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Generic Schemes for Single-Molecule Kinetics. 3: Self-Consistent Pathway Solutions for Nonrenewal Processes

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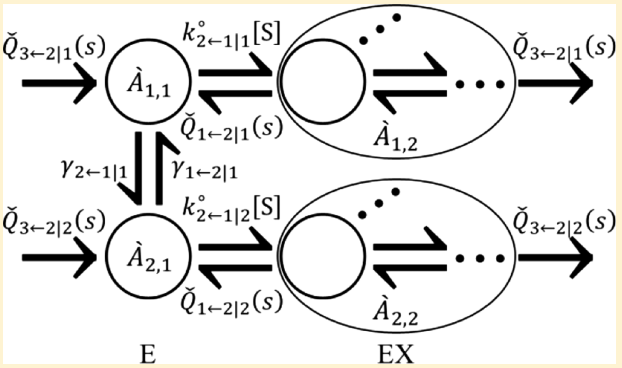
Supporting Information

ABSTRACT: We recently developed a pathway analysis framework (paper 1) for describing single-molecule kinetics for renewal (i.e., memoryless) processes based on the decomposition of a kinetic scheme into generic structures. In our approach, waiting time distribution functions corresponding to such structures are expressed in terms of self-consistent pathway solutions and concatenated to form measurable probability distribution functions (PDFs), affording a simple way to decompose and recombine a network. Here, we extend this framework to nonrenewal processes, which involve correlations between events, and employ it to formulate waiting time PDFs, including the first-passage time PDF, for a general kinetic network model. Our technique does not require the assumption of Poissonian kinetics, permitting a more general kinetic description than the usual rate approach, with minimal topological restrictiveness. To demonstrate the usefulness of this technique, we provide explicit calculations for our general model, which we adapt to two generic schemes for single-enzyme turnover with conformational interconversion. For each generic scheme, wherein the intermediate state(s) need not undergo Poissonian decay, the functional dependence of the mean first-passage time on the concentration of an external substrate is analyzed. When conformational detailed balance is satisfied, the enzyme turnover rate (related to the mean first-passage time) reduces to the celebrated Michaelis−Menten functional form, consistent with our previous work involving a similar scheme with all rate processes, thereby establishing further generality to this intriguing result. Our framework affords a general and intuitive approach for evaluating measurable waiting time PDFs and their moments, making it a potentially useful kinetic tool for a wide variety of single-molecule processes.



1. INTRODUCTION

Condensed-phase spectroscopic advances have afforded the observation of real-time biomolecule trajectories at the single-molecule (SM) level.[1,2] Such time traces offer information on microscopic kinetics that is often unobtainable from ensemble-averaged measurements, such as the effect of dynamic disorder due to conformational interconversion on enzyme turnover.[3−5] An SM experiment permits the measurement of probability distribution functions (PDFs) of the waiting times between detectable molecular events, those of which can be used for kinetic analysis. One such PDF of interest, the first-passage time PDF $\phi(t)$, can be employed to evaluate several key quantities, including the mean first-passage time, which is related to the turnover rate (in the context of enzyme kinetics),[6,7] velocity (for molecular motors),[8] or flux through an ion channel (for ion transport).[9] From a theoretical perspective, it is thus useful to build kinetic models characterized by such measurable PDFs to make suitable connections to SM experiments.

The underlying transitions of an SM kinetic scheme, by definition, are (first-order) rate processes (i.e., linear kinetic transitions), with the underlying states each undergoing Poissonian decay characterized by a monoexponential decay time (i.e., dwell time) PDF. It is often the case in SM experiments, however, that some states in a proposed scheme undergo non-Poissonian decay (characterized by, for instance, multiexponential, stretched exponential, or power law decay time PDFs[10]) due to hidden internal dynamics and thus represent aggregates of states,[11] rather than underlying ones. Transitions out of such aggregated states cannot be properly treated as rate processes,[12] so it is advantageous to describe them by waiting time distribution functions of arbitrary form.[13] The waiting time distribution formalism is based on such transition waiting time distribution functions and can be used to formulate measurable PDFs, including $\phi(t)$, without necessitating the assumption of Poissonian kinetics.[10] This permits a more general time/probability kinetic description, which has fewer topological restrictions than the typical rate approach. This alternative description has been developed by

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Ninio for the mean first-passage time,[14] but the waiting time distribution framework extends it to the entire PDFs.

Recently, we developed a self-consistent pathway approach (paper 1),[10] equivalent to the waiting time distribution formalism, based on the decomposition of an SM scheme into generic structures (motifs). In our framework, waiting time distribution functions corresponding to such motifs are written in terms of self-consistent pathway solutions and concatenated to construct measurable PDFs in an intuitive manner, permitting a straightforward means of decomposing and recombining a network. Subsequently, we employed this approach to study stochastic fluctuations in enzyme turnover events for a generic scheme containing an aggregated intermediate state with an arbitrary internal topology (paper 2).[12] Unlike a predetermined scheme, a generic scheme can generate all possible kinetic models with the same basic topological connectivity, requiring minimal assumptions on the form of the underlying scheme. These analyses, however, were restricted to renewal processes, which are characterized by independent and identically distributed events.[15] At the SM level, processes are often nonrenewal in nature (with measurable memory effects[3,16,17]), particularly those involving enzymes, which undergo conformational fluctuations on many time scales.[18] Here, we extend our self-consistent pathway framework to nonrenewal processes and apply it to generic schemes for single-enzyme turnover. The hallmarks of our approach are its incorporation of self-consistency, focus on measurable waiting time PDFs, suitability for a more general time/probability kinetic description, and connection to SM networks that may involve non-Poisson decay processes.

This article is organized as follows: In Section 2, we introduce the established waiting time distribution framework, which can be used to formulate measurable PDFs,[10] in the context of nonrenewal SM processes. In Section 3, we develop kinetic motifs involving transitions between SM state manifolds and discuss how they can be employed to equivalently construct measurable waiting time PDFs in a more mathematically compact fashion. By examining a general kinetic network model, we demonstrate in Section 4 how such measurable PDFs can be expressed in terms of self-consistent pathway solutions based on these motifs. In Section 5, we present explicit calculations for such a model, which we adapt to two generic schemes for enzyme turnover with conformational interconversion. For each generic scheme, we examine the functional dependence of the mean first-passage time on the concentration of an external substrate.

2. NONRENEWAL WAITING TIME DISTRIBUTION FORMALISM

In this section, we make some basic definitions and introduce the established waiting time distribution formalism[10] in the context of nonrenewal SM processes. This is equivalent to the novel self-consistent pathway framework we develop in Sections 3 and 4, but as we explain in Section 3, our pathway technique is more mathematically compact and less redundant. Herein, we refer to an SM scheme as being composed of discrete states, each of which may or may not be aggregated, connected by unmonitored and monitored kinetic transitions. To avoid non-Markovian memory,[11] which is different from nonrenewal memory, we require that all transitions into an aggregate proceed into the same underlying (i.e., intra-aggregate) state. We refer to a kinetic step as a set of forward and reverse transitions connecting two states. A step can be reversible or irreversible, and two states may be connected by more than one step. We restrict our analysis to schemes with irreversible monitored steps and consider an event to be the occurrence of a monitored transition. Driving sources are assumed to be time-independent. Thus, because the monitored steps are irreversible, for nontrivial kinetics, the system eventually reaches a nonequilibrium steady state as the inherent thermodynamic inconsistency of the scheme precludes equilibrium behavior. We refer to an event state as a state into which at least one monitored transition proceeds. A scheme with a single event state must correspond to a renewal process (even if it includes multiple monitored transitions); however, a renewal process can involve multiple event states (example in Section 5.2) as long as the consecutive-event difference distribution function vanishes (see the Supporting Information for details). A nonrenewal process necessarily involves multiple event states and is characterized by memory effects[3,16,17] (i.e., correlations between events), which often arise due to the presence of multiple types of transitions (e.g., chemical and conformational transitions). As shown in paper 1,[10] a renewal process is completely specified by $\phi(t)$ (the PDF of the waiting time between consecutive events [first-passage time]), i.e., all quantities corresponding to SM measurements can be readily determined once $\phi(t)$ is known. A nonrenewal process is described by a set of PDFs, which can be inhomogeneously averaged to obtain measurable PDFs, including $\phi(t)$. However, $\phi(t)$ does not describe multievent kinetics for nonrenewal processes (discussed in the Supporting Information).

