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Stochastic dynamics of small ensembles of non-processive molecular motors: The parallel cluster model

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Non-processive molecular motors have to work together in ensembles in order to generate appreciable levels of force or movement. In skeletal muscle, for example, hundreds of myosin II molecules cooperate in thick filaments. In non-muscle cells, by contrast, small groups with few tens of non-muscle myosin II motors contribute to essential cellular processes such as transport, shape changes, or mechanosensing. Here we introduce a detailed and analytically tractable model for this important situation. Using a three-state crossbridge model for the myosin II motor cycle and exploiting the assumptions of fast power stroke kinetics and equal load sharing between motors in equivalent states, we reduce the stochastic reaction network to a one-step master equation for the binding and unbinding dynamics (parallel cluster model) and derive the rules for ensemble movement. We find that for constant external load, ensemble dynamics is strongly shaped by the catch bond character of myosin II, which leads to an increase of the fraction of bound motors under load and thus to firm attachment even for small ensembles. This adaptation to load results in a concave force-velocity relation described by a Hill relation. For external load provided by a linear spring, myosin II ensembles dynamically adjust themselves towards an isometric state with constant average position and load. The dynamics of the ensembles is now determined mainly by the distribution of motors over the different kinds of bound states. For increasing stiffness of the external spring, there is a sharp transition beyond which myosin II can no longer perform the power stroke. Slow unbinding from the pre-power-stroke state protects the ensembles against detachment. © 2013 AIP Publishing LLC.

I. INTRODUCTION

Numerous processes in single cells and tissues require the generation of mechanical force and directed motion. Most of these processes are based on the activity of molecular motors interacting with the filaments of the cytoskeleton, that is, motors from the dynein-, kinesin-, and myosin-families interacting with microtubule or actin filaments. Examples include separation of chromosomes and closure of the constriction ring during cell division, intracellular transport of cargo vesicles and organelles, contraction of muscle cells, large-scale rearrangements in a developing tissue, and wound closure after tissue injury. The mechanical energy used in these processes is gained by hydrolysis of ATP (adenosine triphosphate) and drives a cycle of conformational changes in the allosteric motor molecules. Over the last decades, the way single motor molecules work has been dissected in great quantitative detail. However, it remains a formidable challenge to understand how molecular motors work in the physiological context of cells and tissues, where they usually collaborate in groups. Here we theoretically address one crucial aspect of this situation, namely, force generation in small ensembles of non-processive motors.

A large research effort has been focused on processive motors which stay attached to the substrate sufficiently long as to not loose contact for many motor cycles. For example, the two motor heads of conventional kinesin typically take more than 100 steps of 8 nm length before the motor unbinds from its microtubule track. Although this property would enable processive motors to work alone, experimental evidence suggests that also processive motors in a physiological context often collaborate in small groups. The main benefit here is that attaching several motors to the same cargo increases the walk length dramatically and allows the cargo to pass over defects on the track and change reliably between tracks of finite length. Furthermore, because the velocity of a processive motor typically decreases with the applied load, groups of motors sharing an external load are able to transport cargo at larger velocities or to exert larger forces on a cargo or an elastic element. As an example for the latter, it has been shown that the force necessary to pull membrane tubes from a lipid vesicle can only be produced by groups of processive motors.

In contrast to processive motors, non-processive motors cannot do useful work single-handedly and therefore necessarily have to operate in groups in order to generate persistent motion or appreciable levels of force. The paradigm for a non-processive motor acting in ensembles is myosin II in cross-striated skeletal muscle. In the muscle sarcomere, hundreds of myosin II motors are assembled in so-called thick filaments. The arrangement of myosin II in a thick filament is of bipolar order, that is, the myosin II motors in the two halves of a thick filament are oriented in opposing directions. The myosin II motors in either half of a thick filament walk as an ensemble on so-called thin filaments, actin filaments which
are anchored in the two Z-discs bounding the sarcomere. The arrangement of thick and thin filaments is symmetric with respect to the mid-plane of the sarcomere so that motor activity leads to muscle contraction. Investigation of the structure of muscle has been facilitated by the remarkable precision of the spatial arrangement of myosin II motors in the sarcomere. For example, in frog skeletal muscle each half of a thick filament contains 294 myosin II motors which are arranged at a very regular distance of 14.5 nm.14

Even before myosin II had been biochemically characterized, the first theoretical description of muscle contraction was already based on the non-equilibrium binding and unbinding kinetics of myosin to actin which effectively rectified thermal fluctuations of an elastic element.15 In this early model, myosin was described as an actin binding site fluctuating in a harmonic potential and binding preferentially ahead of and unbinding preferentially behind its equilibrium position, thus inducing a net force displacing the actin filament. Precise measurements of contraction speed as function of force in combination with X-ray diffraction and detailed modeling allowed to identify the chemical and mechanical details of the myosin II hydrolysis cycle and led to the development of the crossbridge model.16, 17 which provided a molecular mechanism for the principle of preferential binding and unbinding proposed by Huxley.15 Those advances inspired theoretical models analyzing the statistical physics of large ensembles of myosin II motors simultaneously pulling on a single filament.18, 19 With these models it has been shown that in order to describe the response of skeletal muscle to varying loading conditions it is essential that the unbinding rate of myosin II from actin is a decreasing function of the applied load. In contrast to, e.g., the processive motor kinesin, this myosin II from actin is a decreasing function of the applied load. In contrast to, e.g., the processive motor kinesin, this myosin II from actin is a decreasing function of the applied load.

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present such a model for the non-processive molecular motor myosin II. A short account of some of our results has been given previously.38

Our work is motivated by the long tradition in modeling force generation in skeletal muscle. Generic models for molecular motors investigate the fundamental conditions for the generation of directed motion in a thermal environment but do not take specific properties of molecular motors into account. In ratchet models, a particle switches between diffusive movement in a flat potential and in a periodic potential.39

By breaking detailed balance for the transitions between the two potential landscapes, directed motion of the particle can ensue. Ratchet models allow to study generic effects of cooperativity of a large number of motors such as the emergence of directed motion in symmetric systems or spontaneous oscillations.39, 40 Diffusion and switching of the particles is usually described in the framework of a Fokker-Planck equation. Within this framework, the effect of a finite ensemble size has been included by assuming a fluctuating drift velocity with a noise intensity that increases with decreasing ensemble size.36 This approach allows to observe effects specific for finite sized ensembles, such as the reversal of the direction of motion.41 Beginning with the work of Huxley,15 some molecular characteristics were introduced by assuming different conformational states of the motor molecules. With the focus on the large assemblies of motors in muscle, analytical progress was usually made using mean-field approximations. A mean-field model for molecular motors with three conformational states, in which the bound motors moved with given velocity was used to calculate the force-velocity relation of an ensemble of motors.18 Adapting the transition rates between the conformational states allowed to study processive as well as non-processive motors. Onset of oscillatory behavior of the ensembles was investigated in a generic two-state model, in which conformational changes of the motors upon binding and unbinding allowed bound motors to exert force on their environment.42 Here, the binding an unbinding rates could be adapted to describe different types of motors and different force-velocity relations. In a generalization, an ensemble of molecular motors working against a visco-elastic element was investigated.43 Using a similar mean-field approach as Leibler and Huse18 for a two-state model, the dynamic behavior of ensembles was investigated, revealing limit cycle oscillations induced by the coupling to the visco-elastic element. To describe specific properties of muscle fibers, crossbridge models with varying degree of detail have been used. Using computer simulations on large ensemble of myosin II with a crossbridge model including explicitly the power stroke and load dependent unbinding from the post-power-stroke state, details of the force-velocity relation for muscle fibers could be fitted very accurately to experimental results and collective phenomena such as the synchronization of the power stroke under load or the transient response of muscle to a step change of the external load were investigated.19, 44

Coupling to an external elastic element allowed to observe oscillations for the crossbridge model.45 Recently, a detailed crossbridge model for myosin II was used to describe also the activity of small myosin II ensembles in the cytoskeleton.46

The activity of small groups of myosin II in motility assays was studied using computer simulations. Large ensembles were described in a mean-field approach. By comparison of the results to experiments, parameters of the model could be determined. The focus of the model was on the description of the ATP dependence of the transition rates. Therefore, the crossbridge model included two separate post-power-stroke states of the bound motors but did not include a bound pre-power-stroke state so that it did not explicitly describe the power stroke.

In order to study effects of molecular details for ensembles of myosin II motors, we use a crossbridge model with three states as a starting point, which was originally used for skeletal muscle.44, 45 Unlike Walcott et al.,46 we use a pre- and a post-power-stroke state as the two bound states, so that the power stroke of myosin II is included explicitly. To reduce the complexity of the analytical description, we make two approximations: (i) we assume that molecular motors in equivalent conformational states have equal strain and (ii) we exploit a separation of time scales in the myosin II cycle and assume that there is thermal equilibrium of the bound states. The partial mean-field approximation of the first assumption still distinguishes between the two different bound states. It can be justified by the small duty ratio of myosin II motors which leads to a narrow distribution of the strains of bound motors. The assumption of local thermal equilibrium between bound states reduces the system to a two-state model. The effective properties of these states, however, still depend on the distribution over the two bound states. The two approximations allow us to derive a one-step master equation for the binding dynamics of the motors, which explicitly includes the effects of strain-dependent rates and small system size. A one-step master equation has been introduced before for transport by finite-sized ensembles of processive motors with slip bond behavior,9 but not for non-processive motors with catch bond behavior. Together with rules for the displacement of an ensemble upon binding and unbinding, the one-step master equation fully characterizes the dynamics of ensembles. We investigate two paradigmatic loading conditions for the ensemble: constant loading and linear loading, in which the external load depends linearly on the position of the ensemble. For constant external load, we can solve the one-step master equation for the stationary states and derive binding properties and force-velocity relation from these. For linear external load, the movement of the ensemble feeds back to the load dependent binding rates, so that we have to use computer simulations to analyze this case. In both loading scenarios, we find that the motor ensemble adapts its dynamical state to the external conditions in a way which is reminiscent of its physiological function.

II. MODEL

A. Crossbridge model for single non-processive motors

To describe the mechanism of force generation by non-processive molecular motors, we use a crossbridge model for myosin II16, 19 Variants of cross-bridge models differ by number and type of conformational states they include,19, 44, 46 depending on the focus of the modeling approach. Here, we
distinguish two bound conformations of the motors and one
unbound state. The essential mechanical elements of myosin
II in our model are depicted schematically in Fig. 1(a). The
motor head binds the motor to the substrate, which in the case
of myosin II is an actin filament. The motor head also is the
active domain of myosin II which binds ATP or the prod-
ucts of ATP hydrolysis—ADP (adenosine diphosphate) and
a phosphate group Pi. Hinged to the motor head is the rigid
lever arm which can exist either in the primed (gray) or the
stretched (black) conformation. The lever arm amplifies small
conformational changes in the head domain of the motor so
that the tip of the lever arm swings forward by a distance d
in the transition from primed to stretched conformation. This
movement stretches the elastic neck linker, which is modeled
as a linear elastic element with spring constant \( k_m \). Elastic
forces in the neck linker are transmitted to the anchor, through
which a myosin II motor can integrate firmly into myosin II
motor filaments such as cytoskeletal minifilaments.

Driven by the hydrolysis of ATP, myosin II cycles
through a sequence of mechanical and chemical conforma-
tions to generate force and directed motion. The exact se-
quence of reaction steps, the rates of transitions as well as the
molecular parameters of myosin II are subject of debate. The
basic sequence of conformations we use in our model, how-
ever, is well supported by experimental observation. In partic-
ular, the reversal of the power stroke under load has been ob-
served for similar types of single headed myosin molecules.48
In Table I, we list the values for the most important param-
eters as determined experimentally or used in earlier models.
These parameters depend on the exact experimental condi-
tions, e.g., ATP concentration and spatial arrangement of mo-
tors, and also on the exact type of myosin II.20,31 In the main
body of our paper we use the parameters of the last column of
Table I. These are taken from Vilfan and Duke.45 As shown
schematically in Fig. 1(b), we model the myosin II motor cy-
cle by three discrete mechano-chemical states44 with stochas-
tic transitions between them. In the unbound state (0), the
motor head is loaded with ADP and Pi, and the lever arm is in its
primed conformation. The primed conformation is a high en-
ergy state, which stores part of the approximately 80 pN nm
of energy released in ATP hydrolysis. The motor then re-
versibly transitions to the weakly-bound state (1) with on-
rate \( k_{01} \) and off-rate \( k_{10} \). Concomitant with the release of Pi,
the lever arm swings to the stretched conformation, thereby

![Crossbridge model for non-processive myosin II motors molecules. (a) Mechanical elements of myosin II. (b) Myosin II motor cycle with three discrete mechano-chemical states. In the unbound state (0), the motor head binds ADP and Pi, and the lever arm is in the primed conformation (gray in (a)). Binding to the substrate brings the motor to the weakly-bound (pre-power-stroke) state (1) with unchanged mechanical conformation. After release of the Pi group, the lever arm swings forward into the stretched conformation (black in (a)). This power stroke brings the motor to the post-power-stroke state (2). Replacing ADP by ATP, unbinding from the substrate and ATP hydrolysis brings the motor back to the unbound state (0). Because of the consumption of ATP, the last transition is irreversible. All other transitions are reversible.](https://scitation.aip.org/content/aip/journal/jcp/139/17/10.1063/1.4794135?casa_token=C3Vlpry4A38XXXGBu-MbV4xJQHxtC8CzqC8d6OOGnO-zPU7-XHfYiMn7o72A6L0PaNtZJiQWxXm3EA)

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<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Values</th>
<th>Model value</th>
</tr>
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<tr>
<td>Thermal energy</td>
<td>( k_B T )</td>
<td>—</td>
<td>4.14 pN nm</td>
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<tr>
<td>Power-stroke distance</td>
<td>( d )</td>
<td>8 nm, 10 nm</td>
<td>8 nm</td>
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<tr>
<td>Motor elasticity</td>
<td>( k_m )</td>
<td>0.3 pN nm(^{-1}), 19,46 2.5 pN nm(^{-1}), 45 3.0 pN nm(^{-1}), 47</td>
<td>2.5 pN nm(^{-1})</td>
</tr>
<tr>
<td>Transition rates</td>
<td>( k_{01} )</td>
<td>6 s(^{-1}), 46,47 40 s(^{-1}), 45,46</td>
<td>40 s(^{-1})</td>
</tr>
<tr>
<td></td>
<td>( k_{10} )</td>
<td>0 s(^{-1}), 46 2 s(^{-1}), 45</td>
<td>2 s(^{-1})</td>
</tr>
<tr>
<td></td>
<td>( k_{02} )</td>
<td>~18 s(^{-1}), 46 80 s(^{-1}), 45</td>
<td>350 s(^{-1}), 46</td>
</tr>
<tr>
<td></td>
<td>( k_{12} )</td>
<td>10(^3) s(^{-1}), 45</td>
<td>10(^3) s(^{-1})</td>
</tr>
<tr>
<td>Post-power-stroke bias</td>
<td>( E_{pp} )</td>
<td>~60 pN nm(^{-1}), 45</td>
<td>~60 pN nm</td>
</tr>
<tr>
<td>Unbinding distance</td>
<td>( \delta )</td>
<td>0.328 nm, 45 1.86 nm, 46 2.60 nm(^{-1})</td>
<td>0.328 nm</td>
</tr>
<tr>
<td>Unbinding force</td>
<td>( F_0 )</td>
<td>—</td>
<td>12.62 pN</td>
</tr>
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</table>

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releasing most of the energy stored on the primed neck linker, and the motor enters the post-power-stroke state (2). With the stretched lever arm, the motor molecule is close to its conformational ground state and there is a strong free energy bias $E_{pp}$ favoring the post-power-stroke state. Compared to the binding transitions, transitions between the bound states are relatively fast, with unloaded transition rates $k_{12}^0 \simeq k_{21}^0$. Replacing ADP by ATP, unbinding from the substrate and hydrolysis of ATP completes the motor cycle and brings the motor back to the unbound state (0) with primed lever arm. The unloaded off-rate for this last step is $k_{20}^1$. Due to the energy released in ATP hydrolysis this transition is considered as irreversible, thus defining the direction of the motor cycle. Most importantly in our context, both power stroke and unbinding from the post-power-stroke state depend on load. The power stroke $(1) \rightarrow (2)$ moves the lever arm forward by the power-stroke distance $d$ and strains the elastic neck linker of a motor. Replacing ADP by ATP and unbinding from (2) requires an additional movement of the lever arm in the same direction as the power stroke, thus straining the neck linker further by the unbinding distance $\delta$ making unbinding slower under load. The load dependent rates for these transition are denoted without the superscript (see Fig. 2).

