Product and host engineering for low-cost manufacturing of therapeutic proteins in the yeast *Komagataella phaffii*

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The COVID-19 pandemic revealed a global need for affordable, accessible biologic medicines such as prophylactic vaccines and antiviral monoclonal antibodies. Indeed, two years after the emergence of SARS-CoV-2, access to vaccines and therapeutic proteins is still limited in low- and middle-income countries (LMICs). Long development timelines, expensive manufacturing requirements, and stringent storage conditions all contribute to the high cost of biologic medicines generally. While therapeutic proteins may have higher stability and less stringent storage requirements than newer modalities like therapeutic RNAs, complex therapeutic proteins like antibodies are typically produced in mammalian cells, which increase costs due to sterility requirements, expensive feedstocks, and long cell line development timelines. Additionally, there is limited total global capacity for manufacturing therapeutic protein in mammalian hosts.

Alternative hosts have potential to increase the accessibility of therapeutic proteins by shortening development timelines, lowering manufacturing costs, and increasing the global manufacturing capacity. Single-celled eukaryotes such as yeasts represent a "sweet spot" for protein manufacturing—yeasts grow to high cell densities on inexpensive feedstocks like bacteria, and are capable of post-translational modifications and secretion of products into the extracellular space like mammalian cells. One yeast, *Komagataella phaffii* (*Pichia pastoris*), is especially attractive due to a highly developed secretory pathway. *K. phaffii* is currently used for manufacturing of insulin and subunit vaccines in LMICs, and has recently been approved by the FDA for the manufacture of several complex therapeutic proteins, including a monoclonal antibody. Wider adoption of alternative hosts such as *K. phaffii* in both high-income countries and LMICs has been hampered by bespoke manufacturing challenges for unique therapeutic proteins such as subunit vaccines, and low upstream titers of complex therapeutic proteins with stringent quality requirements such as monoclonal antibodies.

In this thesis, we explore two engineering strategies to improve the manufacturing of therapeutic proteins in *K. phaffii*. First, we engineered the product sequences of several subunit vaccine antigens and monoclonal antibodies to improve quality and secreted titer without sacrificing therapeutic function. Second, we developed tools for genome engineering in *K. phaffii* and applied these tools to engineer strains with improved secreted productivity. These strains along with predictive knowledge of small modifications to product sequences will enable rapid development of manufacturing processes for a wide range of therapeutic proteins.

Lastly, we discuss an illustrative case study of integrated product and manufacturing process design. We applied product engineering and host strain engineering to enable low-cost manufacturing of a subunit vaccine candidate for COVID-19. Design of the drug substance for manufacturability enabled rapid technology transfer and scale up, and the vaccine candidate is currently in clinical trials. This success exemplifies the immediate impact of manufacturing in *K. phaffii* in LMICs, where the need for therapeutic protein interventions is greatest.