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Tuning ensemble-averaged cargo run length via fractional change in mean kinesin number

John O. Wilson¹, Arturo D. Zaragoza², Jing Xu¹

¹Physics, University of California, Merced, CA, USA

²Mechanical Engineering, University of California, Merced, CA, USA

Abstract

The number of motors carrying cargos in biological cells is not well-defined, instead varying from cargo to cargo about a statistical mean. Predictive understanding of motility in cells therefore requires quantitative insights into mixed ensembles of cargos. Toward this goal, here we employed Monte Carlo simulations to investigate statistical ensembles of cargos carried by a Poisson-distributed number of motors. Focusing on the key microtubule-based motor kinesin-1, our simulations utilized experimentally determined single-kinesin characteristics and alterations in kinesin's on- and off-rates caused by cellular factors and/or physical load. We found that a fractional increase in mean kinesin number enhances the ensemble-averaged cargo run length and amplifies run-length sensitivity to changes in single-kinesin on-rate and off-rate. These tuning effects can be further enhanced as solution viscosity increases over the range reported for cells. Together, our data indicate that the physiological range of kinesin number sensitively tunes the motility of mixed cargo populations. These effects have rich implications for quantitative and predictive understanding of cellular motility and its regulation.

Keywords

kinesin-1; motor number; on-rate; off-rate; run length; Monte Carlo simulation

1. Introduction

Kinesin-1 is a key microtubule-based motor that drives long-range delivery of cargos in cells [1, 2]. This intracellular motility is critical for eukaryotic cell function and survival; dysfunctions in this process are linked to human diseases including neurodegeneration [3, 4]. While the single-molecule characteristics of kinesin are reasonably well understood, many important questions still remain, including how the number of kinesins carrying a cargo impacts the distance over which the cargo travels before unbinding from the microtubule ("run length").

In cells, individual vesicular cargos are often carried by one or two kinesins, with the motor number varying among cargos in a population [5-7]. Although intuitively run length

should increase as more motors carry a cargo, it is unclear how substantial such an increase would be, given that only a limited fraction of the cargo population is carried by more than one kinesin *in vivo* [5-7]. Further, for a given cargo carried by multiple kinesins, intra-motor strain can hinder cooperative function between kinesins and limit the contribution of additional kinesins to run length [8-10]. Additionally, the microtubule binding and unbinding kinetics of kinesin can be altered by cellular factors such as microtubule-associated proteins [11-17]. How the interplay of these single-motor alterations with a fractional change in mean kinesin number impacts cargo delivery is not currently understood.

Here we employed Monte Carlo-based simulations to investigate statistical ensembles of cargos, with each cargo carried by a Poisson-distributed number of kinesins. Our assumption of a Poissonian motor number is consistent with random processes [18-20] and with data available for biological scenarios [5-7]. We varied the mean number of kinesins on motile cargos between 1 and 1.3, corresponding to a modest range of 0-25% of the cargos in the population carried by two or more motors (figure 1(a)). We then characterized the run length of the cargo population as the ensemble average of the mean run lengths of cargos carried by one or more kinesins. Our simulations employed experimentally determined single-kinesin characteristics [18, 20-29], including the load-dependent off-rate [28, 29] that limits the cooperative function of kinesins in a team. We varied the single-kinesin on-rate and unloaded off-rate over a large parameter space, capturing how these characteristics are altered by cellular factors [11-17]. Our simulations also included cargo diffusion [30] and viscous drag [31-34], two sources of physical load that are important at the molecular level and present in all live cells. We found that a fractional change in mean kinesin number has important ramifications for the ensemble-averaged cargo run length, suggesting that the physiological range of kinesin number [5-7] provides crucial regulation of cargo delivery in cells.

2. Methods

2.1 Monte Carlo-based simulations

2.1.1 Single-kinesin characteristics and other simulation parameters—

Kinesin's load-dependent off-rate is as previously determined by experimental [25, 28, 29] and modeling [30, 35] studies:

$$\epsilon(F) = \begin{cases} \epsilon_0 \cdot \exp(|F| / F_-), & F \leq 0 \\ \epsilon_0 \cdot (1 + 3.825 \cdot F), & 0 < F \leq 2 \\ \epsilon_0 \cdot 7.4 \cdot \exp(F / F_+), & F > 2 \end{cases} \quad (1)$$

where ϵ_0 is the unloaded off-rate and F is the load on the motor. Positive values of F indicate load oriented in the direction of kinesin motion; negative values of F indicate load oriented in the direction opposite to motor motion. F is in units of pN. $F_+ = k_B T / \delta_+$ and $F_- = k_B T / \delta_-$ are unbinding forces of kinesin, $k_B T$ is the thermal energy at room temperature (4.11 pN·nm), $\delta_+ = 0.32$ nm, and $\delta_- = 0.60$ nm [25, 29].

