To analyze how Ase1 accumulates at shrinking microtubule ends, and the effects this has on shrinkage speed, we considered a one-dimensional microtubule made of lattice sites corresponding to tubulin heterodimers, starting at the plus end (see Methods). Although it includes a single microtubule, this model is applicable to antiparallel pairs, by adapting the on/off rates of Ase1 binding to the lattice. Events such as Ase1 binding, unbind, and hopping to neighboring sites are stochastic with constant rates (Fig. 4A) that were determined experimentally (see 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 microtubule by hopping at its plus ends, as shown experimentally (Fig. 4A, red arrow on the left)(refs). MT shrinkage is also modelled stochastically by detachment of the terminal subunit, at a rate that is affected by Ase1 (Fig. 4B top). 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 mean depolymerization speed measured *in vitro* without Ase1 (Table S1). The parameter specifies the effect of Ase1 on disassembly (Fig. 4B bottom). 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 shrinking tip and a concomitant decrease of the shrinkage speed (Fig. 4D, 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 shrinking 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. Specifically, we aimed to reproduce the timescale of accumulation of Ase1, and the total amount of Ase1 accumulated and shrinking speed reached at steady state (Table S2). For , the model predicted shrinkage speeds that are comparable to the experimentally observed ones (Fig. 4E), but when comparing the timescale and number of Ase1 molecules accumulated at steady state we found that they were respectively 2.5× and 8× higher than experimentally observed (Fig. 4F, G). Therefore, despite recapitulating the experimental phenomenology qualitatively (Fig. 4D), this first model was insufficient to quantitatively reproduce our experimental results.

The failure of Model 1 indicated that Ase1 should affect microtubule shrinkage at lower density. We thus hypothesized that Ase1 molecules located at lattice sites other than the terminal one could affect shrinkage. 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 (Fig. 4C). This rate at steady state would then be , where is the probability of site being occupied by Ase1. For and this model (Model 2) reproduced microtubule growth speed at steady state and accumulation timescale, but the accumulation of Ase1 at steady state was more than double of the experimentally observed (Fig. 4E-G). Higher values of gave improved fit to the experiments, but it is unclear how Ase1 could influence the unbinding of the terminal tubulin heterodimer from a distance. Instead, it seemed more natural to assume that Ase1 molecules in neighboring protofilaments would also affect the unbinding, since they are closer. To avoid the complexity of modelling the 13 microtubule protofilaments with their longitudinal offset, we turned to a phenomenological model (see Methods) in which the rate of loss of subunits at the plus end is . Here, the exponent 3 represents the number of protofilaments that are considered sufficiently close to affect depolymerization of one protofilaments (*i.e.* this protofilament, and the two flanking ones). This model (Model 3) with produced the best agreement with the experimental data from single microtubules (Fig. 4E-G). The data from crosslinked microtubules could be reproduced by reducing the diffusion rate of Ase1 (VALUE) and its unbinding rate (VALUE) (Fig. 4SXX), following the experimentally known retention and slower diffusion of Ase1 in microtubule overlaps (refs).

In summary, stochastic modelling of the system showed that the observed effects of Ase1 on shrinking microtubule ends could be explained simply. The failure of the simplest model suggested that Ase1 acts cooperatively near the terminal tubulin units. Modifying the assumptions in this direction indeed improved the agreement obtained, coming close to reproducing the experimental data quantitatively. The effects of Ase1 on microtubule shrinkage speed and the timescale and extent of the accumulation of Ase1 molecules could all be reproduced within a factor 2.

|  |  |  |
| --- | --- | --- |
| Parameter | Value(s) | Source |
| microtubule depolymerization speed at 0 Ase1 | k0d = 300 nm/s | Figure S4A (leftmost box) |
| Ase1 off-rate | koff = 0.016 s-1 | Figure S4C |
| Ase1 diffusion coefficient | D = 0.093 µm2/s | Figure S4D |
| Ase1 on-rate | kon = 0.001 s-1 | Not directly measured. on-rate of 0.001 s-1 matches single microtubule density, while ~0.02 s-1 matched overlaps (compare Figure 4E, Figure S2B) |
| Tubulin dimer/binding site length | 8nm | The anatomy of flagellar microtubules: polarity, seam, junctions, and lattice.  JCB 1995 |

|  |  |  |
| --- | --- | --- |
| Measurement | Value(s) | Source |
| Timescale of accumulation | = 10.8 s | Fit of Fig. S3A to |
| Ase1 molecules accumulated at steady state | = 4.8 molecules per protofilament | Fit of Fig. S3A to |
| Shrinkage speed at steady state | =0.123 μm/s | Fit of Fig. S3B to |