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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA,
IRVINE

Intracellular cargo transport is determined by motors, cargo, and environment

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Physics

by

Matthew Jacob Bovyn

Dissertation Committee:
Associate Professor Jun Allard, Chair
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2021

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TABLE OF CONTENTS

	Page
LIST OF FIGURES	vi
LIST OF TABLES	viii
ACKNOWLEDGMENTS	ix
VITA	xi
ABSTRACT OF THE DISSERTATION	xix
1 Introduction	1
2 Background	4
2.1 Introduction	4
2.2 Microtubules	5
2.3 Molecular Motors	6
2.3.1 Kinesin	6
2.4 Cargos	9
2.5 Previous models of cargo transport	9
3 Cargo Routing at Microtubule Intersections	12
3.1 Abstract	13
3.2 Introduction	13
3.3 Results	17
3.3.1 Experimental Setup	17
3.3.2 Final navigational outcomes depend on 3D geometry	18
3.3.3 Characterization of Tug-of-War events	19
3.3.4 Mechanisms of cargo routing	21
3.3.5 A mathematical model of cargo transport reproduces observed switch probabilities	22
3.3.6 The influence of intersection geometry on ToW probability	25
3.3.7 The influence of intersection geometry on navigational outcomes, given ToW	26
3.3.8 The limits of geometric regulation	28
3.3.9 Low filament separations	30

3.3.10	Acute angles / intermediate separations	30
3.4	Discussion	32
3.5	Materials and Methods	34
3.5.1	Optical trapping and 3D motility experiments	34
3.5.2	Super-resolution imaging	35
3.5.3	Statistical analysis	35
3.6	Mathematical model for routing at intersections	36
3.6.1	Model description	36
3.6.1.1	Stochastic ordinary differential equations for cargo motion .	37
3.6.1.2	Poisson processes	41
3.6.2	Numerical simulation of the model	43
3.6.2.1	Model parameters	44
3.6.2.2	Initial conditions	44
3.6.2.3	Special case of 0 μm geometries	46
3.6.2.4	Fitting unknown parameters to data	46
3.6.3	Model Results	50
3.6.3.1	Categorization of trajectories	51
3.6.3.2	Encounter outcome dependance on MT geometry	51
3.6.3.3	Explanation of ToW probability	54
3.6.3.4	Explanation of conditional probability of switching given ToW	55
3.7	Acknowledgements	60
4	Diffusion of kinesin motors on cargo can enhance binding and run lengths during intracellular transport	61
4.1	Abstract	61
4.2	Introduction	62
4.3	Results	66
4.3.1	A computational model of cargo transport including freedom of motors to diffuse in the cargo membrane	66
4.3.2	Cargos with free motors bind to the microtubule faster than those with rigidly anchored motors	70
4.3.3	Cargos with free independent motors form dynamic clusters which increase travel distance	73
4.3.4	Cargos with free independent motors are better able to transport against a load compared to cargos with rigid dispersed motors	77
4.4	Discussion	78
4.4.1	Cargo size provides a potential sorting mechanism and avenue for studying motor-cargo attachment	79
4.4.2	Comparison with previous studies	81
4.4.3	Influence of cargo surface mobility on transport	83
4.4.4	Quantitative model provides framework to infer motor organization .	83
4.4.5	Future model extensions	85
4.5	Methods	86
4.5.1	Chemomechanical model of cargo transport with viscous membranes .	86
4.5.2	Estimate for viscosity experienced by cargos rotating in the cytoplasm	87

4.5.3	Extrapolation of rebinding time from previous work <i>in vitro</i>	87
4.5.4	Quantification of rebinding time for lipid droplets	88
4.5.5	Time for a motor to reach the microtubule	89
4.5.6	Range of accessible motor diffusion coefficients	90
4.5.7	Model for free clusters	90
4.5.8	Curve fitting	91
4.5.9	Inference on numbers of motors from published data	92
4.6	Mathematical model of cargo transport with motor freedom	98
4.6.1	Model description	98
4.6.1.1	Stochastic ordinary differential equations for cargo motion	99
4.6.1.2	Construction and discretization of the stochastic ordinary differential equations	104
4.6.1.3	Poisson processes	106
4.6.2	Numerical simulation of the model	108
4.6.2.1	Model parameters	110
4.6.2.2	Initial conditions	113
4.6.3	Validation of the simulation	115
4.6.3.1	Drag of anchors through the cargo membrane	115
4.6.3.2	Anchor diffusion in the cargo membrane	117
4.7	Acknowledgments	117
5	Cargo rotation plays an important role in binding to the microtubule	118
5.1	Abstract	119
5.2	Introduction	119
5.3	Results	121
5.3.1	Quantification of rebinding times	121
5.3.2	Rebinding data is not explained by a previous 3D model of cargo and motor	123
5.3.2.1	3D model predicts binding events which are not detectable	123
5.3.2.2	Simulated events are only detectable when motor heads pass a threshold	127
5.3.3	Beads which rotate more slowly also rebind more slowly	127
5.3.4	Compartmental model	129
5.3.4.1	Compartmental model can match rebinding times from simulation at physically reasonable parameters	131
5.3.5	Motors must sometimes leave the microtubule	133
5.4	Discussion	134
5.4.1	Relationship between motor on rate, cargo rotation, and rebinding time	134
5.4.2	Bead heterogeneity	135
5.4.3	Detectability of binding events	135
5.4.4	More complex models	136
5.4.5	Connections between on and off rates	138
5.5	Methods	138
5.5.1	Bead assay	138
5.5.2	Description of data and uncertainty estimation	139

5.5.3	Curve fitting	141
5.5.4	Manual annotation of force traces	141
5.5.5	3D model and simulation	142
5.5.6	Adjustments to previous model for this experimental system	142
5.5.6.1	Motor length	142
5.5.6.2	Surface effects	143
5.5.6.3	Inclusion of optical trap	143
5.5.6.4	Parameter values	144
5.5.7	Three state compartmental model	144
5.5.7.1	Derivation of mean and distribution of rebinding times	144
5.5.7.2	Parameterization	145
5.5.7.3	Determination of the area ratio parameter r	146
5.5.8	Mean first passage time out of a spherical cap by cargo rotation	147
5.6	Acknowledgments	149
6	Discussion	150
6.1	Motors, cargo and environment dictate transport outcomes	151
6.1.1	At microtubule intersections	151
6.1.2	In the context of the cell	152
6.1.3	In rebinding	153
6.2	Towards bridging in vitro measurements into insights inside the cell	155
6.3	Science this work enables	157
	Bibliography	159

LIST OF FIGURES

	Page
2.1 Super-resolution image of a MT network	5
2.2 Microtubule structure	6
2.3 Composition of transport kinesins	8
2.4 Bidirectional motion and cargo delivery	10
3.1 3D MT crossings and cargo navigational outcomes	15
3.2 Identification and quantification of ToW	20
3.3 Restoring force on bead handles	21
3.4 Cargo navigation flow chart, with associated outcome probabilities	23
3.5 Mathematical model of navigational outcomes	24
3.6 Solutions to the heuristic model for ToW probability	26
3.7 Empirical cumulative probability distributions for ToW times	27
3.8 Number of motors engaged on MTs during ToW	29
3.9 Mechanisms that elevate switch probabilities	31
3.10 Diagrams of simulated cargo dynamics	37
3.11 ToW probabilities for varying combinations of the unknown parameters	48
3.12 Experimental observations inform selection of unknown parameters	49
3.13 Snapshots from simulated cargo trajectories	52
3.14 Probabilities of ToW and switching given ToW combine to explain switch probability	53
3.15 A heuristic model matches qualitative features of ToW probability	55
3.16 Numbers engaged, forces and ToW times explain conditional probability of switching	57
4.1 Mean first passage times to the microtubule	64
4.2 Transport outcomes and motor organization modes	68
4.3 Cargo time to bind	71
4.4 Run lengths and dynamic clustering	74
4.5 Run length against load	78
4.6 Qualitative comparison of transport properties for cargos in different organization modes	80
4.7 Mean reach elevation	93
4.8 Cargo time to bind distributions	94
4.9 Run length distributions	95
4.10 Forces on cargo	96

4.11	Model cartoon	99
4.12	Comparison of simulated with analytical results	116
5.1	Diagram of rebinding interpretation	122
5.2	Example section of an annotated trace	122
5.3	Rebinding time distributions for individual 560nm diameter beads in standard assay buffer	124
5.4	Examples of experimental and simulated traces	125
5.5	Comparison of measured and predicted rebinding time distributions	125
5.6	Comparison of simulated true and annotated rebinding time distributions	126
5.7	Comparison of simulated true and recovered mean rebinding times	126
5.8	Example trace with cargo center and motor heads locations	127
5.9	Means and distributions of rebinding times for beads with varying diameters	128
5.10	Means and distributions of rebinding times for beads in glucose solutions of different viscosities	128
5.11	Mean rebinding times of all conditions plotted against mean rotational diffusion coefficient	129
5.12	Rebinding time distributions for all beads	130
5.13	Three state model	131
5.14	Compartmental model can match simulated rebinding times	132
5.15	Qualitative features of median rebinding time	133
5.16	Geometric constraints on surface area of bead	148

LIST OF TABLES

	Page
3.1 List of parameter values used	45
4.1 Measured rebinding times for lipid droplets	89
4.2 List fit values	97
4.3 List of parameter values used	114
5.1 Computational model parameters	144
5.2 Accessible parameter ranges	147

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- M. J. Bovyn, B. J. N. Reddy, S. P. Gross, and J. F. Allard, “Roles of motor on-rate and cargo mobility in intracellular transport”, bioRxiv, 2020.07.13.201434 (2020)

- M. J. Bovyn, “Geometry Matters for Cargos Navigating 3D Microtubule Intersections”, Master’s Thesis (University of California, Irvine, 2019), p. 79
- D. E. Chapman, B. J. N. Reddy, et al., “Regulation of in vivo dynein force production by CDK5 and 14-3-3 ϵ and KIAA0528”, *Nature Communications* **10**, 228 (2019)
- J. P. Bergman, M. J. Bovyn, et al., “Cargo navigation across 3D microtubule intersections.”, *Proceedings of the National Academy of Sciences* **115**, 537–542 (2018)
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- W. M. Grundy, S. J. Morrison, M. J. Bovyn, S. C. Tegler, and D. M. Cornelison, “Remote sensing D/H ratios in methane ice: Temperature-dependent absorption coefficients of CH₃D in methane ice and in nitrogen ice”, *Icarus* **212**, 941–949 (2011)
- S. C. Tegler, D. M. Cornelison, et al., “Methane and nitrogen abundances on Pluto and Eris”, *The Astrophysical Journal* **725**, 1296–1305 (2010)

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CONTRIBUTED TO APPLICATION FOR NIH R01 awarded to Jun Allard and Steve Gross (NIGMS R01 GM123068), which funded my work.

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ABSTRACT OF THE DISSERTATION

Intracellular cargo transport is determined by motors, cargo, and environment

By

Matthew Jacob Bovyn

Doctor of Philosophy in Physics

University of California, Irvine, 2021

Associate Professor Jun Allard, Chair

Eukaryotic cells are divided into a complex system of compartments, with their spatial organization ever-changing to enable cell functions. For example, in neurons, packages of neurotransmitters are transported from the cell body, down axons, to synapses, where their delivery mediates learning and memory. Phagosomes are transported to meet lysosomes, which fuse to kill pathogens. We know a great deal about the kinesin, dynein, and myosin molecular motors which do mechanical work to drive this motion, as well as the microtubule and actin tracks they are transported along. However, how the many different cargos in a cell are transported using largely the same sets of motors and tracks, but to different outcomes, remains largely a mystery. To understand how cargos can be directed, we build a model of cargo movement at the scale of a cargo. We build a simple model of how motors work from the details known in vitro, then incorporate the physics of a small solid body in a fluid. We apply this model to give insight into several problems. We show that when two microtubules cross at an intersection, they interact with motor and cargo dynamics to dictate outcomes that depend on geometry of the intersection. In another study, we show that organization of motors on the cargo surface can drive different transport outcomes. When motors are attached rigidly to the surface of a cargo, the cargo faces a tradeoff between fast binding and efficient transport. However, when motors are free to diffuse in the cargo membrane, both are possible. In a third study, we take and analyze data on how cargos bind in vitro,

and show that a mismatch between model prediction and experimental outcomes provides insight into the behavior of motor molecules. Overall, these studies show that looking at intracellular cargos as 3D objects can provide insight into how cargo transport functions in the cell, as well as providing a bedrock for understanding how and why cells may manipulate the behaviors that emerge from the individual components when they are put together.

Chapter 1

Introduction

In our search to understand the world around us, the first step is often to catalogue and organize the things we observe. In the study of the living world, our cataloging and organization has led to the view that living things, when painted with the broadest possible brush, are best organized into three domains: bacteria, archaea, and eukaryota [9]. Eukaryotes, the domain that includes humans, have cells which display rich internal structure, with compartments that separate different chemical environments from each other using membranes. This organization was present in the last common eukaryotic ancestor [10]. A subset of these compartments which occur across cells of different tissues and in different organisms are called organelles: mitochondria, lipid droplets, lysosomes, endoplasmic reticulum, peroxisomes, Golgi, and others. Recent advances in fluorescence microscopy have allowed us to view multiple organelles together in the same cell at the same time [11]. Analysis of the spatial distribution of organelles shows both that different organelles are distributed differently and that contacts between the various organelles are not random [11]. From observations like this, we gather that the organization of organelles in space and time is controlled by the cell.

Membranes are not the only way for cells to organize themselves. Reaction-diffusion systems and phase separated compartments are other examples of ways cells can localize molecules and change local chemical composition (for examples, see the *e. coli* min system [12] and P granules in *d. melanogaster* embryos [13]). Why use membranes? One evocative example is that of the lysosome. Lysosomes are filled with molecules that are able to break down biological material. This allows, for example, macrophages to engulf and destroy bacteria which have invaded a human body. The membrane which separates the molecules on the inside of the lysosome from the rest of the cell allows cells to keep those molecules around without digesting themselves.

The dynamic organization of these compartments in space and time is essential for cells to live, as well as to fulfill their function in the greater context of an organism. For example, neurons communicate with each other via signals passed back and forth at points called synapses. Chemical signals passed back and forth at these synapses bind to receptor molecules on the cell's surfaces. The receptors then pass signals into the cell. Therefore controlling the number of receptor molecules at a synapse is a way of controlling how neurons communicate — one which is essential for the processes of learning and memory [14]. For another example, Herms, Bosch, et al. [15] examine monkey kidney cells grown in culture. When these cells are fed a diet with glucose, mitochondria are localized near the nucleus and lipid droplets are localized near the cell periphery. Lipids from the lipid droplets are used at the cell periphery to make more membrane, allowing the cells to grow and divide. However, when these cells are starved of glucose, the mitochondria move out and the lipid droplets move in, allowing them to contact each other. When this happens, the mitochondria are able to take in the lipids from the lipid droplets and use them to keep up the cell metabolism.

The examples in the preceding paragraph are referred to as intracellular transport — we can understand the compartments as holding some important resources (receptors or lipids in the examples), which need to be transported to the correct place in the cell. The neuron example

reveals that cargo transport can be precise: the right amount of receptors are dropped off at particular synapses, each $1/1000$ the length of the neuron in which they reside. The starved cells example reveals that cargo transport is multiplexed: cargos can directly differently at the same time.

In the work in this dissertation, I examine how cells are able to perform this function of directing cargo transport. I first give some background on the systems cells use to perform the transport in chapter 2. Then, I detail three studies on how cargos are transported in chapters 3, 4 and 5. Finally, I tie lessons from the three studies together in chapter 6.

Chapter 2

Background

This chapter is adapted from [2], written by Matthew Bovyn.

2.1 Introduction

The advent of video microscopy brought the dynamic nature of the organelle environment into focus in the early 1980s [16, 17]. Soon after, Ron Vale and colleagues discovered the identity of one of the proteins responsible for the observed transport, kinesin [18]. In the more than 30 years since this discovery, we have learned a great deal about each of the components of the transport system from a combination of careful in vivo and in vitro experimental work, as well as enlightening modeling efforts.

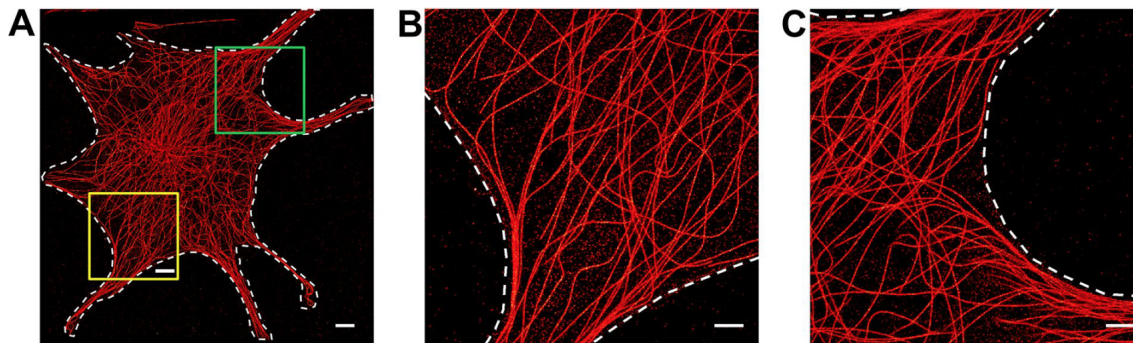


Figure 2.1: An example of a super-resolution image of an entire MT network of a cell (A), adapted from [1], licensed under CC BY-NC-SA 3.0. Single MTs are identifiable from fluorescence labelling in red. Region in yellow box magnified in (B), region in green box magnified in (C). Scale bars are $5\mu\text{m}$ in panel A and $2\mu\text{m}$ in panels B and C.

2.2 Microtubules

Eukaryotic cells possess a microtubule (MT) network, composed of hundreds of individual MT filaments. Recently, super-resolution microscopy has allowed imaging and mapping of the entire MT network with single MT resolution [1], as shown in figure 2.1. Individual MTs are dynamic, assembling and disassembling stochastically [19]. This allows the MT network to be reorganized as cells move and change shape, as well as in response to external cues [1, 15, 20].

The MT network serves many functions in the cell. Most importantly for the work in this document, MTs serve as the “roads” along which cargos are transported. In addition to this role, MTs are a component of the cytoskeleton, which gives cells shape and structure. Furthermore, MTs bear the forces exerted to divide chromosomes between the daughter cells in cell division.

The structure of MTs allow them to perform these functions. MTs are tubular, consisting of a number of linear protofilaments assembled into a ring [21] as shown in figure 2.2. MTs commonly have 13 or 14 protofilaments [22], but that number can vary from fewer than 9 to more than 15 depending on organism and cell type [23]. Each protofilament is a repeating

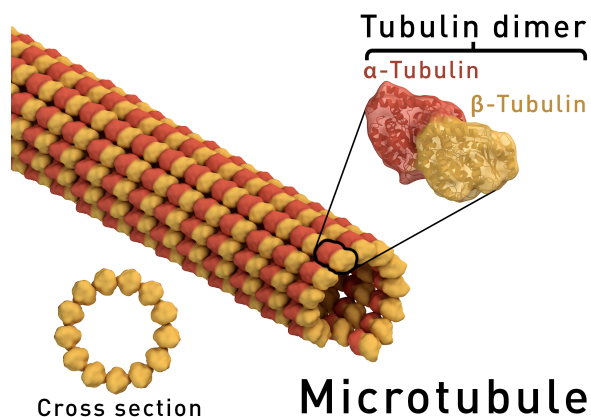


Figure 2.2: The structure of a microtubule is shown, demonstrating the tubular structure composed of linear protofilaments made up of repeating dimers of α and β subunits. Illustration “Structure of a microtubule” by Thomas Splettstoesser is licensed under CC BY-SA 4.0.

polymer of dimers of α and β tubulin subunits. This repeating structure makes it possible for kinesin and dynein to move processively along the MT outer surface. MT structures can have defects, and it has been shown that these defects can influence transport [24].

The tubular structure makes MTs rigid, with persistence lengths near 1 mm, many times the size of an entire cell. Rigidity can vary with MT length and repeated bending [25].

2.3 Molecular Motors

Molecular motors are a class of enzymes (specifically ATPases), which convert the chemical energy of ATP into mechanical work. There are three classes of molecular motors involved in cargo transport: kinesin superfamily motors and cytoplasmic dynein which walk along microtubules, and myosin superfamily motors which walk along actin. Here we’ll focus on kinesin.

2.3.1 Kinesin

The defining feature of a kinesin is the kinesin motor domain, which binds to MTs and is responsible for ATP hydrolysis [26]. Hundreds of genes which include the kinesin motor

domain have been identified across a variety of different organisms; together these genes make up the kinesin superfamily. This superfamily has been divided into 14 families based on function and sequence similarity [27]. In mice, there are 45 individual kinesins which perform a variety of tasks in the cell, from transporting cargo to MT depolymerization [28]. The motors which have established roles in transport are the members of the kinesin-1 (KIF5), kinesin-2 (KIF3) and kinesin-3 (KIF1) families [26]. Kinesin-1 is the most well studied family, also called conventional kinesin. It is a heterotetramer of a kinesin heavy chain homodimer and two kinesin light chain subunits, as shown in figure 2.3. Kinesin-2 can exist either as a homodimer or heterodimer of different motor domain containing subunits, with each form associated with different motility properties. However, the motility mechanism of kinesin-2 is similar to kinesin-1 [29]. Kinesin-2 motors also tend to switch protofilaments while walking, resulting in a spiral path around the MT [30], where kinesin-1 motors walk a straight line [31]. Kinesin-3 motors exhibit limited motility as a monomer, but likely work as dimers in vivo, similar to kinesin-1 and kinesin-2 motors [32]. We will focus on kinesin-1 for the rest of this section and will refer to it simply as kinesin.

Kinesin motors step processively along MT tracks in a hand-over-hand fashion, with each motor domain taking 16 nm steps [33] that move the center of mass of the motor forward by 8 nm [34]. When unloaded, kinesin steps quickly, moving about $1 \mu\text{m s}^{-1}$. Times between steps are exponentially distributed [35]. Velocity reduces under increasing resistive load until stall, which occurs at $\approx 6 \text{ pN}$. The nature of this decrease has been found to be superlinear in some studies [36–39], while others find more linear behavior [40, 41].

Kinesin unbinds from the microtubule at a rate that also depends on the load experienced by the motor. Several reports agree that unbinding rate increases with load exponentially up to the stall force, but disagree about behavior above stall. One study claims unbinding rate increases only slowly above stall [42], while another claims unbinding rate continues to increase exponentially [41]. The unbinding rate has been found to depend on the direc-

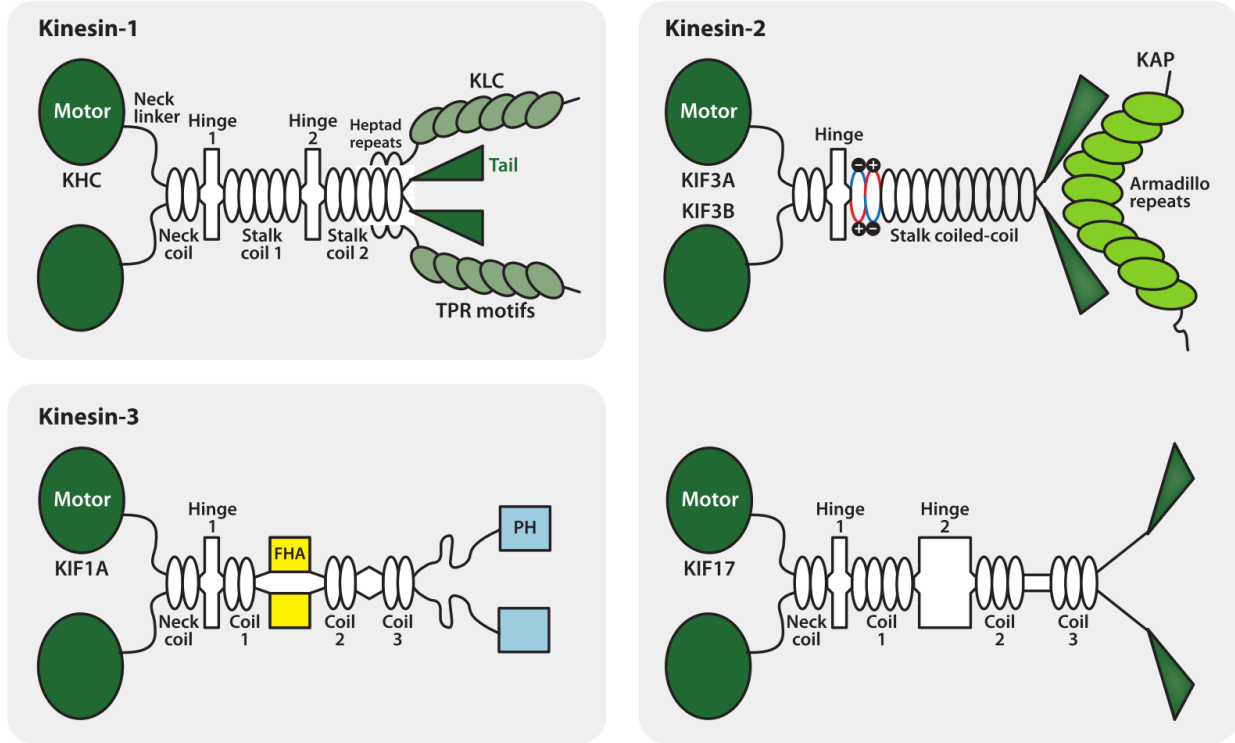


Figure 2.3: Subunit composition of transport kinesins. Kinesin-1 or conventional kinesin is the most well studied motor family. Kinesin-2 and kinesin-3 are less studied, but also known to transport cargos in cells. Kinesin motor domains are the defining feature of the kinesin superfamily and are shown as green ovals. Kinesin motors domains bind to the MT, which the other end of the motors bind cargos or linkers. Abbreviations: FHA, forkhead associated; KAP, kinesin-associated protein; KHC, kinesin heavy chain; KIF, kinesin family; KLC, kinesin light chain; PH, pleckstrin homology; TPR, tetratricopeptide repeat. Figure from [26].

tionality of the load applied. Hindering loads result in the aforementioned behavior, while assisting loads result in an increased unbinding rate [41, 43]. Sideways loads result in slightly asymmetrical unbinding behavior [44].

The stiffness of the kinesin stalk is has been measured in tension [45] and also resists compression to a lesser degree [46]. It has little resistance to torsion [47, 48].

2.4 Cargos

A wide variety of organelle cargos such as lipid droplets, mitochondria, melanosomes, peroxisomes, pigment granules, endosomes, secretory vesicles, RNA granules, and virions are transported bi-directionally by molecular motors [49, 50]. Long distance cargo transport is also particularly important in neurons. Cargos such as dense core vesicles carrying neuropeptide and synaptic vesicles carrying neurotransmitter must be transported down the length of axons, often millimeters to centimeters long, and delivered at presynapses [51]. These observations lead to a picture of cargo transport where bidirectional motion is controlled to correctly localize cargos, as shown in figure 2.4.

2.5 Previous models of cargo transport

An early model of multiple motor cargo transport posed the problem as a Markov chain, with state transitions representing binding and unbinding of the motors [52]. It was shown this model was able to reproduce a variety of the behaviors observed in vivo [53]. Despite its success, it was difficult to interpret how experimentally observed parameters translated to model parameters, including how forces are shared between multiple motors and the stochastic nature of motor stepping.

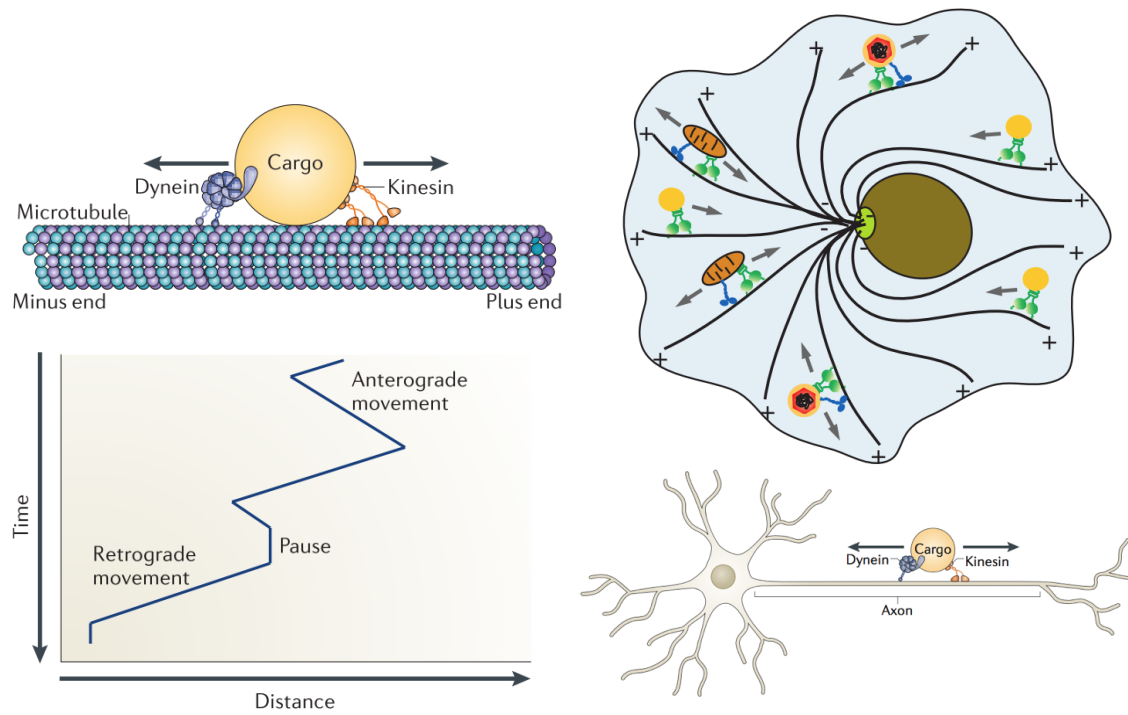


Figure 2.4: A variety of cargos have been shown to move bidirectionally on MTs in cells, as shown on the left. By biasing this bidirectional motion, the cell may be able to deliver cargos to specific locations in neurons and other cell types, as shown on the right. Figures on left and bottom right from [49]. Figure on top right from [50].

A subsequent model represented motors as two points, with forces in each generated by the stretch in spring-like motor stalks [54]. While only one dimensional spatially, this model was able to elucidate many details of how multiple motors might work together [36]. In a surprising negative result, the Gross lab showed that neither model was able to match the behavior of cargos in vivo when supplied with realistic single motor behavior [42].

Three dimensional models of cargo transport have also since been constructed [55–57]. They have been used to investigate the impact of how motors are arranged on a cargo [56] and cargo switching between filaments [58]. Recently, a model of cargo transport included the ability for motors to diffuse in the cargo membrane in 3D [57].

Chapter 3

Cargo Routing at Microtubule Intersections

The following chapter is adapted from a manuscript created by Jared Bergman, Matthew Bovyn, Manasa Gudheti, Steven Gross, Jun Allard and Michael Vershinin. The project was initiated by Michael Vershinin. Michael Vershinin and Jared Bergman developed the experiments. Cargo navigation experiments were performed by Jared Bergman. Manasa Gudheti performed super-resolution microscopy. The model was created by Matt Bovyn, Steve Gross, and Jun Allard. Matt Bovyn wrote and performed simulations. The model was modified and developed to fit the experimental situation by Matt Bovyn with advice from Jun Allard, Steve Gross, Jared Bergman and Michael Vershinin.

Jared Bergman, Matt Bovyn and Michael Vershinin wrote the description of the main findings of the work. Matt Bovyn and Jun Allard wrote the description of the model and model results. The manuscript was published as [3]. A similar adaptation was included in [2].

3.1 Abstract

Eukaryotic cells transport cargos along microtubules to control their distribution within the cell and deliver them to distant locations. While we understand how molecular motors can transport cargos along individual microtubules, the cell’s microtubules are usually arranged in a complex 3D network. While traversing this network, cargos need to navigate intersections where microtubules cross at a wide variety of separation distances and angles. To gain insight into how cargos navigate these intersections, we have used a recently established 3D construction technique based on holographic optical trapping to build single 3D microtubule intersections in vitro with relevant nanoscale precision. We then used these fully suspended microtubule structures to perform motility assays on kinesin-1 coated cargos. We find that some intersection geometries influence cargos to pass along their current microtubule, while other geometries influence them to switch to the intersecting one. To understand how, we use a 3D Brownian dynamics simulation of cargo transport to investigate the mechanisms which give rise to the observed switching probabilities. Using these stochastic simulations, we find that switching probability is often determined by a competition between a stronger motor team on the primary microtubule and the intersecting microtubule sterically hindering that team’s progress. This understanding of the basic mechanisms of switching at single intersections in 3D helps lay a foundation for understanding how the cell may regulate switching to control how cargos navigate the MT network and ultimately their spatial organization.

3.2 Introduction

The microtubule (MT) network in eukaryotic cells is typically a dense, highly variable, three-dimensional (3D) mesh (Fig. 3.1A). MT network topologies are known to vary widely between cells [59] and even between cells of the same type and lineage [60]. Within a given

network, MTs converge to form crossings at a variety of filament separations, and angles of intersection [61]. Often, the crossings feature inter-filament separations that are comparable to the scale of the cargos found within the cell (Fig. 3.1A). At these “intersections,” a cargo, with multiple motors on its surface, can potentially interact with several MTs simultaneously; a scenario known as Tug-of-War (ToW) [53, 62]. The cargo can then either pass along the original MT, switch to the intersecting filament, pause, or detach. The probabilities of these outcomes are known to be sensitive to the 3D layout of the filaments [58, 63, 64] but the mechanistic details of this phenomenon are unclear. Given that the architecture/topology of the MT cytoskeleton serves as a persistent and pervasive backdrop for cargo transport [65], its role in cargo routing warrants a thorough investigation.

The importance of MT cytoskeletal architecture is underscored by the fact that MT network remodeling occurs often in various diseases and during normal cellular processes. For example, neuritic de-arborization or restructuring is often encountered in neurodegenerative diseases [66, 67]. Microtubule remodeling is also observed in many neoplasias [68] and is associated with pathways often disturbed in cancers [69]. There is also evidence that suggests the geometry of the MT network, itself, acts as a regulator to tune insulin granule secretion in mouse pancreatic β -cells [20].

The cell can use multiple mechanisms to modulate its MT architecture. MTs can be locked in parallel [70] or antiparallel [71] alignment via cross-linking. Axonal branching generally shows a preference for normal angles [72]; low branch angles can arise from MT nucleating factors [73]. The cell can set overall MT spacing by simply controlling the amount of polymerized tubulin, either via regulation of expression levels [74] or filament stability [75]. Filament spacing can also be controlled via MAPs that cross-link microtubules [76]. Microtubule and actin networks are often coupled and remodeling of one can drive structural changes in the other [77, 78]. External factors which affect cell shape can also regulate MT network topology [79]. Finally, MT crossings are known to be special loci for intracellular regulation

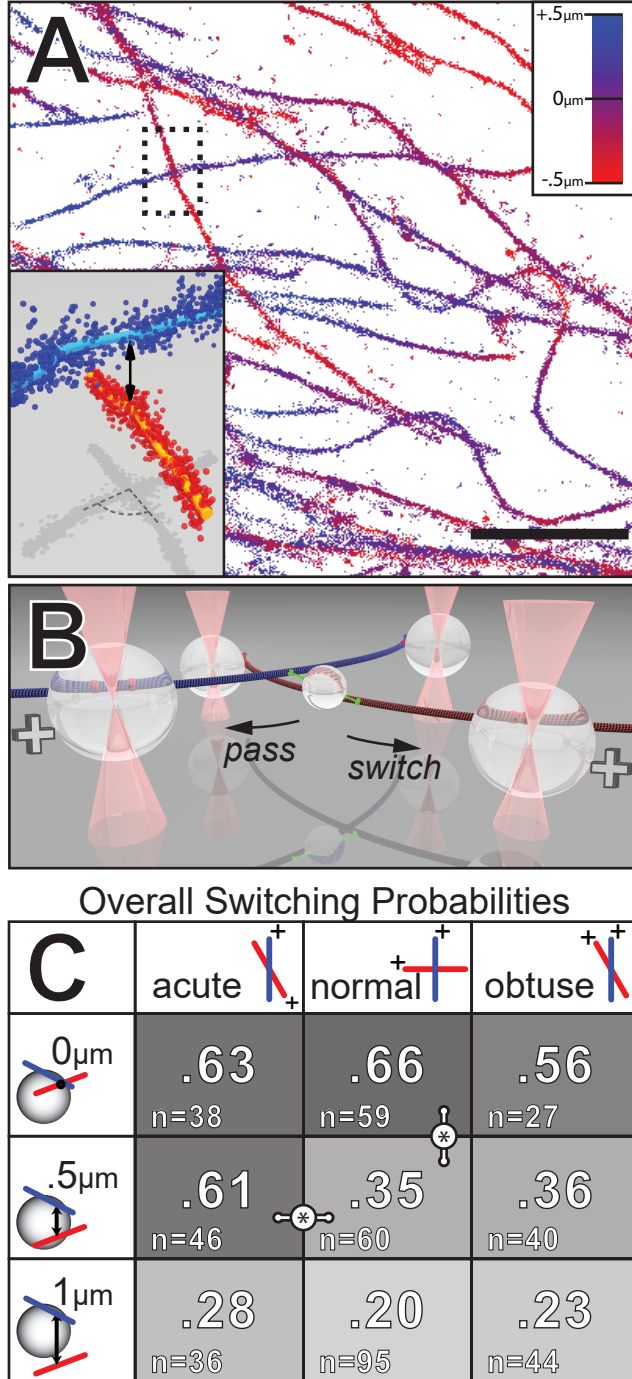


Figure 3.1: 3D MT crossings and cargo navigational outcomes.

(A) 3D STORM image of MT cytoskeleton from BSC-1 cell. Color coding indicates relative depth. Scale bar: 4μm. (Inset) Perspective image of the MT crossing highlighted in dashed box. MT position fits (light blue and orange) are shown along with the registered photon origins (red and blue). MT separation at point of closest approach (double arrow) and MT-MT angle (protractor, dashed lines) are annotated for clarity. A few photon origins near MT-MT intersections are not shown for clarity of annotation.

(B) Illustration of in vitro 3D MT crossing with an MC undergoing ToW. BHs (large spheres) are permanently bound to MTs (blue and red). BHs are held in 3D via HOTs (light pink cones). MT plus ends are indicated (+ signs). For clarity, we only depict two motors on the MC (small bead), engaged on either MT. Navigational choices are indicated with arrows.

(C) Table showing switching probabilities as a function of 3D MT arrangement. Higher switch rates are highlighted by darker background. Significant differences ($p < 0.05$, Barnard's test) are indicated by links.

[80]. Currently, the implications of these topology variations for cargo logistics and overall biomechanics are not quantitatively understood.

Decoupling the influence of the MT network’s 3D topology from regulatory protein factors is challenging. The same pathways that drive network remodeling can also couple to motor regulation. The result is that, to date, the impact of geometric changes in MT networks on intracellular cargo transport has been difficult to isolate and quantitate. It is common in biology to think in terms of chemical regulation. However, to truly understand how intracellular cargo transport functions, it is critical to gain a baseline understanding of how the 3D MT geometry alone impacts cargo distribution, starting at the most fundamental level of the MT network: individual MT-MT intersections. Studying cargo navigational behavior as a function of 3D network geometry poses considerable experimental challenges. In vivo investigations cannot easily decouple chemical and topological regulation, as discussed above. Moreover, although theoretical work highlights its importance [58], in vitro bead assays that use traditional MT-glass deposition techniques are also unable to model MT-MT crossings with controlled filament separation [64, 81].

To address this question, we developed an in vitro system to suspend and dynamically manipulate multiple individual MT filaments in 3D. Each MT is manipulated individually via holographic optical trapping (HOT) of two or more bead handles (BH), as previously described [82]. We used this bottom-up approach to systematically construct MT-MT intersections featuring various angles and separations. We then measured the statistics of kinesin-1 driven model cargo (MC) transport on these model MT geometries, and used this data to constrain 3D simulations. We show from experimental data and theoretical analysis that navigational outcomes exhibit systematic variation based on 3D MT intersection geometry. Further, we propose dynamic mechanisms that explain the observed preferences.

3.3 Results

3.3.1 Experimental Setup

The broad aim of this work is to understand the impact of cytoskeletal geometry on intracellular transport. A comprehensive experimental model of all possible geometries (e.g. Fig 3.1A) is well beyond the scope of any singular study, so we restricted our scope to representative model scenarios. We focused on the simplest type of intracellular MT intersections where just two filaments cross (Fig. 3.1A, inset). We used silica microspheres, driven by full length KHC homodimers, as our MC. This is a common, albeit simplified, model for in vitro work. We chose to focus on assays outside the single-molecule regime because there is substantial evidence that cargos in cells are often driven by multiple motor ensembles, and indeed multiple-motor ensembles are essential for a ToW to develop.

We used in vitro 3D MT manipulation via HOT [82] to examine three filament spacings: zero, radius, and diameter of the MC. Our MCs were 1 μm in diameter, hence we constructed and observed MC transport across model intersections featuring 3 separation distances (0 μm , 0.5 μm , and 1 μm). We decided to only parametrize this geometric range because the probability of a cargo interacting with both MTs for crossing separations greater than the cargo diameter quickly becomes negligible. For each separation distance, we examined three different angles of intersection, acute (MT polarities nearly counter aligned), normal (90°), obtuse (MT polarities nearly aligned), for a total of nine geometric conditions.

We chose silica microspheres as our MCs because their density is 2.2x that of water. This biased the cargo to hang below the MT to which it was engaged, although Brownian motion along all three axes was both expected and observed. Thus, in our setup, we always deposited the MC (via HOT) onto the “overpass” MT, such that the hanging cargo would be likely to encounter the lower, crossing MT (Fig. 1B). With this setup, our 0 μm separation

experiments resemble the “underpass” MT geometry in prior crossing experiments, in which MTs were attached to a glass substrate [64, 81]. However, our experimental model allows for MT bending, twisting, and vibrations which cannot be recapitulated when MTs are firmly attached to a solid substrate. The absence of the solid glass substrate in our work is a major difference since the cargo can explore many more three-dimensional paths as it negotiates the intersection.

3.3.2 Final navigational outcomes depend on 3D geometry

For each of the nine MT network arrangements in our assays, we quantified final cargo routing outcomes strictly in terms of, “switching” or “passing”, because detachment at intersections was negligible. In addition, we do not report pause events, because we can characterize the entire navigational event, even in cases where the MC navigational choice takes several seconds to make. Below, we report switching probability only, as passing probability is complementary.

Our results suggest that 3D MT network topology alone can be an effective regulator of cargo routing (Fig. 3.1C). Geometries in the upper left corner of the table promote switching while those in the lower right corner promote passing. Therefore, routing outcomes are determined by multiple geometric factors interacting in non-trivial ways. Disentangling these factors by intuition alone is challenging, therefore we relied upon *in silico* modeling. Helpfully, many fine mechanistic details are resolved by our experimental approach (see below), which constrain the *in silico* model.

3.3.3 Characterization of Tug-of-War events

A cargo that does not engage in a ToW, does not switch; hence precisely distinguishing between ToW and non-ToW events is critical to dissect the mechanisms that lead to differential switching probabilities. Our spatiotemporal resolution is sufficient not only to establish whether a ToW took place, but also to precisely determine ToW durations.

We can readily identify ToW start and end by observing significant MC displacements away from MT axes and associated MT deflections (Fig. 3.2A). MC tracks for representative pass (Fig. 3.2B), and switch (Fig. 3.2C) events are shown, along with their displacements projected along the axes of the intersecting MTs. A ToW start can be identified with the beginning of a sustained displacement upon the crossing MT's axis. ToW end can then be identified when the cargo snaps back upon dissociation from either the primary or crossing MT. BH displacements can also be used as an indicator of a ToW because the MC motors engaged in a ToW will exert force on the MTs that ultimately displaces the BHs (Fig. 3.1B, 3.2A *middle*, D).

Although BH displacements can help confirm ToW presence and duration, their greatest benefit is that they are an indicator of how many motors were exerting force on the bead. We set the trap stiffness at ~ 1 pN/100 nm, so that a single kinesin motor could not pull the BH out of the trap (escape event) but two or more motors working together could do so readily (Fig. 3.3). Quantifying collective activity of multiple motors via trap escape forces is a well-established approach both in vivo [83, 84] and in vitro [85] and has been validated in silico [85]. This setup allowed us to control the surface density of motors on MCs by discarding assays in which BH escape fraction exceeded 25% of total events. This provided refined control on top of the more crude and variable approach of controlling motor concentration at incubation time. It also provided confidence that ToWs in our assays were dominated by forces from 1-2 kinesin motors; more motors are likely engaged on the MT but

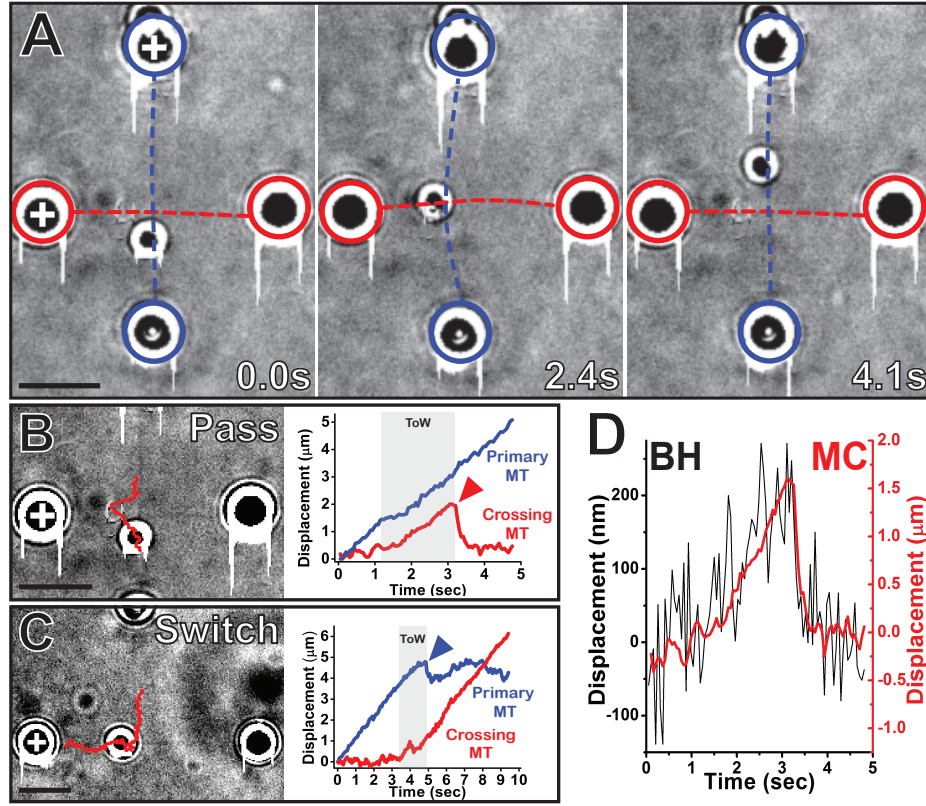


Figure 3.2: Identification and quantification of ToW.

