Kinetics of a multistate enzyme in a large oscillating field

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ABSTRACT A simple, general, and efficient method for calculating the response of a set of coupled first-order (or pseudo-first-order) chemical reactions to an arbitrarily large periodic field is described. The method is applied to a four-state membrane transport enzyme that is electroconformationally coupled to an ac field, i.e., the enzyme has electric charges that move concomitantly with a conformational transition. The calculation is done both for enzymes in a planar membrane and for enzymes in the spherical membrane of a cell or vesicle in suspension.

INTRODUCTION

When a membrane transport enzyme is electroconformationally coupled to an oscillating electric field it can transduce energy from the field to transport substrate against a concentration gradient. This has been shown experimentally (1) and theoretically (2, 3). The theoretical work is restricted to a two-state model, which cannot describe as many features of a transport enzyme as a multiple-state model. Numerical work has also been done to calculate the response of a four-state enzyme to an oscillating electric field. In one case (4), the nonlinear differential equations (with no steady-state approximation for the concentration of substrate and product) were solved by integrating forward in time, and the field-induced concentration gradient was calculated as a function of time. This technique uses excessive amounts of CPU time and is impractical for systematic investigation of the response of a realistic system. Subsequent steady-state numerical calculations (5) were done starting from field-off initial conditions. The method failed at higher frequencies because too many cycles had to be integrated. Up to now no simple general method has been described for calculating the steady oscillating response of a multistate enzyme in a periodic field of arbitrary amplitude.

We describe an easily understandable but rigorous general method for solving realistic linear kinetic models in a periodic field without having to approximate. This represents an extension of classical relaxation kinetic methods (6) to allow for arbitrarily large-amplitude periodic perturbations of coupled linear chemical reactions. Such large-amplitude perturbation techniques are particularly important for membrane enzymes subjected to external oscillating electric fields because even a small applied field leads to large fields within the lipid bilayer. The induced nonlinear behavior contains a wealth of kinetic information. Our computational method can be used e.g. to analyze a multistate enzyme in an oscillating field of arbitrary frequency, which could not be done by previous methods.

The field could be e.g. a periodic ligand concentration that modulates the activity of a receptor (7), an oscillating substrate concentration that supplies energy to an ion pump (8), or an externally applied electric field that causes an enzyme to transduce free energy from the field to its output reaction (9, 10). Here we solve a four-state model for an enzyme-catalyzed transport through a membrane in an ac electric field. Our approach brings into focus the physical reasons for energy transduction from the ac field.

To simplify exposition, we first consider an enzyme in a planar bilayer membrane. At the end of the paper we extend the calculation to the spherical symmetry relevant to suspensions of spherical cells or vesicles.

FOUR-STATE MODEL

Consider a large number of identical enzyme molecules in a planar bilayer membrane. Each is oriented in the same way in the membrane, and the average distance between the molecules is large enough that enzyme–enzyme interactions may be ignored. The enzyme catalyzes the transport reaction $S_1 \rightleftharpoons S_2$. It has two electrically distinct conformational states (10), E with the binding site for the substrate S_1 on one side of the membrane, and E^* with the binding site for the product S_2 on the other side. For simplicity we assume that S_1 and S_2 are constant concentrations and that the reaction $S_1 \rightleftharpoons S_2$ is not electrogenic. The interaction with the electric field occurs only by electroconformational coupling, i.e., the E^* forms have a different arrangement of charges than the E forms. Our

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analysis can easily be generalized to electrogenic reactions.

We assume that all interconversions between states may be treated as either unimolecular or pseudo-first-order transitions. Then, a simple cycle for the enzyme can be described by the four-state kinetic model,

$$\begin{tikzcd}[column sep=huge, row sep=large]
 & SE_2 \arrow[rr, shift left, "c\phi"] \arrow[dd, shift left, "a"] & & E_3^*S \arrow[ll, shift left, "bc/\phi"] \arrow[dd, shift left, "ab"] \arrow[dr] & \\
S_1 \arrow[ur] & & & & S_2 \\
 & E_1 \arrow[uu, shift left, "ab"] \arrow[rr, shift left, "bc\phi"] & & E_4^* \arrow[uu, shift left, "a"] \arrow[ll, shift left, "c/\phi"] &
\end{tikzcd} \qquad (1)$$

We describe the parameters of this model first without an electric potential turned on, so that for the moment ϕ reduces to 1. We have chosen the remaining rate coefficients to have reflection symmetry for simplicity. The parameter a is a scaling factor for the association–dissociation steps, b is a bias factor that is a measure of the difference in affinity of the enzyme for substrate on the two sides of the membrane, and c is a scaling factor for the conformational change steps. This is a reasonable model for a membrane transporter.

