**Ase1 selectively increases lifetime of antiparallel microtubule overlaps**

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**Abstract**

Microtubules (MTs) are dynamically instable polar biopolymers switching between periods of polymerization and depolymerization. In presence of MT-crosslinking proteins, MTs form overlaps and self-assemble reversibly into complex indispensable networks, such as the bipolar mitotic spindle. Differential regulation of MT stability in parallel and antiparallel overlaps is essential for the integrity of these networks. Diffusible MT crosslinkers of the Ase1/MAP65/PRC1 family associate with different affinities with parallel and antiparallel overlaps providing a basis for this differential regulation. How Ase1 regulates MT stability in parallel and antiparallel bundles is however unknown. Here, we show that Ase1 selectively promotes antiparallel MT overlap longevity, predominantly by selectively accelerating rescues. We also observed Ase1 accumulating at the retracting ends of depolymerizing MTs, concomitant with slower depolymerization, for crosslinked MTs as well as isolated MTs. Theoretically, assuming that Ase1 hinders detachment of tubulin subunits is sufficient to enrich Ase1 at MT ends. A mathematical model built on this idea shows good agreement with the experiments. We propose that differential regulation of MT dynamics by Ase1 contributes to mitotic spindle assembly by specifically stabilizing antiparallel overlaps in the midzone, compared to parallel overlaps or isolated MTs.

**Introduction**

Spatial regulation of microtubule (MT) organization and dynamics is critical for the assembly of structures such as the mitotic spindle (Nédélec, Surrey and Karsenti, 2003). MTs are dynamic polymers, undergoing stochastic switching between phases of polymerization and depolymerization, with the switch from the polymerization to the depolymerization phase termed catastrophe and the reverse transition termed rescue (Kirschner and Mitchison, 1986). Precise regulation of MT dynamics is essential for spindle assembly, chromosome segregation, cytokinesis, and polarization of interphase arrays in many cell types. MT-based structures are formed by interconnected MTs and often by lateral association of several MTs into bundles. As MTs are polar filaments, they can be bundled in a parallel or antiparallel manner. Within bipolar structures, like mitotic spindles, parallel and antiparallel MT overlaps coexist. While MTs near the spindle poles tend to be associated parallelly, MTs in the spindle mid-zone form antiparallel overlaps that are essential to the mechanical integrity of the spindle (Mastronarde *et al.*, 1993). Importantly, parallel and antiparallel overlaps have different roles, and related to this role, often differ in their stability. For example, antiparallel overlaps in yeast spindles are stable such as to prevent spindle collapse (Löiodice, 2005; Yamashita, 2005). A common and crucial component of such a mechanism is a MT crosslinker that can distinguish between parallel and antiparallel MTs. Diffusive MT-bundling proteins of the conserved Ase1/MAP65/PRC1 family are a prime example of molecules with this capacity. The geometry of Ase1/MAP65/PRC1 MT binding sites favors antiparallel MT, which results in increased Ase1/MAP65/PRC1 affinities for antiparallel MT overlaps and preferential antiparallel crosslinking activity (Janson *et al.*, 2007; Gaillard *et al.*, 2008; Bieling, Telley and Surrey, 2010; Subramanian *et al.*, 2010; Kellogg *et al.*, 2016; She *et al.*, 2019). Ase1/MAP65/PRC1 proteins are found *in vivo* preferentially at the spindle midzone, and are involved in spindle integrity and regulation of spindle elongation (Löiodice, 2005; Yamashita, 2005; She *et al.*, 2019). Ase1 deletion mutants, although viable, exhibit interphase MTs with reduced bundling and mitotic spindles that often fall apart as they attempt to elongate in anaphase (Löiodice, 2005; Yamashita, 2005).  The preferred binding of Ase1/MAP65/PRC1 family proteins to antiparallel MTs leads to the recruitment of other proteins at the midzone that can locally alter MT dynamics, such as CLASP (Bratman and Chang, 2007; Liu *et al.*, 2009; Kitazawa *et al.*, 2014) or kinesin-4 (Bieling, Telley and Surrey, 2010; Mani *et al.* 2021). By recruiting these additional factors, Ase1 family proteins can differentially regulate the dynamics of bundled MTs, specifically affecting the dynamics of antiparallel bundles, without changing the polymerization dynamics of single MTs (Bieling, Telley and Surrey, 2010; Mani, Wijeratne and Subramanian, 2021). Additionally, Ase1 family members themselves are also known to have direct effects on MT dynamics. In vitro experiments have shown that MAP65-1, upon crosslinking MTs, promotes rescues (Stoppin-Mellet *et al.*, 2013). Based on the modeling of their observed overlap dynamics, Stoppin-Mellet *et al*. predicted MAP65-1 to have more effect on antiparallel MTs compared to parallel ones. This is in line with the aforementioned preferential binding of MAP65-1, PRC1 and Ase1 to antiparallel overlaps. However, experimental evidence showing how Ase1/MAP65/PRC1 regulates MT dynamics in parallel and antiparallel MT bundles is still lacking.

We here show that Ase1 alone is sufficient to extend the lifetime of antiparallel overlaps, in conditions where single and parallel MTs remained highly dynamic and short lived. This differential regulation is due to a promotion of MT rescue and a decrease of MT depolymerization velocity, specifically in antiparallel overlaps. As we observed that Ase1 density at MT ends during MT depolymerization correlates with a decrease of MT depolymerization, we propose that Ase1 reduces the dissociation rate of terminal tubulin subunits from MTs. Since Ase1 binds more strongly to antiparallel microtubules, its propensity to oppose depolymerization is amplified for antiparallel MTs, leading to their specific stabilization, compared to parallel overlaps and single MTs.

**Results**

**Ase1 selectively promotes persisting antiparallel MT overlaps**

To study the interactions between diffusible MT crosslinkers and depolymerizing MT ends, we employed total internal reflection (TIRF) time-lapse imaging of immobilized, GMPCPP-stabilized MT seeds in the presence of 30µM free tubulin and 42 nM Ase1 (Methods). Under these conditions, we observed dynamic, Ase1-decorated MT extensions polymerizing from the MT seeds (Figure 1A, B, Movie S1). When two MT plus ends, emanating from different seeds and polymerizing towards each other, encountered, these MTs either bundled or crossed, depending on the angle of incidence. Typically, at high angles the MTs crossed and only interacted at the crossing point, while at small angles either parallel or antiparallel association could be formed. As previously reported (Janson *et al.*, 2007), antiparallel bundles formed even at large initial angles of incidence (up to 40°), while parallel bundles only formed at initial angles below 20° (Figure S1A). Quantitative analysis revealed increased lifetimes of antiparallel overlaps compared to parallel ones (Figure 1C). Notably, at 42 nM Ase1 in solution, the Ase1 density on antiparallel overlaps was an order of magnitude higher than on parallel ones (Figure 1D), consistent with the previously reported differential affinities (Janson *et al.*, 2007). Repeating these experiments with 420 nM Ase1, we observed the density of Ase1 to be similar on antiparallel and parallel bundles, roughly twice the density found on single MTs (Figure 1D). This possibly indicated that, at this high concentration, a similar number of Ase1 molecules was present within parallel and antiparallel overlaps. Note, however, that this value represents the total density of Ase1 at the bundle, which might differ from the density of Ase1 molecules directly engaged in MT crosslinking by being bound simultaneously to both MTs. Despite similar decoration levels by Ase1, antiparallel overlaps were still significantly more stable than parallel ones (Figure 1C). Given the low polymerization velocity of minus ends, we very rarely observed antiparallel overlaps formed by two minus ends encountering each other, and we thus could not meaningfully quantify the associated lifetime. The relative stability of antiparallel overlaps may at least partly owe to the fact that antiparallel overlaps grow with twice the speed of parallel overlaps (since both MTs polymerize in opposite directions); hence, there is more opportunities for rescues to occur during depolymerization. However, our kymographs suggested that microtubule dynamics may also be different for antiparallel overlaps (Figure 1A,B, Figure S1C), and we set out to quantify this issue next.

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**Figure 1. Ase1 selectively promotes persisting antiparallel MT overlaps.**

**A**Kymographs of two MTs polymerizing towards each other in the presence of 42nM (respectively 420 nM) Ase1-mNeonGreen, subsequently forming a persisting region of antiparallel MT overlap. In sketches, dynamic extensions with GDP lattices are grey, and stabilized GMPCPP seeds are white. The teal bars next to kymographs indicate the presence of regions of overlap (we only counted regions where the two partaking MT regions are constituted by GDP-tubulin, i.e., a seed stabilized by GMPCPP did not count). The pink bars indicate a termination of the overlapping period, as evaluated for D. **B**Kymographs of two MTs polymerizing in a parallel configuration in the presence of 42nM (respectively 420 nM) Ase1-mNeonGreen, sometimes forming a region of overlap. **C**Survival probability of antiparallel and parallel overlaps, showing the probability that an overlap formed by two dynamic MT extensions persists at a given time after its formation (Methods). Semitransparent regions indicate 95% lower and upper confidence bounds. **D**Quantification of the density of Ase1-mNeonGreen in (anti)parallel overlaps (Methods), normalized by the median of the Ase1 density on single MTs. Three stars denote p<0.001 compared to any other population, as detected by analysis of variance (ANOVA). “n.s.” denotes no difference between populations (p>0.05). The numbers below the boxes denote the number of analyzed microtubule bundles. Panels show data for MT plus ends (minus ends generally were not analyzed).

