"Gliding Assays" for Motor Proteins: A Theoretical Analysis

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Much information about the workings of molecular motors can be gleaned from motility assays in which cytoskeletal filaments are observed as they glide across a surface coated with motor proteins. The statistical properties of the paths followed by the filaments depend both on the motors (their surface concentration and mode of action) and on the filaments (their length and rigidity). By analyzing the dynamics of this system, we determine these dependencies and suggest how they may be used to infer more accurate quantitative information about motor proteins than is currently extracted from gliding assays.

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Motor proteins are enzymes that convert chemical energy derived from adenosine triphosphate (ATP) hydrolysis into mechanical work. Their ability to generate force and to undergo directed motion forms the basis of muscle contraction and is fundamental to a wide range of cellular functions such as locomotion, the transport of organelles, and the segregation of chromosomes during mitosis. These "molecular motors" travel along linear cytoskeletal polymers of remarkably long persistence length. Well-studied examples include kinesin and dynein, which move on microtubules, and myosin which moves on actin filaments. The prevailing belief is that they operate by "stepping" between successive binding sites each time they go through their biochemical cycle, moving preferentially in a particular direction by virtue of the polarity of the polymers.

Our expanding knowledge of the way that molecular motors work owes much to the recent development of *in vitro* motility assays [1] which permit the examination of the interaction between a small number of enzymes and individual filaments. One of the most versatile involves the microscopic observation of the motion of filaments as they glide across a surface coated with molecular motors [2–5] (Fig. 1). In such an assay, purified motor proteins are first carefully adsorbed or chemically attached to a glass substrate. Then a solution of stabilized filaments is introduced. The motors grasp the polymers and, by moving along them in one direction, propel them in the opposite sense across the surface. Imaging of the filaments by fluorescent or dark-field microscopy allows their motion to be observed in real time. Interpretation of gliding assays has been hampered by inaccurate knowledge of the surface density of active motors and the mean number of enzymes interacting with a filament at a given instant [6–9]—quantities required in order to ascertain the physical properties of a single motor from observation of the combined effect of many. In this Letter we show how the value of these important quantities, as well as certain details of the motor-filament interaction, can be deduced from experimental observations of the polymer trajectories.

We assume the motor proteins to be deposited randomly on the substrate with surface concentration σ and suppose that they can reach isotropically over a distance w to interact with a filament. The filaments have length L and a rigidity most conveniently described by their persistence length p. Their dynamics is characterized by an average speed v and by a parameter of thermal diffusion, $\gamma \sim kT/\eta$ (where η is the viscosity of the solution) [10]. The fraction f of the biochemical cycle (of total duration τ) that the motors spend bound to a filament is known as the "duty ratio" and two broad classes may by distinguished: We first consider motors with a high duty ratio ($f \approx 1$) and then generalize our arguments to include those with low duty ratios ($f \ll 1$).

High duty ratio.—In the case $f \approx 1$ in which a motor releases a filament so briefly that it does not have the opportunity to diffuse away, the polymer conformation is effectively pinned at the locations of successive motor proteins. The filament's only translational mode is along its contour. As it is propelled forward, it locates new motors at its leading end and other motors are left behind at the trailing end. If the number of bound motors gets reduced to 1, an additional mode becomes available: The filament can pivot about the motor as it moves forward [11]. It will rotate until it comes within range of another motor, at which instant it gets pinned and is once

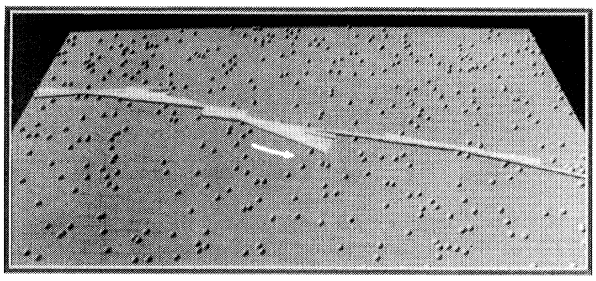

FIG. 1. A cytoskeletal filament (cylinder), propelled by motor proteins (spheres), glides across a substrate. The trajectory of the filament is shown as a series of snapshots, with the arrow indicating the direction of motion.

