

## Abstract

GOEDECKE, DAVID MICHAEL Stochastic Modeling of the Behavior of Dynein.  
(Under the direction of Dr. Timothy C. Elston).

Molecular motors are proteins that convert stored energy into physical work inside cells, and thus are the engines that drive many cellular functions. An individual motor can be studied using a laser trap to measure its response to working against an external force. Axonemal dynein is the molecular motor responsible for the rhythmic beating of eukaryotic cilia and flagella. An individual axonemal dynein molecule is capable of both unidirectional, processive motion and bidirectional motion when placed under a load (Shingyoji et al., 1998). This capability may be an important underlying factor in the mechanism for flagellar and ciliary motion. A detailed stochastic model is proposed which links the physical motion of a two-headed dynein molecule to the biochemical steps of its ATP hydrolysis cycle. Forward motion is driven by ATP hydrolysis, while backward motion is due to a passive process of biased diffusion. The model exhibits both processive and bidirectional behaviors. A simplified model which can be more easily analyzed is derived, as is an alternate version which steps backward actively, rather than sliding passively. The simplified models are then used to predict motor characteristics such as the load-velocity profile, the stall force, and the effective diffusion coefficient, which can be determined experimentally and used to distinguish among competing mechanisms.

# **Stochastic Modeling of the Behavior of Dynein**

by

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To my wife, María Antonieta Caicedo

## Biography

I was born March 22, 1965 to Jerome E. and Nancy E. Goedecke. I grew up in Hudson, Massachusetts, where I graduated from Hudson High School in 1983. I received a Bachelor of Science degree in Biology from the California Institute of Technology in 1988. After graduation, I worked for the biotechnology firm Amgen, Inc. in California for almost 10 years, at first in Manufacturing and then in Process Development. It was at Amgen that I met my wife, María Antonieta, whom I married in 1997.

We moved to North Carolina in 1998, and I started taking classes at North Carolina State University that fall. I formally entered the Biomathematics program in 1999 and received my Master of Biomathematics degree in 2001. My outside interests include travelling, gardening, and spoiling our Portuguese Water Dogs, Siena and Firenze.

## Acknowledgements

I could not have completed this dissertation without the help and support of many other people. My advisor, Tim Elston, has always been positive and supportive, and has worked hard to keep me motivated and on track. Dr. Chikako Shingyoji, whose experimental results I have tried to model, provided valuable insight and background information about her work. Thanks also to my other committee members, Sharon Lubkin, Carla Mattos, John Monahan, and Kevin Gross, for their patience, flexibility, and advice. Thanks to my fellow Biomathematics students, especially Jason Pirone, Dan Dougherty, and Stacy Sherling. Most importantly, I want to thank my wife, María Antonieta, who has given me the love and support I needed to get this done.

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# Chapter 1

## Introduction

### 1.1 Molecular Motors

Living creatures, from single-celled bacteria to humans, are composed of complex biological systems that must function in a coordinated way in order to maintain life, even at cellular and subcellular levels. Nutrients must be brought into the system, new compounds and structures must be synthesized, and waste products must be excreted. Some of the processes involved are passive and spontaneous, depending only on the proximity and concentrations of the components involved, but most require active work to be done in order to be completed in a reasonable length of time and in a coordinated fashion. Molecular motors are the key to running many of these active processes.

Molecular motors are proteins or protein complexes which convert chemical energy into force and motion, in order to perform work at the cellular or subcellular level. Some motors obtain their chemical energy by harnessing a concentration gradient across a membrane. One example is the eukaryotic protein ATP synthase, which uses the proton gradient found across the inner membrane of mitochondria to produce molecules of adenosine triphosphate (ATP), the fuel for many other reactions within

cells. Other motors hydrolyze ATP or other nucleotides to power their main functions. RNA polymerase, for example, synthesizes a new RNA strand from a DNA template by hydrolyzing the polyphosphate bond of a ribonucleotide. The released energy is used to attach the ribonucleotide base to the growing RNA polymer and move the enzyme to the next position on the DNA template.

Some molecular motors are specifically responsible for generating motion, propelling the cell itself, the medium surrounding the cell, or cargo within the cell. The bacterial flagellar motor is an extremely complicated structure made up of many proteins which drives the propeller-like motions of bacterial flagella. This allows the bacteria to swim from one region to another in search of more favorable living conditions, such as higher nutrient concentrations or lower toxin levels. The sliding of myosin along actin filaments causes the muscle contraction necessary for vertebrate movements, although actin and myosin also play many other structural and contractile roles within cells, and are found in almost all eukaryotes. Kinesin is a eukaryotic protein that is important for directed intracellular cargo transport, as are cytoplasmic forms of dynein. Other dyneins are responsible for the movement of eukaryotic cilia and flagella. In the respiratory tract, irritants are cleared by a flow of mucus caused by the coordinated movement of cilia. Flagellar motion propels a swimming protozoan or sperm cell through its environment.

## 1.2 The System to be Modeled

Ciliary dynein was the first microtubule-based molecular motor to be isolated (Gibbons and Rowe, 1965), roughly 20 years before kinesin or cytoplasmic dynein (Vale et al., 1985; Brady, 1985; Paschal et al., 1987). Dyneins are much larger, more complicated motors than either kinesin or myosin, making them harder to understand and model than the other cytoskeletal motor proteins. In recent years however, technological advances such as the laser trap have allowed the physical properties of individual protein molecules to be studied. Their static and dynamic responses to challenges such as pulling against an outside force can be recorded and analyzed. With such

physical response data added to the still growing body of structural and biochemical information, more realistic, detailed models of how dynein functions are now possible.

Using a laser trap to study dynein extracted from sea urchin sperm flagella, Shingyoji et al. (1998) observed processive motion along a microtubule under small external loads. Surprisingly, at larger loads a back and forth or “oscillatory” motion was observed. Neither behavior had been observed before for dynein, and the oscillatory motion appears to be unique to dynein.

The focus of this dissertation, modeling the behavior of a two-headed flagellar dynein, was chosen for several reasons. Dynein has not been as well-studied and understood as other molecular motors. The problem was intellectually attractive because the experimental behavior observed by Shingyoji et al. (1998) had not been seen before, and is perhaps unique. Learning how dynein moves may someday lead to help for those with certain pulmonary diseases caused by dysfunctional respiratory cilia or infertility problems caused by immotile sperm.

Information about the biochemical and mechanical properties of the protein as well as other physical and thermodynamic constraints are considered in the models presented. The remainder of this introductory chapter will discuss the biological details of dynein and describe the physical details of the experimental system that was used. Chapter 2 will present some of the mathematical background for the modeling, focusing on an explanation of the physical phenomenon of Brownian motion and on the analysis of chemical reaction systems. A detailed model to explain and predict the dynein’s behavior will be developed and discussed in Chapter 3, and then a simplified version of that model will be presented in Chapter 4. While the simplified model necessarily loses some level of detail, it gains significantly in computational speed and analytic tractability. An alternative model using an active back movement mechanism will be explored briefly, with the results and predictions compared to those of the preferred model in Chapter 5. The main proposed model will also be compared and contrasted with a model published by Brokaw (2000). Finally, the dissertation will close with conclusions and suggestions for future experimental and modeling work, in Chapter 6.

### 1.3 Biological Background

Eukaryotic cilia and flagella have very similar, well-conserved structures. An outer membrane covers a central shaft, the axoneme, which is composed of nine sets of doublet microtubules surrounding a central sheath and two single microtubules. Each doublet microtubule has the cross-section of a figure eight. The smaller, circular shaft is known as subfiber A and the larger, incomplete shaft is known as subfiber B. The outer pairs of microtubules are each connected by flexible links to the central sheath and to the neighboring doublet microtubules. Arrays of dynein molecules are anchored along the length of each subfiber A. Each dynein molecule has either two or three arms which are able to interact transiently with subfiber B of the adjacent microtubule doublet, moving from binding site to binding site. See Figure 1.1 for a cross-sectional view of an axoneme.

Flagellar beating is generated by the coordinated actions of these arrays of dynein molecules within the axonemes. Dynein hydrolyzes ATP and uses the released energy to move along the adjacent microtubule, thereby sliding the microtubule doublets past each other. Because the microtubules are anchored at the base of the flagellum, the sliding motion is converted into a bending of the flagellar shaft. Waves of flagellar bending propel the sperm, alga, or other swimming cell through its environment.

Cytoplasmic dyneins are similar to axonemal dyneins. They usually have two identical arms which can bind to sites along a microtubule track, and they hydrolyze ATP to power their motion. However, rather than being anchored at their base to a single microtubule, cytoplasmic dyneins bind and transport a variety of intracellular cargos. Cytoplasmic dynein is responsible for separating chromosomes during mitosis as well as the minus-end-directed transport of organelles and vesicles along the microtubule tracks of the cytoskeleton.

Dyneins are large proteins, generally with a molecular weight of 1 to 2 MDa, made up of several subunits. Each dynein arm is composed of a large heavy chain subunit with a molecular weight of 500 kDa or more, and various smaller intermediate chain and light chain subunits. Each heavy chain has a large globular head containing the ATP binding and hydrolysis sites. Extending from the head is a large stem

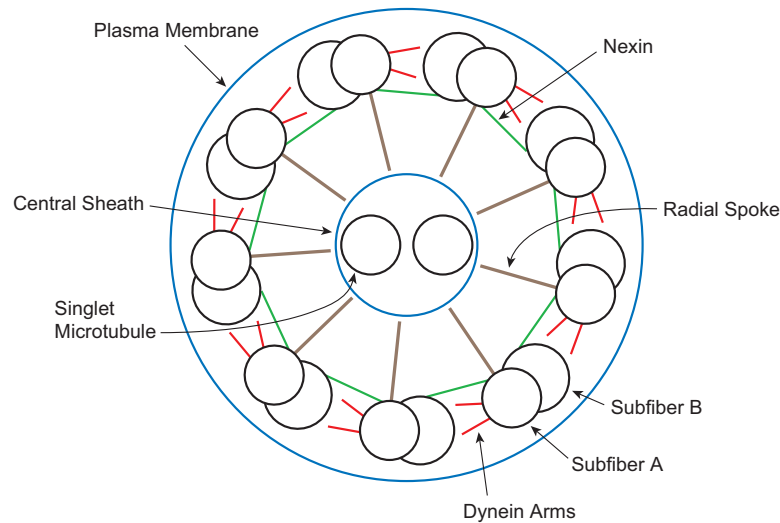


Figure 1.1: Schematic cross-section of an axoneme. Nine sets of doublet microtubules are arranged around a central sheath containing two singlet microtubules. Dynein molecules are anchored to subfiber A of each doublet and can interact with subfiber B of the adjacent microtubule. The microtubule doublets are flexibly connected to the central sheath by radial spokes, and to the neighboring doublet by nexin.

that interacts with other heavy chains and with the intermediate and small chain subunits to hold the dynein together and anchor it to the microtubule (in the case of axonemal dynein) or attach it to the intracellular cargo to be transported (in the case of cytoplasmic dynein). Also extending from the head is a small stalk, composed of two antiparallel coiled coils with a small globular region between them which is responsible for microtubule binding (Gee et al., 1997). A complete dynein molecule has the shape of a bunch of flowers held together by the stems.

The structure of the head region causes dynein to be classified as part of the AAA (ATPases associated with various cellular activities) family of proteins (Neuwald et al., 1999). These proteins form a large and diverse group with a high degree of sequence conservation and structural similarity in their ATP hydrolyzing domains (Vale, 2000). They have six nucleotide binding regions, which assemble into a hexameric ring structure. Dynein is unusual in that all six AAA domains are found within one polypeptide chain, rather than in separate subunits. The head forms a heptameric ring from the six AAA domains and a seventh domain from which the

microtubule binding stalk extends (Samsó et al., 1998). The amino acid sequences of two of dynein's AAA domains are much less conserved than the others, and do not have the ability to bind nucleotide. Dynein's four other AAA domains are better conserved. At least two of them, and possibly all four, are able to bind nucleotides at physiological concentrations, although apparently only one binding site is able to hydrolyze ATP at an appreciable rate (Mocz and Gibbons, 1996). It is likely that the other three sites have some regulatory function (Mocz et al., 1998; Shiroguchi and Toyoshima, 2001; Reck-Peterson and Vale, 2004). Regulation of dynein activity may also be achieved by  $\text{Ca}^{2+}$ -binding or by phosphorylation of certain amino acid residues within the dynein heavy chain (King and Patel-King, 1995).

## 1.4 Experimental System and Results

The mechanical properties of dynein and other motor proteins can be studied *in vitro* at the level of individual protein molecules. A laser trap, or 'optical tweezers', is a device which allows an experimenter to capture a molecule in a microscope field and measure the load force against which that molecule or a motor attached to it can perform work (Block, 1992). The trap functions by tightly focusing a laser beam in the specimen plane of a microscope in such a way that a light gradient is set up. This gradient attracts a bead, usually made of glass, polystyrene, or latex, which is attached to a molecule of interest. When the bead is displaced from the center of the trap, the light gradient induces a restoring force. For small displacements, the laser trap behaves as a linear spring, obeying Hooke's Law:  $F = -\kappa x$ , where  $F$  is the force generated,  $\kappa$  is the spring constant of the trap, and  $x$  is the displacement of the bead. Because physical quantities such as the position of the bead can be measured repeatedly at high resolution over time, other quantities such as the velocity of the bead, the variance of the bead's position, and the load imposed by the trap can also be determined. From those data and an estimation of the spring constants of the connections between the bead and the attached molecules, the movements of the molecules themselves can be derived. Data from such tests can be used to infer and

model the underlying mechanisms of the molecular motors' mechanochemical cycles.

Shingyoji et al. (1998) performed such experiments on dynein molecules obtained from sperm flagella of the sea urchin *Hemicentrotus pulcherrimus*. In their experimental setup, diagrammed in Figure 1.2, a microtubule doublet with exposed dynein arms was attached to a microscope slide. A singlet microtubule with an attached latex bead was then brought into contact with the dynein. When ATP was released into the system, the dynein activated and began moving along the singlet microtubule. Because the dynein was anchored to the microscope slide, this motion caused the microtubule and the bead to move against the restoring force of the laser trap.

Shingyoji's group observed that at loads close to zero, the forward motion of the bead was processive — that is, the dynein made multiple consecutive steps in the same direction without detaching from the microtubule. As the resulting trap force increased, however, the bead's motion began to fluctuate, with the dynein repeatedly moving several steps forward before changing direction under the influence of the trap and moving a distance equivalent to several steps backward. Neither the processive motion nor the multiple step fluctuations had been reported previously for axonemal dynein.

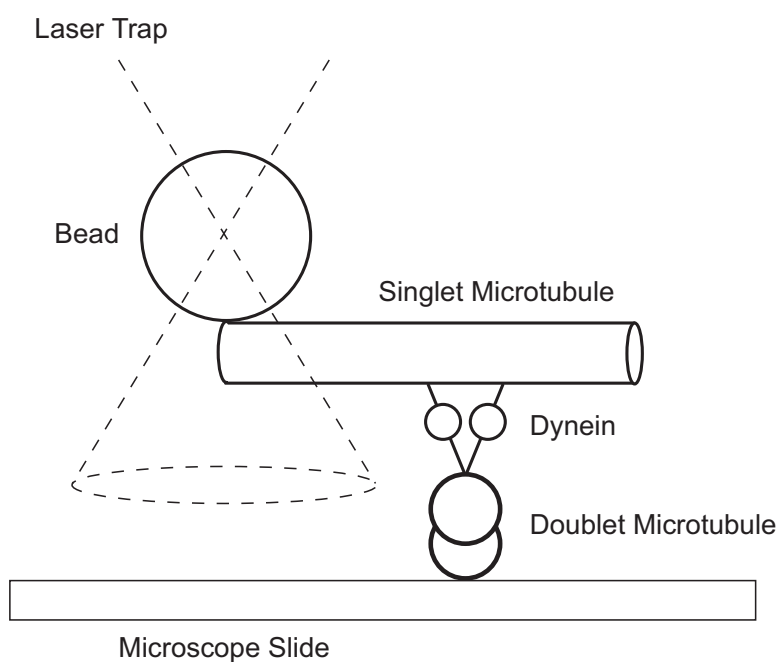


Figure 1.2: Experimental setup. An anchored doublet microtubule with dynein molecules is attached to a glass coverslip. A singlet microtubule is positioned so that both arms of a single dynein molecule can bind to it. Attached to the singlet microtubule is a  $1\text{ }\mu\text{m}$  diameter bead which can be caught and manipulated by a laser trap. When ATP is released into the system, the dynein is activated. Because the dynein is anchored, it moves the microtubule and bead against the resistive force of the laser trap.

# Chapter 2

## Mathematical Analysis of

## Brownian Motion and Chemical

## Reaction Systems

On the molecular scale at which dynein and other motor proteins operate, the physical phenomenon of Brownian motion greatly affects the mechanics of the motor and must be included properly in any model at this scale. The series of chemical steps in dynein's ATP hydrolysis cycle must also be accounted for correctly. This chapter will therefore provide mathematical background for modeling both Brownian motion and chemical reaction systems, laying much of the theoretical foundation for the work presented in the remaining chapters of this dissertation.

## 2.1 Brownian Motion

Brownian motion is the name applied to the rapid, seemingly random movement of particles suspended in a fluid that is in thermal equilibrium. This phenomenon can be seen most easily at a macroscopic scale by watching dust particles in a shaft of sunlight. It is named for the botanist Robert Brown, who studied it extensively in 1827 (Brown, 1828), although the phenomenon had been discovered at least as early as 1784, when it was described by Jan Ingenhousz. In 1905, Albert Einstein provided the first satisfactory mathematical explanation of Brownian motion. He showed that it was related to diffusion, which was already mathematically well understood (Einstein, 1905). Marian von Smoluchowski independently derived the same analysis, publishing it in 1906 (von Smoluchowski, 1906). In 1908, Paul Langevin published a different, more direct approach that confirmed Einstein's results. In his analysis, Langevin introduced the first stochastic differential equation (SDE), the general form of which now bears his name (Langevin, 1908).

### 2.1.1 Einstein's Analysis of Brownian Motion

Physically, Brownian motion occurs because a small particle is buffeted by the frequent, uncorrelated impacts of the much smaller particles making up the surrounding fluid, as shown in Figure 2.1. Einstein focused on describing the position of such a Brownian particle. He began by assuming that the motions of all the particles are independent of each other, and that the motions of the same particle within two different time intervals are also independent provided that the intervals are not chosen to be too small.

Let  $\tau$  be the length of a time period that is very small compared to observable intervals, but large enough that movements of the same particle within two consecutive periods of length  $\tau$  are mutually independent. Assume that there are  $N$  total suspended particles in the fluid. Then in a period of length  $\tau$ , the  $x$  position of each particle will change by some amount  $\delta$ . For each particle,  $\delta$  will have a different value and may be either positive or negative. Within the interval  $\tau$ , the number of particles

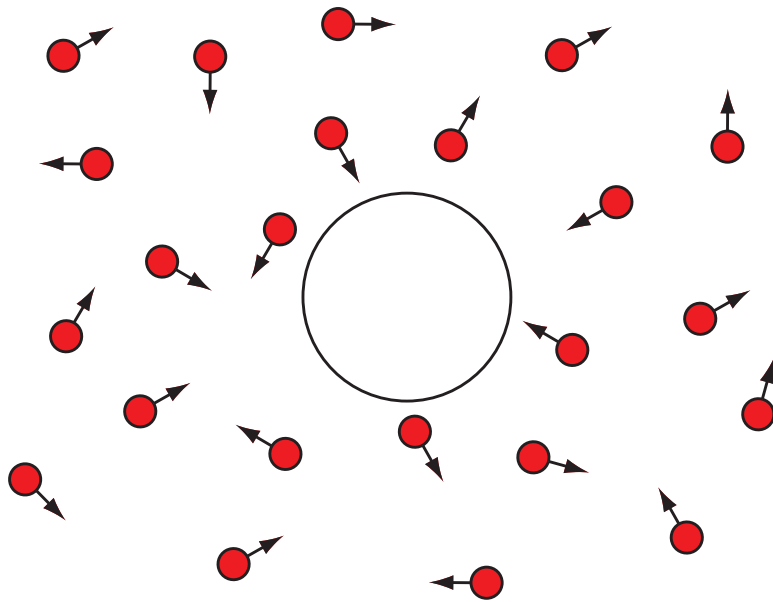


Figure 2.1: Cartoon of Brownian motion. A macroscopically small, but microscopically large, particle is continually buffeted by independent impacts of the much smaller particles making up the surrounding fluid. Einstein showed that the mean squared displacement of the larger particle in the  $x$  direction is  $E[x^2] = 2Dt$ , where  $D$  is the diffusion coefficient of the particle.

$dN$  with a change in position of between  $\delta$  and  $\delta + d\delta$  can be written as

$$dN = Np(\delta) d\delta \quad , \quad (2.1)$$

where  $p(\delta)$  is a symmetric probability density function centered at  $\delta = 0$ . Thus  $p(\delta) \neq 0$  only for very small values of  $\delta$ ,  $p(-\delta) = p(\delta)$ , and

$$\int_{-\infty}^{\infty} p(\delta) d\delta = 1 \quad . \quad (2.2)$$

Now let  $n = f(x, t)$  be the number of particles per unit volume. Then at time  $t + \tau$ , the number of particles which are between two planes perpendicular to the  $x$ -axis located at  $x$  and  $x + dx$  is given by

$$f(x, t + \tau) dx = dx \int_{-\infty}^{\infty} f(x + \delta, t) p(\delta) d\delta \quad . \quad (2.3)$$

Because  $\tau$  is very small, we can expand the Taylor's series for  $f(x, t + \tau)$  and write

$$f(x, t + \tau) = f(x, t) + \tau \frac{\partial f(x, t)}{\partial t} + O(\tau^2) \quad , \quad (2.4)$$

where  $O(\tau^2)$  denotes terms of order  $\tau^2$  and higher, which are negligible compared to terms of order  $\tau$  and can be dropped.

We can also expand the Taylor's series for  $f(x + \delta, t)$  to get

$$f(x + \delta, t) = f(x, t) + \delta \frac{\partial f(x, t)}{\partial x} + \frac{\delta^2}{2!} \frac{\partial^2 f(x, t)}{\partial x^2} + \dots \quad . \quad (2.5)$$

Only very small values of  $\delta$  contribute to the series, which can thus be brought under the integral sign. Substituting equations 2.4 and 2.5 into equation 2.3,

$$\begin{aligned} f(x, t) + \tau \frac{\partial f(x, t)}{\partial t} &= f(x, t) \int_{-\infty}^{\infty} p(\delta) d\delta + \frac{\partial f(x, t)}{\partial x} \int_{-\infty}^{\infty} \delta p(\delta) d\delta \\ &\quad + \frac{\partial^2 f(x, t)}{\partial x^2} \int_{-\infty}^{\infty} \frac{\delta^2}{2} p(\delta) d\delta + \dots \quad . \end{aligned} \quad (2.6)$$

On the right-hand side, the second, fourth, etc., terms vanish because  $p(-\delta) = p(\delta)$ . At the same time, the first, third, fifth, etc., terms each decrease rapidly compared with the preceding term. Substituting from equation 2.2 and keeping only the  $f(x, t)$  and  $\partial^2 f(x, t)/\partial x^2$  terms, we obtain

$$\tau \frac{\partial f(x, t)}{\partial t} = \frac{\partial^2 f(x, t)}{\partial x^2} \int_{-\infty}^{\infty} \frac{\delta^2}{2} p(\delta) d\delta \quad . \quad (2.7)$$

If we now set

$$D = \frac{1}{\tau} \int_{-\infty}^{\infty} \frac{\delta^2}{2} p(\delta) d\delta \quad , \quad (2.8)$$

we obtain

$$\frac{\partial f(x, t)}{\partial t} = D \frac{\partial^2 f(x, t)}{\partial x^2} \quad . \quad (2.9)$$

Einstein recognized this as the diffusion equation, which was already well understood, with the diffusion coefficient  $D$  given by equation 2.8. He thus concluded that Brownian motion corresponds to diffusion. For a group of  $N$  particles diffusing out from a single point with an initial position of  $x(t=0) = 0$ , the probability density function of the particles at time  $t$  is given by the solution of equation 2.9,

$$f(x, t) = \frac{N}{\sqrt{4\pi Dt}} \exp\left(-\frac{x^2}{4Dt}\right) \quad . \quad (2.10)$$

From this equation, the variance of the particle's displacement  $x$  is

$$\sigma^2(x) = 2Dt \quad , \quad (2.11)$$

so that the root-mean-square displacement of the particles is  $\sigma(x) = \sqrt{2Dt}$ .

