Theory of antiparallel microtubule overlap stabilization by motors and diffusible crosslinkers

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Abstract
Antiparallel microtubule bundles are essential structural elements of many cytoskeletal structures, for instance, the mitotic spindle. Sliding of microtubules relative to each other can lead to an overall elongation of the bundle. However, such sliding must be accompanied by microtubule growth, to maintain the overlap, which is a landmark of anaphase. Diffusive crosslinkers of the Ase1/PRC1/MAP65 family are able to form stable overlaps in combination with kinesin-14 motors. This process is thought to arise through a balance of forces between motors and crosslinkers, the latter effectively producing an entropic pressure. We provide a continuous theory to explain the formation of stable overlaps, in which steric effects caused by the finite number of binding sites available on the microtubule lattice play a leading role. We confirmed the validity of this approach using discrete stochastic simulations performed with the Open Source simulation engine Cytosim. From the densities of motors and crosslinkers, their diffusion rates, and the velocities of motors, the theory predicts the sliding speed of microtubules and explains the force production and breaking effect of crosslinkers and motors containing diffusible microtubule-binding domains. Finally, we discuss a mechanism by which sliding and microtubule growth can be coordinated without the need for fine-tuning the parameters of the system, in line with the known robustness of mitosis.

KEYWORDS
cell biology, cytoskeleton, mathematical modeling, mitotic spindle, molecular motors

1 INTRODUCTION

Arrays of parallel microtubules are indispensable in cells, appearing for instance in mitotic spindles (Ward, Roque, Antony, & Nedelec, 2014), neuronal dendrites (Yau et al., 2016), or marginal bands of blood platelets (Mathur et al., 2019). Observed in cross-section by electron microscopy, these arrays have a regular organization that is composed either of square, triangular (Ward et al., 2014), or even hexagonal unit cells (Tilney, 1971). These bundles are formed by specialized molecular crosslinkers that mechanically connect adjacent microtubules. Some crosslinkers only bind when the microtubules are oriented in the same direction (Roque, Ward, Murrells, Brunner, & Antony, 2010), while others bind only when the microtubules are antiparallel (Ward et al., 2014). Molecular motors can also connect microtubules and slide them relative to each other, resulting in overlap shortening, and these changes can be opposed by crosslinkers.

Interplay between motors and crosslinkers is particularly relevant in the case of the central spindle. During anaphase, interpolar microtubules emanate from opposing spindle poles and interdigitate forming an antiparallel square lattice known as the midzone, which is organized by motors and crosslinkers (Ward et al., 2014). In certain species, once chromosomes have been segregated, interpolar microtubules remain as the only connection between spindle poles. In that case, microtubule growth and motor sliding in the midzone must be coordinated to allow...
for separation of the poles while maintaining a stable overlap (Scholey et al., 2016). How this coordination is achieved is still an open question.

In vitro assays have been used to understand how taxol-stabilised microtubules can form stable overlaps in the presence of motors and crosslinkers. Importantly, it was found that two microtubules were sufficient to form a stable antiparallel overlap, if the correct combination of motors and crosslinkers is used. In the presence of crosslinkers from the MAP65/Ase1/PRC1 family, kinesin-14 forms stable overlaps (Braun et al., 2011), in contrast to kinesin-5, which moves antiparallel microtubules apart at constant speed (Shimamoto, Forth, & Kapoor, 2015). Noteworthy, the kinesin-14 family members Ncd and HSET, in addition to a motor head, contain a diffusible tail (Braun et al., 2017), making them able to form asymmetric connections with motor and diffuse head bound to different microtubules. Interestingly, Kinesin-14 can only reach sub-picoNewton forces when crosslinking antiparallel microtubules (Lüdecke, Seidel, Braun, Lansky, & Diez, 2018), even though its motor domain is able to exert picoNewton forces when pushing antiparallel microtubules (Lüdecke, Seidel, Braun, Lansky, & Diez, 2018), even though its motor domain is able to exert picoNewton forces when pushing antiparallel microtubules (Lüdecke et al., 2018), even though its motor domain is able to exert picoNewton forces when crosslinking antiparallel microtubules (Lüdecke, Seidel, Braun, Lansky, & Diez, 2018). This suggests that the diffusible tail of kinesin-14 limits the force exerted by its motor domain, in an antiparallel bridge.

Crosslinkers of the MAP65/Ase1/PRC1 family preferentially crosslink antiparallel microtubules (Janson et al., 2007), and can diffuse longitudinally along single microtubules and microtubule overlaps (Kaptein et al., 2008). They are required for the organization of the midzone during anaphase in yeast (Loliodice et al., 2005) and human cells (Zhu & Jiang, 2005), and PRC1 has recently been observed to locate to the bridging fibers connecting sister k-fibers, suggesting that this protein may also have a role during metaphase (Polak, Risteski, Lesjak, & Tolić, 2017). Fission yeast cells expressing excess Ase1 exhibit slower mitotic spindle elongation (Krüger, Sanchez, Paoletti, & Tran, 2019; Rincon et al., 2017), suggesting these molecules can oppose microtubule sliding in vivo.

In vitro, diffusible crosslinkers can also act as breaks (Braun et al., 2011; Lansky et al., 2015; Wijeratne & Subramanian, 2017). Being passive in nature, one might have expected the force required to move a head to be solely proportional to the sliding speed. It was found however that the resistance to sliding can increase dramatically with the density of the molecules on the antiparallel overlap (Braun et al., 2011), suggesting the existence of a critical density of crosslinkers above which the system jams. These crosslinkers are also able to widen an overlap in vitro, in the absence of any motor (Lansky et al., 2015), leading to the idea that they could be regarded as a gas confined within the overlap.

Such observations were understood by considering the discrete nature of the microtubule lattice with its well-known periodicity of 8 nm, given that only one head, at most, may bind to a tubulin heterodimer. In these experiments, diffusible crosslinkers remain associated preferentially with the overlap region, where the two binding domains can be bound. Statistically, overlap extension creates more possibilities for the crosslinkers to bind, resulting in an entropic pressure effectively pushing the microtubules ends apart. Inversely, when sliding results in the densification of the crosslinkers in the overlap, the resistance to sliding increases, eventually reaching a steady state overlap length, which can remain stable for several minutes (Braun et al., 2011; Hannabuss et al., 2019; Lansky et al., 2015; Wijeratne & Subramanian, 2018).

