

Φ -Value Analysis of a Linear, Sequential Reaction Mechanism: Theory and Application to Ion Channel Gating

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ABSTRACT We derive the analytical form of a rate-equilibrium free-energy relationship (with slope Φ) for a bounded, linear chain of coupled reactions having arbitrary connecting rate constants. The results confirm previous simulation studies showing that Φ -values reflect the position of the perturbed reaction within the chain, with reactions occurring earlier in the sequence producing higher Φ -values than those occurring later in the sequence. The derivation includes an expression for the transmission coefficients of the overall reaction based on the rate constants of an arbitrary, discrete, finite Markov chain. The results indicate that experimental Φ -values can be used to calculate the relative heights of the energy barriers between intermediate states of the chain but provide no information about the energies of the wells along the reaction path. Application of the equations to the case of diliganded acetylcholine receptor channel gating suggests that the transition-state ensemble for this reaction is nearly flat. Although this mechanism accounts for many of the basic features of diliganded and unliganded acetylcholine receptor channel gating, the experimental rate-equilibrium free-energy relationships appear to be more linear than those predicted by the theory.

INTRODUCTION

Allosteric proteins are large, multimeric polymers that alternatively adopt more than one stable conformation. In experiments, such conformational changes often appear to be simple, two-state reactions. However, given the size and complexity of proteins, it is reasonable to assume that short-lived intermediate states exist during the course of a global isomerization.

One way to probe the intermediate states of an overall reaction (the transition-state ensemble; TSE) is by Φ -value analysis (1–3). A localized perturbation (e.g., a point mutation) that alters the equilibrium constant of a two-state reaction can do so by changing the forward rate constant, the backward rate constant, or both. The value Φ is the slope of the rate-equilibrium free energy relationship (REFER), which is a log-log plot of the forward rate constant versus the equilibrium constant for a perturbation series. The value Φ is a fraction that quantifies the relative extent to which the forward and backward rate constants were altered. A Φ -value of 1 indicates that only the forward rate constant changed and a Φ -value of 0 indicates that only the backward rate constant changed with the perturbation.

The nicotinic acetylcholine receptor (AChR) is an allosteric membrane protein that regulates the flow of ions at the nerve-muscle and other cholinergic synapses. It has five subunits (total MW \sim 290 kDa) and is roughly cylindrical in shape (\sim 170 Å \times 70 Å) (4). The ground states of the AChR gating isomerization are called *closed* (*C*; ions cannot readily pass through the channel) and *open* (*O*; monovalent cations

flow rapidly). In single-channel patch-clamp recordings with a time resolution of \sim 25 μ s, AChR gating appears to be a two-state process (5) and the transition from a nonconducting to a conducting conformation occurs within the time resolution of the instrumentation (\leq 3 μ s; (6)). Nonetheless, both structural and mutagenesis studies suggest that many thousands of atoms move in the course of the gating conformational change.

Recently, numerical methods were used to show that Φ -values depend on the position of the perturbation within a linear reaction chain, with higher values indicating earlier positions in the sequence (7). In addition, the diffusion of energy across a flat TSE landscape was shown to account for many of the essential features of AChR gating. These include the two-state kinetic behavior in patch-clamp recordings, the presence of discrete domains within which residues all have the same Φ -value (8), the organization of these domains approximately along the long axis of the protein (9), and an apparent upper limit to the channel-opening rate constant of \sim 1 μ s⁻¹ (10). The numerical analysis was limited because only the simplest reaction mechanism was examined (a single rate constant connecting all of the intermediate states), and because this mechanism was probed only by using simulations.

Here, we develop the analytical form of a REFER in the case of a bounded, linear chain of coupled reactions of arbitrary length and having arbitrary connecting rate constants. Although analytical solutions for the net rate-constant of a linear reaction chain have previously been described for specific cases (11,12), we are unaware of a general solution to this problem. We then apply the theory to the pattern of fractional Φ -values that has been observed for diliganded AChR gating.

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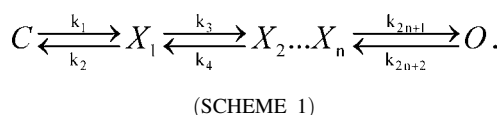
METHODS

The numerical calculations were done by using MAPLE 9.5 (Waterloo Associates, Waterloo, Ontario, Canada).