Equation 2.9 describes diffusion from a stationary point. If the particles are drifting with some velocity  $v(x, t)$  as well as diffusing, the equation becomes

$$\frac{\partial f(x, t)}{\partial t} = -v(x, t) \frac{\partial f(x, t)}{\partial x} + D \frac{\partial^2 f(x, t)}{\partial x^2} \quad . \quad (2.12)$$

The diffusion equation is actually a special case of the Fokker-Planck equation, which can be used to describe a large class of stochastic processes with continuous sample paths. Section 2.5 will discuss using the Fokker-Planck equation to approximate the solution of a master equation in order to model a chemical reaction system.

Although we will not present details here, it should be noted that in his Brownian motion paper, Einstein also derived the relationship  $D = k_B T / \zeta$  that links the diffusion coefficient  $D$  with the frictional drag coefficient  $\zeta$  (Einstein, 1905). This equation is often known as the ‘‘Einstein-Smoluchowski relation’’, and is also important in modeling molecular motor behavior.

### 2.1.2 Langevin's Analysis of Brownian Motion

In contrast to Einstein, Langevin started his analysis by focusing on the velocity of the Brownian particle rather than its position. He assumed that the net force acting on a particle of mass  $m$  in the  $x$  direction at time  $t$  could be resolved into two effective forces: a frictional drag force,  $-\zeta v(t)$ , due to the viscosity of the surrounding fluid, and a randomly fluctuating force,  $F(t)$ , due to the incessant impacts of the fluid molecules on the particle. Here  $\zeta > 0$  is the drag coefficient, and  $v(t)$  is the velocity component of the particle in the  $x$  direction at time  $t$ .  $F(t)$  is assumed to have a mean value of zero, with  $F(t')$  and  $F(t)$  uncorrelated for all  $t' \neq t$ , and  $F(t)$  and  $v(t')$  statistically independent for all  $t' \leq t$ . By Newton's second law, the equation of motion for the particle is

$$m \frac{dv(t)}{dt} = -\zeta v(t) + F(t) \quad . \quad (2.13)$$

Multiplying this equation by  $x$ , we have

$$mx \frac{d^2x}{dt^2} = -\zeta x \frac{dx}{dt} + xF(t) \quad . \quad (2.14)$$

If we now calculate  $d(x^2)/dt$  and  $d^2(x^2)/dt^2$  and rearrange terms, we find that

$$\frac{d(x^2)}{dt} = 2x \frac{dx}{dt} \quad \Rightarrow \quad x \frac{dx}{dt} = \frac{1}{2} \frac{d(x^2)}{dt} \quad (2.15)$$

and

$$\frac{d^2(x^2)}{dt^2} = 2x \frac{d^2x}{dt^2} + 2\left(\frac{dx}{dt}\right)^2 \quad \Rightarrow \quad x \frac{d^2x}{dt^2} = \frac{1}{2} \frac{d^2(x^2)}{dt^2} - v^2 \quad . \quad (2.16)$$

Substitution into equation 2.14 yields

$$\frac{1}{2}m \frac{d^2(x^2)}{dt^2} - mv^2 = -\frac{1}{2}\zeta \frac{d(x^2)}{dt} + xF(t) \quad . \quad (2.17)$$

This equation, however, still contains the stochastic term  $xF(t)$ . To average out any unusual effects of a particular trajectory and find the mean behavior of the Brownian particle's motion, we take the expected value of each of the terms in equation 2.17 to obtain

$$\frac{1}{2}m \frac{d^2(E[x^2])}{dt^2} - E[mv^2] = -\frac{1}{2}\zeta \frac{dE[x^2]}{dt} + E[xF(t)] \quad . \quad (2.18)$$

Langevin set the term  $E[xF(t)] = 0$  because of the “irregularity” of  $F(t)$ . Implicitly, he assumed that  $x(t)$  and  $F(t)$  are statistically independent. He also took advantage of a result from statistical mechanics (as did Einstein) that at equilibrium, the mean kinetic energy of a Brownian particle is

$$E\left[\frac{1}{2}mv^2\right] = \frac{1}{2}k_B T \quad \Rightarrow \quad E[mv^2] = k_B T \quad . \quad (2.19)$$

After substitution and rearrangement, equation 2.18 then becomes

$$\frac{d^2(E[x^2])}{dt^2} + \frac{\zeta}{m} \frac{d(E[x^2])}{dt} = \frac{2k_B T}{m} \quad . \quad (2.20)$$

Integrating this equation twice over the period  $(0, t)$  with  $x(0) = 0$  yields

$$\frac{d(E[x^2])}{dt} = \frac{2k_B T}{\zeta} (1 - \exp(-\frac{\zeta}{m}t)) \quad (2.21)$$

$$E[x^2] = \frac{2k_B T}{\zeta} \left( t - \frac{m}{\zeta} [1 - \exp(-\frac{\zeta}{m}t)] \right) \quad . \quad (2.22)$$

A large protein molecule such as dynein has a mass of  $m \approx 10^{-18}$  g, and its frictional drag coefficient in water is on the order of  $\zeta \approx 10^{-7}$  g/s. We can define a time constant  $\tau = m/\zeta$ , which then has a value of  $\tau \approx 10^{-11}$  s.

If we expand Taylor’s series of the exponential term for very short times ( $t \ll \tau$ ), we obtain

$$E[x^2] = \frac{2k_B T}{\zeta} \left( t - \tau \left[ 1 - \left( 1 - \frac{t}{\tau} + \frac{t^2}{2\tau^2} + O(t^3/\tau^3) \right) \right] \right) \quad , \quad (2.23)$$

which then simplifies to

$$E[x^2] = \frac{2k_B T}{m} t^2 \quad \text{for } t \ll \tau \quad . \quad (2.24)$$

The particle moves ballistically with a velocity of  $v = \sqrt{k_B T/m} \approx 2 \text{ m s}^{-1}$ , but only for  $\tau \approx 10^{-11}$  s, and so it travels a distance of only  $v\tau \approx 0.01 \text{ nm}$  before its next collision. It therefore quickly comes to thermal equilibrium with the surrounding fluid. For long times ( $t \gg \tau$ ), the exponential term goes to zero and equation 2.22 reduces to

$$E[x^2] = \frac{2k_B T}{\zeta} t \quad \text{for } t \gg \tau \quad . \quad (2.25)$$

If we now substitute  $D = k_B T / \zeta$ , we again see that the mean square displacement of the Brownian particle is

$$\mathbb{E}[x^2] = 2Dt \quad \text{for } t \gg \tau, \quad (2.26)$$

in agreement with Einstein's result from equation 2.11. Langevin's method is more direct than Einstein's, however, and provides a simple way to generalize a deterministic equation to a stochastic one.

If there is an external force  $F_{\text{ext}}$  acting on the particle, it can be added to equation 2.13 to obtain

$$m \frac{dv(t)}{dt} = -\zeta v(t) + F_{\text{ext}}(x, t) + F(t) \quad . \quad (2.27)$$

Internal forces generated by a molecular motor such as dynein can be added in the same way. Such forces can be characterized by a potential:  $F_{\text{ext}}(x, t) = -\partial\phi_{\text{ext}}(x, t)/\partial x$ , so the general form of the Langevin equation describing Brownian motion becomes

$$m \frac{dv(t)}{dt} = -\zeta v(t) - \frac{\partial\phi(x, t)}{\partial x} + F(t) \quad . \quad (2.28)$$

This corresponds to the diffusion with drift described by equation 2.12.

Like Einstein's, Langevin's analysis of Brownian motion has been generalized and extended to study a larger class of stochastic processes. As will be shown in Section 2.6, one application is to model a chemical reaction system by approximating the solution of its master equation.

## 2.2 Chemical Reaction Systems

There are two basic ways to mathematically analyze systems of coupled chemical reactions: the deterministic approach and the stochastic approach. Deterministic methods model the concentrations of the chemical species, treating them as continuous variables that evolve according to ordinary differential equations (ODEs). These ODEs are often referred to as rate equations. Deterministic methods therefore model

the average behavior of the system. Stochastic methods capture the inherent randomness of biochemical reactions. These models consider the number of molecules of each chemical species rather than the concentrations, treating them as discrete random variables. The time-dependent probabilities for the numbers of molecules evolve according to a differential equation known as a ‘master’ equation.

The traditional approach to modeling a well-mixed system of  $n$  chemical species  $\{\mathcal{X}_1, \mathcal{X}_2, \dots, \mathcal{X}_n\}$  which can undergo a specified set of reactions is to create a set of rate equations for the concentration of each of the chemical species:

$$\begin{aligned} \frac{d[\mathcal{X}_1]}{dt} &= f_1([\mathcal{X}_1], [\mathcal{X}_2], \dots, [\mathcal{X}_n]) \\ \frac{d[\mathcal{X}_2]}{dt} &= f_2([\mathcal{X}_1], [\mathcal{X}_2], \dots, [\mathcal{X}_n]) \\ &\dots \\ \frac{d[\mathcal{X}_n]}{dt} &= f_n([\mathcal{X}_1], [\mathcal{X}_2], \dots, [\mathcal{X}_n]) \quad , \end{aligned} \tag{2.29}$$

in which the functions  $f_i$  are determined by the forms and rates of the reactions which can occur in the system, and  $[\mathcal{X}_i]$  represents the concentration of substance  $\mathcal{X}_i$ . Solving the ODEs gives the concentration of each species as a function of time.

The deterministic approach often provides important information about the mean behavior of the chemical species in the system over time, although it provides no information about the statistical variability of the system. It is frequently considered easier to implement and is therefore more commonly used than the stochastic approach. However, deterministic methods are not appropriate when fluctuations are important, or when the number of molecules is very small, as often happens in biological systems. Inside a cell for example, there may be only a few copies of a particular regulatory protein, so small fluctuations in number may have a large impact on the state of the system. For single molecule experiments, such as those performed by Shingyoji et al., it clearly does not make sense to talk about a concentration.

Even in chemical reaction systems whose mean behavior is well-described by ordinary differential equations, the behavior of a real system will deviate from the mean because the underlying processes are actually discrete and stochastic. For example,

in the chemical reaction



the number of molecules of substances  $\mathcal{X}_1$  and  $\mathcal{X}_2$  can change only by integer amounts. Also, by tracking only the number of molecules of each species and not the position and velocity of each molecule, we discard information about the system which is necessary to calculate whether particular molecules react with each other, and at what times. The complete system of molecular quantities, positions, and velocities may be deterministic, but the reduced system is not. To compensate, and to keep the problem tractable, we generally assume that the system is well-mixed — that nonreactive collisions between molecules occur much more frequently than reactive collisions and that individual reactions happen randomly throughout the system.

The assumption of a well-mixed system allows us to state that the probability of a particular reaction  $R$  happening within an infinitesimal time interval  $[t, t + dt)$  is approximately proportional to  $dt$ . That is,

$$\Pr(\text{reaction } R \text{ occurs in } [t, t + dt)) = a_R \cdot dt + o(dt) \quad , \quad (2.31)$$

where the reaction rate  $a_R$  is independent of  $dt$  but may depend on the present number of molecules  $X_i(t) = x_i$  of each species  $\mathcal{X}_i$ <sup>1</sup> and on the current time  $t$ , and  $o(dt)$  signifies terms that are negligible for small values of  $dt$ , such as the probability of more than one reaction happening within the time period  $[t, t + dt)$  (Gillespie, 1992b). Let the vector  $\mathbf{x} = (x_1, x_2, \dots, x_n)$  denote the state of the system at time  $t$ , and let  $\mathbf{x}'$  be the state of the system at some other time  $t'$ . Note that the reaction probabilities in equation 2.31 describe the transitions between states of the system, and that while they may depend on the present state, they do not depend on any past state. This property of being ‘memoryless’ defines a stochastic process as a Markov process. It is a powerful property to have, as we can then define everything about the future probabilities of the system in terms of the current state,  $\mathbf{x}$ , and the transition probabilities,  $\Pr(\mathbf{x}', t' \mid \mathbf{x}, t)$ , given that  $t' > t$  (Gardiner, 1985).

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<sup>1</sup>When referring to random variables, uppercase letters (e.g.,  $X$ ) will denote the variable and lowercase letters will denote specific values (e.g.,  $X(t) = x$ ).

## 2.3 The Master Equation

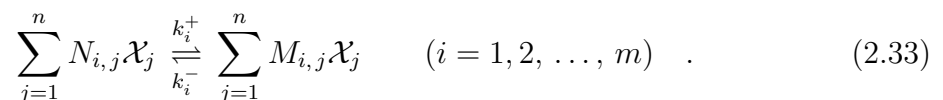
Stochastic modeling approaches directly account for the discrete, random nature of the variables involved in a system, whether it is a set of chemical reactions or some other system of interacting components. For concreteness, we will focus on a chemical reaction system in this section.

The master equation is a differential equation which exactly describes the time evolution of the probability of the system's being in a given state  $\mathbf{x}$  at time  $t$ . It does so by summing the probabilities of all possible one-step transitions into and out of state  $\mathbf{x}$ . For the chemical reaction in equation 2.30, the master equation is

$$\frac{\partial P(x_1, t)}{\partial t} = k(x_1 + 1)P(x_1 + 1, t) - kx_1P(x_1, t) \quad , \quad (2.32)$$

where  $P(x_1, t)$  is the probability that there are  $x_1$  molecules of  $\mathcal{X}_1$  in the system at time  $t$ , and  $k$  is the average rate of converting one molecule from  $\mathcal{X}_1$  to  $\mathcal{X}_2$ . In terms of the variables from equation 2.31, in this case  $a(x_1) = kx_1$ .

Now consider a general, well-mixed system composed of  $n$  species  $\{\mathcal{X}_1, \mathcal{X}_2, \dots, \mathcal{X}_n\}$  which can chemically interact through a set of  $m$  reaction pairs  $\{R_1, R_2, \dots, R_m\}$  with forward rates  $k_i^+$  and backward rates  $k_i^-$  ( $i = 1, 2, \dots, m$ ) (note that irreversible reactions would simply have backward rates equal to zero):



Here  $N_{i,j}$  is the number of molecules of  $\mathcal{X}_j$  involved as reactants in the forward reaction  $R_i^+$ , and  $M_{i,j}$  is the number of molecules of  $\mathcal{X}_j$  involved as products in  $R_i^+$ . Let  $X_j(t) = x_j$  be the number of molecules of species  $\mathcal{X}_j$  in the system at time  $t$ , and let

$$\begin{aligned} \mathbf{X} &= (X_1, X_2, \dots, X_n) \\ \mathbf{x} &= (x_1, x_2, \dots, x_n) \\ \mathbf{N}_i &= (N_{i,1}, N_{i,2}, \dots, N_{i,n}) \\ \mathbf{M}_i &= (M_{i,1}, M_{i,2}, \dots, M_{i,n}) \quad . \end{aligned} \quad (2.34)$$

If we let  $\boldsymbol{\delta}_i = \mathbf{M}_i - \mathbf{N}_i$ , then when reaction  $R_i^+$  occurs,  $\mathbf{x} \rightarrow \mathbf{x} + \boldsymbol{\delta}_i$ , and when  $R_i^-$  occurs,  $\mathbf{x} \rightarrow \mathbf{x} - \boldsymbol{\delta}_i$ . The rate constants for the reactions, in the notation of equation 2.31, are given by

$$a_i^+(\mathbf{x}) = k_i^+ \prod_{j=1}^n \frac{x_j!}{(x_j - N_{i,j})!} \quad (2.35)$$

$$a_i^-(\mathbf{x}) = k_i^- \prod_{j=1}^n \frac{x_j!}{(x_j - M_{i,j})!} \quad (2.36)$$

Note that  $a_i^+(\mathbf{x})$  and  $a_i^-(\mathbf{x})$  are proportional to  $\prod_j \binom{x_j}{N_{i,j}}$  and  $\prod_j \binom{x_j}{M_{i,j}}$ , respectively, which are the number of ways of choosing combinations of  $\mathbf{N}_i$  or  $\mathbf{M}_i$  molecules from  $\mathbf{x}$  molecules. For such a system, the master equation has the form

$$\begin{aligned} \frac{\partial P(\mathbf{x}, t)}{\partial t} = & \sum_{i=1}^m \left[ a_i^-(\mathbf{x} + \boldsymbol{\delta}_i) P(\mathbf{x} + \boldsymbol{\delta}_i, t) - a_i^+(\mathbf{x}) P(\mathbf{x}, t) \right. \\ & \left. + a_i^+(\mathbf{x} - \boldsymbol{\delta}_i) P(\mathbf{x} - \boldsymbol{\delta}_i, t) - a_i^-(\mathbf{x}) P(\mathbf{x}, t) \right] \quad , \quad (2.37) \end{aligned}$$

where  $P(\mathbf{x}, t) = \Pr\{\mathbf{X}(t) = \mathbf{x}\}$ .

The master equation is usually easy to write out, but difficult or impossible to solve analytically, unless it is for a fairly simple system. Numerical solutions to equation 2.37 also quickly become impractical as the number of molecules in the system increases. For example, if  $0 \leq x_j \leq 9$  for  $j = 1, 2, \dots, n$ , then there would be  $10^n$  possible states of the system to be tracked.

## 2.4 Gillespie's Direct Method

One alternative to solving the master equation is to use a stochastic simulation approach such as Gillespie's Direct Method. This method is a stochastic numerical algorithm which exactly simulates the time evolution of a system. It is rigorously equivalent to the master equation approach, as it is also derived directly from the assumption of a well-mixed system and equation 2.31 (Gillespie, 1977). Implementation of the algorithm is straightforward, even for systems with a large number of species.

Given a system in a particular state, the Direct Method calculates which reaction occurs next, and at what time that reaction occurs. The system is updated with this information, and the steps of the algorithm are repeated for the next time step. Let the system be in state  $\mathbf{x}$  at time  $t$ . Then the next reaction,  $\mu$ , and the time step,  $\tau$ , are chosen by drawing a pair of random numbers  $(\mu, \tau)$  from the following joint probability density function (Gillespie, 1977):

$$P(\mu, \tau) = \begin{cases} a_\mu(\mathbf{x}) \exp(-a_0(\mathbf{x})\tau) & \text{if } 0 \leq \tau < \infty \text{ and} \\ & \mu = 1, 2, \dots, 2m \\ 0 & \text{otherwise} \end{cases}, \quad (2.38)$$

where

$$a_0(\mathbf{x}) = \sum_{\mu=1}^{2m} a_\mu(\mathbf{x}) = \sum_{i=1}^m [a_i^+(\mathbf{x}) + a_i^-(\mathbf{x})] \quad (2.39)$$

is the total reaction rate constant out of state  $\mathbf{x}$ , and  $\mu \in \{1, 2, \dots, 2m\}$  because there are  $m$  pairs of forward and backward reactions. The probability density  $P(\mu, \tau)$  in equation 2.38 thus depends on the rates of all the reactions and the number of molecules of all the chemical species in the system, not just the single reaction  $\mu$  and its reactants (Gillespie, 1977).

The marginal probability distribution for choosing reaction  $\mu$  can be found by integrating  $P(\mu, \tau)$  over all  $\tau \geq 0$ :

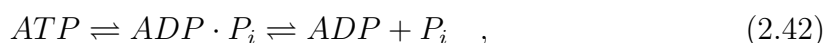
$$P(\mu) = \int_0^\infty P(\mu, \tau) d\tau = a_\mu(\mathbf{x})/a_0(\mathbf{x}) \quad (\mu = 1, 2, \dots, 2m) \quad . \quad (2.40)$$

Similarly, the probability density function for choosing time step  $\tau$  is found by summing  $P(\mu, \tau)$  over all  $\mu$ :

$$P(\tau) = \sum_{\mu=1}^{2m} P(\mu, \tau) = a_0(\mathbf{x}) \exp(-a_0(\mathbf{x})\tau) \quad (0 \leq \tau < \infty) \quad . \quad (2.41)$$

Note that  $P(\mu)$  and  $P(\tau)$  are independent, so that  $P(\mu, \tau) = P(\mu) \cdot P(\tau)$ .

As an example, consider a simplified version of the hydrolysis of ATP into ADP and phosphate by an enzyme:



where  $ADP \cdot P_i$  represents the complex of ADP and inorganic phosphate which have been generated from the ATP but not yet released by the enzyme. For notational convenience, let  $\mathcal{W}$ ,  $\mathcal{X}$ ,  $\mathcal{Y}$ , and  $\mathcal{Z}$  denote the  $ATP$ ,  $ADP \cdot P_i$ ,  $ADP$ , and  $P_i$ , respectively. Then



where  $k_1$ ,  $k_2$ ,  $k_{-1}$ , and  $k_{-2}$  are the rates of the reactions.

Letting  $\mathbf{w} = (w, x, y, z)$  denote the number of molecules of each chemical species in reaction 2.43, we have

$$a_0(\mathbf{w}) = k_1 w + (k_{-1} + k_2)x + k_{-2}yz \quad . \quad (2.44)$$

$P(\mu)$  is given by

$$P(\mu = 1) = \frac{k_1 w}{k_1 w + (k_{-1} + k_2)x + k_{-2}yz} \quad (2.45)$$

$$P(\mu = 2) = \frac{k_{-1}x}{k_1 w + (k_{-1} + k_2)x + k_{-2}yz} \quad (2.46)$$

$$P(\mu = 3) = \frac{k_2 x}{k_1 w + (k_{-1} + k_2)x + k_{-2}yz} \quad (2.47)$$

$$P(\mu = 4) = \frac{k_{-2}yz}{k_1 w + (k_{-1} + k_2)x + k_{-2}yz} \quad , \quad (2.48)$$

and for  $P(\tau)$  we find

$$P(\tau) = (k_1 w + (k_{-1} + k_2)x + k_{-2}yz) \exp \left( - (k_1 w + (k_{-1} + k_2)x + k_{-2}yz) \tau \right) . \quad (2.49)$$

To generate values for  $\mu$  and  $\tau$  from  $P(\mu)$  and  $P(\tau)$ , we first choose two independent random numbers between 0 and 1 from a uniform random number generator. The numbers,  $r_1$  and  $r_2$ , are then transformed in the following manner (Gillespie, 1977): given  $r_1$ , choose  $\mu$  to be the integer for which

$$\sum_{\nu=1}^{\mu-1} a_\nu(\mathbf{x}) < r_1 a_0(\mathbf{x}) \leq \sum_{\nu=1}^{\mu} a_\nu(\mathbf{x}) \quad , \quad (2.50)$$

and given  $r_2$ , choose

$$\tau = \frac{1}{a_0(\mathbf{x})} \ln \frac{1}{r_2} \quad . \quad (2.51)$$

Once  $\mu$  and  $\tau$  have been chosen, the system is updated by changing the time from  $t$  to  $t + \tau$ , and changing the system state from  $\mathbf{X}(t) = \mathbf{x}$  to  $\mathbf{X}(t + \tau) = \mathbf{x} + \delta_\mu$ , where  $\delta_\mu$  is the net effect of reaction  $\mu$  on  $\mathbf{X}(t)$ .

