Published in final edited form as:

Bull Math Biol. 2018 August ; 80(8): 2088–2123. doi:10.1007/s11538-018-0448-9.

Assessing the impact of electrostatic drag on processive molecular motor transport

J. Darby Smith and

Department of Mathematics, University of Florida, Gainesville, FL

Scott A. McKinley

Department of Mathematics, Tulane University, New Orleans, LA

Abstract

The bidirectional movement of intracellular cargo is usually described as a tug-of-war among opposite-directed families of molecular motors. While tug-of-war models have enjoyed some success, recent evidence suggests underlying motor interactions are more complex than previously understood. For example, these tug-of-war models fail to predict the counterintuitive phenomenon that inhibiting one family of motors can *decrease* the functionality of opposite-directed transport. In this paper, we use a Stochastic Differential Equations modeling framework to explore one proposed physical mechanism, called *microtubule tethering*, that could play a role in this “co-dependence” among antagonistic motors. This hypothesis includes the possibility of a tradeoff: weakly-bound trailing molecular motors can serve as tethers for cargoes and processing motors, thereby enhancing motor-cargo run lengths along microtubules; however, this introduces a cost of processing at a lower mean velocity. By computing the small- and large-time mean-squared displacement of our theoretical model and comparing our results to experimental observations of dynein and its “helper protein” dynactin, we find some supporting evidence for microtubule tethering interactions. We extrapolate these findings to predict how dynein-dynactin might interact with the opposite-directed kinesin motors and introduce a criterion for when the tradeoff is beneficial in simple systems.

Keywords

Intracellular transport; Molecular motors; Electrostatic drag; Microtubule tethering

PACS

87.10.Mn; 87.16.Ka; 87.16.Nn

1 Introduction

Cytoplasmic dynein is a processive molecular motor that is essential for intracellular transport in most animal cells. Dynactin is a protein associated with dynein that has long been considered a *helper protein* because overall transport by dynein motors increases in its presence (Gill et al, 1991). How exactly dynactin accomplishes this has been a source of uncertainty, but in the last decade, analysis of dynactin sub-components has revealed some

clues. For example, dynactin is thought to act as a connector both between intracellular cargo and dynein motors, as well as between motor-cargo complexes and microtubules (Ayloo et al, 2014). Specifically, for the latter function, a component of dynactin called p150^{Glued} is thought to enhance dynein-dynactin's affinity for microtubules, making motor-cargo complexes attach to microtubules more quickly and detach from microtubules more slowly. There seems to be a trade-off, though, in that the average velocity of dynein-driven cargo might be decreased when dynactin is present (Culver-Hanlon et al, 2006). For these reasons, dynactin has been called "both a dynamic tether and brake during dynein-driven motility" (Ayloo et al, 2014).

Intracellular transport is typically bidirectional: cargoes exhibit bursts of anterograde (toward the cell periphery) and retrograde (toward the cell body) motility with significant pauses in between. Microtubules are polarized, and in the cellular systems we consider (the axons of neurons, for example), microtubules are aligned so that their plus-ends and minus-ends are respectively in the anterograde and retrograde directions. For this reason, anterograde transport is generally driven by the plus-end oriented kinesin family of motors, while retrograde transport is conducted by minus-end oriented dynein. Kinesin motors tend to take smaller, but more frequent steps than dynein motors (Carter and Cross, 2005), and are much more resilient with respect to the application of opposing forces (Mallik et al, 2004; Carter and Cross, 2005). As a result, when both kinesin and dynein motors are attached to a single cargo, it is hypothesized that anterograde transport will dominate.

From a mathematical modeling point-of-view, the bidirectional movement of cargo along microtubules has been described as a "tug-of-war" between kinesin and dynein motors. Under the tug-of-war hypothesis, motor conflict is resolved according to a balance-of-forces calculation, and this is translated into cargo mobility in one of two ways: either 1) the motors pull each other to stall force leading the motor-cargo complex to have essentially zero velocity, sitting in a paused state (Segal et al, 2012); or 2) the motor-cargo complex has a variable mean velocity that depends on the respective numbers of plus- and minus-end directed motors that are attached to the cargo and microtubule simultaneously (Klumpp and Lipowsky, 2005; Müller et al, 2008). While the tug-of-war modeling regime can successfully describe cargo motion at a qualitative level, there have been multiple critiques when the model is confronted with data. For example, Kunwar et al (2011) observed that tug-of-war models do not accurately predict the frequency and duration of pauses when the number of plus-end and minus-end motors are altered. In particular, Kunwar et al (2011) noted that as the number of available motors increases, the tug-of-war model predicts that the frequency of pauses that arise from motor conflict should also increase. Experimentally, this was not the case for the transport of lipid droplets. Moreover, in a review of *in vivo* experiments, Hancock (2014) observed that it is a general phenomenon that when one family of motors is inhibited, transport by opposite-directed motors is reduced rather than increased. He termed this phenomenon the *paradox of co-dependence among antagonistic motors* and proposed several possible mechanisms that could explain it. In this work, we focus on what has been called the *microtubule tethering hypothesis*.

The microtubule tethering hypothesis stems from the observation that molecular motors can be weakly bound to microtubules through electrostatic forces while *not* being engaged in

force-generation through stepping (Hancock, 2014). For the sake of clarity, suppose that there are two motors attached to a cargo – one kinesin motor and one dynein-dynactin complex – and that the dynactin is weakly bound to the microtubule while dynein is not engaged. In this scenario, it is hypothesized that the dynein-dynactin pair can be dragged toward the plus end by the kinesin motor through their respective connections to the cargo. If the kinesin motor were to detach, the dynein-dynactin pair could remain weakly bound to microtubule, and the motor-cargo complex would diffuse along the length of the microtubule. In this state, the kinesin motor would remain closer to the microtubule than would be the case if dynactin were not in the weakly bound state. As such, the kinesin motor could rebind to the microtubule rapidly and resume its plus-end motility. In this sense, we say that a weakly bound dynein-dynactin pair can *rescue* a plus-end run. This would have the effect of increasing *anterograde processivity*, i.e. the length of motor-cargo runs in anterograde direction. However, there is a potential cost for this rescue: when a kinesin motor processes with a weakly bound dynactin, this could introduce an *electrostatic drag* force that would diminish the mean velocity. Quantifying this trade-off is a major goal of this work.

The concept of tethering has appeared throughout the molecular motors literature and the concept is not restricted to the impact of helper proteins like dynactin, or to non-functionalized motors. In fact, the term “tether” has been used to describe dynamics for multiple agents and multiple scales. At the smallest scale, there is the well-established concept that dimeric motors use one head to tether the motor to the microtubule while the second head undergoes a diffusive search for a new binding site (see Hackney (1994), for example). At a larger physical scale, multiple functionalized motors can act as tethers for each other: when one motor unbinds, there can be other active motors that remain engaged with the microtubule to prevent detachment. Cooperative transport arising from tethering effects (even among opposite directed motors) was observed in a series of tug-of-war papers, (Klumpp and Lipowsky, 2005; Müller et al, 2008, 2010). This boost in overall transport is largely due to increased run lengths as it has been noted that the multiple same-direction oriented motors can actually decrease the velocity of the system (Kunwar et al, 2008; Bieling et al, 2008). A third tethering mechanism arises when considering potential secondary binding sites motors utilize to tether themselves to the microtubule while stepping (see Kincaid and King (2006)). For instance, the K-loop of monomeric kinesin-3 allows the single-headed motor to remain tethered to the microtubule while the motor head searches for the next site (Okada and Hirokawa, 2000).

In this work, we focus on passive, mobile tethers and are motivated by data that suggests that diffusion along microtubules is somehow hindered by the interaction, but that the effect on motor processivity is relatively modest. For example, the existence of a weakly bound state for dynein-dynactin was observed by King and Schroer (2000) and Culver-Hanlon et al (2006). Moreover, through a series of experiments on dynein, dynactin, and dynactin sub-components, Culver-Hanlon et al (2006) observed a version of the tradeoff we described above. By tracking the location of tagged cargos, the authors noted that dynein run lengths were nearly tripled on average in the presence of dynactin and certain dynactin sub-components. Meanwhile, for these same events, the mean velocity was diminished by roughly 10%. In addition to characterizing processivity and velocity, the authors documented

what they termed *microtubule skating events*. These events were said to occur when dynein motility was knocked out, but dynactin or a dynactin sub-component could still bind to a microtubule. In these events, motor-cargo complexes were observed to diffuse in one dimension, along the length of a microtubule. As we explore in Sections 2 and 3, there is direct evidence in their Mean-Squared Displacement data (Figure 2b) that the long-term diffusivity of the cargo-motor complex is less than if the cargo was diffusing freely.

A similar phenomenon arises in kinesin-1 and myosin-V interactions. Ali et al (2008) observed that beads coated with myosin-V could weakly bind and diffuse along the length of a microtubule. When beads were coated with active kinesin-1 and myosin-V, they observed that run lengths were more than doubled when measuring the run lengths of beads coated with kinesin-1 alone. The authors also noted that kinesin-1 can weakly bind to actin. Performing an analogous run length experiment, the authors found that run lengths were also improved when kinesin-1 was bound to beads with myosin-V traveling on actin. There was a notable difference in how the active motor's average velocity was affected by the presence of the trailing motor. While myosin-V experienced a 15% knockdown in velocity with a weakly bound kinesin-1, the velocity of a processing kinesin-1 motor was unaffected by trailing myosin-V.

This kinesin-myosin dynamic was investigated in two separate efforts by Berger et al (2009) and Posta et al (2009). Both investigations focused on the major effect – the doubling of run length – and on inferring what the rate of rescue must be to make the long run length possible. Here, we focus on the second effect – the knockdown in velocity, possibly through *electrostatic drag* – and, among other goals, establish why kinesin-1 might be less affected by drag than the other motors.

1.1 Overview and Summary of Results

Our work is organized in terms of increasing model complexity. We begin in Section 2 with a model for *pinned diffusion*. Using an SDE model built on Langevin dynamics, we calculate the Mean-Squared Displacement (MSD) of a cargo's position, assuming it is pinned to a microtubule through a Hookean spring to a fixed microtubule site. Using experimentally observed MSD behavior for such events from Culver-Hanlon et al (2006), we estimate fundamental model parameters. In Section 3, we move on to skating events, introducing a model for the interaction between the microtubule and the contact point on the motor-complex, which we generally refer to as the *anchor*. Recently Minoura et al (2010) investigated weak binding interactions with microtubules and we used their findings as a guide for our modeling decisions. The model introduces three new parameters, and we can gain some new information from the large time asymptotics of cargo MSD during skating events. The evaluation of the large time MSD behavior is a bit more challenging in this setting and in Theorem 1 we provide a general result on MSDs that includes the one-cargo- n -anchor example.

In Section 4, we study the impact of the electrostatic drag in the context of a standard model for force-dependent motor stepping. In Theorem 2, we provide a general result for the asymptotic velocity of a cargo that is simultaneously attached to n anchors and m motors assuming a linear force-velocity relationship. A major question in the model development

for anchor diffusion along microtubules is whether it is reasonable to assume that the force of friction should be related to the magnitude of the thermal fluctuations through the usual Fluctuation-Dissipation relationship (Kubo, 1966). In Section 4.3, we think of Fluctuation-Dissipation as something like a null hypothesis for analyzing cargo data. We calculate an expected relationship between the percentage knockdown in cargo diffusivity when attached to an anchor and the percentage knockdown in motor velocity when attached to a trailing motor through the cargo. In the only two instances we can find for such a comparison, we note that the velocity knockdown is significantly different than what one would expect under Fluctuation-Dissipation and linear dynamics.

In Section 4.4, we return to the original question, assessing the theoretical circumstances in which there is a net benefit in the trade-off between enabling rescues by tethered anchors and the knockdown in velocity due to electrostatic drag. Specifically, using estimated parameters and a four-state Markov chain model, we assess the impact of a single trailing weakly bound dynein-dynactin complex on a processing kinesin motor. Ultimately, we find that electrostatic drag forces may be significant when observing skating events on microtubules, but not so much as to make the fundamental trade-off not worthwhile.

