Identifiability of aggregated Markov models of single ion channels

Frank Ball

Frank.Ball@nottingham.ac.uk

University of Nottingham

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Glycine receptor models

"Flip"  "Jones and Westbrook"

States with an asterisk are open, other states are closed. $a$ denotes concentration of agonist glycine.

(Burzomato et al. (2004))
Glycine receptor schemes

(Burzomato et al. (2004))
Outline of talk

- Brief background on aggregated Markov models of single ion channels and associated inference.
- Identifiability problems
  - Method for investigating identifiability and distinguishability of schemes.
  - Application to glycine receptor schemes.
- Concluding comments/future challenges.
Single channel modelling

Model

INFERENCE

Actual record

Minimum detectable sojourn

Idealised record

Inferring

Filtered record

Reconstructed record

Identifiability of aggregated Markov models of single ion channels – p.5
Aggregated Markov Processes

- Single channel gating modelled as an irreducible continuous-time Markov chain \( \{X(t)\} \) with state space \( E = \{1, 2, \ldots, n\} \).

- \( E = O \cup C \)
  - \( O = \) open states \( n_O = |O| \)
  - \( C = \) closed states \( n_C = |C| \)

- \( \{X(t)\} \) has transition rate matrix \( Q = [q_{ij}] \), where

\[
q_{ij} = \lim_{t \downarrow 0} \frac{1}{t} P(X(t) = j | X(0) = i) \quad (i \neq j), \quad q_{ii} = -\sum_{j \neq i} q_{ij}.
\]

- Assume \( q_{ij} = 0 \iff q_{ji} = 0 \) (satisfied by all time reversible models).

- Partition

\[
Q = \begin{bmatrix}
Q_{oo} & Q_{oc} \\
Q_{co} & Q_{cc}
\end{bmatrix}
\]
Equilibrium sojourn time distributions

\[ f_O(t) = \pi_O \exp(Q_{Oot})Q_{OC} \]

\[ f_C(t) = \pi_C \exp(Q_{Cot})Q_{CO} \]

\[ f_{OC}(t, s) = \pi_O \exp(Q_{Oot})Q_{OC} \exp(Q_{CC}s)Q_{CO} \]

\[ f_{OCO}(t_1, s_1, t_2) = \]

\[ \pi_O \exp(Q_{Oot_1})Q_{OC} \exp(Q_{CC}s_1)Q_{CO} \exp(Q_{Oot_2})Q_{OC} \]

\[ \pi_O = (\pi_1^O, \pi_2^O, \ldots, \pi_n^O): \text{equilibrium open entry state distribution} \]

(Colquhoun and Hawkes (1977), Fredkin et al. (1985))
Parameter estimation

Channel record \((t_1, s_1, t_2, s_2, \ldots, t_m, s_m)\)

Estimate \(Q\) (or parameters \(\theta\) describing \(Q\)) by maximising the likelihood

\[
L(Q) = \pi_O \left[ \prod_{i=1}^{m} \{ \exp(Q_{OQt_i})Q_{OC} \exp(Q_{CCs_i})Q_{CO} \} \right]^{1}
\]

to yield the maximum likelihood estimator (MLE) \(\hat{Q}(\hat{\theta})\).

Incorporate time interval omission and bursts

Identifiability problems

(Ball and Sansom (1989), Qin et al. (1997), Colquhoun et al. (2003))
Identifiability problems

- Over-parameterised models
- Equivalent models
- Identifiability and distinguishability of schemes
- Time interval omission induced near-unidentifiability
- Poorly resolved (near unidentifiable) models

\[
\text{scheme} = \text{set of allowable transitions between states} \\
\text{model} = \text{scheme} + \text{transition rates}
\]
Equilibrium sojourn time PDFs

If $Q_{OO}$ and $Q_{CC}$ are diagonalisable with eigenvalues $-\lambda_1, -\lambda_2, \ldots, -\lambda_{n_O}$ and $-\mu_1, -\mu_2, \ldots, -\mu_{n_C}$, respectively, then

$$f_O(t) = \pi_O \exp(Q_{OO}t)Q_{OC}1 = \sum_{i=1}^{n_O} \alpha_i \exp(-\lambda_i t)$$

and

$$f_C(t) = \pi_C \exp(Q_{CC}t)Q_{CO}1 = \sum_{j=1}^{n_C} \beta_j \exp(-\mu_j t)$$

\{X(t)\} time-reversible implies that $Q_{OO}$ and $Q_{CC}$ are diagonalisable, the $\alpha_i, \lambda_i, \beta_j, \mu_j$ are all real, and $\alpha_i \geq 0$ and $\beta_j \geq 0$.