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again driven along its axis. The general motion is thus characterized by periods of advance along the polymer contour, punctuated by periods of diffusive rotation. The relative frequency of these two elements depends on the mean number of motors attached to the filament. Our analysis, then, proceeds by two steps. First, we calculate the mean distance $\langle d \rangle$ between bound motors. Then, we determine the statistical properties of the path followed by the filament as a function of $\langle d \rangle$.

Separation between motors attached to the filament.— How does a polymer find additional motors? As it moves ahead, its leading end fluctuates laterally, so that when it has advanced a distance l from the position of the most recently bound motor it will have explored an area $A(l)$ of the surface. Assuming a diffusion-limited interaction, any motor located within this area will attach to the filament. The degree of fluctuation depends on the polymer's flexibility and also on the time, so that three different scaling regimes for $A(l)$ can be distinguished (we assume here $p^{-2} \ll \sigma \ll w^{-2}$). (i) For short l, there is little appreciable bending, and only motors which are located within w of the path of the filament can attach to it. Thus, $A \sim wl$. (ii) For intermediate l, the end section of the polymer can fluctuate laterally by a distance considerably larger than w. The typical angle through which it bends by thermal agitation is $\theta_{\rm eq} \sim (l/p)^{1/2}$, so that the area covered in this is $A \sim l^2\theta_{\rm eq} \sim l^{5/2}p^{-1/2}$. (iii) For long l, the fluctuating end portion may not fully equilibrate as it advances. The available time $t = l/v$ should be compared with the equilibration time $t_{\rm eq} \sim \theta_{\rm eq}^2 D_\theta$, where $D_\theta \sim \gamma/l^3$ is the rotational diffusion coefficient of the leading end [12]. When $t < t_{\rm eq}$, the polymer has time to bend only through an angle $\theta \sim (D_\theta t)^{1/2}$ smaller than $\theta_{\rm eq}$. Thus in this case $A \sim l^2\theta \sim (\gamma/v)^{1/2}l$. In each of these three regimes, the mean distance $\langle d \rangle$ between bound motors is specified by the condition $\sigma A(l = \langle d \rangle) = 1$. So, we obtain the following scaling behavior [13]:

$$\langle d \rangle \sim \begin{cases} \sigma^{-1}w^{-1}, & \sigma \gg \sigma^*, \quad (1a) \\ \sigma^{-2/5}p^{1/5}, & \sigma^* \gg \sigma \gg \sigma^{**}, \quad (1b) \\ \sigma^{-1}(\gamma/v)^{-1/2}, & \sigma^{**} \gg \sigma, \quad (1c) \end{cases}$$

where the boundaries between the different regimes are given by

$$\sigma^* \sim w^{-5/3}p^{-1/3}, \qquad \sigma^{**} \sim (\gamma/v)^{-5/6}p^{-1/3}. \quad (2)$$

So far we have assumed that the fluid friction does not affect lateral diffusion. This may not be so when the bound motors are widely spaced. If the leading end deviates by angle θ as it advances, it suffers a lateral force $F_{\rm fluid} \sim \zeta v\theta$, where $\zeta \sim kTl/\gamma$ is its translational frictional coefficient. This should be compared with the elastic restoring force $F_{\rm el} \sim kT\theta/\theta_{\rm eq}^2 l$. When $F_{\rm fluid} > F_{\rm el}$, the end section of the molecule will be buckled by the frictional force. This condition is satisfied when $l > (\gamma/v)^{1/3}p^{1/3} = l_{\rm crit}$. Inspection of Eqs. (1b) and (2) shows that $l_{\rm crit} = \langle d(\sigma^{**}) \rangle$, indicating that buckling will occur when $\sigma < \sigma^{**}$. Thus the nonequilibrated regime (1c) does not exist in practice. It is replaced by a regime in which the filament buckles once it has advanced a distance $l_{\rm crit}$ from the most recently bound motor. If the buckling occurs in the plane of the surface so that another motor is encountered, $\langle d \rangle \sim l_{\rm crit}$ (by dimensional argument) and Eq. (1c) should be replaced by

$$\langle d \rangle \sim (\gamma/v)^{1/3}p^{1/3}, \qquad \sigma^{**} > \sigma. \quad (1c')$$

If, on the other hand, buckling out of the plane is favored, the polymer will lose contact with the surface; then σ^{**} is the *critical motor concentration* below which filaments will not glide across the surface.