Kinesin's load-dependent stepping rate is as previously determined by experimental [23, 25, 29] and modeling [30, 36, 37] studies:

$$k_{step}(F) = \begin{cases} 0, & F \leq -F_s \\ (v_0 / \Delta x) \cdot (1 - (F / F_s)^2), & -F_s < F \leq 0 \\ v_0 / \Delta x, & F > 0 \end{cases} \quad (2)$$

where v_0 is the unloaded velocity, Δx is the step size, F_s is the stall force, and F is the load on the motor. Load directions are as described for equation (1).

The same values for single-kinesin stiffness (0.32 pN/nm [21, 22, 26]), rest length (40 nm [20, 27]), step size (8 nm [18, 24]), stall force (7 pN [28]), and unloaded velocity (0.8 $\mu\text{m/s}$ [18, 24]) were used for all simulations. The values of single-kinesin on-rate and unloaded off-rate are indicated in the text.

A simulation time step of 10 μs was used for all simulations. This time step is shorter than the time scale of the fastest process in kinesin's mechanochemical cycle [36]. A cargo diameter of 0.5 μm , which is within the ranges measured for intracellular cargos [5, 34, 38, 39], was used for all simulations. Values of solution viscosity are indicated in the text.

2.1.2 Individual cargo runs—Individual cargo runs were simulated along a one-dimensional microtubule as described previously [36, 37]. Briefly, each simulated cargo is carried by one or more kinesins. Each kinesin is modeled as a non-interacting, non-compressible spring that stochastically binds to or unbinds from the microtubule. Each kinesin is attached to the cargo at the same point and experiences load only when its displacement from the cargo is larger than the motor's rest length.

At the start of a simulated run, all kinesins on the cargo are unbound from the microtubule. At each simulation time step, an individual unbound kinesin binds the microtubule with a probability determined by its on-rate, within a region on the microtubule that can be explored by its rest length. The simulation time step is incremented, and the above evaluations are repeated until at least one kinesin on the cargo binds the microtubule, at which point the run is initiated.

At each time step during the run, the motile state of each kinesin on the cargo is evaluated and updated as follows. A currently unbound kinesin binds with a probability determined by its on-rate, within a region on the microtubule that can be explored by its rest length. A currently bound kinesin unbinds with a probability determined by its load-dependent off-rate (equation (1)) or remains bound and advances one step with a probability determined by its load-dependent stepping rate (equation (2)). The load on each bound kinesin is calculated based on its current position relative to that of the cargo.

Once the motile states of all kinesins on the cargo are updated, the position of the cargo is updated by summing the deterministic drift motion and the random thermal motion of the cargo during the time step and along the one-dimensional microtubule. The drift motion of the cargo opposes kinesin motion and is determined as $-\tau \cdot F / \xi$, where τ is the simulation

time step, F is the net load on the cargo, and $\xi = 3\beta\pi\eta d$ is the friction constant determined by the hydrodynamic drag coefficient β , the solution viscosity η , and the cargo diameter d . β is unity in bulk solution, and increases per Faxen's law near surfaces [40]. Based on a typical experimental geometry in which the cargos move on surface-anchored microtubules, β was estimated as ~ 3 , in good agreement with a recent experimental report [41]. Our simulations are not strongly sensitive to this scaling (for example, figure 3). The thermal motion of the cargo is uncorrelated with kinesin motion and is drawn from a normal distribution with mean square displacement $2D \cdot t$, where the cargo diffusion constant D is related to the friction constant ξ by the Einstein relation $\xi = k_B T/D$ [42]. The simulation time step is then incremented, and the above evaluations and updates are repeated until all kinesins on the cargo stochastically unbind from the microtubule, at which point the run is terminated.

2.1.3 Cargos carried by the same number of kinesins—Each cargo in a simulated population was carried by the same number of kinesins (1, 2, 3, 4, or 5). Simulations of individual cargo runs were carried out as described in section 2.1.2.

