Integration of Metabolomics and Flux Balance Analysis: Applications and Challenges

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Abstract

This book chapter presents an in-depth analysis of the integration of metabolomics and flux balance analysis (FBA) as powerful tools for understanding metabolic processes and their applications in various scientific disciplines. The potential applications of metabolomics in these fields were discussed, highlighting the valuable insights it offers into metabolic pathways and networks. The subsequent sections delve into the different techniques employed in metabolomics research, including targeted and untargeted approaches using "LC–MS, GC–MS, and NMR". The chapter also explores important tools utilized in flux balance analysis, such as OptKnock, OptGene, OptStrain, COBRA Tools, MetaboAnalyst 4.0, OptFlux, CellNetAnalyzer, SBRT, and Escher-FBA. Furthermore, the chapter discusses metabolomics integration using FBA and highlights the methodologies for identifying and annotating metabolites, including the use of metabolite databases and spectral libraries. The integration of metabolomics data with genome-scale metabolic models was explored, along

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with the estimation of metabolic fluxes from metabolomics data using the "Constraint-Based Reconstruction and Analysis (COBRA) Toolbox". The chapter presents case studies and applications that demonstrate the utility of metabolomics and FBA in various contexts, including therapeutic and diagnostic applications. It explores the application of metabolomics in blood, urine, and saliva, highlighting their potential as non-invasive diagnostic tools. Moreover, the chapter addresses the challenges and limitations associated with integrating metabolomics and FBA, providing insights into future perspectives and directions for further research.

10.1   Introduction

Many disorders include hypoxia, which can be extremely harmful to cells. Designing medicines to improve cellular defenses against hypoxia stress is a key objective of medical research, as a prerequisite, additional basic research into the defense systems and processes of hypoxia cell death is needed. The low oxygen level causes a sharp decline in mitochondrial respiratory activity due to the consequence of metabolic (Feala et al. 2009). Due to its-known genome, easily accessible methods for genetic alteration, fecundity, short lifespan, and inherent tolerance to considerable oxygen level changes, *Drosophila melanogaster* has been a popular model organism for systems biology techniques as well as hypoxia investigations. Because of widely recognized genetic makeup, availability of genetic manipulation tools, high reproductive rate, short lifespan, and natural ability to withstand significant variations in oxygen levels, *Drosophila melanogaster* has emerged as a favored model organism for studying systems biology and investigating hypoxia (Krishnan et al. 1997). It is possible for organisms to adapt to shifting environmental conditions while still carrying out vital survival processes from an economic standpoint, it is critical to investigate the effects of these metabolic adaptations, but they can also have major effects on health and disease (Kitano 2004). The modification of fluxes in metabolic networks is one example of molecular adaptation in action. Flux alterations in affected metabolic pathways occur when there are changes in substrate availability. According to Stelling (2004), these flux variations can offer crucial insights into cellular physiology, output, and how organisms react to disruptions. Since intracellular fluxes cannot be observed directly, concentration measurements must be used to quantify them. To achieve this, various experimental and computational methods, including kinetic models, are employed to approximate dynamic fluxes within metabolic networks (Teusink et al. 2000). Dynamic flux balance analysis (DFBA) and ^{13}C-metabolic flux analysis (MFA) have been developed.

Novel methodologies such as ^{13}C-metabolic flux analysis (MFA) and dynamic flux balance analysis (DFBA) have been devise to advance the field (Van Winden et al. 2005). A constraint-based modeling technique called flux balance analysis (FBA) is used to find fluxes in a steady state (Willemsen et al. 2015). The networks of metabolic consist of a larger number of reactions compared to metabolites, resulting in a situation where the stoichiometry of the network imposes mass balance constraints. As a result, an under-determined system of linear equations is created (O'Grady et al. 2012; Wiechert 2001). Capacity constraints, which specify the top and lower bounds of the fluxes, are additionally imposed to condense the solution space. The range of feasible flux values is constrained by these restrictions. The ideal flow distribution within the specified restrictions is then sought after by solving the under-determined linear system as an optimization problem (Förster et al. 2003). The phenotype is described by this objective function as a biological aim like biomass production (e.g., maximal growth yield or energetic efficiency). Each reaction's relative contribution to the phenotype is quantified by the objective function. FBA might be used to calculate fluxes for various steady state conditions in a perturbed system, however this method would not account for transient behavior following a disturbance. By maximizing the objective function over the desired time period, DFBA calculates the transient behavior of the fluxes following a disturbance (Mahadevan et al. 2002). Both capacity constraints and dynamic mass balance constraints were apply to the objective function in flux balance analysis (FBA). The alterations in metabolite concentrations dependent on fluxes, biomass, and other kinetic parameters are described by the dynamic mass balance constraints as differential equations. The maximum rate of change for the fluxes over specific periods of time can be specified using additional restrictions, provided they are available (Varma and Palsson 1994). Dynamic flux balance analysis (DFBA), in contrast to flux balance analysis (FBA), entails evaluating the derivatives (changes) of metabolite concentrations over time. Compared to FBA, where the change in metabolite concentrations is assumed to be zero and only the fluxes are in doubt, this introduces additional unknown parameters. Consequently, the system in DFBA becomes more complex with a higher number of unidentified parameters (Willemsen et al. 2015). To address the dynamic optimization challenge, orthogonal collocation on finite elements is utilized to parameterize the dynamic equations. This approach enables the estimation of dynamic flux profiles. However, it is important to note that this method may be less suitable for modeling extracellular dynamics over extended timeframes or larger metabolic networks. In situations where the driving objective of an organism under varying conditions is known, such as during a diauxic shift, classical dynamic flux balance analysis (DFBA) is employed to estimate the dynamic flux profiles (Mahadevan et al. 2002).

When there are several parameters to estimate, dynamic flux balance analysis (DFBA) is frequently used. Its primary application is for bigger structures to generate dynamic flux profiles, which involve both internal and external fluxes. DFBA is particularly useful when studying the response of an organism to specific perturbations, where external flows through time following the perturbation are of interest. Additionally, DFBA is employ to assess objective functions that influence

transient cellular behavior after disturbances. Experimental techniques like mass spectrometry and liquid or gas chromatography (LC/GC–MS) enable direct measurements of metabolite concentration profiles, allowing for the determination of how concentrations change over time. In DFBA, these measured concentration profiles can be used to determine time derivatives and metabolite concentrations, rather than relying solely on estimation. However, measured concentration profiles cannot be directly incorporate into DFBA. A new technique called MetDFBA is created for overcoming this restriction. MetDFBA constructs a system of linear equations via derivatives that directly calculated from observed concentration profiles and then replacing them into the mass balance equation (Willemsen et al. 2015). This method significantly lessens the computational difficulty of DFBA (Schuetz et al. 2007). This paradigm is especially appropriate for larger systems because of the lower complexity and fewer unknowns. We used time-resolved metabolomics data from a feast-famine experiment employing *Penicillium chrysogenum* to produce these estimates. By leveraging this data, we were able to assess and validate the flow changes predicted by MetDFBA against experimental measurements. This comparison serves to demonstrate the accuracy and reliability of our method in capturing the dynamics of metabolic fluxes in response to perturbations (Canelas et al. 2008; Willemsen et al. 2015).

10.1.1 Metabolomics Overview

A scientific activity called "metabolome analysis" focuses on finding and quantifying every metabolite that exists in a biological system. The metabolome encompasses a broad range of metabolites, varying in terms of concentration and physio-chemical characteristics. Due to the extensive nature of the metabolome, it is not feasible for a single technology to enable the simultaneous assessment of all metabolites at once. Researchers use a number of platforms and analytical methods, each with strengths and weaknesses, to address this problem. These methods include gas chromatography (GC), liquid chromatography (LC), nuclear magnetic resonance (NMR) spectroscopy, and others. The metabolome can be covered more thoroughly by the use of several complimentary approaches, which enables researchers to discover and quantify a wider variety of metabolites inside the biological system under study (2015) Töpfer et al. As a result, the word "metabolomics" refers to a group of technologies that study various metabolome components (Redestig et al. 2011). Metabolomics research produces multivariate data, which can make statistical analysis difficult, especially when there are more variables than experimental samples. In such cases, the high dimensionality of the data can make it difficult to extract meaningful insights. To address this issue, principal component analysis (PCA) is commonly employed. The dimensionality of multivariate data can be decreased by using the vector transformation technique known as PCA. By relocating the data "cloud" onto fresh axes in the multivariate space, it does this. These new axes, called principle components, are an orthogonal set of basis vectors and are weighted combinations of the original variables (in this case, metabolites).

The original multivariate data can be represented in a lower-dimensional space using PCA, preserving as much information as feasible. This transformation simplifies the data visualization and analysis, enabling the identification of patterns, clusters, and relationships among the metabolites and samples. PCA serves as a valuable exploratory tool in metabolomics research, aiding in the interpretation and understanding of complex datasets (Coquin et al. 2008). Metabolomics is a supplementary approach to genomics and proteomics for investigating the responses of complex biological systems to environmental, physical, and genetic influences (Griffin and Bollard 2004). Metabolomics studies frequently provide relative quantifications of metabolites, which are evaluated based on the fold-change in peak size between two samples. However, absolute metabolite quantifications must be obtained in order to compare metabolite concentrations precisely. Measurements expressed in moles per unit weight of tissue, such as mol per gram (g) of fresh weight (FW), are the result of calibration curves utilizing standards for each metabolite. There is an increasing emphasis on determining the absolute amounts of metabolites, even if relative changes in metabolite levels are frequently adequate for many uses (Dettmer et al. 2007). In-depth conversations and consultations have been held to provide a thorough understanding of the different metabolic techniques. The complex nature of metabolites, which are integral to a network structure, allows the metabolome to be regarded as a distinct cellular level. Furthermore, the metabolome serves as a crucial bridge connecting genotype and phenotype (Töpfer et al. 2015). The metabolome (Nielsen and Jewett 2007) refers to the full set of metabolites, non-genetically encoded substrates, intermediaries, and products of metabolic pathways that are coupled with a cell as a result of advances in complex network research (Kueger et al. 2012).

Significant advancements have been achieved recently in the creation and application of metabolomics technologies, which make it easier to identify and measure metabolites in their whole. Due to these developments, it is now possible to examine metabolites on a massive scale, which has allowed researchers to better understand metabolic processes and their effects (Töpfer et al. 2015). These tools add to the tried-and-true methodology used in studies on e-nomics, transcriptome, and proteomics, which stand out for a careful analysis of the pertinent cellular components (Romero et al. 2008; Töpfer et al. 2015). Metabolite fluxes were measured during hypoxia and recovery phases in order to look into the processes underlying the age-related drop in hypoxia tolerance. After incorporating these flux estimates into a model, network simulations were run to look at changes in important fluxes including ATP, H, and glucose. This method was used to create theories to explain the observed decline in hypoxia tolerance with aging. The consistency and concordance of these assumptions with the experimental findings was further confirmed by comparing them to transcription patterns seen in young and old flies (Griffin and Bollard 2004). The systematic examination of every metabolite present in a biological sample is the focus of the biological area of metabolomics. It primarily emphasizes the characterization and description of metabolites that are soluble in water. By examining the water-soluble metabolites, metabolomics aims to provide insights into the metabolic processes and pathways occurring within the biological

system under investigation. This branch of study plays a crucial role in understanding the biochemical changes and metabolic profiles associated with various biological phenomena, including disease states, drug responses, and environmental interactions (Xia et al. 2013). Metabolomics is widely regarded as a crucial tool in the study of systems biology because of its relationship to genomes and proteomics. The manipulation of gene and protein expression levels controls biological processes, and metabolomics, along with proteomics and transcriptomics, provides a thorough understanding of a system's behavior. Biopsies are taken from two or more experimental groups, and the samples' metabolites are then isolated and evaluated in metabolomics investigations. This makes it possible for researchers to examine and contrast the metabolic profiles of various groups, making it easier to identify important metabolites linked to particular circumstances or experimental variables (Narad et al. 2022). Numerous experimental methods, including NMR (nuclear magnetic resonance), MS (mass spectrometry), and LCMS (liquid chromatography-mass spectrometry), are used to evaluate metabolites. By applying these methods, metabolites are located, and the acquired information is used to build metabolic pathways. In terms of precision, sensitivity, quantification, and dependability, tailored metabolomics is superior to untargeted metabolomics and has a reduced percentage of false positives. Enzymes, which play a crucial role in metabolomics, can be affected by factors like chemical stability and temperature, leading to fluctuations in metabolomics samples. In order to assure reliable results, the sample preparation procedure needs to be adjusted. Metabolomics is a relatively new field that seeks to recognize and measure low-molecular-weight exogenous and endogenous compounds in biological systems. Its close relationship with physiology and genotype enables the exploration of how genotype and environment interact. The field of metabolomics focuses on understanding an organism's full metabolome, which is the collection of tiny compounds that interact inside biological systems and have an impact on diet, genetics, and the environment. It has significant applications in areas such as molecular and personalized medicine, toxicology, and other related disciplines (Narad et al. 2022). The metabolome, which includes variations in gene and protein expression, serves as the organism's final downstream result. It serves as the molecular phenotype reflecting both health and disease states. The "Human Metabolome Database (HMDB)" is a valuable resource containing extensive data on various substances, includes lipid- and water-soluble metabolites, organic acids, nucleotides, lipids, steroids, carbohydrates, and amino acids. Metabolomics can be divided generally into two categories: "targeted" and "untargeted." Targeted metabolomics involves the systematic quantification and identification of specific metabolites based on a predetermined hypothesis or set of target compounds. On the other hand, untargeted metabolomics takes a hypothesis-driven approach, aiming to comprehensively identify and analyze metabolites without predefining specific targets. In metabolomics, consideration is given to both the exo-metabolome (metabolites released outside the cell) and the endo-metabolome (metabolites within the cell). This holistic approach allows for a comprehensive understanding of the metabolic processes and interactions within biological systems. Overall, the field of metabolomics can be divided into targeted and untargeted approaches, with the aim

of quantifying and identifying metabolites, including considerations of both exo-metabolome and endo-metabolome, to unravel the molecular complexities of biological systems (Narad et al. 2022). The systematic quantification also includes the exo- and endo-metabolome and metabolite identification (Putri et al. 2013), Untargeted metabolomics are discovered through a hypothesis-driven approach that permits complete metabolome scanning, also known as metabolic fingerprinting, and pattern recognition. According to Toya and Shimizu (2013) and Narad et al. (2022), the main goal of targeted metabolomics, which is based on hypothesis testing, is to confirm the results of the untargeted study.

