TOPICAL REVIEW

Motor proteins and molecular motors: how to operate machines at the nanoscale

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TOPICAL REVIEW

Motor proteins and molecular motors: how to operate machines at the nanoscale

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Abstract
Several classes of biological molecules that transform chemical energy into mechanical work are known as motor proteins or molecular motors. These nanometer-sized machines operate in noisy stochastic isothermal environments, strongly supporting fundamental cellular processes such as the transfer of genetic information, transport, organization and functioning. In the past two decades motor proteins have become a subject of intense research efforts, aimed at uncovering the fundamental principles and mechanisms of molecular motor dynamics. In this review, we critically discuss recent progress in experimental and theoretical studies on motor proteins. Our focus is on analyzing fundamental concepts and ideas that have been utilized to explain the non-equilibrium nature and mechanisms of molecular motors.

(Some figures may appear in colour only in the online journal)

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1. Introduction

Biological cells are very complex dynamic and heterogeneous systems that operate under non-equilibrium conditions, supporting a large number of biochemical and biophysical processes, including gene replication, transcription, translation, cellular transport, cell motility and cell division [1–4]. An important role in sustaining cellular functions is played by several classes of active enzymatic molecules, generally called motor proteins or molecular motors [1–10, 16, 17]. Despite significant research efforts in recent years, the fundamental mechanisms of their functioning remain not fully understood [5, 8–10].

There are many different types of molecular motors that are expressed in living cells [1, 2, 5, 7]. The first motor proteins, myosins, which are important for muscle contraction, were discovered in 1940s [1, 2, 5, 11]. Another class of motor proteins, dyneins, which are responsible for propelling sperm, bacteria and other cells, were first reported in 1963 [12]. Surprisingly, the most experimentally studied kinesin motor proteins, which support cellular transport processes, were first purified and analyzed only in 1985 [13–15]. A different type of rotating molecular motors, ATP synthase proteins, was fully analyzed in the early 1990s [18]. Since then many classes of molecular motors have been discovered and classified, and new motor protein systems are constantly being added [10]. However, it is widely believed that all these nanoscale machines convert

chemical energy into mechanical work probably using similar principles, although it is still not clear if there is one mechanism or several different mechanisms, and in most cases the microscopic details of the underlying processes are still not clear [8, 9]. Motor proteins typically consume chemical energy, which can be transformed into mechanical work, by accelerating various biochemical reactions such as the hydrolysis of ATP (adenosine triphosphate) or related compounds, and polymerization processes in DNA, RNA and other protein molecules. During these catalytic processes, a fraction of the released chemical energy is somehow channeled by molecular motors into mechanical motion. Understanding the microscopic details of these processes is one of the most important fundamental scientific problems.

Analyzing the dynamics of motor proteins, one can clearly view them as tiny engines that consume fuel (energy of biochemical reactions) to produce mechanical work useful for their biological functions [4, 5]. However, their working conditions are very different from the environment of macroscopic engines: motor proteins operate in stochastic non-equilibrium isothermal systems that are also crowded by a large number of other chemically active biological molecules. At the same time, these molecular motors display a very efficient and robust performance, and any major malfunction in them will most probably lead to cell death. Significant advances in the experimental studies of motor protein dynamics and their functions have been achieved [19–69]. The application of advanced spectroscopic and microscopic methods have allowed researchers to visualize and manipulate motor proteins with a single-molecule precision and high temporal resolutions, providing important information on how molecular motors operate. This success has led to the development of multiple theoretical approaches that discuss different aspects of motor protein dynamics [8, 10, 70–114].

In this review I present a brief progress report based on some recent experimental and theoretical investigations that provide important information on the fundamental mechanisms governing dynamic behavior and functioning of motor proteins. It should be noted that there are several excellent reviews on molecular motors that have appeared in recent years [7–10, 16, 17]. However, most of them discuss only the biological or experimental aspects of motor protein motility, while my intention is to concentrate more on the main theoretical concepts and ideas in the field. As a result, I will not be able to cover all the subjects related to molecular motors, but rather the goal of this review is to present an emerging unified theoretical picture of motor protein dynamics consistent with the basic laws of chemistry and physics. Artificial molecular motors have also been developed in recent years as a way of mimicking and copying the useful properties of highly efficient, versatile and robust biological motor proteins [115–119]. There is much promise in the application of these synthetic molecular motors for fundamental scientific and technological purposes. However, they are still not well investigated and even much less is understood about the mechanisms that control them. In this review I will discuss only the advances and developments in experimental and theoretical studies of biological motor proteins. Furthermore, this review on molecular motors obviously represents a subjective theoretician's view of the field, which might not always agree with other existing ideas surrounding motor protein dynamics.

2. Classification of motor proteins

Motor proteins usually become active enzymes after binding to some static or dynamic cellular structures such as cytoskeleton protein filaments, cellular membranes, nucleic acids or other protein complexes. This observation allows us to divide motors into several groups.

(1) *Cytoskeleton filaments motor proteins*, such as dyneins, kinesins and myosins, start to work by associating with and moving along the cytoskeleton filaments (actin filaments and microtubules). These motor proteins utilize the energy of hydrolysis of ATP (adenosine triphosphate) or related compounds, and they are the main players in cellular transport processes. Cytoskeleton-bound motor proteins are the most studied systems from the dynamic and structural points of view, and our current understanding of the mechanisms of energy transduction in molecular motors comes mainly from experiments on kinesin and myosin motor proteins [5, 8–10, 17].

(2) *Nucleic acids motor proteins*, such as polymerases, topoisomerases, gyrases, helicases and many others, usually function by associating with DNA and RNA molecules, and the source of chemical energy for these motors is the polymerization reactions of nucleic acids, synthesis of proteins and/or ATP hydrolysis. These motor proteins are important for the maintenance and processing of genetic information, as well as for the synthesis of all protein molecules in cells [10, 40, 120, 121]. These enzymes are currently at the center of intense experimental investigations; however, the available information on how they work is still quite limited, especially in comparison with cytoskeleton motor proteins, most probably due to the very high complexity of biological systems.

(3) *Rotary motor proteins*, such as bacterial flagella (essential for bacterial migration) and F_0F_1-ATP synthase (which is used to synthesize ATP molecules, the main source of energy in biological systems, in mitochondria), are usually bound to cellular membranes. They are involved in circular motions in the membrane or outside of the cell; although, to be more exact, only parts of the molecule outside of the membrane usually move. Some of rotary motors utilize the electrochemical energy of various ion gradients that exist across cellular membranes. These molecular motors are very important for cell motility and chemotaxis (i.e., for the cellular motion in the direction of available nutrients) [27, 49, 50, 122, 123], but the mechanisms of these rotary motor proteins are understood much less in comparison with linear motor proteins.

