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INTERNATIONAL YEAR OF LIGHT 2015

"Although there's much we don't know, we also must discuss, understand, promote, and ask new questions."

Prof. Dr Thomas C. Südhof, Avram Goldstein Professor at the School of Medicine at Stanford University, was awarded the 2013 Nobel Prize in Physiology or Medicine jointly together with James E. Rothman and Randy W. Schekman for their work on vesicle trafficking.

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Combating multi-resistance – a Sisyphean work?

Prof. Dr med. Karsten Becker
Institute of Medical Microbiology,
University Hospital Münster,
Münster, Germany

Karsten Becker is an Associate Professor and Chief Senior Physician at the Westphalian Wilhelms-University of Münster. He studied medicine at the Magdeburg Medical School followed by a research period at the Hannover Medical School. After receiving his doctorate in 1991, he completed his habilitation in medical microbiology in Münster in 2002 and qualified as medical specialist for Microbiology, Virology and Infectious Disease Epidemiology in 2006. Since 2008, he is chairman of the Standing Working Group "Diagnostic Procedures" of the German Society for Hygiene and Microbiology (DGHM). He is particularly interested in the epidemiology, pathogenesis, diagnosis, prevention and chemotherapy of staphylococcal infections. In particular, he has done extensive research on the intracellular lifestyle of the staphylococcal small colony-variant (SCV) phenotype. Further interests comprise the detection, identification and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA). An additional main focus is on the development and improvement of microbiological diagnostic tests. He is the author of several medical textbooks and encyclopedias and more than 200 scientific publications.

Is it far-fetched to draw this analogy? Since antiquity, Sisyphean work describes hard and useless efforts eternally imposed. Applying this metaphor to activities for the prevention of resistant, particularly, multi-resistant microorganisms, those who are professionally acquainted with infectious diseases might be tempted to fully agree with this statement, at least in "hours of weakness".

At first glance, the use of this term may seem accurate in view of globally increasing rates of pathogens being resistant to antibiotic key drugs, often as multi-, extensively or even pan-drug-resistant strains. The world-wide exploding consumption of antibiotics for medical, but also for non-medical purposes such as in conventional livestock breeding maintains this alarming development by a continuous selection pressure on the microbial world. One can be absolutely certain; the microbes are well-equipped with an only partly discovered arsenal of genetically encoded mechanism enabling them to resist antibiotic substances. Of note, these defense mechanisms are really thoroughly tested and evolutionarily improved in a "biological war" waged in microbial ecosystems such as natural biofilms, where natural antibiotic compounds are applied in the struggle for living space and nutrients. These circumstances are aggravated in the hospital environment by several factors. Moreover, global migration processes, unbounded tourism and many other medical, demographic and live style changes impact negatively the prevention and combating of infectious diseases. In consequence, from a global perspective, we experienced increasing rates of different kinds of multi-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), extended spectrum ß-lactamases (ESBL)-producing and carbapenem-resistant Gram-negative bacteria and multi-up to pan-resistant *Mycobacterium tuberculosis* strains.

Does resistance matter? Every use of antibiotics – justified or not – causes a selection pressure on both the target organism and susceptible parts of the microbiota (physiological flora), however, misuse, multiplies the effects. Every kind of resistance is diminishing our therapeutic options, i.e. the patient might be deprived of the most effective drug (from pharmacodynamics and pharmacokinetics view points) and/or of the drug with least side effects. Many resistance mechanisms are encoded on mobile genetic elements. Thus, they will be exchanged between microbes; and the "bugs" are very promiscuous, ignoring species barriers. Moreover, resistance genes are often part of complex elements (e.g. cassettes) comprising multiple resistance genes, but also genes mediating resistance to biocides (antiseptics) and/or heavy metals and metalloids. Thus, co-selection even may occur without primary involvement of antibiotics. Last, but not least, the activities of the pharmaceutical global players in terms of the development of novel antimicrobial drugs had declined greatly in the past decade.

What is necessary? In contrast to other pharmaceuticals, antibiotics are "societal" drugs because their improper use makes them worthless also for other patients. In addition, being colonized or infected by a multi-resistant pathogen is not a personal matter because the nosocomial pathogens are easily transferable between patients, medical personnel and facilities. Thus, we have learned that only joined efforts across disciplines are able to contain the development and spread of multi-resistant microorganisms. A good example is the establishment of antibiotic stewardship or even infection disease teams at the hospital. However, disparate efforts at a given healthcare facility are finally condemned to failure. The interdependencies of those hospitals exchanging patients, often across administrative boundaries, require their precise determination followed by joined regional efforts to establish networks of all health care players to combat multi-resistance. That this will work, at least for MRSA, was demonstrated by the success of the MRSA-net/EurSafety Health-net networks. A key to success was the joint generation of different grades of seals of quality. Their content focused on risk-adapted screening procedures and a consistent implementation of the MRSA prevention measures ("search and destroy"). Not to forget, there is an urgent need to reinforce the research on antibiotic active substances. If used for prevention such as MRSA decolonization, they should be target-specifically tailored (e.g. recombinant phage endolysins).

To come back to the beginning, combating multi-resistance was, still is and will forever be hard Sisyphean work – but surely not for nothing! The respective efforts are based on effective prevention measures without the possibility of an "ultimate victory", but – rather in the sense about a neck-and-neck race - with the necessity to control, contain and - as far as possible - reverse the development and spread of multi-resistant pathogens inside and outside of the hospital. This remains a task for the whole society and its realization will require adequate funding. The German Center for Infection Research (DZIF) with the thematic translational units "Healthcare-associated and Antibiotic-resistant Bacterial Infections" and "Novel Anti-infectives" is a recent response to these challenges.

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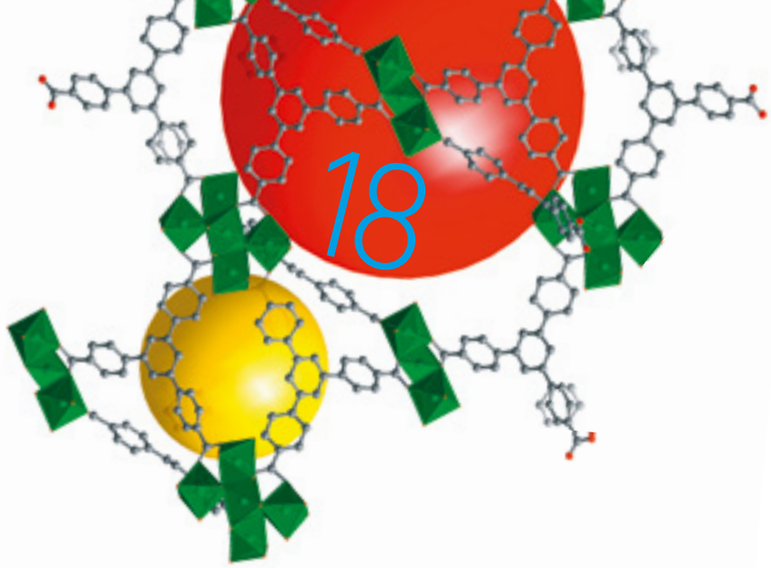
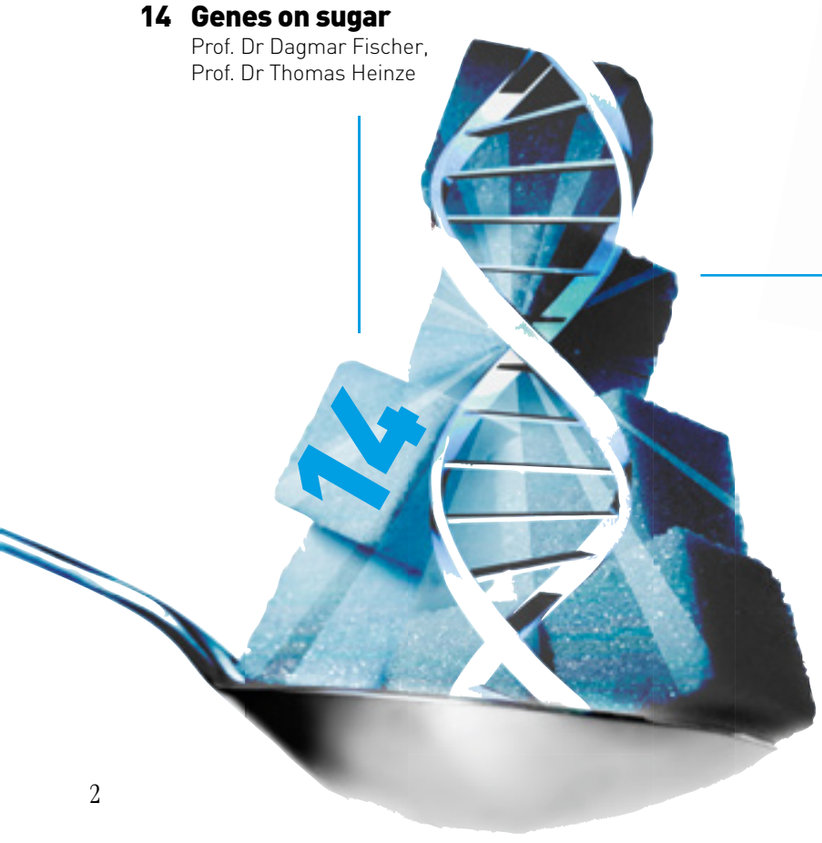


INTERNATIONAL YEAR OF LIGHT 2015



Cover: © picture alliance/APA/picturedesk.com, Karl Schöndorfer
Cover quote: The quote from Thomas Südhof is taken from an interview with The Journal of Clinical Investigation: J Clin Invest. 2013 Dec 2; 123(12): 4984–4985. Published online 2013 Dec 2. doi: 10.1172/JCI74014

Nanomachines for neurotransmitter release

Prof. Dr Paul G. Layer

Thomas C. Südhof, since 2008 at the Howard Hughes Medical Institute at Stanford University, was awarded in 2013 jointly together with James E. Rothman and Randy W. Schekman the Nobel Prize in Physiology or Medicine in the fields of cell physiology and neurophysiology "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells" [1]. Südhof, a native German having received his MD in Göttingen, Germany, began his endeavor into the neurochemistry of synapses as a PhD student under Victor Whittaker at the Göttingen Max Planck Institute. The synapse as the central unit of neural activities has fascinated neuroscientists as from the beginning of the last century. In the seventies, when Neurochemistry became a field of neurosciences by its own, the molecular machinery at a cholinergic synapse appeared to be still relatively simple, consisting of the neurotransmitter acetylcholine (ACh), its postsynaptic ACh receptor (AChR), its degrading enzyme acetylcholinesterase (AChE), a presynaptic choline re-uptake system, and choline acetyltransferase (ChAT) to resynthesize the NT. Quite simple and straight-forward, it seemed: three, four proteins appeared to do the job.

As soon as molecular biology techniques had entered neurosciences research, things, however, changed dramatically. The molecular machinery at synapses turned out to be much more complex, with a myriad of individual proteins being involved in the NT release process, which all play roles by formation and/or disintegration of distinct protein complexes at specific times and locations during NT release. After having moved to the Southwestern Medical School of the University of Texas in Dallas, Südhof's research focussed on the question what happens after calcium uptake into the presynaptic terminal. Using brain cells from mice, by 1995 the Südhof laboratory had not only shown that Synaptotagmin-1 is a synaptic vesicle Ca^{2+}-binding protein, but it is indispensable for fast Ca^{2+}-triggered NT release. Lots of questions remained; e.g. mammals express sixteen synaptotagmins, of which eight do not bind calcium! Which ones are essential for NT release, and what are the others for? They found that in fact multiple pathways of Ca^{2+}-triggered exocytosis (e.g., hormone release, etc.) are controlled by different synaptotagmins. Thus, synaptotagmins as Ca^{2+}-sensors function in most if not all Ca^{2+}-dependent membrane fusion reactions, in complex with other proteins representing a molecular release nanomachine.

It was for these discoveries that Südhof was awarded the Nobel Prize. But along his successful scientific journey, he has made several other remarkable discoveries; one of them being the "neurexin-neuroligin story" [2]. Through alternative splicing of transcripts of their genes, a multitude of effective protein isoforms could (at least theoretically) be produced. It was speculated that a combinatorial pairing of particular isoforms of neurexins and neuroligins could function as a specifying cellular code during wiring of brain networks. Neuroligins belong to a so-called cholinesterase-domain family of adhesion proteins (ChED), whereby all of them present a domain of high identity with AChE, while not exerting any enzymatic activity. This was most fascinating to me, since in 1993 we had suggested nonenzymatic adhesive functions of cholinesterases [3], which was supported by the detection of ChED proteins [4].

In recent years, Südhof has focussed much on medical implications of diseased synaptic proteins, e.g. their possible roles in schizophrenia, autism, Parkinson's or Alzheimer's disease. For instance, neuroligins are genetically linked to autism, and deletion of various neuroligins in the mouse led to quite severe changes of cerebellar neural circuits [5].

Personal: Thomas C. Südhof was born on 22 December 1955 in Göttingen. He received his education at a Waldorfschule (in the U.S. called Steiner Schools). As their alumni, he is the first Nobel laureate of these anthroposophical schools. Südhof has seven children from two marriages, two with his second wife Lu Chen, who is a Neurosurgery professor also at Stanford. The Max Planck Society has tried hard to bring him back to Germany for good, however, could not compete with the exceptional academic, administrative and financial American conditions. In keeping close connections with Germany, since 2014 Südhof acts as Visiting Fellow at the Berliner Institut für Gesundheitsforschung (BIG). I am expecting much more exciting research from Südhof's laboratory: much more for lab&more!

→ layer@bio.tu-darmstadt.de

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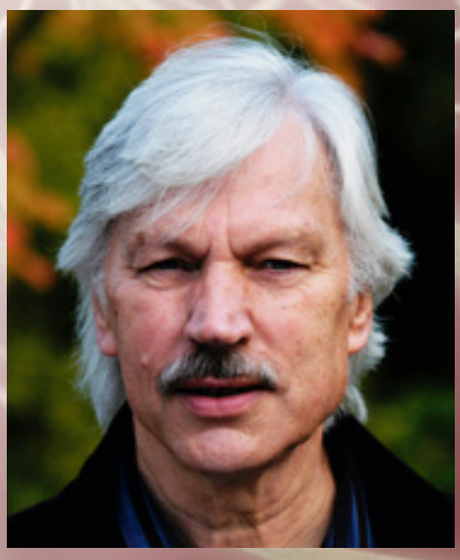
[5] Zhang B, et al. (2015) Neuroligins sculpt cerebellar Purkinje-cell circuits by differential control of distinct classes of synapses. Neuron 87, 781–796

Picture: © istockphoto.com | 4X-image



market view

Mettler Toledo wins 2015 Control Design Readers' Choice Award

Picture: www.controldesign.com

Control Design readers selected Mettler Toledo, the leading global supplier of precision instruments and services, in their 2015 Readers' Choice Awards survey as the winner in the Measurement, Load Cell/ Weighing product category.

Control Design 2015 Readers' Choice Awards recipients are featured in the September 2015 issue of the magazine, which includes automation leaders in 61 product categories.

