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Universality of Poisson Indicator and Fano Factor of Transport Event Statistics in Ion Channels and Enzyme Kinetics

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Abstract:
We consider a generic stochastic model of ion transport through a single channel with arbitrary internal structure and kinetic rates of transitions between internal states. This model is also applicable to describe kinetics of a class of enzymes in which turnover events correspond to conversion of substrate into product by a single enzyme molecule. We show that measurement of statistics of single molecule transition time through the channel contains only restricted information about internal structure of the channel. In particular, the most accessible flux fluctuation characteristics, such as the Poisson Indicator (P) and the Fano Factor (F) as function of solute concentration, depend only on three parameters in addition to the parameters of the Michaelis-Menten curve that characterizes average current through the channel. Nevertheless, measurement of Poisson indicator or Fano factor for such renewal processes can discriminate reactions with multiple intermediate steps as well as provide valuable information about the internal kinetic rates.

Keywords: Single molecule events, counting statistics, waiting time distribution, Michaelis-Menten kinetics, renewal theory, shot noise.

Introduction

Quantitative biology is aimed to develop mathematical/theoretical tools for quantitative predictions of biochemical system dynamics. This field has always been influenced by the problem of a large diversity of biochemical processes. Even if one develops a very precise description of some kinetic biochemical processes in one organism, it is usually unlikely to find exactly the same biochemical process in another organism. Hence, the unifying laws that
encounter in wide range of biochemical systems are of particular importance for this field. One widely known example is called the Michaelis-Menten (MM) law. According to it, the average rate of product creation $\langle J \rangle$ and the substrate concentration $[S]$, in enzymatic reaction, are related by

$$\langle J \rangle = \frac{k[S]}{[S] + K_{MM}},$$

where $K_{MM}$ and $k$ are constant parameters. The MM-law was initially derived for a simple reaction, $E + S \xrightleftharpoons[k_{-1}]{k_1} ES \rightarrow P + E$, in which the substrate $S$ is converted into product $P$ via an intermediate complex $ES$ that the substrate creates with enzyme molecule $E$. Interestingly, the MM law was found in a much wider class of enzymatic reactions, with possibly many internal substeps. Recently, this observation was explained by showing that a large class of passive (i.e. driven only by difference of substrate and product concentrations) enzymatic reactions, with multiple states of enzyme-substrate complex, follows the Michaelis-Menten law.

Eq. (1) is also encountered in biochemistry beyond the context of enzyme kinetics. For example, traditionally, the key measurable quantity in ion channel transport has been the steady state flux in a single channel through a membrane that separates two compartments with different solute concentrations. MM law was found to describe the transport of solute molecules through a class of ion channels, in which $\langle J \rangle$ represents the ion flux through the channel and $[S]$ is the solute concentration on one side of the channel, assuming that $[S]=0$ on the other side. Recent theoretical studies shed light on the origin of observed wide applicability of the MM-formula (Eq. 1) in ion channels. Bezrukov et al showed that a general model of transport through a chain of $N$ neighboring sites with $2(N-1)$ rate constants, as well as its continuous 1D diffusion limit, produce the same dependence of average flux on solute concentrations as in an effective MM-model with appropriately chosen transition rate constants. To avoid a mixture of ion channel and enzyme terminology, in this article, we will use the ion channel interpretation of our models throughout the text, and return to enzyme applications only in the discussion.
The simplicity of eq. (1) implies that information provided by measurements of average flux is intrinsically limited. Only two constants can be obtained experimentally by measuring MM-curve for a renewal process. Additional terms will contribute to the substrate-dependence of turnover rate if a multiple-channel reaction is driven away from equilibrium\textsuperscript{4,5}. The advance of single molecule techniques allowed researchers to alleviate this restriction by studying not only average currents but also the statistics of single molecule transitions in the channel-facilitated transport through biological membranes\textsuperscript{2,3,6-8}. For example, single-channel ion current measurements have been used to study the translocation dynamics of single-stranded RNA and DNA through α-haemolysin channel in lipid bilayer membrane\textsuperscript{9}. During the translocation, the single stranded polymer partially blocks the channel. This leads to transient blockades in α-haemolysin single channel current and the current is restored to its original value when the DNA exits from the other side of the membrane. By detecting time moments of such events of DNA exit, one can study not only mean DNA transition times but also characterize fluctuations of those time intervals. Such high-resolution transition events recording from single ion channels were shown to provide information that is hidden in ensemble-averaged experiments. The most accessible characteristics of fluctuations in molecular transport are related to second moment of turnover time statistics and current distribution. Those include the Poisson indicator ($P$), defined by

