Tau directs intracellular trafficking by regulating the forces exerted by kinesin and dynein teams

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Organelles, proteins, and mRNA are transported bidirectionally along microtubules by plus-end directed kinesin and minus-end directed dynein motors. Microtubules are decorated by microtubule-associated proteins (MAPs) that organize the cytoskeleton, regulate microtubule dynamics and modulate the interaction between motor proteins and microtubules to direct intracellular transport. Tau is a neuronal MAP that stabilizes axonal microtubules and crosslinks them into bundles. Dysregulation of tau leads to a range of neurodegenerative diseases known as tauopathies including Alzheimer's disease (AD). Tau reduces the processivity of kinesin and dynein by acting as an obstacle on the microtubule. Single-molecule assays indicate that kinesin-1 is more strongly inhibited than kinesin-2 or dynein, suggesting tau might act to spatially modulate the activity of specific motors[1-4]. To investigate the role of tau in regulating bidirectional transport, we isolated phagosomes driven by kinesin-1, kinesin-2, and dynein and reconstituted their motility along microtubules[5]. We find that tau biases bidirectional motility towards the microtubule minus-end in a dose-dependent manner. Optical trapping measurements show that tau increases the magnitude and frequency of forces exerted by dynein through inhibiting opposing kinesin motors. Mathematical modeling indicates that tau controls the directional bias of intracellular cargoes through differentially tuning the processivity of kinesin-1, kinesin-2, and dynein. Taken together, these results demonstrate that tau modulates motility in a motor-specific manner to direct intracellular transport, and suggests that dysregulation of tau might contribute to neurodegeneration by disrupting the balance of plus- and minus-end directed transport.

The motor proteins kinesin and dynein transport organelles, proteins, and mRNA along microtubules. Kinesin motors move towards the cell periphery and dynein moves towards the cell center. Many intracellular cargoes associate with both kinesin and dynein motors to enable movement in both directions along microtubules and to navigate around obstacles through the dense cellular environment[6-8]. Further, regulatory factors modulate the activity of kinesin and dynein to target cargoes to specific locations in the cell[9, 10]. Proper regulation of intracellular transport is required to maintain biosynthetic, degradative, and signaling pathways, and defects in trafficking are associated with developmental and neurodegenerative diseases[11, 12].

One potential mechanism for regulating intracellular transport is through microtubule-associated proteins (MAPs) that bind along microtubules and modulate the interaction of motors with the

microtubule surface. Tau is a neuronal MAP that bundles and stabilizes microtubules in the axon[13, 14]. Tau inhibits kinesin-1 motility in vitro by acting as an obstacle on the microtubule surface[1, 2]. In contrast, kinesin-2 and dynein are better able to navigate in the presence of obstacles and are only inhibited at high levels of tau[1, 3, 15]. By exerting differential effects on the motility of kinesin and dynein, tau might act to tune the balance of plus- and minus-end directed transport.

In support of a central role in regulating axonal transport, overexpression of tau in epithelial and neuronal cells results in mislocalization of mitochondria and other vesicular cargoes to the cell center by preferentially inhibiting plus-end directed movement[15-18]**.** Tau knock-out mice exhibit age-dependent loss of neurons and defects in microtubule organization and axonal trafficking[14, 19]. In humans, tau mutations result in neurodegenerative disease[20] and tau dysregulation is implicated as a key driver of Parkinson's[19] and Alzheimer's[21] diseases.

To examine the influence of tau on organelle transport in the absence of cytosolic components, post-translational modifications, or signaling events present in the cell, we isolated intracellular cargoes, along with their native transport machinery, and reconstituted their bidirectional motility along microtubules[5, 22]. Latex-bead-containing phagosomes are a useful model system for studying bidirectional transport. Their uniform size and density enables facile, high-purity isolation[23] and manipulation with an optical trap[5, 24]. The motor proteins and adaptors that transport phagosomes have been extensively characterized through proteomic and biophysical assays[5, 23-26]. Late phagosomes, as studied here, are transported by the microtubule motors kinesin-1 (KIF5B), kinesin-2 (KIF3A/B), and dynein-dynactin[5, 25, 26] and are positive for late endosomal markers such as LAMP1, Rab5, Rab7 [23] and motor adaptors including dynactin and Lis1[5] and KAP3[27]**.**

