N-Glycosylation optimization of recombinant antibodies in CHO cell through process and metabolic engineering - DTU Orbit (15/06/2019)

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Thanks to the recent advances in Chinese hamster ovary (CHO) “omic” revolution, the development of recombinant therapeutic protein bioprocessing using CHO cell factory started to merge with the new biological mindset called systems biology. In order to produce a CHO-derived recombinant therapeutic protein with ensured safety, efficacy and cost-effectiveness, holistic understanding of titer and N-glycosylation of the protein in relation to cell culture process as well as genomic, proteomic, metabolic and physiological status of the cells becomes a superior approach. Combining the knowledge of CHO cell culture technology, upstream process engineering, metabolic engineering, and glycobiology into a systematic framework allow us to improve the production of recombinant therapeutic protein towards an optimal balance between quantity and quality.

In the presented work, recent know-how on impact, analysis, control and optimization of N-glycosylation were thoroughly reviewed. In particular, how to control and optimize N-glycosylation in CHO cells was exclusively studied. The main focus of this PhD project is to find effective approaches of modulating N-glycosylation of CHO-derived recombinant monoclonal antibody (mAb) towards desired patterns, and at the same time try to understand the underlying mechanisms of that from a systems biology perspective. Two different strategies were used and achieved great success in glyco-optimization: 1) optimize media and culture process; 2) Genetically optimize CHO cell factory.

In the early part of the thesis, the first strategy was displayed by a number of successful case studies, in which process and media engineering approach was successfully used to direct N-glycosylation. Controlling the balance between glucose and amino acid metabolism, using galactose as feed additives, changing process parameters such as seeding density and cultivation duration are all demonstrated to be effective. The causal explanation of their impact on glycosylation can be various, including product, metabolism, proteome and physiology-associated mechanism.

In the middle part of the thesis, both literature reviews and experimental applications were provided to demonstrate how to use omics data and implement systems biology to understand biological activities, especially N-glycosylation in CHO cells.

In the last part of the thesis, the second strategy that apply genetic and metabolic engineering approach to improve N-glycosylation capability of CHO cells was also presented promising results. Overexpression of either N-acetylglucosaminyltransferase I (GnTI) in CHO cells was confirmed to improve the maturation of glycans in mAb.

In conclusion, integrating the concept of systems biology with process and metabolic engineering has been demonstrated through a number of studies to be a superior way of controlling and optimizing N-glycosylation of CHO-derived recombinant therapeutic protein.