

**Advancements in Analytical Methods and Opto-Mechanical
Techniques to Study Molecular Motors and Mutations**

A THESIS

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Dedication

To my family - mother, father and brother - for their unwavering belief in me

Abstract

Transport of important cargo inside the cell occurs through ‘motor-proteins’: nanoscale biological machines that carry cargo over tracks formed by filaments called microtubules. Defects in intracellular transport mechanisms are linked to numerous neuro-degenerative disorders such as Alzheimer’s and Huntington’s disease. Understanding motor-protein functionality and related diseases at the cellular level requires detailed investigation of motor protein behavior. My thesis enables the study through the following key contributions:

1. Innovation of new experimental and computational methods to analyze transport inside cells, with unprecedented resolution and accuracy.
2. Deployment of the new methods to study motor-protein behavior and how mutations in proteins affect intracellular transport, leading to neuro-degenerative diseases.

My research makes these contributions through analytical and experimental approaches.

In the first part, I have developed analytical tools that are capable of probing how the motor proteins function while carrying cargoes inside the cell, with a particular focus on the transport of cargors by teams of motors. Utilizing the properties of Markov processes, I have developed a computational engine that can provide exact probabilities of the various configurations adopted by motors in the team containing single or multiple species. This methodology helps in answering previously unanswered questions on the mechanisms adopted by motors in response to external events, such as obstructions or impediments along the path of cargo travel, changing load conditions and varying ATP concentrations. The analysis predicts that motors develop a cooperative mechanism and cluster together while carrying progressively heavier loads; but tend to adopt a non-clustered configuration in response to sudden spikes in loads (which is akin to sudden obstacles along the path of travel). A further extension of the computational tool enabled the investigation of the impact of disease causing mutations on team based transport, where the mutations are known to impair single motor behavior. I analyzed the consequence of having a minority

of mutated motors in a team, with the mutation that has been implicated in Huntington's disease. Under certain conditions, the teams with even a few mutated motors are seen to gain a significant competitive advantage, potentially contributing to the dysregulation of cargo transport, impairment of neuronal function and the onset of neurodegeneration. A separate contribution of this work is the underlying analytical technique, which facilitates exact computations, detection of rare events, ease of usage and a reduction in computational resources needed for its functionality, each of which is a notable improvement over existing approaches.

The above analytical conclusions need to be supported by experimental evidence, which is not possible without commensurate improvements in the available platforms of experimental interrogation. Among the existing instruments, the 'Optical Tweezer' and the associated principle of 'Optical Trapping' (whose inventor, Arthur Ashkin, won the 2018 Nobel Prize for Physics) has been pivotal in the experimental analysis of molecular motors. Despite their numerous advantages, optical tweezers suffer from a lack of utilization of a modern controls paradigm and a systems engineering based approach leading to a constrained performance. In the second part of my thesis, I developed a platform for disturbance estimation based on multi-objective synthesis. This facilitated the analysis of multi-input and multi-output systems where the regulation of a certain variable is the objective, in the presence of an external disturbance while simultaneously providing estimates of the disturbances in real time. The platform is designed to function in situations where the disturbance is altered by the noise, whose impact needs to be mitigated. I tested the efficacy of the platform on an 'Optical Tweezer' instrument using live samples of the protein Kinesin-I and demonstrated over 50% improvement over previous studies, while working in extremely low force ranges of femto-newtons and offering a unique ability to regulate load force on the motor-protein itself. This platform can be utilized to experimentally probe the conclusions borne out of the previous analytical studies involving multiple motors and mutations and can also be extended to study faster motors in their native environment, something which was not possible using the earlier approaches.

While numerous techniques exist that can study motor proteins through the dynamics of the cargo they carry, there are far fewer experimental techniques that can effectively probe

the motors themselves. This is mainly due to the restrictions imposed by the extremely small dimensions of the motors and the limited probing capacity of optical instruments such as fluorescent microscopes. To tackle this issue, I have developed a unique and low cost instrument called 'TIRF-Tweezer', that combines the capabilities of total internal reflection fluorescence (TIRF) microscopy and optical tweezers. This greatly enhances the probing capacity of optical tweezers and facilitates the investigation of, amongst other things, the previously drawn conclusions on the relative configurations adopted by motors while transporting cargo under varying conditions. To further bolster such investigations, I have also developed robust biological protocols that can recreate the required cellular environments in an *in-vitro* environment and help provide experimental evidence to the analytical conclusions.

With these analytical and experimental tools, I intend to equip biologists and biophysists with useful tools that can assist in the task of analysis of molecular motor behavior.

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Chapter 1

Introduction

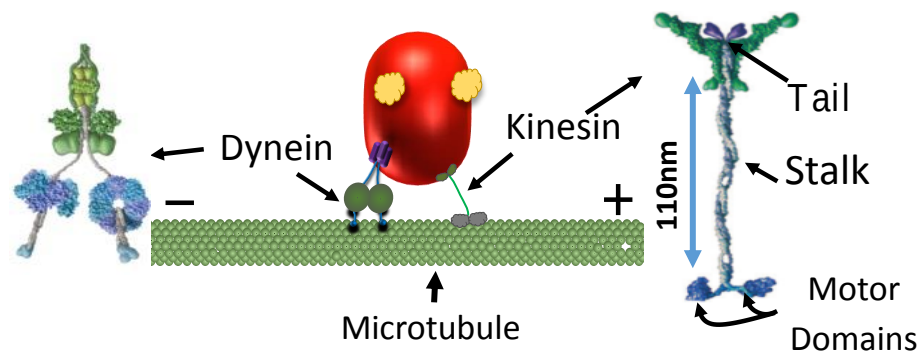


Figure 1.1: Schematic showing intracellular cargo carried by motor proteins kinesin and dynein over a section of the microtubule inside the cell. Kinesin pulls the cargo towards the ‘plus’ end of the microtubule while dynein primarily pulls the cargo towards the ‘minus’ end of the microtubule

Inside a human cell, nanometer sized biological machines called ‘*motor-proteins*’ (Fig. 1.1) carry important intracellular cargo such as organelles and vesicles from source to destination. These motors operate by converting chemical energy to mechanical work through the hydrolysis of ATP molecules and are critical for maintaining healthy functioning of the cell. Each motor transports the cargo by taking steps as small as 8 nanometers, and works against forces as tiny as a tens of pico newtons. However, often multiple motors of same or different types work in teams to ferry a common cargo. Disruption of this transport mechanism due to malfunctioning motors, possibly due to mutations affecting individual motors, has been implicated in severe neuro-degenerative diseases such as Huntington’s, Alzheimer’s disease, muscular disorders and uterine complications. It is highly likely that some of the

motors in a team of motors carrying the common cargo may be affected by such disease-causing mutations, whose impact on team-based transport is not well understood. How the motor-protein functions, how its various components interact with each other and how multiple types of motor proteins aid or hinder one another while transporting a common cargo is an area of active ongoing research.

Existing studies involve utilizing numerical simulations to make predictions about motor protein behavior, which are then tested experimentally. However, these approaches either use simplifying assumptions that hinder a detailed characterization of important transport characteristics; or are computationally inefficient and require supercomputers or computer clusters to be implemented. Furthermore, the experimental investigations performed using existing state-of-art currently lack sufficient resolution and precision. Thus, improvement on the computational as well as experimental front is necessary to enable advanced investigation of molecular motors.

In this thesis I have followed a two-fold approach. Firstly, I have utilized various analytical and mathematical tools to model the transport of cargo by a team of multiple motor proteins, where the team consists of motors of the same or different types. The work has enabled the development of a highly efficient computational tool to simulate the various transport modalities of team-based transport. The analysis has allowed for an intricate study of the various strategies and schemes deployed by teams of motors while carrying a cargo in the inherently temperate environment inside the cell. Furthermore, the model is used to analyze the impact of having one or more motor-proteins that are affected by disease causing mutations, in a team carrying a common cargo. Together, the study has revealed numerous outcomes that describe the mechanisms underlying intracellular transport and how these mechanisms are impacted by disease causing mutations. These outcomes can be effectively tested through controlled *in-vitro* experimental studies.

Secondly, following the contributions to the analytical aspects, I have worked on developing novel experimental approaches that are complementary to the computational studies and can aid the corroboration of the analytical conclusions. This involves developing new biological protocols that can robustly replicate the environment inside the cell onto an *in-vitro* platform. Along with the protocols, I have proposed and implemented advancements

that improve upon the existing experimental capabilities of Opto-Mechanical instruments used to probe motor proteins, something that is central to the testing of various team-based transport modalities proposed in the earlier analytical studies. This involves development, testing and a proof-of-concept demonstration of an improved experimental paradigm that enables studies of motor proteins under constant force on '*Optical Tweezers*'. Lastly, I have developed a new instrumentation platform that combines optical trapping capabilities of optical tweezers with the high resolution imaging capability of total internal reflection fluorescence (TIRF) microscopy, through the development of a *TIRF-Tweezer* platform. The *TIRF-Tweezer* provides the latest state-of-art capability that can be used to investigate, among numerous other things, the various modalities predicted for team based transport as the team of motors navigates through the chaotic environment inside the cell. Together, these experimental advancements provide tools to investigate how motors in a team function to facilitate regulated cargo transport and how the disruption of cargo traffic inside the cell due to mutations can lead onset neurodegenerative disorders such as Huntington's and Alzheimer's disease.

The thesis is organized as follows :

- Chapter 1 provides an introduction to the work and a general outline of the thesis.
- Chapter 2 presents the analytical methodology developed to study transport by team of multiple motor proteins, with one or more motors in the team affected by disease causing mutations. An accompanying simulation engine is also developed, which is more computationally efficient and provides an exact analysis of the team-based transport. The approach is instantiated for a team of kinesin motor proteins and a mutation that has been implicated in Huntington's disease. It reveals interesting and hitherto unknown insights into the impacts of a minority of mutations on cargo transport properties such as average run-length and velocity . The underlying model also finds applications beyond the specific example demonstrated in the chapter.
- Chapter 3 provides a mathematical approach towards understanding how a team of finite number of motors navigate the turbulent conditions inside a cell while transporting a common cargo. This is done through a detailed study of a steady state

transition probability matrix that is used to define the underlying system. Specifically, the study investigates how the motors in the team respond to changing ATP concentrations and various hindering load forces on the cargo. This chapter draws numerous unique conclusions that suggest the existence of a cooperative mechanism among the motors in the team, which facilitates robust functioning under the changing intracellular environment.

- Chapter 4 details the development and experimental verification of a constant force clamping mechanism on 'Optical Tweezers', that improves upon existing state-of-art methods. Here, principles of system identification and mixed objective modern control synthesis are utilized to overcome the limitations of existing force clamps. The efficacy of the method is demonstrated using extensive simulations and is corroborated with experiments using live samples of the motor protein 'Kinesin-I'. This chapter demonstrates (to the best of my knowledge) a first-of-its-kind regulation of sub-pico newton forces on cargo carried by live motor protein with regulation error 10%, while simultaneously estimating the stepping motion of the motors.
- Chapter 5 provides a brief overview of the development of the combined *TIRF-Tweezer* system, along with a demonstration of total internal reflection fluorescence (TIRF) microscopy on fluorescently labeled beads.
- Chapter 6 details the conclusions and discussions
- Appendix A provides the protocols used for the Kinesin bead motility assay that was used to perform the experimental corroboration of the optical force clamp in Chapter 4
- Appendix B provides brief information on the miscellaneous collaborations that I was a part of during the course of my research.

Chapter 2

Interrogating Emergent Transport Properties for Molecular Motor Ensembles: A Semi-analytical Approach

Intracellular transport is an essential function in eucaryotic cells, facilitated by motor proteins - proteins converting chemical energy into kinetic energy. It is understood that motor proteins work in teams enabling unidirectional and bidirectional transport of intracellular cargo over long distances. Disruptions of the underlying transport mechanisms, often caused by mutations that alter single motor characteristics, are known to cause neurodegenerative diseases. For example, phosphorylation of kinesin motor domain at the serine residue is implicated in Huntington's disease, with a recent study of phosphorylated and phosphomimetic serine residues indicating lowered single motor stalling forces. In this article we report the effects of mutations of this nature on transport properties of cargo carried by multiple wild-type and mutant motors. Results indicate that mutants with altered stall forces might determine the average velocity and run-length even when they are outnumbered by wild type motors in the ensemble. It is shown that mutants gain a competitive advantage and lead to an increase in the expected run-length when the load on the cargo

is in the vicinity of the mutant’s stalling force or a multiple of its stalling force.

A separate contribution of this article is the development of a semi-analytic method to analyze transport of cargo by multiple motors of multiple types. The technique determines transition rates between various relative configurations of motors carrying the cargo using the transition rates between various absolute configurations. This enables an exact computation of biologically relevant quantities like average velocity and run-length. It can also be used to introduce alterations of various single motor parameters to model a mutation and to deduce effects of such alterations on the transport of a common cargo by multiple motors. Our method is easily implementable and we provide a software package for general use.

2.1 Introduction

Motor proteins- kinesin, dynein and myosins- are nanoscale machines that are the main effectors of intracellular transport. They play a critical role in the growth and sustenance of healthy cells by enabling a transport of intracellular cargo over networks of microtubules [4]. Disruption of the functions performed by these molecular motors is linked to neurodegenerative diseases such as Huntington’s, Parkinson’s and Alzheimer’s Disease [5, 6], muscular disorders such as heart disease, uterine complications and high blood pressure. The mechano-chemical behavior of a single motor moving along the microtubule substrate to transport a cargo is relatively well understood [7]. There is considerable prior work on understanding how a single-motor transports a cargo on a microtubule. In [8] the Fokker–Planck equation for a single particle moving in a one-dimensional potential is obtained and used to determine the probability distribution of the position of the particle in a single repeat length of the polymer track via discretization. The underlying approach was extended to a motor connected to a cargo by a linear spring [9, 10] while traveling under the influence of a tilted periodic potential. *In vivo* it is known that multiple motors work in teams to transport a common cargo [11]; how multiple motor-proteins coordinate to transport a common cargo is not well understood [11, 12, 13]. Many studies employ a probabilistic description of the behavior of a single motor protein to construct models that describe how multiple motors

transport a common cargo [11, 14, 15, 16]. In [17], Gross and coworkers employed Monte-Carlo simulation studies built on a model of a single kinesin in to explore how multiple identical kinesin motors might interact to transport a cargo against a hindering load force. Their work indicates, counter intuitively, the existence of a form of strain-gating, where the motors of an ensemble share loads unequally enabling cargo transport over longer distances. Xu and coworkers examined the effects of ATP concentration on the transport of cargo carried by single and two motors in [18]. At decreased levels of ATP concentration, the velocities of cargoes transported by single and two motor proteins decreases. Coincidentally at decreased ATP concentration there was an appreciable increase in the run-length of cargoes transported by two motors, while no such effect was seen in the case of transport by one motor. The authors proposed that the increased run-length observed in the presence of two motors results from the lowered dissociation of each motor from the microtubule at decreased ATP concentrations and the increased probability that the cargo stayed bound to the microtubule. Studies such as [16, 19] using probabilistic models of single motors have also predicted in that an ensemble of kinesin motors is a robust system and the robustness increases under high loads [20].

The study of cargo transport by a heterogeneous ensemble of motor proteins composed of both wild type and mutant motors is important to inform our understanding of how mutant motors impact intracellular transport and lead to an onset of diseases. Recent studies have implied that alterations in the kinesin-1 motor domain may have a role in impaired axonal transport. Phosphorylation of a mammal kinesin motor domain by kinase c-Jun N-terminal kinase-3 (JNK3) at a conserved serine residue (Ser-176 in A and C isoforms and Ser-175 in B isoform) is implicated in Huntington's disease [21]. However the mechanisms affected by Ser-175 phosphorylation are not well understood. An experimental study by Selvin and coworkers in [22] reported that a negative charge at Ser-175, acquired through mutation or phosphorylation, leads to a lower stall force and decreased velocity under external loads of 1 pN or more, while leaving the ATPase, microtubule-binding affinity and processivity unchanged.

Using a semi-analytical method, we reveal surprising emergent transport behaviors arising when a cargo is transported by multiple motor proteins, some of which are mutated and

some are not. In particular we analyze the impact of Ser-175 kinesin mutation such as those reported in [22] on cargo transport in the presence of wild type motors. The detailed investigation made possible by our method leads us to hypothesize that under certain conditions, a ‘*mooring mechanism*’ is activated where proteins *moor* the cargo to the microtubule and prevent it from being lost. While these *mooring proteins* do not contribute to the motion of the cargo, they enhance the probability of attachment of other cargo-bound motor proteins to the microtubule that subsequently contributes to an increase in average cargo displacement. The activation of mooring mechanism depends on a number of external factors such as load force and ATP concentration. However, it is also determined by intrinsic properties of the motor protein such as the stall force of the individual motors. Remarkably, mutant motors that have stalling forces matched to the external load force can act dominantly and determine emergent transport properties such as longer run-length, even when they are outnumbered in the ensemble by wild-type motors. Such mechanisms could potentially point to how diseased states emerge and progress coincident with the accumulation of the mutant motor species.

A separate contribution of the article is a semi-analytical method for determining the probability distribution of various configurations of a cargo carried by multiple number and types of motor-protein. A detailed experimental study of the various modalities of transport by multiple motor proteins (homologous or otherwise) requires significant instrumental resolution than what is needed to investigate single motor behavior. As a consequence, observing the transport dynamics of multiple motors is experimentally challenging [23, 24]. It is further compounded by the combinatorial complexity introduced by the multitude of scenarios possible when many motors and motor types participate in transport. Such challenges motivate the use of analytical and computational tools. The mean-field approach in [14, 16] makes use of simplifying assumptions, such as equal load sharing among all motors, to achieve analytical results, thereby sacrificing significant detail for computational benefits. The Monte Carlo approach in [17], provides better fidelity where complex models can be employed; however, the accuracy of results depend on the number of iterations and on the rarity of the events that occur.

Unlike the Monte-Carlo simulations or any currently implemented simulation methods

that study multi-motor ensembles, our Master Equation based method [25] analytically solves for the probability distributions of all possible scenarios at any time point. The methodology is uniquely powerful and enables the calculation of various biologically relevant quantities such as average velocity and run-length, for reasonably sized ensembles and with high accuracy while using lesser computational resources and time as compared to Monte Carlo simulations (the methodology proposed in [9, 10] also obtains results without resorting to Monte Carlo simulations with a single motor attached to the cargo). The underlying concepts behind our methodology are motivated by earlier work reported in [19]. The key enabling concept is that of ‘relative configuration’ of motor proteins, determining the transition probabilities between relative configurations from the transition probabilities of the absolute configuration space and subsequently determining the biologically relevant quantities from the relative configuration space. The computation engine is implemented using MATLAB and can be used to simulate cargo transport by any two unidirectional species.

Our method provides a general platform to study the transport of cargo by multiple motors of two different types where each type of motor protein can be individually characterized by a probabilistic model describing its stepping, detachment and attachment rates. For this article, the technique has been utilized to introduce alterations of various parameters from the nominal ones to model a mutation of the serine residue and compute the effect of such a mutation on cargo carried by a mixture of wild-type and mutant motor-proteins. In summary, we developed a simulation engine to study the transport of cargo by multiple motor proteins with distinct properties that in concert can exhibit emergent transport behaviors.

In summary, we developed a simulation engine to study the transport of cargo by multiple motor proteins with distinct properties that in concert can exhibit emergent transport behaviors. A recently reported mutation is incorporated into the model and the computation platform is used to study its effects on coordinated cargo transport to arrive at non-trivial and, to the authors’ knowledge, yet undiscovered features in the transport mechanisms resulting from the coupling of multiple wild-type and mutated motors via a common cargo. Primarily it is inferred that the presence of mutant motors affects average runlength

and velocity of cargoes, with the nature and magnitude of the effect dependent on external loading conditions and population of mutant motors in the ensemble. The computational model used to obtain results of such nature is described under *Methods*. Results of biological significance obtained from its instantiation for a group of wild-type and mutated kinesin carrying a common cargo are presented under *Results and Discussions*, followed by a summary of related conclusions.

In this study, we investigate the impact of a previously reported kinesin mutation on the transport of a motor ensemble and its attached cargo. The mutation, located within the motor domain of the kinesin, mimics the phosphorylated state of Ser-175 [22]. The motor domain phosphomimetic mutation does not affect the ATPase, microtubule-binding affinity or processivity of the motor, but does reduce the stall force and velocity of kinesin under a load force. The in vitro phosphorylation of Ser-175 for a full length kinesin similarly reduces stall force and velocity of the motor. Both the mutant and in vitro phosphorylated kinesin showed no other aberrant single motor behavior.

Here we use computational modelling to analyze the impact of the Ser-175 mutant kinesin on a heterogeneous ensemble of motors and its transported cargo. The wild-type and mutant kinesin motors have a different stalling force; the wild type kinesin has a stall force of $F_s = 6pN$ and the mutated kinesin has a reduced stall force of $\bar{F}_s = 5.5pN$ (see [22] for more detail). In our analysis we considered cargoes transported by the following motor ensembles : cargo with two wild-type (WW) motors , one wild-type and one mutant (WM) motors, two mutant (MM) motors , three wild-type (WWW) motors , two wild-type and one mutant (WWM) motors, one wild-type and two mutant (WMM) motors, and three mutant (MMM) motors.

2.1.1 How External Loading Conditions Affect Behavior of Heterogeneous Motor Ensemble

Motor configuration when engaged to the microtubule approaches steady state

For cargoes carried by multiple motor proteins, transport occurs as each motor steps

along the microtubule lattice, sequentially binding, translocating and releasing from the microtubule in a well defined mechanochemical cycle [11, 16, 26, 2]. Eventually, the condition where no motors are attached to the microtubule will be reached and the cargo will diffuse away from the microtubule. Thus it is evident that the only steady state is the condition when none of the motors of the ensemble carrying the cargo remain attached to the microtubule. Indeed, in all the cases considered where there are three or less motors on the cargo (i.e. $M \leq 3$) it is seen that for any specified initial probability distribution of motors engaged to the microtubule, the steady-state probability distribution has no motors attached to the microtubule i.e. cargo is eventually lost. Figure 2.1(a) shows the exactly computed probability distribution for the WM case, where with increasing time the probability of no motors attached (red curve) increases and eventually reaches one.

Given that the steady-state probability distribution is the trivial case of no motors attached, nothing much of value can be derived regarding the motor behavior from the trivial steady-state probability distribution. However the *conditional* probability distribution of the various configurations of motor proteins carrying a common cargo, *given that at least one motor is attached to the microtubule* (i.e. when the motor-cargo complex is not completely detached from the microtubule), is of significant interest (see analogous description in [27] on discretization that separates the effects of attachment and cycling through the kinetic steps of a motor). Here it is apriori not evident whether the conditional probability distribution has a steady state.

Remarkably, the results show that under a constant load force on the cargo the conditional probability distribution of the various motor configurations reaches a steady state. The steady state distribution is independent of the initial probability distribution chosen (see for example, Figure 2.1(b) for the WM case). This indicates that the cargo-motor ensemble consisting of motors with different stalling forces is a robust system which, starting from any arbitrary initial condition, behaves according to a fixed distribution after some transient period. Furthermore, the existence of such a steady-state distribution, termed here as the *steady-state conditional pdf*, allows the computation of most of the variables of biological interest. For a homologous ensemble of two molecular motors, it is proven that the associated Markov chain is ergodic [28], implying that the states eventually reach a

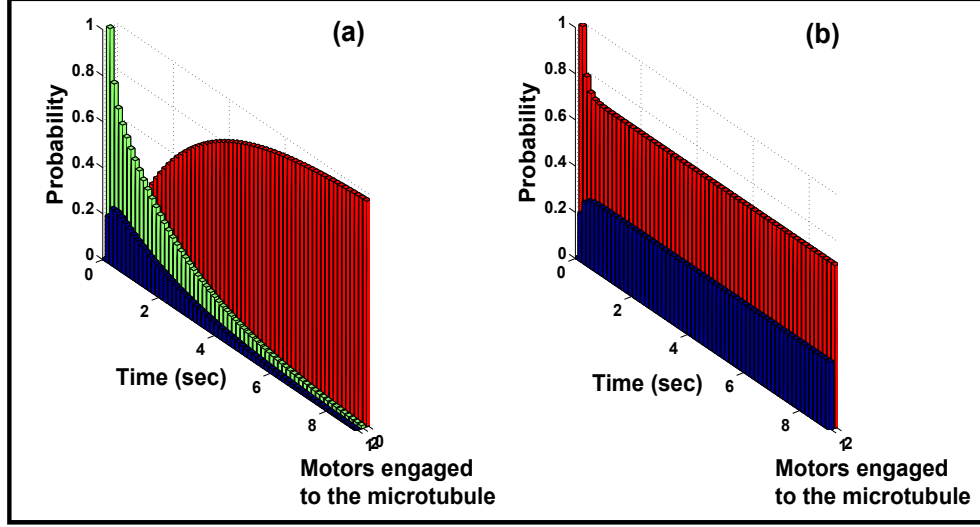


Figure 2.1: **Probability of motors being bound to the microtubule at time t under load force of $1.2 pN$ for the WM case.** In (a) the probability of one bound motor (blue) and two bound motors (green) approaches 0 as time $t \rightarrow \infty$. Probability of no bound motors (red) (i.e. the cargo dissociates from the microtubule) approaches 1 as $t \rightarrow \infty$. In (b) the probability of one bound motor (blue) and two bound motors (red) under the condition that cargo is not lost, reaches a steady state.

unique steady state distribution.

Load forces on cargo affect conditional steady state distribution of motor ensemble

The conditional steady-state distribution is used to analyze transport of cargo with two attached motors and assumes that at least one motor is attached (Figure 2.2). Figure 2.2 shows the probabilities of the number of wild-type and mutated motor proteins that remain attached to the microtubule under varying load forces. The analysis indicates that when the cargo has two wild type motors attached (WW case), the probability that both motors remain attached to the microtubule peaks when load force is close to $12pN$. This equals twice the stalling force of a single wild type motor; indicating a relationship between number of motors attached (obtained using the steady state distribution) and applied load. Thus at these loads there exists a propensity to retain and not loose the cargo. Similarly, in the MM case (where the cargo is attached to two mutant motors) the probability that both motors are attached to the microtubule is maximum near $11pN$ which is twice the stalling force

of the mutated motor. The WM ensemble displays lower peaks for the dual attachment of motors at both load forces of $11pN$ and $12pN$.

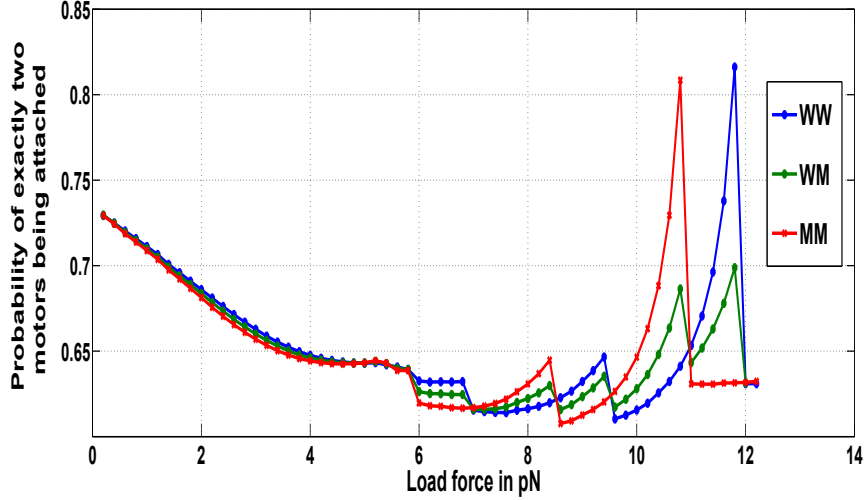


Figure 2.2: **Probability of exactly two motors attached to the microtubule for ensembles WW, WM and MM.**

It is therefore evident that for ensembles containing two motors, the conditional steady state distributions of the number of motors attached is not significantly affected until the load force on the cargo is close to twice the stalling force values of the wild -type or mutant motor ($2F_s = 12pN$ or $2\bar{F}_s = 11pN$). A similar observation is made for ensembles with three motors and for load force values close to twice and thrice the stalling force of a single motor or $2F_s$, $2\bar{F}_s$, $3F_s (= 18pN)$ and $3\bar{F}_s (= 16.5pN)$. We hypothesize a ‘*mooring mechanism*’ to explain the observations where the probability of a motor remaining attached to the microtubule is increased when the load force on the motor is close to its stalling force, thereby mooring the motor to the microtubule. The moored motor prevents the motor-cargo complex from being detached from the microtubule. The mooring mechanism is evident in the case of a wild-type motor, where the probability of the wild-type motor remaining attached to the microtubule is high when the load force on the motor is close to $F_s = 6 pN$ (Figure 2.11 , blue curve). The WW curve in Figure 2.2 thus indicates that when load force is close to $12pN$, both the motors face a load force less than but close to $F_s = 6 pN$ allowing for the cargo to be ‘*moored*’ to the microtubule through both the motors. A similar reasoning can explain the MM curve in Figure 2.2 at load force close

to $11pN$, where the mooring mechanism for both the mutant motors is activated when the load force on each motor is close to $\bar{F}_S = 5.5 pN$ (Figure 2.11 , green curve). It indicates that for load forces close to multiples of single motor stalling forces, equal sharing of loads is preferred by the motors in the ensemble. Thus a cooperative mode between an ensemble of motors is encouraged when the load forces are close to the stall-force or multiples of the stall forces. For example, if a WW ensemble is subjected to load force close to $2F_s = 12 pN$, unequal load sharing would subject one of the motors to a force greater than its stalling force $F_s = 6 pN$. This will lead to its detachment and eventual transfer of the entire load onto the remaining motor. This, in turn, would gradually lead to the remaining single motor being detached from the microtubule, and the resultant loss of cargo attachment to the microtubule substrate. On the other hand, an equal load sharing will stall both the motors but ensure the retention of the cargo. A similar inference can be made for cargo transport by three motors and load forces approaching the values F_s , $\bar{F}_s$, $2F_s$ or $2\bar{F}_s$ (figure not shown).

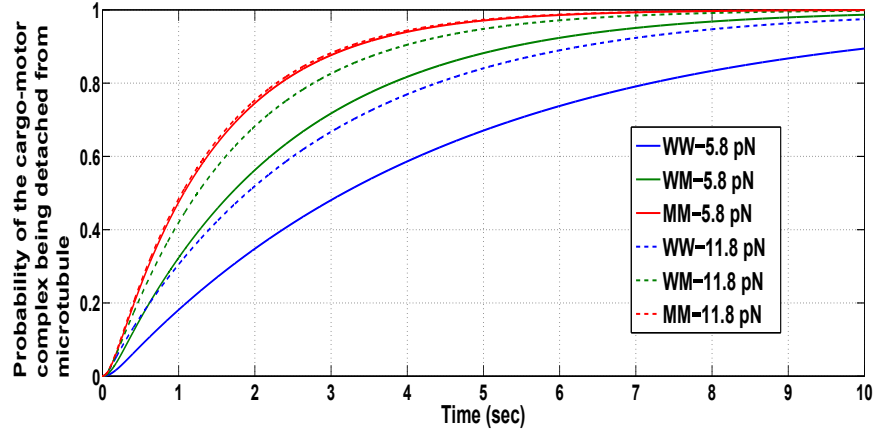


Figure 2.3: **Probability of the cargo-motor complex getting detached from the microtubule, for ensembles WW, WM and MM at load forces close to but less than F_s and $2F_s$ ($F_s = 6 pN$).**

Note that, it is possible when the load force is close to twice the stalling force, the cargo detaches from the microtubule with a high probability. In such a case, the previously mentioned conclusions reached about the probabilities by applying the condition that the cargo-motor complex does not detach from the microtubule (i.e. at least one motor remains

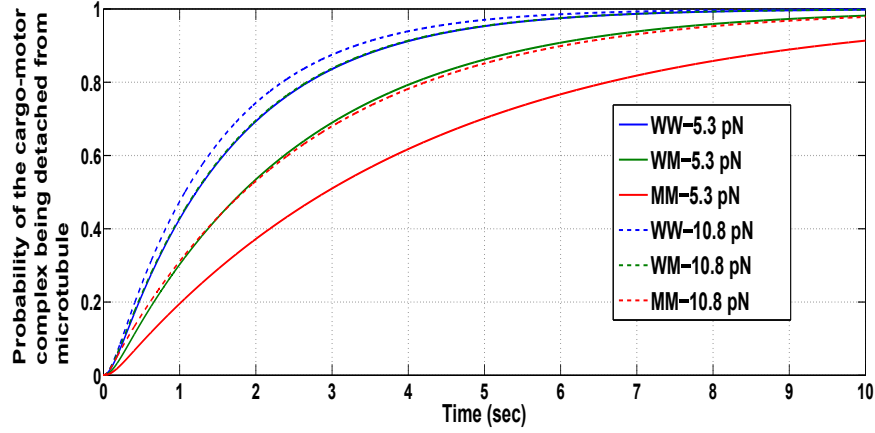


Figure 2.4: **Probability of the cargo-motor complex getting detached from the microtubule, for ensembles WW, WM and MM at load forces close to but less than F_s and $2F_s$ ($F_s = 5.5$ pN).**

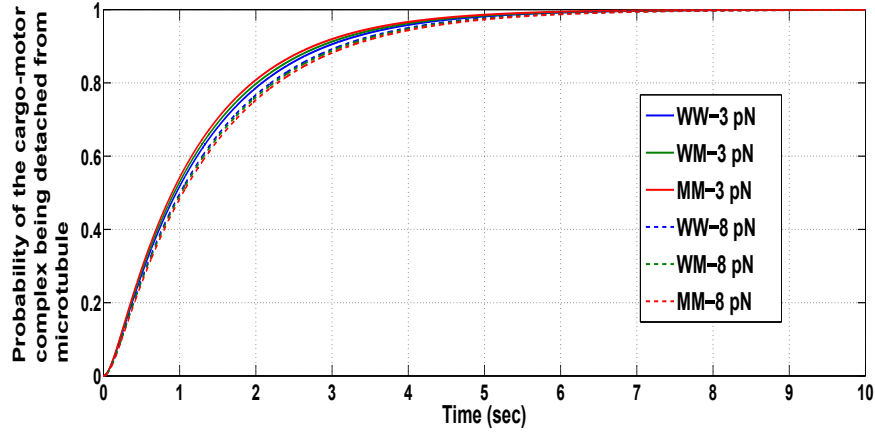


Figure 2.5: **Probability of the cargo-motor complex getting detached from the microtubule, for ensembles WW, WM and MM at load forces 3 pN and 8 pN**

engaged to the microtubule) will not carry much significance. We then utilized the probabilities obtained without the conditioning to reach similar conclusions. Figures 2.3 and 2.4 show that the time taken for the cargo to be lost (i.e. time taken for the probability of detachment of the cargo to be close to one) is higher when the load force is close to but less than the appropriate stalling force or twice the appropriate stalling force value. The time taken for detachment is lesser for a load force of an intermediate value (compare with Figure 2.5). The figures show that if the load force is matched to an appropriate stall force or its multiples (for example, close to $2F_s$ for WW or $2\bar{F}_s$ for MM ensemble), the probability

of detachment of the cargo from the microtubule becomes appreciable only after a long time has passed. Thus, the conclusions reached using the conditional distribution are also supported by results inferred from the entire configuration space with no restriction that the cargo remains attached. It implies that the study on the condition that ‘the cargo-motor complex is not detached from the microtubule’ provides important insights.

In conclusion, load force on the cargo has a significant effect on the steady state distribution of motor ensembles and can provide an insight into how multiple motor ensembles function under varying loads. In particular, when a cargo is subjected to load forces that are multiples of the stalling force for individual motors, a cooperative behaviour in the form of equal load sharing is observed. This averts a scenario where unequal load sharing would subject some of the motors to loads greater than their stall forces, leading to their detachment and the eventual loss of cargo from the microtubule. Such cooperative behavior possibly helps retain the cargo. It is thus more probable that cargoes attached to a higher number of wild-type motors are retained under load forces close to multiples of the stalling force of a wild-type motor, making the presence of more mutants disadvantageous in these load force regimes.

2.1.2 Hindering Load Force on the Cargo can Tune Multiple Motor Travel

The knowledge of the steady state conditional probability distributions allows one to compute biologically relevant quantities such as average velocity and run-length. The results obtained for ensembles containing two and three motors for varying load forces are reported.

