Computer Methods and Programs in Biomedicine 263 (2025) 108672

Contents lists available at ScienceDirect

Computer Methods and Programs in Biomedicine

journal homepage: www.sciencedirect.com/journal/computer-methods-and-programs-in-biomedicine

MInfer: Bridging MetaboAnalyst and Jacobian analysis for metabolomic networks

Jana Schwarzerova [a,b,c,*], Erdő Gabor Mate [b], Jakub Idkowiak [d], Dominika Olesova [e,f], Ales Kvasnicka [g], Dana Dobesova [h], David Friedecky [h], Valentyna Provaznik [a], Jozef Skarda [c], Wolfram Weckwerth [b,i,*], Thomas Nägele [j,*]

[a] Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Czech Republic
[b] Molecular Systems Biology (MOSYS), University of Vienna, Vienna, Austria
[c] Department of Molecular and Clinical Pathology and Medical Genetics, University Hospital Ostrava, Ostrava, Czech Republic
[d] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Czech Republic
[e] Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Slovak Republic
[f] Institute of Neuroimmunology, Slovak Academy of Sciences, Slovak Republic
[g] Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway
[h] Department of Clinical Biochemistry, Palacký University Olomouc and University Hospital Olomouc, Czech Republic
[i] Vienna Metabolomics Center (VIME), University of Vienna, Vienna, Austria
[j] Faculty of Biology, Plant Evolutionary Cell Biology, Ludwig-Maximilians-Universität München, Germany

ARTICLE INFO

Keywords:
Metabolomic data analysis
Systems biology
Metabolite interactions
Dynamic relationships

ABSTRACT

Background and Objective: Metabolomic interaction networks provide critical insights into the dynamic relationships between metabolites and their regulatory mechanisms. This study introduces MInfer, a novel computational framework that integrates outputs from MetaboAnalyst, a widely used metabolomic analysis tool, with Jacobian analysis to enhance the derivation and interpretation of these networks.
Methods: MInfer combines the comprehensive data processing capabilities of MetaboAnalyst with the mathematical modeling power of Jacobian analysis. This framework was applied to various metabolomic datasets, employing advanced statistical tests to construct interaction networks and identify key metabolic pathways.
Results: The application of MInfer revealed significant metabolic pathways and potential regulatory mechanisms across multiple datasets. The framework demonstrated high precision, sensitivity, and specificity in identifying interactions, enabling robust network interpretations.
Conclusions: MInfer enhances the interpretation of metabolomic data by providing detailed interaction networks and uncovering key regulatory insights. This tool holds significant potential for advancing the study of complex biological systems.

1. Introduction

Metabolomic analysis has emerged as a cornerstone of systems biology, providing unprecedented insights into the molecular underpinnings of cellular processes, disease mechanisms, and metabolic regulation [1]. Metabolites play a key role in reflecting physiological states, with fluctuations in their levels often signaling shifts in cellular function [2] or pathology [3]. As a result, metabolomic data hold immense potential for biomarker discovery [4], disease diagnosis [5], and therapeutic targeting [6]. However, interpreting these complex data in the context of highly interconnected metabolic networks remains a significant challenge.

Predictive models are at the forefront of diagnostic methods aimed at aiding the rapid detection of disease diagnosis [6], which ultimately plays a critical role in treatment. Recent studies [7–9] demonstrate that metabolomics data can detect subtle changes in the immune system that

* Corresponding authors.
E-mail addresses: Jana.Schwarzerova@vut.cz (J. Schwarzerova), wolfram.weckwerth@univie.ac.at (W. Weckwerth), thomas.naegele@biologie.uni-muenchen.de (T. Nägele).

https://doi.org/10.1016/j.cmpb.2025.108672
Received 9 December 2024; Received in revised form 5 February 2025; Accepted 13 February 2025
Available online 18 February 2025

may play a key role in early symptoms detection. This raises new opportunities for the development of prediction tools specifically tailored for metabolomics data.

The rapid advancement of experimental metabolomics analysis has opened new avenues for understanding cellular processes at the molecular level. Metabolites serve essential functions in various biological processes and are often indicative of both physiological [10] and pathological states [5]. Despite the wealth of data generated by modern metabolomic technologies, one of the key challenges remains the interpretation of this information within the context of metabolic networks.

Existing tools for analyzing metabolomic data, such as MetaboAnalyst [11], have become essential for processing and visualizing metabolomic datasets. MetaboAnalyst provides comprehensive workflows for data normalization, statistical analysis, and pathway mapping, facilitating the identification of metabolic pathways and functional insights. Despite these capabilities, current tools often limited in their ability to infer the dynamic interactions between metabolites, which constrains their utility in elucidating the underlying network behavior that drives cellular processes. Moreover, manually curating metabolic networks using databases like KEGG [12] can be a tedious process, susceptible to errors, particularly when dealing with the intricate complexity of biological systems. To overcome these limitations, advanced computational methods are required to capture the dynamic interactions within metabolomic network. One promising approach is to use Jacobian analysis, a mathematical technique used in systems biology to model the stability and dynamic behavior of biochemical networks [13]. By quantifying how small perturbations in metabolite concentrations affect other components of the network, Jacobian analysis provides a robust framework for understanding the intricate dependencies within metabolic systems [13–15].

In this study, we introduce MInfer (https://github.com/cellbiomaths/MInfer), a novel computational platform that integrates the data-processing strengths of MetaboAnalyst [11] with the analytical power of Jacobian analysis [15]. MInfer is designed to construct metabolomic interaction networks that incorporate both metabolic pathways and the dynamic metabolites relationships, offering a deeper level of biological insight.

We apply MInfer to a series of metabolomic datasets, showcasing its ability to reveal critical metabolic interactions and pathways. Our findings highlight the potential of MInfer as a valuable tool for the comprehensively analyzing of metabolomic data, enhancing the interpretation of complex biological systems and advancing our understanding of disease mechanisms and metabolism. In this paper, we detail the development of MInfer, its methodological framework, and the biological discoveries enabled through its application.

1.1. Related work

The analysis of metabolomic data, characterized by its high dimensionality and complexity, requires a comprehensive computational approach to extract meaningful biological insights. A variety of computational tools have been developed to address this challenge, among which COVAIN (Matlab tool for **Cova**riance **In**verse) [15] stands out. COVAIN provides a fully integrated workflow for the statistical analysis of metabolomic data, supporting both uni- and multivariate analyses, time-series evaluation, and correlation network analysis [15].

One of the most innovative aspects of COVAIN [15] is its algorithm for reconstructing an inverse differential Jacobian matrix from metabolomics data, a mathematical approach that is crucial for analyzing dynamic interactions within metabolic networks. The algorithm integrates covariance data from metabolomics with a stoichiometric matrix representing metabolic pathways, allowing researchers to pinpoint potential perturbation sites within a network. This feature enables the inference of changes in network behavior under different metabolic conditions, offering insights into the regulatory mechanisms that control metabolism.

COVAIN introduced this foundational concept of using of Jacobian [15] and Lyapunov analyses [13] for studying the stability and dynamic properties of biochemical networks. This technique is especially powerful for modeling the non-linear dynamics characteristic of biological systems. A closely related approach, based on the stochastic Lyapunov matrix equation, has been used to unify system-theoretical principles across both biology and ecology [13]. This method bridges forward and inverse modeling strategies, allowing researchers to analyze system dynamics around quasi-steady states by leveraging statistical properties of large-scale multi-omics data [16]. By integrating stochastic simulations, this approach offers insights into how perturbations propagate through a system, contributing to a more predictive understanding of genotype-environment-phenotype relationships [17].

The stochastic Lyapunov matrix equation, also widely applied in control theory and machine learning, extends beyond biology into systems engineering. In ecological studies, similar mathematical frameworks, such as community matrices, have been used to examine the stability and complexity of ecosystems [13]. The hybridization of these approaches—spanning from biological systems to ecological models—holds promise for improving our understanding of both individual organism function and broader ecological interactions. By leveraging eigenvalues, matrix algebra, and stochastic differential equations, this framework offers a robust method for assessing the dynamic behavior of complex biological networks [13]. In a recent study, we applied inverse differential Jacobian calculation from metabolomics data combined with multi-omics analysis to investigate macrophage dynamics [16]. This approach identified a novel metabolomic checkpoint in macrophage polarization. In a follow-up study this checkpoint was confirmed to be essential for tumor growth inhibition by tumor-associated macrophages [18]. The integration of these advanced computational techniques holds the potential to transform metabolomi data interpretation, facilitating the discovery of new biomarkers and therapeutic targets and ultimately improving clinical outcomes.

1.2. Benchmark dataset

Firstly, we employed datasets from a previous study on *Arabidopsis thaliana*, which explored metabolic adjustments at two distinct temperature conditions (6 °C and 16 °C) [19]. These datasets encompass 37 metabolites that represent the core metabolome of 241 *A. thaliana* ecotypes.

Additionally, a red blood cell metabolism model (http://www.ebi.ac.uk/biomodels-main/BIOMD0000000070) from the BioModels database [20] was used to illustrate the inverse differential Jacobian approach, as demonstrated in the dataset from a study [15] by Sun and Weckwerth. Simulated metabolite data were generated for two states, one with high glucose import and the other with low glucose import, leading to the calculation of the corresponding covariance matrices. The inverse differential Jacobian was subsequently derived from these covariance data to pinpoint possible perturbation sites within the biochemical network [2]. This approach facilitates the identification of differential regulation in reactions triggered by changes in metabolite concentrations, as shown in the perturbed red blood cell model.

To further validate the inverse differential Jacobian approach, we utilized data from the study by Cífková et al. [21] and the study by Karlíková et al. [22]. The study by Cífková et al. [21] analyzed metabolic profiles in lung tumor tissues and adjacent nonmalignant tissues from 23 patients with non-small cell lung cancer (NSCLC). This comprehensive dataset encompassed 500 compounds, including 309 lipids and 191 metabolites, with isomeric molecules such as PC, PC—O, PC-P, PE, PE-O, and PE-P resolved using RP-UHPLC/MS. By focusing on a selected group of 44 metabolites that demonstrated the most significant variations, we applied the inverse differential Jacobian method to this NSCLC dataset to pinpoint metabolic pathways and reactions potentially disrupted by tumor development. This approach allowed us

to map sites of regulatory perturbations within the lung cancer metabolic network, highlighting its potential utility in identifying biochemical mechanisms underlying cancer metabolism.

1.3. Methods

The aim of this study was to establish a foundation for further research using our R package, MInfer. This package extends the analytical capabilities of MetaboAnalyst [11] by incorporating Jacobian analysis, enabling the derivation and interpret metabolomic interaction networks, see Fig 1. This schematic highlights the seamless integration of MInfer into existing workflows, providing a robust framework for advanced metabolomic studies.

Firstly, the proposed R package provides a function that accepts a matrix of metabolites to create a metabolomics interaction network. The package includes a library of metabolomic pathways derived from MetaboAnalyst. Furthermore, the set of metabolomic pathways is extended by incorporating information from the red blood cell model obtained from BIOM0000000070 [15], the lung metabolomic interaction network created by the authors of the tools, and data from the Weiszmann study [19] on *A. thaliana*.

For the analysis, metabolite data were prepared by organizing them into distinct groups (control vs case). The covariance matrix C_k for each group M_k was computed to represent the variability among metabolites, defined as:

$$C_k = cov(M_k) = \frac{1}{N-1}(X - \overline{X})^T(X - \overline{X}),$$

where X is the matrix of metabolite data and $\overline{X}$ is the mean value. The Jacobian matrix J as matrix of first partial derivatives that describes how the outputs of a model change in response to changes in its inputs. For a function $f: \mathbb{R}^n \rightarrow \mathbb{R}^m$, the Jacobian is defined as:

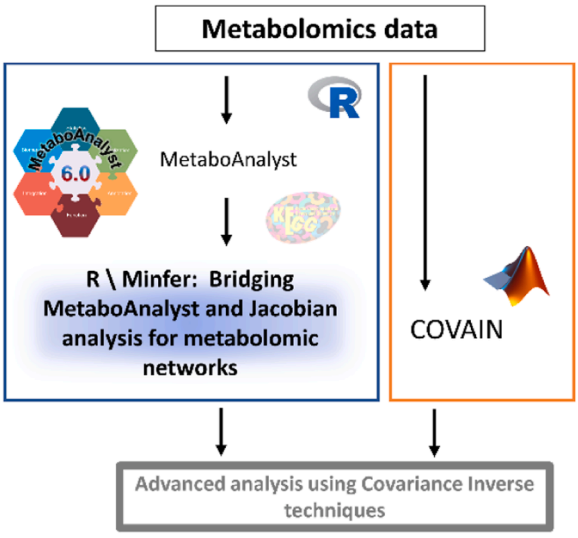

Fig. 1. Schematic representation of MInfer integration into advanced metabolite analysis via inverse covariance techniques. At the start, metabolomic data are analyzed using MetaboAnalyst, which employs KEGG ID nomenclature for metabolite identification. This step ensures consistent and accurate annotations, serving as a foundation for downstram analyses. Following this, data can be preprocessed through two pathways: (Blue) MInfer: Leveraging the Jacobian matrix-based approach, MInfer enables dynamic modeling of metabolic networks, uncovering regulatory patterns and interactions at the systems level. (Orange) COVAIN: Alternatively, data can be analyzed using the covariance-based approach in combination with the inverse differential Jacobian implemented in COVAIN, a tool programmed in Matlab. This schematic highlights the seamless integration of MInfer into existing workflows, providing a robust framwork for advanced metabolomic studies.

$$J(f) = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} & \cdots & \frac{\partial f_1}{\partial x_n} \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} & \cdots & \frac{\partial f_2}{\partial x_n} \\ \cdots & \cdots & \cdots & \cdots \\ \frac{\partial f_m}{\partial x_1} & \frac{\partial f_m}{\partial x_2} & \cdots & \frac{\partial f_m}{\partial x_n} \end{bmatrix}$$

The estimation of the Jacobian matrix J begins with the equation [15]:

$$JC + CJ^T = -2D,$$

which establishes the foundational relationship among matrices C, A and D. This equation implies that, at the system's fixpoint, A approximates the Jacobian J, simplifying the solution process. The Total Least Squares (TLS) was applied here, using singular value decomposition (SVD) to solve the system of equations for matrices A and D [15]. The matrix D is constructed by random noise $\sigma \sim N(0, \sigma^2)$ for each iteration, which was incorporated into the diagonal elements of matrix D:

$$D_{ii} = -0,5\sigma_i^2$$

The matrix A was constructed using the Kronecker product:

$$A_{left} = C^T \otimes I_N$$

$$A_{right} = I_N \otimes C$$

with I_N being the identity matrix of size N. The matrix D was vectorized to yield $D_{vec} = vec(D^T)$.

Using SVD, the system of equations represented by $[A_2, D_{vec}]$ was solved to estimate the Jacobian vector as:

$$J_{vec} = -\frac{V_{1:(N^2-1),N^2}}{V_{N^2,N^2}}.$$

Statistical analysis, including the calculation of median and quantiles, was performed on the estimated Jacobian. The normalized median Jacobian vector was reshaped back into a matrix from $J = reshape(J_{vec\ norm\ median}, N, N)$ to facilitate the interpretation of dynamic interactions among metabolites. This comprehensive methodological framework effectively integrates statistical techniques and the Jacobian matrix to analyze interrelationships in metabolomics.

Through the application of inverse covariance methods, MInfer enables a deeper exploration of metabolomic data, facilitating the construction of interaction networks that capture dynamic relationships between metabolites. This framework thus supports advanced analyses, providing insights into complex biological systems and potential regulatory mechanisms.

2. Results

In this section, we present the results of applying the MInfer framework to a variety of metabolomic datasets, showcasing its effectiveness in constructing metabolomic interaction networks and capturing dynamic relationships between metabolites. The R package, developed based on the biochemical Jacobian as outlined earlier [23], supports differential calculations essential to this analysis.

We begin by evaluating Jacobian calculations using benchmark datasets to identify key metabolic pathways and assess MInfer's capability in mapping complex metabolite interactions. These validation experiments confirm the robustness of our approach, highlighting MInfer's versatility in interpreting metabolomic data across diverse biological contexts. Finally, we demonstrate MInfer's application in a lung cancer case study, where it successfully maps disease-specific

metabolic disruptions.

2.1. Validation experiments

To validate the inverse differential Jacobian approach, we referenced prior research on *A. thaliana*, particularly the study by Weiszmann et al. [19] and analytics findings by Nägele et al. [23]. These studies demonstrate how differential Jacobians can be derived from metabolite variance data collected under different growth conditions, such as temperatures of 6 °C (Fig. 1 A1) and 16 °C (Fig. 1 A2), highlighting critical regulatory responses in metabolomics pathways. Nägele et al. [23] presented a heatmap of Jacobian matrix entries, illustrating the changes in enzymatic reaction rates between these conditions. The heatmap (Fig. 2, A1 an A2) represents the ration of Jacobian entries as 6 °C versus 16 °C, with a color scale indicating the magnitude of perturbations in biochemical reaction rates. This method is crucial because it highlights the dynamic regulation of specific metabolites in response to environmental changes. Notably, fumarate exhibited substantial changes in sensitivity to aspartate, succinate, and ornithine, while malate showed significant changes with respect to fumarate. These findings align with GWAS analysis, where a SNP marker near the gene FUM2 (encoding fumarase) was strongly associated with fumarate levels in the cold stressed plants [19]. This demonstrates the high variance of fumarate metabolism as a cold response effect in different Arabidopsis ecotypes, further supported by wet-lab validation in the study by Weiszmann et al. [19].

The increased Jacobian ratios for metabolites such as citrate, fumarate, glutamate, raffinose, and leucine under low-temperature stress underscore the sensitivity of metabolic networks to environmental changes. These findings provide compelling evidence for the utility of the inverse differential Jacobian approach in capturing biologically relevant regulatory patterns and identifying key metabolites involved in adaptive responses. The entries in the Jacobian matrix serve to indicate the sensitivity of enzymatic reaction rates to fluctuations in particular metabolites, providing insights into how metabolic pathways adapt to varying conditions.

At lower temperatures, the study observed substantial regulatory shifts in metabolites such as citrate, fumarate, glutamate, raffinose, and leucine. The increased Jacobian ratios for these metabolites suggested an intensified regulatory role under cold stress. These findings illustrate how temperature variations can significantly influence the regulatory mechanisms within metabolic pathways, impacting processes from citrate metabolism to nitrogen assimilation.

This evidence not only supports the validity of our approach but also underscores its effective implementation within our R package, MInfer. For visual representation, we can showcase these dynamics in a figure (Fig 2: A1, A2), illustrating how the Jacobian entries correlate with the observed shifts in metabolite regulation across different temperatures. This figure will help to further elucidate the relationship between environmental factors and metabolic regulation, reinforcing the applicability of the inverse differential Jacobian approach.

To further validate this approach, we applied it to a published model of red blood cell metabolism from the BioModels database (BIOM0000000070), as demonstrated by Sun and Weckwerth [15]. Simulated metabolite data reflecting experimental scenarios with two distinct conditions—high and low glucose import—were generated. Covariance matrices and Jacobian matrices were derived for each condition, mirroring typical experimental designs. The comparison of Jacobian matrices across these two biological systems (Fig. 2) demonstrates the flexibility and robustness of our approach, bridging computational analysis with experimental validation and biological insights.

2.2. Case study I: metabolite profiling of the chronic myeloid leukemia

By leveraging advanced metabolomic analysis, researchers can identify key metabolic pathways disrupted in Chronic Myeloid Leukemia (CML), paving the way for potential diagnostic and therapeutic

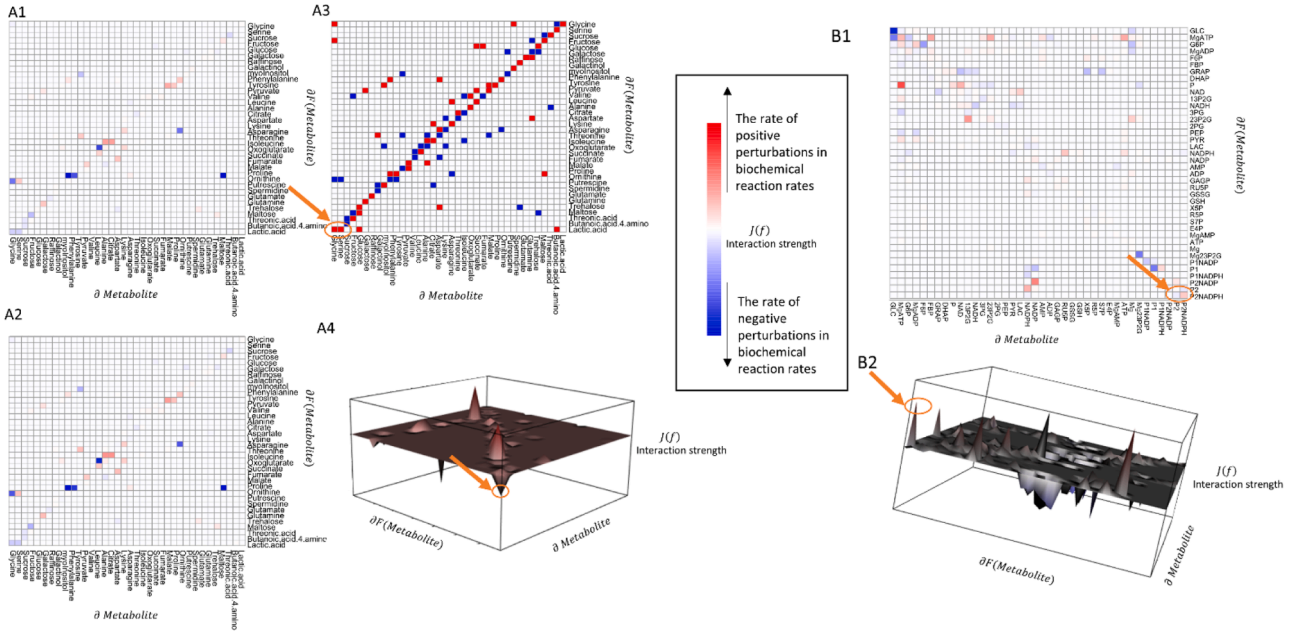

Fig. 2. Comparison of Jacobian matrices from different biological models: On the left side, the Jacobian matrices calculated from the benchmark dataset of A. thaliana are presented, while the right side features those for red blood cells. (A1): Jacobian matrix entries from A. thaliana at 6 °C according to Nägele [23]. (A2): Jacobian matrix entries at 16 °C according to Nägele. Significant changes are observed in fumarate's interactions with aspartate, succinate, and ornithine, as well as malate's interaction with fumarate confirmed by wet-lab experiments in [19]. (A3): Jacobian matrices from different biological models (6 °C/16 °C). Color bar indicates ration of Jacobian entries (6 °C/16 °C), with dark red an dark blue representing strong pertubation in biochemical reaction rates. (A4): 3D visualization of Jacobian matrix entries from A. thaliana (B) Jacobian matrix of red blood cells.. (B1): Jacobian matrices from different biological models of case and control groups from red blood cells [15]. (B2): displaying a 3D represenation

advancements. This case study focuses on the exploration of metabolomic networks in CML, utilizing data from the KEGG databases [12].

Fig. 3 illustrates the covariance inverse analysis of selected metabolites and the metabolic interactive network (MIN) associated with CML. This visualization highlights the interconnected pathways and metabolites. Notably, Fig. 3A showcases metabolites involved in energy metabolism, nucleotide synthesis, and amino acid metabolism, all of which play crucial roles in the proliferation and survival of leukemic cells. The Jacobian differential matrix, depicted in Fig. 3B, is a powerful tool for assessing the relationships and interactions between metabolites in the context of CML. This matrix provides a quantitative measure of how changes in metabolite concentrations can affect one another, reflecting the dynamic nature of metabolic pathways in leukemia. By analyzing the Jacobian matrix, researchers can gain insights into the regulatory mechanisms governing metabolite interactions in CML. Fig. 3C presents a 3D visualization of the differential matrices, offering a more intuitive understanding of the complex interactions within the CML metabolomic network.