Results were gathered through questionnaires sent to more than 17,000 subscribers who have buying authority or influence for industrial control and automation products. The questionnaires included unaided ballots that allowed tem to rank their first, second and third choices for suppliers in each of the 61 product categories.

Mettler Toledo once again took the top spot in the Measurement, Load Cell/Weighing category, having received this honour for 15 years now – ever since Control Design began polling readers in 2001.

→ **www.mt.com**

Glycotope and Octapharma enter exclusive worldwide licence agreement on human blood coagulation factors

Glycotope GmbH, a global leader in the glyco-optimisation of biopharmaceuticals, and Octapharma AG, one of the world's largest human protein product manufacturers, announced that they have entered into an exclusive worldwide licensing agreement for Glycotope's portfolio of preclinical human blood coagulation factors based on its unique and proprietary GlycoExpress™ (GEX™) technology platform. In addition, Octapharma will take a stake in the company by purchasing new Glycotope shares generated through a capital increase at a premium price. Under this agreement, Glycotope receives an initial payment of EUR 80 million from Octapharma.

Glycotope has developed a novel long-lasting technology basket for generating proteins with largely improved pharmacokinetic properties. So far significant improvements in key pharmacokinetic properties could be achieved for complex blood coagulation factors. This technology is suitable for half-life extension of blood coagulation factors as well as other proteins for improved patient compliance, and represents the newest member in the Glycotope platform technologies.

→ **www.glycotope.com**

Evotec awarded contract to manage NCI Chemical Biology Consortium Screening Libraries Center

Evotec AG has entered into a multi-year compound management agreement with the US National Cancer Institute (NCI), Department of Health and Human Services. Under the terms of the agreement, Evotec will provide compound management services to the NCI Chemical Biology Consortium (CBC) for a period of five years, with a total estimated value of up to EUR 4.5 million ($ 4.9 million).

The NCI Chemical Biology Consortium contract will continue to provide for the ongoing acquisition, storage, maintenance and distribution of the current library collection. This repository will form part of the CBC's drug discovery and development platform for new oncology therapeutics being researched by a consortium of primarily academic and non-profit institutions.

→ **www.evotec.com**

VDGH calls for a sense of proportion in connection with new IVD Regulation

The EU's deliberations for the drafting of a new Regulation on In Vitro Diagnostics (IVD) are entering the crucial phase. The objective is the creation of a new legal framework for the marketing and market supervision of products for laboratory diagnosis.

The Managing Director of Verband der Diagnostica-Industrie (VDGH) [the Diagnostics Industry Association], Dr Martin Walger, has complained that a number of additional measures called for by the Council are lacking in a sense of proportion, and are only designed to generate bureaucracy. Thus the VDGH has voiced the criticism that in the final outcome the new Regulation breaks the logical link between risk class and test procedure. In other areas, the Council wants to adopt regulations from the Ordinance on Medical Devices which have no bearing on laboratory diagnostics.

A total of 40,000 laboratory tests would be affected. For the European IVD industry, this would mean an additional liability in coming years amounting to over EUR 2 billion.

→ **www.vdgh.de**

Roche wins the first HPV primary screening tender in Europe

Roche announced that it has been awarded a 5-year contract by the Dutch National Institute for Public Health and the Environment (RIVM) for implementation of the cobas® HPV Test as the first-line, primary screening test in the national cervical cancer screening program.

The Netherlands is expected to be the first country in the world with an organised cervical screening programme to complete the transition from the Pap test to primary HPV screening. Using HPV as the primary test is based on the overwhelming scientific evidence that it offers significant improvement over more traditional Pap cytology screening due to its ability to detect more pre-cancerous disease.

→ **www.roche.com**

Analytik Jena AG expands Executive Management Board

The Supervisory Board of Analytik Jena AG has decided to expand the company's Executive Board. This shows the company responding to its growth and the ever-increasing challenges associated with its operational business. The restructuring also lays the groundwork for appointing a successor to Klaus Berka, the company founder and Chief Executive Officer, whose contract expires in March 2017.

Ulrich Krauss, who has more than two decades of experience in a variety of international management positions at Carl Zeiss, will be taking charge of the newly established Executive Board department of Marketing and Sales on 1 November 2015.

There will also be a new Chief Operating Officer (COO) at Analytik Jena AG. Dr Peter Juschitz will now be in charge of Group manufacturing, purchasing and logistics. Dr Juschitz was most recently Technical Director at AHT Cooling Systems in Rottenmann, Styria, Austria, and, before that, CEO of Epcos Kft, Szombathely, Hungary. Juschitz launched his career at Philips, where he held a variety of management positions for many years.

→ **www.analytik-jena.de**

LDC and Infinity Pharmaceuticals in partnership for the joint development of cancer medicaments

Lead Discovery Center GmbH (LDC) and Infinity Pharmaceuticals, Inc. of Cambridge, USA, have entered into partnership with the aim of jointly identifying exceptionally promising projects for the development of cancer medicaments. The project proposals come from the portfolio of LDC and from its extensive academic network.

Based on the partnership agreement, Infinity will learn about projects from the multifarious project pipeline of LDC, as well as being informed about new project ideas emanating from LDC's research network. This comprises links to leading universities as well as to highly regarded institutes of Germany's internationally renowned research organisations, the Max Planck Society and the Helmholtz Association. The main emphasis of the partnership will be on the field of oncology.

Infinity will be evaluating promising formulations of effective ingredients by LDC, with a view to identifying one or more projects for an ongoing partnership or for licensing. For the further development of selected projects, LDC will place its expertise and resources in the early phase of research into effective ingredients at Infinity's disposal.

→ **www.lead-discovery.de**

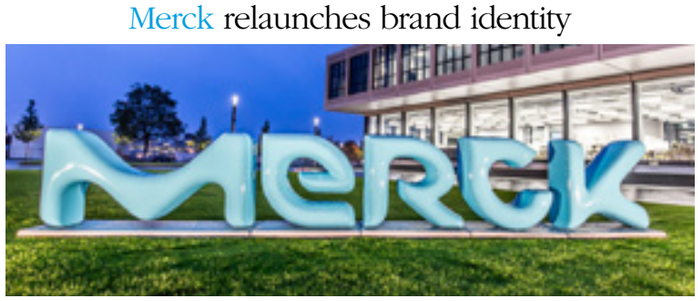
Qiagen launches new bioinformatics solution for hereditary diseases

Qiagen N.V. has announced the launch of a new Qiagen hereditary disease solution for research labs to accelerate solve rates in diagnostic odyssey cases, while freeing up time and resources by enabling researchers to focus directly on the right causal candidates. The offering includes Qiagen's Biomedical Genomics Workbench, Biomedical Genomics Server Solution, Ingenuity® Variant Analysis™, and HGMD® Human Gene Mutation Database.

"Qiagen continues to expand our solutions to enable the incredible advances that clinical research labs are making every day, particularly in next-generation sequencing for hereditary diseases," said Dr Laura Furmanski, Head of Qiagen's Bioinformatics Business Area. "By providing the market's most comprehensive biomedical content, more than 10 million findings in our Qiagen Knowledge Base, and the benefits of 16 years of experience in expert curation, we ensure the highest-quality analysis and interpretation – helping customers move from sample to insight."

→ **www.qiagen.com**

Merck relaunches brand identity



Merck, a leading science and technology company, announced the relaunch of its brand identity. The fundamental revision of the visual appearance as well as the introduction of a new logo reflect the transformation into a global science and technology company. At the same time, the brand architecture at business level has been simplified. Outside the United States and Canada, the company will operate uniformly as Merck.

"Merck has fundamentally changed over the past ten years," emphasized Karl-Ludwig Kley, Chairman of the Executive Board and CEO. "We have developed from a classic supplier of pharmaceuticals and chemicals into a global technology company. With our unique combination of highly specialized biopharmaceutical, life science and materials businesses, we are in a position today to offer solutions to support global megatrends such as health and digitization. The complete overhaul of our brand identity is to communicate this new direction vis-à-vis our customers, partners and applicants. We want to be recognizable and remain visible as Merck worldwide so as to strengthen our well-known brand name. For this we have deliberately rid ourselves of outdated features and will be focusing on a young and eye-catching image."

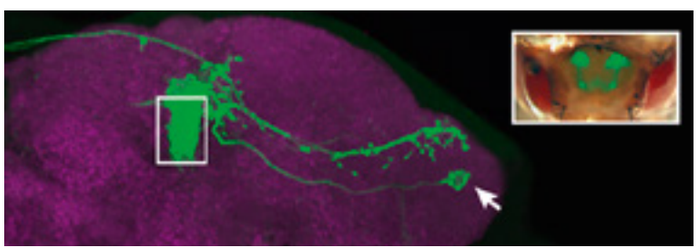
The investment in the Merck brand is part of the "Fit for 2018" strategic transformation and growth program, which includes the focus on innovative, technology-driven businesses as well as the modernization and expansion of global headquarters in Darmstadt, Germany.

→ **www.merckgroup.com**

researched

Neurobiology

Brain cells in fruit fly hold secret to individual odor preferences



Responding appropriately to the smell of food or the scent of danger can mean life or death to a fruit fly, and dedicated circuits in the insect's brain are in place to make sure the fly gets it right.

In studies designed to better understand how the brain processes information, scientists led by Cold Spring Harbor Laboratory (CSHL) Associate Professor Glenn Turner have identified an important component in these circuits: the point at which incoming sensory information begins to be transformed into a neural signal that instructs a fly's response. The cells, called mushroom body output neurons (MBONs), appear to distill nuanced information about an odor into clear instructions: approach or flee.

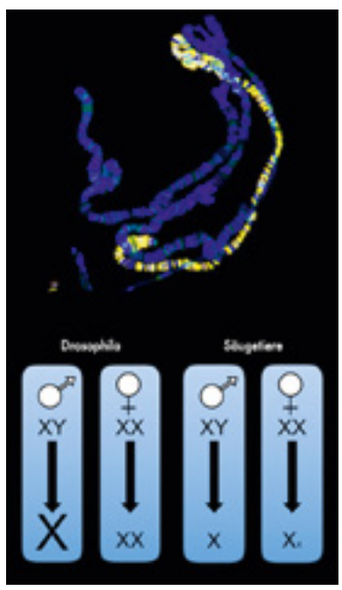
By genetically labeling and following the activity of the same MBONs in multiple flies (there are precisely 34 in each brain, situated in known locations), the scientists found that each cell had a characteristic response pattern in each individual. The pattern, in other words, differed between flies. This suggests that MBONs may underlie individual odor preferences that develop as flies learn to associate smells with positive or negative experiences.

Original Publication: Hige, T. et al. (2015) Nature 526, 258–262
Source: http://www.cshl.edu/news-and-features/approach-or-buzz-off-brain-cells-in-fruit-fly-bold-secret-to-individual-odor-preferences.html; Cold Spring Harbor Laboratory, New York

Drosophila genetics

The logistics on the drosophila X chromosome

Researchers decode molecular mechanism facilitating dosage compensation in flies: If we place an order in an online store we are often thrilled how fast the parcel is delivered to our doorstep. This is possible because logistic companies have established a very reliable and efficient system to distribute goods. Scientists at the Max Planck Institute of Immunobiology and Epigenetics in Freiburg now uncovered a similar distribution system in flies to achieve dosage compensation. By combining state-of-the-art molecular and imaging techniques the researchers revealed a molecular mechanism that allows the protein complexes that regulate dosage compensation to spread over the entire X chromosome. They observed that the so-called high affinity sites (HAS), which are binding regions for the protein complexes, often occur at X chromosomal regions with enriched long-range contacts to each other and further positions on the X chromosome. These central logistics hubs then facilitate the distribution of the dosage compensation machinery towards nearby locations.



Chromosomes of the fruit fly: The MSL-complex (yellow) mediates dosage compensation and decorates specifically the X chromosome.
© MPI f. Immunobiology and Epigenetics

Original Publication: Ramírez, F. et al. (2015) Mol. Cell 60, 1–17
Source: http://www.ie-freiburg.mpg.de/4610903/news_publication_9678505?c=723600 Max Planck Institute of Immunobiology and Epigenetics, Freiburg

Evolution

Plant helps show how living things adapt

A freshwater plant that has evolved to live in seawater is shedding light on how living things adapt to new environments. The findings could help scientists better understand how species have been able to adapt to major shifts of circumstances in the past, such as transferring from water to land, or from light to dark environments. In adapting to new surroundings, organisms must develop ways to perform everyday functions, such as securing food and oxygen, and reproducing. The latest study is one of the first to track such a significant lifestyle transition in the lab, instead of relying on fossil clues.

Researchers studied successive generations of a common freshwater algae, *Chlamydomonas reinhardtii*, in increasingly salty water. These plants have a key role in providing nutrients and removing carbon dioxide from the atmosphere, so understanding how they can evolve from freshwater to seawater aids understanding of the history and diversity of life on Earth. They found that freshwater algae adapted to seawater in two stages.

Original Publication: Lachapelle, J. et al. (2015) Evolution 69 (10), 2662 DOI: 10.1111/evo.12760
Source: http://www.ed.ac.uk/news/2015/algae-151015; The University of Edinburgh

Brain Research

Silver bullet for neurodegenerative diseases

Scientists have unraveled how mutant molecules damage the nervous system of people with Charcot-Marie-Tooth (CMT) disease, a group of disorders that hinder people's ability to move and feel sensation in their hands and feet.

In laboratory testing, the researchers were able to improve symptoms of the disease in mice, raising hopes that they may have found an avenue for treating people with CMT.

Symptoms of the disease, which typically appear in adolescence or early adulthood, include muscle weakness and decreased muscle size, loss of sensation and deformities in the feet and legs. The symptoms typically first appear in the lower extremities, but eventually may move into the hands.

Original Publication: He, W. et al. (2015) Nature doi:10.1038/nature15510
Source: http://www.salk.edu/news/pressrelease_details.php?press_id=2122; The Salk Institute, La Jolla, CA

Reproduction

Sex lives of male worms: One gene makes a big difference

For tiny nematode worms of the species *Caenorhabditis elegans*, males are rare and generally irrelevant in nature. That's because the vast majority of *C. elegans* individuals are self-fertilizing hermaphrodites. But NYU biologist Matthew Rockman and his colleagues have made a discovery that points to a previously unknown dynamic among these worms: Natural variation in a single gene produces males with excretory pores that attract the sexual attentions of other males.

Scientists had known that some *C. elegans* males are attracted to other males. Rockman and his co-authors were intrigued based on a general interest in understanding how and why individuals differ from one another when it comes to sexual behaviors as well as other traits.

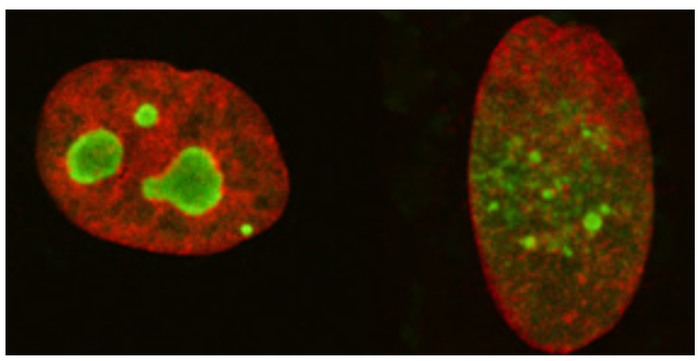
They've now traced much of the variation in this behavior to a single gene known as plep-1. Males carrying two copies of a plep-1 mutation attract other males for reasons that remain mysterious. When males mate with the excretory pore of another male, they leave an injurious plug behind. Males with plugged excretory pores have trouble in mating and they don't live as long as either.