(A) Three video frames: before, during, and after ToW (left to right). Circles show BH positions in the left panel and are positioned identically in middle and right panels. Dashed lines highlight positions of MTs inferred from video for each frame. Blue and red color scheme represents over- and underlying components respectively ($0.5 \mu\text{m}$ separation). Displacement of the top BH relative to its fiducial circle indicates presence of ToW (middle panel); its original position is restored once ToW has ended (right panel). White plus signs indicate the MT plus ends. Frame timings shown in lower right corner.

(B) Analysis of the pass event shown in panel (A). Left panel: Trajectory of MC (red) overlaid on one cropped video frame. Right panel: MC displacements projected along the primary (blue) and crossing (red) MT directions. Red arrowhead highlights the snapback event which is typical of a ToW conclusion. Gray band: ToW temporal extent (~ 1.9 s).

(C) Analysis of a switch event. Left panel: Trajectory of MC (red) overlaid on one video frame. Right panel: MC displacement projected along the primary (blue) and crossing (red) MT's axes ($0.5 \mu\text{m}$ separation). Blue arrowhead highlights when the MC undergoes a “snapback”, an event which is typical of a ToW conclusion. Gray band: ToW temporal extent (~ 1.6 s).

(D) MC displacement along the crossing (red) MT axis shown in (B) overlaid onto trace of BH displacement from trap center, due to ToW (for the top BH in (A)). All scale bars are $5 \mu\text{m}$.

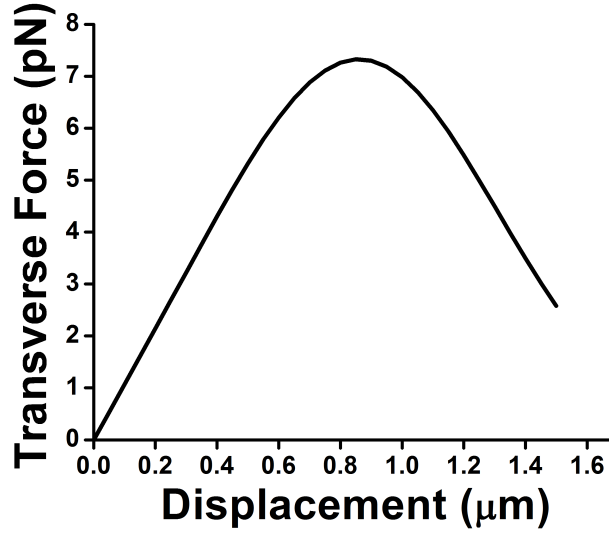


Figure 3.3: Restoring force on a glass bead (index of refraction 1.55, 1000 nm radius) exerted by the HOT for various displacements from trap center. Calculations were performed using previously published software[86].

not all are positioned to exert force during the ToW.

3.3.4 Mechanisms of cargo routing

The ability to sensitively detect ToW events allowed us to quantify their probability. We could then also examine the probability for the cargo to pass or switch, conditional on ToW occurrence. This analysis is informative because these probabilities reveal different aspects of cargo dynamics (Fig. 3.4). Our data indicates that ToW probability is sensitive to 3D geometry: it is higher for narrower filament separations, and for acute/obtuse angles (Fig. 3.4A). For 0 μm separations, ToW probability is so high that significant differences as a function of angle may not be practical to measure. A different pattern of navigational outcomes emerges when trivial passes (no-ToW events) are omitted (Fig. 3.4B vs Fig. 3.1C). Four out of nine geometries show switch probabilities close to 50%. We also record switch probabilities that are significantly higher than 50% for the following geometries: 0 μm normal, 0 μm acute, and 0.5 μm acute ($p < .05$ Barnard’s test). We conclude that geometric

constraints can promote or inhibit switching outcomes for ToW events.

3.3.5 A mathematical model of cargo transport reproduces observed switch probabilities

As mentioned above, it is difficult to disentangle the mechanism through which each factor acts to (in concert) determine ToW and switching probabilities. Therefore, to gain further insight into the mechanism determining cargo routing, we constructed an *in silico* model of cargo transport. This model allows us to examine experimentally unobservable details, such as how motor locations, bound states and force states change with time.

The model incorporates relevant experimental details including: the well-established properties of kinesin-1 motors, and cargo translational and rotational diffusion, however, simulated MTs do not move, twist, or bend (Fig. 3.5A). 500 cargo trajectories were simulated independently for each assay geometry, and probabilities of ToW and switching were then determined analogously to the experimental data. For full simulation details and parameter fitting procedure, see 3.6.1 and 3.6.2. Briefly, there were two parameters that could not be established from current experiments or prior literature: average motor number attached to MCs and each motor’s on-rate. These two parameters were constrained by matching three experimental observations: ToW probability as a function of geometry (Fig. 3.11), fraction of BH escape events and MC run lengths (Fig. 3.12). Thus, the model is fully constrained and therefore predictive.

We found good agreement between experimentally observed and theoretically predicted probabilities of ToW’ing and switching (Fig. 3.5B-D). We now turn to investigation of the quantitative details of ToW and switching (next two sections), and their implications for the mechanisms of cargo navigation at MT intersections.

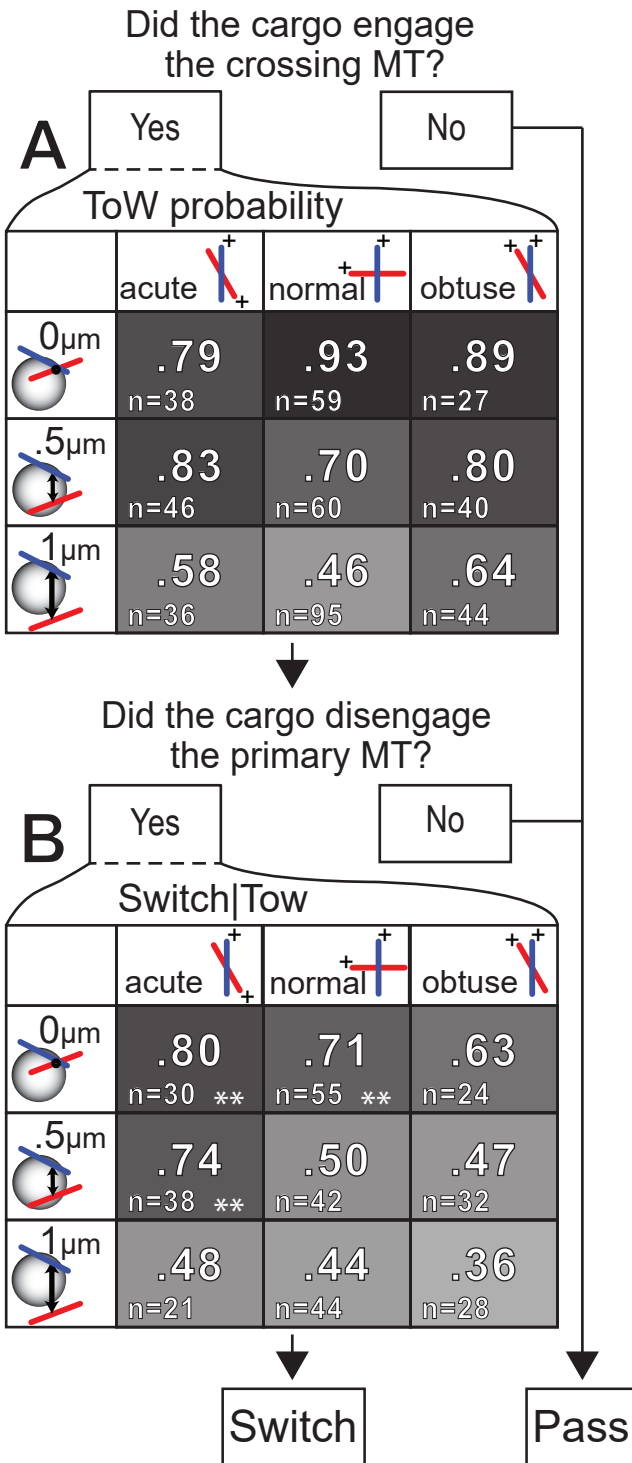


Figure 3.4: Cargo navigation flow chart, with associated outcome probabilities.

(A) Probabilities of ToW, as a function of 3D MT network geometry.

(B) Probabilities of switching conditional on ToW taking place, as a function of 3D MT network geometry. ** indicate probability is significantly higher than 50% ($p < 0.05$, Barnard's test).

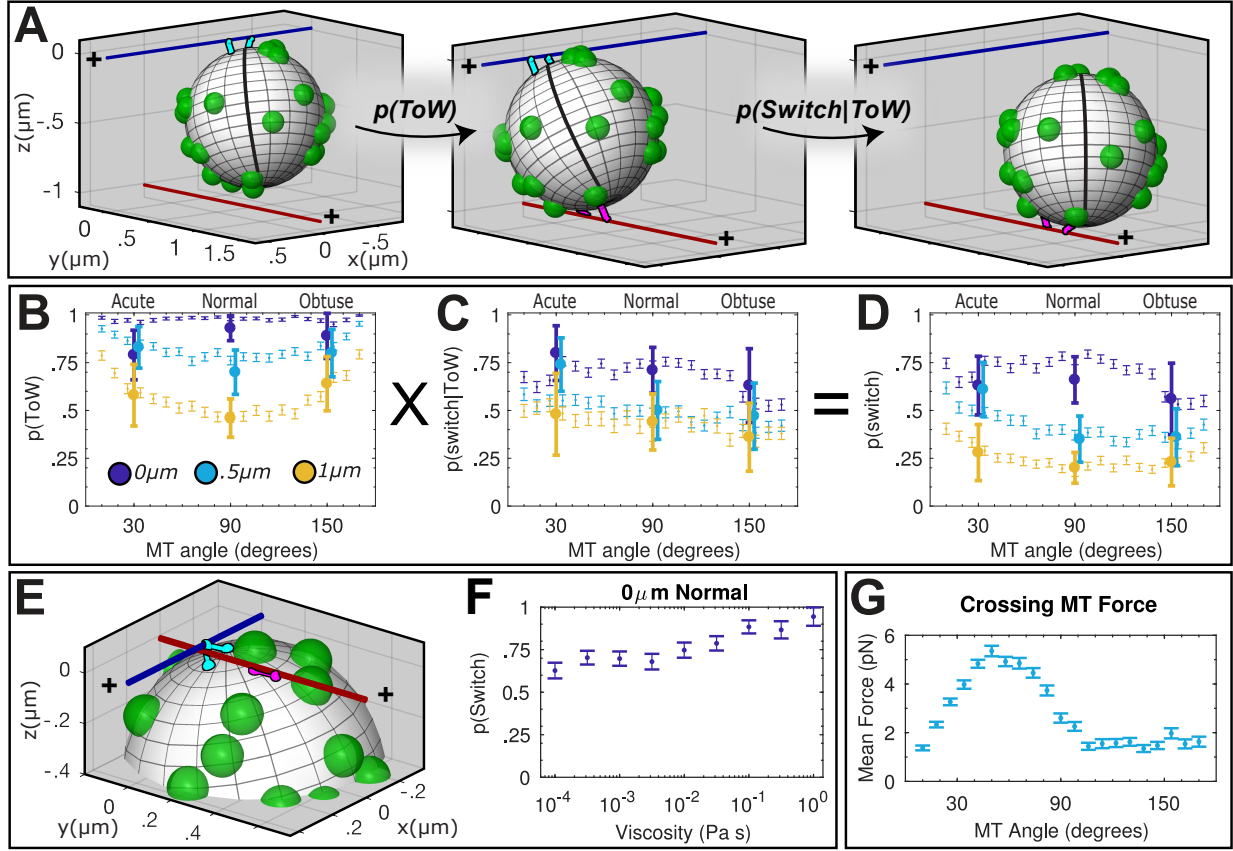


Figure 3.5: Mathematical model of navigational outcomes.

(A) Snapshots of simulated cargo activity at an intersection (color coding as in Fig. 3.4). Examples of Pre-ToW, ToW, and post-ToW states are shown (left, middle, right respectively). Motors bound to the primary MT are shown in cyan. Motors bound to the crossing MT are shown in magenta. Unbound motors are shown as green spherical protrusions from MC; sphere radius represents motor's maximal reach. Bead's "prime meridian" demarcated to emphasize rotational movement. (B) Simulated probabilities to engage in ToW. Probability of undergoing ToW for each geometry is shown as thin symbols (error bars: SEM). Experimental data is shown as large symbols (error bars: 95% C.I.). Experimental data points for 0.5 μm geometries shifted slightly horizontally to aid the eye. (C) Simulated probability to switch, given the cargo engaged in ToW. Simulated and experimental data represented as in (B). (D) Simulated probability to switch, overall, i.e. the product of probability to ToW (B), and probability to switch, given ToW (C). Simulated and experimental data represented as in (B). (E) Snapshot of a ToW for 0 μm , 90° geometry. Here, primary MT motors are blocked from passing the intersection by the crossing MT at 0 μm . Bound and unbound motors represented as in (A). (F) Probability to switch increases with increasing simulated fluid viscosity. Error bars: SEM. (G) Force exerted by crossing MT on the cargo for 0.5 μm geometries (mean $\pm$ SEM).

3.3.6 The influence of intersection geometry on ToW probability

The longer a cargo spends within reach of the primary and intersecting MTs (henceforth, the ToW zone), the more chances unbound motors on the cargo have, to engage the intersecting MT. This phenomenon can be understood from a simple, heuristic model. If we consider the cargo as having a single rate of binding to the crossing MT given by $k_{\text{on}}^{\text{macro}}$, the probability the cargo binds to the crossing MT before leaving the ToW zone is given by

$$p(\text{ToW}) = \frac{k_{\text{on}}^{\text{macro}}}{k_{\text{on}}^{\text{macro}} + v/d_{\text{ToW}}}, \quad (3.1)$$

where v is the cargo velocity and d_{ToW} is the length of the ToW zone. This simple model can accurately reproduce experimental ToW probabilities for both the 0.5 μm and 1 μm separation distance geometries (Fig. 3.6), but not for the 0 μm separation geometries. This strongly suggests that there are mechanisms at play for 0 μm separations which are not present at other distances (see below).

On the other hand, our full model closely recapitulates all experimental ToW probabilities, including 0 μm separation data. It is encouraging that the model captures several *a priori* intuitive features of the system. For 0 μm geometries, if a cargo is driven by multiple motors then there is guaranteed to be a pool of motors able to bind the crossing MT (namely, the already engaged motors). Thus, we *a priori* expect the ToW probability for all angles to be close to 1, which our model reproduces. For 0.5 μm and 1 μm geometries, we expect higher ToW probability for longer ToW zones (acute and obtuse angles). Indeed, simulated ToW probabilities were smallest for 90° intersections (Fig. 3.5B). Also, as expected, they were symmetric about the normal angle (since kinesin binding is not affected by MT polarity). Simulated ToW probabilities also increased when MT separation decreased from 1 μm to 0.5 μm , as expected. When the intersecting MT encounters the MC's midsection, at 0.5 μm separations, it samples more of the bead's surface area (especially when rotational diffusion

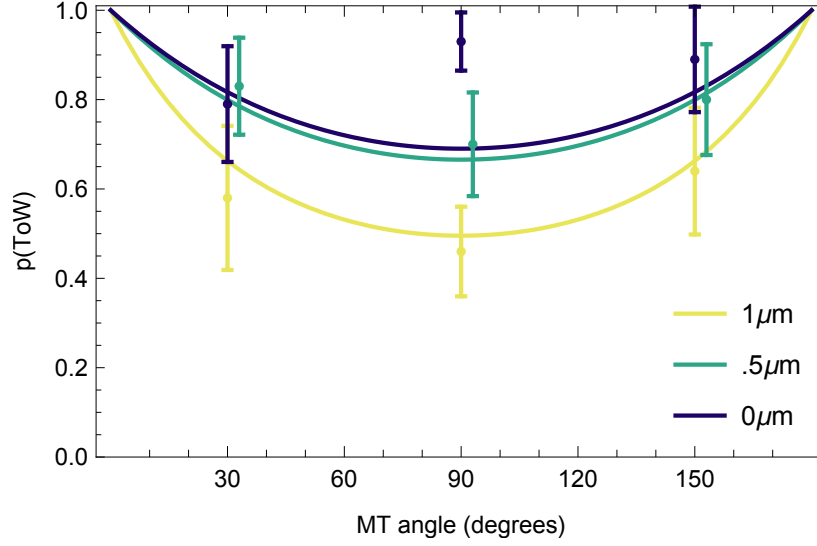


Figure 3.6: Solutions to the heuristic model for ToW probability

A heuristic model (given in equation 3.1) poses the probability of undergoing ToW as the the probability of the cargo binding (with constant binding rate) to the crossing MT before leaving the ToW zone (see equation 3.25 and figure 3.15). Solutions shown as solid curves. Bars represent 95% confidence intervals for corresponding experimental data. Bars for data at $0.5\mu\text{m}$ shifted slightly to aid the eye. Solutions plotted with $k_{\text{on}}^{\text{macro}}$ set to 1 s^{-1} .

is taken into account). Hence, at $0.5\mu\text{m}$ separation, more motors are given a chance to engage on the crossing MT, which increases ToW probability.

3.3.7 The influence of intersection geometry on navigational outcomes, given ToW

Experimentally, we observe a large range of geometries (most $0.5\mu\text{m}$ and $1\mu\text{m}$ geometries) where the conditional probability to switch is $\sim 50\%$. At first glance, this appears to be a relatively intuitive result: if ToW lasts $\sim 1\text{ s}$ or more (our experimental ToW durations average $\sim 3\text{ s}$, Fig. 3.7), then the motor team engaged on the crossing MT should be able to reach steady state [56], and become equal in number to the primary motor team. With two equivalent ways to proceed, the probability of either choice would indeed be $\sim 50\%$. However, such a consideration is too simplistic as we discuss below.

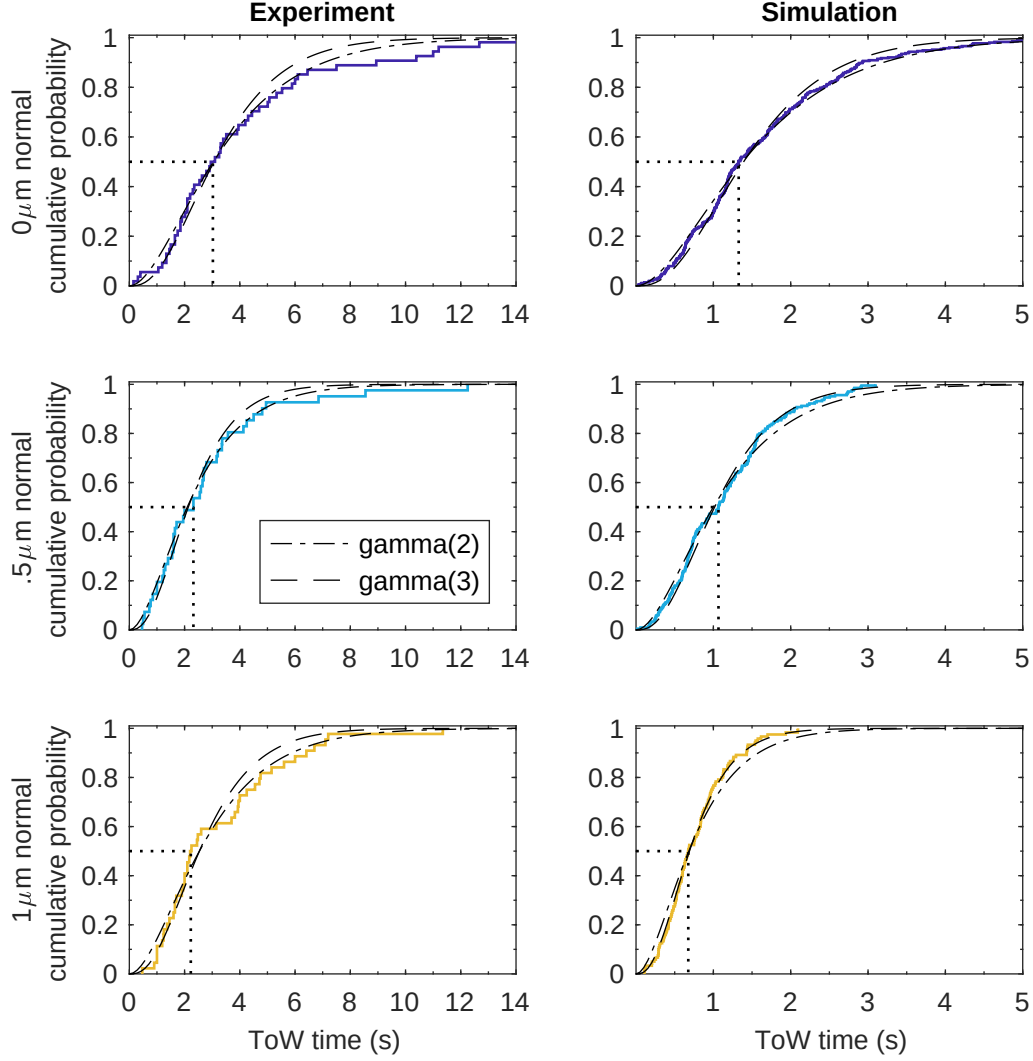


Figure 3.7: Empirical cumulative probability distributions for ToW times

The time for which cargos underwent ToW in 90 degree (normal) geometries was measured in experiments and simulations. Cumulative distributions are shown for each geometry. To aid interpretation, medians are highlighted with dotted lines. Fits to gamma cumulative distribution functions are shown in dashed lines. Good fits to gamma distributions with shape parameter 2-3 indicate distributions are well approximated as generated from 2-3 independent exponential events which we interpret as motor unbinding events.

Experimental results allow for sensitive identification of ToW, so that ToW events can be analyzed separately from trivial “no-ToW” intersection passages (Fig. 3.4). The same analysis can be performed for simulated events (Fig. 3.5B-D). A careful analysis of the simulations helps us shed light on the mechanistic details of cargo navigation.

We first assessed whether the number of motors in the two ensembles is in fact equal in our simulations. We find that contrary to naïve expectation, the number of engaged motors on the secondary MT is comparable but consistently lower than that on the primary MT (ratio of ~ 0.7 ; Fig. 3.8). The reason is that the motors already engaged on the primary MT constrain the bead from full range of linear and rotational diffusion. Once a ToW starts, the bead diffusion is even more constrained; this curtails the number of motors that can reach the secondary MT. Thus, the team of motors pulling along the primary MT generally has an advantage. However, the two paths to proceed are not equivalent either. The crossing MT itself can serve as an obstacle for bead progress and exert a force which hinders the MC from passing (Fig. 3.5G). The smooth decrease from passing to switching prevalence across our experimental geometries (Fig. 3.4B) therefore reflects the balance between net motor activity (which is biased in favor of moving along the primary MT) and steric hindrance from the crossing MT.

3.3.8 The limits of geometric regulation

Our results establish that a single MT intersection can significantly bias cargo routing towards more switching or more passing. Can a single MT intersection produce a near 100% bias for switching or passing? What are the mechanisms which affect these limits? It is easy to see that $\sim 100\%$ passing naturally occurs for intersections with filament separation much greater than MC diameter. Is 100% switching attainable? To address this, we consider two special cases which lead to elevated probability to switch, and their broader implications

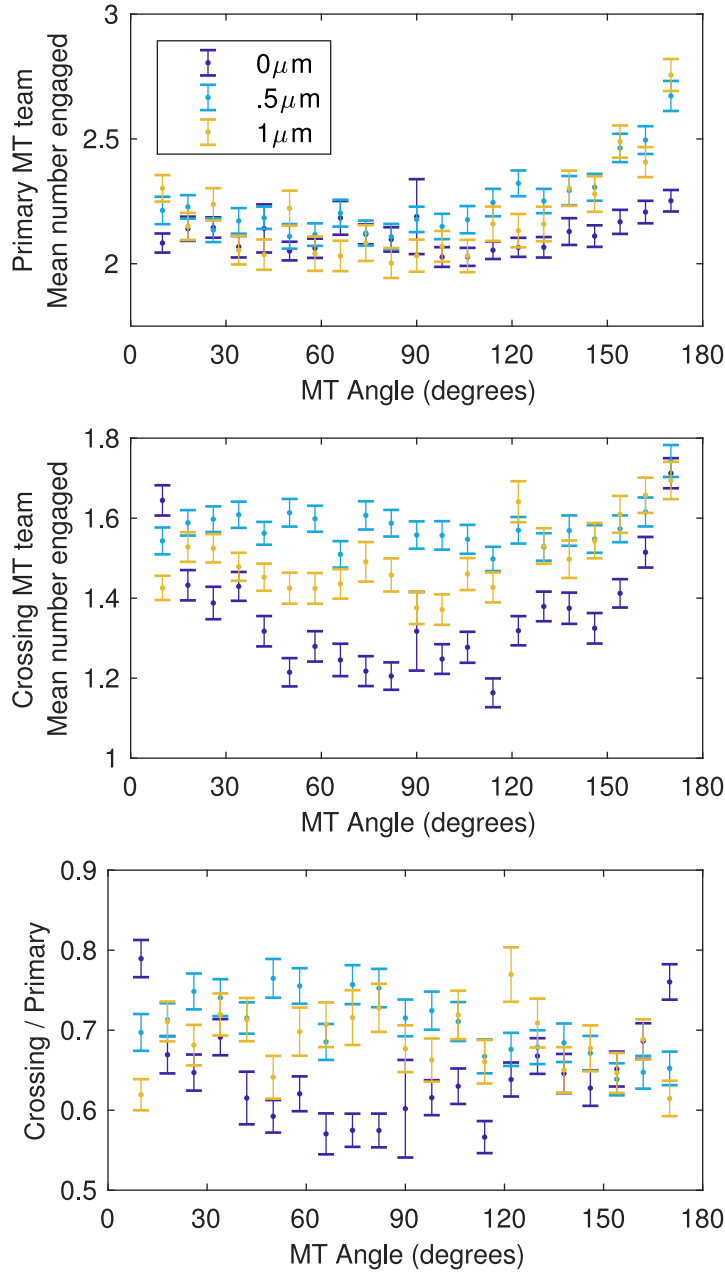


Figure 3.8: Number of motors engaged on MTs during ToW

The mean number of motors engaged on the primary (*top*) and crossing (*center*) MTs during simulated ToWs are shown for each geometry. Additionally, the ratio of the mean number of motors engaged on the crossing MT to the mean number engaged on the primary MT is shown (*bottom*). Data are represented as mean $\pm$ SEM. Separation distances are colored as specified in the legend in the top panel.

for geometric regulation. For completeness, we also discuss each experimental geometry individually in 3.6.3.

3.3.9 Low filament separations

A closer look at the geometric setup (Fig. 3.5E) makes it clear that the 0 μm case is qualitatively distinct. At 0 μm separation, the crossing MT sterically hinders the motors engaged on the primary MT, but not for the MC itself. To pass, the cargo can “hurdle” over the crossing MT due to Brownian motion but this is improbable for our large silica MCs (density $\approx 2.2\times$ water). Hurdling would also be unlikely in the viscous cytosolic environment (even for smaller cargos). The only other way to pass is by a mechanism we refer to as “monkey-barring.” To pass via monkey-barring, the MC must first diffuse underneath the crossing MT so that some of the unengaged motors on its surface could bind to the distal side of the primary MT (Fig. 3.9A). The motors proximal to the crossing MT must then gradually disengage. The above suggests that passing at 0 μm separation should have a sensitive dependence on the diffusion properties of the medium. This is indeed seen in our simulations (Fig. 3.5F). In fact, for viscosities close to intracellular range, where cargo diffusion is suppressed, the preference to switch approaches 100%.

3.3.10 Acute angles / intermediate separations

Why is switching more prevalent for 0.5 μm acute geometry? Again, this must be due to the crossing MT acting as a strong hindrance, but the mechanism cannot be the same as in the previous section. Here, monkey-barring is not feasible but hurdling is. Evidently, in this case, for switching probability to be elevated, hurdling must be suppressed. The vertical forces associated with the ToW for 0.5 μm acute geometry pull the MC between MTs. This effectively wedges the cargo between the two MTs, which indeed prevents hurdling. We

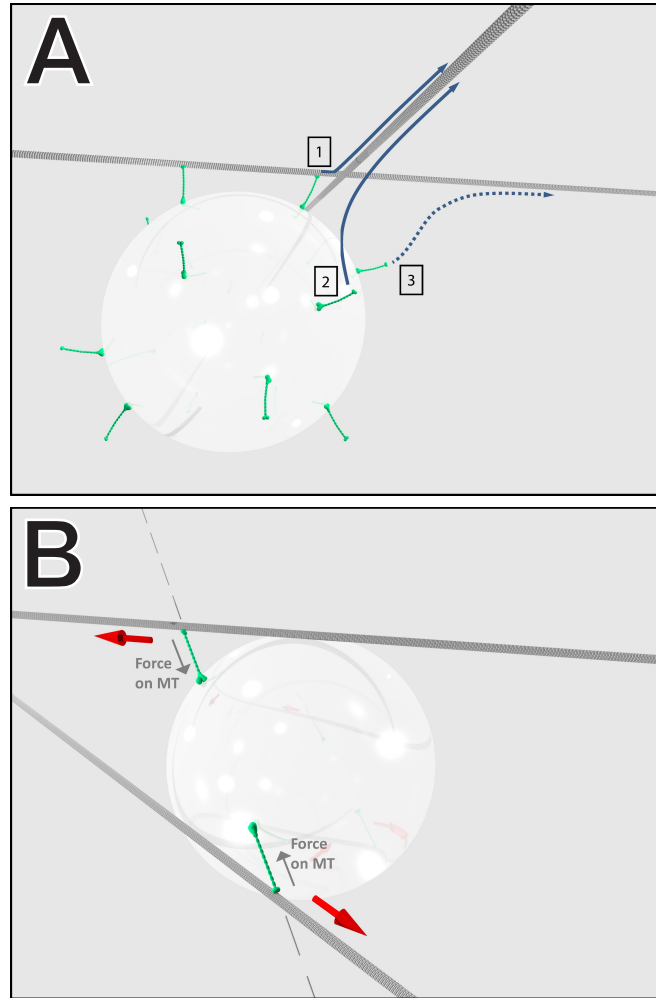


Figure 3.9: Mechanisms that elevate switch probabilities.

(A) Illustration of MC approaching a normal intersection with a separation of $0\ \mu\text{m}$.

1) Motors engaged on the primary MT can detach, then rebind to the crossing MT.

2) Unengaged motors can bind the crossing MT.

3) Unengaged motors can bind the primary MT at a site distal to filament intersection - the first step in the monkey-barring mechanism.

All motors are in green regardless of engagement status.

(B) Illustration of MC engaged in ToW at an acute $0.5\ \mu\text{m}$ intersection. A minimal ToW with only two motors is shown for visual clarity. The forces exerted by motors are counter aligned (grey arrows) and serve to wedge the MC between the MTs. Red arrows point to MT plus ends.

refer to this mode of steric hindrance as “chop-sticking” (Fig. 3.9B). Note that in our simulations, MTs were not allowed to bend, which would likely be a factor under experimental conditions. In reality, the two motor teams engaged in a ToW during chop-sticking would be expected to pull the two MTs closer together, which would make the crossing MT an even stronger obstacle to passing. This is likely the reason why experimentally observed switching probability for $0.5\mu\text{m}$ acute geometry is somewhat higher than theoretical prediction.

3.4 Discussion

Cargos driven by multiple motors can exhibit incredibly complex behavior. Even when one considers this for a single cargo moving along a single MT, the system complexity is sufficient to lead to highly non-trivial emergent behaviors [54, 87]. The number of motors in biologically relevant systems is typically small which necessitates highly detailed experiments and modeling to account for not only averaged behavior but also the effect of fluctuations. The addition of just one more filament adds such complexity that the emergent behaviors can dramatically diverge to give rise to discrete outcomes: passing and switching. It is therefore a fascinating model system to study emergent behaviors in biology.

We have modeled this problem in a highly controlled experimental environment in which we imposed very specific restrictions on cargo size and other experimental variables. We then performed highly detailed modeling of our system *in silico* to generalize our experimental results. We were thus able to infer the key processes which underlie our observations. This enables us to extrapolate how cargo routing might function in cells (and other environments).

Our analysis shows that the team of motors driving the cargo along the primary MT is generally at an advantage, even when the ToW is prolonged. This implies that there is an inherent bias to pass. However, our data shows that for many geometric conditions passing

and switching is balanced, and in some cases, switching dominates. The missing factor which shifts the balance between passing and switching is the extent to which the crossing MT acts to sterically hinder the motors progressing along the primary MT.

We show that the crossing MT can indeed be an effective obstacle, especially when it intersects at an acute angle with intermediate separation, or near-zero separation. Both types of geometries significantly inhibit passing but via different mechanisms: intermediate separations with acute angles lead to chop-sticking, while near-zero filament separations mean that passing is only accessible by means of monkey-barring (which is an unlikely event). These results imply that the cell could selectively route cargos of different sizes by controlling MT intersection angle and spacing. Our simulations closely follow the experimental results but two important deviations are seen: the conditional probability to switch given that ToW already started is higher in experiments than in simulations. Also, the time a cargo spends at the intersection is higher in the experiment than in simulation (Fig. 3.7). In both cases, the discrepancy is clearly linked to the fact that we model MTs as an infinitely rigid rod. To date, MT rigidity has been mostly studied separately from MT-based transport. Our work suggests that MT bending and more generally biomechanics of the MT cytoskeleton must be taken into account in future studies of intracellular motility as they are a non-negligible contributor to cargo routing.

A faithful and detailed *in silico* model of cargo motility and the ToW process enables us to then speculate about cargo navigation patterns beyond our specific conditions. First, we show that if viscosity increases, then switching would be further favored for near-zero filament separations. In effect, our simulations lead us to speculate that not only MT geometry but also local microrheology can be a regulator of cargo routing.

We also predict that extremely short motors should find reaching across the crossing MT for near-zero separations particularly difficult, so cargos driven by very short motors would preferentially switch at intersections. It is tempting to speculate that overall kinesin length

evolved to reduce the probability of cargos getting trapped in filament-filament switching loops.

On a more architectural level, our results suggest that for each cargo type and size there is a critical MT density at which efficient transport can be inhibited by too much switching at intersections. This prediction from the single molecule level dovetails with the more qualitative observations for the insulin secretion process [20].

Our work opens many new directions for future work, including studies of more complex intersections, more complex cargos and motor complexes. Together these baseline studies can then serve as a basis for studying chemical regulation of motility in the context of cytoskeletal network geometry. A gradual from-the-bottom-up increase in complexity can then gradually lead to comprehensive quantitative understanding of intracellular cargo fluxes, from a single-molecule mechanistic perspective.

3.5 Materials and Methods

3.5.1 Optical trapping and 3D motility experiments

Our holographic optical trapping and bead assays were performed as previously described [82]. However, in present work, enzymatically dead KIF5A heavy chain dimers were adsorbed onto bead handles non-specifically. Switch/pass outcomes were assessed live during the experiments from the video feed and recordings were conducted until definitive outcome was attained. Video records of bead positions were tracked using custom software (MATLAB, MathWorks, Natick, MA).

3.5.2 Super-resolution imaging

African green monkey kidney epithelial (BSC1) cells were labeled with primary antibody, alpha-tubulin, against microtubules followed by secondary antibody labeling with Alexa Fluor 647. Super-resolution images were recorded with a Vutara commercial microscope (Bruker, Salt Lake City, UT) based on the single molecule localization (SML) biplane FPALM technology [88, 89].

Microtubules were imaged using a 647 nm excitation laser, and 405 nm activation laser in photoswitching buffer comprising of 20 mM cysteamine, 1% betamercaptoethanol, and oxygen scavengers (glucose oxidase and catalase) in 50mM Tris+10 mM NaCl+10% glucose buffer at pH 8.0. Images were recorded using a 60x/1.2 NA Olympus water immersion objective and Photometrics Evolve 512 EMCCD camera with gain set at 50, frame rate at 50 Hz and maximal powers of 647 nm and 405 lasers set at 8 and 0.05 kW/cm² respectively.

Data was analyzed by the Vutara SRX software (version 6.00). Single molecules were identified by their brightness frame by frame after removing the background. Identified particles were then localized in three dimensions by fitting the raw data in a customizable region of interest (typically 12x12 pixels) centered around each particle in each plane with a 3D model function which was obtained from recorded bead data sets. Fit results were stored as data lists for further analysis.

3.5.3 Statistical analysis

Much of our data is in the form of contingency tables. Barnard's exact tests were used to assess significance of differences between outcomes.

3.6 Mathematical model for routing at intersections

3.6.1 Model description

We present a three dimensional mesoscale model for the dynamics of the cargo, which takes into account rotational and translational diffusion, as well as stochastic stepping and binding of the motors. The locations of the cargo bead and motors attached to it are governed by a set of stochastic ordinary differential equations. Binding state and location of the motor head along the microtubule (MT) are considered discrete states and transitions between them occur stochastically, modeled as Poisson processes. We construct a Monte Carlo numerical simulator, based on a hybrid Euler-Maruyama-Gillespie scheme, and simulate an ensemble of stochastic trajectories, from which we derive probabilities for tug of war and switching.

Motors in the model have two states:

Bound: A motor i is defined by two points: one represents the the motor domains, which we call the head $\vec{h}^i$; the other represents the location at which the motor is bound to the bead, which we call the anchor $\vec{a}^i$. The motor has a rate of stepping along the MT k_{step}^i and a rate of unbinding from the MT k_{off}^i .

Unbound: A motor i is defined by one point, the anchor location $\vec{a}^i$. The motor has a rate of binding to MT j , $k_{\text{on}}^{i,j}$, that depends on its location relative to the MT as described below.

We track the anchor locations, head locations of bound motors, the location of the cargo center $\vec{c}$, and the cargo orientation $\vec{\theta}$.

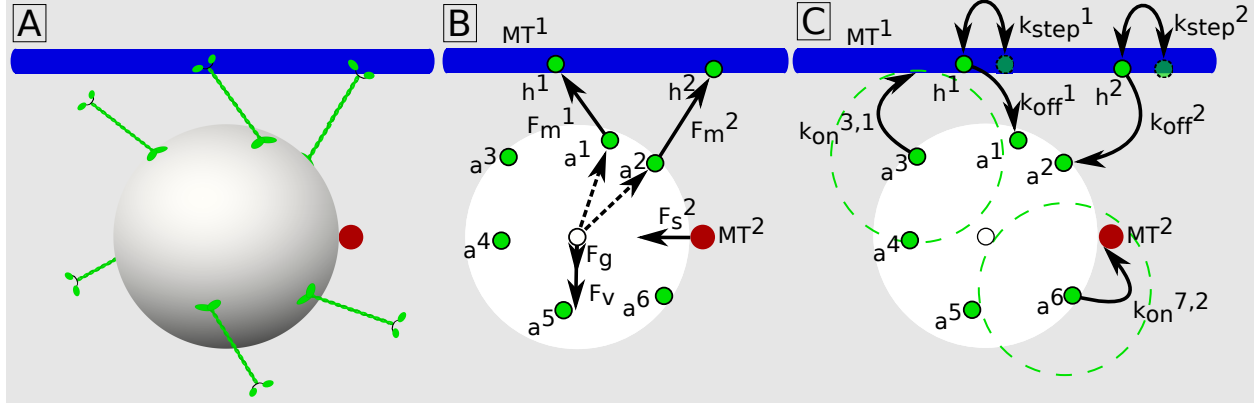


Figure 3.10: Diagrams of cargo dynamics

A) Schematic of a model cargo in motion. Kinesin motors are shown in green, primary MT in red, crossing MT in blue, and bead in white.

B) Free body diagram of the cargo corresponding to the cartoon in A. Anchor locations $\vec{a}^i$ and head locations $\vec{h}^i$ are shown with green dots. Cargo center location $\vec{c}$ is shown with a white dot with black outline. Forces are shown with solid arrows. Dashed arrows show the lever arm through which the motor forces exert torque.

C) Diagram showing the possible state changes corresponding to the cartoon in A. Unbound motors have on rates greater than 0 if their anchor is less than 1 motor length from a MT. Bound motors have rates of stepping and unbinding.

3.6.1.1 Stochastic ordinary differential equations for cargo motion

We first impose force-balance on the cargo. The motion of the cargo is the inertialess regime (Reynolds number $\sim 10^{-6}$), so we omit the inertia term. A free body diagram showing the deterministic forces acting on model cargo is shown in figure 3.10B. All forces must balance, so

$$\sum \vec{F} = \underbrace{\sum_i \vec{F}_m^i}_{\text{motor forces}} + \underbrace{\sum_j \vec{F}_s^j}_{\text{steric forces}} + \underbrace{\vec{F}_g}_{\text{force of gravity}} + \underbrace{\vec{F}_b}_{\text{Brownian force}} + \underbrace{\vec{F}_v}_{\text{viscous force}} = 0. \quad (3.2)$$

Similarly, we can write the balance of torques on the model cargo

$$\sum \vec{\tau} = \underbrace{\sum_i \vec{\tau}_m^i}_{\text{torque from motor forces}} + \underbrace{\vec{\tau}_b}_{\text{Brownian torque}} + \underbrace{\vec{\tau}_v}_{\text{viscous torque}} = 0. \quad (3.3)$$

By specifying the forms of each of these forces below, we construct the equations of motion of the cargo center $\vec{c}$ and cargo orientation $\vec{\theta}$.

Motor forces We model the force that the motor exerts on the cargo as originating from the stretch in spring-like motor stalks, based on experimental measurements [45, 46] and in line with previous models [54–56]. The form of this force is that of a one-way (tension-only) spring with rest length equal to the length of the motor. The force $\vec{F}_m^i$ exerted by motor i on the cargo bead is given by

$$\vec{F}_m^i(\vec{a}^i, \vec{h}^i) = \begin{cases} -\kappa_m \left(|\vec{h}^i - \vec{a}^i| - L \right) \left(\frac{\vec{h}^i - \vec{a}^i}{|\vec{h}^i - \vec{a}^i|} \right) & |\vec{h}^i - \vec{a}^i| > L \\ 0 & \text{otherwise} \end{cases}. \quad (3.4)$$

This force also exerts a torque on the cargo bead, given by the cross product of the lever arm and force vector

$$\vec{\tau}_m^i(\vec{a}^i, \vec{h}^i, \vec{c}) = (\vec{a}^i - \vec{c}) \times \vec{F}_m^i(\vec{a}^i, \vec{h}^i). \quad (3.5)$$

Steric forces The cargo bead and MTs are prevented from overlapping in space by a steric force with the form of a one-way (compression-only) spring, given for MT j by

$$\vec{F}_s^j(\vec{c}) = \begin{cases} -\kappa_s \left(R - |\vec{r}(\vec{c}, j)| \right) \left(\frac{\vec{r}(\vec{c}, j)}{|\vec{r}(\vec{c}, j)|} \right) & |\vec{r}(\vec{c}, j)| < R \\ 0 & \text{otherwise} \end{cases}, \quad (3.6)$$

where $\vec{r}(\vec{p}, j)$ is a vector from point $\vec{p}$ to the nearest point on the MT. This is found by computing the perpendicular distance from $\vec{p}$ to the MT surface, given by

$$\vec{r}(\vec{p}, j) = \left(1 - \frac{R_{\text{MT}}}{\left| (\vec{x}_{\text{MT}}^j - \vec{p}) - \left((\vec{x}_{\text{MT}}^j - \vec{p}) \cdot \vec{d}_{\text{MT}}^j \right) \vec{d}_{\text{MT}}^j \right|} \right) \left((\vec{x}_{\text{MT}}^j - \vec{p}) - \left((\vec{x}_{\text{MT}}^j - \vec{p}) \cdot \vec{d}_{\text{MT}}^j \right) \vec{d}_{\text{MT}}^j \right). \quad (3.7)$$

Force of gravity The experimental system uses a silica bead with density greater than water as the cargo. Therefore the model cargo experiences a small but significant force due to gravity given by the buoyancy of the bead,

$$\vec{F}_g = \frac{4}{3}\pi R^3(\rho_b - \rho_w)\vec{g}, \quad (3.8)$$

where ρ_b and ρ_w are the mass densities of the silica bead and water, respectively.