Most experimental and theoretical studies concentrate on molecular motors that transform chemical energy into linear

motion. These proteins typically translocate along the protein filaments and/or nucleic acids in an effectively 1D fashion, which helps to quantify their dynamic properties [5, 8, 10]. The properties of rotary molecule motors, which are generally more complex systems, are not so well quantified. However, one could argue that the mechanisms of energy conversion in rotary motor proteins are probably similar to linear motor proteins [8]. Thus, theoretical ideas developed for linear motor proteins can be easily extended to rotary molecular motors, although one still has to be careful in the application of these methods [8]. For this reason, in this review we concentrate on molecular motors involved in linear translocation.

Linear molecular motors could also be divided into two groups depending on how many enzymatic cycles can be performed for each encounter with the cytoskeleton filament or nucleic acid. There are several classes of motor proteins that are strong enough to function as single independent species, similar to railway locomotives or heavy tracks on highways. They translocate along cytoskeleton filaments or nucleic acids in preferred directions by repeatedly hydrolyzing ATP molecules or polymerizing DNA, RNA or other protein molecules, taking hundreds of discrete steps before finally dissociating. These motor proteins are called *processive molecular motors*. The most known examples include kinesin and dynein motor proteins, which move in opposite directions along microtubule filaments. Similarly, myosins V and VI motor proteins translocate in opposite directions along actin filaments. RNA and DNA polymerases move with a high fidelity over long distances along DNA molecules. It is important to note here that protein filaments, as well as nucleic acids, can be viewed as polar tracks for motor proteins, effectively breaking the symmetry and specifying the preferred direction of the motor's motion. This directionality of molecular motors is critically important for their biological functions. In simple terms, different cellular vesicles and organelles must be transported fast in all directions in the cell and versatile motor proteins are able to do this.

There are other motor proteins, most notably the muscle myosins [5], that function in cells only via working together in large groups. However, details of the cooperative mechanisms of these *non-processive molecular motors* are currently much less understood. Such non-processive motors typically make only one or few steps before detaching from their tracks. It is widely believed that the specific processivity of motor proteins is closely related to their structure [10, 17, 124, 125]. Non-processive molecular motors are often monomers, while processive motors mostly exist in multi-domain dimeric or oligomeric forms [3, 5]. It is frequently argued that these structural properties of processive motors explain why these motor proteins could stay attached to the filamentous track for long times while performing their functions. For active oligomeric motor proteins the probability that all motor heads (where enzymatic reactions are taking place) dissociate from the track simultaneously is much lower than the probability for a monomer molecular motor to detach. In nucleic motor proteins the increased processivity is frequently due to non-motor domains of the molecules that remain attached to the nucleic acid track in a 'clamp-like' fashion, keeping the catalytic domains of the motor close to the nucleic acid for a significant amount of time.

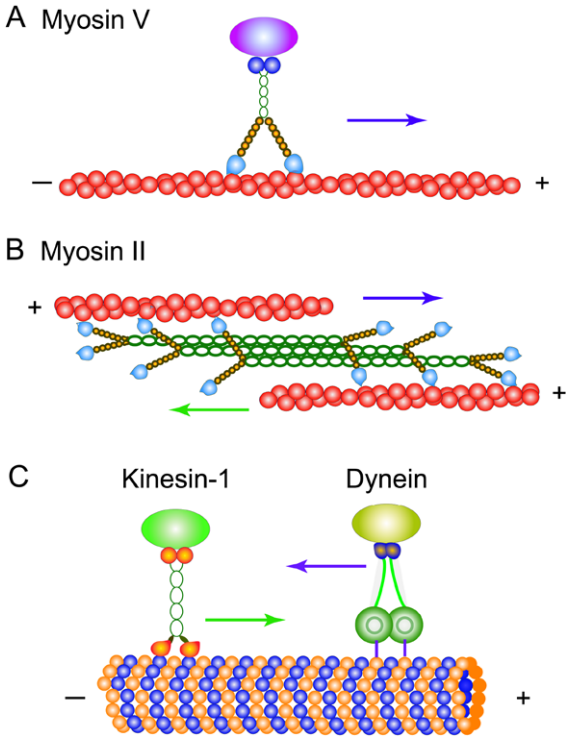

Figure 1. Schematic view of some of the most important linearly translocating motor proteins. (A) Dimeric myosin V motor proteins step unidirectionally along actin cytoskeleton filaments. (B) A group of monomeric myosin-II motor proteins combined in the filament can move together along several actin filaments. (C) Conventional kinesin motor proteins translocate along the microtubules in the positive direction, while the dynein motors step along the microtubules in the opposite direction.

All motor proteins have a multi-domain structure to support their successful functioning in the complex cellular environment—see figure 1. The regions of the molecule where biochemical reactions are catalyzed are known as motor domains. There are also domains that responsible for binding to cellular cargoes, as well as regions that provide necessary mechanical flexibility and chemical stability. It is interesting to note that for some motor proteins various domains might strongly interact with each other, modifying their individual enzymatic activities. For example, experiments indicate that, for some kinesins and myosins, partial unfolding of a cargo-binding domain might completely inhibit the catalytic activities of the motor head domains [126–128]. In other words, these nanoscale trucks move only when there is an available load, and there is little futile consumption of the fuel from the cellular point of view. Full structural information is naturally very important for understanding the microscopic details of motor protein functioning.

3. Experimental investigations

Motor proteins have been investigated by a variety of experimental methods that provide complementary informa-

tion on their mechanisms. Bulk biochemical experiments have measured rates of chemical reactions associated with motor protein activity [28, 32, 129–132]. The application of well-established chemical kinetic methods, including stopped flow, isotope exchange, fluorescent labeling and temperature quenching, has shown that motor protein catalytic activities involve a complex network of biochemical states and conformations [129, 131], although for many myosin and kinesin motor proteins one or few dominating biochemical pathways related to ATP hydrolysis have been identified [28, 32, 64, 129, 130]. But the problem with bulk chemical experiments is that the average properties of a large number of molecular motors (both active and inactive) are measured, complicating the determination of the mechanisms of motility at the single-molecule level. Structural information has been obtained by utilizing x-ray crystallography and cryomicroscopy methods [5, 26, 133, 134]. These experiments provide images of various biochemical states of single molecular motors with atomic-scale resolutions. However, it is not clear how realistically these measurements describe motor protein activity, since in many cases the experimental conditions differ significantly from the cellular conditions. In addition, these experiments probe only static properties, whereas many intriguing questions are related to the dynamics of motor proteins.

