DESIGNING AN ARTIFICIAL GOLGI REACTOR FOR CELL-FREE GLYCOSYLATION[1]

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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 can allow strict control over the reaction conditions while addressing enzyme promiscuity. Both should enhance product quality. Furthermore, because the enzymes are immobilized, we can achieve a one-step protein purification/immobilization. The latter as well as the modularity of our design, makes the system more sustainable and easily tailored for each application, thus producing any desired glycoform to homogeneity.

A detailed approach to the design and optimisation of such a reactor based on mathematical modelling focusing on monoclonal antibody (mAb) therapeutics has been published[1]. Optimisation of reactor design and operational parameters directs the whole process towards the desired glycan structure.

In this PhD research, 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 were 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).