Motoring alone and gating together – theoretical models of biological transport

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Jestem świadom, że niezgodność niniejszego oświadczenia z prawdą ujawniona w dowolnym czasie, niezależnie od skutków prawnych wynikających z ww. ustawy, może spowodować unieważnienie stopnia nabytego na podstawie tej rozprawy.

Kraków, dnia .................... ..................................................
Abstract

This doctoral thesis is about the motion at the most fundamental level of life: the cell. Without the coordinated and effective ways of transporting organelles, organic building blocks, signaling molecules, ions and everything, that needs to be moved from one place of the cell to the other, biosphere would not develop on any moment in Earth’s history. The same is true for the mechanisms of exchanging information between cells and their surroundings. The main focus is put on the intracellular active transport (IAT), in which specialized mechanical machines, the so-called motor proteins, carry their cargos along cytoskeletal tracks. It is believed that they do so by literally walking along polymeric filaments (microtubules or actin) and pulling vesicles or organelles.

Results presented in the thesis are based on four published articles. Three of them explore different aspects of single molecular motor dynamics. It is shown how the protein’s structure, especially the part known as the neck linker, which tethers the diffusion of motor’s subunits, may affect its performance. By combining recent experimental observations, an alternative model of motor’s action is introduced. According to “cargo-mooring model”, motor protein and its cargo interact cooperatively to move along the track. The simplicity of this idea led to the exact analytical results for the force-velocity relation - an important measure of the protein’s ability to operate even against opposing loads. Being aware that motors move in a complex environment, the cytoplasm, part of the work attempts to describe the behavior of a motor under Brownian and, more important, non-Brownian noise.

Many experiments suggest some form of team-work between coupled molecular motors. To address this issue, the last article introduces a generic model of coupled oscillators in which a phase transition between unsynchronized and synchronized “phase” may occur. In this setup competition between phase synchronization and diffusive effects shapes the collective behavior and leads to a “synchronized pulse” - a low-entropy structure that facilitates the production of more entropy by the system as a whole. It is shown that such system may be used to capture collective features of, e.g. ion channels, which are coupled by cell membrane. But it may also be used to model a group of motor proteins, coupled by a shared cargo.
Streszczenie


Białka te poruszają się wzdłuż filamentów cytoszkieletu i - zgodnie z powszechnie uznaną teorią - ciągną cargo (organelle lub pęcherzyki wypełnione substancjami chemicznymi).


 Wyniki prac eksperymentalnych sugerują, że białka motoryczne wewnątrz komórki działają w grupach. W ostatnim z załączonych artykułów wprowadzono model sprzężonych oscylatorów, w których można wyróżnić rodzaj przejścia.
fazowego między stanem nieuporządkowanym a takim, w którym oscylatory za-
czynają działać wspólnie - synchronizują sposób zmiany stanu. Dla tego układu,
w którym synchronizacja i efekty dyfuzyjne rywalizują ze sobą w sposób nasuwa-
jący skojarzenie z solitonem, przeprowadzano analizę w ujęciu termodynamiki
stochastycznej, określając warunki synchronizacji. Układ taki może posłużyć do
modelowania zarówno kanałów jonowych, połączonych błoną komórkową, jak i
białek motorycznych, przyczepionych do tego samego cargo.
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Chapter 1

Introduction

Biological complexity arises from - and depends on - a symphony of processes that form an interconnected network. In this network, every transition depends on the others. As an example let us take signal transduction through a neuron: membrane ion transporters, some of them driven by chemical energy in the form of ATP, need to produce an optimal electrochemical gradient between the cell and the environment; a stimulus needs to be received to start depolarization; in a complicated process of neurotransmission, electrical signal is decoded to chemical activity in the junction of two nerve cells (the so-called synapse), and transmitted further. Then, the neuron needs to restore its primary electrochemical gradient and load its pre-synaptic membrane with neurotransmitters. Those, in turn, need to be produced in the vicinity of the cell nucleus and transported, often to a distance larger than one meter, by specialized proteins, which need to be synthesized and controlled by other proteins, which... The story could go on and on, leaving us with the impression of something beyond our reach.

Fortunately, due to an immense progress in the field of biophysics we can now study even very complicated processes individually and trace their mutual relations and links. We still may never get a total understanding of a single cell, but thanks to a combination of experimental techniques and quantitative methods we are no longer weaponless in this endeavor.

Below we will briefly introduce some of those techniques which proved to be useful in building our understanding of one of the most complex systems known to the world: the eukaryotic cell. The focus will lie on the phenomenon of cellular active transport - a set of processes which involve different agents and structures, and connects all the other nodes and links of the aforementioned living network.

1ATP - adenosine triphosphate; a molecule composed of a nucleoside adenosine bound to three phosphate groups; hydrolysis of the bonds that connect adjacent phosphates is a source of energy that fuels a great number of metabolic processes within the cell.
1.1 Inside a cell

There is not a lot of free space inside a living cell. Cytosol - a mixture of water, macromolecules and their building blocks, inorganic ions, metabolites and other chemicals - is highly crowded. It can be estimated that in prokaryotes - i.e. cells which, like bacteria, do not have a separated, membrane-bound nucleus - the mean spacing between proteins is comparable to their size (i.e. around 10 nm). Eukaryotes are different in that they have specialized compartments - regions usually surrounded by membrane to optimize their functionality. Also, most of their genetic material stored in DNA is enclosed in a unique type of organelle: the nucleus. Despite those forms of organization, eukaryotic cytosol is also packed with macro- and micromolecules, and hence crowded. Molecular crowding has a lot of serious consequences for the cell.

Cytosol together with all the organelles, except nucleus, forms a complex medium, called the cytoplasm. It sets the scene for most of cellular activities, including intracellular transport. This observation sounds trivial, but it was only in the late 1970s that people started to realize the important differences between biological dynamics in macro- and microscale. The milestone in developing a new perspective was set by Edward M. Purcell in his lecture, which later was turned into an article “Life at Low Reynolds Number”. What he proposed was a beautiful discussion of the consequences of applying basic tools of hydrodynamics - the Navier-Stokes equations - to a bacterium moving in its natural environment.

Navier-Stokes equations describe the motion of a fluid. They can be derived by applying Newton’s second law for a fluid element. The acceleration of this element in an arbitrary velocity field \( \mathbf{v} \) is \( \mathbf{a} = \frac{D \mathbf{v}}{Dt} \). The material time derivative, \( D/ Dt \), reflects change in acceleration due to time and/or space inhomogeneities of the velocity field. It is defined as:

\[
\frac{D}{Dt} = \frac{\partial}{\partial t} + \mathbf{v} \cdot \nabla.
\]

Fluid element - for simplicity we assume that it is a cuboid of dimensions \( \Delta x \times \Delta y \times \Delta z \) and mass \( \Delta m \) - is subject to the action of two types of force: pressure force and viscous force. The pressure field \( p(x, y, z, t) \) is a source of opposing forces, acting on the parallel walls of the fluid element. After obtaining its components in \( x-, y- \) and \( z- \)-direction, the resulting pressure force may be written as:

\[
\delta F_{\text{pressure}} = -\left( \frac{\partial p}{\partial x}, \frac{\partial p}{\partial y}, \frac{\partial p}{\partial z} \right) \Delta x \times \Delta y \times \Delta z = -\nabla p \Delta x \times \Delta y \times \Delta z.
\]

It was Isaac Newton who first showed that the viscous force on each wall of the considered cuboid is proportional to the rate of change of the fluid velocity.

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2Here, **bold** indicates vectors.
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in the direction perpendicular to this wall and to its surface. With that in mind one can derive the formula for this force.

\[ \delta F_{\text{viscous}} = -\eta \nabla^2 v \Delta x \Delta y \Delta z. \]  
(1.2)

Inserting Eqs. (1.1) and (1.2) into Newton’s second law one obtains:

\[ \Delta ma = \delta F_{\text{pressure}} + \delta F_{\text{viscous}}, \]  
(1.3)

with mass being the product of density, \( \rho \), and volume (\( \Delta x \Delta y \Delta z \)). Finally, from Eq. (1.3) and noticing that \( a(x, t) = \partial v(x, t)/\partial t + v(x, t)\partial v(x, t)/\partial x \), one obtains the Navier-Stokes equations:

\[ \frac{\partial v}{\partial t} + v \cdot \nabla v = -\frac{1}{\rho} \nabla p + \frac{\eta}{\rho} \nabla^2 v. \]  
(1.4)

1.1.1 Low Reynolds number

Navier-Stokes equations hide two physical realities. This becomes obvious when one writes them in a dimensionless form (for details please see 9, p. 496):

\[ \frac{\partial v'}{\partial t'} + v' \cdot \nabla v' = -\nabla p' + \frac{\eta}{\rho u L} \nabla^2 v'. \]  
(1.5)

Let us recall that the parameters \( v', p', t' \) are the re-scaled velocity, pressure (or, more precisely, pressure variation, since \( p' \) is used as a gradient) and time, respectively, \( u \) and \( L \) are the characteristic velocity and length, \( \rho \) is the fluid’s density, \( \eta \) - (kinematic) viscosity and \( \nabla' \equiv L \nabla \). Left-hand side (LHS) of Eq. (1.5) gives the information about the inertia of the system. The second term on the right-hand side (RHS) depends on the viscosity. The dimensionless quantity \( (\rho u L)/\eta \), expressing the ratio of inertial forces to viscous forces, is called the Reynolds number (Re). For situations where the inertia dominates over viscosity, i.e. for \( Re > 1 \), the motion of a fluid or an object merged in it is turbulent. But with decreasing Re, when the inertia becomes less significant for the net dynamics, the situation changes dramatically. We call this situation an overdamping. In an overdamped reality the flow is linear and Navier-Stokes equations reduce to a much simpler Stokes equation: \( \nabla p = \eta \nabla^2 v \). In what is coming next we will be talking about objects - motor proteins moving in a buffer or a cytoplasm - for which the Reynolds number is of the order \( 10^{-9} \). This will have serious consequences for their performance.

1.1.2 From Brownian motion to active transport

Cell’s morphology shapes its physiology. The bigger the cell, the longer the distances that need to be covered by metabolites or proteins, which are often used far from the places of their origin. This is one of the greatest differences that shaped the divergent evolutionary paths of bacteria and eukaryotes.

\[ ^3\text{For an elegant discussion and derivation see } [9] \text{ pp. 485-490.} \]
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Prokaryotes are small creatures. *Escherichia coli*, one of the well-known model organisms, is a bacteria that lives in human intestines. It has a rod-like shape with typical length of 1 – 2 μm and a diameter of 0.5 – 1 μm. We will use this length - 1 μm - as a “standard ruler” for the purpose of building up our intuition about biological scales of interest. And so, it would take a chain of around 50 such cells to measure out the width of a human hair. Going down towards smaller objects - macromolecules - one would need a palisade built of 500 parallel DNA strands to connect two poles of *E. coli*.

From our point of view, a question of interest is how fast a molecule - a globular protein of radius $R = 3$ nm - can be transported to distance of 1 micron only by a Brownian diffusion? Let us start with an idealized model, in which cell’s cytoplasm has the same viscosity as water at $20^\circ C$, which means $\eta = 1$ Pa s. For this protein, the diffusion coefficient, $D$, can be obtained from the Einstein-Stokes relation:

$$D = \frac{k_B T}{\gamma},$$

where $k_B = 1.38 \times 10^{-23}$ J/K is the Boltzmann constant, $T$ is temperature in Kelvins and $\gamma = 6\pi \eta R$ is the drag coefficient of a spherical probe. For $N$-dimensional isotropic diffusion the mean squared displacement is given by: $\langle x^2 \rangle = 2NDt$. From this, in a 1-dimensional case, we can estimate the time in which the protein diffuses to a distance of 1 micron. It is around 5 ms - short enough to satisfy physiological needs. Of course, cytoplasm is not water. Intracellular diffusion is not 1-d. And, as mentioned before, molecular crowding is a phenomenon which cannot simply be neglected. Surprisingly, thanks to their small sizes bacteria tend to overcome those difficulties without the need of any mechanisms that allow for intracellular transport. Prokaryotic cells function well, relying on diffusion - passive transport - as their main mechanism of segregation and transport of reagents.

How about the eukaryotic cells? First, let us note that their size spans from 10 μm to even more than 1 m. For example, recurrent laryngeal nerve (RLN) of an adult giraffe can have 4.6 m. This is a huge distance for some of the neurotransmitters, e.g. neuropeptides, or enzymes that are created in the cell body on one pole of the neuron and need to be transported to the other pole. Diffusive motion of a $R = 3$ nm protein from one end of the 1 m long nerve cell to the other (even in an idealized scenario from the previous example) would take approximately 150 years. That would not work well for a giraffe.

But nature found its way. In the course of evolution, when the cells begun to grow and specialize, an ingenious mechanism emerged. A class of specialized proteins formed what is now called a cytoskeleton - an intracellular network of filaments, which form tracks for even more specialized motor proteins. The latter may literally walk along cytoskeleton with a constant speed of around

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*In general, diffusion coefficient depends on the system under consideration and is different, for example, when one considers inertia or when it is assumed that the motion is overdamped. Reference [2] gives an elegant discussion of this important issue.*
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1 \(\mu m/s\) and carry a cargo from the place of its synthesis to even the furthest regions of the cell. The whole process, known as intracellular active transport (IAT), is driven by a chemical energy, obtained from e.g. ATP hydrolysis, which in turn is transformed into a mechanical work of the motor protein. But is this 1 \(\mu m/s\) sufficient to ensure the right concentrations of all transported cargos in the right regions of the cell?

1.1.3 Two is better than one

Motor proteins (or molecular motors, as they are often called) do not work alone. They form ensembles - or teams - of multiple motors, usually coming from different protein families (e.g. kinesins and dyneins). It is one of the cell’s most important tasks to maintain a sufficient number of different types of motor proteins to sustain the constant transport of resources [4, 5]. But this effort pays off. Since the discovery of the first processive (i.e. walking along cytoskeletal filaments in a given direction) motor proteins: kinesin-1, described by Ron Vale and colleagues in 1985 [4], and of cytoplasmic dynein by Paschal et al. [11] in 1987, each year was bringing - and still is! - astonishing discoveries of great efficiency, importance and flexibility of all the IAT components.

But the cooperation of motor proteins is not the only example of biological collective phenomena [2, 4, 5]. However, despite the fact that the actors may differ, the general mechanisms behind cooperative, collective action of, e.g., different classes of proteins are often strikingly similar, as will be mentioned in Chapter 4.

1.2 What is this thesis about?

After years of intensive research we know a lot about transport occurring in living cells. Spectacular experiments done in vitro and in vivo let us see the agents involved in moving cargos or sorting ions on both sides of the cell membrane and to manipulate them with a single molecule precision. Numerous theoretical models have been proposed to bridge the gap between knowing the microscopic structure of the protein and understanding how it functions. Yet, every new discovery brings new questions.

One of the biggest challenges is now to put together everything we know about different sub-systems of the cell and describe their mutual dependencies and relations. For example, despite the fact that Brownian diffusion for years served as a model that explains the motion of objects - i.e. proteins, organelles, molecules - inside cells, recent findings questioned this approach. Researchers observed that the action of motor protein causes their tracks - cytoskeleton - to move, which in turn leads to the propagation of “fluctuating random forces” through the cytoplasm [4]. Even that the characteristic of the motion may suggest “normal diffusion” (i.e. mean square displacement depends linearly on time), after a careful analysis the fluctuations turn out to be non-Gaussian (see Sec. 5.1). In other words it has been shown that the best model to describe the
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motion of proteins in cytoplasm would be anomalous diffusion \[16,18\]. Those findings also highlight the need of understanding the impact of non-Brownian noise on intracellular processes.

In this thesis we will try to address some of the issues of cellular transport which are still not fully understood.

- **Stepping molecular motor amid Lévy white noise [A1]** - in this paper, which starts with a simple representation of a kinesin-like molecular motor as two coupled Brownian-particles, we are asking about the impact of noisy environment on motor’s dynamics. It is now becoming clear that cytoplasm is a much more complex medium than previously assumed. Non-Brownian fluctuations are not anomalies - they are the norm. We describe the behavior of a motor under different types of noise by numerical simulating its motion.

- **Understanding Operating Principles and Processivity of Molecular Motors [A2]** - in this paper we look at a motor protein from the perspective of three complementary approaches: molecular dynamics, chemo-mechanical modeling and thermodynamics. We ask two questions: how the protein’s structure affects its function and what are the ways of describing the efficiency of a motor? We extend some of the popular models (chemomechanical model of kinesin proposed by Liepe et al. \[18\] or Brownian particle model introduced by Żabicki et al. in \[20\]) and highlight some of the key difficulties that one faces when trying to combine different approaches in successful modeling of motor proteins. For example, it is impossible to simulate chemical reactions with molecular dynamics, while chemomechanical approach is blind to some subtle, yet important, structural properties of the protein.

- **“Cargo-mooring” as an operating principle for molecular motors [A3]** - in this paper we broaden our perspective by studying the impact of cargo on kinesin walk along a polymeric track. We propose a mechanism - “cargo-mooring” - according to which motor protein and its cargo interact cooperatively to move along the microtubule. We work out how chemical energy from ATP hydrolysis can be used to rectify the cargo’s diffusion. This approach allows to derive the velocity as a function of load force, cargo size and buffer viscosity. All the parameters come from experiments and the results are obtained analytically.

- **Phase transitions and entropies for synchronizing oscillators [A4]** - in this paper we investigate a generic model of coupled oscillators in which a phase transition between unsynchronized and synchronized “phase” occurs when the coupling parameter is varied. We describe a competition between phase synchronization and diffusive effects, which shapes the collective behavior of the system, and leads to a “synchronized pulse”. It is a low-entropy structure that facilitates the production of more entropy by the system as a whole. With the increasing number of states we apply a
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continuum approximation and derive a potential Burgers’ equation for a propagating pulse. In that case the phase transition does not occur. This system may be used to model some biophysical systems, like ion channels, which are coupled by cell membrane, or motor proteins, which share the same cargo.

We start with a simple idea of a motor protein modeled as two coupled particles. They move in a potential which mimics a periodic polymeric track, such as a microtubule. This approach originates from the known crystallographic structure of the canonical example of a motor protein: kinesin-1 (see Sec. 2.2.1) [21, 22]. In short, kinesin-1 is a homodimer, which means that it is composed of two identical structures. Each of these structures consists of a big, globular “head”, made out of folded amino acid strand. The second end of this strand is loose and sticks out from the head, like a single thread from a ball of wool. Loose ends from both subunits curl around each other, forming the stalk. It is therefore convenient to model kinesin’s heads as two identical particles, coupled by an elastic linker.

We build up on this approach by adding more and more features, which experiments tell us are important for understanding motor-cargo-track dynamics (protein’s structure, length of a step and waiting time between subsequent conformational changes, to name only few). In the end we discuss possible consequences of coupling such agents together into groups. This progressive way from “simple to more complex” and from “one to many” is a leading motif of this work.
Chapter 2

Dramatis personae - on motors, tracks and cargos

Intracellular active transport rests upon four pillars:

1. Tracks - objects that set routes for the motors.
2. Motors - objects that carry the cargo.
3. Cargos - objects that need to be moved from one place of the cell to the other.
4. The environment - a source of energy (ATP), chemicals which may affect the transport and of noise, resulting from e.g. temperature variations or active processes that happen in the cell.

In this chapter we will briefly characterize all of those elements. We will also take a glimpse of experimental methods used for studying cytoskeletal molecular motors.

2.1 Cytoskeleton - intracellular highways

Eukaryotic cell membranes are not rigid. Especially in the case of animal cells, which lack cell walls, their morphological structure and mechanical properties rely on a protein scaffold, called the cytoskeleton. This is a magnificent structure, that not only saves the cell from collapsing, but also enables it to change its shape and move. It is constantly reorganized in response to stimuli and physiological needs, one of them being the organization of active intracellular transport.

Eukaryotic cytoskeleton is build of three major types (families) of filaments: actin filaments (or microfilaments), intermediate filaments and microtubules. The building blocks of those filaments are the actin monomer, the intermediate filament dimer and the $\alpha\beta$ tubulin heterodimer, respectively. Each type of
filaments has its own group of associated proteins and factors which regulate their orientation, pace of growing and shrinking and other properties.

2.1.1 Actin filaments

Actin participates in a huge number of cellular processes. It establishes cell shape, allows for muscle contraction, cell migration (motility) and division, plays a major role in cell signaling and helps in organizing intracellular transport, especially in the vicinity of the membrane, where it forms the disordered network, known as cell cortex.

Actin monomers have a protein mass of around 45-kDa and a diameter of roughly 6 \( \text{nm} \). They are found in two forms: globular G-actin and filamentous F-actin, the latter being a building block of linear polymers. There are two types of actin filaments: cytoskeletal microfilaments, which set a track for molecular motors myosins, and those involved in muscle contraction.

Typical microfilament is a left-handed helix of two interlaced actin monomers with a full period of 72 \( \text{nm} \). One of its ends is pointed (one-start or minus-end), while the other is barbed (two-start or plus-end). The whole structure is dynamic: actin monomers from the minus-end slowly dissociate from the filament to the cytoplasm, when at the same time new monomers bind to the barbed end. The filament, with one end shrinking in length and the other growing, seems to be moving across the cytosol or a buffer. This phenomenon is called \textit{treadmilling}.

2.1.2 Intermediate filaments

A diameter of roughly 10 \( \text{nm} \) places them between microfilaments and microtubules. A single filament resembles a stiff rod with globular structures on both ends. \( \text{[2]} \). Like actin filaments, they provide a structure support, especially further from the membrane and in cell nucleus. They also support cell organization by anchoring organelles and play a role in cell division and signaling. Each tissue has its own set of intermediate filaments, for example there are neurofilaments in nerve cells, vimentin filaments in mesenchymal cells or keratin filaments in hair, nail and epithelial cells.

2.1.3 Microtubules

Microtubules are polymeric tubes, consisting of alternately arranged \( \alpha \)- and \( \beta \)-tubulin dimers and empty inside. \( \text{[1]} \). They can grow up to 50 \( \mu \text{m} \) of length. An outer diameter of 25 \( \text{nm} \) makes them the widest cytoskeletal filaments.

Microtubules are periodic structures. The distance between neighboring dimers is constant and equal to 8.2 \( \text{nm} \). A place where the \( \alpha \)-tubulin connects with \( \beta \)-tubulin forms an asymmetric gap - a binding place for molecular motors.

Microtubules are also polarized. The so called minus-end of the tube, associated with the structure called microtubule-organizing center (MTOC), like centrosomes close to cell nucleus or the basal bodies in cilia and flagella, is
stable, while the second, called the plus-end, probes the cytoplasm in a random direction. It grows by adding new tubulin dimers. It may happen that an already attached dimer would fall off the plus-end. This initiates a series of similar events, leading to a “catastrophe”, i.e. rapid disassembling of the filament. Shrinking microtubule can still be “rescued” when the rate of polymerization will prevail that of depolymerization. This switching between assembling and disassembling is called the dynamic instability \[\text{[24]}\].

It is worth noting that, according to a “search and capture” model proposed by Kirschner and Mitchison in 1986 \([23]\), when a growing microtubule captures kinetochore or some protein complexes during cell division, it may be stabilized. This mechanism allows to form long-lasting structures, like mitotic spindle.

Besides building up the cellular scaffold, microtubules are involved in many crucial processes, such as cell migration, propelling cilia and flagella, development of main axes and polarizing developing embryos \([24]\) and mitosis. They also serve as polymeric tracks for cytoskeletal processive motor proteins: dyneins and kinesins.

### 2.2 Cytoskeletal molecular motors - intracellular trucks

Cytoskeletal motor proteins, or mechanoenzymes, transform the free energy, mostly obtained from ATP hydrolysis, into mechanical work while connected with cytoskeletal filaments. They are amongst the oldest and the most sophisticated components of eukaryotic proteome. They consist of three superfamilies: kinesins, myosins and dyneins.

Due to their similar structure, it was suggested that kinesins and myosins have common ancestry (see \([27]\) for a review). However, they are walking on different filaments - microtubules and F-actin, respectively - what makes all the speculations about their origin more subtle. The most plausible scenario is that both motor families evolved independently from the same group of NTPases \([24]\).

None of the cytoskeletal motor superfamilies is ubiquitous to all eukaryotes. There are organisms without any representatives of dyneins and/or myosins. Kinesins seem to be encoded by all known genomes, however a given organism has only some, out of many, types of this protein.

This diversity of motor repertoires reflects the long evolutionary history of eukaryotes: it can be estimated that the last eukaryotic common ancestor (LECA) had at least 9 families of dyneins, 11 families of kinesin and 3 families of myosin \([24]\).

One of the key features of cytoskeletal motor proteins is processivity. A motor is processive if its single molecule can move continuously along cytoskeletal filament. The velocity of a processive motor does not depend on the density of

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\(^{1}\)NTPase is an enzyme that catalyzes the decomposition of a nucleoside triphosphate. For example, kinesins, dyneins and myosins are all ATPases, because they take the chemical energy from ATP hydrolysis.
CHAPTER 2. DRAMATIS PERSONAE - ON MOTORS, TRACKS AND CARGOS

other motors from the same family, transporting the same cargo \[^2\]. On the other hand, a motor is nonprocessive when the motion is observed only for a high density of motors. When the number of nonprocessive motor molecules does not exceed a certain threshold, no continuous movement can be observed. According to this, conventional kinesin-1, cytoplasmic dynein and myosin V are processive, while NcKIn3 kinesin, myosin II and the so-called outer-arm dynein are not.

The other feature that we will often refer to is the directionality. Moving on a polarized filament, some motors show a strong affinity for a given direction. For example, kinesin-1 moves from microtubule’s minus-end towards the plus-end, while cytoplasmic dynein prefers the opposite direction. This preference is caused by the asymmetry of both, the motor and the track. It is worth noting that kinesins and dyneins may sometimes take a single step in the “wrong” direction. Those rare events are called backsteps \[^3\].

2.2.1 Kinesins

Kinesin superfamilly members play two important roles in the cell. They take care of a cell’s internal transport needs by carrying liposomes containing various substances (e.g. neurotransmitters, building blocks of the membrane and cytoskeletal filaments) and also organelles (e.g. mitochondria). They do so by literally walking on microtubules, fueled by the ATP hydrolysis. The other role is organizing cell division. Kinesins are responsible for shaping microtubules, so that they form mitotic spindle, for chromosome congresion to the spindle equator and their positioning. They also take part in cytokinesis.

In what’s coming next we will often use kinesin-1 as an example of a “typical” cytoskeletal processive motor. It is why we will now give some more details about this protein.

Kinesin-1

Single kinesin-1 molecule consists of two motor domains, or heads, binding to the microtubule, a stalk responsible for attaching the cargo and over a dozen amino acids long neck linkers, linking the heads with the stalk and transferring strain between them. The neck linker - a sequence of 14-18 amino acids adjacent to the catalytic core of the head - is important for motor’s dynamics. Experiments done by William O. Hancock’s group showed that when neck linkers of kinesins from diverse families were all replaced by the same 14-residue long sequence, their processivity became the same \[^3\]. And when researchers extended the length of those neck linkers, they observed a drop in both, processivity and velocity.

\[^2\]Yet the net velocity of the cargo transported by a group of motors may change due to their cooperation. This change is caused by allowing for longer or shorter steps, as in the case of dynein \[^4\], or by changing kinesin’s dissociation rate from the microtubule \[^4\], and not by speeding up or slowing down a single motor.
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Motor domains are those parts of the protein where the catalyzed ATP hydrolysis - the source of energy - takes place. Such reaction releases around 22 $k_B T$ of energy. This energy can drive a conformation change of kinesin’s head that leads to a mechanical step, i.e. moving the rear head before the leading one. This mechanism, called hand-over-hand, resembles human’s walking pattern [31, 35].

Stepping, or as is often called walking of a kinesin molecule is a cyclic process. It starts with the two heads docked to the microtubule’s neighboring binding places, one (leading) in front of the other (rear). As mentioned before, binding places lie around 8 nm away from each other.

