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editorial

Angela Made in Germany

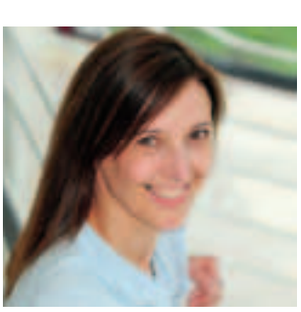
If we, as Germans, have to think about what our country has to offer, there's actually a whole lot of things that come to mind. Even the most conservative of us couldn't have failed to note that Angela Merkel is now being presented like a top quality product of German performance. One other, by no means minor reason why politics in our country is working so well – and so successfully in many people's minds – is that the German economy is breaking sales records with its products and skills, and so providing record tax revenue for our country.

The quality of German products can look back on a long tradition. "Made in Germany" is a quality seal that has actually meant something for quite some time now and that still fills us with pride. As a result, German companies are also entering the international markets with self confidence, and I believe we may also claim that, as a rule, we are a very reliable partner.

The major brands like BASF, Merck, SAP, and all the others are known all over the world. In the special fields of research and development that we cover in our specialist magazine labor&more, a unique publication on this sector with editions in German, English, and Russian, there are likewise many companies that are now exporting to all of the key countries in the world. Some of these you will, of course, find in this issue too, and they are our customers because they too value the quality of our magazine.

So why not take our recommendation, and come visit us in Europe, in Germany and Switzerland. The next few months will see some important events like ILMAC in Basel, Biotechnica in Hannover, and (next year) analytica in Munich – perhaps you can then combine your trip to the Bavarian capital with an FC Bayern München match. This Triple Champion club plays at a beautiful stadium – in Adidas brand shoes, of course. Everything Made in Germany.

→ **Jörg Peter Matthes**
CEO, Publisher



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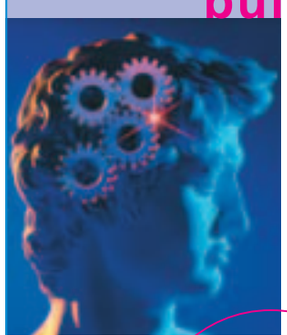

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market view

JULABO Introduces New Company Vision

JULABO staff and management established company guidelines with the support of external moderators. They were introduced to all employees at the end of April. The new company guidelines are based on the first vision statement developed by JULABO in 2007. The new version is considerably shorter with simpler wording. The design emphasizes two graphic elements for recognition: red focal circles on the front page and the JULABO house. Ralf Wurth, member of the executive management and – among other things – responsible for personnel, compared the vision with a wrist watch: "Only the clock face and hands and the resulting time are outwardly visible. In a company these are the homepage, catalog and products. As a perfectly interlocking clockwork is operating behind the clock face, committed employees support JULABO's objectives and values. The company guidelines boost functioning cooperation and meshing of the company's wheels."

→ **www.julabo.de**

Roche Launches First Sugar-Transferase

Today announced the launch of its new alpha-2,6-Sialyltransferase for in vitro sialylation of glycoproteins and complex molecules as human monoclonal antibodies (mAbs). The enzyme, which is offered for research and pilot scale applications, is produced under animal-origin free conditions and offers a very high lot-to-lot consistency. "This launch is the first in a series to offer a complete glyco-engineering portfolio of key enzymes and activated sugars covering a broad spectrum of applications," said Ruedi Stoffel, Head of Biochemical Reagents & Custom Biotech. "The initial feedback from bio-manufacturing customers showed that our continuous scientific and technical support throughout the up-scaling and development process differentiates Roche as a strong partner."

The alpha-2,6-Sialyltransferase delivers up to 95% bi-antennary sialylation of N-Glycan chains within 6–8 hours, a performance which is currently not offered by competitor products. It is based on a human genome sequence and expressed in mammalian expression systems. Over the coming months, Roche plans to complete the portfolio through launches of additional Sialyl-and Galactosyltransferases.

→ **www.roche.com**

Merck Millipore: Conformity of Water Purification Systems

Merck Millipore presents its Millitrack Compliance software. This is an e-solution for the optimisation of laboratory performance and the conformity of Milli-Q Advantage and Milli-Q Integral water purification systems with official regulations. The Compliance software comes preinstalled and can be activated with a special code. Equipment qualification is mandatory in regulated laboratories. For users of laboratory water systems in regulated industries all over the world, it is becoming ever more important to observe the official guidelines as they are set down in FDA Regulation 21 CFR Part 11 and similar regulations issued by global supervisory bodies.

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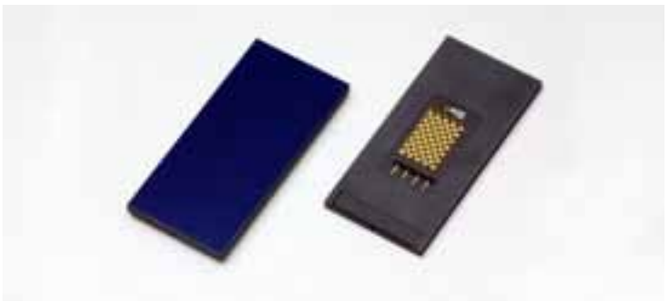
Hamamatsu develops CCD image sensors for the Subaru Telescope's Hyper-Suprime Cam



Hamamatsu Photonics K.K., in conjunction with the National Astronomical Observatory of Japan (NAOJ), Osaka University, and Kyoto University, has developed CCD image sensors for use in the Hyper-Suprime Cam, an ultra-wide field of view prime focus camera installed in the Subaru Telescope at the summit of Mauna Kea, Hawaii. Compared to the CCD image sensors used in the first generation Suprime-Cam, the new CCD image sensors additionally provide extended sensitivity in the near-infrared region and highly uniform product quality. Hamamatsu mass produced 116 of these large-area (3 cm x 6 cm) back-illuminated deep-depletion CCD image sensors for this project.
To see ist and to find out more about the Subaru Telescope's Imaging Discovery visit: www.naoj.org/j_index.htm

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Mettler Toledo: Heat Flow Calorimeter

The heat flow calorimeter Optimax HFCal from Mettler Caledo is intuitive to use and allows the operator to conduct experiments faster for considerable savings in resource costs. Fast and reliable heating and cooling achieve time savings. The device allows simple and fast characterisations of safety relevant process parameters including heat transfer, precise heating of the reaction mixture, isothermal and non-isothermal heat flow, enthalpy, and thermal conversion rates in all reaction stages. The electrical heating technology and the Peltier cooling system provide precise temperature control from –40 to 180 °C – and that without cryostats and ice baths.

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Royalty Visits Max Planck Institute

Her Royal Majesty Maha Chakri Sirindhorn visits the Max Planck Institute of Colloids and Interfaces in Potsdam – On 27 June 2013, the Thai Princess, Her Royal Highness Maha Chakri Sirindhorn, was a guest at the Max Planck Institute of Colloids and Interfaces in the Potsdam-Golm Science Park. Her visit concentrated on the generation and utilisation of secondary resources gained from biomass. Fig: Diverse types of biomass (grape rind and seeds, sugar beet rind, violet root, patchouli leaves, empty palm fruit clusters) can be used to produce secondary resources. © MPI Colloids and Interfaces. One of the most pressing tasks of our times is the search for new technologies in realising a sustainable economy and climate protection. The Thai Princess, Her Royal Highness Maha Chakri Sirindhorn, met with scientists at the Max Planck Institute of Colloids and Interfaces to look into the details of this topical issue of social policy. With a Bachelor of Arts in History and a PhD in the development sciences, Princess Sirindhorn was interested above all in new technologies for regional development. The focus was on various fields of colloid chemistry based on biological raw materials: the production and application of carbons for alternative energy technologies and soil conditioning as well as the production of key polymers and chemicals up to medicines.

Source: www.bib-tuebingen.de
Original publication:
Cell Stem Cell (2013),
DOI: dx.doi.org/
10.1016/j.stem.2013.01.008.

Photo: © panthermedia \ Olexandr Moroz

Ann Ehrenhofer-Murray Takes Up Post of Albert Einstein Professor

In early July, the excellent biologist Ann Ehrenhofer-Murray took up her post as Albert Einstein Professor for Molecular Cell Biology at the HU Berlin Institute of Biology. The Einstein Foundation Berlin supports this appointment with a subsidy for equipping the laboratories.

Ehrenhofer-Murray's prime interest is epigenetic research, a burgeoning field of biology and the constituent of genetics that combines molecular and systems biology. By experimenting on bakers' and fission yeast, she investigated how changes in chromatin, the "packaging" around DNA, affect the deactivation of genes, and made some spectacular discoveries.

Ann Ehrenhofer-Murray, born in 1966, is a British and Swiss citizen. She studied biochemistry at the Swiss Federal Institute of Technology Zurich where she also received her doctorate. During a subsequent research period at the University of California in Berkeley (USA), she discovered epigenetics for herself. Afterwards she set up her study group at the Max Planck Institute of Molecular Genetics in Berlin. Between her habilitation at HU Berlin and her appointment at the University of Duisburg-Essen, she also worked for a brief period at Gießen University.

Source: www.einsteinfoundation.de

Professor Ann Ehrenhofer-Murray
Photo: University of Duisburg-Essen

Analytik Jena Receives Major order from Pakistan

Jena, 24 May 2013 – Via its Leipzig subsidiary AJ Roboscreen GmbH, Analytik Jena AG has received a major order to supply eleven Pakistani clinical laboratories with systems for preparing samples and identifying various viruses. In response to a major public invitation, the tender submitted by AJ Roboscreen GmbH via its regional sales partner won through over those of renowned fellow competitors. The order will take effect in the third quarter of the current business year for Analytik Jena AG and will be worth nearly half a million euros. The order is first limited to one year, when the equipment will be delivered before the end of the current business year.

"We're pleased with this order. It serves to underscore our strategy of moving reagent sales to the focus of our marketing activities," explained Alexander Berka, Head of the Life Science Business Unit of Analytik Jena AG. "Based on our own technologies and patents and certified under CE IVD, the test kit can be used not only to identify viruses, but also to prepare the reagents for sample preparation."

AJ Roboscreen GmbH, a 100% subsidiary of Analytik Jena GmbH with head office in the north of Leipzig, specialises in the development of diagnostic kits, reagents, fine chemicals, and single-use consumables for the standardised cleanup and detection of nucleic acids and for the identification of specific proteins.

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Signals from the Depths of the Brain

Even supposedly simple movements like reaching for a cup of coffee demand the interfunctional collaboration of many brain areas. Brain researchers in Tübingen could verify that in this process the superior colliculus of the Lamina tecti or midbrain – a neuronal structure that forms the tectum of the human brainstem – functions as a control module for arm and hand movements. Of particular relevance are the recent research findings with respect to the high density of networks performing the most diverse functions in the brainstem. When these malfunction, they promote e.g. dystonias, a group of neurodegenerative movement disorders.

Assisted with functional magnetic resonance tomography, or fMRT for short, the brain researchers investigated the superior colliculus of the midbrain in healthy test subjects. Developed in Tübingen, this highly sensitive fMRT system recorded measurements showing obvious signals of active neurons in the superior colliculus when the test subjects moved their arms. The researchers then proved for the first time that the superior colliculus not only participates in the processing of visual stimuli and acts as a stopover for commands to move the eyes. It also functions as a control module for the movements of the upper limbs.

Original publication: Himmelbach M, Linzenbold W, Ilg UJ (2013), Neuroimage 82, 61–67
Source: www.bih-tuebingen.de
Photo: © panthermedia | michelangelus

Modified Peptides

Radiopharmaceuticals represent a modern class of drugs that are used for diagnostic and therapeutic purposes in nuclear medicine. High image quality and effective tumour therapy require compounds that can accumulate quickly and specifically in tumour tissue. However, a number of highly promising peptides are often stable for only a few minutes in the body before they can be broken down by enzymes (proteases).

Scientists headed by Professor Thomas Mindt at Basel University and University Hospital systematically exchanged a building block in a peptide's backbone. Developed at the University and University Hospital, this method allows an efficient synthesis of peptidomimetics with enhanced biological properties.

Source: www.unibas.ch

Hiking Trails in Activity Landscapes

Data Visualization in Medicinal Chemistry

Prof. Dr. Jürgen Bajorath
Department of Life Science Informatics, B-IT, LIMES Program Unit Chemical Biology and Medicinal Chemistry, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany

The massive growth of compound activity data provides opportunities and challenges for medicinal chemistry. Conventional approaches for the analysis of structure-activity relationships (SARs) are not suitable for the exploration and exploitation of this unprecedented knowledge base. Recently, new computational methodologies have been introduced for large-scale SAR analysis that put emphasis on visualization to provide an intuitive access to complex SAR patterns and identify key compounds.

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Jürgen Bajorath studied biochemistry and obtained diploma and Ph.D. degrees from the Free University Berlin (West). He is Professor and Chair of Life Science Informatics at the Bonn-Aachen International Center for Information Technology (B-IT) and the LIMES Institute of the University of Bonn. He is also an Affiliate Professor in the Department of Biological Structure at the University of Washington in Seattle. His research focuses on chemoinformatics and the development of computational methods for medicinal chemistry and chemical biology.

