

Back-stepping, hidden substeps, and conditional dwell times in molecular motors

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(Received 6 November 2006; published 20 February 2007)

Processive molecular motors take more-or-less uniformly sized steps, along spatially periodic tracks, mostly forwards but increasingly backwards under loads. Experimentally, the major steps can be resolved clearly within the noise but one knows biochemically that one or more mechanochemical substeps remain hidden in each enzymatic cycle. In order to properly interpret experimental data for back-to-forward step ratios, mean conditional step-to-step dwell times, etc., a first-passage analysis has been developed that takes account of hidden substeps in N -state sequential models. The explicit, general results differ significantly from previous treatments that identify the observed steps with complete mechanochemical cycles; e.g., the mean dwell times τ_+ and τ_- prior to forward and back steps, respectively, are normally *unequal* although the dwell times τ_{++} and τ_{--} between successive forward and back steps are equal. Illustrative ($N=2$)-state examples display a wide range of behavior. The formulation extends to the case of two or more detectable transitions in a multistate cycle with hidden substeps.

DOI: 10.1103/PhysRevE.75.021909

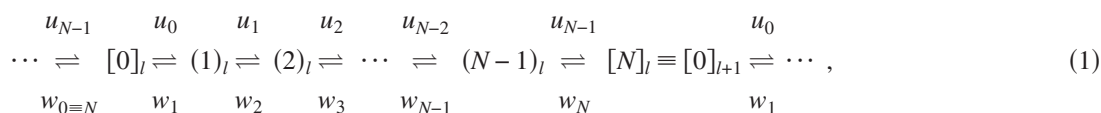
PACS number(s): 87.16.Nn, 05.20.Dd, 05.40.-a, 82.37.-j

I. INTRODUCTION

Processive motor proteins or molecular motors [1–3] (such as kinesin, cytoplasmic dynein, and myosin V) “walk” along molecular tracks (microtubules and actin filaments) taking *observed mechanical steps* of well-defined (mean) spacing d , each step being of “negligibly short” ($\lesssim 100 \mu\text{s}$) duration relative to the mean time(s) between steps that are of order 1 to 20 ms [1–18]. Steps may be taken *forwards* (+) or *backwards* (–). The mean velocity (observed over tens to hundreds) of steps, V , is a *function* of the load $\mathbf{F} = (F_x, F_y, F_z)$ exerted on the motor and the fuel concentration

[ATP] (for most cases) [19–21] and, in general, of other features of the aqueous solution including the $p\text{H}$, ionic strength, temperature T , and other reagents and/or reactants such as [ADP], [Pi], [AMP·PNP], [BeF₂], etc.) [1,7,14,17].

Motor proteins are enzymatic *catalysts* that, following biochemical knowledge and principles, turn over one “fuel molecule” (usually ATP) for each full step via (in the simplest case) a *linear sequence of reversible kinetic transitions* (or *reactions*) embodying N (bio)chemical states per turnover [19–25]. This situation is embodied in the following *basic sequential model*:



which is understood to repeat periodically as the motor moves processively along its track. The subscript $l=1, 2, \dots$ labeling the N basic states $[0], (1), \dots, (N-1)$, denotes the sites on the linear track spaced at distance d apart.

By convention the state $(i)=[0]$ is “bound” or “nucleotide-free” so that the transition $[0] \rightarrow (1)$ represents the *binding* of one fuel molecule to the awaiting motor. Thus we write

$$u_0 = k_0[\text{ATP}], \quad (2)$$

where the pseudo-first-order rate constant k_0 and all the remaining rate constants u_i, w_j depend also on \mathbf{F} . But under fixed conditions ($\mathbf{F}, [\text{ATP}], \dots$), the rates do not change. Note that this formulation embodies the *tight coupling principle* of one fuel molecule being consumed per (forward) step [1,19,20,25]. This is *assumed* in the basic model, which also neglects irreversible *detachments* from the track (which, however, can be included readily in principle [19,22,23]).

When convenient we will allow the state labels i, j, \dots to take values outside the basic range $[0, N-1]$; for that reason we adopt the periodicity convention

$$u_{i+lN} \equiv u_i, \quad w_{j+lN} \equiv w_j, \quad l = 0, \pm 1, \pm 2, \dots \quad (3)$$

Now, in the simplest experimental situation, as observed for kinesin, no *mechanical substeps* are detected [6,14] to

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within the noise level (which amounts to $\Delta x \lesssim 1$ nm). Furthermore to within the resolution time ($\lesssim 100$ μ s), successive steps occur at times, say, $\dots, t_{k-1}, t_k, t_{k+1}, \dots$. Thus, between the identifiable mechanical steps of (mean) magnitude d , the motor *dwells* in a *mechanical state* that, within the noise level Δx , appears well defined with no systematically detectable *substeps*, forwards or backwards. Then, individual *dwelt times* in the mechanical states, namely,

$$\tau_k = t_k - t_{k-1}, \quad (4)$$

can be measured to reasonable precision and averages may be computed, over “many” observations encompassing, say, n steps, to yield an *overall mean dwelt time*

$$\tau = \langle \tau_k \rangle \approx \frac{1}{n} \sum_{k=1}^n \tau_k. \quad (5)$$

Here and below we use the “asymptotically equals” symbol \approx to indicate an approximate equality that becomes exact in a long run under steady-state conditions.

Given a (sufficiently long) sequence of n observed steps with n_+ forward steps and n_- backward steps, we can also define the (steady-state) *step splitting probabilities* or back-step and forward-step fractions

$$\pi_+ \approx n_+/n, \quad \pi_- \approx n_-/n, \quad (6)$$

where, since $n = n_+ + n_-$, one has

$$\pi_+ + \pi_- = 1. \quad (7)$$

Furthermore, dwell times before a $+$ or $-$ step can be (and have been [14]) measured separately leading to distinct *prior dwell times*

$$\tau_+ = \frac{1}{n_+} \sum^+ \tau_k \quad \text{and} \quad \tau_- = \frac{1}{n_-} \sum^- \tau_k, \quad (8)$$

the restricted sums including just $+$ or $-$ steps, respectively.

To the degree that the runs are long so that π_+ and π_- may be accurately considered as probabilities one must evidently also have

$$\pi_+ \tau_+ + \pi_- \tau_- = \tau. \quad (9)$$

As discussed recently in some detail [21,24], each individual biochemical state (i), may be characterized by a definite (mean) *longitudinal location* in physical space, i.e., *along* the track, which we supposed aligned with the x coordinate, and, possibly, *transverse* to the track, the y coordinate, or *normal* to the track, the z coordinate. Hence the basic model implies the existence of *substeps*, say, of magnitude

$$d_j = x_{j+1} - x_j, \quad (10)$$

between successive mechanochemical states [26]. However, the great majority of these mechanical displacements will be *hidden* by noise and so *unobservable*. This is the crucial issue.

The evidence (in particular for kinesin [6,14]) reveals the existence of one principal or *major mechanical substep* of magnitude

$$d_M = x_{M+1} - x_M \approx d, \quad (11)$$

that corresponds to a specific transition (M) \rightarrow ($M+1$) for a *forward* or $+$ *step*. Such a unique forward-step is sometimes called a “power stroke.” Then, clearly, within the basic model a back-step ($-$) corresponds to the specific transition ($M+1$) \rightarrow (M).

For simplicity we will initially suppose that there is *only one* such single, well defined and observable principal mechanical transition in the processive reaction cycle: it will be referred to as a *major transition* while all other smaller, unobservable displacements, presumed “hidden,” will be termed *substeps*.

It is of interest, all the same, to analyze situations in which, within the full cycle, there are a number of *visible* (or observable) *substeps*. Indeed, an initial substep large enough to be readily observable was predicted for myosin V by Kolomeisky and Fisher [20] on the basis of dwell-time data obtained at different [ATP] and force levels [4]; it was later observed unambiguously by Uemura *et al.* [11]. Thus, in Sec. V below, the case of K ($< N$) distinct major substeps is considered explicitly [27].

Nevertheless, since most of the forward and reverse transitions, (i) \rightarrow ($i-1$) and ($i-1$) \rightarrow (i), are *not* observable, one does *not* know (and *cannot* tell) the (bio)chemical (sub)state of the motor *during an observed dwell time*, τ_k , between steps $k-1$ and k : see (4). Indeed, the biochemical state will change as time progresses and not necessarily in a uniform sense, e.g., ATP might bind and then be released (or unbind) without undergoing a hydrolysis step. For this *crucial reason* once the basic model has $N=2$ or more states the expressions for τ_+ and τ_- in terms of the basic rates u_i and w_j *cannot be trivial*—and the same goes for the splitting or backwards and forwards probabilities π_+ and π_- .

The basic theoretical problem is thus to find *explicit expressions* for π_+ , π_- , and for the *partial dwell times* as well as for *conditional* or *pairwise* stepping fractions, π_{++} , π_{+-} , \dots , and dwell times, τ_{++} , τ_{+-} , \dots , that it is natural to introduce (as seen below). Indeed, it is clear that these general statistical concepts are not restricted to linear or translocational motors, on which we have focussed; in fact, they apply equally to *rotary stepping motors* like F₁-ATPase [28–31], F₁F₀-ATPase [32,33], and bacterial flagellar motors [2,34]. However, since, in these respects, kinesin and myosin V have been studied more extensively, we will retain the language appropriate for processive motor proteins walking on linear tracks.

In previous theoretical studies [20,35–37] splitting probabilities and conditional mean dwell times have been introduced in the context of molecular motors *via* the following definitions (which are distinguished from those used in the discussion above by haccs) specifically: $\check{\pi}_+ \equiv \pi_{0,N}$ and $\check{\pi}_- \equiv \pi_{0,-N}$ are the probabilities that a motor starting at a well-defined physical site l along the track in the binding state (j)= $[0]$ will arrive at the next site $l+1$ or back at the previous site $l-1$ [in both cases in state (j)= $[0]$] without having undergone the opposite transition to complete a full backward or forward cycle, respectively. Then, similarly, $\check{\tau}_\pm \equiv \tau_{0,\pm N}$ are the average times a motor spends at site l (starting at $j=0$) before completing a full forward or backward cycle to site $l \pm 1$ [in state (j)= $[0]$]. Exact results for such

