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Detachment of molecular motors under tangential loading

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Abstract. – We introduce a general framework to study the processivity of molecular motors moving along a polar filament and discuss the average time spent attached to the filament as a function of a tangentially applied load. Our study of specific models suggests that the attachment time of a motor decreases with increasing ATP concentration and that double-headed motors such as kinesins lose their processivity under forcing conditions while processive single-headed motors are less sensitive to tangential forcing.

Introduction. – Experiments on motor proteins crucially depend on an important property of such systems, called processivity. Motor proteins such as kinesins, which can hydrolyse a large number of ATP molecules before detaching from the microtubule, are called processive, whereas those like myosin II, which typically interact just once with actin filaments and detach, are called non-processive [1]. Physiologically, processivity is important since it permits transport involving a small number of motors. Non-processive motors, like myosin II in muscles, are found to work in large assemblies. After having collected important experimental information on elementary steps, the velocity as a function of load and the stall force of molecular motors such as kinesin [2–4], there are now efforts to quantitatively study the processivity. As a result, there is now ample experimental evidence that applying a force tangential to the cytoskeletal filaments and opposing the natural motion of molecular motors considerably decreases the processivity [4–6].

A priori, one can define processivity in three different ways. First, it can be chosen as the average number of chemical cycles before detachment from the filament; second, it can be defined as the attachment lifetime of the motor to the filament; third, it can be, as in ref. [6], chosen to be the mean length spanned by the motor on the filament in a single run. The first definition is intrinsic to the process but extremely difficult to measure. The other two are directly accessible to experiment. For the sake of conciseness we focus our discussion on the attachment lifetime and only give an example for the run length.

The existence of a disruptive force normal to the cytoskeletal filament could potentially explain the decrease in processivity, since it is clear that tangential forces applied on a bead or

on a rod attached to the motor produce a normal component on it. The detachment produced by normal forces is similar to the detachment of adhesion proteins under load, investigated in refs. [7,8]. However, in the case of processive molecular motors there is an additional physical mechanism leading to detachment due to applied tangential forces. This effect is the subject of the present letter.

Our calculations show that applying a force strictly tangential to the filament in general reduces the average attachment time of molecular motors. This effect is particularly pronounced for a model motivated by double-headed processive motors, which provides a natural explanation for the loss of kinesin processivity observed under loading conditions [4–6]. It is interesting to remark that for a model adapted to single-headed processive motors we find a less marked force dependence of the average attachment time.

Model. – These results are obtained by taking into account the possibility of detachment in N-state models of molecular motors [9–16]. In these models, the motor is represented by a point particle which can be in one of N internal states (representing the steps of the chemical cycle) and is placed at point x in the one-dimensional space representing the filament. For each state i, the particle is subject to a periodic potential $W_i(x)$ of period l. The transition rates $\omega_{ji}(x)$ depend on ATP concentration as discussed in ref. [16]. Its hydrolysis drives the motion along the filament.

Detachment can be represented in this picture by allowing the particle to disappear with a local detachment rate $\alpha_i(x)$ which depends both on its state and on its position. It is reasonable to expect that $\alpha_i(x) \sim \exp[W_i(x)/k_{\mathrm{B}}T]$, since the "attempt rates" for detachment should not depend very strongly on position. The exponential dependence is so strong that, to a first approximation, one may consider high detachment rates to be concentrated near the maxima of the potential; elsewhere we assume a constant, much smaller, detachment background.

We assume that the particle attaches to the filament at $t = 0$ with a probability distribution $P_i^{(0)}(x)$. The behavior of the system can be described by the probability density $P_i(x,t)$ to find the particle at position x and state i at time t. This quantity satisfies, for $t > 0$, the evolution equation

$$\partial_t P_i + \partial_x J_i = \sum_j (\omega_{ij} P_j - \omega_{ji} P_i) - \alpha_i P_i, \tag{1}$$

where the currents J_i are defined by

$$J_i = -\mu_i \left[k_{\mathrm{B}} T \partial_x P_i + (\partial_x W_i - f_{\mathrm{ext}}) P_i \right], \tag{2}$$

in which μ_i is the particle mobility and f_{ext} the externally applied tangential force.