Chemical tradition and subjectivity

Chemists are trained on the basis of two-dimensional representations of molecular structure, i.e., molecular graphs. In medicinal chemistry, the exploration of structure-activity relationships (SARs), a cornerstone of compound optimization efforts, is largely based on comparisons of molecular graphs of active compounds. Traditionally, structure-activity data is recorded and monitored in R-group tables that list chemical core structures and substituents (R-groups) of active compounds together with their potency information. To this date, such R-group tables are indispensable tools for practicing medicinal chemists.

Traditionally, medicinal chemistry efforts are centered on individual compound series. Analogs of active compounds are made and tested and compound potency and other optimization relevant properties (such as, for example, solubility or metabolic stability) are attempted to be optimized. Compound series are considered on a case-by-case basis, usually one series at a time. Individual compound series can be conveniently represented in R-group tables to deduce SAR information as long as compound numbers do not become too large. For example, it is hardly possible to subjectively analyze, with a chemist's eye, and understand SAR information associated with more than 100 or so compounds. Although there are individual differences, we quickly strike our limits in analyzing and comparing larger numbers of chemical structures and their activities to derive SAR rules.

Despite these constraints, subjective criteria, chemical intuition, and experience continue to play a major role in compound analysis and design, more so than one might anticipate in the era of virtually unlimited information resources. Furthermore, despite the undisputed role of chemical ingenuity, it is well documented that even seasoned and successful medicinal chemists rarely agree in their assessment of chemical characteristics that render compounds 'drug-like' and attractive for further optimization [1]. In addition, our perception of chemical structure and properties is strongly context-dependent and conclusions drawn about preferred candidate compounds typically change with the ordering of molecules presented to us [1]. Chemical experience and intuition have a successful history in medicinal chemistry, but there is ample room for more systematic and 'objective' data analysis and compound design concepts.

The compound activity data deluge

In recent years, we have been experiencing a nearly exponential growth in compound activity data (there is no end in sight) and currently available data volumes are beginning to impede traditional medicinal chemistry strategies. Compound activity data do not only grow in pharmaceutical companies at unprecedented rates, they also grow in the public domain. For example, PubChem [2], the major public domain repository for biological screening data, and ChEMBL [3], a major public source of compound activity data from medicinal chemistry projects, currently already contain more than 10 million active molecules, the majority of which is annotated with activities against multiple biological targets.

In addition to increasing data volumes, the heterogeneity of SAR data has become a substantial complication for SAR exploration. For attractive therapeutic targets, many chemically different compound series are typically available that have originated from diverse sources and have been subjected to different types of biological activity measurements. This equally applies to public domain data, which are collected from the scientific and patent literature, and proprietary compound data that accumulate within large pharmaceutical companies. For high-profile target families such a G protein coupled receptors in the central nervous system or protein kinases implicated in various forms of cancer, the pharmaceutical industry has been generating large amounts of increasingly heterogeneous compound data in the course of drug discovery projects. Learning from this information for medicinal chemistry applications has become a challenge that is as of yet largely unmet.

Computational analysis and predictions

Increasing compound data volumes and heterogeneity conflict with the traditional 'one compound series at a time' focus of practical medicinal chemistry and make subjective case-by-case analysis very difficult. The tasks at hand go much beyond of what could possibly be handled with the aid of molecular graphs and R-group tables. In light of this situation, should their not be a push to complement and support practical medicinal chemistry efforts with more systematic and 'objective' data analysis schemes? Yes, indeed. However, computational methods have traditionally played a different role in medicinal chemistry.

In order to assess the role of computational analysis and predictions in medicinal chemistry, we should distinguish between new computer-aided drug design methods that are typically promoted and applied by computational chemistry groups, often fairly remote from practicing medicinal chemists (which presents a problem for drug discovery), and computational techniques that have long been considered a more or less integral part of medicinal chemistry. First and foremost, this applies to the Quantitative SAR (QSAR) paradigm that has dominated computational approaches in medicinal chemistry since the 1960s [4].

Fig. 1 Section of a prototypic SAR network of a large compound data set. In a so-called network-like similarity graph (NSG), compounds are displayed as nodes and edges indicate molecular similarity relationships. Nodes are coloured according to compound potency using a continuous colour spectrum from green (lowest potency in the data set) over yellow to red (highest potency). In addition, nodes are scaled in size according to their contribution to local SAR discontinuity.

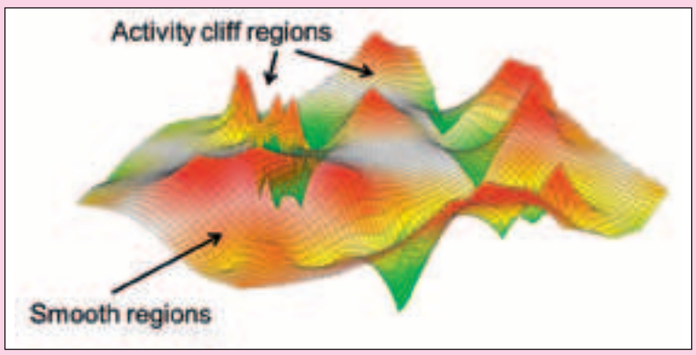

Fig. 2 Three-dimensional activity landscape. A 3D model of a compound data set is displayed that is reminiscent of 'true' activity landscapes containing gently sloped and rugged regions. This activity landscape view is obtained by a 2D projection of chemical reference space with an interpolated biological activity surface added as the third dimension. The surface is colored by compound potency according to Figure 1. White/transparent surface areas are interpolated and not populated with active compounds. Activity cliff and smooth regions are indicated. In activity cliff regions, small chemical modifications of compounds (i.e., very short 'moves' in chemical space) have a profound effect on biological activity. By contrast, in smooth regions, structurally diverse compounds have similar activity.

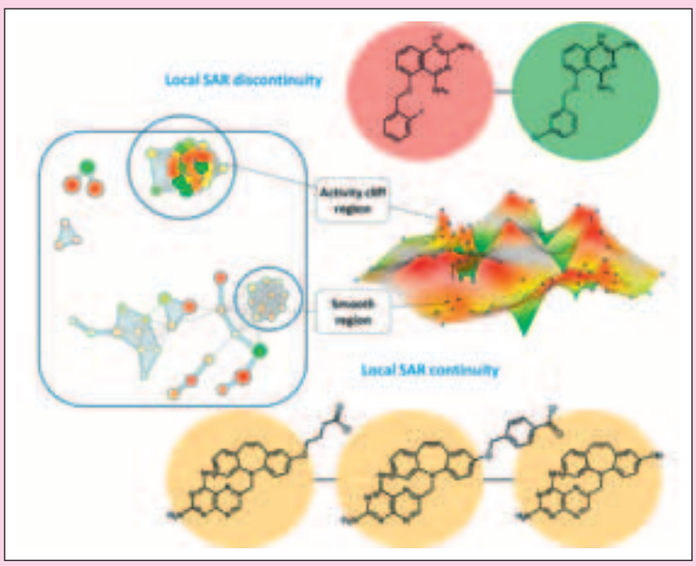

Fig. 3 Correspondence of SAR features in alternative landscape representations. On the right, a three-dimensional activity landscape is shown. Compound positions are represented as dots and the surface is colored according to compound potency. On the left, the corresponding network-like similarity graph is shown (represented according to Figure 1). Corresponding regions in these activity landscape views are indicated. In addition, molecular graphs of active compounds are shown that form an activity cliff (top) or map to regions of SAR continuity (bottom).

QSAR analysis generally attempts to derive linear models of biological activity on the basis of known sets of structurally similar compounds that are represented by various two- or three-dimensional descriptors of molecular structure and properties. Then, the resulting series-specific QSAR models are utilized to predict the potency of new analogs. Although QSAR methods often substantially differ in their computational details, they share the traditional medicinal chemistry focus on individual compound series and, even more importantly, try to answer the cardinal question that governs the efforts of a practicing medicinal chemist: 'Which compound to make next?'

This question is usually more important to a medicinal chemist than any other that might conceivably be addressed by computational analysis. It is therefore not surprising that QSAR-based predictions have long been the major focal point of computational medicinal chemistry and, in addition, that medicinal chemists are generally much more interested in compound activity predictions than in compound data mining and knowledge extraction. This presents a conundrum for medicinal chemistry that is beginning to be addressed.

New computational concepts

Given the large volumes of heterogeneous compound activity data that are becoming available, there has recently been increasing awareness -inside and outside the pharmaceutical industry- that medicinal chemistry must go beyond conventional (Q)SAR paradigms and learn from these large volumes of available proprietary as well as public data. Given declining drug approval rates and stellar budgetary requirements of pharmaceutical R&D, one cannot possibly afford not to make use of such data as a knowledge base to learn from the past (considering both successes and failures) and make more data-driven decisions going forward.

To these ends, computational methodologies are required for systematic large-scale SAR analysis, taking structural heterogeneity of active compounds and different types of activity measurements into account. This is an area where data mining and SAR exploration meet and where new questions are addressed that go beyond individual compound activity predictions. For example, one would like to monitor the evolution of SAR information in the context of lead optimization projects involving different compound series over time and revisit decisions made by project teams to select one or the other analog or series for further exploration. In addition, one would like to compile and compare compound and SAR information that is currently available for a given therapeutic target and view newly identified active compounds in the context of this information to select the most promising candidates for further development.

Such tasks bring along new computational requirements. For example, numerical SAR analysis functions have been developed to systematically compare compound structures and potencies in very large data sets and make it possible to quantify SAR information on a large scale [5]. Furthermore, algorithms have been introduced to systematically identify compound pairs that are only distinguished by a single substructure exchange [6] and associate these so-called matched molecular pairs with SAR information [7]. Moreover, many results of large-scale SAR analysis are made accessible through visualization techniques.

SAR visualization

For the practice of medicinal chemistry, a numerical description of SAR characteristics is usually insufficient. Rather, results of data mining and analysis efforts must be presented to chemists in an intuitive manner. For large data sets, this ultimately requires the consideration of SAR visualization methods, which are becoming increasingly popular [8].

The concept of 'activity landscapes' is particularly suitable for visualization purposes. An activity landscape is generally defined as any graphical representation that systematically integrates compound similarity and potency relationships [9]. In a particularly intuitive form, an activity landscape can be rationalized as a two-dimensional projection of chemical space with biological activity added as the third dimension. Computationally, this requires the application of dimension reduction techniques as well as the interpolation of a

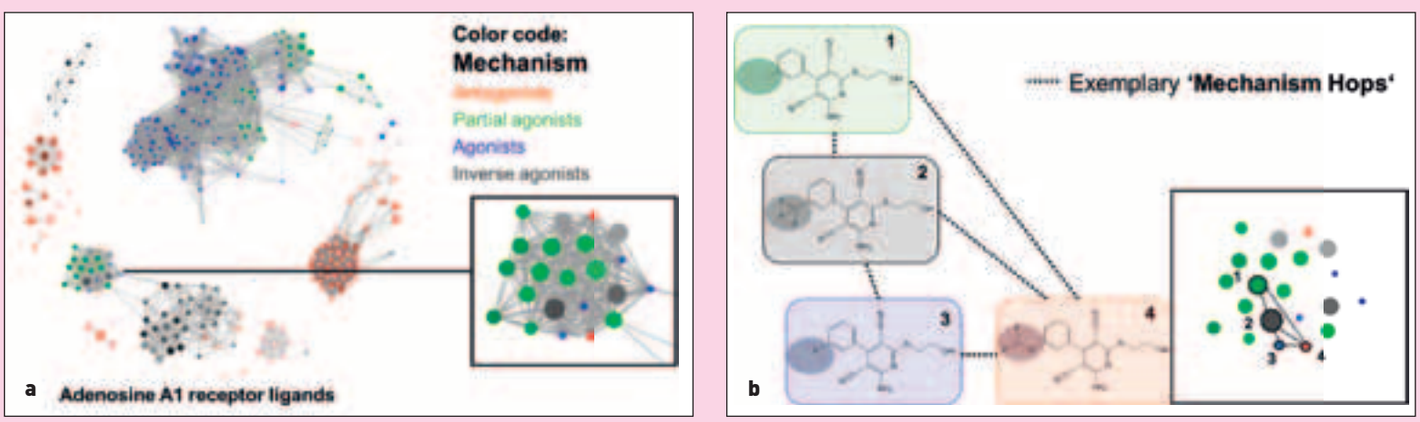

Fig. 4 Exploring the basis of chemical modifications of receptor ligands leading to changes in the molecular mechanism-of-action. In **(a)**, An NSG variant is shown for a large set of receptor ligands with different mechanism-of-action in which the potency-based color code according to Figure 1 is replaced by mechanism-based coloring. As expected, compounds with the same mechanism are often more similar to each other than compounds with different mechanisms and thus form clusters in the network. However, there are exceptions such as the enlarged subgraph on the right that consists of very similar compounds with different mechanisms. In **(b)**, the structures of analogs from this subgraph are compared, which reveals small chemical modifications leading to 'mechanism hopping'.

coherent activity surface from arrays of compound potency values. So generated activity landscapes remind us of geographical maps where smooth and rugged regions have a concrete SAR meaning. For example, in gently sloped and smooth regions, propagating structural changes of compounds (corresponding to moves in chemical space) are accompanied by only small to moderate changes in activity. Thus, structurally diverse compounds retain similar activity, a phenotype often referred to as 'SAR continuity'. By contrast, in rugged landscape regions containing mountains and peaks, small chemical changes lead to significant potency alterations, a phenotype rationalized as 'SAR discontinuity'. Pairs or groups of structurally very similar compounds (closely related analogs) with large potency differences (e.g., two to three orders of magnitude or more) represent the extreme form of SAR discontinuity and are termed 'activity cliffs' [10]. These activity cliffs are the most prominent feature of activity landscapes and rich in SAR information, because small chemical modifications of active compounds lead to large-magnitude biological effects. Not surprisingly, once identified in large compound data sets, activity cliffs often become an immediate focal point of medicinal chemistry efforts.