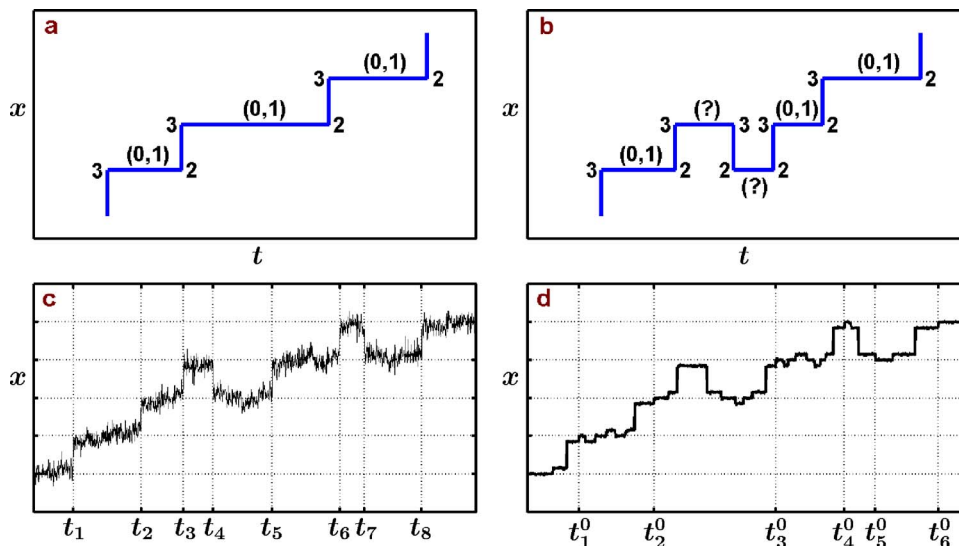


FIG. 1. (Color online) Schematic graphs illustrating why the full-cycle interpretation is not adequate for describing stepping data: Plot (a) depicts a hypothetical time series of forward steps for an ($N=4$) cycle with $M=2$. The (0,1)'s indicate that the states $[0]_l$ and $(1)_l$ are both certain to occur at least once between the pairs of states $(3)_{l-1}$ and $(2)_l$. (See the text.) Then (b) represents a similar time series but with one major back step. The question marks between the forward-back and back-forward steps indicate that one does not know if the motor has ever passed through the states $[0]_l$ and/or $(1)_l$ in these intervals. Finally, (c) and (d) depict two equivalent time series for a motor with $N=3$ and $M=1$ but with different noise levels. The first plot allows one to identify only major forward and backward transitions at times t_k (marked on the axis) while the states $[0]_l$ and corresponding substeps are hidden in the noise. However, the second plot reveals all the transitions and substeps, so that the t_k^0 , marking the beginning (or end) of each full cycle, can be determined. These schematic examples demonstrate that detectable transitions do not necessarily correspond to a full biochemical cycle so that a proper statistical analysis must take account of substeps hidden in the noise. Notice, indeed, that the three major transitions identified as forward and back steps at times t_3, t_4 , and t_5 (on the left) are associated with only a single complete cycle from t_2^0 to t_3^0 .

statistics can be derived by mapping into a Markov renewal process [36,37].

With these definitions the explicit formulas obtained [20,35] for $\tilde{\pi}_\pm$ and $\tilde{\tau}_\pm$ correspond to a *full mechanochemical cycle* during which a complete forward or backward step is certainly taken. However, the resulting expressions can be applied to the analysis of experimental stepping data *only* if these data allow one to identify each full cycle. If, instead, the observed noise hides one or more of the $N > 1$ biochemical or mechanochemical substeps, while only major mechanical transitions are detectable, one *cannot* in general decide unambiguously whether a motor executed the detectable steps (or power strokes) with or without completing a full cycle. In such cases, the previous expressions cannot be applied to account for the observed step fractions and dwell times π_\pm and τ_\pm . Instead, the results must be modified to allow for the ambiguity arising from the hidden substeps. It transpires, as we show below, that this rather subtle and at first-sight inconsequential small difference actually leads to significant changes in the load dependence especially (but not exclusively) when the fractions of back steps and forward steps are similar in frequency, i.e., on approaching stall conditions when the velocity, V , becomes small relative to its load-free value [19–21].

To clarify the issues involved, suppose the cycle has four states ($N=4$) and the major transition occurs between states $(2)_l$ and $(3)_l$ (i.e., $M=2$). Then in stepping time series such as illustrated in Figs. 1(a) and 1(b), one can identify all the moments of time at which the motor leaves state $(2)_l$ and

reaches state $(3)_l$ on moving forwards or when it leaves state $(3)_l$ for state $(2)_l$ on moving backwards. When successive forward steps are realized, as illustrated in Fig. 1(a), one knows that the motor must pass through the remaining two states, $[0]$ and (1) , at some points between the major transitions [see Fig. 1(a)] because state (2) cannot otherwise be reached *following* state (3) at the same site l . Thus in a sequence of three successive + steps one can conclude that the middle step is associated with a complete (forward) cycle. The corresponding observations are equally true for successive back steps. On the other hand, when the overall stepping sequence encompasses *both* back steps *and* forward steps, which is the interesting (and usual) situation [see Fig. 1(b)], it is *impossible*, for example, to be sure that the motor has completed a full forward cycle when a (detectable) forward step is followed by a back step; likewise, one cannot tell if a full back cycle was completed. In such cases, the full-cycle assumption is not valid.

The full-cycle assumption can be inadequate even when only a run of forward steps is seen, as in Fig. 1(a), in that back steps may merely be infrequent. This is somewhat counterintuitive since one might well argue that each + step does then correspond to a full cycle. However, if the enzymatic cycle is reversible there is always the possibility of a completed back step; thus explicit expressions in terms of the basic rates $\{u_j, w_j\}$ will differ when potentially hidden substeps are allowed for. Nevertheless, as we demonstrate in Sec. IV, there are various cases in which the quantitative differences may be small.

To demonstrate further the consequences of different conceivable interpretations, consider an ($N=3$)-state motor with two possible substeps. Figure 1(c) illustrates a stepping series with a relatively high noise level so that only the major transitions, say $(1)_l \rightleftharpoons (2)_l$ for $M=1$, at times t_k (with the corresponding dwell times $\tau_k = t_k - t_{k-1}$) can be measured. On the other hand, Fig. 1(d) shows *exactly the same* series of steps, but with a much lower noise level revealing the previously obscured small substeps, $[0]_l \rightleftharpoons (1)_l$ and $(2)_l \rightleftharpoons [0]_{l+1}$. In the latter case, one can determine the times t_k^0 when the motor reaches the bound state $[0]_l$ for the first time (i.e., when a cycle is completed). And then one can reliably determine the number \check{n}_+ of full forward, and \check{n}_- of full backward cycles. In general, when both forward and back steps are present the mean values of the cycle times $\check{\tau}_i = t_i^0 - t_{i-1}^0$ (and so $\check{\tau}$, $\check{\tau}_+$, and $\check{\tau}_-$) are quite different from the mean step-to-step dwell times τ , τ_+ and τ_- , that one can obtain from the noisy stepping series in Fig. 1(c). The difference between the splitting probabilities, $\check{\pi}_\pm$ and π_\pm , is even more obvious. For example in Fig. 1(d) one has *only* one back cycle since one must not consider the major transitions at times t_3 and t_4 as indicating full stepping cycles because the motor never actually reached the next bound state $[0]$: hence from this sequence one should estimate $\check{\pi}_+ \approx 5/6$ and $\check{\pi}_- \approx 1/6$ and $\check{\tau}_+/\check{\tau}_- \approx 5$. Conversely in Fig. 1(c) one would count six forward and two back steps (or to be more precise, major transitions) leading to the estimates $\pi_+ \approx 3/4$ and $\pi_- \approx 1/4$ so that $\pi_+/\pi_- \approx 3$.

From a mathematical viewpoint, although most of the transitions and biomechanicochemical states remain unseen, there is one bright spot. Specifically, in light of the basic feature or model assumption embodied in Eq. (1), at the instant of time before the moment, say t_k , at which a + step occurs, one can be *sure* the motor was in state (M) while in the *instant just after* t_k the motor is in state ($M+1$); and, likewise, just *before a backward* ($-$) *step* the state ($M+1$) is occupied, while just *after a back step* the state (M) is definitely occupied. Together with the standard Markovian premise of chemical kinetics, namely, that once in a well-defined chemical state the subsequent departures are independent of the mode of arrival, this crucial observation enables the systematic calculation of splitting probabilities and conditional dwell times for general $N > 1$ via the *Theory of First Passage Times*: specifically, as we now explain, we may use the analysis as formulated by van Kampen [38].

II. CONDITIONAL SPLITTING PROBABILITIES AND DWELL TIMES

Before undertaking explicit calculations to obtain expressions for π_+ , π_- , and τ_+ , τ_- , in terms of the u_i and w_j for general N and M , we introduce some further statistical properties that are straightforward to observe experimentally and might prove mechanistically informative. At the same time, they enter naturally in to the first-passage analysis that is presented in Sec. III.

In addition to the prior dwell times defined in (8) one may *separately observe post dwell times* by measuring intervals *following after* + or $-$ major steps: we will label the corre-

sponding mean dwell times $\tau_{+\diamond}$ and $\tau_{-\diamond}$, where the subscript \diamond is read as “diamond” and denotes, here and below, a + or a $-$ step. However, such dwell times may be *truncated* by *detachments* (or dissociations or disconnections) in which the motor leaves the track (essentially irreversibly) so ending a run. The *rates of detachment from states* (i), say δ_i , can certainly be included in the basic sequential kinetic model [19,22,23]; but in the *first instance* they may be *neglected* provided, as we will suppose, only time intervals between observed + or $-$ mechanical steps are considered. (Their effects, however, would be significant if dwell times *prior to detachments or immediately following attachments* were considered which might, indeed, prove informative.)

Neglecting such “initial” and “final” dwell times (although the former have been examined by Veigel *et al.* for myosin V in seeking *observable* mechanical substeps [39]) one may still observe the *four distinct conditional mean dwell times*,

$$\begin{aligned} \tau_{++}: & \text{ between two successive forward (+) steps,} \\ \tau_{+-}: & \text{ between a + step followed by a back-step,} \\ \tau_{-+}: & \text{ between a back-step followed by a + step,} \\ \tau_{--}: & \text{ between two successive back-steps,} \end{aligned} \quad (12)$$

defined, as in (8), in terms of the observed intervals $\tau_k^{++} = t_k^{(+)} - t_{k-1}^{(+)}$, averaged over n_{++} pairs of successive + steps, and likewise for n_{+-} pairs of + steps followed by $-$ steps, etc.

Another aspect is to note that for realistic runs of limited length, deviations of order $1/n$ will arise. Thus, for example, for a run of length $n = n_+ + n_-$ *starting* with a + step the overall mean dwell time is given by

$$\tau = [(n_+ - 1)\tau_+ + n_- \tau_-] / (n - 1), \quad (13)$$

there being only $(n-1)$ measurable (prior) dwell times $\tau_l = t_l - t_{l-1}$. Using the definitions (6) then yields

$$\tau = \pi_+ \tau_+ + \pi_- \tau_- - \frac{\pi_- (\tau_+ - \tau_-)}{n - 1}. \quad (14)$$

In fitting asymptotic ($n \gg 1$) expressions to real data from finite runs such systematic deviations should be recognized. Here, however, we will neglect such finite- n or end effects.

To proceed further it is also helpful to introduce the *pairwise step probabilities* π_{++} and π_{+-} defined as the probability that a + step is followed by a + or, respectively, by a $-$ step, and likewise, π_{-+} and π_{--} . These then satisfy

$$\pi_{++} + \pi_{+-} = 1 \quad \text{and} \quad \pi_{-+} + \pi_{--} = 1. \quad (15)$$

Again, in a finite run of n steps one can divide the $n-1$ successive pairs into n_{++} of + steps followed by a + step, and so on, and use $\pi_{++} \approx n_{++} / (n_{++} + n_{+-})$, $\pi_{+-} \approx n_{+-} / (n_{+-} + n_{++})$, etc. Noting that in a given run one must have $|n_{+-} - n_{-+}| \leq 1$, and neglecting finite- n corrections, leads to the valuable relation

$$\pi_+ \pi_{+-} = \pi_- \pi_{-+}. \quad (16)$$

From this follows the connections

$$\pi_+ = 1 - \pi_- = \pi_{-+}/(\pi_{+-} + \pi_{-+}), \quad (17)$$

$$\frac{1}{\pi_+} = 1 + \frac{\pi_{+-}}{\pi_{-+}} \quad \text{and} \quad \frac{1}{\pi_-} = 1 + \frac{\pi_{-+}}{\pi_{+-}}. \quad (18)$$

Together with (15) these relations show that the pair π_{+-} and π_{-+} or, equivalently, π_{++} and π_{--} serve to determine all the backward and forward or splitting probabilities.

