

SUPPLEMENTARY METHODS

Methods Text 1: Analytical Model: Derivation of the expansion forces

We consider two filaments with overlap length L , exhibiting l discrete binding sites of length δ on each filament in the overlap (with $L = l\delta$). Motivated by our experimental observation that the dissociation constant from the overlap regions is one order of magnitude lower than from single filaments (Figure S3A and S3B) and considering the experimental crosslinker concentration in solution (much lower than the dissociation constant on single filaments), we assume that crosslinkers do not bind to single microtubules but exclusively bind to the overlap region to both filaments with a dissociation constant K_D^d as measured in the overlap. The experiments indicate that the cooperativity of binding is weak (Figure S3A), and we thus make the simplifying assumption that the crosslinkers bind non-cooperatively. The partition function for n crosslinkers in the overlap is then given by:

$$Q(l, n) = \binom{l}{n} \left([X] / K_D^d \right)^n, \quad (1)$$

where $[X]$ is the concentration of the crosslinkers in solution. Motivated by the near constant number of crosslinkers in expanding overlaps (Figure S1B), we first consider the case where no crosslinkers bind into or unbind from an overlap ($n = \text{constant}$, scenario as in Figures 1 and 2). The force acting on the transport microtubule ('expansion force') is then given by:

$$F = \frac{k_B T}{\delta} \ln[Q(l+1, n)/Q(l, n)] \quad (2)$$

$$= -\frac{k_B T}{\delta} \ln\left[\frac{l+1-n}{l+1}\right] \quad (3)$$

$$\cong \frac{k_B T}{\delta} \frac{n}{(l+1)} \cong \frac{k_B T}{\delta} \frac{n}{l} \quad (4)$$

where we used $\ln(1 - x) \approx -x$, for $x = n/(l+1) \ll 1$. This shows that the force decreases as the overlap length δl increases and the crosslinker density n/l decreases. Equation 4. is the one-dimensional analog of the ideal gas law $pV = nk_B T$ for a gas in a cylinder. It can also be regarded as a special, discretized case of a Tonks gas.

The maximum entropic force arises when the crosslinkers are maximally compressed, that is when they occupy all lattice sites ($l = n$):

$$F^{\max} = \frac{k_B T}{\delta} \ln(n+1) \quad (5)$$

Because the number of crosslinkers is constant, the potential energy of binding is constant and the force is completely determined by the entropy of the crosslinkers in the overlap region. When $l = n$, the number of states is one, and the entropy is zero. The force is thus determined by the entropy of the system when $l = n + 1$. When $l = n + 1$, one hole is created and the number of positions for this hole is $n + 1$. The entropy of this state is thus $k_B T \ln(n + 1)$, which increases logarithmically with n . Considering a lattice spacing of $\delta = 8$ nm and $n = 10$, the maximum expansion force is about 1 pN. For $n = 100$ the maximum expansion force rises to about 2.4 pN.

Allowing for crosslinker binding into and unbinding from an overlap (with rates fast on the timescale on which the filaments move) the partition function becomes:

$$Q(l) = \sum_{n=0}^l Q(l, n) \quad (6)$$

$$= \left(1 + [X]/K_D^d\right)^l \quad (7)$$

The free energy

$$\mathcal{F} \equiv -k_B T \ln Q(l) \quad (8)$$

$$= -k_B T l \ln \left(1 + [X]/K_D^d\right) \quad (9)$$

then decreases linearly with the number of binding sites l in the overlap and the expansion force $F = -\partial \mathcal{F} / \partial L$ is constant:

$$F = \frac{k_B T}{\delta} \ln \left(1 + [X]/K_D^d\right) \quad (10)$$

If the overlap increases, additional binding sites become available. The binding of a crosslinker to a site in the overlap decreases the free energy of the system not only by the energy of binding, but also by increasing the entropy of the crosslinkers in the solution. The latter effect explains why the binding occupancy increases with concentration, and why the expansion force increases with concentration. While the expansion force decreases as the overlap increases at constant n (see Eq. 4), crosslinker condensation into the overlap allows the system to sustain a constant expansion force as the overlap length increases (Eq. 10). The expansion force, now independent of the overlap length, is 0.3 pN for $[X] = K_D^d$.

The average number of crosslinkers that bind to an overlap depends on the crosslinker concentration and is given by

$$\langle n \rangle = \frac{\sum_{n=0}^l n Q(l, n)}{Q(l)} = \frac{l [X] / K_D^d}{1 + [X] / K_D^d} \quad (11)$$

which is simply the number of sites l times the probability that a site is occupied.

Including crosslinker binding to and unbinding from single filaments into our analytical model can lead to a reduction in the estimated force

$$F = \frac{k_B T}{\delta} \ln \left(1 + \frac{[X] / K_D^d}{(1 + [X] / K_D^s)^2} \right) \quad (12)$$

where K_D^d and K_D^s are the dissociation constants for Ase1 binding to an overlap and a single filament, respectively. For the regime $[X] < K_D^s$ present in our experiments the above equation reduces to Eq. 10 and crosslinker binding to single filaments can be ignored.

A microtubule consists of 13 protofilaments, and it is conceivable that crosslinkers between two microtubules can bind to more than one protofilament. To account for this possibility, we modeled the force generation by a constant number of crosslinkers n in an overlap of length l consisting of d protofilaments. The total number of binding sites in such an overlap is ld and the partition function is

$$Q(ld, n) = \binom{ld}{n} ([X] / K_D^d)^n \quad (13)$$

The force is then given by

$$F = \frac{k_B T}{\delta} \ln \left[\frac{(d(l+1))! (dl-n)!}{(dl)! (d(l+1)-n)!} \right] \quad (14)$$

In the low-density limit, this expression reduces to the ideal-gas expression of Eq. 4: in this limit, the force is given by the overall density $\rho = n/l$, irrespective of the number of protofilaments. However, at higher densities, the force depends on the number of protofilaments. Indeed, the maximum force, which is reached when the overlap is fully occupied ($n = ld$), depends on the number of protofilaments and is given by:

$$F^{\max} = \frac{k_B T}{\delta} \ln \left[\frac{(d(l+1))!}{(dl)! d!} \right] \quad (15)$$

The maximum force increases approximately linearly with the number of protofilaments. For 10 crosslinkers (a typical number of crosslinkers in our experiments) and for $d = 3$ protofilaments the maximum force is predicted to be ~ 4.5 pN.

Methods Text 2: Analytical Model: Derivation of the sliding velocity

To elucidate the effective friction coefficient γ_{MT} on the transport filament (top filament) that is connected to the fixed template filament (bottom filament) via diffusible crosslinkers with two filament interaction sites (heads), we use a simple force-balance argument (neglecting the viscous drag exerted by the solution (Hunt et al., 1994; Tawada and Sekimoto, 1991)). We first consider a top filament that is connected via a single crosslinker to the bottom filament. In steady state, where the

top filament and crosslinker move at constant but different speeds, the drag on the top filament is given by

$$\gamma_{\text{MT}} v_{\text{F}} = \gamma (v_{\text{F}} - v_{\text{p}}) \quad (16)$$

where γ is the frictional drag coefficient between a head and a filament, v_{p} is the relative velocity of the crosslinker with respect to the bottom filament, and v_{F} is the velocity of the top filament with respect to the bottom filament. To determine v_{p} we note that in steady state the drag force exerted by the bottom filament on the crosslinker must balance the drag force exerted by the top filament:

$$\gamma v_{\text{p}} = \gamma (v_{\text{F}} - v_{\text{p}}) \quad (17)$$

Hence, it follows that in steady state

$$v_{\text{p}} = v_{\text{F}} / 2 \quad (18)$$

Combining this expression with Eq. 16 yields the effective friction

$$\gamma_{\text{MT}} = \gamma / 2 \quad (19)$$

This argument can straightforwardly be extended to n crosslinkers assuming that they hop independently. This gives

$$\gamma_{\text{MT}} = n\gamma / 2 \quad (20)$$

Assuming crosslinker binding and diffusion is in quasi-equilibrium on the timescale of filament sliding, the sliding velocity of the top filament is given by:

$$v = \frac{F}{\gamma_{MT}} \quad (21)$$

Using Eq. 20 and Eq. 3 we then obtain the equation for the velocity in response to an expansion force by a constant number of crosslinkers:

$$v = \frac{-k_B T \ln \left[\frac{l+1-n}{l+1} \right]}{\delta n \gamma / 2} \quad (22)$$

In the limit of $l \gg n$, (expansion force given by Eq. 4) the velocity reads

$$v = \frac{2k_B T}{l \delta \gamma} = \frac{2D_{Ase1}^{MT}}{L} \quad (23)$$

The velocity for overlap expansion with additional condensation of crosslinkers to microtubule overlaps is given by combining Eq. 10, 11, 20, 21 and the Einstein equation:

$$\begin{aligned} v &\approx \frac{2k_B T (1 + [X] / K_D^d) \ln(1 + [X] / K_D^d)}{l \delta \gamma [X] / K_D^d} \\ &= \frac{2D_{Ase1}^{MT} (1 + [X] / K_D^d) \ln(1 + [X] / K_D^d)}{L [X] / K_D^d} \end{aligned} \quad (24)$$

This expression is independent of the number of protofilaments d because both the condensation force and the average number of crosslinkers (and hence the friction) increase linearly with the number of protofilaments. It is seen that the velocity decays as $1 / L$, as in the case with entropic expansion without condensation (Eq. 23).

