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On the Computation of Minimal Cut Sets in Genome Scale Metabolic Networks

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Abstract— **A cut set for an objective reaction in a metabolic network is a set of reactions whose knockout disables flux through that reaction at steady state. Cut sets represent a particular type of failure mode of a metabolic network and may correspond to novel drug targets. In this paper, we demonstrate how cut sets can be obtained from the computation of sub-Elementary Modes (sub-EM). The sub-EM's of a metabolic network are the Elementary Modes (EM) of a submatrix of the stoichiometry matrix formed by taking a subset of its rows. Sub-EM's emerge naturally in the intermediate steps of the standard tableau algorithm for computation of EM, and are thus obtainable for a network of any size. By employing properties of the feasible flux cone, we show how cut sets for a reaction can be constructed by enumerating minimal hitting sets for the sub-EM's containing that reaction. Though the resulting cut sets are not guaranteed to be minimal, they can be reduced to minimality via a second linear programming pruning step. We demonstrate the applicability of this approach to a recent genome scale metabolic model of *E.coli*.**

I. INTRODUCTION

The metabolic network is the biochemical machinery with which a cell transforms a limited set of nutrients in its environment into the multitude of molecules required for growth and survival. It consists of hundred to thousands of small molecule species intricately linked by an even larger set of biochemical reactions. The expansive and highly connected nature of this important cellular system greatly limits the degree of insight that may be gained from the isolated study of a single component or module. The first step towards systems-level understanding of metabolism is the construction of a model that captures what is known regarding an organism's small molecule biochemistry and its underlying genetics. The advent of sequencing technology combined with general improvements in the organization of biological information [9], [14] has allowed the building of such *genome-scale metabolic models* for numerous microbial organisms, including *E. coli*, *S. cereviseae*, *H. pylori*, and S. aureus [17], [19], [4], [5], [15], [16], [10].

Current approaches to the study of genome-scale metabolic networks employ an analysis of feasible and optimal reaction fluxes through the network at steady state, subject to structural, thermodynamic, and flux capacity constraints [14], [16]. Given these constraints, the flux configuration through the network is limited to a polyhedral cone, which can be checked for non-emptiness to characterize the production capacity of the network [7], [8], analyzed via flux balance analysis (FBA) to find points that maximize biomass production [21], [14], and probed using mixed-integer linear programming to find minimal reaction sets that support biomass production [3]. Alternatively, one can directly identify the elementary functional units of a metabolic network using a convex decomposition of the polyhedral cone representing feasible network states [18]. The most prevalent notions of such units are elementary flux modes (EM) and extreme pathways (EP) [20], [17], [13].

A gene or reaction is considered *essential* for a given physiological function if its knockout abolishes that function. Relatively few metabolic genes or reactions can be considered essential for growth of an organism in rich media. However, a gene or reaction that that may not be essential "alone" may be still be part of one or more "essential sets". Klamt and Gilles refer to such sets of reactions as *minimal cut sets*, since knockout of that set of reactions abolishes (i.e. "cuts") the physiological function, while knock out of any of its subsets preserves it [12]. Unlike minimal reaction sets and EM / EP, which represent the basic functional subunits of a metabolic network, minimal cut sets represent its most simple "failure modes" [3], [11].

Though execution of a linear program can validate whether a given reaction set is a cut set for an objective reaction, use of linear programming for the design of novel cut sets requires a brute force search through all reaction subsets (i.e. brute force FBA). The latter approach is reasonable for determining essential reactions (i.e. single reaction cut sets) but scales poorly in the search for more complex (i.e. higher cardinality) cut sets. For example, a network of 1000 reactions yields 1000 potential single knockouts, however the set of potential double knockouts approaches 1 million, and the set of potential triple knockouts approaches 1 billion.

A more systematic approach for generating cut sets is the minimal cut set (MCS) algorithm of Klamt and Gilles [12], which employs the elementary modes (EM) of a metabolic network to determine minimal cut sets for a particular objective reaction. Underlying the MCS algorithm is the principle that a minimal cut set R for an objective reaction j is a minimal hitting set for all j-containing EM [11]. As a result, MCS can be enumerated for a network through the simple application of a minimal hitting set algorithm to the collection of j-containing EM. The main limitation of this approach is the difficulty of calculating EM for large networks (i.e. larger than 200 reactions) given current computing resources and algorithms. This limitation prohibits the application of Klamt and Gilles method to genome-scale networks, rendering brute-force FBA the only current method

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available for genome-scale cut set computation.

In this paper, we demonstrate how cut sets can be obtained from the computation of sub-elementary modes (sub-EM's). The sub-EM's of a metabolic network are the elementary modes of a submatrix of the stoichiometry matrix formed by taking a subset of its rows. Alternatively stated, sub-EM's are flux configurations that place only a subset of species in the system at steady state. Sub-EM's naturally emerge in the intermediate steps of the tableau algorithm for EM computation [20], and are thus obtainable for a network of any size. By employing properties of the feasible flux cone, we show how cut sets for a reaction j can be constructed by enumerating minimal hitting sets for j-containing sub-EM's. Though the resulting cut sets are not guaranteed to be minimal, they can be reduced to minimality via a second linear programming based step. As we show, this method offers a practical approach for minimal cut set computation in genome-scale metabolic networks.

We demonstrate the applicability of this approach to a recent genome scale metabolic model *E. coli* iJR904 [16]. Our results reveal many complex (i.e. 2 or more reactions) minimal cut sets for the biomass reaction in each model. Knockouts of these reaction sets is predicted to be lethal by the *in silico* model, while knockouts of any of their subsets are predicted to be viable. These results lend insight into the function and vulnerabilities of several microbial metabolic networks. Furthermore, our results suggest important experiments for model validation and represent potential targets for drug design.

II. PROBLEM FORMULATION AND APPROACH

We represent a mass-balanced metabolic network of n chemical reactions involving m metabolites in a stoichiometry matrix $S \in \mathbb{R}^{m\times n}$ [14]. In our formulation, the matrix S incorporates stoichiometric information about all exchange reactions (uptake and secretion) and about the maintenance and growth reactions. Each entry S_{ij} specifies the stoichiometric coefficient for metabolite i in reaction j, which is negative for substrates and positive for products. We represent the flux distribution through the reactions of the network by $v \in \mathbb{R}^n$, where a component v_j corresponds to the flux of reaction complex passing through reaction j. The concentrations of species in the system at time t are denoted by $x(t) \in \mathbb{R}^m_+$. Finally, thermodynamic constraints restrict a subset of reactions $T \subseteq N$ to be irreversible. Under these assumptions, the rate of change in time of species concentrations is given by:

$$\dot{x} = Sv,\ v_T \geq 0. \tag{1}$$

Metabolic reactions occur at a fast rate with respect to cell regulatory and environmental changes. When modeling at the slower time scale it is reasonable to apply the *quasi-steady state assumption*, under which we have:

$$Sv = 0,\ v_T \geq 0. \tag{2}$$

A (steady state) metabolic network with stoichiometry S and set of irreversible reactions T will be denoted for short by (S, T). The set of all *feasible fluxes*

$$K = \{v \in \mathbb{R}^n \,|\, Sv = 0,\ v_T \geq 0\} \tag{3}$$

is a polyhedral cone in $\mathbb{R}^n$.

Definition 1: A set of reactions $C \subseteq N$ is a *cut set* for an objective reaction $j \in N$ in system (S, T) if

$$v_C = 0 \rightarrow v_j = 0, \forall v \in K. \tag{4}$$

A cut set C is minimal if no proper subset of C is a cut set.

In this paper we consider the following problem:

Problem 1: Compute minimal cut sets for a target reaction $j \in N$ in a metabolic network (S, T).

A "brute force" approach commonly used to solve the latter problem involves using flux balance analysis (FBA) to test every possible reaction combination to determine whether it "cuts" the objective reaction j. To determine all minimal cut sets of cardinality one (essential reactions), one has to decide the non-emptiness of the set $\{v \,|\, v \in K, v_i = 0, v_j \neq 0\}$ for all $i \in N$, $i \neq j$, which can be achieved by solving linear programs. For example, if the solution to both linear programs $\max_v v_j$ and $\min_v v_j$ with constraint set $\{v \,|\, v \in K,\ v_i = 0\}$ is zero, then this is equivalent to i being an essential reaction for j (minimal cut set of cardinality 1). To determine all minimal cut sets of cardinality two, all pairwise combinations $\{i, k\} \in N$ $(i \neq k \neq j \neq i)$ not containing essential reactions have to be considered. Again, $\{i, k\}$ is a minimal cut set for j if and only if the solution to both linear programs $\max_v v_j$ and $\min_v v_j$ with constraint set $\{v \,|\, v \in K, v_i = v_k = 0\}$ yields 0. The procedure continues for higher cardinality combinations.

Though applicable to the study of single and double knockouts, the brute-force FBA approach fails to be useful for generation of higher order cut sets in genome-scale metabolic models, which generally have greater than 1000 reactions. The solution we propose in this paper is a "rational" approach for discovering cut sets that employs computation of generators of a polyhedral cone and the subsequent application of a minimum hitting set algorithm. The idea is not new - this approach was proposed by Klamt and Gilles in [12], [11]. However, because of limitations due to complexity, their approach also cannot be applied to genome scale metabolic models. In this paper, we propose a two step procedure. In a first step, we determine cut sets based on the computation of generators for polyhedral cones over-approximating K. In the second, we use linear programming to prune these sets and make them minimal. We cannot guarantee that we compute all the minimal cut sets for j with our approach. However, as shown in Section V, our approach leads to the computation of a large set of non-trivial minimal cut sets at genome scale.

III. PRELIMINARIES

A. Tableau algorithm for elementary mode computation

The tableau algorithm described in [20] is the standard approach for computing generators of polyhedral cones as-

sociated with metabolic networks. In this case, the generators are called *elementary modes* (EM). This algorithm is a modification of the classical Gaussian elimination algorithm used to compute the null space of a matrix. Here we give a brief description of the algorithm, which is necessary to understand the rest of the paper.

The tableau algorithm takes as arguments the matrix $S \in \mathbb{R}^{m \times n}$ and the index set $T \subset N$, and returns a set of elementary modes $E(K)$ of K, corresponding to a collection of rays and lines that generate K. If $T = N$, the output corresponds to the extreme rays of K.

The tableau algorithm proceeds in an iterative fashion by computing generators $E(K^i)$ for a series of cones $K^i \subset \mathbb{R}^n$, $i \in \{0, \ldots, m\}$ given by:

$$K^i = \{v \mid S_{M_i} v = 0,\ v_T \geq 0,\ A_i \subseteq M,\ |M_i| = i\} \quad (5)$$

The algorithm is seeded with the initial cone $K^0 = \{v \in \mathbb{R}^n \mid v_T \geq 0\}$. The initial collection of generators $E(K^0)$ consists of rays pointing in the directions of Euclidean basis vectors $e^j \in \mathbb{R}^n, j \in T$ and lines corresponding to the remaining Euclidean basis vectors. At each iteration $i \in \{1, \ldots, m\}$, the generators $E(K^i)$ are computed from the analysis of rays and lines in $E(K^{i-1})$ in three steps. In the first step, each ray / line in E^{i-1} is tested to determine whether it belongs to the hyperplane $S_i v = 0$. Rays and lines in $E(K^{i-1})$ that belong to this hyperplane are added to the collection $E(K^i)$. In the second step of iteration i, the remaining rays and lines in $E(K^{i-1})$ are paired to compute intersections of cone K^{i-1} with the hyperplane $S_i v = 0$. The final step of each iteration involves pruning of "decomposable" or dependent generators from $E(K^i)$. This step ensures minimality or near-minimality of the set $E(K^i)$ as a V-representation for the cone K^i.

Following iteration m, the tableau algorithm terminates, having computed a set of rays and lines (elementary modes) that generate the polyhedral cone $K = K^m$. The costliest part of the tableau algorithm is the pruning step applied at the end of each iteration. Furthermore, the memory requirements of this algorithm are prohibitive for application to large metabolic networks, given the large number of generators for many polyhedral cones [6].

B. Minimal hitting set for a collection of lines and rays

In combinatorics, a *hitting set* of a collection of sets $\mathcal{C}$, each taken from a discrete universe of items U, is a set $H \subset U$ that intersects every set in $\mathcal{C}$. H is a *minimal hitting set* if none of its subsets are hitting sets of $\mathcal{C}$.

In this paper, we refer to hitting sets $H \subset N$ of a collection of elementary modes $E \subset \mathbb{R}^n$ [11].

Definition 2: A set $H \subset N$ is a hitting set for for a collection of elementary modes $E \subset \mathbb{R}^n$ if H intersects $NZ(r)$ for every $r \in E$. In addition, H is a *minimal hitting set* for E if none of its subsets are hitting sets.

Algorithm 1, a modification of the Berge algorithm for hypergraph traversal, provides a simple and fast procedure for computing the set $\mathcal{H}$ of all minimal hitting sets of cardinality k or less of a collection E of generating vectors (i.e. EM or sub-EM) [2].

Algorithm 1 $\mathcal{H} = MinHit(E, k)$

```
/* Input: E = {r^1, ..., r^|E|}, a collection of lines and/or rays in R^n */
/* Output: H, a collection of minimal hitting sets of E with cardinality k or less*/
/* H is initialized with the empty set.*/
H = {∅}
for all j ∈ 1, ..., |E| do
  for all i for which r_i^j ≠ 0 do
    /* Expand sets in H so that they "hit" nonzero */
    /* components of ray / line j of E */
    for all H ∈ H for which i ∉ H do
      replace H with H ∪ {i} in H
    end for
  end for
  /* Prune duplicate, non-minimal, and high cardinality sets from H */
  for all H ∈ H do
    if |H| > k OR there exists Ĥ ∈ H for which Ĥ ⊆ H then
      remove H from H
    end if
  end for
end for
```

IV. COMPUTATION OF MINIMAL CUT SETS

Klamt and Gilles [12], [11] showed that a minimal hitting set $C \subset N$ for a collection of elementary modes E (see Definition 2) with positive entry in position j (such an elementary mode is said to contain reaction j) is a minimal cut set for reaction j in system (S, T) (see Definition 1).

In this section, we demonstrate how application of Klamt and Gilles' cut set criteria to intermediate results of the tableau algorithm for EM computation (described in Section III-A) allows identification of cut sets for (S, T).

At each iteration $i \in M$, the tableau algorithm determines a collection of generators $E(K^i) \subset \mathbb{R}^n$ for the cone K^i in equation (5). $E(K^i)$ corresponds to a collection of non-decomposable lines and rays that obeys the quasi steady state assumption for a *subset* $A_i \subseteq M$ of species in the system. K^i can also be seen to correspond to the feasible flux cone of the system (S_{A_i}, T) with elementary modes $E(K^i)$. The rays and lines in $E(K^i)$ are therefore called *sub-elementary modes* (sub-EM).

By applying Klamt and Gilles' criteria to $E(K^i)$, a minimal hitting set R for the collection of j-containing sub-EM in $E(K^i)$ is a minimal cut set for j in the system (S_{A_i}, T). This means that R is a minimal set of reactions satisfying the relation:

$$v_R = 0 \implies v_j = 0, \quad \forall v \in K^i \quad (6)$$

However, from Equations (3) and (5), it is clear that K^i over-approximates K, i.e.

$$K \subseteq K^i. \tag{7}$$

R thus also satisfies equation (4) and is a cut set for j in the full system (S, T). As a result, the application of $MinHit$ (Algorithm 1) to a collection of j-containing sub-EM taken from any iteration i of the tableau algorithm is guaranteed to yield valid cut sets for an objective reaction j.

Algorithm 2 $MCS = BruteCut(\mathcal{H}, j, S, T)$

```
/* Input: H, a collection of cut sets for reaction j in
system (S,T) */
/* Output: MCS, a collection of minimal cut sets for j
*/
MCS = empty collection
push sets in H onto stack ToDo
while ToDo is not empty do
  H = pop set from ToDo
  /* Run linear programs (i.e. flux balance analysis) or
  check lookup table to find any subsets of H that are
  also cut sets for j */
  D = collection of size |H| − 1 subsets of H that are
  cut sets for j in system (S,T)
  if D is empty then
    add H to collection MCS
  else if D contains one set then
    add sole set in D to MCS
  else
    push sets in D onto stack ToDo
  end if
end while
```

A collection of sub-EM arises from an incomplete analysis of the metabolic network, and thus only contain partial information about its dynamics. As a result, analysis of sub-EM leads to a sufficient but *not necessary* criterion for determining whether a reaction set C constitutes a cut set for reaction j. This results in two caveats regarding the "quality" of cut set obtained from the analysis of pre-elementary modes: 1) not all cut sets are guaranteed to be found and 2) cut sets that are found are not guaranteed to be minimal.

As may be intuitively expected, the quality of cut sets directly depends on the "quality" of sub-EM. The latter, in turn, depends on the iteration i of the tableau algorithm from which the sub-EM are gathered, and the row order employed by the tableau algorithm (see Section III-A). A general rule of thumb for the quality of sub-EM is that later iterations yield "higher quality" sub-EM; in other words, "naive" sub-EM gathered from an early iteration of the algorithm will yield fewer cut sets that are farther from being minimal, while "mature" sub-EM gathered from a later iteration will yield larger numbers of cut sets that are closer to being minimal.

Since minimality of cut sets obtained from sub-EM is not guaranteed, we propose a brute-force post processing algorithm for 1) checking minimality of cut sets and 2) reducing them to their minimal subsets if they are not minimal. The procedure is iterative (Algorithm 2). For a given cut set H, subsets of cardinality $|H| - 1$ are tested to see if they are cut sets. If none is found, then the initial cut set H is minimal (see Definition 1). Otherwise, the new found cut sets are tested for minimality in the same way. To check whether a set $H \subset N$ is a cut set for reaction $j \in N$, we need to check the feasibility of the set $\{v \in K \,|\, v_H = 0, v_j \neq 0\}$, for which we can use linear programming (i.e. flux balance analysis) as described in Section II.

V. RESULTS

We use the computational framework developed in this paper to compute minimal cut sets for biomass production in the *E. coli* iJR904 genome scale model [16], which has 761 metabolites involved in 1835 chemical reactions (*i.e.,* the stoichiometry matrix S in equation (3) is 761×1835). These reactions represent the inflow, outflow, and inter-conversion of small-molecule chemical species in an *E. coli* cell grown in a rich nutrient media. For each species in the model, there exists a "sink" reaction that represents its dilution during growth and consumption by macromolecular processes. Of the 761 species, 49 correspond to "biomass components" that are considered to be essential substrates for survival and growth [16]. In this model, knock out of biomass production (and thus growth and survival) corresponds to "cutting" flux through *at least one* of the sinks corresponding to an essential biomass component.

We computed sub-EM's using the tableau algorithm and applied a local greedy optimization strategy at each iteration to minimize computation as described by Bell *et al.* [1]. Sub-EM's were collected once progress in the algorithm reached memory limit or suffered very significant slow down. Cut sets for each biomass component sink j were computed by applying the $MinHit$ (Algorithm 1) to the collection of j containing sub-EM. For computational ease and biological relevance we limited $MinHit$ to generating cut sets of size 10 or below. Minimal cut sets for each j were computed by applying algorithm $BruteCut$ (Algorithm 2) to the output of $MinHit$. To solve the linear programs involved in $BruteCut$, we used the semidefinite programming package SeDuMi (http://sedumi.mcmaster.ca/).

In a preliminary computation, we used brute-force FBA to determine that there are 92 "essential" reactions whose knockout disables production of at least one biomass component in rich media (i.e. single reaction cut sets).

Execution of the tableau algorithm to iteration 709 (of 761) on *E. coli* iJR904 in rich media yields 157207 sub-EM. 1375 complex MCS of cardinality 2 to 10 emerged from the application of $MinHit$ and $BruteCut$ to these results. In this process, execution of $BruteCut$ involved only 79,328 linear optimization steps. In comparison, brute force FBA required over 1.2 million linear optimizations just to generate minimal cut sets of cardinality 2. The number required to compute larger cardinality sets would likely be exponentially higher.

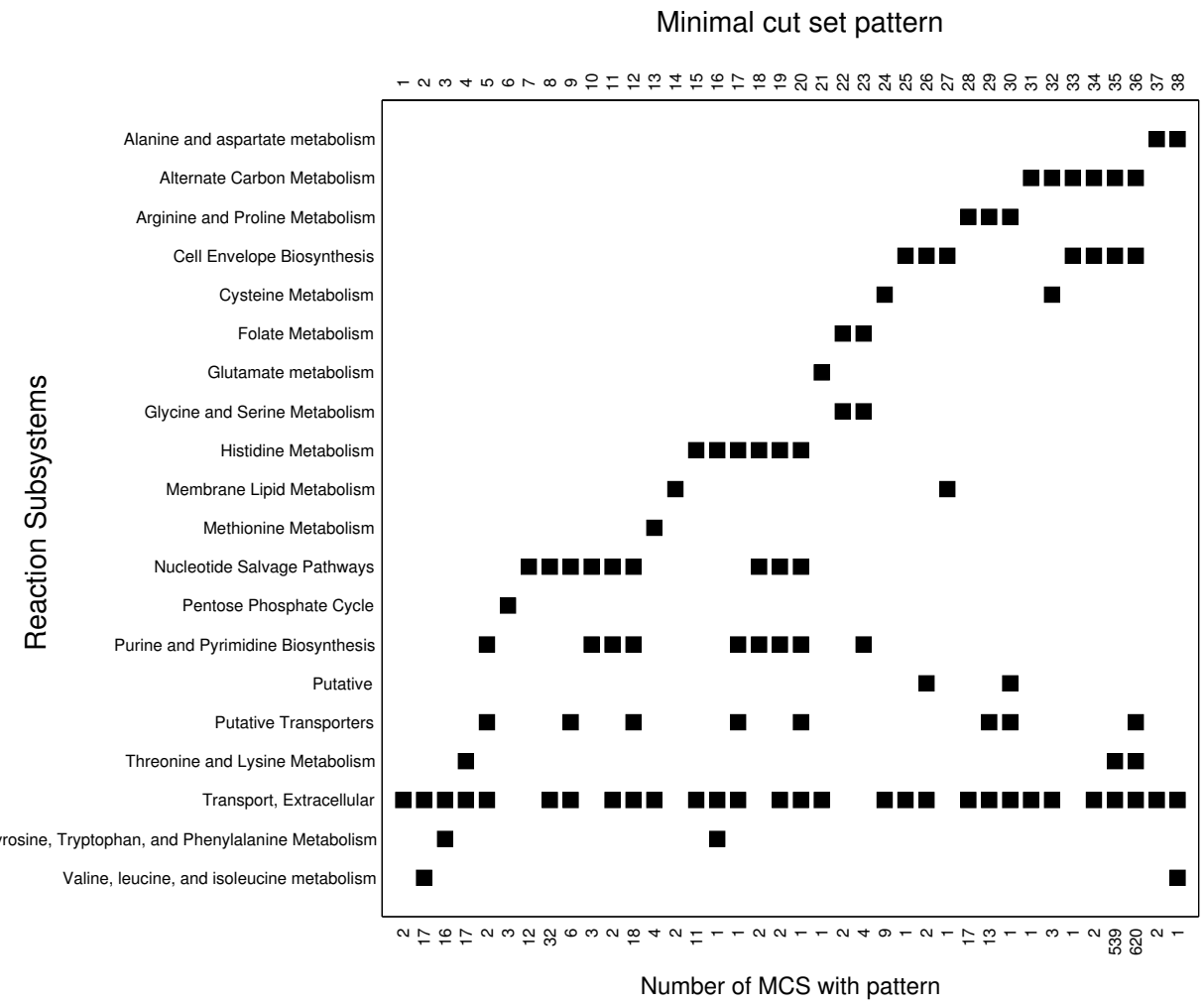

Fig. 1. Subsystem involvement of 1375 complex MCS for rich media production of *E. coli* biomass. MCS employ reactions from a wide variety of subsystems to target specific biomass components. Each column in this figure represents a unique pattern of *E. coli* subsystems targeted by one or more of the 1375 MCS. The row of numbers below the figure specifies how many cut sets obey the given subsystem pattern. Reactions subsystem grouping taken from the *E. coli* iJR904 model annotation [16].

MCS were successfully obtained for 34 to 49 of the biomass component sinks, which includes 17 components that are immune to attack by any of the 92 single reaction knockouts of biomass. These MCS range in cardinality between 2 and 10 and employ 223 reactions in the *E. coli* genome scale metabolic network (in contrast with the 92 essential reactions). These results show that though relatively few reactions are essential for biomass production in rich media, many more emerge as potential targets for knockout when included in the context of a multi-pronged attack.

For further analysis of MCS mechanisms, we used linear programming to determine the spectrum of reactions knocked out by each MCS. Despite the fact that most MCS (1235 of 1375) consist of 6 or more reactions, we find that the vast majority (1317 of 1375) of the corresponding knockouts appear to carry out a ”surgical strike” on biomass production, disabling the producibility of only a single biomass component (e.g. L-threonine, lipolysaccaride, GTP).

Reactions contribution to the 1375 MCS span 20 reaction subsystems (compared to only 10 subsystems spanned by the 92 single knockouts). Not surprisingly, a vast majority of the MCS discovered employ transporter reactions (subsystems ”Transport, Extracellular” and ”Putative Transporter”). Expression of MCS in terms of reaction subsystems yields 38 unique ”cut set patterns”, shown in Figure 1. The predominant two patterns (35 and 36, comprising 1160 of 1375 MCS) amongst the collection of cut sets consists of least one reaction from each of the following reaction subsystems: ”Transporter, Extracellular” or ”Putative Transporter”, ”Cell Envelope Biosynthesis”, ”Threonine and Lysine metabolism”, and ”Alternate Carbon Metabolism”. Other subsystems significantly represented amongst the MCS are ”Nucleotide Salvage Pathways” (78 minimal cut sets), ”Purine and Pyrimidine Biosynthesis” (35 cut sets), and ”Arginine and Proline Metabolism” (31 minimal cut sets).

VI. CONCLUDING REMARKS

This study illustrates the first genome-scale application of the "rational" approach to minimal cut set (MCS) computation outlined by Klamt and Gilles [12]. We achieve this application by extending Klamt and Gilles' theoretical results to intermediate outputs of the elementary mode algorithm, which we refer to as sub-EM. With application to a genome-scale model of *E. coli*, we demonstrate that we are able to generate large numbers of genome-scale cut sets for a given metabolic objective. Though our method employs linear programming in a post-processing step, the number of optimizations required to compute large cardinality minimal cut sets with our approach (80K) is extremely small in comparison to brute-force FBA ($O(10^{30})$). However, since our method is not guaranteed to find all minimal cut sets of a given cardinality, it can be seen as complementary to brute-force FBA for certain applications.

Our results show that though *E. coli* is robust to single reaction deletion in the context of rich media, it is susceptible to compromise via higher-order knockouts. Complex MCS provide an approach to undermine this robustness through a multi-pronged attack. As a result, MCS can be potentially used to identify novel targets for drug design. In additional to pharmaceutical applications, each MCS makes a number of important experimentally verifiable assertions regarding the viability of an organism; namely, it asserts that knockout of the MCS is lethal, while the knockout of each of its subsets should be viable. From a model validation perspective, the biological insight offered from knockout of immediate subsets of MCS, i.e. *sub-minimal* cut sets, may be the most important. In each such mutant, the metabolic network is forced to use a particular route to complete a metabolic function that is normally distributed among a set of redundant pathways. The study of such mutants can be used characterize the function of individual enzymes whose role is normally obscured by network redundancy. In particular, the performance of a sub-minimal cut set can determine how well a given enzyme can handle the entire load of flux for a given cell function. This experimental approach can be more generally applied to determine the role of enzyme pairs, triplets, etc. It also gives a novel use for higher order MCS (i.e. containing 5 or more reactions) that may be impractical as pharmaceutical targets.

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