To characterize the type and number of microtubule motors associated with isolated phagosomes, we used immunofluorescence and quantitative photobleaching. We performed three-color immunofluorescence on isolated phagosomes and found that 47% of phagosomes were positive for kinesin-1, kinesin-2, and dynein; while 17% were positive for kinesin-2 and dynein and 11% were positive for kinesin-1 and dynein. For a small fraction of phagosomes, we observed a positive signal for a single motor type **(Fig. 1b-d)**. Taking into account the likelihood that in some cases motors are present but not detected, these results indicate that most late phagosomes are transported by kinesin-1, kinesin-2, and dynein, in agreement with previous studies[5, 25]. To estimate the number of each type of motor bound to the phagosomes, we used stepwise photobleaching. For these assays, we labelled each motor using Alexa 647 in separate flow chambers to ensure similar conditions for each motor. We identified steps in the intensity traces using a step detection algorithm based on a two-sample t-test[28]. Single kinesin-1 motors were imaged with identical conditions to estimate the number of fluorophores conjugated to each motor **(Fig. S1d)**. These results indicate that approximately 2 kinesin-1, 3 kinesin-2, and 6 dynein motors are bound to each cargo on average **(Fig. 1e)**, similar to the estimates for late endosomes isolated from mouse brain[7].

The effect of tau on the motility of kinesin and dynein has been characterized for individual motors and multiple motors of one type [1, 2, 4, 18, 29]. Here, we investigate the potential role for tau in directing the motility of bidirectional cargoes driven by teams of both kinesin and dynein motors. Similar to their motility in living cells, isolated phagosomes move bidirectionally along microtubules[5] **(Fig. 1, Fig. S2)**. Using TIRF microscopy and subpixel resolution tracking [30, 31],

phagosomes were imaged with high spatiotemporal resolution (~5 nm, 80 ms; and **SI Appendix, Fig. S2c)**. In the absence of tau, phagosomes exhibit approximately equal fractions of movement towards the microtubule plus- and minus-ends **(Fig. 1 f,k)**.

We focused on the shortest isoform of tau, 3RS, as it has been shown to have the strongest effects on motility[1]. At low concentrations (1 nM), tau forms small clusters that exhibit both static and diffusive motility along the microtubule [32, 33]. At higher concentrations (10 nM), tau decorates the entire microtubule lattice **(Fig. 1g,h)**. Tau alters the net directionality of transport by shifting motility towards the microtubule minus end. Phagosomes move towards the microtubule minus end 49% of the time in the absence of tau compared to 59% (1 nM) and 65% (10 nM) when tau is present **(Fig. 1),** indicating that the density of tau on the microtubule surface determines the relative activity of kinesin and dynein.

To examine how tau modulates motor processivity, we analyzed the length of individual runs between each reversal in the trajectories. Previous in vitro studies show that tau acts as an obstacle to decrease motor processivity, and that kinesin is inhibited more strongly than dynein by tau. When applied to bidirectional cargoes, these results suggest the hypothesis that tau will decrease kinesin run lengths sharply while dynein run lengths will also decrease but to a lesser extent. Instead, we observe that the mean run length of plus-end directed runs is unchanged while the frequency of long processive runs towards the minus end increases in the presence of tau **(Fig. 2a).** This mechanism of regulation is specific to cargoes driven by both kinesin and dynein – tau reduced the minus-end directed run lengths of mRNA complexes driven by dynein alone[34]. Together these results suggest that for bidirectional cargoes, tau reduces the activity of opposing kinesin motors to enhance dynein processivity.

We next analyzed the off-axis displacements to investigate how teams of motors navigate on tau-decorated microtubules. Off-axis displacements increase in the presence of tau for both plus- and minus-end directed runs, indicating that both kinesin and dynein teams switch protofilaments to avoid obstacles on the microtubule **(Fig. 2b)**. Individual dynein and kinesin-2 motors can step to adjacent protofilaments[29, 35, 36]. For cargoes driven by multiple motors, sideways steps could also result from the binding and unbinding of motors to multiple protofilaments. Average velocities of phagosomes along the microtubule axis decrease with increasing tau concentration in both directions, consistent with tau acting as an obstacle to transport **(Fig. 2c)**.