10.1.2 Applications of Metabolomics

Metabolomics has demonstrated its widespread applicability in the fields of health, synthetic biology, and food sciences, demonstrating its significance in a variety of fields. The following is a discussion of the numerous metabolomics applications: (Putri et al. 2013; Narad et al. 2022).

10.1.2.1 Microbial Science

Microbes are a prominent resource in metabolomics due to their amenability to experimental modifications. However, high-resolution analysis, regulated ambient conditions for metabolite identification, and improved sample preparation methods are required for microbial metabolomics. When preparing samples for microbial metabolomics, extraction and quenching are the two crucial procedures. In order to stop biological reactions in cells, a procedure known as quenching must first be used to collect metabolites from the cells. To ensure accurate and reproducible results, sample quenching is performed at a specific time point during the metabolomics workflow. This quenching step allows for the determination of the actual quantity of metabolites present at that particular moment, enhancing the reliability of the results obtained. Two key aspects—short-term biological reaction halting and minimal metabolite leakage—validate quenching. The microbial cells are subjected to the extraction process depending on the chemical characteristics of the target analyte, the reactivity of the enzymes, the cell characteristics, and the durability of the cell membrane. Depending on how effectively microbial cells can tolerate the demanding environment, high temperature, methanol, chloroform, or free thawing are utilized. Stable isotopes are typically used in microbial metabolomics. For instance, ^{14}C glucose was utilized to investigate the connection between overall control and cellular metabolism in *Saccharomyces cerevisiae*. MFA has also been used to analyze the central metabolism using ^{13}C-labeled intermediates. The application of microbial metabolomics holds significant potential for advancing the study of higher organisms. The knowledge gained from microbial metabolomics can be extrapolated and applied to better understand and analyze the metabolomes of higher organisms. By leveraging the insights and techniques derived from microbial metabolomics, researchers can enhance their understanding of complex metabolic processes in

higher organisms, contributing to advancements in fields such as medicine, ecology, and agriculture (Putri et al. 2013; Narad et al. 2022).

10.1.2.2 Plant Science

In order to understand the complicated biological processes and decipher the roles of numerous important genes, metabolomics is crucial for plant science (Toya and Shimizu 2013). Plant metabolic status is significantly influenced by transcriptional control under a variety of developmental and environmental circumstances. It was reported that the, the mechanism behind the regulatory roles in metabolic phenotype and gene expression remains enigmatic. Metabolomics enables botanists to conduct detailed investigations into the dynamic behavior of plant metabolic systems. By analyzing plant metabolomics, researchers can assess both metabolites and gene expression, providing valuable insights into plant physiology. The AtMetExpress dataset has been utilized to study the metabolic profiles and gene expression patterns of the *Arabidopsis thaliana* plant, contributing to a deeper understanding of its metabolic pathways and regulatory mechanisms (Putri et al. 2013). Based on a report, the genome of *Arabidopsis thaliana* contains a substantial number of vital metabolic genes involved in the production of commercially significant plant compounds. The data revealed the presence of 1589 metabolic signals related to various phytochemicals and a total of 167 distinct metabolites within the plant. This information highlights the rich metabolic potential of *Arabidopsis thaliana* and its significance in the production of essential compounds (Putri et al. 2013), the diversity of plants' secondary metabolism and the source of dynamics are determined by the transcription of metabolites and the regulation of those transcripts. It is also admirable how metabolomics is being used in breeding and agriculture sciences. It is important to identify the genetic components that play a crucial role in controlling metabolic levels in order to increase the nutritional value of the crops. Metabolomics is frequently used to explore these linkages since the relationship between biomass/yield and metabolite composition controls plant metabolism (Putri et al. 2013). Metabolomics has emerged as a valuable tool in breeding and crop sciences, particularly for enhancing the nutritional value of crops. By identifying genetic factors that influence metabolic levels, researchers can make targeted improvements. Understanding the intricate relationship between biomass, yield, and metabolite composition is crucial for manipulating plant metabolism. Metabolomics provides a comprehensive approach to studying these relationships, enabling researchers to explore and optimize crop traits related to metabolite composition and overall crop quality (Narad et al. 2022). Metabolomics is important for both defining the necessary level of risk management and for the effective production of genetically modified crops. Researchers can now produce enormous amounts of phytochemicals thanks to improvements in metabolomics technology, which opens up a wide range of potential uses. These developments contribute to the exploration and utilization of plant metabolites for agricultural purposes, including the development of genetically modified crops and effective risk assessment strategies (Narad et al. 2022). For plant metabolomics, three main approaches are required: a method to calculate false discovery rates, a vast mass spectral library of

phytochemicals, and MS spectrum data for elucidating metabolite structure (Toya and Shimizu 2013; Narad et al. 2022).

10.1.2.3 Animal Science

With the use of metabolomics technology, it is now possible to investigate the biological processes of important model organisms like fruit flies and zebrafish. These species' metabolism can be thoroughly study to learn a great deal about their pathogenic, physiological, and developmental processes.

A popular model organism for investigating different biological, behavioral, and biomedical processes connected to organogenesis and embryogenesis in vertebrate development is the zebrafish (*Danio rerio*). Its genetic resemblance to humans, quick development, clear embryos, and simplicity of genetic modification all contribute to its popularity. Researchers extensively study zebrafish to gain insights into fundamental developmental processes, investigate disease mechanisms, and screen potential therapeutic compounds. It is a useful tool for enhancing our understanding of vertebrate development and human health due to its usefulness as a model organism. Due to its ease of breeding in high numbers and relatively low maintenance cost compared to other model organisms, it has been extensively utilized for research into the development of drugs and diseases (Riekeberg and Powers 2017). The association between embryogenesis and metabolome can be ascertained with the help of the metabolomics approach, which acts as a fingerprint for analyzing the embryonic process and helps with medication therapies. *Caenorhabditis elegans*, a different model organism, is frequently used to research aging, genetic disorders, physiology, lifespan, and medication toxicity screening (Narad et al. 2022). Due to their short lifespan, similarity to human aging, and ease of breeding, fruit flies, also known as *Drosophila melanogaster*, are extensively employed to study the physiology and genetics of aging.

A useful model organism for metabolomics research on topics like embryonic biology, the effects of phenobarbital, pesticide resistance, oxidative stress, and more is the zebrafish (*Danio rerio*). Its resistance to mutations, hypoxia, and cold shock further enhances its suitability for these research areas (Putri et al. 2013; Narad et al. 2022).

10.1.2.4 Medical Science

Numerous medical fields have utilized metabolomics. It frequently used to examine the biomarkers found in physiological fluids and to ascertain how drugs work. The use of metabolomics, which tracks metabolite changes in biofluids, is common in pharmacological therapy and medical therapy (Toya and Shimizu 2013). Metabolites serve as biomarkers for illnesses, therefore a change in their content in bodily fluids denotes the presence of a disease. As a result, a variety of metabolites provide data on treatment response with a high degree of selectivity and sensitivity (Narad et al. 2022). Additionally, a lot of people utilize metabolomics to forecast how a drug will react to a certain condition and to gauge how the disease may develop in the future. Furthermore, the zebrafish is utilized in predicting personalized treatment options for patients. Precision medicine, single cell metabolic

phenotyping, personalized medicine, metabolome-wide association studies (MWAS), and epidemiological population research are a few areas where metabolomics has applications. These diverse applications highlight the value of metabolomics in monitoring and understanding health and disease. The identification and analysis of metabolites, the characterization of tiny molecules, and the high-dimensional profiling of individual cells all depend on metabolomics. It is extensively utilized for the discovery of clinical biomarkers through various approaches such as metabolomics fingerprinting, profiling, foot printing, and metabolome-wide association studies (MWAS). These methodologies enable the identification and utilization of metabolomics signatures for diagnostic, prognostic, and therapeutic purposes in various clinical applications (Putri et al. 2013). It is also used to research a variety of metabolic syndromes, including serious conditions brought on by sugar and lipid metabolism, like cancer, heart disease, and cerebrovascular disease. Through the pathophysiological study of metabolites and biomarkers, it assists in the early detection of fatal diseases (Narad et al. 2022). The pathophysiological investigation of metabolites and biomarkers using metabolomics aims to detect life-threatening disorders early. Using LCMS-based metabolomics techniques, biomarkers such as trimethylamine oxide have been identified as indicators of cardiovascular diseases. This highlights the potential of metabolomics in uncovering valuable biomarkers for timely diagnosis and intervention in critical medical conditions (Toya and Shimizu 2013). It could function as a biomarker for illnesses like myocardial infarction, coronary artery disease, and peripheral artery disease. It is frequently employed in cancer research, early cancer diagnosis, and accurate prognosis. In order to measure metabolic flux in lung cancer cells, the metabolomics method based on ^{13}C stable isotopes has been used. It revealed an excess of alanine, lactate, and glutamine, three substances crucial to the growth of cancer (Putri et al. 2013). In order to comprehend the biochemical alterations in cancer cells, isotopomer-based metabolomics is employed. It is a promising, non-invasive, and extremely sensitive cancer diagnostic technique. Additionally, it is used to understand neurological disorders and psychological issues (Putri et al. 2013). As a potential biomarker for brain metabolomics, cerebral spinal fluid has been studied using 1H NMR-based metabolomics. This method is used to study neurodegenerative conditions including Alzheimer's and Parkinson's. Furthermore, neural metabolomics offers insights into psychiatric conditions like depression and schizophrenia, where alterations in neurotransmitter systems and phospholipids in neuronal membranes are implicated in the pathogenesis of schizophrenia (Narad et al. 2022). Since alterations in lipid metabolism are a contributing factor in schizophrenia, lipidomic analysis is regularly carried out to identify the likely biomarkers underlying pathophysiology. Drug toxicity testing, early diagnosis, therapy, and research of biochemical alterations in mood disorders all make use of metabolomics. Our comprehension of the relationship between pathological diseases and molecular abnormalities in the body is improved by combining metabolomics with other omics approaches. This comprehensive approach offers valuable insights into disease mechanisms and potential therapeutic approaches (Putri et al. 2013; Narad et al. 2022).

10.1.2.5 Food and Herbal Medicines

A promising method to evaluate the safety and quality of food and herbal treatments is metabolomics. Food product quality can be influenced by milling, atmospheric storage, and genetic modifications. The quality control of finished food products and the safety assurance of herbal remedies can be successfully implemented by sensory evaluation and metabolomics (Putri et al. 2013). The five senses—touch, hearing, sight, taste, and smell—are employed in sensory evaluation, a scientific method, to examine, evoke, interpret, and quantify product quality. The preservation of quality standards and cost management are crucial in the food sector. To evaluate the quality of different food products such fruits, cereals, crops, and drinks, MS-based metabolomics approaches are used. The field of food metabolomics encompasses the organization of flavor-active chemicals and the simulation of human senses, contributing to advancements in food quality assessment (Narad et al. 2022). Sensomics, a subfield of food metabolomics, mimics human hearing, taste, sight, smell, and touch to assess the quality of food. In food metabolomics, methods like NMR and GCMS are used. These are also utilized for industrial, pharmaceutical, and research related to herbal medicines. They are employed in the analysis of pharmacological and toxic effects. Consequently, metabolomics is developing into a strong, trustworthy, useful, and promising instrument for quality assurance and sensory chemistry. It provides valuable insights into the chemical composition and sensory characteristics of various products, enabling effective quality assessment and control measures. Metabolomics contributes to enhancing the overall understanding and evaluation of product quality, reinforcing its importance in the field of sensory chemistry (Putri et al. 2013; Narad et al. 2022).

10.2 Flux Balance Analysis

Metabolic networks are modelled using a variety of mathematical techniques that are based on the determination of a single solution specifying all the fluxes via a metabolic network. FBA, or constraint-based analysis, provides a fundamental understanding of how a metabolic network is made up and functions. Mathematical formulas are used to reflect these constraints (Kauffman et al. 2003). The annotated genomic sequences provide information on the enzymes involved in these processes. In order to find the enzymes involved in metabolism, Because of the advancement of homology searches, it is now possible to compare known genes to those that are unknown. Table 10.1 lists a few useful databases for genetic and metabolic information (Narad et al. 2022).