RESULTS

First-passage rate across an arbitrary, bounded landscape

Consider a linear chain of reactions in which two stable end-states (C and O) are separated by an arbitrary number (n) of intermediate states (X):



Let k_f be the inverse of the mean first latency for a complete $C \rightarrow O$ passage under the condition that $k_1, k_{2n+2} \ll$ all of the other rate constants. Specifically, we assume that the aggregate lifetime of the intermediates, $[X_1 \dots X_n]$, is vanishingly short compared to the lifetimes of the ground states, C and O .

Define r_j as the ratio of the exit rate constants (backward/forward) from X_j ,

$$r_j = \frac{k_{2j}}{k_{2j+1}}, \quad (1)$$

where r_j is the ratio of the probabilities that an exit from X_j will occur to either of its neighbors. Because there is only one exit rate constant from C , we define

$$r_0 = 1. \quad (2)$$

We can use linear algebra methods (13) to solve for k_f for $n \leq 2$. For $n = 1$,

$$k_f = \frac{k_1 k_3}{k_1 + k_2 + k_3}, \quad (3)$$

and, when $k_1 \ll (k_2 + k_3)$,

$$k_f \approx \frac{k_1}{1 + r_1}. \quad (4)$$

Similarly, for $n = 2$,

$$k_f = \frac{k_1 k_3 k_5}{k_2 k_4 + k_2 k_5 + k_3 k_5 + k_1 k_4 + k_1 k_5 + k_1 k_3}, \quad (5)$$

and when k_1 is small,

$$k_f \approx \frac{k_1}{1 + r_1 + r_1 r_2}. \quad (6)$$

For n -intermediate states (see Appendix),

$$k_f \approx \frac{k_1}{1 + r_1 + r_1 r_2 + \dots + (r_1 r_2 \dots r_n)} = \frac{k_1}{1 + \sum_{i=1}^n \prod_{j=1}^i r_j}. \quad (7)$$

In absolute-rate theory, a rate constant is the product of a transmission coefficient, a partition coefficient, and an exponential function of the activation energy. The transmission coefficient (κ) quantifies the fraction of trajectories in the TSE that actually reach the product. In the high-friction limit of Kramers' formalism (14), $\kappa = \omega_b/\gamma$, where ω_b is the curvature of the (parabolic) barrier and γ is a continuum frictional coefficient. Experimental values for κ , ω_b , and γ are difficult to obtain and these parameters remain essentially unknown for allosteric proteins.

The denominator of Eq. 7 is the average number of times the $C \rightarrow X_1$ transition occurs before the stable state O is reached. This number is a function only of the ratios of exit rate constants from the intermediate states. Hence, the inverse of the denominator of Eq. 7 gives κ as a function of the rate constants (the r -values) of an arbitrary, discrete, finite Markov chain:

$$\kappa_{C \rightarrow O} = \left(1 + \sum_{i=1}^n \prod_{j=1}^i r_j \right)^{-1}, \quad (8)$$

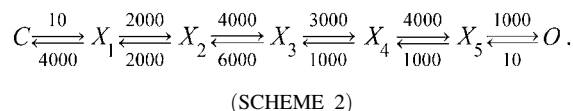
$$\kappa_{O \rightarrow C} = \left(1 + \sum_{i=n}^1 \prod_{j=i}^1 r_j \right)^{-1}. \quad (9)$$

If we impose a length scale on the intermediate transitions and make all of the rate constants equal ($r = 1$), k_f is the mean first-passage rate for isotropic diffusion between two absorbing boundaries. Under this condition, Eq. 7 predicts that this rate is linear with n :

$$k_f \approx \frac{k_1}{n + 1}. \quad (10)$$

This agrees with the laws of diffusion, which predicts that the time to reach an absorbing boundary across an interval x is of the order x^2 when the particle starts in the interior of the interval but of the order x when the particle starts near a boundary (15). Other limiting conditions of Eq. 7 also make sense—i.e., $k_f \rightarrow 0$ as the r -values $\rightarrow \infty$ and $k_f \rightarrow k_1$ as the r -values $\rightarrow 0$.

We use the following reaction sequence as a numerical example:



The rate constant units are inverse time (e.g., ms^{-1}). For this reaction chain $r_1 = 2$, $r_2 = 0.5$, $r_3 = 2$, $r_4 = 0.25$, and $r_5 = 1$. We calculate from Eq. 7 that $k_f = 1.42 \text{ ms}^{-1}$, which is in excellent agreement with the value calculated using Q-matrix methods. Increasing the magnitudes of the intermediate rates 10-fold did not alter k_f . In Scheme 2, the $C \rightarrow X_1$ transition occurs, on average, approximately seven times before state O is occupied ($\kappa_{C \rightarrow O} \approx 0.14$).