Entropic expansion was previously modelled using both a continuous analytical model and lattice-based stochastic simulations (Lansky et al., 2015). Interestingly, while the analytical model did not quantitatively match the experimentally observed behaviors, the computational model did, as it exhibited a drag that increased exponentially with the number of crosslinkers. Thus, a discrete approach seemed to be necessary to explain the observed behavior. We show here however that an extended analytical theory, that includes the steric effects caused by the discrete lattice of binding sites, exhibits good agreement with their experiments.

Previous theoretical work examined the formation of stable overlap in a regime where crosslinkers exert a pushing force on the overlap, and although the effect of having a finite lattice was considered, the behavior of the system did not change qualitatively with or without a lattice (Johann, Goswami, & Kruse, 2015). We suggest however that these effects are essential to determine the extent of the overlap.

Aiming to understand how motors and diffusible microtubule binders can be combined to form stable overlaps, we study here four related systems (Figure 1) inspired by the in vitro work precited. All systems contain two antiparallel microtubules and we only consider their motion in one dimension. We also presume a constant overlap length, assuming that microtubule growth matches the sliding exactly. This point will be discussed. The four systems differ in the way the diffusible and motor heads are associated to form connecting molecules. In System A, crosslinkers bind and unbind but do not diffuse along microtubules, while sliding is produced by bivalent motors. System B is similar, except that the crosslinkers can diffuse along microtubules and never unbind. It was realized in vitro using PRC1 and kinesin-5 (Subramanian et al., 2010). System C corresponds to experiments using Kinesin-14 (Lüdecke et al., 2018), in which the sliding is produced by motors composed of a diffusible tail and a motor head, without crosslinkers. Finally, in System D, diffusible motors pull against diffusible crosslinkers. This was explored with Kinesin-14 and Ase1 (Braun et al., 2011; Lansky et al., 2015). Another system in which Kif4A motors directly pulled on PRC1 crosslinkers was modelled previously by us (Hannabuss et al., 2019). It is thus omitted here. These systems offer gradual complexity and different outcome. After defining a common set of assumptions, we predict the sliding speed of the microtubules in each system.

## 2 | ASSUMPTIONS

The general assumptions are the same for all systems. Motor and crosslinking entities are made of two heads, binding to different microtubules. Unbound entities are uniformly distributed in space, and their heads can bind with equal rates $k_b$ if they reach a microtubule. A bound head may unbind with constant rate $k_u$. If one head is attached, the other head can attach to the other microtubule if it is overlapping at this position, also with rate $k_b$. An entity bound to two
microtubules exerts an elastic force of stiffness $\kappa$ and zero resting length (Figure 1a). At the time of second binding, the gap $\delta$ between the two heads is null, but if microtubules slide, a tension $f = \kappa \delta$ will build up. This tension is relieved if the heads move appropriately along the microtubules, or if the microtubules slide relative to each other. The movement of the heads along the microtubule is affected by the tension $f$ in the associated link, differently for motors and passive heads. We consider three types of heads (Table 1). Motor heads move continuously, since we are considering situations where jamming of motors does not occur. Attached motor heads move toward the plus-end with a speed $v_m = v_0(1 - f_m/f_s)$, depending on the force against which the motor is pulling $f_m$, its unloaded speed $v_0$ and stall force $f_s$. Thus, an antagonistic force reduces motor speed linearly, as shown experimentally (Meyhöfer & Howard, 1995). We define $\gamma_m = f_s/v_0$, the characteristic drag coefficient of the motor head, such that $v_m = v_0 - f_m/\gamma_m$. This equation determines the force-velocity relationship of the motor. We note that Kinesin-14 moves toward the minus end of microtubules, but as microtubule assembly dynamics are ignored here and only one type of motor is present, we can ignore microtubule polarity as the system is unchanged by swapping "plus" and "minus" throughout. Nondiffusible passive heads do not move along microtubules, and must unbind to relocate on a filament, releasing the associated linker tension immediately. We define $\gamma_c = \kappa/k_u$, the effective drag coefficient of the crosslinkers. Diffusible passive heads are modeled following (Lansky et al., 2015). They bind at discrete sites on the microtubule lattice, separated by $a = 8$ nm. Passive heads can diffuse on this lattice by hopping to adjacent sites with a rate $k_0$. However, a crosslinker head may not move to a position that is already occupied, nor step out of the microtubule at its ends. In the absence of external force, passive heads hop equally in both directions, undergoing pure one dimensional (1D) diffusion with a coefficient $D_1 = k_0a^2$. When the tension $f_d$ in the linker between the heads builds up, the upstream ($k^+$) and downstream ($k^-$) rates differ (Figure 1b). How these rates vary is not known, but thermodynamic consideration dictates that for any pair of states ($a$, $b$) with potential energies ($U_a$, $U_b$), the transition rates should satisfy Arrhenius law: $k_{b \rightarrow a} = \epsilon' e^{-\epsilon'/k_BT}$ with $\epsilon = (U_b - U_a)/k_BT$, and this is fulfilled by assuming (Wang, Peskin, & Elston, 2003):
\[ k_{a-b} = \frac{\epsilon}{1-e^{-\epsilon}} k_0 \quad \text{and} \quad k_{b-a} = \frac{\epsilon}{e^\epsilon-1} k_0 \quad (1) \]

Since \( U = \frac{1}{2} k \Delta^2 \), the hopping rates read:

\[ k^+ = \frac{a - \beta}{1-e^{-a}} k_0 \quad \text{and} \quad k^- = \frac{a + \beta}{e^{\epsilon a} - 1} k_0, \quad (2) \]

where \( a = a_d/k_0 T \) expresses the bias caused by force and \( \beta = xa^2/2k_0 T \) echoes the difficulty of reaching a neighboring binding site due to the stiffness of the linker. The diffusion rate of a crosslinker that is bound to two overlapping microtubules is defined by \( a, k_0 \) and \( \beta \) and the microtubule’s own movements. In this article, we adopt the continuum limit that is obtained by neglecting the contribution of \( \beta \). The drift speed along a microtubule, under a given force, then reads:

\[ v_d = a(k^+ - k^-) = \frac{f_d}{\gamma_d} \quad (3) \]

with \( \gamma_d = k_0 T/k_0 a^2 \), the characteristic drag coefficient of a diffusible head. Accordingly, we adopted \( \kappa = 100 \text{ pN/\mu m} \), a value for which the continuum limit is valid, for forces in the pN range (see Section 5). Microtubules are incompressible lines oriented in opposite directions. The orientation of each microtubule dictates the natural movement of attached motors. The estimated viscous drag \( \gamma_{fil} \leftrightarrow 3 \pi \mu H/[\log(H/d) + 0.312] \) depends on the length of the microtubule \( H \), its diameter \( d \), and the viscosity of the fluid \( \zeta \), following (Tirado & García De La Torre, 1979).

