CMP 610b Lecture 7
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Matrix formulation of kinetic schemes

In lecture 6 we considered the interpretation of molecular events as a Markov process, and looked at the statistics of open times for a simple two-state scheme. In this lecture we will generalize this sort of analysis to multistate kinetic schemes, and introduce some mathematical concepts that are useful in handling the complexities that arise.

THE OPEN TIME WITH MULTIPLE STATES

Suppose we have a channel that can close in two different ways, modelled as

$$C_1 \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O \underset{\delta}{\overset{\gamma}{\rightleftharpoons}} C_3$$

How do we compute the open time distribution in this case? It is helpful to recall our definition of a rate constant, as the probability per unit time of making a transition. The equation for $F'(t)$, the open-time 'survivor function' is

$$\frac{dF'(t)}{dt} = -\frac{\text{Prob}\{\text{O at } t \text{ and O}\rightarrow C_1 \text{ or O}\rightarrow C_3 \text{ during } (t, t+dt)\}}{dt}$$

meanwhile we have

$$\beta = \frac{\text{Prob}\{\text{ O}\rightarrow C_1 \text{ during } (t, t+\delta t) \mid \text{ O at time } t \}}{\delta t}$$

$$\gamma = \frac{\text{Prob}\{\text{ O}\rightarrow C_3 \text{ during } (t, t+\delta t) \mid \text{ O at time } t \}}{\delta t}$$

so the probability of making a transition from O to either of the closed states in an infinitesimal time dt is $(\beta + \gamma)$ times the probability of being in O at time t, and the equation for F' becomes

$$\frac{dF'(t)}{dt} = -(\beta + \gamma) F'(t)$$

That is, the rate of leaving the open state is simply the sum of the rates leading away from that state. The solution to this equation is a single exponential function but with a faster decay than in the case with one closed state. Here is an interesting phenomenon: there is no way to distinguish dwell times in O that terminate in C_1 from those that end by a transition to C_3. Even if β is much larger than γ, the openings that are terminated by a transition to C_1 will be no shorter.

TIME COURSE OF THE STATE PROBABILITIES

Now we will consider another sort of problem. Supposing that the channel is in state C_1 at time zero, what will be the probability that it is in state O at a given time *t*? To make the notation easier, let us number the states and rename the rate constants in this way:

$$C_1 \underset{k21}{\overset{k12}{\rightleftharpoons}} O_2 \underset{k32}{\overset{k23}{\rightleftharpoons}} C_3 \qquad (1)$$

Now we can write an equation for the probability p_1(t) of being in the first state,

$$\frac{dp_1}{dt} = -p_1 k_{12} + p_2 k_{21}.$$

Here what we are saying is that the occupancy of state 1 decreases as transitions 1→2 occur, and increases as transitions from 2→1 occur. Likewise we can write two more equations for the other two states,

$$\frac{dp_2}{dt} = p_1 k_{12} - p_2 (k_{21}+k_{23}) + p_3 k_{32}$$

$$\frac{dp_3}{dt} = p_2 k_{23} - p_3 k_{32}$$

We can solve these equations to obtain the three time courses $p_1(t)$, $p_2(t)$ and $p_3(t)$. A shorthand way of writing these equations uses vector and matrix notation. For example these equations become

$$\frac{d\begin{pmatrix} p_1 & p_2 & p_3 \end{pmatrix}}{dt} = \begin{pmatrix} p_1 & p_2 & p_3 \end{pmatrix} \times \begin{pmatrix} -k_{12}-k_{13} & k_{12} & k_{13} \\ k_{21} & -k_{21}-k_{23} & k_{23} \\ k_{31} & k_{32} & -k_{31}-k_{32} \end{pmatrix}$$

Here I have included two more rates, k_{13} and k_{31}, for generality; in the case of our scheme (1) these are zero. This matrix equation can be rewritten in the compact form

$$\frac{d\mathbf{p}(t)}{dt} = \mathbf{p}(t)\,\mathbf{Q} \qquad (2)$$

where $\mathbf{p}(t)$ is the *probability vector* and **Q** is the *transition rate matrix*. The vector **p** contains the probability of being in each state as a function of time; note that because of the way we have set up the problem that **Q** does *not* depend on time. This is a characteristic of a "time homogeneous Markov process", which is the sort of process we will be talking about. The elements of **Q** are called q_{11}, q_{12}, and so on. They are specified by

$$q_{ij} = \begin{cases} k_{ij}, & i \neq j \\ -\sum_{m \neq i} k_{im}, & i = j. \end{cases} \qquad (3)$$

