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Separation via binding

Selective enrichment of proteins

Cell technology

Star charts and Petri dishes

Stress research

Molecular consequences

Prof. Dr Stefan Hell –

"We have not reached the end."
Prof. Dr. Stefan W. Hell, department head NanoBiophotonic at Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany. Nobel prize laureate for chemistry in 2014.

Sensing the real world …

By Prof. Dr Boris Mizaikoff
Director, Institute of Analytical and Bioanalytical Chemistry,
University of Ulm, Germany

Boris Mizaikoff joined the faculty at the University of Ulm, Germany, as a Chaired Professor and Director at the Institute of Analytical and Bioanalytical Chemistry in 2007 with prior appointments at the Vienna University of Technology (Vienna, Austria), and at the Georgia Institute of Technology (Atlanta, USA). His research interests focus on optical chem/bio sensors, tailored (bio) molecular recognition interfaces, molecularly imprinted materials, system miniaturization and integration, and multifunctional (nano)analytical techniques with applications in environmental analysis, process monitoring, and biomedical diagnostics.

Modern analytical chemistry is on a continuous quest towards more sensitive, reliable, and versatile measurements for probing atoms, ions, and molecules in increasingly complex measurements providing spatially and temporally resolved information on their presence, diversity, and concentration. In addition, rather than collecting discrete samples for subsequent laboratory analysis, which involves sample collection, transport, storage, and preparation strategies prior to the actual analysis, an increasing global demand for in-situ, on- or in-line, and in-field applicable analytical tools is clearly evident. This trend currently translates into a wide range of application scenarios ranging from environmental and atmospheric monitoring to process analysis and control, safety/security/surveillance settings, military applications, and clinical and (bio)medical diagnostics.

In particular, molecular analysis strategies address species as small as volatile organic constituents (VOCs) or as large as biomacromolecules including proteins, enzymes, and aggregates thereof. Consequently, molecular diagnostics have been the recent focus of an entire analytical device category promising innovative solutions for the 'analytical wish list' mentioned above: optical chemical sensors and biosensors.

While conventional chem/bio sensors based on electrochemical, thermal, or mass-sensitive transduction schemes have readily matured into commercial devices during past decades, optical chem/bio sensors – and in particular waveguide-based sensing schemes – may be considered the 'young kid on the block'. Notwithstanding, optical chem/bio sensing schemes gain increasing popularity across the analytically relevant wavelength regime from the ultraviolet into the mid-infrared and even at THz frequencies. This may be attributed to the telecommunication revolution facilitating the development and dramatic cost reduction of (fiber)optic waveguide technology, semiconductor-based light sources including lasers and light emitting diodes, and integrated optics.

In any chem/bio sensing system, versatile molecular, biomolecular, and biological recognition schemes are responsible for selectively recognizing or preconcentrating target analytes of interest from complex real-world matrices. The main hardware components specific to optical sensing schemes usually comprise a broad- or narrowband light source, a waveguide/transducer, and an optical detector. These components ideally lend themselves for miniaturization using advanced micro- and nanofabrication techniques for achieving on-chip integration at a systems level.

A key component from the practical and analytical perspective is the waveguide, which frequently serves as the actual signal transducer responsible for the reproducible interaction between photons and molecules – an active research area at the Institute of Analytical and Bioanalytical Chemistry at the University of Ulm. Next to serving as the substrate for immobilizing chemical or biological molecular recognition and enhancement schemes, waveguides provide ideal photon conduits enabling the transition of delicate optical sensing schemes into real-world environments with the required robustness. Last but not least, by smartly tailoring the waveguide/transducer geometry (e.g., using tapered fibers, resonating structures, etc.), their analytical signal may be optically enhanced in addition to the chem/bio amplification, which finally gives rise to waveguide-enhanced chem/bio diagnostics.

Reflecting on the 'analytical wish list', it is evident that even the most sophisticated optical chem/bio sensing schemes will not replace modern analytical instrumentation, but should be considered innovative and frequently specialized analytical devices that are complementary to classical laboratory techniques. Yet, serving as 'first responders' in environmental, process or clinical application scenarios, optical chem/bio sensors may do what they do best – provide direct in-situ analytical information by sensing the real world …

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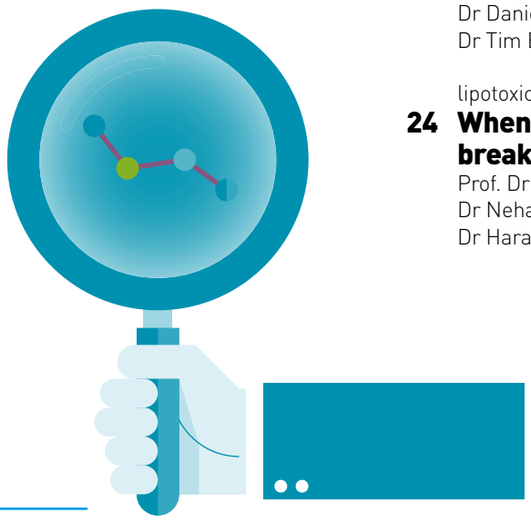
content



Cover: © dpa | Sven Pförtner

"And let there be light!"

The lab&more team has its finger on the pulse of science – always working on the principle that "What interests us will interest other people as well". In a time when rigid subject boundaries, like those between chemistry, biology and the technical disciplines, are increasingly being broken down, interdisciplinary communication is coming to be crucially important for science and research. New ideas often come into being when you widen your horizons and think outside the box.

So our principal concern is to engage with current research and trend-setting themes, and to present them in such a way that they are accessible for a broad international readership and may be found useful for people's own projects. Our publisher Peter Matthes has more than 40 years of experience behind him – and on that basis his company succidia AG has been setting standards in the field of professional media with an approach unique to the industry since 2005. Our authors are highly regarded international experts, and their articles are laid out in reader-friendly format and with an exceptionally attractive design. Lavish and elaborate illustrations encourage readers to get to grips with the specialist information conveyed.

Our recipe for success is also based on close cooperation and teamwork. The graphic agency 4t works independently but forms part of the publishing house, so ideas can be realised flexibly and in short order. Our editors, graphic artists and marketing and sales staff work hand in hand, and so are highly efficient in detecting the latest developments and realising them in close conjunction with our partners in science and industry.

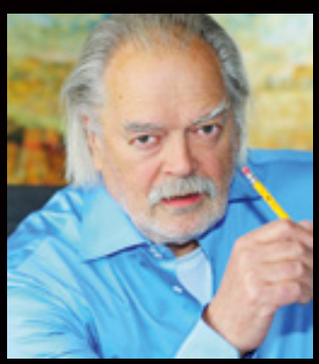
The German edition labor&more occupies a leading position among natural science specialist periodicals. We make our mark on the international stage, with cooperative projects and activities at professional events, congresses and trade fairs, and through partnerships with research institutes. Our growing international network gives us access to the foremost scientists and players in industry. In addition to our ten German issues, lab&more brings out four English issues and two Russian ones aimed at Eastern European readers – enabling us to reach an audience all over the world.

In the year 2015 we will be devoting particular attention to the importance of light. This year has been proclaimed the International Year of Light by the United Nations General Assembly, with the aim of reminding us that light is an elementary requirement for the life of human beings, plants and animals – which also makes it a central component of science and culture. Research into light facilitates a better understanding of the cosmos, and opens up a wide range of innovative possibilities for the application of optical technology. With this first issue, a reputed head in the research world faces us: Prof. Dr Stephan Hell, Nobel laureate and lab&more author is co-developer of the STED technology and has relevantly revolutionized microscopy. His research provides new insights into the innermost secrets of life. Also, the content kicks off with a guest editorial by Professor Mizaikoff, who explains the importance of optical chemical sensors and biosensors for analytics.

Other hot topics in food research, in the biosciences and in connection with important subject areas like water and the environment reflect current challenges, and we are confident that the interest of our readers will be fully engaged.

Sincerely,
The lab&more team

Picture: www.istockphoto.com | kimeveruss, UKRT



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CEO, Publisher

Claudia Schiller
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Editorial Staff

Parliament backs GMO opt-out for EU member states

A new legislation to allow EU member states to restrict or ban the cultivation of crops containing genetically modified organisms (GMOs) on their own territory, even if this is allowed at EU level, was passed by MEPs in January. The legislation, informally agreed by Parliament and Council in December, was originally tabled in 2010 but was then deadlocked for four years due to disagreement between pro- and anti-GMO member states. The new rules would allow member states to ban GMOs on environmental policy grounds other than the risks to health and the environment already assessed by the European Food Safety Authority (EFSA). Member states could also ban GMO crops on other grounds, such as town and country planning requirements, socio-economic impact, avoiding the unintended presence of GMOs in other products and farm policy objectives. Bans could also include groups of GMOs designated by crop or trait.

→ **www.europarl.europa.eu**

European BIOTECHNICA Award 2015 – Apply now!

Companies can now apply for the 2015 European BIOTECHNICA Award – the application deadline is March 31st, 2015. This year's prize will be awarded to a European company offering a "disruptive" technology – i.e. one that has revolutionised the fields of biotechnology or the life sciences – and which the company has demonstrably established as a successful business model. Awarded by Deutsche Messe AG and its partners since 2003, this prize is now viewed as one of Europe's most distinguished accolades for the biotech industry. The prize will be awarded on Monday, 5 October, during the BIOTECHNICA opening ceremony and in the presence of around 500 guests from business, research and politics.

→ **www.biotechnica.de**

European Institute of Innovation and Technology – Rhein-Neckar biotech cluster leads InnoLife consortium to success

The Rhein-Neckar biotech cluster (BioRN) is a member of the winning InnoLife consortium, selected as the knowledge and innovation community (KIC) for the Healthy Living and Active Ageing (EIT Health) project put out to tender by the European Institute of Innovation and Technology (EIT). With funding of up to € 700 million and a total budget of over € 2 billion, EIT Health is one of the largest publicly-financed healthcare initiatives worldwide. The 50 primary partners and 90 other associated members from nine EU countries include Roche Diagnostics in Mannheim, AbbVie in Ludwigshafen and the University of Heidelberg (all Germany) as representatives of the BioRN cluster, plus key institutions and companies from the BioRN partner regions of Cambridge (UK) and Leuven (Belgium) (Health Axis Europe Alliance). Over the next seven years, consortium partners will be working together as a Knowledge and Innovation Community (KIC) with the goal of developing innovative products, services and conceptual models able to improve quality of life and contribute to sustainable healthcare provision all over Europe.

→ **www.BioRN.org**

KPMG expects M&A business to be brisk in 2015

In the year just past, the chemicals and pharmaceutical industry posted mergers and acquisitions totalling USD 214 billion. Compared to 2013 (USD 123 billion), this represents an increase of 74%. Several very large pharma sector deals were responsible for the lion's share of this increase. In this sector, the total value of M&A deals closed in 2014 amounted to USD 162 billion – the highest figure since 2009. Vir Lakshman, Head of Chemicals and Pharmaceuticals at KPMG Germany, expects this upward trend to continue in 2015, predicting that many pharmaceuticals companies will be using targeted deals to pursue their strategic re-orientation.

→ **www.kpmg.de**

market view

Sartorius sells Industrial Technologies division to Minebea

Sartorius, a leading international supplier to the lab and pharma sector, is to sell its Industrial Technologies (Intec) division to the Japanese Minebea Co., Ltd. (Minebea) and its partner, the Development Bank of Japan, Inc. The sale price is linked to the division's operating profit made in the 2014 financial year and will be determined in early 2015; the parties have agreed the price as 7.5 times EBITDA earned in the 2014 financial year. Successful conclusion of the deal is subject to the usual conditions, including approval by M&A regulators, and is expected to be finalised in the first quarter of 2015. With revenue of € 102 million in 2013, the Intec division is the smallest division in the Sartorius Group and achieved an operating margin of 10.1% (underlying EBITDA) at the end of the previous year. Worldwide, the division employs around 700 people, of which some 350 work at the German sites in Hamburg, Aachen and Bovenden. At Minebea, the Intec business will complement the company's Measurement Components division. The company will continue to employ the entire Intec workforce.

→ **www.sartorius.de**

Biomedical technology Production-line proteins

When cultivating cells, protein-coated Petri dishes are now increasingly utilised in order to accelerate growth. Researchers from Fraunhofer IBMT have joined forces with Saueressig to develop a roll-to-roll printing press capable of printing protein patterns onto film. This offers a cost-effective and efficient route to bulk pattern production. The protein patterns are necessary because the kinds of cells naturally occurring in organisms such as humans, animals and plants are able to sense their own surroundings. A foreign environment can lead to changes in the cell's form or function, for example. This, in turn, would lessen the reliability of cultured cells.

→ **www.ibmt.fraunhofer.de**

Roche acquires Trophos

Roche has announced the signing of an agreement to acquire Trophos, a privately-held biotechnology company headquartered in Marseille, France. With the aid of a technology platform developed in-house, Trophos has discovered the active ingredient olesoxime (TRO19622), which is being developed to treat spinal muscular atrophy (SMA), a rare and debilitating hereditary neuromuscular disease most frequently diagnosed in children. Findings from a decisive clinical Phase II study with olesoxime in SMA have revealed a positive effect on retention of neuromuscular function for SMA type II and SMA type III requiring the use of a wheelchair, plus a lessening of the medical complications that are associated with the disease.

→ **www.roche.com**

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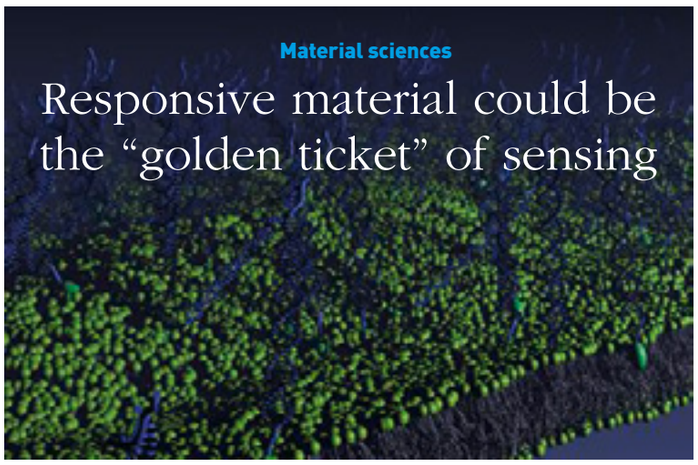


Material sciences

Responsive material could be the "golden ticket" of sensing

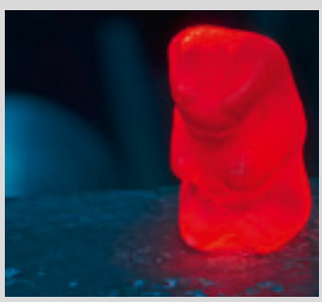
A lipid membrane functionalised with DNA-linkers
Picture © Lorenzo Di Michele

Researchers from the University of Cambridge have developed a new self-assembled material, which, by changing its shape, can amplify small variations in temperature and concentration of biomolecules, making them easier to detect. The material, which consists of synthetic spheres "glued" together with short strands of DNA, could be used to underpin a new class of biosensors, or form the basis for new drug delivery systems. The interplay between the lipid spheres, called giant vesicles, and the strands of DNA produces a unique response when the material is exposed to changes in temperature. Instead of expanding when heated – as is normally the case – the material contracts, a phenomenon known as negative thermal expansion.

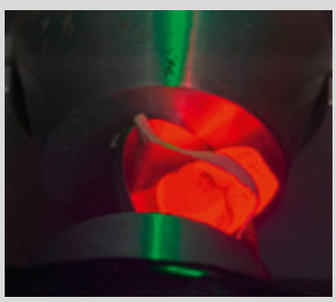
Source: www.cam.ac.uk
Originally published in: Nat. Commun., 2015, DOI:10.1038/ncomms6948

Physics

Bombarding gummy bears with antiparticles



A gummy bear in the experiment setup



Setup with fixated gummy bear
Pictures: Wenzel Schürmann/TUM

By conducting experiments on gummy bears, researchers from TU Munich have now perfected a previous method to the extent that they can determine the free volume of gelatine preparations. Tailor-made gelatine preparations are used in pharmaceutical applications to make drugs easier to swallow by packing them inside a gelatine capsule. Gelatine also protects sensitive active ingredients from oxidation. With other drugs, however, there is a need to ensure that they are released only slowly. Here, gelatine can be used, as it takes time to dissolve. These applications are influenced by the nanopores in the material. Using red gummy bears as their model for a gelatine capsule that dissolves slowly in the stomach, scientists bombarded the bears in various dry states with positrons – the electron's antiparticle. If a positron and an electron collide, the result is the brief existence of an exotic particle, the "positronium". This particle then vanishes in a flash of light. Measurements showed that the emergent positronium survives just 1.2 ns on average in dry gummy bears, but 1.9 ns in aqueous gummy bears. Researchers can now draw conclusions about pore size and number by looking at the lifetime of positronium particles in the material.

