Identifiability of Aggregated Markov Models of Single Ion Channels

Ball, Frank
University of Nottingham, School of Mathematical Sciences
University Park
Nottingham NG7 2RD, UK
E-mail: frank.ball@nottingham.ac.uk

1 Introduction

Ion channels are protein molecules embedded in cell membranes. They are fundamental units of the nervous system and contain aqueous pores that may be open or closed. When open, ion channels permit selective flow of ions across the membrane. An understanding of the processes governing opening and closing of ion channels provides important insights into diseases caused by channel disorders, the modes of actions of drugs (for example local anaesthetics) which perturb ion channels and hence the rational design of drugs acting on a nervous system.

The patch clamp technique enables the experimenter to record the current flowing across a single ion channel. Over the past 30–35 years, there has been considerable interest in the development and analysis of stochastic models to describe the opening and closing of ion channels, and in methods of inference for such models; see, for example, Sakmann and Neher (1995) and Hawkes (2005).

The gating mechanism of a single ion channel is usually modelled as a finite state continuous-time Markov chain. The state space is partitioned into two classes, termed open and closed, corresponding to the receptor channel being open or closed, and it is possible to observe only which class, rather than which state, the process is in. A consequence of this aggregation of states is that distinct underlying processes may be equivalent, in that they yield probabilistically indistinguishable aggregated processes. Single-channel models are usually specified in terms of a kinetic scheme which gives the allowable transitions between states. The above equivalence means that (i) a scheme may be unidentifiable, in that there exist equivalent models that satisfy the constraints imposed by the scheme, and (ii) two distinct schemes may be indistinguishable, in that there exist models from the two schemes that are equivalent. Experimenters often wish to discriminate between rival schemes, which have different biophysical interpretations, and it is important to know whether or not this is in principle possible and also whether a given scheme is identifiable.

In this paper, we give a brief introduction to ion channel modelling and associated identifiability problems. We also outline a method (described in detail in Ball *et al.* (2011)) for investigating identifiability and distinguishability of a range of practically relevant single-channel gating schemes, together with its application to schemes that have been proposed for glycine receptor channels.

2 Models and inference

We assume that the gating behaviour of a single ion channel is modelled as an irreducible continuous-time Markov chain $\{X(t)\} = \{X(t) : t \geq 0\}$, with finite state space $E = \{1, 2, \ldots, n\}$ and transition-rate matrix $Q = [q_{ij}]$. Thus, q_{ij}, for $i \neq j$, is the transition rate of the channel from state i to state j, and $q_{ii} = -\sum_{j \neq i} q_{ij}$. The state space E is partitioned into $O = \{1, 2, \ldots, n_O\}$ and $C = \{n_O + 1, n_O + 2, \ldots, n\}$, which are respectively classes of n_O open states and n_C $(= n - n_O)$ closed states, and at any given time it is possible to observe only which class of states the channel is in.

A single-channel model of this type is usually specified by a kinetic scheme, which indicates the allowable transitions between states (i.e. which off-diagonal q_{ij} may be non-zero). Sometimes the q_{ij} for allowable transitions are functions of parameters, having biophysical meaning; see, for example,

Figure 1, which gives two such kinetic schemes that have been considered by Burzomanto *et al.* (2004) for a single heteromeric $\alpha 1 \beta$ glycine receptor channel. In both of these schemes, the 3 states indexed with an asterisk are open and the other 7 states are closed. Further, a denotes concentration of the agonist glycine, which is controlled in experiments, and all other quantities are parameters which need to be estimated. See Burzomanto *et al.* (2004) for further details, including the biophysical rationale behind the two models and interpretation of the parameters.

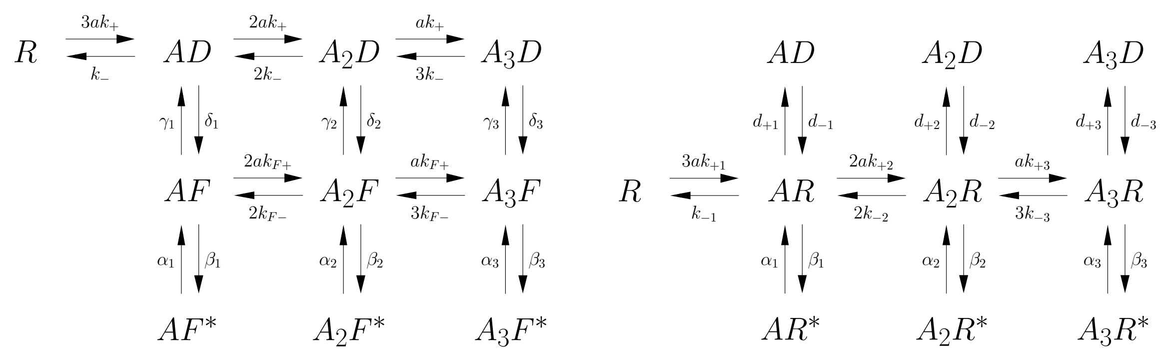

Figure 1: Models for glycine receptor channel.

