Motility states in bidirectional cargo transport

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Abstract – Intracellular cargos which are transported by molecular motors move stochastically along cytoskeleton filaments. In particular for bidirectionally transported cargos it is an open question whether the characteristics of their motion can result from pure stochastic fluctuations or whether some coordination of the motors is needed. The results of a mean-field (MF) model of cargo-motors dynamics proposed by Müller *et al.*[Müller, M. J. *et al.*. (2008). PNAS, 105(12), 4609-4614.], suggest the existence of states which are characterized by a symmetric bimodal distribution of cargo velocities. These states would result from a stochastic tug-of-war. Here we analyze the influence of the MF assumption on the cargo motion by considering a model that takes explicitly the position of each motor into account. We find that those states with symmetric bimodal distributions then disappear. As the MF model implicitly assumes some stepping synchronization between motors, we introduce a partial synchronization via an artificial mutual motor-motor activation, and show that the results of the MF model are then recovered but, even in this favorable case, only in the limit of a strong motor-motor activation and of a high number of motors. We conclude that the MF assumption is not relevant for intracellular transport.

Introduction. – Almost every cellular function is related to transport processes. These are necessary in order to maintain concentration gradients, but also in order to built and adapt cellular structures. Many of these transport issues are carried out by molecular motors, i.e. proteins which perform a directed stochastic motion along the polar filaments of the cytoskeleton. The cytoskeleton of eukaryotic cells is composed of three different kinds of filaments: actin, intermediate filaments and microtubules. These filaments give a cell its characteristic shape, but also play a key role in intracellular transport. Microtubules (MTs) have well-defined plus- and minus-ends, and are tracks for transport from the nucleus to the periphery and *vice versa* [1].

Two families of proteins called molecular motors walk along the MTs - kinesin and dynein. Kinesins walk from the minus-end, which is in general located close to the nucleus, to the plus-end which grows in the vicinity of the cell membrane, while dyneins walk in the opposite direction [1]. After this processive motion, motors detach stochastically from the filament and possibly diffuse around before attaching again.

Molecular motors are able to transport very different types of cargo, either individually or by teams of molecular motors which are attached to the same cargo. Transport by teams of molecular motors is particularly relevant for large objects like vesicles or cell organelles [2]. Transporting cargo by many motors in a crowded environment is obviously beneficial since it increases the processivity along the MT dramatically and also allows the motor cargo complex to withstand larger friction forces.

For several types of cargo, however, a bidirectional motion along a filament was observed both *in vivo* [3] and *in vitro* [4]. These observations suggest that the cargo is transported both by kinesin and dynein motors which are attached to the cargo at the same time. The number of motors is usually estimated to be in the order of up to five motors per team [5,6]. One key question is if the observed cargo motion is a result of unknown coordination mechanisms between motors or is driven by fluctuations.

Various theoretical models have been suggested, which aim at describing the origin of the complex dynamics

of bidirectionally transported cargo [7–9]. A mean-field model (MF-model) which describes bidirectional cargo motion [7] driven by two teams of molecular motors, was introduced a few years ago by Müller *et al.*. They assume that two equally strong teams of molecular motors with opposed walking directions are bound to a cargo at the same time. The model is based on a mean-field assumption stating that the force exerted by the cargo is equally shared among motors moving in the same direction. An equivalent formulation of the MF assumption is that all the motors of the same team have the same linkage stretching. This property can only be maintained if the motors step synchronously and allows one to formulate the MF model without any explicit reference to the motor positions. The forces exerted on the motors by the cargo determine their attachment and detachment rates. The cargo's velocity also depends on these forces. Assuming a force balance at every time, this velocity is uniquely determined for a given number of attached "+" and "−" motors. In the frame of this MF assumption, the model by Müller *et al.* predicts the existence of states, which are characterized by a symmetric bimodal (or trimodal) distribution of cargo velocities around both single motor velocities (and zero velocity). In this case, also the distribution of the number of attached motors is bi-/trimodal. In the following we shall refer to these distributions as *symmetric bimodal distributions, SBD*. In the MF-model, these states originate from a purely stochastic tug of war between the oppositely directed motors without requiring any regulatory mechanism.