3 | RESULTS

3.1 | System A: conventional motors and crosslinkers

We consider first nondiffusible crosslinkers that can bind and unbind from the antiparallel microtubules with constant rates \( k_b \) and \( k_u \) (Figure 1a). The motors are of the Kinesin-5 family, sliding antiparallel microtubules apart. Given that all binding/unbinding rates and the overlap length are constant, there is a steady number of active motors in the system, producing an average force \( F \) between the microtubules. There is also a steady number of crosslinkers \( c \), and their combined force must balance the motor force. The average force per crosslinker is then \( f_d = F/c = \kappa \delta \). Microtubules slide when one crosslinker unbinds, as the force of the motor is redistributed on a smaller number of crosslinkers. To evaluate the sliding associated with an unbinding event, we can consider the balance of forces after detachment: \( F/(c-1) = \kappa \delta_{\text{after}} \). The maximum displacement of the microtubule is therefore \( \delta_{\text{after}} - \delta = \delta/(c-1) \). This will be the actual displacement, if the timescale of binding is sufficiently slow to allow the system to reach equilibrium, corresponding to \( k_{b/u} \ll \kappa \). This condition holds true for realistic parameter values. In this regime, each filament will move at speed \( v_{fil} = k_0 \zeta(c/(c - 1)) \), since \( 2c \) heads can unbind, and each unbinding event leads to a translation of both filaments by \( \delta/2(c - 1) \). We can now calculate the force of the motors. Fast processive motors that are able to reach stall force \((v_0/k_0 > k_u) \) (see Table 2), will have reached a steady state defined by the force-velocity relationship \( v_0/v_0 = 1 - f_m/f_u \). If \( m \) is the average number of bound motors, \( f_m = F/m = \kappa \delta/c/m \), and by substituting \( v_{fil} \) we finally derive:

\[ \frac{v_0}{v_{fil}} = 1 + \frac{c-1 \gamma_c}{
\kappa m}
\quad (4) \]

The filament speed \( (v_{fil}/v_0) \) is simply determined by the ratio of bound crosslinkers to motors (Figure 2a) and the associated drag coefficients. As the system is symmetric, the filaments slide apart at speed \( 2v_{fil} \), not exceeding \( 2v_0 \) as expected. Speed is independent of the absolute density of the molecules on the microtubule. It follows that such system cannot form stable overlaps, since it slides \( (v_{fil} > 0) \) for any number of motors \( (m > 0) \).

3.2 | System B: bivalent motors and diffusible crosslinkers

We now consider diffusible crosslinkers, that however do not bind or unbind for simplicity (Figure 1b). This setup is comparable to the PRC1/kinesin-5 system (Subramanian et al., 2010). To account for the fact that only one crosslinker head may occupy each lattice site \( (a = 8 \text{ nm}) \), we introduce the probability \( \rho_c \in [0,1] \) for a lattice site to be occupied, and treat this value as if it was uniform along the lattice. In reality, since filament ends act as diffusion barriers, crosslinkers may accumulate at filament ends (Braun et al., 2011; Lansky et al., 2015). However, in our case where the overlap is kept constant by microtubule growth, the crosslinkers remain equidistributed, and this parameter is effectively uniform. Therefore, \( \rho_c \approx ca/L \), and Equation (3), becomes

| TABLE 2 | Parameters of simulations |
|-------------------|-----------------------------|
| **Common**        | **Linke stiffness** |
| Lattice size      | \( a \) \quad 8 \text{ nm} |
| Rigid crosslinker | Unbinding rate \( k_u \) \quad 2.38 \text{ s}^{-1} |
|                   | Binding rate \( k_b \) \quad 1 \text{ s}^{-1} |
| Bivalent motor    | Unbinding rate \( k_u \) \quad \*0.01 \text{ s}^{-1} |
|                   | Binding rate \( k_b \) \quad \*0.01 \text{ s}^{-1} |
|                   | Unloaded speed \( v_0 \) \quad 0.05 \text{ \mu m/s} |
| Stall force       | \( f_u \) \quad 6 \text{ pN} |
| Diffusive crosslinker | 1D diffusion rate \( D_1 \) \quad 0.1 \text{ \mu m}^2/\text{s} |
| Diffusive motor   | Unloaded speed \( v_0 \) \quad 0.2 \text{ \mu m/s} |
|                   | Stall force \( f_u \) \quad 6 \text{ pN} |
|                   | 1D diffusion rate \( D_1 \) \quad 0.1 \text{ \mu m}^2/\text{s} |

*Note: Parameters used in the computer simulations, unless specified.