Let $\boldsymbol{\pi} = (\pi_1, \pi_2, \ldots, \pi_n)$ denote the equilibrium distribution of $\{X(t)\}$. Partition

$$(1) \qquad Q = \begin{bmatrix} Q_{OO} & Q_{OC} \\ Q_{CO} & Q_{CC} \end{bmatrix} \quad \text{and} \quad \boldsymbol{\pi} = (\boldsymbol{\pi}_O, \boldsymbol{\pi}_C)$$

in the obvious fashion.

Suppose that $X(0) \in O$ and let $T_O = \min\{t > 0 : X(t) \in C\}$ denote the time that the channel first enters the closed class of states. Then T_O has (matrix) probability density function (pdf) given by

$$(2) \qquad \boldsymbol{f}_{OC}(t) = \exp(Q_{OO}t)Q_{OC} \qquad (t > 0) ,$$

where $\boldsymbol{f}_{OC}(t) = [f_{ij}^{OC}(t)]$ with

$$f_{ij}^{OC}(t) = \frac{d}{dt} P(T_O \leq t \text{ and } X(T_O) = j \mid X(0) = i) \qquad (i \in O, j \in C) ;$$

see, for example, Colquhoun and Hawkes (1977). Define $\boldsymbol{f}_{CO}(s) = \exp(Q_{CC}s)Q_{CO}$ $(s > 0)$ similarly for closed sojourns.

Let $\boldsymbol{\pi}^O = (\pi_1^O, \pi_2^O, \ldots, \pi_{n_O}^O)$, where, for $i = 1, 2, \ldots, n_O$, π_i^O is the equilibrium probability that an open sojourn of the channel begins in state i. Then, as shown for example in Colquhoun and Hawkes (1977), $\boldsymbol{\pi}^O = \boldsymbol{\pi}_C Q_{CO} / \boldsymbol{\pi}_C Q_{CO} \mathbf{1}$. (Throughout the paper $\mathbf{1}$ denotes a column vectors of ones, whose dimension, in the present case n_O, is apparent from the context.) Define $\boldsymbol{\pi}^C$ similarly for closed sojourns.

Suppose that the channel is in equilibrium and a sequence $t_1, s_1, t_2, s_2, \ldots, t_m, s_m$ of successive open and closed sojourns is observed, starting with an open sojourn. Then, see Fredkin *et al.* (1985), the likelihood of these data is given by

$$(3) \qquad L(Q) = \boldsymbol{\pi}^O \left[\prod_{i=1}^{m} \{\boldsymbol{f}_{OC}(t_i) \boldsymbol{f}_{CO}(s_i)\} \right] \mathbf{1}$$

and Q, or the parameters governing Q, can in principle be estimated, for example by maximum likelihood (see, for example, Ball and Sansom (1989)).

In practice, the current flowing across a single ion channel is recorded using the patch clamp technique. Moreover, the record is corrupted by noise and low-pass filtering, and is sampled at finite intervals. The sequence of open and closed sojourns of the channel is then reconstructed, often using some kind of threshold-crossing algorithm. Such a reconstruction results in the loss of very brief sojourns in either the open or closed classes of states. This phenomenon is known as *time interval omission* and is usually modelled by assuming that any open or closed sojourn of $\{X(t)\}$ having length less than some (assumed known) critical value τ fails to be detected. Thus, for example, an observed open sojourn commences with an actual open sojourn of length at least τ, which is followed by a number of pairs of closed and open sojourns with the closed sojourns all having length less than τ, and terminates as soon as there is a closed sojourn of length at least τ.