One might naively write the traditional solution to eqn. (2) as

$$\mathbf{p}(t) = \mathbf{p}(0)\, e^{\mathbf{Q}t}$$

which, as it turns out, makes mathematical sense and is the way one would solve the equation on a computer, for example. More about this later.

INTERESTING THINGS YOU CAN DO WITH THE PROBABILITY VECTOR

Channel current. One thing you would want to do is to find out the probability the channel is in the open state, state 2. This is just given by p_2. If you wanted to be fancy you could obtain p_2 by constructing the vector **u** = (0 1 0) and forming the vector inner product

$$\mathbf{p}.\mathbf{u} = \sum_{i=1}^{3} p_i u_i$$

which in this case just yields p_2. This fancy approach works nicely if you have multiple open states, perhaps with different conductances. Then you can construct the **u** vector with the different current levels and the product will give you the average current.

Charge transfer. There are several situations in which transitions among states result in the transfer of charge across a cell membrane. One case are the gating currents or conformation currents due to rearrangement of charged entities in a membrane protein. Another case is in computing the current due to a transporter. In either case if you have a kinetic scheme for the steps involved, you can calculate the current that flows.

The basic idea is that with each state can be associated a charge c_i, so that the total charge movement can be written

$$\langle c \rangle = \sum_i c_i p_i \, .$$

What is measured experimentally is the current $J = d\langle c \rangle / dt$, that is the time derivative of the charge. Making use of the fact that $d\mathbf{p} / dt = \mathbf{pQ}$, it is easy to see that

$$\begin{aligned} J(t) &= \sum_{ij} p_i(t) q_{ij} c_j \\ &= \mathbf{pQ}\ \mathbf{c} \end{aligned} \qquad (4)$$

where the sums are taken over all states.

Dwell times. Suppose we want to compute the probability density function of closed times, for comparison with a histogram of closed times obtained from a single-channel recording. A closed time is the dwell time between leaving an open state of the channel and returning to an open state. Suppose in our three-state model a closed time starts by a transition 2→1. Then the closed time will be the time before the channel returns to state 2. The probability distribution (survivor function) of these closed times $F'(t)$ will equal the probability $p_1(t)$ in the scheme

$$C_1 \xrightarrow{k12} O_2$$

since in this scheme, once the channel opens nothing further happens; the closed time is terminated.

In general, one can compute the closed time distribution function as the dwell time in closed states of a scheme in which all of the rates from open to closed states are set to zero. You prepare the system in each closed state and then see how the probability of remaining closed evolves with time. You then weight each of these survivor functions by the probability of starting a closed interval in each closed state, and sum them. In matrix notation, the distribution function of closed times is given by

$$F'_c(t) = \pi_c e^{\mathbf{Q}'t} \cdot \mathbf{u} \qquad (5)$$

where π_c is a vector of probabilities whose i^{th} element is the probability of landing in state i when the channel closes (assuming that state i is a closed state; otherwise the element is zero). **Q'** is the same as **Q** except that all elements q_{ij} are zero in the cases that i is an open state and j is a closed state. The vector **u** is a row vector of ones, so that the dot product has the effect of summing all the elements of the product of the first two terms in the equation. Finally, by taking the time derivative of F'_c you obtain the probability density function f_c which you can superimpose over an experimental closed-time distribution:

$$f_c = -\frac{d}{dt} F'_c$$
$$= \pi_c e^{\mathbf{Q}'t} \mathbf{Q}' \mathbf{u}$$

By the same process, just reversing the roles of closed and open states, one can obtain the open time distribution and probability density functions as well.

