Bayesian Inference of Differential Metabolic Fluxes in Ulcerative Colitis using High-throughput Transcriptomics Data

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Abstract

Advances in high-throughput technology have enabled the measurement of transcriptomic responses to a broad range of conditions. Our increased understanding of human metabolic reactions has also enabled the construction of large enzymatic network models. With the availability of detailed models and data to fit them to, various methods for analyzing the effects of transcriptional regulation on metabolism have been proposed. However, for conditions under which our understanding of the transcriptional response is limited, constructing a quantitative model for metabolic changes can prove challenging. In this work, we suggest a novel Bayesian approach that combines systems-level modeling of metabolism with Bayesian inference of the transcriptional response. Our method combines Bayesian reasoning with flux balance constraints that are used in mechanistic models of metabolism, and is thereby able to incorporate both uncertainty and systems-level metabolic knowledge into the model. It models only metabolic differences between two conditions, thereby simplifying the model to capture only effects that are derived from the transcriptomic response to a specific stimulus. We demonstrate the usefulness of our approach by studying the transcriptional regulation of metabolism in two independent ulcerative colitis datasets.

Keywords: Transcriptional Regulation, Bayesian Inference, Differential Metabolic Flux

1 Introduction

Transcriptional regulatory mechanisms are a key factor in determining the metabolic activity of cells under different conditions. For example, cancer cells, which rely on energy produced via glycolysis, modify the expression of glycolytic enzymes [1]. To prevent DNA damage from reactive oxygen species (ROS), they must keep intracellular ROS below a toxic threshold, otherwise cell death will result [2]. Therefore, cancer cell also regulate the pentose phosphate pathway to combat oxidative stress in addition to necessary anabolic processes [3]. While cancer cells differ from healthy ones by other characteristic modifications such as sustained proliferative signaling, changes in metabolism are recognized as a one of the hallmarks of cancer[4]. For a review of metabolic pathway activity in cancer see [5]. Transcriptional regulation of metabolism has also been studied in normal cells. For example, distinct expression profiles corresponding to different gastric sites have been identified, reflecting different metabolic processes [6]. The influence between transcription and metabolism is likely bidirectional. For example, the metabolite alpha-ketoglutarate has been observed to induce transcriptional programs in cancer cells that resemble those of pre-cancer cells [7]. Hence, in order to further our understanding of biological networks and their role in disease it is of high importance to model the interactions between transcription and metabolism.

Metabolomics data are difficult to interpret using traditional approaches. Lee et al. describe the limitations of using metabolomics data in pathway analysis, and call for new algorithms that accommodate the unique properties of metabolites [8]. On the other hand, high-throughout transcriptomics has been used extensively for explaining regulatory changes under various conditions, including those that affect metabolism [9]. Enrichment analysis identifies significant over-representation of predefined categories of genes in those genes selected as significantly changing in expression[10]. Gene Set Enrichment Analysis (GSEA) identifies high ranking gene pathways using the ranking obtained by testing association with a condition without a cutoff threshold [11]. Both approaches do not evaluate interactions between genes and are therefore applicable to a broad range of datasets, but they do not model metabolic processes directly. Pathway structure has also been used in tandem with gene expression. SPIA scores a pathway based on both overrepresentation of its genes in the differentially expressed set and the significance of their interactions in the pathway graph [12]. BRAS models a pathway using a Bayesian network, a directed acyclic graphical mode, using nodes to represent expression levels and edges to represent relationships between genes [**?**]. clipper uses graph decomposition of pathways to identify those pathways associated with gene expression differences and the signal paths that have the greatest association with the change [13]. A more metabolism-specific approach, Flux Balance Analysis (FBA) models the metabolic network by assuming flux balance and maximizing an objective such as biomass production, with additional constraints on reaction rates [14, 15]. This requires a detailed knowledge of metabolic reactions at the genome scale, but not of their rates, which are estimated by solving the optimization problem. FBA models have been expanded to account for transcriptional regulation [16, 17]. If an explicit gene regulatory network model is used, both knowledge of the metabolic network and the gene regulatory network are required. Expression data can also be