The Direct Method has several advantages. It is an exact method, based on the underlying fluctuation and probabilities of the system, rather than an approximation. It does not approximate infinitesimal time steps of length  $dt$  by a finite  $\Delta t$ , but rather samples the probability distribution  $P(\tau)$ . In addition, it provides a complementary approach to the master equation by focusing on individual sample paths of the system, rather than looking at the system as a whole.

Disadvantages of the Direct Method, however, are that Monte Carlo simulations tend to be computationally expensive, and that multiple simulation runs must be done to estimate average behaviors and other statistics. If there are very different time scales in the system, then fast reactions dominate. Smaller time steps are chosen, and a simulation must take many more steps to complete a time series of a given length. This can be a problem if, as in the dynein model presented in Chapter 3, the slow reactions govern the behavior of interest. In such cases, other methods may be more useful, such as using Langevin equations (described below in Section 2.6) to model the fast reactions while using the Direct Method to model the slow reactions, or using a quasi-equilibrium assumption to eliminate the fast variables from the system. Both of these approaches were used in the dynein modeling described in Chapters 3 and 4.

## 2.5 The Fokker-Planck Equation

Methods do exist to approximate the solution of the master equation. If there are large numbers of molecules in the system (i.e.,  $x_j \gg 1$ ), then the  $x_j$  can be treated as continuous variables, simplifying analysis and increasing simulation speed. In this case, equation 2.37 can be approximated by a partial differential equation (PDE) known as the Fokker-Planck equation (FPE). The Fokker-Planck equation describes the time evolution of the probability density function of the variable(s) being modeled. A second-order differential equation is usually used, but higher-order Fokker-Planck

equations do exist and are sometimes necessary (Gillespie, 1980).

As stated in Section 2.1.1, the Fokker-Planck equation is a generalized form of the diffusion equation. For a one dimensional system, the FPE has the form

$$\frac{\partial P(x, t)}{\partial t} = -\frac{\partial}{\partial x}[A(x, t)P(x, t)] + \frac{\partial^2}{\partial x^2}[D(x, t)P(x, t)] \quad , \quad (2.52)$$

where  $P(x, t)$  is the probability of  $x$  at time  $t$ ,  $A(x, t)$  is the drift coefficient, and  $D(x, t)$  is the diffusion coefficient. In multidimensional systems such as a set of chemical reactions, the Fokker-Planck equation takes the form (Gardiner, 1985)

$$\frac{\partial P(\mathbf{x}, t)}{\partial t} = -\sum_{j=1}^n \frac{\partial}{\partial x_j} A_j(\mathbf{x})P(\mathbf{x}, t) + \frac{1}{2} \sum_{j, k=1}^n \frac{\partial^2}{\partial x_j \partial x_k} B_{j, k}(\mathbf{x})P(\mathbf{x}, t) \quad , \quad (2.53)$$

where

$$A_j(\mathbf{x}) = \sum_{i=1}^m \delta_{i,j} [a_i^+(\mathbf{x}) - a_i^-(\mathbf{x})] \quad (2.54)$$

$$B_{j, k}(\mathbf{x}) = \sum_{i=1}^m \delta_{i,j} \delta_{i,k} [a_i^+(\mathbf{x}) + a_i^-(\mathbf{x})] \quad . \quad (2.55)$$

In equations 2.54 and 2.55,  $\delta_{i,j}$ , the  $j$ th component of the vector  $\boldsymbol{\delta}_i$ , describes the net effect of reaction  $R_i^+$  on the number of molecules  $x_j$ . The  $A_j(\mathbf{x})$  are drift terms that describe the mean behavior of the system, and are the same terms that occur in the conventional reaction rate equations derived from the law of mass action. The  $B_{j, k}(\mathbf{x})$  are diffusion terms which capture the variability of the system around the mean. The second-order Fokker-Planck equation is thus an extension of the traditional deterministic model. The master equation (2.37) can be recovered from the Fokker-Planck equation (2.53) by an appropriate second-order discretization (Gillespie, 1992a).

The ATP hydrolysis system described by the reactions in equation 2.43 leads to

the following Fokker-Planck equation:

$$\begin{aligned}
\frac{\partial \boldsymbol{\rho}}{\partial t} = & -\frac{\partial}{\partial w}(-k_1 w + k_{-1} x) \boldsymbol{\rho} - \frac{\partial}{\partial x}(k_1 w - (k_{-1} + k_2)x + k_{-2} y z) \boldsymbol{\rho} \quad (2.56) \\
& -\frac{\partial}{\partial y}(k_2 x - k_{-2} y z) \boldsymbol{\rho} - \frac{\partial}{\partial z}(k_2 x - k_{-2} y z) \boldsymbol{\rho} \\
& + \frac{1}{2} \frac{\partial^2}{\partial w^2}(k_1 w + k_{-1} x) \boldsymbol{\rho} + \frac{1}{2} \frac{\partial^2}{\partial x^2}(k_1 w + (k_{-1} + k_2)x + k_{-2} y z) \boldsymbol{\rho} \\
& + \frac{1}{2} \frac{\partial^2}{\partial y^2}(k_2 x + k_{-2} y z) \boldsymbol{\rho} + \frac{1}{2} \frac{\partial^2}{\partial z^2}(k_2 x + k_{-2} y z) \boldsymbol{\rho} \\
& - \frac{\partial^2}{\partial w \partial x}(k_1 w + k_{-1} x) \boldsymbol{\rho} - \frac{\partial^2}{\partial x \partial y}(k_2 x + k_{-2} y z) \boldsymbol{\rho} \\
& - \frac{\partial^2}{\partial x \partial z}(k_2 x + k_{-2} y z) \boldsymbol{\rho} + \frac{\partial^2}{\partial y \partial z}(k_2 x + k_{-2} y z) \boldsymbol{\rho} \quad ,
\end{aligned}$$

where  $\boldsymbol{\rho} = \text{Pr}\{w, x, y, z, t\}$ .

While a Fokker-Planck equation is generally more mathematically tractable than the corresponding master equation, it also quickly becomes difficult to solve exactly as the number of chemical species in the system increases. Furthermore, as in the case of the deterministic reaction rate equations, if the numbers of molecules in the system are small, the approximation that the chemical reactions behave like diffusion processes may not be valid. In this case, the chemical reactions must be treated as jump-type processes, and a fourth-order or higher Fokker-Planck equation is often necessary to accurately approximate the master equation (Gillespie, 1980).

## 2.6 The Langevin Equation

Another method to approximate the solution to the master equation is to use a stochastic differential equation, which is an ODE with a rapidly varying noise term. Although the noise can arise in many different ways, choosing an appropriate form based on the fluctuations in the underlying system yields a particular type of SDE known as a Langevin equation. As mentioned in Section 2.1.2, a Langevin equation is a generalization of the equation of motion used by Langevin in his analysis of Brownian motion.

A properly defined Langevin equation corresponds with a particular Fokker-Planck equation and therefore with the associated master equation. While the FPE describes the evolution of the probability density function of a variable, the Langevin equation follows a single trajectory of that variable. The two approaches provide equivalent but complementary information about the stochastic process being modeled.

Solutions to Langevin equations can often be numerically simulated faster and more easily than solutions to the corresponding Fokker-Planck equations. Interestingly, while there is only one valid Fokker-Planck representation of a given order for a particular master equation, there may be more than one valid Langevin representation for the same process (Gillespie, 1996).

Using somewhat different notation from Section 2.1.2, the standard form of a one dimensional Langevin equation is

$$x(t + dt) = x(t) + A(x(t), t) dt + \sqrt{D(x(t), t)} \cdot N(t) \sqrt{dt} \quad , \quad (2.57)$$

where  $A(x, t)$  and  $D(x, t)$  are any two smooth functions with  $D(x, t) \geq 0$ .  $N(t)$  is a standard normal random variable with  $N(t')$  and  $N(t)$  statistically independent for all  $t \neq t'$ .

By rearranging equation 2.57 and taking the limit as  $dt \rightarrow 0$ , the Langevin equation can be cast in the commonly seen white-noise form,

$$\frac{dx}{dt} = A(x, t) + \sqrt{D(x, t)} \cdot \Gamma(t) \quad (j = 1, 2, \dots, n) \quad , \quad (2.58)$$

where  $\Gamma(t) \equiv \lim_{dt \rightarrow 0} \left[ \text{Normal}(0, \frac{1}{dt}) \right]$  is a Gaussian white-noise process, although strictly speaking,  $x(t)$  is not differentiable (Gillespie, 1996).

The standard form of a multivariate Langevin equation for the chemical reaction system from Section 2.3 is

$$X_j(t + dt) = X_j(t) + A_j(\mathbf{X}(t)) \cdot dt + \sum_{i=1}^m \delta_{i,j} \sqrt{a_i^+(\mathbf{X}(t)) + a_i^-(\mathbf{X}(t))} \cdot N_i(t) \sqrt{dt} \quad (j = 1, 2, \dots, n) \quad , \quad (2.59)$$

Note that in this case, we must have  $N_{i1}(t)$  and  $N_{i2}(t)$  statistically independent for  $i1 \neq i2$ , as well as  $N_i(t)$  and  $N_i(t')$  independent for  $t \neq t'$ , which we had in the univariate case.

For the example system given by equation 2.43, the appropriate Langevin equations are

$$\frac{dW}{dt} = -k_1w + k_{-1}x - \sqrt{k_1w + k_{-1}x} \cdot \Gamma_1(t) \quad (2.60)$$

$$\begin{aligned} \frac{dX}{dt} = & k_1w - (k_{-1} + k_2)x + k_{-2}yz + \sqrt{k_1w + k_{-1}x} \cdot \Gamma_1(t) \\ & - \sqrt{k_2x + k_{-2}yz} \cdot \Gamma_2(t) \end{aligned} \quad (2.61)$$

$$\frac{dY}{dt} = k_2x - k_{-2}yz + \sqrt{k_2x + k_{-2}yz} \cdot \Gamma_2(t) \quad (2.62)$$

$$\frac{dZ}{dt} = k_2x - k_{-2}yz + \sqrt{k_2x + k_{-2}yz} \cdot \Gamma_2(t) \quad . \quad (2.63)$$

Solving stochastic differential equations analytically is often not possible, and so solutions must be simulated numerically. The Euler method is often used to perform the simulations because of its simplicity. More accurate numerical simulation methods exist, but can be significantly more difficult to implement because of the complexities of dealing with stochastic Taylor's series expansions. The standard form of the Langevin equation is already in the form of an update equation, making it convenient to use with the Euler method simply by replacing  $dt$  in equation 2.59 with a finite time step  $\Delta t$ :

$$\begin{aligned} X_j(t + \Delta t) = & X_j(t) + A_j(\mathbf{X}(t)) \cdot \Delta t + \sum_{i=1}^m \delta_{i,j} \sqrt{a_i^+(\mathbf{X}(t)) + a_i^-(\mathbf{X}(t))} \cdot N_i(t) \sqrt{\Delta t} \\ & (j = 1, 2, \dots, n) \quad , \end{aligned} \quad (2.64)$$

Care must be taken to choose  $\Delta t$  small enough to avoid numerical instability of the simulation results but large enough to minimize round-off errors.

## Chapter 3

# A Model of the Mechanochemical Cycle of Dynein

We propose a mechanochemical model of a two-headed dynein which links the motions observed by Shingyoji et al. with the steps in dynein's ATP hydrolysis cycle. The major assumption of the model is that while the motor moves forward actively, powered by ATP hydrolysis, it slides backward passively as a result of biased diffusion along the microtubule. The model assumes that the dynein is always attached to the microtubule by at least one of its two heads. If both heads were to release the microtubule while working against the force of the laser trap, the bead and microtubule would be pulled back very rapidly to the center of the trap. Predictions from this model will be compared in Chapter 5 with those from other models, including an active back-stepping model by Brokaw (2000).

For simplicity, the dynein is assumed to be composed of two arms with identical physical and biochemical properties. This is appropriate for cytoplasmic dyneins, but may be less accurate for axonemal dyneins, whose heavy chains are often heterodimers. The model also does not include the potential regulatory effects of phosphorylation or of  $\text{Ca}^{2+}$ -binding to the dynein, or of the binding of ATP or ADP

at AAA sites other than the main hydrolysis site, AAA1. These factors are undoubtedly important for a better understanding of how dynein functions, but the details are only now being discovered (Yagi, 2000; Silvanovich et al., 2003; Kikushima et al., 2004; Reck-Peterson and Vale, 2004). Models including such effects may be the subject of future work.

### 3.1 Model Description

The model contains a main chemical cycle in which the energy of ATP binding and hydrolysis drives forward motion, and a branch pathway in which the dynein can slide backward along the microtubule under the pull of the laser trap (see Figure 3.1). The key evidence to support this backsliding mechanism is the observation that when ADP is bound to both heads, dynein undergoes one-dimensional diffusion along the microtubule (Vale et al., 1989). As discussed in Section 3.1.2 below, this diffusive set of states provides a mechanism for releasing the tension of the laser trap and leads to a possible explanation of the oscillatory movement described by Shingyoji et al. (1998).

#### 3.1.1 The Main Hydrolysis Pathway

The model’s main pathway, shown as states 1 to 5 in Figure 3.1, corresponds to dynein’s chemical cross-bridge cycle, which is shown in Figure 3.2. The details of the kinetic steps in the cycle are as follows:

1. ATP binds to the empty hydrolysis site on a dynein head, causing that head to rapidly detach from the microtubule (Porter and Johnson, 1983), and the dynein enters state 1.
2. The ATP-containing head is then able to interact with the “front” of the other dynein head. This interaction stimulates ATP hydrolysis, leading to state 2.
3. Phosphate is released by the dynein, leading to state 3.

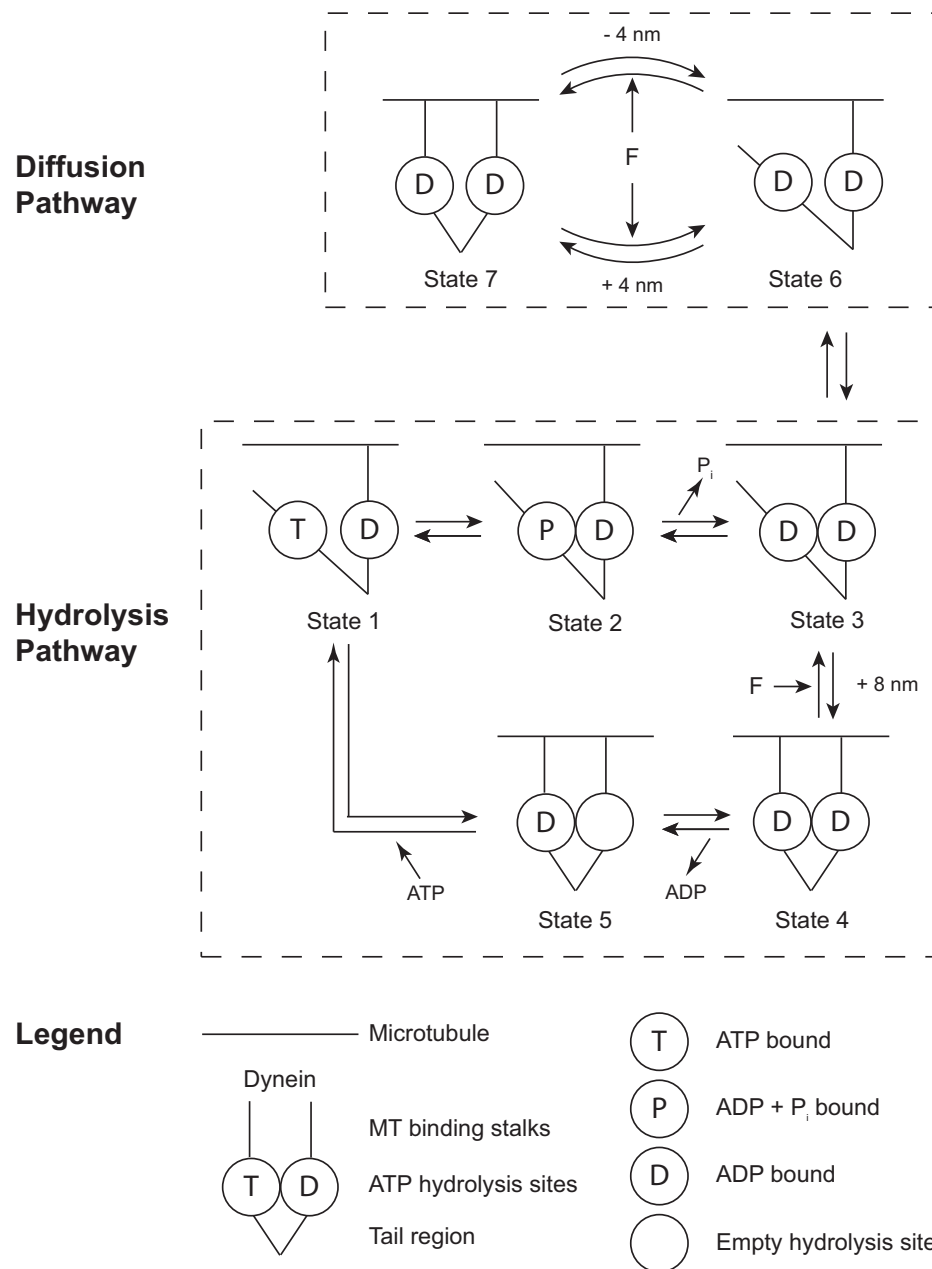


Figure 3.1: Mechanochemical cycle. The proposed cycle consists of a main hydrolysis pathway which drives dynein movement and a branch diffusion pathway in which dynein can slide freely along the microtubule. Force from the laser trap would bias the dynein to slide backward.

4. The dynein is now positioned to undergo a conformational change in which the front head attaches to the next binding site on the microtubule, and the dynein enters state 4.
5. The binding of the front dynein head to the microtubule stimulates ADP release (Omoto and Johnson, 1986; Holzbaur and Johnson, 1989b) by the rear head, leading to state 5.
6. A new ATP molecule can now bind to the empty hydrolysis site, completing the cycle to state 1.

This proposed cycle is consistent with (1) the dynein chemical cross-bridge cycle (Johnson, 1983; Porter and Johnson, 1983), (2) an isomerization of the dynein-ADP complex in the cycle (Holzbaur and Johnson, 1989a), and (3) dynein's power-stroke being associated with product release (Burgess et al., 2003; Johnson, 1985), and in particular with the dynein-ADP complex (Tani and Kamimura, 1999). Interaction between the two dynein heads is also consistent with other work (Iyadurai et al., 1999; Shimizu and Johnson, 1983a), coordinates the action of the heads, and would explain the unidirectional motion of dynein movement toward the minus-end of the microtubules that had been observed previously (Sale and Satir, 1977; Fox and Sale, 1987; Gibbons, 1988; Vale and Toyoshima, 1988).

### 3.1.2 The Diffusion Pathway

The laser trap used in Shingyoji et al.'s experiments provides a spring-like resistance to movement. Each physical step forward by the dynein increases the force against which the motor must work to make the next forward step, slowing the rate of that transition. If the main ATP hydrolysis cycle shown in Figure 3.1 were the only pathway available to dynein, the molecule would eventually stop moving when the load of the optical trap reached the stall force of the motor. However, dynein is capable of one-dimensional diffusion along a microtubule when ADP and vanadate are bound (Vale et al., 1989). Vanadate acts as a phosphate analog which blocks the

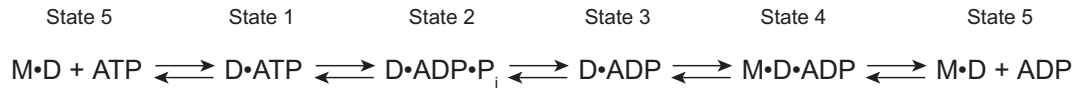


Figure 3.2: Chemical cross-bridge cycle. The states in dynein's ATP hydrolysis cycle are labelled to correspond with the states shown in Figure 3.1.

release of ADP by dynein, and thus has the effect of stabilizing dynein's ADP-bound state (Shimizu and Johnson, 1983b). The proposed diffusion pathway is represented by states 6 and 7 of Figure 3.1.

Transitions from state 3 to state 4 require dynein to undergo a conformational change. Therefore, this step is force dependent. The model assumes that the tension developed by the laser trap eventually forces the dynein molecule into a chemical state (state 6) in which both heads contain ADP, but are no longer interacting with each other as they do in state 3 of the main hydrolysis cycle. This shift is not necessarily a result of the heads being pulled apart, but rather is because the tension-induced slowing of the transition rate from state 3 to state 4 allows the heads more time, and therefore more opportunity, to take the branch path from state 3 to state 6. In state 6, if the heads do not immediately reassociate, the unbound head has greater freedom of movement than it does in state 3, and potentially can attach to the microtubule binding site located either in front of or behind the bound head. These two possibilities are represented in Figure 3.1 by the two sets of transition arrows connecting state 6 to state 7. From state 7, either one of the two heads can release the microtubule, returning the dynein to state 6. The dynein is therefore free to diffuse along the microtubule by repeatedly moving between states 6 and 7. However, its motion is biased by the force of the laser trap, so that the dynein tends to move toward the center of the trap. As the dynein moves backward along the microtubule,

strain is released and the bias for backward movement is reduced. The heads then have more time to reassociate, allowing the dynein to go from state 6 back to state 3, reentering the hydrolysis pathway. Because the load has been reduced, the transition from state 3 to state 4 is again favorable, and the dynein can walk forward until sufficient resistance has built up to enter the branch pathway once more.

If an experiment were done in which the dynein was forced into state 6, for example by vanadate inhibition of the hydrolysis cycle, but there were no force being imposed by the laser trap, then the rates of forward and backward steps between states 6 and 7 in the model would be equal, and the dynein would execute a random walk along the microtubule, as observed by Vale et al. (1989).

## 3.2 The Mathematical Model

The experimental system shown in Figure 1.2 can be modeled as a series of bodies representing the bead, the microtubule, and the dynein, connected by springs to account for the elasticity in the linkages, as shown in Figure 3.3.

