THESIS ABSTRACT

Regulation of complex biological processes aims to achieve goals essential for an organism's survival or to exhibit specific phenotypes in response to stimuli. This regulation can occur at several levels, such as cellular metabolism, signaling pathways, gene transcription, mRNA translation into proteins, and post-translational modifications. Systems biology approaches can facilitate integrating mechanistic knowledge and high-throughput omics data to develop quantitative models that can help improve our understanding of regulations at various levels. However, computational modeling of biological processes is challenging due to the vast details of the control processes with unknown details. The cybernetic modeling approach accounts for unknown control mechanisms by defining a biological goal that the system aims to optimize, and subsequently mathematically formulates the cybernetic goal.

This thesis aims to develop a mathematical framework that integrates a cybernetic model with novel information-theoretic methods to study the inflammatory response in mammalian macrophage cells. The inflammatory response of the body is a protective mechanism that fights off infecting pathogens by inducing the production of immune signaling proteins called cytokines and chemokines, as well as specific lipids known as eicosanoids. However, excessive levels of cytokines and eicosanoids may result in chronic inflammatory diseases such as hyper-inflammation syndrome, COVID-19, and asthma. Only a few studies have focused on modeling AA metabolism, and the developed models utilize Michaelis-Menten kinetics, or assume the linear form and can, at best, include control at the gene expression level only. The distinguishing feature of a cybernetic model is that by defining a cybernetic objective, it can account for control at multiple levels, including transcriptional, translational, and post-translational modifications.

The following paragraphs address a specific research problem, outline the approaches employed to study it, and summarize the key findings.

First, we studied the cellular response to inflammatory stimuli that produce eicosanoids—prostanoids (PRs) and leukotrienes (LTs)—and signaling molecules—cytokines and chemokines—by macrophages. A few studies suggest that targeting eicosanoid metabolism could be a promising new approach to regulating cytokine storm in COVID-19 infection. We developed a cybernetic model combined with novel information-theoretic approaches to study the integrated system of eicosanoids and cytokines. Our cybernetic model formulates a cybernetic goal, which requires the causal relationship between the eicosanoid and cytokine secretion processes; however, this causal relationship is unknown due to insufficient mechanistic information. We developed novel information-theoretic approaches (discussed later in detail) to understand the causality between eicosanoids and cytokines. The causality result from information theory suggests that Arachidonic acid (AA) may be the cause for initiating the secretion of cytokine TNFα. The model captured the data for all experimental conditions, including control, treatment with Adenosine triphosphate (ATP), (3-deoxy-d-manno-octulosonic acid)2-lipid A (KLA), and a combined treatment of ATP and KLA in mouse bone marrow-derived macrophages (BMDM). The cybernetic model also enhanced our understanding of enzyme dynamics by predicting their profiles. The results indicated that the dominant metabolites are PGD_2 (a PR) and LTB_4 (an LT), aligning with their corresponding known prominent biological roles during inflammation. Based on the causality and cybernetic model result and using heuristic arguments, we also infer that AA overproduction can lead to increased secretion of cytokines/chemokines. Consequently, a potential clinical implication of this study is that modulating eicosanoid levels could lower TNFα expression, suggesting eicosanoids could be a viable strategy for managing hyperinflammation in widespread inflammatory diseases.

Second, we studied the dynamics of the anti-inflammatory lipid mediators from eicosapentaenoic acid (EPA) metabolism, which can be beneficial in reducing the severity of diseases such as cancer, cardiovascular effects, and promoting visual and neurological development. This study employed a cybernetic model to study the enzyme competition between AA and EPA metabolism in murine

macrophages. The cybernetic model adequately captured the experimental data for control and EPA-supplemented conditions in RAW 264.7 macrophages. The cybernetic variables provide insights into the competition between AA and EPA for the COX enzyme. Predictions from our model suggest that the system undergoes a switch from a predominantly pro-inflammatory state in control to an anti-inflammatory state with EPA supplementation. A potential application of this study is utilizing the model estimation of the ratio of $[AA]_{switch}$ and $[EPA]_{switch}$ concentrations required for the switch to occur as 2.2, which aligns with the experimental observations and falls within the recommended range of 1-5 needed to promote anti-inflammatory response.

Third, we focused on predicting novel causal connections between AA and cytokines using time series analysis as mechanistic information connecting AA and cytokines is unknown. In this work, we developed Time delay Renyi Symbolic Transfer Entropy (TDRSTE), a novel model-free information-theoretic metric. We computed it from high-throughput omics datasets for bivariate non-stationary time series to quantify causal time delays. The TDRSTE method adequately estimated time delay for the synthetic dataset, captured causality for the real-world biological dataset of the AA metabolic network with a prediction accuracy of 80.6%, where it correctly identified 25 out of 31 connections, and detected novel connections between non-stationary lipidomics and transcriptomics profiles for eicosanoids and cytokines, respectively. The results indicate that AA may initiate the secretion of cytokines like TNFα, IL1α, IL18, and IL10. Conversely, cytokines such as IL6 and IL1β may have an early causal impact on AA. These findings suggest a potential causal link between AA and cytokines, paving the way for further exploration with more extensive experimental data in future investigations.

This thesis develops a theoretical framework integrating the cybernetic modeling technique with novel information-theoretic approaches to study the inflammatory response in mouse macrophages. As described in previous paragraphs, the success of the cybernetic framework in capturing the dynamic behavior of multiple processes serves to validate the idea that regulation is driven toward achieving cellular goals. The cybernetic framework can be applied to better understand the mechanisms underlying the normal and diseased states, and to predict the behavior of the inflammatory system given a perturbation.