Reading the metabolic fine print

The application of metabolomics to diagnostics, drug research and nutrition might be integral to improved health and personalized medicine

Barely a decade has passed since the term metabolomics—the systemic analysis of the chemical 'fingerprints' of specific cellular processes—made its debut in the scientific literature (Oliver *et al*, 1998), but its application in medicine goes back to the Middle Ages, when doctors used to diagnose diseases by the colour and smell of the patient's urine. However, modern-day metabolomics started in the late 1960s with the development of chromatographic separation techniques that allowed the detection and analysis of various compounds in complex mixtures. The first publication that described the use of these techniques appeared in the early 1970s and analysed the content of urine and breath (Pauling *et al*, 1971).

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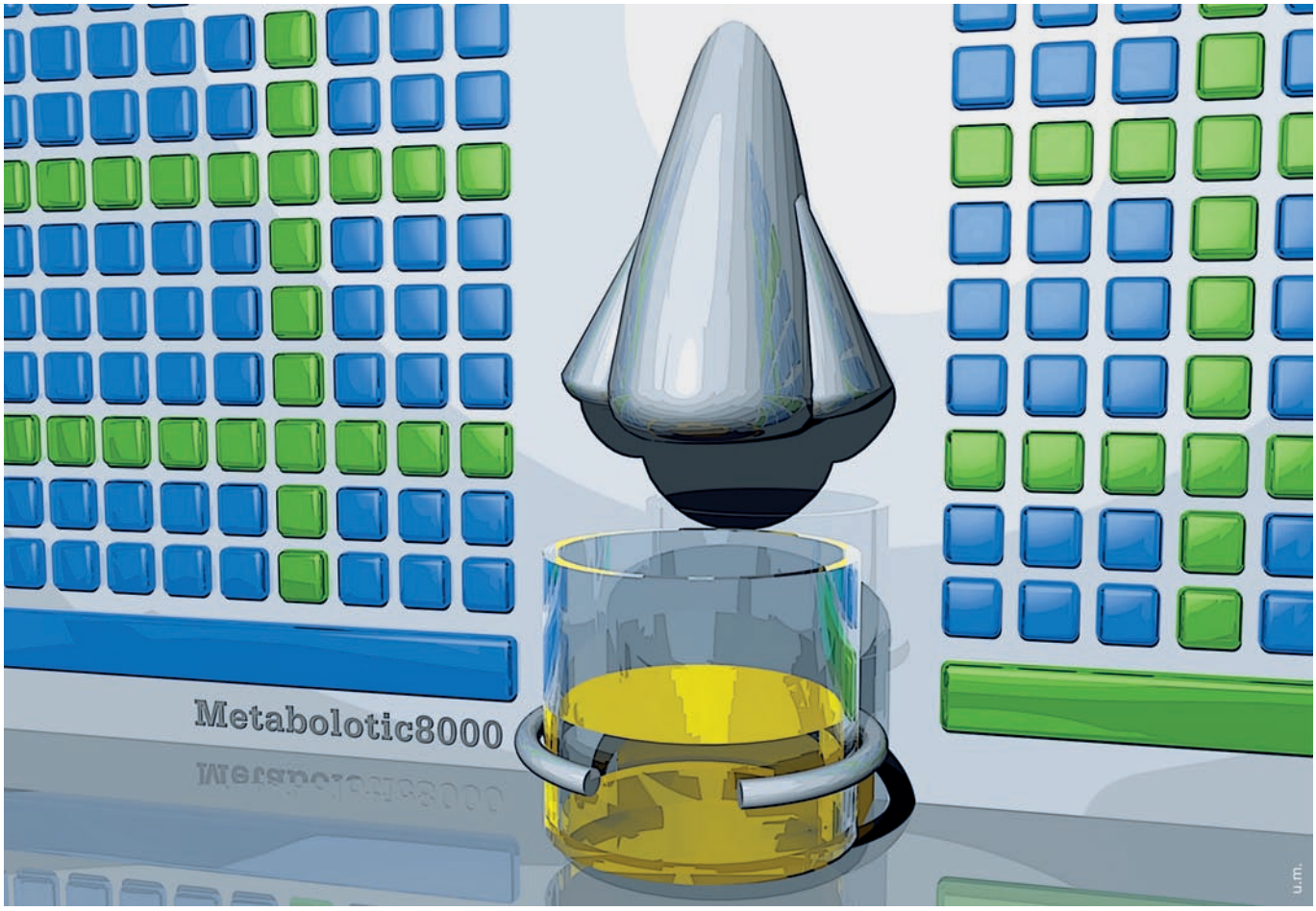
Metabolomics is a powerful diagnostic tool that provides a snapshot of an organism's metabolic state through the systemic analysis of its metabolites. Any deviations from the normal range indicate possible pathological aberrations; yet, the big question remains whether metabolomics has the necessary predictive power to become a tool for clinical use, in particular, in personalized medicine.

