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CHO-specific recombinant protein glycosylation reaction network

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CHO-SPECIFIC RECOMBINANT PROTEIN GLYCOSYLATION REACTION NETWORK

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Protein glycosylation is one of the most important product quality attributes and impacts efficacy, half-life, and immunogenicity. Previous glycosylation models effectively simulated the key parts of the N-glycosylation pathway. Building upon these prior efforts as well as recent CHO-K1 and Chinese hamster (CH) genome sequencing efforts, we share a new model for CHO- and CH- glycosylation. The model contains all N-glycosylation-related genes and all of the metabolic genes associated with central carbon metabolism, nucleotide sugar precursor synthesis, and nucleotide sugar transport annotated from the CHO-K1 and CH genomes. The model predicts both intracellular and secreted glycans for both mAb and non-mAb biotherapeutics, making it applicable for many experimentally-relevant applications. By including user-definable pseudo-kinetic parameters, the model predicts all potential glycans for a given condition, including the likely glycoform. We scanned the literature for experimental observations that could be used to test our model. We found 10 publications that included 15 gene modifications that reported impact on the glycoform for mAb and non-mAb molecules. We successfully modeled glycosyltransferase gene overexpression (St6Gal1, Ggta1)and knockouts of glycosyltransferase, nucleotide sugar precursor synthesis, and nucleotide sugar transport genes (Fut8, Mgat1, β4Galt, St3Gal3, GMDS, SLC35A3) with a >93% accuracy, sensitivity, and specificity across the entire range of literature.. Moreover, we tested model predictions for a model non-mAb protein by modifying additional glycosyltransferase genes (Mgat2 knockdown, Mgat4 overexpression, and Fuca1 overexpression). We believe the model's simple design and relevance will support systems biology related efforts to design and control product quality attributes in the future.