In addition to three-dimensional landscape models, there are many different -and equally informative- ways to represent activity landscapes of compound data sets and visualize SAR information. Among others, these include molecular network representations in which nodes represent active compounds and edges pairwise similarity relationships. Such SAR networks can be annotated with additional layers of information. For example, in so-called 'network-like similarity graphs' (NSGs) [9], large red and green nodes connected by edges represent activity cliffs that are easy to spot. Compounds forming such activity cliffs can be interactively selected from NSGs for further analysis. NSGs are designed to explore relationships between global and local SAR features in large and heterogeneous compound data sets. They enable the identification of information-rich SAR microenvironments in compound data sets having varying levels of SAR information content as well as data sets yielding heterogeneous activity landscapes.

Methods for large-scale SAR visualization based upon the activity landscape concept and data mining techniques add a new dimension to computational medicinal chemistry and complement traditional QSAR analysis. They are designed to explore and exploit the rapidly growing amounts of compound activity data for the practice of medicinal chemistry.

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Bibliography

[1] Lajiness, M.S. et al. (2004) J. Med. Chem. 47, 4891-4896
[2] Wang, Y. et al. (2012) Nucleic Acids Res. 40, D400-D412
[3] Gaulton, A. et al. (2012) Nucleic Acids Res. 40, D1100-D1107
[4] Esposito, E.X. et al. (2004) Methods Mol. Biol. 275, 131-214
[5] Peltason, L. & Bajorath, J. (2009) Future Med. Chem. 1, 451-466
[6] Hussain, J. & Rea, C. (2010) J. Chem. Inf. Model. 50, 339-348
[7] Wassermann, A.M. et al. (2012) Drug Develop. Res. 73, 518-527
[8] Stumpfe, D. & Bajorath, J. (2012) RSC Adv. 2, 369-378
[9] Wassermann, A.M. et al. (2010) J. Med. Chem. 53, 8209-8223
[10] Stumpfe, D. & Bajorath, J. (2012) J. Med. Chem. 55, 2932-2942

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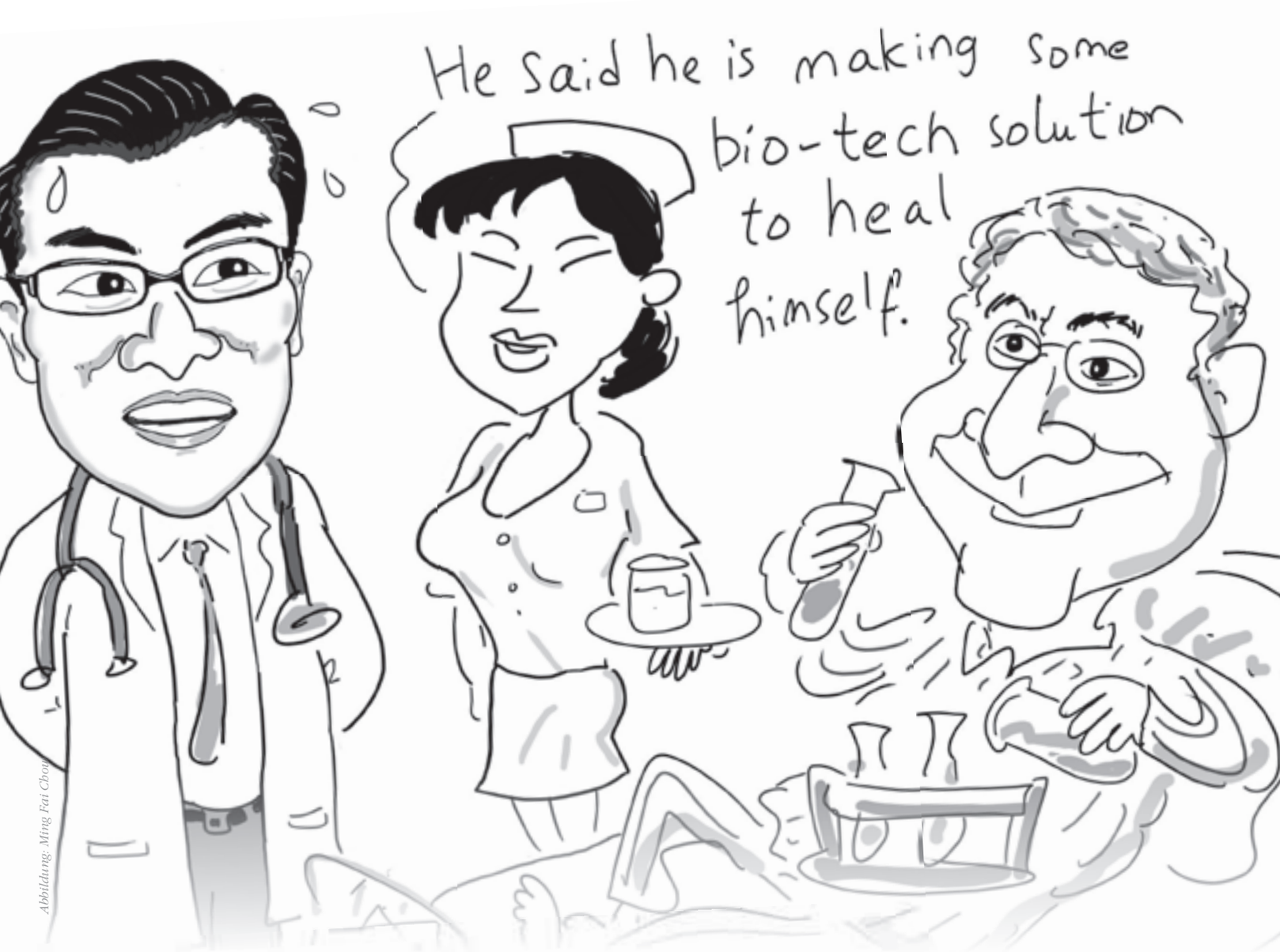

Abbildung: Ming Fai Chow

Fabulous FABP

The earliest plasma marker for myocardial infarction

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Prof. Dr. Jan F.C. Glatz
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The application of Fatty Acid-Binding Protein (FABP) as a plasma marker for the diagnosis of acute myocardial infarction was first suggested in 1988. Currently, FABP is proven to have added value for the diagnosis of patients presenting with chest pain suggestive of myocardial infarction, especially in the early hours after onset of symptoms. The routine application of FABP for this purpose not only will improve patient outcome but also markedly reduce costs for those cases in which an infarction can be excluded. This article describes the almost 25 years of fascinating history between discovery and commercial application.

"Time saves heart muscle"

Suspected heart attack? Rapid triaging of patients admitted to hospital with chest pain is needed to include positive cases for immediate application of appropriate therapy. An acute myocardial infarction (AMI) is caused by obstruction of one or more coronary arteries leading to lack of oxygen supply and ultimately dysfunction of that area of the heart. The sooner the arteries can be re-opened – by installing thrombolytic therapy – the less cardiac muscle will die and the better cardiac performance is maintained ("time saves muscle"). However, rapid triaging is equally important to exclude low-risk patients who can safely then be sent home. The latter not only adds to proper patient care but also to a marked reduction of costs for health care.

AMI markers

Biochemical plasma markers for myocardial injury have now for years been accepted as the primary tool for AMI diagnostics. These markers include creatine kinase isoenzyme MB (CK-MB), troponin T, and troponin I. The advantage of these markers is that an elevation of their plasma concentration, together with typical complaints of the patient, is almost absolute proof of the occurrence of an AMI. Changes in the electrocardiagram (typically, S-T segment elevation) may also be seen after an AMI but, importantly, some 25% of patients do not show such changes so that this parameter alone cannot be applied for making a final diagnosis.

The biochemical markers, however, have the disadvantage that, following an AMI, their plasma concentration does not start to increase until about 4 (CK-MB) or about 6 hours (troponins) after onset of symptoms. As a result, for a significant number of patients presenting with chest pain their final diagnosis is delayed, while for saving lives in the early hours after onset of AMI symptoms speed is the key!

The corollary is that the ideal biochemical marker for AMI would show a much more rapid appearance in plasma after cardiac injury. Both myoglobin and Fatty Acid-Binding Protein (FABP) have been found to be elevated in plasma within 2 hours after symptom onset. Clinical studies have revealed that of these, FABP performs more specifically than myoglobin, making FABP the preferred plasma marker for early diagnosis or exclusion of AMI.

The discovery of a "smart dwarf", FABP

The cytoplasmic protein FABP functions as an intracellular fatty acid carrier in parenchymal cells, thus supplying essential substrates for energy production in the myocytes. It comprises as much as 1–2% of total cardiac cytosolic proteins, making it one of the most abundant cytosolic proteins. This is very good for a potential biomarker.

It was as early as in 1988 that Jan Glatz suggested FABP as a plasma biomarker [1]. He was studying the biological function of FABP in the transport of long-chain fatty acids across the cellular cytoplasm. When performing experimental studies with the isolated perfused rat heart subjected to ischemia and reperfusion, he observed that FABP was released from damaged myocytes into the perfusion buffer. This serendipitous finding led him to suggest FABP as a potentially superior marker of myocardial injury in humans. It was reasoned by him that its relatively small size (14.5 kDa) and abundance in the heart could allow a rapid release of significant amounts of FABP from injured myocardium into the circulation. Subsequent mechanistic studies disclosed that these assumptions indeed were correct.

Biochemical AMI markers compared

Table 1 compares the characteristics of proteins that can serve as a plasma biomarker for AMI. It is clear that FABP stands out from the other marker proteins in that it is detectable in plasma within 1–2 h after myocardial injury, which is much earlier than any of the other marker proteins. In fact, some studies have reported its elevated presence in plasma as soon as 30 min after AMI onset. Although referred to as heart-type FABP, this protein – unlike CK-MB and the troponins – is not absolutely heart-specific but is expressed only in relatively low amounts in skeletal muscles. Peaks of FABP are reached at 6–8 hours, and the blood plasma level returns to normal within 24–36 hours due to rapid elimination by the kidneys.

The latter is the 'beauty' of FABP: the rapid renal clearance keeps the normal (reference) plasma concentration very low so

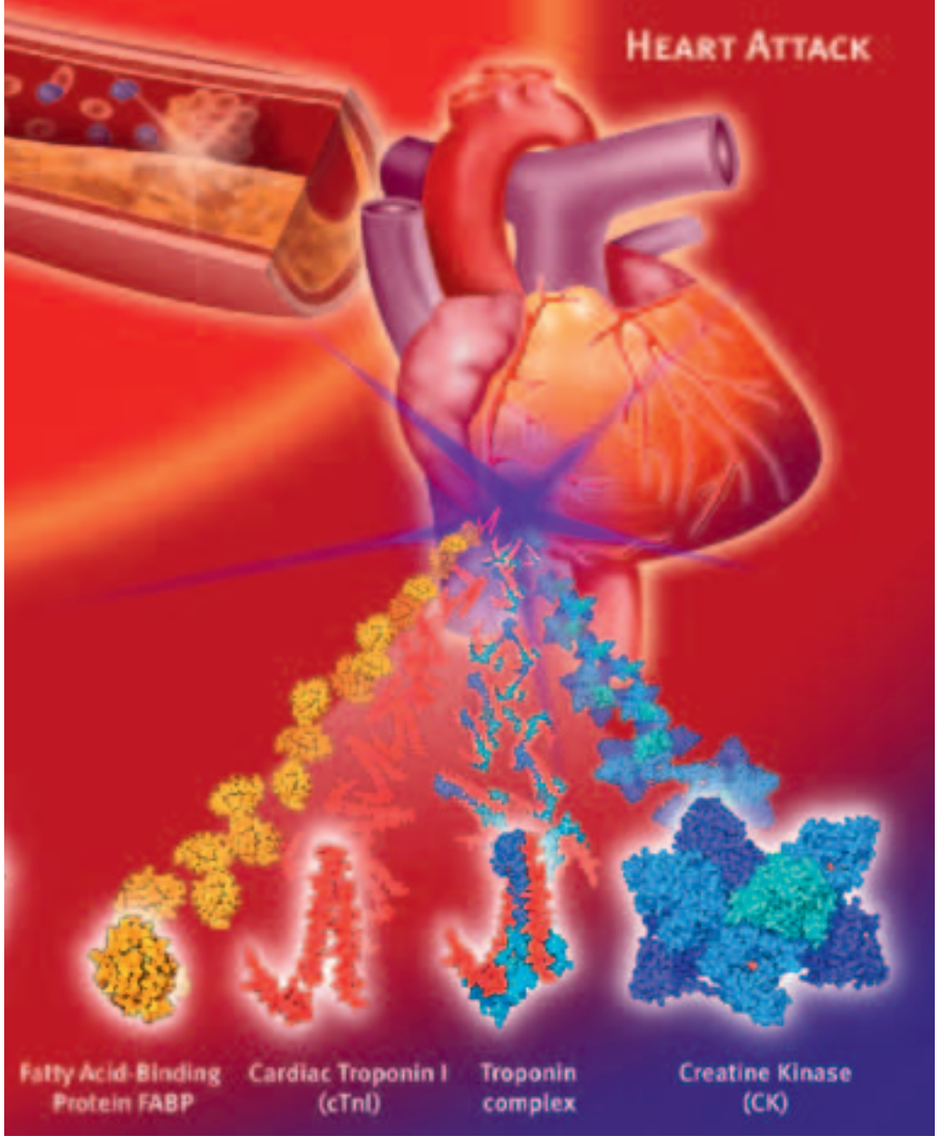


Fig.2 Proteins that upon a myocardial infarction will be released into plasma (in the order in time from left to right).