used to calibrate the metabolic network in FBA [18].
Other data types, like growth rate of cells, can be useful for modeling metabolic changes. While it is possible to measure both the transcriptome and growth rate of single cells [19], such datasets are not common.
In this work we present a novel approach that applies Bayesian inference to the inference of differential metabolic fluxes between two conditions, i.e. only those metabolic fluxes by which the conditions differ. Our approach does not require an estimate of exact reaction rates, and can use posterior error probabilities from Bayesian differential expression analysis to select the most likely fluxes. By combining Bayesian inference with a metabolic constraints we are able to eliminate solutions that are not mechanistically sound and at the same time obtain inferential rigor. We demonstrate the power of the method by modeling differential fluxes in two independent expression datasets with a high degree of agreement. The next section details how an optimal model for differential fluxes is computed. The Results section then describes application to data from ulcerative colitis patients and healthy controls.

2 Materials and Methods

We wish to associate each enzyme with a probability of either performing a reaction more often in cases than in controls, or conversely with a probability of performing a reaction less in cases than in controls. We can compare the mean expression level of each enzyme under the two conditions, where a higher mean indicates increased activity. However, we need to associate the difference with a probability. Karlebach and Robinson [20] have previously proposed a method for differential expression and splicing analysis that produced posterior error probabilities (PEP). That is, the probabilities of no effect and its complement, the probability that the effect/difference is biologically meaningful, are both generated by the analysis. Briefly, a mixture prior with two components, each representing the distribution of parameter value under one of the two hypotheses (effect and no effect), is defined based on the region of practical equivalence (ROPE), a posterior summary method which separates the possible parameter values into regions of effect and no effect. The PEP is the density of the posterior that belongs to the region of no effect. The weights of the mixture components have a uniform prior and are inferred from the data. Hence in a dataset with a small number of true effects the prior will drive the posterior towards the component of no effect, and when the fraction of true effects is larger so will the weight of the prior component of true effects be. Given the probabilities associated with each enzyme, one could choose the set of enzymes with probability of activity greater than 0.5, assuming that the group of enzymes that are over expressed in cases will perform metabolic reactions more in cases than in controls and vice versa. However, this combination of enzymes may correspond to fluxes that do not constitute a viable metabolic response, and is likely to include many false positives from the differential expression analysis. Requiring that the solution preserves flux balance restricts the solution space to biological plausible responses. In order to find the most likely set of reactions that are differential between cases and controls and preserve flux balance, we require that the product of the stoichiometric matrix S and the

reaction rates vector v is equal to the zero vector, i.e $S \cdot \vec{v} = \vec{0}$. Furthermore, since some of the enzymes that carry out the reactions are expressed at a higher level in cases and some in controls, we reverse the direction of the reactions corresponding to the latter. Formally, we multiply the column in S which corresponds to that reaction by -1. The reason for this choice is that if an enzyme is chosen by the model as having a significantly decreased activity in cases, then the products of the reaction it carries out are produced less and the metabolites that it consumes are consumed less compared to controls. In order to preserve flux balance other enzymes need to consume more of the metabolites the down-regulated enzyme would have otherwise consumed, and less of the metabolites it would have otherwise produced, and so at least one of these enzymes would need to be up-regulated in cases. One should note that we only model the changes in metabolic fluxes between controls and cases, not the absolute fluxes in either of these groups.
In order to choose the enzymes/reactions that preserve flux balance and form the metabolic network with the highest probability, we need to ensure that if an enzyme/reaction is not chosen, its reaction rate will be 0. This is accomplished by formulating the problem of selecting the differential reactions between cases and controls as a Mixed-Integer Linear Programming (MILP) optimization problem. We define a Boolean variable rho_i for each reaction, and a continuous variable eta_i for each reaction rate. If reaction i is chosen as contributing to fluxes that are differential between cases and controls, then its rho_i variable is set to 1, and the logarithm of $1 - PEP_i$, i.e. the logarithm of the probability that the enzyme is differentially expressed between cases and controls, is added to the objective function. If the reaction is not selected , its rho_i variable will be set to 0, and the logarithm of its PEP will be added to the objective. The objective for all the genes $1..N$ is thus:

$\sum_{i=1}^{N} (1 - rho_i) \cdot log(PEP_i) + rho_i \cdot log(1 - PEP_i)$.

