Smoluchowski Gating Theory and its Applications to Viral Blocking

John L. Spouge

National Center for Biotechnology Information
National Library of Medicine
Bethesda MD 20894

Phone: (301) 402-9310
Fax: (301) 435-2433
Email: spouge@nih.gov

For Recent Research Developments in Physical Chemistry

Running Title: Protein Gating and Viral Blocking

Version Date: January 7, 2003
ABSTRACT
This article gives an overview of recent developments in the theories of protein-ligand gating and reversible blocking of chemical reactions, with emphasis on applications to virology in general and to HIV in particular. In gating theory, reversible conformational fluctuations of a protein $P^* \leftrightarrow P$ or its ligand $L^* \leftrightarrow L$ ($P$ and $L$ unreactive) modulate the kinetics of irreversible complex formation $P^* + L^* \rightarrow PL$. Reversible chemical blockers $B$ cause similar interconversions, e.g., $P^* + B \leftrightarrow P$ or $L^* + B \leftrightarrow L$. Thus, the Smoluchowski mean-field theories for these phenomena are similar, and in a single stroke, gating theory can be applied to the reversible blocking, and in particular reversible blocking of viral attachment. Protein gating ($P^* \leftrightarrow P$) and ligand gating ($L^* \leftrightarrow L$) appear symmetric, but a protein usually appears in a much smaller concentration than the corresponding ligand. The difference between the concentrations creates an asymmetry between protein and ligand gating. As a consequence, for comparable gating kinetics ($P^* \leftrightarrow P$ vs. $L^* \leftrightarrow L$), the reaction $P^* + L^* \rightarrow PL$ proceeds more slowly under protein gating than ligand gating. Solutions to ligand gating follow directly from the corresponding gated isolated pair problems (IPPs), so this article gives two formalisms for solving Markovian (memoryless) gated IPPs. Solutions to protein
gating are more difficult but can be approximated, so this article gives a simple derivation of the Zhou-Szabo approximation for solving protein gating. Without assuming any specialized knowledge of virology, this article also applies Smoluchowski gating theory to the reversible blocking of viral attachment. Some of the resulting observations are new and potentially important, indicating that the physical chemistry of viruses is a fertile field for further exploration.

Keywords: Smoluchowski Mean-Field Theory, Protein-Ligand Gating, Reversible Chemical Blocking, Viral Attachment, HIV, sCD4, Soluble Chemokine Receptors

Abbreviations:
CR  Cellular Receptor
HIV  Human Immunodeficiency Virus
IPP  Isolated Pair Problem
MOI  Multiplicity of Infection
MOA  Multiplicity of Attachment
sCD4  Soluble CD4
VAP  Viral Attachment Protein
:= definition  ≡ identity

1. INTRODUCTION

In the 1940’s and 1950’s, the phage physicists such as Delbrück (1) studied bacterial genetics using viruses, and in the process, they enriched virology with a wealth of ideas from physical chemistry. Preeminent among these ideas, the concept of “multiplicity of infection” (to be explained later) is now indispensable to experimental virologists (2). Since the 1960’s, immunology and molecular biology have come to play an increasingly dominant role in virology, while the study of the physical chemistry of viruses has been correspondingly subordinated.

Recent research on the human immunodeficiency virus (HIV) has forcefully reaffirmed the importance of physical chemistry to virology, however. Soluble CD4 (sCD4) is a chemical that reversibly blocks HIV attachment to cells, among other effects (3-7). Despite test-tube successes, clinical trials using sCD4 as a therapeutic agent against HIV infection failed (8,9). Kinetic analyses of HIV attachment and sCD4 blocking, some of
which displayed semi-quantitative agreement with the clinical results, showed that the clinical failure was predictable (10-13). The analyses have become even more pertinent as HIV therapies similar to sCD4 (e.g., soluble chemokine receptors) move forward from test-tube success to clinical trials (14-16).

Kinetic analyses of HIV have an immediate specific importance in view of the worldwide AIDS epidemic. Because they derive from the generalities of physical chemistry and not from the specifics of biology, however, they may be applied to many different viruses. Thus, the importance of the analyses may be expected to endure beyond any immediate need.

With these considerations as motivation, my collaborators and I have developed mean-field theories of viral attachment and its blocking (10,11,17-19). Under certain circumstances, the resulting theories happened to be mathematically equivalent to Smoluchowski theories of protein gating (20-22). Thus, in a single stroke, Smoluchowski theory for protein gating can be applied to viral blocking. Moreover, blocking viral attachment is itself just a special case of blocking a chemical or biological reaction, and so the Smoluchowski theory applies to any reversible blocker, as long as the blocker under study is in chemical excess. Thus, the potential applications of gating theory are much wider than previously expected.

To examine the physical contexts of the various theories more closely, consider gating theory in its original context, a ligand binding to a protein (23,24). Conformational fluctuations often influence binding kinetics. In some cases, protein conformation is important. Consider, e.g., an oxygen molecule entering the heme pocket of myoglobin. Oxygen molecules have no obvious point of entry into the static X-ray structure of myoglobin (25). The myoglobin side chains that appear to block the heme pocket are postulated to “act as a gate”, swinging out of the way and permitting oxygen molecules to enter the heme pocket. In other cases, ligand
conformation is important. Consider, e.g., a peptide binding to a major histocompatibility protein. Here, the major histocompatibility protein only binds the peptide if the peptide takes a specific conformation (26-28).

Symbolically, the conformational fluctuations of a protein $P^* \leftrightarrow P$ ($P$ unreactive) or its ligand $L^* \leftrightarrow L$ ($L$ unreactive) sometimes modulate the kinetics of an irreversible binding reaction $P^* + L^* \rightarrow PL$. In the context of protein-ligand interactions, the interconversion $P^* \leftrightarrow P$ is called protein gating, while the interconversion $L^* \leftrightarrow L$ is called ligand gating. For ease of exposition, this article considers protein and ligand gating as separate, mutually exclusive phenomena, since if necessary, the corresponding mathematical solutions can be superimposed.

Reversible chemical blockers (e.g., sCD4) cause interconversions similar to gating (11,18). Reversible protein blockers $B$ cause the interconversion $P^* + B \leftrightarrow P$ (where $P$ is the blocked protein); reversible ligand blockers, the interconversion $L^* + B \leftrightarrow L$. If the blocker $B$ is in excess and has no other significant effects, blocking interconversions are mathematically equivalent to gating.

Although theories of gating and reversible blocking are sometimes equivalent, they need to be distinguished from theories of dynamic trapping (29-33). In gating theory, reactivities evolve independently of the reactants’ spatial positions. In dynamic trapping, the reactivities depend on the positions. This article does not examine dynamic trapping.

Thus far, protein and ligand gating appear to be symmetrical in gating theory. In the typical physical context, however, a protein has a much smaller concentration than the corresponding ligand. To anticipate other applications of gating theory (e.g., to viral blockers), we probably should call the protein and ligand the “minority” and “majority” species (although we shall not). In any case, the difference in relative concentrations creates an asymmetry between protein gating and ligand gating that has only recently been
appreciated. Perhaps surprisingly, for comparable gating kinetics ($P^* \leftrightarrow P$ vs. $L^* \leftrightarrow L$), the reaction $P^* + L^* \rightarrow PL$ proceeds more slowly under protein gating than ligand gating (20,34,35). Using Jensen’s inequality, Section 2 demonstrates that this “minority vs. majority gating effect” is a general phenomenon, extending even to non-Markovian gating (gating with memory).

Once the qualitative distinction between protein and ligand gating has been demonstrated, we proceed to specific gating solutions. Solutions to ligand gating are directly accessible after solving isolated pair problems (IPPs) that contain a gate. Section 3 therefore describes two methods for solving IPPs with Markovian gates. One method uses Green’s functions and is particularly simple if only one gating state permits reaction (22). The other method uses trapping rates and is useful if only one gating state prevents reaction (21). The two methods are therefore pleasantly complementary. In contrast, solutions to protein gating are not directly accessible after solving gated IPPs, but Zhou and Szabo found a clever approximate solution (34). Their approximation involves solving a set of simultaneous ordinary differential equations whose terms incorporate results from a gated IPP. Section 4 gives a simple zero-correlation derivation of the Zhou-Szabo approximation and avoids hierarchy of reduced distribution functions in the original derivation (20).

