TOPOLOGICAL METABOLIC ANALYSIS: A ROBUST OPTIMIZATION-BASED FRAMEWORK FOR METABOLIC PROCESS SYSTEMS ENGINEERING

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ABSTRACT

The number and diversity of high-value industrial applications for both prokaryotic and eukaryotic organisms continues to expand. Since the first recombinant protein therapy manufactured in mammalian cell culture was licensed for human use in 1986 (t-PA) the use of eukaryotic cell-cultures for the biological manufacture of high-value therapeutic compounds has become commonplace in biotechnology. Microbial cell-cultures also continue to gain relevance for the industrially valuable commodities such as biogas, hydrogen, bioethanol, and bioplastics. In each of of these cases, the network of metabolic reactions within the chosen organism is responsible for completing the desired chemical synthesis. It is therefore desirable to systematically engineer these networks to improve manufacturing processes. It is, however, well known that the complexity and robustness of metabolic networks (particularly eukaryotic networks) makes it difficult to ensure that any particular manipulation of the network actually elicits its intended effect. Quantitative modeling techniques for metabolic networks of arbitrary size and complexity are therefore necessary tools to better guide engineering strategies.

We introduce a new optimization-based modeling framework for the analysis of metabolic networks of arbitrary size and complexity. This framework derives from the state-space approaches for modeling of chemical process networks originally developed by Manousiouthakis and co-workers. Because a defining aspect of this approach is a comprehensive mathematical treatment of network topology, we name this new modeling technique topological metabolic analysis (TMA).

TMA defines a set of material-balance constraints describing the fundamental ways in which material (metabolites) may be distributed and transformed within a metabolic network whose reactions have known (or assumed) stoichiometries. The manner in which these balances are constructed not only permits quantitative modeling of reaction rates and the overall metabolite uptake and secretion rates, but also of the manner by which every network metabolite is distributed among and shared between each network reaction. This constraint model, like existing techniques, is consistently underdetermined and therefore must be paired with an objective function to identify a particular solution.

We therefore design a generalized quadratic "aggregate" objective function (AOF) offering a number of unique mathematical advantages. First, it does not require that any particular amount of experimental data be known in advance, enabling metabolic model-

ing even under circumstances where experimental data is scarce. Second, the convexity (or, more-precisely, semi-convexity) of the objective enables the identification and characterization of a potentially infinite number of topologically distinct network configurations, all of which are globally optimal solutions to the given modeling problem. Careful characterization of this family of solutions can offer insights into the robustness of the metabolic network.

We initially demonstrate the utility of our TMA modeling framework using a case study of bacterial metabolism (*Actinobacillus succinogenes*). We show that our TMA framework, using only external metabolite uptake and secretion measurements, identifies modeling solutions that both offer useful biological insight and compare favorably against solutions obtained using much less-convenient ^{13}C isotope tracing measurements.

We then show how TMA can be used to interrogate metabolic networks in ways not previously demonstrated using classical stoichiometric modeling methods. Employing a case-study of hybridoma metabolism, we examine the topology of a sample metabolic network, and show that multiple topologically-distinct network configurations (each of which is equivalently optimal in reproducing experimental observations) can be identified using TMA. We further prove that these alternate network configurations are not mathematically distinguishable using existing stoichiometric modeling approaches. We explore matters of both under- and over- completeness in metabolic reconstructions, and present tools offered within our TMA framework to address each. We demonstrate how the use of a linear term within our quadratic aggregate objective function (AOF) can be implemented to combine experimental observations with either known biological motives or purely engineering-based goals to generate modeling solutions of use if experimental data is scarce, or when probing a particular metabolic network for fundamental engineering limitations.

We then apply our TMA modeling framework to characterize the differential metabolism of three Chinese Hamster Ovary (CHO) clonal variant cell lines experimentally known to exhibit substantial differences in nutrient uptake and secretion, growth rate, biomass composition, and productivity of a recombinant humanized IgG antibody product. Since no suitable metabolic reconstruction for this species is yet available, we develop our own comprehensive metabolic reconstruction based upon available genomic data for *Mus musculus*. Using TMA, we accurately model the experimentally observed behavior of the three variant CHO cell lines. We are further able to use our modeling results to develop

insight into the fundamental differences in both carbohydrate and amino acid metabolism among these cell lines. Finally, by exploiting relative weighting of terms within our TMA aggregate objective, we characterize the most metabolically "relaxed" state for each cell line, and show each to be substantially different from its corresponding experimentally observed state.

Finally, we explore a unique application of our TMA framework; the rational design of an aerobic microbial fuel cell. Using a basic metabolic reconstruction describing the growth of a model microbe (*Pseudomonas putida*) within such a fuel cell, we evaluate a number of both aerobic and anaerobic operating conditions utilizing variety of substrates. We ultimately show that coulombically efficient aerobic operation of such a device can, in theory, be made possible through the inactivation of electron-transport chain complex IV.