$$P = \frac{\langle t^2 \rangle - 2\langle t \rangle^2}{\langle t \rangle^2},$$

which is also known as the Mandel parameter in the context of photon counting statistics\textsuperscript{10-12}, and the Fano factor ($F$), defined by

$$F = \frac{\langle J^2 \rangle - \langle J \rangle^2}{\langle J \rangle^2},$$

where $t$ is the time between successive molecular transitions through the channel and averaging is over a large number of such observed transitions; $J$ is the total number of molecules transferred through the ion channel during a specified measurement time interval. In the context of photon statistics, the Fano parameter and the Poisson indicator are related to the Mandel’s parameter which describes the bunching and antibunching of emitted photons.
If ion channels were just “windows” without internal structure for instantaneous molecular transitions through a membrane, the statistics of turnover times would be exponential and statistics of currents would be Poissonian. This corresponds, respectively, to $P = 0$ and $F = 1$. Functional dependences of $P([S])$ and $F([S])$ on the solute concentration, $[S]$, on one side of a membrane provide valuable information about structure of an ion channel. In our present paper we will consider the model of ion channel kinetics with the possibility of multiple closed loops in kinetic network, as shown in Figure 1. We will show that, similarly to the universality of average flux characteristics, the complexity of $P$ and $F$ for transport through ion channels reduces to the universal functions that depend on, maximum, three additional constant parameters. We will also show that one can derive a connection between the Fano factor and Poisson indicator for such renewal kinetic processes. While the universality of $P$ and $F$ will be the main focus of our work, we will perform calculations on the level of the full statistics of turnover events, so that our method can be used to explore similar properties of higher order correlators, if needed.

This paper is organized as follows. In Section II, we introduce our general kinetic model for transport through an ion channel and derive expressions for the first passage time distribution and related observables in fluctuation statistics such as the second moment of the distribution and the Poisson indicator. We also concentrate on simple kinetic models with only two internal states and use the self consistent pathway formalism proposed by Cao and Silbey\textsuperscript{15} to derive expressions for the waiting time distribution in terms of the elementary kinetic rates and show how these models influence parameters in the general expression for the Poisson indicator. In Section III, we calculate the Fano factor by exploring a connection between the turnover probability distribution and the cumulant generating function, which is directly related to the Fano factor. We derive expressions for the parameters that influence Fano factor in terms of the kinetic rates for two state models. We summarize our results in Section IV.

II. First passage time distribution

In our model of ion channel, shown in Figure 1, we consider transport through a singly occupied channel with arbitrary number of internal states. The channel is assumed to be capable of having
maximum one molecule inside, i.e. even if the molecule is smaller than the length of the ion channel, we assume that it creates a potential that blocks other molecules from entering the channel. The left side compartment contains solute particles at concentrations \([S]\) while the right compartment has negligible solute concentration. \(E\) represents the single empty channel state, and \(P_1, P_2, \ldots P_N\) are possible solute occupied states at the entrance to the channel. We assume that after the solute molecule leaves the channel, the internal degrees of freedom of the channel relax quickly so that the empty state \(E\) of the channel can be represented in our model by a single state. We also assume that the solute concentration on the left of the channel is set to a constant value. Hence, evolution repeats, in the statistical sense, each time the channel becomes empty. If we understand the dynamics between only two successive moments at which the channel becomes empty, we can reconstruct all other statistical characteristics of the process. This type of reaction scheme is referred to as a renewal process\(^{16}\).

