# Metabolome

The metabolome refers to the complete set of small-molecule chemicals found within a biological sample. The biological sample can be a cell, a cellular organelle, an organ, a tissue, a tissue extract, a biofluid or an entire organism. The small molecule chemicals found in a given metabolome may include both endogenous metabolites that are naturally produced by an organism (such as amino acids, organic acids, nucleic acids, fatty acids, amines, sugars, vitamins, co-factors, pigments, antibiotics, etc.) as well as exogenous chemicals (such as drugs, environmental contaminants, food additives, toxins and other xenobiotics) that are not naturally produced by an organism. In other words, there is both an endogenous metabolome and an exogenous metabolome. The endogenous metabolome can be further subdivided to include a "primary" and a "secondary" metabolome (particularly when referring to plant or microbial metabolomes). A primary metabolite is directly involved in the normal growth, development, and reproduction. A secondary metabolite is not directly involved in those processes, but usually has important ecological function. Secondary metabolites may include pigments, antibiotics or waste products derived from partially metabolized xenobiotics. To qualify as a metabolite, or to be considered to be part of the metabolome, a small molecule must typically have a molecular weight <1500 Da. This means that molecules such as glycolipids, polysaccharides, short peptides (<14 amino acids) and small oligonucleotides (<5 bases) can be regarded as metabolites or constituents of the metabolome. On the other hand, very large macromolecules such as proteins, messenger RNA, ribosomal RNA, microRNA and DNA are definitely not metabolites and are not considered to be part of the metabolome. The study of the metabolome is called **metabolomics.**

Metabolomics has developed into a major tool in functional genomics and plant systems biology. The various methods used for metabolomic analysis will be discussed from the analytical methods back to the preanalytical phase and the biological experiment. Particularly aspects of the preanalytical phase of the analysis is dealt with, including the risks of artefact formation with the various commonly used solvents. Metabolomics is like a snap shot, and conclusions from dynamic systems must be drawn with great care as demonstrated with a biosynthetic study of salicylate in Catharanthus roseus cell cultures. With the ever-increasing speed of sequencing of genes, the emphasis of research is moving towards the function of genes. The focus was first on proteomics, as there is a logical link between the sequence of a gene and the amino acid sequence of a protein. However, such a sequence does not give much information about the function of the protein. For most proteins the function is best reflected by the phenotype. The characterization of the phenotype thus has become an important objective, and here metabolomics comes into the picture. Once a gene sequence is determined it stands for ever as a fact that can be used over and over again. The same applies for amino acid sequences. By proper storage of these results, data mining becomes possible in any place at any time. Analytical methods The ultimate goal of metabolomics is the qualitative and quantitative analysis of all metabolites in an organism. A quite ambitious goal, which none of the methods applied at present can meet. One has to deal with a large number of compounds with totally different chemical properties, which might be unstable and present in a wide range of quantities. Concerning the number, our estimation is that an organism contains about the same number of compounds as it has genes.

## Metabolomics And Metabonomics.

Metabolomics is the scientific study of chemical processes involving metabolites. Specifically, metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles. The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. mRNA gene expression data and proteomic analyses reveal the set of gene products being produced in the cell, data that represents one aspect of cellular function. Conversely, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to provide a better understanding of cellular biology.
Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification".

The term “omics” refers to any type of specific study that provides collective information on a biological system. Representative omics includes genomics, proteomics, and metabolomics, and new omics is constantly being added, such as lipidomics or glycomics. Each omics technique is crucial to the understanding of various biological systems and complements the information provided by the other approaches. The main strengths of metabolomics are that metabolites are closely related to the phenotypes of living organisms and provide information on biochemical activities by reflecting the substrates and products of cellular metabolism.

The transcriptome does not always correlate with the proteome, and the translated proteome might not be functionally active. Therefore, their changes do not always result in phenotypic alterations. Unlike the genome or proteome, the metabolome is often called the molecular phenotype of living organisms and is easily translated into biological conditions and disease states. Here, we review the general strategies of mass spectrometry-based metabolomics. Targeted metabolome or lipidome analysis is discussed, as well as nontargeted approaches, with a brief explanation of the advantages and disadvantages of each platform. Biomedical applications that use mass spectrometry-based metabolomics are briefly introduced.

Metabolites are typically low molecular weight biomolecules. They are the building blocks of the genome, proteome, and cell membranes and play key roles in biology as signalling molecules, energy sources, and metabolic intermediates. Their levels provide integrative information on biological functions and define the phenotypes of biological systems in response to genetic or environmental changes. Metabolite analysis involves the use of different analytical techniques to identify and quantify intracellular and extracellular metabolites. Successful metabolomic studies rely on proper sample preparation, innovative instrumentation, and bioinformatic tools. Metabolites show broad variations in physicochemical properties, such as polarity, acidity, and volatility. Thus, it is difficult to simultaneously analyze a broad range of metabolites. Metabolomics has developed along with innovative analytical instrumentation. Various analytical techniques have all been applied for metabolite analysis, including mass spectrometry (MS), nuclear magnetic resonance (NMR), Fourier transform infrared (FT-IR) spectroscopy, and Raman spectroscopy. (fig 2.)