Statistical properties of the path.—The statistical properties of the path taken by the polymer are governed by the mean angle $\langle \Delta\theta \rangle$ through which the filament swivels when fastened to a single motor and the mean distance $\langle S \rangle$ that it travels between successive rotations. $\langle \Delta\theta \rangle$ is calculated from the condition $\sigma A = 1$, where here A is the area covered by the filament as it rotates. We shall neglect correlations and assume that the pivot is positioned anywhere on the filament with equal probability, in which case $A = \frac{1}{3}L^2\langle \Delta\theta \rangle$. So

$$\langle \Delta\theta \rangle = 3\sigma^{-1}L^{-2}. \quad (3)$$

To calculate $\langle S \rangle$ we consider the stochastic evolution of the number n of motors bound to the filament. The polymer acquires a new motor at its leading end, on average, each time it advances a distance $\langle d \rangle$, given by Eq. (1). At the trailing end, by contrast, a motor is lost each time the filament moves through a distance L/n equal to the average separation between currently bound motors. Hence the evolution of n may be described approximately by a master equation with transition rates $w_{n\to n+1} = k_+$ and $w_{n\to n-1} = k_- n$, where $k_+ = v/\langle d \rangle$ and $k_- = v/L$. The period during which the filament is driven along its contour begins with two motors attached and ends when just one is bound. Its average duration is, therefore, the mean first passage time for going from $n = 2$ to $n = 1$, which can be calculated exactly: $\langle T_{2\to 1} \rangle = (k_-/k_+^2)[\exp(k_+/k_-) - 1 - k_+/k_-]$. There is a small correction to this expression owing to the particular way in which the initial state arises: When pivoting, the filament may locate an additional motor anywhere along its length, rather than only at the leading end as it does when being driven forward. The rate of transit from the initial state directly to the state $n = 1$ is thus altered from $w_{2\to 1} = 2k_-$ to $w'_{2\to 1} = 3k_-$. This changes the first passage time by a constant factor $(k_+ + 2k_-)/(k_+ + 3k_-)$. The average curvilinear distance traveled by the filament in between periods of rotation is given by $\langle S \rangle =$

$v\langle T_{2\to 1}\rangle$, so we obtain

$$\langle S\rangle = \frac{L + 2\langle d\rangle}{L + 3\langle d\rangle}\frac{\langle d\rangle^2}{L}\left(e^{L/\langle d\rangle} - 1 - \frac{L}{\langle d\rangle}\right). \quad (4)$$

In this analysis we have neglected certain correlations that are actually present (e.g., when the filament swivels it is more likely to encounter a second motor close to its ends than nearby the pivot point). In order to evaluate the significance of these approximations we have performed a numerical simulation of a rigid polymer ($p \to \infty$) moving across a random array of motors. The polymer is propelled longitudinally when bound to two or more motors and rotates diffusively when attached to only one (Fig. 1). In Fig. 2 are compared the values of $\langle\Delta\theta\rangle$ and $\langle S\rangle$, obtained from the simulation and the predictions of Eqs. (3) and (4), with $\langle d\rangle$ given by Eq. (1a). A small systematic discrepancy is discernible in the values of $\langle S\rangle$, but Eq. (4) is found to be accurate to within 20% across the entire range of filament length.

