Expanding the Chemical Toolkit for Studying Microtubule Acetylation

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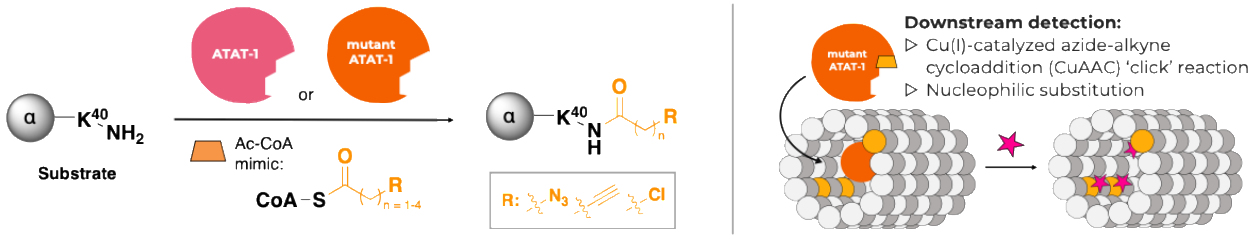
Tubulin acetylation is an abundant post-translational modification of stable microtubules, including the ciliary axoneme, yet its functional role remains poorly understood[1]. To elucidate the role of axonemal acetylation in both wild-type and ciliopathy cells, we aim to visualize acetylation events through the incorporation of bioorthogonal cofactor mimics along microtubules. We first evaluated the tolerance of ATAT1, the enzyme responsible for acetylating lysine 40 on α-tubulin, toward seven synthetic mimics of its native cofactor, acetyl coenzyme A (Ac-CoA). These mimics carried bioorthogonal ligation handles of different sizes to enable subsequent fluorophore conjugation. Only one mimic supported partial α-tubulin acylation, whereas the others partially or fully competed with Ac-CoA without substrate modification, revealing limited promiscuity of ATAT1 toward unnatural cofactors. To overcome this limitation, we performed structural docking to guide engineering of the ATAT1 cofactor-binding pocket[2]. Mutation of leucine 163 to alanine (L163A) enhanced binding of six mimics while reducing affinity for Ac-CoA. Among these, five successfully acylated α-tubulin on microtubules *in vitro*, whereas two acted as competitive inhibitors. Furthermore, HDAC6, the main α-tubulin deacetylase, selectively removed labeling installed by ATAT1-L163A with one mimic out of four tested, indicating that engineered acylation events differ in their susceptibility to deacetylation. Together, this work establishes a foundation for developing bioorthogonal mutant–cofactor pairs capable of site-specific labeling at the native α-tubulin acetylation site. This strategy opens new possibilities for directly visualizing microtubule acylation events without relying on antibody-based detection.



References:

[1] A. Iuzzolino, F.R. Pellegrini, D. Rotili, F. Degrassi, D. Trisciuoglio. *Cell. Mol. Life Sci*. **2024**, *81(1)*, 193.
[2] A.M. Davenport, L.N. Collins, H. Chiu, P.J. Minor, P.W. Sternberg, A. Hoelz. *J. Mol. Biol.* **2014**, *426(14)*, 2605-2616.