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Dr Maria Riedner, Prof. Dr Sascha Rohn

From the genome via the proteome to the understanding of life

Prof. Dr Michalel Hecker
Prof. Dr Barbara Bröker

Light from fungi

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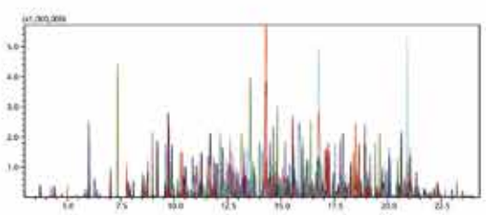

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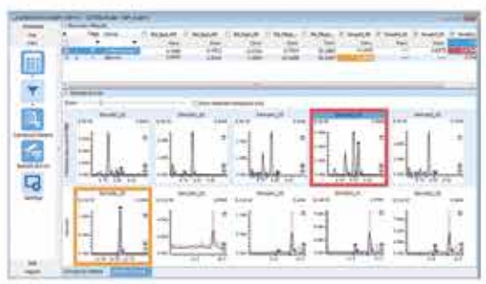

www.shimadzu.eu

Fear is in the air

The heart of Europe – the European Union is no longer working properly, and Brussels, its capital, is still trembling. Bombs, threats, dead and injured. Things that we never used to have to worry about, because they were happening in countries in Africa and Asia, are now on our own doorstep. – I too have a page from a newspaper with the "Je suis Charlie" logo hanging up in my office.

What we are experiencing is dreadful, and casts a shadow over Europe that has been noticeably absent for decades. We were and are busy with economic development, and the figures are looking good in most countries. Nobody can complain, especially in Germany. It runs and runs and runs – as the famous advertising slogan for the Volkswagen Beetle used to say. Although they have now thought out a slightly different strategy and things are not running quite as smoothly. This engine is misfiring.

Things change. The Rockefeller family is joining forces with climate protestors. Who would have thought it? Such immense wealth coming from oil. It went well for generations and now they are going in a new direction. Respect. But they certainly cannot ignore the fact that in the United States, there can be some serious compensation claims if companies or organisations deliberately conceal the risks. Prevention is better than paying again and again.

And then there is Donald Trump, the blond hooligan who could become the next US President. Hopefully it will remain hypothetical. We have also just heard that data in the US is no longer secure – if it ever was? The FBI has hacked Apple and now they have a tool that will give them access to all Apple data.

And all this to get you in the mood for a year that has hardly even begun. And there are some great events happening too. The Olympic Games, European Football Championships and as mentioned, the elections on 8 November 2016 to choose a new President of the United States. If the famous futurologist Matthias Horx is to be believed, retro is all the rage. The future and looking ahead are not so popular. Fashion, music, ideas – everything revolves around an idealised view of a past that was supposedly better than the present. – But according to the polls, more than two-thirds of the German people are looking ahead with optimism and that is our wish, is my wish, for you all.

→ **Jörg Peter Matthes**
CEO, Publisher

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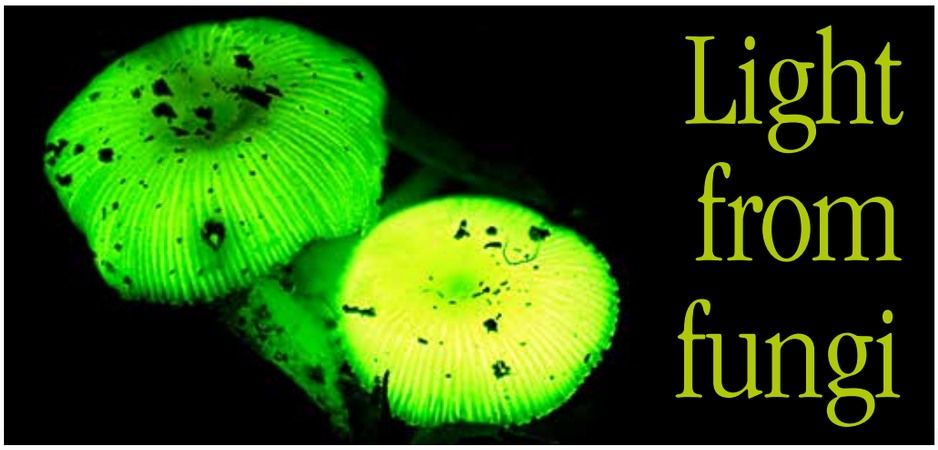

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profile

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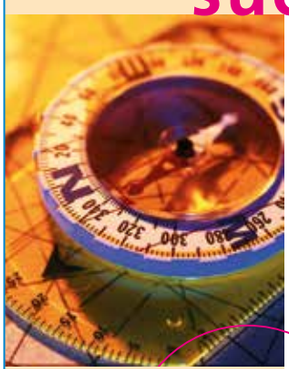

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market view

Companion Diagnostics

New collaborative venture between R-Biopharm AG and Merck KGaA

R-Biopharm AG has announced the signing of a collaboration agreement with Merck for the development of companion diagnostics. This agreement green lights the first collaborative venture between the two companies for the research, development and marketing of new companion diagnostics products. The project also establishes a general framework supportive of future partnerships with a large potential palette of therapeutic areas and a broad spectrum of technologies.

Companion diagnostics play a major role in the field of personalised medicine: they help to identify new, specific treatments that match the individual needs of the patient, so as to not only improve patient care but also reduce the overall costs involved in healthcare provision.

Financial aspects of this agreement were not disclosed.

→ **www.dgap.de**

Mergers and Acquisitions I

Bristol-Myers Squibb to Acquire Padlock Therapeutics, Inc.

Bristol-Myers Squibb Company and Padlock Therapeutics, Inc. announced that the companies have signed a definitive agreement under which Bristol-Myers Squibb will acquire all of the outstanding capital stock of Padlock, a private, Cambridge, Massachusetts-based biotechnology company dedicated to creating new medicines to treat destructive autoimmune diseases. The acquisition will give Bristol-Myers Squibb full rights to Padlock's Protein/Peptidyl Arginine Deiminase (PAD) inhibitor discovery program focused on the development of potentially transformational treatment approaches for patients with rheumatoid arthritis (RA). Padlock's PAD discovery program may have additional utility in treating systemic lupus erythematosus (SLE) and other autoimmune diseases.

The transaction includes upfront and near term contingent milestone payments of up to $225 million and additional contingent consideration of up to $375 million upon the achievement by Bristol-Myers Squibb of certain development and regulatory events.

→ **www.news.bms.com**

TB Screening

Qiagen partners with Taiwan in nationwide TB screening effort

Qiagen N.V. announced that QuantiFERON®-TB Gold, the modern standard for accuracy in diagnosing latent tuberculosis (TB) infection, was selected by the Taiwan Centers for Disease Control (Taiwan CDC) to replace the tuberculin skin test for screening at-risk individuals five years and older.

Starting this month, Taiwan's nationwide TB control effort will use QuantiFERON-TB Gold to test close contacts of patients with active tuberculosis, a contagious and life-threatening disease. In addition to treating patients with active TB, Taiwan will provide antibiotic treatment for patients identified as having latent TB infection (which if untreated can remain dormant and be activated years later). Only children younger than five years of age will be screened with the skin test.

→ **www.qiagen.com**

Spin-Off

Evotec spins off auto-immune disease company as 'Topas Therapeutics GmbH'

Evotec AG announced the formation of a spin-off company in the field of nanoparticle-based therapeutics to treat immunological disorders. Epidarex Capital, EMBL Ventures and Gimv participated together with Evotec in the EUR 14 m ($ 15.75 m) Series A round of Topas Therapeutics GmbH. Evotec will remain the largest shareholder after the financing round.

Topas emerges from the neuro portfolio of Bionamics GmbH which was acquired by Evotec in March 2014 and is an early stage therapeutics company using ground breaking nanoparticle technology to target autoimmune and inflammatory diseases via the induction of antigen specific immune tolerance in the liver. The platform has been exclusively licensed from the University Medical Center Hamburg-Eppendorf. It is anticipated that Topas will advance their initial programme targeting multiple sclerosis into clinical development in 2017.

→ **www.evotec.com**

Julabo Management

Markus Juchheim is now the sole Managing Director at Julabo GmbH

The company founder and shareholder Gerhard Juchheim has handed over the complete executive leadership responsibilities of Julabo GmbH to his son Markus after almost 50 years as its Managing Director. This is not a new responsibility for Markus Juchheim, who has led the company along with his father for the past nine years. Markus Juchheim will lead Julabo GmbH as the sole Managing Director starting immediately.

→ **www.julabo.com**

Mergers and Acquisitions II

Affymetrix to engage in discussions with Origin Technologies

Affymetrix, Inc. announced that the Company's Board of Directors has informed Origin Technologies Corporation, LLC that the Company will engage in discussions with Origin regarding its unsolicited merger proposal submitted on March 22, 2016 to acquire the Company for $17.00 per share in an all-cash transaction.

Affymetrix has communicated to Origin and its representatives that the following key deliverables are critical to the Company's evaluation of the Origin proposal:

- Drafts of a merger agreement and other transaction documents containing the specific terms of the Origin proposal;
- Complete copies of certain funding and financing documents; and
- Details on Origin's plans to obtain all regulatory approvals that are required or will be sought, including CFIUS approval.

The Affymetrix Board continues to recommend that its stockholders vote in favor of the adoption of the merger agreement with Thermo Fisher Scientific Inc.

→ **www.investor.affymetrix.com**

Carl Zeiss Lecture 2016

Prof. Thomas Pollard receives Carl Zeiss Lecture Award from DGZ and ZEISS

Presentation of the 2016 Carl Zeiss Lecture Award. From left to right: Dr Richard Ankerhold (Carl Zeiss Microscopy GmbH), Prof. Thomas D. Pollard (Yale University, USA), Prof. Klemens Rottner (TU Braunschweig).

The German Society for Cell Biology (DGZ) and ZEISS have presented the Carl Zeiss Lecture Award to Professor Thomas D. Pollard in Munich. The Award recognises outstanding work in cell biology and microscopy methods that establishes international research landmarks in issues of interest to the field of cell biology.

Pollard is Sterling Professor of Molecular, Cellular and Developmental Biology and Professor of Cell Biology and of Molecular Biophysics and Biochemistry at Yale University in New Haven, USA. His research work focuses on the molecular basis of cellular motility and cytokinesis. Pollard receives the accolade not only as a result of his outstanding work in the field of cell biology but also in recognition of his laboratory's exemplary combination of the techniques of modern microscopy with biochemical and biophysical methods to provide quantitative explanations of the molecular basis of cellular movement.

→ **www.zeiss.de**

Process Industries

Achema will be taking place in Frankfurt through 2024

Achema, the world forum and leading show for the process industries, will be staying in Frankfurt. The organiser, Dechema Ausstellungs-GmbH, and Messe Frankfurt have agreed to continue their successful collaboration for at least the next three events. The contract has been extended until 2024.

Achema has been taking place on Messe Frankfurt's exhibition grounds since 1937, one of the international guest events in Frankfurt with a rich tradition. The trade fair is held once every three years. At last year's event, some 3,800 exhibitors from around the world presented their products, processes and services. 166,444 participants from around the globe visited Achema in 2015. The next Achema will take place from 11 to 15 June 2018.

→ **www.messefrankfurt.com**

researched

Virus research

Scientists eliminate HIV-1 from genome of human T-Cells

A specialized gene editing system designed by scientists at the Lewis Katz School of Medicine at Temple University is paving the way to an eventual cure for patients infected with HIV, the virus that causes AIDS. In a study published online in the Nature journal, Scientific Reports, the researchers show that they can both effectively and safely eliminate the virus from the DNA of human cells grown in culture.

Kamel Khalili, PhD, Laura H. Carnell Professor and Chair of the Department of Neuroscience and colleagues decided to try a different approach, specifically targeting HIV-1 proviral DNA (the integrated viral genome) using uniquely tailored gene editing technology. Their system includes a guide RNA that specifically locates HIV-1 DNA in the T-cell genome, and a nuclease enzyme, which cuts the strands of

Prof. Dr Kamel Khalili

T-cell DNA. Once the nuclease has edited out the HIV-1 DNA sequence, the loose ends of the genome are reunited by the cell's own DNA repair machinery.

Source: www.medicine.temple.edu
Original publication: Kaminski, R. et al (2016) Scientific Reports 6, Article number: 22555, DOI:10.1038/srep22555

Cancer research I

Novel molecular processes controlling key genes in prostate cancer uncovered

Researchers at Karolinska Institutet and the University of Oulu in Finland have elucidated gene regulatory mechanisms that can explain how known genetic variants influence prostate cancer risk. The findings reveal widespread deregulation of androgen receptor function, a key player in prostate cancer. The vast majority of the three billion base-pairs in the human genome are identical across individuals. Nevertheless, genome sequence variation that does occur in the population has a profound effect on an individual's predisposition for developing various diseases. In the case of prostate cancer, 100 regions of genetic variation have been identified through comparative genetic studies. Each have a small but significant influence on prostate cancer risk. Previous studies have demonstrated an association of these genomic regions with disease, but the molecular processes accounting for the disease association have not yet been uncovered for most of these 100 regions.

Source: www.ki.se
Original publication: Whitington, T. et al (2016) Nature Genetics, DOI: 10.1038/ng.3523

Attention deficits

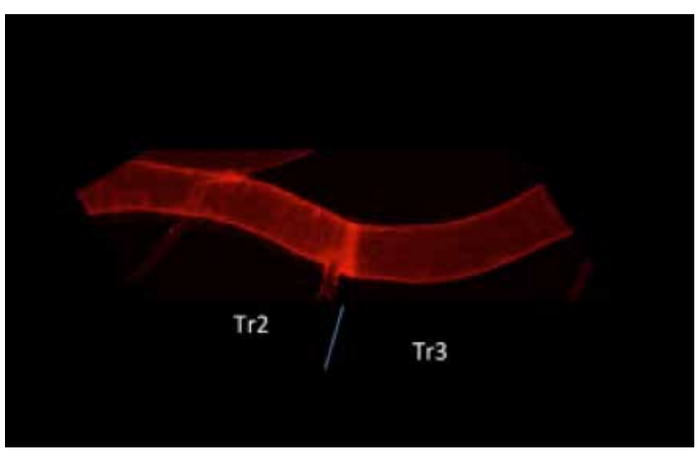
Study reveals a basis for attention deficits

More than 3 million Americans suffer from attention deficit hyperactivity disorder (ADHD), a condition that usually emerges in childhood and can lead to difficulties at school or work. A new study from MIT and New York University links ADHD and other attention difficulties to the brain's thalamic reticular nucleus (TRN), which is responsible for blocking out distracting sensory input. In a study of mice, the researchers discovered that a gene mutation found in some patients with ADHD produces a defect in the TRN that leads to attention impairments. The findings suggest that drugs boosting TRN activity could improve ADHD symptoms and possibly help treat other disorders that affect attention, including autism.

Source: www.news.mit.edu
Original publication: Wells, M.F. et al. (2016) Nature, DOI:10.1038/nature17427

Stem cells

Researchers dig up new molecular details on "the other type" of stem cells



Drosophila trachea fragment. Externally, there is no difference between the Tr2 segment, where facultative stem cells are found, and Tr3, which indicates the rest of the cells in the tissue. (N.J. Djabrayan, IRBBarcelona)

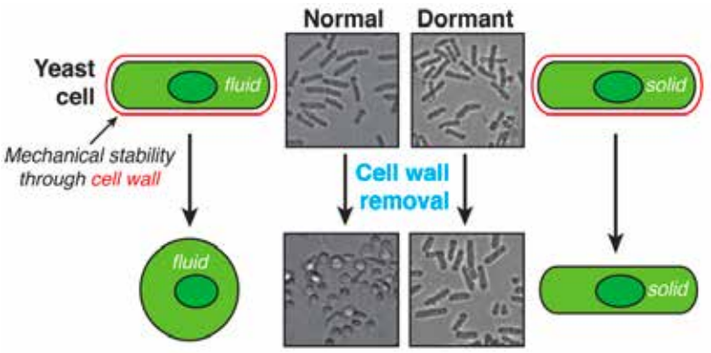
In a study published in PLos Genetics, scientists have identified two molecular signals and the pathway of events that allows cells in a tissue that are already specialized to regain their behaviour as stem cells. The study offers new information about how cells become differentiated and how "this other type" of stem cells, called facultative, get activated, which is of particular interest in cell reprogramming, regenerative medicine, and in understanding cancer. Facultative stem cells are being identified more and more often in human tissues and organs, but much less is known about them compared to typical stem cells, which have distinct morphological traits.

Source: www.irbbarcelona.org
Original publication: Djabrayan, N.J.-V. & Casanova, J. (2016) PLoS Genet., DOI: 10.1371/journal.pgen.1005909

Cell biology

Cells in stand-by mode

Normally, cells are highly active and dynamic: in their liquid interior, called the cytoplasm, countless metabolic processes occur in parallel, proteins and particles jiggle around wildly. If, however, those cells do not get enough nutrients, their energy level drops. This leads to a marked decrease of the cytoplasmic pH – the cells acidify. In response, cells enter into a kind of stand-by mode, which enables them to survive. How cells switch on and off this stand-by mode is unknown. Now, a team of researchers from Dresden, Germany, might have found the answer: The cytoplasm of these seemingly dead cells changes its consistency from liquid to solid, thereby protecting the sensitive structures in the cellular interior.



Source: www.mpi-cbg.de
Original publication: Munder, M.C. et al. (2016) eLife, DOI: dx.doi.org/10.7554/eLife.09347

Cancer research II

New gene identified as cause, early indicator of breast cancer

The gene GT198, whether mutated by genetics and/or environmental factors, has strong potential as both as a way to diagnose breast cancer early and as a new treatment target, said Dr. Lan Ko, cancer biologist in the Department of Pathology at the Medical College of Georgia at Augusta University and at the Georgia Cancer Center at Augusta University.

Mutations of the gene are known to be present in both early onset breast and ovarian cancer. Now scientists have shown that the stem, or progenitor cells, which should ultimately make healthy breast tissue, can also have GT198 mutations that prompt them to instead make a perfect bed for breast cancer. Their studies were done on an international sampling from 254 cases of breast cancer in pre- and postmenopausal women.

GT198, which is also a coactivator of receptors for steroid hormones such as estrogen, is normally regulated by estrogen, Ko said. But once mutated, GT198 can enable tumor production without estrogen. "Regardless of how much hormone you have, it's out-of-control growth," Ko said of the resulting classic, rapid growth of cancer.

(From left) Drs. Nahid Mivechi, Nita Maihle and Lan Ko.

Source: www.agwire.augusta.edu
Original publication: Yang, T. et al. (2016) Am. J. Pathol., DOI: dx.doi.org/10.1016/j.ajpath.2016.01.006

Microbiota

Mother's gut microbiota strengthens newborn's immunity

Already during pregnancy, microbes in the mother's gut shape the baby's immune system. This effect is brought about by microbial molecules that are transmitted to the baby across the placenta or via antibodies in the mother's milk. Scientists from Bern University Hospital, the University of Bern, the German Cancer Research Center (DKFZ) and ETH Zurich have now reported this finding in an article published in Science. Babies are born with immature immune systems. Up until now, scientists have assumed that newborns start after birth to adapt to the host of microorganisms that compose their own intestinal microbiome.

Source: www.dkfz.de
Original publication: Gomez de Agüero, M. et al. (2016) Science Vol. 351, Issue 6279, pp. 1296-1302, DOI: 10.1126/science.aad2571

immunoproteomic

From the genome via the proteome to the understanding of life

The pathogen *Staphylococcus aureus* as a model

Prof. Dr Michael Hecker[1] and Prof. Dr Barbara Bröker[2]

[1] Institute of Microbiology, University Medicine Greifswald, Germany
[2] Department of Immunology, Greifswald University Hospital, Germany

Fig. 1 *Staphylococcus aureus* colonies on a blood agar plate

immunoproteomic

Multi-resistant strains of *Staphylococcus aureus* and other bacteria constitute a growing threat to humankind. Medical professionals, scientists and politicians all agree: new antibiotics, approaches to immunisation and alternative anti-infection strategies are all required if we are to avoid regressing to the era before the introduction of antibiotics. With the aid of the novel possibilities offered by modern genome research, we wish to arrive at a comprehensive understanding of the physiology and pathophysiology of Staphylococcus – to improve both our knowledge and our arsenal of countermeasures. We conduct research jointly with our colleagues from Greifswald, Münster, Tübingen and Würzburg as part of Transregional Collaborative Research Centre 34, which is funded by the German Research Foundation (DFG). This article presents initial results from this highly ambitious and important undertaking.

Multi-resistant bacteria – a threat to humankind

Multi-resistant strains of *Staphylococcus aureus* constitute a growing threat to humankind (Fig. 1). These dangerous bacteria are not only responsible for a third of the feared hospital-acquired infections but can also trigger other serious conditions such as endocarditis or sepsis. What is especially problematic is that their increasing – and extremely worrying – resistance to a range of antibiotics means that often, only a handful of drugs actually have the desired effect. While experts in the field have consistently warned of this development for some time now, a remedy has yet to appear. Indeed, we now know of bacteria that cannot be treated by any of the antibiotics we have available – a situation that is a stark reminder of the time before the introduction of antibiotics. Recently, politicians have also finally come around to agreeing with expert opinion: urgent action is now needed if we are to avoid a catastrophe for humankind. Interest is focusing not only on new antibiotics but also approaches to immunisation and alternative anti-infection strategies, as well as a general boosting of the immune system [1].

For us at Greifswald, one vision has remained uppermost in our minds in the era of genomics and post-genomics: with the aid of the novel opportunities presented by genomics research, we want to achieve an entirely new and comprehensive understanding of the life processes of pathogenic bacteria – and not only in the lab but also in the hospital infection process. If we can better understand bacterial life, then we will also learn to combat these infectious agents more effectively. This was the starting point for the launch of DFG Transregional Collaborative Research Centre 34 on the topic of "Pathophysiology of staphylococci in the post-genomic era" (2006–2018) some years ago in Greifswald, together with infection biologists and medical specialists in Würzburg (Hacker), Tübingen (Götz and Peschel) and later also in Münster (Peters). With the targeted application of the new arsenal of methods from functional genomics research and proteomics in particular, this Centre aims to achieve a more comprehensive understanding of the life of pathogens – their metabolism, their adaptation to the growth inhibition factors encountered in their hosts, their virulence potential with which they attempt to attack their hosts, their strategies for bypassing and shielding themselves from the human immune system, and many other aspects of their pathophysiology. Armed with this new knowledge, we can then derive new countermeasures. This is naturally a very ambitious project that requires considerable staying power!

The genomic revolution: seeing life's bigger picture

We are currently witnessing a development that has been fittingly termed the "genomic revolution" and which has led to a paradigm shift in the life sciences. The starting point of this new development was the publication of the first complete genome sequence of the bacterium *Haemophilus influenzae* in 1995. The human genome sequence followed only six years later, presented to the public at the White House in Washington as "Decoding the Book of Life". The aptly-chosen title holds out promise of a new dimension: for the first time, scientists were in a position to understand life in its entirety and not merely its various aspects. The initial euphoria soon gave way to a certain amount of disillusionment, however, since the genome sequence is itself merely the blueprint for life and more is still required to understand life's processes [2]. How this blueprint is applied – how the blueprint for life is transformed into a living thing – is a question to which the field of "functional genomics" must provide the answers. We know that the mechanism of differential gene expression decides on the point in time and intensity with which each gene is expressed, which in turn manufactures the right quantity of each

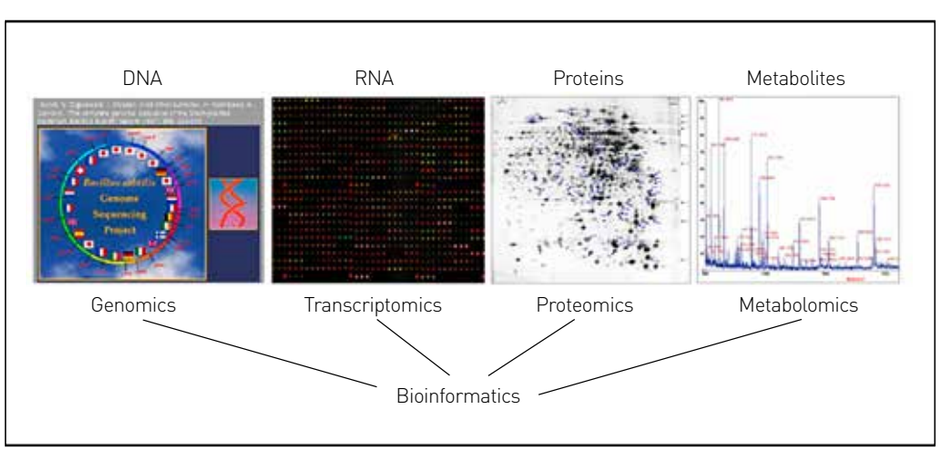


Fig. 2 From the genome sequence via proteins to life. The genome sequence is merely a blueprint for life: functional genome research must now work on translating the blueprint for life into life itself. Proteomics must lead the way in decoding the "virtual life" of the gene into the "real life" of the protein, since proteins – not genes – are the musicians in the symphony of life.

protein, so as to ultimately construct the complex protein network typical for and essential to every living organism. In this context, the multi-omics techniques that enable us to record the totality of transcripts (specifically including the wealth of non-coding RNA), proteins and metabolites in a cell now offer us a decisive advantage. To derive new knowledge from this cornucopia of data, bioinformatics and systems biology have a major role to play in the processing and post-processing of the prodigious amounts of data generated by omics techniques (Fig. 2).

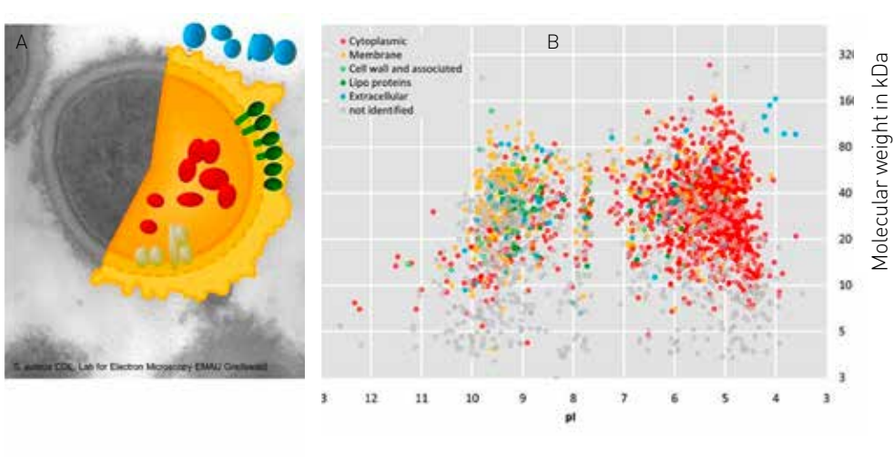
On the difficult and typically rocky road from genome to life, proteins are the focus of interest in particular since it is the proteins, not the genes, that are the primary tools within all of life's processes. The life of a simple bacterium consists of only a hundred or few thousand different proteins, which can be captured almost in their entirety with modern techniques in proteomics. A protein's amino acid sequences grants it an unmistakable structure and thus not only its unique function but also, ultimately, its singular role in the process of life. As a result of the above, the low complexity of single-cell bacteria makes them ideal model systems for studying and better understanding the journey from the genome via proteins to life.

Our analysis work got underway by looking at *Bacillus subtilis*, the model organism for gram-positive bacteria [3]. More than a decade ago, we decided we would attempt to transfer the scientific approach of physiological proteomics and the insights thereby gained to a related pathogenic organism. Following extensive discussions with Jörg Hacker, we decided on *S. aureus*, which is today our most important model organism for pathogens.

How can proteomics lead to an improved understanding of the pathophysiology of *S. aureus*?

