda_+ - \lambda_-}\Big[\left(\alpha\nu + \mu(\lambda_+ + \alpha + \delta)\right)e^{\lambda_+ t} + \left(-\alpha\nu - \mu(\lambda_- + \alpha + \delta)\right)e^{\lambda_- t}\Big]$$

Note that

$$\lambda_+ - \lambda_- = \sqrt{\left((\alpha+\delta)-(\mu+\nu)\right)^2 + 4\nu\delta}$$

which we abbreviate as $\sqrt{}$. Therefore

$$\rho(t) = [C]_0 \left[\frac{\alpha}{2}\left(e^{\lambda_+ t} + e^{\lambda_- t}\right) + \frac{\mu\delta - \frac{\alpha}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{\sqrt{}}\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)\right]$$

$$+ [I]_0 \left[\frac{\mu}{2}\left(e^{\lambda_+ t} - e^{\lambda_- t}\right) + \frac{\alpha\nu + \frac{\mu}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{\sqrt{}}\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)\right]$$

$$= \left([C]_0 \frac{\alpha}{2} + [I]_0 \frac{\mu}{2}\right)\left(e^{\lambda_+ t} + e^{\lambda_- t}\right)$$

$$+ \left([C]_0 \frac{\mu\delta - \frac{\alpha}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{\sqrt{}} + [I]_0 \frac{\alpha\nu + \frac{\mu}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{\sqrt{}}\right)\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)$$

Note that $\rho(t)$ is of the form

$$\rho(t) = [C]_0 \rho_C(t) + [I]_0 \rho_I(t)$$

and moreover that the following substitutions

$$\alpha \to \mu$$
$$\mu \to \alpha$$
$$\delta \to \nu$$
$$\nu \to \delta$$

convert ρ_C into ρ_I and vice versa. It is obvious that this should be the case from the symmetry of the original problem.

As a check, we now show that $\rho(t)$ has the properties required of a probability density function supported on $[0,\infty)$, that is, that

$$\rho(t) \geq 0 \quad \text{for} \quad 0 \leq t < \infty$$

$$\int_0^\infty \rho(t)\,dt = 1$$

for all $[C]_0, [I]_0$ such that $[C]_0 \geq 0$, $[I]_0 \geq 0$, and

$$[C]_0 + [I]_0 = 1$$

For this to be true it is necessary and sufficient that

$$\rho_c(t) \geq 0 \quad , \quad \rho_I(t) \geq 0$$

for $0 \leq t < \infty$, and

$$\int_0^\infty \rho_c(t)dt = \int_0^\infty \rho_I(t)dt = 1$$

Because of the symmetry between ρ_c and ρ_I that was pointed out above, however, we need only consider ρ_C. Of course, we assume in the following that all of the rate constants are positive.

To prove that $\rho_c(t) \geq 0$, we first recall that

$$\lambda_- < \lambda_+ < 0$$

from which it follows that

$$e^{\lambda_+ t} - e^{\lambda_- t} \geq 0$$

for $t \geq 0$.

Therefore
$$\frac{\mu\delta}{r}\left(e^{\lambda_+ t} - e^{\lambda_- t}\right) \geq 0$$

and
$$\rho_c(t) \geq \frac{\alpha}{2}\left(e^{\lambda_+ t} + e^{\lambda_- t}\right)\left(1 - \frac{(\alpha+\delta)-(\mu+\nu)}{r}\,\frac{e^{\lambda_+ t} - e^{\lambda_- t}}{e^{\lambda_+ t} + e^{\lambda_- t}}\right)$$

But
$$0 \leq \frac{e^{\lambda_+ t} - e^{\lambda_- t}}{e^{\lambda_+ t} + e^{\lambda_- t}} \leq 1$$

and
$$\left|\frac{(\alpha+\delta)-(\mu+\nu)}{\sqrt{((\alpha+\delta)-(\mu+\nu))^2 + 4\nu\delta}}\right| = \sqrt{\frac{((\alpha+\delta)-(\mu+\nu))^2}{((\alpha+\delta)-(\mu+\nu))^2 + 4\nu\delta}}$$

$$< 1$$

It follows that $\rho_c(t) \geq 0$, and similarly that $\rho_I(t) \geq 0$.

