Theoretical Investigation of Intracellular Transport by Molecular Motors

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Abstract

Molecular motors are protein nanomachines that organize the internal order of all eukaryotic cells by shuttling intracellular cargos. Kinesins, dyneins, and myosins are three identified superfamilies of molecular motors which often function together within the cells. All of these motors power cellular motility using energy derived from adenosine triphosphate (ATP) hydrolysis. Molecular biology has revealed that the functional impairments of molecular motors would contribute to various human diseases, such as Alzheimer and cancer. Engineering developments have also emerged regarding the utilization of molecular motors in nanorobotics with a variety of missions, such as molecular communications. Despite this progress, the properties of intracellular cargo transport are not well understood. Motivated by the recent experimental findings, this thesis proposes computational and mathematical frameworks to investigate two different modes of intracellular cargo transport driven by (i) a single motor and by (ii) an assembly of two coupled identical motors. We focus on the cargo transport by kinesins because kinesin stepping kinetic scheme has been developed previously, and recent experiments have further measured input parameters for our theory. Nevertheless, our models are rather general and can be applied to other types of cytoskeletal molecular motors.

To study the first mode of transport, we develop two types of models: a mathematical model for analysing the dynamics of kinesin stepping, and computational models for simulating the processivity and information processing unit of kinesin. The mathematical model employs a kinetic scheme underlying kinesin stepping and determines the probabilities of forward and backward steppings of the motor and a stall force of ~ 7.3 pN independent of ATP concentration ([ATP]). The backward stepping of kinesin is shown to be powered by ATP hydrolysis, rather than ATP synthesis, while
backward stepping is related to the both ATP hydrolysis and synthesis pathways. We then develop a stochastic algorithm, based on a probabilistic timed automaton, to simulate the processivity of kinesin. The algorithm estimates the mean run length of kinesin, which is in agreement with experimental data, and suggests that the mean run length of kinesin can be determined by the number of ATP hydrolysis and ATP synthesis cycles taken by the motor before becoming inactive. The kinetics of kinesin stepping is further modelled as a digital circuit to demonstrate, by using logic representation, how the cellular inputs sensed by kinesin are transformed into mechanical steps. It is shown that external load directs the cargo transport, whereas [ATP] affects the duration and velocity of the transport.

To investigate the second mode of transport, we develop a general mathematical framework using master equation approach based on the single kinesin properties. It is shown that weakly coupled kinesins produce larger mean run length compared to that of single kinesin, whereas strongly coupled motors generate a mean run length almost equal to that of the single motor. The mean run length of the assembly under assisting loads above ~ 2 pN is found to be less dependent on the coupling strength between the motors, compared to that under resisting loads. Weak coupling between the motors enhances the cargo velocity under resisting forces relative to the velocity of single kinesin and strongly coupled motors.
Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

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List of Publications

This thesis contributed to the following publications:


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Chapter 1

Introduction

This chapter provides a brief introduction to the superfamilies of molecular motors and also highlights the scope of this thesis. Motivation and objectives of this research are presented in Section 1.2, along with the significance of studying intracellular cargo transport by molecular motors. Section 1.3 outlines the contributions of this thesis. This chapter concludes with the organization of the thesis in Section 1.4.

1.1 Molecular Motors

Organisms, from human beings to bacteria, move to adapt to changes in their environments. Cells, themselves, are dynamic sites of bustling mechanical functions, such as transporting materials from one site to another, assembling and disassembling of structures, communicating, and, in many cases, the entire cell moves itself. These types of activities are based on dynamic changes within cells mainly initiated by molecular motors which move along the cytoskeleton of the cell (Karp 2010). Eukaryotic cells possess a skeletal system, a cytoskeleton, which is a dynamic protein scaffold with diverse functions, such as providing structural support for the cell, organizing the cytoplasm, responding to external stimuli, and generating motility. The cytoskeleton of a cell is composed of a network of microtubules (MTs), actin filaments, and intermediate filaments associated with hundreds of different molecular motors specialized for distinct activities. MTs and actin filaments are polar structures distributed in all eukaryotic cells, while intermediate filaments are non-polar and have only been identified in animal cells (Karp 2010). Molecular motors are enzymatic nanomachines that utilize the energy derived from the hydrolysis of adenosine
triphosphate (ATP), into adenosine diphosphate (ADP) and inorganic phosphate (P_i), to generate force or to move cellular cargos within the cytoplasm of the cells (Keller et al. 2013). Collectively, cytoskeletal molecular motors can be grouped into three major superfamilies: kinesins, dyneins and myosins (Khataee and Khataee 2010a; Mallik and Gross 2004). These motor proteins fall into two categories based on the cytoskeletal tracks utilized (Khataee and Khataee 2010a; Mallik and Gross 2004):

I. MT-based kinesin and dynesin motors which generate motion along MTs.

II. Actin-based myosin motors that move along actin filaments.

Most kinesins, like conventional kinesins, ferry cargos from the cell centre to the cell periphery by stepping along MTs. Dynein motors are divided into two groups (Mallik and Gross 2004):

I. Cytoplasmic dyneins which transport various cargos from the periphery of the cell toward the cell centre by walking along MTs.

II. Axonemal dyneins which power the motion of cilia and flagella.

Myosins are also generally divided into two broad groups (Mallik and Gross 2004; Khataee and Khataee 2009):

I. Conventional myosins, first identified in muscle tissue, drive muscle contractions,

II. Unconventional myosins, e.g. myosin-V, involved in organelle transport along actin filaments very similar to the mechanism of kinesin motors.

Collectively, conventional kinesin, cytoplasmic dynein, and unconventional myosin molecular motors are self-guiding systems that traverse unidirectionally on their cytoskeletal filaments and shuttle cellular cargos (Chowdhury 2013; Karp 2010; Mallik
In vivo, these molecular motors often function together in the cellular cargo transportation (Vershinin et al. 2007). This means that cargoes may move bidirectionally along MTs if transported by both kinesin and dynein motors and also switch from MT-based transport to actin-based transport if myosin motors are attached to cargo too (Rodionov et al. 2003) (see Figure 1.1).

**Figure 1.1.** Examples of cargo transport by multiple molecular motors. (A) Electron microscopy image of four molecular motors transporting a mitochondrion. Motors are indicated by arrows (Ashkin et al. 1990). (B) Electron micrograph showing a vesicle (yellow) is bound to a MT (green) in the vicinity of an actin filament (red). Schematics of kinesin (K), dynein (D), and myosin-V (M) motors are drawn approximately to scale (Holzbaur and Goldman 2010).

The types of cellular cargo transported by these motors include vesicles (e.g. Golgi-derived and ER-derived vesicles), organelles (e.g. mitochondria, lysosomes and endosomes), and cytosolic components (e.g. proteins, ribonucleoprotein (RNP) particles, RNAs, mRNAs, cytoskeletal tracks, and viruses) (Karp 2010; Mandelkow and Mandelkow 2002). The passive transport of these cellular cargoes driven by diffusion has been shown to be insufficient for the cell, as in the crowded cytoplasm-like environment diffusion is too slow and its direction is difficult to be regulated (Berger 2012; Driver 2011; Howard 2001). Thus, an active cargo transport system is required to create the internal order of eukaryotic cells, and this transport system is actuated by conventional kinesin, cytoplasmic dynein, and unconventional myosin molecular motors (Vogel 2005).
1.2 Motivation

The significance of studying the mechanism underlying dynamics of molecular motors becomes apparent when considering the important roles of molecular motors ranging from human diseases to synthetic nanotechnological applications. Molecular biology has revealed critical roles for motor proteins in brain wiring and formation, neuronal survival, higher brain function, and control of central nervous system (Hirokawa et al. 2010). Therefore, the impairment of these functions of motor proteins has been found as a contributing factor to neuronal disease pathogenesis, such as hereditary spastic paraplegia (Ebbing et al. 2008) and Alzheimer (Stokin et al. 2005), and other diseases, such as cancer (Yu and Feng 2010). Engineering developments have also emerged with the applications of molecular motors in nanorobotics systems, as these proteins can exert localized forces on nanostructures (Hess 2006), as well as in a controlled propagation architecture of nanonetworks in which information molecules are carried by these motors between transmitter and receiver nanomachines (Nakano et al. 2012; Pierobon and Akyildiz 2010). Molecular motors are shown to be able to communicate with each other by performing computational tasks using sophisticated biochemical circuits which have evolved as their processing units to transfer input signals perceived from the environment into output signals (Bray 1995; Seelig et al. 2006). The ability of biological nanomachines to communicate with each other is anticipated to contribute in medical domains such as monitoring the condition of a human body, regenerating biological tissues and interfacing artificial devices with neural systems (Nakano et al. 2012). Inspired by the function of these molecular machines, engineers have begun to mimic the functions of proteins capable of processing information to invent synthetic nanodevices (De Silva and Uchiyama 2007) with potential applications in medical diagnostics and engineering (Fischer et al. 2009; Goodman et al. 2012; Hess 2011; Wang 2009; Wang and Manesh 2010), such as drug delivery systems (Cohen et al.
molecular shuttles (Schmidt and Vogel 2010), medical diagnostic devices (Kumar et al. 2013), lab-on-chip devices (Hess 2006; Kumar et al. 2013), and molecular communication systems (Nakano et al. 2012; Pierobon and Akyildiz 2010). Molecular biology further aims to manipulate the formation elements of the molecular motors by changing their amino acid sequence to construct novel motors (Karp 2010). To achieve these promising applications, the long-term goal of nanorobotics (Dubey et al. 2004; Karp 2010), a precise temporal and spatial control of synthetic nanodevices is required to direct the transportation along predefined paths and the load/unload of specific cargoes. Meanwhile, precise temporal and spatial control of synthetic nanodevices is a challenging problem because of the stochastic motion, high complexity and small size of molecular motors (Khataee and Ibrahim 2012; Khataee et al. 2013; Wang and Manesh 2010). Thus, to investigate the roles of molecular motors in human diseases and also achieve a precise motion control of synthetic nanodevices, one needs to understand the biophysical fundamentals of intracellular mechanisms of cargo transport by molecular motors.

Following quantitative data resulting from single-molecule experiments (Andreasson et al. 2015; Milic et al. 2014; Carter and Cross 2005; Clancy et al. 2011; Hackney 2002; Nishiyama et al. 2002; Schnitzer et al. 2000; Seitz and Surrey 2006; Taniguchi et al. 2005; Visscher et al. 1999; Yajima et al. 2002), the recent experiments have addressed the collective mechanism of molecular motors via studying the motility of an assembly of two coupled conventional kinesin motors carrying a common cargo (Jamison et al. 2012; Jamison et al. 2010; Rogers et al. 2009). In parallel to experimental studies, theoretical investigations have also addressed various aspects of molecular motors (Berger et al. 2015; Berger et al. 2012; Driver et al. 2010; Klumpp and Lipowsky 2005; Kunwar and Mogilner 2010; Liepelt and Lipowsky 2007; Nam and Epureanu 2015;
Conventional kinesin, which will be referred to as kinesin hereafter, is the best-studied member of kinesins (Kawaguchi 2013; Verhey et al. 2011) and has a simpler structure compared to cytoplasmic dynein and unconventional myosin (Burkhardt 1998; Mallik and Gross 2004). Theoretical frameworks proposed in this thesis are developed based on the single motor dynamics and thus, can be easily adjusted to study the motility of other molecular motors acting as a single motor or a team of motors. Such theoretical approaches not only provide new mechanistic insights and experimentally testable predictions, but can also make discoveries which cannot be achieved by using the current experimental technologies. Theoretical findings would then help us to describe the roles of molecular motors in human diseases and engineering applications.

1.3 Research Contribution

The contribution of this thesis is the development of novel mathematical and computational frameworks to investigate the intracellular cargo transport driven by single kinesin and by a team of two coupled kinesins over various loads and ATP concentrations ([ATP]). This contribution is addressed in two phases. First, the stepping dynamics of single kinesin is mathematically modelled. The main contributions of this
mathematical model, obtained by utilizing the kinetic scheme of single kinesin stepping, are as follows:

- The calculation of the stall force of kinesin.
- The calculation of the probabilities of forward and backward steppings.
- The description of the dependence of backward stepping to both ATP hydrolysis and ATP synthesis kinetic pathways.

These fundamentals of kinesin stepping are then modelled as a digital circuit to simulate processing unit actuating kinesin steps. Using digital circuits to model the behaviour of kinesin motor would provide new insights for the potential applications of kinesin stepping in the molecular communication and computing (Nakano et al. 2012; Pierobon and Akyildiz 2010). In this area, the issue of connectivity of molecules is frequently raised when the output from one molecule is used to control another (De Silva and Uchiyama 2007). Digital circuit modelling allows us to employ logic representation to demonstrate how the cellular inputs sensed by kinesin are transformed into mechanical steps. This is particularly important for representing the information handling in an assembly of molecular motors or other molecules, each with its own function, where the output from one molecule is used to control another. Furthermore, the mechanical kinetics driving kinesin motion is fundamentally different from those of macroscopic motors (Wang 2008). Since digital circuits are used to describe the control flow of macroscopic systems’ behaviours, representing the stepping properties of kinesin using digital circuits allows us to provide a clear presentation of the motor’s processing unit for a broad readership.
The cellular interactions of kinesin are further modelled as a probabilistic timed automaton used to simulate the processivity of the motor. The principle contribution of this automaton model is the development of a stochastic simulation algorithm used to:

- Calculate the mean run length of kinesin.
- Analyse the frequency of backward stepping powered by ATP hydrolysis and ATP synthesis kinetic pathways.

The second phase of the contribution of this thesis is the development of a mathematical framework to study the motility of two elastically coupled kinesins carrying a common cargo. The main contributions of this framework, obtained by using the dynamics of single kinesin, are the calculations of:

- The distribution of load between two motors.
- The mean run time and the mean run length of the cargo.
- The velocity of the cargo.

1.4 Organization of Thesis

Chapter 1 provides a preview to molecular motors along with the motivation and the contribution of this thesis.

Chapter 2 provides the relevant background needed to comprehend the topic of this thesis. It covers the structure and the function of kinesin motor, theoretical models reported to study the motility of kinesins, and biological significance of the motor. This chapter further describes how the research questions arise from the literature.
Chapter 3 presents research questions raised from the literature of the dynamics of single kinesin stepping and the motility of two-kinesin complex. This chapter proposes a research methodology to answer the research questions.

Chapters 4 and 5 present the development of mathematical and computational models proposed to study the dynamics of single kinesin molecular motor. In Chapter 4, we develop a mathematical model to investigate the mechanical kinetics of single kinesin stepping. Then, computational models developed to simulate the processivity and the information processing unit of single kinesin are presented in Chapter 5.

Chapter 6 presents the development of a mathematical framework proposed to investigate the motility of two coupled kinesins carrying a common cargo. To develop this framework, we use the dynamics of single kinesin.

Chapter 7 provides conclusions drawn from all chapters. It also discusses potential directions of future research.

Appendix provides supplementary material of this thesis including detailed calculations with respective discussions.
Chapter 2

Studies of Kinesin Motor Protein

2.1 Introduction

This chapter outlines the background knowledge of kinesin motor required to understand the topic of this thesis. First, the superfamily of kinesins is introduced. Then, in Section 2.3 the structure of kinesin is described. Section 2.4 discusses the stepping mechanism and the regulation of single kinesin as well as the cargo transport by two coupled kinesins. It is followed by a discussion of the theoretical approaches reported to study the dynamics of single kinesin and multiple kinesins. The biological significance of kinesin is presented in Section 2.6. This chapter concludes with a summary in Section 2.7.

2.2 Kinesin Superfamily Proteins

Kinesin superfamily proteins (KIFs) have been classified into three major groups depending on the position of the motor domain in the molecule (Hirokawa et al. 2009):

I. N-kinesins (N-KIFs): have a motor domain in the amino-terminal region. This type of kinesin motors move toward MT plus ends.

II. C-kinesins (C-KIFs): have a motor domain in the carboxy-terminal region. This class of kinesins move toward MT minus ends.

III. M-kinesins (M-KIFs): have a motor domain in the middle. M-kinesins do not move in similar way with N-kinesins and C-kinesins. M-kinesins diffuse to the plus or minus end of MTs to depolymerize MTs (Hunter et al. 2003).
Kinesin was first discovered by Ronald Vale and colleagues (Vale et al. 1985) while studying axonal transport in squid, subsequently referred to as kinesin-1 or conventional kinesin (Karp 2010). Experimental observations have shown that all kinesins have a motor domain, i.e. the catalytic core for ATP hydrolysis, and the binding site for MTs (Cochran and Kull 2008; Rice et al. 1999). Among KIFs, kinesin-1 is one of the best-studied members in terms of its motion mechanism at the single molecular level, its binding proteins, its regulation of axonal transport, and its association with diseases (Kawaguchi 2013; Verhey et al. 2011).

2.3 Structure

Kinesin is a heterotetramer protein, meaning that it possesses four polypeptide chains which associate tightly to form the molecule, consisting of two heavy chains (KHCs) and two light chains (KLCs) (Figure 2.1). Each KHC is composed of four domains (Mandelkow and Mandelkow 2002):

I. The N-terminal motor domain that allows kinesin to bind to MT and convert chemical energy into mechanical work as it contains the catalytic site for ATP hydrolysis and binding site for MTs (Cochran and Kull 2008; Rice et al. 1999).

II. The neck domain that determines the movement direction (Endow and Waligora 1998; Yildiz et al. 2008).

III. The coiled-coil stalk domain which is responsible for dimerization of KHCs and their association with KLCs. The stalk domain also contains hinge segments that enable the autoinhibition of kinesin to a folded (inactive) conformation (Verhey and Rapoport 2001).

IV. The C-terminal tail domain involved in cargo binding and regulation of the motor (Verhey et al. 2011).
Each KLC contains two subunits (Mandelkow and Mandelkow 2002; Manning and Snyder 2000; Verhey et al. 2011):

I. A heptad repeat region that is responsible for the association with KHCs.

II. Six tetratricopeptide repeat (TPR) motifs that play role in cargo binding and regulation of the motor.

![Figure 2.1](image.png)

*Figure 2.1. A simplified structure of kinesin motor protein with KHCs, a coiled-coil stalk, and KHCs (Hirokawa et al. 2010).*

### 2.4 Function

*In vivo,* a large proportion of kinesins are not bound to cargos, existing as soluble motors in the cytosol (Verhey and Rapoport 2001). The activity of soluble kinesin motors is switched off by autoinhibition process which will be discussed in Section 2.4.2. Kinesin becomes active by binding to cargo in the presence of MTs (Friedman and Vale 1999), walks along MT in a directed way, and detaches from MT at the end of run (Berger 2012).

Kinesin motors associate with their cargos directly or through adaptor proteins. The direct binding of the motor to cargos is accomplished through TPR motifs of KLCs or the tail domain of the motor (Hirokawa et al. 2009). The association of the motor with cargo can also be mediated by adaptor proteins such as JIPs and APP for binding to
vesicles, syntabulin for binding to mitochondria and syntaxin vesicles, and Milton-Miro complex for binding to mitochondria (Hirokawa et al. 2010). Nevertheless, how kinesin motor recognize cargos, such as mRNAs, has not been fully elucidated (Hirokawa et al. 2010). After cargo binding in the presence of MTs, kinesin motor acts as an ATPase, releasing stored energy through hydrolysing of ATP, to transport cargos within the cell by stepping along MT tracks (Friedman and Vale 1999; Khataee and Khataee 2010b). The remaining of this section describes how kinesin motors step along MT and how the motors regulate their activities as a single motor or as a pair of motors.

### 2.4.1 Stepping Mechanism

Kinesin with a cargo binds to and walks along MT. The widely accepted stepping mechanism for the motion of kinesin motor along MTs is hand-over-hand (Clancy et al. 2011; Yildiz et al. 2008). Kinesin motor starts hand-over-hand stepping with binding of the trailing head to MT that causes it to release its ADP (Figure 2.2A and B). Because the neck linker of the trailing empty-head cannot be docked, the length of neck linker is not long enough to allow the leading ADP-head binding appropriately to MT. ATP binding to the trailing empty-head leads to a conformational change that docks its neck linker, ATP is hydrolysed to ADP and $P_i$ which releases about $20k_BT$ energy (where $k_B$ is the Boltzmann constant and $T$ is the temperature), the leading ADP-head rapidly and appropriately binds to MT, and ADP is released from the leading head (Carter and Cross 2005; Hackney 2002; Kawaguchi 2008; Xie 2010) (Figure 2.2C-E). Strong bindings of both heads to MT lead to the strain between two neck linkers that accelerates ATP-hydrolysis and $P_i$ release in the trailing head, while reduces the rate of ATP binding to the leading empty-head (Xie 2010). After $P_i$ release, the trailing ADP-head moves to the leading position due to the affinity between the two heads and very
weak affinity between the trailing ADP-head and MT (Xie 2010) (Figure 2.2 F). Then, the cycle repeats. Therefore, the hand-over-hand movement is accomplished similar to the way of human’s walk and the motor pulls the cargo towards the plus-end of MT. Kinesin makes a forward step of about $d \approx 8.2$ nm with the consumption of one ATP molecule per cycle (Clancy et al. 2011). This motor is able to take approximately 100 steps along MT before detaching from MT, where at least one head of kinesin is attached to MT at all times during the stepping cycles, meaning that kinesin is a processive motor (Clancy et al. 2011; Schliwa 2003; Toprak et al. 2009).