$Q$: set of all partitioned $Q$-matrices for which $Q_{OO}$ and $Q_{CC}$ each have distinct eigenvalues, and $\alpha_1, \alpha_2, \ldots, \alpha_{n_O}$ and $\beta_1, \beta_2, \ldots, \beta_{n_C}$ are all nonzero.
Over-parameterised models

For $Q \in Q$,

$$f_{OC}(t, s) = \pi_O \exp(Q_{OO} t) Q_{OC} \exp(Q_{CC} s) Q_{CO} 1$$

$$= \sum_{i=1}^{n_O} \sum_{j=1}^{n_C} \alpha_{ij} \exp(-\lambda_i t + \mu_j s)$$

$$f_{OCO}(t_1, s_1, t_2) = \pi_O \exp(Q_{OO} t_1) Q_{OC} \exp(Q_{CC} s_1) Q_{CO} \exp(Q_{OO} t_2) Q_{OC} 1$$

$$= \sum_{i=1}^{n_O} \sum_{j=1}^{n_C} \alpha_{ijk} \exp(-\lambda_i t_1 + \mu_j s_1 + \lambda_k t_2)$$
Over-parameterised models

For \( Q \in \mathcal{Q} \),
\[
\begin{align*}
  f_{OC}(t, s) &= \pi_O \exp(Q_{Oot})Q_{OC} \exp(Q_{CC}s)Q_{CO}1 \\
  &= \sum_{i=1}^{n_O} \sum_{j=1}^{n_C} \alpha_{ij} \exp(- (\lambda_i t + \mu_j s))
\end{align*}
\]
\[
\begin{align*}
  f_{OCO}(t_1, s_1, t_2) &= \pi_O \exp(Q_{Oot_1})Q_{OC} \exp(Q_{CC}s_1)Q_{CO} \exp(Q_{Oot_2})Q_{OC}1 \\
  &= \sum_{i=1}^{n_O} \sum_{j=1}^{n_C} \sum_{k=1}^{n_O} \alpha_{ijk} \exp(- (\lambda_i t_1 + \mu_j s_1 + \lambda_k t_2))
\end{align*}
\]

Parameters of \( f_{OCO}(t_1, s_1, t_2) \), \( f_{CCO}(s_1, t_2, s_2) \) and all higher order joint pdfs are determined by the parameters of \( f_{OC}(t, s) \) and \( f_{CO}(s, t) \).

This implies that \( Q \) is unidentifiable if it depends on more than \( 2n_O n_C \) independent parameters (\( n_O n_C + n_O + n_C - 1 \) if \( \{X(t)\} \) is time reversible).

For models with rank \( R (= \min\{\text{rank}(Q_{OC}), \text{rank}(Q_{CO})\}) \), these bounds are reduced to \( 2R(n_O + n_C - R) \) and \( (n_O + n_C)(R + 1) - R^2 - 1 \), respectively.

(Fredkin et al. (1985), Fredkin and Rice (1986))
Overparameterised model - example

\[ n_O = 2, n_C = 2, R = 1 \] so maximum number of identifiable parameters is \( 2R(n_O + n_C - R) = 6 \), hence model is **unidentifiable** as it has 8 transition rates.

(Wagner et al. (1999))
Equivalent models

Two models with

\[
Q = \begin{bmatrix}
Q_{OO} & Q_{OC} \\
Q_{CO} & Q_{CC}
\end{bmatrix}
\quad \text{and} \quad
Q' = \begin{bmatrix}
Q'_{OO} & Q'_{OC} \\
Q'_{CO} & Q'_{CC}
\end{bmatrix}
\]

are equivalent (written \(Q \sim Q'\)) if \(\{X(t)\}\) and \(\{X'(t)\}\) have probabilistically indistinguishable equilibrium aggregated processes, i.e. if

\[
\begin{align*}
fo(t_1) &= f'_O(t_1), \\
f_C(s_1) &= f'_C(s_1), \\
f_{OC}(t_1, s_1) &= f'_{OC}(t_1, s_1), \\
f_{CO}(s_1, t_1) &= f'_{CO}(s_1, t_1), \
\end{align*}
\]

If \(Q \sim Q'\) and both \(Q\) and \(Q'\) belong to \(Q\) then \(n_O = n'_O\) and \(n_C = n'_C\).

