the Analytical Scientist

SPECIAL SERIES:

Omics

EDITORIAL

The Buzz About (Prote)Omics

Is it time for proteomics to take a spotlight in scientific engagement?

Protein biochemistry tells a tale of diverse protein function. These roles – which range from structural stability to catalysis – are not the only source of variability surrounding these molecules, though. Proteins can also be present in vastly differing amounts, and can be altered in terms of their amino acid sequence and co- and post-translational modifications; the depth of the known proteome covers over 10,000 quantified proteins and 10,00010 proteoforms.

With the above in mind, it's probably no surprise to hear that capturing protein complexity with accuracy and high throughput is a great challenge. Yet, the technologies behind proteomics are geared to capture this immense landscape of complexity. In fact, the past five years have ushered in many proteomics advances, leading to improved protein coverage, sensitivity, and throughput; we can now cover extremes from single-cell analyses to the processing of hundreds of samples in a single day.

Most excitingly, proteomics, despite its maturity, continues to grow in new ways. As an example: top-down proteomics approaches are expanding to capture the functionally important patterns of protein post-translational modifications. Further examples: the ways in which we can probe three-dimensional protein structure in vivo (important for determining structural changes as a function of disease) are improving; quantification of a protein interaction partners in cells is leading to growing acknowledgement that proteins can choose to stay at home or travel the world; and single-cell proteomics is emerging as a serious tool for studying cell differentiation, evolution, and interactions.

What's more, high-throughput methods with automated and highly QCed workflows are allowing proteomics to boom into a large-scale human population science. In these cases, we can conduct consistent analyses of thousands of samples. Using such methods, we are able to study our natural history, disease progression, and responses to therapies (Amanda Hummon discusses a more personalized approach to the latter application here).

The future is bright, but there's still some space for improvement and innovation in our toolbox. Proteomics promises to answer burning questions about mechanisms in biology, and increased ease of application means that the proteomics buzz continues to grow. Along with other omics approaches (think metabolomics and lipidomics; see "Gurus of Omics"), previously untold knowledge regarding genetic, metabolomic, and environmental influences lies in wait. Perhaps that's why The Analytical Scientist team was so keen to devote the February issue to the magic of omics… Let's explore!

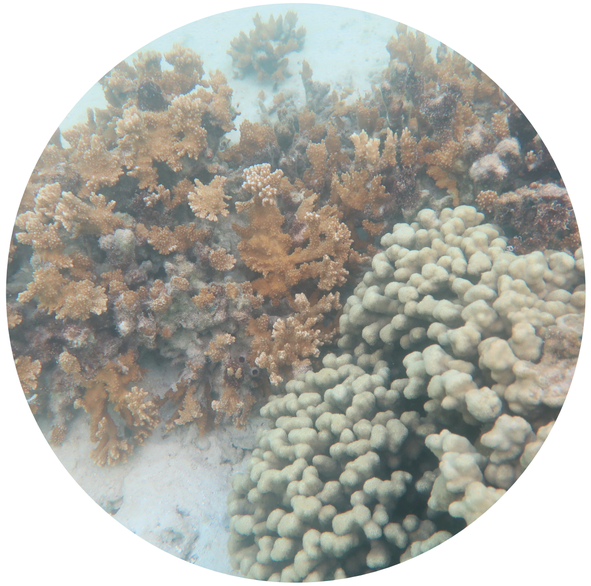
Jennifer Van Eyk, Director of the Advanced Clinical Biosystems Research Institute and the Precision Biomarker Laboratories at Cedars-Sinai Medical Center, Los Angeles, California, USA

John Yates is Ernest W. Hahn Professor of Chemical Physiology and Molecular and Cellular Neurobiology at The Scripps Research Institute, LaJolla, California, USA. He was recently named Editor of the Journal of Proteome Research

UPFRONT

The Coral Doctors

Researchers dive into the metabolomics behind bleaching



Our coral reefs are dying. Climate change and local stressors are recognized as the key contributors, causing higher sea surface temperatures that lead to widespread bleaching events. Now, a team of researchers has added to our understanding by uncovering the metabolomic shifts associated with heat stress in our planet's most biodiverse ecosystems (1).

"The goal of our study was to identify metabolite markers that can be used to assess coral health in time to enact conservation efforts before reefs are seriously damaged," says Debashish Bhattacharya, co-author of the paper.

The team used untargeted hydrophilic interaction LC-MS to study the stress-resistant coral species Montipora capitata and stress-sensitive Pocillopora acuta – both from reefs in Hawaii. "Given the large number of 'dark' metabolites in marine systems, we decided to conduct untargeted analysis with the goal of identifying both known and novel markers in the coral system – broadening our knowledge about these species," says Bhattacharya.