It is worthwhile to carry these considerations a stage further by recognizing the Markovian character of the basic N -state model (1). Thus, neglecting detachments, the four division or splitting probabilities π_{++} , π_{+-} , π_{-+} , and π_{--} satisfying (15) can be regarded as the elements of a 2×2 stepping matrix, $[\pi_{\alpha\beta}]$, that stochastically determines the transitions from one (major) step, $+$ or $-$, to the next. By virtue of the conservation of probability, the largest eigenvalue is $\lambda_0=1$; but the second eigenvalue, which determines the decay per step of step-step correlations, is just

$$\lambda_1 = 1 - \pi_{+-} - \pi_{-+} = \pi_{++} - \pi_{--} = \pi_{++} + \pi_{--} - 1. \quad (19)$$

This vanishes when $\pi_{+-} = \pi_{-+} = \frac{1}{2}$ which corresponds to $\pi_+ = \pi_-$ and hence, to *stall conditions* in which the mean velocity, V , vanishes. However, in general λ_1 need not vanish at stall.

Counting arguments similar to those yielding (13)–(16) also lead to relations for the conditional mean dwell times. For completeness and consistency with later expressions, we utilize the $+/-$ “diamond” notation introduced before. For the prior dwell times, we thus find

$$\tau_+ \equiv \tau_{\diamond+} = \pi_{++}\tau_{++} + \pi_{+-}\tau_{-+}, \quad (20)$$

$$\tau_- \equiv \tau_{\diamond-} = \pi_{-+}\tau_{-+} + \pi_{--}\tau_{--}, \quad (21)$$

which, in turn, are fully consistent with the relation (9) for τ in terms of τ_+ and τ_- where we should note

$$\pi_+ \equiv \pi_{\diamond+} \equiv \pi_{+\diamond} \quad \text{and} \quad \pi_- \equiv \pi_{\diamond-} \equiv \pi_{-\diamond}. \quad (22)$$

Then the *post dwell times* likewise satisfy

$$\tau_{+\diamond} = \pi_{++}\tau_{++} + \pi_{+-}\tau_{-+}, \quad (23)$$

$$\tau_{-\diamond} = \pi_{-+}\tau_{-+} + \pi_{--}\tau_{--}, \quad (24)$$

while the overall mean dwell time is given by

$$\tau \equiv \tau_{\diamond\diamond} = \pi_+\tau_{+\diamond} + \pi_-\tau_{-\diamond}. \quad (25)$$

Each of these pairwise fractions and dwell times can be obtained from the same experimental data (i.e., stepping time series) that have been used experimentally to obtain the step splitting probabilities and the prior dwell times in the course of studying the dynamics of a motor as a function of load and [ATP], etc. But by observing such further independent statistical parameters one can test the basic theory more com-

pletely and hope to obtain more reliable and constrained fitting values for the rates determining the full mechanochemical cycle.

At a more detailed level it is also useful to define $n_{i,+}^{\rho\sigma}$ and $n_{i,-}^{\rho\sigma}$ with $\rho, \sigma = \diamond, +, \text{ or } -$, as the mean number of forward and backward transitions, possibly hidden, from states (i) and (j) , respectively, in the intervals between (major) steps *subject* to the conditions specified by the pair (ρ, σ) . If these transitions prove to be detectable, they can be counted and used in fitting parameters; but if they pertain to hidden transitions (e.g., the hydrolysis of ATP, etc.), it is of interest to estimate how often they occur given specific rates. The appropriate calculations on the basis of the model (1) are developed below in Sec. III E.

It is appropriate here to mention various hidden-Markov methods, etc. [40–43], that have been derived and employed to locate steps in the presence of noise (and to fit their amplitudes, or kinetic parameters, etc.). These approaches require an input stochastic model [40,43,44]; we believe the present approach could provide a valuable complement in the extraction of kinetic parameters from such experimental data since, as we will see, it reveals the kinds of behavior different models can generate.

III. EXPLICIT CALCULATIONS

A. Formulation and notation

The various stepping fractions, dwell times, etc., introduced in Secs. I and II can be derived explicitly in terms of the basic kinetic rates by using van Kampen’s analysis for one-dimensional, nearest-neighbor first-passage processes [38]. Accordingly, following the basic sequential model (1), with the N -periodicity conventions (3) for the sequential forward and backward rates, u_i and w_j , we envisage a random walker on a one-dimensional lattice with sites labeled $i, j = 0, \pm 1, \pm 2, \dots$, corresponding, in turn, to the motor states (i) , (j) , etc. (again subject to the periodicity convention). If the single major or observable step per cycle corresponds to the transitions $(M) \rightleftharpoons (M+1)$ with $M \in [0, N-1]$ we introduce (following Ref. [38]) absorbing boundaries on the left and the right via

$$L \equiv M \quad \text{and} \quad R \equiv M + 1 + N. \quad (26)$$

If, for given initial conditions at time $t=0$ (to be selected below), $q_i(t)$ is the probability that the motor and/or walker is in state (i) at time t we may construct the $N \times N$ transition matrix $\mathbf{A} = [A_{ij}]$ with elements

$$A_{i,j} = u_j \delta_{i,j+1} + w_j \delta_{i+1,j} - (u_j + w_j) \delta_{i,j}, \quad (27)$$

where $i, j \in [L+1, R-1] = [M+1, M+N]$. Then if $\mathbf{q}^T = [q_{M+1}, q_{M+2}, \dots, q_{M+N}]$ is the state vector, the governing rate equations are

$$\frac{d\mathbf{q}(t)}{dt} = \mathbf{A}\mathbf{q}(t), \quad (28)$$

This completes the first-passage formulation [38]. Before proceeding, however, we record some convenient notation

for the various products and sums of the rate constants that enter the analysis. To that end, our first definition [45] is of the ($m \geq 1$)-term product

$$\Gamma_{l,m} = \prod_{j=1}^m \frac{w_{l+j}}{u_{l+j}}, \quad (29)$$

which, by periodicity, is invariant under $l \Rightarrow l \pm N$. Likewise, the N -term product $\Gamma_{l,N}$ is independent of l yielding, specifically,

$$\Gamma_N = \Gamma_{l,N} = \prod_{j=0}^{N-1} \frac{w_j}{u_j} \quad (30)$$

[45] Then for all $l=0, \pm 1, \pm 2, \dots$ a central role will be played by the $(n-1)$ -term sum

$$\Delta_{l,n} = \sum_{m=1}^{n-1} \Gamma_{l,m} \quad (n \geq 1), \quad (31)$$

where, for the empty sum, we set $\Delta_{l,1} \equiv 0$. Indeed, these sums appear in previous analyses [19,22,23,45] via “renormalized” inverse forward rates (or transition times)

$$r_l = u_l^{-1}(1 + \Delta_{l,N}). \quad (32)$$

Specifically, these enter into the expression [19,22,23]

$$\frac{V}{d} = \frac{(1 - \Gamma_N)}{\sum_{l=0}^{N-1} r_l} \quad (33)$$

for the *mean velocity* V , which we recall here for convenience of reference. (Note that d is the mean spacing of sites along the track.) One sees directly from this that *stall conditions*, i.e., $V=0$, are determined by $\Gamma_N(u_i, w_j)=1$. The situation near stall will be a major focus for our discussions in Sec. IV.

The analysis of van Kampen [38] may now be put to work. Readers uninterested in the details may skip to the next section or peruse the main results, namely, (37)–(41) for π_{++} , etc., (54)–(57) for τ_{++} , etc., and (60)–(62) for τ_+ , τ_- and τ .

B. Pairwise step splitting probabilities

To proceed, let π_k^L be the total probability that a motor starting at $t=0$ in state (k) with $L < k < R$, so that $q_i(0) = \delta_{ik}$, eventually reaches the left absorbing state (L) for the first time, i.e., without having been absorbed at sites L or R . A moment’s thought confirms

$$\pi_k^L = w_{L+1} \int_0^\infty q_{L+1}(t) dt; \quad (34)$$

likewise, for reaching the boundary state (R) for the first time, one has

$$\pi_k^R = u_{R-1} \int_0^\infty q_{R-1}(t) dt. \quad (35)$$

Then we may appeal to Ref. [38], Chap. XII, Eq. (2.8) which, using the notation (29), states

$$\pi_k^L = 1 - \pi_k^R = \frac{\sum_{m=k-L}^{R-L-1} \Gamma_{L,m}}{1 + \sum_{m=1} \Gamma_{L,m}}. \quad (36)$$

For L and R we have (26). If the motor starts just after a (major) forward or $+$ step it is in the initial state ($k = (M+1)$); then π_k^L and π_k^R correspond, respectively, to π_{+-} and π_{++} . Conversely, just after a (major) back or $-$ step the motor is in state (M) which, by periodicity is equivalent to starting $k=M+N$; then we may identify π_k^L and π_k^R with π_{--} and π_{-+} , respectively. On using the notation (30) and (31) we thus obtain

$$\pi_{++}(M, N) = 1 - \pi_{+-} = \frac{1}{1 + \Gamma_N + \Delta_{M,N}}, \quad (37)$$

$$\pi_{-+}(M, N) = 1 - \pi_{--} = \frac{1 + \Delta_{M,N}}{1 + \Gamma_N + \Delta_{M,N}}. \quad (38)$$

It is interesting to note the cross relation

$$\pi_{--} = \Gamma_N \pi_{++}. \quad (39)$$

At stall this implies $\pi_{++} = \pi_{--}$ and $\pi_{+-} = \pi_{-+}$ which reflects the expected $+/-$ symmetry. The results (37) and (38) also yield, via the identities (17) and (18) the explicit step fraction expressions

$$\pi_+ = 1 - \pi_- = \frac{1 + \Delta_{M,N}}{1 + \Gamma_N + 2\Delta_{M,N}}, \quad (40)$$

$$\pi_- = \frac{\Gamma_N + \Delta_{M,N}}{1 + \Gamma_N + 2\Delta_{M,N}}. \quad (41)$$

C. Pairwise and prior dwell times

Now, following van Kampen [38], Chap. XII, Eqs. (1.7)–(1.8) the *conditional mean first-passage times* for arriving either at the left or right absorbing boundaries starting from a state point $k \in [L+1, R-1] = [M+1, M+N]$ as before, are

$$\tau_k^L = \frac{\int_0^\infty t q_{L+1}(t) dt}{\int_0^\infty q_{L+1}(t) dt}, \quad \tau_k^R = \frac{\int_0^\infty t q_{R-1}(t) dt}{\int_0^\infty q_{R-1}(t) dt}, \quad (42)$$

where the $q_i(t)$ are the solutions of (28) subject, for our purposes, to the two, alternative initial conditions,

$$q_i^+(0) = \delta_{i,M+1} \quad \text{and} \quad q_i^-(0) = \delta_{i,M+N}, \quad (43)$$

as discussed in deriving (37) and (38).