Suppose that the channel is in equilibrium and $t_1, s_1, t_2, s_2, \ldots, t_m, s_m$ is now a sequence of successive observed open and closed sojourns. Then the likelihood is given by (3) but with $\boldsymbol{\pi}^O$, $\boldsymbol{f}_{OC}$ and $\boldsymbol{f}_{CO}$ replaced by their time interval omission counterparts $\tilde{\boldsymbol{\pi}}^O$, $\tilde{\boldsymbol{f}}_{OC}$ and $\tilde{\boldsymbol{f}}_{CO}$, respectively. Although calculation of $\tilde{\boldsymbol{\pi}}^O$ is straightforward, calculation of $\tilde{\boldsymbol{f}}_{OC}$ and $\tilde{\boldsymbol{f}}_{CO}$ is considerably more difficult. A recursive exact expression is available for $\tilde{\boldsymbol{f}}_{OC}(t)$ (and hence also for $\tilde{\boldsymbol{f}}_{CO}(s)$), but it can be numerically unstable for large values of t. However, an asymptotic approximation is available, which turns out to be very good, even for small values of t (of the order of 2τ or 3τ), for which the exact expression can be evaluated accurately. Thus $\tilde{\boldsymbol{f}}_{OC}$ and $\tilde{\boldsymbol{f}}_{CO}$ can be evaluated using a combination of the exact and asymptotic expressions; see Hawkes *et al.* (1992) for details.

Another difficulty is that there there may be more than one channel contributing to a recording. However, channel recordings often show periods of repetitive open activity, known as *bursts*, which are noticeably separated from other such periods. This may be modelled by specifying a critical time t_{crit} and classifying any observed closed sojourn as short ($\leq t_{\text{crit}}$) or long ($> t_{\text{crit}}$). The long observed closed sojourns are then used to partition the channel record into bursts. One can usually be almost certain that activity within a burst comes from a single channel, so only information from within bursts is used to calculate the likelihood. See Colquhoun *et al.* (1996), (2003) for further details and examples of such inference, and Burzomanto *et al.* (2004) for application of these methods to glycine channels, including estimation of the parameters in the two models in Figure 1.

An attractive alternative to using sojourn time reconstructions is to base inference directly on the current record and several authors have developed such methodology; see, for example, Fredkin and Rice (1992), (2001), who use likelihood-based hidden-Markov techniques, and Ball *et al.* (1999), de Gunst *et al.* (2001) and Gin *et al.* (2009), who use Bayesian Markov chain Monte Carlo methods.

3 Identifiability

Suppose that the single-channel process $\{X(t)\}$ is in equilibrium. Let $f_O(t)$ $(t \geq 0)$ and $f_C(s)$ $(s \geq 0)$ denote the pdfs of the lengths of typical (actual) open and closed sojourns, respectively. Then it follows from (2) and the equivalent equation for closed sojourns that

$$(4) \quad f_O(t) = \boldsymbol{\pi}^O \exp(Q_{OO}t)Q_{OC}\mathbf{1} \quad (t \geq 0) \qquad \text{and} \qquad f_C(s) = \boldsymbol{\pi}^C \exp(Q_{CC}s)Q_{CO}\mathbf{1} \quad (s \geq 0).$$

Similarly, the joint pdf of a typical open sojourn and its subsequent closed sojourn is

$$f_{OC}(t,s) = \boldsymbol{\pi}^O \exp(Q_{OO}t)Q_{OC} \exp(Q_{CC}s)Q_{CO}\mathbf{1} \qquad (t, s \geq 0),$$

and the joint pdf of three successive sojourns, starting with an open sojourn, is

$$f_{OCO}(t_1, s_1, t_2) = \boldsymbol{\pi}_O \exp(Q_{OO}t_1)Q_{OC} \exp(Q_{CC}s_1)Q_{CO} \exp(Q_{OO}t_2)Q_{OC}\mathbf{1} \qquad (t_1, s_1, t_2 \geq 0).$$

Let $\{X(t)\}$ and $\{X'(t)\}$ be two aggregated continuous-time Markov chains, with transition-rate matrices Q and Q', respectively, each partitioned as in (1). The processes $\{X(t)\}$ and $\{X'(t)\}$ are said to be *equivalent*, and we write $Q \sim Q'$, if when in equilibrium they yield probabilistically indistinguishable aggregated processes, i.e. if $f_O = f'_O, f_C = f'_C, f_{OC} = f'_{OC}, f_{CO} = f'_{CO}, f_{OCO} = f'_{OCO}, f_{COC} = f'_{COC}, \ldots$, where $f'_O, f'_C, f'_{OC}, f'_{CO}, f'_{OCO}, f'_{COC}, \ldots$ are the equilibrium sojourn time joint pdfs for $\{X'(t)\}$.