Since the probability that the particle detaches anywhere on the filament between time t and $t + \mathrm{d}t$ is given by $\int_{-\infty}^{+\infty} \mathrm{d}x \sum_i \alpha_i(x) P_i(x,t)$, we can evaluate the average attachment time $\tau = \int_0^\infty \mathrm{d}t\, t \int_{-\infty}^{+\infty} \mathrm{d}x \sum_i \alpha_i(x) P_i(x,t)$. It is straightforward to show that this definition is equivalent to

$$\tau = \int_0^\infty \mathrm{d}t \int_{-\infty}^{+\infty} \mathrm{d}x \sum_i P_i(x,t)\,. \tag{3}$$

Similarly we can define the mean run length as

$$\lambda = \int_0^\infty \mathrm{d}t \int_{-\infty}^{+\infty} \mathrm{d}x\, x \sum_i \alpha_i(x) P_i(x,t)\,. \tag{4}$$

Introducing the Laplace transform $\tilde{P}_i(x,s) = \int_0^\infty \mathrm{d}t\, e^{-st} P(x,t)$, we obtain

$$\tau = \int_{-\infty}^{+\infty} \mathrm{d}x \sum_i \tilde{P}_i(x, s{=}0)\,, \tag{5}$$

where $\tilde{P}_i(x,s)$ is, for each s, a solution of the ordinary differential equation

$$s\tilde{P}_i + \frac{\mathrm{d}\tilde{J}_i}{\mathrm{d}x} + \sum_j (\omega_{ji}\tilde{P}_i - \omega_{ij}\tilde{P}_j) + \alpha_i \tilde{P}_i = P_i^{(0)}(x), \tag{6}$$

which we only need to solve for $s = 0$.

Method. – Since eq. (6) is linear, we can solve it for $P_i^{(0)}(x) = \delta_{ii_0}\delta(x - x_0)$ without loss of generality. This implies that a motor is initially placed at position x_0 and in state i_0. In our numerical analysis, we choose $x_0 = 0$ and $i_0 = 1$. Then, for $x > x_0$ and $x < x_0$, the solution can be found by a transfer matrix technique. The initial solution imposes the matching conditions

$$\begin{aligned} P_i(x_0 + \epsilon, s) &= P_i(x_0 - \epsilon, s)\,, \\ J_i(x_0 + \epsilon, s) &= J_i(x_0 - \epsilon, s) + \delta_{ii_0}\,. \end{aligned} \tag{7}$$

Assuming that the vector $\vec{Y} = (y_1, y_1', \ldots, y_N, y_N')$ contains the values of the solution and its first derivatives at point x, one can evaluate the solution at point $x + nl$ as $\mathcal{M}^n \vec{Y}$, where the transfer matrix $\mathcal{M}$ is evaluated by explicitly solving eq. (6) within a period. Notice, however, that, since the boundary conditions imply that $P(x,t)$ vanishes as $x \to \pm\infty$ at all times, this must be also true for $\tilde{P}(x,s)$ for all s. Therefore, when $x > x_0$ ($x < x_0$), Y must belong to the N-dimensional space spanned by the eigenvectors of $\mathcal{M}$ ($\mathcal{M}^{-1}$) whose absolute value is smaller than 1. The $2N$ matching conditions (7) are thus in general sufficient to fix the $2N$ coefficients which identify the solution.

The evaluation of the transfer matrix is difficult in general, but can be made tractable by assuming that the $\omega_{ij}(x)$ and the $\alpha_i(x)$ are piecewise constant, and that the $W_i(x)$ are piecewise linear. Then the coefficients of eq. (6) are piecewise constant, and the solution can be expressed by exponentials, which must be smoothly connected at the joining points, ensuring the continuity of the probability density P and of the current J. Therefore, at the price of a special treatment for the period containing x_0, the solution of eq. (6) reduces to a set of transcendental equations whose solution can be numerically evaluated [17, 18]. In particular, the infinite-force limit [17] and the simplest case with only one state can be fully solved analytically [18].

Examples. – We discuss two simple examples with $N = 2$ and asymmetric sawtooth potentials: i) *Identical shifted states* (ISS): This system involves two identical potentials shifted by half a period ($W_1(x) = W_2(x + l/2)$); ii) *Diffusive steps* (DS): In this case we choose a flat excited state ($W_2(x) = \text{const}$) and all transition rates for this state are constant. This simplified situation is valid as long as the variations of W_2 do not exceed kT [11, 13]. The potentials and the transition rates for these situations ISS and DS are defined in fig. 1. We denote the potential amplitude by U and characterize the potential asymmetry by a/l, where a denotes the position of the maximum of W_1.