"We focused on the polar metabolites because they have fast turnover and they are more closely related to the central carbon and nitrogen metabolic network," adds Xiaoyang Su, co-author of the paper. "To confidently identify these metabolite markers, we synthesized more than two dozen chemical standards and used these to compare the retention time on the LC and tandem MS spectra."

For both species, the team discovered a variety of co-regulated dipeptides that had the highest differential accumulation under thermal stress. These, along with other metabolites identified by the study, can be used as markers for thermal stress and thus health in wild coral – the diagnostic beginnings of what the team calls the "Coral Hospital."

Furthering its coral health mission, the team has now secured funding to create a microfluidic device that can be deployed by reef managers and members of the public alike to monitor the levels of different stress-related metabolites and proteins.

REFERENCES AVAILIABLE ONLINE

ONLINE

Clues from a Watery Grave

Bone proteomics could help investigators get to the bottom of drowning deaths

ONLINE

Search for the Unknown

A new NMR spectroscopy system could help researchers get to grips with the metabolome

IN MY VIEW

Down to a Single Cell

To decipher the functionality of complex biological systems, laboratories should sidestep bulk cell measurements in favor of single-cell proteomics

Biological system functionality is defined by the intricate interplay of its diverse cell types. Yet, cells are often studied in bulk, obscuring intracellular variability and single-cell contributions. This can hinder our ability to study complex diseases like cancer, in which cellular heterogeneity is a significant driver of prognosis. The scientific community needs to step away from averaged protein readouts and towards examining single-cell behavior.

The field of single-cell proteomics is still in its infancy, but is already delivering on its promise to capture transient cellular changes in dynamic systems by capitalizing on key advances in MS/MS-based approaches – particularly those affording improved sensitivity. This is key to make the most of biological samples' limited protein content (because, although present in quantities an order of magnitude greater than mRNA, cellular protein cannot be amplified!). This is particularly tricky when working with samples which are themselves limited, such as patient biopsies.

So how do we boost our proteomic outputs in light of limited sample? There are two main ways: i) by minimizing sample loss with meticulous preparatory steps, and ii) by adopting the most appropriate instrumentation to enhance experimental accuracy.

When it comes to minimizing sample loss, proteins' "sticky" nature is an issue; they cling to the sides of pipette tips and tubes at every stage of sample preparation, making conservation tricky. Robotic liquid handlers with non-contact pipetting can help – as can minimizing sample volumes to an absolute minimum (less than 1 µl). Single cell samples can be isolated through fluorescence-activated cell sorting or laser capture microdissection, which allows for the study of primary tissues in situ.

"Though it may seem overwhelming to transition from the safe familiarity of bulk protein analyses into the novel and technically demanding world of single-cell proteomics, I would say the time is now."

Experimental accuracy can be improved by including "booster" samples in the multiplexing mix. These increase the number of ions available, allowing us to enhance the lower limit of detection of MS analyses. In our lab, we use tandem mass tags (TMTs; isobaric labels for the accurate quantification of peptides and proteins in MS/MS-based analysis) for this. We dedicate a single TMT channel to tag a group of up to 200 booster cells, leaving other channels available for single cells. This boosts the protein identification rate by providing optimal ion levels for identifying and quantifying peptides that represent a significant portion of the cellular proteome.

New-generation MS systems are also equipped with real-time search features in which MS3 scans are only triggered for peptide precursors identified at the MS2 level. Real-time data acquisition vastly improves proteome coverage, with up to 95 percent of peptides quantified at MS3 level. Matching MS2 fragments with the correct peptide sequence provides improved accuracy. By not wasting runtime on unnecessary MS3-level scans, real-time searching also significantly improves the productivity of single-cell proteomics workflows as a whole. Field asymmetric ion mobility spectrometry (FAIMS) can also filter out background ions and provide deeper sample coverage. "How effective are these interventions?" I hear you ask. According to a proof-of-concept study we conducted in a heterogeneous acute myeloid leukemia model system (1), very! In fact, we were able to apply TMT multiplexing with a FAIMS-optimized workflow and precision robotics to conduct an entire sample preparation workflow on more than 3,000 cells. Our in-house data processing workflow, SCeptre (single-cell proteomics readout of expression), allowed us to visualize the data and pinpoint protein expression levels within each individual cell type from the sample.

Though it may seem overwhelming to transition from the safe familiarity of bulk protein analyses into the novel and technically demanding world of single-cell proteomics, I would say the time is now. The ability to examine a biological system down to a single cell can pave new paths in research – and clinics. Laboratories can set themselves up for future successes now by venturing into simple proof-of-concept experiments or collaborative efforts that eventually develop into a tailored single-cell workflow.

