Binary Markov Chains for Single Molecule Kinetics

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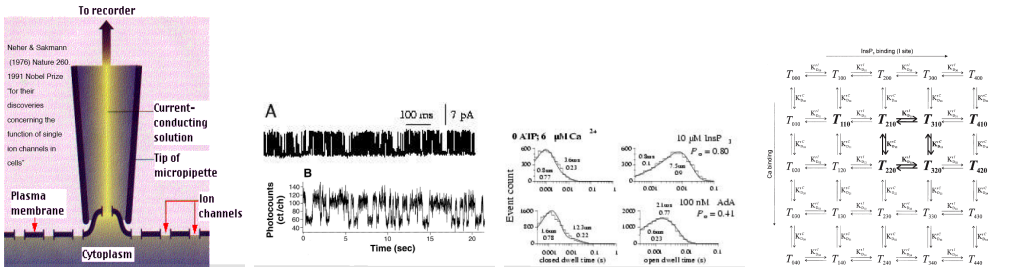
Abstract

In this lecture we will develop the theory of identification of aggregated Markov models for single molecule kinnetics.

1 Introduction

The theory of aggregated Markov models as developed by Colquhoun and others has been used to model ion channels since soon after Sakmann and Neher invented the patch-clamp recording. The essential prediction of the theory is that the dwell-time distributions are sums of decaying exponentials. Considerable effort has gone into extracting kinetic gating mechanisms from the statistics of patch-clamp recordings. There are two primary computer programs written for this task; HJCFIT, developed at the Colquhoun lab, and QUB developed at the Sachs lab. Considerable time and expertise has gone into the development of these codes.

We will formulate the theory of aggregated binary Markov models in which we stress the notion of probability *flux*. This formulation is mathematically equivalent to the standard formulation as developed by Colquhoun



(a) Patchclamp recorder (b) Current trace (c) Distribution Functions (d) Reaction Kinetics

Figure 1: (a) (s)The patchclamp was invented by Neher and Sakmann in the mid 1970's. With it they were able to observe the first current traces from single ion channels. (b) The two time series are from single molecules. The upper trace is an ion channel current trace. The lower one is the fluorescense signal emitted by an intermediate of the reaction catalyzed by a single molecule of the enzyme cholesterol oxidase (by groups at Harvard and Pacific Northwest National Lab [9]).(c)The statistics of these signals are analyzed in terms of "dwell time" distributions. (d)From the statistics of the signals researchers try to reconstruct the transition diagram that the protein undergoes as the molecule undergoes it's stochastic time dynamics.

and others [1, 2, 3, 4, 5, 6, 7, 8] but the physical content of the theory is highlighted.

We use our formulation to derive new results on modal gating, latency distributions, and on multimeric channels with identical indpendent subunits. We develop a fairly complete theory of the models with identical independent subunits. Including e We also discuss identifiability of Markov models in light of ligand dependence. Throughout, we stress the concept of detailed balance, or *microscopic reversibility*. We will show how to calculate the ligand dependence of the equilibrium open and closed probabilities and of the mean open and closed times by inspection when detailed balance holds. Similar results can also be obtained when detailed balance doesn't hold, but they are slightly more difficult. We also will derive some old results in a new way that is simpler and more intuitive. There is some pleasure in seeing old results in a new light. For example we will show that the higher dimensional distribution functions contain no additional information beyond the two-dimensional ones. Our derivation of this result is short and transparent compared to Fredkin *et al*'s original derivation [4]. We will also show when detailed balance is satisfied the coefficients of the decaying exponential in the one-dimensional dwell-time distributions are positive. Our derivation of

this result is short and transparent compared to Kijima and Kijima's original derivation [5].

2 Background

We consider binary Markov chains in which there are N states. Of these states, N_O are denoted "O" (open) and N_C are denoted "C" (closed). We assume current flows through the channel when it is in one of the open states and that it passes no current when it is in one of the closed states. We assume that the transitions from open to closed and *vice versa* are instantaneous. The current takes on only two values, 0, and 1, say, with no intermediate values. Although real channels are more complex than this, with the baseline current drifting, the binary assumption is still a reasonable first approximation to the observed dynamics.

2.1 The Master Equation and Transition Rates

We assume that the channel switches randomly between states with exponentially distributed waiting times. The probability $p(t) = (p_1(t), p_2(t), \ldots p_N(t))$ for being in the various states evolves according to the "master equation"

$$\frac{dp}{dt} = pQ \tag{1}$$

with solution $p(t) = p_o \exp(Qt)$. p_o is the vector of initial probabilities and Q is called the "generator matrix" with Q_{ij} giving the probability per unit time of making a transition from state i to state j. The diagonal elements satisfy $Q_{ii} = -\sum_{j \neq i} Q_{ij}$ which is a statement of conservation of probability. A convenient way to see this is to denote a column vector containing all ones by u. Then we have $dp/dt\ u = pQu = 0$. Multiplication of a row vector on the right by u is an "inner product" that corresponds to summing the components of the row vector. We denote the vector of equilibrium probabilities p^{eq}:

$$p^{eq}Q = 0\ . \tag{2}$$

We also have

$$Qu = 0 \tag{3}$$

These probabilities must sum to 1 so that $p^{eq}u = 1$. Normalization of the initial probabilties is written the same way: $p_o u = 1$. Because we are going to consider binary Markov chains we partition Q:

$$Q = \begin{pmatrix} Q_{OO} & Q_{OC} \\ Q_{CO} & Q_{CC} \end{pmatrix} \tag{4}$$

where Q_{OO} describes the open to open transitions, Q_{OC} the open to closed transitions, *etc.* We also partition the equilibrium probability p^{eq} and the initial probabilities accordingly:

$$p^{eq} = (p_O^{eq}, p_C^{eq}) \tag{5}$$

$$p_o = (p_{Oo}, p_{Co}) \tag{6}$$

and similarly

$$u = \begin{pmatrix} u_O \\ u_C \end{pmatrix} . \tag{7}$$

Combining equations 2-7 yields:

$$\begin{aligned} Q_{CO}u_O + Q_{CC}u_C &= 0 \\ Q_{OC}u_C + Q_{OO}u_O &= 0 \\ p_C^{eq}Q_{CC} + p_O^{eq}Q_{OC} &= 0 \\ p_O^{eq}Q_{OO} + p_C^{eq}Q_{CO} &= 0 \end{aligned} \tag{8}$$

which we shall refer to collectively as the "null vector" equations.

The key statistical distributions are the open and closed dwell-time distributions. The open time distribution is the probability density for a channel that opened at time 0 to close for the first time at time t_O. The open time distribution is solved by assuming that the channel is initially open but that once closed it stays closed. Then the probability that the channel is in any of the open states is a decreasing function of time. The probability that the channel first closed at time t_O is given by

$$\frac{dp_O}{dt} = p_O Q_{OO} \tag{9}$$

which has solution $p_O(t_O) = p_{0o}\exp(Q_{OO}t_O)$. The probability, F_O, that the channel remains open at time t_O is the sum of the probability over all the open states:

$$F_O(t_O) = p_{Oo}\exp(Q_{OO}t_O)u_O \tag{10}$$

where we've used the fact that multiplication on the right by an "all ones" vector is a summation. (Multiplication on the left by $u^T, or u_C^T or u_O^T$ is also a summation.) The probability, G_C, that the channel closes for the first time at time t_O is $G_C(t_O) = 1 - F_O(t_O)$. The open-time distribution $f_O(t_O)$ is defined by:

$$\int_0^{t_O} f_O(t)dt = G_C(t_O) \tag{11}$$

or $f_O(t_O) = dG_C(t_O)/dt_O$ so that

$$f_O(t_O) = -p_{Oo} \exp(Q_{OO} t_O) Q_{OO} u_O \tag{12}$$

which can be combined with the null vector equations to yield:

$$f_O(t_O) = p_{Oo} \exp(Q_{OO} t_O) Q_{OC} u_C \ . \tag{13}$$

The closed time distribution is similar.

$$f_C(t_C) = p_{Co} \exp(Q_{CC} t_C) Q_{CO} u_O \ . \tag{14}$$

The above formulae for the open and closed time distributions are valid for all initial probability distributions. The appropriate initial distribution depends on the experiment. In the next subsection we discuss that initial probabilities, p_{Co} and p_{Oo} in detail.

2.2 The Initial Probabilities p_{Oo} and p_{Co}

The form for the distribution function in Eq(13) is the same for *all* initial probabilities. Specifically, that formula is valid for both the equilibrium open time distribution and for latency distributions. The only difference between the two is the initial probability vector.

2.2.1 Latency versus equilibrium: a simple example

Here we consider the model

$$C_0 + L \underset{k_{10}}{\overset{k_{01}L}{\rightleftharpoons}} C_1 \underset{k_{O1}}{\overset{k_{1O}}{\rightleftharpoons}} O \tag{15}$$

in which the step from C_0 to C_1 requires ligand binding and the step from C_1 to the open state is a conformation change that requires no ligand binding.

The concentration of the ligand is denoted "L". For this model

$$Q_{CC} = \begin{pmatrix} -k_{01}L & k_{01}L \\ k_{10} & -k_{10} - k_{1O} \end{pmatrix} . \tag{16}$$

We shall consider the distribution function of closed time for two different experiments, (1)a "ligand jump" experiment and (2)an equilibrium experiment. In the ligand jump experiment the channel is in a preparation containing no ligand until time $t = 0$ at which time the ligand concentration is instantaneously increased to $L > 0$. The time for the channel to go open during a single experiment is called "the latency". By performing such an experiment repeatedly one can build up the "latency distribution" from data. In the equilibrium experiment, the channel is exposed to ligand concentration L and flickers on and off. The only difference in the distribution functions for these two cases is the initial probability vector p_{Co}. In the ligand jump experiment $o_{Co} = p_{Co}^{jump} = (1, 0)$. In the equilibrium experiment $p_{Co} = p_{Co}^{eq} = (0, 1)$ (why?). For convenience we can write the closed time distribution as $f_C(t_c) = p_{Co} S e^{-\Lambda t_c} \Lambda u_C$ where $-\Lambda$ is the diagonal matrix containing the eigenvalues of Q_{CC} and S is the matrix of eigenvectors of Q_{CC} with amplitudes chosen so that $Su = u$. Thus the distribution function can be written:

$$f_C(t_c) = \tilde{p}_{Co} \begin{pmatrix} \lambda_+ e^{-\lambda_+ t_c} \\ \lambda_- e^{-\lambda_- t_c} \end{pmatrix} \tag{17}$$

where $\tilde{p}_{Co} = p_{Co} S$ and $-\lambda_\pm$ are the eigenvalues of Q_{CC}:

$$\lambda_\pm = \frac{1}{2}(-T \pm \sqrt{T^2 - 4\Delta}) \tag{18}$$

where T and Δ are the trace and determinant of Q_{CC} respectively:

$$T = -k_{01}L - k_{10} - k_{1O} \tag{19}$$

$$\Delta = k_{01}Lk_{1O} . \tag{20}$$

(**NB:** $\lambda_\pm$ are the negatives of the eigenvalues of Q_{CC} so thet are positive numbers and with our definition above: $\lambda_+ > \lambda_- > 0$.) Thus we have:

$$f_C^{lat}(t_c) = (1, 0) S \begin{pmatrix} \lambda_+ e^{-\lambda_+ t_c} \\ \lambda_- e^{-\lambda_- t_c} \end{pmatrix} \tag{21}$$

$$f_C^{eq}(t_c) = (0, 1) S \begin{pmatrix} \lambda_+ e^{-\lambda_+ t_c} \\ \lambda_- e^{-\lambda_- t_c} \end{pmatrix} \tag{22}$$

and S is given by:

$$S = \begin{pmatrix} 1 & 1 \\ 1 + \frac{-\lambda_+}{k_{01}L} & 1 + \frac{-\lambda_-}{k_{01}L} \end{pmatrix} \begin{pmatrix} \alpha_+ & 0 \\ 0 & \alpha_- \end{pmatrix} \tag{23}$$

where

$$\begin{pmatrix} \alpha_+ \\ \alpha_- \end{pmatrix} = \begin{pmatrix} 1 & 1 \\ 1 + \frac{-\lambda_+}{k_{01}L} & 1 + \frac{-\lambda_-}{k_{01}L} \end{pmatrix}^{-1} \begin{pmatrix} 1 \\ 1 \end{pmatrix} = \frac{1}{\lambda_+ - \lambda_-} \begin{pmatrix} -\lambda_- \\ \lambda_+ \end{pmatrix} \tag{24}$$

so that $Su = u$. We have then:

$$f_C^{lat}(t_c) = \frac{\lambda_+\lambda_-}{\lambda_+ - \lambda_-}(e^{-\lambda_- t_c} - e^{-\lambda_+ t_c}) \tag{25}$$

$$f_C^{eq}(t_c) = \frac{\lambda_-\lambda_+}{\lambda_+ - \lambda_-}\Big((1 + \frac{-\lambda_-}{k_{01}L})e^{-\lambda_- t_c} - (1 + \frac{-\lambda_+}{k_{01}L})e^{-\lambda_+ t_c}\Big) \tag{26}$$

The coefficients of the decaying exponentials in $f_C^{lat}(t_C)$ are clearly equal and of opposite sign. The coefficients of the two decaying exponentials in $f_C^{eq}(t_C)$ are both positive. To see this note that $(2k_{01}L + T)^2 + (T^2 - 4\Delta) > 0$ and $(2k_{01}L + T)^2 - (T^2 - 4\Delta) < 0$. It follows that $\lambda_+ \geq k_0 1L \geq \lambda_-$ so that the amplitudes of the decaying exponentials of $f_C^{eq}(t_C)$ are both positive. We shall show a more general version of this result later.

2.3 Equilibrium Entry Probabilities 1

The quantities p_{Co} and p_{Oo} are the initial (or entry) probabilities for the various states being occupied. At *equilibrium*, the entry probabilities are determined from the equilibrium occupancies and the flux of probability from open to closed. Intuitively, one can understand the equilibrium entry probabilities p_{Co}^{eq} and p_{Oo}^{eq}, in terms of an ensemble of current traces. We take the ensemble and in each member of the ensemble, choose an open to closed transition. Then $(p_{Co}^{eq})_j$ is the fraction of the open-closed transitions that resulted in the channel entering the jth closed state. Similarly $(p_{Oo}^{eq})_i$ is the fraction of the closed-open transitions that resulted in the channel entering the ith open state. We now show how to calculate the entry probabilities in terms of probability fluxes.

2.3.1 Probability Flux

The most intuitive way to determine appropriate initial conditions is in terms of probability flux. The instantaneous probability flux $\mathcal{J}_{ij}$ from state i to state j is given by the probability of being in state i p_i times the transition rate from state i to state j, k_{ij}:

$$\mathcal{J}_{ij} = k_{ij} p_i \ . \tag{27}$$

We'll denote the total flux out of state i by: $\mathcal{J}_i = \sum_j \mathcal{J}_{ij} = k_{ij} p_i$. The probability that the state entered state j from state i, given that it just left state i is given by:

$$p_{ij} = \frac{\mathcal{J}_{ij}}{\mathcal{J}_i} = \frac{k_{ij}}{\sum_j k_{ij}} \tag{28}$$

2.3.2 Equilibrium Entry Probabilities 2

We now consider the flux from open to closed at equilibrium. The equilibrium flux from the ith closed state to the jth open state is denoted J_{ij} and is given by:

$$J_{ij} = (p_C^{eq})_i k_{ij} \ . \tag{29}$$

The flux from all closed states into the ith open state is given by $(p_C^{eq} Q_{CO})_i$. In vector notation the equilibrium probability flux into the various open states is given by $p_C^{eq} Q_{CO}$. The total flux from closed to open, denoted J, is given by $J = p_C^{eq} Q_{CO} u_O$. It is easy to show that at equilibrium, the total flux from open to closed, $p_O^{eq} Q_{OC} u_C$, is equal to the total flux from closed to open. That is,

$$J = p_C^{eq} Q_{CO} u_O = p_O^{eq} Q_{OC} u_C \tag{30}$$

The entry probabilities for channels that just transitioned from closed to open, p_{Oo}^{eq} (or open to closed p_{Co}^{eq}) are given by

$$p_{Oo}^{eq} = \frac{p_C^{eq} Q_{CO}}{J} \tag{31}$$

$$p_{Co}^{eq} = \frac{p_O^{eq} Q_{OC}}{J} \ . \tag{32}$$

In the example above we said that p_{Co}^{eq}. If you've understood the intuitive notion of equilibrium entry probability it should be clear that $p_{Co}^{eq} = (0, 1)$. We show it here using Eqs(30,32. We have that $Q_{OC} = (0, k_{OC})$ and $J =$