The aim of this work is to test whether those states with SBD persist beyond mean-field. We use a more explicit modeling approach (explicit position-based, EPB-model) inspired by the model introduced by Kunwar *et al.* [8]. In contrast to the MF-model we explicitly consider the motor's positions on the filament, and the couplings between motors and cargo which are modeled as linear springs. As we want to discuss here the consequences of the mean-field assumptions rather than modeling an explicit experimental setup, we consider that "+"- and "−"-directed motors have the same response to applied forces as in the MF-model [7]. The EPB-like approach has already been analyzed in different aspects and it was shown that differences between MF- and EPB-model exists [8, 10, 11]. But the fundamental question whether these famous MF-states with SBD exist for symmetric teams of motors has not been yet addressed as such.

Here we shall concentrate on the case of equal teams as in [7]. Our main result is that SBDs cannot be observed anymore when the MF assumption is released. We introduce an artificial mutual motor activation to quantify the degree of motor synchronization needed to be in the MF limit. We show that even if partial motor synchronization could be induced (we do not expect that this is the case in real cells), the MF assumption would be relevant only in the (biologically rather improbable) case of many motors.

Model description. – In the EPB-model we assume that two teams of motors are tightly bound to a cargo. Each team consists of N "+"- and "−"-motors, respectively (see Fig. 1 for a sketch).

To determine the load force applied on the cargo at position $x_C(t)$ at time t we take every single motor position x_i into account. We model the motor linker as a linear spring with spring constant α and untensioned length L_0, such that motors experience no force when located at a distance from $x_C(t)$ smaller than L_0. So the force $F_i(x_C(t), \{x_i\})$ on the cargo caused by the i-th motor is given by

$$F_i(x_i - x_C(t)) = \qquad\qquad\qquad\qquad (1)$$
$$\begin{cases} \alpha(x_i - x_C(t) + L_0), & x_i - x_C(t) < -L_0 \\ 0, & |x_i - x_C(t)| < L_0 \\ \alpha(x_i - x_C(t) - L_0), & x_i - x_C(t) > L_0. \end{cases}$$

We assume that no force is exerted if the motor is not attached to the filament. When attached the motors can perform steps along the filament with a rate depending on the force F_i chosen here so as to allow a direct comparison with the MF results [7]. It is defined as

$$s_+(F_i) = \begin{cases} \frac{v_F}{d}, & F_i < 0 \\ \frac{v_F}{d}\left[1 - \frac{F_i}{F_S}\right], & 0 \le F_i \le F_S \\ \frac{v_B}{d}, & F_i > F_S \end{cases} \qquad (2)$$

for "+"-motors and symmetrically for "−"-motors.

If no force is applied on the motors or if the force is in the direction of its motion the motor moves with its force-free velocity v_F divided by the step length d. For a force opposite to motion, "+/−"-motors continue stepping in their preferred direction if the force $|F_i|$ is smaller than the stall force F_S but the velocity decreases linearly with increasing force. If the force (in absolute value) exceeds the stall force the motors step backwards with $v_B \ll v_F$.

If not explicitly stated we don't consider exclusion of the motors on the filament but discuss its effects in the results section. Similarly, we shall also investigate the influence of the force-free region L_0 on the cargo's motion. Our results show that states with SBD exist in very extreme (and biologically irrelevant) cases only.

The motors' detachment rates $k_d(F_i)$ are also force-dependent and explicitly given by [7]

$$k_d(F_i) = k_d^0 \exp\left(\frac{|F_i|}{F_D}\right), \qquad (3)$$

where k_d^0 gives the force-free detachment rate and the detachment force F_D gives the force scale. Once a motor is detached it attaches again with a rate k_a within the tension-free area $x_C(t) \pm L_0$.