**Ase1 differentially regulates MT dynamics**

To investigate how antiparallel overlaps are stabilized by Ase1, we quantified MT dynamics as function of Ase1 density (Figure 2, Figure S2). We found that, at 42 nM Ase1 concentration, polymerization velocities and catastrophe rates were similar for all MTs, either single or bundled (Figure 2B, D). However, antiparallel MTs displayed a marked decrease in depolymerization velocity and a pronounced increase in rescue rate compared to single and parallel MTs (Figure 2A, C). We hypothesized that this observation might be due to the observed increased Ase1 density on antiparallel MTs at 42 nM Ase1 (Figure 1D). Indeed, differences in Ase1 density can partially explain this observation, as at a higher Ase1 concentration (420 nM), we observed reduced polymerization and depolymerization velocities in all configurations: single MTs and overlapping MTs (Figure 2A). However, antiparallel MTs exhibited again reduced depolymerization velocity and increased rescue rate compared to single and parallel MTs (Figure 2A, C) despite the fact that at 420 nM Ase1 concentration, the Ase1 decoration levels of antiparallel and parallel bundles were comparable (Figure 2E, F). This result suggests that density of Ase1 is not the sole factor affecting MT dynamics. Altogether, these results show that Ase1 is able to differently modulate MT dynamics in parallel and antiparallel overlaps, with a high specificity for stabilizing antiparallel MT bundles.

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**Figure 2. Ase1 differentially regulates MT dynamics.**

The depolymerization velocity (**A**), polymerization velocity (**B**), rescue rate (**C**), and catastrophe rate (**D**) of dynamic MT plus ends in different configurations and in the presence of 42 or 420 nM Ase1-mNeonGreen. **E** Depolymerization velocity (see A) versus Ase1-mNeonGreen density (see Figure 1D) in a given region divided by number of MTs (i.e., density is divided by 2 in the case of bundles).  **F** Rescue rate (see C) versus Ase1-mNeonGreen density (see Figure 1D). All plots show results for the same experiments as shown in Figure 1.  Boxplots are weighted by the distance an MT end covered during a sampled period of polymerization or depolymerization. In boxplots, the numbers indicate the number of recorded events, in bar plots, the numbers indicate the number of catastrophes or rescues. Non-overlapping notches of boxes in the boxplots indicate that medians differ between the respective populations, at a 95% confidence level. Further, in bar plots, the height of the bar indicates the catastrophe/rescue rate as determined from all time lapses (number of total events divided by total duration of depolymerization), while the error bars indicate the lowest and highest rates as determined from each individual time lapse (Methods). For p-values for A-D, see Table S1.

**Accumulation of Ase1 at depolymerizing MT ends reduces depolymerization velocities**

To investigate the mechanism of Ase1-dependent MT stabilization, we next examined Ase1 at MT ends. On polymerizing plus ends, Ase1 did not exhibit any specific localization (Figure 1A, B, Figure 3A, Figure S2A). We observed however that Ase1 accumulated at depolymerizing MT ends, proximal of the retracting ends (Figure 3A-C). We found that these Ase1 molecules, swept by the MT end, accumulated within 20 seconds after catastrophe, after which Ase1 densities saturated (Figure 3D, Figure S3A). The accumulation and the resulting elevated levels of Ase1 at the retracting MT end coincided with a slowdown of depolymerization (Figure 3E, Figure S3B). For antiparallel MTs, the local accumulation of Ase1 at the MT end coincided with increased rescue rates (Figure 3F). For single MTs and parallel MTs, the rescue rate was too low to observe this correlation (Figure 2C).

To exclude the possibility that Ase1 could bind to tubulin in solution and change the chemical equilibrium of MT depolymerization, or that it could bind directly to MT ends, we removed both Ase1 and tubulin from solution. As MTs started to depolymerize Ase1 still accumulated at the depolymerizing ends, further indicating that this increase is due to sweeping of Ase1 molecules that were already bound to the MT lattice before catastrophe (Figure S3C, Movie S2). As has recently been observed with a synthetic MT crosslinker (Drechsler *et al.*, 2019), we observed that a depolymerizing MT end would drag other MTs, indicating that substantial forces could be transmitted by this mechanism (Figure 3G). In summary, these experiments indicate that lattice bound Ase1 molecules hinder MT depolymerization, and are swept by the depolymerizing MT ends. The resulting specific accumulation of Ase1 at MT ends can then further lead to their stabilization.

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**Figure 3. Ase1 is swept by depolymerizing MT ends.**

**A**Kymographs of the plus end of depolymerizing single MTs at 6 nM Ase1-GFP in solution (left) and antiparallel MTs at 1 nM Ase1-GFP (right).  The stabilized GMPCPP-MT seeds were labeled with 15% rhodamine or 15% Alexa647, while the free tubulin in solution was labelled with 7% rhodamine. In sketches, dynamic extensions with GDP lattices are colored red, and stabilized GMPCPP seeds are colored blue. **B**Density of Ase1-mNeonGreen at single MT ends during depolymerization at 420 nM, minus the equilibrium density (as determined by measuring the density at the respective spot on the MT before catastrophe). Each colored thin line is the median signal distribution of one depolymerization event (median of all observed frames). The thick red line is the median of all the thin colored lines. Results from same experiments as analyzed in Figures 1, 2, and 3C. **C**Boxplot showing the estimated median number of additional Ase1 molecules at MT ends per depolymerization event, which was derived inferred from data as shown in A. Results from same experiments as analyzed in Figures 1, 2, and 3B.  **D** Number of additional Ase1 molecules at the end of depolymerizing MTs for single MTs (left) and antiparallel MTs (right), divided by the equilibrium density (as determined by measuring the density at the respective spot on the MT/bundle before catastrophe). The first bin in the left panel is statistically different from all the others, the first bin the right panel is different from the bins between 20s and 40s. **E** Instantaneous depolymerization velocity plotted over number of additional Ase1 molecules at the MT. Data points recorded during the first 5 seconds after catastrophe were discarded as they were affected by limits in temporal resolution. The first bin in the right panel is statistically different from all the others except for the last bin. **F**Rescue rate plotted over number of additional Ase1 molecules at the MT end. The duration depolymerized at a respective x-value was added to the respective bin. The number of rescues observed in the same bin (N) was then divided by the sum of depolymerized durations (shown in min, the number in parentheses refers to the number of MTs) to yield the rescue rate. The blue line shows a linear fit to the data (where data points had been weighted by the sums of durations). **G** A time series of micrographs (Ase1-GFP channel) showing an event where the accumulated Ase1 at one depolymerizing MT end (indicated by yellow arrows) causes the end to “drag” a MT it crosses with it, thereby bending it. In the frames where bending occurs, the arrows are colored red. The blue arrows indicate the (depolymerizing) end of the formerly bent MT. Same experiments as shown in S3C.  Results shown in A and D-F are from a different dataset than results shown in Figures 1, 2, and B,C (see Methods).

**Stabilization of terminal tubulin subunits by Ase1 is sufficient to reproduce Ase1 sweeping and reduced MT depolymerization velocity**

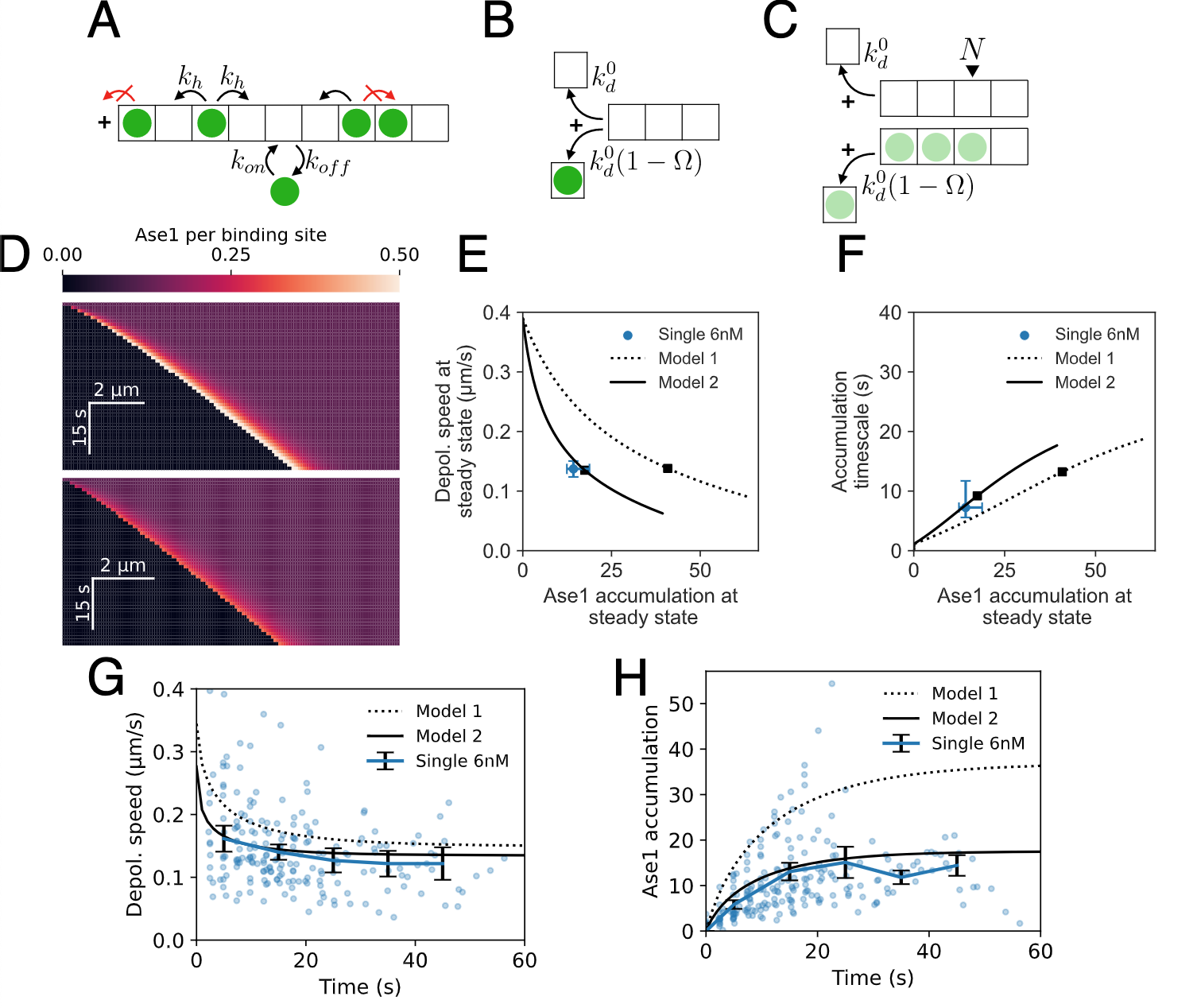
To analyze how Ase1 accumulates at depolymerizing MT ends, and the effects this has on depolymerization velocity, we developed a simple mathematical model that considers a one-dimensional MT made of lattice sites corresponding to tubulin heterodimers, starting at the plus end (see Methods). The model neglects the interaction between protofilaments, and for simplicity we focused on the effect of Ase1 on the depolymerization velocity of single MTs. Events such as Ase1 binding, unbinding, and hopping to neighboring sites are stochastic with constant rates (Figure 4A) that were determined experimentally (Table S1). Importantly, only one Ase1 molecule can be attached to any one tubulin heterodimer, and Ase1 can thus only hop to unoccupied neighboring sites. We also assume that Ase1 does not fall off from the MT by hopping at its plus ends, as shown experimentally (Braun *et al.*, 2011). MT depolymerization is modelled stochastically by detachment of the terminal subunit, at a rate that is affected by Ase1 (Figure 4B). Specifically, this rate is when the first tubulin subunit is free of Ase1, and , if Ase1 is bound at the terminal site. The value of is set by the MT depolymerization velocity we experimentally measured without Ase1 (Table S1). The parameter specifies the effect of Ase1 on depolymerization. If , Ase1 has no effect, while if , the terminal subunit cannot unbind if Ase1 is bound. For any value , this simple model leads to an accumulation of Ase1 near the depolymerizing end and a concomitant decrease of the depolymerization velocity (Figure 4D top, S4A). The accumulation occurs because subunits without Ase1 are more likely to be lost at the plus-end, so depolymerization increases the density of Ase1 at the depolymerizing end. At steady state, the system can be characterized by the probability of the terminal site to be occupied, and the rate of subunits loss is .

Since all parameters of this model (Model 1) were set from experimental measurements, except for , we first tested whether any value of could quantitatively recapitulate the experimental behavior of single MTs with 6nM of Ase1. Specifically, we aimed to reproduce the timescale of accumulation of Ase1, and the total amount of Ase1 accumulated and depolymerizing velocity reached at steady state (Table S2). For , the model predicted depolymerization velocities that are comparable to the experimentally observed ones (Figure 4E), but when comparing the timescale and number of Ase1 molecules accumulated at steady state we found that they were respectively 2× and 4× higher than experimentally observed (Figure 4F). Therefore, despite recapitulating the experimental phenomenology qualitatively (Figure 4D, top), this first model was insufficient to quantitatively reproduce our experimental results.

The failure of Model 1 indicated that Ase1 should affect MT depolymerization at lower density. We thus hypothesized that Ase1 molecules located at lattice sites other than the terminal one could affect depolymerization. We initially tested the possibility that the rate of tubulin subunits loss at the plus end would be reduced by a factor if any of the terminal sites were occupied (Figure 4C). This rate at steady state would then be , where is the probability of site being occupied by Ase1. is not experimentally constrained, but the range of possible values is small, since it is unlikely that distant tubulin subunits could affect the detachment of the terminal subunit. For and this model (Model 2) reproduced MT polymerization velocity and Ase1 accumulation at steady state and Ase1 accumulation timescale (Figure 4E-F), and recapitulated the dynamics of the system (Figure 4G, H). The model predicts that at steady state Ase1 density should decay exponentially from the plus end with a length scale of ~600nm (Figure S4B). Experimentally, we observed a decay of Ase1 signal from the plus end with length scale of ~200 nm (Figure S4D). However, this signal is hardly comparable with our single-protofilament model, not only because it comes from multiple protofilaments that may not be in register, but also because shrinking protofilaments are likely curved outwards (Ref?).

Using Model 2 with the same value of , we could recapitulate the Ase1 accumulation timescale and steady state accumulation in single microtubules with 1nM of Ase1 (Figure S4D), but the model predicted a ~15% decrease with respect to the maximum velocity while the experimentally observed decrease was between 25% and 60% (Figure S4C, Table S2). The reason for this disagreement is likely the low density of Ase1 molecules at 1nM concentration (<1% of tubulin dimers bound to Ase1 in the body of single MTs vs. ~12% at 6nM of Ase1). At this very low density, the system may not be well captured by our mean field approach (see Methods). The model also fails to reproduce the behavior of antiparallel overlaps (Figure S4E-F). This is not surprising given that overlaps are not symmetric, and some protofilaments have almost no Ase1, while others have extremely high Ase1 density (76% tubulin dimers bound to Ase1 in the MT body assuming 2 protofilaments crosslinked, 50% assuming 3, Figure XYZ, see “Mathematical modelling” in Methods). A more complex model accounting for protofilament interactions would be needed for overlaps, but it would need to be informed by experimental measurements of such interactions.

In summary, this simple model recapitulated quantitatively the behaviour of the system for 6nM of Ase1, and within an order of magnitude for 1nM of Ase1. Importantly, the model offers non-obvious insights about the effect of Ase1 on shrinking microtubules, as it shows that Ase1 preventing depolymerization at plus ends (Fig. 4B) -a necessary assumption- drives Ase1 sweeping at shrinking microtubule ends without further assumptions. Sweeping occurs because subunits without Ase1 are more likely to be lost at the plus-end, so depolymerization increases the density of Ase1 at the depolymerizing end.

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**Figure 4: Stabilization of terminal tubulin subunits by Ase1 is sufficient to reproduce Ase1 sweeping and reduced microtubule shrinkage velocity**

**A-C** cartoons representing model assumptions (see Methods): **A** Ase1 binding, unbind, and hopping to neighboring sites are stochastic events with constant rates **.** Only one Ase1 molecule can be attached to any one tubulin heterodimer, so moving or binding to an occupied binding site is not allowed (red crossed arrow on the right). Ase1 does not fall off from the microtubule by hopping at its plus ends (red crossed arrow on the left). **B** In Model 1, we assume that the detachment rate of the tubulin terminal subunit is when the first tubulin subunit is free of Ase1, and if Ase1 is bound at the terminal site. **C** In Model 2, we assume that the detachment rate of the tubulin terminal subunit is when all the first tubulin subunit are free of Ase1, and if any of those sites is occupied. **D** Distribution of Ase1 density in time in Model 1 with , represented as a simulated kymograph (see scalebar). **E** Shrinkage velocity in time as predicted by Model 1, 2 and 3, using the parameters indicated in the main text, which best fitted the experimental observations (Model 1: , Model 2: and , Model 3: and ). **F** Values of shrinkage velocity and Ase1 accumulation at steady state measured from experiments (blue dot) or predicted by Model 1, 2 and 3 (lines). The lines represent all the solutions of each model from to . Black squares mark the value of represented in (E). **G** Same as (F), with accumulation timescale in the x axis.

**Discussion**

In this work, we investigated the effects of the diffusible MT crosslinker Ase1 on MT depolymerization, both when crosslinking MTs and on single MTs. Our results show that Ase1 decreases MT depolymerizing velocities, and, by selectively promoting the rescue rates of MTs in antiparallel overlaps, selectively stabilizes antiparallel overlaps, while not substantially affecting the dynamics of single MTs or MTs in parallel overlaps. In bipolar MT arrays, like mitotic spindles, this activity of Ase1 can enable selective stabilization of the array’s central regions, while keeping the rest of the array dynamic and pliable. An earlier study on a plant Ase1 analogue, MAP65-1, found increased rescue rates of MTs within bundles compared to single MTs (Stoppin-Mellet *et al.*, 2013). This study, however, did not experimentally distinguish between parallel and antiparallel bundles. Our methods allowed us to directly distinguish between different bundling orientations, where we did not observe parallel MTs to display more rescues than single MTs.

We imaged Ase1 sweeping, the accumulation of lattice-bound, diffusible Ase1 at the retracting ends of depolymerizing MTs, and found that accumulated Ase1 can transduce forces to other MTs. This accumulation, termed ‘protein sweeping’ or 'herding', is analogous to the *in vitro* behavior of microtubule-severing enzyme spastin and the kinetochore associated Ndc80 and Dam1 complexes (Lombillo, Stewart and Richard McIntosh, 1995; Franck *et al.*, 2007; Grishchuk *et al.*, 2008; Powers *et al.*, 2009; Umbreit *et al.*, 2012; Grishchuk, 2017; Al-Hiyasat *et al.*, 2021). Like Ase1, Dam1- and Ndc80- complexes accumulate at depolymerizing MT ends and decrease depolymerization velocity. We produced a simple model based on the assumption that Ase1 prevents the detachment of terminal tubulin subunits (Figure 4A, B). This assumption alone leads to a decrease in MT depolymerization velocity and accumulation of Ase1 at the tip of shrinking microtubules (Figure 4D, E). Assuming Ase1 to stabilize multiple tubulin dimers, we were able to quantitatively reproduce the experimental results (Figure 4F, G). Stabilization of adjacent protofilaments might be explained by Ase1 binding to a tubulin dimer allosterically stabilizing the adjacent protofilament either by Ase1's intrinsically disordered N-terminus directly binding to the adjacent protofilament (Subramanian *et al.*, 2010) or indirectly through the tubulin lattice, such as kinesin-1 (Morikawa *et al.*, 2015; Peet, Burroughs and Cross, 2018).

We observed that the decrease in depolymerization velocity was stronger in antiparallel overlaps compared to parallel overlaps and single MTs (Figure 2A, Figure S2C). In addition, MT rescue rate was increased specifically in antiparallel overlaps (Figure 2C, Figure S2E). This might be due to the fact that Ase1 diffusion and unbinding rates are lower in microtubule overlaps (Kapitein *et al.*, 2008; Lansky *et al.*, 2015), due to protein avidity resulting from the multivalent interactions of Ase1 with the MTs (Braun, Diez and Lansky, 2020; Erlendsson and Teilum, 2021). Our model predicts that a molecule with lower diffusion rate is more likely to remain close to the shrinking end during depolymerization, preventing the unbinding of subunits. This stabilizing effect will be present for longer time for lower unbinding rates. Additionally, in overlaps, individual protofilaments of a depolymerizing MT are coupled to the other MT by Ase1. In order to bend during depolymerization, protofilaments likely have to work against the lattice of the other MT, which might lead to further stabilization. Finally, Ase1 is known to display ‘multimerization’ within antiparallel MT overlaps, potentially driven by cooperative MT binding behavior (Kapitein *et al.*, 2008). A combination of these factors could explain the stronger stabilizing effect of Ase1 on antiparallel overlaps. In respect to Ase1 multimerization, it is interesting to note that a recent study found PRC1 to slow down motor-driven MT sliding more strongly when PRC1 appeared to multimerize at the edges of MT overlaps (Alfieri, Gaska and Forth, 2021) (it appears unclear how this multimerization might be related to the Ase1 multimerization observed by Kapitein *et al.*, 2008). Though the cases of MT depolymerization and MT sliding are different in that MT depolymerization does not involve MT movement (and thus no movement of Ase1 binding sites), ‘herding-driven Ase1 multimerization’ appears plausible. The possibility of Ase1 molecules acting cooperatively to promote rescues for antiparallel MTs specifically is intriguing and would offer the cell a lever for modulating the rescue-promoting effect of MT crosslinking.

Our modelling further suggests that the ability of Ase1 to both diffuse and reduce tubulin subunit detachment at depolymerizing plus ends confers interesting biological properties. Not only can Ase1 reduce the depolymerization velocity of MTs, but this makes Ase1 capable of tracking depolymerizing ends, since subunits without Ase1 are more likely to be lost. Our findings thus suggest that any diffusing molecule that prevents tubulin unbinding will track depolymerizing ends, and therefore may exert forces on objects that the molecule has affinity for on accessible regions as the MT shrinks. Conversely, these forces will drag the molecule in the opposite direction of MT depolymerization, making it more likely to be at the terminal subunits, amplifying its braking effect on depolymerization velocity. It may be interesting to measure the forces which Ase1 sweeping is capable of transducing, which would also shed light on the question on whether protofilament powerstrokes are an important component of Ase1 sweeping. Interestingly, starPEG-(KA7)4, a synthetic MT crosslinker with multivalent MT-binding interfaces has recently been shown to also drag MTs when being swept by depolymerizing MTs (Drechsler *et al.*, 2019), even though it did not hinder MT depolymerization of isolated MTs. It would be interesting to scrutinize the dynamics of bundles crosslinked by starPEG-(KA7)4 in future studies, as well as the depolymerization speed of “pulling” MTs. It may also be interesting to investigate the interplay of stabilization by Ase1 with other factors which influence MT dynamics. For instance, does Ase1 stabilization introduce nonlinearities to the linear relationship between rescue frequency and tubulin concentration (Walker *et al.*, 1988)? If that were the case, then the spindle XYZ (just brainstorming here: If the free tubulin concentration were to decrease during spindle formation (is this the case?), then it would be interesting for Ase1 to disproportionately increase rescue frequency at low free tubulin concentrations – i.e., the spindle would be fortified even though free tubulin concentration is low). UPDATE: Actually, this is super boring, since the tubulin concentration in fission yeast seems rather constant during the cell cycle, see table 1 of https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6468777/

Our results show that the presence of diffusible MT crosslinkers can suffice to establish enduring antiparallel MT overlaps, such as those found in the midzone of mitotic spindles. In such context Ase1 can work cooperatively with other MT rescue factors such as CLASP (Bratman and Chang, 2007) or provide an alternative mechanism for selective life time enhancement of antiparallel overlaps. We speculate that the impact of diffusible crosslinkers on MT dynamics may be tunable by posttranslational modifications of either the crosslinkers or the MT surface. Such a tunability has recently been proposed for a seemingly related capacity of Ase1, namely the braking of MT sliding caused by molecular motors (Fu *et al.*, 2009; Thomas, Ismael and Moore, 2020). For actin filament overlaps, it has been observed that F-actin crosslinkers slow down actin depolymerization (Maul *et al.*, 2003; Schmoller, Semmrich and Bausch, 2011), suggesting that crosslinker dependent stabilization of filaments may be a fundamental mechanism in cytoskeletal systems.

# **References**

Al-Hiyasat, A. *et al.* (2021) ‘Herding of proteins by the ends of shrinking polymers’. doi: https://doi.org/10.48550/arXiv.2112.07757.

Alfieri, A., Gaska, I. and Forth, S. (2021) ‘Two modes of PRC1-mediated mechanical resistance to kinesin-driven microtubule network disruption’, *Current Biology*, 31(12), pp. 2495-2506.e4. doi: 10.1016/j.cub.2021.03.034.

Bieling, P., Telley, I. a. and Surrey, T. (2010) ‘A minimal midzone protein module controls formation and length of antiparallel microtubule overlaps’, *Cell*, 142(3), pp. 420–432. doi: 10.1016/j.cell.2010.06.033.

Bratman, S. V. and Chang, F. (2007) ‘Stabilization of Overlapping Microtubules by Fission Yeast CLASP’, *Developmental Cell*, 13(6), pp. 812–827. doi: 10.1016/j.devcel.2007.10.015.

Braun, M. *et al.* (2011) ‘Adaptive braking by Ase1 prevents overlapping microtubules from sliding completely apart’, *Nature Cell Biology*, 13(10), pp. 1259–1264. doi: 10.1038/ncb2323.

Braun, M., Diez, S. and Lansky, Z. (2020) ‘Cytoskeletal organization through multivalent interactions’, *Journal of Cell Science*, 133(12), pp. 1–8. doi: 10.1242/jcs.234393.