B. Parallel cluster model for ensembles of non-processive motors

Because non-processive molecular motors are bound only during a small fraction of the motor cycle they have to cooperate in groups to generate sustained levels of force or persistent motion against an external load. A sufficiently large number of motors ensures permanent attachment of the group, while individual motors continuously unbind and rebind as they go through their motor cycle. Fig. 2(a) illustrates the coupling of myosin II motors in an ensemble working against an external load. With their anchors the motors are firmly integrated into the rigid backbone of the motor filament, whereas the motor heads bind to the substrate. In Fig. 2(a), the motors are oriented such that the lever arm swings towards the right during the power stroke, so that the motors exert force on the motor filament towards the right. The external load pulls the motor filament towards the left, against the motor direction. Because the motors are attached directly to the motor filament, they are working effectively in parallel against the external load. Such parallel arrangement was confirmed experimentally for the myosin II motors in the muscle sarcomere.30, 49 We will discuss two paradigmatic situations for the external load: (i) a constant external load, which is independent of the position of the motor filament, and (ii) an elastic external load, which increases linearly with the displacement of the motor filament. For constant external load, the ensemble will eventually reach a steady state of motion with load dependent velocity. For a linear external load, an isometric state with vanishing velocity is expected. Experimentally, the unipolar ensemble of myosin II motors in Fig. 2(a) would represent one half of a thick filament in the muscle sarcomere or of a minifilament in the cytoskeleton. In this case, the external load is generated by the motors in the other half of the bipolar motor filament or is due to the tension in a surrounding actin network. In reconstituted assays, a constant load could be realized through viscous forces in a flow chamber or applying active feedback control; a linear load might be realized using elastic elements such as optical traps.

For an ensemble of parallel motors in mechanical equilibrium, the external load $F_{ext}$ is balanced by the sum of elastic...
forces \( F_n = k_m \xi_n \) in the neck linkers of all bound motors:

\[
F_{ext} = \sum_{\text{bound}} F_n = k_m \sum_{\text{bound}} \xi_n.
\]  

(1)

In this expression, \( k_m \) is the elastic constant of the neck linkers, \( \xi_n \) is their elongation (or strain), and the index \( n \) runs over all motors which are bound to the substrate. An imbalance of forces induces a change of the position of the motor filament. This changes the strain \( \xi_n \) of all bound motors simultaneously until the balance of forces in Eq. (1) is restored. In addition, a linear external load would be changed by the displacement of the motor filament. In the following, we assume that the relaxation time towards mechanical equilibrium is negligible compared to the time scale for stochastic transitions, so that the mechanical state of an ensemble always obeys the force balance in Eq. (1). Anchoring of the motors to the rigid motor filament in combination with the condition of the force balance introduces a tight mechanical coupling between motors: a stochastic transition of one motor changes the force balance and hence the strain of all bound motors. Thus, the strain \( \xi_n \) of a bound motor does not only depend on the state of the motor itself but results from the past activity of the motor ensemble. Specifically, \( \xi_n \) is determined by the displacement of the motor filament after binding of a motor. Therefore, Eq. (1) determines the sum but not the individual strains of the motors: while the sum over the strain of the bound motors vanishes at vanishing external load, the individual strains \( \xi_n \) will in general not. The distribution of the \( \xi_n \) will be determined by the randomly distributed times during which a motor remains bound to the substrate and the displacement of the motor filament during these times.

Because the rates for the stochastic transitions \( (1) \leftrightarrow (2) \) and \( (2) \rightarrow (0) \) depend on the strain of a motor, the mechanical coupling leads to a dynamical coupling of the motors, as illustrated in Fig. 2(a). A stochastic description of the ensemble dynamics as a Markov process would thus require not only the mechano-chemical state but also the strain of every motor as state variables. Denoting the total number of motors in an ensemble by \( N_e \), the state space for an ensemble with \( N_e \) motors would then encompass \( 3^{N_e} \) discrete states and \( N_e \) independent, continuous variables (\( N_e - 1 \) considering Eq. (1)). This complexity prohibits analytical solutions and previous approaches either used mean field models or computer simulations (see, e.g., Refs. 18, 19, and 44). Here, we use mean-field elements to arrive at an analytically tractable model, which preserves the molecular details contained in the crossbridge model and allows to study stochastic effects due to finite ensemble size. We make the assumption that all motors in the same mechano-chemical state have the same strain. This assumption is the essence of our parallel cluster model (PCM); its validity will be discussed in Sec. III A 5 and demonstrated by comparison with computer simulations. As illustrated in Fig. 2(b), the PCM effectively describes the ensemble of molecular motors as an adhesion cluster of parallel bonds.50–52 In this picture, the power stroke shortens the closed bonds by the power-stroke distance \( d \). Therefore, closed bonds can be in two conformations with different lengths in which they carry different loads. All motors in a given conformation, however, carry an equal share of the external load and have the same strain. Thus, all motors in a given mechano-chemical state are mechanically equivalent within the PCM so that the state of a motor ensemble of \( N_1 \) motors can be characterized by the number of motors in each of the mechano-chemical states.

We use the number \( i \) of bound motors \((0 \leq i \leq N_1)\) and the number \( j \) \((0 \leq j \leq i)\) of motors in the post-power-stroke state. The number of motors in the weakly-bound state then follows as \( i - j \) and the number of unbound motors is \( N_1 - i \). The strain of the motors in the weakly bound state is referred to as \( \xi_{ij} \), where the indices indicate the dependence on the ensemble state \((i, j)\). Since the power stroke stretches the neck linker by \( d \), the strain of the motors in the post-power-stroke state \( (2) \) is given by \( x_{ij} + d \). With \( i - j \) motors with strain \( x_{ij} \) and \( j \) motors with strain \( x_{ij} + d \), the force balance in the PCM reads

\[
F_{ext} = k_m[(i - j)x_{ij} + j(x_{ij} + d)] = k_m[ix_{ij} + jd].
\]  

(2)

This expression can be solved for the strain \( x_{ij} \) of the weakly bound motors. For constant external load, \( F_{ext} = \text{const} \), Eq. (2) yields

\[
x_{ij} = \frac{(F_{ext}/k_m) - jd}{i}.
\]  

(3)

For linear external load, we have to introduce an external coordinate describing the position of the motor ensemble. We define \( z \) as the average position of the bound motor heads. The position of the motor filament then is given by \( z - x_{ij} \). The definition of ensemble position is described in detail in Sec. II E. With the external elastic constant \( k_e \), the linear external load is \( F_{ext} = k_e(z - x_{ij}) \). Inserting this into Eq. (2) yields

\[
x_{ij} = \frac{(k_e/k_m)z - jd}{i + (k_e/k_m)}.
\]  

(4)

for the strain of the weakly bound motors. Here, we define the strain of a weakly bound motor to be positive when the neck linker is stretched in the direction of the external load (towards the left in Fig. 2(a)), whereas the average position \( z \) of bound motor heads increases in the motor direction (towards the right in Fig. 2(a)) (see Sec. II E). If all bound motors are in the weakly-bound state \( (j = 0) \), the strain \( x_{ij} = x_{00} \) is positive, that is, the neck linkers pull on the motor filament against the external load. When the external load is not too large, the strain of the weakly bound motors can become negative, if sufficiently many motors have gone through the power stroke. In this case, the neck linkers of the weakly bound motors pull the motor filament against the motor direction, thereby supporting the external load. Because \( j \leq i \), the strain \( x_{ij} + d \) of motors in the post-power-stroke state is always positive. It is this pulling of post-power-stroke motors which eventually drives force generation and motion by the ensemble.

The major benefit of the PCM lies in the fact, that it eliminates the history dependence of the strain and introduces \( x_{ij} \) as a state function. Within the PCM, the strain of all motors follows from the current ensemble state \((i, j)\) and the external load \( F_{ext} \). For constant external load, \( F_{ext} = \text{const} \) takes the role of a parameter and the ensemble dynamics is fully characterized by \((i, j)\). For linear external load, \( F_{ext} = k_e(z - x_{ij}) \) is changed through the activity of motors so that additional
rules for the position $z$ of the ensemble are required to fully characterize the dynamics of an ensemble.

C. Local thermal equilibrium of bound motors

For an ensemble with $N_t$ myosin II motors, the number $i$ of bound motors ranges from 0 to $N_t$. The number $j$ of motors in the post-power-stroke state ranges from 0 to $i$. Fig. 3(a) shows the corresponding network of states $(i,j)$ which are connected by the possible stochastic transitions. Binding to and unbinding from the weakly bound state change $i$ by $\pm 1$ without changing $j$ (vertical transitions). The power stroke and its reversal change $j$ by $\pm 1$ but leave $i$ constant (horizontal transitions). Unbinding from the post-power-stroke state reduces $i$ and $j$ simultaneously (diagonal transitions). This is the only irreversible transition in the network and is marked by an arrow. In total, there are $(N_e + 1)(N_e + 2)/2$ states and $3N_e(N_e + 1)/2$ transitions (of which $N_t(N_t + 1)$ are reversible) in this network.

To further reduce the complexity of the model, we take advantage of the strong separation of time scales between the slow binding and unbinding transitions and the transitions between the bound states, which are at least an order of magnitude faster \cite{19,45} (see Table I). Following previous modeling approaches, we assume that a local thermal equilibrium (LTE) is maintained within the bound states. \cite{45} For a given number $i$ of bound motors, the conditional probability to find $j$ motors in the post-power-stroke state and $i − j$ in the weakly bound state is given by the Boltzmann distribution

$$p(j|i) = \frac{1}{Z_i} \exp(-E_{ij}/k_BT).$$

Here, $Z_i$ is the appropriate partition sum for given $i$,

$$Z_i = \sum_{j=0}^{i} \exp(-E_{ij}/k_BT).$$

The energy $E_{ij} = j E_{pp} + E_{el} + E_{ext}$ of an ensemble in state $(i,j)$ is the sum of the free energy bias $E_{pp} \simeq -60$ pN nm $< 0$ towards the post-power-stroke state for $j$ motors, the elastic energy $E_{el}$ stored in the neck linkers and a possible external contribution $E_{ext}$. The elastic energy of the neck linkers is given by

$$E_{el} = \frac{k_m}{2} [(i − j)x_{ij}^2 + j(x_{ij} + d)^2].$$

The strain $x_{ij}$ is given by Eq. (3) for constant and Eq. (4) for linear external load. The external contribution to the energy vanishes for constant external load, $E_{ext} = 0$. For linear external load, $E_{ext}$ is given by the energy stored in the external harmonic potential,

$$E_{ext} = \frac{k_e}{2}(z − x_{ij})^2,$$

where $x_{ij}$ is given by Eq. (4) and $z − x_{ij}$ is the position of the motor filament.

Combining Eqs. (3) and (7) reveals that for constant external load, the elastic energy of the neck linkers is identical in states $(i,j)$ and $(i, i − j)$. $E_{el}$ is minimal when all bound motors are either weakly bound ($j = 0$) or in the post-power-stroke state ($j = i$). Intermediate states with $0 < j < i$ have a larger elastic energy because bound motors in opposite conformations are pulling against each other. Because of this symmetry of $E_{el}$, the free energy bias $E_{pp}$ translates directly into a strong bias of the LTE distribution towards the post-power-stroke state so that almost all bound motors are in the post-power-stroke state. The symmetry of the elastic energy is not affected by the exact value of the constant external load, which merely changes the absolute values of $E_{el}$, so that the bias of the LTE distribution persists for arbitrary values of a constant external load.

A linear external load is increased by the power stroke of a motor. Therefore, elastic energy $E_{el}$ and external energy $E_{ext}$ tend to increase with an increasing number of post-power-stroke motors and introduce a bias towards the weakly bound state, opposite to the free energy bias $E_{pp}$. To demonstrate this, we compare the energy in the extreme states $(i,0)$ and $(i, i)$. For a given $z$, $E_{el}$ and $E_{ext}$ take the smallest value in state $(i,0)$, in which all bound motors are weakly bound with strain $x_{i0} = (k_i/k_m)z/(i + k_i/k_m)$. In state $(i, i)$, all bound motors are in the post-power-stroke state with the strain $x_{ii} = (k_i/k_m)(z + d)/(i + k_i/k_m)$. Because $x_{i0} < x_{ii}$ and $z$, the elastic energy in state $(i,0)$ is smaller than in state $(i, i)$. $E_{el}$ is smaller in state $(i,0)$ than in state $(i, i)$. $E_{ext}$ is smaller in state $(i,0)$ than in state $(i, i)$. The total energy difference is

$$\left( E_{el} + E_{ext} \right)_{i=0} - \left( E_{el} + E_{ext} \right)_{i=i} = \frac{ik_mk_i}{k_m + k_i} \frac{d(d + 2z)}{2}. \quad (9)$$

It increases with increasing $k_i$ and $z$ so that the bias of the LTE distribution will shift from the post-power-stroke state to the weakly bound state at large values of the external elastic constant $k_i$ or the ensemble position $z$. This transition eventually will stall ensemble movement, because movement is driven by post-power-stroke motors and requires passage through the motor cycle.
D. One-step master equation for binding dynamics

As illustrated in Fig. 3(b), the assumption of a LTE within the bound motors effectively projects all ensemble states \((i, j)\) with different \(j\) but given \(i\) onto a single variable. Thus, the state of an ensemble is described by the number \(i\) of bound motors alone. In this effectively one-dimensional system, the probability \(p_i(t)\) to find \(i\) motors bound to the substrate at time \(t\) follows the one-step master equation

\[
\frac{d}{dt} p_i = r(i + 1)p_{i+1} + g(i - 1)p_{i-1} - \left[r(i) + g(i)\right] p_i. \tag{10}
\]

Once \(p_i(t)\) is known, the probability \(p_{ij}(t) = p(j|i)p(t)\) to find the ensemble in the state \((i, j)\) at time \(t\) is obtained as the product of \(p_i(t)\) with the time independent LTE distribution \(p(j|i)\) from Eq. (5). In the one-step master equation, the effective reverse rate \(r(i)\) describes the rate at which bound motors unbind from the substrate, that is, \(r(i)\) is the rate of the transition \(i \rightarrow i - 1\). The effective forward rate \(g(i)\) describes the rate at which free motors bind to the substrate, that is, \(g(i)\) is the rate of the transition \(i \rightarrow i + 1\). The effective transition rates \(r(i)\) and \(g(i)\) with their dependence on \(i\) and \(F_{\text{ext}}\) define the stochastic dynamics of binding and unbinding in the motor ensemble.

