

A model for DNA helicase mechanism based on a flashing ratchet

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Helicases are molecular motors that consume energy supplied by chemical reactions to unwind double-stranded nucleic acids (like DNA and RNA) and to translocate along one of the single-strands. Motivated by the recent claims, based on experimental observations on the helicase NS3 of hepatitis C virus (HCV), that monomeric helicases are governed by a Brownian ratchet mechanism, here we develop a quantitative model. Our Brownian ratchet model, which is a somewhat new reformulation of the Betterton-Jülicher theory of helicases, is generic two-state model and is applicable to all helicases which follow the Brownian ratchet mechanism. We illustrate the predictive power of the model by calculating some experimentally testable motor properties of a few monomeric helicases. Specifically, we predict the speed of unwinding of the double-stranded DNA and fluctuations around the average drift of the helicase. Our predictions are in excellent quantitative agreement with the corresponding experimental data.

PACS numbers:

I. INTRODUCTION

Helicases [1] are enzymes that unwind double-stranded nucleic acids and translocate along one of the two single-strands. These proteins consume chemical energy (typically, supplied by the hydrolysis of ATP) and perform mechanical work. Therefore, these nucleic acid translocases are molecular motors [2, 3, 4, 5, 6, 7], which share common features with cytoskeletal molecular motors [8, 9, 10]. Helicases are broadly divided into the hexameric group (which consist of an hexameric arrangement of six ATPase domains) and non-hexameric (mostly dimeric and a few monomeric) group. Two alternative processes, called the rolling (or hand-over-hand) and inchworm mechanisms have been suggested for the helicase activity of non-hexameric helicases [11]. For hexameric helicases, at least three different alternative mechanisms of enzymatic activities have been suggested; these include, activities of all the ATP-binding domains in (a) parallel, (b) ordered sequential manner and (c) random-sequential manner [12].

However, other mechanisms for helicase translocation have been suggested. Analyzing the data from a series of experiments, Patel and coworkers [13] have suggested a flashing-ratchet mechanism [14, 15], for the monomeric helicase NS3 of the hepatitis C virus (HCV) [16]. They also proposed a qualitative two-state model for the ratchet. We note that the experiments of Patel group [13] focus on DNA unwinding by monomers of the NS3 helicase domain, but different experimen-

tal results have been found when studying unwinding of RNA by full-length NS3 or HCV replication complexes [17, 18, 19]. The mechanism of the NS3 helicase may vary under different experimental conditions.

Motivated by the proposed flashing-ratchet mechanism for NS3 helicase, we develop and solve a flashing ratchet model of helicases. The model significantly extends the original Betterton-Jülicher (BJ) model [20] to incorporate the two-state scenario suggested in ref.[13] and thereby make a direct contact with the flashing ratchet mechanism. A two-state model for the helicase was also considered by Betterton and Jülicher [20], but the nature of the two states in that formulation and the mechanism of translocation of the helicase is different from those developed here.

Our paper is structured as follows. In section II we describe the discrete version of a flashing ratchet (the helicase) which acts to push a fluctuating obstacle (the DNA ss-ds junction). Section III contains the basic equations which describe the model, the transformation of the equations using midpoint and difference variables, and the general solutions for the velocity and diffusion coefficient of unwinding. We describe the results for a hard-wall interaction between helicase and junction in section IV, including limiting cases of various parameters being large or small. Using the rate constants extracted from earlier empirical data on HCV NS3 helicase, in section IV we also predict our theoretical estimate for the speed of unwinding by this helicase. Finally, in section V we summarize our conclusions.

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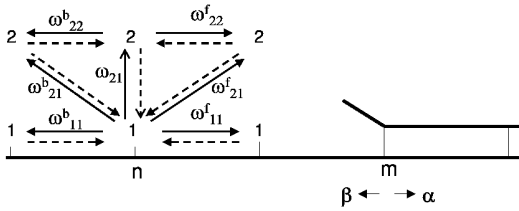


FIG. 1: A schematic representation of the model.

II. THE MODEL

We use a model inspired by the work of Levin et al. [13] which proposed that the HCV helicase switches between 2 states: one is tightly bound to the ssDNA, while the other is weakly bound. This scenario is referred to in the physics literature as a flashing ratchet [14]. The flashing ratchet is a special case of a two-state model [14] because the helicase can be found in either of the two allowed chemical states, namely, a state S in which it is strongly bound to the ssDNA strand and another state W in which it is weakly attached to the same strand. When applying the flashing ratchet scenario to HCV helicase, the tightly bound state is represented by a periodic sawtooth potential (with periodicity of one ssDNA base pair) and the weakly bound state is represented by a uniform (position-independent) potential.

In the traditional continuous models of Brownian ratchets, one first writes a Fokker-Planck equation; because we use a discrete model our approach is based on master equations. The discrete approach can be useful when comparing to experiments. In the Fokker-Planck approach, one needs the explicit functional form of the fluctuating potential. Although most often a sawtooth-like form is assumed to incorporate the asymmetric, periodic potential, the actual form of the potential experienced by a real molecular motor has not been measured or calculated. We bypass this difficulty by capturing the Brownian motor mechanism effectively through a judicious choice of rate constants (or transition probabilities), many of which can be obtained from experiments [21]. A similar strategy has been followed recently in developing a Brownian ratchet model for the single-headed kinesin KIF1A [22], although realistic implementation of the strategy is more difficult here because of the intrinsic heterogeneity of the ssDNA track [23].

