

# Collective alignment of polar filaments by molecular motors

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We study the alignment of polar biofilaments, such as microtubules and actin, subject to the action of multiple molecular motors attached simultaneously to more than one filament. Focusing on a paradigm model of only two filaments interacting with multiple motors, we were able to investigate in detail the alignment dynamics. While almost no alignment occurs in the case of a single motor, the filaments become rapidly aligned due to the collective action of the motors. Our analysis shows that the alignment time is governed by the number of bound motors and the magnitude of the motor force fluctuations. We demonstrate that the timescale is in the order of seconds, much faster than for passive crosslink-induced bundling. *in vitro* experiments on multiple-motor alignment of microtubules are in good agreement with these results.

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Fascinating self-organizing behavior of polar biofilaments such as microtubules (MTs) and actin, that build up the cytoskeleton of most cells, continues to attract enormous attention both among biologists and physicists. Recent experimental studies revealed a variety of self-organized structures like three-dimensional gels, two-dimensional networks, as occurring underneath the cell membrane, aster-like patterns similar as formed during mitosis, as well as one-dimensional bundled structures, as found in stress fibers. While *in vivo* these structures are controlled by a plethora of proteins and signaling pathways [1], it has been established [2] that such structures also form *in vitro* in the absence of regulatory proteins.

Bundles of polar biofilaments as actin and MTs play an important role in the cytoskeleton's functioning and elasticity. Bundle contraction has been investigated [3] as well as the bundle elasticity [4], however the dynamics of the bundle formation is not fully understood. There are some situations, e.g. when the filaments are growing from organizing centers in a specified direction, where regulation and the confined geometry lead to bundling. *In vitro* experiments [5] showed that crosslinks induce bundling, and such a transition has been recently discussed theoretically [6]. However, it is generally believed that linear molecular motors like kinesin, myosin etc., which convert chemical energy from adenosine triphosphate (ATP) hydrolysis into directed mechanical motion, play an important role in the alignment of individual filaments, which can be considered as a precursor of bundle formation. Practically nothing is known about the dynamics of bundle organization due to motor activity; in particular, what are the relevant parameters (e.g. motors concentration, processivity, etc.) determining the characteristic time of the alignment.

In this Letter we theoretically predict and demonstrate by complementary *in vitro* experiments that filaments can be aligned due to collective action of multiple motors. Taking advantage of a highly simplified yet non-

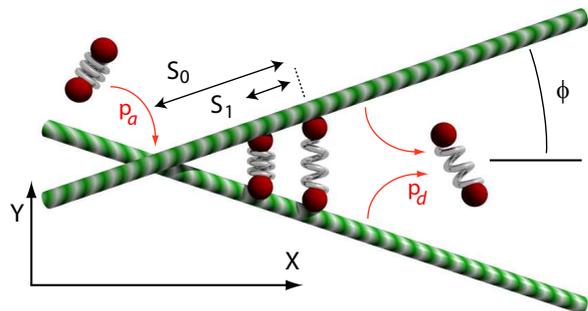


FIG. 1: (Color online) Sketch of two MTs interacting with multiple motors. Motors attach with probability  $p_a$  at the intersection point  $S_0$ , move along the tubules (in  $+x$  direction) while exerting a spring-like force and detach with a rate  $p_d$ .

trivial micromechanical model, containing only two filaments interacting with multiple motors, we were able to investigate in detail the dynamics of the filaments' alignment (or zipping in the terminology of Ref. [7]). Both theory and experiment revealed that the polar filaments become aligned on a timescale of seconds due to the collective action of the motors, which is faster by two orders of magnitude than the alignment by passive crosslinks (several minutes [5]). In contrast, a single motor, even if it is highly processive, is known to lead to practically no alignment [8]. Our model also indicates that the alignment time is related to the number of attached motors and the stochastic nature of motor motion: the alignment time is directly related to the randomness in the motor stepping and force.

To investigate the collective alignment of polar filaments by multiple motors we focus first on the simplest situation of a pair of two perfectly rigid rods of fixed length  $L$  interacting with molecular motors attached to both tubules. The number of motors  $N$  is not fixed: motors can attach with a probability  $p_a$  at the intersection point of the two rods, as well as detach from any place with a fixed rate  $p_d$ . We consider either motor oligomers

with multiple heads, as used in Refs. [2], or multiple motors attached to a nanoparticle as in Ref. [9]. The motors are modeled by massless linear springs attached perpendicular to the bisector, see Fig. 1. Due to the great disparity in the size of biofilaments and molecular motors ( $\simeq 10 - 40\mu\text{m}$  vs.  $\simeq 100\text{nm}$ ), the marching of a motor attached to only one filament produces no noticeable displacement of the filament. Thus we focus on the motors attached to both filaments. As a further simplification we consider the symmetric case, i.e. the distance  $S$  (with  $-L/2 < S < L/2$ ) of the intersection point from the center of mass of one MT is the same for both MTs. It is easy to see that even if motors attach asymmetrically to two rods, it will soon assume a symmetric position [8].

The motion of the rods follows from the balance of forces and torques exerted on the rods by the motors and the environment (viscous drag). The torque balance leads to an equation for the intersection angle  $\phi$ ,  $\eta_r \dot{\phi} = \tau$ , with  $\eta_r \simeq \frac{\pi\eta L^3}{3 \ln(L/d)}$  the rotational friction coefficient [10]. The total torque  $\tau$  created by the motor forces  $F_n$  (neglecting the motors' bending rigidity  $F_n$  acts exactly along the  $y$ -axis, see Fig. 1) and the drag force is of the form  $\tau = \sum F_n S_n \cos \phi$ , with  $S_n$  the position of the  $n$ -th motor. The motor force  $F_n$  is described by a linear spring,  $F_n = -\kappa l = -\kappa(S_n - S_0) \sin \phi$ , where  $\kappa$  is the spring constant,  $S_0$  is the position of the intersection point of the two tubules and  $l = (S_n - S_0) \sin \phi$  is the spring extension. Finally we arrive at the torque balance

$$\dot{\phi} = \frac{\kappa}{\eta_r} \cos \phi \sin \phi \sum_{n=1}^N S_n (S_0 - S_n). \quad (1)$$

The equation of motion for the  $n$ -th motor is provided by the force-velocity relation ( $v = dS_n/dt$ ),

$$\dot{S}_n = V [1 - \kappa(S_n - S_0) \sin^2 \phi / F_{st}] + \xi_n(t). \quad (2)$$

Here we used a well-accepted linear force-velocity relation [11, 12],  $V(F) = V(1 - F_l/F_{st})$ , with a stall force  $F_{st}$ .  $F_l$  is the projection of the force  $F_n$  opposing the motor motion on the direction of the rod. The stochastic term  $\xi_n$  describes random fluctuations in motor stepping and force. We assume  $\langle \xi_n(t) \rangle = 0$  and  $\langle \xi_n(t) \xi_m(t') \rangle = 2D\delta(t-t')\delta_{nm}$ . The noise strength  $D$  resulting from fluctuations in force and stepping of the motors can be estimated from experimental measurements of the so-called "randomness parameter" [13], defined as  $r = \lim_{t \rightarrow \infty} \frac{\langle x(t)^2 \rangle - \langle x(t) \rangle^2}{d \langle x(t) \rangle} = \frac{2D}{dV}$ , where  $d = 8\text{nm}$  is the motor step size. From Ref. [13] we obtain  $r \simeq 0.4$ , which results for  $V \simeq 0.2\mu\text{m}/s$  to the estimate  $D \simeq 3 \cdot 10^{-4} \mu\text{m}^2/s$ .

Before proceeding with our analysis, we would like to illustrate a nontrivial effect: Eqs. (2) imply an *effective attraction* between the motors. Indeed, any difference in the attachment positions has the tendency to decrease,  $dS/dt = -V\kappa S \sin^2 \phi / F_{st} + \xi$ . This effect has a simple interpretation: the forward motor ( $S_i > S_j$ ) is stretched

more and thus experiences a larger opposing force than the trailing one. According to the force-velocity relation, the forward motor will move slower than the trailing one, and the distance between them will decrease. Thus, Eqs. (2) describe two competing processes: localization of the motor distribution near the intersection point due to attractive interaction and spreading of the distribution due to the random force  $\xi$ .

