

GALMAX: MODEL-INSPIRED GLYCOENGINEERING FOR BIOPHARMACEUTICAL QUALITY ASSURANCE

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β 4-galactosylation is a critical quality attribute (CQA) of monoclonal antibodies (mAbs) because of its influence on their oncolytic activity [1] and because it is a major source of product heterogeneity [2]. mAb β 4-galactosylation variability arises from both metabolic and cellular machinery bottlenecks: (i) limited availability of uridine diphosphate galactose (UDP-Gal) and (ii) reduced availability/activity of β -1,4 galactosyltransferase (β 4GalT), the enzyme which catalyses β 4-galactosylation. Here we present GalMAX, a simple and robust cell engineering strategy that eliminates the above bottlenecks to maximise mAb β 4-galactosylation and to, thereby, address the quality assurance challenges posed by this CQA.

A compact metabolic model for CHO cells, which accounts for nucleotide sugar demand towards cellular and product glycosylation [3], was used to identify serine (O)-linked cellular glycosylation as a major sink of UDP-Gal. CRISPR-Cas9 was used to abrogate cellular O-galactosylation by knocking out COSMC, the core 1 β 3-Gal-T-specific molecular chaperone, from two mAb-producing CHO cells, CHO-DP12 and CHO-VRC01. COSMC knockout cells were enriched using lectin-aided fluorescence cell sorting and transfected with a plasmid containing the human β 4-galactosyltransferase I gene to produce DP12 and VRC01 GalMAX cells. COSMC-KO, β 4GalT1+, and GalMAX variants of DP12 and VRC01 were cultured in batch and fed-batch mode for characterisation. mAb product glycoprofiling was performed with the LC-MS method developed by Carillo et al., 2020 [4].

Under batch cultivation, the DP12-GalMAX cells produced 96% galactosylated mAb glycoforms (40% above parental cells), and the VRC01-GalMAX cells yielded 98% galactosylated species (a 2.2-fold increase over parental cells) (Figure 1). Under fed-batch culture, DP12-GalMAX achieved 92% galactosylated glycoforms (14.7% more than the DP12-GalT+ cells and 44% more than the parentals), and VRC01-GalMAX produced 95% galactosylated glycoforms (11% higher than the VRC01-GalT+ cells and 50% higher than the parentals) (Figure 2). The differences observed when comparing GalMAX cells with the GalT+ variants demonstrate how the COSMC knockout frees UDP-Gal consumption towards mAb β 4-galactosylation (Figures 1 and 2). The cell engineering events had no negative impact on cell growth, metabolism, or mAb titre. By yielding extremely high levels of β 4-galactosylation, our model-inspired GalMAX strategy reduces product variability and has the potential of enhancing the oncolytic activity of mAbs. Our GalMAX

platform has also been used to develop strategies for real-time control of mAb glycosylation and for facile and robust mAb glycoprofiling based on lectin-aided flow cytometry measurements. GalMAX therefore has great potential in contributing to biopharmaceutical quality assurance.

References:

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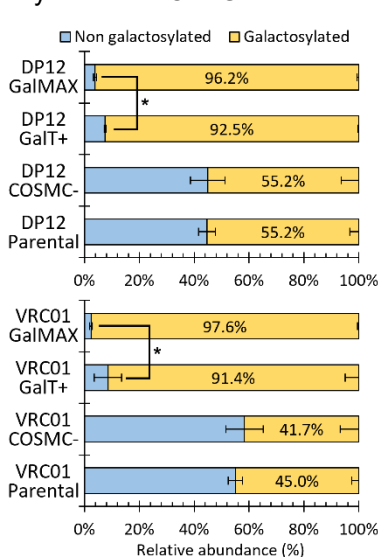


Figure 1. mAb β 4-galactosylation in batch culture

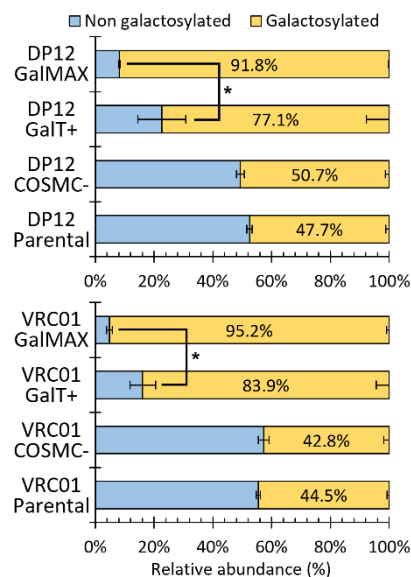


Figure 2. mAb β 4-galactosylation in fed-batch culture