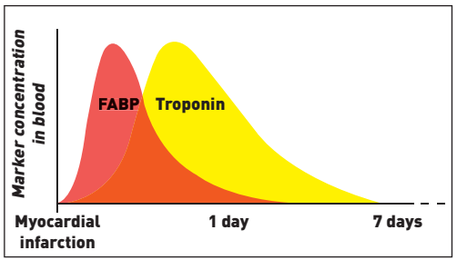


Fig.1 Release of FABP, myoglobin, CK-MB and cardiac troponins from the injured heart into plasma after acute myocardial infarction (AMI).

that upon release of FABP from cardiac muscle after AMI the upper reference level (established for groups of individuals) is soon surpassed, enabling a very early diagnosis. In contrast to this 'dwarf protein', all other marker proteins (except the small size muscle molecule myoglobin) are larger in size: troponin I (TnI) is 1.5-fold larger, troponin T (TnT) 3 times larger, and CK-MB 5 times larger. Myoglobin has the disadvantage that it is more abundant in skeletal muscle than in cardiac muscle (up to 3-fold higher content in skeletal muscle), resulting in a relatively high plasma reference concentration which hampers an early diagnosis.

The troponins, cardiac TnI and cardiac TnT, are structurally bound proteins that upon AMI first must be dissociated from the myofibrillar structures before they can be released into the circulation. Therefore, their release from injured myocardium follows a different pattern with elevated plasma concentrations occurring from approximately 6 h to more than 1 week after infarction. Hence, the so-called diagnostic window of the various marker proteins differs significantly with FABP being the preferred early marker and cTnI/cTnT the preferred late marker (Fig. 1) [2,3]. A panel of marker proteins, e.g., FABP together with cTnI or with cTnT, would cover the entire range and allow the proper diagnosis of patients with chest pain from almost immediately until more than a week after AMI onset. FABP has a further diagnostic advantage in that due to the early normalisation of its plasma concentration after AMI, a re-infarction (second AMI) occurring within a few days from the first event can be monitored from a new rise of plasma FABP, which is not the case for CK-MB nor the troponins as these would just remain elevated.

An early marker needs a rapid test

Because FABP, unlike CK-MB, is a non-enzymatic protein, its detection and quantification must be performed with an immunochemical assay. A large number of immunoassays for FABP have been described and have been successfully applied for retrospective analyses of plasma FABP in patient samples. However, the implication of FABP for clinical decision-making in the case of suspected AMI would require tests with a performance time < 10 min. For immunoassays this represents quite a challenge.

In June 1993, Jan Glatz presented his novel findings on FABP in the diagnosis of AMI for the first time to an international conference of the Belgian Society for Clinical Chemistry, held in Brugge, Belgium. He was approached by Dr. Hans-Georg Eisenwiener, head of the Research Division at Roche Diagnostics, Basel, Switzerland. In the same year they embarked on a collaborative project to develop a microparticle-enhanced turbidimetric assay to be performed on a conventional clinical chemistry analyzer (performance time 8 min) [4]. For this, specific monoclonal antibodies showing exceptionally high affinities for FABP were generated, and much attention was paid to standardization of measurements using recombinant human heart-type FABP produced under strictly controlled conditions. Both this turbidimetric assay and an extremely accurate and reproducible ELISA

Characteristics of plasma biomarkers for acute myocardial infarction (AMI)

Marker protein	Molecular mass (kD)	Elevation in plasma after AMI (h)	Peak plasma concentration (h)	Normalisation of plasma level * (days)
FABP	14.5	1 – 2	6 – 12	1 – 1.5
Myoglobin	17.8	2 – 3	6 – 12	1 – 2
Cardiac troponin I	22.5	3 – 8	12 – 24	7 – 10
Cardiac troponin T	37.0	3 – 8	12 – 24	7 – 10
Creatine kinase MB	86	2 – 6	12 – 24	2 – 3

* Dependent on (time of) reperfusion of the occluded vessels.

method for FABP have been instrumental to revealing the added value of plasma FABP as early biomarker for AMI diagnosis in a number of large trials. For instance, the European multicentre clinical trial EuroCardi, comprising 4 hospitals in 3 countries, revealed the superiority of FABP [5]. It unanimously revealed a significantly better performance of FABP over the other small biomarker, myoglobin, for early AMI detection as well as early estimation of infarction size. It also showed its advantages compared with the 'slow' troponins.

At this time, the German company Boehringer Mannheim offered immunochemical tests for myoglobin, CK-MB and also a test for the new cardiac marker, troponine T. The troponine test was a milestone in cardiology, developed by Dr. Hugo Katus in Germany. In spring 1998, Roche and Boehringer Mannheim merged. Roche decided after this merger to concentrate on the troponine T test taken over from Boehringer Mannheim. This was understandable as the TnT test was well-appreciated and well-introduced to physicians internationally already and had been adopted as a 'golden standard' by cardiologists. Unfortunately, the cardiac marker division of the new company (based at the former Boehringer plant in Mannheim) did not appreciate the performance of FABP, especially the added value for early infarction diagnostics, whereby the combination of FABP and TnT could markedly extend the diagnostic window. The consequence was that the turbidimetric FABP test that had been developed with so much care was not launched to the market. This regrettable event also made other (smaller) biotech companies to reconsider their efforts to build an FABP test.

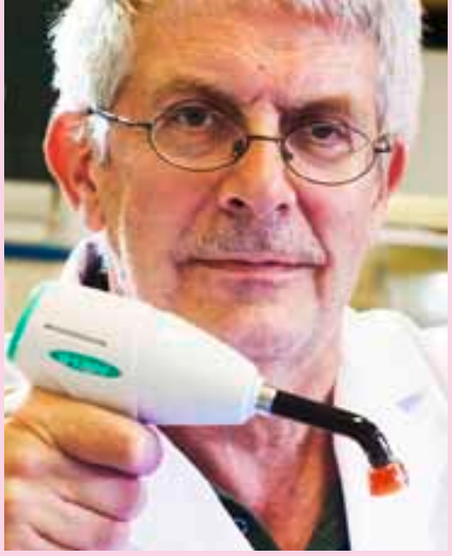
Biosensor technology for FABP

In the same period the Fraunhofer Institute of Chemical and Biosensors (ICB) in Münster had started a project to develop a biosensor for FABP. This project was supervised by Reinhard Renneberg who had moved in 1991, after German unification, from the Central Institute of Molecular Biology (Berlin-Buch) to the ICB to become head of the Department of Immunosensors. Like Jan Glatz, he immediately took an enthusiastic view of the new biomarker. His ICB-Department constructed an immunosensor-based instrument. This was the first bioelectrochemical approach worldwide to quantify FABP in plasma samples. Unfortunately, it could not work with full blood due to electrochemical interferences with red blood cells.

"Crisis means danger plus chance"

While the stage for FABP was far from promising, events developed internationally in different directions. This follows the Chinese character for "crisis" (Fig. 4) which wisely combines danger and chance. Reinhard Renneberg accepted a professorship at the Hong Kong University of Science and Technology (HKUST), currently (2012) ranking as the top Asian university. Together with Dr. John Sanderson of Prince of Wales Hospital and the small Chinese company EY labs Inc. he managed to get a government grant of 4.5 million HKD (450 k€) to develop a reliable and cheap full-blood FABP rapid test for the huge Chinese market.

In parallel, in Berlin-Buch (Germany) Ilka Renneberg started in 2000 the first FABP company, 8sens biognostic Ltd., targeting Europe. Together with the producer and distributor rennesens GmbH, they produced a credit card style FABP rapid test [6]. The



Reinhard Renneberg, born 1951, studied chemistry at Lomonossov University, Moscow. After obtaining a diploma he moved to the Zentralinstitut für Molekularbiologie (ZIM) [Central Molecular Biology Institute] in Berlin-Buch, where he acquired a doctorate in 1978 and became a Professor of Biosensorics in 1991. From 1991 to 1995 he directed the Immunosensorics Division of the Fraunhofer Institute for Chemical and Biosensorics (ICB) in Münster. In 1994 he followed the call of the Hong Kong University of Science and Technology (HKUST), becoming a Full Professor of Analytical Biotechnology. Professor Renneberg has also been active in setting up companies and as the Scientific Director of R&C Biogenius Ltd. He is the author of 'Bioanalytics for Beginners' and 'Biotechnology for Beginners', for which he won the Literary Prize of the Fonds der Chemischen Industrie [Chemical Industry Fund] in 2008.

Jan Glatz, born 1955, studied chemistry and biochemistry in Nijmegen and Utrecht, acquiring a doctorate in 1983. After a post-doctorate stay at the University of Wageningen he moved to the University of Maastricht in 1986, where he worked in the field of molecular medicine in conjunction with the lipid metabolism of the heart, in particular with the function and significance of fatty-acid-binding proteins. During this period he made the lucky discovery that the cytoplasmatic protein FABP is a biomarker for myocardial infarction. Glatz is Professor of Cardiac Metabolism at the Cardiovascular Research Institute of Maastricht (CARIM), University of Maastricht, Netherlands. His present research work is focuses on membrane substrate transporters and diseases of the cardiac metabolism.

card needed three hanging drops of blood and it took 15 min to get a reliable YES or NO result. The design was great in style, however, but quite expensive in production. 8sens is currently marketing a follow-up test, a simpler cassette-type rapid test for FABP and a second test for troponin I. In this way an early and a late marker can be evaluated at the same time and be quantified with a reader.

More recently, in 2008 the small biotech company FABPulous was started in the Netherlands (www.fabpulous.com). FABPulous combines an ultra-rapid manual plasma preparation method with a rapid and sensitive FABP lateral flow immunoassay thus providing a point-of-care test with a performance time of < 5 min, to be used in primary care especially to rule-out AMI in patients presenting with acute chest pain.

The Chinese character for 'crisis'.

Several other companies now also offer FABP plasma tests. For instance, Randox Laboratories Ltd. has included FABP in their Biochip Array Technology panel which enables the simultaneous assessment of multiple AMI biomarkers in a single patient sample using the MultiStat rapid testing platform. Results are available within 30 min. In China, Reinhard Renneberg launched in 1995 a university-based biotech company, Renneberg & Caughterley Biogenius Ltd (R&C Biogenius) at HKUST. It was supported generously by the Small Company Development scheme (SERAP) of the Hong Kong government. R&C developed cassette style tests for FABP, troponin I, CRP and neopterin, licensed and transferred the technology across the border to KSB (Shenzhen Kang Sheng Bao Bio-Technology Co, Ltd), a producer company in Shenzen (PR China). KSB and R&C Biogenius in turn applied for a Sino-FDA approval in Beijing which was granted in 2009. This means: the FABP test is now available commercially in China [7]. A quantitative version of the FABP rapid test is available worldwide except in the USA through Concile GmbH (Germany, www.concile.de) and 8sens.biognostic (Germany, www.biognostic.de).

FABP is more than a fabulous marker

FABP is a sensitive marker that detects minor myocardial injury like that occurring in selected patients with unstable angina pectoris. In a prospective study comprising more than 1,400 patients it was shown that FABP predicts long-term mortality after acute coronary syndromes (ACS, which includes AMI and unstable angina pectoris) and identifies patients at risk for subsequent cardiovascular events [8]. Importantly, FABP is able to distinguish between low-risk and high-risk patients across the wide range of plasma troponin values and at all parts of the ACS spectrum, thus showing that FABP offers independent prognostic information [8]. Similarly, other studies have disclosed that in patients with congestive heart failure plasma FABP identifies those at high risk for future cardiac events.