We take the origin of the main coordinate system to be the center of the laser trap ( $x_t \equiv 0$ ). We let  $x_b(t)$  denote the position of the bead and  $x_m(t)$  denote the position of the left edge of the microtubule at time  $t$ . The dynein molecule is anchored to the coverslip at position  $x_a$ . We denote the position of the leftmost, or first, dynein head as  $\tilde{x}_1$  and the position of the second dynein head as  $\tilde{x}_2$ . Because the dynein is anchored, a dynein step to the left in fact moves the microtubule to the right, and the apparent dynein position in this frame of reference is always  $x_a$ . It is therefore more convenient to measure the positions of the two dynein heads and the dynein molecule as a whole relative to the dynein's initial binding site on the microtubule rather than to the center of the laser trap, so that the movement of the dynein along the microtubule can be tracked. Let  $x_1(t)$  be the position of the first dynein head and  $x_2(t)$  be the position of the second dynein head in the dynein coordinate system. Dynein binding sites are located every 8 nm along the microtubule. As described in Section 3.3, we assume that this is also dynein's step size. The position of the

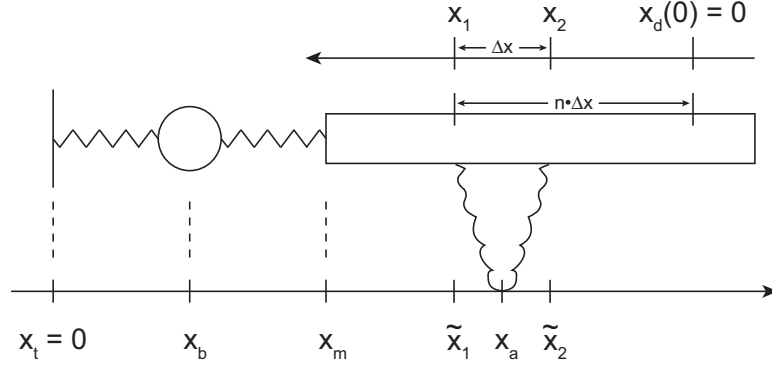


Figure 3.3: Physical model. The experimental system can be represented as a series of bodies connected by springs. The origin of the main coordinate system is taken to be the center of the laser trap. The dynein head positions are measured to the left of the dynein's starting position on the microtubule. The positions at time  $t$  of the bead, the microtubule, and the dynein are  $x_b(t)$ ,  $x_m(t)$ , and  $x_d(t)$ , respectively. The dynein is anchored at  $x_a$ .

leftmost dynein head at time  $t$  on this discrete scale is then given by  $x_1(t) = n \cdot \Delta x$ , where  $n = 0, \pm 1, \pm 2, \dots$  indexes the binding sites, with positive  $n$  to the left of the dynein's original position at time  $t = 0$ , and  $\Delta x = 8$  nm. If both heads are attached to the microtubule, then the second head's position is given by  $x_2(t) = x_1(t) - \Delta x$ ; otherwise, its position is  $x_2(t) = x_1(t)$ . The position of the dynein molecule as a whole is taken to be the mean of the positions of its two heads,  $x_d(t) = (x_1(t) + x_2(t))/2$ .

The series of bodies and springs shown in Figure 3.3 can be further reduced to a single body representing the dynein, connected by a single spring to the laser trap, as shown in Figure 3.4. This reduction forms the basis of the simplified model described in Chapter 4. By utilizing the information given by Shingyoji et al. (1998) and the force balances in the system, numerical values can be derived for most of the spring constants necessary in both the detailed and simplified versions of the model. Note that Shingyoji et al. did not distinguish between the positions of the microtubule and the dynein, i.e.,  $x_{m, \text{Shingyoji}} = x_{d, \text{Shingyoji}}$ . Because they used the "bead-to-dynein linkage stiffness" with this displacement, in this dissertation we take  $x_d \equiv x_{m, \text{Shingyoji}}$ , and therefore, generally  $x_m \neq x_{m, \text{Shingyoji}}$ .

The following force balance relationships exist among the springs of the system at

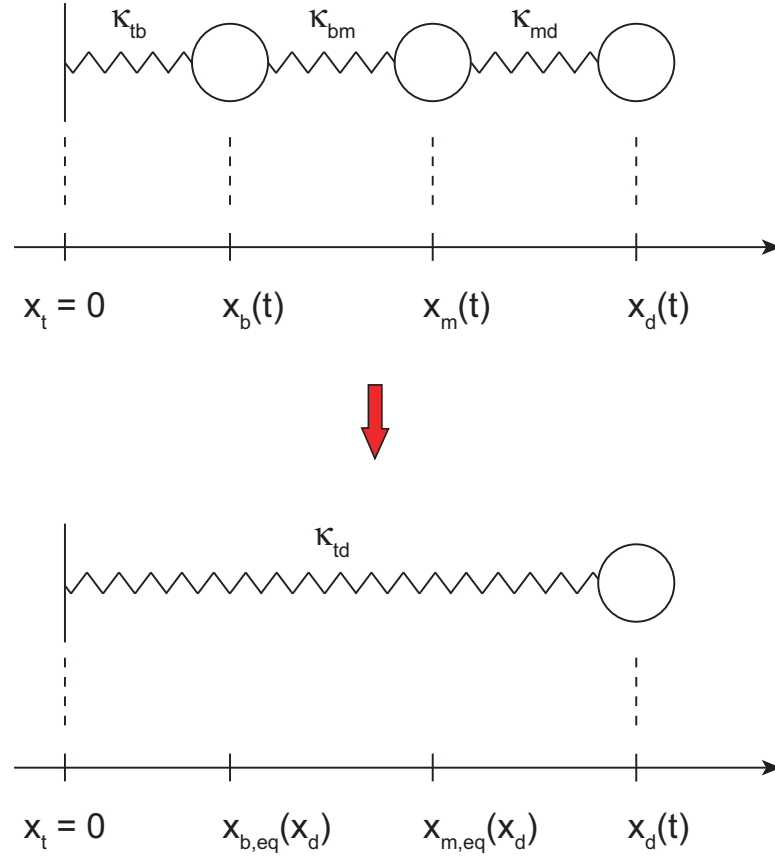


Figure 3.4: Simplified physical model. The series of bodies and springs representing the experimental system can be reduced to a single body representing the dynein molecule connected by a single spring to the laser trap. As in Figure 3.3, the origin is at the center of the trap,  $x_t \equiv 0$ , and the dynein position at time  $t$  is  $x_d(t)$ . The bead and microtubule are taken to be at their equilibrium positions relative to dynein,  $x_{b,eq}(x_d)$  and  $x_{m,eq}(x_d)$ .

mechanical equilibrium:

$$\begin{aligned} F = \kappa_{tb}x_{b,eq} &= \kappa_{bm}(x_{m,eq} - x_{b,eq}) = \kappa_{md}(x_d - x_{m,eq}) \\ &= \kappa_{tm}x_{m,eq} = \kappa_{bd}(x_d - x_{b,eq}) = \kappa_{td}x_d \quad , \end{aligned} \quad (3.1)$$

where  $\kappa_{tb}$ ,  $\kappa_{tm}$ ,  $\kappa_{td}$ ,  $\kappa_{bm}$ ,  $\kappa_{bd}$ , and  $\kappa_{md}$  are the spring constants for the trap-to-bead, bead-to-microtubule, microtubule-to-dynein, trap-to-microtubule, bead-to-dynein, and trap-to-dynein connections, respectively, and  $x_{b,eq}$  and  $x_{m,eq}$  are the equilibrium positions of the bead and microtubule relative to  $x_d$ . The spring constant  $\kappa_{md} = (1+p)\kappa_d$ , where  $\kappa_d$  is the spring constant of a single dynein arm. The factor  $p$ , where  $0 \leq p \leq 1$ , represents the probability that both dynein heads are attached to the microtubule at the same time. The factor  $(1+p)$  is then the average number of microtubule-bound dynein heads. Numerical values are derived from the experimental data for most of these spring constants in Section 3.3.

The motions of the bead and microtubule are affected not only by the dynein and the laser trap, but also by the thermal fluctuations, or Brownian motion effects, of the surrounding medium. At microscopic scales, viscous forces dominate inertial forces, leading to overdamped dynamics, and so the motion of a general Brownian particle can be described by the following version of the Langevin equation (??) (Wang et al., 2003):

$$\zeta \frac{dx}{dt} = -\frac{\partial \phi(x)}{\partial x} + \sqrt{\zeta 2k_B T} \cdot f_B(t) \quad , \quad (3.2)$$

where  $\zeta$  is the friction coefficient,  $\phi(x)$  is the potential energy, and  $x$  is the position of the particle. The value  $k_B$  is the Boltzmann constant and  $T$  is absolute temperature, so that  $k_B T$  is a measurement of thermal energy. At a temperature of 25 C,  $k_B T \approx 4.12$  pN nm. The function  $f_B(t)$  is taken to be a Gaussian white-noise process representing the thermal fluctuations of the particle. The friction coefficient,  $\zeta$ , is associated with the diffusion coefficient,  $D$ , through the Einstein-Smoluchowski relation,  $D = k_B T / \zeta$  (Berg, 1993). For the dynein experimental system, the Langevin

equations for the bead and the microtubule are given by

$$\zeta_b \frac{dx_b}{dt} = -\kappa_{tb}x_b + \kappa_{bm}(x_m - x_b) + \sqrt{2\zeta_b k_B T} \cdot f_b(t) \quad (3.3)$$

$$\zeta_m \frac{dx_m}{dt} = -\kappa_{bm}(x_m - x_b) - \kappa_d(\tilde{x}_1 - x_a) - \kappa_d(\tilde{x}_2 - x_a) + \sqrt{2\zeta_m k_B T} \cdot f_m(t) . \quad (3.4)$$

Equation 3.4 can be rewritten in terms of  $x_1$  and  $x_2$  as

$$\zeta_m \frac{dx_m}{dt} = -\kappa_{bm}(x_m - x_b) + \kappa_d(x_1 - x_m) + I \cdot \kappa_d(x_2 - x_m) + \sqrt{2\zeta_m k_B T} \cdot f_m(t) , \quad (3.5)$$

which is the form that will be used. In equation 3.5,  $I$  is an indicator function which takes the value  $I = 1$  when both dynein heads are bound and the value  $I = 0$  when only one head is bound.

Because the dynein position,  $x_d(t)$ , is measured on a discrete scale, the motion of the dynein molecule is modeled as a continuous time Markov chain, based on tracking the chemical state and the positions of the two heads of the dynein as the molecule moves around the chemical cycle.

The chemical state of the dynein (see Figure 3.1) at time  $t$ ,  $S(t)$ , can be modeled with the following set of ordinary differential equations:

$$\frac{dp_1}{dt} = -(k_{12} + k_{15})p_1 + k_{21}p_2 + k_{51}[\text{ATP}]p_5 \quad (3.6)$$

$$\frac{dp_2}{dt} = k_{12}p_1 - (k_{21} + k_{23})p_2 + k_{32}[\text{P}_i]p_3 \quad (3.7)$$

$$\frac{dp_3}{dt} = k_{23}p_2 - (k_{32}[\text{P}_i] + k_{34} + k_{36})p_3 + k_{43}p_4 + k_{63}p_6 \quad (3.8)$$

$$\frac{dp_4}{dt} = k_{34}p_3 - (k_{43} + k_{45})p_4 + k_{54}[\text{ADP}]p_5 \quad (3.9)$$

$$\frac{dp_5}{dt} = k_{15}p_1 + k_{45}p_4 - (k_{54}[\text{ADP}] + k_{51}[\text{ATP}])p_5 \quad (3.10)$$

$$\frac{dp_6}{dt} = k_{36}p_3 - (k_{63} + k_{67l} + k_{67r})p_6 + (k_{76l} + k_{76r})p_7 \quad (3.11)$$

$$\frac{dp_7}{dt} = (k_{67l} + k_{67r})p_6 - (k_{76l} + k_{76r})p_7 , \quad (3.12)$$

where  $p_i(t)$  is the probability that  $S(t) = i$ , and  $k_{ij}$  is the rate constant for the transition from state  $i$  to state  $j$ , for  $i, j \in \{1, 2, \dots, 7\}$ . The letters  $l$  and  $r$  are used in the rate constants for the transitions between state 6 and state 7 to indicate

whether the moving dynein head is doing so to the left or to the right side of the stationary head. Equations 3.6 – 3.12 can be written more simply as

$$\frac{d\mathbf{p}}{dt} = \mathbf{M}\mathbf{p} \quad , \quad (3.13)$$

where  $\mathbf{p}(t) = (p_1(t), p_2(t), \dots, p_7(t))^T$  is a 7 x 1 column vector of the chemical state probabilities and  $\mathbf{M}$  is a 7 x 7 sparse matrix governing transitions between the states. Equation 3.13 will be used later, in Section 4.2.1. The positions of the dynein heads,  $x_1(t)$  and  $x_2(t)$ , and the dynein itself,  $x_d(t)$ , are updated appropriately as  $S(t)$  changes and the dynein moves from state to state. A schematic version of Figure 3.1 highlighting the transition rates between chemical states is shown in Figure 3.5.

### 3.3 Theoretical and Experimental Model

#### Constraints

A number of constraints can be placed on the parameters of the dynein model based on theoretical considerations and experimental data. In this section, we will constrain the step size taken by a dynein molecule, the transition rate constants between chemical states, the spring constants of the various links among the laser trap, the bead, the microtubule, and the dynein, and their diffusion coefficients.

#### Dynein Step Size

Thermodynamic considerations limit the step size taken by the dynein during active motion. In a chemical reaction, the change in free energy,  $\Delta G$ , depends on the nature and concentrations of the reactants and products. In the case of ATP hydrolysis by dynein,

$$\Delta G = \Delta G^0 + k_B T \ln \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]} \quad , \quad (3.14)$$

where  $\Delta G^0$  is the free energy change per molecule of ATP hydrolyzed, under the standard condition of  $[\text{ATP}]$ ,  $[\text{ADP}]$ , and  $[\text{P}_i]$  each being equal to 1 M. The  $\Delta G$

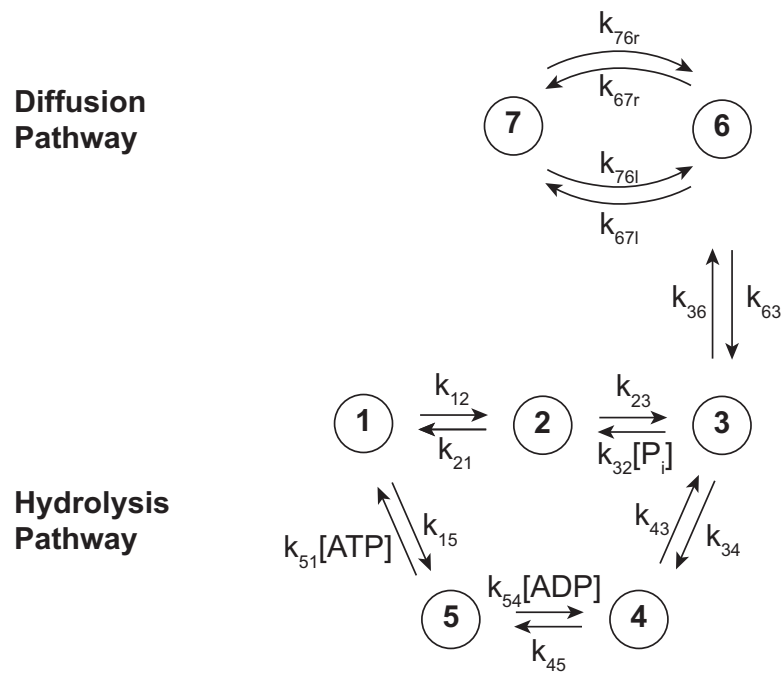


Figure 3.5: Schematic mechanochemical cycle. The transition rate constant from state  $i$  to state  $j$  is  $k_{ij}$ . Physical steps occur when moving between states 3 and 4 and between states 6 and 7. The rate constants for these transitions therefore must be force-dependent.

associated with the hydrolysis of one ATP molecule represents the maximum amount of energy available to the dynein to perform work in one cycle.

At physiological conditions of approximately  $10^{-3}$  M ATP,  $10^{-5}$  M ADP, and  $10^{-3}$  M  $P_i$ , the free energy released by ATP hydrolysis is  $\Delta G = -14.4$  kcal mol $^{-1}$ , which is equivalent to  $\sim 100$  pN nm for each ATP molecule hydrolyzed. Shingyoji et al. (1998) reported the average force generated by dynein during oscillatory motion to be  $\sim 6$  pN. The step size taken by dynein must therefore be  $\lesssim 16.7$  nm per cycle (100 pN nm/6 pN). Dynein binding sites are located every 8 nm along the microtubule, the repeat length of the component tubulin dimers. While a step size of 16 nm is thermodynamically possible, a step size of 8 nm seems more likely and was used in the model.

### Detailed Balance Constraints

The property of detailed balance states that a system of reactions that is at thermal equilibrium has all of its individual reactions at equilibrium. For example, for the first step of dynein's hydrolysis cycle,

$$k_{12} p_{1,eq} = k_{21} p_{2,eq} \quad . \quad (3.15)$$

Enforcing this property ensures that there is no net chemical flux through the hydrolysis cycle and no average motion of the dynein at thermal equilibrium. Combining equation 3.15 with similar constraints on the other steps of the hydrolysis cycle, chemical equilibrium therefore implies

$$\frac{k_{12}k_{23}k_{34}k_{45}k_{51}}{k_{15}k_{21}k_{32}k_{43}k_{54}} = \frac{[\text{ADP}]_{eq}[\text{P}_i]_{eq}}{[\text{ATP}]_{eq}} = K_{eq} \quad . \quad (3.16)$$

Because  $\Delta G^0 = -7.53$  kcal mol $^{-1}$  for ATP hydrolysis and  $K_{eq} = \exp\left(\frac{-\Delta G^0}{k_B T}\right)$ , we can then calculate that

$$\frac{k_{12}k_{23}k_{34}k_{45}k_{51}}{k_{15}k_{21}k_{32}k_{43}k_{54}} = 3.3 \times 10^5 \text{ M at } T = 298 \text{ K} \quad . \quad (3.17)$$

Detailed balance also places constraints on force dependent kinetic steps. The transitions between states 3 and 4 involve a change in the position of the dynein

molecule. The model assumes a single step of  $\Delta x = 8$  nm per hydrolysis cycle, so that these transitions are the only force dependent steps around the main hydrolysis pathway. The rate constants for these transitions must satisfy the constraint

$$\frac{k_{34}}{k_{43}} = \frac{k_{34}^0}{k_{43}^0} \exp\left(\frac{-\Delta\phi_{34}}{k_B T}\right) \quad , \quad (3.18)$$

where  $k_{34}^0$  and  $k_{43}^0$  are the transition rates between states 3 and 4 when there is no load force,

$$\Delta\phi_{34} = \phi_3(x_1 + \Delta x) - \phi_3(x_1) \quad (3.19)$$

is the change in potential energy resulting from completing one cycle, and

$$\phi_3 = \frac{1}{2}\kappa_{tb}(x_b)^2 + \frac{1}{2}\kappa_{bm}(x_m - x_b)^2 + \frac{1}{2}\kappa_d(x_1 - x_m)^2 \quad (3.20)$$

is the potential energy associated with dynein in state 3. Thus,

$$\Delta\phi_{34} = \frac{1}{2}\kappa_d[(x_1 - x_m + \Delta x)^2 - (x_1 - x_m)^2] \quad . \quad (3.21)$$

The change in potential for the reverse transition, from state 4 to state 3, is given by  $\Delta\phi_{43} = -\Delta\phi_{34}$ . However, it is written as

$$\Delta\phi_{43} = \frac{1}{2}\kappa_d[(x_1 - x_m - \Delta x)^2 - (x_1 - x_m)^2] \quad (3.22)$$

because of the convention of having  $x_1$  denote the position of the leftmost head, rather than always being the position of the same head.

Equation 3.18 does not uniquely determine the rate constants  $k_{34}$  and  $k_{43}$ ; instead, following Chen (2000),

$$k_{34} = k_{34}^0 \exp\left(-\alpha \frac{\Delta\phi_{34}}{k_B T}\right) \quad (3.23)$$

$$k_{43} = k_{43}^0 \exp\left(+ (1 - \alpha) \frac{\Delta\phi_{34}}{k_B T}\right) \quad , \quad (3.24)$$

where  $0 \leq \alpha \leq 1$  is a constant which partitions the load effect between the two rates. For simplicity we assume  $\alpha = 1/2$ , so that the rate constants have the following forms:

$$k_{34} = k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}}{k_B T}\right) \quad (3.25)$$

$$\begin{aligned} k_{43} &= k_{43}^0 \exp\left(+\frac{1}{2} \frac{\Delta\phi_{34}}{k_B T}\right) \\ &= k_{43}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{43}}{k_B T}\right) \quad . \end{aligned} \quad (3.26)$$

Other values of  $\alpha$  were tried, but did not lead to improvements in the model.

An argument might be made that other transitions in the mechanochemical model should have load dependent rates, while the force dependence of the transitions between states 3 and 4 is reduced. For example, Figure 3.1 suggests that transitions between states 5 and 1, or between states 3 and 6, could be force dependent. Shifting the entire force dependence to another transition rate pair implies that dynein takes its physical step at a different point during the chemical cycle. Splitting the force dependence among more than one transition rate pair is equivalent to assuming that the dynein takes multiple substeps which sum to 8 nm during each hydrolysis cycle. Although it has not yet been observed for dynein, there is evidence that kinesin takes such substeps (Coppin et al., 1996; Schnitzer et al., 2000).

The diffusion pathway involves transitions between model states 6 and 7. One cycle of this pathway either forward or backward moves the dynein by  $\Delta x = 8$  nm, so that in one step from state 6 to state 7 or from state 7 to state 6 (a half-cycle), the dynein position changes by  $\Delta x/2 = 4$  nm. If we denote the one-dimensional diffusion coefficient of dynein along the microtubule for this process by  $D_d$ , then each of the rate constants  $k_{67l}^0$ ,  $k_{67r}^0$ ,  $k_{76l}^0$ , and  $k_{76r}^0$  must be equal to  $D_d/(\Delta x/2)^2 = 4D_d/\Delta x^2$ . In these transitions, however, either the second head is binding  $\pm\Delta x$  from the first head (state 6  $\rightarrow$  state 7), or one head is releasing the microtubule (state 7  $\rightarrow$  state 6), and the changes in potential must be distributed appropriately. The potential energy of the system when dynein is in state 6 is

$$\phi_6 = \frac{1}{2} \kappa_{tb} x_b^2 + \frac{1}{2} \kappa_{bm} (x_m - x_b)^2 + \frac{1}{2} \kappa_d (x_1 - x_m)^2 \quad , \quad (3.27)$$

while the potential energy of state 7 is

$$\phi_7 = \frac{1}{2} \kappa_{tb} x_b^2 + \frac{1}{2} \kappa_{bm} (x_m - x_b)^2 + \frac{1}{2} \kappa_d (x_1 - x_m)^2 + \frac{1}{2} \kappa_d (x_1 - x_m - \Delta x)^2 \quad . \quad (3.28)$$

Thus the changes in potential energy for transitions between state 6 and state 7 are

as follows:

$$\Delta\phi_{67l} = +\frac{1}{2}\kappa_d(x_1 - x_m + \Delta x)^2 \quad (3.29)$$

$$\Delta\phi_{67r} = +\frac{1}{2}\kappa_d(x_1 - x_m - \Delta x)^2 \quad (3.30)$$

$$\Delta\phi_{76l} = -\frac{1}{2}\kappa_d(x_1 - x_m)^2 \quad (3.31)$$

$$\Delta\phi_{76r} = -\frac{1}{2}\kappa_d(x_1 - x_m - \Delta x)^2 \quad (3.32)$$

Note that no ‘ $+\Delta x$ ’ term appears in either equation 3.28 or 3.31, again because  $x_1$  represents the position of the leftmost head, rather than always the position of the same head. For each of the  $\Delta\phi$ ’s in equations 3.29 - 3.32, we assume that the associated rate constants follow the same pattern as in equations 3.25 and 3.26 above, so that the ratio of each pair of opposite transition rates has the appropriate Boltzmann distribution, as in equation 3.18. Thus,

$$k_{67l} = k_{67l}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67l}}{k_B T}\right) \quad (3.33)$$

$$k_{67r} = k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}}{k_B T}\right) \quad (3.34)$$

$$k_{76l} = k_{76l}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{76l}}{k_B T}\right) \quad (3.35)$$

$$k_{76r} = k_{76r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{76r}}{k_B T}\right) \quad (3.36)$$

## Experimental Constraints on Transition Rates

The experimental data reported by Shingyoji et al. (1998) place bounds on some of the transition rate constants  $k_{ij}$ . From the reported maximum velocity of  $6.4 \mu\text{m s}^{-1}$  and a dynein step size of  $\Delta x = 8 \text{ nm}$ , the minimum effective rate constant for the entire ATP hydrolysis cycle must be  $\geq 800 \text{ s}^{-1}$  ( $6400 \text{ nm s}^{-1} / 8 \text{ nm}$ ). However, this minimum can be further constrained, and the second order rate  $k_{51}$  can be estimated, by analyzing the reported velocity vs. ATP concentration data and comparing that to the theoretical velocity of the hydrolysis cycle in the model. The dynein hydrolysis cycle has one ATP binding step, and when there is no applied force, forward movement

is tightly coupled to hydrolysis. Therefore, the ATP concentration and the velocity of untrapped sliding microtubules should follow Michaelis-Menten kinetics when there is no load:

$$V = \frac{V_{\max}[\text{ATP}]}{K_M + [\text{ATP}]} \quad (3.37)$$

Here  $V$  is the microtubule velocity,  $V_{\max}$  is the theoretical maximum velocity, and  $K_M$  is the Michaelis constant, the ATP concentration at which  $V = \frac{1}{2} V_{\max}$ . When  $[\text{ATP}] \ll K_M$ , equation 3.37 reduces to

$$V \approx \frac{V_{\max}}{K_M} [\text{ATP}] \quad (3.38)$$

In this region, the velocity is approximately a linear function of the ATP concentration, and ATP binding to the dynein is the rate-limiting step in the cycle. In the data presented by Shingyoji et al. (1998), the velocity of the untrapped sliding microtubules and the ATP concentration were linearly related, so the dynein was operating in this regime.