When an electric potential ψ is turned on, the energy of the state E_3^*S is changed relative to the state SE_2 by $q\psi$, where q is the effective enzyme charge that moves across the membrane during a conformational change and ψ is the potential difference across the membrane. Thus the electric potential causes the equilibrium constant for the reaction $SE_2 \rightleftharpoons E_3^*S$ to be multiplied by $\exp(q\psi/kT)$. The equilibrium constant equals the ratio of the rate coefficients. For simplicity we apportion half of the exponential factor to each rate coefficient. Thus the effect of the electric potential on the transition $SE_2 \rightleftharpoons E_3^*S$ in Eq. 1 is given by the factor,

$$\phi = \exp(\tfrac{1}{2}q\psi/kT), \qquad (2)$$

in the rate coefficients for that transition. Similarly, the energy of the state E_4^* is decreased relative to the state E_1 by $q\psi$, and the effect of the electric potential on this transition is also given by Eq. 2 in the rate coefficients.

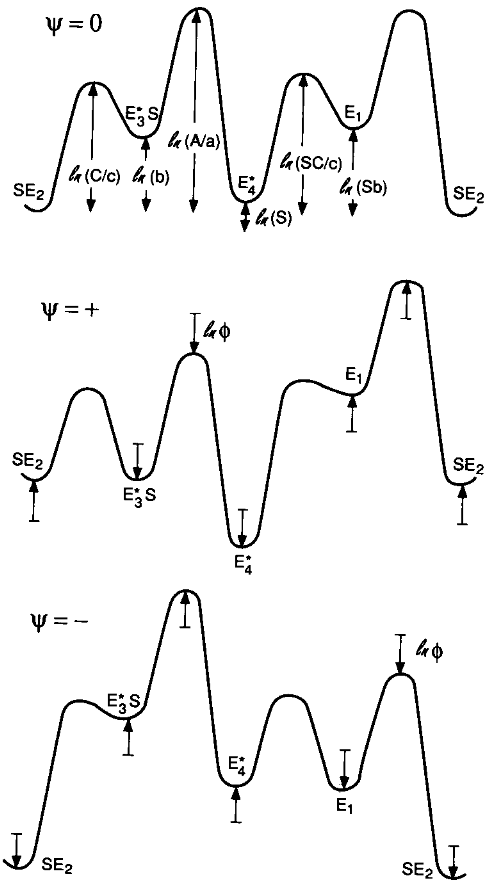

FIGURE 1 Energy of the enzyme vs. reaction coordinate for (*a*) zero potential, (*b*) positive potential, and (*c*) negative potential. The rate constants a, b, and c are from the model in the text. We have used $S_1 = S_2 = S$, and A and C are frequency factors, so that $\ln(A/a)$ and $\ln(C/c)$ are activation energies in kT units. $\ln \phi = q\psi/2kT$ is the energy of interaction with the electric field. These graphs show qualitatively why net pumping occurs (see analysis in text).

ANALYSIS OF MODEL

Fig. 1 illustrates qualitatively how an ac field can cause the enzyme to pump substrate against a concentration gradient. The basic free energy of this enzyme is plotted vs. the reaction coordinate in Fig. 1 *a* for zero electric potential. The curve shown describes a very poor enzyme, with large activation barriers and low-energy intermediate states (11, 12). When the potential is positive, the energies shift as shown in Fig. 1 *b*, where E_4^* is seen to be more stable than SE_2. The system must relax toward a new equilibrium corresponding to the new energy levels, and so SE_2 must be converted to E_4^*. Because the activation barrier for the transition via E_3^*S is now lower than for the transition via E_1, the reaction goes mostly through E_3^*S, thus releasing more S_2 than S_1. When the potential is negative, the energies shift as shown in Fig. 1 *c*, where SE_2 is more stable than E_4^*, and so E_4^* must be converted to SE_2. Because the activation barrier for the transition via E_1 this time is lower than for the transition via E_3^*S, the reaction goes mostly through E_1, binding more S_1 than S_2. This process repeats with the net transport of S_1 to S_2 accumulating on every cycle.

Uphill pumping occurs clockwise only when the affinity of E for S_1 is greater than the affinity of E^* for S_2. This occurs when $b > 1$. If $b < 1$, uphill pumping by the ac electric potential can occur only in the counterclockwise direction. No uphill pumping occurs when $b = 1$.