In addition to the flux balance constraints, we add the following two constraints for each reaction:

$eta_i \leq rho_i$

$eta_i \geq \frac{rho_i}{10^5}$

Consequently, if rho_i is set to 0, eta_i must also be 0. If rho_i is set to 1, eta_i can vary over five orders of magnitude. It should be noted that our goal is to find which reactions and metabolites are active due to the conditions that are present in cases but not in controls. This qualitative knowledge will be used to derive insights into the regulation of metabolism under the conditions of the study, as will be demonstrated in the Results section. If two enzymes can carry out the same reaction, each will have its own rho and eta variables. We used the Gurobi solver to solve this MILP problem [21].
In order to obtain the probabilities of no-effect (PEP) for each enzyme, we modified the model of Karlebach and Robinson [22] such that it would only include differential expression parameters, i.e. the parameter β but not the parameter α which modeled

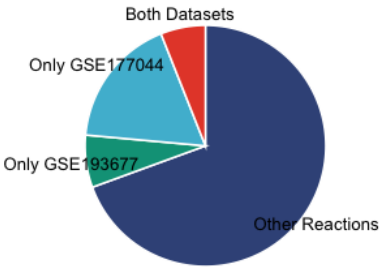
changes in isoform proportions. β models the difference between two distributions of log-transformed counts, one in case and one in controls. The density of the posterior of beta in the region of practical equivalence is the posterior error probability (PEP), the probability of no-effect.
We refer to the reactions that are selected for the solution ($rho = 1$) as differential reactions, and to the fluxes that they generate as differential flux. The next section describes an application of this method to high-throughout transcriptomic datasets.

3 Results

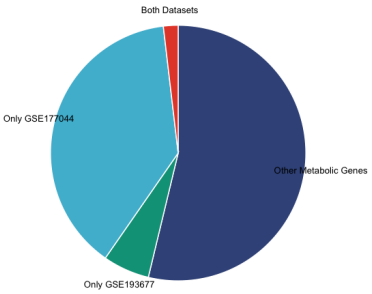
We obtained two independent transcriptomics datasets from ulcerative colitis patients and healthy controls from the Gene Expression Omnibus [23]: GSE177044 submitted in Wacker et al. [24], and GSE193677 submitted in Argmann et al. [25]. Since GSE177044 utilized whole-blood RNA-Sequencing and GSE193677 obtained samples using biopsy, we expect to see correlated though not identical metabolic changes [25]. The Human-GEM [26] model was used for the differential metabolic reactions between cases and controls, excluding enzymes that were not expressed, separately for each dataset.
When examining the metabolic reactions that constitute to the differential flux between cases and controls, we expected to see a high overlap between the two datasets. Indeed, figure 1a shows that the size of the overlap is about half the total number of the differential reactions in GSE193677. It would be incorrect to assume that the set of reactions in each datasets are selected independently, as they maintain flux balance. However, we can estimate how often this magnitude of overlap will occur for two unrelated datasets by randomly generating different sets of differential genes, finding the differential fluxes for them and computing a statistic to estimate the overlap between pairs of these simulated conditions. The values of the statistic can be compared to its values for the overlap between the reactions of GSE177044 and GSE193677. We permuted the gene labels for the differential expression results of GSE177044, and for each permutation solved the differential flux MILP problem to obtain a set of reactions. The hypergeometric CDF computed using the overlap between differential fluxes in 1,000 simulated dataset pairs was always smaller than the one computed for the ulcerative colitis datasets. Next, for each of the two datasets we chose a set of genes such that the mean PEP is 0.05, which sets the expected fraction of false positives to 0.05. Figure 1b shows the overlap between differentially expressed metabolic genes in the two datasets, compared to the total number of metabolic genes. As can be seen in the figure, the overlap is smaller than the overlap between reactions that were chosen for the model of differential fluxes. This is an indication that the metabolic model captures condition-specific properties more accurately. We further computed the Pearson correlation between reaction rates in the models for the two datasets, and compared it to correlation between reaction rates in pairs of randomly generated datasets. The correlation between GSE177044 and GSE193677 was higher than 0.01 of the simulated dataset pairs, indicating that the agreement between the models reflects a common biological condition.