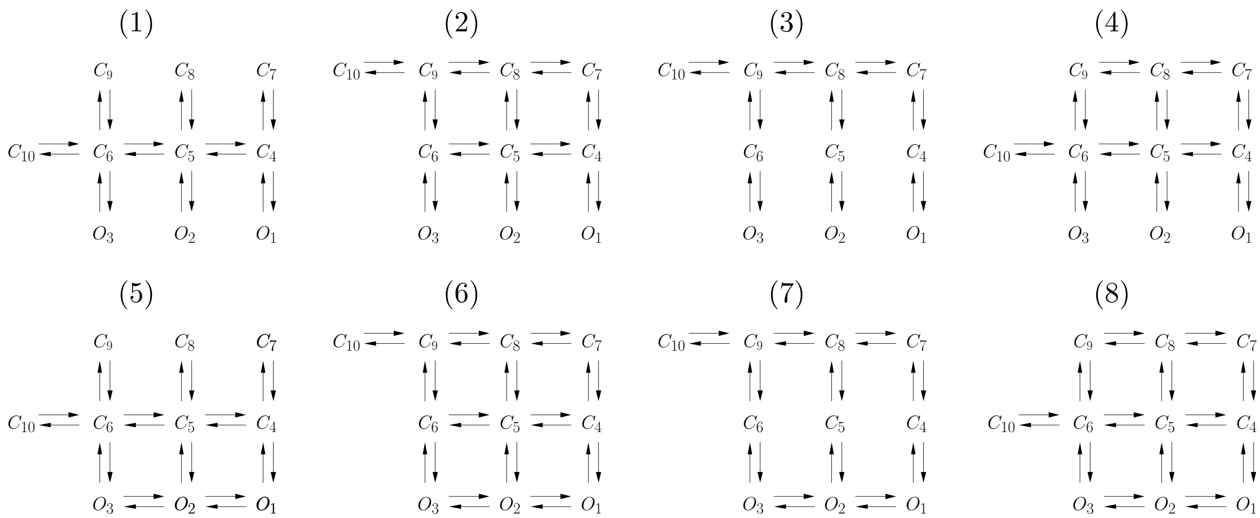

Figure 2: Schemes for glycine receptor channel.

Recall that a single-channel model is often described by a kinetic scheme, indicating allowable transitions between states. Examples of such schemes for a heteromeric $\alpha 1\beta$ glycine receptor channels are shown in Figure 2. Each scheme has 3 open states (O_1, O_2, O_3) and 7 closed states $(C_4, C_5, \ldots, C_{10})$. A scheme is said to be *identifiable* if there do not exist distinct transition-rate matrices Q and Q' satisfying its constraints such that $Q \sim Q'$, otherwise the scheme is *unidentifiable*. Two schemes are said to be *distinguishable* if there do not exist Q satisfying the constraints of one scheme and Q' satisfying the constraints of the other scheme such that $Q \sim Q'$, otherwise the schemes are *indistinguishable*. An example of two indistinguishable schemes is given in Figure 3; see Wagner *et al.* (1999) and Bruno *et al.* (2005) for details.

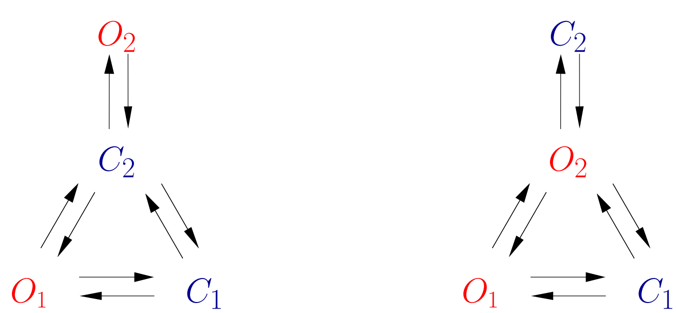

Figure 3: Example of indististinguishable schemes.

3.1 Over-parameterised models

Suppose that Q_{OO} and Q_{CC} are diagonalisable, as is the case if $\{X(t)\}$ is time reversible. (Equilibrium channel gating is usually a time-reversible phenomenon; see Laüger (1995).) Then the equilibrium sojourn time pdfs take the form

