Metabolomics

From small molecules to big possibilities

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Metabolomics. From small molecules to big possibilities.
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From small molecules to big possibilities

Lecture delivered on Metabolomics in acceptance of the post of professor, at Leiden University College of Applied Sciences on Wednesday 10 April 2019 by Dr P.W. Lindenburg


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METALLICA

Table of contents

Dear Members of the Executive Board of the Leiden University College, the director of the Faculty of Science & Technology, teachers, students, colleagues, friends, family and highly valued listeners.

1. The heel prick: an early form of metabolomics assessment

When I mention the type of work I do at a get together, I am often met with raised eyebrows. I develop analytical-chemical methods for metabolomics research. Without realising it, almost everyone in the Netherlands comes into contact with both analytical chemistry and a simple form of metabolomics at a very young age.

Figure 1, Blood collection from a new-born's heel on a special type of tissue paper

If you have children, you will probably remember the heel prick to which your new-born child was subjected. With a small puncture to the heel, a few drops of blood are collected within a few days after birth on a special type of tissue paper [1]. On the photo you can see how this procedure is carried out on my daughters Yfke and Rosa, when they were five days old. Since 1974 the heel prick procedure has been carried out on almost every single child in the Netherlands.

The tissue paper with the blood sample is sent to a laboratory where, by an analytical-chemical method, metabolites are measured to determine if the baby is suffering from a metabolic disorder. Phenylketonuria is an example of such a metabolic disorder.

Phenylketonuria is a hereditary metabolic disorder. The small phenylalanine molecule occurs naturally in the human body. This amino acid is a metabolic product, also known as metabolite. Phenylalanine is needed, among other things, for the production of neurotransmitters which operate our brains. It is also the component of another important metabolite, tyrosine. The conversion of phenylalanine to tyrosine is carried out by the phenylalanine hydroxylate enzyme. It is this enzyme that does not function in children with phenylketonuria, who are born with a defect in the gene encoding it. As a result, phenylalanine is no longer converted and therefore starts building up. In high concentrations Phenylalanine is toxic, the consequences of which are disastrous to the baby: irreversible brain damage occurs within a few weeks.

So it's imperative that we determine as early as possible in the life of a new-born whether it suffers from phenylketonuria. Fortunately, this can be realised by measuring the concentration of phenylalanine in the blood. If the concentration is above a certain value, it confirms phenylketonuria. If the baby then follows a strict diet which contains as little phenylalanine as possible (a low-protein diet), phenylalanine will not result in a toxic concentration, damage is therefore prevented and the new-born has a much healthier life ahead of it.

Phenylketonuria is a relatively common disorder: its cause, diagnosis as well as treatment are clear. The reason for this, is that phenylketonuria can be traced back to a single defective gene, whereby it prevents the conversion of a single metabolite; also known as monofactorial disease.

I am definitely not saying that it's easy to have phenylketonuria and we were very pleased with the good result of both our daughters' analysis from the heel prick.

2. The system perspective

However, there are quite a few diseases hidden behind a much more complex issue. An example of such is asthma. This chronic respiratory inflammatory disease is a common occurrence. Caused by a combination of complex and poorly understood genetic and environmental factors. There is therefore no ‘asthma gene’, which by being defective is responsible for phenylketonuria [2]. There is no cure for asthma; its symptoms however can be treated. Other examples of these types of disorders include diabetes, Alzheimer’s and vascular diseases. These are multifactorial disorders and can only be understood if we study all the affected underlying processes in conjunction.

Figure 2 presents a simplified metabolic scheme in the form of a metro network. Although this is a highly simplified representation of human metabolism, its complexity is still impressive. Hidden issues occur in one part of the metro network, as a result of which some subways do not arrive or arrive late; this can result in unpredictable effects to other parts of the network. The relationship between cause and consequence of such type of effect can only be linked if the entire metro network is considered.

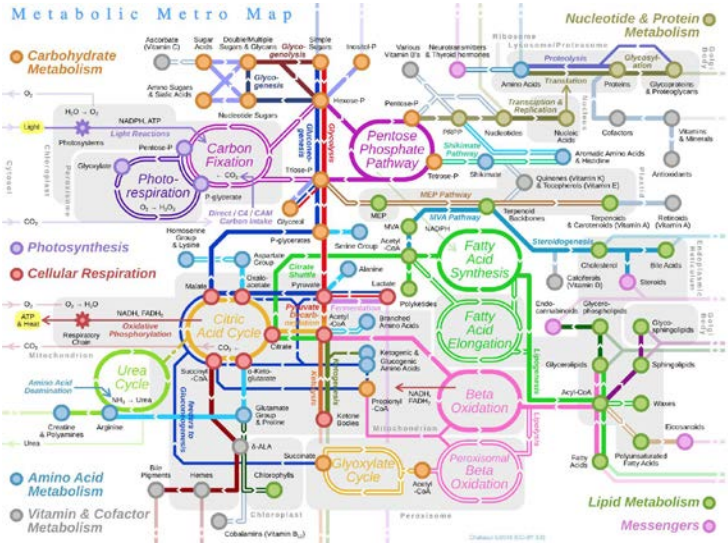

Figure 2 The ‘Metabolic Metro Map’, a schematic representation of human metabolism (source: www.wikipedia.org [3])

To illustrate an example from the world of birds, something that has fascinated me my entire life. Figure 3 presents a flock of common starlings *(Sturnus vulgaris)*. They are disrupted by a bird of prey, the peregrine falcon *(Falco peregrinus)*. It emerges out of nowhere, and dive bombs the starlings, which immediately start to swarm. By forming a constantly changing swirling cloud, they make it very difficult for the peregrine falcon to concentrate on a single target.

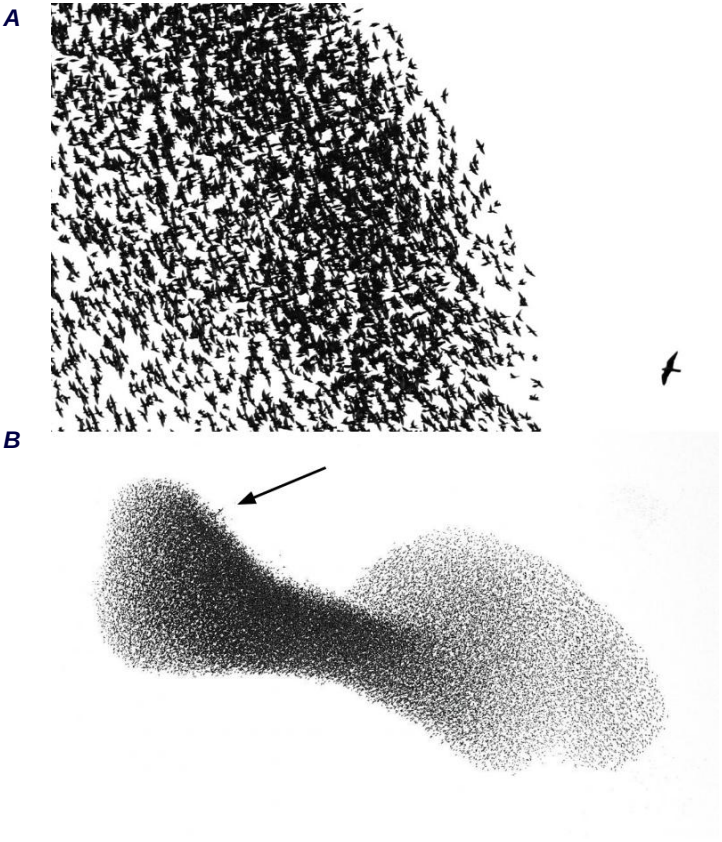

Figure 3 presents a flock of starlings (Sturnus vulgaris) *reaction to a peregrine falcon*

(Falco peregrinus)

A) part of a starling swarm with a peregrine falcon. Photo: Tobias Dansen.

