

Intracellular cargo transport along multiple filaments with lane switching

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Cargo transport mostly involves multiple molecular motors that move along cytoskeletal filaments. Such motors have the ability to detach and reattach on a filament and possibly switch onto another filament. Cargo transport by multiple motors on a single filament has been widely studied; however, cargo transport involving motors switching between filaments remains poorly understood. Here, we use lattice-based Monte Carlo simulations to investigate the properties of transport on multiple filaments, such as velocity, run length, and mean first passage time, compared with transport on a single filament. We provide an analytical expression for cargo velocity for multiple lanes as an extension of the expression for a single lane with a limited number of binding sites. We find this expression is sufficient to describe the force-velocity curves of clusters of motors on multiple lanes in our simulations. Our results show that cargoes being transported on multiple filaments move faster and further than those transported by a single filament. The time taken to deliver cargo to a given target distance is characterized by the mean first passage time, and our results showed a decrease in the time taken for transport on multiple filaments compared with single filaments. Moreover, we consider the effect of molecular motors' intrinsic properties in binding kinetics on their transport. Highly nonprocessive molecular motors with fast binding on and off rates, which are less persistent on filaments, transport cargo more slowly and for shorter run lengths compared with the same number of more processive motors with slow binding on and off rates. Consequently, nonprocessive motors with fast binding kinetics require more time than motors with slow binding kinetics to reach the same target.

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I. INTRODUCTION

Cargo transport of many organelles, such as mitochondria and synaptic vesicles in cells, requires molecular motor proteins that move along cytoskeletal filaments [1,2]. Molecular motor proteins drive active transport along cytoskeletal filaments that form a complex network in cells [3]. Individual filaments have a polarity sensed by the molecular motors, such that the motors have directed motion. They play a role in delivering the cargo to its destination, for example, from the cell center to the cell periphery by kinesin, which is directed to the plus end of microtubules (MTs). In the reverse direction, cargo is carried by dynein to the minus end of MTs.

In general, transport in a cell is mediated by more than one motor protein, which are simultaneously bound to a cargo [4–8]. This raises the question of how many motors can attach to the same cargo and how they are organized on the cargo's surface. A recent study demonstrated that the shape of the contact zone on a cargo surface affects the number of

motors that can simultaneously bind to MTs [9]. The typical number of motors found to be present on the cargo, such as endosomes or vesicles from *in vivo* experiments, is between 1 and 10 [6,10]. The organization of motors binding on the cargo surface is also important for cargo transport. A previous study showed that having multiple motors improves transport robustness in crowded environments [8]. Most modeling work in this area assumes that the motors are aligned along the filament [2,5,7,11]. Some results have assumed that the motors are organized randomly on the cargo surface [6,12–14]. This supports the idea that the molecular motors are more likely to bind to many filaments [15], and that, *in vivo*, many filaments exist. One recent paper [16] considers the intersection between two MTs that can cause traffic jams when there are many motors. They thus find an optimal number of motors for efficient transport. In our work, we focus on transport along bundles of many MTs with motors that can (un)bind to switch which filament they are on. The many filaments act as many lanes, where the “lanes” terminology is an analogy to driving on a motorway with several lanes.

The motors' processivity is also important for cargo transport [1,17]. There are two different classes of molecular motors classified by their processivity: processive and nonprocessive motors [2,18]. In some modeling work,

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kinesin-1 is considered a fully processive motor that never unbinds from the filaments [2]. Some theoretical work considers kinesin-1 a slightly nonprocessive motor that can rarely unbind from the filament, although it still remains on the filament for a long time [5]. *In vivo* experiments have shown that a single kinesin-1 motor can perform 100 steps on average before detaching from MTs [18]. Therefore, cargo transported by multiple kinesin-1 can stay attached for more steps and achieve longer run lengths. This indicates that the distance traveled along the filament depends on the number of motors. Kinesin-14 (Ncd) is a kind of nonprocessive motor that often unbinds from the filaments but rebinds to them rather fast [5,19–21]. It is difficult to measure collective transport by identifying the number of motors in an experiment *in vivo*. However, important work has been done constructing the collective transport of kinesin-1 and Ncds by defining a number of motors *in vitro* and showing that the velocity and run length increase with the number of motors [19].

Studies have shown that cargo transport is not confined to a single protofilament but instead involves multiple protofilaments within the same MT [8,9]. If MTs are closely spaced, this might influence how motors switch between filaments [8]. The organization of the cytoskeletal network also plays a critical role in regulating cargo transport dynamics. A recent study investigated how cytoskeletal network mesh size impacts cargo transport [22]. The researchers experimentally created MT networks with different mesh sizes for quantum dot cargoes and found that mesh size significantly influences transport efficiency by affecting cargo run length and trajectory persistence. Their study confirms the impact of MT network structure on cargo transport, further supporting the need to study transport mechanisms involving multiple filaments. An experimental study [23] shows that the distance of a cargo transport can be longer than the length of a single MT, which is about 10 – 40 μm . This supports the idea that the transport occurs on more than one filament. This suggests that the molecular motors attached to the cargo can move along several filaments at the same time and can switch lanes in the case of nonprocessive motors [1,5,23]. The mechanism underlying this process is unclear because molecular motors switching lanes are of the size and timescales that are very difficult to observe even with modern super-resolution microscopy techniques. Most *in vivo* studies of transport by multiple motors investigate this behavior using cargo tracking, and it is still a challenge to track the dynamics of individual motors and lane-switching in real time [1]. Therefore, simulations are necessary in investigating this process.

Our work is mainly focused on cargo transport pulled by several motors with the ability to switch between several MTs. We aim to investigate the efficiency of the transport of molecular motors switching between several filaments (MTs). We study molecular motors in two different classes of MT binding kinetics, which are classified by slow and fast detachment and reattachment rates. Kinesin-1 attachment is intrinsically slow because the motors can roughly bind to MTs in a small area, which relates to the area of one tubulin dimer [24], whereas Ncd has inherently high binding on and off rates. The intrinsically different properties of the binding kinetics of molecular motors are then compared to study the effect of transport with switching filaments.

II. MATHEMATICAL MODELS: EXTENDING THE ONE-LANE TO THE MANY-LANE MODEL

The theoretical investigation of motor transport following a simple exclusion process (SEP) was pioneered by Evans *et al.* [25]. Later, Campas *et al.* [2] developed the theoretical and simulation model of transport by multiple molecular motors, assuming that the force is exerted on the leading motor. Our previous work explored cargo transport by multiple molecular motors, with the ability to attach and detach on a single filament, through a comparison of analytical calculations with simulations in Ref. [26,27]. The analytical expressions for nonprocessive motors in the case of finite binding sites along a single lane are validated by simulations of the corresponding system in Ref. [26,27]. Now, we extend the system to multiple lanes, allowing the motors to switch between lanes. The analytic expression in the case of multiple lanes based on the leading motor model is discussed in this section.