Analysis showed spermine as a potential target in the chronic myeloid leukemia dataset. Spermine acts as a negative regulator of macrophage differentiation in myeloid leukemia cells, with this regulation linked to the modulation of polyamine catabolic enzymes. Polyamines pathway metabolites, including spermine, have been shown to regulate leukemia stem cell function. Reducing polyamine concentrations helps alleviate leukemia burden [24,25]. The connection between spermine and L-arginine in polyamine metabolism should be verified in the appropriate heatmap or dataset (see Fig 3B).

2.3. Case study II: lung cancer metabolomic networks

This study investigates metabolomic networks in lung cancer using the MInfer R packages, integrating pathway data from MetaboAnalyst resources like KEGG and SMP databases. The Jacobian analysis was applied to 122 metabolites, with Fig. 4 illustrating interactions for the first 22. One of the metabolites with multiple positive perturbations with other metabolites in biochemical rates was L-arginine. Alteration in L-arginine metabolism has been previously associated with lung cancer [26] and the tumour microenvironment [27]. The analysis highlights key metabolic players, including ADP, FAD, and S-Adenosylmethionine, which are critical for energy metabolism, nucleotide synthesis, and amino acid regulation—processes essential for tumor progression.

The Jacobian matrix quantifies how fluctuations in metabolite concentrations influence others, shedding light on the dynamic regulation within the network. Notable findings include L-Carnitine's role in enhanced mitochondrial metabolism supporting tumor growth and L-Arginine's involvement in cell signaling and angiogenesis. Additionally, glutathione emerges as vital for managing oxidative stress, which is crucial for cancer cell survival.

A 3D visualization enhances understanding of metabolic interactions, identifying key network hubs and potential intervention points in lung cancer metabolism. This approach underscores the potential of Jacobian analysis to identify biomarkers and therapeutic targets. By mapping dynamic metabolomic changes, the study advances our understanding of metabolic dysregulation in lung cancer, paving the way for more precise diagnostic and treatment strategies.

2.4. Basic statistics of network analysis for metabolomics interaction network

Table 1 provides a comparative summary of the basic statistics for the metabolomic interaction networks associated with CML and lung cancer. These metrics offer valuable insights into the structural and functional characteristics of the networks, shedding light on their complexity and dynamics.

For the CML network, the analysis reveals a relatively modest structure comprising 44 nodes (metabolites) and 52 edges (interactions). Each metabolite interacts, on average, with 2.3 other metabolites, indicating a moderately interconnected network. The network's diameter and radius are 7 and 4, respectively, suggesting a structure with a reasonable balance of connectivity and reach. The characteristic path

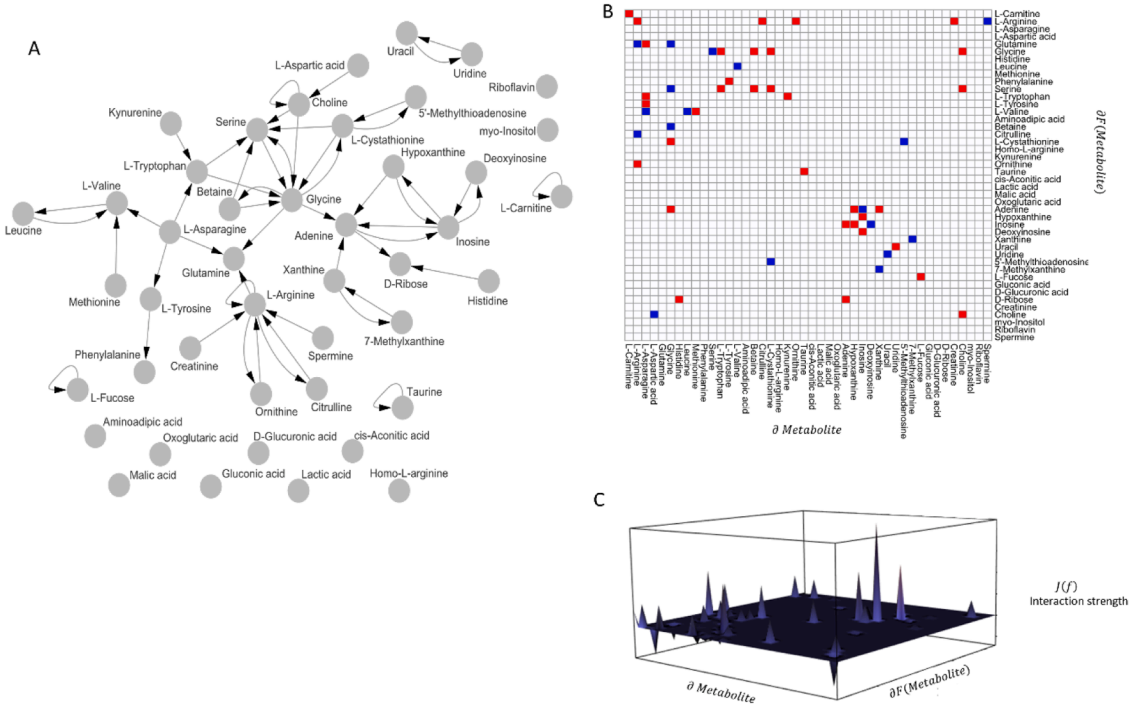

Fig. 3. Metabolomic Interaction Networks (MIN) and Jacobian Analysis in Chronic Myeloid Leukemia (CML): A) MIN illustrating metabolic pathways in human metabolism derived by KEGG; B) Jacobian matrix showcasing the differential relationships between metabolites; C) 3D visualization of the Jacobian matrix, revealing dynamic interactions and clustering of metabolites in the context of lung cancer.