3.1. Single-molecule experimental methods

The largest fraction of information concerning the microscopic mechanisms of motor proteins come from single-molecule experimental techniques that have been developed in the past 20 years [5, 8, 9, 135]. These methods, including optical-trap and magnetic tweezers spectroscopy, Förster resonance energy transfer (FRET), single-molecule AFM (atomic force microscopy), fluorescent labeling and super-resolution spectroscopy, have allowed researchers to observe and modify the activity of individual motor protein molecules with unprecedented spatial and temporal resolution [19, 20, 22, 24–27, 29–31, 33–53, 55–66], providing powerful tools for investigating the dynamics of molecular motors. The latest advances in single-molecule experimental methods and their applications have been described in detail in several recent reviews [9, 135–137], so here I will present only a brief discussion of several techniques especially important for studying molecular motors.

Optical-trap spectroscopy is one of the most powerful single-molecule experimental methods that have been widely applied for studying the dynamic properties of different classes of molecular motors [24, 25, 30, 33–38, 42, 44, 48, 52, 57, 60, 63, 64, 66]. This approach uses a laser beam to monitor the displacements of a bead to which a single motor protein molecule is chemically attached [5, 22, 25, 30, 85, 136, 137]. The bead experiences a trapping force that pushes it closer to the focus region of the non-uniform laser field, while the connected motor protein drags the bead in the direction of its motion along the cellular track [5]. The effective potential of interactions created by the optical tweezers setup is very close to a harmonic potential and can be calibrated with a high precision. The bead motion can be visualized and monitored with a high precision, allowing one to quantify the dynamic properties of motor proteins. This experimental method measures nanometer displacements and piconewton forces, which is very appropriate for studying mechanochemical couplings in motor protein systems [9]. An example of typical particle trajectories observed in optical-trap spectroscopic measurements is presented in figure 2. The original setups have also been modified to include force clamps via feedback control and to add vectorial forces (parallel to the filament tracks) that allow the measurement of long runs of molecular motors at constant external forces [136, 137]. It has been argued that the spatial resolution of improved optical-trap systems could reach ∼1 nm, while conformational transitions lasting not less than ∼100 μs could also be successfully resolved [9, 137]. Another advantage of optical tweezers is the ability to be coupled with other methods, such as fluorescent labeling approaches [9]. Thus, optical trapping is one of the most successful single-molecule methods in uncovering the dynamic properties of motor proteins in *in vitro* systems. However, the method is very difficult to apply for studying dynamic processes in live cells due to the heterogeneous cellular environment and complex geometry of the experimental setup [9].

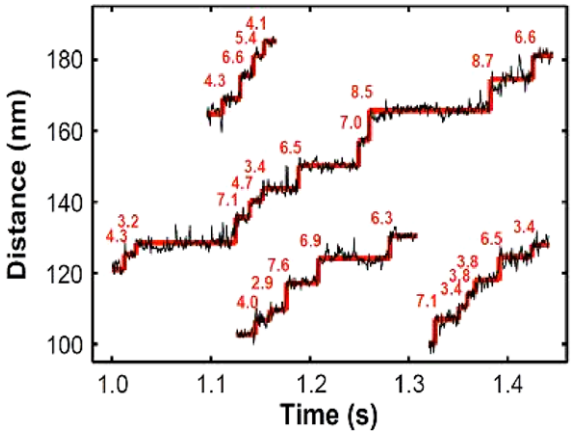

Figure 2. Typical particle trajectories obtained in optical-trap spectrometry experiments. In this case, the dynamics of two-kinesins assemblies at high ATP concentration to visualize discrete steps is measured. Note that observed step size in many cases is a fraction of the single kinesin step size $d = 8.2$ nm, as expected, since only one motor molecule can step at a given time. Courtesy of M R Diehl.

Closely related to optical-trap spectrometry is a method known as magnetic tweezers. It utilizes the magnetic field for manipulating single molecules [40, 45, 137, 138]. In these experiments the motor protein molecule is simultaneously chemically bound to the surface and to the magnetic bead. Tracking the vertical position of the bead as well as the lateral (parallel to the surface) fluctuations allows one to calibrate the force acting on the bead via the equipartition theorem. One of the biggest advantages of magnetic tweezers is the ability to apply relatively large torques (up to ∼1000 pN nm^{-1}) [137, 138]. It makes this method especially powerful for investigating nucleic acid motor proteins, such as

topoisomerases, helicases, polymerases, gyrases and others, for which the rotational degrees of freedom are critically important for their proper functioning [40, 45, 137–139]. These DNA and RNA bound molecular motors must unzip, untangle, or remove topological defects during their motion, and it cannot be accomplished without the rotation. The method has been successfully used to analyze many nucleic acid motor proteins [40, 139]. However, the magnetic tweezers method has a low temporal resolution, limiting its application to slow dynamic processes associated with molecular motors [137].

Light microscopy plays a very important role in clarifying the structural and dynamic properties of motor proteins [9, 136, 137]. In typical experiments fluorescent dyes are directly attached to enzyme molecules, which allows one to localize and track single molecular motors in a microscope with a high precision. However, there is a fundamental barrier in spatial resolution due to diffraction that limits the size of the object that can be observed by the wavelength of the light used to interrogate the system [140–142]. For the microscopy methods currently utilized it means that objects smaller than ∼100 nm cannot be clearly observed, which is far larger than the sizes of most motor proteins [140–142]. This problem has stimulated significant efforts to push the resolution below the diffraction limit, resulting in the development of several successful experimental methods, generally known as super-resolution spectroscopy [140–142].

There are three main super-resolution techniques that are currently used to a large degree in studies of biological systems: structured illumination microscopy (SIM), stimulated emission depletion (STED) and single-molecule localization and composition methods, as realized in photoactivated localization microscopy (PALM) and in stochastic optical reconstruction microscopy (STORM) [140–142]. In SIM experiments a biological sample is projected with a fine pattern of stripes that is varied in space and time. After processing all the images and analyzing the signal variations, a high-resolution view of the studied object can be obtained. However, the resolution improvement is limited and the best method of SIM can only visualize objects larger than ∼50 nm, while the temporal resolution is rather low. Another popular super-resolution method, STED, utilizes a second donut-shaped laser beam that stimulates the deactivation of the excited fluorophores back into the ground state, except for the central hole area [140–142]. Thus STED effectively switches off fluorophores at the periphery of the studied object, significantly increasing the spatial resolution (up to 10–20 nm). The disadvantage of this method is a limited choice of appropriate fluorophore groups and the difficulty in developing multi-color experiments that use fluorophores with different wavelengths. A different approach is exploited in super-resolution PALM/STORM methods, where the idea that the increasing number of photons emitted from the source allows one to localize the center of mass of the image with a high precision is applied [140–142]. In these experiments fluorophores are randomly switched on and off, and the analysis of images collected at different times leads to improved spatial resolutions (∼20–30 nm) in many biological systems. Again, the drawback of these methods is a special choice of the fluorophore groups and low temporal resolution. Methods related to the PALM/STORM approach have been successfully applied for tracking different motor proteins *in vivo* and *in vitro* [9, 39, 46, 143–145].