Publication of the genome sequence of *S. aureus* meant that we were now able to identify virtually all of its proteins – its "protein inventory" [4, 5]. As with other bacteria, the known proteins in this sequence were also accompanied by others that had never been described and whose function was therefore unknown. The nearly 2,000 proteins were classified according to a range of criteria by Dörte Becher. First of all, we separated the cytosolic and transmembrane proteins from those surface-associated proteins that protrude from the cell or which are transported to the exterior (the secretome). Following this, we then assigned all proteins to (their known) functional units (Fig. 3 and 4). One result of this work was the near-complete reconstruction of metabolism, which involves almost half of all proteins – and not merely from the somewhat vague genomic prediction but derived from the real life processes of the bacteria. In addition, a great many proteins were also assigned to the basic functions of life such as gene expression (including its regulation, translation and protein quality control), signal transduction and many other processes. The end result presented the life of simple organisms at the level of proteins to a degree of completeness virtually never encountered before. With the aid of quantitative proteomics, one can go a step further and calculate the investment for the life processes described, and therefore answer such questions as how "expensive" glycolysis or translation is for the cell (see Table 1).

As a next step, we considered the question of the conditions that a bacterium encounters during an infection event in the human host, since adaptation to these typically growth-inhibiting – or even (from a bacterium's perspective) life-threatening situations – is decisive for the former's survival in the host and thus for the overall infection process. In this lab work, we were able to both identify and quantify the proteins whose synthesis is promoted as a response to being deprived of nutrition, oxygen or iron, or subjected to oxidative, osmotic, heat shock, acidic and many other kinds of stress. From this work, Stephan Fuchs and Susanne Engelmann derived a proteome signature library in response to infection-relevant stimuli. These signatures are a valuable resource from which the physiology and living conditions can be derived for bacteria that have been isolated from infected cell cultures or directly from the host (e.g. taken from the nasal cavity [6]). A signature for oxidative stress (an increased synthesis of catalase, superoxidase and many others), for example, signals to the experimenter that *S. aureus* has encountered reactive oxygen radicals – which are used by immune cells to kill bacteria – or at least attempt to do so. This signature library is also an important instrument for predicting the function of unknown proteins – at least as a



C

Localization	Number of theoretically predicted proteins	Identified proteins: Total	Identified proteins: Percentage
Cytosolic proteins	1,795	1,424	79
Membrane proteins	580	373	64
Lipoproteins	66	63	95
Sortase substrate	20	19	95
Cell wall-associated proteins	14	13	93
Secreted proteins	143	113	79
All proteins	**2,618**	**2,005**	**76**

Fig. 3 Diagram of the complete proteome of *Staphylococcus aureus*
A) The most important proteome subfractions from *S. aureus*. B) A virtual 2D protein gel: each dot represents a protein. The location of the protein on the gel is determined by its size and charge. C) Overview of the proteins predicted and actually detected. The proteome coverage is 76%. Accounting for the fact that not all genes are expressed at the point in time of measurement, coverage is actually higher (modified after Becher et al., PloS One 4, 2009, e8176; Hecker et al., Laborwelt 15, 2014, 5)

first-order approximation. As a result, we have been able to identify numerous previously-unknown proteins that are probably involved in surmounting the problems of protein stress or heat shock, oxidative stress and glucose or oxygen deficiencies. Jörg Bernhardt has used a Voronoi tree map to provide a clear and vivid visualisation of the kinetics of the complete protein inventory, i.e. the increase or decrease in the quantities of individual proteins in response to hunger or stress, so as to provide a highly detailed and complete picture of simple life processes as a "symphony of proteins" (Fig. 4).

As a consequence, Uwe Völker and his team were able to apply these insights to describe and trace the "lifestyle" of staphylococci directly within the infection process. This work shows that bacteria exhibit a significant inhibition to growth rate and therefore an associated induced "stringent response" when they penetrate into human epithelial or endothelial cells; they display a low oxygen concentration or even an iron deficiency, to name just two examples, and they encounter, as expected, oxidative stress in infected macrophages. Lastly, with their discovery of alternative RNA polymerase sigma factor, SigB, Uwe Völker and his team have identified a truly decisive regulator that is of central importance in the invasion or intracellular proliferation of bacteria in human epithelial cells [7]. An accurate understanding of the lifestyle of the pathogen in the infected host is likely to be a key requirement for developing new treatment strategies – although it will be a long and laborious journey towards this goal.

Cell surface-associated proteins and proteins present in the extracellular medium are of particular importance for pathogenic bacteria. Surface-associated proteins are those that establish the initial, direct contact with the host and its immune system following infection – via the formation of microcolonies or biofilms, for example, to the invasion of human cells; secreted proteins, on the other hand, accommodate the majority of virulence factors – well over 20 are estimated in the case of *S. aureus*. For both surface-associated and secreted proteins, we have, as expected, found numerous examples already described in the literature in our high-coverage proteome repository. In addition, we have also identified many proteins not previously encountered in research and which are likely to play a central role in the infection process. It is well known that the secreted proteins constitute a reservoir for virulence factors: these are used to cause damage to the host (toxins, enterotoxins, etc.), to commandeer nutrients and to bypass or sabotage the host's immune system. Uncovering the precise role of these as-yet unknown virulence factors in causing damage to the host as part of the various disease conditions is likely to result in a new and comprehensive understanding of disease genesis and progression. Indeed, Susanne Engelmann's work in analysing the virulence factors from defined clinical isolates taken from various patient cohorts (wounds, sepsis, osteomyelitis) was able to show that only eight virulence factors occur in all isolates, and that each isolate possesses numerous secreted proteins previously unknown to scientific research. Such patient isolates are a treasure trove for the identification and later functional characterisation of previously unknown virulence factors involved in disease genesis and progression [5, 8]. Susanne Engelmann's work has in fact already identified a new virulence factor that is probably involved in the circumvention of the host's immune response.

Our extensive knowledge of *S. aureus* virulence factors is also invigorating immunological research, since the immune system also has a "vested interest" in these bacterial proteins [8, 9]. Immune proteomics thus offers us a view of the immune response at a previously unattainable level of detail and completeness (Fig. 5). Since the efficacy of vaccines relies on the formation of an immune memory for the infectious agent's proteins, we hope that this research initiative will generate ideas for the development of an *S. aureus* vaccine [10]. Yet immune proteomics has even greater potential: as our understanding of the immune system's "rulebook" for controlling *S. aureus* improves, we will be able to identify phenomena that break these rules – and are therefore of particular interest – at an earlier stage. This approach has led to our discovery that *S. aureus* (and perhaps other bacteria as well?) can produce allergens that induce asthma in mice. Whether this means we now hold the key to certain severe forms of asthma, whose causes have previously been searched for in vain, can only be answered by further research.

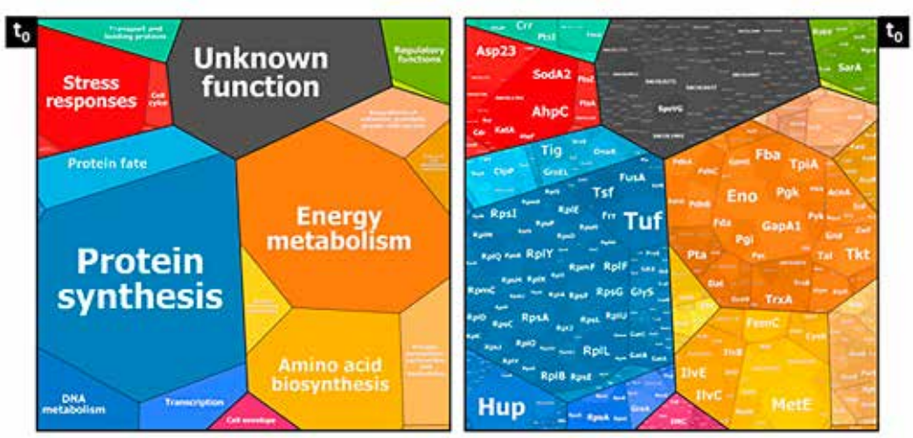


Fig. 4 Assignment of proteins from *S. aureus* to functional units. The size of each area is proportional to the quantity present (after Bernhardt et al., unpublished)

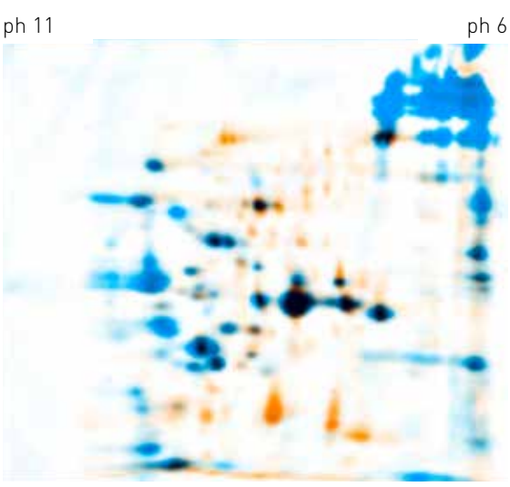
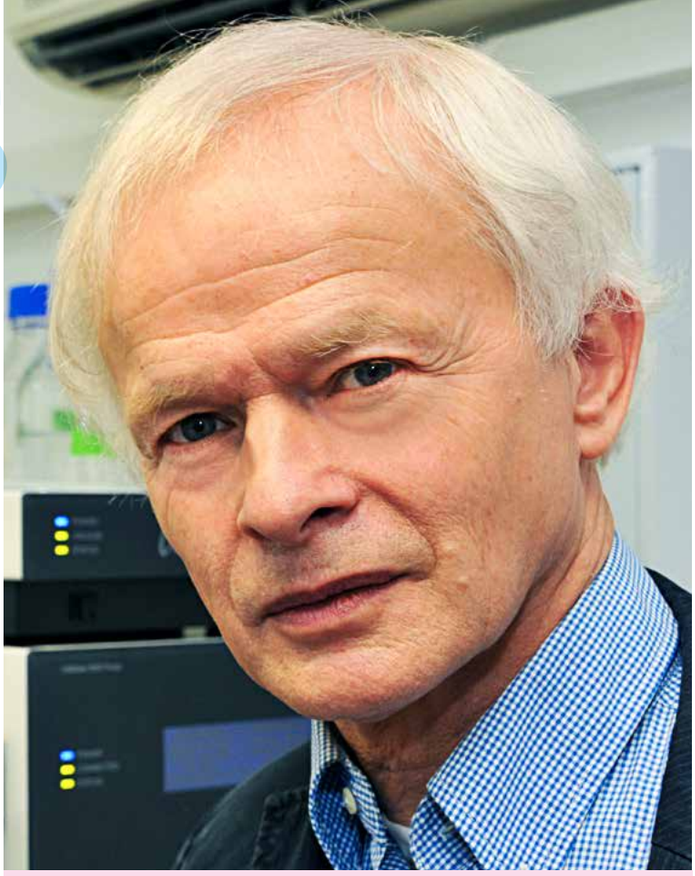


Fig. 5 The *S. aureus* human immune proteome – an example of work in progress
The proteins secreted by *S. aureus* (strain 8425) were separated using a two-dimensional gel electrophoresis method and appear as orange-coloured spots. After applying the bacterial proteins to a membrane, these were incubated with serum from 16 adult humans and the binding of IgG antibodies was made visible (blue). The immune system clearly exhibits a strong response to some proteins, while showing a weak or zero response to others. The sum total of bacterial proteins that trigger an antibody or T-cell response is referred to as the "immune proteome".



Michael Hecker studied biology at the University of Greifswald, where he also received his doctorate. He has been a professor at the University of Greifswald since 1986 and was Director of the University's Institute for Microbiology up until 2014. He is a co-initiator of Transregional Collaborative Research Centre 34 (TR-CRC 34) and was its spokesman until 2012. He was President of the German Association for General and Applied Microbiology (VAAM) until 1999, and a member of several national and international academies. He sits on the Senate of the German National Academy of Sciences Leopoldina. *Picture: Peter Binder*

Barbara M. Bröker studied medicine and philosophy in Münster, Vienna and Bristol (UK). She completed her habilitation in immunology in 1996 and has been Professor for Molecular Immunology at the University of Greifswald since 2000. She is the Director of the Department of Immunology at Greifswald University Hospital and has been the spokeswoman for TR-CRC 34 since 2012. She was also a member of the DFG Senate Committee on Collaborative Research Centres from 2008 to 2012, and has been a member of the DFG Review Board Microbiology, Virology and Immunology since 2016.

Proteins in the symphony of life – thoughts beyond the infection biologist's perspective

The global, sophisticated mechanisms of gene expression control guarantee that each individual protein is provided in the required quantity and at the right point in time, as we have demonstrated in a quantitative model study on the response of *S. aureus* to oxygen deprivation – an extremely common occurrence in the host during an ongoing infection. From the massive induction of proteins as a result of oxygen deficiency, we were not only able to detect those that initiate a changeover to fermentation process (e.g. lactate dehydrogenase) but also those whose function is yet unknown and whose detailed study offers important insights into previously unknown mechanisms of adaptation to the course of infection. With our publication and description of the protein inventory, an important step has been taken along the path from the genome via the proteome to life itself: the life processes of simple bacteria can now be traced and described at a level of detail that we would have considered unthinkable 20 years ago. What lies ahead on this path and what is logically the next course of action? Life isn't simply about a jumble of proteins – life's symphony requires these proteins to be orchestrated. The challenge in the years to come will be to understand how we can close the gap in our knowledge from the protein inventory to cell physiology – how the proteins released at the ribosome in precisely coordinated quantities work to organise the life of the organism. Bernd Bukau (Heidelberg) has shown us that proteins locate partners even during their "birth" on the ribosome and proceed to form a dynamic, highly-sensitive and presumably highly orderly protein network that is influenced by environmental conditions and itself controls almost all life processes. Advanced knowledge of its protein inventory makes *S. aureus* into a popular model organism that is not merely of interest for research issues within infection biology but which can be of help in answering Schrödinger's famous book-length question "What Is life?"