B) the entire starling swarm with peregrine falcon indicated by the arrow. Photo: Manuel Presti.

Only a portion of the starlings directly react to the peregrine falcon (Figure 3A). Most of the starlings have however not noticed the peregrine falcon. They are only reacting in response to their neighbour's behaviour. Rapid communication takes place within the cloud of starlings. There are countless interactions which lead to a swirling ball of starlings, which as a result create many fantastic shapes, as presented in Figure 3B. If we only studied one or a few individual starlings we would never fully understand the complexity of the interactions within a starling cloud. In fact, we would not be able to even see the starling cloud. We can only study starling swarms by looking at the entirety of it.

Incidentally, there is much research being carried out on starling swarms by biologists, engineers, physicists and mathematicians [4]. It is thought that the starling swarms patterns are universal and that understanding them, can provide fundamental insights on collective movements on other levels, such as cell migration, behaviour of bacterial colonies and even complex physical and chemical systems.

3. Metabolism is a complex chemical system

Our metabolic process, our metabolism, is a complex chemical system. Metabolites are small molecules that are both intermediate compounds as well as end products of metabolism. The metabolome can be defined as the complete complement of all metabolites found in a specific cell, organ or organism. Every cell, tissue, organ and organism as well as every community has its own metabolome. The composition of the metabolome varies and is depended on the state in which the biological system is located [5]. Metabolomics is the scientific study of the metabolome of a given biological system. In this context, much attention is often paid to other small molecules which influence the metabolism, such as medicines, food components and contaminants [6]. Furthermore, analytical chemistry enables us to examine complex multifactorial processes within the metabolome. In other words, the metabolome is a collection of small molecules possessing great potential.

Figure 4 presents the generic workflow of metabolomics. Social issues are translated into a specific biological question. Later on in my lecture I will be touching on various examples. Next, an experiment is designed to adequately examine this question. Samples are taken which can be prepared so they can be subsequently measured. A large amount of data is processed so that it can be analysed. Eventually, the results of the research will be interpreted and the research question can then hopefully
be answered. In any event, it's a certainty that new questions will always arise when one is searching for an answer.

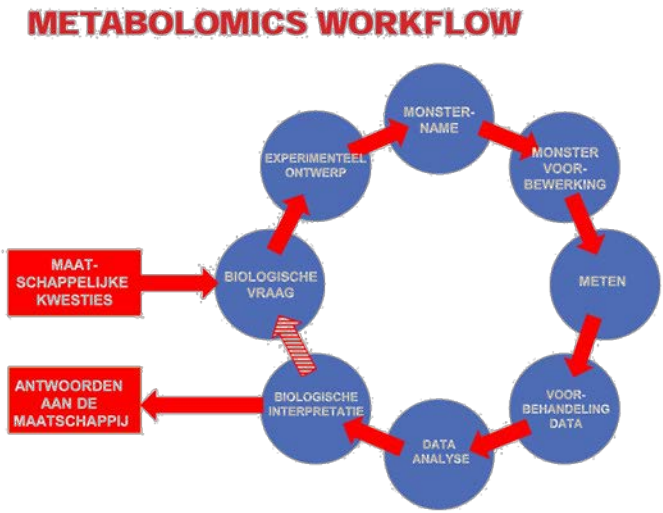

Figure 4 The metabolomics workflow

A very important area of research that will benefit from metabolomics is *'personalised medicine*'. Simply take a look around you and it's evidently clear that every individual is unique. However, we not only differ on the outside, but also on the inside. We can examine this with metabolomics. Your metabolome is the result of your DNA and your characteristics, such as age and gender, as well as your walk of life, such as your lifestyle and diet (Figure 5). For this reason, the metabolome is also referred to as the chemical phenotype, and tells us what we chemically 'look' like on the inside. This includes a wealth of information which answers fundamental questions e.g., 'Will this medicine work for this patient?', 'What is the optimal dose for this patient?' and 'What is the most likely course the disorder will take with this patient?'

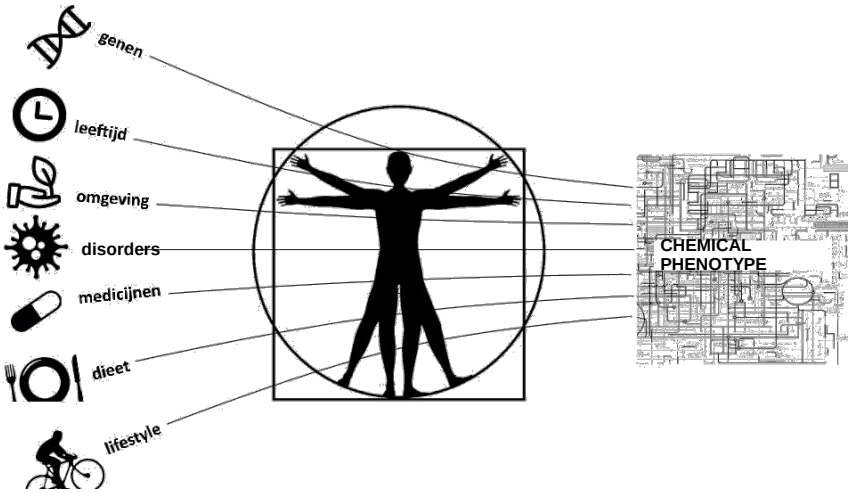

Figure 5 The metabolome, or chemical phenotype, expresses genes, age, environment, disorders, medicines, diet and lifestyle, among other things.

Other areas of research that are boosted by metabolomics are forensic science, ecology as well as environmental and nutritional science. I will elaborate on this later on during this lecture.

4. Analytical chemistry: the driving force behind metabolomics

The crucial question is: how can we measure the metabolome?
The challenges were enormous. The latest update on the Human Metabolome Database contains no less than 114,000 various metabolites [6]. These are all small molecules, but their diversity is enormous. Some of these occur in very high concentrations, others in very low concentrations. Imagine throwing a lump of sugar in a salt pool; some metabolites in our body are just as diluted as the sugar in the pool! Others come up noticeably in the salt concentration. Imagine that this is an outdoor swimming pool that has not been cleaned for years, that has accumulated waste and which has established all types of life forms. Somewhere in this molecule soup there is a hidden sugar cube...