To describe the pumping quantitatively, we write the differential equations that the enzyme state probabilities must satisfy, as specified by Eq. 1. Since the four probabilities must sum to unity,

$$\mathrm{E}_1 + \mathrm{SE}_2 + \mathrm{E}_3^*\mathrm{S} + \mathrm{E}_4^* = 1, \tag{3}$$

we can work with just three of them. We express the rate equations in matrix form. The state probability vector,

$$\mathbf{E} = \begin{vmatrix} \mathrm{E}_1 \\ \mathrm{SE}_2 \\ \mathrm{E}_3^*\mathrm{S} \end{vmatrix}, \tag{4}$$

satisfies the matrix equation,

$$\mathrm{d}\mathbf{E}/\mathrm{d}t + G\mathbf{E} = \mathbf{F}, \tag{5}$$

where G is the rate coefficient matrix,

$$G = \begin{vmatrix} ab\mathrm{S}_1 + bc\phi + c/\phi & c/\phi - a & c/\phi \\ -ab\mathrm{S}_1 & c\phi + a & -bc/\phi \\ a\mathrm{S}_2 & a\mathrm{S}_2 - c\phi & bc/\phi + ab + a\mathrm{S}_2 \end{vmatrix}, \tag{6}$$

and the right side is the column vector,

$$\mathbf{F} = \begin{vmatrix} c/\phi \\ 0 \\ a\mathrm{S}_2 \end{vmatrix}. \tag{7}$$

The matrix Eq. 5 is convenient for expressing subsequent calculations in a concise way.

SOLUTION FOR COUPLED REACTIONS IN A PERIODIC POTENTIAL

We have described a specific four-state kinetic model of a transport enzyme in order to have a concrete example for the application of our method. However, the following is valid for an arbitrary system of linear equations, including, but not restricted to, the n simultaneous equations for $n + 1$ coupled chemical reactions in an arbitrarily large periodic field.

Applying an ac electric field normal to a planar membrane gives rise to an oscillating membrane potential. If the frequency of the field is small compared with the relaxation rate of the membrane double layer ($\approx 10^7$ Hz), the potential is given by

$$\psi = \psi_0 + \psi_1 \cos \omega t, \tag{8}$$

where ψ_0 is the average or dc potential and ψ_1 is the amplitude of the ac potential, which oscillates at frequency $\omega/2\pi$. Although to be specific we have chosen a sinusoidal potential, the following also holds for any periodic potential.

When the potential (Eq. 8) is substituted into Eq. 2, the coefficient of **E** and the right side of Eq. 5 will then be periodic with period equal to $2\pi/\omega$. Hence after any transients have decayed, the steady oscillation in the state probability vector **E** will also be periodic with the same period. We need to solve these equations over only one period. We divide the period into m equal time increments,

$$\Delta t = 2\pi/m\omega, \tag{9}$$

and substitute

$$t = k\Delta t, \quad k = 1, 2, \ldots, m \tag{10}$$

for the time. Then Eq. 5 becomes the set of algebraic equations,

$$\frac{\mathbf{E}[(k+1)\Delta t] - \mathbf{E}[(k-1)\Delta t]}{2\Delta t} + G(k\Delta t)\mathbf{E}(k\Delta t) = \mathbf{F}(k\Delta t), \tag{11}$$

where $k = 1, 2, \ldots, m$. Because $\mathbf{E}(t)$ is periodic, $\mathbf{E}[(m+1)\Delta t]$ in Eq. 11 can be replaced by $\mathbf{E}(\Delta t)$, and $\mathbf{E}(0)$ can be replaced by $\mathbf{E}(m\Delta t)$. This reduces the number of unknowns to m, which is the number of equations relating the unknowns.

Eq. 11 is a set of m linear algebraic equations for the m unknown column vectors $\mathbf{E}(k\Delta t)$. In super matrix form they become

$$\begin{vmatrix} G_1 & H & 0 & 0 & \cdots & 0 & -H \\ -H & G_2 & H & 0 & \cdots & 0 & 0 \\ 0 & -H & G_3 & H & \cdots & 0 & 0 \\ 0 & 0 & -H & G_4 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ 0 & 0 & 0 & 0 & \cdots & G_{m-1} & H \\ H & 0 & 0 & 0 & \cdots & -H & G_m \end{vmatrix} \begin{vmatrix} \mathbf{E}_1 \\ \mathbf{E}_2 \\ \mathbf{E}_3 \\ \mathbf{E}_4 \\ \vdots \\ \mathbf{E}_{m-1} \\ \mathbf{E}_m \end{vmatrix} = \begin{vmatrix} \mathbf{F}_1 \\ \mathbf{F}_2 \\ \mathbf{F}_3 \\ \mathbf{F}_4 \\ \vdots \\ \mathbf{F}_{m-1} \\ \mathbf{F}_m \end{vmatrix} \tag{12}$$

where $\mathbf{E}_k$, $\mathbf{F}_k$, and $\mathbf{G}_k$ are abbreviations for $\mathbf{E}(k\Delta t)$, $\mathbf{F}(k\Delta t)$, and $G(k\Delta t)$, respectively,

$$H = h\mathrm{I}, \quad h = 1/(2\Delta t), \tag{13}$$

and I is the $n \times n$ unit diagonal matrix. Because of the periodic boundary conditions, the time derivative of **E** at $t = \Delta t$ causes the submatrix $-H$ to appear in the upper right corner as well as the H just to the right of G_1, and the time derivative of **E** at $t = m\Delta t$ causes the submatrix

H to appear in the lower left corner as well as the $-H$ just to the left of G_m.

The time dependence of $\mathbf{E}(t)$ is obtained by solving the linear algebraic Eqs. 12. For small *m* and *n*, the $m \cdot n$ equations could be solved numerically without taking advantage of the sparseness of the matrix. However, the method of Appendix A avoids the memory and computation time limitations that this brute force approach entails and permits calculations of more complicated kinetic models than Eq. 1. This solution can be performed easily by a subroutine, which receives the successive $n \times n$ submatrices $\mathbf{G}_k$ in turn, so that memory for the supermatrix in Eqs. 12 need never be assigned.

FREE ENERGY TRANSDUCTION FROM AN AC FIELD

The resulting time dependence of the state probabilities for our four-state example is shown in Fig. 2. There we see that the enzyme state probabilities oscillate, reaching peaks successively clockwise. First E_1 reaches its peak, then SE_2, then E_3^*S, then E_4^*, and the cycle repeats. This is the sequence that corresponds to transport of S_1 into S_2. Transport occurs in this direction only if $b > 1$. If $b < 1$, the sequence would reverse, and S_2 would be transported into S_1, as shown in Appendix B.

The curves in Fig. 2 are highly distorted from sinusoidal shape by the nonlinearity. The shapes depend on the values of the parameters in the model. If the concentration gradient is so large that the energy in the field is not enough to cause the enzyme to pump against the gradient, the probability densities of states E_1 and SE_2 oscillate together and 180° out of phase with states E_3^*S and E_4^*, which oscillate together.

Transport is demonstrated explicitly by the rates. The instantaneous net rates of the four transitions in the model 1 are given by

$$J_{12} = abS_1 \cdot E_1 - aSE_2 \tag{14}$$

$$J_{23} = c\phi SE_2 - bc\phi^{-1} E_3^*S \tag{15}$$

$$J_{34} = abE_3^*S - aS_2 \cdot E_4^* \tag{16}$$

$$J_{41} = c\phi^{-1}E_4^* - bc\phi E_1. \tag{17}$$

These can easily be calculated from the time dependence of the state probabilities and are plotted vs. time in Fig. 3. This figure shows that the net rates peak in the same sequence as the state probabilities. The positive peaks are larger than the negative ones, and the rates are positive more often than negative. This demonstrates net pumping caused by the field.

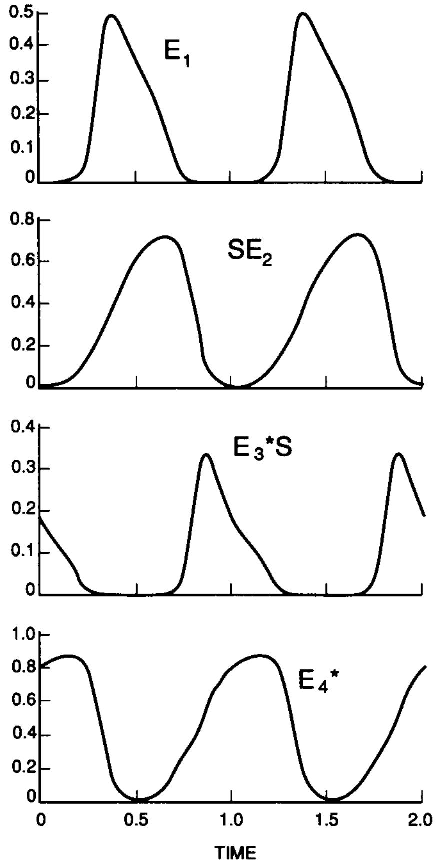

FIGURE 2 Probabilities of the four enzymes states vs. time for two cycles with $a = 1$, $b = 10$, $c = 1{,}000$, $S_1 = 0.5$, $S_2 = 1$, $q\psi_1 = 5$, and $\omega/2\pi = 1$. This figure along with Fig. 3 shows the sequence of events associated with pumping of the substrate against a concentration gradient.