Average velocity typically reduces with increasing number of mutant motors, but varies as load forces approach multiples of stall forces

The effect of load force on the average velocity of a cargo is described in Figures 2.6 and 2.7 for a total of two and three attached motors, respectively. It is evident that for any given ensemble, average cargo velocity mostly reduces with increase in the hindering load force on the cargo. It is further evident from Figure 2.6 that for a given load force on the cargo, the average velocities are highest for the WW ensemble, followed by WM and the

lowest for MM ensemble. For the same load force and number of motors in the ensemble, it follows that the average velocity decreases with increased participation of mutant motors (which have a lower stall force). A similar conclusion is reached when three motors are attached to the cargo (see Figure 2.7). Thus, for a fixed load force and total number of motors, average cargo velocity reduces with increasing number of mutant motors in the ensemble. Inferences of this nature can be verified *in-vitro* by using laser-trap experiments to exert appropriate load forces on a cargo being carried by a quantified number of motor proteins and measuring the average cargo velocity for varying proportion of wild-type and mutant motors carrying the cargo.

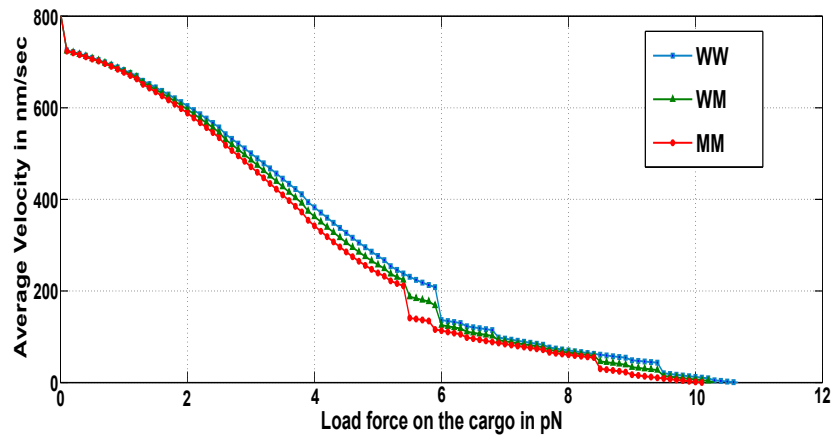


Figure 2.6: Average velocity with varying load forces on the cargo for ensembles WW, WM and MM.

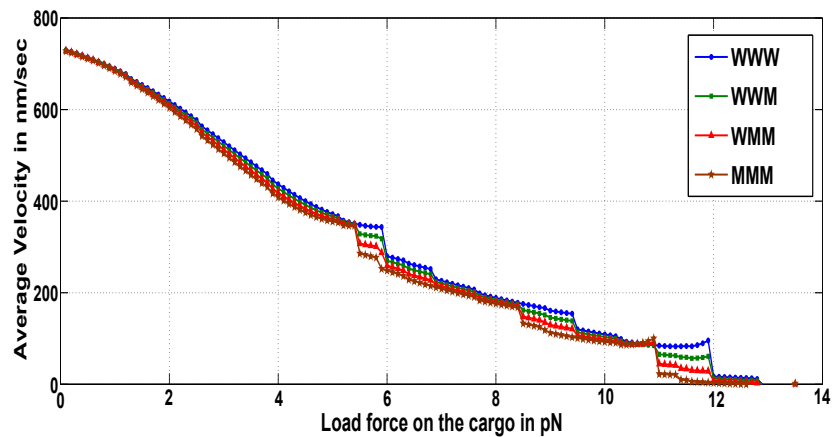


Figure 2.7: Average velocity with varying load forces on the cargo for ensembles WWW, WWM, WMM and MMM.

However, for some motors ensembles the trend of decreasing cargo velocity at elevated load forces is not observed. Figure 2.8 shows the average velocity for load forces between 10.4 pN and 12.1 pN . In the case of WWW ensemble, as the load forces increase to 12 pN (which is equal to $2F_s$) the average velocity *increases* with increasing load force. Our semi-analytic method allows for a fine analysis of such counter-intuitive observations where it is possible to extract the precise motor ensemble configurations that contribute to the observed effect.

Upon analysis it is observed that as the load force approaches the appropriate multiples of stalling force ($2F_s$ or $2\bar{F}_s$ in Figure 2.8), detachment of motors from the microtubule becomes less likely and the attached motors tend to cluster close together on the microtubule. This is similar to an observation made in [17, 19] where under high loads, the motors carrying a common cargo tend to remain clustered together while under low loads they tend to spread apart. Since the detachments are less likely, backward transitions of the cargo induced by single motor detachment are less frequent. Moreover, if a detachment were to occur, the magnitude of load induced backward transition of the cargo is less due to the clustering of motors under high loads. On average, this contributes to an increase in average velocity despite increasing load. This explains the increase in average velocities of the three motor ensembles at load forces approaching 12 pN .

Interestingly, the increase in average velocities at load forces approaching $2F_s = 12 \text{ pN}$ is more prominent in the WWW ensemble than in MMM ensemble, indicating that it is the detachment of the wild-type motors in the ensemble that becomes less unlikely. Thus, as the number of wild type motors increases, detachment events at load forces close to $2F_s = 12 \text{ pN}$ become less likely. Similarly, at load forces approaching $2\bar{F}_s = 11 \text{ pN}$ the average cargo velocity increases with increasing load force; with the increase becoming more prominent for ensembles having more numbers of mutant motors (i.e. higher for MMM ensemble than WWW ensemble).

Significantly, this observation suggests that by tuning the load forces on a cargo to values near the multiples of the stall force, one could counter a decreased velocity resulting from an increase in load force or the presence of a higher number of mutant motors.

An important feature of the semi-analytic method to be emphasized is the ease with

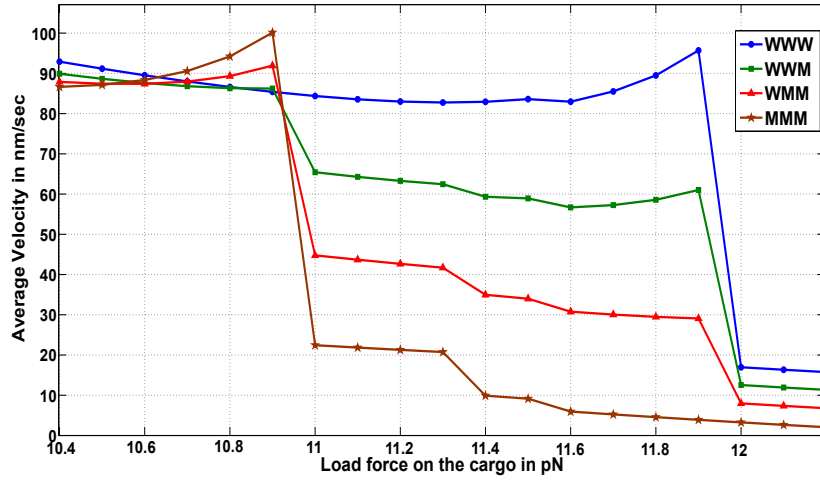


Figure 2.8: Figure showing variation of average velocity with load force on the cargo for ensembles containing 3 motors, for load forces between 10.4 pN and 12.1 pN .

which detection of rare events such as those described above are made possible. Monte Carlo based methodology, for example, would not only take significant computations to simulate rare events with a high degree of confidence, but would also be unable to determine the incidence causes of rare events. Thus our model allows the user to easily unearth the cause of a rare event.

In conclusion, load forces approaching multiples of the appropriate stall force for a cargo decrease the probability of detachment of associated motors from the microtubule. Since detachment of motors in a multiple motor ensemble can lead to backward motion of the cargo (because kinesin doesn't actively step backward i.e. towards minus end), a decrease in the detachment probability leads to higher forward motion of cargo on average, leading to an increase in average velocity even though the load increases to approach multiples of stalling forces. If load forces are close to multiples of F_s (or $\bar{F}_s$) detachment of wild-type (or mutant) motors in the ensemble decreases and cargoes with higher wild-type (or mutant) motors demonstrate greater increase in average velocity.

Cargo run-lengths impacted by heterogeneity in the motor ensemble

The dependence of run-length on the load force when the cargo has two and three

motors attached to it is shown in Figures 2.9 and 2.10 respectively. A general trend is that the average run-length reduces with an increase in the antagonistic load force on the cargo. Moreover, for a fixed number of motors in an ensemble, at most values of load force, the run-length is *reduced* with an increasing number of mutant motors in the ensemble (Figure 2.9 inset(A) and Figure 2.10 inset(A)).

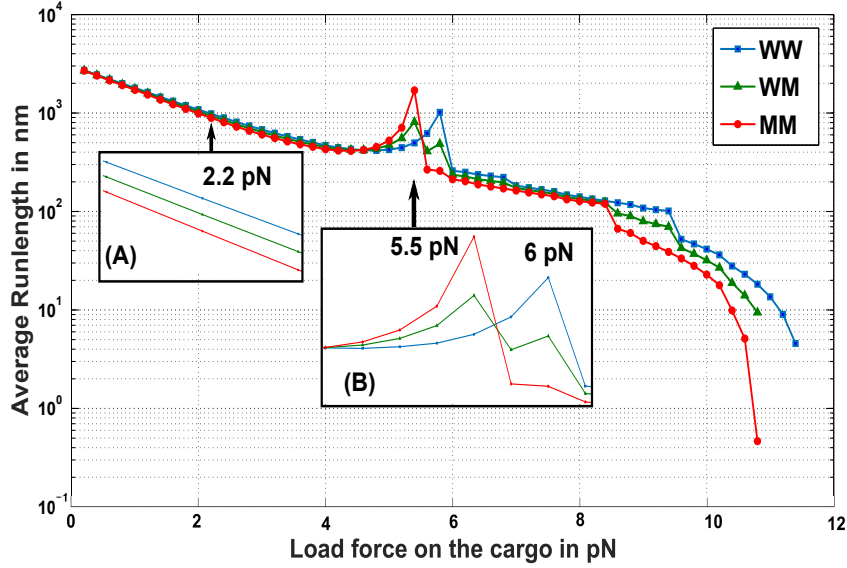


Figure 2.9: **Average Run-length under different load forces on a cargo with ensembles of two motors, wild type and mutant (WW, WM and MM).**

Surprisingly, under a load force approaching 6 pN (Figure 2.9 inset(B)), there is a surge in the average distance traveled by the cargo, peaking close to 6 pN and falling for larger load forces. The average run-length when load force is close to 6 pN increases with the number of wild-type motors and decreases with the number of mutant motors (note that, at load force equal to 5.8 pN , ensemble WW travels $\approx 1000 \text{ nm}$, WM travels $\approx 500 \text{ nm}$ while MM travels $\approx 270 \text{ nm}$). Similar behavior is seen in Figure 2.10 inset(B). Note that 6 pN is the stalling force F_s of the wild-type motor.

Similarly, when the load force is close to 12 pN (Figure 10, inset C), the average run-length of a cargo is higher when the number of wild-type motors is greater than the number of mutant motors in the ensemble. Note that the overall run-length values for the three motor ensemble are lower here, since the load force on the cargo is higher (compare 12 pN , Figure 2.10 inset(C) versus 6 pN , Figure 2.10 inset(B)). Also note that 12 pN is twice the

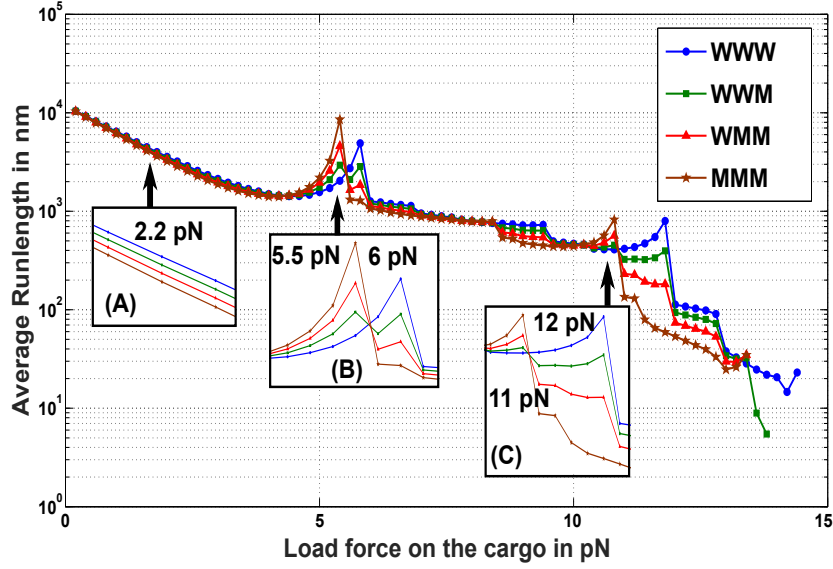


Figure 2.10: **Average Run-length** under different load forces on a cargo with ensembles of three motors, wild type and mutant (WWW, WWM, WMM and MMM).

wild-type motor stalling force F_s .

In Figure 2.9 inset(B), when the load force is close to 5.5 pN , a surge in the run-length is observed, peaking close to 5.5 pN and falling for larger load forces. However, the order of increase in run-length seen here is opposite to that seen near 6 pN , i.e. the run-length is higher for larger number of mutant motors and is lower for a larger number of wild-type motors in the ensemble. Similar behavior is seen in Figure 2.10 inset(B). Note that 5.5 pN is the stalling force $\bar{F}_s$ of the mutant motor.

In Figure 2.10 inset(C), for a load force close to 11 pN , the average run-length is higher when a larger number of mutant motors is present in the ensemble. Note that 11 pN is twice the mutant motor stalling force $\bar{F}_s$.

To explain the reason behind such an observation, we examine the run-length observed for heterogeneous motor ensembles (e.g. WM). In Figure 2.11 we determined the probability for the wild type and mutant motor remaining attached to the microtubule using the conditional steady state probability distributions for various load forces. From Figure 2.11(blue curve) and (2.17), it is evident that the probability of the wild type motor remaining engaged with the microtubule lattice is high when the load force is close to its

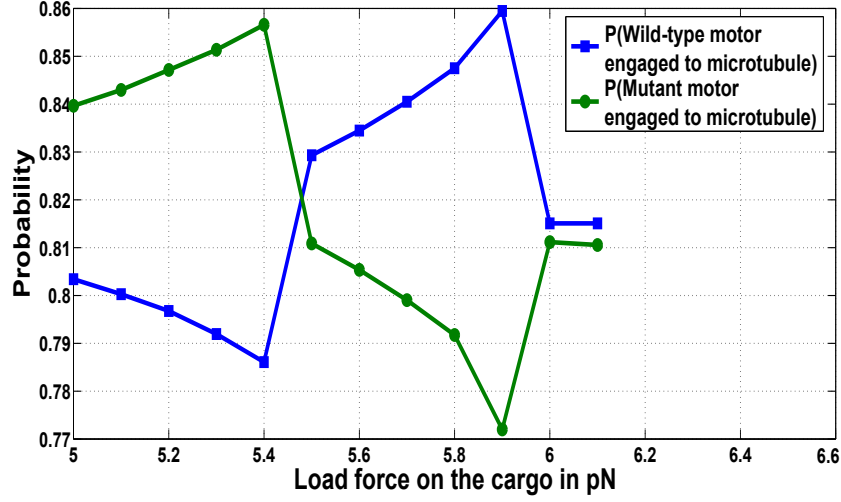


Figure 2.11: Probability of wild-type and mutant motor being attached for ensemble WM.

stalling force $F_S = 6 \text{ pN}$. It can thus be inferred that under this condition, the *mooring mechanism* is activated by the wild-type motor and the cargo remains bound to the microtubule with a higher probability. As a consequence, the other disengaged mutant motor has a greater opportunity to reattach to the microtubule, overcoming the stalled condition of the leading motor and contributing to the increase in run-length. Thus the run-length under these conditions will increase with an increase in the number of wild-type motors, since it is the wild-type motors that will tether the cargo to the microtubule lattice and favoring its continued translocation. The peak in the run-length observed in Figure 2.7 inset(C) for a load force close to 12 pN can similarly be explained. As the two leading wild-type motors stall and remain attached to the microtubule, the third motor will reattach, overcome the stalled condition and promote an extension of the run-length.

The same load dependency of cargo run-lengths is also observed for mutant motors with reduced stall forces. At load force equal to the reduced stall force of $\bar{F}_S$, a mutant motor exhibits an increased probability for remaining attached to the microtubule lattice (from (2.18)) and activates the *mooring mechanism*, preventing the cargo from diffusing away from the microtubule (Figure 2.11, see peak in green trace at 5.5 pN). The tethering by the mutant motor increases the probability that the disengaged wild type motor will reattach, overcome the stalled mutant motor and extend the cargo runlength. The same effect also

explains the positive correlation between run-length and number of mutant motors at load forces close to $\bar{F}_s$ and multiples of $\bar{F}_s$, as the probability of having mutant motors that are stalled increases making the loss of cargo more unlikely.

Significantly, in these load force ranges and for a fixed number of motors attached to the cargo, the cargo with more mutant motors attached will travel further than other cargoes with fewer or no mutant motors attached. Remarkably, the run-length of the cargo is predicted by the single motor characteristics, namely the stalling force, of the mutant motor. One can test these inferences *in-vitro* by attaching a quantified number of wild-type and mutant motors to a cargo and using an optical tweezer to exert appropriate load forces to measure the effect of ensemble heterogeneity on average distance traversed by the cargo. The relevance of studying cargo under various load forces to in-vivo functionality is detailed in [29].

ATP Concentration can Tune Multiple Motor Travel

It is known that kinesin hydrolyzes one ATP per step [26]. Thus, the rate at which kinesin steps depends on its rate of ATP hydrolysis. It was shown experimentally in [18] that under no load conditions, average velocity for ensembles having one and two motors decreases with decreasing ATP concentration. It was also shown that run-length was not appreciably affected by changing ATP concentrations when the cargo had only one kinesin attached. However, when the cargo has two kinesin motors attached, run-length was demonstrated to have a strong negative correlation with single motor velocity (and single motor velocity was shown to have a positive correlation with ATP concentration). Our semi-analytic method was used to assess the conclusions reached in [18] and to further understand how the presence of mutant motors (with a different stalling force $\bar{F}_s$) can impact the ATP dependence of run length. The altering of this behavior due to the presence of more than two motors in the ensemble is also studied.

Average run-length for multiple motor ensemble negatively correlates to ATP concentration when restricted to certain regimes

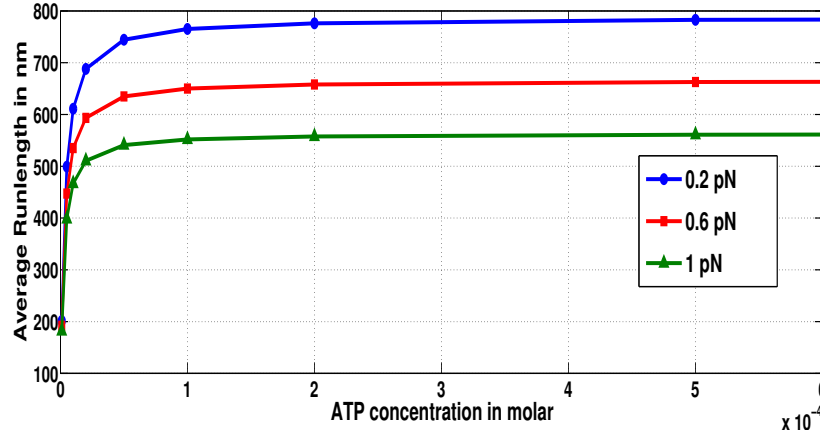


Figure 2.12: **Effect of ATP concentration on run-length for ensemble containing one wild-type motor, for various hindering load forces on the cargo.**

We first considered the affect of ATP concentration on run-length of a cargo containing only a single wild type motor (Figure 2.12). Our analysis shows that as ATP concentration increases, the distance traveled by the cargo also increases monotonically until ATP concentration saturates. When the cargo carried by a single wild-type motor is subjected to 0.2 pN load force, the effect of ATP concentration on run-length is not substantial for concentrations above 10 μM (611 nm at 10 μM ATP and 786 nm at 2 mM ATP). Thus run-length of a cargo with a single wild-type motor is not substantially affected by ATP concentration, agreeing with the trend observed in [18].

However, the effect of ATP concentration is more pronounced for ensembles containing more than one motor. For ensembles comprised of multiple motors, decreased ATP concentrations under certain conditions leads to increased average run-length of the cargo. For example, in both the WW (Fig 2.13) and WWW (Figure 2.17) ensembles, we observe that with reducing ATP concentration, the run-length first increases, peaks at a certain value and then decreases. The average velocity of cargo transport will decrease with decreasing ATP concentration (see Figure 2.15 for WW and Figure 2.16 for WWW), indicating that the increased run-length is not due to increasing velocity but instead reflects the increased probability that the cargo remains attached to the microtubule for a longer time. As is evident from Figure 2.14 for WW ensemble, not only does the probability of attachment increase in the presence of two motors by comparison to one motor, but the probability

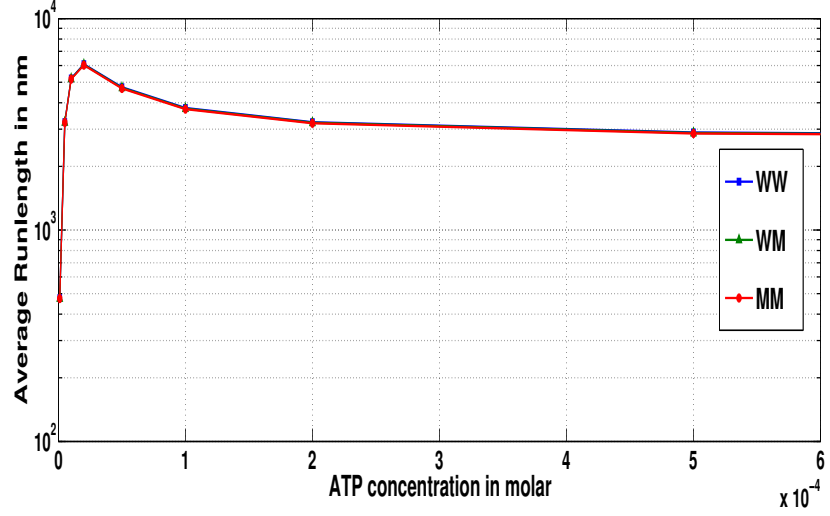


Figure 2.13: **Effect of ATP concentration on run-length for ensembles WW, WM and MM against load force of 0.2 pN.**

that the motors remain attached to the microtubule also increases as the ATP concentration is decreased. A similar argument is provided for the experimental observations made by Xu and team in [18] for ensemble WW, that reported a negative correlation between single-motor velocities and run-length of cargo transport by ensemble WW.

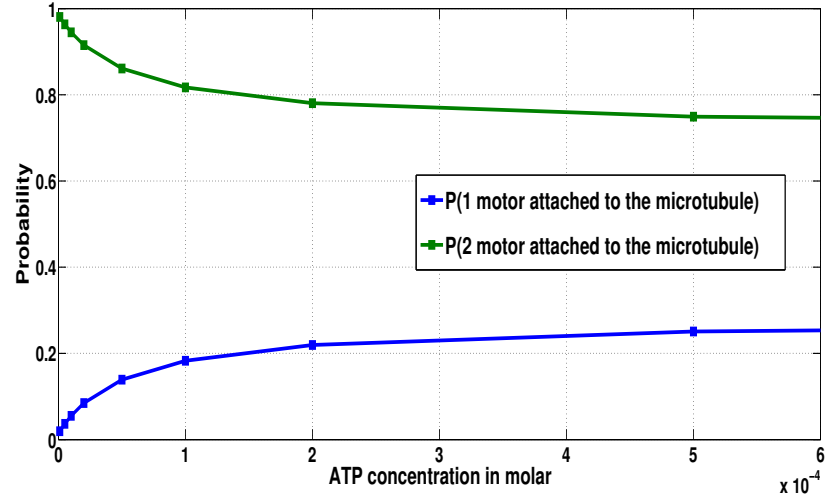


Figure 2.14: **Effect of ATP concentration on run-length for ensemble WW.**

Furthermore, the results also predict a negative correlation will persist until a certain minimum ATP concentration, below which the average run-length starts decreasing. To summarize, the runlength cannot continue to increase as ATP concentration falls, since at a

certain threshold the low ATP concentrations will hinder ATP hydrolysis and the stepping of the motor domains along the microtubule lattice. At this limiting ATP concentration, regardless of how long the cargo remains attached, the motors will no longer be able to translocate along the microtubule lattice and runlength will decrease.

In conclusion, the ATP concentration in certain regimes negatively correlates with cargo run-length, as long as there are more than one motor attached to the cargo. Such behavior observed for a two motor ensemble agrees with results reported in [18].

The heterogeneity of motors within an ensemble does not alter the effect of ATP concentration on run-length

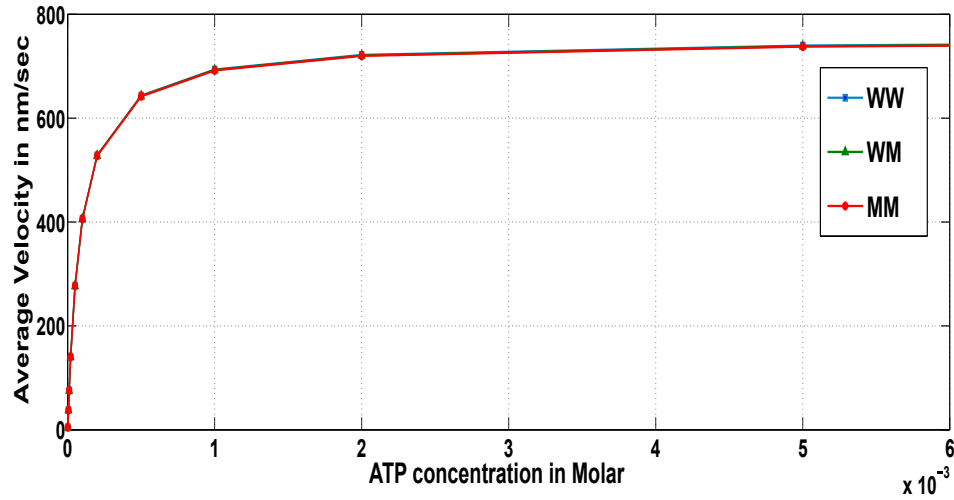


Figure 2.15: Effect of ATP concentration on average velocity for 2-motor ensembles WW, WM and MM against load force of 0.2 pN .

To analyze the impact of ATP concentration on the behavior of ensembles with multiple motors, we characterized the average run-length and velocity for ensembles with two motors (Figure 2.13 and Figure 2.15) and three motors (Figure 2.17 and Figure 2.16).

Ensembles with mutant motors also exhibited a similar trend of increasing run-length as ATP concentration was reduced under fixed load forces (Figure 2.18, WM ensemble). When compared with other two-motor ensembles (Figure 2.13, load force is 0.2 pN), it is seen that at constant load force and ATP concentrations, the cargoes carried by ensembles WW, WM

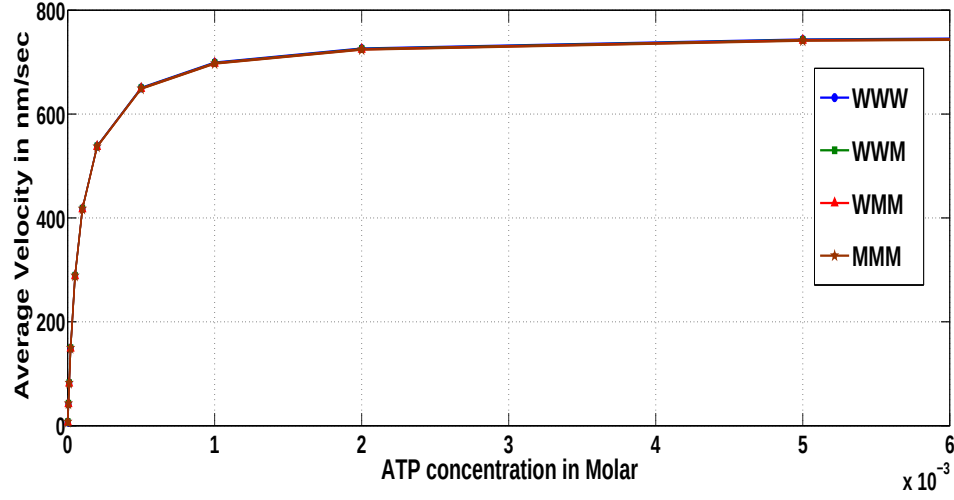


Figure 2.16: **Effect of ATP concentration on average velocity for 3-motor ensembles WWW, WWM, WMM and MMM against load force of 0.2 pN.**

and MM exhibit almost the same average run-length. Thus, if the ATP concentration and number of motors within the ensemble is constant and if the load force does not approach the stall force (or multiples of stall force) for either wild type or mutant (i.e. F_s or $\bar{F}_s$), then the average run-length will not be affected by the number of mutant motors in the ensemble. Similar conclusions hold for ensembles with three motors.

When load forces are close to, but less than, the stalling force F_s for the same number of motors in the ensemble, then irrespective of the ATP concentration the ensembles with more wild-type motors will dominate cargo transport and exhibit longer run-lengths. In contrast, if load forces are close to, but less than $\bar{F}_s$, then ensembles with more mutant motors will dominate cargo transport and exhibit longer run-lengths.

2.2 Biological Impact

2.2.1 Single motor mutation influences the emergent properties of cargo transport

We developed a simulation engine to study the transport properties of a cargo carried by multiple motor proteins of different types. To demonstrate the efficacy of our methodology we capitalized on a previous study by Selvin and coworkers [22] which characterized a kinesin

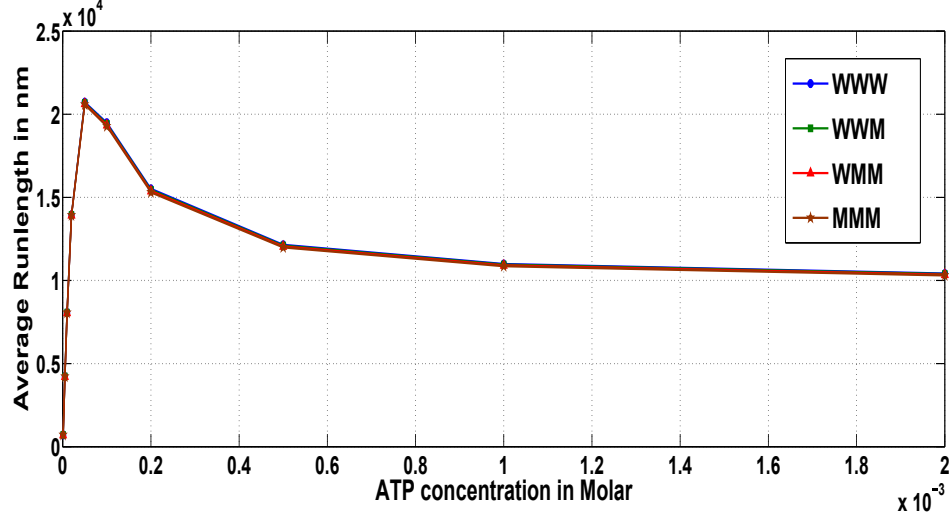


Figure 2.17: **Effect of ATP concentration on average runlength for 3-motor ensembles WWW, WWM, WMM and MMM against load force of 0.2 pN**

motor that exhibits a reduced stall force. The study reported that placing a negative charge at the Ser-175 position of the kinesin motor domain, through either mutation or phosphorylation, reduces the stalling force of the motor and decreases its average velocity under external loads. We incorporate this into our method to enable an analysis of the behavior of ensembles comprising of several wild-type and mutant kinesin motors, where the mutant motors have lower stalling forces. The analysis provides support for the following conclusions :

1. A mutation that modulates stalling force of the motors impacts the emergent transport properties of a molecular motor ensemble as follows :

- (a) For a cargo bound to a fixed number of motors and under a constant load force away from the mutant motor stalling force $\bar{F}_s$ (or its multiples), the average velocity and run-length decreases with an increase in the number of mutant motors in the ensemble. A similar trend is observed for a load force close to the wild-type motor's stalling force F_s (or its multiples).

Such a behavior is expected, since cargoes carried by ensembles having a higher proportion of mutant motors (that have a reduced stalling force) would traverse shorter run-lengths on average. Under these conditions, ensembles with a higher

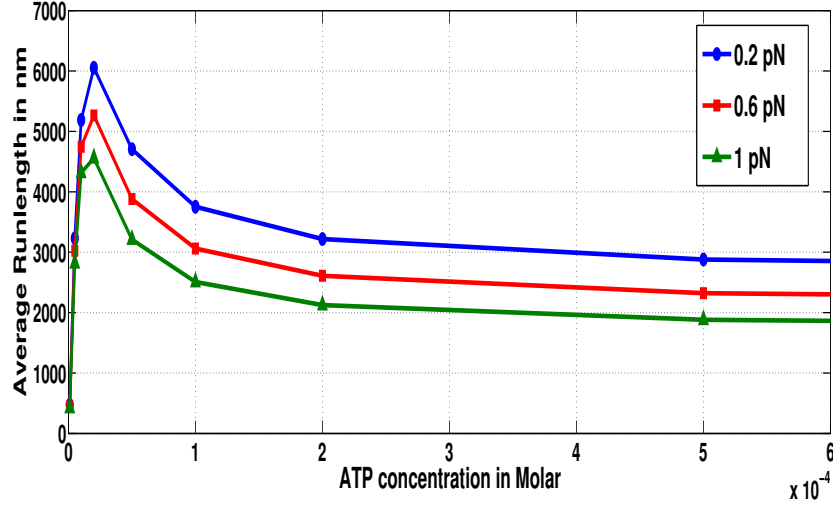


Figure 2.18: **Effect of ATP concentration on run-length for ensemble WM, for various hindering load forces on the cargo.**

mutant population will eventually lag behind similar-sized ensembles with a lower mutant population.

- (b) Remarkably, for a cargo subjected to load forces close to the mutant motor's stalling force $\bar{F}_s$ (or its multiples), the average run-length increases with an increase in the number of mutant motors in the ensemble. We hypothesize the existence of a *mooring mechanism*, where mutant motors tether the cargo to the microtubule, providing the other detached motors with a greater opportunity to reattach to the microtubule and move the cargo forward. It consequently leads to a higher average run-length of the cargo. Thus, surprisingly under these conditions, ensembles with a higher population of mutants gain an aggressive edge over similar sized ensembles with a lower mutant population.

These results are similar to the observations in [30, 31], where transport of microtubules by varying populations of two kinesin motors, that have a large disparity in average velocities and force dependent dissociation rates, is dictated by a small proportion of faster motors.

2. ATP concentration modulates cargo transport by multi-motor ensembles as follows :

- (a) ATP concentration in specific ranges negatively correlates with average run-length of the cargo under a constant load force, if the cargo has more than one motor attached. This finding agrees with the experimental observation made by Xu and coworkers for a 2-motor ensemble in [18].
 - (b) For a fixed number of motors bound to the cargo, the effect of ATP concentration on run-length is independent of the ratio of wild-type and mutant motors.
3. While we used a kinesin phosphorylation mutant [22] to explore the consequences of a multi-motor ensemble containing motors of lower stall forces, similar analysis and the computational methodology can be utilized to obtain insights on the effect of different types of motors modeled by different sets of parameters on the transport of a common cargo. Moreover, mutations that impact other single motor parameters, including on/off rates, directionality, and elasticity, could be evaluated for impacts on ensemble behavior.

It is likely that the aberrant behavior caused due to the presence of mutant motors with lower stalling forces, when occurring over multitudes of ensembles, can contribute to the disruption of cargo traffic, impediment of neuronal function and the emergence of neurodegeneration.

2.2.2 Simulation engine developed for evaluating the impact of single motor parameters on behavior of motor ensembles

The computational methodology proposed by Gross and coworkers in [17] employed Monte Carlo simulations to offer several insights regarding transport properties of molecular motor ensembles. In this article, we have developed a novel, semi-analytical approach to study the effect of single motor mutations on the behavior of motor ensembles and cargo transport. This methodology allows us to compute exactly, the biologically relevant quantities such as average run-length and velocity for an ensemble of motors using experimental parameters derived from single motor experiments. The single motor parameters chosen in this article for kinesin-1 motors are obtained from experimental data in [17]. The model correctly

reproduces the force-velocity curve and run lengths as a function of load for single kinesin-1 motor as reported in [32]. The results for ensembles WW and WWW (i.e. all wild-type motors) are in exact quantitative agreement with those obtained via Monte Carlo simulations as presented in [17] as well as [19], fully validating the methodology. For a two-motor ensembles, the results obtained using our model agree with the inferences made in [33, 29]. Unlike Monte Carlo approaches, this model is computationally less extensive and its efficiency is independent of the number of iterations. The model also offers ways to investigate rare events and can be extended to any species of motor proteins, given the knowledge of their individual parameters. It is also easily possible to interrogate specific single motor parameters and to determine their effect on ensemble behavior, thus making it a useful tool to study the contribution of other single motor mutations as well as post translational modifications to the transport behavior of cargoes.