Section 5 applies Smoluchowski gating theory to the reversible blocking of viral attachment. It does not assume any specialized knowledge of mathematics or virology, so it sketches the relevant virological basics. In principle, gating theory could have been applied to the reversible blocking of general chemical reactions, but I chose the viral context because I am familiar with the physical constants for HIV and helped analyze some pertinent experimental data.

The overview of gating and blocking in this article is intended to be brief and intuitive. When necessary, the reader is encouraged to refer to the references, which generally contain
complete explanations and rigorous demonstrations. Some of the observations about viruses in Section 5 are new, and some could be called fundamental. Thus, they indicate that the physical chemistry of viruses is a fertile field for further exploration.

We begin by examining gating theory in the context of proteins and their ligands.

2. PROTEIN AND LIGAND GATING

This section develops the Smoluchowski theory for protein-ligand gating. For simplicity, we examine an irreversible protein-ligand reaction whose products are inert, the annihilation reaction $P^* + L^* \rightarrow \phi$. As the Introduction explains, the mathematical theory presented can easily be extended to certain other applications, e.g., to a chemical reaction undergoing reversible blocking, as long as the blocker is in excess. As indicated below, Poisson distributions also extend the theory to reactions that do not render the protein inert. Among these reactions are the enzymatic reaction $P^* + L^* \rightarrow P + \phi$ (e.g., where the protein $P^*$ is an enzyme; $L^*$, a ligand), the trapping reaction $PL^*_n + L^* \rightarrow PL^*_{n+1}$ for $n = 0,1,2,...$ (e.g., where the “protein” $P^*$ might be a cell; the “ligand” $L^*$, a virus (36)), etc.

To develop a Smoluchowski theory for protein-ligand gating, let us first review Smoluchowski theory without gating (37-40). Consider one particular protein molecule. Make the Smoluchowski approximation: the protein molecule under consideration sees no effective change in its local ligand concentration, if a ligand molecule somewhere else reacts with some other protein molecule. The Smoluchowski approximation is essentially an independence assumption, permitting reconstruction of reaction kinetics from the fate of individual protein molecules. The Smoluchowski approximation is reasonable if diffusion is faster than the protein-ligand reaction, so the ligand molecules re-equilibrate their positions between reactions. This assumption is certainly accurate if the protein is dilute and the ligand concentrated.
Let us choose coordinates so the protein molecule is at the origin \((r = 0)\), so the ligands are located relative to the protein molecule. Before reaction, let the equilibrium concentration of the ligands at position \(r\) be \(\rho(r)\), with \(\rho(r)\) normalized so that at infinity, the ligand concentration is \(\rho(\infty) = 1\). Assume that at time \(t\), the rate of reaction for a single ligand molecule starting at \(r_0\) is \(\sigma(t|r_0)\). The quantity \(\sigma(t|r_0)\) is derived from the isolated pair problem (IPP), in which a single ligand molecule reacts with a single protein molecule. Because of the Smoluchowski approximation, the reaction rate can be derived from the IPP as

\[
k(t) = \int_V \sigma(t|r_0) \rho(r_0) dr_0,
\]

where \(V\) is the reaction volume. (This article uses “:=” to denote a definition.) The reaction rate-constant

\[
k_\infty := \lim_{t \to \infty} k(t)
\]

(if it exists) is often of primary interest, since after a transient, it effectively determines the reaction rate for all time. Let \(c\) be the ligand concentration. Section 5 emphasizes the importance of the multiplicity of reaction

\[
m(t) := c \int_0^t k(\tau) d\tau
\]

to theories of blocking (10). Kinetic theory often subordinates \(m(t)\) and substitutes Eq (3) directly into Eq (4) below.

For the trapping reaction \(PL_n^* + L^* \rightarrow PL_{n+1}^*\), the multiplicity of reaction in Eq (3) has two physical interpretations, one obvious and one subtle. First, \(m(t)\) is the mean number of ligands trapped up to time \(t\). Second, \(m(t)\) is the mean number of ligands that must be blocked to prevent the protein from reacting up to time \(t\). For the annihilation reaction \(P^* + L^* \rightarrow \phi\), the first interpretation no longer applies, but the second interpretation is very important to the blocking theory presented in Section 5.

Smoluchowski theory for the annihilation reaction \(P^* + L^* \rightarrow \phi\) shows that the probability that the protein molecule has not reacted and has survived up to time \(t\) is

\[
S(t) = e^{-m(t)}.
\]
Eq (4) has an attractive heuristic based on the Poisson distribution. Eq (3) gives the mean number of potential reactions up to time $t$, with each potential reaction having a small probability $ck(\tau)d\tau$ and the correlation between the potential reactions being negligible. Thus, the Poisson distribution pertains, its parameter is given by the multiplicity of reaction $m(t)$ in Eq (3), and Eq (4) gives the Poisson probability that the actual number of reactions is 0 (41,42). This heuristic for Eq (4) with the annihilation reaction $P^* + L^* \rightarrow \phi$ is easily generalized to the trapping reaction $PL_n^* + L^* \rightarrow PL_{n+1}^*$. There, the probability that the protein has trapped $n$ ligands up to time $t$ is just the Poisson probability

$$S_n(t) = e^{-m(t)} \frac{[m(t)]^n}{n!}. \quad (5)$$

We now superimpose gating on the annihilation reaction $P^* + L^* \rightarrow \phi$. Consider again a system consisting of a single protein molecule surrounded by a large number of ligand molecules. We could develop a theory for continuous gating states, but for simplicity, we assume that the gating states are enumerated by a finite set $\Omega_Q = \{q_1, q_2, q_3, \ldots\}$. The gating history of the protein-ligand system can be described by vector $Q = \{Q_1, Q_2, Q_3, \ldots\}$, with the component $Q_i$ corresponding to the $i^{th}$ ligand molecule surrounding the protein. **Protein gating** is the extreme where the components of $Q$ are equal (protein gating is equivalent to making the ligands gate in unison). **Ligand gating** is the other extreme, where the components of the vector $Q$ are probabilistically independent (each ligand gates independently). The component $Q_i$ gives the gating history for the $i^{th}$ ligand, because it specifies the $i^{th}$ ligand’s reactivity over all times as follows. The equation $Q_i(t) = q_j$ states that the $i^{th}$ ligand is in the $j^{th}$ gating state at time $t$. Thus, each component of $Q$ is a function $Q_i: [0, \infty) \rightarrow \Omega_Q$ from the time interval $[0, \infty)$ into the set of gating states $\Omega_Q$.

Let the complete gating history of the protein-ligand system be $Q$, and given $Q$, let the protein survival probability be $S(Q;t)$. If
on one hand, the protein is gating, all components of $Q$ are slaved to the protein gating history $Q = Q_1 = Q_2 = Q_3 = \ldots$. For the moment, we will consider $Q$ fixed, and thus deterministic. In principle, complete knowledge of the protein gating history $Q$ determines the multiplicity of reaction, just as in Eq (3):

$$m(Q; t) = c \int_0^t k(Q; \tau) d\tau.$$  

(6)

In Eq (6), $k(Q; t)$ is the reaction rate for the IPP corresponding to the gating history $Q$. Note that in the IPP, the gating history $Q$ has an unnatural symmetry: Eq (6) does not distinguish between protein and ligand gating.

Eq (4) then shows that $S(Q; t) = \exp[-m(Q; t)]$. The overall protein survival probability is $S_P(t) = \langle S(Q; t) \rangle_Q$, where $\langle \bullet \rangle_Q$ denotes the average over all protein gating histories $Q$. Thus, for protein gating,

$$S_P(t) = \langle \exp[-m(Q; t)] \rangle_Q.$$  

(7)

If on the other hand, the ligands are gating independently, all components of $Q$ are probabilistically independent copies of a single ligand gating history, again denoted by $Q$. Define $m(Q; t)$ in Eq (6) from the IPP and average it over the ligand gating history $Q$. The result, the multiplicity of reaction $\langle m(Q; t) \rangle_Q$, provides the mean of the Poisson distribution in Eq (4). Thus, the protein survival probability for ligands that are gating independently is

$$S_L(t) = \exp[-\langle m(Q; t) \rangle_Q].$$  

(8)

We can now demonstrate that if the protein gates, its survival is more probable than if the ligands gate independently, given comparable reaction and gating kinetics. The demonstration rests on Jensen’s inequality (43, p. 237): for any convex function $\varphi(X)$ and random variable $X$, $\varphi(\langle X \rangle) \leq \langle \varphi(X) \rangle$. Because $\varphi(X) = e^{-X}$ is convex, Jensen’s inequality gives

$$S_L(t) = \exp[-\langle m(Q; t) \rangle_Q] \leq \langle \exp[-m(Q; t)] \rangle_Q = S_P(t),$$  

(9)

as asserted.
Now, compare the solutions for protein and ligand gating in Eqs (7) and (8). Ligand gating is easier to solve than protein gating, because in ligand gating, the multiplicity of reaction \( m(Q;t) \) is averaged over the gating histories before the nonlinear exponentiation. Thus, solutions for ligand gating can be determined directly from solutions to gated IPPs by direct averages over the gating histories. Section 3 therefore gives two complementary methods for solving gated IPPs. Explicit solutions for protein gating are more problematic and are covered in Section 4.