In the model, the rate of switching to the diffusion loop is negligible when there is no load, implying that transitions between states 3, 6, and 7 can then be ignored. Holding the  $[\text{ADP}]$  and  $[\text{P}_i]$  concentrations in the system constant, all of the transition rates in the hydrolysis cycle except  $k_{51}[\text{ATP}]$  are constant, and we can estimate the velocity of the cycle. The assumption of constant rates allows states 1 through 4 to be collapsed into one effective state  $1_{\text{eff}}$ , and so the cycle collapses into a two-state cycle with the transition rate from state 5 to state  $1_{\text{eff}}$  equal to  $k_{51}[\text{ATP}]$  and the transition rate from state  $1_{\text{eff}}$  to state 5 equal to an effective rate,  $k_{\text{eff}}$ , derived from all the other rates in the 5-state cycle (see Figure 3.6). The time constant for completing a cycle is equal to the sum of the time constants for the individual steps, and each time constant is the inverse of the corresponding rate constant. Thus we have

$$T_{\text{cycle}} = t_{51_{\text{eff}}} + t_{1_{\text{eff}}5} \quad (3.39)$$

and

$$\frac{1}{K_{\text{cycle}}} = \frac{1}{k_{51}[\text{ATP}]} + \frac{1}{k_{1_{\text{eff}}5}} \quad (3.40)$$

where  $t_{51_{\text{eff}}}$  and  $t_{1_{\text{eff}}5}$  are the expected times to complete a transition between state  $1_{\text{eff}}$  and state 5,  $T_{\text{cycle}}$  is the expected time to complete the cycle, and  $k_{51}[\text{ATP}]$ ,  $k_{1_{\text{eff}}5}$ , and

$K_{\text{cycle}}$  are the associated transition rate constants. Equation 3.40 can be rearranged to give

$$K_{\text{cycle}} = \frac{k_{1_{\text{eff}5}}[\text{ATP}]}{k_{1_{\text{eff}5}}/k_{51} + [\text{ATP}]} \quad . \quad (3.41)$$

Finally, the expected velocity of the cycle is equal to the product of the step size and the rate constant:

$$V = \Delta x \cdot K_{\text{cycle}} = \frac{\Delta x \cdot k_{1_{\text{eff}5}}[\text{ATP}]}{k_{1_{\text{eff}5}}/k_{51} + [\text{ATP}]} \quad . \quad (3.42)$$

Comparing equations 3.37 and 3.42, we have  $V_{\text{max}} = \Delta x \cdot k_{1_{\text{eff}5}}$  and  $K_M = k_{1_{\text{eff}5}}/k_{51}$ , so that  $V_{\text{max}}/K_M = \Delta x \cdot k_{51}$ . By combining this with equation 3.38,

$$k_{51} = \frac{V}{[\text{ATP}]\Delta x} \quad . \quad (3.43)$$

Thus,  $k_{51}$  can be estimated as the slope of the velocity vs.  $[\text{ATP}]$  curve divided by  $\Delta x$ . Substituting  $\Delta x = 8 \text{ nm}$  and  $V/[\text{ATP}] = 7.8 \times 10^6 \text{ nm M}^{-1} \text{ s}^{-1}$ , as calculated from Shingyoji et al.'s (1998) Figure 3b, we find  $k_{51} \approx 9.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ . A rate of  $k_{\text{ATP}} = 1.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  was previously reported for a three-headed dynein from *Tetrahymena* cilia (Omoto and Johnson, 1986). At 1 mM ATP, the pseudo-first-order rate constant  $k_{51}[\text{ATP}] = 980 \text{ s}^{-1}$ . Because this is the rate-limiting step under no-load conditions, all forward rate constants in the main 5-step hydrolysis cycle must be  $\geq 980 \text{ s}^{-1}$ .

## Spring Constants

Numerical values for several of the spring constants in the dynein model can be derived from experimental data reported by Shingyoji et al. (1998) and the force balances of equation 3.1. Refer to Figure 3.4 for a diagram of the springs involved.

A numerical value of  $\kappa_{td} = 0.063 \text{ pN nm}^{-1}$  can be found for the effective spring between the optical trap and the dynein by comparing the force and displacement scales from Figure 2a of Shingyoji et al. (1998). By combining this value for  $\kappa_{td}$  with the reported value of  $x_d = 1.51 \cdot x_b$  from the Methods section of Shingyoji et al. (1998) and the force balance  $\kappa_{td}x_d = \kappa_{tb}x_b$  from equation 3.1, we next obtain  $\kappa_{tb} = 0.095 \text{ pN nm}^{-1}$ .

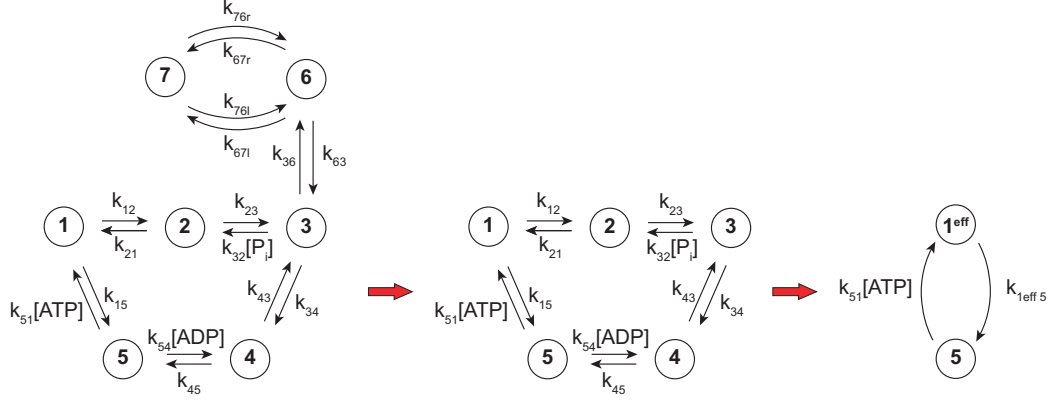


Figure 3.6: Effective collapsed cycle. By assuming zero load and constant ADP and  $P_i$  concentrations, the mechanochemical cycle from Figure 3.1 can be collapsed into a simple, two-state cycle.

Equation 3.1 also leads to the relationship

$$\frac{1}{\kappa_{td}} = \frac{1}{\kappa_{tb}} + \frac{1}{\kappa_{bd}} \quad , \quad (3.44)$$

which can be rearranged to give

$$\kappa_{bd} = \frac{\kappa_{tb} \kappa_{td}}{\kappa_{tb} - \kappa_{td}} \quad , \quad (3.45)$$

yielding  $\kappa_{bd} = 0.187 \text{ pN nm}^{-1}$ . These calculated values for  $\kappa_{tb}$  and  $\kappa_{bd}$  are approximately in the middle of their respective reported ranges of  $0.04 - 0.14 \text{ pN nm}^{-1}$  and  $0.1 - 0.3 \text{ pN nm}^{-1}$ .

A similar manipulation of equation 3.1 gives

$$\frac{1}{\kappa_{bd}} = \frac{1}{\kappa_{bm}} + \frac{1}{\kappa_{md}} \quad , \quad (3.46)$$

which in turn yields

$$\kappa_{bm} = \frac{\kappa_{md} \kappa_{bd}}{\kappa_{md} - \kappa_{bd}} \quad . \quad (3.47)$$

The value of  $\kappa_{md}$  must be estimated to use equation 3.47, because Shingyoji et al. did not determine separate microtubule and dynein positions. Recalling that  $\kappa_{md} = (1 + p)\kappa_d$ , assuming that the second dynein head is bound to the microtubule 50% of the time, so that  $p = 0.5$ , and assuming that the dynein arms each have the same stiffness of  $\kappa_d = 1.0 \text{ pN nm}^{-1}$ , as did Brokaw (2000), we have  $\kappa_{md} = 1.5 \text{ pN nm}^{-1}$ . Then we find from equation 3.47 that  $\kappa_{bm} = 0.213 \text{ pN nm}^{-1}$ .

## Diffusion Coefficients

The diffusion coefficient of a spherical body of radius  $a$  moving through a fluid with viscosity  $\eta$  is given by  $D_{\text{sphere}} = k_B T / (6\pi\eta a)$  (Berg, 1993). The beads used by Shingyoji et al. (1998) had a radius of  $a = 0.5 \mu\text{m}$ . Assuming the bead was moving through a medium with a viscosity of  $\eta = 0.01 \text{ g cm}^{-1} \text{ s}^{-1}$  and a temperature of  $T = 298 \text{ K}$ , its diffusion coefficient is  $D_b = 4.3 \times 10^5 \text{ nm}^2 \text{ s}^{-1}$ . The values of the diffusion coefficients of the microtubule,  $D_m = 6.7 \times 10^5 \text{ nm}^2 \text{ s}^{-1}$ , and of the microtubule-bound dynein,  $D_{md} = 0.9 \times 10^4 \text{ nm}^2 \text{ s}^{-1}$ , were taken from Vale et al. (1989).

## Switching Between the Hydrolysis and Diffusion Pathways

Fitting the model to the experimental oscillation data requires balancing the transition rates out of states 3 and 6 to achieve appropriate switching between the hydrolysis and diffusion pathways, which in turn constrains the parameters  $k_{36}$  and  $k_{63}$ .

Let  $x_h$  be the approximate high limit of the observed fluctuations of the dynein and let  $x_l$  be the approximate low limit. Given a dynein molecule in state 3 located at  $x_d = x_l$ , the model should tend to follow the hydrolysis cycle by moving to state 4, rather than enter the diffusion pathway by jumping to state 6. From equation 2.40, the probability of a transition from state  $i$  to state  $j$  is  $P(i \rightarrow j) = k_{ij} / \sum_j k_{ij}$ . This implies that the transition rates  $k_{34}$  and  $k_{36}$  have the following relationship:

$$k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}(x_l)}{k_B T}\right) > k_{36} \quad . \quad (3.48)$$

If the dynein is in state 3 at  $x_d = x_h$ , however, the spring force should have increased to the point that the model is more likely to switch to the diffusive pathway than to continue in the hydrolysis pathway, so that

$$k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}(x_h)}{k_B T}\right) < k_{36} \quad . \quad (3.49)$$

Combining these two inequalities places upper and lower bounds on the value of  $k_{36}$ :

$$k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}(x_h)}{k_B T}\right) < k_{36} < k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}(x_l)}{k_B T}\right) \quad . \quad (3.50)$$

Substituting the expression for  $\Delta\phi_{34}$  from equation 3.21 and the appropriate values from Table A.1, we obtain

$$8.23 \times 10^3 \text{ s}^{-1} < k_{36} < 5.84 \times 10^4 \text{ s}^{-1} \quad . \quad (3.51)$$

To fit the experimental data, the actual value chosen was  $k_{36} = 2.19 \times 10^4 \text{ s}^{-1}$ , the geometric mean of the boundaries.

Similarly, if the dynein molecule is in state 6 and located at  $x_d = x_l$ , the model should be more likely to switch out of diffusion and go back to state 3 than to continue diffusing by moving to state 7, so that

$$k_{63} > k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}(x_l)}{k_B T}\right) \quad , \quad (3.52)$$

while in state 6 at  $x_d = x_h$ , the model should tend to take a backward diffusive step, moving from state 6 to state 7 instead of reentering the hydrolysis pathway:

$$k_{63} < k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}(x_h)}{k_B T}\right) \quad . \quad (3.53)$$

Thus,

$$k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}(x_l)}{k_B T}\right) < k_{63} < k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}(x_h)}{k_B T}\right) \quad . \quad (3.54)$$

Substituting the expression for  $\Delta\phi_{67r}$  from equation 3.30 and parameter values from Table A.1, we obtain

$$6.21 \times 10^3 \text{ s}^{-1} < k_{63} < 1.65 \times 10^4 \text{ s}^{-1} \quad . \quad (3.55)$$

The actual value used to fit the experimental data was  $k_{63} = 1.01 \times 10^4 \text{ s}^{-1}$ , the geometric mean of the boundaries.

### Other Experimental Constraints

The overall rate of ATP consumption in the experiments,  $\lambda = 2.86 \text{ s}^{-1}$ , was determined by fitting the exponential decay equation

$$\text{ATP}(t) = \text{ATP}(0) \exp(-\lambda t) \quad (3.56)$$

to the ATP concentration data presented in Figure 3a of Shingyoji et al.. The approximate high and low limits of the oscillatory motion of the dynein,  $x_h = 112$  nm and  $x_l = 80$  nm, were taken from the Figure 2a of that paper. See Table A.1 for the full list of parameter values used in the detailed model.

### 3.4 Numerical Methods

The Brownian motion of the bead and the microtubule occurs on a smaller distance scale, and on a much faster time scale, than the motion imposed by the dynein and the laser trap. The Langevin equations 3.3 and 3.5 were used to update  $x_b(t)$  and  $x_m(t)$ . The forward Euler method was used to generate sample paths from the equations for the bead and microtubule. Substituting  $1/\zeta = D/k_B T$  from the Einstein-Smoluchowski relation, equations 3.3 and 3.5 were discretized as follows:

$$x_b(t + \Delta t) = x_b(t) + \frac{D_b}{k_B T} [-\kappa_{tb}x_b(t) + \kappa_{bm}(x_m(t) - x_b(t))] \Delta t + \sqrt{2D_b \Delta t} \cdot Z_1(t) \quad (3.57)$$

$$x_m(t + \Delta t) = x_m(t) + \frac{D_m}{k_B T} [-\kappa_{bm}(x_m(t) - x_b(t)) - \kappa_d(x_1(t) - x_m(t)) - I \cdot \kappa_d(x_2(t) - x_m(t))] \Delta t + \sqrt{2D_m \Delta t} \cdot Z_2(t) , \quad (3.58)$$

where values of  $Z_1$  and  $Z_2$  were chosen from independent standard normal distributions.

A Monte Carlo method based on Gillespie's Direct Method was used with equations 3.6 – 3.12 to update the chemical state of the dynein by determining whether a state change occurred during the time period  $[t, t + \Delta t)$ , and if so, which change occurred. Given a dynein molecule in state  $S(t) = i$ , equations 2.31 and 2.39 together imply that the probability of some transition out of state  $i$  occurring in a time interval of length  $\Delta t$  is

$$\Pr[i \rightarrow \text{not } i] = \sum_{j \neq i} k_{ij} \cdot \Delta t \quad , \quad (3.59)$$

and the probability of a transition from state  $i$  to a particular state  $j$  is

$$\Pr[i \rightarrow j] = k_{ij} \cdot \Delta t \quad . \quad (3.60)$$

Which transition occurred, if any, was then chosen by using equation 2.50.

The size of the time step was chosen to ensure the accuracy and the numerical stability of the solutions of both the stochastic and the ordinary differential equations. Choosing the largest possible value for  $\Delta t$  reduces the possibility of round-off errors affecting the accuracy of the calculations, as well as decreasing the number of iterations necessary to obtain a time series of a given length. However, the Euler method becomes unstable if  $\Delta t$  is too large. Equations 3.57 and 3.58 have critical time step values of  $\Delta t_{b,crit} = \Delta x_b^2 / (2D_b)$  and  $\Delta t_{m,crit} = \Delta x_m^2 / (2D_m)$  respectively. Equation 3.59 requires  $\sum_{j \neq i} k_{ij} \cdot \Delta t \leq 1$  in order for  $\Pr[i \rightarrow \text{not } i]$  to be a valid probability, implying that  $\Delta t \leq 1 / \sum_{j \neq i} k_{ij}$ . The characteristic time used to determine  $\Delta t$  in the Direct Method (from equation 2.51) is  $t_{char} = 1 / \sum_{j \neq i} k_{ij}$ , and therefore is valid to use in equation 3.59. Recall that the Direct Method chooses values for  $\Delta t$  such that the probability of more than one state transition happening within the time period  $[t, t + \Delta t)$  is negligible.

The fact that some of the  $k_{ij}$  are force dependent and therefore not constant implies that choosing a constant value for  $\Delta t$  would not be the best choice. To avoid stability problems with equation 3.59,  $\Delta t$  would have to be less than or equal to the smallest reasonably expected value of  $t_{char}$ , and thus smaller than necessary for the many iterations of the model when  $t_{char}$  is not near its lower limit. As stated above, this would increase the number of iterations needed to complete a time series, and increase the potential for accumulation of round-off errors.

Given these constraints, at the beginning of a simulation run we defined  $\Delta t_{SDE}$  to be a fixed value slightly less than the smaller of  $\Delta t_{b,crit}$  and  $\Delta t_{m,crit}$ . At each iteration, we defined  $\Delta t_{MC}$  be the tentative time step for the Monte Carlo part of the simulation, chosen according to equation 2.51. The actual time step used for the iteration was then chosen as

$$\Delta t = \min(\Delta t_{SDE}, \Delta t_{MC}) \quad (3.61)$$

to ensure the stability of the solutions to all of the equations.

The algorithm used at each time step to generate the sample path was as follows:

1. Update  $[\text{ATP}]$ ,  $[\text{ADP}]$ , and  $[\text{P}_i]$  if necessary.
2. Update the forces and transition rates as necessary.
3. Generate  $Z_1(t)$  and  $Z_2(t)$  from independent standard normal distributions.
4. Generate  $\Delta t$  according to equation 3.61.
5. Calculate  $x_b(t + \Delta t)$  and  $x_m(t + \Delta t)$ .
6. Generate  $r(t)$  from a uniform  $[0,1]$  distribution.
7. Decide which chemical state transition occurred, if any, according to equation 2.50.
8. Calculate  $S(t + \Delta t)$ ,  $x_1(t + \Delta t)$ ,  $x_2(t + \Delta t)$ , and  $x_d(t + \Delta t)$ .
9. Record the updated data values.
10. Repeat the above steps to achieve a time series of the desired length.

### 3.5 Model Results

A typical time series generated by the detailed model is shown in Figure 3.7. The dynein moves processively along the microtubule to a distance of  $\sim 80\text{--}110$  nm, then begins to move backward and forward somewhat irregularly with fluctuations of  $\sim 30\text{--}35$  nm in amplitude, as in Shingyoji et al.'s (1998) experimental data. The average velocity of the model dynein's initial rise from 0 to 80 nm is  $v_{init} = 6.96 \pm 2.24 \mu\text{m s}^{-1}$ , (mean  $\pm$  sd,  $n = 100$  runs). Shingyoji et al. reported an initial velocity of  $5.3 \mu\text{m s}^{-1}$ .

Although it is much faster to run the hybrid SDE model described in the previous section than to run an equivalent model based entirely on the Direct Method, it is

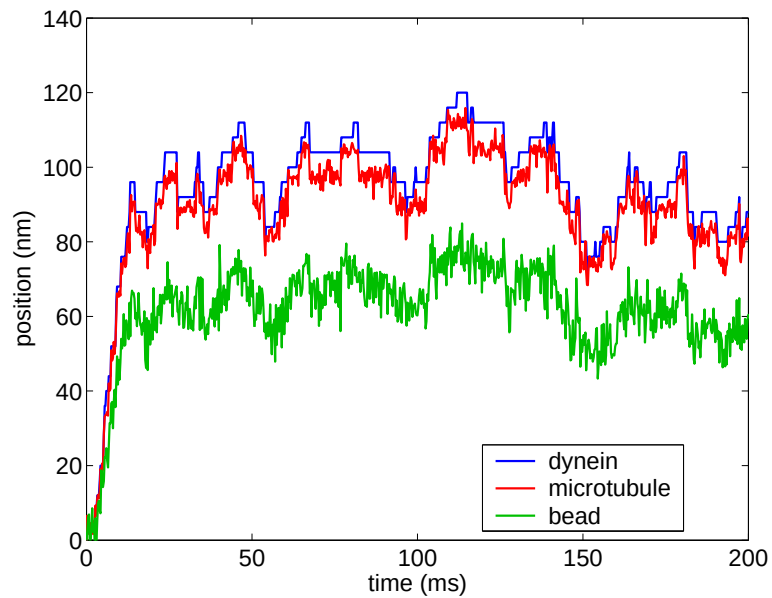


Figure 3.7: A typical time series generated by the detailed model. As in the plot of experimental data in Figure 2a of Shingyoji et al. (1998), the dynein and microtubule displacements rise steadily to  $\sim 80 - 110$  nm, and then fluctuates with an overall peak-to-peak height of  $\sim 30 - 35$  nm. Over time, the ATP concentration declines and the ADP and  $P_i$  concentrations rise at rates consistent with the rate of ATP consumption measured by Shingyoji et al.

still very computationally expensive. Therefore, the determination of characteristics of the model requiring a large number of long time series to estimate accurately, such as the power spectrum, the long-term average velocity, and the effective diffusion coefficient of the motor, was deferred to the simplified model presented in Chapter 4.

## Chapter 4

# Simplified Dynein Model with Diffusion

The detailed model described in Chapter 3 can be reduced to a model which tracks only the position of the dynein. This is done by assuming that the Brownian motion of both the bead and the microtubule occurs on a very fast time-scale compared to the motions caused by the dynein. The bead and microtubule are then taken to be located at their equilibrium positions relative to the position of the dynein at each time step. This simplification of the model eliminates equations 3.3 and 3.5, the SDEs governing the bead and microtubule, and allows a much longer time step based on the slower time scale of dynein motion to be used in the simulations. Simulation speed for a given time series length is therefore greatly increased, and sample paths can be generated efficiently using Gillespie's Direct Method, as described in Section 2.4.

The simplified model also permits the average velocity and the effective diffusion coefficient of the dynein motor to be computed directly and exactly from the probability transition matrix governing the model, rather than estimating their values by averaging over multiple Monte Carlo simulations of the dynein motor's behavior. The value of the motor's randomness parameter therefore also can be calculated exactly.

See Section 4.2.1. The simplified and detailed versions of the model give qualitatively the same results, as shown below in Section 4.3. Quantitative differences in the results can be reduced by adjusting some parameters in the simplified model.