To test the hypothesis that tau reduces the forces exerted by kinesin motors to enhance dynein motility, we measured forces exerted by phagosomes using an optical trap. Teams of dynein and kinesin motors exert forces up to 9 pN, with an approximately equal fraction of plus- and minus-end directed events consistent with their motility **(Fig. 1f, 3a, S3)**. The plus-end directed forces show a peak ~4 pN similar the unitary stall force for kinesin-1 and kinesin-2 **(Fig. 3a, S3c)**. Lower force events (~1.4 pN and 2.3 pN) may be due to detachments before the motors reached stall or opposing loads from dynein[37]. Dynein forces exhibit peaks at ~1 pN intervals consistent with events driven by multiple dynein motors, each exerting ~1 pN [5, 24]. Tau decreases the frequency and magnitude of kinesin-driven forces, indicating that both fewer kinesins are able to engage [2] and that the engaged kinesins exert less force in the presence of tau **(Fig. 3b, S3d)**. Correspondingly, minus-end directed force events are more frequent and shifted towards higher magnitudes, suggesting that dynein teams are able to exert higher forces when force generation by opposing kinesin motors is inhibited by tau.

Previously, a mathematical model demonstrated that a stochastic tug of war between teams of opposing motor proteins in the absence of external regulation results in bidirectional motility similar to the trajectories we observe[38]. We extended this model to simulate the motility of cargoes transported by kinesin-1, kinesin-2, and dynein **(SI Appendix)**. Force measurements indicate phagosomes are transported by up to 3 kinesin motors and 10 dynein motors, similar to the total number of motors estimated by quantitative photobleaching **(Fig. 1b-e)**. We modeled the motility of cargoes transported by one kinesin-1, two kinesin-2, and ten dynein motors and found similar trajectories to those observed for isolated phagosomes **(Fig. 4, and Table S1)**. We estimated the effect of tau on the unbinding rates of kinesin-1, kinesin-2, and dynein using data from in vitro experiments[38, 39] **(Fig. 4e, and SI Appendix, Fig. S4a)**. Consistent with our experimental results, the number of minus-ended trajectories increases with increasing tau concentration **(Fig. 4a, d)**. Further, the model accurately describes the observation that the plus-end directed run lengths are not largely affected, but minus-end directed run lengths increase due to tau **(Fig. 4b, c)**. For comparison, we modeled the effect of tau on cargoes where only kinesin-1 or kinesin-2 is active. While the results are similar **(SI Appendix, Fig. S4)**, the model predicts that tau has a strong effect on kinesin-2 processivity, inconsistent with single-molecule experiments[4] **(Table S2)**. Thus, recruiting kinesin-1 to a cargo may be a way to confer sensitivity to regulation by tau.

By reconstituting the bidirectional motility of endogenous organelles in the presence of MAPs, we show that tau tunes the balance of plus- and minus-end directed transport through motor-specific regulation of processivity**.** On bidirectional cargoes driven by both kinesin and dynein, preferential inhibition of kinesin results in fewer engaged kinesins such that the kinesin team exerts less force. Dynein processivity is enhanced in response to reduced opposing forces, shifting transport towards the microtubule minus end. Regulation of transport by tau suggests a general mechanism, where small perturbations in the force generation or processivity of opposing motors results in biasing the net direction of transport.

Intracellular cargoes are often transported by dynein and multiple kinesin members. Kinesin-1, kinesin-2, and dynein interact to transport late endosomes[7, 25]. Kinesin-1, kinesin-3, and dynein associate with mitochondria and synaptic vesicle precursors[40, 41]. Employing multiple kinesin members on a single cargo might be a way to target tau regulation to specific cargoes. Mathematical modeling suggests that for cargoes transported by kinesin-1, kinesin-2, and dynein, regulation by tau is primarily mediated through kinesin-1 due to its high sensitivity to tau **(Fig. 4 and S4)**. These results imply that cargoes transported primarily by kinesin-1 and dynein, such as early endosomes, might be more strongly regulated by tau than cargoes where kinesin-2 is also present like late endosomes and phagosomes.

Further, the interaction between tau, motors, and microtubules is regulated through tau isoform expression and post-translational modifications. Six tau isoforms, generated through alternative splicing, differ in the number of microtubule-binding repeats and length of the acidic projection domain that extends from the microtubule surface[20, 42]. The shortest isoform of tau inhibits kinesin and dynein motility more strongly than longer isoforms[1, 2, 43]. Tau is modified by a variety of post-translational modifications, and over 85 putative phosphorylation sites have been identified [44-46]. While the function of most of these sites is unknown, phosphorylation of tyrosine 18 weakens tau's affinity to microtubules and in turn the inhibition of kinesin-1 motility[19, 47]. Thus,

multiple layers of regulation allow the cell to tune the local density and dynamics of tau to control trafficking in specific subcellular regions[17, 48].

