**Combined computational and experimental analysis to characterize impact of bioprocess conditions on Chinese Hamster Ovary (CHO) cell metabolism and site-specific N-linked glycosylation of an IgG with distinct Fab and Fc glycans**

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Monoclonal antibodies (mAbs) have revolutionized the field of medicine over the last two decades. MAbs have demonstrated extraordinary ability to treat diseases such as cancer, autoimmune diseases, and viral infections. The $100^{th}$ mAb was approved by the US FDA in 2021, with many more in clinical development. The increase in demand for mAbs, increase in number of mAbs in development, and the development of novel biotherapeutic modalities requires innovation in biomanufacturing for supply to keep up with demand. Biotherapeutic proteins are produced via biological processes that involve growing cells in bioreactors. Chinese Hamster Ovary (CHO) cells have emerged as the leading platform for producing glycosylated therapeutics. Development of these processes has traditionally relied on experimentation to determine optimal operating conditions. Over the last several years, mathematical models have demonstrated capabilities to aid with biotherapeutic process development. However, many of these mathematical models do not consider the effect of bioreactor parameters such as pH, dissolved oxygen, and temperature. The major focus of this thesis is to utilize mathematical models to elucidate the effect of these process parameters on CHO cell metabolism and N-linked glycosylation. This mechanistic understanding is further used to develop predictive mathematical models that have various applications to aid with process intensification.

Chapter 2 involves cultivating CHO cells at various bioreactor pH conditions and measuring the concentrations of viable cells, glucose, lactate, amino acids, mAbs, and ammonia. These measurements were utilized to perform flux balance analysis. The resulting fluxes calculated from flux balance analysis provided mechanistic insights into the effect of bioreactor pH on CHO cell metabolism. The mAb used in this study contains glycosylation sites in the Fab and Fc regions. The effect of any process parameter on site specific N-linked glycosylation has not been reported. Hence, we decided to utilize glycopeptide mapping to elucidate the effect of bioreactor pH on Fab and Fc glycosylation. Bioreactor pH had a complex effect on glycosylation. Certain glycan fractions were impacted at one site but not the other.

Several mathematical models for glycosylation have been developed over the last two decades. However, none of these models address site-specific N-linked glycosylation differences that can fundamentally arise from the glycosylation process. The work performed in chapter 3, led to the development of a site-specific N-linked glycosylation model. This model was used to elucidate the effect of bioreactor pH on Fab and Fc glycosylation. The model was also subsequently used to predict the effect of galactose and $MnCl_2$ supplementation on Fab and Fc glycosylation. These predictions were validated by performing fed-batch experiments in shake flasks.

Chapter 4 involved developing a mathematical model that can predict concentrations of viable cells, glucose, lactate, ammonia, amino acids, and mAb if provided with the bioreactor pH, seeding cell density, media composition, and feed addition time. A complex dynamic metabolic flux analysis model was constructed to achieve this aim. Chapter 4 also involves demonstrating the application of this model to predict intensified fed-batch and perfusion bioreactor performance by using a model trained only on fed-batch data. These predictions were successfully validated. This thesis involves utilizing computational and experimental approaches to develop tools for bioprocess development.