*Binding and unbinding of bivalent motors is disabled in System B.*
In vitro, the viscous drag of the filament is small compared to the drag of diffusible crosslinkers. For example, with $\xi < 0.01$ Pa s, the viscous drag per unit length for microtubules is $\sim 0.015$ pN s $\cdot$ μm$^{-2}$ whereas $\gamma_d = 0.04$ pN s $\cdot$ μm$^{-1}$ for $D_1 = 0.1$ μm$^2$/s. Hence, at densities above 1 crosslinker/μm, the force exerted by the viscous drag of the solution remains negligible, such that the force in the motor links should equal the force in the crosslinker links. With $m$ motors and $c$ crosslinkers, and calling $f_m$ and $f_d$ the forces per molecule, this means $F = mf_m = cf_d$. In the steady state, because of the symmetry, motors and crosslinkers are immobile in space, and the speed of the heads is equal to the speed of the filament: $v_{fil} = v_m = v_d$. Using the motor force-velocity relationships ($v_m = v_0 - f_m/f_d$) and Equation (5), we derive:

$$v_0 = \frac{(1 - \rho_c) f_d}{\gamma_d}$$

(5)

In addition to the ratio of motor to crosslinkers and their drag coefficients, the density of crosslinkers on the microtubule lattice also sets the filament speed (Figure 2b). Higher occupancy leads to lower speeds (see Figure 2b, black line obtained for $\rho_c = 0$). In the regime where the second term of the right hand side dominates, the speed is proportional to the number of motors, and also affected by the amount of crosslinkers (terms $c$ and $\rho_c$). Notably, this system can form stable overlaps whenever all lattice sites are occupied by crosslinkers ($\nu_c$ = 1), since in that case sliding will stall for any number of motors.

### 3.3 System C: diffusible motors

We now consider diffusible motors composed of a motor head linked with a diffusible head (Table 1, Figure 1c). We focus on the low density regime, and model the diffusible tail on a lattice without occupancy limits such that multiple heads can bind to the same site. A diffusible head unbinds immediately upon reaching the end of a filament.

At steady state, motors move toward the plus-end of their microtubule. Diffusible heads follow their motor at a distance $\delta$ behind, effectively moving toward the minus-end of the microtubule to which they are bound. With all links pulling in the same direction, the forces $f$ of the links add up and the movement of the filament is $v_{fil} = m f f_{fil}$, with $m$ the number of links. The mean speeds of motor heads ($v_m$) and diffusible heads ($v_d$) are relative to their microtubules, which move in opposite directions, and the steady state requires $v_m - v_{fil} = v_d + v_{fil}$. From the motor force-velocity and Equation (3) we then derive $v_0 - f f_{fil} = f / f_d + 2 v_{fil}$ and finally:

\[ \text{FIGURE 2} \quad \text{Systems A and B, bivalent motor and diffusible or nondiffusible crosslinkers. (a) Steady state speed for System A as a function of the ratio of the number of crosslinkers to motors (c/m), with motors ($f_m = 6$ pN, $v_0 = 0.05$ μm/s) pulling nondiffusible crosslinkers ($k = 100$ pN/μm, $k_u = 2.38$ s$^{-1}$), resulting in $\rho_c/f_m = 0.35$ (see Table 1). Dots represent the results of individual simulations containing 30 (blue circles), 60 (orange squares), or 90 (grey triangles) motors and a random number of crosslinkers ($1 - 375$). The line indicates Equation (4). (b) Steady state speed for System B as a function of the ratio of the number of crosslinkers to motors (c/m), with bivalent motors ($f_m = 6$ pN, $v_0 = 0.05$ μm/s) and diffusible crosslinkers ($D_1 = 10^{-5}$ μm$^2$/s), resulting in $\gamma_d/f_m = 0.35$ (see Table 1). Dots represent the results of individual simulations containing 60 (blue circles), 120 (orange squares), 240 (grey triangles) motors, and a random number of crosslinkers (1 - 380). Colored lines show the corresponding predictions of Equation (6). Black line represents the prediction if occupancy of the lattice is ignored ($\nu_c = 0$). Speed becomes zero when $\rho_c = 1$. (c) Steady state sliding speed for System B as a function of the occupancy of crosslinkers ($\rho_c$), in simulations containing equal number of crosslinkers and motors, for three different values of crosslinker diffusion rates ($D_1$). Dots represent the results of individual simulations, with $D_1 = 3.5 \times 10^{-5}$ (blue discs), $D_1 = 3.5 \times 10^{-4}$ (orange squares), and $D_1 = 3.5 \times 10^{-3}$ μm$^2$/s (grey triangles), resulting in $\gamma_d/f_m = 1; 10^{-1}$ and $10^{-2}$, respectively (See Table 1). These simulations included an equal amount of crosslinkers and motors, randomly chosen between 5 and 375. Since motors and crosslinkers do not unbind, the mean occupancies of crosslinkers and motors are equal. Colored lines show the corresponding predictions of Equation (6), $L = 3$ μm for all the simulations on this figure, and the horizontal and vertical positions of simulation dots are calculated from the simulation results (see 4: Simulation Methods) [Color figure can be viewed at wileyonlinelibrary.com] \]
\[
\frac{v_0}{v_{th}} = 2 + \frac{\gamma_m}{m} \left(\frac{1}{\gamma_d} + \frac{1}{\gamma_m}\right).
\]  

(7)

This formula can be compared to the sliding speed obtained in a gliding assay in which immobilized motors are pulling directly on the microtubule: \(v_0/v_{th} = 1 + \gamma_m/(m\gamma_d)\). First, the factor 2 in Equation (7) indicates that diffusible motors, since they only contain one motor domain, can only slide microtubules at half their unloaded speed, unlike tetrameric kinesin-5 motors, which can slide microtubules at their unloaded speed. Second, part of the work produced by the motors is necessarily wasted in moving the diffusible head. Optimal microtubule transport is obtained for \(1/\gamma_d \rightarrow 0\), but if the passive head can move, only a fraction of the motor force is transmitted to the link. This effect can be understood by considering immobile microtubules, for which the force in the link \(f\) is set by:

\[
\frac{v_0}{f} = \frac{v_0}{f_{th}} + \frac{1}{\gamma_d}
\]  

(8)

If \(f \ll v_0\gamma_d\) (the tail is hard to move), all the motor work is transmitted (Figure 3a), but in any case the transmitted force is limited to \(v_dv_0\) (Figure 3a, dashed line). If \(\gamma_d < \gamma_m\) a significant fraction of the motor work will be used in sliding the diffusible head, rather than the microtubules. Indeed, if \(\gamma_d \ll \gamma_m\) (which is expected from the measured values), the force required to transport the diffusive tail on the microtubule is negligible compared to the stall force, and the motor heads move nearly at their unloaded speed. This means that the force produced is \(\gamma_dv_0\) (Figure 3a, dashed line), corresponding to the drag force produced by diffusive tails moving at the motor’s unloaded speed.