We next turned to examine insights into the differential metabolic reactions under ulcerative colitis by comparing the models generated for the two experimental datasets. The first question the models can address is whether there is a difference in the frequency by of metabolite production in differential fluxes compared to the complete metabolic network. In other words, do the models indicate that some metabolites are significantly over- or under-utilized. We counted the fraction of reactions that produce each metabolite in each of the two ulcerative colitis differential flux models and compared it to their fractions in the complete metabolic network. Out of the 10 most under-utilized metabolites in each model/dataset, 4 were common to both models/-datasets. The same overlap computed between pairs of simulated datasets was always lower, again suggesting that the overlap in the ulcerative colitis datasets reflects a common biological condition. The fractions of reactions producing these four metabolites compared to the same fractions in the full model are shown in figure 2. The total number of metabolites used by the models was 3,527 and 1,529 for GSE177044 and GSE193677. Lower-than expected utilization of these metabolites in the differential fluxes could be a result of their lower availability for the modeled reactions in patient samples. We next examined the literature in order to interpret this finding.

PAPS (Phosphoadenosine-5'-phosphosulfate) is an intermediate in the production of hydrogen sulfide that is in turn used in biosynthesis. Hydrogen sulfide has been suggested as contributing to the pathology of ulcerative colitis by interrupting the maintenance of the colonic epithelial barrier [27]. Lower availability of PAPS for cellular metabolism could reflect a metabolic tuning in response to higher-levels of hydrogen sulfide. In randomly generated datasets, the same reduction in PAPS production occurs in only 0.01 of the cases, suggesting that most regulatory tuning will not cause such an extreme reduction. Since the mechanism of pathology involves hydrogen sulfide interrupting beta-oxidation, we tested whether inhibiting beta-oxidation will have an effect of PAP metabolism by down-regulated the ACADM gene [28] *in-silico* in both GSE177044 and GSE193677. Down-regulation can be applied to the model by removing the expression of the gene altogether from the dataset. In both down-regulation models, the fraction of reactions that generate PAPS significantly increased. Figure 3 shows this increase. In arbitrary gene down-regulations, this level of increase occurs in less than 1% of the cases, suggesting that this response does not commonly follow changes in metabolic fluxes. This may indicate that the low utilization of PAPS in the differential fluxes is coupled to the transcriptional control of beta-oxidation, and that the regulatory responses in the two models signal an increase in beta-oxidation.
Malonylation of the protein GAPDH (glyceraldehayde-3-phosphate dehydrogenase) leads to dissociation of GAPDH from TNF and therefore promotes inflammation [29]. Malonyl-CoA serves as a donor for this reaction, and therefore its decreased utilization by the metabolic network may reflect a metabolic reprogramming that makes it more available for the malonylation of TNF, a reaction that is not part of the metabolic model. We therefore sought a gene whose inhibition would increase utilization of malonyl-CoA by the metabolic network and whose protein product can be targeted by known compounds. Inhibiting GSTP1 results in an increase in differential metabolic reactions that process malonyl-CoA to a level observed in the complete



(a) Overlap between metabolic reactions in the differential fluxes of the two datasets, GSE177044 and GSE193677. The overlap is about half the number of the all the reactions in GSE193677. The dark blue area denoted as 'Other Reactions' corresponds to reactions that had expressed genes that can carry them out in both datasets, but were not selected for either differential flux.



(b) Overlap between differentially expressed genes in the two datasets, GSE177044 and GSE193677. The dark blue area denoted as 'Other Metabolic Genes' corresponds to genes that are expressed n both datasets, but were not selected for the set of differentially expressed genes.

metabolic network. It is therefore possible that such inhibition will reduce the metabolite's availability for pro-inflammatory activity. The alkaloid piperlongumine is an inhibitor of GSTP1 that has been to alleviate inflammation in other disease models [30, 31]. An interesting follow-up experiment would be to measure its effect on a disease model. Currently, we have not developed a method for predicting adverse effects of metabolic changes, and therefore it is not possible to predict if inhibition of GSTP1 has detrimental effects in this context. We can test the metabolic impact of the inhibition by comparing metabolite utilization in GSE177044 before and after inhibition and other randomly generated datasets. Figure 4 shows that after inhibition the network's metabolite utilization is still closer to its state before inhibition than to its state for random datasets. However, the model does not include effects of metabolic changes on gene expression, which may follow more slowly than metabolic network tuning. Additionally, it does not include the reactions that directly regulate inflammation. Nevertheless, the ability to predict changes in uptake or utilization of metabolic compounds can serve as a first step in a series of experiments that test such hypotheses.