2 Pinned Diffusion: Modeling and Inference

In the pinned diffusion scenario, the cargo diffuses about a fixed anchor point (see Figure 1a). When there is no mean velocity, as is the case for any unbiased diffusion, a natural summary statistic for particle paths is the *pathwise Mean-Squared Displacement* (MSD). If $X(t)$ is the position of a cargo at time t , then the theoretical Mean-Squared Displacement at time t is given by the formula

$$\text{MSD}(t) = \mathbb{E}[(X(t) - X(0))^2].$$

The pathwise version of the MSD is an average expressed in terms of time lags. If δ is the length of the time increment between cargo position observations and N is the total number of data points, then, for the j th cargo, we write the n th MSD time lag as

$$\mathcal{M}_j(n\delta) = \frac{1}{N - n + 1} \sum_{k=0}^{N-n} |X_j((k+n)\delta) - X_j(k\delta)|^2. \quad (1)$$

In their work on dynein and dynactin, Culver-Hanlon et al (2006) reported pathwise MSDs for several categories of movement, two of which appear in Figure 2. In the top left panel, Figure 2a, we see several pathwise MSDs (with their average displayed in thick black) for cargos that are attached to a microtubule through a bond that is immobile. We refer to these events as *pinned diffusion*. In the top right panel, Figure 2b, we see pathwise MSDs and their average for skating events. For pinned diffusion, the MSD saturates at large time, while for skating diffusion, the MSD continues to increase.

We use a Langevin Stochastic Differential Equation (SDE) framework for the cargo position process $X(t)$ in the overdamped (zero-mass) limit. When the cargo is tethered to an anchor and/or motor (see Figure 1c), this takes the form:

$$\gamma_c dX(t) = (F_{\text{motor}} + F_{\text{anchor}})dt + \sqrt{2k_B T \gamma_c} dW(t). \quad (2)$$

The forces F_{motor} and F_{anchor} are exerted by the motor and anchor on the cargo, respectively, and $W(t)$ represents a standard Brownian motion. This is a one-dimensional version of the type of model introduced by Korn et al (2009) that has been used, for example, by McKinley et al (2012) and Bouzat (2016). Throughout this work, we will assume that the tether for both the anchor and the motor are linearly elastic and obey Hooke's law. That is, the force contributed is proportional to the tether extension length and the constant of proportionality is the effective spring constant. We use κ_m to denote the effective spring constant for the motor-cargo tether and use κ_a to represent the anchor-cargo tether. When the motors and anchors used are explicit, we replace the m and a with the rst letter of the motor or anchor. For example, κ_k is the effective spring constant for kinesin and κ_d is the effective spring constant for dynein/dynactin. As such, using $Y(t)$ and $Z(t)$ to denote the locations of the motor and anchor, respectively, we have

$$F_{\text{motor}} = \kappa_m(Y(t) - X(t)), \text{ and } F_{\text{anchor}} = \kappa_a(Z(t) - X(t)).$$

It is also common for authors to use non-linear models for the cargo-motor connection: for example, worm-like chain (WLC) models, which stiffen as they extend, and finitely extensible non-linear elastic (FENE) models, which have very low resistance until stretched near their maximum length (Hariharan and Hancock, 2009; Hughes et al, 2012). We opted to use a linear model not only because it makes explicit analysis possible, but also because there is some reasonable support for it in the literature; specifically in the low drag force regime we will be working in. See Kutys et al (2010), for example.

Since there is no active motor force for pinned diffusion paths (see Figure 1a), we have

$$\text{Pinned Diffusion : } dX(t) = -\frac{\kappa_a}{\gamma_c}(X(t) - z)dt + \sqrt{\frac{2k_B T}{\gamma_c}}dW(t) \quad (3)$$

where z is the fixed location of the anchor along the microtubule. The solution to this SDE is (see Appendix A)

$$X(t) = X(0)e^{-\frac{\kappa_a}{\gamma_c}t} + (1 - e^{-\frac{\kappa_a}{\gamma_c}t})z + \sqrt{\frac{2k_B T}{\gamma_c}} \int_0^t e^{-\frac{\kappa_a}{\gamma_c}(t-u)} dW(u).$$

There is some question as to what are appropriate initial conditions for the SDE (3). From an experimental point-of-view, cargo position is the observed quantity, and so it is natural to set

$X(0) = 0$. The position of the cargo relative to the anchor fluctuates, and, as time progresses, the law of the spatial distance between them approaches the stationary distribution of (3). Since this is an Ornstein-Uhlenbeck SDE of the form $dY(t) = -a(Y(t) - m)dt + \sqrt{\sigma}dW(t)$, the stationary distribution is Gaussian with mean m and variance $\sigma=2a$ (see Wiersema (2008), for example). It follows that if we assume that the system is in its stationary distribution at time zero, then the anchor position should be considered to be random relative to the cargo's initial condition, with $z \sim N\left(0, \frac{k_B T}{\kappa_a}\right)$. Using Itô's formula, we can calculate the theoretical MSD for the cargo position:

$$\text{MSD}(t) = \mathbb{E}[X^2(t)] = \frac{2k_B T}{\kappa_a} \left(1 - e^{-\frac{\kappa_a}{\gamma_c} t}\right). \quad (4)$$

If it were possible to directly observe the anchor position, say $z = 0$, then the leading coefficient would be half of this value (see Appendix A and Equation 35).

We can use the small- and large- t asymptotics of the MSD to estimate the parameters of our model. As t tends to zero, the MSD is linear; as it tends to infinity, the MSD reaches a plateau:

$$\lim_{t \rightarrow 0} \frac{1}{t} \text{MSD}(t) = \frac{2k_B T}{\gamma_c}, \quad \lim_{t \rightarrow \infty} \text{MSD}(t) = \frac{2k_B T}{\kappa_a}. \quad (5)$$

If we assume that $k_B T = 0.0041$ pN μm as in Kojima et al (1997) or Muthukrishnan et al (2009), then we can use Figure 2a to infer the parameters γ_c and κ_d . (Note that we switch from writing κ_a to κ_d here because this inference is specifically for the dynactin tether spring constant.) Consistent with the observations in (5), in the small- t regime we observe that the empirical MSD is roughly linear, with a slope estimated to be $0.05 \mu\text{m}^2/\text{s}$. The linear nature of the MSD in the small- t regime is reminiscent of the MSD for free diffusion. Intuitively, one can interpret this as what the MSD would be if the cargo were not tethered to the microtubule. Meanwhile, the MSD at larger times appears to reach a plateau at approximately $0.015 \mu\text{m}^2$. In this asymptotic region, the MSD is capturing the long-term variance of the cargo position relative to the anchor position. Taken together, we estimate that

$$\gamma_c \approx 0.16 \text{pNs}/\mu\text{m}, \quad \text{and} \quad \kappa_d \approx 0.55 \text{pN}/\mu\text{m}. \quad (6)$$

These are physiologically reasonable values. If we assume the Stokes-Einstein drag law for spherical particles holds for the cargo, then $\gamma_c = 6\pi r\eta$, where r is the effective radius of the cargo and η is the dynamic viscosity of the fluid. Culver-Hanlon et al (2006) use beads of radius $.105 \mu\text{m}$. If the fluid were similar to water, then the viscosity would be $0.001 \text{pN}/\mu\text{m}^2$.

It would follow that $\gamma_c \approx 0.002$ pN s/ μ m. This is consistent with the expectation that the viscosity of buffer would be greater than that of water. We could not find not much information on cargo-dynactin interaction, making it difficult to validate the estimate for κ_d . We can, however, compare this value to one obtained by Bruno et al (2011). In this experiment, aggregation and dispersion of melanosomes by kinesin-2 and cytoplasmic dynein was observed, finding an effective spring constant for *dynein* to be approximately 60 pN/ μ m, much greater than our estimate for the dynactin constant. However, to demonstrate that our model does indeed emulate the observed data, we simulated fifty cargo paths for the same length of time as in the Culver-Hanlon et al (2006) experiments and computed the associated pathwise MSDs. We display the ensemble average of the pathwise MSDs in black in Figure 2c. The pathwise MSD of six sample paths are displayed as thin gray curves, showing that the path-by-path variation is similar to what was reported by Culver-Hanlon et al. The theoretical MSD is displayed in blue.

3 Skating Diffusion: Modeling and Inference

3.1 Skating Model Development

In this section we describe our model for the impact of electrostatic drag on the anchor. We assume that the cargo position $X(t)$ satisfies the same SDE as for pinned diffusion (3), except that here the anchor position z is dynamic and denoted $Z(t)$. In what follows, let $V_a(t)$ denote the velocity of the anchor at time t . We will derive an SDE for $V_a(t)$ based on a Newtonian force-balance framework, and then take the mass parameter m_a to zero (the overdamped limit) to yield an SDE for $Z(t)$ that can be expressed in terms of the cargo position.

Suppose that the velocity of the anchor along the length of the microtubule can be described by the equation

$$m_a dV_a(t) = (F_{\text{applied}} + F_{\text{MTdrag}})dt + \sigma_a dW_a(t),$$

where m_a is the mass of the anchor, σ_a is the magnitude of the thermal fluctuations experienced by the anchor near the microtubule, and $W_a(t)$ is a standard Brownian motion. We assume there are two independent sources of external drag: first, F_{applied} represents the force on the anchor induced through the tether by separation from the cargo; and second, F_{MTdrag} represents the weak binding interaction with the microtubule. To decide on appropriate forms for the terms σ_a and F_{MTdrag} requires some discussion.

The most thorough investigation we are aware of concerning the electrostatic interaction of charged particles with microtubules appears in Minoura et al (2010). In that work, the authors describe the interaction as one-dimensional diffusion (along the length of the microtubule) interspersed with a rapid sequence of transient binding and unbinding events. The transitions between the diffusing state and the immobilized state are assumed to be so rapid that the immobilized states are unobserved. The evidence of the interaction is that the diffusivity of the charged particle is diminished near the microtubule, with magnitude of the change from standard diffusion being increased with increased charge on the surface of the particles. A similar model for transient immobilization events for a diffusive particle appears

in Chen et al (2014), describing the behavior of viruses whose diffusivities in mucus are decreased by the presence of certain antibodies. (See also a review by (Witten and Ribbeck, 2017)). In both settings, the authors use a two-state model: in one state the particle of interest diffuses with the expected Stokes-Einstein drag; in the other state, the particle is immobilized. The switching is modeled by a two-state Markov chain with binding and unbinding rates k_{on} and k_{off} . Both rates are taken to infinity in such a way that the ratio $k_d = k_{\text{off}}/k_{\text{on}}$ is constant. Therefore the fraction of time that the particle is freely diffusing is $\alpha = k_{\text{off}}/(k_{\text{on}} + k_{\text{off}}) = k_d/(1 + k_d)$. The stochastically averaged effective diffusion constant is then $D_{\text{eff}} = \alpha D$.

This approach does not translate to a Newtonian force-balance framework though, because there are multiple interpretations for how the temporary binding events might impart a force on the mobile particle. Because our present interest is to evaluate *whether or not* electrostatic drag forces are significant, we refrain from analyzing multiple potential models. Rather, we adopt a phenomenological description of the transient binding interactions, and simply assume that the relevant constants are modified by three constants q , q' and q'' , which we will describe presently.

As a baseline model, consider the Langevin equation for the velocity of the anchor when it is freely diffusing

$$\text{Freely diffusing anchor: } m_a dV_a(t) = -\gamma_a V_a(t)dt + \sqrt{2k_B T \gamma_a} dW_a(t).$$

Here, γ_a denotes the Stokes drag coefficient for the anchor when freely diffusing. It is not possible to know γ_a directly since no experimental data exists for freely diffusing motors or motor components, and the shape of any generic anchor is unknown, but we do assume that the system satisfies the Fluctuation-Dissipation relationship. Near the microtubule, the magnitude of thermal fluctuations and associated drag might be modified by wall effects or other issues that arise from the local geometry. Because we cannot directly observe anchor behavior – near the microtubule, near the wall, or otherwise – we introduce a single multiplicative factor q meant to summarize these effects. It follows that the diffusivity of the anchor near the microtubule would be $k_B T / q \gamma_a$ if there were no electrostatic interactions. Particle diffusivities tend to decrease near walls (see Johnson (1998), for example), so we expect q to be greater than one.

For our model of F_{MTdrag} , we assume that it is comprised of two forces that act in superposition: 1) the force due to fluid drag near the microtubule $[-q\gamma_a V_a(t)]$; and 2) the force due to transient binding and unbinding events $[-\gamma_E V_a(t)]$, where γ_E is the drag coefficient due to electrostatic interactions. We summarize the microtubule drag force by writing

$$F_{\text{MTdrag}} = -(q\gamma_a + \gamma_E)V_a(t) = -q'\gamma_a V_a(t).$$

We emphasize that a modeling choice is being made here and note that it is not clear that the binding and unbinding events will result in a force that is proportional to the velocity. In a

“stick-slip” modeling framework, for example, there is an initial resistance to force and then the dynamic friction is a constant that does not depend on velocity (see Urbakh et al (2004)).