Source: www.tum.de, Originally published in: J. Phys. Chem. B, 2014, DOI: 10.1021/jp504504p

Biophysics

Mode of action of protein channelrhodopsin-2 decoded

Researchers have shed light upon the mode of action of the light-controlled channelrhodopsin-2 with high spatiotemporal resolution. This biomolecule is used in optogenetic applications, which is deployed to control the activity of living cells with light. With time-resolved vibrational spectroscopy and bio-molecular simulations, the Bochum-Berlin team could find out what is actually happening inside a protein and ultimately triggers its activation. The EHT (E90-Helix2-tilt) model describes the mode of action of channelrhodopsin-2 as follows: the light-sensitive group of the protein, i.e. the retinal, is twisted under incidence of light. This twist then continues in the protein and opens a pore ultra-fast, which is closed by amino acid E90 in the dark. E90 marks the narrowest place in the pore and opens it through a downward move, similar to the motion of a swing door, so that water can enter an empty vestibule above the narrowest place in the pore. The entering water then tilts the protein helix H_2, which eventually triggers a protein-traversing open ion channel. When forming this model, the Bochum researchers benefitted from their comprehensive experience that they had gained resolving the mechanism of light-driven proton pump bacteriorhodopsin in detail.

Source: aktuell.rubr-uni-bochum.de
Originally published in: Angew. Chem. Int. Ed., 2014, DOI: 10.1002/anie.201410180

Evolutionary biology

New blood stems cells thanks to interferon gamma

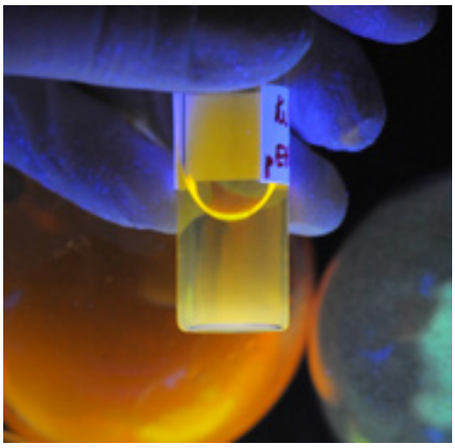
In the early stages of embryonic development, differentiation gradually produces stem cells with precisely-defined tasks, such as the production of blood. Scientists at the Max Planck Institute for Heart and Lung Research in Bad Nauheim (Germany) have now discovered how blood stem cells are created in the embryo. Although normally involved in inflammatory processes, the molecule interferon gamma also plays a decisive role in the genesis of this cell type during the early stages of embryonic development. In the future, this research could be used to significantly improve the creation of such blood stem cells in the lab.

Source: www.mpg.de
Originally published in: Dev. Cell, 2014,

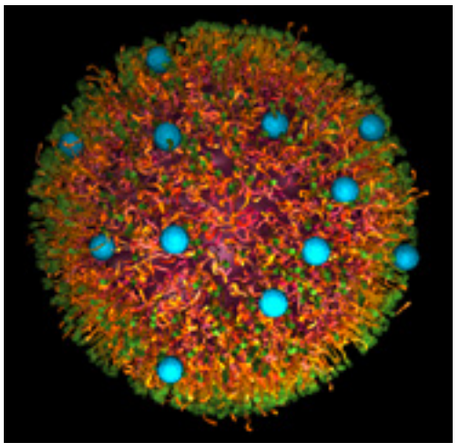
Nanomedicine

Colourful nanoguides to the liver

Researchers have managed to create nanoparticles capable of transporting an active ingredient (AI) package to liver or kidney tissue as instructed by novel “guide” dyes. Tagging with the dyes also enables the transport process to be monitored. The functional proof of the principle is delivered by the reduction in cholesterol production, mediated by siRNA (small interference RNA). This siRNA can silence specific genes and thereby prevent the production of the proteins coded for by these genes. The process involves introducing the genetic material into the target cells, where it acts without being excreted again or – even worse – damaging healthy tissue. Medical practitioners and chemists from Jena and Munich (Germany) and the US have now managed to create genetic material nanotransporters that make their way to a selected cell type, where they then release their AI package.



Researchers at Jena have managed to create highly specialised nanoparticles capable of transporting an AI package to liver or kidney tissue as instructed by novel "guide" dyes.
Picture: Jan-Peter Kasper/FSU Jena



Schematic diagram of a nanoparticle with an AI package in its interior (purple) and specific dye markings on the particle surface (blue spots).
Picture: JCSM/SmartDyeLivery GmbH

Source: www.uni-jena.de, Originally published in: Nat. Commun., 2014, DOI: 10.1038/ncomms6565.

What makes something "authentic"?

Food safety as a global challenge

Prof. Dr Markus Fischer,
Hamburg School of Food Science,
University of Hamburg

Markus Fischer studied food chemistry at Munich Technical University (TUM), receiving his doctoral degree in 1997 in molecular biology/protein chemistry. In 2003, he completed his habilitation in the departments of food chemistry and biochemistry. Director of the Institute for Food Chemistry at the University of Hamburg since 2006, he is the founder (2011) and Director of the Hamburg School for Food Science (HSFS). Markus Fischer's many active engagements include positions held on the Federal Institute for Risk Assessment (BfR) Scientific Advisory Board and Research Association of the German Food Industry (FEI) Scientific Committee, and (from 2014) the Board of the Food Chemistry Society (LChG), and his role as German delegate to the European Food Chemistry Division.

Whether a foodstuff is "authentic" – i.e. whether it is genuine or original – is of considerable importance not only for the complex and global procurement chain that drives the food processing/manufacturing industries but also for consumer safety.

Nor is the counterfeiting of food products – often referred to in common parlance as "food fraud" or "food fakery" – by any means a modern problem. In centuries past, flour was adulterated with chalk or other powdered substances – some of them actually poisonous – honey with starch syrup and butter with synthetic butter (margarine). In the present day, profit-hungry food fakers continue to make headline news, with horrifying examples including the melamine affair, the horsemeat scandal that recently came to light and cases involving the processing of meat no longer deemed fit for human consumption. These cases not only constitute food fraud but also involve the repeated and deliberate endangerment of consumer health. Experience of such incidents has shown us that food fraud is a hot-button topic for consumers. In extreme cases, consumers may even respond to the scandal by avoiding certain kinds of products entirely, thereby posing an existential threat not only to the affected company but to entire sectors of the industry. For companies in the food industry, safeguarding themselves against potential involvement in a food scandal is therefore an essential precondition to remaining both productive and competitive.

In Europe, consumers are protected by legislation that prescribes the end-to-end traceability of food and raw materials throughout all of the various stages within food production, processing and distribution. While this quality control process often uses shipping papers as a common denominator, the scandals mentioned above show that these papers alone offer no guarantees for the authenticity of the shipment content, since the deliberate relabelling or mislabelling of cheap goods as premium products can easily undermine these well-intentioned attempts at ensuring traceability. The problem is aggravated by the fact that many raw materials cannot be procured from within the European Union or are preferentially sourced from outside the European Economic Area for commercial reasons. Worldwide, food fraud most commonly affects goods such as olive oil, fish and organic foods, as well as commodities for example spices, tea, cocoa, coffee or nuts. Globally, revenue from counterfeit or adulterated raw materials and foods amounts to tens of billions of euros every year. This figure highlights the fact that the quality control strategies practiced to date are unequal to the problem. In addition, some imported commodities are produced by manufacturing processes that do not meet European standards, and are therefore subject to specific customs regulations in certain cases. One way of circumventing these regulations is to fake the country of origin on the commodity label.

Compared to centuries past, contemporary challenges are therefore considerably more complicated and, due to the global material cycles now in place, include determining the commodity type (e.g. variety), identifying the exact geographical origin (e.g. to verify a product as a regionally-protected food) and distinguishing between specific types of production (ecological and sustainable vs. conventional agriculture).

To give the food industry peace of mind in the contexts mentioned above, more reliable strategies and solutions are required, capable of facilitating the unique characterisation of raw materials. This process must also attend to the fact that many of our "modern-day fraudsters" also have a scientific background and a sound working knowledge of the methods used within corporate quality control or regulatory surveillance. As a consequence, product fakery can be modified and refined to an extent where detection becomes ever more problematic.

As a general rule, the authenticity or originality of commodities can be determined using a sufficient quantity of valid and stable biomarkers – especially in terms of interaction with the environment.
Three fundamental prerequisites can be summarised for this approach.

- Each individual (microorganism, animal or plant) – and thus every commodity – can be described by its endogenous endowment. Endogenous processes are coded using the platform of DNA and expressed as proteins (the proteome), which are in turn involved in the formation and breakdown of metabolic end products (the metabolome).
- The levels of genetic expression mentioned above, including the isotopic signature and the identification of rare earths characteristic for certain geographical locations can be influenced by exogenous, natural factors (solar radiation, composition of the subsoil, etc.) or anthropogenic factors (pesticides, fertilisers, etc.) across a wide range of timescales. The degree to which a raw material is influenced by exogenous factors depends on its surroundings and exposure, i.e. the effect duration.
- The profile, consisting of various elements, isotopes and molecules – and comparable with the uniqueness of a human fingerprint – unambiguously defines both the type of raw material involved and its source/origin (variety, provenance, environment, climate and soil quality) and the type of cultivation involved (organic/conventional agriculture).

While already deployed within food analysis, the individual technologies necessary (genomics, proteomics, metabolomics and isotopolomics) tend to produce results that are rarely unambiguous and occasionally rather hard to decipher. Accordingly, consistent application followed by the correlation of the various perspectives is the only way to ensure a system-wide overview of a biological system and its responses to both internal and external influences, such as can then be applied in order to determine the authenticity of foods and raw materials.

As a first step in differentiating a selection of sample populations, one option is to perform non-target screening for the individual component groups. This hypothesis-free approach enables the identification of marker substances by a process whereby the comparably large volumes of data obtained are reduced via multivariate analysis to the analytes exhibiting the greatest variance, contributing to distinctions between the sample populations. Ultra-high-resolution instrumentation-based methods are also applied, so as to maximise the quality of the data and thus increase the likelihood of teasing out divergences between the individual sample populations. Targeted analyses can then be run on the biomarkers identified by the above process to achieve their absolute quantification.

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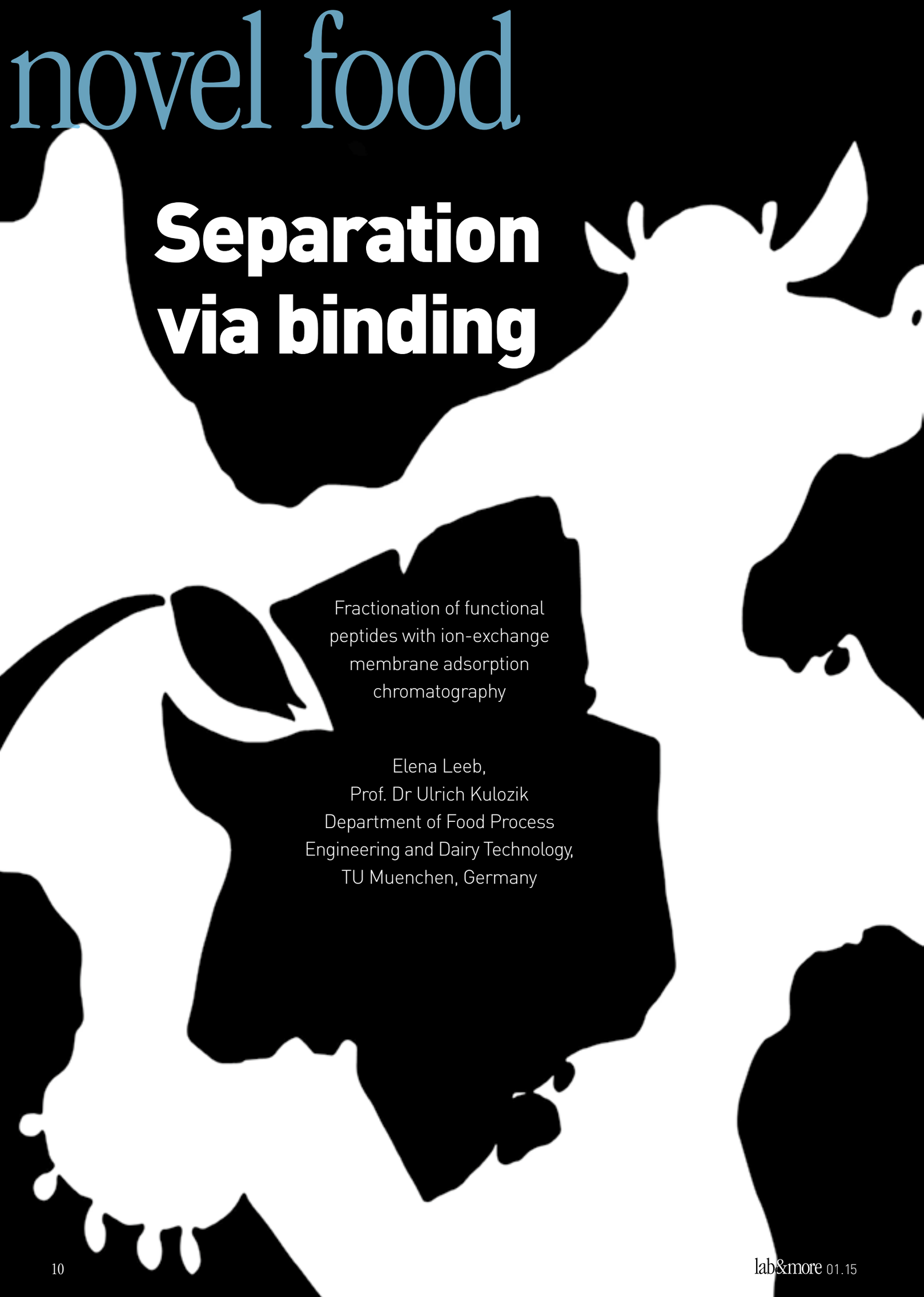
Picture: © istockphoto.com | olvas

Separation via binding



Fractionation of functional peptides with ion-exchange membrane adsorption chromatography

Elena Leeb,
Prof. Dr Ulrich Kulozik
Department of Food Process Engineering and Dairy Technology,
TU Muenchen, Germany

By deploying specific proteases for the enzymatic hydrolysis of proteins, the release of peptides can be controlled. This process can be used to produce functional peptides, which may exhibit an ability to reduce blood pressure or have a surface-active function. The deployment of biofunctional peptides in food as an "added value" is only possible at sufficiently effective concentrations, however. Accordingly, processes are required that enable the production and selective concentration of such functional peptides.

Arterial hypertonia – or high blood pressure in common parlance – is one of the primary risk factors for cardiovascular disease. One contemporary treatment for this disorder involves blocking the angiotensin converting enzyme (ACE), a key enzyme that is ascribed a significant role in the renin-angiotensin system. By cleavage of the decapeptide angiotensin I, it catayses its conversion to angiotensin II, thus causing an increase in blood pressure. Over the last few years, the use of ACE inhibitors has proved to be a successful treatment for arterial hypertonia. Since these drugs often produce side effects, the inclusion of ACE inhibitors in low concentrations in foodstuffs has attracted a great deal of interest. Termed "functional foods", these not only provide nutrition but also offer an additional positive benefit for the health of the person consuming them. To be able to achieve such an effect, however, sufficient concentrations of the functional peptides are required in the relevant types of foods.

Production of functional peptides

The release of functional peptides from proteins can be achieved in a number of ways (fig. 1). First of all, the use of a suitable protein precursor is necessary, embedding the functional peptides in its amino acid sequence. In recent years, milk proteins – and whey proteins in particular – have distinguished themselves in research as protein precursors for a wide range of functional peptides. These proteins release a series of active peptides during the process of digestion in the gastrointestinal tract, although the concentration of these peptides is too low to achieve a significant positive effect.

One method that is designed to achieve the targeted release of functional peptides from milk proteins is fermentation with specialised starter cultures. In this process, enzymes from the microorganisms lead to an increase in the production of the ACE-inhibiting peptides IPP and VPP. In one case, involving the Finnish market, a range of fermented milk products were developed that had proven capable of achieving a slight reduction in blood pressure in clinical trials. If there is a need to include such functional peptides in other foodstuffs, however, then these need to be added to the food product. One option for harvesting functional peptides from natural raw materials is enzymatic hydrolysis. As one example, tryptic hydrolysis of the major whey protein β-lactoglobulin enables the release of the ACE-inhibiting peptides f(9–14) and f(142–148) [1].

Fractionation of hydrolysates

Enzymatic hydrolyses always generate a complex mixture of various peptides, however, even when deploying specific enzymes and maintaining constant reaction conditions. In addition, the hydroly-

Elena Leeb graduated with an M.Sc. in Nutrition and Biomedicine from the School of Life Sciences, TU München. She is currently studying for her doctorate as a research assistant in the Department of Food Process Engineering and Dairy Technology, where her work focuses on the targeted enzymatic hydrolysis of proteins and the subsequent fractionation of the hydrolysates to obtain peptides for technological and biofunctional applications.

Ulrich Kulozik studied food technology at TU München, obtaining his doctorate in the field of membrane separation technology. In 1991, he completed his habilitation in the specialist fields of food process engineering and bioprocess engineering. Following a position as Department Manager for Research/Technology Transfer at Kraft Foods R&D in München from 1992 to 1999, he was appointed Head of the Department of Food Process Engineering and Dairy Technology at TU München in 2000.

sates may contain not only the desired peptides but also peptides with negative properties such as a bitter flavour, for example. Accordingly, the production of functional components also requires procedures that permit the separation of other kinds of peptides while concentrating the bioactive peptides. Since the peptides of the total tryptic hydrolysate of β-lactoglobulin have similar molecular weights, however, fractionation of the peptides using established separation procedures such as membrane filtration is barely feasible. Methods are therefore required that enable a fractionation of the molecules based on their specific physical and chemical properties. Currently, there is only one approach to this kind of selective fractionation of individual components from a complex peptide mixture: packed chromatography columns. This approach is very limited in its throughput, however. Membrane adsorption chromatography (MAC) offers a new and more powerful alternative to traditional liquid chromatography (see sidebar).

