Stochastically Gated Chemical Reactions

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This article derives a Smoluchowski theory for the irreversible, diffusion-influenced, stochastically gated reaction P* + L* → ∅ (inert) between a protein P* and its ligand L*, with the ligand in excess, [P] ≪ [L]. Protein gating P* ⇄ P (P unreactive) is contrasted with ligand gating L* ⇄ L (L unreactive). It is shown explicitly, even for non-Markovian gating or a finite number N of ligand molecules (without $N \rightarrow \infty$), that if the reaction and gating kinetics are comparable, ligand-gated reactions always proceed faster than the corresponding protein-gated reactions. The reaction P* + L* → ∅ is mathematically equivalent to the special case $n = 0$ of $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$ ($n = 0, 1, 2, ...$). The reaction $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$ might, for example, model reactions between an enzymatic protein molecule and its ligands, where the subscript n on P* counts the number of ligands irreversibly processed by the protein. A simple zero-correlation approximation is used to derive and generalize the Zhou−Szabo approximation for protein-gated reactions from P* + L* → ∅ to $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$. The zero-correlation approximation naturally suggests a variance-reduction technique for simulating gated reactions in a computer.

I. Introduction

Conformational fluctuations can influence the reaction kinetics between a macromolecule and its ligand. Macromolecular conformation may be important, e.g., when an oxygen molecule enters the heme pocket of myoglobin. Although the static X-ray structure of myoglobin shows no point of entry for oxygen molecules, the myoglobin side chains that block the heme pocket may "act as a gate[1]", swinging out to permit oxygen molecules to enter. In other cases, ligand conformation may be important, e.g., when a peptide binds to a major histocompatibility complex protein. Here, the antigen-binding site of a histocompatibility protein may bind only specific peptide conformations.[2−4]

Chemical blockers also influence reaction kinetics. For example, soluble CD4 blocks human immunodeficiency virus (HIV) infection in the test tube. Soluble CD4 (among its other effects) reacts reversibly with proteins on the surface of HIV, preventing the virus from attaching to target cells. Therefore, even though the kinetic analyses were not encouraging,[5−8] soluble CD4 was tried as a therapy for HIV infection in clinical trials[9,10] and failed.

Thus, the conformational fluctuations of a protein P* ⇄ P (P unreactive) or its ligand L* ⇄ L (L unreactive) sometimes modulate the formation of a chemical complex, P* + L* → PL, and consequently can influence reaction kinetics. The interconversion P* ⇄ P is called "protein gating", and the interconversion L* ⇄ L, "ligand gating". For linguistic simplicity, this article considers protein gating and ligand gating as separate, mutually exclusive phenomena, since if necessary, their mathematical solutions can be combined.[11] Extrinsic chemical blockers like soluble CD4 may cause interconversions similar to gating (e.g., P* + B ⇄ P or L* + B ⇄ L). If a blocker B has no other significant effects, however, blocking interconversions are mathematically equivalent to gating and require no further special mention.

Theories of gating, as opposed to theories of dynamic trapping,[12−14] generally assume that the gating interconversions evolve independently of the spatial positions of the molecules. The first gating study[15] analyzed deterministic two-state gating P* ⇄ P, but surprisingly, stochastic gating turned out to be easier to analyze.[16] Time-dependent rate coefficients[17−19] for several multistate gating processes[20,21] have now been derived for the isolated pair problem, in which a single ligand molecule binds to a protein molecule. The isolated pair problem has an unnatural symmetry, however, because protein and ligand gating have equivalent effects on the reaction kinetics. If a single protein molecule is surrounded by many ligand molecules, however, the symmetry breaks down:[11] if the ligands are gating, their reactivities are independent, whereas if the protein is gating, its gating state forces the ligand reactivities to correlate.

Zhou and Szabo[11] examined the reaction P* + L* → ∅ (inert) when the ligand is in excess, [P] ≪ [L]. They permitted the protein (and, separately, the ligand) to have multiple gating states, subject to the significant restriction that all the conversions from one gating state to another are Markov processes. They found that for Markovian-gated ligands, L* ⇄ L, time-dependent rate coefficients for a mean-field Smoluchowski theory[17−19] can be derived as usual from the isolated pair problem and merely incorporate the gated rate coefficient. For the Markovian-gated protein, P* ⇄ P, however, time-dependent rate coefficients can not be found analytically, although they can be approximated.