REFERENCES AVAILIABLE ONLINE

Erwin Schoof, Associate Professor, Department of Bioengineering, Technical University of Denmark, Lyngby, Denmark

ONLINE

Making Sense of Metabolite Mixtures

Substructure analysis provides a means to decipher complex metabolic samples

Justin J.J van der Hooft, Bioinformatics Group, Wageningen University, Wageningen, the Netherlands

ONLINE

Ethical Considerations in Clinical Proteomics

Conversations about ethical issues with proteomics data need to be had – and we are here to start the conversation

Sebastian Porsdam Mann, Faculty of Law, University of Oxford, UK. Philipp Geyer, OmicEra Diagnostics, Munich, Germany. Peter Treit, Department of Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Munich, Germany. Matthias Mann, Department of Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Munich, Germany, and the Novo Nordisk Foundation Center for Protein Research, the University of Copenhagen, Denmark

FEATURE

The Call of Coronal Duty

An insider's perspective on the proteomic battlegrounds of COVID-19

With March 11, 2021 marking one year since COVID-19 was officially classified as a pandemic by the WHO, it goes without saying that many of us – not least those on the frontline of the fight – are feeling a bit war weary. And yet, for so many in the analytical chemistry community who answered the call of duty back in 2020, the battle rages on.

With so much uncharted ground still to cover when it comes to this novel coronavirus and our response to it, there is an abundance of work ongoing across all sub disciplines of our field. For now, we decided to share some of the spoils from one battlefront in particular: proteomics. Here, experts Jeroen Demmers, Perdita Barran, and Manfred Wuhrer tell us about their work in the fight against COVID-19, and provide an insider's perspective on some of the developments we can expect to see in the coming months.

COVID-19 Detection: Hitting the Mark

How we successfully used targeted proteomics for the detection of SARS-CoV-2 proteins

By Jeroen Demmers, Director of the Proteomics Core Facility and Associate Professor of Proteomics, Erasmus University Medical Centre, the Netherlands

Early last year, once it became clear that COVID-19 had started to spread out across the world, there was a general sentiment in Europe that it wouldn't happen that easily here. Just like the SARS and MERS coronavirus pandemics that came before (in 2003 and 2013, respectively), many people thought that this novel virus would be kept out of the region as well. Soon enough, the pandemic hit northern Italy hard, and it wasn't long before there were messages of infected people in the southern province of Noord-Brabant in the Netherlands.

The first official outbreaks were reported in early March, and things developed quickly from there. In the second half of March our institute was shut down – like many others across Europe – and only research on COVID-19 was allowed to continue. For us, this work was happening at the Viroscience department at Erasmus MC, where several research groups had been focusing on coronaviruses for decades. My research lab and core facility had a choice: shut the lab, or grab this opportunity to contribute to SARS-CoV-2 containment by adapting our technology for use in virus detection and – if successful – diagnostics.

The journey to discovery

Thanks to our previous work during the MERS coronavirus pandemic (our lab had identified the MERS-CoV human receptor protein using MS-based proteomics; see "Gone Fishing" for more information), we had already established connections with the Viroscience department. I decided to contact coronavirus specialists Bart Haagmans and Mart Lamers as I knew they were working day and night on SARS-CoV-2 assays to answer questions about the mechanism of infection. For one of their assays, they were interested in analyzing the response of the host cell proteome to viral infection in a recently developed organoid-derived bronchioalveolar tissue culture. Using our technology, we were able to help them monitor up- and downregulation of large numbers of proteins upon viral infection and learn more about the intracellular pathways that are turned on or off as a result of infection. Because of this work, and our connections with the Viroscience department, we were also granted access to some of their interesting SARS-CoV-2 samples – meaning we could test whether it was possible to measure viral proteins in complex samples, such as cell lysates.

We started off with samples from an infected Vero E6 cell line derived from the African green monkey – this cell line is used to propagate viruses and serves as a rich source of viral material. A dilution series was then created to demonstrate the limit of detection of specific viral proteins. As the virus was already genotyped, the protein amino acid sequences that we needed for the analysis of proteins based on peptide fragmentation or MS/MS data were already available. Also, we were quite lucky (or unlucky?) in that just a few days before most of the institute was shut down, a brand new Orbitrap Eclipse MS was installed in the lab. The first proteins analyzed on that machine were SARS-CoV-2 proteins!

The output of a standard proteomics experiment is usually a table of identified proteins, which is generated in the final step of a database search using software tools that may take up to several hours. The progression of this process (at least in the tool that we use) is indicated by a green bar. I clearly remember the anxiety and excitement that we felt when, after the very first database search, we saw the bar hit 100 percent and the list of identified proteins popped up: the first time we identified SARS-CoV-2 proteins really felt like looking the monster directly in the eye.