**Waiting time distribution functions**

We assume that experimentally only some specific events, called monitored transitions, are observable. For example, in ion channels, monitored transitions can be events when a transported molecule leaves the ion channel. We will assume that the evolutions of the system are statistically identical after each monitored event. The central object of the renewal theory is the first passage time distribution \(\phi(t)\) between two successive monitored events. More precisely, given the moment of one monitored transition, \(\phi(t)dt\) is the probability of observing the next monitored transition between time \(t\) and \(t+dt\) after this time moment. In this article, we will assume that monitored transitions correspond to events when solute molecules are leaving ion channel to the right compartment. Such events were shown to be detectable in ion channel experiments\(^2,^3\).

It is generally assumed that the kinetic rate for entering the “empty” ion channel is proportional to the solute concentration \([S]\). Hence let \(k_{Ej}[S]\) be the rate for making a transition from empty state \(E\) into the state with a solute molecule inside the ion channel at site \(j\), with constant parameter \(k_{Ej}\) independent of \([S]\). In correspondence to this process, we introduce the
probability per unit time, \( Q_{E_j} (t) \) of the event that at time \( t \), after the channel becomes empty, the new solute molecule enters the channel for the first time at site \( j \). Explicitly, \( Q_{E_j} (t) \) is exponentially distributed:

\[
Q_{E_j} (t) = k_{E_j} [S] \exp \left( -t \sum_{j=1}^{N} k_{E_j} [S] \right).
\]  

This type of probability is known as the waiting time distribution function, which accounts not only for fundamental rate processes or their combinations but also non-exponential decay processes. The waiting time distribution formulation allows us to condense a large class of complex reactions into a generic scheme which is the irreducible representation of measurements\(^{15,17}\). In the current setting, the accessible measurements are substrate binding and enzymatic turnover, which define the basic elements of the waiting time analysis presented below.

Let then \( Q_{R_j} (t) \) be the probability per unit time of the event that the molecule that just entered the site \( j \) will leave the channel at time \( t \) to the right, i.e. by making a monitored transition. We assume that all elementary reactions, except the monitored ones are, in principle, reversible, so there is also a finite probability per unit time, \( Q_{L_j} (t) \), that the solute molecule, being initially at site \( j \) will leave the channel to the left at time \( t \) without making the monitored transition. In both cases, after leaving to the left or to the right, the channel becomes open again and process renews. Probability \( \phi(t) \) then satisfies a formal convolution law:

\[
\phi(t) = \sum_{j=1}^{N} \int_{0}^{t} dt_1 Q_{E_j}(t_1) \left[ Q_{R_j} (t-t_1) + \int_{t_1}^{t} dt_2 Q_{L_j}(t_2) \phi(t-t_2) \right].
\]  

This equation can be transformed into algebraic equation, which is satisfied by the Laplace transform of \( \phi(t) \), i.e. by \( \phi(s) = \int_{0}^{\infty} e^{-st} \phi(t) \, dt \). The result is

\[
\phi(s) = \sum_{j=1}^{N} Q_{E_j}(s) \left[ Q_{R_j}(s) \phi(s) + Q_{L_j}(s) \right]
\]  

(6)
Here and in what follows, we distinguish probability distributions and their Laplace transforms by writing in their arguments, respectively, \( t \) or \( s \).

Eq 6 can be formally solved as

\[
\phi(s) = \frac{\sum_{j=1}^{N} Q_{E_j}(s) Q_R'(s)}{1 - \sum_{j=1}^{N} Q_{E_j}(s) Q_L'(s)}.
\]  

\[ (7) \]

This compact expression generalizes the distribution function derived for chain reactions and exemplifies the self-consistent pathway method formulated for the first passage time distribution of generic enzymatic networks\(^{15,17}\). The introduction of \( Q_R \) and \( Q_L \) simplifies such analysis and can generate hierarchical distribution functions for chain reactions.