For a Markovian-gated protein, a superposition approximation[22] truncates the infinite hierarchy of equations for the reduced distribution functions at the pair level. Zhou and Szabo pointed out that this approximation is exact in several extremes, e.g., when reaction proceeds much faster or much slower than gating, and in the reaction-controlled limit, when the reaction rate depends only on the present gating state. They then simulated a chemical reaction in three dimensions to demonstrate that their approximation remained accurate between the extremes of fast and slow reaction. Graphs from their numerical simulations display quite nicely the differences between protein- and ligand-gating rates.

Most of this article treats the reaction P* + L* → ∅ as a special case $n = 0$ of the reaction $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$ for $n = 0, 1, 2, ...$ The reaction $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$ could, for example, model an enzymatic protein P* processing its ligand

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substrates L* irreversibly, where the subscript n on P* counts the number of ligands processed by the protein. Alternatively, it could model viruses L* irreversibly attaching to a cell P*, where the subscript n counts the number of viruses attached to the cell.[23] The survival probability for P_0^* in the reaction $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$ then can provide the survival probability for P* in the reaction $P^* + L^* \rightarrow \varnothing$.

The plan of this article is as follows. Section II restricts itself to the reaction $P^* + L^* \rightarrow \varnothing$ and gives a Smoluchowski gating theory that explicitly includes non-Markovian gating. Jensen's inequality,[24] $\phi(\langle X \rangle) \leq \langle \phi(X) \rangle$ for convex ϕ, shows that for comparable reaction and gating kinetics ligand-gated reactions always proceed faster than the corresponding protein-gated reactions, even for a finite number of ligands, on the basis of (but correcting) previous techniques.[25] Section III generalizes the Smoluchowski gating theory for $P^* + L^* \rightarrow \varnothing$ to the reaction $P_n^* + L^* \rightarrow P_{n+1}^* + \varnothing$ by examining the whole Poisson distribution $e^{-\lambda}\lambda^n/n!$ instead of just $e^{-\lambda}$, the $n = 0$ term. Section IV shows that the Smoluchowski gating theory given here is equivalent to previous Smoluchowski theories for Markovian gating. A generalization of the zero-covariance approximation $\langle YZ \rangle \approx \langle Y \rangle \langle Z \rangle$ then derives the Zhou−Szabo approximation without the use of reduced distribution functions. The zero-correlation approximation, when exact, naturally suggests a variance-reduction technique for simulating gated reactions in a computer. Section V gives the discussion.

II. Smoluchowski Gating Theory for P* + L* → ∅

Up to eq 8, this section merely augments the standard elements of Smoluchowski theory with gating. Gating introduces extra dependencies on a variable $\mathbf{Q}$, which summarizes the complete gating history of the protein−ligand system. After specializing $\mathbf{Q}$ to protein-gated and ligand-gated systems, and then comparing the corresponding protein survival probabilities, Jensen's inequality gives eq 11: for comparable reaction and gating kinetics, ligand-gated reactions always proceed faster than the corresponding protein-gated reactions, even for non-Markovian gating or a finite number of ligands.

Let us begin by considering the kinetics of a protein and ligand reacting to form an inert product: $P^* + L^* \rightarrow \varnothing$. If the ligand is in excess, $[P] \ll [L]$, protein molecules are approximately independent, so only the fate of a single protein molecule needs to be examined. Consider accordingly a single protein molecule surrounded by N ligands in a closed volume V in d dimensions. In a standard approximation, the protein is fixed at the origin, while each ligand diffuses relative to it.[17−19] Let the operator $L_{\mathbf{r}_i}$ specify the motion of the ith ligand; for example, $L_{\mathbf{r}_i}$ might be the d-dimensional diffusion operator $D\nabla_{\mathbf{r}_i}^2$.

We now superimpose gating on this system. The following theory can be extended to continuous gating states if necessary,[11] but let us assume that a countable set $\Omega_Q = \{q_1, q_2, q_3, ...\}$ enumerates the gating states. The gating history of the protein−ligand system can be described by a vector $\mathbf{Q} = (Q_1, Q_2, ..., Q_N)$, which has one component for every ligand. This description is quite flexible: for ligand gating, the components $Q_1, Q_2, ..., Q_N$ of $\mathbf{Q}$ are probabilistically independent (each ligand gates independently), and for protein gating, they are equal, i.e., $Q_1 = Q_2 = ... = Q_N = Q$ (protein gating is equivalent to having the ligands all gate in unison). The gating history for the ith ligand is described by Q_i, which specifies the ligand's reactivity over all times. Thus the gating state of the ith ligand at time t is $Q_i(t)$, where Q_i: $[0,\infty) \rightarrow \Omega_Q$ is a function from the time interval $[0,\infty)$ into the set of gating states Ω_Q.