To obtain the pairwise conditional mean dwell times we proceed in two steps. First, we integrate the kinetic equations (28) over all times recognizing that the $q_j(t)$ approach zero exponentially fast for all $j \in [M+1, M+N]$, since the walker must eventually be absorbed at either L or R . This yields

$$-\mathbf{q}^\pm(0) = \mathbf{A}\mathbf{T}^\pm, \quad (44)$$

where the superscripts identify the alternative initial conditions (43). The elements of the vector \mathbf{T}^\pm have the dimensions of time and are given simply by

$$T_k^\pm = \int_0^\infty q_k^\pm(t) dt. \quad (45)$$

By the definitions (34) and (35) with (26), we also have the relations

$$T_{M+1}^+ = \pi_{+}/w_{M+1}, \quad T_{M+1}^- = \pi_{-}/w_{M+1}, \quad (46)$$

$$T_{M+N}^+ = \pi_{++}/u_{M+N}, \quad T_{M+N}^- = \pi_{-}/u_{M+N}. \quad (47)$$

Since \mathbf{A} is a tridiagonal matrix the equations (44) can be inverted recursively to obtain

$$T_j^+ = \pi_{++} \frac{\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M}}{u_j \Gamma_{M,j-M}}, \quad (48)$$

$$T_j^- = \pi_{-} \frac{1 + \Delta_{M,j-M}}{u_j \Gamma_{M,j-M}}. \quad (49)$$

One general approach to this inversion can be found in Ref. [38], Chap. XII, Sec. II. On recalling $\Delta_{l,1} \equiv 0$, one may check that these solutions verify the relations (46) and (47).

The next step is to multiply (28) by t and again integrate over all time which yields

$$-\mathbf{T}^\pm = \mathbf{A}\mathbf{S}^\pm, \quad (50)$$

where, with the same superscript conventions, etc., we have

$$S_k^\pm = \int_0^\infty t q_k^\pm(t) dt. \quad (51)$$

From (42) and following the arguments above (37) and (38), we can now make the identifications

$$\tau_k^{L+} = \tau_{+-} = S_{M+1}^+ / T_{M+1}^+, \quad (52)$$

$$\tau_k^{R-} = \tau_{-+} = S_{M+N}^- / T_{M+N}^-, \quad (53)$$

and similarly for τ_{-} and τ_{++} . Inverting (44) finally leads to the basic pairwise dwell time expressions

$$\tau_{++} = \sum_{j=M+1}^{M+N} T_j^+ (1 + \Delta_{M,j-M}), \quad (54)$$

$$\tau_{-+} = \sum_{j=M+1}^{M+N} T_j^- \frac{1 + \Delta_{M,j-M}}{1 + \Delta_{M,N}}, \quad (55)$$

$$\tau_{+-} = \sum_{j=M+1}^{M+N} T_j^+ \frac{\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M}}{\Gamma_N + \Delta_{M,N}}, \quad (56)$$

$$\tau_{--} = \sum_{j=M+1}^{M+N} T_j^- \frac{\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M}}{\Gamma_N}. \quad (57)$$

By using (37) and (38) for π_{++} and π_{-} in (48) and (49) we may establish the unanticipated, general equality

$$\tau_{++} = \tau_{--}, \quad \text{all } M, N. \quad (58)$$

En route to the prior dwell times τ_+ and τ_- it is convenient to introduce

$$T_j = \pi_+ T_j^+ + \pi_- T_j^- = \frac{(1 + \Delta_{j,N})/u_j}{1 + \Gamma_N + 2\Delta_{M,N}}, \quad (59)$$

where we have used (40) and (41) and may recall that the numerator is the inverse rate defined in (32) and used in the past. Note also that by virtue of the periodicity we have $T_{j\pm N} \equiv T_j$ and, likewise, for the T_j^\pm . Then, by utilizing (20), (21), (37), and (38), we obtain the prior dwell times in the form

$$\tau_+ \equiv \tau_{\diamond+} = \frac{1}{\pi_{+j=M+1}} \sum_{j=M+1}^{M+N} T_j \frac{1 + ta_{M,j-M}}{1 + \Gamma_N + \Delta_{M,N}}, \quad (60)$$

$$\tau_- \equiv \tau_{\diamond-} = \frac{1}{\pi_{-j=M+1}} \sum_{j=M+1}^{M+N} T_j \frac{\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M}}{1 + \Gamma_N + \Delta_{M,N}}. \quad (61)$$

Finally, with the aid of (9), the overall mean dwell time is simply

$$\tau = \sum_{j=M+1}^{M+N} T_j \equiv \sum_{i=0}^{N-1} T_i. \quad (62)$$

Although, we have introduced the various step fractions via the pairwise fractions π_{++} , etc., this was not a necessary move from the mathematical point of view. Indeed, one can find the results and (40) and (41) for π_+ and π_- , and the present expressions for τ_+ , τ_- , and τ , directly by solving the basic rate equations (28) with the initial conditions

$$q_k(0) = \pi_+ \delta_{k,M+1} + (1 - \pi_+) \delta_{k,M+N}, \quad (63)$$

together with the relations (44) and (50) and appropriate boundary conditions. By this route one need not mention the pairwise splitting probabilities or pairwise dwell times. Nevertheless, the pairwise stepping fractions and dwell times can be useful in data analysis and to test theory, since they represent additional force and [ATP] dependent parameters that can be measured without significant extra experimental effort.

D. Individual and post dwell times

Now the form (62) for τ strongly suggests that $T_j \equiv T_j^{\diamond}$ is actually the overall *individual mean dwell time* spent in state (j) irrespective, as indicated by the use of the $+/-$ or diamond notation [see (20)–(25) of the stepping sequence. This conclusion is, indeed, justified since it follows from (45) that we may identify $T_j^+ = T_j^{\diamond}$ and $T_j^- = T_j^{\diamond}$ as *individual post +* and *- step mean dwell times* in state (j),

respectively. Consequently, the mean overall *post dwell times*, are given by

$$\tau_{+\diamond} = \sum_{i=0}^{N-1} T_i^{+\diamond} \quad \text{and} \quad \tau_{-\diamond} = \sum_{i=0}^{N-1} T_i^{-\diamond}. \quad (64)$$

From these results one may verify that (25) is satisfied.

Likewise, we anticipate relations like

$$\tau_{++} = \sum_{i=0}^{N-1} T_i^{++}, \quad (65)$$

etc., and, hence, from (54) and (57) we surmise that the *conditional individual state dwell times* are

$$T_j^{++} = T_j^{--} = \frac{(1 + \Delta_{M,j-M})(\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M})}{u_j \Gamma_{M,j-M} (1 + \Gamma_N + \Delta_{M,N})}, \quad (66)$$

while (56) and (55) yield

$$T_j^{-+} = \frac{\Gamma_N (1 + \Delta_{M,j-M})^2}{u_j \Gamma_{M,j-M} (1 + \Delta_{M,N}) (1 + \Gamma_N + \Delta_{M,N})}, \quad (67)$$

$$T_j^{+-} = \frac{(\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M})^2}{u_j \Gamma_{M,j-M} (\Gamma_N + \Delta_{M,N}) (1 + \Gamma_N + \Delta_{M,N})}. \quad (68)$$

In terms of these we can define the *prior individual* (or *partial dwell times* via

$$T_j^{\diamond+} = \pi_{++} T_j^{++} + \pi_{+-} T_j^{-+} = \frac{r_j (1 + \Delta_{M,j-M})}{(1 + \Delta_{M,N}) (1 + \Gamma_N + \Delta_{M,N})}, \quad (69)$$

$$T_j^{\diamond-} = \pi_{-+} T_j^{+-} + \pi_{--} T_j^{--} = \frac{r_j (\Gamma_N + \Delta_{M,N} - \Delta_{M,j-M})}{(\Gamma_N + \Delta_{M,N}) (1 + \Gamma_N + \Delta_{M,N})}, \quad (70)$$

where the r_j are defined in (32). Hence one can check the expressions for the mean overall *prior dwell times*, $\tau_{\diamond+}$ and $\tau_{\diamond-}$, given in (60) and (61).

Evidently, the analysis presented does not fully justify the inferences regarding (66)–(70). However, these expressions have been checked by direct computation for $N=2$ (as recorded in the Appendix) and the various cross-checks for general M and N also serve as validation. However, a complete justification requires a more elaborate calculation that we hope to present in the future. By the same route one can derive the mean conditional counts, $n_{j,+}^{\rho\sigma}$ and $n_{j,-}^{\rho\sigma}$ [see after Eqs. (23)–(25)] of the *hidden substeps* as we now proceed to demonstrate. Corresponding results for $N=2$ are also presented in the Appendix.

E. Counting the hidden substeps

As touched on briefly in the penultimate paragraph of Sec. II, it is surely of interest in light of our basic premise to estimate for a particular model how many hidden substeps actually arise on average in the typical intervals $\tau \equiv \tau_{\diamond\pm}$, $\tau_{+\diamond}$, τ_{++} , etc., between specified successive observable steps, i.e., major transitions between states (M) and ($M+1$).

Granted the results obtained in the preceding section for the $T_j^{\rho\sigma}$, where henceforth, (ρ, σ) runs through the nine combinations

$$\{\rho, \sigma\} = \{\diamond\diamond; +\diamond, -\diamond, \diamond+, \diamond-; ++, +-, -+, --\}, \quad (71)$$

this is a reasonably straightforward exercise.

Notice, first, that the mean dwell time in a given state (j), say $\bar{\tau}_j$, is, by virtue of the Markovian character of the relevant biochemical reactions *independent* of whether the state was reached from state ($j-1$) or ($j+1$) and of whether the motor departs to states ($j+1$) or ($j-1$): formally, we may write

$$\bar{\tau}_j = (u_j + w_j)^{-1} = \tau_j^{(-)\rightarrow(+)} = \tau_j^{(-)\rightarrow(-)} = \tau_j^{(+)\rightarrow(+)} = \tau_j^{(+)\rightarrow(-)}, \quad (72)$$

where the superscripts have an obvious interpretation.

Then, if $n_{j\rightarrow j+1}$ and $n_{j\rightarrow j-1}$ are the number of transitions, i.e., substeps, forwards or backwards, respectively, from state (j) in an interval between observable steps we desire the conditional mean values

$$n_{j,+}^{\rho\sigma} = \langle n_{j\rightarrow j+1} \rangle_{\rho\sigma} \quad \text{and} \quad n_{j,-}^{\rho\sigma} = \langle n_{j\rightarrow j-1} \rangle_{\rho\sigma}. \quad (73)$$

In terms of these we will have for the overall mean numbers of + or – hidden transitions per step-to-step interval

$$\bar{n}_+^{\rho\sigma} = \frac{1}{N-1} \sum_{j=M+1}^{M+N-1} n_{j,+}^{\rho\sigma}, \quad (74)$$

$$\bar{n}_-^{\rho\sigma} = \frac{1}{N-1} \sum_{j=M+2}^{M+N} n_{j,-}^{\rho\sigma}, \quad (75)$$

for all nine pairs (ρ, σ) . As a moments thought reveals, the limits on the summations here must be carefully set: thus, after a forward step to state ($M+1$) any putative forward hidden substep transformation from state ($M+N$) would represent a full *observable* + step (i.e., a major transition) and so is to be excluded from the sum of *substeps*; equally, any back transition from state ($M+1$) would represent an observable (back)step whereas back *substeps* from state ($M+N$), prior to the final forward step, are to be counted: see, for example, Fig. 2 which can be regarded as a noise-free version of Fig. 1(d) [but for an ($N=4$)-state model].