**Substrate Dependence of Poisson Indicator**

We note that eq 7 is still a formal solution because only the functional form of \( Q_{E_j}(s) \), at this stage, is known:

\[
Q_{E_j}(s) = \frac{k_{E_j}[S]}{s + \sum_{j=1}^{N} k_{E_j}[S]},
\]

\[ (8) \]

while \( Q'_L(s) \) and \( Q'_R(s) \) remain unknown yet. However, to achieve our goals, their explicit form is not needed. Importantly, we know that neither \( Q'_L(s) \) nor \( Q'_R(s) \) depends on the external solute concentration \([S]\). Substituting \( Q_{E_j}(s) \) from eq 8 into eq 7 and taking the derivative of eq 7,

\[
\langle t \rangle = \frac{\langle -1 \rangle}{\lim_{s \to 0} \frac{\partial \phi(s)}{\partial s}}
\]

we obtain the average first passage time

\[
\langle t \rangle = \frac{A}{[S]} + B,
\]

where
$$A = 1/k_{E1}a, \quad B = -(b+c)/a$$

where $a = \sum_j Q^j_R(0), 1-a = \sum_j Q^j_L(0)$, $b$ and $c$ are constants that are the first derivatives of, respectively, $Q^j_R(s)$ and $Q^j_L(s)$ at $s=0$.

Thus, we obtained a linear relation between the mean first passage time $\langle t \rangle$ and the inverse of solute concentration $[S]^{-1}$. This is equivalent to the relation in eq 1 obtained for $\langle J \rangle = 1/\langle t \rangle$.

For example, parameters of MM-curve, $K_{MM}$ and $k$, can be expressed via $A$ and $B$ as $K_{MM} = A/B$ and $k = 1/B$. As we mentioned, this universality, i.e. independence of functional form of $\langle J \rangle$ on the detail of the internal kinetic model of the channel, was previously discussed in a series of previous work.

Next, by analogy with average turnover rate we consider higher moments of the turnover time distribution. Substituting eqs 7 and 8 into

$$\langle t^n \rangle = \int_0^\infty dt^n \phi(t) = (-1)^n \lim_{s \to 0} \partial^n \phi(s)/\partial s^n,$$

we find that the Poisson indicator $P$ defined in eq 2 reads:

$$P = \frac{[S][q[S]-\eta]}{(\delta+[S])^2},$$

where the $q, \eta, \delta$ are all constants that are different combinations of the different rate constants $k_{Ej}$ and the first and second derivatives of $Q^j_L(s)$ and $Q^j_R(s)$ at $s=0$, which do not depend on solute concentration $[S]$. Explicitly, we obtained: $A = 1/k_{E1}a, B = -(b+c)/a,$

$q = -2b^2 - 2bc + a(d+f)/\left(b+c\right)^2, \eta = 2b/k_{E1}\left(b+c\right)^2, \delta = -1/k_{E1}(b+c);$

$d$ and $f$ are the second derivatives of, respectively, $Q^j_R(s)$ and $Q^j_L(s)$ at $s=0$.

Eq 11, as well as the similar expression for the Fano factor that we will derive in the following section, are the central results of our work. Eq 11 shows that the Poisson indicator $P$
has a universal functional dependence on the solute concentration. It is parameterized only by three constant parameters irrespective of the number of internal states and kinetic rates inside the ion channel.

The parameter $P$ is, generally, a non-monotonous function of $[S]$, and at high solute concentrations, $\lim_{[S] \to \infty} P = q$, i.e. generally at high concentration the statistics of turnover time distribution is non-Poisson. Note that eq 11 is derived under the assumption that substrate binding as described by eq 8 is a rate step. A general functional form of $[S]$-dependence can be obtained by incorporating the non-Poissonian distribution of the substrate binding.

**C. Kinetics with two-internal states**

Recently Cao and Silbey$^{15,17}$ proposed a self consistent approach, which is based on the theory of renewal processes, for studies of turnover time statistics in single molecule kinetics. This theory is equally well suited for applications to ion channels. It provides a straightforward way to express waiting time distributions $Q(t)$ via the elementary kinetic rates of a kinetic model. While generally explicit expressions would be complex, such expressions can be easily written for simplest models with only one and two internal states.