4 Conclusion

This work presented a novel approach for genome-scale metabolic analysis of high-throughput gene expression data. Our method uses Bayesian inference to infer the differential metabolic fluxes between two conditions, obtaining a model that maximizes the probability of the differential fluxes and maintains flux-balance constraints. We showed its application to two datasets of ulcerative colitis patients and healthy controls, obtaining a high degree of agreement and deriving insights about regulation of

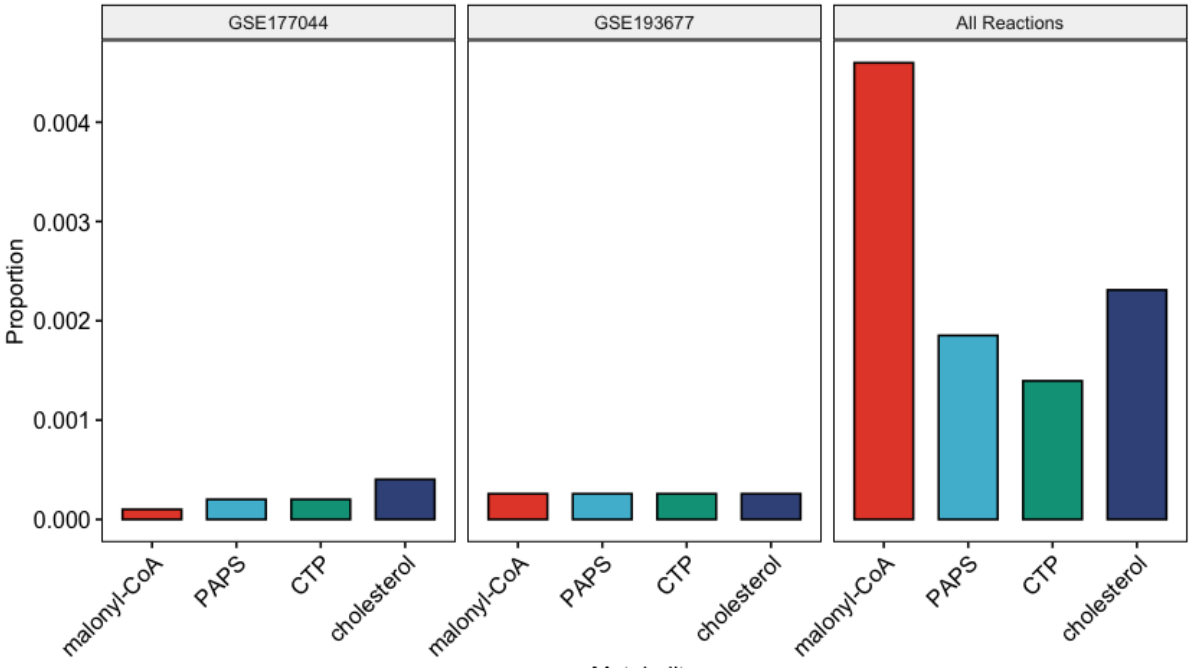


Fig. 2: The fraction of reactions that generate the metabolites malonyl-CoA, PAPS, CTP and cholesterol, in the models of differential fluxes for GSE177044 and GSE193677, and in the complete metabolic network(All Reactions). These 4 metabolites are among the 10 metabolites that are produced significantly less than expected in both the GSE177044 model and the GSE193677 model.

metabolism under the condition. The method has the potential to improve our understanding of metabolic regulation in a broad range of conditions, since it only requires high-throughout transcriptomics data and a metabolic network model as inputs. Currently, the reactions included in the model are limited to the metabolic level, but the hypotheses generated using the model linking metabolism to other processes can be tested in the lab.

Declarations

4.1 Competing Interests

The authors declare that they have no competing of interests.

4.2 Data Availability

4.3 Ethics Declaration

Not applicable.

