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A flexible state-space approach for the modeling of metabolic networks II: Advanced interrogation of hybridoma metabolism ☆

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ABSTRACT

Having previously introduced the mathematical framework of topological metabolic analysis (TMA) – a novel optimization-based technique for modeling metabolic networks of arbitrary size and complexity – we demonstrate how TMA facilitates unique methods of metabolic interrogation. With the aid of several hybridoma metabolic investigations as case-studies (Bonarius et al., 1995, 1996, 2001), we first establish that the TMA framework identifies biologically important aspects of the metabolic network under investigation. We also show that the use of a structured weighting approach within our objective provides a substantial modeling benefit over an unstructured, uniform, weighting approach. We then illustrate the strength of TAM as an advanced interrogation technique, first by using TMA to prove the existence of (and to quantitatively describe) multiple topologically distinct configurations of a metabolic network that each optimally model a given set of experimental observations. We further show that such alternate topologies are indistinguishable using existing stoichiometric modeling techniques, and we explain the biological significance of the topological variables appearing within our model. By leveraging the manner in which TMA implements metabolite inputs and outputs, we also show that metabolites whose possible metabolic fates are inadequately described by a given network reconstruction can be quickly identified. Lastly, we show how the use of the TMA aggregate objective function (AOF) permits the identification of modeling solutions that can simultaneously consider experimental observations, underlying biological motivations, or even purely engineering- or design-based goals.

1. Introduction

Since the first recombinant protein therapy manufactured in mammalian cell culture was licensed for human use in 1986 (t-PA) (Vehar et al., 1986), the use of eukaryotic cell cultures for the biological manufacture of high-value therapeutic compounds has become commonplace in biotechnology. Mammalian cell cultures, particularly Chinese hamster ovary (CHO) and NS0 or Sp2/0 murine-myelomas, are the most industrially prevalent platforms for therapeutic protein production (Chu, 2001), primarily due to their ability to generate the glycosylation patterns needed for human efficacy. While there has been some recent success in obtaining similar glycosylation patterns in plant (Bakker et al., 2001; Ko et al., 2008; Jamal et al., 2009) and yeast (Hamilton and Gerngross, 2007; Potgieter et al., 2009) cells, these technologies are still largely developmental.

The development of cell-culture-based manufacturing processes has been characterized over the years principally by "trial-and-error" experimentation aimed towards identifying growth-medium formulations or growth conditions capable of sustaining high viable cell concentrations for as long as possible (Liu et al., 2001; Altamirano et al., 2006). Metabolic models and other quantitative strategies have only more recently been employed for the rational selection of these same parameters (Altamirano et al., 2004; Liu and Chang, 2006; Xie and Wang, 2006). Even still, medium- and condition-based process improvements are notoriously cell-line-specific, and often do not generate similar improvements for different cell-lines secreting different proteins. Moreover, methods for improving viable cell density address only one half of the overall productivity problem; it is equally important to improve the per-cell product yield.

As methods in genetic engineering have improved, process improvements through direct gene manipulation have become possible. Techniques that improve protein yield by amplifying the copy number of target genes (e.g. dihydro-folate-reductase, DHFR) have already become commonplace. Genetic techniques directed towards promoting the transcription rate of target genes have also been reported (Koduri et al., 2001; Running Deer and Allison, 2004).

☆ Mathematical symbols: All mathematical symbols appearing in this paper are as defined in the previous work (Baughman et al., this issue).