Consider a closed, X-state SM kinetic network described by $A(\Xi)$, where Ξ is a state coordinate with the discrete representation Ξ_j for $1 \leq j \leq X$. The state corresponding to Ξ_j is thus represented by $A(\Xi_j) = A_j$. The waiting time matrix for the scheme $\mathbf{Q}(t)$ is defined as $Q_{i,j}(t) = \sum_\mu Q_{i,j}^{(\mu)}(t)$, where $Q_{i,j}^{(\mu)}(t)$ is the waiting time distribution function for transition μ from A_j to A_i because the two states may be connected by multiple steps. For instance, in the SM version of the Michaelis−Menten (MM) model for enzyme turnover (discussed in Section 5.2), the unbound and substrate-bound enzymatic states are connected by a substrate binding/dissociation step as well as a catalytic step. When transition μ from A_j to A_i is a rate process, $Q_{i,j}^{(\mu)}(t) = k_{i,j}^{(\mu)} \exp[-k_j t]$, with corresponding rate $k_{i,j}^{(\mu)}$ and A_j depletion rate $k_j = \sum_{i\neq j,\mu} k_{i,j}^{(\mu)}$. In the waiting time distribution formulation, however, $Q_{i,j}^{(\mu)}(t)$ can take on an arbitrary form; thus, the A_j decay time PDF $\sum_i Q_{i,j}(t)$ need not be monoexponential. For a scheme with all rate processes, the waiting time distribution formalism becomes equivalent to the transition rate matrix formalism, wherein the kinetics can be described by the rate matrix $\mathbf{K}$, where $K_{i,j} = \delta_{i,j} k_j - (1 - \delta_{i,j}) \sum_\mu k_{i,j}^{(\mu)}$, with the master equation, $\dot{\mathbf{P}}(t) = -\mathbf{K}\mathbf{P}(t)$, for the population distribution vector $\mathbf{P}(t)$. In the remainder of this section, we focus on the waiting time distribution framework for formulating measurable PDFs for nonrenewal processes (as we did in paper 1 for renewal processes[10]), but we indicate the forms that key quantities reduce to in this special case of equivalence.

The waiting time matrix can be separated into monitored and unmonitored parts as follows: $\mathbf{Q}(t) = \mathbf{Q}_0(t) + \mathbf{Q}'(t)$, with $Q'_{i,j}(t) = \sum_\mu Q'^{(\mu)}_{i,j}(t)$, where $Q'^{(\mu)}_{i,j}(t)$ is the waiting time distribution function for monitored transition μ from A_j to A_i, and $\mathbf{Q}_0(t)$ represents the contribution of the unmonitored transitions. Note that $Q'_{i,j}(t)$ may differ from $Q_{i,j}(t)$ because not all A_j-to-A_i transitions are necessarily monitored. We note that in the rate

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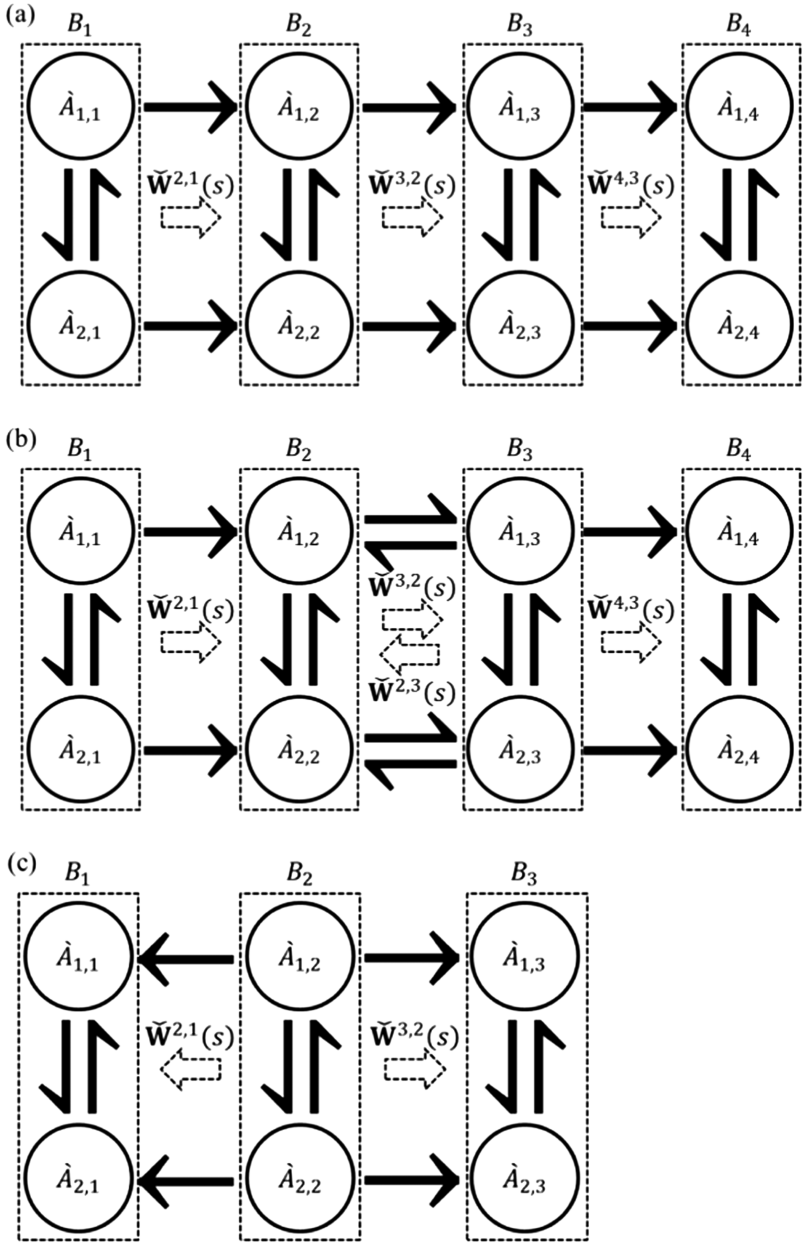

Figure 1. Examples of higher-order kinetic motifs based on transitions between state manifolds. The transition from manifold B_j to B_i is described by the waiting time matrix $\mathbf{W}^{i,j}(t)$. (a) Sequential motif corresponding to a three-link chain of irreversible manifold steps. (b) Sequential motif similar to that of (a) but with manifold back-branching due to the reversible manifold step between B_2 and B_3. (c) Branching motif corresponding to a two-channel manifold decay process.

matrix formalism, $\mathbf{K}$ can be written as $\mathbf{K} = \mathbf{K}_0 - \mathbf{K}'$, with $K'_{i,j} = (1 - \delta_{i,j})\sum_\mu k'^{(\mu)}_{i,j}$, where $k'^{(\mu)}_{i,j}$ is the rate for monitored transition μ from A_j to A_i, and $\mathbf{K}_0$ represents the remaining part of $\mathbf{K}$. Next, we define the first-passage time matrix as

$$\check{\mathbf{\Phi}}(s) = \check{\mathbf{Q}}'(s)[\mathbf{I}_X - \check{\mathbf{Q}}_0(s)]^{-1} \tag{1}$$

where the Laplace transform of a function $h(t)$ is given by $\check{h}(s) = \int_0^\infty dt\, e^{-st} h(t)$, and $\mathbf{I}_X$ is the identity matrix of order X. Here, $\check{\Phi}_{i,k}(s)$ is the waiting time distribution function for (i) traveling from A_k to a state A_j without making a monitored transition ($[\mathbf{I}_X - \check{\mathbf{Q}}_0(s)]^{-1}_{j,k}$ contribution) and then (ii) making a monitored transition from A_j to A_i ($\check{Q}'_{i,j}(s)$ contribution), and it is only nonzero for nonzero $\sum_j Q'_{i,j}(t)$ (although it need not be). Thus, $\sum_i \Phi_{i,k}(t)$ represents the waiting time PDF for beginning in A_k and making a monitored transition. We note that in the rate matrix formalism, $\mathbf{\Phi}(t) = \mathbf{K}'\mathbf{G}_0(t)$. In general, the Green's function $\mathbf{G}(t)$ describes the population evolution as $\mathbf{P}(t) = \mathbf{G}(t)\mathbf{P}(0)$, where, in the rate matrix formalism, $\mathbf{G}(t) = \exp[-\mathbf{K}t]$, with $\check{\mathbf{G}}(s) = [s\mathbf{I}_X + \mathbf{K}]^{-1}$ and $\mathbf{G}_0(t) = \exp[-\mathbf{K}_0 t]$.