In the MF-model [7] the velocity of the cargo is simply a function of attached motors, and is thus constant between two at-/detachment events. All motors are implicitly assumed to move with the same velocity as the cargo. By

v_F	1000 nm/s	F_D	3 pN	α	0.1 pN/nm
v_B	6 nm/s	k_d^0	1 s^{-1}	L_0	110 nm
F_S	6 pN	k_a	5 s^{-1}	d	8 nm

Table 1: Simulation parameter taken from [7] and [8].

assuming that one team feels exactly the same force as the other one in opposed direction (if no external force), Müller *et al.* determine this constant velocity between two motor events.

In contrast, here we take the individual motor steps into account. The force applied on the cargo is determined separately for each motor (Eq. (1)). We move the cargo of mass m with radius R along its equation of motion in a viscous medium with viscosity η (see [12] for details)[1]

$$m\frac{\partial^2 x_C(t)}{\partial t^2} = -6\pi\eta R\frac{\partial x_C(t)}{\partial t} + \sum_{i=1}^{n_++n_-} F_i(x_C(t), \{x_i\}).$$

(4)

Here n_+ and n_- count respectively the "+"- and "$-$"-motors, which are attached to the filament, such that $0 \leq n_\pm \leq N_\pm$.

Results. – In this work we analyze whether a model which takes every single motor position into account produces the same state with SBDs as it was seen for strong[2] motors in the MF-model [7]. Therefore, we have considered the same kind of strong motors, as defined in TABLE 1, and we measure the velocity distribution and the probability of a given number of attached motors of each kind $p(n_+, n_-)$.

In contrast to the MF-model [7] where the cargo velocity is constant, the cargo in the EPB model moves according to its equation of motion and, therefore, not with constant speed. In our analysis we will consider the statistics of the cargo velocity $\bar{v}(t) := \frac{x_C(t+\Delta t)-x_C(t)}{\Delta t}$ over a time interval Δt. Here we use $\Delta t = 0.16$ s [7,13].

With the chosen set of parameters given in TABLE 1 the MF-model produces fast cargo motion with SBDs (FIG. 2(**a**)). For the EPB-model, however, these states with SBDs are not observed (FIG. 2(**b**)). This result is rather robust and we checked that it does neither depend on the particular realization of the motor-cargo coupling nor the details of the motors' response to external forces or the chosen set of parameters. This result gives strong evidence that fluctuations of the motor positions play a crucial role in the cargo dynamics, and that the MF assumption of an equal sharing of forces between motors of the same kind qualitatively changes the results.

[1] $\eta = 10$ mPas, $m = 10^{-14}$ kg, $R = 1000$ nm. We keep for the reasons of generality the mass term in our model since it doesn't aggravate the numerical requirements even though it is not necessarily needed.

[2] In the MF model strong motors correspond to the case $F_S > F_D$ such that one team can pull off the other one.
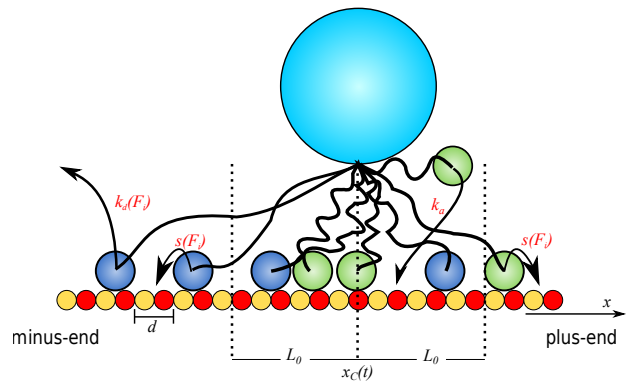

Fig. 1: Schematic drawing of the motor kinetics. A cargo (light blue) is moved by two teams of motors pulling in "+" (green) and "-" (blue) direction, respectively. The single motors can walk on and detach from the filament. Once they are detached, they can attach again within the force free area $[x_C(t) - L_0, x_C(t) + L_0]$.