ONLINE

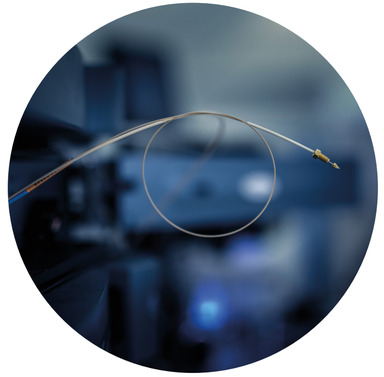
How can proteomics help in the fight against COVID-19?

"Understanding the role that proteins play in the SARS-CoV-2 infection process and disease progression is vital to the development of therapeutic and preventative strategies. In this way, proteomics has proven to be an indispensable tool in COVID-19 research, and its role will no doubt be expanded in the future."

ONLINE

Gone Fishing

"For our work on the MERS-CoV human receptor protein, we designed a relatively simple 'Fishing' experiment that was performed using in vitro synthesized MERS-CoV Spike 1 protein immobilized on magnetic beads."



The right sample

For a few months, we worked on nothing but COVID-19. Virtually all other projects were put on hold and since most meetings at work were cancelled and there were no teaching duties, it really felt like a postdoc project where the full focus is on basic science. I truly relished this lack of distraction, despite the troubling situation we – as citizens of the world – were in. On a personal level, we were building a new house and we weren't sure whether we could still sell our old house – what with the threats of a housing market collapse together with the crashing stock markets and other doomsday scenarios that circulated. It was truly both an exciting and troubling time.

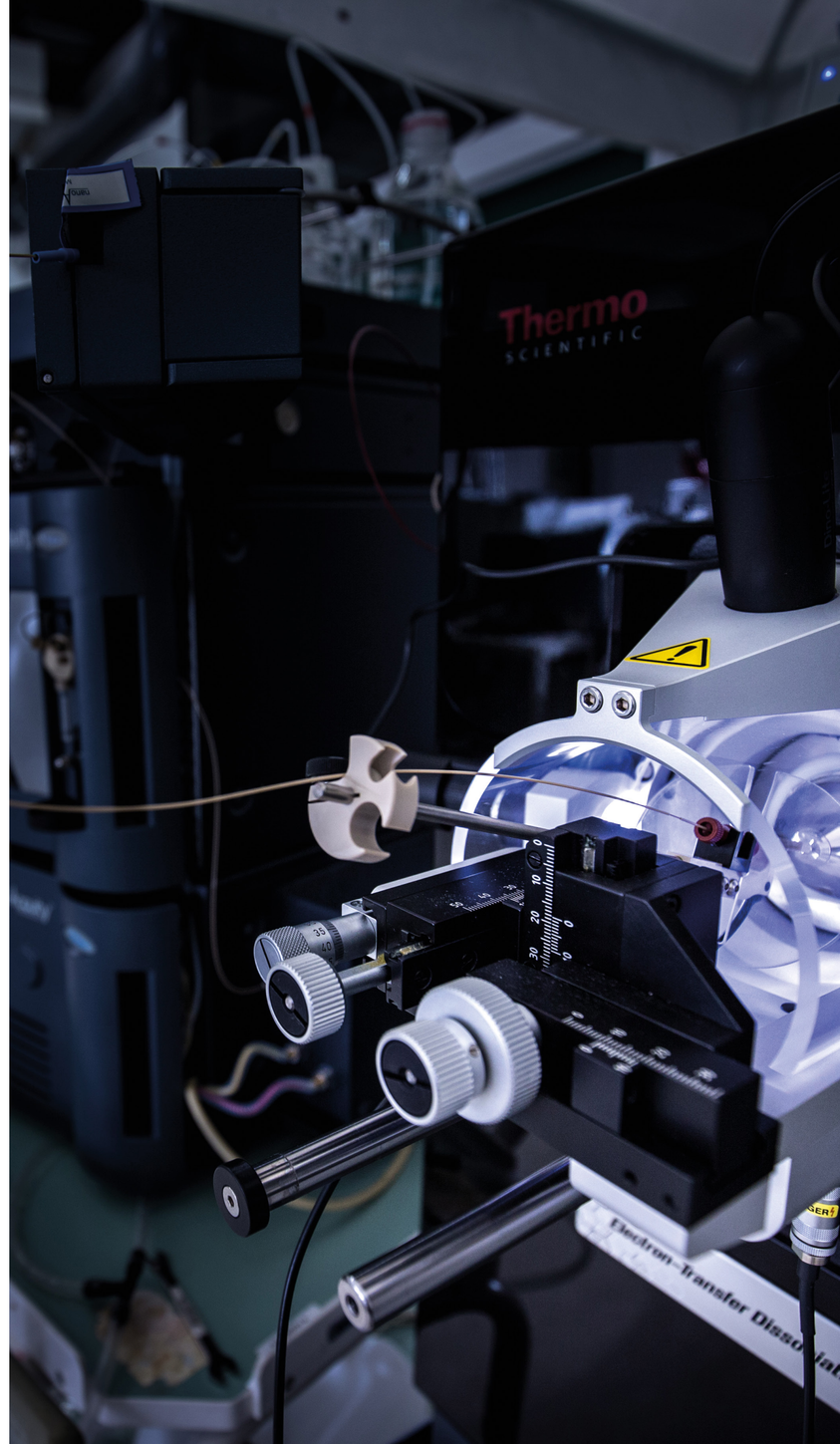
We soon identified a set of proteolytic peptides that could serve as the target peptides in follow-up experiments. Also, we were able to calculate limits of detection for viral proteins in our proteomics assays. Under ideal conditions, we could go down to the mid- to low attomole range in targeted experiments, just like the numbers we had seen before in another project on non-related proteins.