$p_O^{eq} Q_{OC} u_C = p_O^{eq} k_{OC}$ so that $p_{Co}^{eq} = \frac{p_O^{eq} Q_{OC}}{J} = \frac{p_O^{eq}(0, k_{OC})}{p_O^{eq} k_{OC}} = (0, 1)$ in accord with the intuitive claim.

2.3.3 Non-Equilibrium Entry Probabilities

In the example that began this section we calculated both the equilibrium closed time distribution function and the distribution of latencies for an experiment in which the ligand concentration was zero until time t=0 at which time it was jumped to some nonzero value. Since the model had only a single state with no ligand bound (C_0) the entry probability was given by: $p_{Co}^{lat} = (1, 0)$. More generally, in such an experiment, in which the system is allowed to equilibrate under one condition and then jumped rapidly to another condition the entry probability should be given by the equilibrium occupancies for the first condition. Thus in terms of the example discussed at the beginning of this section we see that $p_{Co}^{lat} = p_C^{eq}(L = 0) = (1, 0)$.

2.4 Moments of the Equilibrium Distribution Functions

We can now write an explicit formula for the dwell-time distributions in terms of the transition matrices and the equilibrium occupancies.

$$f_O(t_O) = \frac{1}{J} p_C^{eq} Q_{CO} e^{Q_{OO} t_O} Q_{OC} u_C = \frac{1}{J} p_O^{eq} Q_{OO} e^{Q_{OO} t_O} Q_{OO} u_O \quad (33)$$

$$f_C(t_C) = \frac{1}{J} p_O^{eq} Q_{OC} e^{Q_{CC} t_C} Q_{CO} u_O = \frac{1}{J} p_C^{eq} Q_{CC} e^{Q_{CC} t_C} Q_{CC} u_C \quad (34)$$

The moments of the open time distribution are given by:

$$< \tau_O^n > \equiv \int_0^\infty t^n f_O(t) dt = (-1)^{n+1} n! p_O^{eq} Q_{OO}^{1-n} u_O / J \ . \quad (35)$$

Using the null vector equations (8) it can be shown that the zeroeth moment is unity (as it must be if the distribution functions are to be properly normalized. Moreover we have the following for the important "mean open time": $\tau_O = \frac{p_O^{eq} u_O}{J}$. The quantity in the numerator, $p_O^{eq} u_O$, is of fundamental importance. It is the equilibrium open probablity denoted by P_O ($P_O \equiv p_O^{eq} u_O$).

We also have the equilibrium closed probability $P_C \equiv p_O^{eq} u_O$ so that

$$\tau_O = \frac{P_O}{J} \tag{36}$$

$$\tau_C = \frac{P_C}{J} \tag{37}$$

which has a simple physical interpretation. The mean open time is simply the equilibrium open probability divided by the flux of probability from the open states to the closed states. Note that $\tau_O + \tau_C = 1/J$.

2.4.1 Example: Latency Distribution for the linear chain

Here we consider a model of the form:

$$C_O \underset{k_{10}}{\overset{k_{01}}{\rightleftharpoons}} C_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C_2 \ldots C_{N-1} \underset{k_{N(N-1)}}{\overset{k_{(N-1)N}}{\rightleftharpoons}} C_N \underset{k_{OC}}{\overset{k_{CO}}{\rightleftharpoons}} O \tag{38}$$

We are going to consider the distribution of latencies for the channel to open given that it began in the state C_0 so that $p_{Co} = (1, 0, 0, \ldots, 0, 0)$. For convenience we label the elements of these vectors $0, 1, 2, \ldots, N$. Similarly we label the elements of Q_{CC} as $0, 1, \ldots N$. We assume that $N \geq 1$ so that there are at least two closed states. We now derive a closed-form expression for the latency distribution in terms of the decays.

$$Q_{CC} = \begin{pmatrix} -k_{01} & k_{01} & 0 & 0 & \ldots & 0 \\ k_{10} & -k_{10} - k_{12} & k_{12} & 0 & \ldots & 0 \\ 0 & k_{21} & -k_{21} - k_{23} & k_{23} & \ldots & 0 \\ & & \vdots & & & \\ 0 & \ldots & k_{i(i-1)} & -k_{i(i-1)} - k_{i(i+1)} & k_{i(i+1)} & 0 \\ 0 & \ldots & 0 & 0 & k_{N(N-1)} & -k_{N(N-1)} - k_{CO} \end{pmatrix} \tag{39}$$