Various single-molecule methods have reliably measured many structural, biochemical and dynamic properties of molecular motors. It has been shown for conventional kinesins that these processive motor proteins move along a single protofilament in the plus direction of the microtubule with speeds up to 1 μm s^{-1} and making on average around a hundred steps (each of the size $d = 8.2$ nm) before detaching into the solution [20, 22, 23, 25, 30]. It is also known that the chemical cycles of these motor proteins are tightly coupled to the mechanical motion (one ATP molecule is consumed to make one forward step), and to stop these motors one has to exert a force of the order of 7–8 pN, which is known as the stall force [23, 25, 30]. Cytoplasmic dynein motor proteins move along microtubules in the opposite direction with the same step sizes, probably exerting smaller forces, although these observations are still controversial [52, 53, 146]. Another well-studied class of processive motor proteins, myosins V, hop along actin cytoskeleton filaments with $d =$ 36 nm steps and stall forces of up to 2–3 pN. Single-molecule particle-tracking methods clearly prove that kinesins, myosins and dyneins utilize a hand-over-hand mechanism in their motion by alternating leading and trailing positions of their motor heads [38, 39, 46, 147]. Single-molecule experiments explicitly determined the responses of various motor proteins to external forces via measuring the force–velocity curves [9, 25, 30, 42, 54, 146]. These experiments also measured the fluctuations of each motor protein during their motion along linear tracks via a so-called randomness parameter [8, 30, 148]. One of the most important fundamental observations obtained via single-molecule experimental methods is the fact that all associated chemical and mechanical transitions in motor proteins are fully reversible [9, 48, 67].

4. Theoretical studies

4.1. General remarks

Experimental studies have provided a significant amount of quantitative information that has stimulated strong theoretical discussions on the mechanisms of motor protein functioning [5, 8, 10, 70, 72, 87, 88, 95, 103, 110, 148]. The main goal of theoretical models for molecular motors is to explain the coupling between biochemical transitions and mechanical motions in order to understand the energy conversion at the microscopic level. It is known that all chemical processes can proceed in both directions, i.e., they are reversible, although available experimental data might not provide direct evidence for this reversibility. At given experimental conditions backward transitions could be very slow and not observable during the limited time of the experimental measurement. However, for molecular motors the reversibility of the involved chemical reactions cannot be neglected, since it might lead to unphysical conclusions and

wrong assumptions about the mechanisms [8, 48, 75, 92]. From the chemical point of view, motor proteins are catalytic molecules that, by definition, accelerate both forward and backward chemical reactions. This observation suggests that molecular motors which help to hydrolyze ATP when moving forward at one set of conditions could also accelerate the synthesis of ATP at another conditions. This conclusion has been experimentally shown for F_0F_1 ATP synthase rotary motors [45, 50] and for some other kinesin motor proteins [152, 153]. It will be impossible to explain fully the mechanisms of molecular motors without properly taking into account the reversibility of associated biochemical processes. From the theoretical point of view, it is also important to note that although ATP hydrolysis is a complex biochemical process that involves multiple reaction steps, often a single transition can limit progression through the enzymatic cycle.

A large number of processive motor proteins, which are most studied experimentally, typically function in cells by moving in a linear fashion along cytoskeleton proteins such as actin filaments and microtubules [1, 3, 5]. Because of the polymeric structure of these filaments the dynamics of molecular motors can be viewed as effectively one-dimensional periodic biased motion [8, 10, 81]. All existing theoretical approaches adopt this view, although the implementation of this picture is rather different. In the so-called continuum ratchet models [8, 10, 72, 87, 88, 95] the motion of motor proteins along some continuum potentials is assumed. A different approach argues that the motion of motor proteins can be described by a network of discrete stochastic transitions between specific biochemical states with variable spatial positions [8].

It is important to note that to develop a successful theoretical framework for describing motor proteins one has to follow several rules. Theoretical models should take into account the symmetries of the system, such as the periodic structure, polarity and chirality of molecular motor tracks. They also cannot violate the basic laws of physics and chemistry. A successful theoretical method should also provide a reasonable quantitative description of available experimental observations that can shed the light on the microscopic mechanisms of the underlying processes, as well as predictions that can be tested in future experiments. These arguments present a set of criteria that should be used in evaluating the applicability of different theoretical approaches for analyzing motor proteins dynamics. Unfortunately, many proposed theoretical models do not satisfy these conditions, and their results cannot be fully trusted [8].

4.2. Continuum ratchet potentials methods

In this continuum method a molecular motor is viewed as a particle that moves along several spatially parallel, periodic but generally asymmetric free-energy potentials as shown in figure 3 [8, 10, 71–73, 81, 87, 88, 95]. The different potential surfaces are the result of the interactions of motor proteins with the filaments, fuel molecules (ATP) and hydrolysis products in different biochemical states, and the molecular motor can stochastically switch between these states due to the released chemical energy. At each free-energy potential surface the molecule mainly diffuses in the direction of the local minimum. The sustained unidirectional motion of the particle requires a constant supply of chemical energy that underlies the non-equilibrium nature of molecular motors dynamics. No net motion can be observed at equilibrium conditions. One can introduce a function $P_i(x, t)$ that defines the probability density for the motor protein to be found at location x at time t at the potential surface $W_i(x)$: see figure 3. The temporal evolution of the system can be described by a set of Fokker–Planck equations with source terms [10, 72, 88, 95],

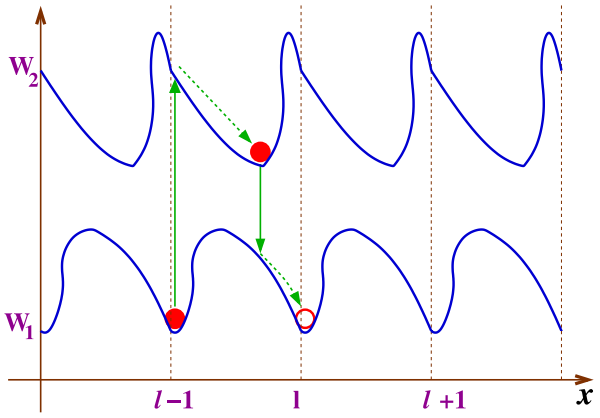

Figure 3. A schematic representation of the motion of molecular motors in continuum thermal ratchet models. The simplest situation with two periodic asymmetric potentials is shown. Solid vertical lines correspond to stochastic transitions driven by chemical reactions, e.g., ATP hydrolysis. Dashed arrow lines describe the diffusional motion along each potential surface.