We obtain $\phi(t)$ by averaging the set of PDFs $\{\sum_i \Phi_{i,k}(t)\colon 1 \le k \le X\}$ inhomogeneously with the set of weights $\{\overline{P}^{\mathrm{ev}}_k\colon 1 \le k \le X\}$. That is

$$\phi(t) = \sum_{i=1}^{X} [\mathbf{\Phi}(t)\overline{\mathbf{P}}^{\mathrm{ev}}]_i \tag{2}$$

where the event-averaged population distribution $\overline{\mathbf{P}}^{\mathrm{ev}}$ is given by the solution to

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$$\bar{\mathbf{P}}^{\mathrm{ev}} = \int_0^\infty \mathrm{d}t\ \mathbf{\Phi}(t)\bar{\mathbf{P}}^{\mathrm{ev}} = \check{\mathbf{\Phi}}(0)\bar{\mathbf{P}}^{\mathrm{ev}} \tag{3}$$

with the constraint

$$\sum_{k=1}^{X} \bar{P}_k^{\mathrm{ev}} = 1 \tag{4}$$

Here, $\bar{\mathbf{P}}^{\mathrm{ev}}$ reflects how the population is distributed, on average, immediately following an event, serving as the event-averaged initial condition. Note $\int_0^\infty \mathrm{d}t\, h(t) = \check{h}(0)$ for a function $h(t)$. Quantities presented herein employing $\bar{\mathbf{P}}^{\mathrm{ev}}$ are referred to as being event-averaged. Alternatively, the time-averaged initial condition can be used, which is discussed in the Supporting Information. We note that $\bar{P}_k^{\mathrm{ev}}$ is only nonzero for nonzero $\delta_{k,i}\sum_j Q'_{i,j}(t)$. Thus, for a renewal process with a single event state, $\bar{P}_k^{\mathrm{ev}}$ reduces to $\delta_{k,i}$ and $\phi(t)$ becomes equal to $\Phi(t)_{i,i}$, consistent with our formulation in paper 1.[10] Note that $\bar{\mathbf{P}}^{\mathrm{ev}}$ corresponds to the eigenvector of $\int_0^\infty \mathrm{d}t\, \mathbf{\Phi}(t)$ with an eigenvalue of unity. Additionally, $\phi(t)$ is properly normalized, such that $\int_0^\infty \mathrm{d}t\, \phi(t) = 1$. In the Supporting Information, we use the framework presented in this section to formulate expressions corresponding to four different types of SM measurements.

3. KINETIC MOTIFS

An SM kinetic scheme can be decomposed into subschemes[14] corresponding to sequential and branching motifs, each of which can be described by a waiting time distribution function.[10] The waiting time distribution function for the basic sequential motif corresponding to a chain of irreversible steps connecting A_l to A_i is given by $\check{Q}_{i,l}^{\mathrm{seq,irr}}(s) = \Pi_{j=l}^{i-1}\check{Q}_{j+1,j}(s)$. In general, for A_l separated from A_i by at least three links, the waiting time distribution function for traveling from A_l to A_i via a path wherein the only transitions involving these two states are the A_l-to-A_k and A_j-to-A_i transitions, i.e., the A_l-to-A_i sequential motif, is expressed as $\check{Q}_{i,l}^{\mathrm{seq}}(s) = \check{Q}_{i,j}(s)\underline{\check{Q}}_{j,k}(s)\check{Q}_{k,l}(s)$. Here, $\underline{\check{Q}}_{j,k}(s)$ is the waiting time distribution function for traveling from A_k to A_j via all possible paths within the motif and is based on the solution to a self-consistent equation when this set of paths contains at least one reversible step (i.e., "back-branching"). The waiting time PDF for the motif corresponding to the A_j decay process, i.e., branching out of A_j, is given by $Q_j^{\mathrm{branch}}(t) = \sum_i Q_{i,j}(t)$. For a renewal process with a single event state, the waiting time distribution functions corresponding to these sequential and branching motifs can be concatenated to form $\phi(t)$ (see ref 10 for examples); we note that this approach requires adaptation to a given generic scheme. For a process with multiple event states, however, we seek to use motifs to construct a matrix composed of waiting time distribution functions that can be employed to obtain measurable PDFs via inhomogeneous averaging.

As mentioned in Section 2, $\Phi_{i,k}(t)$ is only nonzero for nonzero $\sum_j Q'_{i,j}(t)$ (although it need not be) and as seen from eq 2, it only contributes to $\phi(t)$ when $\bar{P}_k^{\mathrm{ev}}$ is nonzero. It would thus be mathematically advantageous to have a matrix $\mathbf{\Omega}(t)$ containing fewer zero elements than $\mathbf{\Phi}(t)$ as well as fewer nonzero elements that do not contribute to $\phi(t)$. Here, we present the underpinnings for formulating $\mathbf{\Omega}(t)$, the first-passage time pathway matrix, via higher-order kinetic motifs. We begin by introducing an event coordinate Θ with the discrete representation Θ_l for $1 \le l \le Y$, where Y is the number of event states (i.e., the number of rows in $\mathbf{\Phi}(t)$ with nonzero elements, or equivalently, the number of nonzero elements of $\bar{\mathbf{P}}^{\mathrm{ev}}$). On the basis of the topology of the scheme, we introduce a secondary coordinate Λ with the discrete representation Λ_j. We then map the single-coordinate network described by $A(\Xi)$ to a dual-coordinate network described by $\grave{A}(\Theta,\Lambda)$ (see Section 4 for an example), wherein the state corresponding to Θ_l, Λ_j is represented by $\grave{A}(\Theta_l,\Lambda_j) = \grave{A}_{l,j}$. We refer to the manifold of states corresponding to Λ_j as $B(\Lambda_j) = B_j$, which consists of at most Y states. In essence, Θ describes the internal topologies of such manifolds, and Λ distinguishes the manifolds and defines the direction of effective propagation (e.g., the horizontal direction for the model discussed in Section 4), which involves transitions between manifolds. We describe the B_j-to-B_i manifold transition by its corresponding waiting time matrix $\mathbf{W}^{i,j}(t)$ and can identify higher-order kinetic motifs based on such transitions between manifolds, with $\mathbf{W}^{i,j}(t)$ representing a higher-order analogue of $Q_{i,j}(t)$. Here, $\mathbf{W}^{i,j}(t)$ is a square matrix of order Y, with element $W_{k,l}^{i,j}(t)$ representing the waiting time distribution function for traveling (i) from $\grave{A}_{l,j}$ to $\grave{A}_{k,j}$ via Θ-direction transitions within B_j and then (ii) from $\grave{A}_{k,j}$ to $\grave{A}_{k,i}$ via a Λ direction transition. An SM scheme with multiple event states can be decomposed into higher-order sequential and branching motifs (examples in Figure 1), each of which can be described by a corresponding waiting time matrix. We now express the higher-order analogues of the motifs presented earlier. Additionally, we refer hereafter to transitions between manifolds simply as transitions, where one is monitored (contains the monitored-state transitions) and the rest are unmonitored. Whether a transition is a state or manifold transition should be clear from context.