Deterministic gating, which fixes the vector $\mathbf{Q}$, permits a standard Smoluchowski theory, as follows. Let $P_N = P_N(\mathbf{Q};\mathbf{r}_1, ..., \mathbf{r}_N, t)$ be the probability of finding the ith ligand at position $\mathbf{r}_i$ ($i = 1, 2, ..., N$) relative to the protein. If $\kappa(q,\mathbf{r})$ denotes the reactivity at a given position $\mathbf{r}$ when the system's gating state is q, then

$$\frac{\partial P_N}{\partial t} = \sum_{i=1}^{N} [L_{\mathbf{r}_i} - \kappa(Q_i(t),\mathbf{r}_i)] P_N \tag{1}$$

If the ligands are initially distributed independently with distribution $\rho_N(\mathbf{r})$, where $\int_V \rho_N(\mathbf{r}) V^{-1}\, d\mathbf{r} = 1$, then $P_N(\mathbf{Q};\mathbf{r}_1,...,\mathbf{r}_N,0) = \prod_{i=1}^{N} [\rho_N(\mathbf{r}_i) V^{-1}]$.

Since eq 1 is separable,

$$P_N(\mathbf{Q};\mathbf{r}_1,...,\mathbf{r}_N,t) = \prod_{i=1}^{N} p_N(Q_i;\mathbf{r}_i,t) \tag{2}$$

where

$$\frac{\partial p_N}{\partial t} = [L_{\mathbf{r}} - \kappa(Q(t),\mathbf{r})] p_N \tag{3}$$

with the initial conditions $p_N(Q;\mathbf{r},0) = \rho_N(\mathbf{r}) V^{-1}$. For a closed volume V, eq 3 has a reflecting boundary condition at V's surface. The survival probability that the protein has not reacted by time t is

$$S_N(\mathbf{Q};t) := \int_{V^N} P_N(\mathbf{Q};\mathbf{r}_1,...,\mathbf{r}_N,t)\, d\mathbf{r}_1 ... d\mathbf{r}_N = \prod_{i=1}^{N} [\int_V p_N(Q_i;\mathbf{r}_i,t)\, d\mathbf{r}_i] \tag{4}$$

where eq 2 gives the second equality.

To obtain results in the simplifying limit $V \rightarrow \infty$, Smoluchowski theory assumes the following limiting behavior (":=" denotes a definition): $c := \lim_{N\rightarrow\infty} NV^{-1}$, $\rho(\mathbf{r}) := \lim_{N\rightarrow\infty} \rho_N(\mathbf{r})$, and $p(Q;\mathbf{r},t) := \lim_{N\rightarrow\infty} p_N(Q;\mathbf{r},t)V$, where

$$\frac{\partial p}{\partial t} = [L_{\mathbf{r}} - \kappa(Q(t),\mathbf{r})] p \tag{5}$$

with initial conditions $p(Q;\mathbf{r},0) = \rho(\mathbf{r})$ and no boundaries (because $V \rightarrow \infty$).

Because the operator $L_{\mathbf{r}}$ is conservative (i.e., $L_{\mathbf{r}} \int_V p_N(Q;\mathbf{r},t)\, d\mathbf{r} = 0$), integrating eq 3 over the volume V and then over the time interval $[0,t]$ gives $\int_V p_N(Q;\mathbf{r},t)\, d\mathbf{r} = 1 - \int_0^t k_N(Q;\tau)\, d\tau$ in eq 4, where $k_N(Q;t) := \int_V \kappa(Q(t),\mathbf{r})\, p_N(Q;\mathbf{r},t)\, d\mathbf{r}$.

If $k(Q;t) := \lim_{N\rightarrow\infty} k_N(Q;t)$, then under mild regularity conditions

$$k(Q;t) = \int_{R^d} \kappa(Q(t),\mathbf{r})\, p(Q;\mathbf{r},t)\, d\mathbf{r} \tag{6}$$

is the limiting reaction rate per unit initial ligand concentration. Define the useful[8] intermediate quantity

$$m(Q;t) := c \int_0^t k(Q;\tau)\, d\tau \tag{7}$$

The limiting survival probability can be derived from the foregoing by algebraic manipulation:

$$S(\mathbf{Q};t) := \lim_{N\rightarrow\infty} S_N(\mathbf{Q};t) = \lim_{N\rightarrow\infty} \prod_{i=1}^{N} [1 - N^{-1} m(Q_i;t)] = \exp[-\lim_{N\rightarrow\infty} N^{-1} \sum_{i=1}^{N} m(Q_i;t)] \tag{8}$$

where the final equality follows from taking logarithms and expanding in powers of N^{-1}.