3. GATED ISOLATED PAIR PROBLEMS

Since the solutions to ligand gating problems can be generated directly from the solutions to gated IPPs, we now examine general techniques for solving gated IPPs. The techniques are shown in overview, since the references give the complete calculations (21,22).

In the IPPs of interest, a single particle moves about in some space until it reacts with and is absorbed by a single stationary trap. The trap corresponds to the protein molecule in Section 2 above; the particle corresponds to the ligand. For applications to gating, we need to consider a single particle moving about in a space with multiple traps.

Our first method for solving gated IPPs is based on Green’s functions. Let \( G(r,t|r_0) \) be any Green’s function describing time-homogeneous Markovian particle movement through a volume \( V \) without any traps. Thus, \( G(r,t|r_0) \) is the probability density that a particle initially at position \( r_0 \) will be at position \( r \) at time \( t \) later. The initial condition for the Green’s function in \( V \) is \( G(r,0|r_0) = \delta(r-r_0) \), where \( \delta(r-r_0) \) is a Dirac \( \delta \) function. Also, because there is no trapping, \( G(r,t|r_0) \) satisfies a normalization condition \( \int_V G(r,t|r_0)dr \equiv 1 \) for all \( t \). (This article uses “\( \equiv \)” to denote equality for all values of a variable. In this case, e.g., the equation holds for all \( t \geq 0 \).)
As a specific example, $G(r,t|r_0)$ could be the Green’s function for particle diffusion in a potential $\phi(r)$. This particular Green’s function satisfies the evolution equation

$$\frac{\partial G}{\partial t} = L G,$$

where $L$ is the second-order linear differential operator $Lf(r) = -\left(\nabla_r \cdot J\right)f(r)$, and $J$ is the flux defined by the vector-valued operator $Jf(r) = -e^{-\beta\phi(r)}D(r)\nabla_r \left[e^{\beta\phi(r)} f(r)\right]$.

The presence of traps introduces sink terms into an evolution equation. If, e.g., every position $r$ has a trap of strength $c(r) \geq 0$ associated with it, the evolution equation becomes

$$\frac{\partial p}{\partial t} = L p - c(r)p.$$  
Here, the propagator $p(r,t|r_0)$ is the probability density that a particle initially at position $r_0$ will be at position $r$, untrapped, at time $t$ later. The propagator $p(r,t|r_0)$ satisfies the same initial condition as the Green’s function $G(r,t|r_0)$,

$p(r,0|r_0) = \delta(r - r_0)$,

but does not satisfy a normalization condition, because in general

$$\int_V p(r,t|r_0) d\mathbf{r} \leq 1.$$  

A gated particle-trap system adds extra structure to a simple particle-trap system. It also has a gating state $q$ that evolves in a gating state space $\Omega_Q = \{q_1,q_2,q_3,\ldots\}$. Again for convenience, $\Omega_Q$ is assumed finite. If the system contains many traps with individual gates, then we just assume that the system’s gating state $q$ summarizes the states of all the individual gates. In the previous paragraph, the strength of the ungated traps depended only on their position $r$. In a gated system, the strength of a trap now also depends on the system’s gating state $q$. Thus, when the gating state becomes $q_j$, the strength of trap at position $r$ becomes $c_j(r), j = 1,2,\ldots,N$. The gated trap strengths $c_j(r) = c(q_j,r)$ are therefore a function of the “gated state” of the system $x = (q,r)$, an ordered pair specifying both the gating state and particle position. The gated state $x$ now evolves in a “gated state space” $\Omega = \Omega_Q \times V$. Note that we use the terms “gating state” and “gated state” to distinguish between $q$ and $x = (q,r)$.

Gating models can be either stochastic or deterministic. Although deterministic gates were the first examined (24), stochastic gates...
are easier to solve, for reasons that will become apparent (23). The simplest of the stochastic gates are Markovian gates. For a Markovian gate, transition rates between gating states depend only on the present gating state, not on the gating history. The simplest of the Markovian gates are Poisson gates. For a simple Poisson gate, e.g., there are two gating states, one active \( q_1 = (+) \) and the other blocked \( q_2 = (−) \). The particle can be trapped in the active state \((+)\), but not in the blocked state \((−)\). The gating interconversions \((+) \leftrightarrow (−)\) are Poisson processes, activation \((−) \rightarrow (+)\) having rate constant \(\alpha\); and blocking \((+) \rightarrow (−)\), rate constant \(\beta\).

The essential insight for solving Markovian gating is that when the gating and particle movement are both Markovian, movement of the state \(x = (q, r)\) in the gated state space \(\Omega = \Omega_Q \times V\) is also Markovian (29,30,32,34,44). Because of gating, a single trap \(a\) in the volume \(V\) corresponds to several traps \((q_i, a)\) in the gated space \(\Omega\). Each \((q_i, a)\) has the same spatial coordinate \(a\) but corresponds to a different gating state \(q_i\). The correspondence reduces the Markovian gating problem to a problem that can be solved by standard Markovian trapping methods (45), at the expense of enlarging the state space and possibly increasing the number of traps.

We now examine a single ungated trap at the point \(a = 0\). So that the trap actually traps, the volume \(V\) must be either a discrete lattice in any dimension or a subset of a line. The lattice application justifies the use of vector notation in the following, but the following common chemical approximation makes the linear application more important. Consider a spherical particle moving in a three-dimensional sphere with a spherical trap at the sphere’s center. After removal of spherical symmetries, the approximation becomes exactly the linear application (37,38,40).

When gating structure is added, a single gated trap at the point \(a = 0\) corresponds to a finite number of point traps, positioned at points \(a_i = (q_i, 0)\) in the gated space. As we noted, the equations of ordinary Markovian
trapping can now be applied to gated Markovian trapping. For example, if \( G(x,t|x_0) \) is the Green’s function describing the particle’s movement through the gated state space \( \Omega = \Omega_G \times V \), if \( a_i \) are the corresponding traps of strength \( \kappa_i \) in \( \Omega \) \( (i = 1,2,\ldots,M) \), and if \( p(x,t|x_0) \) is the corresponding propagator in \( \Omega \), then
\[
G(x,t|x_0) = p(x,t|x_0) + \int_0^t \sum_{i=1}^M \kappa_i p(a_i, \tau|x_0)G(x,t-\tau|a_i)d\tau.
\]
(10)

Eq (10) states that every path in the absence of trapping corresponds to either an untrapped path or a path that terminated at time \( \tau \) at the trap \( a_i \).

In theory, Eq (10) can be solved for arbitrary \( M \) with the Laplace transform
\[
\hat{f}(s) = \int_0^\infty e^{-st}f(t)dt \quad (46, \text{Ch. 29}).
\]
Since the Laplace transform of a convolution is the product of Laplace transforms, i.e.,
\[
\int_0^\infty e^{-st}\left[\int_0^t f(t-\tau)g(\tau)d\tau\right]dt = \hat{f}(s)\hat{g}(s),
\]
the transformation of Eq (10) evaluated at \( x = a_i \) \( (i = 1,2,\ldots,M) \) yields a system of \( M \) simultaneous linear equations in \( \hat{p}(a_i,s|x_0) \).

When \( \hat{p}(a_i,s|x_0) \) is substituted back into the transformed Eq (10), \( \hat{G}(x,s|x_0) \) determines \( \hat{p}(x,s|x_0) \).