Protein molecules per bacterial cell	... of which	Proportion in %
1,200,000	Total investment	100%
190,000	Ribosomal proteins (53)	16%
114,000	Amino acid metabolism	10%
83,000	Glycolysis	7%
52,000	Protein quality control (chaperones, etc.)	4%
15,000	Tricarboxylic acid cycle during glucose excess	1%

Tab. 1 Investments and costs for the "simple life" of *S. aureus* (D. Zühlke, J. Bernhardt and S. Fuchs, unpublished)

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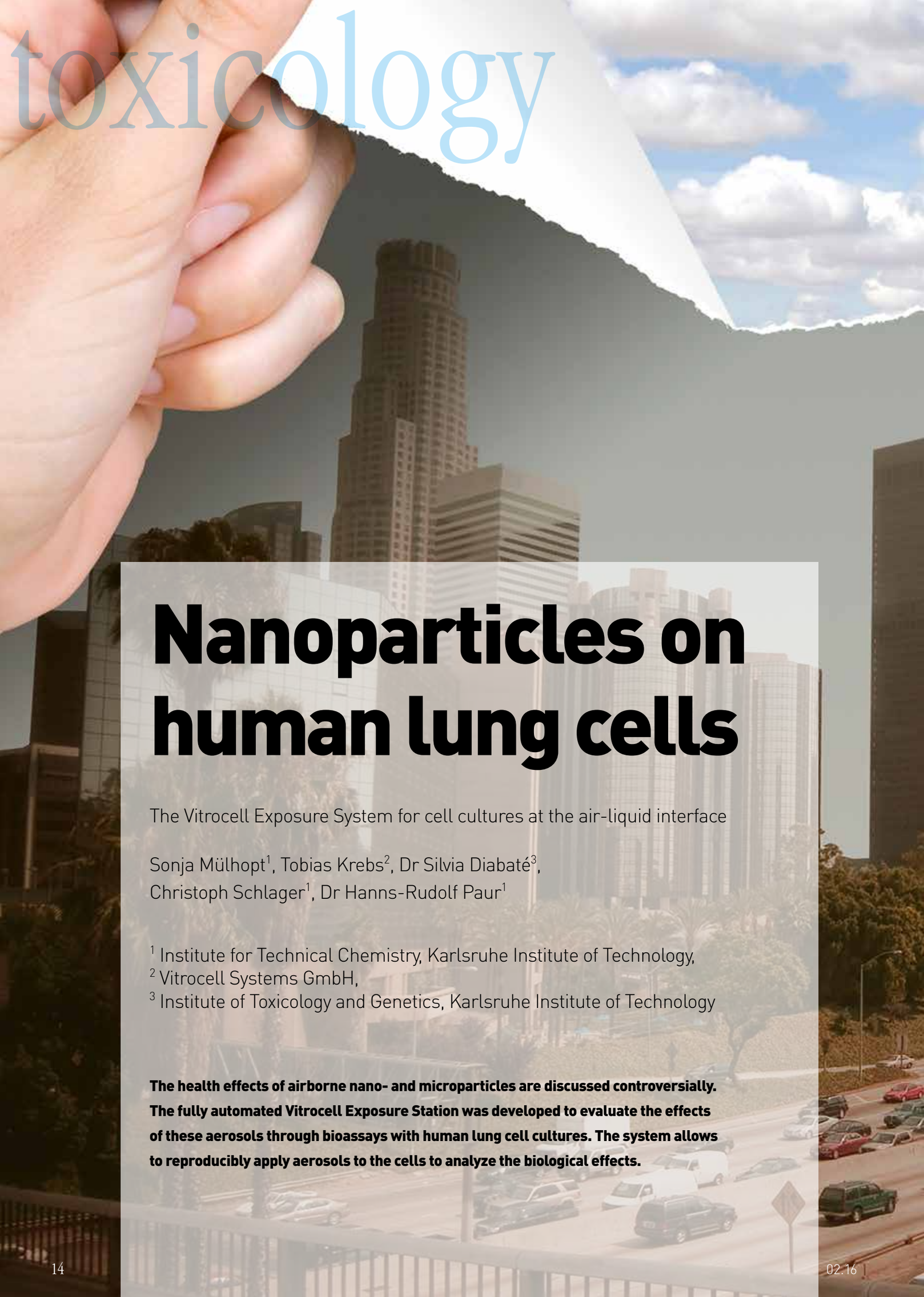

Nanoparticles on human lung cells

The Vitrocell Exposure System for cell cultures at the air-liquid interface

Sonja Mülhopt[1], Tobias Krebs[2], Dr Silvia Diabaté[3],
Christoph Schlager[1], Dr Hanns-Rudolf Paur[1]

[1] Institute for Technical Chemistry, Karlsruhe Institute of Technology,
[2] Vitrocell Systems GmbH,
[3] Institute of Toxicology and Genetics, Karlsruhe Institute of Technology

The health effects of airborne nano- and microparticles are discussed controversially. The fully automated Vitrocell Exposure Station was developed to evaluate the effects of these aerosols through bioassays with human lung cell cultures. The system allows to reproducibly apply aerosols to the cells to analyze the biological effects.

New Materials – New Opportunities, New Risks? During the past two decades, measuring methods with ever higher resolutions and the resulting increasing understanding of the submicron regime have strongly influenced both the use and the risk assessment of nanoscale substances and systems: Whereas nanoparticle technology opens up new possibilities in the field of materials science, large-scale technical applications are generating new issues regarding occupational health and safety and environmental protection. The attractiveness of nanoparticles i.e., particles which according to EU standards are smaller than 100 nanometers (= $100*10^{-9}$ m) in at least one dimension, consists in the fact that most of their atoms are not located any more inside the molecules but on the surface and that the macroscopic properties, therefore, may change. The nanoparticles, for example, may have an increased solubility and chemical reactivity as well as reduced melting points. Besides, superparamagnetism and higher refractive indices and, hence, size-dependent chromaticity were observed. In addition to the often desired "new" physical properties, nanoparticles can have new biological properties i.e., in a biological system, they can cause so far unknown or untypical biological responses such as inflammations. Due to their small nanoparticle size, substances which so far have been classified as harmless hence can turn into potentially harmful products.

Undesirable nanoparticles

In spite of the above advantages, unwanted nanoparticles are becoming more and more of a problem: Although atmospheric loads have strongly decreased since the end of the eighties due to the improvement of combustion systems and filtering techniques in industry and traffic, particulate matter has become a quasi-measurable problem: The threshold value for particulate matter emissions of 50 µg/m³, which must not be exceeded on more than 35 days per year, was still often surpassed in 2014 in the big cities in spite of the introduction of low-emission zones and in spite of the fact that 50 µg/m³ still is far above of what has been recommended by the WHO. It was found in epidemiological studies by Dockery and Pope that environmental pollution with particulate matter correlates with the relative risk of diseases and death [1]. It was proved in several studies also by German scientists that the number of respiratory and cardiovascular diseases increases with the concentration of fine and ultrafine particulate matter [2]. Ultrafine particulate matter can deeply

penetrate the human respiratory tract and remain there for up to one year before being removed by the cleaning mechanism of the lung (Fig. 1).

The smaller the deeper

Whereas particles that are 1 to 10 µm in diameter are deposited mainly in the nasopharyngeal zone (green curve) and in the upper bronchia (yellow curve), particles smaller than one micrometer in diameter penetrate deep into the secondary and tertiary bronchia (blue curve) and the alveolae (red curve), where they have a mean residence time of ca. 400 days before they are removed by the cleaning mechanism of the lung. In adults, the alveolae, where the gas exchange from atmospheric oxygen to the blood and carbon dioxide to the respiratory air takes place, have a mean gas exchange surface of 140 m². Since there are hardly any air movements in this area, the gas and particle behavior is mainly characterized by diffusion.

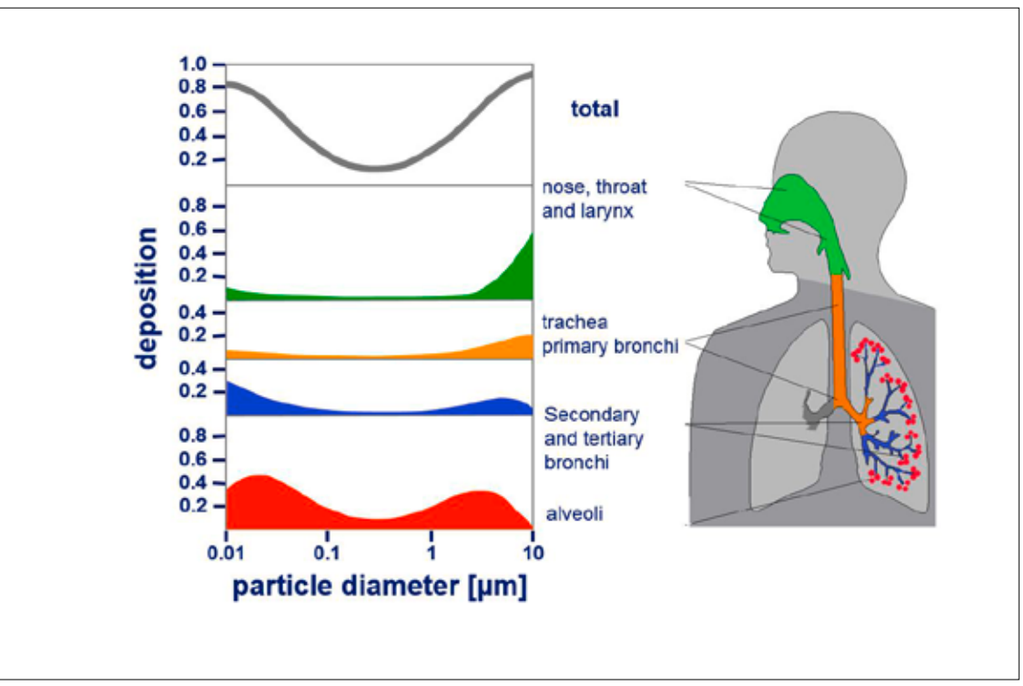

Fig. 1 Degree of separation as a function of particle size for the different regions of the human respiratory tract [9].

Fig. 2 Automated exposure system for reproducible exposure of bioassays at the air-liquid interface. Left: Schematic view of the main process components. Right: Photograph of Vitrocell system.
Picture: KIT, Vitrocell Systems GmbH

Investigation of nanoparticles

The correlation between particle emissions, residence time of particles in the human body, and biological effects of particles is the subject of intensive investigations. In addition to epidemiological studies, animal tests are carried out to be able to analyze systemic effects such as cardiovascular diseases. Screening tests and method development increasingly are carried out on the basis of cell cultures. During these in vitro studies, the cell cultures are exposed to the particles to be investigated and are analyzed for biological reactions after a defined incubation period. The responses can either be detected at a very early stage, for example metabolic changes in cells, or may occur only after some time, for example the release of cytokines (messengers) which are known as markers of inflammatory processes.

ALI processes

In the case of toxicological standard methods, particles are suspended in the culture medium, which is needed for cell cultivation, and are then applied onto the cultures. Whereas this so-called “submerged” (= covered with a liquid) method is well-suited for analysis of cells from organs that can be exposed to the particles without air admission e.g., intestinal cells, it is less suited for inhalation toxicology of air-borne particles. On the one hand, complete covering of the lung cells with liquid is not physiologic because the cells in the lung are covered only with a thin liquid film. On the other hand, the particles both during sampling and during application to the cells in culture medium are strongly influenced and hence the biological effectiveness may change considerably. Since the particles in the liquid are colloidal in character or partially agglomerated, the amount deposited on the cells cannot be determined precisely. A different technique where the cells are exposed at

Tab. 1 Survey of successively applied and analyzed aerosols, cell cultures, and biological effects

aerosols	**industrial nanoparticles**	titanium dioxide, silicon dioxide, silver, platinum	
	combustion aerosols	emissions from wood stoves, marine diesel engines, wood-fired boilers, pellet boilers, municipal waste incinerators	
cell cultures	**human lung epithelial cells**	A549, BEAS-2B, SK-MES-1	co-cultures from epithelial cells and macrophages and/or endothelial cells
	macrophages	THP-1, RAW264.7	
	human endothelial cells	HUVEC	
biological effects	**markers for inflammatory processes**	release of IL-8, IL-6, MCP-1, expression of ICAM-1	
	markers for cytotoxicity	release of LDH, reduction of AlamarBlue	
	markers for oxidative stress	expression of HMOX-1	
	markers for metabolism of foreign substances	expression of CYP1A1	