Brownian force The Brownian force $\vec{F}_b$ is a random variable with mean 0 and variance $2k_B T \xi$, where ξ is the drag coefficient and $k_B T$ is the thermal energy unit. Specifically,

$$\langle \vec{F}_b \rangle = \vec{0} \quad (3.9)$$

$$\langle \vec{F}_b(t) \cdot \vec{F}_b(s) \rangle = 2(k_B T)(6\pi\eta R)\delta(t - s) \quad (3.10)$$

where we have inserted the translational drag coefficient of a sphere at low Reynold's number and $\delta(x)$ is the Dirac delta function.

Similarly, the Brownian torque on the bead is a random variable characterized by

$$\langle \vec{\tau}_b \rangle = \vec{0} \quad (3.11)$$

$$\langle \vec{\tau}_b(t) \cdot \vec{\tau}_b(s) \rangle = 2(k_B T)(8\pi\eta R^3)\delta(t-s). \quad (3.12)$$

Viscous force In the low Reynolds limit, linear drag dominates. The drag on the cargo is thus given by Stokes' Law,

$$\vec{F}_v = -6\pi\eta R \frac{d\vec{c}(t)}{dt}. \quad (3.13)$$

The viscous torque is given by the rotational analogue to Stokes' Law,

$$\vec{\tau}_v = -8\pi\eta R^3 \frac{d\vec{\theta}(t)}{dt}. \quad (3.14)$$

Construction and discretization of the stochastic ordinary differential equations

With the forms of forces known, we rewrite equations 3.2 and 3.3 as a pair of coupled ordinary stochastic differential equations

$$\frac{d\vec{c}(t)}{dt} = \frac{1}{6\pi\eta R} \left(\sum_i \vec{F}_m^i(\vec{a}^i(t), \vec{h}^i(t)) + \sum_j \vec{F}_s^j(\vec{c}(t)) + \vec{F}_g \right) + \frac{1}{6\pi\eta R} \vec{F}_b(t) \quad (3.15)$$

and

$$\frac{d\vec{\theta}(t)}{dt} = \frac{1}{8\pi\eta R^3} \left(\sum_i \vec{\tau}_m^i(\vec{a}^i(t), \vec{h}^i(t), \vec{c}(t)) \right) + \frac{1}{8\pi\eta R^3} \vec{\tau}_b(t), \quad (3.16)$$

Equation 3.15 is a specific implementation of the overdamped Langevin equation, used in Brownian dynamics. Equation 3.16 is its rotational counterpart.

We discretize these equations according to the Euler-Maruyama method. For an update from

the n th timestep at time t_n to the next time t_{n+1} with $\Delta t \equiv t_{n+1} - t_n$, the discretization is

$$\begin{aligned} \vec{c}(t_{n+1}) = \vec{c}(t_n) + \frac{1}{6\pi\eta R} \left(\sum_i \vec{F}_m^i \left(\vec{a}^i(t_n), \vec{h}^i(t_n) \right) + \sum_j \vec{F}_s^j(\vec{c}(t_n)) + \vec{F}_g \right) \Delta t \\ + \sqrt{2 \frac{k_B T}{6\pi\eta R}} \Delta t \vec{G}_c(t_n) \end{aligned} \quad (3.17)$$

and

$$\vec{\theta}(t_{n+1}) = \vec{\theta}(t_n) + \frac{1}{8\pi\eta R^3} \left(\sum_i \vec{\tau}_m^i \left(\vec{a}^i(t_n), \vec{h}^i(t_n), \vec{c}(t_n) \right) \right) \Delta t + \sqrt{2 \frac{k_B T}{8\pi\eta R^3}} \Delta t \vec{G}_\theta(t_n). \quad (3.18)$$

where $\vec{G}_c^n$ and $\vec{G}_\theta^n$ are two mutually uncorrelated vectors of three independently and identically distributed gaussian random variables with mean 0 and variance 1.

Update of anchor locations Since the motors are statically bound to the bead, the change in their locations is fully defined by the change in the location of the center of the bead $\vec{c}(t_{n+1}) - \vec{c}(t_n)$ and the change in the orientation of the bead $\vec{\theta}(t_{n+1}) - \vec{\theta}(t_n)$.

The change in the bead orientation $\vec{\theta}(t_{n+1}) - \vec{\theta}(t_n)$ corresponds to a vector pointed along the axis of rotation of the bead with a length corresponding to the magnitude of the rotation in radians. This vector can be converted into a rotation matrix $\mathbf{M}_R(t_n)$. The next location of anchor i is then calculated by finding

$$\vec{a}^i(t_{n+1}) = \vec{a}^i(t_n) + (\vec{c}(t_{n+1}) - \vec{c}(t_n)) + (\mathbf{M}_R(t_n) (\vec{a}^i(t_n) - \vec{c}(t_n)) + \vec{c}(t_n)). \quad (3.19)$$

3.6.1.2 Poisson processes

We model all state transitions in the system as Poisson processes, diagrammed in figure 3.10C. Experiments have reported exponential distributions of times between steps [35] and

times before unbinding [42]. This is also the most basic assumption we can make for times before binding.

Stepping Kinesin motors step processively along MT tracks in a hand-over-hand fashion, with each motor domain taking 16 nm steps [33] that move the center of mass of the motor forward by 8 nm [34]. The rate at which the motor steps, and thus the motor velocity, depends on the load the motor is stepping against in a way well modeled as

$$k_{\text{step}}^i = \begin{cases} \frac{v}{d} (1 - F_m^i/F_s)^w & F_m^i < F_s \\ 0 & F_m^i \geq F_s \end{cases}, \quad (3.20)$$

as described in [36].

When a motor steps, it is moved forward along the direction of the MT j to which it is bound by the step distance d . This translates to an update of the head position

$$\vec{h}^i(t_{n+1}) = \vec{h}^i(t_n) + d \left(\vec{d}_{\text{MT}}^j \right). \quad (3.21)$$

Unbinding Kinesin unbinds from the MT with a rate dependent on the force experienced by the motor. Measurements have shown that unbinding rate increases exponentially below stall [43]. Above stall, measurements have shown that unbinding rate increases only slowly with increasing force [42]. Therefore, we state the unbinding rate as

$$k_{\text{off}}^i = \begin{cases} \epsilon_0 \exp(F_m^i/F_d) & F_m^i \leq F_s \\ a + bF_m^i & F_m^i > F_s \end{cases}. \quad (3.22)$$

When a motor unbinds, it is simply put into the unbound state as defined at the beginning of this section.

Binding The conditions which govern the rate of binding to the MT are not well known. In the absence of detailed experimental elucidation, we make only the most basic assumptions possible: the motor domains must be able to reach the MT to bind, and, if this condition is met, the motor binds with a constant rate. This translates to an on rate for motor i to MT j given by

$$k_{\text{on}}^{i,j} = \begin{cases} \pi_0^{\text{micro}} & |\vec{r}(\vec{a}^i, j)| \leq L \\ 0 & \text{otherwise} \end{cases} \quad (3.23)$$

where $\vec{r}(\vec{a}^i, j)$ is given by equation 3.7.

When a motor i binds to MT j , the head location is placed at the location on the MT nearest the anchor location $\vec{a}^i$, given by

$$\vec{h}^i(t_{n+1}) = \vec{a}^i(t_n) + \vec{r}(\vec{a}^i(t_n), j). \quad (3.24)$$

3.6.2 Numerical simulation of the model

Section 3.6.1 outlines a numerical scheme for updating for the model's dynamic variables in equations 3.17, 3.18 and 3.19 over a timestep Δt . We simulate the model forward in time using these equations. Time steps are chosen dynamically. The largest stable time step for the Euler-Maruyama scheme is given by ξ/κ , where ξ is the drag coefficient and κ is the spring constant of the stiffest operating spring. The maximum time step is chosen based on the springs active during that step. The equivalent stiffness of multiple active motor springs is taken into account, but the steric spring in equation 3.6 remains by far stiffest in the system if it is active.

For each timestep, we generate exponential random variables from distributions with means

set by each Poisson rate, given in equations 3.20, 3.22, and 3.23, as in the Gillespie (next-event) algorithm. If any of these times are smaller than the maximum stable time step, the smallest generated time is chosen. If the smallest generated time came from an unbinding rate, a state change is implemented at the end of the update step by setting the motor to the unbound state. If this time came from a stepping rate or binding rate, the update occurs by equation 3.21 or 3.24, respectively. If no generated time is shorter than the maximum stable time step, the update is done with the maximum stable time step and there is no state change. The memoryless property of the exponential distributions which underlie the Poisson processes ensure that these substeps do not change the overall dynamics.

The numerical simulation is written in C. It takes approximately 0.5 s to simulate 1 s of cargo motion with a 3.3 GHz Intel Core i5 processor (single thread).

3.6.2.1 Model parameters

The kif5A motors used in experiments are members of the well studied kinesin-1 family. As such, many of the model parameters have been estimated in previous experiments. The parameters used to simulate the model are given in table 3.1. There are two parameters in the model not well constrained by experiments in the literature: the number of motors on a cargo, N , and the on rate of a motor given that it is close enough to the MT to bind, π_0^{micro} .

3.6.2.2 Initial conditions

Beads are incubated with motors in solution to produce experimental cargos. We assume this process produces beads with motors uniformly distributed on their surface as supported by experimental evidence in [94]. Therefore, we generate random positions for motor anchors uniformly distributed on the surface of the bead.

Parameter	Description	Value	Notes
N	Number of motors on the bead	30	Determined by fit to experiments in this paper
κ_m	Kinesin stalk spring constant	320 pN μm^{-1}	Measured in [45, 46]
L	Kinesin length	80 nm	Measured in [90, 91]
π_0^{micro}	Base binding rate (microscopic)	10 s $^{-1}$	Determined by fit to experiments in this paper. Lower bound estimated in [52, 92]
ϵ_0	Base unbinding rate	0.7 s $^{-1}$	Estimated from run lengths [43, 93, 94]
a	Superstall unbinding rate intercept parameter	1.07 s $^{-1}$	Measured in [42]
b	Superstall unbinding rate slope parameter	0.186 s $^{-1}$ pN $^{-1}$	Measured in [42]
F_s	Stall force	5 pN	Measured in [37, 42, 43]
F_d	Critical detachment force	4 pN	Measured in [42, 43, 95]
v	Unloaded motor velocity	1 $\mu\text{m s}^{-1}$	Velocities measured here
d	Step size	8 nm	Measured in [34]
w	Curvature index	2	Measured in [37, 38]
κ_s	Steric spring constant	40 000 pN μm^{-1}	Set high enough to ensure the cargo spends no more than 5 time steps in a row intersecting a MT
R	Cargo bead radius	0.5 μm	Provided by manufacturer
ρ_b	Cargo bead density	2.0 g/cm 3	Provided by manufacturer
ρ_w	Density of experimental medium	1.0 g/cm 3	Density of water
$\vec{g}$	Vector for gravitational acceleration	(0,0,-9.8) m/s 2	
η	Viscosity of fluid	8.5 $\times 10^{-4}$ Pa s	Viscosity of water
T	Temperature of the fluid	293 K	Experiments performed at room temperature
R_{MT}	MT radius	12 nm	Measured in [21]
$\vec{x}_{\text{MT}}^1$	Defining point for primary MT	(0,0,0)	
$\vec{d}_{\text{MT}}^1$	Defining direction vector for primary MT	(1,0,0)	
$\vec{x}_{\text{MT}}^2$	Defining point for crossing MT		Varied in simulations
$\vec{d}_{\text{MT}}^2$	Defining direction vector for crossing MT		Varied in simulations

Table 3.1: List of parameters. Values listed are used for all simulations unless explicitly stated.

Simulated cargos are initially placed just below the primary MT and far enough from the intersection that the cargo can not reach both MTs simultaneously (section 3.6.3.3 and figure 3.15). This distance is a function of the MT geometry and is given by equation 3.25. All cargos begin the simulation with one motor attached to the MT. This motor is selected at random and the cargo is rotated so its anchor is next to the MT.

3.6.2.3 Special case of $0\text{ }\mu\text{m}$ geometries

As discussed in the main text, in $0\text{ }\mu\text{m}$ geometries the crossing MT presents a steric barrier to the progress of the primary MT motor team. To modify the simulation to incorporate these effects, motors walking along the primary MT were prevented from stepping into the volume occupied by the crossing MT. Unbound motors were also prevented from binding if their head would be placed in the volume occupied by the crossing MT. These restrictions were lifted if the cargo was above the plane of the MTs.

3.6.2.4 Fitting unknown parameters to data

While the behavior of bound kinesins under load has been intensely investigated, the binding rate of kinesin to the MT is less understood. A binding rate of 5 s^{-1} has been estimated based fits to other models [52, 92], but these estimates include diffusion processes and do not directly translate to the microscopic binding rate we use here. We define the microscopic binding rate as the rate at which a motor binds the MT given it is possible for the motor heads to reach the MT, thus 5 s^{-1} represents a lower bound.

It was unfeasible to directly determine the total number of motors bound to experimental beads. Modeling binding of motors to beads in solution using Poisson statistics has enabled estimation of motor number when there are few motors (binding fraction $< .9$)[94], but the experiments in this work are done in the many motors regime (binding fraction indistinguish-

able from 1) so motor number cannot be determined from binding fraction. Simple binding of motors to cargo beads in solution predicts a total numbers of motors which is Poisson distributed [40]. We simulate with only a single number of total motors, which we identify with the mean of the experimental Poisson distribution. We do not expect this model simplification to impact the results due to the peaked nature of the Poisson distribution.

Fit to tug of war probability To determine values for the unknown parameters (total number of motors N and microscopic on rate π_0^{micro}), we fit to the probability of cargos to undergo ToW.

Ensembles of cargo trajectories were simulated for combinations of total number of motors and off rate and assayed for ToW as outlined in section 3.6.3.1. We survey the fit to ToW probability for experimental conditions and compare the simulated probability with the experimentally measured one. We exclude $0\text{ }\mu\text{m}$ separation geometries, as prominent steric effects which might obscure the fit are present (see figure 3.15). Furthermore, ToW probability is symmetric about the normal (see figures 3.14A and 3.15), so angled geometries were grouped to increase statistical power. This leaves us with four experimental conditions to fit, as shown in figure 3.11.

The experimental geometries provide redundant information about best fit parameters, with many parameter combinations fitting all four. Therefore we examine other experimental observations to select a set of parameters.

Robust transport constraint Experimental cargos are often observed to travel along the entire length of microtubules ($10\text{ }\mu\text{m}$ or greater). Because single motor off rates are constrained by single molecule run length experiments [43, 93, 94], we can constrain acceptable combinations of motor number and on rate by looking at simulated cargo run length. An ensemble of cargos were simulated without the crossing MT and allowed to walk until either

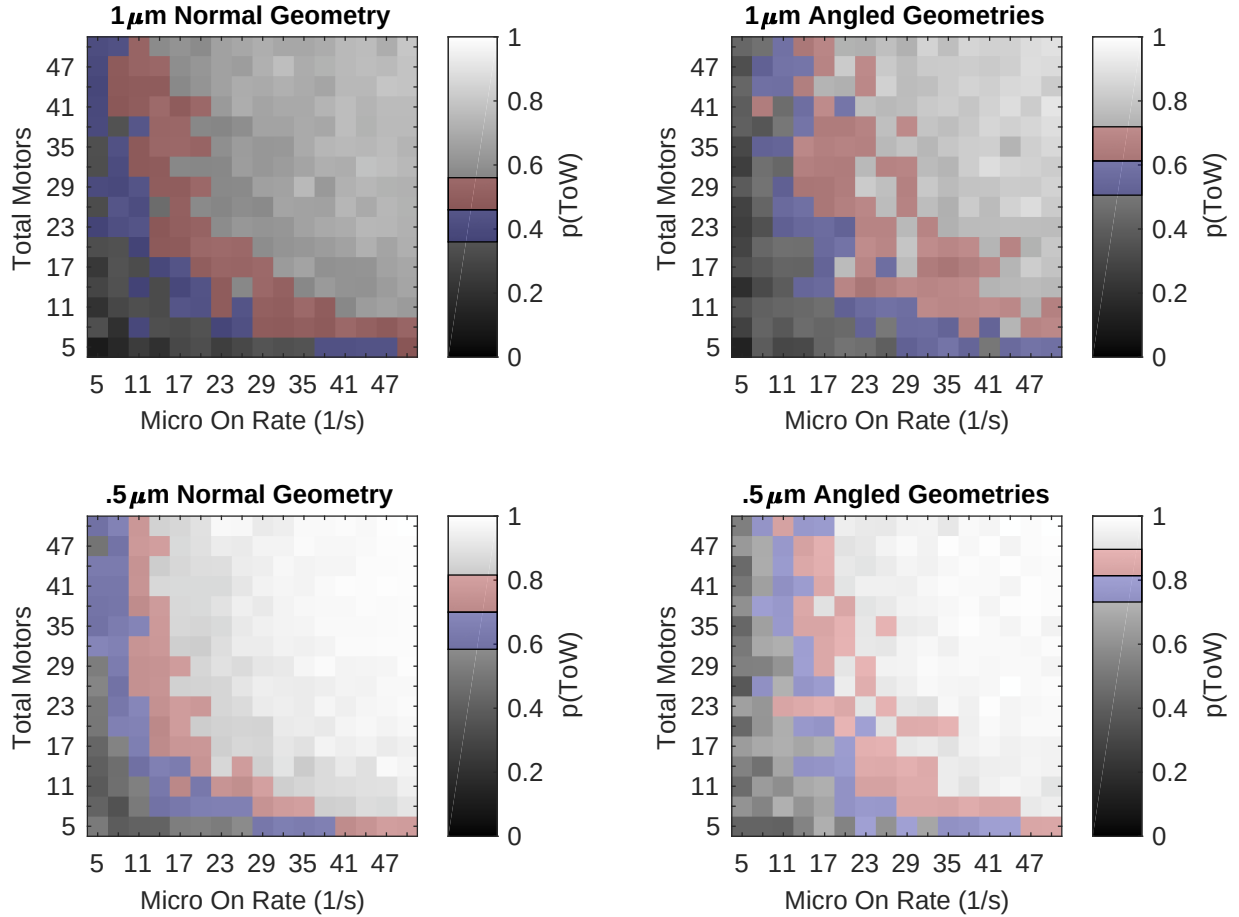


Figure 3.11: ToW probabilities for varying combinations of the unknown parameters. ToW probabilities are shown as grey scale heat maps for a range of parameter combinations. Those which yielded ToW probabilities within the experimental 95% confidence interval are highlighted in color. Red highlighting indicates a probability above the experimental mean, blue highlighting below. All probabilities gathered from 300 cargos. For angled geometries, 150 acute runs were grouped with 150 obtuse runs.

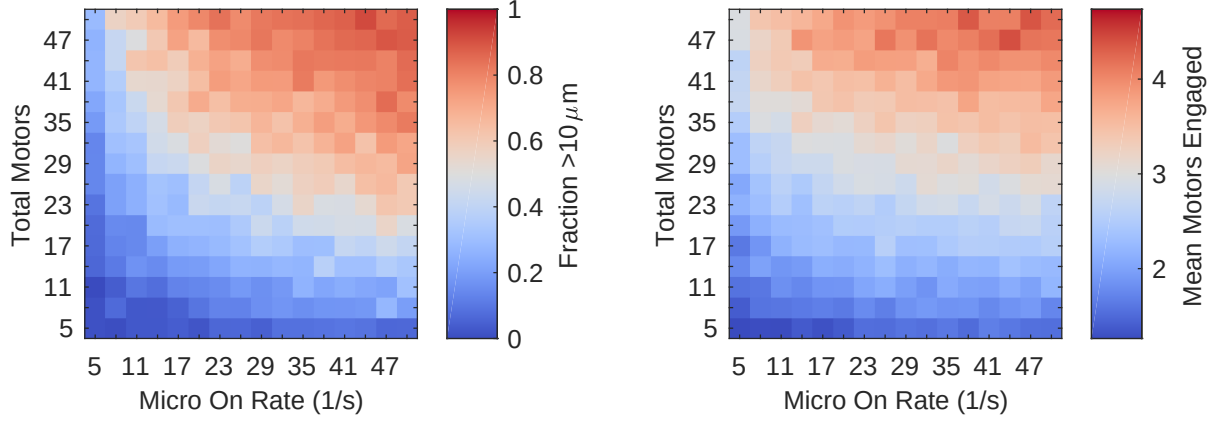


Figure 3.12: Experimental observations inform selection of unknown parameters

Left: Fraction of cargos that travelled more than 10 microns are shown as a heat map for a range of parameter combinations. Fractions determined by simulation of 300 cargos.

Right: Mean number of motors engaged on the MT at steady state is shown as a heat map for a range of parameter combinations. Values represent mean over 200 cargos. The number of motors engaged was assessed 1.5 s after the run began for each cargo. This time was long enough for cargos of all parameter combinations to reach steady state.

all motors detached from the MT or a motor head passed a point 10 μm from where the cargo started. Results are shown in figure 3.12. In experiments, roughly half of cargos were observed to travel the entire length of the MT.

Force constraint In experiments, force production of cargos was limited by the strength of the optical traps securing the bead handles. If generated forces were large enough, the forward bead handle was pulled out of its optical trap. Data for these cargos was discarded. The maximum force the bead handles could withstand was about 7 pN, greater than the single motor stall force but less than the maximal stall force of two motors. Because experimental cargos often do not stall and geometric effects tend to keep all motors from exerting their maximal force on the bead handle at once, we expect there to be 2-3 motors exerting force on the bead handle.

Because simulated MTs are not dynamic, average or maximal simulated forces do not necessarily correspond to those one would expect to see experimentally. Therefore we investigate

the number of motors simultaneously engaged on the MT at steady state, which estimates an upper limit for the number of motors simultaneously producing force.

To find the number of motors engaged on the MT, we simulate cargos walking along the primary MT without interference from a crossing MT. First, we simulate an ensemble of cargos and examine the average number of motors engaged over the ensemble as time progresses. After an initial transient, there is a period of time where neither the number of engaged motors nor the standard deviation thereof changes much. We call the average number of engaged motors during this time the steady state number. A survey of parameter combinations revealed that 1.5 s was long enough for cargos to come to steady state. Then, we check the average number of engaged motors at steady state for many parameter combinations. The results are shown in figure 3.12.

Parameter selection A survey of figures 3.11 and 3.12 reveals that only parameter combinations with high numbers of total motors and low on rate fit the ToW probability and also match estimated run length and number of engaged motors. A manual search of parameter combinations in this region yielded $N = 30$, $k_{\text{on}}^{\text{micro}} = 10 \text{ s}^{-1}$ as the best fit.

3.6.3 Model Results

Cargo trajectories were simulated forward in time as described in sections 3.6.1 and 3.6.2. Simulations were performed using the parameter values listed in table 3.1. All parameter values used except microscopic on rate and total number of motors on the cargo are established in the literature or set by the experiment. Section 3.6.2.4 details how values for the unknown parameters were fit.

An ensemble of trajectories was simulated for a given geometry. Trajectories were categorized as described below, and probabilities of ToW and switching were derived. As shown in figure

3.13, the general behavior of simulated cargos was the same as experimental ones.

3.6.3.1 Categorization of trajectories

Simulated trajectories were categorized using rules intended to emulate experimentally observed markers. Simulations began with the cargo outside of the ToW zone (section 3.6.3.3 and figure 3.15) and ended one of three ways: by the cargo detaching from the MT, by the cargo leaving the ToW zone on the primary MT, or by the cargo leaving the ToW zone on the crossing MT. The latter two cases were categorized as passes and switches, respectively. If the cargo detached from the primary MT before going by the intersection point, the run was discarded. If the cargo passed the intersection on the primary MT, but detached before leaving the ToW zone, the run was counted as a pass. Similarly, if the cargo was walking on the crossing MT but detached before leaving the ToW zone, the run was counted as a switch. Experimental runs were categorized using the same criteria.

Simulated cargos were marked as undergoing ToW when motors were attached to both MTs simultaneously for more than 0.25 s. This time was selected as the minimum time in which a ToW would be detectable experimentally, given the camera frame rate. ToW time was measured as the total time during which motors were attached to both MTs and average values during ToW were accumulated during this period.

3.6.3.2 Encounter outcome dependance on MT geometry

Ensembles of trajectories were simulated for varying separations and angles between the MTs. MT separations were varied from 0 μm to 1 μm , corresponding to 0 to 1 times the cargo diameter. Angles between the MTs were varied from nearly parallel to nearly anti-parallel. Figure 3.14 shows the resulting ToW and switch probabilities, along with the conditional probability of switching, given the cargo underwent ToW.

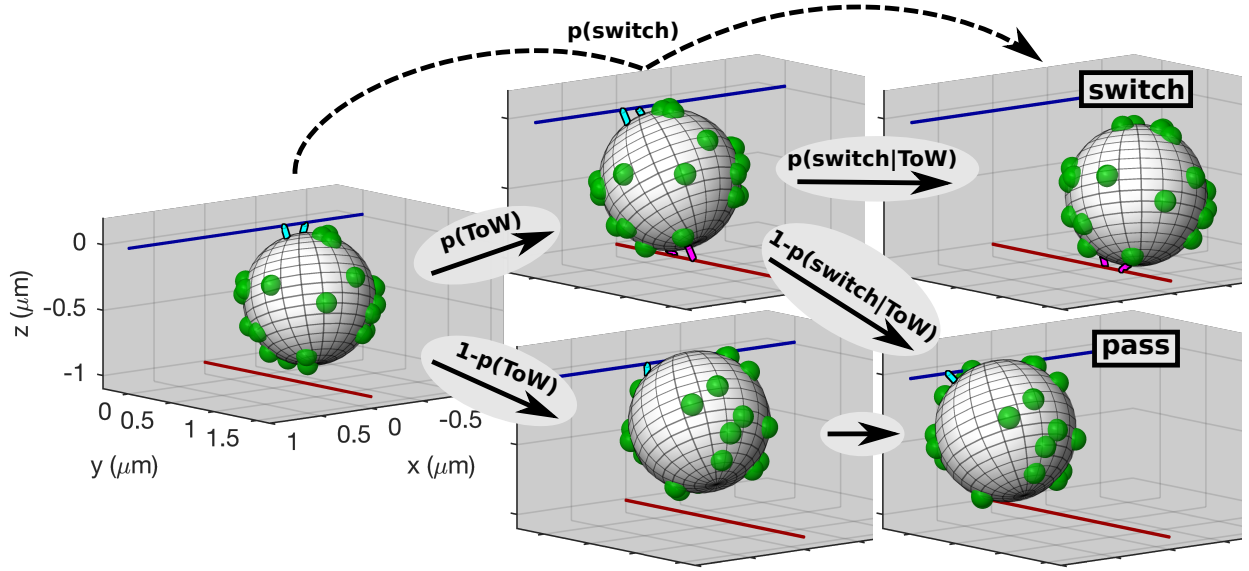


Figure 3.13: Snapshots from simulated cargo trajectories

During a simulation run, cargos begin outside the ToW zone with a single motor attached to the MT. Motors step stochastically, moving the cargo along the MT (*left*). The cargo diffuses translationally as well as rotationally. When the cargo encounters the crossing MT, motors may bind and induce a ToW with $p(\text{ToW})$ (*center, top*) or the cargo may go by the MT without any motors binding (*center, bottom*). Cargos which do not ToW always pass. ToWs may end in the primary MT motor team becoming detached, resulting in a switch, with probability $p(\text{switch}|\text{ToW})$ (*right, top*). Otherwise, the motor team on the crossing MT detaches, resulting in a pass (*right, bottom*). To switch, a cargo must undergo ToW, then the crossing MT team must win the ToW. Therefore, the overall probability of switching $p(\text{switch})$ is given by $p(\text{ToW}) \times p(\text{switch}|\text{ToW})$.

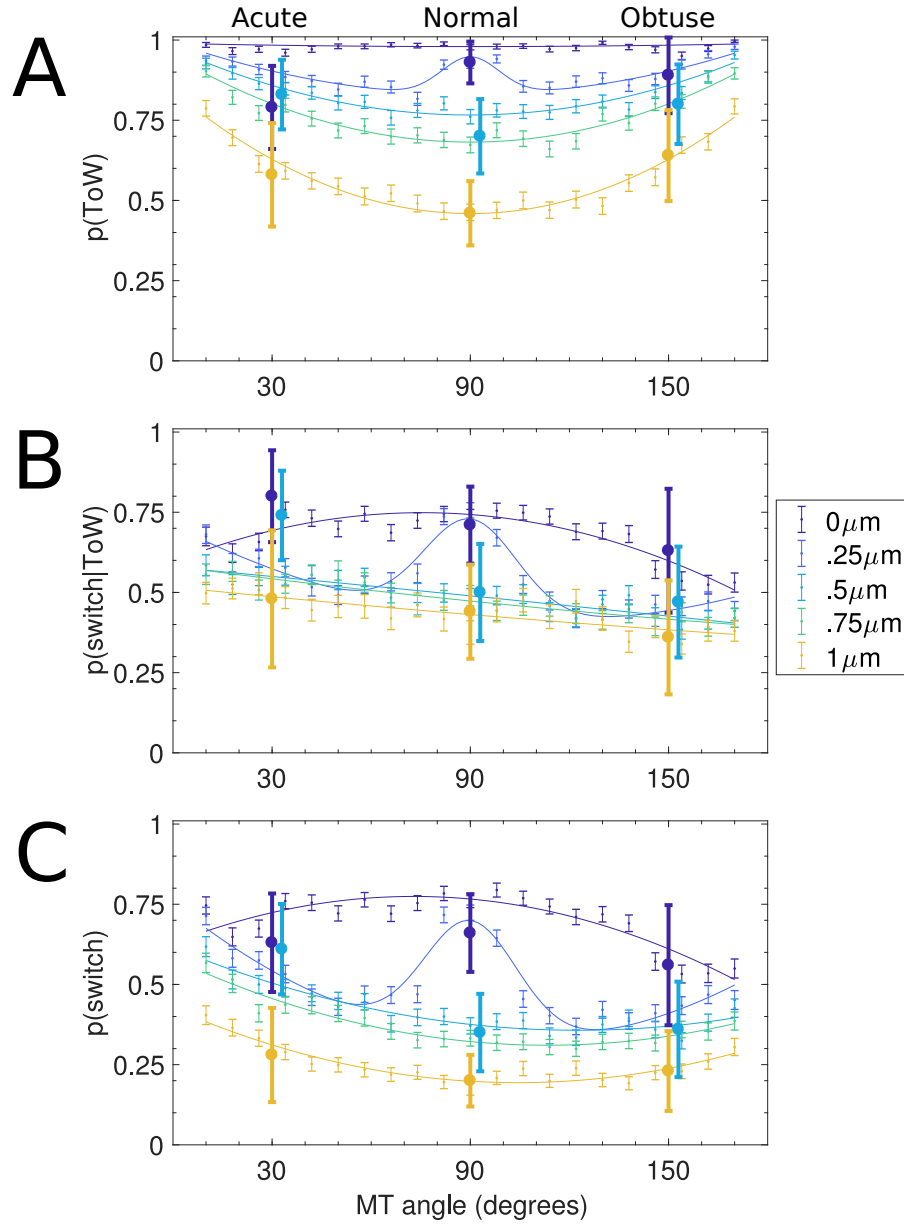


Figure 3.14: Probabilities of ToW and switching given ToW combine to explain switch probability

A: ToW probability as a function of the angle between the MTs is shown for separation distances corresponding to 0, 1/4, 1/2, 3/4 and 1 times the cargo diameter. Simulated data shown with thin bars representing standard error. Curves represent quadratic fits to simulated data for all but 0.25 μm separation distance, which is fit to a quadratic with an overlaid gaussian. Experimental data shown with thick bars representing 95% confidence interval.

B: Conditional probability of switching, given the cargo undergoes ToW. Data represented as in *A*.

C: Overall switch probability. Data represented as in *A*

3.6.3.3 Explanation of ToW probability

In agreement with intuition, ToW probability increases with the distance the cargo must travel while able to reach both MTs simultaneously, which we call the ToW zone, denoted x_{ToW} . The extent of the ToW zone was calculated from geometry diagrammed in figure 3.15, and is given by

$$x_{\text{ToW}} = 2 \frac{\sqrt{4(L + R + R_{MT})^2 - ((\vec{x}_{\text{MT}}^1 - \vec{x}_{\text{MT}}^2) \cdot \hat{z})^2}}{\left\| \vec{d}_{\text{MT}}^1 \times \vec{d}_{\text{MT}}^2 \right\|} = 2 \frac{\sqrt{4(L + R + R_{MT})^2 - (\text{separation distance})^2}}{\sin(\text{MT angle})}. \quad (3.25)$$

The probability a cargo will undergo ToW can then be understood as a competition between the rate at which the cargo binds to the crossing MT, which happens at a rate which we denote $k_{\text{on}}^{\text{macro}}$, and the rate it leaves the ToW zone, which happens at a rate given by the cargo velocity (equal to the maximum single motor velocity v) divided by the ToW zone length. Thus,

$$p(\text{ToW}) = \frac{k_{\text{on}}^{\text{macro}}}{k_{\text{on}}^{\text{macro}} + \frac{v}{x_{\text{ToW}}}}. \quad (3.26)$$

The rate at which the cargo attaches to the crossing MT, $k_{\text{on}}^{\text{macro}}$, is in general a complex function of geometry as well as motor and cargo parameters. However, for this heuristic analysis we assume it is constant across different geometries. If we fit the macro on rate to the experimental data on ToW probability, we recover the qualitative features of the full simulation result as shown in figure 3.15.

Steric effects strongly influence ToW probabilities in $0\text{ }\mu\text{m}$ and $0.25\text{ }\mu\text{m}$ geometries

A comparison of figures 3.15 and 3.14A reveals that the heuristic model captures the features

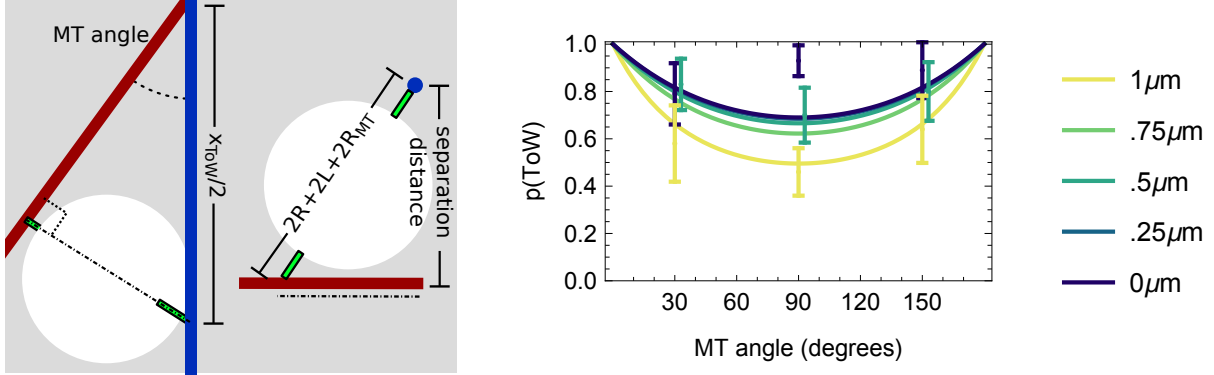


Figure 3.15: A heuristic model matches qualitative features of ToW probability

Left: Diagram of the ToW zone. The ToW zone is defined as the distance along the primary MT in which the cargo can reach the crossing MT. Its length was calculated geometrically and is given by equation 3.25. Primary MT is shown in blue, crossing MT in red, motors in green and cargo in white.

Right: Solutions to the heuristic model for ToW probability. The heuristic model poses the probability of undergoing ToW as the the probability of the cargo binding (with constant binding rate) to the crossing MT before leaving the ToW zone and is given in equation 3.26. Experimental probabilities are shown with error bars representing 95% confidence interval. Bars for data at $0.5\mu\text{m}$ shifted slightly to aid the eye. Solutions plotted with $k_{\text{on}}^{\text{macro}}$ set to 1 s^{-1} .

of ToW probability for MT separations $0.5\mu\text{m}$ and greater very well. However, it fails to reproduce features of the simulated data at $0.25\mu\text{m}$ and $0\mu\text{m}$. This failure shows that the peak in ToW probability near the normal for $0.25\mu\text{m}$ separation and the overall high ToW probability at $0\mu\text{m}$ must result from effects not considered in the heuristic model. As discussed in the main text, steric interactions play a large role.

3.6.3.4 Explanation of conditional probability of switching given ToW

The complex dependence of conditional probability of switching on geometry is not intuitive and warrants further investigation. To this end, we show the mean number of motors engaged on each MT during ToW, the mean forces exerted on the motor teams during ToW, the ToW time, and how each of these depends on the MT geometry in figure 3.16. Below we explain the behavior of the simulated conditional probability of switching on geometry using these

results.

1 μm near-normal geometries At 1 μm separation distance, the cargo can not sterically interact with both MTs at once. Since forces exerted by the MTs are minimal (figure 3.16C), motor teams in these ToWs pull directly on each other, as evidenced by high forces on the motor teams and a ratio near 1, shown in figure 3.16B. Furthermore, ToW times are short (figure 3.16D), meaning the crossing MT team often only gets one chance to cause a switch. One might expect, then, that since the ratio of crossing MT to primary MT motor number is $\approx .7$ (figure 3.16A), switches would happen about 30% of the time. However, a ToW between motor teams of different numbers is not directly determined by motor numbers. The slow increase of unbinding rate above stall gives the smaller team a significant chance to win the ToW, leading to an overall probability of switching higher than 30%, but still below 50%.

1 μm acute geometries Conditional probabilities of switching at 1 μm are slightly higher for acute geometries than they are near the normal. As in the latter, motors pull directly on each other during ToWs, evidenced by high forces in figure 3.16B. However, an asymmetry exists between a ToW won by the crossing MT motor team and the primary MT motor team in acute geometries. When the primary MT motor team wins a ToW, the cargo continues on through the ToW zone (which is longer for more acute geometries, see equation 3.25). During this time motors may again bind to the crossing MT, giving another chance for a switch. Evidence of this effect can be seen in the longer ToW times shown in figure 3.16D. However, when the crossing MT team wins a ToW, the cargo is likely to diffuse down and away from the primary MT and avoid rebinding. This effect drives switching up slightly for acute geometries compared to near-normal.

1 μm obtuse Unlike in near-normal and acute geometries at 1 μm , motors in obtuse geometries feel low average forces, as shown in figure 3.16B. ToW times are long, as seen in

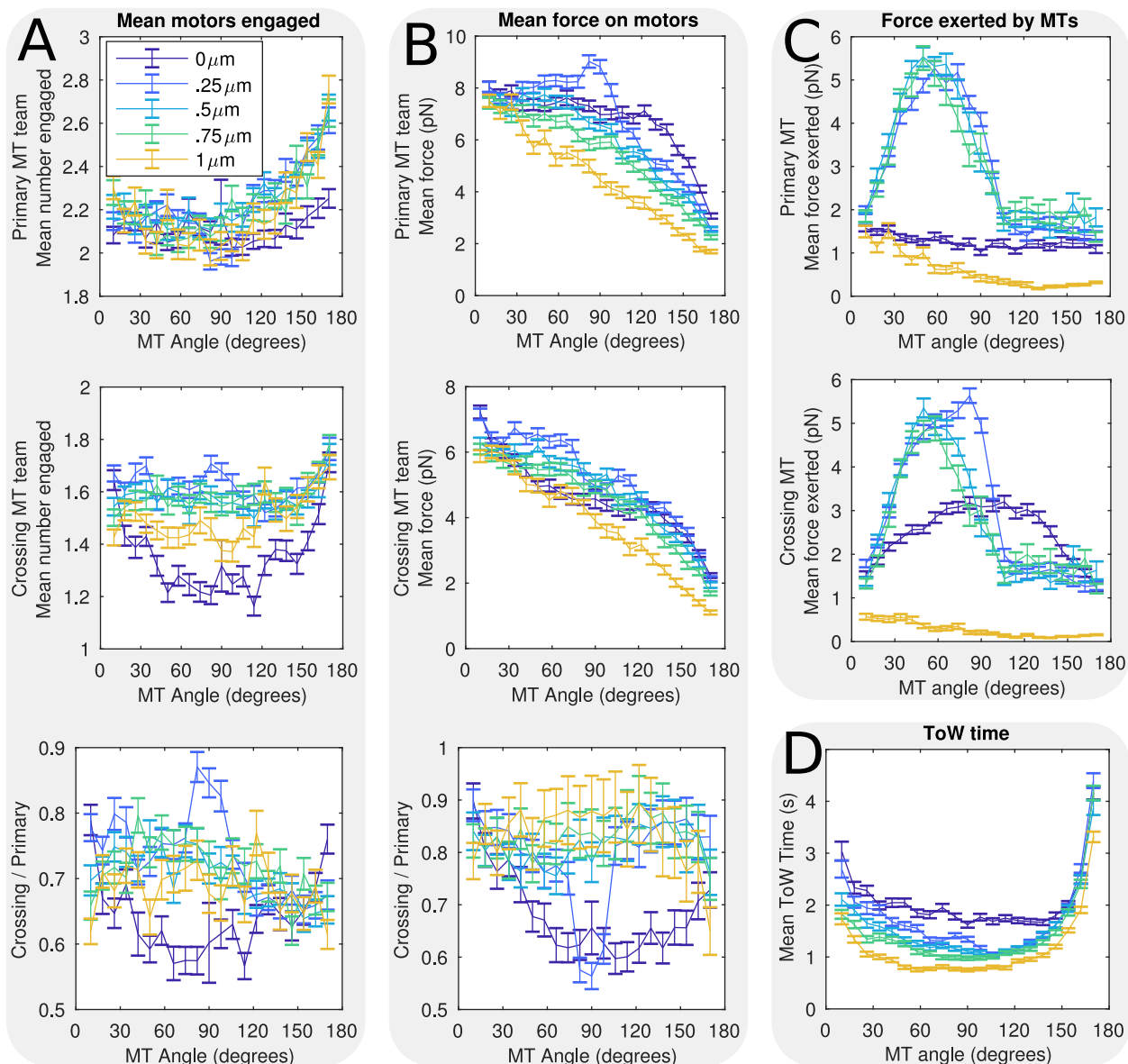


Figure 3.16: Numbers engaged, forces and ToW times explain conditional probability of switching

A: Mean number of motors engaged during ToW for primary and crossing MT teams. The numbers are shown as a function of MT angle. Below, the ratio of mean motors engaged on the crossing MT to the primary MT is shown. Separation distances are colored as described in the legend in the top panel of *A*. Error bars represent standard error of the mean.

B: Mean force experienced by the motor teams on the primary and crossing MTs during ToW. Forces are shown as a function of angle. Below, the ratio of the forces on the crossing MT team to the forces on the primary MT team is displayed. Data represented as in *A*.

C: Mean steric force exerted by the primary and crossing MTs during ToW. Forces shown as a function of angle and represented as in *A*. *D*: Mean ToW time as a function of angle between the MTs. ToW time is the total time motors are engaged on both MTs. Data represented as in *A*.

figure 3.16D, because MT alignment is close to parallel and motors walk in nearly the same direction. Motors do not feel significant load until the cargo has moved far enough from the intersection that the distance from MT to MT is greater than twice the bead diameter. These long periods of walking under low force turn the ToW into a competition of processivity as much as force. While a smaller motor team can compete against a larger motor team in a force competition, processivity grows quickly with motor number, so larger motor teams are more likely to win a processivity competition. Because there are on average more motors on the primary MT team, the probability of switching goes down as the distance walked goes up with more obtuse angles.

0.5 μm near-normal geometries In 0.5 μm geometries, the cargo must hurdle up and over the crossing MT to pass. If the cargo is underneath the primary MT, the primary MT motors are loaded as they pull the cargo into the crossing MT, but the cargo cannot pass the intersection until it is out from underneath the primary MT. In near-normal geometries, the crossing MT team assists this process by pulling the cargo out toward the plus end of the crossing MT. While this is taking place, the primary MT motors continue to be loaded. This load sometimes causes primary MT motors to unbind, leading to a weaker team on average. As crossing MT motors walk, the force from the primary MT motors is shifted from the crossing MT to the crossing MT motor team. In the end, this interaction has similar dynamics to the ones described in the 1 μm near-normal geometries, albeit with a primary MT motor team that is now weaker on average. This slightly biases the ToW toward the crossing MT team relative to 1 μm near-normal geometries, resulting in a ToW probability near 50%.

0.5 μm acute geometries Unlike in near-normal geometries at 0.5 μm , the crossing MT motors do not aid hurdling behavior in acute geometries. In fact, they impede hurdling; when the crossing MT motors engage, they act to wedge the cargo between the MTs, described

as "chop-sticking" in the main text. This effect can be seen in the high forces exerted by the MTs at these geometries shown in figure 3.16C. Because crossing MT motors impede hurdling and the cargo must travel beyond the intersection to pass, crossing MT motors often get multiple chances to cause a switch, indicated by long ToW times in figure 3.16D. This leads to the conditional probability of switching rising 50%.

0.5 μm obtuse geometries As in 0.5 μm near-normal geometries, crossing MT motors aid hurdling in 0.5 μm obtuse geometries. Additionally, like obtuse geometries at 1 μm separation distance, ToW times are long (figure 3.16D) and forces are low (figure 3.16B). As described, this biases the ToW toward the larger motor team on the primary MT and results in less switching.

0.25 μm and 0.75 μm geometries The conditional probability of switching in 0.25 μm and 0.75 μm geometries is similar to that of 0.5 μm geometries, indicating the same mechanisms are at play. There is a significant deviation at angles near the normal in 0.25 μm geometries. At these angles, the crossing MT becomes a very effective barrier to progress of the cargo. Because the crossing MT is above the cargo equator, steric forces from the crossing MT tend to push the cargo back down, causing it to get stuck below the intersection. This effect can be seen in the high MT forces near normal in figure 3.16C. Stuck cargos are more likely to switch, leading to an overall high switching probability.

0 μm near-normal geometries As explained in the main text, cargos may only pass two ways in 0 μm geometries: by the cargo hurdling up and over the crossing MT, or by monkey-barring. Since cargos experience a gravitational force, they are likely to encounter the intersection below the crossing MT and not be able to hurdle. Because successful monkey-barring is a process which requires two rare events, this leads to a high probability of switching.