Now analytical chemistry comes into the picture.
Analytical science or analytical chemistry is the part of chemistry that deals with the analysis of chemical compounds in mixtures [7]. This involves examining which substance is present as well as the concentration of such substance. Over the years, its analytical-chemical techniques have increasingly improved. The differentiation has become so great that thousands of substances can be measured in a single sample. Modern analytical equipment is so sensitive that we are able to measure less than one nanogram per litre. A nanogram represents one billionth of a gram. Furthermore, analytical techniques have progressed and are so much faster; in the near future we will be able to virtually measure in real-time.
The results of such developments were not overlooked in the heel prick. Earlier on, I mentioned the cause of phenylketonuria and that we can determine whether or not a new-born is healthy by testing the new-borns' blood. In 1962 Robert Guthrie, an American scientist, developed a test with which phenylketonuria could be diagnosed [8]. A blood sample was introduced to a specially grown bacterium during this test. As soon as this bacterium comes in contact with the blood from a phenylketonuria patient, it will start growing and can be determined under a microscope within 24 hours. Phenylketonuria, however, is not the only congenital metabolic disorder. There are a few dozen, each with its own unique cause and accompanying metabolites. The bacteria of Robert Guthrie only responds to phenylalanine and are therefore not suitable for the detection of other metabolic disorder which concern other small molecules. With modern analytical-chemical techniques, the small amount of blood collected from the heel prick can now be examined for no less than 19 different metabolic disorders, including phenylketonuria [9]. I don't expect it will only remain on a total of 19 disorders.

Thanks to advances in analytical chemistry which develop even faster chemical analysis, more accurate and sensitive measurement systems have appeared with which we can now measure thousands of metabolites in a single metabolomics sample. Analytical chemistry is thus the driving force behind metabolomics. Developments, particularly in mass spectrometry and separation techniques have been crucial. Allow me to explain.

Metabolites are small molecules. Molecules consist of atoms. Every atom has a unique mass and therefore almost all molecules, including metabolites, have a unique mass. In recent decades, better devices have been introduced which enable us to determine the exact mass of metabolites: mass spectrometers. A mass spectrometer can determine significantly small differences in mass between molecules. Conversely, a mass spectrometer is capable of measuring the difference between a car with a traffic fine under its windscreen wiper to a car without, in other words a single gram per thousand kilograms. Thanks to the capability of mass spectrometry, we can identify metabolites and be informed as to what type of molecule a particular metabolite is. We call this a qualitative analysis.

When a number of metabolites are introduced into a mass spectrometer all at the same time, they disrupt each other's signals and the results become unreliable. Thanks to modern separation techniques, i.e., ultra-performance liquid chromatography, capillary gas chromatography and capillary electrophoresis, we are able to introduce metabolites from a sample in the mass spectrometer one by one. This allows us to determine in which concentration metabolites occur. We call this quantitative analysis.

5. The Metabolomics research group: the connection between the analytical chemistry of the university college and that of the university

Since 2006, at the Leiden Academic Centre for Drug Research (LACDR) of the Leiden University, in the Analytical BioSciences & Metabolomics department of Professor Thomas Hankemeier, I have been developing analytical chemical methods to increase the effectiveness of metabolomics.

The Leiden University College established the Metabolomics research group in 2016. Marja Krosenbrink-Gruijters, the Chemistry Educational Manager, has been an important driving force in this regard. She noted the importance of metabolomics and the role that modern analytical chemistry plays and thus the relevance of metabolomics in the Chemistry program.
For myself, it was a golden opportunity to form a research group together with students and teachers from Leiden University of Applied Sciences and turn it into a successful endeavour that is part of a network that elevates metabolomics to a higher level, both scientifically and educationally.
On 7 September 2016, before dawn, I woke up in a holiday home in Schiermonnikoo where I was staying with my family. Seven hours later, after a boat trip, car ride, bike ride and change of clothes, I arrived at room G3.073 to give a presentation as part of my application for the position of Metabolomics lecturer. And now I stand before you to present my lecture. A fantastic feeling. As a lecturer, I find myself on the cutting edge between education, science and the practice thereof. I work with teachers, doctoral candidates, students, companies, universities and other colleges. I feel like a kid in a candy store.

Before the research group was established, there was little connection between the university's analytical chemistry and the actual university, even though they were a mere stone's throw apart from each other. That connection is currently well established.

The Metabolomics research group's objective is to develop and use new analytical methods to answer complex questions my means of detailed chemical information.

6. Metabolomics research carried out at the Leiden Academic Centre for Drug Research (LACDR)

The collaboration between the research group and the Analytical BioSciences & Metabolomics department of the university is of great importance to realise the research group's objective. I am scientifically rooted in this department. Working at the LACDR offers me an extremely inspiring environment, full of cutting edge technology and ambitious as well as intelligent people from all over the world. The Analytical BioSciences and Metabolomics department's ambition is to develop innovative analytical strategies for metabolomics-driven health monitoring and system biology studies. This is very socially relevant and I consider myself lucky to have been able to contribute to this for over 12 years and that I can continue to do so as a Metabolomics lecturer.

Over the past 12 years, much attention was paid to the use of electric fields to manipulate metabolites in such a way that they can be properly measured. A large part of the metabolites are loaded, and will thereby move when they are exposed to an electric field This phenomenon is called electromigration and it offers all kinds of interesting options. For example, we can separate metabolites in a thin glass tube, whereby they are delivered to the detector one by one allowing them to be properly measured. This is capillary electrophoresis. By allowing the electromigration of metabolites in different immiscible liquids, we can concentrate them at lightning speed, thereby making it possible to measure them in extremely low concentrations. This is electrical extraction. The potential of electrical extraction was first recognised in the 90s by Ubbo Tjaden, my co-supervisor [10]. My doctoral research focused on developments in the metabolomics electrical extraction method [11-14].

We can measure metabolites in extremely small samples with electrical extraction assistance, even if they occur in a low concentration. Furthermore, electrical extraction is a high-speed technique, and therefore useful in studies in which many samples must be measured. This is the basis for an important part of my research at LACDR/UL. Over the years we have built various innovative set-ups, i.e., automated electrical extraction [15] and electrical extraction in a chip [16]. Figure 6A presents the principle of electrical extraction on a chip. The process that metabolites undergo in this chip is made visible with a crystal violet purple dye (Figure 6B). A liquid containing crystal violet passes through a thin channel in a chip. As soon as this liquid passes through the electric field, the crystal violet molecules migrate at lightning speed to another liquid, which is stagnant. This results in the crystal violet accumulating there. The implication is that this

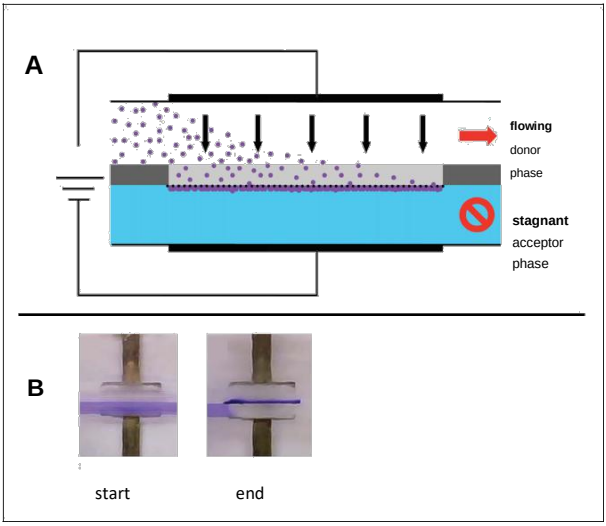

Figure 6 A) the operating principle of electrical extraction on a chip B) electrical extraction of the crystal violet dye. From [16]

the process can be used to concentrate metabolites, which makes them easier to measure.