Ion-exchange membrane adsorption chromatography

To enable a selective separation of the ACE-inhibiting peptides from the complex β-lactoglobulin hydrolysate, the process of ion-exchange membrane adsorption chromatography (IE-MAC) was used. While analyte separation in MAC follows the same principles as used in conventional ion exchange chromatography, MAC offers the key advantages of significantly higher flow rates and a reduced process time [3]. It was also possible to demonstrate this in the fractionation of hydrolysates, using a selection of anion exchange chromatography columns in the form of a conventionally packed column and a membrane adsorber. Figure 2 presents the chromatograms of the two fractionation methods.

While the fractionation of the hydrolysate using the packed chromatography column method completed only after about 80 minutes, separation of the fractions using the membrane adsorber method was achieved after just 40 minutes. In addition, MAC also achieved superior selectivity of the individual fractions, despite higher flow rates. As a result of these properties and the membrane adsorber's modular construction, which makes it simple to scale up the procedure, MAC offers the food industry a selective and economically appealing separation technique.

Fractionation of the ß-lactoglobulin tryptic hydrolysate

The starting substrate for the fractionation process was provided by the total tryptic hydrolysate of β-lactoglobulin, which contains 17 other peptides in addition to the ACE-inhibiting peptides mentioned. The optimised procedure for the fractionation of the hydrolysate using IE-MAC requires the deployment of an anion exchanger (AE) as its first step. As a result of the hydrolysate's complex composition and the correspondingly broad spectrum of isoelectric points, not all peptides bind to the AE, and are collected as flow-through (AE FT). Peptides with a

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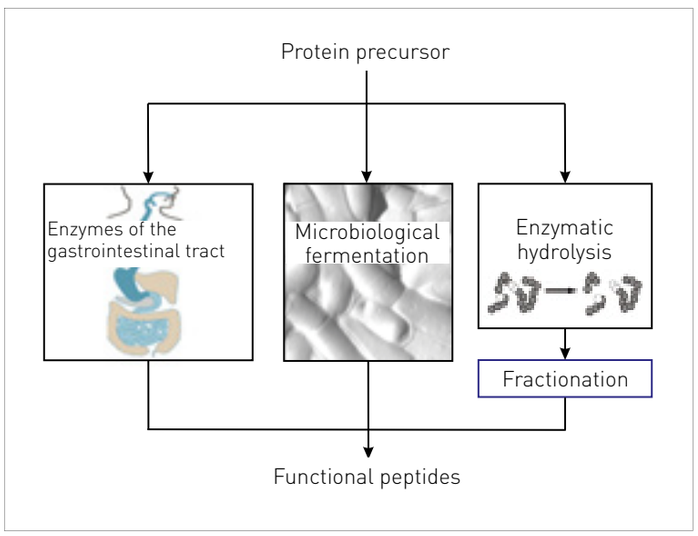

Fig. 1 Production of functional peptides via proteolysis.

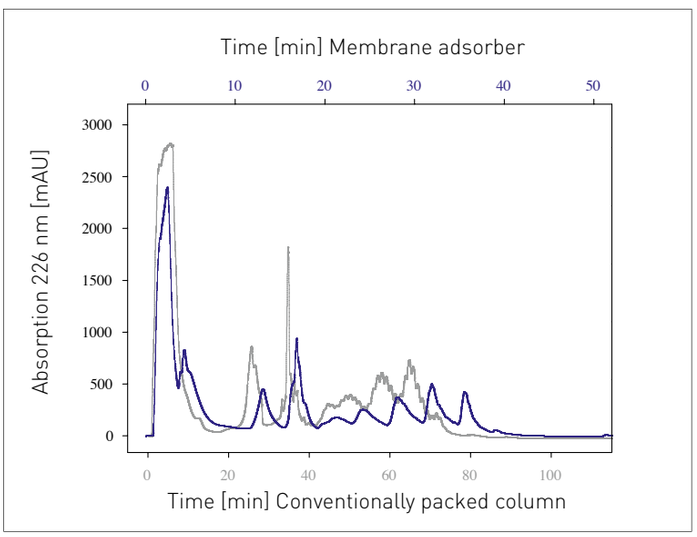

Fig. 2 Comparison of the fractionation of a ß-lactoglobulin hydrolysate by utilising a conventionally packed anion exchanger column "Mono Q 5/50 GL" (grey line) and the membrane-based anion exchanger "Sartobind Q" (blue line); fractionation conditions: 0.03 M phosphate buffer, pH 7, flow rate Mono Q: 1 ml/min, flow rate Sartobind Q: 5 ml/min.

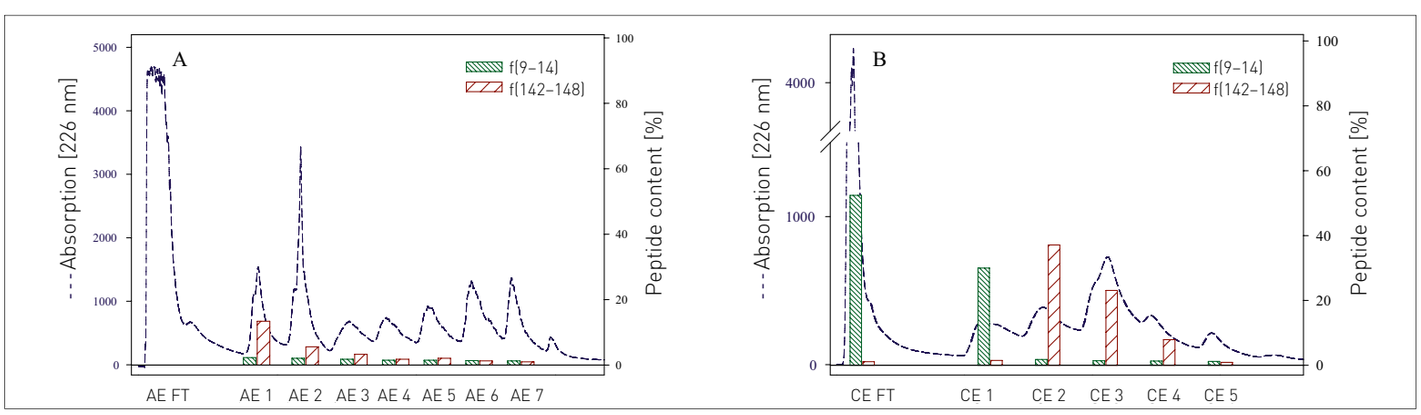

Fig. 3 Chromatograms of the fractionation process developed using coupled anion (A) and cation ion-exchange (B) membrane adsorption chromatography, also showing the percentage content of ACE-inhibiting peptides f(9–14) and f(142–148) obtained from the fractions harvested (modified after [4]).

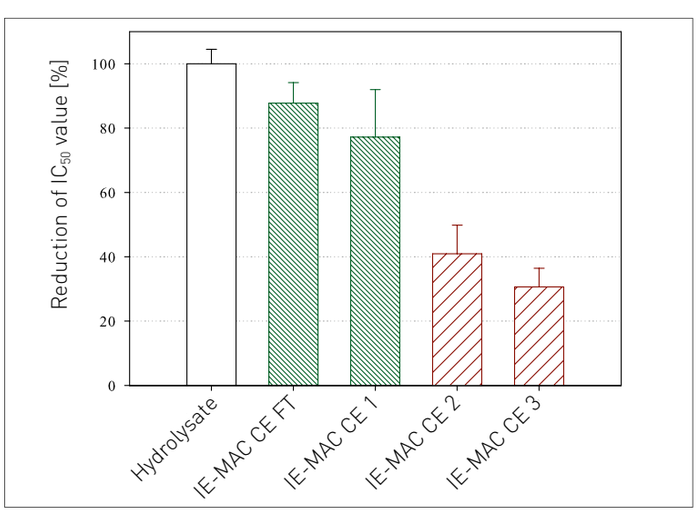

Fig. 4 Reduction of IC_{50} values in the fractions obtained with the cation exchanger compared to the hydrolysate originally deployed; CE FT: anion exchanger flow through, CE 1 to CE 3: fractions 1–3 of the anion exchange process (modified after [5]).

Membrane adsorption chromatography

In contrast to conventional packed chromatography columns, membrane adsorption chromatography utilises a porous cellulose membrane as the carrier material for the functional groups. The membrane itself resembles a conventional spiral wound module and is wound around a solid core, so as to offer a large membrane surface area and thus a large number of binding sites. As the fluid to be separated flows along the membrane, thus promotes convective transport of the molecules. Mass transfer here is several magnitudes faster than in the diffusion-limited mass transport of conventionally packed columns. In addition, the membrane modules are specially designed to offer only low levels of backpressure, permitting flow rates of a tenfold column volume per minute to be achieved. In contrast to conventional columns, separation processes utilising membrane adsorption chromatography can therefore be completed tens or even hundreds of times faster [2].

negative charge bind to the AE, however, and can be separated into a total of seven fractions (AE 1 to AE 7) by means of an ionic strength gradient. In a second step, the AE FT is passed through a cation exchanger (CE) and a total of five fractions (CE 1 to CE 5) are thereby obtained, again by utilising an ionic strength gradient. In addition, this step also obtains a further fraction with non-binding peptides in the flow-through of the cation exchanger (CE FT). Overall, then, the use of the two ion exchangers enables the fractionation of the hydrolysate into 13 fractions. To investigate the results of fractionation, it was first necessary to characterise the peptide composition of the individual fractions. Mass spectrometry was used to quantify the peptides contained in the fractions, and the volume of ACE-inhibiting peptides f(9–14) and f(142–148) harvested was calculated as a percentage proportion of the hydrolysate deployed. Figure 3 presents the chromatograms obtained from the chromatography processes, plus the percentage proportion of the target peptides in the individual fractions [4].

Clearly discernible is the fact that successful concentration of the ACE-inhibiting peptides was achieved, especially in the cation exchanger fractions. Here, the peptide fragment f(9–14) was concentrated primarily in the flow-through (CE FT) – i.e. in the fraction of non-binding peptides – and in the first fraction (CE 1), while the peptide f(142–148) is principally found in the second and third fractions (CE 2–3) of the cation exchanger. In order to exclude antagonistic effects of other peptides contained in the fractions on the potency of ACE inhibition, the ACE-inhibiting activity of the fractions was investigated in vitro.

In vitro analysis of ACE-inhibiting activity

This analysis applied the calculation of IC_{50} values to determine the inhibitory potency of the hydrolysate overall and of the fractions obtained with the maximum volume of ACE-inhibiting peptides. This IC_{50} value is the concentration at which half-maximal inhibition of the ACE is achieved. As the IC_{50} values decrease, this corresponds to an increase in the potency of ACE inhibition. Figure 4 presents the reduction in IC_{50} values achieved by the fractions investigated with a maximum volume of the ACE-inhibitory peptides f(9–14) and f(142–148) compared to the total hydrolysate.

As can be seen, the fractionation of the hydrolysate by means of IE-MAC leads to a considerable enhancement in the ACE-inhibitory properties. For fraction CE 2, for example, the IC_{50} value is reduced to 40% of its initial value, with fraction CE 3 achieving a reduction of 30%.

The findings presented here clearly demonstrate that IE-MAC can be successfully deployed for the fractionation of ACE-inhibiting peptides from the complex β-lactoglobulin hydrolysate. The fractions thereby harvested could then be deployed following clinical testing or safety approvals as foodstuffs (nutraceuticals or e.g. novel foods) containing peptides for lowering blood pressure.

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Molecular consequences

The effects of psychotherapy on DNA damage caused by traumatic stress

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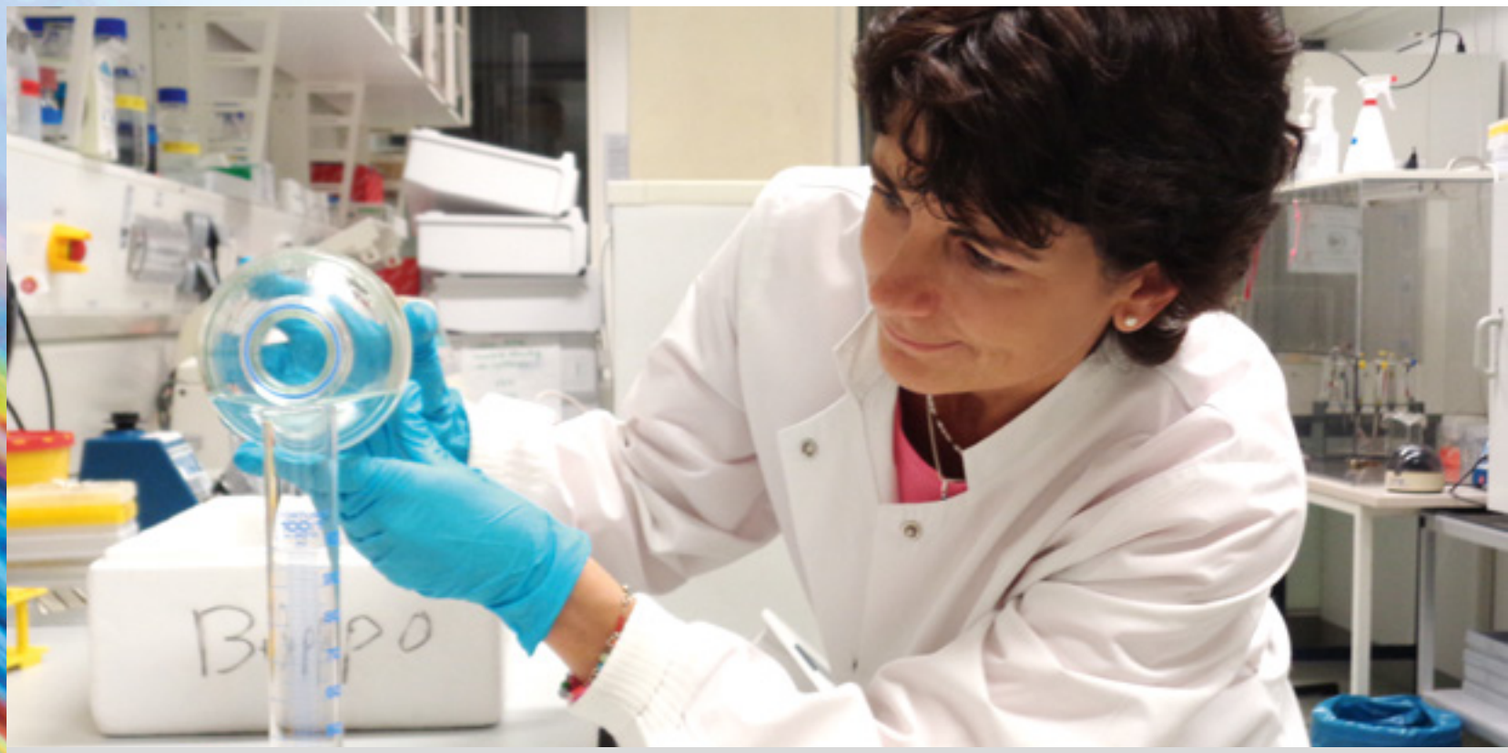
The natural and social environment plays a decisive role in the life of an individual, and has significant repercussions for his or her physical and mental health. Factors in the social environment such as a high population density or noisy cities with traffic congestion and light pollution are considered to be potential stress factors. Social relationships and the working environment also exert a considerable influence on a person's psyche and state of well-being. In today's society, stress research is a field that has attracted increasing interest in recent years, fuelled by the growing incidence of striking neurological problems.



Maria Moreno-Villanueva commenced her studies in biology at the University of Murcia in Spain. After arriving in Germany, she first completed a course of training as a cytology assistant in Tübingen. Following this, she worked in the medical laboratory of Dr Böhm in Friedrichshafen, where she was responsible for cytology diagnosis conducted in the course of cancer screenings. After three years here, she then moved to Konstanz, where she completed her biology degree while working part-time for Dr Stocker. In 2008, she obtained her doctorate magna cum laude from the Molecular Toxicology department. Maria Moreno-Villanueva has been a manager in the EU project MARK-AGE (www.mark-age.eu) since 2008, and a research assistant at the University of Konstanz since 2011, where her work in the Molecular Toxicology Group involves repair mechanisms for damaged genetic material. She has received numerous accolades for her work, including the Ursula M. Händel Animal Welfare Prize (2011, shared with her doctoral supervisor Prof. Alexander Bürkle), the "Mujer del Año" ("Woman of the Year") prize from the Spanish region of Murcia (March 2014) and the Ecology Prize from the University of Konstanz's "Environment and Living" foundation (July 2014). On March 1st, 2015, she will begin a research year abroad at the NASA Johnson Space Centre in Houston, USA, funded by the German Research Foundation.

Research findings show that chemical environmental pollutants such as dioxins, PCBs (polychlorinated biphenyls), pesticides, particulate matter or asbestos in dirty or industrialised residential areas all pose a hazard to our health. Turning to social factors, however, much research has yet to be completed on the ways that issues related to population, education, employment, income, lifestyle, social class and the family trigger psychological disorders, and on the biological mechanisms that are responsible.