The time-averages of J_{12}, J_{23}, J_{34}, and J_{41} can easily be computed by averaging Eqs. 14–17 over one cycle, i.e., over $k = 1, 2, \ldots m$. These averages equal each other as they must, and they equal the average rate $\bar{J}$ of pumping substrate from S_1 to S_2. For the conditions of Fig. 3, the average net rate is 0.43, which, since it is positive, is the rate of pumping clockwise up the concentration gradient $S_2/S_1 = 2$. The concentration gradient at which the average net flux disappears is static head. For the parameters used here, this value is $S_2/S_1 = 16.8$. At larger concentration gradients, the flux becomes negative, i.e., downhill.

A necessary condition for detailed balance,

$$(c\phi)(ab)(c/\phi)(abS_1) = (a)(bc\phi)(aS_2)(bc/\phi), \tag{18}$$

holds at equilibrium, i.e., when $S_2 = S_1$ in the absence of an ac field (even with an arbitrary dc field), and the average net flux is identically zero. When an ac field is switched on, condition (Eq. 18) continues to hold at every instant in time. Nevertheless, the field causes net clockwise flux.

The average power transduced by the enzyme against

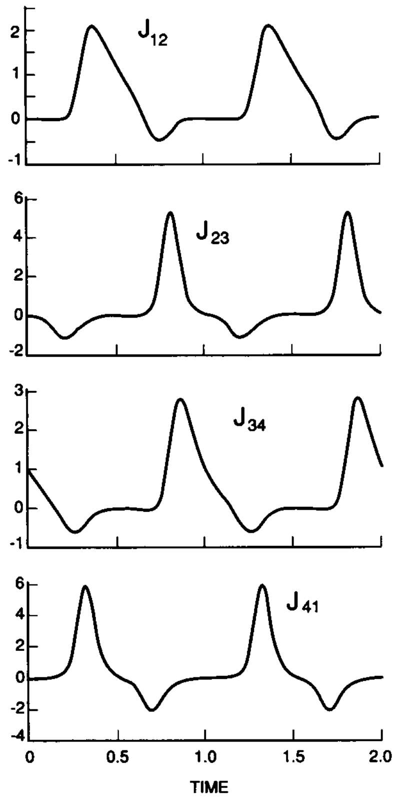

FIGURE 3 Instantaneous net rates of the four transitions vs. time for the same conditions as in Fig. 2. Positive rates are in the clockwise direction. The time average of each rate is equal to 0.43. Because this is greater than zero, net pumping occurs up the concentration gradient $S_2/S_1 = 2$.

the concentration gradient is the average rate of pumping the substrate times the free energy increase per molecule pumped:

$$\text{Power produced} = \overline{J_{23}\Delta G} = \bar{J}kT\ln(S_2/S_1). \qquad (19)$$

Here J_{23} is the instantaneous net rate for the transition that transports substrate from SE_2 to E_3^*S, and $\bar{J}$ is its time average. The power produced is positive when the enzyme is pumping uphill. This is the average power transduced from the ac field by the enzyme to maintain the concentration gradient.

The average power input from the ac field is the time average of the current times the ac potential.

$$\text{Power absorbed} = \overline{q(J_{23} + J_{14})\psi_1 \cos(\omega t)}. \qquad (20)$$

Here qJ_{23} is the instantaneous electric current across the membrane between state SE_2 and state E_3^*S, and $qJ_{14} = -qJ_{41}$ is the instantaneous current between state E_1 and state E_4^*. As before, the overbar represents a time average.

The efficiency of energy transduction from the ac field is equal to the power produced by the enzyme, pumping up the gradient, divided by the power it absorbs from the alternating potential. For the conditions of Fig. 2, the efficiency is 14%. As noted elsewhere (13), the efficiency can exceed 50% for other conditions. Also in that reference several results for the energy transduction properties of the model enzyme are given as a function of frequency, concentration gradient, ac and dc field amplitude, and kinetic parameters of the system.