Methods Text 3: Computational Model

In the simulations we model a bottom protofilament of length l_B that is fixed and a top protofilament of length l_T that can move. The length l is expressed in the number of binding sites for crosslinker heads. A crosslinker consists of two heads that are connected by a spring of stiffness k . The heads mutually exclude each other and cannot pass each other on a filament.

A crosslinker can bind from solution to a single filament via one of its two heads with a rate $k_{\text{on}}cl_\alpha(1-n_\alpha)$, where k_{on} is the crosslinker association rate, c is the concentration of crosslinkers in solution, l_α is the length of filament $\alpha = B, T$ and n_α is the occupancy of the lattice sites on filament α ; $l_\alpha(1 - n_\alpha)$ is thus the total number of free binding sites on filament α to which a crosslinker can bind. When a crosslinker is connected to a single filament only, it can dissociate from it into the solution via a rate k_{off} . Binding from solution is thus characterized by the dissociation constant $K_D^s = k_{\text{off}}/k_{\text{on}}$. A crosslinker i that is bound to one filament via one of its two heads can, via its other head, bind to an empty site on the other filament with rate $k_a = k_d^0 \exp[-\beta\Delta U/2]$ (see also footnote ¹), where $\Delta U = 0.5k(x_i^F - x_i^B)^2$ is the potential energy of the spring with x_i^F and x_i^B the positions of the heads on the top and bottom filament, respectively. A head of a crosslinker bound to two filaments can dissociate from a filament with rate $k_d = k_d^0 \exp[\beta\Delta U/2]$. Filament binding of a head that is already connected to one filament via its other head is thus characterized by the equilibrium constant $K_{\text{eq}} = k_a / k_d = k_a^0 / k_d^0 \exp[-\beta\Delta U]$. Note that, as detailed balance dictates, the equilibrium constant depends on the tension in the spring connecting the

¹ Detailed balance implies that $k_a = k_a^0 \exp[-\beta(1 - \lambda)\Delta U]$, while $k_d = k_d^0 \exp[\beta\lambda\Delta U]$. Here we have taken $\lambda = 1/2$.

two heads; a higher tension increases the potential energy and thus decreases the affinity. If the spring is not under tension, then the equilibrium constant is given by $K_{\text{eq}}^0 = k_a^0 / k_d^0$. The dissociation constant for the binding of a crosslinker from solution to two filaments is given by $K_D^d = K_D^s / K_{\text{eq}}$, which indeed depends on the tension in the spring after binding; when the spring is not under tension, the dissociation constant is $K_D^{d,0} = K_D^s / K_{\text{eq}}^0$.

A head of a crosslinker connected to a single filament—a singly-bound crosslinker—can hop from one lattice position to a neighboring vacant lattice position with rate $k_{\text{hop}}^0 = D_s / \delta^2$, where D_s is the diffusion constant of a crosslinker connected to a single filament and δ is the lattice spacing. Note that hopping does not involve the unbinding of the head since crosslinkers can diffuse along filaments without detaching. The head of a crosslinker bound to two filaments—a doubly-bound crosslinker—can hop with a hopping rate $k_{\text{hop}} = k_{\text{hop}}^0 \exp[-\beta \Delta U / 2]$, where ΔU is the change in the potential energy of the spring connecting the two heads. Clearly, the effective diffusion constant D_d of a crosslinker bound between two filaments depends on the spring stiffness k . We exploited this dependence to match D_d with the value measured experimentally. The equation-of-motion of the top filament is given by

$$\gamma_0 \frac{dx_T}{dt} = - \sum_{j=1}^N k (x_j^T - x_j^B) + \eta(t) \quad (25)$$

Here, $\gamma_0 = k_B T / D_F$ is the viscous drag experienced by a filament moving through the solution, with D_F the diffusion constant of the filament, x_T is the position of the top filament, N is number of doubly-bound crosslinkers, and $\eta(t)$ is a Gaussian white noise term of magnitude $\sqrt{2k_B T \gamma_0}$.

