Metabolomics: What's Happening Downstream of DNA

The decoding of the human genome gave rise to genomics and proteomics—"global" studies of genes and proteins, respectively—which are often touted in terms of their enormous clinical potential. In the midst of a growing shift toward translational studies in today's biomedical research scene, yet another "-omics" science has come to the fore. The science is called metabolomics, and its protagonists say it offers a cheap, rapid, and effective way to diagnose illness and monitor patient therapy.

Metabolomics is the study of metabolite profiles in biological samples, particularly urine, saliva, and blood plasma; scientists are interested in all, rather than some, of the metabolites in a given sample. Metabolites are the by-products of metabolism, which is itself the process of converting food energy to mechanical energy or heat. The number of different metabolites in the human is unknown; estimates range from a low of 2,000–3,000 to a high of around 20,000, compared to an estimated 30,000 genes and 100,000 proteins. Of particular interest to metabolomics researchers are small, low-molecular-weight compounds that serve as substrates and products in various metabolic pathways. These "small molecules," as they are called, include compounds such as lipids, sugars, and amino acids that can provide important clues about the individual's health.

The metabolome—the collection of all metabolites in a cell at a point in time—reveals much about that cell's physiological state at the time of sampling, and humans have trillions of cells of many different types, all with potentially different metabolomes. Whereas genes and proteins set the stage for

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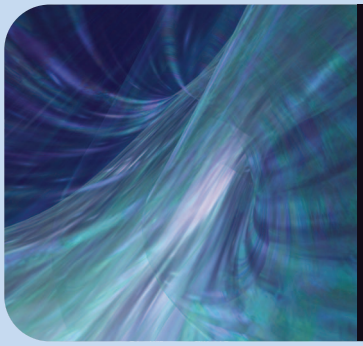
what happens in the cell, much of the actual activity is at the metabolite level: cell signaling, energy transfer, and cell-to-cell communication are all regulated by metabolites. Furthermore, gene and protein expression are closely linked, but metabolite behavior more closely reflects the actual cellular environment, which is itself dependent on nutrition, drug and pollutant exposures, and other exogenous factors that influence health. Explains Bill Lasley, a professor in the Department of Population Health and Reproduction at the University of California (UC), Davis, "Genomics and proteomics tell you what might happen, but metabolomics tells you what actually *did* happen."



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–Bill Lasley
University of California, Davis

As in the other "-omics," metabolomics data are gathered with high-throughput methods; nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (MS) using robotic automation are the dominant analytical techniques used in the field today. "Metabolomics is a beautiful approach for rapidly acquiring a vast amount of information about the molecular composition of a sample," says Mark Viant, a research fellow in the School of Biosciences at the University of Birmingham, United Kingdom. "If you have a disease, it's likely that your metabolism is going to be affected. The same is true if you get hit with a toxicant. To be honest, the diagnostic potential is staggering."

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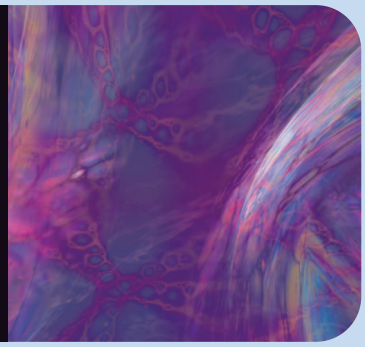

Other researchers apparently agree: metabolomics research activities are now becoming more widespread. The NIH Roadmap for Medical Research, a broad set of initiatives intended to focus the organization's agenda for the next several years, includes an initiative called Metabolomics Technology Development, which is headed by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). This initiative, currently in the planning stages, will fund several extramural research projects this year, says Maren Laughlin, who directs the NIDDK Metabolism and Structural Biology Program. A new international association established to promote the field, the Metabolomics Society, was announced in March 2004. This international organization of experts from academia, government, and industry is headed by Rima Kaddurah-Daouk, cofounder and vice president for biological research at Metabolon, a company that applies metabolomics techniques to clinical uses. According to Kaddurah-Daouk, the society will bring together leaders from different disciplines with the ultimate goal of building the metabolomics technology and integrating metabolomics with the broader universe of systems biology. "The activities included under the umbrella of the Metabolomics Society will encompass metabolic profiling, metabolite flux analysis, biochemical modeling, and more," she says.