SPHERICAL SYMMETRY

When the enzyme is imbedded in the membrane of a cell or vesicle in suspension, an additional step must be performed in the calculation. If an ac field of amplitude E_1 is applied to a suspension of spherical cells or vesicles, the resulting amplitude of the membrane potential oscillation is (10)

$$\psi_1 = 1.5RE_1 \cos\Theta. \qquad (21)$$

Here R is the radius of the cell or vesicle, and Θ is the angle between the field and the normal to the membrane. We assume no dc field is applied so the dc potential is entirely due to the selective permeability of the membrane to various ions. We also assume that the enzymes remain uniformly distributed over the surface of the membrane when the ac electric field is applied. Then the effect of the enzymes being in a spherical membrane can be calculated by averaging the previous results over the surface of a sphere. The quantities to be averaged are the average state probability, the rate of pumping, the power produced, and the power absorbed, but not the efficiency, which is the average power produced divided by the average power absorbed. The spherical average is done by putting the calculation of these quantities into a loop and integrating over $\frac{1}{2}\sin\theta\, d\theta$ from 0 to π.

The rate of pumping does not average to zero. The ac potential at angle θ has the same amplitude as the potential at angle $\pi - \theta$ and is merely of the opposite phase. Because the net rate is averaged over a cycle, it is independent of the phase of the ac potential. Hence the rate, e.g., at $\theta = 0$ is the same as the rate at $\theta = \pi$. The only difference in pumping at these two angles is that, e.g., while an enzyme at $\theta = 0$ is on average going from SE_2 to E_3^*S pumping substrate into the cell, another enzyme at $\theta = \pi$ is on average going from E_4^* to E_1 without transporting any product back out of the cell.

SUMMARY

We have given a general method for solving kinetic equations for the time-dependent state probability of a multistate membrane enzyme in an ac electric field. We have applied it to a four-state membrane transport enzyme in a planar membrane and also to an enzyme in the spherical membrane of a cell in suspension. We show that an enzyme can transduce free energy from a periodic electric field to drive a reaction away from equilibrium if two conditions are fulfilled. First the enzyme must have electric charge that moves concomitantly with its conformational change. Second the affinity of the enzyme for substrate must be different than its affinity for product.

APPENDIX A

In this appendix we show how Eqs. 12 are solved efficiently using Gaussian elimination and back substitution. There are two differences from the elementary discussion in textbooks (14): We want to take advantage of the sparseness of the matrix to reduce computation time and memory usage considerably, and we must be careful with the noncommutivity of the submatrices. The efficiency of this method makes possible the solution of large kinetic problems that could not otherwise be accomplished.

A related calculation is described by Newman (15). However, his method will not work on our problem with periodic boundary conditions, which require submatrices in the remote corners away from the diagonal. These remote submatrices generate six out of the eight recursion relations we will derive.

We first show how Eqs. 12 are converted to the triangular form,

$$\begin{vmatrix} \mathrm{I} & \mathrm{A}_1 & 0 & \cdots & 0 & 0 & \mathrm{B}_1 \\ 0 & \mathrm{I} & \mathrm{A}_2 & \cdots & 0 & 0 & \mathrm{B}_2 \\ 0 & 0 & \mathrm{I} & \cdots & 0 & 0 & \mathrm{B}_3 \\ \vdots & \vdots & \vdots & & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & \mathrm{I} & \mathrm{A}_{m-2} & \mathrm{B}_{m-2} \\ 0 & 0 & 0 & \cdots & 0 & \mathrm{I} & \mathrm{A}_{m-1} + \mathrm{B}_{m-1} \\ 0 & 0 & 0 & \ldots & 0 & 0 & \mathrm{C}_m \end{vmatrix} \cdot \begin{vmatrix} \mathbf{E}_1 \\ \mathbf{E}_2 \\ \mathbf{E}_3 \\ \vdots \\ \mathbf{E}_{m-2} \\ \mathbf{E}_{m-1} \\ \mathbf{E}_m \end{vmatrix} = \begin{vmatrix} \mathbf{P}_1 \\ \mathbf{P}_2 \\ \mathbf{P}_3 \\ \vdots \\ \mathbf{P}_{m-2} \\ \mathbf{P}_{m-1} \\ \mathbf{Q}_m \end{vmatrix}, \quad (\mathrm{A1})$$

by Gaussian elimination, and we obtain recursion relations for the submatrices and subcolumn vectors that appear here.

