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# Development and application of glycosyltransferases for in vitro glycoengineering

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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 Δ108 variant produces mainly mono-sialylated N-glycans. In contrast, Δ89 – 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 Δ89 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 FcγRI, 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.