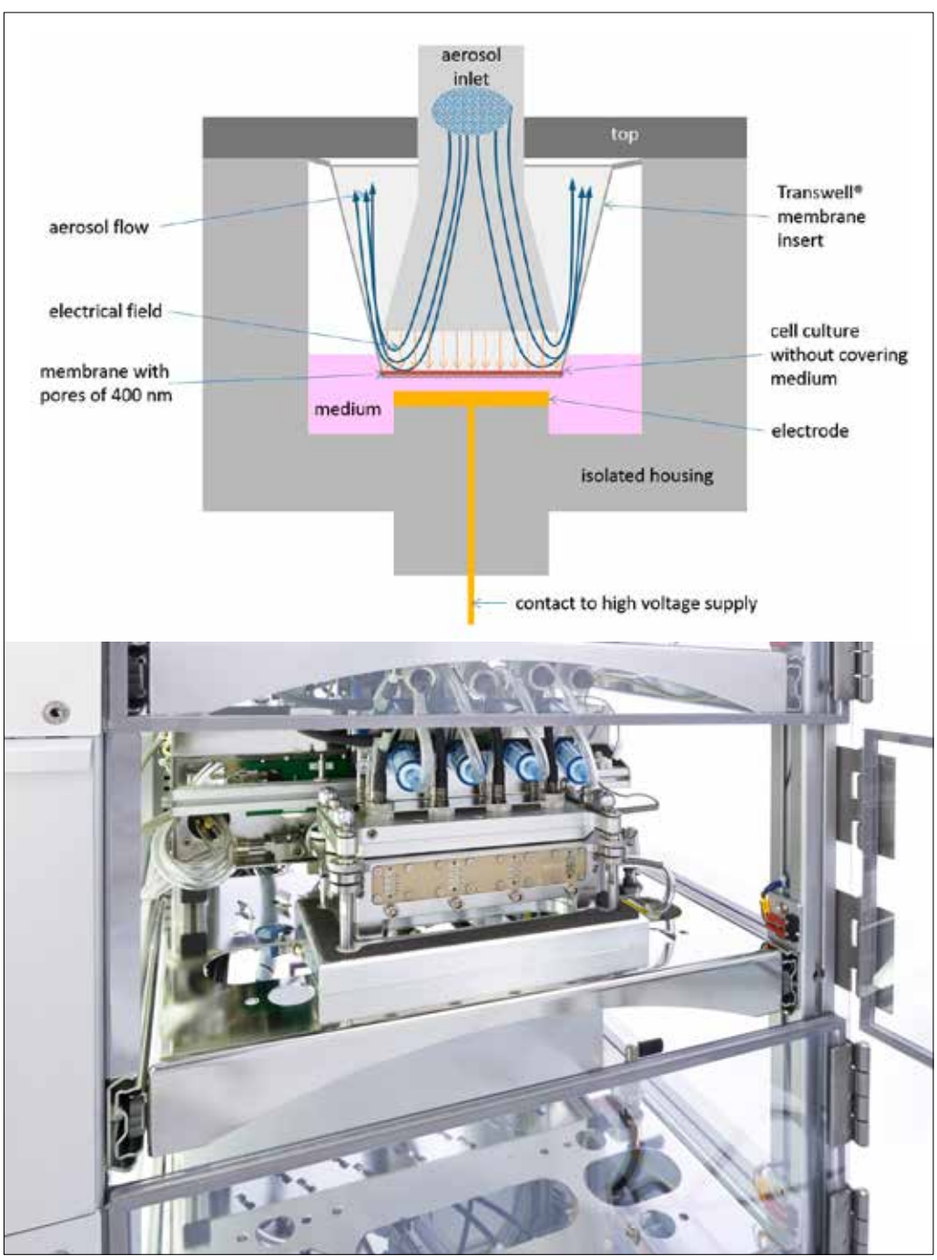

Fig. 3 Exposure chamber with Transwell culture dish. upper: Schematic diagram with electrode under the culture medium and flow lines of the aerosol flow above the cell culture. lower: Photograph of a quadripartite Vitrocell module for three 6-well inserts and a quartz crystal microbalance in the drawer of the exposure system. *Picture: KIT, Vitrocell Systems GmbH*

their air-liquid interfaces i.e., where the cells are covered only with a thin liquid film, has been used therefore for several years. This so-called ALI exposure (ALI = Air-Liquid Interface) is more realistic, can be reproduced more easily, and dose, in particular, is defined more precisely [3, 4].

The user-friendly automated exposure system

At KIT, an automated exposure system for ALI exposures was developed in cooperation with Vitrocell Systems GmbH (Waldkirch, Germany) (Fig. 2). This system allows both reproducible sampling and conditioning of aerosols and exposure of the cell cultures under conditions imitating those of the human lung. In addition, the relevant dose can be determined online [5]. For ALI exposure, bioassays were developed and used for toxicological analysis of particulate emissions from the industry [6, 7] as well as of nanoparticles [8].

Firstly, a sample of the aerosol to be analyzed is taken from the respective process at a volume flow of 1 m^3/h and is conducted through a PM2.5 low-volume impactor. The objective of the preliminary separation of larger particles is to simulate deposition in the upper respiratory tract and avoid that individual large particles make a non-reproducible contribution to the deposited mass and thus impede analysis by bioassays. Subsequently, the relative humidity is adjusted to 85 % r.H. through water vapor dosing to protect the cell cultures from drying out. Once stabilized, the humidified aerosol flows into a particle reactor. On each of the three levels of the reactor, there are isokinetic sampling probes from which the conditioned aerosol is conducted into the exposure chambers of the Vitrocell modules. Additional sampling points e.g., mobility analyzers, are available for external particle measurement or for filter-based sampling for electron microscopy. The Vitrocell modules are the heart of the system: Inside of them, the cell cultures that have been cultivated on the membrane inserts are apically exposed to the aerosol and are supplied basally with the culture medium (Fig. 3). All components are uniformly heated to 37 °C. To increase deposition efficiency, high voltage can be applied by an electrode below the culture medium. It is due to the electrical field, generated between aerosol inlet and cell culture, that charged particles are increasingly deposited on the cell culture by the electrical forces.

All flows are controlled by integrated mass flow controllers operated via touch screen

From left to right: Christoph Schlager, Sonja Mülhopt, Hanns-Rudolf Paur, Tobias Krebs *Picture: KIT*

Christoph Schlager He studied mechanical engineering / process engineering at Baden-Wuerttemberg Cooperative State University. During his studies and since their completion in 2012, he has been working on the development of the exposure method at KIT's Institute for Technical Chemistry and, in particular, has been supervising the use of the exposure system during large conjoint measurement campaigns conducted by the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) on e.g., wood-fired boilers and marine diesel engines.

Sonja Mülhopt She completed her studies in process engineering with a diploma degree and received her master's degree in chemical engineering in 2014. Since 2000, together with Vitrocell Systems GmbH and Institute of Toxicology and Genetics, she has been developing the method for aerosol exposure of cell cultures at the gas-liquid interface with integrated online dose measurement at KIT's Institute for Technical Chemistry. Since 2012, she has been heading the group Exposure Methods.

Hanns-R. Paur He obtained his doctorate in chemistry from LMU Munich and worked as a postdoc at UC Riverside in California. Currently, Dr. Paur heads the Division of Aerosol and Particle Technology at KIT's Institute for Technical Chemistry. His scientific fields of work comprise the formation, separation, and effects of ultrafine particles. Dr. Paur is vice president of Gesellschaft für Aerosolforschung – GAeF (Association for Aerosol Research) and appointed member of the VDI ProcessNet Gas Treatment Group.

Tobias Krebs studied industrial engineering. After having gained comprehensive entrepreneurial experience, he has been self-employed since 1997 in the development and marketing of technologically advanced products. In 1999, he started to work on in vitro inhalation toxicology and founded VITROCELL Systems GmbH as an independent company in 2007. Today, VITROCELL is a leading supplier of equipment for in vitro exposure of cells of the respiratory tract and of dermal tissue for research institutes, contract laboratories, regulatory authorities, and industrial companies throughout the world.

Silvia Diabaté She studied biology at Martin-Luther University Halle-Wittenberg and obtained her doctorate from Gießen University in 1984. Since 1998, Dr. Diabaté has been carrying out toxicological investigations of nanomaterials at Institute of Toxicology and Genetics at Karlsruhe Institute of Technology. In cooperation with Institute for Technical Chemistry, she developed the in vitro procedure for exposure of lung cells at the air-liquid interface.

monitors. The intuitive HMI (human-machine interface) surface for all control and data acquisition functions has been developed specifically for this device. The system can be integrated in a network.

The system is being used already in two EU projects (NanoMILE and QualityNano) and at the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE), where considerable experience has been gained already with nano-aerosols.

Conclusion

The experience gained so far with the automated exposure system shows that the effects of nanoparticles on human lung cells can be analyzed reproducibly. Numerous groups from European laboratories have gained experience already in using the new technology. Since the new system allows realistic exposures, it is expected that a valid data base for evaluation of particulate matter emissions and nanomaterials can be created for the first time through in vitro experiments and that the number of animal experiments in that field can be reduced.

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AchemAsia 2016: Leading trade show for the process industries in China for the tenth time

From May 9–12, 2016, AchemAsia will open its gates in Beijing. For the tenth time, experts from all over the world will meet in order to present products and processes for plant engineering and chemical processing, get information on the latest developments in the process industries, and make new contacts. With exhibitors and participants from 17 countries, AchemAsia is the most international trade show for the process industries in China. It covers apparatus and plant engineering as well as process technology, petrochemistry, pharma and food processing, agrochemistry and laboratory and packaging techniques. Environmental technology and water treatment are also in the focus. The exhibition is accompanied by sseveral praxis-oriented symposia on process intensification, current challenges in the petrochemical industry, and single-use technologies.

The tenth anniversary of AchemAsia occurs at a time of economic change: China's economic growth is slowing down and the government strives for a structural change from an export-oriented economy to a strong domestic market. With the strategy "Made in China 2025" it has initiated an ambitioned program to transform China to a high-tech location. German and international companies have to prepare for more competition from China. On the other hand, the high-investment program opens great opportunities for German technologies in production, plant engineering and automation as well as in providing equipment for a wide range of sectors.

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Molecular typing

New possibilities in the diagnosis of pathogenic *E. coli*

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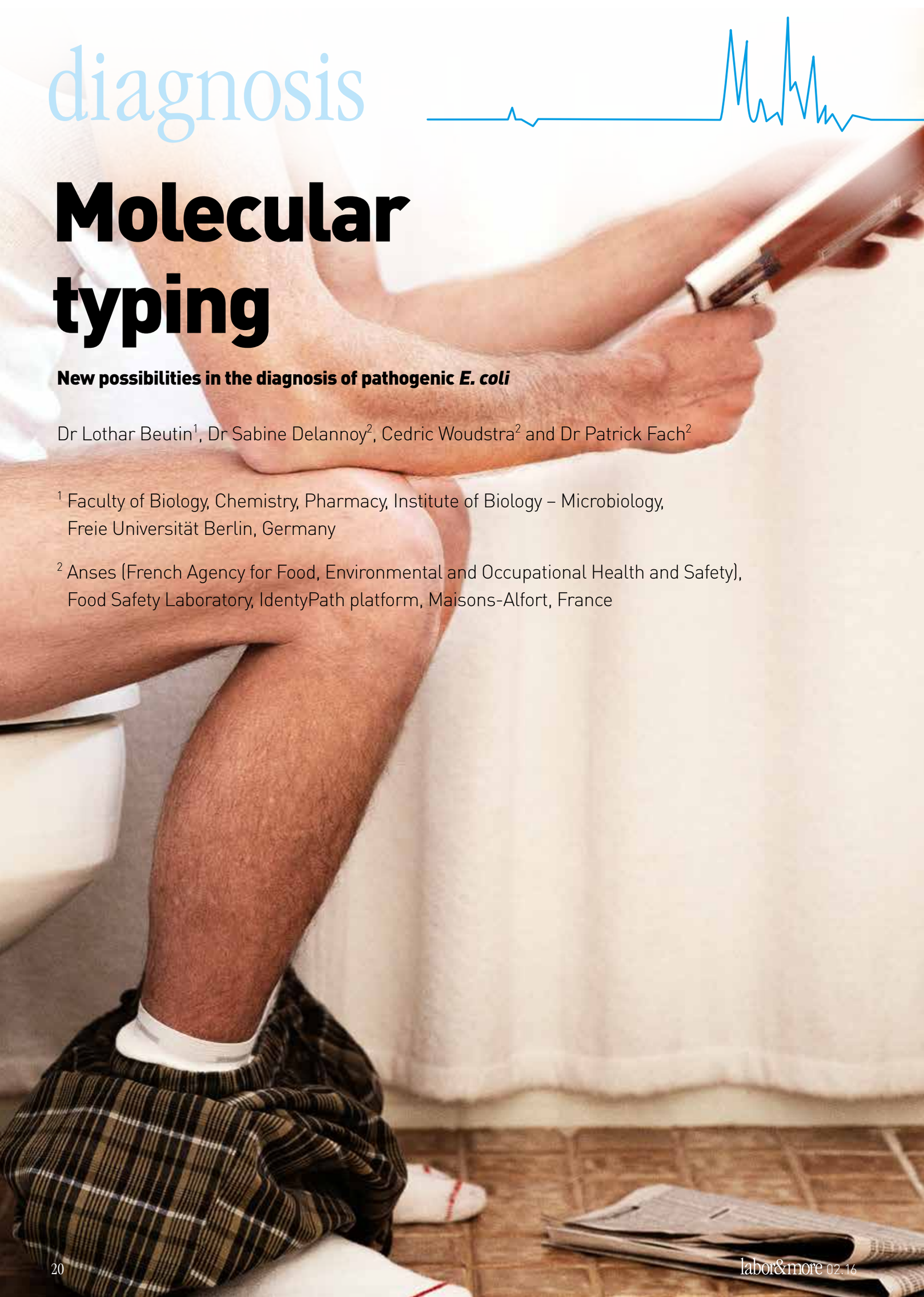

Strains of the bacterial species *Escherichia coli* occur not only as useful members of the gut flora of humans and warm-blooded animals but also as dangerous pathogens, whose properties are responsible for the genesis of urinary tract infections, sepsis and meningitis, and can even lead to bloody diarrhoea and kidney failure.
Since dangerous *E. coli* cannot be distinguished from harmless strains with the use of conventional microbiological methods, molecular-genetic methods with a high sample throughput have a major role to play in the diagnosis and prevention of *E. coli* infections.
This approach proved its worth during the EHEC 0104 outbreak in summer 2011 with over 4,000 severely ill patients. Methods such as next-generation DNA sequencing combined with real-time PCR arrays offer the potential required for the rapid, cost-effective processing of large numbers of samples – such as occur during outbreaks, food sampling and in the field of hospital hygiene.

"The good, the bad and the ugly" – the useful, troublesome and downright deadly members of the species *E. coli*

Strains of the bacterial species *Escherichia coli* are naturally-occurring, symbiotic members of the gut flora of humans and warm-blooded animals. Not all strains of *E. coli* are harmless, however: some subgroups of this species possess properties that can lead to illness in both humans and animals. Extra-intestinal pathogenic *E. coli* (ExPEC), which colonises the gut of its hosts without producing symptoms, can cause urinary tract infections, sepsis and meningitis if it passes into parts of the body outside the gut. Other pathogenic strains of *E. coli* can cause intestinal infections and diarrhoea, and, in the case of enterohaemorrhagic *E. coli* (EHEC), bloody diarrhoea, kidney failure (HUS) and neurological damage.