2.3 Methods

The Master Equation based methodology used to obtain the aforementioned results is described in this section. This method is used to study emergent properties of an ensemble of multiple motors of two types, that can each take a step on, detach from or reattach onto the microtubule. The knowledge of transition probabilities of stepping, detachment and attachment enable the determination of transition rates between various *absolute configurations* of the motors, allowing for the calculation of transition rates between the corresponding *relative configurations*. These rates enable the calculation of the probability distribution of the various ensemble configurations, thereby facilitating the computation of several biologically relevant quantities such as average velocity, run-length and number of attached motors.

We begin with the construction of the relative state space, along with calculations necessary to arrive at several biologically relevant quantities. Then, a general methodology to obtain the transition rates between absolute configurations given the knowledge of the probability rates of stepping, detachment and reattachment to the microtubule for a wild-type motor(P_S, P_D, P_A) and probability rates for a mutant motor($\bar{P}_S, \bar{P}_D, \bar{P}_A$) is presented.

Finally, the model used to determine the probability rates for kinesin motor proteins is detailed.

2.3.1 Construction of Relative Configuration Space

Consider a cargo that is carried by both wild-type and mutant motor proteins on a microtubule. The microtubule is modeled as directed linear lattice formed by equally sized dimers with dimension d . Here the k^{th} dimer is located at location $\bar{a}_k = kd$ and indexed by the set of integers $I = \{\dots, -2, -1, 0, 1, 2, \dots\}$. Each motor protein bound to the cargo can attach, take a forward step or detach from the microtubule. The *absolute configuration* $\Omega := \left\{ \begin{matrix} \Omega_{h,k} \\ \Omega_{d,k} \end{matrix} \right\}_{k \in I}$ of motor-protein arrangement on the microtubule specifies the number, $\Omega_{h,k}$, of wild-type motor proteins and the number, $\Omega_{d,k}$, of mutant motor proteins at the k^{th} location on the microtubule.

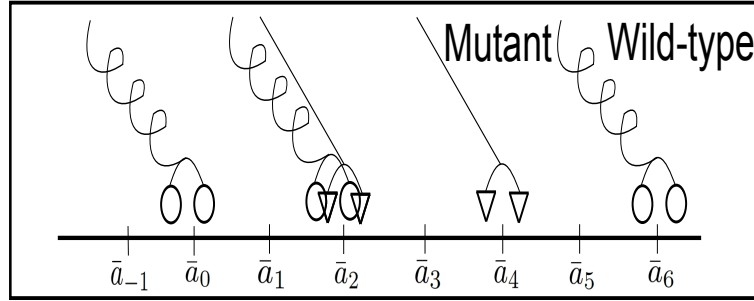


Figure 2.19: **Locations of wild-type and mutant motors on a section of the microtubule lattice.** The microtubule is modeled as a directed linear lattice $\bar{a}_k = \bar{a}_0 + kd$, where $\bar{a}_k$ is the position of the k^{th} location. If all the motors carry a common cargo i.e. the wild-type motor at a_0 is the rearguard motor, the string representation is $|M_h||M_h M_d||M_d||M_h|$

For example, the absolute configuration of the ensemble of motors illustrated in Figure 2.19 is given by

$$\Omega = [\dots \Omega_{-1} \Omega_0 \Omega_1 \Omega_2 \Omega_3 \Omega_4 \Omega_5 \Omega_6 \dots] = \begin{bmatrix} \dots & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 1 & \dots \\ \dots & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & \dots \end{bmatrix}.$$

The *relative configuration* of an ensemble of motors is represented using a string of three symbols. Given an absolute configuration we first identify the *rearguard motor* which is the motor that is attached to the microtubule and lags behind all the other motors on the microtubule. Using the location of the rearguard motor as a reference, the relative

configuration ϑ is obtained as a string of three symbols ' M_h ', ' M_d ' and '|', where ' M_h ' and ' M_d ' denote wild-type and mutant motors respectively, with '|' denoting a separator that distinguishes different microtubule locations. The motor located the farthest from the rearguard motor on the microtubule is identified as the *vanguard motor*.

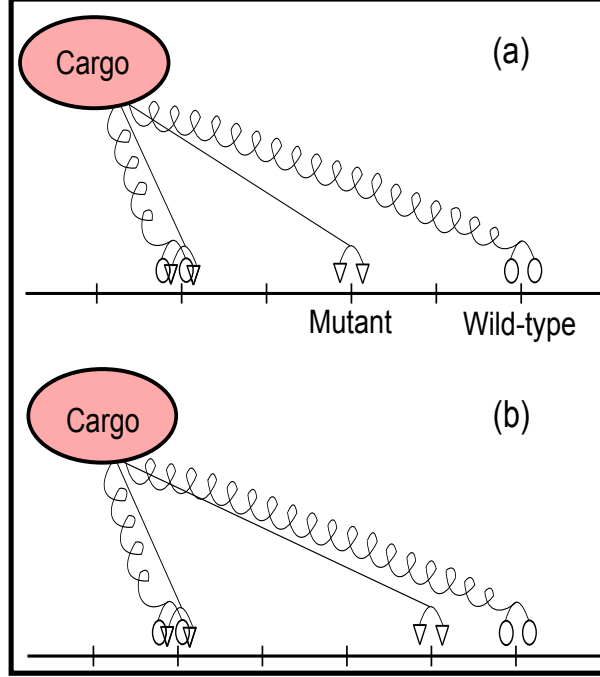


Figure 2.20: **Locations of wild-type and mutant motors on a section of the microtubule lattice.** The string representation for the two configurations is (a) $|M_h M_d||M_d||M_h|$ and (b) $|M_h M_d|||M_d|M_h|$

For example, the relative configuration of the ensemble in Figure 2.20(a) is the string ' $|M_h M_d||M_d||M_h|$ '. The configuration that results after the furthestmost mutant motor in Figure 2.20(a) takes a step is shown in Figure 2.20(b) which has a relative configuration given by ' $|M_h M_d|||M_d|M_h|$ '.

Both the mutant as well as the wild-type motor proteins are characterized via their own set of stepping, attachment, and detachment probabilities (for wild-type (P_S, P_D, P_A) and for mutant $(\bar{P}_S, \bar{P}_D, \bar{P}_A)$). The individual motors for both species are modeled as hookean springs when stretched that offer no resistance when compressed. A single motor is assumed to have a linkage rest length L_0 and spring stiffness constant K_e . Motors of both the species are assumed to not step backward and are bound to the cargo particle irreversibly. More complex models of motor-proteins can be easily accommodated. It is further assumed that

there exists a force F_{stall} called the *stalling force*, where if the force on the motor protein $F \geq F_{stall}$ then the motor does not take a forward step and stepping probability is zero [2]. The stalling force can be that is also used to estimate how many motors are carrying the cargo. For an ensemble of wild-type and mutant motors carrying a cargo, the following result holds:

Result 1 : Given an ensemble of M molecular motors attached to a common cargo that is subjected to a load force F_{load} , the distance between the rearguard and the vanguard motor is bound by

$$n = \max \left\{ \frac{(M+1)\max(F_s, \bar{F}_s) - F_{load}}{K_e} + d, \frac{F_{load}}{K_e} \right\} + 2L_0 \quad (2.1)$$

where F_s is the minimum load force for which the stepping probability of the wild-type motor protein becomes zero (i.e. the stalling force for the wild-type motor protein), $\bar{F}_s$ is the minimum load force for which the stepping probability of the mutant motor protein becomes zero (i.e. the stalling force for the mutated motor protein), L_0 is the rest length of the motor linkage, K_e is the linkage stiffness and d is the step-size of the motor.

A detailed derivation is provided below.

Proof :

Consider an ensemble of wild-type and mutant motors bound to a cargo with the following assumptions :

- There are a total of M (wild-type and mutant) motors bound to the cargo. The wild-type motor stalling force is F_s and the mutant motor stalling force is $\bar{F}_s$.
- The motor linkages have a rest length of L_0 and stiffness K_e . The linkages are modelled as hookean springs when stretched that offer no resistance when compressed.
- There exists a constant load force F_{load} on the cargo.
- Z_{eq} is the mean position of the cargo, which is the equilibrium position determined by the forces exerted on the cargo by the motors through their linkages and the load force F_{load}

- Any unattached motor bound to the cargo can attach to only those locations on the microtubule that are within L_0 distance of the cargo position.

For the discussion below, it is assumed that there is at least one motor on the cargo opposing its motion (for other cases, similar arguments can be used). Suppose there be f motors assisting the cargo motion and r motors opposing it ($f + r \leq M$). Let the locations of the f motors on the microtubule assisting the cargo motion be $Z_1^+, Z_2^+, \dots, Z_f^+$. The linkage length of a motor located at Z_i^+ is given by $L_i^+ = Z_i^+ - Z_{eq}$ and the force it exerts on the cargo is $F_i^+ = K_e(L_i^+ - L_0)$. If the motors are located in such a way that $0 < L_0 \leq L_1^+ \leq L_2^+ \leq \dots \leq L_f^+$, then $F_1^+ \leq F_2^+ \leq \dots \leq F_f^+$ and the total force exerted on the cargo in the forward direction is $F_{fwd} = \sum_{i=1}^f F_i^+$.

Let the locations of the r motors on the microtubule opposing the cargo motion be $Z_1^-, Z_2^-, \dots, Z_r^-$. The linkage length of a motor located at Z_j^- is given by $L_j^- = Z_{eq} - Z_j^-$ and the force it exerts on the cargo is $F_j^- = K_e(L_j^- - L_0)$. If the motors are located in such a way that $0 < L_0 \leq L_1^- \leq L_2^- \leq \dots \leq L_r^-$, then $F_1^- \leq F_2^- \leq \dots \leq F_r^-$ and the total force exerted on the cargo in the backward direction is $F_{back} = \sum_{j=1}^r F_j^-$. The separation between the vanguard and rearguard motor can be found as $S = L_f^+ + L_r^-$ where L_f^+ and L_r^- are the linkage lengths of the vanguard and rearguard motor respectively.

Suppose the vanguard motor (thereby all the motors) is not stalled. Then $F_{fwd} < M \max(F_s, \bar{F}_s)$. If the vanguard motor is a wild-type motor, $F(L_f^+) < F_s$ thus $L_f^+ < \frac{F_s}{K_e} + L_0$ and if it is a mutant motor, $F(L_f^+) < \bar{F}_s$ thus $L_f^+ < \frac{\bar{F}_s}{K_e} + L_0$. In general $L_f^+ < \frac{\max(F_s, \bar{F}_s)}{K_e} + L_0$. At equilibrium, $F_{back} = F_{fwd} - F_{load}$, thus $\sum_{j=1}^r F_j^- = F_{fwd} - F_{load}$ which implies that $F(L_r^-) + \sum_{j=1}^{r-1} F_j^- = F_{fwd} - F_{load}$.

It follows that $F(L_r^-) \leq F_{fwd} - F_{load}$. Substituting for $F(L_r^-)$ it follows that $K_e(L_r^- - L_0) < M \max(F_s, \bar{F}_s) - F_{load}$ and thus $L_r^- < \frac{M \max(F_s, \bar{F}_s) - F_{load}}{K_e} + L_0$. Thus the separation S between the vanguard and rearguard motor when none of the motors are stalled is given as,

$$S < \frac{(M+1)\max(F_s, \bar{F}_s) - F_{load}}{K_e} + 2L_0. \quad (2.2)$$

Let $n_{max} := \frac{(M+1)\max(F_s, \bar{F}_s) - F_{load}}{K_e} + d + 2L_0$. Thus, if none of the motors are stalled then the separation $S < (n_{max} - d)$.

Consider a current configuration where the separation $S' < n_{max}$. In the current configuration, the vanguard motor is either in stalled (Case I) or not stalled (Case II) condition.

Case I : If the vanguard motor is stalled, then any ensuing change in the configuration will only decrease the separation since the vanguard motor cannot step forward and the rearguard motor cannot step backward. Thus the separation remains less than n_{max} .

Case II : If the vanguard motor is not stalled, then using (2.2) it can be stated that the separation $S' < (n_{max} - d)$. Any change in the current configuration that can increase the separation is if the vanguard motor takes a forward step of length d , where the new separation $(S' + d) < n_{max}$. Thus the separation remains less than n_{max} .

It can thus be stated that if in a current configuration the separation is less than a bound n_{max} then for any subsequent configuration the bound is obeyed.

It is to be noted that the absolute configuration space admits infinitely many representations as there is always a small probability of finding the cargo at any location on the microtubule. However, the above result concludes, that given a stall force for both wild-type and mutant motors the relative configuration space is finite, since there are no motors beyond n units away from the rearguard motor in any relative configuration.

Transition Probabilities between Relative Configurations

Next, we determine the probability of transitioning from a relative configuration to another from the transition probabilities in the absolute configuration space.

It is evident that an absolute configuration can be associated with a unique relative configuration. For example, the relative configuration associated with Figure 2.19 is $|M_h||M_hM_d||M_d||M_h|$. Thus the absolute configuration

$$\Omega = \begin{bmatrix} \cdots & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 1 & \cdots \\ \cdots & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & \cdots \end{bmatrix}$$

is mapped to the relative configuration $|M_h||M_hM_d||M_d||M_h|$. We denote the projection operator Υ that maps an absolute configuration Ω to a corresponding relative configuration by $\Upsilon(\Omega)$.

The probability that the absolute configuration is Ω' at time $t + \Delta t$ conditioned on an initial configuration of Ω at t is represented as $P_\Omega(\Omega', t + \Delta t | \Omega, t)$. It is assumed that the

transition probability $P_{\Omega}(\Omega', \Omega)$ between the two absolute configurations Ω and Ω' is given by $\nu_{\Omega}(\Omega', \Omega)\Delta t$ for a small time interval Δt , where the notation $\nu_{\Omega}(\Omega', \Omega)$ represents the *probability rate of transition* between Ω and Ω' . The underlying assumption that the rate $\nu_{\Omega}(\Omega', \Omega)$ is independent of the time instant t holds true, since the ν_{Ω} only depends upon the initial configuration and the type of transition (motor stepping, detachment or attachment) from Ω to Ω' .

In a similar manner, the transition probability that the relative configuration is ϑ' at time $t + \Delta t$ given that it is ϑ at time t is denoted by $P_{\vartheta}(\vartheta', t + \Delta t | \vartheta, t)$. The *transition probability* $P_{\vartheta}(\vartheta, \vartheta')$ between two relative configurations ϑ and ϑ' is expressed as $\nu_{\vartheta}(\vartheta', \vartheta)\Delta t$ where $\nu_{\vartheta}(\vartheta', \vartheta)$ denotes the probability rate of transition between ϑ and ϑ' .

The knowledge of the transition rate ν_{Ω} in the absolute configuration space enables the following result :

Result 2 : The rate of transition between relative configurations ϑ and ϑ' is given by :

$$\nu_{\vartheta}(\vartheta', \vartheta) = \sum_{0 \leq \beta \leq \lceil \frac{n}{d} \rceil} \nu_{\Omega}(\tau^{\beta} \Omega', \Omega) \quad (2.3)$$

where Ω is any absolute configuration that satisfies $\Upsilon(\Omega) = \vartheta$, Ω' is any absolute configuration that satisfies $\Upsilon(\Omega') = \vartheta'$, $(\tau^{\beta} \Omega')$ is an absolute configuration obtained after linearly shifting all the motors in Ω' by β locations on the microtubule towards the right and d is the dimension of a single microtubule dimer

A detailed derivation of (2.3.1) is provided below :

Transition rates between relative configurations

Here the validity of Result 2 is established.

Result 2 : The rate of transition between relative configurations ϑ and ϑ' is given by

$$\nu_{\vartheta}(\vartheta', \vartheta) = \sum_{0 \leq \beta \leq \lceil \frac{n}{d} \rceil} \nu_{\Omega}(\tau^{\beta} \Omega', \Omega),$$

where $\Upsilon(\Omega) = \vartheta$, $\Upsilon(\Omega') = \vartheta'$, $(\tau^{\beta} \Omega')$ is an absolute configuration obtained after linearly shifting all the motors in Ω by β locations on the microtubule towards the right and d is the

Let Ω and Ω' be a pair of absolute configurations such that $\Upsilon(\Omega) = \vartheta$ and $\Upsilon(\Omega') = \vartheta'$. It is assumed that the system is translation invariant with its stochastic behavior unaffected if the cargo and the ensemble of motors bound to it shift to a new location along the microtubule. The translation invariance property enables the construction of a set $\Omega(\vartheta)$ of all absolute configurations that have the same relative configuration ϑ as $\Omega(\vartheta) = \{\tau^\beta \Omega : \beta \in I\}$, where I is the set of integers. In a similar manner, $\Omega(\vartheta') = \{\tau^{\beta'} \Omega' : \beta' \in I\}$

Using the definition of transition probability, $P_{\vartheta}(\vartheta', t + \Delta t | \vartheta, t)$ can be expressed as follows :

$$\begin{aligned}
P_{\vartheta}(\vartheta', t + \Delta t | \vartheta, t) &= \frac{P_{\vartheta}(\vartheta', t + \Delta t, \vartheta, t)}{P_{\vartheta}(\vartheta, t)} \\
&= \frac{\sum_{\Omega \in \Omega(\vartheta)} \sum_{\Omega' \in \Omega(\vartheta')} P_{\Omega}(\Omega', t + \Delta t, \Omega, t)}{\sum_{\Omega \in \Omega(\vartheta)} P_{\Omega}(\Omega, t)} \\
&= \frac{1}{\sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t)} \sum_{\beta} \sum_{\beta'} P_{\Omega}(\tau^{\beta'} \Omega', t + \Delta t, \tau^{\beta} \Omega, t) \\
&= \frac{1}{\sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t)} \sum_{\beta} \sum_{\beta'} P_{\Omega}(\tau^{\beta'} \Omega', t + \Delta t | \tau^{\beta} \Omega, t) P_{\Omega}(\tau^{\beta} \Omega, t) \\
&= \frac{1}{\sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t)} \sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t) \sum_{\beta'} P_{\Omega}(\tau^{\beta'} \Omega', t + \Delta t | \tau^{\beta} \Omega, t) \\
&= \frac{1}{\sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t)} \sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t) \sum_{\beta'} P_{\Omega}(\tau^{(\beta' - \beta)} \Omega', t + \Delta t | \Omega, t) \\
&= \frac{1}{\sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t)} \sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t) \sum_{\beta'} P_{\Omega}(\tau^{\beta'} \Omega', t + \Delta t | \Omega, t) \\
&= \sum_{\beta'} P_{\Omega}(\tau^{\beta'} \Omega', t + \Delta t | \Omega, t) \\
&= \sum_{\beta'} \nu_{\Omega}(\tau^{\beta'} \Omega', \Omega) \Delta t.
\end{aligned}$$

In the third equality, $\sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t)$ is performed over all shifts β of an absolute configuration Ω such that its projection is the relative configuration ϑ i.e. $\Upsilon(\Omega) = \vartheta$. Similarly while determining $\sum_{\beta} \sum_{\beta'} P_{\Omega}(\tau^{\beta'} \Omega', t + \Delta t, \tau^{\beta} \Omega, t)$, the absolute configuration Ω' satisfies $\Upsilon(\Omega') = \vartheta'$.

In the sixth equality, translation invariance property is applied wherein both the absolute

configurations at t and $t + \Delta t$ are shifted by β places to the left (via the operation $\tau^{-\beta}$). For the seventh equality, the set $\{\tau^{(\beta' - \beta)}\} = \{\tau^{\beta'}\}$, since β' is any integer and β is fixed. Moreover, since Ω has been arbitrarily chosen with the only condition that $\Upsilon(\Omega) = \vartheta$, this will hold for every $\Omega \in \Omega(\vartheta)$. Using the definition that $P_{\vartheta}(\vartheta', t + \Delta t | \vartheta, t) = \nu_{\vartheta}(\vartheta', \vartheta) \Delta t$, $\nu_{\vartheta}(\vartheta', \vartheta) \Delta t = \sum_{\beta'} \nu_{\Omega}(\tau^{\beta'} \Omega', \Omega) \Delta t$ and thus,

$$\nu_{\vartheta}(\vartheta', \vartheta) = \sum_{\beta'} \nu_{\Omega}(\tau^{\beta'} \Omega', \Omega).$$

Since for a finite number of motors bound to a cargo the maximum distance between the vanguard and rearguard motor is finite (and equal to $\lceil \frac{n}{d} \rceil$ locations on the microtubule),

$$\nu_{\vartheta}(\vartheta', \vartheta) = \sum_{0 \leq \beta \leq \lceil \frac{n}{d} \rceil} \nu_{\Omega}(\tau^{\beta} \Omega', \Omega).$$

As n is finite for a finite number of molecular motors attached to the cargo (from (2.1)), (2.3.1) involves only a finite number of computations. The transition rates between absolute configurations are obtainable using the chemical kinetics of the motor protein as it steps, detaches or attaches to the microtubule. The rate $\nu_{\vartheta}(\vartheta', \vartheta)$ is obtainable using *Result 2* and the knowledge of the rates ν_{Ω} between corresponding absolute configurations.

Results 1 and 2 together imply that given a finite number of molecular motors carrying a common cargo, the relative configuration space is finite. Thus, by projecting or aggregating the states of the infinite dimensional model (consisting of absolute configuration Ω' s) into ‘macro-states’, one can obtain a finite dimensional model (see [34] for related work). Thus, if H is the set of all the possible relative configurations, the knowledge of transition rates from (2.3.1) can be used to show that the probability $P_{\vartheta}(\vartheta, t)$ of the relative configuration being ϑ at time t satisfies the master equation,

$$\frac{\partial}{\partial t} P_{\vartheta}(\vartheta, t) = \sum_{\vartheta' \in H} \nu_{\vartheta}(\vartheta, \vartheta') P_{\vartheta}(\vartheta', t) - P_{\vartheta}(\vartheta, t) \sum_{\vartheta' \in H} \nu_{\vartheta}(\vartheta', \vartheta). \quad (2.4)$$

Evolution of Probability Distribution of Relative Configuration

Solution for the master equation (2.4) determines the time evolution of probabilities of all the relative configurations. Note that (2.4) is an *exact description* of how the probability distribution evolves in the relative configuration space without any approximations. Consider a ordering of all relative configurations given by $\vartheta_1, \dots, \vartheta_N$ (where N is the finite

number of relative configurations) where the probability of finding the motors in a relative configuration ν_i at time t is denoted by $P_i(t)$ and let $P(t) = [P_1(t), \dots, P_N(t)]^T$. Using the expression of transition rates $\nu_\vartheta(\vartheta_j, \vartheta'_i)$ and (2.4), it can be shown that the dynamics of the model describing the vector $P(t)$ is given by :

$$\frac{d}{dt}P(t) = \Gamma P(t),$$

where $\Gamma \in \mathcal{R}^{N \times N}$ is a sparse stochastic matrix which is determined by the transition rates $\nu_\vartheta(\vartheta_j, \vartheta_i)$ ($\Gamma_{ji} = \nu_\vartheta(\vartheta_j, \vartheta_i)$ if $i \neq j$, $\Gamma_{ii} = 1 - \sum_{i \neq j} \nu_\vartheta(\vartheta_j, \vartheta_i)$). Given a specified initial probability vector $P(t_0)$, it follows that

$$P(t) = e^{\Gamma(t-t_0)} P(t_0). \quad (2.5)$$

When specified for kinesin-1 motors and realistic values of number of motors ($M \leq 5$) and system parameters, the dimension of Γ is $N \times N$ where N lies between 10^5 and 10^7 , making the evaluation of $e^{\Gamma(t-t_0)}$ manageable using a standard computer (the results in this article are obtained using Intel quad core i5 processor, 3.4 GHz, RAM 8 GB). The software is easily implementable using platforms like MATLAB and is faster and computationally more efficient than Monte-Carlo based approaches. Computer clusters can be used to manage more complex scenarios involving larger number of motors.

Calculating Biologically Relevant Quantities

Once the probability vector $P(t)$ is known, expressions of several biologically relevant quantities can be obtained in the following manner.

Average Number of Engaged Motors (Wild-type/Mutant)

Average number $m_h(t)$ of wild-type motors attached to the microtubule is given by

$$\langle m_h(t) \rangle = \sum_{i=1}^N m_h(\vartheta_i) P_i(t), \quad (2.6)$$

where $m_h(\vartheta_i)$ represents the number of wild-type motors in the relative configuration ϑ_i or the number of M_h symbols in the representation ϑ_i . In a similar manner, the average number of mutant motors $m_d(t)$, attached to the microtubule is given by

$$\langle m_d(t) \rangle = \sum_{i=1}^N m_d(\vartheta_i) P_i(t), \quad (2.7)$$

where $m_d(\vartheta_i)$ represents the number of mutant motors in the configuration ϑ_i or the number of M_d symbols in the representation ϑ_i .

Average Velocity and Average Run-length

Average velocity $v(t)$ of the cargo being carried by M motors is determined as

$$v(t) = \sum_{\vartheta \in H} \sum_{\vartheta' \in H} d_{avg}(\vartheta', \vartheta) \nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t), \quad (2.8)$$

where $d_{avg}(\vartheta', \vartheta)$ is the expected change in cargo position, when the initial and final relative configurations at t and $t + \Delta t$ are restricted to being ϑ and ϑ' respectively and is given by

$$d_{avg}(\vartheta', \vartheta) = \frac{1}{\nu_{\vartheta}(\vartheta', \vartheta)} \sum_{0 \leq \beta \leq n_{max}} d(\rho^{\beta} \Omega', \Omega) \nu_{\Omega}(\rho^{\beta} \Omega', \Omega). \quad (2.9)$$

The expression $d(\Omega', \Omega)$ in (2.9) is the change in cargo equilibrium position when the absolute configuration changes from Ω to Ω' . The average velocity of the cargo (2.8) and (2.9) is determined by undertaking the following steps :

Step I : The expected change in cargo position when the initial and final relative configurations at t and $t + \Delta t$ are restricted to being ϑ and ϑ' respectively, denoted by $d_{avg}(\vartheta', \vartheta)$, is obtained.

Step II : The expected change in the cargo position in time Δt , denoted as $D_{\Delta t}$ is obtained by removing the restriction of the relative configurations being ϑ and ϑ' at t and $t + \Delta t$. The average velocity of the cargo at time t follows as,

$$v(t) = \frac{D_{\Delta t}}{\Delta t}$$

To obtain $d_{avg}(\vartheta', \vartheta)$ the following steps are undertaken :

1. The change in cargo equilibrium position,

$$d(\Omega', \Omega) = Z_{eq}(\Omega') - Z_{eq}(\Omega),$$

is determined for every possible transition from an initial absolute configuration Ω at time t to a final absolute configuration Ω' at time $t + \Delta t$, where Ω and Ω' are chosen such that $\Upsilon(\Omega) = \vartheta$ and $\Upsilon(\Omega') = \vartheta'$.

2. The probability $P(\Omega', t + \Delta t, \Omega, t | \vartheta', t + \Delta t, \vartheta, t)$ of transitioning from Ω to Ω' in time Δt is determined for every such pair of absolute configurations (Ω, Ω') , conditioned on the fact that the relative configuration also transitions from ϑ to ϑ' in the same time Δt .
3. A weighted sum of $d(\Omega', \Omega)$ with the weights given by the probabilities $P(\Omega' t + \Delta t, \Omega, t | \vartheta', t + \Delta t, \vartheta, t)$ is obtained.

Starting with any pair of absolute configurations Ω and Ω' satisfying the conditions $\Upsilon^{(e)}(\Omega) = \vartheta$ and $\Upsilon^{(e)}(\Omega') = \vartheta'$, the expected change in cargo position $d_{avg}(\vartheta', \vartheta)$, when the initial and final relative configurations at t and $t + \Delta t$ are restricted to being ϑ and ϑ' respectively, is given by

$$\begin{aligned}
d_{avg}(\vartheta', \vartheta) &:= \sum_{\Omega \in \Omega(\vartheta)} \sum_{\Omega' \in \Omega(\vartheta')} d(\Omega', \Omega) P(\Omega', t + \Delta t, \Omega, t | \vartheta', t + \Delta t, \vartheta, t) \\
&= \sum_{\beta} \sum_{\beta'} d(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) P(\tau^{\beta'} \Omega', t + \Delta t, \tau^{\beta} \Omega, t | \vartheta', t + \Delta t, \vartheta, t) \\
&= \sum_{\beta} \sum_{\beta'} d(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) \frac{P(\tau^{\beta'} \Omega', t + \Delta t, \tau^{\beta} \Omega, t, \vartheta', t + \Delta t, \vartheta, t)}{P(\vartheta', t + \Delta t, \vartheta, t)} \\
&= \sum_{\beta} \sum_{\beta'} d(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) \frac{P(\tau^{\beta'} \Omega', t + \Delta t, \tau^{\beta} \Omega, t)}{P(\vartheta', t + \Delta t, \vartheta, t)} \\
&= \sum_{\beta} \sum_{\beta'} d(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) \frac{\nu_{\Omega}(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) P_{\Omega}(\tau^{\beta} \Omega, t)}{\nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t)} \\
&= \frac{1}{\nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t)} \sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t) \sum_{\beta'} d(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) \nu_{\Omega}(\tau^{\beta'} \Omega', \tau^{\beta} \Omega) \\
&= \frac{1}{\nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t)} \sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t) \sum_{\beta'} d(\tau^{(\beta' - \beta)} \Omega', \Omega) \nu_{\Omega}(\tau^{(\beta' - \beta)} \Omega', \Omega) \\
&= \frac{1}{\nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t)} \sum_{\beta} P_{\Omega}(\tau^{\beta} \Omega, t) \sum_{\beta'} d(\tau^{\beta'} \Omega', \Omega) \nu_{\Omega}(\tau^{\beta'} \Omega', \Omega) \\
&= \frac{1}{\nu_{\vartheta}(\vartheta', \vartheta)} \sum_{\beta'} d(\tau^{\beta'} \Omega', \Omega) \nu_{\Omega}(\tau^{\beta'} \Omega', \Omega).
\end{aligned}$$

In the seventh equality, translation invariance property is applied wherein both the absolute configurations at t and $t + \Delta t$ are shifted by β places to the left (via. the operation $\tau^{-\beta}$).

For the eighth equality, the set $\{\tau^{(\beta' - \beta)}\} = \{\tau^{\beta'}\}$, since β' is any integer and β is fixed. The result is identical for any choice of absolute configuration Ω that satisfies $\Upsilon^{(e)}(\Omega) = \vartheta$. Thus,

$$d_{avg}(\vartheta', \vartheta) = \frac{1}{\nu_{\vartheta}(\vartheta', \vartheta)} \sum_{\beta'} d(\tau^{\beta'} \Omega', \Omega) \nu_{\Omega}(\tau^{\beta'} \Omega', \Omega).$$

After obtaining the expected value $d_{avg}(\vartheta', \vartheta)$, the expected change $D_{\Delta t}$ in cargo position in time Δt is determined to be,

$$\begin{aligned} D_{\Delta t} &= \sum_{\vartheta \in H} \sum_{\vartheta' \in H} d_{avg}(\vartheta', \vartheta) P_{\vartheta}(\vartheta', t + \Delta t, \vartheta, t), \\ &= \sum_{\vartheta \in H} \sum_{\vartheta' \in H} d_{avg}(\vartheta', \vartheta) \nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t) (\Delta t). \end{aligned}$$

where H is the set of all possible relative configurations. It enables the calculation of average velocity as,

$$\begin{aligned} v(t) &= \frac{D_{\Delta t}}{\Delta t} \\ &= \sum_{\vartheta \in H} \sum_{\vartheta' \in H} d_{avg}(\vartheta', \vartheta) \nu_{\vartheta}(\vartheta', \vartheta) P_{\vartheta}(\vartheta, t). \end{aligned}$$

Average run-length is then calculated by summing the average velocity over time

$$Average \text{ Runlength} = \int_0^{+\infty} v(t) dt \quad (2.10)$$

2.3.2 Transition rates between absolute configurations

In this section we present a general scheme for determining transition rates between absolute configurations. We begin with a structural model for single motor protein that consists of motor head, stalk and cargo binding tail domain. The linkage between the motor-heads and tail for single motor is modeled as a hookean spring when stretched, that has a rest length L_0 . It offers no resistance when compressed [17]. The motor heads move along the microtubules exerting a force F on a cargo that is expressed as a function of its length L by,

$$F(L) = \begin{cases} K_e(L - L_0) & \text{if } L \geq L_0, \\ 0 & \text{if } |L| < L_0, \\ K_e(L + L_0) & \text{if } L \leq -L_0. \end{cases}$$

Z_{eq} is the mean position of the cargo that is its equilibrium position determined by the forces exerted by the motors on the cargo through their linkages and the load force F_{load} on the cargo. The mean cargo position for a fixed $F_{load} > 0$ is a function of the absolute configuration i.e. $Z_{eq} = Z_{eq}(\Omega)$. If the cargo position is assumed to follow a truncated Gaussian distribution $\Theta(z)$ with variance σ , its probability density $\Theta(z)$ for $|z| < 3\sigma$ is given by,

$$\Theta(z) = (e^{-\frac{z^2}{2\sigma^2}}) / (2 \int_0^{3\sigma} e^{-\frac{z^2}{2\sigma^2}} dz).$$

The effect of thermal noise can be incorporated by determining the steady state variance σ of the cargo position. We assume that when a motor in the ensemble takes a forward steps or detaches, the probability distribution of the cargo position reaches a new distribution with negligible transient. Thus the time scale of the cargo dynamics is assumed to be faster than the rate of change of motor configurations.

A transition to another configuration Ω' occurs if either the wild type or mutant motor at a location $\bar{a}_k$ steps forward to $\bar{a}_{k+1}$, detaches from the location $\bar{a}_k$ or reattaches to the location $\bar{a}_k$ on the microtubule. By representing Ω' as $\Omega + S$, S is a sequence that corresponds to the type of transition (step, detach or attach) and the type of motor (wild-type or mutant) that has transitioned. The transition rate from Ω to Ω' is determined by averaging the associated probability rate over the position of the cargo.

The model of a single motor-protein is specified via the probability $P_S(F)$ of the motor taking a step, the detachment probability, $P_D(F)$, of the motor detaching from the microtubule, and the probability of attachment P_A of an unattached motor-protein to the microtubule, per second. Here F is the force acting on the motor which is considered positive if it is directed opposite to the motor stepping direction (e.g. kinesin *forward* stepping is towards the microtubule + end). Here in order to calculate the transition rates between absolute configurations it is assumed that the probability rates of step, detachment and attachment are known; later we illustrate a way to compute these probabilities for kinesin motors.

Transition Rate for Stepping

The stepping transition of a wild-type motor from the location $\bar{a}_k$ to $\bar{a}_{k+1}$ is represented as

$$\Omega \xrightarrow{STEP_h} \Omega + S_{h,k}^{(step)}, \text{ i.e.}$$

$$\Omega = \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} \xrightarrow{STEP_h} \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} + \begin{bmatrix} \cdots & -1 & +1 & \cdots \\ \cdots & 0 & 0 & \cdots \end{bmatrix}.$$

As the transition $\Omega \xrightarrow{STEP_h} \Omega + S_{h,k}^{(step)}$ occurs if any of the $\Omega_{h,k}$ wild-type motors located at $\bar{a}_k$ step forward to the position $\bar{a}_{k+1}$, the associated transition rate for stepping, $\nu_\Omega(\Omega + S_{h,k}^{(step)}, \Omega)$ is determined by averaging over the position of the cargo as,

$$\nu_\Omega(\Omega + S_{h,k}^{(step)}, \Omega) = \Omega_{h,k} \int_{Z_{eq}(\Omega)-3\sigma}^{Z_{eq}(\Omega)+3\sigma} P_S(F(z - \bar{a}_k)) \Theta(z - Z_{eq}(\Omega)) dz$$

where $\Omega_{h,k}$ is the number of wild-type motors located at $\bar{a}_k$ and P_S is the probability of a wild type motor taking a step from the location $\bar{a}_k$ on the microtubule per second.

