Ars Separatoria Acta 6 (2008) 85-99

ARS SEPARATORIA ACTA

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METABOLOMICS – NEW TRENDS IN LIFE CHEMISTRY

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ABSTRACT

Metabolomics is reviewed in relation to functional genomics and systems biology. A historical account of the introduction and evolution of metabolite profiling into modern comprehensive metabolomics approach is provided. Many of the techniques used in metabolomics, including Fourier transform infrared (FT-IR) spectroscopy, nuclear magnetic resonance (NMR), chromatography and mass spectrometry are surveyed. Applications and various methods of data visualization are summarized.

Keywords: Metabolomics, Metabonomics, Analytical instrumentation, QSRR, Bioinformatics

INTRODUCTION

We live in an exciting time where technology continues to push back the frontiers of science, and the search to better understand holistic biological systems has spawned the 'omics' revolution [1]. For a deeper perception of molecules and relationships at a cellular level, we use of several research methods which lay out the sense of investigations in bioanalytics. These modern technologies of bioanalytics defined the name 'omics'. They characterize the miniaturization and the automation of measuring processes, simultaneous registration of hundreds or thousands pieces of information as well as the use of complex chemometrics tools in analytical results. The general aim is to obtain information that can explain and identify the differences between certain sets of organisms, such as differences in genotypes or between people affected by various diseases and disease-free controls or elucidate factors that influence biochemical events.

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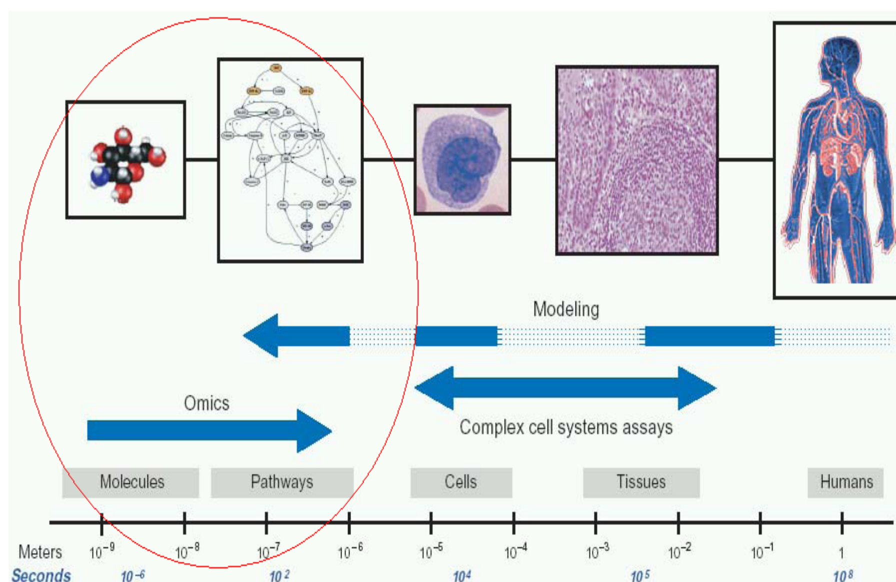

Fig. 1. Approaches to systems biology and 'omics' [2].

'Omics' technologies are based on comprehensive biochemical and molecular characterizations of an organism, tissue or cell type. Approaches to systems biology and 'omics' are shown in Figure 1.

Functional studies (Figure 2) have thus emphasized analyses at the level of gene expression (transcriptomics), protein translation (proteomics) including post-translational modifications, and the metabolic network (metabolomics), with a view to a 'systems biology' approach of defining the phenotype and bridging the genotype. Even the representation in Figure 2 is simplistic since whilst in our linear conception of the cell the general flow of information goes from gene to transcript to protein to metabolite to phenotype, there are multiple feedback loops from metabolites to proteins and/or transcripts, as well as others ones [3-7]. Changes in the metabolome occur in consequence of those changes in the transcriptome that result in changes in the levels or catalytic activities of enzymes. Therefore, metabolome analysis is a valuable tool for explanation the gene function [8].

In this review advances in the way we both gather and use metabolomic data for the large-scale reconstruction of biological systems and for the generation of both testable hypothesis and the predictive models that lie at the heart of systems biology are highlighted.

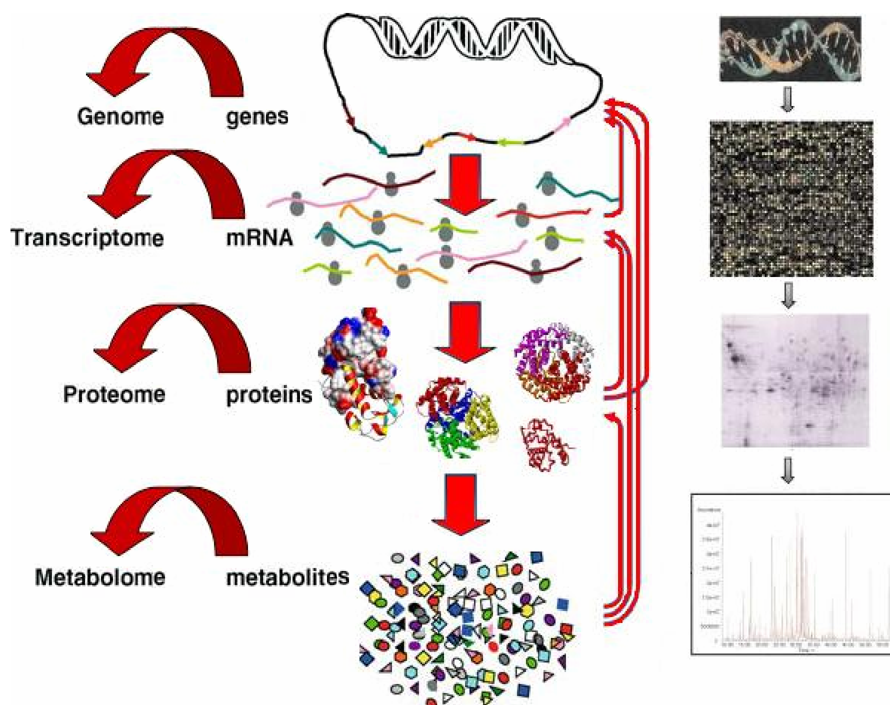

Fig. 2. General schematic of the 'omic' organization. The general flow of information is from genes to transcripts to proteins to metabolites to function (or phenotype) [4].

THE DEVELOPMENT OF METABOLOMICS

Metabolomics developed from metabolic profiling. In the early 1970's Gas Chromatography-Mass Spectrometry (GC-MS) techniques were used to analyze steroids, acids, and neutral and acidic urinary drug metabolites [9,10]. Soon afterwards, the concept of using metabolic profiles in screening and diagnosing began to spread [11-12]. During the early 1980's, results of the application of HPLC and NMR for metabolite profiling began to appear in the literature [13]. Metabolic profiling research remained stable in the 1980's with approximately 10 to 15 publications a year. With this increase in publications, came a divergence in the use of the new technology. However, it was not until the early 1990's that metabolic profiling was first used as a diagnostic technique in plant systems by Sauter [14]. However, application of metabolome analysis was discussed first in 1998 by Oliver [15]. His team estimated the number of yeast metabolites to be approximately 600 and proposed the concept of metabolomics. Later, Fiehn introduced a clear definition for metabolome analysis and terms for other approaches to measure cellular metabolites [16-17]. At the turn of the century, many

genome sequencing efforts were underway or near completion, and it soon became clear that a large number of the genes that were being sequenced could be assigned a function [18]. It then became apparent that a closer study of proteins (proteomics) might also be an effective means with which to study gene function [7]. The same applies to metabolomics. Since then, the number of groups using and entering this field has grown up. This interest is demonstrated in the publication record. A SciFinder Scholar search on metabolomics yields an increasing number of hits for each year since 2000 [19]. It is shown in Figure 3.

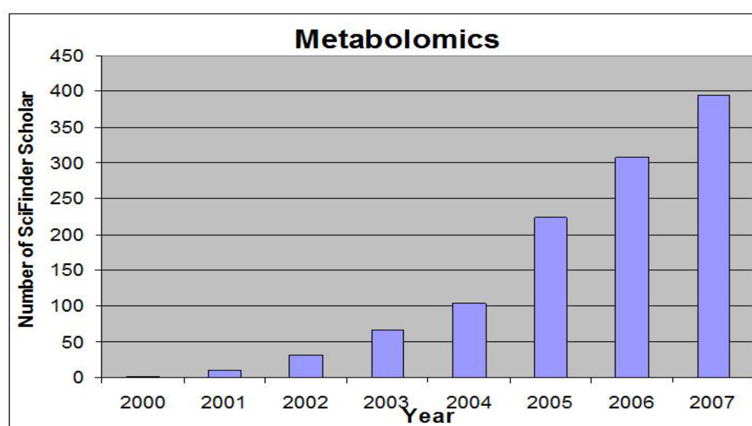

Fig. 3. SciFinder Scholar search results document the continuously growing research area of metabolomics based on the numbers of publications per year.

METABOLOMICS OR METABONOMICS

Before any discussion of metabolomics is initiated, some explanation: What is the difference between metabonomics and metabolomics, and when is the use of either term appropriate? The answer, unfortunately, is that, depending on whom you read or talk to, both terms may be appropriate in most cases and the distinctions are more a matter of historical usage than meaningful scientific definition. Metabonomics is defined as 'the quantitative measurement of the timerelated multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification' [44]. The actual term was devised from the Greek roots 'meta' (change) and 'nomos' (rules or laws) with reference to chemometric models that have the ability to classify changes in metabolism. While not expressly defined, the term metabolomics was indicated by Fiehn [16] to be the 'comprehensive and quantitative analysis of all metabolites.

Cases for the definition and differentiation of the terms target analysis, metabolite/metabolic profiling, metabolomics, and metabolic fingerprinting

have been made (Fiehn, 2002) with the suggestion that metabonomics has been erroneously used to describe comprehensive analysis of the metabolome, and that a more correct terminology for metabonomics would be metabolic fingerprinting. We have also seen a similar misuse of the term metabolomics for less comprehensive methods such as biomarker analysis. We propose that any technology of which the output is processed with pattern recognition software and without differentiation of individual metabolites should be termed 'metabolic fingerprinting' and not 'metabolomics' or 'metabonomics'.

METABOLOMIC TECHNOLOGIES

The genome and transcriptome consist of linear polymers of four nucleotides with highly similar chemical properties. The proteome is more complex, but is still based on a limited set of 22 primary amino acids. When surveying the metabolome, the chemical complexity is rather significant [4,8]. Lipid soluble chemicals that are normally found in membranes, polar chemicals from aqueous parts of the cell, acidic and basic ions, stable structures and structures that oxidize at the slightest mistreatment are included in the analysis. Until a universal measuring machine materializes, anyone working in metabolomics will have to make compromises [7]. The chemical diversity and complexity of the metabolome make it extremely challenging to profile the metabolome simultaneously. Currently, no single analytical technique provides the ability to profile the metabolome [8].

Standard methodology for metabolomics has not yet been established because a typical metabolomics experiment involves multiple steps: sampling, extraction, pretreatment, derivatization, analysis, data conversion and data mining. Each step is a potential source of experimental error. The complicated nature of data acquisition and subsequent analysis is the main reason why metabolomics is not yet widely used [20,21].

Sample preparation is perhaps the most underestimated part of metabolomics analyses. The composition and the quantity of metabolites detected depend to a large extent on the sample preparation chosen. In order to make measurements were reproducible, the conditions of the biological material should be as uniform as possible, in terms of environment (e.g., light, temperature, humidity, nutrients, time of sampling), ideally leaving the biological variation as the only inherited variation. For metabolomics applications, a fast, reproducible, unselective extraction method is preferred for detecting the wide range of metabolites that occur in a plant, avoiding unforeseen chemical modifications [22].

Currently, there is no method to extract all metabolites and measure them all. Additionally, there is always a risk that some metabolites will be lost along the way [7]. An extraction method and machine must be carefully

chosen to suit particular interests. However, various methodologies are available for extracting compounds from biological materials:

– liquid extraction (temperature- or pressure-assisted),
– solid-phase extraction (SPE),
– solid-phase microextraction (SPME).

In general, metabolites of interest are extracted by liquid extraction with one solvent, aqueous or organic, or with a combination of solvents (liquid-liquid extraction), implying that the type of metabolites extracted depends on the chemical properties of the solvent used.

For a certain class of metabolites, a particular solvent can be more adequate, yet not unique for its extraction. The choice of solvent should also be compatible with the analytical instruments used [22].

Metabolites are chemical entities and can be analysed by standard tools of chemical analysis such as nuclear magnetic resonance (NMR) [23], Fourier transform infrared (FT-IR) spectroscopy [24], and mass spectrometry often combined with chromatography [25,26]. The resolution, sensitivity and selectivity of these technologies can be enhanced or modified by coupling them to gas chromatography (GC) or liquid chromatography (LC) steps [3]. We can also use different methods such as high performance liquid chromatography (HPLC) with ultraviolet and photodiode array detection, capillary electrophoresis coupled to absorbance detection (CE/UV) or capillary electrophoresis coupled to laser induced fluorescence detection (CE/LIF). The techniques commonly used in different metabolomic strategies are shown in Figure 4. Selection of the most suitable technology is a compromise between speed, selectivity and sensitivity. Tools such as NMR are rapid and selective, but have relatively low sensitivity. Other methods such as capillary electrophoresis coupled to laser induced fluorescence detection, although highly sensitive, are deficient in selectivity. Methods such as GC/MS and LC/MS offer good sensitivity and selectivity, but also relatively longer analysis times. Generally the technology platform of choice depends on the type of sample to be analysed. Biofluid can be analysed by nuclear magnetic resonance (NMR) with little or no sample preparation, whereas tissues and cells from animal, plant or microbial systems necessarily require some sample pretreatment.

For metabolite target analysis and metabolite profiling, studies can be geared to monitor specific metabolites by selective analysis and well-developed calibration methods.

As suggested in Figure 1, this can be achieved by conventional techniques, such as separation by GC or high performance liquid chromatography coupled to a suitable detection system [3,6,27].

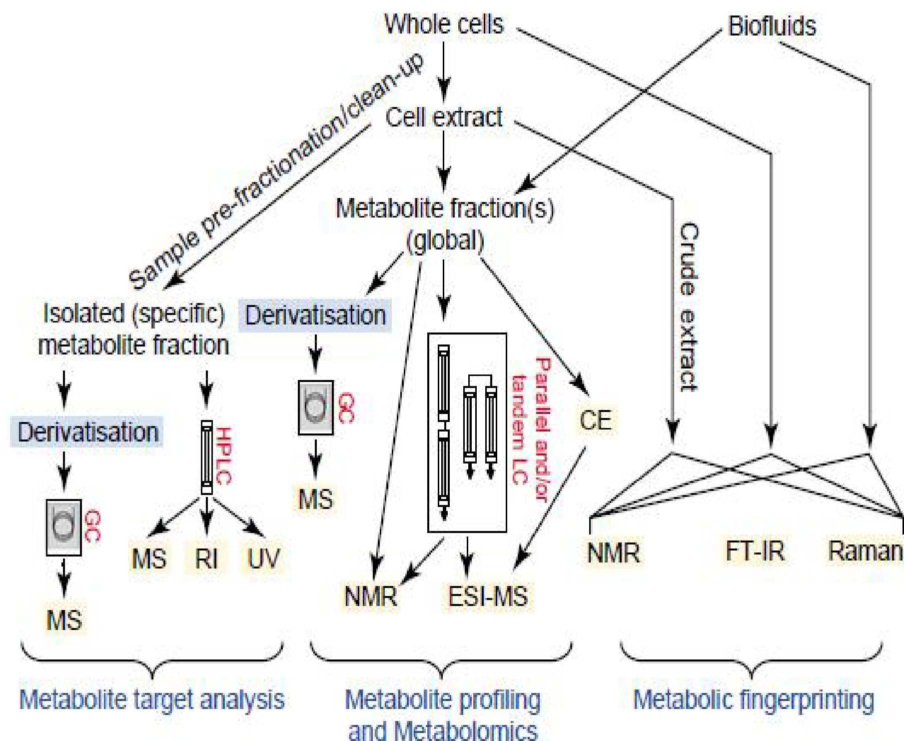

Fig. 4. The techniques commonly used in metabolomic strategies [3].

APPLICATION OF QSRR IN METABOLOMICS

One of the initial steps of metabolomic analysis is metabolite separation. However, little information from HPLC, usually employed for the separation, is utilized in metabolomics. Meanwhile, prediction of the retention time for a given metabolite, combined with routine MS/MS data analysis, could help to improve the confidence of metabolite identification.

The ultimate goal is to understand and to predict the behaviour of complex systems, such as plants, by using the results obtained from data mining tools for subsequent modeling and simulation. We can try to apply quantitative structure-retention relationships (QSRR) that predict a given physicochemical or biological property of metabolites and try to identify unknown substances or estimate their relative bioactivities. QSRR are statistically derived relationships modeling the analyte (metabolite) retention as a function of properties (descriptors) related to the analyte molecular structure and the physicochemical properties of both the stationary and mobile phase. The presently applied methodology and goals of QSRR studies are schematically presented in Figure 5. To undertake a QSRR study, one needs a set of quantitatively comparable retention parameters for a

sufficiently large series of analytes and a set of their structural descriptors. Through the use of computer-aided statistical chemometric techniques, retention parameters are characterized in terms of various analyte descriptors. If statistically significant and physically meaningful QSRR are obtained, then they can be applied to: identify the most useful structural descriptors in regard of properties; to predict of chromatographic retention parameters for a new analyte and to identify unknown analytes (for example metabolites); to gain insight into molecular mechanism of separation operating in a given chromatographic system; to quantitatively compare separation properties of individual types of chromatographic columns; to evaluate properties other than chromatographic physicochemical properties of analytes, such as lipophilicity and dissociation constants; to estimate relative biological activity within sets of drugs and other xenobiotics as well as material properties of members of a family of chemicals [28-32].

To obtain reliable QSRR, appropriate input data are necessary and a stringent statistical analysis must be carried out. Chromatography can readily provide large amounts of proper input data because in a chromatographic analysis conditions may be kept constant for many separated analytes. Thus, analyte structure can be the single variable in the system.

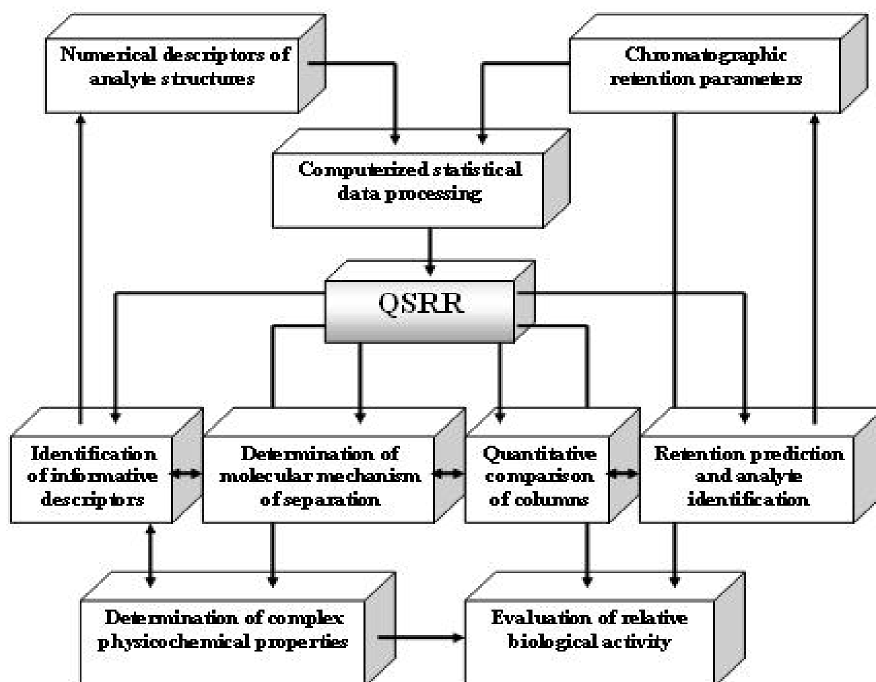

Fig. 5. Methodology and goals of QSRR studies [29].

In Figure 6 the flow-chart of the entire task of model-building is presented.

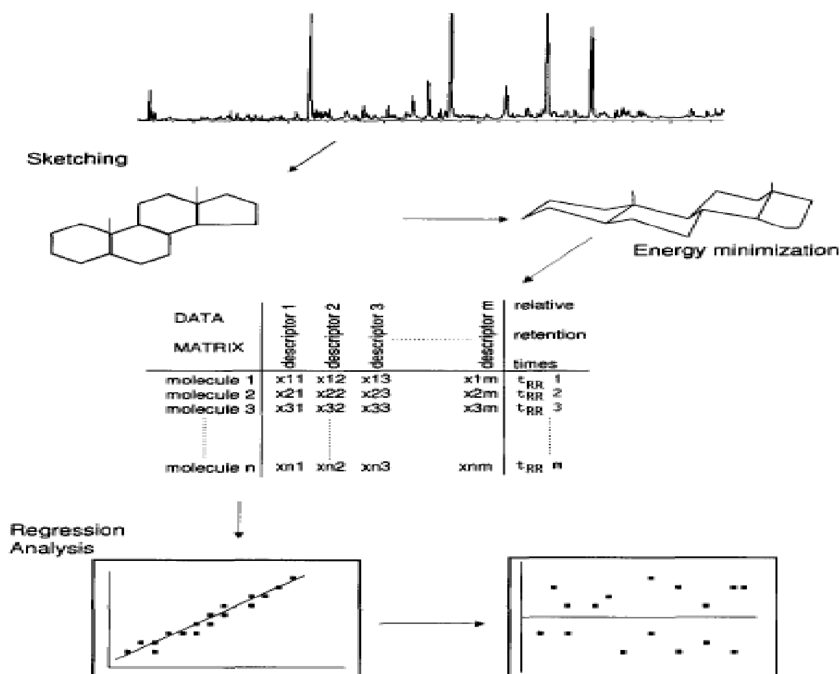

Fig. 6. Flow-chart of the procedure for a QSRR model generation [28].

CHEMOMETRICS

No review of metabolomics technology can be complete without at least some mention of chemometrics. In metabolomics, raw data from instrumental analysis, such as chromatogram, spectra, *etc.*, should be converted to appropriate data matrices. Only essential components should be selected from the raw data for subsequent mining steps (Fig. 7). In metabolomics, identification of differences between samples has normally involved different multivariate tools such as principal component analysis (PCA), hierarchical cluster analysis (HCA), discriminate analysis (DA), or artificial neural networks (ANN) [21,33,34].

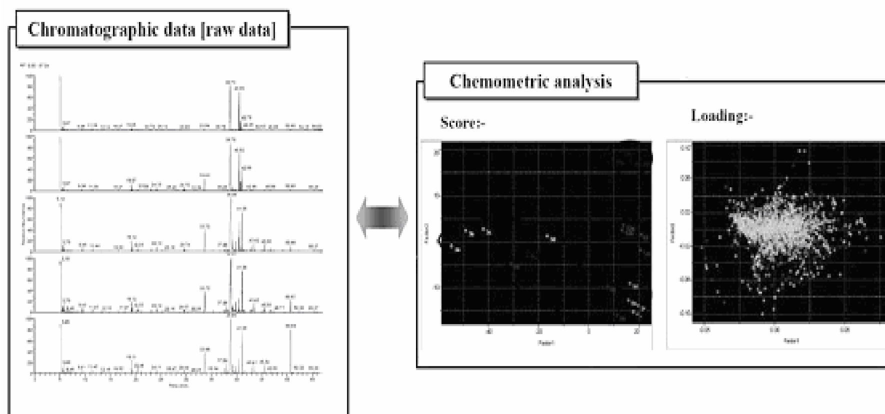

Fig. 7. General schematic of data mining [21].

APPLICATIONS OF METABOLOMICS

Systems biology requires the integration of biology, medicine, mathematics and chemistry with biostatistics and bioinformatics to transform complex and diverse datasets into useful knowledge, and systems biology approaches are being increasingly applied in the fields of microbiology, and plant and medical sciences (Fig. 8). We focus here on analytical sciences in medical systems biology; in particular, on the status and challenges for metabolomics and proteomics. Data pre-processing before the important steps of biostatistics, bioinformatics and modeling is briefly considered and notable references for data evaluation and integration steps are given [27].

Plant metabolomics is still a field in its infancy, but the opportunities are almost endless. Metabolomics offers the unbiased ability to characterize and differentiate genotypes and phenotypes based on metabolite levels [7]. Biological systems can be examined at several levels, including cellular, tissue, organ, or even whole organism in response to environmental stressors [36]. The following is just a subset of the possible applications, like characterization of metabolism, identification of regulated key sites in networks [7].

Metabolomics, or large-scale metabolite pathways (Fig. 9), has a potentially important role as a complement to functional genomics and proteomics in discovery of biomarkers for different diseases, signaling pathway relationships, disease mechanisms, pharmaceutical drug development and drug effects [36,37].

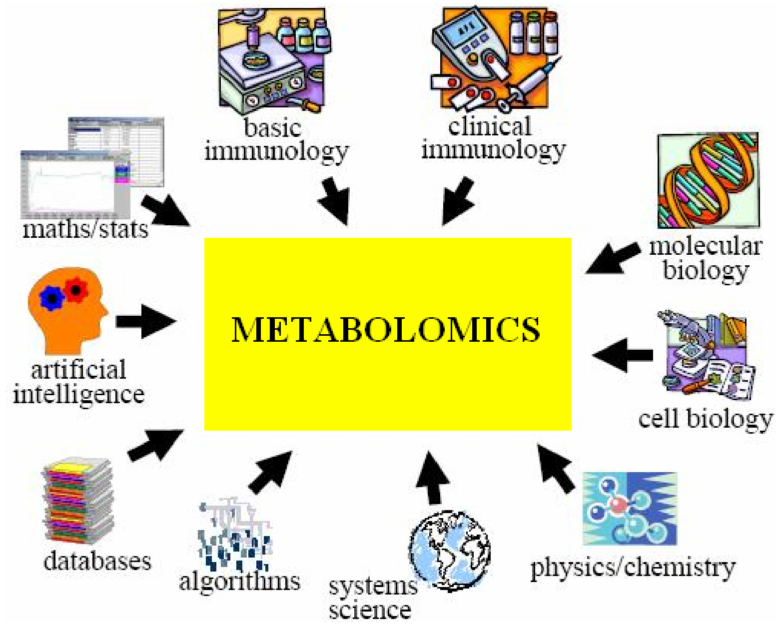

Fig. 8. Applications of metabolomics.

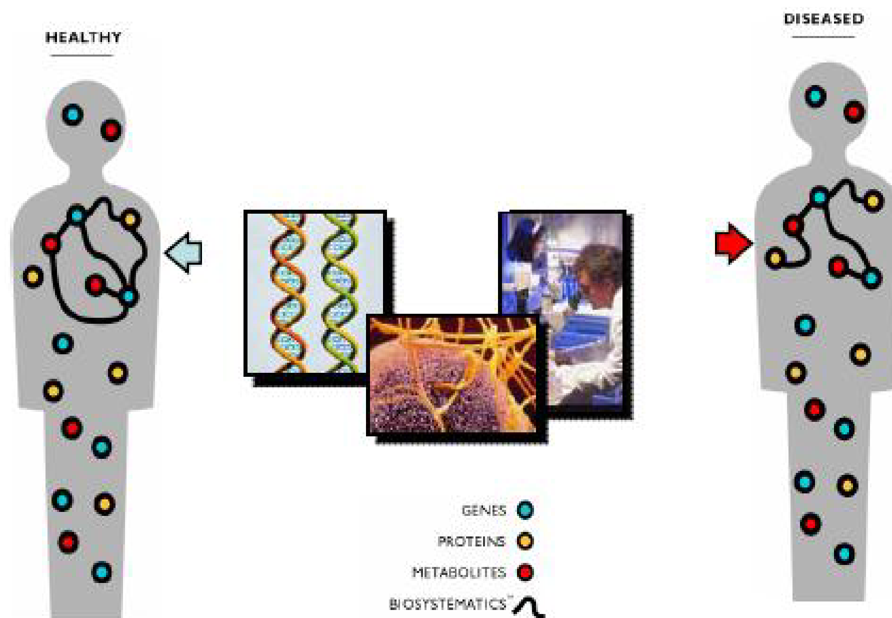

Fig. 9. Schematic of difference metabolite pathways for a healthy and a diseased individual.

Metabolomics has been widely adopted in toxicology but is relatively new in human nutrition. This new area of research has found applications also in food assessment and safety, technological advances in analytical chemistry [38].

Another field of interest of metabolomics is environmental. Some of the most significant efforts in the area of metabolic profiling have been made in the area of the botanical sciences [25,39-43].

Several recent publications demonstrate quite convincingly the power of clinical metabolomics. While earlier studies on inborn errors of metabolism focused on molecular identification of the relevant metabolic pathways [44], a more recent effort details methods for rapid identification of inborn errors of metabolism using pattern recognition techniques [45]. Even more compelling was recent work demonstrating that metabolomics could be used for the rapid and non invasive assessment of the severity of coronary heart disease (CHD) in a clinical population [46]. The same group demonstrated a relationship between serum metabolic profiles and hypertension [47]. Beyond disease diagnostics, metabolomics has also been shown to be an effective tool for assessing lifestyle markers of health, particularly related to nutritional variation [48].

Other biomedical applications of metabolomics include the identification of a unique bimolecular associate with a parasite infection in mice [49] and metabonomic assessment of adrenal lipids in the hypoxic neonatal rat [50].

FUTURE PERSPECTIVE AND CONCLUSION

Metabolomics is a burgeoning science that brings together analytical technology, genomics and computation, and lies at the core of systems biology. Major areas of development will involve improving the sensitivity, universality and discrimination of instruments, and this will involve new approaches and better deconvolution [51]. New analytical techniques need to be developed that can increase the amount of the metabolome that can be sampled in each step.

Structural, or chemical, identification of the many uncharacterized metabolites is still a very important and yet unsolved problem [51]. Therefore, it will also be beneficial to create automated procedures that can identify unknown peaks by combining information from MS fragmentation patterns, isotope ratios, exact masses, structure generators, and biochemical databases. Once the analytical and extraction procedures have been perfected and the bioinformatics tools developed, there are endless metabolic linkage networks that need to be defined.

On the other hand, in combination with other techniques such as proteomics and transcriptomics, it is hoped that a general picture of

metabolism can be formed. On the horizon there are computer models of cell biochemistry incorporating all levels of gene expression. It is envisioned that such 'virtual cells' will help explain and illustrate many of the more challenging details of molecular cellular systems such as metabolite channeling, compartmentalization and transport. It will also has a impact on the ability to use plants as bioreactors producing tomorrow's medicines and chemical stocks [8].

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