Proceeding with this model, there is a second choice to be made: namely whether the anchor’s diffusion *along the length of a microtubule* should satisfy the Fluctuation-Dissipation relationship. If the system is in complete thermal equilibrium, then the drag coefficient in the noise should match the coefficient that appears in F_{MTdrag} . However, one could view the force generated by the microtubule (and the fact that it is attached to a glass plate) as external to the cargo-anchor complex. In the language of thermodynamics, the microtubule plays the role of connecting the particle to a cooler external heat bath that, on average, removes energy from the heat bath in which the cargo-anchor complex moves. In this case, it is reasonable for the drag coefficient in the friction term to be larger than the coefficient that appears in the magnitude of thermal fluctuations.

Rather than make a claim one way or another, we will introduce a second factor q'' for the thermal fluctuations experienced by the anchor while simultaneously attached to the cargo and experiencing weak electrostatic forces by the microtubule:

$$m dV_a(t) = F_{\text{applied}} - q' \gamma_a V_a(t) dt + \sqrt{2k_B T q'' \gamma_a} dW_a(t).$$

So, there are now three unknown parameters in the above equation: q' , q'' and γ_a . Notably, the available data allows us to make two inferences concerning the magnitude of the drag force and the thermal fluctuations: the first is through measuring the MSD of skating diffusion events; the second is through measuring the velocity of a processive motor-cargo-anchor system and comparing it to a motor-cargo system. As we will see, the first quantity will depend on the magnitude of the fluctuations while the second will not. If the system is in thermal equilibrium, then the estimated parameters q' and q'' should be very close. If they are not the same (which is what we find), then this is perhaps evidence that Fluctuation-Dissipation is not satisfied for anchor diffusion along the microtubule.

Taking the mass to zero and assuming that F_{applied} comes from the tether to the cargo, we have our model for position of the anchor $Z(t)$ in terms of the cargo position $X(t)$:

$$\begin{aligned} dX(t) &= \frac{\kappa_d}{\gamma_c} (Z(t) - X(t)) dt + \sqrt{\frac{2k_B T}{\gamma_c}} dW(t) \\ \text{Skating Diffusion:} \quad dZ(t) &= \frac{\kappa_d}{q' \gamma_c} (X(t) - Z(t)) dt + \sqrt{\frac{2k_B T q''}{(q')^2 \gamma_d}} dW_d(t). \end{aligned} \quad (7)$$

Using techniques established in Section 3.2, we establish that the MSD of the cargo then satisfies

$$\lim_{t \rightarrow \infty} \frac{1}{t} \mathbb{E}[X^2(t)] = 2k_B T \frac{(\gamma_c + q''\gamma_d)}{(\gamma_c + q'\gamma_d)^2}. \quad (8)$$

By inspection of Figure 2b, we estimate the large-time asymptotic slope to be $0.0035 \mu\text{m}^2/\text{s}$ (Culver-Hanlon et al, 2006). Recalling the value $k_B T = 0.0041 \text{ pN } \mu\text{m}$, we find that

$$\frac{(\gamma_c + q''\gamma_d)}{(\gamma_c + q'\gamma_d)^2} \approx 0.43 \frac{\mu\text{m}}{\text{pN s}}. \quad (9)$$

This result is already significant. If there were no force due to electrostatic drag, nor a correction to the coefficient of the noise in (7), then this ratio would be $1/\gamma_c = 1/0.16 = 6.25$, an order of magnitude greater than 0.43. On the other hand, if the skating diffusion system does satisfy Fluctuation-Dissipation, with $q' = q''$, then we find that the electrostatic drag coefficient is $q'\gamma_d = 2.17$. This is much larger than the fluid drag coefficient. If Fluctuation-Dissipation is not satisfied, we do not have enough information to parametrize the system. In Section 4, we propose using data from motor processing events to obtain an independent assessment of the system to yield greater insight into this issue.

3.2 A Limit Theorem for the Large-Time Behavior of Skating Event MSDs

In this section, we establish a theorem characterizing the asymptotic MSD of a system of interacting particles that are connected pairwise through Hookean springs. As a special case, one can derive the formula given in Equation 8 in the previous section when there is one cargo and one anchor. The theorem also applies when there is one cargo and an arbitrary number of anchors.

We summarize the connections between particles by an $n \times n$ matrix $A = (a_{ij})$, whose entries are the spring constants associated with the connection between particles i and j . We note that the matrix arising from the system (7) is not symmetric because of the different drag coefficients γ_c and $q'\gamma_d$. We therefore introduce a diagonal matrix Γ with entries $(\gamma_1, \gamma_2, \dots, \gamma_n)$ to include these multiplicative factors. The system of SDEs governing the vector of particle positions $\mathbf{X}(t) = (X_1, X_2, \dots, X_n)^\top$ can then be written

$$d\mathbf{X}(t) = \Gamma A \mathbf{X}(t) dt + \sum d\mathbf{W}(t), \quad (10)$$

where $\mathbf{W}(t) = (W_1(t), W_2(t), \dots, W_n(t))^\top$ is a vector of standard Brownian motions and Σ is a diagonal matrix with strictly positive diagonal entries $(\sigma_1, \dots, \sigma_n)$. Let $\tilde{A} = \Gamma A$. Then each component α_{ij} of $\tilde{A}$ is given by

$$\alpha_{ij} = \gamma_i a_{ij}. \quad (11)$$

We write the i th row of $\tilde{A}$ as $\tilde{\mathbf{A}}_i$. Then, for each $i \in \{1, 2, \dots, n\}$, we have

$$dX_i(t) = \tilde{\mathbf{A}}_i \mathbf{X}(t) dt + \sigma_i dW_i(t).$$

By Itô's rule, for $i \neq j$ we have

$$d(X_i X_j) = \left(X_i \tilde{\mathbf{A}}_j + X_j \tilde{\mathbf{A}}_i \right) \mathbf{X} dt + \sigma_j X_i dW_j(t) + \sigma_i X_j dW_i(t), \quad (12)$$

and

$$d(X_i^2) = \left(2X_i \tilde{\mathbf{A}}_i \mathbf{X} + \sigma_i^2 \right) dt + \sigma_i X_i dW_i(t), \quad (13)$$

Let $\mathbf{Y}(t)$ represent the vector of products $X_i X_j$ where $i \neq j$. That is, define

$$\mathbf{Y} = (X_1^2, X_1 X_2, X_1 X_3, \dots, X_1 X_n, X_2^2, X_2 X_3, \dots, X_2 X_n, X_3^2, \dots, X_{n-1} X_n, X_n^2)^\top.$$

Then, the system of SDEs given by (12) and (13) has the form

$$d\mathbf{Y} = (M\mathbf{Y} + F)dt + S(t)d\mathbf{W}(t), \quad (14)$$

where M is a square matrix of constants determined from Equations 12 and 13, $S(t)$ is a matrix containing only zeros and the $\sigma_i X_i$ terms from Equations 12 and 13, and F is the constant vector:

$$F = (\sigma_1^2, 0, 0, \dots, 0, \sigma_2^2, 0, \dots, 0, \sigma_3^2, 0, \dots, 0, \sigma_n^2)^\top.$$

If we identify the product term $X_i X_j$ with the double index (i, j) for $i \neq j$, then we can summarily write the entries of M , denoted $m_{(i,j),(k,\ell)}$, as

$$m_{(i,j),(k,\ell)} = \begin{cases} 2\alpha_{ii}, & \text{if } i = j = k = \ell \\ \alpha_{ii} + \alpha_{jj}, & \text{if } i = k \text{ and } j = \ell \\ 2\alpha_{j\ell}, & \text{if } i = j = k \\ 2\alpha_{ik}, & \text{if } i = j = \ell \\ \alpha_{j\ell}, & \text{if } i = k \text{ and } i \neq j \\ \alpha_{ik}, & \text{if } j = \ell \text{ and } i \neq j \\ 0, & \text{if } i \neq k \text{ and } j \neq \ell. \end{cases} \quad (15)$$

When a matrix has nonnegative entries off the diagonal, with rows that sum to 0, then it can be interpreted as the rate matrix of a continuous-time random walk on an associated directed graph G . If i and j are two vertices of G and $a_{ij} > 0$, then we say that i has an edge directed toward j . We say that there exists a *path from i to j* in G if there exist vertices $k_1, \dots, k_m$ such that i has an edge directed toward k_1 , k_1 has an edge directed toward $k_2, \dots$, and k_m has an edge directed toward j . By definition, G is called *strongly connected* if for every pair of vertices i and j , there exists a path from i to j and from j to i . It is a standard result that a matrix with non-negative entries irreducible if and only if its associated directed graph is strongly connected (Berman and Plemmons, 1994).

With this notation and terminology defined, we are now ready to state and prove a general theorem for the second moments of linear systems of connected particles.

Theorem 1—Let $\mathcal{G}$ be a connected graph with vertices $\{1, \dots, n\}$ and edges ε labeled accordingly to the connected pairs $(i, j) \in \varepsilon$. Associate to each edge a positive value k_{ij} if i connects to j . Define the matrix $A = (a_{ij})$ to have the values

$$a_{ij} = \begin{cases} k_{ij} & \text{if } (i, j) \in \varepsilon \\ 0, & \text{if } i \neq j \text{ and } (i, j) \notin \varepsilon \\ -\sum_{\ell=1, \ell \neq i}^n k_{i\ell} & \text{if } i = j. \end{cases}$$

Let Γ be a diagonal matrix with strictly positive diagonal entries $\gamma_1, \dots, \gamma_n$.

Suppose that $\mathbf{X}(t)$ satisfies Equation 10 for this A and Γ . Construct M and F as above. Then θ is a simple eigenvalue of M with real, positive, left row eigenvector $\mathbf{u}_0$, and for any i, j we have

$$\lim_{t \rightarrow \infty} \frac{1}{t} \mathbb{E}[X_i(t), X_j(t)] = \mathbf{u}_0 F.$$

The proof of this theorem, and of Theorem 2 below, rest on the following lemma.

Lemma 1—Let $\xi(t) = (\xi_1(t), \xi_2(t), \dots, \xi_n(t))^T$ satisfy

$$\dot{\xi}(t) = M\xi(t) + F, \quad \xi(0) = \xi_0, \quad (16)$$

where F is an $n \times 1$ constant vector and M is an $n \times n$ constant matrix. Suppose that

1. M has non-negative off-diagonal entries;
2. the rows of M sum to zero; and
3. the directed graph associated with M is strongly connected.

Then θ is a simple eigenvalue of M with real left row eigenvector $\mathbf{u}_0$ and, for $j \in \{1, \dots, n\}$,

$$\lim_{t \rightarrow \infty} \frac{1}{t} \xi_j(t) = \mathbf{u}_0^T F.$$

The proof of this lemma rests on the same ideas necessary to show that the law of an irreducible, finite state space, continuous-time random walk on a graph converges to its stationary distribution. The only distinction is the introduction of the forcing term F . For completeness, we include the proof of this lemma in the Appendix.

Proof (of Theorem 1): Note that for any given $i \neq j$, $X_i(t)X_j(t)$ is a component of $\mathbf{Y}(t)$, and the expected value of $\mathbf{Y}(t)$ obeys

$$\mathbb{E}[\dot{\mathbf{Y}}(t)] = M\mathbb{E}[\mathbf{Y}] + F. \quad (17)$$

Therefore, to obtain the result it suffices to prove that M has the three properties required by Lemma 1.

Inspection of M (defined component-wise in Equation 15) shows that each row of M will be either twice the sum of the row $\tilde{\mathbf{A}}_i$ for some i , or the sum of the entries in rows $\tilde{\mathbf{A}}_i$ and $\tilde{\mathbf{A}}_j$ for some $i \neq j$. Since the rows of $\tilde{\mathbf{A}}$ sum to zero, this implies the rows of M sum to zero. Similarly, the non-zero off-diagonal entries of M are sums of off-diagonal entries of $\tilde{\mathbf{A}}$, so they are non-negative.

It remains to argue that the directed graph associated with M is strongly connected. To do so, we first note that the directed graph associated with $\tilde{\mathbf{A}}$ is strongly connected. This is because the graph associated with $\mathbf{A}$ is connected and, for every i and j , if $a_{ij} > 0$, then $a_{ji} > 0$ (see the definition in Equation 11).