Note that every row of Q_{CC} save the last one sums to zero so that the only nonzero element of $Q_{CC} u_C$ is the last element. Since $f^{lat}(t = 0) = p_{Co} Q_{CC} u_C$ and the only non-zero element of p_{Co} is element 0 so that $f^{lat}(t = 0) = 0$. This result is quite general. A similar arguement can be used to show that the $0^{th} - (N-1)^{th}$ derivatives of the latency distribution are all zero:

$$\frac{d^i f^{lat}}{dt^i} = p_{Co} Q_{CC}^{1+i} u_C = 0 \; i = 0, \ldots, N-1 \text{ for the linear chain} \tag{40}$$

The latency distribution distribution can be written

$$f^{lat}(t) = \sum_i \alpha_i e^{-\lambda_i t} \tag{41}$$

and combined with the normalization condition $\int f^{lat} dt = 1$ and Eq(40) to yield a system of $N+1$ equations for the $(N+1)$ unknown amplitudes $\alpha_0, \alpha_1 \ldots \alpha_N$:

$$\begin{aligned} 1 &= \sum_i \frac{\alpha_i}{\lambda_i} \\ 0 &= \sum_i \alpha_i \\ 0 &= \sum_i \alpha_i \lambda_i \\ 0 &= \sum_i \alpha_i \lambda_i^2 \\ &\vdots \\ 0 &= \sum_i \alpha_i \lambda_i^{N-1} \ . \end{aligned} \tag{42}$$

These Vandermonde equations can be inverted to yield:

$$\alpha_i = \frac{\prod_j \lambda_j}{\prod_{j=1\ j\neq i}^{N} (\lambda_j - \lambda_i)} \tag{43}$$

The result that the $0-(N-1)$ derivatives of $f^{lat}(0)$ are all zero is valid for more complex reaction networks. The general result is that if there are N-links in the shortest path between the closed states that are initially occupied to the closest open state, then the $0-(N-1)$ derivatives of $f^{lat}(0)$ are all zero.

3 Identifiability

Our goal is the identification of a reaction network (as well as reaction rates) such as that shown in Fig. 1d from experimental data. Obviously, whether

this is possible or not depends on the nature of the data. We begin with another example. We consider the two topologies:

$$\begin{array}{lcccccc} I: & C_0 & \underset{k^I_{10}}{\overset{k^I_{01}}{\rightleftharpoons}} & C_1 & \underset{k^I_{O1}}{\overset{k^I_{1O}}{\rightleftharpoons}} & O \\ II: & C_0 & \underset{k^{II}_{O0}}{\overset{k^{II}_{0O}}{\rightleftharpoons}} & O & \underset{k^{II}_{1O}}{\overset{k^{II}_{O1}}{\rightleftharpoons}} & C_1 \end{array} \tag{44}$$

If the data consists only of the steady-state equilibrium open-closed time-series obtained at a single value of the rate constants, it isn't possible to distinguish between these two topologies; for any set of rates $k^I_{10}, k^I_{01}, k^I_{O1}, k^I_{1O}$ in the first topology, we can find a set in the second topology that will yield identical distribution functions. Indeed in the example given in "latency versus equilibrium" subsection, we actually constructed most of the transformation that maps from topology I to topology II. It was simply the similarity transformation that diagonalizes Q_{CC}. Kienker showed that it is possible to find transformations between model topologies such that two different topologies with appropriately chosen rates have identical dwell-time distributions [7]. The transformations that accomplish this task are similarity transformations which map the transition matrices and entry probabilities between different topologies provided the topologies have the same number of open and closed states.

3.1 Kienker Transformations

Kienker showed that any two models with generator matrices, Q and $\widetilde{Q}$ with the same number of open and closed states, that satisfy some mild technical conditions will yield the same distribution functions if and only if there exist similarity transformations, S between Q and $\widetilde{Q}$. The similarity transformation S has the following form:

$$S = \begin{pmatrix} S_{OO} & 0 \\ 0 & S_{CC} \end{pmatrix} \tag{45}$$

and

$$Su = u \tag{46}$$

with u a column vector containing all ones, as usual. The parts of the generator matrices transform according to:

$$\begin{array}{ll} \widetilde{Q}_{OO} = S^{-1}_{OO} Q_{OO} S_{OO} & \widetilde{Q}_{OC} = S^{-1}_{OO} Q_{OC} S_{CC} \\ \widetilde{Q}_{CO} = S^{-1}_{CC} Q_{CO} S_{OO} & \widetilde{Q}_{CC} = S^{-1}_{CC} Q_{CO} S_{OO} \end{array} \tag{47}$$

and the initial distributions transform according to:

$$\widetilde{p}_{Oo} = p_{Oo} S_{OO} \quad \widetilde{p}_{Co} = p_{Co} S_{CC} \tag{48}$$

In the next subsection we introduce the multi-time distribution functions. It is easy to check that they are invariant under these transformations.