In the state \((i, j)\) of an ensemble, weakly bound motors unbind with off-rate \(k_0(i, j)\) and post-power-stroke motors unbind with off-rate \(k_20(i, j)\). Following previous modeling approaches,\(^{19,44}\) we assume that the off-rate from the weakly bound state is independent of the load on a motor and therefore independent of the state of an ensemble, \(k_{20}(i, j) = k_{20} = \text{const}\). Unbinding from the post-power-stroke state requires the lever arm to work against the external load over the unbinding distance \(\delta\). Assuming a Kramers-type load dependence, the off-rate from the post-power-stroke state decreases exponentially with the load \(F_{ij} = k_a(x_{ij} + d)\) on the neck linker

\[
k_{20}(i, j) = k_{20}^0 \exp(-F_{ij}/F_0). \tag{11}
\]

The unbinding force scale \(F_0 = k_B T/\delta \simeq 12.6\) pN is set by the thermal energy \(k_B T\) and the unbinding distance \(\delta\). In state \((i, j)\), there are \(i - j\) weakly bound motors and \(j\) post-power-stroke motors. Because all stochastic transitions proceed independently, the rate for unbinding of a motor in state \((i, j)\) is the sum over the single-motor transition rates

\[
\begin{align*}
\gamma(i, j) &= (i - j)k_{10}(i, j) + jk_{20}(i, j). \tag{12}
\end{align*}
\]

The effective reverse rate \(\gamma\) for the transition \(i \rightarrow i - 1\) in the one-dimensional system is then obtained by averaging \(\gamma(i, j)\) over \(j\) with the LTE distribution from Eq. (5).

\[
\gamma(i) = \sum_{j=0}^{i} \frac{\gamma(i, j)p(j|i)}{p(i)}. \tag{13}
\]

Considering the exponential dependence of \(k_{20}(i, j)\) on strain and the strain dependence of the LTE distribution, \(\gamma\) is a strongly nonlinear function of the external load and the number of bound motors. Since the off-rate \(k_{20}(i, j)\) decreases under load (see Eq. (11)), the post-power-stroke state of myosin II behaves as a catch bond. Due to the strong bias of the LTE distribution towards the post-power-stroke state, myosin II motors predominantly unbind from the post-power-stroke state. This implies that also the effective reverse rate \(r(i)\) decreases under load and the myosin II ensemble as a whole behaves as a catch bond. For constant external load, the bias persists for arbitrary values of \(F_{\text{ext}}\), so that the catch bond character of myosin II motors is found for all values of the external load. For elastic external load, the bias of the LTE distribution passes over to the weakly bound state for very stiff external springs. This means that myosin II behaves as a catch bond at small values of \(k_t\) and \(z\), but unbinds with load-independent rate at large values of \(k_t\) or \(z\). For very large loads, it is expected that unbinding of motors is accelerated under load as for slip bonds,\(^{49}\) but such large loads will not be considered here.

The only pathway for binding is the transition \((0) \rightarrow (1)\) from the unbound state to the weakly bound state of a motor. Because unbound motors are not subject to any load, the on-rate is assumed to be constant, \(k_{01} = \text{const}\). With \(N_i - i\) motors binding independently, the effective forward rate is given by

\[
g(i) = (N_i - i)k_{01}. \tag{14}
\]

The forward rate \(g(i)\) increases linearly with the number \(N_i - i\) of unbound motors but is otherwise independent of the state of the ensemble. Because there is no dependence on \(j\), averaging with the LTE distribution is not required.

With the definition of the effective transition rates in Eqs. (13) and (14), the one-step master equation of Eq. (10) for the binding dynamics in an ensemble of myosin II motors is fully characterized for the case of constant external load. If the external load depends on the position of the ensemble, as for a linear load, Eq. (10) has to be solved together with additional rules for the movement of the motor ensemble, which will be introduced in Sec. II E.

E. Ensemble movement

With the introduction of the PCM, we have focused on modeling the dynamics of binding and unbinding of molecular motors in an ensemble. The displacement of the ensemble, on the other hand, seems to be eliminated by the analogy to a cluster of parallel adhesion bonds. Nevertheless, there are clear prescriptions for the transformation of binding and unbinding of motors to a displacement of the ensemble. In order to derive these prescriptions and to elucidate the inherent approximations, we take a step back and consider the general case of an ensemble of molecular motors without the approximation of the PCM, that is, we consider an ensemble in which every motor is characterized by an individual value of the strain. The spatial coordination of the motors is schematically depicted in Fig. 2(a). The anchors are integrated into the motor filament at fixed positions. Because the motor filament is rigid, the relative positions of the anchors are constant and we can assume that all anchors are at the same position \(\xi_{\text{fix}}\), which is identified with the position of the motor filament. In the following, we consider a reference state in which \(i\) motors are bound to the substrate and \(N_i - i\) are unbound. The neck linkers of the bound motors have the strains \(\xi_{\text{fix}}\), where the
index $n \in \{1, \ldots, i\}$ labels the bound motors. The position $z_n$ of a bound motor head on the substrate is related to the position $z_{fil}$ of the motor filament via its strain $\xi_n$ as $z_n = z_{fil} + \xi_n$ for weakly bound and $z_n = z_{fil} + \xi_n - d$ for post-power-stroke motors. To abbreviate notation, we define the offset of a motor head from its anchor as $x_n := \xi_n$ for weakly bound and $x_n := \xi_n - d$ for post-power-stroke motors. Using this definition, the position of a bound motor head can be written as

$$z_n = z_{fil} + x_n$$  \hspace{1cm} (15)

for all bound motors with $n \in \{1, \ldots, i\}$. We now define the position of an ensemble as the average position of the bound motor heads

$$\bar{z} := \frac{1}{i} \sum_{n=1}^{i} z_n.$$  \hspace{1cm} (16)

With this definition, the ensemble position $\bar{z}$ can only change through binding or unbinding of motors, because motor heads are bound at fixed positions on the substrate. By contrast, the position $\xi_{fil}$ of the motor filament also changes through transitions within the bound states which change the balance of forces in Eq. (1). Only for completely detached ensembles, in which no motor is bound to the substrate, we have to use the position $\xi_{fil}$ of the motor filament as the ensemble position. Because unbound motors have vanishing strain and unbound motor heads are at the same position as the anchors, $\bar{z}_{fil}$ is identical to the average position of the unbound motor heads. Inserting $z_n$ from Eq. (15) into Eq. (16), the average position of the bound motor heads is

$$\bar{z} = \bar{z}_{fil} + \bar{x}_{ij}.$$  \hspace{1cm} (17)

The average offset $\bar{x}_{ij}$ between anchors and motor heads is related to the external load $F_{ext}$ via the balance of forces in Eq. (1) as

$$\bar{x}_{ij} := \frac{1}{i} \sum_{n=1}^{i} x_n = \frac{(F_{ext}/k_m) - j d}{i}. \hspace{1cm} (18)$$

Unlike the absolute position $z$ and the individual values of $x_n$ or $\xi_n$, which all result from the history of the ensemble, $\bar{x}_{ij}$ follows from the current state of the ensemble alone. In particular, it depends on the external load $F_{ext}$, the number $i$ of bound motors, and the number $j$ of post-power-stroke motors.

We now calculate how the average position of bound motor heads changes through binding of one additional motor. Assuming that $i$ motors are bound initially with average position $\bar{z}$ and that the new motor binds with vanishing strain at $z_{fil}$ to the substrate, the new average position $\bar{z}'$ of $i+1$ bound motor heads is

$$\bar{z}' = \frac{i \bar{z} + z_{fil}}{i+1} = \bar{z}_{fil} + \frac{i}{i+1} \bar{x}_{ij}.$$  \hspace{1cm} (19)

Thus, binding of a motor changes ensemble position by

$$\Delta \bar{z}_{ij} := \bar{z}' - \bar{z} = \frac{i}{i+1} - 1 \bar{x}_{ij} = -\frac{\bar{x}_{ij}}{i+1}. \hspace{1cm} (20)$$

Like $\bar{x}_{ij}$, the binding step $\Delta \bar{z}_{ij}$ is a function of the ensemble state before binding. After binding, the average offset of the motors is adjusted to

$$x'_{ij} = \frac{i}{i+1} \bar{x}_{ij}. \hspace{1cm} (21)$$

Combining this with $\bar{z}'$ confirms that the position of the motor filament remains unchanged, $z_{fil}' = \bar{z}' - \bar{x}'_{ij} = \bar{z}' - \bar{x}_{ij}$ and $\bar{x}_{ij}' = \bar{x}_{ij}$. This is required for consistency, because the balance of forces is not affected by a motor binding with vanishing strain. In a more general description, motors could be allowed to bind with a finite value of the strain chosen from a random distribution with vanishing mean. In this case, Eq. (21) for $\Delta \bar{z}_{ij}$ would remain valid in the ensemble average.

Unlike the binding step $\Delta \bar{z}_{ij}$, the change of the ensemble position upon unbinding depends on which of the motors unbinds. Assuming that a motor head with offset $x_n$ unbinds from the position $z_n = z_{fil} + x_n$ on the substrate, the average position $\bar{z}''$ of the $i-1$ remaining motor heads is

$$\bar{z}'' = \frac{i \bar{z} - z_n}{i-1} = \frac{i(z_{fil} + \bar{x}_{ij}) - (z_{fil} + x_n)}{i-1} = z_{fil} + \frac{i \bar{x}_{ij} - x_n}{i-1}. \hspace{1cm} (22)$$

Thus, unbinding of a motor from $z_n$ changes the position of the ensemble by

$$\Delta \bar{z}''_{ij,n} = \bar{z}'' - \bar{z} = \frac{i \bar{x}_{ij} - x_n}{i-1} - \bar{x}_{ij} = \frac{\bar{x}_{ij} - x_n}{i-1}. \hspace{1cm} (23)$$

Assuming that all motors are equally likely to unbind, the average of the unbinding step over all bound motors vanishes

$$\Delta \bar{z}''_{ij} = \frac{1}{i} \sum_{n=1}^{i} \Delta \bar{z}''_{ij,n} = \frac{\bar{x}_{ij} - \bar{x}_{ij}}{i-1} = 0. \hspace{1cm} (24)$$

For weakly bound motors this assumption is valid, because the off-rate $k_{10} = \text{const}$ is independent of strain. Post-power-stroke motors, on the other hand, are catch bonds with an off-rate $k_{20}(i,j)$ decreasing exponentially with increasing strain. Therefore, post-power-stroke motors unbind preferentially with small strain and small offset, $x_n < \bar{x}_{ij}$. Averaging the unbinding step $\Delta \bar{z}''_{ij,n}$ with the actual off-rates would then lead to a positive displacement $\Delta \bar{z}''_{ij} \geq 0$. The size of the unbinding step depends on the distribution of strains of the bound motor heads and vanishes when all motors have the same offset. In addition, unbinding of a motor with non-zero strain changes the balance of forces so that the position $z_{fil}$ of the motor filament is changed.

When the last bound motor unbinds and the ensemble detaches completely from the substrate, the motor head relaxes instantaneously from its position $\bar{z} = z_1 = z_{fil} - x_1$ on the substrate to the position $z_{fil} = \bar{z} - x_1$ of the motor filament. Because the position of the detached ensemble is described by the position $z_{fil}$ of the motor filament, unbinding of the last motor changes the ensemble position by

$$\Delta \bar{z}''_{x1} = -x_j \text{ for } j = 0, 1. \hspace{1cm} (25)$$

The dependence on the state of the unbinding motor is included in the definition of the offset $x_j$. Within the PCM, weakly bound motors have the strain $x_j$ and the strain of post-power-stroke motors is $x_j + d$, so that all bound motors are characterized by the same offset $x_j$. Therefore, all bound motor heads are at the same position.
z = z_{fil} + x_{ij} on the substrate. To apply the general expressions for the displacement to the PCM, the averages $\bar{z}$ and $\bar{x}_{ij}$ are replaced by the quantities $z$ and $x_{ij}$ which are the same for all motors in the PCM ensemble. In a given state $(i, j)$, binding of a new motor changes the position $z$ of the ensemble by

$$\Delta z_{ij}^{\text{on}} = -\frac{x_{ij}}{i+1}. \quad (26)$$

This is the actual change of the average position of the bound motor heads assuming that the new motor has bound with vanishing strain at the position $z_{fil}$. As illustrated in Fig. 4, in order to implement the assumption of the PCM, all $i + 1$ bound motor heads have to be shifted to the new common position $z' = z + \Delta z_{ij}^{\text{on}}$ on the substrate after binding. This shift is not meant to correspond to an actual physical process but is a theoretical procedure required to maintain the PCM assumption of identical strains of bound motors. As in the general case, the position of the motor filament does not change upon binding of a motor because the balance of forces is unchanged. Because all bound motor heads have the same offset $x_{ij}$ and are at the same position $z$ on the substrate, the position $z$ of the ensemble is unchanged by the unbinding of a motor

$$\Delta z_{ij}^{\text{off}} = 0 \quad \text{for} \quad i \geq 2. \quad (27)$$

This is the same result as for the average in Eq. (24). Comparison with the general case reveals that the PCM predicts too small a displacement upon unbinding and will underestimate the velocity of an ensemble when there is a wide distribution of the strains of motors. When the last motor unbinds from the substrate, according to Eq. (25) the position of the motor ensemble changes by the unbinding step

$$\Delta z_{ij}^{\text{off}} = -x_{ij} \quad \text{for} \quad j = 0, 1. \quad (28)$$

Equations (26)–(28) completely specify the rules for ensemble movement resulting from the binding dynamics in an ensemble. The ensemble moves forward, when a motor binds while the strain $x_{ij}$ of the weakly bound motors is negative and moves backwards when $x_{ij}$ is positive. Unbinding of a motor does not change the position unless the last motor unbinds. In this case, the position of the unbinding motor head relaxes to the position of the motor filament.