Observable features.—At long times, the trajectory followed by the filament has the statistics of a random walk. The most readily observable quantities are *the persistence length P of its path* and the *overall translational diffusion coefficient D* of the polymer. Two processes contribute to P: the curving of the filament between adjacent bound motors, and the sudden changes in direction when the polymer swivels. The former contribution is

$$P_{\text{curve}} = \begin{cases} p, & \sigma > \sigma^{**}, \quad (5a) \\ p(\sigma/\sigma^{**})^2, & \sigma^{**} > \sigma, \quad (5b) \end{cases}$$

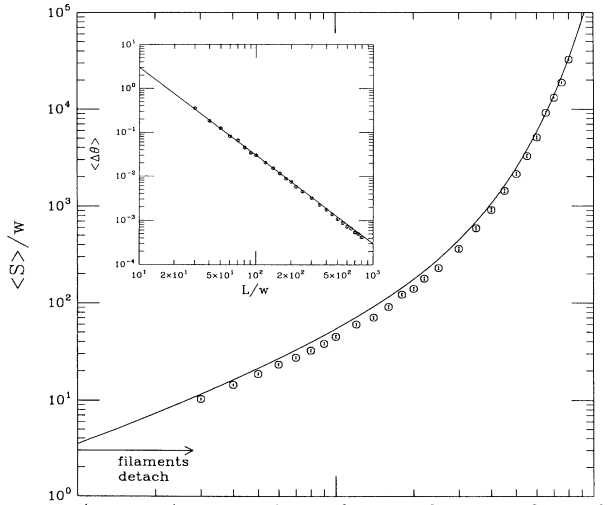

FIG. 2. The mean distance $\langle S\rangle$ traveled by a filament between successive rotations and the mean angle of rotation $\Delta\theta$ as a function of filament length L. Solid lines are the predictions of Eqs. (3), (4), and (1a); data points are from a simulation of a rigid filament. Motor density is set at $\sigma = 0.01w^{-2}$.

where the shorter persistence length of (5b) compared to (5a) is due to the leading end buckling, rather than bending between attached motors. The contribution from pivoting is

$$P_{\text{pivot}} = \langle S\rangle/\langle\Delta\theta\rangle^2. \quad (6)$$

The observed persistence length of the path is given by $P = 1/(P_{\text{curve}}^{-1} + P_{\text{pivot}}^{-1})$, and the translational diffusion coefficient is $D = vP$. These quantities increase very rapidly with the filament length; as one progresses from short to long polymers, D and P vary first as L^5, then as $\exp(L)$ [Eqs. (3), (4), and (6)] before saturating.

Another observable quantity is the size of the *shortest filament that remains bound to the substrate.* A short filament, when pivoting, may be unable to find a second motor before it get driven off the first, in which case it will diffuse away from the surface. This will happen if there are no other motors located within a range L. Alternatively it may occur because the filament has time to explore just a limited area of the surface $A = (\gamma/v)^{1/2}L$. Thus, only filaments longer than $L_{\min} \sim \min[\sigma^{-1/2}, (\gamma/v)^{-1/2}\sigma^{-1}]$ will travel across the surface.

Kinesin is reported to have a duty ratio close to unity, detaching for only a very brief instant each cycle [6,14]. In kinesin motility assays, microtubules—extraordinarily stiff polymers [15] with persistence length $p \approx 5$ mm—are typically driven at speeds $v \sim 0.5$ μm/s. Supposing the reach of kinesin to be $w \approx 20$ nm, one obtains from Eq. (2): $\sigma^* \approx 50$ μm^{-2} and $\sigma^{**} \approx 0.05$ μm^{-2}. We estimate that the intermotor spacing is $\langle d\rangle \approx 1$ μm at σ^* and $\langle d\rangle \approx 20$ μm at σ^{**}—microtubules shorter than this will be observed frequently to swivel (and to dissociate if $L < 0.1$ μm at σ^* and $L < 20$ μm at σ^{**}), while longer ones will appear to move smoothly along their contour. Comparison of these figures with the observations of Howard *et al.* [6] indicates that the concentration of working motors on the substrate may be *at least an order of magnitude lower* than the value estimated from the adsorbed quantity of enzymes [16].