In practice, however, only the equation corresponding to a single trap \( (M = 1) \) in the gated space \( \Omega \)
\[
G(x,t|x_0) = p(x,t|x_0) + \int_0^t \kappa p(a, \tau|x_0)G(x,t-\tau|a)d\tau.
\]
(11)
is easily solved. The restriction \( M = 1 \) appears too stringent to solve gating problems, because gating splits a single trap at \( a = 0 \) into several traps at \( a_i = (q_i,0) \) in the gated space. If, however, only one gating state \( q_1 \) permits trapping at \( a \) (e.g., as in simple Poisson gating), then of the traps \( a_i \), only the first \( a_1 = (q_1,0) \) is really a trap. Eq (11) provides a direct solution for this important case.

The equation analogous to Eq (1), but with gating, is
\[
k(t) = \int_\Omega \sigma(t|x_0)\rho(x_0)dx_0.
\]
(12)
Because \( \hat{G}(x, s|x_0) \) determines \( p(x, t|x_0) \), the trapping rate \( \sigma(t|x_0) \) relevant to Eq (1) can be determined from the time derivative of the survival probability of a particle starting at \( x_0 \):

\[
S(t|x_0) := \int_{\Omega} p(X,t|x_0) dX = 1 - \int_0^t \sigma(\tau|x_0) d\tau.
\]

(13)

In the absence of trapping, before reaction starts, the equilibrium density of the particle satisfies

\[
\rho(x) = \int_{\Omega} G(x,t|x_0) \rho(x_0) dx_0 \quad (14)
\]

for any \( t \geq 0 \). Thus, through Eqs (12), (13), and (14), the Green’s function \( \hat{G}(x, s|x_0) \) determines the reaction rate-constant \( k_x := \lim_{t \to \infty} k(t) \) (if it exists). Alternatively, the Tauberian relation (43, p. 442) \( k_x = \lim_{s \to \infty} s \delta_k(s) \) determines \( k_x \) without inverting any Laplace transforms, as long as \( \hat{G}(x, s|x_0) \) is known.

To determine \( \hat{G}(x, s|x_0) \), let \( x = (q, r) \) and \( x_0 = (q_0, r_0) \). For ease of analytic solution, let us assume that the gating transitions are independent of the spatial position. Then

\[
G(x,t|x_0) = G_q(q,t|q_0)G_r(r,t|r_0). \quad (15)
\]

In Eq (15), \( G_q(q,t|q_0) \) and \( G_r(r,t|r_0) \) are the separate Green’s functions for the gating state and the spatial position, respectively. Explicit Laplace transforms for \( G_r(r,t|r_0) \) have been tabulated for many physically important cases (47), so if \( G_q(q,t|q_0) \) is a linear combination of exponential functions \( \sum c_j e^{-\gamma_j t} \), the shift property of Laplace transforms

\[
\hat{f}(s + \alpha) = \int_0^\infty e^{-st} \left[ e^{-\alpha t} f(t) \right] dt
\]

and Eq (15) explicitly yield the required Laplace transform,

\[
\hat{G}(x, s|x_0) = \sum c_j \hat{G}_r(r, s + \gamma_j|r_0).
\]

Fortunately, in the case of Markovian gating, \( G_q(q,t|q_0) \) has exactly the form \( \sum c_j e^{-\gamma_j t} \). Hence, solutions to Markovian gating are more accessible than solutions to deterministic gating. As an example, recall that in simple Poisson gating, the interconversions \(+ \leftrightarrow (-)\) are Poisson processes, activation \(+ \to (-)\) having the rate constant \( \alpha \); blocking \((- \to (+))\), the rate constant \( \beta \). The equilibrium probabilities for the two gating states, active (+) and blocked (−), are \( \alpha := \rho(+) = \alpha (\alpha + \beta)^{-1} \) and
$b:= \rho(-) = \beta(\alpha + \beta)^{-1}$. The Green’s function $G_q^{(pg)}(q,t|q_0)$ for Poisson gating satisfies

$$
\frac{dG_q^{(pg)}(+,t|+)}{dt} = -\beta G_q^{(pg)}(+,t|+) + \alpha G_q^{(pg)}(-,t|+)
$$

$$
= -\beta G_q^{(pg)}(+,t|+) + \alpha \left[1 - G_q^{(pg)}(+,t|+)\right]
$$

Thus,

$$
G_q^{(pg)}(+,t|+) = a + be^{-(\alpha+\beta)t}, \quad (17)
$$

so explicit Laplace transforms for the gated Green’s function $G(x,t|x_0)$ in Eq (15) can be found if the Laplace transform for the spatial Green’s function $G_r(r,t|r_0)$ is known.

Simple Poisson gating has a generalization, serial Poisson gating, that also gives explicit gating solutions through the Green’s function method (22). Let a gate have $J$ independent components, each of which must be active for the gate to be active: $(+)= (+_1,+_2,\ldots,+_J)$. Assume each interconversion $+_j \leftrightarrow -_j$ is a Poisson process, activation $-_j \rightarrow +_j$ having the rate constant $\alpha_j$; and blocking $+_j \rightarrow -_j$, the rate constant $\beta_j$. Applied to each component, Eq (17) yields

$$
G_q^{(pg)}(+,t|+) = \prod_{j=1}^{J} \left[a_j + b_j e^{-(\alpha_j+\beta_j)t}\right], \quad (18)
$$

where $a_j:= \rho(+) = \alpha_j (\alpha_j + \beta_j)^{-1}$ and $b_j:= \rho(-) = \beta_j (\alpha_j + \beta_j)^{-1}$. Again, because the gating Green’s function for serial Poisson gating is a linear combination of exponential functions, explicit Laplace transforms for the gated Green’s function $G(x,t|x_0)$ can be found.

Laplace transforms can also be determined for an analog of serial Poisson gating called parallel Poisson gating (21). In parallel Poisson gating, the gate has $J$ independent components, but unlike serial Poisson gating, the gate is active if any of its components are active. Thus, in parallel Poisson gating, the gate is blocked only if all of its components are blocked:
\((-) = (-1, -2, \ldots, -j)\). As before, assume each interconversion \((+j) \leftrightarrow (-j)\) is a Poisson process, activation \((-j) \rightarrow (+j)\) having the rate constant \(\alpha_j\); and blocking \((+j) \rightarrow (-j)\), the rate constant \(\beta_j\). Thus, e.g., with \(a_j\) and \(b_j\) defined as for serial Poisson gating above, the equilibrium probability the parallel Poisson gate is blocked is \(b := b_1b_2\ldots b_J\).

Because \(2^J-1\) gating states permit trapping in parallel Poisson gating, a single trap in the volume \(V\) produces \(M = 2^J - 1\) traps in the gated space \(\Omega = \Omega_0 \times V\). The Green’s function method given above becomes too complicated to be practical in parallel Poisson gating. We therefore turn to our second technique for solving gated IPPs, which is based more directly on the trapping rates.

In the ungated system, where the trap is always active, let \(\sigma_r(t|\mathbf{r}_0)\) be the trapping rate at time \(t\). In the gated system, let \(\sigma(t|q_0,\mathbf{r}_0)\) be the trapping rate for a particle initially in gating state \(q_0\) at position \(\mathbf{r}_0\). Because in parallel Poisson gating, an active trap is occasionally blocked, our second technique essentially perturbs the active trapping rate \(\sigma_r(t|\mathbf{r}_0)\) to determine \(\sigma(t|q_0,\mathbf{r}_0)\). Let \(\sigma_-(t|q_0,\mathbf{r}_0)\) be the rate of “failed first trapping opportunities” (10). A failed first trapping opportunity occurs when a particle at the trap would have been trapped, except that gating has blocked the trap (thus, “\(\sigma\)” for trapping, and “\(-\)” for failure because of the \((-\) gating state). The various trapping rates are related by the following equation:

\[
\sigma(t|q_0,\mathbf{r}_0) = \sigma_r(t|\mathbf{r}_0) - \sigma_-(t|q_0,\mathbf{r}_0) + \int_0^t \sigma_-(\tau|q_0,\mathbf{r}_0)\sigma(t-\tau|-,\mathbf{a})d\tau.
\]

(19)

In the gated system \((\sigma(t|q_0,\mathbf{r}_0))\), the trapping rate is the same as the ungated trapping rate \((\sigma_r(t|\mathbf{r}_0))\), except for two perturbations. First, failed first trapping opportunities occur \((\sigma_-(t|q_0,\mathbf{r}_0))\); and second, a particle may survive a failed first trapping opportunity but be trapped later (the integral). In the second case, the particle survived a first trapping opportunity at some time \(\tau\) \((\sigma_-(\tau|q_0,\mathbf{r}_0))\), but then it started from the blocked trap in gated state \((-\), \(\mathbf{a}\)) and was trapped at time \(t-\tau\) later \((\sigma(t-\tau|-,\mathbf{a}))\).
The solution of Eq (19) is similar to the solution of Eq (10). Again, for ease of analytic solution, let us assume that the gating transitions are independent of the spatial position. Take Laplace transforms and solve for $\hat{\sigma}(s|q_0, r_0)$ in terms of the other quantities. The rate of failed first trapping opportunities $\sigma_-(t|q_0, r_0)$ is given by

$$\sigma_-(t|q_0, r_0) = G_q(-, t|q_0)\sigma_r(t|r_0),$$

(20)

because a failed first trapping opportunity occurs only if the particle would have been trapped ($\sigma_r(t|r_0)$), but because of gating the trap was blocked ($G_q(-, t|q_0)$).

Eqs (19) and (20) determine the Laplace transform of $\sigma(t|q_0, r_0)$, but solution becomes particularly simple if the initial state $q_0$ is an equilibrium mix of active and blocked states. For the case of equilibrium $q_0$, the probability of the trap being blocked in Eq (19) is $G_q(-, t|q_0) = b$, because there is no information about the gating state until the particle is vulnerable to trapping.

The equilibrium solution $G_q(-, t|q_0) = b$ is particularly useful, because Eq (12) shows that the reaction rate $k(t)$ depends $\rho(x_0) = \rho(q_0, r_0) = \rho(r_0)\rho(q_0)$, the equilibrium distribution of both gating and position. The solution of Eq (19) also requires the value of $\sigma(t|q_0, r_0)$, where $(q_0, r_0) = (\cdot, \cdot)$, i.e., it requires $\sigma_-(t|\cdot, \cdot) = G_q(-, t|\cdot)\sigma_r(t|\cdot)$. Since the spatial trapping rate $\sigma_r(t|\cdot)$ is assumed known, only $G_q(-, t|\cdot)$ is required. By analogy with Eq (18) for serial Poisson gating, the gating Green’s function for parallel Poisson gating is

$$G_q^{(PPR)}(-, t|\cdot) = \prod_{j=1}^J \left[ b_j + a_j e^{-\gamma_j t} \right]$$

(21)

(just interchange quantities associated with the active and blocked states in Eq (18)). Again, Eq (21) has the form $\sum c_j e^{-\gamma_j t}$ required to give explicit Laplace transforms for the trapping rates pertinent to parallel Poisson gating.

Just as Eq (10) generalizes Eq (11) from a single trap $\cdot$ to $M$ traps $\cdot, a_2, \ldots, a_M$, Eq (19) can be generalized by introducing trapping rates $\sigma(a_i, t|q_0, r_0)$ and failed first trapping rates $\sigma_-(a_i, t|q_0, r_0)$ specific to a particular trap $a_i$. As with Eq (10), the generalization can be
solved in theory by setting up simultaneous linear equations for the Laplace transformed trapping rates, but again in practice, only the equation corresponding to a single trap is easily solved.

We note an important result for Section 5. Consider an unmoving sphere of radius $R$ surrounded in an unbounded three-dimensional volume by some point particles whose diffusion constant is $D$. The sphere is perfectly absorbing for the particles except for a simple Poisson gate $(+ \leftrightarrow (-))$. Without the gate ($\beta = 0$), the reaction rate-constant is $k_x = 4\pi DR$, but with the simple Poisson gate it becomes (23,45)

$$k_{\infty}^{(pg)} = k_x \left[ 1 + \frac{\beta}{\alpha} \left( 1 + \sqrt{\frac{(\alpha + \beta) R^2}{D}} \right)^{-1} \right]^{-1}$$  \hspace{1cm} (22)

If the sphere has radius $R_1$ but now moves with diffusion constant $D_1$, whereas the particles now have radius $R_2$ and move with diffusion constant $D_2$, a standard equivalency shows that Eq (22) still holds with $R$ replaced by $R_1 + R_2$ and $D$ by $D_1 + D_2$ (11,40).

In Eq (22), if $R^2 D^{-1} \gg (\alpha + \beta)^{-1}$, the gate has almost no effect on the rate of reaction ($k_{\infty}^{(pg)} = k_x$). On the other hand, if $R^2 D^{-1} \ll (\alpha + \beta)^{-1}$, the gate reduces the reaction rate by a factor $a := \alpha(\alpha + \beta)^{-1} (k_{\infty}^{(pg)} = k_x a)$. At equilibrium, $a$ is the fraction of the time the gate is active, corresponding to the smallest possible gated reaction rate.

Some qualitative reasoning clarifies these results. The quantities $R^2 D^{-1}$ and $(\alpha + \beta)^{-1}$ can be interpreted as characteristic times. The capture time $R^2 D^{-1}$ is the time during which a diffusing particle remains near the surface of the sphere. The switch time $(\alpha + \beta)^{-1}$ is the approximate time required (within a factor of 2) for the gate to switch from activated to blocked or back again, whichever is faster. (The proof follows. The switch from activated to blocked takes time $\beta^{-1}$; the switch back takes $\alpha^{-1}$; and $\gamma^{-1} = \min\{\alpha^{-1}, \beta^{-1}\} \Rightarrow \frac{1}{2} \gamma^{-1} \leq (\alpha + \beta)^{-1} \leq \gamma^{-1}$.)

Thus, if a diffusing particle approaches and leaves the surface of the sphere before any activation or blocking occurs ($R^2 D^{-1} \ll (\alpha + \beta)^{-1}$), Eq (22) shows that the
gate has its maximum effect \((k_{(pg)}^\infty = k_x a)\). On the other hand, if several switches occur \((R^2D^{-1} >> (\alpha + \beta)^{-1})\), then the gate has almost no effect on the reaction rate \((k_{(pg)}^\infty = k_x)\), regardless of the equilibrium fraction \(a\) (23).

In summary, the forward and backward rates \(\alpha\) and \(\beta\), and not just the equilibrium fraction \(a\), determine the gate’s influence on reaction rates. If the switch time is much shorter than the capture time, blocking has almost no effect.

4. THE ZHOU-SZABO APPROXIMATION

This section examines protein gating in the important case of Markovian gating, where the rate of transition to other gating states is determined solely by the present gating state. Zhou and Szabo developed an ingenious approximation for solving protein gating (20,34). Although their approximation depends on neglecting correlations, it is exact in three extreme cases (given below). The three exact extreme cases are broad enough, apparently, to enforce a good approximation across the entire range of interest.

Before giving a relatively simple derivation of the Zhou-Szabo approximation, let us define some intermediate quantities. The random variable \(1_{Q(t) = q}\) equals 1 if the protein gating state \(Q(t)\) is \(q\) and 0 otherwise. The equation \(1 = \sum_{(q)} 1_{Q(t) = q}\), where the sum is over all gating states \(q\), therefore expresses the idea that the protein must be in some definite gating state at time \(t\). Define

\[
S_p(q; t) := \langle 1_{Q(t) = q} S(Q; t) \rangle_Q. \tag{23}
\]

Eq (23) is the average probability over all gating histories that the protein survives and is in gating state \(q\) at time \(t\). The overall survival probability is just

\[
S_p(t) = \sum_{(q)} S_p(q; t). \tag{24}
\]

Let \(a_{q_0 q} = a_{q_0 q}(t)\) be the (memoryless Markovian) rate for the transition from gating state \(q_0\) to gating state \(q\). The rate \(a_{q_0 q}\) is permitted to depend on the time \(t\), but for brevity, our notation suppresses the
dependence. The following expression for the change in $S_p(q; t)$ is exact:

$$\frac{dS_p(q; t)}{dt} = \sum_{(q_0)} a_{q_0q} S_p(q_0; t) - \left(1_{Q(t)=q} S(Q; t)c k(Q; t)\right)_Q.$$

(25)

The first term represents the conversion of the gating state $q_0$ to the gating state $q$, while the second term represents the reaction of the protein in gating state $q$ with a ligand, averaged over all gating histories.