$$
\begin{aligned}
f_O(t) &= \sum_{i=1}^{n_O} \alpha_i \exp(-\lambda_i t) \quad (t \geq 0), \\
f_C(s) &= \sum_{j=1}^{n_C} \beta_j \exp(-\mu_j t) \quad (s \geq 0), \\
f_{OC}(t,s) &= \sum_{i=1}^{n_O}\sum_{j=1}^{n_C} \alpha_{ij} \exp(-(\lambda_i t + \mu_j s)) \quad (t, s \geq 0), \\
f_{OCO}(t_1, s_1, t_2) &= \sum_{i=1}^{n_O}\sum_{j=1}^{n_C}\sum_{k=1}^{n_O} \alpha_{ijk} \exp(-(\lambda_i t_1 + \mu_j s_1 + \lambda_k t_2)) \quad (t_1, s_1, t_2 \geq 0),
\end{aligned}
\tag{5}
$$

where $\lambda_1, \lambda_2, \ldots, \lambda_{n_O}$ and $\mu_1, \mu_2, \ldots, \mu_{n_C}$ are the eigenvalues of Q_{OO} and Q_{CC}, respectively.

Let $\mathcal{Q}$ denote the set of all partitioned transition-rate matrices such that Q_{OO} and Q_{CC} each have distinct eigenvalues (and hence are diagonalisable) and the coefficients $\alpha_1, \alpha_2, \ldots, \alpha_{n_O}$ and $\beta_1, \beta_2, \ldots, \beta_{n_C}$ in the above representations of $f_O(t)$ and $f_C(s)$ are all nonzero. Fredkin *et al.* (1985) showed that for $Q \in \mathcal{Q}$, the parameters of $f_{OCO}(t_1, s_1, t_2), f_{COC}(s_1, t_2, s_2)$ and all higher order joint pdfs are determined by the parameters of $f_{OC}(t, s)$ and $f_{CO}(s, t)$. Thus, by determining the maximum number of free parameters in $f_{OC}(t, s)$ and $f_{CO}(s, t)$, they showed that $Q \in \mathcal{Q}$ is not identifiable if it depends on more than $2n_O n_C$ independent parameters. Fredkin and Rice (1986) showed that this bound is reduced to $2R(n_O+n_C-R)$, where $R = \min\{\text{rank}(Q_{OC}), \text{rank}(Q_{CO})\}$. Further, these bounds are reduced to $n_O n_C + n_O + n_C - 1$ and $(n_O + n_C)(R + 1) - R^2 - 1$, respectively, if $\{X(t)\}$ is time reversible.

These bounds can be used to show that some schemes are unidentifiable. For example, the scheme in Figure 4 is unidentifiable (Wagner *et al.* (1999)) as it contains 8 transition rates but $n_O = n_C = 2$ and $R = 1$, so $2R(n_O + n_C - R) = 6$.

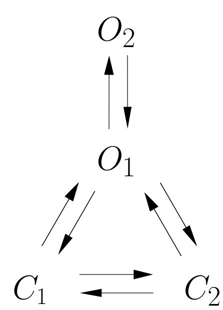

Figure 4: Example of an unidentifiable scheme.

3.2 Equivalent models

Recall that $\{X(t)\}$ and $\{X'(t)\}$ are aggregated continuous-time Markov chains, with transition-rate matrices Q and Q', each partitioned as at (1). Suppose that $Q, Q' \in \mathcal{Q}$ and $Q \sim Q'$. Then the open sojourn time pdfs imply that $\{X(t)\}$ and $\{X'(t)\}$ have the same number of open states, i.e. $n_O = n'_O$; similarly, $n_C = n'_C$. Kienker (1989) proved that, if $Q, Q' \in \mathcal{Q}$, then $Q \sim Q'$ if and only if there exists a nonsingular (similarity) matrix

$$
S = \begin{bmatrix} S_{OO} & 0 \\ 0 & S_{CC} \end{bmatrix},
$$

where S is partitioned as in (1), such that $S\mathbf{1} = \mathbf{1}$ and

$$Q' = S^{-1}QS.$$

A necessary and sufficient condition for $Q \sim Q'$ for general Q was proved by Larget (1998) but this is much less transparent than Kienker's condition.

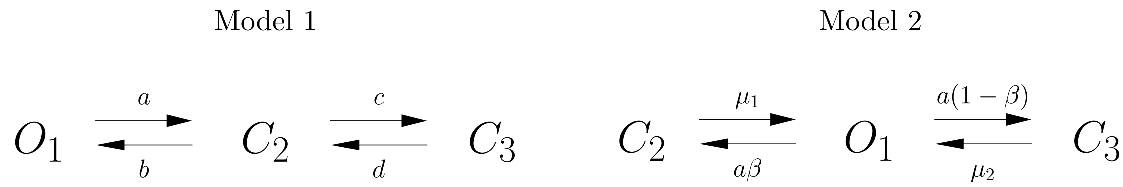

Figure 5: Equivalent three-state models.