3.2 The Multi-time Distribution functions

There is an infinite hierarchy of multi-dimensional distribution functions for the densities of being open for a time t_{O1} then closed for a time t_{C1} open for a time t_{O2}, closed for a time t_{C2}, open closed, etc.. These are denoted f_{CO}, f_{OC}, f_{OCO}, f_{COC}, etc.

$$\begin{aligned}
f_O(t_O) &= p_{Oo} \exp(Q_{OO} t_O) Q_{OC} u_C \\
f_C(t_C) &= p_{Co} \exp(Q_{CC} t_C) Q_{CO} u_O \\
f_{OC}(t_O, t_C) &= p_{Oo} \exp(Q_{OO} t_O) Q_{OC} \exp(Q_{CC} t_C) Q_{CO} u_O \\
f_{CO}(t_O, t_C) &= p_{Co} \exp(Q_{CC} t_C) Q_{CO} \exp(Q_{OO} t_O) Q_{OC} u_C \\
f_{OCO}(t_{O1}, t_{C1}, t_{O2}) &= p_{Oo} \exp(Q_{OO} t_{O1)} Q_{OC} \exp(Q_{CC} t_{C1}) Q_{CO} \exp(Q_{OO} t_{O2)} Q_{OC} u_C \\
f_{COC}(t_{C1}, t_{O1}, t_{C2}) &= p_{Co} \exp(Q_{CC} t_{C1}) Q_{CO} \exp(Q_{OO} t_{O1}) Q_{OC} \exp(Q_{CC} t_{C1}) Q_{CO} u_O \\
&\vdots
\end{aligned} \tag{49}$$

These distribution functions are sums of decaying exponentials and can be written:

$$\begin{aligned}
f_O(t_O) &= \sum_{i=1}^{N_O} \alpha_i \exp(-\lambda_i t_O) \\
f_C(t_C) &= \sum_{i=1}^{N_C} \beta_i \exp(-\omega_i t_O) \\
f_{OC}(t_O, t_C) &= \sum_{i=1}^{N_O} \sum_{j=1}^{N_C} A_{ij} \exp(-\lambda_i t_O - \omega_j t_C) \\
f_{CO}(t_O, t_C) &= \sum_{i=1}^{N_O} \sum_{j=1}^{N_C} B_{ji} \exp(-\lambda_i t_O - \omega_j t_C) \\
&\vdots
\end{aligned} \tag{50}$$

where the $\lambda_i > 0$ and $\omega_i > 0$ are the open and closed time decay rates respectively. We define row vector α with components, $\alpha_i,\ i = 1, 2, \ldots N_O$, and β with components $\beta_i,\ i = 1, 2, \ldots N_C$. A and B can be written as $N_O \times N_C$ and $N_C \times N_O$ matrices respectively. It will prove convenient to define diagonal matrices containing the open time decays $\Lambda_{ii} = \lambda_i$ and $\Omega_{jj} = \omega_j$. Note that $\exp(-\Lambda t)$ is a diagonal matrix with $\exp(-\Lambda t)_{jj} = \exp(-\lambda_j t)$. The distributions can be compactly written

$$\begin{aligned}
f_O(t_O) &= \alpha \exp(-\Lambda t_O) u_O \\
f_C(t_C) &= \beta \exp(-\Omega t_C) u_C \\
f_{OC}(t_O, t_C) &= u_O^T \exp(-\Lambda t_O) A \exp(-\Omega t_C) u_C \\
f_{CO}(t_O, t_C) &= u_C^T \exp(-\Omega t_C) B \exp(-\Lambda t_O) u_O \\
&\vdots
\end{aligned} \tag{51}$$

Distribution functions written as explicit sums of decaying exponentials have a direct interpretation in terms of a specific transition diagram known as the uncoupled model or BKU form (for Bauer-Kienker-Uncoupled) Form. In BKU form every open state is linked to every closed state (and vice versa) but no closed state is linked to any other closed state, and no open state is linked to any other open state. Topology II in Eq(44) is an example of BKU form in the case that there are two closed states and a single open state. Provided the open and closed time decays are nondegenerate, there is a unique similarity transformation that maps any model into uncoupled form. It is simply the transformation that diagonalizes Q_{CC} and Q_{OO}. **NB:** There is no guarantee that the transformations that do this will preserve positivity of rates. *i.e.* some of the rates in $\widetilde{Q}_{OC}$ and $\widetilde{Q}_{CO}$ might be negative. We'll discuss this more later.

$$\begin{aligned}
f_O(t_O) &= u_O^T \Pi_O^{unc} \Lambda \exp(-\Lambda t_O) u_O \\
f_C(t_C) &= u_C^T \Pi_C^{unc} \Omega \exp(-\Omega t_C) u_C \\
f_{OC}(t_O, t_C) &= u_O^T \exp(-\Lambda t_O) \Pi_O^{unc} \widetilde{Q}_{OC} \Omega \exp(\Omega t_C) u_C \\
f_{CO}(t_O, t_C) &= u_C^T \exp(-\Omega t_C) \Pi_C^{unc} \widetilde{Q}_{CO} \Lambda \exp(\Lambda t_O) u_O \\
f_{OCO}(t_{O1}, t_{C1}, t_{O2}) &= u_O^T \exp(-\Lambda t_{O1}) \Pi_O^{unc} \widetilde{Q}_{OC} \exp(\Omega t_{C1}) \widetilde{Q}_{CO} \exp(\Lambda t_{O2}) \Lambda u_O \\
f_{COC}(t_{C1}, t_{O1}, t_{C2}) &= u_C^T \exp(-\Omega t_{C1}) \Pi_C^{unc} \widetilde{Q}_{CO} \exp(\Lambda t_{O1}) \widetilde{Q}_{OC} \exp(\Omega t_{C2}) \Omega u_C \\
&\vdots
\end{aligned} \tag{52}$$

[4, 6, 10, 7]