Castro, E. R. *et al.* (2018) ‘Determination of dynamic contact angles within microfluidic devices’, *Microfluidics and Nanofluidics*, 22(5), p. 51. doi: 10.1007/s10404-018-2066-0.

Coimbatore Balram, K. *et al.* (2016) ‘The Nanolithography Toolbox’, *Journal of Research of the National Institute of Standards and Technology*, 121, p. 464. doi: 10.6028/jres.121.024.

Drechsler, H. *et al.* (2019) ‘Multivalent electrostatic microtubule interactions of synthetic peptides are sufficient to mimic advanced MAP-like behavior’, *Molecular Biology of the Cell*. Edited by T. Surrey, 30(24), pp. 2953–2968. doi: 10.1091/mbc.E19-05-0247.

Erlendsson, S. and Teilum, K. (2021) ‘Binding Revisited—Avidity in Cellular Function and Signaling’, *Frontiers in Molecular Biosciences*, 7. doi: 10.3389/fmolb.2020.615565.

Franck, A. D. *et al.* (2007) ‘Tension applied through the Dam1 complex promotes microtubule elongation providing a direct mechanism for length control in mitosis’, *Nature Cell Biology*, 9(7), pp. 832–837. doi: 10.1038/ncb1609.

Fu, C. *et al.* (2009) ‘Phospho-Regulated Interaction between Kinesin-6 Klp9p and Microtubule Bundler Ase1p Promotes Spindle Elongation’, *Developmental Cell*, 17(2), pp. 257–267. doi: 10.1016/j.devcel.2009.06.012.

Gaillard, J. *et al.* (2008) ‘Two Microtubule-associated Proteins of Arabidopsis MAP65s Promote Antiparallel Microtubule Bundling’, *Molecular Biology of the Cell*, 19(10), pp. 4534–4544. doi: 10.1091/mbc.e08-04-0341.

Grishchuk, E. L. *et al.* (2008) ‘The Dam1 ring binds microtubules strongly enough to be a processive as well as energy-efficient coupler for chromosome motion’, *Proceedings of the National Academy of Sciences*, 105(40), pp. 15423–15428. doi: 10.1073/pnas.0807859105.

Grishchuk, E. L. (2017) *Biophysics of Microtubule End Coupling at the Kinetochore*, *Progress in molecular and subcellular biology*. doi: 10.1007/978-3-319-58592-5\_17.

Janson, M. E. *et al.* (2007) ‘Crosslinkers and Motors Organize Dynamic Microtubules to Form Stable Bipolar Arrays in Fission Yeast’, *Cell*, 128(2), pp. 357–368. doi: 10.1016/j.cell.2006.12.030.

Kapitein, L. C. *et al.* (2008) ‘Microtubule-Driven Multimerization Recruits ase1p onto Overlapping Microtubules’, *Current Biology*, 18(21), pp. 1713–1717. doi: 10.1016/j.cub.2008.09.046.

Kellogg, E. H. *et al.* (2016) ‘Near-atomic cryo-EM structure of PRC1 bound to the microtubule’, *Proceedings of the National Academy of Sciences of the United States of America*, 113(34), pp. 9430–9439. doi: 10.1073/pnas.1609903113.

Kirschner, M. and Mitchison, T. (1986) ‘Beyond self-assembly: From microtubules to morphogenesis’, *Cell*, 45(3), pp. 329–342. doi: 10.1016/0092-8674(86)90318-1.

Kitazawa, D. *et al.* (2014) ‘Orbit/CLASP is required for myosin accumulation at the cleavage furrow in Drosophila male meiosis’, *PLoS ONE*, 9(5). doi: 10.1371/journal.pone.0093669.

Lansky, Z. *et al.* (2015) ‘Diffusible Crosslinkers Generate Directed Forces in Microtubule Networks’, *Cell*, 160(6), pp. 1–10. doi: 10.1016/j.cell.2015.01.051.

Liu, J. *et al.* (2009) ‘PRC1 cooperates with CLASP1 to organize central spindle plasticity in mitosis’, *Journal of Biological Chemistry*, 284(34), pp. 23059–23071. doi: 10.1074/jbc.M109.009670.

Löiodice, I. (2005) ‘Ase1p Organizes Antiparallel Microtubule Arrays during Interphase and Mitosis in Fission Yeast’, *Molecular biology of the cell*, 16(1), pp. 1756–1768. doi: 10.1091/mbc.E04–10–0899.

Lombillo, V. A., Stewart, R. J. and Richard McIntosh, J. (1995) ‘Minus-end-directed motion of kinesin–coated microspheres driven by microtubule depolymerization’, *Nature*, 373(6510), pp. 161–164. doi: 10.1038/373161a0.

Mani, N., Wijeratne, S. S. and Subramanian, R. (2021) ‘Micron-scale geometrical features of microtubules as regulators of microtubule organization’, *eLife*, 10, pp. 1–26. doi: 10.7554/eLife.63880.

Mastronarde, D. N. *et al.* (1993) ‘Interpolar spindle microtubules in PTK cells’, *Journal of Cell Biology*, 123(6 I), pp. 1475–1489. doi: 10.1083/jcb.123.6.1475.

Maul, R. S. *et al.* (2003) ‘EPLIN regulates actin dynamics by cross-linking and stabilizing filaments’, *Journal of Cell Biology*, 160(3), pp. 399–407. doi: 10.1083/jcb.200212057.

Morikawa, M. *et al.* (2015) ‘X-ray and Cryo-EM structures reveal mutual conformational changes of Kinesin and GTP-state microtubules upon binding’, *The EMBO Journal*, 34(9), pp. 1270–1286. doi: https://doi.org/10.15252/embj.201490588.

Nédélec, F., Surrey, T. and Karsenti, E. (2003) ‘Self-organisation and forces in the microtubule cytoskeleton’, *Current Opinion in Cell Biology*, 15(1), pp. 118–124. doi: https://doi.org/10.1016/S0955-0674(02)00014-5.

Peet, D. R., Burroughs, N. J. and Cross, R. A. (2018) ‘Kinesin expands and stabilizes the GDP-microtubule lattice’, *Nature Nanotechnology*, 13(5), pp. 386–391. doi: 10.1038/s41565-018-0084-4.

Powers, A. F. *et al.* (2009) ‘The Ndc80 Kinetochore Complex Forms Load-Bearing Attachments to Dynamic Microtubule Tips via Biased Diffusion’, *Cell*, 136(5), pp. 865–875. doi: 10.1016/j.cell.2008.12.045.

Ruhnow, F., Zwicker, D. and Diez, S. (2011) ‘Tracking Single Particles and Elongated Filaments with Nanometer Precision’, *Biophysical Journal*, 100(11), pp. 2820–2828. doi: https://doi.org/10.1016/j.bpj.2011.04.023.

Schindelin, J. *et al.* (2012) ‘Fiji: an open-source platform for biological-image analysis’, *Nature Methods*, 9(7), pp. 676–682. doi: 10.1038/nmeth.2019.

Schmoller, K. M., Semmrich, C. and Bausch, A. R. (2011) ‘Slow down of actin depolymerization by cross-linking molecules’, *Journal of Structural Biology*, 173(2), pp. 350–357. doi: 10.1016/j.jsb.2010.09.003.

She, Z. Y. *et al.* (2019) ‘Mechanisms of the Ase1/PRC1/MAP65 family in central spindle assembly’, *Biological Reviews*, 94(6), pp. 2033–2048. doi: 10.1111/brv.12547.

Song, Y. H. and Mandelkow, E. (1995) ‘The anatomy of flagellar microtubules: polarity, seam, junctions, and lattice.’, *Journal of Cell Biology*, 128(1), pp. 81–94. doi: 10.1083/jcb.128.1.81.

Stoppin-Mellet, V. *et al.* (2013) ‘MAP65 Coordinate Microtubule Growth during Bundle Formation’, *PLoS ONE*, 8(2). doi: 10.1371/journal.pone.0056808.

Subramanian, R. *et al.* (2010) ‘Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein’, *Cell*, 142(3), pp. 433–443. doi: 10.1016/j.cell.2010.07.012.

Thomas, E. C., Ismael, A. and Moore, J. K. (2020) ‘Ase1 domains dynamically slow anaphase spindle elongation and recruit Bim1 to the midzone’, *Molecular Biology of the Cell*. Edited by K. Bloom, 31(24), pp. 2733–2747. doi: 10.1091/mbc.E20-07-0493-T.

