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Engineering of the Chinese Hamster Ovary (Cho) Cell Lines to Investigate Cellular Bottlenecks during Recombinant Protein Expression

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Cultured mammalian cells such as Chinese hamster ovary(CHO)have become the host of choice to produce and secrete biotherapeutic recombinant proteins as they encompass the potential to perform human-like PTMs and correctly fold and assemble complex proteins . However, currently one of the limitations that are observed in the production of immunoglobulins (Igs) is the low degree of N-glycans terminating in sialic acid. In order to overcome this impediment, we focussed on carrying out the below reaction so that the amount of sialic acid can be improved and so as to enhance the complete assembly of N-glycans.

R-Galactose(R-Gal)+Sialic acid(CMP-Neu5Ac)-(Sialyltransferase;STGal) → R-Gal-SA.

The study also focussed on investigating the various bottlenecks which obstruct the sialic acid pathway, such as under expression of α(2,6)STGal, decreased CMP-SA(Cyclic Monophosphate-sialic acid) and improper glycan attachments and faulty galactosylation in N,O-glycans. To investigate these features, we were successful in cloning the genes (B4GalT1 and C1GalT1 chaperone) and transfecting constructs for these into the CHO cells hence our results demonstrate that the cells expressing the genes of interest in the PCR, however did not show expression in the blots. Hence, engineering these systems enabled us to study the various bottlenecks and provide a suitable solution.