Table 10.1 Database and tools used in metabolic network analysis (Narad et al. 2022)

Sr.	Section	Databases/tools	Description	References
1	Tools	MetaFluxNet	Metabolic flux analysis	Lee et al. (2003)
2		Yana	Network reconstruction, analysis, and visualisation	Schwarz et al. (2005)
3		System Biology Research Tool	Analysis of stoichiometric networks through multiple methods	Wright and Wagner (2008)
4		Constraint Based Reconstruction and Analysis Toolbox	Works with MATLABs for metabolic network analysis, gene deletions, etc., using FBA	Becker et al. (2007)
5		PathwayAnalyzer	Uses MoMA, FBA for gene deletion studies and metabolic networks	Raman and Chandra (2009)
6		BML Software Guide	Model databases	Hucka et al. (2015)
7		CellNetAnalyzer	Databases of functional and structural analysis	Klamt et al. (2007)
8		SNA—Stoichiometric Network Analysis	Mathematic toolbox for metabolic networks	Urbanczik (2006)
1	Database	BRENDA	Information of molecular and biochemical pathways on enzymes	Schomburg et al. (2002)
2		BioCyc	Databases of pathways for several organisms	Karp et al. (2019)
3		Reactome	Curated databases of biological processes in humans	Fabregat et al. (2017)
4		PEDANT	Genome annotations	Riley et al. (2007)
5		Biomodels	Databases of kinetic models of pathways	Li et al. (2010)

10.3 Metabolomics Techniques

In this book chapter we give you, an overview of metabolomics, which is mainly segregated into two main, approaches targeted and untargeted. Below we discuss some frequently used techniques such as, LCMS, GCMS and NMR and their consequent data analysis procedure.

10.3.1 Targeted and Untargeted Metabolomics Techniques

The Targeted approach is mainly focused on identification and quantification of specific class of metabolites or metabolites. These might be substances from a certain class, direct products of a protein, enzyme substrates, or participants in a certain pathway. Normally, the targeted approach is hypothesis-driven, aiming to test

specific hypotheses. Another metabolome technique, the untargeted analysis involves measuring metabolites within biological system.

10.3.1.1 LC–MS

LC–MS, a "Liquid Chromatography–Mass Spectrometry" combined the principles of both LC and MS. LC is mainly used to separate the available molecule in liquid mobile phase by using solid stationary phase (Pitt 2009). The high resolving power of LC based analysis is used to determine the structure and quantified degradation of compounds and impurities in different materials. By combining, LC–MS makes it possible to identify and characterize individual components within a complex mixture based on their mass-to-charge ratios (Karpievitch et al. 2010). This yields important information on the make-up and standard of the analyzed materials. An ion source, a mass analyzer, and a detector are the three crucial parts of a mass spectrometer. Sample molecules are turned into ions by the ion generator, and then utilizing an electromagnetic field, the mass analyzer separates the ions according to their mass-to-charge ratios. The detector then captures and measures the separated ions, providing valuable information about their abundance and mass properties (Fuerstenau and Benner 1995). Once these ions have been separated by the mass analyzer, the detector finally measures them. Electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and fast atom bombardment (FAB) are just a few of the flexible ion sources that can be used with mass spectrometry. These different ion sources provide diverse ionization mechanisms and are selected based on the specific requirements of the analysis or sample type in mass spectrometry (Agarwal and Goyal 2017). ESI stands out among these other ion sources because of its gentle ionization capabilities, which makes it easier to produce plenty of ions by charge exchange in solution. The first identification of analytes is aided by this property. For the measurement of polar and semi-polar metabolites, LC–MS is a flexible analytical method that is often used in metabolomics investigations (Xiao et al. 2012). It works well for profiling small molecules, including amino acids, organic acids, nucleotides, carbohydrates, lipids, and other water-soluble compounds (Sato et al. 2004). LC–MS enables the comprehensive characterization and quantification of these compounds, providing valuable insights into the metabolic profile and composition of biological samples (Fig. 10.1). Metabolites are essential components involved in crucial cellular processes, signaling pathways, energy metabolism, and various disease-related pathways. LC-ESI-MS has emerged as the preferred technique for analyzing and profiling metabolites in complex biological samples. By incorporating chromatographic separation, the complexity of the sample can be reduced, and any potential matrix effects during ionization can be minimized. This approach allows for enhanced sensitivity, specificity, and accuracy in metabolite analysis, making LC-ESI-MS a powerful tool in metabolomics research (Böttcher et al. 2007). Reverse phase liquid chromatography (RPLC) is often employed, with the use of C18 columns, to effectively separate semi-polar compounds such as phenolic acids, flavonoids, glycosylated steroids, alkaloids, and other glycosylated species in LC-ESI-MS (Lu et al. 2008). RPLC is a popular technique for effective semi-polar chemical separation in LC-ESI-MS,
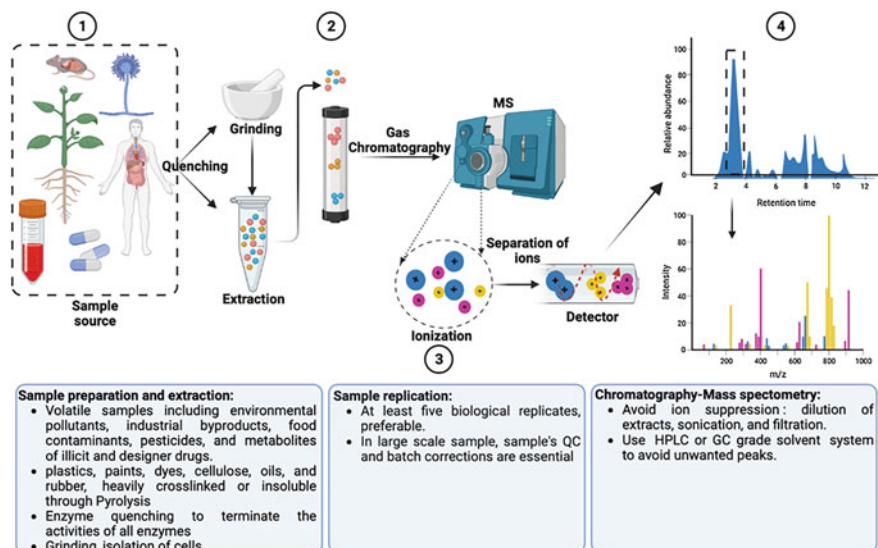
Fig. 10.1 General flowchart of LC–MS for metabolomics

frequently utilizing C18 columns. Different substances, such as phenolic acids, flavonoids, glycosylated steroids, and other glycosylated species, can be separated using this method. These semi-polar metabolites can be thoroughly analyzed and identified in complex biological samples using RPLC and LC-ESI-MS (Zhou et al. 2012). In LC–MS data, variations can be observed not only in the spectra obtained from different instruments but also in the MS/MS spectra generated under different experimental conditions. These discrepancies arise from the use of diverse combinations of ionization sources, collision energies, mass analyzers, and detectors. These variations in instrument types and experimental settings can impact the characteristics and interpretation of the MS/MS spectra, highlighting the need for careful consideration and standardization in data analysis and comparison. As a result, several MS/MS spectra can be seen for the same metabolite, highlighting how experimental variables can affect the final spectrum features.

The raw LC–MS data must undergo a number of preprocessing steps in order to create a peak list that facilitates analysis and comparison of different runs. Outlier identification, peak matching, baseline correction, filtering, outlier screening, and retention time alignment, ion annotation, normalization, transformation, and use of software tools are some of these stages that are listed below (Castillo et al. 2011). Every one of these preprocessing procedures is essential for cleaning up the data and getting it ready for insightful analysis and future comparisons. In order to account for matrix effects and time-dependent changes brought on by instrument sensitivity variations, detected peaks are normalized using an internal reference peak. Gradient elution in LC–MS allows high-resolution spectrum data of metabolites, permitting specialized metabolite study (Griffiths and Wang 2009). Various equipment

configurations cause variations in LC–MS and MS/MS spectra, resulting in various spectra for the same metabolite. Currently, manual verification is utilized after mass-based search to identify metabolites in untargeted metabolic research. Because there exist chemicals with extremely similar molecular weights, it has been shown that even with an accuracy of less than 1 ppm, which is substantially more precise than most analytical systems can attain, it is still insufficient for unambiguous metabolite identification (Calderón-Santiago et al. 2017). Secondly, isomers with the same elemental content but distinct structures cannot be distinguished by mass-based metabolite identification. Third, there is a dearth of information in all metabolite databases (Sleno 2012). A significant fraction of the detected ions in a typical LC–MS-based metabolomics experiment is still unidentified or has numerous plausible identifications. Through mass-based searches, less than 30% of these ions may be accurately identified. But the use of QqQ-based LC–MS, LC-SRM-MS, and LC-HRMS full scan analysis has shown how crucial metabolite quantification is for comprehending the response to illnesses, treatments, and environmental factors. These techniques enable accurate and precise measurement of metabolite levels, providing valuable insights into metabolic changes and their implications (Dowling 2017). Additionally, some analytes, including those that show the neutral loss of H_2O or CO_2, may exhibit non-specific transitions that are frequently seen in matrix interferences. This lack of specificity can impact the accuracy of quantification in the selected reaction monitoring (SRM) method, leading to incorrect results. It is important to consider and address these challenges in order to ensure reliable and precise quantification of analytes in metabolomics studies (Pozo et al. 2006). A global MS detection employing HRMS, such as FTICR, Orbitrap, TOF, or QTOF, can get beyond these limitations in SRM analysis (Amer et al. 2023). In full-scan mode, high-resolution mass spectrometry (HRMS) enables the identification of almost all compounds present in a sample. With the advancements in HRMS technology, such as fast scan rates, it is possible to capture an ample number of data points across chromatographic peaks. By producing extracted ion chromatograms (EICs) within a small mass window (e.g., 5–10 mmu) centered on the theoretical *m/z* value of each analyte, this permits accurate quantification. This approach enhances the accuracy and sensitivity of quantification in metabolomics studies (Alygizakis et al. 2023). In summary, LC–MS has revolutionized the field of metabolomics, enabling researchers to comprehensively study the dynamic and intricate world of small molecules. With its ability to provide detailed insights into metabolic pathways, biomarker discovery, and understanding of disease mechanisms, LC–MS continues to drive groundbreaking discoveries and advancements in various scientific disciplines. It's potential to transform healthcare, agriculture, environmental studies, and personalized medicine is immense, making it an indispensable tool in the quest to unravel the complexities of the metabolome.

10.3.1.2 GC–MS

By separating molecules based on their volatility, gas chromatography. Its initial use was explained in 1952 (Bartle and Myers 2002). The analytes are initially adsorbed to a GC column's surface at a slightly raised temperature in order to accomplish

separation. The GC column can be quickly heated and cooled since it is housed inside an oven. The temperature is increased once the analytes are bonded, which causes them to leave the column surface in decreasing order of volatility (McNair et al. 2019). Once the analytes have been thermally desorbed, they are transported down the column surface toward the detector using a carrier gas (mobile phase, often helium). With its unmatched capabilities for the investigation of tiny molecules, GC–MS has completely transformed the area of metabolomics (Cui et al. 2018). A crucial technique for comprehending the metabolome is gas chromatography with mass spectrometry (GC–MS), which combines the separation power of gas chromatography with the sensitive and focused detection of mass spectrometry (Smart et al. 2010). Chemical derivatization is required to improve the volatility of metabolites containing polar functional groups, such as carboxylic and amino groups, in contrast to LC–MS and NMR-based metabolomics. This modification is essential to enhance their vaporization properties for improved detection and analysis in techniques such as GC–MS (Zeki et al. 2020). Pre- or post-derivatization GC–MS analysis and data collection are performed on the volatile compounds. To understand the complex mass signals, a data processing technique should be used to recognize the real signals, classify the signals into different compounds, and align these compounds from different samples (Ràfols et al. 2018). The metabolic route linked to particular physiological or pathological abnormalities can be discovered after peak annotation. As a result, the complete procedure entails the following steps: collecting the samples, extracting the metabolites, derivation the compounds, analyzing the instruments, analyzing the data, and annotating the metabolites and the pathways. The following graphic illustrates the basic steps of GC–MS-based metabolomics, which can be used to analyze the metabolites in biofluids, tissues, or cell samples (Fig. 10.2).

One of the main factors contributing to the adoption of GC–MS in metabolomics investigations is accurate and repeatable chemical identification. In GC-based metabolomics applications, MS detection with electron ionization (EI) is commonly employed in combination with GC. The use of EI mode allows for non-discriminatory identification of all compounds suitable for GC analysis, as the scan response is generally proportional to the injected compound quantity. Various GC columns are utilized to separate fatty acids, amino acids, sugars, and monosaccharides, with the 5% phenyl, 95% methyl siloxane column being frequently used due to its broad selectivity for untargeted metabolomics applications (Zaikin and Halket 2009). Peaks and retention time were given to the same variable in each sample after being separated from the raw data. Three approaches in particular—target analysis, peak selecting, and deconvolution—have proven to be beneficial for this purpose (Koek et al. 2011). In gas chromatography-mass spectrometry (GC–MS), many detector types can be employed to examine and locate chemicals that have been separated by the gas chromatograph. Four typical GC–MS detectors are listed below:

1. Flame Ionization Detection (FID): In GC–MS, the FID detector is a popular and very sensitive one. It works by creating ions from the chemicals that elute from
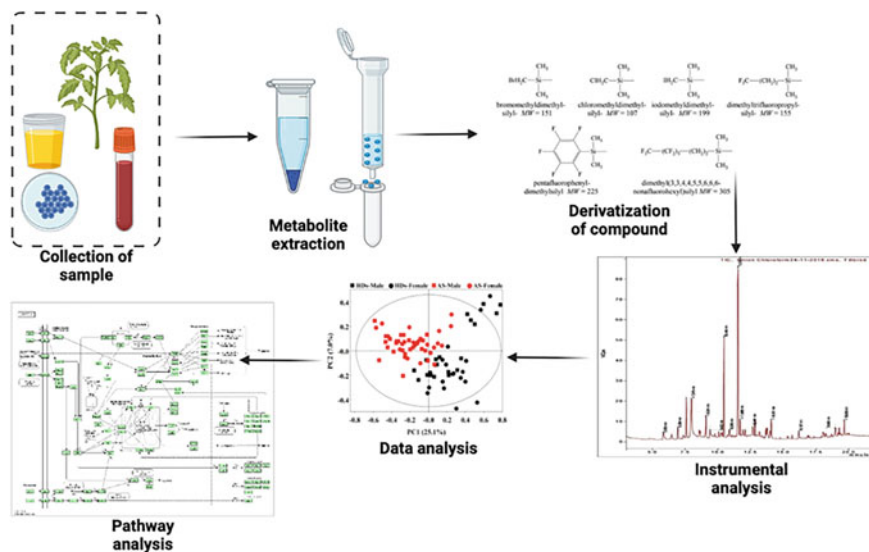
Fig. 10.2 General working flow of GC–MS for metabolomics

the gas chromatograph by burning them in a flame of hydrogen and air (Poole 2015). After being collected and tested, the produced ions reveal how many of the chemicals are present in the sample. The investigation of a variety of chemicals, including hydrocarbons, is made possible by the FID's outstanding sensitivity, large linear dynamic range, and resilience (Jalili et al. 2020).