The ISS model is motivated by the motion of double-headed kinesins. Each of the two states could correspond to a situation where one of the heads is strongly bound to the filament.

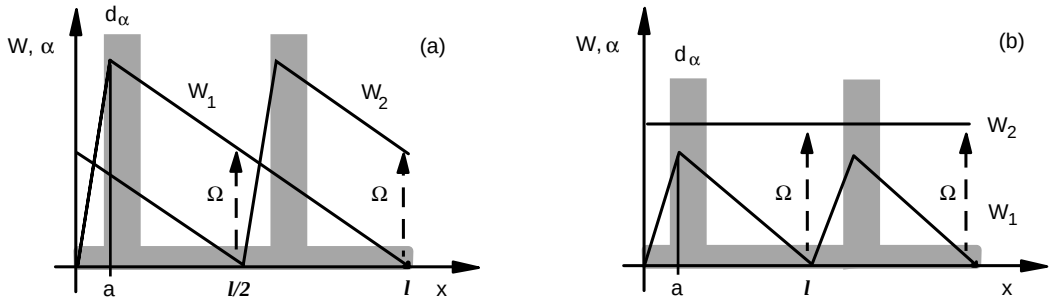

Fig. 1 – Schematic representation of two specific models discussed in the text. (a) Identical shifted states (ISS): Transitions are localized in a region of size d_ω. The detachment rates α are piecewise constant with the maximal value $\alpha_{\max}$ localized in a region of size d_α. Elsewhere $\alpha = \alpha_{\min}$. (b) Diffusive steps (DS): Excitations are localized at the minima of W_1, deexcitations are homogeneous. The detachment rates α are maximal ($\alpha_{\max}$) near the potential maximum and $\alpha_{\min}$ otherwise. The potential amplitudes are denoted by U, the asymmetry of the sawtooth potentials is characterized by the length a, and l denotes the potential period. The value of W_2 can satisfy $W_{1\min} < W_2 < W_{1\max}$, but the line has been displaced upwards for graphical clarity.

Because the two kinesin heads are identical, the two states are identical up to a relative shift. We choose transitions between the states localized within a region of size d_ω near the potential minima. At the minimum of $W_1(x)$, excitations occur with a rate $\omega_{21} = \Omega$ while $\omega_{12} = \omega$. Because of the symmetry of the states $\omega_{21} = \omega$ and $\omega_{12} = \Omega$ at the minima of $W_2(x)$. Elsewhere, ω_{12} and ω_{21} vanish. The rates of detachment from the filament $\alpha_i(x)$ are chosen piecewise constant with a large value $\alpha_{\max}$ located in a region defined by $W_i(x) > U - k_{\mathrm{B}}T$ of size d_α around the potential maxima, and with a small value $\alpha_{\min}$ elsewhere, see fig. 1(a).

Note that this simple choice captures the essential exponential dependence of the detachment rate but is more convenient to use for practical calculations.

The use of the DS model is motivated by the recently observed processive motion of artificially constructed individual kinesin heads [19, 20]. The particle, initially lying in the potential minima in state 1, gets excited into state 2, where it diffuses. It then falls back into state 1, and advances if diffusion has brought it past the nearest potential maximum. For this system, we choose excitations that are localized in the potential minimum where $\omega_{21} = \Omega$, $\omega_{21} = 0$ elsewhere. The de-excitation rate $\omega_{12} = \omega$ is constant along the period. The detachment rates $\alpha_1(x)$ are large ($\alpha_1 = \alpha_{\max}$) in a region of width d_α around the potential maxima, and have a small value ($\alpha_1 = \alpha_{\min}$) elsewhere. We choose α_2 to be a constant, related to the value of the potential energy in state 2. The fact that the observed attachment times of an artificially constructed single-headed kinesin is similar to those characteristic for double-headed kinesins [19] suggests that in this case the potential energy of state 2 is not larger than the potential maximum of state 1, and thus that $\alpha_2 < \alpha_{\max}$. If the potential energy of state 2 is large, one has $\alpha_2 \geq \alpha_{\max}$, and the corresponding motor is non-processive: indeed, it is likely to leave the filament as soon as it gets excited. This situation could apply, therefore, to myosin II.