Taken together, these studies suggest that in addition to its role in organizing the microtubule cytoskeleton[49, 50], tau has a central role in controlling the motility of motor proteins to direct trafficking. Further, different motors have varying sensitivity to tau, enabling tau regulation to be targeted to specific cargoes. Our observations indicate that defects in tau expression, localization, and binding dynamics would significantly alter the balance of plus- and minus-end directed transport, contributing to tau's role in neurodegenerative diseases[51].

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Author Contributions:
A.R.C and A.G.H designed research. A.R.C performed the experiments. F.B. wrote the mathematical model. C.L.B contributed reagents. A.G.H and A.R.C analyzed the data, and wrote the manuscript. A.G.H conceptualized the project and supervised the work.

Supplementary Information:
Supplemental Information for this article includes four supplemental figures, two supplemental tables, and four movies.

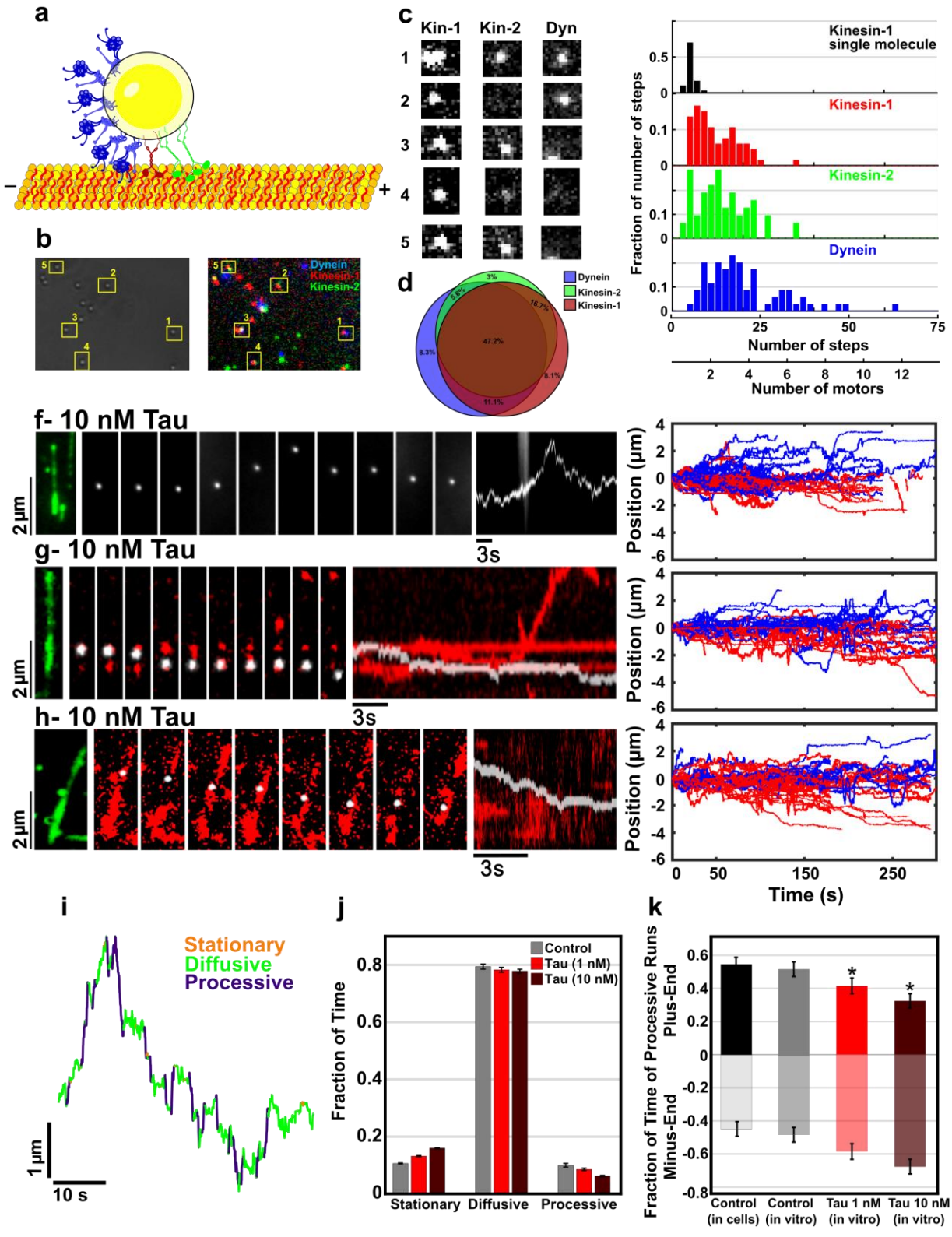

Figure 1. Tau directs the motility of isolated bidirectional cargoes towards the microtubule minus end.