The stepping transition of a mutant motor from the location $\bar{a}_k$ to $\bar{a}_{k+1}$ is represented as

$$\Omega \xrightarrow{STEP_d} \Omega + S_{d,k}^{(step)}, \text{ i.e.}$$

$$\Omega = \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} \xrightarrow{STEP_d} \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} + \begin{bmatrix} \cdots & 0 & 0 & \cdots \\ \cdots & -1 & +1 & \cdots \end{bmatrix}.$$

As the transition $\Omega \xrightarrow{STEP_d} \Omega + S_{d,k}^{(step)}$ occurs if any of the $\Omega_{d,k}$ mutant motors located at $\bar{a}_k$ step forward to the position $\bar{a}_{k+1}$, the associated transition rate for stepping, $\nu_\Omega(\Omega + S_{d,k}^{(step)}, \Omega)$ is determined by averaging over the position of the cargo as,

$$\nu_\Omega(\Omega + S_{d,k}^{(step)}, \Omega) = \Omega_{d,k} \int_{Z_{eq}(\Omega)-3\sigma}^{Z_{eq}(\Omega)+3\sigma} \bar{P}_S(F(z - \bar{a}_k)) \Theta(z - Z_{eq}(\Omega)) dz$$

where $\Omega_{d,k}$ is the number of mutant motors located at $\bar{a}_k$ and $\bar{P}_S$ is the probability of a mutant motor taking a step from the location $\bar{a}_k$ on the microtubule per second.

Transition Rate for Attachment/Detachment

The attachment/detachment transition of wild-type motor at location $\bar{a}_k$ is represented as $\Omega \xrightarrow{ATT_h/DET_h} \Omega \pm S_{h,k}^{(att)}$ i.e.

$$\Omega = \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} \xrightarrow{ATT_h/DET_h} \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} \pm \begin{bmatrix} \cdots & 1 & 0 & \cdots \\ \cdots & 0 & 0 & \cdots \end{bmatrix},$$

where the plus sign is for attachment and minus sign is for detachment.

As the transition $\Omega \xrightarrow{ATT_h/DET_h} \Omega \pm S_{h,k}^{(att)}$ occurs if any of the $\Omega_{h,k}$ wild-type motors located at $\bar{a}_k$ detach from the microtubule, the associated transition rate for detachment, $\nu_\Omega(\Omega - S_{h,k}^{(att)}, \Omega)$ is calculated by averaging over the position of the cargo as,

$$\nu_\Omega(\Omega - S_{h,k}^{(att)}, \Omega) = \Omega_{h,k} \int_{Z_{eq}(\Omega)-3\sigma}^{Z_{eq}(\Omega)+3\sigma} P_D(F(z - \bar{a}_k)) \Theta(z - Z_{eq}(\Omega)) dz$$

where $\Omega_{h,k}$ is the number of wild-type motors located at $\bar{a}_k$ and P_D is the probability of a wild type motor detaching from the location $\bar{a}_k$ on the microtubule per second.

The attachment/detachment transition of mutant motor at location $\bar{a}_k$ is represented as

$$\Omega \xrightarrow{ATT_d/DET_d} \Omega \pm S_{d,k}^{(att)} \text{ i.e.}$$

$$\Omega = \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} \xrightarrow{ATT_d/DET_d} \begin{bmatrix} \cdots & \Omega_{h,k} & \Omega_{h,k+1} & \cdots \\ \cdots & \Omega_{d,k} & \Omega_{d,k+1} & \cdots \end{bmatrix} \pm \begin{bmatrix} \cdots & 0 & 0 & \cdots \\ \cdots & 1 & 0 & \cdots \end{bmatrix}.$$

As the transition $\Omega \xrightarrow{ATT_d/DET_d} \Omega \pm S_{d,k}^{(att)}$ occurs if any of the $\Omega_{d,k}$ mutant motors located at $\bar{a}_k$ detach from the microtubule, the associated transition rate for detachment, $\nu_\Omega(\Omega - S_{d,k}^{(att)}, \Omega)$ is determined by averaging over the position of the cargo as,

$$\nu_{\Omega}(\Omega - S_{d,k}^{(att)}, \Omega) = \Omega_{d,k} \int_{Z_{eq}(\Omega)-3\sigma}^{Z_{eq}(\Omega)+3\sigma} \bar{P}_D(F(z - \bar{a}_k)) \Theta(z - Z_{eq}(\Omega)) dz$$

where $\Omega_{d,k}$ is the number of mutant motors located at $\bar{a}_k$ and $\bar{P}_D$ is the probability of a mutant motor detaching from the location $\bar{a}_k$ on the microtubule per second.

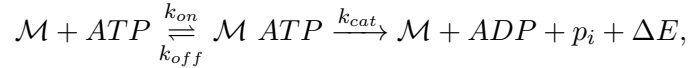
For both the wild-type and mutant motors, a constant probability rate of reattachment to the microtubule, P_A and $\bar{P}_A$, are assumed. If a motor is linked to the cargo, it is assumed to attach to the microtubule without stretching its linkage. Thereby, the only locations where the motor reattachment can occur are located within a distance L_0 of the cargo.

Probability Rates for Kinesin

The results put forth in this article correspond to an instantiation of our methodology for kinesin motor protein. The probability rates of stepping, detachment and attachment of a single kinesin motor are determined using several available studies [11, 16, 26, 2].

Probability of Stepping, per second

Kinesin takes a step on the microtubule by hydrolyzing an ATP molecule [35] in the following manner :



where ΔE is the energy released. From [36], the ATP hydrolysis rate predicted using Michaelis-Menten dynamics relates to the probability rate stepping for a wild-type kinesin by assuming that the free motor head binds to the microtubule location with defined probability η . P_S is then expressed as

$$P_S = \frac{k_{cat}[ATP]}{[ATP] + k_m} \eta, \quad (2.11)$$

where $k_m = \frac{k_{cat} + k_{off}}{k_{on}}$.

From [17], the force F exerted by the cargo on the motor affects motor dynamics by

modifying η as,

$$\eta(F) = \begin{cases} 1 & \text{if } F = 0, \\ 1 - \left(\frac{F}{F_s}\right)^2 & \text{if } 0 < F < F_s, \\ 0 & \text{otherwise.} \end{cases} \quad (2.12)$$

Furthermore [17] assumes that the force F influences the kinetics of ATP hydrolysis by affecting k_{off} in the following manner :

$$k_{off}(F) = k_{0,off} e^{\frac{F\delta_l}{K_b T}},$$

where $k_{0,off}$ is the backward reaction rate of hydrolysis when $F = 0$, δ_l is an experimentally determinable parameter, T is the temperature and K_b is the Boltzmann constant. Thus, the probability rate of stepping for a wild-type motor under a constant force F is given by,

$$P_S(F) = \frac{k_{cat}[ATP]}{[ATP] + \frac{k_{cat} + k_{off}(F)}{k_{on}}} \eta(f). \quad (2.13)$$

In the case of a mutant motor protein with an altered stalling force $\bar{F}_s$, the probability rate of stepping under a force F is given by,

$$\bar{P}_S(F) = \frac{k_{cat}[ATP]}{[ATP] + \frac{k_{cat} + k_{off}(F)}{k_{on}}} \bar{\eta}(F), \quad (2.14)$$

where the probability $\bar{\eta}$ that the free mutant motor head binds to the microtubule location is written as

$$\bar{\eta}(F) = \begin{cases} 1 & \text{if } F = 0 \\ 1 - \left(\frac{F}{\bar{F}_s}\right)^2 & \text{if } 0 < F < \bar{F}_s \\ 0 & \text{otherwise} \end{cases} \quad (2.15)$$

Probability of Detachment, per second

Kinesin motors are processive species that take a certain number of steps during their ATP driven movement along microtubule before dissociating from it. The *processivity* (denoted by $\mathcal{L}$) represents how far the motor can move, on average, before its detachment from microtubule. From definition of $\mathcal{L}$ in [32],

$$\mathcal{L} = \frac{d[ATP] A e^{-F\delta_l/K_b T}}{[ATP] + B(1 + A e^{-F\delta_l/K_b T})},$$

where A, B and δ_l are experimentally determinable parameters. The relation between the probability rate of stepping P_S and detachment P_D is given by,

$$\frac{P_S(F)}{P_D(F)} = \frac{\mathcal{L}}{d}. \quad (2.16)$$

As long as the wild-type motor is not stalled i.e. $F < F_s$, the probability rate of detachment is given by,

$$P_D(F) = \frac{[ATP] + B(1 + Ae^{-F\delta_l/K_bT})}{[ATP]Ae^{-F\delta_l/K_bT}} P_S(F). \quad (2.17)$$

When the load force on the wild-type motor equals to or exceeds its stalling force i.e. for $F \geq F_s$, a constant detachment rate is assumed in [17] as,

$$P_D(F) = P_{back} = 2/sec.$$

For a mutant motor that is not stalled i.e. $F < \bar{F}_s$, the probability rate of detachment is given by,

$$\bar{P}_D(F) = \frac{[ATP] + B(1 + Ae^{-F\delta_l/K_bT})}{[ATP]Ae^{-F\delta_l/K_bT}} \bar{P}_S(F). \quad (2.18)$$

When the load force on the mutant motor equals to or exceeds its stalling force i.e. for $F \geq \bar{F}_s$, the constant detachment rate is assumed to be

$$\bar{P}_D(F) = P_{back} = 2/sec.$$

For $F \geq F_s$ or $F \geq \bar{F}_s$, an assumption that the single motor detachment rate increases exponentially with F as opposed to a discontinuous jump to a constant value of $2/sec$ does not alter the nature of the results obtained.

Probability of Attachment, per second

Probability of attachment is experimentally found to be $P_A \approx 5/sec$ [37, 38]. In this article it is assumed that the probability of attachment for a mutant motor, $\bar{P}_A \approx 5/sec$.

Numerical parameters for Kinesin

The numerical parameters considered for wild-type motors in this article are the same as that used in [17], all of which have been experimentally verified. Specifically $k_{on} = 2.10^6 M^{-1}s^{-1}$, $k_{off} = 55 s^{-1}$, $k_{cat} = 105 s^{-1}$, $F_s = 6 pN$, $d = 8 nm$, $d_l = 1.6 nm$, $\delta_l = 1.3 nm$, $A = 107$, $B = 0.029 \mu M$, $[ATP] = 2 mM$, $T = 300K$ and $K_{el} = 0.32 pN/nm$. All the parameters for mutant motors are the same, except for its stalling force which is taken as $\bar{F}_s = 5.5 pN$.

Chapter 3

Steady State Dynamics of Molecular Motors Reveal Load Dependent Co-operativity

Transport of intracellular cargo is often mediated by teams of molecular motors that function in a chaotic environment and varying conditions. We show that the motors have unique steady state behavior which enables transport modalities that are *robust*. Under reduced ATP concentrations, multi-motor configurations are preferred over single motors. Increasing load force drives motors to cluster, but very high loads compel them to separate in a manner that promotes immediate cargo movement once the load reduces. These inferences, backed by analytical guarantees, provide unique insights into the strategies adopted by motors.

3.1 Introduction

Intracellular cargo such as vesicles, filaments and organelles are transported inside the cell by molecular motors like kinesin, dynein and myosin. The motors convert chemical energy to mechanical energy through ATP hydrolysis [35], while operating individually as well as in homologous or heterologous teams [12, 39]. Numerous experimental studies have analyzed multi-motor ensembles and have demonstrated the benefits of working in teams, such as enhanced run-lengths [17, 40] and robustness in a turbulent environment

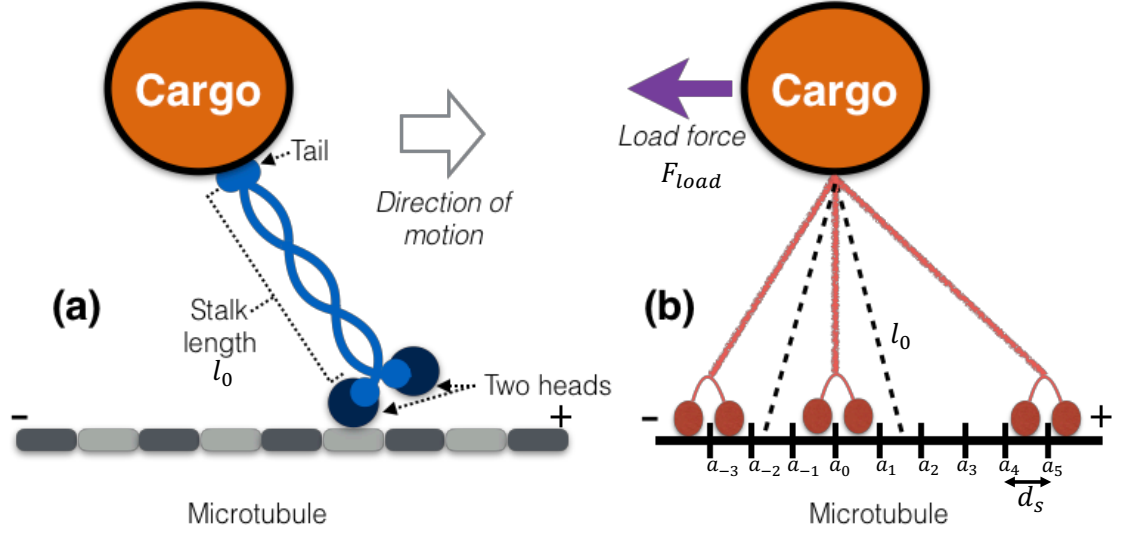


Figure 3.1: (a) Structure of the molecular motor Kinesin (b) Representation of an ensemble of three motors carrying a cargo. F_L is the load force opposing the cargo. The absolute configuration is $Z = [\dots 1\ 0\ 0\ 1\ 0\ 0\ 0\ 1\ \dots]$ and relative configuration is $\sigma = M|||M|||M$, where M denotes motor location on the MT (microtubule) and $|$ denotes the MT locations.

[16]. On the analytical side, there are numerous studies that investigate cargo transport by two motors [41, 42], more than two motors [16] and analyze average velocity, run-length and distributions of bound motors [14] that shed light on team behavior. Several of these studies utilize a probabilistic description of single motor behavior to build models that describe transport of cargo by a team [11, 14, 15, 16]. These models coupled by Monte-Carlo methods offer numerous benefits in terms of simulating behavior of teams of motors that help guide tedious experiments [17]. While Monte-Carlo (MC) simulations have proven helpful, they suffer from (i) the inability to detect modalities of transport that are rare, (ii) a lack of deductive capability of understanding a specific mode seen in a realization of the MC simulation easily and (iii) the inability to provide insights into the asymptotic behavior of the team of motors. On the other hand, simplified models that encapsulate the dynamics of team of motors provide insights analytically but these models lie on the other end of the spectrum to MC simulations where a detailed description is not addressed.

In this chapter we adopt a semi-analytic methodology [19] that, while capturing the detailed description of MC methods, is able to provide conclusions on asymptotic behavior and a deductive capability lacking in MC methods. The method utilizes a finite dimensional reduction of a Markov model to enable an *exact* calculation of the probability distribution

function of the relative behavior of motors in a team, through a computationally efficient semi-analytic approach. We prove (for a generalized case of a team of finite motors) that the relative configurations of the motors in the team while transporting cargo, have a *unique and non-trivial* steady state distribution. This implies that the system of multiple motors carrying a cargo, is a *highly robust system* where irrespective of the initial orientation, the motors in the team assume a unique steady state distribution. Moreover, the unique steady state distribution dependent on external environmental factors is determinable; providing a means to reach conclusions on how the team of motors overcomes an adverse environment. Here the efficacy of the approach is demonstrated by investigating how teams deal with changing ATP concentrations and external load forces by examining two and three motor ensembles. Key analytical results reported here are that, as ATP concentrations are lowered, teams tend to favour multi-motor configurations over single motor configurations; with the cargo more probable to be connected to the microtubule filament by more than one motor than only one motor. The implications are that the average run-length of multi-motor ensembles is increased even though average ensemble velocity decreases as the ATP concentration reduces. It is known that reduced ATP concentration lead to reduced single motor velocity, which is hypothesized as a reason for increased runlengths when cargo is transported by two motors [18]. Such a mechanism also provides a possible regulating mechanism by which transport occurs by an ensemble containing a mixture of different motors [43].

The approach in this chapter also establishes that when load forces on the cargo change, motors adopt a form of cooperation by clustering together in order to handle increasing load forces. The propensity to cluster increases with load force. However the trend (of closer clustering to handle higher load forces) does not hold for all loads. Indeed, for very high values of load forces (possible when cargo encounters obstacles along the path of travel), the teams abandon clustering and resort to spreading out in a manner where the entirety of the load falls on few motors as possible, with the rest of the motors assuming configurations where they are subjected to forces that are near stall-force of a single motor. We hypothesize that if the load force can be shared in a manner that prevents any of the motors from being loaded beyond stall, clustering is preferred as it aids motion. Moreover, in the case that the

load force is very high where even with equitable load sharing the forces on each motor is beyond its stall force, the motor ensemble abandons clustering and prefers configurations where most of the load is taken by a few motors while other motors are only loaded to be near stall and are primed to take steps on a small reduction of load force. Thus separation of motors at very high loads is preferred in order to enable higher probability of immediate cargo translation in the forward direction, once the high forces (e.g. due to impediment in transport) have passed and the high load phenomenon has subsided.

The chapter is organized as follows. We provide a brief overview of the semi-analytic model and introduce terminology to establish the finite-dimensional model. We then establish the existence and uniqueness of the steady state distribution of various configurations of the motors in a team, by instantiating the model for molecular motors involving stepping, detachment and reattachment probabilities. We then instantiate for Kinesin-I and analyze the impact of changing ATP concentrations and load forces on multi-motor ensembles by studying their effect on the steady state distribution. We leverage the detailed information provided by the complete knowledge of the distribution of motor configurations to give an explanation for the observed behaviors of motor ensembles.

3.2 Semi-analytic Model

We model a single motor as a hookean spring when stretched, with a rest length l_0 and stiffness constant K_e (both parameters determined experimentally), that offers no resistance when compressed [17]. The stalling force F_S captures the maximum load bearing capacity of the motor. A team has finite $\bar{m}$ motors attached to the cargo, while the cargo bears constant load force F_L . Floating motors attached to the cargo can only reattach to those locations on the microtubule within l_0 distance of the cargo. The microtubule (MT) is modeled as a sequence of equally spaced dimers $a_q = a_0 + qd_s$, where a_q is the location of q^{th} dimer and d_s is the length of the dimer. Based on [19], the locations of the motor on the MT are represented by $Z := \{z_q\}, q \in I$ where z_q motors are located on the a_q^{th} location of the MT and I is the set of integers. For e.g., the configuration in Fig. 3.1 is $Z = [... 1 0 0 1 0 0 0 0 1 ...]$ As a motor in the ensemble steps, detaches or reattaches to the

MT, the configuration changes from Z to Z' . If the probability rate of transition $\lambda_Z(Z', Z)$ from Z to Z' is known, we can define an infinite dimensional Markov model, similar to [44, 45]. Furthermore, the probability of going from Z to Z' in time Δt is represented by $\lambda_Z(Z', Z)\Delta t$. Thereby, the probability that the configuration is Z' at t given that it was Z at initial time t_0 , represented by $P_Z(Z, t|\bar{Z}, t_0)$, satisfies the probability Master Equation where the rates are given by λ_Z . However, as the typical MT filament is much longer (μm) than the average run-lengths of motors (nm), it effectively makes Z a bi-infinite sequence and the subsequent master equation intractable.

The issue is resolved in [19] by showing that the maximum distance between the forward most (vanguard) and rear most (rearguard) motor is bounded, thus enabling a projection of the infinite dimensional model to a finite dimensional model and preserving the Markov property. Thus, instead of an absolute configuration Z we can now define a relative configuration σ that captures the locations of the motors in an ensemble relative to the rearguard motor. The symbolic representation consists of M and $—$, with M denoting motor location on the MT and $—$ denoting the MT locations. For e.g. Fig. 3.1 is denoted by $\sigma = M|||M||M$. Subsequently, in the finite dimensional model, $\lambda_\sigma(\sigma', \sigma)$ denotes the transition rates between the relative configurations σ and σ' . In a manner similar to the absolute configurations, the probability $P_\sigma(\sigma, t)$ of being σ at time t obeys the Master Equation, $\frac{\partial}{\partial t}P_\sigma(\sigma, t) = \sum_{\sigma' \in \bar{S}} \lambda_\sigma(\sigma, \sigma')P_\sigma(\sigma', t) - P_\sigma(\sigma, t) \sum_{\sigma' \in \bar{S}} \lambda_\sigma(\sigma', \sigma)$. Here, $\bar{S}$ is the set of finite relative configurations. It is evident that for a finite number of motors $\bar{m}$ carrying a common cargo under a finite load force F_L , the space of relative configurations is finite. Let the set be $\bar{S} = \{\sigma_1, \dots, \sigma_n, \sigma_{n+1}, \dots, \sigma_{n'}\}$. Let the probability vector $P(t) = [P_1(t), \dots, P_{n'}(t)]$ where each element $P_i(t)$ is the probability of the system being in the i^{th} relative configuration σ_i at time t . In [19] it is shown that the probability vector $P(t)$ satisfies the master equation as,

$$\frac{d}{dt}P(t) = \bar{\mathbf{\Gamma}}P(t) \quad (3.1)$$

where $\bar{\mathbf{\Gamma}} \in \mathbf{R}^{(n') \times (n')}$ is defined by the transition rates $\lambda_\sigma(\sigma_j, \sigma_i)$. Solving for $P(t)$, we get $P(t) = e^{\bar{\mathbf{\Gamma}}(t-t_0)}P(t_0) = \bar{\mathbf{J}}P(t_0)$, where $P(t_0)$ is an initial probability vector and $\bar{\mathbf{J}} \in \mathbf{R}^{(n') \times (n')}$.

3.3 Ergodicity of underlying system:

Consider $\tilde{S} = \sigma_{n+1}, \dots, \sigma_{n'}$ as the set of relative configurations with at most $m - 1$ motors attached to the microtubule (i.e. $\tilde{S}$ contains relative configurations with exactly $0, 1, 2, \dots, m - 1$ motors attached to the microtubule) and let $\sigma_{n+1} = \phi$ denote the relative configuration where no motors are attached to the MT i.e. cargo is permanently lost. By propagating (3.1) for known models of molecular motors such as Kinesin-I and using any value of initial distribution $P(t_0)$, it is seen that the eventual distribution $P(t)$ reflects the condition of cargo being permanently disengaged from the microtubule. Thus, as $t \rightarrow \infty$, $P(t) = [0, 0, \dots, 0, 1, 0, \dots, 0]$ where $P_{n+1}(t) = 1$. Although it represents an intuitive result, since loss of cargo is an inevitable eventual outcome of transport of cargo, this represents a trivial steady state distribution which provides no information whatsoever regarding transport by teams of motors. However, it is much more relevant to consider cargo transport under a condition that a fixed number of motors, say $m > 0$, *remain attached* to the microtubule. In this manner it is possible to analyze the transport of cargo under the condition that the cargo is not lost, thus enabling us to analyze the mechanisms employed by the motors while undertaking intracellular transport.

When conditioned on the ensemble having at least m motors attached to the MT, the new state space reduces from $\tilde{S}$ to $S = \{\sigma_1, \dots, \sigma_n\}$, where S contains all the relative configurations with at least m motors attached to the microtubule. Again, based on [19], the probability vector $P(t) = [P_1(t), \dots, P_n(t)]$ satisfies the master equation as,

$$\frac{d}{dt}P(t) = \mathbf{\Gamma}P(t) \quad (3.2)$$

where $\mathbf{\Gamma} \in \mathbf{R}^{(n) \times (n)}$ is defined by the transition rates $\lambda_\sigma(\sigma_j, \sigma_i)$. Solving for $P(t)$, we get $P(t) = e^{\mathbf{\Gamma}(t-t_0)}P(t_0) = \mathbf{J}P(t_0)$, where $P(t_0)$ is an initial probability vector and $\mathbf{J} \in \mathbf{R}^{(n) \times (n)}$.

The distribution $P(t)$ contains information regarding the manner in which multiple motors transport a common cargo. It is not evident apriori what the nature of $P(t)$ would be like; whether the value will have a steady state or will vary between various transient values. By using known model for molecular motors consisting of stepping, detachment and reattachment probabilities and a constant load force on the cargo, we utilized the properties of the underlying Markov model to prove the following statement :

Result 1: For $m \in [0, \bar{m} - 1]$, the Markov chain with state space S and transition matrix $\mathbf{J}$ has a $[\pi^T \mathbf{0}^T]^T$ as a *unique steady state distribution*.

The proof above result s provided at the end of the chapter.

Owing to the ergodicity of the underlying system, under constant load force, the probability $P(t)$ of the numerous relative configurations reaches a *unique steady state* P_{ss} (see mathematical proof for *Result 1* provided at the end of the chapter). It implies that the ensemble of molecular motors carrying a common cargo is a highly robust system and behaves in a fixed manner after an initial transient period has passed. The manner of the behavior is dependent upon the external conditions such as load force on the cargo, indicating that the distribution $P(t)$ contains information on how the various molecular motors arrange themselves to handle various types of load forces. Furthermore, the steady state distribution $P(t)$ is independent of the initial distribution $P(t_0)$ at the time instant t_0 , indicating that no matter how the motors are oriented prior to the initiation of the cargo transport, they prefer to align themselves in a fixed matter that is dependent on external conditions.

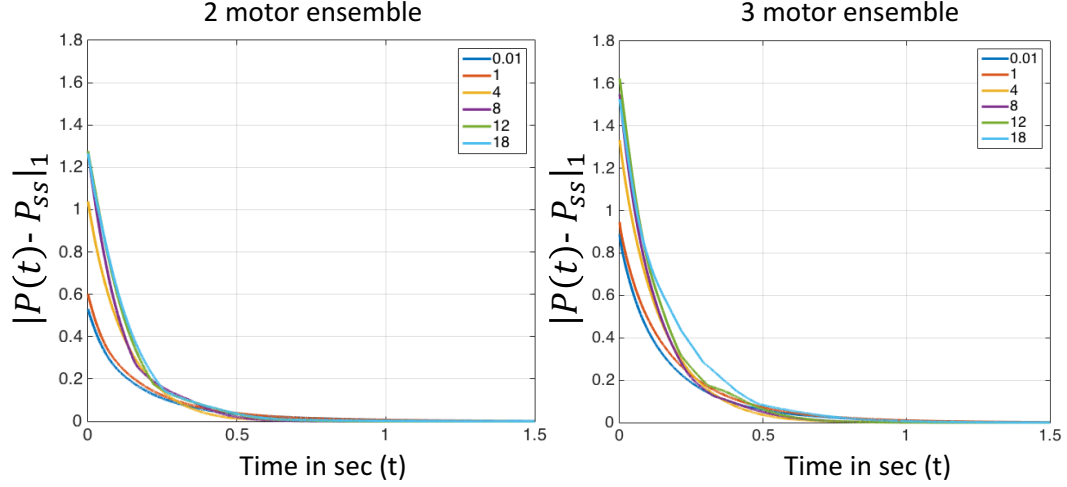


Figure 3.2: Convergence to steady state for 2 and 3 motors in 1-norm. The x-axis denotes time t and y-axis denotes $\|P(t) - P_{ss}\|_1 = \sum_{\sigma} |P(\sigma, t) - P_{ss}(\sigma)|$, where P_{ss} satisfies $P_{ss} = \mathbf{J}P_{ss}$. The legends denote load forces in pN.

One way to obtain the steady state distribution P_{ss} is to algebraically solve for $P(t) = \mathbf{J}P(t_0)$ at the steady state i.e. given the number and type of motors and load force (meaning, for a known $\mathbf{J}$), obtain P_{ss} such that $P_{ss} = \mathbf{J}P_{ss}$. In Fig.(3.2) it is seen that the value of $P(t)$ obtained by propagating Eq. (3.1) quickly approaches P_{ss} obtained by solving

$P_{ss} = \mathbf{J}P_{ss}$. Thus by propagating Eq.(3.1) we can not only conveniently obtain a reasonable estimate for P_{ss} but can also analyze the dynamics of the process of orientation of the motors in the ensemble as time progresses, thus making the short and long term behavior of the ensemble tractable.

3.4 Analysis of Steady State Distributions

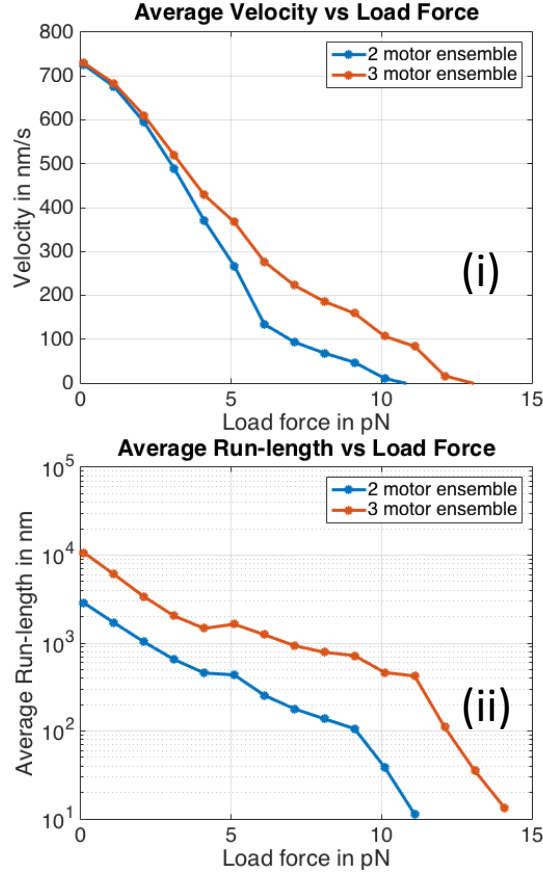


Figure 3.3: (i) Variation of average velocity with load force for a 2 motor ensemble (ii) Variation of average run-length with load force for a 3 motor ensemble

The steady state distribution P_{ss} can be utilized to compute important biological quantities governing intracellular traffic, such as average cargo velocity, run-length and average number of engaged motors. An instantiation for ensemble of finite Kinesin-I (2 and 3-motor) yields average velocity (Fig. 3.3(i)) and run-length (Fig. 3.3(ii)) values that are in good agreement with existing computational [17] and experimental studies [18, 29]. The model is also able to confirm some of the known observations of multi-motor based cargo transport,

such as the monotonic decrease in run-length and velocity with increasing load force; and the significant increase in run-length but not in velocity as the number of motors in an ensemble increases. The model also effectively captures the consequence of changing ATP concentration, where decreasing ATP concentrations led to reduced velocity but increased run-length for multi-motor ensembles (as compared to single motor behavior), confirming existing experimental [18] and computational [46] observations. It implies that a majority of the cargo-ensemble performance is governed by the unique steady state behavior and not the transient characteristics. The good agreement between results obtained using the analytic model and the existing literature justifies the usage of the analytic framework to arrive at conclusions about the steady state dynamics of the ensemble and how it responds to changing external conditions. Subsequently, we utilized the computational model to quantify the effect of external conditions on the behavior of the multiple motors, which we observe is captured by the steady state P_{ss} . In particular, we analyze the effect of varying ATP concentration and load forces on transport of cargo by multi-motor ensembles.

3.5 Effect of ATP concentration:

As is seen in Fig. 3.4(a) and (c), ATP concentration does impact the steady state probability distribution of the multi-motor ensembles. However, a mere observation of the probabilities is not enough to indicate the nature the trend. Upon a further detailed analysis, we analyzed the probabilities of interest as the probability of 1 motor being attached to the microtubule (denoted by p_{1mot}) and the probability of more than 1 motor being attached to the microtubule (equal to $1 - p_{1mot}$). It is seen that for both 2 and 3 motor ensembles, the respective p_{1mot} reduces whereas the probability of more than 1 motor being attached increases, with reducing ATP concentration. Changes in load force on the cargo have no impact on these observed trends.

A direct impact of this behavior can be observed on the variation of average velocity and run-length of multi-motor ensembles with ATP concentration. It is seen that, with reducing ATP concentrations, the average velocity for multi-motor ensembles *reduces* (Fig. 3.5 and Fig. 3.6) but the runlength *increases* (Fig. 3.7 and Fig. 3.8). The increase in

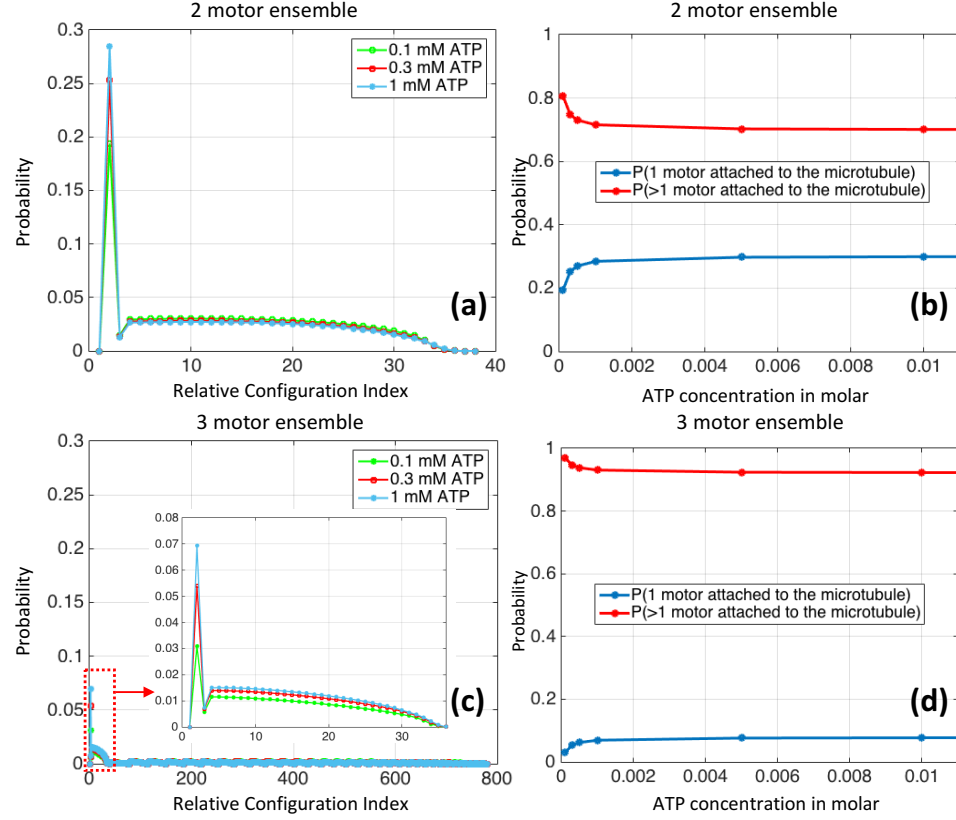


Figure 3.4: Variation of probability distribution function of 2 and 3 motor ensembles with ATP concentration

runlength can be attributed to the fact that, even though the velocity has diminished with reducing ATP concentration, the probability of more than 1 motor remaining attached to the microtubule has increases. Thus, the cargo has a higher probability of remaining engaged to the microtubule through more than 1 motor, than through just 1 motor. This enables the second motor (and third motor for 3 motor ensemble) to contribute to the cargo motion, allowing for a higher probability of the cargo being linked to the microtubule during the course of cargo travel. This enhances the overall distance covered by the multi-motor ensemble. The increase in the probability of more than one motor attached to the microtubule can be attributed to the inverse correlation of the single motor detachment rates with ATP concentration, based on the single motor detachment model used for the analysis in this chapter. The observations of average velocity and runlength corroborate experimental observations in [18], while providing a possible explanation based on steady state probability distributions of the multi-motor ensembles.