System C was simulated for a motor with the characteristics of kinesin-14: \(v_0 \approx 0.2 \mu m/s, f_s \approx 5 \text{pN}\) (Lüdecke et al., 2018) and \(D_1 = 0.1 \mu m^2/s\) (Braun et al., 2017; Norris et al., 2018) (Figure 3a, dots). We recover Equation (7) and increasing viscosity reduces the sliding speed as anticipated (Figure 3b). With \(\gamma_d = 0.04 \text{pN} \cdot s/\mu m\) and \(\gamma_m = 25 \text{pN} \cdot s/\mu m\), certainly \(\gamma_d \ll \gamma_m\), and the reduced sliding speed is set by \(v_0/v_{th} = 2 + \gamma_m/(m\gamma_d)\). An interesting prediction can be derived from this formula. One can expect the drag of a filament to be roughly proportional to its length \(H\) (as predicted for \(H > 2 \mu m\) (Tirado & García De La Torre, 1979)), and if the linear density of active motors is constant, the sliding speed will be independent of the length of the microtubule (since \(\gamma_m/m\) is constant). This is what has been experimentally observed (Lüdecke et al., 2018).

3.4 System D: diffusible motors and diffusible crosslinkers

We now add symmetric diffusible crosslinkers to System C (Figure 1d). As shown experimentally, this slows down the sliding speed (Braun et al., 2011; Lüdecke et al., 2018). In these experiments, one microtubule is fixed while an antiparallel shorter one is free to move and crosslinked by the diffusible crosslinker Ase1 and the motor Ncd. Sliding occurs at a constant speed set by the ratio of motors to crosslinkers. When the transported microtubule reaches the end of the fixed

![Figure 3](https://example.com/Figure3.png)

**FIGURE 3** Systems C and D, sliding by diffusible motors. (a) The maximum usable force of a diffusible motor is limited by the drag coefficient of its diffusible head \(\gamma_d\) (see Table 1). Dots represent the average force exerted per motor on fixed microtubules at steady state divided by motor stall force \((f/f_s)\) as a function of the parameter \(\gamma_m/\gamma_d\) (see Table 1) in individual simulations with different diffusion rates of motor tail for Kinesin-14 like motors \((f_s = 6 \text{ pN}, v_0 = 0.2 \mu m/s)\) and \(D_1 \in [10^{-7}, 1] \mu m^2/s\). The line represents the prediction of Equation (8). The dashed line represents the upper limit \(\gamma_d/f_s\). (b) Sliding speed for System C as a function of density of motors, with Kinesin-14 like diffusive motors \((f_s = 6 \text{ pN}, v_0 = 0.2 \mu m/s, D_1 = 0.1 \mu m^2/s)\), for different viscosities \((\rho, \text{in Pa-s}): 0.01\) (blue discs), 0.1 (orange squares), and 1 (grey triangles). Dots represent the results of individual simulations containing a random number of motors in \([1, 100]\). Colored lines show the corresponding predictions of Equation (7). (c) Sliding speed for System D as a function of crosslinker occupancy \(\langle \rho \rangle\), with diffusible motors, as in (b), and diffusible crosslinkers \((D_1 = 0.1 \mu m^2/s)\). Dots represent the results of individual simulations with varying number of motors: 100 (blue circles, \(\rho_m = 0.06\)), 200 (orange squares, \(\rho_m = 0.12\)), and 300 (grey triangles, \(\rho_m = 0.18\)). The number of crosslinkers is randomly chosen in \([1, 300]\). Colored lines show the corresponding predictions of Equation (9). Note that simulations cannot yield negative speeds because overlap is kept constant by growth. For all simulations, \(L = 3 \mu m\). All dots are placed according to the values of the relevant quantities averaged after the system has reached steady state (see 4: Simulation Methods) [Color figure can be viewed at wileyonlinelibrary.com]
microtubule, the sliding stalls and eventually a stable overlap is established. During this time where the overlap decreases, the density of Ase1 increases but the density of Ncd remains unchanged. This suggests that Ncd turnover is faster than sliding, and that Ase1 does not compete with Ncd for binding sites. We make corresponding assumptions, with diffusible crosslinkers that do not unbind, and diffusible motors that bind and unbind with constant rates. Diffusible crosslinkers are modelled as in System B and the diffusible motors as in System C, and they do not interfere with each other for binding (Figure 1d). The diffusible heads from Ase1 and Ncd are distinct, and we note their drag coefficients \( \gamma_a \) and \( \gamma_c \) respectively ("\( T' \)" for tail of Ncd). Given the observed parameters of kinesin-14 (\( v_0 = 0.2 \, \mu m/s, D_1 = 0.1 \, \mu m^2/s \) [Lüdecke et al., 2018; Braun et al., 2017]) we expect forces produced by diffusible motors (Equation (8), with \( D_1 = 0.1 \, \mu m^2/s \)) to be in the same range as entropic pressure. The main force opposing the motor is thus the drag of the diffusible crosslinkers, as in System B, while the filament drag is negligible. We can use the contribution of the (positive) entropic pressure directly from (Lansky et al., 2015): 
\[
P = - (k_B T/a) \log(1 - \rho_c).
\]
The force balance becomes \( c_f d + P = m r_m \). We can calculate the filament sliding speed \( v_{fil} \) given that \( v_d = v_{fil} \) and \( v_m = v_{fil} \). Using Equation (5), we obtain the following relation:
\[
v_{fil} = \left[ v_0 - \frac{P}{m r_m} \right] / \left[ 2 + \frac{c_f d}{(1 - \rho_c) m r_m} \right].
\]
where, we have defined \( \frac{1}{r_m} = \frac{\rho_c}{\rho_c} + \frac{1}{\rho_c} \). The denominator of the right hand side resembles the previous equations, while the numerator accounts for the entropic pressure. Speed decreases with crosslinker drag \( c_f d \) and increases with motor tail drag \( (m r_m) \) as expected. For low densities \( \rho_c < 1 \), the sliding speed depends on the ratio of motors to crosslinkers (Braun et al., 2011). Interestingly, the model predicts negative speeds if the entropic pressure is sufficient (Figure 3c). Thus, stable overlaps may form for which \( \rho_c < 1 \). The result can be expressed from the density of species in the overlap \( \rho_m = ma/L \) and \( \rho_c = ca/L \) as:

\[
v_{fil} = \left[ v_0 + \frac{k_B T \log(1 - \rho_c)}{m r_m} \right] / \left[ 2 + \frac{c_f d}{(1 - \rho_c) m r_m} \right].
\]