For the EPB-model, the distribution of bound motors $p(n_+, n_-)$ in FIG. 2(**b**) has a peaked structure on the diagonal, i.e. most of the time the same number of motors of both teams are bound to the filament. Furthermore, in the frame of the EPB-model, we can determine how many of those bound motors are actually engaged in the tug-of-war, i.e. apply a non-zero force to the cargo. This quantity $\tilde{p}(n_+, n_-)$ shown in FIG. 2(**c**) demonstrates that not all motors which are bound to the filament exert a force on the cargo, a fact which, again, contradicts the mean-field assumption.

When a motor detaches due to a high load, this results in the MF-model into a new cargo velocity and a sharing of the high load between the remaining motors of the team, which most probably will also detach within a very short time. In the EPB-model, motors have different positions. As illustrated in FIG. 3, the motor that detaches is most probably the most distant from the cargo, with a reservoir of bound motors of the same team behind. After detachment, the cargo can move backwards, and as a result, first, the load between both teams is partly relaxed. Second, due to the backward motion of the cargo, some bound motors which were not exerting any force will become involved in the pulling of the cargo and replace the detached motor. This effect makes obvious why we cannot detect the detachment cascades as they are observed within the mean-field description, and why states with SBDs can be sustained only in the MF-model.

Mutual motor activation In the previous discussion we pointed out that, in a given parameter range, the MF-model can exhibit some states with SBDs that are not present in the EPB-model [3]. This is because the MF-

[3] We exclude the cases where only one motor at a time is pulling, for example in the case of a detachment rate much higher than the attachment rate (which anyhow does not give SBDs). In these trivial cases no collective effect occurs anyhow.
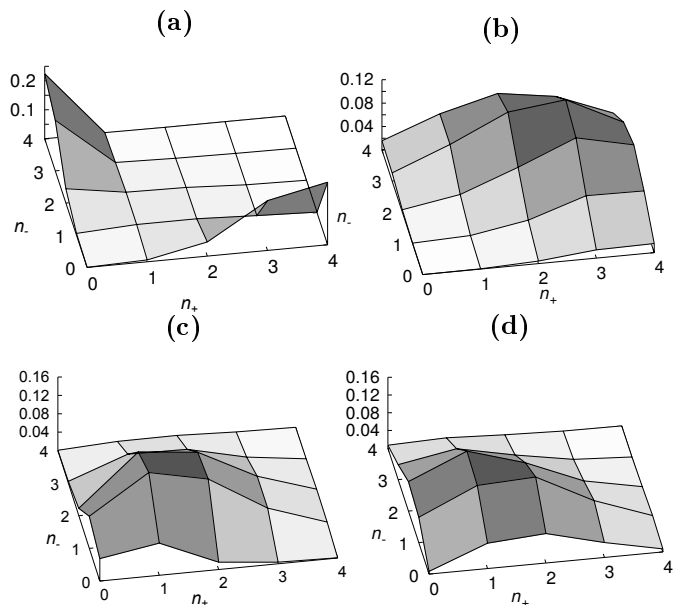
(a) **(b)**

(c) **(d)**

Fig. 2: The probability $p(n_+, n_-)$ of n_+ "+"-motors and n_- "−"-motors bound to the filament **(a)** in the MF-model and **(b)** for the EPB-model in the case of no activation and for $N = 4$. The probability $\tilde{p}(n_+, n_-)$ of +"-motors and n_- "−"-motors which are actually engaged in the tug-of-war (i.e. which apply a non-zero force on the cargo) in the EPB-model is shown without activation in **(c)** and with a mutual activation with $a = 5$ and $R_A = 32$ nm in **(d)**.

assumption implicitly corresponds to a perfect stepping synchronization between all attached motors of a given team. In order to analyze the degree of synchronization needed to observe SBD states, we shall now explicitly model some mutual motor activation in the EPB-model which leads to an artificial synchronization of the motors.