As an example, consider the model with only two internal states, shown in Figure 2a. $E$ represents the empty state, $ES$ and $EP$ are the two interconvertible internal states, which correspond e.g. to two internal states of a molecule inside an ion channel. By applying the theory$^{15,17}$, the self consistent equation for the first passage time distribution in the Laplace space is

$$\phi(s) = \frac{Q_{E1}(s)Q_{12}(s)Q_{23}(s)}{1 - Q_{E1}(s)Q_{12}(s) - Q_{12}(s)Q_{21}(s)}$$

(12)

where $Q_{23}(s)$ describes the monitored transition, $Q_{E1}(s)$ and $Q_{12}(s)$ describe the transitions from the state $E$ to $ES$ and the state $ES$ to $EP$, respectively. $Q_{12}(s)$, and $Q_{21}(s)$ are the backward transition rates from the intermediate state $ES$ to the empty state $E$ and the transition from the state $EP$ to $ES$, respectively. In analogy to eq 7, $Q_{23}(s)$ and $Q_{12}(s)$ represents $Q_{k}^{i}(s)$ and the
backward transitions $Q_22(s)$ and $Q_2j(s)$ represents $Q_j^L(s)$. Eq. 12 for this two state kinetics is thus a special case for eq 7.

Following eq 8, the waiting time distributions in terms of the kinetic rate constants are given by

$$Q_{21}(s) = k_{E1}/(s + k_{1e} + k_+),$$
$$Q_{12}(s) = k_1/(s + k_{1e} + k_+),$$
$$Q_{23}(s) = k_2/(s + k_+ + k_2),$$
$$Q_{2j}(s) = k_{j}/(s + k_+ + k_2).$$

Taking the derivative of eq 12 and using eq 13 we found that parameters $q$, $\eta$ and $\delta$ in eq 12 in terms of kinetic rates of Figure 2a can be expressed as:

$$q = -\frac{2k_k k_2}{\left(k_2 + k_+ + k_2\right)^2},$$
$$\eta = \frac{2k_{1e}k_+}{k_{E1}\left(k_2 + k_+ + k_2\right)^2},$$
$$\delta = \frac{k_2 k_+ + k_{1e}\left(k_2 + k_+\right)}{k_{E1}\left(k_2 + k_+ + k_2\right)}.$$

The negative value of the parameter $q$ means that fluctuations of turnover times for this two state model are always sub-Poisson, i.e. they are suppressed in comparison to the ones in the Poisson process. In contrast, for the reaction process shown in Figure 2b, in which the second internal state is an idle state, using the self consistent approach as before, we find that $q = \frac{2k_k k_2}{\left(k_+ + k_2\right)^2}$.

This positive value corresponds to super-Poissonian statistics.

We note that kinetic models with more than two internal states may still be distinguishable from 2-state models if variances of fluxes are measured. For example, for the reaction scheme given in Figure 2a, the minimum of the Poisson indicator is achieved at
Minimizing this expression further over the choice of kinetic rates, we find the minimum possible value of the Poisson indicator is -2/3 and it is achieved for reaction in Figure 2a when all elementary reactions are irreversible and have the same kinetic rates \( E \xrightarrow{k} ES \xrightarrow{k} EP \xrightarrow{k} P \). Hence the value of \( P_{\min} \) lower than -2/3, if observed, would indicate that the reaction mechanism involves more than two intermediate states.