0 μm acute geometries Because the ToW zone is longer in acute geometries than near-normal, cargos are more likely to undergo several ToW events. This is reflected in the long ToW times at acute geometries shown in figure 3.16D. In the opposite effect of that in 0.5 μm geometries, these longer ToWs lead to less switching in 0 μm geometries. This is because these longer ToWs give the cargo more chances to monkey-bar and pass the intersection.

0 μm obtuse geometries Conditional probability of switching falls as angles become closer to parallel for 0 μm separation distance, and approaches 50%. The forces on the cargo in these geometries are such that motors on the crossing MT tend to pull the cargo up and over the top of the intersection. This leads to greater ease of passing because the crossing MT no longer blocks progress of the primary MT motor team when the cargo is above the plane.

3.7 Acknowledgements

We are grateful Dr. Erik Jorgensen for helpful discussions and guidance on super-resolution imaging of microtubules. This work was supported by NSF grant number ENG-1563280 to M.V., NIH T32 EB009418-07 to M.B. and NIH R01 GM123068 to J.A. and S.G.

Chapter 4

Diffusion of kinesin motors on cargo can enhance binding and run lengths during intracellular transport

Material in this chapter is adapted from a manuscript created by Matthew Bovyn, Babu Reddy, Steven Gross and Jun Allard. The project was initiated by Matthew Bovyn, Jun Allard, and Steven Gross. Matthew Bovyn and Jun Allard created the model. Matthew Bovyn wrote the code and performed simulations. Babu Reddy performed experiments and analyzed experimental data. Matthew Bovyn, Jun Allard and Steven Gross wrote the text.

The manuscript was published as [4].

4.1 Abstract

Cellular cargos, like lipid droplets and mitochondria, are transported along microtubules (MTs) using molecular motors such as kinesins. Many experimental and computational

studies have been done on cargos with rigidly attached motors. We recently reported that cargo attachment and transport initiation happen on 1-10s timescales *in vivo*. However, cargo rotation is expected to occur on timescales of 10^2 - 10^3 s at cytoplasmic viscosities. Many biological cargos have lipid membranes that may allow surface mobility of motors, leading us to consider motor diffusion on the cargo as a potential resolution to this discrepancy. To compare different motor arrangements and mobilities, we developed a coupled-viscosity model for the movement of cargo and motors and integrated it with our mechanochemical 3D computational model of cargo transport. Using it, we show that organizational changes can optimize for different objectives. Cargos with clustered motors are transported efficiently, but are slow to bind to MTs. On the other hand, those with motors dispersed rigidly on their surface bind MTs quickly, but are transported inefficiently. Finally, cargos with freely-diffusing motors have both fast binding and efficient transport, although less efficient than clustered motors. These results suggest that experimentally observed changes in motor organization may be a control point for transport.

4.2 Introduction

To establish and maintain their internal organization, eukaryotic cells employ molecular motors in the kinesin, dynein and myosin superfamilies to transport organelles and other cargo along microtubule (MT) and actin filaments. Cargo transport is a complex, multi-step process at both the cell scale [15, 96] and cargo scale [56, 97]. The mechanisms by which motor proteins generate forces and move cargos have often been studied [34, 43, 52, 54, 95]. However, much less is understood about how cargos bind to the MT in the first place. We recently reported that the time for a cargo to reattach to the MT after detaching in an optical trap *in vitro* is on the order of 1 second at viscosities 1x-10x water [5] (chapter 5). Furthermore, this time depends strongly on the size of the cargo, indicating that cargo

rotation plays a significant role. How does this extrapolate to the cytoplasmic environment, where viscosities are estimated to be orders of magnitude larger (see 4.5.2)? We begin with a simple calculation, presented in Fig 4.1A. We assume a cargo with a motor bound rigidly to it, oriented such that the motor is opposite the MT. We ask how long it would take for rotational diffusion to bring the motor within binding range of the MT. For cytoplasmic viscosity estimates (gray line), and a cargo of 500nm-1000nm in diameter, we estimate the time to be $10^2 - 10^3$ s. (For details on the calculation see section 4.5.5.) Extrapolations from data in [5] (chapter 5) also yield times to bind of hundreds of seconds (see 4.5.3). Yet, *in vivo*, in COS1 cells, after detachment in an optical trap [98], we found rebinding times were ~ 10 s for dynein-driven transport and ~ 7 s for kinesin-driven transport ([98] and section 4.5.4). Strikingly, the rebinding time for dynein-driven transport went down as lipid droplets made repeated attempts at escaping the optical trap [98], likely due to active regulation [6]. This hints that the binding process is important.

Why is there such an extreme difference between the time predicted by this simple calculation and the measured time? While the calculation doesn't try to correctly reflect details of the lipid droplet's behavior in the optical trap such as the multiple motors likely attached to it or the orientation from which it starts, the extreme time difference led us to consider mobility of the motors on the cargo. Many cellular cargos are encased by lipids, raising the possibility that motors diffuse in those lipids. As a first step, in Fig 4.1B we perform the equivalent of the rigid rotation calculation in Fig 4.1A, now assuming the motor is free to diffuse in a fluid membrane. For diffusion coefficients typical of membrane-anchored proteins (thick line, BioNumbers BNID:114189 [99]), and a cargo of 500nm-1000nm in diameter, we estimate the mean time for the motor to enter the binding radius to be 1-10s. Therefore, the diffusion is fast enough that it might help accelerate motor binding; membrane fluidity might in principle be a key factor in cargo binding and transport initiation.

As mentioned, however, the calculation shown in Fig 4.1 is highly simplified. The on rate of

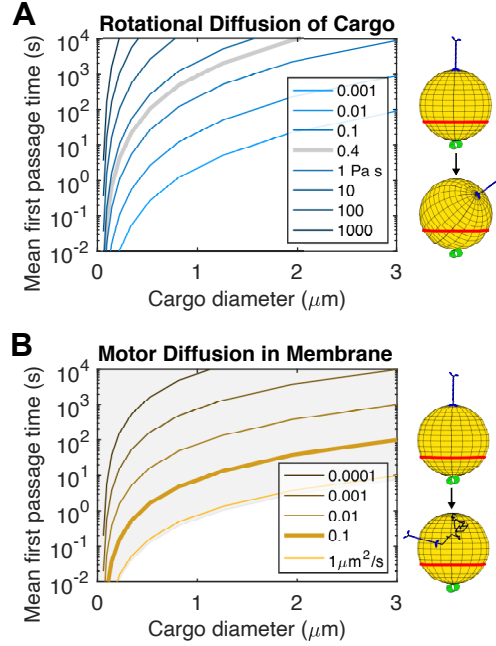


Figure 4.1: A motor (blue in right schematic) initially located opposite a microtubule (green) must move (to below red line) in order to bind the microtubule. Mean first passage times (MFPTs) for this movement are shown for:

A: rotational diffusion of the cargo. Shown for various values of viscosity of the surrounding fluid. Values are chosen to span the range estimated for cytoplasm. Grey curve is at our best guess for viscosity experienced by intracellular cargos, see 4.5.2.

B: diffusion of the motor in the cargo surface. Shown at various values of the diffusion coefficient. MFPTs for diffusion coefficients achievable when the surrounding fluid has viscosity of 0.4 Pa s are shaded in grey, see 4.5.6.

For details on calculation, see 4.5.5.

the motor once it is close to the MT is ignored. Might the motor diffuse away again before it is able to bind, leading to binding slower than predicted above? The presence of multiple motors on the cargo [100, 101] may also explain the fast binding of the lipid droplets, as the angle the cargo has to rotate through to bring a motor near the MT decreases if motors are spread around the surface. On the other hand, several motors often engage the MT simultaneously on lipid droplets [98, 102], and rigidly dispersed motors are unlikely to be able to bind simultaneously [56].

The influence of motor diffusion on transport after binding has been recently studied, but much remains to be understood. In the actin-myosin transport system, both the velocity

and run-lengths of lipid vesicles has been shown to depend on membrane composition [103]. Several papers have compared the collective action of kinesin motors which are free to diffuse in membranes to cases where they are rigidly attached to a surface, both in MT gliding assays [104] and bead assays [105]. These studies show that motor freedom in membranes can influence transport properties, but it is unclear how to translate the *in vitro* results into the context of the cell, especially since transport is slowed in the gliding assay, while it is sped up in the bead assay. Furthermore, Li et al. [105] find that, when motors are allowed to diffuse, there is a small increase in velocity, with no difference in how far cargos travel overall. Thus, the impact which freedom to diffuse may have on cargo transport in the cell is unclear.

In this paper, we carry out a comprehensive computational study of how cargos with different motor organizations are transported with a focus on the less-explored case of cargos which have motors that are free to diffuse in the membrane. This is particularly relevant since some cargos appear to switch motor distribution as they progress through their life cycle [97]. This requires us to compare transport properties over a wide range of membrane fluidities, from fluid to solid. We construct a computational model of cargo transport which includes multiple motors that are able to diffuse in the cargo membrane. While there is a rich history of theoretical studies of motor-based transport [52, 54], the ability to describe the full range of membrane fluidity represents a significant advance, including over 3D models with rigidly attached motors [3, 56]. Since there are two viscosities in the system (the membrane and the cytoplasm), forces translate into motion in a non-trivial, coupled manner. Furthermore, the two viscosities induce diffusion (thermal fluctuations) with different amplitudes, so motors experience statistically correlated noise. Overcoming these computational challenges required that we separately formalize the stochastic equations of motion for all motors and cargo, analytically manipulate these in full generality into a frame of reference in which the motion can be simulated using an Euler-Maruyama scheme, and combining this with the chemomechanics of motor binding and unbinding. This allowed the computationally efficient

simulation needed for the large parameter sweeps we report, in contrast to lookup-table approaches or numerical iteration methods. (Several recent models have included motors that rearrange on the cargo [57, 106]; however, they limited their studies to parameter regimes in which the complicating factors above were absent.) This software is openly available at github.com/mbovyn/Motor-Cargo-Simulator (DOI:10.5281/zenodo.4325111). The results of our computational model reveal that the changes in motor organization and mobility have significant impact on MT binding, transport and force generation. We made a particular effort to simulate behavior at parameters relevant to the cellular environment. Our work points to parts of cargo transport that are not well understood, and informs the picture of overall transport.

4.3 Results

4.3.1 A computational model of cargo transport including freedom of motors to diffuse in the cargo membrane

We wish to explore the impact of different classes of surface organization of motors on the transport steps of binding and running (Fig 4.2A). We choose four extreme cases, as shown in Fig 4.2B, which we term organization modes. The first two modes have motors bound rigidly to the cargo. They differ in how motors are spaced; the first mode we term “rigid clustered” places all the motor anchors at the same point, while in the second mode, which we term “rigid dispersed”, the motor locations are random, drawn from a uniform distribution over the surface. The other two modes have motors which are free to diffuse in the cargo surface. In the “free independent” mode, the motor anchors do not interact with each other, other than through forces they exert on the cargo. In the final mode, termed “free clustered”, all motor anchors are bound together, but the ensemble is able to diffuse in the cargo membrane.

In this mode, we assume the cluster of motors diffuses with a reduced diffusion coefficient so that $D \sim 1/\sqrt{N}$ where N is the number of motors, which is consistent with motors being arranged in roughly a disk (rather than, e.g., in a row, see 4.5.7).

To investigate transport outcomes of cargos in these four different organization modes, we construct a three dimensional model of a cargo, the motors, and a MT. This model includes properties of the cargo, such as size and shape, properties of the motors, such as length and binding rate, and properties of the environment, such as viscosity. For every property we estimate ranges and typical values for cargos in the cell. Descriptions of how these values were estimated and relevant information can be found in section 4.6.2.1. Values used can be found in table 4.3.

We model the cargo as an undeformable sphere. We attach motors to the cargo at points which we term the anchors. We model these motors using the well studied chemomechanics of kinesin-1. These motors can bind the MT when the anchor is within reach of the MT (blue hemispheres in Fig 4.2A represent motor reach length). Once bound, they step along the MT and unbind from it with rates that have been studied *in vitro*. We draw our chemomechanical model for kinesin from recent experimental [41] and modeling [107] efforts. For more details, see supplement sections 4.6.1.1 and 4.6.1.3.

As motors step, they exert forces on the cargo which would both tend to pull the cargo along through the surrounding fluid, and drag the anchor through the cargo membrane. In our model, forces which would drag the anchor through the cargo membrane result both in displacement of the anchor in the membrane and rotation of the cargo, in proportion to the anchor diffusion coefficient. Forces acting to move the cargo body do so against viscous drag, as we model the surrounding fluid as Newtonian. While recent measurements suggest the cytoplasm is an actively-driven, complex fluid with significant elasticity [108, 109], methods for simulating diffusion and the effect of further active forces in this environment are still in development. Modeling the cytoplasm surrounding the cargo as Newtonian allows us to

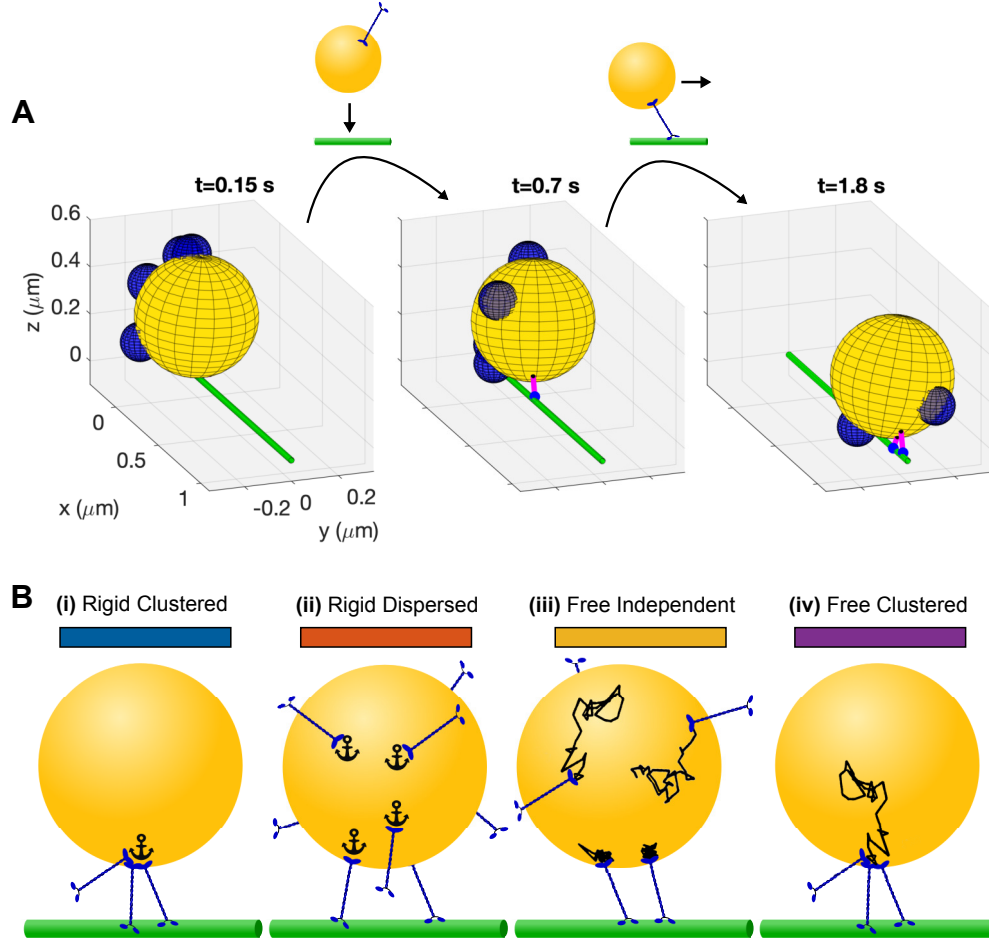


Figure 4.2: Transport outcomes and motor organization modes to be investigated.

A: We separate the cargo transport process into binding and run steps. Before binding, first bound, and transported states are represented by simulation snapshots. The cargo is yellow and the microtubule is green. The blue hemispheres represent the reach length of unbound motors, which have their anchor point at the center of the hemisphere. Bound motors are represented with a magenta stalk, small black anchor point and larger blue sphere at the center of mass location of the motor heads.

B: Motor anchoring modes to be investigated. Cargo shown in yellow, motors in blue and microtubule in green. We name the states: rigid clustered (**i**), rigid dispersed (**ii**), free independent (**iii**), and free clustered (**iv**). Solid color bars indicate the color used for this mode in the rest of the text.

qualitatively capture that some cargos in cells may be relatively free to move, while others may be significantly impeded by their local environment. For more information, see 4.6.2.1.

Both cargo and motors diffuse in their respective (3D or 2D) fluids. The cargo diffuses both rotationally and translationally with statistics governed by the Fluctuation-Dissipation Theorem, as implied by the viscosity of the surrounding fluid. Motors diffuse in the cargo surface with statistics governed by a diffusion coefficient. In general, complex movement may result from the interaction of motor anchors with different lipid domains [97] or other structures (for example, diffusion of cell surface proteins is influenced by the underlying actin cortex [110]).

A full description of the model and derivation of the equations we simulate can be found in supplemental section 4.6. The simulation framework is detailed in supplemental section 4.6.2 and parameter values listed in table 4.3 are used for all simulations, unless otherwise indicated.

To obtain transport properties of cargos in different organization modes, we simulate 100 or more stochastic trajectories and examine the resulting distributions. We simulate trajectories using a hybrid Euler-Maruyama-Gillespie scheme, and report a series of tests which show that the code reproduces expected results in some simplified situations in supplemental section 4.6.3. Snapshots from a single trajectory are shown in figure 4.2A. As time progresses, motors diffuse around the surface of the cargo, motors bind and unbind from the MT, and cargo orientation changes as it is pulled along by forces generated by the motors, as shown in video 1 of [4].

4.3.2 Cargos with free motors bind to the microtubule faster than those with rigidly anchored motors

For a cargo to be transported, one of its motors must first bind the MT. In this section, we use our model to investigate the time it takes for a cargo located near a MT to bind to it. Visualizations of example simulations for each organization mode are shown in videos 2–5 of [4].

We first compare the four organization modes as a function of the number of motors on the cargo, as Fig 4.3A shows for a $0.5\text{ }\mu\text{m}$ diameter cargo. Simulated cargos are allowed to diffuse rotationally, but not translationally, with no gap between the cargo and microtubule. Initial motor locations are picked from a uniform distribution over the surface of the cargo. We find that for cargos in the rigid clustered mode, the mean time to bind is long when there is a single motor, and stays constant as the number of motors on the cargo increases. This can be understood by considering the timescales in the problem; cargos in this mode spend most of their time waiting until the motors are near enough to the MT for them to bind, as rotation is slow compared to the characteristic binding time of a single motor. A cargo in the rigid dispersed mode with only one motor is identical to a rigid clustered cargo with one motor. For rigid dispersed cargos, however, we find the time to bind decreases drastically as more motors are added. The average angle though which these cargos must rotate before a motor comes within reach of the MT decreases as motors are added. This change is most drastic for the first few motors, with the time to bind of these $0.5\text{ }\mu\text{m}$ diameter cargos decreasing by an order of magnitude with the addition of only 5 motors.

We find that $0.5\text{ }\mu\text{m}$ diameter cargos with a single free motor with diffusion coefficient $0.1\text{ }\mu\text{m}^2\text{ s}^{-1}$ bind more than an order of magnitude faster than cargos of the same size with a single rigidly attached motor. This also can be understood by considering timescales: diffusion on the surface is much faster than rotational diffusion of the cargo, so less time is

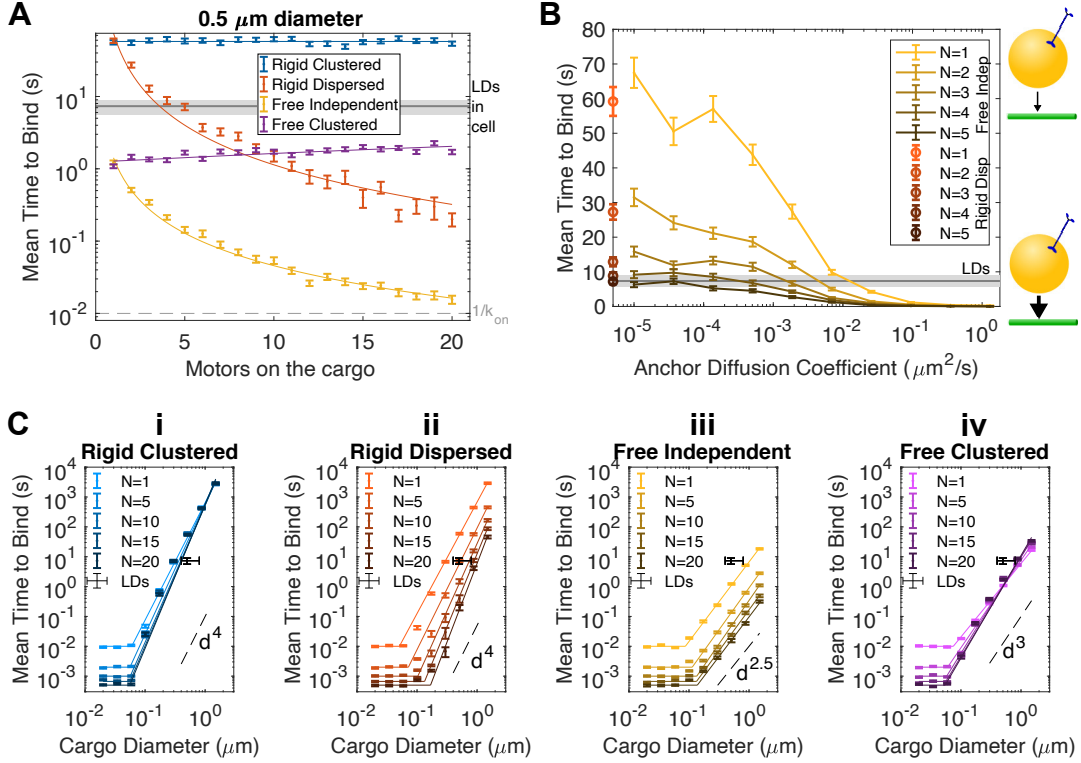


Figure 4.3: Times for cargos in different organization modes to bind to the microtubule. Simulated cargos are allowed to diffuse rotationally, but not translationally, with no gap between the cargo and microtubule. Initial motor locations are picked from a uniform distribution over the surface of the cargo. In all panels, error bars represent standard error of the mean of 300 simulations. Underlying distributions are approximately exponential (see figure 4.8). The experimentally measured time for lipid droplets (LDs) to bind in the cell [98] is represented in each panel: Grey rectangles in panels **A** and **B** represent the measured mean $\pm$ standard error of the mean. In **C**, additional error bars in diameter represent the approximate range of sizes of lipid droplets measured.

A: Time for the cargo to bind to the microtubule for the four anchoring modes, as a function of the number of motors on the cargo. The characteristic time for a single motor to bind, assuming it is near the microtubule, is shown in dashed grey. Distributions of times to bind in the different modes are shown in figure 4.8A. Overlaid curves are fits, detailed in table 4.2.

B: Dependence of the cargo binding time on the diffusion coefficient of the motor anchors in the cargo membrane for the free independent mode. Red error bars located on the left vertical axis are for the rigid dispersed mode. Distributions of binding times at the indicated diffusion coefficients are shown in figure 4.8Bi. Distributions of binding times for the lowest diffusion coefficient and rigid dispersed cargos are shown together in figure 4.8Bii. Lines between points are guides for the eye.

C: Time for the cargo to bind as a function of the cargo radius for the four anchoring modes (**i-iv**), shown for various values of the number of motors on the cargo, N . Dashed lines indicating scaling with diameter d to the indicated power are shown for comparison. Overlaid curves are fits, detailed in table 4.2.

spent waiting for a motor to come near the MT. When free motors are added in a cluster, we find that time to bind does not decrease with more motors in that cluster. In fact time to bind increases slightly. This is due to our assumption that clusters with more motors have a diffusion coefficient that decreases with the number of motors in the cluster (more drag through the cargo surface), see 4.5.7. This indicates that the time spent waiting for motors to come near the MT is the slowest process. When anchors are independent, time to bind goes down drastically as the number of motors is increased. This effect includes the decreased time to bind from the spread out initial locations of the motors, as well as the fact that each motor performs its own search. While anchor motions are all subject to the same contribution from the rotational diffusion of the cargo, the rotation timescale is much slower than surface diffusion, making each search almost independent at this cargo size and diffusion coefficient.

The results of Fig 4.3A indicate that at $0.1 \mu\text{m}^2 \text{s}^{-1}$, surface diffusion is much faster than cargo rotation. At some diffusion coefficient, surface diffusion should become slower than cargo rotation, and the time to bind for free independent cargos should approach that of rigid dispersed cargos. We find that the motor diffusion coefficient must be decreased by orders of magnitude to obtain significant changes in the time to bind, as shown in Fig 4.3B. For the $0.5 \mu\text{m}$ diameter cargos shown, diffusion coefficient of the motors must be lower than $10^{-4} \mu\text{m}^2 \text{s}^{-1}$ for free independent cargos to have times to bind similar to rigid dispersed cargos.

We find that times to bind for cargos in each of the organization modes depend differently on the cargo size. When cargos are small enough that all motors can simultaneously reach the MT ($\sim 50 \text{ nm}$ diameter), the time to bind for cargos in all organization modes is the same. (We note our model assumes no direct, e.g., steric, interaction between motors, which could influence this result strongly for small cargo and many motors.) As cargo size increases, time to bind remains dependent only on motor number until cargos reach $\sim 100 \text{ nm}$ in diameter.

For cargos larger than this, scaling of time to bind with size is drastically different for cargos in the different organization modes. For cargos with rigidly attached motors, time to bind scales with approximately the fourth power of the cargo diameter. For cargos in the free independent mode, time to bind scales with between the second and third power of the diameter. Free clustered cargos with one motor are identical to free independent cargos with one motor, and have the same scaling. As the number of motors increases, however, scaling becomes more severe, nearing the scaling of rigid motors at high motor number.

4.3.3 Cargos with free independent motors form dynamic clusters which increase travel distance

We next investigate the distance that cargos travel, after initial attachment to the MT. To do so, we begin simulations with a single motor bound to the MT and simulate until the cargo reaches a state in which all motors are detached from the MT. A few stochastic trajectories, along with the mean position over many cargos are shown as a function of time in Fig 4.4A (top). Once bound to the MT, rigid clustered cargos and free clustered cargos behave similarly. Hereafter, we show only results for rigid clustered cargos and refer to them as “clustered” to reflect this. Visualizations of example simulations for each organization mode are shown in videos 6–8 of [4].

We find that cargo run lengths depend strongly on motor organization mode. For cargos with clustered motors, just four motors working together give run lengths on the order of the size of a cell. Motors in this mode work together very well, as if any motor is bound the rest of the motors are located where they are also able to bind the MT. This contrasts with the dispersed mode, where many motors are necessary to achieve run lengths of a few microns. For the $0.5\text{ }\mu\text{m}$ diameter cargos plotted in Fig 4.4B, 25 motors are required to achieve a mean run length of $3\text{ }\mu\text{m}$. These results are consistent with previous work comparing these two

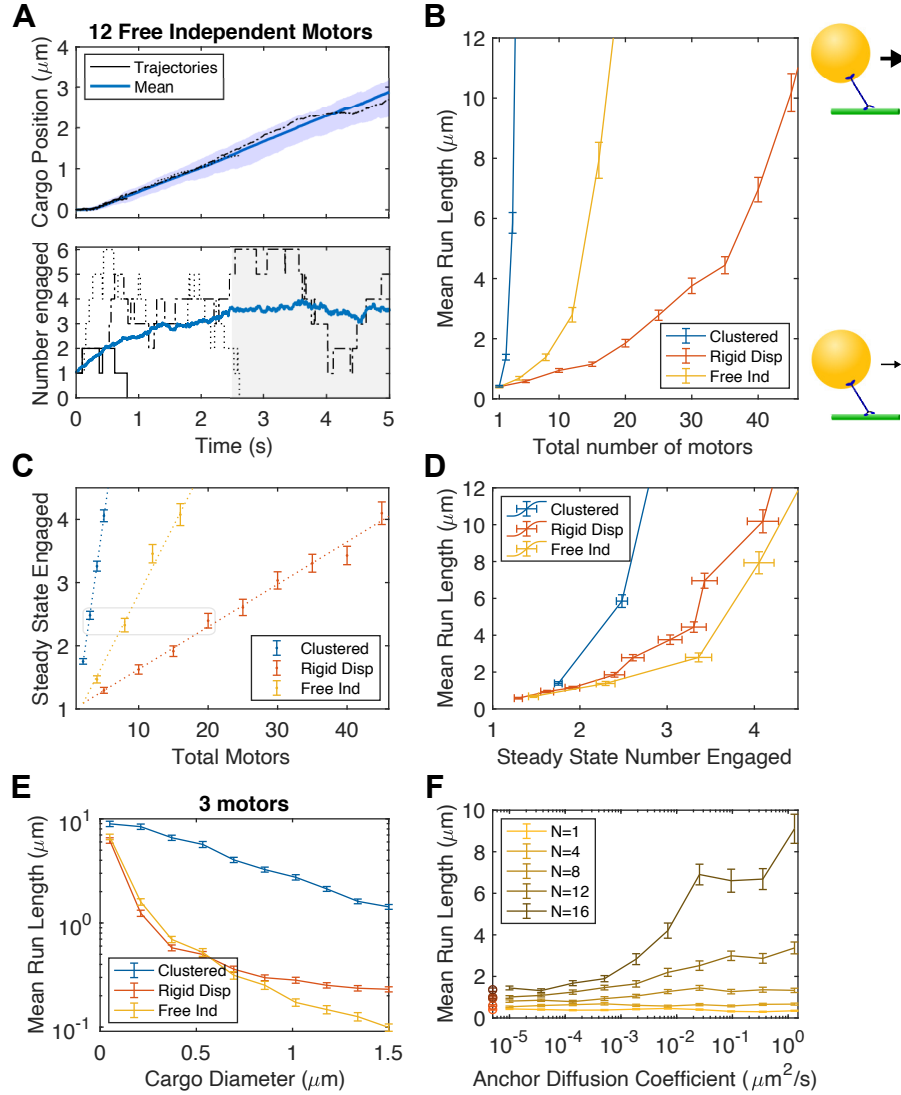


Figure 4.4: Free independent cargos have longer run lengths than rigid dispersed cargos due to dynamic clustering

A: Position (top) and number of motors engaged (bottom) with time for free independent cargos with 12 total motors. Trajectories of three cargos are shown in black and the mean over 100 cargos is shown in dark blue. Cargos are excluded from the calculation after they fall off the microtubule. The area between the 5th and 95th percentile positions is shaded in blue in the top panel. Times greater than the time to steady state are shaded in grey in the bottom panel.

B: Mean run lengths for cargos in each of the three anchor modes as a function of the total number of motors on the cargo. Distributions of run lengths are shown in figure 4.9A.

C: Mean number of motors engaged at steady state versus motor number for the 3 anchor modes. Linear fits are shown as dotted lines. For values, see table 4.2. Grey box indicates conditions with similar mean number engaged at steady state which will be compared in later figures.

Caption continues on next page.

Figure 4.4:

D: Mean run length as a function of steady state number of engaged motors for the three anchoring modes.

E: Mean run length for cargos with three total motors as a function of cargo size for each of the three anchoring modes.

F: Mean run length for cargos with free independent motors as a function of the diffusion coefficient of the motors in the cargo membrane, for different numbers of total motors. Red error bars overlaid on the left axis are for rigid dispersed cargos of the same total numbers. In **B**, **E** and **F**, error bars are SEM of 300 cargos. In **C**, error bars are SEM of 100 cargos. In **B**, **D**, **E** and **F** lines between points are guides for the eye.

modes [56].

For cargos with free independent motors, we find run lengths which are longer than those of dispersed cargos, but not as long as those of clustered cargos. One possibility is that the run lengths are due to the number of motors which are instantaneously bound to the MT at a given time, which we term the number engaged. We therefore query this quantity in our simulations. The number of motors engaged on the MT fluctuates with time. Several stochastic trajectories are shown in Fig 4.4A (bottom), along with the mean over 100 cargos at each time. We find that the mean number of motors engaged rises from the initial condition of 1 to a steady state value over a period of time. In Fig 4.4C, we show that free independent cargos have more motors engaged than rigid dispersed cargos. The initial locations of motors on the surface of cargos in these two modes is the same, i.e., uniformly random on the surface. Therefore, the increased steady state number of engaged motors on free independent cargos indicates that motors are diffusing to the MT, binding, and remaining bound for longer than they would if simply placed randomly. In other words, the motors cluster near the MT. These clusters are dynamic, with motors diffusing in and binding, as well as unbinding and diffusing away, as can be seen in videos 1 and 8 of [4].

How strong is the clustering effect? In the range of total motor numbers investigated, the number of engaged motors is 25 % to 30 % of the total number on the cargo. This is more than the 10 % to 15 % of motors engaged on rigid dispersed cargos, but less than the ~ 80 %

of motors engaged on clustered cargos (Fig 4.4C). So, while dynamic clusters contain more motors than would be available to bind the MT if motors were distributed randomly on the surface, they do not contain all or even most of the motors on the cargo.

We hypothesized that dynamic clustering is responsible for free independent cargos' enhanced run length over rigid dispersed cargos. To test this hypothesis, we plot mean run length vs. steady state number engaged. If dynamic clustering is responsible for the enhanced run length, we expect the free independent and rigid clustered modes to have the same run length when the number of engaged motors is the same. We see in Fig 4.4D that data from the two modes is similar. Run lengths for free independent cargos are in fact slightly lower once corrected for number of engaged motors. This is surprising because cargos with similar mean numbers of motors engaged at steady state have similar distributions of motors engaged, as shown in figure 4.9B. In supplementary figure 4.9C, we show that the lower run length is explained by a longer time to steady state and resultantly more cargos which fall off the MT at early times.

The three modes also differ significantly in their dependence on cargo size. In Fig 4.4E, we show that cargos with clustered motors have a run length that depends only weakly on cargo size, while free independent and rigid dispersed cargos have a more complex dependence.

The run length advantage of free independent cargos over rigid dispersed ones should, like the binding time advantage, be reduced to zero at low anchor diffusion coefficients. In Fig 4.4F, we show that the diffusion coefficient must be reduced by orders of magnitude to have significant impacts on run length. We find that diffusion coefficients below $10^{-4} \mu\text{m}^2 \text{s}^{-1}$ are effectively rigid, which is a similar threshold we found for time-to-bind.

4.3.4 Cargos with free independent motors are better able to transport against a load compared to cargos with rigid dispersed motors

A cargo's ability to generate a sustained force is also important for navigating the crowded environment of the cell. In this section, we examine the run lengths of cargos in the different organization modes against a constant force.

As expected, we find that that increasing force decreases the run length of cargos, no matter what the organization mode or number of motors, as can be seen in 4.10, A. We find that 7 pN of force is sufficient to reduce the run lengths from 20 μm or more to nearly zero in every organization mode.

We now compare the run length of cargos in different organization modes under force. In Fig 4.5A, we show that cargos with 20 free independent motors have significantly longer run lengths than cargos with the same number of rigid dispersed motors, when subject to forces up to 7 pN. At higher forces, run lengths for these cargos are effectively zero. At this high number of motors, cargos with clustered motors travel long distances, even when loaded with 12 pN or more (Fig 4.5A, arrows). When the number of total motors is 5, we can see that cargos with clustered motors outperform both rigid dispersed and free independent cargos (4.10, B). At this low number of total motors, cargos in rigid dispersed and free independent modes are almost always driven by a single motor, so differences between the two modes are not apparent.

Is the increase in run lengths under force also solely due to changes in the number engaged? To test this hypothesis, we plot run lengths under force for cargos in the three organization modes with total numbers of motors which give the cargos matching steady state numbers of engaged motors (at 0 force, indicated by box in Fig. 4.4C). Like in the zero force case, when we

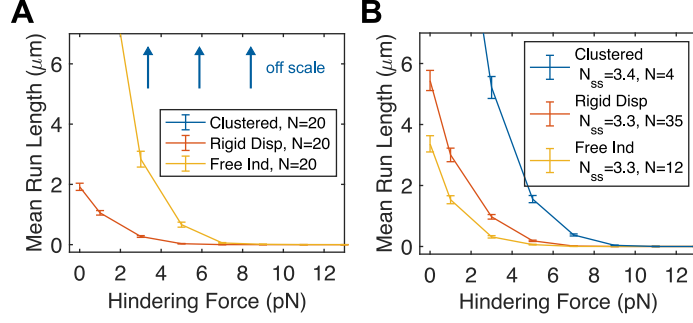


Figure 4.5: Cargos with free independent motors travel farther under load due to dynamic clustering.

Mean run lengths as a function of external hindering load on the cargo for cargos in each of the three modes, with

A: matched total number of motors. Cargos with 20 clustered motors travel farther than 20 μm on average, and are not shown to clarify the difference between rigid dispersed and free independent modes.

B: matched number of motors engaged at steady state (at 0 external load). Distributions of run lengths for these cargos can be found in figure 4.10C.

Error bars are standard error of the mean over 300 cargos in both **A** and **B**. Lines between points are guides for the eye.

compare at the same steady state number, we find that cargos with free independent motors have similar, but slightly lower run lengths than cargos with dispersed motors. Therefore, the enhancement in run length of free independent motors comes from the ability of motors to form dynamic clusters, like in the unloaded case.

A priori, we hypothesized that free motors share the load more equally, and that this would lead to longer run lengths under load, after correcting for number engaged. However, as shown in Fig. 4.4B, we do not find this effect is strong enough to outperform cargos with rigid dispersed motors on a per-motor-engaged basis.

4.4 Discussion

While transport of subcellular cargo by molecular motors is increasingly understood, the extension of this understanding to control of cell internal organization will involve studying

the three-way interplay between the cargo, the MT and local environment, and the motors. In this work, we made a particular effort to simulate behavior at parameters relevant to the cellular environment. Our work points to parts of cargo transport that are not well understood, and informs the picture of overall transport. We developed a computational model of the motors' interaction with the cargo, assuming different modes of organization. These modes varied both in the positions of the motors on the surface of the cargo and the freedom of those positions to change via movement in a fluid membrane. Combining Fig. 4.3 and 4.4, we arrive at our main conclusion, summarized in Fig. 4.6. First, cargos with rigidly attached motors face a tradeoff between, on the one hand, time for the cargo to bind to a nearby microtubule and, on the other, the run length of cargos once one motor is bound. Rigid clustered motors bind slowly, but have long run lengths. Rigid dispersed cargos bind faster than rigid clustered cargos, but have short run lengths. Second, depending on parameters, cargos with free motors can overcome this tradeoff. Cargos with free independent motors bind faster than cargos in either of the rigid modes, and have run lengths which are longer than those of rigid dispersed cargos, but not as long as clustered cargos. When motors are arranged in a free cluster, cargos have the same long run lengths as rigid clusters, as well as binding to a nearby microtubule more quickly. The time to bind is not as fast as a free independent cargo, however, or even a rigid dispersed cargo with many motors.

4.4.1 Cargo size provides a potential sorting mechanism and avenue for studying motor-cargo attachment

We find that time to bind and run length are sensitive to cargo size in different ways for the four organization modes (Fig. 4.3C). Rigid clustered cargos have a time to bind which scales up strongly as cargo size increases, but run length is relatively insensitive to cargo size. The time to bind for rigid dispersed cargos also scales strongly, but their run length is more sensitive to cargo size in comparison to rigid clustered cargos. Free independent cargos

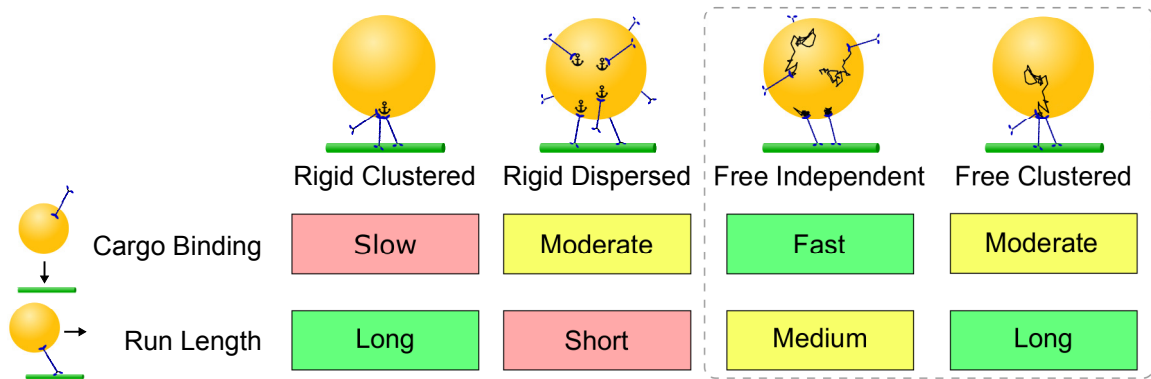


Figure 4.6: We find that different organizations of the motors on the cargo have different implications for how rapidly the cargo will bind to a nearby microtubule and the cargo’s ability to travel along the microtubule. For cargos with motors rigidly bound, (first two columns) there is a tradeoff between clustered motors, which are slow to bind the microtubule, but travel long distances, and dispersed motors, which bind the microtubule quickly but are have poor travel distances. Cargos with freely diffusing motors (third column), at reasonable surface diffusion coefficients, cargo sizes and motor numbers, overcome this tradeoff. They bind the microtubule at least as fast as rigidly dispersed motors, and faster for large and realistic estimates of diffusion coefficient. They travel farther than rigid dispersed motors because of the formation of dynamic clusters. Because these clusters have high internal turnover, travel distances are lower than that of a rigid cluster. For a cargo with a freely diffusing cluster of motors (fourth column), cargo binding may behave more like a rigid cluster or freely diffusing motors, depending on parameters. Once it has bound the microtubule, it behaves indistinguishably from a rigid cluster in both run length and force generation.

have a much weaker scaling of time to bind, as well as an intermediate dependence of run length on cargo size. These different scalings raise the possibility that the cell could use the size dependent behaviors to differentially direct otherwise similar cargos.

Binding time scaling found in Fig. 4.3C could be used to identify the organization of motors on cargos from the cell. Using the natural variability of cargo sizes to uncover different scaling exponents could inform whether the motors are free or not.

4.4.2 Comparison with previous studies

This work adds to a growing body of evidence that, first, the position of motors on the cargo (whether free or not) impacts transport [56, 111]; second, that mobility on the cargo surface further influences transport [57, 104–106, 112]; and, third, that there are significant differences in the arrangement of motors on cargo in cells [97, 106], for example via changes to lipid content [113, 114]. The closest direct experimental study related to our work is by the Xu Lab [105]. *In vitro*, they find broad agreement with our simulations that fluid membranes enhance transport. However, the details are different: they find increased velocity for membrane cargos where we predict none, and do not find a run length increase. We note that our simulations predict significantly longer run lengths for free motors only when total motor numbers are more than about 5, a regime not tested in [105]. Our work thus highlights the difficulty extrapolating *in vitro* results into the cellular environment.

Our work is in broad agreement with Chowdary, Kaplan, Che, and Cui [106], who combined advanced microscopy and tracking with a computational model to give insight into the motion of endosomes in axons. They report the emergence of dynamic clustering in their simulation via preference for binding the MT track. Their simulation allows motors to move on the surface of a cargo independent of each other and of rotations of the cargo itself. Our work extends this by considering a model in which motor anchor position, cargo rotation and

cargo translation are all coupled with both cytoplasmic viscosity/fluctuations and membrane viscosity/fluctuations, via a force-balance relationship at every time point. Particularly interesting complexity arises, e.g., the two viscosities give rise to fluctuations that are, in the lab frame of reference, no longer uncorrelated (see supplemental). Doing so allowed us to explore a wide range of parameters, from the limit in which the cargo membrane is nearly inviscid and the motor can step along the MT without significantly moving the cargo, all the way to a static anchoring. It also allowed us to explore the competition between cargo rotation and diffusion of motors to drive binding time – of particular importance when the cargos are small. The coupling via force-balance also led to an emergent inter-motor communication via how each motor is loaded, which influenced predicted run lengths. Interestingly, we find that dynamic clustering is not a strong effect for all parameters regimes. For example, at best guess parameters, we find only 25% of the motors are clustered (e.g., Fig 4.4).

Our work contrasts with Jiang, Vandal, et al. [115], which predicts that hundreds of motors are necessary to achieve long run lengths. Jiang, Vandal, et al. [115] measure the accumulation of diffusing motors on microtubules in a gliding assay configuration, and find that motors bind slowly. They interpret a model where kinesin binds at $\sim 5 \text{ s}^{-1}$ (which this paper also uses), but has only a short reach. The model for kinesin binding in this paper uses the length of kinesin-1 in electron microscopy for the reach parameter. We consider this to be a lower bound for the maximum possible reach of kinesin in cells, since adaptor proteins likely extend this length somewhat. It is clear that in some situations kinesin binds quickly [5] (chapter 5), while in others it binds slowly [115]. It is unclear why, and even more unclear how to model this process in the context of the cytoplasm. Measurements of the number of kinesins on cargos have been made. Photobleaching indicates that just a few kinesins are present on mouse macrophage phagosomes [101] and phagosomes from mouse brain [100]. Quantitative mass spectrometry on synaptic boutons finds that 1-2 kinesins are present per vesicle [116] (see 4.5.9). While few motors are present on these cargos, it remains unclear

how motor numbers vary between cargos of different types and sizes.