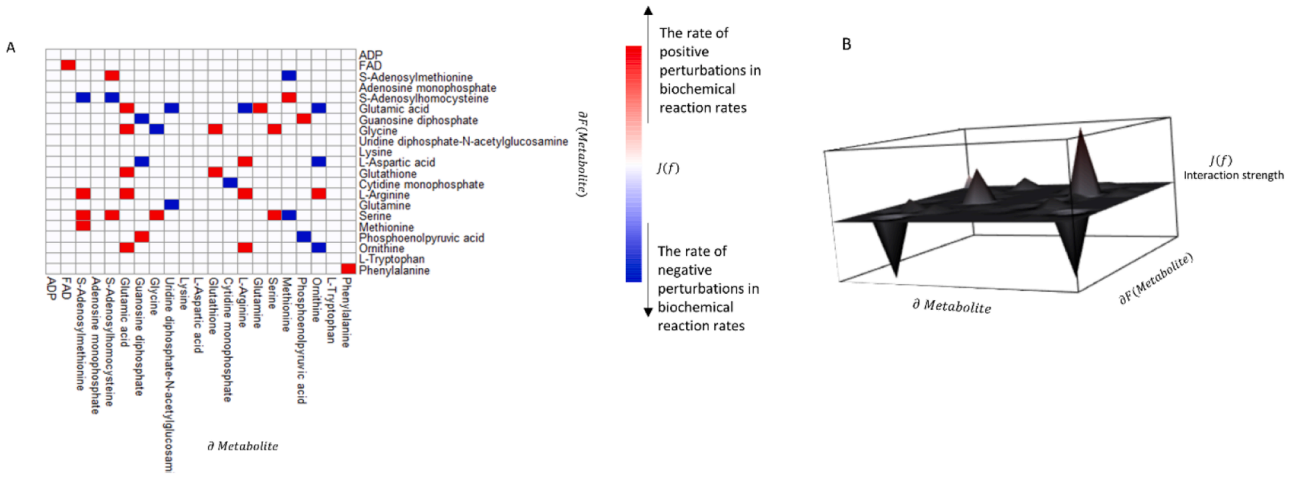

Fig. 4. Jacobian Analysis in lung cancer; illustration of interaction for the first analysed 22 metabolites: A) Jacobian matrix showcasing the differential relationships between first 22 metabolites; C) 3D visualization of the Jacobian matrix, revealing dynamic interactions and clustering of metabolites in the context of lung cancer.

Table 1
Basic statistics of network analysis for Metabolomic interaction network.

	CML	Lung cancer
Number of nodes	44	121
Number of edges	52	187
Avg. number of neighbors	2.345	4.652
Network diameter	7	7
Network radius	4	4
Charatristic path length	3.682	3.232
Clustering coefficient	0.133	0.616
Network density	0.084	0.103

length of 3.7 indicates that most metabolites are separated by about four intermediaries, reflecting an efficient exchange of metabolic information. However, the clustering coefficient of 0.133 and network density of 0.084 highlight the network's sparse nature, typical of disease-specific metabolic pathways with selective interaction patterns.

In contrast, the lung cancer network demonstrates a higher level of complexity, with 121 nodes and 187 edges. This greater connectivity is evident from the higher average number of neighbors per node (4.7), indicating that each metabolite is connected to more interactions. While the network's diameter and radius remain consistent with the CML network (7 and 4, respectively), the characteristic path length is shorter at 3.2, suggesting even more efficient communication between metabolites. The lung cancer network also displays a higher clustering coefficient (0.616) and network density (0.103), indicating more pronounced clustering and interaction among metabolites, which may reflect the diverse metabolic alterations characteristic of tumorigenesis.

3. Discussion

The development of MInfer marks a significant advancement in metabolomic data analysis, providing a streamlined tool that enhances the capabilities of widely used platforms like MetaboAnalyst by integrating them with the mathematical rigor of Jacobian analysis. Metabolomics has gained traction as a pivotal area within systems biology, enabling researchers to decode the intricate relationships among metabolites and their roles in biological processes. By integrating MInfer within R, researcher can now extend the utility of MetaboAnalyst and other R packages such as erah [28], fostering the integration of targeted and untargeted analysis approaches. This combined approach provides a comprehensive framework for constructing metabolomic interaction networks that encapsulate the subtleties of metabolic regulation.

Our validation experiments with benchmark datasets, particularly from *A. thaliana* and red blood cell metabolism models, underscore MInfer's efficacy in revealing critical metabolic pathways. The differential Jacobians calculated from these datasets illustrate how environmental conditions, such as temperature variations, can significantly influence metabolic regulation. This ability to capture dynamic interactions positions MInfer as a valuable tool in both basic research and clinical applications, where understanding metabolic shifts is essential for disease diagnosis and treatment strategies.

The versatility of MInfer extends beyond lung cancer, as demonstrated by its application in metabolomic studies of Chronic Myeloid Leukemia (CML). CML is characterized by specific metabolic reprogramming, driven in part by the BCR-ABL1 fusion protein, which promotes abnormal cell proliferation and survival. Our analysis of CML metabolomic networks revealed critical disruptions in energy metabolism, amino acid biosynthesis, and nucleotide synthesis, all of which are vital for sustaining the rapid growth of leukemic cells. Notably, metabolites such as L-Carnitine, L-Arginine, and Kynurenine were identified as significant players in the altered metabolic landscape of CML.

Elevated levels of L-Carnitine, a key metabolite involved in fatty acid oxidation and mitochondrial energy production, suggest an increased reliance on mitochondrial metabolism to support leukemic proliferation. This mirrors findings in lung cancer, where mitochondrial upregulation supports tumorigenesis, highlighting the broader relevance of L-Carnitine as a marker and therapeutic target in oncology [29]. However, these potential hypotheses need further validation with large prospective cohorts of patients with multicentric clinical data concerning survival and response to targeted therapy, immunotherapy and chemotherapy.

L-Arginine, essential for nitric oxide synthesis and cell signaling, also displayed altered metabolism in CML. Changes in its regulation may influence angiogenesis and immune modulation, two factors crucial for both leukemic cell survival and progression.

Additionally, Kynurenine, derived from L-Tryptophan metabolism, emerged as a metabolite of interest in CML [30]. Its elevation may reflect immune evasion strategies employed by leukemic cells, similar to its role in the tumor microenvironment of NSCLC [30]. The accumulation of Kynurenine in CML underscores its potential utility as a surrogate marker for assessing disease progression and therapeutic response, particularly in immune-targeted therapies.

By identifying regulatory perturbations within intricate biochemical networks, MInfer aids in discovering biomarkers and therapeutic targets, thereby deepening our understanding of cancer metabolism. This knowledge can potentially inform treatment strategies and improve patient outcomes.