### III Current distribution function and Fano factor

A different type of single molecule measurement is the probability distribution for the number of events observed within a time bin.18,19 In this measurement approach, the number of molecules transferred through the channel is measured during time intervals \( t \) and the probability \( P_n(t) \) of the number \( n \) of transitions is obtained after many repetitions of the measurement. A convenient way to study current distribution theoretically is by introducing the probability generating function (pgf) 20-22

\[
Z(\omega, t) = \sum_{n=0}^{\infty} P_n(t) e^{i\omega n} = e^{\omega \chi(t)},
\]

where \( \chi \) is called the counting parameter, \( \omega(\chi) \) is the cumulant generating function. Its derivatives with respect to \( \chi \) give the cumulants of the distribution \( P_n \), such as the mean \( \langle n \rangle \) and the variance \( \sigma^2 \):

\[
\langle n \rangle = -i \frac{\partial \omega(\chi)}{\partial \chi} \bigg|_{\chi=0}, \quad \sigma^2 = \langle n^2 \rangle - \langle n \rangle^2 = \left(-i\right)^{2} \frac{\partial^2 \omega(\chi)}{\partial \chi^2} \bigg|_{\chi=0}.
\]

The Fano factor is defined to be the ratio of the variance to the mean, i.e.
Recently, the Fano factor in most general enzymatic models with two internal states, which also correspond to our models of ion channels with two internal nonempty states, was studied by Mugler et al.\textsuperscript{13} It was shown that measuring both the average current and the Fano factor as function of solute concentration is sufficient to distinguish among all possible 2-state enzymatic kinetics models and, moreover, to determine values of all kinetic rates quantitatively.

The natural next question is whether measurements of the Fano factor can be used to extract information about more complex enzymatic mechanisms. To resolve this question, we will first demonstrate the connection between the turnover probability functions and the cumulant generating function, from which the Fano factor can be readily obtained for renewal processes.

Let $\phi(t)$ be the probability that a turnover event takes place in time $t$ and $\psi(t)$ is the probability that no monitored transitions happen during time $t$ after the last monitored event. Then the event averaged probability distribution function $P_n(t)$, after the Laplace transform over time, is given by

$$P_n(s) = \phi^n(s) \psi(s).$$

Using eq 19, the generating function in the Laplace space becomes

$$Z(\chi, s) = \sum_{n=0}^{\infty} \phi^n(s) \psi(s) e^{i\chi n} = \frac{\psi(s)}{1 - e^{i\chi} \phi(s)},$$

which is the discrete Fourier Transform of $P_n(s)$ over $n$-index and its Laplace transform over time. Returning to real time,
At large measurement time, we look for the dominating exponential part in eq 21. This happens at \( s = s^* \) where \( s^* \) is the pole in the denominator in eq 21 which is provided by the solution of the equation

\[
1 - e^{s^*} \phi(s^*) = 0. \tag{22}
\]

As a function of counting parameter, the solution of eq 22 also coincides with the cumulant generating function, defined in eq 16, because at large measurement times \( t \), according to eqs 21-22, the generating function behaves as \( Z(\chi, t) \sim e^{\chi x^N} \). This form corresponds to linearly growing current cumulants, and hence a constant value of the Fano factor. For illustration, in Appendix A, we perform calculation of all functions for a simple Michaelis-Menten model explicitly using this approach.

The relation between the turnover time probability distribution \( \phi(t) \) and the cumulant generating function suggests that one can express the Fano factor in terms of derivatives of \( \phi(t) \) and obtain a similar universality to the Poisson indicator. Indeed, from eq 22 we have

\[
\phi(s) = e^{-i\chi} \tag{23}
\]

The inverse of this function can be written as

\[
s = \phi^{-1}(e^{-i\chi}) = \omega(\chi). \tag{24}
\]

Using the properties of inverse functions and its derivatives as shown in Appendix B, the Fano factor \( F \) is given by

\[
F = \left. \frac{\partial^2 \phi(s)}{\partial s^2} \right|_{s=0} - \left( \left. \frac{\partial \phi(s)}{\partial s} \right|_{s=0} \right)^2 \left( \left. \frac{\partial \phi(s)}{\partial s} \right|_{s=0} \right)^2.
\tag{25}
\]
Applying eq 6 to eq 25 we obtain

\[ F = \frac{\alpha' + \beta'[S] + \gamma'[S]^2}{(\mu + [S]^2)^2} - 1. \]  \hspace{1cm} (26)