Industry research is also on the rise, as pharmaceutical and biotechnology companies investigate metabolite profiles as potential tools for drug development. Their efforts have been guided in part by researchers at London University's Imperial College of Science, Technology, and Medicine, including Jeremy Nicholson, a professor of biological chemistry who is widely regarded as one of the field's leading figures. Nicholson was among the first to apply the tools of metabolite analysis—first NMR and now also MS—to the assessment of metabolite changes in biofluids over time.

A Debate over Terms

Nicholson refers to the field as "metabonomics"—the term he and his colleagues coined in 1996 to describe studies of metabolite profiles in the biofluids of whole organisms. "Metabonomics" specifically covers the integrated approach of looking at the effects of all the cellular metabolomes at one time, says Nicholson.

This research had actually been ongoing since the late 1980s, explains John Lindon, a visiting professor at Imperial College and one of Nicholson's long-time collaborators. "We did the first crucial analysis back in 1988," Lindon says. "This was analysis of NMR spectra from rat urine given different toxicants using pattern recognition techniques. We immediately realized the power of the approach and have been working in the field ever since." Lindon says the term "metabonomics" complements "genomics" and "proteomics."

In recent years, "metabolomics," with its greater similarity to "metabolite," has emerged as the more widespread term, particularly at the NIH and among its affiliated scientists. But Nicholson says metabolomics can be regarded as a subset of metabonomics—the latter, he says, covers classifying samples, understanding biochemical mechanisms, identifying biomarkers, quantitatively analyzing concentrations and fluxes, and probing molecular dynamics and interactions.

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Confusion over what to call the field is a persistent problem; sources interviewed for this article universally described the debate as a distraction from the science itself that must be resolved. Kaddurah-Daouk says the Metabolomics Society will dedicate itself in the early stages to defining appropriate terminology as a top priority. "We respect the niche that Nicholson wants to define as 'metabonomics,' which we believe will form a subset of the broader field," she says.

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–Bruce Hammock
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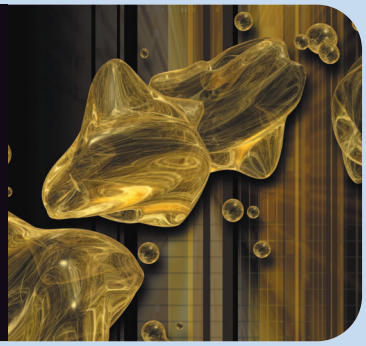


Metabolite Biomarkers

In the long run, scientists are looking to metabolomics to fill important gaps in systems biology, a research paradigm focused on all the interconnected molecular pathways in cells and organisms. Short-term clinical goals for the field are more concerned with the search for biomarkers, or molecular indicators of pathology.

Individual metabolites have already been used as disease biomarkers for years. Elevated glucose, for instance, is indicative of diabetes mellitus. And cholesterol is a metabolite long associated with heart disease and stroke. Metabolomics enables the identification of biomarkers based on entire groupings of metabolites that are up- or downregulated in unison under specific conditions.

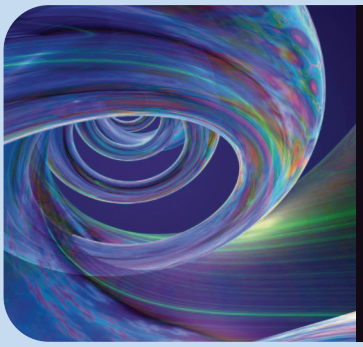
Bruce Hammock, a distinguished professor of entomology in the UC Davis Cancer Research Center and director of the NIEHS–UC Davis Superfund Basic Research Program, says these metabolic profiles could broaden insights into the cause of disease. "High cholesterol might tell you that you have a problem, but if you supplement with five other measures, you could determine why you have the problem," he explains. "We might be able to say it's because your transport proteins are poor, or because you're eating too much fat, and so on. The profile will give you knowledge and information rather than just data."