CHARACTERISTIC RELAXATIONS AND EIGENVECTORS

Now let us take our three-state scheme and give it some specific rate constants in units of s^{-1}:

$$C_1 \underset{1}{\overset{1}{\rightleftharpoons}} O_2 \underset{100}{\overset{100}{\rightleftharpoons}} C_3$$

This might represent a channel with a very slow opening step from the closed state C_1, but with a rapid blocking step. What will be the steady-state probability vector? At steady state the occupancy of each state is equally probable, so

$$\mathbf{p}_\infty = \left(\frac{1}{3} \quad \frac{1}{3} \quad \frac{1}{3} \right)$$

If we were to somehow prepare a population of channels with this distribution among the states, then there would be no change with time of the distribution. (Of course, individual channels will be flipping from one state to another, but the expectation value of the occupancy of each state will remain constant.)

Now suppose we are at steady state but then perturb the distribution, at time zero, by an amount **p'**:

$$\mathbf{p}(0) = \mathbf{p}_\infty + \mathbf{p}'$$

The perturbation is such that we increase the occupancy of state 1 but decrease the occupancies of states 2 and 3,

$$\mathbf{p}' = \delta\,(\,1.0 \quad -0.5 \quad -0.5\,) \qquad (7)$$

where δ is an arbitrary small factor that sets the size of the perturbation. You can see what would happen after the perturbation: the equilibrium between states 2 and 3 won't be disturbed, but a slow relaxation with a rate $\lambda' \approx -1.5\ s^{-1}$ (time constant of about 0.7 s) will occur between them and state 1. In fact you could guess that the time course of the occupancy probabilities will be approximately

$$\mathbf{p}(t) \approx \mathbf{p}_\infty + \mathbf{p}'\, e^{\lambda' t},$$

that is, the probabilities will relax with a slow time constant to the equilibrium value $\mathbf{p}(\infty)$. Notice that the exponential is decaying in time because λ' is negative.

Consider another kind of perturbation,

$$\mathbf{p}'' = \delta\,(\,0.0 \quad 1.0 \quad -1.0\,). \qquad (8)$$

Here we are changing the relative occupancies of states 2 and 3. We expect a very rapid equilibration, which will occur with a rate $\lambda'' \approx -200 s^{-1}$ (a time constant of about 5 ms) and follow

$$\mathbf{p}(t) \approx \mathbf{p}_\infty + \mathbf{p}''\, e^{\lambda'' t}.$$

Decomposition into characteristic modes. What we have picked in eqns. (7) and (8) are characteristic probability vectors that correspond to special relaxation modes of the kinetic scheme. We can use these to obtain the time course of the probability vector given an arbitrary starting configuration if we can decompose the starting probabilities into these modes. The problem is, given a starting vector $\mathbf{p}(0)$, we would like to write it in the form

$$\mathbf{p}(0) = \mathbf{p}_\infty + a_1\,\mathbf{p}' + a_2\,\mathbf{p}'' \qquad (9)$$

because if we are successful, we could treat this like the superposition of perturbations and say that the solution to eqn. (2) will then be approximately

$$\mathbf{p}(t) \approx \mathbf{p}_\infty + a_1\,\mathbf{p}'\, e^{\lambda' t} + a_2\,\mathbf{p}''\, e^{\lambda'' t}. \qquad (10)$$

As an example, suppose that the starting vector is $\mathbf{p}(0) = (\,1 \quad 0 \quad 0\,)$. That is, we start in state 1. The coefficients that satisfy eqn. (9) are

$$a_1 = 0.667$$
$$a_2 = 0$$

(try it for yourself!). On the other hand, if we started with $\mathbf{p}(0) = (0 \quad 0 \quad 1)$ then the coefficients would be

$$a_1 = -0.333$$
$$a_2 = -0.5$$

and now, armed with these coefficients, we can obtain an approximate solution to the time course of the probability in each state.

Characteristic values and vectors. What we have introduced here in disguise are the characteristic values and characteristic vectors of the matrix **Q**. The values λ' and λ" are called *eigenvalues* of the matrix, and the vectors **p'** and **p"** are *eigenvectors*, as is $\mathbf{p}_\infty$. The elements of **p'** and **p"** given here are approximate; they can be found to high precision using standard computer algorithms. Thus for any **Q** that represents a reasonable kinetic scheme, a computer can find for you the eigenvalues and the elements of each eigenvector. There is also a straightforward way to perform the decomposition of an arbitrary vector **p** into the eigenvectors as in eqn. (9). Thus we can solve for the time course of the probabilities as in eqn. (10) in this way.