$$\frac{\partial P_i(x,t)}{\partial t} + \frac{\partial J_i}{\partial x} = \sum_j u_{ji} P_j(x,t) - \sum_j u_{ij} P_i(x,t), \qquad (1)$$

where u_{ij} are transition rates between states i and j. The particle current has contributions from diffusion, from the interaction potential and from the action of possible external fields [72],

$$J_i = \mu_i \left[-k_B T \frac{\partial P_i(x,t)}{\partial x} - P_i(x,t) \frac{\partial W_i(x)}{\partial x} - P_i(x,t) \frac{\partial W_{\text{ext}}(x)}{\partial x} \right], \qquad (2)$$

with μ_i describing a mobility of the molecular motor in the state i. These equations in principle can be solved if the potential functions are known. Several simple cases have been analyzed [72].

These chemically driven ratchet models [72, 88] are also known as Markov–Fokker–Planck models [95]. They provide a simple and consistent description of the motor protein's dynamics and mechanochemical coupling with a relatively small number of parameters. Continuum models are well suited for mathematical treatments using established analytical and numerical tools. The ratchet models are also a starting point of fundamental studies on the nature of non-equilibrium phenomena in molecular motors [154, 155].

However, there are several properties of these continuum models that complicate their application for modeling molecular motor dynamics. With the exception of a few oversimplified and unrealistic potential surfaces, general analytical results cannot be obtained. For most situations numerical calculations should be performed, but they are typically also quite demanding. Furthermore, it is almost impossible to derive the realistic potentials from the available structural information on motor proteins, and approximations must be employed in the computation of the dynamic properties of molecular motors. In addition, ratchet models are not very flexible in analyzing systems with complex biochemical networks. As a result, it is hard to estimate the reliability and applicability of ratchet models for uncovering the mechanisms of real motor proteins. This analysis suggests also that continuum models can be reasonable utilized now only for the description of some qualitative features of molecular motor dynamics as well as for discussing the general fundamental features of non-equilibrium systems [8, 154]. Despite this relatively pessimistic conclusion, it can be argued that the increased amount and quality of structural information and improving the computational power of full atomistic simulations should make this continuum approach much more valuable and attractive for motor proteins in the future.

4.3. Discrete-state stochastic models

The catalytic activities of motor proteins involve a variety of biochemical reactions. Stimulated by the importance of these chemical processes in relation to the dynamics of molecular motors, a different theoretical approach, based on discrete-state stochastic models of traditional chemical kinetics, has been developed [8, 10, 77–80]. It argues that the motion of molecular motors can be described as a network of chemical transitions between discrete biochemical states with variable spatial positions. In the simplest linear sequential model, as shown in figure 4, it is assumed that during the enzymatic cycle the motor protein moves from the binding site l on the filament to the identical binding site $l+1$ via a sequence of N intermediate biochemical states that might have different spatial locations. Two identical binding sites are separated by a distance d, which corresponds to the step size of the molecular motor. It is known that for kinesin and dynein motor proteins translocating along microtubules in opposite directions d is equal to 8.2 nm, while for myosins V proceeding along actin filaments the step is larger, $d \approx 36$ nm. The motor protein in the mechanochemical state j_l ($j = 0, 1, \ldots, N-1$) can step forward to the state $(j+1)_l$ with a rate u_j, or it might move backward to the state $(j-1)_l$ with a rate w_j. Discrete states j_l describe different stages of ATP hydrolysis catalyzed by the action of the motor protein molecules. For example, one might assume that 0_l corresponds to the state when the motor protein is strongly bound to the molecular track, awaiting the arrival of the ATP molecule. In these discrete-state models reverse transitions are explicitly taken into account, in agreement with the fundamental concepts

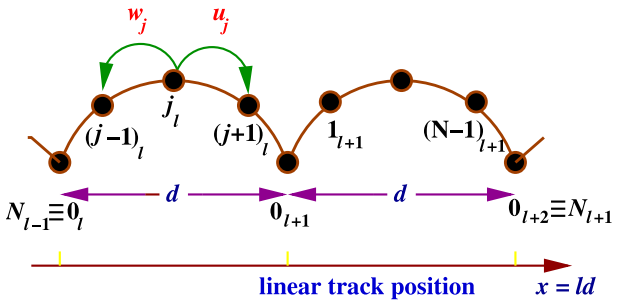

Figure 4. A schematic view of a linear sequential discrete-state stochastic model for analyzing the dynamics of single molecular motors. Transition rates u_j and w_j describe the forward and backward rates for the motor protein to step out of the site j. The distance between neighboring binding sites is d.

of physics and chemistry, and supporting the experimental observations on backward steps [8, 48, 64, 67, 68, 148].

In discrete-state stochastic models the dynamics of molecular motors can be described by analyzing the function $P_j(l, t)$, which is the probability to find the molecule in the state j_l at time t. Its temporal evolution is governed by master equations [8],

$$\frac{\mathrm{d}P_j(l,t)}{\mathrm{d}t} = u_{j-1}P_{j-1}(l,t) + w_{j+1}P_{j+1}(l,t) - (u_j + w_j)P_j(l,t). \tag{3}$$

These expressions can be easily understood, since they are kinetic equations for motor protein-related biochemical transitions, and they reflect the conservation of the probability to find the motor protein molecule in the given state. It can be shown also that the same equations also describe the motion of a single random walker on a periodic (with a period of size N) one-dimensional infinite lattice [8]. Thus, this mapping allows one to utilize the mathematical formalism, developed by Derrida in 1983 [149], to obtain exact and explicit expressions for all the dynamic properties, such as the mean asymptotic large-time velocity

$$V = V(\{u_j, w_j\}) = \lim_{t\to\infty} \frac{\mathrm{d}\langle x(t)\rangle}{\mathrm{d}t}, \tag{4}$$

and the mean dispersion (or effective diffusion constant)

$$D = D(\{u_j, w_j\}) = \frac{1}{2}\lim_{t\to\infty} \frac{\mathrm{d}}{\mathrm{d}t}\left[\langle x^2(t)\rangle - \langle x(t)\rangle^2\right]. \tag{5}$$