A simple example of two equivalent models, examined in more detail in Kienker (1989), is given in Figure 5. In Model 1, the underlying continuous-time Markov chain $\{X(t)\}$ is time-reversible (since its associated graph is a tree; see Kelly (1979), Lemma 1.5), so β_1, β_2 in (5) are nonnegative (Kijima and Kijima (1987)). Hence, the closed sojourn time distribution is a mixture of two exponential distributions and its pdf takes the form $f_C(s) = \beta\mu_1 \mathrm{e}^{-\mu_1 s} + (1-\beta)\mu_2 \mathrm{e}^{-\mu_2 s}$ $(s \geq 0)$, where $\beta \in (0,1)$. It is then immediate that Models 1 and 2 are equivalent since, in both models, successive open and closed sojourn times are mutually independent (as there is only one open state) and open sojourn times follow an exponential distribution with mean a^{-1}.

3.3 Identifiability and distinguishability of schemes

It is straightforward to use Kienker's condition to determine whether two given models (i.e. Qs) are equivalent but generally it is a much harder task to determine whether a given scheme is identifiable or whether two given models are distinguishable. In practical applications transition-rate matrices usually belong to $\mathcal{Q}$ (possibly after state-space reduction to remove symmetries), so we now restrict attention to such Q.

Bruno *et al.* (2005) introduced Manifest Interconductance Rank (MIR) form, a canonical form for aggregated Markov models which greatly aids determining identifiability and distinguishability of schemes. We now assume that, for $i, j \in E$, $q_{ij} > 0$ if and only if $q_{ji} > 0$, a condition that is satisfied by any plausible steady-state single-channel model (and by all time-reversible models). An open state, i say, is termed an open gateway state if $q_{ij} > 0$ for some closed state j (so the channel can leave and enter the open class O via state i) and a closed gateway state is defined similarly. A scheme is in MIR form if and only if (i) all gateway states have precisely one link to the other class and (ii) there is no link between non-gateway states. Bruno *et al.* (2005) proved that amost every model can be expressed in MIR form, i.e. for almost all $Q \in \mathcal{Q}$, there exists a Q' in MIR form such that $Q \sim Q'$. However, some of the transition rates in Q' may be negative (or even complex if $\{X(t)\}$ is not time reversible). They also proved that MIR form is identifiable for almost all parameters, i.e. for almost all Q and Q' in MIR form, if $Q{\sim}Q'$ then $Q = Q'$ (possibly after permutation of states). The exceptional cases in the above results consist of degeneracies, such as certain matrices not being diagonalisable.

Bruno *et al.* (2005) described how to transform a model to MIR form, when it is possible. Thus to determine whether or not two schemes are distinguishable it is sufficient to transform a general model from each scheme into MIR form. This may not be easy in practice and there is also the issue of exceptional cases. However, many schemes in the ion channel literature are close to being in MIR form and investigating distinguishability of some such schemes motivated Ball *et al.* (2011) to define the following simple extension of MIR form.

A scheme is in open semi-MIR form if (i) every open state is a gateway state and (ii) every gateway state has precisely one link to the other class. Thus all the schemes in Figure 2 are in open semi-MIR form (indeed schemes (1) and (5) are in MIR form).

Suppose that Q is in open semi-MIR form. Let A denote the set of closed gateway states and let $B = C \setminus A$. Then Q can be expressed in the partitioned form

$$Q = \begin{bmatrix} Q_{OO} & Q_{OA} & 0 \\ Q_{AO} & Q_{AA} & Q_{AB} \\ 0 & Q_{BA} & Q_{BB} \end{bmatrix}.$$

Let $\{\tilde{X}(t)\}$ be the aggregated continuous-time Markov chain, having state space $C = A \cup B$, that is derived from $\{X(t)\}$ by setting all closed-to-open transition rates to zero. Then $\{\tilde{X}(t)\}$ has transition-rate matrix $\tilde{Q}$, given in partitioned form by

$$\tilde{Q} = \begin{bmatrix} Q_{AA} + Q_{AO} & Q_{AB} \\ Q_{BA} & Q_{BB} \end{bmatrix}.$$

Ball *et al.* (2011) showed that, under mild conditions, if $\{X(t)\}$ and $\{X'(t)\}$ are equivalent, then so are the corresponding derived processes $\{\tilde{X}(t)\}$ and $\{\tilde{X}'(t)\}$, when viewed as aggregated continuous-time Markov chains on the state space C partitioned into $A \cup B$, thus reducing the dimension of the problem. Further, for schemes with a block structure, such as the schemes in Figure 2, such dimension reduction can be done progressively. Ball *et al.* (2011) used such arguments to show that schemes in Figure 2 (and some other schemes for glycine channels) are distinguishable and identifiable, except for very few explicitly-stated exceptional cases.