Chemical process of releasing the ADP from the leading head triggers the rear head to dissociate from the microtubule. Then, by a mechanism called the power stroke [36], combined with a diffusive search for a binding place [33], the unbounded head moves before the now-rear attached head and the stable connection with the filament is reestablished. Meanwhile, new ATP molecule attaches to the microtubule-bound head, is hydrolyzed and the products of this reaction are released. Or, at least, this is what the most popular interpretation of empirical data says is happening. After more than 30 years since the discovery of kinesin-1 new articles that challenge the consensus are still being published [34, 37, 38]. We will come back to this once again, while discussing chemomechanical models in the next chapter.

Altogether, most characteristic features of a kinesin-1 can be summarized:

- kinesin-1 is a processive molecular motor;
- its preferred direction is towards the plus-end of the microtubule;
- sometimes (with $p \approx 0.05$ [32]) it makes a “mistake” and back-steps towards the minus-end;
- exactly 1 ATP molecule is hydrolyzed for every step, forward or backward;
- every step moves a detached head by around 16 nm (two times the length of $\alpha - \beta$-tubulin dimer), while the center of mass of the molecule moves by 8 nm;
- in physiological condition single kinesin may carry a cargo with a constant speed of around 800 nm/s;
- stepping is synchronized and coordinated: after one head unbinds and rebinds again, the other one moves, and then they change again.

2.2.2 Myosins

They are the only known actin-based motor proteins [4]. The very name “myosin” emphasizes the most important role of those motors: some of them (especially myosin II) enable the muscle contraction. However, there are also non-muscular myosins, which utilize their ability of interacting with actin filaments while transporting cargos in the cytoplasm (myosin I and V), enable
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hearing (myosin XV) and seeing (myosin III). They also play a huge role in cell migration and immobilize cargoes, like vesicles, in the actin-rich regions of cell cortex. This allows the cell to store chemicals close to the places where they will be later used. Myosins from different classes differ in the directionality (e.g. myosin V walks towards the plus-end of actin filament, while myosin VI-towards the minus-end), step size (36 nm for myosin V and 10 nm for myosin I) and processivity.

Despite those differences, the members of most classes are built similarly to kinesin-1. They consist of the tail domain, which interacts with cargos, the neck linker used during force generation and the head (or heads) which interacts with actin filaments. The head shows enzymatic activity - it is an ATPase which enables ATP hydrolysis.

2.2.3 Dyneins

Dyneins are different from the previously described superfamilies in structure and performance. Among the 9 classes, 8 are associated with a specific cell’s motile and/or sensory organelle: the cilium. Cytoplasmic dynein-1 operates outside cilia, e.g. in neurons, where it works antagonistically to kinesins. Responsible for retrograde transport, it carries the organelles and vesicles along the microtubules from the presynaptic region towards the cell bodies.

All dyneins come from a large class of NTPases, called the AAA+ proteins. The characteristic part of their molecules are two hexameric rings built out of six AAA+ domains each. This structure is much more complicated than motor domains of kinesins and myosins.

Cytoplasmic dynein coming from different organisms differs significantly. For example, the one extracted from yeast cells is slower than the mammalian dynein (velocity of 100 nm/s vs 800 nm/s, respectively), but can keep moving under higher opposing load (stall force of 7 pN vs 2 pN, respectively) \(^3\). Mammalian cytoplasmic dyneins make steps of various lengths, while yeast dyneins take 8 nm long steps only. But the general feature which distinguishes dyneins from other types of motors is that their microtubule binding domains, called stalks, work more independently. There is no visible coordination of stepping and the “hand-over-hand” pattern is not visible in the observed trajectories.

2.3 Cargos - intracellular goods

The complex machinery that enables active intracellular transport has only one reason: to guarantee fast and efficient flow of resources from the place of their origin to where they are needed. Most cargos are packed in small (tens to

\(^3\)Every external force that pulls the cargo, e.g. exerted by an optical tweezer, in the direction opposite to that of the motor will be called an opposing load. The lowest force that immobilizes the motor - in other words, a force which a motor cannot oppose any longer and stops - is called a stall force.
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hundreds of nanometers in diameter) vesicles, covered with lipid membrane. The process of connecting a given cargo with a given type of motor is not random, but highly regulated (see [16, 17] for a review).

Cargo molecules are proteins, fragments of biological membranes, that will, for example, form synaptic vesicles or help to rebuild synaptic membrane, cytoskeletal filament monomers, mRNA and many more [16]. It happens that the ensembles of motors transport the whole organelles, like mitochondria, lysosomes and melanosomes.

Dyneins that walk in the direction of neuron’s cell body return exogenous material, trophic factors and used fragments of bio-membranes. Those cargos may then by recycled or utilized by lysosomes [18].

In the in vitro experiments researchers often use polystyrene or silica beads as cargos. They can have various sizes, from 50 nm to more than 1 μm. In [A3] we investigate how the size of the cargo affects the performance of a kinesin-1 molecular motor.

2.4 The environment

The last element we cannot ignore when discussing intracellular transport is the environment. By this we understand all the factors that affect the functioning of molecular motors, the stability of cytoskeletal filaments and the properties of the cargo.

For example, molecular motors are regulated by a huge range of regulatory proteins and factors. They may change the affinity of a given motor for a given type of cargo or increase its probability of dissociating from the filament [16, 17]. Regulation is a very complicated issue, which we still, after years of research, do not fully understand. As Ahmet Yildiz, one of the leading researchers working in the field of intracellular transport, told during a conference on molecular motors: “In biology, when you know that something is important but you don’t know what it is doing, you say it regulates something”. This is, and will stay true for many years to come.

But regulation is not the only way in which cytosol affects the transport. The others are, for example, crowding and “fluctuating random forces”, which originate from active cellular processes, such as the activity of molecular motors. Recently it has been shown that when myosin motors move the actin filaments, the motion propagates through a cytoplasm and stirs it [15]. This affects the organelles, but also vesicles that move - actively or passively - inside the cell. Including such phenomena into theoretical models of molecular motors is still challenging and probably more work, also experimental, needs to be done in this direction.
2.5 Single-molecule techniques for studying molecular motors

Joel E. Cohen was right, when he wrote in [49] that “Mathematics is biology’s next microscope, only better”. But it does not mean, that the time of traditional microscopes has gone - on the contrary. The field of active intracellular transport is one of the biggest playgrounds for researchers who are constantly improving accuracy and resolution, not only in space, but also in time. They also find brilliant ways of combining different techniques to enable new types of experiments, like the force spectrum microscopy [15] or super-resolution microscopy [10].

When kinesin-1 was discovered, biology and biophysics still relied on static methods, like electron microscopy. But only couple of years later a new way of doing experiments was made possible by the technical progress and ground-breaking ideas. This led to a whole new discipline, single-molecule manipulation, where the action of single molecule could be observed - and modified! - dynamically.

For molecular motors, this approach led to the idea of gliding-filament assays [11]. Motors were immobilized on a cover slip and filaments suspended in a buffer were put on top of them. Video microscopy was capturing the movement of single filaments. From this movies some basic parameters, like kinesin’s velocity or directionality could be inferred.

Shortly after the bead assays have been introduced [12]. Now the filaments were immobilized on a cover slip and motors were walking along them. Yet it was impossible to see a single motor protein, we could observe the motion of a cargo - silica or polystyrene bead. It was even possible to prepare the assay in such a way that only one molecule of a motor was carrying the cargo.

Another milestone was the application of optical tweezers. Thanks to them researchers could manipulate the cargos by applying external forces. One of the first successes was demonstrating that kinesin-1 can work against opposing loads not larger than 6-8 pN [32, 34, 58].

Nowadays optical tweezers are a standard method for studying molecular motors in action. They can be used not only in in vitro assays, but also in vivo, that is: not in an artificial buffer, but in a fully operational living cell. Together with other single-molecule techniques, like AFM, fluorescence microscopy, force spectrum microscopy and the whole biotechnological toolkit, they still provide new and exciting data [14].

With the experimental data in hand, it became possible to propose theoretical models that were aimed to explain why do the experimenters see what they were seeing. As one could expect, this approach has led to new discoveries, but also to new questions.
Chapter 3

Modeling Molecular Motors

Soon after Svoboda et al. published their results [52], theoreticians provided models that were explaining how a single molecule can utilize the free energy from ATP hydrolysis by changing it into mechanical work. Over time, two different approaches dominated the field.

The first one was based on an idea that when a motor gets smaller and smaller, the effect of fluctuations (i.e. thermal noise) gets larger and larger [53]. When we compare the power that drives the kinesin walking along microtubule with the power of thermal motion associated with this walk, we will find that the latter is roughly 9 orders of magnitude larger. Astumian and Hänggi compared that to a man trying to walk in a hurricane [54]. And yet, tiny proteins perform well despite - or, as ratchet models teach us: thanks to - the blows of this thermal hurricane.

The other approach originated from an observation that the energy released during ATP hydrolysis is not released in a single act. Instead, the whole process is a set of steps and each of those steps corresponds to a different “molecular situation” of a motor domain. More precisely, every reaction step is associated with a specific conformation of a protein [54, 57, 58, 40]. For example, kinesin-1 head may be strongly or weakly bounded with the microtubule. It can also be unbounded and diffuse, tethered by a neck linker. As a consequence, the whole molecule may be in a two-heads-bound (2HB) state, one-head-bound (1HB) state or it may dissociate from the microtubule, when both heads will unbound.

Before we will give some more details on those two methods, let us invoke a classical textbook example, that would prove its usefulness shortly after.

3.1 Toy Model - Poisson Stepper

Let us imagine a uni-directional molecular motor, which never makes any back-steps, walking along a one dimensional filament [57]. Every step moves its center of mass over a distance of $d$ between neighboring binding sites. If that motor would be kinesin-1 on microtubue, then $d = 8$ nm, approximately.
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The motor starts in the origin of the filament, which implies that the position in time \( t = 0 \), \( x(t = 0) \), is equal to 0. Then it begins to walk, according to a master equation:

\[
\frac{d}{dt} p_n(t) = kp_{n-1}(t) - kp_n(t).
\]

Here \( p_n(t) \) is the probability that the motor occupies \( n \)-th binding site at time \( t \) and \( k \) is a stepping rate, associated with ATP hydrolysis. That means the motor waits for some time after each step, before it can move to the next binding site. From this, the normalization condition and knowing that

\[
\frac{d}{dt} p_0(t) = -kp_0(t),
\]

we may obtain equations of motion for the moments:

\[
\begin{align*}
\langle n(t) \rangle &= kt \\
\langle n^2(t) \rangle &= k^2t^2 + kt.
\end{align*}
\]

The variance\(^1\) linearly increases in time, as \( \sigma_n^2(t) = kt \). For this simple case we can analytically solve the master equation, obtaining the Poisson distribution:

\[
p_n(t) = \frac{(kt)^n}{n!}e^{-kt}.
\]

Such model was among the first that were used to explain experimentally observed behavior of processive cytoskeletal motors \(^8\). Unfortunately, reality quickly turned out to be a little more demanding and new approaches had to be taken to tackle the challenge.

3.2 Ratchet Models

In 1993, less than two months before the data on kinesin’s motility showed up in *Nature*, Magnasco published a seminal paper \(^9\). It started with a statement that “there are situations in which an ambient noise cannot be reduced”. The case of molecular motors operating in the “Brownian domain”, Magnasco argued, is an example of such situation.

Inspired by an idea of Feynman’s “ratchet and pawl”, he then defined a ratchet as a periodic potential with a broken (spatial or dynamical) symmetry and an associated Langevin equation of the form

\[
\dot{x} = f(x) + \xi(t) + F(t).
\]

Here cyclic coordinate \( x \) describes the state of the ratchet, e.g., a position of a particle moving in a force field resulting from the assymetric potential \( f(x) = -\partial V(x) \). Gaussian noise with vanishing mean and satisfying Einstein’s

\(^1\) Variance is obtained from \( \sigma_n^2 = \langle n^2 \rangle - \langle n \rangle^2 \).
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fluctuation-dissipation relation, \( \langle \xi(t)\xi(s) \rangle = 2k_B T \delta(t-s) \), reflects thermal fluctuations and \( F(t) \) is any driving force, stochastic or deterministic.

In the absence of thermal noise, \( x(t) \) may change when a driving will be big enough to overcome the potential barriers. But what Magnasco showed was that slow enough fluctuations of a force around a zero average can also change \( x(t) \). In other words, noise does not necessarily destroy the system, but it can even act as an additional driving.

Magnasco suggested that a similar approach could be used to explain the motion of a molecular motor along a periodic track. Indeed, shortly thereafter Astumian and Bier proposed a model in which a Brownian particle was moving in a periodic potential \[ \xi(t) = 2k_B T \delta(t-s) \]. Even in the absence of the net force, they could obtain a directional flow when the height of the energy barrier was changing in time due to fluctuations.

Rescaling their model to fit the biological reality described by Svoboda et al. in \[ \xi(t) \] and identifying the ATP binding and hydrolysis with the source of fluctuations of the energetic barrier, they could for the first time reproduce the observed kinesin-1 flux and ATP turnover rate with a reasonable accuracy.

After the first successes, other researchers started proposing the modified models. Their efforts were stimulated by the constant progress made by the experimentalists. For example, it became evident that fluctuations in motor displacement result not from the irregular step lengths, but rather from the chemical nature of the driving. The timing of ATP hydrolysis may vary from reaction to reaction and the only thing we know is the average time of such a cycle. This led to the concept of randomness - an experimental observable, defined as a dispersion (Fano factor) obtained in a long-time limit:

\[
r = \lim_{t \to \infty} \frac{\sigma_n^2(t)}{\langle n(t) \rangle}.
\]

For an experiments done with the optical trap, randomness may be introduced in a slightly different way:

\[
r = \frac{\sigma_x^2(t) - 2 < \eta^2 >}{d < x(t) >},
\]

with \( x(t) \) being the displacement of the motor, \( d \) - the step size and \( < \eta^2 > = k_B T / (\kappa_{tr} + \kappa_{mot}) \). Empirical parameters \( \kappa_{tr} \) and \( \kappa_{mot} \) are the stiffnesses of the optical trap and of the bead-microtubule connection, respectively. Randomness of a perfect Poisson stepper would be equal to 1. If the motor would work as a “molecular clock”, moving absolutely regular, it would have a randomness equal to 0. However, for kinesin-1, this measure was close to 0.5 for an unloaded motor and it was increasing with load.

To explain this observation, let us go back to the toy model of a Poisson stepper. In the aforementioned example the stepper was moving from one binding site to the other, without any intermediate steps. But what experiments suggest, this idealization goes too far from what’s really going on. Previously we have assumed that changing position is a one-step process. Staircase-like
trajectories of kinesin-1, with quick 8nm jumps and a long waits in-between, suggest that this assumption is not good enough. ATP hydrolysis consists of several reactions (ATP binding, chemical bond breaking and the release of products). They all take time (flat parts of trajectories), but are crucial for the final act: making of a single step. To capture this, without losing generality and simplicity of the original toy model, we add an additional intermediate step, in which the Poisson stepper is “assimilating” chemical fuel. During this, no change of position is observed.

Let us take \( \alpha \) for the rate of both: assimilation and mechanical motion. If those rates would differ significantly, the model would reduce to a single-step case. Denoting the probability of finding the stepper \textit{without} fuel at position \( n \) at time \( t \) by \( p_n(t) \) and the probability of finding the stepper \textit{assimilating} fuel at position \( n \) at time \( t \) by \( q_n(t) \), we can write down the new master equation:

\[
\frac{d}{dt} p_n(t) = \alpha q_{n-1}(t) - \alpha p_n(t)
\]

\[
\frac{d}{dt} q_n(t) = \alpha p_n(t) - \alpha q_n(t).
\]  

(3.2)

The solution is [51]:

\[
p_n(t) = \frac{(\alpha t)^{2n}}{(2n)!} e^{-\alpha t}
\]

\[
q_n(t) = \frac{(\alpha t)^{2n+1}}{(2n+1)!} e^{-\alpha t}.
\]  

(3.3)

With that, following the same reasoning as in Sec. 3.1, we find that the randomness is equal to 1/2 - close to the one obtained experimentally.

This idea of stepping as a cycle of intermediate steps, chemical and mechanical, led to a complimentary approach to modeling molecular motors.
3.3 Chemomechanical Models

Motor proteins are, in fact, enzymes: they catalyze chemical reactions. For example, kinesin-1, which is an ATPase, binds ATP and accelerates its hydrolysis. Chemical products of this multi-step reaction are inorganic phosphate, $P_i$, ADP and energy, while its mechanical effect is the change in position of the motor’s heads. With every chemical step (i.e. binding of ATP, hydrolysis, $P_i$ and ADP release) the state of the whole enzyme changes. Its both heads may be bound with the microtubule (two-heads-bound conformation, 2HB), or only one (one-head-bound conformation, 1HB), and the binding may be tight or weak [19, 40]. This tight coupling of chemical and mechanical properties drove the development of the so-called chemomechanical cycle models.

Chemomechanical cycle shows motor protein in different states and specifies the possible transitions between them. Every state represents a given chemical and mechanical conformation of a molecule, for example “rear head releasing $P_i$ and un-occupied (empty) binding site on leading head; un-docked neck linker” [4]. It is thus easy to manipulate states and rates and to incorporate new experimental findings. Transition rates come directly from the experiments or at least can be estimated by the methods of chemical kinetics [4, 19, 39]. Some of them depend on reagents’ concentrations or on external force. For example, to see how an external force, $F$, from an optical tweezer will affect motor’s processivity, one can simply modify the rates of force-sensitive transitions of an unloaded (i.e. measured when no force is acting on the system) motor, $k_0$, by a Boltzmann factor to obtain the force-dependent rates $k$, since:

$$k \propto k_0 \exp \left[ \frac{F d}{k_B T} \right],$$  \hspace{1cm} (3.4)$$

where $d$ is the step length.

Chemomechanical cycle can be easily modeled. In this approach motor’s walk along a polymeric track emerges as a Markov process in the chemomechanical state space. Numerical simulations, based predominantly on the Gillespie algorithm [33, 34], are fast and provide basic informations about motor’s velocity, processivity and its response to force variations. Another advantage is that experimental findings can be directly implemented into the model by changing parameters or subsequent steps of the cycle. When one uses ratchet models, every new feature or information needs to be “translated” into the form of a potential. This is both, mathematically and computationally more challenging.

Ratchet and chemomechanical models are the two methods used most frequently for modeling molecular motors [33]. Among others, molecular dynamics should be mentioned. After the crystallographic structure of kinesin was obtained with a good resolution, it was possible to perform computer simulations of the molecule in a Lennard-Jones potential. Despite the fact that molecular dynamics cannot capture the effects of chemical reactions, even the purely

\footnote{Of course, to determine which transitions of the chemomechanical cycle depend on the force and which are insensitive to it, one needs to perform proper experiments [4, 19, 34].}
mechanical properties of, e.g. a tethered diffusion of the free head of a 1HB kinesin proved to be interesting. For example, Hyeon and Onuchic showed how the binding of ATP is regulated by the molecular strain, transmitted via the neck linker [58]. They also combined their approach with chemomechanical modeling and showed all the structural conformations that kinesin takes for every step of the cycle [57]. Researchers from Cross’s group used molecular dynamics to examine the role and significance of electrostatic interactions between the head and the microtubule [58].

As usual, the best models are those which combine different approaches. For example, to capture the link between structure and function and, at the same time, the stochastic nature of the molecular processes, like the diffusion of the unbound head in a 1HB state in search of a microtubule’s binding site, one can construct a model of a chemomechanical ratchet [80]. We advocate for this approach, as discussed in [A3].
Chapter 4

Thermodynamical properties of synchronized (bio-)oscillators

Mesoscopic systems range from molecules to objects measuring micrometers. This is a natural scale for most of the cellular processes: chemical reactions, signal transduction, structural rearrangement, and biosynthesis of macromolecules [7]. In the late 90’s and early 2000’s, with developing novel and more accurate experimental methods, scientists gained access to data about sub-cellular biological systems, like single molecules. It quickly turned out that the then theoretical methods were not sufficient to explain those findings, and help to design new experiments.

4.1 Stochastic thermodynamics - a bridge between thermodynamics and stochastic dynamics

One of the important questions was formulated (and answered) by Ken Sekimoto in his seminal paper “Langevin Equation and Thermodynamics” [1]: how is stochastic dynamics related to thermodynamics? In other words, how to describe energetics of dynamical systems like molecular ratchets, where noise is unavoidable, for which one can formulate a Langevin equation?

The problem was known since the founding works on statistical mechanics by Einstein and Smoluchowski [2,3], but it was Sekimoto who elegantly showed the link between Langevin equation and the first law of thermodynamics. He called his approach “stochastic energetics”.

Another important step was made by Udo Seifert, who noticed that entropy does not necessarily have to be a property of an ensembles of particles. For a
CHAPTER 4. THERMODYNAMICAL PROPERTIES OF SYNCHRONIZED (BIO-)OSCILLATORS

single particle (e.g. a biomolecule or a colloidal particle) unbalanced chemical potentials or external mechanical forces become the source of non-equilibrium dynamics. Overdamped motion in such systems can be described by the stochastic Langevin equation:

\[ \dot{x}(t) = \frac{D}{T} F(x, \{\lambda\}) + \xi(t), \]

(4.1)

where \( D \) represents the diffusion coefficient, \( T \) stands for the temperature of the heat bath (i.e. solutions in which the particle is embedded), \( \{\lambda\} \) is a set of parameters controlling the dynamics and \( \xi(t) \) describes the effect of uncorrelated fluctuations which fulfill the condition \( <\xi(t)\xi(t')>-2D(t-t') \). In Eq. (4.1) the force \( F(x, \{\lambda\}) \) can be composed of both, conservative and “external” drivings. The Smoluchowski-Fokker-Planck equation associated with this dynamics reads:

\[ \partial_t p(x, t) = -\partial_x \left( \frac{D}{T} F(x, \{\lambda\})p(x, t) - D\partial_x p(x, t) \right). \]

(4.2)

As discussed by Seifert \[74\], the exchanged heat \( Q \) and applied work \( W \) can be meaningfully defined at the level of individual trajectories in Langevin dynamics expressed by Eq. (4.1) by noting that \( Q = \int_{t_0}^{t} \dot{x}(t) d\tau \) and \( W = \int F dx \). Accordingly, also the entropy can be identified at this level: the increase of entropy in the medium (the heat dissipated into the bath) is determined by the relation:

\[ \Delta s_r(x(t)) = \frac{Q(x(t))}{T}, \]

(4.3)

whereas a stochastic (trajectory dependent) entropy is ascertainment as \( s(t) = -\ln p(x(t), t) \). By use of the corresponding Smoluchowski-Fokker-Planck equation, the total trajectory-dependent entropy production can be written in the form: \( s_{tot}(t) = \dot{s}(t) + s_r(t) \), thus clearly separating the system entropy from the heat dissipated into the bath.

A version of this approach (for discrete states characterizing the system) is used in publications \[A2\] and \[A4\].

This line of research led to a field which is now called “stochastic thermodynamics” \[75, 76\]. It proved useful for understanding the energetics and thermodynamics of stretching of biopolymers \[74\], particles moving in viscous media \[78, 79\], ATP synthesis and hydrolysis by F1-ATPase \[80\], chemical reaction networks \[74, 81\], and ion channels \[82, 83\], to name only few examples. A promising new field emerged from the application of stochastic thermodynamics to study the flow of information in multi-state systems, like quantum dots \[84\], biosensors \[85\] and other examples of the so-called bipartite systems \[86, 87\].

4.2 Collective phenomena in biological transport

Biology is about coordination and cooperation. The characteristic principle of life - its ability to exist in improbable states - relays on the simultaneous action

\footnote{For the sake of clarity, we are assuming the Boltzmann constant \( k_B = 1 \).}
of dozens of components: metabolic reactions, communication between an organism and the environment, distribution of intracellular goods and resources and so on [5]. From this perspective it may be surprising that it took biologists more than two and a half centuries to seize on Huygens’ concept of synchronization and other collective phenomena [4]. Situation changed after the first publications of Arthur Winfree (a physicist by training) on synchronization of coupled oscillators [9, 10], a concept that was brilliantly explored by Yoshiki Kuramoto [14, 15].

Kuramoto proposed a model which describes the long-term dynamics of any set of $N$ weakly coupled limit-cycle oscillators:

$$\dot{\theta}_i = \omega_i + \sum_{j=1}^{N} \Gamma_{ij} (\theta_j - \theta_i).$$  \hspace{1cm} (4.4)

Here $\theta_i$ denotes the phase of oscillator $i$, $\omega_i$ its natural frequency and $\Gamma_{ij}$ the interaction function. Kuramoto confirmed Winfree’s numerical results by showing that a population of such oscillators can exhibit a kind of phase transition: from an incoherent state, in which each unit keeps its natural frequency, into synchrony, where the whole system, or at least its part, acts like one oscillator.

In the simplest case oscillators are coupled all-to-all (which allows to apply the mean-field approach) and the coupling is sinusoidal:

$$\Gamma_{ij} (\theta_j - \theta_i) = \frac{K}{N} \sin(\theta_j - \theta_i),$$

where $K$ is the non-negative coupling strength.

To visualize the behavior of such system, Kuramoto introduced the so-called complex order parameter:

$$r \exp i\psi = \frac{1}{N} \sum_{j=1}^{N} \exp i\theta_j,$$ \hspace{1cm} (4.5)

where $\psi$ is the average phase and $r$ measures the coherence of the oscillators.

Kuramoto model has been successfully applied to many problems from different fields, ranging from biological and chemical oscillators, through neuroscience, to Josephson junctions and lasers [3, 4, 13, 14, 15].

In [A4] we use it to explain the phase transition between disordered and synchronized states in a set of coupled oscillators, which try to capture some of the features of ion channels connected by an elastic cell membrane. In living cells ion channels do not work independently of one another. Collective behavior has been observed in numerous experiments [13, 16]. Usually it manifests in the change of the opening/closing probabilities of channels when one of their neighbors opens or closes. Although a lot of “hand-waving” arguments is given, the underlying mechanism by which the membrane proteins “communicate” is

\footnote{It should be mentioned that the first person to apply the notion of synchronization to biological problem was Norbert Wiener [9].}
still not obvious. One way of propagating the signal about the given channel’s could be via the membrane. Opened channel changes the structure of nearby lipids in a different way than does the closed one [17, 22]. Hence, membrane deformation can be a realization of a coupling, but only on short distances. Another possibility is the change of ion gradient between both sides of the membrane. With the opening of channels the ions start to flow from and/or inside the cell, changing the transmembrane potential, $V_{\text{mem}}$. The more open channels, the bigger the change in the electric field and the collective response of other channels. The same effect can be also achieved experimentally by applying the external potential [10].

Ion channel clusters, i.e. regions on a membrane where the action of one channel is amplified by the actions of the neighboring ones [101, 104], have been found in many cell types and have been documented to play an important role in signal transduction. For example, specific targeting, clustering and maintenance of $Na^+$ and $K^+$ channels in myelinated nerve fibers are essential to achieve high conduction velocities of action potential propagation [102]. It has been also reported that distributions of receptors in clusters of variable sizes optimize the response to small stimuli and the sensitivity to signal amplitudes [101]. Similar conclusions have been drawn by Shuai and Jung [103] who, in their studies of inositol 1,4,5-triphosphate receptors in the plasma membrane, have shown that channels' clustering can dramatically enhance the cell’s capability of creating a large $Ca^{2+}$ response to weak stimulation. Hopefully, another findings will result in better understanding of this fascinating issue.

Motor proteins also show collective behavior when operating in groups. For them the natural coupling is the cargo they share. It has been shown that teams of dynein motors can generate large persistent forces [28]. Each dynein molecule tunes its step-size to share load in an optimal way. Under high load, team members can all bind to microtubule to resist detachment. This ability to coordinate the action of many dynein motors is important not only for cargo transport, but also for a flagellar motility [103].

Also myosins work “better” (i.e. they achieve higher velocities) in groups than solo [108]. Surprisingly, the data for kinesin is unclear. Some papers, especially theoretical ones, propose models of synchronously operating kinesin motors [12, 109, 110]. But recent experiments show that wild type kinesins do not work cooperatively [28, 108, 111]. This can change after modifying some structural properties of the motor [112].