While setting up these assays, I had already started asking around for patient material to see whether we could detect proteins in clinical specimens (such as nasal swabs) and to determine if it could be used as a diagnostic tool. However, getting patient samples turned out to be more difficult than I had anticipated. For conventional PCR-based testing, samples are usually stored in a "transport medium." This medium contains a lot of protein, the signals of which dramatically mask the signals of viral proteins in our assay. Unfortunately, adaptation of standard protocols in diagnostic departments is virtually impossible, and as research scientists who are used to changing protocols if something doesn't work, this was quite frustrating.

But one day we got a message from a collaborating clinical virologist who had collected a different type of sample from a COVID-19 patient. This was a sputum sample, deposited on a little glass slide with no addition of transport medium or any other buffer solution. Upon inactivation of the virus in 80 percent acetone, we could take the sample from the BSL lab to our own lab and subject it to our standard bottom-up proteomics protocols – which basically means digesting all the proteins into peptides. This sample was in fact the first clinical specimen in which we could clearly detect SARS-CoV-2 peptides.

We used a targeted proteomics assay, which means that we set the MS in such a way that it only detects viral peptides that were selected a priori. The quadrupole in the Orbitrap hybrid MS then acts as a filter that lets only the peptides (or m/z values) of interest pass through. Upon fragmentation of the peptide to determine the amino acid sequence, the fragment ions are measured in the Orbitrap with high selectivity and sensitivity – the high mass accuracy of the Orbitrap is a clear advantage over such targeted methods in a triple quadrupole instrument here. The fragment ion fingerprint that is thus obtained is highly specific for the selected peptide. These fingerprints are then computationally compared with the specific fingerprints that were defined in the experiments on infected Vero E6 cells. Using targeted MS, the sensitivity can be increased and the limit of detection is at least 10-fold lower compared with data-dependent acquisition (untargeted) MS.

Next, we contacted clinical virologists from a hospital in the south of the Netherlands, which was located in the center of the area that was hit by the first COVID-19 wave in the spring of 2020. The clinicians there used so-called Eswabs, for which no protein rich transport medium is necessary. This results in much less background in our analyses and therefore increased sensitivity. Despite the excess of red tape, we managed to get an Eswab sample cohort to our lab. This sample set contained various swabs within a wide range of PCR Ct values and we could see a nice inverse correlation between Ct value and peak intensities of target peptides in the mass spectra, reflecting the abundance of proteins. Later, in a second sample cohort, we managed to get similar results and could detect SARS-CoV-2 peptides at fairly high Ct values (i.e. low viral counts).



Winning the war…

Where are we now? We have established the proof-of-concept and have shown that it is definitely possible to detect SARS-CoV-2 proteins using MS. The challenge now is to translate this methodology from the R&D stage to the clinical diagnostic lab. For the analyses we have performed so far, we used state-of-the-art, ultra-sensitive Orbitrap mass spectrometers – which are typically not present in clinical diagnostic labs. Still, the basic technology is comparable to triple quad MS and these are readily available in many clinical labs.

There is still a debate around the level of sensitivity we really need in COVID-19 testing. The limit of detection of PCR based methods is unsurpassed, but do we really need that sensitivity? It is unclear whether infected individuals, whose nasal swab PCR Ct values are in the high 20s or low 30s, are infectious. Although no viral proteins could be detected in most swabs with associated Ct values of >26, we have to test whether the sensitivity that can be reached by MS-based approaches is sufficient to differentiate between infectious and non-infectious people. Only then will we be able to assess the potential value of MS-based COVID-19 testing.

One clear advantage of this technology over other testing methods however, is that proteins of multiple different viruses can be targeted in one assay. If peptide signatures for a given virus are defined, these can be relatively easily included in the target list. This way, samples can be screened for multiple viruses simultaneously. This is not only useful now, but also in the future when differentiation between different pathogens will be needed.

