SYSTEMS ENGINEERING N-GLYCANS OF RECOMBINANT THERAPEUTIC PROTEINS

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Protein N-glycosylation reactions form a distributed reaction network spanning over different compartments of Golgi apparatus. The resulting glycan structures are influenced by glycosylation enzymes, the supply rate of nucleotide sugars, as well as competition among extending glycan substrates for a common enzyme and among different enzymes for a common substrate. Controlling the glycan profile of a therapeutic protein product is important for product quality for both innovative drugs as well as biosimilars. Metabolic engineering of the glycosylation pathway offers a venue for modulating the glycan profile. We have taken a systems engineering approach to identify, through model assisted design, the genetic manipulations that may steer the glycan flux to the desired path. However, unlike the energy metabolism pathway for which a small number of enzymes play pivotal roles in controlling the flux, the glycosylation pathway lacks key regulated steps as easily identifiable targets for genetic alteration to re-direct the flux. The model prediction thus serves only as a imprecise guide rather than a clear beacon. Furthermore, very likely multiple genetic alterations are needed in order to steer glycan flux distribution. A scheme of rapid construction of gene combinations to facilitate genetic engineering of the cell is necessary.

We establish a golden gate assembly workflow for production of multi-gene constructs for engineering the glycan biosynthesis pathway. Libraries containing promoters of varying strengths, terminators, and glycosylation related coding sequences of interest, all refactored to be devoid of type IIS restriction sites, were synthesized. In the first level of assembly, an additional library of single gene constructs were formed from these base components with single reactions. In the second level of assembly, these monocistrons were then combinatorically combined to form a multi-gene cassette library.

In an application of this approach, the N-glycosylation pattern of a recombinant IgG produced in CHO cells was examined with a stoichiometric network visualization tool (GlycoVis) to track the reaction paths which lead to the product glycans and identify galactosylation as potentially limiting glycan maturation. Cassettes consisting of sequences coding for nucleotide sugar synthesis enzymes, nucleotide sugar transporters, and glycosyltransferases were then selected to engineer the IgG producing cell. Multiple cassettes successfully directed the glycosylation to produce antibody with desirable glycoforms. These results served to refine our model parameters and sharpen its predictive capabilities. This combination of systems analysis and synthetic glycoengineering can be broadly applied and enhances our capability to steer N-Glycan patterns and control the quality of therapeutic proteins.