The velocity $v_{ij}$ of an ensemble in state $(i, j)$ is given by the product of the displacement step induced by a binding or unbinding transition with the rate at which this transition proceeds. Unbound motors bind with the effective forward rate $g(i)$ defined in Eq. (14). Unbinding only changes ensemble position when the last motor unbinds. Weakly bound motors unbind with the constant off-rate $k_{10} = \text{const}$; post-power-stroke motors unbind with the off-rate $k_{20}(1, 1)$. Thus, the velocity of the ensemble in state $(i, j)$ is

$$v_{ij} = g(i)\Delta z_{ij}^{\text{on}} + \left[k_{10} \Delta z_{ij}^{\text{off}} x_{ij} + k_{20}(1, 1) \Delta z_{ij}^{\text{off}} \delta_{ij} \right] \delta_{ij} \quad (29)$$

$$= -g(i)\frac{x_{ij}}{i+1} - \left[k_{10} x_{10} \delta_{ij} + k_{20}(1, 1) x_{11} \delta_{ij} \right] \delta_{ij}. \quad (30)$$

The last term applies only to unbinding from the state $i = 1$ and distinguishes between weakly bound motors ($j = 0$) and post-power-stroke motors ($j = 1$).

To combine the expressions for ensemble displacement and velocity with the solutions of the one-step master equation for the binding dynamics, we have to average over the variable $j$ using the LTE distribution. The offset of the bound motors in state $i$ is given by

$$x_i = \sum_{j=0}^{i} x_{ij} p(j|i) = \frac{1}{Z_i} \sum_{j=0}^{i} x_{ij} \exp(-E_{ij}/k_B T). \quad (31)$$

The binding step of the ensemble due to the transition $i \rightarrow i + 1$ then becomes

$$\Delta z_{ij}^{\text{on}} = \sum_{j=0}^{i} \Delta z_{ij}^{\text{on}} p(j|i) = -\frac{1}{i+1} \sum_{j=0}^{i} x_{ij} p(j|i) = -\frac{x_i}{i+1} \quad (32)$$

for $i \geq 1$. The unbinding step is $\Delta z_{ij}^{\text{off}} = 0$ for $i \geq 2$ and $\Delta z_{1j}^{\text{off}} = -x_1$ for $i = 1$. Averaging the velocity $v_{ij}$ from

![FIG. 4. Change of ensemble position z upon binding of a motor within the PCM. (a) Before binding, all bound motor heads are at the ensemble position z. In the illustrated case, the strain of the weakly bound motor is negative, $x_{ij} < 0$, so that motor heads will bind at $z_{fil} = z - x_{ij} > z$ ahead of the current ensemble position. The post-power-stroke motors have positive strain, $x_{ij} + d > 0$, and work against the external load and the elastic force from the weakly bound motor. For the illustration, motors are depicted with length $\ell_0$ so that the anchors are at $z_{fil} - \ell_0$. (b) After a motor has bound, all bound motor heads are shifted to the new ensemble position z to implement the PCM assumption of equal $x_{ij}$ of all bound motors. Because the external force is distributed over a larger number of bound motors, the position $z_{fil}$ of the motor filament also shifts to larger values.](image-url)
Eq. (29) over \( j \) yields the velocity in state \( i \),
\[
v_i = g(i) \Delta z_i^{an} - [k_{11}x_{10}p(0)1 + k_{20}(1, 1)x_{11}p(1|1)] \delta_{1i}.
\]
(33)
The expression for the velocity applies to attached ensembles with at least one bound motor, that is, \( i \geq 1 \). To complete the description of ensemble movement, the velocity of detached ensembles with \( i = 0 \) has to be defined. The position of the detached ensemble is described by the position of the motor filament, \( z_{0i} \), which is identical to the position of the unbound motor heads. We assume that the external load \( F_{ext} \) moves the motor filament through the viscous environment with effective mobility \( \eta \). For constant external load, detached ensembles move with constant velocity
\[
v_0 = -\eta F_{ext}.
\]
(34)
The negative sign follows from the definition of the direction of the external load opposite to the working direction of the motors. Detached ensembles attach to the substrate with forward rate \( g(0) = N_i k_{01} \) so that the random attachment times follow an exponential distribution with average \( g^{-1}(0) \). For constant velocity \( v_0 = \text{const} \), this implies an exponential distribution also for the size of the backsteps. The average backstep size is then given by
\[
\Delta z_{0i}^{an} = \frac{v_0}{g(0)} = -\frac{\eta k_i}{N_i k_{01}}.
\]
For a linear external load, \( F_{ext} = k_i z_{0i} \), the velocity of the detached ensemble depends on the position \( z_{0i} = z \) of the ensemble
\[
v_0(z) = z_{0i} = -\eta k_i z_{0i}.
\]
(36)
Therefore, the average backstep size for linear external load depends on the position \( z_{0i} \) of the motor filament at detachment
\[
\Delta z_{0i}^{an} = -\frac{\eta k_i z_{0i}}{N_i k_{01} + \eta k_i}.
\]
(37)
For a large mobility with \( \eta k_i \gg N_i k_{01} \), the average backsteps size is \( \Delta z_{0i}^{an} \approx z_{0i} \), that is, the ensemble is effectively reset to the initial position \( z = 0 \).

Together, the master equation of Eq. (10) for the stochastic binding dynamics and the rules for the displacement upon binding and unbinding of motors fully characterize dynamics and movement of an ensemble of molecular motors. For constant external load, ensemble movement is slaved to binding and unbinding of motors, because on- and off-rates are independent of the position \( z \) of the ensemble. In this case, the master equation can be solved independently for the probability distribution \( p_i(t) \) and the average velocity of an ensemble can be inferred from this solution. The average bound velocity, that is, the average velocity of ensembles with at least one bound motor, is given by
\[
v_b(t) = \sum_{i=1}^{N_i} v_i \hat{p}_i(t) = \sum_{i=1}^{N_i} \frac{v_i p_i(t)}{1 - p_0(t)}.
\]
(38)
The probability distribution \( \hat{p}_i(t) \) is normalized over the attached states \( i \in \{1, \ldots, N_i\} \) of the ensemble. The effective velocity of an ensemble, which includes the backward motion (slips) of the unbound ensemble, is given by the average
\[
v_{\text{eff}}(t) = \sum_{i=0}^{N_i} v_i p_i(t)
\]
(39)
over all states \( i \in \{0, \ldots, N_i\} \). Because \( v_0 = -\eta F_{ext} \leq 0 \), the effective velocity is smaller than the bound velocity, \( v_{\text{eff}}(t) \leq v_b(t) \). From the average ensemble velocity as function of time, the position \( z(t) \) can be calculated as
\[
z(t) = z_0 + \int_0^t v_{\text{eff}}(t') dt'.
\]
(40)
For linear external load, the transition rates characterizing the master equation depend on the position of the motor ensemble, so that the master equation has to be solved together with the displacement of the ensemble, which usually has to be done numerically.

### III. RESULTS

#### A. Constant load

**1. Analytical solutions of the one-step master equation**

Mathematically, the reduction of the stochastic binding dynamics on the two-dimensional network of states of Fig. 3(a) to the one-dimensional system of Fig. 3(b) described by Eq. (10) is a dramatic advance, because many general results are known for one-step master equations. For constant external load, the transition rates are independent of the position \( z \) of the ensemble and stationary solutions of the one-step master equation can be derived analytically. For a single variable and in the absence of sources and sinks, stationarity implies detailed balance, that is, \( r_{i+1} p_{i+1} = g(i) p_i \). Iterating this condition yields the stationary probability \( p_i(\infty) \) to find \( i \) bound motors in an ensemble
\[
p_i(\infty) = \frac{\prod_{j=0}^{i-1} g(j)}{1 + \sum_{k=1}^{N_i} \prod_{j=0}^{k-1} g(j) / r_{j+1}}.
\]
(41)
This distribution immediately allows to calculate the average number of bound motors as
\[
N_b = \langle i \rangle = \sum_{i=0}^{N_i} i p_i(\infty).
\]
(42)
In order to calculate averages restricted to attached ensembles with \( i \geq 1 \), the stationary probability distribution \( p_i(\infty) \) has to be re-normalized for the \( N_i \) attached states
\[
\hat{p}_i(\infty) = \frac{p_i(\infty)}{1 - p_0(\infty)} = \frac{\prod_{j=0}^{i-1} g(j)}{\sum_{k=1}^{N_i} \prod_{j=0}^{k-1} g(j) / r_{j+1}}.
\]
(43)
The average detachment time \( T_{10} \) of an ensemble is defined as the mean first passage time of the ensemble from the initial attached state, in which only a single motor is bound \( (i = 1) \), to complete detachment of the ensemble, where all motors have dissociated \( (i = 0) \). The mean first passage time \( T_{10} \) can be calculated analytically using the adjoint
master equation\textsuperscript{53}

$$T_{10} = \sum_{j=1}^{N_i} \frac{1}{r(j)} \prod_{k=1}^{j-1} g(k).$$

(44)

The average attachment time of an ensemble is defined as the mean first passage time $T_{10}$ from the detached state ($i = 0$) to the initial attached state ($i = 1$). This transition involves only a single binding step so that the mean first passage time is given by the inverse of the forward rate $g(0)$,

$$T_{01} = \frac{1}{g(0)} = \frac{1}{N_i k_0}.$$  

(45)

A measure for the ability of ensembles of non-processive motors to generate force and directed motion is the duty ratio of an ensemble. For a single molecular motor, the duty ratio is defined as the fraction of time in the motor cycle, during which the motor is attached to its substrate. Processive motors usually are characterized by large duty ratios close to unity, which allows them to walk along the substrate for many motor cycles. Non-processive motors, on the other hand, are characterized by small duty ratios, which reduces the interference between cooperating motors. For ensembles of molecular motors, we define the ensemble duty ratio $\rho_d$ as

$$\rho_d = \frac{T_{10}}{T_{10} + T_{01}}.$$  

(46)

This is the ratio of detachment time $T_{10}$, which is the average time during which an ensemble remains attached to the substrate before detaching again, to the average time it takes to complete one attachment-detachment cycle of an ensemble, which is the sum $T_{10} + T_{01}$ of detachment and attachment time. To allow for efficient motion and force generation, the duty ratio of an ensemble of non-processive motors should be close to unity, comparable to that of processive motors.

The average bound velocity of an ensemble in the stationary state is given by Eq. (38) with the stationary probability distribution from Eq. (43) replacing $\hat{p}_i(t)$, that is,

$$v_b = \sum_{i=1}^{N_i} v_i \hat{p}_i(\infty).$$  

(47)

Correspondingly, the average effective velocity in the stationary state is found by inserting $p_i(\infty)$ from Eqs. (41) in (39) as

$$v_{\text{eff}} = \sum_{i=0}^{N_i} v_i p_i(\infty).$$  

(48)

The effective velocity can be also expressed using the ensemble duty ratio

$$v_{\text{eff}} = \rho_d v_b + (1 - \rho_d) v_0 = \frac{T_{10} v_b + T_{01} v_0}{T_{10} + T_{01}}.$$  

(49)

Because $v_0 \leq 0$, the effective velocity of an ensemble is always smaller than the bound velocity. The closer the ensemble duty ratio is to unity, the closer is the effective velocity to the bound velocity.

Processive motors can be characterized by their processivity, that is, the average number of steps a motor takes on a substrate before unbinding. For ensembles of non-processive motors, we can use the average walk length $d_w$ between attachment and complete detachment as a measure for the effective processivity. Assuming that relaxation to the stationary distribution $\hat{p}_i(\infty)$ is fast compared to the detachment time $T_{10}$, the ensemble moves with constant bound velocity $v_b$ from Eq. (47) over the detachment time $T_{10}$ so that the average walk length is given by the product

$$d_w = v_b T_{10}.$$  

(50)

Unlike processive motors, $d_w$ does not correspond to a fixed number of binding and unbinding steps of motors because the displacement steps depend on the state of the ensemble.

2. Binding dynamics

To demonstrate the stochastic effects resulting from the finite number of motors in an ensemble and the influence of the catch bond character of the post-power-stroke state, we first study the dependence of the binding dynamics on ensemble size $N_i$ and external load $F_{\text{ext}}$.

Fig. 5(a) shows the average detachment time $T_{10}$ (see Eq. (44)) of an ensemble of myosin II motors as function

![Figure 5](image-url)
of ensemble size \( N_i \) for different values of the external load per motor \( F_{\text{ext}}/N_i \). \( T_{10} \) appears to increases exponentially with \( N_i \), where prefactor and scale of the exponential increase with \( F_{\text{ext}}/N_i \). An approximation for \( T_{10} \) can be derived for vanishing external load under the assumption that all bond motors are in the post-power-stroke state, which is justified by the strong bias of the LTE distribution towards the post-power-stroke state. For \( F_{\text{ext}} = 0 \), the dynamics of the bound motors is not coupled so that not only the on-rate \( k_{01} \) but also the off-rate \( k_{20} = k_{20}^0 \) is independent of the ensemble state \((i,j)\). A series expansion of Eq. (44) for \( T_{10} \) then leads to

\[
T_{10} \approx \frac{1}{k_{01}N_t} \left[ \exp \left( \ln \left[ \frac{k_{20}^0 + k_{01}}{k_{20}} \right] N_t \right) - 1 \right]
= T_{01} \left[ \exp \left( \ln \left[ \frac{k_{20}^0 + k_{01}}{k_{20}} \right] N_t \right) - 1 \right].
\]  

(51)

Comparison with the exact results for \( F_{\text{ext}}/N_i = 0.013 \) pN in Fig. 5(a) shows excellent agreement. For finite load, no closed form can be found, because the off-rate \( k_{20}(i,j) \) is a strongly nonlinear function of the ensemble state \((i,j)\). A fit of \( T_{10} \) to a function of the type of Eq. (51) with adapted prefactor and scale of the exponential yields a better approximation than a pure exponential but deviations at small \( N_i \) are still observed, in particular for large external load (not shown).