Here $x(t)$ defines a position of the molecular motor on linear track at time t. These expressions directly connect the transition rates u_j and w_j, which can be obtained *independently* from bulk chemical kinetic experiments, with the dynamic properties (V and D) of motor proteins measured in single-molecule experiments. This is a significant advantage in comparison with the continuum ratchet approach, since the input parameters in discrete-state models are independently measured experimental quantities [8]. For the simplest model with $N = 2$ states this theoretical approach gives the following

expressions for the mean velocity and dispersion [8, 86]:

$$V = d\frac{u_0u_1 - w_0w_1}{u_0 + u_1 + w_0 + w_1},$$
$$D = \frac{d^2}{2}\frac{(u_0u_1 + w_0w_1) - 2(V/d)^2}{u_0 + u_1 + w_0 + w_1}. \quad (6)$$

The big advantage of the discrete stochastic method is the ability to obtain analytical expressions for any dynamic property of motor protein systems. The molecular motor catalyzes hydrolysis of ATP or related compounds and it utilizes part of the released chemical energy to exert a force in the direction of its motion. This driving force can be explicitly estimated as outlined in the discrete-state stochastic approach [75, 76]. It was shown that for the simplest sequential model (see figure 4) the exerted force is equal to

$$F_S = \frac{k_BT}{d}\ln\prod_{j=0}^{N-1}\frac{u_j}{w_j}. \quad (7)$$

This simple result can be easily understood by using standard thermodynamic arguments. One can define a function $K = \prod_{j=0}^{N-1}(\frac{u_j}{w_j})$ and it can be shown that it corresponds to an effective equilibrium constant for the process of moving the motor protein molecule from the binding site l to the binding site $l + 1$. Then, the expression $\Delta G_0 = -k_BT\ln K$ gives the free energy released in the system when the protein molecule moves between two neighboring binding sites. This difference is a result of hydrolyzing one ATP molecule after making one forward step via N intermediate transitions. All this free energy might be converted into mechanical work to move the motor protein by the step size d, thus exerting a force equal to $F_S = \Delta G_0/d$. This is a stall force, since it is equal to the external force needed to stop the molecular motor. For linear sequential models (see figure 4) the stalling condition corresponds to equilibrium, while for more complex biochemical networks this might not be the case. It should be noted that neglecting any of the backward transitions, i.e., assuming that even one specific rate $w_j = 0$, leads to the unphysical prediction of the diverging stall force and the infinite free-energy change. This argument clearly shows that theoretical models that assume irreversible transitions cannot be reliably utilized for understanding the fundamental mechanisms of motor proteins [8].

Molecular motors in cellular environment are subjected to many external forces and fields. Single-molecule experiments are able to impose a measured force F directly to single motor protein molecules [5, 148, 150, 151]. In discrete-state stochastic models the effect of external forces can be easily incorporated by introducing load distribution factors, $\theta_j^{\pm}$ [8, 75, 76]. These parameters quantitatively describe how the work performed by external forces is distributed between various biochemical transitions. It also provides a measure of the change in the free-energy landscape of the system under the influence of the external field. Assuming that the external force acts parallel to the filament, a single molecular motor produces a work Fd during one enzymatic cycle. It can be shown using reaction-rate theories [8] that transition rates are modified under the effect of external forces in the following way,

$$u_j(F) = u_j(0)\exp(-\theta_j^+Fd/k_BT),$$
$$w_j(F) = w_j(0)\exp(\theta_j^-Fd/k_BT), \quad (8)$$

with the additional requirement that

$$\sum_{j=0}^{N-1}(\theta_j^+ + \theta_j^-) = 1. \quad (9)$$

It also should be mentioned that the products $\theta_j^{\pm}d$ correspond to projections of free-energy landscape extrema along the reaction coordinate, defining the substeps for the motion of molecular motors [8]. This is a valuable theoretical prediction of the discrete-state approach, since the substeps for dynamical motion of various motor proteins have been observed in single-molecule experiments [42, 54, 156]. It is important to note that this method of taking into account the effect of external forces on transition rates is not exact. It implicitly assumes that the position and energy of the transition-state complex for each chemical transition do not change with the external force, making the parameters $\theta_j^{\pm}$ independent of F. One could argue that this approximation should work well for large energy barriers between individual states, which is a reasonable description for most motor proteins. However, there are theoretical arguments that point out the danger of using such oversimplified pictures for some chemical processes in biopolymers [159]. It will be important to investigate this issue in more detail in the future.

As illustrated in figure 2, the single-molecule experiments provide comprehensive information on the residence times of molecular motors in different spatial positions associated with some specific biochemical states. These quantities are also known as dwell times, and they are frequently measured with a high precision [29, 34, 48]. From the theoretical point of view, they are related to the concept of first-passage processes, which is a well developed and widely utilized theoretical method in chemistry, physics and biology [160, 161]. It has been argued that full distributions of dwell times (or first-passage times) might help to identify details of the microscopic mechanisms of motor proteins [8, 89, 90]; however, in experiments, this approach is rarely used. To illustrate the method, let us consider a linear sequential model (see figure 4) and introduce a function $F_{j,N}(t)$, which is defined as a probability for the molecular motor to reach the state N for the first time at time t if it started at the site j at $t = 0$ [160, 161]. This probability function can be found by analyzing a set of backward master equations that control its temporal evolution,

$$\frac{dF_{j,N}(t)}{dt} = u_jF_{j+1,N}(t) + w_jF_{j-1,N}(t) - (u_j + w_j)F_{j,N}(t). \quad (10)$$

Note that these expressions are different from the normal master equations (as in equation (3)), since they discuss the arrival probability densities [160, 161]. Solving these equations allows one to determine all the dynamic properties

of molecular motors. Theoretical analyses of mean first-passage times and their application to motor proteins have been performed for various systems [8, 10, 89, 90, 162]. One of the most surprising results is a prediction that full-cycle dwell times before the forward and backward steps, τ_+ and τ_- correspondingly, are the same for motor proteins in linear sequential models, although the probabilities of forward and backward steps are very different [90]. One would naively think (and many researchers still do!) that these times are different. This relation is a consequence of a fundamental law, known as a principle of microscopic reversibility, that applies for all chemical processes. For $N = 2$ models it was shown that

$$\tau_+ = \tau_- = \frac{(u_0 + u_1 + w_0 + w_1)}{(u_0 w_0 + u_1 w_1)}. \tag{11}$$

It is important to note that these theoretical predictions have been confirmed in several experiments on motor proteins [34, 48]. In addition, extending the theory of first-passage processes for specific motor protein systems it has been argued that more detailed analysis of the statistics of events is a powerful but still infrequently applied tool for uncovering the mechanisms of molecular motors [8, 162].