The waiting time matrix for the sequential motif corresponding to a chain of irreversible manifold steps connecting B_l to B_i with only B_j-to-B_{j+1} (for $l \le j < i$) transitions (example in Figure 1a) is given by

$$\check{\mathbf{W}}_{\mathrm{seq,irr}}^{i,l}(s) = \prod_{l}^{j=i-1} \check{\mathbf{W}}^{j+1,j}(s) \tag{5}$$

Here, the product index j counts down from $i-1$ to l, such that $\check{\mathbf{W}}^{l+1,l}(s)$ is the rightmost matrix in the product. For B_l separated from B_i by at least three transitions, the waiting time matrix for traveling from B_l to B_i via a path wherein the only transitions involving these two manifolds are the B_l-to-B_k and B_j-to-B_i transitions, i.e., the B_l-to-B_i sequential motif (example in Figure 1b), is expressed as

$$\check{\mathbf{W}}_{\mathrm{seq}}^{i,l}(s) = \check{\mathbf{W}}^{i,j}(s)\underline{\check{\mathbf{W}}}^{j,k}(s)\check{\mathbf{W}}^{k,l}(s) \tag{6}$$

Here $\underline{\check{\mathbf{W}}}^{j,k}(s)$ is the waiting time matrix for traveling from B_k to B_j via all possible paths (involving transitions between manifolds) within the motif and is based on the solution to a self-consistent matrix equation when this set of paths contains at least one reversible step between manifolds (i.e., manifold back-branching). For the motif in Figure 1b, $\check{\mathbf{W}}_{\mathrm{seq}}^{4,1}(s) = \check{\mathbf{W}}^{4,3}(s)\underline{\check{\mathbf{W}}}^{3,2}(s)\check{\mathbf{W}}^{2,1}(s)$, where $\underline{\check{\mathbf{W}}}^{3,2}(s)$ is expressed in the recurrence relation $\underline{\check{\mathbf{W}}}^{3,2}(s) = \check{\mathbf{W}}^{3,2}(s) + \underline{\check{\mathbf{W}}}^{3,2}(s)\ \check{\mathbf{W}}^{2,3}(s)\check{\mathbf{W}}^{3,2}(s)$ [or equivalently, $\underline{\check{\mathbf{W}}}^{3,2}(s) = \check{\mathbf{W}}^{3,2}(s) + \check{\mathbf{W}}^{3,2}(s)\ \check{\mathbf{W}}^{2,3}(s)\underline{\check{\mathbf{W}}}^{3,2}(s)$], which has the self-consistent solution $\underline{\check{\mathbf{W}}}^{3,2}(s) = \check{\mathbf{W}}^{3,2}(s)[\mathbf{I}_2 - \check{\mathbf{W}}^{2,3}(s)\check{\mathbf{W}}^{3,2}(s)]^{-1}$ [or $\underline{\check{\mathbf{W}}}^{3,2}(s) = [\mathbf{I}_2 - \check{\mathbf{W}}^{3,2}(s)\ \check{\mathbf{W}}^{2,3}(s)]^{-1}\check{\mathbf{W}}^{3,2}(s)$].

The waiting time matrix for the motif corresponding to the B_j manifold decay process, i.e., manifold branching out of B_j (example in Figure 1c), is given by

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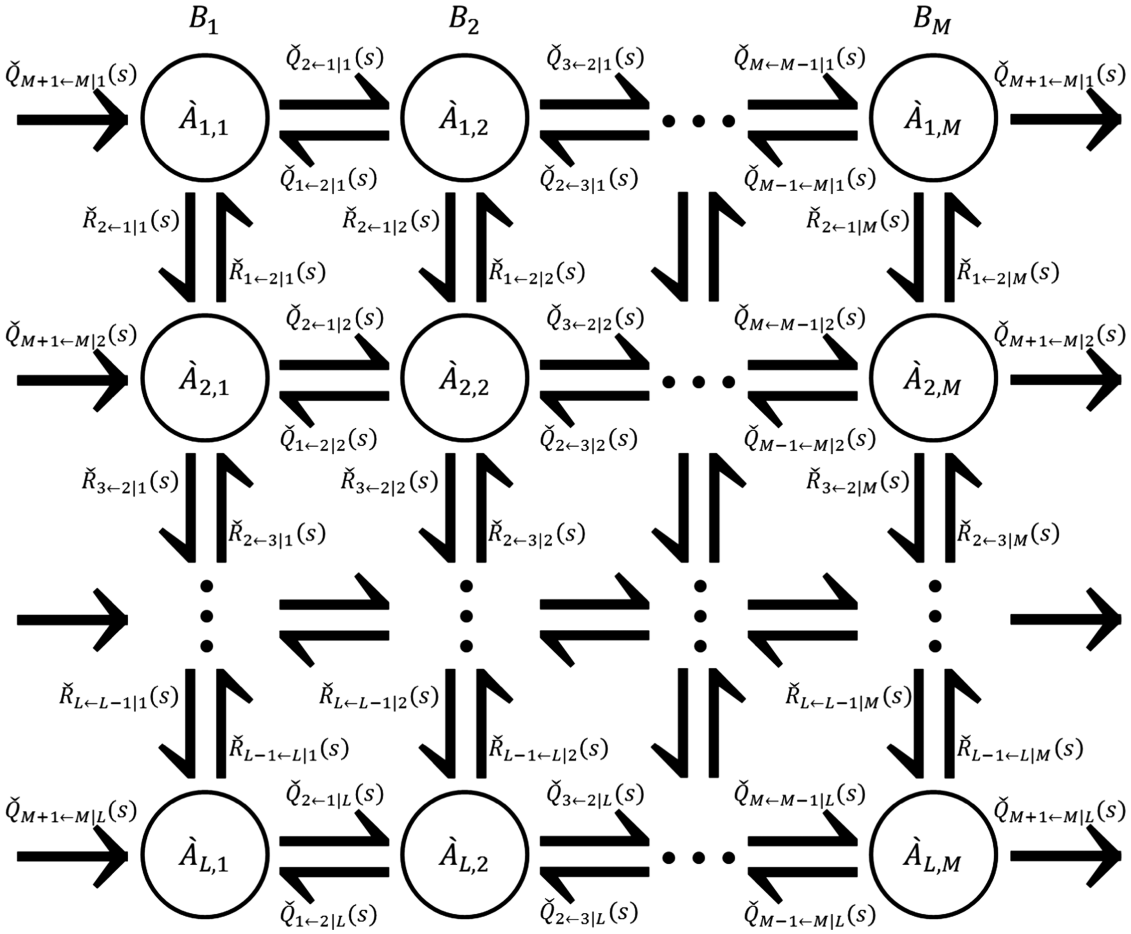

Figure 2. General $L \times M$ SM kinetic network model (see the text for details). Transitions are described by waiting time distribution functions of arbitrary form.

$$\mathbf{W}^{(j)}_{\text{branch}}(t) = \sum_i \mathbf{W}^{i,j}(t) \tag{7}$$

For a renewal process with a single event state, $Y = 1$, and eqs 5−7 reduce to their scalar analogues. The waiting time matrices corresponding to these higher-order sequential and branching motifs can be concatenated (see Section 4 for an example) to form $\mathbf{\Omega}(t)$ (a square matrix of order Y); we note that this approach requires adaptation to a given generic scheme. We obtain $\phi(t)$ by averaging the set of waiting time PDFs $\{\sum_k \Omega_{k,l}(t)\colon 1 \le l \le Y\}$ inhomogeneously with the set of weights $\{F^{\text{ev}}_l\colon 1 \le l \le Y\}$. That is, $\phi(t) = \sum_{k=1}^{Y}[\mathbf{\Omega}(t)\mathbf{F}^{\text{ev}}]_k$, where the integrated event survival probability flux distribution vector $\mathbf{F}^{\text{ev}}$ is given by the solution to $\mathbf{F}^{\text{ev}} = \int_0^\infty \mathrm{d}t\, \mathbf{\Omega}(t)\mathbf{F}^{\text{ev}}$ with the constraint $\sum_{l=1}^{Y} F^{\text{ev}}_l = 1$. The survival probability distribution is discussed in the Supporting Information. Here, $\mathbf{F}^{\text{ev}}$, a dimensionless measure of the stationary event population flux distribution, reflects how the flux via the monitored transitions is distributed amongst the Y event states, with its elements corresponding to the nonzero elements of $\bar{\mathbf{P}}^{\text{ev}}$. Note that $\mathbf{F}^{\text{ev}}$ corresponds to the eigenvector of $\int_0^\infty \mathrm{d}t\, \mathbf{\Omega}(t)$ with an eigenvalue of unity. Additionally, $\mathbf{\Omega}(t)$ and $\mathbf{F}^{\text{ev}}$ can be employed to obtain the event-averaged joint PDF for a sequence of events (formulated in the Supporting Information), which can be used to characterize the correlations between events.[16] In the following section, we use the higher-order motifs introduced here to formulate $\mathbf{\Omega}(t)$, $\mathbf{F}^{\text{ev}}$, and $\phi(t)$ for a general kinetic model.