4.4.3 Influence of cargo surface mobility on transport

Our results show that changing the diffusion coefficient of motors can change transport. As shown in Fig. 4.4F, a large change in diffusion coefficient is necessary to significantly change transport (potentially explaining why [115] did not observe a change in their assay, which also had a different geometry that will significantly influence motor behavior). It is tempting to speculate that this could be used to modulate transport. In Sec. 4.5.6, we discuss theoretical estimates for upper bounds on diffusion coefficients. Diffusion coefficients several orders of magnitude lower have been measured for transmembrane proteins [117], showing that a wide range is achievable in the cell. Briefly, this could be achieved by changes in lipid composition [105, 115], changes in linker molecules [114, 118, 119], membrane tension [120] or by membrane crowding. Whether any of these modulators would lead to a sufficiently large change to modulate transport remains to be determined. However, in a potential analogous system, we note a 5-fold change in membrane diffusion of *cdc42* in the yeast outer membrane has been suggested to be under regulation during yeast mating [121]. Another possibility is by changing anchoring to a structure like a lipid raft [97, 114] or cytoskeletal structure [110], which could (if the anchor were long-lived) reduce diffusion coefficient to nearly zero.

4.4.4 Quantitative model provides framework to infer motor organization

It is possible to combine various measurements of cargo and transport properties made in cells with the results of our model to learn about those quantities which are unknown. Data on forces generated in optical traps, which can be used to infer numbers of active motors, is

abundant ([98, 102, 122–125], many others). Binding time has been quantified *in vivo* in [98] and *in vitro* in [98, 101]. We are aware of direct reports of (total) motor numbers for two cargos [100, 101], and indirectly for one more ([116], see 4.5.9). We know of motor organization reported for one case [97]. It is rare that several of these quantities are reported for the same cargos. Chaudhary, Berger, Berger, and Hendricks [101][126] report motor numbers, forces, and binding times *in vitro*, from which it may be possible to infer organization. Reddy, Mattson, et al. [98] report binding times and forces in cells, which we comment on below. Combining measurements of binding time, force generation, and motor number would allow for significant insights into both how cargos are transported and how kinesin and other motors function in the cell. Since each of these quantities has been measured individually, they need only be combined to allow inference on organization. Combining optical trapping with super-resolution microscopy [127] could allow for even better, individual cargo specific characterization. We point out that super-resolution measurements would likely not be able to fully define organization, since free independent motors may look the same as rigid dispersed motors (or possibly clustered motors) in a snapshot. However, using our results, it would be possible to combine an organization snapshot with force measurements and/or binding times to determine organization.

Both binding times and forces for COS-1 lipid droplets can be obtained from the experiments conducted in [98]. In Sec. 4.5.4, we revisit data collected in [98] to extract kinesin-driven binding times. The measured 7 s binding time is consistent with either ~ 5 rigid dispersed motors, or 5 or fewer free independent motors (at varying diffusion coefficients). However, the mean forces generated by the lipid droplets indicate they are often driven by multiple kinesins. We find that for the parameters chosen, both free independent and rigid dispersed cargos with 5 motors are almost always driven by a single motor (Fig. 4.4C). Extrapolating from the results of our model, two resolutions to this conundrum present themselves. First, if kinesins were organized into groups of 2 which were then rigidly dispersed on the cargo, they would have similar binding times to 5 single motors rigidly dispersed, as binding times

are rotation limited. They would also engage 2 motors much more often, resulting in higher forces. Second, free independent motors could form stronger dynamic clusters and engage more motors simultaneously if the binding rate of subsequent motors was higher than 5 s^{-1} , possibly allowing for both 7 s binding time and multiple motor forces. Since MAP7 is known to increase kinesin binding rate [126, 128, 129], a higher binding rate is possible for the kinesins on the lipid droplets in COS-1 cells.

4.4.5 Future model extensions

That the transport machinery is sensitive to motor organization opens several possible directions of future work. First, cargos in the cell exist in a microtubule network with a specific architecture [3, 58, 130, 131]. The current work focused on exploring interaction with a single MT, but could readily be extended. Second, many cargos are deformable [132] and this might lead to significant differences in transport. While deformability of a large cargo will lead to transport changed by interacting with the environment, e.g., spacing of nearby cytoskeletal elements and organelles, our work suggests cargo deformability might also impact transport more directly via changes to motor organization. Finally, motors are sensitive to MT-associated proteins (MAPs) [81, 133]. The accessibility of the motors to MAPs is expected to exhibit similar behavior to the accessibility of motors to the MT, which we have shown to vary widely depending on organization mode.

4.5 Methods

4.5.1 Chemomechanical model of cargo transport with viscous membranes

In [3] (chapter 3), we presented a chemomechanical model for cargo transported by multiple motors. The cargo is modeled as a sphere in a Newtonian fluid with cytoplasmic viscosity, that can translate and rotate under deterministic forces and thermal (stochastic) forces. Motors bind, unbind and step in stochastic, force-dependent and location-dependent manner.

Here, to simulate motors diffusing on the surface of the cargo, we extend the model from [3] (chapter 3) in the following ways. First, we allow the motor anchor points to move on the surface of the cargo, experiencing a second viscosity in the local plane of the spherical cargo. This increases the number of degrees of freedom in the computation from 6 (translation and rotation of the cargo) to $6 + 2N$ where N is the number of motors (in addition to the location of the motor heads). To describe motor diffusion, we also must now consider stochastic thermal forces in the second viscosity. Finally, we also develop new force-dependence for stepping and unbinding in response to experimental evidence, e.g., [41, 107].

To numerically simulate this new model, in which motors experience two viscosities and two distinct modes of thermal fluctuations, we first formalize the equations of motion (not including binding, unbinding and stepping) and solve these analytically, then input these into the numerical scheme that uses an Euler-Maruyama time-stepping for motion and a Gillespie-type scheme for binding, unbinding and stepping.

Full description of the model is in chapter 4.6, and the code is openly available at github.com/mbovyn/Motor-Cargo-Simulator (DOI:10.5281/zenodo.4325111).

4.5.2 Estimate for viscosity experienced by cargos rotating in the cytoplasm

The cytoplasm is known to have complex rheology, with estimates for its viscosity varying orders of magnitude [134]. In section 4.6.2.1, we provide an in-depth literature review and discussion of estimates for cytoplasmic viscosity. We conclude that the most relevant study is Wilhelm, Gazeau, and Bacri [135], who used active, magnetic rotational microrheology to study chains of magnetic endosomes in HeLa cells. The authors report a value for the viscous component of the resistance to rotational motion as 2 Pa.s. Since the chains of endosomes are several microns long and are possibly experiencing resistance from being dragged through the cytoskeleton, it is unclear if spherical cargos would experience similar resistance. Wilhelm et al. then repeat their measurement on cells with cytoskeletons perturbed by either latrunculin A or nocodazole, finding that in either case the viscous component is reduced to 0.4 Pa.s. We select this value as our best guess for the viscosity experienced by rotating cargos. For further discussion, see section 4.6.2.1.

4.5.3 Extrapolation of rebinding time from previous work *in vitro*

In [5] (chapter 5), we measure the time it takes for beads with single kinesin motors to bind to the microtubule after detaching in an optical trap. We find the time increases with viscosity, from 0.8 s in water to 4.4 s at 10x water viscosity (0.01 Pa.s). We construct and fit two models of the binding process, one assuming motor binding is reaction limited and the other assuming motor binding is diffusion limited. When we evaluate these two models to 0.4 Pa.s, we find times of 100 s and 300 s, respectively. We additionally fit a line to rebinding time as a function of viscosity and extrapolate to 0.4 Pa.s to find 190 s.

4.5.4 Quantification of rebinding time for lipid droplets

We use data from experiments originally conducted in [98], which focused on dynein-driven cargo, to estimate kinesin-associated binding times. Forces produced by the motors on the lipid droplets (LDs) from COS1 cells was measured using an optical trap (700 mW, 980 nm single mode diode laser, EM4) and Position Sensitive Diode (PSD, First Sensor CA) assembled onto an high resolution DIC Nikon microscope, as described in [98]. High resolution real time particle tracking and automated translation stage were used to stall a linearly moving LD inside the cell by positioning the optical trap on to its center. The motors on the trapped LDs produce a series of stalls and detachments from MTs in the cell. MT orientation in the COS1 cells is majority plus ends out. So whenever LDs moved linearly outward from the cell center they are predominantly moved by kinesin motors (for quantification they are designated as MT Plus end or P events) and dynein motors move the LDs towards the cell center (MT Minus end or M events).

In COS1 cells motor detachment and rebinding events can be observed as clear peaks and sharp step like falling of PSD voltage signal in the PSD (2 kHz). Particle tracking of LDs in DIC video (30 Hz) also showed similar position displacements. A trap stiffness of 5 pN to 8 pN/100 nm and cell thickness of $\sim 1\text{ }\mu\text{m}$ to $3\text{ }\mu\text{m}$ are found to be optimum conditions for force measurements. For *in vitro* force measurements, LD motion was reconstituted after purification from COS1 cells using a five step sucrose gradient centrifugation [98].

In table 4.1, we report summary statistics for the measured binding times. PP represents the average time between LD plus end detachment and resumption of its motility in plus end. MP is the average time between LD minus end detachment and resumption of its plus end motility inside the optical trap.

	Mean (s)	SEM (s)	N
<i>in vivo</i>			
PP	7.3	1.7	26
MP	7.0	1.1	15
<i>in vitro</i>			
PP	.19	.06	89

Table 4.1: Measured rebinding times for lipid droplets in COS1 cells and for lipid droplets purified from COS1 cells and with their motility reconstituted *in vitro*. Mean and standard error of the mean (SEM) taken from N binding events. Quantification was done on data taken for [98].

4.5.5 Time for a motor to reach the microtubule

In figure 4.1 we show an estimate of the time it would take for a motor, initially opposite the MT, to come within reach of it. These times come from analytical expressions for mean first passage time to a spherical cap,

$$\frac{8\pi\eta R^3}{k_B T} \log \left(\frac{1 - \cos(\pi)}{1 - \cos(\theta)} \right) \quad (4.1)$$

for rotational diffusion of the cargo and

$$\frac{R^2}{D} \log \left(\frac{1 - \cos(\pi)}{1 - \cos(\theta)} \right) \quad (4.2)$$

for diffusion in the cargo surface [136]. Here η represents viscosity (of the fluid surrounding the cargo), R is the cargo radius, k_B is Boltzmann's constant, T is temperature, and D is diffusion coefficient (of the motor in the cargo surface). To apply these equations, we had to first estimate θ , the extent of the spherical cap to which the motor must diffuse before binding. To do so, we simulated motors diffusing from the north pole of the cargo and recorded the first anchor location where the motor was able to bind with its maximum rate as described in supplemental section 4.6.1.3, referred to as a max reach location. We then used the mean elevation of these points as our estimate for θ .

Max reach locations and mean elevations are shown for various cargo sizes in figure 4.7.

4.5.6 Range of accessible motor diffusion coefficients

As discussed in Introduction and Discussion, the diffusion coefficient of motors in the cargo surface is unknown. In Discussion, we mention molecular phenomena that could influence motor mobility.

Here, we perform a reductionist estimate. Saffman and Delbrück described that the diffusion coefficient of a disk in a membrane depends on the size of the disk, the viscosity of the membrane and the viscosity of the surrounding fluid [137]. The highest achievable diffusion coefficient for any membrane viscosity is $k_B T / (16a\eta)$, where a is the radius of the disk and η is the viscosity of the surrounding fluid. The lowest achievable diffusion coefficient approaches 0 as membrane viscosity increases (see [138, 139]). Because both the motor anchor and membrane viscosity are likely highly variable for different cargos, we annotate figure 4.1B with an estimate for the highest achievable diffusion coefficient ($1.3 \mu\text{m}^2 \text{s}^{-1}$ for $a = 0.5 \text{ nm}$), and the lowest possible ($0 \mu\text{m}^2 \text{s}^{-1}$).

We explore this full range of surface mobilities in the Results.

4.5.7 Model for free clusters

Keeping with the Saffman and Delbrück model [137], we model a free cluster as having motors grouped together diffusing in a membrane with the same viscosity as motors in the free independent mode. A diffusion coefficient of $0.1 \mu\text{m}^2 \text{s}^{-1}$ with a hydrodynamic radius of 1 nm corresponds to a membrane viscosity of $0.009 \text{ Pa s } \mu\text{m}$ with the closed form expression of [139]. We are then left to model how the hydrodynamic radius changes as a function of the motor number. We model each new motor in the cluster as adding an additional 2 nm disk

to the cluster. We then model the arrangement of these disks as the densest packing possible and take the hydrodynamic radius of the cluster to be the radius of the smallest circle which can circumscribe these disks, given in [140]. For 20 motors, this gives a hydrodynamics radius of 5 nm. Since the resulting spread is small compared to the length of the motor's reach (80 nm), we approximate all the motors as overlayed at the same point in space. To simulate this, we give a single motor a binding rate N times the base binding rate.

Since neither linkers nor clusters are well understood [114], there is no model for cluster arrangement suggested by data. While an individual linker could be bound to more than one motor, reducing this size, it is unlikely a single linker could effectively transmit large forces created by many motors. Packing could also be less dense than ideal, increasing the hydrodynamic radius.

4.5.8 Curve fitting

All fits were accomplished with the fit function in Matlab.

Data in figure 4.3A were fit to the functional forms in table 4.2.

Data in figure 4.3C exhibit two regimes, for small cargos binding time is insensitive to cargo size, while depending strongly on cargo size for bigger cargos. To reflect this, two curves are represented. For small diameters, $1/(Nk_{\text{on}})$ is plotted. Power laws are fit to all cargos above a certain size for each motor number, and the two curves are joined where they intersect. Power law fits were made to cargos above 50 nm, for rigid clustered and free clustered modes, while fits were made to cargos above 200 nm diameter for rigid dispersed and free independent modes. Power law exponents are given in table 4.2.

4.5.9 Inference on numbers of motors from published data

Wilhelm, Mandad, et al. [116] use quantitative mass spectrometry to report numbers of proteins in synaptic boutons. Present in this data are measurements for kinesin proteins. To estimate the number of motors present per vesicle, we used the instructions included in table S3 to combine their measurements with molecular masses (from UniProt) to find numbers of proteins per bouton. We then divided this total number by the number of vesicles per bouton reported in the paper. Kif5b is present at .1/vesicle, while the most abundant is Kif2a, a 1/vesicle. Kif2a is a kinesin-13 protein reported to transport neuronal vesicles [141].

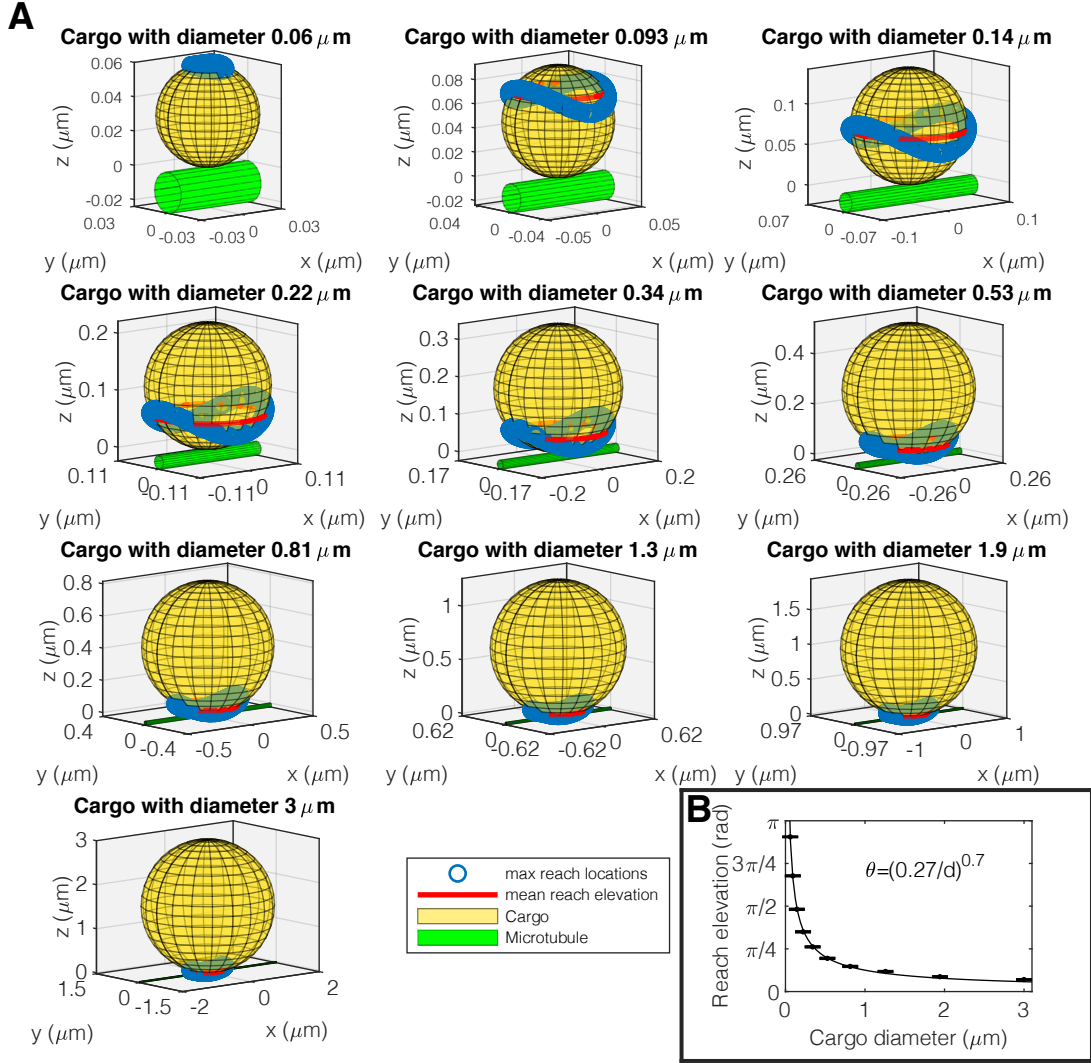


Figure 4.7: Mean reach elevation, the approximate location on the cargo that a motor can be bound while able to reach the microtubule, used in calculations shown in Fig. 4.1.

Cargo and microtubule were situated as shown in **A**. For details on how we model the mechanics of a motor and its attachment to the cargo, see supplemental section 4.6.1.1.

A: Mean reach elevation for cargos of various sizes, as labeled in each panel. Legend in final panel applies to every other panel.

B: Mean reach elevation for each cargo size shown in **A**. Bars represent standard error of the mean. Data are well fit to reach elevation $\theta = (.27/d)^{.7}$, where d is cargo diameter (black curve).

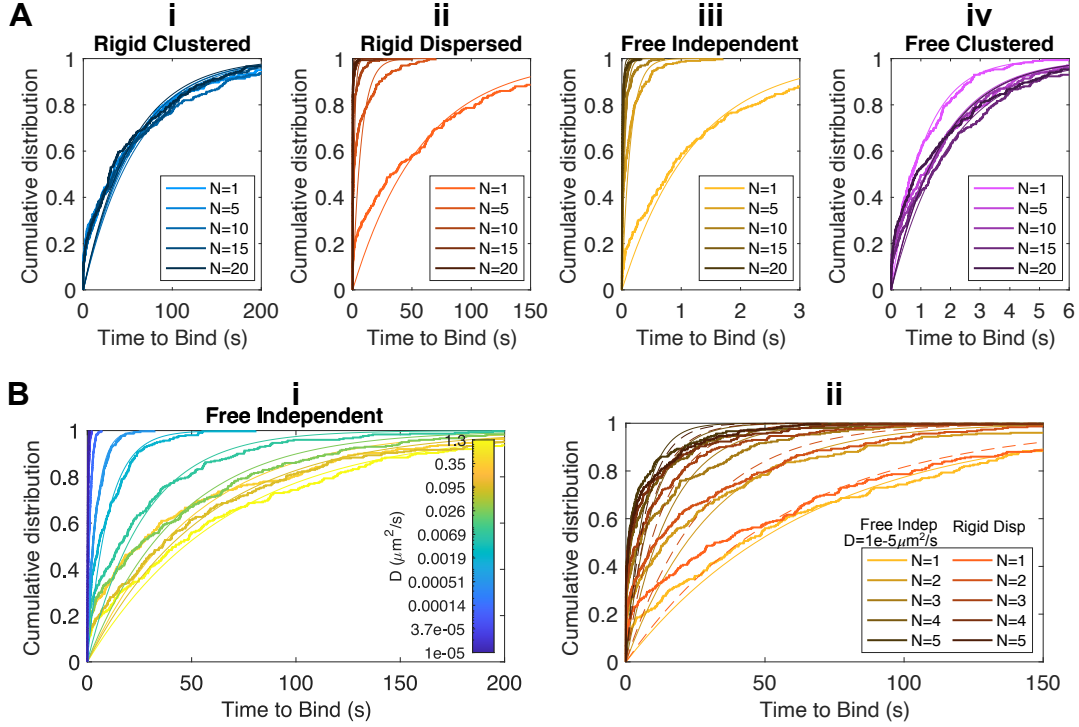


Figure 4.8: Distributions of times for cargos to bind to the microtubule

Ai-iv: Simulated empirical cumulative distributions of time to bind for cargos in each of the four organization modes. Thinner curves of the same color are exponential fits to the data.

Bi: Simulated empirical cumulative distributions for free independent cargos with a range of motor diffusion coefficients D . Diffusion coefficients are log spaced, values of D for each distribution shown are labeled on the colorbar. Thinner curves of the same color are exponential fits to the data.

Bii: Simulated empirical cumulative distributions of times to bind for free independent cargos at diffusion coefficient $D = 1 \times 10^{-5} \mu\text{m}^2 \text{s}^{-1}$ for several numbers of motors on the cargo (yellows). Also shown are distributions for rigid dispersed cargos at the same numbers of total motors (reds). Thinner curves of the same color are exponential fits to the data. Dashed curves are fits for rigid dispersed cargos.

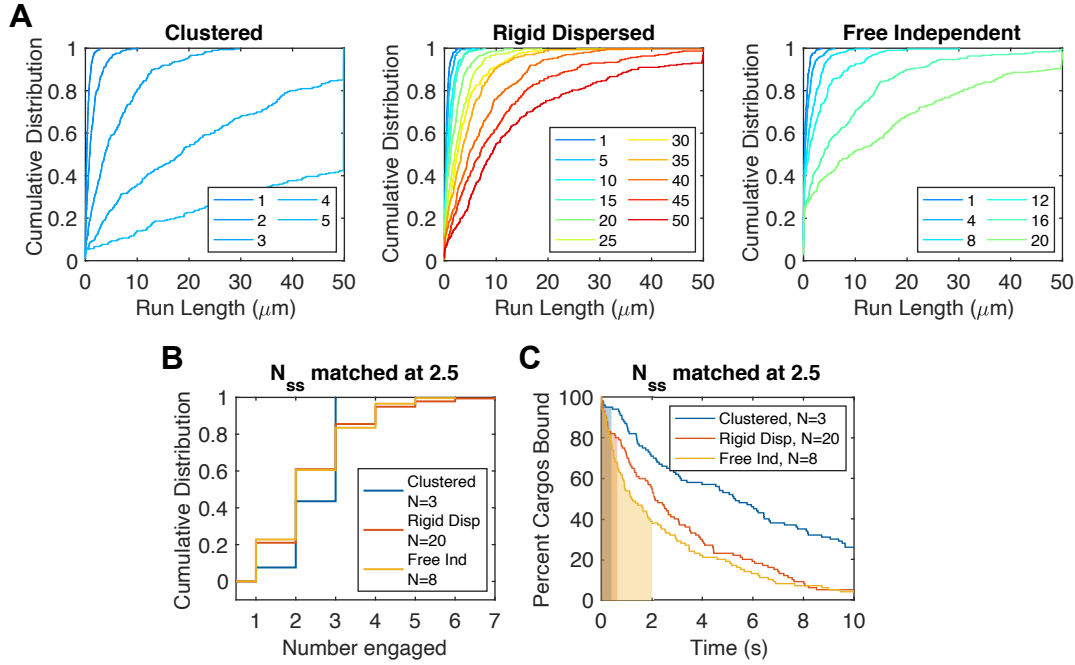


Figure 4.9: Distributions of run lengths

A: Simulated empirical cumulative distribution of run lengths for cargos in the clustered, rigid dispersed and free independent modes. Different total numbers of motors are shown on a common color axis.

B: Simulated empirical cumulative distributions of cargos with closely matched mean numbers of motors engaged at steady state (N_{ss}). Total numbers of motors N was picked for each mode to match an N_{ss} of 2.5.

C: Percent of cargos bound as a function of time for cargos with the same total numbers of total motors N as in **B**, picked to match mean number of motors engaged at steady state, N_{ss} . The time before steady state is reached is filled in color below the curve for each organization mode.

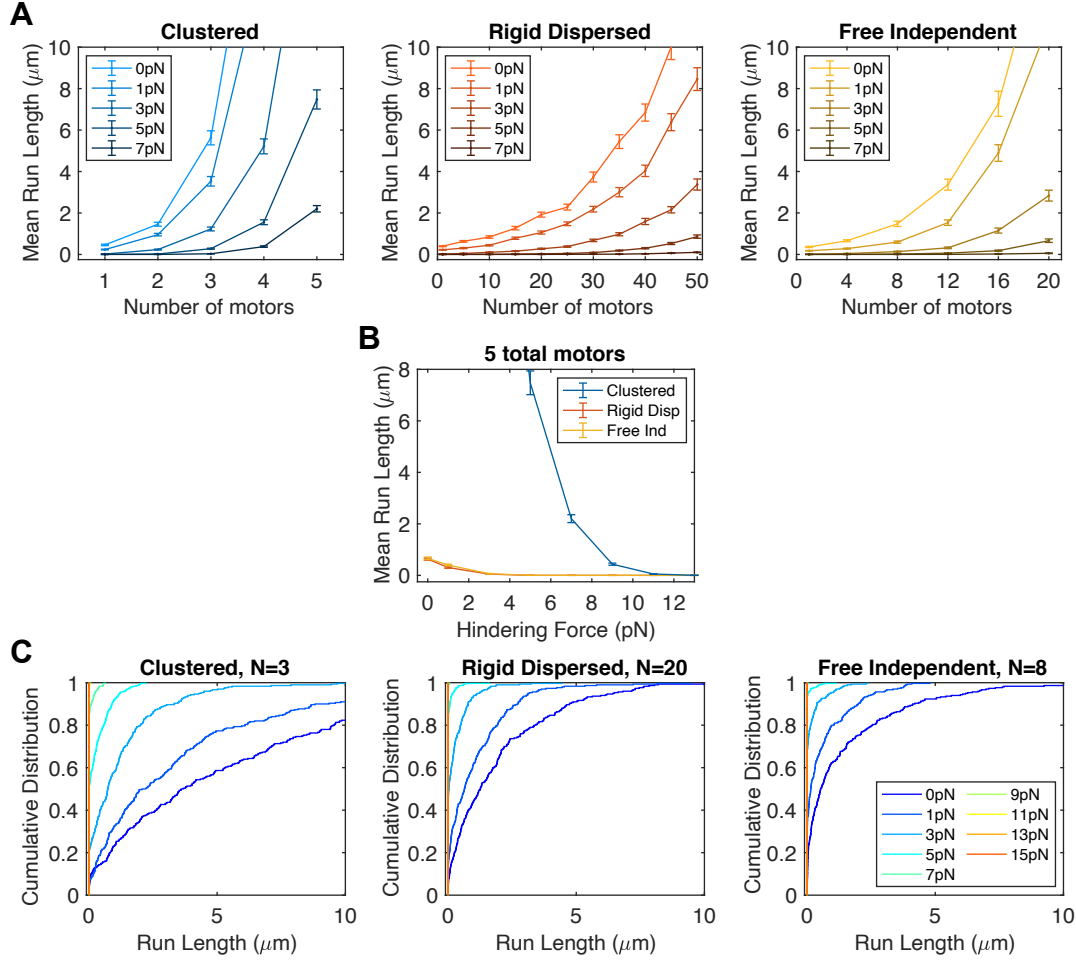


Figure 4.10: Means and Distributions of run lengths under force

A: Run lengths as a function of the number of total numbers on the cargo for several values of hindering force on the cargo. Error bars are standard error of the mean.

B: Mean run lengths as a function of external hindering load on the cargo for cargos in each of the three modes, with the total number of motors on the cargo matched at 4.

C: Simulated empirical cumulative distributions of run lengths of cargos under different loads. Numbers of motors are chosen to match figure 4.5B. Distributions are colored on a common axis corresponding to the legend in the right (free independent) panel.

Figure	Data	Equation	Values	95 % CI	Notes
4.3A	Rigid Clustered	aN^0	a : 57	56, 60	
	Rigid Dispersed	$(a/N)^b$	a : 10 b : 1.8	9, 12 1.7, 2.0	
	Free Independent	$(a/N)^b$	a : 1.3	1.2, 1.4	
	Free Clustered	$(a/N)^b + cN$	b : 1.5 a : 5.5E7 b : 0.01 c : 0.04	1.4, 1.6 -1.5E10, 1.5E10 -.2, .2 0.007, 0.08	
4.3Ci	$N = 1$	ad^b	b : 3.9	3.7, 4.1	
	$N = 5$		b : 4.4	4.0, 4.7	
	$N = 10$		b : 4.6	4.2, 5.0	
	$N = 15$		b : 4.7	4.3, 5.1	
	$N = 20$		b : 4.8	4.4, 5.3	
4.3Cii	$N = 1$	ad^b	b : 3.7	3.4, 4.0	
	$N = 5$		b : 4.0	3.4, 4.7	
	$N = 10$		b : 4.5	4.0, 5.0	
	$N = 15$		b : 4.9	4.2, 5.6	
	$N = 20$		b : 5.3	4.0, 6.4	
4.3Ciii	$N = 1$	ad^b	b : 2.7	2.2, 3.1	
	$N = 5$		b : 2.8	2.7, 2.9	
	$N = 10$		b : 2.9	2.6, 3.2	
	$N = 15$		b : 2.7	2.3, 3.2	
	$N = 20$		b : 2.7	2.1, 3.2	
4.3Civ	$N = 1$	ad^b	b : 2.5	2.2, 2.7	
	$N = 5$		b : 2.9	2.7, 3.2	
	$N = 10$		b : 3.2	3.0, 3.5	
	$N = 15$		b : 3.3	3.0, 3.7	
	$N = 20$		b : 3.4	3.1, 3.7	
4.4C	Clustered	$a + bN$	a : 0.1954 b : 0.7693	$\Rightarrow$ Adding 4 motors leads to ≈ 3 more motors engaged.	
	Rigid Dispersed	$a + bN$	a : 0.9553 b : 0.0673	$\Rightarrow$ Adding 15 motors leads to ≈ 1 more engaged.	
	Free Independent	$a + bN$	a : 0.6585 b : 0.2157	$\Rightarrow$ Adding 5 motors leads to ≈ 1 more engaged	

Table 4.2: List of fit values

4.6 Mathematical model of cargo transport with motor freedom

This section was adapted from material published as the supplemental material of [4]

4.6.1 Model description

We present a three dimensional mesoscale model for the dynamics of the cargo and motors. The model takes into account translational diffusion of motors in the cargo membrane, rotational and translational diffusion of the cargo body, as well as stochastic stepping and binding/unbinding of the motors. The locations of the cargo and the anchors where motors are attached to it are governed by a set of stochastic ordinary differential equations. Binding state and location of the motor head along the microtubule (MT) are considered discrete states and transitions between them occur stochastically, modeled as Poisson processes. A cartoon showing general aspects of the model is shown in figure 4.11. We construct a Monte Carlo numerical simulator, based on a hybrid Euler-Maruyama-Gillespie scheme, and simulate an ensemble of stochastic trajectories, from which we derive transport statistics.

Motors in the model have two states:

Bound: A motor i is defined by two points: one represents the motor domains, which we call the head $\vec{h}_i$; the other represents the location at which the motor is attached to the cargo, which we call the anchor $\vec{a}_i$. The motor has a rate of stepping along the MT k_i^{step} and a rate of unbinding from the MT k_i^{off} .

Unbound: A motor i is defined by one point, the anchor location $\vec{a}_i$. The motor has a rate of binding to the MT, k_i^{on} , that depends on its location relative to the MT as described below.

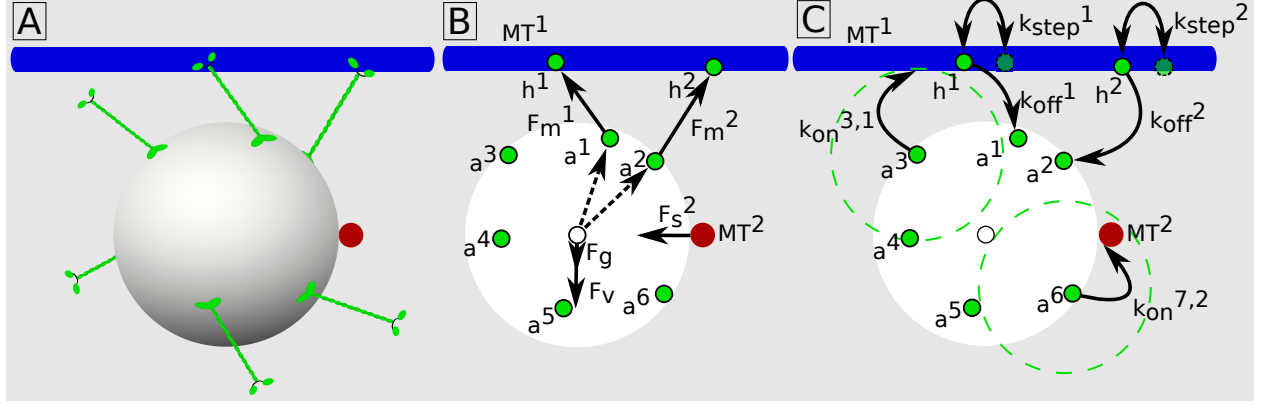


Figure 4.11: Cartoon diagramming various aspects of the model

A: We model the cargo as a 3D sphere which diffuses rotationally and translationally. Attached to the cargo are a number of motors which are free to diffuse along the surface of the cargo.

B: Motors which are bound to the MT exert forces as tension-only springs with a rest length equal to the length of the motor. These forces act to drag the anchors through the membrane, as well as to translate and rotate the cargo.

C: Motor which are bound to the MT have rates of stepping and unbinding that depend on the force experienced by the motor. Unbound motors have a non-zero binding rate only if the MT is within the motor's reach.

We track the anchor locations $\vec{a}_i(t)$, head locations of bound motors $\vec{h}_i(t)$, the location of the cargo center $\vec{c}(t)$, and the cargo orientation $\vec{\theta}(t)$. We note that these quantities are time dependent and suppress the (t) below where appropriate to simplify notation.

4.6.1.1 Stochastic ordinary differential equations for cargo motion

We first impose force-balance on the cargo and anchors. The motion of the cargo is in the inertialess regime (Reynolds number $\sim 10^{-6}$), so we omit the inertia term. Similarly, we assume anchor motion is dominated by linear drag and also in the inertialess regime. All forces must balance, so for each anchor

$$\sum \vec{F} = \underbrace{\vec{M}_i^{\parallel}}_{\text{motor force tangent to surface}} + \underbrace{\vec{D}_i^{\text{anchor}}}_{\text{drag force on anchor}} + \underbrace{\vec{B}_i^{\text{anchor}}}_{\text{Brownian force on anchor}} = 0 \quad (4.3)$$

For the cargo body,

$$\begin{aligned} \sum \vec{F} = & \underbrace{\vec{E}}_{\text{external force}} + \underbrace{\vec{S}}_{\text{steric force}} + \underbrace{\vec{B}^{\text{cargo}}}_{\text{Brownian force on cargo}} + \underbrace{\vec{D}^{\text{cargo}}}_{\text{drag force on cargo}} \\ & + \sum_i \underbrace{\vec{M}_i^\perp}_{\text{motor force normal to surface}} - \underbrace{\left(\vec{D}_i^{\text{anchor}} + \vec{B}_i^{\text{anchor}} \right)}_{\text{reaction forces}} = 0. \end{aligned} \quad (4.4)$$

Similarly, we can write the balance of torques on the cargo

$$\sum \vec{\tau} = \underbrace{\vec{\beta}^{\text{cargo}}}_{\text{Brownian torque on cargo}} + \underbrace{\vec{\delta}^{\text{cargo}}}_{\text{drag torque on cargo}} - \sum_i \underbrace{\vec{\delta}_i^{\text{anchor}} + \vec{\beta}_i^{\text{anchor}}}_{\text{torque from reaction forces}} = 0. \quad (4.5)$$

By specifying the forms of each of these forces below, we construct the equations of motion of the cargo center $\vec{c}$, cargo orientation $\vec{\theta}$, and anchor locations $\vec{a}_i$.

Motor mechanics and forces We model the attachment at the motor anchor as a free hinge, in both rotation and bending. Therefore we calculate the distance from the anchor to the MT as the shortest distance which does not imply the stalk would pass through the cargo (see equation 4.30). We do not include any torque at the anchor.

In stretching, we model the force that the motor exerts as originating from the stretch in spring-like motor stalks, based on experimental measurements [45, 46, 142] and in line with previous models [54–56]. The form of this force is that of a tension-only spring with stiffness κ^{motor} and rest length L . The force $\vec{M}_i$ exerted by motor i is given by

$$\vec{M}_i(\vec{a}_i, \vec{h}_i) = \begin{cases} \kappa^{\text{motor}} \left(\left| \vec{h}_i - \vec{a}_i \right| - L \right) \left(\frac{\vec{h}_i - \vec{a}_i}{\left| \vec{h}_i - \vec{a}_i \right|} \right) & \left| \vec{h}_i - \vec{a}_i \right| > L \\ 0 & \text{otherwise} \end{cases}. \quad (4.6)$$

We then break the motor force into components normal and tangent to the sphere at the

point of the anchor. We model the cargo as undeformable, so the normal component of the motor force, $\vec{M}_i^\perp$, acts to translate the cargo body. The tangential component, $\vec{M}_i^\parallel$, acts to drag the anchor through the cargo membrane. We calculate these components as

$$\vec{M}_i^\perp = \vec{M}_i \cdot \left(\frac{\vec{a}_i - \vec{c}}{|\vec{a}_i - \vec{c}|} \right) \quad (4.7)$$

$$\vec{M}_i^\parallel = \vec{M}_i - \vec{M}_i^\perp \quad (4.8)$$

Steric forces We model the MT as an infinite cylinder of radius R_{MT} . The cylinder location is defined by a point $\vec{p}^{\text{MT}}$ and direction vector $\vec{d}^{\text{MT}}$. The cargo and MT are prevented from overlapping in space by a steric force with the form of a compression-only spring of stiffness κ^{steric} and rest length 0, given by

$$\vec{S}(\vec{c}) = \begin{cases} -\kappa^{\text{steric}} \left(R - |\vec{r}(\vec{c}, j)| \right) \left(\frac{\vec{r}(\vec{c}, j)}{|\vec{r}(\vec{c}, j)|} \right) & |\vec{r}(\vec{c}, j)| < R \\ 0 & \text{otherwise} \end{cases}, \quad (4.9)$$

where $\vec{r}(\vec{x}, j)$ is a vector from point $\vec{x}$ to the nearest point on the MT. This is found by computing the perpendicular distance from $\vec{x}$ to the MT surface, given by

$$\vec{r}(\vec{x}, j) = \left(1 - \frac{R^{\text{MT}}}{\left| (\vec{x}_{\text{MT}} - \vec{x}) - \left((\vec{x}_{\text{MT}} - \vec{x}) \cdot \vec{d}_{\text{MT}} \right) \vec{d}_{\text{MT}} \right|} \right) \left((\vec{x}_{\text{MT}} - \vec{x}) - \left((\vec{x}_{\text{MT}} - \vec{x}) \cdot \vec{d}_{\text{MT}} \right) \vec{d}_{\text{MT}} \right). \quad (4.10)$$

Drag forces We model the fluid surrounding the cargo as a Newtonian fluid with viscosity η . The drag on the cargo is thus given by Stokes' Law,

$$\vec{D}^{\text{cargo}} \left(\frac{d\vec{c}}{dt} \right) = -6\pi\eta R \frac{d\vec{c}}{dt}. \quad (4.11)$$

The viscous torque is given by the rotational analogue to Stokes' Law,

$$\vec{\delta}^{\text{cargo}} \left(\frac{d\vec{\theta}}{dt} \right) = -8\pi\eta R^3 \frac{d\vec{\theta}}{dt}. \quad (4.12)$$

The motion of the anchor in the membrane is also assumed to be dominated by linear drag. The drag force is proportional to the difference in velocity between the anchor and the fluid that makes up the cargo membrane, given by

$$\vec{D}_i^{\text{anchor}} \left(\vec{c}, \vec{a}_i, \frac{d\vec{c}}{dt}, \frac{d\vec{\theta}}{dt}, \frac{d\vec{a}_i}{dt} \right) = \left(\frac{k_B T}{D} \right) \left(\frac{d\vec{c}}{dt} + \frac{d\vec{\theta}}{dt} \times (\vec{a}_i - \vec{c}) - \frac{d\vec{a}_i}{dt} \right). \quad (4.13)$$

This leads to a torque on the cargo given by the cross product of the lever arm and the force,

$$\vec{\delta}_i^{\text{anchor}} \left(\vec{c}, \vec{a}_i, \frac{d\vec{c}}{dt}, \frac{d\vec{\theta}}{dt}, \frac{d\vec{a}_i}{dt} \right) = (\vec{a}_i - \vec{c}) \times \left(\frac{k_B T}{D} \right) \left(\frac{d\vec{c}}{dt} + \frac{d\vec{\theta}}{dt} \times (\vec{a}_i - \vec{c}) - \frac{d\vec{a}_i}{dt} \right). \quad (4.14)$$

Brownian forces We model the fluid surrounding the cargo as a Newtonian fluid with viscosity η . The Brownian force on the cargo, $\vec{B}^{\text{cargo}}(t)$, is a vector of three random variables in time (one per spatial dimension) with mean 0 and variance $2k_B T \xi$, where ξ is the drag coefficient and $k_B T$ is the thermal energy unit. Specifically, for the Brownian force on the cargo body,

$$\langle \vec{B}^{\text{cargo}}(t) \rangle = \vec{0} \quad (4.15)$$

$$\langle \vec{B}^{\text{cargo}}(t) \cdot \vec{B}^{\text{cargo}}(s) \rangle = 2(k_B T)(6\pi\eta R)\vec{\delta}(t-s) \quad (4.16)$$

where we have inserted the translational drag coefficient of a sphere at low Reynold's number and $\vec{\delta}(x)$ is a vector of three Dirac delta functions. Similarly, the Brownian torque on the cargo, $\vec{\beta}^{\text{cargo}}$, is characterized by

$$\langle \vec{\beta}^{\text{cargo}}(t) \rangle = \vec{0} \quad (4.17)$$

$$\langle \vec{\beta}^{\text{cargo}}(t) \cdot \vec{\beta}^{\text{cargo}}(s) \rangle = 2(k_B T)(8\pi\eta R^3)\vec{\delta}(t-s). \quad (4.18)$$

where we have inserted the rotational drag on a sphere at low Reynold's number.

We model the membrane of the cargo as a Newtonian fluid, in which motor anchor diffuse with coefficient D . The Brownian force on anchor i in the membrane is characterized by

$$\langle \vec{B}_i^{\text{anchor}}(t) \rangle = \vec{0} \quad (4.19)$$

$$\langle \vec{B}_i^{\text{anchor}}(t) \cdot \vec{B}_i^{\text{anchor}}(s) \rangle = 2(k_B T) \left(\frac{k_B T}{D} \right) \vec{\delta}(t-s) \quad (4.20)$$

where we have expressed the drag coefficient as the ratio of the thermal energy unit and the diffusion coefficient of the motor in the membrane via the Einstein relation. This force

causes a torque on the cargo equal to the cross product of the lever arm and the force,

$$\vec{\beta}_i^{\text{anchor}} = (\vec{a}_i - \vec{c}) \times \vec{B}_i^{\text{anchor}}. \quad (4.21)$$

4.6.1.2 Construction and discretization of the stochastic ordinary differential equations

With the forms of forces specified, equations 4.3, 4.4, and 4.5 represent a set of $3(N + 2)$ coupled ordinary stochastic differential equations, where N is the total number of motors on the cargo. The forms of these equations are, for each motor,

$$0 = \vec{M}_i^{\parallel}(\vec{a}_i, \vec{h}_i) + \frac{k_B T}{D} \left(\frac{d\vec{c}}{dt} + \frac{d\vec{\theta}}{dt} \times (\vec{a}_i - \vec{c}) - \frac{d\vec{a}_i}{dt} \right) + \vec{B}_i^{\text{anchor}}, \quad (4.22)$$

for translation of the cargo,

$$0 = \vec{E} + \vec{S}(\vec{c}) + \vec{B}^{\text{cargo}} - 6\pi\eta R \frac{d\vec{c}}{dt} + \sum_{i=1}^N \vec{M}_i^{\perp}(\vec{a}_i, \vec{h}_i) - \left(\frac{k_B T}{D} \left(\frac{d\vec{c}}{dt} + \frac{d\vec{\theta}}{dt} \times (\vec{a}_i - \vec{c}) - \frac{d\vec{a}_i}{dt} \right) + \vec{B}_i^{\text{anchor}} \right), \quad (4.23)$$

and for rotation of the cargo

$$0 = \vec{\beta}^{\text{cargo}} - 8\pi\eta R^3 \frac{d\vec{\theta}}{dt} - \sum_{i=1}^N (\vec{a}_i - \vec{c}) \times \frac{k_B T}{D} \left(\frac{d\vec{c}}{dt} + \frac{d\vec{\theta}}{dt} \times (\vec{a}_i - \vec{c}) - \frac{d\vec{a}_i}{dt} \right) + (\vec{a}_i - \vec{c}) \times \vec{B}_i^{\text{anchor}}. \quad (4.24)$$

Equations 4.22 and 4.23 are specific implementations of the overdamped Langevin equation, used in Brownian dynamics. Equation 4.24 is the rotational counterpart of 4.23. The dynamic variables of these equations are the $3N$ anchor position components, the 3 cargo position components, and the 3 cargo orientation components. The system of stochastic

ordinary differential equations is linear, allowing us to solve for the derivative terms. The resulting equations have many terms and are not amenable to display.