The researchers say that the persistence of this gene – despite its detrimental effects on the individuals who carry it – may be explained by the distinctive reproductive mode in this species. Most of these worms have only one parent, a self-fertile hermaphrodite. Because matings between males and hermaphrodites are rare, sex has little chance to generate new combinations of genes – unlike in species with males and females. With few genetic combinations circulating in the population, natural selection has little ability to precisely weed out harmful mutations, particularly if they happen to occur on otherwise healthy chromosomes.

Original Publication: Noble et al. (2015), Current Biology 25, 1–8
Source: http://www.nyu.edu/about/news-publications/news/; The New York University

Cancer research

RNA glue for the protein assembly line



Lowering the level of aluRNA induces the dispersion of nucleolar compartments into smaller nucleolar domains, which are less efficient. Increasing the level of aluRNA forces fusion into larger and more active nucleolar domains. *© dkfz.de*

Scientists from the German Cancer Research Center (DKFZ) have discovered how RNA molecules regulate the structure of the nucleolus and drive the synthesis of the cellular machinery needed for protein production. When cells grow and divide rapidly, they need to run up the production of proteins. The cellular machinery for this task is synthesized and assembled in a special compartment of the cell nucleus called the nucleolus. The nucleolus constantly adapts its shape if the cell needs to produce more or less protein. Accordingly, fast dividing tumor cells often have bigger nucleoli.

Original Publication: Caudron-Herger, M. et al. (2015) EMBO J., doi: 10.15252/embj.201591458
Source: http://www.dkfz.de/en/presse/ German Cancer Research Center, Heidelberg

RNA Stability

'Bodyguard' Protein Helps Protect Young RNAs

RNA molecules perform key functions in the cell. They transmit genetic information as blueprints for proteins and fulfil other essential tasks. For this purpose, they undergo a multi-step process during which they must be protected from premature degradation. Scientists from the Max Planck Institute (MPI) of Biochemistry in Martinsried have now identified a new mechanism that stabilizes specific RNA molecules and guides their processing. "The molecular complex associated with the protein NCBP3 we identified is primarily important in cellular stress situations such as virus infections," says Andreas Pichlmair, research group leader at the MPI of Biochemistry. "Our findings may open up new therapeutic approaches in the future."

Original Publication: Gebhardt, A. et al. (2015) Nature Communications 6, 8192 DOI: 10.1038/ncomms9192
Source: http://www.biochem.mpg.de/5164404/20150918_pichlmair_ncbp3; MPI of Biochemistry, Martinsried

Antibiotic resistance

Novel Theoretical Approach to Reduce Antibiotic Resistance

It is estimated that each year in the United States 2 million people become infected with bacteria that are resistant to one or more types of antibiotics, and at least 23,000 people will die because of these infections. This problem is being exacerbated by overuse of antibiotics for livestock and also in community clinical practice. This overuse, combined with the slow pace of novel drug discovery is a growing threat to public health. In response to this, Moffitt Cancer Center researchers have developed a novel mathematical method inspired by Darwinian evolution to use current antibiotics to eliminate or reduce the development of antibiotic-resistant bacteria.

According to the Centers for Disease Control, one of the core actions that can be taken to fight antibiotic-resistant infections is to improve the use of antibiotics that currently exist. One approach to achieve this is by using different combinations or sequences of antibiotics; however, given the high number of antibiotics in existence, it would be extremely difficult to experimentally identify the best combination or sequence of drugs.

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molecular pharm

Behind the doorman's back

How biopharmaceutical drug substances use the nose as a pathway to the brain

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According to WHO estimates, over 1 billion people worldwide suffer from disorders of the central nervous system (CNS). Some of the most familiar are Alzheimer's, Parkinson's and multiple sclerosis. Although they have long been the focus of research, effective pharmacotherapies are still unavailable for many of these diseases. What is it about CNS disorders that makes it especially difficult to develop drug products that target pathophysiological mechanisms – so as to retard, halt, change or even reverse them? What new solution strategies are available in this field of research?

The first major obstacle to the development of new active ingredients for the treatment of CNS disorders is presented by the pathophysiological mechanisms that underlie the various diseases. Mechanisms for CNS disorders are often complex and in many cases (such as Alzheimer's) remain inadequately elucidated to date. There are, however, several highly promising candidate compounds such as insulin that significantly improve cognitive performance for Alzheimer's in animal models. To be able to assess these potential drug substances in terms of their use in treating human patients in clinical trials, we need a practicable system that facilitates the safe transport of these candidate compounds in their biologically active form to their site of action.

Strict exclusivity

Depending on drug substance structure, this can often be a difficult undertaking, however: just like a club with VIP access, the human brain/CNS is an extremely exclusive location. To avoid this physiologically sensitive organ being affected by undue turmoil and general commotion, the body uses a careful process of selection to

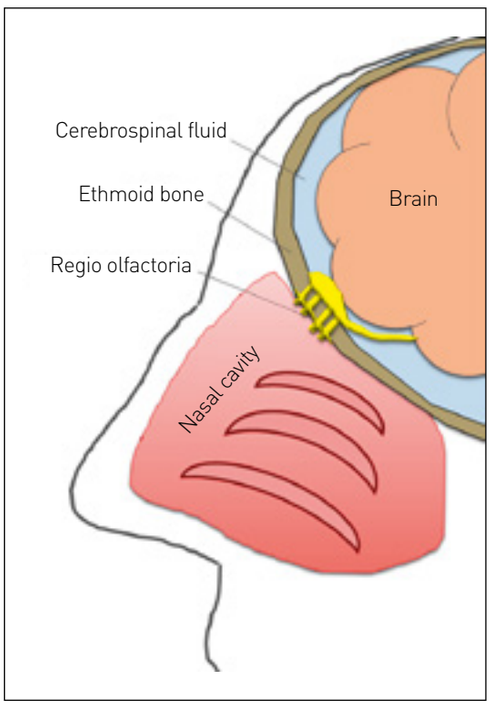

Fig. 1 Section through the human skull, illustrating the *regio olfactoria*

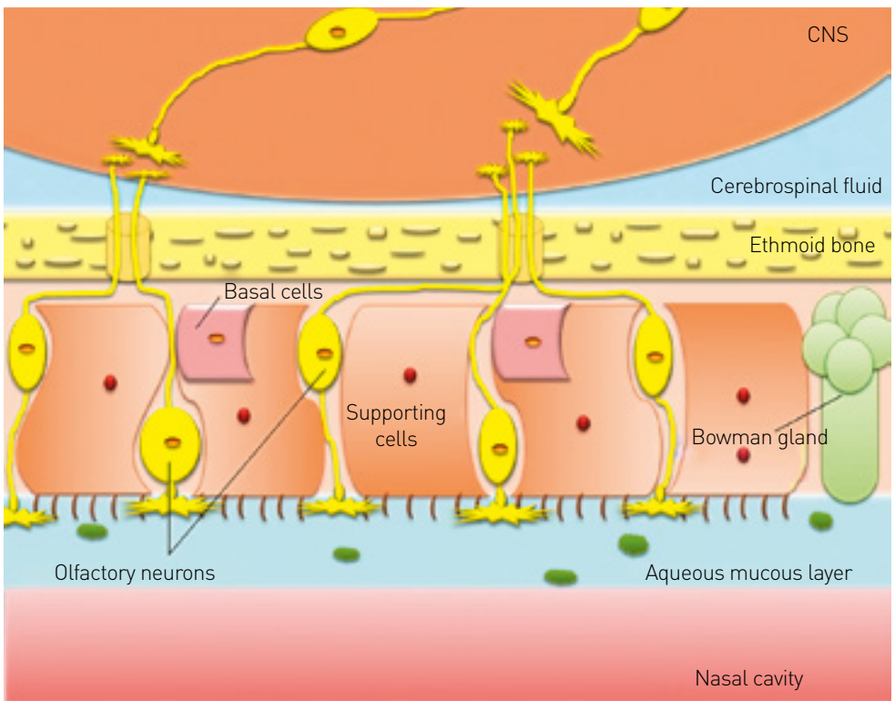

Fig. 2 Structure of the olfactory epithelium and the *regio olfactoria*

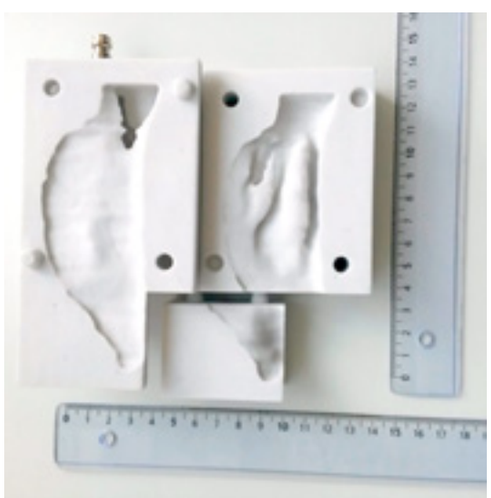

Fig. 3 Section through a 3D print of the nasal cavity

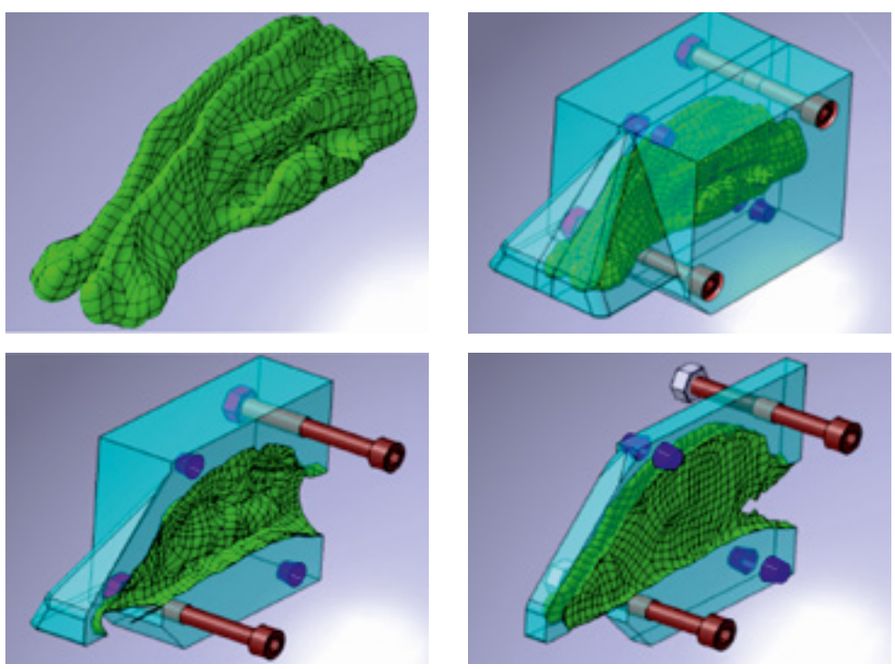

Fig. 4 Computer simulation of the human nasal cavity
(Rolf Pfäffle, Fa. Beiter)

decide which molecules are granted access and which have to "wait outside". The bouncer employed by this exclusive club is the blood-brain barrier (BBB). This is a physiological barrier between the circulating blood and the central nervous system. As a doorman, the BBB has the job of letting in "staff" (proteins such as transferrin) and "paying guests" (nutrients such as glucose) while escorting "patrons who've had enough for today" (degradation products such as CO_2) out of the brain. All of this keeps the brain's VIP bouncer very busy.

While its colleagues (the capillary tubes at the body's periphery) are constructed of endothelial cells equipped with openings (described as being "fenestrated") and intracellular gaps – thus permitting them to allow dissolved and suspended molecules to pass much more easily – the blood-brain barrier consists of a continuous epithelium that is especially tightly sealed. This continuous epithelium presents an impenetrable barrier to most of the molecules transported in the bloodstream. For selected molecules, specialized transport proteins enable an exchange of substances between blood and the CNS.

For a candidate compound who is new in town – and who therefore doesn't have a transporter to call on – it is very difficult to gain access to the exclusive CNS VIP Club. But it's not impossible. Small, lightweight molecules – the "size zero models" among molecules – in particular have a good chance of slipping by, especially if they (unlike their human counterparts) are lipophilic, i.e. easily dissolved in fats (e.g. antidepressants). Big and heavy molecules that are hydrophilic, i.e. easily dissolved in water, have little chance of making it past the bouncers. And in cases where we can see from afar that these big molecules carry a charge, their chance of being waved through by the doormen is drastically reduced.

This state of affairs is especially frustrating when we recall how many new and innovative drug substances are being produced in the field of biopharmaceuticals alone. Products manufactured using biotechnology include, first and foremost, therapeutic proteins such as hormones (e.g. insulin), growth factors (e.g. erythropoietin, "epo") and therapeutic antibodies. Of these, therapeutic antibodies are now a primary focus in drug development: as a key element of the immune system, they exhibit high-affinity binding – the "lock-and-key" principle – to pathogens, for example, and thus help to eliminate them.

The nose as a backdoor

Since biotechnological techniques allow us to design the structure of antibodies so that they can specifically recognize almost any molecule, they have the potential to offer targeted therapies for a wide variety of disease mechanisms while causing few side effects on account of their specificity. The problem with these therapeutic proteins is that they belong to the group of macromolecules for which the blood-brain barrier presents an insurmountable obstacle. Yet the obstacle can be bypassed instead of tackled head-on.

Alongside its front door, the CNS VIP Club also has a kind of rear entrance, namely the nose – or the regio olfactoria, to be more precise (Fig. 1). This region is located right at the top of the nasal cavity. At this location, the brain and the (cerebrospinal) fluid that surrounds it is separated from the outside world by only a single bone (the ethmoid bone) and a few layers of cells (the olfactory epithelium) (Fig. 2). Since the ethmoid bone is pervaded by nerve fibers, this barrier can be overcome by a wide variety of drug substances, including therapeutic proteins. This has already been demonstrated by several groups of researchers.

The research group in the Institute of Pharmaceutical Biotechnology at Biberach University of Applied Sciences, working together with Prof. Mavoungou and Prof. Schafmeister under the direction of Prof. Katharina Schindowski, has set itself the task of developing an intranasal drug delivery system that is capable of introducing drug substances in their biologically active form and with demonstrable kinetics into the brain via the regio olfactoria. The aim of this research project is to develop a system that enables the safe transportation of various active ingredients targeting disorders of the CNS to their site of action, and thus open the (back) door to the treatment of a wide range of illnesses. One well-known intranasal drug delivery system is the nasal spray, which turns drug substances into a fine mist, allowing them to penetrate deep into the nasal cavity as gas-borne aerosols. While this system works brilliantly for the small chemical molecules intended to reduce inflammation in the nasal mucosa during colds or allergy attacks, for example, it cannot be adapted to drug substance heavyweights like proteins quite so easily – since the rear entrance to the CNS VIP Club is tucked away on the roof of the nose.