Memories are still fresh of the EHEC O104 outbreak of summer 2011, with over 4,000 patients, 800 cases of HUS and 53 deaths. The search for the contaminated food that was the source of the infection proved to be difficult and time-consuming [1]. One reason for this situation was the fact that both harmless and pathogenic *E. coli* are widespread as indicators of faecal contamination in the environment (soil, water, agricultural implements). Food of both plant and animal origin can contain *E. coli* bacteria as a result of contamination during cultivation (fertilisers, irrigation) and production (milking, slaughtering). The diagnosis of pathogenic *E. coli* is problematic since the harmless and pathogenic strains of this species cannot be distinguished by applying phenotypic criteria (metabolic performance, morphology, culturing on differential media). Accordingly, this constitutes a fundamental difference to the diagnosis of obligate pathogens such as *Salmonella* or *Shigella*. Since *E. coli* are de facto present in all samples, suitable diagnostic instruments must be made available to distinguish pathogenic from harmless strains of this species as part of a rapid, reliable process.

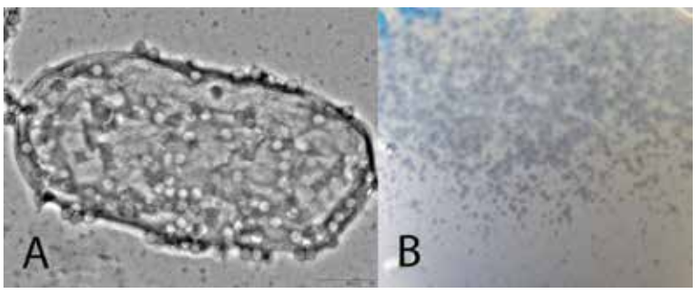

Fig. 1 Transfer of Shiga toxins by bacteriophages
A) Electron micrograph of an *E. coli* bacterium (micrograph taken by Jochen Reetz, BfR, Berlin) infected by Stx-phages (round particles on the surface of the bacterium).
B) Plaque formation (bacterial lysis) by Stx-phages on an *E. coli* culture on nutrient agar (image L. Beutin). Via horizontal gene transfer by bacteriophages, which carry the genes for the production of Shiga toxins, the property required for Stx formation can be continuously transferred to new strains of *E. coli*. Some of these novel reactions, such as the outbreak strain EHEC 0104:H4, have proven to have the makings of "superbugs": pathogens that carry virulence properties in novel combinations and are more dangerous than their initial strains [2, 3].

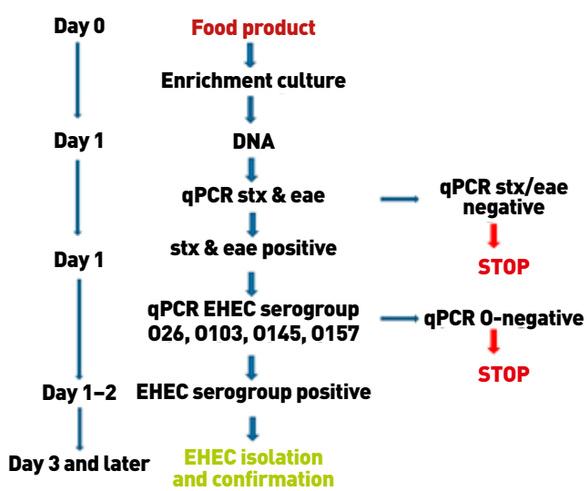

Fig. 2 Schematic workflow of the application of ISO/TS 13136 to EHEC analysis
Principle of ISO/TS 13136:2012, "Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups;
German version CEN ISO/TS 13136:2012. The method is designed
a) for products intended for human consumption and for use as animal feed; b) for environmental samples taken from food production and food processing; and c) for environmental samples taken from primary production (agriculture).
Execution: A quantity of the foodstuff to be investigated (at least 25 g/25 ml) is diluted with an enrichment broth for *E. coli* and this enrichment culture is then incubated for 18–24 h at 37 °C. On the next day, a DNA preparation is made from 1 ml of enrichment culture. A qPCR for the Shiga toxins (stx) and attachment factors (eae) of the EHEC is performed with the DNA obtained. Only if both PCRs react positively is a second PCR then performed, which attempts to detect the five EHEC-typical serogroups (O26, O103, O111, O145, O157). If one or more of these groups is also detected, then the corresponding EHEC strain must be isolated from the culture and its markers subsequently confirmed. All other findings – only stx-positive, only eae-positive, stx + eae positive but EHEC O-groups negative – are not followed up.

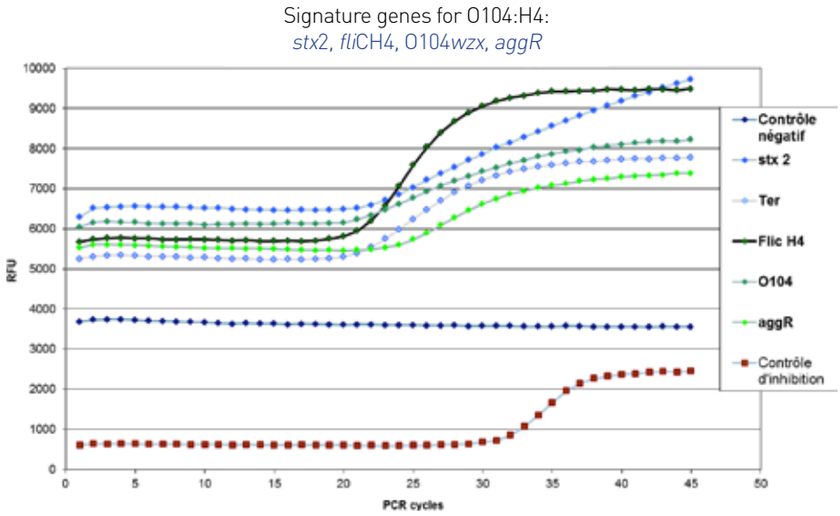

Fig. 3 Multi-parametric detection of EHEC O104:H4 signature genes with qPCR
Multiplex qPCR performed on a GeneDisc® Cycler (Pall GeneSystems) for the detection of EHEC O104:H4 from complex test material. The pathogen's signature genes (Shiga toxin: stx2, flagellar type: fliCH4, O-antigen: wzxO104 and enteroaggregative properties: aggR) are detected in parallel. The generous loan of a GeneDisc Cycler from Pall GeneSystems (Bruz, France) substantially supported EHEC O104:H4 diagnostic work at the Federal Institute for Risk Assessment during the EHEC O104:H4 outbreak, and enabled the processing of over 600 food samples in a short space of time (Adolphs & Alt et al. 2012).

Show me your face and I'll say who you are

Of the various options available for differentiating bacterial strains within a single species, the serological typing of *E. coli* and *Salmonella* has been the global standard for many years now. In principle, this approach is based on immunologically detectable differences in the bacterial surface structures, which can be used as antigens for manufacturing diagnostic sera. With serotyping, *E. coli* bacteria can be distinguished from one another by a kind of "face recognition" process that looks at their outer surface for features such as lipopolysaccharides (O-antigens), capsules (K-antigens) and flagellar antigens (H-antigens). The literature currently describes over 180 O-antigens, 50 K-antigens and 53 H-antigens for *E. coli*, and these can occur in various combinations. Serotyping is an important instrument for detecting the presence of potentially pathogenic *E. coli* strains, since certain serotypes are often associated with disease-causing properties. This is another reason why serotyping has remained the global gold standard for describing *E. coli* isolates to the present day. It is a key resource in the identification of epidemics, and saw recent use in the major EHEC 0104:H4 outbreak in summer 2011 in Germany and other affected countries.

The wolf in sheep's clothing

Viewed in and of itself, however, the serotype of an *E. coli* bacterium says nothing about its disease-causing propensities. From the 1970s onwards, increasing numbers of virulence factors have been discovered for *E. coli* and other bacteria. Bacterial virulence factors promote the colonisation and proliferation of their carriers to the detriment of the host and competing microflora – with the result typically being disease. Often, a pathogen is only created in the first place by a combination of virulence factors.

Prior to the actual disease, the bacteria successfully colonise and proliferate in specific organs of the host's body. Various colonisation factors enable pathogenic *E. coli* to colonise cells of the bladder, kidney and small intestine – parts of the body that are not accessible to harmless *E. coli* bacteria. This leads to urinary tract infections and/or diarrhoea.

Normally, *E. coli* are also unable to live (or even survive) in blood – unless the bacteria protect themselves by expressing certain LPS structures and polysaccharide capsules. Such encapsulated bacteria can proliferate in their host's blood and then spread to a range of organs via the circulatory system. Systemic disorders such as sepsis and meningitis (nosocomial infections) can occur, often associated with severe progression and fatalities.

With the formation of various poisonous substances (toxins), pathogenic *E. coli* can reprogram the functions of certain cells in their host (enterotoxins) or destroy the cells entirely (Shiga toxins and other cytotoxins). The effects of toxins on the host organism are multifarious: with EHEC, which produces Shiga toxin, they range from crippling diarrhoea to renal failure (HUS) and brain damage.

The characterisation of genes that are responsible for the bacteria's various virulence factors has opened the way to the molecular diagnosis and typing of pathogenic strains of *E. coli* and other disease-causing agents. By developing appropriate genetic detection methods (DNA hybridisation, PCR, DNA sequencing), increasingly simpler and cheaper techniques that are also quicker to carry out can be used to determine the "virulotype", a concept that describes the totality of a pathogen's disease-causing characteristics.

Such investigations reveal that the virulotype doesn't always match the serotype, since many virulence properties can – unlike the serotype – be spread by horizontal gene transfer right across a wide number of strains of the *E. coli* species. One example of this is the transfer of the genes for Shiga toxins by bacterial viruses (phages) (Fig. 1, [2, 3]). As a result of this efficient transfer mechanism, more than 400 serotypes of *E. coli* have now been identified as producers of Shiga toxins (STEC) [4]. That said, only few STECs have the potential virulence of an EHEC and the capability to cause severe disorders in their hosts such as bloody diarrhoea and HUS. Accordingly, the successful diagnosis of highly pathogenic EHECs from contaminated food requires the virulotype to be established as the sum of EHEC-typical properties, so as to be able to distinguish EHECs from other, less dangerous STECs.

Using "biometric face recognition" to identify pathogenic *E. coli* in food and clinical samples

Food of plant and animal origin, as well as clinical material and environmental samples, often contain complex mixtures of bacteria, in which small numbers of potential pathogens can also be found. To detect EHECs in foodstuffs, step-by-step diagnostic methods have been developed in Europe and the USA, with which the plethora of over 400 STEC serotypes can be analysed to identify those strains classifiable as the dangerous EHECs. Quantitative PCR (qPCR,

Lothar Beutin studied biology at Freie Universität Berlin, also receiving his doctorate from the same university and completing his habilitation in microbiology in 1992. He was a long-standing Director of the National Reference Centre for *E. coli* at the Robert Koch Institute and the Reference Laboratory for *E. coli* at the Federal Institute for Risk Assessment. His research interests include R&D work on the diagnosis, characterisation and detection of virulence factors in pathogenic strains of *E. coli*. He collaborates actively with numerous international research groups. He has collaborated closely with the Patrick Fach lab (Anses Maisons-Alford, France) since 2009, in a partnership that has produced numerous joint publications and patents.

Patrick Fach received his Ph.D. from Compiègne University of Technology (France). In 1999, he was appointed Director of the Biotechnology Unit in the Research Laboratory for Food and Food Safety run by the French food safety agency AFFSA. He works closely with research groups in Europe and the USA. He joined Anses (French Agency for Food, Environmental and Occupational Health & Safety) in 2010, where he manages the genomic analytical technology platform IdentyPath.

Sabine Delannoy received her Ph.D. in molecular and cell biology in 2006 from Southern Methodist University in Dallas, Texas (USA). Sabine Delannoy has worked as part of the IdentyPath platform team since 2010. One focus of her work is the development of methods for the detection and characterisation of the human pathogen Shiga toxin-producing *E. coli* (STEC), and the development of diagnostic instruments for use with STEC infections. Since 2012, she has co-authored twenty publications on this and related topics.

Cédric Woudstra works at Anses as a development engineer on the IdentyPath analytical platform, where he specialises in work on *Clostridium botulinum*. This also involves close collaboration with the French National Reference Laboratory for Botulism in Ploufragen (Bretagne, France). In his work, Cédric Woudstra develops methods for molecular detection and typing based on quantitative real-time PCR with a high sample throughput. In addition, Cédric Woudstra is also involved in next-generation sequencing projects.

real-time PCR) has established itself as an investigative technique, since it is specific and sensitive enough to reliably detect a few target organisms (here: EHECs) among accompanying flora several magnitudes more numerous. Figure 2 offers a schematic representation of the investigative method for the EU Directive (ISO/TS 13136) [5].

The molecular typing of pathogenic *E. coli* is flexible in its application. With the increasing number of publicly-available genome sequences of pathogenic *E. coli*, new genetic markers can be identified that indicate the presence of a highly virulent microorganism in a complex sample with even greater precision. It has also been easily possible to include newly-occurring EHEC variants such as the enteroaggregative EHEC O104:H4 in the test schema by means of their typical markers (Fig. 3).

The collaboration in place between the research groups of Lothar Beutin (previously BfR, Berlin) and Patrick Fach (Anses, Paris) since 2009 has resulted in the development of molecular diagnostic methods for the detection of EHEC and other diarrhoea pathogens, which have already seen practical application in EU Directives such as ISO/TS13136 (Fig. 2) and in the diagnosis of EHEC O104:H4 (Fig. 3) [6] (Beutin & Fach 2014). The future – and ambitious – objective of this collaborative work is a complete molecular serotyping of *E. coli* that is more sensitive, more specific and more comprehensive than conventional typing with agglutinative antisera.

The molecular detection of pathogenic *E. coli* offers new opportunities for diagnostics. This affects in particular the targeted search for sources of infection such as contaminated food but also has relevance for the rightly feared nosocomial infections. Alongside *S. aureus* and *P. aeruginosa*, extra-intestinal pathogenic *E. coli* (ExPECs) are the primary cause of nosocomial infections such as pneumonia, urinary tract infections, sepsis and meningitis. ExPECs are found in the gut flora of many people and can be introduced by means of patient contacts, nursing staff, hospital utensils or food products. The genetic signature of many ExPECs is already known. Molecular diagnostics enables the identification of specific markers for the nosocomial microbe in question, and a targeted search for these markets can rapidly and reliably identify the source of infection. The investigation of complex mixtures of microbes and a large number of samples is no longer an insurmountable obstacle. Modern analysis equipment such as the IdentyPath platform (Patrick Fach lab, Anses, Paris) enable the automated execution of up to 9,000 qPCR reactions in three hours at a cost of 10–20 euro cents/sample. Looking to the future, the technical requirements have already been met for the improved analysis and real-time detection of complex epidemiological circumstances.