To close the description we need an equation for the intersection point of the rods. This we derive from the constraint  $Y = -S_0 \sin \phi$  and the equation of motion for the  $y$ -coordinate of the center of mass of the tubules  $\eta_t \dot{Y} = F_d$ , with the viscous drag force  $F_d = \sum F_n$  and  $\eta_t \simeq \eta_{\perp} = \frac{4\pi\eta L}{\ln(L/d)}$  [10]. For simplicity we have neglected the anisotropy of the translational friction by letting  $\eta_{\parallel} = \eta_{\perp} = \eta_t$ . This results immediately in  $\partial_t X = 0$ , i.e. there is no overall translation of the center of mass. Finally, the equation for the intersection point coordinate  $S_0$  is

$$\dot{S}_0 = -\kappa \sum_{n=1}^N \left( \frac{\cos^2 \phi S_0}{\eta_r} S_n (S_0 - S_n) + \frac{S_0 - S_n}{\eta_t} \right). \quad (3)$$

Eqs. (1)-(3) with the attachment/detachment kinetics can be solved by a standard Langevin scheme, the angle between the tubules evolving in time as shown by the dashed lines in Fig. 2, where the following known parameters have been used: kinesin velocity  $0.01-1\mu\text{m}/s$  (depending on ATP concentration) [13]; kinesin spring constant  $200-400\text{pN}/\mu\text{m}$  [14]; stall force of kinesin  $5-8\text{pN}$  [13]; attachment/detachment rates  $50\text{s}^{-1}$  and  $1\text{s}^{-1}$  [15, 16]; solvent viscosity  $\sim 0.005\text{pNs}/\mu\text{m}^2$ ; MT length  $L = 10\mu\text{m}$ , and diameter  $24\text{nm}$ .

A *continuum description* can be obtained in the limit of a large number of motors attached to the two rods. In this case, from Eq. (2) one can derive a Fokker-Planck equation for the probability distribution function  $P(S, t)$  to find a motor at position  $S$  at time  $t$ ,

$$\partial_t P = D \partial_S^2 P - \partial_S (\tilde{V} P) - p_d P + p_a \delta(S - S_0(t)), \quad (4)$$

with  $\tilde{V} = V[1 - \kappa(S - S_0(t)) \sin^2 \phi / F_{st}]$  and the  $\delta$ -function ensuring attachment at the intersection point  $S_0$  only. The equations for the angle and the intersection point can be obtained by generalizing Eqs. (1),(3):

$$\dot{\phi} = \frac{\kappa}{\eta_r} \cos \phi \sin \phi \int dS P(S) S (S_0 - S), \quad (5)$$

$$\dot{S}_0 = -\kappa \int dS P(S) (S_0 - S) \left( \frac{\cos^2 \phi S_0}{\eta_r} S + \frac{1}{\eta_t} \right). \quad (6)$$

Eqs. (4)-(6) can be solved if one assumes that the attachment/detachment kinetics is sufficiently fast compared to the relative movement of the motors and the intersection point, i.e.  $p_d \gg V/L$ . Then the motor distribution  $P(S)$  is slowly varying in the frame moving along with

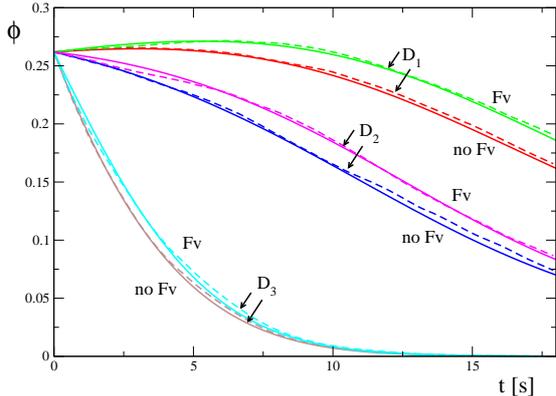


FIG. 2: (Color online) The angle  $\phi$  vs. time  $t$  during the motion of multiple motors.  $D_1 = 5 \cdot 10^{-6} \mu\text{m}^2/\text{s}$  (with and without force-velocity (Fv) dependence),  $D_2 = 10^{-5} \mu\text{m}^2/\text{s}$  and  $D_3 = 5 \cdot 10^{-5} \mu\text{m}^2/\text{s}$ . The dashed lines are Langevin simulations, averaged over 10 runs, for  $V = 0.2 \mu\text{m}/\text{s}$ ,  $p_a = 50 \mu\text{m}/\text{s}$ ,  $p_d = 1/\text{s}$ ; initial conditions  $\phi_i = \pi/12$ ,  $S_i = -3 \mu\text{m}$ , see also movies 1 & in [18]

$S_0$ ,  $P(S, t) = P(S - S_0(t))$ . Introducing  $\tilde{S} = S - S_0(t)$  one obtains a quasi-stationary problem,

$$0 = D \partial_{\tilde{S}}^2 P - (\delta V - \alpha \tilde{S}) \partial_{\tilde{S}} P - (p_d - \alpha) P + p_a \delta(\tilde{S}), \quad (7)$$

with  $\delta V = V - \dot{S}_0$  and  $\alpha = V \kappa \sin^2 \phi / F_{st}$ . Eq. (7) can be solved exactly in the absence of a force-velocity dependence, i.e. if the motor force is much smaller than the stall force,  $F/F_{st} \rightarrow 0$ . The solutions to the left and right of  $\tilde{S} = 0$  [17], have to be matched by continuity  $P_l(0) = P_r(0)$  and by a derivative jump condition from the  $\delta$ -function. Obtained distributions are shown in [18].

