

Molecular, Genetic, and Process Approaches for Improving Secreted Pharmaceutical Protein Quality in *Komagataella phaffii*

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Biopharmaceutical products constitute a significant portion of the global bioeconomy. Compared to traditional synthetic small-molecule drugs, recombinant therapeutic proteins offer advantages like enhanced specificity and reduced side effects, and there has been tremendous growth in their innovation thanks to modern DNA technologies and AI-driven algorithms. While mammalian platforms such as Chinese Hamster Ovary (CHO) cells are commonly used for their high production titer and capability for complex post-translational modifications, their high cost of goods manufactured can greatly constrain biopharmaceutical global accessibility. The yeast *Komagataella phaffii* is the prime candidate for next-generation biomanufacturing for reasons including simpler host biology, reduced time to market, and better sustainability. Nevertheless, product quality, such as size/charge variants and non-human glycosylation, can be of major concern for proteins secreted from this host organism.

This thesis explores three different engineering approaches aimed at improving the quality of both aglycosylated and glycosylated proteins, with a particular focus on monoclonal antibodies, the leading class of protein biopharmaceuticals by both sales and innovation.

Firstly, we demonstrated significant quality improvements through molecular sequence engineering of aglycosylated monoclonal antibody backbones. By making informed, conservative mutations to two or three amino acid residues, we greatly reduced product-related variants from proteolysis and N-terminal variations. We further showed the comparability between yeast- and CHO-secreted products, providing a framework for rapid product development with this unconventional yeast.

Secondly, we applied CRISPR-Cas9 gene editing technology to humanize the glycosylation pathway of *K. phaffii*. We achieved homogeneous G0 glycosylation on a reporter peptide by resolving a previously unreported synthetic lethality via a transcriptomics-informed approach. Key challenges for monoclonal antibody glycosylation were also identified through further comprehensive pathway engineering.

Lastly, we examined the performance of glycoengineered *K. phaffii* strains under varied process conditions. Employing a machine learning algorithm, we improved the desired glycan abundance on a subunit vaccine candidate. The process-robustness of engineered strains suggests the potential of this host as a viable commercial biomanufacturing host.