**Figure 2.2.** A simplified schematic of kinesin hand-over-hand movement mechanism. D, T, and DP stand for ADP, ATP, and ADP-P$_i$, respectively. The nucleotide-free state has been shown by $\phi$ (Schliwa 2003).

Kinesin motor uses gating mechanisms to coordinate the activities of the two heads and keep them out of phase (Clancy, et al., 2011; Yildiz, et al., 2008). In gating mechanism, a chemical or structural transition in one head is inhibited until the partner head proceeds through a critical step in its cycle (Yildiz, et al., 2008). Several theories have been conducted to study how one head waits for its partner:
I. Chemical gating: proposes that either ATP binding to the nucleotide-free head is inhibited until the partner head dissociates from MT or that ADP release from the partner head is repressed until it is propelled to a forward position by ATP binding/neck linker docking in the nucleotide-free head.

II. Tubulin binding gating: the motor waits in a one-head-bound intermediate and the detached stepping head cannot bind to the next tubulin-binding site until the partner head binds ATP.

III. Tension gating: proposes that the detachment of the rear head requires or is facilitated by the tension generated from a power stroke in the front head.

Kinesin motor may also undergo futile mechanical cycles. After ATP hydrolysis in the trailing head, if the leading ADP-head does not rapidly attach to MT, P, will release from the trailing head and both heads will contain ADP (Figure 2.3E). This intermediate state can lead to two possible pathways (Hackney 2002):

I. Restart the cycle through releasing ADP from the trailing head (Figure 2.3B). In this futile mechanical cycle, no mechanical step is made.

II. Diffuse of the motor from MT (Figure 2.3A).

For the ATPase activity of kinesin several kinetic schemes have been reported (Carter and Cross 2005; Clancy et al. 2011; Gilbert et al. 1998; Hackney 2002; Kawaguchi 2008; Mandelkow and Johnson 1998; Schlwa 2003; Seitz and Surrey 2006; Xie 2010; Yajima et al. 2002), due to the existence of several different sequences of states that define a complete ATP hydrolysis cycle (Chowdhury 2013).
Figure 2.3. A simplified schematic of kinesin futile cycle. D, T, and DP stand for ADP, ATP, and ADP-P$_i$, respectively. The nucleotide-free state has been shown by Ø (Hackney 2002; Schliwa 2003).

2.4.2 Stepping Regulation

The detachment of kinesin motor from MT is regulated by autoinhibition mechanism (Hirokawa et al. 2010). The autoinhibition is a mechanism that the tail domain binds to the motor domain which inhibits ATPase activity of the motor domain and the binding of the motor to MT (Friedman and Vale 1999; Verhey et al. 2011). This may prevent wasteful hydrolysis of ATP by the motor when it is not attached to cargo (Khataee and Khataee 2010b; Friedman and Vale 1999; Verhey et al. 2011). The detachment of kinesin motor from MT may be caused by various regulatory factors, such as long pausing (Schnitzer et al. 2000), high resisting loads (Carter and Cross 2005), obstacles on MT (Verhey et al. 2011), and the release of cargo (Hirokawa et al. 2009).

There is evidence showing that kinesin cargo release is regulated by phosphorylation and Ca$^{2+}$ signalling (Hirokawa et al. 2009). The TPR domain of KLCs binds to JNK-
interacting protein 1 (JIP1) which serves as a cargo receptor for kinesin and a scaffolding protein for Active c-Jun N-terminal kinase (JNK) pathway kinases (Hirokawa et al. 2009; Stagi et al. 2006). JNK pathway can phosphorylate kinesin (Horiuchi et al. 2007; Morfini et al. 2006; Stagi et al. 2006). Therefore, the activation of JNK pathway kinases disrupts the binding between kinesin and JIP1 which causes the release of the cargo and thus, the autoinhibition of the motor (Horiuchi et al. 2007). Ca\textsuperscript{2+} signalling also regulates mitochondria delivery of kinesin which leads to the recruitment of mitochondria at Ca\textsuperscript{2+} regions of cell (Hirokawa et al. 2010). Ca\textsuperscript{2+} signalling regulates kinesin stepping through two pathways (Hirokawa et al. 2010):

I. Miro protein, the mitochondria adaptor, binds directly to the motor domain and inhibits the interaction between the motor domain and MTs.

II. Binding of Ca\textsuperscript{2+} to the Miro detaches mitochondria from the motor.

The motility of kinesin can be reactivated by binding to cargo in the presence of MTs (Friedman and Vale 1999).

Another factor that might affect kinesin motility is obstacles on MTs (Verhey et al. 2011). In vivo, kinesin motors compete with a multitude of molecular motors and non-motile MT-associated proteins (MAPs) for binding to MTs. These competitors might block the activity of kinesin (Dixit et al. 2008). Studies revealed that tau, a neuronal MAP, can increase the detachment rate of kinesin from MT (Dixit et al. 2008; Vershinin et al. 2007). It has been shown that abnormal accumulation of tau in soma disrupts cargo transportation leading to neurodegenerative diseases (Dixit et al. 2008). Kinesin usually detaches from MT when encountering obstacles on MTs (Telley et al. 2009) or bypasses such obstacles after temporary pausing (Dreblow et al. 2010). Kinesin function has also been examined at MT intersections where the motor was more likely to pass the
intersection on the same MT (Ross et al. 2008). The cargo attached to kinesin may also be snagged on cytoskeletal filaments causing the load to increase significantly. In general, when faced with an insuperable obstacle, the motor will become detached to avoid futile ATP hydrolysis. This mechanism enables vesicles carried by single motors (or multiple motors) to be released and diffused away from barriers briefly, then to rebind to MT via kinesins and resume forward progress without wasting energy (Schnitzer et al. 2000).

Experiments have shown that the detachment of kinesin from MT occurs after ATP hydrolysis (Gilbert et al. 1995). Due to the tight affinity between the motor head and MT in both ATP and nucleotide-free states, it is unlikely that detachment occurs from these kinetic states or any state when both heads are bound to MT (Schliwa 2003). The detachment most likely occurs when the bound trailing head is in ADP·P\(_i\) state, while the unbound leading head contains ADP (Schliwa 2003; Seitz and Surrey 2006) (Figure 2.2D). This intermediate state shown in Figure 2.2D also provides an alternative route for the detachment of kinesin from MT through a futile cycle (Hackney 2002) (Figure 2.3E). Other models have suggested an intermediate state for the detachment of kinesin from MT where the both heads are in ADP state (Hackney 2002; Yajima et al. 2002). The detachment state of kinesin is also affected by [ATP]. It has been observed by Schnitzer et al. (2000) that at limiting [ATP] the detachment probability of kinesin from MT in MT-bound (i.e. kinesin-MT) intermediate state (in Figure 2.2B) increases with the increase of the duration of this state, meaning that the motor is paused at this intermediate state, waiting for ATP binding (Clancy et al. 2011; Hackney 1994). Accordingly, at high [ATP] the detachment probability from intermediate state kinesin-MT diminishes (Schnitzer et al. 2000). After detaching from MT, both heads of
soluble kinesin are tightly bound to ADP (Kawaguchi 2008; Mandelkow and Johnson 1998) (see Figure 2.2A).

Recent experiments from Block laboratory (Andreasson et al. 2015) used single molecule optical trapping assay to measure the detachment rate of single kinesin under resisting and assisting loads parallel to MT at saturating [ATP]. Resisting (or backward) and assisting (or forward) loads are taken toward minus and plus ends of MT, respectively. Kinesin unbinding rate was found to be dramatically asymmetric with respect to the direction of load (see Figure 2.4). It is yet unclear what happens to the motor after it has been inactivated at its destination. Several studies have suggested that motors are degraded at the synapse (Verhey and Rapoport 2001). Detached kinesin motor remains in the inactive folded form until the binding of an appropriate cargo reactivates its motility (Verhey et al. 2011).

![Figure 2.4. Unbinding rate of single kinesin against resisting and assisting loads parallel to MT at [ATP] = 2000 µM (Andreasson et al. 2015).](image-url)
2.4.3 Forward and Backward Steppings

Kinesin motor moves primarily in the forward direction (i.e. toward the plus end of MT). However, with increasing external load applied to the motor, the backward steps (i.e. toward the minus end of MT) start to occur (Nishiyama et al. 2002). Experimental observations reported that the stepping direction of kinesin motor changes at stall force ($F_{\text{stall}}$), ranging between 5-7.6 pN (Carter and Cross 2005; Nishiyama et al. 2002; Visscher et al. 1999; Yildiz et al. 2008). It was observed in (Carter and Cross 2005; Nishiyama et al. 2002) that:

I. Below stall force: forward stepping of the motor is dominant.

II. At stall force: forward and backward steppings are equally likely.

III. Above stall force: backward stepping is more frequent.

Experimental observations by Nishiyama et al. (2002) have shown that at loads lower that 4 pN kinesin stepping is made in the forward direction. The occurrence of backward stepping increases with an increase in load and dominated the occurrence of forward stepping at a load of 7-8 pN, giving rise to a stall force of about 7.6 pN. Their results showed that the ratio of forward and backward stepings is dependent on the load, but not on [ATP]. Recent optical trap experiments performed by Jamison et al. (2010) further measured stall force of single kinesin at 7.3 pN. Carter and Cross (2005) has also reported that both forward and backward steppings of kinesin motor are triggered by ATP binding with step length of almost 8 nm. They investigated kinesin stepping at loads from -15 pN to 15 pN, and measured the stall force at 7.2 pN where the ratio of forward and backward steppings was equal. According to their observations, the choice between forward and backward stepping was dependent only on load, not on [ATP]. In discussing backward stepping under high resisting loads, Carter and Cross (2005) stated that backward stepping requires ATP binding, while no evidence has been
observed for the coupling of backward stepping to ATP turnover. They also argued that the model in which backward stepping synthesizes ATP, proposed by Fisher and Kolomeisky (2001), is unlikely. However, Fisher and colleagues (2005) remarked that the experimental observations in (Carter and Cross 2005) rest on a misconception of the significance of dwell times before forward and backward stepings, and that backward stepping could result in ATP synthesis.

2.4.4 Processivity and Velocity

Mechanical processivity of kinesin has revealed that the motor takes multiple steps in the forward direction, and occasionally in the backward direction (Carter and Cross 2005; Nishiyama et al. 2002; Yildiz et al. 2008) before detaching from MT in the presence of ATP (Howard et al. 1989; Yajima et al. 2002), or in the absence of ATP if external load is applied to the motor (Yildiz et al. 2008). Since kinesin uses gating mechanisms to coordinate the activities of its two heads, the gating mechanisms are shown to be requirements for the accomplishment of kinesin mean run length (i.e. the distance that the motor travels before detaching from MT) (Toprak et al. 2009). This processivity of kinesin ensures that cellular cargos are transported reliably over long distances. In analysing the processivity and velocity of single kinesin, Schnitzer et al. (2000) used a single molecule bead assay to measure the mean run length and velocity of the motor over a range of resisting loads and [ATP]. The run lengths and velocity of kinesin motor were observed to be exponentially distributed under fixed loads or [ATP] (Schnitzer et al. 2000; Yajima et al. 2002) (see Figure 2.5A-D).
In modelling kinesin processivity, Block and colleagues in Schnitzer et al. (2000) used an energy landscape formalism, where the kinetic rates followed a Boltzmann-type relationship, and showed that the mean run length of kinesin, $L$, exhibits Michaelis–Menten kinetic behaviour, $L = L_0[ATP] / ([ATP] + L_M)$, where $L_0$ is the mean run length at zero load and $L_M$ is Michaelis constant. Using a global analysis of kinesin processivity data carried out from Michaelis–Menten functions at different [ATP] and loads and then, fitted to kinetic rates, $L$ was calculated as follows:

$$L = \frac{d \times [ATP] \times A \exp \left( - \frac{F \delta}{k_B T} \right)}{[ATP] + B \left( 1 + A \exp \left( - \frac{F \delta}{k_B T} \right) \right)}$$

(2.1)

where $d$ is the step size (equal to 8.2 nm), $A = 107 \pm 9$ gives the average number of catalytic cycles before detachment, $\delta = 1.3 \pm 0.1$ nm is the characteristic distance associated with load dependence, and $B = 0.029 \pm 0.009$ mM is Michaelis constant (data
are shown in Figure 2.5A and B). The energy landscape model proposed in Schnitzer et al. (2000) predicts that kinesin motility should remain tightly coupled to ATP hydrolysis even under load. Meanwhile, a theoretical model derived from this energy model describes the dynamics of kinesin stepping by both the ATP hydrolysis and the ATP synthesis kinetic cycles at various [ATP] and loads (Fisher and Kim 2005; Fisher and Kolomeisky 2001; Kolomeisky and Fisher 2007). It was found by Toprak et al. (2009) that processivity of kinesin motor and thus, its mean run length is correlated to the cellular interactions of the motor. Yet, little is known about how this motor can remain attached to MT through the hundreds of stepping cycles (Toprak et al. 2009).

Schnitzer et al. (2000) also used their energy landscape formalism method to model the load- and [ATP]-dependent velocity of single kinesin as follows:

$$v = \frac{d \times k_{cat}(F) \times [ATP]}{[ATP] + \frac{k_{cat}(F)}{k_b(F)}}$$  \hspace{1cm} (2.2)

where $k_{cat}$ is the catalytic-turnover rate constant, and $k_b$ is the apparent second order rate constant for ATP binding calculated as:

$$k_{cat}(F) = \frac{k_{cat}^0}{P_{cat} + q_{cat} \cdot \exp \left(\frac{F \delta_{cat}}{k_b T}\right)}$$  \hspace{1cm} (2.3)

$$k_b(F) = \frac{k_b^0}{P_b + q_b \cdot \exp \left(\frac{F \delta_b}{k_b T}\right)}$$  \hspace{1cm} (2.4)

where $k_{cat}^0 = 103 \pm 2 \text{ s}^{-1}$ and $k_b^0 = 1.3 \pm 0.1 \mu\text{M}^{-1} \text{ s}^{-1}$ are the load-free catalytic-turnover rate constant and the load-free ATP binding rate constant, $\delta_{cat} = \delta_b = 3.7 \pm 0.3 \text{ nm}$ denotes the load characteristic distance, $p$ and $q = (1 - p)$ are the fractions of the load-free catalytic cycle required for biochemical and mechanical transitions, respectively,
\[ q_{\text{cat}} = 6.2 \pm 2.7 \times 10^3 \text{ and } q_b = 4.0 \pm 1.4 \times 10^{-2}. \] Data for the velocity of single kinesin is shown in Figure 2.5C and D.

Recent experiments from Block laboratory used single molecule optical trapping assay to measure the mean run length and velocity of single kinesin under both resisting and assisting loads at different [ATP] (Andreasson et al. 2015; Milic et al. 2014). Mean run length data has showed that under small assisting loads the mean run length of kinesin was an order of magnitude shorter than that under resisting loads. In addition, no difference was observed in the mean run length measured under low [ATP] compared to that in the presence of high [ATP] (see Figure 2.6A). Analysis of velocity data also indicated that the velocity of kinesin remained unchanged over assisting forces (see Figure 2.6B).

**Figure 2.6.** Mean run length and velocity of single kinesin. (A) Mean run length against resisting and assisting loads at [ATP] = 2000 µM (Andreasson et al. 2015; Milic et al. 2014). (B) Velocity against resisting and assisting loads at [ATP] = 2000 µM (Andreasson et al. 2015).
2.4.5 Cargo Transport by Two Coupled Kinesins

*In vivo*, cargoes are usually transported by small groups of motors which may belong to the same or to different motor superfamilies (Keller et al. 2013; Vershinin et al. 2007). In the simplest case, cargo transport is usually performed by a group of identical motors, for example multiple kinesins. Recent experiments from Diehl laboratory (Jamison et al. 2010; Rogers et al. 2009) used optical trap experiment to investigate the load-dependent transport properties of two elastically coupled kinesins carrying a common cargo along MT. Their results showed that the stepping of two-kinesin assembly against applied load was primarily asynchronous due to interactions between motors, i.e. the communication of forces between two motors. Inspection of the two-motor assemblies showed that kinesins frequently detached from and rebound to MTs during two-motor runs. Their main findings showed that the mean run length of two-motor system was larger than that of single kinesin, whereas the velocity measurements of the two-motor complex and single motor were not significantly different (Jamison et al. 2010; Rogers et al. 2009). The applied load was also shown not to be shared equally as the load on the leading motor increases with the increase in intermotor distance (Jamison et al. 2010) (see Figure 2.7).

![Figure 2.7](image)

*Figure 2.7.* Load shared between the leading ($F_{le}$) and trailing ($F_{tr}$) motors against the separation distance between the motors at fixed resisting loads ($F_{ex}$) (Jamison et al. 2010).
2.5 Theoretical Models

2.5.1 Single Motor Models

The combination of appropriate theory with experimental observations of molecular motors would provide new insights to uncover and describe the mechanisms underlying functions of the motors. The central task of theoretical models is to connect the mechanochemical processes of molecular motors to their directed motions (Kolomeisky and Fisher 2007). One of the basic principles of these theoretical models is that all biochemical transitions in the kinetic schemes of the motors are reversible, even when there is no direct evidence in available data (Kolomeisky and Fisher 2007). Two main approaches have been developed for the theoretical modelling of a single motor protein (Fisher and Kolomeisky 2001; Kolomeisky and Fisher 2007) as follows:

I. Continuum ratchet model.
II. Discrete stochastic model.

In the ratchet model, motor protein is viewed as diffusing on two or more spatially parallel, periodic and asymmetric coarse-grained energy surfaces (i.e. potentials). The corresponding potentials describe distinct biochemical states of the motor. Under the input of chemical energy the motor switches stochastically between different potentials and the system evolves, so that a biased diffusion ensues. The ratchet model has been shown to have several troublesome aspects (Kolomeisky and Fisher 2007):

I. General analytical results cannot be found. It can be resorted to a full numerical approach, but the computations are relatively demanding and many functional parameters are entailed. Thus, determining the range and uniqueness of fits to real data is a nontrivial task.
II. Deriving appropriate realistic potential functions which are meaningfully detailed presents significant challenges as it is hard to judge the reliability and instructiveness of the resulting implications for real motor proteins.

Thus, continuum ratchet models can profitably be utilized to describe various qualitative features rather than quantitative features of motor dynamics (Kolomeisky and Fisher 2007).

Discrete stochastic models, however, provide a flexible theoretical framework for understanding motor protein mechanisms and were shown to be effective in describing the available experimental data (Kolomeisky and Fisher 2007). The discrete stochastic model supposes that during each stepping cycle, a motor passes through a sequence of intermediate kinetic states. Mathematically, this simple sequential model describes a particle that hops randomly on a one-dimensional periodic lattice of sites. For kinesin’s processive stepping, a principle discrete stochastic model reported by Fisher and Kolomeisky (2001) defines a single stepping cycle of kinesin as passing through a sequence of \( n \) kinetic states where state \((j)\) may transit to states \((j + 1)\) and \((j - 1)\) with forward and backward transition rates \(u_j\) and \(w_j\) \((j = 0, 1, \ldots, n-1)\), respectively:

\[
\begin{align*}
  &u_0 \text{↔} w_1 \quad u_1 \text{↔} w_2 \quad u_2 \text{↔} w_3 \quad \ldots \quad u_{n-1} \text{↔} w_0 \\
| \quad d_0 | \quad \ldots \quad d_{N-1} \quad | \\
\end{align*}
\]

where \(d_j\) represents the sub-step lengths for the centre of the motor along MT. The total step length is then calculated as follows:

\[
d = \sum_{j=0}^{n-1} d_j \quad (2.5)
\]
It has been shown that the transition rates between kinetic states are influenced by the temperature (Leibler and Huse 1993) and external load $F$ (Fisher and Kolomeisky 1999, 2001), but not sub-step lengths (Fisher and Kolomeisky 2001), as:

$$u_j^{(F)} = u_j^{(0)} \exp \left( -\frac{\theta_j^+ Fd}{k_B T} \right)$$  \hspace{1cm} (2.6)$$

$$w_j^{(F)} = w_j^{(0)} \exp \left( +\frac{\theta_j^- Fd}{k_B T} \right)$$  \hspace{1cm} (2.7)$$

where $u_j^{(0)}$ and $w_j^{(0)}$ are the transition rates at zero load, $\theta_j^+$ and $\theta_j^-$ are the characteristic distances for the load and $d$ is the kinesin step size. The load distribution factors demonstrate how the external load affects the individual kinetic rates. It is assumed that (Fisher and Kolomeisky 1999, 2001):

$$\sum_{j=0}^{n-1} \theta_j^+ + \theta_j^- = 1$$  \hspace{1cm} (2.8)$$

$$d_j = (\theta_j^+ + \theta_{j+1}^-)d$$  \hspace{1cm} (2.9)$$

By fitting to experimental data of Block et al. in (Schnitzer et al. 2000; Visscher et al. 1999) at room temperature, Fisher and Kolomeisky (2001) defined the simplest discrete stochastic model for an individual stepping cycle of single kinesin as passing through a sequence of four kinetic states (i.e. $n = 4$) as follows:

$$u_0 \leftrightarrow u_1 \leftrightarrow u_2 \leftrightarrow u_3 \leftrightarrow [0]$$  \hspace{1cm} (Scheme 2.2)$$

where the kinetic states are denoted as:

$$[0] = \text{M-K} \hspace{1cm} [1] = \text{M-K-ATP}$$

$$[2] = \text{M-K-ADP-P_i} \hspace{1cm} [3] = \text{M-K-ADP}$$
where M·K denotes the MT-kinesin complex, and the ADP-Pi complex stands for the products of ATP hydrolysis: ADP and P$_i$. The estimated parameters of the Equations (2.6) and (2.7) for the simplest four-state model at room temperature are summarized in Table 2.1. Consequently, the load-free [ATP]-dependent kinetic rates are also defined by:

$$u_{00}^0 = k_0[\text{ATP}]$$  \hspace{1cm} (2.10)$$

$$w_{00}^0 = \frac{k_0'[\text{ATP}]}{\sqrt{1 + \frac{\text{ATP}}{c_0}}}$$  \hspace{1cm} (2.11)$$

where $k_0 = 1.8$ µM$^{-1}$s$^{-1}$, $k_0' = 0.225$ µM$^{-1}$s$^{-1}$, and $c_0 = 16$ µM (Fisher and Kolomeisky 2001). This theoretical kinetic scheme asserts that forward stepping of kinesin motor is associated with ATP hydrolysis, while backward stepping could give rise to ATP synthesis (Kolomeisky and Fisher 2007). Even though, Carter and Cross (2005) stated that ATP synthesis is unlikely in backward stepping, Fisher and Kim (2005) remarked that the experimental observations in (Carter and Cross 2005) rest on a misconception of the significance of dwell times before forward and backward stepping, and that backward stepping could result in ATP synthesis.