Background

DNA stores all of our genetic information that codes for the proteins essential to life: as a result of this fact, damage to the genetic molecule DNA can endanger the entire organism. DNA damage can occur spontaneously within our cells or can be triggered by external physical factors such as irradiation, reactive oxygen species (ROS) or chemical substances – the latter originating in both food constituents and drugs (chemotherapy). While DNA damage can be repaired in principle, damage left untouched or inadequately remediated leads to mutations, loss of control over cell division (cancers) and even to cell death.

N-glycans are heterogeneous chains of sugar molecules. Glycans are bound to proteins via glycosylation. They occur as cell membrane-bound glycoproteins (e.g. receptors, channel proteins, histocompatibility antigens) or in soluble forms (e.g. serum proteins, transport proteins, immunoglobulins, protein hormones). Since the glycosylation of proteins is an age-dependent process, certain glycosylation patterns in human plasma have therefore been described as biomarkers for a person's biological age [1] (fig. 1).

Consequences of traumatic stress

Experiencing a traumatic event such as rape, abuse, natural disasters or traffic accidents can trigger a post-traumatic stress disorder (PTSD). Current scientific research work is uncovering a connection between

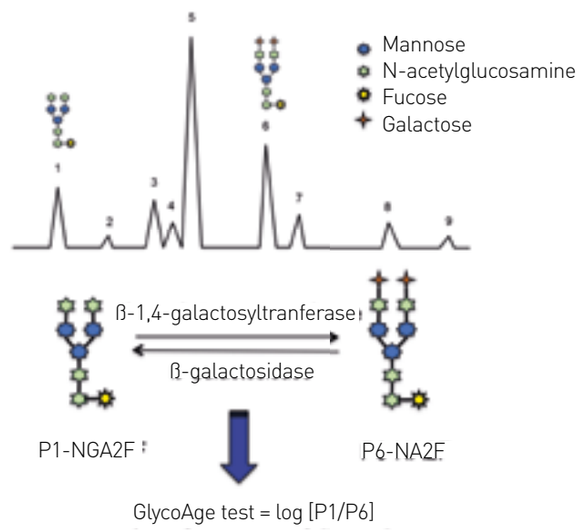


Fig. 1 GlycoAge Test (modified from Vanhooren, V. et al. Exp Gerontol. 2010). Schematic diagram illustrating a number of glycans (peaks P1 to P9) in blood serum. The logarithm of P1-NGA2F (agalactosylated, core-α-1,6-fucosylated biantennary) divided by P6-NA2F (bigalactosylated, core-α-1,6-fucosylated biantennary) constitutes the GlycoAge Test.

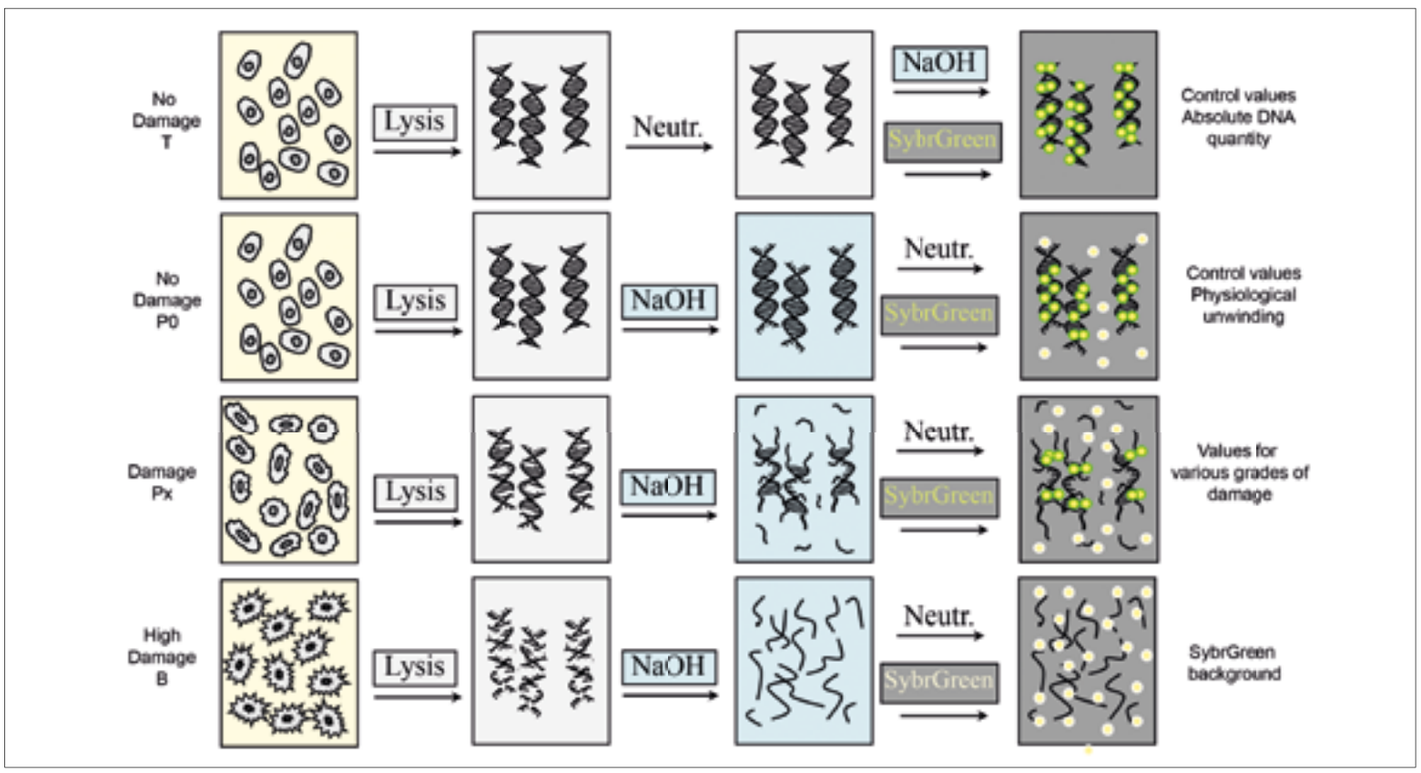


Fig. 2 Schematic diagram of the FADU method (from Moreno-Villanueva, M., Bürkle, A. (2012) High-throughput assays to quantify the formation of DNA strand breaks. In: P. Steinberg (ed.), High-throughput screening methods in toxicity testing. John Wiley & Sons, Inc., Hoboken, NJ, USA). The cells are shown in the yellow boxes. The light grey boxes show double-stranded DNA with increasing levels of damage while the blue boxes show double-stranded DNA with an increasing degree of unwinding. The small circles in the dark grey boxes represent dye molecules (yellow = no fluorescent signal, and green = fluorescent signal).

traumatic stress and an increased risk of health problems such as heart failure, stroke, high blood pressure, cardiac arrhythmias, obesity and diabetes [2].

Study design

In our recently-published interdisciplinary study, we analysed DNA strand breaks and DNA repair in 65 study participants: 34 PTSD patients and 31 control subjects, whereby the control group was subdivided into 11 traumatised individuals and 20 healthy volunteers sharing the same ethnic origin. The group of PTSD and traumatised patients consisted of women and men who had fled to escape war, torture and rape. All test subjects were recruited at the Reichenau Centre for Psychiatry, Konstanz.

To investigate the effects of psychotherapy on DNA strand breaks, the PTSD patients were randomly assigned to two separate groups: a treatment group and a wait-list control group. Patients in the treatment group received treatment in the form of narrative exposure therapy (NET) for four months, while patients in the wait-list control group received no NET.

Blood samples were taken, and blood cells and plasma extracted from whole blood. The endogenous DNA strand breaks and capability for cellular DNA repair were investigated in key cells of the immune system, in what are termed peripheral mononuclear blood cells (PMBCs). The DNA strand breaks and a repair period of 90 minutes following ex vivo (i.e. in the laboratory test tube) irradiation were quantified with the aid of a fluorescence-detected alkaline DNA unwinding (FADU) assay (fig. 2). The N-glycosylation patterns were identified in plasma using DSA-FACE technology.

Findings

Our results show that both patients with a post-traumatic stress disorder and chronically traumatised patients exhibit an elevated number of DNA strand breaks, while irradiation-induced DNA repair reveals no significant differences between the patient group and the control group [3] (fig. 3). The clinician-administered PTSD scale (CAPS) is a clinical interview conducted to record the frequency and intensity of symptoms stemming from the post-traumatic stress disorder. We found a significant decrease in CAPS scores with patients receiving NET and – interestingly – a reduction in accumulated DNA strand breaks after receiving psychotherapy [3]. Other studies have already shown a reduction in PTSD symptoms following NET. On the other hand, the positive effect of psychotherapy on molecular-level changes with potential benefits to physical health – such as DNA strand breaks – had not been demonstrated to date.

One possible cause for the accumulation of DNA strand breaks is an increased concentration of adrenalin in the blood. In stressful situations, adrenalin is released into the blood, binding to adrenergic receptors in cells, where it triggers the rapid provisioning of energy reserves. New research findings show that the chronic stimulation of adrenergic receptors leads to the loss of the "tumour suppressor" protein p53, and, as a consequence, to the accumulation of DNA strand breaks [4]. Furthermore, the immune system responds to the frequent and prolonged occurrence of stress responses by increasing the rate of cytokine production and activating inflammatory processes, in the course of which reactive oxygen species (ROS) are formed. At the molecular level, these ROS lead to a variety of cellular changes. One especially common consequence is the occurrence of DNA strand breaks. Yet the mechanism by which psychotherapy brings about a reduction in DNA strand breaks remains unclear, however.

The concentration of certain chains of sugar molecules – the N-glycans – changes in blood

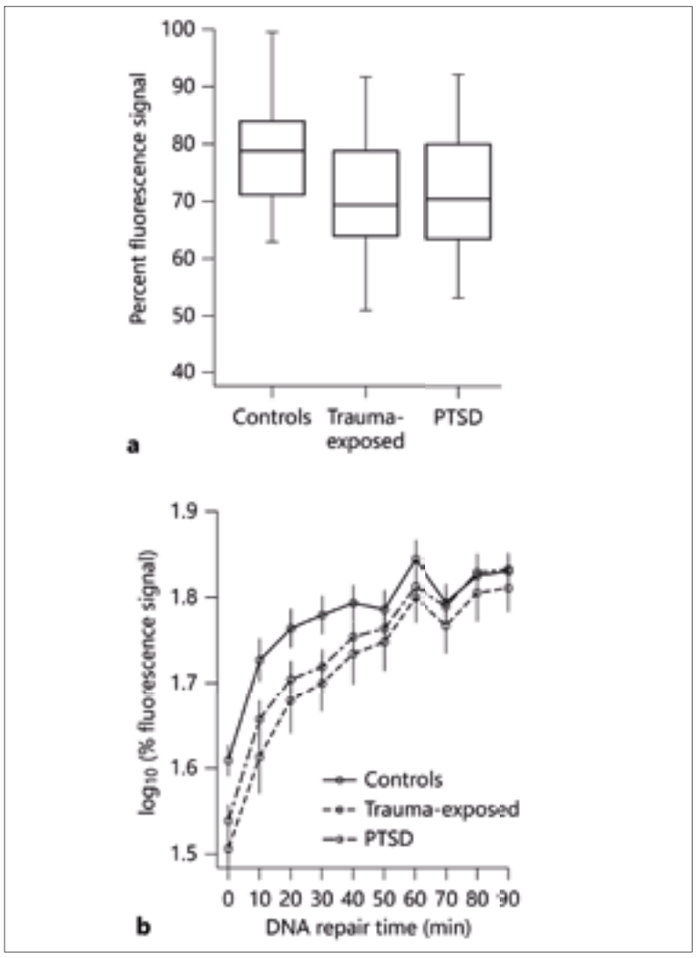


Fig. 3 DNA strand breaks and DNA repair (from Morath and Moreno et al., Psychother Psychosom. 2014). **a)** Low fluorescence values indicate a high number of DNA strand breaks. **b)** DNA repair following X-ray irradiation (= 3.8 Gy at 0 min). Cells were incubated for 10 to 90 minutes at 37 °C to allow them time to repair. As periods grow longer, DNA repair also increases.

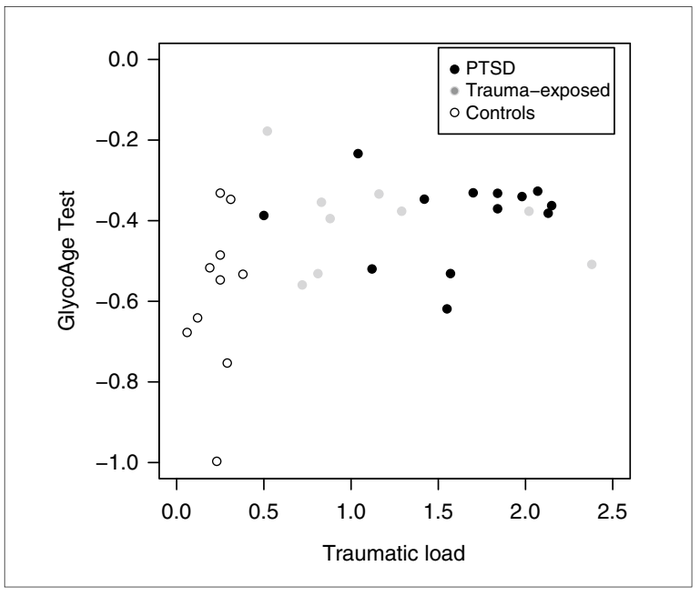


Fig. 4 GlycoAge score (from Moreno and Morath et al., Translational Psychiatry 2013). Scatterplot between GlycoAge test and traumatic stress. High values in the GlycoAge test correlate positively with an increase in traumatic stress.

plasma with increasing age [1]. In our study, PTSD patients exhibit an N-glycosylation pattern matching that of a healthy individual some 15 years older [5] (fig. 4). Researchers have established a link between the immune system, stress and age. Moreover, the N-glycosylation pattern is associated with inflammatory processes in older people, and an increased level of inflammatory immune activity was observed in PTSD patients. Ultimately, changes in N-glycosylation patterns are not merely a biomarker for a person's physiological age, but are also involved in the genesis of age-associated illnesses.

Psychological stress is not only harmful to our health but is also a negative factor affecting our communities. Further studies are needed to improve our understanding of the molecular mechanisms of psychological stress. Research strategies need to be developed to help us overcome the stresses of the social milieu on the one hand while also improving our understanding of the molecular mechanisms of psychiatric stress and thus leading to the development of effective treatments.

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FADU method for the quantification of DNA strand breaks

FADU stands for the "fluorometric analysis of DNA unwinding". This method exploits the unwinding of double-stranded DNA under controlled alkaline conditions. The DNA is partially unwound in an alkaline solution. The degree of unwinding depends on time, the pH value, the temperature and the number of strand breaks. The extent of fully-unwound DNA is a function of the number of DNA strand breaks. An automated version of the FADU method has been developed and established by our laboratory. The method is currently undergoing validation with an industrial partner (fig. 5).

DSA-FACE technology for the identification of N-glycosylation patterns

The first step is to separate the chains of sugar molecules (glycans) from the proteins and tag these with a fluorophore. The various glycans are separated and identified by using DSA-FACE (= DNA sequencer-assisted, fluorophore-assisted carbohydrate electrophoresis).

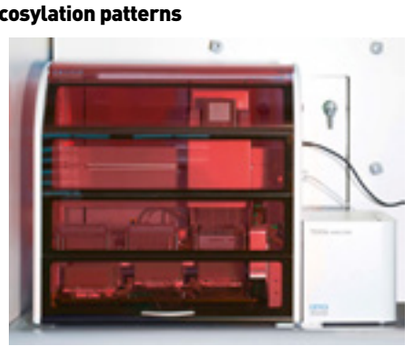

Fig. 5 FADU Genotox hardware (from CETICS Healthcare Technologies GmbH).