I am currently the co-supervisor of five doctoral candidates from the Analytical BioSciences and Metabolomics department.

For example, I assist Yupeng He, a doctoral candidate, with developing a metabolomics method which can be used to better understand muscle ageing. We are building a device that can measure extremely sensitive metabolites in very small muscle samples. The device concentrates metabolites using electrical extraction, separates them using capillary electrophoresis and detects them with mass spectrometry. Amar Oedit, a doctoral candidate, has carried out crucial preliminary work on this subject.

Zhengzheng Zhang, a doctoral candidate, is developing advanced technology to measure fats which appear in our body. You may think of fat as being the fat you eat, is in oily hair or obesity. However, fat is not just fat. There are thousands of different types of fat molecules [17]. Fats form all the important processes in our body [18].

Disruptions in one's fat metabolism may lead to illnesses. There is evidence that disruptions in the transport of fat molecules are involved in the development of (onset of) Alzheimer's disease. To be able to examine this, new analytical-chemical technology is required which details the transportation of fats through our body. This is what Zhengzheng, together with postdoc Yulia Shakalisava, are developing. Simon Leygeber, a doctoral candidate, in collaboration with DSM, is developing, among other things, metabolomics methods to unravel the chemistry of taste. By combining metabolomics data with data from taste panels, we develop a pipeline to discover flavour substances.

Farideh Hosseinkhani, a doctoral candidate, develops methods to reliably study the metabolism of the intestinal flora. Our gastrointestinal tract is inhabited by one to two kilograms of microorganisms, an estimated one hundred thousand million (in numbers this is the number one followed by 11 zeros; 100,000,000,000) [19]. This is more bacteria than you have in your body’s cells! There are several thousand different types of microorganisms known in the human gastrointestinal tract, most of which are bacteria. This is the intestinal flora (bacteria), also referred to as the microbiome. This intestinal flora plays a very important role in digesting our food. All this bacteria form a diverse variety of metabolites on a large scale. However, it has become evident in the last few years that the composition of microbiome is related to various disorders of the host cell, therefore ours. Examples of such are obesity, immune disorders and even central nervous system disorders such as Alzheimer's disease [20]. The activity of the microbiome can be examined in detail with the help of metabolomics and connections to the development of such disorders can be confirmed. Later on, we will further discuss the intestinal flora.

7. Metabolomics at the Leiden Centre of Applied Bioscience (LCAB)

The Metabolomics research group was formed two and a half years ago. The most important research themes that the research group focuses on are forensic science, biodiversity and personalised medicine. Since September 2017, the research group has been part of the Leiden Centre for Applied Bioscience, the research centre of the Science & Technology faculty. Within this research centre there are four research groups who carry out research. Advanced measuring and detection techniques are used for analysing samples from various types of environments and/or organisms: air, water, soils, humans, animals, plants and foods. Over the past year and a half, the LCAB has grown into a close-knit team working together on issues in the area of biodiversity and health. The office garden, where we are all seated, both students and the lecturer, ensures for an easily accessible, social, pleasant, efficient, and yes, noisy workplace. Here university research is carried out as university research was intended to be carried out: apply practically-oriented questions on the cutting edge of education and research from the MKB, conducted by teachers and students and supported by an analyst (Figure 7).

Figure 7 Chemistry lecturer Maarten van der Horst and analyst Richard van Rijn with an LC-MS

The research groups' lab became extremely well equipped in a short period of time. Manager of Applied Science Danny Dukers played a crucial role in this. We currently have two liquid chromatographs linked to mass spectrometry, a gas chromatograph linked to olfactory detection and mass spectrometry as well as a capillary electrophoresis device linked to fluorescence detection. You don't have to remember all this. The bottom line is that we can carry out our objective: to develop and use new analytical methods to answer complex questions with detailed chemical information.

8. Metabolomics in the microbiome

The following is an example of such a complex question: what is the influence of the intestinal flora on the performance of endurance athletes? Earlier on, I told you about the microorganisms in our intestinal flora: it's a complex ecosystem inside there! Approximately 40% of endurance athletes suffer from gastrointestinal disorders during or in the hours after exercise. Cycling fans among you will probably still remember Tom Dumoulin, who on 23 May 2017 in the Giro d'Italia's Queen Stage suddenly jumped from his bicycle to relieve himself on the roadside. His victory in the Giro seemed to be in danger, but fortunately it all worked out in the end.

The primary objective of the 'With help of my little friends?' research project, which was set up by Dionne Noordhof, a Scholarly Scientist, to examine whether there is a relationship between the composition and activity of the intestinal flora and the development of gastrointestinal disorders during endurance exertion activities. By analysing the DNA in faeces, we are able to determine which microorganisms are present in it. Every type of micro-organism has an unique DNA. We are able to obtain a picture of the metabolic activity of the microbiome during exercise by analysing certain metabolites (amino acids and derivatives) in the athletes' faeces. We are very curious to find out if the microbiome of runners who suffer from gastrointestinal complaints differs from that of complaint-free runners and whether the microbiome can be influenced in such a way that the gastrointestinal disorders of the athletes are reduced through diet.

9. Metabolomics in nutrition

Metabolomics is well suited for research concerning nutrition and preparation of food.
Karsten Kaspers, a chemistry lecturer, together with Sarah Rothman and Frans Zweipfenning, scholarly students, have developed a method to determine the content of minerals in cherry vine tomatoes. It appears that tomatoes on the vine differ from each other due to their positions. This type of information is important for tomato growers. The subsequent step with metabolomics is to examine which are the underlying biochemical processes.
My research group works closely with the university's newly established Foodlab, where the history, physics and chemistry of preparing foods are examined.
The taste experience of food is largely determined by our sense of smell. Gas chromatography is a separation technique that is ideally suited for the separation of fragrances. The mass spectrometer can identify the fragrances. However, a mass spectrometer cannot smell and therefore we use an olfactory detector, or a human nose (Figure 8). After the fragrances are separated, they can be smelled one by one and then identified by the mass spectrometer.
The first results, obtained by analysts Richard van Rijn and Sietse Kuipers in collaboration with Martin Brussee, a scholarly student, are promising.

Figure 8 Student Martin Brussee working as an olfactory detector at a GC-MS.

10. Metabolomics in the fingerprint

There are quite a few situations in which only very small samples are available and detailed chemical information is needed as much as possible. A striking example is the fingerprint.

Fingerprints are iconic for forensic investigation. The line patterns in a fingerprint are unique to every person. However, perfect fingerprints that lead to a perfect match are rare occurrences. A fingerprint contains a range of small molecules which provide information on the person who left it behind. It is plausible that every individual has a unique chemical profile in their fingerprints [21]. A certain type of metabolite, namely the fatty acids, is already known to be in fingerprints, although its exact composition is not known. This information can be crucial in solving crimes. Obtaining as much metabolic information as possible from a fingerprint is a major challenge which requires an innovative analytical-chemical approach. Figure 9 presents the overall purpose of the study.