We capture the physics of the flashing ratchet in a discrete hopping model. We represent the ssDNA by a one-dimensional lattice each site of which corresponds to a single base. We label each site by the integer index i . As in the BJ model [20], we neglect the sequence in-

homogeneity of the ssDNA (in principle, the model can be extended to capture this feature). The position of the helicase is denoted by the integer n . Every known helicase has a fixed direction of translocation, i.e., either 3' to 5' or 5' to 3' along the left-right asymmetric ssDNA [?]. In our model the helicase is assumed to translocate towards increasing n (from left to right). The junction between ssDNA-dsDNA is located at site m (see fig. 1). At any spatial position n , the helicase can be either in state 1 (strongly bound, labeled S), or 2 (weakly bound, labeled W).

The model is fully described by the allowed transitions between states and the corresponding reaction rates. We use notation where $\omega_{\mu\nu}^f$ is the rate of the transition *to* state μ at an arbitrary spatial location n *from* the state ν located at $n + 1$ where both μ and ν can be either 1 (S) or 2 (W). The corresponding backwards transition from $n + 1$ to n has rate $\omega_{\nu\mu}^b$. In general, we could have all transitions sketched in fig. 1.

Helicase “sliding” corresponds to transitions along the ssDNA without a change in biochemical state of the protein. In the 1 (S) state, these sliding transitions occur at rate ω_{11}^f (for increasing n) and ω_{11}^b (for decreasing n). When the helicase is in the 2 (W) state, the forward/backward sliding rates are ω_{22}^f and ω_{22}^b . Physically, these transitions occur because of Brownian motion of the protein, decoupled from any biochemical state change. The transitions associated with ω_{22}^f and ω_{22}^b can be interpreted to be caused by one-dimensional diffusion of the helicase in the weakly-bound state; unbiased diffusion would correspond to $\omega_{22}^f = \omega_{22}^b$. Even in the strongly-bound state the helicase will be significantly affected by thermal fluctuations; the transitions associated with ω_{11}^f and ω_{11}^b can be interpreted as thermally-activated Kramers-like processes. In general, we would expect the sliding rates for the 2 (W) state to be much larger than for the tightly bound (1) state.

The helicase can undergo “chemical” transitions which correspond to a change in biochemical state without physical translocation along the ssDNA. At fixed n , the rate of transition *to* state 1 (S) *from* 2 (W) occurs at rate ω_{12} , while the reverse transition occurs at rate ω_{21} . If one of these reactions is coupled to ATP hydrolysis, then the forward/reverse transitions may be out of equilibrium and break the detailed balance relation. For example, the Levin et al. model of HCV helicase suggests that ATP hydrolysis is required to remove the helicase from the tightly bound state, implying that the $1 \rightarrow 2$ transition at rate ω_{21} is out of equilibrium.

The final type of helicase transitions are those where a change of biochemical state and physical translocation occur together. If the helicase is located at n and is in state 1 (S), then it can make a transition to state 2 (W) while moving forward to site $n + 1$ at rate ω_{21}^f ; the same change of state coupled to a backwards displacement to site $n - 1$ occurs at rate ω_{21}^b . The corresponding reverse transitions occur at rates ω_{12}^b (transition from state 2 at

$n + 1$ to state 1 at n) and ω_{12}^f (transition from state 2 at $n - 1$ to state 1 at n).

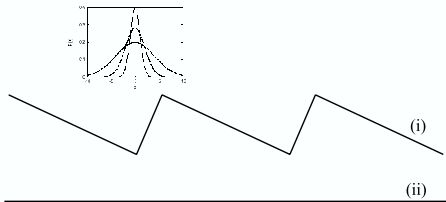


FIG. 2: Schematic description of the Brownian-ratchet mechanism

We can associate the discrete transition rates with the physical process and develop intuition for the relative magnitudes of the rates by comparing the discrete rates to the continuous picture illustrated in fig.2. The physical processes captured by the parameters ω_{21}^f and ω_{21} can be understood as follows by comparison with the flashing ratchet. For the sake of simplicity, suppose that the potential seen by the motor periodically oscillates between the sawtooth shape and the flat shape shown in fig. 2. When the sawtooth potential is on for some time, the particle settles at the bottom of a well; this is the location of a potential minimum for state 1 at site n . Then, when the potential is switched off, the particle is switched to state 2. The probability distribution of the position of the particle (initially a delta function) begins to spread symmetrically. (The spread is symmetric assuming free diffusion in the absence of any force). After some time, the Gaussian probability distribution spreads such that it overlaps with the potential minimum of state 1 at site $n + 1$, in addition to the overlap it has with the original well. When the sawtooth potential is again switched on, there is a non-vanishing probability that the particle will

move to the potential minimum at site $n + 1$. In our discrete model, this transition corresponds to a transition from state 2 and site n to state 1 at site $n + 1$, which occurs at rate ω_{12}^f . There is also a significant probability that the particle will fall back into the original well; this is captured in our model by the parameter ω_{12} . As the motor efficiency increases, we expect ω_{12}^f to increase relative to ω_{12} . For a motor that is unbiased (which would occur, for example, if the sawtooth potential is symmetric), we would have $\omega_{12}^f = \omega_{12}^b$.

The dsDNA opens and closes due to thermal fluctuations. When the helicase and junction are far apart, the opening rate is α and the closing rate β . We assume that these rates are independent of the NA base sequence and that the only fluctuations are those for which the NA opens or closes at the ss-ds fork only. Since the NA breathing results from thermal fluctuations, the rates α and β satisfy detailed balance: $\frac{\alpha}{\beta} = e^{-\Delta G}$, where ΔG is the free energy of one base-pair bond.