2. "Single Quadrupole Mass Spectrometry (SQ-MS)": SQ-MS mass spectrometers are frequently utilized in GC–MS. It is made up of a single quadrupole mass filtering ions with a preference for those with a higher mass-to-charge ratio (m/z) (Morain 2013). SQ-MS can analyze various m/z values to identify and quantify specific chemicals in a sample (Modisha et al. 2018). It helps with structural elucidation and compound identification and quantification by giving details about the mass fragments created during ionization.

3. "Time-of-Flight Mass Spectrometry (TOF-MS)": An additional mass spectrometer type employed in GC–MS is TOF-MS. It determines the ion's flight duration from the ion source to the detector based on their m/z values (Guilhaus 1995). The mass resolution, sensitivity, and accuracy of TOF-MS are all quite excellent. It is frequently employed for untargeted analysis and is capable of providing thorough details on the full mass range contained in a sample, enabling the identification of unidentified chemicals and the discovery of trace-level analytes (Hird et al. 2014).

4. "Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS)": It traps ions with a magnetic field and detects the oscillation frequencies, which enables extremely precise mass determinations (Heck et al. 2011). For complicated mixture analysis and isobaric chemical identification, FT-ICR-MS is a great choice due to its high mass resolution, mass accuracy, and

sensitivity. But FT-ICR-MS equipment is expensive and complicated, and it's frequently employed in high-end research settings (Seger et al. 2013).

A target analysis list is created, detailing each metabolite expected to be found in the data file's *m/z* value and defined retention time window. The instrument vendor's software uses the target list to calculate each metabolite's peak area, enabling quantification (Fiehn 2016). In addition, the purity and quality of isolated peaks should be controlled by utilizing internal standards that have been isotopically labeled for extraction and derivatization. In conclusion, GC–MS has become a potent and crucial technique in metabolomics, providing accurate and sensitive investigation of polar and semi-polar metabolites. With its broad selection of detectors, including FID, SQ-MS, TOF-MS, and FT-ICR-MS. GC–MS also facilitates thorough metabolite profiling, identification, and quantification, with greater insights into biological processes, disease causes, and the identification of new biomarkers. An important technique advancing metabolomics research and its applications in areas including personalized medicine, environmental studies, and agriculture, GC–MS is able to handle a variety of sample types and provide high-resolution data.

10.3.1.3 NMR

NMR spectroscopy, a non-destructive analytical method, is a cornerstone of metabolomics because it offers priceless insights into the structure, dynamics, and interactions of metabolites (Cheng et al. 2013). The field of metabolomics can benefit from NMR's major properties, which are listed below (Trimigno et al. 2015).

1. NMR has excellent quantitative and reproducibility.
2. The target list is used by the instrument vendor's software to determine each metabolite's peak area, allowing for quantification.
3. As analytical technological advances have resulted in the detection of an increasing number of signals in complex biological mixtures, many of which remain unidentified, NMR allows for the identification of unknown metabolites, which is crucial.
4. There is no need for sample preparation or separation because to NMR's ability to examine entire biofluids and tissue, which is critical because these operations greatly increase analytical variability.
5. NMR preserves the sample integrity after analysis, allowing for potential reanalysis using NMR or other techniques such as MS in the future.
6. NMR allows for the tracing of metabolic pathways and the measuring of metabolic fluxes by using precursors that have been stable isotope-labeled.
7. Using one or more atomic nuclei, such as ^{1}H, ^{13}C, ^{31}P, or ^{15}N, NMR can detect metabolites.
8. NMR analysis is advantageous for sensitive metabolites like glutamine and coenzymes, as it does not require harsh sampling or ionization voltage treatment.
9. The NMR workflow for metabolomics involves key steps such as signal detection, metabolite identification using 1D and 2D NMR methods, database
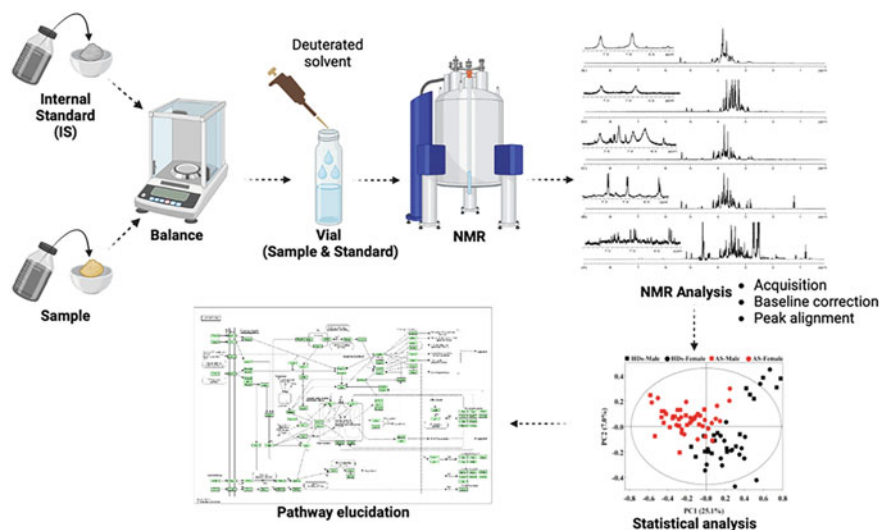
Fig. 10.3 General workflow of NMR for metabolomics

searching and verification, and quantification of identified metabolites. These procedures are essential for the NMR-based metabolomics examination of biological material (Fig. 10.3).

NMR offers some advantages over metabolomics that are unmatched. NMR offers a window into seeing and precisely measuring all of the more prominent compounds found in biological fluids, cell extracts, and tissues without the requirement for time-consuming sample preparation or separation. For compounds that are challenging to ionize or require derivatization for MS analysis, NMR has advantages. It enables the identification of substances, even those with different isotopomer distributions, that have identical masses, providing valuable insights in metabolomics research (Markley et al. 2017). NMR serves as the primary method for elucidating the structures of unknown substances. It enables the investigation of metabolic pathway compartmentalization and provides insights into the kinetics and mechanisms of metabolite conversions through the utilization of stable isotope labels (Fan and Lane 2016). A variety of cutting-edge techniques are being used with NMR-based metabolomics to produce fresh and in-depth data. The next section goes through a few of these techniques.

10.3.1.3.1 Isotope Enhanced NMR to Track Metabolism

This approach utilizes NMR spectroscopy, which possesses the unique ability to identify atom-specific positional isotopomer distributions that arise from the utilization of stable isotope-enriched precursors (Lane et al. 2008). Numerous stable isotope-rich materials, such ^{13}C, ^{15}N, and ^{2}H, have been thoroughly studied (Yu et al. 2023). In order to quantitatively assess the downstream metabolic products

of several pathways, including as glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway (PPP), ^{13}C-labeled glucose is frequently used to follow metabolism (Antoniewicz 2018). This tracking doesn't provide an active metabolic pathway but they also give rates of metabolism step, where labeled substrate gets consumed and their product formed. Therefore, the isotope-enhanced NMR becomes useful in cancer metabolism or cellular metabolism investigation (Tavares et al. 2015).

10.3.1.3.2 Micro-Coil NMR

In this NMR approach, liquid chromatography is used to separate metabolites from complex mixtures, and then direct online detection is used, frequently after pre-concentration online. Micro-coil NMR is a common technique utilized for this purpose (Nagana Gowda and Raftery 2019). This approach is not characterized by high throughput but is better suited for analyzing metabolites in samples with limited mass. Due to the increasing interest and demand for NMR miniaturization, major instrument suppliers now offer micro-coil probes with multinuclear capabilities (Badilita et al. 2012).

When utilizing micro coil NMR, caution must be exercised when concentrating samples, as metabolites with low solubility and sample matrices containing high levels of salt or proteins can negatively impact the relative and absolute quantities of metabolites. It is important to consider these factors to ensure accurate and reliable results during micro coil NMR analysis of samples (Anderson et al. 2012). A recent assessment of the impact of sample concentration on commonly used serum and urine samples revealed that the sensitivity improvement achieved varied for different metabolites and sample matrices. The observed sensitivity enhancement did not follow a linear trend as expected, highlighting the complex relationship between concentration and sensitivity in metabolite analysis.

10.3.1.3.3 Fast NMR Method

High-throughput analysis is yet another crucial requirement in metabolomics, the fastest in terms of data gathering. Numerous developments in 2D NMR have made it possible to obtain data quickly (Croasmun and Carlson 1996). HMQC and HSQC are acronyms for heteronuclear single and multiple quantum coherence and are widely used in 2D NMR experiments in metabolomics. These strategies can greatly speed up data collection by using forward maximum entropy reconstruction and non-uniform sampling, cutting down on the time needed for thorough metabolite profiling (Rouger et al. 2017).

In order to increase the steady-state magnetization, a shorter T1 relaxation time and a better flip angle are combined in the SOFAST NMR (selective optimal flip angle short transient) method. This makes it possible to acquire data quickly, especially for the SOFAST-HMQC 2D experiment, which may be finished in about 10–15 s. Real-time monitoring of metabolism in living cells is made possible by such quick data collecting, giving important insights into cellular processes (Sibille et al. 2012). Single-scan acquisition methods have shown great potential in metabolomics, particularly in 2D experiments. These methods allow for the

acquisition of data from a single scan, offering advantages such as reduced acquisition time and improved sensitivity. With their ability to provide valuable information in a shorter time frame, single-scan 2D experiments have proven to be valuable tools in metabolomics research.

10.3.1.3.4 Hyperpolarization Method

There is a significant interest in utilizing hyperpolarization of nuclear spins to enhance sensitivity for real-time in vivo metabolism research. Hyperpolarization techniques enable the generation of highly polarized nuclear spins, resulting in enhanced signal intensity in NMR experiments. This enhanced sensitivity allows for the detection of metabolites in real time, opening up new possibilities for studying dynamic metabolic processes in living organisms (Wang et al. 2019). By introducing hyperpolarized substrates into biological systems, downstream metabolites can be identified with high sensitivity while preserving the polarized nuclear spin state. Unlike the PHIP method, the DNP approach allows for the hyperpolarization of various metabolites without encountering major challenges (Gowda and Raftery 2015). Real-time metabolic investigations benefit most from the dissolution DNP method, which involves quickly melting and injecting a hyperpolarized solid containing the target substrate, a glassing agent, and a polarizing agent into cells, tissue, or organs (Hurd et al. 2012). Overall, although these technologies are still in their infancy, they hold great promise for the sector. In conclusion, NMR spectroscopy is essential to metabolomics research because it provides important information on the intricate metabolic patterns of biological materials (Zhang et al. 2013). It is an essential tool for researching metabolic changes in health and illness since it may offer metabolite identification, quantitative analysis, and information on metabolic pathways. NMR data may be used with other omics methods to help researchers fully comprehend metabolic networks and find new biomarkers for diagnostic and therapeutic uses.

10.3.2 Important Tools of Flux Balance Analysis

10.3.2.1 OptKnock

Genes make up a sizable portion of a standard metabolic model (B1000). Therefore, as the set size increases, it takes more processing resources to do an exhaustive search of knockout sets. OptKnock is based on the duality theory, which claims that there is a single dual LP problem for each primal LP problem that equals the primal's objective function. By setting the objective functions equal to one another, the dual problem is used to increase constraint while maximizing biomass output (Burgard et al. 2003). A single mixed integer linear programming (MILP) problem is used to combine the growth-maximizing problem and the maximum product yield problem. The Optx and OptKnock algorithms can be used independently as a programmed to predict knockouts (Narad et al. 2022).

10.3.2.2 OptGene

OptKnock's disadvantage is that nonlinear objective functions are not optimized. With the MILP problem, it becomes a computationally demanding process when there are many knockouts (Burgard et al. 2003). OptGene gets around these limitations. With the following genetic algorithm:

1. It generates a collection of arbitrary optimal conditions.
2. The metabolic model for each set is solved using MOMA and FBA.
3. Each member of the set is given a score based in part on their metabolic status.
4. The condition with the highest score is chosen as the ideal one.
5. Up until the best score is obtained, step 2 is repeated (Burgard et al. 2003; Narad et al. 2022).