## 4.1 Simplification Steps

The model is simplified by assuming that the Brownian motion of the bead and the microtubule occurs on a much faster time scale than the dynein motion, and can therefore be ignored. In this approximation the microtubule and bead positions are set to their equilibrium values relative to the position of the dynein at each time step. Physically, the system is reduced from a series of bodies connected by springs, to a single body representing the dynein which is attached to the optical trap by one effective spring, as shown in Figure 3.4. This reduction allows us to write one Langevin equation for the system, similar to equations 3.3 and 3.5:

$$\zeta_{bmd} \frac{dx_d}{dt} = -\kappa_{td} x_d + \sqrt{2\zeta_{bmd} k_B T} \cdot f_{bmd}(t) \quad , \quad (4.1)$$

where  $\zeta_{bmd}$  is the effective friction coefficient and  $f_{bmd}(t)$  represents the thermal fluctuations of the system. The effective spring constant,  $\kappa_{td}$ , is given in terms of the original springs in the system by

$$\kappa_{td} = \frac{1}{\frac{1}{\kappa_{tb}} + \frac{1}{\kappa_{bm}} + \frac{1}{\kappa_{md}}} \quad . \quad (4.2)$$

Recall from Section 3.2 that  $\kappa_{md} = (1 + p)\kappa_d$ , where  $0 \leq p \leq 1$  describes the percent of time that both dynein heads are bound to the microtubule, so that  $(1 + p)$  is the average number of bound dynein heads. A value of  $p = 0.5$  was assumed for the model.

From equation 3.1, the equilibrium positions of the bead and the microtubule are found to be

$$x_{b,eq}(t) = \kappa_{td} \left( \frac{1}{\kappa_{tb}} \right) x_d(t) \quad (4.3)$$

$$x_{m,eq}(t) = \kappa_{td} \left( \frac{1}{\kappa_{tb}} + \frac{1}{\kappa_{bm}} \right) x_d(t) \quad . \quad (4.4)$$

The changes in potential used in the force dependent rates, which were given by equations 3.21, 3.22, and 3.29 – 3.32 for the detailed model, become

$$\Delta\phi_{34} = \frac{1}{2}\kappa_{td}((x_d + \Delta x)^2 - x_d^2) \quad (4.5)$$

$$\Delta\phi_{43} = \frac{1}{2}\kappa_{td}(x_d^2 - (x_d + \Delta x)^2) \quad (4.6)$$

$$\Delta\phi_{67l} = \frac{1}{2}\kappa_{td}\left(\left(x_d + \frac{\Delta x}{2}\right)^2 - x_d^2\right) \quad (4.7)$$

$$\Delta\phi_{67r} = \frac{1}{2}\kappa_{td}\left(\left(x_d - \frac{\Delta x}{2}\right)^2 - x_d^2\right) \quad (4.8)$$

$$\Delta\phi_{76l} = \frac{1}{2}\kappa_{td}\left(x_d^2 - \left(x_d + \frac{\Delta x}{2}\right)^2\right) \quad (4.9)$$

$$\Delta\phi_{76r} = \frac{1}{2}\kappa_{td}\left(x_d^2 - \left(x_d - \frac{\Delta x}{2}\right)^2\right) \quad (4.10)$$

for the simplified model.

The quasi-equilibrium assumption means that  $x_b$ ,  $x_m$ , and  $x_d$  are changing together in the simplified model, rather than separately, as in the detailed model. This affects the transition rates for the state changes in the model in which the physical position of the dynein changes, that is, transitions between states 6 and 7 and those between states 3 and 4.

The diffusion coefficient for the dynein,  $D_d$ , should be replaced by an effective diffusion coefficient,  $D_{\text{bmd}}$ , for the bead-microtubule-dynein combination, in the diffusion cycle rates  $k_{67l}^0$ ,  $k_{67r}^0$ ,  $k_{76l}^0$ , and  $k_{76r}^0$ . Thus we set

$$D_{\text{bmd}} = \frac{1}{\frac{1}{D_b} + \frac{1}{D_m} + \frac{1}{D_d}} \quad (4.11)$$

and

$$k_{67l}^0 = k_{67r}^0 = k_{76l}^0 = k_{76r}^0 = \frac{4D_{\text{bmd}}}{\Delta x^2} \quad (4.12)$$

In practice, because both  $D_b$  and  $D_m \gg D_d$ ,  $D_{\text{bmd}} \approx D_d$ , and this adjustment has only a small effect on the behavior of the model.

The effect of the quasi-equilibrium assumption is larger on the transition rates  $k_{34}$  and  $k_{43}$ , which govern the physical movement of the dynein during the ATP hydrolysis

cycle. In fact, the rates  $k_{34}^0$  and  $k_{43}^0$  must be modified in the simple model. The changes in potential given by equations 3.21 and 4.5 can be algebraically rearranged to give

$$\Delta\phi_{34,detailed} = \kappa_d(x_1 - x_m)\Delta x + \frac{1}{2}\kappa_d\Delta x^2 \quad (4.13)$$

and

$$\Delta\phi_{34,simplified} = \kappa_{td}x_d\Delta x + \frac{1}{2}\kappa_{td}\Delta x^2 \quad . \quad (4.14)$$

Assuming that  $x_m = x_{m,eq}$ , and utilizing the force balance  $\kappa_d(x_1 - x_{m,eq}) = \kappa_{td}x_d$  from equation 3.1, we can rewrite equation 4.13 as

$$\Delta\phi_{34,detailed} = \kappa_{td}x_d\Delta x + \frac{1}{2}\kappa_d\Delta x^2 \quad . \quad (4.15)$$

Then we have that

$$\Delta\phi_{34,detailed} - \Delta\phi_{34,simplified} = \frac{1}{2}(\kappa_d - \kappa_{td})\Delta x^2 > 0 \quad . \quad (4.16)$$

Thus, the change in potential  $\Delta\phi_{34}$  is always smaller in the simplified model than in the detailed model for  $\Delta x \neq 0$ . A smaller change in potential per step allows the dynein to move farther from the center of the trap before feeling an equivalent restoring force. This effect is minor when the displacement is small and ATP binding is the rate-limiting step of the hydrolysis cycle. It becomes a significant factor as the displacement increases and the transition from state 3 to state 4 becomes rate-limiting.

To correct for this effect, we adjusted the value of the rate constant  $k_{34}^0$  in the simplified model to match the behavior of the simple model with that of the detailed model. The value of  $k_{43}^0$  was adjusted by the same factor to maintain the condition of detailed balance. The adjustment factor was determined through a process of trial and error. Based on the empirical results, we set

$$k_{34}^0 = k_{34,detailed}^0/5 \quad (4.17)$$

$$k_{43}^0 = k_{43,detailed}^0/5 \quad (4.18)$$

in the simplified model.

### Switching Between the Hydrolysis and Diffusion Pathways

As shown for the detailed model in Section 3.3, the rate constants  $k_{36}$  and  $k_{63}$  are constrained in the simplified model to achieve proper switching between the hydrolysis and diffusion pathways. However, because of the differences in the two versions of the model described above, these constraints must also be modified. Equation 3.50,

$$k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}(x_h)}{k_B T}\right) < k_{36} < k_{34}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{34}(x_l)}{k_B T}\right) \quad , \quad (4.19)$$

still holds, but now we substitute the expression for  $\Delta\phi_{34}$  from equation 4.5 and the appropriate values from Table A.2 to obtain

$$1.65 \times 10^3 \text{ s}^{-1} < k_{36} < 1.17 \times 10^4 \text{ s}^{-1} \quad . \quad (4.20)$$

To fit the experimental data, the actual value chosen was  $k_{36} = 4.39 \times 10^3 \text{ s}^{-1}$ , the geometric mean of the boundaries.

Similarly, starting from equation 3.54,

$$k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}(x_l)}{k_B T}\right) < k_{63} < k_{67r}^0 \exp\left(-\frac{1}{2} \frac{\Delta\phi_{67r}(x_h)}{k_B T}\right) \quad , \quad (4.21)$$

we substitute the expression for  $\Delta\phi_{67r}$  from equation 4.8 and parameter values from Table A.2 to obtain

$$6.00 \times 10^3 \text{ s}^{-1} < k_{63} < 1.60 \times 10^4 \text{ s}^{-1} \quad . \quad (4.22)$$

The value used to fit the experimental data was  $k_{63} = 1.57 \times 10^4 \text{ s}^{-1}$ , about 2% below the upper limit.

## 4.2 Statistical Properties of the Dynein Motor

The statistical properties of different models can often be estimated and compared with experimentally measurable motor characteristics to determine whether a particular model is a good fit. These properties include the mean, the variance, and the covariance of the motor's position, which can be used to characterize the dynein motor, as described in the following sections.

### 4.2.1 Velocity, Diffusion, and Randomness

Experimentally measurable characteristics of a molecular motor include the average velocity, the effective diffusion coefficient, and the randomness parameter, which provide information about the mean behavior of the motor and the variability of that behavior. These parameters can be calculated from the mean and the variance of the motor position,  $E[x(t)]$  and  $\sigma^2(x(t)) = E[x(t)^2] - (E[x(t)])^2$ , respectively.

The average velocity of the dynein is the mean change in the dynein position over time:

$$v = \lim_{t \rightarrow \infty} \frac{E[x(t)]}{t} \quad . \quad (4.23)$$

The effective diffusion coefficient describes the variability of the dynein position over time, and can be calculated as

$$D_{\text{eff}} = \lim_{t \rightarrow \infty} \frac{\sigma^2(x(t))}{2t} \quad . \quad (4.24)$$

The randomness parameter (Schnitzer and Block, 1995) is a dimensionless number defined as

$$r = \lim_{t \rightarrow \infty} \frac{\sigma^2(x(t))}{E[x(t)] \cdot \Delta x} = \frac{2D_{\text{eff}}}{v \cdot \Delta x} \quad . \quad (4.25)$$

Thus  $r$  also describes the variability of the motor, but in a way which gives more insight into the underlying motor mechanism. If  $r = 0$ , there is no variability and the motor takes completely regular steps, moving like clockwork. A value of  $r = 1$  implies that the motor behaves according to a Poisson process, with one rate-limiting step in its chemical cycle per physical step. Assuming that motor backstepping and reverse transitions in the ATP synthesis cycle are negligible, the value  $1/r$  measures the number of rate-limiting steps in the motor's cycle.

The simplified model was analyzed using the method of Wang et al. (2003) to compute the average velocity, effective diffusion coefficient, and randomness parameter as functions of applied force and ATP concentration. This method is briefly described here. The simplified dynein model derived above is a discrete Markov process in two dimensions. The first state variable,  $N(t)$ , characterizes the position of the dynein molecule along the microtubule and the second variable,  $S(t)$ , determines the chemical state of the molecule. Let  $p_i(n, t) = \Pr[N(t) = n, S(t) = i]$  ( $i = 1, \dots, 7$ ) be the

seven elements of the column vector  $\mathbf{p}(n, t)$  and let  $\mathbf{M}$  be the transition matrix governing changes in  $\mathbf{p}(n, t)$ , as in Section 3.2. The transition matrix  $\mathbf{M}$  can be written as  $\mathbf{M} = \mathbf{L} + \mathbf{L}_+ + \mathbf{L}_-$ , where  $\mathbf{L}$ ,  $\mathbf{L}_+$ , and  $\mathbf{L}_-$  contain the transitions in which  $n$  does not change, those in which  $n \rightarrow n + 1$ , and those in which  $n \rightarrow n - 1$ , respectively. Then the master equation which governs  $\mathbf{p}$  can be written as

$$\frac{d\mathbf{p}(n, t)}{dt} = \mathbf{L}\mathbf{p}(n, t) + \mathbf{L}_+\mathbf{p}(n - 1, t) + \mathbf{L}_-\mathbf{p}(n + 1, t) \quad . \quad (4.26)$$

It is possible to show that the average velocity is given by

$$v = \Delta x \sum_{j=1}^7 [(\mathbf{L}_+ - \mathbf{L}_-)\mathbf{p}^s]_j \quad , \quad (4.27)$$

where the stationary probability  $\mathbf{p}^s$  satisfies the equation

$$\frac{d\mathbf{p}^s}{dt} = \mathbf{M}\mathbf{p}^s = 0 \quad , \quad (4.28)$$

subject to the constraint that the elements of  $\mathbf{p}^s$  must sum to one. The effective diffusion coefficient is found from the formula

$$D_{\text{eff}} = \frac{\Delta x^2}{2} \sum_{j=1}^7 [(\mathbf{L}_+ + \mathbf{L}_-)\mathbf{p}^s + 2(\mathbf{L}_+ - \mathbf{L}_-)\mathbf{r}]_j \quad , \quad (4.29)$$

where the vector  $\mathbf{r}$  satisfies the equation

$$\mathbf{M}\mathbf{r} = \sum_{j=1}^7 [(\mathbf{L}_+ - \mathbf{L}_-)\mathbf{p}^s]_j \mathbf{p}^s - (\mathbf{L}_+ - \mathbf{L}_-)\mathbf{p}^s \quad , \quad (4.30)$$

subject to the constraint that the elements of  $\mathbf{r}$  must sum to zero. For a detailed derivation of these equations, see Wang et al. (2003).

## 4.2.2 Autocovariance Function

As described in Section 4.2.1, the mean and variance of a stochastic process provide important information about the state of that process at some time  $t$ . The covariance of the process, meanwhile, can provide more information about the dynamics of the process, by describing how the process value at time  $t$  is related to the value at some

other time  $s = t + \tau$ . The autocovariance function of a random variable  $X$  is defined as the covariance of the values of the variable at two different times:

$$\begin{aligned} \text{ACV}_X(t, \tau) &= \text{Cov}\left(X(t), X(t + \tau)\right) \\ &= \text{E}\left[\left(X(t) - \text{E}[X(t)]\right)\left(X(t + \tau) - \text{E}[X(t + \tau)]\right)\right] \end{aligned} \quad (4.31)$$

It is called the autocovariance function to emphasize that it is a function of a single random variable measured at different times, rather than a function of two different random variables. The autocovariance function is also sometimes referred to as the autocorrelation function, although that term more properly applies to the autocovariance divided by the variance of  $X(t)$ .

If the statistics of  $X(t)$  such as the autocovariance depend only on the size of the time interval between measurements,  $|\tau|$ , and not on the actual times of the measurements, then  $X$  is termed a stationary process. In this case, we can let

$$R(\tau) = \text{Cov}(X(t), X(t + \tau)) \quad , \quad (4.32)$$

and it can be shown that  $\text{E}[X(t)]$  is constant and that  $R(\tau)$  is an even function of  $\tau$ , that is,  $R(-\tau) = R(\tau)$ , under stationarity.

If  $X(t)$  is a stationary process, then the autocovariance can be decomposed to look for dominant frequency components in the behavior of  $X(t)$ . The spectral density or power spectrum of  $X(t)$  is given by

$$S(\omega) = \int_{-\infty}^{\infty} R(\tau) \cos(\omega\tau) d\tau \quad , \quad (4.33)$$

the Fourier transform of  $R(\tau)$ . Note that there are no  $\sin(\omega\tau)$  terms in equation 4.33 because  $R(\tau)$  is even. The inverse Fourier transform of  $S(\omega)$  then gives

$$R(\tau) = \frac{1}{2\pi} \int_{-\infty}^{\infty} S(\omega) \cos(\omega\tau) d\omega \quad , \quad (4.34)$$

from which it is easy to see that there will be a peak in  $S(\omega)$  at any component frequency  $\omega = \omega_0$  which strongly contributes to the behavior of  $X(t)$ .

For a stationary process  $X(t)$ , we also expect the autocovariance to decrease as the time interval between measurements increases. That is,

$$\lim_{\tau \rightarrow \infty} R(\tau) = 0 \quad . \quad (4.35)$$

$R(\tau)$  often asymptotically goes to

$$R(\tau) \sim R_0 \cdot \exp(-\tau/\tau_c) \quad , \quad (4.36)$$

where  $R_0$  and  $\tau_c$  are constants (Gardiner, 1985). The correlation time,  $\tau_c$ , is a parameter which characterizes how quickly the correlation decays between measurements of  $X$ . That is,  $X(t)$  and  $X(t+\tau)$  will appear to be correlated unless  $\tau \gg \tau_c$ . Thus, both the peak pattern in the power spectrum and the correlation time help characterize the behavior of  $X(t)$ , and can be used to distinguish between different mechanisms for generating  $X(t)$ .

## 4.3 Results

### 4.3.1 Time Series

A typical time series generated by the simplified model is shown in Figure 4.1. Here, as in the time series from the detailed model shown in Figure 3.7, the dynein moves systematically to a displacement of  $\sim 80-110$  nm, then fluctuates irregularly with an amplitude of  $\sim 30-35$  nm. The initial velocity of the dynein is  $v_{init} = 5.02 \pm 1.08 \mu\text{m s}^{-1}$  ( $n = 100$  runs).

The benefits of using the simplified model are that it greatly increases simulation speed and ease of analysis compared to using the detailed model. Because of these advantages, the simplified model was used to simulate dynein behavior and study motor characteristics over a wider range of conditions than was the detailed model.

### 4.3.2 Velocity, Diffusion, and Randomness

The model predicts a load-velocity curve that is concave-down, with the velocity being almost constant at small loads and then decreasing sharply as the force increases (Figure 4.2). The velocity increases as a function of the ATP concentration at a given load, but goes to zero at loads of approximately 6.1 to 6.2 pN, because there

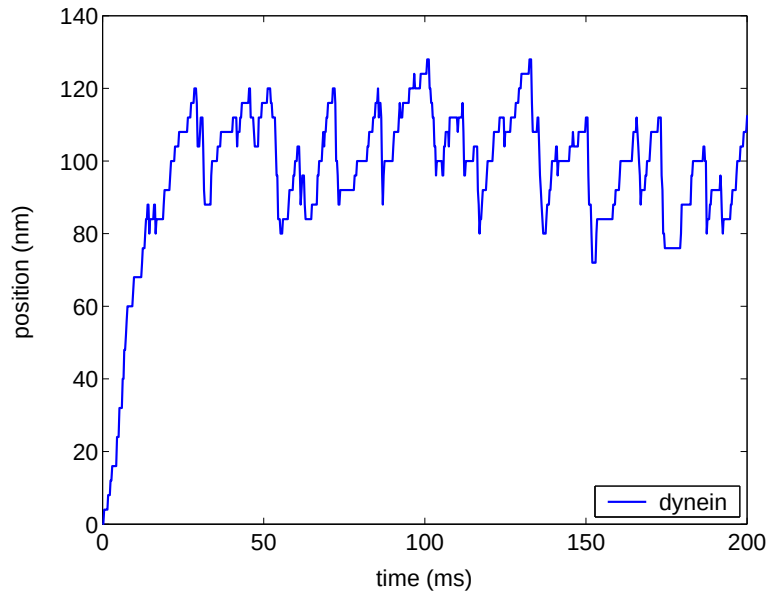


Figure 4.1: A typical time series generated by the simplified model. The results are similar to those of the detailed model (shown in Figure 3.7).

is then a significant probability of entering the diffusion pathway. This occurs well below the thermodynamic stall force, which for the ATP levels shown ranges between approximately 10.9 pN and 12.5 pN. A key model assumption is the existence of the diffusion pathway. To investigate how this pathway affects the behavior of the motor we removed it from the model by setting the transition rates  $k_{36} = k_{63} = 0$ , so that the dynein had to remain in the main hydrolysis pathway at all times. In this case, the velocity decreases more slowly and does not reach zero until the thermodynamic stall force (Figure 4.2). The plot of velocity vs [ATP] shows that at zero force, the velocity is directly proportional to the ATP concentration (Figure 4.3). It is not until the force approaches 5 to 6 pN that there is a significant deviation from a linear relationship between the velocity and the ATP level.

The effective diffusion coefficient in the normal model is almost constant at low forces, declines to a minimum as the force increases, and then spikes upward as the force increases past 6 pN (Figure 4.4). This rapid increase in the effective diffusion coefficient is due to the dynein's moving backward as the model shifts into its diffusion pathway. The effective diffusion coefficient increases as the ATP concentration

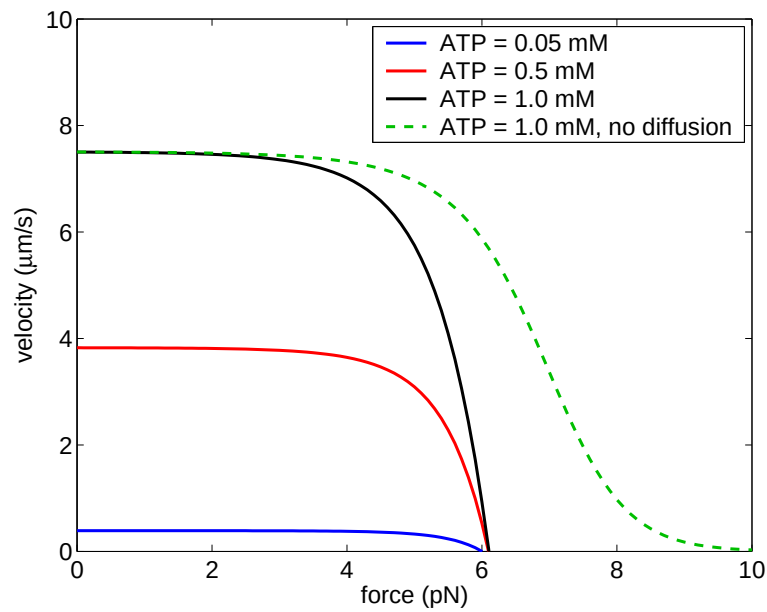


Figure 4.2: The predicted velocity vs. force curves at constant levels of  $[ATP]$ : For these traces,  $[ADP]$  and  $[P_i]$  were held constant at their physiological levels of  $10^{-5}$  M and  $10^{-3}$  M, respectively. The dynein velocity is predicted to remain relatively constant at low forces, then curve sharply downward as the load approaches  $\sim 5 - 6$  pN. The velocity goes to zero at a much smaller load than the thermodynamic stall force of  $\sim 11 - 12.5$  pN. In contrast, if the diffusion pathway is removed from the model the velocity changes much more gradually as the force increases. The dynein finally reaches zero velocity at the thermodynamic stall force.

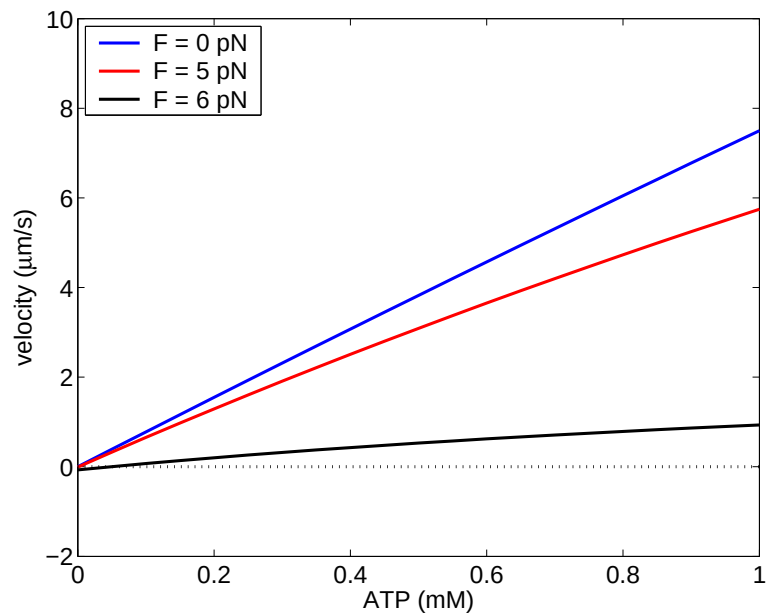


Figure 4.3: The predicted velocity vs. [ATP] at constant force levels: [ADP] and  $[P_i]$  were held constant as in Figure 4.2. At  $F = 0$  pN, the velocity is a linear function of [ATP]. The velocity does not significantly deviate from a straight line until  $F \sim 5$  pN. Although it appears that the three curves pass through the origin, the dynein velocity actually reaches zero while the ATP concentration is still positive, because of the constraint of detailed balance.

increases at a given load. If the diffusion pathway of the model is absent, the effective diffusion coefficient does not suddenly rise, but rather continues decreasing until the motor stalls.