Chivany Soemai, a chemistry student, guided by chemistry lecturers Anton Heemskerk and André van Roon are developing an innovative method which 1) facilitates working with very small samples and 2) concentrates the substances to be measured so that they can be measured properly.

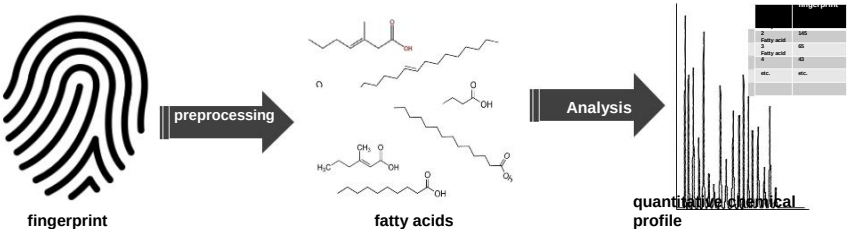

Figure 9 Schematic representation of the "Fingerprint 2.0" study

The method is based on three chemical phenomena:
1) some liquids form emulsions when mixed,
2) liquids have different freezing points,
3) fatty acids dissolve much easier in one type of liquid than in another.

The method works as follows. Fingerprints are first collected with a cotton swab. We then dissolve the fatty acids in a liquid, such as acetone, and add water. As a result, they become highly diluted, which will complicate the measurement. However, we add a small drop of the liquid undecanol, which has two special properties: fatty acids dissolve extremely well in it and it freezes at 19˚C. The combination of acetone, water and undecanol results in a special effect: an emulsion is formed. Two normally immiscible liquids nevertheless form a stable mixture thanks to the presence of a third liquid. A well-known emulsion is mayonnaise, in which oil and vinegar mix well due to the egg yolk. In an emulsion, molecules can very easily ‘interchange’ from one liquid to another. In such a case, the fatty acids end up in the undecanol. The emulsion is then cooled and the undecanol freezes. An easy to work with ‘ice cube’ is created in which the fatty acids are concentrated. Figure 10 details this procedure. The ice cube is then analysed. This technique is referred to as the *‘dispersive liquid-liquid microextraction based on the solidification of the floating organic phase’,* abbreviated as DLLME-SFO.

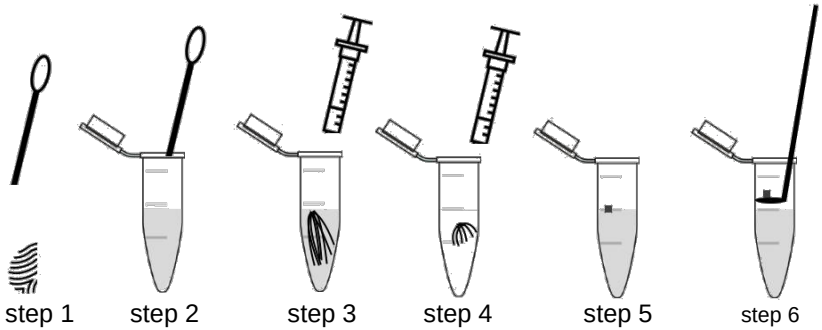

Figure 10 Schematic renditions of fatty acids in a fingerprint with DLLME-SFO.
Step 1: collect fingerprints with a cotton swab.
Step 2: dissolve the sample in acetone.
Step 3: add water.
Step 4: add undecanol.
Step 5: freeze undecanol.
Step 6: collect undecanol ice cube with fatty acids.

A KIEM-HBO project, *'Vingerafdruk 2.0: het meten van chemische patronen in vingerafdrukken' ['Fingerprint 2.0: measuring chemical patterns in fingerprints,']*, was started by the research group, to examine the potential of such an approach to the chemical analysis of fingerprint. We conduct such studies in collaboration with the Netherlands Forensic Institute, Leiden University (the LACDR) and Interscience Ltd. The project is funded by the Regieorgaan Praktijkgericht Onderzoek SIA [SIA Directorate for Practice-oriented Research].

11. Metabolomics in livers-on-a-chip

We also apply a similar procedure when determining medicines and their metabolites in small samples obtained from organs-on-a-chip. These are microorganisms that grow in microfluidic channels [22]. Microorganisms are an extremely suitable means for testing medicines. In the near future, it will be possible to cultivate cells from your own blood
from the micro-particles of your choice and subject them to tests and analysis. This offers enormous possibilities for putting personalised medicine into practice. In 2016, this technology was recorded as one of the top 10 breakthrough technologies by authoritative World Economic Forum [23].

The company MIMETAS developed organs-on-chips, which resulted in the OrganoPlate®, in which organs can be exposed to medicines. The Organoplate® contains up to 96 micro-organs and is extremely suitable for testing many types of medicines quickly. One of the micro-organs grown herein is the liver: the liver-on-a-chip. The liver is a crucial organ for drug related research. Various types of medicines are converted to metabolites in the liver. For both the safety and efficacy of a potential new medicine, it is important to know how quickly it is broken down in the liver and which metabolites are produced thereby. There is currently no reliable method available to study the biotransformation of drugs in livers-on-a-chip. Chemistry lecturers Natasja Carol-Visser and Maarten van der Horst are developing an analytical-chemical method which makes this possible. Hereby we initially focus on the commonly prescribed painkillers diclofenac and ibuprofen. A functional liver-on-a-chip will be able to convert diclofenac and ibuprofen into their metabolites. Figure 11 presents a diagram of how this works. There are extremely small channels in an Organoplate®, in which livers are grown in the centre channel. A liquid containing nutrient is passed on either side of each tissue. This creates an extremely real-life micro-environment [24]. The medication is added via the channels. The liver converts such medicine into metabolites and excretes them in the fluid that is collected for analysis. These samples are only 30 microliters in size and contain low concentrations of metabolites.

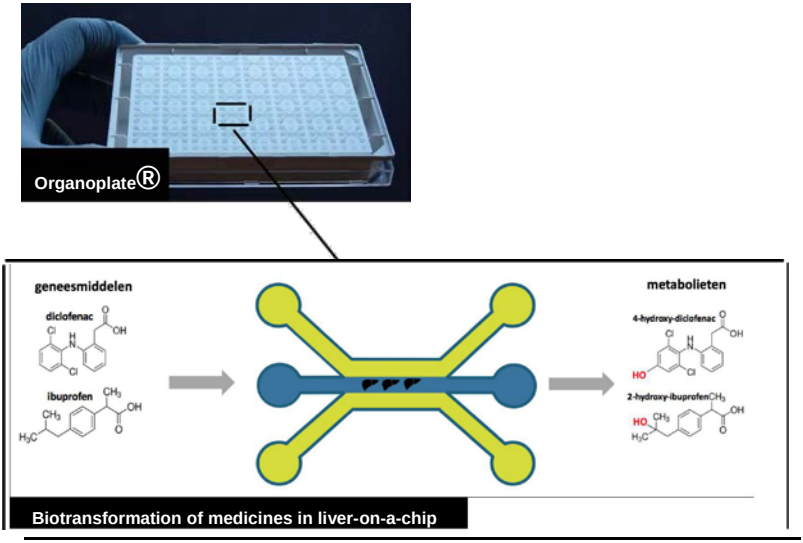

Figure 11 An Organoplate® and a schematic representation of diclofenac and ibuprofen biotransformation in a liver-on-a-chip.