4. PATHWAY SOLUTIONS FOR A GENERAL KINETIC MODEL

Consider an $L \times M$ SM kinetic network consisting of unmonitored, reversible nearest-neighbor steps with monitored, irreversible periodic steps on one end (shown in Figure 2). Such a network can be used to model a number of (potentially) nonrenewal processes, including enzyme turnover with conformational interconversion (i.e., conformation-modulated enzyme turnover),[6,7] and transport through a discretized ion channel with multiple empty states.[9] Here, we use transition waiting time distribution functions of arbitrary form to express $\mathbf{\Omega}(t)$ for this general network in terms of self-consistent pathway solutions. The matrix $\mathbf{\Omega}(t)$ can be used to obtain measurable waiting time PDFs, including $\phi(t)$, which we formulate here as well. The key results of the section, eqs 12−15, are equivalent to eqs 1−4 for this scheme.

In our model, we employ the dual-coordinate system described in Section 3, with $\Theta = V$ and $\Lambda = H$, where H and V correspond to the horizontal and vertical directions, respectively, in Figure 2; note that $Y = L$ here. Presumably, each coordinate corresponds to a different type of transition, e.g., for conformation-modulated enzyme turnover, transitions in the H and V directions represent chemical and conformational transitions, respectively. As mentioned in Section 2, we require all transitions into an aggregate to proceed into the same underlying state. Consider $\grave{A}_{l,m}$: the waiting time distribution function for the unmonitored transition from this

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state to $\grave{A}_{l,n}$ in the H direction is $\check{Q}_{n\leftarrow ml|}(s)$ and that to $\grave{A}_{k,m}$ in the V direction is $\check{R}_{k\leftarrow |lm}(s)$. The monitored $\grave{A}_{l,M}$-to-$\grave{A}_{l,1}$ H direction transition corresponds to $\check{Q}_{M+1\leftarrow Ml|}(s)$. We expand $\check{Q}_{n\leftarrow ml|}(s)$ and $\check{R}_{k\leftarrow |lm}(s)$ about $s = 0$ in terms of their moments as $\check{Q}_{n\leftarrow ml|}(s) = q_{n\leftarrow ml|}[1 - \langle t_{Q_{n\leftarrow ml|}}\rangle s + O(s^2)]$ and $\check{R}_{k\leftarrow |lm}(s) = r_{k\leftarrow |lm}[1 - \langle t_{R_{k\leftarrow |lm}}\rangle s + O(s^2)]$, where the corresponding branching probabilities $q_{n\leftarrow ml|}$ and $r_{k\leftarrow |lm}$ account for proper normalization and are constrained by the normalization condition

$$\sum_n q_{n\leftarrow ml|} + \sum_k r_{k\leftarrow |lm} = 1 \tag{8}$$

We note that local detailed balance, which is violated in the monitored steps, results in the constraint $q_{m+1\leftarrow ml|}r_{l\leftarrow l+1|m}/(r_{l+1\leftarrow l|m}q_{m\leftarrow m+1|l}) = r_{l\leftarrow l+1|m+1}q_{m+1\leftarrow ml|l+1}/(q_{m\leftarrow m+1|l+1}r_{l+1\leftarrow l|m+1})$ for $m < M$. However, it is unnecessary to impose this relation for the purposes of our kinetic analysis, as our principal results hold, regardless of whether it is satisfied.

We begin our formulation by defining a waiting time matrix $\check{\mathbf{U}}^{(m)}(s)$ for V direction transitions within B_m as $\check{U}^{(m)}_{k,l}(s) = \check{R}_{k\leftarrow |lm}(s)$. Next, we define a matrix $\check{\mathbf{T}}^{n,m}(s)$ describing H direction transitions as $\check{T}^{n,m}_{j,k}(s) = \delta_{j,k}\check{Q}_{n\leftarrow ml|k}(s)$. These matrices are then used to construct the waiting time matrix

$$\check{\mathbf{W}}^{n,m}(s) = \check{\mathbf{T}}^{n,m}(s)[\mathbf{I}_L - \check{\mathbf{U}}^{(m)}(s)]^{-1} \tag{9}$$

which corresponds to the unmonitored B_m-to-B_n transition for $n \leq M$ and the monitored B_M-to-B_1 transition for $m = M$ and $n = M + 1$. The waiting time matrix $\check{\mathbf{Z}}^{(m)}(s)$, which accounts for manifold back-branching, corresponds to the passage out of and back to B_m via all possible paths (involving transitions between manifolds) in the region $H_n : n \leq m$ and is expressed in the recurrence relation

$$\check{\mathbf{Z}}^{(m)}(s) = \mathbf{I}_L + \check{\mathbf{Z}}^{(m)}(s)\check{\mathbf{W}}^{m,m-1}(s)\check{\mathbf{Z}}^{(m-1)}(s)\check{\mathbf{W}}^{m-1,m}(s) \tag{10}$$

which has the self-consistent solution

$$\check{\mathbf{Z}}^{(m)}(s) = [\mathbf{I}_L - \check{\mathbf{W}}^{m,m-1}(s)\check{\mathbf{Z}}^{(m-1)}(s)\check{\mathbf{W}}^{m-1,m}(s)]^{-1} \tag{11}$$

with $\check{\mathbf{Z}}^{(1)} = \mathbf{I}_L$. We note that the right-hand side of eq 11 can be expanded as $\sum_{i=0}^{\infty}[\check{\mathbf{W}}^{m,m-1}(s)\check{\mathbf{Z}}^{(m-1)}(s)\check{\mathbf{W}}^{m-1,m}(s)]^i$, where $[\check{\mathbf{W}}^{m,m-1}(s)\check{\mathbf{Z}}^{(m-1)}(s)\check{\mathbf{W}}^{m-1,m}(s)]^i$ corresponds to the passage of and back to B_m i times via paths (involving transitions between manifolds) in the region $H_n : n \leq m$. We use these pathway solutions to obtain $\check{\mathbf{\Omega}}(s)$ via concatenation as

$$\check{\mathbf{\Omega}}(s) = \prod_{1}^{m=M} \check{\mathbf{W}}^{m+1,m}(s)\check{\mathbf{Z}}^{(m)}(s) \tag{12}$$

The first-passage time PDF is then given by

$$\check{\phi}(s) = \sum_{j=1}^{L} [\check{\mathbf{\Omega}}(s)\mathbf{F}^{ev}]_j \tag{13}$$

where $\mathbf{F}^{ev}$ is given by the solution to

$$\mathbf{F}^{ev} = \check{\mathbf{\Omega}}(0)\mathbf{F}^{ev} \tag{14}$$

with the constraint

$$\sum_{l=1}^{L} F^{ev}_l = 1 \tag{15}$$

5. EXPLICIT CALCULATIONS

5.1. General Four-State Model. Here, we provide explicit calculations for the kinetic model considered in Section 4 with $L = M = 2$. In Section 5.1, rather than using the typical rate description, we employ a more general time/probability description,[14,19] which has the added advantage of more compact expressions. We begin by considering $\mathbf{F}^{ev}$ and the mean first-passage time $\langle t\rangle = \int_0^\infty dt\, \phi(t)t = -d\check{\phi}(s)/ds|_{s=0}$, which can be expressed as (derivations in the Supporting Information)

$$\mathbf{F}^{ev} = D\begin{pmatrix} q_{3\leftarrow 2|1}[q_{2\leftarrow 1|1}r_{1\leftarrow 2|1} + r_{1\leftarrow 2|2}q_{2\leftarrow 1|2}] \\ q_{3\leftarrow 2|2}[q_{2\leftarrow 1|2}r_{2\leftarrow 1|1} + r_{2\leftarrow 1|2}q_{2\leftarrow 1|1}] \end{pmatrix} \tag{16}$$

and

$$\begin{aligned}\langle t\rangle = D(&\grave{\tau}_{1,1}[(q_{1\leftarrow 2|1} + q_{3\leftarrow 2|1})r_{1\leftarrow 2|2} + r_{1\leftarrow 2|1}(q_{1\leftarrow 2|2} + q_{3\leftarrow 2|2})] + \grave{\tau}_{2,1}[(q_{1\leftarrow 2|2} + q_{3\leftarrow 2|2})r_{2\leftarrow 1|2} \\ &+ r_{2\leftarrow 1|1}(q_{1\leftarrow 2|1} + q_{3\leftarrow 2|1})] + \grave{\tau}_{1,2}[q_{2\leftarrow 1|1}r_{1\leftarrow 2|1} \\ &+ r_{1\leftarrow 2|2}q_{2\leftarrow 1|2}] + \grave{\tau}_{2,2}[q_{2\leftarrow 1|2}r_{2\leftarrow 1|1} + r_{2\leftarrow 1|2}q_{2\leftarrow 1|1}])\end{aligned} \tag{17}$$