10.3.2.3 OptStrain

Although the OptGene and OptKnock algorithms are thought to be quite effective at predicting knockouts, their range of use is restricted to changes in metabolic processes. By creating a library of biotransformation to enhance the prediction of heterologous routes, OptStrain solves this issue (Burgard et al. 2003). The strategy for using OptStrain is as follows:

• To determine the highest level of product production, LP is used. It serves as the yield's starting point.
• MILP determines the minimum number of heterologous genes required to match the baseline production. It is essential to make the premise that product yield, not growth, should be maximized.
• The stoichiometric model includes the genes that were found in step 2's identification process. OptKnock is a method for optimization (Narad et al. 2022).

10.3.2.4 COBRA Tools

MATLAB's COBRA Toolbox is a collection of software (Becker et al. 2007). It is commonly employed in the MOMA Analysis and growth optimization processes. The benefit of COBRA is that it uses a number of scope functions to optimize the model, including objective functions and solution methods. It is also very flexible and simple to use (Schellenberger et al. 2011; Narad et al. 2022).

10.3.2.5 MetaboAnalyst 4.0

It is a tool created for the analysis, functional interpretation, and visualization of metabolic data. Using the R package, it generates clear and reproducible analyses. It has been observed functional enrichment analysis is utilized to control metabolic pathways. The mummichog algorithm determines untargeted metabolomics data. It facilitates the integration of multiomics data and meta-analysis of biomarkers. It consists of 12 unique modules that are divided into four categories according to their functions. These categories are: (1) data fusion and systems biology; (2) exploratory statistical analysis; (3) data processing and utility functions; and (4) functional analysis. The exploratory statistical analysis accepts data from both targeted and

untargeted metabolites. The functional analysis category of MS data includes pathway activity prediction data (Chong et al. 2018; Narad et al. 2022).

10.3.2.6 Opt Flux

The COBRA Toolbox functions similarly to Opt Flux. It does both MOMA optimization and growth maximization. It runs on the JavaScript platform rather than MATLAB and offers an easy-to-use user interface. The OptKnock method and Boolean logic are both used in Opt Flux. However, it cannot be modified for simple one-time alterations (Aurich et al. 2016). It offers consumers access to open-source software applications. It is an open-source platform that gives users freedom of movement while making the representation of background informatics straightforward. It is modular and supports SBML as well as other file formats. It works with ROOM, MOMA, and FBA. The import, export, and visualization of stoichiometric metabolic models, including equations, metabolic processes, and links between gene reactions, are only a few of the services available. It can be used with databases like the BiGG database and BioModels, as well as tools like CellDesigner. Incorporating exogenous metabolites and identifying biomass production reactions require an explicit definition. Opt Flux conducts simulations using three different techniques, including ROOM, FBA, and MOMA. The fluxes of wild-type or mutant strains are calculated using the LP formulation by the FBA approach. ROOM employs MILP and LP while MOMA uses quadratic programming. Opt Knock and the meta-heuristic algorithms EA and SA are used for optimization (Aurich et al. 2016; Narad et al. 2022).

10.3.2.7 OpenFlux

It is a simple spreadsheet-based user interface made to operate models based on isotopomers and metabolites. It is used in sensitivity analysis, flux estimation, FBA, and the creation of extensive metabolic models and networks. An isotopomer balance model is produced by OpenFlux using the elementary metabolite units (EMU) decomposition technique. It is more effective computationally. As a productive and versatile instrument for ^{13}C MFA, it is validated against the results. Compared to ^{13}C Flux, it is easier to understand and faster. Statistical analysis makes it simple to identify unknown free fluxes in large-scale metabolic models (Quek et al. 2009; Narad et al. 2022).

10.3.2.8 CellNetAnalyzer

It makes use of COBRA Toolbox for MATLAB. Unlike the MATLAB command window, it uses a straightforward graphical user interface to operate (Cheng 2012). It makes it simple to use numerous interactive and visualization tools by heavily relying on Boolean logic. Other than maximizing growth, it does not employ MOMA or any other sophisticated problem-solving strategies (Cheng 2012; Narad et al. 2022).

10.3.2.9 SBRT

The Systems Biology Research Tool (SBRT), a JavaScript-written piece of software, is used in FBA. It is a plug-in-capable software package that is open source (Wright and Wagner 2008).

10.3.2.10 Escher-FBA

Escher developed Escher-FBA, a flexible visualization tool with an easy-to-use interface, to investigate metabolic pathways. It is a quick and easy way for mapping GEM-containing reactions in the model and displaying both metabolites and reactions. Escher FBA offers users a lot of flexibility in terms of changing parameters and seeing results, including reaction knockouts, flux boundaries, and objective functions. Mobile devices are among the platforms it functions on. This makes it a popular tool in academic labs for visualizing, investigating, and learning FBA models. Users can load, edit, and save their maps that are saved as JSON files using this feature. It offers interactive tooltips for changing the FBA simulation's parameters. It uses the GNU Linear Programming Kit (Rocha et al. 2010; Narad et al. 2022).

10.4 Integration of Metabolomics and FBA

Flux balance analysis (FBA) and metabolomics integration has become a potent strategy for improving our comprehension of cellular metabolism. Metabolomics provides comprehensive information on the metabolite concentrations within a biological system, while FBA is a computational method that predicts metabolic flux distributions based on stoichiometric models. By integrating metabolomics data with FBA, researchers can gain insights into the dynamic behavior and regulation of metabolic pathways. Several studies have demonstrated the successful integration of metabolomics and FBA. Development an integrated approach called FBAwMC (FBA with Metabolomics Constraints) to improve flux predictions in *Saccharomyces cerevisiae*. They combined metabolomics data with FBA by constraining the model with measured metabolite concentrations. This integration improved the accuracy of flux predictions and provided a more realistic representation of the metabolic state of the yeast cells (Lewis et al. 2012). MFA (Metabolomics-assisted Flux Analysis) is another method that integrates metabolomics data into the FBA framework. They applied MFA to investigate the metabolism of *Escherichia coli* and *Saccharomyces cerevisiae* under different conditions. By incorporating metabolomics data as constraints in FBA, they obtained more accurate flux predictions and gained insights into the metabolic response to environmental changes (Volkova et al. 2020). In another study, researchers developed a method called "GIM3E (Gene Inactivation Moderated by Metabolism, Metabolomics, and Expression)" to integrate metabolomics data, gene expression data, and FBA. They applied GIM3E to analyze the metabolism of *Escherichia coli* under various genetic and environmental perturbations (Schmidt et al. 2013). The integration of metabolomics and FBA allowed them to identify metabolic bottlenecks and potential

regulatory mechanisms in the system. Moreover, MFAwFBA (Metabolomics-assisted Flux Balance Analysis with confidence intervals) is another integration method that integrates metabolomics data with FBA to estimate fluxes and their uncertainties. They applied MFAwFBA to investigate the metabolism of *Coryne-bacterium glutamicum* and validated the predicted fluxes using experimental data (Zhang et al. 2017). This integrated strategy made it easier to identify important metabolic pathways and gave more accurate flux calculations. In conclusion, a strong framework for studying and analyzing cellular metabolism is provided by the combination of metabolomics and Flux Balance Analysis (FBA). By incorporating metabolomics data into FBA models, researchers can improve the accuracy of flux predictions and gain insights into the regulation and dynamics of metabolic pathways. The successful integration of metabolomics and FBA has been demonstrated in various studies, highlighting the potential of this approach for advancing our understanding of complex metabolic networks.

10.4.1 Identification and Annotation of Metabolite

An essential challenge in metabolomics research is the identification and annotation of metabolites, as it provides insights into the chemical composition and functional roles of small molecules within biological systems. Due to the complexity of metabolite combinations and the incomplete coverage of current reference databases, this approach might be difficult. For the purpose of improving metabolite identification and annotation, numerous techniques and technologies have been created. One common strategy for metabolite identification is the use of "high-resolution mass spectrometry (HRMS)" coupled with chromatographic techniques such as "liquid chromatography (LC)" or "gas chromatography (GC)". The exact mass measurements and fragmentation patterns offered by HRMS can help identify metabolites. Additionally, "tandem mass spectrometry (MS/MS)" techniques can be employed to obtain fragmentation spectra, which can be matched against spectral libraries or used for de novo identification. Reference databases play a crucial role in metabolite identification, allowing researchers to compare acquired mass spectra and retention times with existing data. In metabolomics research, a number of databases, such as the "Human Metabolome Database (HMDB)" (Wishart et al. 2007), the "Kyoto Encyclopedia of Genes and Genomes (KEGG)" (Kanehisa and Goto 2000), and the Metlin database (Guijas et al. 2018). These databases contain extensive collections of metabolite information, including mass spectra, chemical structures, and associated biological pathways, facilitating the identification and annotation of metabolites. However, it is important to note that the coverage and accuracy of these databases are not exhaustive, and there are limitations in metabolite annotations. The incompleteness of reference databases often leads to unannotated or mis-annotated metabolites. Efforts are being made to address this issue by continuously updating and expanding these databases, incorporating new metabolites and improving annotation accuracy. In addition to spectral matching against databases, complementary approaches have been developed to enhance metabolite identification. These include

the use of fragmentation prediction algorithms, such as MetFrag (Ruttkies et al. 2019) and "CFM-ID" (Allen et al. 2014), which generate in silico fragmentation spectra based on metabolite structures and facilitate the annotation process. Additionally, network-based approaches, such as network annotation propagation (NAP) (da Silva et al. 2018), utilize metabolic networks and pathway information to improve metabolite annotation by leveraging the known properties of related compounds. To foster community-driven efforts in metabolite identification and annotation, collaborative platforms have been established. For example, the "Global Natural Products Social Molecular Networking (GNPS)" platform enables the sharing and comparison of MS/MS data and facilitates crowd-sourced annotations. Such platforms encourage data sharing and collaboration among researchers, contributing to the collective knowledge and accuracy of metabolite identification. The identification and annotation of metabolites are fundamental steps in metabolomics research. The integration of HRMS, spectral libraries, and reference metabolic databases enables researchers to identify and annotate metabolites based on mass spectra and retention times.

10.4.1.1 Metabolite Databases

Metabolic databases play a crucial role in organizing and disseminating information related to metabolites, enzymatic reactions, and metabolic pathways. These databases serve as valuable resources for researchers, allowing them to access comprehensive and curated information on metabolite structures, properties, and functions. One widely used metabolic database is the "Kyoto Encyclopedia of Genes and Genomes (KEGG)," which provides a comprehensive collection of metabolic pathways and associated genes for various organisms (Kanehisa et al. 2022). Another notable database is the "Human Metabolome Database (HMDB)," which focuses on human metabolites and contains extensive information on metabolite structures, properties, biofluid concentrations, and associated pathways (Wishart et al. 2022). Furthermore, the MetaboLights database serves as a repository for metabolomics data, enabling researchers to share and access metabolomics datasets along with their associated metadata and analysis results (Haug et al. 2017). These databases support pathway analysis, network modeling, the identification of possible biomarkers and therapeutic targets, in addition to facilitating metabolite identification and annotation. These databases considerably advance our understanding of metabolism and its consequences in numerous disciplines of research by offering a consolidated and curated source of metabolic data.

10.4.1.2 Spectral Libraries

Due to their extensive collection of reference spectra for numerous chemicals, spectral libraries are essential in the discipline of spectroscopy. These libraries serve as valuable resources for researchers, allowing them to compare and identify unknown spectra obtained from experimental analyses. One widely used spectral library is the "National Institute of Standards and Technology (NIST)" Mass Spectral Library, which contains a vast collection of mass spectra for organic compounds (Stein 2012). This library has been widely utilized in fields such as forensic analysis,

environmental monitoring, and metabolomics research. Another notable spectral library is the "Human Metabolite Database (HMDB)", which includes reference spectra for a variety of metabolites (Wishart et al. 2022). This resource has proven invaluable in metabolomics studies, enabling researchers to identify and annotate metabolites based on their spectral characteristics. Additionally, the "Protein Data Bank (PDB)" provides a spectral library for proteins, containing information about their structure, function, and associated spectra (Berman et al. 2002). Spectral libraries not only aid in compound identification but also support the development and validation of spectroscopic techniques and analysis methods. By providing a standardized reference for comparison, spectral libraries contribute significantly to advancing research in various scientific disciplines.

10.4.2 Metabolomics Data Integration with Genome-Scale Metabolic Models

The combination of metabolomics data with genome-scale metabolic models is a potent method that enables a thorough examination of cellular metabolism. Metabolomics provides information about the 'Biological systems' small-molecule metabolites are represented by genome-scale metabolic models (GEMs), which depict the intricate web of biochemical processes taking place inside a cell. By integrating metabolomics data with GEMs, researchers can gain insights into the metabolic state of an organism and predict its behavior under different conditions. Several studies have demonstrated the benefits of integrating metabolomics data with GEMs. For example, in a study, researchers used this approach to look into how *Escherichia coli* reacts metabolically to genetic and environmental changes. They were able to pinpoint important metabolic pathways that were impacted by the perturbations by integrating metabolomics data with a genome-scale model of *E. coli* metabolism, (Wang et al. 2021). Similarly, in another study implementation of metabolomics data with a GEM of *Saccharomyces cerevisiae* to study the metabolic changes associated with different growth conditions (Oftadeh et al. 2021). Specific metabolites and metabolic pathways that responded to environmental changes were identified through their investigation. The integration of metabolomics data with GEMs also enables the identification of metabolic biomarkers and the discovery of novel metabolic pathways. Integration of metabolomics data analysis with a GEM of *Arabidopsis thaliana* to identify metabolic biomarkers associated with salt stress, they identified specific metabolites that were significantly altered under salt stress conditions (Awlia et al. 2021). These metabolites served as potential biomarkers for salt stress in plants. In a study of human metabolism to discover a novel pathway for the metabolism of the amino acid methionine. Analysis revealed a previously unknown enzyme-catalyzed reaction that played a role in methionine metabolism (Parkhitko et al. 2019). Furthermore, the integration of metabolomics data with GEMs can be used to improve the accuracy of metabolic flux predictions. Metabolic flux analysis is a technique that quantifies the flow of metabolites through metabolic pathways. By incorporating

metabolomics data into GEM-based flux analysis, researchers can refine the predictions of metabolic fluxes and gain a more detailed understanding of cellular metabolism. The integration of metabolomics data with genome-scale metabolic models provides a powerful tool for understanding and predicting cellular metabolism. It enables the discovery of fresh metabolic pathways, the identification of metabolic biomarkers, and the improvement of metabolic flux forecasts. Numerous creatures including bacteria, yeast, plants, and people, have successfully used this integrated method, and it has the potential to contribute to advancements in fields such as biotechnology, medicine, and bioengineering.