Fig. 5(b) shows the average detachment time \( T_{10} \) for different ensemble sizes as function of the external load per motor. \( T_{10} \) increases exponentially for not too small values of \( F_{\text{ext}}/N_i \) where the scale of the exponential increases with \( N_i \). This exponential increase is a consequence of the catch bond character of the post-power-stroke state: for all values of a constant external load, the LTE distribution is strongly biased towards the post-power-stroke state, that is, \( p(ii) \propto 1 \) and \( 0 \propto p(j \neq i|i) \). Hence, unbinding will occur predominantly from the post-power-stroke state so that the effective reverse rate \( r(i) \approx p(i|i)k_{20}(i,i) \propto \exp(F_{\text{ext}}/F_0) \) decreases exponentially under load. This induces the exponential increase of \( T_{10} \) observed in Fig. 5(b). Only for very large loads beyond \( F_{\text{ext}}/N_i \approx 20F_0 \), unbinding from the post-power-stroke state would become slow enough to make unbinding from the weakly bound state significant so that \( T_{10} \) would reach a plateau. At this level of force, however, forced unbinding would have to be taken into account and we do not consider such large forces in our model. The average attachment time \( T_{01} \) of a myosin II ensemble involves only a single binding step, so that the dependence on \( F_{\text{ext}} \) and \( N_i \) is rather weak: \( T_{01} = (k_{01}N_i)^{-1} \) is independent of external load and decreases inversely with ensemble size.

The ability of a molecular motor to generate sustained levels of force or continuous motion depends crucially on its duty ratio. Due to the increase of the detachment time \( T_{10} \) and the decrease of the attachment time \( T_{01} \) with \( N_i \), the ensemble duty ratio can be adjusted via the ensemble size \( N_i \). The minimal number of motors, which could allow for a duty ratio close to unity and almost continuous attachment of an ensemble, is determined by the inverse of the duty ratio of a single motor. For \( F_{\text{ext}} = 0 \), the off-rate of myosin II can be approximated as \( k_{20} \approx k_{20}^0 \approx 80 \text{ s}^{-1} \) because myosin II unbinds almost exclusively from the post-power-stroke state. With the on-rate \( k_{01} = 40 \text{ s}^{-1} \), the duty ratio of a single myosin II is

\[
\rho_{\text{single}} = \frac{T_{01}}{T_{01} + T_{10}} = \frac{k_{01}}{k_{01} + k_{20}^0} \approx 0.33.
\]

(52)

This value is significantly larger than the observed duty ratio of skeletal and smooth muscle myosin II but comparable to the duty ratio (\( \approx 0.23 \)) of non-muscle myosin II.\(^{31}\) For \( \rho_{\text{single}} \approx 0.33 \), a minimum of \( N_i \approx 3 \) motors is required for continuous attachment. However, due to the stochastic binding dynamics and the lack of coordination of individual motors, the ensemble duty ratio will be smaller than the sum over the single motor duty ratios and a larger number of motors will be required to ensure continuous attachment. For vanishing external load, the approximation of Eq. (51) for the average detachment time \( T_{10} \) can be used to derive an approximation for the duty ratio. Inserting Eq. (51) for \( T_{10} \) in Eq. (46) for the ensemble duty ratio yields

\[
\rho_d \approx 1 - \exp \left( -\ln \left[ \frac{k_{20}^0 + k_{01}}{k_{20}} \right] N_t \right).
\]

(53)

With increasing \( N_i \), the duty ratio saturates exponentially from the single motor value \( \rho_d = 1 - k_{20}^0/(k_{20}^0 + k_{01}) \) for \( N_i = 1 \) towards \( \rho_d \approx 1 \) for large \( N_i \). Fig. 6(a) shows \( \rho_d \) as

![Fig. 6. Analytical results for the parallel cluster model with constant external load: ensemble duty ratio \( \rho_d \). (a) \( \rho_d \) as function of ensemble size \( N_i \) for the values \( F_{\text{ext}}/N_i = 0.0132 \) pN, 1.262 pN, 3.787 pN, and 8.835 pN of the external load per motor. The black, dashed-dotted curve is the approximation of Eq. (53). (b) \( \rho_d \) as function of the external load per motor \( F_{\text{ext}}/N_i \) for ensemble sizes \( N_i = 4, 6, 9 \), and 15. Constant parameters are listed in Table I.](image-url)
function of $N_t$ for different values of the external load per motor. Equation (53) provides an excellent approximation for near-vanishing load. For $F_{\text{ext}}/N_t = 0.013$ pN, the duty ratio is $\rho_d \simeq 0.33$ for a single motor. For $N_t = 3$, the duty ratio is $\rho_d \simeq 0.7 < 1$ and reaches unity for ensemble sizes beyond $N_t \simeq 15$. With increasing external load, the duty ratio is elevated already for $N_t = 1$ and a smaller number of motors is required to reach a duty ratio of $\rho_d \simeq 1$. Fig. 6(b) shows the ensemble duty ratio (see Eq. (46)) as function of the external load per motor. For $N_t = 4$, $T_{10} \simeq 0.025$ s and $T_{01} \simeq 0.006$ s so that $\rho_d \simeq 0.8$ at $F_{\text{ext}}/N_t = 0$. Due to the exponential increase of $T_{10}$ with $F_{\text{ext}}/N_t$, the duty ratio increases quickly and saturates at $\rho_d \simeq 1$ above $F_{\text{ext}}/N_t \simeq F_0 \simeq 12.6$ pN. With increasing ensemble size $N_t$ the duty ratio increases and the limiting value $\rho_d \simeq 1$ is reached at smaller values of $F_{\text{ext}}/N_t$. For $N_t = 15$ the duty ratio is practically unity for all values of the external load, because $T_{10} \simeq 0.73$ s and $T_{01} \simeq 0.0017$ s so that $\rho_d \simeq 0.998$ at $F_{\text{ext}} = 0$.

Fig. 7(a) shows the average number of bound motors $N_b$ as function of ensemble size $N_t$ for different values of the external load. For all values of the load, $N_b$ increases linearly with $N_t$, where the increase becomes steeper under larger external load. Fig. 7(b) shows $N_b$ as function of the external load per motor for different ensemble sizes. At vanishing load, $N_b \simeq \rho_d^\text{single} N_t \simeq 0.33 N_t$. For $F_{\text{ext}} = 0$, the motors bind independently, because the PCM assumes that motors in equivalent states have equal strains and because most bound motors are in the post-power-stroke state, so that no internal stress is built up between motors in different states. With increasing $F_{\text{ext}}/N_t$, the average number of bound motors increases sub-linearly and plateaus towards $N_t$ for large loads. The recruitment of additional bound motors under increasing load was described theoretically$^{19}$ and has been observed experimentally for myosin II in muscle.$^{14}$ The increase of the average number of bound motors under load observed in Fig. 7(b), as well as the increase of $T_{10}$ and $\rho_d$ under load, confirms that myosin II as a whole behaves as a catch bond over a large range of values of a constant external load. Thus, the efficiency of an ensemble of non-processive motors for the generation of motion and force, which is determined by detachment time, duty ratio, and number of bound motors, can be adjusted by changing the ensemble size $N_t$ or by using the force sensitivity of the motors.

### 3. Stochastic trajectories

To gain more insight into the movement of an ensemble of non-processive molecular motors and the relation between binding and movement, it is instructive to look at single, stochastic trajectories. We use the Gillespie algorithm$^{54}$ to simulate stochastic binding and unbinding trajectories according to the one-step master equation of Eq. (10) and apply the rules for the displacement upon binding and unbinding to implement ensemble movement. Within the Gillespie algorithm, the transition rates $r(i)$ and $g(i)$ are used to choose time and type of the next stochastic transition from an exponential probability distribution. After a binding transition $i \rightarrow i + 1$ with $i \geq 1$, the position $z$ of the ensemble is changed by $\Delta z^\text{on} = -x_i/(i + 1)$. For $i = 0$, the position of the detached ensemble is changed by $\Delta z^\text{off} = -\eta F_{\text{ext}} \tau$ before attachment. Here, $\tau$ is a random attachment time with average $T_{01}$. After an unbinding transition $i \rightarrow i - 1$, $z$ is unchanged for $i \geq 2$. If the last motor unbinds, that is for $i = 1$, ensemble position is changed by $\Delta z^\text{off} = -x_1$. After adjusting ensemble position, the new value of the strain $x_1$ of weakly bound motors is determined from the balance of forces in Eq. (1) and the position $z_{\text{fil}}$ of the motor filament is set to $z_{\text{fil}} = z - x_i$. With the updated LTE distribution $p(j|i)$, the average strain of weakly bound motors, $\bar{x}_i = \sum_{j=0}^i x_j p(j|i)$, and the transition rates $r(i)$ and $g(i)$ are calculated and new random time and type of the next reaction are chosen.

Fig. 8(a) shows a stochastic trajectory of an ensemble with $N_t = 4$ motors working against the constant external load $F_{\text{ext}}/N_t = 0.126$ pN. The lower panel shows the fluctuating number of bound motors $i$, the upper panel the ensemble position $z$. The external load is below the stall force so that the attached ensemble moves forward with a velocity fluctuating around the average bound velocity $v_0 > 0$. The number of bound motors fluctuates strongly and the ensemble frequently detaches completely from the substrate. Unlike $z$, the position $z_{\text{fil}} = z - x_i$ of the motor filament is also changed by a
change of the strain $x_i$ of the motors. When a motor binds, the external load is distributed over a larger number of motors, so that the strain is reduced and the motor filament slides forward in addition to the change of $z$. When a motor unbinds, the strain $x_i$ of the remaining bound motors increases, so that the motor filament slides backwards, while $z$ remains constant. Trajectories of $z_{fil} = z - x_i$ therefore are rather close to $z$ but show stronger fluctuations (not shown). Complete detachment of the ensemble leads to backward steps of average size $\Delta z_{det} = -v_0 T_{fil}$. For a small load as in Fig. 8(a), however, backsteps are too small to be resolved so that detachment events appear as pauses in the trajectory. Fig. 8(b) shows a stochastic trajectory for an ensemble with $N_t = 4$ motors but at larger external load. At this load, the average bound velocity is positive so that the net movement of the attached ensemble is forward, although the strain $x_1$ is positive for a single bound motor so that binding of the second motor leads to a backward step in $z$. Detachment of the ensemble has become only marginally less frequent under the larger load, but the size of the backward steps has increased (note the larger $z$ scale in (b) compared to (a)) such that the effective velocity is close to zero. Hence the value of the external load is close to the effective stall force, at which the forward movement of the attached ensemble balances the backward slips of the detached ensemble.

Fig. 9(a) shows a stochastic trajectory of an ensemble at larger ensemble size $N_t = 8$. The external load is below the stall force, so that the attached ensemble moves forward with slightly fluctuating velocity. Due to the larger ensemble size, complete detachment occurs less frequently but the large external load leads to large backsteps. In Fig. 9(b), the ensemble size is the same as in (a) but $F_{ext}$ is increased to a value close to the stall force with $v_{th} \geq 0$. Due to the catch bond character of myosin II, the typical number of bound motors is increased and complete detachment is rare. Nevertheless, the ensemble position $z$ fluctuates strongly because binding at small $i$ leads to backsteps $\Delta z_{det}^{i,m} \leq 0$, whereas binding at larger $i$ leads to forward steps $\Delta z_{det}^{i,m} \geq 0$ of the ensemble. At the stall force these two effects balance so that the average bound velocity vanishes, $v_{th} = 0$. 

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**Fig. 8.** Stochastic trajectories for constant external load. Ensemble position $z$ (upper panel) and number $i$ of bound motors (lower panel) as function of time $t$ for ensemble size $N_t = 4$ and external load per motor (a) $F_{ext}/N_t = 0.126$ pN and (b) $F_{ext}/N_t = 0.835$ pN. In (a) and (b), the detached ensemble slides backwards with mobility $\eta = 10^3$ nm pN$^{-1}$ s$^{-1}$. Constant parameters are listed in Table I.

**Fig. 9.** Stochastic trajectories for constant external load. Ensemble position $z$ (upper panel) and number $i$ of bound motors (lower panel) as function of time $t$ for ensemble size $N_t = 8$ and external load per motor (a) $F_{ext}/N_t = 1.26$ pN and (b) $F_{ext}/N_t = 12.6$ pN. In (a) and (b), the unbound ensemble slides backwards with mobility $\eta = 10^3$ nm pN$^{-1}$ s$^{-1}$. Constant parameters are listed in Table I.
4. Velocity and walk length

Fig. 10(a) shows analytical results for the average bound velocity \( v_b \) (see Eq. (47)) as a function of the external load per motor for different ensemble sizes. For \( F_{\text{ext}} = 0 \), the average bound velocity is \( v_b(F_{\text{ext}} = 0) \simeq 640 \, \text{nm} \, \text{s}^{-1} \) and is independent of ensemble size \( N_t \). From its maximal value, \( v_b \) decreases with increasing \( F_{\text{ext}}/N_t \) in a concave fashion and becomes negative for loads above the stall force \( F_s \). The concave shape of the force-velocity relation has been explained before by the increase of the average number of bound motors under load\(^{19,44} \), which is a consequence of the catch bond characteristic of myosin II. Compared to a system with constant \( N_b \), the increase of \( N_b \) reduces the load on the individual bound motors and reduces their strain \( x_i \). According to Eq. (33) for \( v_i \), this increases the velocity at given external load and hence the stall force of the ensemble. Beyond sufficiently large ensemble sizes of \( N_t \geq 15 \), the force-velocity relation \( v_b(F_{\text{ext}}/N_t) \) hardly changes with further increasing \( N_t \). At \( N_t = 15 \), the stall force per motor is \( F_s/N_t \simeq 12.4 \, \text{pN} \). With increasing ensemble size, it increases slightly to \( F_s/N_t \simeq 13.5 \, \text{pN} \) for \( N_t = 50 \) because the force-velocity relation becomes very shallow near \( F_s \). Smaller ensembles show a more rapid decrease of \( v_b \) from the value at \( F_{\text{ext}} = 0 \) as well as a smaller \( F_s/N_t \). The inset in Fig. 10(a) shows \( v_b \) as a function of the absolute load \( F_{\text{ext}} \) for the same values of \( N_t \) as in the main panel. The analytical results from the model are compared to the effective, Hill-type force-velocity relation\(^{55} \)

\[
v_{\text{hill}}(F_{\text{ext}}) = v_b(F_{\text{ext}} = 0) - \frac{F_s - F_{\text{ext}}}{F_s/(N_t/\alpha)}. \tag{54}
\]

For the comparison, the values of \( v_b(F_{\text{ext}} = 0) \) and \( F_s \) are taken from the model results. The parameter \( \alpha \) is dimensionless and is used to fit the curvature of the force-velocity relations. The comparison shows that Eq. (54) describes the force-velocity relation for all \( N_t \) extremely well. The parameter \( \alpha \) is almost identical for ensemble sizes \( N_t = 8-50 \) with a typical value of \( \alpha \simeq 0.21 \) and differs significantly only for \( N_t = 4 \). For \( N_t = 4 \), the ensemble detaches frequently so that the off-step \( \Delta x_{i,j}^{\text{off}} \) from Eq. (28), in which the strain of the last unbinding motor is released and changes the ensemble position, contributes significantly to the bound ensemble velocity. This causes the markedly different dependence on \( F_{\text{ext}}/N_t \) with smaller curvature of the force-velocity curve for \( N_t = 4 \). For \( N_t = 1 \), this off-step upon unbinding can still generate forward movement.

