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Development and Application of Glycosyltransferases for In Vitro Glycoengineering

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Glycosylation is an important posttranslational modification of proteins influencing protein folding, stability and regulation of the biological activity, *e.g.* IgG featuring terminal sialic acids were shown to suppress inflammatory response (ADCC) and increase serum half-life. Fully galactosylated structures seem to improve binding to the complement system (CDC).

Despite all efforts in cell-line engineering and process optimization to increase glycosylation homogeneity, today therapeutic proteins still show a heterogeneous glycosylation pattern, with large variations between bioprocesses and even from batch to batch. The use of glycosyltransferases for enzymatic synthesis of well-defined glycan structures will become an essential tool – at least in order to provide clear conclusions on the structure-function relationship of different glycan variants.

Therefore, highly active derivatives of human

- beta-galactoside alpha-2,6 sialyltransferase 1 (ST6Gal-1),
- beta-1,4 galactosyltransferase 2 (B4Gal-T2), and
- beta-galactoside alpha-2,3 sialyltransferase 6 (ST3Gal-6)

are currently developed for secreted expression by transient gene expression (TGE) as well as CHO-K1 for future GMP production.

To our surprise, N-terminally truncated variants of human ST6Gal-1 allow directed G2+1SA and G2+2SA mab glycoengineering in sialylation experiments using bi-antennary glycans (mabs) as well as tetra-antennary glycans (EPO) as substrate: The $\Delta 108$ variant produces mainly mono-sialylated N-glycans. In contrast, $\Delta 89$ – which is commercially available – produces a high degree of bi-sialylated glycans during the first 8 hours followed by sialydase activity leading to a continuously decreasing overall sialylation level. Consequently, dependent on the incubation time different sialylation patterns can be achieved. The x-ray structure of the $\Delta 89$ variant is described in Kuhn *et al.* (2013) Acta Crystallography 69:1826-38.

We used *in vitro* glycoengineering (IVGE) to investigate the impact of IgG1 Fc glycans on effector functionality: After creating a unique set of Fc glycan variants of an IgG1 with different levels of galactose and sialic acid, respectively, we analyzed their impact on Fc γ RI, IIa and IIIa binding by Surface Plasmon Resonance (SPR), and on ADCC activity. Furthermore, we supported an early stage project by IVGE to prepare material for mouse PK studies.