Some experts believe metabolomics could provide clinical uses sooner than either genomics or proteomics. Several factors contribute to this view. First, metabolite profiles are comparatively cheap to generate, assuming the requisite instruments have already been purchased—once purchase costs are subtracted, the standard instruments, particularly NMR, can identify a sample's metabolite spectrum quickly for a few dollars. In contrast, the DNA microarrays used in genomics research cost hundreds to thousands of dollars and are often unavailable to clinicians, while protein analysis is time-consuming and hindered by the much larger size and complexity of the molecules, which have more functional components. Furthermore, the functions of most genes and proteins remain unknown, whereas metabolites can often be assigned to particular tissues and disease categories, which allows fairly easy extrapolation of their functions.

Finally, metabolomics is noninvasive and allows for repeated sampling over time. Gene expression profiles, on the other hand, can be generated only from cells that have been impacted by disease, such as tumor cells. Proteins can be obtained from tissues and blood plasma, but not from urine, where they generally only appear as symptoms of illness. But metabolites are present in tissues, blood, saliva, *and* urine. Some biofluid samples can be linked to anomalies in particular tissues. For instance, urine is more likely to reflect renal disease, whereas saliva may more accurately reflect lung disease.

Metabolomic biomarkers do have their limitations, however. Donald Robertson, a scientist at Pfizer, says that in some cases metabolic responses—which vary greatly in terms of their dynamic range—are so far removed from the source of pathology that they are almost impossible to interpret. "Usually drugs or disease unleash a cascade of biomolecular effects throughout the body," he explains. "Many of these are subtle and below analytical detection limits." But Robertson adds that most metabolic changes could be detected if researchers knew what to look for.

In a sense, Robertson says, the limitations of metabolomics are the exact opposite of those posed by genomics. Whereas the genetic source of a disease might be too far "upstream" of the pathology to identify, metabolic changes might be too far "downstream," and diluted by the activities of proteins, the environment, and other intermediate biochemical events. Metabolomic profiles are also subject to random fluctuations, and can be influenced by diet, sleep patterns, age, smoking, and many other variables that mask the effects of disease or toxicity. Teasing biomarkers out from this background noise is a complex analytical and statistical challenge, scientists



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University of Louisville, Kentucky

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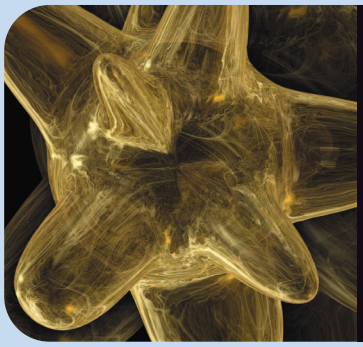
say, although one that ultimately should be achievable.

It is for these reasons and others, stresses Teresa Fan, an associate professor of chemistry at the University of Louisville, Kentucky, that scientists should view all the "-omics" sciences as complementary. "The bottom line is that you're not going to get the full picture with any one '-omic' technique," she says. "Looking at the genome won't tell you much about the downstream function, but looking at the metabolome won't tell you much about the underlying regulation. It's the whole integration that's important."



We need to show the field produces something of value that can help us understand and monitor disease. Metabolomics has to show it can be used to identify new therapeutic targets, streamline drug discovery, and identify the best drug candidates.

–Rima Kaddurah-Daouk
Metabolon

Metabolomics and Environmental Health

Metabolomics applications in environmental health, now in their early stages, may yield important benefits to the field. Some scientists believe metabolomics can fill key data gaps in environmental toxicology and enable more informed risk assessment decision making. And because metabolomics studies may yield early-stage toxicity screens, the science could lessen the number of animals needed for research.

There currently are a few different environmental health projects going on in several research settings. Lasley, for instance, is using metabolomics to investigate how dioxin and other endocrine-disrupting compounds alter lipid chemistry. And in collaboration with the NIEHS, scientists at the biotechnology company Paradigm Genetics are investigating metabolomic changes in animals following exposure to acetaminophen.

Brenda Weis, Toxicogenomics Research Consortium coordinator for the NIEHS, organized a conference on metabolomics and environmental health that convened at the institute last May. With equal participation from industry, academia, and the government, the conference defined the state of the science for metabolomics. A brief write-up of the meeting highlights was published in the October 2003 issue of *EHP*, with a full meeting report expected this year. According to Weiss, conference participants explored the issue of whether metabolomics technologies are ready for environmental health research applications, and discussed appropriate strategies for developing the science in this respect. Their conclusions highlight a set of needs for advancing the technology: namely, databases, bioinformatics tools, and multidisciplinary teams and training, among others.

Despite this apparent forward movement, Weis says the field of environmental health has yet to embrace metabolomics as a significant tool for research. The NIEHS's activities in the field are minimal, and no formal extramural projects have been funded. The attitude of institute scientists, Weis says, is "cautious," and they are watching how the science develops and advances through developments that are largely occurring elsewhere.

The Holy Grail for us is the ability to measure biologically active concentrations of metabolites in both a spatial and time-dependent manner. This will allow us to understand metabolite fluxes in biochemical pathways.

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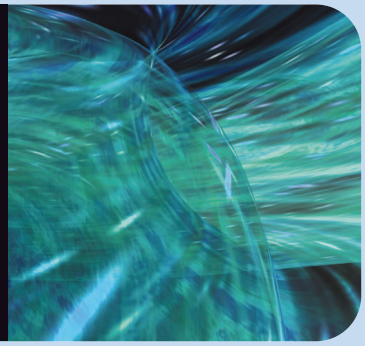

However, progress in the NIH Roadmap's Metabolomics Technology Development initiative is of particular interest to the NIEHS, Weis adds. This initiative was designed with input from the institute, which helped prepare the request for applications with an eye toward technology innovations. Now, Weis says, institute scientists want to see what kind of new technologies emerge from the Roadmap. "Then we can determine how to build on the science with specific applications to environmental health, which really hasn't been done yet."

Industry Pushes Forward

Today, the bulk of progress in metabolomics is coming out of industry. Perhaps the biggest venture in the field—funded with tens of millions of dollars—is a collaboration among Imperial College and the pharmaceutical companies Pfizer, Pharmacia (which has since been purchased by Pfizer), Hoffman-La Roche, Novo Nordisk, Bristol-Myers Squibb, and Eli Lilly and Company. Run by a steering committee co-chaired by Lindon and Nicholson, this group, known as the Consortium for Metabonomic Toxicology (COMET), is developing screening tools for use in drug discovery.

According to Nicholson, COMET recently wrapped up the first phase of its research: the assessment and prediction of liver and kidney toxicity from exposure to 80 model compounds in rats and mice. "We've developed a toxicity screening system based on NMR data that is at least as good as anything coming out of genomics or proteomics," Nicholson says. "We're writing up a paper that shows this,

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and we'll be submitting to a peer-reviewed journal soon."

The approach taken by Imperial College researchers involves linking peak patterns on the NMR spectrum to toxicity or drug efficacy using statistical pattern recognition. The COMET researchers rely on statistical pattern recognition in part because changes are difficult to compare by eye. In short, the spectrum becomes a "fingerprint" for the pathology in which the identity of the actual metabolites is deemed unnecessary, at least at the outset. "The pattern is what's important in terms of distinguishing normal from abnormal responses," Nicholson says. "We use the pattern recognition intensity signature as a method of discriminating which parts of the spectrum are carrying the information, and then solve the molecular structures of the biomarkers."

Experts in the field generally agree that fingerprinting is useful in the short term. Fingerprinting advances metabolomics by stimulating interest and funding. Furthermore, it generates screening tools for toxicity evaluation and disease diagnosis that lay the groundwork for more detailed studies.