This reformulation highlights that the contribution of entropic pressure decreases with overlap length \( L \), because it only depends on density, while the other forces exercised by motors and crosslinkers scale with \( L \). Moreover, at high occupancies where, \( \rho_c < 1 \), the speed tends to zero as:

\[
v_{fil} \approx \frac{k_B T}{L d} \left( \log(1 - \rho_c) \right).
\]

Thus, System D can form stable overlaps above total occupancy \( \rho_c < 1 \), when the motor-generated forces compensate the entropic pressure caused by crosslinkers.

### 3.5 Entropic overlap expansion

We wondered if System D could recapitulate entropic overlap expansion, resulting from confinement of crosslinkers. This was measured experimentally by first applying hydrodynamic flow, to compress overlaps, and subsequently stopping the flow and measuring the expansion speed (Figure 3B from [Lansky et al., 2015]). The expansion is purely driven by entropic forces, and can be analyzed by omitting the motors from System D. We considered pairs of microtubules of length 20 μm, with different initial overlap lengths. Assuming that the force under which these overlaps were formed is the same, the density of crosslinkers prior to the release of the force should be similar, since entropic forces depend only on density. Following these assumptions, the force per crosslinker is \( f_d = \frac{P}{d} \) and using Equation (2) we would predict a sliding speed:

\[
v_{fil} = a (1 - \rho_c) \left[ k^+ (f_d) - k^- (f_d) \right].
\]

Assuming that \( k_0 \) could be different on microtubule overlaps and on single microtubules, the measured diffusion constant on overlaps (0.011 μm²/s) can be matched by multiple combinations of \( k \) and \( k_0 \) (Figure 4a). For such combinations, stochastic simulations show good agreement with the experimental data (Figure 4b, c). For \( k < 300 \) pN/μm the theory remains in good agreement with the stochastic model, provided that one uses Equation (2) to evaluate Equation (11), taking into account the contribution of \( \beta \). It seems that multiple parameter combinations could be adequate to model these results.

### 3.6 Steady overlaps

In this last section, we consider the situation where sliding results in overlap shrinkage. Specifically, we aim to understand in vitro experiments that showed overlaps remaining for several minutes (Braun et al., 2011; Lansky et al., 2015; Wijeratne & Subramanian, 2017). This phenomenon occurs when the turnover of crosslinkers is slower than sliding, such that sliding results in crosslinker accumulation in the overlap. On the contrary, if crosslinker turnover is sufficiently fast, the density of crosslinkers does not increase, and stable overlaps do not form (Subramanian et al., 2010). Equation (6) predicts that sliding stops when \( \rho_c = 1 \). For values of \( r_m \gg r_d \) (the motors are stronger than the crosslinkers), the sliding indeed stops when crosslinkers are totally compacted at \( L = ca \) (Figure 5a). However, for a diffusible motor, the entropic pressure can promote \( L > ca \) (Figure 5b). From Equation (9), an equilibrium between entropic pressure and motor force is reached if:

\[
\frac{1}{L} \left[ \frac{1}{L} \log(1 - \frac{ca}{L}) \right] = - \frac{v_{fil} a m r_m}{k_B T}
\]

This result is confirmed by simulations (Figure 5b), showing that even if entropic forces are smaller than the typical stall force of a single motor head, they are able to stabilize overlaps at densities above...
Also, from Equations (6) and (9), we predict that, once a steady state length is reached, it can still decrease if crosslinkers unbind, or increase if more crosslinkers bind. Interestingly, diffusion rate of the crosslinkers does not affect final overlap length, but rather the speed at which this steady state is reached.

### STOCHASTIC SIMULATION METHODS

We used the Open Source project Cytosim in 1D (https://gitlab.com/f.nedelec/cytosim). The top (resp. bottom) microtubule is represented by an ordinate $p$ (resp. $p_0$) and a direction $d = +1$ (resp. $d = -1$). The
locations of the heads are recorded by their distance from the minus-end, a.k.a the abscissa $x_i$, such that the position in space is $p + dx_i$. An array of boolean values $T$ is used for each microtubule to represent lattice occupancy, where $T[i]$ corresponds to abscissas in $[a, a + a]$. The system is evolved using a time step of $\tau = 10^{-5}$ s. Hopping to neighboring sites are stochastic events, simulated using a random number generator: a rate $R$ is simulated by testing $\theta < 1 - e^{-\kappa T}$ at every time step. Hopping is forbidden if the lattice is occupied, and the lattice is updated at each molecular binding, unbinding or displacement. The force in a link is $\kappa \theta$ with $\delta = x_i - x_j$. The movement of motors is represented by updating the abscissas: $x_i = x_i + \nu$. The total force on each microtubule is calculated by summing all link forces. A Brownian dynamic approach using an overdamped Langevin equation is used to model the system, with an implicit numerical integration scheme (Nedelec & Foethke, 2007). The steady state speeds in Figures 2 and 3b,c were calculated from 40 s of simulated time. The sliding speed was obtained by regression of the distance between the microtubule minus ends, from 8 to 40 s. Steady state speed measurements in Figure 4c were calculated similarly from 100 s of simulated time. The fitting for bivalent motors (blue dots) was done using data from 70 to 100 s, and from 40 to 100 s for diffusive motor (orange squares). The steady state force in Figure 3a was measured from 40 s of simulated time. The microtubules (as shown on Figure 1c), were immobilized by a Hookean element of stiffness $\kappa$. For each simulation, $x_i$ was adjusted to ensure that it would always have a similar stretch at steady state: $x_i \propto \alpha D_{av}/\nu k T + 1/v$. The steady state overlap length for bivalent motors (Figure 5a) was taken as the final overlap length after 100 s of simulated time, while for diffusive motors, the average overlap length was calculated from 80 to 200 s. The diffusion rate of crosslinker in overlaps (Figure 4a) was calculated from the mean squared displacement (MSD/2 t) of 1,000 crosslinkers bound to two microtubules after 1 s of simulated time, in simulations with an infinite capacity lattice. The expansion speed (Figure 4b) was measured from 15 s of simulated time by regression of the distance between microtubule plus ends.

