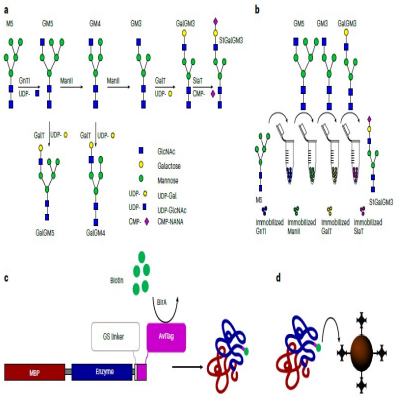
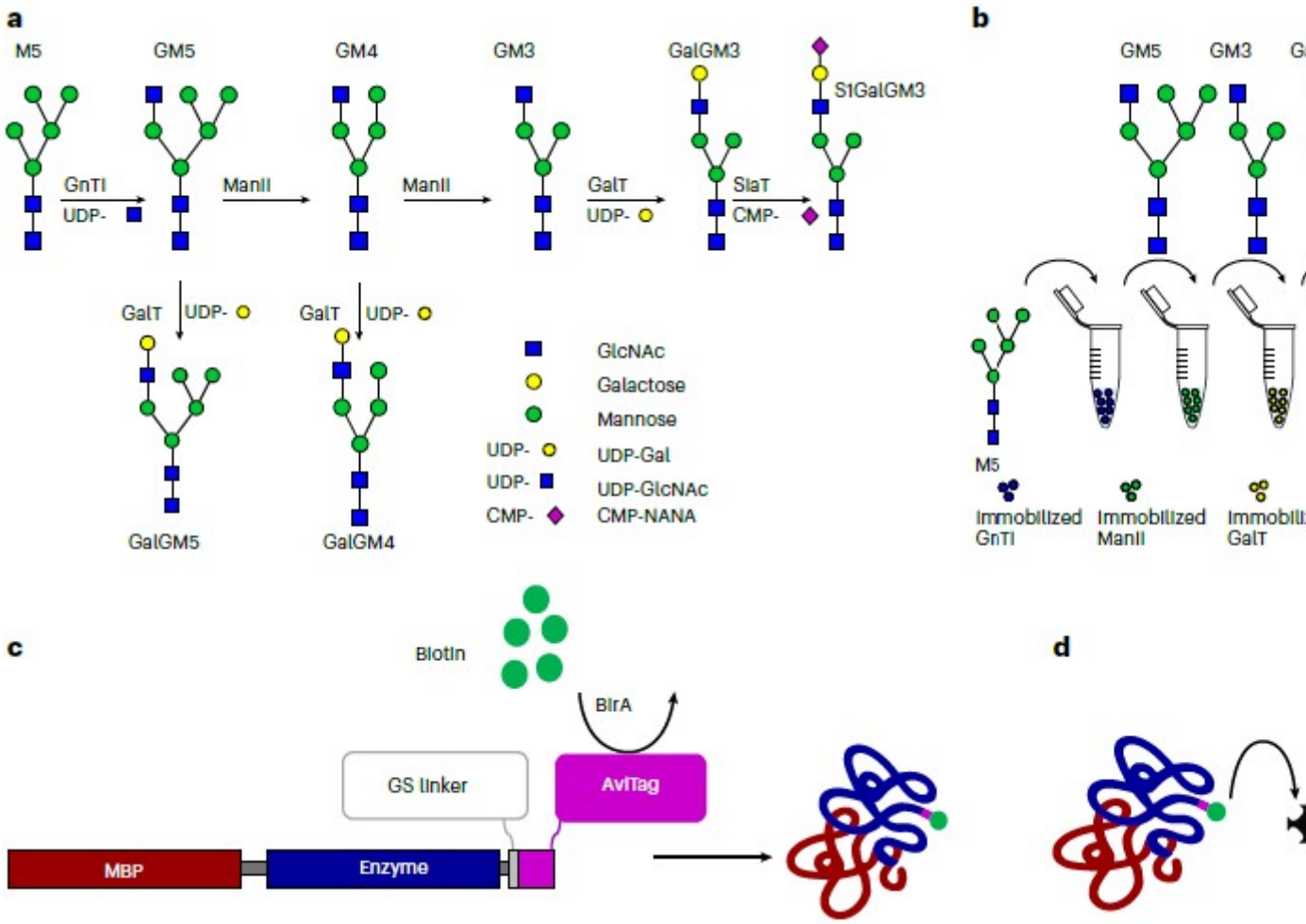


Immobilized enzyme cascade for targeted glycosylation

Description

/Glycosylation stands as a pivotal post-translational protein modification, exerting profound effects on protein folding, half-life, and functionality. Its non-templated and heterogeneous nature stems from the inherent promiscuity of the enzymes involved. In their study, the authors present a novel platform termed Sequential Glycosylation Reactions for Tailored Sugar Structures (SUGAR-TARGET), which facilitates bespoke and controlled N-linked glycosylation in vitro. This platform is made possible by immobilized enzymes generated through a streamlined one-step immobilization/purification method.



Strategy and design of artificial Golgi reactions (SUGAR-TARGET) for targeted glycosylation. a, N-Linked glycosylation pathway as regulated by GnTI, ManII, GalT and SiaT. GalT simultaneously recognizes the products of GnTI and ManII, making it a highly promiscuous enzyme. **b**, Sequential glycosylation reactions using immobilized enzymes. The facile enzyme recovery and thus the spatiotemporal separation will yield the desired product, S1GalGM3, in a homogeneous state. **c**, In vivo biotinylation strategy. The catalytic domain of a target enzyme is fused to an MBP on the N terminus and AviTag on the C terminus. To ensure functionality, a small two-residue glycineâ??serine (GS) linker was inserted before the AviTag. The biotin ligase BirA recognizes AviTag and can perform enzymatic biotinylation. **d**, Biotinylated enzyme is subsequently immobilized on streptavidin-coated solid supports.

The researchers construct a reaction cascade that emulates a glycosylation pathway, where enzyme promiscuity naturally operates, to humanize a spectrum of proteins sourced from diverse cellular systems. The outcome is the production of nearly homogeneous glycoforms. Utilizing immobilized Î²-1,4-galactosyltransferase, the authors enhance the galactosylation profile of three IgGs, resulting in terminal galactosylation levels ranging from 80.2% to 96.3%. Moreover, they demonstrate enzyme recycling capabilities, sustaining reaction times exceeding 80 hours. This platform boasts ease of implementation, modularity, and reusability, enabling the generation of homogeneous glycan structures from various host organisms. These structures can be subjected to functional and clinical evaluations, underscoring the versatility and potential of the SUGAR-TARGET platform.

Category

1. News