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Caseins in fresh milk

HPTLC-MS imaging of proteins and protein derivatives

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In protein analysis, research has now expanded beyond topics such as the identification and quantification of proteins to take a particularly strong interest in protein modifications (known as "post-translational modifications", PTM). Importantly, these modifications do not merely determine the effects of specific proteins, since modified proteins can also be utilised as biomarkers in physiology or as process markers in food analysis.

Current analysis work is based on separation methods utilising chromatography and electrophoresis, which often produce less than satisfactory results in terms of protein modifications, since the underlying reactions that lead to these PTMs can be very complex and are often insufficiently well characterised.

The aim of coupling thin-layer chromatography (HPTLC) with mass spectrometry (MS) is to exploit the advantages of both techniques. This approach neo-proteomics aims to enable even the identification of PTMs that are very complex in terms of the number and diversity of their chemical structures. One example that can be mentioned is non-targeted reactions of proteins with other food constituents – and also including those that take place in vivo. Numerous protein glycations occur, for example, which can be utilised as biomarkers for the metabolic disorder diabetes mellitus. In this context, the decision to use the technique of HPTLC-MS imaging brings additional benefits: Alongside direct detection on/from the thin-layer plate, elution behaviour can enable statements to be made about selected physical and chemical properties of the proteins/peptides (such as polarity) and the molecular weight can be determined. The imaging enables the detection of modifications and the visualisation of the protein/peptide distribution on the thin-layer plate. Overviews can thus be readily obtained and individual samples compared.

An analysis of caseins in fresh milk was used to test a procedure using this methodology. The aim was to demonstrate detection of a range of caseins after chromatographic separation on a thin-layer plate, followed by the use of mass spectrometry for the charting (imaging) of the individual peptides.

Background

The term "proteomics" describes the field of research concerned with the analysis of any and all proteins – whether these are formed by an organism, a piece of tissue or a cell. The aim is to improve our understanding of the properties of proteins and their associated biological processes [1]. Two approaches have been used to date to analyse these complex research issues: the top-down method and the bottom-up method. In the bottom-up approach, the proteins are split into smaller subunits using proteolysis – typically with the aid of the enzyme trypsin – before analysis with mass spectrometry. The constituent peptides are chromatographically separated so that the subsequent mass spectrometry can identify and characterise the peptide sequences and modifications (as applicable) [2]. In the top-down approach, however, the intact proteins are separated from one another and then analysed using mass spectrometry. With this technique, individual smaller fragments are created during mass spectrometry itself by ionisation. The spectra of these fragment ions, in turn, enables the determination of the protein sequence and modifications. While separation and identification is considerably more difficult to achieve here, one can record a complete set of information about the protein without major losses – which is not possible when using the bottom-up approach [2].

Although it is a separation method with a long history, there has previously been little use for thin-layer chromatography in modern high-performance analytics due to its lack of efficiency and power. In the form of HPTLC (High-Performance Thin Layer Chromatography), however, this method is now increasingly been deployed as a highly-sensitive, precise and rapid technique with potential applications in many areas of analytical science [3]. With the use of high-performance equipment for the application, development, documentation and improvement of sorbents, it was possible to increase separation efficiency while improving both precision and reproducibility. Major benefits of this technology include the possibility of analysing multiple samples in parallel, as well as multimodal and multidimensional techniques. Other advantages include the wide range of detection and coupling options, and especially the coupling of HPTLC with Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry (HPTLC-MALDI-MS), with Electrospray-Ionisation Mass Spectrometry (HPTLC-ESI-MS) and with Desorption Electrospray Ionisation Mass Spectrometry (HPTLC-DESI-MS) [4, 5, 6]. One new development involves specialised MS-grade plates for coupling TLC with mass spectrometry. This enables the achievement of high sensitivity with low background signals and thus excellent signal/noise ratios [7].

Knut Behrend studied chemistry at the University of Hamburg and completed his studies in 2015 with an M.Sc. following dissertation work completed at Merck KGaA in Darmstadt, Germany. He is studying for his Ph.D. since May 2015 under Professor Sascha Rohn, with his topic being the "development and characterisation of new thin-layer preparations for use in coupled TLC-MS." He is completing his thesis work in the R&D Group for Instrumental Analytics at Merck KGaA in Darmstadt.

Michael Schulz studied technical chemistry at the Georg-Simon-Ohm University of Applied Sciences in Nuremberg. He began working at Merck KGaA in 2000, where he was employed until 2007 as a laboratory engineer in the R&D lab for thin-layer chromatography. In the period from 2007 to 2014, he was laboratory manager for the Thin-Layer Chromatography and Particulate HPLC Materials R&D labs, and has been responsible for the R&D Group for Instrumental Analytics at Merck KGaA since 2014.

Katerina Matheis studied chemistry at the University of Karlsruhe and received her doctorate in 2010 from the Karlsruhe Institute of Technology (KIT). She was a research assistant in the Central Analytics unit at Merck KGaA before being appointed to her position as manager of the Mass Spectrometry Analysis of Small Molecules lab in 2013.

Methodology

The HPTLC-MALDI imaging workflow can essentially be subdivided into three steps: (I) Sample preparation and chromatographic separation; (II) measurement using mass spectrometry; and (III) the imaging itself – i.e. the presentation of the mass spectrometry data and their analysis (Fig. 1).

I) Sample preparation and thin-layer chromatography

Thanks to their solubility at pH 4.6, the caseins can be easily separated from milk and whey proteins, after unwanted fat has been removed via centrifugation. Various caseins are present in milk (and dependent to an extent on the milk's origin, i.e. "cow vs. sheep"), whereby αS1-casein, αS2-casein, -casein and -casein are the best-known of their type. For the analytical procedure utilised, the caseins are split proteolytically with trypsin and without prior separation. The separation of the resulting peptides takes place on a thin-layer plate (here: an HPTLC silica gel, MS-grade 60 F254 for MALDI, Merck KGaA, Darmstadt, Germany). If the sample is applied as a band, separation can take place in one dimension; if applied spot-wise, separation can take place in two dimensions (Fig. 2). For two-dimensional separation, the HPTLC plate is initially developed in the first dimension with 2-butanol/pyridine/aqueous ammonia/water as the mobile phase. Once the plate is completely dry, it is rotated 90° and subsequently developed in the second dimension with 2-butanol/pyridine/acetic acid/water as the mobile phase. Apart from solvent ratios, the two mobile phases differ primarily in terms of their choice of modifying agent ("modifier", typically acetic acid, aqueous ammonia, tetrahydrofuran, etc.). In the first dimension, aqueous ammonia is used for a basic pH value, while acetic acid is used in the second dimension for an acidic ph value. Separation in thin-layer chromatography is a complex interaction involving the interplay of the analytes with the stationary and the mobile phase. As a result of differences in the pH values, the charge and thus the polarity of the peptides is influenced, whereby a range of different retardation factors (Rf) is achieved. This signifies different migration speeds experienced by a peptide and thus different distances of the peptide from the separation origin, which is dependent on the particular modifier added.

The use of thin-layer plates developed in parallel furthermore permits a direct comparison between the detection methods (Fig. 1). In the field of protein and peptide analysis in particular, traditional colour reagents can supply initial data about the properties and – to an extent – about the chemical structure. In this context, colour reagents such as ninhydrin or fluorescamine are deployed, whose interaction with the analytes on the thin-layer plate correspondingly indicates their presence – as well as their distribution on the plate (Fig. 2). Here, the broad spectrum of derivatisation reagents available can clearly be viewed as an advantage of thin-layer chromatography.

II) Coupling of thin-layer chromatography with MALDI mass spectrometry

With MALDI-TOF-MS coupling, the chromatographic separation stage is followed by completely coating the surface of the HPTLC plate with a suitable MALDI matrix. This can be completed by dipping or spraying. The plate to be measured is then fitted into a (commercially-available) adapter and placed in the mass spectrometer for measurement (Fig. 3). The fully-automated

Maria Riedner studied biochemistry at the Freie Universität Berlin and completed her doctoral studies from 2007 to 2010 at Hamburg-Eppendorf University Hospital, where she continued to work as postdoc in the Core Facility Mass Spectrometry Proteome Analysis until 2011. She is managing the Mass Spectrometry unit in the Faculty of Chemistry at the University of Hamburg since 2012. Her research works focuses on the identification of therapeutically relevant proteases and the development of analytical methods for the characterisation of proteins and proteoforms.

Sascha Rohn studied food chemistry at Goethe University, Frankfurt (Main), and received his doctorate in 2002 from the Institute of Nutritional Science at the University of Potsdam. He completed his habilitation from 2004 to 2011 in the Institute of Food Chemistry at TU Berlin. He was appointed Professor of Food Chemistry in the HAMBURG SCHOOL OF FOOD SCIENCE, University of Hamburg, in 2009. Research in his laboratory focuses on analytics, and the stability and reactivity of bioactive ingredients during the processing and production of foodstuffs and animal feeds of plant origin.

measurement is performed in tracks in the case of 1D separation, while when 2D separation has been used, the laser moves across the measurement area of the plate using a raster of squares of a defined resolution. At each raster point, a mass spectrum is recorded. From the sum total of data provided by all raster points, a mass spectrometry image is then generated, in which the masses detected can be depicted in colour, depending on their intensity in the various spectra (Fig. 4).

III) Analysis and findings

By comparing the MALDI image with the "image" of a traditionally coloured plate, peptides previously detected only due to their fluorescence colouration or following derivatisation can now be identified based on their mass. Accordingly, the use of standards during separation as typical in thin-layer chromatography is no longer strictly necessary, since mass spectrometry makes the unambiguous identification of each band (1D) or each spot (2D) possible in the majority of cases. In the experiment conducted for demonstration purposes using caseins obtained from fresh milk, this methodology was able to detect 89 peptides on a single plate following 2D development. Due to the size of this number – and also to simplify analysis – the annotation is performed in two arbitrary mass ranges for one and the same plate (here: 600 – 1500 Da and 1500 – 4800 Da in Figs. 4A and B). This also enables a good visualisation of the peptides' distribution on the plate.

The casein peptide example shows that the separation is independent of the peptide mass. In general, large and small peptides can be detected across the entire separation area, since this is – as has been described above – primarily determined by the polarity and can be selectively influenced by the chromatographic conditions. Multiple forms of interaction occur between the peptides, the stationary phase and the mobile phase, which are strongly dependent on the amino acid sequence of the individual peptide. The individual amino acids also determine, among other aspects, the peptide's charge, which can be influenced by the pH value (of the eluents). As a result, peptides with a very small difference in mass of 1 Da can nonetheless exhibit different Rf values (examples marked with "#" in Fig. 5).

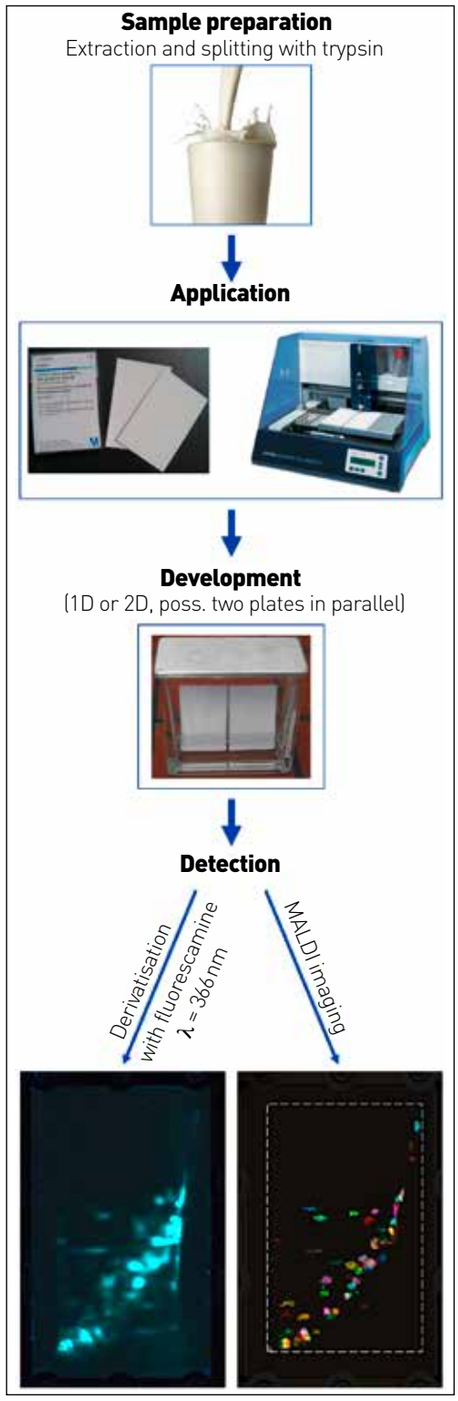


Fig. 1 Schematic workflow of the analysis of a sample by applying 2D-HPTLC-MALDI mass spectrometry.

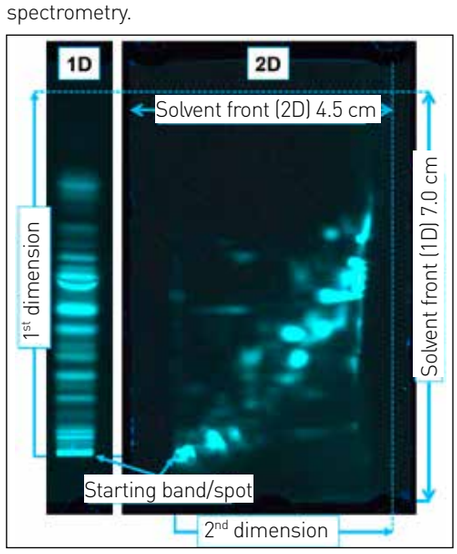


Fig. 2 Peptides obtained by splitting milk proteins with trypsin after one and two-dimensional development. Derivatised with fluorescamine and visualised under UV light (366 nm).