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Nomenclature

Metabolite abbreviations

ACoA	acetyl-coenzyme-A
ATP	adenosine-triphosphate
ARG	arginine
ALA	alanine
MAB	monoclonal-antibody
ASN	asparagine
ASP	aspartate
TC	carbohydrate
CHOL	cholesterol
CIT	citrate
CO_2	carbon-dioxide
CYS	cysteine
DNA	deoxy-ribonucleic-acid
E4P	erythrose-4-phosphate
$FADH_2$	reduced-flavin-adenine-dinucleotide
FA	fatty-acid
F6P	fructose-6-phosphate
GLC	glucose
GAP	glyceraldehyde-3-phosphate
GLN	glutamine
GLU	glutamate
GLY	glycine
HIS	histidine
HYP	hydroxy-proline
ILE	isoleucine
LAC	lactate
LEU	leucine
PL	phospholipid
LYS	lysine
MET	methionine
NADH	reduced-nicotinamide-adenine-dinucleotide
NADPH	reduced-nicotinamide-adenine-dinucleotide-phosphate
NH_3	ammonia
O_2	oxygen
OMA	oxaloacetate/malate
PHE	phenylalanine
PRO	proline
PROT	cellular-protein
PYR	pyruvate
PEP	phospho-enol-pyruvate
R5P	ribose-5-phosphate
RNA	ribonucleic-acid
Ru5P	ribulose-5-phosphate
S7P	sedoheptulose-7-phosphate
SER	serine
THR	threonine
TRP	tryptophan
TYR	tyrosine
VAL	valine
X5P	xylulose-5-phosphate

There have also been efforts towards improved process yield by manipulation of genes and enzymes involved in metabolic processes other than transcription and translation of the target genes (Paredes et al., 1999; Wong et al., 2006; Wlaschin and Hu, 2007).

As a result of such efforts, it is 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 (Bailey et al., 2002; Park et al., 2008). Quantitative modeling techniques for metabolic networks of arbitrary size and complexity are therefore appealing as tools to better guide such engineering strategies.

The majority of modeling approaches currently in use, including metabolic flux analysis (MFA) and flux balancing analysis (FBA), derive from a fundamental stoichiometric balancing technique originally described by Palsson and co-workers (Savinell and Palsson, 1992b,a; Varma and Palsson, 1994b,a).

While these approaches have proven useful, they nonetheless suffer from a number of mathematical limitations. Among these is a sensitivity to the completeness of the reconstructed metabolic network used to model the system of interest. Because the modeling constraints in a stoichiometric balancing method rely directly upon the matrix of stoichiometric coefficients describing the reactions present, the ability of the model to adequately capture certain metabolic behaviors is contingent upon inclusion of the proper reactions (Kauffman et al., 2003; Puchalka et al., 2008; Raman and Chandra, 2009). This holds particularly true when stoichiometric models are integrated with data from isotopic tracing experiments (e.g. ^{13}C NMR or MS), since omitting certain reactions in that case can substantially alter interpretation of the data (van Winden et al., 2001; Zamboni et al., 2009).

Another limitation of classical stoichiometric modeling approaches is an inherent inability to quantitatively describe the topology of metabolic networks. By quantifying only network stoichiometry ($\underline{\underline{s}}$), reaction rates ($\underline{\mathcal{R}}$), and overall metabolite uptake and/or secretion rates ($\underline{k}$), these models can reveal the relative activities of each reaction in the network, but not the manner in which the reactions are interconnected by the sharing of metabolites.

In this work, we demonstrate how topological metabolic analysis (TMA), a novel optimization-based modeling framework previously introduced (Baughman et al., this issue), can be used to interrogate metabolic networks in ways not previously demonstrated using classical stoichiometric modeling methods. Employing illustrative case-studies of hybridoma metabolism (Bonarius et al., 1995, 1996, 2001), 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 show that these alternate network configurations are not mathematically distinguishable using existing stoichiometric modeling approaches.

We then discuss the completeness of metabolic network reconstructions, and how incomplete reconstructions can negatively impact model quality. Specifically, by exploiting the unique manner in which metabolite inputs and outputs are represented within TMA, we easily identify metabolites whose metabolism is not adequately described by the given reconstruction, and which thereby prohibit the reconstruction from adequately modeling experimental observations.

Lastly, we demonstrate how the use of an aggregate objective function (AOF) can 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.

2. Theory and methods

2.1. Topology of metabolic networks

We note that the mathematics underlying topological metabolic analysis (TMA) as a modeling framework have been previously