The **algorithm** to simulate the model is similar to that used in Albada et al. 2009 (van Albada et al., 2009). The equation-of-motion of the filament, Eq. 25, is propagated via a Heun scheme (Greiner et al., 1988). The algorithm to determine when the next hopping, binding or unbinding event of a crosslinker will occur is essentially a kinetic Monte Carlo algorithm (Bortz et al., 1975). It is based on the observation that the *survival* probability $S(t)$, i.e. the probability that no crosslinker event has happened before a time t after the last event, is given by

$$S(t) = \exp(-a(t)) \quad (26)$$

where $a(t)$ is the cumulative total propensity function:

$$a(t) = \int_0^t dt' k_T(t') \quad (27)$$

with $k_T(t)$ being the total propensity function, which is obtained by summing up all the rates over all tentative crosslinker events, i.e. all possible hopping, binding and unbinding events.

In practice, right after hopping, binding or unbinding event of a crosslinker, a random number, ξ , between zero and one is drawn. The equation-of-motion of the top filament, Eq. 25, is then integrated together with the equation that describes the temporal evolution of $a(t)$:

$$\frac{da(t)}{dt} = k_T(t) \quad (28)$$

Integrating Eq. 28 since the last event leads to an estimate for $a(t) = \int_0^t dt' k_T(t')$. The

next event then occurs at a time t after the last event when

$$a(t) > \log(1/\xi) \quad (29)$$

The event i — either a hopping, binding or unbinding event — is subsequently chosen with a probability p_i as given by

$$p_i(t) = k_i(t) / k_T(t) \quad (30)$$

where $k_i(t)$ is the rate of event i .

Methods Text 4: Exponential friction

The origin of the exponential dependence of the friction on the number of crosslinkers is that the transport filament has to overcome an energy barrier in order to move. Consider a top filament that is precisely in register with the bottom filament. The lowest potential energy configuration is one in which the crosslinkers are not under tension. Suppose that out of the N crosslinkers one head of one crosslinker, say the bottom head, makes a hop to the right. After the hop, this crosslinker will pull the top filament to the right. The lowest potential energy configuration is now one in which the force exerted by this crosslinker pulling the top filament to the right is precisely balanced by the force of the other crosslinkers pulling it to the left. However, in this new state the potential energy stored in the strained crosslinkers is higher than it was originally.

We can now ask what is the lowest potential-energy path by which the top filament can move from one lattice position to the next. Starting from the configuration above, with $N_R = 1$ crosslinker pulling the filament to the right and the other $N_L = N - 1$ crosslinkers pulling it to the left, the lowest path is one in which a

head of one of the N_L crosslinkers makes a hop: either the bottom head making a hop to the right or the top head making a hop to the left. In either case, we have $N_R = 2$ crosslinkers pulling the top filament to the right and $N_L = N - N_R$ crosslinkers pulling it to the left. The next step along the lowest potential-energy path is one in which this process is repeated, until we have $N_L = N/2$ crosslinkers pulling the top filament to the left and $N_R = N/2$ crosslinkers pulling it to the right. Importantly, during this whole process, the potential energy of the system rises. The state with $N_L = N/2$ crosslinkers pulling the top filament to the left and $N_R = N/2$ linkers pulling it to the right is the transition state, the state of the system at the top of the barrier, after which the energy falls again. One can show that the height of the potential-energy barrier is given by

$U^* = \frac{k\delta^2 N}{8}$. This means that the diffusion constant of the top filament

$D = \delta^2 k_0 e^{-\beta k\delta^2 N/8}$ decreases exponentially with the number of crosslinkers N and the total crosslinker friction $\gamma = k_B T / D$ increases exponentially with N .

Table S1: Parameters for the model used in the simulations.

Parameter	Value	Description	Source
D	$0.1 \mu\text{m}^2\text{s}^{-1}$	Diffusion constant single Ase1 head	Figure S3C
k	$5 \times 10^4 k_B T \mu\text{m}^{-2}$	Spring stiffness of Ase1 dimer	Fit ^(a)
k_{on}	$0.01 \text{nM}^{-1}\text{s}^{-1}$	Ase1 binding rate to a single microtubule	From K_D^s and k_{off}
k_{off}	0.1s^{-1}	Ase1 unbinding rate from a single microtubule	Measured ^(b)
k_a^0	0.5s^{-1}	Association rate 2 nd Ase1 head	From K_D^d, K_D^s, k_d^0
k_d^0	0.05s^{-1}	Dissociation rate 2 nd Ase1 head	Measured ^(c)
$K_{\text{eq}}^0 = k_a^0 / k_d^0$	10	Equilibrium constant	From k_a^0 and k_d^0
$K_D^s = k_{\text{off}} / k_{\text{on}}$	10 nM	Dissociation constant of Ase1 from single microtubule	Figure S3A
$K_D^{d,0} = K_D^s / K_{\text{eq}}^0$	1 nM	Dissociation constant of Ase1 from overlap (double microtubule)	Figure S3B
D_F	$0.01 \mu\text{m}^2\text{s}^{-1}$	Diffusion constant of microtubule in solution	(Hunt et al., 1994)
δ	$0.01 \mu\text{m}$	Lattice spacing	Tubulin dimer length