One challenge to overcome will be improving the analysis time: the sample preparation for proteomics assays takes a while, mainly because of the protein digestion step. This can be dramatically reduced by microwave irradiation. Furthermore, LC gradients could be much shorter than they are now: we have managed to reduce the gradients lengths threefold and could still detect most of the SARS-CoV-2 peptides. Running clinical samples using LC gradients of only up to a few minutes should be possible.

As a final note, I'd like to mention that part of our early work was published on biorXiv. Although manuscripts are not peer reviewed, they can be downloaded by the scientific community and the general public for free. For COVID-19 research, this has been a tremendous help in the dissemination of data, knowledge and protocols. Even though our manuscript has not yet been published in a scientific journal, our selection of target peptides and MS data sets have been used by others and already proven useful. I believe this is a beautiful illustration of the power of open access scientific knowledge – a trend I hope to see continue in the future.

I've demonstrated my own work using MS to detect viral proteins, but this is just one application of this versatile technology. It is clear to me that by studying proteins, both from SARS-CoV-2 and the human host cell, proteomics has profoundly changed the way we study viral infection and disease progression at the molecular level. I am excited to see the many potential novel applications that will no doubt come to fruition in this fast moving field.

READ THE FULL ARTICLE ONLINE

ONLINE

Collaboration and Determination

Perdita Barran, Professor of Mass Spectrometry at the University of Manchester, UK, shares a targeted approach to SARS-CoV-2 proteomics

ONLINE

A structural and systems biology view

Manfred Wuhrer, Professor of Proteomics and Glycomics at Leiden University and Head of the Center for Proteomics and Metabolomics in the Netherlands, shares his view on how MS-based proteomics can contribute to COVID-19 research in the clinical lab

SOLUTIONS

The Promise of eMetabolomics

Understanding specific interactions within and between organisms and their environment feeds into a much bigger endeavor: protecting the planet

"For the first time in history, the stability of nature can no longer be taken for granted […] Never has it been more important to understand how the natural world works and how to help it."

The opening lines of the critically acclaimed Netflix series "Our Planet," narrated by the famous British natural historian Sir David Attenborough, should really make us all stop and think. To play our part, we believe we can contribute to a better understanding of nature with environmental metabolomics.

For the uninitiated, metabolomics is the scientific study of the metabolome – the sum of all small-molecule intermediates and end-products of metabolism found in a given biological sample. Crucially, the composition of the metabolome is not only dependent on the biological system's current state but is also affected by many exogenous factors, including chemicals – medicines, food components, contaminants, and so on (1, 2).

Intelligent study designs, alongside modern analytical chemistry and sophisticated data analysis, enable us to extract valuable information from the metabolome – a point proven by the application of metabolomics to the area of human health and its significant contribution to the development of personalized medicine (3). It is now beyond any doubt that metabolomics adds an important layer of information to genomic and proteomic analyses.

At the Research Group Metabolomics of the Leiden Centre for Applied Bioscience, our ambition is to apply metabolomics in the fields of ecology, biodiversity, and environmental science. When metabolomics is applied to environmental questions – in other words, used to study the interaction between organisms and external stressors – it is fittingly called environmental metabolomics, or eMetabolomics for short (4). We broaden this definition and include interactions within and between species and their environment, with a view to addressing ecological and biodiversity issues.

The field of eMetabolomics offers promise for the following reasons:

- Changes in the metabolome are likely to be a more sensitive indicator of external stressors than information at the gene and protein levels (4).
- eMetabolomics is sensitive to the environmental effects of (emerging) chemical pollutants, including those not yet routinely monitored and/or with unknown modes of action. eMetabolomics may offer the first mechanistic insights into unknown modes of action.
- eMetabolomics can be used to study specific processes between organisms.
- And eMetabolomics can aid in the rapid identification of organisms.

Community service

To stretch the promise of eMetabolomics even further, we focus on the "community metabolome" – the collection of metabolites that are secreted into their environment by all the organisms within a given ecosystem. In essence, the community metabolome reflects the entire influence of various factors (climate, pollution, and invasive species) on the ecosystem in which we live (see Figure 1).

Currently, we are investigating the strength of eMetabolomics at the community level in a multi-omics investigation into ecological water quality. Acknowledging the importance of information layers, we also measure the unique traces of DNA generated by each organism

in the aquatic ecosystem – dubbed environmental DNA or eDNA – as part of the project. Combining these two layers of information creates a multi-omics image (see Figure 2). The eDNA analysis provides us with information on which species are present in an aquatic ecosystem, while eMetabolomics provides us with information on which processes are taking place. For example, with the help of eDNA, we can detect the presence of water fleas; with eMetabolomics, we can demonstrate that the water fleas reproduce.