It is however clear that groups of different motors which move the same cargo may coordinate their action. Bidirectional intracellular transport, during which multiple kinesins and dyneins change turns in moving the cargo in a series of back-and-forth runs, oftentimes interrupted by pauses, starts and changes of direction [113]. This saltatory motion may seem as random, but the result is not accidental. Reinhard Lipowsky proposed a model which he called “tug-of-war” [114], in which the group that at the moment generates larger force wins and moves the cargo in its direction. However this elegant idea has been questioned by the recent experiments [115].

Another example of collective effects that shape active intracellular transport
is the so-called “trafficking”. Every microtubule is a track for numerous groups of molecular motors. The analogy to a highway jammed with cars during rush-hours is a good analogy. Trafficking, together with molecular crowding, is a challenge and may become dangerous when the precious resources will get stuck in a molecular traffic jam [116]. This is why so many control mechanisms are involved to control and coordinate the action of different motors and the motion of different cargos [117].

Studies on collective biological phenomena are complicated and involve the combination of experimental techniques and theoretical modeling. We know so much about single agents - tracks, biomembranes, motors or ion channels - but bringing this knowledge together is not enough to predict the behavior of teams that try their best to achieve the common goal: sustaining life. It is clear that the coming years will bring more fascinating discoveries, but probably also new difficult questions.
Chapter 5

Included papers: summary and comments

This chapter consists of short summaries of 4 articles. In order to anticipate new trends in the research performed by the author so far, a set of open problems has been included at the end of each of them.

5.1 Stepping molecular motor amid Lévy white noise

The first paper presents the behavior of a simple model of two Brownian particles coupled by an elastic linker, which move in a periodic “ratchet” potential with a broken symmetry. We formulate the equations of motion (Eq. (2) in [A1]) for each of the heads separately, but since they are coupled, we choose to investigate the behavior of the center of mass. The heads switch alternately, which means that at a given time only one of them is moving, while the other is “switched off” and immobile. This approach captures some of the characteristics of one-head-bound state of kinesin-1. The crucial point is that this two-headed stepper is influenced by external bursting fluctuations, modeled in the form of symmetric Lévy white noise.

Random motion in cytoplasm is usually attributed to thermal fluctuations, which contribute to the dynamics of objects as irregular forcing occurring randomly in time. The latter are commonly modeled as a Gaussian noise source. However, under the non-equilibrium conditions in which the natural molecular motors operate, surroundings and viscoelastic properties of the cytoplasm are known to be a source of non-thermal noises.

In a beautiful paper published recently in Cell, Guo et al. describe a set of simple experiments which led to spectacular conclusions [3]. They have injected fluorescent beads into the cytoplasm of A7 melanoma cells. Their diameter was larger than the average cytoskeletal mesh size (100 – 500 nm vs 50 nm,
respectively). Thus, beads’ motion reflected the fluctuations of the cytoplasm. Then scientists have observed the random motion of each bead for around 2 minutes. The positions of the beads were measured with high precision (the error was less than 25 nm!), which led to accurate results for a mean square displacement (MSD).

At timescale shorter than 0.1 s, the MSD was nearly constant. At longer timescales, however, MSD was found to depend linearly on time. Results were qualitatively identical for all tested bead sizes. The amplitude of the fluctuations was found to scale with the probe’s diameter, $d$, as $1/d$. Guo et al. noted that such motion could be interpreted as thermal Brownian motion only if the environment would be purely viscous liquid and at thermal equilibrium. But cytoplasm is a viscoelastic medium and the conditions are far from equilibrium.

In the second stage of the experiment, researchers pharmacologically blocked (1) myosin II - a molecular motor which binds actin filaments together and takes part in remodeling the actin cortex - and (2) active processes due to ATP synthesis inhibition. After both interventions (i.e., inactivation of myosin II and ATP depletion) the resulting MSD was significantly different from the control. In the latter case it was almost constant even for a long timescales.

Researchers concluded that the observed motion of a small probe in a cytoplasm “is not a result of thermally-induced fluctuations, but is instead the result of the random forces due to the aggregate motor activity in cells” \cite{1}. The very act of moving the cytoskeletal filaments by molecular motors, such as myosins II, is a source of fluctuating random forces, which propagate in the cytoplasm and affect other objects (i.e., proteins, vesicles or polystyrene probes). Other biologically relevant situations where non-thermal fluctuations were shaping the behavior of moving objects, like proteins diffusing in cell membrane \cite{117} or molecular motors’ cargos \cite{118}, were also reported.

Our paper addresses the problem of maintaining the structural integrity of a motor under the action of impulsive and non-Gaussian fluctuations \cite{A1}. We considered environmental fluctuations modeled by white Lévy noise, i.e. uncorrelated random forcing $\xi(t)$ whose characteristic function reads:

$$\phi(k,t) = \begin{cases} 
\exp \left[-\sigma^\alpha |k|^\alpha \left(1 - i\beta \text{sgn}k \tan \frac{\pi \alpha}{2}\right) + i\mu k\right], & \text{for } \alpha \neq 1, \\
\exp \left[-\sigma |k| \left(1 + i\beta^2 \pi \text{sgn}k \ln |k|\right) + i\mu k\right], & \text{for } \alpha = 1,
\end{cases} \quad (5.1)$$

where $\alpha \in (0,2]$, $\beta \in [-1,1]$, $\sigma \in (0,\infty)$, $\mu \in (-\infty,\infty)$ are parameters characterizing distribution of kicks. In particular, tails of the distribution are characterized by power law asymptotics $|\xi|^{-(1+\alpha)}$ governed by the stability index $\alpha$. For $\beta = 0$, the distribution becomes symmetric with its bulk width scaled with the parameter $\sigma$, cf. \cite{A1}.

As a measure of motor’s performance under different types of noise we have chosen the displacement of the median, $q_{0.5}(t)$, of the midpoint between the positions of two particles coupled with an elastic linker for 1000 independent trajectories. The “group velocity” of the particle “packet” is given by:

$$v(t) = \frac{dq_{0.5}(t)}{dt}.$$
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We highlight possible mechanisms of taming the bursting fluctuations by different modes of the system’s response to perturbations and demonstrate impact of structural modifications of the motor on its transport properties. Those modifications included numerical modifications of walker’s mechanical properties (i.e., linker’s maximal and equilibrium length) as well as coordination between alternating activation and deactivation of both heads (switching time, $\tau$).

The main result presented in the paper [A1] is that the noise controls the motion in a nontrivial way. The group velocity tend to decrease for noise departing from Gaussianity (cf. Fig. 4 in [A1]). But is for non-Gaussian noise that we have observed a feature interesting from a practical point of view: current reversal. Figure 5 shows that for short linkers the directionality of the motor can be reversed by a change of the noise type. That could be used as a control mechanism for artificial molecular motors. We have also found that the optimal turnover times, $\tau$, are different for different types of noise. The general tendency is that the optimal $\tau$, for which the velocity is maximal, is longer for non-Gaussian noises, where bursting fluctuations occur more frequently.

Open problems:

- we know that a probe moving in a visco-elastic medium, like cytoplasm, is subject to non-Gaussian fluctuations. But how to capture their nature? And are they identical in time, or change from one stage of cell’s life to the other?

- we can use noise to control the motion of a molecular motor - but can we modify the type of fluctuations that shape the diffusion in the cytoplasm without disturbing the whole organism?

5.2 Understanding Operating Principles and Processivity of Molecular Motors

The second paper, [A2], presents the same issue - structural and operational principles, that govern the behavior of processive molecular motors - from three different perspectives.

After a general introduction which lists the fundamental features of kinesin-1, we describe an in silico experiment in which two polymeric chains: alanine and kinesin’s neck linker are subject to a stretching force. We wanted to check how their structure - i.e. amino acid composition - affects the elastic properties. Structures of those polymers were previously known from the atomic-level crystallographic pictures and were obtained from the Protein Data Bank (PDB).

We have used Gromacs environment to perform molecular dynamics simulations of stretching of the two chains [119]. The results showed significant difference between the two polymers: while the neck linker was very well following expectations derived from a harmonic spring model, in the case of alanine a notable deviation from this model was observed (cf. Fig. 2 in [A2]). Another result, quite obvious, but often ignored, was that different parts of the same
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polymer had different elastic properties. For example, one of the halves of the
linker was able to stretch to a longer distance than the other. Biopolymers are
not necessarily uniform springs.

The second approach concentrates on comparing the predictions of different
chemomechanical models, which are often used to fit the experimental data [21, 22]. As a comparison we choose the stepping ratio, or the forward-to-backward
steps rate, \( n_f/n_b \). We simulate the models and show that the predicted \( n_f/n_b \)
differs significantly. More serious problem is that these predictions depend
greatly on the proper definition of mechanical transition: an exact sequence of
steps that would be counted as one step. After almost three decades of inten-
sive research prestigious journal still publish papers which give the “universal”,
or “consensus” models of kinesin’s “ultimate” chemomechanical cycle [23, 24].
At the same time, there is a lot of models that are useful in explaining the
experiments, but one still should be careful in trusting them without critical
consideration of other possibilities.

In the last part we discuss the energetics of a system originally proposed by
Żabicki et al. in [24]. It consists of two particles coupled by an elastic linker,
moving in a ratchet potential. The key difference between this model and the
one presented in [A1] is that the motion is driven by the energy coming from an
external depot (cf. Eq. (14) in [A2]). To fully understand the link between the
observed dynamics of the motor and the energy flow, the inertia of the heads
is also taken into account. This approach, different from treating the motion
as over-damped, was developed for analyzing the group of mobile particles with
external energy uptake and storage (depot) [21, 22]. Active Brownian motion
(ABM), as it is called, may be also useful in the case of artificial nano-robots
and the groups of molecular motors [23, 24].

In [A2] we concentrate on the notion of efficiency of a motion of a two-headed
walker. This is a troubling issue, because the motion in mesoscale is so much
different from what we know from macroscale - the scale, for which the very
concept of efficiency was tailored. Because of different reality in which they
are operating and by the fundamental difference between the way in which the
energy is transformed into mechanical work motor protein is not a steam engine.
In the paper we compare three different efficiencies. One of them describes how
well the motor resists the external, opposing force. The second - generalized
efficiency introduced by Derényi et al. in [25] - compares the minimal energy
that is needed for the accomplishment of a given task with the actual energy that
was spent by the system. The third one, so-called Stokes efficiency, proposed
by Wang and Oster in [20] takes into account the viscous drag that the motor
needs to overcome. Depending on the definition, motor’s efficiency may increase
or decrease with an external load, as we compare in Fig. 7 of [A2].

We conclude that only by addressing the problem of intracellular transport
from different angles and using different, yet complimentary, approaches one
can appreciate the beauty and take the challenge of exploring new features of
tiny motors that constantly sustain our functioning and life.
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Open problems:

- how to include mechanical and chemical properties into the ratchet models?
- how to translate experimental data into a ratchet potential? One way is to use specific set of boundary conditions, as we propose in [A3]. The important matter is the applicability of any model to experimentalists, who wish to test their hypotheses quickly and easily.

5.3 “Cargo-mooring” as an operating principle for molecular motors

In the third paper we go beyond the standard approach, which focuses on the motor protein. We start by noting that in the vast majority of experiments the observed object is the cargo carried by the “hidden” motor. It is only during data analysis when one can infer about the behavior of the mechanoenzyme, which molecular properties (stepping pattern, ATP turnover rate and so on) are known. And when the motion is perturbed with an optical tweezer, the external force is directed not on a motor, but on a cargo. Most typical “hand waving” argument for treating the cargo and the motor as one object is that the latter fully controls the first. The force, it is said, is somehow transduced from the cargo via the stalk to motor domains, probably in the form of molecular strain - and so the force of 5 pN applied by a trap can be used to calculate the rates of the load-dependent steps of the chemomechanical cycle.

Although we cannot rule out this possibility, we work out an alternative mechanism of interaction between the motor and its cargo. We call it “cargo-mooring”, since the motor is assumed to hold the vesicle in the proximity of the microtubule, allowing for a tethered thermal motion in a range limited by the elasticity of the protein. This approach is supported by the staircase-like shape of experimentally obtained trajectories, in which cargo spends most of the time wiggling around a certain position. Displacement in a directed, active manner is possible due to rapid “jerks”, which correspond to single steps of the molecular motor that transports the cargo. One needs to realize that this is not the most effective way of pulling an object through a viscous medium. Our brief estimation shows, that the energy released during ATP hydrolysis can be insufficient to drive the directed motion - and yet the motion is an empirical fact. So maybe the motor is not pulling the cargo at all?

We recall that the stalk that connects kinesin heads and cargo has been found to be important for intraprotein communication. “Cargo-mooring” is the first model to account for the stalk’s functionality on the computational level. Its main assumption is that the motor “knows” the position - i.e. if it is tilted to the plus or minus end of the microtubule - of the cargo. We show that the geometry of the cargo-motor-microtubule system allows for such a relative orientation that the motor can make a step without the need of pulling the
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cargo. Then the energy would be utilized only for unbinding the head, but not for pulling the cargo over 8 nm. The observed effect is a change in position of the cargo, and the resulting trajectory has the well-known staircase-like shape.

We analytically derive the force-velocity relation for a kinesin-like motor working under the assumptions of the “cargo-mooring” model - Eqs. (12) and (15) in [A3]. This is the main result presented in the paper. Though our "holistic" approach involves some parameters, like the viscosity of the solution and the size of the cargo, we can still understand and analytically evaluate many of the model’s consequences. All the parameters may be found experimentally.

It is surprising that no-one ever performed a simple in vitro experiment in which the impact of cargo’s size on the processivity of a single kinesin molecule would be studied systematically. We believe that such an experiment would verify our hypothesis.

Open problems:

- is the cargo-mooring a universal model, that explains the motion of any type of cytoskeletal motor protein? Unpublished results for a dynein-like motor were promising, but with a more complex dynamics, for example different possible step length, the model required more assumptions. And what about myosin, that can switch from one actin filament to the other?

- is it possible to model a group of motor proteins that share a cargo? We have approached this problem by noting, that the geometric orientation of motors matters. If one of them is maximally deflected, what is the deflection of the other group members? Can one motor limit the range that the diffusing cargo explores between mechanical steps?

5.4 Phase transitions and entropies for synchronizing oscillators

In the last paper we make a transition from focusing on only one agent - a single kinesin motor or ion channel - to the more physical reality of interacting agents. It starts with a generic model of a cyclic, four-state oscillator with irreversible transitions, coupled with \( N - 1 \) other identical units. Coupling modifies transition rates from state \( i \) to \( i + 1 \), such that:

\[
k_{i \rightarrow i+1} = k_0 \exp[\alpha(p_{i+1} - p_i - 1)].
\]  

(5.2)

Here \( \alpha \geq 0 \) represents the coupling strength, \( k_0 \) is a positive constant and \( p_i = n_i/N \) is the probability of finding a population of \( n_i \) oscillators in state \( i \) at a given moment.

One of the interesting features of this system is the competition between diffusion and synchronization. Coupling modifies the rates in such a way that it is possible that the populations in two neighboring states will get significantly bigger than in the other two, forming a “high probability pulse”. Diffusion of the oscillators to the less populated states is counteracted by the synchronized pull
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towards the pulse (cf. Fig. 2 in [A4]). This situation is similar to the well-known Burgers’ shock [27].

We show a simple way to calculate the amplitude of the pulse, \( \eta \), as a function of coupling strength, \( \alpha \):

\[
\eta \approx \sqrt{\frac{3}{2}(\alpha - 2)}.
\]

At \( \alpha = 2 \) there is a phase transition from a disordered set of independent oscillators to a synchronized state. Stochastic and deterministic numerical simulations of the system prove this prediction.

Next we consider the entropy production associated with the formation of a pulse. When the probabilities for all states are no longer equal, the moving pulse is associated with a net production of entropy. This is a manifestation of a breaking of symmetry and a self-organization of a higher entropy disordered system into lower entropy synchronized one.

With increasing number of states from 4 to \( n \), one can build a more general, continuous model. The resulting equation, describing the propagation of a robust synchronized pulse traveling around the cyclic setup of \( n \) states, is an analog of the so-called potential Burgers’ equation [28]. It is worth noting that in the continuous case the phase transition easily found for the four-state model does not exist. The amplitude of a propagating front is no longer related to the coupling strength, \( \alpha \). We recall that when a classical model of synchronized oscillators proposed by Kuramoto is taken to its continuum limit, the phase transition from disordered to ordered state remains present. But in this case the coupling is still global (all-to-all), while for our model, expressed in the form of Burgers’ equation, it becomes more and more local (i.e., transition rates are affected only by the population in two neighboring states). However, even in the vicinity of phase transition our \( n \)-state system still produces entropy.

The paper closes with a discussion of the possible application of our findings in a more physical setup, like a population of ion channels coupled by a cell membrane. It has been shown that by changing its state (i.e., by opening or closing), ion channel affects other membrane proteins, which become more likely to change their state [24, 27]. The question about the possibility of phase transition and synchronized opening and closing of groups of channels remains open.

Open problems:

- do molecular motors work cooperatively in the same way as synchronizing ion channels, fireflies or heart’s pacemaker cells?
- how does the efficiency of synchronized objects change with respect to the efficiency of a single object?

5.5 Final remarks

Transport of resources, whether by the cytoskeletal motor proteins or through the ion channels, is a complicated process that needs to be constantly sustained
and regulated by every living cell. It depends on different agents, that need to cooperate in the pursuit of the ultimate goal: cell’s proper functioning.

In the described papers we have tried to address some of the open questions:

- what are the cons and pros of different methods used in modeling ([A1]) and is it possible to combine them in such a way that the model is still reliable and practically useful ([A1] and [A3])?

- how the environment affects the transport and how can the random fluctuations be used in engineering artificial molecular walkers ([A2])?

- how does the energetics of a coupled system of transporters change when they all start working as one team ([A4])?

Despite almost three decades of intense research in the field these questions still are a subject of an intense debate in the community. I am privileged to complete this thesis shortly after the significance of some of those problems has been recognized by the Nobel prize committee. In 2016 Jean-Pierre Sauvage, J. Fraser Stoddart and Bernard L. Feringa were awarded the Nobel Prize in Chemistry for the design and synthesis of molecular machines. We may only hope that the most spectacular discoveries are yet to come.
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Stepping molecular motor amid Lévy white noise

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We consider a model of a stepping molecular motor consisting of two connected heads. Directional motion of the stepper takes place along a one-dimensional track. Each head is subject to a periodic potential without spatial reflection symmetry. When the potential for one head is switched on, it is switched off for the other head. Additionally, the system is subject to the influence of symmetric, white Lévy noise that mimics the action of external random forcing. The stepper exhibits motion with a preferred direction which is examined by analyzing the median of the displacement of a midpoint between the positions of the two heads. We study the modified dynamics of the stepper by numerical simulations. We find flux reversals as noise parameters are changed. Speed and direction appear to very sensitively depend on characteristics of the noise.

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I. INTRODUCTION

Since the late 1980s it has become possible to follow and manipulate moving motor proteins at a molecular level [1]. More recently, such motor proteins have become the moving parts in actual nanotransport machines [2]. In eukaryotic cells the motor protein kinesin is responsible for the transport of organelles and vesicles filled with chemicals. This motor protein literally walks along a biopolymer called “microtubule” as it is fueled by the conversion of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate. The microtubule filaments are periodic structures of a distinct polarity, different at each end.

In a minimal model it is possible to reconstruct the motion of a motor protein on a biopolymer as just the motion of a Brownian particle on an array of dipoles. In such a minimal model the ATP hydrolysis causes the motor to have a fluctuating charge and/or charge distribution. If the array of dipoles is anisotropic, then these nonequilibrium fluctuations will make the motor drift through a “ratchet” mechanism [3–11]. Typically, one assumes that the overall force acting on the particle is a superposition of the Gaussian thermal noise with another periodic or stochastic force due to the ATP hydrolysis [9]. In those cases all moments of the distribution of the noisy force exist, and the existence of moments of the distribution of the particle’s velocity is guaranteed.

However, as it has been documented in numerical and analytical studies [12–14], the minimal setup for directed transport can also be obtained when taking just the periodic anisotropic potential (see Fig. 1) with added symmetric Lévy noises. Here the term Lévy noise, $L(t)$, is used to denote a natural extension of a standard (Gaussian) Brownian-Wiener process [15–17], which includes (a) a general family of stochastic processes with stationary independent increments whose (b) probability distribution belongs to the class of infinitely divisible distributions and satisfies the stability (self-similarity) criterion, i.e., $L(\sigma t) \sim \sigma^\alpha L(t)$ with the stability exponent $\alpha \in (0, 2]$. The latter property reflects invariance of the probability density of a random variable under convolution and can be easily rephrased in terms of the Fourier transform of the corresponding probability density function. In analogy to the white Gaussian noise, which is formally represented as the time derivative of the Wiener process, the general Lévy white noise can be defined as the time derivative of the symmetric Lévy process, $\xi(t) = L(t)$. Asymptotic (tail) properties of the probability distribution function (PDF) of the increments $\Delta L(t) = L(t + h) - L(t)$ are governed by the stability index $\alpha$, i.e., $p(l) \sim 1/(|l|^{\alpha+1})$. Unlike Gaussian noises, Lévy processes may contain random jump discontinuities of arbitrary size (loosely, the intensity measure of its Poissonian jumps [14] is proportional to $|l|^{-\alpha-1}dl$) and therefore are well adapted to account for pulsatory or discrete behavior of natural signals.

It is now known that Lévy noise is useful in understanding the behavior of many biological systems, like bacteria [18],...
FIG. 1. (Color online) Model system: The heads are subject to an external potential (a) from the filamentous track (e.g., microtubule) and an internal potential (b) due to sterical interactions within the motor. The internal potential is the sum of a harmonic and a Lennard-Jones potential [cf. Eqs. (2) and (3)]. In the absence of a constraint on the distance $|y - x|$ and in the presence of “bursting fluctuations,” the two heads can be pulled apart to an unphysically large distance, i.e., a catastrophe as shown in (c) can occur. Parameter values are $a = 0.25$, $\epsilon = 0.1$, $\tau = 5$, $k = 0.8$, $L = 1$, $dt = 0.01$.

predatory fish [19], spider monkeys [20], or human beings [21]. But it came as a great surprise that Brownian diffusion—until recently a model of choice for describing any subcellular processes—was unable to explain a huge amount of experimental data on intracellular motion. Anomalous diffusion, i.e., an unusual nonlinear time dependence of the mean-square displacement (MSD) accompanied by an anomalous scaling behavior [22–25], has become a commonly observed phenomenon in intracellular transport [26–30].

In cell biology, molecular diffusion is recognized as a main form of transport within the cells. Random motion in cytoplasm is usually attributed to thermal fluctuations, which contribute to the dynamics of objects as irregular forcing occurring randomly in time. The latter are commonly modeled as a Gaussian noise source. In a Gaussian distribution the tails of the probability density fall off exponentially and the MSD in the passive motion grows linearly with time.

In contrast to the aforementioned canonical Brownian ratchet powered by Gaussian white noise, we here investigate transport under the action of white Lévy noise. The “heavy” or “fat” tail of the distribution of Lévy noise amplitudes means that large jumps occur more likely than for the Gaussian case. As a consequence, we face a challenge when trying to characterize the ensuing directed motion on the ratchet. This is because the statistical moments of the examined flux of particles can be divergent. In particular, for $\alpha \in (0, 1)$ neither the dispersion nor even the mean of the corresponding displacements exist. Therefore, the statistical analysis of the induced flux is no longer possible in terms of standard notions such as mean velocity, standard deviation, and Péclet number [31–33]. New measures to characterize the transport must be introduced [12, 13, 34].

The $\alpha$-stable Lévy noise is associated with systems that are out of thermal equilibrium, and it can occur when conditions leading to the standard fluctuation-dissipation theorem are violated [35–38]. In particular, the Lévy-type statistics is observed in various scientific areas where scale-invariant phenomena take place or can be suspected (for a recent short review see Ref. [16] and references therein). This statistics allows one to describe real situations in which the evolution shows abrupt jumps, called Lévy flights.

The interplay of deterministic dynamics and perturbative Lévy-type noises has been addressed in the literature in various scenarios, including several noise-induced effects like resonant activation [36, 37], stochastic resonance [39, 40], noise-enhanced stability in Josephson junctions [37], and dynamical hysteresis [39, 41]. It has also been studied in the context of population dynamics [40, 42], escape from bounded intervals [38, 43], barrier crossing problems [44–47], stationary distributions and steady states in confining potentials [48–51]. Also, noise-induced directed motion in spatially periodic potentials has been investigated, although mostly in the presence of Gaussian noise [3, 4, 7]. In contrast, relatively few research efforts [12, 13, 34, 52, 53] have been undertaken

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to understand motion in a ratchet potential under the action of jumpy Lévy fluctuations.

On the other hand, increasing experimental evidence has been gathered over the last few years documenting that particles in a living cell perform not only thermal diffusion [23,24,26,27] and significant understanding of the nonequilibrium processes that underlie anomalous diffusion has been gained. It has become possible by developing new microscopy techniques which allow us to follow and identify [54,55] the responsible random forces. According to those studies the cytoskeleton appears to be a very dynamic viscoelastic structure [56] that is subject to a lot of mechanical activity through, for instance, the motor proteins that are connected to it. This nonequilibrium activity has been found to generate random fluctuating forces large enough to literally “stir” the cytoplasm [54,57]. The use of Lévy noise in motor protein models is then warranted by the ample evidence of relatively frequent large fluctuations. Their presence, as previously observed, is in fact a signature of Lévy noise.

Here we present a study on a two-headed motor model subject to action of the external Lévy random forcing. We start out, in Sec. I, with the characterization of the noise and formulation of a basic ratchet model. In Sec. II we describe the systems response to external forces. Summary, conclusions, and a discussion of our results are the contents of Sec. III.

II. METHODS

We let our Lévy noise [15,58,59] consist of subsequent random kicks $\xi(t)$. We restrict ourselves to a case of symmetric stable noises, $L_\alpha(t) = \int_0^t \xi(t')dt'$, where for the characteristic function we have

$$\phi(k,t) = \exp[i\int_0^t \xi(t')dt'] = \exp[-\sigma^2|k|^\alpha t].$$

Here the parameter $\alpha$ ($\alpha \in (0,2]$) denotes the stability index of the distribution whose “fat” power-law tails of the PDF are characterized by $|\xi|^{-1+\alpha}$ asymptotics. The parameter $\sigma$ stands for a scale parameter that measures the intensity of the noise. For the special case $\alpha = 2$, the Gaussian noise is retrieved with $\sigma^2$ representing the variance of the corresponding fluctuations. In what follows we will use $\alpha \in [0,1,0.5]$, i.e., we consider symmetric, strictly stable distributions for different scale parameters $\sigma$ [60]. We used the Weron algorithm [61] to generate the Lévy distributed variables in our simulations. The time step in all the simulations is set to $dt = 0.01$.

