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seminar

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Host: Dr. Niels C. Reichardt

Chemical precision tools to understand protein O-glycosylation

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O-GalNAc glycosylation is a major constituent of the cell surface glyco-code. Glycosylation is primed by 20 GalNAc transferase (GalNAc-T) isoenzymes that introduce the first, Ser/Thr-linked GalNAc residue using UDP-GalNAc as a sugar donor.[1] Despite partial redundancy, GalNAc-Ts have been differentially associated with disease, suggesting a pivotal role of isoenzyme-specific protein substrates. However, studying these substrates is complicated by the cross-talk of different isoenzymes with each other.
Here, a chemical biology method termed "bump-and-hole engineering" is used to dissect the details of GalNAc-T isoenzyme specificity in the living cell.[2–4] In a structure-guided process, the active site of a GalNAc-T is enlarged by mutation, creating a "hole" that renders the enzyme compatible with a chemical functionality ("bump") in a synthetic UDP-GalNAc derivative. Structural and functional characterization ensures viability of the orthogonal enzyme-substrate pair to glycosylate native protein substrates. A traceable chemical handle in the bump allows for the specific detection of glycoproteins by bioorthogonal ligation. The GalNAc salvage pathway is re-programmed to deliver bumped UDP-GalNAc derivatives to the cell, and MS glycoproteomics enables the characterization of GalNAc-T isoenzyme-specific glycosylation sites and glycan structure in a single experiment. We further show that the chemical handle can be tailored to suppress epimerization to the corresponding UDP-GlcNAc derivatives, thereby considerably reducing the complexity of glycoprotein labeling. Bump-and-hole engineering yields precision tools to investigate the biology of O-GalNAc glycans.[5]

(1) de las Rivas, M. et al. *Curr. Opin. Struct. Biol*. **2019**.
(2) Choi, J., Wagner, L. J. S. et al. *J. Am. Chem. Soc.* **2019**.
(3) Schumann, B. et al. *Mol. Cell* **2020**.
(4) Debets, M. F., Tastan, O. Y. et al. *Proc. Natl Acad. Sci. USA* **2020**.
(5) Calle et al. *J. Amer. Soc. Mass Spectrom.* **2021**