where

$$\begin{aligned}D^{-1} = &q_{3\leftarrow 2|1}(q_{2\leftarrow 1|1}r_{1\leftarrow 2|1} + r_{1\leftarrow 2|2}q_{2\leftarrow 1|2}) \\ &+ q_{3\leftarrow 2|2}(q_{2\leftarrow 1|2}r_{2\leftarrow 1|1} + r_{2\leftarrow 1|2}q_{2\leftarrow 1|1})\end{aligned} \tag{18}$$

and the mean $\grave{A}_{l,m}$ decay time (discussed in the Supporting Information) is given by $\grave{\tau}_{l,m} = \sum_n q_{n\leftarrow ml|}\langle t_{Q_{n\leftarrow ml|}}\rangle + \sum_k r_{k\leftarrow |lm}\langle t_{R_{k\leftarrow |lm}}\rangle$. Equations 17 and 18 show that, in its most general form, $\langle t\rangle$ is compactly expressed in terms of branching probabilities and mean decay times, consistent with the approach developed by Ninio.[14] Thus, for our first-order (in t) analysis, it is unnecessary to expand the transition waiting time distribution functions beyond their first-order (in s) terms. In general, these expansions can be truncated at the order corresponding to that of the highest-order waiting time moment being calculated. Note that the branching probabilities are constrained by eq 8, such that only 6 are independent here (5 if the aforementioned constraint resulting from local detailed balance (see Section 4) is satisfied); thus, 10 parameters (or 9) are needed to specify $\langle t\rangle$, which corresponds to the number of rates necessary to specify the kinetics in the rate description, i.e., the number of independent (based on the local detailed balance constraint) transitions in the scheme. In fact, even when the kinetics are non-Poissonian, this one-to-one correspondence between independent transitions and parameters necessary for specification of the kinetics holds to first order in t (but not higher[12]). Also, note that $\mathbf{F}^{ev}$ is expressed in terms of only branching probabilities in this description.

The mean first-passage time can be written as[6,7,20] $\langle t\rangle = \sum_l^L\sum_m^M \grave{\tau}^{res}_{l,m}$, where $\grave{\tau}^{res}_{l,m}$ is the mean $\grave{A}_{l,m}$ residence time (discussed in the Supporting Information). For $L = M = 2$,

$$\begin{aligned}\grave{\tau}^{res}_{1,1} &= D\grave{\tau}_{1,1}[(q_{1\leftarrow 2|1} + q_{3\leftarrow 2|1})r_{1\leftarrow 2|2} + r_{1\leftarrow 2|1}(q_{1\leftarrow 2|2} + (q_{3\leftarrow 2|2})] \\ \grave{\tau}^{res}_{2,1} &= D\grave{\tau}_{2,1}[(q_{1\leftarrow 2|2} + q_{3\leftarrow 2|2})r_{2\leftarrow 1|2} + r_{2\leftarrow 1|1}(q_{1\leftarrow 2|1} + (q_{3\leftarrow 2|1})] \\ \grave{\tau}^{res}_{1,2} &= D\grave{\tau}_{1,2}[q_{2\leftarrow 1|1}r_{1\leftarrow 2|1} + r_{1\leftarrow 2|2}q_{2\leftarrow 1|2}] \\ \grave{\tau}^{res}_{2,2} &= D\grave{\tau}_{2,2}[q_{2\leftarrow 1|2}r_{2\leftarrow 1|1} + r_{2\leftarrow 1|2}q_{2\leftarrow 1|1}]\end{aligned} \tag{19}$$

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J. Phys. Chem. B 2018, 122, 4601−4610

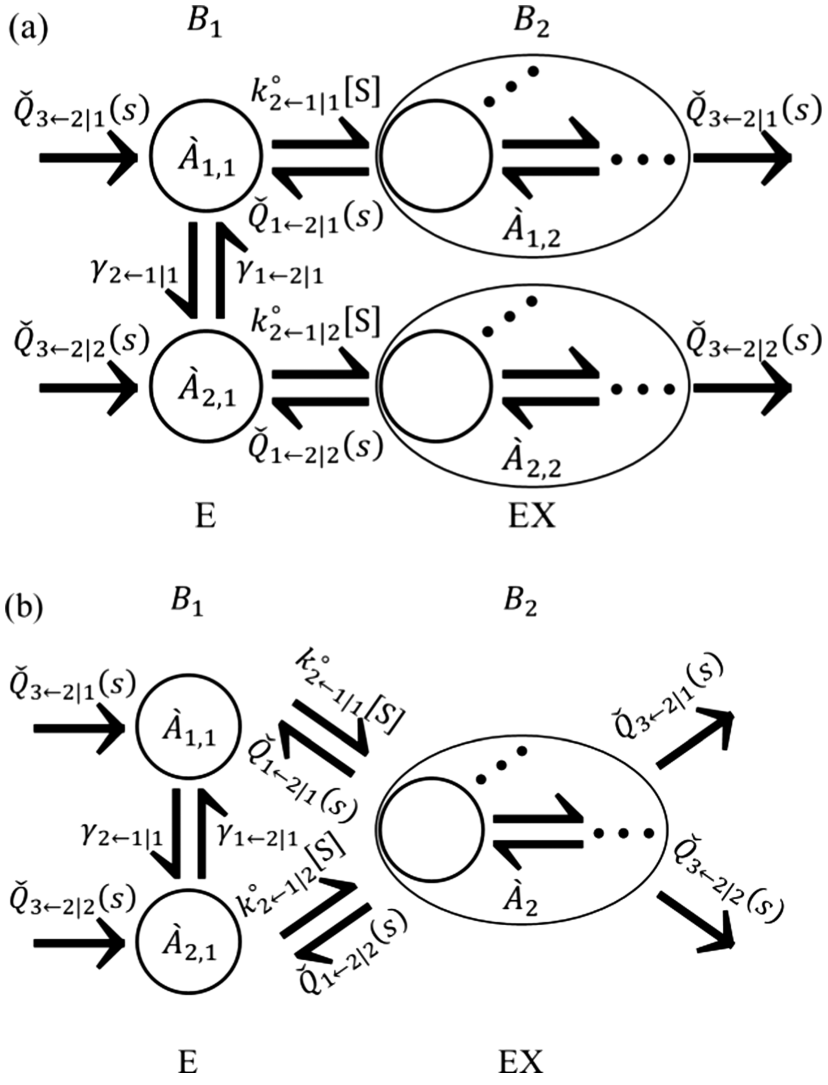

Figure 3. Generic schemes I (a) and II (b) for single-enzyme turnover with conformational interconversion. Each unbound enzymatic state is unaggregated, so transitions out of it are rate processes. Transitions out of the possibly aggregated bound enzymatic state(s) may be non-Poissonian and are thus described by waiting time distribution functions of arbitrary form. We note that generic scheme II (b) corresponds to a renewal process even though it involves a nontrivial event-averaged initial condition.