Let (i, j) and (k, ℓ) be two distinct vertices on the directed graph associated with M . Suppose that $i \neq k$. Since the graph associated with $\tilde{\mathbf{A}}$ is connected, there exists a path through nodes $i = \beta_1, \beta_2, \dots, \beta_s = k$ with positive edge values

$$i = \beta_1 \xrightarrow{\alpha_{\beta_1\beta_2}} \beta_2 \xrightarrow{\alpha_{\beta_2\beta_3}} \dots \xrightarrow{\alpha_{\beta_{s-1}\beta_s}} \beta_s = k.$$

If $\beta_w = j$ for any $w \in \{1, \dots, s\}$, then using Equation 15, there exists a path on the directed graph associated with M with positive edge values

$$(i, j) = (\beta_1, j) \xrightarrow{\alpha_{\beta_1\beta_2}} (\beta_2, \beta_3) \xrightarrow{\alpha_{\beta_2\beta_3}} \dots \xrightarrow{\alpha_{\beta_{s-1}\beta_s}} (\beta_s, j) = (k, j).$$

On the other hand, if $j = \beta_w$ for some w , then we have the path with positive edge values

$$(i, j) \xrightarrow{\alpha\beta_1\beta_2} (\beta_2, j) \xrightarrow{\alpha\beta_2\beta_3} \dots \xrightarrow{\alpha\beta_w-1\beta_w} (j, j) \xrightarrow{2\alpha\beta_w\beta_w+1} \dots \xrightarrow{\alpha\beta_s-1\beta_s} (k, j).$$

Thus when $i = k$, there exists a path between (i, j) and (k, j) . Analogously, a path may be found from (k, j) to (k, ℓ) when $j = \ell$. Hence, by connecting these two paths, there exists a path between (i, j) and (k, ℓ) .

Since M has all four necessary properties for Lemma 1, we can apply it to Equation 17 and the stated result follows. $\square$

The theorem can be readily applied to the one cargo, one motor system (7). For this system,

$$\Gamma = \begin{pmatrix} \frac{1}{\gamma_c} & 0 \\ 0 & \frac{1}{q'\gamma_d} \end{pmatrix}, \text{ and } A = \begin{pmatrix} -\kappa_d & \kappa_d \\ \kappa_d & -\kappa_d \end{pmatrix}.$$

The associated graph of A consists of two vertices connected with an edge with value κ_d . Thus the graph of A is connected. The diagonal entries of Γ are strictly positive. So, Theorem 1 applies.

To quickly obtain M and F , we calculate the second moments of System 7 using Itô's rule, finding

$$\begin{aligned} d(X_t^2) &= \left[-\frac{2\kappa_d}{\gamma_c} X_t^2 + \frac{2\kappa_d}{\gamma_c} X_t Z_t + \frac{2k_B T}{\gamma_c} \right] dt + 2X_t \sqrt{\frac{2k_B T}{\gamma_c}} dW(t) \\ d(X_t Z_t) &= \left[\frac{\kappa_d}{q'\gamma_d} X_t^2 - \left(\frac{\kappa_d}{\gamma_c} + \frac{\kappa_d}{q'\gamma_d} \right) X_t Z_t + \frac{\kappa_d}{\gamma_c} Z_t^2 \right] dt \\ &\quad + Z_t \sqrt{\frac{2k_B T}{\gamma_c}} dW(t) + X_t \sqrt{\frac{2k_B T q''}{(q')^2 \gamma_d}} dW_d(t) \\ d(Z_t^2) &= \left[\frac{2\kappa_d}{q'\gamma_d} X_t Z_t - \frac{2\kappa_d}{q'\gamma_d} Z_t^2 + \frac{2k_B T q''}{(q')^2 \gamma_d} \right] dt + 2Z_t \sqrt{\frac{2k_B T q''}{(q')^2 \gamma_d}} dW_d(t). \end{aligned} \quad (18)$$

Hence,

$$M = \begin{pmatrix} -\frac{2\kappa_d}{\gamma_c} & \frac{2\kappa_d}{\gamma_c} & 0 \\ \frac{\kappa_d}{q'\gamma_d} & -\left(\frac{\kappa_d}{\gamma_c} + \frac{\kappa_d}{q'\gamma_d} \right) & \frac{\kappa_d}{\gamma_c} \\ 0 & \frac{2\kappa_d}{q'\gamma_d} & -\frac{2\kappa_d}{q'\gamma_d} \end{pmatrix}, \quad F = \begin{pmatrix} \frac{2k_B T}{\gamma_c} \\ 0 \\ \frac{2k_B T q''}{(q')^2 \gamma_d} \end{pmatrix}.$$

The left row eigenvector associated with the eigenvalue zero is:

$$\mathbf{u}_0 = \begin{pmatrix} \gamma_c^2 & 2q'\gamma_d\gamma_c & (q')^2\gamma_d^2 \\ (q'\gamma_d + \gamma_c)^2 & (q'\gamma_d + \gamma_c)^2 & (q'\gamma_d + \gamma_c)^2 \end{pmatrix}.$$

So, we have

$$\lim_{t \rightarrow \infty} \frac{1}{t} \mathbb{E}[X_t^2] = \mathbf{u}_0 F = 2k_B T \frac{q''\gamma_d + \gamma_c}{(q'\gamma_d + \gamma_c)^2},$$

justifying Equation 8.

4 Motor Procession Inhibited by Electrostatic Drag

4.1 Force and Velocity of Molecular Motor Stepping

Thus far we have used a Langevin framework to describe the balance of forces that influence the motion of the cargo and anchor, but this type of model is not appropriate when considering motor stepping. Generally speaking, modelers use a phenomenological and empirically informed *force-velocity relationship* summarizing the effect that opposing force has on the mechano-chemical processes that constitute motor stepping. Similar to McKinley et al (2012), we will use an SDE approximation for the motor position along the microtubule:

$$dY(t) = \nu_m \left(1 - \frac{f(t)}{F_m^*} \right) dt + \sigma_m(f(t)) dW(t). \quad (19)$$

Here, $f(t)$ represents the instantaneous opposing force felt by the motor at time t . The form of the drift term arises from assuming that the force-velocity relationship is linear, with an average velocity of ν_m when there is no opposing force, and zero average velocity when the opposing force is the *stall force* F_m^* . We note that experimental evidence suggests that this force-velocity relationship is ATP concentration dependent, and roughly linear at low to medium loads (Svoboda et al, 1993; Kojima et al, 1997; Visscher et al, 1999; Carter and Cross, 2005; Toba et al, 2006; Kunwar et al, 2008). The effects of nonlinearity are likely to be more significant for loads at near stall-force, but the dynamics presented here are generally near zero load.

The coefficient of the noise, σ_m , arises from the use of an SDE to approximate a discrete state-space Markov chain. Indeed, the drift term itself is, in principle, the difference of a forward stepping rate $\lambda(f)$ and a backward stepping rate $\mu(f)$. The corresponding state-dependent diffusivity would be $\sqrt{\lambda(f) + \mu(f)}$. (See Kurtz, Thomas J. (1981), for example, for a rigorous discussion of the approximation of continuous-time discrete state space Markov chains by SDEs.) There is not a unique way to write a force-diffusivity curve that

corresponds to the drift term shown in (19). However, in the analysis that follows we only compute the mean of this system. As a result, we do not need an explicit form for the force-diffusivity relationship, and we will write it simply as $\sigma_m(X(t), Y(t))$.

4.2 Dynein Motility Inhibited by Electrostatic Drag of Dynactin

The exact physical arrangement of dynein and dynactin with respect to the cargo and the microtubule is still relatively uncertain. It is known that dynactin typically binds directly to the cargo at its so-called *pointed end* while dynein and dynactin bind together through the common use of a coiled coil protein BICD2 (Urnavicius et al, 2015). Urnavicius et al (2015) describe dynactin as having a *shoulder/sidearm projection*, comprised primarily of p150^{Glued} which was shown by Waterman-Storer et al (1995) and later by Culver-Hanlon et al (2006) to be the subunit that binds to microtubules. Relating this to our previous model discussion, we consider the shoulder/sidearm complex to be the anchor. Urnavicius et al (2015) reported that the length of the shoulder/sidearm complex is about 40 nm and the length of the pointed end of dynactin is about 20 nm. The length of dynein is on the order of 45 nm (Burgess et al, 2003). In Figure 1d we illustrate the relative positions of dynein, dynactin and the cargo in the model that follows.

Because it simplifies the analysis somewhat without significantly affecting the results, we simplify the geometry for the cargo-motor-anchor complex to consider the situation where dynein and dynactin attach to the cargo through separate tethers at the same point, as in Figure 1b. (This is as opposed to the two sharing a branch point as seen in Figure 1d.) Using (2), (7), and (19) we model this system as

$$\begin{aligned} dX(t) &= -\frac{\kappa_d}{\gamma_c}(X(t) - Z(t)) + \frac{\kappa_m}{\gamma_c}(Y(t) - X(t))dt + \sqrt{\frac{2k_B T}{\gamma_c}}dW_c(t) \quad (20) \\ dY(t) &= \nu_d(1 - \frac{\kappa_m(Y(t) - X(t))}{F_m^*})dt + \sigma_m(X(t), Y(t))dW_m(t) \\ dZ(t) &= \frac{\kappa_d}{q'\gamma_d}(X(t) - Z(t))dt + \sqrt{\frac{2k_B T q''}{(q')^2 \gamma_d}}dW_d(t). \end{aligned}$$

Let $\sigma_c = \sqrt{2k_B T \gamma_c}$ and $\sigma_a = \sqrt{2k_B T q'' \gamma_d}$. In general, for m identical motors, Y_i , and n identical anchors, Z_j , we have the system

$$\begin{aligned} dX(t) &= \left(\sum_{i=1}^m \frac{\kappa_m}{\gamma_c}(Y_i(t) - X(t)) - \sum_{j=1}^n \frac{\kappa_a}{\gamma_c}(X(t) - Z_j(t)) \right)dt + \frac{\sigma_c}{\gamma_c}dW_X(t) \quad (21) \\ dY_i(t) &= \left(\nu_d - \frac{\nu_d \kappa_m}{F_m^*}(Y_i(t) - X(t)) \right)dt + \sigma_m(X(t), Y_i(t))dW_{Y_i}(t) \\ dZ_j(t) &= \frac{\kappa_a}{q'\gamma_a}(X(t) - Z_j(t))dt + \frac{\sigma_a}{q'\gamma_a}dW_{Z_j}(t). \end{aligned}$$

The following theorem establishes the mean asymptotic velocity for this general system.

Theorem 2—Suppose a cargo $X(t)$ is attached to n identical anchors, $n \geq 0$, and m identical motors, $m \geq 1$, as described by System 21. Then,

$$\lim_{t \rightarrow \infty} \frac{\mathbb{E}[X(t)]}{t} = \frac{\nu_m}{1 + \frac{\nu_d q' \gamma_a n}{F_m^* m} + \frac{\nu_d \gamma_c}{F_m^* m}}.$$

Proof: We first handle the case when $n \geq 1$. Let $\tilde{Y}(t) = \frac{1}{m} \sum_{i=1}^m Y_i(t)$ and $\tilde{Z}(t) = \frac{1}{n} \sum_{i=1}^n Z_i(t)$. Rewrite System 21 as

$$dX(t) = \left(\frac{m\kappa}{\gamma_c} (\tilde{Y}(t) - X(t)) - \frac{n\kappa}{\gamma_c} (X(t) - \tilde{Z}(t)) \right) dt + \frac{\sigma_c}{\gamma_c} dW_X(t)$$

$$d\tilde{Y}(t) = \left(\nu_d - \frac{\nu_d \kappa m}{F_m^*} (\tilde{Y}(t) - X(t)) \right) dt + \frac{1}{m} \sum_{i=1}^m \sigma_m(X(t), Y_i(t)) dW_{Y_i}(t)$$

$$d\tilde{Z}(t) = \frac{\kappa_a}{q' \gamma_a} (X(t) - \tilde{Z}(t)) dt + \frac{1}{n} \sum_{j=1}^n \frac{\sigma_a}{q' \gamma_a} dW_{Z_j}(t).$$

Let $V(t) = (\mathbb{E}[X(t)], \mathbb{E}[\tilde{Y}(t)], \mathbb{E}[\tilde{Z}(t)])^\top$ and $F = (0, \nu_d, 0)^\top$. For clarity of calculation, let

$$a = \frac{m\kappa}{\gamma_c}, \quad b = \frac{n\kappa}{\gamma_c}, \quad c = \frac{\nu_d \kappa m}{F_m^*}, \quad d = \frac{\kappa_a}{q' \gamma_a}.$$

Then, $V(t)$ satisfies

$$\dot{V}(t) = MV(t) + F,$$

where

$$M = \begin{pmatrix} -(a+b) & a & b \\ c & -c & 0 \\ d & 0 & -d \end{pmatrix}.$$

Note that M has right eigenvector $\mathbf{v}_0 = (1, 1, 1)^\top$ and left row eigenvector

$$\mathbf{u}_0 = \left(\frac{cd}{ad+bc+cd}, \frac{ad}{ad+bc+cd}, \frac{bc}{ad+bc+cd} \right).$$