First simulate, then formulate

Such particle flows through the nasal cavity are now being simulated in collaboration with the University of Ulm and Ulm University of Applied Sciences. The simulation data is intended to produce key findings such as the optimum particle size, for example. The simulations show

From left to right:

Stefan Carle studied pharmaceutical biotechnology at Biberach University of Applied Sciences. For his master's dissertation, he is using a model of the nasal epithelium to study the transport of biopharmaceuticals.

Martina Stützle studied pharmaceutical biotechnology at Biberach University of Applied Sciences. She is a doctoral candidate in the Prof. Zimmermann lab in the Institute of Pharmaceutical Biotechnology at Biberach University of Applied Sciences. Her thesis topic is the "Development of protein aerosols for intranasal nose-to-brain drug delivery".

Katharina Zimmermann studied biochemistry and received her doctorate in 2001 on biomarkers in Alzheimer's disease. This was followed by positions at the University of Heidelberg and at Sanofi-Aventis in France. She led an independent group of researchers at Inserm (Lille, France) until 2007, followed by a position at Boehringer Ingelheim Pharma. In early 2010, she accepted a professorship in Molecular Pharmacology at Biberach University of Applied Sciences.

Johannes Flamm studied pharmacy in Freiburg and has been a doctoral candidate since 2015 in the Prof. Katharina Zimmermann lab in the Institute of Pharmaceutical Biotechnology at Biberach University of Applied Sciences. His thesis topic is "The intranasal nose-to-brain transport mechanism and approaches for the controlled application of active ingredients by means of intranasal delivery".

that very small aerosol particles must be generated if we intend to penetrate into the regio olfactoria. This simulated experiment was repeated in the lab with a 3D printed model of the human nasal cavity: the lab experiment confirmed the finding (Figs. 3 and 4). This tells us that the rear entrance is not merely well-hidden: the drug substances must also be packed in vehicles for transport that are small enough to navigate the arduous path to the back door.

The particle size of such transport vehicles can be controlled with a range of techniques for atomizing the solution or altering its viscosity. Alongside the familiar pump-action nasal sprays, aerosols can also be generated with ultrasound, propellants or vibrations.

To create an aerosol, energy is introduced into the medium we want to turn into a mist. Unfortunately, this procedure is sub-optimal for highly sensitive protein substances, since the force required often destroys the crucial spatial structures that the proteins need to function. By modifying the formulation (i.e. the liquid in which the proteins are dissolved), however, we have managed to identify formulations for a range of proteins that are appropriate for protecting the proteins from destructive forces during the process of aerosol generation.

Our next goal is now to develop a system that allows a therapeutic drug substance to reach sufficient concentrations at the site of the regio olfactoria and within the CNS. A number of obstacles must still be overcome. In this context, one important goal for the intranasal application of therapeutic proteins is to ensure their release in a way that does not activate the immune cells present in the nasal mucosa. This would effectively vaccinate the person against the drug substance administered.

If you want to get into the CNS VIP Club by the back door, it's best to be quiet so as not to alert the security guys. There's still a lot to do – but anyone wanting a seat in the CNS VIP Lounge has to work hard to earn it.

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profile

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polysaccharide ch

Genes on sugar

Developing bio-based carrier systems for gene transfer

Prof. Dr Dagmar Fischer[1,2] and Prof. Dr Thomas Heinze[2,3]

[1]Institute of Pharmacy, Faculty of Biology and Pharmacy, Friedrich Schiller University Jena
[2]Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena
[3]Institute of Organic Chemistry and Macromolecular Chemistry, Center of Excellence for Polysaccharide Research, Friedrich Schiller University Jena, Germany

The targeted transport of DNA and RNA using vectors (mostly made from synthetic polymers) in cell cultures has become part of routine practice in biological R&D – a fact highlighted by the multitude of commercial kits now available. To date, however, obstacles relating to use in patients have beset many laboratory studies and – in particular – the transfer to clinical practice. In many cases, such issues are related to safety concerns and limited biodegradability of such carriers. At FSU Jena, new bio-based and natural systems using polysaccharides are being developed with the aim of solving these specific problems.

Vectors used to transfer DNA and RNA must be "all-rounders", capable of performing a wide range of discrete tasks. These include maintaining the stability of their nucleic acid "cargo", achieving an efficient and selective uptake in the target cells, and with high efficiency (transfection) while simultaneously ensuring excellent tolerability. Particularly in terms of use in patients, both their safety and their biocompatibility must be assured at all times [1].

Dextrans: familiar sugars, rediscovered

Dextrans have been successfully deployed in pharmacy and medicine for many decades now as plasma expanders, tableting excipients, and stabilizers for colloidal formulations: both their toxicology and biodistribution are therefore adequately well-studied. The macromolecular biopolysaccharides are formed biosynthetically as a branched or unbranched molecule in sucrose-rich media by the activity of the dextransucrase enzyme, which is produced from various *Leuconostoc* strains. Dextrans are degraded in the organism and metabolized in the body [2]. Since they are not themselves capable of interaction with DNA and RNA, the logical next step was the covalent binding of cationic pendant groups to accomplish an electrostatic interaction with the negatively charged DNA and RNA [3]. To date, however, such side chains have often consisted once again of synthetic polymers rather than biogenic functionalities. Such polymers are neither biodegradable nor excretable and occasionally exhibit high toxicity.

Sugars and amino acids: two natural partners

In contrast to the above, introducing naturally-occurring functionalities with amine and ammonium groups appeared to be a highly promising approach. Work at Jena on polysaccharide chemistry has resulted in the development of efficient reactions under moderate conditions that permit the introduction of almost any carboxylic acid into polysaccharides [4, 5]. One pioneering achievement was the reproducible production of dextran amino acid esters with controllable molar mass and configurable content (average degree of substitution, DS) for the ester substituents – a synthesis variant that can easily be upscaled. In this approach, amino acids are activated with commercially available N,N-carbonyldiimidazole and undergo a homogeneous reaction to produce the corresponding dextran derivatives with a high level of purity and without appreciable polymer degradation (the average degree of polymerization (DP) of the initial dextran is preserved) or toxic byproducts. Based on this synthesis model, we gain access to a library of mono- and multifunctional dextran amino acid esters, which can also act as carriers of other functional groups: a construction kit for a practically unimaginable number of dextran derivatives offering considerable structural diversity. Products selected to date have exhibited outstanding characteristics as DNA transporters and interest is currently focused on ß-alanine-substituted dextrans.

Alanine-substituted dextrans as DNA transporters

Alanine-substituted dextrans are excellent water-soluble, can be stored permanently at room temperature following freeze-drying, and can be reconstituted quickly and completely in buffers and cell culture media. In standardized toxicity tests (according to DIN ISO 10993-5) using L929 mouse fibroblasts, they exhibited outstanding levels of tolerability that

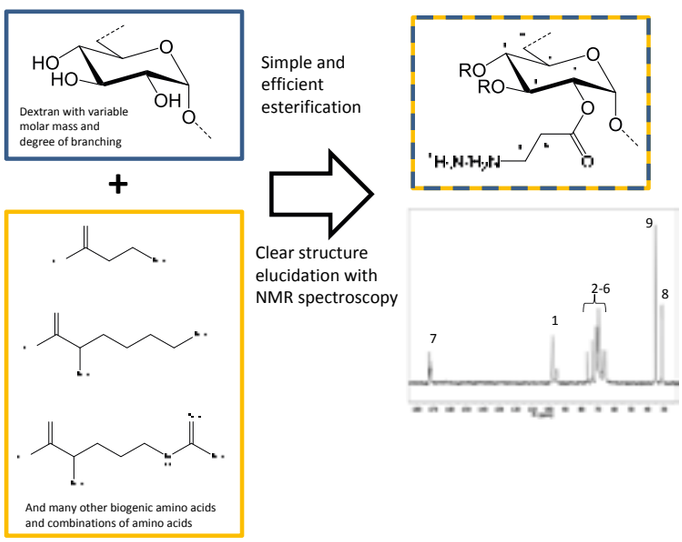

Fig. 1 Development of a dextran amino acid ester library with controllable, high-quality characteristics

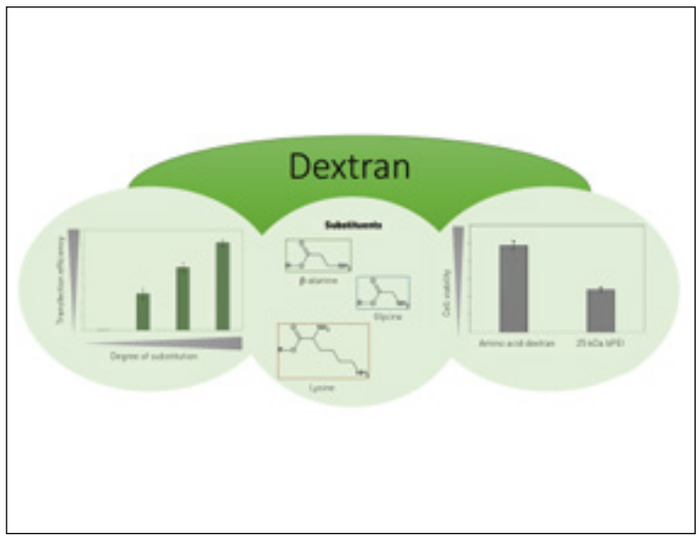

Fig. 2 Amino-acid substituted dextrans as biocompatible and highly efficient transfection systems

Dagmar Fischer studied pharmacy before then obtaining her doctorate in pharmaceutical technology and biopharmacy from the Philipps University of Marburg in 1997. After a period spent at Texas Tech University Health Sciences Center (USA), she gained several years' experience as Director of Preclinical Research & Development at Antisense Pharma GmbH before accepting a professorship in Pharmaceutical Technology at Friedrich Schiller University Jena in 2008. Her work concentrates on the development of nanoparticulate carrier systems from natural and synthetic polymers with an especial focus on inflammation, infection and cancer.

Thomas Heinze studied chemistry at FSU Jena. After receiving his doctorate there in 1985 and subsequent postdoc work, he completed his habilitation at KU Leuven (Belgium) in 1997. In 2001, he accepted a professorship in Macromolecular Chemistry at the University of Wuppertal (Germany). He has held a chair in Organic Chemistry at FSU Jena since 2002, where he is the Director of its Center of Excellence for Polysaccharide Research. His research focuses on the development of polysaccharide materials for applications in biology, medicine and technology.

were higher by a factor of two than those for known synthetic polymers such as the commonly used polyethyleenimines [6]. If one investigates interactions with blood cells such as erythrocytes, which are the first contact partners within the organism following injection, no hemolytic effects or aggregations of blood cells – i.e. no thrombotic events – are observed in any therapeutically relevant quantities.

The simple pipetting of DNA or RNA into the solutions of alanine-substituted dextrans causes the spontaneous formation of complexes that are deployable within a few minutes. With an overall cationic charge and a size of approx. 100 nm under optimum conditions and batch ratios, the complexes meet all of the requirements for good uptake via the cell membrane. They can be deployed in serum-rich media and protect the nucleic acids against enzymatic attack from nucleases in blood just as well as they stabilize against the uncontrolled and undesirable displacement of the nucleic acids by serum proteins. They are not only capable of transfecting mammal cells but have the unusual property of causing no effects of toxicological concern even at high doses.

What does the future hold?

Modification of the complexes based on dextran-alanine for the intended application cannot be achieved merely by varying the production conditions and varying the formulation of the complex. The model can be re-used for a range of cationic amino acids and their combination. Properties such as the binding strength of the complexes with DNA and RNA, stability, toxicity and transfection efficiency can be controlled by the selection and combination of various types of amino acid. While lysine is more advantageous for binding DNA, for example, an excess of alanine has an especially positive effect on the transfection of cells. To sum up, the new synthesis strategy offers numerous approaches for structural variation. Amino acid-modified dextrans offer a simple way of bypassing numerous obstacles such as poor biodegradability, unacceptable toxicity and time consuming instructions for synthesis experiments.

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Center of Excellence for Polysaccharide Research Friedrich Schiller University Jena

The Center of Excellence for Polysaccharide Research is a leading research institution originally formed by six multinationals at the Friedrich Schiller University Jena and the Thuringian Institute of Textile and Plastics Research (TITK) at Rudolstadt in 2002, and which received financial support from this consortium of companies until 2007. The Center's activities focus on joint R&D work on polysaccharides as functional raw materials for the future. Products and methods are investigated and developed both within basic research and application-oriented research. Here, the Center pursues various strategies of biopolymer derivatization under homogeneous and heterogeneous reaction conditions, and of regioselective functionalization. The systems available also permit methods to be upscaled to the level of the process laboratory. The work of the Center also guarantees the provision of initial and advanced training to students in the field of bio organic chemistry.

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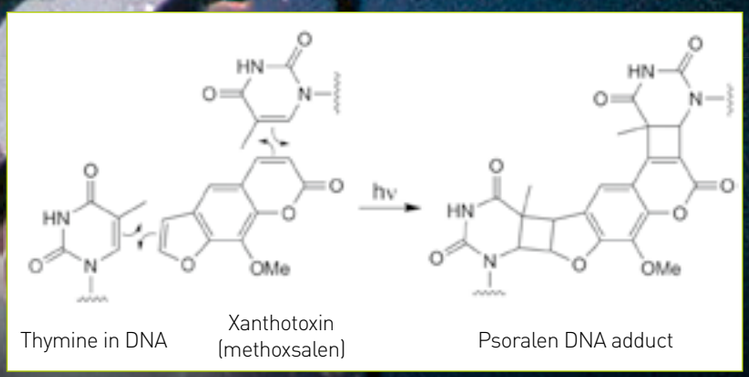
Heribert Warzecha is Professor for Plant Biotechnology and Metabolic Engineering at TU Darmstadt.

Rock around the Hog(weed)

Prof. Dr Heribert Warzecha



Methoxsalen cycloaddition reactions with DNA thymine bases, leading to covalent interstrand cross-linking.

When rock stars grab hold of a mike, they usually sing about love in all its many shades and meanings. Or they give us their take on the latest developments in politics and society at large. Plants have a very minor role to play in their lyrics – at most serving to set the scene for the first topic mentioned (that of love) with their flowers. On the other hand, they can also serve as the source of mind-expanding substances whose effects are then reflected on at considerable length (see Eric Clapton's cover of "Cocaine" from 1977).

In 1971, however the well-known rock band Genesis published a song that dealt with a quite different topic: the introduction of an invasive plant species into Europe and the various problems that this proceeded to cause. "The Return of the Giant Hogweed", a track on the album "Nursery Crime", also provides copious details of the toxic principles behind its constituent chemicals as well as options for controlling this invader. Although the song is now almost half a century old, its words are – perhaps unlike the musical style in which it was written – as relevant as ever.