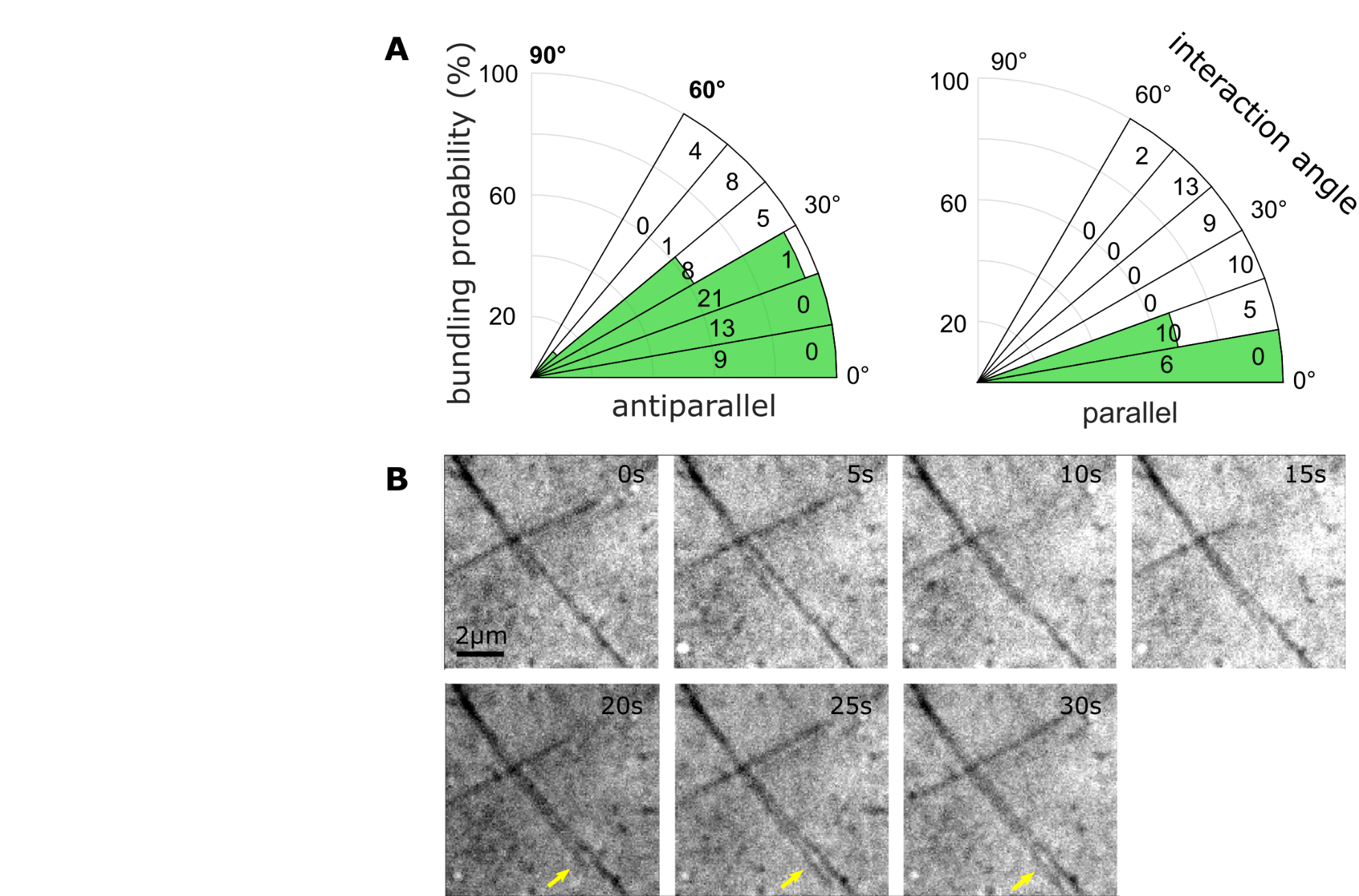
Umbreit, N. T. *et al.* (2012) ‘The Ndc80 kinetochore complex directly modulates microtubule dynamics’, *Proceedings of the National Academy of Sciences*, 109(40), pp. 16113–16118. doi: 10.1073/pnas.1209615109.

Walker, R. a. *et al.* (1988) ‘Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies.’, *Journal of Cell Biology*, 107(4), pp. 1437–1448. doi: 10.1083/jcb.107.4.1437.

Yamashita, A. (2005) ‘The Roles of Fission Yeast Ase1 in Mitotic Cell Division, Meiotic Nuclear Oscillation, and Cytokinesis Checkpoint Signaling’, *Molecular Biology of the Cell*, 16(3), pp. 1378–1395. doi: 10.1091/mbc.E04-10-0859.

Zhang, H. *et al.* (2020) ‘<scp>nanolithography toolbox</scp> —Simplifying the design complexity of microfluidic chips’, *Journal of Vacuum Science & Technology B*, 38(6), p. 063002. doi: 10.1116/6.0000562.

# **Supplementary figures**



**Figure S1. A** Bundling probability for situations when MT plus ends encountered other MTs, in either parallel or antiparallel orientation, versus the initial angle of interaction (results pooled for all Ase1-mNeonGreen concentrations). The outer numbers denote the numbers of recorded crossings at the respective angle, while the inner numbers denote the numbers of bundling events. **B** A MT polymerizing close to another, parallel MT at 0nM Ase1 (imaged with Interference Reflection Microscopy). As can be seen, at our buffer conditions, there is no significant MT bundling if Ase1 is absent even in the case of very shallow angles.

Diagram

Description automatically generated

**Figure S2. A** Full kymographs of the MTs shown in Figure 3A. **B** Equilibrium density of Ase1-GFP on MTs and within overlaps for the set of experiments which is evaluated in Figure 3A, D-F. Isolated MTs at 6nM and antiparallel MTs at 1nM are statistically different from all other populations (p<0.001), and parallel MTs are in addition different from isolated MTs at 0nM (p<0.05). There are no other statistically significant differences. We did not observe parallel overlaps at 1pM Ase1-GFP, and did not generate overlaps at 6nM since the motivation here was to assess the dynamics of isolated MTs with high Ase1 densities. **C-F** The depolymerization velocity (**C**), polymerization velocity (**D**), rescue rate (**E**), and catastrophe rate (**F**) of the dynamic MT plus ends for set B experiments (see Methods). The rescue rate of antiparallel MTs is different from all other populations (p<0.005), while there are no statistically significant differences between catastrophe rates. The Ase1 density on antiparallel MTs at 1 pM was less than 0.001 molecules per nm (a minimum amount was required for forming overlaps). The numbers in the boxes in B represent the number of MTs, the numbers in C+D represent the number of depolymerization respectively polymerization periods analyzed, the numbers in E+F represent the total time in minutes spent depolymerizing respectively polymerizing which had been analyzed. Boxplots C+D are weighted by the distance a MT end covered during a sampled period of polymerization or depolymerization. Further, in bar plots, the height of the bar indicates the catastrophe/rescue rate as determined from all time lapses (number of total events divided by total duration of depolymerization), while the error bars indicate the lowest and highest rates as determined from each individual time lapse.

**Graphical user interface, chart

Description automatically generated**

**Figure S3. A** The number of additional Ase1 molecules at the end of depolymerizing MTs for single MTs (left) respectively antiparallel MTs (right), plotted over the time passed since the catastrophe, for the set of experiments as shown in Figure 3A, D-F. Each data point represents data extracted from one line scan, the data points recorded during each event are connected by lines and share the same color. Analysis as performed for Figure 3D. **B** The frame-to-frame depolymerization velocity of MTs over time, for experiments as shown in Figure 3A, D-F (analogous to A). Because the exact time of catastrophe is unknown due to limits in temporal resolution, the velocity measurement right after catastrophe underestimates the actual velocity. **C** Kymograph (Ase1- mNeonGreen channel) of depolymerizing MT. In this experiment, Ase1 and tubulin had been removed from the assay buffer during the time frame indicated by the pink bar next to the kymograph, prompting subsequent MT depolymerization and concomitant Ase1 accumulation at the end. Same assay buffer as in Figure 3A,D-F, data from same experiments as shown in Figure 3G.

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**Figure S4. A-C** Top: Distribution of Ase1 density in time for each model represented as a simulated kymograph (see scalebars), with parameters as in Figure 4E. Bottom: Distribution of Ase1 density in time for the same data, represented as Ase1 density on terminal sites for the indicated timepoints (see legend). **D** Fitted values for the length scale of the decay of Ase1-GFP signal at the MT tip toward the MT body (exp(-x/length scale), see Methods) over time after catastrophe, for single MTs. Representation analogous to Figure 3D. **E** RecordedFRAP recovery times on single MTs (see Methods) – the median value was used as koff for modeling. **F** Mean square displacement of single Ase1 molecules diffusing on single MTs during the first second (see Methods) and fitted line – the slope of the fitted line was used for modeling (number of molecules = 2008).