Φ-values

The value Φ is the differential coefficient of $\log k_f / \log K_{\text{eq}}$, where K_{eq} is the equilibrium constant for the overall,

$C \leftrightarrow O$ reaction ($[O]/[C]$ at equilibrium), which is the product of the intermediate equilibrium constants:

$$K_{eq} = \frac{k_1 k_3 k_5 \dots k_{2n+1}}{k_2 k_4 k_6 \dots k_{2n+2}} = \frac{k_1}{k_{2n+2}} \prod_{i=1}^n r_i. \quad (11)$$

Equation 7 gives the overall, $C \rightarrow O$ forward rate constant and Eq. 11 gives the overall equilibrium constant. These are the components of a REFER, the first derivative of which is called Φ .

We see from Eq. 11 that K_{eq} will change equally for an equivalent change in any single r -value. However, Eq. 7 predicts that a change in r_1 will have the greatest effect on k_f , while a change in r_n will have the least effect on k_f . Thus, a change in r_1 will produce a high Φ -value, while an equivalent change in r_n will produce a low Φ -value. Qualitatively, the Φ -value for a perturbation series provides information about the position of the perturbed intermediate in the overall reaction sequence, with higher Φ -values indicating earlier positions in the chain.

We derive a closed-form expression for Φ for a perturbation series that changes a single r -value. The changes in $\log(k_f)$ and $\log(K_{eq})$ consequent to a change in r_1 are

$$\begin{aligned} \frac{\partial(\log k_f)}{\partial(r_1)} &= \frac{1}{k_f} \frac{\partial(k_f)}{\partial(r_1)} \\ &= -\frac{1 + \sum_{i=2}^n \prod_{j=2}^i r_j}{1 + \sum_{i=1}^n \prod_{j=1}^i r_j} \\ &= -\frac{\sum_{i=1}^n \prod_{j=1}^i r_j}{r_1 \left(1 + \sum_{i=1}^n \prod_{j=1}^i r_j\right)} \end{aligned} \quad (12)$$

and

$$\frac{\partial(\log K_{eq})}{\partial(r_1)} = \frac{1}{K_{eq}} \frac{\partial(K_{eq})}{\partial(r_1)} = -\frac{1}{r_1}. \quad (13)$$

Hence, by the chain rule,

$$\begin{aligned} \Phi_{r_1} &= \frac{\partial \log(k_f)}{\partial \log(K_{eq})} = \frac{\partial \log(k_f)}{\partial r_1} \frac{\partial r_1}{\partial \log(K_{eq})} = \frac{\frac{\partial \log(k_f)}{\partial r_1}}{\frac{\partial \log(K_{eq})}{\partial r_1}} \\ &= \frac{\sum_{i=1}^n \prod_{j=1}^i r_j}{1 + \sum_{i=1}^n \prod_{j=1}^i r_j}. \end{aligned} \quad (14)$$

By combining this equation with that for r_0 (Eq. 2), we get

$$M = 1 + \sum_{i=1}^n \prod_{j=1}^i r_j = \sum_{i=0}^n \prod_{j=0}^i r_j. \quad (15)$$

Thus, Eq. 14 can be simplified to $\Phi_{r_1} = (M - r_0/M)$. In general, for perturbations of r_m (the m^{th} intermediate state),

$$\Phi_{r_m} = \frac{M - \sum_{i=0}^{m-1} \prod_{j=0}^i r_j}{M}. \quad (16)$$

We calculate from Eq. 16 that for the reaction shown in Scheme 2, $\Phi_1 = 0.857$, $\Phi_2 = 0.571$, $\Phi_3 = 0.428$, $\Phi_4 = 0.143$, and $\Phi_5 = 0.071$.