It's certainly true that the giant hogweed (Heracleum mantegazzianum) – also known by the colorful names of "hogsbane" or "cartwheel flower" – was brought to England (initially to Kew Gardens in London) from the Caucasus in 1817. Viewed as a decorative plant that readily attracted bees, it was planted by many a gardener in Victorian England, but quickly ran wild and is now found throughout Europe and even in North America. The spread of this invasive species, especially along riverbanks and roadsides, results in the displacement of native species and itself constitutes a major ecological problem. But the giant hogweed has another trick up its sleeve: growing as much as 5 m tall, every part of the plant contains toxic constituents that pose an immediate threat to both humans and animals. As with many plants in the Apiaceae (or parsley) family Heracleum mantegazzianum contains linear furanocoumarins (psoralens, see Figure). These chemicals have the property of triggering a condition called phytophotodermatitis when exposed to UV light. If parts of the skin come into contact with the plant sap and are then exposed to sunlight, the affected skin reddens and forms blisters after only 15 minutes. The blistering is so extreme that it often resembles burn injuries. Even after healing – which often takes several weeks – scars remain, accompanied by areas of hyperpigmented skin.

The effect can be explained by the fact that psoralens like xanthotoxin can intercalate very well into DNA on account of their planar structure. Activation by long-wave UV radiation then triggers a cycloaddition reaction between the DNA's pyrimidine bases and the furan and the adjoining pyron ring of the furanocoumarin. This cross-linking of the DNA strands is the cause of the multifarious toxic reactions at the cellular level.

Nor are cases of phytophotodermatitis limited only to contact with giant hogweed: many other members of the parsley family can also cause similar reactions – as can citrus plants. The substances found in these species, such as bergapten, then trigger the typical dermatitis symptoms. As one example, many perfumes used to contain large amounts of this substance, as a constituent of aromatic bergamot oil. Elegant ladies who sprayed themselves liberally with perfume and then exposed their décolleté to the sun frequently had to cope with Eau de Cologne or berloque dermatitis (skin hyperpigmentation).

Despite all of these detrimental effects, however, the phototoxic substances also have their uses in medicine. In what is known as "PUVA" therapy (psoralen + UVA), patients with vitiligo (depigmentation of the skin) or psoriasis are given methoxsalen and the affected area of the skin is then subjected to UVA radiation. As long as treatment is applied very precisely, the results can be very positive.

So we can see that the giant hogweed is not all bad – and perhaps that was Peter Gabriel's message all along. On the other hand, containment of the uncontrolled spread of the plant has had to be practiced for a great many years, in order to avoid inadvertent and painful "encounters" with the plant.

At the end of the day, we can only guess at the reasons why the group chose to deal with this very un-rocker-like topic. But perhaps their work will inspire others. Let's hear it for the chamomile ballad – the laxative blues!

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The Return of the Giant Hogweed lyrics

Songwriters: GABRIEL, PETER / BANKS, ANTHONY/ HACKETT, STEVEN / RUTHERFORD, MICHAEL
Recommended listening: www.spotify.com, www.youtube.com

Picture: © wikipedia.com | Rodhullandemu

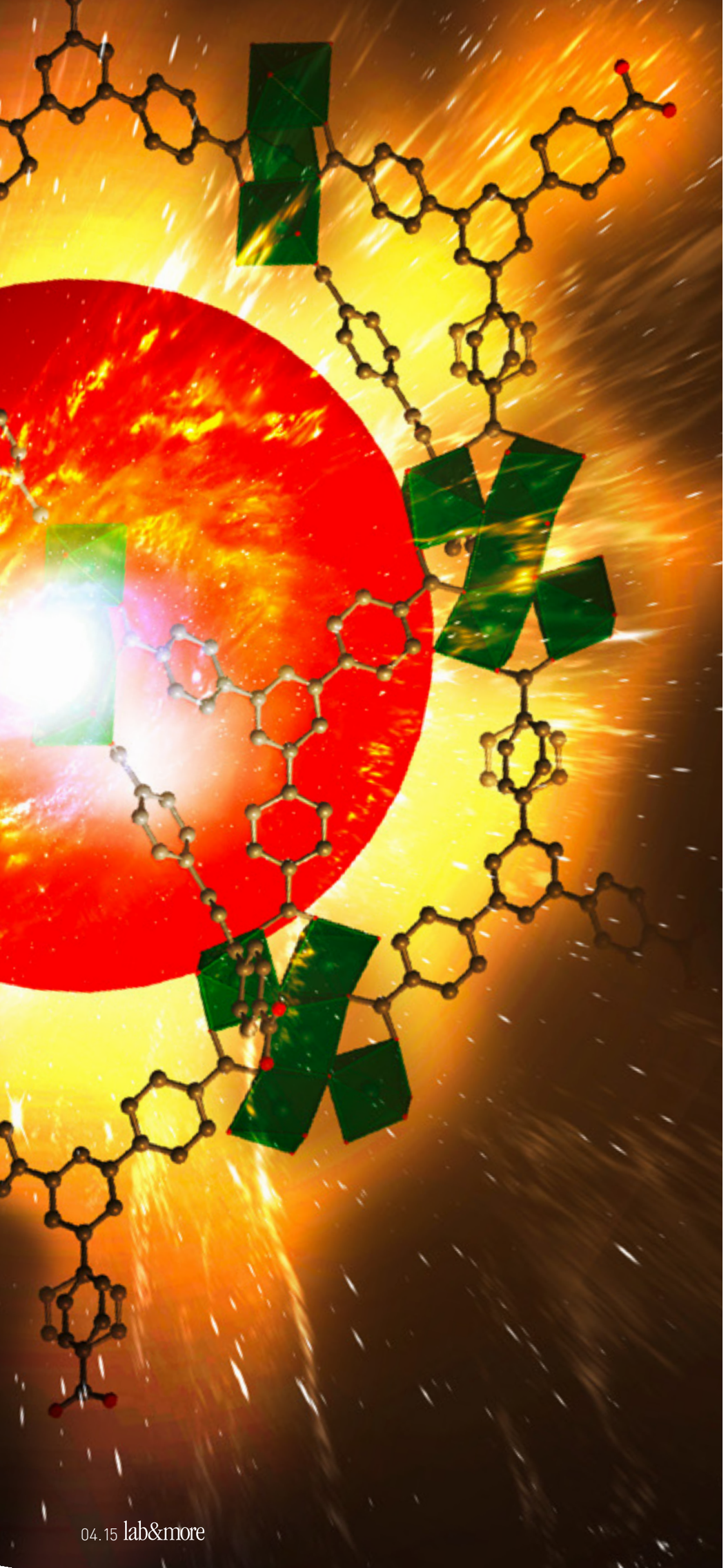
MOFs

Chemical Research in Germany

The search for endless emptiness

Metal-organic frameworks: record-breakers in porosity

Prof. Dr Stefan Kaskel
Inorganic Chemistry, TU Dresden, Germany



In recent years, metal-organic frameworks have been setting one record after another in relation to specific surface area, with over 7,000 m^2/g now being achievable. Their modular construction and the large number of functionalities that can be built into the lattice make them both interesting and highly promising for a wide variety of applications.

Metal-organic frameworks: Lego building blocks for inorganic chemists

The structure of metal-organic frameworks (MOFs) is deceptively simple: one simply reacts a di- or tricarboxylic acid – such as terephthalic acid – with transition metal salts such as zinc or copper nitrate. In the solution, inorganic clusters then form such as Zn_4O clusters or $Cu_2(OOC\text{-})_4$ paddle-wheel units (Fig. 1) [1–3], which function as nodes and position the carboxylic acid molecules at the corners of highly symmetrical polyhedra (octahedra, cubes). Due to the bi- or trifunctional structure of the carboxylic acids (linker molecules), the clusters can now be cross-linked endlessly in three dimensions. This structural principle, a pioneering discovery made by Omar Yaghi, Susumu Kitagawa and Gérard Férey, has resulted in a multitude of new compounds. These compounds exhibit extremely high porosity, tailor-made pore sizes and functional groups on the inner surface, which can be utilized by a wide variety of applications [4]. Today, over 10,000 metal-organic frameworks are now known. The current record is now approx. 7,000 m^2/g for the inner surface (BET) – comfortably exceeding the specific surface areas achieved for conventional adsorbents (activated charcoal and zeoliths).

The first materials were synthesized at the end of the 1990s. Today, we can now say that the number of metal-organic frameworks has virtually exploded. After about 15 years of academic research, the first potential applications are now starting to take shape in a wide variety of fields, including energy storage, gas separation techniques, and catalysis, etc. A number of startups have also been formed in the meantime: these are now offering metal-organic frameworks for research purposes or as samples for industrial use.

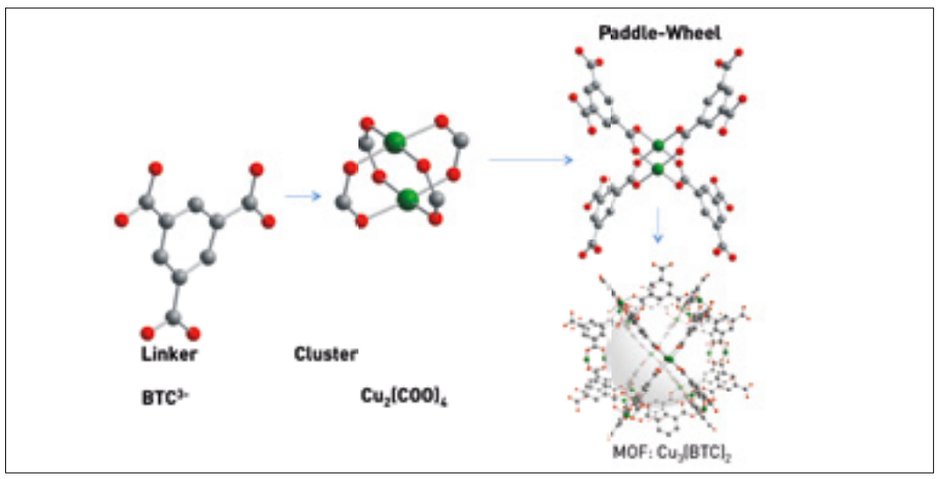


Fig. 1 Modular structure of MOFs using $Cu_3(BTC)_2$ as an example

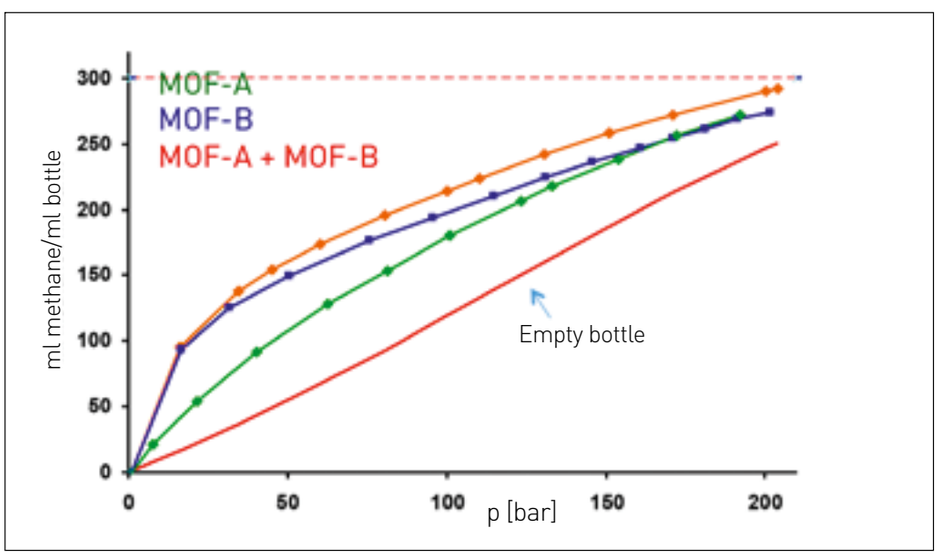


Fig. 2 Methane charge curve for MOF-filled fuel bottles (at 20 °C)

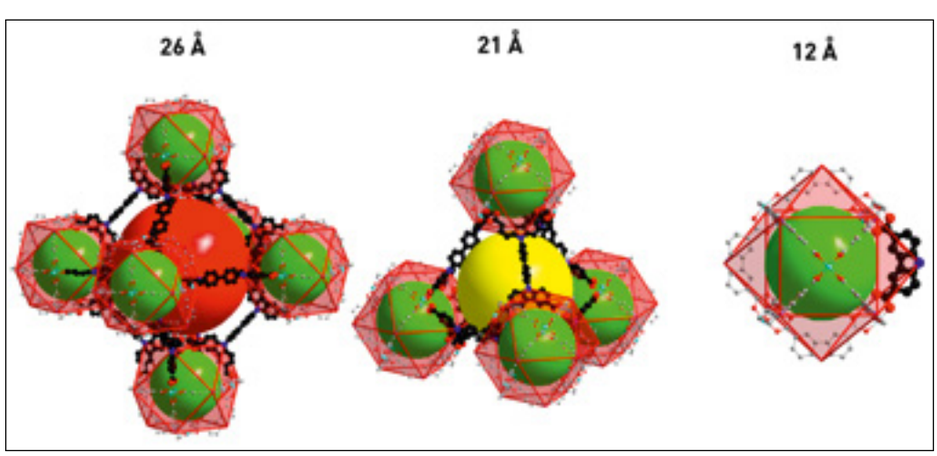


Fig. 3 Multimodal pore structure in DUT-49 (DUT = Dresden University of Technology), with pore size specified

Applications in energy storage

The use of MOFs in energy storage systems for gases such as hydrogen and methane was postulated at an early stage [5]. Since hydrogen can be stored only at low temperatures (-195 °C), however, MOF-based hydrogen storage remains a long-term research topic. In contrast, methane (natural gas) can be stored at room temperature and pressures between 30 and 100 bar [6–10]. This offers significant advantages in terms of capacity: by using a MOF-based storage system, up to three times the volume of methane can be stored in comparison to an empty compressed gas cylinder (Fig. 2). One such MOF, DUT-49, is currently one of the record holders in terms of gravimetric methane storage capacity (Fig. 3) [7]. Despite this, numerous challenges must still be met in turning this kind of MOF storage into marketable systems. As one example, filling generates large quantities of heat (enthalpy of adsorption) that need to be dissipated. Here, suitable thermal management models need to be developed. Volume markets are also difficult starting markets for new materials, due to intense price pressure. BASF is currently pioneering this commercialization work, having developed prototype vehicles that use the kinds of MOF-based natural gas systems described above. In this context, markets that envisage a distribution structure at the medial pressure range (60 bar) are especially attractive. The market for natural gas-powered vehicles is also gaining ground in the US, where shale gas production has resulted in low gas prices.

Moreover, the large volumes of heat generated by adsorption processes can also be used for latent heat storage. Such systems are currently being deployed in air-conditioning units and heat pumps (Fig. 4) [11–13]. Although these applications typically use zeoliths and SiO_2 materials, MOFs are also ideally suited for such uses, due to their high water adsorption capacity. In addition, the partial pressure (relative air humidity) at which the MOF stores or releases water can be regulated in a flexible manner by configuring the pore sizes and integrating hydrophobic groups on the inner surface. This can be used for the effective optimization of energy needs.