A. The model

We consider the following overdamped Langevin system:

$$\frac{dx}{dt} = -\xi_1(t) V(x) + k (r - a) + U_{LJ}(r) + \xi_1(t)$$

$$\frac{dy}{dt} = -\xi_2(t) V(y) - k (r - a) - U_{LJ}(r) + \xi_2(t).$$

(2)

The coordinates $x$ and $y$ represent the positions of the two heads of the motor and $r = |x - y|$ stands for the distance between them. The two heads are coupled by a harmonic spring of a natural length $a$ and an elasticity constant $k$. The functions $\xi_i(t)$, where $i = 1,2$, are the independent random forces modeled by white Lévy noises. As was explained in the Introduction, this non-Gaussian white noises can be thought of as a result of nonequilibrium activity in a viscoelastic cytoskeleton. In order to prevent the two heads from overlapping their positions, we introduce a Lennard-Jones potential as in Refs. [62,63],

$$U_{LJ}(r) = 4e\left[\left(\frac{s}{r}\right)^{12} - \left(\frac{s}{r}\right)^6\right],$$

(3)

which becomes strongly repulsive when the heads are too close. Here $s = 2^{-1/a}a$, where $a$ is the location of the minimum of $U_{LJ}(r)$. The alternating action of the heads is represented by a dichotomous variable $z(t)$, as proposed formerly by Dan et al. [63],

$$z_i(t) = \begin{cases} 0 & \text{for } 0 \leq t < \tau/2 \\ 1 & \text{for } \tau/2 \leq t < \tau. \end{cases}$$

(4)

Here $i = 1,2$ denotes the two heads and $\tau$ is the period of the periodic functions $z_i(t)$. The variables $z_1$ and $z_2$ are in antiphase: If one of the heads is active (e.g., $z_1 = 1$), then the other one is turned off (e.g., $z_2 = 0$). The two heads of the motor interact with the underlying structure of the track (reminiscent of a microtubular trail for kinesins) via a “ratchet” potential $V(x)$ [see Fig. 1(a)]

$$V(x) = \frac{1}{2\pi} \left[\sin(\frac{2\pi x}{L}) + \frac{1}{4} \sin(\frac{4\pi x}{L})\right].$$

(5)

In Eq. (5) we set the period $L = 1$. It is easily seen that this potential is anisotropic: Going from left to right the slope is characterized by a steeper increase and a slower decrease. According to Curie’s principle [64], net directed flux in a particular direction can only occur if there is a symmetry-breaking feature in the setup. In our case that symmetry-breaking feature is the anisotropy of the potential $V(x)$.

The motor protein heads take turns in being attached to the track. In Eqs. (2) this is represented by $V(x)$ and $V(y)$, which alternately take the form of Eq. (5). When a head is unattached, the potential is zero. This switch between heads thus involves an energy difference $\Delta E = V(x) - V(y)$. Had the system been at equilibrium, $\Delta E$ would have determined the ratio of the switching rates. In that case there would have been a Boltzmann relation:

$$\frac{\gamma(x \rightarrow y)}{\gamma(y \rightarrow x)} = \exp[\Delta E/k_BT]$$

(6)

between the switching rates $\gamma$. Here the rate $\gamma(x \rightarrow y)$ is for the transition where the $y$ head attaches and the $x$ head detaches. The rate $\gamma(y \rightarrow x)$ is for the transition where the $x$ head attaches and the $y$ head detaches. Furthermore, $k_B$ is Boltzmann’s constant and $T$ represents the absolute temperature. Note that the harmonic potential $\frac{1}{2}k(r-a)^2$ and the Lennard-Jones potential $U_{LJ}(r)$ do not affect Eq. (6) as these energies depend on $r = |x - y|$ and do not change when a switch occurs. Equation (6) describes the detailed balance that we ought to have at thermal equilibrium. However, in a processive motor protein the attachment-detachment cycle is coupled to ATP hydrolysis. The role of ATP hydrolysis is to break detailed balance and bring the system from Eq. (6) to a situation where the forced switching of Eq. (4) applies.
Equation (4) is a good approximation if the energy that is released in the ATP hydrolysis is significantly larger than $\Delta E$ [65]. It is valid if the ATP hydrolysis “overwhelms” the $\Delta E$ that rules the equilibrium behavior.

The forced switching of Eq. (4) would have been the only energy input into the system, if the noise, $\zeta(t)$ [cf. Eqs. (2)], had been thermal, equilibrium noise. In that case, ATP hydrolysis would be 100% accountable for the net motion of the motor. A nonthermal $\zeta(t)$ is a complication since it represents another energy input into the system. Below we will examine the effects of such a $\zeta(t)$, adhering, however, to the situation when the increments of random forces $\Delta \zeta(t)$ are stationary and statistically independent (i.e., $\zeta$ has a character of white noise perturbing the system’s dynamics). It should be noted that this approach stands in a clear difference to the treatment of white noise perturbing the system’s dynamics. It should be noted that this approach stands in a clear difference to the treatment of white noise perturbing the system’s dynamics. It should be noted that this approach stands in a clear difference to the treatment of white noise perturbing the system’s dynamics. It should be noted that this approach stands in a clear difference to the treatment of white noise perturbing the system’s dynamics.

Initially, the two heads of the motor are located so that the distance between them is equal to $a$. For time $t > 0$, the distributions of the positions $x, y$ evolve due to the presence of the stochastic forces and the deterministic potentials. As was mentioned before, the mean may diverge when Lévy noise is involved. We therefore characterize the motion of the center of mass of the heads, i.e., $X_n(t) = [x(t) + y(t)]/2$, with the median $q_{0.5}$, defined as $\text{Prob}[X_n(t) \leq q_{0.5}(t)] = 1/2$. Accordingly, the time derivative of the median,

$$v(t) = \frac{dq_{0.5}(t)}{dt},$$

may serve as an estimate of the group velocity of the particle “packet” [12,13,34]. With $v(t)$ we have a quantity that allows us to characterize ensemble transport even in case of unbounded average currents.

III. STRUCTURAL AND FUNCTIONAL CUTOFFS OF THE FLUCTUATIONS

A. Response to bursting fluctuations

With decreasing $\alpha$, the probability of longer jumps increases. Consequently, a “bursting fluctuation” may occur that pulls the coupled heads apart over a very long distance [see Fig. 1(c)]. The model is supposed to describe a prototypical nanomotor and it is obvious that constraints must exist to secure the motor’s integrity and function.

The variables $x$ and $y$ [cf. Eq. (2)] represent the positions of the two heads of the motor protein. It is the relative distance $|y - x|$ that has to be limited in course of the action. In order to prevent a “rupture,” as depicted in Fig. 1(c), a response to catastrophic fluctuations has to be imposed in the model.

The elastic properties of a linker structure in typical molecular motors, like kinesins, have been formerly addressed in experimental studies and molecular dynamics simulations using GROMACS software [68,69]. Based on accumulated evidence, it is unlikely that a molecular machine would respond instantaneously to an elongation with an elastic counteraction that resets the linker structure to its equilibrium conformation of length $a$ or shorter (see Fig. 2).

B. The effect of noise intensity on the motor’s velocity

We have checked how changing the noise intensity $\sigma$ affects the motor’s velocity. Figure 4 shows the results of the simulations. The maximal group velocity of the independent motors notably drops for noise departing from Gaussianity. However, the higher the stability index $\alpha$ of the noise, the lower the intensity $\sigma$ for which the velocity reaches its maximum value. Also, for $\alpha = 1.5$ the peak around the maximum is significantly broader than for $\alpha = 2.0$, thus reflecting that the maximum velocity range is wider for lower $\alpha$. This could be interesting when we use noise as a possible control mechanism for an artificial molecular motor.

C. Varying the inner structure

As was pointed out in the Introduction, motor proteins carry cargo, like organelles and vesicles with chemicals, from the cell’s interior to its periphery. Speedy delivery is important for the cell’s survival and it is likely that speed has been optimized in the course of evolution. The linker length $d_{\text{max}}$ is a variable that is subject to natural selection and likely to have been subject to such optimization.

FIG. 2. (Color online) A sketch illustrating the model for the system’s response to bursting fluctuations. If there were no constraint, the leading head would have been in the dotted position. For explanation, see the main text.

Following these observations, we simply “freeze” the distance between the heads when it reaches $|y - x| = d_{\text{max}}$, i.e., we leave it at $|y - x| = d_{\text{max}}$ until a next iteration reduces the distance $|y - x|$ again. Simulations following this scenario are shown in Figs. 3(a) and 3(b).

Parameters of our model can be categorized as those describing the inner structure and mechanics of the molecular motor and those associated with its chemical activity. Processive motility of molecular motors like kinesins depends on the mechanical transmission of stress through a neck linker which connects the two heads of the molecule. This tension-transmitting element is an unstructured protein segment. In case of kinesin it is 30 amino acids long. It is a simple flexible polypeptide polymer and it is responsible for the internal potential in our model [1,68–70]. In particular, the equilibrium length $a$ and elasticity constant $k$ can be seen as characteristics of a neck linker polymer. The distance $d_{\text{max}}$ represents that maximum allowable extension of the neck linker. The oscillations $z_i(t)$ [cf. Eq. (4)] are associated with the chemical activity of the motor protein. The mechanical stepping cycle is coupled to the hydrolysis of ATP. The parameter $\tau$ [cf. Eq. (4)] controls the time for the pertinent conformational changes to occur.
Figure 5 shows the results of simulations in which the velocity of the median \( q_{0.5} \) was determined for different values of \( d_{\text{max}} \). We took 1000 independent motors acting under the constraints of the model and recorded their velocity in the course of 100 time units.

In line with findings discussed in Secs. IIIA and IIIB there is a clear nonmonotonic relation between the velocity of the stepper and the maximum linker length \( d_{\text{max}} \). With an increasing linker length, the group velocity drops rapidly, assumes a minimum, and eventually tends to zero. For an equilibrium linker length \( a = 0.25 \) (left panel of Fig. 5) and for Gaussian noise, i.e., stability index \( \alpha = 2 \), the induced current does not change direction as \( d_{\text{max}} \) is varied. In this case derived velocities of the median remain positive within the domain of change of \( d_{\text{max}} \in (0,8) \). In contrast, for approximately twice shorter length \( a = 0.1 \) (right panel of Fig. 5) the current becomes inverted in the negative direction (cf. Fig. 6).

What is most remarkable about Fig. 5 is that it shows the possibility of current reversal when a parameter describing noise or internal structure is changed. At small values of \( d_{\text{max}} \), the directionality can be reversed with a mere change of the stability index \( \alpha \). On the other hand, for transport subject to non-Gaussian noises of \( \alpha = 1.5 \) and \( \alpha = 1.8 \), the current can reverse its direction as the maximum elongation of the linker \( d_{\text{max}} \) is varied. This is a current inversion induced by non-Gaussian Lévy noise, which is akin to that found with non-Gaussian colored noise [71,72]. It is furthermore observed that at \( a = 0.1 \) (right panel of Fig. 5) the transport is slowed down and eventually stopped at increasing linker length \( d_{\text{max}} \). For a longer equilibrium distance between the heads, \( a = 0.5 \), the curves are similar to those observed for \( a = 0.25 \), although velocities are significantly smaller (data not shown).

Altogether, described currents \( v(t) = dq_{0.5}(t)/dt \) are higher for motors subject to Gaussian noises (see Fig. 6) than for steppers influenced by impulsive Lévy fluctuations. The movement depends also on allowable extension of the linker with respect to its equilibrium elongation and becomes suppressed at excessive extensions.

**D. Varying the enzymatic rate**

To examine the impact of the chemical activity on the motor’s velocity, we have simulated the motion of 1000 independent walkers under different noise parameters and for different values of \( \tau \). We have next analyzed the displacement of the median and calculated the group velocity.

In Fig. 7 the normalized velocity as a function of the period \( \tau \) for different values of the stability index \( \alpha = 1.5,1.8,2 \) is displayed. To estimate the group velocity, the maximum reached by the median \( q_{0.5} \) in course of 100 steps has been divided by the time in which it has been achieved. Furthermore, for each value of the index \( \alpha \) separately, the derived velocity has been normalized with respect to its maximum value. Such
FIG. 5. (Color online) Velocity of the median $q_{0.5}$ of 1000 independent walkers after 100 time units as a function of $d_{\text{max}}$ for different values of the stability index $\alpha = 1.5, 1.8, 2.0$ and for different lengths of the fully relaxed linker: $a = 0.25$ (a) and $a = 0.1$ (b). The variable $d_{\text{max}}$ represents the maximum allowable distance $|y - x|$ between the heads. The parameter values are $\epsilon = 0.1, \tau = 5, k = 0.8, dt = 0.01$, and $\sigma = 0.5$. The duration of the individual motor trajectories has been set to $t = 100$. Lines are drawn to guide the eye.

FIG. 6. (Color online) Trajectories of the median $q_{0.5}$ of 1000 independent walkers. Graphs have been presented for different values of the linker extension $d_{\text{max}}$ close to and beyond the minimum depicted in Fig. 5. Top (bottom) panels refer to linkers characterized by natural (relaxed) lengths $a = 0.25$ ($a = 0.1$). Stability index is $\alpha = 2.0$ and $\alpha = 1.5$ for left and right panels, respectively.

The analysis shows (cf. Fig. 7) a clear maximum in the normalized velocity as a function of $\tau$, thus indicating an optimal value for the chemical activity rate. As it has been discussed in the existing literature [2,28], fast intracellular transport is important for a living cell and in the course of evolution the motor protein is likely to have sought out a maximum, similar to the one depicted in Fig. 7. Apparently, the location of the maximum is very sensitive to the noise type: As it can be deduced from Fig. 7, for smaller values of the stability index $\alpha$, i.e., when more bursting fluctuations act on the system, the
 FIG. 7. (Color online) The velocity of the median \( q_{0.5} \) of 1000 independent walkers as a function of the period \( \tau \), for different values of the stability index \( \alpha \), namely \( \alpha = 1.5, 1.8, 2 \). The parameter values are \( \alpha = 0.25, \epsilon = 0.1, d_{\text{max}} = 0.5, k = 0.8, \sigma = 0.5, \) and \( dt = 0.01 \). Lines, obtained by curve fitting, are drawn to guide the eye.

optimum value of the velocity shifts towards longer turnover times \( \tau \).

IV. DISCUSSION AND CONCLUSIONS

In this paper we have considered a simple model for a stepping motor protein acting under the influence of white stable noises. The model contains parameters that represent the structure and the chemical activity of the motor system. Structural features are denoted by the parameters \( k, a, \) and \( d_{\text{max}} \) of the system and have been preliminary analyzed in different dynamic responses, as proposed in Sec. III A. In order to study noise-induced flux, we have focused on the dynamic response of the model (cf. Fig. 2).

It is further shown that noise characteristics and motor parameters control the speed and even the direction of the stepper motion in a nontrivial way. Our results can be significant for understanding of the evolution of motor proteins and for the design of artificial molecular machines.

Dimers, like kinesin, are commonly described by a system of two coupled equations like Eq. (2). The coupling between \( x \) and \( y \) in Eq. (2) should be a reflection of the structure of a real protein. For kinesin the two heads are linked through a polymeric chain that is commonly known as the neck linker. We can examine the model for different equilibrium lengths \( a \) and for different maximum lengths \( d_{\text{max}} \) of the fully extended linker. There is an optimal equilibrium length \( a \) for which the processivity and speed of the motor are maximized. In the case of our motors, that optimal length is around \( a = 0.25 \). A similar result has been reported for Gaussian noise [62].

Results presented in Figs. 4 and 5 document a rich scenario of behaviors emerging when Gaussian noise is replaced by Lévy noise with \( \alpha < 2 \). Specifically, in Fig. 5 current reversals are shown to occur when noise parameters are changed. Additionally, for motors working under the action of impulsive Lévy noises, multiple current reversals are observed when the structure parameter \( d_{\text{max}} \) is varied.

The parameters \( d_{\text{max}} \) and \( a \) characterize the elastic properties of the linker that connects the two heads of the motor. The induced current appears to depend very sensitively on \( d_{\text{max}} \) and \( a \). This suggests the possibility of a control mechanism through the linker. Experimental results on natural kinesin motors appear consistent with this idea [70].

In a changing environment such as a living cell, it is possible to imagine that noise parameters, such as \( \alpha \), may differ for different metabolic stages. This leads to an interesting possibility: by changing \( \alpha \) in the course of development, the direction of motor motion may reverse. Natural cytoskeletal molecular motors, like dynein, kinesin, or myosin, walk one way without ever changing direction. However, there are mutants that switch the direction of their motion stochastically [73]. Noise as a possible control mechanism for the motor’s flow may also be an important feature for those trying to synthesize artificial molecular motors.

We have shown how motor motion, as described by Eq. (2), depends on chemical properties. Figure 7 shows that there is an optimal time after which the heads should interchange their activity. This value depends on the noise characteristics—the closer the noise is to Gaussian, the faster the reactions should be.

After 20 years of intensive research in the field of molecular transport, we know a lot about the structure of molecular motors [1]. We also know a lot about rates, speeds, step sizes, and load-velocity characteristics [2,4,28,31,69,73]. Yet we have only a limited knowledge of how molecular motors work, especially under the action of nonequilibrium, non-Gaussian noises. The ultimate goal of studies like ours is therefore to find and understand relations between motor structure and function. Such insight would be invaluable in, for instance, drug design or constructing artificial molecular motors.

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[58] Other choices in the ranges $\sigma \in [0,1]$ and $\alpha \in [0,2]$ have also been pursued in our numerical studies. For the purpose of this presentation we have chosen only the set $\sigma = [0,1,0.5]$ and $\alpha = [1.5,1.8,2.0]$, which yield the most significant qualitative differences.

UNDERSTANDING OPERATING PRINCIPLES AND PROCESSIVITY OF MOLECULAR MOTORS*

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Motor proteins, sometimes referred to as *mechanoenzymes*, are a group of proteins that maintain a large part of intracellular motion. Being *enzymes*, they undergo chemical reactions leading to energy conversion and changes of their conformation. Being *mechanodevices*, they use the chemical energy to perform mechanical work, leading to the phenomena of motion. Over the past 20 years a series of novel experiments (*e.g.* single molecule observations) has been performed to gain the deeper knowledge about chemical states of molecular motors as well as their dynamics in the presence or absence of an external force. At the same time, many theoretical models have been proposed, offering various insights into the nano-world dynamics. They can be divided into three main categories: mechanochemical models, ratchet models and molecular dynamics simulations. We demonstrate that by combining those complementary approaches a deeper understanding of the dynamics and chemistry of the motor proteins can be achieved. As a working example, we choose kinesin — a motor protein responsible for directed transport of organelles and vesicles along microtubule tracts.

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1. Introduction

Problem of transport is fundamental for understanding efficient functioning of complex systems. Regardless of whether one studies social interactions among ants, networks of municipal delivery of goods, coupled systems of electrical conductors or “machinery” of living cells, a comprehensive insight into their structure and operation properties can be gained only by unraveling the dynamical features of a system under consideration.

For modern cell biology and biotechnology, understanding how cells maintain and control their inner environment is one of the most fundamental goals. For physicists, chemists and nano-engineers studying the most optimal and evolution-optimized bio-machines creates an unique way for conceptual advancement of design, fabrication and manipulation of synthetic nano-devices, such as lab on a chip or dedicated nano-robots [1, 2].

Chemical models of molecular motors focus on the Markov chain, kinetic description of the reaction cycles responsible for the mechanical transitions. So-called ratchet models are mostly based on sets of Langevin equations and treat the kinesin dimer as two linked Brownian particles moving in a periodic potential. Molecular dynamics (MD) models approach the problem from the low level dynamics of single or grouped molecules, based on information obtained from crystallographical data.

In this work, we briefly review results of our studies aimed to understand processivity of a molecular motor by combining results of simulations of a statistical mechanochemical device with a ratchet model and molecular dynamics investigations of an elastic motor structure.

1.1. Molecular motors

Molecular motors are a class of highly specialized molecules, present in both prokaryotic and eukaryotic cells. Their role is to convert energy into mechanical work. It is this unique feature that makes them invaluable for essential biological processes such as coordinated intracellular transport, muscle contraction, transcription, mitosis and ATP$^1$ synthesis, to name only few. The source of energy they use, as well as their function, differ between distinct classes of motors. Here, we focus on an eukaryotic cytoskeletal motor protein, kinesin-1 (so-called conventional kinesin).

Kinesins take care of internal transport needs of a cell: they carry liposomes containing various substances (e.g. neurotransmitters) and also organelles (e.g. mitochondria). They do so by literally walking on the biopolymer microtubule, an important part of the cytoskeleton, forming a molecular tract. Microtubule is a polymeric tube, consisting of alternately arranged $\alpha$- and $\beta$-tubulin dimers [3]. The whole structure is polarized: the so-called minus end of the tube, associated with the structure called microtubule-organizing center (MTOC) and localized most often near the nucleus, is stable while the second, called plus end is labile and may be assembled or disassembled, depending on the cell’s needs. This variability of the plus end is called dynamic instability [4]. Single kinesin-1 molecule (see Fig. 1) consists of two motor domains (heads), binding to the microtubule, a stalk

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$^1$ Adenosine-5’-triphosphate.
responsible for attaching the cargo and elastic neck linkers, connecting the heads with the stalk. Heads are those parts of the protein, where the production of the source of energy to the motor (catalyzed ATP hydrolysis) takes place.

Fig. 1. Schematic representation of a kinesin-1 motor protein. It consists of two motor domains (heads), binding to the microtubule; a stalk responsible for attaching a cargo and neck linkers — polypeptides containing over a dozen of amino acids each, connecting both heads with the stalk.

Accordingly, molecular motors are powered by the chemical energy, stored in the form of phosphate bonds in ATP molecules. Hydrolyzing those bonds triggers the changes of their conformation and allows them to perform mechanical work. In the case of kinesin-1, each successfully hydrolyzed ATP molecule allows to perform one step towards the plus end of the microtubule or, extremely rarely, towards its minus end. This broken symmetry between the frequency of forward and backward steps is an effect of kinesin’s directionality. For many mechanoenzymes, observed over the long time scale, the number of steps taken in one direction is greater than the number of steps taken in the other one. Directionality is a consequence of a structure of both, motor and its track, and can be also observed in coordinated actions of many collaborating groups of motors. One of the best examples is the bidirectional axonal transport, where plus-end oriented kinesins and minus-end oriented dyneins move their cargos from cell body (perikaryon) to axon back and forth.

The regular structure of microtubule affects the dynamics of kinesin in yet another way: because the head may dock itself to the microtubule only in a specific manner (most of the head attaches to β-tubulin monomers), each step is of the same size, i.e. about 8 nm for the movement of the center-of-mass, which corresponds to microtubule’s periodicity.
2. Molecular dynamics

The behavior of a protein amino acid chain is roughly similar to that of a biopolymer. As such, it comes as no surprise that a specific fragment of a kinesin chain is responsible for spring-like relative movement of kinesin heads. Such a fragment, present in each kinesin head, is known as a neck linker. One typical kinesin molecule consists of two identical amino acid chains\(^2\). N-terminus of each chain is the core of each kinesin head that forms around a single Mg\(^{2+}\) ion. More than 300 residues belongs to a kinesin head. Towards the C-terminus, the amino acid chain takes upon a shape of a helix. The helixes of two different chains intertwine, forming a stalk (cf. Fig. 1). The neck linker is a part of the amino acid chain situated between a head and a stalk. Only when it forms the outer edge of the kinesin head central \(\beta\)-sheet, does it develop a rigid conformation. Otherwise, a neck linker attached to the free kinesin head remains unstructured. In a typical kinesin structure, neck linker spans from 338th to 354th residue \([5]\) and has a mass of about 2 kDa\(^3\). In most biomechanical models, those two linkers are treated as harmonic springs. Nevertheless, since the amino acid sequence is often significantly varied, it is unclear if such an approach is correctly justified. Our aim was, therefore, to determine whether and to what extend, the structure of the linker can be approximated by the elasticity of the worm-like-chain (WLC) model \([6]\).

The elasticity of unstructured biopolymers is frequently described by the WLC model which has been claimed to predict correctly the force extension properties of polypeptide chains (see discussion in \([7]\)). Within this approach, the force required to extend a polymer with a given contour length \(L_c\)\(^4\) and persistence length \(L_p\) to a given end-to-end distance \(x\) can be approximated by the formula

\[
f = \frac{k_B T}{L_p} \left[ \frac{1}{4} \left( 1 - \frac{x}{L_c} \right)^{-2} + \frac{x}{L_c} - \frac{1}{4} \right], \tag{1}
\]

where \(k_B T\) is energy measured in Boltzmann units of temperature. In order to perform a suitable test, we have studied elasticity of the linker by performing MD simulations (GROMACS 4.5.4 \([8]\)) on a structure of an

\(^2\) Amino acids owe their name to the presence of two characteristic groups: an amine group \((-\text{NH}_2\)) and a carboxyl group \((-\text{COOH})\). When forming a peptide, a peptide bond is created by joining the amine group of one amino acid to the carboxyl group of the subsequent one. A final product of this condensation reaction is a chain that has a free amine group on one end and a free carboxyl group on the other. Those ends are called N-terminus and C-terminus, respectively.

\(^3\) An atomic mass unit of 1 dalton (Da) corresponds to \(1.661 \times 10^{-27}\) kg.

\(^4\) The contour length of a polypeptide is equal to the number of amino acids times distance along the chain per amino acid \([6]\).
idealized polymer (a model alanine structure) and on a sequence of a neck linker domain obtained from the Protein Data Bank (PDB). Starting coordinates for a linker structure have been obtained from the sequence of a kinesin-like protein KIF3B, downloaded from the existent PDB database (ID: 3B6U). Both structures (alanine and the 3B6U linker) have been further used to determine an effective mechanic potential function $V$ of a system containing $N$ atoms, described as

\[
V = \sum_{\text{bonds}} \frac{k_i}{2} (x_i - x_{i,0})^2 + \sum_{\text{angles}} \frac{k_i}{2} (\theta_i - \theta_{i,0})^2
+ \sum_{\text{torsions}} \frac{V_n}{2} \left[ 1 + \cos (n\omega - \gamma) \right]
+ \sum_{i}^{N} \sum_{j=i+1}^{N} \left( 4\epsilon_{ij} \left[ \frac{\sigma_{ij}}{r_{ij}} \right]^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right) + \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}. \tag{2}
\]

Here, $x_i$ is a symbol of an $i$th bond length, $\theta_i$ is a symbol of an $i$th angle value while $x_{i,0}$ and $\theta_{i,0}$ are their respective reference values. $V_n$ is a parameter that gives information about rotation barriers of torsion angle $\omega$, while $k_i$ refers to a $i$th force constant. $\epsilon_{ij}$ is a minimal value of the Van der Waals potential between atoms $i$ and $j$, $r_{ij}$ represents a distance between these centers and $\sigma_{ij}$ is a distance between them when Van der Waals potential value is 0. Symbols $q_i$ and $q_j$ refer to charges of $i$th and $j$th atoms and $\epsilon_0$ stands for a dielectric constant. Since bond lengths, bond- and dihedral angles can be rephrased in terms of position vectors $\vec{r}$, changes in the position of atoms are evaluated by solving Newton equation of motion with forces $\vec{F}_i = -\frac{\partial V}{\partial \vec{r}_i}$.

For the purpose of modeling, in course of simulations, both linker and alanine sequences have been put into boxes filled (uniformly) with water and subject to periodic boundary conditions. Equilibrium runs of 50 ps have been carried out for each sequence. A single simulation step has covered $2 \times 10^{-3}$ ps and each simulation has been running for 100 ps. The simulations have been performed at constant pressure of 100 kPa and at temperature of 300 K. The methods used to control temperature (separately, for solvent and solute) and pressure of the system have been adjusted to the Berendsen and Parinello–Rahman procedures [8], respectively. Results shown in Fig. 2 have been averaged over 6 simulations for each molecule.

Only that part of the KIF3B structure which contained a sequence identified as a neck linker [5] has been simulated. Additionally, constant force $f$ ($f = 216$ pN) has been applied, pointing from the neck linker central residue’s center-of-mass towards either one of the ends. Similar procedure has been used for a simulation of a comparative sequence consisting of alanine residues. The results of those simulations are depicted in Fig. 2.
Our aim was to study elasticity of the linker by performing MD simulations of a protein head’s fragment obtained from KIF3B structure stored in PDB database (ID: 3B6U). The (constant) external forces applied are set to 216 pN. Simulation steps are $2 \times 10^{-3}$ ps and length of each simulation is 100 ps. Results shown in the graph are averaged over 6 simulations for each molecule. The radius of a circle around each point corresponds to the standard deviation from the mean.

Left plot of Fig. 2 displays distance changes for alanine consisting of 19 amino acid residues. Here, “f direction 10–1” denotes the force applied to 1st residue, pushing towards that position from the 1th residue while force “f direction 10–19” denotes action of the force applied to 19th residue pushing from the same center (position of the 10th residue). Analogously, right plot illustrates distance changes for a sequence consisting of 18 amino acids of the neck linker. Label “f direction 9–1” denotes the force applied to 1st residue from the 9th residue while “f direction 9–18” denotes the force of the same intensity applied to 18th residue and pushing from the 9th one. The radius of a circle around each point corresponds to the standard deviation. Distance changes are calculated for distances between $C_\alpha$ atoms of residues being pushed away and atoms $C_\alpha$ of those residues that are a reference for applied forces (9th and 10th residues for alanine and neck linker sequences, respectively).

If the contour length $L_c$ is made of $n$ units of an approximately same length $l$, then by the Kuhn’s formula $l = 2L_p$, so that in the linear regime of the extension, one can approximate Eq. (1) with the Hook force,

$$f \approx \frac{3k_B T}{l L_c} x .$$ (3)

The motion of the linker center-of-mass can be then written as

$$M \frac{dv}{dt} = \frac{3k_B T}{l L_c} (x_0 - x) - \gamma L v + \xi(t) ,$$ (4)
with $M$ standing for the mass of the extended polymer/linker, $\xi$ being the Brownian white noise mimicking thermal fluctuations, $x_0 - x$ denoting a relative position and $\gamma_L$ describing the drag coefficient. With the extension satisfying $dx/dt = v$, and by assuming the overdamped motion in the center of linker’s mass, this equation predicts a linear $x(t)$ dependence. As visualized in Fig. 2, stretching applied to the alanine sequence results, in both its parts, in a semi-linear response typical for a harmonic spring curve, but only up to a certain point in time (about 50 ps), after which a leveling-off is observed. In contrast, for times up to about 30 ps, stretching of each half of the neck linker structure results in extensions very well following expectations derived from a harmonic spring model. Still, both linker halves do not behave identically and their elasticity coefficients are systematically different. One of the halves is able to stretch to a longer distance than the other, even though they consist of the same number of residues.

Altogether, these results show that our method can detect spring-like behavior of a neck linker and that such behavior is not an universal feature in amino acid chains. Furthermore, they suggest that distance changes between kinesin heads in models describing kinesin movement should be represented in more complex way than stretching of a single spring.

3. Chemical models

Conformational changes within the kinesin’s structure influence chemical kinetics of reaction cycles, catalyzed by the motor. For this reason, one of the approaches towards motor proteins modeling is a chemomechanical one. It is based on the results of experiments, in which the enzymes’ working cycles can be determined. Knowing all the reactions that may occur on the active sites of kinesin’s motor domains, it is possible to estimate the rate of the sequence of transitions. One can then quantitatively describe the chemomechanical coupling, that is a connection between energy providing chemical reactions and conformational changes they trigger, leading to directed stepping along microtubule.

Fig. 3 shows a schematic representation of an exemplary kinesin’s chemomechanical cycle. Ovals represent two kinesin’s heads, left — the rear one, right — the leading one. Such representation is consistent with the hand-over-hand walking model [9], where heads interchange their positions sequentially. Each of the heads may be found in four chemical states:

- $E$, when the motor domain is empty,
- $D$, when the motor domain is occupied by ADP$^5$,

$^5$ ADP — adenosine diphosphate.
• T, when the motor domain is occupied by ATP, and
• P, when the motor domain is occupied by an ADP–P complex\(^6\).

State P may be neglected, since its lifetime is very short (that is, inorganic phosphate is released rapidly after the ATP decay) [3]. Transitions among states are related to binding and unbinding the ATP, ADP and P molecules. For example, transition 1 → 2 from Fig. 3 may be deciphered as the binding of one ATP molecule to the leading head of kinesin-1, and the reverse transition 2 → 1 is a release of one ATP molecule from the leading head.

Fig. 3. Kinesin’s working cycle, from [10], changed. Thin arrows represent chemical reaction, thicker one — a mechanical step. Ovals stand for kinesin’s heads at chemical states E (empty head), D (head with attached adenosine diphosphate ADP) and T (head with adenosine triphosphate ATP), respectively. Clouds represent chemical substrates — ADP, ATP and P (inorganic phosphate). During transition 2 ↔ 5 kinesin takes one mechanical step: a forward step, towards the plus end of the microtubule, when (2 → 5) or a backstep (5 → 2).

It is known that in states T and E the head is tightly bound to the microtubule, while in state D the binding is weak and may be easily broken. This and the results of studies on how the concentration of ATP and ADP impacts on motor’s dynamical properties led to conclusion that the mechanical transition (a step) is possible only from DT to TD states and in reverse. Another important factors that have to be taken into account when constructing schemes of the kinesin working cycle, are the mechanical constraints. It has been shown that the access to motor domain’s active place is controlled by the strain of the whole molecule, conditioned by the strain of neck linker [11]. For each chemical state the neck linker’s stiffness must be determined and taken into account. It is why the studies similar to those presented in Sec. 2 are so important.

\(^6\) P — inorganic phosphate.
Motor proteins are subjected to many forces of distinct origin, that affect their behavior. In general, for our purpose we may divide them, after Howard [3], into chemical forces, resulting from the formation of chemical bonds, and external mechanical forces, as the load force exerted on motor-cargo system in optical tweezer experiments. While the first ones may drive kinesin to move in one direction, the latter may favor the opposite direction. A net result of competing forces depends on which force has greater effect on the protein’s chemomechanical cycle.

Chemical forces reflect the probability of each reaction and their effects, leading to conformational changes and performing mechanical work. As for mechanical forces, the standard way of reflecting their impact on reaction rates originates from the Arrhenius rate theory. In the case of walking kinesin which covers in each mechanical step a distance of $d \approx 8$ nm, the external force $F$ modifies the reaction rate $k$ by a force-dependent transition, so that $k \propto k_0 \exp\left[\frac{Fd}{k_BT}\right]$.

While constructing chemomechanical models, one has to include all available biologically relevant data. It sets the background and defines the constraints for the model. Below, we will briefly comment on some ambiguities often found when comparing different chemomechanical models.

After hydrolyzing one ATP molecule and detaching from microtubule, the rear head is subjected to random Brownian motion, that moves it back and forth around the still attached second head. When the head finds itself near the microtubule’s next binding site, new bounds may form and dock the head, finishing the forward step. Occasionally it may happen that, after hydrolyzing one ATP molecule, the leading head will detach. The probability that it will move back and dock to the rear free binding place is much smaller than that of rebinding to the same place. However, it is non-zero and such backsteps can occur. What is important, both forward and backward steps (and unbinding–rebinding events, ending with futile hydrolysis and no step, as well) require hydrolysis, or at least attachment of one ATP molecule [12].

Since there are some convincing suggestions that backstepping may play an important role in maximizing motor’s speed by increasing its entropy [13], and considering that the stepping ratio, $\frac{m_f}{m_b}$, is experimentally achievable, our goal was to investigate three different theoretical models, used frequently to describe kinesin’s behavior: 2-state and 4-state models from [14] and 6-state model from [10] (see Fig. 3).

As can be seen in Fig. 4, the results differ significantly.

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7 It seems likely that the conformation change, which accompanies the detachment of the rear head, can also move this head in a directed manner at a small distance, decreasing the distance it has to cover due to thermal motion and increasing the probability of successful binding in front of the second head. This mechanism is called a power stroke.
Fig. 4. Step ratio as a function of load force for three (2-state, 4-state and 6-state) models. Parameters of the 2-state and 4-state models are the same as in [14], and those of the 6 state models are from [10] with [ADP] = [P] = 10 µM, [ATP] = 1 mM. $n_f$ denotes the number of forward steps, $n_b$ — number of backward steps. Each point symbolizes the step ratio obtained after $10^5$ transitions in the chemomechanical cycle for different values of the force (from 0 to 10 pN, increasing by 0.5 pN for subsequent cycle’s series).

In the case of the 2- and 4-state models from [14], the backstepping issue has not been discussed. For the 6-state model [10] the step ratio $q$ has been introduced and defined as $q = \frac{P_2\omega_{25}}{P_5\omega_{52}}$, where $P_i$ is the probability of being in state $i$ of the chemomechanical cycle (see Fig. 3) and $\omega_{ij}$ is the rate of a transition from state $i$ to $j$. When calculating motor’s velocity, authors of [10] have also used the parameters connected with the transition from state 2 to state 5 of the proposed chemomechanical cycle, cf. Fig. 3. However, this treatment seems rather ambiguous, since the experimental data suggest, that after advancing the rear head in front of the leading one (transition 2 $\rightarrow$ 5 in Fig. 3), ADP has to be released (transition 5 $\rightarrow$ 6), allowing the now-leading head to attach firmly to the microtubule. Otherwise, before releasing ADP, a weakly bounded head may easily detach with no additional ATP molecule being hydrolyzed and the head will continue its diffusive search of a free binding site. Such an event should not, in our opinion, be counted as one step. Similar concerns can be brought up with regard to the backsteps (transitions 5 $\rightarrow$ 2 in Fig. 3).

To check how the definition of a single step, both forward and backward, affects the predictions of the model, we have compared the results of simulations of the chemomechanical cycle of the 6-state model from [10] (see Fig. 3) for three different cases, differing only in the definition of what should be counted for “one step”.
Accordingly, in model 1 all the transitions $2 \rightarrow 5$ were identified as one forward step, while the transitions $5 \rightarrow 2$ as one backward step. In model 2 we have counted one forward step for the sequence of transitions being $2 \rightarrow 5 \rightarrow 6$ or $2 \rightarrow 5 \rightarrow 4$ (that is, the kinesin’s head has been assumed docked after interchanging position with the other head and after another chemical, but not mechanical, transition from state 5 has taken place). Furthermore, one backward step has been identified with the transition sequences $5 \rightarrow 2 \rightarrow 1$ or $5 \rightarrow 2 \rightarrow 3$. In model 3, the most rigorous one, a forward step has been counted after each $2 \rightarrow 5 \rightarrow 6$ sequence, while the backward one, after the $5 \rightarrow 2 \rightarrow 1$ sequence has been concluded. This has been in line with the analysis of experimental data described recently by Block’s group in [15].

Illustration of our results is displayed in Fig. 5. Number of forward and backward steps performed by the motor influenced by different loads, while simulating models 1, 2 and 3, is shown in Fig. 6.

![Fig. 5. Step ratio as a function of load force for three models: Model 1, Model 2 and Model 3 (see the text). Parameters of all the models are the same, all as in [10] with $[ADP] = [P] = 10 \mu M$, $[ATP] = 1 \text{ mM}$. Step ratio is $\frac{n_f}{n_b}$, where $n_f$ denotes the number of forward steps, $n_b$ — number of backward steps. Each point symbolizes the step ratio obtained after $10^5$ transitions in the chemomechanical cycle for different values of the force (from 0 to 10 pN, increasing by 0.5 pN for subsequent cycle’s series).](image)

All simulations were based on the Gillespie algorithm for chemical state transitions [16, 17]. We have treated kinesin’s stepping as a Markov chain in which the probability $P_i(t)$ that the motor occupies state $i$ at time $t$ is evolving according to the Master equation

$$
\dot{P}_i = -\sum_j P_i \omega_{ij} - P_j \omega_{ji}.
$$

(5)
Fig. 6. Number of forward steps (left plot) and backward steps (right plot) as a function of load force for three models: Model 1, Model 2 and Model 3 (see the text). Parameters of all the models are the same, all as in [10] with $[\text{ADP}] = [\text{P}] = 10 \, \mu\text{M}$, $[\text{ATP}] = 1 \, \text{mM}$. Each point was obtained after $10^5$ transitions in the chemomechanical cycle for different values of the force (from 0 to 10 pN, increasing by 0.5 pN for subsequent cycle’s series).

Rate of a transition from state $i$ to $j$, $\omega_{ij}$, as well as other parameters were taken from [14] (for the 2-state and 4-state model) and [10] (for the 6-state model and for models 1, 2 and 3), unless the text states differently

$$\omega_{ij} = \kappa_{ij}I_{ij}([X])\phi_{ij}(F_0). \quad (6)$$

Here, $\kappa_{ij}$ is rate constant of the transition from $i$ to $j$, $I_{ij}([X]) = [X]$ and $X = \text{ATP, ADP or P}$ and $I_{ij}([X]) = 1$ for the transitions without chemical reactions. $\phi_{ij}(F_0)$ describes the effect of load force $F_0$ which assumes the following form [10]:

$$\phi_{25}(F_0) = \exp\left(-\theta \frac{F_{0d}}{k_B T}\right) \quad \text{and} \quad \phi_{52}(F_0) = \exp\left((1 - \theta) \frac{F_{0d}}{k_B T}\right)$$

for the mechanical transitions and $\phi_{ij}(F_0) = 2\left(1 + \exp\left[\chi_{ij}\frac{F_{0d}}{k_B T}\right]\right)^{-1}$ and $\chi_{ij} = \chi_{ji}$ for the chemical transitions.

Results show that the stepping ratios’ ($n_f/n_b$) comparison made for different kinesin models may be used as a method of their evaluation and validation of parameters (e.g. rate constants) that cannot be obtained experimentally. Moreover, they reveal the importance of defining the critical elements of chemomechanical cycles, especially the sequence of transitions involved in a definition of a forward and backward steps. As justified above, moving the rear head in front of the leading one is not enough to count this action as one forward step. For this, subsequent docking of the head to the microtubule and following chemical transitions are required. One way of solving the ambiguity of the definition of step is to identify it with an irreversible transition as in [15]. However, such an approach depreciates effects connected with molecular crowding, where Brownian movement of undocked...
head may be of greater importance than in buffer solutions used for in vitro experiments. For example, it is a common knowledge that in overcrowded environment of cell’s interior, reaction rates differ significantly from those measured in vitro [18].

As presented in Fig. 6, the number as well as the definition of backsteps, significantly affect the value of the stepping ratio and, as a consequence, the estimated motor’s velocity. This tendency is observed even for values of load smaller than the stall force $F_S \sim 7$ pN. It is an argument for the theories similar to the one presented by Bier and Cao in [13].

4. Ratchet models

A mechanic motor is a machine that converts some kind of energy into useful mechanical work, and as such it is what we call an engine. Key features of an engine are:

(a) fuel — the kind of energy that is used to perform work;
(b) power — which gives information what kind of work can by done by an engine in a given period of time;
(c) efficiency — the measure of how effectively the provided energy is converted into mechanical work.

For an average macro-motor like a car, an engine is a gas-driven, 120 kW strong device of an efficiency of about 20%. In this work, we discuss efficiencies of much smaller motors operating in a nano-scale of the living cells. The size of molecular motors and their overcrowded environments have a crucial impact on the key features of their dynamics. Kinesin, dynein and other motor proteins work in a dense cell environment, named sometimes “Brownian domain” after work by Magnasco [19]. That statement has been the foundation of using Smoluchowski’s idea (a fluctuation-driven ratchet) as an explanatory model for molecular motors [3, 6, 20, 21, 22]. Obviously, in biological realms it is hard to achieve thermal gradients large enough to drive directed motion [20]. There are, however, other ways of providing energy that can result in net movement of a particle. We briefly review them in the forthcoming sections.

4.1. Fluctuation driven ratchets

The first kind of models are based on the external fluctuations of the ratchet-shaped potentials [21]. Those include cyclically turning on/off potentials ("flashing ratchets") or rocking (tilting) potentials mitigated by fluctuating forces (so-called “rocking” or “tilting” ratchets) [23]. This group of

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8 Stall force, $F_S$, is such a load force, under which the motor cannot operate and stops.
models can be jointly described by a Langevin equation in the form

\[
m\dddot{x} + \gamma \dot{x} + \frac{dV(x,t)}{dx} = \xi(t) ,
\]

where \( V(x,t) \) is a periodic, asymmetric potential that changes in time and \( \xi(t) \) stands for the random Gaussian forcing. In general, for the group of particles with external energy uptake and storage in the form of a depot, the active Brownian motion (ABM) can be modeled by a varying friction coefficient \([24,25,26,27,28]\). In this class of models friction may become negative at low body’s velocity. Langevin equation under those circumstances reads

\[
m\dddot{x} + \gamma (\dot{x}) \dot{x} = \xi(t) ,
\]

Depending on an approach two different, nonlinear velocity-dependent friction functions are usually postulated. The first one proposed by Schweitzer \textit{et al.} \([24]\) reads

\[
\gamma (\dot{x})_{\text{SET}} = \gamma_0 \left(1 - \frac{\beta}{1 + \dot{x}^2}\right)
\]

with \( \beta \) being a control bifurcation parameter. The model implies negative friction for low velocities within the range \(|\dot{x}| < \sqrt{\beta - 1}\) and a “standard” positive value outside this region. The active motion corresponds to the regime \( \beta > 1 \). Somewhat simpler version of the model is offered by the Rayleigh–Helmholtz friction function \([24]\)

\[
\gamma (\dot{x})_{\text{RH}} = \gamma_0 \left(\dot{x}^2 - \alpha\right) .
\]

Here, friction is negative within the region of \(|\dot{x}| < \sqrt{\alpha}\) and becomes zero for \( \alpha = \dot{x}^2 \). Accordingly, the friction force attains negative values at small speed (it is pumping energy into the system) while at large velocities a cubic term \(\dot{x}^3\) dominates. Both models of frictional forces have been used to describe systems with an energy depot \([24]\), which acquires energy from the environment with a rate \(q(r)\), stores it as an internal energy \(e(t)\) and then provides it for conversion into kinetic energy with a rate \(d(v)\)

\[
\frac{de(t)}{dt} = q(x) - ce(t) - d(\dot{x})e(t) .
\]

After taking into account mechanical energy balance, Langevin equation for the depot-based active Brownian particle reads

\[
m\dddot{x} + \gamma \dot{x} + \nabla V(x) = d_2 e(t) \dot{x} + \xi(t) ,
\]
where \( d_2 = v^2 / d(\dot{x}) \). Comparing to Eq. (7), the most evident difference is the new term \( d_2 e(t)\dot{x} \), which is responsible for coupling of energy depot on the particle motion and *vice versa*.

Majority of works on the subject of molecular motor relate to the overdamped case, *i.e.* the inertia term in the Langevin equation is usually skipped. This is a perfectly legitimate practice as a single Langevin equation in most of those works models behavior of a whole Brownian motor moving in a dense, viscous medium. However, inertial effects can be crucial for understanding processivity of non-biological nano-machines, as *e.g.* atomic motors moving in optical lattices [21]. There are also approaches which establish a connection between ABM dynamics and stochastic dynamics of a group of coupled molecular motors [28]. In contrast to models of overdamped dynamics, they incorporate more than just one equation of motion [29, 27, 28] while preserving also the inertia term. It should be stressed that including inertia \( m\ddot{x} \) term in the Langevin equation implicates possible occurrence of chaotic behavior which has been documented in former studies on “inertia ratchet” models [30, 22]. The latter exhibit interesting performance characteristics, like negative mobility and current reversal.

In the case of motor proteins it seems also to be a wise choice to first model compartments of the protein with distinct Langevin equations and then, analyze the convergence of such a description to a single equation governing the motion of the center-of-mass. As a result, the center-of-mass dynamics can be associated with the single equation of motion considered in other models. The friction coefficient estimated in the center-of-mass implies a low ratio of the inertial and the viscous forces. In consequence, a low Reynolds number\(^9\) is then the reason for skipping an inertia term.

The models discussed in this work have included inertia in the original equations of motion. Accordingly, for the purpose of achieving directed motion, values of initial conditions and other parameters controlling the motion have to be carefully adjusted. In the following section, an integral presentation of the models will be focused on defining range of parameters for which the motor works.

### 4.2. Ratchet model and motor efficiency

The relative motion of two heads (each of mass \( m \)) of the motor is given by [31, 32]

\[
m\ddot{x} = -m\gamma_0 \dot{x} - U'(x_c + x/2) + U'(x_c - x/2) + TS'(x) + mde(t)\dot{x} + m\sqrt{2D_v}\xi,
\]

(13)

---

\(^9\) The Reynolds number is equal to \( R = \frac{\rho L \dot{x}}{\mu} \), where \( \rho \) is the density of the medium, \( L \) is a characteristic length of the moving object, \( \dot{x} \) is the speed of the movement relative to the medium and \( \xi \) stands for the viscosity.
where $U(x)$ is a ratchet-type periodic potential, friction frequency $\gamma_0$ is acting on the relative velocity $v$ of the heads $x$, $x_c$ stands for the position of kinesin’s center-of-mass, $\xi(t)$ is a white Gaussian noise of intensity $m\sqrt{2D_v}$ with $D_v = \gamma_0 k_B T / m$ and $TS'(x)$ incorporates entropic force exerted by an extended elastomer that links motor heads, where $TS(x) = s_0 + ax^2 - bx^4$. The mechanical energy of motion is powered by the energy flow

$$\frac{de(t)}{dt} = q - ce(t) - mdv^2e(t),$$

(14)

with $v \equiv \dot{x}(t)$, $q$ standing for energy accumulated in the depot container, $c$ representing dissipation rate and $d$ denoting coupling between the motor and the depot. An analogous equation for the motion of the center-of-mass reads

$$v_c \equiv \dot{x}_c = \frac{1}{\Gamma} \left( F_0 - U' \left[ x_c + \frac{x}{2} \right] - U' \left[ x_c - \frac{x}{2} \right] \right) + \sqrt{2D_{x_c} \xi_0},$$

(15)

where the total motor mass $M \gg 2m$ with $\Gamma \dot{x}_c$ denoting a viscous drag opposing the motion (here $\Gamma = 2M\Gamma_c$). In the above equation, $F_0$ stands for an additional force (load) disfavoring the movement of the motor and $\Gamma_c$ denotes the friction coefficient in the center-of-mass [31]. For the ratchet potential $U(x)$ we use the standard model [33]

$$U(x) = h [0.499 - 0.453(\sin(2\pi(x + 0.1903))) + \frac{1}{3}(\sin(2\pi(x + 0.1903)))],$$

(16)

where $h$ is the height of the barriers. In this simple approach, efficiency of the motor can be estimated [34, 35] as a fraction of power exerted by the motor working against external force $F_0$ divided by the quanta of chemical energy $q$ provided by the hydrolysis of ATP

$$\eta_C \equiv \frac{|F_0 \langle v_c \rangle|}{q}.$$  

(17)

The problem is, however, that in the absence of external force the efficiency is by definition zero. The way out is to follow the recipe presented by Derenyi et al. [36]. Those authors introduce the concept of generalized efficiency, defined as a ratio of minimal energy needed for the task to be accomplished ($E_{in}$) and the actual energy used to perform this task ($E_{in}$)

$$\eta_{gen} \equiv \frac{E_{min}}{E_{in}}.$$  

(18)

For molecular motors the minimal energy is used when the motor (interpreted as one body) is moving uniformly along the track at the average velocity $\langle v_c \rangle$. In this case the minimum power reads

$$P_{min} = \frac{dE_{min}}{dt} = F_0 \langle v_c \rangle + \Gamma \langle v_c \rangle^2.$$  

(19)
Consequently, the generalized efficiency for the presented model is defined as

$$\eta_{\text{gen}} \equiv \frac{|F_0\langle v_c \rangle| + \Gamma \langle v_c \rangle^2}{q}, \quad (20)$$

where $\langle v_c \rangle$ is an average velocity of the motor in the center-of-mass.

Yet another approach towards definition of the efficiency of molecular motors has been proposed in the papers by Wang et al. and Oster [35, 34]. Authors introduce so-called Stokes efficiency, viewed as a cost-effective measure of utilization by the motor of the chemical Gibbs free energy in generating a unidirectional motion and transporting a load through a viscous medium

$$\eta_S \equiv \frac{\Gamma \langle v_c \rangle^2}{A\langle r \rangle + F_0 \langle v_c \rangle}, \quad (21)$$

Here, $A$ represents chemical free energy consumed in one motor cycle and $\langle r \rangle$ stands for the rate of the chemical reaction cycle. In terms of presented model the Stokes efficiency can be estimated by substituting $A\langle r \rangle = q$ in Eq. (21). To further inspect percentage of the free energy used by moving motor heads, we have also analyzed

$$\eta_{\text{heads}} \equiv \frac{m \gamma \langle |v| \rangle^2}{q + F_0 \langle v_c \rangle}, \quad (22)$$

where $\langle |v| \rangle$ stands for average absolute relative velocity of the motor heads. Figure 7 displays results of this analysis for a set of parameters guaranteeing the progressive motor’s motion. The classical efficiency $\eta_C$ gradually increases from a zero value to a maximum registered at $F_0 = 0.18$ followed by a subsequent drop to zero at a stall force $F_0 = 0.2$. Similar dependence of the efficiency with a maximum observed at moderate loads has been described in the experimental work of Nishiyama et al. [37].

In contrast, within the same range of load forces the Stokes efficiency $\eta_S$ remains very low whereas the generalized definition Eq. (20) results in non-monotonous efficiency-load dependence. Altogether, this analysis shows importance of a proper definition of efficiency to be used when exploring energetics of molecular motors and making use of available experimental data. In particular, for motors with internal degrees of freedom, other channels for storage and dissipation of energy can be envisioned [38]. In such situations, the generalized definition of efficiency has to be tailored to take into account the average input power as the change in both, the potential and chemical energy of the motor cycle.
Fig. 7. Thermodynamical efficiency $\eta_C$, generalized efficiency $\eta_{\text{gen}}$ and Stokes efficiency $\eta_S$ as a function of the load force $F_0$, given in dimensionless units, for $\Gamma \approx 0.102$.

5. Conclusions

In general, molecular motors operate in groups performing dedicated tasks via synchronized action. In intracellular transport there is about several motors [3] which coordinate motion along microtubules. Many experiments and models have attempted to unravel basic features of load sharing and bi-directionality of the transport performed by higher assemblies of motors. Understanding collective behavior of molecular motors and nature of the protein friction associated with a directed motor motion are nowadays the most intriguing and important issues in this field.

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REFERENCES


“Cargo-mooring” as an operating principle for molecular motors

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HIGHLIGHTS

• Experiments suggest that cargo dynamics play a role for kinesin’s motion.
• We work out how chemical energy can be used to rectify the cargo’s diffusion.
• Kinesin and its cargo interact cooperatively to move along the polymeric track.
• We derive the velocity as a function of load force, cargo size and buffer viscosity.
• Experimental observations appear consistent with our results.

ABSTRACT

Navigating through an ever-changing and unsteady environment, and utilizing chemical energy, molecular motors transport the cell’s crucial components, such as organelles and vesicles filled with neurotransmitter. They generate force and pull cargo, as they literally walk along the polymeric tracks, e.g. microtubules.

What we suggest in this paper is that the motor protein is not really pulling its load. The load is subject to diffusion and the motor may be doing little else than rectifying the fluctuations, i.e. ratcheting the load’s diffusion. Below we present a detailed model to show how such ratching can quantitatively account for observed data.

The consequence of such a mechanism is the dependence of the transport’s speed and efficacy not only on the motor, but also on the cargo (especially its size) and on the environment (i.e. its viscosity and structure). Current experimental works rarely provide this type of information for in vivo studies. We suggest that even small differences between assays can impact the outcome. Our results agree with those obtained in wet laboratories and provide novel insight in a molecular motor’s functioning.

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1. Introduction

For eukaryotic cells, free diffusion is simply too slow and too uncontrollable to take care of the transport needs. For this reason the motion of certain vesicles and organelles involves motor proteins and their filamentous tracks. This guarantees proper cell functioning. Intracellular active transport is something that every eukaryotic cell has to coordinate, maintain and constantly shape. It involves many players, like different types of filaments, molecular motors and cargos that are moved from one place to another. Many of these components can be studied in isolation in vitro. Milestone works on kinesin-1, experimental (Svoboda et al., 1993; Visscher et al., 1999; Howard, 1997) as well as theoretical (Astumian and Bier, 1994; Astumian, 1997; Jülicher et al., 1997), have brought many scientists from different fields to the topic of active intracellular transport. A lot of questions have been both asked and answered. We now know for instance that the walking pattern is “hand-over-hand” and not “inchworm” (Asbury, 2003; Yildiz et al., 2004). Also the role of the neck linker that connects the two heads has been cleared up to a large extent (Guyodh and Block, 2006; Kozielski et al., 1997; Tomishige et al., 2006; Mogilner et al., 2001; Shastry and Hancock, 2011). We have, furthermore, attained a good understanding of the relation between mechanics and chemistry (Mori et al., 2007). But there are still unsolved problems.