Typically, perturbations may change a single intermediate equilibrium constant, which will in turn alter two adjacent r -values. We can reformulate Eqs. 7 and 16 to reflect changes in the i^{th} microstate transition equilibrium constants, K_i ,

$$K_{eq} = \prod_{i=1}^{n+1} K_i. \quad (17)$$

If k_1 is greatly less than the other rate constants, then the approximate mean latency (L) from C to O is

$$\begin{aligned} L &\approx \frac{1}{k_1} + \frac{k_2}{k_1 k_3} + \frac{k_2 k_4}{k_1 k_3 k_5} \dots + \left[\frac{k_2 k_4 \dots k_{2n}}{k_1 k_3 \dots k_{2n+1}} \right] \\ &= \frac{1}{k_1} + \frac{1}{K_1 k_3} + \frac{1}{K_1 K_2 k_5} \dots + \left[\frac{1}{K_1 K_2 \dots K_n k_{2n+1}} \right] \\ &= \sum_{i=1}^{n+1} \left(\frac{1}{k_{2i-1}} \prod_{j=0}^{i-1} \frac{1}{K_j} \right), \end{aligned} \quad (18)$$

where $K_0 = 1$.

By the application of the chain rule, the Φ -value for a perturbation of a single, intermediate equilibrium constant (K_m) for the m^{th} step of the reaction chain is

$$\Phi_{K_m} = \frac{\frac{\partial(\log k_f)}{\partial K_m}}{\frac{\partial(\log K_{eq})}{\partial K_m}} = \frac{-K_m \frac{\partial L}{\partial K_m}}{L} = \frac{\sum_{i=m}^{n+1} \left(\frac{1}{k_{2i-1}} \prod_{j=0}^{i-1} \frac{1}{K_j} \right)}{\sum_{i=1}^{n+1} \left(\frac{1}{k_{2i-1}} \prod_{j=0}^{i-1} \frac{1}{K_j} \right)}. \quad (19)$$

Based on Eqs. 16 and 19, it is easy to prove that $\Phi_{K_i} = \Phi_{r_{i-1}}$.

The Φ -values are not constants but are functions of the specific rate-constants of the model. Φ_m will vary from 0 to 1 as K_m decreases ($\infty \rightarrow 0$). However, for any given value of K_m , the value of Φ_m always becomes smaller as m increases. Thus, for all values of K_{eq} , the relative value of Φ gives the relative position of the perturbation in the reaction chain.

We can estimate the curvature of a rate-equilibrium free-energy relationship for a bounded, linear sequence of reactions, i.e., the second derivative of $\log(k_f)$ versus $\log K_{eq}$, as

$$\begin{aligned} c_m &= \frac{\partial(\log k_f)}{\partial(\log K_{eq}) \partial(\log K_{eq})} \\ &= \frac{\frac{\partial(\log k_f)}{\partial K_m} \frac{\partial(\log K_{eq})}{\partial K_m} - \frac{\partial(\log k_f)}{\partial K_m} \frac{\partial(\log K_{eq})}{\partial K_m} \frac{\partial(\log K_{eq})}{\partial K_m}}{\left[\frac{\partial(\log K_{eq})}{\partial K_m} \right]^3} \\ &= \ln(10) (\Phi_m^2 - \Phi_m). \end{aligned} \quad (20)$$

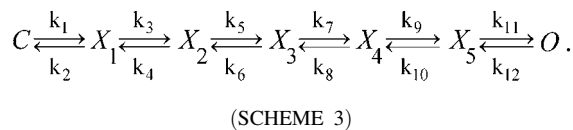
For a linear chain of reactions, the magnitude of the curvature is parabolic and depends only on Φ . As was

previously shown by using numerical methods (7), the absolute value of curvature is maximal when $\Phi = 0.5$.

Application to diliganded AChR gating

To demonstrate the utility of this theory, we now apply Eq. 16 to the experimentally determined values for diliganded AChR gating. In AChRs, residues appear to be organized into discrete domains of constant Φ . So far, six such domains have been identified, with $\sim\Phi$ -values of 0.93, 0.80, 0.65, 0.54, 0.35, and 0.0. Moreover, these domains are spatially organized, according to Φ -value, approximately along the long axis of the protein.

For the purposes of this analysis, we assume that these six domains move sequentially during AChR diliganded gating:



Because the lifetime of $[X_1 \dots X_5]$ is vanishingly brief, we have no information regarding whether the pore is conducting or nonconducting in these intermediate states. Given this reaction mechanism, we use Eq. 16 to solve for four of the five r -values for the intermediate state transitions. The results are $r_2 = 1.15$, $r_3 = 0.73$, $r_4 = 1.72$, and $r_5 = 1.84$. From Eq. 16, we can only conclude that $r_1 = 0.13(k_1/k_f)$.