The main point is that an organism's metabolome—its full and unique cocktail of metabolites—changes in response to stimuli and environmental conditions. This is in contrast to its genome, representing the organism's sum total of genes, and its transcriptome, which is the varying range of messenger RNA molecules expressed by those genes in each cell type. Except when mutations occur, both the genome and transcriptome are fixed for life.

It is this strong interaction between the metabolome and the environment that unites the various definitions of metabolomics. It also includes a definition of the sister term metabonomics, which describes the complete metabolic state of an organism, and was coined by one of its pioneers, Jeremy Nicholson, Chair in Biological Chemistry at Imperial College, London, UK. "Metabolomics has about 20 published definitions, conflicting but all analytical, all about measuring some stuff in some other stuff," Nicholson said. More specifically, the overall aim is to measure the small molecules in biological samples—be they urine or blood samples from a patient, or a small cluster of yeast cells. The molecules in question are involved in metabolic processes occurring within the organism and might be small proteins, lipids or other organic compounds; indeed, lipidomics is a subfield of metabolomics. Where metabolomics and metabonomics differ, however, is that although metabolomics is concerned with measuring the metabolites in one sample, which might be derived from only one cell type, metabonomics is the global study of the systems that regulate metabolism, including variations over time.

The measurement process itself is a huge technological challenge given the large and diverse range of metabolites present in even a simple organism. Bacteria have at least 600 metabolites, whereas most plants produce an estimated 200,000 or more (Oldiges *et al*, 2007). Even single-celled eukaryotes such as yeast have a range of different cellular compartments, each with a unique set of metabolites. Yet, even this complexity pales in comparison with multicellular eukaryotes, which consist of many cell types that produce different metabolites in varying quantities at different times. "In humans there are 500 different cell types, with therefore 500 different metabolomes interacting in space



and time," Nicholson explained. The specific metabolome also varies, in part determined by epigenetic factors that cause varying levels of gene expression.

Metabolomics rests on a combination of analytical techniques—mainly mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy—to identify the compounds and their quantities, coupled with mathematical or statistical modelling to identify significant peaks and troughs.

Given the diversity of metabolic compounds, no single technique is able to obtain all the information down to the precise concentrations of each molecule, but this is not usually necessary. In fact, the main objective of metabolomic analysis is to reduce the cost and time of analytical techniques by analysing a range of metabolites of interest for a given application. One of the most promising developments is the Orbitrap™ mass spectrometer (Scigelova & Makarov, 2006), invented by Alexander Makarov, the Director of Global Research for Life Sciences Mass Spectrometry at Thermo Fisher Scientific (Cheshire, UK). The Orbitrap contains two electrodes—a barrel-shaped cylinder with a spindle inside it—that form an electrostatic field. The ionized sample is injected tangentially into the electric field and becomes trapped as its electrostatic attraction to the inner electrode is balanced by centrifugal forces. The particles are thus separated into rings according to their mass-to-charge ratio, and the rings oscillate laterally along the Orbitrap tube with harmonic periods. Ultimately, the measurement of these periods by conventional fast Fourier transformation yields the atomic mass of the components and their intensities. "The Orbitrap technology, which appeared one or two years ago, allows higher resolution mass spectrometry, and we are now ready to accelerate the process of discovery to understand the chemical nature of metabolites," commented Eric Ezan, leader of the Metabolome, biomarkers and therapeutic proteins group at the Institute de Biologie et de Technologies de Saclay in Gif-sur-Yvette, France. Ezan's group studies human and animal metabolomes, looking for biomarkers associated with metal toxicity and the metabolism of drugs.