Equations 5−8 are the operational Smoluchowski approximations. Equation 8 confirms that Smoluchowski gating theory holds for any fixed deterministic gating history $\mathbf{Q}$.

Smoluchowski theory without gating can be recovered by suppressing all the gating dependencies in eqs 5−8. Equation 8 then simplifies because $N^{-1}\sum_{i=1}^{N} m(t) = m(t)$. Similarly, when the protein is gating, $Q_1 = Q_2 = ... = Q_N = Q$, so $N^{-1}\sum_{i=1}^{N} m(Q_i;t) = m(Q;t)$. Thus in eq 8, taking averages $\langle\,\rangle_Q$ over the gating history Q gives the expected protein-gated survival probability,

$$S_{\mathrm{P}}(t) := \langle S(\mathbf{Q};t)\rangle_Q = \langle \exp[-m(Q;t)]\rangle_Q \tag{9}$$

On the other hand, if the ligands gate independently, Kolmogorov's strong law of large numbers shows that $\lim_{N\to\infty} N^{-1}\sum_{i=1}^{N} m(Q_i;t) = \langle m(Q;t)\rangle_Q$ with probability 1.[26] Thus if $\langle\,\rangle_{\mathbf{Q}}$ (with bold subscript $\mathbf{Q}$) denotes the average over the independent ligand-gating histories $\mathbf{Q} = (Q_1, Q_2, ..., Q_N)$, eq 8 yields

$$S_{\mathrm{L}}(t) := \langle S(\mathbf{Q};t)\rangle_{\mathbf{Q}} = \exp[-\langle m(Q;t)\rangle_Q] \tag{10}$$

For ligand gating, the intermediate $\langle\,\rangle_{\mathbf{Q}}$ (bold $\mathbf{Q}$) in eq 10 is superfluous in the limit $N \to \infty$, since the large number of ligands then performs the required averaging with probability 1. For a finite number N of ligands (as below), however, eq 10 must retain the averaging $\langle\,\rangle_{\mathbf{Q}}$ to be correct.

A previous demonstration[25] purported to show that a ligand-gated reaction proceeds faster than the corresponding protein-gated reaction. Using gating histories (instead of gating states) corrects an inaccuracy in that demonstration and simultaneously extends it to non-Markovian gating as follows. Jensen's inequality $\phi(\langle X\rangle) \le \langle\phi(X)\rangle$ with $\phi(x) = \mathrm{e}^{-x}$ gives

$$S_{\mathrm{L}}(t) = \exp[-\langle m(Q;t)\rangle_Q] \le \langle \exp[-m(Q;t)]\rangle_Q = S_{\mathrm{P}}(t) \tag{11}$$

even for non-Markovian gating histories Q.

Equation is also true for N ligands in a finite volume V: use eq 4 instead of eq 8, use the N-subscripted analogs of all quantities, and apply Jensen's inequality with $\phi(x) = (1 - x)^N$ ($N \ge 2$) instead of $\phi(x) = \mathrm{e}^{-x}$.

III. Smoluchowski Gating Theory for $\mathbf{P}_n^* + \mathbf{L}^* \to \mathbf{P}_{n+1}^* + \varnothing$

This section develops a Smoluchowski gating theory for the reaction $\mathrm{P}_n^* + \mathrm{L}^* \to \mathrm{P}_{n+1}^* + \varnothing$ for $n = 0, 1, 2, ...$ Linguistically, this reaction is treated as a model for an enzymatic protein P* processing its ligand substrates L* irreversibly, where the subscript n on P* counts the number of ligands processed by the protein.