Now consider $T_j^{\rho\sigma}$, the mean time spent during a (ρ, σ) step interval in a state (j) that is *neither* an initial *nor* a final state of a major transition, so that $j \neq M, M+1$. On average this state will be visited on $T_j^{\rho\sigma} / \bar{\tau}_j = (u_j + w_j) T_j^{\rho\sigma}$ separate occasions during the interval; thus it entails $n_{j,+}^{\rho\sigma}$ forward substep *departures* and $n_{j,-}^{\rho\sigma}$ back substep *departures*. Equally, it entails, on average, $n_{j-1,+}^{\rho\sigma}$ forward substep *arrivals* and $n_{j+1,-}^{\rho\sigma}$ back substep *arrivals*. As a result we have the *frequency relations*

$$\omega_j^{\rho\sigma} \equiv (u_j + w_j) T_j^{\rho\sigma} = n_{j,+}^{\rho\sigma} + n_{j,-}^{\rho\sigma}, \quad (76)$$

for $j \in [M+2, M+N-1]$ and, similarly,

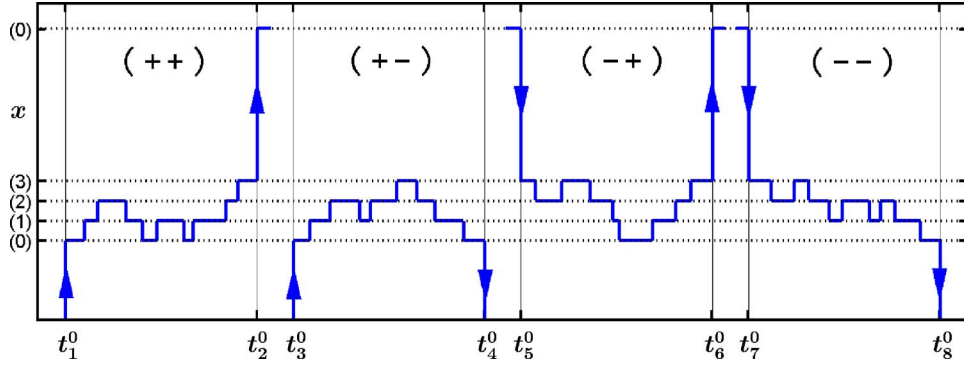


FIG. 2. (Color online) Schematic plots illustrating substeps and their counting rules (see text) in the $N=4$ case with $M=3$ for $(++)$, $(+-)$, $(-+)$, and $(--)$ stepping intervals. Thus, for example, from the first plot one confirms $n_{0,+}^{++} = n_{1,-}^{++} + 1$ and, correspondingly, that the motor visited the state $[0]$ three times in this realization; and, likewise, $n_{1,+}^{++} + n_{1,-}^{++} = n_{0,+}^{++} + n_{2,-}^{++}$ is, here, equal to 4 so that, correspondingly, the motor spent time in the substate (1) on four occasions before undergoing the forward major transition $(3) \rightarrow (4 \equiv 0)$. Similarly, the other plots serve to establish the frequency relations (76)–(85).

$$\omega_j^{\rho\sigma} = n_{j-1,+}^{\rho\sigma} + n_{j+1,-}^{\rho\sigma}, \quad (77)$$

where the units of ω_j may be regarded as substeps per interval.

For the reasons explained after (75) and illustrated in Fig. 2 the boundary states $j=M+1$ and $j=M+N$, require special consideration. As noted, one cannot have substeps that go backwards from state $(M+1)$ and, in the case of a prior forward step, the certain incoming arrival must be included in the individual mean dwell time. Thus we are led to the four boundary frequency relations

$$\omega_{M+1}^{++} = n_{M+1,+}^{++} = n_{M+2,-}^{++} + 1, \quad (78)$$

$$\omega_{M+1}^{+-} = n_{M+1,+}^{+-} + 1 = n_{M+2,-}^{+-} + 1, \quad (79)$$

$$\omega_{M+1}^{-+} = n_{M+1,+}^{-+} = n_{M+2,-}^{-+}, \quad (80)$$

$$\omega_{M+1}^{--} = n_{M+1,+}^{--} + 1 = n_{M+2,-}^{--}. \quad (81)$$

Complementary arguments apply for the opposite boundary state ($j \equiv M+N$), yielding

$$\omega_{M+N}^{++} = n_{M+N,-}^{++} + 1 = n_{M+N-1,+}^{++}, \quad (82)$$

$$\omega_{M+N}^{+-} = n_{M+N,-}^{+-} = n_{M+N-1,+}^{+-}, \quad (83)$$

$$\omega_{M+N}^{-+} = n_{M+N,-}^{-+} + 1 = n_{M+N-1,+}^{-+} + 1, \quad (84)$$

$$\omega_{M+N}^{--} = n_{M+N,-}^{--} = n_{M+N-1,+}^{--} + 1. \quad (85)$$

The frequency relations (76) and (77) with the boundary relations (78)–(85) constitute, together with the definition $\omega_j^{\rho\sigma} = (u_j + w_j)T_j^{\rho\sigma}$ in (76), a complete set from which we may derive explicit general expressions for all the $n_{j,+}^{\rho\sigma}$ and $n_{j,-}^{\rho\sigma}$. To proceed, we note first that the purely counting arguments involving π_+ , π_- , and π_{++} , π_{--} , etc., that led to the reduced relations (20), (21), (23), and (24), apply equally to ω_j^{++} , ω_j^{+-} , etc. Accordingly, we obtain the *post* boundary frequency relations

$$\omega_{M+1}^{+\diamond} = n_{M+2,-}^{+\diamond} + 1 = n_{M+1,+}^{+\diamond} + \pi_{+-}, \quad (86)$$

$$\omega_{M+N}^{+\diamond} = n_{M+N-1,+}^{+\diamond} = n_{M+N,-}^{+\diamond} + \pi_{++}, \quad (87)$$

$$\omega_{M+1}^{-\diamond} = n_{M+2,-}^{-\diamond} = n_{M+1,+}^{-\diamond} + \pi_{--}, \quad (88)$$

$$\omega_{M+N}^{-\diamond} = n_{M+N-1,+}^{-\diamond} + 1 = n_{M+N,-}^{-\diamond} + \pi_{-+}, \quad (89)$$

while the *prior* relations are

$$\omega_{M+1}^{\diamond+} = n_{M+1,+}^{\diamond+} = n_{M+2,-}^{\diamond+} + \pi_{++}, \quad (90)$$

$$\omega_{M+N}^{\diamond+} = n_{M+N,-}^{\diamond+} + 1 = n_{M+N-1,+}^{\diamond+} + \pi_{+-}, \quad (91)$$

$$\omega_{M+1}^{\diamond-} = n_{M+1,+}^{\diamond-} + 1 = n_{M+2,-}^{\diamond-} + \pi_{-+}, \quad (92)$$

$$\omega_{M+N}^{\diamond-} = n_{M+N,-}^{\diamond-} = n_{M+N-1,+}^{\diamond-} + \pi_{--}, \quad (93)$$

Finally, in analogy to (25), we obtain

$$\omega_{M+1}^{\diamond\diamond} = n_{M+2,-}^{\diamond\diamond} + \pi_+ = n_{M+1,+}^{\diamond\diamond} + \pi_-, \quad (94)$$

$$\omega_{M+N}^{\diamond\diamond} = n_{M+N-1,+}^{\diamond\diamond} + \pi_- = n_{M+N,-}^{\diamond\diamond} + \pi_+. \quad (95)$$

Now (94) clearly implies the result

$$n_{M+1,+}^{\diamond\diamond} = \omega_{M+1}^{\diamond\diamond} - \pi_- = (u_j + w_j)T_{M+1}^{\diamond\diamond} - \pi_-, \quad (96)$$

where π_- and $T_{M+1}^{\diamond\diamond} \equiv T_{M+1}^{\diamond\diamond}(\{u_j, w_j\}; M, N)$ are given explicitly in (41) and (59). A similar result follows from (94) for $n_{M+2,-}^{\diamond\diamond}$. But by appealing to (77) for $j=M+2$ we also obtain

$$n_{M+3,-}^{\diamond\diamond} = \omega_{M+2}^{\diamond\diamond} - n_{M+1,+}^{\diamond\diamond}, \quad (97)$$

which, with the aid of (96), yields an explicit expression in terms of T_{M+1} and T_{M+2} . Substituting $j=M+3$ in (76) then leads to an expression for $n_{M+3,+}^{\diamond\diamond}$. By proceeding recursively in this fashion we eventually obtain, for $k=1, 2, \dots$, the general expressions

$$n_{M+2k,-}^{\diamond\diamond} = \sum_{j=M+1}^{M+2k-1} (-)^{j-M-1} (u_j + w_j) T_j^{\diamond\diamond} - \pi_+, \quad (98)$$

$$n_{M+2k+1,-}^{\diamond\diamond} = \sum_{j=M+1}^{M+2k} (-)^{j-M} (u_j + w_j) T_j^{\diamond\diamond} + \pi_-, \quad (99)$$

for the average number of hidden *back substeps* from the $N-2$ hidden intermediate states ($M+2$) to ($M+N-1$) and from the prestep state ($M+N$) \equiv (M). We may also note the sum rule

$$\sum_{l=0}^{N-1} (-)^l (u_l + w_l) T_l^{\diamond\diamond} = \begin{cases} 0 & \text{for } N \text{ even,} \\ 1 & \text{for } N \text{ odd,} \end{cases} \quad (100)$$

and the special relations $\pi_+ = u_{M+N} T_{M+N}^{\diamond\diamond}$ and $\pi_- = w_{M+1} T_{M+1}^{\diamond\diamond}$, so that $n_{M+1,+}^{\diamond\diamond} = u_{M+1} T_{M+1}^{\diamond\diamond}$ and $n_{M+N,-}^{\diamond\diamond} = w_{M+N} T_{M+N}^{\diamond\diamond}$.

More generally (98) and (99) can be extended to arbitrary (ρ, σ) if, following the sequence (71), π_+ and π_- are replaced by

$$\tilde{\pi}_+ = \{\pi_+; 1, 0, \pi_{++}, \pi_{-+}; 1, 1, 0, 0\}, \quad (101)$$

$$\tilde{\pi}_- = \{\pi_-; \pi_{+-}, \pi_{--}, 0, 1; 0, 1, 0, 1\}, \quad (102)$$

respectively. Finally, one finds that the mean number of *forward substeps* from the $N-2$ hidden intermediate states and the post-step state ($M+1$) in specified intervals can be written

$$n_{M+2k,+}^{\rho\sigma} = \sum_{j=M+1}^{M+2k} (-)^{j-M} (u_j + w_j) T_j^{\rho\sigma} + \tilde{\pi}_+, \quad (103)$$

$$n_{M+2k-1,+}^{\rho\sigma} = \sum_{j=M+1}^{M+2k-1} (-)^{j-M-1} (u_j + w_j) T_j^{\rho\sigma} - \tilde{\pi}_-, \quad (104)$$

for $k=1, 2, \dots$. Some examples of these various expressions for small N are listed in the Appendix.

IV. DISCUSSION AND ILLUSTRATIONS

First, it is appropriate to look more closely at the difference between our present results [see (40), (41), and (60)–(62) and those of the previously presented analysis [20,35]. As in Sec. I [after (11)] we use a haček to distinguish the results that presuppose the completion of a full enzymatic cycle between all pairs of successive major, i.e., observable steps. In the present notation one then has [35]

$$\check{\pi}_+ = \frac{1}{1 + \Gamma_N}, \quad \check{\pi}_- = \frac{\Gamma_N}{1 + \Gamma_N}, \quad (105)$$

for the forward and back fractions while the mean, prior, and post dwell times are all given by

$$\check{\tau} = \check{\tau}_+ = \check{\tau}_- = \sum_{n=0}^{N-1} r_n / (1 + \Gamma_N), \quad (106)$$

where r_n is defined in (32). On the other hand, when allowing for hidden substeps, these three times are all distinct: see (60)–(62).