Further, inspection of M shows that it has all necessary properties for Lemma 1 to apply. Thus,

$$\lim_{t \rightarrow \infty} \frac{\mathbb{E}[X(t)]}{t} = \frac{\nu_d ad}{ad+bc+cd} = \frac{\nu_d}{1 + \frac{\nu_d q' \gamma_a^n}{F_m^* m} + \frac{\nu_d \gamma_c}{F_m^* m}}.$$

When $n = 0$, define $\tilde{Y}$ as above and let $V(t) = (\mathbb{E}[X(t)], \mathbb{E}[\tilde{Y}(t)])^\top$ and $F = (0, \nu_d)^\top$. Let a and c be defined as above. Then $V(t)$ will satisfy $V(t) = MV(t) + F$ where

$$M = \begin{pmatrix} -a & a \\ c & -c \end{pmatrix}.$$

We note that M satisfies all conditions necessary for Lemma 1 and has left row eigenvector

$$\mathbf{u}_0 = \left(\frac{c}{a+c}, \frac{a}{a+c} \right).$$

Thus,

$$\lim_{t \rightarrow \infty} \frac{\mathbb{E}[X(t)]}{t} = \frac{\nu_d a}{a+c} = \frac{\nu_d}{1 + \frac{\nu_d \gamma_c}{F_m^* m}}.$$

This is consistent with the previous calculation when $n = 0$. $\square$

Applying Theorem 2 to system (20), we have for a single dynactin anchor and a single dynein motor,

$$\lim_{t \rightarrow \infty} \frac{E[X(t)]}{t} = \frac{\nu_d}{1 + \frac{\nu_d q' \gamma_d}{F_d^*} + \frac{\nu_d \gamma_d}{F_d^*}}. \quad (22)$$

Mallik et al (2004) and Gross et al (2000) found that a single cytoplasmic dynein motor without dynactin has zero mean velocity when the load or opposing force is about 1.1 ± 0.04 pN. We use this as our estimate for the stall force of dynein. Culver-Hanlon et al (2006) found that under the influence of only dynein, motor-cargo complexes have an average velocity of $0.27 \pm 0.11 \mu\text{m/s}$. We will denote this value by θ . Further, they found that under the influence of both dynein and dynactin, cargo have an average velocity of $0.24 \pm 0.09 \mu\text{m/s}$

(Culver-Hanlon et al, 2006). We will denote this value by θ' . (The benefit of dynactin is that the average run length is tripled.) We note that θ' is the quantity on the right-hand side of (22). On the other hand, θ takes its value from the reduced system

$$\begin{aligned} dX(t) &= \frac{\kappa_m}{\gamma_c}(Y(t) - X(t))dt + \sqrt{\frac{2k_B T}{\gamma_c}}dW_c(t) \\ dY(t) &= \nu_d \left(1 - \frac{\kappa_m(Y(t) - X(t))}{F_m^*} \right) dt + \sigma_m dW_m(t). \end{aligned} \quad (23)$$

Theorem 2 applies to the system (23) with $m = 1$ and $n = 0$. Hence

$$\theta = \lim_{t \rightarrow \infty} \frac{E[X(t)]}{t} = \frac{\nu_d}{1 + \frac{\gamma_c \nu_d}{F_d^*}} \quad (24)$$

From (24), we can use the values $\gamma_c = 0.16$ s/ μ m and $F_d^* = 1.1$ pN s/ μ m to estimate that $\nu_d \approx 0.32$ μ m/s. Notably, this value does not contribute to our estimation of the electrostatic drag parameters. Instead, using (22) and (24), we find that

$$\frac{1}{\theta'} - \frac{1}{\theta} = \frac{q' \gamma_d}{F_d^*}.$$

If we use the mean values $\theta = 0.27$ μ m/s, $\theta' = 0.24$ μ m/s, we have the estimate

$$q' \gamma_d \approx 0.51 \frac{\text{pN s}}{\mu\text{m}}.$$

This value is more than three times the coefficient for fluid drag on the cargo, but smaller than it would be if Fluctuation-Dissipation holds (see the end of Section 3 for comparison).

Returning to (9) we can estimate for $q'' \gamma_d$

$$q'' \gamma_d \approx 3.03 \text{ pNs}/\mu\text{m}, \quad (25)$$

which implies

$$\frac{q''}{q'} \approx \frac{1}{17} \quad (26)$$

If this estimate for the ratio of q' and q'' is accurate, then this is strong evidence against the idea that Fluctuation-Dissipation holds when we include electrostatic drag in our model. However, it is important to note that the data for motor velocity is quite noisy. In fact, the p -value reported by Culver-Hanlon for rejecting the null hypothesis (that the velocity of dynein with and without dynactin is the same) is 0.18. That is to say, the difference between 0.27 and 0.24 is not statistically significant given the number and noisiness of the observations. This does not imply that the effect is not real. The reported results are exactly what we would expect to see if there truly is a 10% drop in velocity. This is simply a comment that more data is needed to confirm the phenomenon and, unfortunately, we know of no better estimates from experiments of this kind at this time.

In order to qualitatively validate our parameter estimation, we simulated 50 cargo paths, displayed in Figure 2d. For the ratio $q''/q' = 1/17$, we set $q'' = 1$ and $q' = 17$. We note that the small-time and large-time dynamics agree, however the middle portion of the results from Culver-Hanlon et al (2006) seems to have a flatter average pathwise MSD. While our model can capture dynamics in the $t \rightarrow 0$ and $t \rightarrow \infty$ regions, it is difficult to model the middle portion as we do not have enough data. It may be possible to understand the dynamics in this region with path data, but the current pathwise MSD data set is insufficient. Ultimately, however, we are interested in quantifying the long-term tradeoff between velocity loss and processivity gain. Hence only the long-term dynamics will be of consequence in this analysis.

4.3 Understanding departures from Fluctuation-Dissipation

The approaches thus far have demonstrated the ability to gain information on electrostatic drag through two dynamics: knockdown in diffusivity of cargo when attached to an anchor, and knockdown in asymptotic velocity when a motor/cargo complex is attached to an anchor. For the data from Culver-Hanlon et al (2006), a 93.2% knockdown in diffusivity occurs when cargo are attached to dynactin, and an 11.1% knockdown in velocity is observed when cargo and dynein is attached to dynactin. In this section, we seek to provide context for pairing these two knockdown values assuming that Fluctuation-Dissipation for anchor diffusion along the microtubule holds. Ultimately, we find that the data suggest the knockdown in velocity is significantly different than what would be expected if Fluctuation-Dissipation was assumed.

To that end, let us assume $q'' = q'$. Then, the diffusivity of free cargo is given by

$$D_{\text{free}} = \frac{k_B T}{\gamma_c}, \quad (27)$$

and the diffusivity of cargo subject to an anchor is given by

$$D_{\text{anch}} = \frac{k_B T}{\gamma_c + \gamma' q_a}. \quad (28)$$

Define the *fractional knockdown in velocity*, p_v , as

$$p_v = 1 - \frac{\theta'}{\theta},$$

and the *fractional knockdown in diffusivity*, p_d as

$$p_d = 1 - \frac{D_{\text{anch}}}{D_{\text{free}}}.$$

Using Equations 22, 24, 27, and 28, p_v can be expressed as

$$p_v = \frac{p_d \chi}{1 - (1 - \chi)p_d}, \quad (29)$$

where $\chi = \gamma_c \theta / F_m^*$ is a dimensionless parameter. Values of $\chi < 1$ form a sub-linear curve in the p_d - p_v plane as exhibited in Figure 3.

Recall that for the Culver-Hanlon et al (2006) data, $\gamma_c = 0.16 \text{ pN s}/\mu\text{m}$, and for dynein in this context, $\theta = 0.27 \mu\text{m/s}$, and $F_d^* = 1.1 \text{ pN}/\mu\text{m}$. Hence, $\chi = 0.04$. Using the initial and asymptotic slopes of Figure 2b, we can calculate $p_d = 0.93$. Then, Equation 29 implies that $p_v = 0.347$. However, using the observed values of $\theta = 0.27 \mu\text{m/s}$ and $\theta' = 0.24 \mu\text{m/s}$, we directly calculate $p_v = 0.111$. Note that this observed value is noticeably less than what is expected when Fluctuation-Dissipation is assumed.

Thus far, we have focused our efforts only on a single dynactin based MSD data set. However, the effect of dynactin on kinesin-1, kinesin-2, and dynein bound cargo has been experimentally examined by King and Schroer (2000), Berezuk and Schroer (2007), and Kardon et al (2009) to name a few. These labs report the knockdown in velocity and increase in run length, but do not provide any data on the diffusivity of cargo. By providing the diffusional data and MSD plots, Culver-Hanlon et al (2006) have provided a way to relate and analyze the effects of drag due to dynactin.

While dynactin can function as a skating anchor for purposes of tethering, it is not the only structure that can do so. Any non-force generating microtubule associated protein that can bind to cargo and diffuse along the length of the microtubule has the potential to create drag terms and affect the diffusivity of the cargo. In particular, Ali et al (2008) observed that myosin V is diffusive on microtubules and increases processivity of kinesin-1 driven cargo. They do not directly report free diffusive data for their $0.0075 \mu\text{m}$ radius Qdots, however, with the information provided it is possible to estimate γ_c by way of the Stokes-Einstein drag law. Assuming that the viscosity of the buffer is similar to Culver-Hanlon et al (2006), then the viscosity is about 100 times greater than water. This yields an estimate $\gamma_c \approx 0.014 \text{ pNs}/\mu\text{m}$. This allows us to estimate the diffusivity of free Qdots to be $0.5857 \mu\text{m}^2/\text{s}$. Ali et al (2008) report that the diffusivity of the Qdots along the microtubule when attached to a

single myosin V is $0.18 \mu\text{m}^2/\text{s}$. Using these diffusivities, we calculate $p_d = 0.69$. Ali et al (2008) do not provide the stall force of their kinesin-1 motors, but we instead can use the widely accepted value $F_k^* = 7.0 \text{ pN}$ (Visscher et al, 1999). Further, they also report that the average velocity of their Qdots when attached to a single kinesin-1 motor is approximately $\theta = 0.88 \mu\text{m}/\text{s}$. It follows that $\chi = 0.0176$. Using Equation 29, we see that the “null model” predicts that the associated velocity knockdown would be $p_v = 0.0039$. However, Ali et al (2008) report that the average velocity of Qdots attached to a processing kinesin-1 motor and with possible sliding myosin V motors attached is $\theta' = 0.84 \mu\text{m}/\text{s}$. This knockdown, $p_v = 0.05$, is an order of magnitude larger than the predicted value.

We would like to emphasize that the estimates provided here are based on relatively few paths from experiments that were not designed to directly measure these effects, and with parameters that are gathered from different experimental set-ups. Rather than speculate on why the observed behavior is distinct from the predictions of the null model, we suggest that this can be a framework for future inquiries and a guide for determining whether nonlinear effects might be in play.

4.4 Predicting the Impact of Electrostatic Drag on Kinesin-1 Motility

The model proposed in Section 4.2 may not change substantially if the processive motor is *from the kinesin family* instead of dynein. Because kinesin is a separate motor, it should have a separate contact point with the cargo, as depicted in Figure 1c. Kinesin motors will have different spring constants and stall forces, but this does not alter the system of SDEs (20) and the formula for the asymptotic velocity is the same as (22) and (24).

To fill in estimated parameter values, we consulted the literature for kinesin-1. For example, Visscher et al (1999) reported the stall force of kinesin-1 to be about 7.0 pN , and also reported the unburdened velocity of kinesin-1 is about $0.81 \mu\text{m}/\text{s}$. This latter estimate was verified by experiments conducted by Carter and Cross (2005) and was used in simulations performed by Kunwar et al (2008). With these parameter choices, along with our choice for γ_c , we can use (24) to predict the asymptotic velocity of cargo when being transported by a solitary kinesin-1 motor to be about $0.80 \mu\text{m}/\text{s}$. Using (24) and our estimate for $q'\gamma_d$ yields a predicted asymptotic velocity of $0.75 \mu\text{m}/\text{s}$. This is a reduction of about 6%.