(E. g. \(\sum_{i=0}^{n_o} \alpha_i e^{-\lambda_i t} = \sum_{i=0}^{n'_o} \alpha'_i e^{-\lambda'_i t}\) for all \(t > 0 \implies n_O = n'_O\).)
Equivalent models

**Theorem 1** (Kienker (1989))
Suppose \( \{X(t)\} \) and \( \{X'(t)\} \) each have \( n_O \) open and \( n_C \) closed states, and both \( Q \) and \( Q' \) belong to \( \mathcal{Q} \). Then, \( Q \sim Q' \) if and only if there exists a nonsingular matrix

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

with \( S1 = 1 \), such that \( Q' = S^{-1}QS \).

Larget (1998) obtained a necessary and sufficient condition for \( Q \sim Q' \) **without** requiring \( Q, Q' \in \mathcal{Q} \).
Example of equivalent models

\[ O_1 \xrightarrow{a} C_2 \xleftarrow{b} C_3 \]
\[ C_2 \xrightarrow{c} O_1 \xleftarrow{d} C_3 \]

\[ f_{O}(t) = ae^{-at} \quad f_{C}(t) = \beta \mu_1 e^{-\mu_1 t} + (1 - \beta) \mu_2 e^{-\mu_2 t} \]
\[ (0 < \beta < 1) \]

is equivalent to

\[ C_2 \xrightarrow{\mu_1} O_1 \xleftarrow{a\beta} C_3 \]
\[ C_2 \xleftarrow{a(1 - \beta)} O_1 \xrightarrow{\mu_2} C_3 \]
Identifiability of a scheme

Given a scheme and transition rates, e.g.

\[
\begin{array}{ccc}
C_{10} & C_9 & C_8 \\
q & i & k \\
r & j & i \\
f & e & C_6 \\
f & d & c \\
O_3 & O_2 & O_1 \\
\end{array}
\]

\[
\begin{array}{ccc}
C_5 & C_4 \\
m & i & h \\
p & j & g \\
C_{10} & C_6 & C_5 \\
q & o & m \\
r & p & n \\
C_{10} & C_9 & C_8 \\
q & i & k \\
r & j & i \\
O_3 & O_2 & O_1 \\
\end{array}
\]

does there exist another set of transition rates \((\alpha' - r')\) such that \(Q \sim Q'\)?
Example of an unidentifiable scheme

(Wagner et al. (1999))
Distinguishability of schemes

Given two mechanisms, e.g.

(1) \[C_{10} \xrightarrow{q} C_6 \xrightarrow{o} C_5 \xrightarrow{m} C_4 \]

(2) \[C_{10} \xrightarrow{q} C_6 \xrightarrow{o} C_5 \xrightarrow{m} C_4 \]

and a set of transition rates \((a-r)\) for (1) does there exist a set of transition rates for (2) such that \(Q_1 \sim Q_2\), and vice versa?
Example of indistinguishable schemes

(Wagner et al. (1999), Bruno et al. (2005))
Gateway states: states that can be entered directly from the other class.

A model is in MIR (Manifest Interconductance Rank) form if and only if

(i) all gateway states have precisely one link to the other class,
(ii) there is no link between non-gateway states.

Model (1) is in MIR form, models (2) and (10) are not.
MIR Form

**Theorem 2** (Bruno et al. (2005))

(a) **Almost every** model can be expressed in MIR form, i.e. for almost all $Q$ belonging to $Q$, there exists a $Q'$ in MIR form such that $Q \sim Q'$. 

[Some transition rates in $Q'$ may be **negative** (or **complex** if $Q$ is not time reversible)]

(b) **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).