In the following, the summation index I_n runs over all subsets of $\{1, 2, ..., N\}$ containing exactly n elements. The limiting probability that the protein has processed n ligands is given by

$$\begin{aligned} S_{(n)}(\mathbf{Q};t) &:= \lim_{N\to\infty} \sum_{I_n} \prod_{i\notin I_n} [1 - N^{-1} m(Q_i;t)] \prod_{j\in I_n} [N^{-1} m(Q_j;t)] \\ &= \lim_{N\to\infty} \sum_{I_n} \prod_{i=1}^{N} [1 - N^{-1} m(Q_i;t)] \prod_{j\in I_n} [N^{-1} m(Q_j;t)] \\ &= \exp[-\lim_{N\to\infty} N^{-1} \sum_{i=1}^{N} m(Q_i;\tau)] \lim_{N\to\infty} N^{-n} \sum_{I_n} \prod_{j\in I_n} m(Q_j;t) \end{aligned} \tag{12}$$

In the second expression in eq 12, the first product $\prod_{i\notin In}$ represents unprocessed ligands; the second product $\prod_{j\in In}$, processed ligands. The second equality follows from $\lim_{N\to\infty} \prod_{i\in I_n} [1 - N^{-1} m(Q_i;t)] = 1$; the third equality, from eq 8.

In eq 12, there are $N(N-1)...(N-n+1)/n!$ terms in any sum $\sum_{I_n}$, since this is the number of ways of choosing n objects out of N. Note that $\lim_{N\to\infty} N^{-n}[N(N-1)...(N-n+1)/n!] = 1/n!$

Define

$$X_n(Q;t) := \exp[-m(Q;t)] \frac{[m(Q;t)]^n}{n!} \tag{13}$$

For protein gating, $Q_1 = Q_2 = ... = Q_N = Q$, so eq 12 shows that $S_{(n)}(\mathbf{Q};t) = X_n(Q;t)$. Thus for any particular protein gating history Q, the number of ligands processed is Poisson distributed. Since taking averages in eq 12 gives

$$S_{\mathrm{P}(n)}(t) := \langle S_{(n)}(\mathbf{Q};t)\rangle_Q = \langle X_n(Q;t)\rangle_Q \tag{14}$$

protein gating just mixes these Poisson distributions.

On the other hand, if the ligands gate independently, there is a single Poisson distribution with parameter $\langle m(Q;t)\rangle_Q$:

$$S_{\mathrm{L}(n)}(t) := \langle S_{(n)}(\mathbf{Q};t)\rangle_{\mathbf{Q}} = \exp[-\langle m(Q;t)\rangle_Q] \frac{[\langle m(Q;t)\rangle_Q]^n}{n!} \tag{15}$$

where eq 15 follows from eq 12 just as eq 10 followed from eq 8.

With the convex function $\phi(x) = \mathrm{e}^{-x}\cosh x = \sum_{n=0}^{\infty} \mathrm{e}^{-x} x^{2n}/(2n)!$ in Jensen's inequality $\phi(\langle X\rangle) \le \langle\phi(X)\rangle$,

$$\sum_{n=0}^{\infty} S_{\mathrm{L}(2n)}(t) = \phi[\langle m(Q;t)\rangle_Q] \le \langle\phi[m(Q;t)]\rangle_Q = \sum_{n=0}^{\infty} S_{\mathrm{P}(2n)}(t) \tag{16}$$

Equation 16 implies that at any fixed time t an even number of ligands are more likely to have processed by a protein-gated reaction than by the corresponding ligand-gated reaction. Equation 16 therefore provides a quaint counterpart to eq 11. Like eq 11, it can also be extended to N ligands in a finite volume V: use N-subscripted analogs of all quantities, and apply Jensen's inequality with $\phi(x) = {}^1/_2[(1+x)^{2N} + (1-x)^{2N}]$ ($N \ge 2$) instead of $\phi(x) = \mathrm{e}^{-x}\cosh x$.

IV. Markovian Gating Theory for $\mathbf{P}_n^* + \mathbf{L}^* \to \mathbf{P}_{n+1}^* + \varnothing$

In this section, the gating history Q is assumed to be a continuous time Markov process. Thus the probability that $Q(t + \Delta t) = q$ given $Q(t) = q_0$ is

$$P(Q(t+\Delta t) = q | Q(t) = q_0) = \delta_{q_0 q} + a_{q_0 q}(t)\Delta t + o(\Delta t) \tag{17}$$

as $\Delta t \to 0$, where $\delta_{q_0 q}$ is Kronecker's delta, i.e., $\delta_{q_0 q} = 1$ if $q_0 = q$, and $\delta_{q_0 q} = 0$ otherwise. The gating operator L_{g} appearing in a previous notation[11] has the matrix entries $a_{q_0 q}(t)$ in the present notation.