Results. – Figure 2 shows the average attachment time τ as a function of the externally applied force for the ISS model for two different values of the excitation rate Ω which is an increasing function of the ATP concentration. The maximal attachment time is independent

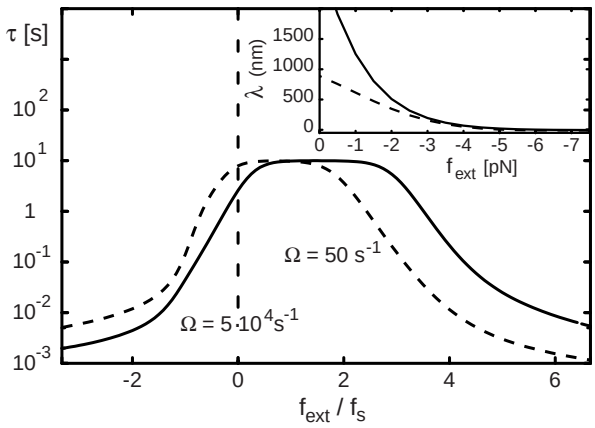

Fig. 2 – Average attachment time to a filament τ as a function of the normalized force $f_{\rm ext}/f_{\rm s}$, where $f_{\rm s}$ is a typical value of the stall force. Identical shifted states (ISS) with $U/k_{\rm B}T = 20$, $l = 16$ nm, $a/l = 0.2$, $\mu_1 = \mu_2 = 171$ nm pN^{-1}s^{-1}, $\omega = 10^{-4}$ s^{-1}, $d_\omega/l = 0.02$, $d_\alpha/l = 0.05$, $\alpha_{\rm min} = 0.1$ s^{-1}, $\alpha_{\rm max}/\alpha_{\rm min} = 4.8 \cdot 10^8$ for $\Omega = 5.0 \cdot 10^4$ s^{-1} and $\Omega = 50$ s^{-1}. For these parameter values, the system has, for large Ω, a saturated spontaneous average velocity $v_0 = 1.1$ μm/s in the absence of external forces and the force is normalized with $f_{\rm s} = 6.0$ pN. Inset: mean run length as a function of the external force $f_{\rm ext}$, in the ISS with identical parameters. Note the similarity with experimental results of ref. [6].

of Ω and occurs in the absence of applied forces. In this regime, the system is processive and spends an important amount of time attached. The time τ decreases if an external force which opposes motion is applied. At stalling conditions, the time of attachment has dropped by almost two orders of magnitude: it decreases by several orders of magnitude for larger forces. The situation for the DS model is different, as shown in fig. 3. Forces up to 10-20 times larger than the stall force are necessary for reducing the bound time by a factor of 10^2 for $\Omega = 10^2$ s^{-1}; for $\Omega = 10^5$ s^{-1} the reduction does not exceed a factor of 2 even for large forces. The reduced sensitivity of the processivity to external forces in the DS model is due to the fact that the x-independent detachment rate α_2 governs this regime. Our calculations show that the processivity in the absence of external forces decreases for increasing Ω (cf. fig. 3(a)). This effect occurs in both models but is very significant in the DS model. For the case of large Ω we find in the DS model a weak dependence of the attachment time as a function of tangential load. Figure 3(b) exhibits the behavior of the DS model for the same parameters as in fig. 3(a), but with $\alpha_2 \geq \alpha_{\rm max}$ (*i.e.*, $W_2 \geq W_1$). The same general trends are visible, but the excitation-rate dependence is strongly increased. The processivity and force sensitivity are totally lost for high excitation rate Ω.

Discussion and conclusion. – Our study shows that the processivity of a molecular motor is influenced by an externally applied force tangential to the filament. This mechanism applies to all situations in which a molecule moves along a linear structure. The details of the initial condition $P_i^{(0)}$ will not be relevant if the motor undergoes on average several cycles and moves a few periods before detaching, as in the case of kinesins. However, for non-processive motors, knowledge of the probability $P_i^{(0)}$ is required for making detailed predictions.