<table>
<thead>
<tr>
<th>State $j$</th>
<th>$u_{j0}^0$ (s$^{-1}$)</th>
<th>$w_{j0}^0$ (s$^{-1}$)</th>
<th>$\theta_j^+$</th>
<th>$\theta_j^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0.120</td>
<td>0.430</td>
</tr>
<tr>
<td>1</td>
<td>580</td>
<td>40</td>
<td>0.020</td>
<td>0.130</td>
</tr>
<tr>
<td>2</td>
<td>290</td>
<td>1.6</td>
<td>0.020</td>
<td>0.130</td>
</tr>
<tr>
<td>3</td>
<td>290</td>
<td>40</td>
<td>0.020</td>
<td>0.130</td>
</tr>
</tbody>
</table>
In this four-state discrete stochastic model, kinetic states are mechanically indistinguishable, meaning that the model can be summarised to a mechanically two-state discrete stochastic model (Fisher and Kim 2005). Meanwhile, Fisher and Kim (2005) stated that no purely two-state model could fit the experimental data in (Schnitzer et al. 2000; Visscher et al. 1999). Using the two- and four-state kinetic models of kinesin, the velocity and the mean run length of single kinesin was obtained (Fisher and Kolomeisky 1999) as follows:

\[
V = \frac{d}{\sum_{j=1}^{2} r_j \left( 1 - \prod_{j=0}^{n-1} \frac{w_{j+1}^{F}}{u_j^{F}} \right)}
\]

(2.12)

where \(d\) is the step size and \(r_j\) is obtained as:

\[
r_j = \frac{1}{u_j^{F}} \left( 1 + \sum_{k=1}^{n-1} \prod_{i=1}^{k} \frac{w_{j+i}^{F}}{u_{j+i}^{F}} \right)
\]

(2.13)

and,

\[
< L > = \frac{V}{P_0 \delta_0 + P_1 \delta_1}
\]

(2.14)

\[
P_1 = \frac{u_0 + w_0}{u_0 + w_0 + u_1 + w_1}
\]

(2.15)

where \(P_1 = (1 - P_0)\) is the steady-state probability that the motor is in a state processing ATP (i.e., kinetic state [1]) and the parameters obtained by fitting to Schnitzer (2000) run length data are \(\delta_0^0 = 0.025\) s\(^{-1}\), \(\delta_0^1 = 1.3\) s\(^{-1}\), \(\theta_0^0 = 0\), and \(\theta_0^1 d = 0.7\) nm (see Figure 2.8).
Figure 2.8. Velocity and mean run length of kinesin (Fisher and Kolomeisky 2001). (A) Velocity as a function of load at fixed [ATP]. (B) <L> against [ATP] at fixed loads. (C) <L> against load at fixed [ATP]. The data points are obtained by Visscher et al. (1999) and Schnitzer (2000).

Four-state discrete stochastic model provides an [ATP]-dependent stall force given by (Fisher and Kolomeisky 2001):

\[
F_{\text{stall}} = \left( \frac{k_B T}{d} \right) \ln \left( \prod_{j=0}^{n-1} \frac{H_j}{W_j} \right)
\]  

(2.16)

Equation (2.16) implies that the stall force rises as the [ATP] increases, which is consistent with experimental observations by Visscher et al. (1999). However, the later experimental observations of Nishiyama et al. (2002) and Carter and Cross (2005) showed that stall force seems to be independent of [ATP], and dependent only on load.

Liepelt and Lipowsky (2007) also proposed a seven-state kinetic scheme for an individual stepping cycle of single kinesin and calculated velocity and mean run length of single kinesin that are in agreement with the results of the four-state model developed by Fisher and Kolomeisky (2001). They pointed out a limitation of the four-state model arguing that the four-state model determines the stall force by the flux balance between ATP hydrolysis and ATP synthesis, implying that the ATP hydrolysis rate vanishes at the stall force in the limit of small [ADP] (Liepelt and Lipowsky 2007), whereas experiments in (Carter and Cross 2005) have provided evidence that the ATP hydrolysis
rate is finite in this limit. Using Equation (2.6), we calculate ATP hydrolysis rate at stall force of 7.3 pN as $u_{i}^{(F=7.3)} \approx 431.42 \text{ s}^{-1}$, indicating that this rate does not vanish at stall force. In addition, the seven-state kinetic scheme does not address the kinetic state ADP·P$_i$ in an individual stepping cycle of kinesin, which is experimentally shown to be involved in the stepping cycle of kinesin (Andreasson et al. 2015; Milic et al. 2014).

### 2.5.2 Two Motors Models

To study the cargo transport by multiple molecular motors, a general theoretical framework was proposed by Klumpp and Lipowsky (2005). That work studied unidirectional transport by one team of identical motors assuming that all motors share the load equally. However, the recent experimental findings conducted on an assembly of two coupled kinesins showed that MT-bound kinesins within the assembly most likely will not be able to share the applied load equally (Jamison et al. 2010). In parallel to these experimental findings (Jamison et al. 2010; Rogers et al. 2009), recent theoretical works have focused on the assembly of two coupled kinesins. In a set of theoretical works, Diehl and colleagues have developed discrete state transition rate model based on the strain energy and the kinetic rates of single kinesin to study and analyse the motility of two-kinesin assembly (Driver et al. 2011; Driver et al. 2010; Uppulury et al. 2012b, 2012a). Their main results have shown that: (i) the assembly does not tend to advance to configurations with high intermotor distance, (ii) the load distribution between MT-bound kinesins depends on the intermotor distance, and load sharing only occurs if the motors are attached to closely spaced MT binding sites, and (iii) velocity of the assembly follows the velocity of single kinesin.
In another set of theoretical works, Lipowsky and colleagues considered kinesin stalks as linear elastic springs with stiffness (i.e. elastic strength) $k_{motor}$ coupled to a common cargo (Berger et al. 2015; Berger et al. 2012; Keller et al. 2013) (see Figure 2.9). In their model, the discrete stepping of two MT-bound motors leads to the discrete extension of the elastic linkers and thus, the development of strain force between the motors. Accordingly, the cargo state is described by a discrete microstate space where every microstate ($i$), where $i = 0, \ldots, N$, is associated with the extension $i$ of the linkers and elastic force $F_i$ exerted on one motor and $-F_i$ on the other, as required by Newton’s third law. It has been assumed that the two-motor run starts from microstate (0), where both motors are bound to MT with relaxed linkers, i.e. there is no elastic force between the motors. If one of the motors steps, each elastic linker is stretched by $d/2$ (where $d$ is the step size of kinesin), cargo state stretches to microstate (1), and $\pm F_1$ forces are exerted on the motors. For two such linear springs in series in the presence of external force $F_{ex}$, they defined the loads exerted on the leading ($F_{le}$) and trailing ($F_{tr}$) motors in each microstate ($i$) as follows (Berger et al. 2015):

\[
F_{le}(i) = F_{ex} + k_{motor} \frac{di}{2} \quad (2.17)
\]

\[
F_{tr}(i) = -(k_{motor} \frac{di}{2}) \quad (2.18)
\]

where the term $(k_{motor} di/2)$ is the elastic force between the motors.

**Figure 2.9.** Cargo transport by two kinesins (Berger et al. 2012). $\epsilon_1$ and $\epsilon_2$ are the detachment rates of single motor and one of the motors in a two MT-bound configuration of motors, respectively. $\pi$ is the binding rate of single kinesin to MT.
Transitions of cargo state between the microstates occur whenever a motor steps. In each microstate, elastic linkers are stretched due to either a forward step of the leading motor or a backward step of the trailing motor. Conversely, a forward step of the trailing motor or a backward step of the leading motor relaxes the linkers (Driver et al. 2010). Therefore, the load-dependent stretching and relaxation rates are connected to the stepping rate of single kinesin as follows (Berger et al. 2012):

\[ k_s(i) = \frac{v(F_{le}(i))}{d} \quad (2.19) \]

\[ k_r(i) = \frac{v(F_{tr}(i))}{d} \quad (2.20) \]

where \( v \) denotes the velocity of single kinesin given by Equation (2.2) and \( d \) is the step size of single kinesin. The highest microstate \( N \) corresponds to the largest force that can be exerted on motors without detaching them from MT, i.e. \( F_N \). This maximum force has been considered as stall force by Berger (Berger 2012). Thus, by solving equation \( F_{stall} = F_N \), one can calculate \( N \). Using this discrete state model for cargo transport by two kinesins, they obtained explicit equations for the mean run time, velocity, and the mean run length of cargo as follows (Berger et al. 2012) (see Figure 2.10):

\[ <\Delta t_{ca}> = \frac{\pi + \varepsilon_2}{\varepsilon_1 \varepsilon_2} \quad (2.21) \]

\[ <\Delta x_{ca}> = \frac{\pi v_2 + \varepsilon_2 v_1}{\varepsilon_1 \varepsilon_2} \quad (2.22) \]

\[ v_{ca} = \frac{\Delta x_{ca}}{\Delta t_{ca}} \quad (2.23) \]

In Equations (2.21)-(2.23) \( \varepsilon_1 \) and \( \varepsilon_2 \) are the detachment rates of single motor and one of the motors in a two MT-bound configuration of motors, respectively, \( \pi \sim 5 \text{ s}^{-1} \) (Koster et
al. 2003) is the binding rate of kinesin, \( v_1 \) is the velocity of single kinesin given by Equation (2.2) and \( v_2 \) is the velocity of cargo transported by two MT-bound kinesins (see Figure 2.9). Using the average stepping rates of the motors, they calculated \( v_2 \) as (Berger et al. 2012):

\[
v_2 = \frac{1}{2} \sum_{i=0}^{N} (v_1(F_i) + v_1(F_i)) P_i
\]

(2.24)

where \( P_i \) is the probability of finding cargo in microstate \( i \) obtained from the kinetic scheme of two-motor assembly.

![Figure 2.10. Dynamics of two coupled kinesins carrying a cargo (Berger et al. 2015). (A) Cargo velocity, (B) mean run time, and (C) mean run length of cargo as a function of resisting load at fixed motor stiffness.](image)

In the calculations of all these theoretical works (Berger et al. 2015; Berger et al. 2012; Driver et al. 2010; Fisher and Kolomeisky 2001; Keller et al. 2013; Klumpp and Lipowsky 2005; Kunwar and Mogilner 2010; Nam and Epureanu 2015; Uppulury et al. 2012b, 2012a), the detachment rate of kinesin was calculated symmetrically with respect to the direction of load as follows:

\[
\varepsilon_1(F) = \varepsilon_0 \exp \left( \frac{|F|}{F_d} \right)
\]

(25)

where \( \varepsilon_0 \) is the unloaded unbinding rate, \( |F| \) is the absolute value of the load \( F \), and \( F_d \) is the detachment force expressed as \( F_d = k_B T / d \). The force \( F_d \) depends on the thermal energy \( k_B T \) and the potential barrier between the attached and detached states of kinesin.
measured as $d \sim 1.3$ nm (Schnitzer et al. 2000), so that the detachment force is $F_d \sim 3$ pN (Klumpp and Lipowsky 2005). However, the recent experimental data from Block laboratory have shown that the detachment rate of kinesin is asymmetric with respect to the direction of load (Andreasson et al. 2015) (see Figure 2.4). The dramatic asymmetry of the detachment rate has direct consequences for the stochastic models of collective cargo transported by a group of motors under resisting and assisting loads (Milic et al. 2014). Therefore, studying the motility of two coupled kinesins by considering the asymmetric detachment rate of kinesin with respect to the load direction is still lacking.

### 2.6 Biological Significance

Kinesin motor is best known for its role in axonal transport because most of the proteins required in the axon and nerve terminals have to be transported from the cell body over large distances. This large distance cargo transport is vital for neuronal function and survival (Hirokawa et al. 2009). The involvement of kinesin motors in the directed axonal transport and homeostasis, which causes the generation of the neural polarity and synapses, is fundamental for brain wiring and formation (Hirokawa et al. 2010). Kinesin motors can also be regarded as neural signal transduction molecules merely by stepping along MTs because the motors transport signalling molecules or their associated proteins. Therefore, they can modulate neural behaviours, e.g. survival or apoptosis (Hirokawa et al. 2010). Consequently, the impairment of kinesin function is associated with neurodegenerative diseases. Neurodegenerative diseases are often accompanied by the accumulation of proteins in the cell processes, caused by impaired axonal transport, which eventually leads to neuronal dying-back. These diseases are generally classified into two groups (Mandelkow and Mandelkow 2002): (i) cases where physiological cargoes are not delivered appropriately, e.g. Alzheimer, and (ii) cases where non-
physiological cargoes (e.g. viruses) make use of the transport system. Experiments have shown that the brains of Alzheimer disease patients contain neurofibrillary tangles (Stokin et al. 2005). The tangles contain accumulated abnormal amounts of tau, motor proteins, organelles, vesicles, and amyloid plaques. Tau impairs kinesin mediated transport of APP which leads to the accumulation of APP. Amyloid plaques derived from APP then degenerate neurites and cause Alzheimer disease (Stokin et al. 2005).

An example for the second class of neurodegenerative diseases is the neurotropic herpes viruses that utilize kineisn motor to traverse long distances from the cell body to axon terminals (Diefenbach et al. 2002).

To diminish impairment and disruptive roles of kinesin motors several biological and engineering techniques have been proposed. Molecular biology aims to manipulate the formation elements of the motor proteins by changing their amino acid sequence, and also to discover novel motors with various capabilities through the genome projects (Karp 2010). Engineers have also begun to mimic the functions of motor proteins and invent synthetic nanodevices with medical diagnostic capabilities. To achieve these goals, the first step is to understand the biophysical fundamentals of intracellular mechanisms of cargo transport by molecular motors.

2.7 Chapter Summary

This chapter provided a background to the topic and also introduced the arguments in the literature that aroused due to the discrepancies among previous findings. Based on these arguments, the following points require further studies for single kinesin: (i) the association of forward and backward steppings of kinesin with ATP hydrolysis and
ATP synthesis pathways, (ii) the dependence of the stall force of kinesin to [ATP] and load, (iii) the role of cellular interactions of kinesin in its processivity, and (iv) the association of ATP hydrolysis and ATP synthesis pathways with the mean run length of kinesin. Even though single kinesin motor has been shown to be capable of processing information (Khataee et al. 2013; Pierobon and Akyildiz 2010), studying the processing unit that actuates its steppings has not been considered yet. For cargo transport by an assembly of two coupled kinesins, the following points require more investigation: (i) the distribution of load between the motors under various external loads and (ii) the velocity and the mean run length of the assembly over resisting and assisting loads at various coupling strengths by considering the asymmetric detachment rate of single motor with respect to the direction of load.
Chapter 3

Research Design and Methodology

3.1 Introduction

This chapter first presents the research questions raised from the biophysical fundamentals of intracellular mechanisms of cargo transport by kinesins. Then, Section 3.3 presents the theoretical approach proposed in this thesis to answer these research questions.

3.2 Research Questions

From the experimental and theoretical findings discussed in the previous chapter, it is known that there are several research gaps and discrepancies among previously reported findings in studying the dynamics of cargo transport by single or multiple kinesins. As we discussed in Section 2.5, the central task of theoretical modelling of molecular motors is to link the biochemical processes of the motors and their mechanics (Kolomeisky and Fisher 2007). This task raises several prominent research questions on the cargo transport mechanism driven by single kinesin as follows:

I. What is the dependency between the stall force of kinesin to [ATP] and external load?

II. How is the backward stepping of kinesin associated with the ATP hydrolysis and ATP synthesis kinetic pathways?

III. How are the ATP hydrolysis and ATP synthesis kinetic cycles associated with the processivity of kinesin?

IV. How is the control flow of kinesin motion related to its cellular interactions?
Studies of the intracellular cargo transport by a team of two coupled kinesins has also revealed that so far little is known about the combined cellular mechanisms of molecular motors. The primary research questions identified from the motility of two coupled kinesins carrying a common cargo are as follows:

I. How is the external load distributed between the motors?

II. What are the force-velocity and force-run length relationships under assisting and resisting loads when considering the asymmetric detachment rate of single kinesin with respect to the direction of load?

### 3.3 Research Methodology

This thesis aims to focus on the poorly understood aspects of intracellular mechanisms of cargo transport by kinesin motors addressed in the previous section. Since in cells many cargoes are carried by more than one motor simultaneously, it is necessary to investigate the relation between the cargo transport by single motor and by an assembly of motors. This bottom-up strategy will develop a systematic understanding of the cargo transport in vivo. Therefore, we investigate intracellular cargo transport by developing a theoretical approach in two phases:

- First phase studies the cargo transport by single kinesin via developing mathematical and computational models. We develop a mathematical model to investigate the mechanical kinetics underlying individual stepping cycles of single kinesin. Based on the results of this mathematical model, we develop computational models to simulate kinesin processivity and the information processing unit actuating stepping cycles of the motor. This phase would address all four research questions raised from the intracellular mechanisms of cargo transport by single kinesin.
Second phase studies the cargo transport by an assembly of two coupled kinesins via developing a mathematical framework. We develop a mathematical framework based on master equation approach that integrates dynamics of single kinesin into a predictive theory for the motility of a cargo carried by an assembly of two kinesins. This phase addresses two research questions related to the intracellular mechanisms of cargo transport by two coupled kinesins.

This two-phase approach would enable us to investigate the intracellular cargo transport driven by single kinesin and by a team of two coupled kinesins over various loads and [ATP]. We show that while our theoretical frameworks are capable of describing the reasons of discrepancies among previous works, the models also provide results consistent with the resent experimental data along with testable predictions. Even though Yildiz and colleagues (2008) have shown that kinesin motor can step in the direction of the applied load in the absence of ATP, this thesis focuses on the ATP-driven cargo transport by kinesin motors to study the effect of [ATP] and loads on the dynamics of the transport. [ATP] is considered as a factor that affects the velocity of kinesin at various loads (Schnitzer et al. 2000; Visscher et al. 1999) and thus, it affects the duration of stepping cycles of kinesin. [ATP] is also proposed as a mean for temporal control of synthetic nanodevices powered by kinesin motor (Dinu et al. 2007; Wang and Manesh 2010).

3.4 Chapter Summary

This chapter detailed the issues in the dynamics of cargo transport by single kinesin or by two coupled kinesins. Then, a two-phase theoretical methodology based on
mathematical and computational models is proposed to provide answers to these issues. The first phase (in Chapters 4 and 5) focuses on the mechanism of cargo transport by single kinesin, whereas in the second phase (in Chapter 6) the motility of cargo carried by two coupled kinesins is investigated.
Chapter 4

Mathematical Modelling of Mechanical Kinetics of Kinesin Stepping

4.1 Introduction

This chapter describes the mathematical model proposed in this thesis to study the mechanical kinetics actuating individual stepping cycles of single kinesin. In Section 4.2, we develop our mathematical model and present analysis and discussion of the results. Section 4.3 summarises this chapter.