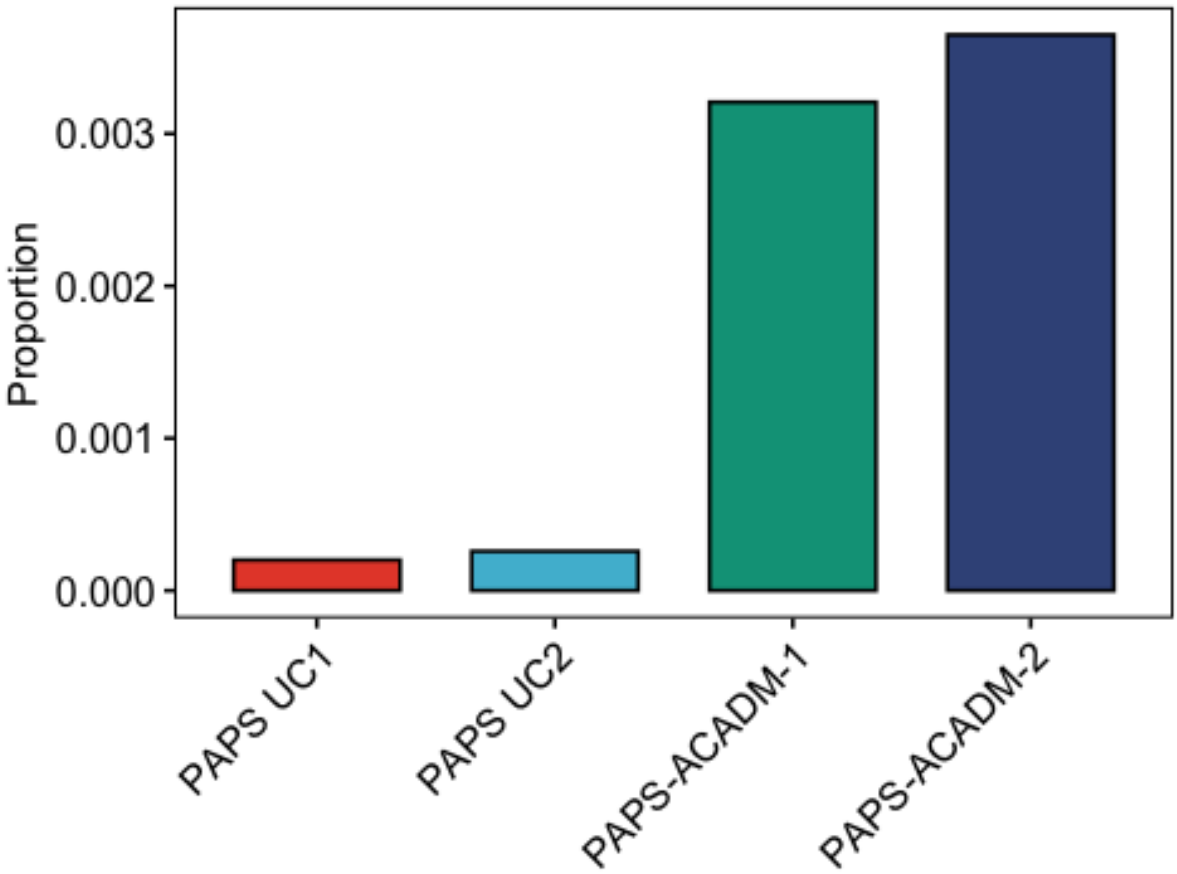


Fig. 3: Reactions that produce PAPS increase significantly in the model of differential fluxes when ADADM has been down-regulated. The bar marked PAPS ACADM-1 shows the proportion of PAPS-producing reactions after ACADM is down-regulated in GSE177044, and bar marked PAPS ACADM-2 shows the same value for the ACADM down-regulation in GSE193677. The bars marked PAPS UC-1 and PAPS-UC2 show the same proportions before the ACADM down-regulation in GSE177044 and GSE193677, respectively.

4.4 Consent to Publish Declaration

Not applicable.

4.5 Consent to Participate Declaration

Not applicable.

4.6 Author Contributions

G.K. performed all the work described in this manuscript.