The ISS model suggests that for double-headed motors the time of attachment is particularly sensitive to the presence of tangential forces. Indeed, in the presence of tangential forces,

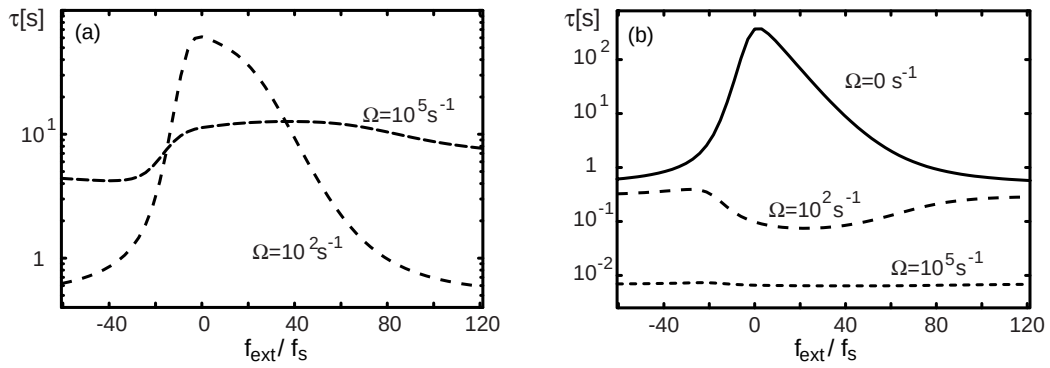

Fig. 3 – (a) Average attachment time to a filament τ as a function of the normalized force $f_{\rm ext}/f_{\rm s}$, where $f_{\rm s}$ is a typical value of the stall force. Diffusive steps (DS) with $U/k_{\rm B}T = 10$, $l = 8$ nm, $a/l = 0.2$, $\mu_1 = \mu_2 = 5 \cdot 10^2$ nm pN^{-1}s^{-1}, $\omega = 100$ s^{-1}, $d_\omega/l = 0.02$, $d_\alpha/l = 0.1$, $\alpha_{\rm min} = 10^{-3}$ s^{-1}, $\alpha_{\rm max}/\alpha_{\rm min} = 2.2 \cdot 10^4$, $\alpha_2/\alpha_{\rm min} = 67$ (*i.e.*, $W_2 \simeq 4k_{\rm B}T$), for $\Omega = 1.0 \cdot 10^5$ s^{-1} and $\Omega = 1.0 \cdot 10^2$ s^{-1}. In this system, $v_0 \simeq 157$ nm/s and $f_{\rm s} = 0.33$ pN. (b) Average attachment time to a filament τ as a function of the normalized force $f_{\rm ext}/f_{\rm s}$, where again $f_{\rm s} = 0.33$ pN (with the exception of the case for $\Omega = 0$ s^{-1} for which the stall force vanishes). Same model as in (a), but $\alpha_2/\alpha_{\rm min} = 1.63 \cdot 10^5$ (*i.e.*, $W_2 \simeq 12k_{\rm B}T$).

the motor is more likely to be found in high-energy configurations, where the detachment rate is large. The DS model demonstrates a different behavior. In this case, the system spends an important fraction of time in the second state with a conformation-independent detachment rate α_2 that dominates the detachment probability and sets the order of magnitude of the lifetime τ. Therefore, the time of attachment depends only weakly on the applied force.

It has been observed experimentally that the time of attachment of kinesin molecules to microtubules decreases as a load is applied tangentially to the filament [5]. These observation are consistent with our calculations corresponding to the ISS model (fig. 2). Equivalently, calculations of the mean run length based on the ISS model are fully consistent with the results of ref. [6] (fig. 2, inset). As a result, kinesins lose their processivity before their direction of motion is reversed at stalling conditions and in general reverse motion cannot be detected. Furthermore, the attachment time of double-headed kinesins increases by about a factor of eight when the ATP concentration is decreased at constant load of 1 pN from 2 mM to 5 μM [6]. With the choice of fig. 1(a) we find an increase of the attachment time by a factor of 4.5 when decreasing ω by a factor that corresponds to the change in ATP concentration. If we slightly increase the width of the detachment region $\alpha_{\rm max}$, this factor can be further increased to match the observed ratio. Our calculations predict that single-headed kinesins, which move processively in the absence of external forces [19], should, in contrast to double-headed kinesins, remain processive in the presence of tangential forces. Therefore, the reversal of the direction of motion could be observable under loading conditions. It is further gratifying to remark that the DS model is successful in explaining the different processivities of KIF1A (fig. 3(a)), single-headed conventional kinesin and myosin II (fig. 3(b)): the W_2 binding energy of KIF1A has to differ by $8k_{\rm B}T$ from that of myosin and by $4k_{\rm B}T$ from that of kinesin. The $4k_{\rm B}T$ corresponds indeed to the difference measured in [20]. In the myosin-II case, processivity is totally lost and the calculated behavior in the absence of ATP is fully consistent with known experimental data, with and without external force [21–23].

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