The Zhou-Szabo approximation is essentially a zero-correlation approximation, conditional on the present protein gating state $q$. If $Y$ and $Z$ are any random variables, and $\langle \bullet \rangle_{Q(t)=q}$ represents an average over all gating histories $Q$, conditional on $Q(t) = q$, then

$$\langle 1_{Q(t)=q} YZ \rangle_Q = P(Q(t) = q) \langle YZ \rangle_Q |_{Q(t)=q}$$

$$\approx P(Q(t) = q) \langle Y \rangle_Q |_{Q(t)=q} \langle Z \rangle_Q |_{Q(t)=q}$$

$$= \langle 1_{Q(t)=q} Y \rangle_Q \langle Z \rangle_Q |_{Q(t)=q},$$

(26)

where “$\approx$” is the zero-correlation approximation. Substitute $Y = S(Q; t)$ and $Z = k(Q; t)$ into Eq (26) and apply the resulting approximation to Eq (25) to yield the Zhou-Szabo approximation

$$\frac{dS_p(q; t)}{dt} = \sum_{(q_0)} a_{q_0q} S_p(q_0; t) - S_p(q; t)c \langle k(Q; t) \rangle_Q |_{Q(t)=q}$$

(27)

Eq (27) can also be generalized to the trapping reaction $PL_n^* + L^* \rightarrow PL_{n+1}^*$ (20).

In the form above, Eq (27) is a system of simultaneous differential equations, one for each gating state. The parameters $a_{q_0q}$ and $c$ characterize the gating system under consideration, while the averages $\langle k(Q; t) \rangle_Q |_{Q(t)=q}$ can be determined from simulations of the gated IPP.

Zhou and Szabo found Eq (27) to be a close approximation to their results from Monte Carlo simulations. They also noted that Eq (27) is exact in three important extremes: (1) if
gating interconversions are much faster than the reaction rate \( (c \to 0) \); (2) if gating interconversions are much slower than reaction rate \( (c \to \infty) \); and (3) if the reaction rate depends only on the present gating state \( (k(Q; t) = k(Q(t); t)) \).

5. THE REVERSIBLE BLOCKING OF VIRAL ATTACHMENT

This section applies the theory of protein gating to reversible blocking reactions, specifically reversible reactions that block viral attachment. Although gating theory has not yet found its way from physical chemistry to the biological mainstream, it has many direct implications for virology.

First, we need to state a few basic facts about viruses. Successful infection requires a virus to attach, penetrate, uncoat, and replicate within a target cell (48). Attachment is mediated by viral attachment proteins (VAPs), present in multiple copies and unique to each type of virus. Viral attachment usually requires the attachment proteins to bind to a specific cell receptor or receptors (CRs), also present in multiple copies on the cells that are viral targets (49).

Attachment occurs in two stages. First, contact is made between a single VAP and a single CR. Second, additional VAPs and CRs are recruited into a contact area, rendering viral detachment from the target cell impossible. For the purposes of this article, viral attachment is therefore considered irreversible. Thus, attachment has occurred only when the virus can no longer detach from the target cell.

Phenomenologically, viral attachment to cells can be thought of as a chemical reaction (11,19). Denote a virus by \( V^* \) and a target cell by \( C^* \). Since many viral particles may attach to a single cell, the relevant chemical reaction is trapping: \( CV_n^* + V^* \to CV_{n+1}^* \) (36). Once attached, however, a virus does not always cause infection in the target cell. For simplicity, mathematical models usually make the standard (if often tacit) assumption that once attached, only a fixed fraction of viruses go on to infect (50,51). The value of \( f \)
depends on the particular virus and the particular cell under study.

We now consider Eq (3) and its application to a cell surrounded by viruses. Assume the Smoluchowski approximation applies to the cell under consideration. In Eq (3), \( k(t) \) is the rate of viral attachment to cells; and \( c \) is the concentration of viruses. The paragraphs following Eq (3) assign two possible physical interpretations. First, it is the mean number of viruses attached to a cell. Second, it is the mean number of attachment events that must be blocked to ensure that no virus attaches to the cell. To extend the first interpretation, let \( f \) be the fraction of attached viruses that go on to infect. The mean number of viruses infecting a cell is \( f \times m_C(t) \), a quantity familiar to virologists as the cellular multiplicity of infection (MOI) (2).

MOI is a venerable quantity with a modern counterpart. Consider Eq (3) again, this time not from the cell’s perspective, but symmetrically, from the perspective of a single viral particle. Assume the Smoluchowski approximation applies to the viral particle under consideration. As before, \( k(t) \) is the rate of viral attachment to cells; \( c \) now represents the concentration of cells. The paragraphs following Eq (3) again assign \( m(t) = m_V(t) \) two possible physical interpretations. Only the second interpretation pertains to viruses, however, because each viral particle attaches to only one cell. Thus, the first interpretation, “the mean number of cells attached to a viral particle”, makes no sense. On the other hand, the second interpretation describes \( m_V(t) \) as the mean number of attachment events that must be blocked to ensure that the viral particle does not attach to a cell. In analogy to cellular MOI, this quantity \( m_V(t) \) for viruses is called the viral multiplicity of attachment (MOA) (10).

Using the simplest approximation \( k(t) \approx k_\infty \) in Eq (3), the viral MOA becomes \( m_V(t) \approx k_\infty c t \). The rate-constant approximation is consistent with many experimental data (52,53), including those for laboratory strains of HIV infecting CEM-SS cells (10,18).
(although it may not be valid in all viral systems (54-56)). With the rate-constant approximation, the concept of MOA has some simple, practical consequences for HIV therapies that reversibly block viral attachment.

On one hand, laboratory studies of HIV blocking are often done with a virus-cell incubation period of 1 hour, at T cell concentrations of about $10^6$ ml$^{-1}$. Measured rates for HIV attaching to CEM-SS T cells were about $10^{-6}$ ml h$^{-1}$ (10,18). Thus, in the laboratory, $m_f(t) \approx 10^{-6} \text{ml h}^{-1} \times 10^6 \text{ml}^{-1} \times 1 \text{h} = 1$. On the other hand, lymph nodes are the primary repositories of HIV infection in the human body (57,58). The lymph nodes contain T cell concentrations of about $10^8$ ml$^{-1}$. In laboratory studies, HIV has about 10 hours to attach to cells before losing infectivity (59), which we take as an approximate limit to the longevity of HIV particles in a lymph node. Thus, in a lymph node, $m_f(t) \approx 10^{-6} \text{ml h}^{-1} \times 10^8 \text{ml}^{-1} \times 10 \text{h} = 10^3$.

The reversible blocker sCD4 showed some test-tube success against HIV infection (3-7). When it was tried as a therapy for HIV infection in clinical trials, it failed (8,9). In retrospect, the failure is unsurprising: to block infection in the test-tube, sCD4 had to block 1 viral attachment event ($m_f(t) \approx 1$), whereas in a lymph node, it had to block 1000 viral attachment events ($m_f(t) \approx 10^3$) (10). Thus, these simple back-of-the-envelope calculations indicate the enormous difficulties facing any HIV attachment blocker, including the ones currently under investigation as potential therapeutic agents (e.g., soluble chemokine receptors (14-16)).

Blocker efficacy under lymph node conditions can be estimated from test-tube data. Eq (4) shows that at time $t$, the fraction of viruses not attached to cells is $e^{-m_f(t)}$. Because experimentally measured infection $i(t)$ is proportional to the fraction of attached viruses, $i(t)i_{\infty}^{-1} = 1 - e^{-m_f(t)}$. Under the rate-constant approximation, if $k_{\infty}$ is the viral attachment rate and $c$ is the cell concentration, the viral MOA is $m_f(t) \approx k_{\infty}ct$. Thus, $i(t) = i_{\infty}(1 - e^{-k_{\infty}ct})$. My collaborators measured
infection for different concentrations of cells and sCD4 (18). For each fixed concentration of sCD4, a two-parameter fit to $i_\infty$ and $k_\infty$ permitted infection to be extrapolated to lymph node conditions ($c=10^8\text{m}l^{-1}; t=10\text{ h}$) (10). The extrapolation was in semi-quantitative agreement with the clinical results (8,9).

Current virological practice usually measures infection over a range of blocker concentrations, rather than over a range of cell concentrations. Because HIV is harbored in the lymph nodes, however, and because lymph node cell concentrations are difficult to duplicate in the laboratory, the extrapolation over cell concentrations is much more relevant.