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Mass spectroscopy uses electric fields to separate compounds, whereas NMR relies on magnetic fields. Quantum mechanical effects mean that only atomic nuclei with odd numbers of protons are perturbed by the magnetic field, including hydrogen, which is fortunately present in almost every metabolite. Moreover, molecules respond differently to the resonating magnetic field according to the electron density distribution around the nuclei, which generates

highly refined spectral patterns that yield a great deal of data about the metabolites in a sample. NMR provides the most comprehensive information about a wide range of metabolites and, as a crude generalization, is preferred for large-scale applications. However, it is less sensitive than mass spectrometry, which is the method of choice for analysing complex samples containing many metabolites in varying concentrations.

The chemical analysis is difficult enough, but the mathematical modelling needed to make useful deductions is even more challenging, owing to the colossal amount of information produced. In fact, it is often the combination of many measurements that yields a unique signature that can be associated with a particular disease or response to a drug. Such a signature is a pattern of spectral peaks and troughs of varying shape that also differ in intensity and duration between different metabolites. When a candidate drug is given to a laboratory mouse, for example, some metabolites will show a simple relationship—their concentration in the blood might rise to a sharp peak and then decline back to their normal level—but the behaviour of other metabolites can be more complex—perhaps oscillating with decreasing amplitude and eventually settling down as the impact of the drug fades. There are also many intermediate responses that defy precise definition, yet, when combined, yield a unique signature that defines a specific event such as the reaction to a drug.

The principle is the same as that involved in the recognition of a handwritten signature or a face based on a characteristic combination of various features. For example, a particular drug might cause the production of three metabolites, the levels of which increase at various times but always in the same order and to roughly the same magnitude. An 'abnormal' response would then be one in which the increase in one metabolite occurred out of turn or to an abnormally high level.

One of the most powerful methods for analysing metabolic data has come from the Consortium of Metabonomic Toxicology (COMET), which includes several large pharmaceutical companies and is led by Imperial College. The initial objective of COMET was to conduct physiological experiments on rats to build a database of metabolic responses at successive time intervals. This work was used to characterize and identify the various responses of individual animals to the same drug or toxin using both NMR and mass spectrometry, followed by the application of probability theory and pattern-matching methods to identify unique response signatures. This led to a sequel, COMET II, described by Nicholson as a metabolic expert system for drug toxicology screening.

As Nicholson pointed out, drug companies hate unpredictability, such as when the response to a given drug varies significantly, even between genetically identical individuals. All too often such unpredictability fails to show up until the early clinical testing phase of drug development, and it therefore contributes to the continuing failure of the quest to reduce drug development costs and times; currently, only 11% of drugs that proceed into development end up on the market (Kola & Landis, 2004).

There have been many false dawns in drug development during the past few decades, and many analysis platforms—such as early *in silico* systems to narrow the search for candidate molecules—have withered away without having any noticeable impact on the process. But, Paul Watkins, Director of the Clinical Research Centre at the University of North Carolina, Chapel Hill, USA, thinks that metabolomics will be different: "I believe we are on the verge of great advances in understanding disease and making better drugs thanks to metabolomics, even though there has been little impact to date," he said.

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The reason for his optimism lies in the ability of mathematical analysis platforms such as COMET II to associate complex time-stamped metabolic signatures with variations in responses to a drug, even among genetically identical individuals. Of course, many variations in responses to a drug are pharmacogenetic—resulting from genetic differences—but, as Nicholson pointed out, often the differences are extra-genomic and are therefore undetectable using genomics or even proteomics, which cannot readily take into account environmental factors. "One thing interesting to us is that there is a lot of variation in toxicological and metabolic responses due to variation in gut microbes," he said. "If you take 100 rats that are genetically near identical, in the same cage, and give them a particular dose, you might get 30 really sick, 20 quite sick, and 20 get nothing at all."