_exceptional_ **Cases consist of degeneracies such as certain matrices not being diagonalisable.**
Application of MIR form

Models (1) and (5) are in MIR form, so they are identifiable and distinguishable (for almost all parameters)
Identifiability of glycine schemes

In the next part of my talk I described an as yet unpublished method for investigating identifiability and distinguishability of a range of practically relevant single channel gating schemes and used the method to show that the glycine receptor schemes 1-10 are distinguishable and identifiable (apart from perhaps schemes 9 and 10, which may not be identifiable).
Time interval omission unidentifiability

\[ O_1 \xrightarrow{\mu_O^{-1}} C_2 \xleftarrow{\mu_C^{-1}} \]

Log-likelihood of \((\mu_O, \mu_C)\) from 10,000 simulated pairs of observed open and closed sojourns, with \(\mu_O = 0.2990, \mu_C = 0.8787\) and \(\tau = 0.2\) ms

(Yeo et al. (1988), Ball and Davies (1995))
Two-state model

Likelihood has two maxima

Slow \( (\hat{\mu}_O^S, \hat{\mu}_C^S) = (0.2975, 0.8896) \)

Fast \( (\hat{\mu}_O^S, \hat{\mu}_C^S) = (0.1070, 0.2194) \)

Slice of Log-likelihood along line joining two maxima
Poorly resolved models

Model 1

\[ O_1 \xrightarrow{94} C_2 \xrightarrow{4} C_3 \]

Model 2

\[ O_1 \xrightarrow{94} C_2 \xrightarrow{50} C_3 \]

Model 3

\[ O_1 \xleftarrow{50} C_2 \xleftarrow{91} C_3 \]

(Fredkin and Rice (1992))
Fredkin and Rice Model 1

Fredkin and Rice Model 1

\[
O_1 \quad \overset{94}{\rightarrow} \quad C_2 \quad \overset{4}{\rightarrow} \quad C_3
\]

MLEs of \( Q_{CC} \) based on 10,000 closed sojourns
Fredkin and Rice Model 2

\[ O_1 \xrightarrow{94}{4} C_2 \xrightarrow{50}{91} C_3 \]

MLEs of \( Q_{CC} \) based on 10,000 closed sojourns
Fredkin and Rice Model 3

\[ O_1 \xrightarrow{94\;\text{to}\;50} C_2 \xrightarrow{91\;\text{to}\;4} C_3 \]

MLEs of \( Q_{CC} \) based on 1,000 closed sojourns
Fredkin and Rice Models 1 and 3

Estimated closed time pdfs using MLE based on 10,000 closed sojourns, 1000 simulations

Model 1

Model 3
Fredkin and Rice Models 1, 2 and 3

\[ f_C(t) = \beta \mu_1 e^{-\mu_1 t} + (1 - \beta) \mu_2 e^{-\mu_2 t} \]

Model 1

\[
\begin{array}{ccc}
O_1 & \xrightarrow{94} & C_2 \\
& \xleftarrow{50} & \\
C_2 & \xrightarrow{4} & C_3 \\
C_3 & \xleftarrow{91} & \\
\end{array}
\]

\[
\begin{array}{cccc}
\beta & \mu_1 & 1 - \beta & \mu_2 \\
\hline
Model 1 & 0.9233 & 45.92 & 0.0767 & 99.08 \\
Model 2 & 0.9897 & 2.56 & 0.0103 & 142.44 \\
Model 3 & 0.6582 & 1.39 & 0.3418 & 143.61 \\
\end{array}
\]
Concluding comments/future challenges

- Open semi-MIR form yields a method of investigating identifiability and distinguishability applicable to a range of practically relevant single channel gating schemes.

- Systematic method of determining identifiability and distinguishability of more general schemes?

- Equivalence of models with concentration dependent rates?

- Equivalence of models incorporating time interval omission and/or from burst data?
Concluding comments/future challenges

Discrimination of models in practice?

- Design of experiments that maximise difference between predicted outcomes of competing schemes.

- Testing of non-nested schemes
  - Bayesian model choice using Markov chain Monte Carlo (Hodgson and Green (1999))
  - Likelihood-based model selection - embed schemes in more general scheme (Wagner and Timmer (2001))
  - Above methods applied to only very simple schemes (4 states, 6 rates).


References