This reduction in cargo velocity attached to kinesin-1 is less than the $\sim 11\%$ reduction in cargo velocity attached to dynein reported by Culver-Hanlon et al (2006) and similar to the knockdown observed by Ali et al (2008) (with a myosin V anchor). This is consistent with model prediction: increased stall force implies that a motor is less sensitive to various forms of drag. To demonstrate this, we consider the fractional knockdown in velocity, p_v , as a function of the motor stall force F_m^* :

$$p_v(F_m^*) = \frac{q'\gamma_d\nu_m}{F_m^* + q'\gamma_d\nu_m + \gamma_c\nu_m}. \quad (30)$$

The knockdown factor is inversely proportional to both the motor stall force F_m^* and the cargo drag coefficient γ_c . As expected, for larger particles or a more viscous environment, the cargo drag dominates the effect of electrostatic drag. When electrostatic drag is dominant, larger stall force motors will be more resilient. This is showcased in Figure 4a. We display two distinct curves, one for dynein and one for kinesin-1 because Equation 30 is also dependent on the unburdened velocity of the motor. The curves are marked in the appropriate places for the stall force values of 7 pN and 1.1 pN observed for kinesin-1 and dynein, respectively. We note that different values for the stall forces have been observed in vivo. For example Shubeita et al (2008) found that kinesin-1 and dynein exhibit stall forces of 2.5 pN each. In a more recent investigation, Jun et al (2014) demonstrated there is considerable variation among in vivo measurement techniques and gave a final estimate of the kinesin-1 stall force to be closer to 5 pN. They did not investigate dynein. Assuming this value for dynein and either of these two estimates for kinesin-1 in vivo, Figure 4a predicts that the percent loss in velocity for kinesin-1 will slightly exceed the percent loss in velocity for dynein. This arises from the fact that the kinesin curve lies above the dynein curve and that the difference in stall force of 2.5 pN and 2.5 or 5 pN is closer than the distance of 7 pN and 1.1 pN.

Before moving on, we should note a potentially countervailing experimental observation by Berezuk and Schroer (2007). In that work, the authors reported essentially no velocity knockdown in the transport of 200nm beads by kinesin-1, kinesin-2, and dynein in the presence of dynactin. As noted above, such differences can easily happen if the fluid environments have different viscosities. For kinesin-1, an $100\times$ increase in cargo drag will reduce the knockdown factor to 2 %.

Up to this point, we have considered force-dependent models to derive results about the average velocity of motor-cargo-anchor systems. Whether or not the predicted reduction in asymptotic velocity is “worth it” for the motor-cargo complex depends on how much more rapidly a kinesin-1 motor rebinds when it is tethered to the microtubule by dynein-dynactin. To assess the trade-off in the simplest possible situation, we calculate the average velocity for a complex consisting of one kinesin-1 motor and one dynein-dynactin complex, where *the dynein motor in non-functionalized*, that are simultaneously bound to a cargo while reversibly binding to and unbinding from a microtubule of infinite length. We suppose that the system has four states, each with an associated mean-velocity,

00: Free Diffusion (Mean velocity 0 $\mu\text{m/s}$);

01 : Dynactin bound, but not kinesin-1 (Mean velocity 0 $\mu\text{m/s}$);

10 : Kinesin-1 bound, but not dynein (Mean velocity $\theta_k \mu\text{m/s}$);

11 : Both kinesin-1 and dynactin bound (Mean velocity $(1 - \phi)\theta_k \mu\text{m/s}$),

where ϕ represents the factor of velocity loss, and θ_k is the asymptotic velocity of cargo attached to a single kinesin-1 motor. For emphasis, we note again that we are mimicking the experimental set up of Culver-Hanlon et al (2006) and assuming dynein is non-functionalized. In this way, the main feature is that dynactin functions as a diffusive anchor.

It follows that the mean velocity of the “01” state is zero and the knockdown in velocity in the “11” state is entirely due to electrostatic drag. The transition rate matrix is given by

$$Q = \begin{pmatrix} -(d_{\text{on}} + k_{\text{on}}) & d_{\text{on}} & k_{\text{on}} & 0 \\ d_{\text{off}} & -(d_{\text{off}} + \rho k_{\text{on}}) & 0 & \rho k_{\text{on}} \\ k_{\text{off}} & 0 & -(k_{\text{off}} + \rho d_{\text{on}}) & \rho d_{\text{on}} \\ 0 & k_{\text{off}} & d_{\text{off}} & -(k_{\text{off}} + d_{\text{off}}) \end{pmatrix}$$

where ρ is the factor by which kinesin-1 binding is enhanced when tethered to the microtubule by dynein-dynactin.

By the ergodic theorem for Markov chains, we can compute the asymptotic velocity of the system by first finding the stationary distribution of the state of the complex, multiplying the time spent in each state by its associated mean velocity and summing. The binding and unbinding rates for kinesin and dynein are not generally agreed upon. In particular, binding rates are usually reported in the units of $\text{s}^{-1}[\text{M}]^{-1}$ where $[\text{M}]$ is the concentration of the binding substrate - in this case, the concentration of microtubule binding sites. However, this concentration is almost never reported and it is therefore very difficult to compare across experimental setups. As a starting point for our parametrization, we used the values presented by Müller et al (2008) as a benchmark. Müller et al (2008) were studying a tug-of-war among motors that regularly bind and unbind while being held close to the microtubule. Viewing these held motors as being pinned, their values would be considered *rebinding* rates in our context Hancock (2018). Assuming that the rebinding rate is greater than initial binding rates, and for lack of a clear initial binding rate in the literature, we chose the lower binding rate of 1 s^{-1} for both motors. We adopt their value for k_{off} , but modify the value of d_{off} based on more recent studies of dynein’s behavior in the presence of dynactin. Namely, from a recent study by Ayloo et al (2014), the average run length of dynein-dynactin is roughly $2.5 \mu\text{m}$, and the average velocity is $0.5 \mu\text{m/s}$. We can then calculate the value to be closer to 0.2 s^{-1} . This is similar to the rates used by Bouzat (2016).

Using these attachment rates, which we record in Table 2, we iterated through values of $\phi \in (0, 0.2)$, and calculated the asymptotic velocity for all values of $\rho \in (0, 2)$ of the four-state system. We then compared these velocities to the asymptotic velocity of the two-state system with kinesin-1 alone, governed by the transition matrix

$$Q' = \begin{pmatrix} -k_{\text{on}} & k_{\text{on}} \\ k_{\text{off}} & -k_{\text{off}} \end{pmatrix}.$$

We then plotted the first value of ρ that exceeded the two-state asymptotic velocity, resulting in the curve displayed in Figure 4b. All (ϕ, ρ) combinations that lie above the curve have an

asymptotic velocity larger than what would be the case if there were no effect due to tethering. As expected, the threshold for whether the tradeoff is productive increases with knockdown in velocity. We chose to illustrate the graph for $\phi \in (0, 0.2)$ since this region contains the loss in velocity for both dynein and kinesin. Feng et al (2018) recently estimated that the rebinding factors for kinesin-1 and kinesin-2 are very close to one, while that value remains unknown for dynein. The aforementioned velocity loss factors for kinesin-1 (6%) and dynein (11%), both of which are close to the values observed by Ali et al (2008) (for a myosin V anchor) and Culver-Hanlon et al (2006), respectively, means that these systems are very close to the counterproductive region.

5 Discussion

Experimentalists and theoreticians have repeatedly observed that there can be complex and unexpected interactions among molecular motors that are simultaneously attached to a single intracellular cargo. Among these observations is that the presence of motors that tend to process in one direction can enhance the performance of motors that process in the opposite direction (Hancock, 2014). There are several possible mechanisms that could explain this “co-dependence among antagonistic motors.” In this work we have developed and analyzed a mathematical model for one such mechanism: *microtubule tethering*.

At the core of the microtubule tethering hypothesis is the idea that there is an intermediate, weakly-bound state for certain molecular motors in which a motor stays close to a microtubule, but is not tightly bound to one location. Motors and other particles in such a state have been observed to “skate” along the length of a microtubule as a one-dimensional diffusion, but with a diffusivity much less than what it would be if the particle were free. We conjecture that this loss of mobility is due to “electrostatic drag” and develop a system of SDEs that emulate this behavior. Using experimental data, we parameterized our model and observed that the electrostatic drag force is comparable to other forces that are commonly included in motor-cargo models. In fact, under the most basic set of assumptions, we find the electrostatic drag coefficient to be ten times larger than the fluid drag coefficient for the cargo (see discussion surrounding Equation 9).

In order to perform an exact mathematical analysis, we assumed linear forces throughout this work. However, since the forces we describe in this experimental setting are all “small,” we believe that our main results should hold if one employs, for example, a nonlinear spring model or a nonlinear force-velocity curve for processing motors. Such modifications would, however, require a computational approach to analyze and parameterize the system. Moreover, because the available data is strictly in the form of an MSD curve, it is not possible at present to establish whether these nonlinear models are necessary to capture essential behavior. While it is inevitable that more refined particle trajectory data will become available in the future, we note that using such data to improve parameter estimation or investigate model comparison will be quite challenging.

We also stress that although our model produces behavior similar to what is observed, we have not (and cannot at the moment) eliminate the possibility that alternative hypotheses might explain the behavior described here. For example, we have not considered the role of

steric hindrances, the possible impact of reducing cargo motion to one dimension, or the fact that these dynamics take place near a “wall,” i.e. the glass plate that serves as a base for the experiments. (See Korn et al (2009) for an example of a physical model incorporating the effects of a wall on cargo.)

As an explicit part of our analysis, we investigated whether it is appropriate to assume the Fluctuation-Dissipation relationship for weakly bound, skating motors diffusing along a microtubule. We found that the existing data does not support the Fluctuation-Dissipation hypothesis, but because relevant data is so scarce, we do not consider the issue closed. To provide a framework for future investigations, we developed an analysis that uses Fluctuation-Dissipation as something like a “null hypothesis” that experimental observations can be compared against.

Placing these caveats aside, it is natural to extrapolate the skating diffusion findings to predictions among multiple motors. To this end, we have generalized our asymptotic velocity and mean-squared displacement results to situations involving multiple identical motors and identical anchors. In doing so, we have demonstrated that it is indeed possible for electrostatic drag to have significant impact on MSD of cargo attached to skating motors (see Theorem 1), but nevertheless have minimal impact when being dragged by a processing motor that is attached to the same cargo (see Theorem 2). This is important because it lends support to the idea that a weakly bound motor can act as a tether - serving to “rescue” the processing motor when it detaches - without meaningfully diminishing the average velocity of the system.

Acknowledgments

The authors would like to thank Will Hancock, John Fricks, Pete Kramer, Veronica Ciocanel, and Erkan Tüzel for their thoughtful and very informative feedback in the development of this work.

This work was supported by NIH Grant Number R01GM122082-01.

Appendix

A Solution for Pinned Cargo System

The SDE used for pinned diffusion is the Ornstein-Uhlenbeck equation with non-zero mean. For notational efficiency, we write it as follows

$$dX(t) = -\alpha(X(t) - \mu)dt + \sigma dW(t), \quad (31)$$

with $X(0) = x$. Because this SDE is linear, with additive noise, we can write the solution in convolution form using Duhamel’s formula

$$\begin{aligned} X(t) &= e^{-\alpha t}x + \int_0^t e^{-\alpha(t-u)}(-\alpha\mu)du + \int_0^t e^{-\alpha(t-u)}\sigma dW(u) \\ &= e^{-\alpha t}x + (1 - e^{-\alpha t})\mu + \sigma \int_0^t e^{-\alpha(t-u)}dW(u). \end{aligned}$$

Allowing for the possibility that the initial condition x and the center μ can be random variables, two quantities of interest follow immediately:

$$\mathbb{E}[X(t)] = e^{-\alpha t} \mathbb{E}[x] + (1 - e^{-\alpha t}) \mathbb{E}[\mu] \quad (32)$$

$$\mathbb{E}[X(t)\mu] = e^{-\alpha t} \mathbb{E}[x\mu] + (1 - e^{-\alpha t}) \mathbb{E}[\mu^2]. \quad (33)$$

To compute the MSD of $X(t)$, we apply Itô's formula to (31) using the function $f(x) = x^2$ to find that

$$dX^2(t) = -2\alpha X(t)(X(t) - \mu) + \sigma^2 dt + 2\sigma X(t) dW(t).$$

It follows that

$$\frac{d}{dt} \mathbb{E}[X^2(t)] = -2\alpha \mathbb{E}[X^2(t)] + 2\alpha \mathbb{E}[X(t)\mu] + \sigma^2,$$

which, again by Duhamel's formula has the solution

$$\begin{aligned} \mathbb{E}[X^2(t)] &= e^{-2\alpha t} \mathbb{E}[x^2] + \int_0^t e^{-2\alpha(t-u)} (2\alpha \mathbb{E}[X(u)\mu] + \sigma^2) du \\ &= e^{-2\alpha t} \mathbb{E}[x^2] + 2\alpha \int_0^t e^{-2\alpha(t-u)} \mathbb{E}[X(u)\mu] du + (1 - e^{-2\alpha t}) \frac{\sigma^2}{2\alpha}. \end{aligned}$$

Substituting in 33, the second term on the right-hand side evaluates to

$$2\alpha \int_0^t e^{-2\alpha(t-u)} \mathbb{E}[X(u)\mu] du = 2e^{-\alpha t} (1 - e^{-2\alpha t}) \mathbb{E}[x\mu] + (1 - e^{-\alpha t})^2 \mathbb{E}[\mu^2]. \quad (34)$$

We conclude that

$$\mathbb{E}[X^2(t)] = \mathbb{E}\left[e^{-\alpha t} x + (1 - e^{-\alpha t}) \mu\right]^2 + (1 - e^{-2\alpha t}) \frac{\alpha^2}{2\alpha}. \quad (35)$$

Expressing this in terms of the parameters used in the main text

$$\alpha = \frac{\kappa}{\gamma}, \sigma = \sqrt{\frac{2\kappa_B T}{\gamma}}, x = 0, \mu \sim N(0, \frac{\kappa_B T}{\kappa}),$$

we have

$$\mathbb{E}[X^2(t)] = 2(1 - e^{-\kappa t/\gamma}) \frac{k_B T}{\kappa}. \quad (36)$$

B Proof omitted from the main text

Proof (of Lemma 1)

Since the rows of M sum to zero, $\lambda_0 = 0$ is an eigenvalue of M with right eigenvector $\mathbf{v}_0 = (1, 1, \dots, 1)^\top$. Let $\mathbf{u}_0$ be the left eigenvector associated with 0 for M .