3.4 Time interval omission induced near-unidentifiability

Although it is intuitively plausible, and fairly easy to show formally, that if $Q \sim Q'$ then the corresponding observable processes incorporating time interval omission and/or bursts are probabilistically indistinguishable, it is not known whether or not the converse is true. However, it is well known that time interval omission can cause near-unidentifiability. This occurs even in the simple two-state model shown in Figure 6; see, for example, as Yeo *et al.* (1988) and Ball and Davies (1995).

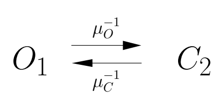

Figure 6: Two-state model.

The left panel of Figure 7 shows, for the two-state model of Figure 6, the log-likelihood function of the mean (actual) open and closed sojourn times (μ_O, μ_C) based on $10,000$ simulated pairs of observed open and closed sojourns, with $\mu_O = 0.2990, \mu_C = 0.8787$ and $\tau = 0.2$ ms. The likelihood function has two local maxima of similar heights, a *slow* peak $(\hat{\mu}_O^S, \hat{\mu}_C^S) = (0.2975, 0.8896)$, which in this case corresponds to the actual model, and a *fast* peak $(\hat{\mu}_O^F, \hat{\mu}_C^F) = (0.1070, 0.2194)$, which here is an artifact of time interval omission. The right panel of Figure 7 shows a slice of the log-likelihood surface in the vertical plane through the two maxima. A similar phenomenon has been observed in more complicated models; see, for example, Colquhoun *et al.* (2003). Distinguishing between the corresponding two candidate models can sometimes be done by examining the underlying single-channel record, otherwise they can be discriminated between by observing the same channel under different experimental conditions (for example, different agonist concentrations). Clearly such multimodal likelihood surfaces make designing MCMC samplers that move freely in their associated parameter spaces challenging. Similar identifiablity issues may also arise when making inference directly from single-channel recordings.

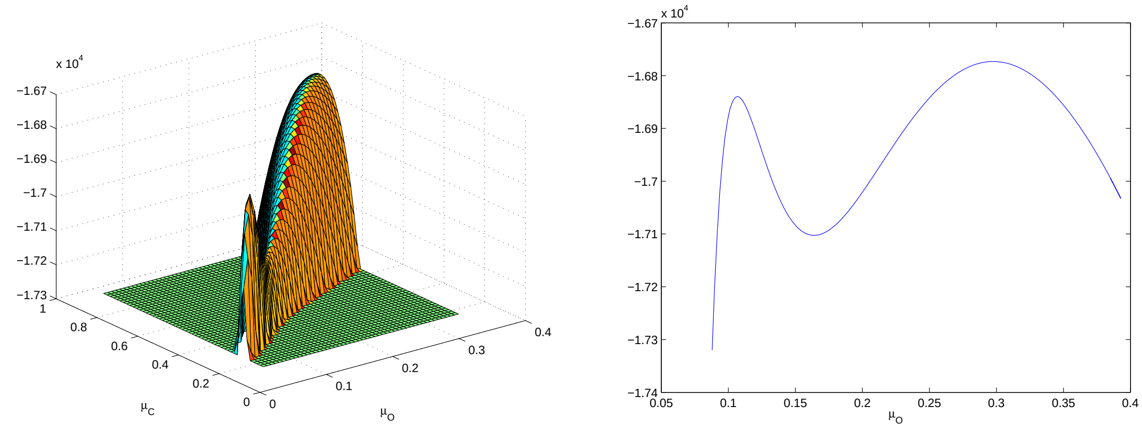

Figure 7: Log-likelihood of (μ_O, μ_C) for two-state model.

3.5 Poorly resolved models

Although, in theory, two or more schemes may be distinguishable, in practice, their predictions may be sufficiently similar to make discriminating between them difficult. Similarly, a scheme may be identifiable in theory but hard to resolve in practice. A striking example of the latter was given by Fredkin and Rice (1992), who considered amongst others the two three-state models shown in Figure 8.