This section shows that for Markovian gating the present gating theory reduces to previous theories. In particular, eq 21 following provides some insight into the Zhou−Szabo approximation[11] for protein-gated reactions. Linguistically, we shall ascribe the gating to the protein, since eqs 6, 7, and 15 reduce ligand gating to the isolated pair problem in eq 5, in which the source of the gating, protein or ligand, is immaterial.

The following uses $\mathbf{1}_A$ to denote an indicator random variable; that is, $\mathbf{1}_A = 1$ if the event A occurs, and $\mathbf{1}_A = 0$ otherwise. Note that

$$\sum_{(q)} \mathbf{1}_{Q(t)=q} = 1 \tag{18}$$

since the protein is always in some gating state q. In addition, the following uses $\dot{F}(t)$ to denote the time derivative of a function $F(t)$.

Some standard conditions on a Markov process[27] are imposed on $a_{q_0q}(t)$ in eq 17: (1) $\sum_{(q)} a_{q_0q}(t) = 0$; (2) $a_{q_0q}(t) \geq 0$, $q \neq q_0$; and (3) $\sum_{(q)} |a_{q_0q}(t)| \leq M < \infty$. Condition (3) is sufficient[28] to avoid the pathology of passing through an infinite number of gating states in a finite time.[29] Note that $a_{q_0q}(t)$ is explicitly permitted to vary with the time t, although the following suppresses its time dependence.

We now derive a known[30] but central result, eq 21. Since the remainder of this article applies eq 21 several times, the derivation uses a generic notation. Let $X(Q;t)$ be any real random variable whose value depends only on the gating states $Q(\tau)$ $(0 \leq \tau \leq t)$ up to and including the time t.[31] Define

$$S^*(q,t) := \langle \mathbf{1}_{Q(t)=q} X(Q;t) \rangle_Q \tag{19}$$

Starred quantities then denote averages of $\mathbf{1}_{Q(t)=q}$ times some real random variable (this paper uses them only when the gating is Markovian). Because of eq 18, the factor $\mathbf{1}_{Q(t)=q}$ serves to break up the average into contributions from the different gating states q.

If Y is any real random variable depending only on the gating states $Q(\tau)$ $(0 \leq \tau \leq t)$ up to and including the time t, then conditioning on $Q(\tau)$ $(0 \leq \tau \leq t)$ and applying the Markov property and eqs 18 and 17 show that $\langle [1_{Q(t+\Delta t)=q} - 1_{Q(t)=q}] Y \rangle_Q = \{\sum_{(q_0)} a_{q_0q} \langle \mathbf{1}_{Q(t)=q_0} Y \rangle_Q + o(1)\} \Delta t$. This argument, applied below to both $X(t)$ and $\dot{X}(t)$, gives

$$\begin{aligned} S^*(q,t+\Delta t) &= \langle \mathbf{1}_{Q(t+\Delta t)=q} X(Q;t+\Delta t) \rangle_Q \\ &= \langle \mathbf{1}_{Q(t+\Delta t)=q} [X(Q;t) + \dot{X}(Q;t)\Delta t] \rangle_Q + o(\Delta t) \\ &= S^*(q,t) + \{\sum_{(q_0)} a_{q_0q} S^*(q_0,t) + \langle \mathbf{1}_{Q(t)=q} \dot{X}(Q;t) \rangle_Q + o(1)\} \Delta t \end{aligned} \tag{20}$$

Taking limits as $\Delta t \to 0$,

$$\frac{\partial S^*(q,t)}{\partial t} = \sum_{(q_0)} a_{q_0q} S^*(q_0,t) + \langle \mathbf{1}_{Q(t)=q} \dot{X}(Q;t) \rangle_Q \tag{21}$$

To show that eqs 17−21 yield results equivalent to other Markovian gating theories, let

$$p^*(q,\mathbf{r},t) := \langle \mathbf{1}_{Q(t)=q} p(Q;\mathbf{r},t) \rangle_Q \tag{22}$$