4. Conclusion

In summary, MInfer represents a novel computational framework that effectively facilitates the transition from MetaboAnalyst to Jacobian analysis, enhancing the exploration of metabolomic networks. This integration allows researchers to harness the strengths of both platforms, enabling a comprehensive analysis that captures the dynamic interactions between metabolites. By providing a seamless pathway for fluent inference of interaction networks derived from the MetaboAnalyst library, MInfer reflects the intricate and evolving nature of metabolite relationships. This capability significantly improves the interpretation of metabolomic data within the context of biological systems, allowing for a more nuanced understanding of metabolic processes. The ability to visualize and analyze the intricate relationships between metabolites will empower researchers to develop predictive models that can anticipate metabolic changes associated with various physiological conditions or diseases.

Moreover, we anticipate that MInfer will stimulate further research and collaboration within the metabolomics community, driving innovation and discovery in this rapidly growing field. By facilitating interdisciplinary partnerships, MInfer has the potential to enhance our collective knowledge and foster the development of new methodologies that address the challenges inherent in metabolomic data analysis.

While MInfer provides a powerful framework for target-based analysis, this also comes with certain limitations. It relies on a predefined list of metabolites, which can restrict the breadth of analysis. However, there are other R packages that offer untargeted analysis, focusing on the identification of metabolites without a fixed target list. This approach allows for the exploration of a wider range of metabolites, potentially uncovering novel interactions and pathways.

By providing a streamlined tool that focuses on target-based analysis in R language, MInfer offers a highly reliable and specific approach for understanding metabolomic networks. However, this reliance on target metabolites highlights a potential opportunity for future integration with untargeted methods, where tools like erah could complement MInfer to broaden the scope of metabolomic research.

Ethical approval

This study does not require ethical approval as it is based entirely on previously published datasets. No new data involving human or animal subjects were collected or generated for this research.

Funding

This research was supported by the Ministry of Health of the Czech Republic through the conceptual development of research organization funding (FNOs/2024).

This article has been produced with the financial support of the European Union under the LERCO project (Project No. CZ.10.03.01/00/22_003/0000003) via the Operational Programme Just Transition.

Computational resources were supplied by the Ministry of Education, Youth, and Sports of the Czech Republic through the CESNET project (Project No. LM2015042) and the CERIT-Scientific Cloud project (Project No. LM2015085), under the program Projects of Large Research, Development and Innovations Infrastructures.

Ethics statement

This study does not require ethical approval as it is based entirely on previously published datasets. No new data involving human or animal subjects were collected or generated for this research.

CRediT authorship contribution statement

Jana Schwarzerova: Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. **Erdő Gabor Mate:** Validation, Investigation. **Jakub Idkowiak:** Validation, Investigation. **Dominika Olesova:** Writing – review & editing, Formal analysis, Conceptualization. **Ales Kvasnicka:** Writing – review & editing, Formal analysis, Conceptualization. **Dana Dobesova:** Conceptualization. **David Friedecky:** Writing – review & editing, Conceptualization. **Valentyna Provaznik:** Supervision. **Jozef Skarda:** Writing – review & editing, Investigation, Formal analysis. **Wolfram Weckwerth:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Thomas Nägele:** Writing – review & editing, Validation, Software, Methodology, Formal analysis.

Declaration of competing interest

The authors have declared no conflict of interest

Acknowledgment

We express our sincere gratitude to Michal Holčapek, Eva Cífková, and their co-authors for their invaluable contributions to the field, which served as foundational data for this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cmpb.2025.108672.

References

[1] Sharma, M., Singh, A. (2024). Systems biology for metabolic disorder and disease. In: Joshi, S., Ray, R.R., Nag, M., Lahiri, D. (eds) Systems Biology Approaches: Prevention, Diagnosis, and Understanding Mechanisms of Complex Diseases. Springer, Singapore. https://doi.org/10.1007/978-981-99-9462-5_4.

[2] W. Weckwerth, Unpredictability of metabolism—The key role of metabolomics science in combination with next-generation genome sequencing, Anal. Bioanal. Chem. 400 (2011) 1967–1978.

[3] J. Kang, L. Zhu, J. Lu, X. Zhang, Application of metabolomics in autoimmune diseases: insight into biomarkers and pathology, J. Neuroimmunol. 279 (2015 Feb 15) 25–32.

[4] R. González-Domínguez, A. Sayago, Á. Fernández-Recamales, Metabolomics in Alzheimer's disease: the need of complementary analytical platforms for the identification of biomarkers to unravel the underlying pathology, J. Chromatogr. B 1071 (2017) 75–92.

[5] V.R. Varma, A.M. Oommen, S. Varma, R. Casanova, Y. An, R.M. Andrews, R. O'Brien, O. Pletnikova, J.C. Troncoso, J. Toledo, R. Baillie, Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: a targeted metabolomics study, PLoS Med. 15 (1) (2018) e1002482.

[6] D.K. Trivedi, K.A. Hollywood, R. Goodacre, Metabolomics for the masses: the future of metabolomics in a personalized world, New. Horiz. Transl. Med. 3 (6) (2017) 294–305.

[7] A. Mujalli, W.F. Farrash, K.S. Alghamdi, A.A. Obaid, Metabolite alterations in autoimmune diseases: a systematic review of metabolomics studies, Metabolites. 13 (9) (2023) 987.

[8] S. Rodriguez-Muguruza, A. Altuna-Coy, V. Arreaza-Gil, S. Castro-Oreiro, M. Mendieta-Homs, N. Del Castillo-Piñol, M.J. Poveda-Elices, R. Fontova-Garrofé, M R.Chacón, Metabolomic dataset - EAR study (Verze 1) [Data set], Zenodo (2023), https://doi.org/10.5281/zenodo.8131016.

[9] J. Fu, F. Zhu, C.J. Xu, Y. Li, Metabolomics meets systems immunology, EMBO Rep. 24 (4) (2023) e55747.

[10] A. Price-Whelan, L.E. Dietrich, D.K. Newman, Rethinking'secondary'metabolism: physiological roles for phenazine antibiotics, Nat. Chem. Biol. 2 (2) (2006) 71–78.