The topology that the sums of decaying exponentials correspond to is known as the "uncoupled" topology or what we called "Bauer-Kienker" uncoupled form (BKU form)[8]. BKU form corresponds to every open state being linked to every closed state and vice versa, with no links between open states and no links between closed states. We showed previously that there is a unique (up to permutations of the labels) BKU form representation for every model except those which have multiple equal decays, (*i.e.* equal λ_{Oi}s). Applying the Kienker transformations to uncoupled form results in:

$$
\begin{aligned}
Q_{OO}^{unc} = -\Lambda_{OO} &= S_O^{-1} Q_{OO} S_O && (53)\\
Q_{CC}^{unc} = -\Lambda_{CC} &= S_C^{-1} Q_{CC} S_C && (54)\\
Q_{OC}^{unc} &= S_O^{-1} Q_{OC} S_C && (55)\\
Q_{CO}^{unc} &= S_C^{-1} Q_{CO} S_O && (56)\\
p_{Oo}^{unc} &= p_{Oo} S_O && (57)\\
p_{Co}^{unc} &= p_{Co} S_C && (58)\\
u_O &= S_O u_O && (59)\\
u_C &= S_C u_C && (60)\\
& && (61)
\end{aligned}
$$

where S_O and S_C are the matrices containing the right eigenvectors of Q_{OO} and Q_{CC} respectively. The matrices $-\Lambda_{OO}$ and $-\Lambda_{CC}$ are diagonal and contain the open and closed decay times respectively.

We introduce the "diag" operator which is equivalent to the diag operator in Octave or Matlab. It maps a vector x into a diagonal matrix $diag(x)$ via $diag(x)_{ii} = x_i$. Before writing the dwell time distributions in this form, we introduce two diagonal matrices that are contructed from the initial probability vectors:

$$
\begin{aligned}
\Pi_O^{unc} &= diag(p_{Oo}^{unc}) && (62)\\
\Pi_C^{unc} &= diag(p_{Co}^{unc}) && (63)
\end{aligned}
$$

and apply the transformation rules (Equations 56) to the distributions as written in Eq(49). For example we have

$$f_O(t_O) = -p_{Oo} S_O S_O^{-1} \exp(Q_{OO} t_O) S_O S_O^{-1} Q_{OO} S_O S_O^{-1} u_O = u_O^T \Pi_O^{unc} exp(-\Lambda t_O) \Lambda u_O$$

finally we write the distribution functions

$$\begin{aligned}
f_O(t_O) &= u_O^T \Pi_O^{unc} exp(-\Lambda t_O)\Lambda u_O \\
f_C(t_C) &= u_C^T \Pi_C^{unc} exp(-\Omega t_C)\Omega u_C \\
f_{OC}(t_O, t_C) &= u_O^T \Pi_O^{unc} exp(-\Lambda t_O)\widetilde{Q}_{OC} \exp(\Omega t_C)\Omega u_C \\
f_{CO}(t_O, t_C) &= u_C^T \Pi_C^{unc} exp(-\Omega t_C)\widetilde{Q}_{CO} \exp(\Lambda t_O)\Lambda u_O \\
f_{OCO}(t_{O1}, t_{C1}, t_{O2}) &= u_O^T \Pi_O^{unc} exp(-\Lambda t_{O1})\widetilde{Q}_{OC} \exp(\Omega t_{C1})\widetilde{Q}_{CO} \exp(\Lambda t_{O2})\Lambda u_O \\
f_{COC}(t_{C1}, t_{O1}, t_{C2}) &= u_C^T \Pi_C^{unc} exp(-\Omega t_{C1})\widetilde{Q}_{CO} \exp(\Lambda t_{O1})\widetilde{Q}_{OC} \exp(\Omega t_{C2})\Omega u_C \\
&\vdots
\end{aligned} \tag{64}$$

Note that the matrices Π_C^{unc}, Π_O^{unc}, Λ, and Ω, are diagonal while $\widetilde{Q}_{OC}$ and $\widetilde{Q}_{OC}$ are not diagonal (or necessarily even square). Although matrices do not generally commute diagonal matrices do. (*i.e.* in general $AB \neq BA$ for matrices A and B but if both A and B are diagonal then $AB = BA$. Consequently we can write Eqs(64) as

4 Detailed Balance

At thermodynamic equilibrium all reactions must balance. This condition is known as detailed balance. Ion channel gating kinetics are generally believed to obey detailed balance. Detailed balancing of reactions imposes constraints on reaction rates. These constraints have manifestations that are relevant to data-driven model reconstruction. We show here that at equilibrium, the coefficients of the decaying exponentials in the one dimensional dwell-time distributions are all positive (providing DB holds). We also show that $f_{AB}(t_A, t_B) = f_{BA}(t_A, t_B)$ providing DB holds. If the steady state corresponds to thermodynamic equilibrium it follows that

$$w_i Q_{ij} = w_j Q_{ji} \ . \tag{65}$$

(Remember that w is the vector of equilibrium probabilities: $wQ = 0$. After defining the diagonal matrix W, $W_{ii} = w_i$

$$\begin{aligned}
W_{ij} &= 0 \ if \ i \neq j \\
W_{ii} &= w_i
\end{aligned}$$

Note that

$$w = u^T W \tag{66}$$

Eq(65) can be written

$$WQ = (WQ)^T = Q^T W \tag{67}$$

where ^{T} denotes the tranpose $Q^T_{ij} = Q_{ji}$. From Eq(67) we find

$$W^{1/2} Q W^{-1/2} = W^{-1/2} Q^T W^{1/2} = (W^{1/2} Q W^{-1/2})^T \tag{68}$$

which means that Q is similar to a symmetric matrix, $Q^{sym} = W^{1/2} Q W^{-1/2}$.