In case of the load-dependent motor speed, although the homogeneous part of Eq. (7) has an analytical solution in terms of confluent hypergeometric functions, this solution is impractical to proceed with. By transforming to the form without first derivative, for not too high  $\alpha$  the function  $\bar{P}(S)$  can be obtained approximately.

The integrals in Eqs. (5),(6) can be evaluated analytically, and keeping in Eq. (6) only terms in linear order in the small variable  $\delta V$ , the effect of the motion of multiple motors on a pair of MTs can be described by a single effective equation for the mutual angle:

$$\dot{\phi} = -\cos \phi \sin \phi \frac{\eta \left( V S_0 + 2D \frac{\kappa}{\eta_t} f \right)}{1 + \eta \cos^2 \phi S_0^2}. \quad (8)$$

Here  $S_0 = S_i + Vt$ , with  $S_i$  the initial condition for the intersection point  $S_0$  at  $t = 0$ , and we have introduced  $\eta = \eta_t / \eta_r$ . Remarkably, this equation captures both the single motor case (corresponding to  $f = 0$  [8]), as well as the multiple motor case ( $f = p_a / p_d^2$  in the absence

of a force-velocity relation, while becoming a function of  $\phi$ , to leading order  $f = \frac{p_a}{p_d^2} \left( 1 - 2 \frac{V \kappa \sin^2 \phi}{p_d F_{st}} \right)$ , in the presence). For a single motor the change in the angle is totally due to the motion of the intersection point - governed by the term  $V S_0$ , but the angle decreases if the intersection point (the motors) is on the right side with respect to the center of rod ( $S_0 > 0$ ), while increasing if at the opposite side ( $S_0 < 0$ ). A global (averaged) change in the mutual angles is thus a purely statistical effect in this case. However, for multiple motors the small diffusion due to force fluctuations yields the term  $\sim D$ , which always adds a negative contribution and thus reduces the angle. If this term dominates, there is fast alignment. A comparison between the Langevin simulations of Eqs. (1)-(3) and the analytical result, Eq. (8), is shown in Fig. 2. For a not too high mutual angle, load-dependence only slightly decreases the zipping rate.

A simple expression for the characteristic alignment time can be obtained from Eq. (8) for small initial angles and in the limit of dominating diffusion term, yielding  $\tau_0 = \eta_r p_d^2 / 2D \kappa p_a$ . This can be rewritten as

$$\tau_0 = \eta_r / r d \kappa N l_m, \quad (9)$$

where  $N \simeq 2-4$  is the number of motors attached to both filaments and  $l_m \simeq 1 \mu\text{m}$  is the (average) single motor runlength. For the given parameters, this evaluates to  $\tau_0 \simeq 1-3$  s, faster by two orders of magnitude than alignment by passive crosslinks, which is typically in the order of several minutes [5].

To investigate also the effect of filament flexibility, we performed molecular dynamics simulations [18] with filaments characterized by a bending stiffness. While single motors can already lead to loose bundles for such semi-flexible filaments [19] since the motor force locally brings the filaments closer together, multiple motors however induce very fast zipping, see movies 1 & 2 in [18]. Depending on the diffusivity of motors on the filaments, buckling [20] can also occur for low randomness, while it is suppressed for large randomness.