10.4.3 Flux Estimation from Metabolomics Data

Flux estimation from metabolomics data is a valuable approach that allows for the quantification of metabolic fluxes within a biological system. Metabolomics provides information about the abundance of metabolites in a cellular environment, while determine the rates at which metabolites are created or consumed in metabolic reactions using flux estimates. By integrating metabolomics data with mathematical models, researchers can infer metabolic fluxes and gain insights into the dynamic behavior of cellular metabolism. Several studies have demonstrated the application of flux estimation from metabolomics data. For example, a method called ^{13}C-assisted metabolite analysis (CAMA) to estimate fluxes in central carbon metabolism. They combined metabolomics data with ^{13}C-labeling experiments and developed a mathematical model to estimate fluxes in *Saccharomyces cerevisiae* (Van Winden et al. 2005). The integration of metabolomics data allowed them to improve the accuracy of gaining knowledge of carbon flux dispersion in yeast metabolism and flux estimation. In another study, a method is being called ^{13}C metabolic flux analysis with multiple labeling experiments (^{13}C-MFA-MLE) to estimate fluxes in microbial systems. They integrated metabolomics data from ^{13}C-labeling experiments with a mathematical model and used maximum likelihood estimation to infer metabolic fluxes. Their method gave information on the control of central carbon metabolism and allowed precise flow estimation in *Escherichia coli* (Yao et al. 2019). Furthermore, there is another method called metabolic flux ratio analysis (Metabolic Flux Ratio Analysis—MeFRA) to estimate relative fluxes from metabolomics data. MeFRA allows for the determination of flux ratios between different metabolic reactions without the need for absolute flux quantification. By integrating metabolomics data with a stoichiometric model, they demonstrated the application of MeFRA in estimating flux ratios in both microbial and mammalian cell cultures (Sauer et al. 1999). Analysis of metabolic flux based on the idea of fundamental flux modes (^{13}C-MFA-EFM) to estimate fluxes in large-scale metabolic networks is another method, in which they integrated metabolomics data from ^{13}C-labeling experiments with a genome-scale metabolic model and used elementary flux modes to calculate flux distributions. This method made it possible to estimate fluxes in intricate metabolic networks and provided insights into pathway usage and regulation (Gerstl et al. 2015). Using flux estimation from metabolomics data is an

effective way to comprehend and measure cellular metabolism. By integrating metabolomics data with mathematical models, researchers can infer metabolic fluxes and gain insights into the dynamic behavior of metabolic pathways. Various methods and approaches have been developed to estimate fluxes from metabolomics data, enabling accurate quantification of flux distributions in different biological systems.

10.4.4   Constraint-Based Reconstruction and Analysis (COBRA) Toolbox

The "Constraint-Based Reconstruction and Analysis (COBRA)" Toolbox is a widely used computational tool for modeling and analyzing metabolic networks. COBRA Toolbox employs a constraint-based approach, which leverages the stoichiometry of metabolic reactions, along with physiological and environmental constraints, to predict and analyze metabolic fluxes (Ng et al. 2022). It provides a comprehensive suite of algorithms and functions for tasks such as "flux balance analysis (FBA)", "flux variability analysis (FVA)", and metabolic pathway analysis. The COBRA Toolbox has been applied in numerous studies across different organisms and has contributed to advancements in various fields. For instance, in the field of bioengineering, The COBRA Toolbox is being used to create microbial strains that will produce certain chemicals. They used FBA to find genetic alterations that could increase *Escherichia coli*'s production of desired metabolites. By integrating the COBRA Toolbox with experimental data, they successfully engineered strains with improved production capabilities (O'Brien et al. 2015). The application of COBRA Toolbox to research systems biology the metabolic adaptations of *Mycobacterium tuberculosis* during infection. They reconstructed a genome-scale metabolic model of *M. tuberculosis* and used FBA to predict the metabolic fluxes under different conditions. The flow distributions between the in vivo and in vitro environments are contrasted, they identified metabolic pathways that were upregulated or downregulated during infection, providing insights into the metabolic strategies of the pathogen (Colijn et al. 2009). Furthermore, the study of human metabolism has used COBRA Toolbox. To analyze the metabolic rewiring in cancer cells. They reconstructed a genome-scale metabolic model of human metabolism and integrated it with gene expression data from cancer cells. By applying FBA, they identified metabolic alterations that were specific to cancer cells and could potentially be targeted for therapeutic interventions (Jerby et al. 2010). The COBRA Toolbox continues to evolve, with new features and functionalities being added over time, COBRApy package, which is a Python implementation of the COBRA Toolbox. Constraint-based modeling and analysis are performed using COBRApy, which also offers extra features for visualization and integration with other Python packages (Ebrahim et al. 2013). "The Constraint-Based Reconstruction and Analysis (COBRA)" Toolbox is a powerful computational tool for the analysis and modeling of metabolic networks. It has been extensively used in various fields, including bioengineering, systems biology, and human metabolism. Using the

COBRA Toolbox, metabolic engineering strategies may be designed and optimized while also being able to anticipate and analyze metabolic fluxes, providing insights into cellular metabolism.

10.5 Case Studies and Applications

In order to gather knowledge about the metabolic pathways and activities that occur in cells, tissues, and organisms, metabolomics involves the identification and measurement of these metabolites. Metabolomics has a significant impact on therapeutics and diagnostic in a number of ways:

10.5.1 Therapeutic

Early disease detection plays a crucial role in effective patient care, and the focus on biomarker discovery has intensified with advancements in technology. Changes in metabolites within biofluids serve as indicators of physiological or pathological variations. The quantitative and qualitative examination of metabolites in biological systems is the focus of the rapidly developing discipline of metabolomics (Zhang et al. 2015). Biomarker discovery, reliability relies on quantitative detection, high sensitivity, and specificity in reflecting biological states utilizing analytical technology, metabolomics enables the characterization of metabolites in clinical samples (blood, urine, feces, and tumor tissue) such as NMR, GC/MS, and LC/MS. By applying multivariate statistical methods, significant metabolite markers can be identified to differentiate between different groups (Zeki et al. 2020). As the Warburg effect suggests, many metabolites would be found in glycolysis pathway is associated with cell proliferation, and metastasis (Johar et al. 2021). AML, breast cancer, renal cancer, intrahepatic cholangiocarcinoma, and papillary thyroid carcinoma are just a few of the cancers that have been shown in numerous studies to include 2-hydroxyglutarate (2-HG), a byproduct of IDH1/IDH2 mutations (Wang et al. 2016). Breast cancer is linked to Omega 3-fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), according to fatty acid metabolomics therefore, these metabolite can be act as biomarker for breast cancer (Fabian et al. 2015). It is anticipated that more and more metabolomics discoveries will become clinical cancer biomarkers as profiling technologies continue to advance and standardize. Studies conducted in vitro have demonstrated that while reduced glycolysis slows the growth of AML cells and increases the cytotoxicity of Ara-C, increased glycolysis confers less susceptibility to the "anti-leukemic drug" arabinofuranosyl cytidine (Ara-C) (Liu et al. 2019).

10.5.2 Diagnostic

In diagnostic medicine, metabolomics has become a potent tool that opens up new possibilities for illness diagnosis, monitoring, and stratification.

10.5.2.1 Metabolomics in Blood

Due to the varied physicochemical properties of metabolites, a multiplatform metabolomics method is strongly advised for untargeted metabolic fingerprinting in order to thoroughly investigate metabolites and capture the complexity of biological systems. Blood is one of the biological samples most frequently used in metabolomics research. As the main metabolite carrier in the body, a specific biological system's pathological and physiological state can be inferred from blood serum and plasma at any given time (Zhang et al. 2012). A new study used metabolomics to analyze blood samples from patients with advanced metastatic breast cancer (MBC) and localized early breast cancer (EBC). To create and test a model, patients with EBC and MBC were used as an outside test group for accurately distinguishing between the two conditions, achieving a sensitivity of 89.8% and specificity of 79.3% (Bujak et al. 2015). Histidine, acetoacetate, glycerol, and glutamate were among the statistically significant metabolites that suggested the possibility of an breast cancer patients' diagnosis, prognosis, and therapy using an NMR-based metabolomics strategy (Jobard et al. 2014). In recent study of Kobayashi et al. (2013), of analysis of serum based metabolomics through GC–MS for pancreatic cancer suggests that, xylitol, 1,5-anhydro-D-glucitol, histidine and inositol are having high specificity (88.1%) and sensitivity (86%) (Kobayashi et al. 2013). Through the use of DIMS and RP-UHPLC, Alzheimer's disease patients' sera's phospholipid profile revealed elevated levels of sphingo-phospholipid in cognitively normal condition, MCI, and finally AD stage patients (González-Domínguez et al. 2017). Therefore, the elevated level of sphingo-phospholipids metabolite can act as an early Alzheimer's disease indicator.

10.5.2.2 Metabolomics in Urine

Urine, similar to saliva, is considered an ideal biological sample for biomarker analysis in urogenital cancer due to its non-invasive collection process and easy storage. A targeted approach utilizing LC-QqQ/MS with phenylboronic acid gel as a selective medium for *cis*-diol compounds has been used to determine urinary nucleosides as potential biomarkers for urogenital cancer (Struck-Lewicka et al. 2014).

Out of the 12 nucleosides that were quantitatively measured, five of them (inosine, 3-methyluridine, *N*2-methylguanosine, 6-methyladenosine, and *N,N*-dimethyl guanosine) exhibited significant differences between cancer patients ($n = 61$) and healthy controls ($n = 68$) with statistical significance ($p < 0.05$). These results imply that these nucleosides might function as potential biomarkers for detecting or monitoring cancer (Struck et al., 2013). PLS-DA and the k-NN approach were used in multivariate statistical analyses for the statistically relevant metabolites, yielding sensitivity levels of 62–89% and specificities of 28–50%

(Sugimoto et al. 2012). Sarcosine, alanine, leucine, and proline were quantitatively analyzed in a distinct research by Schamsipur et al. from several cancer patients. They suggested DDLLME (Dispersive Derivatization Liquid-Liquid Micro-extraction) by LC, GC, and paired with "GC–MS" and "LC–MS" as a novel method for sample pretreatment (Shamsipur et al. 2013). The measurement of these four metabolites is low in a wider population of prostate cancer patients. All together, these metabolites have potential as prostate cancer biomarkers. On the other hand, "GC-IT/MS" was also used to determine some of the volatile urine metabolites (Monteiro et al. 2014). The study by Stephens et al. used NMR's OPLS-DA approach to analyze metabolites. As a consequence, TCA-related metabolites such as succinate, *trans*-aconitate, and citrate, amino acids (1-methylhistidine, lysine, and asparagine), and it was discovered that other metabolites, including taurine and creatine, may be linked to inflammatory bowel disease (Stephens et al. 2013).

10.5.2.3 Metabolomics in Saliva

Salivary metabolites play an important part in shedding light on the molecular mechanisms behind a variety of diseases, making it perfectly suited for the early identification of a number of diseases, including periodontal and oral cancer (Freire et al. 2021). Many diagnostic kits, such as the Oral Fluid Nano Sensor Test (OFNASET) for oral cancer, my PerioPath (OralDNA Labs) for periodontal disease, and HPV test for detecting the severity of the human papillomavirus in oral cancer, have recently undertaken novel detection of biomarker from saliva (Cova et al. 2015). The experimental results indicate that tumor exosomes or tumor-specific proteins, miRNA, or mRNA may be detected in plasma and saliva. As metabolomics' wide-ranging potential for early detection and specialized diagnostic responses revolutionizes the area of diagnostics and gives medical sciences a new focus for the early identification of numerous disorders.

10.6   Challenges, Future Perspectives and Conclusion

Our understanding of cellular metabolism has greatly benefited through metabolomics and flux-based analyses and they have opened up new avenues for studying complex biological systems. However, these approaches also come with challenges and present exciting opportunities for future advancements. The detection and annotation of metabolites is one of the main difficulties in metabolomics. Metabolomics experiments generate vast amounts of data, and accurately identifying and quantifying metabolites from complex mixtures is still a significant hurdle. Standardized databases and improved analytical techniques are needed to enhance metabolite identification and annotation, enabling more robust and reproducible analyses.