Since the diagonal of M is negative, there exists some positive real number r such that $M' = M + r\mathbb{I}$ is a non-negative matrix. Note that $\lambda, \mathbf{v}_\lambda$ are an eigenvalue-eigenvector pair for M if and only if $\lambda + r$ and $\mathbf{v}_\lambda$ are an eigenvalue-eigenvector pair for M' .

Now we show that M' is irreducible. M' differs from M only on the diagonal. Therefore, the associated directed graph of M' will have the same edge values between distinct vertices as that of M . Since the associated graph for M is strongly connected, the one for M' is strongly connected as well and consequently M' is irreducible.

Since M' is a non-negative, irreducible matrix, the Perron-Frobenius theorem holds (Berman and Plemmons, 1994). Thus, there exists a simple real eigenvalue $\lambda_p > 0$ for M' such that the spectral radius of M' is λ_p , all other eigenvalues of M' must have real part strictly less than λ_p , and the only eigenvectors whose components are all real and positive are those eigenvectors associated with λ_p . Note that

$$M'\mathbf{v}_0 = M\mathbf{v}_0 + r\mathbf{v}_0 = r\mathbf{v}_0.$$

Ergo, since $\mathbf{v}_0$ is a eigenvector whose components are all real and positive, we must have that $\lambda_p = r$. Further,

$$\mathbf{u}_0 M' = \mathbf{u}_0 M + \mathbf{u}_0 r = \mathbf{u}_0 r.$$

Since $\mathbf{u}_0$ is an eigenvector associated with $\lambda_p = r$, its components are all real and positive.

As r is a simple eigenvalue for M' and all other eigenvalues of M' have real part strictly less than r , it now follows that 0 is a simple eigenvalue for M and all other eigenvalues of M have real part strictly less than 0.

Write the remaining eigenvalues of M as

$$0 = \lambda_0 < |\lambda_1| \leq |\lambda_2| \leq \dots \leq |\lambda_k|,$$

where we assume that λ_i has multiplicity m_i . Write M in Jordan normal form. That is write $M = PJP^{-1}$ where

$$J = \begin{pmatrix} 0 & & \\ & J_1 & \\ & & \ddots \\ & & & J_k \end{pmatrix},$$

and J_i is an $m_i \times m_i$ matrix of the form

$$J_i = \begin{pmatrix} \lambda_i & 1 & & \\ & \lambda_i & 1 & \\ & & \ddots & \ddots \\ & & & \lambda_i & 1 \\ & & & & \lambda_i \end{pmatrix}.$$

Since zero is a simple eigenvalue, the first column of P is $\mathbf{v}_0$ and the first row of P^{-1} is $\mathbf{u}_0$. The solution of Equation 16 is given by

$$\begin{aligned} \mathbf{Y}(t) &= e^{Mt} Y_0 + \int_0^t e^{M(t-s)} ds \cdot F \\ &= P e^{Jt} P^{-1} Y_0 + P e^{Jt} \int_0^t e^{-Js} ds P^{-1} F. \end{aligned} \quad (37)$$

Consider e^{Jt} . Write $J = D + N$, where D is a diagonal matrix with diagonal coinciding with J . Then N is a matrix with zeros everywhere except for some 1's on the superdiagonal. Specifically, N is the block diagonal matrix

$$N = \begin{pmatrix} 0 & & \\ & N_1 & \\ & & \ddots \\ & & & N_k \end{pmatrix},$$

where each N_i is an $m_i \times m_i$ nilpotent matrix of the form

$$N_i = \begin{pmatrix} 0 & 1 & & \\ & 0 & 1 & \\ & & \ddots & \ddots \\ & & & 0 \end{pmatrix}.$$

Notably, N commutes with D . Hence

$$e^{Jt} = e^{(D+N)t} = e^{Dt} e^{Nt}.$$

Further, e^{Nt} has the form

$$e^{Nt} = \begin{pmatrix} 1 & & \\ & E_1(t) & \\ & & \ddots \\ & & & E_k(t) \end{pmatrix},$$

where

$$E_i(t) = \begin{pmatrix} 1 & t & t^2/2 & \cdots & \frac{t^{m_i-1}}{(m_i-1)!} \\ & 1 & t & \cdots & \frac{t^{m_i-2}}{(m_i-2)!} \\ & & 1 & \cdots & \frac{t^{m_i-3}}{(m_i-3)!} \\ & & & \ddots & \vdots \\ & & & & 1 \end{pmatrix}$$

Hence,

$$e^{Jt} = \begin{pmatrix} 1 & & & \\ e^{\lambda_1 t} & E_1(t) & & \\ & & \ddots & \\ & & & e^{\lambda_k t} E_k(t) \end{pmatrix}.$$

Since $\operatorname{Re}(\lambda_i) < 0$ for all $i \geq 0$, we have

$$\lim_{t \rightarrow \infty} \frac{1}{t} e^{Jt} = \begin{pmatrix} 1 & & \\ & 0 & \\ & & \ddots \\ & & & 0 \end{pmatrix}.$$

It follows that

$$\lim_{t \rightarrow \infty} \frac{1}{t} P e^{Jt} P^{-1} Y_0 = 0. \quad (38)$$

We now turn our attention to the integral $\int_0^t e^{J(t-s)} ds$. Note that for any integer $a \geq 1$,

$$\int_0^t (t-s)^a e^{\lambda_i(t-s)} ds = \frac{\Gamma(a+1, -\lambda_i t) - a(a!)}{\lambda_i^{a+1}},$$

where $\Gamma(s, x)$ represents the incomplete gamma function. For large x , $\Gamma(s, x) \sim x^{s-1}e^{-x}$ (Abramowitz and Stegun, 1964). So, for sufficiently large t ,

$$\int_0^t (t-s)^a e^{\lambda_i(t-s)} ds \sim \frac{(-\lambda_i t)^a e^{\lambda_i t} - a(a!)}{\lambda_i^{a+1}}.$$

In particular, since $\text{Re}(\lambda_i) < 0$,

$$\lim_{t \rightarrow \infty} \frac{1}{t} \int_0^t (t-s)^a e^{\lambda_i(t-s)} ds = 0. \quad (39)$$

Now,

$$\int_0^t e^{J(t-s)} ds = \begin{pmatrix} \tilde{E}_1(t) & & \\ & \ddots & \\ & & \tilde{E}_k(t) \end{pmatrix},$$

where $\tilde{E}_1(t)$ is an upper triangular matrix for which the entry on the j th row and $(j+k)$ th column (where $k \geq 0$) is

$$\tilde{E}_i(t) = \int_0^t (t-s)^k e^{\lambda_i(t-s)} ds$$

It follows now from Equation 39 that

$$\lim_{t \rightarrow \infty} \frac{1}{t} \int_0^t e^{J(t-s)} ds = \begin{pmatrix} 1 & & \\ 0 & \ddots & \\ & & 0 \end{pmatrix}. \quad (40)$$

Equations 38 and 40, together with the fact that $\mathbf{v}_0 = (1, 1, \dots, 1)^\top$ is the first column of P and $\mathbf{u}_0$ is the first row of P^{-1} imply

$$\lim_{t \rightarrow \infty} \frac{1}{t} P e^{Jt} P^{-1} Y_0 + P \int_0^t e^{J(t-s)} ds P^{-1} F = \mathbf{v}_0 \mathbf{u}_0 F.$$

Hence $\lim_{t \rightarrow \infty} \frac{1}{t} Y_j(t) = \mathbf{u}_0 F$ for any $j \in \{0, \dots, n-1\}$ as claimed. $\square$

References

- Abramowitz M, , Stegun IA. Handbook of mathematical functions: with formulas, graphs, and mathematical tables Vol. 55. Courier Corporation; 1964
- Ali MY, Lu H, Bookwalter CS, Warshaw DM, Trybus KM. Myosin v and kinesin act as tethers to enhance each others' processivity. *Proceedings of the National Academy of Sciences*. 2008; 105(12):4691–4696.
- Ayloo S, Lazarus JE, Dodda A, Tokito M, Ostap EM, Holzbaur EL. Dynactin functions as both a dynamic tether and brake during dynein-driven motility. *Nature communications*. 2014; 5:4807.
- Berezuk MA, Schroer TA. Dynactin enhances the processivity of kinesin-2. *Traffic*. 2007; 8(2):124–129. [PubMed: 17181772]
- Berger F, Müller M, Lipowsky R. Enhancement of the processivity of kinesin-transported cargo by myosin v. *EPL (Europhysics Letters)*. 2009; 87(2) 28,002.
- Berman A, Plemmons RJ. Nonnegative matrices in the mathematical sciences. SIAM. 1994
- Bieling P, Telley IA, Piehler J, Surrey T. Processive kinesins require loose mechanical coupling for efficient collective motility. *EMBO reports*. 2008; 9(11):1121–1127. [PubMed: 18802450]
- Bouzat S. Models for microtubule cargo transport coupling the langevin equation to stochastic stepping motor dynamics: Caring about fluctuations. *Physical Review E*. 2016; 93(1) 012,401.
- Bruno L, Salierno M, Wetzler DE, Despósito MA, Levi V. Mechanical properties of organelles driven by microtubule-dependent molecular motors in living cells. *PLoS One*. 2011; 6(4):e18,332.
- Burgess SA, Walker ML, Sakakibara H, Knight PJ, Oiwa K. Dynein structure and power stroke. *Nature*. 2003; 421(6924):715–718. [PubMed: 12610617]
- Carter NJ, Cross R. Mechanics of the kinesin step. *Nature*. 2005; 435(7040):308–312. [PubMed: 15902249]
- Chen A, McKinley SA, Wang S, Shi F, Mucha PJ, Forest MG, Lai SK. Transient antibody-mucin interactions produce a dynamic molecular shield against viral invasion. *Biophysical journal*. 2014; 106(9):2028–2036. [PubMed: 24806935]
- Culver-Hanlon TL, Lex SA, Stephens AD, Quintyne NJ, King SJ. A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. *Nature cell biology*. 2006; 8(3):264–270. [PubMed: 16474384]
- Feng Q, Mickolajczyk KJ, Chen GY, Hancock WO. Motor reattachment kinetics play a dominant role in multimotor-driven cargo transport. *Biophysical Journal*. 2018; 114(2):400–409. [PubMed: 29401437]
- Gill SR, Schroer TA, Szilak I, Steuer ER, Sheetz MP, Cleveland DW. Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. *J Cell Biol*. 1991; 115(6):1639–1650. [PubMed: 1836789]
- Gross SP, Welte MA, Block SM, Wieschaus EF. Dynein-mediated cargo transport in vivo a switch controls travel distance. *The Journal of cell biology*. 2000; 148(5):945–956. [PubMed: 10704445]
- Hackney DD. Evidence for alternating head catalysis by kinesin during microtubule-stimulated atp hydrolysis. *Proceedings of the National Academy of Sciences*. 1994; 91(15):6865–6869.
- Hancock WO. Bidirectional cargo transport: moving beyond tug of war. *Nature Reviews Molecular Cell Biology*. 2014; 15(9):615–628. [PubMed: 25118718]
- Hancock WO. Mechanics of bidirectional cargo transport. In: King SM, editor *Dyneins: structure, biology and disease: dynein mechanics, dysfunction, and disease* Vol. 2. Academic Press; 2018 152171chap 7
- Hariharan V, Hancock WO. Insights into the mechanical properties of the kinesin neck linker domain from sequence analysis and molecular dynamics simulations. *Cellular and molecular bioengineering*. 2009; 2(2):177–189. [PubMed: 21544223]
- Hughes J, Hancock WO, Fricks J. Kinesins with extended neck linkers: a chemomechanical model for variable-length stepping. *Bulletin of mathematical biology*. 2012; 74(5):1066–1097. [PubMed: 21997362]
- Johnson RW. Handbook of Fluid Dynamics CRC Press; 1998