Assume we have already performed the elimination on the first $k - 1$ columns. We will use the kth equation to perform the elimination on the kth column of the subsequent equations. The only equations that have nonzero elements in the kth column below the diagonal are the $k + 1$th and mth equations. The three equations are

$$\begin{vmatrix} \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ \cdots & \mathrm{I} & \mathrm{A}_k & 0 & \cdots & 0 & \mathrm{B}_k \\ \cdots & -H & G_{k+1} & H & \cdots & 0 & 0 \\ & \vdots & \vdots & \vdots & & \vdots & \vdots \\ \cdots & \mathrm{D}_k & 0 & 0 & \cdots & -H & \mathrm{C}_k \end{vmatrix} \cdot \begin{vmatrix} \vdots \\ \mathbf{E}_k \\ \mathbf{E}_{k+1} \\ \vdots \\ \mathbf{E}_m \end{vmatrix} = \begin{vmatrix} \vdots \\ \mathbf{P}_k \\ \mathbf{F}_{k+1} \\ \vdots \\ \mathbf{Q}_k \end{vmatrix}, \quad (\mathrm{A2})$$

where we have shown all the nonzero elements of these equations. The new symbols in these equations will be defined by recursion relations and initial conditions to be derived. To determine the initial conditions, multiply the first of Eqs. 12 by the inverse G_1^{-1} of the submatrix G_1 and compare these equations with Eqs. A2 for $k = 1$, to get

$$\mathrm{A}_1 = hG_1^{-1} \quad (\mathrm{A3})$$

$$\mathrm{B}_1 = -\mathrm{A}_1 \quad (\mathrm{A4})$$

$$\mathrm{C}_1 = G_m \quad (\mathrm{A5})$$

$$\mathrm{D}_1 = \mathrm{H} \quad (\mathrm{A6})$$

$$\mathbf{P}_1 = h^{-1}\mathrm{A}_1\mathbf{F}_1 \quad (\mathrm{A7})$$

$$\mathrm{Q}_1 = \mathbf{F}_m. \quad (\mathrm{A8})$$

To perform the elimination, multiply the kth equation of Eqs. A2 on the left by H and add to the $k + 1$th equation. Then multiply the original kth equation by D_k and substract from the mth equation. This gives

$$\begin{vmatrix} \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ \cdots & \mathrm{I} & \mathrm{A}_k & 0 & \cdots & 0 & \mathrm{B}_k \\ \cdots & 0 & G_{k+1} + h\mathrm{A}_k & H & \cdots & 0 & h\mathrm{B}_k \\ & \vdots & \vdots & \vdots & & \vdots & \vdots \\ \cdots & 0 & -\mathrm{D}_k\mathrm{A}_k & 0 & \cdots & -H & \mathrm{C}_k - \mathrm{D}_k\mathrm{B}_k \end{vmatrix} \cdot \begin{vmatrix} \vdots \\ \mathbf{E}_k \\ \mathbf{E}_{k+1} \\ \vdots \\ \mathbf{E}_m \end{vmatrix} = \begin{vmatrix} \vdots \\ \mathbf{P}_k \\ \mathbf{F}_{k+1} + h\mathbf{P}_k \\ \vdots \\ \mathbf{Q}_k - \mathrm{D}_k\mathrm{C}_k \end{vmatrix}, \quad (\mathrm{A9})$$

Multiply the $k + 1$th equation by the inverse of its $k + 1$th coefficient to get

$$\begin{vmatrix} \vdots & \vdots & \vdots & \vdots & & \vdots & \vdots \\ \cdots & \mathrm{I} & \mathrm{A}_k & 0 & \cdots & 0 & \mathbf{B}_k \\ \cdots & 0 & \mathrm{I} & \mathrm{A}_{k+1} & \cdots & 0 & \mathbf{B}_{k+1} \\ & \vdots & \vdots & \vdots & & \vdots & \vdots \\ \cdots & 0 & \mathrm{D}_{k+1} & 0 & \cdots & -H & \mathrm{C}_{k+1} \end{vmatrix} \cdot \begin{vmatrix} \vdots \\ \mathbf{E}_k \\ \mathbf{E}_{k+1} \\ \vdots \\ \mathbf{E}_m \end{vmatrix} = \begin{vmatrix} \vdots \\ \mathbf{P}_k \\ \mathbf{P}_{k+1} \\ \vdots \\ \mathbf{Q}_{k+1} \end{vmatrix}, \quad (A10)$$