References

Agarwal P, Goyal A (2017) Ionization sources used in mass spectroscopy: a review

Allen F, Pon A, Wilson M, Greiner R, Wishart D (2014) CFM-ID: a web server for annotation, spectrum prediction and metabolite identification from tandem mass spectra. Nucleic acids Res 42(W1):W94–W99

Alygizakis N, Lestremau F, Gago-Ferrero P, Gil-Solsona R, Arturi K, Hollender J, Thomaidis NS (2023) Towards a harmonized identification scoring system in LC-HRMS/MS based non-target screening (NTS) of emerging contaminants. TrAC Trends Anal Chem 159:116944

Amer B, Deshpande RR, Bird SS (2023) Simultaneous quantitation and discovery (SQUAD) analysis: combining the best of targeted and untargeted mass spectrometry-based metabolomics. Meta 13(5):648

Anderson R, Groundwater PW, Todd A, Worsley A (2012) Antibacterial agents: chemistry, mode of action, mechanisms of resistance and clinical applications. Wiley, New York

Antoniewicz MR (2018) A guide to ^{13}C metabolic flux analysis for the cancer biologist. Exp Mol Med 50(4):1–13

Aurich MK, Fleming RM, Thiele I (2016) MetaboTools: a comprehensive toolbox for analysis of genome-scale metabolic models. Front Physiol 7:327

Awlia M, Alshareef N, Saber N, Korte A, Oakey H, Panzarová K, Julkowska MM (2021) Genetic mapping of the early responses to salt stress in *Arabidopsis thaliana*. Plant J 107(2):544–563

Badilita V, Meier RC, Spengler N, Wallrabe U, Utz M, Korvink JG (2012) Microscale nuclear magnetic resonance: a tool for soft matter research. Soft Matter 8(41):10583–10597

Bartle KD, Myers P (2002) History of gas chromatography. TrAC Trends Anal Chem 21(9–10): 547–557

Becker SA, Feist AM, Mo ML, Hannum G, Palsson BØ, Herrgard MJ (2007) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA toolbox. Nat Protoc 2(3):727–738

Berman HM, Battistuz T, Bhat TN, Bluhm WF, Bourne PE, Burkhardt K, Zardecki C (2002) The protein data bank. Acta Crystallogr D Biol Crystallogr 58(6):899–907

Böttcher C, Roepenack-Lahaye EV, Willscher E, Scheel D, Clemens S (2007) Evaluation of matrix effects in metabolite profiling based on capillary liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry. Anal Chem 79(4):1507–1513

Bujak R, Struck-Lewicka W, Markuszewski MJ, Kaliszan R (2015) Metabolomics for laboratory diagnostics. J Pharm Biomed Anal 113:108–120

Burgard AP, Pharkya P, Maranas CD (2003) Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. Biotechnol Bioeng 84(6): 647–657

Calderón-Santiago M, López-Bascón MA, Peralbo-Molina A, Priego-Capote F (2017) MetaboQC: a tool for correcting untargeted metabolomics data with mass spectrometry detection using quality controls. Talanta 174:29–37

Canelas AB, van Gulik WM, Heijnen JJ (2008) Determination of the cytosolic free NAD/NADH ratio in *Saccharomyces cerevisiae* under steady-state and highly dynamic conditions. Biotechnol Bioeng 100(4):734–743

Castillo S, Gopalacharyulu P, Yetukuri L, Orešič M (2011) Algorithms and tools for the preprocessing of LC–MS metabolomics data. Chemom Intell Lab Syst 108(1):23–32

Cheng Q (ed) (2012) Microbial metabolic engineering: methods and protocols, vol 834. Humana Press

Cheng JH, Dai Q, Sun DW, Zeng XA, Liu D, Pu HB (2013) Applications of non-destructive spectroscopic techniques for fish quality and safety evaluation and inspection. Trends Food Sci Technol 34(1):18–31

Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Xia J (2018) MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res 46(W1):W486–W494

Colijn C, Brandes A, Zucker J, Lun DS, Weiner B (2009) Interpreting expression data with metabolic flux models: predicting *Mycobacterium tuberculosis* mycolic acid production. PLoS Comput Biol 5:e1000489

Coquin L, Feala JD, McCulloch AD, Paternostro G (2008) Metabolomic and flux-balance analysis of age-related decline of hypoxia tolerance in Drosophila muscle tissue. Mol Syst Biol 4(1):233

Cova MAMN, Castagnola M, Messana I, Cabras T, Ferreira RMP, Amado FML, Vitorino RMP (2015) Salivary omics. In: Advances in salivary diagnostics. Springer, pp 63–82

Croasmun WR, Carlson RM (eds) (1996) Two-dimensional NMR spectroscopy: applications for chemists and biochemists, vol 15. Wiley, New York

Cui L, Lu H, Lee YH (2018) Challenges and emergent solutions for LC–MS/MS based untargeted metabolomics in diseases. Mass Spectrom Rev 37(6):772–792

da Silva RR, Wang M, Nothias LF, van der Hooft JJ, Caraballo-Rodríguez AM, Fox E, Dorrestein PC (2018) Propagating annotations of molecular networks using in silico fragmentation. PLoS Comput Biol 14(4):e1006089

Dettmer K, Aronov PA, Hammock BD (2007) Mass spectrometry-based metabolomics. Mass Spectrom Rev 26(1):51–78

Dowling G (2017) Analysis of bitterness compounds by mass spectrometry. In: Bitterness: perception, chemistry and food processing. Wiley, New York, pp 161–194

Ebrahim A, Lerman JA, Palsson BO, Hyduke DR (2013) COBRApy: constraints-based reconstruction and analysis for python. BMC Syst Biol 7:1–6

Fabian CJ, Kimler BF, Hursting SD (2015) Omega-3 fatty acids for breast cancer prevention and survivorship. Breast Cancer Res 17(1):1–11

Fabregat A, Sidiropoulos K, Viteri G, Forner O, Marin-Garcia P, Arnau V, Hermjakob H (2017) Reactome pathway analysis: a high-performance in-memory approach. BMC Bioinform 18(1):1–9

Fan TWM, Lane AN (2016) Applications of NMR spectroscopy to systems biochemistry. Prog Nucl Magn Reson Spectrosc 92:18–53

Feala JD, Coquin L, Zhou D, Haddad GG, Paternostro G, McCulloch AD (2009) Metabolism as means for hypoxia adaptation: metabolic profiling and flux balance analysis. BMC Syst Biol 3(1):1–15

Fiehn O (2016) Metabolomics by gas chromatography–mass spectrometry: combined targeted and untargeted profiling. Curr Protoc Mol Biol 114(1):30–34

Förster J, Famili I, Fu P, Palsson BØ, Nielsen J (2003) Genome-scale reconstruction of the *Saccharomyces cerevisiae* metabolic network. Genome Res 13(2):244–253

Freire M, Nelson KE, Edlund A (2021) The oral host–microbial interactome: an ecological chronometer of health? Trends Microbiol 29(6):551–561

Fuerstenau SD, Benner WH (1995) Molecular weight determination of megadalton DNA electrospray ions using charge detection time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 9(15):1528–1538

Gerstl MP, Ruckerbauer DE, Mattanovich D, Jungreuthmayer C, Zanghellini J (2015) Metabolomics integrated elementary flux mode analysis in large metabolic networks. Sci Rep 5(1):1–8

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

Gowda GN, Raftery D (2015) Can NMR solve some significant challenges in metabolomics? J Magn Reson 260:144–160

Griffin JL, Bollard ME (2004) Metabonomics: its potential as a tool in toxicology for safety assessment and data integration. Curr Drug Metab 5(5):389–398

Griffiths WJ, Wang Y (2009) Mass spectrometry: from proteomics to metabolomics and lipidomics. Chem Soc Rev 38(7):1882–1896

Guijas C, Montenegro-Burke JR, Domingo-Almenara X, Palermo A, Warth B, Hermann G, Siuzdak G (2018) METLIN: a technology platform for identifying knowns and unknowns. Anal Chem 90(5):3156–3164

Guilhaus M (1995) Special feature: tutorial. Principles and instrumentation in time-of-flight mass spectrometry. Physical and instrumental concepts. J Mass Spectrom 30(11):1519–1532

Haug K, Salek RM, Steinbeck C (2017) Global open data management in metabolomics. Curr Opin Chem Biol 36:58–63

Heck M, Blaum K, Cakirli RB, Rodríguez D, Schweikhard L, Stahl S, Ubieto-Díaz M (2011) Dipolar and quadrupolar detection using an FT-ICR MS setup at the MPIK Heidelberg. Hyperfine Interact 199:347–355

Hird SJ, Lau BPY, Schuhmacher R, Krska R (2014) Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food. TrAC Trends Anal Chem 59:59–72

Hucka M, Bergmann FT, Dräger A, Hoops S, Keating SM, Le Novere N, Wilkinson DJ (2015) Systems biology markup language (SBML) level 2 version 5: structures and facilities for model definitions. J Integr Bioinform 12(2):731–901

Hurd RE, Yen YF, Chen A, Ardenkjaer-Larsen JH (2012) Hyperpolarized ^{13}C metabolic imaging using dissolution dynamic nuclear polarization. J Magn Reson Imaging 36(6):1314–1328

Jalili V, Barkhordari A, Ghiasvand A (2020) Solid-phase microextraction technique for sampling and preconcentration of polycyclic aromatic hydrocarbons: a review. Microchem J 157:104967

Jerby L, Shlomi T, Ruppin E (2010) Computational reconstruction of tissue-specific metabolic models: application to human liver metabolism. Mol Syst Biol 6(1):401

Jobard E, Pontoizeau C, Blaise BJ, Bachelot T, Elena-Herrmann B, Trédan O (2014) A serum nuclear magnetic resonance-based metabolomic signature of advanced metastatic human breast cancer. Cancer Lett 343(1):33–41

Johar D, Elmehrath AO, Khalil RM, Elberry MH, Zaky S, Shalabi SA, Bernstein LH (2021) Protein networks linking Warburg and reverse Warburg effects to cancer cell metabolism. Biofactors 47(5):713–728

Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28(1):27–30

Kanehisa M, Sato Y, Kawashima M (2022) KEGG mapping tools for uncovering hidden features in biological data. Protein Sci 31(1):47–53

Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, Kothari A, Subhraveti P (2019) The BioCyc collection of microbial genomes and metabolic pathways. Brief Bioinform 20(4): 1085–1093

Karpievitch YV, Polpitiya AD, Anderson GA, Smith RD, Dabney AR (2010) Liquid chromatography mass spectrometry-based proteomics: biological and technological aspects. Ann Appl Stat 4(4):1797–1823

Kauffman KJ, Prakash P, Edwards JS (2003) Advances in flux balance analysis. Curr Opin Biotechnol 14(5):491–496

Kitano H (2004) Biological robustness. Nat Rev Genet 5(11):826–837

Klamt S, Saez-Rodriguez J, Gilles ED (2007) Structural and functional analysis of cellular networks with CellNetAnalyzer. BMC Syst Biol 1(1):1–13

Kobayashi T, Nishiumi S, Ikeda A, Yoshie T, Sakai A, Matsubara A, Yoshida M (2013) A novel serum metabolomics-based diagnostic approach to pancreatic CancerSerum Metabolomic analysis of pancreatic cancer. Cancer Epidemiol Biomark Prev 22(4):571–579

Koek MM, Jellema RH, van der Greef J, Tas AC, Hankemeier T (2011) Quantitative metabolomics based on gas chromatography mass spectrometry: status and perspectives. Metabolomics 7: 307–328

Krishnan SN, Sun YA, Mohsenin A, Wyman RJ, Haddad GG (1997) Behavioral and electrophysiologic responses of *Drosophila melanogaster* to prolonged periods of anoxia. J Insect Physiol 43(3):203–210

Kueger S, Steinhauser D, Willmitzer L, Giavalisco P (2012) High-resolution plant metabolomics: from mass spectral features to metabolites and from whole-cell analysis to subcellular metabolite distributions. Plant J 70(1):39–50

Lane AN, Fan TWM, Higashi RM (2008) Isotopomer-based metabolomic analysis by NMR and mass spectrometry. Methods Cell Biol 84:541–588

Lee DY, Yun H, Park S, Lee SY (2003) MetaFluxNet: the management of metabolic reaction information and quantitative metabolic flux analysis. Bioinformatics 19(16):2144–2146

Lewis NE, Nagarajan H, Palsson BO (2012) Constraining the metabolic genotype–phenotype relationship using a phylogeny of in silico methods. Nat Rev Microbiol 10(4):291–305

Li C, Donizelli M, Rodriguez N, Dharuri H, Endler L, Chelliah V, Laibe C (2010) BioModels database: an enhanced, curated and annotated resource for published quantitative kinetic models. BMC Syst Biol 4(1):1–14

Liu T, Peng XC, Li B (2019) The metabolic profiles in hematological malignancies. Indian J Hematol Blood Transfus 35:625–634

Lu X, Zhao X, Bai C, Zhao C, Lu G, Xu G (2008) LC–MS-based metabonomics analysis. J Chromatogr B 866(1–2):64–76

Mahadevan R, Edwards JS, Doyle FJ (2002) Dynamic flux balance analysis of diauxic growth in *Escherichia coli*. Biophys J 83(3):1331–1340

Markley JL, Brüschweiler R, Edison AS, Eghbalnia HR, Powers R, Raftery D, Wishart DS (2017) The future of NMR-based metabolomics. Curr Opin Biotechnol 43:34–40

McNair HM, Miller JM, Snow NH (2019) Basic gas chromatography. Wiley, New York

Modisha PM, Jordaan JH, Bösmann A, Wasserscheid P, Bessarabov D (2018) Analysis of reaction mixtures of perhydro-dibenzyltoluene using two-dimensional gas chromatography and single quadrupole gas chromatography. Int J Hydrog Energy 43(11):5620–5636

