Substrate-controlled Enzymatic Glycosylation for Complex Oligosaccharide Synthesis

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Carbohydrates play a vital role in numerous physiological processes, such as cellular adhesion, pathogen infection, immune responses, and the development of autoimmune diseases. To get an insight into carbohydrates in living systems, considerable efforts have thus focused on developing practical methods for preparative access. Bacterial glycosyltransferases (GTs) are considered particularly promising biocatalysts for facilitating oligosaccharide syntheses because of their broad substrate tolerance, high production levels in *E. coli* overexpression systems, and ease of handling. However, a potential limitation of these inherently multifunctional bacterial GTs is the control of a desired catalytic activity on glycan synthesis. We focused on acceptor preference profiling of fucosyltransferases (FucTs) to predict the products formed on a given oligosaccharide with multiple fucosylation sites. We demonstrated that the addition of α1,2-linked Fuc or β1,3-GlcNAc on the nonreducing end Gal increased the substrate-binding affinity for FucTs, which resulted in a higher turnover number and enhanced catalytic activity.[1,2] In addition to sugar portion modification, the outcome for a GT-catalyzed reaction could be controlled by reducing end aglycone, which would render site-selective fucosylation possible under controlled conditions.[3] A library of of *para*- and *iso*-human milk oligosaccharides could be synthesized by our strategies. Having these well-defined structures provides valuable standards for the characterization and quantification of complex glycans isolated from nature and enables comprehensive screens for new prebiotic activities and advances in exploring the HMO−gut microbiome relationship.

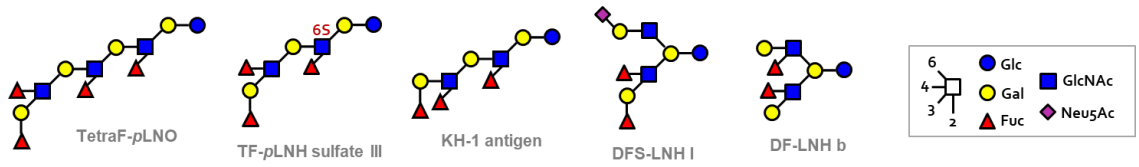

Figure 1. Structures of *para*- and *iso*-human milk oligosaccharides (partial).

References

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