To demonstrate the fast collective alignment, we performed *in vitro* experiments in a similar geometry: a bead assay was investigated as previously described [9]. Carboxylated polystyrene beads (200-nm diameter; Polysciences, Warrington, PA) were incubated with kinesin-1 motors (0.9 nM). At this concentration, the beads spontaneously bound to and moved along glass-bound MTs, which are often more than  $15 \mu\text{m}$  long. The beads had a very long ( $> 8 \mu\text{m}$ ) runlength (while for individual motors it is  $\sim 1 \mu\text{m}$ ), suggesting that the surface of the beads was decorated with a high density of kinesin motors. MT zipping events were observed to happen when loose MT were present. A time series of such an alignment is shown in Fig. 3a-c, where one MT is fixed to the substrate and another, mobile one is connected to the former by the multiple-motor-covered beads. The mutual angle be-

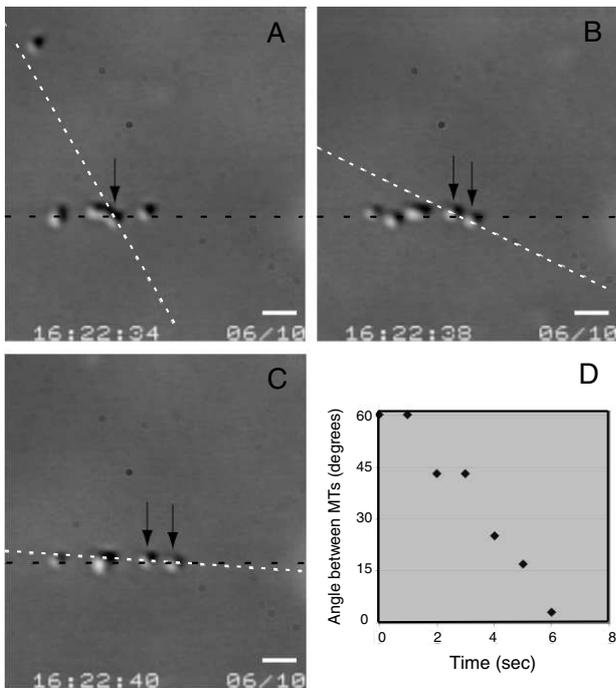


FIG. 3: MTs “zip” together due to multiple motor activity. (A-C) A sequence (for a movie see [18]), showing one MT (black dashed line) fixed on a glass slide with a second mobile MT (white dashed line) bound to the fixed one via cargos (200 nm diameter polystyrene beads, arrows) carrying multiple kinesin-1 motors. Panel D shows the angle between the MTs as a function of time, starting at about 60 degrees. The MTs become almost perfectly aligned within 6 seconds, though the actual zipping took approximately 5 seconds. Times are 0s, 4s, and 6s for A-C respectively. The scale bar is  $1\mu\text{m}$ .

tween the MTs as a function of time is shown in Fig. 3d. The alignment time is found to be about 5 s, in agreement with our prediction. Although the experimental setup is not exactly the one of our paradigm model, this shows convincingly that the alignment time is much faster than in the passive, crosslink-induced case.

In conclusion, we have shown that multiple motors may work together in order to align polar filaments, an important precursor of bundle formation. A micromechanical model was proposed, exhibiting that motors form a localized cluster around the intersection point of MTs, propagate with a velocity close to the single motor velocity and lead to fast zipping. The number of attached motors  $N$  as well as the randomness of the motor force appears to be the important parameters determining alignment time and width. Our results also can be easily generalized to the case of motors and static crosslinks, cf. [22], and to two populations of motors walking in opposite directions.

In contrast to the case of multiple processive motors pulling a tube from a membrane [12], in our case a force-velocity relation influences the results only quantitatively. Remarkably, the dense cluster formation *does*

not rely on direct interaction between the motors (e.g. excluded volume) and is thus different from mechanisms based on asymmetric exclusion processes proposed in e.g. [21]. It is also different from the zipping of soft actin filaments by non-processive motors [7]. Together with the experimental demonstration of the fast alignment process, the timescale of several seconds being faster than passive bundling by crosslinks by two orders of magnitude, our work thus suggests another example where motors efficiently achieve a desired goal by working together. Future experiments, e.g. with labeled motors are keenly needed to further explore this fascinating phenomenon of collective alignment. We thank Gijsje Koenderink, George Shubeita and Lev Tsimring for useful discussions. F.Z. and I.S.A. acknowledge support by the US DOE, grant DE-AC02-06CH11357, S.P.G. by the NIH Grant 1RO1GM070676 and M.V. by the NIH Ruth L. Kirschstein National Research Service Award postdoctoral fellowship.

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- [16] Since we only consider motors attached to both filaments, the attachment rate is renormalized.
- [17] To simplify the integrals MTs are assumed infinitely long.
- [18] See EPAPS Document No. for movies. A direct link to this document may be found in the online article’s HTML reference section. The document may also be reached via the EPAPS homepage (<http://www.aip.org/pubservs/epaps.html>) or from <ftp.aip.org> in the directory /epaps/. See the EPAPS homepage for more information.

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