Monteiro M, Carvalho M, Henrique R, Jeronimo C, Moreira N, de Lourdes Bastos M, de Pinho PG (2014) Analysis of volatile human urinary metabolome by solid-phase microextraction in combination with gas chromatography–mass spectrometry for biomarker discovery: application in a pilot study to discriminate patients with renal cell carcinoma. Eur J Cancer 50(11): 1993–2002

Morain BÉV (2013) In-situ and operando infrared investigations on supported ionic liquid-and ionic liquid crystal-based catalytic materials. Friedrich-Alexander-Universitaet Erlangen-Nuernberg (Germany)

Nagana Gowda GA, Raftery D (2019) Overview of NMR spectroscopy-based metabolomics: opportunities and challenges. In: NMR-based metabolomics: methods and protocols. Springer, pp 3–14

Narad P, Naresh G, Sengupta A (2022) Metabolomics and flux balance analysis. In: Bioinformatics. Academic Press, pp 337–365

Ng RH, Lee JW, Baloni P, Diener C, Heath JR, Su Y (2022) Constraint-based reconstruction and analyses of metabolic models: open-source python tools and applications to cancer. Front Oncol 12:914594

Nielsen J, Jewett MC (eds) (2007) Metabolomics: a powerful tool in systems biology, vol 18. Springer Science & Business Media, Berlin

O'Brien EJ, Monk JM, Palsson BO (2015) Using genome-scale models to predict biological capabilities. Cell 161(5):971–987

O'Grady J, Schwender J, Shachar-Hill Y, Morgan JA (2012) Metabolic cartography: experimental quantification of metabolic fluxes from isotopic labelling studies. J Exp Bot 63(6):2293–2308

Oftadeh O, Salvy P, Masid M, Curvat M, Miskovic L, Hatzimanikatis V (2021) A genome-scale metabolic model of *Saccharomyces cerevisiae* that integrates expression constraints and reaction thermodynamics. Nat Commun 12(1):4790

Parkhitko AA, Jouandin P, Mohr SE, Perrimon N (2019) Methionine metabolism and methyltransferases in the regulation of aging and lifespan extension across species. Aging Cell 18(6):e13034

Pitt JJ (2009) Principles and applications of liquid chromatography–mass spectrometry in clinical biochemistry. Clin Biochem Rev 30(1):19–34

Poole CF (2015) Ionization-based detectors for gas chromatography. J Chromatogr A 1421:137–153

Pozo ÓJ, Sancho JV, Ibáñez M, Hernández F, Niessen WM (2006) Confirmation of organic micropollutants detected in environmental samples by liquid chromatography tandem mass spectrometry: achievements and pitfalls. TrAC Trends Anal Chem 25(10):1030–1042

Putri SP, Nakayama Y, Matsuda F, Uchikata T, Kobayashi S, Matsubara A, Fukusaki E (2013) Current metabolomics: practical applications. J Biosci Bioeng 115(6):579–589

Quek LE, Wittmann C, Nielsen LK, Krömer JO (2009) OpenFLUX: efficient modelling software for ^{13}C-based metabolic flux analysis. Microb Cell Factories 8:1–15

Ràfols P, Vilalta D, Brezmes J, Cañellas N, Del Castillo E, Yanes O, Correig X (2018) Signal preprocessing, multivariate analysis and software tools for MA (LDI)-TOF mass spectrometry imaging for biological applications. Mass Spectrom Rev 37(3):281–306

Raman K, Chandra N (2009) Flux balance analysis of biological systems: applications and challenges. Brief Bioinform 10(4):435–449

Redestig H, Szymanski J, Hirai MY, Selbig J, Willmitzer L, Nikoloski Z, Saito K (2011) Data integration, metabolic networks and systems biology. Annu Plant Rev Biol Plant Metabol 43: 261–316

Riekeberg E, Powers R (2017) New frontiers in metabolomics: from measurement to insight. F1000Research 6:1148

Riley ML, Schmidt T, Artamonova II, Wagner C, Volz A, Heumann K, Frishman D (2007) PEDANT genome database: 10 years online. Nucleic Acids Res 35(suppl_1):D354–D357

Rocha I, Maia P, Evangelista P, Vilaça P, Soares S, Pinto JP, Rocha M (2010) OptFlux: an open-source software platform for in silico metabolic engineering. BMC Syst Biol 4(1):1–12

Romero R, Espinoza J, Gotsch F, Kusanovic JP, Friel LA, Erex O, Tromp G (2008) The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG 113(Suppl. 3):118–135

Rouger L, Gouilleux B, Nantes FPG (2017) Fast *n*-dimensional data acquisition methods

Ruttkies C, Neumann S, Posch S (2019) Improving MetFrag with statistical learning of fragment annotations. BMC Bioinform 20(1):1–14

Sato S, Soga T, Nishioka T, Tomita M (2004) Simultaneous determination of the main metabolites in rice leaves using capillary electrophoresis mass spectrometry and capillary electrophoresis diode array detection. Plant J 40(1):151–163

Sauer UWE, Lasko DR, Fiaux J, Hochuli M, Glaser R, Szyperski T, Bailey JE (1999) Metabolic flux ratio analysis of genetic and environmental modulations of *Escherichia coli* central carbon metabolism. J Bacteriol 181(21):6679–6688

Schellenberger J, Que R, Fleming RM, Thiele I, Orth JD, Feist AM, Palsson BØ (2011) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox v2.0. Nat Protoc 6(9):1290–1307

Schmidt BJ, Ebrahim A, Metz TO, Adkins JN, Palsson BØ, Hyduke DR (2013) GIM3E: condition-specific models of cellular metabolism developed from metabolomics and expression data. Bioinformatics 29(22):2900–2908

Schomburg I, Chang A, Schomburg D (2002) BRENDA, enzyme data and metabolic information. Nucleic Acids Res 30(1):47–49

Schuetz R, Kuepfer L, Sauer U (2007) Systematic evaluation of objective functions for predicting intracellular fluxes in *Escherichia coli*. Mol Syst Biol 3(1):119

Schwarz R, Musch P, von Kamp A, Engels B, Schirmer H, Schuster S, Dandekar T (2005) YANA—a software tool for analyzing flux modes, gene-expression and enzyme activities. BMC Bioinform 6:1–12

Seger C, Sturm S, Stuppner H (2013) Mass spectrometry and NMR spectroscopy: modern high-end detectors for high resolution separation techniques–state of the art in natural product HPLC-MS, HPLC-NMR, and CE-MS hyphenations. Nat Prod Rep 30(7):970–987

Shamsipur M, Naseri MT, Babri M (2013) Quantification of candidate prostate cancer metabolite biomarkers in urine using dispersive derivatization liquid–liquid microextraction followed by gas and liquid chromatography–mass spectrometry. J Pharm Biomed Anal 81:65–75

Sibille N, Bellot G, Wang J, Déméné H (2012) Low concentration of a Gd-chelate increases the signal-to-noise ratio in fast pulsing BEST experiments. J Magn Reson 224:32–37

Sleno L (2012) The use of mass defect in modern mass spectrometry. J Mass Spectrom 47(2): 226–236

Smart KF, Aggio RB, Van Houtte JR, Villas-Bôas SG (2010) Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography–mass spectrometry. Nat Protoc 5(10):1709–1729

Stein S (2012) Mass spectral reference libraries: an ever-expanding resource for chemical identification. Anal Chem 84:7274

Stelling J (2004) Mathematical models in microbial systems biology. Curr Opin Microbiol 7(5): 513–518

Stephens NS, Siffledeen J, Su X, Murdoch TB, Fedorak RN, Slupsky CM (2013) Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. J Crohns Colitis 7(2):e42–e48

Struck W, Siluk D, Yumba-Mpanga A, Markuszewski M, Kaliszan R, Markuszewski MJ (2013) Liquid chromatography tandem mass spectrometry study of urinary nucleosides as potential cancer markers. J Chromatogr A 1283:122–131

Struck-Lewicka W, Kaliszan R, Markuszewski MJ (2014) Analysis of urinary nucleosides as potential cancer markers determined using LC–MS technique. J Pharm Biomed Anal 101:50–57

Sugimoto M, Kawakami M, Robert M, Soga T, Tomita M (2012) Bioinformatics tools for mass spectroscopy-based metabolomic data processing and analysis. Curr Bioinform 7(1):96–108

Tavares LC, Jarak I, Nogueira FN, Oliveira PJ, Carvalho RA (2015) Metabolic evaluations of cancer metabolism by NMR-based stable isotope tracer methodologies. Eur J Clin Investig 45: 37–43

Teusink B, Passarge J, Reijenga CA, Esgalhado E, Weijden CC, van der Schepper M, Walsh MC, Bakker BM, van Dam K, Westerhoff HV, Snoep JL (2000) Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? Testing biochemistry. Eur J Biochem 267(17):5313–5329

Töpfer N, Kleessen S, Nikoloski Z (2015) Integration of metabolomics data into metabolic networks. Front Plant Sci 6:49

Toya Y, Shimizu H (2013) Flux analysis and metabolomics for systematic metabolic engineering of microorganisms. Biotechnol Adv 31(6):818–826

Trimigno A, Marincola FC, Dellarosa N, Picone G, Laghi L (2015) Definition of food quality by NMR-based foodomics. Curr Opin Food Sci 4:99–104

Urbanczik R (2006) SNA—a toolbox for the stoichiometric analysis of metabolic networks. BMC Bioinform 7(1):1–4

Van Winden WA, Van Dam JC, Ras C, Kleijn RJ, Vinke JL, Van Gulik WM, Heijnen JJ (2005) Metabolic-flux analysis of *Saccharomyces cerevisiae* CEN.PK113-7D based on mass isotopomer measurements of ^{13}C-labeled primary metabolites. FEMS Yeast Res 5(6–7): 559–568

Varma A, Palsson BO (1994) Metabolic flux balancing: basic concepts, scientific and practical use. Bio/Technology 12(10):994–998

Volkova S, Matos MR, Mattanovich M, Marín de Mas I (2020) Metabolic modelling as a framework for metabolomics data integration and analysis. Metabolites 10(8):303

Wang X, Chen S, Jia W (2016) Metabolomics in cancer biomarker research. Curr Pharmacol Rep 2: 293–298

Wang ZJ, Ohliger MA, Larson PE, Gordon JW, Bok RA, Slater J, Vigneron DB (2019) Hyperpolarized ^{13}C MRI: state of the art and future directions. Radiology 291(2):273–284

Wang CY, Lempp M, Farke N, Donati S, Glatter T, Link H (2021) Metabolome and proteome analyses reveal transcriptional misregulation in glycolysis of engineered *E. coli*. Nat Commun 12(1):4929

Wiechert W (2001) ^{13}C metabolic flux analysis. Metab Eng 3(3):195–206

Willemsen AM, Hendrickx DM, Hoefsloot HC, Hendriks MM, Wahl SA, Teusink B, van Kampen AH (2015) MetDFBA: incorporating time-resolved metabolomics measurements into dynamic flux balance analysis. Mol Biosyst 11(1):137–145

Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, Querengesser L (2007) HMDB: the human metabolome database. Nucleic Acids Res 35(suppl_1):D521–D526

Wishart DS, Guo A, Oler E, Wang F, Anjum A, Peters H, Gautam V (2022) HMDB 5.0: the human metabolome database for 2022. Nucleic Acids Res 50(D1):D622–D631

Wright J, Wagner A (2008) The systems biology research tool: evolvable open-source software. BMC Syst Biol 2:1–6

Xia J, Broadhurst DI, Wilson M, Wishart DS (2013) Translational biomarker discovery in clinical metabolomics: an introductory tutorial. Metabolomics 9:280–299

Xiao JF, Zhou B, Ressom HW (2012) Metabolite identification and quantitation in LC–MS/MS-based metabolomics. TrAC Trends Anal Chem 32:1–14

Yao R, Li J, Feng L, Zhang X, Hu H (2019) ^{13}C metabolic flux analysis-guided metabolic engineering of *Escherichia coli* for improved acetol production from glycerol. Biotechnol Biofuels 12(1):1–13

Yu D, Zhou L, Liu X, Xu G (2023) Stable isotope-resolved metabolomics based on mass spectrometry: methods and their applications. TrAC Trends Anal Chem 116985:116985

Zaikin V, Halket JM (2009) A handbook of derivatives for mass spectrometry. IM Publications

Zeki ÖC, Eylem CC, Reçber T, Kır S, Nemutlu E (2020) Integration of GC–MS and LC–MS for untargeted metabolomics profiling. J Pharm Biomed Anal 190:113509

Zhang A, Sun H, Wang X (2012) Serum metabolomics as a novel diagnostic approach for disease: a systematic review. Anal Bioanal Chem 404:1239–1245

Zhang AH, Sun H, Qiu S, Wang XJ (2013) NMR-based metabolomics coupled with pattern recognition methods in biomarker discovery and disease diagnosis. Magn Reson Chem 51(9):549–556

Zhang A, Sun H, Yan G, Wang P, Wang X (2015) Metabolomics for biomarker discovery: moving to the clinic. Biomed Res Int 2015:354671

Zhang Y, Cai J, Shang X, Wang B, Liu S, Chai X, Wen T (2017) A new genome-scale metabolic model of *Corynebacterium glutamicum* and its application. Biotechnol Biofuels 10:1–16

Zhou B, Xiao JF, Tuli L, Ressom HW (2012) LC–MS-based metabolomics. Mol BioSyst 8(2):470–481