4.7 Funding

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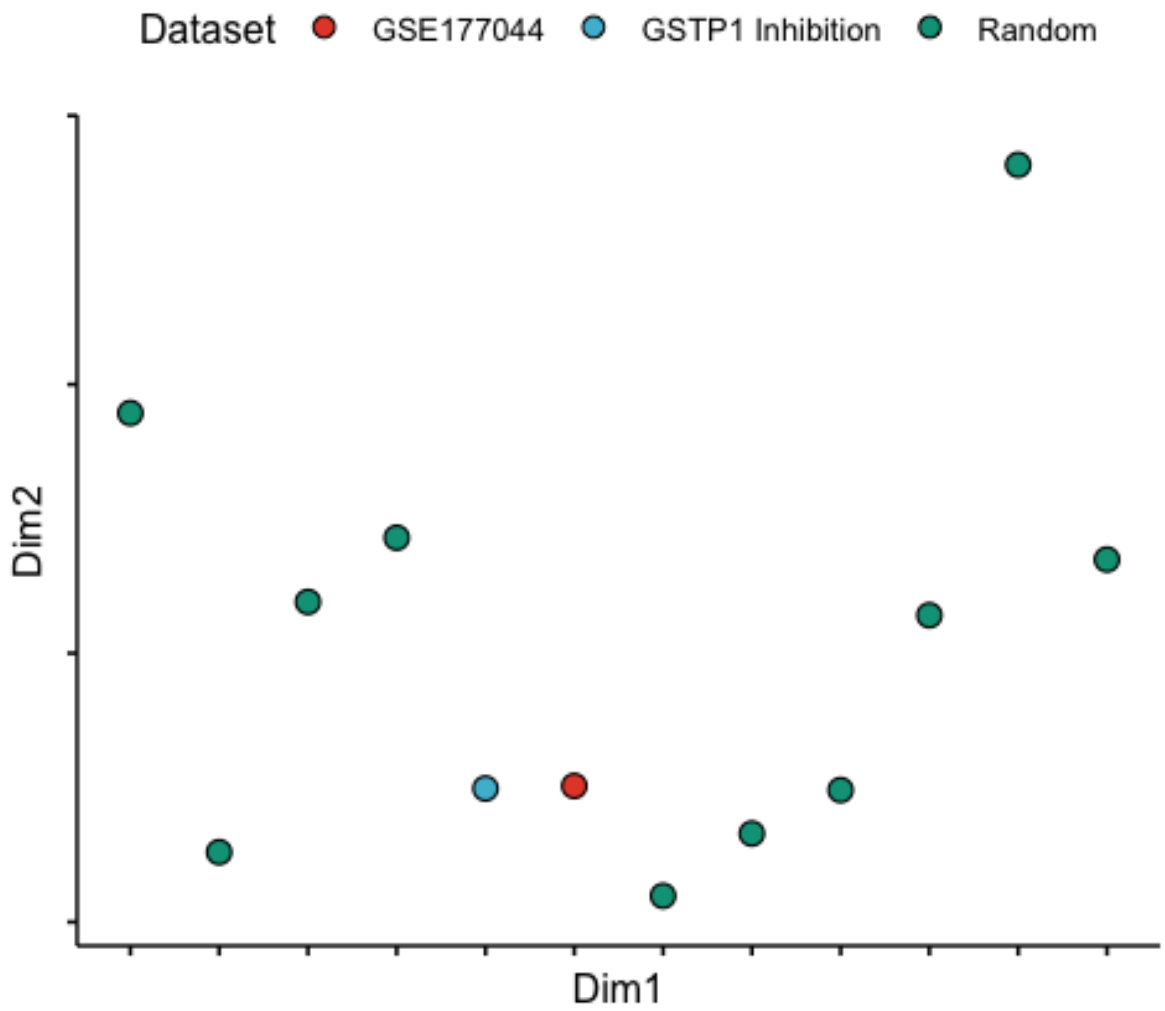

Fig. 4: Similarity in metabolite utilization by the differential flux models constructed for GSE177044, GSE177044 after inhibition of GSTP1 and randomly generated datasets. The fractions of reactions involving each metabolite were mapped to two dimensions using multidimensional scaling. GSE177044 and GSE177044 after GSTP1 inhibition utilize metabolites more similarly than randomly generating datasets, suggesting that inhibition of GSTP1 may have localized effect on the metabolic network.