where the new submatrices can be seen to satisfy

$$\mathrm{A}_{k+1} = h\,(G_{k+1} + h\mathrm{A}_k)^{-1} \quad (A11)$$

$$\mathbf{B}_{k+1} = \mathrm{A}_{k+1}\mathbf{B}_k \quad (A12)$$

$$\mathrm{C}_{k+1} = \mathrm{C}_k - \mathrm{D}_k\mathbf{B}_k \quad (A13)$$

$$\mathrm{D}_{k+1} = -\mathrm{D}_k\mathrm{A}_k \quad (A14)$$

$$\mathbf{P}_{k+1} = h^{-1}\mathrm{A}_{k+1}(\mathbf{F}_{k+1} + h\mathbf{P}_k) \quad (A15)$$

$$\mathbf{Q}_{k+1} = \mathbf{Q}_k - \mathrm{D}_k\,\mathbf{P}_k. \quad (A16)$$

The $k + 1$th and mth equations in Eqs. A10 are the same as the kth and mth equations in Eqs. A2 with k replaced by $k + 1$, so we have progressed one step. Repeat this elimination for $k = 1, 2, 3, \ldots, m - 2$.

When $k = m - 1$, there remain to be considered only the last two equations,

$$\begin{vmatrix} \cdot & \cdot & \cdot \\ \cdots & \mathrm{I} & \mathrm{A}_{m-1} + \mathbf{B}_{m-1} \\ \cdots & \mathrm{D}_{m-1} - H & \mathrm{C}_{m-1} \end{vmatrix} \begin{vmatrix} \cdot \\ \mathbf{E}_{m-1} \\ \mathbf{E}_m \end{vmatrix} = \begin{vmatrix} \cdot \\ \mathbf{P}_{m-1} \\ \mathbf{Q}_{m-1} \end{vmatrix}. \quad (A17)$$

The above-diagonal line of submatrices A_k and the vertical line of submatrices $\mathbf{B}_k$ in Eq. A1 have converged to one submatrix at $k = m - 1$, where the submatrices add. Multiply the next-to-last equation in Eqs. A17 by $\mathrm{D}_{m-1} - H$ and subtract from the last equation to get the last of Eqs. A1 with

$$\mathrm{C}_m = \mathrm{C}_{m-1} - (\mathrm{D}_{m-1} - H)(\mathrm{A}_{m-1} + \mathbf{B}_{m-1}) \quad (A18)$$

$$\mathbf{Q}_m = \mathbf{Q}_{m-1} - (\mathrm{D}_{m-1} - H)\mathbf{P}_{m-1}. \quad (A19)$$

Eqs. A11–A16 and A18–A19 are the desired recursion relations. They can be used along with the initial conditions A3–A8 to calculate all the submatrices in Eqs. A1.

The last of Eqs. A1 gives

$$\mathbf{E}_m = \mathrm{C}_m^{-1}\,\mathbf{Q}_m, \quad (A20)$$

and the back substitution can easily be performed using

$$\mathbf{E}_k = \mathbf{P}_k - \mathrm{A}_k\mathbf{E}_{k+1} - \mathbf{B}_k\mathbf{E}_m \quad (A21)$$

for $k = m - 1, m - 2, \ldots, 2, 1$, thus completing the solution.

This procedure for solving Eqs. 12 is much more efficient than the brute force solution of the $n \cdot m$ simultaneous equations. Only $n \times n$ matrices need to be inverted, and only the A_k, $\mathbf{B}_k$, and $\mathbf{P}_k$ need to be stored.

APPENDIX B

We show that for $b < 1$ pumping occurs in the opposite direction than it does for $b > 1$. In Eq. 1 let

$$a \rightarrow ab \quad (B1)$$

$$b \rightarrow 1/b \quad (B2)$$

$$c \rightarrow cb, \quad (B3)$$

and in Eq. 2 let

$$q\psi \rightarrow -q\psi \quad (B4)$$

so that

$$\phi \rightarrow 1/\phi \quad (B5)$$

in Eq. 1. This gives exactly the same model as the original Eq. 1 except that the arrows all point in the opposite direction. This completes the proof.

For example, if we perform this transformation on the parameters of Figs. 2 and 3, we find that the inverse condition, $a = 10$, $b = 0.1$, $c = 10{,}000$, $S_1 = 1$, $S_2 = 0.5$, $q\psi_1 = 5$, and $\omega/2\pi = 1$, leads to pumping at the same 0.43 rate in the opposite direction at the same 14% efficiency up the concentration gradient $S_1/S_2 = 2$.

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