In this work we assume passive unwinding, which is equivalent to a hard-wall interaction potential in the BJ model [20]. This means that when the helicase and junction are adjacent ($j = 1$), the helicase cannot hop forward and the NA cannot close ($k_1^+ = \beta_1 = 0$). Otherwise, the rates are unaffected by the helicase-junction interaction.

We shall calculate the average speed v of unwinding and fluctuations about the drift of the helicase. While, on physical grounds, it is obvious that ω_{22}^f or ω_{22}^b will not appear in the expression for v , the fluctuations will be affected by the Brownian motion in the weakly-bound state (W) of the helicase.

III. MASTER EQUATIONS

Let $P_\mu(n, m; t)$ denote the probability that, at time t , the helicase is at located at n and is in the “chemical” state μ where $\mu = 1$ and $\mu = 2$ correspond to the states in which the helicase is bound, respectively, *strongly* and *weakly* to the NA, while the ss-ds junction is at m . The master equations governing the time evolutions of $P_\mu(n, m; t)$ are given by

$$\begin{aligned} \frac{dP_1(n, m; t)}{dt} = & -(\alpha_{m-n} + \beta_{m-n} + \omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b + \omega_{21})P_1(n, m; t) \\ & + \omega_{11}^f P_1(n - 1, m; t) + \omega_{12}^f P_2(n - 1, m; t) + \omega_{11}^b P_1(n + 1, m; t) + \omega_{12}^b P_2(n + 1, m; t) \\ & + \omega_{12} P_2(n, m; t) + \alpha_{(m-1)-n} P_1(n, m - 1; t) + \beta_{(m+1)-n} P_1(n, m + 1; t). \end{aligned} \quad (1)$$

and

$$\begin{aligned} \frac{dP_2(n, m; t)}{dt} &= -(\alpha_{m-n} + \beta_{m-n} + \omega_{22}^f + \omega_{22}^b + \omega_{12}^f + \omega_{12}^b + \omega_{12})P_2(n, m; t) \\ &+ \omega_{21}^f P_1(n-1, m; t) + \omega_{22}^f P_2(n-1, m; t) + \omega_{21}^b P_1(n+1, m; t) + \omega_{22}^b P_2(n+1, m; t) \\ &+ \omega_{21} P_1(n, m; t) + \alpha_{(m-1)-n} P_2(n, m-1; t) + \beta_{(m+1)-n} P_2(n, m+1; t) \end{aligned} \quad (2)$$

respectively.

Let us define $j = m - n$ and $l = 2l' = m + n$. Obviously j denotes the separation between the helix and the

junction while l' corresponds to the mid-point between them. In terms of j and l the equations (1) and (2) can be recast as

$$\begin{aligned} \frac{dP_1(j, l; t)}{dt} &= -(\alpha_j + \beta_j + \omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b + \omega_{21})P_1(j, l; t) \\ &+ \omega_{11}^f P_1(j+1, l-1; t) + \omega_{12}^f P_2(j+1, l-1; t) + \omega_{11}^b P_1(j-1, l+1; t) + \omega_{12}^b P_2(j-1, l+1; t) \\ &+ \omega_{12} P_2(j, l; t) + \alpha_{j-1} P_1(j-1, l-1; t) + \beta_{j+1} P_1(j+1, l+1; t). \end{aligned} \quad (3)$$

and

$$\begin{aligned} \frac{dP_2(j, l; t)}{dt} &= -(\alpha_j + \beta_j + \omega_{22}^f + \omega_{22}^b + \omega_{12}^f + \omega_{12}^b + \omega_{12})P_2(j, l; t) \\ &+ \omega_{21}^f P_1(j+1, l-1; t) + \omega_{22}^f P_2(j+1, l-1; t) + \omega_{21}^b P_1(j-1, l+1; t) + \omega_{22}^b P_2(j-1, l+1; t) \\ &+ \omega_{21} P_1(j, l; t) + \alpha_{j-1} P_2(j-1, l-1; t) + \beta_{j+1} P_2(j+1, l+1; t) \end{aligned} \quad (4)$$

Next, let us define the probability distributions of the gap sizes

From equations (3) and (4), the equations for $\mathcal{P}_1(j; t)$ and $\mathcal{P}_2(j; t)$ follow; these are given by

$$\begin{aligned} \mathcal{P}_1(j; t) &= \sum_l P_1(j, l; t) \\ \mathcal{P}_2(j; t) &= \sum_l P_2(j, l; t) \end{aligned} \quad (5)$$

$$\begin{aligned} \frac{d\mathcal{P}_1(j; t)}{dt} &= -(\alpha_j + \beta_j + \omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b + \omega_{21})\mathcal{P}_1(j; t) \\ &+ \omega_{11}^f \mathcal{P}_1(j+1; t) + \omega_{12}^f \mathcal{P}_2(j+1; t) + \omega_{11}^b \mathcal{P}_1(j-1; t) + \omega_{12}^b \mathcal{P}_2(j-1; t) \\ &+ \omega_{12} \mathcal{P}_2(j; t) + \alpha_{j-1} \mathcal{P}_1(j-1; t) + \beta_{j+1} \mathcal{P}_1(j+1; t). \end{aligned} \quad (6)$$

and

$$\begin{aligned} \frac{d\mathcal{P}_2(j; t)}{dt} &= -(\alpha_j + \beta_j + \omega_{22}^f + \omega_{22}^b + \omega_{12}^f + \omega_{12}^b + \omega_{12})\mathcal{P}_2(j; t) \\ &+ \omega_{21}^f \mathcal{P}_1(j+1; t) + \omega_{22}^f \mathcal{P}_2(j+1; t) + \omega_{21}^b \mathcal{P}_1(j-1; t) + \omega_{22}^b \mathcal{P}_2(j-1; t) \\ &+ \omega_{21} \mathcal{P}_1(j; t) + \alpha_{j-1} \mathcal{P}_2(j-1; t) + \beta_{j+1} \mathcal{P}_2(j+1; t) \end{aligned} \quad (7)$$