The mutual motor activation is introduced as follows. If the i-th motor of one team makes a step, the stepping rate of the motors of the same team within the interval $[x_i - R_A, x_i + R_A]$ is multiplied by a factor a. Here we have chosen $a = 5$ and $R_A = 32$ nm, i.e. 4 tubulin subunits. We shall discuss the influence of a later on.

We first analyze the influence of activation for a small number $N_\pm = N = 4$ of motors. FIG. 2**(d)** shows that activation does not change the distribution of attached motors significantly. The probability to have no motor pulling $\tilde{p}(0, 0)$ goes to zero and those for the "two against one"-scenarios $\tilde{p}(2, 1)$ and $\tilde{p}(1, 2)$ are slightly increased. However, we cannot detect a double-peaked distribution.

Now the motor number is increased from $N = 4$ to $N = 40$ motors per team. In column **(A)** of FIG. 4 the probability of engaged motors $\tilde{p}(n_+, n_-)$ (top) and the velocity distribution $p(\bar{v})$ (bottom) without activation ($a = 1$) are shown. The velocity distribution is peaked around zero and the maximal probability of engaged motors is still on the diagonal. If we introduce the mutual motor activation we observe SBDs as shown in column **(B)** of FIG. 4. Apparently, the mean-field assumption is rele-
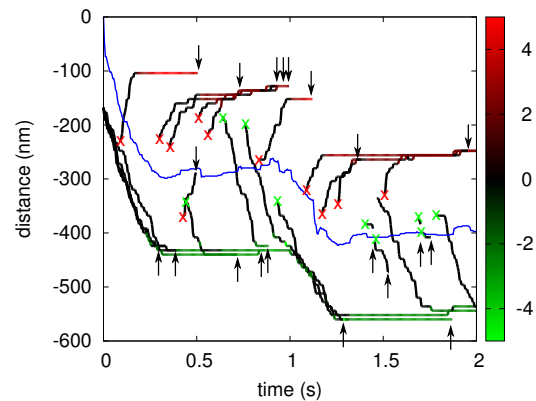

Fig. 3: One specific realization of the trajectories of the cargo (blue) and of all "+"- (red) and "−"- (green) motors. The arrows indicate a detachment event and the crosses an attachment of a "+"- (red) or a "−"- (green) motor. The color code gives the ratio $k_d(F_i)/s_\pm(F_i)$. We choose as initial state a state that would have been stable in the MF case, with only "−"motors pulling on the cargo. We see here that this state is not stable for the EPB-model.

vant only in the limit of a high number of motors (much higher than observed in experiments) and if, in addition, a synchronization mechanism between the motors would exist. Thus we don't expect it to be relevant in real cells.

Influence of exclusion. In the model discussed above we do not consider exclusion along the filament. Since the heads of the molecular motors bind to particular sites on microtubules it would in principle be necessary to consider exclusion effects. For microtubule based cargo transport, however, the number of possible binding sites in close proximity of the cargo is much bigger compared to the number of attached motors [14,15]. Here we discuss its influence on the existence of SBDs.

In Fig. 5 we see that exclusion does not change the behavior qualitatively. Due to exclusive filament positions it becomes impossible to bring the motors at the same position so that they cannot share the force equally. The probability to have several motors engaged in the tug-of-war at the same time even decreases and the system is brought even further from the MF scenario.

Influence of L_0. Further we discuss the influence of the parameter L_0 on the cargo motion. This area around the cargo position $x_C(t)$ is crucial for the way the motors share forces. In the following we analyze two situations: $L_0 = 0$ and $L_0 = L_0^*/2$ where L_0^* is the value of L_0 given in TABLE 1.

In the case of $L_0 = L_0^*/2$ the distribution of attached motors does not change significantly while it broadens for $L_0 = 0$ (Fig. 6 (a,c)). In the later case we also give the velocity distribution (Fig. 6 (e),(f)) which is no longer Gaussian but still unimodal and with fat tails.