A. Distribution of number of bound motors

We first calculated, as in Ref. [26], the distribution of the number of bound motors (n) that can be actively bound, which ranges from 0 to a maximum number of motors N on a single lane within a number of binding sites (M) determined by a cargo width. In our model, the cargo is represented as an extended object with a finite width along the direction of the MTs. This cargo width determines the initial available binding sites for motors. $P_n(N, M)$ is the probability distribution of bound motors (n) out of the total number of motors (N) within M binding sites. This probability can be obtained by the following master equation:

$$\begin{aligned} \frac{\partial P_n(N, M)}{\partial t} &= (N - n + 1)(M - n + 1)k_{\text{on}}^s P_{n-1} + (n + 1)k_{\text{off}} P_{n+1} \\ &\quad - (N - n)(M - n)k_{\text{on}}^s P_n - nk_{\text{off}} P_n, \end{aligned} \quad (1)$$

where $k_{\text{on}} = (M - n)k_{\text{on}}^s$ is the binding rate for an unbound motor and k_{off} is the unbinding rate for a bound motor.

At steady state, $\frac{\partial P_n(N, M)}{\partial t} = 0$, we obtain

$$P_n(N, M) = \frac{1}{n!} \left(\frac{k_{\text{on}}^s}{k_{\text{off}}} \right)^n \frac{N!}{(N - n)!} \frac{M!}{(M - n)!} P_0, \quad (2)$$

where P_0 is the normalization constant given by $\sum_{n=0}^N P_n(N, M) = 1$:

$$P_0 = \left(\sum_{n=0}^N \frac{N!M!}{n!(N - n)!(M - n)!} \left(\frac{k_{\text{on}}^s}{k_{\text{off}}} \right)^n \right)^{-1}. \quad (3)$$

See more details in Sec. II A.

B. Average velocity

The velocity of a cargo pulled by several nonprocessive motors along a single filament is given by

$$\bar{V} = \sum_{n=1}^N \frac{P_n V_n}{1 - P_0}, \quad (4)$$

where P_n is the probability of a number of bound motors and V_n is the velocity when there are n bound motors [5]. We

assume that the velocity of n bound motors, following an SEP in which the motors cannot overtake each other, is limited by the velocity of the leading motor. Their velocity is equal to

$$V_n = p \frac{(1 - e^f(q/p)^n)(1 - q/p)}{e^{f\delta}(1 - q/p) + e^f(q/p - (q/p)^n)}, \quad (5)$$

which is derived in Refs. [2,25]. Thus, the velocity of N nonprocessive motors moving along a single filament with a limited number of binding sites M can be obtained by substituting Eq. (5) and Eq. (2) into Eq. (4):

$$\bar{V} = \sum_{n=1}^N \frac{N!}{n!(N-n)!} \frac{M!}{(M-n)!} \frac{P_0}{1-P_0} \left(\frac{k_{\text{on}}^s}{k_{\text{off}}} \right)^n \times \left(\frac{p(1 - e^f(\frac{q}{p})^n)(1 - \frac{q}{p})}{e^{f\delta}(1 - \frac{q}{p}) + e^f(\frac{q}{p} - (\frac{q}{p})^n)} \right). \quad (6)$$

In the case of multiple lanes, each leading motor shares the force with the leading motors on other lanes, so the leading motors' rates are $p_1 = pe^{-\frac{f\delta}{L}}$ and $q_1 = qe^{\frac{f(1-\delta)}{L}}$, where $f = F dx/k_B T$ is the dimensionless force where F is the load force, dx is the motor step size, L is number of lanes occupied by motors, k_B is the Boltzmann's constant, and $T = 310K$ is the body's temperature; $\delta = 0.5$ is a dimensionless fraction between 0 and 1 that determines how much the forward versus backward stepping rates are affected by the load force. The external load force could potentially be experimentally applied to a cargo *in vitro*. *In vivo*, the force could be from tension in the cargo and/or the drag force of cargo motion moving in the viscous cytoplasmic environment. In our work, we take the force to be a constant value that we vary between simulations to study its effect on the motion. In our model, we assume the external load force acts only on the leading motor on each lane. The following motors step with constant forward and backward stepping rates, denoted as p and q , respectively. Thus, the following motors are affected by the force only indirectly via the SEP, limiting their stepping behind the leading motor. Recent work highlights significant forces that may act on following motors and influence motor detachment rates and stepping dynamics [14]. It may therefore be important to include this in future investigations. Here, we assume that the force makes no difference to the probability of binding, and the cargo velocity in this case is determined by the forwardmost leading motor's velocity. Thus, the analytical solution for the average velocity for multiple lanes is

$$\bar{V} = \sum_{n=1}^N \frac{N!}{n!(N-n)!} \frac{M!}{(M-n)!} \frac{P_0}{1-P_0} \left(\frac{k_{\text{on}}^s}{k_{\text{off}}} \right)^n \times \left(\frac{p(1 - e^{\frac{f}{L}}(\frac{q}{p})^n)(1 - \frac{q}{p})}{e^{\frac{f\delta}{L}}(1 - \frac{q}{p}) + e^{\frac{f}{L}}(\frac{q}{p} - (\frac{q}{p})^n)} \right), \quad (7)$$

where L is the number of bound lanes that can be taken as an average value $\langle L \rangle$ from simulations.

III. COMPUTATIONAL MULTIPLE-LANES MODEL

The system is sketched in Fig. 1 and our model is shown in Fig. 2. We developed a model of transport by multiple

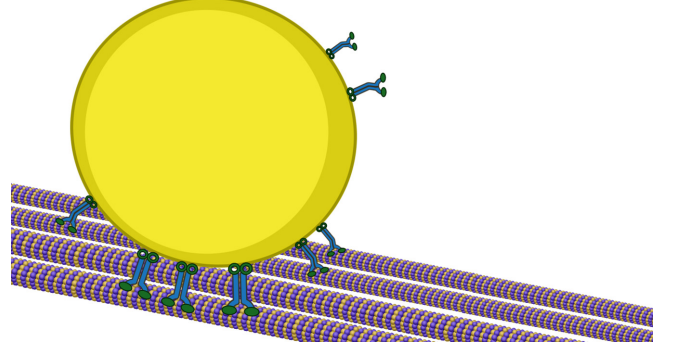


FIG. 1. Sketch of multiple molecular motors randomly distributed on an organelle surface, transporting it along multiple microtubules. The random organization of motors on the cargo surface is supported by Ref. [6]. This figure was created using visual elements from the NIAID BioArt collection [28] and the Reactome Pathway Database [29], available under a Creative Commons Attribution 4.0 International (CC BY 4.0) license.