The KIEM-HBO research group project, *'Liver-on-a-chip: development of measurement methods to determine biotransformation of medicines in livers-on-chips'* is a collaboration project between MIMETAS and Bruker Daltonics as well as Leiden University. This is funded by the Regieorgaan Praktijkgericht Onderzoek SIA [SIA Directorate for Practice-oriented Research].

12. Environmental metabolomics

Until recently, metabolomics has been used primarily for the benefit of humans. My ambition is to also use the great potential of metabolomics in researching complex systems in ecological and environmental sciences as well as to apply these to biodiversity issues. I want to use metabolomics to examine interactions between organisms and their environment, using environmental metabolomics (eMetabolomics).

Ultimately, humans will also benefit from this. Our well-being is not only determined by the processes that take place in our body but also by processes outside of it, via the air we breathe the water we drink and the food we eat. Our health is markedly influenced by our environment. Furthermore, our environment is markedly influenced by our presence. Both aspects can be studied in detail by measuring the community metabolome. This is the collection of metabolites that are secreted into their environment by all the organisms in an ecosystem. This reflects the influence of various factors i.e., climate, pollution and invasive exotic species on the ecosystem in which we live (Figure 12). To measure the community metabolome, innovative on-site sampling techniques and metabolomic methods for new types of samples, i.e., surface water and air as well as samples from all kinds of organisms, must be developed.

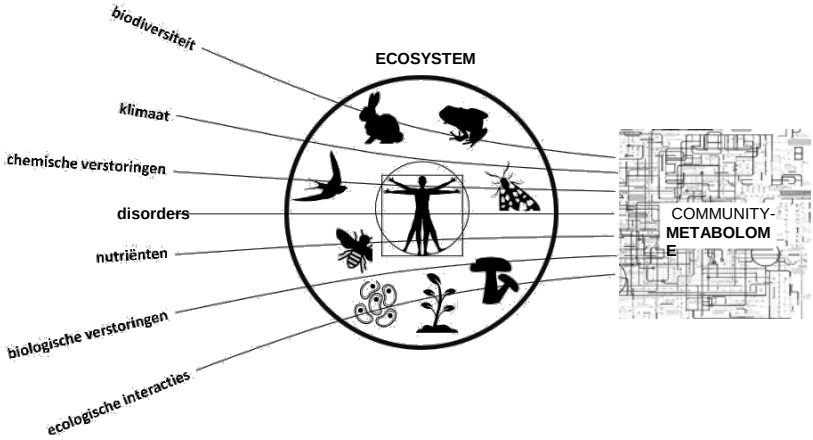

Figure 12 The community metabolome expresses, among other things, biodiversity, climate, chemical disturbances, diseases, nutrients, biological disturbances and ecological interactions.

I believe that with this research, I can contribute to a healthy living environment in which healthy people can coexist with a healthy nature, which includes high biodiversity. The Metabolomics research group forms a link between scientists (analytical chemists, environmental scientists and ecologists) and professional practices (governments, ecological consultancy firms, water boards, etc.). Let me present you with an example of a project in which we apply metabolomics in such a way at Leiden University.

I have mentioned to you before that every cell, every tissue, every organ, every organism, every community contains a metabolome. Well, water from an aquatic ecosystem, i.e., a ditch, also has its own metabolome. There is a large variety of organisms in an aquatic ecosystem which release metabolites into the water [25]. Examining this metabolome is referred to as environmental metabolomics (eMetabolomics). With eMetabolomics we can study the metabolome of the aquatic ecosystem and gain insight into its state, as well as the consequences of disturbances [26]. There is also DNA in the aquatic ecosystem: environmental DNA or eDNA. Every type of organism generates unique traces of eDNA. By analysing eDNA in surface water, we can establish which types of species are present. A molecular image combines information from the eDNA and eMetabolomics analysis (Figure 13).

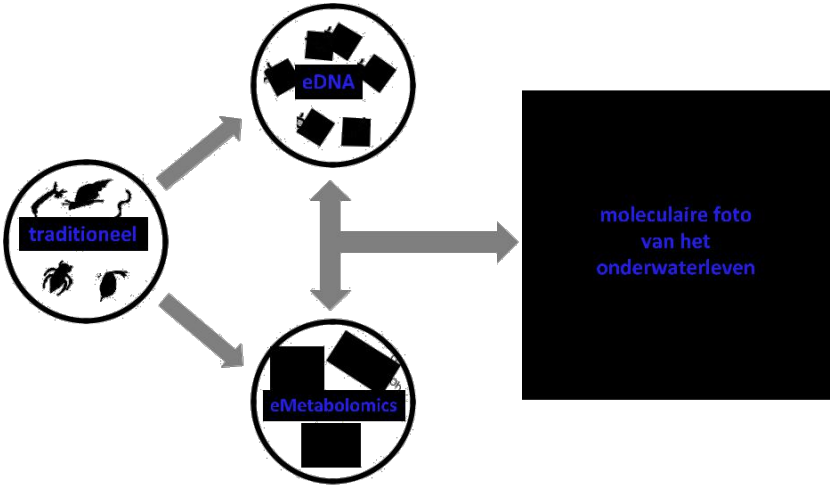

Figure 13 A molecular image combines information collected from an analysis of DNA (eDNA) and metabolic products (eMetabolomics) in the water and can therefore act as an early warning system.

eDNA can provide us with information on which species are present in an aquatic ecosystem. eMetabolomics can provide us with information on which processes are taking place. In a manner of speaking, with the help of eDNA the presence of water fleas can be demonstrated and with eMetabolomics it can be demonstrated that these water fleas reproduce.

We apply this approach in two case studies which examine the effect of biological and chemical disturbances on the ecosystem. In both studies we collect water samples from which we measure the metabolome. Furthermore, chemistry lecturer André van Roon and students Chivany Soemai and Faysal Sbaa are developing the eMetabolomics methods.
For biological disturbance we focus on the red American crawfish *(Procambarus clarkii)*. This is an invasive exotic species which is currently extremely common in many locations, specifically in the Western part of our country. You may have seen one before. The Leiden canal is swarming with them.
It has long been known that crawfish can communicate with each other by using chemical signals, or pheromones [27]. The pheromones are secreted during, among other things, territorial battles and mating. We believe that we can develop an analytical chemical method that can measure the occurrence and behaviour of the red American crawfish.

Figure 14 presents the purpose of the study. Initially we will study the crawfish in aquariums by collecting water samples and analysing them. We will then examine the chemical communication between crayfish in aquariums and mesocosms; these are small ponds that contain a common form of an aquatic ecosystem. We will eventually apply the developed eMetabolomics method to natural surface water.
We hereby focus on 'biogenic amines'. This is a diverse group of biological origin compounds which possess at least one amine group. These are organic bases containing a low molecular weight which play an important role in the metabolism of various types of organisms. The following are the two biogenic amines which are known to play a role in chemical communication in crustaceans: serotonin and octopamine [28, 29].