The analogy of molecular motors with nanoscale machines has stimulated theoretical investigations on the efficiency of these engines, underlining the fundamental difference from macroscopic systems [163–166]. Since molecular motors operate in isothermal conditions, the classical approach to evaluate the efficiency utilizing the Carnot cycle cannot be applied. Motor proteins operate at non-equilibrium, and only for some systems might equilibrium be reached at the stalling conditions when the molecular motor velocity is zero. In this case, the efficiency is close to the thermodynamic limit of 1, but the power output is vanishing since the motor does not move. To better quantify the performance of these nanoscale machines, many researchers have investigated the efficiency at maximum power [163–166]. It has been shown that for weak external forces, when the linear response can be utilized, the efficiency is close to $1/2$, while for more realistic external potentials it can vary between 0 and 1. One interesting observation of these studies is the effect of asymmetry of the underlying free-energy potential surface on the molecular motor efficiency. It has been shown that for systems with the transition state closer to the reactant the efficiency is higher in comparison with the case when the transition state resembles more the product state. Based on experimental observations, it has been argued that single motor proteins operate with a maximal possible efficiency [163–166].

A major advantage of discrete-state stochastic models is their flexibility in handling more complex biochemical networks with a topology that deviates from simple chains [8]. Biochemical experiments on many molecular motors suggest that they do not follow a single linear sequence of states that connects the neighboring binding sites. In many cases, the more realistic picture of underlying biochemical networks for motor proteins includes multiple parallel pathways, loops, branched states that do not lead to directed motion, and effectively irreversible detachments. A theoretical approach that generalizes the original Derrida's method allows one to compute explicitly the dynamic properties of motor proteins with complex networks of biochemical transitions [8, 85, 100, 111–114]. In addition, discrete-state stochastic models have been successfully used to describe interactions between domains of motor proteins and their effect on the overall mechanisms of motility [8, 91]. Furthermore, the original models have been extended to describe explicitly the motion in two-dimensional and three-dimensional free-energy landscapes [8, 92, 93]. Discrete-state stochastic models can account for all the available experimental observations and they provide a flexible and convenient theoretical framework for understanding the mechanism of motor proteins.

4.4. Mechanochemical and structural models

Although discrete-state stochastic models can provide a satisfactory description of the dynamic properties of motor proteins, the weakest point of this approach is a limited connection to molecular structures. In other words, given a free-energy landscape the motion of molecular motors can be well predicted, while the microscopic origins of this specific potential surface, which is the result of complex intra-molecular and inter-molecular interactions of motor proteins with other molecules, are not accessible in this method. It is important to incorporate structural information into a theoretical framework for analyzing molecular motors and understanding the fundamental principles of energy conversion.

The necessity to account for the structural properties of motor proteins has been realized in several recent theoretical developments [96–98, 107–110, 157, 158]. Lan and Sun proposed a mechanochemical approach that calculates explicitly the free-energy landscape for a motor protein's motion [110]. It has two contributions: the chemical energy, which can be obtained from kinetic measurements, and the mechanical part, which is estimated from known structural information on motor proteins using simple mechanical models. This approach has been successfully applied in analyzing the dynamic behavior of myosins V and VI, showing how different domains in these motor proteins interact to transmit forces and to synchronize their motion [96, 97, 110]. A similar method was also productive in analyzing and explaining the complex features of dynein motor proteins [158]. A related structure-based model based on coarse-grained molecular simulations and theoretical ideas from protein folding has also been utilized in investigating the dynamics of kinesin motor proteins [107–109]. Although these structure-related theoretical methods are promising in uncovering the fundamental principles of energy conversion of molecular motors, currently there are several problems that limit their applicability. These include the use of the oversimplified models from polymer physics, the assumption of mechanical (but not chemical) equilibrium, and the use of coarse-grained potentials that are not well justified and tested for motor protein systems. It is expected that this theoretical approach will be significantly improved with

future experimental advances in determining the structures of molecular motors and with the increasing power of modern computer simulations. It is also important to couple these structural methods with more phenomenological discrete-state stochastic models in order to develop a comprehensive multi-scale theoretical analysis of motor proteins.

5. Collective dynamics of motor proteins

One of the main biological functions of motor proteins is to support cellular processes by transporting vesicles and organelles along cytoskeleton filaments [1, 2, 4]. Recent *in vivo* experiments indicate that, during the cellular transport, motor proteins always operate in groups [167–170]. This is a surprising observation, since several *in vitro* studies have shown that some motor proteins are strong enough to function efficiently as single particles [5, 48, 148]. It suggests that the combined action of motor proteins is important in overcoming many challenges during the transportation in the crowded and dynamic cellular environment. It is often assumed that grouping motor proteins should lead to higher force production, longer travel distances and higher speeds, and the efficiency of single motors interacting with other molecular motors is higher [166]. However, the mechanisms of cooperative dynamics of molecular motors are still not well understood, and the impact of the collective motor behaviors on cellular processes remains unclear [103, 171–173].

One of the most serious problems for *in vivo* experimental studies of multi-motor dynamics is the fact that frequently it is very difficult to determine and control the number and structures of motors on transported cellular cargoes [103, 171]. This observation has stimulated the development of several experimental methods that utilized synthetic approaches (via protein or DNA scaffolding) to prepare multi-motor complexes of known composition [60, 174–179], as shown schematically in figure 5. These experimental studies on engineered multi-motor complexes have revealed surprising differences in the collective behavior of various classes of biological molecular motors. Using optical-trap spectrometry it was found that motor complexes consisting of a two-kinesins molecule cooperate negatively, i.e., these two motor proteins do not work together and most of the time the cellular cargo is transported primarily by a single molecular motor [60, 176]. These weak collective responses of kinesins contrast with experimental results on other motor proteins, such as NCD [174] and myosins V [178], where molecular motors cooperate much more productively, sharing the load and increasing the travel distances and speeds.

To explain the complex behavior of molecular motor complexes several theoretical ideas have been proposed [101–103, 172, 173, 180, 181]. Klumpp and Lipowsky introduced a theoretical approach which postulates that a cellular cargo is driven by a system of non-interacting motor proteins that independently bind to their cytoskeleton track or dissociate from it [101]. It can be viewed as an extension of discrete-state stochastic models [8] that have been successfully applied for the analysis of single motor proteins. This theoretical model predicted increased run

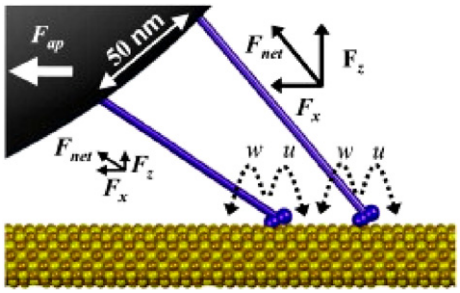

Figure 5. A simplified view of the system of two-kinesins motor proteins that transport the cargo along the microtubule filament track. Reproduced with permission from [103]. Copyright 2011 Elsevier.

lengths of motor complexes as well as a linear dependence of the stall forces on the number of motor proteins. It turns out to be a convenient theoretical picture for analyzing the collective dynamics of molecular motors [179, 180]. However, experimental results on two-kinesins motor protein complexes do not agree with these theoretical predictions [60]. In addition, several weak points of this approach have been criticized [103, 171], namely: unrealistic linear force–velocity for single motors, assumed equal sharing of external loads and a thermodynamically inconsistent description of binding/unbinding events.