molecular motors along multiple lanes, including the motor's ability to switch between lanes. It is performed using discrete lattice-based models in fixed time step Monte Carlo simulations. Motors transporting a cargo move on a square lattice keeping the sequence in the same lane with the hopping rates (forward, p , and backward, q , stepping rates) and can also switch to the other lanes with binding, k_{on} , and unbinding, k_{off} , rates. The lanes are aligned in parallel. Along the lane, the motors perform asymmetric random walks. A motor hops with different rates to the neighboring sites. It makes a forward step with rate p and a backward step with rate q , with values as shown in Table I. The motors follow the SEP such that a motor makes no step if that would involve moving to an occupied lattice site; thus, the motors cannot overtake each other while stepping on the same lane. In the model, the leading motor and the following motors perform stepping at different rates. The leading motor's stepping rates (p_1 and q_1) depends on force transmitted from the cargo, whereas the following motors do not experience the force and step with constant rates p and q . It is important to note for this model that in each simulation, the dimensionless total force f is assumed to be constant over time and is distributed equally among the leading motors on each of the bound lanes L at each time step, $f_L = \frac{f}{L}$, where f is the total dimensionless force transmitted to the leading motors and L is the number of bound lanes. Therefore, the rates of leading motors can be written as $p_1 = pe^{-\frac{f\delta}{L}}$ and $q_1 = qe^{\frac{f(1-\delta)}{L}}$. While performing steps along the lane, the motor can unbind from the filaments at rate k_{off} and rebound to the same lane again or bind to one of the other lanes with rate k_{on} . Also, the motor is allowed to bind on an empty lattice site within a dynamically determined limited number of sites, denoted as M . Motors are initially allowed to bind within the cargo width (M). At each time, the number of binding sites, M , is not fixed and is determined by the sites between the position of the forwardmost leading motor and the last motor, plus one more site in front and behind. As a result, the M binding sites can vary at each step and across different lanes. Binding is rejected if the site is occupied. We performed a simulation of 1×10^8 steps for kinesin-1 and 1×10^7 steps for Ncd

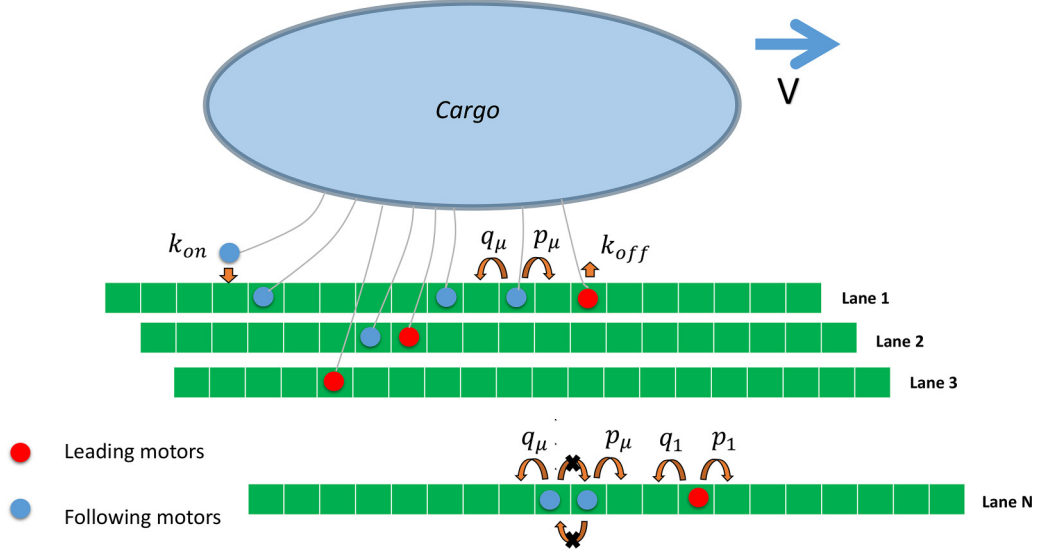


FIG. 2. Diagram of our many-lanes model: an example of eight motors bound to the cargo, three leading and five following, attached on the cargo surface switching lanes with on (k_{on}) and off (k_{off}) rates and performing steps with forward p_μ and backward q_μ rates, where μ is the index of motors. The bound leading motors share the force f equally such that they each experience $\frac{f}{L}$, where L is the number of lanes with bound motors. The motors follow a simple exclusion process such that they cannot be on the same site and cannot overtake each other during stepping along a lane. The green squares and red/blue circles represent lattice sites and motors, respectively. The red circles represent leading motors and the blue circles represent following motors.

in which all motors can possibly unbind from the filament. This is important in calculating run length. The simulation time step is 0.001 s and the simulation procedures are as follows:

- (1) Initially, we have N motors that are not bound to the lane but are attached to a cargo with random positions within a length given by M lattice sites.
- (2) Then, at subsequent time steps, we visit each of the N motors to consider their possible states and positions by generating a random number. Each motor is allowed to either bind to or unbind from the lane according to the relevant probability, $P_{on} = k_{on} dt$ or $P_{off} = k_{off} dt$, respectively. On (re)binding, the position at which an unbound motor binds is random within the available sites. Whilst not explicitly simulated, this mimics the Brownian motion of an unbound motor.
- (3) If it is determined that the unbound motor will bind or rebind, then a second random number is generated to randomly choose which lane or new lane it will rebind or bind to.
- (4) In each step, the number of bound lanes can be changed by the motors switching lanes. This affects the leading motors' stepping rates in the next step, since the force f_L depends on the number of bound lanes.

- (5) After binding, the bound motors are allowed to move along the chosen lane by calculating their tentative steps forward and backward. The forward and backward stepping probabilities are $p_1 dt$ and $q_1 dt$ for leading motors, which depend on f_L , and $p dt$ and $q dt$ for following motors. The step is not allowed if the site is occupied, so the motor stays at its original site.

- (6) We calculate velocity, run length, and mean first passage time (MFPT) for transporting along multilanes from our simulation results.

In this work, we study transport on multiple filaments with two types of molecular motors with slow and fast MT binding kinetics. They are kinesin-1 and Ncds, which have low and high on and off rates, respectively. The parameters of the two motors used in this study are described in Table I. Our main purpose is to investigate the efficiency of transport by several molecular motors on multiple filaments compared with a single filament by calculating the velocity, run length, and MFPT of the transport. Moreover, we show how the different kinetics of molecular motors' binding to MTs affect cargo transport. We used kinesin-1 and Ncds as the representatives of slow and fast binding motors on MTs, respectively.

IV. RESULTS

In this work, the molecular motors are allowed to switch from one filament to another during transport. We study the effect of multiple filaments on the transport of molecular motors by considering the velocity and run lengths. We use the FPT as a measure of the time it takes to deliver a cargo to a target.

We model kinesin-1 as a slightly nonprocessive motor. It is able to bind on and off with low rates of 5 and 1 s⁻¹, respectively, as revealed in some experiments [34–36]. This is

TABLE I. Parameters of kinesin-1 and Ncds.