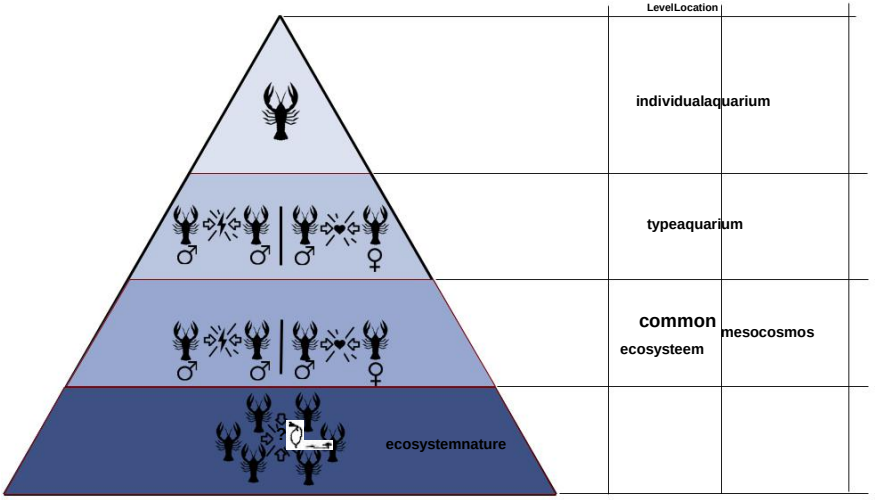

Figure 14 Study design on the development of eMetabolomics methods, which can monitor the occurrence and activity (e.g., reproduction) of crawfish.

If we uncover crawfish-pheromones, we could identify and test these attractants, analogous to how this is already often done with insects. This could be used by water controllers to develop special crawfish traps in which crawfish are lured into using pheromones. These traps can also be used, e.g., to contain pests.

We will also study the chemical disturbance of the surface water by using eMetabolomics: the pesticide thiacloprid.

Alarming reports regularly appear regarding the decline in rural biodiversity [30, 31] this is often associated with pesticides, in particular neonicotinoids (referred to as 'bee poison'), which have a negative effect on the insect population [30] and insect-eating birds [31]. These noticeable changes in the ecosystem are the result of all types of processes which take place over a long period of time, mostly at unnoticed lower levels of the food pyramid. The ecosystem is currently so disturbed that bringing it back to a healthy state will take a considerable amount of effort and time.

There is therefore a need for a measuring system that can detect the disturbances of the aquatic ecosystem in its early stages, which such is still limited. Figure 15 presents the strategy with which we intend to develop an early warning system based on eMetabolomics in the Living Lab. The Living Lab is a test facility of the Centrum voor Milieukunde Leiden (CML) [Institute of Environmental Sciences (IES)] of Leiden University and consists of a testing field with 36 ditches.

Part of these ditches will be disturbed by adding thiacloprid. All the ditches will then be monitored manually and by using the eDNA. Hereby the entire community, from micro-organisms to vertebrates (animals), will be charted in order to gain insight into the changes which take place. The ditches are also regularly sampled for eMetabolomics measurements. At the end of the exposure experiment we will go back to measure the effect of thiacloprid on the aquatic ecosystem by means of eMetabolomics. First, we will identify metabolites in samples collected at the end of the experiment: heavily disturbed ditches. We will then determine these metabolites in much older samples, to identify how early on we are able to detect the effect of thiacloprid. I expect that the effect of thiacloprid can be measured quickly in the metabolome of the ditches [26, 32].

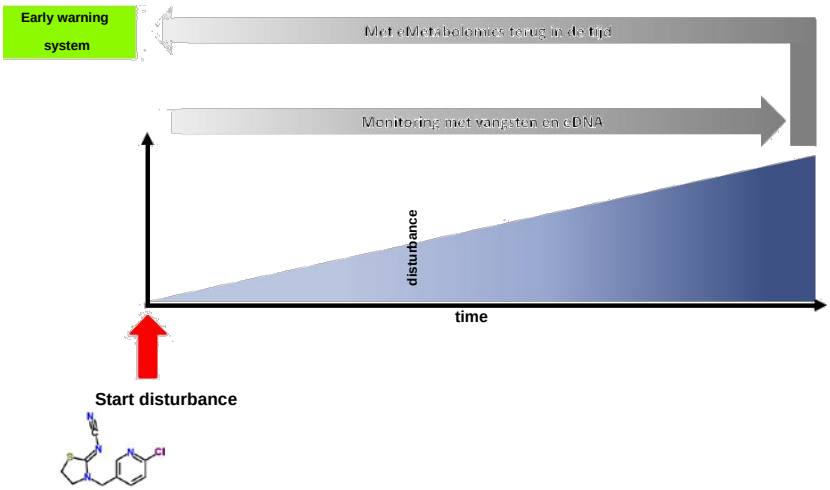

Figure 15 Study design for the development of an early warning system for disruption of the aquatic ecosystem using eMetabolomics, eDNA and catches

The recently launched RAAK-Publiek project, '*eDNA and eMetabolomics: molecular images of underwater life',* funded by Regieorgaan Praktijkgericht Onderzoek SIA [SIA Directorate for Practice-oriented Research], is a collaboration with the Centrum voor Milieukunde Leiden (CML) [Institute of Environmental Sciences (IES)] of Leiden University, Naturalis, the Department of Animal Ecology (VU Amsterdam) the following companies: Baseclear, Bruker Daltonics, Bureau Waardenburg, Witteveen + Bos, the KWR [Watercycle Research Institute], Hoogheemraadschap [municipality or water board] van Rijnland, Holland Rijnland and the Wetsus water institute.

I hope the above examples have convinced you that metabolomics will be of great value to society in a broader sense.

13. Metabolomics and education

However, this can only really be realised if we are able to deliver well-educated students who have the skills to work with technology. For this we require an active learning and working environment. Furthermore, the research group, together with the lecturers from the Chemistry program, developed Beginner ‘Metabolomics’, which is currently being implemented for the second time around and is made accessible to Chemistry, Bioinformatics and Biology & Medical Laboratory Research students. This beginner stage is our platform for translating our research into education. The students are taught in a system by the lecturers of the Chemistry program team
in thinking, modern analytical chemistry and multivariate data analysis. They carry out their beginner internship in a metabolomics lab here in the BioScience Park. After completing this beginner stage, students are ready to work at a metabolomics laboratory, or to move on to Master Biopharmaceutical Sciences: Systems Pharmacology. Our objective is to make the students as autonomous and independent as possible and to stimulate their enthusiasm for the discipline as much as possible, to enable them in making a substantial contribution to its future development.

Thanks to the research group, it is easier for students to move on from the university to an internship or to enrol in a master’s degree at the university, or to start a job as a metabolomics analyst. Expertise of the latest scientific developments in modern analytical chemistry and metabolomics flow from the university to the college and are included and processed in the curriculum. Scientific cooperation is currently applied: Chemistry lecturer Alphert Christina is developing a chemical reaction with PhD student Cornelius Willacey to measure metabolites more sensitively. Everything contributes to developing new methods, new expertise and training enthusiastic competent students. The Leiden BioScience Park is the place to be for metabolomics and analytical chemistry!