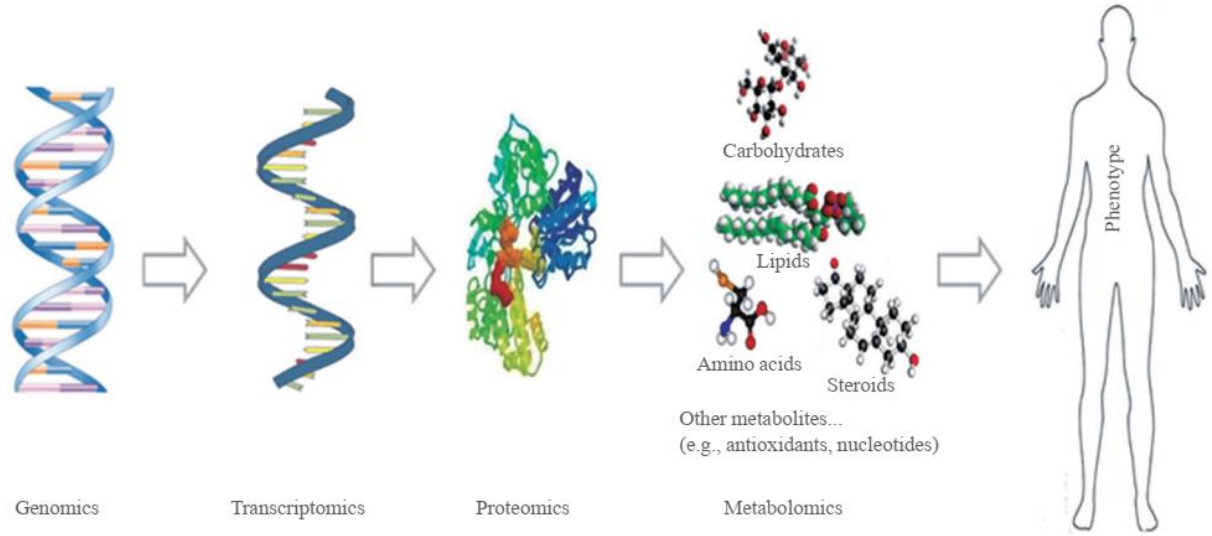

Fig. 1 Conventional omics studies in biology. There are various types of omics, from genomics to metabolomics, and new omics studies are being constantly added, such as lipidomics or glycomics.

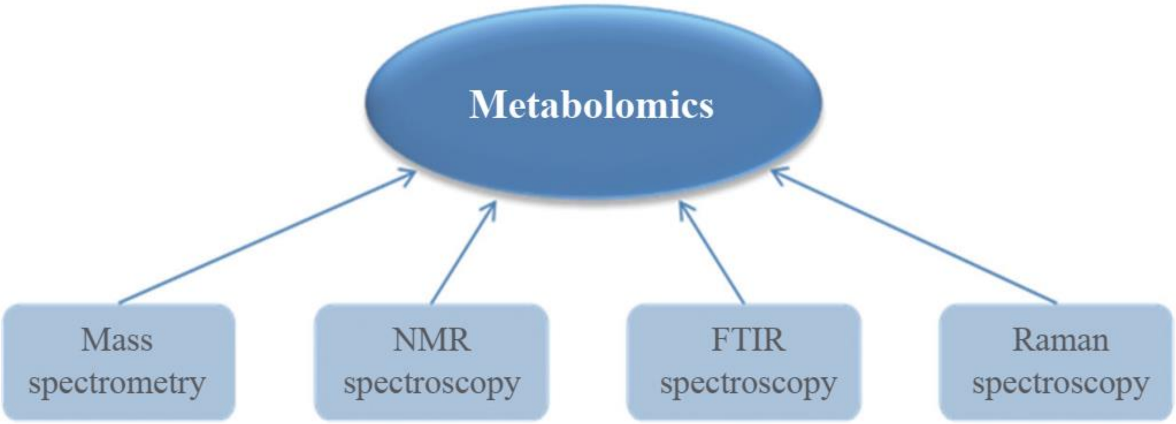

Fig 2. Instrumentation for metabolomics. Analytical instruments for metabolomics should be able to detect various kinds of metabolites present in biological systems.

NMR, **nuclear magnetic resonance**; FTIR, **Fourier transform infrared**.