We discretize these equations according to the Euler-Maruyama method. For an update from the n th timestep at time t_n to the next time t_{n+1} with $\Delta t \equiv t_{n+1} - t_n$, the discretization is

$$\begin{aligned}\frac{d\vec{c}(t)}{dt} &\rightarrow \frac{\vec{c}(t_{n+1}) - \vec{c}(t_n)}{\Delta t} \\ \frac{d\vec{\theta}(t)}{dt} &\rightarrow \frac{\vec{\theta}(t_{n+1}) - \vec{\theta}(t_n)}{\Delta t} \\ \frac{d\vec{a}_i(t)}{dt} &\rightarrow \frac{\vec{a}_i(t_{n+1}) - \vec{a}_i(t_n)}{\Delta t} \\ \vec{B}^{\text{cargo}}(t) &\rightarrow \sqrt{2\frac{k_B T 6\pi\eta R}{\Delta t}} \vec{G}_c(t_n) \\ \vec{\beta}^{\text{cargo}}(t) &\rightarrow \sqrt{2\frac{k_B T 8\pi\eta R^3}{\Delta t}} \vec{G}_\theta(t_n) \\ \vec{B}_i^{\text{anchor}}(t) &\rightarrow \sqrt{\frac{2(k_B T)^2}{D\Delta t}} \vec{G}_i(t_n)\end{aligned}$$

where $\vec{G}_c$, $\vec{G}_\theta$ and the $\vec{G}_i$'s (of which there are N) are mutually uncorrelated vectors of three independently and identically distributed gaussian random variables with mean 0 and variance 1.

We note that the update step moves anchor locations in a direction tangent to the surface of the sphere at point $a_i(t_n)$. The new anchor location, $a_i(t_{n+1})$, then lies outside the surface of the sphere. We force the anchor location back onto the surface of the sphere by correcting it to

$$\vec{a}_i^{\text{corrected}}(t_{n+1}) = \vec{c} + R \left(\frac{\vec{a}_i(t_{n+1}) - \vec{c}}{|\vec{a}_i(t_{n+1}) - \vec{c}|} \right). \quad (4.25)$$

This correction underestimates the true distance the anchor should travel over the surface

of the sphere. The magnitude of this underestimation is small for moves where $\vec{a}_i(t_{n+1}) - \vec{a}_i(t_n) \ll R$. To ensure this is true in simulation, we limit anchor movement distance by choosing a small timestep. To ensure the timestep is small enough, we force the simulation to exit in an error if $|\vec{a}_i(t_{n+1}) - \vec{a}_i(t_n)| > R\pi/5$ (corresponding to an underestimate of 10%), or if $|\vec{a}_i^{\text{corrected}}(t_{n+1}) - \vec{a}_i(t_{n+1})| > 10 \text{ nm}$. Furthermore, we demonstrate in section 4.6.3 that the simulation shows good agreement with analytical calculations of mean first passage times for diffusion on a sphere.

4.6.1.3 Poisson processes

We model all state transitions in the system as Poisson processes. Experiments have reported exponential distributions of times between steps [35] and times before unbinding [42]. This is also the most basic assumption we can make for times before binding.

Stepping Kinesin motors step processively along MT tracks in a hand-over-hand fashion, with each motor domain taking 16 nm steps [33] that move the center of mass of the motor forward by about 8 nm [34]. Unloaded, motors travel at velocity v_0 . When motors are subject to a hindering load, the velocity of the motor decreases with load until it nears 0 at the stall force, $\mathcal{F}^{\text{stall}}$. We use a form for this decrease studied in [36], where exponent w determines if the velocity decreases linearly, sub-linearly, or super-linearly. Asymmetrically, kinesin velocity is unaffected by assisting loads [44]. The overall form of the dependance is given by

$$k_{\text{step}_i} = \begin{cases} \frac{v_0}{d^{\text{step}}} \left(1 - \left(|\vec{M}_i| / \mathcal{F}^{\text{stall}} \right)^w \right) & \vec{M}_i \cdot \vec{d}_{\text{MT}} \leq 0 \quad \& \quad |\vec{M}_i| < \mathcal{F}^{\text{stall}} \\ 0 & \vec{M}_i \cdot \vec{d}_{\text{MT}} \leq 0 \quad \& \quad |\vec{M}_i| \geq \mathcal{F}^{\text{stall}} . \\ \frac{v_0}{d^{\text{step}}} & \vec{M}_i \cdot \vec{d}_{\text{MT}} > 0 \end{cases} \quad (4.26)$$

When a motor steps, it is moved forward along the direction of the MT to which it is bound by the step distance d^{step} . This translates to an update of the head position

$$\vec{h}_i(t_{n+1}) = \vec{h}_i(t_n) + d^{\text{step}} \left(\vec{d}_{\text{MT}} \right). \quad (4.27)$$

Unbinding Kinesin unbinds from the MT with a rate dependent on the force experienced by the motor. Hindering loads increase unbinding rate in an exponential fashion below the stall force [95]. Loads greater than the stall force continue to increase the unbinding rate, but do so more slowly, as found in [42] and analysis of data from [41] in [107]. For hindering loads, we use a piecewise exponential approximation of the theory of [107], in turn based on data from [41]. Unbinding rate is asymmetric, with assisting loads resulting in high unbinding rates [41, 43]. For assisting loads, we use an unbinding rate which is exponential with force, taken from the fit to data in [41]. Each of these exponential functions is parameterized as a function of force F by an unloaded rate ϵ and a characteristic force $\mathcal{F}$, as $\epsilon \exp(F/\mathcal{F})$. Together, we state the unbinding rate as

$$k_{\text{off}i} = \begin{cases} \epsilon_0 \left(\frac{\epsilon^{\text{superstall}}}{\epsilon_0} \exp(\mathcal{F}^{\text{stall}}/\mathcal{F}^{\text{superstall}}) \right)^{|\vec{M}_i|/\mathcal{F}^{\text{stall}}} & \vec{M}_i \cdot \vec{d}_{\text{MT}} \leq 0 \quad \& \quad |\vec{M}_i| < \mathcal{F}^{\text{stall}} \\ \epsilon^{\text{superstall}} \exp(|\vec{M}_i|/\mathcal{F}^{\text{superstall}}) & \vec{M}_i \cdot \vec{d}_{\text{MT}} \leq 0 \quad \& \quad |\vec{M}_i| \geq \mathcal{F}^{\text{stall}} . \\ \epsilon^{\text{assisting}} \exp(|\vec{M}_i|/\mathcal{F}^{\text{assisting}}) & \vec{M}_i \cdot \vec{d}_{\text{MT}} > 0 \end{cases} \quad (4.28)$$

When a motor unbinds, it is simply put into the unbound state as defined at the beginning of this section.

Binding The conditions which govern the rate of binding to the MT are not well known. In the absence of detailed experimental elucidation, we make the assumption that the motor binds at a constant rate if the anchor is closer to the MT than its rest length. The probability of the motor binding at distances greater than the rest length decreases with distance proportionately to the probability of the motor spring stochastically taking on that distance. This translates to an on rate for motor i to the MT given by

$$k_{\text{on}i} = \begin{cases} \pi_0^{\text{micro}} & |\vec{r}(\vec{a}_i, j)| \leq L \\ \pi_0^{\text{micro}} \exp\left(-\frac{\kappa^{\text{motor}}(|\vec{r}(\vec{a}_i)| - L)^2}{2k_B T}\right) & \text{otherwise} \end{cases} \quad (4.29)$$

where $\vec{r}(\vec{x}, j)$ is given by equation 4.10. We note that we prevent the motor from binding when the motor stalk would intersect the cargo (if $\vec{r}(\vec{a}_i) \cdot (\vec{a}_i - \vec{c}) < 0$), unless the motor is long enough to reach around the cargo to that point — that is, if

$$L > R \left(\frac{\pi}{2} - \arccos\left(\frac{(\vec{c} - \vec{a}_i) \cdot \vec{r}(\vec{a}_i, j)}{R|\vec{r}(\vec{a}_i, j)|}\right) - \arccos\left(\frac{(\vec{r}(\vec{a}_i, j) + \vec{a}_i - \vec{c}) \cdot \vec{r}(\vec{a}_i, j)}{|\vec{r}(\vec{a}_i, j)| |\vec{r}(\vec{a}_i, j) - (\vec{a}_i - \vec{c})|}\right) + \arcsin\left(\frac{R}{|\vec{r}(\vec{a}_i, j) - (\vec{a}_i - \vec{c})|}\right) \right) + (\vec{r}(\vec{a}_i, j) - 2\vec{c} + 2\vec{a}_i) \cdot \vec{r}(\vec{a}_i, j). \quad (4.30)$$

When a motor i binds to the MT, the head location is placed at the location on the MT nearest the anchor location $\vec{a}_i$, given by

$$\vec{h}_i(t_{n+1}) = \vec{a}_i(t_n) + \vec{r}(\vec{a}_i(t_n), j). \quad (4.31)$$

4.6.2 Numerical simulation of the model

Section 4.6 outlines a numerical scheme for updating for the model's dynamic variables over a timestep Δt . We simulate the model forward in time using these equations. Time steps are

chosen dynamically. The largest stable time step for the Euler-Maruyama scheme is given by ξ/κ , where ξ is the drag coefficient and κ is the spring constant of the stiffest operating spring. The maximum time step is chosen based on the springs active during that step. The equivalent stiffness of multiple active motor springs is taken into account, but the steric spring in equation 4.9 remains by far stiffest in the system if it is active.

For each time step, we generate exponential random variables from distributions with means set by each Poisson rate, given in equations 4.26, 4.28, and 4.29, as in the Gillespie (next-event) algorithm. If any of the generated times are smaller than the maximum stable time step, the smallest generated time is chosen as the time step. If the chosen time came from an unbinding rate, a state change is implemented at the end of the update step by setting the motor to the unbound state. If this time came from a stepping rate or binding rate, the update occurs by equation 4.27 or 4.31, respectively. If no generated time is shorter than the maximum stable time step, the update is done with the maximum stable time step and there is no state change. Unlike in the Gillespie algorithm, rates are recalculated at each time step as they depend the changing locations and forces in the motors.

On average, many substeps are taken before a Poisson event occurs. The longest maximum stable time available to the system is the one characteristic of a single motor spring, which is $\approx 2.5 \times 10^{-5}$ s. This is much shorter than the mean times between binding (≈ 0.1 s), stepping (≈ 0.01 s) and unbinding, even at several times the stall force (≈ 0.2 s at 20 pN). The shortest maximum stable time step normally experienced is that associated with the steric spring, which is $\approx 2 \times 10^{-7}$ s, about 100 times shorter than the single motor maximum stable time step. During a run of the simulation, the cargo and MT overlap by an average of 2 nm. If the cargo and MT overlap by more than 5 nm for more than 5 consecutive time steps the simulation exits in an error.

The numerical simulation is written in C. It takes approximately 0.5 s to simulate 1 s of cargo motion with a 3.3 GHz Intel Core i5 processor (single thread).

4.6.2.1 Model parameters

We attempt here to estimate the parameters relevant to cargos in the cell. We describe our estimations below. The parameters used to simulate the model are given in table 4.3.

Motor parameters We model our motors based on the well-studied kinesin-1 family. As such, many of the model parameters have been estimated in *in vitro* experiments. While rates of stepping and unbinding may be different in cells, the authors are not aware of any specific motor models from measurements in the cell, and it is not clear how individual motor behavior should be altered to reflect the cellular environment.

The length of the kinesin motor has been imaged in cryo-EM to be 80 nm [90, 91]. It has also been imaged attached to cargos [90, 143], where projections are shorter than 80 nm. It is unclear why. Some kinesin family members are shorter [144]. Since kinesin is floppy [145], molecules could also be folded. On the other hand, reach could also be extended by linker molecules. Since the length parameter represents the distance from the cargo kinesin can reach, not necessarily the most common length it assumes, we use 80 nm as the length parameter.

Motor binding rate has been measured *in vitro* to be about 5 s^{-1} [92, 146]. It is known to be modulated by motor identity [146], presence of microtubule associated proteins such as Tau [81, 133] and MAP7 [128], and microtubule post-translational modification [147]. We have recently found that on rate is much higher for the first binding event than for subsequent events [5] (chapter 5). Since microtubule state is highly variable between transport scenarios and we do not know of any estimations of motor binding rate in the cell, we use the *in vitro* values of 100 s^{-1} for the first motor to bind the microtubule and 5 s^{-1} for subsequent motors.

Cargo parameters Cargos in the cell are highly variable in size, from neuronal vesicles on the order of 100 nm [100, 106], to 3 μ m phagocytosed beads [96]. Other physical parameters, such as number of motors and motor diffusion coefficient are difficult to measure and not well understood. Neuronal vesicles have been measured to have just a few motors [100, 101], but it is unclear if other cargo types have similar numbers of motors, either in surface density or total number.

The surface viscosity of the cargo is discussed in Methods. It is largely unknown, therefore we explore a wide range of values in Results.

Cytoplasm parameters The interior of a eukaryotic cell is increasingly understood to be a complex environment with a complex rheology. Because it holds polymer networks such as those of actin and microtubules, along with numerous membrane-bound organelles, there are many obstacles to the movement of an object through the cytoplasm. The nature of this resistance is both viscous and elastic [108, 109]. Obstacles resist motion in a way that depends on the size and speed of the object being moved [109, 148]. In addition to being obstacles to motion, the cytoskeleton and organelles are active, generating non-thermal motion which pushes other objects in the cell [108, 109, 149]. Further large scale motion can be caused by movement of cell edges during cell motility [150].

In this paper, we seek to understand how molecular motors move cargos in this environment. The complexity of the effects described above, together with the fact that methods to mathematically describe the motion which results are under active development [151], necessitate that we simplify our description of the environment through which cargos are transported. In this paper we model cargos moving through, and diffusing in, a Newtonian fluid. While this description does not allow for elasticity, it does allow for more or less resistance to motion through changing the viscosity.

What viscosity best describes the cytoplasm? A wide range of values have been reported, spanning at least 5 orders of magnitude [134] from about ten times that of water [152] to over one million times [153]. Furthermore, there are a great many experiments — for example, Crick and Hughes estimated 1 Pa s in 1950 [154].

In [135], the authors use magnetic rotation microrheology on HeLa cells which have endocytosed magnetic nanoparticles. Endosomes in which these nanoparticles have gathered form a chain, which the authors rotate with magnets to probe the microrheology of the cytoplasm. The authors estimate a wide spread of viscosities for unperturbed HeLa cells, with a mean around 1 Pa s. However, when the HeLa cells are treated with nocodazole to disrupt microtubules or latrunculin A to perturb microtubules, they estimate a viscosity of 0.4 Pa s, with a much tighter distribution of values. Since much of the motor attachment phenomena we describe in the paper is particularly influenced by cargo rotation, this study of rotating organelles is particularly relevant.

A similar study by Chevy et al. finds viscosities of 0.16 Pa s for NIH/3T3 cells and 1.4 Pa s for MDCK cells, using passive rotation of micron size wires [155]. Berret, using active rotations of similar wires in NIH/3T3 cells, finds viscosities around 30 Pa s for 2 μ m to 3 μ m wires [156].

These three studies are able to learn about the rotation of an object in the cell by exploiting its elongated shape. One might think a spherical object might rotate more freely than an elongated one, as the elongated one could be pushing on other cellular components as it moves. Möller et al. estimate viscosity of J774 mouse macrophages using active rotations of micron size spherical magnetic particles and find viscosities between 10 Pa s and 60 Pa s [157]. As this is the only intracellular viscosity measurement made using spherical particles that the authors know of, we find no evidence to support a lower effective viscosity for spherical particles than for elongated ones. This fact also points out an opportunity for measurements of intracellular rheology using optical manipulation of spherical birefringent particles [158,

159] or tracking of janus particles [160].

Overall, we choose 0.4 Pa s as a representative value for cytoplasmic viscosity. Of the values measured for rotation in the papers listed above, it is toward the low end, being the same order of magnitude as the lowest value measured for rotation.

The cytoplasm also has many microtubules, and cargos can interact with multiple microtubules at once [3] (chapter 3). We limit the scope of this investigation to a single microtubule.

4.6.2.2 Initial conditions

Anchor Placement For cargos in the dispersed or free modes, we place anchors on the cargo by selecting points from a uniform random distribution over the surface of the sphere. For cargos in the clustered mode, all anchors are placed at the same point on the sphere.

Cargo Orientation and Placement Cargos are placed at a set distance from the MT in the $+z$ direction and the cargo is aligned with the MT in the other two directions, i.e. $(0, 0, R + \text{cargo-MT-dist} + R^{\text{MT}})$. The orientation is such that the cargo north pole points in the $+z$ direction.

For simulations where motors pull cargos, one motor is chosen at random and the cargo is rotated so that motor is at the furthest point of the cargo in the $-z$ direction. That motor begins bound to the MT. Cargo-MT distance is set to 0.

For simulations where we measure time for the cargo to bind, the cargo is placed at a set cargo-MT distance and the orientation is not changed. No motors are bound.

Parameter	Description	Value	Notes
κ^{motor}	Motor spring constant	320 pN μm^{-1}	Measured in [45, 46, 142]
L	Motor reach length	80 nm	Length in electron microscopy, measured in [90, 91]
v_0	Maximum velocity	0.75 $\mu\text{m s}^{-1}$	Measured in [41]
d^{step}	Step size	8.2 nm	Measured in [34, 161]
w	Force-velocity curvature index	1.8	Measured in [38]*
$\mathcal{F}^{\text{stall}}$	Stall force	6.1 pN	Measured in [38]*
$\mathcal{F}^{\text{superstall}}$	Characteristic detachment force for superstall loads	7.6 pN	Fit to theory in [107], itself fit to data from [41]
$\mathcal{F}^{\text{assisting}}$	Characteristic detachment force for assisting loads	13 pN	Measured in [41].
ϵ_0	0-force unbinding rate	0.8 s^{-1}	Unloaded velocity divided by unloaded run length. Both quantities measured in [41]
$\epsilon^{\text{superstall}}$	Base unbinding rate for superstall loads	1.6 s^{-1}	Fit to theory in [107], itself fit to data from [41]
$\epsilon^{\text{assisting}}$	Base unbinding rate under assisting load	7.4 s^{-1}	Measured in [41]
π_0^{micro}	Base binding rate (microscopic)	100 s^{-1} (first), 5 s^{-1} (subsequent)	Measured in [5],[92, 146].
N	Number of motors on the cargo		Varied in simulations
D	Diffusion constant of motor anchors in the cargo membrane	0.1 $\mu\text{m}^2 \text{s}^{-1}$	Estimate for generic membrane bound protein from BioNumbers, BNID 114189 [99]
R	Cargo radius	0.25 μm	Approximate size of lipid droplets in COS-1 cells [98]
η	Viscosity of fluid immersing the cargo	0.4 pN $\mu\text{m}^{-2} \text{s}$	Measured in [135] and [108]. See 4.6.2.1
$k_B T$	Thermal energy unit	4.141 95 pN nm	at 300 K
R^{MT}	MT radius	12 nm	Measured in [21]
κ^{steric}	Steric spring constant	40 000 pN μm^{-1}	Set high enough to ensure cargo does not significantly intersect MT
$\vec{x}_{\text{MT}}$	Defining point for MT	(0,0,0)	
$\vec{d}_{\text{MT}}$	Defining direction vector for MT	(1,0,0)	

Table 4.3: List of parameters. Values listed are used for all simulations unless otherwise stated.

*We note that with the form of equation 4.26 and these parameters, the force velocity curve is well matched to force-velocity data in [41].

4.6.3 Validation of the simulation

Simulations of 3D cargo motion coupled with the action of molecular motors have been previously reported [3, 55–57]. In this work we simulate cargos in which motor anchors experience drag through a viscous membrane and diffuse with a finite diffusion constant, which is novel. In this section, we show that our simulation agrees with analytical results in simplified situations.

4.6.3.1 Drag of anchors through the cargo membrane

In our model, anchors experience viscous drag when moving through the cargo membrane. While difficult to find for a spherical cargo geometry, an analytical solution for the anchor equation of motion is readily found for the case of a planar membrane. We begin with the sum of the forces on the anchor

$$\sum \vec{F} = \vec{M}_i^{\parallel} + \vec{D}_i^{\text{anchor}} + \vec{B}_i^{\text{anchor}} = 0 \quad (4.32)$$

To test only the viscous drag, we set the brownian force $\vec{B}_i^{\text{anchor}}$ to 0. Inserting the forms of the motor force and viscous drag from equations 4.6 and 4.13, respectively, we simplify them for the 1D case of a static membrane. We note the motor head moves unidirectionally and restrict the solution to cases where h is constant in time and $h - a(t) \geq L$ (i.e. we start with the motor stretched) to obviate the need for a piecewise defined motor force. With these simplifications, equation 4.32 becomes

$$\kappa^{\text{motor}}(h - a(t) - L) = \frac{k_B T}{D} \frac{da(t)}{dt} \quad (4.33)$$

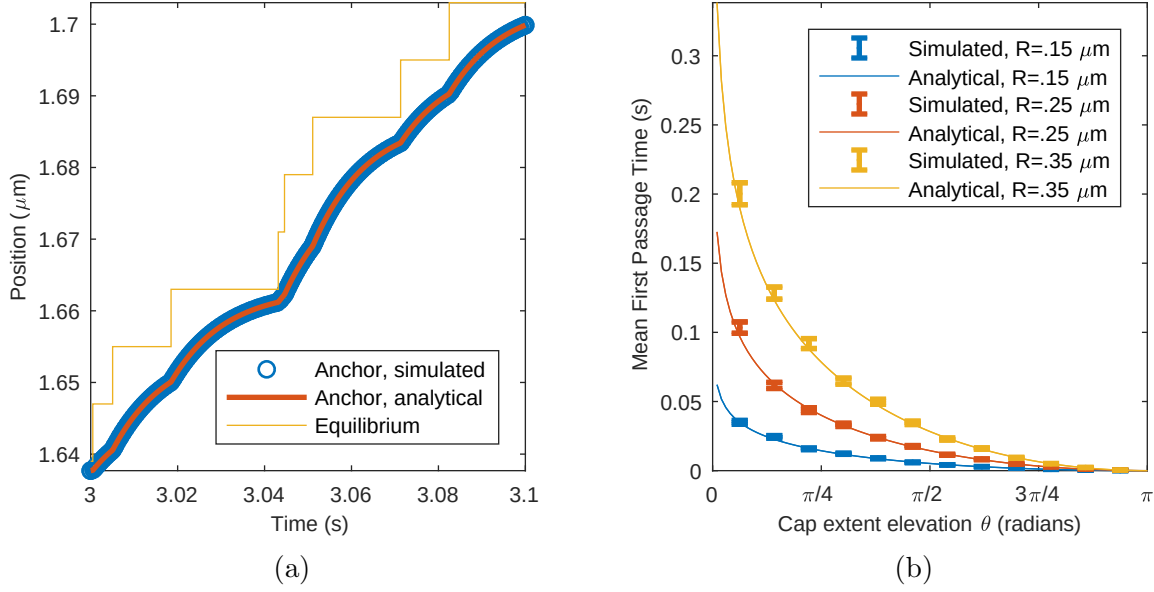


Figure 4.12: Comparison of simulated with analytical results for anchor drag and diffusion (a): Position of an anchor dragged through a 1D membrane by a stepping motor. A representative section of the time course of the anchor location in simulation (blue circles) is shown along with the equilibrium position of the motor spring (yellow). The analytical solution given this equilibrium location is found using equation 4.34 and shown in orange. Simulations done with $D = 0.001 \mu\text{m}^2 \text{s}^{-1}$.

(b): Simulated and analytical mean first passage times for the anchor diffusing from the north pole of the cargo into a cap which extends up to elevation θ . For $\theta = 0$, the cap is a point at the south pole. For $\theta = \pi/2$, the cap is the southern hemisphere of the cargo. Theory is shown in solid curves, simulation data are shown as mean $\pm$ SEM. Simulations done with $D = 3 \mu\text{m}^2 \text{s}^{-1}$.

Solving this differential equation with the initial condition $a(0) = a_0$, we find

$$a(t) = \exp\left(-\frac{\kappa^{\text{motor}} D}{k_B T} t\right) \left((h - L) \left(\exp\left(-\frac{\kappa^{\text{motor}} D}{k_B T} t\right) - 1 \right) + a_0 \right). \quad (4.34)$$

In the limit of a very large cargo with 0 cargo-MT distance, the simulation should produce this behavior. To compare the two, we first generate a simulation in this limit. The head location is constant between steps, allowing us to use the generated head locations to solve equation 4.34 in a piecewise manner over the entire time of the simulation. Analytical and simulated results are compared in figure 4.12a.

4.6.3.2 Anchor diffusion in the cargo membrane

The mean first passage time for a particle to diffuse from the north pole of a sphere into a cap over the south pole which extends up to elevation θ is

$$\frac{R^2}{D} \log \left(\frac{1 - \cos(\pi)}{1 - \cos(\theta)} \right) \quad (4.35)$$

as given in [136]. To compare with this analytical solution, we simulate many trajectories of anchors diffusing from an initial location at the north pole of the cargo into caps extending to different elevations. The results of a comparison between the two is shown in figure 4.12b.

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Chapter 5

Cargo rotation plays an important role in binding to the microtubule

The material in this chapter is adapted from a manuscript created by Matthew Bovyn, Babu Reddy, Steven Gross, and Jun Allard. The project was initiated by Matthew Bovyn, Babu Reddy, Steven Gross and Jun Allard. Babu Reddy performed experiments. Matthew Bovyn performed data analysis. Matthew Bovyn edited simulation code and performed simulations. Matthew Bovyn and Jun Allard created compartmental models. Matthew Bovyn analyzed models and performed parameter inference and model selection. Matthew Bovyn, Steven Gross and Jun Allard wrote the text. Steven Gross and Jun Allard supervised research.

A version of this manuscript was posted on bioRxiv [5] and submitted for peer review. The material has since been heavily revised.

5.1 Abstract

Molecular motors like kinesin are critical for cellular organization and biological function. There is detailed understanding of how they move and how factors such as applied force and the presence of microtubule-associated proteins can alter this single-motor travel. In order to walk, the cargo-motor complex must first attach to a microtubule. This attachment process is less studied. Here, we use a combination of single-molecule bead experiments, modeling, and simulation to examine how cargos with kinesin-1 bind to microtubules. In experiment, we find that increasing cargo size and environment viscosity both significantly slow cargo binding time. We use modeling and simulation to examine how the single motor on rate translates to the on rate of the cargo. This indicates that the mobility of the cargo itself, determined by its size and interaction with the cytoplasmic environment, play a previously underestimated role in determining intracellular transport kinetics.

5.2 Introduction

Eukaryotic cells employ molecular motors to create and maintain order, in part via transporting organelles from one place in the cell to another. At a single-molecule level, motor behavior involves attachment to the microtubule, stepping, and detachment from the microtubule. While the stepping and detachment processes have been extensively studied — including under load in backward, forward [41], left, right [44], and upward [162] directions, and as modified by MAPs [81, 129] and tubulin state [163] — the attachment rate is less well-understood.

Although rate constants can be used to summarize the attachment process, the study of motor attachment is complicated by multiple notions of on-rate: First, there is the “landing rate” of motors from solution onto an immobilized microtubule. Second, there is the overall

time for a cargo adorned with motors to attach to a nearby microtubule. This cargo attachment process involves the subprocess of an individual motor attaching to the microtubule, corresponding to yet a third on-rate (which we refer to as k_{on}). Fourth and finally, for cargo with multiple motors, it is possible that once one motor is attached, subsequent motors bind at a different rate than the first. These different notions of rates of kinesin can be inferred from several studies. Landing rate assays (e.g. [164]) measure binding by directly detecting events where individual motors bind the microtubule. Because binding is detected directly, little interpretation of the data is necessary to quantify the landing rate. However, because the motors are not bound to a cargo, the landing rate is difficult to translate into the context of a motor bound to a cargo. Force generation and run length of systems where two or more motors work together have been measured when linked directly to the bead [81, 165], on antibody linkers attached to beads [166], on DNA linkers without beads [167, 168], and on DNA linkers with beads [169, 170]. Because the motors are necessarily close together in these constructs, the bound or unbound state of each motor is difficult to detect directly (with the notable exception of [146]). Instead, mathematical models have been used to interpret motor binding rate by fitting model parameters to data. The on rates gleaned from these experiments differ from landing rates in two ways. First, the motors are linked together with beads and other molecules, so where the motors are relative to each other and how they are able to move through space is different in each case. Second, the on rate measured is always the rate at which the second motor binds (or perhaps third or fourth, depending on the experiment). The on-rate for kinesin of around 5 s^{-1} was estimated by fitting an ODE model to data on nanotube extraction in [92] and for multiple motor transport on beads in [165]. In an experiment where motor number was controlled using antibody anchoring points, motor on rate was estimated as 0.7 s^{-1} [166]. To match run lengths of multiple motor motor beads, 10 s^{-1} was used in previous work by us [3] (chapter 3). For a two-motor ensemble constructed with a DNA linkage but no cargo bead, a motor on rate of 4.7 s^{-1} was found [146].

Here we use a bead assay, along with biophysical modeling, to investigate the full process of

cargo binding to a nearby microtubule. By analyzing the bead assay time course data, we are able to extract times for the cargo to bind the microtubule. We perform experiments on beads of varying sizes and in mediums of varying viscosity. Putting this data together suggests that binding time is heavily dependent on bead mobility, specifically its rotation. To understand the relationship between binding time and bead rotation, we employ a previously published model and simulation. We find this simulation is not able to match the experimental data. To investigate further, we construct a compartmental model.

5.3 Results

5.3.1 Quantification of rebinding times

To investigate the binding of cargos to microtubules, we use a bead assay [3, 40, 81] altered to ensure the center of the trap is about a bead radius above the microtubule surface, described in methods section 5.5.1. We construct in vitro cargos using 560nm polystyrene beads in the single motor limit (binding fraction $\sim .2$, [94]), and use an optical trap to bring the cargo near the microtubule. We record the force exerted by the trapping laser as a function of time by measuring the deflection of the trapping laser on a QPD (momentum transfer method, [171]). We convert force to position for display purposes, as shown in (figure 5.1, lower). As the motor binds the microtubule and subsequently walks, it displaces the cargo in the trap (figure 5.1i). Because we use an optical trap stronger than the motor's stall force, the motor always eventually detaches and falls back to the trap center (figure 5.1ii), rather than escaping and continuing along the microtubule. There is a period of time after the bead falls back to the center of the trap during which we observe only small fluctuations in bead position (figure 5.1iii). Eventually the motor displaces the bead in the trap once more (figure 5.1v). We monitor beads for several minutes, during which this cycle repeats 10-100

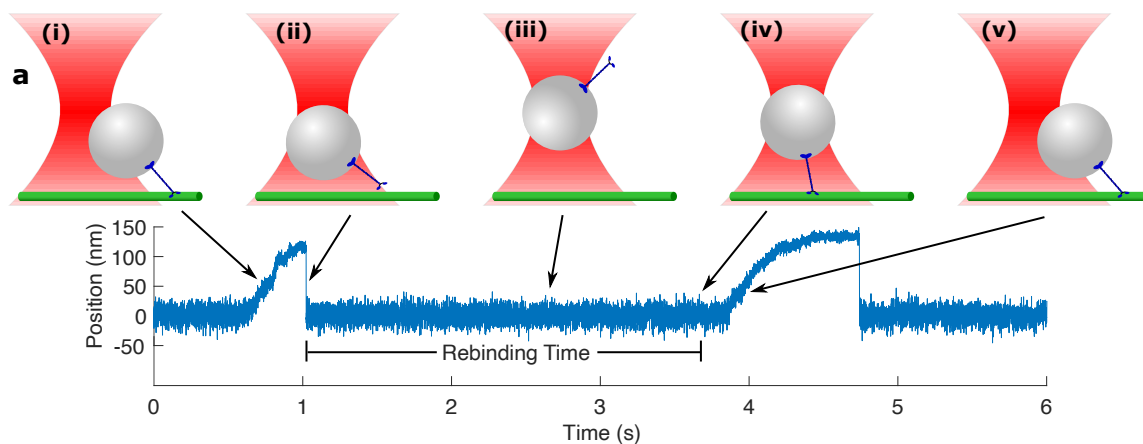


Figure 5.1: Example section of a trace, annotated with interpretation of cargo dynamics.

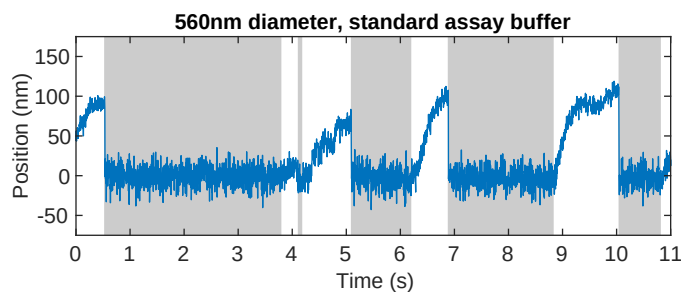


Figure 5.2: Example section of an annotated trace. Rebinding events, found by manual annotation, are shown highlighted in grey.

times.

We begin with the interpretation that the bead beginning to fall back toward the center of the trap indicates a motor unbinding event, after which the cargo is free to rotate. Then, at some point before we observe another excursion from the center of the trap, the motor must bind to the microtubule again. We call this the rebinding time.

To find rebinding times from the experimental data, we manually annotate times when the cargo is observed to fall back toward the trap center and times when an excursion from the trap center begins. We begin with the interpretation that these times are the beginning and end of a rebinding event. An example section of a trace with annotation is shown in figure 5.2.

Rebinding times of individual beads were found to be approximately biexponentially distributed (figure 5.3). We aggregate rebinding events from multiple beads to find a biexponential distribution for the population.

5.3.2 Rebinding data is not explained by a previous 3D model of cargo and motor

To evaluate if this behavior is expected from previous models of motors and cargo, we simulate cargo behavior in a trap using our previously published model [3] (chapter 3) and simulation [doi:10.5281/zenodo.4325116]. We modify the model to better reflect the bead assay here (see methods). When we simulate the model, we find that the model output of cargo center position with time is very similar to the analogous experimental output in a moment to moment sense — cargos spend time fluctuating in the trap, punctuated by events where the motor walks the cargo out of the trap center, creating a detectable signal. However, the amount of time between these events does not match the time between events in experiments even qualitatively. Sample traces are shown in figure 5.4.

To investigate what changes to the model could bring the output more in line with the experimental data, we also simulated the model at higher motor on rates. We found that much higher motor on rates are necessary to bring the model predictions near the experimental data, as shown in figure 5.5. Simulation output was manually annotated to find rebinding times in the same way the experimental data was.

5.3.2.1 3D model predicts binding events which are not detectable

Unlike the experiment, all the details are available in the simulation output, including the position of the motor and its true bound state. When we visualize the 3D positions of motor

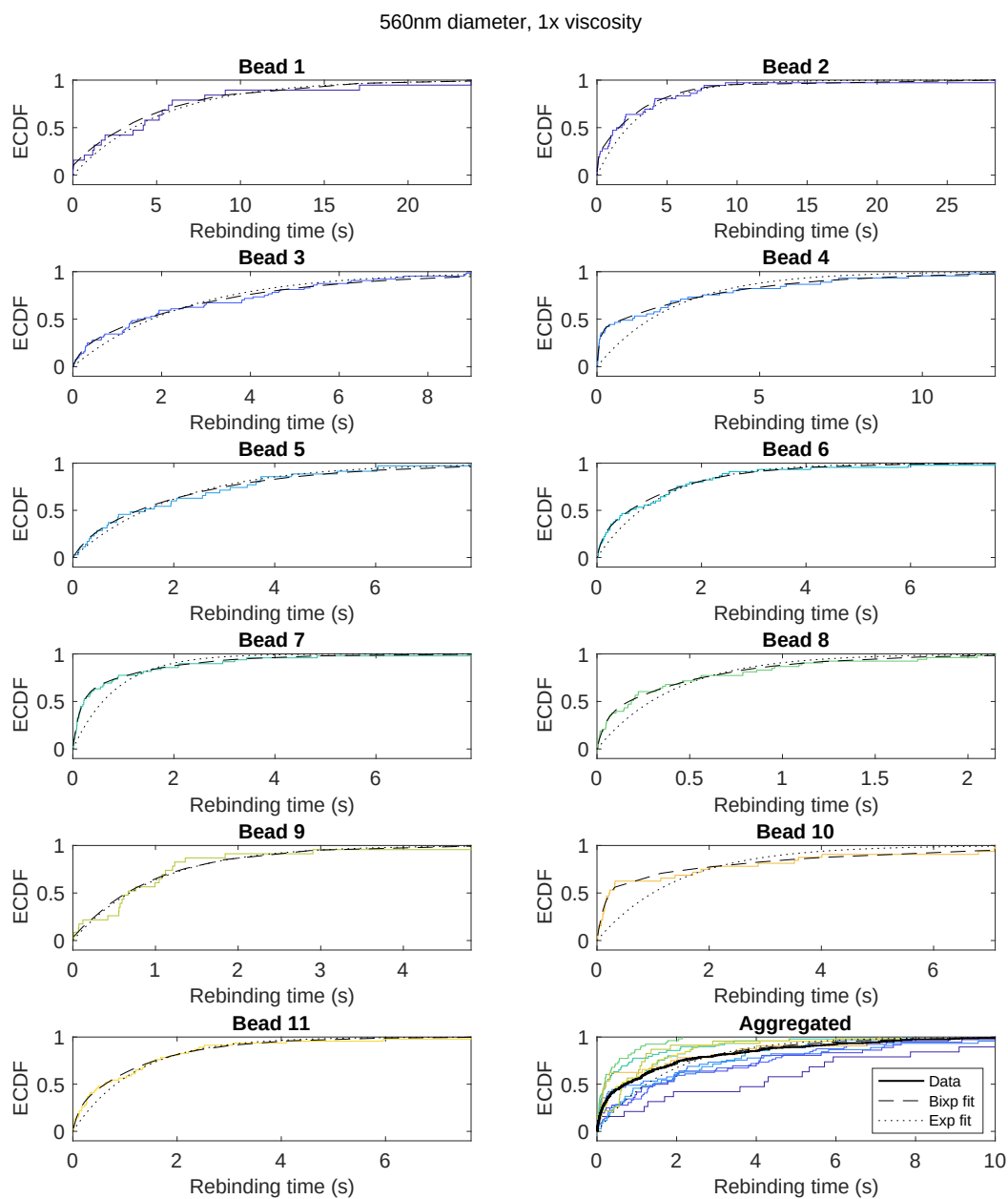


Figure 5.3: Rebinding time distributions for individual 560nm diameter beads in standard assay buffer. Also shown are single exponential and biexponential fits (legend in last panel).

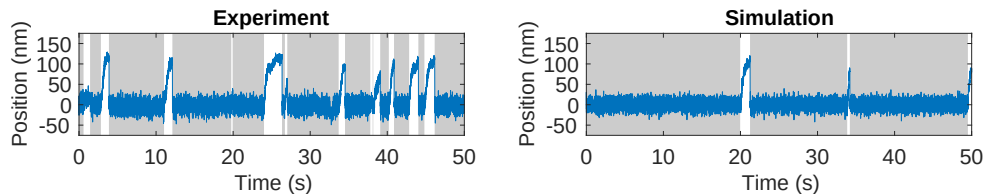


Figure 5.4: Examples of experimental and simulated traces. Parameters used for simulated trace were translated for this experiment from ones established in a previous model [3] (chapter 3). Grey highlighted regions are manually annotated rebinding times.

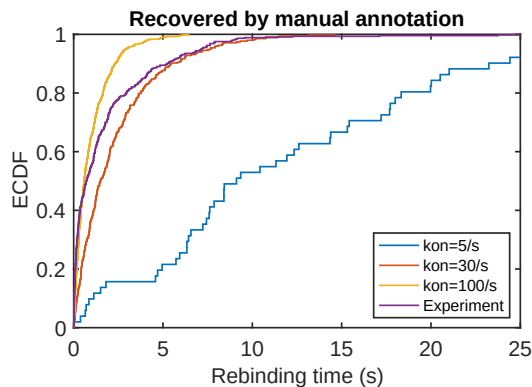


Figure 5.5: Rebinding time distributions for the experimental data with simulation predictions at various values for the motor on rate, given the motor is near the microtubule.

and bead along with the cargo center position, we find that there exist many binding events which happen in the simulation, but are not detectable in the position trace. An example simulation with 3D visualization and position trace are shown in movie 1 of [5].

These undetectable events in the simulation cause the true times between unbinding and binding events to differ from what humans are able to perceive from the position trace. Since this is the way rebinding times are measured for the experimental data, we subjected simulation data to the same manual annotation that the experimental data underwent. We found that manual annotation resulted in a rebinding time distribution with a different shape than the true bound/unbound state within the simulation, as shown in figure 5.6. Means are increased by about 1.5 times, as shown in figure 5.7.

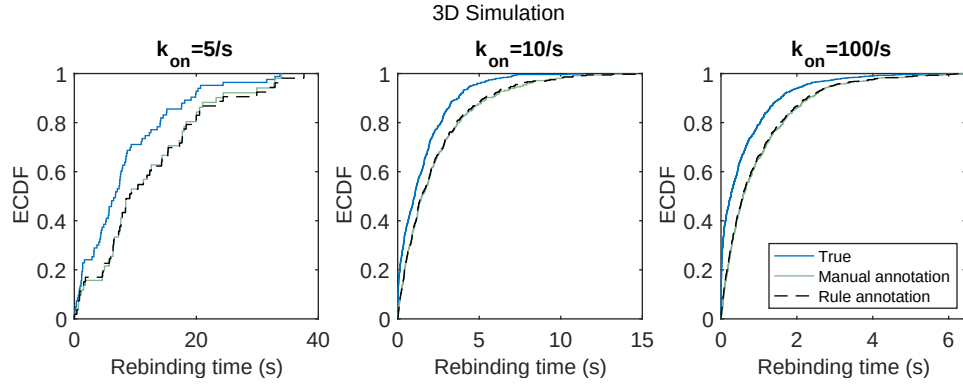


Figure 5.6: Rebinding time distributions from the simulation true state, manual annotation of the trace resulting from the same simulation, and the distribution recovered from the rule.

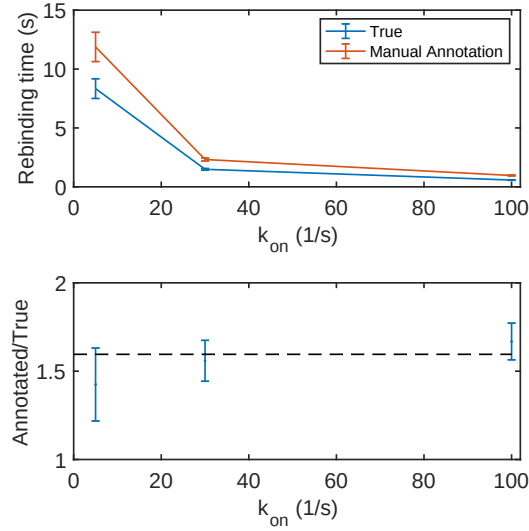


Figure 5.7: Mean rebinding times from the true bound/unbound state in the simulation and manual recovery. The ratio is shown in the bottom panel.

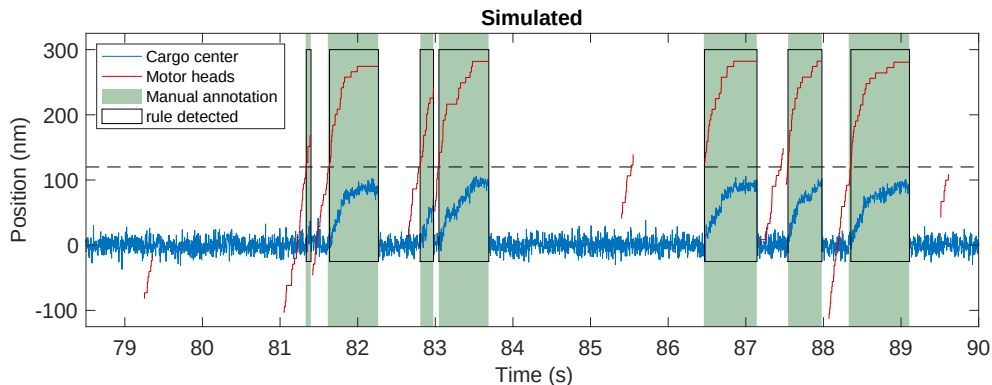


Figure 5.8: Example trace with cargo center and motor heads location plotted. When motor is not bound, the heads position is not shown. Motor bound state from manual annotation shown highlighted in green. Motor bound state inferred from the rule “bound motors are detectable when the heads pass 120 nm from the trap center for more than 0.05 s” is shown highlighted by black boxes.

5.3.2.2 Simulated events are only detectable when motor heads pass a threshold

We seek an understanding of when motor binding events are detectable. In the underlying dynamics of the simulation, the motor heads bind to the position of the microtubule nearest the anchor when a binding event occurs. This results in the motor binding somewhere in the accessible range of the microtubule (about -120 nm to 120 nm from the trap center). Therefore, sometimes the motor heads bind, walk, then fall off before being able to move the cargo significantly in the trap. By comparing the simulation true bound/unbound state, the location of the motor heads, and the annotations, we find a pattern emerges that humans are able to mark a trace when the motor heads pass a threshold of ~ 120 nm for more than 0.05 s. An example trace is shown in figure 5.8. In figure 5.6, we show that detection by this rule recovers very similar rebinding distributions to those recovered by manual annotation.

5.3.3 Beads which rotate more slowly also rebind more slowly

We do experiments on beads at different sizes and in environments with different viscosities (modulated using sucrose).