The above theory is robust, because it is based on the time-tested Eq (3), and because it ignores almost all the detailed structure of viruses and cells. Its validity is therefore independent of whether viral attachment is diffusion-limited, reaction-limited, etc. A desire for more detailed theories now leads us to consider surface structures like VAPs and CRs. Very few theories include these details (60-62).

Recall that VAPs are present in multiple copies on the viral surface; likewise, CRs are present in multiple copies on the cellular surface (11,19,63). The multiplicity of VAPs and CRs is conceptually equivalent to endowing target cells and viruses with multivalent chemical functionalities. Like the analogous multivalency in chemical polymers, the cellular and viral multivalencies have generated many models (64). Under certain conditions and for certain purposes, however, the multivalencies may sometimes be ignored. For example, if viral attachment to cells were diffusion-limited, the multivalency would be irrelevant.

Very few experiments directly address the issue of whether viral attachment is diffusion- or reaction-limited. On one hand, experiments on laboratory strains of HIV (18) indicate that the rate at which an HIV particle attaches to T cells is proportional to the number of VAPs on the viral surface (11,19). Restated,
each VAP contributes equally to the attachment rate. The proportionality is therefore similar to Flory’s time-tested “principle of equireactivity” in polymer chemistry (65-69), a fact that indirectly supports that the idea that HIV attachment is reaction-limited by VAPs. On the other hand, however, experiments on strains of HIV isolated from patients indicated that the number of VAPs is relatively unimportant. They showed that the rate of viral attachment depends on the number of CRs on the target cell surface (70-73). Biological variation possibly explains the experimental discordance, however, since present data are too few to establish broad patterns.

In the absence of sufficient data, different theoretical assumptions may be relevant to different experimental systems. The theoretical possibilities include attachment mechanisms that could be reaction-limited by VAPs, by CRs, or by both, or ones that could be diffusion-limited. Let us begin by assuming, e.g., that attachment is reaction-limited by the VAPs. Consider a blocker $B$ that binds reversibly to a viral attachment protein $A^*$:

$$A^* + B \leftrightarrow AB.$$  \hspace{1cm} (28)

The unblocked VAP $A^*$ is active, whereas a blocked VAP $AB$ is assumed inert and thus unable to react with a CR. If the rate of attachment is reaction-limited by VAPs, then the rate of attachment is proportional to the number of active VAPs on a virus. Symmetric considerations hold if attachment is reaction-limited by the CRs.

Now, let us apply the gating theory in Section 2 to viruses. In the early stages of infection, the virus is the minority species, and the target cells are the majority species. In the language of Section 2, the virus is the “protein”, and the target cells are the “ligands”. Assume that attachment is reaction-limited by both VAPs and CRs. Consider two hypothetical blockers of therapeutic interest, one reversibly blocking VAPs and one reversibly blocking CRs, and assume each blocker have comparable reaction kinetics with its chemical target, the VAPs or CRs.
respectively. Eq (9) indicates that the viral blocker will slow the reaction more than the cell blocker, because the virus (the protein) is the minority species compared to the cells (the ligands).

With few exceptions in virology, basic research and therapeutic interventions are directed against a VAP rather than a CR, because interfering with cells deranges normal physiology more than interfering with viruses. Because of Eq (9), gating theory also supports the standard strategy of interfering with VAPs. Given the assumptions, Eq (9) shows that if all else is equal, blocking the minority VAPs prevents viral attachment more effectively than blocking the majority CRs.

Eq (22) also has some implications for reversible blocking of viral attachment, which we now examine. Let the viral particles have radius and diffusion constant $R_v$ and $D_v$; the cells, radius and diffusion constant $R_C$ and $D_C$ (11). As in Eq (22), $(R_C + R_v)^2(D_C + D_v)^{-1}$ is the characteristic capture time, the time interval during which a viral particle remains near the surface of a cell before diffusing away forever.

Eq (22) will be applied to spheres at room or body temperature ($T=294^\circ$K or $T=310^\circ$K, i.e., $T\approx310^\circ$K) in aqueous solution with a viscosity $\eta\approx2\times10^{-2}$ poise. Under these conditions, the diffusion constant of a sphere is inversely proportional to its radius, with $DR = kT(6\pi\eta)^{-1} \approx 10^{-13}$ s cm$^{-3}$ ($k$ is Boltzmann’s constant) (11,40). Again under the given conditions, the capture time is therefore $(R_C + R_v)R_C R_v 10^{13}$ s cm$^{-3}$.

Assume now that attachment is reaction-limited by viral attachment proteins, and consider a hypothetical reversible blocker of VAPs. Let active VAPs become blocked at rate $\beta$ and blocked VAPs become active at rate $\alpha$. (The blocking rate $\beta$ is the product of the blocker’s intrinsic on-rate and its concentration.)

The characteristic switch time, the time interval required for at least one VAP to change status from activated to blocked or back again, is about $N^{-1}(\alpha + \beta)^{-1}$, where $N$ is the number
of VAPs on a viral surface. (If CRs were being blocked, \( N \) would be the number of CRs on a cell surface.) Consider now the characteristic ratio of capture time to switch time:

\[
r' = \frac{R^2 D^{-1}}{N^{-1} (\alpha + \beta)^{-1}} = \frac{(R_C + R_V) R_C R_V 10^{13} \text{s cm}^{-3}}{N^{-1} (\alpha + \beta)^{-1}},
\]

where the second equality holds in aqueous solutions at room or body temperature.

Let \( a := \alpha (\alpha + \beta)^{-1} \) be the fraction of VAPs remaining unblocked and active at equilibrium; let \( k_\infty \) be the viral attachment rate without blocker; and let \( k_\infty^{(pg)} \) be the attachment rate with blocker. If on one hand the characteristic ratio is large, many VAPs change their blocking status in the time a cell requires to capture a viral particle. Eq (22) shows that blocking is then likely to have little effect on the attachment rate \( (k_\infty^{(pg)} = k_\infty) \). If on the other hand the characteristic ratio is small, VAPs rarely change their blocking status in the time a cell requires to capture a viral particle. Eq (22) then shows that blocking is likely to reduce the attachment rate according to the blocker equilibrium \( k_\infty^{(pg)} = k_\infty a \).

As a concrete example of this calculation, consider the reaction system consisting of an HIV particle, a target T cell, and the VAP blocker sCD4. The viral particle has radius \( R_V = 5 \times 10^{-6} \text{ cm} \); the cell, radius \( R_C = 4 \times 10^{-4} \text{ cm} \) (11). Experiment indicates that for HIV VAPs and sCD4, the activation (off-rate) is \( \alpha = 33 \times 10^{-4} \text{s}^{-1} \) (12). Moreover, measurements of blocking efficacy are usually taken at blocker concentrations ranging near the blocker-VAP dissociation constant \( K_D \). At \( K_D \) (the concentration that exactly balances the disparity between the blocker’s intrinsic on- and off-rate), the on-rate (blocking) equals the off-rate (activation): thus, \( \beta \approx \alpha = 3.3 \times 10^{-4} \text{s}^{-1} \).

An HIV particle has about \( N_V \approx 80 \) VAPs. Thus, for the sCD4/HIV/T cell system, \( 8 \text{ s} \approx R^2 D^{-1} \ll N_V^{-1} (\alpha + \beta)^{-1} \approx 19 \text{ s} \). With a characteristic ratio \( r \approx 8/19 \approx 0.4 \), the blocking of viral attachment should faithfully reflect the equilibrium fraction \( a := \alpha (\alpha + \beta)^{-1} \) over the range of sCD4 concentrations giving \( a \approx \frac{1}{2} \). In
fact, the equilibrium constant of a VAP blocker can be determined from viral infection measurements \((11,18,19)\). The necessity of a small characteristic ratio as a precondition for these measurements has not been stated before.

In typical biological systems, the characteristic ratio in Eq (29) for the reversible blocking of VAPs is smaller than 1, as shown above. The characteristic ratio for the reversible blocking of CRs is usually much larger, however. For example, each T cell has about \(N_C \approx 2 \times 10^4\) CRs \((11)\). In the HIV/T cell system, if a CR blocker had the same values \(\beta \approx \alpha = 3.3 \times 10^{-4} \text{ s}^{-1}\) as sCD4, its characteristic ratio \(r\) would be about \(r \approx 1 \times 10^2\) (because \(N_C\) replaces \(N_V\) in Eq (29)). With this large a characteristic ratio, reversible blocking may not reflect the equilibrium fraction \(a = \alpha(\alpha + \beta)^{-1} \approx \frac{1}{2}\); blocking may leave more (and possibly much more) activity than \(a \approx \frac{1}{2}\).