One might expect that a stiffer linkage and $L_0 = 0$ brings the system closer to the MF limit. Indeed, the stepping of the motors would be synchronized, since the
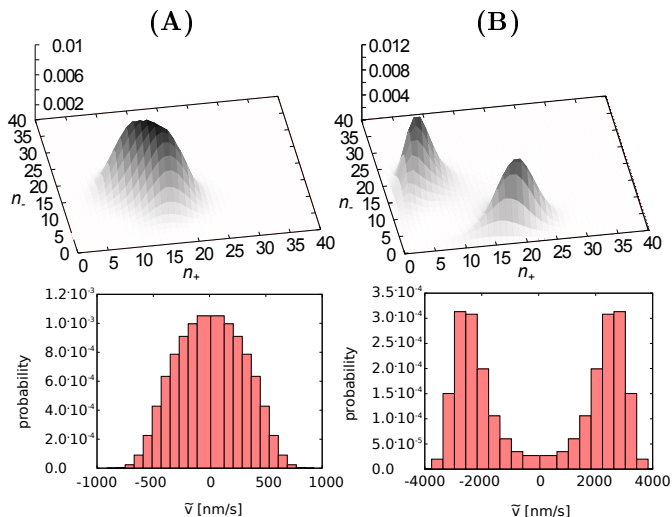
(A)

(B)

Fig. 4: The probabilities $\tilde{p}(n_+, n_-)$ (top) and $p(\overline{v})$ (bottom) are shown for $a = 1$ (without activation) on the left hand side and for $a = 5$ and $R_A = 32$ nm on the right hand side. Note that the cargo's maximal velocity is now $a \cdot v_f$. Here $N = 40$.
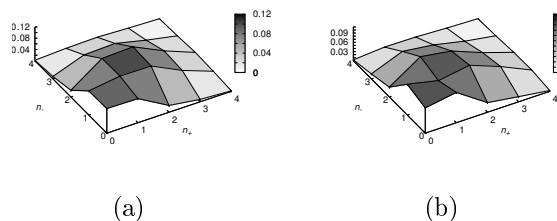

(a) (b)

Fig. 5: Probability of n_+ and n_- motors engaged in the tug-of-war (a) without and (b) with mutual motor activation ($a = 5$, $R_A = 32$ nm) with exclusion on the filament ($N = 4$).

becomes closer to the MF model even though motors do not share the force equally. Furthermore, all motors within one team are closer to each other such that activation is easier to realize. Still, even in this more favorable case, velocity SBDs are not obtained.
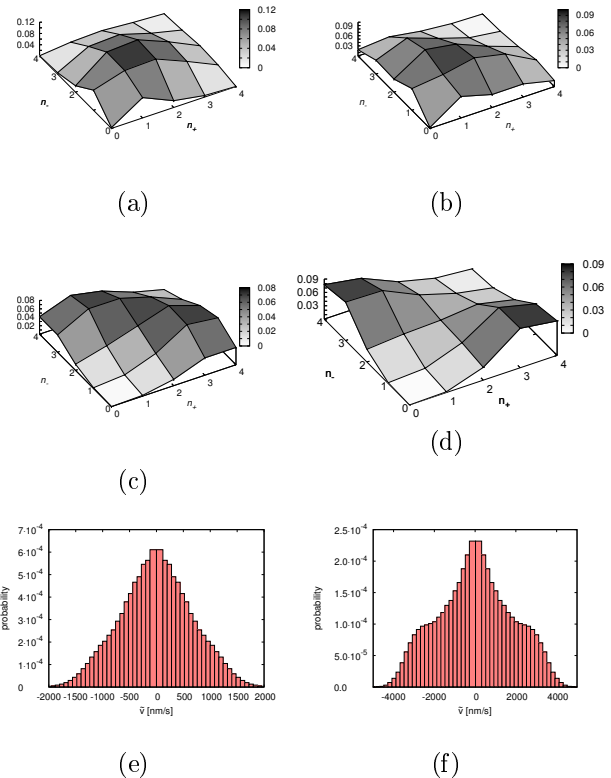