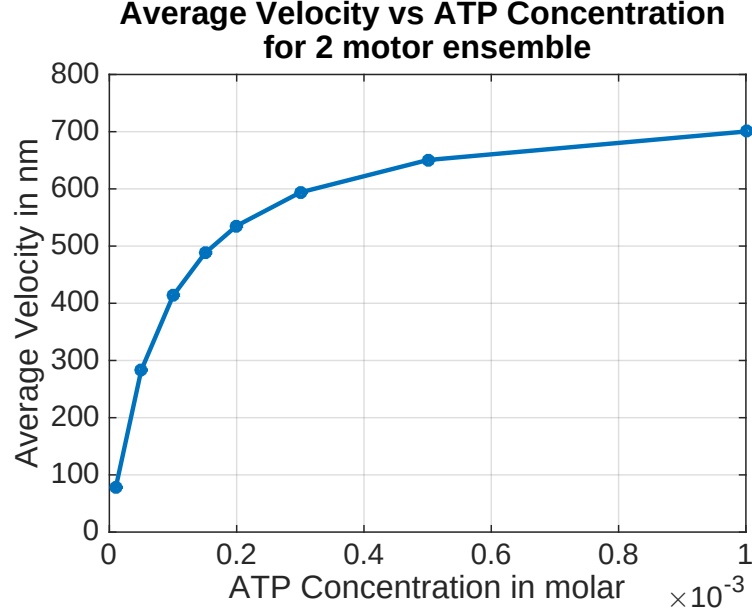


Figure 3.5: Variation of average velocity with ATP concentration for a 2 motor ensemble

3.6 Effect of load force:

The simulation model was further utilized to analyze the impact of varying load forces on the steady state probabilities of relative configurations of 2 and 3 motor ensembles, as shown in Fig. 3.9. It is seen that at very small values of load forces Fig. 3.9 (a) and (d), a significant majority of configurations have low values with little variation. No one relative arrangement is particularly favored, with the variation in probabilities being very gradual. This indicates that the motors do not prefer any particular orientation among themselves at these values of load forces. An intuitive explanation is that at such low loads, since equal or unequal load sharing cause insignificant variations in the load forces balanced by each motor in the ensemble, there wouldn't be a pressing need to adhere to a specific orientation. Thus in these regimes motors tend to spread out more evenly while transporting the common cargo.

However, as the load force is increased, certain configurations start becoming more probable over others. As is seen in Fig. 3.9 (b) for 2 motor ensemble with $F_{load} = 5pN$, the relative configurations $\sigma_2(MM)$ and $\sigma_3(M|M)$ become more probable, with a combined 29.9% share of the total probability (as compared to 4.7% share if all configurations were

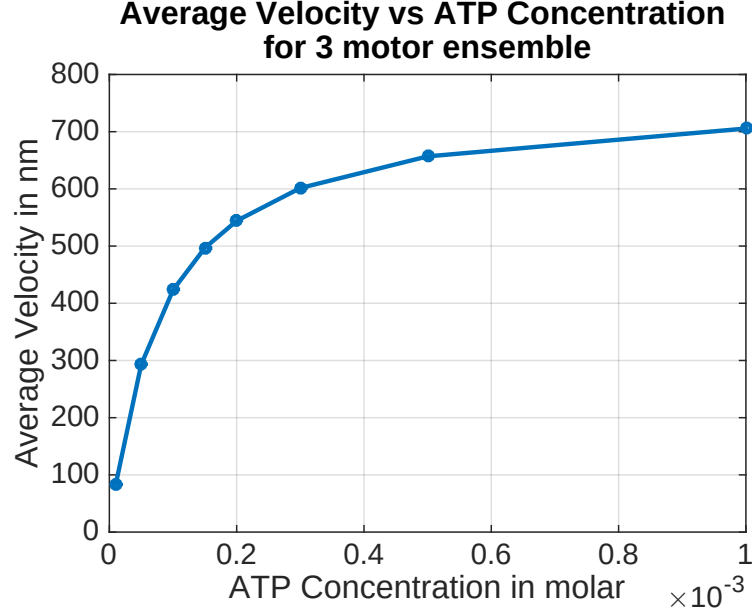


Figure 3.6: Variation of average velocity with ATP concentration for a 3 motor ensemble

equal). For a 3 motor ensemble with $F_{load} = 10pN$ (Fig. 3.9 (e)), the configurations $\sigma_{37}(MMM)$ and $\sigma_{71}(M|MM)$ become more probable, with a 8.51% share of the total probability (as compared to 0.405% share if all configurations were equal). A common attribute connecting these configurations is that these represent arrangements where motors are *clustered* together, with little to no separation between their relative locations on the microtubule.

Let's define probability of clustered states as the sum of probabilities of the clustered states for the 2 and 3 motor ensembles respectively. As is seen in Fig. 3.10, not only is clustered favored at the load forces under consideration, but the probability of clustered states tends to increase with load forces up to a certain value of load force. A possible explanation for the preference to clustering is that, clustered states mean motor extensions for the particular configuration are of similar magnitude. This means that in such configurations, the load force is shared more equally than in other configurations where the motors are far apart and not clustered together. As load force F_{load} increases, a preference to non-clustered states would imply more and more unequal load sharing between the motors of the ensemble. It is likely that one or more motors would have to handle a higher percentage of F_{load} , bringing the value closer to the stalling force F_s ($6pN$ in this case). For example,

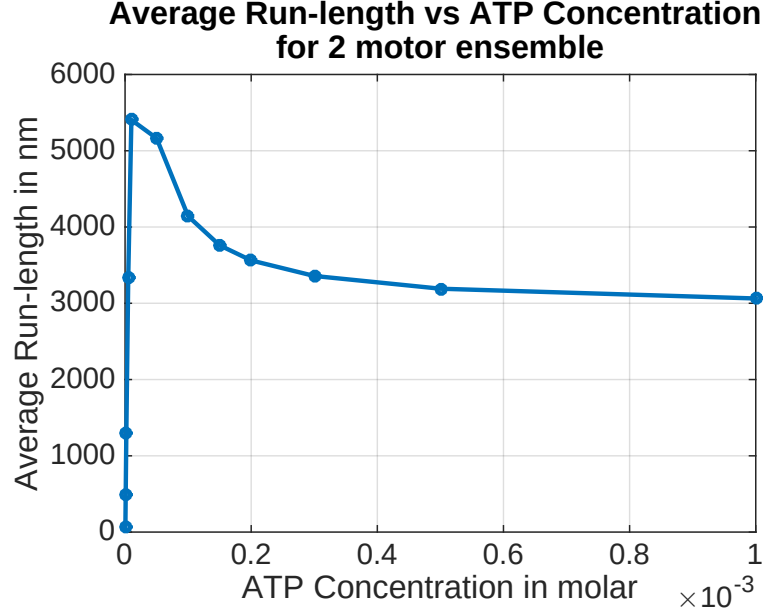


Figure 3.7: Variation of average run-length with ATP concentration for a 2 motor ensemble

for 2 motor ensemble with $F_{load} = 10pN$, in the configuration $\sigma_5 = M||M$ the rearguard motor takes $2.4pN$ while vanguard balances the rest of the $7.6pN$ load. This would put the vanguard motor beyond stall, making it unable to step forward and thus inhibiting the overall cargo propagation. Thus, at higher load forces, it is more disadvantageous to prefer unequal load sharing i.e. configurations with a higher separation between the motors on the microtubule. Thereby, it is beneficial to prefer equal load sharing i.e. clustering, as F_{load} on the cargo increases, as is evidenced by Fig. 3.10.

However, the trend of ‘more clustering’ with ‘higher F_{load} ’ does not hold for all loads forces. From Fig. 3.10 it is seen that beyond a certain high value of load force, there is an abrupt departure from a preference to clustering of motors. In one such case of 2 motor ensemble with $F_{load} = 25pN$ (Fig. 3.9(c)), the clustered states of MM and MMM have zero probability while the state of $\sigma_i = M||||M$ is the most probable (with 27% share of the total probability). For a 3 motor ensemble with $F_{load} = 50pN$ (Fig. 3.9(f)), the state of $\sigma_{64} = MM|||||||M$ is most probable among the 3 motor configurations (with 7.48% share of the total probability). A detailed analysis of the load sharing in these configurations reveals interesting and non-intuitive insights.

In the 2 motor case with $F_{load} = 25pN$, the rearguard motor in the most preferred

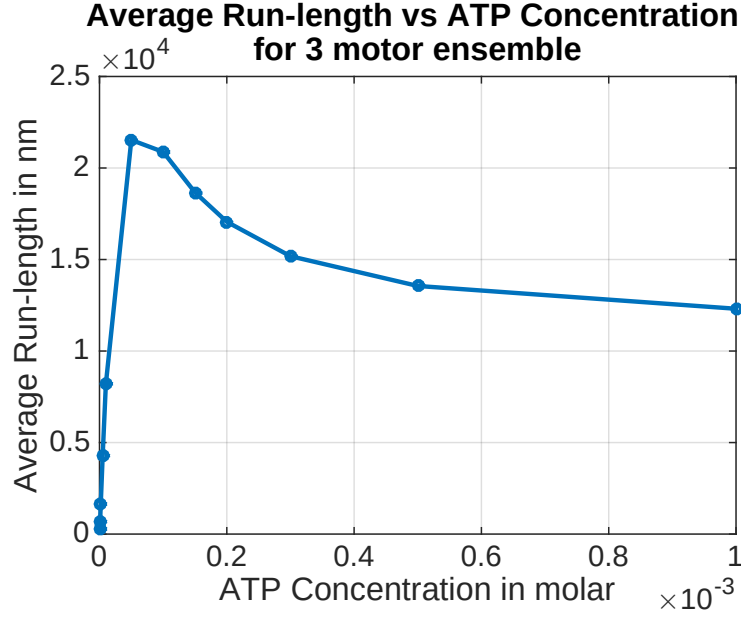


Figure 3.8: Variation of average run-length with ATP concentration for a 3 motor ensemble

configuration of $M||||M$ handles $6.1pN$ of the load force while the vanguard handles the rest $18.9pN$ force. For 3 motor ensemble with $F_{load} = 50pN$, the rearguard motors in the most preferred configuration of $MM||||M$ handle $7.3pN$ of the load force while the vanguard handles the rest $35.4pN$ force. A possible explanation is as follows. As the stalling force for the motors used in this chapter is $F_s = 6pN$, when the load on the motor is beyond F_s , the motor is unable to take a forward step. At these high loads, a common property among the most preferred configurations is that there is one vanguard motor while the rest of the motors are all rearguard motors on the same location on the microtubule. The vanguard and rearguard motors are spread out such that all the rearguard motors are loaded just beyond stall force F_s and the vanguard motor bears the remaining load, whose value is well beyond the stalling force F_s .

Such a high loading event relates to an infrequent yet a possibly debilitating phenomenon inside the cells, such as an unanticipated obstacle along the cargo travel path or an encounter with an oppositely directed cargo. Occurrences like these are most likely sudden events that do not lead to a progressive loading of the load force on the cargo, but a sudden spike. Based on our analysis, the preferred orientation is such that as few motors as possible (i.e. a single vanguard motor) is loaded well beyond stall force. In such an arrangement, once

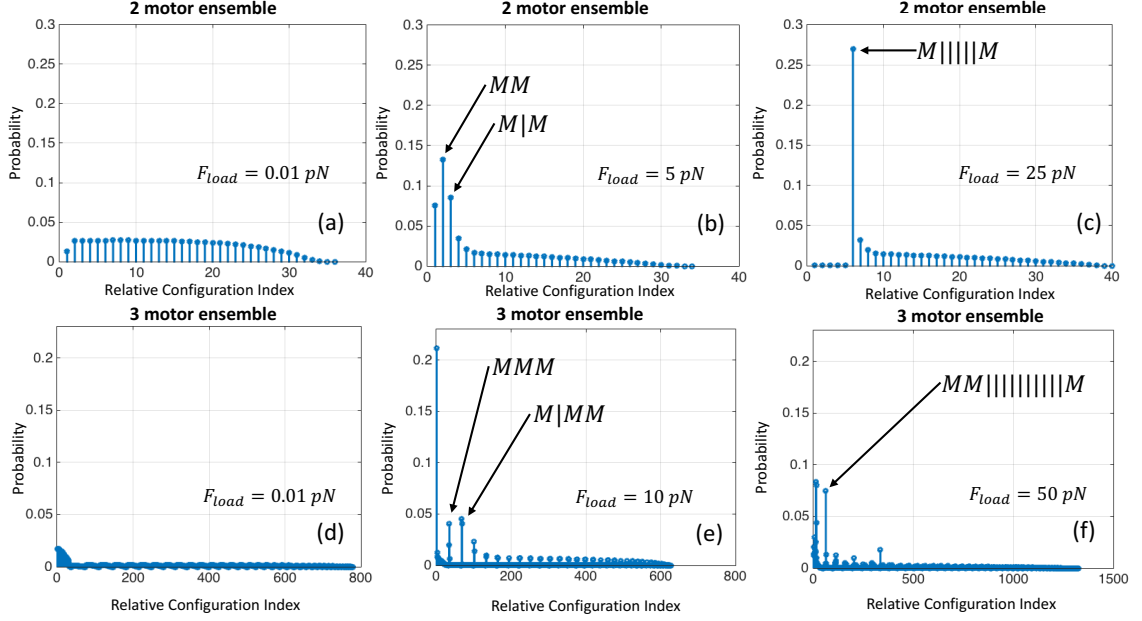


Figure 3.9: Variation of 2 and 3 motor probability distribution with load force. 1.Redo Fig 5 2.Pahile 3 given 2 analyze kar. konte configs jasti probable ahet te bagh ani manuscript madhe lihi 3.(c) change kar to 25 pN

the transient loading event goes away and F_{load} starts to subside, it takes little reduction in F_{load} to reduce the force on each of the rearguard motors and to subsequently enable them to take a step and propel the cargo forward (since force on each motor now falls below F_s). It is in this arrangement that it is possible to mobilize the maximum number of motors by the least reduction in F_{load} , while keeping the load force on the one vanguard motor as low as possible. In contrast, for any other configuration where the rearguard and vanguard motors are closer (like a clustered configuration preferred for lower F_{load}), the non-vanguard motors would be taking a load higher than F_s and there would be less number of motors loaded *just above* their stall force. Thus a larger reduction of load force would be necessary to enable the same numbers of motors to be able to step forward, as in the previous configuration. Another argument would be that, for the same reduction in F_{load} , the preferred orientation enables the maximum number of motors (i.e. all but one vanguard) to be able to walk while maintaining the load force on the vanguard motor as low as possible. Thus, this orientation ensures that once the obstacle has diminished and the high load force has decreased, the motor motion is resumed quicker and the forward propagation of cargo continues towards the destination.

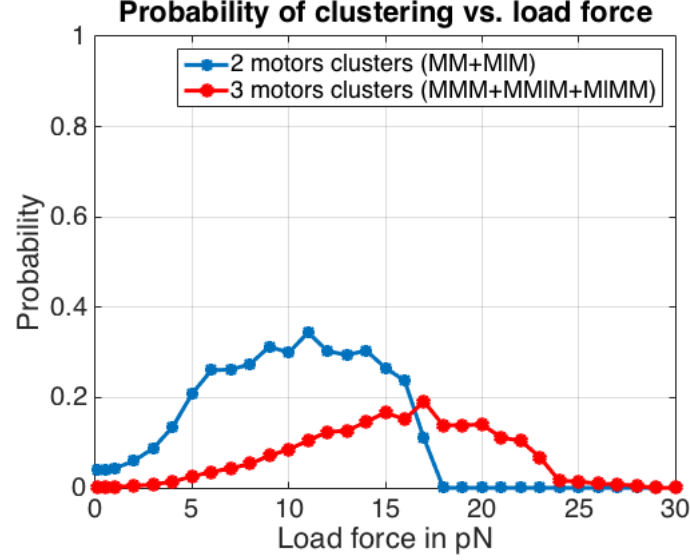


Figure 3.10: Variation of probability of clustering, with load forces for 2 and 3 motors

3.7 Summary:

We utilized a semi-analytic Markov model to analyze intracellular transport of common cargo by teams of molecular motors. We quantified 'states' as the relative arrangement of motors carrying the cargo and proved that an ensemble of finite motors has a *unique steady state distribution*, signifying the robustness of the intracellular transport mechanism. Thus, irrespective of the initial orientation of the motors in the ensemble the motors eventually behave in a fixed manner that is affected by exterior conditions. By instantiating for a team of 2 and 3 kinesin-I motors, our analysis showed that at lower ATP concentrations, the cargo prefers to be connected to the microtubule through more than one motor than just one motor. It is seen that this enhances ensemble run-lengths over single motor, despite reducing ensemble velocities, which is consistent with experimentally observed data [18]. Furthermore, increasing hindering load force on the cargo is tackled by '*clustering*' together. However, at very high load forces the motors abandon clustering and adopt configurations that prefer immediate cargo translocation once the large loading event has subsided. Our approach provides unique insights into the team behaviors of molecular motors, with the analytical approach suitable to study possible mechanisms in teams of other motors as well, such as teams of dynein, myosin or even heterologous ensembles.

3.8 Proof of Result 1:

Here, proof for the following result is provided :

For $m \in [0, \bar{m} - 1]$, the Markov chain with state space S and transition matrix $\mathbf{J}$ has a $[\pi^T \mathbf{0}^T]^T$ as a *unique steady state distribution*.

Proof :

3.8.1 Notations, Definitions and Theorems :

We establish that the Markov chain with state space S and transition matrix $\mathbf{J}$ has $[\pi^T \mathbf{0}^T]^T$ as a *unique steady state distribution*. First we present a few notations, definitions and known theorems.

The symbol $:=$ denotes a definition

$\mathbf{A}$: denotes a matrix

$|\mathbf{A}| < \epsilon$: every element of $\mathbf{A}$ is ϵ

$\mathbf{A}_{j,i}$: $(j, i)^{th}$ entry of matrix $\mathbf{A}$

$\mathbf{A}_{j,i}^k$: $(j, i)^{th}$ entry of matrix $\mathbf{A}^k$

$\mathbf{0}$: zero matrix of appropriate dimension

$\mathbf{1}$: vector of appropriate dimension with all entries 1

$\mathbf{A}^T$: transpose of matrix $\mathbf{A}$

$\rho(\mathbf{A})$: spectral radius of matrix $\mathbf{A}$

ϕ : Null set

Definition 3.8.1 (Markov Chain). Consider the probability space $(S, \mathcal{S}, P)$. A stochastic process X_n on $(S, \mathcal{S}, P)$ is a Markov Chain if $P(X_{n+1} \in B | \mathcal{F}_n) = P(X_{n+1} \in B | \sigma(X_n))$ where $B \in \mathcal{S}$, $\mathcal{F}_n$ is the sigma algebra generated by $\{X_1, X_2, \dots, X_n\}$ and $\sigma(X_n)$ is the sigma algebra generated by X_n . The associated transition matrix $\mathbf{J}$ of a Markov chain has entries $\mathbf{J}_{j,i} := P(X_1 = \sigma_j | X_0 = \sigma_i)$. The p step transition matrix is given by $\mathbf{J}^p$.

Definition 3.8.2 (Column Stochastic Matrix). A matrix $\mathbf{A}$ is said to be column stochastic if $\sum_{j=1}^n \mathbf{A}_{j,i} = 1$ for all $i = \{1, 2, \dots, n\}$. The transition matrix $\mathbf{J}$ is a column stochastic

matrix because $P(X_1 = \sigma_j | X_0 = \sigma_i)$ is a probability measure for a fixed X_0 .

Definition 3.8.3 (Irreducible Markov Chain). If for any $\sigma_i, \sigma_j \in S$, there exist $N \in \mathbb{N}$ such that $(\mathbf{J}^N)_{i,j} > 0$ then the Markov chain is said to be irreducible.

Definition 3.8.4 (Aperiodic Markov Chain). The period of a state $\sigma_i \in S$ is defined as $d(i) := \gcd\{n \geq 1 | (\mathbf{J}^n)_{i,i} > 0\}$. If $d(i) = 1$ then the state is said to be aperiodic. If all states of a Markov chain are aperiodic then the Markov chain is said to be aperiodic.

Definition 3.8.5 (Recurrent and Transient State). Define $T_{\sigma_i} = \inf\{k \geq 1 : X_k = \sigma_i | X_0 = \sigma_i\}$ and if this set is empty then $T_{\sigma_i} = \infty$. If $P\{T_{\sigma_i} < \infty | X_0 = \sigma_i\} = 1$ then σ_i is said to be a recurrent state. If $P\{T_{\sigma_i} < \infty | X_0 = \sigma_i\} < 1$ then σ_i is a transient state.

Definition 3.8.6 (Positive and Null Recurrent State). A recurrent state $\sigma_i \in S$ is said to be positive recurrent if $E[T_{\sigma_i} | X_0 = \sigma_i] < \infty$. It is said to be null recurrent if $E[T_{\sigma_i} | X_0 = \sigma_i] = \infty$.

Definition 3.8.7 (Steady State Distribution). A probability measure π on $(S, \mathcal{S})$ is said to be a steady state distribution if it satisfies $\mathbf{J}\pi = \pi$.

Definition 3.8.8 (Limit Distribution). A probability measure π on $(S, \mathcal{S})$ satisfying $\lim_{n \rightarrow \infty} \mathbf{J}^n = \pi \mathbf{1}^T$ is said to be the limit distribution of $\mathbf{J}$. A limit distribution is a steady state distribution for a Markov chain.

Theorem 3.8.1. [47] A state $i \in S$ is recurrent iff $\sum_{n=1}^{\infty} (\mathbf{P}^n)_{i,i} = \infty$.

Theorem 3.8.2. [47] A finite state irreducible Markov chain is positive recurrent .

Theorem 3.8.3. [48] A limit distribution of a Markov chain is a steady state distribution .

Theorem 3.8.4. [48] Given an irreducible and aperiodic Markov chain the following are equivalent:

1. unique stationary distribution π (such that $\pi > 0$ element wise) exists
2. it is positive recurrent

3. limit distribution exists.

Corollary 3.8.4.1. [48] *A irreducible and aperiodic finite state Markov chain has a unique steady state distribution and limit distribution π such that $\pi > 0$ element wise.*

Theorem 3.8.5. *Let $\mathbf{M}$ be a complex $n \times n$ matrix and $R_j := \sum_{i \neq j} |m_{ij}|$ be the sum of the absolute values of the non-diagonal entries in the j^{th} column. Let $D(m_{jj}, R_j)$ be the closed disc centered at m_{jj} with radius R_j . Every eigenvalue of $\mathbf{M}$ lies within at least one of the discs $D(m_{jj}, R_j)$.*

Lemma 3.8.6. [49] *Consider a matrix $\mathbf{M} \in \mathbb{R}^{n \times n}$ such that $|\rho(\mathbf{M})| < 1$. Then $\sum_{k=0}^{\infty} \mathbf{M}^k = (\mathbf{I} - \mathbf{M})^{-1}$, where $\mathbf{I}$ is the identity matrix of appropriate dimension.*

3.8.2 Main Proof :

Theorem 3.8.7. *Given a cargo attached to $\bar{m}$ motors and subjected to opposing load force F_L , there exists at least one transient state $\sigma_i \in S$.*

Proof. Among the n states, without loss of generality the states σ_n and σ_{n-1} be as described below:

- σ_n describes the relative configuration with all $\bar{m}$ motors attached to the microtubule, of which one is the rearguard motor and the rest $(\bar{m} - 1)$ are vanguard motors (see Fig. 3.11(b)).
- σ_{n-1} describes the relative configuration with has all $\bar{m}$ motors attached to the microtubule, of which one is the rearguard motor, $(\bar{m} - 2)$ are vanguard motors and 1 motor is located at a distance d_s behind the vanguard motors (Fig. 3.11(a)).

We will show that σ_n is a transient state. Let the cargo equilibrium position be X_C . Without loss of generality, let $X_C = 0$. Let the locations of the motors assisting the cargo motion be $X_1, \dots, X_{V-1}, X_V$ such that $X_V \geq X_{V-1} \geq \dots \geq X_1 \geq l_0$. The corresponding forces exerted by the motors at these locations are $F_V^+, F_{V-1}^+, \dots, F_1^+$. Let the location of the rearguard motor be $-X_R$ and force exerted by the motor be F_R^- . Therefore we have $F_R^- = K_e(X_R - l_0)$, $F_V^+ = K_e(X_V - l_0)$, $F_{V-1}^+ = K_e(X_{V-1} - l_0)$. Assume that the system is in

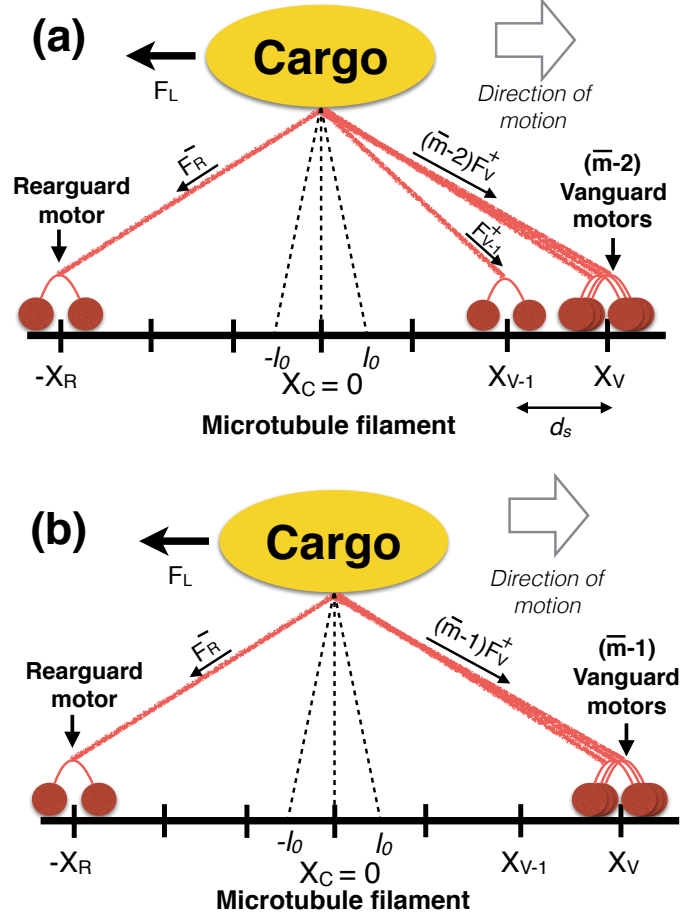


Figure 3.11: Cargo being carried by $\bar{m}$ motors in the relative configuration (a) σ_{n-1} and (b) σ_n

an initial configuration σ_{n-1} as shown in Fig. 3(b) and the distance between the vanguard and rearguard motor is $S_{max} = \max \left\{ \frac{\bar{m}F_s - F_L}{K_e} + d_s, \frac{F_L}{K_e} \right\} + 2l_0$. Using equilibrium of forces it follows from Fig. 3(b),

$$F_{V-1}^+ = F_L + K_e(X_R - l_0) - (\bar{m} - 2)K_e(X_V - l_0)$$

When the extent is S_{max} , the following two cases are possible :

Case I : If $S_{max} = \frac{\bar{m}F_s - F_L}{K_e} + d_s + 2l_0$,

$$\begin{aligned} F_{V-1}^+ &= F_L + K_e(X_R + X_V - X_V + l_0 - 2l_0) \\ &\quad - (\bar{m} - 2)K_e(X_V - l_0) \\ &= \bar{m}F_s + K_e d_s - K_e(\bar{m} - 1)(X_V - l_0) \end{aligned}$$

Since the extent between vanguard and rearguard motor is S_{max} , the vanguard motor

located at X_V cannot take a forward step that is it is stalled (based on the derivation of S_{max} in [19]). Thus $K_e(X_V - l_0) = F_S$. It follows that,

$$\begin{aligned} F_{V-1}^+ &= \bar{m}F_S + K_e d_s - (\bar{m} - 1)F_S \\ &= F_S + K_e d_s > F_S \end{aligned}$$

Therefore, the motor at location X_{V-1} on the microtubule in the configuration σ_{n-1} is stalled that is it cannot take a forward step.

Case II : If $S_{max} = \frac{F_L}{K_e} + 2l_0$, then $S_{max} > \frac{\bar{m}F_S - F_L}{K_e} + d_s + 2l_0$ (from the definition of S_{max} in [19]). Thus,

$$\begin{aligned} F_{V-1}^+ &= F_L + K_e S_{max} - 2K_e l_0 - (\bar{m} - 1)F_S \\ &> F_L + (\bar{m}F_S - F_L + K_e d_s + 2K_e l_0) \\ &\quad - 2K_e l_0 - (\bar{m} - 1)F_S \\ &> F_S + K_e d_s > F_S \end{aligned}$$

Hence, in this case as well, the motor at the location X_{V-1} is stalled. Thus the transition $\sigma_{n-1} \rightarrow \sigma_n$ (that is forward stepping of the one motor located at a distance d_s behind the vanguard motors in σ_{n-1}) will not occur.

The other possible incoming transitions into the configuration σ_n from an initial configuration $\sigma_i, i = \{1, 2, \dots, n-1\}$ are through either (1) simultaneous stepping/reattachment of more than 1 motor, or (2) reattachment of a floating motor that is attached to the cargo but not to the microtubule. Based on the modeling assumptions on Δt , there will be no incoming transition probabilities into the configuration σ_n from an initial configuration σ_i that involves simultaneous stepping/reattachment of more than 1 motor. Furthermore, all the motors in the configuration σ_n have their linkages stretched beyond the rest length l_0 . Based on the modeling assumptions, it is not possible to attain a final configuration σ_n from any initial configuration σ_i through reattachment events. The row $\mathbf{J}_{n,j}, j \in \{1, 2, \dots, n\}$ in the transition matrix $\mathbf{J}$ denotes all the incoming transition probabilities into the configuration σ_n . It is evident from the above discussion that $\mathbf{J}_{n,j} = 0, j \in \{1, 2, \dots, n-1\}$.

Furthermore, the column $\mathbf{J}_{j,n}, j \in \{1, 2, \dots, n\}$ corresponds to all the probabilities of transitioning from initial configuration σ_n to final configuration $\sigma_j, j \in \{1, 2, \dots, n\}$. At

any given instant, a motor walking along the microtubule can detach from it as there is a non zero probability that a motor in the configuration σ_n will detach. It implies $0 < \sum_{j=1}^{n-1} \mathbf{J}_{j,n}$. Furthermore, the sum $\sum_{j=1}^{n-1} \mathbf{J}_{j,n}$ includes probabilities of stepping of the rearguard motor and detachments of any of the $\bar{m}$ motors in σ_n , implying $\sum_{j=1}^{n-1} \mathbf{J}_{j,n} \leq \{\lambda_{step}(F) + \bar{m} \max(\lambda_{detach}(F))\} \Delta t < 1$. Thus $0 < \sum_{j=1}^{n-1} \mathbf{J}_{j,n} < 1$ implying $0 < \mathbf{J}_{n,n} < 1$. The row $\mathbf{J}_{n,j}, j \in \{1, 2, \dots, n\}$ has only one non-zero element $\mathbf{J}_{n,n}$. Let $\mathbf{J}_{n,n} = q$ (with $0 < q < 1$). It follows that, $\sum_{l=1}^{\infty} (\mathbf{J}^l)_{n,n} = \frac{1}{1-q} < \infty$, implying σ_n is a transient state (by Definition 3.8.5).

□

The set of relative configurations of cargo attached to $\bar{m}$ motors with at least m motors attached, denoted by S , can be written as $S = S_m \cup S_{m+1} \cup S_{m+2} \cup S_{m+3} \cup \dots \cup S_{\bar{m}}$, where S_m represents all the relative configurations in S with exactly m motors attached, S_{m+1} represents all the relative configurations with exactly $m+1$ motors attached and so on. From the model it is evident that there always exists a non-zero probability of a motor attached to the microtubule to get detached from it. Thus, given any configuration $\sigma_i \in S_{m_i}$, there exists a configuration $\sigma_j \in S_{m_{i-1}}$ such that $\mathbf{J}_{j,i} > 0$ where $\mathbf{J}_{j,i}$ denotes the probability of reaching σ_j from σ_i in a single hop, which is a detachment event. Thereby, there exists configuration $\sigma_k \in S_m$ such that $\mathbb{J}_{k,i}^{m_i-m} > 0$ i.e. it is possible to reach a configuration in the set S_m from any configuration in S_{m_i} in finite (i.e. $m_i - m$) hops.

Theorem 3.8.8. *Let S_m denote the set of relative configurations in S that have exactly m motors attached to the microtubule. Let n_m denote number of motors attached to the microtubule in the relative configuration $\sigma_p \in S_{n_m}$, where $S_{n_m} = S \setminus S_m$ and $n_m \in \{m+1, m+2, \dots, \bar{m}\}$. Then, there exists configuration $\sigma_k \in S_m$ such that $\mathbb{J}_{k,p}^N > 0$ where $N = n_m - m$ i.e. it is possible to reach a configuration in the set S_m from any configuration in $S_{n_m} = S \setminus S_m$ in finite (i.e. $N = n_m - m$) hops.*

Proof. The set of relative configurations of cargo attached to $\bar{m}$ motors with at least m motors attached, denoted by S , can be written as a disjoint union $S = \cup_{k=m}^{\bar{m}} S_k = S_m \cup S_{m+1} \cup S_{m+2} \cup S_{m+3} \cup \dots \cup S_{\bar{m}}$, where S_k represents all the relative configurations in S with exactly k motors attached to the microtubule.

Consider a configuration $\sigma_i \in S_{m+1}$. It is possible to reach $\sigma_k \in S_m$ from σ_i through detachment of one of the $(m+1)$ motors in the configuration σ_i from the microtubule, that is $\mathbf{J}_{k,i} > 0$. Next consider a configuration $\sigma_j \in S_{m+2}$. Through the detachment of one of the $(m+2)$ motors in $\sigma_j \in S_{m+2}$ from the microtubule, it is possible to reach $\sigma_i \in S_{m+1}$ that is $\mathbf{J}_{i,j} > 0$. Thus, $\mathbf{J}_{k,j}^2 = \sum_{l=0}^n \mathbf{J}_{k,l} \mathbf{J}_{l,j} \geq \mathbf{J}_{k,i} \mathbf{J}_{i,j} > 0$, where $\sigma_i \in S_{m+1}$.

It thus follows that there exists a state $\sigma_t \in S_{n_m-1}$ such that $\mathbf{J}_{k,t}^{n_m-1-m} > 0$. Consider $\sigma_p \in S_{n_m}$. Through the detachment of one of the n_m motors in $\sigma_p \in S_{n_m}$ from the microtubule, it is possible to reach $\sigma_t \in S_{n_m-1}$ that is $\mathbf{J}_{t,p} > 0$. Thus, $\mathbf{J}_{k,p}^{n_m-m} = \sum_{l=0}^n \mathbf{J}_{k,l}^{n_m-1-m} \mathbf{J}_{l,p} \geq \mathbf{J}_{k,t}^{n_m-1-m} \mathbf{J}_{t,p} > 0$. Hence for $N = n_m - m$, $n_m \in \{m+1, m+2, \dots, \bar{m}\}$, $\mathbf{J}_{k,p}^N > 0$ implying that there is a positive probability to reach the state σ_k from σ_p in finite hops. $\square$

Proposition 3.8.9. *The position of the cargo $C(x)$ is a well defined, monotone function of $x = (x_1, \dots, x_{\bar{m}})$ the positions of the motors on the MT, in the sense that $x_i \leq y_i$ for all i implies,*

$$C(x) \leq C(y)$$

Proof. The function

$$F(x, C) = K_e(\mathbb{1}_{x > C+l_0}(x)(x - (C + l_0)) \quad (3.3)$$

$$+ \mathbb{1}_{x < C-l_0}(x)(x - (C - l_0))) \quad (3.4)$$

gives the force of a motor at location x , and cargo at position C . Observe that for fixed C , it is increasing in x , strictly so outside of $(C - l_0, C + l_0)$. Analogously for fixed x , the function is decreasing in C , and strictly decreasing outside of $(x - l_0, x + l_0)$. If we label the motors so that $x_1 \geq x_2 \geq \dots \geq x_{\bar{m}}$ then we can write the force balance equation as

$$F_T = \sum F_i = \sum F(x_i, C) = F_L$$

For $C \geq x_1 - l_0$ we have $F < F_L$, and $F_1 = F(x_1, C)$ to be strictly decreasing in C , and hence F_T (which is decreasing in C due to being a summation of decreasing function) is strictly decreasing as well with $\lim_{C \rightarrow -\infty} F = -\infty$. Thus by the intermediate value theorem $F_T = F_L$ for some value of C , and by the fact that F_T is a strictly decreasing function of C on $(-\infty, x_1 - l_0)$, this value of C is unique.

Now observe that $x_i \leq y_i$ implies $F(x_i, C) \leq F(y_i, C)$, so that $F_T(x, C) \leq F_T(y, C)$. If we suppose that $F_T(y, C) = F_L$, then for $C' > C$ we have

$$F_T(x, C') \leq F_T(y, C') < F_T(y, C) = F_L$$

and from this inequality the monotonicity of C follows. $\square$

Theorem 3.8.10. *Given F_L , and $1 \leq m < \bar{m}$, there exists an ε and N such that for any configuration σ with feasible extent, and at least m motors attached to the MT, there exists a configuration σ_m such that with probability greater than ε , σ_m will reach σ within N steps, and at least m motors will remain attached to the MT during the transport. One can take σ_m to be a single motor balancing the load force F_L and (2) the rest $(m - 1)$ motors at the same location on the MT located at a distance of x ahead of the cargo (i.e. towards the positive end of the MT) uniquely determined by $x \leq l_0 < x + ds$.*

Proof of Theorem 3.8.10. If we start in a configuration with $m + 1$ motors attached to the MT, and label the motors so that their positions at time $t = 0$, $x_i(0)$ satisfy

$$x_1(0) \geq x_2(0) \geq \cdots \geq x_{m+1}(0)$$

and without loss of generality assume that $F_i \geq 0$ for all motors. We will iterate the following algorithm :

1. Detach the second furthest load bearing motor, that is a motor $i \neq 1$ that satisfies $x_i \geq x_j$ for $j \neq 1$, satisfying $x_i > C + l_0$
2. Reattach this motor to MT at the smallest possible location.