Variations in gut microbial populations and numerous metabolizing enzymes secreted by the gut cause these differences, Nicholson explained. "These enzymes didn't evolve to metabolise drugs, of course, but to metabolise natural products and also bacterial toxins, and they come from the gut. So gut microbes and diet are constantly stimulating your drug metabolising enzymes, switching them on and off," he said. "Dietary gut microbe status is a hugely important factor in the biovariability of a drug, and traditional pharmacogenetics completely ignores that." In practice, it is not possible to assess this variability by studying the gut microbial populations directly, but the various signatures can be detected indirectly through the effects on metabolites. In any case, these externally caused individual differences in drug response further highlight the importance of new approaches to analyse the metabolomic state of a patient before prescribing.

Yet, Watkins conceded that modern metabolomics has yet to arrive in the clinic or to have an impact on drug discovery. However, his own laboratory has just completed a project suggesting that it will soon be possible to identify individuals likely to suffer side effects from certain drugs—in this case the popular painkiller paracetamol. "We have done a study that showed that the urine metabolome could predict who will develop mild liver injury when treated for one week with acetaminophen [paracetamol]," Watkins explained. "To my knowledge, this study, which is unpublished, is the only successful application to date of metabolomics in a clinical trial to detect susceptibility."

In addition to drug development and improved treatments, metabolomics also offers the exciting possibility of disease detection far earlier than present methods in toxicology and diagnostics allow. Tools such as COMET can detect the chemical signature associated with a particular disease without needing to know the underlying pathways or metabolites. The key, of

course, lies in associating the early stages of a disease with the combinatorial patterns of many metabolites as revealed, for example, in their NMR spectra.

Metabolomics offers an equally exciting third application in measuring food quality and nutritional value, according to Ezan. "We're trying to go into the food industry, because we may find a lot of applications. For example, many French companies import food from Asia, and China is now a very large provider. We need to be able to assess the quality, especially as imported products can be mixed with domestic ones." Ezan cited the recent scandal in China in which baby milk formula products were found to be contaminated with melamine. More than 5,000 Chinese babies were hospitalized, and the European Union acted quickly to ban imports of baby foods that contain Chinese milk. Ezan is certain that metabolomics could have been used to screen these products effectively: "[y]ou would have been able to see whether melamine was a component of a product containing baby milk," he said.

More positively, metabolomics has the potential to improve the nutritional value of food. The food company Nestlé (Vevey, Switzerland) is already pursuing metabolic research to study the impact of various diets at its Nutrition Research Centre in Lausanne, Switzerland. One aim is to collect data sets on specific metabolites, the role of which is already known, and the other, similar to the COMET approach, involves analysing time-series of large numbers of unknown metabolites. This might not immediately translate into improved food products, but the research by food companies shows their expectations of metabolomics.

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When it comes to developing new foods, rather than assessing the impact on people, the focus of metabolic research switches to the two starkly contrasting nutritional problems of the world: malnutrition and over-consumption. Although these problems require different solutions, both will involve the application of metabolomics to modify foods, according to Robert Hall at the Plant Research International Centre at Wageningen University in the Netherlands. In a recent review, Hall and colleagues argued that metabolomics will shift the focus of food production away from yield towards quality and healthiness, pointing out that relatively minor genetic changes in plants can have a measurable impact on metabolism (Hall *et al*, 2008).

After more than 30 years, metabolomics finally seems to be coming of age. There are high expectations for an impressive range of applications in both diagnostics and drug treatment and discovery, as well as in nutritional research, owing to the ability of metabolomics to analyse cellular events that are not detectable at the level of genomics or proteomics. It remains to be seen, however, whether and when this potential will be realized, particularly as metabolomics uncovers a new level of complexity in the molecular events that take place in cells, organs and whole organisms.

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