Adding the equations (6) and (7) we get

$$\begin{aligned} \frac{d\mathcal{P}(j; t)}{dt} &= -(\alpha_j + \beta_j)\mathcal{P}(j; t) + \alpha_{j-1}\mathcal{P}(j-1; t) + \beta_{j+1}\mathcal{P}(j+1; t) \\ &+ (\omega_{11}^f + \omega_{21}^f)\mathcal{P}_1(j+1; t) + (\omega_{12}^f + \omega_{22}^f)\mathcal{P}_2(j+1; t) + (\omega_{11}^b + \omega_{21}^b)\mathcal{P}_1(j-1; t) + (\omega_{12}^b + \omega_{22}^b)\mathcal{P}_2(j-1; t) \\ &- (\omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b)\mathcal{P}_1(j; t) - (\omega_{22}^f + \omega_{22}^b + \omega_{12}^f + \omega_{12}^b)\mathcal{P}_2(j; t) \end{aligned} \quad (8)$$

for the distribution of gap sizes, irrespective of the “chemical” state of the helicase. Interestingly, the right hand side of the equation (8) does not involve ω_{21} and ω_{12} as the transitions $1 \rightarrow 2$ and $2 \rightarrow 1$ do not change j because the position of the helicase remains unchanged in both these transitions.

We now define the probability current between j and $j + 1$ by

$$\begin{aligned} I(j) &= \alpha_j \mathcal{P}(j) - \beta_{j+1} \mathcal{P}(j+1) \\ &+ (\omega_{11}^b + \omega_{21}^b) \mathcal{P}_1(j) + (\omega_{12}^b + \omega_{22}^b) \mathcal{P}_2(j) \\ &- (\omega_{11}^f + \omega_{21}^f) \mathcal{P}_1(j+1) - (\omega_{12}^f + \omega_{22}^f) \mathcal{P}_2(j+1) \end{aligned} \quad (9)$$

In terms of the probability current (9) the equation (8) can be recast as

$$\frac{d\mathcal{P}(j; t)}{dt} + [I(j) - I(j-1)] = 0 \quad (10)$$

which formally appears as an equation of continuity for the probability. In the steady-state $\mathcal{P}(j)$ is independent of time and we get the condition $I(j) = I(j-1)$. Moreover, since $U(j) \rightarrow \infty$ as $j \rightarrow -\infty$, this constant probability flux must be zero, i.e.,

$$I(j) = 0 \text{ for all } j. \quad (11)$$

Adding the two equations (3) and (4) we get

$$\begin{aligned} \frac{dP(j, l; t)}{dt} &= -(\alpha_j + \beta_j) P(j, l; t) + \alpha_{j-1} P(j-1, l-1; t) + \beta_{j+1} P(j+1, l+1; t) \\ &+ (\omega_{11}^f + \omega_{21}^f) P_1(j+1, l-1; t) + (\omega_{12}^f + \omega_{22}^f) P_2(j+1, l-1; t) \\ &+ (\omega_{11}^b + \omega_{21}^b) P_1(j-1, l+1; t) + (\omega_{12}^b + \omega_{22}^b) P_2(j-1, l+1; t) \\ &- (\omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b) P_1(j, l; t) - (\omega_{12}^f + \omega_{12}^b + \omega_{22}^f + \omega_{22}^b) P_2(j, l; t) \end{aligned} \quad (12)$$

where

$$P(j, l; t) = P_1(j, l; t) + P_2(j, l; t). \quad (13)$$

where $P(j, l; t)$ is the joint probability distribution of the gaps j and midpoints l , irrespective of the “chemical” state of the helicase. We now define the probability distributions of l at time t by

$$\Pi(l; t) = \sum_j P(j, l; t) \quad (14)$$

Note that, by definition, $\Pi(l; t)$ is independent of the chemical state of the helicase, i.e., whether the helicase is in the state 1 or in the state 2. For times much longer than the relaxation time of the difference variable j , we can assume

$$P_\mu(j, l) = \mathcal{P}_\mu(j) \Pi(l) \quad (\mu = 1 \text{ or } 2) \quad (15)$$

Starting from the equation (12), it is straightforward to derive

$$\frac{d\Pi(l; t)}{dt} = -(p+q)\Pi(l; t) + p\Pi(l-1; t) + q\Pi(l+1; t) \quad (16)$$

where

$$p = \sum_j [\alpha_j \mathcal{P}(j) + (\omega_{11}^f + \omega_{21}^f) \mathcal{P}_1(j) + (\omega_{12}^f + \omega_{22}^f) \mathcal{P}_2(j)] \quad (17)$$

and

$$q = \sum_j [\beta_j \mathcal{P}(j) + (\omega_{11}^b + \omega_{21}^b) \mathcal{P}_1(j) + (\omega_{12}^b + \omega_{22}^b) \mathcal{P}_2(j)] \quad (18)$$

Thus, as in the original formulation of BJ [20], the dynamics of the midpoint variable l is, in general, a combination of drift and diffusion. Note that in the special case $p = q$ the drift vanishes and the dynamics of l becomes purely diffusive.