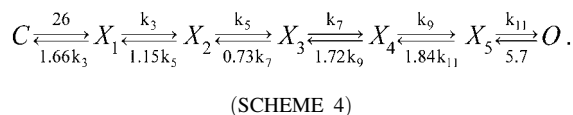
To solve for r_1 , we use the additional experimental result that for perturbations of $C \leftrightarrow X_1$, $k_f = 2 \text{ ms}^{-1}$ when $K_{eq} = 1$ (5,16). Thus, from Eqs. 7–9,

$$2 \text{ ms}^{-1} = \frac{k_1}{\kappa_{C \rightarrow O}}, \quad (21)$$

$$2 \text{ ms}^{-1} = \frac{k_{12}}{\kappa_{O \rightarrow C}}. \quad (22)$$

Solving these equations gives $k_1 = 25.6 \text{ ms}^{-1}$, $k_{12} = 5.71 \text{ ms}^{-1}$. Using the relationship $r_1 = 0.13(k_1/2 \text{ ms}^{-1})$, we calculate $r_1 = 1.66$.

These r -values can be incorporated into a kinetic model of diliganded AChR gating ($K_{eq} = 1$):



An energy landscape derived from this reaction sequence is shown in Fig. 1. Because of the overall tilt in the energy barriers of the TSE, according to this scheme a closed AChR makes $\sim 13 C \rightarrow X_1$ transitions before finally reaching the stable O state, but an open AChR makes only $\sim 2.8 O \rightarrow X_5$ transitions before reaching the stable C state. From Eqs. 8 and

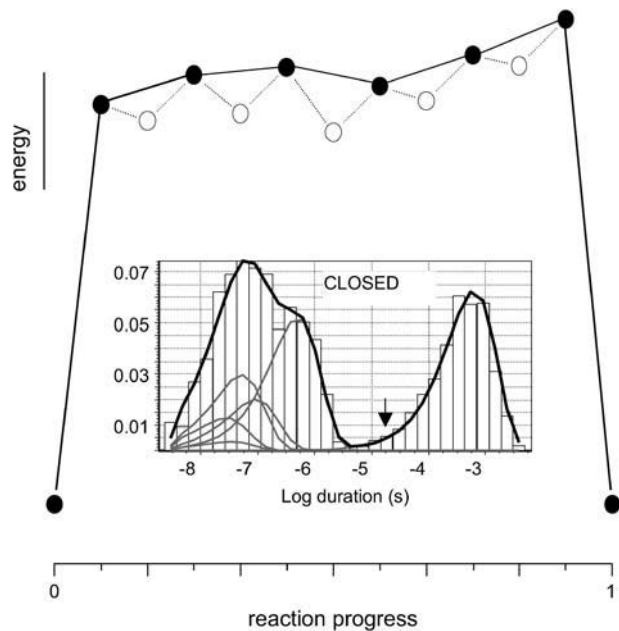


FIGURE 1 Energy landscape for diliganded AChR gating computed from experimental Φ -values (Eq. 16) and an intrinsic gating rate constant of 2 ms^{-1} . The corresponding kinetic model is Scheme 4, with all intermediate states (X_1 – X_5) nonconducting and the forward rate constants from the intermediate states arbitrarily set to $10 \mu\text{s}^{-1}$. The solid line connects the barriers between the intermediate states. The depths of the wells along the reaction surface (open circles) are arbitrary. The transmission coefficients for the forward and reverse overall rate constants are ~ 0.08 and ~ 0.35 , respectively. (Inset) The nonconducting interval duration distribution corresponding to the kinetic scheme. The arrow marks the approximate time resolution of the patch-clamp.

9, we estimate that for diliganded AChR gating, $\kappa_{C \rightarrow O} \approx 0.078$ and $\kappa_{O \rightarrow C} \approx 0.35$.

DISCUSSION

Equation 16 provides a formal relationship between Φ and the sequential position of a transition within a linear reaction chain flanked by absorbing boundaries, under the condition that the aggregate lifetime of the intermediate states is short relative to those of the ground states. Reactions that occur early in this sequence always produce higher Φ -values than those that occur later in the sequence. With this simple reaction mechanism, it is appropriate to classify sites of perturbation that yield higher Φ -values as moving in advance of those that produce lower Φ -values (“early” versus “late”), and sites that generate equivalent Φ -values as “synchronous”.