Also

$$\int_0^\infty \rho_c(t)\,dt = \frac{\alpha}{2}\left(\frac{1}{-\lambda_+} + \frac{1}{-\lambda_-}\right) + \frac{\mu\delta - \frac{\alpha}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{\sqrt{}}\left(\frac{1}{-\lambda_+} - \frac{1}{-\lambda_-}\right)$$

$$= \frac{1}{\lambda_+\lambda_-}\left[-\frac{\alpha}{2}(\lambda_+ + \lambda_-) + \frac{\mu\delta - \frac{\alpha}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{\cancel{\sqrt{}}}\cancel{(\lambda_+ - \lambda_-)}\right]$$

$$= \frac{\frac{\alpha}{2}(\alpha+\delta+\mu+\nu) + \mu\delta - \frac{\alpha}{2}\left((\alpha+\delta)-(\mu+\nu)\right)}{(\alpha+\delta)(\mu+\nu) - \delta\nu}$$

$$= \frac{\alpha(\mu+\nu) + \mu\delta}{\alpha(\mu+\nu) + \delta(\mu+\cancel{\nu}) - \cancel{\delta\nu}} = 1$$

as required

Now consider the problem of determining the six rate constants of the model from patch clamp data. The probability density of the open-time duration is exponential with mean

$$\tau_O = \frac{1}{\beta+\gamma}$$

The probability density for the closed-time duration can be fit to

$$\rho(t) = A\left(e^{\lambda_+ t} + e^{\lambda_- t}\right) + B\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)$$

but A and B are not independent, since $\int_0^\infty \rho(t)\,dt = 1$. Therefore, we get measurements of A, λ_+, λ_-, and this constrains the parameters as follows

$$\frac{1}{2}\left([C]_0\alpha + [I]_0\mu\right) = \frac{1}{2}\left(\frac{\beta\alpha}{\beta+\gamma} + \frac{\gamma\mu}{\beta+\gamma}\right) = A$$

$$(\alpha+\delta)(\mu+\nu) - \delta\nu = \lambda_+\lambda_-$$

$$(\alpha+\delta) + (\mu+\nu) = -(\lambda_+ + \lambda_-)$$

where we have used

$$[C]_0 = \frac{\beta}{\beta+\gamma} \qquad [I]_0 = \frac{\gamma}{\beta+\gamma}$$

Since $\beta/(\beta+\gamma)$ is the probability that the transition by which the open state closes is $O \to C$, whereas $\gamma/(\beta+\gamma)$ is the probabity that the open state closes via $O \to I$.

Overall, we have 4 relationships among 6 parameters, which is not enough to determine the parameters uniquely.

This can be remedied by considering patch clamp data in which the voltage is made to jump either from a very negative voltage or from a very positive voltage to the voltage of interest. At the very negative voltage, the channel is held in the state C, and at the very positive voltage it is held in the state I, so if we consider the time to first opening after the voltage jump, we will find that its probability density function is $\rho_C(t)$ or $\rho_I(t)$ respectively.

All of the other closed times subsequent to the first one should have the probability density $\rho(t)$. Now we may fit the measured $\rho_C(t)$, $\rho_I(t)$, $\rho(t)$ to the forms

$$\rho_C(t) = A_C\left(e^{\lambda_+ t} + e^{\lambda_- t}\right) + B_C\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)$$

$$\rho_I(t) = A_I\left(e^{\lambda_+ t} + e^{\lambda_- t}\right) + B_I\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)$$

$$\rho(t) = A\left(e^{\lambda_+ t} + e^{\lambda_- t}\right) + B\left(e^{\lambda_+ t} - e^{\lambda_- t}\right)$$

Note that the values of λ_+ and λ_- should be the same in all three cases, which is a check on the theory. Now, we have the 6 equations:

$$\tau_0 = \frac{1}{\beta+\gamma}$$

$$\lambda_+\lambda_- = (\alpha+\delta)(\mu+\nu) - \delta\nu$$

$$-(\lambda_+ + \lambda_-) = (\alpha+\delta) + (\mu+\nu)$$

$$A_C = \frac{\alpha}{2}$$

$$A_I = \frac{\mu}{2}$$

$$A = \frac{1}{2}\left(\frac{\beta\alpha}{\beta+\gamma} + \frac{\gamma\mu}{\beta+\gamma}\right)$$

Note that the equations $A_c = \alpha/2$ and $A_I = \mu/2$ immediately determine α, μ

With α and μ known, we then have a pair of equations for β, γ:

$$\left.\begin{aligned} \beta + \gamma &= \frac{1}{\tau_0} \\ \alpha\beta + \mu\gamma &= \frac{2A}{\tau_0} \end{aligned}\right\}$$

and a pair of equations for δ, ν

$$\left.\begin{aligned} \delta + \nu &= -(\lambda_+ + \lambda_-) - (\alpha + \mu) \\ \mu\delta + \alpha\nu &= \lambda_+\lambda_- - \alpha\mu \end{aligned}\right\}$$

Provided that $\alpha \neq \mu$, these equations uniquely determine β, γ and δ, ν

so the inverse problem is solved.