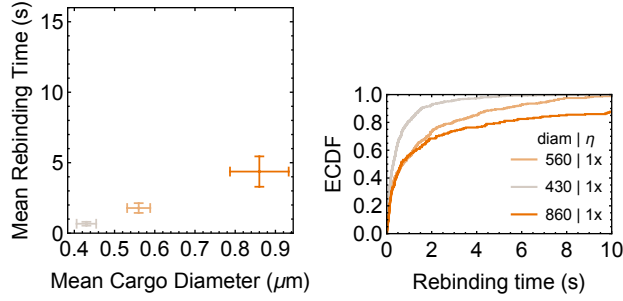


Figure 5.9: Means and distributions of rebinding times for beads with varying diameters

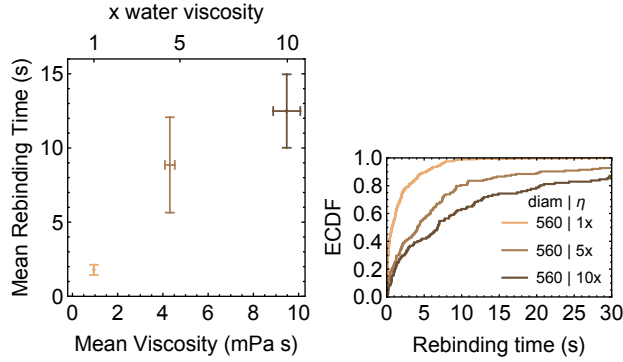


Figure 5.10: Means and distributions of rebinding times for beads in glucose solutions of 0, 35% and 45%, which correspond to viscosities 1x, 5x, and 10x water [172].

We find that the larger beads are, the slower they rebind, as shown in figure 5.9. We find that at higher concentrations of sucrose and therefore higher viscosities, beads bind more slowly, as shown in figure 5.10. The effect of these conditions on bead rotation can be quantified by their impact on the rotational diffusion coefficient of the bead: $k_B T / (8\pi\eta R^3)$, where k_B is Boltzmann's constant, T is absolute temperature, η is viscosity and R is bead radius. When we plot rebinding time against the beads' rotational diffusion coefficients, we find that rebinding time decreases with increasing rotational diffusion coefficient in an evocative way, as shown in figure 5.11.

Distributions of rebinding times for individual beads are shown in figure 5.3. These distributions vary both in mean and in shape.

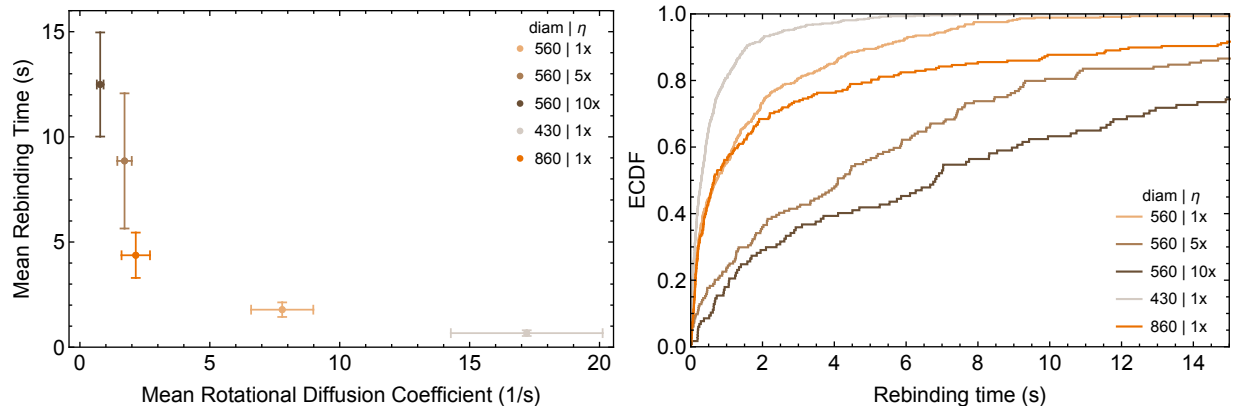


Figure 5.11: Mean rebinding times of all conditions plotted against mean rotational diffusion coefficient. Included below are all rebinding time distributions plotted together.

5.3.4 Compartmental model

We seek an understanding of how motor chemomechanics couple with cargo rotational and translational dynamics to produce the rebinding times we find in experiments. Figure 5.11 is evocative that cargo rotation is important, but a previous model which includes cargo rotation cannot explain the data. Therefore we face a task of model generation, analysis, and comparison. While our simulation is fairly efficient at generating data (~ 1 second of simulated time can be accomplished in 1 second of real time), it is not efficient to modify. This makes testing hypotheses about changes to the core assumptions of the model difficult.

Therefore, we construct a less detailed model where the cargo can exist in one of three states:

Far: the cargo is rotationally oriented such that the motor cannot reach the microtubule

Near: the cargo is rotationally oriented such that the motor can reach the microtubule

Engaged: the motor is bound to the microtubule

We set transition rates between these states as shown in figure 5.13. We include some proportion f of cargos go into the far state directly after the motor unbinds. This allows us to capture uncertainty about how the cargo may change orientation as it goes back to the

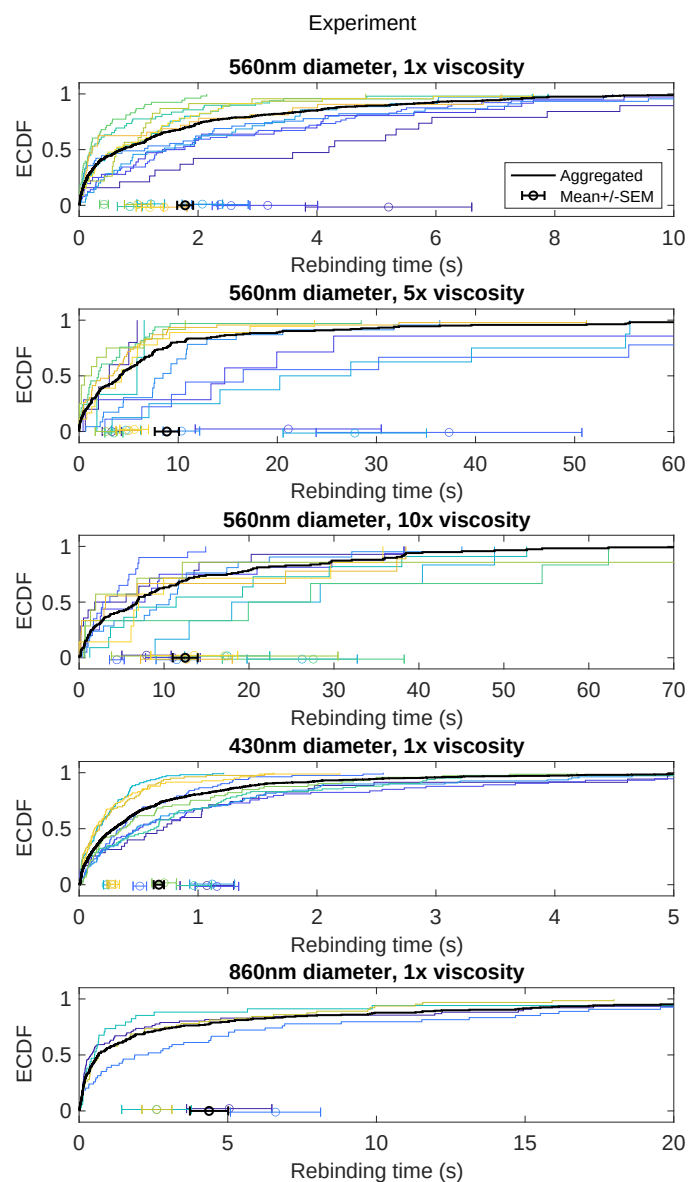


Figure 5.12: Rebinding time distributions for all beads
 Rebinding time distributions for each bead in the experimental data. Beads (curves of different colors) are shown together within a condition. Also shown is the aggregated distribution (black). Means and standard errors of the means are shown along the bottom axis. Y positions of mean and error bar marks are staggered for ease of viewing and are not meaningful.

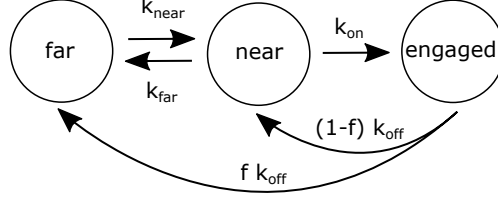


Figure 5.13: Diagram of the three state model

center of the trap. For example, if cargos rolled back along the microtubule surface causing the motor to move away from it, we would expect f to be near one. If motors stuck near the microtubule, we would expect f near zero. We derive the distribution of rebinding times, as well as their mean, for this model.

We then parameterize this model by making two assumptions. First, we assume that the rates of transition between the near and far state are proportional to the rotational diffusion coefficient of the bead. This captures the expected first order behavior of the cargo, and is true even for more detailed descriptions of spheres rotating near surfaces [173–175]. Second, we assume all rotation states of the bead are equally likely. This parameterization allow us to relate parameters in the compartmental model to physical geometry and dynamics of bead orientation.

5.3.4.1 Compartmental model can match rebinding times from simulation at physically reasonable parameters

Our parameterization allow us to relate parameters in the compartmental model to parameters in the 3D simulation, particularly through concerns of geometry. This allows us to find acceptable ranges for those parameters using our 3D simulation, given in table 5.2. We find that the compartmental model can match data from the 3D simulation at a set of parameters within this range, as show in figure 5.14.

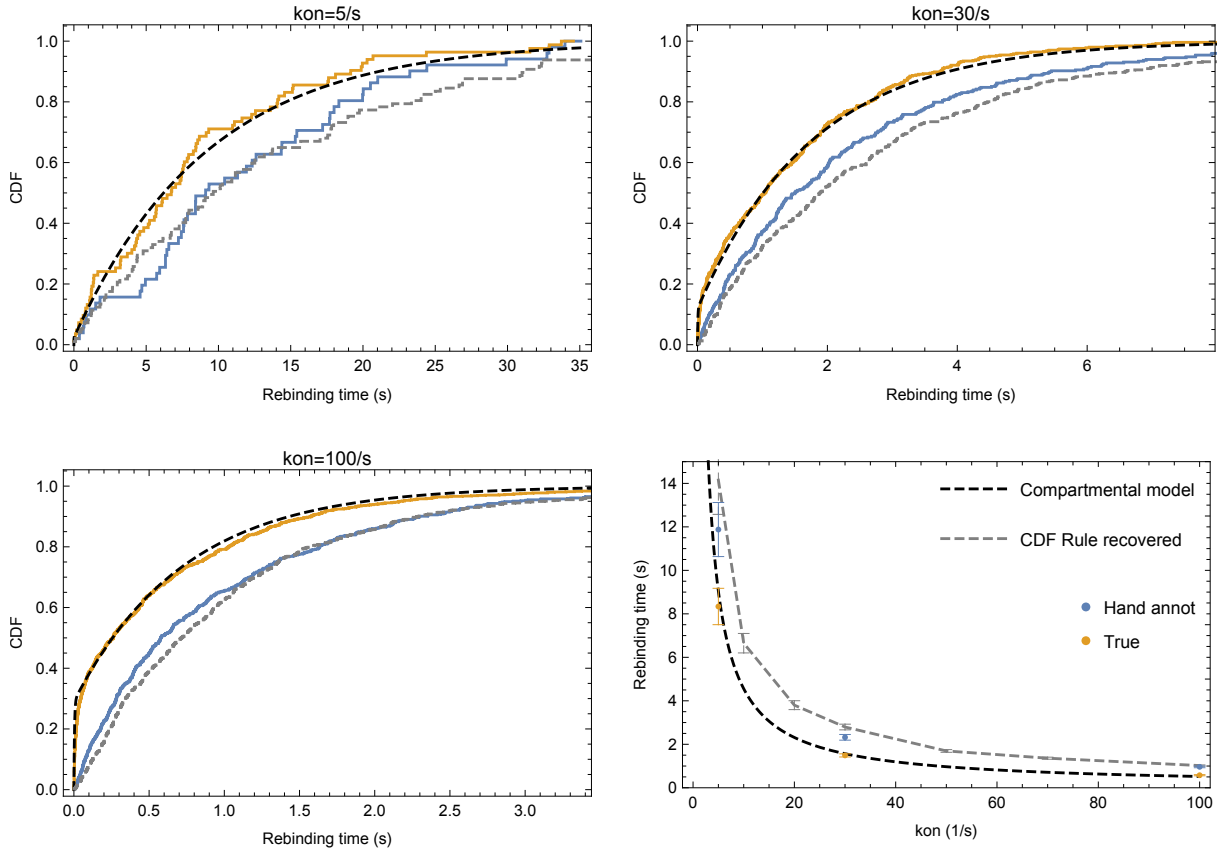


Figure 5.14: Rebinding time distributions generated by the compartmental model can match rebinding time distributions generated by the simulation. Compartmental model parameters are $f=.25$, $a=.42$, $D=8.3$, $r=.022$.

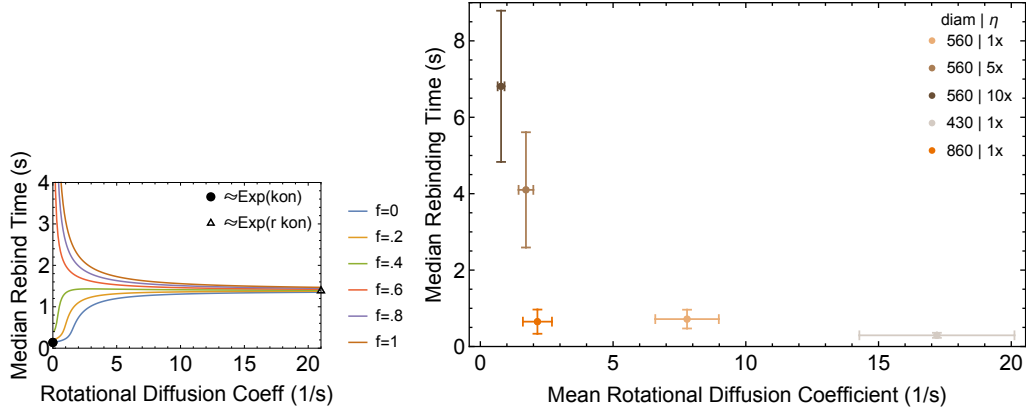


Figure 5.15: Median rebinding time as a function of rotational diffusion coefficient in the compartmental model, for various values of f , the fraction of cargos that go to the far state after the motor unbinds. Shown on the right are medians for the experimental data.

5.3.5 Motors must sometimes leave the microtubule

We observe that rebinding times decrease with increasing rotational mobility of the bead (figure 5.15, right). In our compartmental model, this is not always the case. If we set the fraction of cargos which go to the far state to 0, we find that median rebinding time increases with increasing rotational mobility, as shown in figure 5.15(left). This result can be understood by considering the following: should we expect slower rotation to speed up or slow down binding? When the motor is near the microtubule, it is able to bind. If the cargo is slower to rotate and leave this state, it will bind more quickly on average. When the motor is far from the microtubule, it cannot bind. If the cargo is slower to rotate and enter the near state, binding will be slower on average.

Because our experimental data show that cargos which rotate slower are also slower to rebind, we interpret that cargos in our experiments spend some amount of time in the far state, that is with their motor not able to reach the microtubule. Qualitative comparison with the data suggests that $f > .5$.

5.4 Discussion

While there are many established experimental and theoretical approaches to understand molecular detachment and mobility, the attachment process is less studied, and poorly understood. In this work, we provide a quantitative study of cargo-bound kinesin-1 attaching to a nearby microtubule. We find that attachment is determined by a combination of cargo rotation and the kinesin-1 attachment rate. Cargo rotation, in turn, depends on cargo properties and environment properties.

5.4.1 Relationship between motor on rate, cargo rotation, and rebinding time

A first guess at cargo binding time could be made by simply inverting estimated motor on rates, which yields 0.1 s to 1 s. Why do we measure rebinding times which are significantly longer? Heuristically, this conclusion follows from the inevitability that if the motor is away from the microtubule, it takes time for the cargo to rotate so that the motor comes near it. Even for an infinitely high motor on rate, the mean time for the cargo to bind is 0.1 s to 1 s if the motor starts far away [4] (chapter 4). This effect is exacerbated the fact that the motor is small in comparison to the cargo. Because of this, there are relatively few rotational orientations of the cargo which allow the motor to reach the microtubule (2.5 % to 5.3 % for our cargo sizes, see figure 5.16). This slows down binding in two ways: first by making visiting the region rare as the cargo rotates, and second by making the time which the cargo spends in orientations where the motor can bind short (about 0.005 s for a cargo in water, see 5.5.8). The few acceptable orientations and short timescale for leaving those orientations give context to the importance of rotation in the rebinding process.

5.4.2 Bead heterogeneity

We find that rebinding time distributions vary between beads in the same condition. One explanation for this variability is that the events within all beads are pulled from the same underlying distribution, but are samples from individual beads differ because the number of events is limited. Another explanation for this variability is that individual beads are different in important ways and samples from different beads are effectively pulled from different underlying distributions. We know both that motor behavior can be heterogeneous in important ways [176] and that bead physical dimensions vary from the manufacturer.

We construct beads used in the experiment so that they are as identical as possible. All motors are purified from *e. coli*. Antibodies are used to link motors to beads, controlling potential variation of attachment geometry. In the bead assay we use a low binding fraction to limit the presence multiple motor beads [94].

Therefore, we aggregate all rebinding times within a condition to construct a rebinding time distribution for that condition. We perform parameter inference on this aggregate distribution. This approach is common in studying molecular motors [41].

It would also be possible to perform parameter inference on the rebinding time distribution for each bead individually. This approach could possibly reveal more information about heterogeneity between beads. However, the number of events is limited by the lifetime of any individual motor inside the trap (~ 2 min), and thus power to make inference on parameters is also limited.

5.4.3 Detectability of binding events

In our simulation, we found that some binding events were undetectable using the cargo position trace. One might expect that such events might be observable since the motor

tethers the bead to the microtubule, restricting its motion. This is the case with very weak traps for both the kinesin-microtubule system [46] and the actin-myosin system [177]. However, the trap stiffness used here was adjusted to keep the motor from pulling the bead out of the trap. At this stiffness, bead fluctuations are quite limited. In our model, we assume that kinesin has a relatively short maximum length, and can stretch with a spring constant that has been measured by others [45, 46]. Critically, we also assume that the motor does not resist bending. The combination of a stiff trap with a floppy motor means binding does not detectably change cargo fluctuations.

One might imagine that a stiffer motor would change the fluctuations. Since the bending rigidity of kinesin has not been measured, this is possible in our experiment. However, we do not find sections of data where fluctuations of the bead are constrained. Using a weaker trap could potentially reveal such sections. Such a system would likely be more difficult to investigate. To keep beads in the trap, it might be necessary to build an experimental system where laser power is dynamically controlled to ramp up when the bead starts to move out of the trap. The presence a regime where a single laser power could both allow for enough diffusion of the bead to reveal times when binding is limited and exert enough force to keep single motors trapped could be investigated in our simulation.

5.4.4 More complex models

In our experimental data, we detect “catch” events where motors rebind before the cargo returns to the center of the trap, a process which takes only a few thousandths of a second. These extremely fast rebinding events were recently investigated by Toleikis, Carter, and Cross [178]. They find evidence that similar events are backsteps, distinct from an off event where the motor detaches from the microtubule completely. Since we are interested in investigating the rotation behavior of our cargos, we have excluded these catch events from

the analysis above, under the assumption that unless a catch event occurs, the cargo quickly goes into a state where it rotates.

An even more recent paper [179] finds evidence that motors often stay associated with the microtubule during the entire process of the cargo moving back to center of the trap. This provides an additional avenue for the motor to restart walking which does not go through a state where the cargo can rotate. It suggests that catch events are only a small proportion of “restart” events. This new avenue suggests a very different interpretation for fast rebinding events than the one we have examined here. Further analysis is required to understand how often such sliding events happen in our experimental system and what they mean for our interpretations about the relationship between cargo binding and motor on rate. In the discussion of [5], we noted that if such a state existed the conclusions of that version of this work would need to be reanalyzed. We also noted that at the time there was no evidence for it.

Recent work finds that k560 motors, the same as the kinesin-1 construct used in our experiments, bind to microtubules from solution more slowly than a truncated version of the construct [129]. The authors suggest that k560 possess a self-inhibited state, which binding to a microtubule associated protein disallows. The presence of such a state suggests yet another model of cargo binding with fast and slow on rate states for the motor, and some rates of moving between these states. Further more, moving between these states could be influenced by interactions of the motor with the microtubule. The three compartment model investigated here may effectively yield an average binding rate between such high and low on rate states, or the transitions between those states may happen in such a way that inference on motor on rates and cargo movement are changed significantly. More investigation is necessary to understand the relationship between these models.

5.4.5 Connections between on and off rates

We assume in the model that the motors bind at the same rate independent of their position with regard to the cargo and microtubule. The fact that off rates vary with the direction of the force exerted on the motor [41, 162] suggests that this might not be true. However, since off rates have not been experimentally measured for near-zero assisting forces means it is unclear how unfavorable being bound in an assisting load orientation is. Measurements of off rate with motor angle at near-zero forces could allow detailed balance to be used as a tool to relate on and off rates.

5.5 Methods

5.5.1 Bead assay

Optical trap was assembled on an inverted Nikon microscope using a 980 nm, single mode, fiber coupled diode laser (EM4 Inc). Laser power was set to achieve the trap stiffness, k_{trap} , of $\sim 0.045 \text{ pN nm}^{-1}$ for all the polystyrene beads utilized (0.43 μm , 0.56 μm and 0.89 μm).

Motor dilutions and experiments were carried out in the motility buffer (80 mM Pipes pH 6.9, 50 mM $\text{CH}_3\text{CO}_2\text{K}$, 4 mM MgSO_4 , 1 mM DTT, 1 mM EGTA, 10 μM taxol, 1 mg mL^{-1} casein). Viscosity of the motility buffer was altered (5x and 10x water) by adding appropriate amounts of sucrose. In all the experiments, single motor kinesin coated polystyrene beads were prepared just before the measurements. K-560-His (Kinesin-1, aa 1-560; His tag at c-term) diluted to $\sim 20 \text{ nM}$ was mixed with $\sim 1 \text{ pM}$ of penta-His-biotin antibody conjugated streptavidin polystyrene beads (0.43 μm or 0.56 μm or 0.89 μm diameter, SpheroTech). This ratio produced the bead binding fraction of 10-15% and was maintained to maximize the probability of finding single motor beads in the solution. The bead-motor incubation ($\sim 30 \mu\text{L}$

volume) was carried out at room temperature for 10 min. At the end of incubation, sample chamber with preassembled microtubules was washed with $\sim 30\ \mu\text{L}$ of warm filtered buffer just before injecting the incubated mixture. Experiments were carried out at RT in motility buffer supplemented with 2 mM ATP and oxygen-scavenging system ($0.25\ \text{mg mL}^{-1}$ glucose oxidase, $30\ \mu\text{g mL}^{-1}$ catalase, $4.5\ \text{mg mL}^{-1}$ glucose).

In general, small dust or debris in the solution gets pulled into the trap along with the bead. Trapped dust interferes with motor rebinding to MTs. In our assays, dust particles and aggregates of casein in the buffer were removed using a 100 kD centrifugal filter (Millipore). Another potential issue is the stage drift during measurement and it was minimized with an automated drift correction system using a xyz piezo stage (PI) and custom software.

Activity of the kinesin motors was observed as bead displacements from the trap center in DIC video microscopy (30 fps). The binding events were also registered in photosensitive detector (PSD) at the back focal plane of the condenser. Both these data were digitized and stored into computer hard disk using data acquisition boards and custom LabVIEW software.

5.5.2 Description of data and uncertainty estimation

Our data consists of many individual rebinding times. An individual bead gives 10-100 rebinding times, and about 10 different beads are measured for a given condition of bead diameter and sucrose concentration. Multiple beads come from a single prepared slide.

We aggregate all rebinding times to construct a distribution of rebinding times for that condition. To estimate the error for these measurements, we use a hierarchical bootstrap scheme as laid out in [180].

Beads are variable in size, and experimental preparations are subject to variation in sucrose

concentration and temperature. Because we do not directly measure these quantities, we estimate their value and uncertainty in the following ways.

Bead size Mean bead size is provided by the manufacturer. The manufacturer also provides a distribution of sizes for 560nm beads, from which we learn the distribution of bead sizes has a standard deviation of 95 nm. Standard error of the mean is then calculated using this standard deviation and the number of beads measured.

Viscosity Viscosity of sucrose solutions have been measured [172] as functions of both concentration and temperature. We use 35 % and 45 % solutions to create 5x water and 10x water viscosity solutions respectively. We set our mean viscosity values by interpolating linearly within the tabulated data of [172] for 35 % and 45 % sucrose at 22.5 °C. We estimate an uncertainty of 5 percentage points of sucrose concentration. Since the trapping laser can create local heating near the bead [181], we estimate the temperature of the system to be between the 20 °C room temperature and 25 °C. We estimate 95 % of values fall within a range set by the mean of the four extreme values (± 5 percentage points of concentration at 20 °C and 25 °C).

Viscosity of water without sucrose is also a function of temperature [182]. We linearly interpolate with the tabulated values of [182] at 22.5 °C to find our mean value, and estimate 95 % of values fall within the range set by the mean of the values at 20 °C and 25 °C.

To convert our uncertainty estimates into standard errors of the mean, we divide them by $2\sqrt{n}$, where n is the number of beads.

We use the the above estimates of mean and standard error for bead size (radius R) and viscosity (η) to calculate the mean and standard error for rotational diffusion coefficient, $D = k_B T / (8\pi\eta R^3)$, with $k_B T = 4.1 \times 10^{-3}$ pN μ m. Error was propagated using Mathematica's

ability to do automatic error propagation on values in `Around[]` objects.

5.5.3 Curve fitting

Biexponential fits were accomplished using the `fit` function in Matlab 2019b. Rebinding time empirical cumulative distribution was fit to

$$\text{CDF}(t) = 1 - pe^{-\mu_1 t} - (1 - p)e^{-\mu_2 t}. \quad (5.1)$$

5.5.4 Manual annotation of force traces

Annotators were given only the position traces and were blinded to the identity of the trace (simulation or experiment, as well as the condition).

Participants were given instructions to look through traces for events where cargo positions rose past the baseline noise and created a detectable “kick”. They were instructed to then mark where they thought the trace began rising from the noise and where the bead fell back to the center of the trap. Ambiguous events were rare, and called as could best be determined by collaboration among the annotators. Events were found where the cargo ceased to fall back to the trap center after an initial period of moving back. These “catch” events were excluded from further analysis.

All annotated traces were visually inspected to ensure annotations were a good match to the data. A small set of events were excluded from the analysis (12 of 1932 total) because they included the signature of a piece of dust falling into the trap or a bead diffusing away.

5.5.5 3D model and simulation

A detailed description of the model is given in [3] (chapter 3). Briefly, we model the bead as a sphere which diffuses rotationally and translationally. This motion is coupled to the chemomechanics of the motor, which can bind, unbind and step. Binding is constrained by the ability of the motor to reach the microtubule and unbinding and stepping rates vary with force. Rates are implemented through a Gillespie/Next Event method, which is integrated with an Euler-Maruyama implementation of the stochastic ODE solving for the equations of motion.

5.5.6 Adjustments to previous model for this experimental system

In previous work, we simulated cargos with many motors navigating intersections between microtubules. We chose parameters to match the behavior of cargos navigating intersections between microtubules [3] (chapter 3). Here, we simulate beads with single motors and one microtubule. We have updated the motor model to reflect recent data on differences between assisting and hindering load in kinesin-1 off rate (from given in [4] (chapter 4)). Here we detail further changes made to reflect the experimental setup used to take the data in this paper.

5.5.6.1 Motor length

In [3] (chapter 3), full length KIF5A motors were used. In this work, we use truncated k560 constructs, linked to the bead via anti-his antibodies. We estimate the length of these motors to be 40nm.

5.5.6.2 Surface effects

A sphere in an infinite fluid experiences drag $\gamma_t = 6\pi\eta R$ in translation and $\gamma_r = 8\pi\eta R^3$ in rotation. It diffuses with diffusion coefficient $k_B T / \gamma$. When the same sphere is near a surface, it experiences greater resistance to movement due to the interaction of the fluid, the sphere, and the nearby surface. This effect slows translational diffusion by 2.5-3x [183]. It slows rotation less, by only $\sim 10\%$ [184]. The surface induces differences in the sphere's ability to diffuse perpendicular and parallel to it, and has a similar non-isotropic effect on rotation [183, 184]. The presence of the surface also couples translation to rotation, which can induce rolling. However, the rolling effect is weak, resulting in rotational velocities only $\sim 10\%$ of the translational velocity [185]. The presence of the microtubule below the bead also potentially changes the dynamics of the bead from the cases above, both because it would change fluid flows around the bead, and because the bead may hit it and experience some friction.

Since the beads are highly constrained by the optical trap and do not leave the immediate area of the microtubule often, we do not include increased translational drag on the bead. Since the effect on rotation is weak, we also ignore that. We place a surface in the simulation which disallows the cargo from moving into it.

5.5.6.3 Inclusion of optical trap

We add to the simulation forces exerted by an optical trap. Since the trap is tuned so single motor cargos stay within the linear region of trap stiffness, we model forces exerted by the trap as

$$\vec{F} = \left(-k_x(x_{\text{cargo}} - x_{\text{trap}}), -k_y(y_{\text{cargo}} - y_{\text{trap}}), -k_z(z_{\text{cargo}} - z_{\text{trap}}) \right), \quad (5.2)$$

Parameter	Description	Value
$\vec{k}_{\text{trap}}$	Optical trap stiffness (x,y,z directions)	(.04,.04,.013)pN nm ⁻¹
π_0	motor on rate	varied in simulations
v_0	0 force velocity	0.75 $\mu\text{m s}^{-1}$
d_s	step distance	8.2 nm
$\mathcal{F}_s$	stall force	6.1 pN
w	force-velocity curvature index	1.8
ϵ_0	0 force unbinding rate	0.8 s ⁻¹
ϵ_s	superstall base unbinding rate	1.6 s ⁻¹
$\mathcal{F}_{ss}$	characteristic superstall detachment force	7.6 pN
ϵ_a	assisting base unbinding rate	7.4 s ⁻¹
$\mathcal{F}_a$	characteristic assisting detachment force	12.5 pN

Table 5.1: Parameters used for detailed computational model.

where positions are of cargo and trap centers.

5.5.6.4 Parameter values

Parameters used are listed in Table 5.1.

5.5.7 Three state compartmental model

5.5.7.1 Derivation of mean and distribution of rebinding times

To find the rebinding time, we treat the engaged state as an absorbing state and write down the differential equations which describe this Markov chain:

$$\frac{dp_{\text{far}}(t)}{dt} = k_{\text{far}}p_{\text{near}} - k_{\text{near}}p_{\text{far}} \quad (5.3)$$

$$\frac{dp_{\text{near}}(t)}{dt} = k_{\text{near}}p_{\text{far}} - k_{\text{far}}p_{\text{near}} - k_{\text{on}}p_{\text{near}} \quad (5.4)$$

$$\frac{dp_{\text{eng}}(t)}{dt} = k_{\text{on}}p_{\text{near}}. \quad (5.5)$$

Equations were entered into Wolfram Mathematica 12.0.0.0 and solved analytically using DSolve with initial conditions $p_{\text{far}}(0) = f$, $p_{\text{near}}(0) = 1 - f$, $p_{\text{eng}}(0) = 0$. Binding time cumulative distribution is the same as $p_{\text{eng}}(t)$. We do not print the solution here, as it contains many terms.

Mean time to bind,

$$\langle t_{\text{rebind}} \rangle = \int_0^\infty 1 - p_{\text{eng}}(t) dt \quad (5.6)$$

was found using Integrate. We find

$$\langle t_{\text{rebind}} \rangle = \frac{k_{\text{near}} + k_{\text{far}} + f k_{\text{on}}}{k_{\text{near}} k_{\text{on}}}. \quad (5.7)$$

5.5.7.2 Parameterization

We assume $k_{\text{near}} = aD$ and $k_{\text{far}} = bD$. Substituting this into our expression for the mean rebinding time and taking the limit as $D \rightarrow \infty$ yields the mean rebinding time for very fast rotation. Under the assumption that all rotational state are equally likely, this expression must be equal to $1/(k_{\text{on}}r)$, where k_{on} is the binding rate given the motor is within binding range and r is the proportion of rotational states in which the motor can reach the microtubule. Equating these two expressions yields a relationship between a , b , and r , which we substitute into the mean rebinding time to eliminate b .

This yields a mean rebinding time

$$\langle t_{\text{rebind}} \rangle = \frac{f}{aD} + \frac{1}{r k_{\text{on}}}. \quad (5.8)$$

We perform the same substitution to derive a parameterized rebinding time distribution, but again do not print the expression here.

Descriptions of each parameter are given below, and accessible ranges or guesses of parameters are given in table 5.2.

D : Rotational diffusion coefficient for a sphere in a Newtonian fluid far from a boundary. Set by bead size and environment viscosity.

f : Fraction of cargos that start in the far state. Can range from 0 to 1. In the simulation, we expect f near .5, as a pulling motor is near the edge of the region where it can bind.

a : Slope of time to come near with D . A guess at the parameter can be found through simulation. We find the time for the motor to come into binding range from a random position on the cargo, given the cargo is set as near as possible to the microtubule. The cargo spending time farther away from the microtubule or rotating more slowly would decrease this. Starting from on average closer to the MT than random would increase it.

r : Proportion of rotational states where the motor can reach the microtubule. A maximum is set by bead, motor and microtubule geometry. We use our 3D simulation to find this maximum for the different conditions. The bead spending time away from the microtubule while it fluctuates in the trap could decrease this value.

k_{on} : The binding rate of the motor, given it is near the microtubule. Previous inferences from other experiments are in the range of 1-10/s. We know of no inherent limits.

5.5.7.3 Determination of the area ratio parameter r

The area ratio is introduced in figure 5.16. To find the max reach locations, we began 500 simulations with a single motor located at the north pole of the cargo, positioned directly

Parmeter	560 1x	560 5x	560 10x	860 1x	430 1x
f	(0-1]	(0-1]	(0-1]	(0-1]	(0-1]
a	.42	.084	.042		
D	8.3	1.7	.83	2.3	18
r	(0-.039]	(0-.039]	(0-.039]	(0-.053]	(0-.025]

Table 5.2: Ranges of parameters that are physically accessible to cargos for each condition. For a, we give a guess derived from simulation instead of a limit, as different physical effects could either increase or decrease this.

above a microtubule. We allowed the cargo to diffuse rotationally (but not translationally) until the first time at which the motor was able to bind to the microtubule. Motors were determined to be able to bind if the shortest distance around the surface of the cargo to the microtubule is less than the motor length (for more, see detailed description of simulation in [4] (chapter 4). We recorded this location for each simulation and took the mean to find the mean reach elevation. This process was repeated for each cargo diameter. To the data on mean reach elevation as a function of cargo diameter data we fit the function $1/\log(ad^b + e^{1/\pi})$, and find $a = 28, b = 1.6$ with the function fit in Matlab 2019b.

5.5.8 Mean first passage time out of a spherical cap by cargo rotation

We estimate the time the cargo would spend in the near state if it were freely rotationally diffusing in water. The mean first passage time for a point on a sphere to leave a spherical cap of extent θ by rotation is

$$\langle t \rangle = \frac{1}{D} \log \left(\frac{1 - \cos(\pi)}{1 - \cos(\pi - \theta)} \right) \quad (5.9)$$

as given in [136]. By plugging in the θ value taken from the analysis in figure 5.16, the mean first passage time for a 45 nm motor starting at the south pole of a 560 nm diameter cargo in water is 0.005 s.

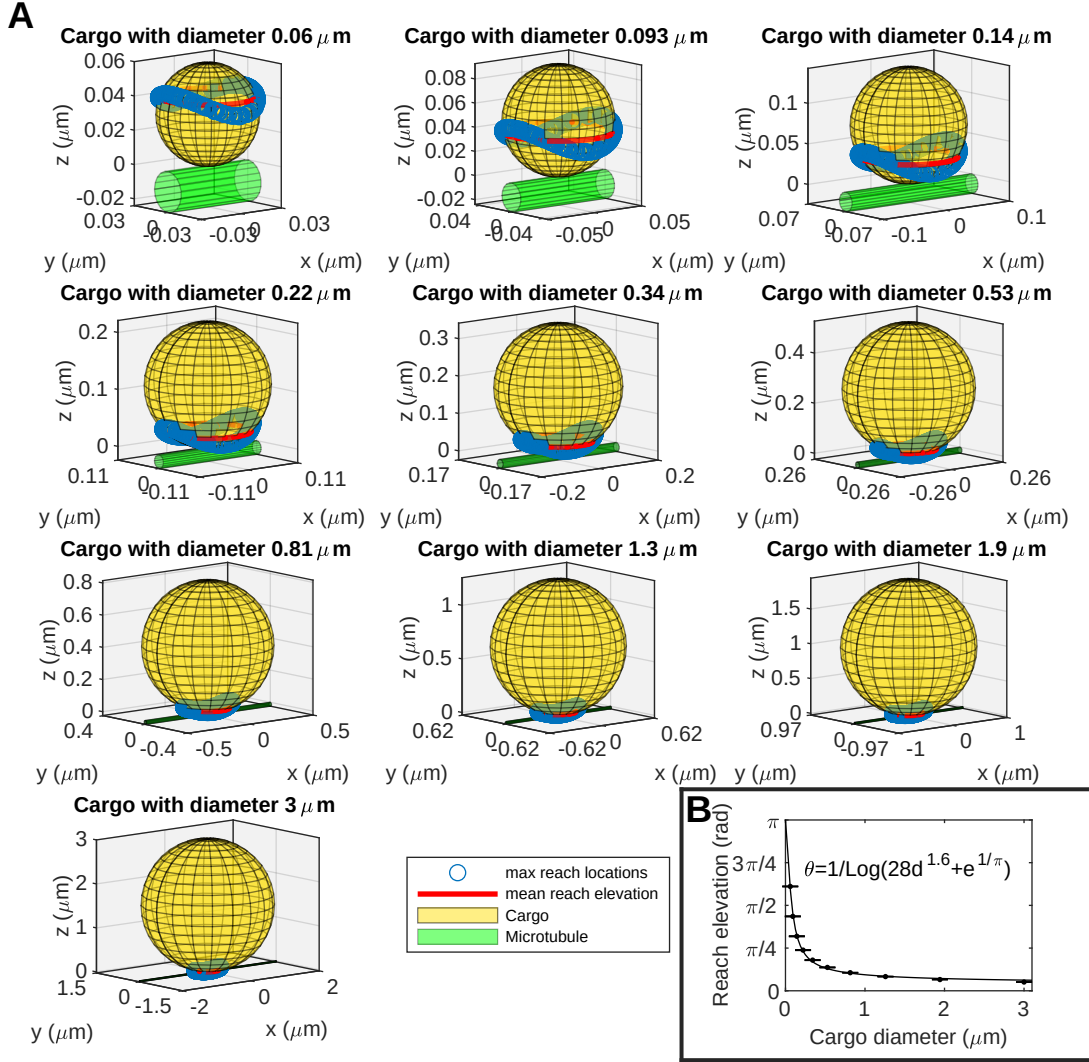


Figure 5.16: Bead and motor geometry determine area ratio

In our compartmental model we introduce the parameter r , a ratio of areas on the cargo surface. The numerator of this ratio is the area on the cargo surface to which a motor can be bound which allows the motor to reach the microtubule. The denominator is the full surface area of the cargo. We use our simulation to determine this ratio as a function of the cargo diameter. In A, we show cargos of various sizes next to a microtubule, along with locations where motors were just barely able to reach the microtubule (blue circles). We take the mean elevation of these locations to find the mean reach elevation, shown in red. Mean reach elevation is shown as a function of cargo diameter in B. Error bars are standard error of the mean. The function $\theta = 1/\log(28d^{1.6} + e^{1/\pi})$ is a good fit to this data. By plugging this in to the ratio of the area of a spherical cap to the total area of a sphere, $(1/2)(1 - \cos(\theta))$, we arrive at the area ratio as a function of cargo diameter.

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Chapter 6

Discussion

The work included in this dissertation attempts to take understanding that has been gained over the last 30 years about the function of molecular motors (especially kinesin-1) and push forward toward an understanding of how cells harness and conduct the actions of those motors to accomplish functional goals. Specifically, we've taken measurements of how kinesin-1 unbinds and steps, and the sometimes complex models created of these processes, and extracted from them a model which attempts to both capture the major features of how things work and be simple enough to compute answers from quickly.

The insights gained in pursuing the projects described in this dissertation mostly stem from integrating this model of motor function with a consideration of cargos as physical objects in a 3D space. The best first step to understanding how things move is often to create models of point objects, which are mathematically and computationally expedient to examine. This work shows that considerations of the shape and size of an object, and how it rotates, can be just as important as thinking about where the object is and how it moves translationally.

6.1 Motors, cargo and environment dictate transport outcomes

This view of the cargo as an extended object moving in 3D space brings into focus a fact about cargo transport: the dynamics of a cargo are determined by the combination of the actions of the motors, properties of the cargo, and the cargo’s environment. When creating and parameterizing a 3D model, each of these things needs to be taken into account explicitly. In this way, creating the model detailed in this dissertation pushed us to consider and investigate each of these.

6.1.1 At microtubule intersections

Our study on how cargos navigate microtubule intersections focused on the environment. We kept the motors and cargo the same as much as possible, while varying properties of the environment — specifically the separation and angle between the two microtubules which create the intersection. We showed that these properties dictate the transport outcome: they can make the difference between cargos almost always switching and almost always passing. We also showed that changing another property of the environment, the viscosity, is predicted to be able to further change outcomes.

These outcomes occur, of course, for cargos of the certain motor and cargo properties that we studied. Different cargo properties in particular could change outcomes drastically. In the case of few motors on the cargo, we would predict little to no switching in any environment — if there are no motors available to bind to the crossing microtubule, switching would be impossible. Similarly, mobility of motors on the surface of cargos could change the dynamics of tug of wars between primary and crossing motor teams as well as the probability of creating a tug of war. To first order, we would expect the effect of changing the cargo size to

mirror the effect of changing the microtubule separation. However, increasing the size of a cargo with a constant number of motors means that those motors are farther separated from each other on average. If instead the number of motors were increased to keep a constant surface density, there would be many more motors available to bind to any section of the microtubule, likely increasing the forces and times of tug of war events.

6.1.2 In the context of the cell

Our study investigating how cargos of different surface organizations are predicted to function in the context of the cell is primarily a study on properties of the cargo. We find that motor organization on the surface of the cargo can create cargos that are transported long distances or short, and bind quickly or slowly. We find in fact that surface organization dictates a specific combination of these two transport properties.

In this work, we attempted to find parameters of the model which are closest to those of cargos in the cell. For the environment, we reviewed the microrheology literature to find measurements of viscosity in the cytoplasm of cells. For cargo properties, we looked at ranges of sizes relevant to lipid droplets, lysosomes, and mitochondria. We also used diffusion coefficients of motors inspired by measured diffusion coefficients of transmembrane proteins. For the properties of motors, we used motor lengths determined in cryo-EM images of purified motors and in situ cargos. Other properties of motors, such as stepping and off rates were taken from in vitro measurements. While these properties might be different in cells, we know of no measurements which show how. However, we can speculate as to how transport outcomes may be different with changes to these properties.

While we modeled the cargo environment as a Newtonian fluid, it is known to be much more complex. It is active, crowded and has significant elasticity [108, 109]. Because of this, cargo translational and rotational behavior could diverge significantly from that exhibited

by cargos in the model. If cargos were to be even less rotationally mobile in the cell than predicted, differences between the time to bind of cargos with rigidly anchored motors and those with diffusing motors would be even greater. These differences would likewise shrink with increased mobility. However, for times to be similar between rigid and free motor cargos, the surrounding environment would need to be nearly equivalent to water.

Time to bind is also, in principle, sensitive to motor microscale on rate. However, we find that cargo binding is well into the rotation limited regime for cargos with rigidly anchored motors. Binding time of these cargos is insensitive to changes in motor on rate over orders of magnitude. Cargos with free motors, on the other hand, are more sensitive to motor on rate. Since they move fast enough at the chosen diffusion coefficients to find the microtubule, then diffuse away again before binding, increasing motor on rate decreases cargo time to bind.

Cargo run length is also sensitive to motor on rate. The motor on rate we used is slower than the time a motor resides near the microtubule while it is diffusing. This leads to cargos with free motors being transported less far than cargos with motors in a rigid cluster. However, if the motor on rate were increased, these two organizations would at some point become similar in run length, because motors which unbind would bind again before diffusing away. However, run lengths at these binding rates would be very long — much longer than observed in cells or in vitro.

6.1.3 In rebinding

In our study on how cargos rebind to the microtubule after detaching in an optical trap in vitro, we vary properties of the cargo while keeping the same motor and environment. We find that rebinding is slower in environments which allow the cargo to rotate less freely. Since many of our motor, cargo and environment properties are known, we then use the variation in rebinding time to infer properties of the motor.

In an environment different from the in vitro environment we constructed for our study, the rebinding process could be quite different. We find that the rebinding process is predicted to depend on rotation quite differently depending on the orientation of the cargo when it returns to the center of the trap. In turn, the orientation of the cargo when it returns to the center of the trap is determined by the fluid dynamic interaction of the cargo, the nearby surface of the coverslip, the microtubule and the trap. While much is known about the fluid dynamics of spheres near surfaces, the interaction of the additional elements present makes it difficult to predict the rotational dynamics of the cargo. If the motor is near the microtubule when the cargo returns to the trap center, the rebinding time is predicted to decrease with increasing viscosity. However, the rebinding time is predicted to increase with increasing viscosity if the motor is far from the microtubule when the cargo returns to the trap center. Our measurements find the rebinding time increase with viscosity, indicating that the motor is often not next to the microtubule when the cargo returns to trap center.