(a) (b)

(c) (d)

(e) (f)

Fig. 6: Probability of n_+ and n_- motors engaged in the tug-of-war (a,b,c,d) and probability of cargo velocity $\tilde{v}$ (e,f), without (a,c,e) and with (b,d,f) mutual motor activation ($a = 5$, $R_A = 32$ nm), for $L_0 = 55$ nm (a,b) and $L_0 = 0$ nm (c,d,e,f), $N = 4$.
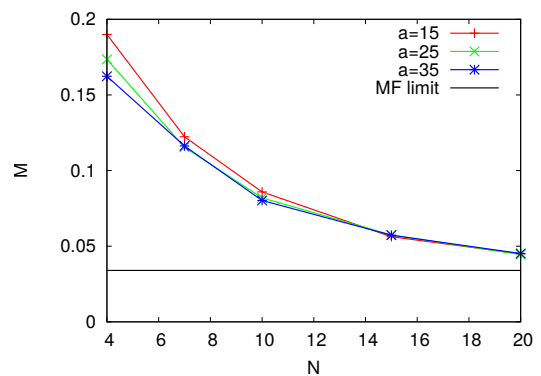
motors under tension slow down considerably. At the same time, however, the processivity of motors goes down dramatically. We checked that for stiffer springs and shorter linkage length, the standard deviation of the cargo velocity decreases, which indicates a narrower velocity distribution: The system is brought further away from the MF results.

If we additionally consider mutual motor activation the distribution of attached motors is in the case $L_0 = L_0^*/2$ not significantly altered (Fig. 6 (b)). Contrarily, the distribution becomes double peaked for $L_0 = 0$ (Fig. 6 (d)). But even in this case the effect on the velocity distribution is only marginal (see Fig. 6 (f)). Indeed, the distribution is still unimodal with a major peak around zero velocity and two shoulders around 2000 nm/s.

The appearance of these shoulders comes from the fact that somehow, taking $L_0 = 0$ brings the system closer to the MF limit and reinforces the effect of activation. One reason is that motors feel a force directly when they are attached to the filament, and in this sense the model

Fig. 7: $M := \sum_i^N \tilde{p}(i, i)$ against the number of motors for different activation factors a. For comparison, the black curve gives the value obtained for $N = 4$ in the MF model.

Influence of (strong) activation. We introduced some mutual activation above as an artificial synchronization between the motors. In this subsection we will analyze the influence of the number of motors and the activation factor in more detail. As a crude estimate of how close we are to a bimodal distribution we define M as the sum of the probabilities to have the same number of motors per team engaged in the tug of war $M := \sum_i^N \tilde{p}(i,i)$. Fig. 7 shows M against N for different activation factors a. One sees that the effective synchronization depends mainly on the number of motors and not on the (arbitrarily chosen) value of a. For the biologically relevant case of small N, even for an activation as strong as $a = 35$, we are not in the MF limit. For $N = 4$ the MF model gives $M = 0.034$, a value still smaller than for a strong activation with 20 motors in the EPB model.

Discussion. – In this work we discuss the influence of the MF assumption used in [7] on the existence of states with SBDs. To compare with the MF-model of Müller *et al.* [7] we introduce the EPB-model which considers explicitly the positions of the motors bound to the filament in the spirit of [8,10]. Within this frame we calculate the forces which act on each individual motor explicitly, rather than just balancing the force between perfectly aligned teams of plus- and minus-directed motors. as implicitly assumed in the MF-model. Significantly, in contrast to the MF-model, we did not find any state with SBDs in the EPB-model for a very large choice of motor characteristics including in particular those used in [7]. While states which generate SBDs are very stable in the MF-model, a realization with only one team pulling the cargo is only a rather rare event with short lifetime in the EPB-model (cf. Fig 3) if the motor teams are equal.