Detailed balance imposes constraints on reaction rates in networks that that contain closed loops. However, it is not necessary to consider loops in constructing the generator matrix. The detailed balance condition implies that the matrix WQ is symmetric. This matrix has a clear physical meaning and we give it a name: Q^{flux}:

$$Q^{flux} \equiv WQ \tag{69}$$

because it is the matrix of probability fluxes between the various states. That is Q^{flux}_{ij} is the equilibrium probability flux from state i to state j. Note that Q^{flux} is a symmetric generator so that $Q^{flux} u = 0$ and $u^T Q^{flux} = 0$.

4.1 Proof that $f_{AB} = f_{BA}$ when detailed balance holds.

We want to show that

$$f_{AB}(t_A, t_B) = \frac{1}{J} w_B Q_{BA} exp(Q_{AA} t_A) Q_{AB} exp(Q_{BB} t_B) Q_{BA} u_A \tag{70}$$

$$f_{BA}(t_A, t_B) = \frac{1}{J} w_A Q_{AB} exp(Q_{BB} t_B) Q_{BA} exp(Q_{AA} t_A) Q_{AB} u_B \tag{71}$$

are equal provided detailed balance holds. To show that $f_{AB}(t_A, t_B) = f_{BA}(t_A, t_B)$ when detailed balance holds we take the transpose of Eq 71 and then apply the detailed balance conditions for the aggregates:

$$\begin{pmatrix} W_A Q_{AA} & W_A Q_{AB} \\ W_B Q_{BA} & W_B Q_{BB} \end{pmatrix} = \begin{pmatrix} Q^T_{AA} W_A & Q^T_{BA} W_B \\ Q^T_{AB} W_A & Q^T_{BB} W_B \end{pmatrix} . \tag{72}$$

Note that the distribution functions are scalar quantities. Taking the transpose of a scalar has no effect; the transpose of a scalar is just the scalar so

that $f_{BA}^T = f_{BA}$. Thus we have

$$\begin{aligned}
f_{BA} &= \frac{1}{J} u_A^T W_A Q_{AB} \exp(Q_{BB} t_B) Q_{BA} \exp(Q_{AA} t_A) Q_{AB} u_B \\
&= \frac{1}{J} u_B^T Q_{AB}^T \exp(Q_{AA} t_A)^T Q_{BA}^T \exp(Q_{BB} t_B)^T Q_{AB}^T W_A u_A \\
&= \frac{1}{J} u_B^T Q_{AB}^T \exp(Q_{AA} t_A)^T Q_{BA}^T \exp(Q_{BB} t_B)^T W_B Q_{BA} u_A \\
&= \frac{1}{J} u_B^T Q_{AB}^T W_A W_A^{-1} \exp(Q_{AA} t_A)^T W_A W_A^{-1} Q_{BA}^T W_B W_B^{-1} \exp(Q_{BB} t_B)^T W_B Q_{BA} u_A \\
&= \frac{1}{J} u_B^T W_B Q_{BA} W_A^{-1} \exp(Q_{AA} t_A)^T W_A Q_{AB} W_B^{-1} \exp(Q_{BB} t_B)^T W_B Q_{BA} u_A \\
&= \frac{1}{J} u_B^T W_B Q_{BA} \exp(Q_{AA} t_A) Q_{AB} \exp(Q_{BB} t_B) Q_{BA} u_A \\
&= \frac{1}{J} w_B Q_{BA} \exp(Q_{AA} t_A) Q_{AB} \exp(Q_{BB} t_B) Q_{BA} u_A \\
&= f_{AB}
\end{aligned} \tag{73}$$

4.2 Proof that the coefficients of the decaying exponentials in the 1-dimensional equilibrium dwell-time distributions are all positive when detailed balance holds.

What we're going to do is show that the equilibrium one-dimensional dwell-time distribution can be written as $x^T \exp(-\Lambda t) x$ where Λ is the diagonal matrix containing the eigenvalues of Q_{AA}. We begin by writing f_A in of the standard forms:

$$\begin{aligned}
f_A(t) &= \frac{1}{J} w_A Q_{AA} \exp(Q_{AA} t) Q_{AA} u_A \\
&= \frac{1}{J} u_A^T W_A Q_{AA} \exp(Q_{AA} t) Q_{AA} u_A \\
&= \frac{1}{J} u_A^T Q_{AA}^T W_A \exp(Q_{AA} t) Q_{AA} u_A
\end{aligned}$$

It is easy to show that $W_A \exp(Q_{AA} t) = W_A^{1/2} S \exp(-\Lambda t) S^T W_A^{1/2}$ where S is the matrix of eigenvectors of the symmetric matrix $W^{1/2} Q_{AA} W^{-1/2}$. Since $W^{1/2} Q_{AA} W^{-1/2}$ is symmetric it's eigenvectors form an orthnormal set so with

appropriate normalization $SS^T = I$. The diagonal matrix $-\Lambda$ contains the eigenvalues of Q_{AA}. Thus we find that

$$\begin{aligned} f_A &= \frac{1}{J} u_A^T Q_{AA}^T W_A^{1/2} S \exp(-\Lambda t) S^T W_A^{1/2} Q_{AA} u_A \\ &= \frac{1}{J} \sum_i \exp(-\lambda_i t) x_i^2 \end{aligned} \tag{74}$$

where $x = S^T W_A^{1/2} Q_{AA} u_A$ so that the amplitudes of $exp(-\lambda_i t)$ are $x_i^2/J > 0$ as was to be shown.

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