I used a program to find the eigenvalues and eigenvectors for our scheme. In case you're interested, the exact values are

$\lambda_\infty = 0$
$\mathbf{p}_\infty = (\ 0.333 \ \ 0.333 \ \ 0.333\ )$

$\lambda' = -1.496$
$\mathbf{p}' = (\ 1.0 \ \ -0.495 \ \ -0.505\ )$

$\lambda'' = -200.5$
$\mathbf{p}'' = (-.005 \ \ 1.005 \ \ -1.0\ )$

Where our guess for the steady-state vector $\mathbf{p}_\infty$ was correct in the first place, and it corresponds to the eigenvalue of zero.

THE MATRIX EXPONENTIAL

Let us return to the equation for the time course of the probabilities,

$$\frac{d\mathbf{p}(t)}{dt} = \mathbf{p}(t)\,\mathbf{Q} \qquad (2)$$

Now let **v** be an eigenvector of the matrix **Q**. The mathematical definition of an eigenvector (here I use "left" eigenvectors) is that it satisfies

$$\mathbf{vQ} = \lambda\mathbf{v}$$

where λ is the eigenvalue associated with **v**. Now notice what happens if we happen to choose

$$\mathbf{p}(0) = a\,\mathbf{v}$$

i.e. **p** starts out being a vector proportional to **v**. Then it can be shown that a solution to eqn. (3) is

$$\mathbf{p}(t) = a\,e^{\lambda t}\,\mathbf{v}.$$

This is exactly the phenomenon we saw above, when we perturbed our system in particular ways corresponding to particular eigenvectors. Our final conclusion was, if we were to express the initial probability vector as an appropriate weighted sum of eigenvectors,

$$\mathbf{p}(0) = \sum_{i=1}^{n} a_i\,\mathbf{v}_i, \qquad (11)$$

Then the solution to equation (3) in this general case will be

$$\mathbf{p}(t) = \sum_{i=1}^{n} a_i \, e^{\lambda_i t} \, \mathbf{v}_i. \qquad (12)$$

where the λ_i are the eigenvalues corresponding to the eigenvectors $\mathbf{v}_i$.

How do we compute the coefficients a_i? One way of looking at the problem is to construct a matrix **V** whose rows are the eigenvectors $\mathbf{v}_i$. Then the a_i are components of the vector **a** that satisfies

$$\mathbf{a}\,\mathbf{V} = \mathbf{p}(0)$$

which has the solution

$$\mathbf{a} = \mathbf{p}(0)\,\mathbf{V}^{-1} \qquad (13)$$

where $\mathbf{V}^{-1}$ is the inverse of the matrix **V**.

A convenient way to use these results is to rewrite **Q** as its so-called *spectral expansion*,

$$\mathbf{Q} = \sum_{i=1}^{n} \lambda_i \, \mathbf{A}_i$$

where the matrices $\mathbf{A}_i$ are derived from **V** and $\mathbf{V}^{-1}$. (Specifically, they are given by

$$\mathbf{A}_i = \mathbf{V}^{-1}\mathbf{D}_i\mathbf{V} \qquad (14)$$

with $\mathbf{D}_i$ being a matrix of all zeroes except for d_{ii}, which is equal to 1.)* With this, the solution to our problem can be written as

$$\mathbf{p}(t) = \mathbf{p}(0)\, e^{\mathbf{Q}t} \qquad (15)$$

with $e^{\mathbf{Q}t}$ given by

$$e^{\mathbf{Q}t} = \sum_{i=1}^{n} e^{\lambda_i t} \, \mathbf{A}_i$$

The matrix exponential is therefore equivalent to decomposing a vector into a weighted sum of eigenvectors (by multiplication with $\mathbf{V}^{-1}$), then including each characteristic exponential decay, and finally reconstructing the original vector by multiplying by the matrix of eigenvectors **V**. We can

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* Our notation here is nonstandard, because we have used **v** to be a "left eigenvector", a row vector, and **V** to be a matrix of these. The usual situation is to use column vectors ("right eigenvectors"). Thus for example in Matlab one writes

[**U**,**D**] = Eig(**Q**)

which delivers a matrix of eigenvectors **U** which is equal to our $\mathbf{V}^{-1}$. Thus by the normal convention, eqn. (14) becomes

$\mathbf{A}_i = \mathbf{U}\mathbf{D}_i\mathbf{U}^{-1}$.

abstract the decomposition and reconstruction by using a set of matrices $\mathbf{A}_i$ in the spectral expansion of **Q**.