Therefore, the average speed of unwinding should be defined as

$$v = \frac{1}{2}(p - q) \quad (19)$$

where the prefactor $1/2$ arises from the fact that the midpoint is actually $l/2$ and not l . Using the expressions (17)

and (18) in (29) we get

$$\begin{aligned}
v &= \frac{1}{2} \sum_j [(\alpha_j - \beta_j) \mathcal{P}(j) \\
&+ (\omega_{12}^f + \omega_{22}^f - \omega_{12}^b - \omega_{22}^b) \mathcal{P}_2(j) \\
&+ (\omega_{21}^f + \omega_{11}^f - \omega_{11}^b - \omega_{21}^b) \mathcal{P}_1(j)].
\end{aligned} \tag{20}$$

Alternatively, the expression for v can also be written as

$$\begin{aligned}
v &= \frac{1}{2} \sum_j [(\alpha_j + \omega_{12}^f + \omega_{22}^f - \omega_{12}^b - \omega_{22}^b - \beta_j) \mathcal{P}_2(j) \\
&- (\beta_j - \omega_{21}^f - \omega_{11}^f - \alpha_j + \omega_{11}^b + \omega_{21}^b) \mathcal{P}_1(j)].
\end{aligned} \tag{21}$$

Similarly, following the same arguments as in ref.[20], we get diffusion constant

$$\begin{aligned}
D &= \frac{p+q}{4} \\
&= \frac{1}{4} \sum_j [(\alpha_j + \beta_j) \mathcal{P}(j) \\
&+ (\omega_{12}^f + \omega_{22}^f + \omega_{12}^b + \omega_{22}^b) \mathcal{P}_2(j) \\
&+ (\omega_{21}^f + \omega_{11}^f + \omega_{11}^b + \omega_{21}^b) \mathcal{P}_1(j)] \\
&= \frac{1}{4} \sum_j [(\alpha_j + \beta_j + \omega_{12}^f + \omega_{22}^f + \omega_{12}^b + \omega_{22}^b) \mathcal{P}_2(j) \\
&+ (\alpha_j + \beta_j + \omega_{21}^f + \omega_{11}^f + \omega_{11}^b + \omega_{21}^b) \mathcal{P}_1(j)].
\end{aligned} \tag{22}$$

Note that if ω_{22}^f and ω_{22}^b are interpreted to be the rate constants corresponding to *unbiased* diffusion of the heli-

case in the weakly bound state 2, then $\omega_{22}^f = \omega_{22}^b$ and the corresponding two terms drop out from the expression for v but not from that for D .

IV. SOLUTION

In order to evaluate v and D we need to get the expressions for $\mathcal{P}_1(j)$ and $\mathcal{P}_2(j)$ in terms of the rate constants. Invoking the principle of detailed balance for the purely “chemical” transitions between the states 1 and 2, while the helicase is located at an arbitrary site n and the fork is at m (i.e., the gap $j = m - n$ remains unchanged), we get

$$\omega_{21} \mathcal{P}_1(j) = \omega_{12} \mathcal{P}_2(j) \tag{23}$$

and, hence,

$$\mathcal{P}_1(j) = \left[\frac{\omega_{12}}{\omega_{21}} \right] \mathcal{P}_2(j). \tag{24}$$

Note that this relation assumes that there is a rapid equilibration of the chemical transitions between the 1 and 2 states at site j . Therefore, this relation is valid in the limit where the rates ω_{21} and ω_{12} are much larger than all the other rates.

Using this detailed-balance relation, the recursion relation for $\mathcal{P}_1(j)$ and that for $\mathcal{P}_2(j)$ are not independent of each other. Using the relation (24) in (??) we find the recursion relation for \mathcal{P}_2 to be

$$\mathcal{P}_2(j+1) = \left\{ \frac{(\omega_{22}^b + \alpha_j + \omega_{12}^b) \omega_{21} + (\alpha_j + \omega_{21}^b + \omega_{11}^b) \omega_{12}}{(\beta_{j+1} + \omega_{12}^f + \omega_{22}^f) \omega_{21} + (\beta_{j+1} + \omega_{11}^f + \omega_{21}^f) \omega_{12}} \right\} \mathcal{P}_2(j) \tag{25}$$

Iterating this recursion relation and, then, using the normalisation condition $\sum_j j [\mathcal{P}_1(j) + \mathcal{P}_2(j)] = 1$, we get

$$\mathcal{P}_2(j) = B c^j \tag{26}$$

with

$$B = \frac{(1-c) \omega_{21}}{c(\omega_{12} + \omega_{21})} \tag{27}$$

and

$$c = \frac{(\alpha + \omega_{11}^b + \omega_{21}^b) \omega_{12} + (\alpha + \omega_{12}^b + \omega_{22}^b) \omega_{21}}{(\beta + \omega_{11}^f + \omega_{21}^f) \omega_{12} + (\beta + \omega_{12}^f + \omega_{22}^f) \omega_{21}} \tag{28}$$