(a) Isolated phagosomes are transported by teams of kinesin-1 (light violet), kinesin-2 (dark violet), and dynein (blue) motors. The microtubule-associated protein tau (red) binds along microtubules (yellow) and alters microtubule stability and motor-based transport.
(b and c) Isolated phagosomes were probed for kinesin-1 (red), kinesin-2 (green) and dynein (blue) antibodies to identify the types of motors associated with each phagosome. Number of phagosomes (n=55).
(d) Immunofluorescence indicates that approximately half of phagosomes are positive for kinesin-1, kinesin-2, and dynein. For other phagosomes, a subset of these motors was detected.
(e) Stepwise photobleaching was used to estimate the number of motors associated with phagosomes. To estimate the number of fluorophores bound to a single motor, single kinesin-1 motors (rkin430-GFP) were analyzed under similar conditions. Photobleaching indicates that ~ 2 kinesin-1, 3 kinesin-2, and 6 dynein motors are associated with each phagosome on average. N = 66 (kinesin-1), 63 (kinesin-2), and 80 (dynein) phagosomes. Example traces of stepwise photobleaching events are shown in **Fig. S1a, S1b, and S1c**.
(f-h) Isolated phagosomes move bidirectionally along microtubules, similar to their motility in cells. At low concentrations, tau binds in discrete clusters that diffuse along the microtubule lattice **(f)**. At higher concentrations tau decorates much of the microtubule lattice **(g)**. In the absence of tau, phagosomes exhibit approximately equal fractions of plus- (blue) and minus- (red) end directed movements. Tau biases the net directionality of cargoes towards the minus end in a dose-dependent manner. Number of trajectories, control (n=88), 1 nM tau (n=70), 10 nM tau (n=75). Number of independent experiments, control (n=4), 1 nM tau (n=6), 10 nM tau (n=6).
(i) Phagosome trajectories include periods of stationary, diffusive, and processive motility, characterized by the displacement between directional reversals (stationary: $L_R < 10$ nm; diffusive 10 nm $\leq L_R <$ 400 nm; processive $L_R \geq 400$ nm). The threshold for stationary runs was determined from the tracking error, and mean-squared displacement (MSD) analysis was used to determine the threshold for processive runs **(Fig. S2f)**.
(j) The fraction of processive runs decreases in the presence of tau.
(k) Tau increases the fraction of minus-end directed motility (*$p<0.05$). Error bars represent SEM.