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Star charts and Petri dishes

Image-based cytometry – a technology conquers cell culture

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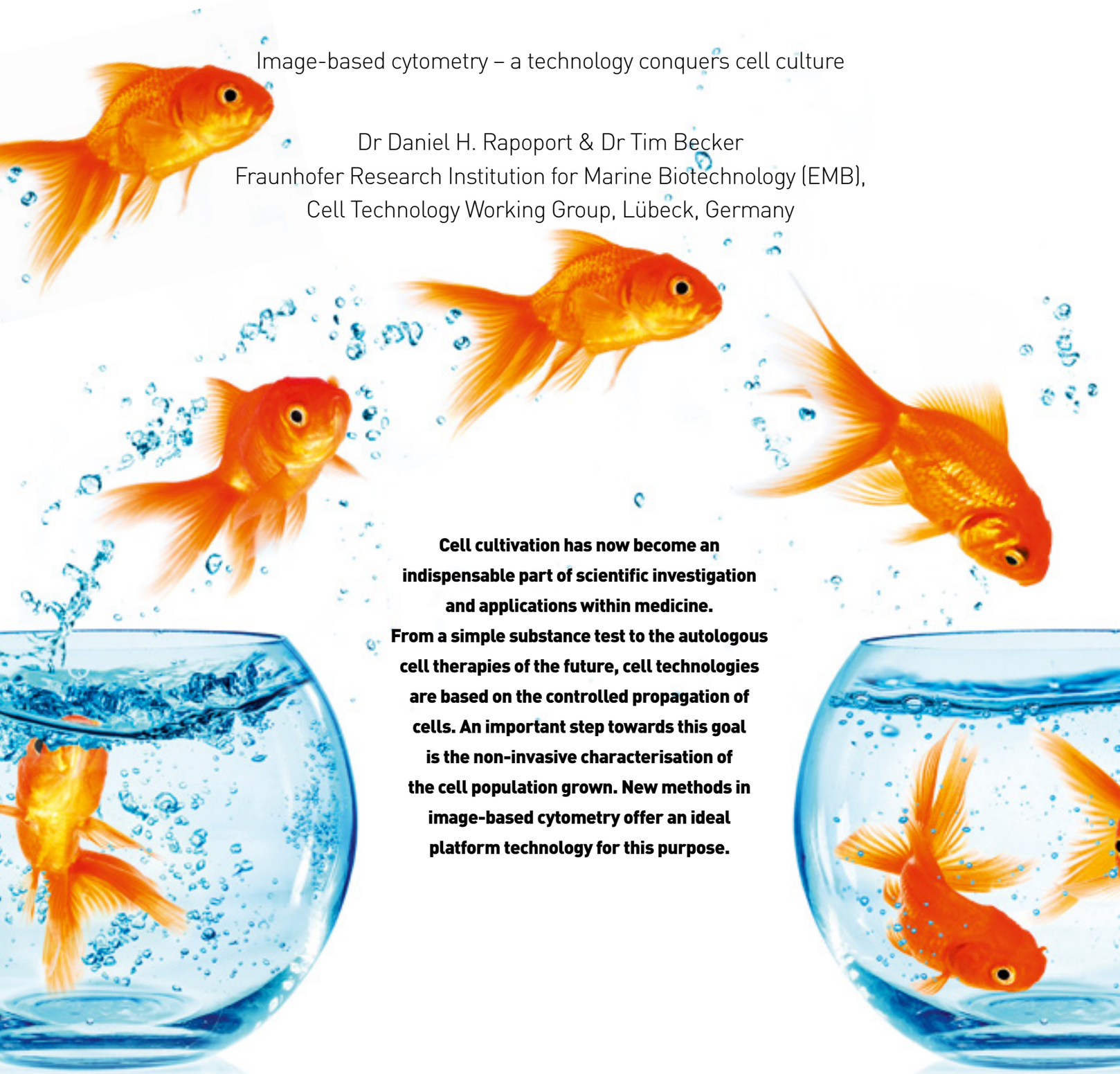


Cell cultivation has now become an indispensable part of scientific investigation and applications within medicine. From a simple substance test to the autologous cell therapies of the future, cell technologies are based on the controlled propagation of cells. An important step towards this goal is the non-invasive characterisation of the cell population grown. New methods in image-based cytometry offer an ideal platform technology for this purpose.

Daniel H. Rapoport studied chemistry at TU Berlin from 1990 to 1994. For his doctoral work, he moved to the Paul Drude Institute for Solid-State Electronics in Berlin. Following an excursion into the multimedia sector as a sound engineer, he joined the Max Planck Institute for Colloids and Interfaces in Potsdam as a postdoc from 2004 to 2006. Following this, he was then able to contribute his extensive chemical and physical expertise to the Lübeck Fraunhofer Working Group for Cell Differentiation and Cell Technology at the Fraunhofer Institute for Biomedical Technology (IBMT). When this was spun off as the Fraunhofer Research Institution for Marine Biotechnology (EMB) in 2008, he accepted the position of Head of the Working Group for Cell Technology. At EMB, he develops technologies for cell isolation, handling and propagation, and has established image-based cytometry as a cell tracking method.

Tim Becker studied Computational Life Science at the University of Lübeck from 2003 to 2008. He completed his doctorate from 2009 to 2013 in the Working Group for Cell Technology at EMB as part of the "Computing in Medicine and Life Sciences" graduate school run by the German Research Foundation. As project lead, he has been coordinating a number of projects at EMB since 2014 involving the ongoing development of technologies and software for image-based cytometry.

Cells are everywhere. Nor do they serve us merely in our bodies – such as when reading these words, for example. They are also taking on an increasingly important role outside their host organism. Cell cultures are used in the laboratory, in medicine and in the cosmetics industry; they are even making inroads into such far-flung regions as sensors and the food industry.

To function properly across this wide variety of applications, cultivation methods require constant improvement. One method that is as old as cell culture itself is that of describing cells by looking at them. The microscope is pretty much the oldest piece of equipment that is required for cell culture and yet – with its most recent models – still one of the most modern.

One of the most exciting trends in cell culture is based on the "smart" use of microscopy and – more precisely – on the marriage of microscopy and computerised image analysis. Today, it's a simple matter to create time series of cells in the Petri dish with the use of automated microscopes. And much can be learnt from these image sequences.

Online monitoring of in vitro cell cultures

The individual images in such series show an identical section of the Petri dish. A new generation of analytical algorithms now allows us to analyse the behaviour of the cells observed over the entire imaging time frame and at the level of single cells. Depending on the scientific issues being investigated and the experimental setup, several application scenarios are conceivable. One interesting application is the evaluation of chemotaxis assays, for example, which are used in drug research and drug testing. Another possible application is the real-time monitoring of proliferating cell populations. This online monitoring approach enables the visualisation of cell growth as a growth or confluence curve in real time. For quality assurance in the field of stem cell therapy, for example, it is imperative to perform quantitative observance, documentation and monitoring of behaviour and growth rates.

To automate these processes, the Fraunhofer Research Institution for Marine Biotechnology (EMB) in Lübeck has developed a purpose-built software application capable of identifying each individual cell in the image data and calculating the cell count. Alongside the cell count, it is also possible to quantify the morphology of the cells identified, as well as determining cell size, length and shape. These morphological parameters can be presented as a function of time (cf. fig. 1). A cell population described using parameters that are sufficiently sound is termed a "parameterised cell culture" [1].

Go forth and multiply: migration and proliferation

Yet image-based cytometry is capable of much more than describing cell cultures in terms of numbers: it also opens up a window onto these cells' dynamic behaviour. Cells are not simply measured: their behaviour is also made capable of analysis. For this to be possible, cells must not only be identified in single images: each of them must also be followed through time. This procedure – termed "cell tracking" – is capable of measuring the cell's pattern of migration, for example. The paths they take provide data on whether the cells move with purpose – such as with the objective of attacking a tumour, for example – or whether they instead just amble aimlessly around the Petri dish. Nor are the shapes of these cell trajectories the only things we can measure: we can also calculate the speed with which the cells move about.

This data provides the starting-point for the kinds of subsequent analysis and modelling performed in chemotaxis assays, for example. Such assays not only enjoy broad-based use in oncological research, as mentioned above, but also in experiments addressing wound healing, which is definitively characterised by the migrational properties of the cells involved. Further scenarios include organogenesis, the investigation of infectious and autoimmune diseases, and research on regenerative processes.

Nor is that all. Cell measurement precision can be improved even further by introducing the separate detection of cell divisions (mitoses). A "mitosis detector" of this kind has been constructed at Fraunhofer EMB. It detects cell divisions by the application of two distinct principles. First, a mitosis event is characterised by the morphological change occurring in the cell cycle just before division. The parent cell is drawn together by the spindle apparatus and

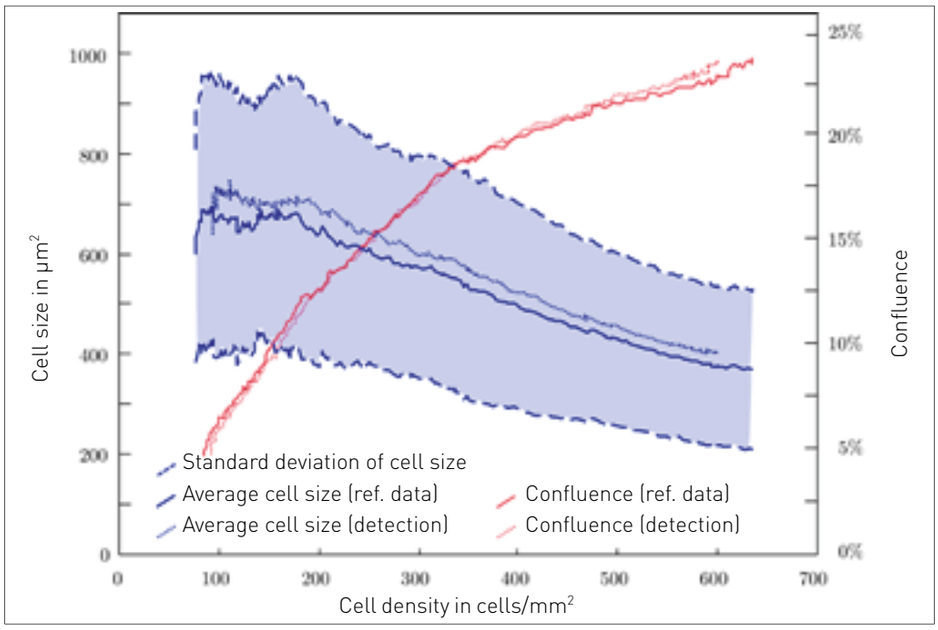


Fig. 1 Change to cell size depending on cell density. Cells in culture shrink to almost half their size when they become confluent.

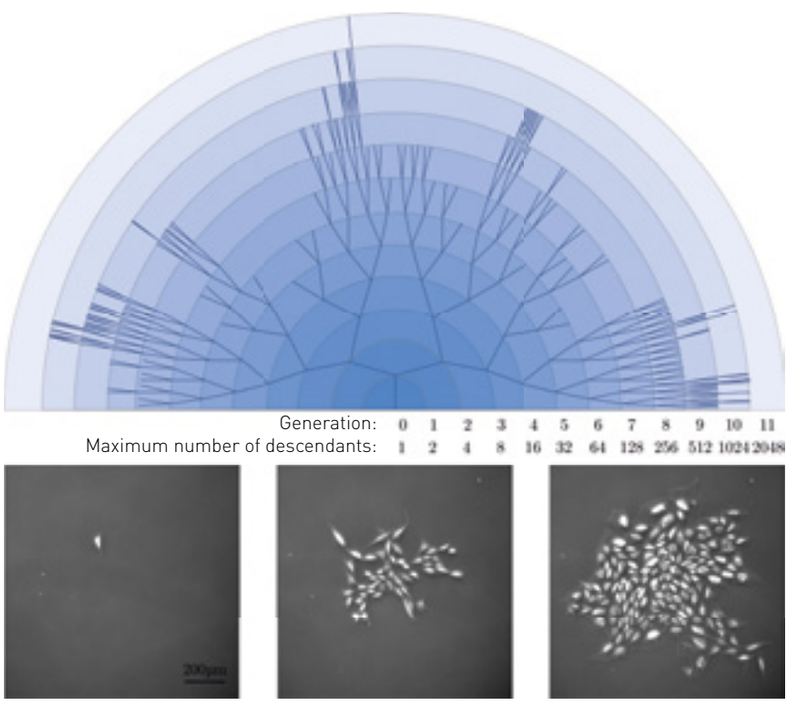


Fig. 2 Family tree of a cell clone. The lower series shows three snapshots, over which the associated family tree is depicted, which classifies all of the cells in the clonal family.

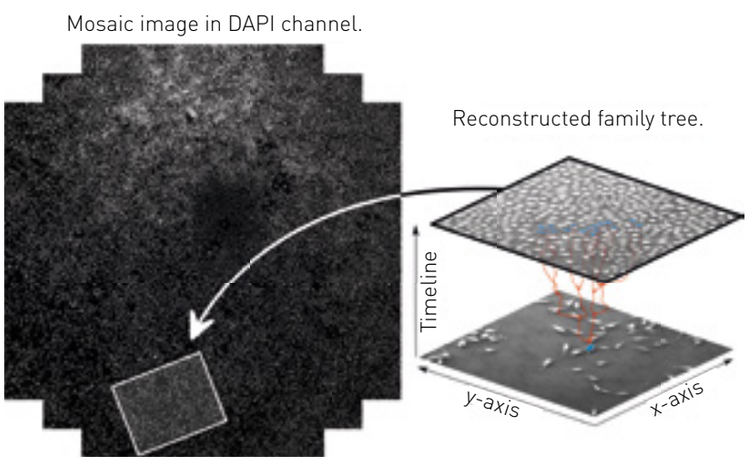


Fig. 3 The star chart problem. The cells whose family relationships were determined using cytometry (right) must be re-identified in the cell image produced by immunocytochemical staining (left). Help is provided by an algorithm adapted from the analysis of photos of stars in the night sky.

assumes a ball-like shape that can be specifically detected [2]. The second possibility consists of searching for the characteristic optical changes present in a mitotic cell using a phase contrast microscope: the spherical cells are not only rounder but are also thicker than the flat adhered cells and so appear brighter in the phase contrast. With the aid of methods for machine-based learning, this mitotic pattern can easily be discovered in the image series [3].

Taken together, both forms of analysis – migration and proliferation – are already capable of delivering a very detailed picture of cell culture events. Complex processes can be investigated such as the metastasis of a tumour or the closure of a wound, for example. These processes are based on the intricate interplay of migration and proliferation, which are now capable of quantitative observation by a non-invasive, dye-free method.

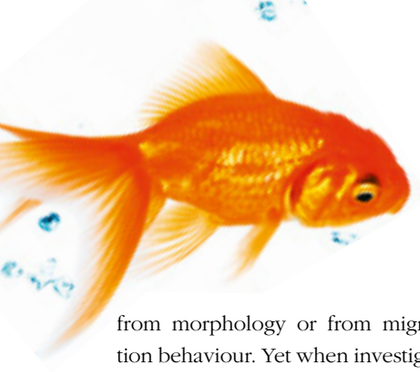
Haven't we met before somewhere? We're relatives, you say?!

So far, we have been considering the behaviour of individual cells. Yet a Petri dish frequently contains millions of cells. Can we learn something about their "social behaviour"? Sure we can – by combining migration analysis (cell tracking) with the mitosis detector! The descendants of every cell undergoing division can be determined. This information can then be used to reconstruct the "family relationships" in the form of a cellular genealogical tree: this records all of the cell's sisters, cousins and grandparents (cf. fig. 2). In addition, cells can also be identified that only "bumped into" each other during their lifetime, i.e. which had cell-to-cell contact – and possibly also exchanged information with one another.

By aggregating all of the morphology, migration and proliferation data described so far, we are then given the unique opportunity to compare cell behaviour in one cell family with the cell behaviour exhibited by other cell families. The reciprocal influence exerted by cell families on each other can also be investigated and visualised in detail.

A star chart search: comparing cytometric data with protein expression patterns

Although the methods described provide a wealth of data, they are still incapable of answering all of the questions posed by cell biology. One such issue is the question of the exact cell type involved, which cannot be determined



from morphology or from migration/proliferation behaviour. Yet when investigating stem cells and questions of developmental biology, however, the resulting cell types are of paramount importance. As a rule, a molecular biologist obtains information about the cell type in question by detecting the presence of known functional proteins. Immunostaining is often used to make such detections. With this technique, a specific antibody binds to the functional protein; the bound antibody can be coupled with a dye that, in turn, renders the functional protein visible.

It would clearly be ideal to combine immunostaining with image-based cytometry. To date, however, the integration of both techniques has faced a technical hurdle: while cytometric data can be recorded as part of the cell culture process, cells for immunocytochemical staining must be fixed – i.e. killed – before then being stained. In short, the two imaging techniques cannot be conducted at the same time: the staining process always gets in the way. How, then, we can find the cells observed with cytometry in the immunostaining images? Since these images are very different and contain a truly vast number of cells, it is not possible to simply pick out individual cells by eye or superimpose one image on top of the other (cf. fig. 3).

To overcome this obstacle, Fraunhofer EMB developed a new method with which identical cells in both types of image could be found and "matched up" by looking at their "cell constellations" [4]. The method resembles the technique developed in astronomy to permit the automated identification of stars and constellations in any random shot of the night sky [5].

To this end, constellations of three, four or five stars – or cells – are described using a unique hash value, which is calculated from the relative geometrical positions of the cells to one another. One condition for hash values is that they are stable, i.e. that each constellation is consistently described using the same hash value, independently of the rotation of the sky or Petri dish and the magnification chosen for the telescope or microscope.

This is used as the basis for a search for the same patterns in both images – the final image in the cytometric image series and an image of the immunocytochemical staining. The basic structure of the search first requires the calculation of the hash values for all constellations of three, four or five cells in the immunostained image. With thousands of cells in the field of view, this might sound like a hugely demanding calculation, but it actually completes much faster than precomputation for astronomical constellations. Calculation of the hash values for the phase contrast image is then completed and the search begins for the most similar constellations in the immunostained image. Finally, the cells that make up these constellations are used to compute an image registry, with which the cell images can be congruently superimposed onto one another.

The star chart search now makes it possible to integrate cytometric data such as degrees of cell relationship with any protein expression patterns chosen. This enables the investigation of truly fundamental questions within developmental biology whose answers previously required a much higher level of effort. In addition, it also permits the general automation of these methods and the execution of the observed processes as high-content analyses, and – in the future – an integration with systems biology.