14. The beauty of complexity

For the past 40 minutes I have enlightened you with information on metabolomics. I have shared with you the importance of analytical chemistry whereby using the heel prick method, could, in the event of phenylketonuria save a life. I have shared with you the more complex, multifactorial disorders that can only be understood, if we are able to measure all the underlying factors.

If one looks closely at the problems and their solutions, then something will stand out. The problem and its solution are often a form of comparable complexity. In other words: common problems have common solutions while complex problems have complex solutions. If you zoom in on just one aspect of a complex problem, you stand a chance of missing the bigger picture and might never understand the system. Unfortunately, this is all too common in politics; I wish politicians would study a cloud of starlings more often, rather than just observing the peregrine falcon. There is beauty in complexity. Finding a solution to a complex problem requires cooperation, empathy and an open mind with regard to different opinions. This also contributes to the beauty of complexity, although unfortunately this is often undervalued.

Thanks to modern analytical chemistry, we are able to contribute to the solution of complex problems with metabolomics in various fields. I have presented you with some examples of such.
The chemistry of a fingerprint.
The metabolic activity of our intestinal flora.
The conversion of medicines through a miniature liver.
The metabolome of the aquatic ecosystem.

By studying small molecules we create large possibilities.

15. Acknowledgments

Dear ladies and gentlemen, I have reached the end of my lecture. I would not be here today without being part of a system. This system, wherein I am able to flourish, consists of colleagues, partners, friends and family.

First of all, I would like to take the time to thank the Executive Board of Leiden University for giving me the opportunity to conduct this lecture and for providing me with a lecturing position at this beautiful university. I feel truly honoured. I would like to extend my thanks to the Faculty Director Patrick Pijnenburg and the Chemistry Department Manager Marja Krosenbrink-Gruijters for their confidence in me upon my application, and thereafter. The environment at Leiden University has been warm and welcoming; people are considerate towards each other and extremely passionate. Danny Dukers, the manager of the Leiden Centre for Applied Bioscience, has been indispensable in setting up the metabolomics lab; with the equipment currently available we are able to carry out challenging research, also in the field of nutrition!

From day one, I have worked very closely with André van Roon. So close in fact, that I almost start missing his humour over the weekend. As an assistant lecturer, André is crucial to the Metabolomics research group. It's thanks to André. There is a beginner metabolomics group, we have excellent internship students and a great eMetabolomics project. And, last but not least, I can talk to André about birds as much I want.

Willeke de Boer made this day possible. This is not the first time she has set up an event, and she focuses on everything down to the smallest detail. She also manages my calendar and keeps chaos at bay. Thank you Willeke! Thank you for your caring nature towards all the employees and students, of the LCAB.
Willem van Leeuwen included me in his research even before I actually started working at the university. I really could not wish for warmer welcome. Walter Zuijderduin is very well versed in the world of grants and subsidies and speaks the language. It would never have been possible to submit three successful research proposals in two months without Walter. Bringing in leads on research projects is of course fun, but they must also be implemented. Fortunately, I am assisted by project managers Marijke Mostert and Aeneas Verrópoulos. I am grateful to Chantal Terstegen for her assistance with writing annual reports. I would like to thank Saskia Kanij for the beautiful website for the research group and Karin Plaatje for her major contribution to all the marketing and communication concerning this day.

The lecturer researchers are the backbone of the research group. André van Roon, Maarten van der Horst, Natasja Carol-Visser, Anton Heemskerk, Alphert Christina and Karsten Kaspers: thank you for your excellent work. Without you, the research would be nothing.

This also goes for the internship students. Sarah Rothman, Nick Jansen, Chivany Soemai, Martin Brussee, Emma Nienhuis, Faysal Sbaa and Frans Zweipfenning, thank you for your enthusiasm and hard work!

A metabolomics lab without working equipment would be waste. Fortunately, analyst Richard van Rijn ensures that students and lecturers are not short of anything for carrying out their experiments. Sietse Kuipers and Jurriaan Beckers have also contributed to this.

Without Regieorgaan Praktijkgericht Onderzoek SIA [SIA Directorate for Practice-oriented Research] funding this would not be possible and I am therefore very grateful to them for funding my research. To get your research funded, a strong consortium of partners from the public sector and SMEs is indispensable. I would like to thank all my cooperation partners for their confidence and willingness to participate in the research with the research group. There are too many to mention,although I would like to mention just a few:

Krijn Trimbos from the Centrum voor Milieukunde Leiden (CML) [Institute of Environmental Sciences (IES)], Berry van Hoorn and Johan Mols from Naturalis, Marcel de Puit from the Dutch Forensic Institute, Rob van der Heijden from Bruker Daltonics and Bas Trietsch from MIMETAS. I would also like to thank Merlijn van Rijswijk of the Dutch Metabolomics Institute for co-organising this day.

As wonderful as the LCAB is, I also cycle to the university while whistling. Working at the Leiden Academic Centre for Drug Research and, in particular, the Analytical BioSciences & Metabolomics group offers me an inspiring environment cramped with cutting edge technology and ambitious people from all over the world. I hugely enjoy being copromoter of Simon Leygeber, Zhengzheng Zhang, Yupeng He, Farideh Hosseinkhani and Amar Oedit. Never a dull moment! Thank you for all the in-depth discussions on various aspects of analytical chemistry, metabolomics and how life is in various places of the world. I also want to thank Ischa Bremer for bringing the potential of DLLME-SFO under my attention while following the first years' Analytical Chemistry course. Isabelle Kohler, thanks for many deep conversations about life while enjoying good coffee. I want to highlight Loes Beijersbergen, who, as office manager, has been crucial for the group for so many years. Thank you for caring so much, Loes.

Thomas, in a way it is a miracle that we are still working together after all those years. I consider myself lucky. I admire your energy, your optimism and your pace. It's sometimes hard to keep up! But when no one sees any possibilities, you still do. Thank you for your vision and for being supportive of my ambitions. I hope I can be a part of the Analytical BioSciences & Metabolomics group for many years to come. Hubertus Irth, thank you for appointing me as guest assistant-professor at the LACDR.

I am where I am thanks to my friends and family.

Ubbo, thank you for your mentorship. I learned much from you. Jan-Willem, I enjoy sparring about woodworking. To the members of Dr Agnaac and his dog Triptych, MSc app group, or Marten, Vivian, Bas and Marten, thanks for your humor, musicality and shared ornithological obsession.

Wouter, together we have been rustling storm clouds in the sky for 20 years, let's keep it up. Joost, you realise system thinking and put it into practice in a different way, I find that very inspiring. Wouter and Joost, it's hard to overstate how much you mean to me.

Coming from, as it is said so beautifully, a good family. Mom and dad, you have always encouraged me in my curiosity and laid the foundation for such. Ruben, Nynke and Irene, how wonderful that we can appreciate our families together. Ruben, I learn from your work experiences.

Maartje, without you I would be less than half what I am.

Yfke and Rosa, I love you very much and I am proud to be your daddy.

I am done speaking now.

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