GENERATING GLYCAN VARIANTS FOR BIOLOGICAL ACTIVITY TESTIG BY MEANS OF PARALLEL EXPERIMENTAL DESIGN AND MULTIVARIATE ANALYSIS

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For more than 20 years, the industry has mainly invested in productivity enhancements. Recently, the focus of cell-culture process development began to shift. The modulation of quality attributes of recombinant therapeutic protein has gained substantial interest as demonstrated by the plethora of recent publications describing the effect of cell culture media on post-translational modifications of recombinant proteins[1]. Focusing on glycosylation, our team has developed a toolbox of media design beyond the commonly known media components and a rational high-throughput experimental design method. We identified and tested a large variety of novel cell culture compatible chemical components in industrial relevant Chinese hamster ovary cell lines (CHO) expressing recombinant antibodies and antibody fusion molecules.

The compounds were evaluated in five different parallel 96-DWP fed-batch experiments, considering their mode of biological action. Viable cell density, viability and product titer were monitored and purified supernatants underwent N-glycan analysis by 2AB-UPLC and site-specific glycan-peptide analysis. Multivariate analysis identified the best performing glycosylation modulators, which were confirmed in spin tubes. Intracellular nucleotide and nucleotide sugar levels were analyzed by capillary electrophoresis, the gene expression by next-generation sequencing technologies, and the impact of the generated glycan variants on the biological activity was assessed. Non-targeted metabolite profiling was carried out to build a multivariate model linking metabolites with the glycan fingerprint.

The screening experiments in 96-DWP produced a large glycosylation distribution diversity[2,3]. Subsequent D-optimal quadratic design in shake tubes confirmed the outcome of the selection process and provided a solid basis for sequential process development at a larger scale. The glycosylation profile with respect to the glycosylation specifications was greatly improved in shake tube experiments: 75% of the conditions were equally close or closer to the specifications than the best 25% in 96-deepwell plates. Further enhancement enabled us to generate extreme glycosylation variants, including high mannose, afucosylated, galactosylated as well as sialic acid species of both a mAb and an antibody fusion molecule with three N-glycosylation sites. The glycan variants induced significant responses in the respective in vitro biological activity assays. Moreover, metabolites correlating with time-dependent glycan profiling data were pinpointed and the glycan distribution of an external data set predicted. Our data highlight the great potential of cell culture medium optimization to modulate product quality and show the feasibility of the generation of a wide range of glycan variants suitable for biological activity testing.

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