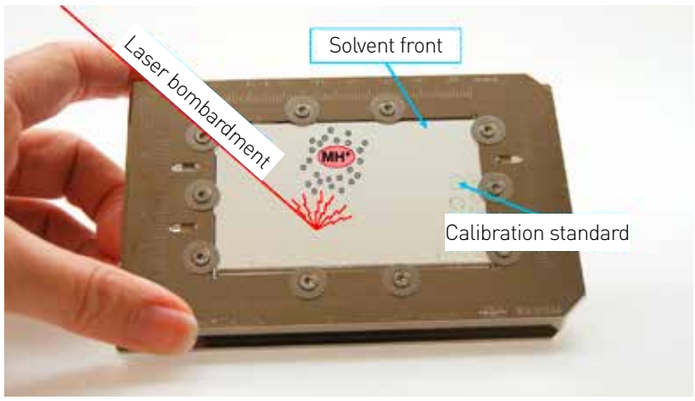
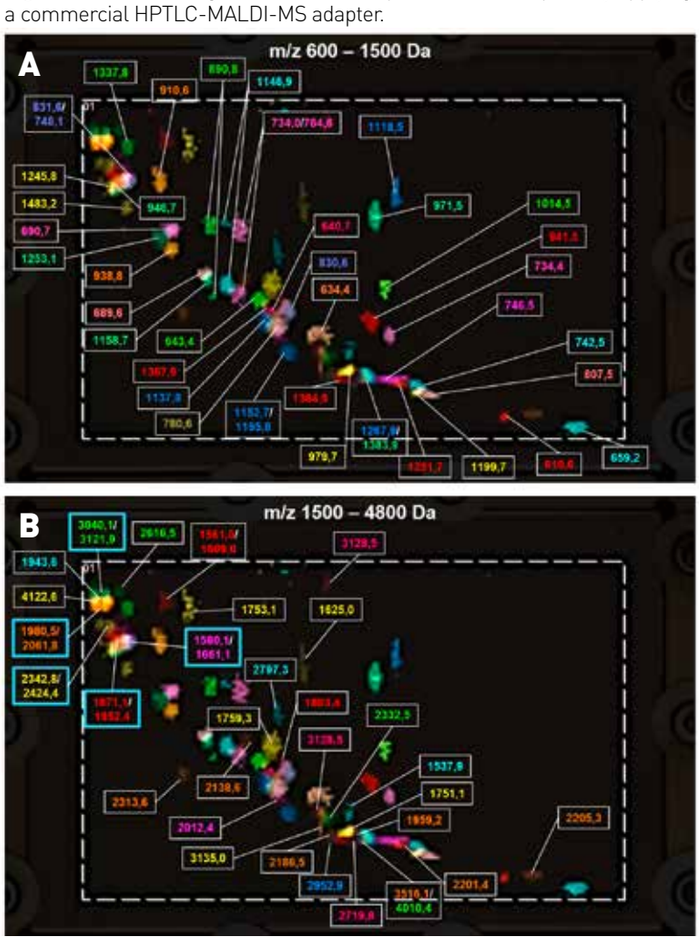


Fig. 3 Schematic diagram of laser desorption on the TLC plate by applying a commercial HPTLC-MALDI-MS adapter.



Figs. 4 A and B Presentation of two-dimensional HPTLC-MALDI imaging with annotation of the masses of all 89 casein peptides detected. (A) mass range 600 – 1500 Da; (B) mass range 1500 – 4800 Da.

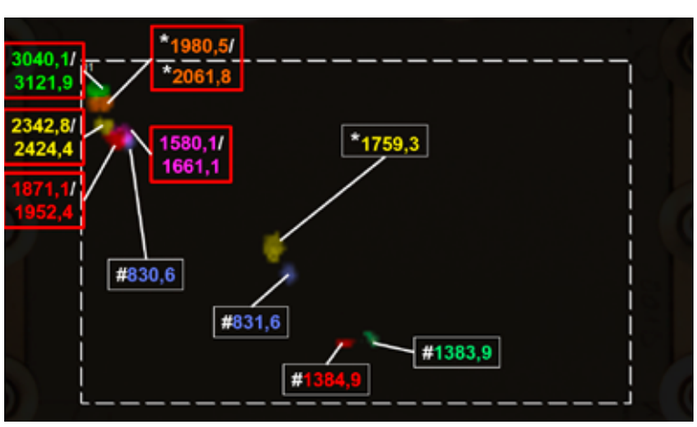


Fig. 5 Presentation of a reduced number of peptides from the 2D-HPTLC-MALDI imaging from Figure 4. Phosphorylated peptides are marked with a red frame, peptides completely sequenced by fragmentation with an asterisk ("*") and peptides with an insignificant mass difference but a different position with a hash sign ("#").

Phosphorylated peptides can often be separated from one another only with difficulty and exhibit only very low Rf values under most of the normal chromatography conditions. Despite this minor separation, however, coupling with mass spectrometry permits the identification of the phosphopeptides by measuring the loss from phosphorylation (neutral loss 80 Da) in the subsequent MS/MS investigations (examples marked with a red border in Fig. 5). As a rule, phosphorylations are typically PTMs of the amino acids serine or threonine. The fragmentation of the peptides in the MS/MS analysis permits the determination of the amino acid sequence and thus the modified amino acid of a peptide. A good example illustrating the sequencing of a phosphorylated peptide is the peptide pair with 1980.5 Da and 2061.8 Da (marked with an asterisk "*" in Fig. 5). These correspond on the one hand to the unmodified peptide FQSEEQQQTEDELQDK with a mass of 1981.9 Da and on the other to the phosphorylated peptide FQS*EEQQQT-EDELQDK with a mass of 2061.9 Da.

Another example of a fully sequenced peptide was measured with a mass of 1759.3 Da. This corresponds to the peptide HQGLPQEVLNENLLR with a theoretical mass of 1759.9 Da.

Summary: from fresh milk to sugars and lipids

The numerous degrees of freedom of the various separation systems offered by HPTLC enable a versatile and comprehensive analysis for the identification and characterisation of various proteins/peptides. When combined with mass spectrometry and imaging, this not only permits the simple determination of the molecular weight of the individual peptides and various modifications, but also provides information about the protein sequence directly from the HPTLC plate "at-a-glance".

With TLC-MALDI imaging, used in the sample experiment for the analysis of fresh milk, it was possible to detect the four most important cows' milk caseins simply and reliably by means of a few specific peptides. This technique can be easily applied not only to other proteins and peptides but also to entirely different analytes such as lipids or sugars. And in the context of physiological changes, i.e. not only modifications of proteins but also changes during the processing and production of foodstuffs, a much wider range of analyses can therefore now be performed.

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Light from fungi

The bioluminescence of higher fungi was probably already known to the Greeks. The fruiting bodies of various species emit light continuously, which is visible to the naked eye. Later, it was realised that the cold aqueous extract of a firefly mass preserves the enzymatic activity of luciferase (luciferase is the name of the enzyme that reacts with the associated luciferin), whereas the hot-water extract leaves only luciferin. Luciferin is oxidised in air with luciferase acting as a catalyst, thereby producing visible bioluminescence.

Working in wooded areas near Krasnoyarsk, a Russian-Japanese team of researchers [1] collected luciferin precursors from five non-luminescent forest specimens. The quantity in the fruiting bodies proved to be 100 times higher than in the mycelium of known bioluminescent fungi such as *Neonothopanus nambi* or *Mycena citricolor*. The researchers proceeded to investigate the fruiting bodies of *Pholiota squarrosa* (shaggy scalycap), isolating six substances that produced the following results in the bioluminescence test [2] (signal/noise): 1 (hispidin 24,000, E-isomer), 2 80 (3, 14-bishispidinyl, E-isomer), 3 6,670 (hispidin Z-isomer), 4 88 (bisnoryangonyl-14-hispidin), Z-isomer 40, 5 (bisnoryangonin, E-isomer) (see Fig. 1) 1,300 and 6 1,000, (bisnoryangonin, E-isomer).

Hispidin is well-known as a styrylpyrone-class compound found in fungal and plant secondary metabolism. It has been isolated as a luciferin precursor from the bioluminescent mycelium of *Neonothopanus nambi*. The researchers then investigated three other species for the presence and specific activity of hispidin. All samples (*M. citricolor, P. stipticus and Armillaria borealis*) contained hispidin, resulting in bioluminescence.

An enzyme substrate – NADPH – is first required for the conversion of hispidin into the hydroxyl compound. An enzyme, namely luciferase, then causes the bioluminescence of the fruiting body in the presence of oxygen, which can is often visible to the naked eye (see Fig. 2).

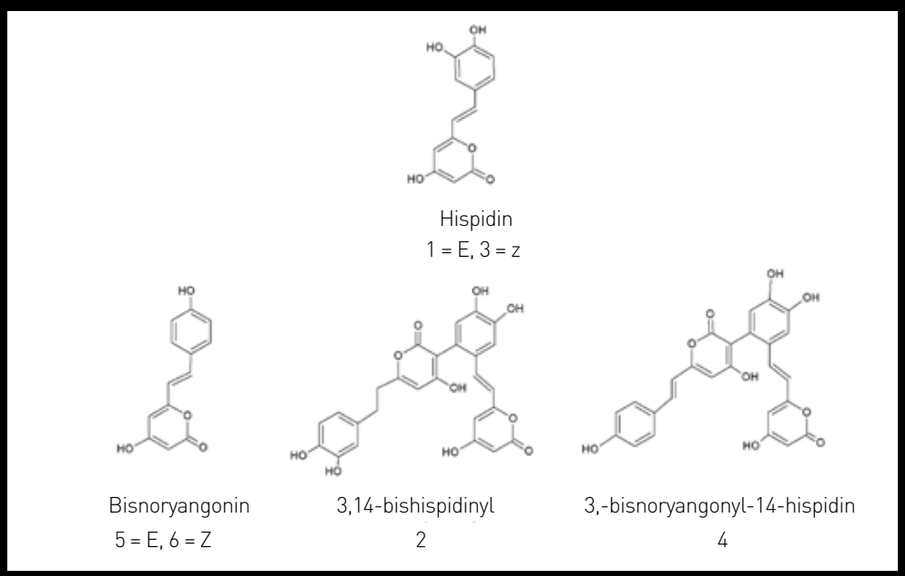

Fig. 1 The Z- and E-hispidines are the most important precursors of the luciferins in fungal secondary metabolism.

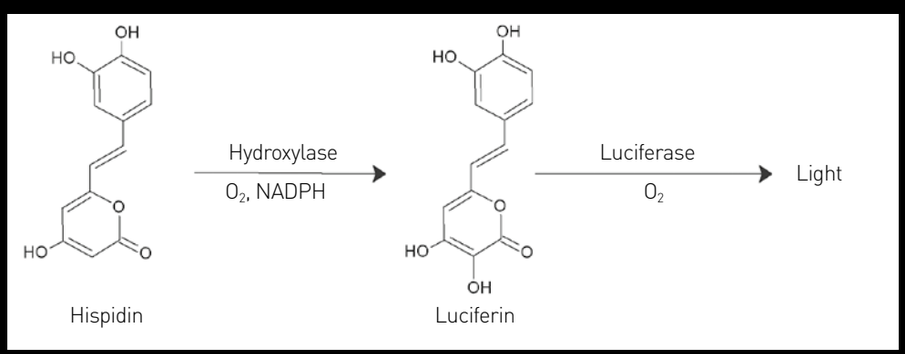

Fig. 2 NADPH first produces 3-hydroxyhispidin. This is then oxidised in the presence of a luciferase.

[1] *Purtov, K. V. et mult. al. (2015) Angew. Chem. 127, 8242–8246, DOI: 10.1002/Anie.201501779*

[2] *Stevani, O. (2009) Photochem. Photobiol. Sci. 8, 1416–1421*

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→ **GS**

Robust proteomic data

Automated sample preparation for protein analysis with mass spectrometry

Mass spectrometric techniques for identification and quantification require proteins in peptides to be split beforehand. This splitting, also known as protein digestion, uses enzymes such as the protease trypsin or the endopeptidase Lys-C.

Analysis using the methods of mass spectrometry can be applied to proteins in solution or proteins that have been isolated by using SDS gel electrophoresis. Sample preparation involves a number of steps. The first step following the denaturation and washing of the sample is a reduction and subsequent alkylation of the sulfhydryl group in the proteins, for which the reagents TCEP and CAA are used. This step is followed by the actual enzymatic digestion and subsequent extraction of the newly-emergent peptides, which are then analysed using mass spectrometric techniques.

To generate robust proteomic data, a reproducible procedure is necessary. The use of automated sample preparation robotics is therefore highly advisable.

Automated protein digestion

The Max Delbrück Centre for Molecular Medicine in the Helmholtz Association worked with Axel Semrau® to develop a standard method for automated protein digestion, and collaborated with leading research institutes to transfer it to PAL RTC sample robotics. For system control, CHRONOS is used, a software package for time-optimised usage of the robotic system and which thereby enables a higher throughput.

Fig. 1 Supervision of the system set-up

Fig. 2 CHRONECT Proteomics Workbench

Automation process

Protein digestion in the solution (in-solution digest)

- Provide protein sample in buffer
- Add TCEP to perform reduction (30 min)
- Alkylate with CAA/20 min
- Add LysC and the first digestion/3 h
- Dilute Sample with buffer
- Add trypsin and second digestion/10 h
- Complete digestion through TFA

The CHRONECT Proteomics Workbench includes a special tray with a vacuum pump connection that enables digestion of the proteins directly within the electrophoresis gel

Protein digestion within electrophoresis gel (in-gel Digest)

- Place gel section into tray with vacuum station
- Wash gel intensively
- Add TCEP to perform a reduction/30 min
- Alkylate with CAA/20 min
- Add trypsin and digest/10 h
- Complete digest with TFA

CHRONECT Proteomics is a development of the Max Delbrück Centre for Molecular Medicine for Axel Semrau®.

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