Then, if one considers the average velocity defined by

$$V = d(\pi_+ - \pi_-) / \tau, \quad (107)$$

one finds that the result is the same in both cases, namely,

$$V = \check{V} = \frac{d(1 - \Gamma_N)}{N-1}, \quad (108)$$

$$\sum_{n=0} r_n$$

as previously [19–23]. This is not really surprising, because the asymptotic ratio of the total distance to the total time should not depend on the way one takes into account (or ignores) substeps. Note that $\Gamma_N=1$ is the condition for stall, when $\pi_+ = \pi_-$, in both analyses.

Now let us take a closer look at the ratio of splitting fractions, because this is a quantity which one can readily obtain from stepping observations to test theory. The effect of an external force, $\mathbf{F} = (F_x, F_y, F_z)$, on the rates can be expressed in leading order [19,24] as

$$u_l = u_l^0 \exp(+d\boldsymbol{\theta}_l^+ \cdot \mathbf{F}/k_B T), \quad (109)$$

$$w_l = w_l^0 \exp(-d\boldsymbol{\theta}_l^- \cdot \mathbf{F}/k_B T), \quad (110)$$

where u_l^0, w_l^0 are the load-free rates while the load-distribution vectors $\boldsymbol{\theta}_l^+$ and $\boldsymbol{\theta}_l^-$ serve to specify how the $2N$ distinct rates respond to the stress. The periodicity of the stepping along the track (which we suppose is in the x direction) implies

$$\sum_{n=0}^{N-1} (\boldsymbol{\theta}_n^+ + \boldsymbol{\theta}_n^-) = \hat{\mathbf{e}}_x = (1, 0, 0). \quad (111)$$

Rearranging these relations leads to

$$\Gamma_N = \prod_{n=0}^{N-1} \frac{w_n}{u_n} = \exp\left(-\frac{dF_x}{k_B T}\right) \prod_{n=0}^{N-1} \frac{w_n^0}{u_n^0} = \exp[-d(F_x - F_S)/k_B T], \quad (112)$$

where the stall force is given by [19,24]

$$F_S = \frac{k_B T}{d} \ln\left(\prod_{n=0}^{N-1} \frac{w_n^0}{u_n^0}\right). \quad (113)$$

From (105) we thus conclude

$$\ln(\check{\pi}_+ / \check{\pi}_-) = -\ln \Gamma_N = d(F_x - F_S) / k_B T. \quad (114)$$

This is a strong prediction since it asserts that the logarithm of the stepping ratio ($\check{\pi}_+ / \check{\pi}_-$) depends linearly on F_x with a slope, $d/k_B T$, determined solely by the step size d . However, this result depends crucially on the assumption that the forward and back steps identified correspond to full enzymatic cycles (as the haček indicate). In a typical experiment, however, the hidden substeps result in a violation of this assumption as we have explained.

Accordingly, let us, instead, compute the stepping fraction (π_+ / π_-) for which (40) and (41) yield

$$\ln(\pi_+ / \pi_-) = d^*(F_x - F_S) / k_B T, \quad (115)$$

where, for the sake of comparison with (114), we have introduced an *effective step size*

$$d^* = \frac{-k_B T}{F_x - F_S} \ln \left(\frac{\Gamma_N + \Delta_{M,N}}{1 + \Delta_{M,N}} \right). \quad (116)$$

In general, this clearly depends on all the rates $\{u_j, w_j\}$ and hence on the force F_x . In the special case $\Delta_{M,N}=0$, however, d^* reduces [via (114)] to d . This condition is, in fact, realized when $N=1$ since $\Delta_{M,1}$ vanishes identically. But an $N=1$ model is unlikely to be adequate. Thus in real systems neither the full linearity vs F_x nor the equality $d^*=d$ are to be expected. In the vicinity of F_S , however, we can estimate d^* by expanding in powers of $\delta F_x \equiv F_x - F_S$. This yields

$$\Gamma_N = 1 - d \delta F_x / k_B T + O(\delta F_x^2), \quad (117)$$

$$\Delta_{M,N} = \Delta_S + \Delta'_S \delta F_x + O(\delta F_x^2), \quad (118)$$

where $\Delta_S = \Delta_{M,N}(F_x = F_S)$ while Δ'_S is the corresponding derivative. Thence we find

$$d^* = \frac{d}{1 + \Delta_S} + O(\delta F_x). \quad (119)$$

It follows from the definition (31) that Δ_S cannot be negative so that, quite generally, one has $d^* \leq d$.

On the other hand, to be concrete, consider an ($N=2$, $M=1$) model for which we have

$$\begin{aligned} \Delta_S &= (w_0/u_0)_S = (u_1/w_1)_S \\ &= (u_1^0/w_1^0) \exp\{d[F_S - (\boldsymbol{\theta}_0^+ + \boldsymbol{\theta}_0^-) \cdot \mathbf{F}_S] / k_B T\}, \end{aligned} \quad (120)$$

where the subscript S denotes evaluation at $\mathbf{F} = \mathbf{F}_S = (F_x = F_S, F_y, F_z)$ in which F_y and F_z need not vanish at stall [24]. It is evident, that Δ_S is not bounded above so that d^*/d is not bounded below. Indeed, the experiments on kinesin of Nishiyama *et al.* [8] and of Carter and Cross on kinesin [14] lead to the estimates $d^* \approx 3.2$ nm and $d^* \approx 4.0$ nm, respectively, whereas the step size is $d \approx 8.2$ nm. This clearly indicates the importance of the hidden substeps in understanding the stepping fractions near stall.

As another concrete example we quote

$$\Delta_S = \left(\frac{w_0}{u_0} + \frac{w_0 w_1}{u_0 u_1} + \frac{w_0 w_1 w_2}{u_0 u_1 u_2} \right)_S = \left(\frac{u_1 u_2 u_3}{w_1 w_2 w_3} + \frac{u_2 u_3}{w_2 w_3} + \frac{u_3}{w_3} \right)_S, \quad (121)$$

for an ($N=4$, $M=3$) model. From this, however, one sees that d^* will be close to d if $(u_3/w_3)_S$ and/or $(w_0/u_0)_S$ are sufficiently small.

More generally it seems likely that both the full cycle assumption and the hidden substep analysis should produce similar results when the rates, u_M and w_{M+1} , for the major transition are slow relative to the substep rates. To demonstrate this explicitly let us suppose that the latter rates satisfy

$$u_M = \eta \tilde{u}_M \quad \text{and} \quad w_{M+1} = \eta \tilde{w}_{M+1}, \quad (122)$$

where η is small while \tilde{u}_M and \tilde{w}_{M+1} and all the substep rates, $u_l \equiv \tilde{u}_l$ ($l \neq M$) and $w_l \equiv \tilde{w}_l$ ($l \neq M+1$), are held fixed. Then one finds

$$\Gamma_{M,s} = \prod_{n=1}^s \frac{w_{M+n}}{u_{M+n}} = \begin{cases} \eta \tilde{\Gamma}_{M,s} & \text{for } s \in [1, N-1], \\ \tilde{\Gamma}_N = \Gamma_N & \text{for } s = N, \end{cases} \quad (123)$$

where $\tilde{\Gamma}_{M,s} = \prod_{n=1}^s (\tilde{w}_{M+n}/\tilde{u}_{M+n})$, and similarly, via (31),

$$\Delta_{M,N} = \eta \sum_{p=1}^{N-1} \tilde{\Gamma}_{M,p} \equiv \eta \tilde{\Delta}_{M,N}. \quad (124)$$

With the aid of these expressions we can rewrite the previous results (40), (41), and (62) as

$$\pi_+ = \frac{1 + \eta \tilde{\Delta}_{M,N}}{1 + \tilde{\Gamma}_N + 2\eta \Delta_{M,N}} = 1 - \pi_-, \quad (125)$$

$$\tau = \check{\tau} \frac{1 + \tilde{\Gamma}_N}{1 + \tilde{\Gamma}_N + 2\eta \tilde{\Delta}_{M,N}}. \quad (126)$$

Evidently to zero order in η , the analyses are equivalent as anticipated.

As seen in earlier investigations that were confined to the velocity vs force relation [19,22], a surprisingly wide range of behavior under varying loads is displayed even by the basic ($N=2$)-state models when the rates are subject to the exponential force distribution laws embodied in (109)–(111) [19,24]. This is illustrated in Fig. 3(a) which depicts the velocity V , normalized by its value $V(0)$ under zero load, as a function of the imposed load F (supposed parallel to the x axis) normalized by the stall force magnitude $|F_S|$. The labeled plots (1)–(5) in Fig. 3 correspond to the selected parameter values listed in Table I. Note that *superstall loads* are included ($F/|F_S| < -1$) which for the parameter sets (1) and (5) results in only a relatively small negative velocity (as uncovered in the kinesin experiments of Carter and Cross [14]). Similarly, *assisting loads* ($F/|F_S| > 0$) are also covered and for sets (2), (4), and (5) result in a saturating or, even, *decreasing* velocity under increasing load. In the *substall* resistively loaded region ($0 > F/|F_S| > -1$) convex, concave, inflected, and even nonmonotonic [see parameter set (5)] behavior is realized.

For these different cases the corresponding forward stepping fractions $\pi_+(F)$ and the logarithmic ratios of forward and/or back steps, (π_+/π_-) , are depicted in Figs. 3(b) and 3(c). (Note that in these figures only the *resisting* range of force, $F < 0$, is displayed.) Although the variation is always monotonically increasing with F (and $\pi_+ = \pi_- = \frac{1}{2}$ when $F = F_S$), a wide range of forms is evident. In the logarithmic plot, Fig. 3(c), one sees linear, concave, and inflected variation close to stall. Furthermore, the value of the effective step size d^* varies markedly: see the last column in Table I.

By using the explicit expressions in the Appendix the nature of other statistical observables such as $\pi_{+-}(F)$, etc., is readily explored. Experiments often measure the overall mean dwell time, $\tau(F)$, between steps. Under assisting loads ($F > 0$), when π_- is negligible, $\tau(F)$ directly mirrors the reciprocal of the velocity $V(F)$; but, in view of the factor $(\pi_+ - \pi_-)$ in (107), it varies somewhat differently under resisting