The randomness parameter also remains relatively constant near  $r \sim 1$  at low forces, indicating a single rate limiting step in the hydrolysis pathway (Figure 4.5). As the force is increased, the transition rate from state 3 to state 4 decreases, and accordingly the randomness parameter also decreases. The randomness parameter then increases rapidly as the force approaches 6 pN. This rapid increase is a consequence of the diffusion pathway. If the diffusion pathway is removed, the behavior of the randomness parameter is significantly different. It decreases to a minimum value of  $r \sim 0.5$  as the rate of the force dependent step slows to that of the ATP binding rate, causing the cycle to have two rate-limiting steps (Figure 4.5). As the force dependent rate continues declining, it becomes rate-limiting by itself, and the value of  $r$  increases again toward one.

### 4.3.3 Autocovariance Function

A typical plot of the autocovariance for the simplified model is shown in Figure 4.6. As expected, the correlation between values of the dynein displacement drops off rapidly as the time interval between them increases. A least squares fit of equation 4.36 to the initial values of this particular data series gives

$$R(\tau) = 212 \cdot \exp(-\tau/3.16) \quad , \quad (4.37)$$

so that the correlation time is  $\hat{\tau}_c = 3.16$  ms. For this fit,  $R^2 = 0.988$ . Based on  $n = 40$  runs,  $\hat{\tau}_c = 3.35 \pm 0.31$  ms for the simplified model with passive backsliding. From equation 4.1, the relaxation time scale should be  $\tau_{bmd} = \zeta_{bmd}/\kappa_{td} = 7.41$  ms.

The Fourier transform of the autocovariance from Figure 4.6 is shown in Figure 4.7. There are no distinct peaks in this power spectrum, indicating that there are no dominant frequencies underlying the observed dynein fluctuations. There were also no dominant frequencies found in the experimental data (Shingyoji et al., 1998).

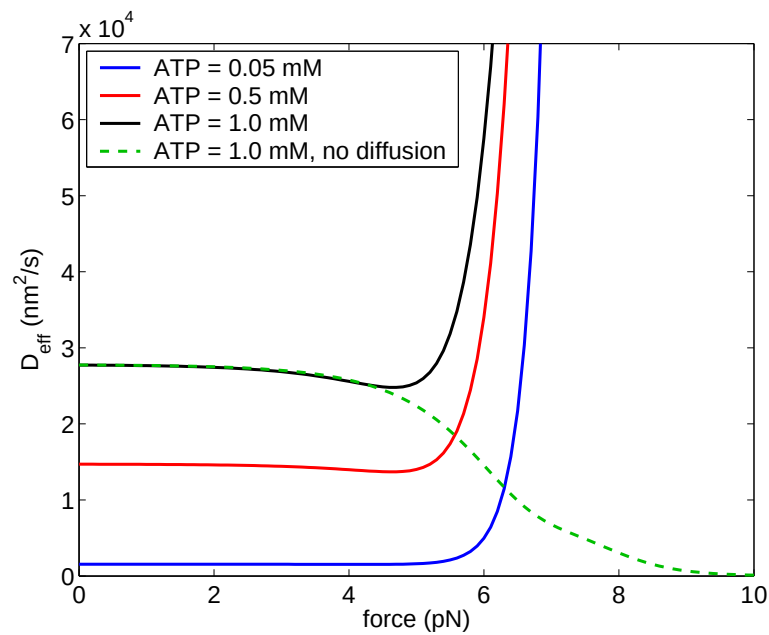


Figure 4.4: The predicted behavior of the effective diffusion coefficient ( $D_{\text{eff}}$ ) as a function of force at constant levels of [ATP]:  $D_{\text{eff}}$  is almost constant at low forces, declines to a minimum as the force increases, and then spikes upward as the force increases past 6 pN. This is due to the dynein's shifting into the diffusion pathway and sliding backward under load. However, if the diffusion pathway is removed, the resulting model predicts that  $D_{\text{eff}}$  will continue declining towards zero as the force increases to the thermodynamic stall force and the dynein stops moving.

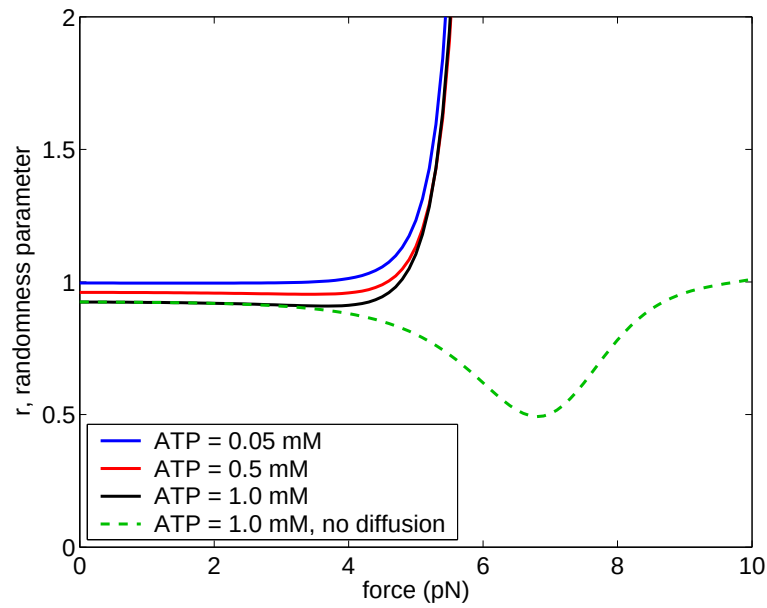


Figure 4.5: The predicted behavior of the randomness parameter ( $r$ ) as a function of force at constant levels of  $[ATP]$ : The randomness parameter remains almost constant near  $r \sim 1$  at low loads. As the force increases past  $F \sim 5$  pN,  $r$  increases rapidly as the dynein shifts more and more into the diffusion pathway, reducing its velocity and increasing the variability of its position. In contrast, if the diffusion pathway is removed from the model, the randomness parameter is predicted to slowly decrease to a minimum value of  $r \sim 0.5$ , indicating that the force-dependent rate is then equal to the rate-limiting ATP binding rate. It increases again toward a value of  $r \sim 1$  as the force-dependent step becomes the single rate-limiting step.

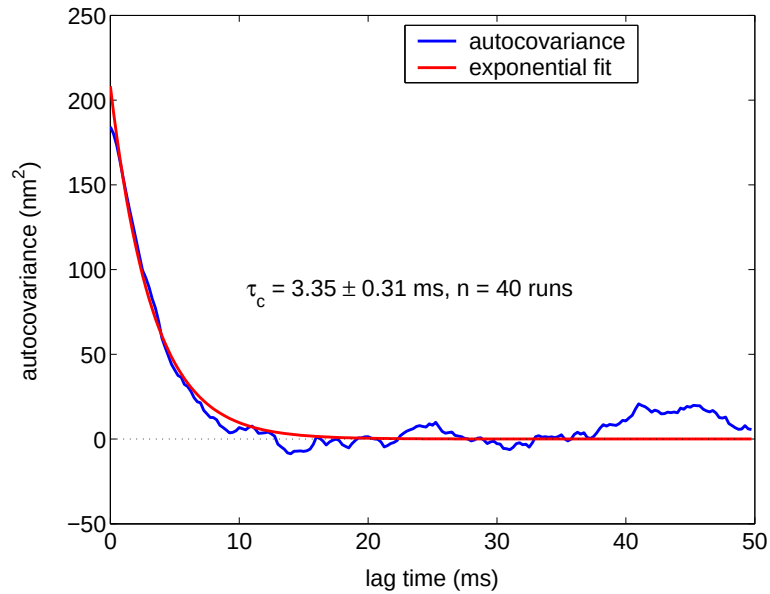


Figure 4.6: Typical autocovariance for a time series from the simplified model with diffusion. The average correlation time for this model is  $\hat{\tau}_c = 3.35$  ms.

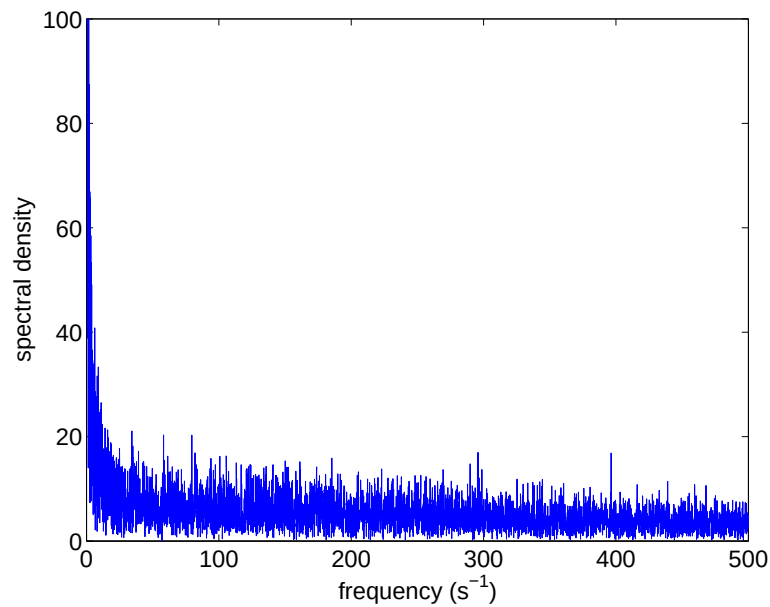


Figure 4.7: Typical power spectrum of a time series from the simplified model with diffusion. The spectral density is the Fourier transform of the autocovariance function. The lack of any peaks indicates that there are no dominant underlying frequencies contributing to the time series data in Figure 4.1.

## Chapter 5

# Alternative Dynein Models

The dynein model presented in Chapters 3 and 4 is based on a mechanism of backward diffusion along the microtubule by the dynein under a sufficient load. At least one other model has been published that is based instead on a mechanism of active, ATP-driven backward motion (Brokaw, 2000). This chapter will discuss one such active backstepping model as an alternative to the passive backsliding model presented in the previous chapters.

### 5.1 Active Backstepping Models

Brokaw (2000) has proposed an alternative model that can also account for the back and forth motion of single dynein molecules, although he does not attempt to correlate the states of the model to particular states in dynein's chemical cycle. The most significant difference between that model and the one presented in this dissertation is that the dynein's backward motion is actively driven by ATP hydrolysis in his model, as the motor switches into reverse under high loads. This mechanism is based on the assumption that both the forward and backward velocities are  $[ATP]$  dependent, which was also proposed by Shingyoji et al. (1998). Both forward and

reverse velocities should then decrease as the ATP is consumed over time. Our model assumes that the backward movement of the dynein is passive and biased by the strain developed in the laser trap, and thus the backward velocity is independent of the ATP concentration. In this case, the forward velocity would decrease with declining ATP levels while the backward velocity should remain constant. This difference provides a method for experimentally distinguishing between the two assumptions.

The simplified model developed in Chapter 4 was modified to create an active back-stepping model in order to compare the effects of the two stepping mechanisms. The mechanochemical cycle of the modified model is diagrammed in Figure 5.1. The diffusion pathway was replaced by a mirror image of the forward hydrolysis cycle, in which the chemical cycle remains the same but the direction of motion along the microtubule is reversed. The rate constants used in the backward cycle were the same as the corresponding rates in the forward cycle (that is,  $k_{i*j*} = k_{ij}$ ), but theoretically could be different if necessary to fit the data.

The transition rates  $k_{33*}$  and  $k_{3*3}$  were balanced with  $k_{34}$  and  $k_{3*4*}$  respectively, to achieve proper switching between the forward and backward hydrolysis loops. Following a similar derivation to that given in equations 4.19–4.22, the switching rates were found to be

$$k_{33*} = k_{34}^0 \exp \left( - \frac{k_{td}}{4k_B T} [(x_s + \Delta x)^2 - x_s^2] \right) \quad (5.1)$$

$$k_{3*3} = k_{3*4*}^0 \exp \left( - \frac{k_{td}}{4k_B T} [(x_s - \Delta x)^2 - x_s^2] \right) \quad , \quad (5.2)$$

where  $x_s = 96$  nm is the desired switching point between the two loops. Substituting appropriate values into equations 5.1 and 5.2, the values of the rates are then  $k_{33*} = 4.39 \times 10^3 \text{ s}^{-1}$  and  $k_{3*3} = 5.59 \times 10^8 \text{ s}^{-1}$ .

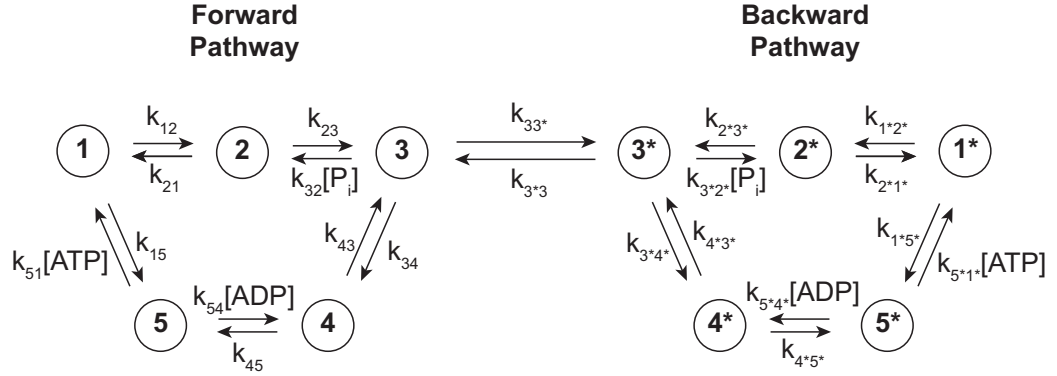


Figure 5.1: Schematic mechanochemical cycle of the active backstepping model. In this model, the dynein can switch its direction of movement on the microtubule. Both forward and backward motion are powered by ATP hydrolysis. Compare this mechanism to the passive backsliding shown in Figure 3.5.

## 5.2 Results

### 5.2.1 Time Series

A typical time series from the active backstepping model is shown in Figure 5.2. As expected, the dynein moves smoothly to a displacement of  $\sim 80\text{--}110\text{ nm}$ . The fluctuations of  $\sim 40\text{--}45\text{ nm}$  made by this model seem to be slightly larger than those made by the previous models, but even if the difference is significant, it might be reduced by further tuning of the model parameters. See the time series in Figures 3.7 and 4.1 for comparison. The initial velocity of the dynein in the active backstepping model is  $v_{init} = 5.00 \pm 1.02\text{ }\mu\text{m s}^{-1}$  ( $n = 100$  runs), in close agreement with the other models.

### 5.2.2 Velocity, Diffusion, and Randomness

The active backstepping model exhibits a velocity vs. force behavior (see Figure 5.3) that is not significantly different from that of the passive diffusion model (see Figure 4.2). The transition from the region of almost constant velocity to the region of rapid decline is somewhat sharper in the active model, and the force at which the

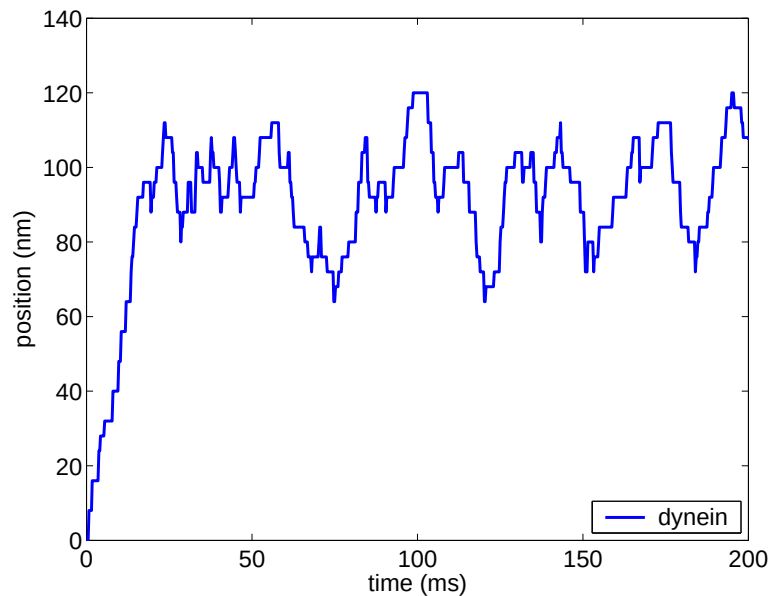


Figure 5.2: A typical time series generated by the active backstepping model. See Figures 3.7 and 4.1 for comparison with the time series of the detailed and simple versions of the backward diffusion model.

dynein velocity reaches zero is only slightly less than the 6 pN force of the passive model. Note that when the backward hydrolysis loop and the diffusion pathway are eliminated from the two models, the forward cycle that remains is the same in both, so the behavior of the two models in that case is identical.

The active model's profile of dynein velocity as a function of [ATP] is also not significantly different from that of the passive model, unless the force is close to the critical value of  $\sim 6$  pN. Compare the behaviors shown in Figures 5.4 and 4.3. At  $F = 0$  pN, the velocity is a linear function of [ATP], indicating that the [ATP] level is rate-limiting.

In contrast, the effect of an applied force on the effective diffusion coefficient of the motor is very different in the two models, and both are predicted to behave differently from a motor which has only a single hydrolysis pathway. Measuring the variability of the motor displacement at appropriate force levels and calculating the effective diffusion coefficient thus could easily distinguish between these mechanisms experimentally. See Figure 5.5 for the active backstepping model and Figure 4.4 for

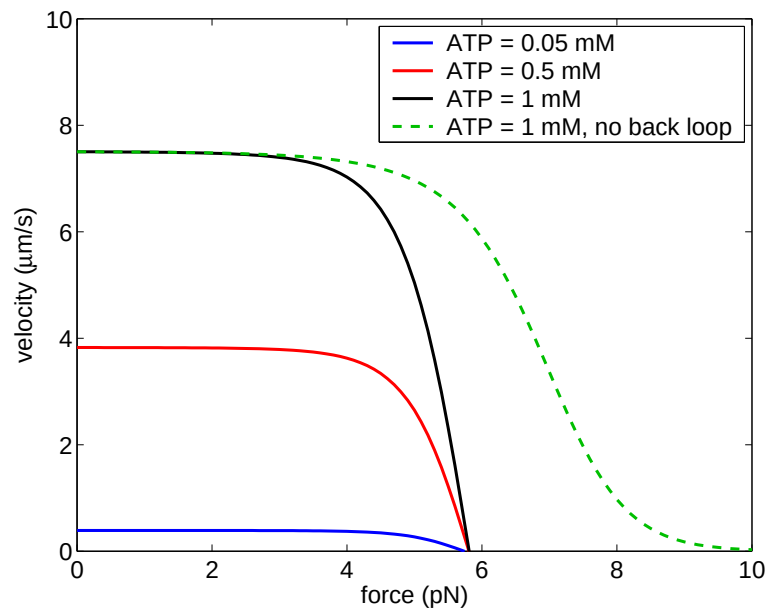


Figure 5.3: The predicted velocity vs. force curves at constant levels of  $[ATP]$  for the active backstepping model: For these traces,  $[ADP]$  and  $[P_i]$  were held constant at their physiological levels of  $10^{-5}$  M and  $10^{-3}$  M, respectively, as in Figure 4.2. The force-velocity relationships are not significantly different between the active and the passive models.

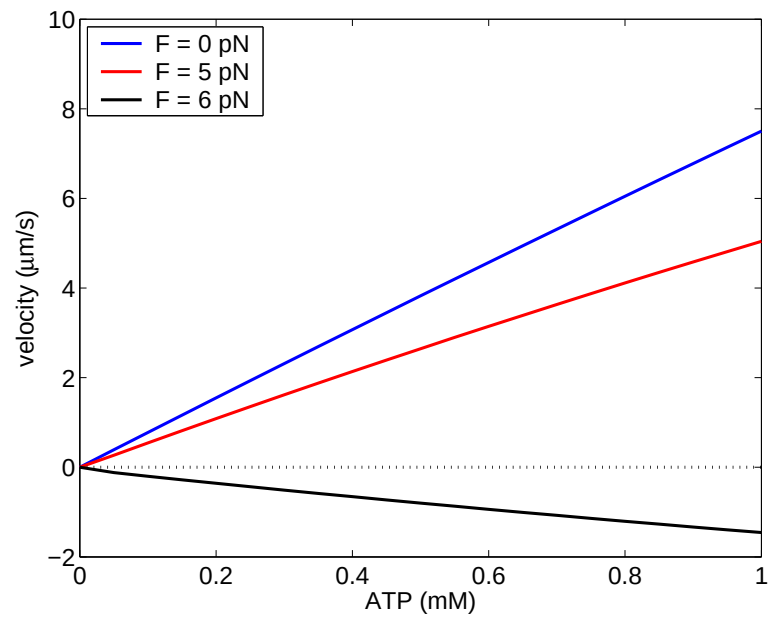


Figure 5.4: The predicted velocity vs.  $[\text{ATP}]$  at constant force levels for the active backstepping model:  $[\text{ADP}]$  and  $[\text{P}_i]$  were held constant as in Figure 4.2. At  $F = 0 \text{ pN}$ , the velocity is a linear function of  $[\text{ATP}]$ . It does not significantly deviate from a linear relationship with  $[\text{ATP}]$  until the force approaches  $F \sim 6 \text{ pN}$ .

the passive diffusion model. In both the passive and the active models,  $D_{\text{eff}}$  is almost constant at low forces, then increases as the force is raised past  $F \sim 5$  pN and the dynein must work harder against the strain.

As the force approaches  $F \sim 6$  pN the two models exhibit completely different behaviors. In the passive backsliding model,  $D_{\text{eff}}$  continues increasing rapidly as the motor spends more and more time in the diffusion pathway. In the active backstepping model, however,  $D_{\text{eff}}$  reaches a maximum at  $F \sim 6$  pN, and then decreases again as the force continues to increase, until it stabilizes near  $F \sim 7$  to 8 pN, at about the same value that it had for  $F < 4$  pN. This is due to the symmetry of the model. At low force levels, the motor is stable in the forward hydrolysis pathway, at high force levels, it is stable in the backward hydrolysis pathway, and at intermediate levels, it is shifting back and forth between them, increasing the overall variability of the system. The active model is also easily distinguished from a system with only a single hydrolysis cycle, in which the motor ceases to move at high force levels.

Like the velocity vs. force profiles, the behavior of the randomness parameter vs. force in the active backstepping model is not significantly different from that in the passive diffusion model (See Figure 5.6 for the active model and 4.5 for the passive model.) The randomness is relatively stable at low force levels near  $r \sim 1$ , indicating a single rate-limiting step (the ATP-binding step). The value of  $r$  increases rapidly as the force increases from  $F \sim 4$  pN to  $F \sim 6$  pN and the motor velocity goes to zero.