Note that we have also used the facts that $\beta_1 = 0$ and $\omega_{12}^f = 0$ at $j = 1$. Thus, finally, we get

$$v = \frac{(\omega_{12} + \omega_{21}) \left[2(\alpha\omega_{12}^f\omega_{21} - \beta\omega_{21}^b\omega_{12}) + (\alpha\Omega_1 - \beta\Omega_2)\omega_{12} + (\alpha\Omega_3 - \beta\Omega_4)\omega_{21} \right] + (\Omega_5\omega_{12} + \Omega_6\omega_{21})(\omega_{11}^f\omega_{12} + \omega_{22}^f\omega_{21})}{2(\omega_{12} + \omega_{21}) \left\{ (\beta + \omega_{11}^f + \omega_{21}^f)\omega_{12} + (\beta + \omega_{12}^f + \omega_{22}^f)\omega_{21} \right\}} \quad (29)$$

where,

$$\begin{aligned} \Omega_1 &= 2\omega_{21}^f + \omega_{11}^f \\ \Omega_2 &= 2\omega_{11}^b - \omega_{11}^f \\ \Omega_3 &= \omega_{22}^f \\ \Omega_4 &= 2\omega_{12}^b + 2\omega_{22}^b - \omega_{22}^f \\ \Omega_5 &= \omega_{11}^f + \omega_{21}^f - \omega_{11}^b - \omega_{21}^b \\ \Omega_6 &= \omega_{12}^f + \omega_{22}^f - \omega_{12}^b - \omega_{22}^b \end{aligned} \quad (30)$$

and

$$D = \frac{(\Omega_{\alpha 1}\omega_{12} + \Omega_{\alpha 2}\omega_{21})(\Omega_{\beta 1}\omega_{12} + \Omega_{\beta 2}\omega_{21}) + (\Omega'_{\alpha 1}\omega_{12} + \Omega'_{\alpha 2}\omega_{21})(\Omega'_{\beta 1}\omega_{12} + \Omega'_{\beta 2}\omega_{21})}{4(\omega_{12} + \omega_{21}) \left\{ (\beta + \omega_{11}^f + \omega_{21}^f)\omega_{12} + (\beta + \omega_{12}^f + \omega_{22}^f)\omega_{21} \right\}} \quad (31)$$

where

$$\begin{aligned} \Omega_{\alpha 1} &= \alpha + \omega_{11}^f + \omega_{11}^b + \omega_{21}^b \\ \Omega_{\alpha 2} &= \alpha + \omega_{22}^f + \omega_{22}^b + \omega_{12}^b \\ \Omega_{\beta 1} &= \beta + \omega_{11}^f + \omega_{21}^f \\ \Omega_{\beta 2} &= \beta + \omega_{12}^f + \omega_{22}^f \\ \Omega'_{\alpha 1} &= \alpha + \omega_{11}^b + \omega_{21}^b \\ \Omega'_{\alpha 2} &= \alpha + \omega_{12}^b + \omega_{22}^b \\ \Omega'_{\beta 1} &= \beta + \omega_{21}^f \\ \Omega'_{\beta 2} &= \beta + \omega_{12}^f \end{aligned} \quad (32)$$

At first sight, it may appear counterintuitive that the average speed v of unwinding depends on ω_{22}^b or ω_{22}^f . In principle, in an infinite system, the unbiased random walk should have no effect on the average speed. But, the fact that this ω_{22}^f or ω_{22}^b -dependence enters the expres-

sion (29) via the condition $I(j) = 0$, makes it very clear that the ω_{22}^b or ω_{22}^f -dependence of v results not from the dynamical equations but from the boundary conditions at $j = 0$. The helicase can reach the fork by Brownian motion only from left side but not from the right; this boundary condition at the fork breaks the left-right symmetry of Brownian motion which, in turn, gives rise to the ω_{22}^f or ω_{22}^b -dependence of v .

A. Alternative method of solution

Here we investigate a method of solving the model without the assumption of rapid equilibrium at site j (as expressed in equation (24)).

We begin from the zero-current relation, equation (9), which gives a relation between the probabilities at site $j + 1$ and those at site j :

$$\beta_{j+1}\mathcal{P}(j+1) + (\omega_{11}^f + \omega_{21}^f)\mathcal{P}_1(j+1) + (\omega_{12}^f + \omega_{22}^f)\mathcal{P}_2(j+1) = \alpha_j\mathcal{P}(j) + (\omega_{11}^b + \omega_{21}^b)\mathcal{P}_1(j) + (\omega_{12}^b + \omega_{22}^b)\mathcal{P}_2(j). \quad (33)$$

Note that $\mathcal{P}(j) = \mathcal{P}_1(j) + \mathcal{P}_2(j)$. Above, this recursion was simplified using the detailed balance relation of equation (24), which relates the probabilities in the 1 and 2 states at site j . Here we suppose that the detailed balance relation does not necessarily apply, but that there is a relationship between the 1 and 2 probabilities given by

$$\mathcal{P}_2(j) = \gamma \mathcal{P}_1(j), \quad (34)$$

where γ is an unknown constant. Here we assume that because the rates do not vary with position, the constant γ is independent of j . We can then use equation (33) and the steady-state version of equation (6) to solve for the recursion relation (that relates $j+1$ to j) and for γ .