The moving motor protein uses chemical energy to overcome the viscous friction of the cytosol and to drive the conformational
changes in the catalytic cycle. In experiments it is possible to apply an external load with an optical tweezer: an external load that the motor has to also work against.

The most efficient way of transporting cargo in a viscous medium would be pulling it with constant velocity from the place where it is formed to its destination. It would lead to trajectories with position linearly dependent on time. However, the “staircase”-like trajectories as obtained experimentally (e.g. as in Svoboda et al., 1993) exclude such a mechanism of smooth active transport. Instead, the experimental results suggest that the cargo is displaced in rapid “jerks”. Those jerks correspond to single steps of the molecular motor that transports the cargo. The energy needed for this can be estimated. It is apparent that evolution lead to a mechanism that is not the most efficient in terms of energy (see Section 1 in SI).

Experimentalists have found that the stalk (the relatively long construct between the heads and the cargo) plays a role in triggering kinesin’s steps (Dietrich et al., 2008; Wong et al., 2009; Yildiz et al., 2008). It appears that kinesin’s heads “communicate” with the cargo through the stalk. New structural data on molecular motors are rapidly becoming available. Many of the more phenomenological models focus on enzymatic activity and describe rather than explain the force generation. Below we present a model where the force is obtained, obtained results agree with experimental data and the construction of the presented model allows for a new insight into the functioning of motor proteins.

2. Model

We consider a system consisting of a motor carrying a spherical cargo along a polymeric track, see Fig. 1A. All the components are embedded in a buffer solution of a known viscosity. The track has a periodic structure, with special domains — binding sites — positioned every $L = 8 \text{ nm}$. This corresponds to the known molecular structure of microtubule (Howard, 2001). The cargo — e.g. a spherical vesicle or a bead of radius $R$ — is subject to diffusion in the surrounding solution. It is attached to the motor, which in turn holds on to the binding site, hence it may move only within a limited range, $2x_{\text{rel}}$ (Fig. 1B). This range is determined by the length and elasticity of the cargo–motor connection. Later in this section we consider the case of $x_{\text{rel}} = L$.

Somewhat like a windswept balloon on a string, the Brownian motion of the cargo makes the motor swing back and forth around the docking point. The deflection, however, will not exceed the maximum value, $\phi_{\text{max}}$ (cf. Fig. 1). Again, this limit is determined by the structural properties (e.g. elasticity) of the setup.

In what follows we will examine the system in terms of the relative position, $x_{\text{rel}}$, between only two points, $x_{\text{rel}} = x_{\text{top}} - x_{\text{bottom}}$. The position of the top point, $x_{\text{top}}$, represents the position of the cargo which we assume to be the same as the position of the motor–cargo link and the cargo’s center. The top point can move to the left and to the right due to thermal diffusion in a continuous range. The position of the bottom point, $x_{\text{bottom}}$, represents the position of the midpoint between motor’s two heads. It can change by $L$ only via discrete steps from one binding site to the next one. In what comes next we also assume that the motor is fully processive. This means that it never detaches from the track.

The stepping process is coupled to a sequence of a chemical reactions. For kinesin-1, as well as for other cytoskeletal motor proteins, this would be the hydrolysis of ATP. Here we do not consider those reactions in detail. For these reactions the motor takes substrates from the buffer solution and acts like an enzyme for the conversion of this substrate into a lower-energy product. The chemical reactions trigger conformational changes of the motor. They let the bottom point detach from the previously occupied site and reattach at the neighboring one. The chemical cycle is evidently not an instantaneous process. It takes $t_c$ to complete the cycle and so no step is possible in a time shorter than $t_c$. However, finishing the cycle is not sufficient for the motor to make a step.

After finishing the chemical cycle and when the position of the cargo allows it, the motor changes its docking point, i.e. takes an $L = 8 \text{ nm}$ jump to the right. Assume that the motor is deflected by $\phi_{\text{max}}$ to the left, so that $x_{\text{rel}} = -L$ (Fig. 1B). The architecture of the microtubule–kinesin–cargo system implies steric constraints that will not permit the cargo to further diffuse to the left. These constraints effectively set up a reflecting barrier for the diffusing cargo. Eventually diffusion will bring the cargo to $x_{\text{rel}} \geq 0$. Then a next step will occur provided that the chemical cycle is also completed.

Imagine that only the first condition (completion of all chemical transitions) has been fulfilled and the relative position between the top and the bottom point stays in the range $-L \leq x_{\text{rel}} < 0$. If the motor would make a step and increase the position of the bottom point by $L$ without any change in the position of the top point (since the motor does not pull the cargo), then it would deflect by an angle greater than $\phi_{\text{max}}$ to the left. This is impossible due to structural limitations.

The motion is conceived as taking place in one dimension. Natural kinesin-1 walks straight along the microtubule and does not wander to the left or right. The cargo however may diffuse sideways. The reason for our 1-dimensional idealization is that the 2-dimensional optical trap methods revealed that sideways loads exerted on the cargo do not significantly affect the directed motion of the motor (Block et al., 2003). This is in contrast with the motor’s high sensitivity for longitudinal loads. If there is any communication between the cargo and the motor, it should be captured by our 1-dimensional approximation.

For simplicity, we first present the derivation without considering backsteps, i.e., without steps back to the binding site on the left (cf. Fig. 1). Then, in the section “Backstepping,” we show how to broaden the analysis and take backsteps into account.

---

**Fig. 1. Cartoon presenting the model’s idea.** (A) The motor is described through the behavior of two points. The top one (blue) represents the cargo. It moves in the continuous range $2x_{\text{rel}}$, driven by Brownian motion. The bottom one (green) stands for the position of the motor on a track (orange). Each step of a motor is a discrete jump of length $L$ between two neighboring binding sites (red circles). The top point reflects the position of the cargo, while the bottom point reflects the position of the midpoint between the two docked heads. The inset in A shows the ideogram of a kinesin-like motor. It consists of two heads (here in violet), connected with spring-like neck linkers, which later interwove to form a stalk (blue wiggly lines). The top of the stalk connects with the cargo (yellow oval). The motor walks on a microtubule (orange). Note that the real microtubule’s binding sites (red squares) are shifted by 4 nm relative to the modeled ones (red circles). (B) A step may occur when the position of the cargo allows for it. After jumping from one binding site to the other (e.g. from green to red, as depicted by the green arrow), the angle between the upper and the lower point must be no larger than the maximal deflection $\phi_{\text{max}}$ (reddish for the red binding site). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)
2.1. Analytical description

In our model the cargo is positioned at a fixed distance from the track and executing diffusive motion parallel to the track. We take \( x = x_t \) for the position of the cargo along the direction of the track. Table 1 lists all the symbols used in this and further sections. The evolution in time of the probability density distribution for \( x \) is described by a Fokker-Planck–Smoluchowski equation:

\[
\frac{\partial}{\partial t} p(x, t | x_0, 0) = \frac{F}{\gamma} p(x, t | x_0, 0) + D \frac{\partial^2}{\partial x^2} p(x, t | x_0, 0).
\]

Here the first term on the right hand side describes the forward drift coming from the optical trap, which holds on to the cargo and applies a load force \( F \). The second term describes the diffusive spreading. The drag coefficient, \( \gamma = 6 \pi \eta R \) (\( R \) is the radius of the cargo and \( \eta \) is the viscosity of the solution), and the diffusion coefficient, \( D \), are connected through the fluctuation–dissipation theorem, \( D = k_B T / \gamma \).

We split the motor’s step into two stages. During the first stage the load diffuses in the range \((-x_m, x_m)\) with reflecting boundary conditions. No mechanical step can be made during this stage. After the time \( t_1 \) the motor is able to make a step if no geometrical obstacles prevent it. For our model it means that positions of cargo and motor fulfill a condition \( x > -x_m + L \). We assume that during the second stage the motor makes a step immediately upon fulfilling the geometrical condition. After \( t_2 \) we thus have the same Fokker-Planck–Smoluchowski equation, but with different boundary conditions. While the left boundary is still reflecting, the right boundary becomes absorbing. We are particularly interested in the mean times needed for the cargo to cross the latter boundary. This will trigger the motor to make one mechanical step: the rear head of the motor will detach from the microtubule and dock at the binding site in front of the leading head, \( 2L \) ahead of its original position. The motion of the rear head is general a combination of diffusion and directed motion, but with a lot of subtleties (Block, 2007).

Our aim is to find force–velocity relations, that is to examine how the external force brought about by the optical tweezer affects the dynamics of the motor. We note that the correct way of measuring the motor’s velocity is through \( v = L / \langle t_{\text{step}} \rangle \). In Section 2 of the SI it is shown how and why \( v = L / \langle t_{\text{step}} \rangle \) leads to wrong results.

2.1.1. Derivation

For \( t_1 \) larger than the timescale of the Brownian jerks, we may write

\[
p(x, t_1 | x_0, 0) \approx p_j(x),
\]

where \( p_j(x) \) stands for the stationary distribution of the cargo’s position for the first stage. As mentioned before, we impose reflecting boundary conditions and obtain a current:

\[
j_s(x_m) = \left. \frac{F}{\gamma} p_j(x_m) \right|_{x_m} - D \frac{\partial p_j}{\partial x}(x_m) = 0 = j_s(-x_m).
\]

From this and from the normalization condition of the probability density function on the interval \((-x_m, x_m)\), an explicit formula for the stationary probability distribution can be obtained. It is an ordinary exponential distribution:

\[
p_j(x) = \frac{ze^{-zx}}{2 \sinh zxm},
\]

where \( z = F / (\gamma D) \).

If after the first stage \( x > -x_m + L \), then the step can be made immediately. Let \( p_1 \) denote the probability of this event:

\[
p_1 = \int_{-x_m + L}^{x_m} \, dx \, p_j(x) = \frac{e^{zx_m}}{2 \sinh zxm} (1 - e^{-zL}).
\]

We will denote by \( p_s \) the probability of the complementary event, i.e. the event in which the motor cannot make a step because of geometrical constraints. For a step to be possible:

\[
p_0 = \int_{-x_m}^{-x_m + L} \, dx \, p_j(x) = \frac{e^{zx_m}}{2 \sinh zxm} (1 - e^{-zL}).
\]

In order to obtain the MFPT we use the formula from the standard textbook by Gardiner (1997, Chapter 5, Section 5.2.7, Eq. (5.2.160)):}

\[
\langle t_p | x \rangle = \frac{1}{D} \int_x^L \, du \, e^{uz} \int_0^u \, dy \, e^{-vy} = \frac{1}{2D} \left( \frac{1}{z^2} (e^{2zL} - e^{2zy}) - (L-x) \right),
\]

where \( x \) denotes the starting position. Now we have to average over the distribution (cf. Eq. (4)) that constitutes our initial condition:

\[
\langle t_p \rangle = \int_0^L \, dx \, \langle t_p | x \rangle p_j(x, 0).
\]

Having done this, we are ready to write down for the mean step time:

\[
\langle t_{\text{step}} \rangle = p_1 t_s + p_0 (t_r + \langle t_p \rangle) = t_r + \int_0^L \, dx \, \langle t_p | x \rangle p_j(x-x_m).
\]

From this and from the normalization condition of the probability density function on the interval \((-x_m, x_m)\), an explicit formula for the stationary probability distribution can be obtained. It is an ordinary exponential distribution:

\[
p_j(x) = \frac{ze^{-zx}}{2 \sinh zxm},
\]

where \( z = F / (\gamma D) \).

If after the first stage \( x > -x_m + L \), then the step can be made immediately. Let \( p_1 \) denote the probability of this event:

\[
p_1 = \int_{-x_m + L}^{x_m} \, dx \, p_j(x) = \frac{e^{zx_m}}{2 \sinh zxm} (1 - e^{-zL}).
\]

We will denote by \( p_s \) the probability of the complementary event, i.e. the event in which the motor cannot make a step because of geometrical constraints (\( x+x_m > L \) then is a condition sine qua non for a step to be possible):

\[
p_0 = \int_{-x_m}^{-x_m + L} \, dx \, p_j(x) = \frac{e^{zx_m}}{2 \sinh zxm} (1 - e^{-zL}).
\]

The last element we need is a mean first passage time (MFPT). If the cargo is in the interval \((-x_m, -x_m + L)\), then the motor has to wait until the cargo will reach the right (absorbing) boundary. We therefore analyze the same system with different boundary and initial conditions. Notice that we shift the interval by \( x_m \) to the right. This will simplify the final formulas:

\[
\begin{cases}
  j(0, t) = -\frac{F}{\gamma} p(0, t) - D(\partial_x p)(0, t) = 0 \\
p(L, t) = 0 \\
p(0, 0) = P(X = x - x_m | -x_m < X < -x_m + L) = \frac{p_j(x-x_m)}{p_0}.
\end{cases}
\]

The result reads as follows:

\[
\langle t_{\text{step}} \rangle = t_r + \frac{e^{zx_m}}{2 \sinh zxm} (\sinh zL - zL).
\]
With $v = L/(t_{\text{step}})$ the resulting force–velocity curve is found to be given by the formula:

$$v = \frac{F L}{t^{\prime} z \sinh 2x_m + e^{\gamma z} (\sinh zL - zL)}.$$  \hspace{1cm} (12)

The important limits are easily derived:

$$\lim_{F \to \infty} v = 0.$$  \hspace{1cm} (13)

We note that it is possible to derive simplified approximations of force–velocity relations for a kinesin-like motor using the approach proposed by Bier et al. (1999). However, Eq. (12) originates directly from the model’s assumptions and is therefore presented in its full form.

3. Results

In a typical in vitro experiment we have kinesins walking along microtubule and carrying a small silica bead. The bead is trapped in the narrow laser of the optical tweezer. How can this force, acting on a bead, be transferred through the long (ca. 110 nm) stalk and neck linkers to the motor domain of the kinesin heads? How can it be that this force interferes with the reaction rates, changing their values by a factor of exp($FL/(k_BT)$)? While we know quite a lot about, for instance, the importance of strain gating (Guydosh and Block, 2006) for a head’s coordination, it is much more challenging to describe how the information about load reaches the binding sites of motor domains. While the chemomechanical approach towards modeling motor proteins (Liepelt and Lipowsky, 2007; Clancy et al., 2011; Fisher and Kolomeisky, 1999; Schilstra and Martin, 2006) explains the impact of external forces on reaction rates through Arrhenius’ law, it does not take into account the cargo or the environment. Recent experiments (see Section 3.4) show that those factors impact the motor dynamics and should not be ignored. In addition, it has been demonstrated that molecular motors, e.g. kinesin, are sensitive to external conditions, like ATP concentration, which change not only the kinetics, but also the whole operating mechanism responsible for mechanical stepping (Guydosh and Block, 2006; Mori et al., 2007). Our approach tries to give a simple physical mechanism behind the force–velocity relation. Below we will elaborate on the model’s parameters and highlight the important consequences of the approach described in the previous sections.

3.1. Model parameters

The presented model has two important parameters: $x_m$, which reflects the motor’s ability to deflect forward and backward while following the diffusing cargo, and $t_\ast$ giving the minimum time needed for chemical and conformational changes between subsequent steps.

$x_m$: Experiments give us no precise values for the maximum deflection of the kinesin motor (Jeney et al., 2004). This parameter can be related to the protein’s elasticity, which appears to play a significant role in the stepping process. After the first experiments on kinesin, it was suggested that the motor stretches the spring-like tether when it makes a step, and that the subsequent elastic relaxation of the tether then pulls the cargo forward (Swooboda et al., 1993).

As shown in Fig. 2C, our model predicts a force–velocity relation for the kinesin-like motor that is almost unaffected by the choice of $x_m$. The mooring model results in the experimentally observed behavior (i.e. trajectories of the cargo are staircase-like, with position fluctuations between the motor’s steps, see Fig. 1 of SI). So the stretching of the tether is not the only possible explanation of the experimental outcome.

The weak dependence of the MFPT, and thus of the motor’s velocity, on $x_m$ may seem counterintuitive, but a good quantitative explanation can be given (see Section 3 in SI).

$t_\ast$: The minimum dwell time, $t_\ast$, is easier to estimate, at least for a kinesin-like motor. From Refs. Visscher et al. (1999) and Carter and Cross (2005) we take 800 nm/s as the velocity of unloaded kinesin. Knowing the length of a single step, $L = 8$ nm, we find $t_\ast = 10$ ms. Even for a fixed $t_\ast$, unaffected by external forces, we observe the decrease in the motor’s velocity in the presence of high loads. In this regime the duration of the chemical cycle is no longer the limiting factor—it is the diffusion that is affected by the force.

To verify our model we have compared the predicted force–velocity curves with the experimental ones from Visscher et al. (1999) and Carter and Cross (2005), see Fig. 2. One observes that even without taking the backstepping into account our model agrees well with the points from Block et al. (2003) for high ATP concentration. With decreasing ATP concentration the time needed for successful hydrolysis increases and so does $t_\ast$. But experiments also show that velocity gets more sensitive to load and starts falling faster at lower ATP concentrations, see Fig. 4A in Block et al. (2003). Our model (Fig. 3B and D), at least in its simple form, cannot explain this increased sensitivity, which to our knowledge has not been widely discussed in the literature (however see Mogilner et al., 2001 for similar observations and insightful discussion).

Another drawback of our approach is associated with the experimental observation that the stall force increases with ATP concentration (Block et al., 2003). The mooring model predicts that the stall force is almost unaffected by the changes of $t_\ast$, as shown in Fig. 3.

We put forth that an explanation might be found in the different ways in which the motor apparently waits for the succeeding chemical transitions under different ATP concentrations. Using FRET, Mori et al. observed that the two-head-bound state predominates for high ATP concentrations (Mori et al., 2007). When there is less chemical fuel in the buffer, kinesin waits for the completion of its chemo-mechanical cycle in a one-head-bound state. It is possible that in the one-head-bound state the strain generated by the load affects the motor differently than in a two-head-bound state.

Another relevant observation is that the speed of an unloaded kinesin differs significantly between Block et al. (2003) and Carter and Cross (2005). Additionally, the motors used by those groups show different backstepping patterns (see Fig. 3). This striking discrepancy between the results from Block et al. (2003) and Carter and Cross (2005) may be, at least to some extent, explained by the difference between the proteins. While Block et al. used kinesin purified from the squid optic lobe, Carter and Cross used kinesin from Drosophila melanogaster. Also, the origins of the microtubules as well as the experimental assays and procedures were slightly different. It is a commonly held view that dynein motors taken from different organisms exhibit different features (see Reck-Peterson et al., 2012 for a review), while kinesins are homogeneous between species. But that view may not be entirely accurate.

In our analysis we did not consider the time of the conformational transition responsible for the step (i.e. moving the rear head to in front of the leading head). Carter and Cross found that the mechanical component of a step takes around 15 μs for kinesin-1 transporting a 0.5 μm bead (Carter and Cross, 2005). This is a negligible amount of time compared to the duration of the entire cycle.

We note that it may be possible to change the times for the different transitions in mutants. An elongated neck linker may, for instance, hinder the docking of the free head and lead to a larger $t_\ast$ (Yildiz et al., 2008).
3.2. Backstepping

The motion of a processive molecular motor is highly directed. However, sometimes the motor takes a step in the backward direction. For kinesin-1, backsteps are steps toward the microtubule’s minus end. Visscher et al. found and Block et al. later confirmed five to ten backsteps for every 100 forward steps (Visscher et al., 1999; Block et al., 2003). This result appeared load independent. In a more recent study Carter and Cross found about one backstep for every 802 forward steps when no load was applied (Carter and Cross, 2005). With an increasing load, the backstep fraction was found to increase rapidly, but the forward-to-backward step ratio turned out to be independent of the ATP concentration (Carter and Cross, 2005). For large loads, backsteps constitute a significant fraction of the total number of steps. This fraction approaches unity for the stall force and falls below unity for higher loads. Therefore, neglecting the backsteps is no longer legitimate for high loads. Below we show how to implement backsteps into the mooring model.

We start by introducing new variables, listed in Table 2. It is easy to see that

\[
\frac{v}{L} = \frac{\eta_0 - \eta_b}{\sum_{i=1}^{n} \tau_i^b(F) + \sum_{j=1}^{m} \tau_j^b(F)} \approx \frac{p_b - p_0}{p_0(F) + p_0(F)} = \frac{1 - 2p_b}{(F(F) + p_0(F(F) - (F(F)))}
\]

(14)

Combining formulas (11) and (14) we arrive at the result:

\[ v = \frac{1 - 2p_b}{Fz_F + \left(\frac{\eta_0}{\sinh z_m} - 2p_b\right) (\sinh z_L - z_L)} \]

(15)

The last step in the derivation is to substitute a phenomenological relationship between the backstep probability \( p_b \) and the other model parameters. To check our model we have used two datasets, one from Block et al. (2003), with constant \( p_b \), and the other from Carter and Cross (2005). In the latter reference Carter and Cross found a logistic curve that links \( p_b \) with the load force \( F \):

\[ p_b = \frac{1}{1 + ae^{-3b}} \]

(16)

with \( a = 802 \) and \( b = 0.95 \) and the load \( F \) given in \( pN \) (Carter and Cross, 2005). The results for those two schemas of backstepping are presented in Fig. 3(A,B) and (C,D), respectively.

3.3. Viscosity and cargo dimension

Most of the molecular motor experiments are conducted in vitro, i.e. in some buffer solution of a homogeneous viscosity. In this study we examine motion in different homogeneous solutions — water, buffer solution, and a reference solution (Fig. 2A). For the buffer we took the effective viscosity \( \eta = 2.4 \times 10^{-3} \) Pa s, as in Beausang et al. (2007). The nearby presence of a wall may lead to the motor and cargo construct “feeling” a higher viscosity. Also the nearby presence of the
motor may cause the cargo to “feel” a higher effective viscosity. So the third solution we used is a hypothetical environment with a viscosity 10 times higher than the aforementioned $\eta = 2.4 \times 10^{-3}$ Pa s of the buffer solution. Results depicted in Fig. 2A show the sensitivity of the velocity to the viscosity. As a general rule, with the same motor, the cargo’s motion slows down as viscosity increases. Since the drag coefficient $\gamma$ depends linearly on viscosity $\eta$, higher $\eta$ implies smaller $D$, i.e. smaller Brownian kicks felt by the cargo. As a consequence, the dwell times in one position are longer — it takes a longer time to reach another no-return point. Also the stall force decreases significantly for higher viscosities. The decrease of the amplitude of the thermal noise makes the cargo less mobile as at the same time the effective force, $F/\gamma$ (cf. Eq. (1)), also decreases.

Interestingly, mooring-model predictions can be fitted to the experimental data even better by increasing the viscosity of the buffer tenfold (Fig. 3 and Fig. 2 in SI). Even the non-monotonic shape of the force–velocity curve for assisting loads, reported by Block et al. (2003), can be reproduced for a viscosity of $\eta = 5.2 \times 10^{-3}$ Pa s, cf. Fig. 2 in SI. Hence, the question about the microscopic velocity “felt” by the diffusing cargo in an in vitro motility experiment arises. There is empirical evidence suggesting that the in vivo effective viscosity is a function of the probe’s size: the bigger the difference in size between crowding agents and the probe, the less viscous the environment (Kalwarczyk et al., 2011; Ochab-Marincek and Holyst, 2011). It is tempting to use a higher “effective” viscosity when comparing the theoretical predictions of the mooring model with experiments. Without going into further detail we conclude at this point that the environment is very important for motor-driven motility. It is regrettable that many experimental reports on motor-protein motion do not include the value of the medium’s viscosity.

The same relation as between viscosity and velocity is observed for cargo size and viscosity. As our base cargo’s radius size we have used $R = 250$ nm, which corresponds with the size of the beads used by Carter and Cross (2005). Additionally, we have also analyzed motor–cargo dynamics for smaller ($R = 25$ nm) and bigger ($R = 500$ nm) cargos, see Fig. 2B. With $\gamma = 6\pi\eta R$ it is obvious that the drag is larger for larger beads. The diffusion coefficient $D = 6\pi\eta R/\gamma$ is smaller for larger beads.

Cargo size and viscosity appear to strongly affect the force–velocity characteristics. These results suggest that it is important to consider viscosity and cargo size when interpreting experimental outcomes.
3.4. Comparison with experimental data

In this section we present some experimental findings, taken from the literature, that show how data involving the environment’s viscosity, cargo size, and the structure-function connection support the mooring model.

As early as in 1994 Hunt et al. (1994) reported in a very elegant paper that the motion of microtubules propelled by single kinesin molecules for gliding assay experiments depends on both the viscosity of the buffer and the microtubule length. That is also what our mooring model would predict, since it is perfectly applicable for gliding assays experiments as well as for bead-pulling against an external force (cf. Fig. 3 in SI).

After analyzing four possible mechanisms of motor action, the authors excluded a “filament-diffusion model” that was somewhat similar to our cargo-mooring model. Their filament-diffusion model predicted a speed in high viscosity solutions that was smaller than the one observed experimentally. Hunt et al. also showed that the microtubule is unable to diffuse in a viscous solution through \(L = 8\) nm during the time in which kinesin takes its mechanical step \(t = 72\ \mu s\) in their paper).

Whereas in the “filament-diffusion model” the diffusion and the chemical changes are separate stages, in the mooring mechanism the cargo diffuses for the entire time needed to complete the chemical cycle of ATP hydrolysis and ADP-Pi release. That time sums up to around 10 ms for all the chemical transitions. The mechanical step takes only \(\mu s\) for an unloaded kinesin motor (Carter and Cross, 2005).

This assumption of our mooring model is consistent with what experimentally obtained trajectories show: Brownian motion of the cargo is observed between steps and not only during them. Within 10 ms, the diffusing cargo (or, as in Hunt et al., 1994, microtubule, cf. Fig. 3 in SI) can easily cover the distance \(L = 8\) nm needed to trigger the step when no external force is applied. This results in different predictions of the two models.

For the filament-diffusion model of Ref. Hunt et al. (1994), the velocity diverges with decreasing viscosity. In our mooring model the velocity of the motor saturates as the viscosity goes down. This is because the time of one step cannot be shorter than the time of the reaction cycle \(t_r\) plus the time of the mechanical transition.

Another difference is the estimation of the maximum force a motor can sustain, i.e., stall force. Hunt et al. show that for the filament-diffusion model this is \(2k_BT/L \approx 1\) pN. This is less than experimental observations and our theoretical predictions. This is again because of the filament-diffusion model’s assumption that the diffusive motion of the cargo takes only a fraction of the hydrolysis time \(t_r\). Our mooring model, built on experimental observations, predicts that the stall force, even for highly viscous buffer, is much higher than the one predicted by the filament diffusion model, see Figs. 2 and 3.

Very recently Efremov et al. reported a series of in vivo experiments that reveal a dependence of the kinesin-based transport on the size of the cargo (Efremov et al., 2014). The velocity of peroxisomes moved by multiple kinesins decreases when these peroxisomes are getting bigger. Interestingly, this is not so obvious for myosin Va. One explanation may be that myosin exhibits complex behavior while interacting with rather disordered actin filaments. Myosin Va can take steps of different lengths while cross-linking between different tracks. This makes observations as well as data analysis problematic. Nevertheless, the observed cargo-size dependence for kinesin-powered peroxisome motion is an important empirical fact that is accounted for by the mechanism that we propose.

The most essential assumption of the mooring mechanism is that there is a kind of communication between the top end of the stalk and, through its bottom end, neck linkers and motor domains. In other words, the deflection of the stalk is assumed to generate a “signal” — i.e. mechanical strain — which can trigger or prevent the motor’s stepping. Yildiz et al. (2008) studied the behavior of kinesins with extended neck linkers. They observed that the velocity of such a modified motor is decreasing with growing length of the artificial amino acid inset. What is even more important, by applying assisting loads with an optical tweezer, the authors were able to speed up the modified motors. They could also trigger steps with a forward load in the absence of ATP. Pulling (that is, deflecting the stalk towards the plus end of the microtubule) alone can make kinesin walk in its natural step-by-step manner! These results prove that the position of the cargo, manifested by the stalk’s deflection, is important for the progressive motion of the motor.