Cliff jumping

When an ecosystem is affected by a chemical (or any other) stressor, it may deteriorate only slowly – almost imperceptibly – at first. But it is known (5) that the slow and steady decline is very often followed by a period of much more rapid deterioration (like walking down a gentle grassy slope towards a cliff). Once the ecosystem has deteriorated too far – or dropped off the edge of the cliff – it takes a considerable amount of effort to restore its healthy state.
Clearly, a measuring system that can detect disturbances in an aquatic ecosystem at these earlier (and slower) stages of deterioration would be highly valuable – a warning shot. In alignment with metabolomics applied to human health issues, we believe that eMetabolomics is capable of detecting changes in the community metabolome earlier than classical methods used to measure ecological water quality (which is to say, the actual identification and counting of species). To return to the example of the water fleas, prior to dying and disappearing, water fleas first become ill – and that results in a change to their metabolome.

The living lab

Our current eMetabolomics project aims to understand the effects of both biological and chemical disturbances on an aquatic ecosystem located in the so-called Living Lab of Leiden University. To increase our chances of success, we have built an exciting multidisciplinary research consortium, involving biologists from the Dutch National Biodiversity Center Naturalis and environmental scientists from the Institute for Environmental Sciences of Leiden University. We've also recruited the assistance of several ecological consultancy companies and governmental organizations.

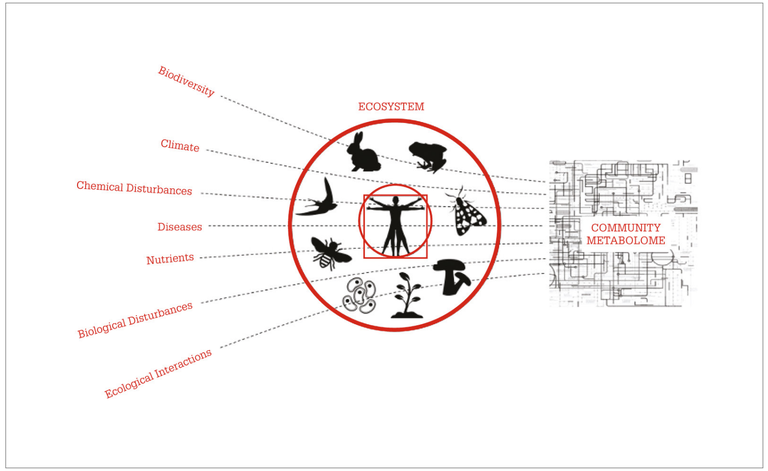
The Living Lab consists of a testing field with 36 ditches. Some of these ditches have been disturbed by adding red swamp crayfish (Procambarus clarkii) or the pesticide thiacloprid. The red swamp crayfish is an invasive exotic species that is currently common in many locations, including the western part of The Netherlands, and it is feared to have a negative effect on aquatic biodiversity. Thiacloprid is a neonicotinoid pesticide, which has been shown to have a very negative effect on the insect population and insect-eating birds (6).

Over the course of several months, all of the ditches have been monitored manually, and samples have been collected for eDNA and community-metabolome analyses. First, we will pinpoint metabolites that are linked to disturbances of the aquatic ecosystem in the samples that were collected at the very end of the field study (when the aquatic ecosystems are disturbed maximally). We will then quantify the same metabolites in much older samples, with a view to identifying how early on we are able to detect disturbances. Our expectations? The negative effects of both thiacloprid and red swamp crayfish can be identified early on – during the slow deterioration phase – in the ditch eMetabolome (4, 7).

Delivering on promises, recognizing weaknesses

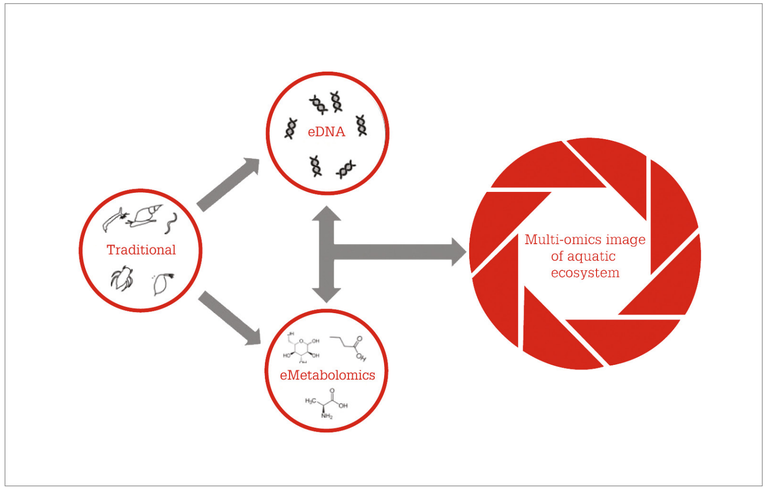
Our study is certainly specific, but we hope the project will demonstrate the strengths of eMetabolomics more broadly. Equally, we know it will challenge us to seek working solutions to four serious challenges:

Figure 1. The community metabolome expresses, among other things, biodiversity, climate, chemical disturbances, diseases, nutrients, biological disturbances and ecological interactions.