2.1.4 Cargos carried by a Poisson-distributed number of kinesins—For each simulated ensemble, the number of kinesins on each cargo in the population was drawn from the discrete Poisson distribution, $\lambda^k e^{-\lambda}/k!$, where the motor number k is a non-negative integer and λ is the Poisson mean. Only cargos assigned one or more kinesins were used to simulate runs. Simulations of individual cargo runs were carried out as described in section 2.1.2.

The mean number of kinesins on motile cargos was varied between 1 and 1.3, corresponding to a range of 0-25% of cargos in the populations carried by 2 kinesins (figure 1(a)). There was a probability of $<0.006\%$ that a cargo is carried by 6 kinesins.

2.2 Analysis of cargo run length

2.2.1 Run length of individual simulated cargos—Run length was determined as the distance that the simulated cargo travels after binding to and before unbinding from the microtubule. Within the uncertainty arising from the thermal diffusion of the cargo, the motion of each simulated run was unidirectional and plus-end oriented (in the direction of kinesin motion). We detected no evidence of minus-end directed (in the direction opposite of kinesin motion) segments within individual simulated runs (data not shown), suggesting no substantial impact of the elastic coupling between motors [43] on the overall direction of cargo motion in our study.

2.2.2 Mean run length of cargos carried by the same number of kinesins—For each simulation condition in which all cargos were carried by the same number of kinesins (section 2.1.3), the cumulative probability distribution of the run lengths was fitted to that of a single exponential distribution $1 - A \cdot e^{-x/d}$. Mean run length was determined as the best-fit decay constant d . The associated standard error of the mean (SEM) was determined via a bootstrap method [44]. The resulting mean run lengths are shown in figure A1(a) in the Appendix.

We verified that the mean cargo run lengths from our simulations, which included the intra-motor strain that hinders motor cooperativity (section 2.1.2), are substantially shorter than those determined from analytical calculations that do not consider intra-motor strain [45] (figure A1(b)).

2.2.3 Ensemble-averaged run length of cargos carried by a Poisson-distributed number of kinesins—For each simulation condition in which cargos were carried by a Poisson-distributed number of kinesins (section 2.1.4), the cumulative probability distribution of cargo run lengths was fitted to the following weighted sum:

$$P(x) = 1 - \sum_{k=1}^5 P_k \cdot e^{-x/d_k}, \quad (3)$$

where P_k is the probability that a cargo is carried by k kinesins and d_k represents the mean run length of cargos carried by k kinesins. Note that the weighted sum considers up to 5 kinesins per cargo because the probability that a cargo is carried by 6 or more kinesins is negligible (<0.006%) over the range of mean kinesins examined in this study. Each probability P_k was determined as the fraction of simulated cargos carried by k kinesins. This approach accounts for stochasticity inherent in simulations of statistical ensembles; the resulting P_k values are in good agreement with those expected from Poisson distributions (data not shown). The value of each fitting parameter d_k was constrained to be within one SEM of the mean run length of cargos carried by a well-defined k number of kinesins (section 2.2.2). This approach accounts for stochasticity inherent in run-length simulations in section 2.1. The resulting best-fit determines the ensemble-averaged cargo run length, $\sum_{k=1}^5 P_k \cdot d_k$. The associated SEM was determined via a bootstrap method [44].

2.3 Data representation

MATLAB functions `corocet.m` [46] and `cmap2pal.m` [47] were used to generate the perceptually uniform color maps in figure 2(a).

3. Results

3.1 A fractional increase in mean kinesin number enhances the ensemble-averaged cargo run length

We first carried out run-length simulations using the commonly reported values for single-kinesin on-rate (5 s^{-1} [48, 49]) and unloaded off-rate (0.8 s^{-1} [24]). Our simulations utilized a solution viscosity of water ($1 \text{ mPa}\cdot\text{s}$), capturing the lower limit of cytoplasmic viscosity [31-34]. Under these conditions, we previously showed that load arising from cargo diffusion shortens single-kinesin runs from the unloaded value of $1 \text{ }\mu\text{m}$ to $0.8 \text{ }\mu\text{m}$ [30].

