Production of Semisynthetic Tubulin with Definable Post-Translational Modification

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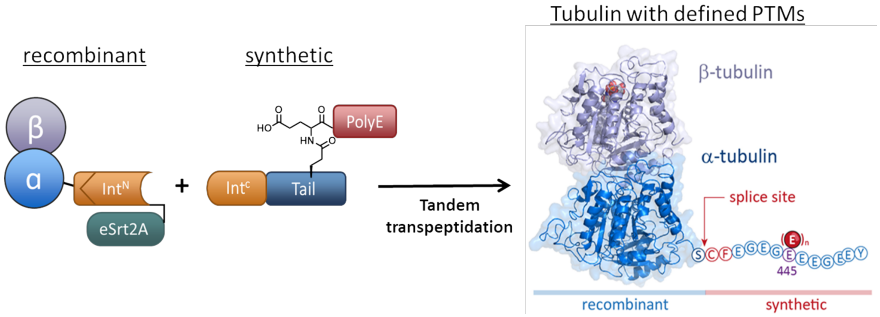
Microtubules, a vital constituent of the cytoskeleton, carry various post-translational modifications (PTMs) that are essential for regulating essential cellular mechanisms. However, the precise mechanisms underlying how PTM patterns govern the functions of microtubules are not well understood, mostly due to a lack of methods to generate tubulin with well-defined PTMs. Our previous work showed a promising semi-synthetic approach to preparing α-tubulin with polyglutamylation (polyE) on their C-terminals. Building upon this, this work aims to improve the method for producing semi-synthetic α- and β-tubulin by incorporating two key advancements. The first improvement involves the use of a building block approach to conjugate the branched polyglutamic acid and the α-tubulin tail via a native amide bond. The second improvement involves the development of a method to modify the β-tubulin tail, offering a potential solution to orthogonally tailor both α- and β-tubulin tails. In future work, we plan to investigate the mechanism behind the activation of the vasohibin complex through polyglutamylation and determine whether polyglutamylation also activates MATCAP. Additionally, we plan to use the semi-synthetic tubulin to identify microtubule-associated proteins (MAPs) that are sensitive to polyglutamylation in cell lysates, by using photoaffinity proteomics.



[1] Ebberink, E.; Fernandes, S.; Hatzopoulos, G.; Agashe, N.; Guidotti, N.; Reichart, T. M.; Reymond, L.; Velluz, M. C.; Pourroy, C.; Janke, C. *bioRxiv,* **2022**.