- Jun Y, Tripathy SK, Narayanareddy BR, Mattson-Hoss MK, Gross SP. Calibration of optical tweezers for in vivo force measurements: How do different approaches compare? *Biophysical journal*. 2014; 107(6):1474–1484. [PubMed: 25229154]
- Kardon JR, Reck-Peterson SL, Vale RD. Regulation of the processivity and intracellular localization of *saccharomyces cerevisiae* dynein by dynactin. *Proceedings of the National Academy of Sciences*. 2009; 106(14):5669–5674.
- Kincaid MM, King SJ. Motors and their tethers: the role of secondary binding sites in processive motility. *Cell Cycle*. 2006; 5(23):2733–2737. [PubMed: 17172850]
- King SJ, Schroer TA. Dynactin increases the processivity of the cytoplasmic dynein motor. *Nature cell biology*. 2000; 2(1):20. [PubMed: 10620802]
- Klumpp S, Lipowsky R. Cooperative cargo transport by several molecular motors. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(48):17,284–17,289.
- Kojima H, Muto E, Higuchi H, Yanagida T. Mechanics of single kinesin molecules measured by optical trapping nanometry. *Biophysical Journal*. 1997; 73(4):2012–2022. [PubMed: 9336196]
- Korn CB, Klumpp S, Lipowsky R, Schwarz US. Stochastic simulations of cargo transport by processive molecular motors. *The Journal of chemical physics*. 2009; 131(24):12,B624.
- Kubo R. The fluctuation-dissipation theorem. *Reports on progress in physics*. 1966; 29(1):255.
- Kunwar A, Vershinin M, Xu J, Gross SP. Stepping, strain gating, and an unexpected force-velocity curve for multiple-motor-based transport. *Current biology*. 2008; 18(16):1173–1183. [PubMed: 18701289]
- Kunwar A, Tripathy SK, Xu J, Mattson MK, Anand P, Sigua R, Vershinin M, McKenney RJ, Clare CY, Mogilner A, et al. Mechanical stochastic tug-of-war models cannot explain bidirectional lipid-droplet transport. *Proceedings of the National Academy of Sciences*. 2011; 108(47):18,960–18,965.
- Kurtz Thomas J. *Approximation of Population Processes*. SIAM. 1981
- Kutys ML, Fricks J, Hancock WO. Monte carlo analysis of neck linker extension in kinesin molecular motors. *PLoS Comput Biol*. 2010; 6(11):e1000,980.
- Mallik R, Carter BC, Lex SA, King SJ, Gross SP. Cytoplasmic dynein functions as a gear in response to load. *Nature*. 2004; 427(6975):649–652. [PubMed: 14961123]
- McKinley SA, Athreya A, Fricks J, Kramer PR. Asymptotic analysis of microtubule-based transport by multiple identical molecular motors. *Journal of theoretical biology*. 2012; 305:54–69. [PubMed: 22575549]
- Minoura I, Katayama E, Sekimoto K, Muto E. One-dimensional brownian motion of charged nanoparticles along microtubules: a model system for weak binding interactions. *Biophysical Journal*. 2010; 98(8):1589–1597. [PubMed: 20409479]
- Müller MJ, Klumpp S, Lipowsky R. Tug-of-war as a cooperative mechanism for bidirectional cargo transport by molecular motors. *Proceedings of the National Academy of Sciences*. 2008; 105(12):4609–4614.
- Müller MJ, Klumpp S, Lipowsky R. Bidirectional transport by molecular motors: enhanced processivity and response to external forces. *Biophysical Journal*. 2010; 98(11):2610–2618. [PubMed: 20513405]
- Muthukrishnan G, Zhang Y, Shastry S, Hancock WO. The processivity of kinesin-2 motors suggests diminished front-head gating. *Current Biology*. 2009; 19(5):442–447. [PubMed: 19278641]
- Okada Y, Hirokawa N. Mechanism of the single-headed processivity: diffusional anchoring between the k-loop of kinesin and the c terminus of tubulin. *Proceedings of the National Academy of Sciences*. 2000; 97(2):640–645.
- Posta F, D'Orsogna MR, Chou T. Enhancement of cargo processivity by cooperating molecular motors. *Physical Chemistry Chemical Physics*. 2009; 11(24):4851–4860. [PubMed: 19506760]
- Segal M, Soifer I, Petzold H, Howard J, Elbaum M, Reiner O. Ndel1-derived peptides modulate bidirectional transport of injected beads in the squid giant axon. *Biology open*. 2012; BIO2012307.
- Shubeita GT, Tran SL, Xu J, Vershinin M, Cermelli S, Cotton SL, Welte MA, Gross SP. Consequences of motor copy number on the intracellular transport of kinesin-I-driven lipid droplets. *Cell*. 2008; 135(6):1098–1107. [PubMed: 19070579]

- Svoboda K, Schmidt CF, Schnapp BJ, Block SM. Direct observation of kinesin stepping by optical trapping interferometry. *Nature*. 1993; 365(6448):721. [PubMed: 8413650]
- Toba S, Watanabe TM, Yamaguchi-Okimoto L, Toyoshima YY, Higuchi H. Overlapping hand-over-hand mechanism of single molecular motility of cytoplasmic dynein. *Proceedings of the National Academy of Sciences*. 2006; 103(15):5741–5745.
- Urbakh M, Klafter J, Gourdon D, Israelachvili J. The nonlinear nature of friction. *Nature*. 2004; 430(6999):525. [PubMed: 15282597]
- Urnavicius L, Zhang K, Diamant AG, Motz C, Schlager MA, Yu M, Patel NA, Robinson CV, Carter AP. The structure of the dynactin complex and its interaction with dynein. *Science*. 2015; 347(6229):1441–1446. [PubMed: 25814576]
- Visscher K, Schnitzer MJ, Block SM. Single kinesin molecules studied with a molecular force clamp. *Nature*. 1999; 400(6740):184–189. [PubMed: 10408448]
- Waterman-Storer CM, Karki S, Holzbaur E. The p150glued component of the dynactin complex binds to both microtubules and the actin-related protein centractin (arp-1). *Proceedings of the National Academy of Sciences*. 1995; 92(5):1634–1638.
- Wiersema UF. *Brownian motion calculus* John Wiley & Sons; 2008
- Witten J, Ribbeck K. *The particle in the spiders web: transport through biological hydrogels*. Nanoscale. 2017

Fig. 1.

(A) Cargo diffuses while tethered to an anchor. The position of the cargo, $X(t)$, is tracked. We denote the anchor's position by $Z(t)$. (B) Cargo diffuses while tethered to a mobile anchor. The cargo is also attached to a retrograde stepping motor, with position $Y(t)$. (C) Cargo diffuses attached to a mobile anchor and an anterograde stepping motor. (D) Displays the orientation of dynactin and dynein relative to the cargo.

Fig. 2.

(A) Observed MSD from Culver-Hanlon et al (2006) of cargo tethered to immobile dynactin (pinned diffusion). The MSD is linear until reaching a threshold. We estimate the slope of the linear portion to be about $0.05 \mu\text{m}^2/\text{s}$ and the horizontal asymptote, corresponding to the threshold, is reported $0.015 \mu\text{m}^2$. (B) Observed MSD for skating events. Cargo is tethered to dynactin which skates along a microtubule in response to cargo. We estimate the asymptotic slope to be about $0.0035 \mu\text{m}^2/\text{s}$. Reprinted by permission from Macmillan Publishers LTD: Nature Cell Biology Culver-Hanlon et al, copyright 2006. (C). Using parameters from Table 1, we simulated fifty 30-second long cargo paths satisfying the pinned diffusion SDE (3), and calculated their associated pathwise MSDs (1). The ensemble average of the pathwise MSDs for all 50 paths is shown in black, with theoretical MSD (4) displayed in dashed blue. Six sample pathwise MSDs are shown in gray. (D). The same procedure of simulation and pathwise MSD analysis is displayed for skating diffusion (7). For purposes of simulation, we choose to set $q' = 17$ and $q'' = 1$, forcing $\gamma_d = 0.03 \text{ pN s}/\mu\text{m}$.

Fig. 3.

Assuming Fluctuation-Dissipation holds, black curves display p_v - p_d relationship for various values of the parameter χ (Equation 29). From bottom to top, $\chi = 0.01, 0.1, 0.5, 1, 5, 10, 100$. The blue dashed curve displays the relationship for the Culver-Hanlon et al (2006) set up: cargo is attached to a dynein motor with and without dynactin; here $\chi = 0.04$. The orange dashed curve is the relationship for the Ali et al (2008) setup: cargo is attached to a kinesin-1 motor, with and without a myosin-V anchor; $\chi = 0.21$. A circle represents the predicted value of p_v under the Fluctuation-Dissipation hypothesis, while an **x** represents the observed value of p_v .

Fig. 4.

(A) Percent loss in asymptotic velocity for motorized cargo when subject to the drag of a dynactin anchor as a function of motor stall force. Values for γ_c and $q' \gamma_a$ are fixed as in Table 1. Dashed orange curve displays percent loss when motor is kinesin-1, using the value of ν_k given in Table 1. The value estimated for F_k^* is marked on the curve. Solid blue curve displays percent loss when motor is dynein, generated using the value of ν_d estimated in Table 1. The value estimated for F_d^* is marked on the curve. **(B)** Curve displays required increase in rebinding factor against percentage decrease in velocity loss for tethering mechanism (ϕ vs. ρ). Shaded region indicates values which do not justify a tethering mechanism.

Table 1

Estimates of parameters for cargo, dynein, dynactin, and kinesin. Values for $k_B T$, cargo velocity, stall force of dynein, and all kinesin-1 values are previously known values. The sources are as follows: [1] corresponds to Kojima et al (1997), [2] to Muthukrishnan et al (2009), [3] to Culver-Hanlon et al (2006), [4] to Mallik et al (2004), [5] to Gross et al (2000), [6] to Visscher et al (1999), [7] to Carter and Cross (2005), and [8] to Kunwar et al (2008). All other parameters are estimated in this paper and the source column gives the section number of their estimation.

Quantity	Label	Value	Source
Cargo Values			
Boltzmann constant-temp	$k_B T$	0.0041 pN μm	[1] [2]
Coefficient of viscous drag	γ_c	$0.16 \frac{\text{pN s}}{\mu\text{m}}$	§2
Velocity (Dynein)	θ	$0.27 \frac{\mu\text{m}}{\text{s}}$	[3]
Velocity (Dynein + Dynactin)	θ'	$0.24 \frac{\mu\text{m}}{\text{s}}$	[3]
Dynactin Values			
Linkage spring constant	κ_d	$0.55 \frac{\text{pN}}{\mu\text{m}}$	§2
Dynein Values			
Stall force	F_d^*	1.1 pN	[4], [5]
Unburdened velocity	v_d	$0.32 \frac{\mu\text{m}}{\text{s}}$	§4.2
Kinesin-1 Values			
Linkage spring constant	κ_k	$340 \frac{\text{pN}}{\mu\text{m}}$	[1]
Unburdened velocity	v_k	$0.81 \frac{\mu\text{m}}{\text{s}}$	[6], [7], [8]
Stall force	F_K^*	7.0 pN	[6]

Table 2

Table of constants used in the “tradeoff model.” Asterisks (*) indicate that these values were modified from the original text based on considerations that appear in Section 4.4. The dagger (†) indicates that the value is calculated from other observations in the paper. Sources are as follows: [9] corresponds to Müller et al (2008), and [10] to Ayloo et al (2014).

Quantity	Label	Value	Source
Kinesin-1 binding rate	k_{on}	1 s^{-1}	[9]*
Kinesin-1 unbinding rate	k_{off}	1 s^{-1}	[9]
Dynein-dynactin binding rate	d_{on}	1 s^{-1}	[9]*
Dynein-dynactin unbinding rate	d_{off}	0.2 s^{-1}	[10]†
Rebinding Rate Factor	ρ	1-2	§4.4
Velocity Loss Factor	ϕ	0-20%	§4.4