Parameters	Kinesin-1 (s ⁻¹)	Ncd (s ⁻¹)
Forward rates (p)	100 [19,30]	22 [19]
Backward rate (q)	10 [2]	2.2 [2]
Binding rate (k_{on})	5 [5,31]	20 [32,33]
Unbinding rate (k_{off})	1 [5]	10 [32,33]

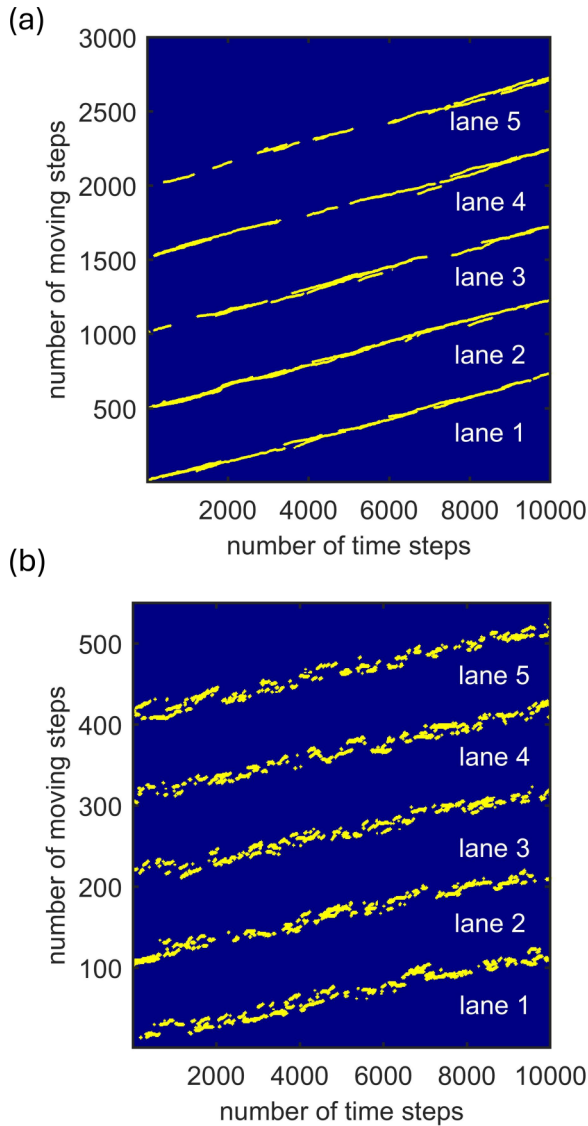


FIG. 3. Example trajectories in yellow of ten motors ($N = 10$) bound on five lanes ($L = 5$) with the initial number of binding sites $M = 20$ in each lane for 10^4 steps with $f = 2$ (a) Trajectories of ten kinesin-1 motors on five lanes while they are active and step on the lanes, where each lane is separated by 500 spatial steps for visibility purposes. (b) Trajectories of ten Ncds on five lanes while they are active and step on the lanes, where each lane is separated by 100 spatial steps for visibility purposes. The parameters used are presented in Table I. The unbound motors are not shown in the graph.

different from most modeling work, including our own in Ref. [26], which models kinesin-1 as a fully processive motor. On the other hand, kinesin-14 or Ncd is a highly nonprocessive motor with higher attachment and detachment rates of 20 and 10 s^{-1} [32,33].

Representative examples of trajectories of ten kinesin-1 and Ncds attached to a cargo with binding kinetics to multiple filaments are shown in Figs. 3(a) and 3(b), respectively. The trajectories of bound motors are plotted as the number of spatial steps against the number of time steps on each lane. We set a gap between lanes to show trajectories on each lane clearly as 500 spatial steps and 100 spatial steps for kinesin

and Ncd, respectively. This is to enable clear visibility of the separated lanes on the graphs and to avoid the overlapping of motors' steps from different lanes if they are too close. The separation is different for each motor type due to their different speeds. The spatial differences within each lane are related to the separation between the forwardmost motor and the last motor on each lane, which is the number of binding sites at each time. One step size represents 8 nm . As can be seen in these results that Ncd is more often unbound from the lane than kinesin-1, and their stepping follows the SEP.

At each time step, unbinding and rebinding events can happen to bound and unbound motors, respectively. In rebinding, the motor can either bind to the same lane or change to other lanes, so the number of bound lanes is changed at each step, as seen in Fig. 4(a). The figure shows an example of the number of lanes bound by ten kinesin-1 and ten Ncds transporting along five lanes at $f = 2$ over a short duration of 500 s, representing only the early stage of the simulation. We also included an occupancy map showing the number of filaments bound by kinesin-1 and Ncd over the entire simulation period. From the results, Ncds switch lanes more often than kinesin-1 because of higher binding and unbinding rates. The probabilities for the number of bound lanes for ten kinesin-1 and Ncds switching between five lanes for 100 runs are plotted in Fig. 4(b). The results show that Ncds and kinesin-1 bind to four lanes most of the time. However, there is a lower probability of Ncds occupying all lanes due to higher unbinding rates. Ncds mostly bind to a few filaments with higher density compared with kinesin-1.

A. Velocity of transport by several motors on multiple lanes

In the simulations, we measured the velocity of a cargo transported by multiple motors along several lanes, which is equal to the velocity of the forwardmost leading motor. The following motors' movement is limited by the leading motor because of the SEP. As a single lane, the velocity of a cluster of N nonprocessive motors working against a force f is equal to the leading motor velocity, which can be analytically expressed by Eq. (6) in which the number of binding sites (M) is limited on a single lane ($L = 1$), but the sequence preservation is not included. The velocities of a cargo transported by ten kinesin-1 and ten Ncds along several filaments from simulations are shown in Figs. 5(a) and 5(b), respectively, compared with the velocity on a single filament. The transport of cargo by multiple motors on one lane is thoroughly demonstrated in Ref. [26,27]. In the absence of force, kinesins and Ncds move at velocities of 720 nm/s and 160 nm/s , as displayed in Fig. 5. In the simulation, we calculate the velocity of the cargo transport by averaging the velocity of the leading motor for a single lane and the forwardmost leading motor for multiple lanes at different forces. Over time, the motors can switch to different lanes. The average velocity is calculated from a particular forwardmost leading motor's movement over the period of time until a new motor becomes the forwardmost leading motor. In Fig. 5, the simulation results correspond to our analytical models on a single lane ($L = 1$) and multiple lanes ($L = 2$ and $L = 3$), following Eqs. (6) and (7), respectively. In the case of multiple lanes, we substitute the averaged number of bound