In the case that j and k both satisfy step 1, choose $i = \min\{j, k\}$. If no motors satisfy step 1, terminate the algorithm. In step 2 recall that the smallest possible location, is $\min\{x \in MT : x > C - l_0\} < C - l_0 + ds$.

After a single iteration, the motors satisfy

$$x_1(1) \geq x_3(1) \geq \cdots \geq x_{m+1}(1) \geq x_2(1),$$

with $x_2(1) < C(0) - l_0 + ds$. Should the algorithm successfully iterate m times, the motors once again satisfy

$$x_1(m) \geq x_2(m) \geq \cdots \geq x_{m+1}(m),$$

In which case there are two possibilities, $x_2(m) > C(m) + l_0$, in which case $C(m) < C(0) - 2l_0 + ds$, or the process has terminated. Iterating km times successfully, thus requires

$$C(km) < C(0) - k(2l_0 - ds) \quad (3.5)$$

. Observe that $x_1(t) = x_1(0)$. By force balance and the fact that none of the motors supply negative force to the cargo

$$F_L = \sum_i K_e(x_i - C - l_0) \geq K_e(x_1 - C(km) - l_0). \quad (3.6)$$

Since $x_1 > C(0) + l_0$, when combined with (3.5), we have

$$F_L > k(2l_0 - ds). \quad (3.7)$$

Thus it follows that the algorithm cannot successfully iterate more than

$$m \left\lceil \frac{F_L}{2l_0 - ds} \right\rceil \quad (3.8)$$

times. Since any configuration with a single motor carrying positive force can clearly advance all of its motors forward the largest position on the MT $\leq C + l_0$, this completes the proof. $\square$

Let the state space $S = \{\sigma_1, \sigma_2, \dots, \sigma_{n-1}, \sigma_n\}$, with σ_1 being the configuration described in Theorem 3.8.10. S can be divided into two sets S_1 and S_2 such that, S_1 is the collection of states that can eventually be reached from the state σ_1 , that is, for every $\sigma_j \in S_1$ there exists $N_j \in \mathbb{N}$ such that $\mathbf{J}_{j,1}^{N_j} > 0$ and S_2 is the collection of states or relative configurations that can never be reached from σ_1 , that is, for every $\sigma_k \in S_2$, $\mathbf{J}_{k,1}^N = 0$, for all $N \in \mathbb{N}$. Note that $S = S_1 \cup S_2$ and $S_1 \cap S_2 = \emptyset$.

Theorem 3.8.11. *If $\sigma_j \in S_1$ and $\sigma_k \in S_2$ then $\mathbf{J}_{k,j} = 0$, that is, there are no transitions from S_1 to S_2 .*

Proof. Suppose $\mathbf{J}_{k,j} > 0$. From the definition of S_1 , there exists $N > 0$ such that $\mathbf{J}_{j,1}^N > 0$ for any $\sigma_j \in S_1$. Then, $\mathbf{J}_{k,1}^{N+1} = \sum_{l=0}^n \mathbf{J}_{k,l} \mathbf{J}_{l,1} \geq \mathbf{J}_{k,j} \mathbf{J}_{j,1} > 0$. Therefore σ_k can eventually be reached from σ_1 . Thus, $\sigma_k \in S_1$, which is a contradiction which implies $\mathbf{J}_{k,j} = 0$, for all $\sigma_j \in S_1, \sigma_k \in S_2$. $\square$

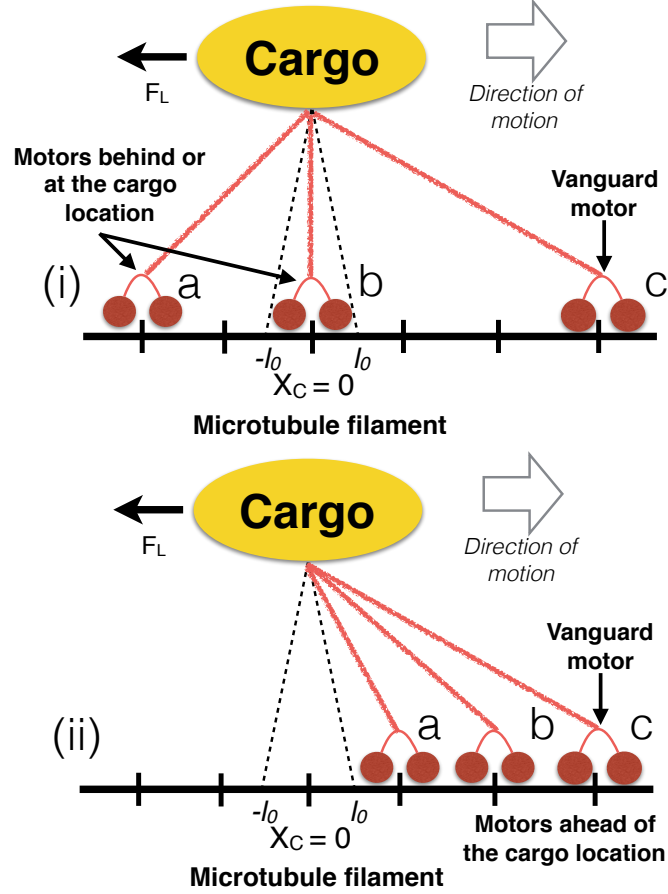


Figure 3.12: (i) Configuration with motors located behind or at the cargo location (motors a and b) (ii) Configuration with motors located ahead of the cargo location (motors a , b and c)

Theorem 3.8.12. *Any state $\sigma_j \in S_2$ is a transient state.*

Proof. Assume $\sigma_j \in S_2$ is recurrent. Then $P(T_{\sigma_j} < \infty | X_0 = \sigma_j) = 1$, implying $P(T_{\sigma_j} = \infty | X_0 = \sigma_j) = 0$. We know from Theorem 3.8.10 that it is possible to reach the state σ_1 from any of the initial states $\{\sigma_2, \dots, \sigma_{n-1}, \sigma_n\}$, that is, there exists $N_j > 0$ such that $\mathbf{J}_{1,j}^{N_j} > 0$. Furthermore from the definition of S_2 , we have $\mathbf{J}_{j,1}^N = 0$ for all $N \in \mathbb{N}$. Thus $P(T_{\sigma_j} = \infty | X_0 = \sigma_j) \geq P(k > 0 : X_k = \sigma_1 \text{ given } X_0 = \sigma_j) \geq P(X_{N_j} = \sigma_1 | X_0 = \sigma_j) > 0$. This contradicts with the assumption. Thus, σ_j cannot be a recurrent state implying $\sigma_j \in S_2$ is a transient state. $\square$

From Theorem 3.8.7 we know that there exists at least one transient state and thus $S_2 \neq \emptyset$. Let us rearrange the states such that $S = \{\sigma_1, \dots, \sigma_r, \sigma_{r+1}, \dots, \sigma_n\}$ where $\sigma_i \in S_1, i = 1, 2, \dots, r$ and $\sigma_j \in S_2, j = r + 1, \dots, n$. The associated transition matrix $\mathbf{J}$ can

now be partitioned as $\mathbf{J} = \begin{pmatrix} \mathbf{A} & \mathbf{B} \\ \mathbf{D} & \mathbf{C} \end{pmatrix}$, where $\mathbf{A}, \mathbf{B}, \mathbf{C}$ and $\mathbf{D}$ represent the probability transition matrices between $S_1 \rightarrow S_1, S_2 \rightarrow S_1, S_2 \rightarrow S_2$ and $S_1 \rightarrow S_2$ respectively. It follows from From Theorem 3.8.11, all elements of the submatrix $\mathbf{D}$ are zero that is $\mathbf{D} = \mathbf{0}$. Thus,

$$\mathbf{J} = \begin{pmatrix} \mathbf{A} & \mathbf{B} \\ \mathbf{0} & \mathbf{C} \end{pmatrix}, \quad (3.9)$$

implying that $\mathbf{A}$ is the probability transition matrix associated with the Markov chain on S_1 . Note that all the elements of the submatrix $\mathbf{B}$ cannot be zero, since it violates the existence of transient states in S_2 .

Theorem 3.8.13. *The Markov chain S_1 is irreducible and positive recurrent.*

Proof. Consider $\sigma_i, \sigma_j \in S_1$. Then there exists $N_1, N_2 \in \mathbb{N}$ such that $\mathbf{J}_{i,1}^{N_1} > 0$ (from the definition of the set S_1) and $\mathbf{J}_{1,j}^{N_2} > 0$ (from Theorem 3.8.10). Thus $\mathbf{J}_{i,j}^{N_1+N_2} = \sum_{l=1}^n \mathbf{J}_{i,l}^{N_1} \mathbf{J}_{l,j}^{N_2} \geq \mathbf{J}_{i,1}^{N_1} \mathbf{J}_{1,j}^{N_2} > 0$. Thus S_1 forms an irreducible Markov Chain. Theorem 3.8.2 implies that S_1 is positive recurrent. $\square$

Theorem 3.8.14. *The transition matrix $\mathbf{J}$ is aperiodic.*

Proof. We first show that the diagonal elements of $\mathbf{J}$ are non-zero.

Consider the configuration σ_i , as described in theorem 3.8.10. The configuration has m motors attached to the MT. Note that the total number of motors attached to the cargo is $\bar{m}$ (with $\bar{m} > m$), meaning that in the configuration σ_1 there are $\bar{m} - m$ floating motors attached to the cargo but unattached to the MT. Based on the model, we have assumed that any floating motor can reattach to any location on the MT within the distance l_0 of the cargo location, with equal probability. $0 < \sum_{j \neq 1} \mathbf{J}_{j,1} \approx (\lambda_{reatt} \Delta t)$. For the Δt chosen in the mathematical model, $\lambda_{reatt} \Delta t \ll 1$. Thus $\mathbf{J}_{1,1} = (1 - \sum_{j \neq 1} \mathbf{J}_{j,1}) > 0$

Consider rest of the diagonal elements $\mathbf{J}_{i,i}, i \neq 1$. Let the initial configuration be $\sigma_i, i \neq 1$. σ_i is a relative configuration with n_m motors attached to the microtubule, with $\bar{m} > n_m \geq m$ and $m \in \{1, 2, \dots, \bar{m} - 1\}$. From the configuration σ_i , there can either be stepping, detachment or reattachment events possible. Thus we can state that for small Δt and $i \neq 1$, $\sum_{j \neq i} \mathbf{J}_{j,i} \leq \{(\lambda_{step} + \lambda_{detach} + \lambda_{reattach})\} \Delta t < \{\bar{m}(\lambda_{step} + \lambda_{detach} + \lambda_{reattach})\} \Delta t$. For

the motors considered, the rates λ_{step} , λ_{detach} and $\lambda_{reattach}$ are non-zero. So the Δt in the mathematical model can be chosen to be small enough so that $\Delta t < \frac{1}{\bar{m}(\lambda_{step} + \lambda_{detach} + \lambda_{reattach})}$ which implies $\sum_{j \neq i} \mathbf{J}_{j,i} < 1$. Note that for $i \neq 1$, $(\mathbf{J}_{i,i} + \sum_{j \neq i} \mathbf{J}_{j,i}) = 1$, meaning for $i \neq 1$, $\mathbf{J}_{i,i} < 1$. Moreover, $\mathbf{J}_{i,i} = (1 - \sum_{j \neq i} \mathbf{J}_{j,i}) > 0$ for $i \neq 1$. Thus, $\mathbf{J}_{i,i} > 0, i = \{1, 2, \dots, n\}$. It implies that $\mathbf{J}_{i,i}^N \geq [\mathbf{J}_{i,i}]^N > 0$ for all $N \in \mathbb{N}$. Thus $\gcd\{N \geq 1 : \mathbf{J}_{i,i}^N > 0\} = \gcd\{1, 2, \dots\} = 1$ implying $\mathbf{J}$ is aperiodic. $\square$

S_1 being irreducible, positive recurrent and aperiodic it follows from Corollary 3.8.4.1 that S_1 has a limit distribution π which is also the unique steady state distribution of S_1 . We now show that, under the condition that the cargo is not lost, the transition matrix $\mathbf{J}$ has a limit distribution and $P(t)$ approaches a unique steady state distribution.

Lemma 3.8.15. *The spectral radius $|\rho(\mathbf{C})| < 1$, $\lim_{l \rightarrow \infty} \mathbf{C}^l = \mathbf{0}$ and $\mathbf{I} - \mathbf{C}$ is invertible.*

Proof. For the transition matrix $\mathbf{J}$ as defined in (3.9), it follows from Theorem 3.8.10 that there exist $k \in \mathbb{N}$ such that $\mathbf{J}_{1,j}^k > 0$ for all $j \in \{1, 2, \dots, n\}$, that is, sum of every column of $\mathbf{C}^k < 1$. The Gershgorin theorem (see Theorem 3.8.5) leads to $|\rho(\mathbf{C}^k)| < 1$, from which it follows that $|\rho(\mathbf{C})| < 1$, $\lim_{l \rightarrow \infty} \mathbf{C}^l = \mathbf{0}$ and $\mathbf{I} - \mathbf{C}$ is invertible. $\square$

Lemma 3.8.16. *Consider a matrix $\mathbf{J}$ as defined in (3.9). Then $\mathbf{1}^T \mathbf{B}(\mathbf{I} - \mathbf{C})^{-1} = \mathbf{1}^T$.*

Proof.

$$\begin{aligned}
 & \mathbf{1}^T \mathbf{B}(\mathbf{I} - \mathbf{C})^{-1} \\
 &= \left[\sum_{i=1}^r \mathbf{B}_{i,1} \quad \sum_{i=1}^r \mathbf{B}_{i,2} \quad \cdots \quad \sum_{i=1}^r \mathbf{B}_{i,n-r} \right] (\mathbf{I} - \mathbf{C})^{-1} \\
 &= \left[1 - \sum_{i=1}^{n-r} \mathbf{C}_{i,1} \quad \cdots \quad 1 - \sum_{i=1}^{n-r} \mathbf{C}_{i,n-r} \right] (\mathbf{I} - \mathbf{C})^{-1} \\
 &= ([1 \ 1 \ \cdots \ 1] - \mathbf{1}^T \mathbf{C})(\mathbf{I} - \mathbf{C})^{-1} \\
 &= \mathbf{1}^T (\mathbf{I} - \mathbf{C})(\mathbf{I} - \mathbf{C})^{-1} = \mathbf{1}^T.
 \end{aligned}$$

Note that the second equality follows from the column stochasticity of $\mathbf{J}$. $\square$

Lemma 3.8.17. *For $N_3 \in \mathbb{N}$ fixed, $\lim_{k \rightarrow \infty} \mathbf{A}^k \mathbf{B} + \mathbf{A}^{k-1} \mathbf{B} \mathbf{C} + \mathbf{A}^{k-2} \mathbf{B} \mathbf{C}^2 + \cdots + \mathbf{A}^{k-N_3} \mathbf{B} \mathbf{C}^{N_3} = \pi \mathbf{1}^T \mathbf{B}(\mathbf{I} + \mathbf{C} + \cdots + \mathbf{C}^{N_3})$.*

Proof. The proof is left to the reader. $\square$

Theorem 3.8.18. *The Markov chain with state space S and associated transition matrix $\mathbf{J}$ has $[\pi^T \ \mathbf{0}^T]^T$ as the limit distribution and unique steady state distribution.*

Proof. Since π is the limit distribution of S_1 , $\lim_{k \rightarrow \infty} \mathbf{A}^k = \pi \mathbf{1}^T$. Using (3.9),

$$\mathbf{J}^k = \begin{pmatrix} \mathbf{A}^k & \mathbf{E}_k \\ \mathbf{0} & \mathbf{C}^k \end{pmatrix}, \quad (3.10)$$

where, $\mathbf{E}_k = \sum_{j=0}^{k-1} \mathbf{A}^{k-1-j} \mathbf{B} \mathbf{C}^j$. Given that $\lim_{k \rightarrow \infty} \mathbf{C}^k = \mathbf{0}$, if we show that $\lim_{k \rightarrow \infty} \mathbf{E}_k = \pi \mathbf{1}^T$, then $[\pi^T \ \mathbf{0}^T]^T$ is the limit distribution of S . Note that $\mathbf{E}_k$ for $k = 1, 2, \dots$ has all entries non negative and bounded by 1.

Given $\epsilon > 0$ there exist $N_1 > 0$ such that for all $k > N_1$, $|\mathbf{C}^k| < \epsilon$. By Lemma 3.8.15, $\mathbf{I} - \mathbf{C}$ is invertible, hence it follows from Lemma 3.8.6, $\lim_{N \rightarrow \infty} \mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^N) = \mathbf{1}^T \mathbf{B} (\mathbf{I} - \mathbf{C})^{-1}$. Thus, there exist an integer N_2 such that for all $k > N_2$, $|\mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^k) - \mathbf{1}^T \mathbf{B} (\mathbf{I} - \mathbf{C})^{-1}| < \epsilon$. Choose $N_3 = \max(N_1, N_2)$. There exist $N_4 > N_3$ such that for all $k > N_4 > N_3$, $|\mathbf{A}^k \mathbf{B} + \mathbf{A}^{k-1} \mathbf{B} \mathbf{C} + \mathbf{A}^{k-2} \mathbf{B} \mathbf{C}^2 + \dots + \mathbf{A}^{k-N_3} \mathbf{B} \mathbf{C}^{N_3} - \pi \mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^{N_3})| < \epsilon$

ϵ (by Lemma 3.8.17). Then,

$$\begin{aligned}
& |\mathbf{E}_{k+1} - \pi \mathbf{1}^T| \\
&= |\mathbf{A}^k \mathbf{B} + \mathbf{A}^{k-1} \mathbf{B} \mathbf{C} + \dots + \mathbf{A}^{k-N_3} \mathbf{B} \mathbf{C}^{N_3} + \\
&\quad \mathbf{A}^{k-N_3-1} \mathbf{B} \mathbf{C}^{N_3+1} + \dots + \mathbf{B} \mathbf{C}^k - \pi \mathbf{1}^T| \\
&\leq |\mathbf{A}^k \mathbf{B} + \mathbf{A}^{k-1} \mathbf{B} \mathbf{C} + \dots + \mathbf{A}^{k-N_3} \mathbf{B} \mathbf{C}^{N_3} - \pi \mathbf{1}^T| + \\
&\quad |\mathbf{A}^{k-N_3-1} \mathbf{B} \mathbf{C}^{N_3+1} + \dots + \mathbf{B} \mathbf{C}^k| \\
&\leq |\mathbf{A}^k \mathbf{B} + \mathbf{A}^{k-1} \mathbf{B} \mathbf{C} + \dots + \mathbf{A}^{k-N_3} \mathbf{B} \mathbf{C}^{N_3} - \\
&\quad \pi \mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^{N_3})| \\
&\quad + |\pi \mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^{N_3}) - \pi \mathbf{1}^T| \\
&\quad + |(\mathbf{A}^{k-N_3-1} \mathbf{B} + \dots + \mathbf{B} \mathbf{C}^{k-N_3-1}) \mathbf{C}^{N_3+1}| \\
&\leq \epsilon + |\pi \mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^{N_3}) - \pi \mathbf{1}^T| \\
&\quad + |\mathbf{E}_{k-N_3} \mathbf{C}^{N_3+1}| \\
&\leq |\pi \mathbf{1}^T \mathbf{B} (\mathbf{I} + \mathbf{C} + \dots + \mathbf{C}^{N_3}) - \pi \mathbf{1}^T \mathbf{B} (\mathbf{I} - \mathbf{C})^{-1}| + \\
&\quad |\pi \mathbf{1}^T \mathbf{B} (\mathbf{I} - \mathbf{C})^{-1} - \pi \mathbf{1}^T| + (n-r+1)\epsilon, \text{ \{by Lemma 3.8.15\}} \\
&\leq |\pi \mathbf{1}^T \mathbf{B} (\mathbf{I} - \mathbf{C})^{-1} - \pi \mathbf{1}^T| + (n-r+2)\epsilon \\
&= (n-r+2)\epsilon \{\text{by Lemma(3.8.16)}\}
\end{aligned}$$

Thus, $\lim_{k \rightarrow \infty} \mathbf{E}_k = \pi \mathbf{1}^T$. It follows that $\lim_{k \rightarrow \infty} \mathbf{J}^k = [\pi^T \mathbf{0}^T]^T \mathbf{1}^T$ which implies $[\pi^T \mathbf{0}^T]^T$ is a limit distribution of $\mathbf{J}$. From Theorem (3.8.3) $[\pi^T \mathbf{0}^T]^T$ is a steady state distribution of S . The irreducibility and aperiodicity of S_1 implies that π is the unique steady state distribution of S_1 which in turn implies that $[\pi^T \mathbf{0}^T]^T$ is the unique steady state distribution of S . $\square$

Chapter 4

Single Molecule Studies Enabled by Model-Based Controller Design

Optical tweezers have enabled important insights into intracellular transport through the investigation of motor proteins, with their ability to manipulate particles at the microscale, affording femto newton force resolution. Its use to realize a constant force clamp has enabled vital insights into the behavior of motor proteins under different load conditions. However, the varying nature of disturbances and the effect of thermal noise pose key challenges to force regulation. Furthermore, often the main aim of many studies is to determine the motion of the motor and the statistics related to the motion, which can be at odds with the force regulation objective.

In this article, we propose a mixed objective H_2/H_∞ optimization framework using a model-based design, that achieves the dual goals of force regulation and real time motion estimation with quantifiable guarantees. Here, we minimize the H_∞ norm for the force regulation and error in step estimation while maintaining the H_2 norm of the noise on step estimate within user specified bounds. We demonstrate the efficacy of the framework through extensive simulations and an experimental implementation using an optical tweezer setup with live samples of the motor protein ‘kinesin’; where regulation of forces below 1 piconewton with errors below 10% is obtained while simultaneously providing real time estimates of motor motion.

4.1 Introduction

Transport of important cargo inside the cells of eucaryotic species occurs through molecular motor proteins: kinesin, dynein and myosin. These motor proteins enable directed transport of cargo by converting chemical energy to mechanical energy and are central to the regulatory mechanisms that maintain the internal organization of the cell [50, 51]. Structurally, the motor proteins are composed of a cargo binding tail domain, a stalk and two motor heads; where the motor heads bind to the microtubule filaments while transporting the cargo as shown in Fig. 4.1. The tail domain attaches to the cargo of interest while the motor heads take discrete steps (or ‘walk’) over the pathways, composed of microtubules, inside the cell [4], thereby enabling directed transport of intracellular cargo. The typical motion of a single motor protein constitutes of series of discrete stepping events [52]. Motor proteins enable crucial intracellular functions, from producing muscle contraction and cellular tension to transport of sub-cellular organelles. Thus, proper functioning of molecular motors is indispensable to maintain a healthy cellular environment. Disruption of transport mechanisms due to impaired motor protein behavior is known to cause a host of maladies, including neurodegenerative diseases such as Huntington’s, Parkinson’s and Alzheimer’s [5, 21], high blood pressure and muscular disorders such as heart disease.

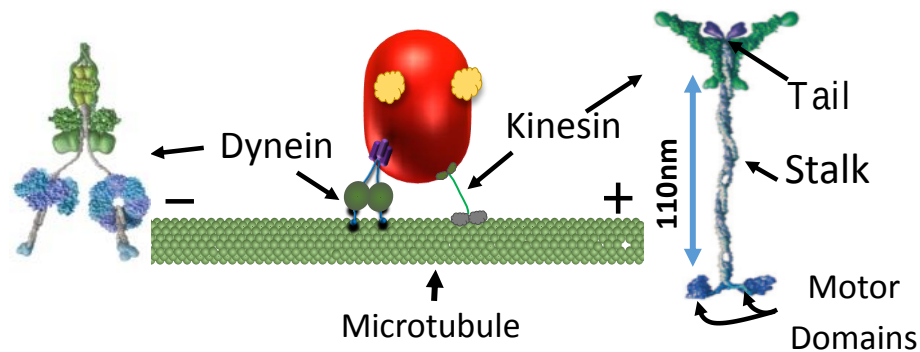


Figure 4.1: Schematic showing intracellular cargo carried by motor proteins kinesin and dynein over a section of the microtubule inside the cell. kinesin pulls the cargo towards the ‘plus’ end of the microtubule while dynein primarily pulls the cargo towards the ‘minus’ end of the microtubule

Challenges in investigation of motor motion through direct observation (using, for example, confocal or transmission electron microscopy) stem from the extremely small dimensions and step sizes of the motor proteins. For example, kinesin is about 110 *nm* in length and

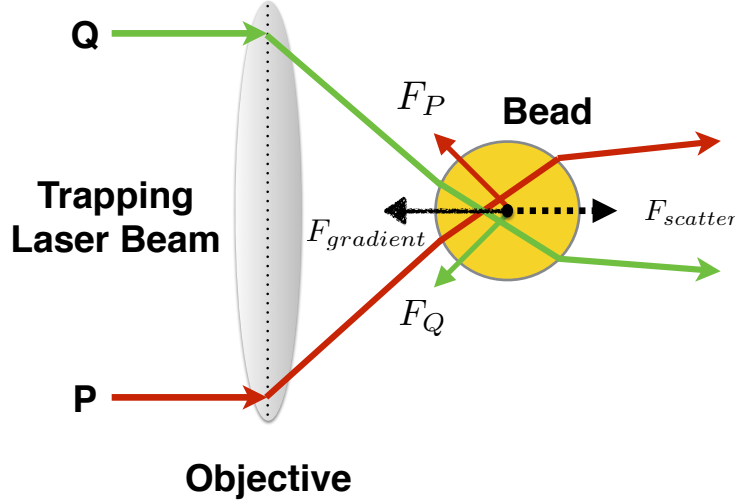


Figure 4.2: Bead in an optical trap, as described in [1]. When the trapping laser beam is passed through a high numerical aperture objective, microscopic particles (such as the spherical bead) in the vicinity of the focus of the objective experience two kinds of forces (scattering and gradient forces) due to the momentum transfer onto the particles from the reflected and refracted rays of the laser. F_P and F_Q are the reaction forces felt by the bead due to the momentum transfer from the rays P and Q respectively. The destabilizing *scattering forces* $F_{scatter}$ generated by the laser beam are balanced by the *gradient forces* $F_{gradient}$ resulting from the Gaussian intensity profile of the laser; thereby creating a stable equilibrium point.

takes 8 *nm* sized steps, while exerting forces in the range of femto newtons on the cargo. An efficient method of probing molecular motors is through the use of optical traps [35, 53], which use a laser beam focused through a high numerical aperture (NA) objective to trap micron-sized dielectric beads suspended in a suitable liquid medium, as shown in Fig. 4.2. When the beam is passed through a high NA objective, the interaction of the bead with the reflected and refracted rays of the laser, results in formation of a stable equilibrium point near the focus of the objective [54]. For small values of bead displacement, Δx , from the equilibrium point, the trap exerts a restoring force F_{trap} on the bead that is directed towards the trap location. F_{trap} varies linearly with the displacement following the relationship $F_{trap} = K_t \Delta x$; where K_t is the stiffness of the trap. The displacement of the bead can be measured up to a limited range but with sub-nanometer resolution using photo diodes whereas high bandwidth cameras yield larger range with resolution in micrometers [55, 56]. The position of the bead can be controlled by manipulating the position of the trapping

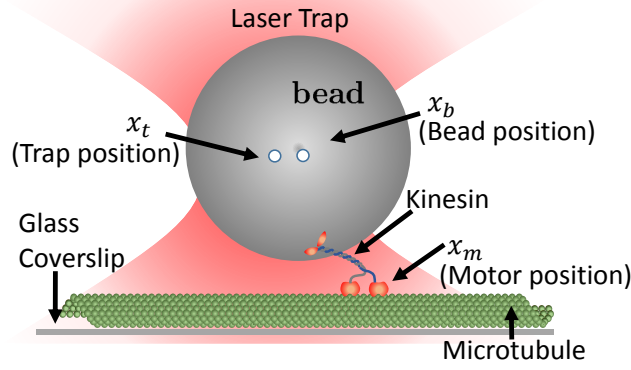


Figure 4.3: Investigation of bead attached to motor protein ‘kinesin’ using an optical trap. x_t , x_b and x_m denote the trap location, bead position and motor position respectively.

laser, using actuators such as galvo mirrors (with bandwidth in Hz) and acousto-optic deflectors (with bandwidth in KHz). Optical traps have enabled force resolution on the order of femto-newtons and position resolution on the order of nanometers, proving successful in the study of a variety of nano-scale systems such as transport inside cells [35, 29], separation of microscopic objects [57, 58], etc.

To facilitate the investigation of motor proteins and detection of the stepping motion (denoted by x_m in Fig. 4.3) using optical traps, motor proteins are attached to spherical dielectric beads using appropriate biochemistry (see [59]). The beads act as cargo that can be trapped using an optical trapping setup. An *in-vitro* environment mimicking the habitat inside the cell is created in a glass chamber, where microtubule filaments are coated at the base of the glass chamber. Using the principles of optical trapping [54, 1], the cargo is trapped and brought close to the microtubules, to allow for the motor proteins connected to the cargo to attach to the microtubule as shown in Fig. 4.3. Once attached, the motor protein takes steps on the microtubule, while pulling the bead. An instance of the bead position measured by a photo-diode sensor is shown in Fig. 4.4. The stepping data collected enables inference of important statistics of molecular motors such as motor detachment rates, stepping rates and reattachment rates, which inform several widely used models of motor proteins. The models have enabled numerous important results on intracellular transport by single and multiple motor proteins [36, 17, 19, 60, 61].

Although measured bead position (such as that shown in Fig. 4.4) yields stepping statistics of the attached motor proteins, the motor linkage stiffens under increasing load

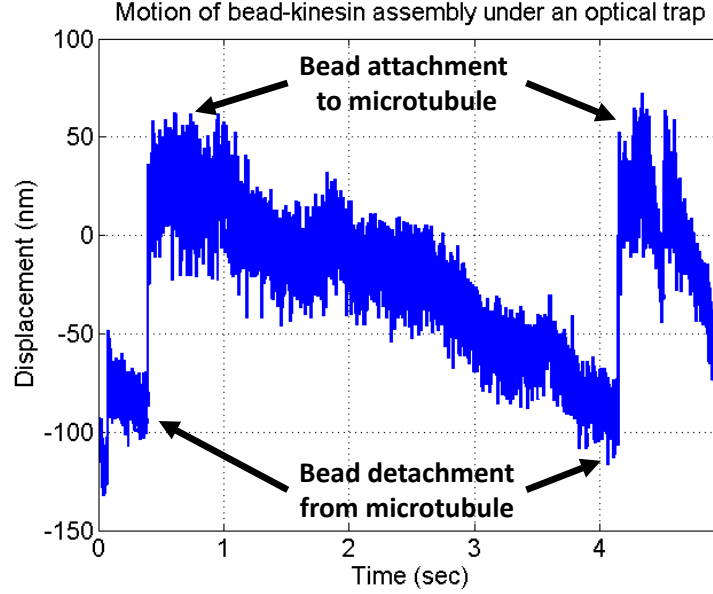


Figure 4.4: Bead position data x_b gathered using position sensor, with the bead in an optical trap while simultaneously traversing along the microtubule via the motor protein kinesin attached to it. When the optical trap is held stationary, it exerts more and more force on the bead as the bead is pulled further away from the trap focus. With increasing force on the bead it is likely that the motors pulling the bead detach from the microtubule, at which point the bead is pulled back towards the focus of the trap due to the restoring force of the trap. This figure shows the sawtooth-like patterns [2] characteristic of the bead motion due to the attached molecular motors.

[62, 3] exhibiting nonlinear force extension characteristics. Thus, bead displacement data yields an inaccurate measure of the motor-protein motion, often necessitating corrections (about 15% for forces beyond 1 pN) to compensate for the effect [3]. Furthermore, for bead position measurements such as those shown in Fig. 4.4, the restoring force on the bead varies as it travels under the influence of the trap. Thus the motor takes every step under changing load conditions. Transport properties of motor proteins are known to be altered when loading conditions change; thus making it difficult to study the effect of load forces on motor protein behavior.

Studies of molecular motors under constant forces using optical tweezers are made possible through the use of *constant force clamps* ([3, 63]). They are designed to make the optically trapped bead follow the motor protein motion by regulating the separation between bead and trap position, $(x_b - x_t)$ to a desired value e_d . allowing for the approximation of motor linkage as a Hookean spring. It is equivalent to maintaining load force on the bead

equal to $F_d = k_t e_d$, thus allowing for constant force studies. Furthermore, it enables the measured bead position to correctly reflect the motor motion; from which the motor stepping statistics can be extracted using a variety of offline step detection techniques [64, 65] without the need for ad-hoc corrections [3]. Constant force clamps manipulate the position of the trap x_t using suitable actuators.

The major challenges in the force regulation objective are due to the high bandwidth disturbance caused by motor motion and the large variance of the thermal noise affecting the cargo. State-of-art force clamps [3, 63] yield low bandwidths and are effective for studying motors moving at slow speeds. As alluded to earlier, elucidating the true motion of the motor protein remains the main objective; however, the measured bead motion is corrupted by thermal noise effects and thus presently the measured position of the bead is processed via offline methods [64, 65] to estimate the motor motion. Furthermore, existing methods regulate the load force on the bead but fail to take into account the affect of motor motion on the force experienced by the molecular motor itself. Thus for studies of motor protein based transport of cargo a method that simultaneously provides, accurate loading of the motor protein while it is in motion, and a high bandwidth and high resolution real time estimate of motor motion, will be a significant enabler. Existing state-of-art [3, 63] is unable to accommodate such a multi-objective design.

Our Contribution In this article, we employ the mixed objective H_2/H_∞ framework described in [66] to address the the desired diverse set of objectives. Using the framework, it is possible to have regulation of load force as well as estimation of stepping motion as design objectives. Through simulations, we demonstrate the ability to regulate forces below 1 pN with errors below 10% on cargoes moving up to speeds of 160 nm/s, which is close to the observed kinesin *in-vitro* speeds. Such a capacity will enable constant force studies where low errors in force regulation is of critical importance [60, 19]. Further simulations demonstrate the ability to regulate forces on motors moving as fast as 1000 nm/s with errors below 17%, resulting in significantly improved force clamp capabilities for probing motors with larger step sizes (hence higher speeds [67]) and motors moving at native speeds [68]. Moreover, we demonstrate real time estimation of motor motion for motors travelling

at speeds up to 160 nm/s with the root mean squared error in step estimation below 5%; while simultaneously maintaining sub pico-newton forces with regulation error below 10% (as mentioned earlier).

We implement the design on a custom designed optical tweezer setup and demonstrate the performance on live samples of motor protein kinesin. Experimentally, we are able to regulate forces below 1 pN with errors below 10% on motors moving up to 200 nm/s using *in-vitro* motility assays. Without sacrificing force regulation, we demonstrate real time stepping estimation of motor motion at 55 nm/s. This constitutes the first such demonstration, in both simulations and experiments, of maintaining load forces below 1 pN with errors less than 10% while simultaneously estimating motor motion in real time. A preliminary simulation study of the force clamps using robust control framework has appeared in [23] and a preliminary experimental demonstration of the force clamp on a system mimicking the motor motion (without using motor proteins) has appeared in [24]. This article builds upon [23, 24] while accommodating the additional objective of reducing the effect of motor motion on the force experienced by the molecular motor. Along with the added objective, the design in this article is able to achieve a notable improvement in the bandwidth of regulation of force on the bead over that reported in [23, 24]. It further presents a detailed computational and experimental implementation of the constant force clamp on live motor protein (kinesin) samples. It thus provides first such demonstration of a model based control design framework on live motor protein samples.

The article is organized as follows. Section II presents the mathematical modeling and characterization of the system. Section III presents the controller design using the mixed objective H_2/H_∞ framework described in [66] for the optical tweezer system. Here, we present simulation results for force regulation and real-time stepping estimation of motor motion in the presence of thermal noise. Section IV presents experimental implementation of the framework and the results associated with testing the design on live kinesin motor proteins. It is followed by conclusions in section V.