The importance of fluctuations in the stepping of motors has also been observed experimentally. In [16] it was found that even within a team of identical motors, individual motors can build up substantial forces in both hindering and assisting directions. Even in the case where the stepping of the motors is strongly coupled the MF model is still of questionable relevance. Indeed we show via the ad hoc assumption of mutual motor activation that SBD states can only be observed if the activation is strong and if a high number of motors is attached to the cargo. To our knowledge this extreme scenario has never been observed in real intracellular transport.

Several characteristics of cargo transport have been observed in *in vivo* experiments [17,18], including anomalous diffusion (sub- or superdiffusion depending on the observation time scales). We have shown indeed in previous work [12,19] that, using more biologically relevant motor characteristics in the EPB-model, these sub- or superdiffusive particle motion at short times can be reproduced. Still, *in vivo* experiments are not so simple to interpret and depending on the system the EPB-model may have to be extended to include system-specific mechanisms. *In vitro* experiments with purified motors would allow to have a better control of the system parameters, and to go even further in the comparison with the models and in the testing of various scenarios. In particular, such experiments could help validate the main claim/result of this work that SBD states are merely an artefact of a specific type of model and therefore probably irrelevant for realistic biological situations.

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REFERENCES

[1] ALBERTS B., JOHNSON A., LEWIS J., RAFF M., ROBERTS K. and WALTER P., *Molecular biology of the cell, 4th ed.* (Taylor and Francis) 2002.

[2] SCHLIWA M. and WOEHLKE G., *Nature*, **422** (2003) 759.

[3] SOPPINA V., RAI A. K., RAMAIYA A. J., BARAK P. and MALLIK R., *Proc Natl Acad Sci U S A.*, **106** (2009) 19381.

[4] HENDRICKS A., PERLSON E., ROSS J., SCHROEDER H., TOKITO M. and HOLZBAUR E., *Current Biology*, **20** (2010) 697.

[5] WELTE M., GROSS S., POSTNER M., BLOCK S. and WIESCHAUS E., *Cell*, **92** (1998) 547.

[6] GROSS S. P., VERSHININ M. and SHUBEITA G. T., *Curr. Biol.*, **17** (2007) R478.

[7] MÜLLER M., KLUMPP S. and LIPOWSKY R., *PNAS*, **105** (2008) 4609.

[8] KUNWAR A., TRIPATHY S. K., XU J., MATTSON M. K., ANAND P., SIGUA R., VERSHININ M., McKENNEY R. J., YU C. C., MOGILNER A. and GROSS S. P., *PNAS*, **108** (2011) 18960.

[9] HANCOCK W. O., *Nature Reviews Molecular Cell Biology*, **15** (2014) 615.

[10] BOUZAT S. and FALO F., *Phys. Biol.*, **8** (2011) 066010.

[11] BOUZAT S., LEVI V. and BRUNO L., *PLOS One*, **7** (2012) e43599.

[12] KLEIN S., APPERT-ROLLAND C. and SANTEN L., *Europhys. Lett.*, **107** (2014) 18004.

[13] GROSS S. P., WELTE M. A., BLOCK S. M. and WIESCHAUS E. F., *J. Cell. Biol.*, **148** (2000) 945.

[14] CAMPÀS O., LEDUC C., BASSEREAU P., CASADEMUNT J., JOANNY J.-F. and PROST J., *Biophysical Journal*, **94** (2008) 5009.

[15] BOUZAT S. and FALO F., *Phys. Biol.*, **7** (2010) 046009.

[16] ARPAG G., SHASTRY S., HANCOCK W. and TÜZEL E., *Biophysical J.*, **107** (2014) 1896.

[17] CASPI A., GRANEK R. and ELBAUM M., *Phys. Rev. E*, **22** (2002) 011916.

[18] KULIĆ I., BROWN A., KIM H., KURAL C., BLEHM B., SELVIN P., NELSON P. and GELFAND V., *P.N.A.S.*, **105** (2008) 10011.

[19] KLEIN S., APPERT-ROLLAND C. and SANTEN L., *Eur. Phys. J. Special Topics*, **223** (2014) 3215.