- Metabolite identification. The human metabolome database currently contains 114,008 metabolites (2). A community metabolome (the result of many organisms living together in an ecosystem) will likely contain many more metabolites – and a database is currently lacking (7).
- Establishing causal relationships between specific metabolites and specific organisms in a highly complex sample, such as the community metabolome, will not be straightforward.
- Measuring the community metabolome. In doing so, we must develop innovative, on-site sampling techniques and specific preparation methods for various (and sometimes new) sample types (surface water, air, organisms).
- Education. Because (e)Metabolomics is a relatively new but rapidly growing research area, educating and training new technicians and researchers is important. At the University of Applied Sciences Leiden, we have developed a degree course in metabolomics specifically to address this final challenge.

Figure 2. A molecular image combines information collected from the measurement of DNA (eDNA) and metabolic products (eMetabolomics)



By forging a stronger link between scientists (analytical chemists, environmental scientists, and ecologists) and professional bodies (governments, ecological consultancy firms, water boards), we believe our research and education can help contribute to a healthier living environment – one in which both people and nature can coexist in the best of health.

REFERENCES AVAILIABLE ONLINE

Peter W. Lindenburg, Research Group Metabolomics, Leiden Centre for Applied Bioscience, Faculty of Science & Technology, University of Applied Sciences Leiden, The Netherlands

André van Roon, Research Group Metabolomics, Leiden Centre for Applied Bioscience, Faculty of Science & Technology, University of Applied Sciences Leiden, The Netherlands

SITTING DOWN WITH

Interrogating Metabolism

Sitting Down With… Timothy Garrett, Director of Experimental Pathology and Associate Professor, Department of Pathology, Immunology and Laboratory Medicine, University of Florida, USA

Can you tell us about your current role?

Since 2019, I have been an associate professor at the University of Florida, where I am also Director of high-throughput metabolomics for the Southeast Center for Integrated Metabolomics (SECIM) and Director of Experimental Pathology. My interests cover both research and clinical work in several areas, including cancer, rare diseases, and diabetes. My lab comprises 12 scientists, including graduate students and post-docs grappling with complex problems and new fields of research. I often think my job is just to facilitate their work! We are asking fundamental questions: what drives human metabolism? Why does it sometimes fail? How does it change throughout the course of a disease? And how can we better characterize disease so that we can make a diagnosis earlier – or more accurately? By understanding how health may be disrupted, we will help find better treatments.

Much of your work is focused around MALDI MS. When did you become aware of this technique?

My interest in MALDI MS began as an undergraduate in Jonathan Amster's lab at the University of Georgia, working on the characterization of bacterial proteins. MALDI fascinated me: such a simple technique, and yet it generates so much information from such tiny samples. Not only did I fall in love with the technique, I also fell in love with the instruments themselves – how to operate them, fix them, and tinker with them to make them better. Right now, we are pushing MALDI to its limits in metabolomics and lipidomics; we are analyzing populations of metabolites and lipids, and applying informatics to determine which ones are important in disease.

How does your research connect with clinical labs?

It may be easiest to demonstrate this with an example. Recently, a clinical pathologist asked for help with a female patient who had symptoms similar to the lysosomal storage disorder, Fabry disease. Interestingly, this is an X-linked condition typically found in males. To investigate her lipid metabolism for defects, we needed to develop a new diagnostic approach, with careful attention to experimental design. It paid off – we found a defect in a non-obvious enzymatic pathway, completely different to those defects typically seen in male Fabry patients. We can't actually declare the patient to have Fabry disease, because our technique is not yet validated as a diagnostic. Our work does, however, suggest ways of better managing these patients, and, as it is MS-based, it is easy for labs to adopt. Ultimately, it may lead to significant improvements in our ability to diagnose and develop new therapies that target the enzymatic defect we identified.

What most satisfies you about your work?

I'm most proud of having built a resource – the SECIM center – that helps address difficult clinical questions both locally and nationally. And it's immensely satisfying to see our work fundamentally affecting patient care. For example, our method of assessing the immune system of transplant recipients, specifically pediatric kidney transplant patients, allows us to predict organ rejection before development of clinical symptoms. And that can improve healthcare management of these children after validation studies are completed. We're always proud to see our systems solving clinical problems.

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