4.2 Mathematical Model

The mathematical model proposed in this section investigates the mechanical kinetics of ATP-driven individual stepping cycles of single kinesin motor at various loads and [ATP] at room temperature. To define individual forward and backward stepping cycles of kinesin motor, we use the simplest four-state discrete stochastic model reported by Fisher and Kolomeisky (2001) (see Scheme 2.2). The four-state discrete stochastic model contains all the biochemical kinetic states which have been experimentally observed in an individual stepping cycle of single kinesin (Milic et al. 2014). In accordance with the central task of theoretical models for molecular motors (Kolomeisky and Fisher 2007), the analysis of all kinetic states and their corresponding kinetic rates associated with kinesin stepping would provide us several new insights into the stepping mechanics of kinesins.
4.2.1 Probabilistic Modelling of Mechanochemicals

We start modelling the mechanochemicals of single kinesin stepping via studying the kinetic rates associated with the kinetic scheme of an individual stepping cycle of the motor. Equations (2.6) and (2.7) show that the forward and backward kinetic rates involved in an individual stepping cycle of kinesin change under different loads, [ATP], and temperatures. We expect this rate would affect the probabilities of triggering state transitions as well as the probability distribution over each kinetic state. We define the probabilities of forward and backward transitions as the ratio of kinetic rates as follows (Khataee and Liew 2014b):

\[
P(u_i) = \frac{u_i^{(F)}}{u_i^{(F)} + w_i^{(F)}}
\]

(4.1)

\[
P(w_i) = \frac{w_i^{(F)}}{u_i^{(F)} + w_i^{(F)}}
\]

(4.2)

where \( i = 0, 1, 2, 3 \), and \( u_i^{(F)} \) and \( w_i^{(F)} \) are given by Equations (2.6) and (2.7), respectively (see Scheme 2.2). To model the probabilities of forward and backward stepping of kinesin motor, we define the mean probabilities of forward and backward kinetic transitions as (Khataee and Liew 2014b):

\[
\langle P(u) \rangle = \frac{\sum_{i=0}^{3} P(u_i)}{4}
\]

(4.3)

\[
\langle P(w) \rangle = \frac{\sum_{i=0}^{3} P(w_i)}{4}
\]

(4.4)

where \( P(u_i) \) and \( P(w_i) \) are the probabilities of forward and backward transitions given by Equations (4.1) and (4.2), respectively. Figure 4.1 shows the mean probabilities of forward and backward kinetic transitions at loads over the force range \(-15 \text{ pN} \leq F \leq 15 \text{ pN}\). To be consistent with the experimental data and the theoretical studies in (Carter
2010; Kolomeisky and Fisher 2007; Liepelt and Lipowsky 2007; Nishiyama et al. 2002;
Taniguchi et al. 2005; Visscher et al. 1999; Yildiz et al. 2008), in this chapter, we
denote resisting and assisting forces by positive and negative signs, respectively. We
define load $F = -10$ pN as the supremum assisting load where at this point and beyond,$\langle P(u) \rangle > 0.99$ and $\langle P(w) \rangle < 0.01$. Similarly, the supremum resisting load is defined at
load $F = 32$ pN where at this point and beyond, $\langle P(w) \rangle > 0.99$ and $\langle P(u) \rangle < 0.01$ (data
are not shown). From Figure 4.1, we observe that with increasing load from assisting to
resisting, the mean forward transition probability $\langle P(u) \rangle$ decreases from almost 1 and
intersects with the mean backward transition probability $\langle P(w) \rangle$ at load between 7-8
pN, beyond which $\langle P(w) \rangle$ becomes dominant. Therefore, the stall force ($F_{stall}$) of
kinesin motor can be obtained by solving equation $\langle P(u) \rangle = \langle P(w) \rangle$ at various [ATP].
Our results show an almost constant and [ATP]-independent stall force of $F_{stall} \sim 7.3$ pN
(Khataee and Liew 2014b), in good agreement with the recent experimentally measured
stall force at 7.3 pN by Jamison et al. (2010) and with the observations of Nishiyama et
al. (2002) in which the probabilities of forward and backward stepping intersect at load
around 7-8 pN and gives $F_{stall} \sim 7.6$ pN. Our obtained stall force is also in agreement
with other experimental data indicating stall force between 5-7.6 pN (Carter and Cross
2005; Nishiyama et al. 2002; Visscher et al. 1999; Yildiz et al. 2008). The stall force
that we obtained here is also consistent with the experimental data by Carter and Cross
(2005) and Nishiyama et al. (2002) that stall force of kinesin motor is independent of
[ATP].
Figure 4.1. Mean probabilities of forward and backward kinetic transitions, $\langle P(u) \rangle$ and $\langle P(w) \rangle$ versus load at fixed [ATP] (Khataee and Liew 2014b).

Figure 4.1 also indicates that the mean probabilities of forward and backward transitions are almost [ATP]-independent. This is consistent with the observed [ATP]-independent but load dependent probabilities of forward and backward stepping by Nishiyama et al. (2002). It was reported that under assisting loads of $-15 \text{ pN} \leq F \leq -2 \text{ pN}$ only forward stepping was observed (Carter and Cross 2005), but with increasing resisting loads the probability of observing backward stepping increases (Nishiyama et al. 2002). These experimental observations agreed with our results in Figure 4.1 as over the load ranging from $-10 \text{ pN} \leq F \leq -2 \text{ pN}$, $\langle P(u) \rangle > 0.98$ and $\langle P(w) \rangle < 0.02$, while with increasing resisting load the probability $\langle P(w) \rangle$ increases. In addition, our calculations show that at loads over the range $0 \leq F \leq 8 \text{ pN}$ and at various [ATP], $\langle P(u) \rangle$ decreases from above 0.95 to about 0.48 (see Figure 4.1). This is in good agreement with the reported probabilities for forward stepping of 1 and 0.5 at the respective loads of $F = 0 \text{ pN}$ and $F = 8 \text{ pN}$ by Taniguchi et al. (2005). These agreements of our calculations with experimental findings indicate that the probabilities of forward and backward stepping of kinesin motor can be modelled well using the mean probabilities of forward and backward transitions defined in Equations (4.3) and (4.4), respectively (Khataee and Liew 2014b).
To further analyse the probabilities of forward and backward stepping of kinesin motor using kinetic transitions, we separately examine the probabilities of forward and backward kinetic transitions given by Equations (4.1) and (4.2) (see Figure 4.2). Since the forward and backward stepping processes of kinesin motor start from kinetic state [0] (Carter and Cross 2005; Clancy et al. 2011), we first study $P(u_0)$ and $P(w_0)$. Figure 4.2 shows that with increasing load from assisting to resisting at all [ATP], $P(w_0)$ is the first backward probability function that intersects its corresponding forward probability function $P(u_0)$. As only transition rates $u_0^{(f)}$ and $w_0^{(f)}$ are [ATP]-dependent, the minimum load at which $P(u_0)$ intersects with $P(w_0)$ at [ATP] = 5 µM is $F = 1.987$ pN (Figure 4.2A). At [ATP] = 100 µM and [ATP] = 2 mM, the crossover load points of $P(u_0)$ and $P(w_0)$ are found to be at $F = 2.754$ pN and $F = 4.034$ pN, respectively (Figure 4.2B and C). These results indicate that at limiting [ATP] the probability of forward stepping is almost equal to 1 when the applied load is lower than 1.987 pN, because at loads $F < 1.987$ pN all the probabilities $P(u_i)$ are greater than $P(w_i)$, $i = 0, 1, 2, 3$ (see Figure 4.2 A). Similarly, at high [ATP] almost all steps are made in the forward direction when the applied load is lower than 4.034 pN (Khataee and Liew 2014b) (see Figure 4.2 C). This is in good agreement with the experimental observations of Nishiyama et al. (2002) that at loads lower than 4 pN the probability of forward stepping is almost equal to 1. The crossover load points of probabilities of [ATP]-independent kinetic transitions are also calculated at loads: (i) $F = 6.515$ pN for $P(u_3)$ and $P(w_3)$, (ii) $F = 8.795$ pN for $P(u_1)$ and $P(w_1)$, and (iii) $F = 17.102$ pN for $P(u_2)$ and $P(w_2)$. However, we are not able to relate these crossover load points with the available experimental data because in current experiments, [ADP] and [P_i] are not separately measured (Fisher and Kolomeisky 2001).
We further examine \( P(u_i) \) and \( P(w_i) \) (where \( i = 0, 1, 2, 3, 4 \)) at \( F > F_{\text{stall}} \), where the backward steps are dominant. Our calculations show that \( P(w_0) > 0.97 \) and \( P(u_0) < 0.03 \) at load range \( F > F_{\text{stall}} \). This implies that the backward stepping of kinesin motor starts when the probability of ADP binding to the motor is much higher than that of ATP binding (Khataee and Liew 2014b). Figure 4.2 also shows that the probability of ATP hydrolysis, \( P(u_1) \), is higher than that of ATP synthesis, \( P(w_2) \), at loads \( F_{\text{stall}} < F < 13 \) pN. At this load range, Figure 4.1 indicates that the backward stepping of the kinesin motor is more probable than forward stepping. This result suggests that the backward stepping of kinesin motor is related to ATP hydrolysis, as experimentally observed in (Carter and Cross 2005; Nishiyama et al. 2002). To further investigate this finding, we compute the kinetic rates at loads \( F > F_{\text{stall}} \) using Equations (2.6) and (2.7) (see Figure 4.3A). Our calculation shows that with increasing resisting load the rates of backward kinetic transitions \( w_i^{(F)} \) increase and crossover their corresponding forward kinetic transition rates \( u_i^{(F)} \) at loads \( F \leq 10 \) pN (where \( i = 0, 1, 3 \)), except for \( w_2^{(F)} \). The rate \( w_2^{(F)} \) crossovers \( u_2^{(F)} \) at \( F \sim 17.1 \) pN. Due to the more frequent detachment of kinesin motor from MT at loads above 10 pN (Carter and Cross 2005), our results in Figure 4.3A indicate ATP synthesis rate as the rate limiting factor for ATP synthesizing cycle. Figure 4.3A and B also indicates that at loads \( F_{\text{stall}} < F \), where the backward stepping of kinesin is dominant, the rate of ATP hydrolysis \( u_1^{(F)} \) is at least 17 times greater than that of ATP synthesis, \( w_2^{(F)} \). Collectively, these results imply that the backward stepping of
single kinesin is mainly related to ATP hydrolysis as in forward stepping (Khataee and Liew 2014b) which is consistent with the experimental and theoretical findings in (Carter and Cross 2005; Liepelt and Lipowsky 2007; Nishiyama et al. 2002). The small rate of ATP synthesis in our analysis, nevertheless, supports the theory in (Fisher and Kim 2005; Fisher and Kolomeisky 2001; Kolomeisky and Fisher 2007) that backward stepping could resynthesize ATP (Khataee and Liew 2014b).

**Figure 4.3. Kinetic rates for the ATP hydrolysis and ATP synthesis cycles (Khataee and Liew 2014a).** Transition rates $u_i^{(F)}$ and $w_i^{(F)}$ at force $F$ are depicted as $u_i$ and $w_i$, respectively, where $i = 0, 1, 2, 3$. (A) Kinetic rates calculated at loads $F_{\text{stall}} < F \leq 10$ pN and $[\text{ATP}] = 100$ µM. (B) ATP hydrolysis and ATP synthesis rates, i.e. $u_1$ and $w_2$ respectively, versus load. ATP synthesis rate gradually increases and crossovers ATP hydrolysis rate at load ~ 19.4 pN.

Probabilistic modelling of kinetic states involved in an individual stepping cycle of kinesin would also provide several new insights into the mechanochemicals of kinesin stepping. We define the stationary probability distribution over each kinetic state of kinesin motor using the Hill’s diagram method (Kurka and Dvorak 1982), where a kinetic scheme is represented as a graph. Let $G$ be a connected and directed graph with vertex set $V(G)$ and edge set $E(G)$ which represent states and transitions of the four-state kinetic scheme of kinesin motor, respectively. The edge weights of $G$ correspond to kinetic rates of the scheme. The stationary distribution of probability over a vertex $v \in V(G)$ is defined as (Khataee and Liew 2014b):
\[ P_v = \frac{\sum_v}{\sum_{\text{total}}} \] (4.5)

where \( \sum_v \) is the sum of products of all directional diagrams of vertex \( v \) and \( \sum_{\text{total}} \) is the sum of all \( \sum_v \) defined as (Khataee and Liew 2014b):

\[ \sum_v = \sum\{ \Lambda_D | D \text{ is a directional diagram of } v \} \] (4.6)

\[ \Lambda_D = \prod\{ e(m, n) | \{ m, n \} \in V(D) \} \] (4.7)

where \( \Lambda_D \) is the product of edge weights of a directional diagram \( D \) and \( e(m, n) \) is the edge weight between vertices \( m \) and \( n \). \( D \) is defined as a directional diagram of a vertex \( v \) if for any vertex \( x \in V(D) \) and \( x \neq v \), there is exactly one path from \( x \) to \( v \). Equation (4.5) enables us to compute the stationary distribution of probability over the kinetic states of kinesin by obtaining the directional diagrams of the states. We derive the directional diagrams of four kinetic states using the definition of directional diagram given in Equations (4.5)-(4.7) (see Figure 4.4).

**Figure 4.4.** Directional diagrams of kinetic states [0] to [3] depicted in parts (A-D), respectively (Khataee and Liew 2014b). For clarity, the transitions rates \( u_i^F \) and \( w_i^F \) are depicted as \( u_i \) and \( w_i \) respectively, where \( i = 0, 1, 2, 3 \).

For example, Figure 4.4A shows four directional diagrams of kinetic state [0], which result in four products and their sum as follows:
\[ \Lambda_0 = \{ w_3 w_2 w_1, u_1 u_2 u_3, u_2 u_3 w_1, u_3 w_2 w_1 \} \] (4.8)

\[ \sum_0 = (w_3 w_2 w_1 + u_1 u_2 u_3 + u_2 u_3 w_1 + u_3 w_2 w_1) \] (4.9)

Similarly, \( \Sigma_i \) and \( \prod_i \) (where \( i = 1, 2, 3 \)) are obtained from Figure 4.4B–D, respectively. \( \Sigma_{\text{total}} \) is the sum of all 16 products obtained from four states (Khataee and Liew 2014b):

\[ \Sigma_{\text{total}} = \Sigma_0 + \Sigma_1 + \Sigma_2 + \Sigma_3 \] (4.10)

Therefore, we define the distribution of probability over four kinetic states ([0] to [3]) as follows (Khataee and Liew 2014b):

\[ P_0 = \frac{(w_3 w_2 w_1 + u_1 u_2 u_3 + u_2 u_3 w_1 + u_3 w_2 w_1)}{\Sigma_{\text{total}}} \] (4.11)

\[ P_1 = \frac{(u_0 w_3 w_2 + w_0 w_3 w_2 + u_0 u_2 u_3 + u_3 u_0 w_2)}{\Sigma_{\text{total}}} \] (4.12)

\[ P_2 = \frac{(u_0 u_1 w_3 + u_3 u_0 u_1 + w_1 w_0 w_3 + u_1 w_0 w_3)}{\Sigma_{\text{total}}} \] (4.13)

\[ P_3 = \frac{(u_0 u_1 u_2 + w_2 w_1 w_0 + u_2 w_1 w_0 + u_1 u_2 w_0)}{\Sigma_{\text{total}}} \] (4.14)

where, the transition rates \( u_i \) and \( w_i \) are given by Equations (2.6) and (2.7) and \( \Sigma_{\text{total}} \) is calculated by Equation (4.10). The distribution of probability over the four kinetic states is plotted in Figure 4.5. As it can be seen from Figure 4.5A, at low [ATP] and low loads the distribution of probability in kinetic state [0] is the highest. This is consistent with experimental findings at limiting [ATP], showing that kinesin is paused at kinetic state [0], waiting for ATP binding (Clancy et al. 2011; Hackney 1994). Figure 4.5A and B shows that the distribution of probability in kinetic state [0] peaks at maximum points of 0.93 and 0.43 at loads \( F = 1.987 \) pN and \( F = 2.754 \) pN, respectively, coincide with the
crossover load points of $P(u_0)$ and $P(w_0)$ at [ATP] = 5 µM and 100 µM. These results imply that the probability of observing kinesin motor in kinetic state [0] will be the highest when the probabilities of ATP and ADP binding to the motor are equal, i.e., $P(u_0) = P(w_0)$. With increasing [ATP] at low loads, the distribution of probability in kinetic states [2] and [3] becomes dominant (see Figure 4.5C). Moreover, at high resisting loads above 10 pN and at all [ATP], the distribution of probability in kinetic states [2] and [3] is greater than that of other kinetic states. This suggests [2] and [3] as the first and second most probable kinetic states that kinesin motor stays at loads above 10 pN (Khataee and Liew 2014b), in agreement with the reported kinetic states for the detachment of kinesin from MT (Schliwa 2003; Seitz and Surrey 2006).

Figure 4.5. Probability distribution over the four kinetic states $[i]$, $P_i$ where $i = 0, 1, 2, 3$, versus load at fixed [ATP](Khataee and Liew 2014b).

4.2.2 Temporal Analysis of Kinetics

We expect the alteration of kinetic rate would change the mean waiting time of kinesin over each kinetic state $[i]$, where $i = 0, 1, 2, 3$ (see Scheme 2.2). Obviously, the mean waiting time of kinesin in kinetic states should agree with the probability distribution over the states. To define the mean waiting time in kinetic states, we use the theory reported by Leibler et al. (1993) in which the kinetic transition rates have reverse relationship with their durations:

$$k_{ij} = \frac{1}{t_{ij}}$$  \hspace{1cm} (4.15)
where $k_{ij}$ and $t_{ij}$ are the transition rate and duration of the transition, respectively, from a kinetic state $i$ to a kinetic state $j$. According to Equation (4.15) and the transition rates in the four-state discrete stochastic, the mean waiting time in kinetic state $[i]$ is defined as (Khataee and Liew 2014b):

$$\langle t_i \rangle = \frac{1}{u_i^{(F)} + w_i^{(F)}}$$

Equation (4.16) enables us to model the mean waiting time of kinesins over the four kinetic states during an individual stepping cycle over different loads and [ATP] (see Figure 4.6). Our results show that at [ATP] = 5 µM and low loads the mean waiting time in kinetic state [0] is greater than that of other states and peaks at a maximum of 0.1055 s at $F = 1.148$ pN in comparison with a maximum of 0.0063 s at $F = 2$ pN and [ATP] = 100 µM (see Figure 4.6A and B). This is consistent with the obtained results of the probability distribution over kinetic state [0] and confirms that at limiting [ATP] kinesin mainly stays in kinetic state [0], waiting for ATP binding (Clancy et al. 2011; Hackney 1994).

It has been observed by Schnitzer et al. (2000) that at limiting [ATP] the probability of detachment of kinesin motor from MT in kinetic state [0] increases with the increase in the duration of this state, while at high [ATP] the probability of detachment from kinetic state [0] diminishes. Our results in Figure 4.6A and B indicates that the load points that maximizes $<t_0>$ are close to the crossover load points of $P(u_0)$ and $P(w_0)$ at [ATP] = 5 µM and 100 µM shown in Figure 4.2A and B. This indicates that at low [ATP] the detachment probability from kinetic state [0] is the highest when the probabilities of ATP and ADP binding to the motor are close (Khataee and Liew 2014b). It also confirms our earlier results about the probability distribution over kinetic state [0] shown in Figure 4.5A and B. Figure 4.6C also shows that at high [ATP] and low loads, the ATP binding rate rises which leads to a reduction in the duration of kinetic state [0].
and an increase in the durations of kinetic states [2] and [3], in agreement with the obtained results of the probability distribution over kinetic states [2] and [3] shown in Figure 4.5C. These findings also agree with the findings of Schnitzer and co-workers (2002) that at high [ATP] the detachment probability from kinetic state [0] diminishes. Accordingly, our results suggest states [2] and [3] as the most probable kinetic states for the detachment of kinesin motor from MT at high [ATP] and low loads (Khataee and Liew 2014b), in agreement with the reported kinetic states for the detachment of kinesin from MT (Hackney 2002; Schliwa 2003; Seitz and Surrey 2006; Yajima et al. 2002).

![Figure 4.6. Mean waiting time of kinesin over the kinetic states \( \langle i \rangle \), \( \langle t_i \rangle \) where \( i = 0, 1, 2, 3 \), versus load at fixed [ATP] (Khataee and Liew 2014b).](image)

To explain the higher durations of kinetic states [2] and [3] over high resisting loads, we analyse their respective forward and backward kinetic transitions. As we discussed in analysing the probabilities of [ATP]-independent kinetic transitions, with increasing load from assisting to resisting (Khataee and Liew 2014b): (i) forward rates \( u_2^{(F)} \) and \( u_3^{(F)} \) fall equally, as they have the same parameters (see Table 1.1), and (ii) backward rate \( w_2^{(F)} \) rises with a rate much smaller than \( w_3^{(F)} \) (see equations (2.6) and (2.7)). Thus, according to Equation (4.16), the slow rise of ATP synthesis rate, i.e. \( w_2^{(F)} \), is the reason that \( \langle t_2 \rangle \) attains its maximum at higher loads and at a time that is higher than that of \( \langle t_3 \rangle \). Figure 4.6 indicates \( F = 10.946 \) pN as the load point that the mean waiting time in kinetic state [2] is the highest, \( \langle t_2 \rangle = 0.0046 \) s for all [ATP], due to the slow rise of
This implies that at high resisting loads $F > F_{\text{stall}}$ the rate limiting factor of kinesin’s backward stepping is ATP synthesis (Khataee and Liew 2014b), confirming our results about kinetic calculations shown in Figure 4.3A.

To compare the duration of kinetic state [2] with that of other kinetic states at load $F = 10.946$ pN, we compute the ratio of $<t_2> = 0.0046$ s to $<t_i>$ using Equation (4.16), where $i = 0, 1, 3$ (see Table 4.1). Table 4.1 shows that the duration of kinetic state [2] is at least 63 times greater than that of kinetic state [0] at load $F = 10.946$ pN and [ATP] = 5 µM. With increasing [ATP], the duration of kinetic state [0] decreases, which leads to a rise in the ratio of $<t_2>$ to $<t_0>$. As the forward and backward kinetic transitions of states [1] and [3] are [ATP]-independent, the ratio of the duration of kinetic state [2] to those of kinetic states [1] and [3] at load $F = 10.946$ pN are constant, at 5.067 and 4.200, respectively (see Table 4.1). Hence, in accordance with the observations of Schnitzer et al. (2000), our results in Table 4.1 suggest $F = 10.946$ pN as the load point where the probability of detachment of kinesin motor from MT in kinetic state [2] is the highest (Khataee and Liew 2014b). This is consistent with the common detachment load $> 10$ pN observed by Carter and Cross (2005). As we showed that at high resisting loads the long mean waiting time in kinetic state [2] is due to the low transition rate $w_{2}^{(F)}$, this low rate of ATP synthesis can be proposed as a reason for the detachment of kinesin motor from MT in kinetic state [2] (Khataee and Liew 2014b). Furthermore, Figure 4.6B and C shows that at loads $F > 10.946$ pN the mean waiting time in state [2] decreases because of the fast rise of ATP synthesis rate, $w_{2}^{(F)}$. Thus, it can be deduced that with an increase in the rate of ATP synthesis at high resisting loads $F > F_{\text{stall}}$, the long duration of being in kinetic state [2] would decrease which could lead to the reduction of the detachment probability of kinesin motor from MT. These results and
the results obtained by separately examining the probabilities of [ATP]-independent
ekineti c transitions (in Figure 4.2) concludes that the backward stepping of kinesin motor
is related to both ATP synthesis and hydrolysis (Khataee and Liew 2014b).