Substitution into eq 21 gives

$$\begin{aligned} \frac{\partial p^*(q,\mathbf{r},t)}{\partial t} &= \sum_{(q0)} a_{q_0q} p^*(q_0,\mathbf{r},t) + \left\langle \mathbf{1}_{Q(t)=q} \frac{\partial p(Q;\mathbf{r},t)}{\partial t} \right\rangle_Q \\ &= \sum_{(q_0)} a_{q_0q} p^*(q_0,\mathbf{r},t) + [L_{\mathbf{r}} - \kappa(q,\mathbf{r})] p^*(q,\mathbf{r},t) \end{aligned} \tag{23}$$

where the second equality follows from eq 5, because $\kappa(Q(t),\mathbf{r})$ there depends only on the *present* gating state, $Q(t) = q$. Equation 23 is merely eq 2.1 in Zhou and Szabo[11] in a different notation. Equation 6 gives

$$\begin{aligned} k^*(q,t) &= \langle \mathbf{1}_{Q(t)=q} \int_{R^d} \kappa(Q(t),\mathbf{r})\, p(Q;\mathbf{r},t)\, d\mathbf{r} \rangle_Q \\ &= \int_{R^d} \kappa(q,\mathbf{r})\, p^*(q,\mathbf{r},t)\, d\mathbf{r} \end{aligned} \tag{24}$$

which after summing over q gives eq 2.7 in Zhou and Szabo.[11] For the isolated pair problem, this demonstrates the equivalence of the Markovian gating theories.

For ligand gating, the equivalence of the theories follows from eqs 6, 7, and 24, when substituted into eq 10 and compared to eq 2.23 in Zhou and Szabo.[11]

For protein gating, from eq 13 the probability that the protein is in gating state q at time t, n ligands having been processed, is

$$S_n^*(q,t) := \langle \mathbf{1}_{Q(t)=q} X_n(Q;t) \rangle_Q \tag{25}$$

Equation 7 and 13 show that $\dot{X}_n = (X_n - X_n) ck(Q;t)$. If we define $X_{-1} := 0$ identically, eq 21 becomes

$$\frac{\partial S_n^*(q,t)}{\partial t} = \sum_{(q_0)} a_{q_0q} S_n^*(q_0,t) + \langle \mathbf{1}_{Q(t)=q} (X_{n-1} - X_n) ck(Q;t) \rangle_Q \tag{26}$$

for $n = 0, 1, 2, \ldots$.

Equation 26 suggests an extension of the zero covariance approximation $\langle YZ \rangle \approx \langle Y \rangle \langle Z \rangle$: if $\langle Y \rangle_{Q/A}$ denotes the average of Y over gating histories Q, conditional on the event A occurring,

$$\begin{aligned} \langle \mathbf{1}_A YZ \rangle_Q &= P(A) \langle YZ \rangle_{Q/A} \\ &\approx P(A) \langle Y \rangle_{Q/A} \langle Z \rangle_{Q/A} \\ &= \langle \mathbf{1}_A Y \rangle_Q \langle Z \rangle_{Q/A} \end{aligned} \tag{27}$$

The approximation in eq 27, when applied to eq 26, yields

$$\begin{aligned} \frac{\partial S_n^*(q,t)}{\partial t} \approx \sum_{(q_0)} a_{q_0q} S_n^*(q_0,t) + \\ [S_{n-1}^*(q,t) - S_n^*(q,t)] \langle ck(Q;t) \rangle_{Q/\{Q(t)=q\}} \end{aligned} \tag{28}$$

The Zhou−Szabo approximation[11] for the survival probability in the protein-gated reaction P + L → ∅ is precisely eq 28 with $n = 0$.

Zhou and Szabo[11] point out that the approximation in eq 18 is exact in three extremes. Fix α_{q_0q} and let $a_{q_0q}(t) = \lambda \alpha_{q_0q}$ where α_{q_0q} is independent of time t. Equation 28 becomes exact as $\lambda \to 0$ (by direct substitution) or as $\lambda \to \infty$ (by semigroup limit theorems[30,32,33]). Moreover, the approximation is also exact in the reaction-controlled limit, in which the reaction rate depends only on the present gating state: $k(Q;t) = k(Q(t),t)$. The transition from eq 26 to eq 28 displays this last property explicitly and suggests a variance-reduction technique for simulating gated reactions in a computer.