4.2 Modeling and Characterization

4.2.1 Experimental Setup

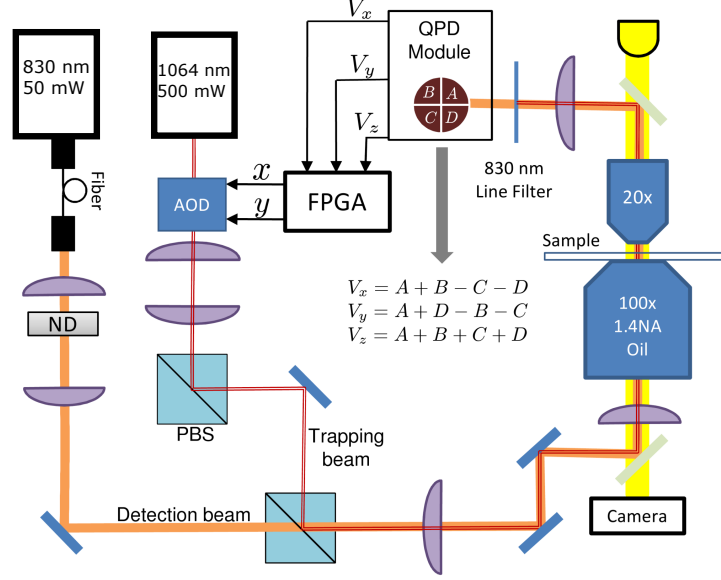


Figure 4.5: Schematic for custom designed ‘Optical Tweezer’ setup

The custom built optical tweezer forms the experimental setup for realizing optical traps (Fig. 4.5) where a Nd:YAG trapping laser (CrystaLaser Inc., $\lambda = 1064$ nm, 500 mW) is expanded using appropriate optics to fill the back aperture of high numerical aperture (NA) objective (Nikon 100x, 1.4 NA, oil immersion). The optical trap is formed at the focal point where spherical polystyrene beads are trapped. The trapping laser passes through 2-axis acousto-optic-detector (AOD, IntraAction Corp., *DTD-274HA6*) that precisely steers the beam in $x - y$ plane in response to appropriate commands. To detect the bead position, a second detection laser (Point Source Inc., *iFLEX 2000*, 50 mW, $\lambda = 830$ nm) is used to map the image of the bead onto a quadrant photodiode (QPD, Pacific Silicon Sensors, *QP50-6SD2*). A neutral density (ND) filter is added in the path of the detection laser to reduce its intensity to ensure that it does not interfere with the trapping phenomenon. The photodiode enables the detection of the bead location by providing three signals V_x , V_y and V_z where V_x and V_y represent the photon distribution on the photodiode along x axis and y axis respectively while V_z corresponds to total light intensity. These signals are captured using FPGA based data acquisition system (National Instruments, *PCI7833R*)

that generates appropriate commands for the AOD. The controller is implemented using Labview-based NI-FPGA.

4.2.2 System Model, Instrument Dynamics and Actuator Characterization

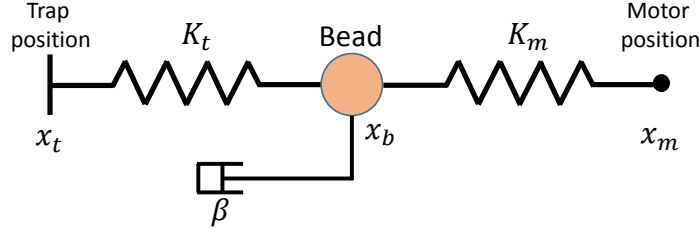


Figure 4.6: Block diagram describing the open loop plant

Let the locations of the trap centre, cargo and motor head (on the microtubule) be denoted by x_t , x_b and x_m respectively. The bead in the optical trap is suspended in an aqueous medium with the coefficient of viscous damping β . For small magnitudes of displacement $x_b - x_t$ of the optically trapped cargo from the trap centre, the force on the bead due to the optical trap is given by $K_t(x_b - x_t)$ [3]. Similarly, for small extensions $x_m - x_b$ of the motor-stalk, the force on the cargo due to the motor protein is modeled as $K_m(x_m - x_b)$ [3], where K_m is the stiffness of the motor-stalk linkage. Under these conditions, the molecular motor carrying an optically trapped cargo (in this case, a spherical dielectric bead) can be modeled as a spring-mass-damper system with trap stiffness K_t , motor stiffness K_m and the coefficient of viscous damping β , as shown in Fig. 4.6. The dynamics of the bead are observed to be highly over-damped [1], thus enabling the equation of motion of the bead in the presence of thermal noise η to be given by the following Langevin equation,

$$m\ddot{x}_b = -\beta\dot{x}_b + K_m(x_m - x_b) + K_t(x_t - x_b) + \eta. \quad (4.1)$$

The mass of the bead m is very small (of the order 10^{-17} kg), so the Eq. (4.1) reduces to,

$$\beta\dot{x}_b = K_m(x_m - x_b) + K_t(x_t - x_b) + \eta. \quad (4.2)$$

Applying Laplace transform to Eq. (4.2),

$$x_b(s) = G(s)(K_mx_m(s) + K_tx_t(s) + \eta(s)). \quad (4.3)$$

where $G(s) = \frac{1}{\beta s + K_t + K_m}$ and there is an abuse of notation with $x_m(s)$ used to represent the time and its Laplace transform as well.

In the absence of the motor (i.e. no motor attached to the bead) and thermal noise, the bead position in open loop follows the equation $x_b(s) = \frac{K_t}{\beta s + K_t} x_t(s)$. Let $G_p(s) = \frac{1}{\beta s + K_t}$. The trap position $x_t(s)$ can be manipulated using the command signal to the actuator $u(s)$ through the relation $x_t(s) = A(s)u(s)$, where the transfer function $A(s)$ models the dynamics of the actuator used to control the trap position. Thus, the position of the bead in open loop, in response to the command signal $u(s)$ is given by,

$$x_b(s) = K_t G_p(s) A(s) u(s), \quad (4.4)$$

One of the primary reasons for the low bandwidth of the state-of-art force clamps is that they ignore dynamics of the actuator i.e. the acousto-optic deflector (AOD) [23, 24]. Given the inherent physics of the AOD, the dynamics need to be modeled accurately in order to design a force clamp that can accommodate slow as well as fast moving motors. In eq. (4.4), the transfer function from the command input $u(s)$ to the measured bead position $x_b(s)$ is given by $G_h(s) = K_t G_p(s) A(s)$. We determine the expression for the actuator transfer function $A(s)$ from the experimentally identified expression for $G_h(s)$ using :

$$A(s) = \frac{G_h(s)}{K_t G_p(s)}, \quad (4.5)$$

where trap stiffness K_t and damping coefficient β are obtained by methods described in [3]. The transfer function $G_h(s)$ specific to the actuator and control hardware used to perform the experiments is identified by performing a chirp wave based system identification. A 3-pole 2-zero transfer function fit yields

$G_h(s) = \frac{0.0396s^2 - 3160s + 1.101 \times 10^8}{1.7 \times 10^{-5}s^3 + 0.9967s^2 + 3.408 \times 10^4s + 1.086 \times 10^8}$. Fig. 4.7 shows that the experimentally obtained step response shows a good match with the response predicted by the transfer function $G_h(s)$. Fig. 4.7 (blue curve) clearly demonstrates that the bead initially traverses in a direction opposite to the commanded direction, which is characteristic of the delays present in the AOD actuation system. The identified transfer function $G_h(s)$ (whose step response is shown in Fig. 4.7, red curve) contains a right half plane zero, that incorporates effects of delays; and by using (4.5) yields the actuator transfer function

$A(s) = \frac{0.66s^2 - 5.267 \times 10^4 s + 1.835 \times 10^9}{s^2 + 5.51 \times 10^4 + 1.81 \times 10^9}$. The magnitude and frequency plots of $A(s)$ are shown in Fig. 4.8 (a) and (b) respectively. Existing force clamps [3, 63] make the simplifying assumption of $A(s) = 1$ which is only appropriate for low frequencies and will lead to diminished performance at higher frequencies.

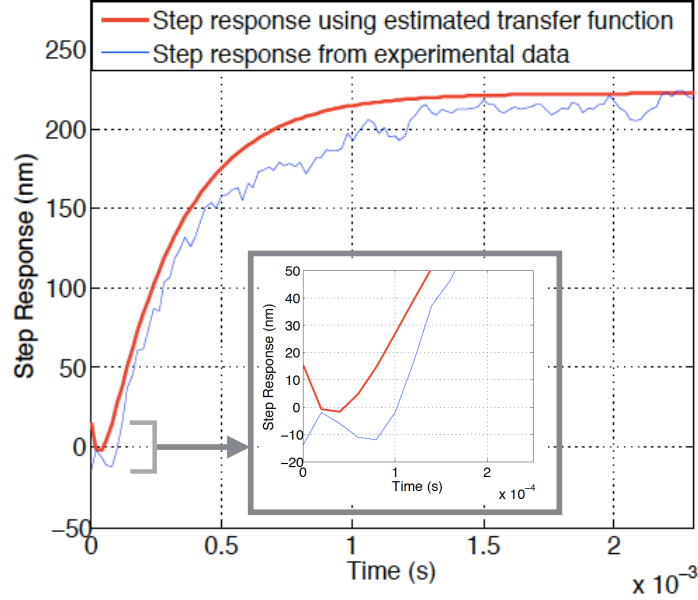


Figure 4.7: Validation of the fitted transfer function $G_h(s)$ by comparing step responses. The experimentally obtained step response shows close agreement with that obtained using the fitted transfer function $G_h(s)$. Note the undershoot seen in both the experimental and simulation data (see inset) is characteristic of the delays present in the AOD system.

Another way to identify the actuator transfer function is to model $G_h(s) = e^{-ts} \frac{C}{s+C}$, where t is the actuator delay and the constant $C = \frac{K_t}{\beta}$ incorporates the plant dynamics. A second order Pade approximation of e^{-ts} yields a transfer function that matches the transfer function $A(s)$ obtained by the chirp wave based identification. The plant $G(s)$, actuator $A(s)$, trap stiffness K_t and motor linkage stiffness K_m , together form the open loop plant P_{OL} , as shown in Fig. 4.9.

In the next section, we present the mixed objective H_2/H_∞ framework utilized in order to design a controller that meets the multiple objectives of maintaining constant force on the bead and estimating the disturbance signal (motor stepping motion).

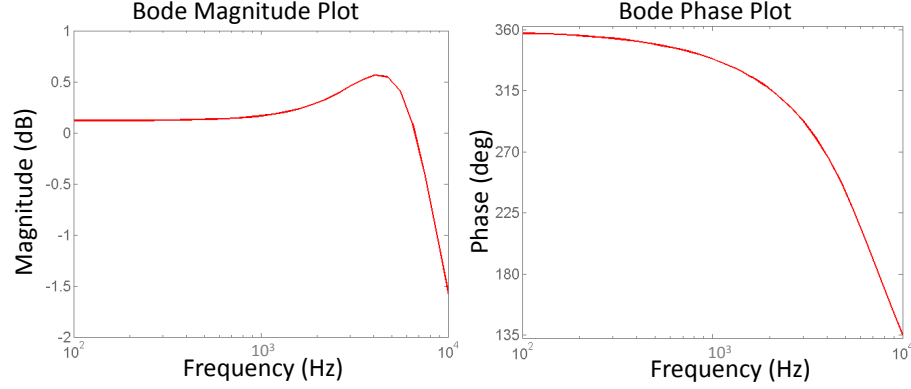


Figure 4.8: Magnitude and phase plots for identified transfer function of the actuator, $A(s)$. Note that at high frequencies, magnitude reduces and phase delay increases, affecting the actuator response for input signals at high frequencies.

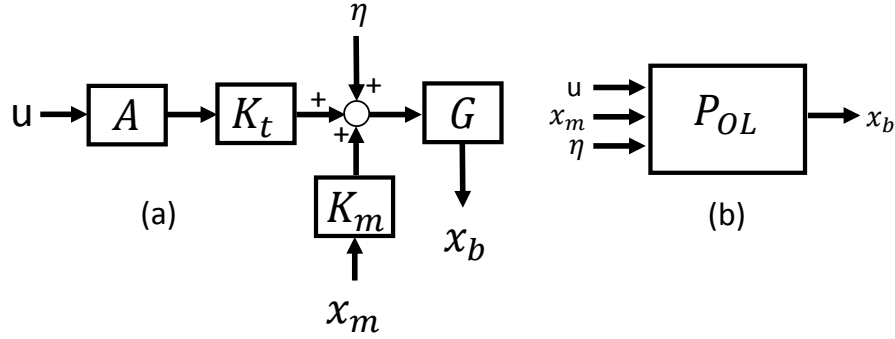


Figure 4.9: Block diagram of the open loop plant P_{OL} . The inputs to the plant are u , x_m and η which denote the trap moment command, motor motion and thermal noise respectively and are related by the (4.3). The output x_b denotes the bead position as measured by the photo diode. As mentioned earlier, $G(s) = \frac{1}{\beta s + k_t + k_m}$ and $A(s)$ denotes the transfer function from trap command signal $u(s)$ to the trap position $x_t(s)$, thereby capturing the dynamics of the actuator (AOD).

4.3 Controller Synthesis

The controller takes the desired force to be maintained, F_d , and the measured bead position, x_b , as its inputs and provides the command signal to the AOD u and the estimate $\hat{x}_m$ of the disturbance x_m as its outputs; thus, the controller $K(s)$ is a two-input two-output system as shown in Fig. 4.10. A block diagram describing the various components of the optical trapping system together with the feedback controller K is shown in Fig. 4.11 (a). The performance objectives on the closed loop system are as follows:

1. Good force regulation in the presence of set point changes and disturbances

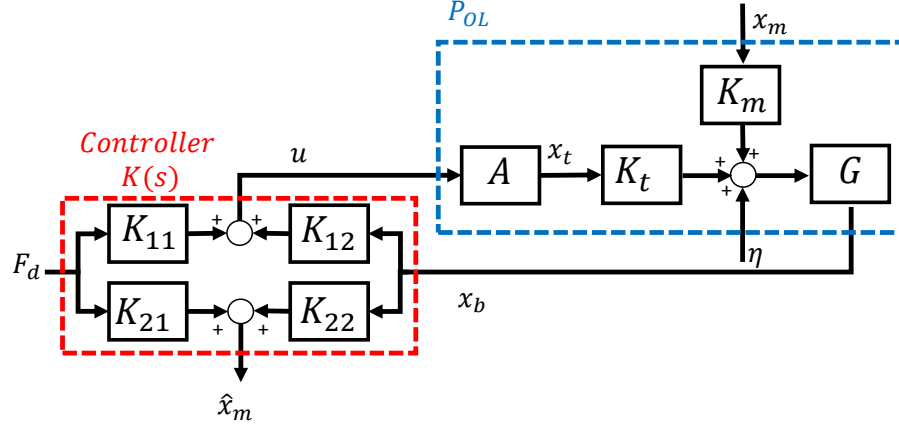


Figure 4.10: Block diagram describing the closed loop plant with open loop plant P_{OL} and controller $K(s)$.

2. Accurate real time estimation of motor motion

The feedback diagram in Fig. 4.11 contains additional signals e_f and $\tilde{x}_m$ corresponding to the regulation error and estimation error, respectively. Input-output transfer functions appearing in this loop are denoted via subscripts e.g. $T_{e_f F_d}(s)$ denotes the transfer function from input F_d to output e_f . The desired performance objectives can be expressed in terms of specific transfer functions. The first objective translates to a requirement of small magnitudes for the transfer function $T_{e_f F_d}(s)$ and $T_{e_f X_m}(s)$.

Furthermore, the gain of the transfer function $T_{e_f x_m}(s)$ is maintained to a small value, enabling the regulation of load force on the motor in the presence of motor motion. It also minimizes the effect of motor motion on extensions of the motor linkage, allowing for the the motor linkage to be approximated as a Hookean spring. The second objective translates to a small gain for the transfer function $T_{\tilde{x}_m x_m}(s)$. The presence of the effect of thermal noise in the plant output x_b necessitates filtering out of the effect of the white noise from the disturbance estimate $\hat{x}_m$. This translates to reducing the effect of η (white noise) on $\hat{x}_m$, that is, minimize the gain of $T_{\hat{x}_m \eta}(s)$ across all frequencies. Note that the disturbance x_m and the noise η enter at the same location as shown in Figure 4.11 (a), so minimizing the H_2 norm of $T_{\hat{x}_m \eta}(s)$ is the same as minimizing the H_2 norm of $T_{\hat{x}_m x_m}(s)$. Another point to be noted is that, in the optical trapping system, the trap position x_t cannot be directly estimated for fast stepping motors (since $\frac{x_t(s)}{u(s)} = A(s) \neq 1$ at high frequencies); hence, it is not possible to design an effective feedback strategy that uses regulation error

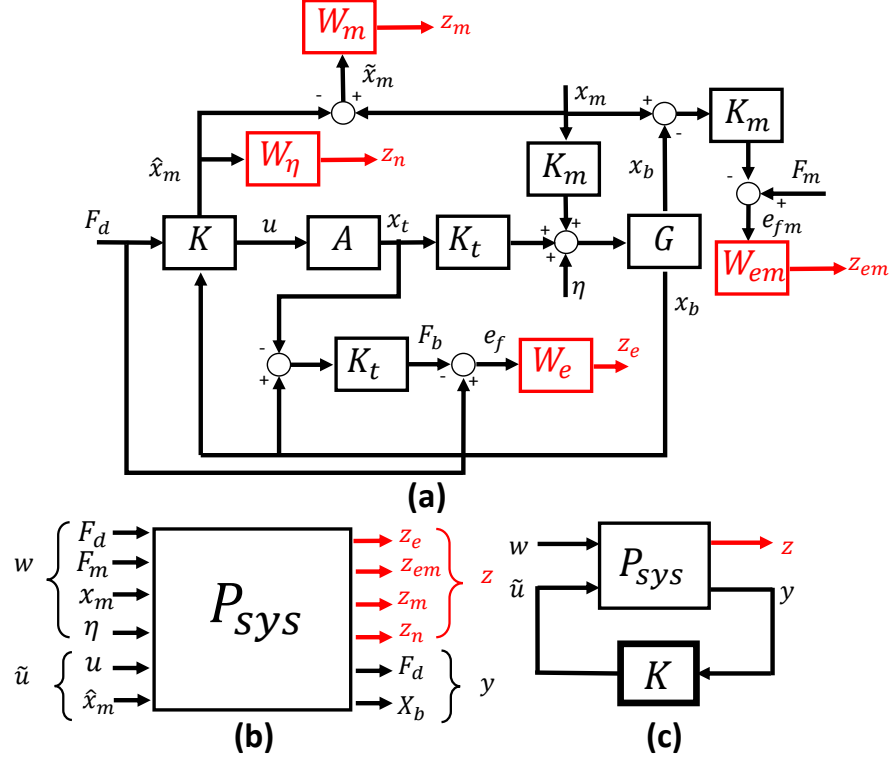


Figure 4.11: (a) Block diagram of the plant with frequency dependent weights W_e, W_{em}, W_m and W_η . (b) P_{sys} denotes the transfer function of the system with inputs $[w; \tilde{u}]$ and outputs $[z; y]$. (c) Closed loop representation of the system with controller K .

$e_f = F_d - k_t(x_b - x_t)$ as an input to the controller. The proposed framework enables desired objectives to be obtained without resorting to the regulation error e_f .

4.3.1 Design Objectives

The multi-objective output feedback control paradigm described out in [66] provides an effective controller synthesis approach to meet the desired objectives. The list below enumerates the desired performance requirements :

1. minimize $\| [T_{e_f F_d} \ T_{e_f x_m}] \|_{H_\infty}$
2. minimize $\| [T_{e_{fm} x_m}] \|_{H_\infty}$
3. minimize $\| T_{\hat{x}_m x_m} \|_{H_\infty}$
4. minimize $\| T_{\hat{x}_m x_m} \|_{H_2}$

We incorporate a constraint on the H_2 norm $\|T_{\hat{x}_m x_m}\|_{H_2}$ as $\|T_{\hat{x}_m \eta}\|_{H_2}^2 < \nu$ in the optimization problem, where ν is specified by the user. Frequency dependent weights $W_e(s)$, $W_{em}(s)$, $W_m(s)$ and $W_\eta(s)$ are introduced to penalize the signals e_f , e_{fm} , $\tilde{x}_m$ and $\hat{x}_m$ respectively as shown in Figure 4.11 (a) and ensure feasibility of the optimization problem. Note that, $W_m(s)$ and $W_\eta(s)$ should emphasize non-overlapping frequency regions since the signals x_m and η enter the plant at the same location. We define the vector $z := [z_e, z_{em}, z_m, z_\eta]^T$ as the generalized output vector, $w := [F_d, F_m, x_m, \eta]^T$ as the vector of generalized input, $y := [F_d, x_b]^T$ as the input to the controller, $\tilde{u} := [u, \hat{x}_m]^T$ as the output of the controller K . P_{sys} is the open loop plant including the weights, as shown in Figure 4.11 (b) and is given as, $P_{sys} =$

$$\begin{bmatrix} W_e & 0 & -W_e K_t K_m G & -W_e K_t G & W_e K_t A(1 - K_t G) & 0 \\ 0 & W_{em} & -W_{em} K_m(1 - K_m G) & W_{em} K_m G & W_{em} K_m G K_t A & 0 \\ 0 & 0 & W_m & 0 & 0 & -W_m \\ 0 & 0 & 0 & 0 & 0 & W_\eta \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & G K_m & G & G K_t A & 0 \end{bmatrix}.$$

The associated optimization problem to synthesize a controller $K(s)$ is given below :

$$\begin{aligned} & \min_{K(s), \gamma} \gamma \\ & \text{such that, } \|[T_{z_e f_d} \ T_{z_e x_m}]\|_{H_\infty} < \gamma \\ & \| [T_{z_{em} x_m}] \|_{H_\infty} < \gamma \\ & \| T_{z_m x_m} \|_{H_\infty} < \gamma \\ & \| T_{z_\eta \eta} \|_{H_2}^2 < \nu \end{aligned} \tag{4.6}$$

Here,

$$\begin{aligned}
T_{zefd} &= W_e(s)(1 - GK_t^2 K_{11}(s)A(s)H(s) \\
&\quad + A(s)K_t K_{11}(s)H(s)), \\
T_{zexm} &= W_e(s)K_t G(s)K_m((A(s)K_{12}(s) - 1)H(s)), \\
T_{zemxm} &= W_{em}(s)K_m G(s)K_t A(s)K_{12}(s)H(s), \\
T_{zmxm} &= W_m(s)(1 - G(s)K_m K_{22}(s))H(s), \text{ and,} \\
T_{z\eta\eta} &= W_\eta G(s)K_{22}(s)H(s).
\end{aligned}$$

where $H(s) = \frac{1}{1 - K_t G(s)A(s)K_{12}(s)}$.

The above optimization problem is a convex optimization problem as shown in [66], the optimal solution to which is obtained by solving the corresponding Linear Matrix Inequalities (or LMI's). The solution scheme in [66] utilizes a common Lyapunov function for all the objectives, thereby bringing some amount of conservatism in the design process. However, the obtained controller is guaranteed to be stabilizing and also is of the same order as the plant, making it suitable for real time implementation. The associated closed loop system is shown in Fig. 4.11 (c).

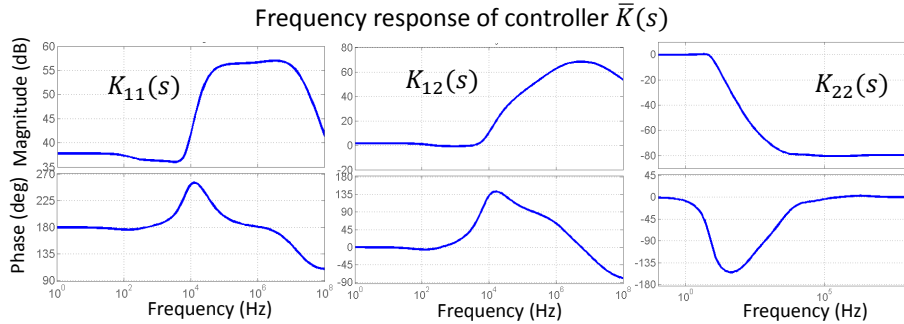


Figure 4.12: Frequency responses of the controller $\bar{K}(s)$

The weight $W_e(s)$ is chosen (Fig. 4.13(a)) to penalize the regulation error e_f , with gains below -16 dB for disturbances upto 700 Hz, corresponding to average motor velocity of motors of 5600 nm/s. It ensures small values of regulation errors at steady state as well as the frequencies of interest, corresponding to the observed native speeds of molecular motors [68]. $W_{em}(s)$ is chosen (Fig. 4.13(b)) to minimize the effect of motor disturbances on error e_{fm} , by maintaining the gain below -5 dB for disturbances upto 100 Hz corresponding to

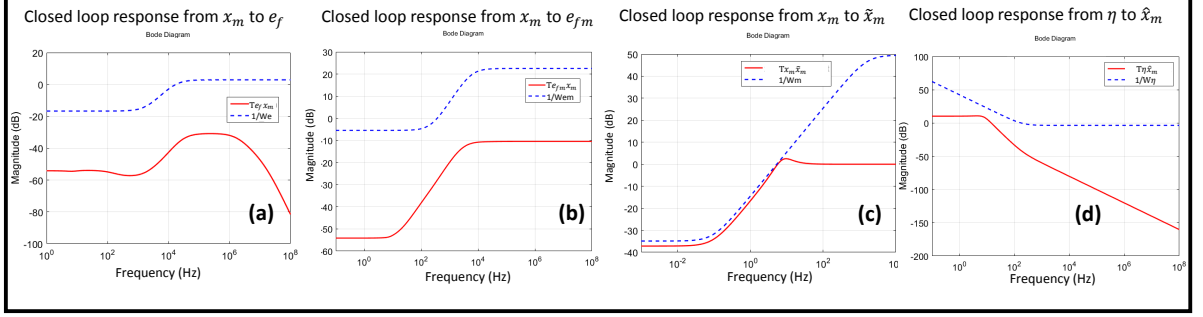


Figure 4.13: Frequency response of (a) $T_{e_f x_m}(s)$ and $W_e^{-1}(s)$, (b) $T_{e_{fm} x_m}(s)$ and $W_{em}^{-1}(s)$, (c) $T_{x_m x_m}(s)$ and $W_m^{-1}(s)$, (d) $T_{x_m \eta}(s)$ and $W_\eta^{-1}(s)$ using mixed objective H_2/H_∞ method. All three closed loop transfer functions meet the desired frequency dependent performance for the chosen weights $W_e = \frac{0.5s+6000}{s+60}$, $W_{em} = \frac{0.075s+2700}{s+1440}$, $W_m = \frac{0.01s+100}{3s+1.8}$ and $W_n = \frac{1.194 \times 10^{-3}s}{7.958 \times 10^{-4}s+1}$.

an average velocity of 800 nm/s, consistent with unloaded speeds of motor protein kinesin [17, 19]. $W_m(s)$ is chosen (Fig. 4.13(c)) to minimize the effect of disturbances on estimation of motor motion, for motor speeds upto 160 nm/s, consistent with observed *in-vitro* speeds using the motility protocol designed in [59] (further details are provided in next section). $W_\eta(s)$ is chosen (Fig. 4.13(d)) to penalize the higher frequencies (> 20 Hz) so that the estimate $\hat{x}_m$ is devoid of high frequency noise. $W_\eta(s)$ is also chosen such that it avoids an overlap of frequencies with the chosen weight $W_m(s)$, while also ensuring the feasibility of the optimization problem. The 2×2 controller $K(s) = \begin{bmatrix} K_{11}(s) & K_{12}(s) \\ K_{21}(s) & K_{22}(s) \end{bmatrix}$ (shown in the closed loop diagram in Fig. 4.10) is determined using the given frequency dependent weights and $\nu (= 2.23)$ by solving the corresponding LMI's shown in [66]. The CVX solver [69] is utilized to solve for the corresponding LMI's (see [66] for more details on solving for LMI's) and returns a stabilizing controller $K(s)$ for the chosen weights and value of ν . The resulting optimal value of γ is obtained to be 2.63.

Note that, $K_{21}(s)$ corresponds to the closed loop transfer function from F_d to $\hat{x}_m$. It is evident that the transfer functions $\| [T_{z_e f_d} \ T_{z_e x_m}] \|_{H_\infty}$, $\| T_{z_{em} x_m} \|_{H_\infty}$, $\| T_{z_m x_m} \|_{H_\infty}$ and $\| T_{z_\eta \eta} \|_{H_2}^2$ in (4.6) do not depend on the the controller transfer function $K_{21}(s)$, implying that the estimate $\hat{x}_m$ does not depend on the reference signal F_d . Thus, setting $K_{21}(s) = 0$ in the optimal controller obtained by solving the set of LMIs and utilizing controller $\bar{K}(s) =$

$\begin{bmatrix} K_{11}(s) & K_{12}(s) \\ 0 & K_{22}(s) \end{bmatrix}$ does not alter the closed loop performances of the desired channels, as defined in (4.6). This simplifies the motor motion estimate $\hat{x}_m(s)$ to be $K_{22}(s)X_b$. $\bar{K}(s)$ is a 6th order controller with frequency response shown in Fig. 4.12. The frequency response of the inverse of the weights $W_e(s), W_{em}(s), W_m(s), W_\eta(s)$ and the corresponding closed loop transfer functions with the optimal controller in loop are shown in Figure 4.13. It is clear from the figure that the closed loop transfer functions meet the desired frequency dependent performance specifications.

4.3.2 Simulation results

The performance comparison metric for regulation is the percentage regulation error, $r := \frac{\sigma(F_d - k_t(x_b - x_t))}{F_d} \times 100\%$ where $\sigma(\cdot)$ denotes standard deviation; and percentage root mean squared (rms) error, $r_{rms} := \frac{e_{rms}}{F_d} \times 100\%$ where e_{rms} is the rms value of the error $e = F_d - F_b$. The performance metric for estimation error, $e_{rms} := \sqrt{\langle \tilde{x}_m^2 \rangle}$, where $\langle \cdot \rangle$ denotes the expectation operator. Table 4.3.2 lists the regulation and estimation error performance of the synthesized controller for $F_d = 0.95$ pN and x_m being a simulated staircase signal of 8 nm steps with frequencies of 5, 10, 15, 20 steps/sec corresponding to average motor speeds of 40, 80, 120, 160 nm/s. It is seen that regulation error is less than 10% and the rms estimation error is less than 6 nm for *in-vitro* motor stepping frequencies upto 160 nm/s. Figure 4.14 (a) shows an example of the estimate $\hat{x}_m$ of x_m using the noisy bead position x_b when the frequency of the steps is 5 Hz and Figure 4.14 (b) shows the error in force regulation.

Thus, the synthesized controller achieves the desired objectives of force regulation and real-time motor motion estimation in simulations. In the next section we demonstrate the experimental performance of the controller using live samples of the motor protein kinesin-I.

Table 4.1: Regulation and Estimation Error for various Motor Stepping Frequencies

Step Rate (steps/s)	Motor Speed (nm/s)	$r(\%)$	$r_{rms}(\%)$	$e_{rms}(\text{nm})$
---------------------	--------------------	---------	---------------	----------------------

5	40	4.85	6.1	5.21
10	80	7.42	8.29	4.83
15	120	8.06	8.87	4.78
20	160	8.40	9.18	4.88

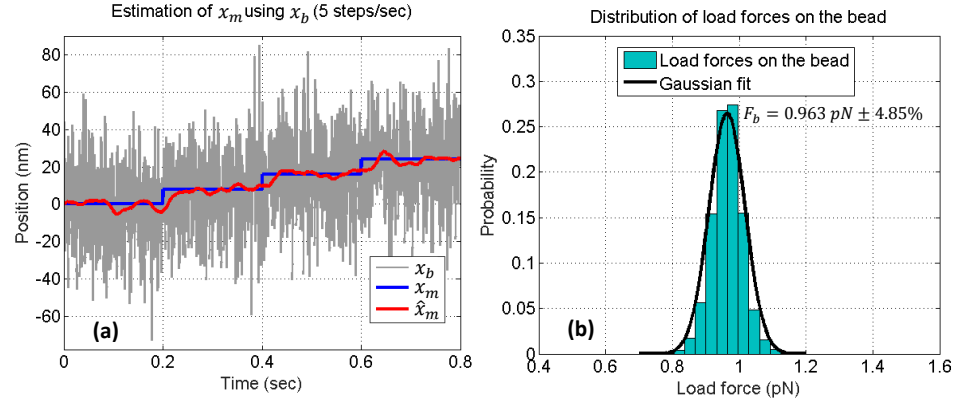


Figure 4.14: (a) A realization of $\hat{x}_m$ computed using x_b when simulated x_m is a staircase signal with stepping frequency 5 Hz and (b) the distribution of the associated regulation error. The force on the bead is $F_b = 0.963 \text{ pN} \pm 4.85\%$ (1 s.d) with 6.1% rms error.

4.4 Experiments with live motor proteins

In this section, we present an experimental implementation of the mixed objective $H_2 - H_\infty$ optical force clamp (designed in the previous section) on our optical tweezer setup. The controller is implemented using an National Instruments *PCI7833R* FPGA (brief description is provided in Appendix A). Furthermore, we develop and implement a bead motility assay carboxylated beads $1\mu\text{m}$ in diameter are attached to Kinesin-I motor protein using appropriate biochemistry. We then test the performance of the mixed objective clamp on the beads carried by live Kinesin-I motors and compare it with the performance of existing state-of-art force clamps.

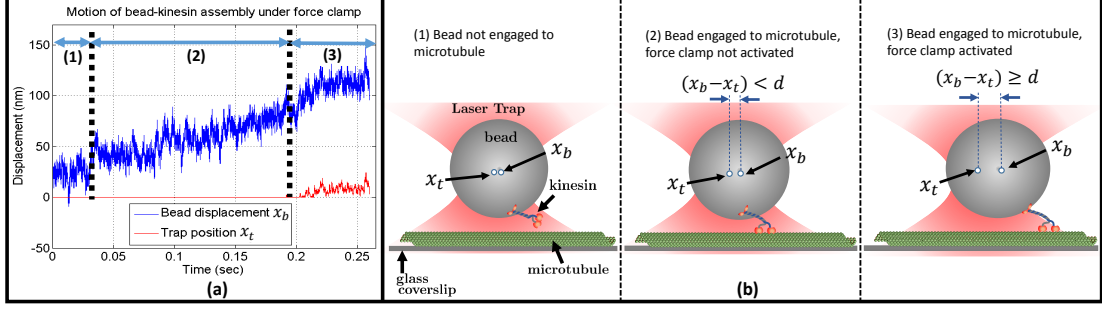


Figure 4.15: (a) Sample trace of bead (X_b) and trap position (X_t) showing optical force clamp in action, divided into three zones. (b) Diagrammatic representation of the behavior of bead-kinesin ensemble in each of the three zones. In zone (1), the bead attached to kinesin is brought close to the glass surface and awaits the attachment of the kinesin to the microtubule filaments coated onto it. No motion of the bead is recorded as the kinesin does not attach to the microtubule. Once the attachment occurs as shown in zone (2), the motor pulls the bead and traverses along the microtubule in a directed manner. However, since the displacement of the bead position from the trap position is less than $d = 100 \text{ nm}$, the force clamp is not triggered and the trap position X_t remains unchanged. Once $X_b - X_t > 100 \text{ nm}$ as seen in zone (3), the force clamp is triggered and the trap position follows the bead position while maintaining $X_b - X_t$ close to 100 nm .

4.4.1 Procedure and demonstration

To investigate motor protein motion using an optical force clamp, the optical tweezer is utilized to trap a polystyrene bead, with the bead being attached to Kinesin-I motors using the protocol described in Appendix A. For the experiment, the trapped bead is brought close to the base of the glass slide where microtubule filaments have been previously coated (See [59] for details) and held stationary at a distance of $\approx 100 \text{ nm}$ from it. As the kinesin protein gradually attaches to the microtubule and begins traversing, a unidirectional motion of the bead towards the positively charged end of the microtubule (similar to Fig. 4.4) is recorded using the photodiode sensor.