Outlook: Time-lapse microscopy as standard

Enough, then, of what can already be achieved by using image-based cytometry. One minor drawback with the approach to date has been the fact that time-lapse microscopes are big, expensive and hard to integrate into routine cell culture. What's stopping image-based cytometry becoming a runaway success in cell culture labs is a lack of inexpensive, rugged instruments that can fit into any incubation chamber and perform their tasks as unobtrusively as the chamber's temperature or CO_2 controllers.

Fraunhofer EMB is already working on an "everyday" miniaturised cell scanner. The question is no longer whether but when image-based cytometry will become part of the standard toolbox used by modern cell culture laboratories.

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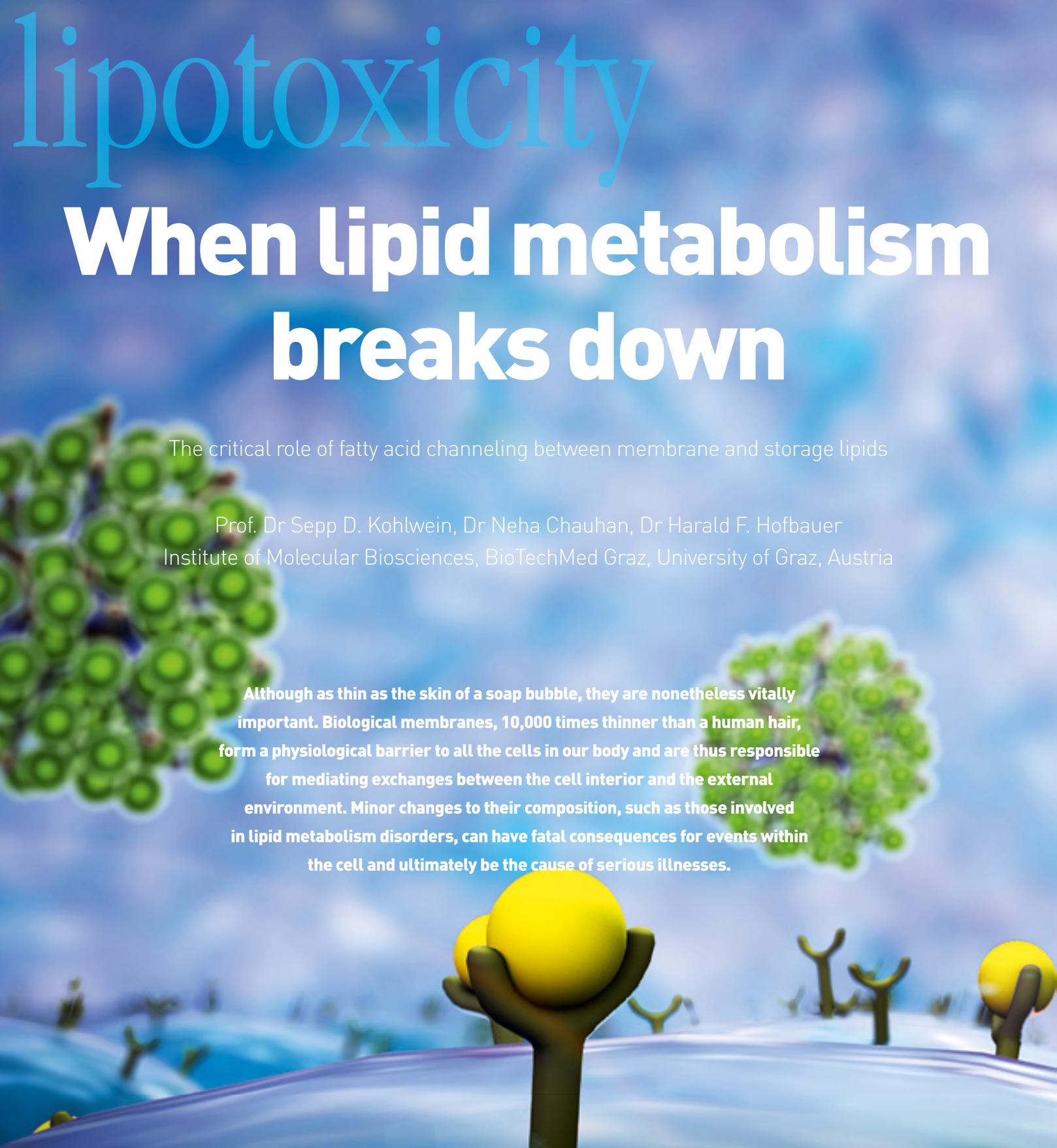
lipotoxicity

When lipid metabolism breaks down

The critical role of fatty acid channeling between membrane and storage lipids

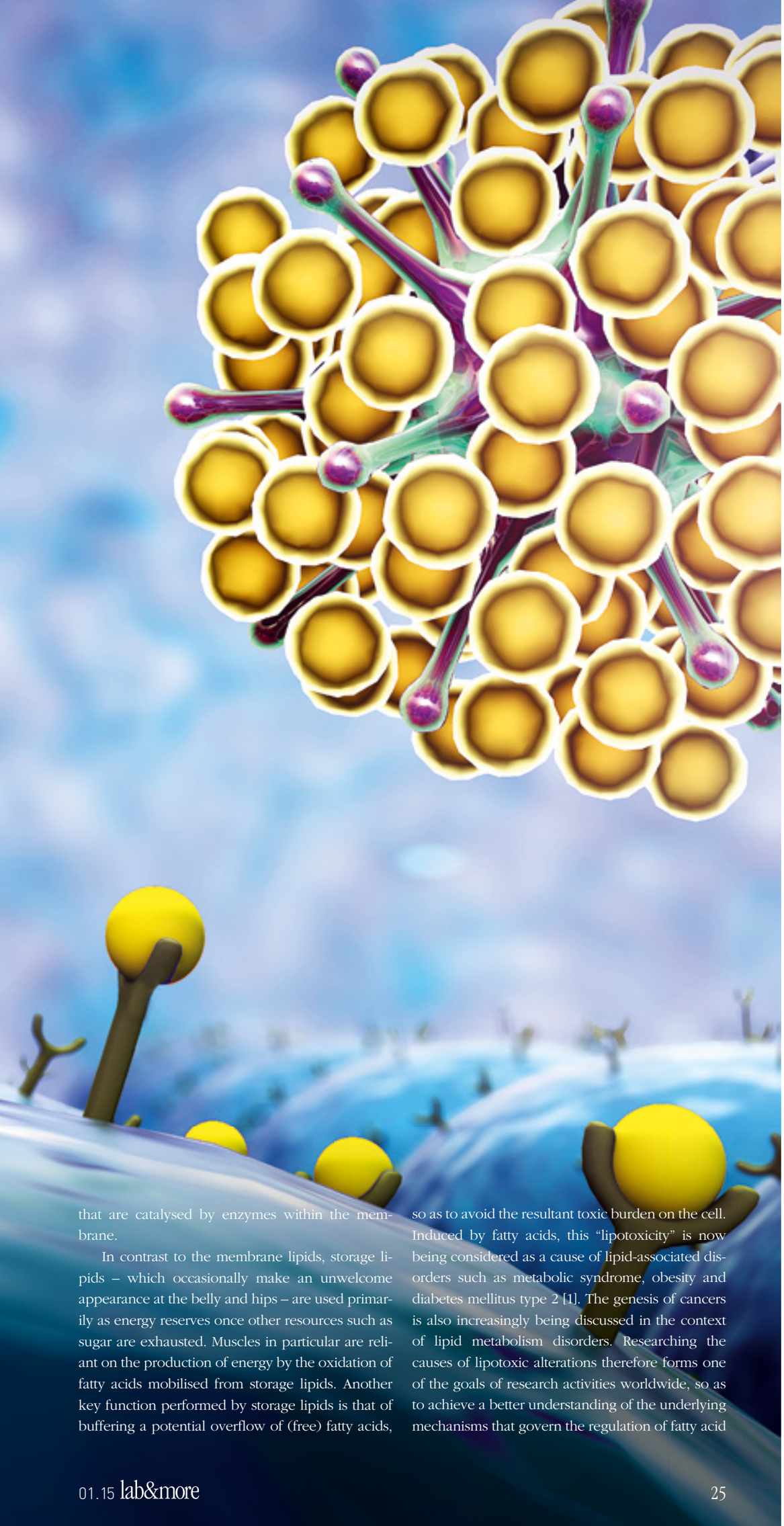
Prof. Dr Sepp D. Kohlwein, Dr Neha Chauhan, Dr Harald F. Hofbauer
Institute of Molecular Biosciences, BioTechMed Graz, University of Graz, Austria



Although as thin as the skin of a soap bubble, they are nonetheless vitally important. Biological membranes, 10,000 times thinner than a human hair, form a physiological barrier to all the cells in our body and are thus responsible for mediating exchanges between the cell interior and the external environment. Minor changes to their composition, such as those involved in lipid metabolism disorders, can have fatal consequences for events within the cell and ultimately be the cause of serious illnesses.

Membrane and storage lipids: a close physiological relationship

The basic structure of all membranes is formed out of a hugely complex mixture of various lipids (from the Greek λίπος/lipos = fat), a class of substances of which cholesterol and lecithin are two of the more familiar members. Biological membranes form the cell's barrier against the environment, thus ensuring that the cellular microcosm remains constant even under changing environmental conditions. In addition, numerous organelles with special-purpose functions within the cell are also enclosed by membranes in more highly-developed cells. Examples include the double nuclear membrane separating the genetic information (in the form of DNA) from cytosol or the cells' "power plants", the mitochondria, which are also enclosed in a double membrane. Other organelles possessing membranes include the endoplasmic reticulum or peroxisomes, for example. This intracellular compartmentalisation ensures that metabolic pathways for synthesis and degradation can exist side-by-side – separated by membranes – as precisely regulated processes. Alongside the membrane-forming lipids, it is the proteins embedded in these membranes in particular that perform specialised functions and whose activity is strongly influenced by the membrane's lipid structure. These functions include (selective) substance transport through the membrane, signal functions and numerous biological reactions



that are catalysed by enzymes within the membrane.

In contrast to the membrane lipids, storage lipids – which occasionally make an unwelcome appearance at the belly and hips – are used primarily as energy reserves once other resources such as sugar are exhausted. Muscles in particular are reliant on the production of energy by the oxidation of fatty acids mobilised from storage lipids. Another key function performed by storage lipids is that of buffering a potential overflow of (free) fatty acids, so as to avoid the resultant toxic burden on the cell. Induced by fatty acids, this "lipotoxicity" is now being considered as a cause of lipid-associated disorders such as metabolic syndrome, obesity and diabetes mellitus type 2 [1]. The genesis of cancers is also increasingly being discussed in the context of lipid metabolism disorders. Researching the causes of lipotoxic alterations therefore forms one of the goals of research activities worldwide, so as to achieve a better understanding of the underlying mechanisms that govern the regulation of fatty acid

lipotoxicity

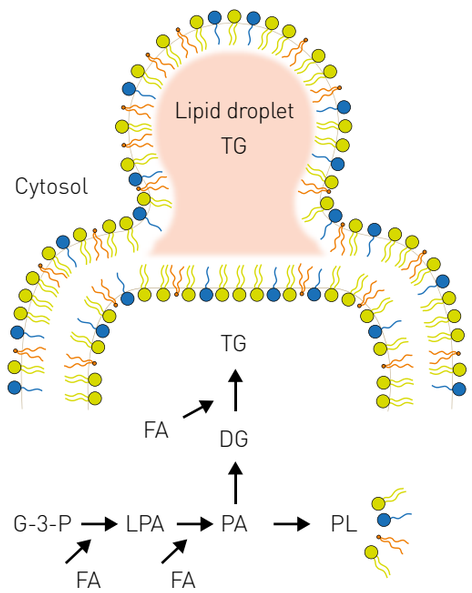


Fig. 1 Bottom: The formation of triglycerides (TG) and membrane phospholipids utilises the same types of precursors – lysophosphatidic acid (LPA) and phosphatidic acid (PA). Depending on fatty acid (FA) availability, a smaller or greater amount of triglycerides is formed, while membrane phospholipids (PL) are maintained at a constant level. If the biosynthesis of TG becomes disrupted, an excess of fatty acids causes changes in the membrane lipids and, in consequence, membrane malfunction and cell death. G-3-P: glycerol-3-phosphate; LPA: lysophosphatidic acid; PA: phosphatidic acid; DG: diglyceride; TG: triglyceride; PL: phospholipid, FA: fatty acids.

Top: Triglycerides (TG) are stored in structures termed "lipid droplets" (LDs). The biosynthetic mechanism for LDs are hotly debated: the current model assumes that TG accumulate between the leaflets of the membrane and that LDs "bud off" into the cytosol once they have reached a certain size.

uptake and its metabolism (see also the special research project LIPOTOX http://lipotox.uni-graz.at).

Membrane-forming lipids and storage lipids are constructed from common precursors, including fatty acids. Some may be sourced from nutrition (examples include saturated fatty acids, trans-fatty acids, omega-3 fatty acids) while some may be produced by cells and tissues themselves as needed. Accordingly, this use of common precursors (fig. 1) requires a sophisticated system of cellular regulation so as to ensure that membrane lipids are produced in the right kinds of qualities and the right kinds of quantities [2]. This is of fundamental importance for cellular growth in particular: every time the cell divides, the cellular membranes must also be duplicated accordingly. Research has already clearly demonstrated that the incorrect synthesis of storage lipids leads to a dramatic sensitivity of cells towards fatty acid excess [3]. On the other hand, the incorrect degradation of storage lipids itself results in impaired cell growth, since membrane lipid synthesis is thereby affected [4].

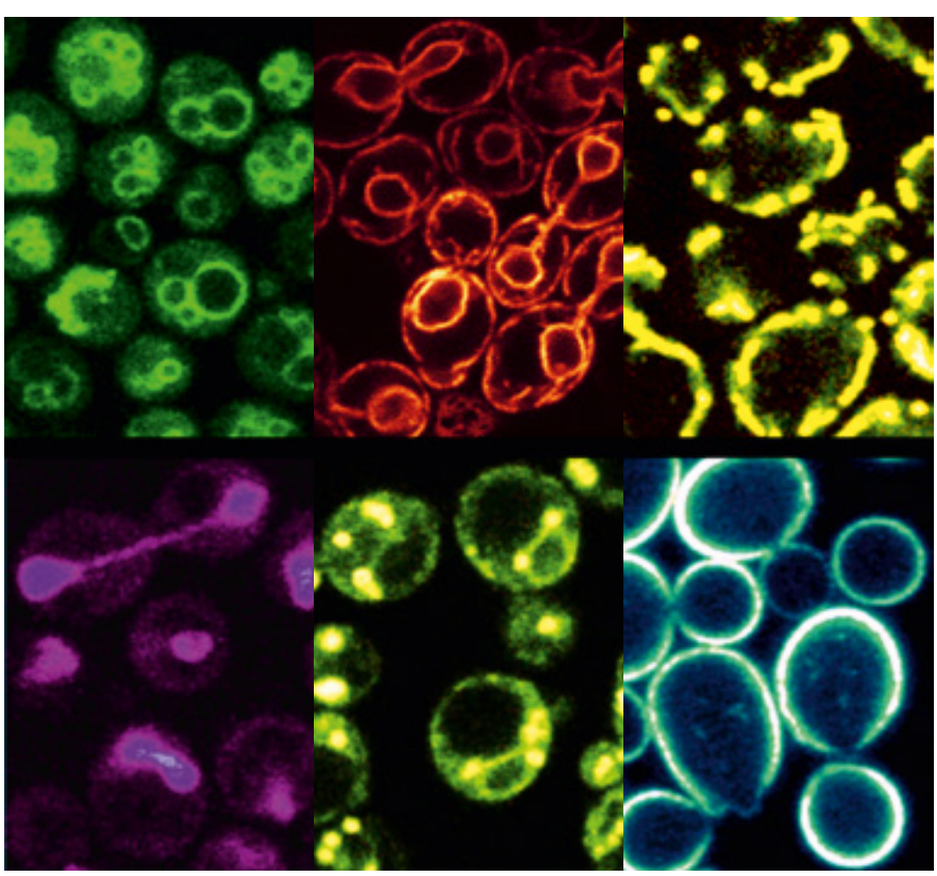


Fig. 2 Subcellular organelles in yeast. The membranes in question are made visible under the fluorescence microscope by staining with specific dyes. The cell size is 5–7 µm. Top row, from left to right: vacuoles, endoplasmic reticulum, mitochondria. Bottom row: cell nucleus, lipid droplet, plasma membrane.

