Title: Design of a cultivation medium for protein production in *Pichia pastoris* based on genome-wide biological understanding

Adoption of non-mammalian host organisms for biologic drug manufacturing could lead to step-changes in cost of manufacturing and volumetric productivity, increasing access to these life-saving drugs for large patient populations. One promising alternative host is *Pichia pastoris*, a methylotrophic yeast that is currently used to manufacture ten approved drugs worldwide. Its fast growth rate and ability to grow to high cell densities can enable fast production cycles, agile process development, and potentially low production costs. While *P. pastoris* has already been engineered to produce antibodies with human-like glycoforms, titers are still lower than those typically achieved with CHO cells. While standard fermentation processes for P. pastoris have been designed, several areas have limited investment to date. Few chemically-defined cultivation media have been reported for *P. pastoris* fermentation and all are minimal salt solutions. While several studies have demonstrated that addition of complex nutrients improves growth and productivity, defined compounds with similar effects have not been identified. Also, methanol feeding protocols for *P. pastoris* have only been developed for fed-batch operation and have not been studied for perfusion cultivation. In this thesis, we describe the design of a rich defined medium (RDM) for cultivation of *P. pastoris* through systematic screening and gene expression analysis. The use of RDM for expression of three proteins yields titers comparable to, or higher than, those in standard complex medium. We then outline a similar methodology for the optimization of individual amino acids and fatty acids in the medium. We also describe how a transcriptomic analysis of methanol feeding strategy in perfusion mode enabled the identification and alleviation of limiting biological processes. This work demonstrates how combining traditional process development strategies with genome-wide sequencing for *P. pastoris* leads to rapid improvement of fermentation processes. Continued progress in this area could lead to a new model for low-cost production of high-quality biologic drugs.