DESIGNING AN ARTIFICIAL GOLGI REACTOR FOR CELL-FREE GLYCOSYLATION

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Key Words: Golgi reactor, cell-free glycosylation, glycosyltransferases immobilization

Glycosylation of therapeutically relevant proteins such as monoclonal antibodies (mAbs), is critical as it can offer increased drug efficiency, efficacy and half-life. Therefore, the production of modern biotherapeutics focuses on controlling the protein glycosylation profile using various methods. Currently, the dominating method is the traditional cell-line engineering of host cells such as mammalian cells. The main goal is to produce mAbs with a human-like glycosylation pattern. However, this approach often struggles due to high sensitivity to the fermentation environment making it difficult to scale up and control. The latter can lead to structural heterogeneity amongst the products which can be immunogenic. In addition to the in vivo methods, there are many in vitro techniques such as chemoselective or enzymatic glycosylation. However, they are often limited by the difficult implementation and, as before, product heterogeneity due to lack of control over the enzymatic reactions.

In line with the need to control glycosylation in the production of therapeutic proteins, we propose an artificial Golgi reactor for in vitro glycosylation. By expressing selected glycosyltransferases and immobilizing them on solid supports we can achieve sequential enzymatic reactions required for protein glycosylation. The spatial separation will allow strict control over the reaction conditions while addressing enzyme promiscuity. Both should enhance product quality. Furthermore, we aim to perform a single-step glycosyltransferases purification/immobilization. Thanks to that, as well as the modularity of our design, the proposed system would be more sustainable and easily tailored for each application, thus producing any desired glycoform to homogeneity. A detailed mathematical approach to design and optimisation of the proposed artificial Golgi reactor focusing on mAb therapeutics has been published [1]. The authors report an optimisation of the reactor design and operational parameters that directs the whole process towards the desired glycan structure.
In this research project, we have achieved expression and in vivo biotinylation of Nicotiana Tabacum GnTI (NtGnTI) and human GalT in E. coli. The biotinylated enzymes were successfully bound to streptavidin beads and used for artificial glycan synthesis. NtGnTI and GalT reacted in a sequential fashion to produce the glycan GalGlcNAcMan5GlcNAc2, as confirmed with MALDI/TOF MS analysis. In the future, we aim in extending the pathway of immobilized enzymes thus demonstrating the importance of this novel platform for in vitro glycosylation.

References:
[1] Klymenko, O. V., Shah, N., Kontoravdi, C., Royle, K. E. & Polizzi, K. M. Designing an Artificial Golgi reactor to achieve targeted glycosylation of monoclonal antibodies. AIChE J. 62, 2959–2973 (2016).