These spectroscopic methods are generally applied to profile metabolic alterations due to their holistic nature, and absorptions at specific wavelengths are sometimes able to provide important clues for the identification of observed metabolic components. However, the sensitivity and selectivity of FT-IR spectroscopy and Raman spectroscopy are lower than those of other methods. NMR spectroscopy, which is a commonly used strategy in metabolomics, is also a rapid and non-destructive method with minimal sample preparation. Chemical shifts in NMR spectra can provide crucial evidence for the identification of specific metabolites. However, NMR requires at least millimolar concentrations of metabolites in samples and its sensitivity is lower than that of MS. Another major disadvantage of NMR is its poor dynamic range. On the other hand, MS remains the most favourable technology for metabolomics due to its wide dynamic range and good sensitivity (nM). In addition, MS is able to detect a diverse range of molecular species comparing to other strategies.

The word origin is from the Greek *μεταβολή* meaning change and *nomos* meaning a rule set or set of laws. Historically, the metabonomics approach was one of the first methods to apply the scope of systems biology to studies of metabolism. There has been some disagreement over the exact differences between 'metabolomics' and 'metabonomics'. The difference between the two terms is not related to choice of analytical platform: although metabonomics is more associated with NMR spectroscopy and metabolomics with mass spectrometry-based techniques, this is simply because of usages amongst different groups that have popularized the different terms. While there is still no absolute agreement, there is a growing consensus that 'metabolomics' places a greater emphasis on metabolic profiling at a cellular or organ level and is primarily concerned with normal endogenous metabolism. 'Metabonomics' extends metabolic profiling to include information about perturbations of metabolism caused by environmental factors (including diet and toxins), disease processes, and the involvement of extragenomic influences, such as gut microflora. This is not a trivial difference; metabolomic studies should, by definition, exclude metabolic contributions from extragenomic sources, because these are external to the system being studied. However, in practice, within the field of human disease research there is still a large degree of overlap in the way both terms are used, and they are often in effect synonymous.

## NONTARGETED METABOLOMICS

Nontargeted metabolomics aims to profile the entire metabolome present in cells, biofluids, or tissues. Nontargeted metabolomics measures as many metabolites as possible to compare biological samples and is useful for hypothesis generation. However, the greatest bottleneck in nontargeted metabolomics is the identification of unknown features. Sample preparation and proper analytical instrumentation are important to detect the maximum number of metabolites.

## CONCEPTS AND FEW APPLICATIONS OF METABOLOMICS

- Toxicity assessment/toxicology. Metabolic profiling (especially of urine or blood plasma samples) detects the physiological changes caused by toxic insult of a chemical (or mixture of chemicals). In many cases, the observed changes can be related to specific syndromes, e.g. a specific lesion in liver or kidney. This is of particular relevance to pharmaceutical companies wanting to test the toxicity of potential drug candidates: if a compound can be eliminated before it reaches clinical trials on the grounds of adverse toxicity, it saves the enormous expense of the trials.

- Functional genomics. Metabolomics can be an excellent tool for determining the phenotype caused by a genetic manipulation, such as gene deletion or insertion. Sometimes this can be a sufficient goal in itself—for instance, to detect any phenotypic changes in a genetically modified plant intended for human or animal consumption. More exciting is the prospect of predicting the function of unknown genes by comparison with the metabolic perturbations caused by deletion/insertion of known genes. Such advances are most likely to come from model organisms such as *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. The Cravatt laboratory at The Scripps Research Institute has recently applied this technology to mammalian systems, identifying the *N*-acyltaurines as previously uncharacterized endogenous substrates for the enzyme fatty acid amide hydrolase (FAAH) and the monoalkylglycerol ethers (MAGEs) as endogenous substrates for the uncharacterized hydrolase KIAA1363.
- Nutrigenomics is a generalised term which links genomics, transcriptomics, proteomics and metabolomics to human nutrition. In general, a metabolome in a given body fluid is influenced by endogenous factors such as age, sex, body composition and genetics as well as underlying pathologies. The large bowel microflora are also a very significant potential confounder of metabolic profiles and could be classified as either an endogenous or exogenous factor. The main exogenous factors are diet and drugs. Diet can then be broken down to nutrients and non-nutrients. Metabolomics is one means to determine a biological endpoint, or metabolic fingerprint, which reflects the balance of all these forces on an individual's metabolism. Environmental metabolomics is the application of metabolomics to characterise the interactions of organisms with their environment. This approach has many advantages for studying organism–environment interactions and for assessing organism function and health at the molecular level. As such, metabolomics is finding an increasing number of applications in the environmental sciences, ranging from understanding organism responses to abiotic pressures, to investigating the responses of organisms to another biota. These interactions can be studied from individuals to populations, which can be related to the traditional fields of ecophysiology and ecology, and from instantaneous effects to those over evolutionary time scales, the latter enabling studies of genetic adaptation.

## CONCLUSION

Metabolomics is a relatively new omics strategy in biomedical research and is gaining attention as a tool for the discovery of biomarkers for disease diagnosis and assessment of disease development and prognosis. In addition, metabolomics may provide evidence for the identification of candidates for novel therapeutic interventions and the elucidation of disease mechanisms. The successful application of metabolomics requires integrated efforts from analytical chemistry, biology, medicine, and biostatistics

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