Microtubule damage sites impact acetylation regulation in the lumen

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Acetylation of lysine 40 on α-tubulin is a post-translational modification (PTM) located in the lumen of microtubules, regulated by the acetyltransferase αTAT1 and the deacetylase HDAC6. In HeLa cells, we recently showed that local microtubule (de)acetylation depends on HDAC6 entry into the microtubule lumen, facilitated by kinesin-1–induced lattice damage[1]. To investigate the mechanisms underlying luminal entry, we reconstituted microtubule damage and (de)acetylation *in vitro*[2]. Using purified αTAT1 and HDAC6 on microtubules with defined lattice conformations, we find that αTAT1 overwrites HDAC6 activity, although its acetylation efficiency decreases upon microtubule damage. Expanded lattices promote αTAT1 activity, whereas compacted lattices impede it. To directly visualize lattice defects, we introduce MTdam, an *in vitro* microtubule damage probe that selectively labels lattice openings. MTdam reveals intrinsic defects in *in vitro*–polymerized microtubules, particularly at annealing sites. It further shows that kinesin-1Δ6 generates additional microtubule damage, including kinks and sheet openings. Together, these results identify lattice damage and conformation as key determinants of microtubule luminal accessibility and (de)acetylation.

References:

(1) Andreu-Carbó, M.; Egoldt, C.; Velluz, M. C.; Aumeier, C. Microtubule Damage Shapes the Acetylation Gradient. *Nat Commun* 2024, *15* (1). https://doi.org/10.1038/s41467-024-46379-5.

(2) Egoldt, C.; Tran, J.; Velluz, M.-C.; Aumeier, C. Microtubule Lattice Conformation and Integrity Regulate α-Tubulin Acetylation. *bioRxiv* 2025.