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Glycosyltransferase Bump-and-hole Engineering to Understand Glycosaminoglycan Biosynthesis

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CIC biomaGUNE - Seminar Room

Glycosaminoglycans (GAGs) are major determinants of proteoglycan function and contribute substantially to the cell-surface glyco-code. While steadily increasing, the number of confirmed proteoglycans is still relatively small, and their functions can be challenging to unravel. New chemical precision tools will enhance our understanding of GAG biology and complement existing methods of molecular and cell biology.

While fully-established GAG chains are structurally highly variable, attachment to the core protein is manifested through a conserved tetrasaccharide linker. The first peptide-proximal monosaccharide is xylose, linked through an O-glycosidic bond predominantly to Ser residues by protein xylosyltransferase (XylT) enzymes. Human cells express the two homologous isoenzymes XylT1 and XylT2 that are individually associated with disease reflecting dysfunctional proteoglycans. Yet, the molecular details underpinning the functions of both isoenzymes are ill-defined.

Here, we develop a chemical biology approach en route to dissect the protein substrate specificities of human XylTs. In a structure-based tactic termed bump-and-hole engineering, we mutate XylTs to incorporate a chemically modified analogue of the natural substrate UDP-xylose that is not accepted by wildtype enzymes. We characterize the kinetic properties of the bump-and-hole system and show that engineered XylT1 retains the peptide substrate specificity of the WT enzyme. We develop a strategy to biosynthesize the UDP-sugar and establish a cellular bump-and-hole system. This precision tool will provide insight into the repertoire and biosynthetic details of proteoglycans.