5 | DISCUSSION

We have examined different ways by which motors and crosslinkers can be combined to make a stable overlap, predicting the sliding speed of the microtubules in each case. The analytical predictions matched the discrete stochastic simulations with $\kappa = 100$ pN/\mu m. The equations resulting from the mean field approximation, without ignoring $\beta$, were solved numerically to improve the fit (Figure 4c). However, for much higher values of $\kappa$ and small forces per crosslinker, the system becomes qualitatively different. Microtubules adopt positions in which their lattices are in register, with an offset between them that is a multiple of the lattice unit. This regime was analyzed recently (Wierenga & ten Wolde, 2019), showing how the jumping rate between two adjacent positions can depend exponentially on the number of crosslinkers. While the stiffness value $\kappa$ is critical in this model (Wierenga & ten Wolde, 2019) as well as in ours, we note that the force may not be Hookean, such that measuring $\kappa$ may be an ill-posed quest. In addition, the dependency of forward and backward rates on the force postulated in (Lansky et al., 2015) is different from ours (Equation 2), but both assumptions seem theoretically valid. Perhaps the most effective way to discriminate between these models is to directly determine the hopping rates of Ase1 under force.

Although we were able to derive our results analytically, this could only be done under conditions where the continuous limit was valid. In future work, a discrete approach may be the preferred way to include effects such as motor biochemical and binding cooperativity and excluded volume interactions, while staying close to the cytoskeletal reality.

The properties of the motors and diffusible crosslinkers operating in bundles are likely tuned for working together. Indeed, diffusible crosslinkers can regulate the sliding of diffusible motors, but they have little effects on the sliding caused by kinesin-5, even when they are in fair excess (Subramanian et al., 2010). Given their biophysical characteristics, we can estimate if a motor would be hindered by crosslinkers or not. Kinesin-5 has a stall force of 1-10 pN (Howard, 2001), and a speed of around 100 nm/s (Howard, 2001). The diffusion rates of individual heads of kinesin-14 and Ase1 have been measured and they seem to be in the range of 0.1 – 0.01 \mu m^2/s (Lansky 2015, Braun 2017). From this, it appears that $\gamma_a \ll \gamma_m$, suggesting that kinesin-5 motors would easily run over diffusible crosslinkers (Figure 2c), which has indeed been observed (Subramanian et al., 2010). Such strong motors can slide microtubules until the crosslinkers reach total compaction. Entropic pressure may be sufficient to stall less efficient force generators. Kinesin-14 ($v_0 = 0.2 \mu m/s$ and $f_s = 1$ pN (Lüdecke et al., 2018)) has a diffusion rate that is comparable to Ase1/PRC1 diffusion. Thus $\gamma_m \gg \gamma_0$, and we predict a significant effect on sliding speed, even at low occupancies (Figure 3c). Thus many qualitative experimental observations are explained from the values of the parameters that have been published.

We have compared two types of molecular breaking: conventional crosslinkers that bind and unbind and diffusible heads. With the first type of breaking, sliding is determined by the ratio between motors and crosslinkers (Figure 2a), while with the second type it depends also on the density of crosslinkers (Figure 2b and 3c). Conventional crosslinkers do not sustain stable overlaps but diffusible crosslinkers can do so with both weak or strong motors. Motors like Kinesin-14 may stall against the entropic pressure (Figure 5b) without compacting the crosslinkers completely. Motors such as Kinesin-5 would stop when crosslinker compaction prevents further sliding (Figure 5a).

System D (Figure 4b, c) represents the experimental setups of (Lansky et al., 2015), and reproduces qualitatively their main observations. Thus, while exponential friction (Wierenga & ten Wolde, 2019) could explain the expansion experiments (Lansky et al., 2015), we propose here that considering lattice occupancy (Equation 10) while adjusting parameters that are otherwise not constrained by experiments (Figure 4) can also lead to the results observed. This alternative theory also explains that, under certain conditions, the sliding speed induced by kinesin-14 in the presence of Ase1 is independent of the
appropriate equations (e.g., 6 or 9 predicting the maximum speed at which the microtubules can slide. Thus, the quantity of sliding: microtubule growth speed should be slower than motor/crosslinker system should be able to keep up with the required assumption. The condition for this to spontaneously occur is that the overlap length, as observed in (Braun et al., 2011). The same has been observed for kinesin-5 and PRC1 (Subramanian et al., 2010).

The equilibrium between motors and an entropic pressure generated by crosslinkers was examined before (Johann, Goswami, & Kruse, 2016), but in this work, simulations were performed assuming similar hopping rates for motors and crosslinkers. We note that the hopping rate of diffusible crosslinkers is 100x faster than that of motors, such that crosslinkers can resist lower forces leading to reduced overlaps as noted (Johann et al., 2016). However, fast diffusion allows crosslinkers to reach full compaction, and in this situation they can resist enormous forces. This suggests that steric hindrance along the whole overlap is important, as captured by Equation (12).

We have assumed that microtubules would grow at the required speed to maintain the overlap steady. We could however relax this assumption in simulations where microtubules were growing at a constant speed $v_g$. They indeed reach a steady state overlap where growth and sliding equalize (Figure 5c). A sharp reduction in speed at high densities of crosslinkers, as predicted for bivalent (Figure 2c) and diffusible motors (Figure 3c), is a key property for this synchronization to happen. It allows for sliding to be conditioned on microtubule growth: microtubule elongation lowers the density of crosslinkers, and motors repack these crosslinkers by sliding the microtubules. Consequently, sliding and growing speeds match without any further assumption. The condition for this to spontaneously occur is that the motor/crosslinker system should be able to keep up with the required quantity of sliding: microtubule growth speed should be slower than the maximum speed at which the microtubules can slide. Thus, the appropriate equations (e.g., 6 or 9 predicting $v_0$) are useful to estimate the conditions under which a stable overlap can be established.

