To understand why Ase1 accumulates at shrinking microtubule ends, and how can this lead to reduced shrinkage speed, we used a simple one-dimensional model (see Methods). For simplicity, we focused on the single microtubule case, and modeled the microtubule as a one-dimensional lattice, with lattice sites corresponding to tubulin dimers, the first site being the plus end. Ase1 can bind from solution, unbind, and diffuse with constant rates (Fig. 4A). These rates were determined experimentally (see Table 1). Importantly, we assume that Ase1 does not “hop out” of the microtubule through diffusion at its plus ends, as shown experimentally (Fig. 4A, red arrow on the left)(refs). We assumed that when the first tubulin subunit is not bound to Ase1, that tubulin subunit can leave the lattice with rate , and this shortens the microtubule (Fig. 4B top). The value of is given by the depolymerization speed measured in the absence of Ase1. However, if the first tubulin subunit is bound to Ase1, the rate at which the tubulin subunit leaves the lattice is reduced, and given by , where is a parameter that goes from zero to one (Fig. 4B bottom). If , the presence of Ase1 has no effect, and if , the first tubulin subunit cannot unbind if it is bound to Ase1. For , this simple assumption produces both a decrease in shrinkage speed and an accumulation of Ase1 at the shrinking tip (Fig. 4D). Since subunits without Ase1 are more likely to be lost at the plus-end, depolymerization increases the density of Ase1 at the shrinking end. At steady state, for a given probability of the first lattice site being occupied, the rate of loss of subunits at the plus end is therefore .

All parameters of the model were set from experimental measurements, except for . Therefore, we next set out to test whether any value of would make the model match the experimental behavior. In particular, we aimed to reproduce the accumulation of Ase1 (timescale and number of Ase1 molecules accumulated at steady state) and the microtubule shrinking speed at steady state (Table 2). We found that for , the model decreased the shrinkage speed at steady state to a value comparable to the experimentally observed (Fig. 4G), but when comparing the timescale and number of Ase1 molecules accumulated at steady state we found that they were respectively 2.5 times and 8 times higher than the experimentally observed for (Fig. 4E, F). Therefore, despite recapitulating the qualitative behavior of the system (Fig. 4D), this simple model was insufficient to quantitatively reproduce our experimental results.

The results from our previous model suggested that Ase1 has a stronger effect on microtubule shrinkage speed than we originally proposed. We hypothesized that this could result from shrinkage speed being affected by Ase1 molecules located at lattice sites other than the first one. We initially tested the possibility that the rate of loss of tubulin subunits at the plus end would be reduced to if any of the first lattice sites in a protofilament was occupied by an Ase1 molecule (Fig. 4C). The rate of loss of subunits at the plus end at steady state would then be , where is the probability of site being occupied by Ase1. For and the model reproduced microtubule growth speed at steady state and accumulation timescale (Fig. 4E, G), but the accumulation of Ase1 at steady state was more than double of the experimentally observed (Fig. 4F). Higher values of still improved the fit of the data, but it seems unlikely that Ase1 molecules that are so distant from the plus end might influence the unbinding of the first tubulin subunit. Instead, it could be that Ase1 molecules in neighboring protofilaments also affect the unbinding. To avoid the complexity of modelling multiple protofilaments in which lattices may not be in register, 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 cooperativity with Ase1 molecules from neighboring protofilaments.

We obtained a good agreement with the experimental data from single microtubules using and (Fig. 4H-J), which suggests that cooperative interactions between Ase1 molecules in neighboring protofilaments might be involved in reducing microtubule growth speed. The data from crosslinked microtubules could be fit by reducing the diffusion rate of Ase1 and its unbinding rate (Fig. 4SXX), mimicking its known retention and slower diffusion in microtubule overlaps (refs).

In summary, mathematical modelling of the system suggests that the observed accumulation of Ase1 at shrinking microtubule ends and the reduction of microtubule growth speed could result from a cooperative effect of Ase1 molecules within and across filaments that suppresses unbinding of tubulin subunits at microtubule ends.

|  |  |  |
| --- | --- | --- |
| 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 | ? |

|  |  |  |
| --- | --- | --- |
| 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 |