But researchers must eventually link the patterns back to biological mechanisms, Weis says. "If you want to understand the underlying biology, you have to understand the peaks," she explains. "You need to know the metabolite concentrations, activity, and structure. It's not enough to just come up with a peak and then say, 'There it is.' You need to go one step further to find out what's behind it. That's how you identify biological pathways."

Understanding the underlying biology is important, Robertson adds, because it helps to confirm that observed profiles are relevant to the process of interest. For example, a drug might cause an animal to lose weight, producing a metabolite profile that reflects nutritional changes rather than toxicity. Without this knowledge, a researcher might erroneously attribute the profile to the drug's toxic mechanism.

The Short-Term Outlook

Metabolomics is in a proof-of-principle phase at the NIH today. Experts agree the field is taking off during a period of "'-omics' fatigue" that has fueled a degree of skepticism among some scientists. Both genomics and proteomics were heavily hyped, and there is some concern over the slow pace of progress in both these fields. Thus, NIH officials are taking a wait-and-see approach to metabolomics, funding small-scale pilot studies designed to produce concrete results.

Kaddurah-Daouk says metabolomics must achieve some important short-term goals in order to garner more funding. First, scientists must validate that the technique is robust, reliable, and reproducible. And second, the field must show that it can generate biomarkers useful for diagnosing disease and monitoring the effects of therapy.

"We need to show the field produces something of value that can help us understand and monitor disease," Kaddurah-Daouk says. "Metabolomics has to show it can be used to identify new therapeutic targets, streamline drug discovery, and identify the best drug candidates. We believe that metabolomics can help in all these respects, but we have to validate them one concept at a time."

Laughlin says that technical improvements are needed to expand the potential of metabolomics. Among those targeted by the Metabolomics Technology Development initiative, she says, are advancements that will allow scientists to identify the biologically active fraction of metabolites, as opposed to those that are "sequestered" in an inactive state and thus irrelevant to whichever process is being studied.

The ability to determine where metabolites are located in the cell is also critically important, Laughlin says. "This is our biggest challenge," she says. "We need *in vivo* measurements in specific areas of the cell. The Holy Grail for us is the ability to measure biologically active concentrations of metabolites in both a spatial and time-dependent manner. This will allow us to understand metabolite fluxes in biochemical pathways."

Many other challenges and needs also face the field. Scientists universally point to the need for a curated, public database for NMR and MS spectra, one that optimally includes profiles for the wide range of populations that make up the human race. Vast databases such as this are key to managing metabolite variability, which is influenced by ethnicity, age, nutrition, and many other factors; comparable databases such as GenBank and Swiss-Prot aid genomics and proteomics researchers in molecular identification. Of course, new bioinformatics methods will be needed to wade through these enormous data sets. The need for innovative advances in bioinformatics is particularly acute with respect to integrating metabolomics data with genomics and proteomics—a top priority for systems biology.

One of the biggest risks to the field, according to Bruce German, a professor of food science and technology at UC Davis, is if people's expectations of the information in NMR spectra are too high. Unlike the genome, which can be sequenced in its entirety, the metabolome varies in a tremendous dynamic range, he says. Promises that NMR will identify all metabolites and deliver yet another "-omics" revolution on this basis must be viewed cautiously, he says.

"High-resolution NMR of intact biofluids does not yet identify all the metabolites," says German. "But the good news is that unlike the genome, which we're only just now beginning to understand, metabolism is well known to scientists. We've spent decades studying metabolism, but ironically very little of this has been brought to a diagnostic application. You could say that metabolism is a mature science looking for a game to play in."

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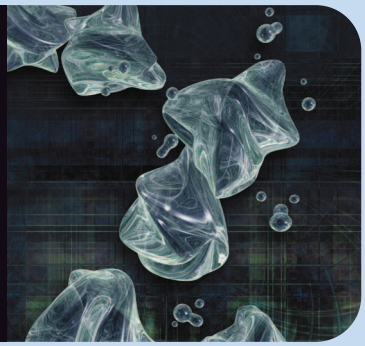

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