Table 4.1. Ratio of $<t_2>/ <t_i>$ at $F = 10.946$ pN, where $i = 0, 1, 3$ (Khataee and Liew
2014b).

<table>
<thead>
<tr>
<th>[ATP] (µM)</th>
<th>$&lt;t_2&gt;$ / $&lt;t_i&gt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$i = 0$</td>
</tr>
<tr>
<td>5</td>
<td>63.654</td>
</tr>
<tr>
<td>100</td>
<td>541.704</td>
</tr>
<tr>
<td>2000</td>
<td>2599.708</td>
</tr>
</tbody>
</table>

4.3 Chapter Summary

This chapter presented a novel but simple mathematical model proposed to study the
mechanical kinetics of kinesin stepping by analysing the kinetics of single stepping
cycle of the motor. The main findings of this chapter are summarized as follows
(Khataee and Liew 2014b): (i) the stall force of kinesin is [ATP] independent and equal
to ~ 7.3 pN, and (ii) even though backward stepping of kinesin is related to both ATP
hydrolysis and ATP synthesis pathways, backward stepping tends to be powered by
ATP hydrolysis, rather than ATP synthesis.
Chapter 5

Kinesin Processivity Simulation

5.1 Introduction

This chapter describes the computational models proposed in this thesis to simulate the processivity and information processing unit of single kinesin motor. In Section 5.2, we propose a stochastic algorithm for simulating kinesin processivity. Then, in Section 5.3, we design a computational model representing the information processing unit actuating kinesin stepping cycles. The simulation results of the models are also discussed in this section. Section 5.4 concludes this chapter.

5.2 Simulating Kinesin Processivity

In this section, we propose a stochastic algorithm to simulate the processivity of single kinesin by modelling its cellular interactions during an individual stepping cycle. To define individual stepping cycles of kinesin, we use the four-state discrete stochastic model reported by Fisher and Kolomeisky (2001) as well as the results of our mathematical model discussed in the previous chapter. First, we discuss the theoretical foundation of the simulation algorithm. Then, the cellular interactions during an individual stepping cycle are modelled as a stochastic automaton used to develop the simulation algorithm for calculating the mean run length of single kinesin under various loads and [ATP].
5.2.1 Theoretical Foundation

Schnintzer et al. (2000) modelled kinesin processivity using an energy landscape formalism and predicted that kinesin motility should remain tightly coupled to ATP hydrolysis even under load. Nevertheless, the four-state discrete stochastic model, derived from this energy model, describes the dynamics of kinesin stepping by both the ATP hydrolysis and ATP synthesis pathways at various [ATP] and loads (Fisher and Kim 2005; Fisher and Kolomeisky 2001; Kolomeisky and Fisher 2007). Since the four-state discrete stochastic model defines both forward and backward stepping cycles, it can be used to count the net number of steps taken by the motor before becoming inactive. Therefore, we can integrate our results obtained by mathematically modelling of mechanical kinetics of kinesin stepping to simulate the processivity of kinesin. We simulate the processivity of single kinesin by computing the number of ATP hydrolysis and ATP synthesis kinetic cycles and thus, the mean number of steps taken by the motor before detachment from MT at different settings of [ATP] and load.

Kinesin has shown a small probability of detachment per mechanical step (Block et al. 1990; Schliwa 2003). The detachment of kinesin from MT in each stepping cycle is also an independent event and does not depend on any previous stepping cycles. Thus, the mean number of forward steps derived from ATP hydrolysis before detachment of kinesin from MT is a geometric distribution given by (Khataee and Liew 2015):

\[ \langle n_h \rangle = \frac{1}{P_{off}} \]  

(5.1)
where $P_{\text{off}}$ is the detachment probability of kinesin from MT per mechanical step. Since kinesin dissociates from MT after each single run, the detachment probability of the motor from MT per step in relation with the mean run length is exponentially distributed as follows (Yajima et al. 2002):

$$P_{\text{off}} = (1 - \exp(-\frac{d}{L}))$$ (5.2)

where $d$ is the step size and $L$ is the mean run length given by Equation (2.1). To obtain the mean number of steps taken by kinesin toward MT plus end per encounter with MT, the mean number of backward steps derived from ATP synthesis should be deducted from $\langle n_b \rangle$. We define the mean number of backward steps derived from ATP synthesis before detaching of kinesin from MT as follows (Khataee and Liew 2015):

$$\langle n_s \rangle = \langle n_b \rangle \times P_s$$ (5.3)

where $P_s$ is the normalised proportion showing the ratio of ATP synthesis pathway to the ATP hydrolysis pathway defined as follows (Khataee and Liew 2015):

$$P_s = \frac{w_3^{(F)}}{u_2^{(F)} + w_3^{(F)}}$$ (5.4)

where $u_2^{(F)}$ and $w_3^{(F)}$ are given by Equations (2.6) and (27). Thus, the mean number of steps taken by kinesin toward MT plus end per encounter with MT is defined as follows:

$$\langle n \rangle = \langle n_b \rangle - \langle n_s \rangle$$ (5.5)

Our theoretical predictions for the processivity of kinesin motor at different [ATP] and loads are obtained from Equation (5.5) (Figure 5.1). Figure 5.1A and B shows that the processivity of kinesin motor exponentially declines with increasing load or decreasing
[ATP] which is in agreement with previous findings in Liepelt and Lipowsky (2007) and Schnitzer et al. (2000). Experimental data in Schnitzer et al. (2000) showed that at low loads and high [ATP], the mean run length $L$ was $\sim 600$ nm. $L$ fell to $\sim 300$ nm when the [ATP] was reduced to 5 µM, and to $\sim 80$nm when load was increased to 5 pN. We show that the computed mean run length, i.e. $<RL>$, is comparable with the experimentally obtained $L$, where $<RL>$ is almost equal to: $\sim 601$ nm at [ATP] = 1000 µM and $F = 1.1$pN, $\sim 300$ nm at [ATP] = 5 µM and $F = 2.4$pN, and $\sim 80$nm when load increases to 5pN at [ATP] $\sim 1$ µM (Khataee and Liew 2015). Results in Figure 5.1A and B also agree with the findings of Liepelt and Lipowsky (2007) in which a 7-state kinetic scheme, based on several motor kinetic cycles, was used to calculate $<RL>$.

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**Figure 5.1.** Mean run length ($<RL>$) and mean backward displacement powered by ATP synthesis ($<BD>$) (Khataee and Liew 2015). $<RL>$, calculated as $<n> \times d$ (see Equation (5.5)) where $d \sim 8.2$ nm, as a function of (A) [ATP] at various resisting load forces $F$ and (B) load at different [ATP]. $<BD>$ calculated as $<n_s> \times d$ (see Equation (5.3)) against (C) [ATP] at different $F$ and (D) load at various [ATP].

At high [ATP], Figure 5.1C and D indicates that the mean backward displacement ($<BD>$) powered by ATP synthesis decreases with rising load. This implies that the falling of $<RL>$ with rising load at high [ATP] observed in Figure 5.1B could only be
due to the rising frequency of backward stepping powered by ATP hydrolysis (Khataee and Liew 2015). This is in agreement with the experimental observations in Carter and Cross (2005) and Nishiyama et al. (2002) and the theoretical results in Khataee and Liew (2014) and Liepelt and Lipowsky (2007) that backward stepplings are mainly generated by ATP hydrolysis at high [ATP]. At limiting [ATP], backward stepping powered by ATP hydrolysis has low frequency due to low [ATP]. At low [ATP], Figure 5.1D shows that $<BD>$ has rising trend, indicating that ATP is increasingly likely to be synthesized during backward movement (Khataee and Liew 2015), as reported in Fisher and Kim (2005), Fisher and Kolomeisky (2001) and Kolomeisky and Fisher (2007). However, the slower falling rate of $<RL>$ at limiting [ATP] than at high [ATP] in Figure 5.1B indicates that the rate of backward stepping powered by ATP synthesis at limiting [ATP] is lower than the rate of backward stepping powered by ATP hydrolysis at high [ATP] (Khataee and Liew 2015). This supports the observations that backward stepplings are more likely to be generated by ATP hydrolysis than by ATP synthesis when [ATP] is not limiting, and that the frequency of backward stepping rises with [ATP] (Carter and Cross 2005). Figure 5.1C further shows that the rise of $<BD>$ slows down with increasing [ATP], implying that backward stepping powered by ATP synthesis is more likely to happen with limiting [ATP]. Together, these theoretical analyses indicate that the processivity of kinesin can be determined by the number of ATP hydrolysis and ATP synthesis kinetic cycles taken by the motor before becoming inactive (Khataee and Liew 2015).

### 5.2.2 Stochastic Timed Automaton Model

To simulate how the cellular interactions of kinesin affect its processivity, we abstract an important set of its cellular interactions as a parametric probabilistic timed automaton (Alur and Dill 1994; Alur et al. 1993; Beauquier 2003; Sproston and Troina 2010), and
construct the automaton model shown in Figure 5.2 with $M_{\text{kinesin}} = (S, s_{\text{initial}}, s_{\text{final}}, I, C, tran, inv, A_s, EC_s)$. The probabilistic timed automata (PTA) are mathematical models augmented with time and discrete probability distributions for modelling the temporal and stochastic properties of stochastic discrete-event systems (Beauquier 2003; Kwiatkowska et al. 2002; Kwiatkowska et al. 2004). The $M_{\text{kinesin}}$ model simulates the ATP hydrolysis and the ATP synthesis kinetic pathways that resulted in the processivity of kinesin. The set $S = \{s_{\text{off}}, H/S\}$ in Figure 5.2A denotes the states of $M_{\text{kinesin}}$ where $s_{\text{off}}$ corresponds to the kinetic state in which kinesin is detached from MT and state $H/S$ stand for an ATP hydrolysis ($H$) or synthesis ($S$) kinetic cycle. State $H/S$ is composed of $H$ and $S$ cycles connected by a state $s_0$ defined to determine which cycle is randomly chosen to be executed. Each $H$ or $S$ cycle is composed of states $s_i$ ($i = 1, 2, 3, 4$) which stand for the kinetic states $[i-1]$ of the four-state discrete stochastic model (see Figure 5.2B and Scheme 2.2). $s_{\text{initial}} = \{s_{\text{off}}\} \subset S$ and $s_{\text{final}} = \{s_{\text{off}}\} \subset S$ are the initial and final states of $M_{\text{kinesin}}$, respectively, because, in vivo, kinesin mainly exists as soluble motor where its activity is switched off and the motor is autoinhibited from binding to and moving on MTs (Kaan et al. 2011; Verhey and Rapoport 2001). The input set $I = \{E, T, D, P, E', T', D', P'\}$denotes the events $E, T, D$ and $P$, which stand for the presence of enabling condition of kinesin (e.g. low external loads, the presence of MT and the absence of regulatory factors), ATP binding, ADP binding and Pi binding, respectively. The complement of an event (i.e. $E'$, $T'$, $D'$ or $P'$) represents the opposite molecular role of that event. To express the temporal properties of kinesin mechanism, we define the clock set $C = \{x\}$ which contains a non-negative real-valued variable (i.e. $x \in \mathbb{R}^+$) that increases at the same rate as real time. Clock $x$ indicates the time elapsed in $M_{\text{kinesin}}$ states, corresponding to the mean waiting time in kinetic states of kinesin. The set $tran$ of transitions which are tuples of the form $e_s = (s, p, a, \dot{C}, s')$ representing a transition
from state $s$ to state $s'$ with probability $p$, event $a \in I \cup \{r\}$ and set $\mathcal{C} \subseteq C$ of clocks which are reset to 0 by transition $e_s$, where $r$ denotes null event.

Figure 5.2. The automaton model, $M_{\text{kinesin}}$ (Khataee and Liew 2015). (A) General representation of $M_{\text{kinesin}}$. $M_{\text{kinesin}}$ starts with incoming unlabeled arrow to state $s_{\text{off}}$ with clock $x$ initialized to 0. As the invariant condition of $s_{\text{off}}$ is true, the control stays in state $s_{\text{off}}$ until perceiving input $E$. By sensing input $E$, $s_{\text{off}}$ changes to state $H/S$ and clock $x$ is reset to 0. In state $H/S$, one of ATP hydrolysis or synthesis pathway is randomly chosen to run or $M_{\text{kinesin}}$ becomes inactivated with probability $P_{\text{off}}$. (B) Detailed representation of $M_{\text{kinesin}}$. By sensing input $E$, ATP hydrolysis or synthesis pathway is chosen to run with probability $P_h$ or $P_s$, respectively. Then, the value of clock $x$ increases uniformly with time. When $x$ is equal to $<t_1>$ the invariant condition $x \leq <t_1>$ requires that the action with event $T$ or $D$ must be taken. Alternatively, if $x$ exceeds $<t_1>$ then the action with event label $E'$ may be taken with probability 1. These behaviors correspond to the attachment of kinesin to MT in the absence of regulatory factors and the start of stepping initiated with kinetic state changing $[0] \xrightarrow{H} [1]$ or $[0] \xrightarrow{W} [3]$. However, if $[ATP]$ is limiting then the motor detaches from MT due to long pausing. For clarity of the graph, transitions of a single action are connected with a dotted line. Also, actions $a_{i3}$ ($i = 1, 2, 3, 4$) are not shown.

The function $\text{inv}: S \rightarrow \zeta$ is defined to assign to each state of $M_{\text{kinesin}}$ an invariant condition from the set $\zeta$ of clock constraints. A state stays invariant, i.e., no change,
when the constraint is true. The invariant condition of state $s_{off}$ is assigned as true because, in vivo, kinesin mainly stays in the detached state without time constraint. As state $s_0$ is defined to determine the kinetic pathway, its invariant condition is assigned as true to show no time constraint. For states $s_i$ ($i = 1, 2, 3, 4$), we assign invariant conditions $x \leq <t_i>$ where clock $x \in C$ and $<t_i>$ denotes the mean wait time of kinesin in kinetic states $[i - 1]$ given by Equation (4.16). The invariant condition of state $H/S$ in Figure 5.2A is defined as the $H$ or $S$ cycle completion time as follows (Khataee and Liew 2015):

$$\langle T_{total} \rangle = \sum_{i=1}^{4} \langle t_i \rangle$$

(5.6)

where $<t_i>$ is given by Equation (4.16). To associate a non-empty set of finite discrete probability distributions with each state $s_i$ ($i = off, 0, 1, 2, 3, 4$), we define action sets $A_i = \{a_{i1}, \ldots, a_{ij}\}$ such that (Khataee and Liew 2015):

$$\sum_{e_i} p(a_{ij}, e_i) = 1$$

(5.7)

where $p(a_{ij}, e_i)$ is the probability of using the edge $e_i$ from source state $s_i$ if the action $j$ has been chosen and $j$ is the number of actions associated with state $s_i$. Two actions $a_{off1}$ and $a_{off2}$ are associated with state $s_{off}$, i.e. $A_{off} = \{a_{off1}, a_{off2}\}$, representing staying in detached state of kinesin and the attachment of kinesin to MT, respectively. For transitions from state $s_0$ to the ATP hydrolysis or synthesis pathway, a single action $a_{01}$ is assigned to with probabilities $P_{s_i}$ in Equation (5.4), and $P_h$ (Khataee and Liew 2015):

$$P_h = \frac{\mu_1^{(F)}}{\mu_1^{(F)} + w_2^{(F)}}$$

(5.8)

where $\mu_1^{(F)}$ and $w_2^{(F)}$ are given by Equations (2.6) and (2.7), and $P_h$ is the normalized ratio of ATP hydrolysis pathway to ATP synthesis pathway. Three actions are associated with each state $s_i$ ($i = 1, 2, 3, 4$), i.e. $A_i = \{a_{i1}, a_{i2}, a_{i3}\}$: $a_{i1}$ and $a_{i2}$ correspond
to the forward and backward kinetic transitions from kinetic state \([i - 1]\) and \(a_{i3}\) expresses the detachment of kinesin from MT in kinetic state \([i - 1]\) or staying in kinetic state \([i - 1]\). To compute the probability of actions \(a_{i3}\), we distribute \(P_{\text{off}}\), given by Equation (5.2), to states \(s_1\) to \(s_4\) according to the probability of being in each state. Therefore, the inactivation probabilities of \(M_{\text{kinesin}}\) in states \(s_i\) \((i = 1, 2, 3, 4)\) per \(H\) or \(S\) cycle, corresponding to the detachment probabilities of kinesin from MT in kinetic states \([i – 1]\) per mechanical step, are defined as follows (Khataee and Liew 2015):

\[
P_{\text{off}}(s_i) = P_{\text{off}} \times P_{i-1} \tag{5.9}
\]

where \(P_{i-1}\) is the probability distribution over kinetic states \([i – 1]\), in Scheme (2.2), defined by Equations (4.11)-(4.14). Finally, the function \(EC_i: a_{ij} \rightarrow \zeta\) is defined to assign an enabling condition to each action \(a_{ij} \in A_{i}\), where \(i = \text{off}, 0, 1, 2, 3, 4\) and \(j\) is the number of actions associated with state \(s_i\). An enabling condition allows the corresponding action to be triggered. The enabling condition of actions associated with state \(s_{\text{off}}\) is assigned as true, \(EC_{\text{off}}: a_{\text{off}, 1}, a_{\text{off}, 2} \rightarrow \text{true}\), meaning that these actions can be triggered at any time. This resembles the cellular mechanism of kinesin which can stay unattached or attached to MT without time constraint. The enabling condition of action \(a_{01}\) associated with state \(s_0\) is also assigned as true, i.e. \(EC_0: a_{01} \rightarrow \text{true}\), meaning that upon the attachment of kinesin to MT, ATP hydrolysis or synthesis pathway can be taken randomly without time constraint. The enabling conditions of actions associated with states \(s_1\) to \(s_4\) and resemble forward or backward kinetic transition are assigned as \(x = <t_i>\), where \(x \in C\) and \(<t_i>\) is computed using Equation (4.16). These conditions enable transitions to be triggered when the mean wait times in their corresponding states are met. For the third actions of states \(s_1\) to \(s_4\), \(a_{i3}\) \((i = 1, 2, 3, 4)\), the enabling condition is assigned as true, meaning that the detachment of kinesin from MT in kinetic state \([i - 1]\) or staying in kinetic state \([i - 1]\) has no time constraint.
5.2.3 Simulation Algorithm and Discussion

To simulate the processivity of kinesin using $M_{\text{kinesin}}$ model at various [ATP] and loads, the function of $M_{\text{kinesin}}$ is analyzed over its states, according to the principles of PTA, where the behavior of a system is modelled as a sequence of system states (or events) called a trace (Alur and Dill 1994). Accordingly, to simulate the processivity of kinesin, we compute the number of the following two traces completed in each run of $M_{\text{kinesin}}$ (Khataee and Liew 2015):

\[
T(h): s_0 \rightarrow s_1 \rightarrow s_2 \rightarrow s_3 \rightarrow s_4 \rightarrow s_0
\]
\[
T(s): s_0 \rightarrow s_1 \rightarrow s_4 \rightarrow s_3 \rightarrow s_2 \rightarrow s_0
\]

where $T(h)$ and $T(s)$ traces correspond to the stepping cycle derived from ATP hydrolysis and ATP synthesis kinetic cycles, respectively. The simulation algorithm for each force and [ATP] setting is as follows:

---

**Algorithm 5.1. Pseudo-code of the algorithm simulating kinesin processivity.**

**Inputs:** $F$ and [ATP].  
**Outputs:** $<RL>$ and $<BD>$.  
1: \textbf{for} Run = 1 to 10000  
2: \hspace{1em} attach kinesin to MT.  
3: \textbf{while} kinesin is attached to MT  
4: \hspace{1em} \textbf{if} kinesin is in kinetic state [0]  
5: \hspace{2em} Randomly select either ATP hydrolysis (i.e. trace $T(h)$) or synthesis cycle (i.e. trace $T(s)$), using Equations (5.4) and (5.8).  
6: \hspace{2em} \textbf{end if}  
7: \hspace{1em} Randomly determine either kinesin detaches from or stays attached in each kinetic state $[i]$ in the chosen cycle, where $i = 0$ to 3, using Equation (5.9).  
8: \textbf{end while}  
9: \hspace{1em} Add up the number of ATP hydrolysis cycles, i.e. $|T(h)|$.  
10: \hspace{1em} Add up the number of ATP synthesis cycles, i.e. $|T(s)|$.  
11: \textbf{end for}  
12: \hspace{1em} Calculate the mean values of $|T(h)|$ and $|T(s)|$, as $<n_h>$ and $<n_s>$, respectively.  
13: \hspace{1em} Calculate $<n>$ using Equations (5.5).  
14: \hspace{1em} Calculate $<RL> = <n> \times d$ and $<BD> = <n_s> \times d$, where $d$ is the step size of Single kinesin.
As Algorithm 5.1 shows, in each run, $M_{\text{kinesin}}$ starts in state $s_{\text{off}}$, transits stochastically between $H$ or $S$ cycles by executing $T(h)$ and $T(s)$ traces until it becomes randomly inactivated in state $s_{\text{off}}$. This run corresponds to the attachment of kinesin to MT, the hydrolysis or the synthesis of one ATP molecule, and the detachment from MT. Hence, the mean numbers of $T(h)$ and $T(s)$ traces after many runs of $M_{\text{kinesin}}$ would represent $\langle n_h \rangle$ and $\langle n_s \rangle$, respectively. Then, using Equation (5.5) $<RL>$ is computed by $\langle n \rangle \times d$, where $d \sim 8.2$ nm. $M_{\text{kinesin}}$ is run for 10000 times and the simulation results of $<RL>$, shown in Figure 5.3A and B, indicate good agreement with experimental observations in (Schnitzer et al. 2000) and theoretical findings in (Liepelt and Lipowsky 2007). Results in Figure 5.3A and B show that the processivity of kinesin exponentially diminishes with increasing load or decreasing [ATP], as showed in (Liepelt and Lipowsky 2007; Schnitzer et al. 2000). Similar to Figure 5.1C and D, simulation results in Figure 5.3C and D show that $<BD>$ is less than $d \sim 8.2$ nm, implying that on average kinesin is not likely to step backward by synthesizing ATP (Khataee and Liew 2015). This supports the observations that backward steppings are more likely to be generated by ATP hydrolysis (Carter and Cross 2005). The low $<BD>$ is due to the low rate of ATP synthesis compared to that of ATP hydrolysis which leads to low $P_s$ in comparison with $P_h$. Therefore, the mean number of $T(s)$ trace is below 1. This low but nonzero mean number of $T(s)$ trace (i.e. very few $T(s)$ traces are executed by $M_{\text{kinesin}}$) supports the theory of the four-state discrete stochastic model in (Fisher and Kim 2005; Fisher and Kolomeisky 2001; Kolomeisky and Fisher 2007) that backward stepping could result in ATP synthesis (Khataee and Liew 2015). Figure 5.3 also indicates a close agreement between the simulation results and the results obtained from theoretical analysis shown in Figure 5.1. This agreement of our simulation and theoretical results indicates that the number of $T(h)$ and $T(s)$ traces executed by $M_{\text{kinesin}}$ can be used to determine the mean run length of kinesin (Khataee and Liew 2015).
Figure 5.3. Stochastic simulation of $\langle RL \rangle$ and $\langle BD \rangle$ (Khataee and Liew 2015). Simulation results are shown by bar graphs. Line graphs are the results obtained from theoretical analysis, as shown in Figure 5.1A-D. $M_{\text{kinesin}}$ simulates $\langle RL \rangle$ as a function of (A) [ATP] at various resisting loads and (B) load at different [ATP]. $M_{\text{kinesin}}$ also simulates $\langle BD \rangle$, computed by the mean number of $T(s)$ traces $\times$ $d$ where $d \sim 8.2$ nm, against (C) [ATP] at different resisting loads and (D) load at various [ATP]. The 25 and 75 percentile values obtained from the simulation are shown in A and B panels. For panels C and D these values are equal to zero showing that more than 75 percent of runs of $M_{\text{kinesin}}$ do not execute $T(s)$ trace.

Temporal analysis of Algorithm 5.1, shown in Figure 5.4, indicates that the inactivation of $M_{\text{kinesin}}$ executing either the $T(h)$ or $T(s)$ trace most probably occurs: (i) in state $s_1$ at low [ATP] and low loads and (ii) in states $s_3$ and $s_4$ at high loads or high [ATP]. These analyses suggest kinetic state [0] (in Scheme 2.2) to be the most probable kinetic state for kinesin detachment from MT at low [ATP] and low loads (see Figure 5.4A). This is consistent with the detachment of kinesin from MT due to a long mean waiting time in kinetic state [0] at limiting [ATP], as observed experimentally by Schnitzer et al. (2000). In accordance with the findings of Schnitzer et al. (2000) that at high [ATP] the probability of detachment from kinetic state [0] diminishes, our simulation further shows that at high [ATP], kinetic states [2] and [3] are the first and second most probable kinetic states for the detachment of kinesin from MT due to their longer durations (see Figure 5.4B). This is in agreement with the reported kinetic states for the detachment of kinesin from MT in (Schliwa 2003; Seitz and Surrey 2006) and in our
mathematical model in Chapter 4. These results are also consistent with the weak affinity of kinesin to MT in kinetic states [2] and [3] (Schliwa 2003; Seitz and Surrey 2006). Simulation results in Figure 5.4 further confirm the small detachment probability of kinesin from MT in kinetic state [1], as reported in (Seitz and Surrey 2006) due to tight affinity of the motor to MT in this state.

Figure 5.4. The number of $M_{\text{kinesin}}$ inactivations in states $s_i$ (where $i = 1, 2, 3, 4$), corresponding to the number of detachments of kinesin from MT in each kinetic states [i-1], respectively, versus load at (A) limiting [ATP] and (B) saturating [ATP] (Khataee and Liew 2015).

In further analyzing the temporal properties of $M_{\text{kinesin}}$, Equation (5.6) further indicates that at low [ATP] and low loads the duration of a stepping cycle is dominated by the duration of kinetic state [0] (i.e. state $s_1$) due to waiting for ATP binding (see Figure 5.5), as reported by Schnitzer et al. (2000). With increasing load, $<t_{\text{total}}>$ decreases suggesting that the duration of a backward stepping cycle at high loads is more likely to be less than that of a forward stepping cycle (Khataee and Liew 2015).
5.3 Simulating Processing Unit Actuating Kinesin Steps

In this section, we propose a computational model for simulating the information processing unit underlying kinesin stepping. Based on the results of our mathematical and computational models presented in the previous sections, we model the mechanochemical kinetics (i.e. processing unit) of kinesin stepping by logical operations and variables to demonstrate how various [ATP] and loads perceived by the motor are transferred into its mechanical steps.

5.3.1 Digital Circuit Model

To model the mechanical kinetics driving the forward and backward stepping cycles of kinesin motor using logical operations, we construct a finite state machine (FSM) as an abstract computational model and use sequential logic to design a digital circuit that simulates the motor stepping behaviour. FSMs are used to demonstrate the control flow of a system’s behaviour, whereas digital circuits map the functional description of a FSM into a logic representation using logic variables and operations (Shiue 2005). The state diagram of the circuit is constructed as a Mealy FSM, $M_{\text{kinesin,motor}}$, due to the correlation of the kinesin motor function to the perceived [ATP] and load from the
cellular environment (Khataee and Liew 2014a). In mealy FSM, the output values are determined by the current state and the current input values (Anderson 2006). To construct $M_{\text{kinesin\_motor}}$, we define and assign binary values to its inputs, outputs, and internal states according to the kinesin motor’s perceived stimuli, stepping direction, and kinetic states, respectively. The $M_{\text{kinesin\_motor}} = (\hat{S}, \hat{I}, \hat{O}, \hat{T}, \hat{s}_0)$ consists of (Khataee and Liew 2014a):

I. The set of states $\hat{S} = \{\hat{s}_0, \hat{s}_1, \hat{s}_2, \hat{s}_3\}$ where $\hat{s}_0$ to $\hat{s}_3$ stand for the kinetic states [0] to [3] in Scheme 2.2, respectively.

II. The input set $\hat{I} = \{x_1, x_2\}$ where $x_1$ and $x_2$ stand for [ATP] and load, respectively.

III. The output set $\hat{O} = \{O_F, O_B\}$ where $O_F$ and $O_B$ stand for the forward and backward steppings, respectively.

IV. The transition function $\hat{T}: \hat{S} \times \hat{I} \rightarrow \hat{S}$ which resembles kinetic rates corresponding to ATP hydrolysis.

V. The output function $\hat{O}: \hat{S} \times \hat{I} \rightarrow \hat{O}$ which represents the steppings.

VI. The initial state $\hat{s}_0$ which represents the initial state of the kinesin cycles, the kinetic state [0].

The binary values 0 and 1 of input $x_1$ are assumed to correspond to $1 \mu \text{M} \leq [\text{ATP}] < 50 \mu \text{M}$ and $50 \mu \text{M} \leq [\text{ATP}] \leq 2 \text{mM}$, respectively, representing low and high [ATP] ranges, as usually applied in experimental and theoretical settings (Clancy et al. 2011; Fisher and Kolomeisky 2001; Liepelt and Lipowsky 2007; Nishiyama et al. 2002; Schnitzer et al. 2000; Yajima et al. 2002; Yildiz et al. 2008). Input $x_2$ is supposed to reset to 0 over assisting and resisting loads below stall force, i.e. $-10 \text{pN} \leq F < F_{\text{stall}}$, and sets to 1 over resisting loads above the stall force, i.e. $F_{\text{stall}} < F \leq 10 \text{pN}$, to denote the forward and backward stepping load ranges of kinesin motor, respectively. As at load $F = F_{\text{stall}}$, forward and backward stepping are equally likely, $F = F_{\text{stall}}$ can be considered to be in
either \( x_2 = 0 \) or \( x_2 = 1 \). Based on the results of our mathematical model proposed in Chapter 4 and also experimental observations in (Carter and Cross 2005; Nishiyama et al. 2002), it is also supposed that kinesin motor only steps forward at loads \( F < F_{\text{stall}} \) and backward at loads \( F > F_{\text{stall}} \), as they are more likely to happen in these load ranges. To represent the states of \( M_{\text{kinesin_motor}} \), we use two Boolean variables to generate four states: \( y_1 y_2 \) and \( Y_1 Y_2 \) to denote the present and the next states of \( M_{\text{kinesin_motor}} \), respectively. We assign binary values to the internal states using Karnaugh map principles (Mano 2001) in such a way that only one state variable (\( y_1 \) or \( y_2 \)) changes when a state transition occurs (see Figure 5.6A).

![Figure 5.6. FSM \( M_{\text{kinesin_motor}} \) and D flip-flop (Khataee and Liew 2014a). (A) \( M_{\text{kinesin_motor}} \): input and output values are shown as \( x_1 x_2 O_F O_B \). States \( \delta_0 \) to \( \delta_3 \) are depicted in red, pink, blue, and green colours, respectively. The incoming unlabelled arrow to state \( \delta_0 \) indicates this state as the initial state. (B) D flip-flop: logic diagram of the D flip-flop consists of two inputs and two outputs. Characteristic table of the D flip-flop shows that the next state of the flip-flop is obtained directly from the input \( D \).

To assign binary values to outputs \( O_F \) and \( O_B \), we follow the kinetic cycles of the forward and backward stepplings. The individual forward and backward stepping cycles start in the kinetic state \([0]\) (Carter and Cross 2005; Clancy et al. 2011), in states \( y_1 y_2 = 00 \) or \( Y_1 Y_2 = 00 \) of \( M_{\text{kinesin_motor}} \). The forward stepping cycle starts from kinetic state \([0]\), and forward jump occurs when the kinetic state \([3]\) changes to \([0]\) at low or high [ATP] and loads \( F < F_{\text{stall}} \). Accordingly, the output \( O_F \) of \( M_{\text{kinesin_motor}} \) is set to 1 when the present internal state \( y_1 y_2 = 10 \) of \( M_{\text{kinesin_motor}} \) changes to next state \( Y_1 Y_2 = 00 \) with input
\( x_1x_2 = X0 \), where the Boolean variable \( X \) is equal to either 0 or 1. For backward stepping, the process initiates similarly from kinetic state \([0]\) and completes with a backward jump when kinetic state changes from \([3]\) to \([0]\) at low or high \([ATP]\) but at loads \( F > F_{stall} \). Accordingly, the output \( O_B \) of \( M_{\text{kinesin\_motor}} \) is set to 1 when the present internal state \( y_1y_2 = 10 \) changes to next state \( Y_1Y_2 = 00 \) with input \( x_1x_2 = X1 \). The state diagram of the circuit is developed in Figure 5.6A, and shows that \( M_{\text{kinesin\_motor}} \) perceives inputs \( x_1 \) and \( x_2 \) and produces outputs \( O_F \) and \( O_B \) while its internal state changes in the sequence of \( \hat{s}_0 \rightarrow \hat{s}_1 \rightarrow \hat{s}_2 \rightarrow \hat{s}_3 \rightarrow \hat{s}_0 \). Therefore, the state table corresponding to the outputs and next states of the sequential circuit in the presence of inputs and present states is shown in Table 5.1. Using Table 5.1, we compute the simplified Boolean functions of state variables \( Y_1 \) and \( Y_2 \) as (Khataee and Liew 2014a):

\[
Y_1 = y_2
\]

\[
Y_2 = y_1'
\]

Equations (5.10) and (5.11) show that the next state \( Y_1Y_2 \) of the circuit depends only on the present state \( y_1y_2 \), not on inputs \( x_1 \) and \( x_2 \). They indicate that the next kinetic state of kinesin motor is determined by the present kinetic state only, not on \([ATP]\) and load \( F \). This is due to that both forward and backward stepping cycles are associated with ATP hydrolysis process. The simplified Boolean functions of outputs \( O_F \) and \( O_B \) can also be obtained from Table 5.1 as (Khataee and Liew 2014a):

\[
O_F = x_2'y_1y_2'
\]

\[
O_B = x_2y_1y_2'
\]

The outputs \( O_F \) and \( O_B \) are set to 1 for forward and backward jumps of kinesin motor, respectively. Equations (5.12) and (5.13) show that the proposed circuit differentiates the stepping direction based on only input \( x_2 \). This agrees with the experimental
observations reported by Carter and Cross (2005) that the choice between forward and backward stepping depends only on load, not on [ATP].

Table 5.1. State table providing the next states and outputs (Khataee and Liew 2014a). For example, the first row of the table shows that the present state $y_1y_2 = s_0 = 00$ of $M_{kinesin\_motor}$ changes to the next state $Y_1Y_2 = s_1 = 01$ in response to the input $x_1x_2 = 00$. This behaviour of $M_{kinesin\_motor}$ corresponds to the kinetic state changing of kinesin motor from [0] to [1] at [ATP] < 50 µM and loads $F < F_{stall}$.

<table>
<thead>
<tr>
<th>Present state</th>
<th>Input</th>
<th>Next state</th>
<th>Output</th>
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<tbody>
<tr>
<td>$y_1$ $y_2$</td>
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<td>1 1</td>
<td>1 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

To design the circuit, we synchronize the changes of the state variables $Y_1$ and $Y_2$ based on the present state variables $y_1$ and $y_2$ (see Equations (5.10) and (5.11)). We use two $D$ flip-flops to synchronously store the 2-bit state variables. The $D$ flip-flop is a basic digital circuit element that consists of one input data ($D$), one control input ($C$), and two outputs ($Y$ and $Y'$). For the $D$ flip-flop, the next state or output is equal to its input data when the control input $C$ is triggered (Mano 2001) (see Figure 5.6B). As the internal states of the circuit stand for the kinetic states of kinesin, the control inputs of the flip-flops should be triggered at intervals equal to the mean waiting times over the kinetic states of kinesin motor during forward and backward stepping cycles, $<t_i>$ where $i = 0, 1, 2, 3$ (see Equation (4.16)). In this way, the active durations of circuit states will be equal to the delay $<t_i>$. In accordance with the theory of communicating mobile
nanomachines in which the actions of each nanomachine are governed by its FSM’s local clock (Wiedermann and Petru 2009), we connect the control inputs of $D$ flip-flops to a digitally programmable delay element (DE) (Mayandi-Nejad and Sachdev 2003) to provide the delay $<t_i>$ in these control inputs. To program DE to provide the delay $<t_i>$, the present state $y_1y_2$ of the flip-flops and inputs $x_1$ and $x_2$ are connected as inputs to the DE since $<t_i>$ depends on the kinetic state of kinesin motor, [ATP], and load (see Equation (4.16)). Therefore, DE triggers the $D$ flip-flops at intervals equal to $<t_i>$. DE is also connected to the outputs of the circuit to synchronize their setting to 1 and resetting to 0 with DE signals, providing a stepping cycle completion time in the outputs. This architecture provides a small active duration of outputs compared to the duration of a stepping cycle as the moving time of kinesin in one step is much shorter (about three orders smaller) than the average time it spends waiting to step, i.e. the ATPase time (Xie et al. 2005). The circuit designed using Equations (5.10)-(5.13) is shown in Figure 5.7A.

**Figure 5.7.** Circuit models (Khataee and Liew 2014a). (A) Circuit for modelling the processing unit actuating stepping cycles of kinesin motor. The applied DE is programmed to provide delay $<t_i>$ (see Equation (4.16)) in the control inputs of the D flip-flops. For clarity of the circuit, the connections between $y_1y_2$ and flip-flops’ inputs are not shown. (B) Logic and block diagram of BB: input EN = 0 leads to reset inputs and complement outputs of the D flip-flops. With input EN = 1, inputs and outputs of BBs are equal to inputs and outputs of the D flip-flops, respectively. (C) Final circuit with BBs.
The experimental observations have shown that in the presence of regulatory factors, the stepping motion of kinesin is deactivated as the motor’s continuous kinetic state change is halted (Friedman and Vale 1999; Hirokawa et al. 2009; Kaan et al. 2011; Verhey et al. 2011). We model all such regulatory factors as an enable ($EN$) input signal which activates/deactivates the circuit function. The binary value of $EN = 0$ denotes the presence of regulatory factors, while $EN = 1$ defines the absence of regulatory factors where kinesin is able to move on MT. To apply the input signal $EN$ to the circuit in Figure 5.7A, $EN$ is connected to the voltage control of DE. It is also connected to the inputs and complement outputs of the $D$ flip-flops through an AND gate, as the complement outputs of the flip-flops are connected to the outputs $O_F$ and $O_B$. We denote the resulting $EN$ regulated flip-flops as a binary block (BB) (see Figure 5.7B). The final circuit in Figure 5.7C indicates that input $EN = 0$ powers off DE and resets the inputs and complement outputs of BBs. Accordingly, it will reset the outputs $O_F$ and $O_B$ of the circuit. On the other hand, with input $EN = 1$, DE triggers active BBs in $<t_i>$ intervals which leads to generate 1/0 signals in outputs $O_F$ and $O_B$ resembling the forward and the backward steps of kinesin motor occur at cycle completion times, respectively.

5.3.2 Simulations and Discussion

The circuit in Figure 5.7C models the mechanical kinetics actuating the forward and backward stepping cycles of kinesin motor using logical operations. The model enables us to simulate the forward and backward steerings of the motor at various [ATP] and loads. We analyse the function of the circuit in Figure 5.7C with different input ranges and compare the results with the kinesin’s mechanism. With input $EN = 1$, depending on the different ranges of inputs $x_1$ and $x_2$ as well as the circuit state, DE provides 16 various delay settings which define the average active duration ranges of the circuit.
states (see Table 5.2). Different combinations of the four inputs of DE show that the highest maximum active duration belongs to state $y_1y_2 = 00$, i.e. $<t_0>$, with input $x_1x_2 = 0X$, having a maximum of $5209 \times 10^{-4}$ s at $F = 0.75$ pN and $[\text{ATP}] = 1$ µM. With increasing $[\text{ATP}]$ and load, the active duration of state $y_1y_2 = 00$ decreases, whereas the active duration of state $y_1y_2 = 11$ becomes dominant with a maximum of $<t_2> = 4636 \times 10^{-6}$ s at $F = 10$ pN. These results are consistent with the obtained results of the mathematical model proposed in Section 4.2.2 and confirm that at limiting $[\text{ATP}]$ kinesin mainly stays in kinetic state [0], while at high resisting loads the duration of being in kinetic state [2] becomes dominant (see Figure 4.6).

Table 5.2. 16 different delay ranges provided by DE, depending on its four inputs (Khataee and Liew 2014a).