Footnotes: ^(a) The spring stiffness k has been chosen such that the diffusion constant of a crosslinker between two filaments is as observed in experiment. ^(b) The crosslinker unbinding rate k_{off} depends on the concentration c of crosslinkers in solution, as observed by Marko and coworkers for the unbinding of proteins from DNA (Sing et al., 2014); the value reported in our table is the unbinding rate for a concentration $c \sim 0.45 \text{ nM}$ as determined experimentally (Braun et al., 2011). We used this value for the simulations of the experiments with Ase1-GFP in solution presented in Figures 4 and 5. As we did not observe any unbinding of crosslinkers from the overlap in experiments when we washed out Ase1-GFP from solution (Figures 1 and S1B), we set the unbinding rate to 0.0001 s^{-1} for the simulation presented in Figure 3. ^(c) The dissociation rate k_d^0 of the “second head” (thus when the crosslinker makes a transition from doubly filament-bound to singly filament-bound) is estimated from the measured effective rate of dissociation of crosslinkers from the overlap region between two filaments, using the procedure described in the Appendix; since k_{off} depends on the concentration, k_d^0 will also depend on the concentration.

Appendix

to footnote ^(c) to the table of parameters for the model used in the simulations.

To determine the effective dissociation rate, we compute the effective time for a doubly-bound crosslinker to completely dissociate. This is a first-passage time problem. Let's denote $\alpha = k_a/(k_a + k_{\text{off}})$; $t_c = 1/k_d + 1/k_{\text{off}}$; $t_r = 1/k_d + 1/k_a$. The average time is given by

$$T = (1 - \alpha) \sum_{i=0}^{\infty} (t_c + it_r) \alpha^i \quad (31)$$

$$= t_c (1 - \alpha) \sum_{i=0}^{\infty} \alpha^i + (1 - \alpha) \sum_{i=0}^{\infty} it_r \alpha^i \quad (32)$$

$$= t_c + t_r \frac{\alpha}{1 - \alpha} \quad (33)$$

$$= \frac{2}{k_{\text{off}}} + \frac{1}{k_d} \left(\frac{k_a}{k_{\text{off}}} + 1 \right) \quad (34)$$

Substituting in the numbers of Supplementary Table 1 yields $T = 80$ s and an effective dissociate rate for a doubly-bound crosslinker of 0.7 min^{-1} . This estimate, based on $k_{\text{off}} = 0.1 \text{ s}^{-1}$ measured at $c \sim 0.45 \text{ nM}$, is within an order of magnitude of the effective dissociation rate for a doubly-bound crosslinker of approximately 0.1 min^{-1} measured using FRAP (Braun et al., 2011).

SUPPLEMENTARY REFERENCES

- Bortz, A.B., Kalos, M.H., and Lebowitz, J.L. (1975). A new algorithm for Monte Carlo simulation of Ising spin systems. *Journal of Computational Physics* 17, 10–18.
- Braun, M., Lansky, Z., Bajer, S., Fink, G., Kasprzak, A.A., and Diez, S. (2013). The human kinesin-14 HSET tracks the tips of growing microtubules in vitro. *Cytoskeleton (Hoboken, NJ)* 70, 515–521.
- Greiner, A., Strittmatter, W., and Honerkamp, J. (1988). Numerical integration of stochastic differential equations. *J Stat Phys* 51, 95–108.
- Sing, C.E., Olvera de la Cruz, M., and Marko, J.F. (2014). Multiple-binding-site mechanism explains concentration-dependent unbinding rates of DNA-binding proteins. *Nucleic Acids Res* 42, 3783–3791.
- van Albada, S.B., Tănase-Nicola, S., and Wolde, ten, P.R. (2009). The switching dynamics of the bacterial flagellar motor. *Mol Syst Biol* 5, 316.