### 5.2.3 Autocovariance Function

A typical plot of the autocovariance for the active backsliding model is shown in Figure 5.7. As in Figure 4.6, the correlation between values of the dynein displacement declines rapidly as the time interval between them increases. The least squares fit in this case gives a value for the correlation time of  $\hat{\tau}_c = 4.43$  ms:

$$R(\tau) = 172 \cdot \exp(-\tau/4.43) \quad , \quad (5.3)$$

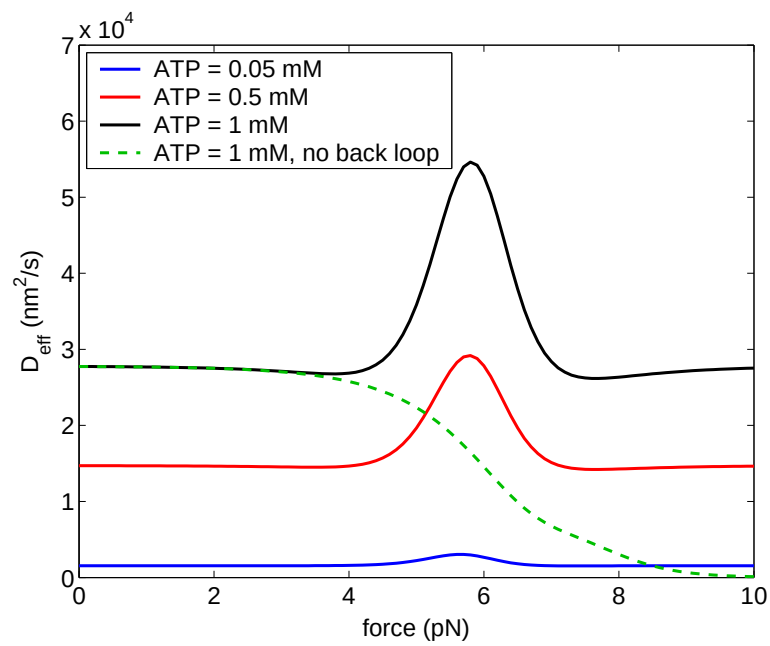


Figure 5.5: The predicted behavior of the effective diffusion coefficient vs. force for the active backstepping model.  $D_{\text{eff}}$  remains approximately constant at both low and high force levels, because the symmetry of the model allows the dynein to be stable in one hydrolysis loop or the other.  $D_{\text{eff}}$  increases to a maximum at  $F \sim 6$  pN as the motor switches more frequently between forward and backward motion.

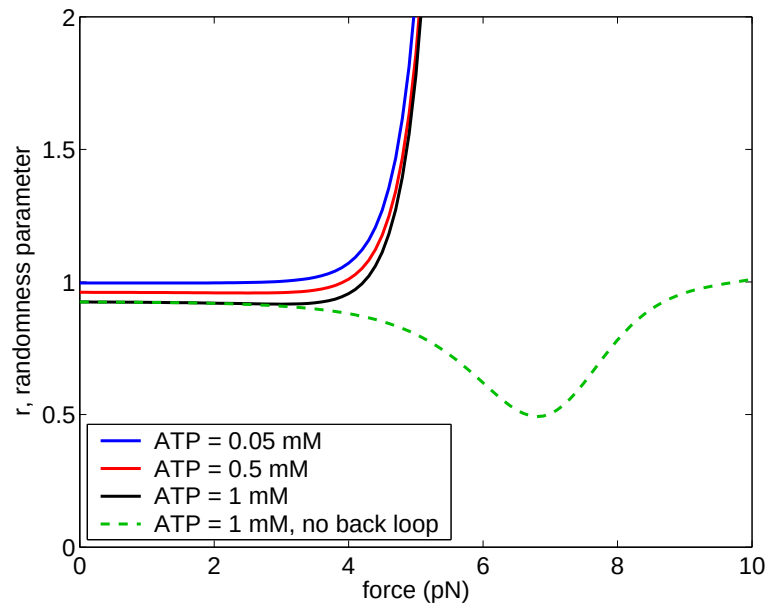


Figure 5.6: The predicted behavior of the randomness parameter ( $r$ ) as a function of force for the active backstepping model: The randomness parameter remains almost constant near  $r \sim 1$  at low force levels. As the force increases past  $F \sim 5$  pN,  $r$  increases rapidly as the dynein shifts more and more into the diffusion pathway, reducing its velocity and increasing the variability of its position. In contrast, if the diffusion pathway is removed from the model, the  $r$  slowly decreases to a minimum value of  $r \sim 0.5$ , indicating that the force-dependent rate is then equal to the rate-limiting ATP binding rate. It increases again toward a value of  $r \sim 1$  as the force-dependent step becomes the single rate-limiting step.

with  $R^2 = 0.965$ . Based on  $n = 40$  runs,  $\hat{\tau}_c = 4.10 \pm 0.36$  ms for the active backstepping model. This value is significantly different from the  $\hat{\tau}_c = 3.35 \pm 0.31$  ms for the passive model, indicating that the dynein position is more highly autocorrelated in this model. However, as shown in Figure 5.8, an exponential fit may not be appropriate for this data. In that case, further analysis may yield more information about the underlying mechanism and properties of the model.

The Fourier transform of the autocovariance from Figure 5.7 is shown in Figure 5.9. As in Figure 4.7, there are no distinct peaks in this power spectrum, indicating that there are no dominant frequency contributions to the observed dynein fluctuations.

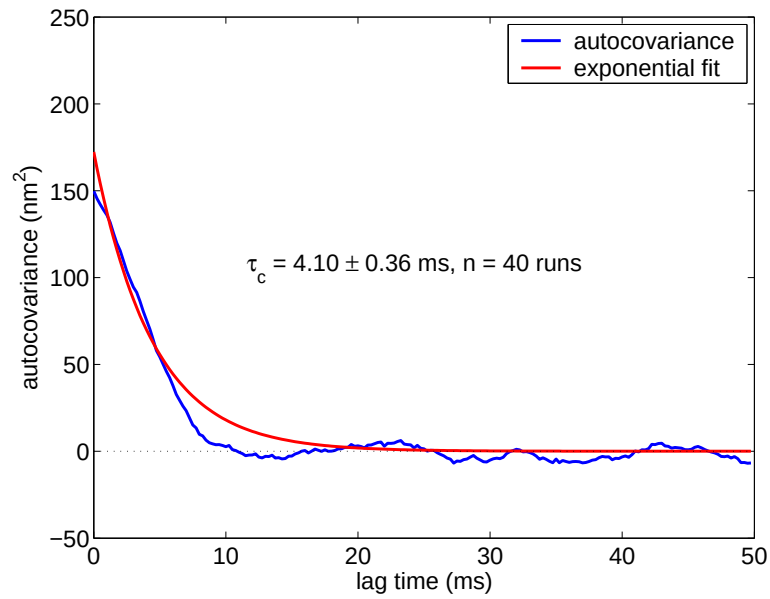


Figure 5.7: Typical autocovariance for a time series from the active backstepping model. The average correlation time for this model is  $\tau_c = 4.10$  ms.

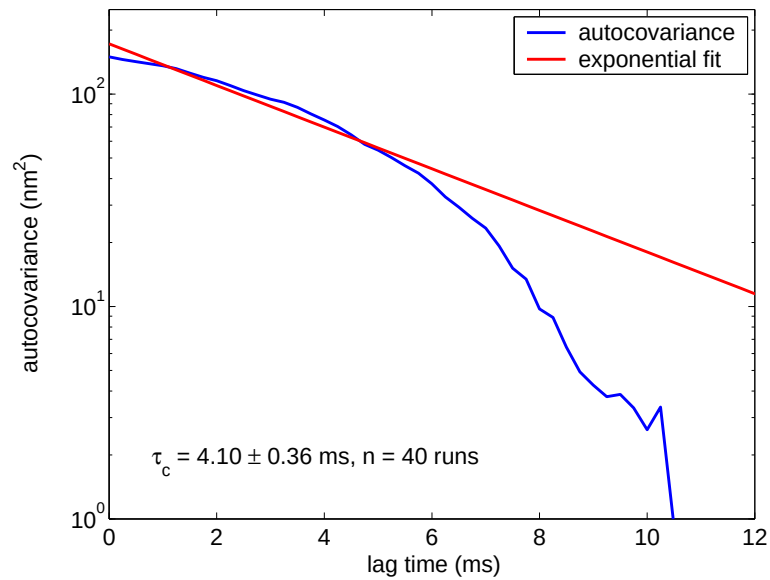


Figure 5.8: The initial part of the time series shown in Figure 5.7 is replotted here with a logarithmic scale on the y-axis.

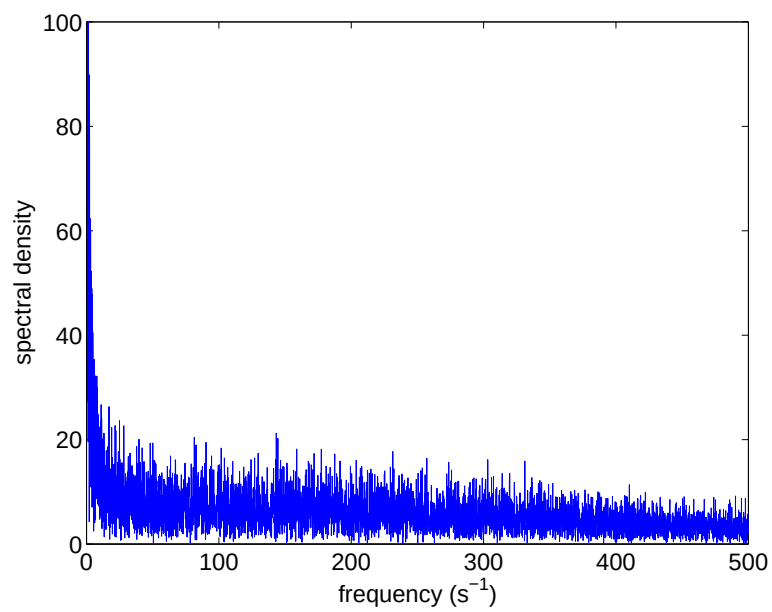


Figure 5.9: Typical power spectrum of a time series from the active backstepping model. The spectral density is the Fourier transform of the autocovariance function. The lack of any peaks indicates that there are not any dominant underlying frequency components contributing to the time series shown in Figure 5.2.

# Chapter 6

## Conclusion

### 6.1 Summary

We have constructed a mechanistic model of dynein that can explain the oscillatory motion observed by Shingyoji et al. (1998). The model is based on the observation that dynein is capable of one-dimensional diffusion along a microtubule when ADP is bound to both heads (Vale et al., 1989). To ensure that the model is consistent with thermodynamics, we required that the model respect detailed balance. This places constraints on the rate constants and the distribution of forces around the hydrolysis cycle. When possible, the values of the model parameters were taken to be consistent with experimental measurements. We have shown how the detailed model, which takes into account the position of the bead, microtubule, and dynein molecule, can be reduced to a simpler, dynein-position-only model. Because the parameters in the simplified model are derived from those used in the detailed model, and because the results are quantitatively similar between the two models, the simplified model was used to analyze and predict the performance of the dynein motor.

The passive backward movement of the dynein under sufficient load, driven by thermal energy rather than ATP hydrolysis, is the key assumption of this model. As

shown in Figures 4.2 — 4.5, the passive sliding assumption leads to several experimentally testable predictions of motor behavior which are different from those made by a model without such a pathway. Specifically, passive sliding would cause the velocity vs. force profile to be exclusively concave down and drop very sharply with the velocity reaching zero at a force of  $F \sim 6.2$  pN, significantly below the thermodynamic stall force of  $F \sim 12.5$  pN at 1 mM ATP. At the same time, the rapid increases in both the effective diffusion coefficient and the randomness parameter as the velocity approaches zero indicate that the variability of the motor position is also increasing.

If the diffusion branch pathway is removed from the model, the predicted motor velocity and effective diffusion coefficient both follow approximately sigmoid declines and do not go completely to zero until the thermodynamic stall force is reached. In this case, the randomness parameter is predicted to decline slowly to a value of  $r \sim 0.5$ , when the rate of the force dependent step and the rate of the ATP binding step are equal and both rate-limiting. The randomness parameter then rises slowly back to  $r \sim 1$  as the force dependent step becomes the sole rate-limiting step, and then increases rapidly when the velocity goes to zero at the stall force.

An alternative model utilizing an active hydrolysis cycle to drive backward motion along the microtubule was also developed, to stand in for such a model by Brokaw (2000). If dynein movement can be measured experimentally with sufficient resolution, it may be possible to differentiate between the mechanisms. Backward motion independent of ATP concentration would mean that the forward velocity would decrease but the backward velocity would not as the ATP is hydrolyzed by the dynein.

The time series of the motor displacement of was found to be similar to that of the passive backsliding model, as were the profiles of the average motor velocity and the randomness parameter as functions of force. The effective diffusion coefficient, however, showed a significantly different profile, and thus could be tested experimentally to distinguish between the two models.

The correlation time  $\tau_c$  between measurements within the same time series is also different between the two models, with the active model having the longer correlation time. Neither model shows any peaks in its power spectrum, indicating that there are no underlying dominant cycles in the fluctuations in the time series. None appeared

either in Shingyoji et al.'s (1998) analysis of their experimental data, although they did report a continually decreasing frequency of oscillations in some of their time series, as measured manually.

We note that the force velocity profile predicted by our model might be important for understanding axonemal dynamics. Flagellar beating requires the coordination of thousands of dynein molecules. Figure 4.2 shows that individual dynein molecules respond to resistive forces in a switch-like manner. At low forces, the velocity is independent of the applied force. At around  $F \sim 5 - 6$  pN, the velocity sharply drops to zero. This sharp drop is due to dynein entering the diffusion pathway. Thus, this pathway provides a mechanism for relieving strain and reducing the time dyneins on opposing microtubule doublets spend working against each other. At the same time, because this mechanism does not require dynein detachment from the microtubule, the molecule can quickly reset in preparation for the next beat. This could be biologically advantageous, allowing the motor to function more efficiently by better coordinating the efforts of many motors, and by allowing the motor to back up and start forward again if its cargo gets temporarily stuck, rather than letting go completely and starting over.

## 6.2 Future Work

There are many opportunities to revise and extend both the experimental work and the modeling done on dynein to date. More biochemical rate data is needed for different dyneins to provide more certainty in the values of the rate constants. In particular, should we expect two-headed and three-headed dynein molecules to function at the same rates? The regulatory effects of phosphorylation of certain amino acid residues of the dynein heavy chains, of  $\text{Ca}^{2+}$ -binding (King and Patel-King, 1995), and of nucleotide binding at dynein's non-hydrolyzing ATPase sites (Mocz et al., 1998; Yagi, 2000; Shiroguchi and Toyoshima, 2001; Silvanovich et al., 2003; Kikushima et al., 2004; Reck-Peterson and Vale, 2004) are being elucidated and should be included in future dynein models.

The models presented in this dissertation have been for a two-headed dynein. Models should also be developed for three-headed dyneins, which may exhibit different behaviors or move at different speeds. Models of individual dynein molecules could also be linked together as components of a larger model to study what is required to obtain coordinated movement within the axoneme.

Finally, the usefulness of any model lies in its explanatory and predictive capabilities. Experiments can be designed and run to test both the assumptions and the predictions of the models. Based on experimental results, the models can then be revised, and new predictions can be made and tested, repeating the cycle and improving our understanding of the systems being modeled.

# Appendix A

## Tables of Parameter Values Used in Models

Table A.1: Parameter values used in the detailed dynein model from Chapter 3.

| Parameter    | Value                                         | Description                                          | Source                     |
|--------------|-----------------------------------------------|------------------------------------------------------|----------------------------|
| $\Delta x_d$ | 8 nm                                          | dynein step size                                     | binding site spacing       |
| $D_b$        | $4.3 \times 10^5 \text{ nm}^2 \text{ s}^{-1}$ | bead diffusion<br>coefficient                        | $= k_B T / (6\pi\eta r)$   |
| $D_m$        | $6.7 \times 10^5 \text{ nm}^2 \text{ s}^{-1}$ | free microtubule<br>diffusion coefficient            | (Vale et al., 1989)        |
| $D_{md}$     | $0.9 \times 10^4 \text{ nm}^2 \text{ s}^{-1}$ | dynein-bound<br>microtubule diffusion<br>coefficient | (Vale et al., 1989)        |
| $D_d$        | $9.1 \times 10^3 \text{ nm}^2 \text{ s}^{-1}$ | dynein diffusion<br>coefficient                      | $= 1 / (1/D_{md} - 1/D_m)$ |

Table A.1: (continued)

| Parameter     | Value                                                 | Description                            | Source                                      |
|---------------|-------------------------------------------------------|----------------------------------------|---------------------------------------------|
| $\kappa_{tb}$ | 0.095 pN nm <sup>-1</sup>                             | trap-to-bead spring<br>constant        | calculated from<br>(Shingyoji et al., 1998) |
| $\kappa_{bm}$ | 0.213 pN nm <sup>-1</sup>                             | bead-to-microtubule<br>spring constant | $= 1/(1/\kappa_{bd} - 1/\kappa_{md})$       |
| $\kappa_{bd}$ | 0.187 pN nm <sup>-1</sup>                             | bead-to-dynein spring<br>constant      | calculated from<br>(Shingyoji et al., 1998) |
| $\kappa_d$    | 1 pN nm <sup>-1</sup>                                 | dynein spring constant                 | assumed<br>(Brokaw, 2000)                   |
| $k_{12}$      | 10 <sup>5</sup> s <sup>-1</sup>                       | ATP hydrolysis rate                    |                                             |
| $k_{23}$      | 10 <sup>5</sup> s <sup>-1</sup>                       | P <sub>i</sub> release rate            |                                             |
| $k_{34}^0$    | 10 <sup>7</sup> s <sup>-1</sup>                       | microtubule binding<br>rate            |                                             |
| $k_{45}$      | 10 <sup>5</sup> s <sup>-1</sup>                       | ADP release rate                       |                                             |
| $k_{51}$      | 9.8 x 10 <sup>5</sup> M <sup>-1</sup> s <sup>-1</sup> | ATP binding rate                       | calculated from<br>(Shingyoji et al., 1998) |
| $k_{15}$      | 100 s <sup>-1</sup>                                   | ATP release rate                       |                                             |
| $k_{21}$      | 1.1 x 10 <sup>5</sup> s <sup>-1</sup>                 | ATP synthesis rate                     | calculated from detailed<br>balance         |
| $k_{32}$      | 1.0 x 10 <sup>6</sup> M <sup>-1</sup> s <sup>-1</sup> | P <sub>i</sub> binding rate            |                                             |
| $k_{43}^0$    | 1000 s <sup>-1</sup>                                  | microtubule release rate               |                                             |
| $k_{54}$      | 2.7 x 10 <sup>5</sup> M <sup>-1</sup> s <sup>-1</sup> | ADP binding rate                       |                                             |
| $k_{36}$      | 2.19 x 10 <sup>4</sup> s <sup>-1</sup>                | rate to switch to<br>diffusion pathway | calculated by fitting<br>data               |

Table A.1: (continued)

| Parameter   | Value                                      | Description                                      | Source                                      |
|-------------|--------------------------------------------|--------------------------------------------------|---------------------------------------------|
| $k_{63}$    | $1.01 \times 10^4 \text{ s}^{-1}$          | rate to switch to<br>hydrolysis cycle            | calculated by fitting<br>data               |
| $k_{67l}^0$ | $570 \text{ s}^{-1}$                       | left-side binding rate                           | $= 4D_d/\Delta x^2$                         |
| $k_{67r}^0$ | $570 \text{ s}^{-1}$                       | right-side binding rate                          | $= 4D_d/\Delta x^2$                         |
| $k_{76l}^0$ | $570 \text{ s}^{-1}$                       | left-side release rate                           | $= 4D_d/\Delta x^2$                         |
| $k_{76r}^0$ | $570 \text{ s}^{-1}$                       | right-side release rate                          | $= 4D_d/\Delta x^2$                         |
| $x_h$       | 112 nm                                     | approximate high limit<br>of dynein fluctuations | (Shingyoji et al., 1998)                    |
| $x_l$       | 80 nm                                      | approximate low limit<br>of dynein fluctuations  | (Shingyoji et al., 1998)                    |
| $k_B$       | $1.38066 \times 10^{-23} \text{ J K}^{-1}$ | Boltzmann constant                               |                                             |
| $T$         | 25 C                                       | temperature of<br>experiments                    | (Shingyoji et al., 1998)                    |
| ATP(0)      | 1.0 mM                                     | initial [ATP]                                    | (Shingyoji et al., 1998)                    |
| $\lambda$   | $2.86 \text{ s}^{-1}$                      | time constant for ATP<br>consumption             | calculated from<br>(Shingyoji et al., 1998) |
| ATP(t)      | $ATP(0) \exp(-\lambda t)$                  | [ATP] at time t                                  |                                             |

Table A.2: Parameter values used in the simplified dynein model with diffusion from Chapter 4. Note that all other parameter values are the same as in Table A.1.

| Parameter     | Value                                         | Description                                                    | Source                                      |
|---------------|-----------------------------------------------|----------------------------------------------------------------|---------------------------------------------|
| $D_{bmd}$     | $8.8 \times 10^3 \text{ nm}^2 \text{ s}^{-1}$ | bead-microtubule-<br>dynein effective<br>diffusion coefficient | $=$<br>$1/(1/D_b + 1/D_m + 1/D_d)$          |
| $\kappa_{td}$ | $0.063 \text{ pN nm}^{-1}$                    | trap-to-dynein spring<br>constant                              | calculated from<br>(Shingyoji et al., 1998) |
| $\kappa_{tb}$ | $0.095 \text{ pN nm}^{-1}$                    | trap-to-bead spring<br>constant                                |                                             |
| $\kappa_{bm}$ | $0.213 \text{ pN nm}^{-1}$                    | bead-to-microtubule<br>spring constant                         | $= 1/(1/\kappa_{bd} - 1/\kappa_{md})$       |
| $\kappa_{md}$ | $1 \text{ pN nm}^{-1}$                        | microtubule-to-dynein<br>spring constant                       |                                             |
| $k_{34}^0$    | $2 \times 10^6 \text{ s}^{-1}$                | microtubule binding<br>rate                                    | $= k_{34, \text{detailed}}^0/5$             |
| $k_{43}^0$    | $200 \text{ s}^{-1}$                          | microtubule release rate                                       | $= k_{43, \text{detailed}}^0/5$             |
| $k_{36}$      | $4.39 \times 10^3 \text{ s}^{-1}$             | rate to switch to<br>diffusion pathway                         | calculated by fitting<br>data               |
| $k_{63}$      | $1.57 \times 10^4 \text{ s}^{-1}$             | rate to switch to<br>hydrolysis cycle                          | calculated by fitting<br>data               |
| $k_{67l}^0$   | $550 \text{ s}^{-1}$                          | left-side binding rate                                         | $= 4D_{bmd}/\Delta x^2$                     |
| $k_{67r}^0$   | $550 \text{ s}^{-1}$                          | right-side binding rate                                        | $= 4D_{bmd}/\Delta x^2$                     |
| $k_{76l}^0$   | $550 \text{ s}^{-1}$                          | left-side release rate                                         | $= 4D_{bmd}/\Delta x^2$                     |
| $k_{76r}^0$   | $550 \text{ s}^{-1}$                          | right-side release rate                                        | $= 4D_{bmd}/\Delta x^2$                     |

Table A.3: Parameter values used in the active backstepping dynein model from Chapter 5. Note that all other parameter values are the same as in Table A.2.

| Parameter    | Value                             | Description                                                         | Source                        |
|--------------|-----------------------------------|---------------------------------------------------------------------|-------------------------------|
| $k_{3*4*}^0$ | $2 \times 10^6 \text{ s}^{-1}$    | backward pathway<br>microtubule binding<br>rate                     | $= k_{34, simple}^0$          |
| $k_{4*3*}^0$ | $200 \text{ s}^{-1}$              | backward pathway<br>microtubule release rate                        | $= k_{43, simple}^0$          |
| $k_{i*j*}$   | various                           | backward pathway<br>transition rates                                | $= k_{ij, simple}$            |
| $k_{33*}$    | $4.39 \times 10^3 \text{ s}^{-1}$ | rate to switch to<br>backward pathway                               | calculated by fitting<br>data |
| $k_{3*3}$    | $5.59 \times 10^8 \text{ s}^{-1}$ | rate to switch to<br>forward pathway                                | calculated by fitting<br>data |
| $x_s$        | 96 nm                             | desired switching point<br>between forward and<br>backward pathways | $= \frac{1}{2}(x_h + x_l)$    |

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