We found that the ensemble average of cargo runs substantially lengthened as the mean number of kinesins on the cargo fractionally increased from one (figure 1). For cargos carried by a single motor (0% with 2 kinesins, figure 1(a)), the run-length distribution was well described by a single exponential fit (figure 1(b) and (c)), with a mean that was in excellent agreement with our prior work ($0.80 \pm 0.04 \text{ }\mu\text{m}$, figure 1(b), inset; compare with $0.8 \text{ }\mu\text{m}$ in [30]). As we increased the fraction of cargos with 2 kinesins (5-25%,

figure 1(a)), the run-length distributions shifted toward longer runs (figure 1(b)), were well-described by a weighted sum of several exponentials (equation (3); solid lines, figure 1(c)), and deviated from a single-exponential fit (grey lines, figure 1(c)). These changes in run-length distributions are consistent with increases in the mean number of kinesins in the cargo population [19, 50]. The reduction in run length due to cargo diffusion was mitigated when the fraction of cargos with 2 kinesins increased to a modest 10%, with the ensemble-averaged cargo run length returning to the unloaded single-kinesin value ($1.07 \pm 0.08 \mu\text{m}$, figure 1(b), inset). Overall, the ensemble-averaged cargo run length increased by 1.9-fold (± 0.2) as the fraction of cargos carried by 2 kinesins increased from 0% to 25% (figure 1(b), inset). For reference, run-length distributions and mean run lengths of cargos carried by exactly 2, 3, 4, or 5 kinesins are shown in figure A2 in the Appendix.

3.2. A fractional increase in mean kinesin number amplifies the impact of single-motor alterations on run length

We next examined how changes in single-kinesin binding and unbinding kinetics impact the ensemble-averaged cargo run length. To allow direct comparison with the simulations in figure 1, we carried out this set of simulations at the same solution viscosity ($1 \text{ mPa}\cdot\text{s}$). We varied the single-kinesin on-rate between 1 s^{-1} and 8 s^{-1} and the unloaded off-rate between 0.5 s^{-1} and 1.6 s^{-1} , guided by how these single-motor rates are altered by cellular factors such as microtubule-associated proteins [11-17].

The run-length increase that we observed in figure 1 was general over a wide parameter space of single-motor on-rates and off-rates (figure 2). For each rate combination, the larger the fraction of cargos with 2 kinesins, the greater the increase in the ensemble-averaged cargo run length over that of single-kinesin cargos (figure 2(a)). Moreover, the sensitivity of cargo run length to these rate changes was amplified by the fraction of cargos carried by 2 kinesins (figure 2(b)). For example, over an 8-fold change in the single-kinesin on-rate and a 3-fold change in its unloaded off-rate, the run-length increase remained largely constant when only a limited fraction of cargos was carried by 2 kinesins (figure 2(b), left). In contrast, the run-length increase was strongly sensitive to changes in single-motor on- and off-rates when the fraction of cargos with 2 kinesins increased to 25% (figure 2(b), right). For reference, the impact of single-motor alterations on the mean run lengths of cargos carried by exactly 2, 3, 4, or 5 kinesins is shown in figures A1(a) and A3 in the Appendix.

3.3. Solution viscosity can enhance the effects of mean kinesin number on run length

We next explored whether our findings in figures 1 and 2 are sensitive to changes in solution viscosity. Thus far, our simulations were conducted at the viscosity of water. In contrast, cytoplasmic viscosities can be up to ~300-fold higher than that of water [31-34]. We previously showed that, due to the interplay between cargo diffusion and viscous drag, the run length of cargos carried by a single kinesin varies non-monotonically with solution viscosity [30].

We found that the effects of kinesin number on run length (figures 1 and 2) can increase as solution viscosity increased over the range reported for cells (figure 3). As in our previous work [30], here we observed a non-monotonic effect of viscosity on the run length of

single-kinesin cargos (dashed line, figure 3(a)). This non-monotonic variation in run length persisted for mixed ensembles of cargos (5-25% with 2 kinesins, figure 3(a)). For each solution viscosity tested, the ensemble-averaged cargo run length increased as the mean number of kinesins on the cargo increased fractionally from one. This run-length increase was sensitive to both solution viscosity and single-kinesin characteristics, for example remaining constant for a combination of 5 s^{-1} on-rate and 0.8 s^{-1} unloaded off-rate (grey scatter, figure 3(b)), but increasing at higher viscosity for a combination of 8 s^{-1} on-rate and 0.5 s^{-1} unloaded off-rate (open scatter, figure 3(b)). These effects were again amplified by the fraction of cargos carried by 2 kinesins (figure 3(b)). For reference, the impact of solution viscosity on the mean run lengths of cargos carried by exactly 2, 3, 4, or 5 kinesins is shown in figure A4 in the Appendix.