Gas scrubbing and separation

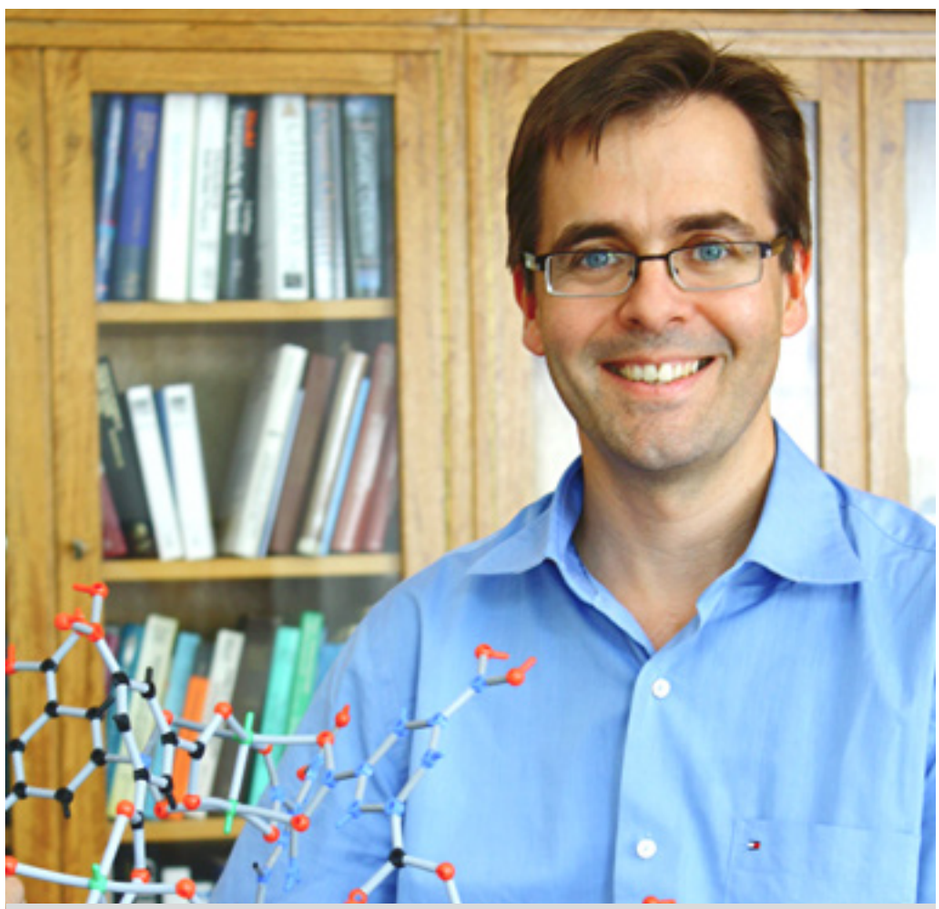
The modular configuration of pore size and functional groups is a key benefit offered by MOFs. This approach can be used to remove certain molecules very selectively from mixtures of gases [14]. Two good examples include the removal of sulfurous compounds (e.g. thio-

phene) from fuels or hydrogen sulfide from air [15, 16]. Such separation techniques are important not only in the purification of fuels and scrubbing of breathable air but also for industrial processes. The use of MOF materials has even been proposed for the implementation of more demanding industrial separation techniques such as propane/propene separation – a task that consumes vast amounts of energy as a distillation process. Initial work here has shown that certain materials are in principle suited for use in implementing such separation processes, due to variations in the speeds at which they absorb propene and propane [17–19].

MOFs could also be deployed in gas filters used for scrubbing toxic gases. This involves integrating MOFs into textiles – by electrospinning, for example (Fig. 5).

MOF sensors and catalysis

MOFs are highly suited for integration into sensors, since their properties – such as color on molecule absorption, or even the actual weight change – can be modified in predefined ways [20, 21]. Thanks to these qualities, they can be used to monitor room air or can even support long-term humidity monitoring within an industrial environment. Research teams have been deploying MOFs as catalysts for a long time: the possibility of anchoring a wide variety of materials in their hierarchical structures is paving the way for numerous potential applications in catalysis. Details of rhodium-based MOFs have now been published [22], which are suitable for hydrogenation. Palladium-based MOFs also exist. Notwithstanding such clear-cut applications, it can still be said that our understanding of the catalytic activity is modest at best. Securing a real grasp of this activity – and thus the targeted use of catalytically active centers in MOFs – can be achieved only if the defect chemistry of these materials can be understood and utilized. Accordingly, we may assume not only that numerous defects are present in MOFs on account of missing metal centers, but that metal centers can be formed that exhibit deviations from the ideal geometry. One problem arises from the fact that catalytically active metals often change their coordination sphere during a catalysis cycle. This is not possible if they are part of a rigid network. Yet if they change their coordination, the network collapses. This inherent problem again shows that catalytically-active centers can be installed only as defective imperfections or foreign atoms. An alternative approach is to use the functional groups of linkers to



Stefan Kaskel studied chemistry at the University of Tübingen, where he also obtained his doctorate in 1997. This was followed by postdoctoral work in the Ames Laboratory at Iowa State University under John Corbett. He then worked as a principal investigator in the field of developing new porous materials at the Max Planck Institut für Kohlenforschung under the directorship of Ferdi Schüth. After receiving his professorial teaching qualification at Ruhr-Universität Bochum in 2004, he accepted a C4-grade professorship in inorganic chemistry at TU Dresden in the same year. Since 2008, he has divided his time between this position and management of the Chemical Surface and Reaction Technology business unit at Fraunhofer IWS in Dresden. His primary research interests are in the fields of porous materials (MOFs, carbon materials, mesoporous materials, zeoliths), nanomaterials, battery materials (with a focus on the field of lithium-sulfur batteries) and coating technologies. At Fraunhofer IWS, development work also includes plasma technologies, CVD systems, and roll-to-roll production methods.

Fig. 4 MOF-coated heat exchanger
Picture: DECHEMA MOF Roadmap

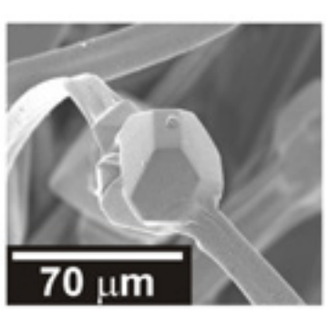


Fig. 5 MOF integration into fibers using electrospinning

coordinate other metals. This model has been used to generate highly-selective and also highly active enantioselective catalysts [23].

Today, the scaling-up of MOFs can be considered unproblematic. At the turn of the millennium, milligram quantities of MOFs were being synthesized as single crystals (Fig. 6). Contemporary processes can synthesize many MOFs at the 10 kg scale while also allowing geometry to be specified (spheres, monoliths, etc.) [24]. While prices are still relatively high due to the small volumes of materials produced, it is foreseeable that the price can be brought under EUR 100/kg once synthesis processes are being performed on a larger scale.

MOF switches

The most fascinating property exhibited by MOFs, however, is that their porosity is also switchable [25]. Accordingly, a range of materials are available with the ability to open or close their pores at certain pressures. These are also termed "breathing" MOFs: as it "breathes", all of the elementary cells in a MOF switch simultaneously from a porous, open structure to a closed structure or vice versa (Fig. 7). This process means we can start with a material that is non-porous and end up with a material that has a specific surface area of several 1,000 m^2 per g/material. These switching operations are triggered by small molecules at a specific partial pressure. This pressure-induced switching behavior is unique and is also referred to MOF "gating" [25, 26].

Outlook

The world of MOFs is currently seeing exponential growth. While work in initial years concentrated on MOF synthesis and fundamental properties, system integration and product development are now the strongest trends in the field. Recent work has focused on integration with electronic systems [27, 28]. A new German Research Foundation priority program was approved for this topic in 2015. This will also require greater efforts to be made in illuminating band structures, charge transport mechanisms and magnetic properties. And the modular structure has also inspired organic and polymer chemists to search for new approaches to constructing metal-free three-dimensional networks using the "Lego" principle. The search for new porous materials and their properties seems to know no bounds. There is still much for us to discover!

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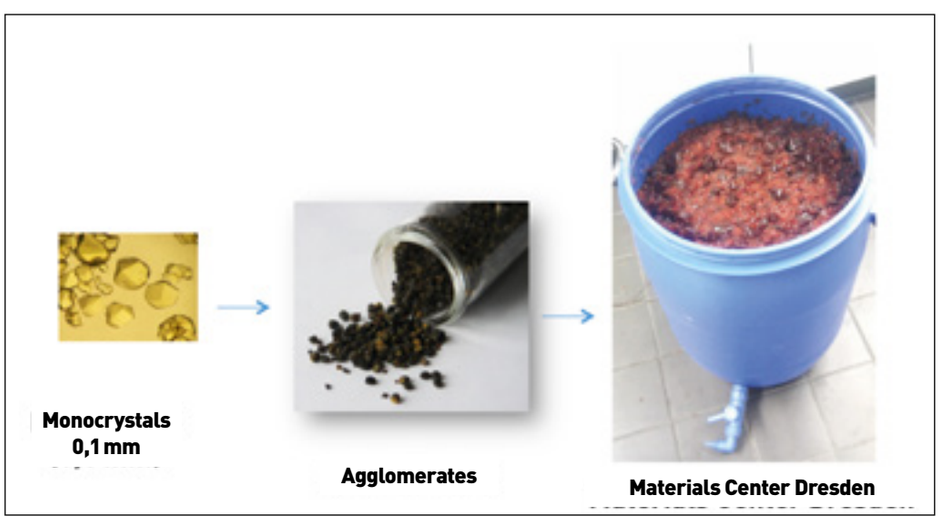

Fig. 6 Scaling-up of MOF production in Dresden up to the 10 kg scale

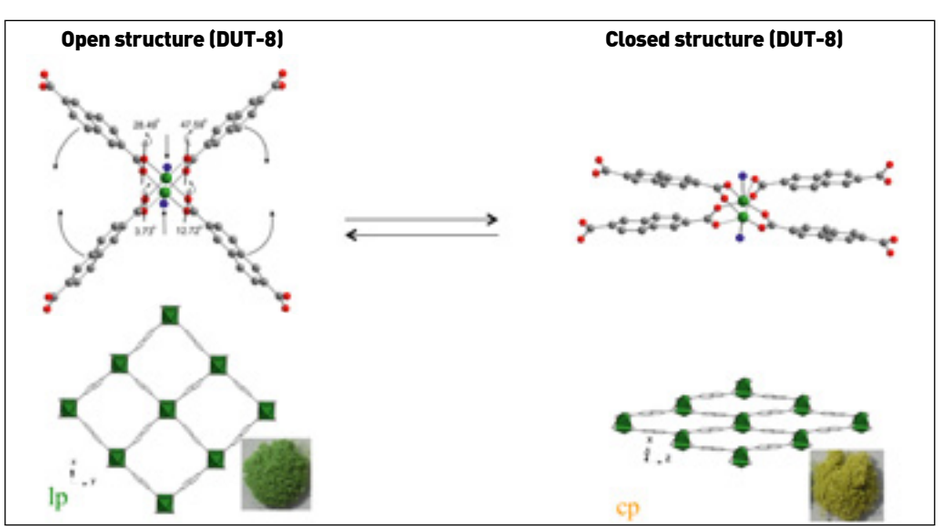

Fig. 7 Switchable MOFs can reversibly change their structure from a closed to an open state.

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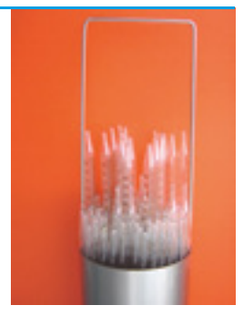

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oleosomes

Functional nanoparticles

From fat droplets in plant cells to novel foods

Dr Birgitta Zielbauer, Prof. Behic Mert, Prof. Thomas Vilgis
Max Planck Institute for Polymer Research, Mainz, Germany

Occurring naturally in oilseeds, oleosomes are particles with special properties. Depending on the plant variety, their size ranges from microns down into the nanoscale. These particles, with their protein-functionalized surfaces, are structurally very stable indeed. This makes them relevant for fundamental research and pharmacology, and also for innovative applications in food science.

Plant-derived fats: oilseeds and oil plants

What do maize, soy, sesame, palm, sunflower, olive, peanut and pumpkin have in common? They are all termed "oil fruits" in agricultural jargon and – depending on the formulation of their fatty acids – have satisfied a huge range of culinary requirements as a source of vegetable oil for many hundreds of years. The botanist makes a further distinction, distinguishing the fruit or fruit pulp (olive, palm) from the seed or seeds, depending on the plant tissue from which the oil is actually isolated. Outside the realm of botany, few details were known until recently about how, why and where plants store their oil. A large body of research on these oil reservoirs can be found for oil seeds such as maize, sunflower seeds and soybeans. These reservoirs are known as "oil bodies" in the relevant literature or oleosomes (Fig. 1). A better understanding of these oleosomes can not only explain the Herculean efforts required by oil producers to extract the oil from the oil seed but also deliver a number of fundamental insights into the function and interaction of phospholipids with proteins. The typical size of an oleosome is at the nanoscale (diameter in soybeans approx. 300 nm), and is essential for seed germination and growth. At the time of germination, when the plant is still unable to resort to photosynthesis, the stored neutral fats are broken down with enzymes into free fatty acids and ultimately monosaccharides, which are required for the construction of carbohydrates [1]. Since a smaller volume leads to an enlarged total surface area, the oleosomes offer a physically larger target to the fat-digesting enzymes (lipases). This is important, as it ensures the greatest amount of energy is available for the seedling during the short period of germination.

To biosynthesize fats, plants make good use of their huge diversity of organelles (cell organs) and their enzyme apparatus. The biosynthesis of fatty acids takes place solely in the plastids, the most familiar of which are the green chloroplasts. According to endosymbiotic theory, these developed from cyanobacteria. Accordingly, their fatty acid constructor enzymes are of prokaryotic origin (descended from bacteria). This is the reason why plants can manufacture fatty acids that are essential for humans – i.e. which the human organism cannot manufacture itself. To do so, the plant uses enzymes such as Δ-1,2-desaturase. This is a desaturating enzyme: it builds carbon/carbon double bonds into fatty acids: saturated fatty acids are turned into unsaturated ones – such as the essentially fatty acids linoleic acid (di-unsaturated) and linolenic acid

Behic Mert studied food technology at Middle East Technical University (METU) (Ankara, Turkey), graduating with a bachelor's degree. He completed his Master of Science in whey protein films in the Department of Food Engineering at Michigan State University (USA). He completed his dissertation in 2004 in the Department of Agricultural and Biological Engineering at Purdue University (West Lafayette, USA). Following this, he was Senior Research Engineer in the Rheology and Physical Properties Lab at the ConAgra Food Company (Omaha, USA). After a period as visiting researcher at Obihiro University (Hokkaido, Japan), he has been a professor in the Food Engineering Department at METU since 2006. He was a Visiting Professor at the MPI for Polymer Research in Mainz from 2014 to 2015.

Birgitta Zielbauer studied physics at the University of Heidelberg and received her doctorate there in 2007 in the field of nanotechnology. After two years as a visiting researcher in France, she has been a research assistant in the "Soft Matter Food Science" lab led by Professor Thomas Vilgis at the Max Planck Institute for Polymer Research in Mainz (Germany) since 2009. Her activities focus in particular on using the methods of material analysis to conduct research into the physical foundations of the properties and structure of foodstuffs. She also has lab management responsibilities and provides support for a wide range of experimental methods in food physics and chemistry at the MPI for Polymer Research in Mainz.