The good fit of \( v_b(F_{\text{ext}}) \) by \( v_{\text{hill}}(F_{\text{ext}}) \) demonstrates the qualitative agreement of the PCM with the experimental force-velocity curve of muscle, for which \( v_{\text{hill}} \) was originally derived. Our model can now be used to estimate the values for load free velocity and stall force and to elucidate their dependence on the model parameters. For vanishing force, the number of bound motors on average is \( N_b \simeq \rho_0 N_t \simeq N_t/3 \). Since almost all of these motors are in the post-power-stroke state, we find \( v_i = -d \) and the binding step of the ensemble is \( \Delta x_{i,j}^{\text{off}} = -x_i/(N_b + 1) \simeq d/N_b \). Together with the forward rate \( g(N_b) = (N_t - N_b)k_{f0} = (2N_t/3)k_{f0} \), we obtain the bound velocity \( v_b(F_{\text{ext}} = 0) = g(N_b)\Delta x_{i,j}^{\text{off}} = d(N_t - N_b)/(N_t/3)k_{f0} = 2dN_t = 640 \, \text{nm} \, \text{s}^{-1} \). To estimate the stall force, we again assume that all \( N_b \) bound motors are in the post-power-stroke state. At the stall force, the strain \( x_N = (F_s/k_{m,N}) - d \) should vanish so that \( \Delta x_{i,j}^{\text{off}} = 0 \). Thus, the stall force follows the relation \( F_s/N_b = k_{m,d}d \simeq 20 \, \text{pN} \). For \( N_t = 15 \) at \( F_s/N_t \simeq 12.4 \, \text{pN} \), the number of bound motors can be read from Fig. 7(b) as \( N_b \simeq 0.68N_t \). This yields the ratio \( F_s/N_b \simeq 18.2 \, \text{pN} \), which is consistent with the estimate. The estimate of the stall force shows that \( F_s \) increases linearly with the number of bound motors. If the number of bound motors remained constant at \( N_b(F_{\text{ext}} = 0) \simeq 0.33N_t \), the stall force would be reduced to one half the actual value. On the other hand, the velocity at vanishing external load decreases with the number of bound motors. If \( N_b \) at \( F_{\text{ext}} = 0 \) had the same value \( N_b \simeq 0.68N_t \) found at \( F_{\text{ext}} = F_s \), the unloaded velocity would be reduced to \( v_b(F_{\text{ext}} = 0) \simeq 160 \, \text{nm} \, \text{s}^{-1} \). In this way, the catch bond character of myosin II motor allows ensembles to adapt the typical number of bound motors to the environmental conditions and to increase the dynamic range of an ensemble. At small external load, a small number of bound motors is able to generate fast movement of the ensemble. At large external load, a large number of bound
The stall force is reduced to \( F_{\text{ext}} \). FIG. 11. Comparison of analytical and numerical results for the parallel cluster model with constant external load: Average walk length exponentially with \( v_{\text{b}} \) and \( v_{\text{eff}} \leq v_{\text{b}} \), which is observed for smaller ensemble sizes, is determined by the frequency \( T_{10}^{-1} \) of detachment and the size \( \Delta_{\text{b}}^{\text{en}} \) of the backsteps. As shown in Fig. 5, \( T_{10}^{-1} \) decreases exponentially with \( F_{\text{ext}} \), while \( \Delta_{\text{b}}^{\text{en}} \) increases linearly with \( F_{\text{ext}} \) (see Eq. (35)). For \( F_{\text{ext}} = 0 \), detached ensembles do not move so that even very small ensembles (including single motors) effectively move forward. Because the frequency of detachment as well as the average duration \( T_{10} = (k_{01} N_{t})^{-1} \) of detachment events increases with decreasing \( N_{t} \), \( v_{\text{eff}}(F_{\text{ext}} = 0) \) is smaller for smaller \( N_{t} \). For \( N_{t} = 4 \) it is \( v_{\text{eff}}(F_{\text{ext}} = 0) \approx 513 \text{ nm s}^{-1} \) and \( v_{\text{eff}} \) decreases very rapidly with increasing external load. The stall force is reduced to \( F_{\text{ext}}/N_{t} \approx 1 \) pN compared with the value \( F_{\text{b}}/N_{t} \approx 7 \) pN for the bound ensemble. For \( N_{t} = 8 \), the effective velocity initially decreases quickly under load. Once the detachment frequency has decreased sufficiently, the force-velocity relation becomes rather shallow. As detachment becomes very rare under further increasing load, the stall force for \( N_{t} = 8 \) is almost identical to the stall force of the bound ensemble with \( v_{\text{b}} = 0 \). The interplay of the linear increase of the size of the backsteps and the exponential decrease of the detachment frequency under load can also lead to a non-monotonous force-velocity relation.

Fig. 11(a) shows the average walk length of an ensemble as function of ensemble size for different values of the external load \( F_{\text{ext}}/N_{t} \). The stationary approximation \( d_{w} = v_{\text{b}} T_{10} \) is compared to numerical results from stochastic simulations. After an initial transient, the average walk length increases exponentially with \( N_{t} \). This increase reflects the exponential increase of the detachment time \( T_{10} \) with \( N_{t} \). The initial transient is due to the variation of \( v_{\text{b}} \) for small \( N_{t} \) at given \( F_{\text{ext}}/N_{t} \). With increasing external load, the walk length increases as long as \( F_{\text{ext}}/N_{t} \) is below the stall force. For the smallest external load, the walk length reaches \( d_{w} \approx 500 \text{ nm} \) for \( N_{t} = 15 \).

For \( F_{\text{ext}}/N_{t} \approx 1.3 \) pN \( \approx 0.1 F_{\text{b}} \), the walk length increases to \( 10^{4} \) nm because the exponential increase of \( T_{10} \) outruns the decrease of the velocity. Fig. 11(b) shows \( d_{w} \) as function of the external load per motor for different ensemble sizes. For \( N_{t} = 4 \), the walk length decreases slowly because the quick decrease of \( v_{\text{b}} \) under load compensates the increase of the detachment time. For larger \( N_{t} \), \( d_{w} \) increases with \( F_{\text{ext}}/N_{t} \) over the range of force shown in the figure. Only when the stall force is reached, the walk length plummets to negative values. The stationary approximation describes the exact numerical results remarkably well over the whole range of ensemble size and external load. This confirms that bound ensembles are characterized by the stationary values for bound velocity \( v_{\text{b}} \) and processivity \( d_{w} \), in analogy to processive motors.

5. Validation of the parallel cluster model

The assumption underlying the parallel cluster model—motors in equivalent mechano-chemical states have identical strains—is not justified a priori. In fact, a finite distribution of strains is expected because motors remain bound at fixed positions on the substrate for random time intervals, while the ensemble moves with fluctuating velocity. When the ensemble moves forward, the bound motor which was bound for the longest time should have the largest strain, while the motor which has bound most recently should have the smallest strain. For groups of processive motors (see Refs. 9, 56, and 57) the load dependence of the velocity seems to provide a natural mechanism for equalizing the load on the motors: as a motor moves ahead of the group it will be subject to a large load; this reduces the velocity of the motor so that the group will catch up with the advancing motor and the loads will be equalized. On the other hand, if a motor trails behind the group it will be subject to the smallest load and will have the largest velocity so that the trailing motor catches up with the group. Such a mechanism does not seem to be at work for ensembles of non-processive motors, which cannot move along the substrate. However, movement of an ensemble of non-processive motors requires continuous unbinding and binding of the motors. Thereby, the motors remain bound.

![FIG. 11. Comparison of analytical and numerical results for the parallel cluster model with constant external load: Average walk length \( d_{w} \). (a) \( d_{w} \) as function of \( N_{t} \) for \( F_{\text{ext}}/N_{t} = 0.013 \text{ pN}, 0.379 \text{ pN}, 1.26 \text{ pN}, \) and 3.79 pN. (b) \( d_{w} \) as function of \( F_{\text{ext}}/N_{t} \) for ensemble sizes \( N_{t} = 4, 8, 10, \) and 15. The approximation \( d_{w} = v_{\text{b}} T_{10} \) (see Eq. (50)) (curves) is compared to results from stochastic simulations (symbols). Constant model parameters are listed in Table I.](http://example.com/figure11)
to the substrate for short time intervals and the strain, which was build up while the motor was bound, is released completely before the motor binds again with vanishing strain. Frequent binding and unbinding of motors as a prerequisite of movement in combination with the release of strain after unbinding should result in a narrow distribution of strains of the bound motors, which is the basis for the PCM. Interestingly, equalizing the load in a group of processive motors requires several step of the motors along the substrate, that is, several unbinding and binding events of the individual motor heads, whereas the strain of a non-processive motors is released in a single step. Due to the catch bond character of myosin II it might occur that motors remain bound for long time intervals and build up excessive strains. This, however, should only possible for a small fraction of bound motors, because the majority of motors in the ensemble is required to displace the ensemble and build up the strain. Therefore, most bound motors will still have a narrow distribution of strains.

To validate the assumptions of the PCM, we compare analytical results obtained within the PCM for constant external load to results from computer simulations which do not use the PCM assumption of equal motor strains. Moreover, these simulations do not apply the LTE of bound states but include stochastic transitions between weakly bound state and post-power-stroke state explicitly. In the simulations, the motor cycle is described by the three distinct mechano-chemical states depicted in Fig. 1. Without LTE, the stochastic transitions in an ensemble with \( N \) motors proceed on the two-dimensional network of mechano-chemical state shown in Fig. 3(a). The mechano-chemical state of the ensemble has to be complemented by the strain \( \xi_n \) of every bound motor in order to calculate strain dependent transition rates. Every bound motor head is assigned an individual position \( z_n \) on the substrate. For given external load \( F_{\text{ext}} \) and positions \( z_n \), the strain \( \xi_n \) for every bound motor is calculated from the balance of forces in Eq. (1). The position \( z \) of the ensemble is defined as the average position of the bound motor heads (see Eq. (16)). As in the PCM, detached ensembles slip backwards in the direction of the external load with mobility \( \eta \).

Transitions between unbound and weakly bound state are independent of strain so that the transitions \((0) \rightarrow (1)\) and \((1) \rightarrow (0)\) proceed with constant transition rates \( k_{01} = \text{const} \) and \( k_{10} = \text{const} \), respectively. The values are listed in Table I. For transitions \((1) \rightarrow (2)\) and \((2) \rightarrow (1)\) between weakly bound and post-power-stroke state we assume constant transition rates \( k_{21} = k_{02}^p \exp(-E_{\text{pp}}/2k_B T)\) and \( k_{12} = k_{21}^0 \exp(+E_{\text{pp}}/2k_B T)\), respectively. For our simulations, we use \( k_{21}^p = k_{21}^0 = 10^7 \text{ s}^{-1}\) but the actual value does not affect the results as long as the forward rate \( k_{12} \) is not smaller than the off-rate \( k_{10} \) from the weakly bound state. Because \( k_{21}/k_{12} = \exp(E_{\text{pp}}/k_B T)\), a Boltzmann distribution for two states with free energy difference \( E_{\text{pp}} < 0\) will establish in equilibrium. Compared to the LTE distribution of Eq. (5), the elastic energy of the motors has been omitted. For constant external load, however, the LTE distribution is dominated by the strong bias \( E_{\text{pp}}\) towards the post-power-stroke state so that the omission will have little effect on results. Unbinding from the post-power-stroke state is irreversible. The transition \((2) \rightarrow (0)\) proceeds with strain dependent transition rate

\[
k_{20}(\xi_n) = k_{20}^0 \exp(-k_E \xi_n/F_0)
\]

for \( \xi_n \geq 0 \) and \( k_{20}(\xi_n) = k_{20}^0 \) for \( \xi_n < 0 \), where \( k_{20}^0 \) and \( F_0 \) from Table I are used. Unbinding is slowed down when the neck linker is stretched in the direction of the external force but remains constant when the neck linker is compressed in the opposite direction. The latter case does not occur in the PCM model (as long as the external load is positive) so that the distinction was not necessary. Results of simulations using the Kramers’ type off-rate for positive and negative strain are discussed in Sec. S.1.2 of the supplementary material.58 As in the simulations with the PCM, we used the Gillespie algorithm54 to simulate the stochastic reactions: the reaction rates for all the motors are used to choose the waiting times between transition and the kind of reaction from the appropriate probability distributions. After every transition, strains \( \xi_n \) and transition rates are updated and the next reaction is determined.

Neglecting the change of the elastic energy in the kinetic description of the power stroke seems necessary because of the large value of the stiffness \( k_m \) of the neck linkers assumed in our model. The neck linker of a motor is stretched when it goes through the power stroke. This step is favorable as long as the increase of the elastic energy of the neck linker is smaller than the (negative) free energy bias \(-E_{\text{pp}}\) towards the post-power-stroke state. For a larger number of bound motors, \( i \gg 1 \), the power stroke stretches the neck linker of a motor approximately by the power-stroke distance \( d \approx 8 \text{ nm}\) and the decrease of the elastic energy of the other bound motors can be neglected. For \( k_m \approx 2.5 \text{ pN nm}^{-1}\), the elastic energy increases by \( k_m d^2/2 \approx 80 \text{ pN nm} > 60 \text{ pN nm} \approx -E_{\text{pp}}\). For the given elastic constant of the neck linkers, individual motors are effectively unable to perform the power stroke when a large number of bound motors holds the motor filament in place. Thus, the motor ensemble is stuck kinetically with most motors in the weakly bound state although the energy of the ensemble as a whole would be reduced by a transition of all bound motors to the post-power-stroke state. To overcome this problem and to make the power stroke favorable also for individual motors in a kinetic description, significantly smaller values of \( k_m \approx 0.3 \text{ pN nm}^{-1}\) have been used.19,44 For larger values \( k_m \approx 2.5 \text{ pN nm}^{-1}\) as in our model, the LTE assumption has been used before.45 For a kinetic description with a large value of the neck linker stiffness, variants of the motor cycle have been used, which do not require an explicit load dependence of the power stroke.46,47 This approach, however, does not allow to describe the transition of the LTE distribution to the weakly bound state when working against very stiff external springs (see Sec. III B 1) or the synchronization of the power stroke against large forces.19

In the following, we compare analytical results from the PCM with numerical results from simulations with individual motor strains. Fig. 12(a) shows the ensemble duty ratio \( \rho_4 \) as function of ensemble size for different values of the external load per motor; Fig. 12(b) shows the average number of bound motors \( N_4 \) as function of \( F_{\text{ext}}/N_4 \) for different values of \( N_4 \). For all ensemble sizes, analytical results from the PCM agree very well with numerical results with individual
motor strains. Significant deviations are only observed for small external load where the analytical results underestimate the duty ratio as well as the number of bound motors. These deviations are caused by the distribution of strains among bound motors. For vanishing and small external load, unbinding of those motors with positive strain will be slowed down while unbinding of those motors with negative strain is unaffected. With increasing external load, the strain of the motors is dominated by the external load and the effects of the distribution of strains become negligible.

