A multi-omics approach to improving productivity of therapeutic proteins in *Pichia pastoris* (*Komagataella phaffii*)

by

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Abstract

Market sizes for novel therapies and growing demand for existing treatments in emerging markets promise to challenge the current capacity for production of therapeutic proteins. Significant reductions in cost and development speed are required to serve patients in the developing world, promote rapid response to disease outbreaks, and pave the way for increasingly personalized medicines. Production of therapeutic proteins in CHO is unlikely to meet future requirements for capacity, cost, and speed. Alternative hosts must be developed to supplement and sustain biomanufacturing. The yeast *Komagataella phaffii* is the only alternative host that blends three critical features: fast growth to high cell density on inexpensive media, secretion of complex human proteins at reasonable titers with minimal host modifications, and regulatory precedent for the production of several marketed biologics. A new approach is needed, however, to convert these opportunities for *K. phaffii* into a meaningful impact on global biomanufacturing. Decades of research in this host have not yet translated into widespread use for complex but important proteins such as monoclonal antibodies (mAbs).

In this thesis, we define a new approach, *strain engineering*, which leverages a multi-omics approach and modern genetic tools to rapidly and rationally understand cell biology and engineer solutions. We apply this strain engineering approach through three levers: smarter molecular design, selection of an optimal starting strain, and modifying the host genome for a product class.

In the first part of this thesis, we engineered the sequences of a trivalent rotavirus vaccine to mitigate glycosylation, aggregation, and truncation variants and improve product titers. Using *in silico* sequence analysis, RNA-Seq, and ribosome profiling, we were able to improve manufacturability without harming antigenicity solely through minimal sequence modifications.

In the second part, we engineered a novel, optimal base strain of *K. phaffii*. We characterized 11 variants of this yeast by whole-genome sequencing and RNA-Seq to identify functional genetic variants that influenced performance as a recombinant host. We combined beneficial features into a new, engineered strain with enhanced transformation and secretion efficiencies.

In the final part of this thesis, we develop tools that are necessary to engineer the host genome for the secretion of more complex proteins. First, we use ATAC-Seq and RNA-Seq to guide selection of optimal sites for integration of heterologous genes. Second, we identify initiation site (TIS) sequences for translational control of protein expression. These two tools, along with CRISPR/Cas9, enabled engineering of a new strain for the production of mAbs.

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