4. Discussion

Kinesin molecular motors, taking care of intracellular transport needs of eukaryotic cells, are among the best known proteins. The energy-consuming active transport in which they are involved is thought to be an evolutionary improvement over free diffusion, the latter being just not sufficiently efficient, fast and reliable for cells bigger than \(1\) \(\mu m\). However, since our knowledge about kinesins comes mainly from in vitro experiments, we are still far from understanding all the factors that impact their behavior in vivo.

Kinesin hydrolyzes one ATP molecule per step. But how the energy is transduced into effective work is still the subject of much debate, experimentation and modeling. The experimental trajectories show that the position of the bead fluctuates around a fixed position and eventually makes a \(8\) nm jump (in the case of kinesin) to the next fixed position, around which it will again fluctuate for some time. This results in a “staircase”-like trajectory as in Svoboda et al. (1993) (cf. Fig. 1 in SI).

Conceiving of the motor as diffusing along an abstract reaction coordinate in a chemical space does not lead to any insight about what is really happening with the motor and its cargo. In the mechanical reality it is the cargo that diffuses in a real physical space while the motor stays attached to the microtubule. Since the analytical results obtained under the assumptions of the proposed cargo-mooring mechanism correspond quite well with experimental data, we suggest that, when studying the intracellular active transport, it is necessary to concentrate not only on the motor, but also on the entire motor–cargo–environment system. This is because the viscosity of the environment and the cargo’s size are seen to significantly affect the motor’s dynamics.

In vivo kinesin transports vesicles of about \(50\) nm in diameter. The stalk that connects the motor and the vesicle has a length of about \(100\) nm. Vesicle and/or stalk can get entangled in the cytoskeletal network. Kinesin that “gets stuck” in such a way will more easily detach from the biopolymer that it is walking on. It is well known that under high loads the detachment rate of a kinesin motor increases (Jamison et al., 2010). There are even more evident clues for the regulatory function of the stalk. By changing the accessibility of the nucleotide pocket on kinesin’s heads, the stalk modulates the dynamics of the entire motor protein (Dietrich et al., 2008; Wong et al., 2009). This means that there is a possibility of “communication” between the cargo and the motor — communication that occurs via the stalk.

If the motor “feels” the impact of diffusive motion on a cargo, the generated strain may slow down or speed up chemical transitions (e.g. the release of ADP) that drive the motility. For example, the catalytic cycle could be accelerated if the cargo can more easily find a free space...
to diffuse a little bit closer towards the microtubule's plus end and thus trigger a next step. This idea gives a possible explanation of the Arrhenius-like dependency of kinesin's dynamics under load. In *in vitro* experiments the external force is applied on the cargo, so it is the cargo's diffusive motion that is affected directly. Since the information about the actual position of the cargo is transmitted to the motor protein via the stalk and since the orientation of the stalk (i.e. its deflection) modulates the chemical transitions in the motor domains, our proposed mooring model is plausible from a structural point of view.

The important role of thermal motion seems universal for many intracellular processes. Already in 1993 Peskin et al. showed how growing polymers may generate forces by rectifying Brownian fluctuations (Peskin et al., 1993). In our model, which actually is a version of the Brownian ratchet, the motor is effectively rectifying the Brownian motion of the cargo to which it is tethered. It is this rectification that requires the chemical energy from ATP hydrolysis. No real “pulling” occurs.

What makes our model different from typical ratchet models is the role it gives to the cargo. We no longer consider the diffusion of a single motor in an abstract chemical space. Instead, we consider the diffusion of a real cargo in a real laboratory space.

A future goal would be to present a more detailed model of the mooring mechanism. It should be extended to describe the action of other cytoskeletal motor proteins: dynein and myosin. Kinesin and dynein are structurally very different. The latter may take not only single 8 nm steps, but also double (16 nm) and triple (24 nm) ones (Reck-Peterson et al., 2012; 2006; Gennerich et al., 2007). Dynein is also load-sensitive, but in a different way than kinesin (Rai et al., 2013). Yet it appears that dynein is like kinesin in that it has also evolved a coordinating activity in the stalk (Ori-McKenney et al., 2010).

The collective behavior of several motors carrying one cargo should also be examined. Summarizing, what we propose is an ability of kinesins, and possibly other types of motors as well, to wait for the cargo to diffuse sufficiently far before they take an actual step. Our suggestion is a more extensive role for the stalk. Strain on the stalk will not merely be a signal for detachment. It can also trigger a next step.

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**Appendix A. Supplementary data**

Supplementary data associated with this paper can be found in the online version at [http://dx.doi.org/10.1016/j.tbi.2015.03.007](http://dx.doi.org/10.1016/j.tbi.2015.03.007).

**References**


1. Energetics of the active transport

The drag force $F_\gamma$ is given by $F_\gamma = \gamma v$, where $\gamma$ is the drag coefficient and $v$ is the average velocity of a probe. The observed average velocity of kinesin-1 is about 800 nm/s, corresponding to about one hundred 8 nm steps per second. The $L = 8$ nm represents the periodicity of the microtubule as well as the kinesin’s step length. Upon closer inspection it appears that the actual mechanical 8 nm step is completed, for an unloaded kinesin, in $t_m = 15 \mu$s, i.e. roughly 0.1 percent of the about 10 ms it takes to complete the entire catalytic cycle [1]. The energy needed to overcome the friction can be evaluated as follows. For a spherical cargo of radius $R = 250$ nm, as in [2], moving in an environment having the viscosity of water $\eta_w = 10^{-3} \text{ Pa s}$ one can write the Stokes’ law: $F_\gamma = 6\pi \eta_w RL/t_m$. The energy $E$ needed for a mechanical step is given by $E = F_\gamma L = \gamma v L$. In the discussed case it is
equal to:

\[ E = 6\pi\eta_w R L^2 t_m \approx 4.9 \, k_B T, \]  

at a temperature of \( T = 300 \) K. Here we have used the thermal energy unit \( k_B T \approx 4.1 \times 10^{-21} \) J. Taking the viscosity of water may be inaccurate and lead to an underestimated of the energy \( E \). Surface effects may be significant in the actual experiments, as discussed in [3].

In optical tweezer experiments, an external load \( F_L \) can be applied to the cargo. The kinesin-1 molecule then pulls the cargo to overcome both the viscous drag and the load force. With an added load force \( F_L \), Eq. (1) becomes:

\[ E = \left(6\pi\eta_w R \frac{L}{t_m} + F_L\right) L. \]  

The range of \( F_L \) in which the motor can still operate (that is, one can observe directed motion of a bead) is between \( 0 - 7 \) pN. For the load \( F_L = 6 \) pN we have (after the unit conversion) \( E \approx 16 \, k_B T \) for a bead of \( R = 250 \) nm. To drive the mechanochemical cycle forward in an efficient manner each major chemical transition is driven by an energy difference of about \( 2 \, k_B T \). This means that about 10 \( k_B T \) is necessary for the chemical part of the entire cycle [4, 5].

At physiological conditions the hydrolysis of one ATP molecule releases an energy of about \( 22 \, k_B T \). This energy must be sufficient to provide for both the mechanical and the chemical part of the cycle.

The calculations presented above set the lower limit for the \textit{in vitro} dynamics of a single kinesin motor. For example bigger beads, having a radius of around \( R = 500 \) nm, have also been used [1, 6]. For these the energy needed to overcome viscous friction is higher. Any modification of this scheme requires more energy input.

In their work, Holzwarth \textit{et al.} estimated the energy needed for pulling the cargo in the buffer solution and in the cytoplasm [7]. Using the data from [8], they concluded that, while active motion in an \textit{in vitro} buffer solution needs less energy than is provided by ATP hydrolysis, the situation changes drastically in a viscoelastic cytoplasm. We base our analysis on the more recent data on kinesin step time from [1] and we find grounds to revise accepted models for both the \textit{in vivo} and \textit{in vitro} case.

\( 26 \, k_B T \) is close to the energy released by ATP hydrolysis. However, as stated above, this estimate can change e.g. for bigger cargo or in the...
cytoplasm. It is possible that the mechanical transition lasts longer when bigger cargos need to be moved (see Fig. 3 of [1]). But this explanation creates more questions. The most serious one is why and how the displacement of the head would be affected by the size of the cargo? Power stroke and/or the diffusion of a head should not depend on the cargo after it is triggered. The only information that can be “transmitted” via stalk is the one about the relative position of the heads and the cargo. Obviously, the heads do not “know” the cargo’s size.

2. Why is $L/\langle t_{\text{step}} \rangle$ a better measure of velocity than $L/\langle 1/t_{\text{step}} \rangle$?

Experiments involving molecular motors often focus on measuring their velocities. One may think, that in order to obtain the average velocity from time measurements it is $L/t_{\text{step}}$ that should be averaged over many trials. We show below that this is not the case. In what follows next we assume that $t_r \gg x_m^2/D$. The right hand side of the inequality is a relaxation time that it takes the system to reach the stationary distribution of cargo’s position in the given range. We will use use this stationary distribution in the second stage of derivation. Otherwise formulas would become cumbersome and a proper choice of initial condition would be crucial.

Molecular motors move along microtubules making many (around 100) steps before reaching a goal. A correct measure of a mean velocity for a given trajectory would then be:

$$v = \frac{nL}{\sum_{i=1}^{n} t_{\text{step}}^{(i)}},$$

where $nL$ stands for a trajectory length and $t_{\text{step}}^{(i)}$ stands for a time of making the $i$-th step.

We underline here that both $v$ and $t_{\text{step}}^{(i)}$ are random variables. Additionally, thanks to our large $t_r$ assumption, $t_{\text{step}}^{(i)}$ are independent and identically distributed (i.i.d.).

For large $n$, however, we can treat $v$ as a number. Exploiting the law of large numbers we may write:

$$\frac{\sum_{i=1}^{n} t_{\text{step}}^{(i)}}{n} \approx \langle t_{\text{step}} \rangle.$$
The variance of a mean of i.i.d. random variables decreases as $n^{-1}$. Therefore, using Eq. (3) and (4), we finally arrive at the proper velocity measure:

$$v = \frac{L}{\langle t_{\text{step}} \rangle}.$$  

3. Weak dependence of MFPT on $x_m$

In a single step case we assume that $L/2 < x_m < L$. Therefore we may write $x_m = qL$, where $q \in (1/2, 1)$.

Let’s first analyze the MFPT for unloaded kinesin, i.e. for $F = 0$. It is easy to show, that $\langle t_{\text{step}} \rangle = t_r + L^2/(6\sigma^2 q)$. Since for typical parameters’ values (as in Fig. 2 of the main text) $L^2/(6Dt_r) \approx 3 \cdot 10^{-3}$, the MFPT is determined by $t_r$. It leads to the week dependence of the MFPT on $q$ (or $x_m$).

General formula for the MFPT can be rewritten into the form:

$$\langle t_{\text{step}} \rangle = t_r + \frac{2\gamma \sinh zL - zL}{Fz} \frac{1}{1 - e^{-2zLq}},$$  

(5)

where $z$ is proportional to $F$. The only dependence on $x_m$ appears in the denominator of the second term. As mentioned before, for small $F$ the first term dominates, while the second term monotonically increases with increasing $F$. For typical parameters’ values, like the one used to generate Fig. 2 of the main text, these two terms have similar values when $zL \approx 10$. It means that the denominator $1 - \exp[-2zLq] \approx 1$ and the dependence on $q$ disappears.

4. Numerical Simulations

Along with the analytical description, it is also possible to perform numerical simulations of the model, using the Langevin dynamics. Sample trajectory for a kinesin-like motor walking when no external force is applied is shown in Fig. 1.

**Numerical procedure**

In the simulation the position of the bead $x$ evolves in time as [3]:

$$x(t + \Delta t) = x(t) + \sqrt{2D\Delta t} \xi(t) - \frac{F_L(x(t))}{\gamma} \Delta t,$$  

(6)

where $\xi(t)$ is uncorrelated Gaussian random noise with zero mean and a standard deviation of one. $D$ is a diffusion constant, related to the drag
coefficient $\gamma$ through the Stokes-Einstein relation: $D = \frac{k_B T}{\gamma}$. $F_L(x(t))$ is the exerted load force. If the load comes from an attached bead in an optimal tweezer we have, effectively a hookean spring:

$$F_L(x(t)) = \kappa x(t),$$

(7)

where $\kappa$ is the tweezer’s stiffness. Equation 6 can now be rewritten in slightly different form:

$$x(t + \Delta t) = x(t) + \sqrt{2D\Delta t} \xi(t) - \kappa x(t) \frac{D}{k_B T} \Delta t.$$  

(8)

Since for a spherical bead of radius $R$ moving in a liquid of viscosity $\eta$ we can write $\gamma = 6\pi \eta R$ and assume the applicability of the Stokes-Einstein relation, we can calculate the diffusion coefficient of the cargo. It is worth noting that the value of $\kappa$ in real optical tweezers is calibrated in respect to the size of the trapped probe, in our case: of the cargo.

A time step should be sufficiently small to catch all the dynamical phenomena discussed here. Note that with decreasing $\Delta t$ the deterministic term in Eq. 8 decreases linearly, while the stochastic term decreases as $\sqrt{\Delta t}$.

If $\Delta t$ is too big, the load force will dominate the dynamics, leading to unrealistic force-velocity curves. Hence a proper choice of the integration time step is essential to catch a good first passage time statistics. In fact, it is a well-known problem that a finite time discretization leads to a systematic error of mean first passage time. Therefore the integration time step has to be of a few orders of magnitudes smaller than the smallest time scale, unless the correction is used [9].


Figure 1: Example of a model trajectory of a kinesin-like motor. Model parameters are: $\eta = 2.4 \times 10^{-3}$ Pa s, as in [3] and $R = 250$ nm and $F = 0$. The time step is $\Delta t = 7.5 \times 10^{-6}$ s and $L = 8$ nm. (Online version in color.)
Figure 2: Force-velocity curves for a backstepping kinesin-like motor with different values of the buffer’s viscosity and for different $t_r$, reflecting different ATP concentrations. ($R = 250$ nm, $x_m = 8$ nm, $t_r = 10$ ms $p_B = 0.07$ and $L = 8$ nm) Extended (also for forward loads) comparison of our model with experimental data from [10]. The non-monotonic shape of the curve for higher viscosity fits well to the experimental data. (Online version in color.)
Figure 3: **Two different types of experiments involving kinesin motors.** In the single molecule motility assays the bead of a known diameter is carried by one motor which walks along microtubule. Motor’s stalk can wiggle around the point in which the two neck linkers coming from the heads intertwine (green dot). Between steps, the cargo is subject to a diffusive motion. In a gliding assay kinesins are stuck to the bottom of a glass dish with the heads sticking into the solution. A microtubule that “floats” on these heads is seen to move in a “wormlike” fashion. Again, the stalk can wiggle around the point in which the two neck linkers coming from the heads intertwine (green dot). Thus, microtubules play a role of the cargo in the mooring model. Solid and dashed blue lines show the extreme positions of the motor. Black and grey circle in the left panel show the extreme positions of the bead. Orange and orangish rectangles in the right panel show the extreme positions of the microtubule. (Online version in color.)
I. INTRODUCTION

The biological world offers many instances of systems where identical oscillators synchronize their phases due to a coupling. Examples include large groups of flashing fireflies [1], pacemaker cells in the heart [2], ensembles of neurons [3], a large number of pedestrians on a bridge [4], and metabolic oscillations of yeast cells in a suspension [5]. These kinds of systems analytical treatment commonly becomes manageable only when the coupling is small and can be mathematically treated as a perturbation [5–7]. Going from incoherent behavior to synchronized oscillators often takes the form of a phase transition as the coupling parameter is increased in value [6].

In this article we study the cyclic, four-state system that is depicted in Fig. 1. We assume a large total population $N$ in the system and $p_i(t) = n_i(t)/N$, where $n_i(t)$ represents the population in state $i$ at time $t$. This means that $p_i(t)$ can be associated with the probability to be in state $i$ at time $t$. As the transitions are irreversible, the system is far from equilibrium. For the rates $k_i = k_{i-1} + k_{i+1}$ we take

$$k_i = k_0 \exp[\alpha(p_{i+1} - p_{i-1})],$$

where $\alpha$ is a non-negative parameter that couples the probabilities (i.e., populations) in the different states.

The competition between diffusion and synchronization in the system depicted in Fig. 1 can be understood as follows. For $\alpha = 0$ there is no coupling and the stochasticity in the exponentially distributed transition times will result in a relaxation to $p_1 = p_2 = p_3 = p_4 = 1/4$ whatever the initial conditions are. However, if $\alpha > 0$ and if we have $p_1 = p_2 = \frac{1}{2}(1 + \varepsilon)$ and $p_3 = p_4 = \frac{1}{2}(1 - \varepsilon)$ (see Fig. 2) and at the same time $k_4 > k_2$ (see Fig. 1), then the diffusive effects can be counteracted. In that case the speeded up rate out of state 4 and the slowed down rate into state 3 will make the pulse in states 1 and 2 keep its shape.

How the coupling strength in Eq. (1) provides the counteraction to the diffusion can be seen as follows. Assume a pulse (high probability) concentrated in one state. If there is a more populated state ahead of the pulse as compared to behind, i.e., $p_{i+1} > p_{i-1}$, then the exponent in Eq. (1) is positive and the forward-moving-rate of the pulse is speeded up. The pulse will then swallow the state ahead. If, on the other hand, a less populated state is ahead and a more populated state is behind, i.e., $p_{i+1} < p_{i-1}$, then the forward rate of the pulse is slowed down and the population behind can merge with the pulse. In both cases there is a pull towards the pulse and the pulse acts like an absorber. The mechanism is somewhat mindful of the well-known Burgers’ shock [8]. Ultimately, in a steady state, diffusion and accumulation balance each other out and the pulse travels around the cycle like a soliton in a time of approximately $4/k_0$.

A three-state version of the setup in Fig. 1 was numerically and analytically studied in Refs. [9–13]. In these works a mean-field approximation was applied and a Hopf bifurcation to synchronized behavior was found. It was furthermore analyzed how the system responds to disorder introduced on the level of the transition rates, demonstrating the rise of synchrony in a population of nonidentical units.

Four-state cycles occur frequently in biological systems. They are seen, for instance, in the ligand-gated and voltage-gated ion channels that maintain homeostasis and facilitate signal transduction [14]. A four-state system similar to the one in Fig. 1 was derived and studied in Ref. [15].

Interestingly, it has been described how different states of an ion channel involve different shapes that imply different stress and strain on the surrounding membrane [16]. Channels that operate in close proximity to each other could thus chemomechanically couple their kinetics. Our coupling parameter, $\alpha$, could then reflect the strength of this interaction. We will come back to this idea in Sec. VI.

The setup of Fig. 1 gives rise to a three-dimensional system of coupled ODEs. A linear analysis [7] around the fixed point $p_1 = p_2 = p_3 = p_4 = 1/4$ leads to eigenvalues

$$\lambda_1 = -2k_0 \quad \text{and} \quad \lambda_{2,3} = \frac{1}{2}k_0(\alpha - 2) \pm i(\alpha + 2).$$

(2)
So, in the $\lambda_1$ direction, the fixed point $p_1 = p_2 = p_3 = p_4 = 1/4$ is an attractor. In the plane perpendicular to this axis, the real part of the complex eigenvalue changes sign, from negative to positive, at $\alpha = 2$, i.e., there is a supercritical Hopf bifurcation at $\alpha = 2$.

It is possible to rigorously derive how the amplitude $\epsilon$ depends on $\alpha$ with an expansion of the aforementioned 3D system of coupled ODEs. But here we give a simple and intuitive derivation to come to the same end result. Further on, we will use the idea behind our simple derivation as a guide to draw conclusions about entropy flow in the system.

Consider the scheme drawn in Fig. 2. For the pulse to stay compact and to not change shape, we need the flow $J_{4 \to 1}$ into the pulse to be equal to the flow $J_{2 \to 3}$ out of the pulse, i.e., $J_{4 \to 1} \approx J_{2 \to 3}$. Writing this as $p_2 k_2 \approx p_1 k_1$, substituting $p_2 = (1 + \epsilon)/4$, $p_4 = (1 - \epsilon)/4$, and taking the expressions for the rates $k_2$ and $k_4$ given in Eq. (1), we obtain:

$$\frac{1}{4}(1 + \epsilon) k_0 \exp[\alpha (p_3 - p_1)] \approx \frac{1}{4} (1 - \epsilon) k_0 \exp[\alpha (p_1 - p_3)],$$

which leads to

$$\frac{1 + \epsilon}{1 - \epsilon} \approx e^{\alpha \epsilon}.$$  (4)

Solving for $\alpha$, we find $\alpha \approx (1/\epsilon) \ln [(1 + \epsilon)/(1 - \epsilon)]$. Next, expanding this expression in $\epsilon$, we get $\alpha \approx 2 + \frac{3}{2} \epsilon^2 + O(\epsilon^4)$. Neglecting the higher-order contributions, we express the pulse amplitude $\epsilon$ in terms of the coupling strength $\alpha$ as:

$$\epsilon \approx \sqrt{\frac{3}{2} (\alpha - 2)}.$$  (5)

In other words, there is a phase transition to a synchronized state at $\alpha = 2$. In Fig. 3 the order parameter, which will turn out to be linearly related to the amplitude $\epsilon$, is depicted as a function of $\alpha$. Equation (5) appears to be in good agreement with the results of simulations. The square-root or pitchfork shape at the bifurcation is found in a simple system such as $\dot{x} = x(\mu - x^2)$, but it also appears more generally in more complicated dynamical systems [17,18].

In Sec. II we study the order parameter that is associated with the transition to the synchronized state. In Sec. III we account for the entropy and the entropy production of the system.

Going back to the setup depicted in Fig. 1, we can identify the sequence $\ldots 1234123412 \ldots$ with a continuous coordinate $x$. We let one unit of $x$ correspond to the distance between two neighboring states.

Suppose that the counterclockwise transition rates in the system of Fig. 1 were all equal to a constant $k_0$. The transition time from one state into the next state then follows an exponential distribution with an average time and a standard deviation both equal to $1/k_0$. To take $n$ steps around the four-step cycle takes an average time of $n/(1/k_0)$. For subsequent steps, the variances $(1/k_0^2)$ add up. So with $n$ steps we have

![FIG. 1. The four-state system that is the subject of this article. The $j \to j + 1$ transition rate depends on the populations in the states $j - 1$ and $j + 1$. We find a phase transition to a synchronized state as the coupling strength $\alpha$ is increased.](image1.png)

![FIG. 2. For a sufficiently large value of the parameter $\alpha$ synchronization occurs in the setup of Fig. 1. (a) The ensuing pulse maintains its shape as it travels around the loop. (b) In the bottom graph the height of the bar indicates the probability to be in the corresponding state.](image2.png)

![FIG. 3. The order parameter $r$ as a function of the coupling strength $\alpha$. The solid line shows the theoretical prediction [cf. Eq. (10)]. The black dots derive from the numerical solution of the system of ODEs that is associated with the kinetics of the system depicted in Fig. 1. We used $k_0 = 1$ and a time step of $\Delta t = 0.001$. The stars represent the results of stochastic simulations with $N = 1000$ units. For each value of $\alpha$ the stochastic simulation ran for $t = 150$ units of time. Error bars indicate the standard deviation.](image3.png)
a standard deviation of $\sqrt{n}(1/k_0)$. In other words, if in time $T$ the system takes an average of $n$ steps, then there is an accompanying standard deviation of $\sqrt{n}$ steps.

In a time $T \gg 1/k_0$ the pulse thus travels an average of about $k_0 T$ units of distance with a standard deviation of $\sigma = \sqrt{k_0 T}$ units of distance. For sufficiently many steps we have, by the central limit theorem, a spreading Gaussian for the probability density that travels in the positive direction:

$$\phi(x,t) = \frac{1}{\sqrt{2\pi k_0 t}} \exp\left[\frac{-(x - k_0 t)^2}{2k_0 t}\right].$$

With a constant drift velocity $k_0$ and a linearly increasing variance $\sigma^2 = k_0 t = 2D t$, we can consider this spreading Gaussian to be the solution of the following diffusion equation with diffusion coefficient $D = k_0/2$.

$$\phi_t = -k_0 \phi_x + D \phi_{xx},$$

where $\phi = \phi(x,t)$ and the subscripts denote partial differentiation, i.e., $\phi_t = \partial_t \phi$, $\phi_x = \partial_x \phi$, and $\phi_{xx} = \partial_x^2 \phi$. Dimensionalizing the time, Eq. (7) can be brought to the simple form

$$\phi_t = -\phi_x + \frac{1}{2} \phi_{xx}.$$  

The speeded up transition behind the pulse and the slowed down transition right ahead of the pulse is what counteracts the diffusive spread. In Sec. IV, we will show how the synchronization adds a nonlinearity to Eq. (7) and how the equation then effectively reduces to a Burgers’ equation. In Sec. V we will derive the order parameter and entropy production associated with this partial differential equation (PDE). In Sec. VI we discuss our findings.

## II. ORDER PARAMETER AS A FUNCTION OF COUPLING STRENGTH

In, for instance, the study of the Kuramoto model, the degree of synchronization is commonly described with the help of an order parameter [19]. In our case the order parameter takes the following form:

$$re^{i \psi} = \sum_{n=0}^{3} p_n e^{i n \alpha}.$$  

(8)

Obviously, $r$ grows from 0 to 1 as the degree of synchronization increases. For the situation in the top panel of Fig. 2, it is easily inferred that

$$re^{i \psi} = \left(\frac{e}{2\sqrt{2}}\right)^{i \pi/4}.$$  

(9)

As the pulse moves around the loop depicted in Fig. 1, $re^{i \psi}$ moves around the complex plane with the same time period.

With Eqs. (5) and (9) we derive for the order parameter as a function of $\alpha$

$$r = \begin{cases} 0 & \text{if } \alpha < 2 \\ \frac{1}{\sqrt{2\sqrt{\alpha - 2}}} & \text{if } \alpha > 2. \end{cases}$$

(10)

Figure 3 shows two simulation results and the theoretical prediction of Eq. (10). For one simulation we numerically solved the ODEs that describe the kinetics of the setup shown in Fig. 1. For the second simulation we performed stochastic simulations using a total of $N = 2000$ coupled units in the entire system and following a Gillespie algorithm [20,21]. In a Gillespie algorithm two random numbers are drawn at each time step. These numbers are used to select (i) the unit that changes its state according to the scheme from Fig. 1 and (ii) the time this transition would take. New values of transition rates [cf. Eq. (1)] and order parameter $r$ are calculated after each time step. The simulations affirm the presence of a phase transition at $\alpha = 2$.