Thomas Vilgis holds a professorship from the University of Mainz and works at the Max Planck Institute for Polymer Research in the physics and chemistry of soft matter, including food systems. Vilgis studied physics in Ulm, where he also received his doctorate in polymers and elastomer systems. This was followed by postdoctoral work at the Cavendish Laboratory in Cambridge under Sir Sam Edwards. His primary field of interest is the molecular properties of food systems and the multiscale physics of foodstuffs. He is the author of many specialist publications and has authored several books on the subject of cooking and science.

(tri-unsaturated). Unsaturated fatty acids are not produced by plants merely for the convenience of the consumer or nutritional scientist, but play a key role in establishing the plant's tolerance to cold weather. Thanks to their low melting point, these fatty acids remain liquid even at low temperatures. Cold-tolerant plants store greater amounts of phospholipids with unsaturated fatty acids in their cell membrane, thus increasing its fluidity (and lowering their crystallization temperature). Quite unlike a tropical plant such as the coconut: in the coconut, the tendency of unsaturated fatty acids to oxidize means saturated fatty acids tend to be stored, and the melting point is controlled merely by chain length. Accordingly, coconut oil is a highly saturated fat, but with short fatty acids.

Thanks to insights obtained from plant physiology and cell biology, we now know how plants manage to distribute – i.e. emulsify – oil within a plant cell in the form of tiny lipid droplets (the oleosomes). In the plant cell, a nanoscale emulsifying process is effectively in place that has been fine-tuned by evolution over millions of years. At a specialized cell organelle (the endoplasmic reticulum), the ready-made fats manufactured by the enzymes accumulate together within this cell's membrane (i.e. between the phospholipid lipid bilayer). This causes the membrane to balloon out into small droplets, which can then detach from the membrane [2]. The size of the droplet is predetermined by the oleosin proteins already mentioned, which simultaneously become stored in the newly-formed oleosome.

Oleosins: special-purpose proteins

These proteins, which stabilize the individual oleosomes, are unique in both their form and function. As an extreme simplification, an oleosin is like an umbrella: the upper part of the umbrella extends into water (and so is hydrophilic) and the handle is rooted in oil (hydrophobic) (see Fig. 1). Oleosins are very stable precisely because of this strong oil "anchor": it consists of a sequence of about 70 hydrophobic amino acids, which form themselves into a "hairpin"-like structure. This sequence is the longest hydrophobic amino acid sequence known to occur naturally [2]. Another characteristic feature of oleosins is the "proline knot", which contains three prolines (an amino acid known as a "helix breaker") and forms the knot-like 180° turn of the hairpin. This medial hydrophobic sequence in the oleosins is identical in all plant seeds. Differences are in fact seen only in the edge sequences –the oleosins' hydrophilic umbrellas – known as the N-terminal and C-terminal domains. These can consist of various hydrophilic domains such as an amphipathic α-helix, for example. The charge present in the outer umbrella portion of the oleosins and the repulsion of two individual oleosomes that results is not only primarily responsible for the stability of soy milk, but also the reason why seeds can retain their power to germinate for centuries at a time.

This also explains why soy milk, tofu and soy whip are extremely efficient emulsifiers. Soy milk contains some of the strongest emulsifiers in existence: phospholipids, oleosins – i.e. proteins that contribute greatly to stability – and oil bodies, which all have their part to play at various orders of magnitude.

This means the surface of the oleosomes is naturally "functionalized": depending on the pH value of its surroundings, the surface bears either a positive or negative charge (Fig. 2).

The net charge on the surface is positive below the isoelectric point and negative when above it. Accordingly, oleosomes have a slight positive charge in the acidic range of pH values around 4 that is of culinary relevance. They form stable emulsions and can be correspondingly encapsulated, e.g. with negatively-charged hydrocolloids.

Extraction

Oleosomes are extracted from raw filtered soy milk in a multi-stage process using a centrifuge. This accelerates the formation of an oleosome "cream", which is then separated from the soy milk's remaining compo-

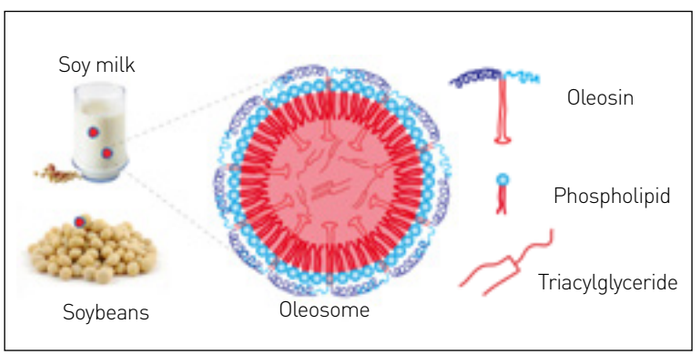

Fig. 1 Simplified illustration of an oleosome from oil seed, e.g. from soybean. Phospholipids form a stable layer but, unlike fat particles of animal origin (LDL and HDL), they do so without cholesterol. Additional stability is achieved via oleosins. These specially-formed proteins with a strongly lipophilic portion, which is bent into the shape of a "hairpin" (top right), are also deposited and act to stabilize the oil bodies. The hydrophilic portions of the protein consist of helices and unstructured polar regions.

nents. The pH value of the raw soy milk and of the medium used in the separate washing stages has a major influence on the degree of success in separating out the storage proteins. With a strongly basic pH value of 11, the separation of oleosomes from the storage proteins glycinin, β-conglycinin and other allergens such as the thiol protease "Gly m Bd 30K" is achieved [4].

Inside an oleosome: neutron scattering

Although various models and simulations exist for the conformation of oleosins within the oleosome, their folding at the molecular level has not been definitively elucidated. It is also a difficult matter to demonstrate small changes in the conformation of the proteins (such as we expect at the beginning of thermal denaturing, for example) by macroscopic measurements of intact oleosomes. Neutron scattering offers us a promising approach to acquiring more precise insights in this context. Neutrons interact with the nuclei of atoms and have the special quality of exhibiting scattering differences when interacting with isotopes of the same element. This is especially pronounced in the case of hydrogen (H) and deuterium (D). This offers the possibility of suppressing or highlighting specific substructures within a sample by substituting hydrogen for deuterium to vary their scattering length densities. In the case of oleosomes, this gives us the option of dispersing them in mixtures with a range of various H_2O/D_2O ratios instead of normal water: this masks the normally dominant signal from the oil while boosting the weak but particularly interesting signal from the protein coat. This is illustrated by Figure 3, where the scatter signals from soy oleosomes are presented with the signals from a model emulsion – also based on soy and lecithin but protein-free (Intralipid) – in both pure H_2O (0% D_2O) and in 12% D_2O.

It can be seen that the signals of the two samples show virtually no differences for pure water, but differ significantly in the case of the 12% D_2O mixture. This is because 12% D_2O is, from a neutron's perspective, virtually indistinguishable from soy oil, and the resulting signal is therefore dominated by the protein coat. In water, however, the oil signal is dominant. If we then compare the scattering curves with those calculated for certain structures and H_2O/D_2O ratios, we can make a statement about the structure of the sample. In the example shown, it can be readily seen that, while a simple sphere model can be used as an approximate description of both oleosomes and Intralipid in the case of pure water, this remains true only for the protein-free Intralipid in the case of the 12% D_2O mixture. To be able to describe the signal generated by the oleosomes, it is necessary to use the more complex model of a sphere with a shell. By adjusting the model to fit the scattering curve, parameters such as oleosome size and shell thickness can be determined. This results in a diameter of 290 nm and a shell thickness of 7 nm, which is compatible with the above-mentioned model representation of the oleosome structure.

Behavior at interfaces

The basic behavior of oleosomes at interfaces is illustrated in Figure 4. If an oleosome reaches

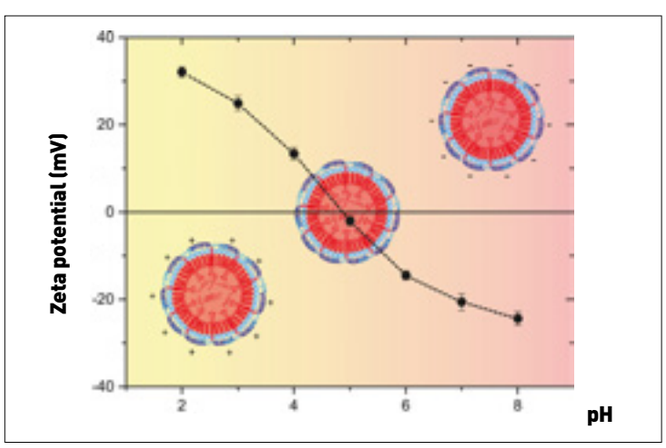

Fig. 2 Measurement of the zeta potential provides information about the surface charge of the oleosomes at various pH values [3].

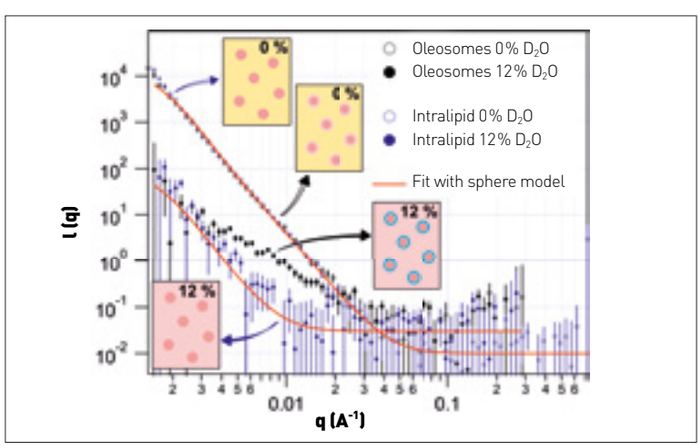

Fig. 3 Small Angle Neutron Scattering (SANS) applied to oleosomes and model emulsions clearly shows the oleosome's protein coat with measurement in 12% D_2O.

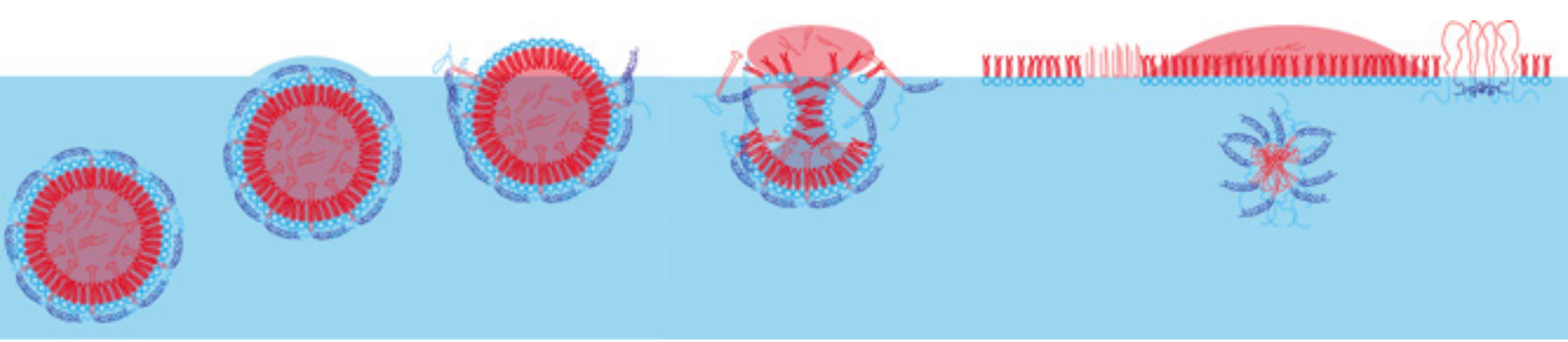

Fig. 4 Schematic diagram of the oleosome interface behavior, as illustrated by Langmuir trough experiments, combined with Brewster angle microscopy [4]. The oleosomes diffuse onto the surface. Once the hydrate water evaporates, the hydrophilic parts of the oleosins denature, the hydrophilic phospholipid heads reorient themselves, the oleosomes burst (hydrostatic pressure). At the air/water interface, various phases of fats, phospholipids and oleosins then form.

the air/water interface, the oleosins on the surface change their conformation as soon as they come into contact with air (which can be viewed as being hydrophobic). This causes the oil body to lose its stability: it ruptures and its components reconfigure themselves into new structures. In the process, the interface-active phospholipids arrange themselves at the surface as shown and the oil itself also collects at the surface. The behavior of the oleosins remains to be fully elucidated. Although the oleosins' amphiphilic character also presents them with the opportunity of arranging themselves along the interface, micelle-like aggregates can also form (as shown), with which the hydrophobic partial sequences are enclosed by the hydrophilic sequences and can thus become water-soluble. An explanation will require further investigation (light scattering, spectroscopy, ...).

This also makes oleosomes interesting in the field of cosmetics. In thickened aqueous solutions, they burst due to the slow evaporation of the solvent and thus release their oil reservoirs equally slowly.

Oleosomes and innovative foods

The functionalized surfaces of the nano- and microparticles can be used to bind the oil bodies into structured aggregates or to process them into solid and semi-solid components for subsequent use in novel foods.

Some initial studies have already explored a number of possibilities. On the one hand, in the basic region and near the isoelectric point, for example, negatively-charged amino acids are predominant in the hydrophilic regions of the oleosins: these could be linked by Ca^{2+} ions, thus leading to a moderate improvement in stability (see Fig. 5). On the other hand, the hydrophilic parts of the protein could be permanently cross-linked with the enzyme transglutaminase at the surfaces. Transglutaminase requires calcium to develop its maximum level of activity: accordingly, maximal stabilization of the emulsion will result only from the interplay of the two substances.

Oleosomes could therefore be used to develop protein-rich foods with an energy content that would be controllable via their fat percentage. This would also be of interest in geriatrics for the prevention and treatment of malnutrition – especially since the texture (and the ease with which food could be swallowed in the event of simultaneous dysphagia) could be precisely modified [5]. If we also up the ante by pointing out that both the surfaces and the oil reservoir can be loaded with bioactive substances of varying solubility (water- and fat-soluble vitamins, phenols, etc.), then oleosomes have every chance of becoming natural biofunctional carrier particles.