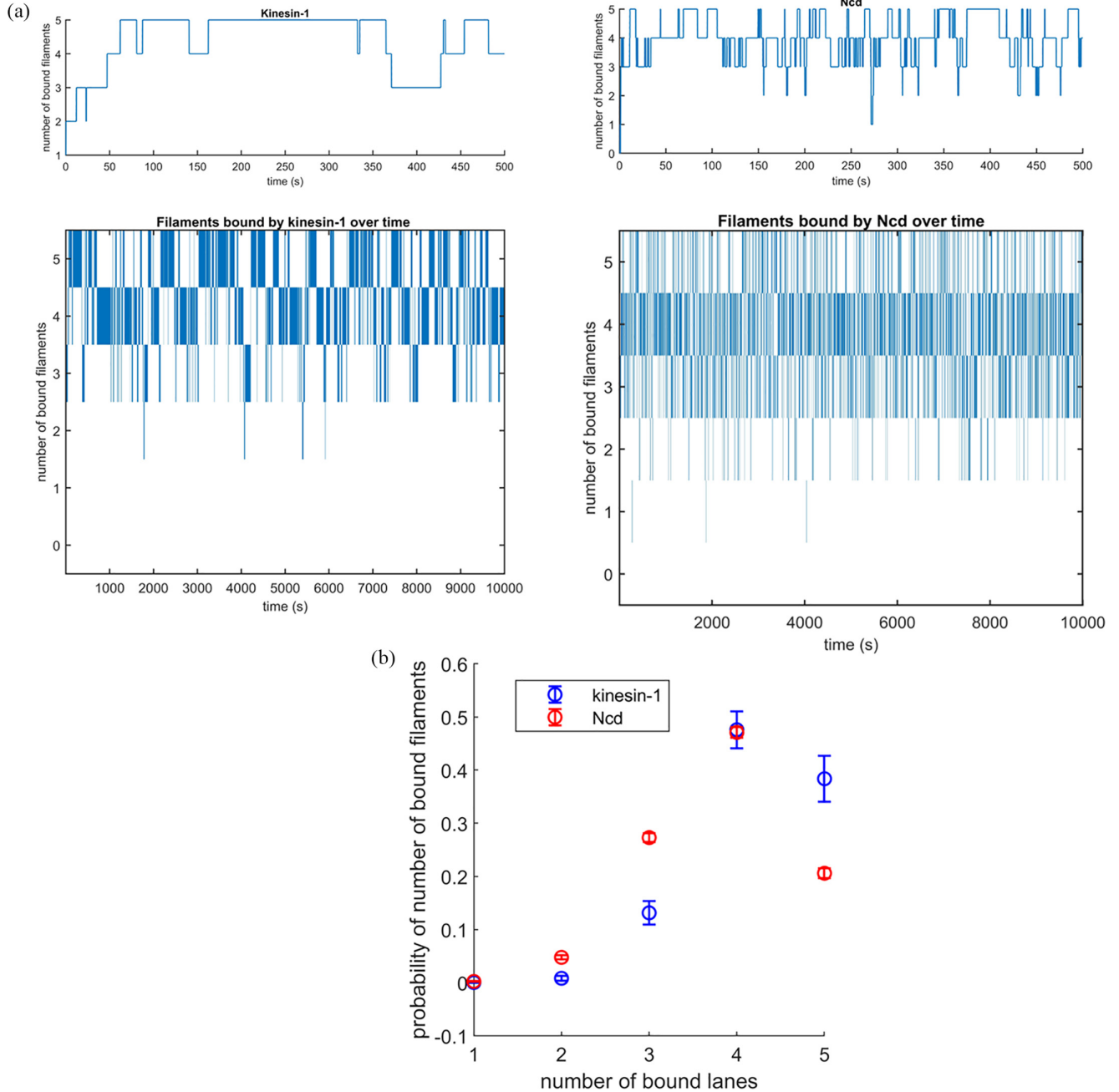


FIG. 4. (a) Time course of changing number of bound filaments for kinesin-1 (top) and Ncd (bottom) at $N = 10$ and $L = 5$, which are related to the trajectories in Figs. 3(a) and 3(b), respectively, for a short time. The entire simulation period has also been shown in an occupancy map where the number of bound filaments is indicated by the color blue for each time step. (b) The probability of the number of bound filaments of ten kinesin-1 (blue symbols) and ten Ncds (red symbols) distributed on five filaments.

lanes $\langle L \rangle$ at each force from simulations into Eq. (7). From the results in Fig. 5, the velocity of the transport carried by ten kinesins and Ncds on single and multiple lanes decreased rapidly with increasing force but the transport on two and three lanes are faster with the same total force compared with a single lane. This is because the transport on multiple lanes can have more than one leading motor that shares the force transmitted from the cargo. Each leading motor experiences a smaller force, which is $f_{\text{lane}} = F/L$, where F is the total force transferred from the cargo and L is the number of lanes. This

implies that having more lanes facilitates transport. Therefore, the cargoes transported on multiple lanes move faster than on a single lane with the same total number of motors and the same total force.

In Fig. 6 we show the velocity of a cluster of ten kinesin-1 and ten Ncd motors on different numbers of lanes (as calculated by the averaged velocity of forwardmost leading motor) compared with the analytical solution given by Eq. (7). From the simulation results of multiple lanes from $L = 1$ to $L = 5$ in Fig. 6, we can see clearly that the velocity increases with the

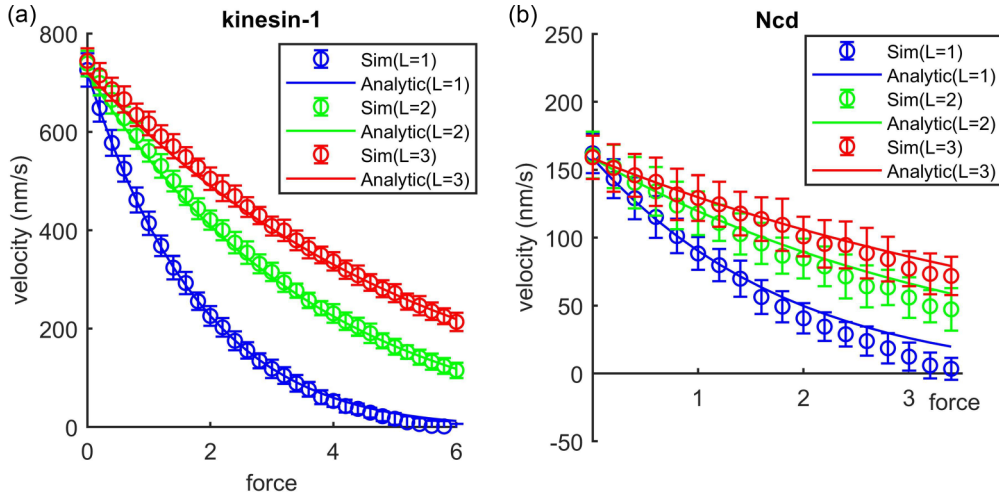


FIG. 5. (a) Force-velocity relation of a cluster of ten kinesin-1 on one (blue), two (green), and three (red) lanes. (b) Force-velocity relation of a cluster of ten Ncds on one (blue), two lanes (green), and three (red) lanes. The solid lines represent the analytical solution for the velocity given in Eq. (6) for a single lane and Eq. (7) for multiple lanes. We substituted the mean value of the number of binding sites $M = \langle M \rangle$ and the mean value of the number of lanes $L = \langle L \rangle$ averaged throughout the simulation time of 100 runs at each force. For example, $\langle M \rangle$ is 12.5 ± 2.0 and $\langle L \rangle$ is 2.93 ± 0.02 for kinesin, and $\langle M \rangle$ is 12.8 ± 0.5 and $\langle L \rangle$ is 2.77 ± 0.01 for Ncd on three lanes ($L = 3$) at $f = 3.4$. The parameters of kinesin-1 and Ncds are given in Table I. The error bars are the standard deviation between the averages of the 100 simulation runs.

number of lanes, which corresponds with the force-velocity relations in Fig. 5.