The optical force clamp is designed to maintain a constant force of F_d on the bead, which is equivalent to maintaining a constant distance of $d = \frac{F_d}{k_t} = x_b - x_t$ between the locations of the bead and the trap. The clamp is designed to trigger only after the bead has moved more than a distance of d away from the trap focus, that is, after $x_b - x_t \geq d$. When $x_b - x_t < d$, the force clamp is not triggered and the trap position does not change in response to changes in the bead position. An instance of the force clamp in action for $d = 100 \text{ nm}$ is demonstrated in Fig. 4.15 (a), where zones 1, 2 and 3 denote the different

stages of bead motion under the optical force clamp. A representation of the bead-motor assembly corresponding to each of the zones is shown in Fig. 4.15 (b). In zone (1), the bead is trapped and being brought close to the microtubule, awaiting attachment of the motor. No directed motion of the bead is recorded. In zone (2), the motor attaches to the microtubule and pulls the bead, yet the force clamp is not triggered since $x_b - x_t < dx$. In zone (3), $x_b - x_t \geq dx$ and the force clamp is triggered, which is seen in the corresponding section in the Fig. 4.15 (a), where the trap position (red trace) follows the bead position (blue trace) and maintains the distance $x_b - x_t$ close to $d = 100 \text{ nm}$. For the trap stiffness $k_t = 0.0095 \text{ pN/nm}$, $d = 100 \text{ nm}$ translates to a desired force of $F_d = 0.950 \text{ pN}$ on the bead.

4.4.2 Results

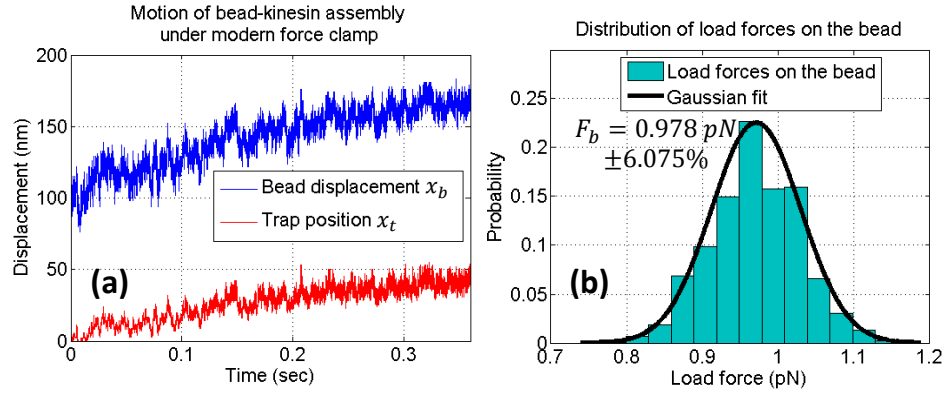


Figure 4.16: (a) Sample trace of bead and trap position showing the modern H_2/H_∞ force clamp in action (b) Distribution of the error in force regulation.

Table 4.2: $H_2 - H_\infty$ Force Clamp Regulation Performance

Motor Speed in (nm/s)	Error in force regulation (%)
59.17	4.67
68.11	5.069
93.2	7.07
107.53	6.075
152.01	5.073

193.3	7.97
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Table 4.3: Performance using traditional force clamp

Motor Speed in (nm/s)	Error in force regulation (%)
45.86	17.806
149.64	17.68

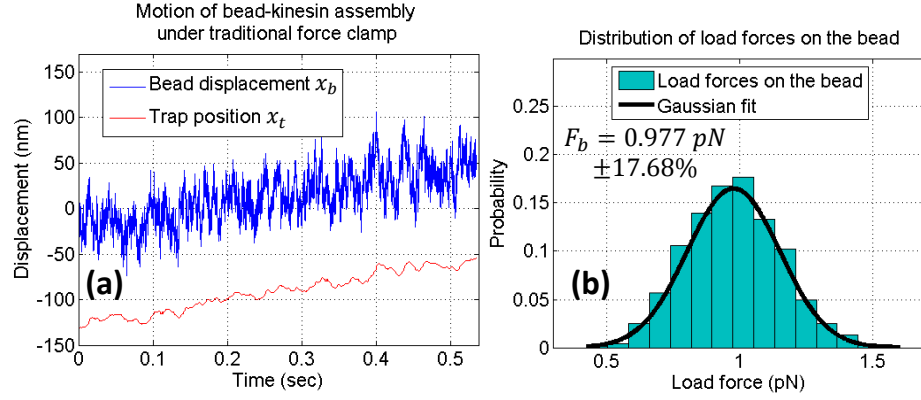


Figure 4.17: (a) Sample trace of bead and trap position showing the traditional force clamp [3] in action (b) Distribution of the error in force regulation.

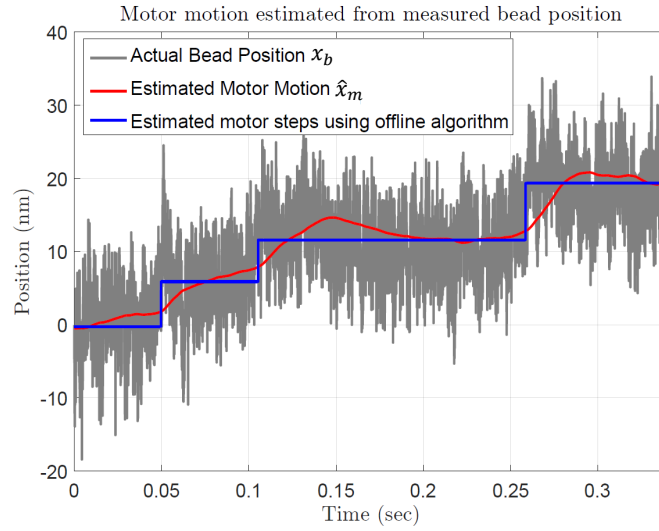


Figure 4.18: Real time estimation of stepping motion, with average bead velocity of 55 nm/s

The mixed objective $H_2 - H_\infty$ force clamp was tested on beads with average velocities ranging from about 59 nm/s to 193 nm/s (see Table 4.4.2 for details). The mixed objective force clamp demonstrated excellent regulation performance, with the error in force resolution ranging from 4.67% to 7.97%. An instance of the force clamp operating on a bead travelling at 107 nm/s is shown in Fig. 4.16 (a), where an error of in force regulation of 6.075% is obtained (as shown in Fig. 4.16 (b)). Note that, the data in table 4.4.2 is the first such demonstration where the force clamp has been able to obtain a force regulation as low as 4.67% while maintaining forces below 1 pN. We compared the performance of the existing state-of-art [3] under similar conditions of load force and velocities, where higher

errors in force regulation were seen (as shown in Table 4.4.2). An instance of the traditional force clamp operating on a bead travelling at 149 nm/s is shown in Fig. 4.17 (a), where an error of 17.68% is obtained (as shown in Fig. 4.17 (b)). It is evident that the mixed objective approach provides an advantage over the existing approaches and is capable of regulating sub pN forces with errors as low as 4.67%.

We further utilized the mixed objective force clamp to provide with a real time estimate $\hat{X}_m$ of the motor motion X_m . The estimate of the motor motion for an average velocity of 55 nm/s ($\approx$ stepping frequency of 7 steps/sec) is shown by red curve in Figure 4.18. Since the actual stepping signal is not known, an estimate obtained using state-of-art offline step estimation algorithm [65] (blue curve) is shown for comparison. It is seen that the high frequency noise is filtered out, providing an estimate $\hat{X}_m$ comparable with state-of-art offline step estimation techniques.

Our method is the first of its kind that demonstrates estimation of motor motion in real time. Moreover, the estimated signal $\hat{X}_m$ can provide starting points or edges that can be used by machine learning or dynamic programming based algorithms to generate statistics in real time or with an acceptable amount of delay. Thus, the force clamp presented in this article facilitates investigation and real time estimation of motor motion under sub pN load forces, which to the best of our knowledge has not been reported in the existing studies.

4.5 Conclusion

This article presents a detailed design for an optical force clamp using a mixed objective H_2/H_∞ framework, along with verifications using simulations as well as experiments using live motor proteins; with extensive applications to investigation of bio-molecules and processive motor proteins. The model-based approach using a systematic design methodology results in notable improvement in force regulation and bandwidth of operation over existing designs [63, 3]. A crucial enabler of the design is a precise understanding and modeling of hitherto un-modeled delays in the existing instruments using right half plane zeros; identified by a data-driven system identification approach. We verify the design through extensive numerical simulations, providing improved force regulation with guaranteed performances

based on user-defined weight functions. The improved design can enable bio-molecular studies with higher resolution through the ability to investigate systems at finer intervals of forces. It will allow researchers to discover events that were not possible due to limitations of current designs, leading to possible refinement of bio-molecular models. The design allows for examining motor proteins at higher velocities close to their native speeds [68] and proteins with larger step sizes [67] with satisfactory force regulation. The design using modern control framework adds to the robustness of the system to parametric uncertainties. We further implemented the design on an optical tweezer setup and tested its efficacy using live samples of the motor protein ‘kinesin’, demonstrating the ability to regulate sub-pico newton forces with errors below 10%; individual attempts are also shown to have errors below 5%.

The article also proposes a scheme for real time stepping estimation of molecular motors, which to the best of our knowledge is absent from current literature. By virtue of the mixed objective H_2/H_∞ design, we are able to reduce the effects of thermal noise on stepping estimate without compromising on the force regulation. We demonstrate the efficacy of the method using simulations as well as experiments involving live ‘kinesin’ samples. Unlike some methods that reduce the effects thermal noise on stepping signal through stiffening the system [70, 3], our method preserves the dynamics of the system and thus avoids impacting the system being examined. The real time step estimation methodology can prove to be of importance to experimental bio-physists, equipping them with the ability to detect and identify motor protein motility in real time. Note that the step estimation bandwidth for our method is limited to about 20 steps/sec (corresponding to low motor speeds of kinesin of about 160 nm/s), due to the trade-off in the H_2/H_∞ cost over the identical channel. It is useful for motor proteins operating in the high load force and low ATP regimes [3, 71]. Future work entails exploiting the knowledge of the noise properties and designing the corresponding weight function (W_η in this article) more efficiently. Nevertheless, our proposed scheme enables improved investigative abilities in molecular biology using system theory tools and provides improvement in force clamping abilities over current methods, with the added contribution of estimation of motor motion in real time.

Chapter 5

Tweezer-TIRF : A single platform for combined Optical Trapping and TIRF imaging study

Optical trapping and fluorescence imaging are two of the central techniques that have been successfully employed in order to perform single molecule research. While investigating molecular motor-based transport *in-vitro*, the motors are attached to fluorescent beads that can be manipulated and controlled using optical trapping while being imaged and visualised using fluorescence microscopy. Optical trapping combined with fluorescence has been crucial in several important discoveries on motor protein functionality.

In many instances, the fluorescence imaging is not limited to the beads functioning as the cargo but is also employed to study specific fluorescently labeled sites on the motors (for example, [72]). Advancements in single molecule fluorescence have enabled the probing of extremely minute and functionally specific sites on the molecules, leading to the unraveling of a host of molecular motor functionalities. A main enabler has been the development of fluorescent dyes of extremely small dimensions and robust stability, that specifically bind to certain locations of the molecule and thereby enable a finer probing. Dyes such as quantum dots (that are approximately tens to hundreds of nanometers in diameter) have revolutionized the field of single molecule fluorescence. However, the probing capabilities enabled

by quantum dots will not be appropriately utilized without commensurate advancements in sensing capabilities as well. These quantum dots are known to emit light signals with very low intensities, posing a significant challenge to their measurement in the midst of a highly noisy environment and the absence of detectors with high sensitivity. These two bottlenecks are resolved by the application of the principle of TIRFM - Total Internal Reflection Fluorescence Microscopy - and the recent advancements in CMOS cameras capable of capturing single photon intensities.

5.1 Total Internal Reflection Fluorescence Microscopy (TIRFM)

Fluorescence microscopy typically involves exciting the entire sample using a light of appropriate wavelength and power, which constitutes the excitation spectrum of the sample to be analyzed. The sample absorbs the light whose wavelength is in its absorption spectrum and emits light with wavelength in its emission spectrum. The emission spectrum has a higher wavelength and lower power than the emission waveform. While performing single molecule studies using fluorescence, one of the drawbacks of the method is a compromised signal-to-noise (SNR) ratio, owing to the fluorescent light illuminating the entire sample around the specimen to be imaged. This is particularly significant factor during single-molecule experiments involving molecular motors labeled with quantum dots, since the motors typically traverse on microtubule filaments (that are coated to the surface of the cover slip). The bulk of the action thus takes place close to the surface of the sample plane, with the excitation wave illuminating the sample plane above it and yielding low SNR and an inability to capture the low intensity signals emitted by the quantum dots.

Instead of illuminating the entire sample plane, TIRFM provides an elegant method to excite an *extremely small optical region* close to the sample surface. This enables a selective imaging of fluorescent molecules that are physically located close to a high refractive index sample surface, such as a coverglass. Consider an interface of two surfaces that have refractive indices (RI) n_1 and n_2 , with $n_1 > n_2$ as shown in Fig. 5.1. When the excitation light is incident on the interface of two surfaces through the medium with higher RI n_1 , the light typically gets refracted into the other medium. At a specific angle of incidence, called

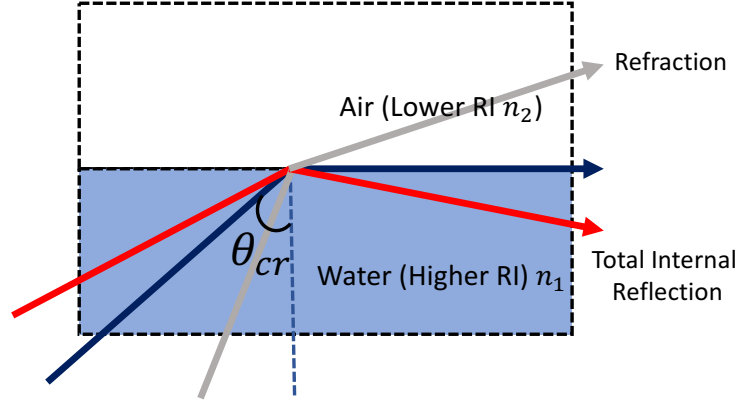


Figure 5.1: Total internal reflection (the ray in red color) when a ray of light travels from a high refractive index (RI) medium to a low RI medium and is incident at an angle greater than the critical angle θ_{cr}

critical angle θ_{cr} , the light does not get refracted but travels along the intersection of the two surfaces. The critical angle is given by,

$$\theta_{cr} = \sin^{-1} \frac{n_2}{n_1}$$

At angles of incidences above θ_{cr} , the light gets reflected back into the same medium, a phenomenon called total internal reflection. When the excitation light is totally internally reflected into the medium n_1 (like coverglass), an electromagnetic field called evanescent wave is formed at the interface; and it extends in the medium n_2 (like a liquid). This wave has the same frequency as the excitation light. The intensity I of the wave reduces exponentially with penetration into the liquid medium l , following the function [TIRF, Fish 2009]

$$I = I_0 e^{-\frac{l}{d}}$$

where d is the small amount of penetration of the evanescent wave and I_0 is the initial intensity at $l = 0$ as shown in Fig. 5.2. The depth d is given by [TIRF, Fish 2009]

$$d = \frac{\lambda}{4\pi(n_2^2 \sin^2 \theta - n_1^2)^{1/2}}$$

where λ is the wavelength of light and θ is the angle of incidence. Together with the exponentially decreasing intensity and limited penetration, this technique enables fluorescent

excitation of only the molecules located in the vicinity of the surface and not of those above. This facilitates high SNR with over 2000-fold improvement than using epi-fluorescencer [Funatsu et al., 1995]. Moreover, the samples are subject to a substantially small amount of light, preventing photo damage while capturing images that are rarely out-of-focus [TIRF, Fish 2009].

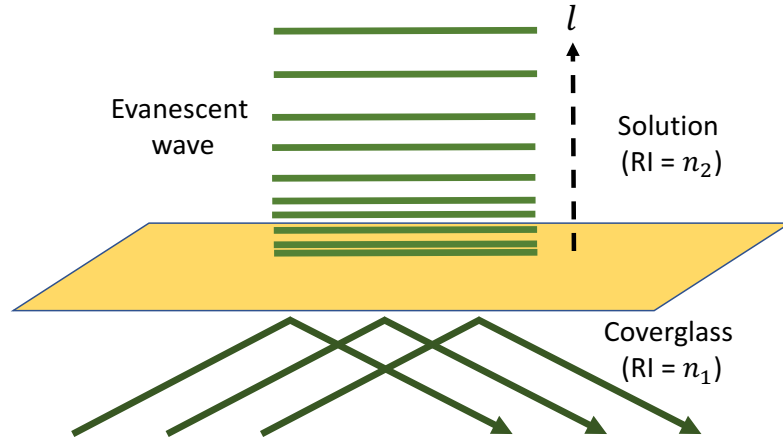


Figure 5.2: Total internal reflection at the boundary between coverglass ($n_1 = 1.52$) and the aqueous solution ($n_2 = 1.33$) results in the formation of evanescent wave, whose intensity decreases exponentially with the penetration l inside the sample

5.2 Motivation

In order to investigate the various modalities and mechanisms deployed by the multiple molecular motors in a team of motors carrynig a common cargo, it is essential to be able to viualize relative locations of the motor heads while traversing on the microtubule filament. For example, one of the main conclusions proposed in Chapter 3 is that under increasing load forces, the motors tend to adopt a mode of cooperation by clustering together and carrying the common cargo. At high load forces however, it is seen that the motors tend to spread apart in a specific manner and adopt a configuration that facilitates immediate cargo translocation when the load subsides. In order to be able to visulize the relative positions of the motors on the microtubule while the common cargo is being subjected to varying load forces, there has to be (1) appropriate biochemical protocols in place that can specifically attach quantum dots to the heads of the motor proteins while simultaneously being able

to attach to spherical beads to the tail domains and obtain robust *in-vitro* motility and (2) appropriate instrumentation (i.e. a combined TIRF-Tweezer system) that can exert controlled forces on the cargo while simultaneously being able to image the quantum dots placed on the heads of the motors carrying the common cargo.

With the knowledge and understanding of TIRFM, we designed the *TIRF-Tweezer* instrument that is capable of implementing TIRFM while simultaneously being able to perform Optical Trapping and thereby exerting controlled optical forces on the cargo. A brief description of the apparatus is given below.

5.3 TIRF-Tweezer Setup

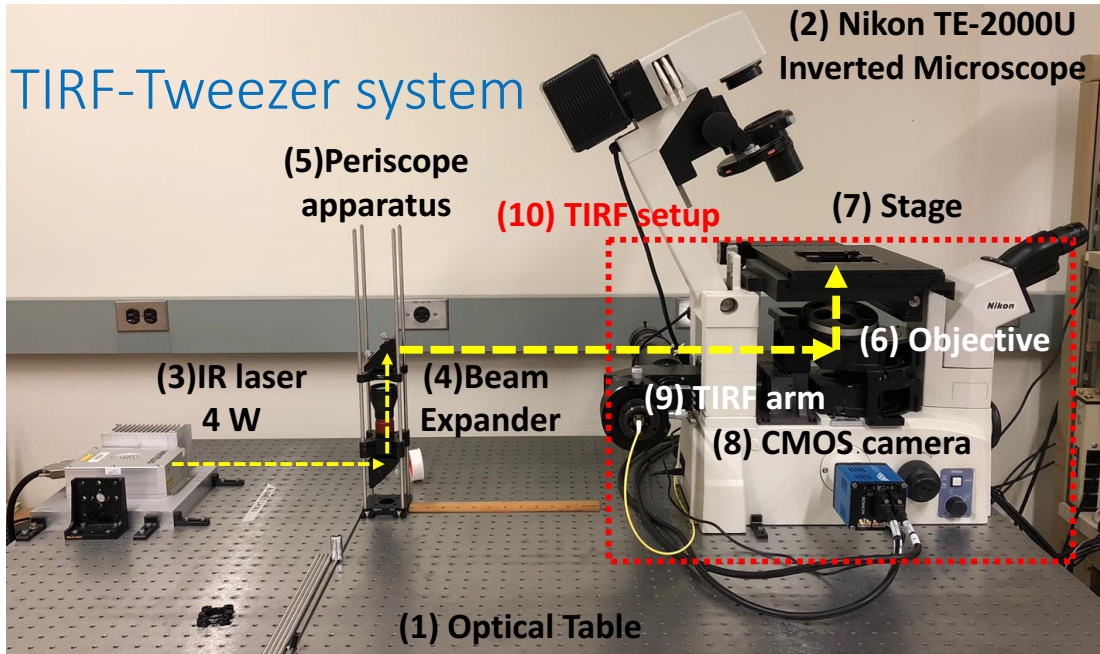


Figure 5.3: Picture of the TIRF-Tweezer setup. (1) Optical Table providing vibration isolation (2) Nikon TE2000U inverted microscope (3) Far infrared laser (LaserQuantum, $\lambda = 1094$ nm, 4 W) (4) Beam expander module (Thorlabs) (5) Periscope apparatus to elevate the laser beam while maintaining the beam expansion (6) Objective (Olympus TIRF 1.4 NA) (7) Nanopositioning stage (Prior Scientific) (8) Zyla 4.2 PLUS sCMOS (PCIe) camera (9) TIRF arm (Nikon) (10) TIRF setup with TIRF arm, TIRF objective and TIRF laser ($\lambda = 450$ nm, 50 mW)

The entire setup is housed on a vibration isolated optical table (Fig. 5.3 (1)), with a

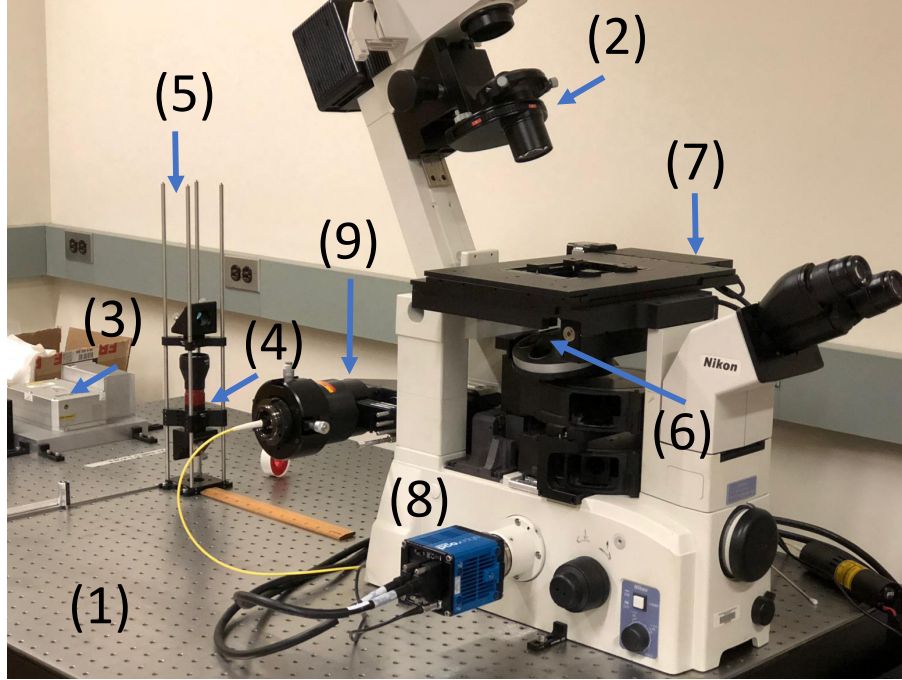


Figure 5.4: TIRF-Tweezer setup from another angle

Nikon TE-2000U inverted microscope (Fig. 5.3 (2)) functioning as the microscopy apparatus.

5.3.1 Optical Tweezer

For the optical trapping portion of the apparatus, a 1094 nm 4 W laser is used as a trapping laser (Fig. 5.3 (3)) and is launched in a beam expander (Fig. 5.3 (4)) which appropriately expands the width of the trapping beam to slightly overfill the back aperture of the objective. The expanded beam is raised at an appropriate height using a periscope apparatus (Fig. 5.3 (5)) and directed into the back focal plane of a high NA TIRF objective (Fig. 5.3 (6)). The sample is housed on a 3 axis nano-positioning stage (Fig. 5.3 (7)) with precise joystick control (not shown in the picture). At the right magnitude of beam expansion and correct incidence of the trapping beam into the objective, a stable optical trap is formed slightly downstream of the focus of the objective.

5.3.2 TIRF

The TIRFM section of the apparatus (Fig. 5.3 (10)) consists of a 488 nm 50 mW coherent laser (not shown in the picture), which is launched into a Nikon TIRF arm (Fig. 5.3 (9)) using a fibre optic cable. Precise vernier caliber controllers present on the TIRF arm enables the positioning of the beam and thereby control the angle of incidence onto the sample plane. A high sensitivity PCO Edge 2 CMOS camera (Fig. 5.3 (8)) is used to collect the single molecule images as well as the brightfield images for optical trapping.

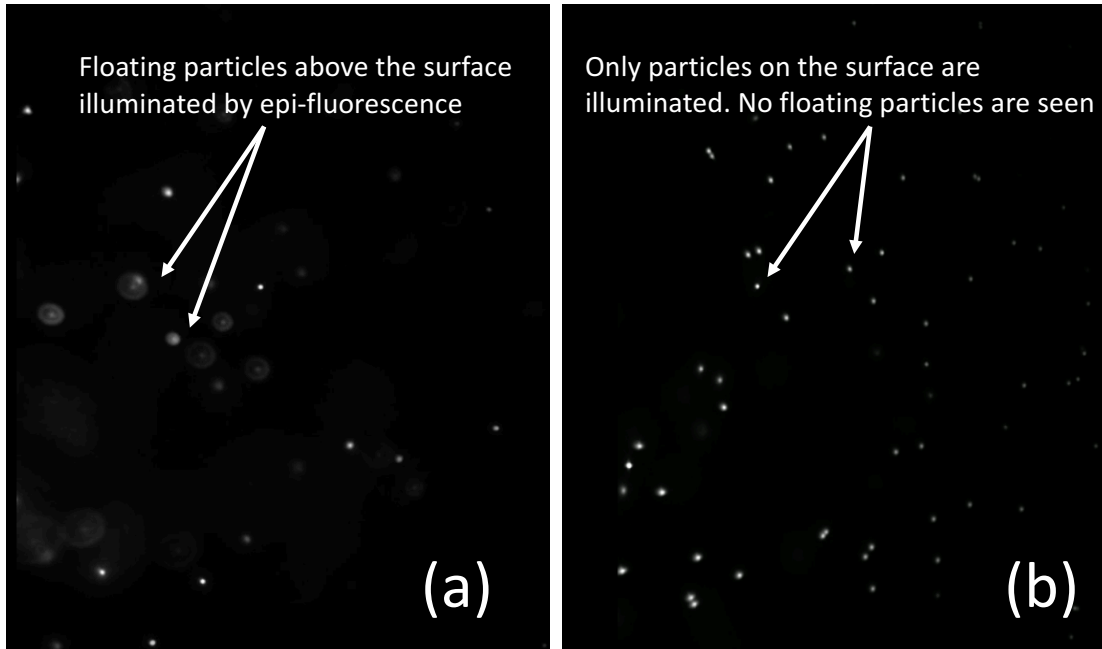


Figure 5.5: 100nm diameter beads imaged using the TIRF-Tweezer setup. (a) Epi-fluorescence mode and (b) TIRF mode

Using the appropriate values for n_1, n_2 and θ_{cr} for the 488 nm laser, we were able to obtain total internal reflection and image fluorescently labeled beads (100 nm diameter, YG, spherotech). As is seen in Fig. 5.5(a), under epi-fluorescence the stationary as well as floating beads are illuminated, with the fluorescence from the floating beads clearly visible as the beads come in and out of the visual plane. However, under total internal reflection (Fig. 5.5(b)), beads stuck to the surface are illuminated while no floating beads are illuminated; meaning a tiny area close to the surface is exposed to the evanescent waves. The increase in signal-to-noise (SNR) is evident in Fig. 5.5(b) in the absence of illumination of the beads

floating in the background.

Chapter 6

Conclusions and Discussions

The main contributions of my research have been two-fold : the development of novel analytical and experimental methods to investigate the transport properties of teams of motor proteins carrying a common cargo and how these properties are impacted by the presence of disease causing mutations that affect these motor motors.

The analytical study predicts that motors affected by mutations (such as those implicated in Huntington's disease that affect the single motor stall force), even when in the minority, can determine emergent transport properties of average velocity and run-length. Under appropriate external loads, the analysis predicts that motor ensembles containing even a minority of mutant motors travel longer distances, potentially contributing to the dysregulation of coordinated cargo transport, impairment of neuronal function and the onset of neurodegeneration. These results are enabled by development of a novel semi-analytic methodology to study cargo transport by multiple motors with distinct transport properties. This method is computationally less extensive than existing Monte-Carlo based approaches, easy to implement, and holds potential for understanding how individual motor proteins and their properties contribute to the coordination of transport by multiple motor ensembles.

The underlying modeling principles are used to perform a further analysis where the system of cargo transport by multiple motors is analyzed using principles of Markov theory. I provide mathematical proof for the existence of transient states in the state space of the Markov chain and a *unique steady state* probability distribution of various configurations

of motors sharing a common cargo. This implies that the system of multiple molecular motors carrying a cargo, a common feature of *in-vivo* intracellular transport, is a robust system that eventually behaves according to a fixed probability distribution irrespective of the initial distribution of the motor configurations. An investigation of the nature of the distribution and its variation with external factors such as ATP concentration and load force reveals that multiple motors possibly coordinate among themselves to function in the turbulent environment inside the cell.

The major experimental contribution of my research has been the development, deployment and demonstration of an indirect imaging scheme that facilitates estimation of disturbances with quantifiable guarantees on regulation of external parameters. The paradigm is applicable to a wide variety of systems where it is desired to regulate certain system variables in the presence of external disturbances, while at the same time providing an estimate of the disturbance in real-time. The framework is instantiated for an 'Optical Tweezer' setup, where an 'Optical Force Clamp' is developed to enable improved tracking of cargoes carried by motors under precisely controlled forces; thus aiding the experimental verification of the numerous conclusions borne out of the analytical studies. At the scale where these experiments are conducted, the disturbance (motor motion) is riddled with thermal noise and the proposed methodology provides a reasonable estimate of the disturbance while simultaneously mitigating the effect of noise and maintaining a constant hindering force. I have utilized a mixed objective H_2/H_∞ framework for this design and verified it, using extensive numerical simulations as well as experiments with live samples of the motor protein 'Kinesin-I', using highly robust protocols for bead motility assays developed and perfected in-house. The application of the scheme is not just limited to single molecule studies and can also be extended to protein and DNA pulling studies with atomic force microscopes, microfluidic flow measurements, high density data storage systems and topography imaging using scanning probe microscopy.

Previous research related to optical force clamps was constrained by lack of utilization of advances in precise modeling of the system as well as a modern control paradigm, with strategies that were unsuitable for studies involving multiple objectives. The approaches

also did not have a handle to regulate the load force directly experienced by the *motor-protein* and were compelled to regulate the force on the cargo itself. Moreover, the resulting controllers did not have any quantifiable guarantees while the outputs had to be processed offline in order to achieve good disturbance estimates. Through this approach, it is possible to regulate both the forces (on the motor as well as the cargo) with the performances guaranteed by user defined weights, while also providing real-time disturbance estimates. The enhanced ability for force regulation allows for investigation of systems at finer force intervals, possibly allowing for biophysists to investigate events in force ranges that was not possible earlier due to limitations of existing designs. Such applications can lead to possible refinement of existing bio-molecular models as well as the ability to investigate faster motors in their native environments. The novel benefit of simultaneous estimation of disturbance estimation in real time, demonstrated by applying the scheme to 'Kinesin-I', can prove impactful when a system is to be analyzed by preserving its dynamics and avoiding forced constraints that can directly impact the system.

The other experimental contribution involves the combining the capabilities of optical trapping with total internal reflection fluorescence (TIRF) microscopy, through the development of a state-of-the-art TIRF-Tweezer instrument. The instrument, developed by using a commercially available inverted microscope (Nikon TE2000-U) and simple optical equipments, is capable of not only trapping spherical cargoes but can also image the heads of the motor-proteins carrying the cargo (which are under 10 nm in size). These intricate experiments are aided by the development of complex biological protocols that involve *in-vitro* bead motility experiments but where the heads of the motor proteins are labeled by quantum dots through appropriate biochemistry. The quantum dots (fluorescently labeled dyes with dimensions in 10's of nanometers) provide single molecule fluorescence that can only be picked up through TIRF microscopy. The combined *TIRF-Tweezer* instrument enables detection of single molecule fluorescence and is thus able to image motor heads with dimensions in 10's of nanometers, while simultaneously performing optical trapping and exerting controlled optical forces on the cargoes. This platform can be extremely useful in validating numerous existing theoretical conclusions, including but not limited to the earlier analytical conclusions on motor behavior under varying load forces, such as the force assisted raveling

and unraveling of proteins such as Utrophin and Dystrophin.

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Appendix A

Protocol : Bead Motility Assay

In the appendix I have detailed the bead motility protocol used to perform the kinesin bead motility experiments mentioned in Chapter 3.

A.1 Coverslip preparation

1. Wash coverslips thoroughly with detergent, followed by ultrapure water
2. Plasma clean the coverslips for 30 sec
3. Air dry, soak in 35% 3-aminopropyl-triethoxysilane in acetone (30 minutes)
4. Briefly rinse with acetone, water
5. Soak in water (15 min), 3 x and air dry

A.2 Reagent Preparation

A.2.1 MTB Buffer

1. Add 984 μl , 1xBRB80
2. Add 5 μl , 0.2 M DTT
3. Add 1 μl , 10 mM Taxol
4. Add 10 μl , 0.1 M GTP

A.2.2 Gloxy

1. Add 36 μl of 1xBRB80
2. Add 6 μl glucose oxidase
3. Add 6 μl catalase
4. Add 6 μl of 50 % 2-mercaptoethanol

A.2.3 Blocking buffer

1. Add 230 μl MTB buffer
2. Add 20 μl 100 mg/ml bovine serum assay (BSA)

A.2.4 BRBCA

1. Add 179 μl 1 x BRB80
2. Add 1 μl 1 mM ATP
3. Add 29 μl , 10 mg/ml casein

A.2.5 Bead motility buffer

1. Add 92.25 μl 1 x BRB80
2. Add 5 μl , 10 mg/ml casein
3. Add 2.5 μl 0.1 M ATP
4. Add 0.25 μl 10 mM Taxol

A.2.6 Dilute bead stock

1. Add avidin coated beads (with 2 % HIS tag), 1 μl
2. Add 14 μl , 1x BRB80

A.2.7 Bead motor stock

1. Add dilute bead stock, 5 μl
2. Add 2 μl K560 kinesin motor protein, incubate over ice for 40 min-1 hr

A.3 Sample Preparation

1. Prepare two rectangular μl chambers on 3 saline coated slides
2. Dilute microtubule (2 μl in 18 μl MTB)
3. FLush 10 μl into the two chambers, incubae 10-15 min
4. Prepare oxidising solution (OS) : Add 2 μl glucose to 18 μl gloxy
5. Prepare blocking buffer : Add 10 μl OS to 50 μl blocking buffer without OS solution
6. Flush 15 μl blocking bufer in both slides, 2x, incubate 5 minutes each

A.3.1 Final stock

1. Add 10 μl bead-motor stock
2. Add 10 μl OS solution
3. Add 5 μl bead motility buffer
4. Flush 12.5 μl solution in each slide and observe under microscope

Appendix B

Miscellaneous Collaborations

Apart from my main research involving molecular motors, I have contributed to two other research projects. The first project involves the study of the limits on energy expenditures while erasing a single bit of memory using a model system. The system was designed using optical tweezers and spherical beads ($1\ \mu m$ in diameter), through the creation of 'double-well' parabolic potentials. The study was performed in close collaboration with my colleagues Saurav Talukdar and Dr. James Melborne and has spanned several articles. Further details can be found in [1, 73, 74, 75, 76].

The second project involves studying the impact of endocytic adaptor protein GIPC on the motor protein 'Myosin VI'. The endocytic adaptor protein GIPC recruits Myosin VI motor to endosomes. Binding of GIPC to Myosin VI leads to faster movement of Myosin VI. One of the mechanisms to increase speed of motor would be through changes in its step size. To probe this, step size of Myosin VI was measured using an optical trap in the presence and absence of the adaptor protein GIPC and the measurement is performed using optical tweezers. Step size of Myosin VI motor was found to be significantly enhanced on binding the adaptor protein GIPC thus confirming the above hypothesis. This study has been performed in collaboration with Dr. Aahim Rai, Professor Shiv Shivramakrishnan and my colleague Rachit Srivastav. The manuscript for this study is currently under preparation.