Fig. 13 compares analytical and simulation results for the bound velocity \( v_b \) in (a) and for the effective velocity \( v_{\text{eff}} \) in (b) as function of the external load per motor for different ensemble sizes. The agreement is quite good for small \( N_t \), but clear deviations are observed for large \( N_t \) at small and intermediate values of \( F_{\text{ext}} \). At vanishing and small load, the simulations show a decrease of the bound velocity \( v_b \) with increasing \( N_t \). In the PCM, \( v_b(F_{\text{ext}} = 0) \) is independent of \( N_t \). The \( N_t \) dependence of \( v_b \) is caused by the increase of the average number of bound motors at small external load observed in Fig. 12(b). As demonstrated in Sec. III A 4, an increasing number of bound motors reduces the bound velocity at vanishing external load. At intermediate values of \( F_{\text{ext}} \), the numerical results for \( v_b \) and \( v_{\text{eff}} \) are larger than predicted by the PCM because the numerical force-velocity relation is less concave than the analytical one. This underestimation of the velocity has been predicted in Sec. II E and is due to the preferential unbinding of post-power-stroke motors with small strain in the presence of a distribution of internal strains. Close to the stall force, the analytical and numerical results again agree very well even for large values of \( N_t \).

B. Linear load

1. LTE distribution and effective reverse rate

For constant external load, the elastic energy stored in the neck linkers was symmetric against exchanging weakly bound and post-power-stroke motors, \( j \leftrightarrow i - j \). This symmetry was not affected by the value of \( F_{\text{ext}} \), so that the LTE distribution remained strongly biased towards the post-power-stroke state for all values of a constant external load. For a linear external load, on the other hand, the elastic energy stored in the neck linkers of bound motors and in the external spring...
favor the weakly bound state (see Eq. (9)). Because this contribution to the elastic energy of the ensemble increases with increasing stiffness of the external spring, the bias of the LTE distribution will shift towards the weakly bound state for large values of $k_l$. This transition between post-power-stroke and weakly bound state has been described as a possible basis for unconventional elastic behavior of muscle fibers.

Fig. 14(a) plots the conditional probabilities $p(i|i)$ (all bound motors in the post-power-stroke state) and $p(0|i)$ (all bound motors in the weakly bound state) from the LTE distribution of Eq. (5) as function of the external spring constant per bound motor, $k_l/i$, for the ensemble position $z = 0$ and different numbers of bound motors, $i$. The probabilities $p(j|i)$ for the intermediate states $0 < j < i$ are negligible due to internal strains built up by bound motors in opposite states working against each other. For small $k_l/i$, most bound motors are in the post-power-stroke state with $p(0|i) \lesssim 1$. At a critical value of the external spring constant, $k_f^c/i \approx 7.5 \, \text{pN nm}^{-1}$, the bias of the LTE distribution shifts rapidly from the post-power-stroke state to the weakly bound state. The ratio $k_f^c/i$ is independent of the number of bound motors but the transition becomes sharper with increasing $i$. The power stroke of a bound motor is driven by the free energy bias $E_{pp} < 0$ towards the post-power-stroke state. Thus, the transition of the LTE distribution from post-power-stroke to weakly bound state occurs, when the increase of the elastic energy of an ensemble upon the transition of $i$ bound motors from weakly bound to post-power-stroke state (see Eq. (9)) exceeds the free energy gain $-jE_{pp}$, upon this transition. Solving the condition

$$\frac{ik_n k_f^c}{i k_m + k_f^c} \frac{d(2z + d)}{2} = -i E_{pp} = i |E_{pp}|$$

(56)

for the critical spring constant yields

$$k_f^c/i = \frac{k_m d (2 z + d)}{2 |E_{pp}|} - 1 \text{.}^{(57)}$$

The ratio $k_f^c(z)/i$ is independent of $i$ for all values of $z$. This is due to the parallel arrangement of the motors under the external load. The inset in Fig. 14(a) plots $k_f^c(z)/i$ as function of $z$. Because $k_l \geq 0$, a finite critical elastic constant exists for all $z \geq 0$ only if $k_m d^2 > 2 |E_{pp}|$. This is the case for our model parameters listed in Table I. In Walcott et al., the parameters $d = 10 \, \text{nm}$ and $k_m = 0.3 \, \text{pN nm}^{-1}$ are used to characterize the power stroke. For these values, $k_m d^2 < 2 |E_{pp}|$ so that the transition of the LTE distribution can only occur above a finite ensemble position $z \geq (|E_{pp}|/k_m d) - d/2$. The corresponding $k_f^c(z)/i$ is also plotted in the inset in Fig. 14(a). For both parameter sets, the critical spring constant decreases as $k_f^c/i \propto z^{-1}$ for large $z$ so that the external load $k_f^c z/i$ at the LTE transition becomes independent of $z$.

The LTE transition from post-power-stroke to weakly bound state affects the binding dynamics of an ensemble quantitatively and qualitatively, because the off-rate from the weakly bound state is significantly smaller than the unloaded off-rate from the post-power-stroke state, $k_{00} \ll k_{20}^0$, and because $k_{ij}$ is independent of the load on a motor. Fig. 14(b) plots the effective reverse rate divided by the number of bound motors, $r(i)/i$, as function of $k_l/i$ for different values of $i$ and $z$. For $k_l/i \to 0$, motors unbind predominantly from the post-power-stroke state ($p(0|i) \lesssim 1$) and $r(i)/i$ approaches the value of the unloaded off-rate from the post-power-stroke state, $r(i)/i \to k_{00}^0 \approx 20 \, \text{s}^{-1}$. From this limit, $r(i)/i$ decreases exponentially with $k_l/i$, because the load on a post-power-stroke motor increases linearly with $k_l/i$ and $k_{20}$ decreases exponentially under load. Below the LTE distribution, it is $r(i)/i \gg k_{10}$. Thus, the effective reverse rate decreases strongly as the LTE distribution shifts towards the weakly bound state and approaches the off-rate from the weakly bound state, $r(i)/i \to k_{10} \approx 2 \, \text{s}^{-1} = \text{const}$ for large $k_l > k_f^c$. The exponential decrease of $r(i)/i$ and the critical elastic constant are independent of $i$ but the transition becomes sharper with increasing $i$. With increasing $z$, the initial exponential decrease of $r(i)/i$ becomes faster. Nevertheless, the drop of $r(i)/i$ at the LTE becomes more pronounced because the LTE transition occurs at smaller values of $k_f^c/i$, as predicted by Eq. (57).
In an ensemble with $N_t$ molecular motors, the number $i$ of bound motors fluctuates continuously. Because the critical elastic constant $k_f^c(z)$ is proportional to $i$, the LTE distribution follows the fluctuations of $i$ and alternates between post-power-stroke state (for large $i$ with $k_f/i < k_f^c(z)/i$) and weakly bound state (for small $i$ with $k_f/i > k_f^c(z)/i$) when the ensemble is in the transition region. Because the transition to the weakly bound state occurs first for the smallest $i$ and unbinding from the weakly bound state is significantly slowed down, onset of the LTE distribution will stabilize the ensemble against unbinding. Furthermore, the critical elastic constant is itself a dynamic quantity, because $k_f^c(z)/i$ reduces with increasing ensemble position. Thus, for linear external load two different mechanisms can stabilize an ensemble as it moves to larger $z$: (i) for ensemble positions below the LTE transition, unbinding is slowed down by the catch bond character of motors in the post-power-stroke state and (ii) above the LTE transition threshold, unbinding is slowed down by the transition to the weakly bound state. Moreover, because ensemble movement relies on the presence of motors in the post-power-stroke state, ensemble movement feeds back negatively on itself and the ensemble will stall as the LTE transition threshold is reached.

2. Stochastic trajectories

As for the case of constant external load, it is instructive to study individual stochastic trajectories in order to gain more insight into the interplay of ensemble movement and binding dynamics. The stochastic trajectories are generated using the Gillespie algorithm as described for the case of constant load. The upper panel of the stochastic trajectories is now used to display the external load $k_f z$, which is proportional to ensemble position but omits the strongly fluctuating contribution of the strain of the motors. The actual external load depends on the number of bound motors and their states: if all bound motors are in the post-power-stroke state, the external load is larger than $k_f z$. If all bound motors are in the weakly bound state, the external load is smaller than $k_f z$.

Fig. 15(a) shows a stochastic trajectory of an ensemble with $N_t = 4$ motors working against a linear external load with elastic constant $k_f/N_t = 0.126$ pN nm$^{-1}$ ($k_f = 1.26$ pN nm$^{-1}$). At $z = 0$, the ratio $k_f/i$ is below the critical elastic constant $k_f^c/i \approx 7.5$ pN nm$^{-1}$ for all $i \geq 1$. Therefore, ensemble position initially increases gradually. During this transient movement, the ensemble occasionally detaches completely, because for all $i \geq 1$ unbinding occurs from the post-power-stroke state with large intrinsic off-rate against small external load. After the initial transient, the ensemble reaches a stationary state in which $k_f z$ fluctuates around a constant average. The typical external load $k_f z \approx 12$ pN in this isometric state corresponds to the ensemble position $z \approx 24$ nm. For this value of $z$, the critical elastic constant is lowered to $k_f^c(z)/i \approx 0.3$ pN nm$^{-1}$. With $k_f = 0.504$ pN nm$^{-1}$, the LTE distribution shifts to the weakly bound state for $i = 1$ but remains in the post-power-stroke state for $i > 1$. Due to the very slow unbinding of the last ($i = 1$) bound motor from the weakly bound state, complete detachment of the ensemble is no longer observed in the isometric state, although $i$ continues to fluctuate between $i = 1$ and $N_t$. This demonstrates the stabilization of the ensemble due to the LTE transition. On the other hand, the LTE transition for $i = 1$ also causes stalling of the ensemble and prevents movement beyond the isometric state. Assuming that all bound motors are in the post-power-stroke state, even for the largest values of $z$ the strain $x_{ij}$ is negative for all $i > 1$ so that the ensemble still steps forward in these states. (Although the size of the steps may be reduced due to the increased probability for weakly bound motors in the proximity of the LTE transition). Because the ensemble can only step backwards when all bound motors are in the weakly bound state, the ensemble alternates between forward stepping for large $i$ ($k_f/i$ below the threshold) and backward stepping for small $i$ ($k_f/i$ above the threshold). The isometric state is reached, when these two contributions balance and the ensemble fluctuates around a constant average position. Excursion to large $z$ will shift the LTE to the weakly bound state for higher values of $i$ and induce quick backward movement and restoring of...
the isometric state. Excursions to small z, on the other hand, will shift the LTE distribution towards the post-power-stroke state for i = 1, thus inducing quick forward movement towards the isometric state. This stalling mechanism is different from the case of constant external load, where the LTE distribution was always in the post-power-stroke state and the stall force was determined by vanishing \( x_{ij} \) under large load. Fig. 15(b) shows a stochastic trajectory of an ensemble with \( N_t = 4 \) motors working against the larger external elastic constant \( k_t/N_t = 1.26 \text{ pN nm}^{-1} \). The isometric load \( k_{iz} \approx 12 \text{ pN} \) is comparable to the trajectory from Fig. 15(a). Due to the larger elastic constant, however, this corresponds to the smaller ensemble position 2.4 nm. Moreover, small fluctuations in z induce strong fluctuations of \( k_{iz} \). The critical elastic constant for the LTE transition is \( k_{iz} / \eta \approx 2.5 \text{ pN nm}^{-1} \). Again, the value of \( k_t \approx 5.04 \text{ pN nm}^{-1} \) is below the critical elastic constant for \( i > 1 \) and above for \( i = 1 \) so that complete detachment is prevented by the LTE transition to the weakly-bound state in the lowest bound state \( i = 1 \). As in Fig. 15(a), the strain \( x_{ij} \) of the bound motors is negative for \( i > 1 \) (assuming they are all in the post-power-stroke state) so that the stalling of the ensemble is caused by the LTE transition.

For the trajectories with \( N_t = 4 \) in Fig. 15, the LTE transition occurred only in the lowest bound state \( i = 1 \). For increasing \( N_t \), more states with \( i \geq 1 \) undergo the LTE transition and fluctuations of \( i \) in the isometric state are effectively restricted to values above the threshold (compare Fig. S4 in the supplementary material). For large ensembles as in Fig. S4 of the supplementary material, the alternating forward and backward motion in the isometric state displays a characteristic pattern of rapid increase of \( k_{iz} \) concomitant with an increase of \( i \), followed by a gradual decrease of \( k_{iz} \) accompanied by a decrease of \( i \). This pattern, which becomes more pronounced for larger values of the external spring constant, is reminiscent of oscillation pattern for ensembles of motors working against an elastic element. A Fourier analysis, however, has not shown any characteristic time scale for the fluctuations.

In the above trajectories, detached ensembles are stationary with mobility \( \eta = 0 \). Fig. 16 demonstrates the effect of resetting an ensemble to \( z = 0 \) with infinite mobility \( \eta = \infty \). Constant parameters are listed in Table I.

3. Binding dynamics

To study the interplay of ensemble movement and binding dynamics, we first analyze the dependence of detachment time \( T_{10} \) on ensemble size \( N_t \) and external elastic constant \( k_t \). Because the one-step master equation Eq. (10) cannot be solved with position dependent transition rates, the detachment time is calculated numerically by averaging the first passage time from \( i = 1 \) to \( i = 0 \) over repeated, stochastic trajectories. The trajectories all start at \( z = 0 \) and are terminated as soon as the ensemble detaches, so that the mobility \( \eta \) has no influence on the result.

Fig. 17(a) plots simulation results for the average detachment time \( T_{10} \) of an ensemble as function of ensemble size \( N_t \) for different values of the external elastic constant \( k_t/N_t \). The largest value of \( k_t/N_t \) is above the critical external elastic constant.
constant at \( z = 0 \) so that unbinding proceeds predominantly from the weakly bound state for all values of \( N_i \) and is independent of load. Thus, \( T_{10} \) increases approximately exponentially with increasing \( N_i \) and reaches \( T_{10} \approx 10^3 \) s already for \( N_i \approx 5 \). For constant load, such detachment times required external loads well beyond the stall force. For the smallest value of \( k_f/N_i \), which is well below \( k_f(z = 0)/i \), a transient regime with a very slow increase of \( T_{10} \) is observed. Here, the average detachment time results from a combination of fast detachment from the post-power-stroke state during the transient increase of \( z \) and slow unbinding from the weakly bound state (for small values of \( i \)) once the isometric state is reached. For sufficiently large \( N_i \), detachment before reaching the isometric state becomes unlikely and \( T_{10} \) is determined by slow unbinding from the weakly bound state. Thus, the increase of \( T_{10} \) with \( N_i \) becomes similar to the exponential increase observed for large \( k_f/N_i \). For increasing values of \( k_f/N_i \), the transient regime of slow increase of \( N_i \) becomes less pronounced and \( T_{10} \) increases exponentially for most values of \( N_i \).