Constant parameters in eq 26 are not independent. Additional constraint on them follows from the fact, which was established in the previous section, that the flux must be Poisson distributed \((F \to 1)\) when \([S] \to 0\). This leads to

\[ F = 1 - \frac{\alpha_B[S][S - K_B]}{(\mu + [S]^2)^2}, \]  \hspace{1cm} (27)

where

\[ \alpha_B = \frac{2b(b+c) - a(d + f)}{(b+c)^2}, \]
\[ K_B = -\frac{2b/k_{iE}}{2b(b+c) - a(d + f)}, \]
\[ \mu = \frac{1}{k_{iE}(b+c)}. \]  \hspace{1cm} (28)

Parameters \(a, b, c, d, f\) in eq 28 were introduced in the previous section. Considering eq 27, we conclude that, similarly to the Poisson indicator, the Fano factor is parameterized only by three independent constants. Eq 27 shows that the Fano factor \(F\) has the same functional dependence on the solute concentration irrespective of the number of internal states inside the ion channel. For the two state model in Figure 2a, parameters \(\alpha_B, K_B\) and \(\mu\) can be explicitly written in terms of elementary kinetic rates:
\[ \alpha_S = \frac{2k_1k_2}{(k_1 + k_2 + k_3)^2}, \]
\[ K_S = \frac{k_{1E} + k_2 + k_3 + k}{k_{21}}, \]
\[ \mu = \frac{k_1k_{1E} + k_2 + k_3 + k}{k_{21}(k_1 + k_2 + k_3)}. \] (29)

Figure 4 shows the dependence of $F$ on solute concentration $[S]$ for the reaction scheme shown in Figure 2a, 2b and for the ion channel model with only one internal state (MM reaction). As in the case with Poisson Indicator, competing reaction schemes can be distinguished based on the value of the Fano factor. For example, the values of $F$ lower than $1/3$ is an indication of a reaction scheme involving more than two intermediate steps.

V Conclusion
In this work, we showed that the Poisson indicator and the Fano factor have simple generic functional dependences on solute concentration irrespective of the number of internal states in the ion channel kinetic model. This observation can be used in practice by analogy with applications of the MM-formula. For example, many biochemical processes favor enzymes with specific values of constants $K_{MM}^{23,24}$ and $k$. In addition, by looking at $k/K_{MM}$ values, one can compare enzyme's preferences for different substrates. We anticipate that measurements of parameters of $P([S])$ and $F([S])$ curves can have similar applications. Noise has been shown to lead to important consequences in biological systems. While some biological processes need to suppress noise, others may need noise as an important part of the reaction mechanism. It should be interesting to explore parameters that characterize the Poisson indicator and the Fano factor curves in a wide class of ion channels and enzymatic reactions. One can expect that evolutionary selection has led to separation of enzymes and ion channels in classes with parameters that either suppress or enhance noise for specific biological reasons. The universality of flux fluctuations imposes restrictions on the information about the structure of a studied ion channel that can be obtained by measuring variance of transport characteristics. On the other
hand, \( P([S]) \) and \( F([S]) \) curves allow us to distinguish reaction schemes and extract some combinations of kinetic rates from experimental data.

In this work we limited our discussion to renewal processes in which empty state of the channel is represented by a single state. Extension of our formalism to other kinetic schemes, such as non-renewal processes with multiple interconvertible empty states, can be a subject of the future research.

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Appendix A

Example: Michaelis-Menten kinetics

Let us consider a MM enzyme catalytic reaction

\[
E + S \xrightarrow{k_{n}} ES \xrightarrow{k} P + E. 
\]

For this simple reaction, following the self consistent approach, the waiting time distributions can be written as

\[
Q_{E1} = \frac{k_{E1}S}{s + k_{E1}S},
\]

\[
Q_{S} = \frac{k_{2}}{s + k_{2} + k_{1E}},
\]

\[
Q_{P} = \frac{k_{1E}}{s + k_{2} + k_{1E}}.
\] (1)
Substituting eq A1 in eq 6 we obtain
\[ \phi(s) = \frac{k_e k_{E1}[S]}{sk_{1E} + (k_2 + s)(k_{E1}[S] + s)}, \]  
(2)

and the Poisson indicator is given by
\[ P = -\frac{2k_2[S]}{k_{E1} \left( k_2 + k_{1E} + [S]\right)}, \]  
(3)