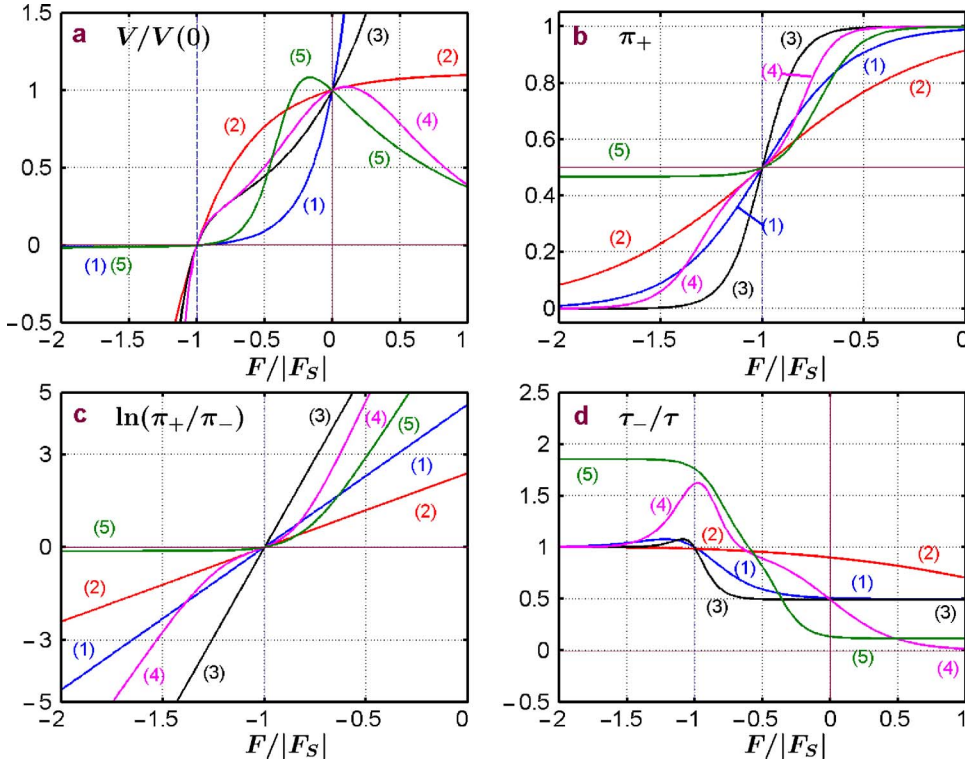


FIG. 3. (Color online) Plots illustrating different dependencies on the normalized force, $F/|F_S|$, of (a) the normalized velocity $V(F)/V(0)$; (b) the forward splitting fraction π_+ ; (c) the logarithm of the splitting fraction ratio (π_+/π_-); and (d) the ratio of mean prior backward to mean total dwell time, $\tau_-(F)/\tau(F)$. The individual plots, labeled (1)–(5), correspond to $(N=2, M=1)$ models under scalar loading, $\mathbf{F}=(F, 0, 0)$, with the selected parameter values presented in Table I.

loads. More interesting is the behavior of the *partial dwell time* $\tau_-(F)$ observed prior to back steps. This is shown in Fig. 3(d) normalized by the overall dwell time $\tau(F) = \pi_+ \tau_+ + \pi_- \tau_-$ [see (9)]. Even though, the ratio τ_-/τ is confined to the range $(0, 2)$ beyond superstall (since $\tau_-/\tau < 1/\pi_-$ and $\pi_- > \frac{1}{2}$ for $V < 0$), striking nonmonotonic and inflected variation arises.

Needless-to-say, many more plots exhibiting unexpected and surprising behavior can be generated; but further exploration seems most useful in connection with specific experimental data. Such applications are planned.

V. MULTIPLE OBSERVED TRANSITIONS

In the preceding sections we have derived expressions only for the case of a *single* major transition in each enzymatic cycle; that, indeed, is the typical situation for experiments on conventional kinesin [6,8,14]. However, our results can be generalized to the case in which several substeps are sufficiently large to be clearly detected, while others remain

hidden in the noise. Suppose there are K visible substeps of (average) magnitudes d_A, d_B, \dots , together totaling

$$d_A + d_B + \dots + d_K \approx d, \quad (127)$$

that occur between states (M_j) and (M_{j+1}) with, in sequence,

$$0 \leq M_A < M_B < \dots < M_K \leq N-1. \quad (128)$$

Then between states $(M_{j-1}+1)$ and (M_j) there are $M_j - M_{j-1}$ hidden states and, consequently, all these states belong (within the noise) to what we may call the same *mechanical level*, J : see Fig. 4.

Now one can count all forward and backward detectable transitions in a long run. Accordingly, let n_{++}^J be the number of pairs of observed substeps that enter the mechanical level J via a $+$ transition, i.e., a step $(J-1)$, and leave via a $+$ transition taking a step J , and similarly for n_{+-}^J , n_{-+}^J , and n_{--}^J . Then, as previously, we can estimate pairwise splitting fractions for level J via

TABLE I. Parameter values for $(N=2, M=1)$ models under scalar loading, $\mathbf{F} \equiv F_x$, employed in Fig. 3 to illustrate different force dependencies. The last column presents the computed values of the effective step size d^* . [See (116).]

	$d F_S /k_B T$	w_0^0/u_0^0	w_1^0/u_0^0	θ_1^+	$\theta_0^- = \theta_1^-$	d^*/d
(1)	9.2	10^{-2}	10^{-2}	0.5	0	0.501
(2)	2.5	10^{-2}	10^{-2}	0	0.5	0.966
(3)	23	10^{-5}	10^{-5}	0.07	0.43	0.503
(4)	23	10^{-5}	10^{-5}	-0.07	0.48	0.113
(5)	10	3.4×10^{-4}	2.5×10^{-3}	-0.1	0.1	0.118

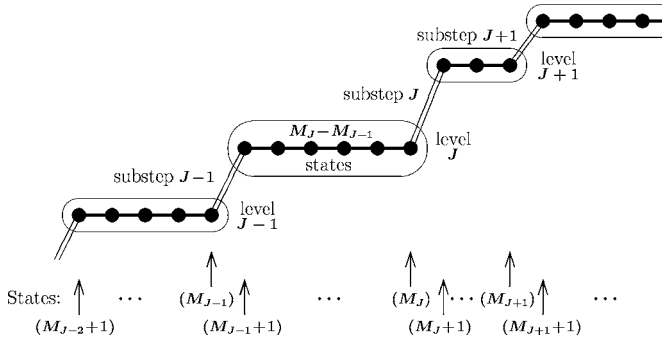


FIG. 4. Schematic depiction of individual biochemical states, (i), organized into mechanical levels, $A, B, \dots, J-1, J, \dots$ when there are $K > 1$ detectable substeps or major transitions with substep J going forward from state (M_J) to state (M_{J+1}) .

$$\pi_{++}^J = \frac{n_{++}^J}{n_{++}^J + n_{+-}^J} = 1 - \pi_{+-}^J, \quad (129)$$

$$\pi_{-+}^J = \frac{n_{-+}^J}{n_{-+}^J + n_{--}^J} = 1 - \pi_{--}^J. \quad (130)$$

Likewise, we can introduce pairwise dwell times, $\tau_{\rho\sigma}^J$ for each mechanical level J as the mean times spent between a ρ and σ substep into and out of that level.

With these definitions we may adopt the same approach used in Sec. III by setting absorbing boundaries at states $(L) = (M_{J-1})$ and $(R) = (M_{J+1})$ around each level J and studying the appropriate first-passage processes. If we set

$$N_J = |M_A - M_B|, \quad |M_B - M_C|, \dots, \\ |M_K - M_{K-1}|, \quad |N - M_K + M_A|, \\ \text{for } J = A, B, \dots, K-1, K, \quad (131)$$

we can then conclude, using the previous results (37) and (38), that

$$\pi_{\rho\sigma}^J = \pi_{\rho\sigma}(M_{J-1}, N_{J-1}). \quad (132)$$

Similarly, recalling the definitions (48) and (49) for $T_j^+(M, N)$ and $T_j^-(M, N)$ and the results (54)–(57), we obtain

$$\tau_{\rho\sigma}^J = \tau_{\rho\sigma}(M_{J-1}, N_{J-1}). \quad (133)$$

The counting of individual hidden substeps developed in Sec. III E can be carried forward to obtain the mean number of substep transitions between two specified major transitions. The results (98)–(104) essentially apply directly with $M \Rightarrow M_{J-1}$ and $N \Rightarrow N_{J-1}$.

In terms of the *conditional individual state dwell times* introduced in (66)–(68) we also have

$$\tau_{\rho\sigma}^J = \sum_{k=M_{J-1}+1}^{M_J} T_k^{\rho\sigma}(M_{J-1}, N_{J-1}) \quad (134)$$

as a measure of the observable mean overall time spent in the mechanical level J subject to the (ρ, σ) conditions.

As regards potential applications of these results, the case $K=2$ may be reasonable for a first analysis of data for myosin V where, as mentioned, a significant observable substep was originally predicted [20] and later observed [11]; however, the experiments also suggest [11, 12] that stepping may proceed through two (or more) alternative pathways so that a purely sequential model (to which our attention has been restricted) may be inadequate [27]. For the F_1 -ATPase motor [29, 30, 33] substeps have also been reported and occasional back steps have been observed. Thus our results should be applicable.

VI. SUMMARY AND CONCLUSION

As explained in the Introduction, the need to develop the hidden substep analysis we have presented arises from the fact that experimentally detectable steps in the motion of a motor protein along its track do not necessarily delineate the completion of full biochemical enzymatic cycles. As a consequence, previous analyses that addressed such observable statistics as back-stepping fractions, π_- , and mean dwell times, τ_+ and τ_- , measured prior to forward (or $+$) and back (or $-$) steps, were not adequate to relate the underlying rates in a biomechanicochemical model, say u_i and w_j to the experimental data.

We have considered the basic N -state sequential kinetic model set out in (1) and specified by N forward rates u_i from biochemical state (i) and N reverse rates w_j from state (j). In general, the rates depend on the concentration of various reagents [see, e.g., (2)] and, in particular, vary experimentally with the load force $\mathbf{F} = (F_x, F_y, F_z)$: see (109) and (110).

The basic problem may then be set up by supposing that as the motor progresses (or retrogresses) along its molecular track only a single “major transition” from state (M) to $(M+1)$ ($0 \leq M < N$), or its reverse, corresponds to a “visible” or detectable “step” in the N -state cycle. All the other transitions are “hidden.” see Fig. 1. [Cases in which more than one major transition or observable (sub)step occur in each full enzymatic cycle are analyzed in Sec. V.] It then transpires that two crucial combinations of the rates $\{u_i, w_j\}$ play a central role, namely, Γ_N and $\Delta_{M,N}$ as defined in (29)–(31).

Indeed, explicit expressions for the forward and backward stepping fractions, π_+ and π_- , are derived in terms of Γ_N and $\Delta_{M,N}$ in Sec. III B and presented in (40) and (41). It proves helpful, furthermore, to relate π_+ and π_- to the *conditional* or *pairwise step probabilities*, π_{++} , π_{+-} , etc., for $+$ steps followed by a $+$ step, or by a $-$ step, etc., that can be defined via counting observations, as explained in (15)–(18) and the associated text. These pairwise probabilities are likewise expressible in terms of Γ_N and $\Delta_{M,N}$: see (37) and (38).

From these results one can see—as explained in further detail in Sec. IV—that only when $\Delta_{M,N} \ll \min\{1, \Gamma_N\}$ can one neglect the hidden substeps without risk of serious error. Particularly instructive is the variation of $\ln(\pi_+/\pi_-)$ as the load F_x passes through stall (at which $\pi_+ = \pi_- = \frac{1}{2}$ so that the mean velocity vanishes). One may then define an *effective step length*, d^* , via (115) and (116). When $\Delta_{M,N} \rightarrow 0$ (or if hidden substeps are ignored) one has the simple *equality* $d^* = d$, where d is the full step size of the motor (per cycle). But, in

fact, d^*/d must always be *less* than unity and, as seen in experiment and illustrated in Table I, this ratio is typically of magnitude only 0.3 to 0.5.

Going beyond simple counts of backward and forward steps, one may also define *conditional mean dwell times*, τ_{++} , τ_{+-} , etc., for the time spent between a pair of successive + steps, or a + step followed by a - step, etc., see (12). These pairwise mean times can likewise be calculated [see (54)–(57)] in terms of *individual-state post dwell times*, $T_j^+(\equiv T_j^{+\diamond})$ and $T_j^-(\equiv T_j^{-\diamond})$, that represent the mean time spent in a state (j) following a + or - step, respectively: see Sec. III D for a fuller explanation of the notation, etc. The corresponding explicit expressions, (48) and (49), involve the basic rate products $\Gamma_{l,m}$ and their sums, $\Delta_{l,n}$, as again defined in (29) and (31). The final results for τ_+ , τ_- , and for the overall mean dwell time τ (between + or - steps) for general M and N entail slightly simpler sums: see (59)–(62).