Yeast, metabolic syndrome and cancer: an authentic experimental system for researching lifestyle diseases

The enormous complexity of lipid metabolism, involving the participation of a great many organs and cell types, is an especial challenge for biomedical research. In research, simpler model systems are therefore used, such as fruit flies, nematode worms or various singled-celled fungi (yeasts). For lab experiments, these not only offer a more multifaceted approach but are considerably easier to handle while also being ethically sound. Alongside the well-known technological applications of (brewer's) yeast in brewing and wine-/bread-making – and also, in recent years, in the fuel industry (bioethanol) – this organism has also established itself as an excellent model system for basic cellular research [5]. In recent years, for example, numerous Nobel prizes have been collected by scientists working in yeast research for the fundamental biological insights thereby achieved, which also have relevance for human cells (2001: Lee Hartwell, Paul Nurse – cell cycle; 2006 Roger Kornberg – transcription; 2009: Elizabeth Blackburn, Carol Greider, Jack Szostak – telomeres; 2013: Randy Schekman – vesicular transport). Yeast is also being successfully deployed in gerontology research and for the investigation of neurodegenerative diseases. Of the 6,000 yeast genes known, around one third

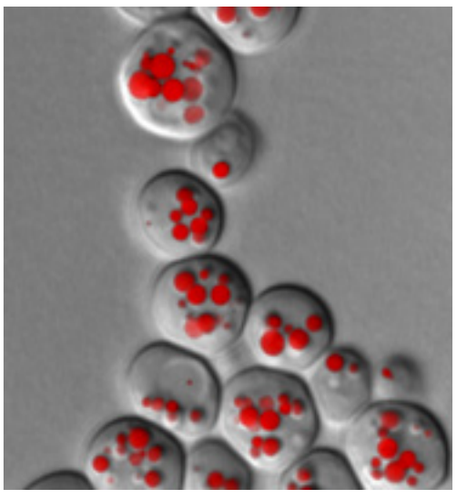


Fig. 3 Fluorescence microscopy image of lipid droplets (LD) in yeast cells. LD are stained using specific fluorescent dyes and can then be made visible under the microscope. LD size and quantity provides information about possible disruptions to lipid metabolism. This yeast culture lacks the two essential lipases that are responsible for the breakdown of triglycerides.

have a counterpart in the human genome, while some 20% of genes that are associated with genetic diseases in humans have a homologue in yeast. Newly-developed pharmaceuticals are routinely tested on yeast cells, a process that simplifies the identification of molecular targets. Furthermore, a highly diverse portfolio of technologies is available not only for the investigation of individual factors but for the systematic study of the cell across the totality of its physiological processes. In the lab, the cells' doubling time of just 90 minutes makes them easy to cultivate in large quantities while facilitating biochemical and genetic manipulation. This short doubling time is all the more astonishing when one considers the cells' highly complex structure, which resembles that of human cells. Alongside the membrane-enclosed cell nucleus, yeast possesses mitochondria, the highly branched membrane system of the endoplasmic reticulum, vesicles, vacuoles and – as a physiological barrier to the outside world – a plasma membrane (fig. 2). As with cells of plant and animal origin, lipid droplets are also present, of the type responsible for lipid storage (fig. 3).

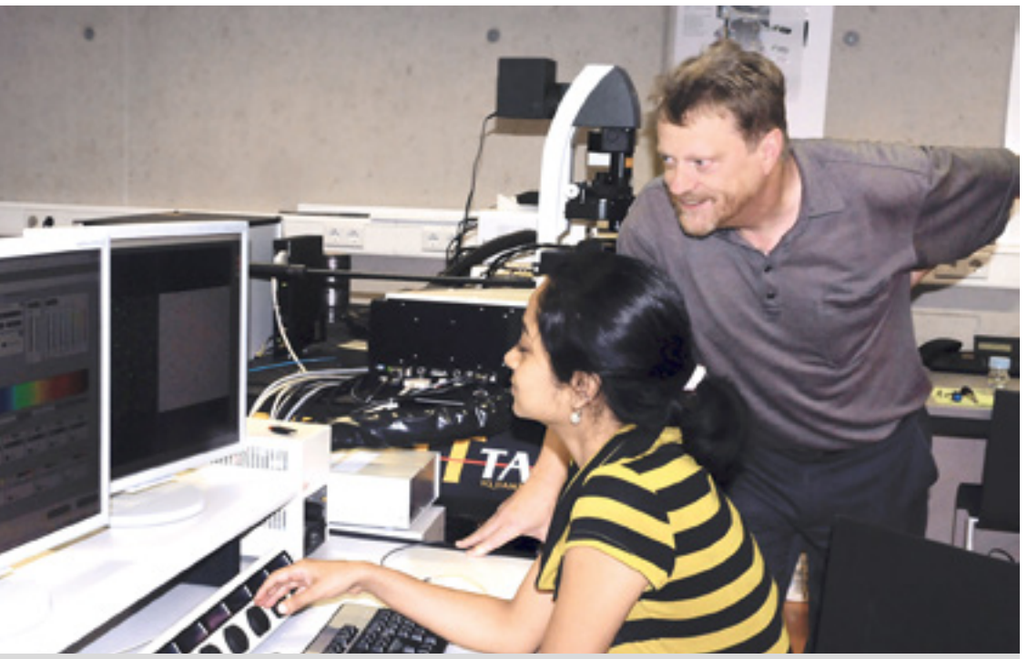
Yeast and cancer cells are especially similar in terms of lipid metabolism [6], which underlines the strength of the yeast system as a model. The primary enzyme involved in human fat breakdown – adipose triglyceride lipase – also has a counterpart in yeast [7]. Disruption to fat breakdown in yeast is accompanied by the development of "obese" yeast cells, which deposit the excess storage lipid (TG) in lipid droplets. The degradation of storage lipids is necessary in order to supply precursors for the synthesis of membrane lipids during cell growth: if enzymes required for the breakdown process are absent, cells not only become fat but also exhibit significant retardation of cell division. Regulatory factors in the cell cycle programme that respond to altered lipid mobilisation are now a major focus of research [8]. If cells lack the ability to respective fat – perhaps due to the deactivation of genes required for fat synthesis in the respective mutants – cells develop a highly sensitive response to an excessive surplus of fatty acids and die off rapidly since the membrane equilibrium is markedly disrupted. This underlines the importance of storage lipids as a "buffer", so as to intercept a surplus of fatty acids – taken up via nutrition, for example – and to render this surfeit harmless for the organism. A malfunction within the fatty acid detoxification process could well be the causative agent of damage to the β cells in the pancreas responsible for insulin production and thus contribute to the genesis of type 2 diabetes in obese patients.

The lipid droplet: the unknown organelle

Until a few years ago, the general opinion was that storage lipids were stored within the cell as inert droplets of fat. Yet this fat droplet is actually a highly complex organelle: lipid droplets (LDs) are enclosed by a membrane lipid layer within the cell (fig. 1), while their surfaces are adorned with a wide variety of proteins [9]. These proteins are used in the synthesis and degradation of storage lipids (or the regulation of such processes) and have a key role to play in interaction with other organelles – such as mitochondria. One result of storage lipid cleavage is the oxidation of the released fatty acids in the mitochondria, for example. In humans and animals, the fatty acids released from adipose tissue are transported via the bloodstream to heart and muscle tissue or the liver, where they are oxidised or converted back into storage lipids.

According to the model currently under discussion, triglyceride is synthesised in the mem-

lipotoxicity



Neha Chauhan studied biology at the University of Bangalore, India. On graduating, she worked as a research assistant in Professor Rajasekharan's lab at the Department of Biochemistry in the Indian Institute of Sciences in Bangalore, India. In 2004, she obtained her doctorate within the "Molecular Enzymology" PhD programme in the Kohlwein lab at the University of Graz. From 2012–2013, she spent several months abroad as a guest researcher at the Department of Biochemistry at the Uniformed Services University of the Health Sciences in Bethesda, USA (Teresa Dunn lab). Neha Chauhan is currently conducting post-doctoral research in the Kohlwein lab on the physiological role of lipid metabolism during cell division.

Sepp D. Kohlwein studied technical chemistry at the Graz University of Technology, obtaining his doctoral degree (Dr. techn.) there in 1982 from the Institute of Biochemistry, where he was associate professor until 2001. Following several periods of research spent at the Albert Einstein College of Medicine in New York and the Carnegie Mellon University in Pittsburgh, he completed his habilitation in biochemistry in 1992. In 2001, Sepp Kohlwein accepted an offer from the Institute of Molecular Biosciences at the University of Graz, where he heads the Yeast Genetics and Molecular Biology Group (http://yeast.uni-graz.at). His primary research interests focus on the investigation of lipid metabolism in yeast as a model for metabolic disorders and on the implementation of live cell and super-resolution microscopy methods for use in cell structure research (http://bioimaginggraz.at).

Harald F. Hofbauer studied chemistry at the University of Graz, majoring in biochemistry and molecular biology, and obtained his doctorate there in 2012 within the "Molecular Enzymology" PhD programme in the Kohlwein lab. Following several periods of research abroad at Cornell University, Ithaca, NY (Susan A. Henry lab) and postdoctoral research work in the Kohlwein lab, Harald Hofbauer is currently working in the Ernst lab at Goethe University Frankfurt's Buchmann Institute for Molecular Life Sciences. Here, his research interests are currently focused on the molecular mechanisms underlying fatty acid-induced lipotoxicity.

brane of the endoplasmic reticulum and stored between the two membrane halves; on reaching a certain size, the developing LD then "buds off" into the cytosol. This model can be used to explain the surface structure of the LDs, which are enclosed by a phospholipid monolayer – unlike all other organelles, which feature a double layer (see fig. 1). Research to date has identified hundreds of proteins associated with lipid droplets, and which potentially play a role in the biogenesis of these organelles or in lipid storage and mobilisation. Although the biogenesis of lipid droplets plays a major part in the storage of fat, a number of fundamental questions remain unanswered. Is the prevailing model – i.e. TG storage between the membrane halves – actually correct? When and how does "budding off" occur? How do LD-associated proteins reach the organelle – and how are these processes regulated? How is the synthesis of membrane lipids and storage lipids regulated?

The close physiological interrelation between membrane phospholipids and triglyceride storage/mobilisation, along with their associated disorders, are considered to be multiply involved in the genesis of our "lifestyle diseases" and now form a major branch of global biomedical research. One may safely assume that the field of yeast research will continue to make significant contributions to our understanding of lipid homeostasis in human cells.

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market view

Pfizer further reduces price in the world's poorest countries

Pfizer Inc. announced new commitments aimed at ensuring the world's most resource-limited countries have access to Prevenar 13 (pneumococcal polysaccharide conjugate vaccine (PCV), 13 – valent, absorbed) through Gavi, the Vaccine Alliance. Pfizer's commitments were announced during Gavi's pledging conference being held in Berlin, Germany, in support of Gavi's 2016 – 2020 strategy. Pfizer announced a 20 cent reduction of its per-dose price for Prevenar 13, from $3.30 per-dose to $3.10 per dose for the new four-dose vial presentation, which is expected to be introduced under the Advanced Market Commitment (AMC) program in 2016.

→ **www.pfizer.com**

Lonza continues to make progress

In 2014 Lonza delivered expected CORE EBIT growth of 11% in constant exchange rates (CER) (+9% in reported currency), compared with 2013. Lonza's overall results confirm the updated guidance communicated during the third-quarter update. Both of Lonza's segments – Specialty Ingredients and Pharma&Biotech – delivered a solid performance despite currency headwinds and the performance of the Water Treatment business. Most of the individual businesses performed according to expectations. Unfavourable weather conditions in the Water Treatment business had a negative impact on revenues for the second consecutive year, as well as time-consuming, complex tech transfers and validations/qualifications in Pharma&Biotech businesses and portfolio optimizations, e.g. the impact of Hopkinton, MA (USA) shutdown.

→ **www.lonza.com**

Eurofins announces successful acquisition

Eurofins Scientific, the global leader in bio-analytical testing, and one of the world leaders in genomic services, announces the successful closing of the transaction to acquire Boston Heart Diagnostics Corp. following review and approval from relevant regulatory bodies. Eurofins signed an agreement to acquire Boston Heart for an initial cash amount US$140 million, plus an earn-out payment that is expected to be in excess of US$60 million upon achievement of certain milestones. Boston Heart utilizes a suite of proprietary diagnostics in combination with additional clinical and genetic tests, extensive cardio-informatics capabilities, and ancillary patient engagement services to provide a leading, advanced diagnostics platform to help identify and reduce the risk of cardiovascular disease, diabetes, and other chronic conditions.

→ **www.eurofins.com**

EU provides data on development of resistant bacteria

The use of certain antimicrobials in animals and humans is associated with resistance to these antimicrobials in bacteria from animals and humans. There are also important differences in the consumption of antimicrobials in animals and in humans between European countries. These are some of the findings of the first integrated analysis of data from humans, animals and food in Europe published jointly by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA). The 'ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals' also identifies data limitations that need to be addressed to allow further analysis and conclusions to be drawn. These include additional data on antimicrobial consumption by animal species, data on antimicrobial consumption in hospitals in more European countries and monitoring of resistant bacteria in the normal flora from both healthy and diseased people.

→ **www.ema.europa.eu**

Wanted: Universal detectability

lab&more in conversation with Dr Stefan Lamotte, Research Scientist, Separation Science & Hyphenated Techniques, Competence Center Analytics, BASF SE

Stefan Lamotte is a research scientist in the Separation Science and Hyphenated Techniques laboratory at the Competence Center Analytics at BASF SE in Ludwigshafen, Germany. This laboratory is responsible for a wide variety of analyses to support R&D for new products, chemical synthesis, process improvement, compound impurity analysis and assaying the toxicological potential for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

Analytical procedures in the laboratory span a variety of chromatographic techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE). Samples analyzed in the laboratory by HPLC are often non-volatile and without a chromophor, thus common UV detectors are not suitable. Alternatively, more universal detectors, based on aerosol techniques such as evaporative light scattering or charged aerosol can overcome this problem and needed to be evaluated especially for demanding surfactant analysis applications.

lab&more in conversation with Dr. Stefan Lamotte, who evaluated Charged Aerosol Detection for the HPLC trace analysis of surfactants.

Dr Lamotte, why did you decide to evaluate the Thermo Scientific Charged Aerosol Detector?

S. Lamotte: Without talking too much about details, we needed to analyze several special surfactants by HPLC. These surfactants are used in advanced petrochemical processes. Our standard separation method is well established and robust, but the samples are quite challenging and the goal is to quantify the relevant surfactants in small elution window on a baseline that is significantly affected by the matrix load. Performing calibration and quantification, we noticed that the response curve was quite different to the ELSD. The ELSD calibration curve is more a quadratic function, while the one for CAD looks more like a root function

Talking about the non-linear response of both detectors. With the Corona Veo RS, you have the possibility to obtain linear calibration curves by using the power function feature? Did you use that feature?

S. Lamotte: In fact we did. Using the power function feature, we were able to nearly obtain linear calibration curves over a wide range. However, it required a significant amount of time to obtain the optimum power function values for our analytes and the power function can be

different for each individual analyte. I would greatly appreciate a software tool to assist in this process. We also were wondering why the peak areas decreased with the increasing power function. This became clear when we understood the need of a normalization factor in the detector firmware to keep the response of high analyte concentrations in the operation range of the detector electronics.

One of the main advantages of Charged Aerosol Detection and evaporative light scattering detection is their universality. Did you see any differences of universality between both techniques?

S. Lamotte: We clearly observed more substances with the Corona Veo than with the other detection techniques including ELSD. In cases where you have highly complex matrices, it highlights the importance of achieving a good chromatographic separation as nearly all components can be observed.

Dr Lamotte, thank you for the interview.

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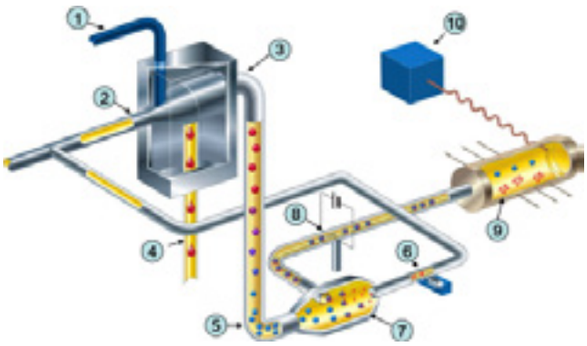


1. Inlet for Mobile Phase Nebulization
2. N_2 Gas for Aerosol Generation
3. Aerosol Inlet to Drying Tube
4. Waste Outlet for Large Drops
5. Solvent Evaporation
6. Corona Needle for N_2 Charging
7. Collision Chamber for particle Charging
8. Ion Trap for Background Removal
9. Electrometer for Current Recording
10. Data Station

Fig. 1

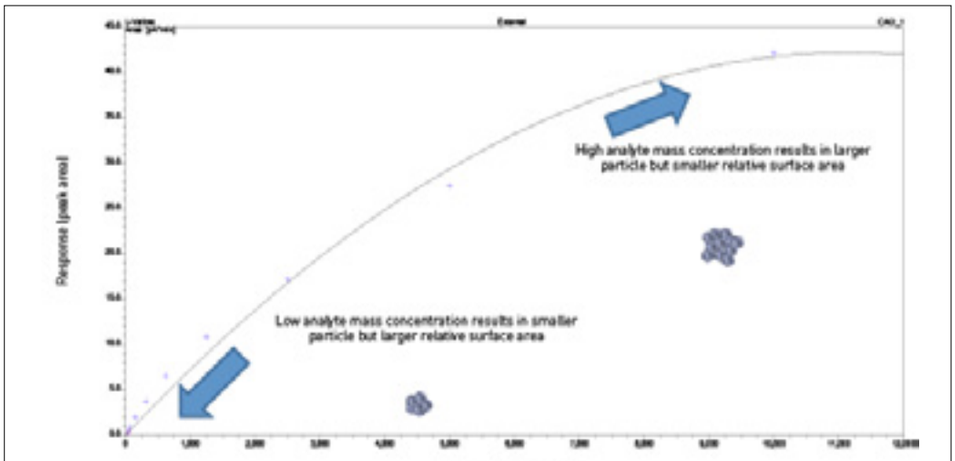


Fig. 2

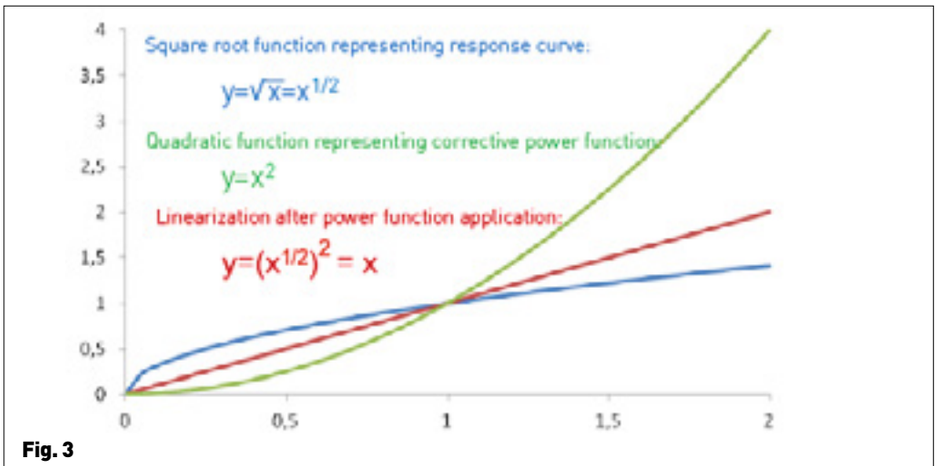


Fig. 3

The signal generation with the CAD (charged aerosol detection) technique is completely different from the ELSD (evaporative light scattering), though both detectors use nebulization to create an aerosol in the first place. In contrast to measuring light scattering properties of this aerosol as the ELSD does, the CAD measures an electric current resulting from a subsequent charging of the aerosol surface. As shown in figure 1, the supporting nitrogen gas is used both for nebulization and for transferring a defined charge to the aerosol surface. This is possible by ionization of the nitrogen in a corona discharge process and bringing it in contact with the aerosol in a collision chamber. With this process the resulting current signal is a strict function of the aerosol surface.