B. Run length of transport by several motors on multiple lanes

During the stepping of motors along a lane, motors have the ability to unbind from the lane and then rebind to the same or a different lane. To obtain run lengths of the transport of a cargo by multiple nonprocessive motors on multiple lanes,

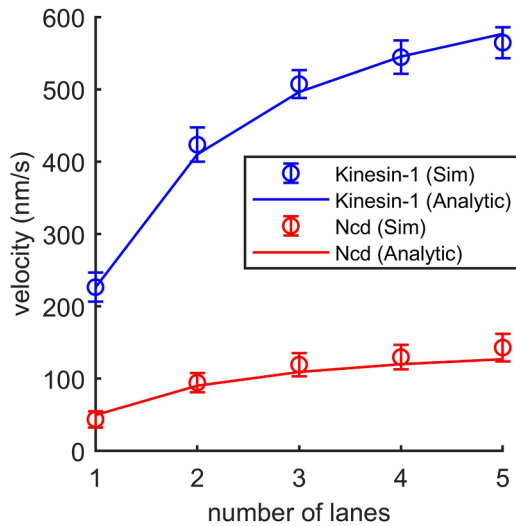


FIG. 6. Velocity of the forwardmost leading motors of ten kinesin-1 (blue symbols) and ten Ncds (red symbols) at $f = 2$ against the number of lanes ($L = 1$ to 5). The solid lines represent the analytical solution for the velocity given by Eq. (7) with the number of lanes substituted by the mean value $L = \langle L \rangle$. For example, for five lanes, $\langle L \rangle$ is 4.23 ± 0.06 and 3.83 ± 0.02 for kinesin-1 and Ncd, respectively. The error bars are the standard deviation between the averages of the 100 simulation runs.

we consider the time from when there is at least one bound motor carrying the cargo until all motors detach from the lanes. The run length is then defined as the difference between the position of the first bound motor and the position of the last bound motor when all motors unbind. Here, we chose to study small clusters with five to ten motors due to computational restrictions.

The average run length against force for five kinesin-1 and Ncds transporting cargo on a single and two lanes are plotted in Figs. 7(a) and 7(b), respectively. The run length of transport by five kinesin-1 and five Ncds on a single and two lanes decreases monotonically with force. This corresponds with past studies on a single filament. Note that the run length of kinesin-1 decreases exponentially with the external load force [11,37], but the run length of Ncd appears to decrease linearly with the force. In our simulations, the run lengths of kinesin-1 and Ncd decrease with increasing forces, with the same trend on two lanes as on one lane. However, the run lengths of motor teams on two lanes are longer than those of a team transporting on a single lane. Importantly, this indicates that intracellular cargoes transported by a team of molecular motors along more than one filament can reach targets further away than if there were only one filament.

We then show in Fig. 8 the effect of the number of lanes on run length for transport by five kinesin-1 and five Ncds on multiple lanes by varying the number of lanes from one to five. The run length of kinesin-1 is longer than that of Ncds, as expected, because kinesin-1 is more processive with lower rates of detachment and stays on the lane for longer periods of time. Experimentally, Bense *et al.* [38] recently found that kinesin-1-driven cargoes tend to maintain straight trajectories at MT intersections rather than frequently switching tracks. For Ncds, the transport is terminated more quickly, with shorter run lengths due to their faster detachment rate. From the results shown in Fig. 8, we see the run lengths of five kinesins increase from 10 μm to 100 μm on one to five

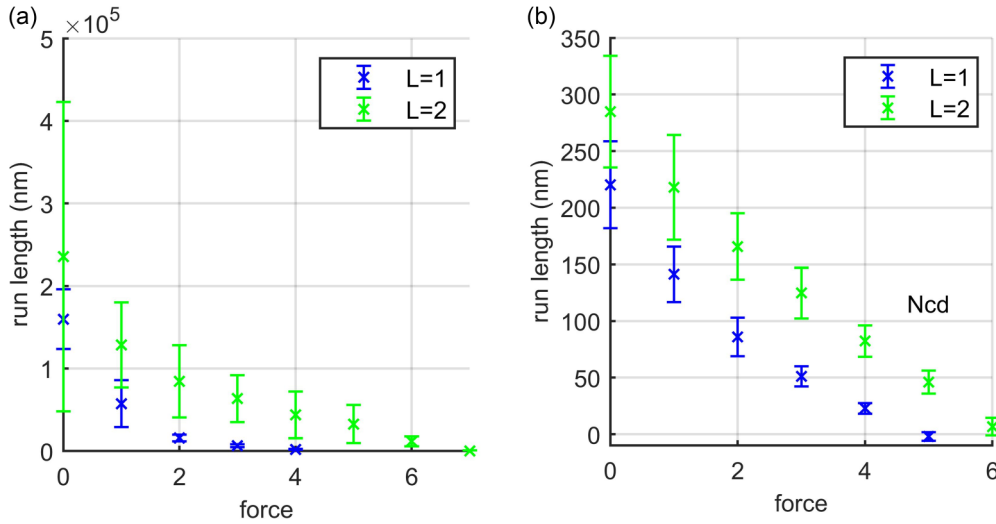


FIG. 7. (a) Run length against total force for five kinesin-1 and (b) five Ncds transporting on a single lane (blue symbols) and two lanes (green symbols). The error bars are the standard deviation between the averages of the 100 simulation runs.

lanes, whereas the run lengths of five Ncds increase from 50 nm to 0.2 μm on one to five lanes. It is clear that the run length increases with the number of lanes. Although, it seems to saturate at a point with a determined maximum number of motors ($N = 5$) attached to the cargo. Our results correspond with past studies that revealed that the distance of cargo transport is longer than typical lengths of MTs (50 μm) [1,5,23]. It is important to note that our simulation does not restrict the length of MTs (lanes), but the simulation is limited by total simulation time or total number of steps. We chose sufficiently long simulation times compared with the unbinding rates of molecular motors such that all motors have the possibility to unbind. This means we can interpret the run length in our simulation results as an intrinsic property of having five

kinesin-1 and Ncds transporting a cargo with specific transition rates.