[11] J. Xia, N. Psychogios, N. Young, D.S. Wishart, MetaboAnalyst: a web server for metabolomic data analysis and interpretation, Nucleic. Acids. Res. 37 (suppl_2) (2009) W652–W660.

[12] M. Kanehisa, M. Furumichi, M. Tanabe, Y. Sato, K. Morishima, KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic. Acids. Res. 45 (D1) (2017) pp.D353-D361.

[13] W. Weckwerth, Toward a unification of system-theoretical principles in biology and ecology—The stochastic lyapunov matrix equation and its inverse application, Front. Appl. Math. Stat. 5 (2019) 29.

[14] X. Sun, B. Länger, W. Weckwerth, Challenges of inversely estimating jacobian from metabolomics data, Front. Bioeng. Biotechnol. 3 (2015) 188.

[15] X. Sun, W. Weckwerth, COVAIN: a toolbox for uni-and multivariate statistics, time-series and correlation network analysis and inverse estimation of the differential jacobian from metabolomics covariance data, Metabolomics. 8 (2012) 81–93.

[16] J.L. Wilson, T. Nägele, M. Linke, F. Demel, S.D. Fritsch, H.K. Mayr, Z. Cai, K. Katholnig, X. Sun, L. Fragner, A. Miller, Inverse data-driven modeling and

multiomics analysis reveals phgdh as a metabolic checkpoint of macrophage polarization and proliferation, Cell Rep. 30 (5) (2020) 1542–1552.
[17] J. Li, S. Waldherr, W. Weckwerth, COVRECON: automated integration of genome- and metabolome-scale network reconstruction and data-driven inverse modeling of metabolic interaction networks, Bioinformatics. 39 (7) (2023) p.btad397.
[18] Z. Cai, W. Li, S. Hager, J.L. Wilson, L. Afjehi-Sadat, E.H. Heiss, T. Weichhart, P. Heffeter, W. Weckwerth, Targeting PHGDH reverses the immunosuppressive phenotype of tumor-associated macrophages through α-ketoglutarate and mTORC1 signaling, Cell Mol. Immunol. 21 (5) (2024) 448–465.
[19] J. Weiszmann, D. Walther, P. Clauw, G. Back, J. Gunis, I. Reichardt, S. Koemeda, J. Jez, M. Nordborg, J. Schwarzerova, I. Pierides, Metabolome plasticity in 241 Arabidopsis thaliana accessions reveals evolutionary cold adaptation processes, Plant Physiol. 193 (2) (2023) 980–1000.
[20] Chelliah, V., Laibe, C. and Le Novère, N., 2013. BioModels database: a repository of mathematical models of biological processes. In Silico Systems Biology, pp.189–199.
[21] E. Cífková, R. Brumarová, M. Ovčačíková, D. Dobešová, K. Mičová, A. Kvasnička, Z. Vaňková, J. Šiller, L. Sákra, D. Friedecký, M. Holčapek, Lipidomic and metabolomic analysis reveals changes in biochemical pathways for non-small cell lung cancer tissues, Biochimica et Biophysica Acta (BBA)-Mol. Cell Biol. Lipids 1867 (2) (2022) 159082.
[22] R. Karlíková, J. Široká, D. Friedecký, E. Faber, M. Hrdá, K. Mičová, I. Fikarová, A. Gardlo, H. Janečková, I. Vrobel, T. Adam, Metabolite profiling of the plasma and leukocytes of chronic myeloid leukemia patients, J. Proteome Res. 15 (9) (2016) 3158–3166.
[23] T. Nägele, A. Mair, X. Sun, L. Fragner, M. Teige, W. Weckwerth, Solving the differential biochemical Jacobian from metabolomics covariance data, PLoS One 9 (4) (2014) e92299, https://doi.org/10.1371/journal.pone.0092299.
[24] I.M. Gavin, D. Glesne, Y. Zhao, C. Kubera, E. Huberman, Spermine acts as a negative regulator of macrophage differentiation in human myeloid leukemia cells, Cancer Res. 64 (20) (2004) 7432–7438.
[25] V. Rondeau, J.M. Berman, T. Ling, C. O'Brien, R. Culp-Hill, J.A. Reisz, M. Wunderlich, Y. Chueh, K.E. Jiménez-Camacho, C. Sexton, K.M. Carter, Spermidine metabolism regulates leukemia stem and progenitor cell function through KAT7 expression in patient-derived mouse models, Sci. Transl. Med. 16 (766) (2024) p.eadn1285.
[26] C. Yang, A. Pataskar, X. Feng, J.M. Navarro, I. Paniagua, J.J. Jacobs, E.A. Zaal, C. R. Berkers, O.B. Bleijerveld, R. Agami, Arginine deprivation enriches lung cancer proteomes with cysteine by inducing arginine-to-cysteine substitutants, Mol. Cell 84 (10) (2024) 1904–1916.
[27] G. Capuano, N. Rigamonti, M. Grioni, M. Freschi, M. Bellone, Modulators of arginine metabolism support cancer immunosurveillance, BMC Immunol. 10 (2009) 1–13.
[28] X. Domingo-Almenara, J. Brezmes, M. Vinaixa, S. Samino, N. Ramirez, M. Ramon-Krauel, C. Lerin, M. Díaz, L. Ibáñez, X. Correig, A. Perera-Lluna, eRah: a computational tool integrating spectral deconvolution and alignment with quantification and identification of metabolites in GC/MS-based metabolomics, Anal. Chem. 88 (19) (2016) 9821–9829.
[29] X. Lyu, Y. Wang, Y. Xu, Z. Zhao, H. Liu, Z. Hu, Metabolomic profiling of tumor tissues unveils metabolic shifts in non-small cell lung cancer patients with concurrent diabetes mellitus, J. Proteome Res. 23 (9) (2024) 3746–3753.
[30] A. Bessede, F. Peyraud, S. Le Moulec, S. Cousin, M. Cabart, F. Chomy, C. Rey, O. Lara, O. Odin, I. Nafia, J.P. Guegan, Upregulation of indoleamine 2, 3-dioxygenase 1 in tumor cells and tertiary lymphoid structures is a hallmark of inflamed non–small cell lung cancer, Clin. Cancer Res. 29 (23) (2023) 4883–4893.