Thus, given the assumptions, gating theory again supports the standard strategy of interfering with VAPs rather than CRs, this time because there are fewer VAPs per HIV viral particle than CRs per T cell \((N_V \approx 80\) vs. \(N_C \approx 2 \times 10^4)\).

Current biological practice generally uses chemical equilibrium binding-constants as a measure of a reversible blocker’s efficacy. The accuracy of this measure depends on the characteristic ratio in Eq (29) being small, however. When a molecular reaction is blocked, the characteristic ratio is usually small, because there is little or no multifunctionality \((N \approx 1)\) and because the reactants have very small radii relative to viruses and cells. In virology, where the characteristic ratio \(r\) has a (large) extra factor of \(N\) and where the radii are relatively larger, the correspondence between binding-constants and blocking efficacy may be less certain. There, the estimation of the characteristic ratio should be a prerequisite to presuming that binding-constants correlate with the efficacy of a reversible blocker.

As a final footnote to the uncertainties as to whether viral attachment is diffusion-limited or reaction-limited, the virological literature
prior to 1960 appears to hold that viral attachment is diffusion-limited (54-56,74-76). I could not locate all the experimental evidence for this belief, although it may be solely based on a simple comparison of experimental attachment rates to Smoluchowski theory (37,38,40). Such a comparison may be misleading. As mentioned above, e.g., HIV attachment rates are about $10^{-6}\text{ml h}^{-1} = 10^{-6}\text{cm}^3\text{h}^{-1}$ (10). Using the parameters given above for HIV and T cells, Smoluchowski theory predicts an upper rate limit to diffusive attachment of about $k_\infty \approx 4\pi D_V R_C = 4\pi \times 4 \times 10^{-4}\text{cm} \times 2 \times 10^{-8}\text{ cm}^2\text{s}^{-1} \approx 3.6 \times 10^{-7}\text{ cm}^3\text{h}^{-1}$. Thus on the one hand, the comparison of experimental data and theoretical rates supports a diffusion-limited mechanism. On the other hand, the same experiment that gave the HIV attachment rate (18) also agreed with Flory’s Principle of Equireactivity, suggesting that attachment was in fact reaction-limited by VAPs (65-68). Even prior to 1960, however, electrostatic interactions were known to be important in

viral attachment (48,74,75), and they can make attachment faster than diffusion. Thus, electrostatic interactions may explain how super-diffusive HIV attachment rates can still be consistent with reaction-limited mechanisms. Further data on this subject would be useful and interesting.
6. SUMMARY

In gating, reversible conformational fluctuations of a protein $P^* \leftrightarrow P$ ($P$ unreactive) or its ligand $L^* \leftrightarrow L$ ($L$ unreactive) may modulate an irreversible binding reaction $P^* + L^* \rightarrow PL$. A reversible chemical blocker $B$ can cause similar interconversions, e.g., $P^* + B \leftrightarrow P$ or $L^* + B \leftrightarrow L$. In the reversible blocking of viral attachment, a blocker $B$ prevents viral attachment $C^* + V^* \rightarrow CV$ by reacting with proteins on the viral or cellular surface. Smoluchowski mean-field theories for all these phenomena are remarkably similar.

Protein gating ($P^* \leftrightarrow P$) and ligand gating ($L^* \leftrightarrow L$) appear symmetric, but a protein usually has a much smaller concentration than the corresponding ligand. The differing concentrations create an asymmetry between protein and ligand gating. For comparable gating kinetics ($P^* \leftrightarrow P$ vs. $L^* \leftrightarrow L$), the reaction $P^* + L^* \rightarrow PL$ proceeds more slowly under protein gating than ligand gating. Moreover, solutions for ligand gating are directly accessible after solving gated isolated pair problems (IPPs). Several formalisms for solving IPPs are now available. Although solutions for protein gating are not so readily accessible, approximate solutions for protein gating are still available through the Zhou-Szabo approximation.

Smoluchowski theory and gating theory can be applied directly to the reversible blocking of chemical reactions, in particular the reversible blocking of viral attachment. For example, the cellular multiplicity of infection (MOI) is the average number of viral particles infecting a cell. A symmetric quantity, the viral multiplicity of attachment (MOA) is the mean number of attachment events that must be blocked to ensure that a viral particle does not attach to any cell. Virologists have long recognized that the concept of MOI is essential when planning and interpreting experiments blocking cellular infection. Only recently, however, has the importance of MOA been recognized.

Most of the viral burden in established HIV infection resides in the lymph nodes,
whose cell concentrations are not easily achieved in the laboratory. Thus, any laboratory estimate of the clinical efficacy of a blocker of HIV attachment automatically requires an implicit extrapolation across a range of cell concentrations (and possibly, viral-cell incubation times as well). The concept of MOA carries out this extrapolation quantitatively and objectively. With first the laboratory success and then the clinical failure of the HIV blocker sCD4 in mind, the MOA extrapolation should be applied to any potential therapeutic agent that reversibly blocks HIV attachment. This includes the ones currently under investigation (e.g., soluble chemokine receptors).

Moreover, current virological practice usually measures infection over a range of blocker concentrations, rather than cell concentrations. Because HIV is harbored in the lymph nodes, however, and because lymph node cell concentrations are difficult to duplicate in the laboratory, extrapolation over cell concentrations is much more relevant.

More generally, with few exceptions, therapeutic interventions in virology are commonly directed against a virus rather than its target cell, probably because interfering with cells deranges normal physiology more than interfering with viruses. Gating theory supports the common practice, but it does so on grounds of effectiveness. First, in the early stages of infection, the virus is the minority species, and the target cells are the majority species. Given certain assumptions, gating theory indicates that blocking the minority species (virus) is probably more effective than blocking the majority species (cells). Second, the viral attachment proteins (VAPs) on a virus are usually less numerous than receptor proteins on cells. Because of this, again given certain assumptions, gating theory indicates that blocking the virus is probably more effective.

Gating theory offers a caution on the current virological practice of using chemical equilibrium binding-constants to estimate the efficacy of a reversible blocker against viral
attachment; however. Blocking probably only reflects equilibrium binding-constants if a diffusing virus approaches and leaves the surface of the cell before any of its VAPS switch between activation and blocking.

This article has achieved its aim if it convinces the reader that Smoluchowski gating theory has many important new applications in virology.

Acknowledgements: I would like to thank my experimental collaborators, Peter L. Nara, Suchin Wu, Michael Merges, and Shawn Conley; my theoretical collaborators, Attila Szabo, George H. Weiss, and Micah Dembo; and finally, Scott P. Layne, who provided the initial impetus for these studies.

REFERENCES

1. Delbrueck, M. 1940 J Gen Physiology 23 631
2. Ellis, E.L. & Delbrueck, M. 1939 J Gen Physiol 22 365
37. von Smoluchowski, M. 1916 *Phys Z* **17** 557
38. von Smoluchowski, M. 1916 *Phys Z* **17** 585
40. Wax, N. 1956 *Selected Papers on Noise and Stochastic Processes* New York Dover
42. Doob, J.L. 1953 *Stochastic Processes* New York John Wiley and Sons
51. Spouge, J.I., Shrager, R.I. & Dimitrov, D.S. 1996 Math Biosci 138 1
54. Valentine, R.C. & Allison, A.C. 1959 Biochimica et Biophysica Acta 34 10
55. Allison, A.C. & Valentine, R.C. 1960 Biochim Biophys Acta 40 400
56. Allison, A.C. & Valentine, R.C. 1960 Biochimica et Biophysica Acta 40 393
58. Fauci, A.S. 1988 Science 239 617
60. Hammer, D.A. & Lauffenburger, D.A. 1987 Biophys J 52 475
65. Flory, P.J. 1941 J Am Chem Soc 63 3083
66. Flory, P.J. 1941 J Am Chem Soc 63 3091


74. Puck, T.T. 1953 Cold Spring Harbor Symp Quant Biol **18** 149