A new development in the field is the generation of high-sensitivity troponin assays (hsTnT and hsTnI). The limit of detection of these assays is about 10-fold lower than that of conventional assays. This suggests their application for diagnosing smaller AMIs otherwise undetected, or for identifying AMI earlier when abnormal troponin levels are too low for detection by conventional assays. While recent reports describe an improved performance of hsTnT when compared to conventional TnT, various studies have also demonstrated frequent elevation of plasma troponin in asymptomatic patients with stable coronary disease, pulmonary embolism, etc. These newer data challenge the application of hsTnT for triaging patients with acute coronary syndromes. Very recent studies have compared the performance of plasma FABP and hsTnT in the emergency room to conclude that FABP performs at least similarly, if not better, than hsTnT, and certainly earlier [9]. As a result, each of the biomarkers has its own characteristics, with FABP being the preferred marker to rule out AMI in the early hours after onset of symptoms and (hs)TnT or (hs)TnI the preferred marker from 3 hours onwards after presentation.

The future is golden

Despite the strong data available for FABP on its performance both as a biomarker for early triaging of patients with chest pain and as a prognostic marker for future cardiac

events, the use of FABP is not yet widespread. We predict that this will change in the near future, when more POC tests and tests for clinical chemistry analyzers become available. FABP will then be adopted as an early plasma marker to be applied besides a late marker such as cTnT or cTnI. In emergency care diagnostics the future focus will be on exclusion of AMI, as this comprises some 80% of patients presenting with chest pain. FABP is excellently suited for this purpose, and in this way will enable a major reduction of costs otherwise spent on hospitalization of non-AMI patients. Given the large numbers of patients who present with chest pain (for instance, in Germany ca. 400,000 annually) it is clear that FABP is destined for becoming a new Golden Standard for life saving.

Acknowledgement

We would like to thank Dr. Hans-Georg Eisenwiener for continued support and stimulating discussions.

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Bibliography

[1] Glatz, J.F.C. et al. (1988) Biochim Biophys Acta 961: 148–152.
[2] Chan, C.P. et al. (2004) Z Kardiol 93: 388–397.
[3] McMahon, C.G. et al. (2012) Am J Emerg Med 30: 267-274.
[4] Robers, M. et al. (1998) Clin Chem 44: 1564–1567.
[5] Pelsers, M.M.A.L., et al. (2005) Clin Chim Acta 352: 15–35
[6] Liao, J. et al. (2009) Int J Cardiol 133: 420-423.
[7] Xie, P.-Y. et al. (2010) J Immunoassay Immonochem 31: 24-32.
[8] Kilcullen, N. et al. (2007) J Am Coll Cardiol 50: 2061–2067.
[9] Inoue, K. et al. (2011) Circ J 75: 2813–2820.

Personal case report

"At 3 o' clock on a Sunday very early morning I felt suddenly a strong chest pain. It did not cease. It irradiated to my heart, my left arm got numb...the classical heart attack symptoms! Ironically, I am 'praying' about these symptoms all the time to my 500 Chinese bioanalytics students at HKUST and in exams always ask them about these symptoms. Now, for the first time, I could watch the symptoms on myself and – most important – in "real time". Great for a passionate experimental scientist like me. So, not being scared at all, even in "good mood", I called the Hong Kong Hot Line for emergencies (1). In the 12 minutes time before the ambulance arrived, I tried our new rapid FABP test. A great experiment. When I arrived at hospital with the ready test, there was no dispute about the diagnosis and I got treated immediately. As you can witness today: I am still alive. Thanks to our new test, thanks to the plasma marker FABP, it saved my life. "

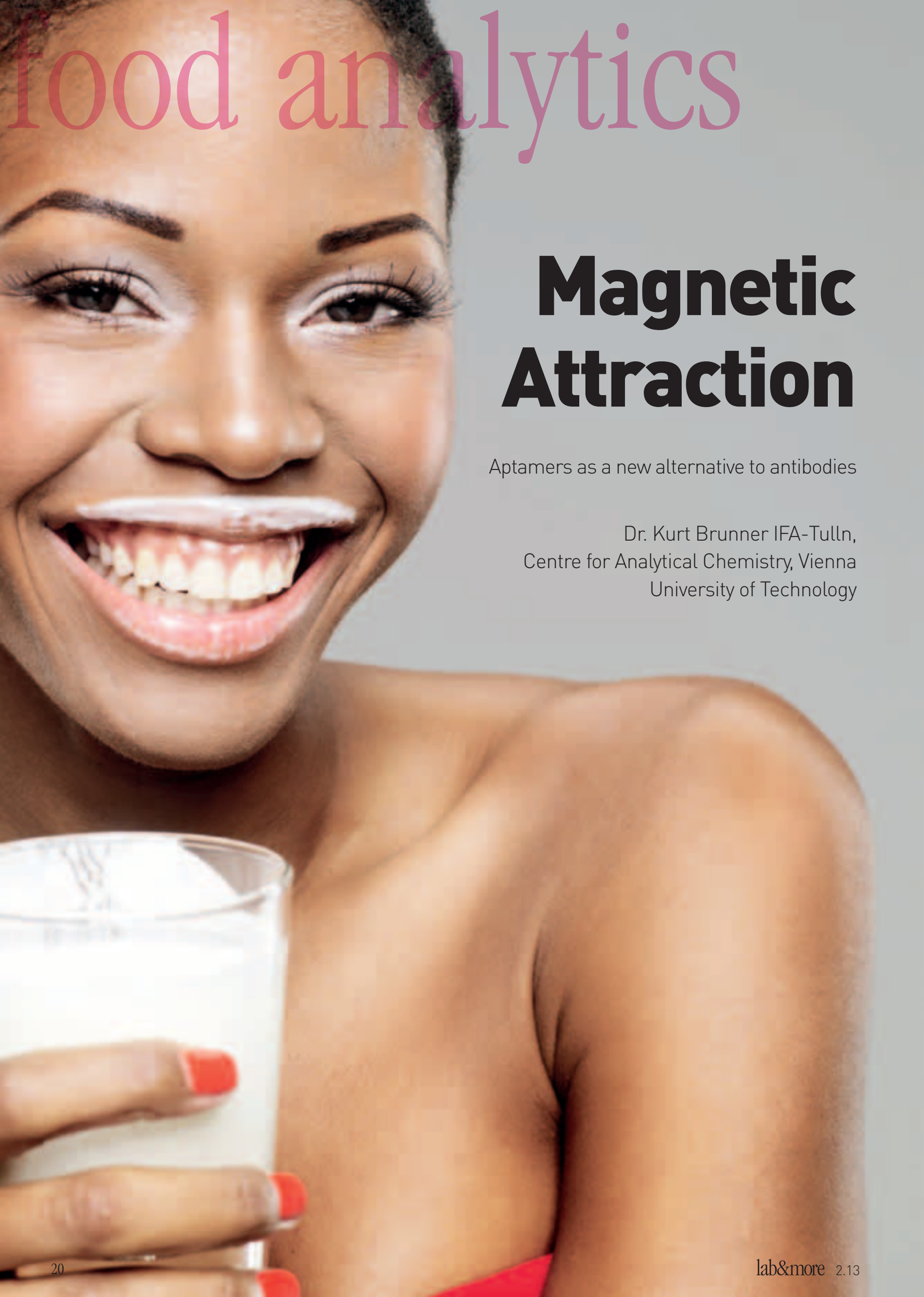
Very lucky!

What better can happen to a scientist than a successful self-experiment?

Reinhard Renneberg, Hong Kong 2008

YouTube

click&watch
www.youtube.com/watch?v=R4w0FK5aGLU



Magnetic Attraction

Aptamers as a new alternative to antibodies

Dr. Kurt Brunner IFA-Tulln,
Centre for Analytical Chemistry, Vienna
University of Technology

Within the European Union, measures to safeguard our food are becoming increasingly stringent: any constituents hazardous to health are either prohibited or permitted only as a trace. For analytics, ensuring compliance and monitoring for all of the statutory provisions safeguarding our food is a hugely challenging task. While immunotests have now been deployed successfully in rapid testing for the last three decades, aptamers have demonstrated increasing potential as a new alternative to antibodies in recent years.

Driven by the high standards demanded by food manufacturers and regulatory authorities, food analytics is pushing into the realms of ever-smaller concentrations: the reliable detection of trace amounts of antibiotics, mycotoxins, pesticides, allergens, hormones and other undesirable substances is already mandatory. In satisfying these requirements, analytics pursues two separate strategies. The first is to deploy high-end methods for trace detection: modern LC-MS applications form the most obvious example of this approach. While these methods represent the peak of sensitivity, they also have an undeniable disadvantage: since the equipment required is extremely expensive and must be operated by specially-trained personnel, this effectively rules out true on-site testing. While samples for analysis are taken on-site, the actual analytical procedures are performed in an appropriately-equipped laboratory.

The rise of rapid tests

To overcome this obstacle, rapid tests have seen increasing deployment over the last few years. These simple test kits are easy to use, very cost-effective and can be performed in a minimally-equipped laboratory or sometimes even carried out on-site. While sensitivity is generally inferior to that of the reference methods, it is still adequate to comply with legal requirements. To develop rapid tests, a common approach is to exploit biomolecules that have the ability to detect a certain target molecule specifically. Antibodies offer the most familiar example of molecules that have highly specific binding properties. Originating in the first immunotests deployed for medical applications, a set of antibody-based rapid tests now dominates application areas throughout analytics. Over the last few decades, rapid immunotests ranging from small molecule and protein testing through to the detection of viruses and microorganisms have been developed and launched on the market. The test delivery systems are generally as varied as the range of molecules targeted. The simplest systems, mostly designed for qualitative analysis, are lateral flow devices (LFDs) or "test strips". Enzyme Linked Immunosorbent Assays (ELISAs) are capable of very high levels of quantification, while modern biosensors produced in recent years represent the cutting edge of immunotest development.

DNA and RNA aptamers as an alternative to antibodies

For a little over a decade, the volume of articles published about aptamers as a new alternative to well-established antibodies has continued to rise. The term "aptamer" is derived from the Latin word "aptus" (= suitable) and the Greek word "meros" (= part) and illustrates the "lock-and-key" relationship between aptamers and their binding partners. Molecules deployed as aptamers are short nucleic acid fragments: either RNA or single-stranded DNA. Their special three-dimensional structure is responsible for their specificity and affinity for a target molecule. The actual binding to the target is effected by a combination of the steric properties, in association with reciprocal electrostatic effects and hydrogen bridge bonds. The procedure deployed for deriving aptamers for a specific target molecule is termed SELEX. SELEX is short for Systematic Evolution of Ligands by Exponential Enrichment and designates the targeted in-vitro evolution of aptamer candidates to derive optimal binding partners. The starting-point for this procedure is a random library of single-stranded nucleic acids. As a rule of thumb, this library should contain no less than 10^{14} separate combinations of the four bases. On account of the many options for structural formation, this approach should

Kurt Brunner, born in 1973 graduated in Technical Chemistry from TU Vienna before obtaining his doctorate from the University's Institute of Chemical Engineering in 2003. While preparing his thesis, he worked on the molecular biology of fungi. Following research work conducted at the University of Naples, he then participated in several projects investigating plant-pathogen interactions as a postdoc at the Vienna University of Natural Resources and Life Sciences and TU Vienna. He has headed the "Molecular Diagnostics" working group at the Department for Agrobiotechnology (IFA-Tulln) since 2008. His activities generally focus on the development of DNA-based test assays for use in food and water analytics.

therefore yield a matching nucleic acid fragment for almost any target molecule. This random sequence is flanked by a constant region at its 5′- and 3′-ends, which later serves as the binding site for PCR primers. This nucleic acid library is then brought into contact with the immobilised target on the carrier medium. This "fishes out" all of the library fragments capable of binding more or less well to the target. These interacting fragments are then multiplied using a PCR procedure on the constant region and subjected to a further round of selection. This process is repeated until the only fragments remaining are the ones that bind most tightly.

To date, the SELEX procedure has now synthesised aptamers matching almost every kind of analyte: bacteria and viruses, polysaccharides, proteins, small molecule substances – even atoms do not present a problem. Since aptamers are enriched in vitro, even highly toxic targets – for which antibody production is a non-trivial procedure – present no difficulties. Two online databases [1, 2] provide the ability to search for previously-discovered aptamers. They provide details of the nucleic acid sequence and offer additional information about the SELEX procedure parameters and association-rate constants for each of the binding partners.