Low duty ratio.—Now we turn to the case $f \ll 1$, corresponding to motor proteins which spend the majority of their biochemical cycle detached from the filament. In this situation, the effective surface concentration of enzymes able to bind to the polymer at a given moment is $\sigma' = f\sigma$. So the average distance $\langle d\rangle$ between attached motors is given by Eq. (1) with σ replaced by σ' [and with a likewise substitution of σ'^* for σ^* in Eqs. (1) and (2)]. The polymer conformation is now only temporarily pinned and the filament starts to diffuse laterally away from any motor as soon as the enzyme releases it. What is the consequence of this freedom of motion? None in the regime $\sigma' \gg \sigma'^*$, for the rigidity of the polymer prevents it bending away from its path by more than w, so the filament cannot escape the range of enzymes that have just let go of it. Each motor lying beneath the filament

will bind again during each subsequent biochemical cycle and the polymer will continue to follow its contour precisely. When $\sigma' \ll \sigma'^*$, on the other hand, the effect of detachment is more significant. In this case the filament fluctuates in between attached motors so that the polymer is able to switch from one ensemble of motors to another. The filament diffuses laterally as it is propelled forward and its tail end does not exactly trace the path taken by the head. Furthermore, if the attached motors are well separated, the polymer may fluctuate so far away from the surface that it escapes the reach of the enzymes and dissociates from the substrate. In order to ensure that the polymer always returns to search over the surface, the equilibration time of the section between attached motors, $t_{eq} \sim \langle d \rangle^4 / \gamma p$, must be longer than $f\tau$, the typical attachment time. Consequently, there is a *critical surface concentration of enzymes* below which *no* filaments (of whatever length) will bend to the substrate: $\sigma'_{min} \sim \min[\sigma'^*, p^{-1/8}(\gamma f \tau)^{-5/8}]$. Above this threshold, only polymers longer than a certain *concentration-dependent critical length* will move continuously across the surface for a substantial distance, since only filaments with $L \gg \langle d \rangle$ are fastened at all times by at least 1 motor. Shorter filaments will travel, on average, a distance $\langle S \rangle$, given by Eq. (4), before detaching.

The muscle force-generating protein myosin is believed [8] to have a low duty ratio $f \approx 0.05$, an overall cycle time $\tau \approx 80$ ms, and a reach $w \approx 20$ nm. The actin filaments with which it interacts have a persistence length $p \approx 17$ μm [15,17,18]. Equation (9) then implies that $\sigma_{min} \approx 500\ \mu m^{-2}$ (in aqueous solution), close to the value reported by Toyoshima *et al.* [7] of the minimum concentration of myosin required to drive actin filaments across a surface. These authors also report a concentration-dependent lower bound on the length of filaments that will move continuously across a densely coated substrate which is in quantitative accord with the above arguments. Uyeda *et al.* [8] have shown that actin filaments will glide across surfaces more sparsely coated with myosin if methylcellulose polymers are dissolved in the solution. It seems likely that the polymer forms a mesh that restricts the lateral diffusion of the filament, so that $\langle d \rangle \sim \sigma' w$ even at low enzyme concentrations. They have also reported a regime in which filament tails do not precisely track the heads, as discussed above.

In conclusion, our analysis permits the inference, from observation of the filament trajectories as a function of length, of the surface concentration of working enzymes and the average number of motor proteins interacting with a polymer. This allows a more accurate quantitative interpretation of motility assays, providing a better characterization of the interaction between molecular motors and cytoskeletal filaments.

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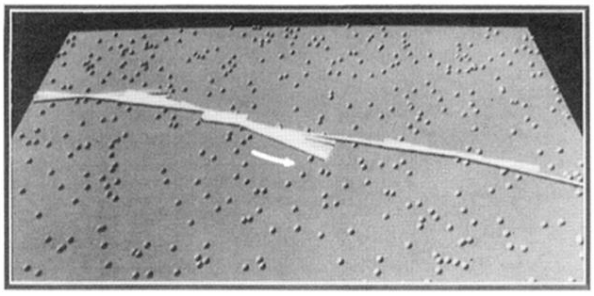

FIG. 1. A cytoskeletal filament (cylinder), propelled by motor proteins (spheres), glides across a substrate. The trajectory of the filament is shown as a series of snapshots, with the arrow indicating the direction of motion.