4. Discussion

Here we present *in silico* evidence that mean kinesin number is an important and sensitive tuning parameter for the ensemble-averaged distance that cargos travel in biological cells. Compared to the single-motor case, a fractional change in mean kinesin number enhances both the ensemble average of cargo run length and its sensitivity to single-kinesin alterations (figures 1 and 2). Thus, although kinesin is thought to function non-cooperatively [8, 9], our study indicates that a modest change in mean kinesin number can have important ramifications for the motility of mixed ensembles of cargos, which reflects the biological scenario. We found that the effects of kinesin number on cargo run length can be further enhanced over the range of solution viscosities reported for living cells (figure 3).

Our results may be understood by considering the interplay between the microtubule-binding and -unbinding kinetics of individual kinesins. The load-dependent off-rate of kinesin [28, 29] limits cooperative function in an ensemble. However, because the on-rate of the motor can be several-fold faster than the unloaded off-rate (5 s^{-1} [48, 49] vs. 0.8 s^{-1} [24]), the presence of an additional motor increases the probability that the cargo remains tethered to the microtubule, thereby lengthening the run without necessarily contributing to the number of kinesins simultaneously driving motility. Thus, under otherwise identical conditions, the faster the on-rate of the motor, the longer the individual runs of cargos carried by 2 kinesins, and the greater the ensemble-averaged run length of the cargo population. Our results capture this interplay for cargos carried by a defined number of kinesins (figures A1 and A3) and for mixed cargo populations (figure 2).

The effects of viscosity (figures 3 and A4) further highlight the interplay between kinesin's binding and unbinding kinetics. Solution viscosity imposes load on the cargo and increases the off-rate of kinesin carrying the cargo [30, 51]. The presence of additional motors on the cargo can reduce the impact of viscous load on kinesin's off-rate by enabling load sharing between kinesins [36]. The higher the solution viscosity and/or fraction of cargos carried by 2 kinesins, the more effective load sharing can be, and the larger the contribution that each additional motor can make to run length. These effects are pronounced when kinesin's on-rate is substantially faster than its off-rate, enabling the load sharing that can only take place between motors simultaneously driving motility.

Taken together, we anticipate that the effects uncovered here will have rich implications for delineating the motility of cellular cargos. In particular, because cellular factors impact kinesin function on the single-molecule level, quantitative insights into how these single-kinesin alterations combine with the biological range of kinesin number are critical for predictive understanding of motility and its regulation in cells. The range of tuning by kinesin number will likely expand as we learn more about the regulation of single-molecule characteristics, including the microtubule binding and unbinding kinetics of the kinesin motor highlighted here.

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Appendix

Figure A1.

Mean run lengths of cargos carried by exactly 1, 2, 3, 4, or 5 kinesins, evaluated for four on-rates and three unloaded off-rates of a single kinesin. (a) Simulation results from the current study. Simulations were carried out using a solution viscosity of 1 mPa·s. Error bars indicate SEM. $N = 5000$ for each simulation condition. (b) Analytical calculations developed by Klumpp et al. [45] describing the mean run length of cargos carried by exactly N motors as $\frac{v}{\epsilon} \left[1 + \sum_{n=1}^{N-1} \prod_{i=1}^n \frac{(N-i)\pi_{ad}}{(i+1)\epsilon} \right]$, where $v = 0.8 \mu\text{m/s}$ is the single-kinesin velocity, ϵ is the motor's unloaded off-rate, and π_{ad} is the motor's on-rate. We verified that the mean

cargo run lengths from our simulations in panel (a), which included intra-motor strain that hinder motor cooperativity, are substantially shorter than those determined from analytical calculations that do not consider intra-motor strain [45] in panel (b). Mean run lengths in (a) were used to determine the ensemble-averaged run length of mixed cargo populations in figures 1-3 in the main text.

Figure A2.

Run lengths of cargos carried by exactly 1, 2, 3, 4, or 5 kinesins. Simulations were carried out using an on-rate of 5 s^{-1} and an unloaded off-rate of 0.8 s^{-1} for a single kinesin, and a solution viscosity of $1 \text{ mPa}\cdot\text{s}$. (a) Cumulative frequency distributions of cargo run length, for five defined kinesin numbers. For reference, run-length distributions for mixed ensembles of cargos are replotted from figure 1(b) in the main text (5, 15, and 25% cargos). Dashed lines indicate best-fit to a single exponential (1, 2, 3, 4, or 5 kinesins) or a weighted sum of several exponentials (equation (4) in the main text) (5, 15, 25% cargos). $N = 1000$ for each distribution. (b) Mean cargo run length, for five defined kinesin numbers. For reference, the ensemble-averaged run lengths of cargos with contributions from 2 kinesins (5, 15, and 25% cargos) are replotted from figure 1(b) in the main text. Error bars indicate SEM. $N = 5000$ for each simulation condition.

