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Trends and approaches in N-Glycosylation engineering in Chinese hamster ovary cell culture

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Summary
Chinese hamster ovary (CHO) cells have become the preferred expression system for the production of complex recombinant glycoproteins. It has been historically successful in industrial scale-up application and in generating human-like protein glycosylation. N-glycosylation of recombinant proteins, in particular, of those as drug substances, is extremely concerned in drug development and approval, as it will largely affect their stability, efficacy, clearance rate and immunogenicity. Therefore to engineering N-glycosylation of CHO cell-derived recombinant proteins are extremely important. Here, we will summarize a group of recent strategies and approaches and come up with case studies for N-glycosylation engineering in CHO cells and show several examples of relevant study cases from our research: 1) media and feed design, 2) culture process optimization, 3) substrate addition, 4) genetic engineering, 5) omics-based characterization, 6) mathematical modelling.

1. Medium and feed design [1]
- The balance of glucose and amino acid concentration in the culture is important for cell growth, IgG titer and high expression.
- Amino acids with the highest consumption (Ser, Leu) rates correlate with the most abundant amino acids present in the produced IgG, and thus require sufficient availability during fermentation.
- Higher specific glucose consumption rate is better for cell growth and maturation of IgG.
- Extracellular glucose consumption and its uptake rate were positively correlated with intracellular UDP-Gal availability, which in turn, resulted in higher galactosylation levels on the complex sugars present on the recombinant product.

2. Culture process optimization
- Strong positive correlation
- Strong negative correlation
- No clear correlation
- Weak positive correlation
- Weak negative correlation

3. Substrate addition [2]
- 8 different substrate additives (glycosylation precursors), including mannose, galactose, fucose, GlcNAc, ManNAc, NeuNAc, uridine, and cystamine were used as feed additives in fed-batch culture run in triplicates in well-controlled bioreactor systems.
- None of the additives caused statistically significant changes to cell growth and IgG productivity.
- Galactose addition increased galactosylation by 11%.
- Mannose addition slightly reduced GlcNAc occupancy.
- ManNAc addition slightly increased fucosylation.

4. Genetic engineering
- Stably overexpress either GnT1 or UDP-GlcNAc transporter in two different IgG-producing cell lines A and B.
- Western blot quantification.
- Localization confirmation using immunostaining: Discrepant GnT1 A and B and UDP-GlcNAc transporter.
- GalNAc (red), Dol-P Marker (green).

5. Omics-based characterization [3]
- Proteinomics analysis on day 2 and day 9 of replicate fed-batch culture.
- mRNA expression analysis at day 2 and day 9.
- Upregulated genes: Glutamine and glutamate metabolism, Cell-cycle, Nucleotide metabolism.
- Downregulated genes: Glucosamine (Ser-GlcNAc) metabolism, Extracellular sensing and signal transduction, Apoptosis, Oxidoreductase.

- Glycosylation mathematical modelling could aid in cell line selection and engineering during the early stages of bioprocess development.
- In-silico prediction of dynamic distribution, kinetics and concentration of glycosylation enzymes along the Golgi space.
- In-silico glycosylation engineering prediction of GnT1 overexpression.
- The overall capabilities of protein secretion machinery from growth phase to stationary phase in the cells gradually exceed the capability of protein glycosylation machinery that is particularly responsible for glycan maturation.

References: