Subject Section

Condition-specific series of metabolic sub-networks and its application for gene set enrichment analysis

Van Du T. Tran¹, Sébastien Moretti¹, Alix T. Coste², Sara Amorim-Vaz², Dominique Sanglard², Marco Pagni¹*

¹Vital-IT Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland, ²Institute of Microbiology, University Hospital Lausanne and University Hospital Center, Lausanne, Switzerland

*To whom correspondence should be addressed.

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Abstract

Motivation: Genome-scale metabolic networks and transcriptomic data represent complementary sources of knowledge about an organism’s metabolism, yet their integration to achieve biological insight remains challenging.

Results: We investigate here condition-specific series of metabolic sub-networks constructed by successively removing genes from a comprehensive network. The optimal order of gene removal is deduced from transcriptomic data. The sub-networks are evaluated via a fitness function, which estimates their degree of alteration. We then consider how a gene set, i.e. a group of genes contributing to a common biological function, is depleted in different series of sub-networks to detect the difference between experimental conditions. The method, named metaboGSE, is validated on public data for Yarrowia lipolytica and mouse. It is shown to produce GO terms of higher specificity compared to popular gene set enrichment methods like GSEA or topGO.

Availability: The metaboGSE R package is available at https://cran.r-project.org/web/packages/metaboGSE.

Contact: marco.pagni@sib.swiss

Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

The advent of high throughput sequencing techniques, especially RNA sequencing, has greatly facilitated the experimental investigation of an organism’s transcriptome under different physiological conditions. RNA-seq data consists of reads mapped onto an annotated genome, which permits quantitation of the transcript abundance of all predicted genes. These values can be used as a proxy to quantify gene expression or may provide hints about protein abundance for protein-coding genes. Differential expression between two conditions or co-expression profiles across many conditions are currently the fundamental statistical approaches to analyze RNA-seq data (Conesa et al., 2016). However, to obtain a biological interpretation from these analyses, genes also need to be carefully annotated with prior biological knowledge. For example, the Gene Ontology (GO) provides genome annotations by grouping genes into sets, each one identified by a unique GO term that corresponds to a process, function or subcellular location. GO terms are hierarchically arranged in a directed acyclic graph (DAG) whose structure can be exploited by computational methods such as topGO (Alexa and Rahnenführer, 2016) together with gene expression data. Another popular method, GSEA (Subramanian et al., 2005) considers two-condition expression profiles and attempts to identify functionally enriched sets of genes by investigating the change in the expression-based orderings of the genes. The incorporation of gene connectivity information has been shown as a way to improve gene set enrichment methods (Alexeyenko et al., 2012; Glaab et al., 2012). Such connectivity information could be provided by a genome scale metabolic network (GSMN).

GSMNs have been successfully used to study and model metabolism in living organisms (Feist and Palsson, 2008; McCloskey et al., 2013;
from the study of Maguire et al. (2014) on the role of the sterol-regulatory element binding protein Sre1 and the transcription factor Upc2 in sterol metabolism in hypoxic and normoxic conditions. A mouse dataset was also investigated, comprising RNA-seq data from macrophages in adipose tissue (Hill et al., 2018) and the metabolic model iMM1415 (Sigurdsson et al., 2010).

2 Materials and Methods

2.1 Datasets

The Y. lipolytica data analyzed is included in the metaboGSE R package from CRAN along with a vignette of the analysis pipeline. The mouse dataset is described in Supplementary Note S1.

2.1.1 RNA-seq data

For Y. lipolytica, 22 RNA-seq samples of normoxic and hypoxic growth with sre1Δ, upc2Δ, sre1Δ/upc2Δ mutants and the wild type strain were obtained from Maguire et al. (2014) (NCBI: PRJNA205557). These data are summarized in Table 1 and processed with standard preliminary RNA-seq data analysis (see Supplementary Note S3).

Table 1. Designation of RNA-seq data obtained from Maguire et al.

| replicate | Code | Genotype | Oxygen   |
|----------|------|----------|----------|
| 1        | WN   | wild type| normoxia 21% |
| 2        | SN   | sre1Δ    |          |
| 3        | UN   | upc2Δ    |          |
| 4        | DN   | sre1Δ/upc2Δ |          |
| 5        | WH   | wild type| hypoxia 1% |
| 6        | SH   | sre1Δ    |          |
| 7        | UH   | upc2Δ    |          |

2.2 Genome-scale metabolic networks

The Y. lipolytica iMK735 model (http://www.ebi.ac.uk/biomodels, Kašvĕk et al., 2015) with a production of 40% lipid content in the biomass was studied. The genome, proteome, GO annotations, and model were integrated within the framework of MetaNetX (Moretti et al., 2016). The external reactions of the model were adapted to approximately simulate growth in Yeast extract Peptone Dextrose (YPD) medium in hypoxic (anaerobic) or normoxic (aerobic) environments (Maguire et al., 2014) by modifying the oxygen supply. For normoxia, oxygen consumption was unrestricted as in the original model and took a value of 244 mmol-gDW-1-h-1 as given by the Minimum Total Flux algorithm where the sum of absolute values of fluxes was minimized. We then arbitrarily limited the available oxygen to 50 mmol-gDW-1-h-1 to simulate hypoxic conditions. Preliminary investigation showed that the model behavior did not significantly depend on the exact value of this setting. The model was cleaned by removing dead-end metabolites, which were only either produced or consumed. Blocked reactions as given by flux variability analysis (FVA) (Mahadevan and Schilling, 2003) were also removed, as explained below. The final investigated model contained 818 reactions, 469 genes, and 605 metabolites after cleaning, and was referred to as the comprehensive model in our analysis.

2.2 Metabolic sub-model construction
Sub-model construction by removing genes refers to the process of (i) removing an initial set of genes (input) and their associated reactions, (ii) determining the blocked reactions (see Supplementary Note S2), (iii) removing the blocked reactions with their associated genes, if any. A similar procedure named deleteModelGenes is available from the COBRA Toolbox (Heirendt et al., 2017). Below, we refer to a particular sub-model with the number of initially removed genes, but all presented analysis results have been obtained after gene removal propagation through the blocked reactions.

2.2.1 Measure of metabolic model fitness
Viability, for instance growth capacity, is crucial for the usability of a metabolic model. Removing a few reactions from a viable network is often sufficient to render it unviable. Here we propose a measure to assess how close an unviable model is to a viable one, which will be defined as the fitness of the model.

2.2.1.1 Principle of growth rescue
In this section, we introduce a procedure to restore the viability of an unviable metabolic network by the introduction of artificial reactions and minimizing the flux on them. It consists first in modifying the input network around the growth reaction as illustrated in Figure 1. An artificial metabolite is created to replace each of those present in the growth reaction except biomass itself. Each artificial metabolite x' is linked to the original metabolite x through a directed help reaction denoted h_x. In addition, x’ can be produced or consumed via a rescue external reaction 0.0

Fig. 1. Schema of GSMN rescue process. M, original GSMN with growth reaction X + Y -> Z + Biomass. M’, expanded GSMN with the full set of rescue (r_x) and help (h_x) reactions for every metabolite x in the biomass reaction. M”, example of a minimal rescued GSMN in the particular case where only metabolite Y needs to be rescued.
denoted \( r \). Note that the purpose of any help reaction is to avoid artificially supplying \( x \) as a side effect of rescuing \( x' \). No constraints are applied on the fluxes of the rescue and help reactions apart from their directions. The same network modifications could be applied to the non-growth associated maintenance reaction that does not allow for a zero flux.

Let \( \mathcal{M}' \) be the expanded version of a GSMN \( \mathcal{M} \) with the modified growth reaction and the full set of rescue and help reactions, \( \mathcal{S}' \) is the corresponding stoichiometric matrix (see basic notions on GSMN in Supplementary Note S2). The flux distribution \( \nu' \) is constrained by the bound vectors \( \mathbf{u}' \) and \( \mathbf{b}' \) that account for the directionality of newly added reactions and constraint the growth reaction at a fixed rate, arbitrarily chosen as 20% of the original model growth objective, i.e., \( \mathbf{lb}^{\text{Biomass}} = \mathbf{ub}^{\text{Biomass}} = 1/5 \mathbf{v}'^{\text{Biomass}} \).

Let \( \mathbf{c}_{\text{Rescue}} \) be a vector of coefficients that are equal to 0 for all but the rescue reactions that receive a coefficient of \( 1/B \), where \( B \) is the stoichiometric coefficient for the metabolite \( x \) in the original growth reaction. The rescue procedure can be stated as the following linear programming (LP) problem:

\[
\begin{align*}
\text{find } & \nu'' = \text{argmin }_{\nu''} (\mathbf{c}_{\text{Rescue}} \cdot \nu'') \\
\text{subject to } & \mathbf{S}' \cdot \nu'' = 0 \\
& \mathbf{lb}' \leq \nu'' \leq \mathbf{ub}'.
\end{align*}
\]

Only rescue reactions for metabolite \( x \) with non-zero flux \( |\nu''_x| > 0 \) are required to restore the model viability. A viable model \( \mathcal{M}'' \) with a minimal flux of rescue reactions could be formulated as shown in Figure 1. It must be noted that the above LP problem could admit more than one solution \( \nu'' \) and different solutions might be expected if \( \mathbf{c}_{\text{Rescue}} \) was set differently. We investigated this problem through simulation with the iMK735 model and observed that most of the solutions are unique and do not depend on the precise values of \( \mathbf{c}_{\text{Rescue}} \).

Figure 2A presents the fraction of metabolites in the growth reaction that need to be rescued after randomly removing genes in the model. This simulation shows the crucial property that the more genes removed, the more the model is damaged. Figure 2B presents the fraction of random models where each individual metabolite needs to be rescued, clearly showing different behaviors among metabolites in the growth reaction. This suggests that the different metabolites should not be treated equally.

2.2.1.2 Weighting scheme for model fitness

A weighting scheme is introduced to account for the variable importance of metabolites in the growth reaction and possible dependencies among them. Model fitness is defined as the realized objective of the following LP problem:

\[
\begin{align*}
\text{find } & F(\mathcal{M}') = 1 - \nu''^{\text{Biomass}}^{-1} \cdot \min_{\nu''} \left( (\mathbf{w}_{\text{Rescue}} \times \mathbf{c}_{\text{Rescue}})^T \cdot \nu'' \right) \\
\text{subject to } & \mathbf{S}' \cdot \nu'' = 0 \\
& \mathbf{lb}' \leq \nu'' \leq \mathbf{ub}'.
\end{align*}
\]

where \( \nu''^{\text{Biomass}} = 1/5 \mathbf{v}'^{\text{Biomass}} \), diag(\( \mathbf{A} \)) is the diagonal of a square matrix \( \mathbf{A} \), \( \mathbf{w}_{\text{Rescue}} \) is a vector of weights that are equal to 0 for all but the rescue reactions and that are normalized to sum to 1. \( \mathbf{w}_{\text{Rescue}} \) is computed in the following procedure. For every gene in the model, a single gene knockout is simulated, and the rescue procedure is performed to determine which metabolites in the growth reaction are affected, using the previously presented LP problem. Hence, every rescue reaction \( r \) can be associated with a binary vector which describes whether the reaction is needed to rescue each of the gene knockouts. These binary vectors are used to compute Euclidean distances between rescue reactions, which are used in hierarchical clustering with average linkage, and the Gerstein method (Gerstein et al., 1994) is then applied to the resulting tree as a means to assign a weight to each rescue reaction. Those rescue reactions with similar binary vectors share weights, while those with unique profile receive a larger weight. The weighting schema has two main effects: (i) it reduces the importance of metabolites that are hardly affected by slightly damaging the model, and (ii) assigns similar importance to metabolites that appear on the same pathway, as shown in Figure 2C. For instance, H2O has the smallest weight, ergosterol and Sulpha-cholestera-8,24-dien-3beta-ol in the same pathway share the same weight. Other sampling schemes were investigated, for example by removing several genes at once, but these did not yield very different metabolite weights. Hence the simpler experimental setting was used. The simulation of random gene removal in Figure 2A and 3 shows that the introduction of this weighting scheme produced the fitness scores that are less dispersed than the fraction of rescued reactions.

2.2.2 Optimal ranking of genes for removal

The proposed fitness function can be used to evaluate a series of condition-specific sub-models constructed by removing genes in any order. The question then arises as to how to optimally rank genes for removal, such as minimizing network disruption i.e. preserving its fitness. In this section, we investigate different metrics to rank genes according to their expression in a given experiment.

We tested the following transformations of the raw expression data to rank the genes:

\[
\begin{align*}
\text{expr} & \quad \text{raw expression in log-counts} \\
\text{pkmExpr} & \quad \text{expression in RPKM (see Supplementary Note S3)} \\
\text{relExpr1} = \text{expr}/\text{expr} & \quad \text{relative expression} \\
\text{zExpr} = (\text{expr} - \text{expr})/\text{sd(\text{expr})} & \quad \text{z-score} \\
\text{revExpr} = 1/(\text{1+\text{expr})} & \quad \text{reverse expression (control)}
\end{align*}
\]

where \( \text{expr} \) and \( \text{sd(\text{expr})} \) denote the average and standard deviation across conditions, respectively.

Let \( \rho \) be a ranking of the genes. Sub-models are constructed by removing genes in the order given by \( \rho \). The resulting fitness scores decrease when more genes are removed, and the decreasing trend depends on \( \rho \). Figure 3 illustrates such reductions obtained by successively removing genes from the comprehensive model iMK735 for the UH condition (up<2A in hypoxic condition, UH2 sample). In this example \( \text{expr} \) is the most fitness-preserving ranking and \( \text{revExpr} \) is the worst one.
Gene set enrichment on metabolic network

We assess a ranking $\rho$ by performance index $Z_\rho$, which indicates the percentage of random draws yielding sub-model fitness higher than that of sub-models created by $\rho$-based removal of the same number of genes and which is weighted by the average random fitness. $Z_\rho$ is computed as follows:

$$Z_\rho = 100 \times \left( \frac{\sum_{i=1}^{k} \left( \frac{F(M_i^{\text{random}})}{\sum_{j=1}^{N} F(M_j^{\text{random}})} \right) - \rho}{N} \right) \sum_{i=1}^{k} \left( F(M_i^{\text{random}}) \geq F(M_i^\rho) \right)$$

where $M_i^\rho$ denotes the rescued sub-model of the comprehensive network $M$ obtained after removing the $i$ genes using ranking $\rho$, and $M_i^{\text{random}}$ the rescued sub-model by randomly removing $i$ genes, $(F(...))$ the average fitness on $N$ draws. The lower the performance index, the better the ranking. The optimal ranking is the one dominating the others by producing the lowest performance index for all investigated RNA-seq samples. For instance, the absolute expression ranking expr is determined as the best one for 19 of 22 the Y. lipolytica samples, whereas it is pkmExpr for the mouse dataset (see Table S1). Other rankings such as $(expr^2/expr)^{2/3}$ and $(expr/expr)^{1/3}$ were also investigated, yet not comparable to the selected ones. In the rest of this article, the expr and pkmExpr rankings will be used in the Y. lipolytica and mouse study, respectively.

2.3 metaboGSE: contrasting gene set enrichment in condition-specific sub-models

We introduce here the metaboGSE method, which aims at identifying gene sets that are differentially enriched. The method consists of three steps depicted in Figure 4 and illustrated in Figure 5 for GO:0006635 – fatty acid beta-oxidation. The first step consists in constructing a series of sub-models for every sample and computing their fitness profile.

In the second step, for a given gene set $g$, we compute the depletion fraction $f(M_i^{\text{expr}}, g)$, i.e. fraction of $g$-associated genes remaining in each sub-model, where $M_i^{\text{expr}}$ denotes the rescued sub-model after the removal of $i$ genes. The evolution of $f(M_i^{\text{expr}}, g)$ is plotted as a function of $(1 - i/k) \cdot F(M_i^{\text{expr}})$, defining a depletion curve, where $k$ denotes the total number of genes in $M$. The depletion curve for each condition is the average curve on all replicates. As shown in Figure 5A, the fraction $f$ of genes associated with fatty acid beta-oxidation decreases rapidly in all conditions, but the depletion curve clearly separates hypoxy wild-type from the other conditions (Figure 5B). The down-regulation of several but not all GO:0006635 genes in the WH condition is illustrated in Figure 5C.

In the third step, we perform a permutation test for the significance of the difference of the given gene set $g$ across conditions. The test statistic is defined as the maximum area between every pair of depletion curves among all conditions. The resampling is performed by permuting replicates between conditions while keeping unchanged the number of replicates in each condition. The resulting $p$-value indicates whether the depletion evolution of $g$ in one condition differs from at least one of the others. For GO:0006635, the discrepancy of WH versus the other conditions is justified with a $p$-value of 0.007 and a test statistic of 0.41 (Figure 5). These $p$-values are subsequently adjusted by Benjamini-Hochberg (BH) correction across all the studied gene sets (Benjamini and Hochberg, 1995). A similar post-hoc permutation test is also implemented for pairwise comparisons between conditions to check for the pairwise differential signal.

3 Results

3.1 Condition-specific sub-model construction on Y. lipolytica data

We applied the selected gene removal strategy to construct sub-models for the seven conditions in Maguire et al. (2014) (Table 1). The expr rankings are different between conditions despite a similar distribution of gene expression values (Figure 6A). The sub-model series constructed based on expr, and thus their fitness, evolve differently across conditions (Figure 6B). Figure 6C-F shows a high degree of variation across conditions in the number of genes and reactions (post propagations through blocked reactions), as well as in the fraction of genes and reactions that are essential. Besides, the erratic evolutions of essential genes and reactions is noteworthy and might be associated with the unexpectedly large spread in essentiality predictions recently reported in Opdam et al. (2017).

To compare the condition-specific sub-model construction from the existing methods with that from our approach, we investigated GIMMME (Becker and Palsson, 2008) and iMAT (Zur et al., 2010). These two methods were benchmarked in Opdam et al. (2017) and could be used with information that is deduced only from GSMN and transcriptomics data. We built a sequence of sub-models with GIMMME using 12 gene expression cut-offs (in log2 RPKM) from 0 to 11 for each of the 22 samples. For iMAT, these cut-offs were used as threshold_{lb} while threshold_{ub} was determined as threshold_{lb} + 2*standard_deviation(expression), as recommended in the Supplementary Data of Zur et al. (2010). The choice of such a limited number of cut-offs was due to highly time-consuming model construction process of the two methods in Matlab. Indeed, the parallelization implemented in metaboGSE using the sybil and parallel R packages allowed us to construct the complete list of all sub-models in almost one hour on a 64-processor Intel Xeon E5-4620 of 2.6 GHz. The MatLab implementations of iMAT and GIMMME were much slower in our hands. Intersections and unions of genes in each 22 sub-models were investigated to evaluate the difference between them. Figure S1 shows 1 – Intersection/Union plotted as a function of $k$ – Union, where $k$ denotes
the number of genes in the comprehensive GSMN. Interestingly, the submodels produced with metaboGSE were quite similar to those produced by iMAT, a method that relies on a MILP algorithm. GIMME produced sub-models that were less distinct across conditions.

3.2 Gene set enrichment on \textit{Y. lipolytica} data

To validate the biological findings produced by our approach, we investigated 135 gene sets defined as GO135 (see Supplementary Note S4). Maguire \textit{et al.} (2014) studied the role of Sre1 and Upc2 in regulating sterol metabolism in hypoxic and normoxic conditions in \textit{Y. lipolytica} by performing GO term enrichment analysis of differentially expressed genes using DAVID. Among the 116 biological-process GO terms they reported, only eight were found in GO135, but none had a reported BH-adjusted \( p \)-value < 0.15. Here we compare our results with those of topGO and GSEA on the iMK735 genes (see Supplementary Note S4).

\textit{Condition contrast is predominantly hypoxia-normoxia.} The depletion curves of the top 50 GO terms found to be significantly enriched by metaboGSE (ordered by permutation test statistic with FDR < 0.05) unite into 99 GO terms and are summarized in Figure S3 and Table S2. These 99 terms also included the eight found in Maguire \textit{et al.} All terms from metaboGSE are of higher or equal specificity, \textit{i.e.} offspring or identical, to those found by topGO and/or GSEA. Thirty-five among the 50 GO terms from metaboGSE are related to those found by the other methods yet include 9 terms of higher specificity: GO:0034637 (cellular carbohydrate biosynthetic process), GO:0015937 (coenzyme A biosynthetic process), GO:0071265 (L-methionine biosynthetic process), GO:0046474 (glycerophospholipid biosynthetic process), GO:0097164 (ammonium ion biosynthetic process), GO:0006656 (phosphatidyl choline biosynthetic process), GO:0001676 (long-chain fatty acid metabolic process), GO:0043649 (dicarboxylic acid catabolic process), and GO:0009098 (leucine biosynthetic process). Six of them are found in the three largest connected DAGs of enriched GO terms depicted in Figure 7.

\textit{Difference in sterol biosynthesis is confirmed.} Ergosterol biosynthetic process (GO:0006696) is enriched by all methods. Along with GSEA and
those simulated (see Table S1). Only four genes of iMM1415 were found to be differentially expressed between PBS and Ly6C (fold-change ≥ 2, FDR < 0.05), resulting in no significantly enriched GO terms with topGO or GSEA. We then applied these two methods to all differentially expressed genes in the genome, but not only to those in iMM1415, to increase the number of differentially expressed genes. We scrutinized a list of 24 GO terms related to inflammatory response, cholesterol and lipid biosynthesis as reported in Hill et al. (2018) and associated to at least one iMM1415 gene. The results shown in Figure S5 reveal that metaboGSE can detect GO terms that are located lower in the Gene Ontology, like GO:0050728 (negative regulation of inflammatory response), GO:0002675 (positive regulation of acute inflammatory response), and GO:1903725 (regulation of phospholipid metabolic process), despite working on a much smaller collection of genes than the other two methods. This confirms the ability of metaboGSE to capture GO terms of higher specificity as observed above with Y. lipolytica.

4 Discussion

We present here a method for gene set enrichment analysis that utilizes a GSMN as an additional source of information and that focuses on genes expressed at low level. Our central working hypothesis is that the correlation between gene expression levels and fluxes on related reactions is very poor in general, but the low expressed genes are plausibly associated with zeroed fluxes. This method is complementary to established methods such as topGO and GSEA that focus on differential expression of sufficiently expressed genes. The introduction of a GSMN restricts the list of investigated genes to those present in the model (i.e. related to metabolism), and thus the list of gene sets that can be discovered. The formulation of the external reactions of the GSMN and how well they represent the experimental system are likely to affect metaboGSE outcome, although this has not been investigated in detail here. Tissue-specific models are not a prerequisite to utilize metaboGSE. The GSMN and the set of RNA-seq data both need to be of high quality and adequate for experimental designs. Our method is capable of producing more informative GO terms (i.e. that are located lower in the Gene Ontology) than those returned by GSEA and topGO for example. This might be because metaboGSE can increase the size of investigated gene sets by considering structural constraints brought by the propagation through blocked reactions, as for example the linearity of the ergosterol biosynthesis pathway in the case reported here. The genes affecting the discrepancy between conditions, which are not necessarily differentially expressed, can be further investigated for each enriched gene set. metaboGSE produces biologically meaningful results to the extent one can interpret them.

Our method does not aim at producing a condition-specific sub-model, but rather integrates on a series of them, thus avoiding the choice of a particular number of genes to remove. A GSMN is a drastic simplification of our understanding and knowledge of biochemistry that neglects most kinetic aspects in its representation of metabolism. On the modeling level, defining a sub-model by removing genes is equivalent to a gene knockout obtained from a molecular construct. It is likely that the metabolism dominating a given physiological state owes more to kinetic regulation than can be accounted for by only the metabolism structure. Moreover, it is very hard to ascertain that a gene is not expressed at all and even in this case, the absence of mRNA does not exclude that the protein is still present at a low concentration, as a remnant of a previous growth phase. Likewise, the presence of the protein does not ensure it is active. The construction of a series of sub-models followed by their rescue is essentially a way to circumvent the hard constraint caused by model viability and exploit the knowledge of the living cell. Such approaches are hard to implement in practice, because the conditions that are likely to be relevant for a particular physiological state are not known in advance. However, we have shown that a GSMN can be used as an additional source of information to improve the quality of gene set enrichment analysis and to discover GO terms that are related to metabolic pathways.
model properties that would be out of range. Our method to construct metabolic sub-networks could also be performed with other omics data, such as proteomics and metabolomics, and applied to other research problems. 

The fitness function is the key component of our method. The proposed measure of fitness shows its capacity to capture the health status of a sub-model and thus suggests some control on our sub-model construction. Despite the biologically meaningful results obtained with the datasets studied, several lines of improvement can be envisaged in future work, including: the formulation of the growth reaction could be improved by considering more metabolites; the proposed weighting scheme is likely suboptimal; and other dynamic properties of the model could be considered separately from the score derived from the LP-based minimization.

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Conflict of Interest: none declared.

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**Fig. 7.** The three largest connected DAGs enriched by either metaboGSE (in red), topGO weight01 (in blue) or GSEA (in yellow). Table S2 provides the full listing of the 99 GO terms resulting from the union of the top 50 GO terms from each method. FDR is reported for each GO term. NA value indicates that the GO term was not reported by the corresponding method. bp: biosynthetic process, cp: catabolic process, mp: metabolic process.
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