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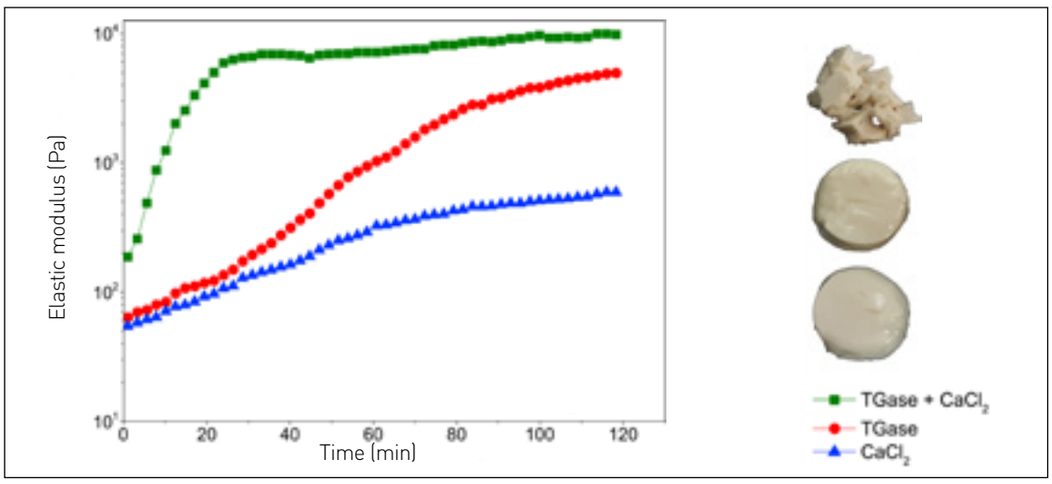

Fig. 5 Increased stability by cross-linking hazelnut oleosomes with calcium chloride, transglutaminase and a combination of the two. The texture is shown on the right-hand edge of the figure. The calcium gels are very soft, almost melting; the oleosomes permanently cross-linked with transglutaminase exhibit a considerably higher shear modulus. The texture is reminiscent of jelly. The combination of TGase and calcium (cofactor) leads to rapid cross-linking and the formation of fragile, yet soft, gels.

raman microscopy

Fitness Test of motile cells

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Analysis of single cells is never easy – and it gets even more difficult when cells are not fixed at a surface but freely moving. In this case, Raman-Trapping-Microscopes arrest motile samples during spectral analysis. These most powerful devices enable novel insights into characteristics and behavior of eukaryotic cells and microorganisms.

Introduction

Raman spectroscopy is an analytical method solely based on the interaction of molecules with light. Specifically, it generates a vibra-tional fingerprint, which is unique for every cell type. Optical trapping allows retaining and manipulation of small particles and motile samples. Combining Raman spectral microscopy with optical trapping allows efficient analysis of motile eukaryotic cells and microorganisms1 - label-free and non-destructive.

There are two types of trapping possibilities:

I. Particles can be trapped in a stretcher, where two divergent counter-propagating laser beams form optical tweezers [2, 3].

II. A single beam optical trap provides a force strong enough to hold and move small particles, like viruses, bacteria and organelles without destroying the trapped specimen [5,6,7,8].

The latter setup is realised in the BioRam® system (CellTool, Bernried, Germany), where the Raman excitation laser is set to allow simultaneously trapping during Raman spectra acquisition. As the combined Raman-Trapping microscope works non-invasively and under physiological conditions, cells remain vital for repeated use.

Challenge 1– Identification of bacteria subtypes

Increasing occurrence of antibiotic resistant bacteria is a heavy threat in modern hospitals. In order to ensure safety of patients it is essential to check for and characterise remaining microorganisms on a routine basis. Current methods like mass spectrometry analyse entire bacterial colonies. Thus, only an overall picture of the bacterial culture can be shown, whereas subpopulations may be underrepresented or even masked in the results.

To show the potential of the BioRam® system, we analysed three different strains of *Pseudomonas* and *Staphylococcus*, which were previously fixed with ethanol. During measurement, single cells were kept in position by optical trapping, enabling the acquisition of significant Raman spectra. Subsequent analysis of Raman spectra using Principal Component Analysis (PCA) – a standard method for reducing dimensionality in multivariate datasets – allowed the separation of bacterial species as well as of different strains. *Pseudomonas* and *Staphylococcus* samples differed in their specific Raman spectra (see Figure 1). A more detailed analysis of *Pseudomonas* and *Staphylococcus* data revealed even more prominent differences between the single strains (data not shown).

Besides the measurement of ethanol fixed bacteria, it is also possible to perform Raman spectroscopy of living microorganisms taken directly from colonies of an agar plate that had been exposed to room air [9].

One of the huge advantages of Raman spectroscopy compared with methods like plaque assays, PCR or mass spectroscopy is its single cell resolution. With this, Raman spectroscopy is able to quickly detect very rare cells within a bacteria colony and will not lose important information due to overgrowing bacterial subtypes.

Challenge 2 – Quality control of blood products

Even today, guaranteeing the quality of all blood products is difficult to achieve. Only about 1% of the blood bags are actually tested for quality and purity. These tests were furthermore conducted during the production of the blood products and the tested bags need to be discarded afterwards. This means that there is so far no control of blood products immediately prior to

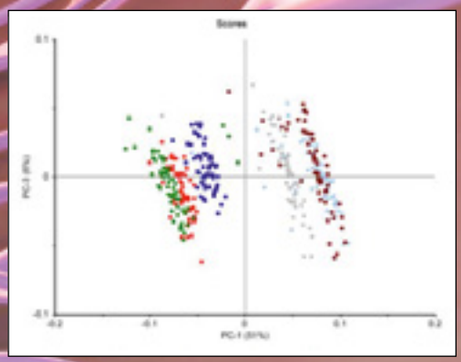

Fig. 1 Results of Raman spectroscopy and subsequent Principal Component Analysis (PCA) of *Pseudomonas* and *Staphylococcus* samples. Scoreplot (PC-1/PC-3) shows Raman spectra of different *Pseudomonas* ●●● and Staphylococcus ●●● strains. Every dot in this score plot represents one measured cell.

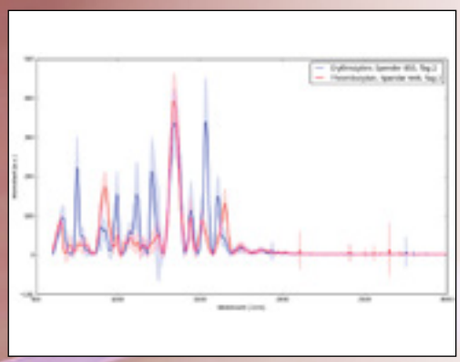

Fig. 2 Raman spectra of erythrocytes and thrombocytes: mean spectra with standard deviations of erythrocytes ● and *thrombocytes* ●. clearly differ. Marked peak differences are mainly related to the haem-group.

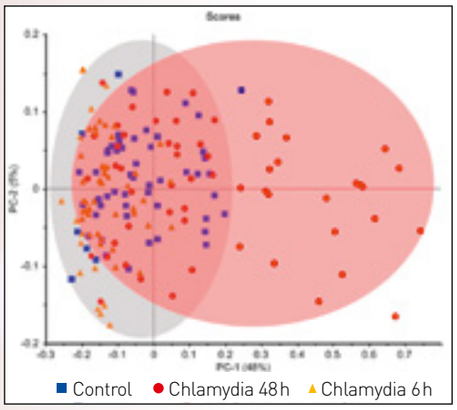

Fig. 4 PCA Scoreplot of *C. pneumoniae* infected monocytes after 6 h ▲, after 48 h ● and the control monocytes ■. About half of the infected monocytes after 48 h appear on the far right side of the Scoreplot due to the infection.

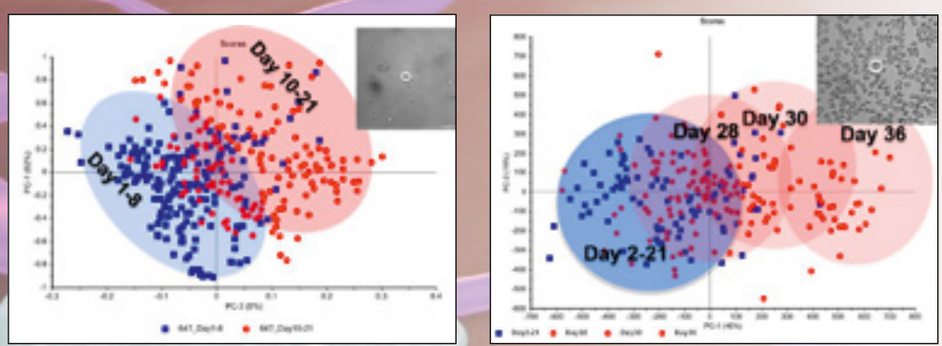

Fig. 3 Raman analyses of blood products: Raman spectra of thrombocytes and erythrocytes from three donors were taken at different time points using the BioRam® device (CellTool, Germany). PCA score plots are shown. Left: score plot of thrombocyte measurements: spectra obtained from cells between day 1 and 8 differ from spectra obtained between days 10 and 21, indicating changes in the thrombocytes after day 8. Right: score plot of erythrocyte measurements: PC-1 scores correlate with cell states of erythrocytes. Up to day 21 all donors are identical, thereafter change in spectra seems donor dependent – "aging" occurs at different time points: day28, day 30, day36, respectively). Inserts show representative light microscopy pictures. Circles depict site of laser spot.

transfusion. The combination of Raman spectroscopy with optical trapping provides a noninvasive, fast and easy approach for ensuring quality of blood products directly before use. This on one hand, enhances patient safety but also saves money as blood products can be tested individually and do not need to be discarded due to empirically determined dates.

In a first experiment, we took Raman spectra of living erythrocytes and thrombocytes using the BioRam® device. Like bacteria, blood cells were kept in the laser focus due to the trapping effect, allowing acquisition of high-quality spectral data. Spectra of different cell types significantly differed, mainly due to the presence of the highly Raman-active haem group in erythrocytes, which allowed easy discrimination of the cells (see Figure 2).

In a further set of experiments we used Raman spectroscopy to follow the aging process of different blood products using thrombocyte and erythrocyte concentrates (Figure 3).

Analysis of thrombocyte concentrates from three different donors up to 21 days after preparation, showed that platelets start to differ in their Raman spectra at day 8, whereas the largest variations were observed at a wavenumber range of 1296–1333cm^{-1} (see Figure 3, left plot). Differences in this range are well known to be associated with apoptotic cell death [10,11].

Analysis of red blood cell concentrates over a period of 36 days after donation, yielded comparable results, however – here the onset of cell decay was donor-dependent and started at different time points (see Figure 3, right plot). The results obtained show that Raman spectroscopy alone or in combination with optical trapping is a valuable tool for monitoring the quality of blood products in an easy, quick and nondestructive way directly before use, and could help to increase safety of blood transfusions.

Challenge 3 – Detection of infection

The combination of Raman spectroscopy and laser trapping can be used as a rapid diagnostic tool for the detection of infected cells. The obligate intracellular pathogen *Chlamydia pneumoniae*, responsible for respiratory infections and a wide range of chronic diseases, uses circulating monocytes as host cells to promote its own replication and dissemination. [12].

In order to demonstrate the power of BioRam® to detect cellular infection, we conducted an experiment, in which monocytes were infected with *C. pneumoniae* and recorded Raman spectra after 6 h and 48 h post infection. The PCA score plot shown in Figure 4 depicts distinct separation between healthy and infected monocytes after 48 h. Monocytes infected with C. pneumoniae for 6 h did not differ from healthy monocytes, while after 48 h post infection, about 50 % of the infected cells showed differences in their Raman spectra, which could be associated with *C. pneumoniae* infection (see Figure 4). Major differences between healthy and infected monocytes could be found in the regions 1645–1660cm^{-1}, 1430–1451cm^{-1}, 1327–1356cm^{-1}, 1290–1306cm^{-1}, and 888–934cm^{-1} of the Raman spectrum, indicating changes in lipids, fatty acids, and nucleic acids [10]. Results from Raman spectroscopy could be verified using an *oligonucleotide* DNA microarray [13], quantitative real-time PCR and immunofluorescence, which emphasized the ability of *C. pneumoniae* to persist but not replicate in human blood cells. Raman analysis combined with optical trapping could therefore become superior to currently used blood cultures for the detection and characterization of cellular infections, as it is highly sensitive to fastidious pathogens and provides immediate results.

Conclusion

Confocal Raman-trapping microscopy, a combination of Raman spectroscopy and the optical trapping of individual cells, is a highly sensitive method for visualising small differences between cell populations in a large variety of samples. We were able to demonstrate that Raman spectroscopy serves as an ubiquitous 'photonic marker' for the fast and efficient identification of pathogens, assurance of cell 'fitness' (i.e. functionality) and early detection of infection. In addition, BioRam®'s nondestructive data acquirement under native cell conditions could become an important device for biologists and physicians to facilitate stem cell identification, to assure quality of cell based therapeutics, or to support cancer diagnosis [9].

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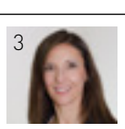

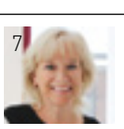

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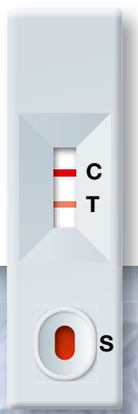

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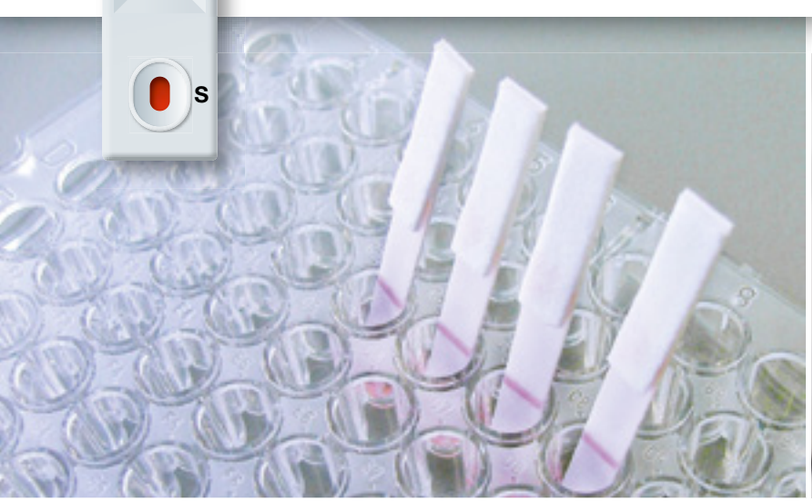
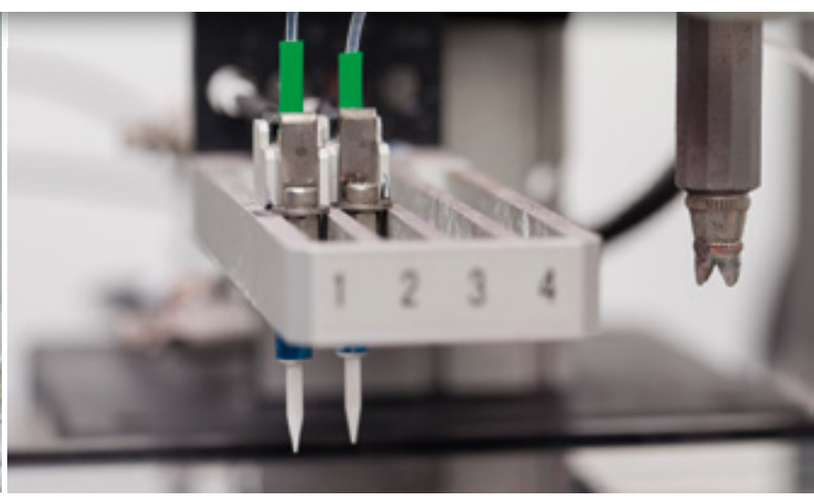

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