Since we have data at a variety of conditions, we have power to fit models of cargo and motor properties. We find that motor on rate needs to be significantly higher in these models than the on rate predicted from studies of two motor run lengths. This is a mystery: why would it be so different? It can be hypothesized that the underlying dynamics of how motor binding occurs is influenced by the presence of the other motor nearby in some way. Unfortunately such a hypothesis is difficult to investigate experimentally.

How might the process of rebinding be different in a different environment? In cells, cargo rotational and translational dynamics are likely different than the dynamics of a bead near a glass surface. Additionally, we expect multiple motors on cargos. Therefore rebinding times are potentially dominated by other factors entirely. Likewise, motor on rates are modulated by the presence of other molecules, both interacting directly with the motors (e.g. dynactin with dynein), transiently with the motors (e.g. tau with kinesin), or indirectly (e.g. by crowding influencing motor head movement).

6.2 Towards bridging in vitro measurements into insights inside the cell

Research on the projects in this dissertation made it clear that while a great deal is known about molecular motors, especially kinesin-1, there remains a great gap between that knowledge and understanding of dynamics of cargos in cells. What would it take to be able to construct a predictive model of a particular cellular cargo? Here I give a set of things which would go a long way toward this goal.

Measurements of total numbers of motors on cargos

It is possible to measure forces and run lengths of cargos in cells. There is a good deal of ambiguity in how these measurements can be interpreted, however. Do motors function significantly differently in cells than they do in vitro? There is reason to think so. Cargos are observed to move significantly faster in cells than single motors do in vitro. If this motion is fully driven by motors, there is no known mechanism through which this could happen [186, 187]. Dynein is known to interact with many other proteins such as bicD and dynactin which alter its function [188]. How do we disentangle unknowns about motors from potential interactions of motors with other things in the cell? One way is to build models which reflect individual cargos observed in cells and see if these models give outputs which reflect the observed motion. If total motor numbers on cargos were better known, it would be a great stride toward being able to match models of cargos with their behavior in cells. Advances in super-resolution microscopy have made it possible to do inference on this, at least in theory. Challenges of incomplete labeling mean there are yet hurdles to be overcome.

Rotational microrheology of spherical particles

The studies in this dissertation have made it clear that cargo rotation is important for transport outcomes. However, rotation is difficult to study in the way that translation

of cargos normally is — by using spherical beads. Rotational microrheology has been performed on elongated structures in cells, but the complexity of the material properties of the cytoplasm makes it difficult to understand how these measurements might translate to a spherical organelle like a lipid droplet or organelles of other shapes. The ability to monitor the rotational orientation of a spherical particle in the cell as it moves would allow better understanding of how cargos move rotationally. Even better would be a set of measurements made on cargos which are not near microtubules. Because motors and other proteins on the cargo may bind the microtubule, without this it is difficult to say what movements are caused by these molecules and which are not. Since these are separate inputs in the model, having a measurement which acts as an input (not near microtubule), and one to match as an output (near microtubule) would be very useful.

Simulation methods for expanded rheologies

While simulation methods for diffusion in a Newtonian fluid are well established, simulating diffusion in non-newtonian fluids is more difficult. Methods must take into account past positions of the cargo. A method for simulating diffusion in a environment with inputs of e.g. g' and g'' would allow simulations to be targeted towards cargos for which detailed microrheology has been measured.

Cargo shape and size distributions

The next frontier of cargo simulation would be to use explicit fluid dynamics techniques to understand how cargos push the fluid around them and are pushed by it. With powerful simulations which take into account the fluid properties and shapes of cargos, it would become useful to know the sizes and shaped of various cargos in cells.

Simultaneous dynamic microtubule and cargo localization

The microtubule intersections project made it clear that the geometry of microtubules in the cell influences transport outcomes. The opposite is also true: cargos move

microtubules around in the cell [62]. Understanding how cargos and microtubules interact in real time through an intersection crossing would give insight into both cargo dynamics and the dynamics of the microtubule cytoskeleton.

Models for stepping, off rates, on rates of dynein and other motors

Having models of the dynamics of these other motors could allow more direct comparison between model predictions and a variety of measurements made on cargos in cells. Run lengths, for example, are much different for cargos in cells than would be expected from multiple motor forces they generate [102]. Models incorporating both kinds of motors have been shown in the past to not be able to explain run length distributions [42]. Much has been learned about motor function in the time since which might allow greater insight into the dynamics which are being influenced.

What would be possible with a predictive model of motors, cargo and environment? One of the enduring questions about cargo transport is that of what activity is a product of the stochasticity of binding and unbinding of the motors, and what behaviors are the result of regulation of motor activity by other molecules. A predictive model of a cargo with matching numbers of motors and environment would allow the identification of regulated behavior. Since regulation requires systems to be set up and maintained by the cell, regulated behaviors are clues to what the cell is trying to accomplish, and how it is going about it.

6.3 Science this work enables

Since most of the work in this dissertation attempts to understand how the components of a system interact outside of their native context, direct takeaways for particular functions in particular cells are difficult to draw out. The contribution is instead a more solid foundation from which studies on such particular cargos can draw. This foundation is important because

it enables more informed questions to be asked. For example, let us take the cases of the cargos mentioned in the introduction.

For packages of receptors in neurons, delivery to the correct synaptic boutons is necessary for learning and memory. An obvious question is: how do cargos decide where to stop? The foundation of work on molecular motors in vitro and models suggests several ways. First, neurons could decorate microtubules near the bouton with a molecule that increases the off rate of motors, such as tau. Detached cargos of neurotransmitters would then potentially be able to diffuse in to the body of the bouton. Microtubule or actin tracks could also be present at the locations of boutons. Then delivery to a specific bouton becomes a matter of switching tracks. Track geometry could be altered to change routing, as seen in the work on intersections. Myosin motors could be attached to or activated on cargos, causing them to move onto actin tracks.

For the mitochondria and lipid droplets which change localization in the starving cells, one might ask how the cells direct these cargos in any of the scenarios. To localize cargos near the periphery or the nucleus, causing one motor team to dominate movement is sufficient to attain this general organization, because microtubule networks in these cells generally have their plus ends pointed out. To get the organelles to find each other, cells have several options. They could activate both motor teams, causing the cargos to move bidirectionally until a mitochondria and lipid droplet meet. Cells could also rearrange their microtubules such that cargos explore the network. This could even be accomplished with only one set of motors activated. Again, both sets of organelles would explore until they met each other. This raises yet another question: what causes them to stop? Are there molecules on the surface of each which bind them together strongly enough that the motor teams are too weak to pull them apart? Are the motors deactivated, or encouraged to fall off the microtubules?

Bibliography

- [1] Z. Zhang, Y. Nishimura, and P. Kanchanawong, “Extracting Microtubule Networks from Superresolution Single-Molecule Localization Microscopy Data”, *Molecular Biology of the Cell* **28**, 333–345 (2016).
- [2] M. J. Bovyn, “Geometry Matters for Cargos Navigating 3D Microtubule Intersections”, Master’s Thesis (University of California, Irvine, 2019), p. 79.
- [3] J. P. Bergman, M. J. Bovyn, et al., “Cargo navigation across 3D microtubule intersections.”, *Proceedings of the National Academy of Sciences* **115**, 537–542 (2018).
- [4] M. Bovyn, B. Reddy, S. Gross, and J. Allard, “Diffusion of kinesin motors on cargo can enhance binding and run lengths during intracellular transport”, *Molecular Biology of the Cell*, mbc.E20–10–0658 (2021).
- [5] M. J. Bovyn, B. J. N. Reddy, S. P. Gross, and J. F. Allard, “Roles of motor on-rate and cargo mobility in intracellular transport”, *bioRxiv*, 2020.07.13.201434 (2020).
- [6] D. E. Chapman, B. J. N. Reddy, et al., “Regulation of in vivo dynein force production by CDK5 and 14-3-3 ϵ and KIAA0528”, *Nature Communications* **10**, 228 (2019).
- [7] W. M. Grundy, S. J. Morrison, M. J. Bovyn, S. C. Tegler, and D. M. Cornelison, “Remote sensing D/H ratios in methane ice: Temperature-dependent absorption coefficients of CH₃D in methane ice and in nitrogen ice”, *Icarus* **212**, 941–949 (2011).
- [8] S. C. Tegler, D. M. Cornelison, et al., “Methane and nitrogen abundances on Pluto and Eris”, *The Astrophysical Journal* **725**, 1296–1305 (2010).
- [9] C. R. Woese, O. Kandler, and M. L. Wheelis, “Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya”, *Proceedings of the National Academy of Sciences of the United States of America* **87**, 4576–4579 (1990).
- [10] J. B. Dacks and M. C. Field, “Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode”, *Journal of Cell Science* **120**, 2977–2985 (2007).
- [11] A. M. Valm, S. Cohen, et al., “Applying systems-level spectral imaging and analysis to reveal the organelle interactome”, *Nature* **546**, 162–167 (2017).
- [12] J. Lutkenhaus, “Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring.”, in, *Annual review of biochemistry* **76**, 539–62 (2007).
- [13] C. P. Brangwynne, C. R. Eckmann, et al., “Germline P granules are liquid droplets that localize by controlled dissolution/condensation”, *Science* **324**, 1729–1732 (2009).

- [14] F. J. Hoernkli, A. J. Kallarackal, and A. V. Maricq, “Mobile AMPARs are required for synaptic plasticity”, *Channels* **9**, 230–232 (2015).
- [15] A. Herms, M. Bosch, et al., “AMPK activation promotes lipid droplet dispersion on detyrosinated microtubules to increase mitochondrial fatty acid oxidation”, *Nature Communications* **6**, 7176 (2015).
- [16] R. D. Allen, N. S. Allen, and J. L. Travis, “Video-enhanced contrast, differential interference contrast (AVEC-DIC) microscopy: A new method capable of analyzing microtubule-related motility in the reticulopodial network of *allogromia laticollaris*”, *Cell Motility* **1**, 291–302 (1981).
- [17] S Inoué, “Video image processing greatly enhances contrast, quality, and speed in polarization-based microscopy”, *The Journal of Cell Biology* **89**, 346–56 (1981).
- [18] R. D. Vale, T. S. Reese, and M. P. Sheetz, “Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility.”, *Cell* **42**, 39–50 (1985).
- [19] J. Howard and A. A. Hyman, “Dynamics and mechanics of the microtubule plus end”, *Nature* **422**, 753–758 (2003).
- [20] X. Zhu, R. Hu, et al., “Microtubules Negatively Regulate Insulin Secretion in Pancreatic β Cells”, *Developmental Cell* **34**, 656–668 (2015).
- [21] A. V. Grimstone and A Klug, “Observations on the substructure of flagellar fibres”, *Journal of Cell Science* **1**, 351–362 (1966).
- [22] G. B. Pierson, P. R. Burton, and R. H. Himes, “Alterations in number of protofilaments in microtubules assembled in vitro.”, *The Journal of Cell Biology* **76** (1978).
- [23] C. Davis and K. Gull, “Protofilament Number in Microtubules in Cells of Two Parasitic Nematodes”, *The Journal of Parasitology* **69**, 1094 (1983).
- [24] W. H. Liang, Q. Li, et al., “Microtubule Defects Influence Kinesin-Based Transport in Vitro”, *Biophysical Journal* **110**, 2229–2240 (2016).
- [25] L. Schaedel, K. John, et al., “Microtubules self-repair in response to mechanical stress”, *Nature Materials* **14**, 1156–1163 (2015).
- [26] K. J. Verhey, N. Kaul, and V. Soppina, “Kinesin Assembly and Movement in Cells”, *Annual Review of Biophysics* **40**, 267–288 (2011).
- [27] C. J. Lawrence, R. K. Dawe, et al., “A standardized kinesin nomenclature”, *The Journal of Cell Biology* **167**, 19–22 (2004).
- [28] N. Hirokawa, Y. Noda, Y. Tanaka, and S. Niwa, “Kinesin superfamily motor proteins and intracellular transport”, *Nature Reviews Molecular Cell Biology* **10**, 682–696 (2009).
- [29] J. O. Andreasson, S. Shastry, W. O. Hancock, and S. M. Block, “The mechanochemical cycle of mammalian kinesin-2 KIF3A/B under load”, *Current Biology* **25**, 1166–1175 (2015).
- [30] M. Brunnbauer, R. Dombi, et al., “Torque Generation of Kinesin Motors Is Governed by the Stability of the Neck Domain”, *Molecular Cell* **46**, 147–158 (2012).

- [31] S. Ray, E. Meyhöfer, R. A. Milligan, and J. Howard, “Kinesin follows the microtubule’s protofilament axis”, *The Journal of Cell Biology* **121**, 1083–1093 (1993).
- [32] N Siddiqui and A Straube, “Intracellular cargo transport by kinesin-3 motors”, *Biochemistry (Moscow)* **82**, 803–815 (2017).
- [33] A. Yildiz, M. Tomishige, R. D. Vale, and P. R. Selvin, “Kinesin walks hand-over-hand.”, *Science* **303**, 676–8 (2004).
- [34] K. Svoboda, C. F. Schmidt, B. J. Schnapp, and S. M. Block, “Direct observation of kinesin stepping by optical trapping interferometry”, *Nature* **365**, 721–727 (1993).
- [35] N. J. Carter and R. A. Cross, “Mechanics of the kinesin step.”, *Nature* **435**, 308–12 (2005).
- [36] A. Kunwar and A. Mogilner, “Robust transport by multiple motors with nonlinear force-velocity relations and stochastic load sharing.”, *Physical Biology* **7**, 16012 (2010).
- [37] K Visscher, M. J. Schnitzer, and S. M. Block, “Single kinesin molecules studied with a molecular force clamp.”, *Nature* **400**, 184–9 (1999).
- [38] T. L. Fallesen, J. C. MacOsco, and G. Holzwarth, “Force-velocity relationship for multiple kinesin motors pulling a magnetic bead”, *European Biophysics Journal* **40**, 1071–1079 (2011).
- [39] A. K. Rai, A. Rai, A. J. Ramaiya, R. Jha, and R. Mallik, “Molecular adaptations allow dynein to generate large collective forces inside cells”, *Cell* **152**, 172–182 (2013).
- [40] K. Svoboda and S. M. Block, “Force and velocity measured for single kinesin molecules”, *Cell* **77**, 773–784 (1994).
- [41] J. O. Andreasson, B. Milic, et al., “Examining kinesin processivity within a general gating framework”, *eLife* **2015**, 1–44 (2015).
- [42] A. Kunwar, S. K. Tripathy, et al., “Mechanical stochastic tug-of-war models cannot explain bidirectional lipid-droplet transport”, *Proceedings of the National Academy of Sciences* **108**, 18960–18965 (2011).
- [43] B. Milic, J. O. L. Andreasson, W. O. Hancock, and S. M. Block, “Kinesin processivity is gated by phosphate release”, *Proceedings of the National Academy of Sciences* **111**, 14136–14140 (2014).
- [44] S. M. Block, C. L. Asbury, J. W. Shaevitz, and M. J. Lang, “Probing the kinesin reaction cycle with a 2D optical force clamp”, *Proceedings of the National Academy of Sciences* **100**, 2351–2356 (2003).
- [45] H Kojima, E Muto, H Higuchi, and T Yanagida, “Mechanics of single kinesin molecules measured by optical trapping nanometry”, *Biophysical Journal* **73**, 2012–2022 (1997).
- [46] S. Jeney, E. H. K. Stelzer, H. Grubmüller, and E.-L. Florin, “Mechanical Properties of Single Motor Molecules Studied by Three-Dimensional Thermal Force Probing in Optical Tweezers”, *ChemPhysChem* **5**, 1150–1158 (2004).
- [47] A. J. Hunt and J Howard, “Kinesin swivels to permit microtubule movement in any direction.”, *Proceedings of the National Academy of Sciences* **90**, 11653–7 (1993).

- [48] B. Gutiérrez-Medina, A. N. Fehr, and S. M. Block, “Direct measurements of kinesin torsional properties reveal flexible domains and occasional stalk reversals during stepping”, *Proceedings of the National Academy of Sciences* **106**, 17007–17012 (2009).
- [49] W. O. Hancock, “Bidirectional cargo transport: moving beyond tug of war”, *Nature Reviews Molecular Cell Biology* **15**, 615–628 (2014).
- [50] S. P. Gross, “Hither and yon: a review of bi-directional microtubule-based transport.”, *Physical Biology* **1**, R1–R11 (2004).
- [51] C. I. Maeder, K. Shen, and C. C. Hoogenraad, “Axon and dendritic trafficking”, *Current Opinion in Neurobiology* **27**, 165–170 (2014).
- [52] S. Klumpp and R. Lipowsky, “Cooperative cargo transport by several molecular motors.”, *Proceedings of the National Academy of Sciences* **102**, 17284–9 (2005).
- [53] M. J. I. Müller, S. Klumpp, and R. Lipowsky, “Tug-of-war as a cooperative mechanism for bidirectional cargo transport by molecular motors.”, *Proceedings of the National Academy of Sciences* **105**, 4609–4614 (2008).
- [54] A. Kunwar, M. Vershinin, J. Xu, and S. P. Gross, “Stepping, Strain Gating, and an Unexpected Force-Velocity Curve for Multiple-Motor-Based Transport”, *Current Biology* **18**, 1173–1183 (2008).
- [55] C. B. Korn, S. Klumpp, R. Lipowsky, and U. S. Schwarz, “Stochastic simulations of cargo transport by processive molecular motors”, *Journal of Chemical Physics* **131**, 10.1063/1.3279305 (2009).
- [56] R. P. Erickson, Z. Jia, S. P. Gross, and C. C. Yu, “How Molecular Motors Are Arranged on a Cargo Is Important for Vesicular Transport”, *PLoS Computational Biology* **7**, edited by A. R. Bausch, e1002032 (2011).
- [57] A. T. Lombardo, S. R. Nelson, et al., “Myosin Va molecular motors manoeuvre liposome cargo through suspended actin filament intersections in vitro”, *Nature Communications* **8**, 15692 (2017).
- [58] R. P. Erickson, S. P. Gross, and C. C. Yu, “Filament-Filament Switching Can Be Regulated by Separation Between Filaments Together with Cargo Motor Number”, *PLoS ONE* **8**, edited by A. J. Kabla, e54298 (2013).
- [59] S. Schnorrenberg, T. Grotjohann, et al., “In vivo super-resolution RESOLFT microscopy of drosophila melanogaster”, *eLife* **5**, 1–11 (2016).
- [60] B. Dong, X. Yang, S. Zhu, D. C. Bassham, and N. Fang, “Stochastic Optical Reconstruction Microscopy Imaging of Microtubule Arrays in Intact Arabidopsis thaliana Seedling Roots”, *Scientific Reports* **5**, 15694 (2015).
- [61] B. Huang, W. Wang, M. Bates, and X. Zhuang, “Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy.”, *Science* **319**, 810–3 (2008).
- [62] O. Osunbayo, J. Butterfield, et al., “Cargo transport at microtubule crossings: Evidence for prolonged tug-of-war between kinesin motors”, *Biophysical Journal* **108**, 1480–1483 (2015).

- [63] Š. Bálint, I. Verdeny Vilanova, Á. Sandoval Álvarez, and M. Lakadamyali, “Correlative live-cell and superresolution microscopy reveals cargo transport dynamics at microtubule intersections.”, *Proceedings of the National Academy of Sciences* **110**, 3375–80 (2013).
- [64] J. L. Ross, H. Shuman, E. L. F. Holzbaur, and Y. E. Goldman, “Kinesin and dynein-dynactin at intersecting microtubules: motor density affects dynein function”, *Biophysical Journal* **94**, 3115–25 (2008).
- [65] I. Verdeny-Vilanova, F. Wehnekamp, et al., “3D motion of vesicles along microtubules helps them to circumvent obstacles in cells”, *Journal of Cell Science* **130**, 1904–1916 (2017).
- [66] E. Y. Van Battum, S. Brignani, and R. J. Pasterkamp, “Axon guidance proteins in neurological disorders”, *The Lancet Neurology* **14**, 532–546 (2015).
- [67] A. Di Polo, “Dendrite pathology and neurodegeneration: Focus on mTOR”, *Neural Regeneration Research* **10**, 559–561 (2015).
- [68] A. L. Parker, M. Kavallaris, and J. A. McCarroll, “Microtubules and Their Role in Cellular Stress in Cancer”, *Frontiers in Oncology* **4**, 1–19 (2014).
- [69] C. M. Galmarini, K. Kamath, et al., “Drug resistance associated with loss of p53 involves extensive alterations in microtubule composition and dynamics”, *British Journal of Cancer* **88**, 1793–1799 (2003).
- [70] G. Fink, L. Hajdo, et al., “The mitotic kinesin-14 Ncd drives directional microtubule–microtubule sliding”, *Nature Cell Biology* **11**, 717–723 (2009).
- [71] R. Subramanian, E. M. Wilson-Kubalek, et al., “Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein”, *Cell* **142**, 433–443 (2010).
- [72] K. Kalil and E. W. Dent, “Branch management: mechanisms of axon branching in the developing vertebrate CNS”, *Nature Reviews Neuroscience* **15**, 7–18 (2013).
- [73] S. Petry, A. C. Groen, K. Ishihara, T. J. Mitchison, and R. D. Vale, “Branching microtubule nucleation in xenopus egg extracts mediated by augmin and TPX2”, *Cell* **152**, 768–777 (2013).
- [74] C. Dumontet, G. E. Durán, et al., “Differential expression of tubulin isotypes during the cell cycle”, *Cell Motility and the Cytoskeleton* **35**, 49–58 (1996).
- [75] A. Desai and T. J. Mitchison, “Microtubule Polymerization Dynamics”, *Annual Review of Cell and Developmental Biology* **13**, 83–117 (1997).
- [76] N. Hirokawa, Y. Shiomura, and S. Okabe, “Tau proteins: the molecular structure and mode of binding on microtubules.”, *The Journal of Cell Biology* **107** (1988).
- [77] M. A. Chesarone, A. G. DuPage, and B. L. Goode, “Unleashing formins to remodel the actin and microtubule cytoskeletons”, *Nature Reviews Molecular Cell Biology* **11**, 62–74 (2010).

- [78] K. G. Young, S. F. Thurston, S. Copeland, C. Smallwood, and J. W. Copeland, “INF1 Is a Novel Microtubule-associated Formin”, *Molecular Biology of the Cell* **19**, 5168–5180 (2008).
- [79] J. M. Gomez, L. Chumakova, N. A. Bulgakova, and N. H. Brown, “Microtubule organization is determined by the shape of epithelial cells”, *Nature Communications* **7**, 13172 (2016).
- [80] O. Hamant, “Cell biology: Cytoskeleton network topology feeds back on its regulation”, *Current Biology* **23**, R963–R965 (2013).
- [81] M. Vershinin, B. C. Carter, D. S. Razafsky, S. J. King, and S. P. Gross, “Multiple-motor based transport and its regulation by Tau.”, *Proceedings of the National Academy of Sciences* **104**, 87–92 (2007).
- [82] J. Bergman, O. Osunbayo, and M. Vershinin, “Constructing 3D microtubule networks using holographic optical trapping”, *Scientific Reports* **5**, 18085 (2015).
- [83] A Ashkin, K Schütze, J. M. Dziedzic, U Euteneuer, and M Schliwa, “Force generation of organelle transport measured in vivo by an infrared laser trap.”, *Nature* **348**, 346–8 (1990).
- [84] S. P. Gross, M. A. Welte, S. M. Block, and E. F. Wieschaus, “Coordination of opposite-polarity microtubule motors”, *The Journal of Cell Biology* **156**, 715–724 (2002).
- [85] R. J. McKenney, M. Vershinin, A. Kunwar, R. B. Vallee, and S. P. Gross, “LIS1 and NudE induce a persistent dynein force-producing state”, *Cell* **141**, 304–314 (2010).
- [86] Y. K. Nahmias and D. J. Odde, “Analysis of radiation forces in laser trapping and laser-guided direct writing applications”, *IEEE Journal of Quantum Electronics* **38**, 131–141 (2002).
- [87] D. K. Jamison, J. W. Driver, A. R. Rogers, P. E. Constantinou, and M. R. Diehl, “Two kinesins transport cargo primarily via the action of one motor: Implications for intracellular transport”, *Biophysical Journal* **99**, 2967–2977 (2010).
- [88] M. F. Juetten, T. J. Gould, et al., “Three-dimensional sub-100 nm resolution fluorescence microscopy of thick samples”, *Nature Methods* **5**, 527–529 (2008).
- [89] M. J. Mlodzianoski, M. F. Juetten, G. L. Beane, and J Bewersdorf, “Experimental characterization of 3D localization techniques for particle-tracking and super-resolution microscopy”, *Optics Express* **17**, 8264–8277 (2009).
- [90] N. Hirokawa, K. K. Pfister, et al., “Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration”, *Cell* **56**, 867–878 (1989).
- [91] J. M. Scholey, J. Heuser, J. T. Yang, and L. S. B. Goldstein, “Identification of globular mechanochemical heads of kinesin”, *Nature* **338**, 355–357 (1989).
- [92] C. Leduc, O. Campas, et al., “Cooperative extraction of membrane nanotubes by molecular motors”, *Proceedings of the National Academy of Sciences* **101**, 17096–17101 (2004).

- [93] S. M. Block, L. S. B. Goldstein, and B. J. Schnapp, “Bead movement by single kinesin molecules studied with optical tweezers”, *Nature* **348**, 348–352 (1990).
- [94] Q. Li, S. J. King, A. Gopinathan, and J. Xu, “Quantitative Determination of the Probability of Multiple-Motor Transport in Bead-Based Assays”, *Biophysical Journal* **110**, 2720–2728 (2016).
- [95] M. J. Schnitzer, K. Visscher, and S. M. Block, “Force production by single kinesin motors.”, *Nature Cell Biology* **2**, 718–23 (2000).
- [96] S. Keller, K. Berghoff, and H. Kress, “Phagosomal transport depends strongly on phagosome size”, *Scientific Reports* **7**, 1–15 (2017).
- [97] A. Rai, D. Pathak, et al., “Dynein Clusters into Lipid Microdomains on Phagosomes to Drive Rapid Transport toward Lysosomes”, *Cell* **164**, 722–734 (2016).
- [98] B. J. N. Reddy, M. Mattson, et al., “Load-induced enhancement of Dynein force production by LIS1–NudE in vivo and in vitro”, *Nature Communications* **7**, 1–14 (2016).
- [99] R. Milo, P. Jorgensen, U. Moran, G. Weber, and M. Springer, “BioNumbers—the database of key numbers in molecular and cell biology”, *Nucleic Acids Research* **38**, 750–753 (2010).
- [100] A. G. Hendricks, E. Perlson, et al., “Motor Coordination via a Tug-of-War Mechanism Drives Bidirectional Vesicle Transport”, *Current Biology*, 10.1016/j.cub.2010.02.058 (2010).
- [101] A. R. Chaudhary, F. Berger, C. L. Berger, and A. G. Hendricks, “Tau directs intracellular trafficking by regulating the forces exerted by kinesin and dynein teams”, *Traffic* **19**, 111–121 (2018).
- [102] G. T. Shubeita, S. L. Tran, et al., “Consequences of Motor Copy Number on the Intracellular Transport of Kinesin-1-Driven Lipid Droplets”, *Cell* **135**, 1098–1107 (2008).
- [103] S. R. Nelson, K. M. Trybus, and D. M. Warshaw, “Motor coupling through lipid membranes enhances transport velocities for ensembles of myosin Va”, *Proceedings of the National Academy of Sciences* **111**, E3986–E3995 (2014).
- [104] R. Grover, J. Fischer, et al., “Transport efficiency of membrane-anchored kinesin-1 motors depends on motor density and diffusivity.”, *Proceedings of the National Academy of Sciences* **113**, E7185–E7193 (2016).
- [105] Q. Li, K.-F. Tseng, S. J. King, W. Qiu, and J. Xu, “A fluid membrane enhances the velocity of cargo transport by small teams of kinesin-1”, *The Journal of Chemical Physics* **148**, 123318 (2018).
- [106] P. D. Chowdary, L. Kaplan, D. L. Che, and B. Cui, “Dynamic Clustering of Dyneins on Axonal Endosomes: Evidence from High-Speed Darkfield Imaging”, *Biophysical Journal* **115**, 230–241 (2018).
- [107] T. Sumi, “Design principles governing chemomechanical coupling of kinesin”, *Scientific Reports* **7**, 1163 (2017).

- [108] M. Guo, A. J. Ehrlicher, et al., “Probing the stochastic, motor-driven properties of the cytoplasm using force spectrum microscopy”, *Cell* **158**, 822–832 (2014).
- [109] W. W. Ahmed, É. Fodor, et al., “Active Mechanics Reveal Molecular-Scale Force Kinetics in Living Oocytes”, *Biophysical Journal* **114**, 1667–1679 (2018).
- [110] A. Kusumi, T. A. Tsunoyama, K. M. Hirose, R. S. Kasai, and T. K. Fujiwara, “Tracking single molecules at work in living cells”, *Nature Chemical Biology* **10**, 524–532 (2014).
- [111] P. Sanghavi, A. D’Souza, et al., “Coin Tossing Explains the Activity of Opposing Microtubule Motors on Phagosomes”, *Current Biology* **28**, 1460–1466.e4 (2018).
- [112] A. Lüdecke, A. M. Seidel, M. Braun, Z. Lansky, and S. Diez, “Diffusive tail anchorage determines velocity and force produced by kinesin-14 between crosslinked microtubules”, *Nature Communications* **9**, 10.1038/s41467-018-04656-0 (2018).
- [113] J. Neefjes, M. M. Jongsma, and I. Berlin, “Stop or Go? Endosome Positioning in the Establishment of Compartment Architecture, Dynamics, and Function”, *Trends in Cell Biology* **27**, 580–594 (2017).
- [114] D. Pathak and R. Mallik, “Lipid - Motor Interactions: Soap Opera or Symphony?”, *Current Opinion in Cell Biology* **44**, 79–85 (2017).
- [115] R. Jiang, S. Vandal, et al., “Microtubule binding kinetics of membrane-bound kinesin-1 predicts high motor copy numbers on intracellular cargo”, *Proceedings of the National Academy of Sciences of the United States of America* **116**, 26564–26570 (2019).
- [116] B. G. Wilhelm, S. Mandad, et al., “Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins”, *Science* **344**, 1023–1028 (2014).
- [117] M. J. Saxton and K. Jacobson, “Single-particle tracking: Applications to membrane dynamics”, *Annual Review of Biophysics and Biomolecular Structure* **26**, 373–399 (1997).
- [118] J. Pu, C. M. Guardia, T. Keren-Kaplan, and J. S. Bonifacino, “Mechanisms and functions of lysosome positioning”, *Journal of Cell Science* **129**, 4329–4339 (2016).
- [119] M. D. Kilwein and M. A. Welte, “Lipid Droplet Motility and Organelle Contacts”, *Contact* **2**, 251525641989568 (2019).
- [120] F. Quemeneur, J. K. Sigurdsson, et al., “Shape matters in protein mobility within membranes”, *Proceedings of the National Academy of Sciences* **111**, 5083–5087 (2014).
- [121] B. D. Slaughter, J. R. Unruh, et al., “Non-uniform membrane diffusion enables steady-state cell polarization via vesicular trafficking”, *Nature Communications* **4**, 1380 (2013).
- [122] P. A. Sims and X. S. Xie, “Probing dynein and kinesin stepping with mechanical manipulation in a living cell”, *ChemPhysChem* **10**, 1511–1516 (2009).
- [123] V. Soppina, A. K. Rai, A. J. Ramaiya, P. Barak, and R. Mallik, “Tug-of-war between dissimilar teams of microtubule motors regulates transport and fission of endosomes”, *Proceedings of the National Academy of Sciences of the United States of America* **106**, 19381–19386 (2009).

- [124] C. Leidel, R. a. Longoria, F. M. Gutierrez, and G. T. Shubeita, “Measuring molecular motor forces in VIVO: Implications for tug-of-war models of bidirectional transport”, *Biophysical Journal* **103**, 492–500 (2012).
- [125] A. G. Hendricks, E. L. F. Holzbaur, and Y. E. Goldman, “Force measurements on cargoes in living cells reveal collective dynamics of microtubule motors”, *Proceedings of the National Academy of Sciences*, 10.1073/pnas.1215462109 (2012).
- [126] A. R. Chaudhary, H. Lu, et al., “MAP7 regulates organelle transport by recruiting kinesin-1 to microtubules”, *Journal of Biological Chemistry* **294**, 10160–10171 (2019).
- [127] F. Cella Zanacchi, C. Manzo, R. Magrassi, N. D. Derr, and M. Lakadamyali, “Quantifying Protein Copy Number in Super Resolution Using an Imaging-Invariant Calibration”, *Biophysical Journal* **116**, 2195–2203 (2019).
- [128] B. Y. Monroy, D. L. Sawyer, et al., “Competition between microtubule-associated proteins directs motor transport”, *Nature Communications* **9**, 1–12 (2018).
- [129] P. J. Hooikaas, M. Martin, et al., “MAP7 family proteins regulate kinesin-1 recruitment and activation”, *Journal of Cell Biology* **218**, 1298–1318 (2019).
- [130] D. Ando, N. Korabel, K. C. Huang, and A. Gopinathan, “Cytoskeletal Network Morphology Regulates Intracellular Transport Dynamics”, *Biophysical Journal* **109**, 1574–1582 (2015).
- [131] M.-V. Ciocanel, B. Sandstede, S. P. Jeschonek, and K. L. Mowry, “Modeling Microtubule-Based Transport and Anchoring of mRNA”, *SIAM Journal on Applied Dynamical Systems*, 10.1137/18M1186083 (2018).
- [132] A. R. Driller-Colangelo, K. W. Chau, J. M. Morgan, and N. D. Derr, “Cargo rigidity affects the sensitivity of dynein ensembles to individual motor pausing”, *Cytoskeleton* **73**, 693–702 (2016).
- [133] R. Dixit, J. L. Ross, Y. E. Goldman, and E. L. F. Holzbaur, “Differential regulation of dynein and kinesin motor proteins by tau”, *Science* **319**, 1086–9 (2008).
- [134] S. Yamada, D. Wirtz, and S. C. Kuo, “Mechanics of living cells measured by laser tracking microrheology”, *Biophysical Journal* **78**, 1736–1747 (2000).
- [135] C. Wilhelm, F. Gazeau, and J. C. Bacri, “Rotational magnetic endosome microrheology: Viscoelastic architecture inside living cells”, *Physical Review E* **67**, 12 (2003).
- [136] D. Coombs, R. Straube, and M. Ward, “Diffusion on a Sphere with Localized Traps: Mean First Passage Time, Eigenvalue Asymptotics, and Fekete Points”, in *SIAM Journal on Applied Mathematics* **70**, 302–332 (2009).
- [137] P. G. Saffman and M. Delbrück, “Brownian motion in biological membranes.”, *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3111–3 (1975).
- [138] A. Naji, A. J. Levine, and P. A. Pincus, “Corrections to the Saffman-Delbrück mobility for membrane bound proteins”, *Biophysical Journal* **93**, L49–L51 (2007).
- [139] E. P. Petrov and P. Schville, “Translational Diffusion in Lipid Membranes beyond the Saffman-Delbrück Approximation”, *Biophysical Journal* **94**, L41–L43 (2008).

- [140] R. Graham, B. Lubachevsky, K. Nurmela, and P. Östergård, “Dense packings of congruent circles in a circle”, *Discrete Mathematics* **181**, 139–154 (1998).
- [141] Y. Noda, R. Sato-Yoshitake, S. Kondo, M. Nangaku, and N. Hirokawa, “KIF2 is a new microtubule-based anterograde motor that transports membranous organelles distinct from those carried by kinesin heavy chain or KIF3A/B”, *Journal of Cell Biology* **129**, 157–167 (1995).
- [142] C. M. Coppin, D. W. Pierce, L. Hsu, and R. D. Vale, “The load dependence of kinesin’s mechanical cycle”, *Proc. Natl. Acad. Sci. USA* **94**, 8539–8544 (1997).
- [143] R. H. Miller and R. I. Lasek, “Cross-bridges mediate anterograde and retrograde vesicle transport along microtubules in squid axoplasm”, *Journal of Cell Biology* **101**, 2181–2193 (1985).
- [144] N. Hirokawa, “From electron microscopy to molecular cell biology, molecular genetics and structural biology: Intracellular transport and kinesin superfamily proteins, KIFs: Genes, structure, dynamics and functions”, *Journal of Electron Microscopy* **60**, 63–92 (2011).
- [145] G. M. Jeppesen and J. K. Heinrich Hoerber, “The mechanical properties of kinesin-1: A holistic approach”, *Biochemical Society Transactions* **40**, 438–443 (2012).
- [146] Q. Feng, K. J. Mickolajczyk, G. Y. Chen, and W. O. Hancock, “Motor Reattachment Kinetics Play a Dominant Role in Multimotor-Driven Cargo Transport”, *Biophysical Journal* **114**, 400–409 (2018).
- [147] N. Kaul, V. Soppina, and K. J. Verhey, “Effects of α -tubulin K40 acetylation and detyrosination on kinesin-1 motility in a purified system”, *Biophysical Journal* **106**, 2636–2643 (2014).
- [148] J. Hu, S. Jafari, et al., “Size- and speed-dependent mechanical behavior in living mammalian cytoplasm”, *Proceedings of the National Academy of Sciences* **114**, 9529–9534 (2017).
- [149] W. Xu, E. Alizadeh, and A. Prasad, “Force Spectrum Microscopy Using Mitochondrial Fluctuations of Control and ATP-Depleted Cells”, *Biophysical Journal* **114**, 2933–2944 (2018).
- [150] E. F. Koslover, C. K. Chan, and J. A. Theriot, “Cytoplasmic Flow and Mixing Due to Deformation of Motile Cells”, *Biophysical Journal* **113**, 2077–2087 (2017).
- [151] R. Metzler, “Gaussianity Fair: The Riddle of Anomalous yet Non-Gaussian Diffusion”, *Biophysical Journal* **112**, 413–415 (2017).
- [152] L. Selvaggi, M. Salemm, et al., “Multiple-Particle-Tracking to investigate viscoelastic properties in living cells”, *Methods* **51**, 20–26 (2010).
- [153] P. A. Valberg and D. F. Albertini, “Cytoplasmic motions, rheology, and structure probed by a novel magnetic particle method”, *Journal of Cell Biology* **101**, 130–140 (1985).
- [154] F. Crick and A. Hughes, “The physical properties of cytoplasm: A study by means of the magnetic particle method Part I. Experimental”, *Experimental Cell Research* **1**, 37–80 (1950).

- [155] L. Chevy, R. Colin, B. Abou, and J.-F. Berret, “Intracellular micro-rheology probed by micron-sized wires”, *Biomaterials* **34**, 6299–6305 (2013).
- [156] J. F. Berret, “Local viscoelasticity of living cells measured by rotational magnetic spectroscopy”, *Nature Communications* **7**, 1–9 (2016).
- [157] W. Möller, S. Takenaka, M. Rust, W. Stahlfhofen, and J. Heyder, “Probing mechanical properties of living cells by magnetopneumography”, *Journal of Aerosol Medicine: Deposition, Clearance, and Effects in the Lung* **10**, 173–186 (1997).
- [158] A. Ramaiya, B. Roy, M. Bugiel, and E. Schäffer, “Kinesin rotates unidirectionally and generates torque while walking on microtubules”, *Proceedings of the National Academy of Sciences* **114**, 10894–10899 (2017).
- [159] S. Zhang, L. J. Gibson, A. B. Stilgoe, T. A. Nieminen, and H. Rubinsztein-Dunlop, “Measuring local properties inside a cell-mimicking structure using rotating optical tweezers”, *Journal of Biophotonics* **12**, 10.1002/jbio.201900022 (2019).
- [160] Y. Gao, S. M. Anthony, Y. Yu, Y. Yi, and Y. Yu, “Cargos Rotate at Microtubule Intersections during Intracellular Trafficking”, *Biophysical Journal* **114**, 2900–2909 (2018).
- [161] A. N. Fehr, C. L. Asbury, and S. M. Block, “Kinesin Steps Do Not Alternate in Size”, *Biophysical Journal* **94**, L20–L22 (2008).
- [162] H. Khataee and J. Howard, “Force Generated by Two Kinesin Motors Depends on the Load Direction and Intermolecular Coupling”, *Physical Review Letters* **122**, 188101 (2019).
- [163] M. Sirajuddin, L. M. Rice, and R. D. Vale, “Regulation of microtubule motors by tubulin isotypes and post-translational modifications”, *Nature cell biology* **16**, 335–44 (2014).
- [164] B. Y. Monroy, T. C. Tan, et al., “A Combinatorial MAP Code Dictates Polarized Microtubule Transport”, *Developmental Cell* **53**, 60–72.e4 (2020).
- [165] J. Beeg, S. Klumpp, et al., “Transport of beads by several kinesin motors”, *Biophysical Journal* **94**, 532–541 (2008).
- [166] J. Xu, Z. Shu, S. J. King, and S. P. Gross, “Tuning Multiple Motor Travel via Single Motor Velocity”, *Traffic* **13**, 1198–1205 (2012).
- [167] A. R. Rogers, J. W. Driver, P. E. Constantinou, D. Kenneth Jamison, and M. R. Diehl, “Negative interference dominates collective transport of kinesin motors in the absence of load”, *Physical Chemistry Chemical Physics* **11**, 4882 (2009).
- [168] N. D. Derr, B. S. Goodman, et al., “Tug-of-War in Motor Protein Ensembles Revealed with a Programmable DNA Origami Scaffold”, *Science* **338** (2012).
- [169] D. K. Jamison, J. W. Driver, and M. R. Diehl, “Cooperative responses of multiple kinesins to variable and constant loads.”, *The Journal of biological chemistry* **287**, 3357–65 (2012).

- [170] K. Furuta, A. Furuta, et al., “Measuring collective transport by defined numbers of processive and nonprocessive kinesin motors.”, *Proceedings of the National Academy of Sciences of the United States of America* **110**, 501–6 (2013).
- [171] Y. Jun, S. K. Tripathy, B. R. J. Narayanareddy, M. K. Mattson-hoss, and S. P. Gross, “Calibration of Optical Tweezers for In Vivo Force Measurements : How do Different Approaches Compare ?”, *Biophysical Journal* **107**, 1474–1484 (2014).
- [172] V. R. Telis, J. Telis-Romero, H. B. Mazzotti, and A. L. Gabas, “Viscosity of aqueous carbohydrate solutions at different temperatures and concentrations”, *International Journal of Food Properties* **10**, 185–195 (2007).
- [173] A. J. Goldman, R. G. Cox, and H. Brenner, “Slow viscous motion of a sphere parallel to a plane wall-I Motion through a quiescent fluid”, *Chemical Engineering Science* **22**, 637–651 (1967).
- [174] R. B. Jones and F. N. Alavi, “Rotational diffusion of a tracer colloid particle. IV. Brownian dynamics with wall effects”, *Physica A: Statistical Mechanics and its Applications* **187**, 436–455 (1992).
- [175] B. Cichocki and R. Jones, “Image representation of a spherical particle near a hard wall”, *Physica A: Statistical Mechanics and its Applications* **258**, 273–302 (1998).
- [176] B. J. Reddy, S. Tripathy, et al., “Heterogeneity in kinesin function”, *Traffic* **18**, 658–671 (2017).
- [177] T. Scholz, S. M. Altmann, et al., “Mechanical properties of single myosin molecules probed with the photonic force microscope”, *Biophysical Journal* **88**, 360–371 (2005).
- [178] A. Toleikis, N. J. Carter, and R. A. Cross, “Backstepping Mechanism of Kinesin-1”, *Biophysical Journal* **119**, 1984–1994 (2020).
- [179] S. Sudhakar, M. K. Abdosamadi, et al., “Germanium nanospheres for ultraresolution picotensiometry of kinesin motors”, *Science* **371**, eabd9944 (2021).
- [180] V. Saravanan, G. J. Berman, and S. J. Sober, *Application of the hierarchical bootstrap to multi-level data in neuroscience*, 2019.
- [181] F. Català, F. Marsà, M. Montes-Usategui, A. Farré, and E. Martín-Badosa, “Influence of experimental parameters on the laser heating of an optical trap”, *Scientific Reports* **7**, 1–9 (2017).
- [182] P. M. Kampmeyer, “The temperature dependence of viscosity for water and mercury”, *Journal of Applied Physics* **23**, 99–102 (1952).
- [183] E. Schäffer, S. F. Nørrelykke, and J. Howard, “Surface forces and drag coefficients of microspheres near a plane surface measured with optical tweezers”, *Langmuir* **23**, 3654–3665 (2007).
- [184] J. Leach, H. Mushfique, et al., “Comparison of Faxén’s correction for a microsphere translating or rotating near a surface”, *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics* **79**, 1–4 (2009).

- [185] J. R. Smart, S. Beimfohr, and D. T. Leighton, “Measurement of the translational and rotational velocities of a noncolloidal sphere rolling down a smooth inclined plane at low Reynolds number”, *Physics of Fluids A* **5**, 13–24 (1992).
- [186] K. Stamer, R. Vogel, E. Thies, E. Mandelkow, and E. M. Mandelkow, “Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress”, *The Journal of Cell Biology* **156**, 1051–1063 (2002).
- [187] S. T. Brady, K. K. Pfister, and G. S. Bloom, “A monoclonal antibody against kinesin inhibits both anterograde and retrograde fast axonal transport in squid axoplasm”, *Proceedings of the National Academy of Sciences of the United States of America* **87**, 1061–1065 (1990).
- [188] J. R. Kardon and R. D. Vale, “Regulators of the cytoplasmic dynein motor.”, *Nature reviews. Molecular cell biology* **10**, 854–865 (2009).