Consider a simulation[11,34] of the protein-gated reaction P* + L* → ∅, with N ligands diffusing around a single fixed protein in a closed volume V. For any particular, fixed realization of the N ligand paths $\mathbf{r}_1, \ldots, \mathbf{r}_N$, the protein's survival probability can be estimated by

$$S^*_{\mathbf{r}_1,\ldots,\mathbf{r}_N}(q,t) = \langle \mathbf{1}_{Q(t)=q} \exp[-\int_0^t \sum_{i=1}^N \kappa(Q(\tau),\mathbf{r}_i(\tau),\mathbf{r}_i(\tau))\, d\tau] \rangle_Q \tag{29}$$

where the average is taken over all the relevant (Markovian) gating histories Q. Simulating the gating histories Q in eq 29 will introduce an error when estimating $S^*_{\mathbf{r}_1,\ldots,\mathbf{r}_N}(q,t)$. The quantity $S^*_{\mathbf{r}_1,\ldots,\mathbf{r}_N}(q,t)$ can be computed exactly, however, since eqs 29 and 21 give

$$\frac{\partial S^*_{\mathbf{r}_1,\ldots,\mathbf{r}_N}(q,t)}{\partial t} = \sum_{(q_0)} a_{q_0 q} S^*_{\mathbf{r}_1,\ldots,\mathbf{r}_N}(q_0,t) - S^*_{\mathbf{r}_1,\ldots,\mathbf{r}_N}(q,t) \sum_{i=1}^{N} \kappa(q,\mathbf{r}_i(t)) \quad (30)$$

Like eqs 23 and 24, eq 30 simplified because $\sum_{i=1}^{N} \kappa(Q(t),\mathbf{r}_i(t))$ depends only on the *present* gating state, $Q(t) = q$. Equation 30 is essentially a dynamic programming technique,[35] in the sense that the extra state variable q keeps exact track of gating's effect on protein survival and eliminates any need to simulate the gating histories Q in eq 29.

This variance-reduction technique is applicable to ligand-gated reactions as well, since eq 30 with $N = 1$ estimates the survival of one gated ligand molecule. Generalization from P + L $\rightarrow \varnothing$ to the reaction P^*_n + L* $\rightarrow P^*_{n+1} + \varnothing$ is straightforward.

V. Discussion

This article has shown that for comparable kinetics the reaction P* + L* $\rightarrow \varnothing$ always proceeds faster with ligand gating than with protein gating, explicitly correcting a previous demonstration[25] and extending it to non-Markovian gating or a finite number of ligands. It also observes that P* + L* $\rightarrow \varnothing$ is mathematically equivalent to the special case $n = 0$ of the reaction P^*_n + L* $\rightarrow P^*_{n+1} + \varnothing$, by examining the whole Poisson distribution $e^{-\lambda}\lambda^n/n!$ instead of just $e^{-\lambda}$, the $n = 0$ term.

The extra generality is inexpensive and potentially useful. The reaction P^*_n + L* $\rightarrow P^*_{n+1} + \varnothing$ could, for example, model viruses L* irreversibly attaching to a cell P*, where the subscript n counts the number of viruses attached to the cell.[23] Alternatively, it could model an enzymatic protein processing ligand substrates irreversibly, where the subscript n on P* counts the number of ligands processed by the enzyme. While its active site is processing a ligand molecule, however, the enzyme is effectively "locked" because other ligands cannot bind. Thus the reaction P^*_n + L* $\rightarrow P^*_{n+1} + \varnothing$ is a realistic enzyme model only if the locking time is negligible. Type I Geiger counters give rise to analogous locking phenomena, however, and locking can be handled mathematically with renewal theory.[36]

In the reaction P^*_n + L* $\rightarrow P^*_{n+1} + \varnothing$, the quantity $m(Q;t)$ defined in eq 7 is the mean number of ligands processed in the time interval $[0,t]$. For the reaction P* + L* $\rightarrow \varnothing$, if a blocker B is causing the gating interconversions, $m(Q;t)$ retains a useful physical interpretation: it is the average number of times the reaction P* + L* $\rightarrow \varnothing$ must be blocked to enable the protein to survive. This interpretation had practical implications in virology for soluble CD4 and other blockers of viral attachment.[8]

A zero-correlation approximation was used to derive directly the Zhou−Szabo approximation for protein-gated reactions. The Zhou−Szabo approximation is exact when the reaction rates depend only on the present gating state. In three dimensions, but not in one or two, ligands eventually ($t \rightarrow \infty$) arrive at the protein at a constant rate.[17,19] Since simulations verified the Zhou−Szabo approximation only in three dimensions,[11] some caution may be indicated. Lower dimensions may require more accurate, and correspondingly more complicated, approximations than the Zhou−Szabo approximation.

Finally, the zero-correlation approximation indicated a variance-reduction technique for simulating gated reactions in a computer. The technique assumes that gating evolves independently of the spatial positions of the molecules, an unrealistic restriction that confines it to theoretical investigations.[34,37] Lifting this restriction would be necessary to make the technique generally useful for simulating reactions between real molecules.[38]

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