C. MFPT of transport by several motors on multiple lanes

The FPT or the first hitting time is defined as the time taken by the first leading motor to reach a given distance. In our simulations, there are multiple motors transporting along the lanes with the ability to unbind and rebind lanes. Each motor is in either a bound or unbound state at any particular time in its trajectory, as shown in Fig. 3. From these trajectories, the FPT is the amount of time that the first motor (out of N motors in the cluster) reaches the given distance. The FPT is recorded for many simulation runs, and the mean average is taken, called the MFPT. We calculate the MFPT for different distances and plot it as a function of the number of lanes in Figs. 9(a) and 9(b) for kinesin-1 and Ncd, respectively. In Fig. 9(c), we show the comparison between kinesin-1 and Ncd for the same distance.

It is clear from Fig. 9 that multiple lanes can enhance the efficiency of transport by decreasing the amount of time taken to reach a target position, that is, MFPT results are a function of the number of filaments. This is consistent with the results in Ref. [3], which characterized the MFPT as a function of the number and length of filaments by simulating a network of cytoskeletal filaments with filaments of different lengths. From Sec. IV B, we know that the kinesin-1 team stays longer on the filaments than Ncds, so the run length by the kinesin team is greater than that by the Ncd team. The set target distance needs to be shorter than the run length at which all motors detach from the lanes. It is reasonable to choose the target destination distance for kinesin at about 10–40 μm , whereas a suitable destination distance is about 80–200 nm for Ncd. At different target distances, both kinesins and Ncds can reach the target point in shorter times as the number of lanes increases. Furthermore, we also show that the kinesin team requires less time to reach the same destination than the Ncd team at different numbers of lanes, as illustrated

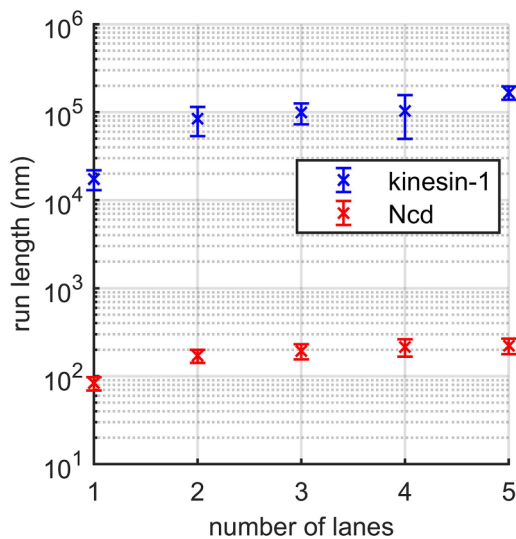


FIG. 8. Run length of five kinesin-1 (blue symbols) and five Ncds (red symbols) on different numbers of lanes ($L = 1$ to 5). The error bars are the standard deviation between the averages of the 100 simulation runs.

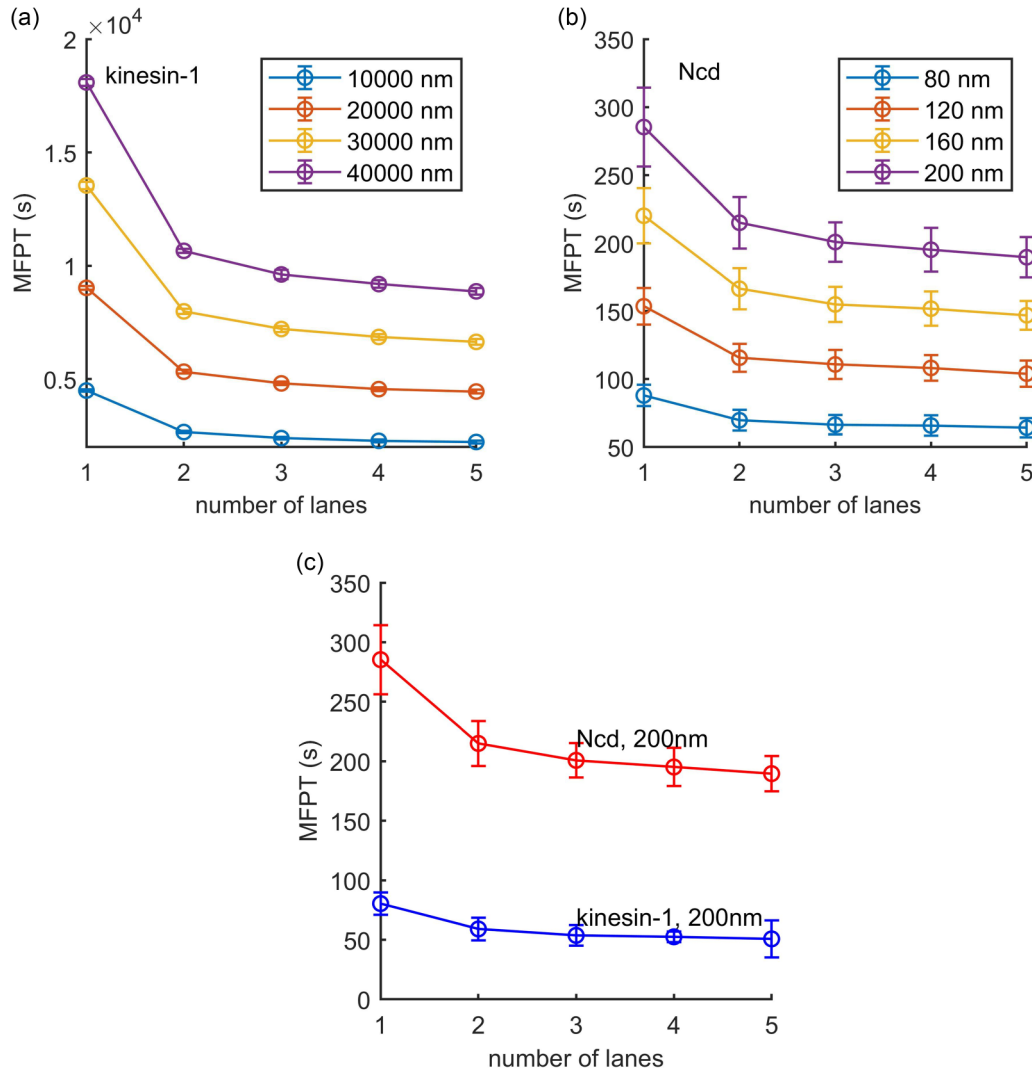


FIG. 9. (a) Mean first passage time (MFPT) of five kinesin-1 on different numbers of lanes reaching a target distance of 10, 20, 30, and 40 μm (blue, red, yellow, and purple, respectively). (b) MFPT of five kinesin-1 on different numbers of lanes reaching a target distance of 80, 120, 160, and 200 nm (blue, red, yellow, and purple, respectively). (c) MFPT of five kinesin-1 compared with that of five Ncds reaching a target distance of 200 nm on different numbers of lanes. The error bars are the standard deviation between the averages of the 100 simulation runs.

in Fig. 9(c), because kinesin-1 is more processive and faster than Ncd.