Fig. 17(b) plots \( T_{10} \) as function of \( k_f/N_i \) for different \( N_i \). The plot reveals three different regimes of the detachment time which corresponds to different detachment mechanisms. At very small \( k_f/N_i \) ensembles detach during the initial increase of \( z \). Here, unbinding of motors proceeds predominantly from the post-power-stroke state under small external load, so that the detachment time is almost independent of \( k_f/N_i \). In an intermediate regime, the detachment time increases significantly. Here, the ensembles can reach the isometric state so that the contribution of slow unbinding from the weakly bound state becomes more prominent. Because the ensembles adjust themselves dynamically to the isometric state, large ensembles display a plateau region with constant \( T_{10} \). When the external elastic constant approaches the isometric state, large ensembles display a plateau region with constant \( T_{10} \). This article is copyrighted as indicated in the abstract. Reuse of AIP content is subject to the terms at: http://scitation.aip.org/termsconditions. Downloaded to IP: 129.206.205.46 On: Tue, 05 Nov 2013 16:53:19
$T_{10}$. For the largest value with $k_f/N_t > k_f^c/N_t$, the duty ratio at $N_t = 1$ is increased significantly with respect to the duty ratio of a single free motor, $\rho_0^{\text{single}} \approx 0.33$. Due to the exponential increase of the detachment time, $\rho_0 \approx 1$ is reached already for $N_t \geq 2$. For smaller $k_f/N_t < k_f^c/N_t$, the duty ratio at $N_t = 1$ is close to the duty ratio of a single free motor. This indicates that unbinding proceeds predominantly from the post-powerstroke state. Due to the rapid increase of $T_{10}$ with $N_t$, permanent attachment with $\rho_0 \approx 1$ is achieved for $N_t \geq 5$. This is significantly smaller than the ensemble size $N_t = 15$ required for permanent attachment under constant external load. Fig. 18(b) plots $\rho_0$ as function of $k_f/N_t$ for different $N_t$. In analogy to the detachment time, $\rho_0$ displays two regimes: a constant duty ratio at small $k_f/N_t$ followed by a rapid increase at intermediate values of $k_f/N_t$. Because $\rho_0 \approx 1$ is already reached here, the strong increase of $T_{10}$ for $k_f/N_t \gtrsim k_f^c(z = 0)/N_t$ cannot be resolved.

To calculate the average number of bound motors $N_b = \langle i \rangle$, we average $i$ over long trajectories in which the ensembles are allowed to detach from the substrate. The detached ensembles are stationary with vanishing mobility, $\eta = 0$, so that the isometric state of the ensembles is probed. Fig. 19(a) plots the average number $N_b$ of bound motors as function of ensemble size $N_t$ for different values of the external elastic constant per motor $k_f/N_t$. For all the values of $k_f/N_t$ the average number of bound motors increases linearly with $N_t$. The slope becomes steeper with increasing $k_f/N_t$ but saturates for very large $k_f/N_t$. Only for small values of $k_f/N_t$ there is a short transient with a slower increase of $N_b$. Fig. 19(b) plots $N_b$ as function of the external elastic constant per motor $k_f/N_t$ for different values of $N_t$. For $k_f/N_t < k_f^c(z = 0)/N_t$, the average number of bound motors is constant. Here, the ensembles adjust themselves to an isometric state at finite ensemble position $z > 0$. As the value of $k_f/N_t$ exceeds the critical values $k_f^c(z = 0)/N_t$, the average number of bound motors increases steeply. Here, the LTE transition to the weakly bound state occurs already at $z = 0$ and for an increasing number of states $i$ (see Fig. S4 of the supplementary material).

4. Average external load

For linear external load, analogous to the force-velocity relation as a characteristic for the dynamic properties of an ensemble under constant external load, is the average external load $\langle F_{\text{ext}} \rangle = (k_f z)$ of the ensemble in the stationary state. As explained in the context of the stochastic trajectories, this quantity differs from the actual load in the external elastic element by leaving out the strain $x_g$ of the motors.

As for the average number of bound motors, the average external load is determined by averaging over long trajectories with multiple unbinding events. In Fig. 20, the detached ensembles are stationary with mobility $\eta = 0$, so that the average load in the isometric state is probed. Fig. 20(a) plots the average external load $\langle F_{\text{ext}} \rangle$ in the stationary state of an ensemble as function of ensemble size $N_t$ for different values of the external elastic constant per motor $k_f/N_t$. Below the critical value for the LTE transition, $k_f/N_t < k_f^c/N_t$, the average external load increases with $N_t$. For very small external elastic constants, $k_f/N_t < 1$ pN nm$^{-1}$, the curves are almost identical for different $k_f$, but begin to decrease for $k_f/N_t > 1$ pN nm$^{-1}$. This decrease of $\langle F_{\text{ext}} \rangle$ for given $N_t$ is observed in the trajectories of Fig. S4 of the supplementary material. At the critical external elastic constant, $k_f/N_t \approx k_f^c/N_t$, the average external load is independent of $N_t$. Above the critical threshold, the LTE transition towards the weakly bound state occurs already at $z = 0$ so that ensemble movement is severely reduced. Here, $\langle F_{\text{ext}} \rangle$ decreases with increasing $N_t$. Fig. 20(b) plots the average external load as function of $k_f/N_t$ for different $N_t$. For small $k_f/N_t < k_f^c/N_t$, the average external load is independent of $k_f/N_t$. Close to the critical value, $\langle F_{\text{ext}} \rangle$ breaks down because ensemble movement is effectively impossible.

Fig. 21(a) plots the average external load as function of ensemble size $N_t$ for different values of $k_f/N_t$ for the case that the ensemble is reset to its initial position $z = 0$ after complete detachment. This corresponds to the limit of large mobility $\eta \to \infty$. The values of $k_f/N_t$ are below the critical value $k_f^c(z = 0)/N_t$ and from a range in which $\langle F_{\text{ext}} \rangle$ at $\eta = 0$ was independent of $k_f/N_t$. For small external elastic constant,
the isometric state corresponds to a large ensemble position \( z \). Therefore, ensembles detach frequently during the long transient movement towards the isometric state. Because detached ensembles are reset to \( z = 0 \), this reduces the average external load, as observed in Fig. 16(a). Because detachment becomes less likely with increasing \( N_t \) and ensembles are stabilized in the isometric state, \( \langle F_{\text{ext}} \rangle \) increases with \( N_t \) and eventually jumps discontinuously to the average load for \( \eta = 0 \). With increasing \( k_f/N_t \), the average external load grows in proportion to \( k_f/N_t \) at given \( N_t \). This means that the movement of the ensemble during the initial transient is hardly affected by the small external load. Because the isometric state corresponds to smaller ensemble position \( z \), however, the discontinuous jump to the curve for \( \eta = 0 \) occurs at smaller values of \( N_t \) and becomes less pronounced. Fig. 21(b) plots the average external load for \( \eta \to \infty \) as function of \( k_f/N_t \) for different \( N_t \). For small \( k_f/N_t \), \( \langle F_{\text{ext}} \rangle \) increases linearly towards the average isometric load. For larger \( N_t \), \( \langle F_{\text{ext}} \rangle \) for given \( k_f/N_t \) increases and jumps discontinuously towards isometric load. The discontinuity becomes more pronounced with increasing \( N_t \) and the position of the jump decreases.

### IV. DISCUSSION AND SUMMARY

In this paper, we have introduced and analyzed a stochastic model for ensembles of non-processive motors such as myosin II working against an external load. The model allows us to investigate in detail the effect of a finite number of motors in an ensemble, most importantly the stochastic binding dynamics of the motors. Introducing the parallel cluster model and using the local thermal equilibrium approximation allowed us to reduce the complexity of the model significantly, eventually leading to efficient numerical simulations and analytical results for stationary properties. In detail, we have analyzed two paradigmatic situations in which the motor-ensemble works against either a constant or a linear external load. Both situations are highly relevant for a large class of experiments.

For constant external load, our results for large ensemble sizes are in good qualitative agreement with previous model results for large assemblies of myosin II.\(^{19,44} \) Due to the local thermal equilibrium assumption, however, our model is not able to describe, e.g., the synchronization of motors under...
large external load which is due to the kinetic hindrance of the power stroke. Average quantities, however, are well represented. In particular, the force-velocity relation of an ensemble follows the characteristic concave shape which is described by a Hill relation and is found experimentally for muscle fibers, as well as for small ensembles of myosin II. The parallel cluster model makes it easy to identify the relevant quantities determining the force-velocity curve. In particular the role of the load-sensitivity of unbinding from the post-power-stroke state and the increase of the number of bound motors under load for the adaption of the dynamic range of an ensemble becomes clear. Due to the strong bias of the LTE distribution towards the post-power-stroke state, myosin II as a whole behaves as a catch bond for the whole range of constant external load considered in this paper. This induces the increase of the number of bound motors under load, which is the basis for the concave shape of the force-velocity relation. At small external load, a small number of bound motors is able to work against the external load and the large number of unbound motors generates fast ensemble movement with little resistance from the bound motors. At large external load, on the other hand, the increase of the number of bound motors allows to increase the stall force of the ensemble relative to the case of a constant number of bound motors. Thus, the mechansensitive response of myosin II to a constant external load greatly increases the dynamic range over which myosin II ensembles can operate and the robustness of ensemble movement. As demonstrated in Sec. S.2.1.1 of the supplementary material, the apparent load-sensitivity of myosin II becomes stronger for small duty ratios, for which a stronger increase of the number of bound motors is observed. The relevance of this mechanism of mechansensitivity for the efficiency of motor ensembles is underlined by the experimental observation of the increase of the number of bound motors in muscle filaments which use myosin II and the recent identification of a similar load-sensitive step in myosin I. This last result indicates that the mechanisms described here might have wider applications. In addition to reproducing previous results for large ensembles, our model in particular allows to identify the range of ensemble sizes in which stochastic detachment of ensembles is relevant and reduces the efficiency of ensembles. As demonstrated in Sec. S.2 of the supplementary material, this range depends strongly on the duty ratio of the single motor. The smaller the single motor duty ratio, the larger is the number of motors in an ensemble that is needed to ensure practically permanent attachment. For the model parameters used in the main part of the paper, the single motor duty ratio is similar but slightly larger than reported for non-muscle myosin II. Here, it turns out that roughly $N_k = 15$ motors are needed for almost permanent attachment. This number is in the range of the size of minifilaments, in particular if one considers that due to spatial restrictions, not all motors which are contained in a minifilament can actually bind to a substrate at the same time. Thus, unbinding will be relevant for cytoskeletal myosin II minifilaments. Interestingly, due to the load-sensitivity of myosin II minifilaments, detachment of the ensembles will occur most frequently for ensembles under small load, whereas ensembles working against large external load are stabilized by the catch bond character of myosin II so that they can form efficient crosslinkers of actin fibers.

For linear external load, it has been shown that the load-sensitivity of the unbinding step is less relevant for the behavior of a motor ensemble. Rather, the reverse transition from the post-power-stroke state to the weakly bound state, which is induced by the stiffness of the external spring or by large external load, eventually stalls the ensemble in the isometric state. As long as the stiffness of the external spring is below the critical threshold, ensembles can move forward until the isometric state is reached. Because ensembles adapt their position dynamically towards the isometric state, characteristic dynamic properties such as the detachment time or the typical number of bound motors in the isometric state are independent of the external elastic constant state and increases significantly only when the critical stiffness is reached. Thus, measuring the change of the number of bound motors with the stiffness of the external force is a characteristic sign of the relative size of the unbinding rates from the different bound states of the motors. The critical value of $k_1$ above which ensemble are no longer able to generate force allows to determine the free energy bias towards the post-power-stroke state. The qualitative change of behavior of the ensemble should allow to observe this experimentally even in noisy data, e.g., in extensions of the three bead assay with better control over the number of motors. Typical stochastic trajectories reveal a behavior which is qualitatively similar to previously predicted or experimentally observed types of behavior. For relatively large ensembles which do not unbind, a sort of irregular oscillation pattern has been observed, similar to the oscillation predicted using a ratchet model for large ensembles of motors. The linear increase of stress followed by a fast stress relaxation which is observed in three bead assays and in active gels has been reproduced for the case that unbinding from the substrate occurs before the ensemble reaches its equilibrium position.

The sequence of reactions in our basic crossbridge model is compatible with experiments. Compared to other models, we have neglected one additional post-power-stroke state, which will be important for detailed descriptions of the force-velocity relation in skeletal muscle or inclusion of the ATP dependence of the motor cycle. The ATP dependence, however, could also be included in our model via an ATP dependent off-rate from the post-power-stroke state. On the other hand, our model explicitly includes the weakly bound state (or pre-power-stroke state) as a load bearing state, which is omitted by Walcott et al. Because for constant external load, the weakly bound state is hardly occupied so that motors bind effectively directly to the post-power-stroke state as in Walcott et al. For linear external load, however, the isometric state of the ensemble is determined by the transition from the post-power-stroke to the weakly bound state, which could not be described without a model for the power stroke. Without the weakly bound state, which is stable under load, this transition would destabilize the ensemble instead of stabilizing it. The choice of the set of parameters used in our model was motivated by previous modeling approaches and did not aim at a description of a specific molecular motor. However, the basic conclusions of the model do not depend on the exact choice
of parameters. The only prerequisite for the application of the model is that transitions between the bound states are fast so that the local thermal equilibrium can establish. Comparison with simulations without PCM or LTE have shown that this requires a forward rate $k_{12}$ for the power stroke which is faster than the off-rate $k_{10}$ from the weakly bound state. Thus, our model can be used with different choices of parameters. This is demonstrated in Sec. S.2 of the supplementary material for different choices of the single motor duty ratio. The discussion of the result has demonstrated the dependence of experimentally measurable quantities such as the number of bound motors, the load free velocity, or the stall force on the model parameters so that the parameters can be adapted to describe a desired behavior of the motor ensemble.

Ensembles of non-processive motors behave very similar to processive motors with a specific force-velocity relation and a typical walk length. The analytical expressions allow to integrate such ensembles in larger systems in a similar manner as it has been done for processive motors. In such a systems, ensembles of ensembles would be multiply coupled either in series or in parallel through forces that they generate. Such model could be used to describe, e.g., tension generation in a stress fibers or the cell actin cortex. Since the relaxation to the stationary values is relatively fast, slow external stimuli, changing for example the motor activity, could be included to mimic signaling events which a cell experiences, e.g., during cell migration.

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