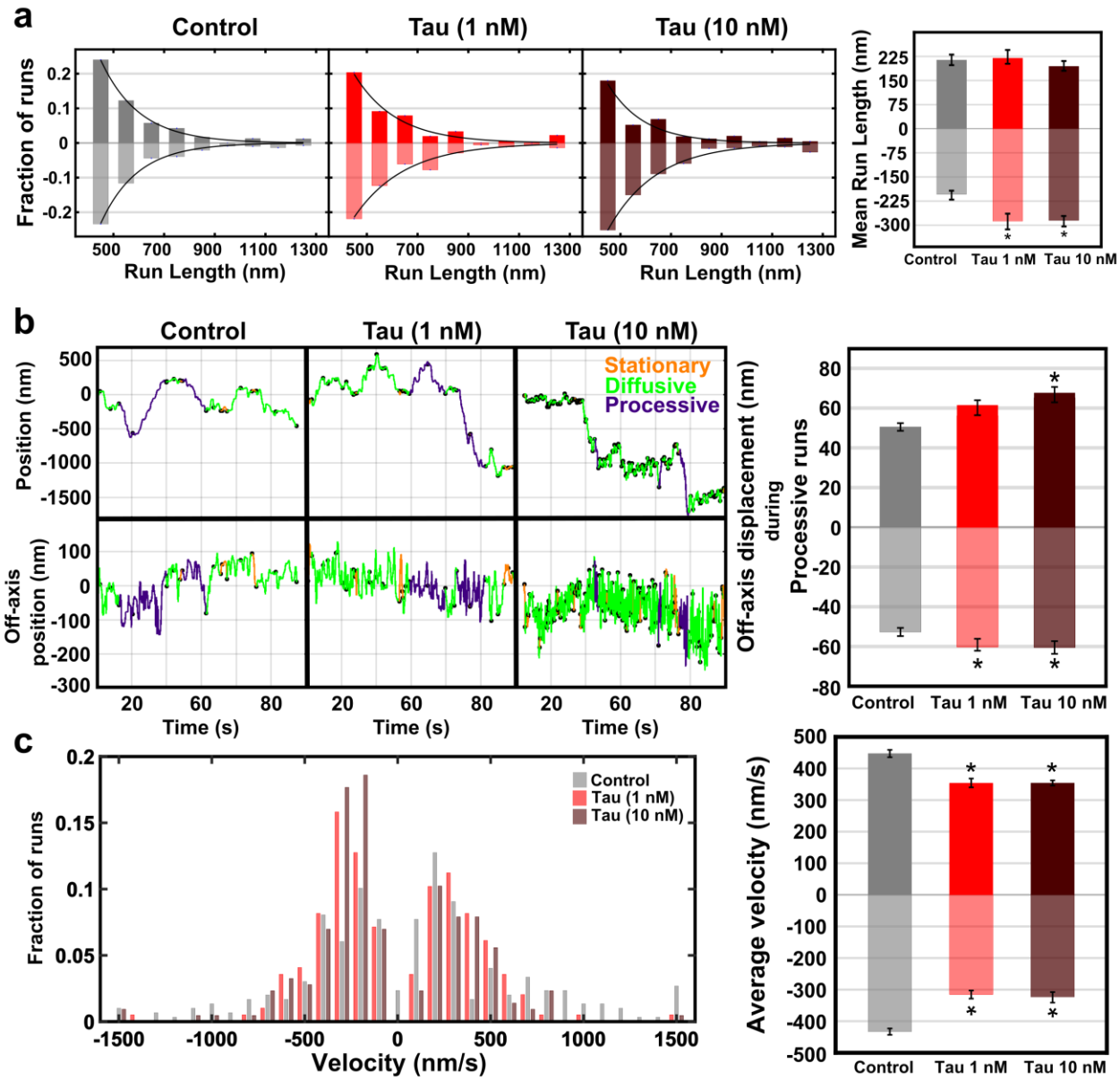

Figure 2. Tau increases the frequency of long dynein-directed runs.
(a) Plus-end directed run lengths are largely unaffected by tau, while long processive runs towards the minus end are more frequent (*p<0.05). Number of processive runs, control (n=298), 1 nM tau (n=207), 10 nM tau (n=215).
(b) Kinesin and dynein navigate around tau obstacles as indicated by increased off-axis displacements (*p<0.05).
(c) Average velocities of phagosomes towards the microtubule plus and minus end are decreased in the presence of tau, accompanied by more frequent off-axis displacements (*p<0.05). Error bars represent SEM.