Outstanding selectors

Compared to antibodies, aptamers have a number of advantages, although these are tempered by limitations. In terms of sensitivity, antibodies and aptamers occupy very similar ranges of values, notwithstanding the fact that the maturity of immunoassay technology makes the latter preferable for detecting very low concentrations. Aptamers have the major advantage of offering outstanding selectivity, however: since the nucleic acid fragments are matched to their target during a complex process of in vitro evolution, undesirable cross-reactions can be virtually excluded. The option of applying the counter-selection technique during the SELEX procedure enables the active exclusion of aptamer candidates with undesirable binding properties from the fragment pool. Just over a decade ago, when aptamer development was still in its infancy, RNA aptamers were a common deployment option. While RNA's multitude of folding options offers substantial benefits when compared to DNA, a high price is paid for this advantage – in the form of substandard stability. In practical terms, however, it is precisely the insensitivity of DNA aptamers to external factors that constitutes their major benefit. DNA is a highly stable biomolecule, capable of surviving unscathed even after exposure to heat, cold, atypical pH environments and high salt concentrations. Hopes for a bright future for aptamers rest precisely on this stability. Even prolonged storage is unable to substantially weaken aptamer activity. Aptamers also offer a further key benefit: they can be synthesised at short notice, both simply and inexpensively. Once its sequence is known, a structurally identical aptamer can be obtained commercially from any manufacturer of oligonucleotides. This not only eliminates batch-to-batch

SELEX – the artificial evolution of aptamers

Aptamers are manufactured using the SELEX procedure. This process involves the iterative improvement of DNA or RNA fragment matching to the target. All SELEX variants share a common starting-point: a library of nucleic acids with random base sequences. The method used for further selection of the highest-affinity fragments can then vary considerably, however. The FluMag-SELEX procedure (Fig. 1) has proved itself to be a particularly uncomplicated and flexible approach, and is now the most widely-available option for generating aptamers for specific analytes.

With this method, the target to which aptamers should bind is immobilised on the surface of magnetised particles. Once functionalised, the particles are then combined with the nucleic acid library and fragments with affinity then bind to the target. A magnet can now be used to separate and rinse off the particles with the first round of candidates. In the next step, the handful of fragments so isolated can be multiplied using PCR and the two strands separated. While we now once again have single-stranded nucleic acids, only those candidates from the initial library with target affinity now remain. The next SELEX round is now started: this time, more stringent conditions are imposed, so as to force further adaptation of potential aptamers to the target. Multiple iterations of this procedure are used until the remaining aptamers all show exceptional binding to the analyte. The last surviving fragments are cloned using plasmids and sequenced.

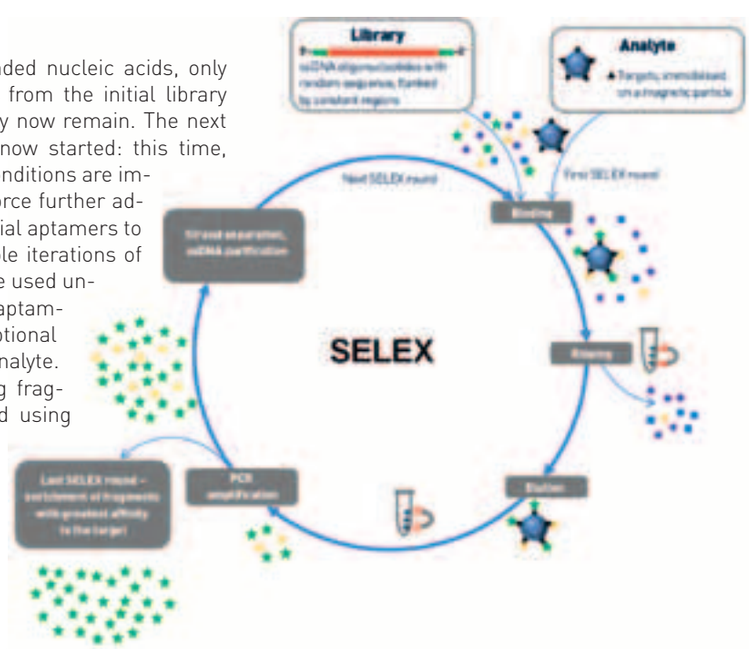


Fig. 1 Overview of the FluMag-SELEX procedure. In recent years, this particular SELEX method has prevailed against other variants, since most targets can be very easily coupled to magnetic particles. Separation of the bound nucleic acid fragments simply requires the use of a permanent magnet.

variations resulting from immunisations but also avoids the need to use animal subjects for antibody harvesting.

Aptamers have been used to develop test formats similar to the forms long since typically used in immunoassays. It is a very simple matter to conjugate aptamers covalently to colloidal gold particles. Such functionalised gold particles have now been developed into a wide range of LFDs for rapid qualitative analysis. A test format essentially reproducing the ELISA technique is also available for quantitative testing. This format, known as an Enzyme Linked Aptamer Assay (ELAA), involves the deployment of an immobilised capture aptamer to "fish" an analyte out of a sample, whilst a second, enzyme-conjugated detection aptamer is deployed as a catalyst for a colour reaction. Since DNA fragments exhibit much simpler and much more widespread surface binding properties than antibodies, this approach opens up a broad spectrum of possible applications for biosensors. Antibodies generally bind to surfaces only via adsorption, whereas aptamer conjugation is generally covalent. The strength of covalent bonds permits the simple regeneration of biosensors. After binding, the analyte can simply be rinsed off the surface of the sensor – even under the harshest environmental conditions.

All of the test formats described above have already seen deployment in food safety detection procedures. Here, aptamers have established themselves in two quite separate disciplines, namely: confirming the presence of pathogens in food and the detection of toxic substances. Table 1 gives an overview of the aptamers developed to date for use in food analytics.

Overview of aptamers developed to date and their corresponding test systems. - Other details of these aptamers, including their sequences and original literature, can be obtained by consulting the aptamer databases [1, 2].

Target class	Target	Aptamer type
Bacteria	Bacillus thuringiensis	DNA
	Campylobacter jejuni	DNA
	Escherichia coli	DNA & RNA
	Francisella tularensis	DNA
	Listeria monocytogenes	DNA
	Salmonella spp.	DNA & RNA
	Staphylococcus aureus	DNA
	Yersinia	RNA
Toxins	Aflatoxin	DNA
	Ochratoxin A	DNA
	Ricin	DNA
Antibiotics	Chloramphenicol	RNA
	Tetracycline	DNA & RNA
	Streptomycin	RNA
	Tobramycin	RNA
	Kanamycin	RNA
	Neomycin	RNA
Pesticides	Atrazine	DNA
	Malachite green	RNA
Inorganic ions	Arsenic	DNA
	Mercury	DNA

The future of aptamers

While many aptamers with the potential for use in foodstuff analysis have been developed to date, commercial exploitation is still a rare occurrence. Since aptamer technology trails immunotest development by about a couple of decades, the key application areas for rapid tests are already occupied by antibody-based detection systems exhibiting a very high degree of optimisation. While aptamer tests must often contend with teething troubles – a frequent criticism is their relative fragility – this class of molecules still offers more development potential than any other technology for the next few years. The unique properties of these nucleic acid fragments benefit the burgeoning field of sensor technology in particular. No other biomolecule can be modified so simply in order to bind it to sensor surfaces – nor at such a low price point. In addition, the stability offered by DNA vis-à-vis external factors permits the regeneration – and hence multiple use – of sensors. The challenge for both researchers and commercial stakeholders is to further improve the wide range of test formats for known and newly-discovered aptamers, while also focusing in particular on robustness for real-world applications.

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Bibliography:
1] The Ellington Lab Aptamer Database: http://aptamer.icmb.utexas.edu
[2] Aptamer Base: http://aptamer.freebase.com/

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green chemistry

Valuable Waste

Biowastes: a new feedstock for the chemicals industry

Dr. Mark Gronnow and Dr. Margaret Smallwood
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The chemicals industry has been wedded to fossil resources for the past hundred and fifty years, but times are a-changing. As the price of oil increases inexorably and environmental impact rises on the policy and regulatory agenda, industry leaders are starting to exploit new, more sustainable feedstocks for the manufacture of fuel and chemicals. Squarely in the frame as potential feedstocks are the vast tonnages of biowastes produced each year from agriculture, brewing and food processing.

Living on a current account

The world's fossil reserves represent stores of "old sunlight" accumulated over millions of years by plants photosynthesising, fixing carbon and deliquescing. We can view these reserves as a deposit account that the humanity has been plundering like teenagers let loose with a parental credit card. The reserves are diminishing and we need to start living on our current account: energy and materials that are renewable year on year.

Whilst there are a range of potential sources for renewable energy, for instance wind, wave, sunlight, geothermal, the resources for chemical manufacture are restricted to carbon fixed by living organisms. The biowastes arising from production of food for the 9 billion people that are anticipated to inhabit our planet by 2050, look an attractive option for producing the chemicals of the future.

Market drivers

The production of chemicals from biorenewable resources is not new: the International Energy Agency (IEA) estimates current annual production of bio-based chemicals and polymers at around 50 million tons [1]. Biorenewables could represent nearly 40% of bulk chemicals by 2050 under favourable market conditions.

The use of biowastes rather than agricultural crops as feedstock both reduces the costs of manufacture of biorenewable chemicals and enhances their sustainability because they are not competing with food production for agricultural resources. Furthermore, it simply seems more sensible to extract molecules which nature has assembled rather than rebuilding chemical structures.

Companies are looking for ways to improve their environmental footprint for reasons of corporate responsibility and compliance with regulations on emissions. Equally important, as the environment moves up the agenda of consumers, green credentials can add value to their brand.

As oil prices rise, these issues come together to motivate the search for alternative, cheaper and more sustainable feedstocks for bio-renewable chemicals – bio-wastes lie squarely in this frame.

Biowaste resources

The volumes of waste arising from agriculture and food production are significant (see table 1 for examples) and they represent a huge reservoir of valuable compounds. For instance the skins of fruit and vegetables are usually discarded, but they are rich in flavour, fragrance and bioactive compounds.

Although many biowastes are mixed, the food industry also produces a number of single-component waste streams. These are the low hanging fruit for processing into high value chemicals. Examples include coffee grounds which are being converted into boards and soil improvers, and brewery wastes which can be transformed into energy products or used as a source of food additives.

Disposal of straw from small grains is a major source of land and air pollution globally. However wheat straw, like many agricultural products, contains a range of valuable compounds including natural waxes. Waxes have uses in products ranging from surface coatings to cosmetics and the compounds found on the surface of wheat straw have properties comparable to a number of waxes currently in widespread use. Technology is already being commercialised to convert the cellulose in straw into ethanol but there is also potential to use the five carbon pentose sugars in the hemicellulose fractions for the manufacture of bulk chemicals such as citric acid.

The first generation biofuel installations produce significant quantities of waste. For instance ten tons of biodiesel makes over a ton of glycerol by-product; bioethanol fermentation from grain creates seven kilograms of dried distillers grains with solubles (DDGS) for every ten litres of ethanol. These waste materials often go to relatively low value applications such as anaerobic digestion for energy but they have potential as feedstocks for the chemicals industry.

Companies are already investing in facilities to exploit waste streams for chemical production. For instance, Archer Daniels Midland is developing a portfolio of chemicals fed by renewable raw materials. They have commissioned a 100,000 ton propylene glycol plant in Illinois which will use glycerol from soybean and canola oil production as feedstock.

Examples of biowaste resources

Waste	Tonnage	Potential Applications
Tomato waste	4.4 mt (Europe) [2]	Production of high value carotenoids, proteins, sugars, fibre and oils
Coffee bean waste	6 mt (Global) [3]	mesoporous carbon, soil improvers, building boards, energy, biofuel,
Agricul-tural biomass Waste	1.4 x 1011 mt (Global) [4]	Fine and bulk chemicals, textiles, paper products, cordage, upholstery and packaging materials, animal feed, insulators and panel boards, energy, biofuels

Interdisciplinary collaboration for technology development

Development of technology for conversion of biowastes into chemicals requires expertise in chemistry, biology and engineering. An interdisciplinary grouping of some 200 scientists focused on biorenewable technology has been established at York in the UK.

At the University of York, the Green Chemistry Centre of Excellence (GCCE) has a track record of working successfully with industry and is internationally recognised for its research on catalysis, materials chemistry, novel reactor technology, platform molecules and clean solvents.

GCCE have established a close collaboration with the Centre for Novel Agricultural Products (CNAP), an award winning, research centre based in the Department of Biology that focuses on research underpinning the development of plants and microbes as green factories

To enable commercialization of their research the two centres have worked with ETDE, a process engineering company, to establish the Biorenewables Development Centre. This is a not-for-profit company offering open access scale up facilities that industry can use to develop processes for converting plants and biowastes into high value products.

- → **www.york.ac.uk/chemistry/research/green/**
- → **www.york.ac.uk/biology/centre-fornovelagriculturalproducts/**
- → **http://www.biorenewables.org/**

Maggie Smallwood, born in 1957, studied Biochemistry and Microbiology at the University of Leeds and completed her doctorate in the Department of Biochemistry, also at the University of Leeds. After a post-doc at the University of Leeds she worked as research manager in a spin-out company from the University of York and then led a strategic research programme at the University of York focused on antifreeze proteins. Since then she has held positions with a number of public private research and development programmes. She is currently employed at the Biorenewables Development Centre as Research Development Manager.