Our analysis indicates that with this model, Φ -values only provide information about the relative exit probabilities from the intermediate states in a linear series of coupled transitions (the r -values). That is, Φ -values reveal the relative heights of the energy barriers between microstates of the TSE, but provide no information about the absolute energies of the wells along the reaction surface. The assumption that the TSE lifetime is negligible renders the depths of these wells irrelevant.

The overall shape of the barriers along the TSE for diliganded AChR gating (Fig. 1) was derived from two experimental observations, the map of Φ -values and the intrinsic gating rate constant. Previously, numerical analyses showed that a flat and isotropic TSE could account, approximately, for the kinetics of diliganded AChR gating (7). The results shown in Scheme 4 and Fig. 1 do not differ greatly from this simple model. The ratios of exit rate constants from each intermediate state are all <2 , and the final barrier of the TS is only $\sim 1.5 k_B T$ higher than the initial barrier.

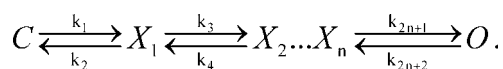
This landscape is provisional because the map of Φ -values is incomplete. The Φ -values that have been reported so far are nearly quantal ($\Delta\Phi \approx 0.15$), with the only deviation being the lack of an experimental Φ -value between $\Phi = 0.35$ and 0.0 . Although one position (in the M4 segment of the β -subunit) has been found with $\Phi = 0.17$ (19), we hesitate to add an intermediate state to the TSE based on only a single residue. If such an intermediate was confirmed, the computed landscape would become flatter still ($r_1 = 1.85, r_2 = 1.15, r_3 = 0.73, r_4 = 1.73, r_5 = 0.95, r_6 = 0.94; \kappa_{C \rightarrow O} = 0.07, \kappa_{O \rightarrow C} = 0.17$; and when $K_{eq} = 1, k_1 = 28.6 \text{ ms}^{-1}, k_{14} = 11.8 \text{ ms}^{-1}$).

With further assumptions, we can infer the depths of the wells of the TSE and a kinetic scheme for physiological gating. The conductances and forward rates of the intermediate states determine the first-passage rate between the first nonconducting intermediate (X_1) and the first conducting state, which is, perhaps, equal to the experimental channel-opening speed-limit (10). Further, if we assume that different agonists perturb only the $C \rightarrow X_1$ rate constant, then we can generate a kinetic scheme for gating of AChRs having two bound molecules of the transmitter, ACh. Scheme 5, in which all of the X -states are nonconducting, predicts the apparent opening speed limit of $\sim 0.86 \mu\text{s}^{-1}$ and the wild-type diliganded gating parameters (with ACh) under standard patch-clamp recording conditions of $k_{\text{opening}} \approx 50,000 \text{ s}^{-1}$, $k_{\text{closing}} \approx 2000 \text{ s}^{-1}$, and $K_{eq} \approx 25$ (rates are μs^{-1}):

(17), is also consistent with the sequential mechanism which predicts that $\Phi \rightarrow 1$ as $K_{eq} \rightarrow 0$. However, one significant deviation between theory with experiment is that the experimental REFERs appear to have less curvature than predicted using the simple linear chain mechanism. Although it is difficult to get accurate experimental estimates of curvature (because of a small range of K_{eq} and experimental scatter), we have not observed the trend in curvature predicted by Eq. 20 in the AChR kinetic data. Indeed, the REFER for position $\alpha 418$, for which $\Phi = 0.5$ over an ~ 100 -fold range of K_{eq} , has very little scatter and is remarkably linear (19). We do not yet know whether this discrepancy between model and experiment indicates that the linear reaction mechanism is fundamentally incorrect for AChR gating, or if this simple mechanism is simply incomplete and can be modified or extended to accommodate all of the features of AChR gating.

APPENDIX

For a linear reaction scheme in which two stable end-states (C and O) are separated by n short-lived intermediate states (X),



(SCHEME 1)

and the mean first latency for a complete $C \rightarrow O$ passage is

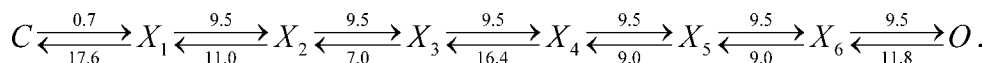
$$L = -\pi_C Q_{CC}^{-1} \mathbf{u}_C, \quad (\text{A1})$$

where

$$\pi_C = \underbrace{(1, 0, 0, \dots, 0)}_{n+1} \quad (\text{A2})$$

and

$$\mathbf{u}_C = \left(\begin{array}{c} 1 \\ 1 \\ \vdots \\ 1 \end{array} \right) \Bigg\} n+1 \quad (\text{A3})$$