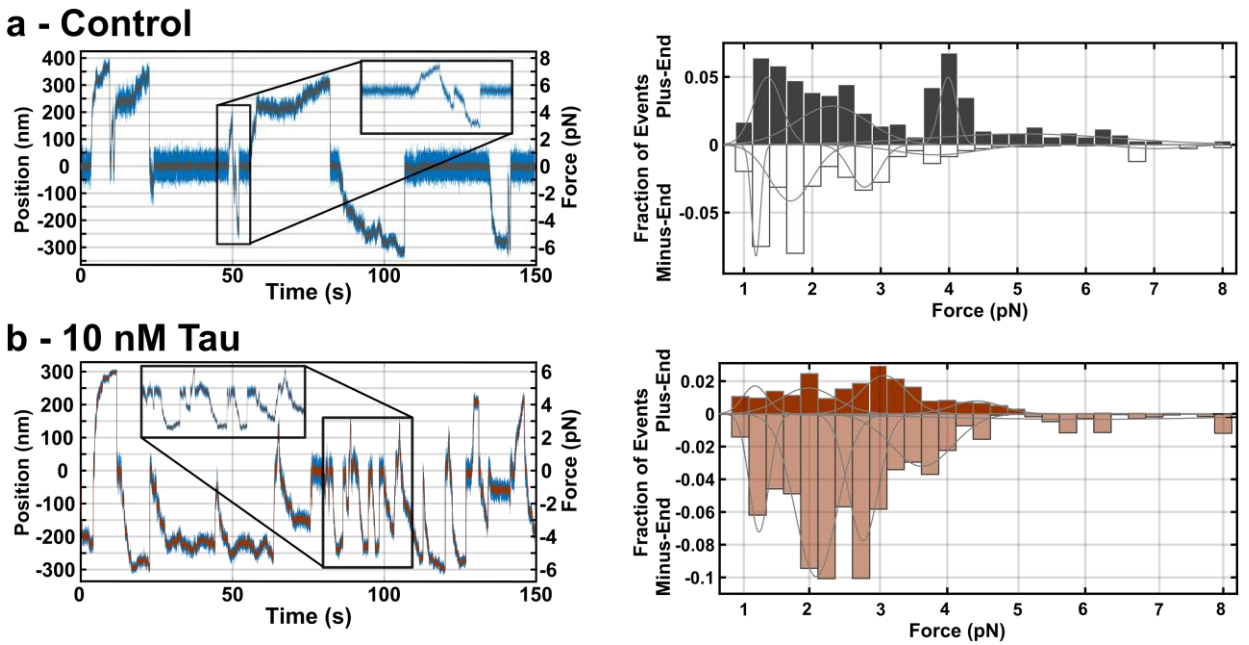

Figure 3. Dynein motors exert higher forces in the presence of tau.

(a) Isolated phagosomes were positioned on polarity-marked microtubules and the forces were measured using an optical trap. Force traces were acquired at 2 kHz and median filtered to 20 Hz. The histograms include the maximum forces for all trap displacements greater than 300 ms in duration (n=949 events from 60 recordings, 7 independent experiments). Plus-end directed force traces are consistent with stall events due to single kinesin-1 and -2 motors ~4-5 pN, as well as events where motors detach before reaching their maximal force and rare events driven by multiple kinesins. Minus-end directed forces indicate events driven by several dynein motors, each exerting ~1.2 pN. The Bayesian Information Criterion was used to determine the optimal number of components to describe the force histograms **(Fig. S3g,h)**. Mean forces of the multicomponent fits for plus-end directed forces are 1.36 pN, 2.3 pN, 4.12 pN, 5.14 pN, and for minus-end directed forces are 1.17 pN, 1.75 pN, 2.75 pN, 3.81 pN, 7.04 pN.

(b) In the presence of 10 nM tau, the frequency of force events in the plus-end direction are greatly reduced (n=1913 events from 81 recordings, 10 independent experiments). Kinesin exerts less force, and events greater than 5 pN are rare. In response, dynein exerts higher forces and dynein-directed forces are more frequent. Mean forces of the multicomponent fits for plus-end directed forces are 1.2 pN, 1.99 pN, 3.07 pN, 4.43 pN and for minus-end directed forces are 1.27 pN, 2.1 pN, 2.8 pN, 3.66 pN, 6.03 pN.