<table>
<thead>
<tr>
<th>DE inputs</th>
<th>Delay (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>$y_1y_2x_1x_2$</td>
<td></td>
</tr>
<tr>
<td>0 0 0 0</td>
<td>$2388 \times 10^{-7}$</td>
</tr>
<tr>
<td>0 0 0 1</td>
<td>$2958 \times 10^{-8}$</td>
</tr>
<tr>
<td>0 0 1 0</td>
<td>$2439 \times 10^{-8}$</td>
</tr>
<tr>
<td>0 0 1 1</td>
<td>$4083 \times 10^{-9}$</td>
</tr>
<tr>
<td>0 1 0 0</td>
<td>$1145 \times 10^{-6}$</td>
</tr>
<tr>
<td>0 1 0 1</td>
<td>$1058 \times 10^{-6}$</td>
</tr>
<tr>
<td>0 1 1 0</td>
<td>$1145 \times 10^{-6}$</td>
</tr>
<tr>
<td>0 1 1 1</td>
<td>$1058 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 0 0 0</td>
<td>$1962 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 0 0 1</td>
<td>$1331 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 0 1 0</td>
<td>$1962 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 0 1 1</td>
<td>$1331 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 1 0 0</td>
<td>$2298 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 1 0 1</td>
<td>$4119 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 1 1 0</td>
<td>$2298 \times 10^{-6}$</td>
</tr>
<tr>
<td>1 1 1 1</td>
<td>$4119 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

To verify that the circuit models the forward and backward stepping cycles of kinesin motor in response to the same biochemical stimuli, we explore the circuit behaviour in response to inputs $[\text{ATP}] = 100$ µM and resisting load $F = 1$ pN in the absence of regulatory factors. As $EN = 1$, DE is active with the initial state $y_1y_2 = 00$ and recognizes the inputs $x_1x_2 = 10$. DE with input 0010 ($y_1y_2x_1x_2$) provides the delay $<t_0> = 6206 \times 10^{-6}$ s.
s over initial state $y_1y_2 = 00$ where $<t_0> \in (2439 \times 10^{-8}, 1207 \times 10^{-5})$. Then, DE triggers BBs which leads to changing the circuit state to $y_1y_2 = 01$ and the DE input to 0110 providing the delay $<t_1> = 1641 \times 10^{-6}$ s over present state $y_1y_2 = 01$ where $<t_1> \in (1145 \times 10^{-6}, 1662 \times 10^{-6})$. After $1641 \times 10^{-6}$ s, the circuit state and the DE input are changed to $y_1y_2 = 11$ and 1110 providing the delay $<t_2> = 3564 \times 10^{-6}$ s in the range of $2298 \times 10^{-6}$ s to $4445 \times 10^{-6}$ s over present state $y_1y_2 = 11$. The accomplishment of the delay $<t_2>$ transitions the state of the circuit to $y_1y_2 = 10$ and accordingly, the input of DE to 1010 providing $<t_3> = 3025 \times 10^{-6}$ s in the range of $1962 \times 10^{-6}$ s to $3032 \times 10^{-6}$ s. Finally, in the next pulse, DE triggers BBs which leads to setting the output $O_F$ to 1, change the circuit state to $y_1y_2 = 00$, and the cycle iterates again. A simulation of the circuit function is shown in Figure 5.8A which is consistent with the obtained results of the durations of kinetic states in Figure 4.6. In the next cycle of the circuit function when the present state is $y_1y_2 = 11$, we increase the input $F$ to 9 pN. It changes the DE input to 1111 and increases delay $<t_2>$ to $4576 \times 10^{-6}$ s, in the range of $(4119 \times 10^{-6}, 4636 \times 10^{-6})$. The next pulse of DE transitions the internal state to $y_1y_2 = 10$ with $<t_3> = 1587 \times 10^{-6}$ s in the range of $(1331 \times 10^{-6}, 2589 \times 10^{-6})$. After $1587 \times 10^{-6}$ s, DE pulses and sets the output $O_B$ to 1 and changes the state to $y_1y_2 = 00$ (see Figure 5.8B). The behaviour of the circuit agrees with our theoretical results, presented in Sections 4.2 and 5.2, about the highest duration of kinetic state [2] under high resisting loads as well as the experimental observations that under loads $F > F_{stall}$ backward stepping are more likely to happen (Carter and Cross 2005; Nishiyama et al. 2002).
Figure 5.8. Simulation of two stepping cycles (Khataee and Liew 2014a). In accordance with the state colours of M\textsubscript{kinesin\_motor} in Figure 5.6A, the durations <t\textsubscript{0}> to <t\textsubscript{3}> are shown in red, pink, blue, and green colours, respectively. (A) First cycle, inputs of the circuit are [ATP] = 100 µM and F = 1 pN. (B) In the second cycle, the input F is increased to 9 pN when the present state is y\textsubscript{1}y\textsubscript{2} = 11.

Table 5.2 also shows the behaviour of the circuit at various thresholds of inputs [ATP] and F ranges. Equation (4.16) indicates that the decreasing of [ATP] to the concentrations of less than 1 µM causes a significant increase in the delay <t\textsubscript{0}>. Similarly, with increasing F to resisting loads above 10 pN, delay <t\textsubscript{2}> rises. Accordingly, when input x\textsubscript{1} (or x\textsubscript{2}) to the circuit crosses the minimum threshold of 1 µM (or maximum threshold of 10 pN), the generated delay <t\textsubscript{0}> (or <t\textsubscript{2}>) overflows its range which would reset EN to 0 and, accordingly deactivates the circuit’s state transition in state y\textsubscript{1}y\textsubscript{2} = 00 (or y\textsubscript{1}y\textsubscript{2} = 11). This is consistent with the high probability of the detachment of kinesin motor from MT in kinetic states [0] at limiting [ATP] (Clancy et al. 2011; Hackney 1994) and in agreement with our mathematical results about the high detachment probability of the motor from MT in kinetic states [2] due to high resisting loads F > 10 pN (Khataee and Liew 2014b). Through rebinding of kinesin motor to MT, the stepping cycle starts in kinetic state [0] (Carter and Cross 2005; Clancy et al. 2011), which is well modelled as the initial state y\textsubscript{1}y\textsubscript{2} = 00 of the circuit when EN is set to 1. In conclusion, our circuit model represents the mechanical kinetics actuating the forward and backward stepplings of kinesin motor as a four-state Mealy
FSM driven by energy released from ATP hydrolysis. This model simulates the stepping behaviour of the motor at various [ATP] and loads, in accordance with the \textit{in vivo} data of kinesin. The proposed computational model suggests that in molecular communication the load that information molecules apply on the motor could be used to directionally transport bionanomachines carrying information molecules over a network of MTs, whereas [ATP] can be manipulated to control the duration and velocity of this transportation (Khataee and Liew 2014a).

5.4 Chapter Summary

In this chapter, we proposed computational models for simulating the processivity and the information processing unit of single kinesin. Then main findings of this chapter are summarized as follows: (i) the processivity of kinesin is coupled to both ATP hydrolysis and ATP synthesis pathways and thus, can be determined by the number of ATP hydrolysis and ATP synthesis kinetic cycles taken by the motor before becoming inactive, (ii) the higher frequency of backward stepping powered by ATP hydrolysis than by ATP synthesis is found to be a reason for the more dramatic falling of kinesin processivity with rising load at high [ATP] compared with that at low [ATP], and (iii) in molecular communication, the load applied to the motor by information molecules could be used to control the transport direction, whereas [ATP] can be manipulated to control the duration and velocity of this transportation.
Chapter 6

Theory of Two Coupled Kinesins Motility

6.1 Introduction

In this chapter, we develop a mathematical framework for investigating cargo transport by two coupled kinesins. This theory utilizes single kinesin properties and predicts the properties of the two-kinesin assembly. In Section 6.2, a kinetic scheme is proposed to describe the different configurations in which the assembly of two kinesins can bind to MT. In Section 6.3, we develop a set of master equations over the kinetic scheme and solve it in terms of parameters obtained from single kinesin properties. Then, Section 6.4 presents our analytical results and also discusses our findings along with the available experimental data. Section 6.5 concludes this chapter.

6.2 Model Description

We propose a kinetic scheme to describe different configurations that an assembly of two kinesins can bind to MT. These configurations are generally classified by the number of MT-bound motors carrying a common cargo, i.e. cargo states 0, 1 and 2 (see Figure 6.1A). The assembly transitions between the cargo states via binding and unbinding of one motor within the assembly. Similar to the theory of Lipowsky and colleagues (Berger 2012; Berger et al. 2015; Berger et al. 2012; Keller et al. 2013), we consider the kinesin stalks as linear elastic springs with stiffness $k_{\text{motor}}$. Therefore, discrete stepping of motors leads to discrete extension of elastic linkers and thus, partitioning cargo state “2” into discrete substates denoted microstates $C_i$, where $i = 0, 1, \ldots, N$. In each microstate $C_i$, $i$ represents the absolute value of the number of $d \sim 8.2$
nm distance units that the assembly is away from the microstate \( C_0 \), where the net elastic force on each motor is zero (see Figure 6.1B).

**Figure 6.1.** Kinetic scheme representation of the discrete state transition rate model for the cargo transport by two kinesins. (A) States of the cargo are defined by the number of motors linking cargo to MT. In state “0”, both motors are detached from MT, in state “1” one motor pulls the cargo, and in state “2” two motors carry the cargo. The unbinding and binding rates of single motor are denoted by \( \omega_1 \) and \( \pi \), respectively. (B) In the extended description of state “2”, the cargo states are defined by discrete microstates \( C_i \). In microstate \( C_0 \), the linkers of the motors are relaxed such that there is no elastic force between the motors. In each microstate \( C_i \), whenever a motor steps, the assembly stretches to microstate \( C_{i+1} \) with higher strain energy (with rate \( k_s(i) \)) or relaxes to microstate \( C_{i-1} \) with lower strain energy (with rate \( k_r(i) \)). The detachment of a motor from MT in a two MT-bound configuration of motors occurs with rate \( \beta_i \).

As Figure 6.1A and B shows, it is assumed that an unbound cargo first binds to MT with one motor, i.e. in cargo state “1” (Berger 2012; Berger et al. 2015; Berger et al. 2012; Driver et al. 2010; Keller et al. 2013). When one motor is active, the other motor can attach to MT with binding rate \( \pi \approx 5 \text{ s}^{-1} \) (Leduc et al. 2004), changing cargo state from “1” to “2”. Cargo is assumed to be stiff, so that it does not stretch or relax, and its position is always at the same vertical distance from MT as kinesins maintain their cargo at a specific height above MT (~ 17 nm) during transport (Kerssemakers et al. 2006). In cargo state “2”, the initial intermotor distance is assumed to be greater than 30 nm as at distances less than 30 nm spacing, molecular crowding reduces the velocities of kinesin motors (Rogers et al. 2009). Steppings of kinesins are not considered to be
simultaneous as experimental observations have shown that most assembly configurations prevent both motors from participating simultaneously in cargo transport (Jamison et al. 2010). Leading and trailing motors may swap their positions on MT while carrying the cargo, due to their stochastic steppings. Leading or trailing motor can also unbind from and rebind to MT while the other motor is attached to MT. The detachment of cargo from MT occurs with 1-motor run in cargo state “1” because it is unlikely that both motors unbind exactly at the same time (Berger 2012).

6.3 Master Equation Approach

6.3.1 Master Equation Formulation

We calculate the mean run time of two-kinesin complex along MT using the following equation:

$$< RT_{\text{complex}} >= \int_0^\infty \partial_t P_0(t) t dt$$

(6.1)

where $P_0(t)$ is the probability of finding cargo in state “0”, shown in Figure 6.1, at time $t$ and $\partial_t P_0(t)$ is the partial derivative of $P_0(t)$ showing the time evolution of the probability of finding two-kinesin complex in state “0”. $P_0(t)$ can be calculated by solving a set of master equations developed over the kinetic scheme in Figure 6.1A or over the extended kinetic scheme in Figure 6.1B. First, we develop a set of master equations over kinetic scheme in Figure 6.1A as follows:

$$\partial_t P_0(t) = \omega_0 P_1(t)$$

(6.2)

$$\partial_t P_1(t) = -(\pi + \omega_1) P_1(t) + \omega_2 P_2(t)$$

$$\partial_t P_2(t) = -\omega_2 P_2(t) + \pi P_1(t)$$
where $P_i(t)$ is the probability of finding cargo in cargo state $i$ (where $i = 0, 1, 2$) at time $t$, $\omega_1$ is the detachment rate of single kinesin from MT, $\omega_2$ is the detachment rate of one motor from MT in a two MT-bound configuration of motors, and $\pi$ is the binding rate of single kinesin to MT. We solve equation (6.2) in Mathematica 10.2 and in agreement with Equation (2.21) proposed in (Berger et al. 2012), we obtain the complex mean run time as follows:

$$< RT_{\text{complex}} > = \frac{\pi + \omega_2}{\omega_1 \omega_2}$$  \hspace{1cm} (6.3)

where the value of parameter $\omega_2$ is unknown and depends on the single kinesin parameters as well as the interactions between the motors, as reported in (Berger et al. 2012). Equation (6.3) would enable us to estimate the maximum run time of the two-motor complex by assigning the minimum value of $\omega_2$ in the equation. It was shown by Lipowsky and colleagues (Keller et al. 2013) that the non-interacting motors ($k_{\text{motor}} = 0$) has the minimum unbinding rate from a two MT-bound configuration equal to $2\omega_1$. Therefore, the maximum run time of the two-motor complex is given by:

$$\max(< RT_{\text{complex}} >) = \frac{\pi + 2\omega_1}{2\omega_1}$$  \hspace{1cm} (6.4)

We then develop a set of master equations over the extended kinetic scheme shown in Figure 6.1B as follows:

$$\partial_t P_0(t) = \omega_1 P_1(t)$$  \hspace{1cm} (6.5)

$$\partial_t P_1(t) = -(\pi + \omega_1) P_1(t) + \sum_{i=0}^{N-1} \left( \beta_i P_{C_i}(t) \right)$$

$$\partial_t P_{C0}(t) = \pi P_1(t) - \left( \beta_0 + k_s(0) \right) P_{C0}(t) + k_r(1) P_{C1}(t)$$

$$\partial_t P_{Ci}(t) = k_s(i-1) P_{C_{i-1}}(t) - \left( \beta_i + k_s(i) + k_r(i) \right) P_{Ci}(t) + k_r(i+1) P_{C_{i+1}}(t) : 1 \leq i \leq (N-1)$$

$$\partial_t P_{CN}(t) = k_s(N-1) P_{CN-1}(t) - \left( \beta_N + k_r(N) \right) P_{CN}(t)$$
where \( P_i(t) \) is the probability of finding cargo in state \( i \) at time \( t \), \( \omega_1 \) is the detachment rate of single kinesin from MT, \( \pi \) is the binding rate of single kinesin to MT, \( k_s(i) \) and \( k_r(i) \) are the stretching and the relaxation rates, respectively, and \( \beta_i \) is the unbind rate of one motor from MT in a two MT-bound configuration of motors. All the parameters of Equations (6.5) can be obtained from single motor properties.

### 6.3.2 Parameter Extraction

In this section, we calculate the parameters of Equations (6.5) via direct utilization of single kinesin properties. First, we obtain an equation for the load-dependent unbinding rate of single kinesin by fitting to the recent data from Block laboratory (Andreasson et al. 2015) at \([\text{ATP}] = 2000 \, \mu\text{M}\) using the Heaviside step function \( \Theta(F) \) as follows:

\[
\omega_1(F) = k_1 \, \Theta(F_c - F) \exp\left\{ \frac{|F| d_{s1}}{k_B T} \right\} + k_2 \, \Theta(F - F_c) \exp\left\{ \frac{|F| d_{s2}}{k_B T} \right\}
\]

(6.6)

where \( F \) is parallel to MT as we only consider forces parallel to MT, \( k_1 \sim 0.897 \, \text{s}^{-1} \) and \( k_2 \sim 8.037 \, \text{s}^{-1} \) are the detachment rates of single kinesin at \( F = 0 \) and \( F = F_c \), respectively, with critical force \( F_c \sim 1.90 \, \text{pN} \) where the rising phase of unbinding rate changes under assisting loads. \( d_{s1} \sim 1.115 \, \text{nm} \) and \( d_{s2} \sim 0.182 \, \text{nm} \) are the characteristic distances for the load, and \( |F| \) indicates the absolute value of \( F \) (see Figure 6.2). To be consistent with the recent experimental findings from Block laboratory (Andreasson et al. 2015; Milic et al. 2014), in this chapter, we denote the resisting and assisting forces with negative and positive signs, respectively. The details of Equation (6.6) are discussed in appendix A.1. Having Equation (6.6), we calculate the mean run time of single kinesin as the inverse of \( \omega_1 \) and thus, the mean run length of single kinesin as \( v / \omega_1 \), where \( v \) is the velocity of single kinesin given by Equation (2.2).
Equation (6.6) is used to calculate the unbind rate of one motor from MT in a two MT-bound configuration of motors $C_i$ (where $i = 0, \ldots, N$) as follows (Berger et al. 2012):

$$\beta_i = \omega_l (F_{le}(i)) + \omega_r (F_{tr}(i))$$

(6.7)

where $F_{le}(i)$ and $F_{tr}(i)$ are the forces exerted on leading and trailing motors in each microstate $C_i$ (where $i = 0, \ldots, N$), respectively. We calculate $F_{le}(i)$ and $F_{tr}(i)$ as follows:

$$F_{le}(i) = \frac{F_{ex}}{2} - k_{motor} \frac{di}{2}$$

(6.8)

$$F_{tr}(i) = \frac{F_{ex}}{2} + k_{motor} \frac{di}{2}$$

(6.9)

where $F_{ex}$ is the external force parallel to MT, $d$ is the step size of single kinesin, and $k_{motor}$ is the stiffness of motors. We discuss the details of Equations (6.8) and (6.9) in appendix A.2. In analysing Equations (6.8) and (6.9), Figure 6.3 indicates that load sharing between two motors bound to MT occurs if the strain energy of the assembly is close to zero, and the load on the leading motor rises with increasing intermotor distance. This is in good agreement with the experimental observations and the theoretical studies from Diehl laboratory (Jamison et al. 2010; Uppulury et al. 2012a) which stated that the distribution of load between two motors is strongly dependent on

Figure 6.2. Detachment rate of single kinesin versus load at [ATP] = 2000 µM calculated using Equation (6.6).
how far apart the motors are bound at MT (compare with Figure 2.7). In contrast, Equations (2.17) and (2.18) proposed by Lipowsky and colleagues (Berger et al. 2015) for $F_{le}(i)$ and $F_{tr}(i)$ do not agree with the experimental findings in (Jamison et al. 2010).

![Figure 6.3. Load shared between the leading ($F_{le}$) and trailing ($F_{tr}$) motors against the separation distance between the motors. Two examples of resisting loads are provided. This example corresponds to two coupled kinesins with $k_{motor} = 0.5$ pN/nm.](image)

Equations (6.5) show that single motor properties are calculated over $N$ microstates, where $N$ indicates the maximum number of $d \sim 8.2$ nm distance units that the assembly is likely to be stretched away from the microstate $C_0$. We calculate $N$ using the steady state probability distribution over microstates $C_i$ with $\beta_i = 0$, where $i = 0, \ldots, N$. The steady state is characterized by time-independent probabilities, i.e. $\partial_t P_i(t) = 0$, and can be obtained using Hill’s diagram method (Berger et al. 2012; Hill 1988; Keller et al. 2013; Khataee and Liew 2014b). Therefore, we derive the directional diagrams of a part of the kinetic scheme in Figure 6.1B which involves two-motor runs using the definition of directional diagram given in Equations (4.5)-(4.7) (see Figure 6.4).
Figure 6.4. Directional diagrams of a part of the kinetic scheme in Figure 6.1B which involves two-motor runs. Directional diagrams are depicted in green colours, as the corresponding part in Figure 6.1B. The stretching and the relaxation rates are denoted by $V_s(i)$ and $V_r(i)$, respectively.

Using the directional diagrams in Figure 6.4, we define the steady state probability distribution over microstates $C_i$, where $i = 0, \ldots, N$, as follows:

$$P_{Ci} = \frac{\Lambda_i}{\sum_{total}}$$

\[ \Lambda_i = \prod_{m=0}^{i-1} k_s(m) \times \prod_{n=i+1}^{N} k_r(n) \]  \hspace{1cm} (6.11)

$$\sum_{total} = \sum_{i=0}^{N} \Lambda_i$$  \hspace{1cm} (6.12)

where $k_s(m)$ and $k_r(n)$ rates are given by Equations (2.19) and (2.20), respectively.

Figure 6.5 shows that the steady state probability distribution over microstates $C_i$ (where $i = 0, \ldots, N$) decreases with increasing intermotor distance. This is consistent with the findings from Diehl laboratory (Driver et al. 2010) indicating a general tendency for the two motor systems to occupy microstate configurations in which the motor linkers are relaxed. Our results also suggest: (i) strongly coupled motors are less likely to be stretched to higher intermotor distances compared to the weakly coupled motors, and (ii) with raising [ATP], the probability that the motors occupy microstate configurations with high strain energy increases. These behaviours of the two-kinesin assembly occur over different resisting and assisting forces.
Figure 6.5. Steady state probability distribution over microstates $C_i$ (where $i = 0, \ldots, N$) versus $i$. Two examples of coupling stiffness values at different [ATP] are provided. This example corresponds to two coupled kinesins carrying a cargo under zero external load.

We calculate $N$ by giving a minimum probability threshold to $P_{Ci}$ indicating a minimum probability that the complex is likely to occupy microstate configuration $C_i$. In what follows, we assume the minimum probability threshold $P_{Ci} > 0.01$.