(SCHEME 5)

The mechanism of Scheme 1, in which the intermediate state transitions are completely coupled, accounts for many of the features of diliganded AChR gating. Moreover, the observation that for unliganded AChR gating, $\Phi \approx 1$ everywhere

and

let

$$\mathbf{x} = \pi_C Q_{CC}^{-1}, \quad (\text{A5})$$

$$Q_{CC} = \begin{pmatrix} -k_1 & k_1 & 0 & 0 & \dots & 0 \\ k_2 & -(k_2 + k_3) & k_3 & 0 & \dots & 0 \\ 0 & k_4 & -(k_4 + k_5) & k_5 & \dots & 0 \\ & & \ddots & & & \\ 0 & \dots & 0 & k_{2(n-1)} & -(k_{2(n-1)} + k_{2n-1}) & k_{2n-1} \\ 0 & 0 & \dots & 0 & k_{2n} & -(k_{2n} + k_{2n+1}) \end{pmatrix}, \quad (\text{A4})$$

where

$$\mathbf{x} = (x_1, x_2, \dots, x_n, x_{n+1}). \quad (\text{A6})$$

The problem of solving the mean first latency L in Eq. A1 is to find the solution \mathbf{x} for

$$\boldsymbol{\pi}_C = \mathbf{x} \mathbf{Q}_{CC}. \quad (\text{A7})$$

From Eq. A7,

$$\begin{cases} 1 = -k_1 x_1 + k_2 x_2 \\ 0 = k_1 x_1 - (k_2 + k_3) x_2 + k_4 x_3 \\ \vdots \\ 0 = k_{2(n-2)-1} x_{n-1} - (k_{2(n-1)} + k_{2n-1}) x_n + k_{2n} x_{n+1} \\ 0 = k_{2n-1} x_n - (k_{2n} + k_{2n+1}) x_{n+1}. \end{cases} \quad (\text{A8})$$

These can be rewritten as

$$\begin{cases} 1 = -k_1 x_1 + k_2 x_2 \\ 1 = -k_3 x_2 + k_4 x_3 \\ \vdots \\ 1 = -k_{2n-1} x_n + k_{2n} x_{n+1} \\ 1 = -k_{2n+1} x_{n+1}, \end{cases} \quad (\text{A9})$$

so that

$$x_i = \frac{(k_{2i} x_{i+1} - 1)}{k_{2i-1}}. \quad (\text{A10})$$

We define $r_i = (k_{2i}/k_{2i+1})$ and solve Eq. A9 by starting with the last equation for x_{n+1} and then solving for the rest by using back substitution. This gives

$$\begin{cases} x_{n+1} = -\frac{1}{k_{2n+1}} \\ x_n = \frac{(k_{2n} x_{n+1} - 1)}{k_{2n-1}} = -\frac{(r_n + 1)}{k_{2n-1}} \\ x_{n-1} = \frac{(k_{2(n-1)} x_n - 1)}{k_{2(n-1)-1}} = -\frac{(r_{n-1}(r_n + 1) + 1)}{k_{2(n-1)-1}} \\ \vdots \\ x_m = -\frac{\left(1 + \sum_{i=m}^n \prod_{j=1}^i r_j\right)}{k_{2m-1}} \\ \vdots \\ x_1 = -\frac{\left(1 + \sum_{i=1}^n \prod_{j=1}^i r_j\right)}{k_1}. \end{cases} \quad (\text{A11})$$

From Eqs. A1, A3, and A5,

$$L = -\sum_{i=1}^{n+1} x_i. \quad (\text{A12})$$

Note that each $x_i \propto 1/k_{2i-1}$ so that $k_1 \ll k_3, k_5, \dots, k_{2n+1}$. Hence, $x_1 \gg x_2, x_3, \dots, x_{n+1}$. Therefore,

$$L = -x_1 - \sum_{i=2}^{n+1} x_i = -x_1 + O(k_1^0) \approx -\frac{\left(1 + \sum_{i=1}^n \prod_{j=1}^i r_j\right)}{k_1}, \quad (\text{A13})$$

and

$$k_f = \frac{1}{L} \approx \frac{k_1}{\left(1 + \sum_{i=1}^n \prod_{j=1}^i r_j\right)}. \quad (\text{A14})$$

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