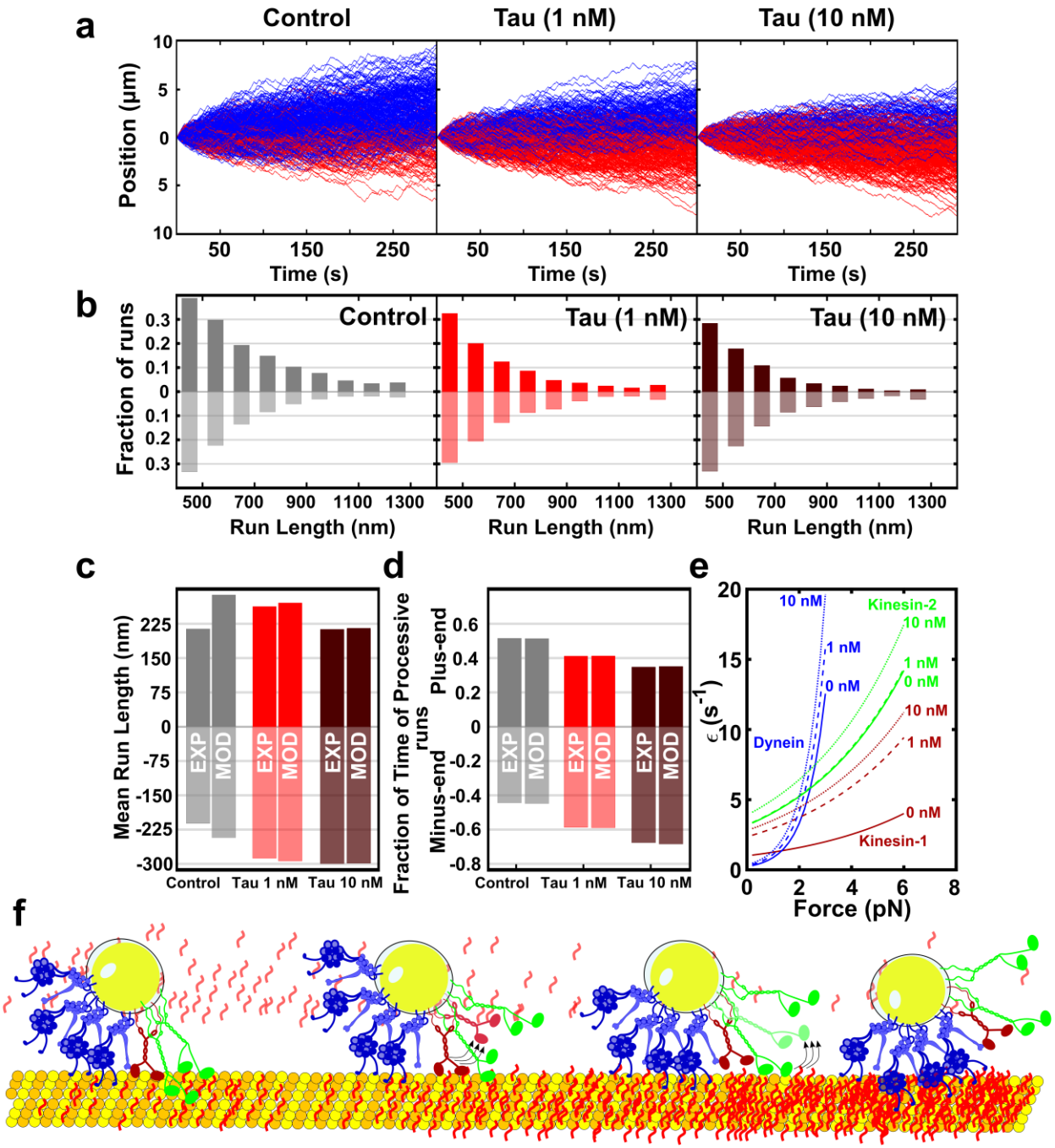

Figure 4. Mathematical modeling indicates that tau biases transport towards the minus end by preferentially inhibiting kinesin-1 processivity.

The model of bidirectional motility developed by Müller, Klumpp, and Lipowsky [38] was modified to describe the interaction of kinesin-1, kinesin-2, and dynein motors. Single motor parameters such as stall force, detachment force, unbinding and binding rates were determined from single molecule data [38, 39]**.** The effect of tau was modeled by increasing the dissociation rate of kinesin-1, kinesin-2, and dynein **([1, 4]; Table S1,2).** Trajectories were simulated for cargoes transported by one kinesin-1, two kinesin-2, and ten dynein motors.

(a) Simulated trajectories show bidirectional motility similar to isolated phagosomes. Lines indicate trajectories with net directionality towards the microtubule plus (blue) and minus (red) end. As tau concentration increases, the fraction of minus-end directed motility increases. Number of trajectories, control (n=400), 1 nM tau (n=400), 10 nM tau (n=400).
(b-c) Similar to the experimental trajectories, the run lengths of dynein-directed runs are increased by tau.
(d) The model indicates that tau regulates the directionality of cargoes by inhibiting the processivity of kinesin-1 more strongly than kinesin-2 and dynein.
(e) Data from single-molecule experiments was used to estimate the sensitivity of motor unbinding rates to load [39, 52-55] and tau [1, 4, 29] **(Fig. S4a)**. Kinesin-1 is more sensitive than kinesin-2 and dynein to tau.
(f) Through preferentially inhibiting kinesin-1, tau reduces the forces exerted by kinesin teams, resulting in enhanced dynein processivity and force generation. By targeting specific motors, tau's effect on motility can be tuned to each cargo depending on the makeup of the motor teams driving its transport.

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