Substituting equation (34) into equation (33), we find

$$\frac{\mathcal{P}_1(j+1)}{\mathcal{P}_1(j)} = \frac{(1+\gamma)\beta + (\omega_{11}^f + \omega_{21}^f) + \gamma(\omega_{12}^f + \omega_{22}^f)}{(1+\gamma)\alpha + (\omega_{11}^b + \omega_{21}^b) + \gamma(\omega_{12}^b + \omega_{22}^b)} = c. \quad (35)$$

Note that this constant c as defined is a function of γ . Next consider the steady-state version of equation (6):

$$0 = -(\alpha + \beta + \omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b + \omega_{21})\mathcal{P}_1(j) + (\beta + \omega_{11}^f)\mathcal{P}_1(j+1) + \omega_{12}^f\mathcal{P}_2(j+1) \quad (36)$$

$$+ (\alpha + \omega_{11}^b)\mathcal{P}_1(j-1) + \omega_{12}^b\mathcal{P}_2(j-1) + \omega_{12}\mathcal{P}_2(j). \quad (37)$$

Plugging in equations (34) and (35) we can rewrite this as

$$\mathcal{P}_2(j) \left[-(\alpha + \beta + \omega_{11}^f + \omega_{11}^b + \omega_{21}^f + \omega_{21}^b + \omega_{21}) + \gamma\omega_{12} + \frac{\alpha + \omega_{11}^b}{c} + c(\beta + \omega_{11}^f) + \frac{\gamma}{c}\omega_{12}^b + \gamma c\omega_{12}^f \right] = 0. \quad (38)$$

Since $\mathcal{P}_2(j) \neq 0$, the expression in brackets must equal zero. This expression allows us to solve for γ in terms of the rate constants.

B. Reduction to BJ model

In order to show the relation between the model we propose here and the BJ model [20], we first consider the special situations where

$$\begin{aligned} \omega_{22}^f &= \omega_{22}^b = 0 \quad \text{and} \\ \omega_{12} &= 1 = \omega_{21}; \\ \omega_{21}^f &= \omega_{12}^b = 0 \quad \text{and} \\ \omega_{11}^f &= \omega_{11}^b = 0 \end{aligned} \quad (39)$$

In such situations $\mathcal{P}_1(j) = \mathcal{P}_2(j)$ for all j and, consequently,

$$v = \frac{1}{2} \sum_j [(\alpha_j - \beta_j)\mathcal{P}(j)] + \frac{1}{4} \sum_j [(\omega_{12}^f - \omega_{21}^b)\mathcal{P}(j)]. \quad (40)$$

Therefore, if we now make the correspondence

$$\omega_{12}^f = 2k^+ \quad \text{and} \quad \omega_{21}^b = 2k^- \quad (41)$$

between the parameters of the two models, the expression (40) reduces to

$$v = \frac{1}{2} \sum_j [(\alpha_j - \beta_j + k^+ - k^-)\mathcal{P}(j)]. \quad (42)$$

which is identical to the corresponding formula for average speed of unwinding in the BJ model [20]. Moreover, in this special case, equation (28) also reduces to the form

$$c = \frac{\alpha + k^-}{\beta + k^+} \quad (43)$$

which is identical to the corresponding expression in the BJ model. Furthermore, in this special case of our model

$$B = A/2 \quad (44)$$

where

$$A = \frac{\beta + k^+ - \alpha - k^-}{\alpha + k^-} \quad (45)$$

so that

$$\mathcal{P}(j) = Ac^j \quad (46)$$

which is identical to the solution for $\mathcal{P}(j)$ in the BJ model [20].

From now onwards let us consider

$$\begin{aligned} \omega_{22}^b &= \omega_{22}^f = \omega_b \\ \omega_{21}^f &= \omega_{12}^b = 0; \quad \omega_{11}^b = \omega_{11}^f = 0 \end{aligned} \quad (47)$$

The variation of v , and D with ω_{21} are shown in fig.3. Clearly, in the limit $\omega_{21} \rightarrow \infty$, v and D saturate to the values given by the expressions

$$v \simeq \frac{2\alpha\omega_{12}^f + \omega_b(\alpha - \beta + \omega_{12}^f)}{2(\omega_{12}^f + \omega_b + \beta)} \quad \text{as } \omega_{21} \rightarrow \infty. \quad (48)$$

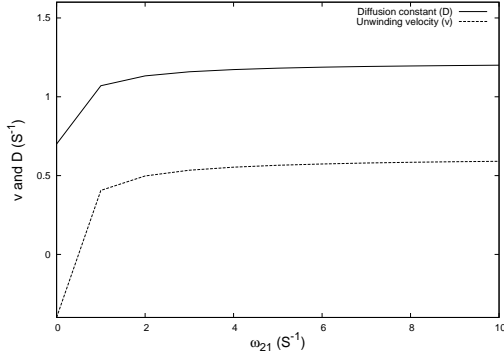


FIG. 3: Variation of v and D with ω_{21} . The numerical values of the parameters are: $\alpha = 1s^{-1}$, $\beta = 7s^{-1}$, $\omega_{12} = 0.4\mu M^{-1}s^{-1}$, $\omega_{21}^b = 0.4\mu M^{-1}s^{-1}$, $\omega_b = 1s^{-1}$, $\omega_{12}^f = 9s^{-1}$.

and

$$D = \frac{\{(\alpha + 2\omega_b)(\omega_{12}^f + \omega_b + \beta)\} + \{(\omega_{12}^f + \beta)(\alpha + \omega_b)\}}{4(\omega_{12}^f + \omega_b + \beta)} \text{ as } \omega_{21} \rightarrow \infty. \quad (49)$$

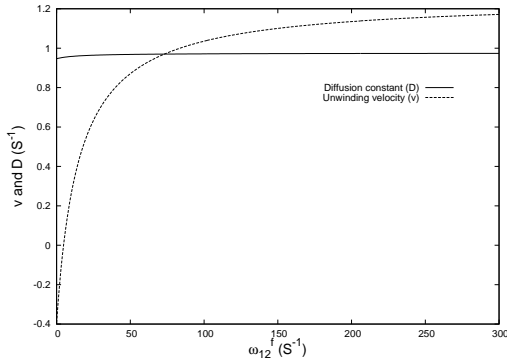


FIG. 4: Variation of v and D with ω_{12}^f . The parameter values are same as in fig.3, except that $\omega_{21} = 0.4\mu M^{-1}s^{-1}$ and ω_{12}^f is the independent variable.