# **Supplementary tables**



|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Value(s) | | Source |
| MT depolymerization velocity at 0 Ase1 | 390 nm/s | | Figure S2C (leftmost box) |
| Ase1 off-rate (koff) | 0.016 s-1 | | Figure S4E |
| Ase1 diffusion coefficient, single MTs | 0.09 µm2/s | | Figure S4F |
| Ase1 diffusion coefficient, antiparallel MTs | 0.011 µm2/s | | 8 times lower than single MTs (Lansky et al. 2015) |
| Ase1 on-rate (kon) | Single 1nM | 0.00012 s-1 | Calculated from experimentally measured koff and equilibrium density of Ase1 in the body of the MT (Fig. S2B) |
| Single 6nM | 0.00224 s-1 |
| Antiparallel (3 protofilaments) | 0.01658 s-1 |
| Tubulin dimer/binding site length | 8nm | | (Song and Mandelkow, 1995) |

**Table S1.** Model parameters that are experimentally constrained.

|  |  |  |  |
| --- | --- | --- | --- |
| Measurement | Value(s) and bootstrap confidence interval | | Source |
| Timescale of accumulation | Single 1nM | 4.5 s [1.9 s, 9.4 s] | Fit of Figure S3A to |
| Single 6nM | 7.2 s [5.6 s, 11.7 s] |
| Antiparallel | 5.4s [3.5 s, 8.8 s] |
| Ase1 molecules accumulated at steady state, per MT | Single 1nM | 22.2 [16.6, 30.2] | Fit of Figure S3A to |
| Single 6nM | 185.5 [161.5, 244.4] |
| Antiparallel | 56.2 [49.0, 64.0] |
| Shrinkage velocity at steady state | Single 1nM | 227 nm/s [155 nms/s, 292 nm/s] | Average value after 20 seconds of shrinkage |
| Single 6nM | 137 nm/s [123 nm/s, 150 nm/s] |
| Antiparallel | 117 nm/s [108 nm/s, 125 nm/s] |

**Table S2.** Experimental measurements that are compared with model predictions.

# **Supplementary movie captions**

Caption for Movie S1:

Movie showing MT dynamics under the presence of 42nM Ase1-mNeonGreen and 30µM free tubulin (Ase1-mNeonGreen channel). The white arrow shows the position of a parallel MT bundle. Same type of experiment as shown in Figure 1."

Caption for Movie S2:

Movie showing accumulation of Ase1 at the tips of depolymerizing MTs (Ase1- mNeonGreen channel). Both Ase1 and tubulin had been removed from the buffer (during the shaky part of the movie). The field of view is 30 times 30 µm, and the time lapse lasts for 100 seconds. Same experiment as shown in Figure S3C."

# **Methods**

Protein purification. Ase1-GFP was expressed and purified as described previously (Janson et al. 2007). Ase1-mNeonGreen was expressed in e. coli cells. After harvesting the cells, the cell pellet was resuspended in 5 mL ice-cold phosphate buffered saline (PBS) and stored at - 80 °C for further use. For cell lysis, the cells were homogenized in 30 mL ice-cold His-Trap buffer (50 mM Na-phosphate buffer, pH 7.5, 5% glycerol, 300 mM KCl, 1 mM MgCl2, 0.1% tween-20, 10 mM BME, 0.1 mM ATP) supplemented with 30 mM imidazole, Protease Inhibitor Cocktail (cOmplete, EDTA free, Roche) and benzonase to the final concentration of 25 units/mL, and centrifuged at 45000 x g for 60 min at 4 °C in the Avanti J-26S ultracentrifuge (JA-30.50Ti rotor, Beckman Coulter). The cleared cell lysate was incubated in a lysis buffer-equilibrated Ni-NTA column (HisPur Ni-NTA Superflow Agarose, Thermo Scientific) for 2 h at 4 °C. The Ni-NTA column was sequentially washed with a wash buffer I (His-Trap buffer supplemented with 60 mM imidazole), a wash buffer II (His-Trap buffer supplemented with 60 mM imidazole and 700 mM NaCl). The beads were re-dissolved with the wash buffer I. The purification tags were cleaved overnight with 3C PreScisson protease. The protein was concentrated using an Amicon ultracentrifuge filter and flash frozen in liquid nitrogen.

In vitro Ase1-MT binding assay. Flow chambers were prepared as described previously (Braun *et al.*, 2011) or fabricated on silicon-on-insulator substrate with a diameter of ≈100 mm and nominal value of the top silicon layer thickness of 50 µm based on a design prepared in Nanolithography toolbox software (Coimbatore Balram *et al.*, 2016; Zhang *et al.*, 2020). Two lithography steps were performed, one defining the flow chamber, the second one for through holes. We etched the top silicon and stopped it at the buried SiO2 layer with no Si residue there, followed by anodic bonding of the silicon wafer with fabricated chambers and through holes to the corning glass type Corning 7740 with nominal thickness of 170 µm, subsequent dicing by a diamond blade dicing saw into individual chips and coating with FAS-17 fluorosilane using method described earlier (Castro *et al.*, 2018). MTs were prepared as described previously (Braun *et al.*, 2011). Biotinylated, GMPCPP-stabilized, fluorescence-labeled MTs in BRB80 (80 mM Pipes/KOH pH 6.9, 1 mM MgCl2, 1 mM EGTA) were immobilized in a flow chamber using biotin antibodies (Sigma B3640, 20 µg ml−1 in PBS). Subsequently, the buffer in the flow cell was exchanged for assay buffer (see below). Then, Ase1 in assay buffer was flushed into the flow cell at the final assay concentration stated in the main text, together with tubulin. Set A experiments (Figures 1, 2, 3B, C, S1) were performed at room temperature and with 30µM unlabeled tubulin present in solution. Set B experiments (Figures 3A, D-G, S2, S3A, B, S4D) were performed at 29°C and with 14µM tubulin, 7% of which was labeled with rhodamine. In the case of set B experiments, non-biotinylated GMPCPP-stabilized MTs were flushed into the flow cell and bound to the template MTs that were sparsely covered sparsely with Ase1 (these steps were performed before the assay buffer had been flushed in). To prevent evaporation in order to avoid changes in component concentrations, the channels were sealed after flushing in the assay buffer.

Assay buffer. The following buffer components common to all used buffers: 20mM PIPES pH 6.9, 10mM HEPES pH 7.2, 0.5mM EGTA, 1mM MgCl2, 0.5mM Mg-ATP, 0.67mM GTP, 0.67% Tween20, 6.7mM DTT, 0.3 mg/ml Casein, 13.5mM D-Glucose, 0.3mg/ml glucose oxidase and 0.03mg/ml catalase. The buffer for set A experiments, in addition to these components, contained 70mM KCl, and 0.1% methylcellulose, 0.1% glycerol, 1mM sodium phosphate and 1µM ATP. The buffer for set B experiments, in addition to the components common to all buffers, contained 116mM KCl and 0.065% methylcellulose.

Imaging. Labeled proteins were visualized sequentially by switching between the corresponding channels (Chroma filter-cubes) using Nikon-Ti E microscope equipped with 100x Nikon TIRF objective in combination with a Hamamatsu Orca Flash 4.0 sCMOS camera (set A experiments) or 63× Zeiss oil immersion TIRF objective in combination with a Andor Ixon DV 897 (Andor Technology) EMCCD camera (set B experiments). The acquisition rate was 5 seconds for set A experiments and 2.5 seconds for set B experiments, the exposure time was 100ms for both cases. For set B experiments, the Alexa647-labeled MT seeds were imaged before the start of the time lapse, and only the Ase1-mNeonGreen channel was imaged during the time lapse. For set B experiments, the rhodamine (tubulin) and the GFP (Ase1) channel where imaged sequentially, whereas every 40th frame the Alexa647 channel was imaged in place of the GFP channel, in order to track the location of the GMPCPP-stabilized seeds (which we with this data determined to not move significantly during experiment time).

Image analysis.Data was analyzed using FIJI 1.52 (Schindelin *et al.*, 2012) and custom-written Matlab (Mathworks) routines.

*Overlap lifetime estimation.* The lifetime of regions of MT overlap was estimated for two different configurations: Antiparallel overlaps, where two dynamic extensions met and formed a dynamic “midzone”, and parallel bundles of two dynamic extensions (as shown in Figure 1). For both antiparallel and parallel overlaps, lifetime was taken to start upon the bundling of the dynamic (GDP) lattices of each involved MT (for antiparallel configurations, we additionally required both plus ends to be within 3 µm to each other upon start of the event). Lifetime was taken to end upon one of the involved MTs to shrink back to its GMPCPP-stabilized region for parallel bundles, and upon the midzone ceasing to exist for antiparallel bundles. The plot is a Kaplan-Meier plot, which adequately accounts for cases where an overlapping region survived until the end of the recorded time-lapse movie (i.e., cases which were right-censored) (Figure 1C was generated by using the Matlab function ecdf with setting “survival” and alpha = 0.05).

*Parameters of MT dynamics,* for set A experiments, have been estimated by generating kymographs and approximating the location of MT plus ends over time and space with straight lines (the Ase1-mNeonGreen signal was used to visually track MT ends, as MT were not imaged directly). For set B experiments, we used FIESTA to determine the locations of MTs (Ruhnow, Zwicker and Diez, 2011). Both methods yielded polymerization and depolymerization velocities. Rescues were identified as events where a MT switches from depolymerization to polymerization before reaching the GMPCPP-stabilized seed, and catastrophes were events where polymerization was followed by depolymerization. Rescue and catastrophe rates were estimated by dividing the number of rescues respectively catastrophes by the sum of the total distance depolymerized respectively polymerized by all plus ends.