V. CONCLUSION

Over the past few decades, our understanding of cargo transport in the cell has been dramatically increased by experimental, simulation, and theoretical studies. However, most studies on the cooperative behaviors and mechanisms of molecular motors are conducted along a single filament. More work is still necessary to understand the transport performed on many filaments and the behaviors of switching filaments. It is difficult to observe these dynamics *in vivo*. Our simulation study contributes to addressing this knowledge gap by focusing on the efficiency of transport by molecular motors along multiple filaments. Our results clearly show that the number of filaments (lanes) can enhance the intracellular cargo transport. This is supported by our measurements of velocity, run length,

and MFPT in our simulations. The velocity and run length of motor clusters on multiple filaments against the force applied to the molecular motors is faster and longer compared with transport on a single filament. The velocity and run length of transport are increased with the number of filaments. We also characterize the multilane transport by the MFPT. Transport of multiple motors along multiple cytoskeletal filaments can substantially decrease the time to deliver a cargo to a target point compared with single-filament transport. Furthermore, our findings show that the intrinsic properties of molecular motors can affect transport. We compared kinesin-1 and Ncd, which have significant differences in MT binding and unbinding rates. Transport by a cluster of kinesin-1 moves faster and further than by Ncds, taking shorter times to reach targets. These findings help to further our understanding of cargo transport by molecular motors in complex cytoskeletal systems within crowded cellular environments [1,23,39,40] and long-range neuronal transport [41–43].

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DATA AVAILABILITY

The data that support the findings of this article are openly available [44]. Codes are available at the public GitHub repository [44].

APPENDIX A: PROBABILITY OF BOUND MOTORS WITH UNLIMITED BINDING SITES

The probability, P_n , of having n motors bound on a single lane within an unlimited number of binding sites can be obtained by the following master equation:

$$\begin{aligned} \frac{\partial P_n}{\partial t} = & (N - n + 1)k_{\text{on}}P_{n-1} + (n + 1)k_{\text{off}}P_{n+1} \\ & - (N - n)k_{\text{on}}P_n - nk_{\text{off}}P_n, \end{aligned} \quad (\text{A1})$$

where N is the total number of motors in the system. At steady state, $\frac{\partial P_n}{\partial t} = 0$, we obtain that

$$\begin{aligned} (n + 1)k_{\text{off}}P_{n+1} - (N - n)k_{\text{on}}P_n \\ = nk_{\text{off}}P_n - (N - n + 1)k_{\text{on}}P_{n-1}. \end{aligned} \quad (\text{A2})$$

At equilibrium, the detailed balance condition holds such that the binding and unbinding processes occur with equal probability, so

$$\begin{aligned} P_n &= \left(\frac{N - n + 1}{n} \right) \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right) P_{n-1} \\ P_n &= \left(\frac{N - n + 1}{n} \frac{k_{\text{on}}}{k_{\text{off}}} \right) \left(\frac{N - n + 2}{n - 1} \frac{k_{\text{on}}}{k_{\text{off}}} \right) (\cdots) \left(\frac{N}{1} \frac{k_{\text{on}}}{k_{\text{off}}} \right) P_0 \\ P_n &= \frac{1}{n!} \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n P_0 ((N - (n - 1))(N - (n - 2))(\cdots)N) \\ P_n &= \frac{N!}{n!(N - n)!} \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n P_0. \end{aligned} \quad (\text{A3})$$

From the normalization $\sum_{n=0}^N P_n(N) = 1$, we obtain:

$$\begin{aligned} \sum_{n=0}^N \frac{N!}{n!(N - n)!} \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n P_0 &= 1 \\ P_0 &= \left(\sum_{n=0}^N \frac{N!}{n!(N - n)!} \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n \right)^{-1}. \end{aligned} \quad (\text{A4})$$

If $k_{\text{on}} < k_{\text{off}}$ this can be written as a convergent series,

$$\sum_{n=0}^N \frac{N!}{n!(N - n)!} \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n = \sum_{n=0}^N \binom{N}{n} \left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n = \left(1 + \frac{k_{\text{on}}}{k_{\text{off}}} \right)^N.$$

Thus,

$$P_0 = \left(1 + \frac{k_{\text{on}}}{k_{\text{off}}} \right)^{-N}$$

and the maximum number of bound motors, N , can be infinitely large.

Therefore, we obtain the distribution of bound motors (n) for unlimited binding sites as follows:

$$P_n(N) = \frac{N!}{n!(N - n)!} \frac{\left(\frac{k_{\text{on}}}{k_{\text{off}}} \right)^n}{\left(1 + \frac{k_{\text{on}}}{k_{\text{off}}} \right)^N}. \quad (\text{A5})$$

APPENDIX B: PROBABILITY OF BOUND MOTORS WITH LIMITED BINDING SITES

The probability P_n of having n motors bound on a single lane within a number of binding sites (M) can be obtained by the master equation written in Eq. (1) and $k_{\text{on}}(M, n) = (M - n)k_{\text{on}}^s$, where $k_{\text{on}}^s = k_{\text{on}}/M$:

$$\begin{aligned} \frac{\partial P_n(N, M)}{\partial t} &= (N - n + 1)(M - n + 1)k_{\text{on}}^s P_{n-1} + (n + 1)k_{\text{off}}P_{n+1} \\ &\quad - (N - n)(M - n)k_{\text{on}}^s P_n - nk_{\text{off}}P_n. \end{aligned} \quad (\text{B1})$$

At steady state, $\frac{\partial P_n(N, M)}{\partial t} = 0$, we obtain that

$$\begin{aligned} (N - n + 1)(M - n + 1)k_{\text{on}}^s P_{n-1} - nk_{\text{off}}P_n \\ = (N - n)(M - n)k_{\text{on}}^s P_n - (n + 1)k_{\text{off}}P_{n+1}. \end{aligned} \quad (\text{B2})$$

For the detailed balance condition, we have

$$\begin{aligned} nk_{\text{off}}P_n &= (N - n + 1)(M - n + 1)k_{\text{on}}^s P_{n-1} \\ P_n &= \frac{(N - n + 1)(M - n + 1)}{n} \frac{k_{\text{on}}^s}{k_{\text{off}}} P_{n-1}. \end{aligned} \quad (\text{B3})$$

Therefore, we obtain the distribution of bound motors (n) for the case of limited binding sites (M) and its normalization constant as follows:

$$\begin{aligned} P_n(N, M) &= \frac{N!M!}{n!(N - n)!(M - n)!} \left(\frac{k_{\text{on}}^s}{k_{\text{off}}} \right)^n P_0 \\ P_0 &= \left(\sum_{n=0}^N \frac{N!M!}{n!(N - n)!(M - n)!} \left(\frac{k_{\text{on}}^s}{k_{\text{off}}} \right)^n \right)^{-1}. \end{aligned} \quad (\text{B4})$$

For $M \gg n$, Eq. (B4) becomes Eq. (A5).

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