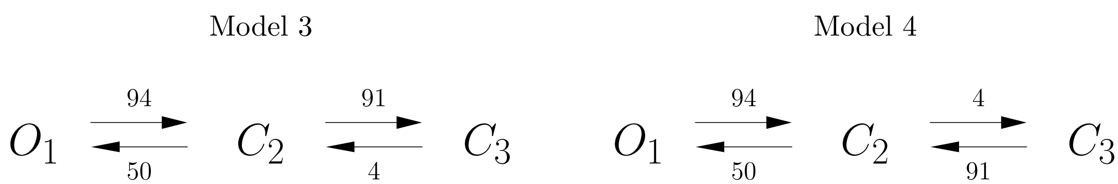

Figure 8: Three-state models.

As shown in Fredkin and Rice (1992), Model 3 is easily resolved but Model 4, which has been proposed for a sodium channel, is not. This is illustrated in Figure 9, which shows scatter plots of maximum likelihood estimates of the parameters (q_{21}, q_{23}, q_{32}) governing closed sojourns from $1,000$ simulations of each model. For Model 3, each simulation comprised of $1,000$ actual closed sojourns, whilst for Model 4, each simulation comprised of $10,000$ actual closed sojourns. (For these models, if actual sojourns are observed, open sojourns provide no information about (q_{21}, q_{23}, q_{32}).) Note that although the estimates for Model 3 are satisfactory, those for Model 4 clearly are not, in spite of being based on appreciably more data. Thus even a very simple model with ideal data may be difficult to resolve.

Related difficulties may also arise when trying to discriminate between alternative schemes, a topic of considerable practical importance when using models to understand ion channel function. Competing schemes are often not nested, which causes problems when using classical likelihood ratio tests. See Hodgson *et al.* (1999) and Wagner and Timmer (2001), respectively, for Bayesian MCMC

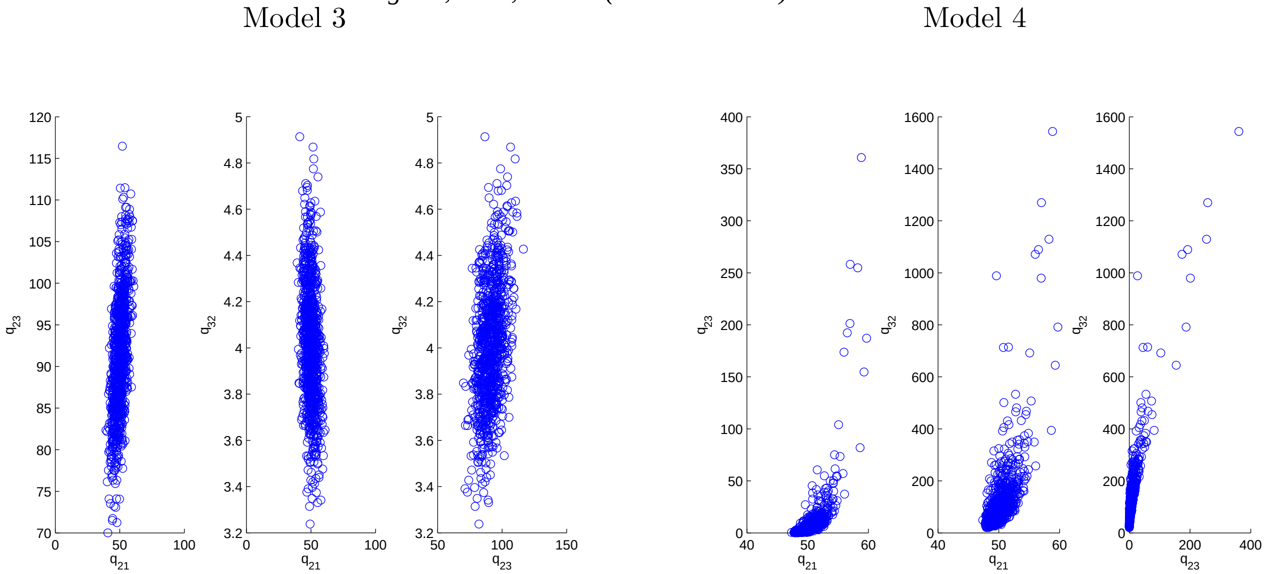

Figure 9: Scatter plots of maximum likelihood estimates of (q_{21}, q_{23}, q_{32}) for Model 3 and Model 4.

and likelihood-based approaches to model choice in Markov models of single ion channels, which is an area that is in urgent need of further research.

Acknowledgements

I thank David Colquhoun and Lucia Sivilotti for helpful discussions. Part of the research outlined here was done whilst I held a Leverhulme Research Fellowship, which is gratefully acknowledged.

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