Mark Gronnow, born in 1979, studied Chemistry at the University of York and acquired a doctorate in 2005 at the Green Chemistry Centre of Excellence. He is a PhD graduate from York with a GSK sponsored project. Having previously worked at the Green Chemistry Centre of Excellence as technical operations manager and at YorkTest Laboratories as technical manager, he is currently the Biorenewables Development Centre (BDC) Process Development Unit Manager. Within the BDC Mark is responsible for the process equipment, supporting new users in developing their application on the wide range of facilities whilst coordinating the best technical and academic support, in addition to ensuring that the equipment is safe to use, regularly serviced and available for users. His research interests focus on biorenewables, biorefining, bioenergy, chemicals from waste/biomass, microwave chemistry, green chemistry and novel extraction systems. He is member of the Royal Society of Chemistry.

Photos: Biorenewables Development Centre (BDC)
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There are also thermochemical options for conversion of lignocellulosic wastes into chemicals or energy products. Torrefaction offers an attractive route to manufacture of a solid energy carrier, biochar. This has a higher energy density than the unmodified biomass and is easier to handle and burn. At higher temperatures (ca. 500°C) pyrolysis can be applied to form liquid energy products such as heavy heating oil or chemicals such as phenols, furans or anhydro- sugars. Research is underway on methods to upgrade these so called "bio-oils" into liquid transport fuels for road and aviation.

New chemical technologies

New technology is needed for reliable extraction, separation and transformation of chemicals from biowastes. Substances of interest are often present at low concentrations in aqueous mixtures or are immobilised in complex bio-polymers.

This challenge has been recognised by the research community and technology is being developed that can up-cycle complex biowastes into chemicals and materials.

Ideally, we would like to access compounds through "natural" solvents such as water, ethanol, and carbon dioxide using minimal energy. CO_2 is particularly useful as a selective extractant for a range of high value natural compounds. It is also a highly tuneable solvent for chemical reactions, particularly those that are enzyme-mediated, as it offers excellent mass transfer with simple product work-up. Software is now available for modelling which "green" and bio-derived solvents are most favourable for particular reactions.

Microwave technology is also finding a place in valorising biowastes. It offers a rapid, flexible, energy-efficient heating method, well adapted for continuous processing to produce liquid and solid fuels as well as chemical products. The microwave system offers a more flexible and lower temperature thermochemical conversion option than those described above (ca. 200°C). The equipment can be installed on mobile units enabling transport between different waste producers. Microwaves can promote novel reaction pathways and accelerate the rate of chemical reactions.

White biotechnology

New industrial biotechnology techniques are critical for the successful exploitation of biowastes for high value chemicals. Scientists now have high throughput "omics" tools that allow them to select strains of micro-organisms that catalyse reactions of choice.

Fig. 1 Arabidopsis plant seedlings

Fig. 2 Hemp oil

Fig. 3 Hemp seed

For instance *Aspergillus* is a versatile filamentous fungus that is capable of metabolising a range of substrates commonly found in waste streams from bioprocessing industries. Researchers from the Centre for Novel Agricultural Products at the University of York together with the BDC and a small technology company, Citration Technology Ltd, have been using genomics to work up potential routes for the production of commercially attractive industrial chemicals using *Aspergillus*.

Most agricultural crops have been optimised for production of food or feed. Scientists are now using molecular breeding approaches to develop crops with improved bio-wastes – for instance straw that is more easily digested to allow its use for production of biofuel and chemicals.

Innovation

Whilst imaginative science has been applied to develop ingenious technology in the laboratory, too often the ideas remain in academic journals rather than being implemented in the real world. Facilities like the Biorenewables Development Centre, adjacent to the University of York in the UK, aim to bridge this gap between laboratory research and commercial application – a gap known as the "valley of death" amongst innovation specialists.

The BDC's open-access facilities are designed for scale up novel technologies to produce an amount of material that industry can test in their own products. Equipment is arranged in a modular fashion and can take crude raw material and refine it through a diverse set of processes. It offers access to analytical and processing technology that industry, especially SMEs, could not otherwise access.

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Bibliography

[1] Biobased chemicals: Value-added products from biorefineries, Report from Task 42, Biorefinery, IEA Bioenergy. Ed DeJong, Adrian Higson, Patrick Walsh, Maria Wellisch

[2] Gerald Ondrey Chemical Engineering. 2004. (http://www.highbeam.com/doc/1G1-114404968.html).

[3] Tokimoto, T., et al. (2005), Removal of lead ions in drinking water by coffee grounds as vegetable biomass, J Colloid InterfSci 281, 56–61

[4] http://www.unep.org/ietc/Portals/136/Publications/Waste%20Management/WasteAgriculturalBiomassEST_Compendium.pdf

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Case study: A greener orange

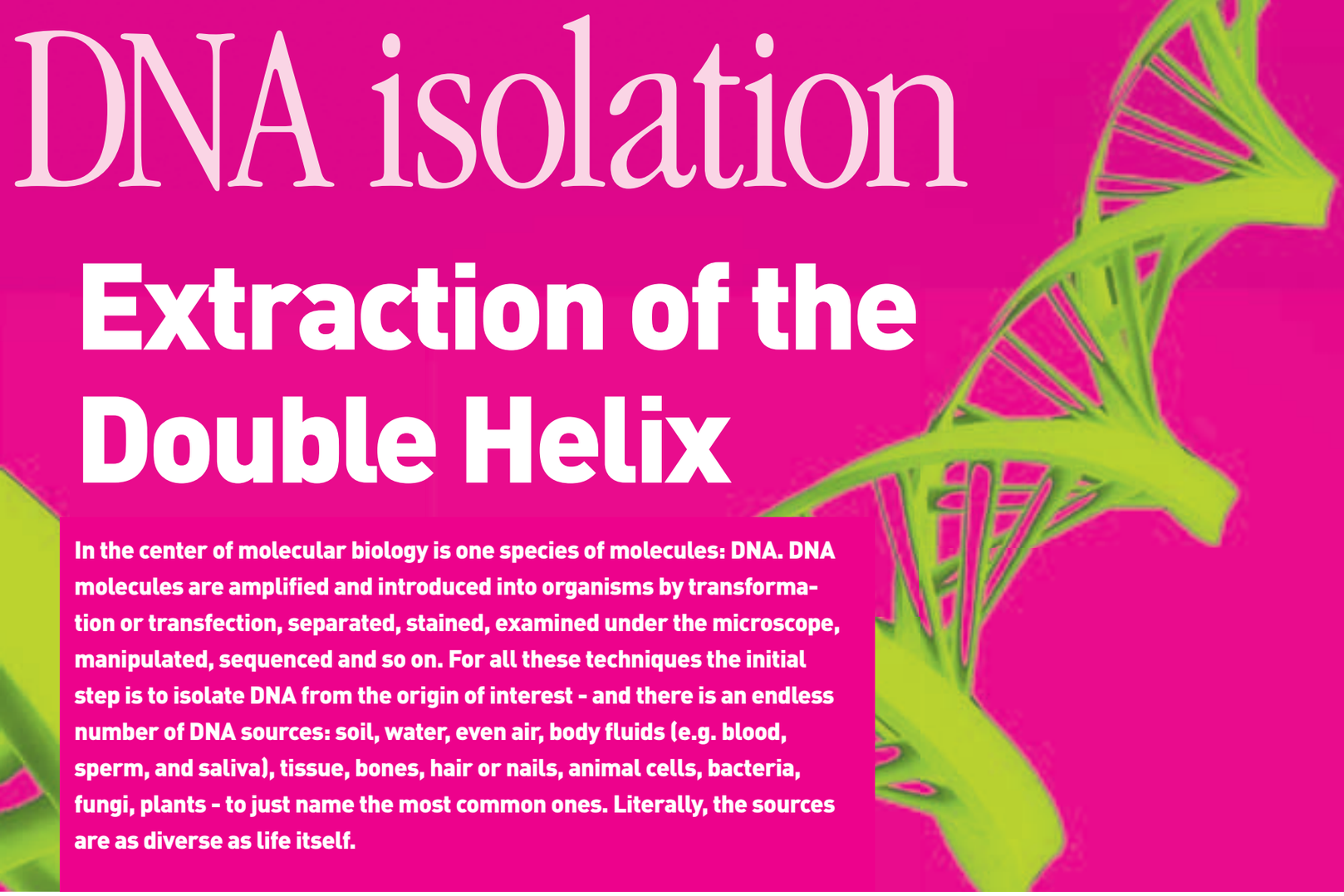
Oranges are an excellent example of a wasted bio-resource: after extracting the juice, around half the fruit is discarded. It is estimated that, in Brazil alone, eight million tons of waste peel is discarded every year. And yet a wide range of commercially valuable compounds can be produced from orange peel.

Researchers in the Green Chemistry Centre of Excellence at the University of York, UK, have joined forces with researchers at the Universities of Sao Paolo and Cordoba to establish the Orange Peel Exploitation Company (OPEC). OPEC is a "zero waste" biorefinery project that will use low temperature microwave technology to extract a range of compounds. Higher value targets include d-limonene, which is a widely used additive in domestic products, and mesoporous carbons that can be used as water purifiers as well as commodity chemicals such as cresol. Residual biomass has potential to be fermented for production of biofuels.

Technology developed by the Green Chemistry group is being scaled up within the Biorenewables Development Centre.

DNA isolation

Extraction of the Double Helix



In the center of molecular biology is one species of molecules: DNA. DNA molecules are amplified and introduced into organisms by transformation or transfection, separated, stained, examined under the microscope, manipulated, sequenced and so on. For all these techniques the initial step is to isolate DNA from the origin of interest - and there is an endless number of DNA sources: soil, water, even air, body fluids (e.g. blood, sperm, and saliva), tissue, bones, hair or nails, animal cells, bacteria, fungi, plants - to just name the most common ones. Literally, the sources are as diverse as life itself.

What does it mean technically when we talk about DNA extraction, isolation or purification - terms often used synonymously for getting preferably pure DNA from a sample? What are the mechanisms behind?

In this article we focus on the isolation of genomic DNA. We give an overview about established DNA isolation techniques, their chemical background and we discuss their respective advantages and limitations. Regarding the basic procedure, DNA extraction is simple and can be done using domestic products. Basically, all you require is a rich source of DNA, salt, water, dishwashing detergent, a coffee filter, high-proof alcohol and a stick to spool the precipitated DNA salt out of solution. For higher demands (regarding quantity and quality), of course, the method requires further refinement. Purity and integrity of the DNA will affect the results of all subsequent applications, so highest quality of DNA is desirable for diagnosis and research.

Not only at home, but also in the lab, the isolation process steps are straightforwarded: First: destroy the cells and bring DNA into solution. Second: remove impurities by non-affinity (DNA remains in solution while everything else is removed) or affinity (DNA selectively binds to a solid matrix) based techniques.

It might be necessary to initially dissociate cells from the sample material (e.g. in case of tissue, bones, and plants). This procedure varies from sample to sample and uses almost every physical method that is finally resulting in a mash of DNA-containing material, including cutting, enzymatic treatment, mixing (using glass beads for example), grinding and powdering in a mortar, freezing in liquid nitrogen and so on. We will skip these details and, for the following pages, assume to have a nice fresh pellet of bacterial cells as extraction source.

1. The lysis

Even if DNA molecules can literally be found in the streets, the DNA of interest is typically enclosed in a cell. To get the DNA, a process called lysis (Greek λύσις, lýsis from lýein "to separate") is required: cell wall (if present) and membrane are broken off, allowing everything formerly locked in the cell to be released into solution: DNA, RNA, proteins, lipids, and metabolites. Lysis buffers for genomic DNA commonly include a detergent, sodium chloride, EDTA and enzymes to degrade proteins and RNA.

Ionic detergents such as SDS strongly destabilize the lipid bilayer of the cell membrane. Solubilizing the membrane lipids, they finally force the breakup of the cellular structure and support precipitation of lipids and proteins. EDTA assists destabilization of the cell wall, but its main task is to inhibit DNase activity by chelating divalent cations such as Mg^{2+}.

When it comes to protein degradation, proteinase K is the most common protease directly employed in the lysis solution. Conditions that promote enzyme inactivation like detergents, chaotropic salts, high temperature and changes in pH are well tolerated by proteinase K. Beside its high stability, proteinase K is characterized by a large number of cleavage sites and therefore perfectly suited to remove cellular and nuclear proteins that are attached to the DNA. Also RNA is degraded enzymatically. The ribonuclease RNase A, which hydrolyses RNA molecules into single nucleotides is heat stable as well, and, like Proteinase K it has no need for cofactors that might be complexed by EDTA.

In contrast, other enzymes such as lysozyme do not withstand the lysis conditions. Lysozyme effectively digests the bacterial cell wall (composed of peptidoglycane), but the enzyme is inhibited by surface-active agents like SDS. This is why for isolation of DNA from (gram-positive) bacteria the lysozyme treatment is performed prior to lysis.

2. Methods of DNA Isolation

When isolating DNA, the aim is of course to achieve maximum purity and quantity in a minimum of time with a minimum of

costs. Unfortunately achieving all of this is rarely possible. Different methods of DNA isolation are employed, depending on sources, type of DNA (genomic, plasmid or organelles, e.g. mitochondrion), sample size, sample age and required purity. But not every method is suitable for every downstream process. The most important DNA isolation techniques today are commercial solid-phase based kits (columns with silica or anion exchange matrices), phenol/chloroform extraction and usage of monophasic reagents followed by ethanol precipitation.