While the signal generation through a light scattering process in the ELSD is ruled by a complex combination of different scattering mechanisms that are mostly analyte-specific, it is controlled by simple geometrical relations in case of the CAD. As the size of the aerosol particles increases with the original analyte concentration (figure 2), the ratio of aerosol particle surface to particle mass decreases respectively. This fact together with the strict relationship between aerosol surface area and resulting electric current has three major consequences:

- 1. Among all HPLC detectors on the market, the CAD shows the most uniform response to a given analyte mass, independent of the specific chemical properties of non-volatile analytes.
- 2. Since the surface to mass ratio increases with decreasing particle size resulting from decreasing analyte concentration, the CAD exhibits increasing sensitivity (slope of response curve) when moving towards trace concentrations.
- 3. The resulting response curve type allows simple linearization by a mathematical signal transformation using a power function (figure 3). Such a straight forward linearization of calibration curves is not possible with an ELSD over a wider calibration range.

Advertorial

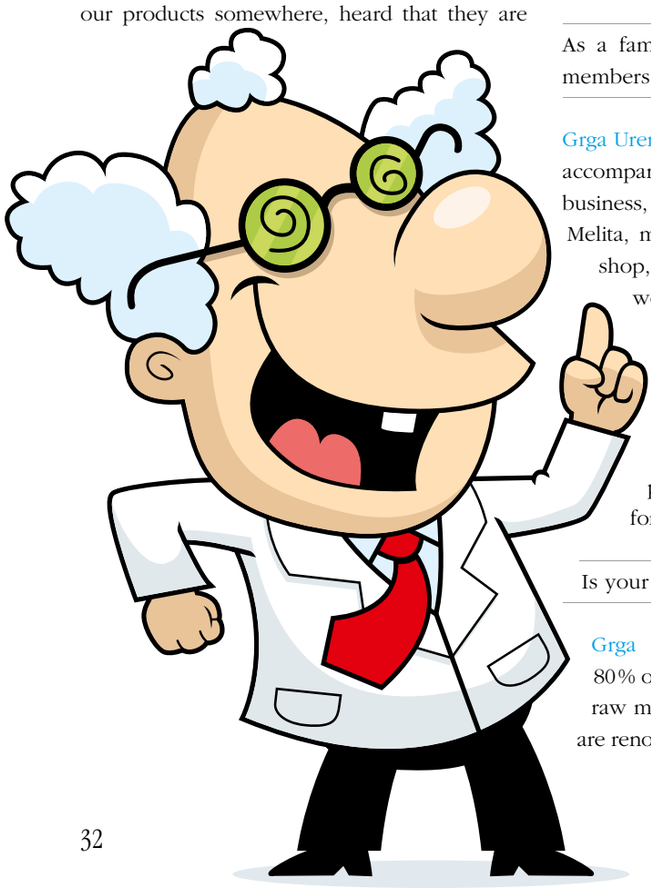
Laboratory furniture & more – A Croatian story

lab&more in conversation with Grga Uremović, Owner of GIMlab – a Croatian manufacturer for laboratory furniture and with Eberhard Keller, Project Manager SO-EE Group Engineering at Merck KGaA

A scientist might enter a lab without ever giving a thought about its furnishing. However, designing a laboratory is a demanding task considering deviations from standard dimensions and having the purpose to use an optimum of the available space. Grga Uremović, from GIMlab Laboratory Furniture, was interviewed by lab&more telling the story of his firm which started 1993 in the middle of the Croatian War of Independence.

Mr. Uremović, did you and your family have a connection to the subject of laboratory before the start of the Company Grga+Melita and how was the start like in these times?

Grga Uremović: We started our work at the time of the last war in Croatia. Professors of the University of Rijeka, Faculty of Medicine, had seen our products somewhere, heard that they are good, and this is how we got started. This was our first laboratory furniture project. Those products, of course, cannot be compared with the today's products. It was rather fighting for our lives, as there was a constant development of products. But we are proud of our results in this period of 22 years of diligent and honest work.



As a family-run company, are all the family members involved in the firm's operations?

Grga Uremović: We have two sons –twins– who accompany us on our way and are with us in business, too. In the beginning it was my wife Melita, my mother and me. In a small workshop, sometimes even outside in the yard we were working but we constantly have expanded the business and further people have been engaged as coworkers. Today, we also have modern CNC technology (computer controlled machines) and provide a premium product quality. We are ready for new challenges.

Is your furniture purely a Croatian product?

Grga Uremović: Good question. More of 80% of the components that we process, the raw materials, is provided by suppliers who are renowned European manufacturers, mainly German. The rest is our own work and innovation. We are proud of our unique complex ergonomic design solutions and our own design. Our motto is - we do not copy our competitors, we respect them.

What are your firm's recent achievements?

Grga Uremović: Soon, we will establish a German branch in Heidelberg. Already, the next step will be a representation in the Near East: Qatar, UAE, KSA, Bahrain or Oman, then in the USA, in New Jersey. We will exhibit GIMlab at this year's ANUGA FOOD TEC in Cologne, ARABLAB in Dubai as well as CPhI worldwide - P- MEC in Madrid and will like to welcome you and your readers there at our stands. Furthermore, we are glad that famous and renowned companies recognize GIMlab laboratory furniture. We can mention some of the recently realized projects: Merck KGaA in Darmstadt and the Ludwig Maximilians University in Munich (Germany), Bakhresa Food Products Ltd. in Dar Es Salaam (Tanzania) and several laboratories in Tanzania and Zanzibar, BICRO Institute in Zagreb (Croatia), amongst others.

→ **grga@gim-lab.com**

Mr. Grga Uremović wants to thank Merck and every other satisfied customer for their trust.

Picture: www.istockphoto.com | cthoman

Lab&more questions to Merck KGaA

What kind of laboratory was furnished by GIMlab?

E. Keller: A new lab for Merck Millipore research. The lab works on biological processes and has to meet the demand for the lowest biological safety level.

Why did Merck decide to choose GIMlab furniture for their laboratory?

E. Keller: Time and flexibility.

What is your experience so far with the furniture, what distinguishes it from others?

E. Keller: They meet the current quality standard. There are no big differences to other furniture's. The flexibility of the people which build up the furniture was impressive.
Story will be continued…

Pictures: X-Team | X-Team GmbH

ms-Pure septum – non-pigmented silicone/PTFE septum

With the increasing sensitivity of analytical instruments there is an increasing demand for pure low bleed septum material to prevent sample contamination with extractable substances from the septum. The ms-Pure silicone/PTFE septum is produced from natural non-pigmented silicone/PTFE. Tests show that it is over 60% cleaner than "standard" silicone/PTFE septums on the market.

→ **www.infochroma.ch**

exhibition

The 40th FEBS Congress – The biochemical basis of life

In 2015, the German Society for Biochemistry and Molecular Biology (GBM) joins forces with the Federation of European Biochemical Societies (FEBS) to organize the 40th FEBS Congress from Saturday July 4th to Thursday July 9th, 2015, at the Estrel Convention Center in Berlin, Germany.

With this joint congress, GBM and FEBS expect to bring together a wide range of researchers from all across Europe and further afield to explore 'The Biochemical Basis of Life'. The organizing team around Prof. Volker Haucke (FMP, Berlin) created an outstanding program:

The congress will cover the entire spectrum of molecular life sciences with symposia on 'Mechanisms of Gene Expression', 'Membranes, Receptors & Bioenergetics', 'Structural Biology and Biophysics', 'Systems Biology, Bioinformatics & Theoretical Biology', 'Molecular Neuroscience' and 'From Chemical Biology to Molecular Medicine'.

In addition, there will be sessions and plenary discussions, e.g. on the future of scientific publishing, women in science, science & society, careers and education, as well as the Young Scientists' Forum (YSF) immediately preceding the congress to promote interactions between pre- and post-doctoral scientists.

The program is available at www.febs2015.org. Also, registration and abstract submission are possible via the congress website.

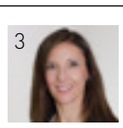
→ **www.febs2015.org**

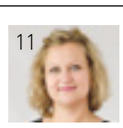
DUPHAT 2015 is the only international pharmaceutical & technology event in the Middle East that gives CME credit points. It serves as the convergence point of pharmacists, manufacturers, distributors, medical representatives, pharmaceutical analysts, consultants and practitioners. The event is held under the patronage of HH Sheikh Hamdan Bin Rashid Al Maktoum, Deputy Ruler of Dubai, Minister of Finance, UAE and President of Dubai Health Authority. DUPHAT 2015, the 20th Dubai International Pharmaceuticals & Technologies Conferences & Exhibition has developed to be one of the most recognized and significant pharmaceutical event in the Middle East and North Africa.

In this conference, it is planned to discuss recent development in pharmaceutical sciences and pharmacy professionals will share knowledge, experience with international experts and enhance scientific cooperation between participants during invited lectures, panels, workshop and certified courses.

All participants interested, we have opened registrations for the conference with special pre-registration rate until 3rd of March 2015.

→ **www.duphat.ae**





lab&more

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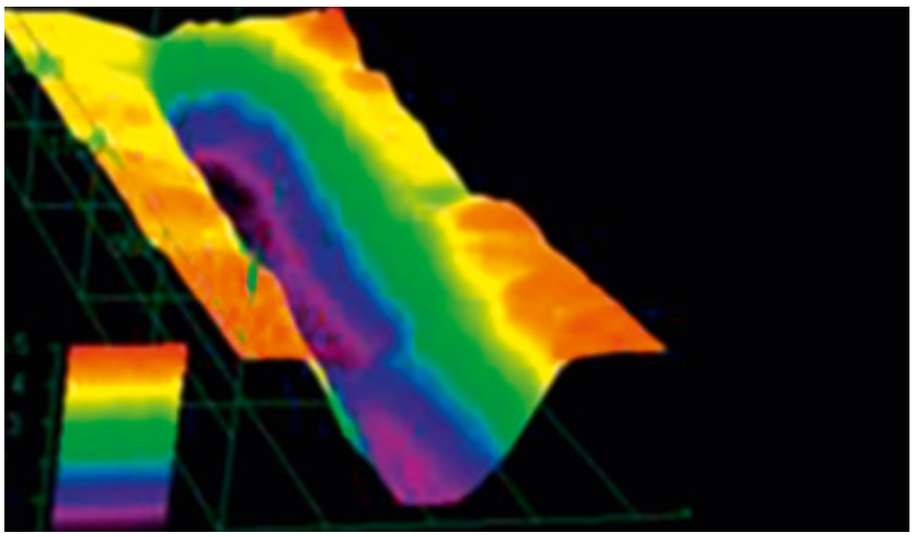

Crime scene investigations with opto-digital microscopy

Fast, efficient and non-destructive towards precious samples, light microscopy plays a vital role throughout forensic science operations. Employing the Olympus DSX100 Opto-digital system, the advanced light microscopy has yielded greater insights into a variety of forensic samples, revealing fingerprints on metal, pen marks on paper and characterising unique designs on seized illicit tablets.

The recovery of latent fingerprints from metals presents great potential for criminal identification, but is also particularly challenging, and although the print effectively 'etches' a pattern into the metal, this can be incredibly faint. In the process of optimising the conditions for print development, detailed visualisation is crucial. The ability to tilt the head of the DSX100 controls the level of oblique lighting, preferentially highlighting the surface to reveal detailed topography, while sophisticated digital technologies have also proved beneficial.

www.olympus-ims.com

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MULTIFUNCTIONAL HIGH SAFETY LABORATORIES

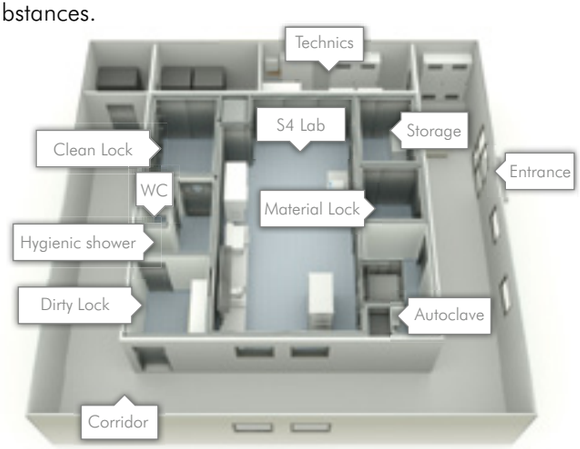
Continuous and new contagious diseases pose a growing threat: Ebola, SARS, bird flu, swine flu, tuberculosis, etc. New high safety laboratories for biological pathogens are being built to prepare for such threats. No matter what type of laboratory you require, HT LABOR + HOSPITALTECHNIK AG is your expert full service supplier for multifunctional high-security laboratories.

The hermetically sealed laboratory systems are hygienically and microbiologically shielded units. Technical barriers, control and monitoring systems ensure compliance, control and traceability of the security policies set by national and international regulations.

All building services parameters are integrated into the control process, the alarm system and monitoring by probes and sensors. The result is a safe research environment for handling highly contagious and dangerous substances.

Types of laboratories

- Medicine
- Microbiology
- Pharmacy
- Tuberculosis lab
- Genetic research
- Biomedicine
- Analysis
- Animal research lab
- Insulating rooms
- Epidemic research
- Epidemic test lab



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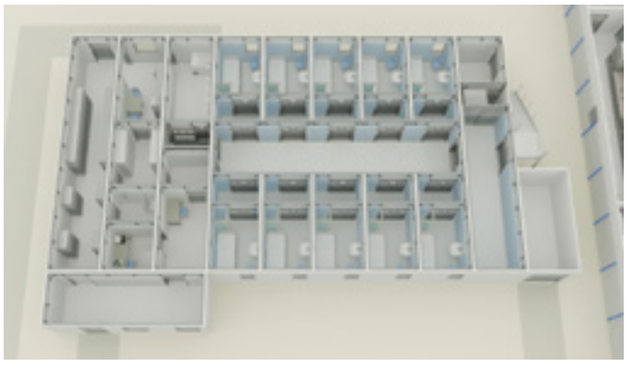
MEDICAL TREATMENT CENTER FOR EBOLA EPIDEMIC

This concept shows a self-sufficient EBOLA ISOLATION STATION, equipped with gas-tight doors, walls, sluice facilities and air filtration systems. Chemical wet-showers ensure the secure decontamination of the medical staff. The access is provided with electronically secured access control systems.



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- TIER-TESTRÄUME / ANIMAL TESTROOMS

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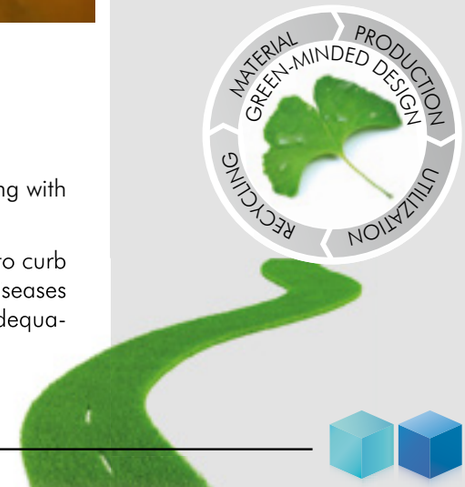
MOBILE LABORATORIES WITH ISOLATION UNIT

Self-sufficient , fully equipped Safety Laboratory for biological safety levels 1 to 3 for dealing with highly infectious germs (e.g. Ebola, SARS, bird flue, ...).

The task is to detect infectious diseases on site, to curb and combat them where the spread of these diseases is greatest, but medical care is not or only inadequately available.

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