If the total population in the system in Fig. 1 is a finite number $N$, then we will not find $r = 0$ at $\alpha < 2$. This is due to the stochasticity in the transitions. We will next estimate what the bottom line value for $r$ is, i.e., the value that we expect for $0 < \alpha < 2$. Taking a total population of $N$, we see that a measurement will, on average, give the value $p = \frac{0.25}{N}$ for each state. In that case the associated variance and standard deviation are $\sigma_1^2 = p(1-p) = \frac{3}{16}$ and $\sigma_1 = \sqrt{\frac{3}{4}}$, respectively. With a total population of $N$, we have $N/4$ for the average population in each state. For the result of many subsequent and independent draws, the variances add up. So we have $\sigma_N^2 = Np(1-p) = \frac{3N}{16}$ and $\sigma_N = \sqrt{\frac{3N}{4}}$. For the order parameter we thus expect an outcome:

$$r e^{i \psi} = \sum_{n=0}^{3} \frac{1}{4} \left(\pm \frac{\sqrt{3}}{4\sqrt{N}}\right) e^{i n \pi/4}.$$  

(11)

The $n = 0$ and $n = 2$ contribution to the sum are multiplied with, respectively, $e^{0i \pi} = 1$ and $e^{i \pi} = -1$. Adding up the averages and the variances of these contributions, we obtain zero for the average real part and $\tilde{\sigma}_N = \frac{3/(8N)}{2}$ for the associated variance. This leads to $\tilde{\sigma}_N = \sqrt{\frac{3}{2N}}$ for the standard deviation. The $n = 1$ and $n = 3$ contributions in Eq. (11) give the same result for the imaginary part. The average value of both real and imaginary part is zero. But the average norm of both real part and imaginary part is not. With a zero-average Gaussian distribution, the average norm is:

$$\rho_{re} = \rho_{im} = \int_0^\infty \frac{2x}{\sqrt{2\pi} \tilde{\sigma}_N} \exp\left[-x^2/(2\tilde{\sigma}_N^2)\right] dx = \frac{2\tilde{\sigma}_N}{\sqrt{2\pi}}.$$  

(12)

For the average value of $r$ we thus come to the result

$$\langle r \rangle \approx \sqrt{\rho_{re}^2 + \rho_{im}^2} = \sqrt{\frac{3}{2\pi N}}.$$  

(13)

We performed a Gillespie simulation with $N = 10000$ and $\alpha = 0$. It led to $r = 0.0090 (\pm 0.0047)$. This is in good agreement with the $r = 0.0069$ that Eq. (13) predicts. Also for $N = 100$ and $N = 1000$ we obtained values for $r$ at $\alpha = 0$ that confirm Eq. (13). The values observed in Fig. 3 for $\alpha < 2$ are well above the $r = 0.022$ that Eq. (13) predicts for $N = 1000$. This is because stochastic fluctuations die out ever more slowly as $\alpha$ is brought from 0 to 2. Simulations closer to the $N \rightarrow \infty$ and $t \rightarrow \infty$ limits should give results closer to the theoretical value of Eq. (13). The Gillespie simulations, however, require a lot of computation time.
III. ENTROPY PRODUCTION OF THE MOVING SYNCHRONIZED PULSE

Following Shannon [22], we take as the entropy per unit in the setup of Fig. 1 and Eq. (1):

\[ S = -\sum_{i=1}^{4} p_i \ln p_i. \quad (14) \]

It is obvious that the situation with the pulse, as shown in Fig. 2, has a lower entropy than the \( p_1 = p_2 = p_3 = p_4 = 1/4 \) situation for which the entropy is maximal. When going from the situation on the left-hand side of Fig. 2 to the situation on the right-hand side of Fig. 2, there is no entropy change according to Eq. (14). However, the pulse has moved forward and what we have effectively done is performing a chemical cycle to move the pulse forward by one unit. We have converted chemical energy into mechanical work and there should be an associated entropy production. In this section we will account for the different entropy contributions.

Realizing that \( \sum p_i = 0 \) as \( \sum p_i = 1 \) at all times, we infer from Eq. (14):

\[ \dot{S} = -\sum_{i=1}^{4} p_i \ln p_i. \quad (15) \]

This sum will not generally turn out equal to zero. This is because the term \( \ln p_i \) adds weights to the flows \( p_i \) and, ultimately, the flows for small \( p_i \) are weighted more heavily.

We take the situation on the left side of Fig. 2, i.e., \( p_1 = p_2 = (1+\varepsilon)/4 \) and \( p_3 = p_4 = (1-\varepsilon)/4 \). We next take \( p_i = J_{i-1,i} = J_{i,i+1} = k_{i-1} p_{i-1} - k_i p_i \) and substitute into Eq. (15) the values for the rates \( k_i \) [cf. Eq. (1)]. We also substitute the \( \alpha \approx (1/\varepsilon) \ln [(1+\varepsilon)/(1-\varepsilon)] \) that we found in the Introduction after Eq. (4). Summing over the four states and after some algebra it is then readily derived that \( \dot{S}_{\text{synch}} = 0 \).

This result is expected as the mechanism leading to Eq. (4) was formulated to lead to a pulse that stays intact.

In the derivation in the previous paragraph leading to \( \dot{S}_{\text{synch}} = 0 \), we did not yet take the stochasticity into account. Each individual transition is a stochastic event and the result of a draw from an exponential distribution. Even with the irreversible transitions in Fig. 1, there is diffusion. The stochasticity in the transitions is giving us the spread of the distribution described in the Introduction and it produces temperaturelike behavior.

In the Introduction we saw that our system has an effective diffusion coefficient of \( D = k_0/2 \). Taking Fick’s diffusion law \( \langle b \cdot P \rangle = D \partial^2 P \) and writing it in the discrete form that is appropriate for the system in Fig. 1, we have

\[ p_i = \frac{k_0}{2} (p_{i+1} - 2p_i + p_{i-1}). \quad (16) \]

When this equation is applied to the situation depicted in Fig. 2, it is found that \( p_1 = p_2 = -\varepsilon k_0/4 \) and \( p_3 = p_4 = \varepsilon k_0/4 \). With again \( p_1 = p_2 = (1+\varepsilon)/4 \) and \( p_3 = p_4 = (1-\varepsilon)/4 \) and applying Eq. (15), the entropy production by the diffusion, at the lowest order in \( \varepsilon \), is found to be

\[ \dot{S}_{\text{diff}} = k_0 \varepsilon^2 = \frac{1}{2} k_0 (\alpha - 2) = 2k_0 r^2. \quad (17) \]

We thus see that, for \( \alpha > 2 \), the moving pulse is associated with a net production of entropy. The entropy production increases with the pulse amplitude \( \varepsilon \) and the associated order parameter \( r \). It needs to be emphasized here again that the entropy production of the moving pulse is not associated with a deterioration of the moving pulse. The pulse stays intact. But as was already explained in the Introduction, it is the fluctuations in the speed of the pulse that make the process nondeterministic and expand the available phase space. Of course, with \( p_1 = p_2 = p_3 = p_4 = 1/4 \) there is no pulse and no such phase space expansion occurs.

For \( 0 < \alpha < 2 \) we have \( p_1 = p_2 = p_3 = p_4 = 1/4 \) and an associated entropy of \( S = 2 \ln 2 \) [cf. Eq. (14)]. With the distribution shown in Fig. 2, the entropy is obviously smaller. Taking \( \ln(1 \pm \varepsilon) \approx \pm \varepsilon - \varepsilon^2/2 \) for the lowest orders in \( \varepsilon \), we find that for \( \alpha > 2 \) and small \( \varepsilon \)

\[ S \approx 2 \ln 2 - \varepsilon^2/2. \quad (18) \]

In other words, the formation of the pulse represents a symmetry breaking and a self-organized establishment of order. However, as we will show in the next paragraphs, the pulse allows the system as a whole to produce more entropy.

The transitions in Fig. 1 dissipate energy and produce entropy. This is obvious for the case of chemical transitions; the more irreversible such a transition, i.e., the larger the ratio of the forward and backward transition rate, the more entropy the forward transition produces.

If \( p_1 = p_2 = p_3 = p_4 = 1/4 \), each transition rate in Fig. 1 equals \( k_0 \) and the average turnover of the system equals \( \sum_{i=1}^{4} p_i k_i = k_0 \). For the situation sketched in Fig. 2 there are two transition rates that take on the value \( k_0 \exp [\alpha \varepsilon/2] \) and two transition rates that take on the value \( k_0 \exp [-\alpha \varepsilon/2] \). This leads to an average transition rate of \( (k) = k_0 \cosh [\alpha \varepsilon/2] \).

The functions \( f(x) = e^x \) and \( f(x) = e^{-x} \) have positive second derivatives, i.e., they are concave up. It is essentially because of Jensen’s theorem \( \langle f(x) \rangle > f(\langle x \rangle) \) if \( f''(x) > 0 \), see Ref. [23] that we have \( \langle k \rangle > k_0 \).

More importantly, also the average turnover for the entire system, i.e., \( \sum_{i=1}^{4} p_i k_i \), evaluates to \( k_0 \cosh [\alpha \varepsilon/2] \) with the synchronized pulse as in Fig. 2.

We observe that not having a flat (maximal entropy) distribution helps the open system to ultimately achieve a higher turnover. The lower-entropy synchronized pulse is essentially an investment; eventually it acts like a catalyst for an enhanced turnover and increased entropy production rate. In this sense the pulse is a self-organized dissipative structure as described by Prigogine [24].

IV. SYNCHRONIZED PULSE AS A BURGERS’ SHOCK

When increasing the number of states in a cycle as in Fig. 1, we encounter ever larger systems of equations. Instead of taking this route with all its analytical and numerical complications, it may be more fruitful to examine the other end. We thus take a cycle with a large number of states \( n \) and go to the continuum limit that we already briefly discussed in the Introduction.

In the cycle depicted in Fig. 4 there are \( n \) states. In the vicinity of the phase transition we have \( \phi(j,t) = O(1/n) \), for the probability in each state \( j \). For a smooth, small-amplitude pulse (in the next section we explore a sinusoidal modulation)}
we have \((\phi_{\text{max}} - \phi_{\text{min}}) = O(1/n)\) for the maximal and minimal \(\phi\). For the horizontal distance between the maximum and the minimum, we, moreover, have \(|x_{\text{max}} - x_{\text{min}}| = O(n)\). This leads to \(\phi_x = O(1/n^2)\) and \(\phi_{xx} = O(1/n^3)\).

Next we enter the \(\phi\) dependence of \(k\) as given in Eq. (1). For the continuous equivalent of Eq. (1) it is found that

\[
k = k_0 \exp[\alpha \phi(x + 1, t) - \phi(x - 1, t)].
\]

For a slowly varying \(\phi(x, t)\) a simple, 1st order approximation is justified and gives \(\phi(x + 1, t) - \phi(x - 1, t) = 2\phi_x(x, t)\). After \(\exp[\alpha \phi(x + 1, t) - \phi(x - 1, t)] \approx 1 + 2\alpha \phi_x(x, t)\), we are thus led to the following nonlinear PDE:

\[
\phi_t = -k_0 \phi_x - 2k_0 \alpha \phi_x^2 + \frac{1}{2} k_0 \phi_{xx} + k_0 \alpha \phi_x \phi_{xx}.
\]

We first notice that \(\phi_x \phi_{xx}\) term is smaller than the other terms on the right-hand side and we therefore neglect it. With a Galilean transformation the \(k_0 \phi_x\) term can be removed. To go to \(\psi'(x', t) = \phi(x, t)\), where \(x' = x - k_0 t\), we formally take

\[
x' = \mu(x, t) = x - k_0 t \quad t' = \nu(x, t) = t.
\]

With the standard Jacobian matrix approach we then have:

\[
\begin{pmatrix}
\phi_x \\
\phi_t
\end{pmatrix} =
\begin{pmatrix}
\mu_s & \nu_s \\
\mu_t & \nu_t
\end{pmatrix}
\begin{pmatrix}
\psi_x \\
\psi_t
\end{pmatrix},
\]

where \(\mu_s = \partial x'/\partial x = 1\), \(\nu_s = \partial t'/\partial x = 0\), \(\mu_t = \partial x'/\partial t = -k_0\), and \(\nu_t = \partial t'/\partial t = 1\). From here it is inferred that \(\phi_x = \psi_x\) and \(\phi_t = \psi_t - k_0 \psi_x\). Substituting this, dropping the primes, and absorbing \(2k_0\) into the timescale, we obtain for the new equation in the new inertial frame

\[
\psi_t = -4\alpha \psi_x^2 + \psi_{xx}.
\]

This is actually a well-known equation. It is generally known as the potential Burgers’ equation [25]. By taking the partial derivative with respect to \(x\) on both sides and next taking \(\psi_t = \varphi\), the original Burgers’ equation is retrieved for \(\varphi = \psi(x, t)\):

\[
\varphi_t = -8\alpha \varphi \varphi_x + \varphi_{xx}.
\]

The potential Burgers’ equation, Eq. (22), is actually linearizable. The linear diffusion equation \(\eta_t = \eta_{xx}\) turns into the above Eq. (22) after taking \(\eta(x, t) = \exp[-4\alpha \psi(x, t)]\).

Equation (23) supports soliton solutions (cf. Fig. 5):

\[
\varphi(x, t) = \frac{\varphi_- + \varphi_+}{2} - \frac{\varphi_- - \varphi_+}{2} \tanh[2\alpha(\varphi_- - \varphi_+)(x - vt)],
\]

where \(v = 4\alpha(\varphi_- + \varphi_+)\). The quantity \((2\alpha(\varphi_- - \varphi_+))^{-1}\) is known as the thickness of the shock. A good explanation of these dynamics is also found in Ref. [26].

Integrating the solution for \(\psi(x, t)\), we obtain \(\psi(x, t)\):

\[
\psi(x, t) = \left(\frac{\varphi_- + \varphi_+}{2} - \frac{1}{4\alpha} \ln[cosh[2\alpha(\varphi_- - \varphi_+)](x - vt)]\right) + \psi(0, t),
\]

where \(\psi(0, t)\) is the constant of integration.

It is important to notice that the phase transition that was identified in the discrete system is not present in the above continuous version. There is no phase transition or bifurcation associated with the soliton depicted in Fig. 5 and described in Eqs. (22)–(25). For \(\alpha = 0\), Eqs. (22) and (23) reduce to an ordinary diffusion equation. As \(\alpha\) is increased from zero, what we see is the development of an ever steeper shock front in the top panel of Fig. 5 and an ever more pronounced synchronization hump in the bottom panel of Fig. 5. With an increasing \(\alpha\), also the speed of the pulse increases. Unlike for the case of the three-state and four-state system, the continuous
case offers no relation between the amplitude of the pulse, \( \langle \phi_0 - \psi > \), and the coupling parameter \( \alpha \).

V. ENTROPY PRODUCTION AND ORDER PARAMETER AS THE SOLITON PROPAGATES

For ordinary diffusion without drift, we have for a particle that starts at \( x = 0 \) at \( t = 0 \):

\[
\Phi(x,t) = \frac{1}{\sqrt{4\pi D t}} e^{-\frac{x^2}{4Dt}}. 
\]  

(26)

For this case the entropy \( S(t) \) is readily evaluated as

\[
S(t) = -\int_{-\infty}^{+\infty} \Phi(x,t) \ln \Phi(x,t) \, dx
\]

\[
= \int_{-\infty}^{+\infty} \frac{e^{-\frac{x^2}{4Dt}}}{\sqrt{4\pi D t}} \left( \frac{x^2}{4Dt} + \ln \sqrt{4\pi D t} \right) \, dx
\]

\[
= \frac{1}{2} \left[ 1 + \ln(4\pi D t) \right]. 
\]

(27)

which leads to \( S(t) = 1/(2t) \). That the diffusion entropy follows \( S \propto \ln t \) can also be understood from more intuitive arguments. Boltzmann’s definition of the entropy, \( S = k \ln \Omega \), tells us that the entropy is proportional to the logarithm of the volume \( \Omega \) of the available phase space. If we identify this available phase space volume with the standard deviation of the Gaussian, then \( \Omega \approx (x^2) \times t \) for normal diffusion, we have \( S \propto \ln \sqrt{t} = \frac{1}{2} \ln t \).

It should be realized that Eqs. (14) and (15) come with a little caveat when we go to continuous \( x \). For a periodic \( \psi(x,t) \) (period \( L \)) the entropy is maximal when the probability distribution is flat, i.e., \( \Phi_0 = 1/L \). Our definition of the entropy is the commonly used Kullback-Leibler distance, which effectively measures how far away we are from this flat distribution [28]:

\[
S(t) = -\int_{x_0}^{x_0 + L} \Phi(x,t) \ln \frac{\Phi(x,t)}{\Phi_0} \, dx. 
\]

(28)

By normalizing the period, i.e., \( x \rightarrow x/L \) and \( \Phi \rightarrow L \Phi \), we can work with entropy as simply \( S = -\int \Phi \ln \Phi \, dx \), where the integration runs over the unity period.

Equation (22), the potential Burgers’ equation, was arrived at after a linear Galilean transformation. It is easy to show that no entropy production is involved in a linear traveling wave. Take the PDE \( \Phi_t = \Phi_x \). To obtain the rate of change for the entropy we perform

\[
\frac{dS}{dt} = -\frac{d}{dt} \int \Phi \ln \Phi \, dx = -\int \Phi(\ln \Phi + 1) \, dx, 
\]

(29)

where the integration runs over one period. For \( \Phi \) periodic in \( x \) we obviously have \( \int \Phi \, dx = 0 \) when the integration runs over one spatial period. For the other term in Eq. (29) we derive

\[
\int \Phi \ln \Phi \, dx = \int \Phi_x \ln \Phi \, dx = \int \ln \Phi \, d\Phi = (\Phi(\ln \Phi - 1) = 0. 
\]

(30)

So the Galilean transformation that removed \( \phi_0 \) from the right-hand side of Eq. (20) is without consequence for the entropy analysis.

Next we take Eq. (22) and write down for the entropy production

\[
\frac{dS}{dt} = -\frac{d}{dt} \int \psi \ln \psi \, dx 
\]

\[
= -\int \psi (\ln \psi + 1) \, dx 
\]

\[
= \left( 4\alpha \psi_x^2 - \psi_{xx} \right) \ln \psi \, dx. 
\]

(31)

We will evaluate both terms.

The integrand \( \psi_x^2 \ln \psi \) is associated with the shock formation. The integrand \( \psi_{xx} \ln \psi \) is associated with the diffusion. The shock term is supposed to give a negative contribution to the entropy production as it accumulates probability density and drives the system away from the homogeneous spread. As we saw before, diffusion produces entropy. So \( \psi_{xx} \ln \psi \) should be positive.

The integral \( \int \psi_x^2 \ln \psi \, dx \) cannot be further simplified. So we have

\[
\dot{S}_{\text{shock}} = 4\alpha \int \psi_x^2 \ln \psi \, dx. 
\]

(32)

The diffusion term can be simplified through integration by parts

\[
\dot{S}_{\text{diff}} = -\int \psi_{xx} \ln \psi \, dx = \int \psi_x d(\ln \psi) = \int \psi_x^2 \left( \frac{1}{\psi} \right) \, dx. 
\]

(33)

As \( \psi_x^2 \) and \( 1/\psi \) are both positive, the integral (33) will indeed always be positive.

The most obvious approximation for the shape of the synchronized pulse in the continuous domain would be a harmonic one. We take a unit period and a small \( \varepsilon \) for the amplitude of the pulse in the vicinity of the phase transition. For the \( t = 0 \) situation we take

\[
\psi(x,0) = 1 + \varepsilon \cos(2\pi x). 
\]

(34)

With Eq. (34) we have at the lowest order for the pertinent terms [cf. Eqs. (32) and (33)]:

\[
\psi_x^2 \approx 4\pi^2 \varepsilon^2 \sin^2(2\pi x), 
\]

\[
\ln \psi \approx \varepsilon \cos(2\pi x) - \frac{\varepsilon^2}{2} \cos^2(2\pi x), 
\]

and

\[
\frac{1}{\psi} \approx 1 - \varepsilon \cos(2\pi x). 
\]

(35)

With these approximations we are led to

\[
\dot{S}_{\text{shock}} \approx -4\pi^2 \varepsilon^4 \quad \text{and} \quad \dot{S}_{\text{diff}} \approx 2\pi^2 \varepsilon^2. 
\]

(36)

We thus observe that for the propagating pulse the negative entropy production occurs at a higher order in the small parameter \( \varepsilon \) than the entropy production. The propagation of a synchronized pulse, we conclude, is associated with a net production of entropy. This is what we would expect as the irreversible chemical transitions that drive the cycle in Fig. 4 dissipate energy and imply a production of entropy.

Recently Nordenfelt applied similar reasoning and techniques to evaluate the entropy production for a synchronizing system described by a PDE [29]. His system, however, is more
complicated as it also involves a continuum of characteristic frequencies.

The order parameter in case of a harmonic approximation as in Eq. \((34)\) is easily evaluated. We find it to be linear in \(\varepsilon\):

\[
r = \int_{-1/2}^{1/2} (1 + \varepsilon \cos[2\pi x]) e^{i2\pi x} \, dx = \frac{\varepsilon}{2}.
\]

The continuous case that we studied in this and the previous section differs from the finite-state model in that there is no emerging simple relation between the amplitude of the pulse and the coupling parameter \(\alpha\). There is also no phase transition for the continuous case. In the case of \(\alpha = 0\) there is no synchronization and the system in Fig. 4 moves in the counterclockwise direction making on average \(k_0\) transitions per unit of time. After the Galilean transformation that we performed [cf. Eq. \((21)\)], the frame moves around the circle with the same speed \(k_0\). It is in that frame that we were led to Fig. 5 and Eqs. \((22)\) and \((23)\).

The variable \(\psi(x,t)\) [cf. Eq. \((22)\)] represents a probability density and therefore has to be positive everywhere. The variable \(\psi(x,t)\) must, furthermore, give unity when integrated over the entire cycle in Fig. 4. But within these restrictions it is still possible, for \(\alpha > 0\), that multiple synchronizations are present. Figure 5 and the solution Eq. \((25)\) make clear how the cusps can be identified with moving solitons. Figure 6 shows how these solitons can propagate at different speeds and in either direction. In other words, the coupling can both speed up and slow down the turnover rate in Fig. 4. Because of the nonlinear character of Burgers’ equation, no simple superposition of solutions applies. Different solitons interact when they collide. If the diffusive part of Burgers’ equation is small relative to the nonlinear term, the solitons appear to collide inelastically and mimic the behavior of sticky gas particles [30].

Schemes with subsequent conformational changes as in Fig. 1 are, of course, simplifications. Going back to the ion channels that we mentioned in the Introduction, we have to realize that an ion channel is a big complicated protein and that what we take to be a discrete transition is, in actuality, continuous diffusive motion through a corridor in a many-dimensional conformational space. An 18-state model has been applied to describe the activity of the \(\alpha 1 \beta\) glycine receptor [31]. At that point discrete models have lost their computational simplicity and intuitiveness. It may well be that biological reality is ultimately better described with a continuous-type model as presented in Secs. IV and V.

**VI. DISCUSSION**

In this article we first studied how diffusion and accumulation compete in a four-state system as described in Fig. 1 and Eq. \((1)\). Like in a three-state system \([9–13]\), a sharp phase transition is observed as the coupling parameter \(\alpha\) is varied. We next went to an \(n\)-state system and studied an \(n \to \infty\) continuum limit.

There is an ongoing and intense research interest in the synchronization behavior of systems like the one in Fig. 1 \([32,33]\). We chose our rates \(k_i\) to depend on the populations \(p_i\) as in Eq. \((1)\), because in chemical kinetics energies commonly are linearly related to logarithms of rates. But other dependencies are possible. Linear dependencies, \(k_i \propto (1 + bp_{i+1})\), have been explored with a smaller population \([34]\). Combinations of rates and fixed waiting times have also been explored \([35]\). In Ref. \([36]\) the replication that is found in biological systems is modeled through an increasing population.

In most works on coupled oscillators the focus is on the bifurcation structure of the system. Much of our focus here has been on the thermodynamics of the synchronizing oscillators and in particular on the production and the flow of entropy. Commonly the mathematics of synchronizing oscillators can get very convoluted. Through approximations we have attempted to keep the formulas concise and intuitive, while still describing the phenomena.

As the transitions in Fig. 1 are irreversible, there is a net production of entropy as the system is cycling. The net production of entropy should also emerge from the synchronization kinetics of the system depicted in Fig. 1. The formation of a phase-synchronized pulse actually involves an increase of the order parameter. However, we have shown that in the vicinity of the phase transition there is still a net positive production of entropy. We have furthermore shown that the phase synchrony can be thought of as a dissipative structure: accumulation of units actually leads to a higher rate for the entropy-producing transitions in Fig. 1.

When a cycle as in Fig. 1 consists of a continuum of states (cf. Fig. 4), the PDEs that are the focus of Secs. IV and V constitute the proper way of description. It is remarkable that the Burgers’ equation that we derive as the appropriate continuization no longer exhibits the phase transition as the coupling parameter \(\alpha\) is varied. No relation between the pulse amplitude \(\varepsilon\) and the system parameters is apparent from the theory.

It may be surprising that the phase transition is no longer present in the continuum limit. When, for instance, the similar and well-known Kuramoto model is taken to its continuum limit \([19]\), a phase transition as in our Fig. 3 remains present. But there is a difference between the continuum Kuramoto model and our ultimate Burgers’-like model. From the governing equation of the Kuramoto model,

\[
\dot{\theta}_i = \omega_i + \frac{K}{N} \sum_{j=1}^{N} (\theta_j - \theta_i),
\]

that biological reality is ultimately better described with a continuous-type model as presented in Secs. IV and V.
it is obvious that every single one of the \( N \) units is coupled to every other unit on the circle. In our setup a unit in state \( i \) is coupled only to units in neighboring states \( i - 1 \) and \( i + 1 \). For the four-state system these two neighbor states still constitute half of the total number of states and interactions can then still be considered global in nature. But as the number of states increases to a large \( n \) (cf. Fig. 4), the neighbor-neighbor interactions take on an ever more local character. It is possible to get global phase transitions with just local interactions. Ising models provide a well-understood example of this. But this is not what happens in Burgers’ equation \([37]\).

In Sec. V we derived how the propagating soliton produces entropy. We see that for a small pulse amplitude \( \varepsilon \), the production of negative entropy associated with the shock-formation term in the PDE is of a higher order in \( \varepsilon \) than the positive entropy production diffusion term.

Soliton propagation is not necessarily always associated with entropy production. Apply, for instance, the transformation \( t \to -t \) to Burgers’ equation \((23)\). In that case the soliton depicted in Fig. 5 will simply move in the reverse direction and with the same speed. Such a soliton, however, would also be a solution to a PDE with antidiffusion (a minus sign in front of the second partial derivative with respect to \( x \)) and shock dissolution (negative \( \alpha \)). For such a soliton the propagation involves the production of negative entropy.

The moving solitons that we derive are still entropy-producing structures. However, they may slow down the turnover rate (cf. Fig. 4) as compared to the setup that has no coupling (i.e., \( \alpha = 0 \)). So these synchronization solitons are no longer the catalysts for enhanced turnover that they were in the finite-state case.

It is instructive to examine the relation of our analysis to the aforementioned conformational coupling of nearby ion channels in a membrane. Multistate models, such as the one introduced in Fig. 1, are commonly used to describe the kinetics of ion channels \([15]\). Collective action of such channels has been observed in numerous experiments \([38–40]\), but the underlying mechanism by which the membrane proteins communicate is still not obvious.

It has been suggested that information about a channel’s state is transmitted via the membrane between the channels. It is indeed not hard to imagine that the different states of a channel are geometrically different. If channels are sufficiently close and if the lipid-bilayer membrane and the cytoskeletal structures in which they are embedded are sufficiently stiff, then channels could, in principle, be coupled chemomechanically and kinetics as in Fig. 1 could ensue \([16,41–44]\).

Of course, such conformational coupling through membrane deformation only works for short distances.

Another possible way to couple closely spaced channels is through the electrochemical gradient between the two sides of the membrane. An open ion channel results in a local change of the transmembrane potential. The more open channels in a small area, the bigger the resulting change in the electric field. As electrochemical potentials can affect opening and closing of a channel \([45]\), feedback loops can be established. This is how action potentials arise and propagate \([46]\). It is also how kinetics like in Fig. 1 can emerge.

Clusters of ion channels, where the action of one channel is amplified by the actions of the neighboring ones, have been found in many cell types. It has even been observed how such clustering is regulated during cell development \([47,48]\). Proper clustering of different kinds of channels in Ranvier nodes of myelinated nerve fibers appears to be essential for achieving a good propagation of action potentials \([49]\). In experimental studies on synaptic transmission it has been shown that having receptor clusters of variable sizes optimizes the response to small stimuli and the sensitivity to signal amplitude \([50]\). In their analysis of a theoretical model of the action of inositol 1,4,5-triphosphate receptors in a plasma membrane, Shuai and Jung found that channel clustering can dramatically enhance a cell’s capability of creating a large Ca\(^{2+}\) response to a weak stimulation \([51]\).

The coupling parameter \( \alpha \) that we introduced could be associated with the density of channels in a cluster and with the average distance of channels in a cluster. Experimental studies to establish an explicit quantitative relation between a coupling parameter and a signal amplitude should be feasible. Our results can be a guideline when performing such studies of the collective action of ion channels. It would be particularly interesting if something as dramatic as a phase transition could be experimentally established.

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