### 6.3.3 Solving Master Equations

To solve the master Equations (6.5), we use numerical methods. We express Equations (6.5) using transition matrix $\bar{A}$ and probability column vector $\bar{P}(t)$ as follows:

$$\partial_t \bar{P}(t) = \bar{A} \times \bar{P}(t)$$  \hspace{1cm} (6.13)

where

$$\bar{A} = \begin{bmatrix}
0 & \omega_\lambda & 0 & 0 & \cdots & 0 \\
0 & -(\pi + \omega_\lambda) & \beta_0 & \beta_1 & \cdots & \beta_N \\
0 & \pi & -(\beta_0 + k_s(0)) & k_r(1) & \cdots & 0 \\
0 & 0 & k_s(0) & -(\beta_1 + k_r(1) + k_s(1)) & \cdots & 0 \\
0 & 0 & \vdots & \vdots & \ddots & \vdots \\
0 & 0 & 0 & 0 & \cdots & -(\beta_N + k_r(N)) \\
\end{bmatrix}_{(N+3) \times (N+3)}$$
We solve Equation (6.13) using the implicit forward time stepping method as follows:

\[
\bar{P}(t + \delta t) - \bar{P}(t) = \bar{A} \times \bar{P}(t + \delta t) \delta t \\
\bar{P}(t + \delta t) \times (I - \delta t \bar{A}) = \bar{P}(t) \\
\bar{P}(t + \delta t) = M^{-1} \bar{P}(t)
\]

(6.14) \hspace{1cm} (6.15) \hspace{1cm} (6.16)

where \(\delta t\) is the time step, \(I_{(N+3)\times(N+3)}\) is the identity matrix, and matrix \(M = (I - \delta t \bar{A})\). \(\delta t\) is calculated to be sufficiently smaller than the fastest characteristic time in the kinetic scheme in Figure 6.1B as follows:

\[
\delta t \leq \min \left\{ \frac{1}{10 \times \max (\omega_1, \pi)}, \frac{d}{v} \right\}
\]

(6.17)

where \(\omega_1\) is the detachment rate of single kinesin from MT, \(\pi\) is the binding rate of single kinesin to MT, \(d\) is the step size of single kinesin and \(v\) is the velocity of single kinesin given by Equation (2.2). Since we assumed that an unbound cargo first binds to MT with one kinesin, then \(P_1(t = 0) = 1\), \(P_0(t = 0) = 0\) and \(P_{C_i}(t = 0) = 0\), where \(i = 0, 1, ..., N\). Therefore, we start solving Equation (6.16) with vector \(\bar{P}(t = 0)\) in which only the second item is equal to 1 and the remaining items of the vector are equal to zero. To ensure that all probability distributions at each time \(t\) are covered during the two-motor complex run time, we repeat the implicit forward time stepping method in Equation (6.16) for the maximum number of time steps, \(N_t\):

\[
N_t = \frac{T_{\max}}{\delta t}
\]

(6.18)
where $\delta t$ is given by Equation (6.17) and $T_{\text{max}}$ is taken to be greater than the maximum run time of the complex. Therefore, $T_{\text{max}}$ is calculated as an order of magnitude of the maximum mean run time as follows:

$$T_{\text{max}} = 10 \times \text{max}(\langle RT_{\text{complex}} \rangle)$$

(6.19)

where $\text{max}(\langle RT_{\text{complex}} \rangle)$ is given by Equation (6.4).

### 6.4 Analytical Results and Discussion

By solving Equations (6.5), we obtain the probability distributions over cargo states “0”, “1”, and “2” in the kinetic scheme in Figure 6.1A. Figure 6.6 shows the cargo run starting from state “1” as $P_1(0) = 1$. As time elapses, the probability that the cargo will be carried by two motors ($P_2(t)$) increases, and eventually cargo detaches from MT in state “0”, i.e. $P_0(T_{\text{max}}) = 1$. Our results in Figure 6.6 show that cargo is more likely to occupy state “2” when the coupling stiffness is weak. This suggests that weakly coupled motors are more likely to carry the cargo with two MT-bound motors, compared to the strongly coupled motors. In addition, the time period taken for the weakly coupled motors to be detached from MT is longer than that of strongly coupled motors, predicting a longer mean run time for the weakly coupled motors relative to the strongly coupled motors. Both resisting and assisting external forces decrease the time taken to find the complex in state “0”, suggesting that the run time of the cargo will peak to its maximum value when no external force applied to the assembly. Collectively, our results in Figure 6.6 suggest weak coupling strength and zero external force to be the factors that increase the probability of cargo transport by two active kinesins.
Figure 6.6. Probability distribution over cargo states “0”, “1”, and “2” against time at [ATP] = 2000 µM with (A-C) $k_{\text{motor}} = 0.05$ pN/nm and (D-E) $k_{\text{motor}} = 5$ pN/nm over different external forces. $P_i(t)$ are shown as $P_i$ (where $i = 0, 1, 2$) in brown, black, and green colours, respectively, as depicted in Figure 1. $K_{\text{motor}}$ is also shown as $k$.

Results of probability distribution over cargo states “0”, enables us to calculate the mean run time, the mean run length, and the average velocity of two-kinesin complex carrying a cargo. The mean run time of the complex is calculated by Equation (6.1) using the trapezoid method as follows:

$$< R T_{\text{complex}} >= \frac{\delta}{2} \sum_{t=0}^{N-1} [(t_{i+1} - t_i ) P_0(t_{i+1}) + (t_{i+1} - t_i ) P_0(t_i )]$$

(6.20)

where $\partial P_0(t_i)$ is calculated using forward, backward and centered approximation methods. Then, we calculate the mean run length of two-kinesin complex using ensemble average velocity of cargo in one and two MT-bound configurations of the motors over time. Cargo states “1” and “2” represent the possible states that the cargo may move along MT at time $t$. Therefore, the mean run length of the complex is given by:

$$< R L_{\text{complex}} >= \lim_{t \to \infty} \int_0^t \tilde{v}(\tau) d\tau$$

(6.21)
where $\bar{v}$ is the average ensemble velocity of the complex in cargo states “1” and “2” calculated as follows:

$$\bar{v}(t) = v \times p_1(t) + \sum_{i=0}^{N} \left( \frac{v(F_{\text{in}}(i)) + v(F_{\text{out}}(i))}{2} \right) p_{Cl}(t)$$  \hspace{1cm} (6.22)

where $v$ is the velocity of single kinesin given by Equation (2.2), $p_1(t)$ is the probability of finding cargo in state “1” obtained from the solution of Equations (6.5), $F_{\text{in}}(i)$ and $F_{\text{out}}(i)$ are the force distributed on the leading and trailing motors, respectively, in microstates $C_i$ given by Equations (6.8) and (6.9), and $p_{Cl}(t)$ is the probability of finding cargo in microstates $C_i$, where $i = 0, .. , N$. Using Equations (6.20) and (6.21), the velocity of the complex is calculated as follows:

$$v_{\text{complex}} = \frac{<RL_{\text{complex}}>}{<RT_{\text{complex}}>}$$  \hspace{1cm} (6.23)

We calculate $<RT_{\text{complex}}>$, $<RL_{\text{complex}}>$ and $v_{\text{complex}}$ with various $k_{\text{motor}}$, as stiffness of the complex can be varied in engineered motor complexes. Our results in Figure 6.7 suggest that weakly coupled kinesins produce larger mean run length and mean run time compared to that of single kinesin, whereas strongly coupled motors generate a mean run length and a mean run time almost equal to that of the single kinesin. The peak value of $<RT_{\text{complex}}>$ at zero external force in Figure 6.7A confirms our earlier results about the time-dependent probability distributions over cargo states shown in Figure 6.6. The general longer mean run length of two-kinesin complex compared to that of single kinesin agrees with the experimental observations stating that the average run length for the two-kinesin systems is larger than that of single motors (Jamison et al. 2010; Rogers et al. 2009). Results in Figure 6.7B are also consistent with the findings of Berger et al. (Berger et al. 2012) showing that an increase of the coupling strength reflects a strong decrease in the mean run length of two-kinesin complex.
Figure 6.7. Mean run time, mean run length and velocity of the two-kinesin complex and single kinesin against external force at [ATP] = 2000 µM with various coupling strength parameters. $k_{\text{motor}}$ is shown as $k$.

Our calculations of the velocity of two-kinesin complex agrees with the previous finding showing that the average velocity measurements of the single- and two-motor constructs were not significantly different over low resisting loads at saturating [ATP] (Berger et al. 2015; Berger et al. 2012; Jamison et al. 2010; Rogers et al. 2009). Our results also suggest that the weak elastic coupling between the motors is an enhancing factor for the complex velocity under high resisting forces relative to the velocity of single kinesin and strongly coupled motors (see Figure 6.7C). This is because weakly coupled motors are more likely to carry the cargo by two-MT bound motors (see Figure 6.6), due to lower detachment rate of single kinesin at low loads. Thus, lowering stuffiness of kinesin increases the probability of two-motor runs and thus, the average ensemble velocity of cargo carried by two-MT bound motors. Furthermore, to compare the dependency of the mean run length and the mean run time of two-kinesin complex to assisting and resisting loads, we calculated the ratio of the mean run length of the complex to that of single kinesin. Our findings in Table 6.1 predict that the mean run length and the mean run time of two-kinesin complex over assisting loads above ~ 2 pN is less dependent on the coupling strength between the motors, compared to that under resisting loads. This is due to the high detachment rate of single kinesin over low assisting forces, compare to that at low resisting forces, which in turn prevents the stretch of kinesins to two MT-bound configurations with larger intermotor distances.
under assisting forces. Over assisting loads, the trailing motor feels only assisting loads (see Equation (6.7)) and due to the higher unbinding rate of single kinesin over assisting forces at saturating [ATP], compare to that over resisting forces, the lagging motor detaches more frequently relative to the leading motor. The behaviour of two-kinesin complex over assisting loads resembles that of single kinesin.

<table>
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<th>-10</th>
<th>-8</th>
<th>-6</th>
<th>-4</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
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<td>4.0</td>
<td>2.9</td>
<td>2.9</td>
<td>2.8</td>
<td>2.2</td>
<td>1.5</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>0.5</td>
<td>26.2</td>
<td>8.0</td>
<td>3.1</td>
<td>1.9</td>
<td>1.7</td>
<td>1.5</td>
<td>1.5</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
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</table>

6.5 Chapter Summary

In this chapter we developed a theoretical framework based on a set of master equations to investigate the cellular mechanism of cargo transport by two elastically coupled motors. The main findings of this chapter are summarized as follows: (i) equal load sharing between two motors is not possible, unless the strain energy of the assembly is almost zero. Load on the leading motor rises with increasing intermotor distance, (ii) by raising [ATP] over different resisting and assisting external forces, the probability that the motors occupy microstate configurations with high strain energy increases, (iii) weakly coupled motors are more likely to carry the cargo with two MT-bound motors, compared to the strongly coupled motors, (iv) both resisting and assisting external forces are factors that decrease the mean run time and the mean run length of the cargo, (v) weakly coupled kinesins produce larger mean run length compared to that of single kinesin, whereas strongly coupled motors generate a mean run length almost equal to that of the single motor, (vi) weak elastic coupling between the motors enhances the
cargo velocity under resisting forces relative to the velocity of single kinesin and strongly coupled motors, and \textit{VII} the mean run length of two-kinesin complex under assisting loads above \(2\) pN is found to be less dependent on the coupling strength between the motors, compared to that under resisting loads.
Chapter 7

Concluding Remarks

7.1 Contributions

The general mathematical and computational frameworks proposed in this thesis allowed us to investigate the intracellular cargo transport driven by single kinesin and by a team of two coupled kinesins over various loads and [ATP]. The contribution of this thesis involved two phases.

In the first phase, the biophysical fundamentals of intracellular mechanisms of cargo transport by single kinesin are studied by developing mathematical and computational models. The main strength of these models is their simplicity. The models have been developed based on the kinetic scheme of the stepping cycle of a single kinesin. They provide results consistent with the recent experimental data, and describe the reasons of discrepancies among previous findings in the literature. The main findings of the models proposed in this phase are as follows: (i) kinesin stalls at force about 7.3 pN independent of [ATP], (ii) backward stepping of kinesin tends to be powered by ATP hydrolysis, while that backward stepping is related to both ATP hydrolysis and ATP synthesis pathways, (iii) processivity of kinesin is associated with both ATP hydrolysis and ATP synthesis pathways, and can be determined by the number of ATP hydrolysis and ATP synthesis kinetic cycles taken by the motor before detaching from MT, and (iv) mechanochemical kinetics of kinesin use the external load to direct the intracellular transport, whereas [ATP] can be used to control the duration and velocity of this transportation.
In the second phase, we studied the intracellular cargo transport by an assembly of two coupled kinesins by developing a mathematical framework based on master equation approach. To develop this framework, we first designed a kinetic scheme to show the different configurations that an assembly of two kinesins can be bound to MT. Then, we constructed a set of master equations over the kinetic scheme of the assembly and defined the parameters of the master equations using single kinesin properties. One of the principle findings of this model is that equal load sharing between the motors is not possible, unless the strain energy of the assembly is almost zero. The load on the leading motor rises with increasing intermotor distance. In addition, the force-velocity and the force-run length diagrams have shown that: (i) weakly coupled kinesins produce larger mean run length compared to that of single kinesin, whereas strongly coupled motors generate a mean run length almost equal to that of the single motor. The mean run length of the complex is found to be less dependent on the coupling strength between the motors, compared to that under resisting loads when external force is above ~ 2 pN, and (ii) weakly coupled kinesins transport the cargo faster than single kinesin and strongly coupled motors under resisting forces.

In summary, this thesis proposed a theoretical approach that integrates the properties of single kinesin motor into a predictive theory for the cooperative cargo transport by two kinesin motors. Our theoretical results are consistent with the available experimental data for single kinesin and two coupled kinesins. To date, little is known experimentally about the coordination of motors within a team, as controlling the number and composition of motors attached to cargo is difficult (Furuta et al. 2013). The comparison of theoretical predictions and experimental observations can provide new insights into mechanistic details which are not directly accessible in experiments, such as the behaviour of multiple kinesins at limiting [ATP] and/or at extreme external load.
forces, and with various motor stiffness values. This would help us to understand the mechanism of cargo transport by several motors \textit{in vivo}.

\section*{7.2 Outlook}

We presented general theoretical frameworks to study the biophysical fundamentals of intracellular mechanisms of cargo transport by molecular motors over various loads and [ATP]. Since these models have been developed based on the kinetic scheme of an individual stepping cycle of single kinesin extracted from experiments, the models can be easily adjusted to study the motility of single unconventional myosin (or cytoplasmic dynein) and a team of unconventional myosins (or cytoplasmic dyneins) using the stepping kinetic scheme of the corresponding motor. Regarding two coupled kinesin motors carrying a common cargo, our theory considered the dynamics of the cargo transport at saturating [ATP], based on the recent experimental data from Block laboratory (Andreasson et al. 2015; Milic et al. 2014). Once experimental data for the detachment rate of single kinesin at limiting [ATP] is available, the proposed model in this thesis can be used to predict the dynamic of cargo transport by two coupled kinesins at low [ATP]. In general, as more single molecule experimental data are produced, our theory can be extended to provide more predictions for the fundamentals of intracellular mechanisms of cargo transport by molecular motors. The results of our proposed bottom-up methodology to study the cargo transport \textit{in vivo}, would be useful for the investigation of pathology of cargo transport related to diseases (Hirokawa and Takemura 2003) and for the construction of hybrid bionanodevices integrating molecular motors (Agarwal 2009; Korten et al. 2010; Van Den Heuvel and Dekker 2007). These bionanodevices are expected to work in a lab on a chip as shuttles for drug delivery within organisms (Müller et al. 2010).
A.  Appendix

A.1 Fit Equation to Kinesin Unbinding Rate Data

In this section, we obtain an equation for the detachment rate of single kinesin over resisting and assisting loads at [ATP] = 2000 µM by fitting to the recent experimental data in (Andreasson et al. 2015). Based on the data shown in Figure 2.4, we assume that the vertical component of the external force leads to a sharp increase in the detachment rate of single kinesin when the load changes from resisting to assisting. Therefore, we use the following exponential form to calculate the detachment rate of single kinesin:

$$\omega_1(F) = \omega_1(0) \exp \left( \frac{\vec{F} \cdot \vec{d}}{k_B T} \right)$$  \hfill (A.1)

where $\omega_1(0)$ is the unloaded detachment rate and, $\vec{F}$ and $\vec{d}$ are vectors of force and its characteristic distances, respectively. The dot product of $\vec{F}$ and $\vec{d}$, denoted as $\vec{F} \cdot \vec{d}$, is extended as follows:

$$\vec{F} \cdot \vec{d} = F_x \times d_x + F_y \times d_y$$  \hfill (A.2)

where $F_x$ (and $F_y$) and $d_x$ (and $d_y$) are the horizontal (and vertical) components of vectors $\vec{F}$ and $\vec{d}$, respectively. $F_x$ is the load parallel to MT shown in the data in Figure (2.4). Thus, we represent $F_y$ as a function of $F_x$ as follows:

$$\vec{F} \cdot \vec{d} = F_x \times d_x + F_x \times \tan(\frac{\theta_F}{F_x}) \times d_y$$  \hfill (A.3)

where $\theta_F$ is the angle between $\vec{F}$ and MT. Inserting Equation (A.3) into Equation (A.1), we obtain:

$$\omega_1(F_x) = \omega_1(0) \exp \left( \frac{F_x}{k_B T} \left( d_x + d_y \times \tan(\frac{\theta_F}{F_x}) \right) \right)$$  \hfill (A.4)

Experimentally obtained data in Figure (2.4) indicates a two-phase rise in the unbinding rate of kinesin over assisting loads. Accordingly, we use Heaviside step function $\Theta(F)$
and define a critical force $F_c$ indicating the force point at which the phase change of unbinding rate occurs. Inserting $\Theta(F)$ into Equation (A.4), we obtain:

$$\omega_t(F_x) = k_1 \Theta(F_x - F_c) \exp \left\{ \frac{|F_x|}{k_B T} \left( d_{x1} + d_{y1} \tan \left( \frac{\theta_1 F_x}{|F_x|} \right) \right) \right\} + k_2 \Theta(F_c - F_x) \exp \left\{ \frac{|F_c|}{k_B T} \left( d_{x2} + d_{y2} \tan \left( \frac{\theta_2 F_c}{|F_c|} \right) \right) \right\}$$  \hspace{1cm} (A.5)

and by fitting Equation (A.5) to experimental data shown in Figure 2.4 we estimate the parameters as: $k_1 \sim 0.897 \text{ s}^{-1}$, $\delta_{x1} \sim 1.115 \text{ nm}$, $\delta_{y1} \sim 1.060 \text{ nm}$, $\theta_1 \sim 1.026^\circ$, $F_c \sim 1.90 \text{ pN}$, $k_2 \sim 8.037 \text{ s}^{-1}$, $\delta_{x2} \sim 0.182 \text{ nm}$, $\delta_{y2} \sim 0.152 \text{ nm}$, and $\theta_2 \sim 0.572^\circ$. The behaviour of our fit Equation (A.5) versus load is shown in Figure A.1.

**Figure A.1.** Fitted function to kinesin unbinding rate data versus load at [ATP] = 2000 $\mu$M. Red points show the experimentally measured data points in (Andreasson et al. 2015) captured from Figure 2.4. The blue curve indicates the behaviour of our fit Equation (A.5).

To avoid the complexity of the different angles of binding of kinesins to MT, we only consider forces parallel to MT similar to a typical gliding assay. Therefore, we define the detachment rate of single kinesin over resisting and assisting forces $F$ parallel to MT at [ATP] = 2000 $\mu$M as follows:

$$\omega_t(F) = k_1 \Theta(F_c - F) \exp \left\{ \frac{|F| d_{x1}}{k_B T} \right\} + k_2 \Theta(F - F_c) \exp \left\{ \frac{|F| d_{x2}}{k_B T} \right\}$$  \hspace{1cm} (A.6)

The behaviour of Equation (A.6) versus load is shown in Figure 6.2.
A.2 Force Distribution between Two Motors

In this section, we describe the distribution of the external force $F_{ex}$ applied to an assembly of two kinesins carrying a common cargo. The difference between our Equations (6.8) and (6.9) and Lipowsky and colleagues’ calculations in Equations (2.17) and (2.18) emerges from the distribution of $F_{ex}$ on two motors when there is no elastic force between the motors. In this configuration, our Equations (6.8) and (6.9) state that $F_{ex}/2$ is exerted onto each motor, whereas Equations (2.17) and (2.18) state that $F_{ex}$ is exerted onto the leading motor and the load exerted onto the trailing motor is zero. In the absence of elastic force between the motors (i.e. none of the motors steps), the assembly of motors is equivalent to a structure in which two identical springs in series with stiffness $k$ are connected to fixed points at both ends (see Figure A.2).

![Two springs in series connected to fixed points 1 and 2 and the external force $F_{ex}$ is applied to the centre of the structure, i.e. the cargo position.](image)

**Figure A.2**. Two springs in series connected to fixed points 1 and 2 and the external force $F_{ex}$ is applied to the centre of the structure, i.e. the cargo position.

In the presence of an external force $F_{ex}$ applied to the centre of the structure (i.e. the cargo position), the force $F_0$ exerted onto the centre of the structure is calculated as follows:

$$F_0 = -F_{ex} - F_{10} + F_{20}$$  \hspace{1cm} (A.7)

where $F_{ij}$ is the force that point $i$ exerts onto point $j$ calculated as follows:

$$F_{10} = k\delta l; \quad \delta l \leq 0$$  \hspace{1cm} (A.8)

$$F_{20} = k\delta l; \quad \delta l \geq 0$$  \hspace{1cm} (A.9)

where $\delta l$ is the deviation of the spring length from its rest length. Considering mechanical force equilibrium, we obtain:

$$-F_{ex} + k\delta l + k\delta l = 0$$  \hspace{1cm} (A.10)
showing that

\[ k \Delta y = \frac{F_{ex}}{2} \]  \hspace{1cm} (A.11)

Equation (A.11) indicates that the force exerted on each of points 1 and 2 in the structure in Figure A.1 is equal to \( F_{ex}/2 \). Therefore, we present the force distribution on each motor as \( F_{ex}/2 \) when the motors linkers are relaxed (see Equations (6.8) and (6.9)).
Bibliography


Berger, F. 2012. Different Modes of Cooperative Transport by Molecular Motors, Max Planck Institute of Colloids and Interfaces.