*Single fluorophore quantification.* Fluorescent signal of a single Ase1-mNeonGreen dimer was determined by generating intensity time-traces of Ase1-mNeonGreen molecules and estimating the height of the occurring steps in change of intensity (only small steps, i.e., steps likely to be bleaching steps). The number of steps was first estimated by eye, and this number was used as input for the *findchangepoints* function of Matlab to determine the position of the steps (by detection of significant changes of the mean value). To yield the intensity per Ase1 dimer, the median of the heights of these steps was calculated and multiplied by two. For the estimation of single Ase1-GFP dimers, the intensity of single diffusive spots of GFP signals on MTs has been taken at very low concentration of Ase1-GFP in the buffer (no bleaching data available for these experiments). For estimation of single fluorophores for set B experiments we did not have bleaching data available, instead we measured the intensity of diffusing molecules (which may overestimate the intensity per single molecule).

*Ase1 density estimation.* Area selections along the MT length (only regions with at least one dynamic extension present were measured) were used to read out the mEGFP or mCherry fluorescent signal and to estimate the integrated signal intensity of GFP- or mNeonGreen-labeled Ase1 bound to the MT. The signal in regions directly adjacent to the MT was subtracted as background signal. The density of GFP- or mNeonGreen -labeled Ase1 bound to the MT was then estimated by dividing the integrated intensity by the estimated intensity per single fluorescent molecule (either GFP or mNeonGreen, see below) and the length of the region. The signal per length (S) measured on isolated MTs was used to correct for the reduced illumination intensity in outer regions of the field of view, in cases where a region of interest (ROI) was located in such a region (Scorrected(ROI)= S(ROI) \* S(isolated MT in center of field of view) / S(isolated MT near ROI).

*Estimation of amount of Ase1 being swept.* To estimate the number of swept Ase1 molecules for Figures 3D-F, S3A (set B experiments), we first obtained density traces for each frame during a MT depolymerization period. These traces were obtained by summing the pixel intensities perpendicular to the MT. For each frame f we analyzed the corresponding density trace Df as follows. (1) We computed Ds by subtracting the density trace Dbefore\_catastrophe of the MT before the catastrophe had occurred from Df (Ds = Df - Dbefore\_catastrophe) (2) We obtained x = 0 = XDsmax, the location of the local maximum of Ds in vicinity of the MT plus end. (3) We obtained XDsright by finding the first local minimum of Ds to the right of XDsright (to reduce the effect of noise, we smoothed Ds for this computation). “Right” of Ds, in our chosen coordinate system, means toward the MT seed (x > 0). (4) XDsleft = XDsmax – 471nm (471 nm = 3 pixels). (5) We computed DA. DA is equal to Df to the left of XDsmax, and equal to Ds + Df(XDsmax) – Ds(XDsmax) to the right of XDsmax. (6) We fitted a distribution YF (shape see below) plus an error function YE to DA between XDsleft and XDsright. We required both YF and the error function to not have any x-offset: YF was a right-sided decaying exponential exp(-x/ λ) (YF = 0 where x < 0, and with λ bounded between 1 and 1000 nm) convolved with a gaussian exp(-x2/ 2σ2) (with σ bounded between 180 to 190 nm to account for the point spread function of our setup; this same σ had been used as input for YE). We also fixed G+E (plus a constant value) to approach the minimum of DA to the left of the end, and the average of DA to the right of XDsright (the average of DA within 5 microns from XDsright, giving more weight to values close to XDsright). (6) We then summed the Ase1 density below YF (as discretized in x by the pixel size), which we took as a proxy for the number of swept Ase1-GFP molecules after dividing by the intensity per Ase1 dimer (obtained as described above). For Figure 3C (set B experiments), the underlying dataset did not allow for such a detailed analysis as described above, instead, the area under curves such as shown in Figure 3B within 500 nM from the MT end (determined by the local maximum in Ase1 density) toward the MT lattice was taken and multiplied by two. The same procedure was repeated for an area on the MT far away from the tip for each frame, and the median of these values was subtracted from the median of the values determined at the tip (to account for the contribution of noise and the slight increase in Ase1 density over time which was present for single MTs at 420 nM Ase1, i.e. the binding of Ase1 to these extensions had not always fully reached equilibrium by the time of catastrophe).

*Fluorescence recovery after photobleaching (FRAP) experiments.* Biotinylated GMPCPP-stabilized MTs were immobilized on the coverslip. We then flushed in the same assay buffer as for set A experiments, incubated until the Ase1 density on MTs reached a steady-state, and subsequently bleached Ase1-mNeonGreen molecules and recorded the recovering Ase1-mNeonGreen signal. We fitted the resulting recovery curve to the expression *D*s – *c* exp(-*bt*), where *D*s is the steady state density, and *c* and *b* are fitting parameters.

*Data representation.* In all boxplots presented in the figures, horizontal midline indicates the median; plus symbols indicate the mean; bottom and top box edges indicate the 25th and 75th percentiles, respectively; the whiskers extend to the most extreme data points not considered as outliers (the function *Alternative box plot* from the IoSR Matlab Toolbox has been used); the numbers indicate the sample size; the notches in Figure 2 A,B are centered on the median and extend to ±1.58\*IQR/sqrt(sample size).

**Mathematical modelling**

**Assumptions**

The model of Ase1 accumulation on depolymerizing MTs, and its effect on depolymerization velocity (Figure 4A) is built on the following assumptions:

1. We neglect interactions between protofilaments and assume that the behaviour of the whole microtubule is determined by a single protofilament. As such, the MT is a one-dimensional lattice, where lattice of size =8nm start at index at the plus end, extending to .
2. Only bound Ase1 molecules are considered by recording the presence or absence (0 or 1) of Ase1 in each lattice site. Bound Ase1 molecules exchange with solution with two constant rates (). Binding is only allowed if the lattice site is empty (Figure 4A). was directly measured, and was adjusted to match the Ase1 equilibrium density on MTs (Table S1).
3. Ase1 particles on the lattice undergo unbiased diffusion characterized by a constant hopping rate (). Hopping is only allowed to an empty site (Figure 4A). The rate is calculated from the experimentally measured diffusion coefficient of Ase1 (Table S1), as .
4. The Ase1 particle in the terminal site (), cannot hop past the MT end (red arrow on the left of Figure 4A), but can detach with rate .
5. The terminal lattice site may dissociate from the MT, with rate which depends on the presence of Ase1, according to each model:
   1. In Model 1, it occurs with rate if the terminal lattice site is not occupied (Figure 4B top), and with rate if it is occupied (Figure 4B bottom). is a parameter between zero and one. If , the presence of Ase1 has no effect, and if , the first tubulin subunit cannot unbind if it is bound to Ase1.
   2. In Model 2, it occurs with rate if the first lattice sites are all not occupied (Figure 4C top), and with rate if any of the terminal sites is occupied (Figure 4C bottom).
   3. For Model 3 (phenomenological model) see below.

is derived from the depolymerization rate of MTs in the absence of Ase1 (), measured experimentally (Table S1), such that .

1. If the terminal lattice site dissociates when a molecule of Ase1 is bound to it, this Ase1 is lost as well (Figure 4B, bottom).

**Simplification to a system of constant size**

Since terminal subunits are more likely to be lost when they are without Ase1 than when they are with Ase1, any dissociation event increases the density of Ase1 remaining on the MT. This effect is only present at the MT end, and away from the end, the probability of a binding site being occupied is only determined by the binding and unbinding constants: .

Therefore, we can restrict the model to a section of the MT with lattice sites, as long as the probability of finding a molecule at position is close to . When a depolymerisation event happens, we shift the lattice indexes such that site becomes site , and set .

**Mean field theory**

The system can be solved using a mean-field approximation, by just considering the ensemble of , the average probability of a site being occupied and neglecting higher-order correlations between neighbouring sites. We can then write a set of discrete differential equations to represent the dynamics of the system:

Specific equations apply at the boundaries and :

The terms of the equation are associated with the rates of diffusion, binding, unbinding () which are constant, and the depolymerization rate (), which is affected by lattice occupancy in a different way in each model (see Assumptions).

For Model 1, .

For Model 2, .

This dynamical system can be evolved from any initial conditions, converging to the unique steady-state solution for a set of given parameters. Assuming that the MT is at binding equilibrium when it starts depolymerizing, we initially set for all sites. From those initial conditions, we integrate the equations numerically using Python’s *odeint* function (see source code).

Code Availability Custom written code is available at: https://github.com/jochenkrattenmacher/Ase1-paper https://github.com/manulera/Krattenmacher\_et\_al\_2021

Data Availability Data is available from the corresponding authors on request.

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