Figure A3.

The impact of single-motor alterations on mean run length, for cargos carried by exactly 2, 3, 4, or 5 kinesins. Simulations were carried out using a solution viscosity of $1 \text{ mPa}\cdot\text{s}$. Single-kinesin on-rates and unloaded off-rates are as indicated. Fold-increase in run length is determined relative to the mean run length of single-kinesin cargos. Error bars indicate SEM. $N = 5000$ for each simulation condition.

Figure A4.

The impact of solution viscosity on mean run length, for cargos carried by exactly 1, 2, 3, 4, or 5 kinesins. (a) Mean cargo run length as a function of solution viscosity, for five defined kinesin numbers. For reference, ensemble-averaged run lengths of mixed cargo populations (5%, 15%, 25% with ≥2 kinesins) are replotted from figure 3(a) in the main text. Simulations were carried out using an on-rate of 5 s⁻¹ and an unloaded off-rate of 0.8 s⁻¹ for a single kinesin. Shaded regions indicate SEM. $N = 5000$ for each simulation condition. (b) Fold-increase in the mean run length of cargos as a function of solution viscosity, for four defined kinesin numbers and three combinations of single-kinesin on-rate and unloaded off-rate. Fold-increase in run length is determined relative to the mean run length of cargos carried by a single motor. Error bars indicate SEM. $N = 5000$ for each simulation condition.

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Figure 1.

The ensemble-averaged cargo run length increases as the fraction of cargos with ≥ 2 kinesins increases. Simulations were carried out using an on-rate of 5 s^{-1} and an unloaded off-rate of 0.8 s^{-1} for a single kinesin, and a solution viscosity of $1 \text{ mPa}\cdot\text{s}$. (a) Fraction of motile cargos carried by the indicated number of kinesins, for mean kinesin numbers between 1.0 and 1.3. Grey regions indicate the fraction of cargos with ≥ 2 kinesins. (b) Cumulative frequency distributions of cargo run length, for the four distributions of kinesin numbers in (a). Dashed lines indicate best-fit to a single exponential (0% with ≥ 2 kinesins) or a weighted sum of several exponentials (equation (4)) (5, 15, 25%). $N = 1000$ for each distribution. Inset: Mean run length of single-kinesin cargos (0% with ≥ 2 kinesins) and ensemble-averaged run length of cargos with contributions from ≥ 2 kinesins (5, 15, and 25%). Error bars indicate SEM. $N = 5000$ for each simulation condition in the inset. (c) Residuals of fits from (b). Grey solid lines indicate deviations from a single-exponential fit (5, 10, 25%).

Figure 2.

The impact of single-motor alterations on the ensemble-averaged cargo run length is amplified by the fraction of cargos carried by ≥ 2 kinesins. Simulations were carried out using a solution viscosity of $1 \text{ mPa}\cdot\text{s}$. Fold-increase in ensemble-averaged run length is determined relative to the mean run length of single-kinesin cargos. (a) Fold-increase in run length as a function of the single-kinesin on-rate and the fraction of cargos carried by ≥ 2 kinesins, evaluated for three unloaded off-rates of a single kinesin. $N = 5000$ for each simulation condition. (b) Fold-increase in ensemble-averaged run length from (a), replotted as a function of single-kinesin on-rate, for three fractions of cargos with ≥ 2 motors and three unloaded off-rates of the single kinesin. Shaded regions indicate SEM.

Figure 3.

Solution viscosity can enhance the effects of kinesin motor number on run length. (a) Ensemble-averaged cargo run length as a function of solution viscosity, for three fractions of cargos with ≥ 2 kinesins. Simulations were carried out using an on-rate of 5 s^{-1} and an unloaded off-rate of 0.8 s^{-1} for a single kinesin. Shaded regions indicate SEM. $N=5000$ for each simulation condition. (b) Fold-increase in the ensemble-averaged run length as a function of solution viscosity, for three fractions of cargos with ≥ 2 kinesins and three combinations of single-kinesin on-rate and unloaded off-rate. Fold-increase in run length is determined relative to the run length of cargos carried by a single motor. Shaded regions indicate SEM. $N=5000$ for each simulation condition.