We observed that the timescale of crosslinker turnover is a critical parameter of the system. If turnover is faster than sliding, overlaps slide apart, as observed experimentally (Subramanian et al., 2010). If turnover is slower than sliding, stable overlaps may form. Interestingly, fission yeast cells reduce the turnover of Ase1 upon anaphase entry, where maintenance of overlaps at the central spindle is important for the separation of spindle poles, suggesting that turnover regulation is active in cells (Fu et al., 2009). Upon the action of only a few motors, the crosslinkers form stable overlaps, as observed experimentally (Hannabuss et al., 2019). The length of such overlap is determined by a simple rule: the overlap decreases until crosslinkers reach their maximal density. This is a remarkably simple and robust mechanism that does not require fine-tuned parameter values or intricate feedback loops. Thus, cells could adjust the length of the overlap by controlling the expression of crosslinkers.

Mechanically, with one molecular link every 8 nm over a few micrometers, such connections between two antiparallel microtubules are very strong. They are strong in the directions orthogonal to their main axis, as needed to maintain the two microtubules aligned. They are also strong in the axial direction, which is essential, particularly during anaphase where these overlaps contribute to spindle elongation. In contrast, any mechanism based on entropic pressure created by a “confined gas of crosslinker” is limited to relatively low forces and bound to result in high longitudinal compliance. This may or may not be desired depending on the operational demands placed upon the bundle.

In conclusion, sliding of microtubules until eventual stalling is robustly achieved in the presence of diffusible crosslinkers (Figure 5a,b), while crosslinkers that only bind and unbind do not seem to sustain similar long-lived overlaps (Equation 4). Perhaps the key property of these systems is to be able to accommodate microtubule assembly while maintaining steady and strong antiparallel connections, a conserved landmark of anaphase. While our theory is directly applicable to microtubules pairs formed in vitro, it will be valuable in the future to pursue geometrically realistic bundles made of more than two microtubules, to comprehend the mechanisms of action of diffusible crosslinkers in vivo.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

All source code for simulation and analysis are available from the authors upon reasonable requests.

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REFERENCES

Braun, M., Lansky, Z., Fink, G., Ruhnow, F., Diez, S., & Janson, M. E. (2011, sep). Adaptive braking by Ase1 prevents overlapping microtubules from sliding completely apart. Nature Cell Biology, 13(10), 1259–1264. https://doi.org/10.1038/ncb2323
Braun, M., Lansky, Z., Szuba, A., Schwarz, F. W., Mitra, A., Gao, M., ... Diez, S. (2017, oct). Changes in microtubule overlap length regulate kinesin-14-driven microtubule sliding. Nature Chemical Biology, 13(12), 1245–1252. https://doi.org/10.1038/nchembio.2495
Fu, C., Ward, J. J., Loiodice, I., Velte-Casquillas, G., Nedelec, F. J., & Tran, P. T. (2009). Phospho-regulated interaction between Kinesin-6 Klp9p and microtubule bundler Ase1p promotes spindle elongation. Developmental Cell, 17(2), 257–267. https://doi.org/10.1016/j.devcel.2009.06.012
Rincon, S. A., Lamson, A., Blackwell, R., Syrovatkina, V., Fraisier, V., Paoletti, A., ... and braking forces generated by ensembles of kinesin-5 crosslinking two microtubules. Developmental Cell, 34(6), 669–681. https://doi.org/10.1016/j.devcel.2015.08.017

Subramanian, R., Wilson-Kubalek, E. M., Arthur, C. P., Bick, M. J., Campbell, E. A., Darst, S. A., ... Kapoor, T. M. (2010). Insights into anti-parallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein. Cell, 142(3), 433–443. https://doi.org/10.1016/j.cell.2010.07.012

Tilney, L. G. (1971). How microtubule patterns are generated: The relative importance of nucleation and bridging of microtubules in the formation of the axoneme of raphidiophrys. Journal of Cell Biology, 51(3), 837–854. https://doi.org/10.1083/jcb.51.3.837

Tirado, M. M., & García De La Torre, J. (1979). Translational friction coefficients of rigid, symmetric top macromolecules. Application to circular cylinders. The Journal of Chemical Physics, 71(6), 2581–2587. https://doi.org/10.1063/1.438613

Wang, H., Peskin, C. S., & Elston, T. C. (2003). A robust numerical algorithm for studying biomolecular transport processes. Journal of Theoretical Biology, 221(4), 491–511. https://doi.org/10.1006/jtbi.2003.3200

Ward, J. J., Roque, H., Antony, C., & Nedelec, F. J. (2014). Mechanical design principles of a mitotic spindle. eLife, 3, 1–28. https://doi.org/10.7554/eLife.03398

Wierenga, H., & ten Wolde, P. R. (2019). Diffusible crosslinkers cause superexponential friction forces. arXiv, 1–12. NY, USA: Cornell University. doi: arXiv:1906.02527

Wijeratne, S., & Subramanian, R. (2017). Geometry of antiparallel microtubule bundles regulates relative sliding and stalling by PRC1 and Kif4A. bioRxiv, 7, e32595. https://doi.org/10.7554/eLife.32595

Wijeratne, S., & Subramanian, R. (2018, oct). Geometry of antiparallel microtubule bundles regulates relative sliding and stalling by PRC1 and Kif4A. eLife, 7, e32595. https://doi.org/10.7554/eLife.32595

Yau, K. W., Schatzle, P., Tortosa, E., Pages, S., Holtmaat, A., Kapitein, L. C., & Hoogenraad, C. C. (2016). Dendrites in vitro and in vivo contain microtubules of opposite polarity and axon formation correlates with uniform plus-end-out microtubule orientation. Journal of Neuroscience, 36(4), 1071–1085. https://doi.org/10.1523/JNEUROSCI.2430-15.2016

Zhu, C., & Jiang, W. (2005). Cell cycle-dependent translocation of PRC1 on the spindle by Kif4 is essential for midzone formation and cytokinesis. Proceedings of the National Academy of Sciences of the United States of America, 102(2), 343–348. https://doi.org/10.1073/pnas.0408438102

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