We can now write the integrated $+H$ direction survival probability flux in the time/probability description as

$$F_{l,m} = q_{m\leftarrow m-1|l}\frac{\grave{\tau}^{\mathrm{res}}_{l,m-1}}{\grave{\tau}_{l,m-1}} - q_{m-1\leftarrow m|l}\frac{\grave{\tau}^{\mathrm{res}}_{l,m}}{\grave{\tau}_{l,m}} \tag{20}$$

which corresponds to the unmonitored step between $\grave{A}_{l,m-1}$ and $\grave{A}_{l,M}$ for $1 < m \leq M$ and the monitored $\grave{A}_{l,M}$-to-$\grave{A}_{l,1}$ transition for $m = M + 1$. Here, $F_{l,1} \equiv F_{l,M+1} = F^{\mathrm{ev}}_l$; thus, $\sum_{l=1}^{L} F_{l,1} = 1$. Because $\grave{\tau}^{\mathrm{res}}_{l,m} \propto \grave{\tau}_{l,m}$, $F_{l,m}$ depends only upon branching probabilities in this description. We note that $F_{l,2} = [(\mathbf{I}_L - \check{\mathbf{W}}^{1,2}(0))\check{\mathbf{Z}}^{(2)}(0)\check{\mathbf{W}}^{2,1}(0)\mathbf{F}_{\mathrm{ev}}]_l$, which reduces to its corresponding form in eq 20. We can similarly write the integrated $+V$ direction survival probability flux for the step between $\grave{A}_{l-1,m}$ and $\grave{A}_{l,m}$ as

$$J_{l,m} = r_{l\leftarrow l-1|m}\frac{\grave{\tau}^{\mathrm{res}}_{l-1,m}}{\grave{\tau}_{l-1,m}} - r_{l-1\leftarrow l|m}\frac{\grave{\tau}^{\mathrm{res}}_{l,m}}{\grave{\tau}_{l,m}} \tag{21}$$

with $J_{1,m} = 0$. We note that the stationary population fluxes corresponding to $F_{l,m}$ and $J_{l,m}$ can be expressed as $F^{\mathrm{s}}_{l,m} = F_{l,m}/\langle t\rangle$ and $J^{\mathrm{s}}_{l,m} = J_{l,m}/\langle t\rangle$, respectively. Thus, $F_{l,m}$ and $J_{l,m}$ are event-normalized measures of their stationary analogues. By population conservation, we can write a flux balance relation for $\grave{A}_{l,m}$ as $F_{l,m} + J_{l,m} = F_{l,m+1} + J_{l+1,m}$. This can be employed, in conjunction with other expressions formulated in this paragraph, to perform the flux balance procedure (previously done using the rate description[6,7]) as an alternative method for evaluating $\langle t\rangle$ in the time/probability description. We note that all expressions presented in this paragraph are applicable to schemes with arbitrarily large L and M, except that for $\grave{\tau}^{\mathrm{res}}_{l,m}$ with $L = M = 2$.

The flux balance relations for $\grave{A}_{l,1}$ and $\grave{A}_{l,2}$ can be combined to show $J_{2,1} = -J_{2,2} = J$, the stationary modulatory population current for our four-state model normalized by $\langle t\rangle^{-1}$, which implies $F_{1,2} + F_{2,2} = 1$. In the context of enzyme turnover with conformational interconversion, modulatory current corresponds to conformational current. We express J as

$$J = D[q_{2\leftarrow 1|2}r_{2\leftarrow 1|1}(q_{1\leftarrow 2|1} + q_{3\leftarrow 2|1})r_{1\leftarrow 2|2} - q_{2\leftarrow 1|1}r_{1\leftarrow 2|1}(q_{1\leftarrow 2|2} + q_{3\leftarrow 2|2})r_{2\leftarrow 1|2}] \tag{22}$$

and this vanishes under the modulatory detailed balance condition

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$$\frac{r_{2\leftarrow 1|1}(q_{1\leftarrow 2|1} + q_{3\leftarrow 2|1})}{r_{2\leftarrow 1|2}q_{2\leftarrow 1|1}} = \frac{r_{1\leftarrow 2|1}(q_{1\leftarrow 2|2} + q_{3\leftarrow 2|2})}{r_{1\leftarrow 2|2}q_{2\leftarrow 1|2}} \quad (23)$$

which is satisfied when the probabilities of traversing the four-state loop in the clockwise and counterclockwise directions are equal. We note that the satisfaction of the aforementioned constraint resulting from local detailed balance still permits the violation of modulatory detailed balance. In addition, the satisfaction of modulatory detailed balance does not imply the satisfaction of (global) detailed balance, which is always violated here for nontrivial kinetics.

5.2. Generic Schemes for Enzyme Turnover. We now consider two generic schemes for single-enzyme turnover with conformational interconversion (illustrated in Figure 3). A generic scheme, rather than a predetermined one, can generate all possible kinetic models with the same basic topological connectivity and thus requires minimal assumptions on the form of the underlying scheme. Generic scheme I (shown in Figure 3a) is an extension of our four-state model from Section 5.1, with $r_{1\leftarrow 2|2} = r_{2\leftarrow 1|2} = 0$. Here, $\grave{A}_{1,2}$ and $\grave{A}_{2,2}$ may be aggregates and thus have arbitrarily complex internal topologies. As a result, the scheme corresponds to all possible models with two parallel (although not necessarily symmetric) pathways. In generic scheme II (illustrated in Figure 3b), $\grave{A}_{1,2}$ and $\grave{A}_{2,2}$ are replaced by a new state $\grave{A}_2$, which may be an aggregate, with B_2 consisting only of $\grave{A}_2$. Within the framework of our formulation in Section 4, this is achieved by making the definitions $[\mathbf{I}_2 - \check{\mathbf{U}}^{(2)}(s)]^{-1}_{k,l} \equiv 1$ and $\grave{\tau}_{1,2} = \grave{\tau}_{2,2} \equiv \grave{\tau}_2$ (for a first-order [in t] analysis), thereby turning our four-state model from Section 5.1 into a three-state generic scheme.

We are interested in the dependence of $\langle t \rangle$ on an external substrate, S, whose chemical conversion to product is catalyzed by a single enzyme. In our models, S can bind (B_1-to-B_2 transition) to the free enzyme, E (state manifold represented by B_1), to form a substrate-bound enzymatic complex, which may be converted to additional bound enzymatic complexes, depending on the underlying mechanism. Here, B_2 represents the state manifold for the bound enzymatic complex(es), EX, which can return to the unbound form via unbinding (unmonitored B_2-to-B_1 transition) or product formation (monitored B_2-to-B_1 transition), with the latter marking the completion of a turnover. Manifold B_1 consists of two unaggregated states, but the state(s) in B_2 may be aggregated. We assume the enzyme undergoes fast and irreversible regeneration, such that another turnover can begin immediately following the formation of a product molecule. In addition, reverse product formation is assumed to be much slower than S binding, making enzyme turnover irreversible here. We assume the enzyme has a single binding site; thus, S binding is the only transition-dependent upon S concentration [S], which is large relative to that of the single enzyme and maintained externally, such that it is effectively time-independent, and the nonlinear kinetic binding process can be treated as pseudolinear. Turnover is modulated by the slow conformational dynamics of E, such that V direction transitions within B_1 represent conformational changes. In generic scheme I, the conformational dynamics of EX are much slower than the chemical transitions within and out of $\grave{A}_{1,2}$ and $\grave{A}_{2,2}$, so they are neglected. In generic scheme II, the conformational dynamics of the first bound enzymatic complex are so fast that the complex (but not necessarily the system) achieves conformational equilibrium, although any subsequent enzymatic complexes need not be in conformational equilibrium. We note that this scheme corresponds to a renewal process, even though it involves a nontrivial event-averaged initial condition, because the enzymatic pathways converge at the first bound enzymatic complex, so the conformational history is "forgotten" between events.

In generic schemes I and II, B_1 consists of unaggregated states. Thus, in both cases, the transitions out of $\grave{A}_{l,1}$ are rate processes, with pseudolinear S-binding rate $k_{2\leftarrow 1|l}$ and conformational interconversion rate $\gamma_{k\leftarrow l|1}$, such that

$$q_{2\leftarrow 1|l} = \frac{k_{2\leftarrow 1|l}}{\sum_k \gamma_{k\leftarrow l|1} + k_{2\leftarrow 1|l}} \quad (24)$$

$$\grave{\tau}_{l,1} = \left[\sum_k \gamma_{k\leftarrow l|1} + k_{2\leftarrow 1|l}\right]$$

and (the nonzero) $r_{k\leftarrow l|1}$ is specified by eq 8. The S-binding rate is proportional to [S]; thus, we introduce [S] as $k_{2\leftarrow 1|l} = k^{\circ}_{2\leftarrow 1|l}[S]$, with rate constant $k^{\circ}_{2\leftarrow 1|l}$. The functional dependence of $\langle t \rangle$ on [S] can now be determined with the state(s) in B_2 possibly being aggregated. This is advantageous because enzyme turnover can proceed through multiple intermediate complexes[6,7,21] (beyond just a single enzyme−substrate complex); thus, we can capture the transition dynamics of these additional intermediates in a simple manner, without loss of generality. Furthermore, we previously showed[6,7] that the effect of slow conformational dynamics on the functional form of $\langle t([S]) \rangle$ for the arbitrarily large model considered in Section 4, with all rate processes, is governed by the interplay between B_1 and the combined manifold corresponding to the set $\{B_n: n > 1\}$. In fact, in the absence of stationary conformational currents between these two manifolds, the turnover rate (i.e., the steady-state rate of product formation), given by $\nu = \langle t \rangle^{-1}$ when the monitored steps are irreversible, reduces to the Michaelis−Menten (MM) functional form (with respect to [S]), consistent with experimental work[18] and additional theoretical analysis.[22] The celebrated MM equation, which corresponds to the scheme in Section 4 with $L = 1$, $M = 2$, and all rate processes, is[23] $\nu_{MM}([S]) = [1/k_{3\leftarrow 2} + K_M/(k_{3\leftarrow 2}[S])]^{-1}$, where $K_M = (k_{1\leftarrow 2} + k_{3\leftarrow 2})/k^{\circ}_{2\leftarrow 1}$. Thus, with only the assumption of fast conformational dynamics for the first bound enzymatic complex, generic scheme II, an $M = 2$ scheme, can capture the interesting effects of modulation (to first order in t) corresponding to a network with M arbitrarily larger than 2, without loss of generality. With the above in mind, we investigate $\nu([S])$ for generic schemes I and II, with a particular emphasis on the role of conformational dynamics.