A more advanced but related theoretical method that takes into account the interactions of motor proteins with cargoes and with cytoskeleton tracks has been proposed recently [103, 171]. This approach enumerates the most relevant discrete states of the system, depending on the chemical conformation of each motor (bound/unbound) and on the distance between bound motors, and then calculates the energies for each state using single-molecule mechanical data and chemical kinetic measurements. The transition rates between different states of the system are estimated using the calculated energy differences between the states and detailed balance arguments, which connect the ratio of forward and backward transition rates to the free-energy difference associated with this transition [103]. The advantages of this method are a consistent thermodynamic description of all the chemical transitions and the use of single-molecule chemical and mechanical data as the input parameters for calculating the dynamic properties of multi-motor complexes [103].

This approach has been tested by analyzing *in vitro* experiments on engineered two-kinesins complexes [60]. In agreement with experiments, it was found that interactions between kinesins reduce the probability for two molecular motors to share the load and to drive the cargo together. The model predicts that geometric and kinetic constraints largely limit how effectively a group of kinesin molecules can cooperate as a team [103]. These theoretical ideas have been generalized to other motor proteins, and it has been argued that fast and efficient motor proteins, such as kinesins, are unlikely to collaborate transporting cellular cargoes. At the same time, less efficient molecular motors whose velocities decrease more rapidly with increasing load are more capable of cooperating productively. To understand this

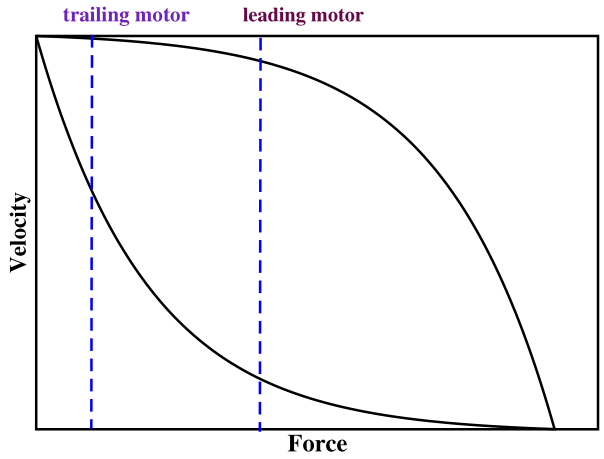

Figure 6. Typical force–velocity curves for single motor proteins. The upper curve corresponds to strong and efficient molecular motors, while the lower curve is for weaker and less efficient motors. Vertical dashed lines correspond to the forces felt by the leading and trailing molecules in two-motor assemblies—see the text for more details.

qualitatively, let us consider typical force–velocity curves for single motor proteins, as shown in figure 6. In the group of motor proteins that move the cargo, the leading molecule experiences the largest share of the load. For strong and efficient motors (upper curve in figure 6), the leading and the trailing molecules move with similar velocities for most loads below the stall force, the trailing particle has a lower probability to catch the leader, and there is a higher probability for the leading motor to unbind, leading to lower cooperativity in the motor protein complex, as observed for kinesins. For weak and less-efficient motors (the lower curve in figure 6), the trailing particle moves significantly faster and has a higher probability to catch the forward molecular motor before the unbinding, increasing the probability of load-sharing configurations and thus leading to a more cooperative behavior. In addition, the relative strength of motor protein interactions with the filament track also has a strong effect on cooperativity. Weak interactions will probably not support cooperative motion, while stronger interacting molecular motors have a higher probability to share the load and to be more cooperative. Based on these arguments, it has been suggested that more cooperative behavior will most probably be observed for dyneins and myosins V. This analysis also leads to a very important prediction, which is still experimentally not tested, that dyneins and myosins V, but not kinesins, serve as strong regulators of cellular transport processes [103].

This theoretical method has been recently extended to account for chemical interactions between bound motor proteins, and it was argued that this effect is important for understanding dynamics in the high-load regime of multiple-motor complexes [172]. In addition, this approach has been also utilized to quantify collaborative behavior in multiple-kinesins transport by varying structural and chemical properties [173]. It was shown that cooperation in kinesins is much more sensitive to changes in chemical rather than mechanical interactions. It seems that this discrete-state stochastic approach currently is probably the most efficient theoretical framework for understanding complex interactions in the dynamics of multiple molecular motors.

6. Perspective: future directions

In recent years the field of molecular motors has seen major developments and strong advances in both experimental and theoretical investigations. It is now possible to visualize and modify the dynamics of single motor proteins with unprecedented spatial and temporal resolutions, while structural studies have provided detailed information on the molecular conformations during the biochemical processes associated with the molecular motor motion. These striking experimental observations have stimulated strong discussions on the functioning and mechanisms of motor proteins, leading to the development of several theoretical methods that were able to successfully explain a large fraction of experimental results. Although many properties of molecular motors are still not fully determined, one could clearly say that the dynamic behavior of single motor proteins in *in vitro* conditions is currently much better understood. However, the situation is very different with regard to understanding the dynamics of multiple-motor proteins. Despite some recent progress, the cooperative dynamics of molecular motors remain not fully specified from both the experimental and theoretical point of views. To understand the complex dynamics of motor protein assemblies one has to take into account many factors, including various chemical and mechanical interactions, the responses to external fields, and relaxation dynamics to the stationary states. It will be important in the future to develop a unified comprehensive theoretical framework that takes into account all these effects.

Another important and very difficult task for the field is to develop quantitative experimental and theoretical methods to understand motor protein dynamics in real cellular conditions. The successful methods must account for complex interactions with other motor proteins and with cytoskeleton filaments as well as with the many other active biological molecules that are present in the cells. It is also important to understand how the cellular signaling system controls the dynamics of molecular motors in strongly non-equilibrium conditions. It is clear that future progress in understanding the complex phenomena associated with motor proteins depends strongly on combined experimental and theoretical efforts.

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