Substituting eq A2 in eq 22, we find
\[ s^* = \frac{1}{2} \left( -k_{E1}[S] + k_{1E} + k_2 + \sqrt{\left(k_{E1}[S] + k_{1E} + k_2\right)^2 + 4k_{E1}[S]k_2(e^{\chi} - 1)} \right). \]  
(4)

This result coincides with the cgf obtained previously by solving the master equation for the generating function\(^{21}\).

**Appendix B**

Let \( y = \phi(s) = e^{-ix} \), then \( s = \phi^{-1}(y) = \omega(\chi) \). The first and second derivatives of the inverse function are given by
\[ \frac{\partial s}{\partial y} = \frac{y}{\omega(\chi)}, \]
\[ \frac{\partial^2 s}{\partial y^2} = -\frac{\partial^2 y}{\partial s^2}. \]

Using eq B1 in the expressions for mean \( \langle n \rangle \) and variance \( \sigma^2 \) in Eq. (17), we find
The ratio of this variance and mean gives the Fano factor defined in eq 25. For the MM scheme, using eq A2 and eq 25, the Fano factor is given by

\[
F = 1 - \frac{\alpha_{a}}{S + K_{a}} \frac{S}{(S + K_{a})^2},
\]

where \( \alpha_{a} = 2k_{1}/k_{e} \), \( K_{a} = k_{e}/k_{e1} \). This is identical to \( F \) obtained previously by solving the master equation for the generating function\(^{13,21}\).

References

Figure captions

Figure 1: The ion channel model. $E$ is the empty state with $N$ internal states for the entry to the ion channel.

Figure 2 Two-state ion channel models: a) $E$ is the empty state, $ES$ and $EP$ are the two internal states. The forward and backward rate constants for transition between $E$ and $ES$ are, respectively, $k_{E1}$ and $k_{1E}$; $k_+$ and $k_-$ are rate constants for intra-channel transitions between $ES$ and $EP$, and $k_2$ is the escape rate from the channel. b) $ES_1$ and $ES_2$ are the internal states. The escape through the channel takes place from $ES_1$. 

(22) van Kampen, N. G. Stochastic processes in physics and chemistry; Elsevier Science: New York, 1992.
Figure 3: Poisson Indicator $P$ as a function of the solute concentration $[S]$ for the two-state ion channel model in Figure 2a. Numerical parameter values are $k_{E1} = 1$, $k_{IE} = 1$, $k_+ = 0.1$, $k_- = 0.01$, $k_1 = 2$ and $q = -0.16$ (blue), $k_{E1} = 1$, $k_{IE} = 1$, $k_+ = 0.1$, $k_- = 0.01$, $k_2 = 2$ and $q = -0.089$ (green). For ion channel model in Figure 2b: $k_{E1} = 1$, $k_{IE} = 1$, $k_+ = 1$, $k_- = 0.7$, $k_2 = 0.5$ and $q = 0.35$ (black), and ion channel model with one internal state (MM model): $k_{E1} = 1$, $k_{IE} = 1$, $k_2 = 1$ and $q = 0$ (red).

Figure 4: Fano Factor $F$ against solute concentration $[S]$. Parameters for Figure 2a are $k_{E1} = 1$, $k_{IE} = 1$, $k_+ = 0.1$, $k_- = 0.01$, $k_2 = 1$ (blue). For Figure 2b: $k_{E1} = 1$, $k_{IE} = 1$, $k_+ = 1$, $k_- = 0.7$, $k_2 = 0.5$ (green) and MM reaction (red): $k_{E1} = 1$, $k_{IE} = 1$, $k_2 = 1$. 