As an example, suppose we want to find the mean time course of the current *I*(t) through a channel that follows a kinetic scheme represented by **Q**. Let **u** be a vector of the channel current corresponding to each state of the scheme. Then

$$I(t) = \sum_{i=1}^{n} e^{\lambda_i t}\ \mathbf{p}(0)\mathbf{A}_i\mathbf{u}$$

$$= \sum_{i=1}^{n} b_i\, e^{\lambda_i t}\ , \qquad (16)$$

and is seen to be a sum of exponential functions of time.

Now you should be able to understand (more or less) some parts of computer programs that make use of these ideas. The following are two procedures from our module "MatCurrents" that compute the expectation value of current through a channel. This module is used in our programs for fitting the time courses of ionic currents. The procedures use the matrix *Q*, the initial state vector *P0* and an array *Currents* which gives the amount of current flowing through the channel for each state. The result of the first procedure is an array (Ampl) of amplitudes (the b_i of eqn. 15) and an array (Lambda) of rates for each exponential component that makes up the result. This procedure makes use of other procedures in a module MatrixD that compute the spectral expansion and perform matrix and vector multiplications.

```
PROCEDURE ComputeRelaxation( Q         : LongMatrixPointer;
                             P0        : LongVectorPointer;
                             NumStates : INTEGER;
                             Currents  : LongVectorPointer;
                             Ampl, Lambda : LongVectorPointer );
  (* Compute the amplitudes Ampl corresponding to the eigenvalues Lambda
     for the mean channel current.  P0 is the vector of
     probabilities at time 0, and Currents is an array giving the channel current
     corresponding to each state. *)
VAR
     i, j      : INTEGER;
     ok        : BOOLEAN;
     A         : MatArrayPointer;
     MatSize   : LONGINT;
BEGIN
   (* we compute Ampl[i] = P0 * A[i] * Currents,
      where Currents is the vector of currents.
   *)
  ok := MatrixD.SpectralExpansion(Q, NumStates, Lambda, A);
  FOR i := 0 TO NumStates-1 DO
     (*  First, compute P = P0 * A[i]   *)
    MatrixD.LeftMul( P0, MatArrayIndex( A, i ), P, NumStates, NumStates );
    Ampl^[i] := MatrixD.InnerProduct( P, Currents, NumStates );
  END; (* FOR *)

END ComputeRelaxation;
```

Now to convert the result of this procedure to a time course that can be plotted, we call another procedure which simply computes the sum of the exponential functions and places it into an array

called *Data*:

```
PROCEDURE ComputeTimeCourse( NumStates      : INTEGER;
                             Ampl, Lambda   : LongVectorPointer;
                             SampleInterval : REAL;
                             NumPoints      : INTEGER;
                             VAR Data       : ARRAY OF REAL );
VAR
  i,j : INTEGER;
  y   : LONGREAL;
  A, dp : LongVectorType;

BEGIN
  A := Ampl^;  (* Copy the amplitudes *)
  FOR j := 0 TO NumStates-1 DO
    dp[j] := ETOX( Lambda^[j] * LONG(SampleInterval) );
  END;
  FOR i := 0 TO NumPoints-1 DO
    y := 0.0;
    FOR j := 0 TO NumStates-1 DO
      y := y + A[j];
      A[j] := A[j] * dp[j];
    END;
    Data[i] := SHORT(y);
  END;
END ComputeTimeCourse;
```

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S. Crouzy and F. J. Sigworth. Yet another approach to the dwell-time omission problem of single-channel analysis. *Biophys. J.* **58**:731-743, 1990.
--Deals with advanced issues in computing dwell-time distributions from kinetic schemes, but also summarizes classical results such as our eqn. (5).