For generic scheme I, $\langle t([S]) \rangle$ is obtained by substituting eq 24, $k_{2\leftarrow 1|l} = k^{\circ}_{2\leftarrow 1|l}[S] \forall l$ and $r_{1\leftarrow 2|2} = r_{2\leftarrow 1|2} = 0$ into eqs 17 and 18 and imposing the constraint in eq 8, resulting in

$$\langle t([S]) \rangle = a + \frac{b}{[S]} \quad (25)$$

where

$$a = \frac{\grave{\tau}_{1,2}k^{\circ}_{2\leftarrow 1|1}\gamma_{1\leftarrow 2|1} + \grave{\tau}_{2,2}k^{\circ}_{2\leftarrow 1|2}\gamma_{2\leftarrow 1|1}}{\alpha}$$

$$b = \frac{\gamma_{2\leftarrow 1|1} + \gamma_{1\leftarrow 2|1}}{\alpha} \quad (26)$$

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J. Phys. Chem. B 2018, 122, 4601−4610

with $\alpha = q_{3\leftarrow 2|1}k^{\circ}_{2\leftarrow 1|1}\gamma_{1\leftarrow 2|1} + q_{3\leftarrow 2|2}k^{\circ}_{2\leftarrow 1|2}\gamma_{2\leftarrow 1|1}$. Note that eight parameters are needed here to specify the non-Poissonian kinetics (to first order in t). Because $J = 0$ here, $v([S])$ follows the MM functional form, consistent with previous predictions involving all rate processes.[6,7] Equations 25 and 26 generalize the mean first-passage time for arbitrary parallel (although not necessarily symmetric) enzymatic pathways.

For generic scheme II, $\langle t([S])\rangle$ is expressed as (derivations in the Supporting Information)

$$\langle t([S])\rangle = a_0 + \frac{b_0}{[S]} + \frac{b_1}{[S] + c_1} \tag{27}$$

where

$$a_0 = \frac{\grave{\tau}_2}{\beta} \tag{28}$$

$$b_0 = \frac{\gamma_{2\leftarrow 1|1} + \gamma_{1\leftarrow 2|1}}{\beta c_1 k^{\circ}_{2\leftarrow 1|1}k^{\circ}_{2\leftarrow 1|2}}$$

$$b_1 = \frac{(1/k^{\circ}_{2\leftarrow 1|1} - 1/k^{\circ}_{2\leftarrow 1|2})(\gamma_{2\leftarrow 1|1}\acute{K}_{M_1} - \gamma_{1\leftarrow 2|1}\acute{K}_{M_2})}{\beta c_1}$$

$$c_1 = \frac{\gamma_{2\leftarrow 1|1}}{k^{\circ}_{2\leftarrow 1|1}} + \frac{\gamma_{1\leftarrow 2|1}}{k^{\circ}_{2\leftarrow 1|2}}$$

with $\beta = q_{3\leftarrow 2|1} + q_{3\leftarrow 2|2}$ and $\acute{K}_{M_i} = (q_{1\leftarrow 2|l} + q_{3\leftarrow 2|l})/k^{\circ}_{2\leftarrow 1|l}$. For transitions out of $\grave{A}_2$, the normalization condition in eq 8 is modified to $\sum_{l,n} q_{n\leftarrow 2|l} = 1$ (although eq 8 still holds for transitions out of $\acute{A}_{i,1}$). Thus, eight parameters are needed here to specify the mean first-passage time; however, if the aforementioned constraint resulting from local detailed balance (see Section 4), which is modified here to $\gamma_{2\leftarrow 1|1}q_{1\leftarrow 2|1}/k^{\circ}_{2\leftarrow 1|1} = \gamma_{1\leftarrow 2|1}q_{1\leftarrow 2|2}/k^{\circ}_{2\leftarrow 1|2}$, is satisfied, then only seven parameters are needed. In addition, a_0, b_0, and c_1 cannot be negative but b_1 can. Equation 27 shows that, for generic scheme II, $\langle t([S])\rangle$ follows the single-conformation functional form with a non-MM correction term. Notably, this is the same basic functional form for $\langle t([S])\rangle$ as that obtained for a conformation-modulated scheme with two symmetric conformational channels of arbitrary length.[6,7] Thus, when $\grave{A}_2$ is unaggregated, we achieve a minimal model for conformational nonequilibrium enzyme kinetics. The conformational current for generic scheme II is given by $J([S]) = (\gamma_{2\leftarrow 1|1}\acute{K}_{M_1} - \gamma_{1\leftarrow 2|1}\acute{K}_{M_2})/[\beta([S] + c_1)]$, which is related to the non-MM correction term in eq 27 as

$$\frac{b_1}{[S] + c_1} \propto \left(\frac{1}{k^{\circ}_{2\leftarrow 1|1}} - \frac{1}{k^{\circ}_{2\leftarrow 1|2}}\right)J([S]) \tag{29}$$

As expected, b_1 vanishes when $J = 0$, which is achieved under the conformational detailed balance condition

$$\gamma_{2\leftarrow 1|1}\acute{K}_{M_1} = \gamma_{1\leftarrow 2|1}\acute{K}_{M_2} \tag{30}$$

Additionally, b_1 can vanish under the alternative condition $k^{\circ}_{2\leftarrow 1|1} = k^{\circ}_{2\leftarrow 1|2}$, even for nonzero J. The general condition for this alternative reduction has been formulated for symmetric conformational channels,[7] but in this case, because the enzymatic pathways converge at the first bound enzymatic complex, it reduces to the simple condition of equal binding rate constants for the two initial conformations. Thus, we see that conformational detailed balance is a sufficient but not necessary condition for MM kinetics. We note that b_1/b_0 represents a unique non-MM parameter for characterizing cooperativity resulting from nonequilibrium conformational dynamics; we refer readers to our previous work[6,7] for a rigorous analysis of predicting and characterizing such behavior and note that this topic has been a point of focus in other recent studies[19,24,25] as well.

6. CONCLUSIONS

In this article, a recently developed pathway technique (paper 1)[10] for describing SM kinetics has been extended to nonrenewal processes, which involve correlations between events. Our approach employs transition waiting time distribution functions of arbitrary form, avoiding the need to assume Poissonian kinetics. This is particularly advantageous for kinetic schemes involving hidden intermediates, the dynamics of which can be accounted for implicitly, without loss of generality. We have formulated measurable waiting time PDFs for a general kinetic network model in terms of solutions to self-consistent pathway equations. Additionally, we have performed explicit calculations for such a model, which we have adapted to two generic schemes for conformation-modulated enzyme turnover. The functional form of the mean first-passage time, with respect to the concentration of an external substrate, has been examined for each generic scheme, wherein the intermediate state(s) need not undergo Poissonian decay. In the absence of conformational current, the enzyme turnover rate reduces to the MM functional form, consistent with previous studies on similar schemes with all rate processes,[6,7,22] thereby establishing further generality to this intriguing finding. In addition to enzyme turnover, our framework may serve as a useful kinetic tool for other SM processes, including molecular motor translocation,[8] ion transport,[9] and fluorescence emission,[10] as well as for single-nanoparticle catalysis.[26]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.7b10507.

Formulation of expressions corresponding to various types of single-molecule measurements and general kinetic model derivations (PDF)

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Notes

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