More transparent formulas for the stepping fractions and dwell times for $N=2$ models (involving only the rates u_0 , u_1 , w_0 , and w_1), and for selected $N=4$ models, are presented in the Appendix. In addition, the parts of Fig. 3 and the associated discussion in Sec. IV, illustrate that a wide range of different types of behavior of $\pi_+(F)$, $\ln[\pi_+(F)/\pi_-(F)]$, and $\tau_-(F)$ as functions of load can be realized even within simple $N=2$ models.

At a higher level of detail, conditional individual-state dwell times, $T_j^{++}, T_j^{+-}, \dots$, can be derived [see (66)–(68)] and, likewise, *post* (as against the previously mentioned *prior*) dwell times, $\tau_{+\diamond}$ and $\tau_{-\diamond}$: see (64). Finally, one can obtain the expressions (98), (99), (103), and (104), for the mean number, $n_{j,+}^{\rho\sigma}$ and $n_{j,-}^{\rho\sigma}$ of hidden, forwards and backwards, substeps from an individual state (j) that occur in a time interval between detectable steps, i.e., major transitions specified by $\{\rho, \sigma\} = \{\dots, +-, -+, \dots\}$: see (71). These results provide quantitative estimates for the number of “lost” or “hidden” transitions occurring in an enzymatic cycle. Such information could be of particular interest for real motor proteins since, when operating in cells to achieve mitosis, etc., they may frequently be in close-to-stall conditions where reverse substeps are likely to be most frequent [46,47].

In conclusion, we have provided a detailed analysis of the statistics of mechanochemical transitions that must be hidden in the experimental noise when a motor protein on its track moves processively via distinct steps, or reaches stall. As experimental resolution at the microsecond and nanometer scales improves, we can expect that such analyses will be increasingly valuable for extracting reliable inferences about motor mechanisms from observational data.

ACKNOWLEDGMENTS

The authors much appreciate correspondence and interactions with Dr. R. A. Cross and Dr. N. J. Carter. Support from the National Science Foundation under Grant No. CHE 03-01101 is acknowledged. One of the authors (M.L.) is grateful for the financial support of the Royal Institute of Technology and the Wallenberg Foundation and for the hospitality of the Institute for Physical Science and Technology at the University of Maryland in Fall 2005.

APPENDIX: EXPRESSIONS FOR TWO-STATE AND FOUR-STATE MODELS

For convenience of reference we provide here explicit expressions for $N=2$ models with $M=1$. First, we recall the full-cycle expressions [20,35]

$$\check{\pi}_+ = 1 - \check{\pi}_- = \frac{u_0 u_1}{u_0 u_1 + w_0 w_1}, \quad (\text{A1})$$

$$\check{\tau}_+ = \check{\tau}_- = \check{\tau} = \frac{u_0 + u_1 + w_0 + w_1}{u_0 u_1 + w_0 w_1}, \quad (\text{A2})$$

the result for general N being given in (105) and (106). Allowing for hidden substeps leads to

$$\pi_+ = 1 - \pi_- = \frac{u_1(u_0 + w_0)}{u_0 u_1 + w_0 w_1 + 2u_1 w_0}, \quad (\text{A3})$$

while the distinct prior dwell (or stepping) times are given by

$$\tau_+ \equiv \tau_{\diamond+} = \frac{(u_0 + w_0)^2 + u_0(w_1 + u_1)}{(u_0 + w_0)(u_0 u_1 + w_0 w_1 + u_1 w_0)}, \quad (\text{A4})$$

$$\tau_- \equiv \tau_{\diamond-} = \frac{(u_1 + w_1)^2 + w_1(u_0 + w_0)}{(u_1 + w_1)(u_0 u_1 + w_0 w_1 + u_1 w_0)}, \quad (\text{A5})$$

with the mean overall dwell time

$$\tau = \pi_+ \tau_+ + \pi_- \tau_- = \frac{u_0 + u_1 + w_0 + w_1}{u_0 u_1 + w_0 w_1 + 2u_1 w_0}. \quad (\text{A6})$$

For general N see (40), (41), and (60)–(62).

The partial or conditional pairwise step probabilities follow, by specializing (37) and (38), as

$$\pi_{++} = 1 - \pi_{+-} = \frac{u_0 u_1}{u_0 u_1 + w_0 w_1 + u_1 w_0}, \quad (\text{A7})$$

$$\pi_{-+} = 1 - \pi_{--} = \frac{u_1(u_0 + w_0)}{u_0 u_1 + w_0 w_1 + u_1 w_0}. \quad (\text{A8})$$

The denominators here, say

$$\mathcal{D}_2 = u_0 u_1 + w_0 w_1 + u_1 w_0, \quad (\text{A9})$$

should be contrasted with those in (A3) and (A6), namely,

$$\mathcal{D}_2^+ = \mathcal{D}_2 + u_1 w_0 = u_0 u_1 + w_0 w_1 + 2u_1 w_0. \quad (\text{A10})$$

At the next level of *individual* (or *partial*) (sub)state properties, the individual or substate dwell times follow from (59), which yields

$$T_0 = (u_1 + w_1)/\mathcal{D}_2^+, \quad T_1 = (u_0 + w_0)/\mathcal{D}_2^+, \quad (\text{A11})$$

which, in accord with (62), satisfy $\tau = T_0 + T_1$. The partial substate dwell times, given generally in (48) and (19), are

$$T_0^+ = (u_1 + w_1)/\mathcal{D}_2, \quad T_1^+ = u_0/\mathcal{D}_2, \quad (\text{A12})$$

$$T_0^- = w_1/\mathcal{D}_2, \quad T_1^- = (u_0 + w_0)/\mathcal{D}_2. \quad (\text{A13})$$

Finally, the conditional or pairwise mean dwell or stepping times are

$$\tau_{++} = \tau_{--} = (u_0 + u_1 + w_0 + w_1)/\mathcal{D}_2, \quad (\text{A14})$$

$$\tau_{+-} = [(u_1 + w_1)^2 + u_0 w_1]/(u_1 + w_1)\mathcal{D}_2, \quad (\text{A15})$$

$$\tau_{-+} = [(u_0 + w_0)^2 + u_0 w_1]/(u_0 + w_0)\mathcal{D}_2. \quad (\text{A16})$$

The mean numbers of *hidden transitions* follow from the results in Sec. III E. For the simplest $N=2$ ($M=1$) case we obtain

$$\begin{aligned} n_{0,+}^{++} - 1 &= n_{0,+}^{+-} = n_{0,+}^{-+} = n_{0,+}^{--} = n_{0,+}^{\diamond-} \\ &= n_{0,+}^{\diamond-} = n_{1,-}^{++} = n_{1,-}^{+-} = n_{1,-}^{-+} \\ &= n_{1,-}^{--} - 1 = n_{1,-}^{\diamond+} = n_{1,-}^{\diamond+} = u_0 w_1 / \mathcal{D}_2, \end{aligned} \quad (\text{A17})$$

$$n_{0,+}^{\diamond+} = n_{0,+}^{\diamond+} = u_0(u_1 + w_1)/\mathcal{D}_2, \quad (\text{A18})$$

$$n_{1,-}^{\diamond-} = n_{1,-}^{\diamond-} = w_1(u_0 + w_0)/\mathcal{D}_2, \quad (\text{A19})$$

$$n_{0,+}^{\diamond\diamond} = u_0(u_1 + w_1)/\mathcal{D}_2^+, \quad (\text{A20})$$

$$n_{1,-}^{\diamond\diamond} = w_1(u_0 + w_0)/\mathcal{D}_2^+. \quad (\text{A21})$$

To provide further insight we quote some results for $N=4$ models with $M=3$, i.e., with the major step as the last transition from state (3) to $[4 \equiv 0]$. Thus we have

$$\pi_{-} = w_0 \mathfrak{N}_0 / \mathcal{D}_4^+, \quad \pi_{+} = u_3 \mathfrak{N}_3 / \mathcal{D}_4^+, \quad (\text{A22})$$

$$\tau = (\mathfrak{N}_0 + \mathfrak{N}_1 + \mathfrak{N}_2 + \mathfrak{N}_3) / \mathcal{D}_4^+, \quad (\text{A23})$$

where, to write the numerator and denominator contributions compactly, we introduce the short-hand product notation

$$\begin{aligned} u_{0,1} &= u_0 u_1, & u_{0,1,2} &= u_0 u_1 u_2, & \dots, \\ w_{0,1} &= w_0 w_1, & w_{0,1,2} &= w_0 w_1 w_2, & \dots, \end{aligned} \quad (\text{A24})$$

etc. Then we have

$$\mathfrak{N}_0 = u_{1,2,3} + w_1 u_{2,3} + w_{1,2} u_3 + w_{1,2,3}, \quad (\text{A25})$$

$$\mathfrak{N}_1 = u_{2,3,0} + w_2 u_{3,0} + w_{2,3} u_0 + w_{2,3,0}, \quad (\text{A26})$$

$$\mathfrak{N}_2 = u_{3,0,1} + w_3 u_{0,1} + w_{3,0} u_1 + w_{3,0,1}, \quad (\text{A27})$$

$$\mathfrak{N}_3 = u_{0,1,2} + w_0 u_{1,2} + w_{0,1} u_2 + w_{0,1,2}, \quad (\text{A28})$$

while the denominator is given by

$$\begin{aligned} \mathcal{D}_4^+ &= w_0 \mathfrak{N}_0 + u_3 \mathfrak{N}_3 = u_{0,1,2,3} + 2w_0 u_{1,2,3} + 2w_{0,1} u_{2,3} \\ &\quad + 2w_{0,1,2} u_3 + w_{0,1,2,3}. \end{aligned} \quad (\text{A29})$$

For the purpose of comparison we quote the result for the velocity, namely,

$$V = d(u_{0,1,2,3} - w_{0,1,2,3}) / (\mathfrak{N}_0 + \mathfrak{N}_1 + \mathfrak{N}_2 + \mathfrak{N}_3), \quad (\text{A30})$$

which, of course, is *not* simply d/τ : see (107). Finally, then we also quote

$$\begin{aligned} \tau_{++} = \tau_{--} &= [\mathfrak{N}_0 + \mathfrak{N}_1 + \mathfrak{N}_2 + \mathfrak{N}_3 \\ &\quad + w_0 u_3 (u_1 + w_1 + u_2 + w_2)] / \mathcal{D}_4, \end{aligned} \quad (\text{A31})$$

and

$$\tau_{\pm} = \frac{u_{2,1,0} \mathfrak{N}_0 + u_{2,1} \mathfrak{D}_1 \mathfrak{N}_1 + u_2 \mathfrak{D}_2 \mathfrak{N}_2 + \mathfrak{D}_3 \mathfrak{N}_3}{\mathcal{D}_4 \mathfrak{N}_3}, \quad (\text{A32})$$

where

$$\begin{aligned} \mathcal{D}_N &= u_{0,1,\dots,N-1} + w_0 u_{1,2,\dots,N-1} + w_{0,1} u_{2,3,\dots,N-1} + \dots \\ &\quad + w_{0,1,\dots,N-1}, \end{aligned} \quad (\text{A33})$$

from which τ_{-} follows by using $\pi_{-} \tau_{-} + \pi_{+} \tau_{+} = \tau$. Of course, results for $M=0, 1$, and 2 and for π_{++}, τ_{++}, T_j , etc., follow from the expressions derived in Sec. III.

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