These saturations are caused by the fact that, in this limit, the unwinding is limited by other smaller rate constants which appear in the formula (48).

Similarly, the variation of v and D with ω_{12}^f are shown in fig.(4); the saturation value obtained by extrapolation from this figure at high ω_{12}^f are consistent with in the expressions

$$v \simeq \frac{2(\omega_{21} + \omega_{12})\alpha + \omega_{21}\omega_b}{2(\omega_{21} + \omega_{12})} \text{ as } \omega_{12}^f \rightarrow \infty \quad (50)$$

and

$$D = \frac{\{(\alpha + 2\omega_b)\omega_{21} + (\alpha + \omega_{21}^b)\omega_{12}\} + \{(\alpha + \omega_b)\omega_{21} + (\alpha + \omega_{21}^b)\omega_{12}\}}{4(\omega_{21} + \omega_{12})} \text{ as } \omega_{12}^f \rightarrow \infty. \quad (51)$$

which we get from equation (29) and (31), respectively, in the limit $\omega_f^+ \rightarrow \infty$.

D approach

In the special limit $\omega_b \rightarrow 0$ the expressions for v and

$$v = \frac{(\alpha\omega_{12}^f\omega_{21} - \beta\omega_{21}^b\omega_{12})}{(\omega_{12}^f + \beta)\omega_{21} + \beta\omega_{12}} \text{ as } \omega_b \rightarrow 0, \quad (52)$$

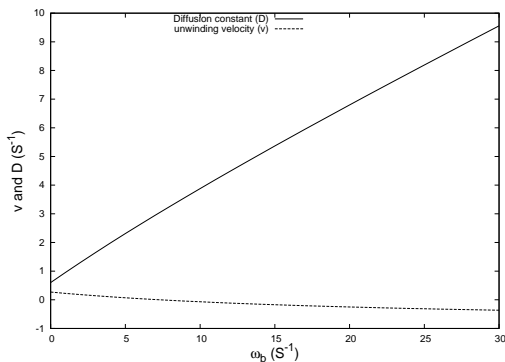


FIG. 5: Variation of v and D with ω_b . The parameter values are identical to those in fig.3 except that $\omega_{21} = 0.4\mu M^{-1}s^{-1}$ and ω_b is the independent variable.

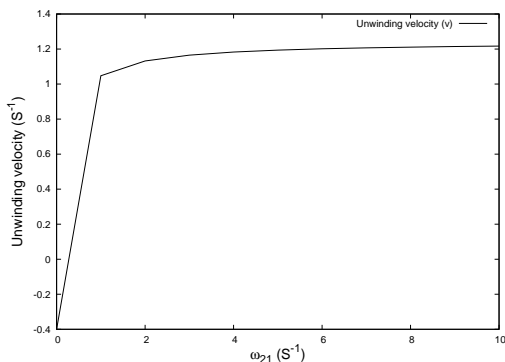


FIG. 6: Variation of v with ω_{21} . The parameter values are identical to those in fig.3 except that $\omega_{12}^f = 50bps^{-1}$ and ω_{21} is the independent variable.

and

$$D = \left(\frac{\alpha}{2}\right) + \frac{\omega_{21}^b \omega_{12}}{2(\omega_{21} + \omega_{12})} \text{ as } \omega_b \rightarrow 0, \quad (53)$$

respectively. In the opposite limit $\omega_b \rightarrow \infty$, the corresponding expressions are

$$v = \frac{\omega_{21}(\alpha - \beta + \omega_{12}^f) + \omega_{12}(\alpha - \beta - \omega_{21}^b)}{2(\omega_{12} + \omega_{21})} \text{ as } \omega_b \rightarrow \infty \quad (54)$$

and

$$D = \left(\frac{\omega_b}{2}\right) \frac{\omega_{21}}{(\omega_{21} + \omega_{12})} \text{ for large } \omega_b. \quad (55)$$

respectively. These limits can be seen on the plots of v and D against ω_b in fig.6.

HCV NS3 helicase is a representative member of the Superfamily-2 of helicases; it is responsible for viral replication and, therefore, a potential drug target. Stepping velocity of NS3 helicase, obtained from *in-vitro* bulk experiments [13] at saturating [ATP], is about 35 ± 4 bp/s. This is an underestimate compared to the stepping velocity of 51 ± 3 bp/s observed in recent single molecule experiments [19]. We now use the approximate estimate $\omega_{12}^f = 50bps^{-1}$, together with the numerical values of the other parameters which we have used so far, to predict the maximum unwinding velocity of the helicase on the basis of our model. The predicted value of the unwinding velocity ~ 1.2 bp/s is very close to the corresponding rate of unwinding by HCV NS3 helicase measured by Patel et al.[13].

V. SUMMARY AND CONCLUSION

In this paper we have developed a general model of unwinding of nucleic acids by helicase motors. In this model, the sites of a discrete lattice denote the positions of the individual bases on the ssDNA. At any spatial position on this discrete lattice, a helicase can exist in one of the two allowed "chemical" states: it can be either strongly or weakly bound to the ssDNA. A special case of this model captures the Brownian ratchet mechanism proposed for HCV NS3 helicase [13]. Solving the master equations for this model in the steady state, we have calculated the speed of unwinding. We have established the consistency of the model by estimating the speed of unwinding using rate constants extracted from the empirical data for HCV NS3 helicase.

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