The traditional method: phenol-chloroform extraction

First described in 1956, the purification of nucleic acids using phenol to remove hydrophobic impurities is still widely employed today. Phenol-chloroform extraction is cheap but effective. Followed by ethanol precipitation to remove salt and organic solvent impurities, the method yields high quantities of pure DNA. On the other hand, the procedure is time consuming, requires multiple tube transfers that bear the risk of contaminations (by foreign nucleic acids but also by phenol/chloroform carry-over) and involves hazardous chemicals.

But still, the phenol extraction is often the method of choice for very small as well as large DNA fragments, and for old, partially degraded DNA. Polar, hydrophilic compounds like DNA, RNA and proteins commonly dissolve best in polar solvents (with water as the solvent offering maximum polarity). But in contrast to nucleic acids, proteins provide a number of non-polar structures as well. The non-polar side chains of phenylalanine, leucine, isoleucine, valine, proline, methionine and alanine enable the protein to stay in solution when exposed to a less polar or even non-polar solvent. The proteins rearrange exposing the non-polar side chains to the surface, while the charged and polar residues become buried inside the protein complex. These features enable the extraction of proteins out of an aqueous phase by a less or even non-polar solvent. Phenol is clearly less polar than water despite its electronegative oxygen atom; because the phenyl ring renders the electron density spread all over the molecule but not concentrated on the oxygen atom.

For DNA isolation, the phenol has to be pH-equilibrated with tris to a final pH of >7.8 to ensure that the DNA is negatively charged and therefore insoluble in the organic phase. Starting with the cell lysate, an equal volume of tris-buffered phenol-chloroform (1:1), or tris-buffered phenol-chloroform-isoamyl alcohol (25:24:1) is added and the solution is mixed by vortexing (small DNA molecules of <10 kb), gently shaking (10-30 kb) or slowly inverting or rotating (>30 kb). Chloroform efficiently denatures proteins, avoids the retention of water in the organic phase and improves the phase separation by increasing the density of the organic phase. The addition of a small volume of isoamyl alcohol reduces foaming during the extraction process, and, aiming at RNA isolation, guarantees the deactivation of RNases (Green & Sambrook, 2012).

Centrifugation accelerates the separation of the two phases, resulting in the aqueous phase with the lower specific gravity on top. But caution: A high salt content or high amounts of sucrose in the aqueous solution can result in an inversion of the two phases! So it is highly recommended to make sure the right solution is further processed. Since equilibrated phenol commonly contains 8-hydroxyquinoline as a stabilizer, the organic phase can be identified by its yellow color. After organic extraction, DNA (and RNA, if no RNase A has been added during lysis) is still in the aqueous phase while denatured proteins have moved into the interphase and lipids have been transferred to the organic phase.

After repeated extractions with the phenol-containing solvent, the DNA-containing aqueous phase is extracted one or more times with chloroform (or chloroform/isoamyl alcohol), to remove residual phenol. Finally, the pure DNA is precipitated from solution using ethanol or isopropanol.

The all in one solution: acidic guanidinium thiocyanate-phenol extraction

Admittedly, this technique does not really aim at DNA isolation in the first place, but nevertheless it is possible to use it on this purpose. And since this technique offers interesting possibilities it should be mentioned in this article. Invented by Chomczynski & Sacchi in 1987 for isolation of RNA, acidic guanidinium thiocyanate-phenol extraction allows cell lysis and successive separation of RNA, DNA and proteins using one reagent. The technique combines the effect of chaotropic salts on structure and solubility of macromolecules with the extraction properties of acidic phenol. Commercialized under names like TRIzol®, TRI REAGENT® and TRItidy™, Chomczynski's reagent conquered the laboratories and still belongs to the number one choices for RNA isolation.

The monophasic solution contains water, phenol, guanidinium thiocyanate (also often referred to as guanidine thiocyanate), β-mercaptoethanol and a detergent. The chaotropic salt guanidinium thiocyanate lyses the cells, denatures the released macromolecules and inactivates RNases and other enzymes. As a detergent, lauroylsarcosine ("sarkosyl") is a good choice, since, in contrast to SDS, lauroylsarcosine shows high solubility in chaotropic high salt buffers. Addition of sarkosyl to cell or tissue homogenates improves the purity of the RNA isolated by guanidinium salts and reduces foaming during homogenisation (MacDonald *et al.*, 1987).

After lysis of the cell in the reagent (e.g. by repetitive pipetting), chloroform (or alternatively bromochloropropane) is added, leading to the generation of a second phase. While DNA and proteins enrich in the newly

Products for Phenol-Chloroform Extraction of DNA	
Phenol equilibrated, stabilized	A1153
Phenol equilibrated, non stabilized	A0971
Phenol equilibrated, stabilized : Chloroform : Isoamyl alcohol 25:24:1	A0889
Chloroform *BioChemica*	A3691
Isoamyl alcohol Molecular biology grade	A2610

Products for guanidinium thiocyanate-phenol extraction	
RNAtidy G	A2867
TRItidy G™	A4051
1-Bromo-3-chloropropane *BioChemica*	A2107

Pure silica-based DNA Purification Spin-Kits & Column Regeneration	
Geno/mini DNA Isolation Spin-Kit	A5185
Plas/mini Isolation Spin-Kit	A5172
DNA Enzyme-free Isolation Spin-Kit	A5266
DNA Isolation Spin-Kit Agarose	A5193

formed organic phase and the interphase, RNA is selectively retained in the aqueous phase. RNA, DNA and proteins are isolated by alcohol precipitation.

Why is hydrophilic DNA transferred into the organic phase? And how is it possible that RNA, in contrast, is not extracted by the phenol-chloroform mixture? DNA and RNA seem to be very similar at first sight and, under neutral conditions (pH 7-8) both molecules remain in the aqueous phase as expected. However, usage of non-equilibrated, and therefore acidic phenol solution, enable the enrichment of DNA molecules in the non-polar organic phase. So why do these two types of nucleic acids behave differently under acidic conditions? The answer mainly lies in the structural differences between DNA and RNA: at the prevailing pH of 4-5, the phosphate groups of the doubled stranded (and less acidic) DNA are protonated and the affinity of the now non-charged (but still double stranded) DNA molecules to the organic solvent strongly increases. The phosphate backbone of the single stranded RNA is largely protonated as well, but due to the exposure of the purine and pyrimidine bases, the RNA is able to form hydrogen bonds with the surrounding water molecules (Zumbo, 2011). As a result, RNA does not lose its hydrophilic properties and still prefers the aqueous phase.

Comment on phenol

Pure phenol is a colorless and crystalline substance. Liquefied phenol is susceptible to oxidation (especially when the pH is equilibrated with Tris) and the phenolic oxidation products introduce strand breaks into nucleic acid molecules and promote cross-linking. Phenol solutions should be clear and colorless, pink or brownish solutions should be discarded. To prevent oxidation, 8-hydroxyquinoline is frequently added to liquefied phenol. The shelf life of equilibrated and 8-hydroxyquinoline-stabilized phenol is approximately 9 months. As a positive side effect, 8-hydroxyquinoline partially inhibits ribonucleases.

The meaning of chaotropic salts in nucleic acid isolation processes

Chaotropic agents like guanidinium salts, urea or lithium perchlorate show a number of positive effects on the isolation process: 1. lysis of the cell membrane, 2. denaturation of proteins, DNA and other macromolecules, 3. inactivation of nucleases, 4. promotion of nucleic acid binding to pure silica material. All these features are based on the same mode of action. The chaotropic substances interfere with intracellular interactions based on hydrogen bonds, hydrophobic effects and van der Waals forces resulting in denaturation of proteins (as a consequence protein activity is significantly reduced) and DNA, but also in reorganization and finally collapse of the membrane. The accumulation of chaotropic salts in the hydrophobic region of the lipid bilayer strongly compromises membrane integrity. By replacing the existing hydrogen bonds with adjacent water molecules by salt bridges, the chaotropic salts destroy the hydration shell that is surrounding the nucleic acids, decrease their solubility, mask charges and mediate adsorption to silica surfaces.

The convenient method: commercial kits

Silica-based spin kits

The ability of DNA to bind rapidly and selectively to silicates at high salt concentrations under alkaline conditions was first described in 1979 (Vogelstein & Gillespie, 1979): DNA was removed from agarose gels and bound to glass in the presence of sodium iodide.

Positively charged ions shield the acidic silica surface promoting adsorption of the DNA molecules through the negatively charged DNA backbone. The type of bridging cation determines the strength of binding (Romanowski *et al.*, 1991). Washing with chaotropic salt solutions removes residual protein impurities without affecting the immobilized DNA. Not only salts, also addition of alcohol influences the interaction between matrix and DNA. The silica-bound DNA withstands washing procedures employing 70% ethanol solutions that displace the excess of salts, metabolites, RNA, carbohydrates, and other alcohol soluble biomolecules. Only elution with pure water or low salt solutions (e.g. TE buffer) releases the DNA. This is because the large excess of water molecules replaces the ionic bonds and rehydrates DNA as well as the surface of the silica particles.

The silica-based spin kits are very popular for isolation of genomic DNA as well as for isolation of plasmids. The purity of the eluted DNA is high, and due to the low salt elution conditions, further desalting of the DNA is not necessary. The method is fast, easy to perform, and provides reproducible quantities and quality. Unfortunately, the columns are not suitable for very small DNA fragments. The smaller the DNA molecules, the tighter the binding between silica matrix and DNA. As a consequence, small DNA cannot effectively be recovered from the column (Green & Sambrook, 2012). A further drawback is that the binding capacity of silica is only moderate. Therefore, these "mini" columns are only suitable for small quantities of DNA, typically up to 20 µg.

Anion exchange-based spin kits

The principle of anion exchange totally differs from pure silica-based DNA purification. In contrast to the negatively charged silicate surface, the matrix material possesses a high density of positive charges. The hydrophilic anion exchange resin consists of large-pored silica beads coated with cationic groups. Maximum binding of DNA takes place under slightly acidic low-salt conditions that enable a direct and undisturbed ionic interaction of the DNA's phosphate groups with the cationic surface of the resin. Impurities are removed by washing with medium salt buffers (~ 0.8–1.0 M NaCl, depending on the pH). The DNA is eluted with high salt solutions at a pH around 8. The excess of anions replaces the bound DNA molecules and saturates the surface of the anion exchange matrix. Anion exchange materials guarantee a very pure DNA and, compared to pure silica, offer a strongly increased binding capacity due to its high charge density. Therefore, anion exchange matrices are preferentially used for large scale DNA isolations. Disadvantage are the elution conditions, namely the requirement of very high salt concentrations to release the DNA from the column. Subsequent desalting is often essential for downstream processes.

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Literature at the author

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Eppendorf Young European Investigators Award 2013

‘Luckily, Individuals Turn out to Be Different.’

labor&more in interview with prize-winning scientist Dr. Ben Lehner

Why are individuals different? Why do the same mutations in the genome have different effects on different individuals? Why does one twin get sick when another does not? How do sicknesses come about through the combination of different mutations? These are the questions which British scientist Dr Ben Lehner – ICREA Research Professor, EMBL-CRG Systems Biology Unit, at the Centre de Regulació Genòmica (CRG) in Barcelona – investigates in his research. In recognition of his trailblazing discoveries he received the Eppendorf Award for Young European Investigators 2013. The prize has been presented by Eppendorf since 1995 in partnership with the scientific journal Nature, and is awarded for outstanding research work in the biomedical sector.

Dr Ben Lehner, winner of the Eppendorf Young European Investigators Award 2013, Natalia Villanueva Gomes and Claudia Schiller of labor&more during the prize-giving ceremony at the EMBL Advanced Training Centre in Heidelberg
Photo: Julie Brahms / Eppendorf AG

Ben Lehner, born 1978, studied biochemistry at the University of Cambridge, obtaining a BA in Natural Sciences and going on to achieve a PhD in 2004. After that he spent a postdoctoral period of residency at the Fraser Lab, The Wellcome Trust Sanger Institute, UK. Since 2006 he has been Group Leader at the EMBL-CRG Systems Biology Unit of the Centre for Genomic Regulation, Barcelona. From 2007 to 2009 he was ICREA Junior Researcher at the Catalan Institute for Advanced Studies (ICREA), becoming a Professor in the faculty of Life and Medical Sciences in 2009. Ben Lehner can already look back on 48 published works and is an expert much in demand. He has received numerous awards, including the European Molecular Biology Organisation (EMBO) Young Investigator Award (2010), the Banc Sabadell Prize for Biomedical Research (National Spanish Prize for Biomedical Research, 2012) and most recently the Eppendorf/Nature Award for Young European Investigators 2013.

l&m: *We know that cancer is associated with a series of mutations. In your highly regarded Nature article (doi:10.1038/nature11273) you were able to show the part played in the process by epigenetics. What exactly did you discover?*

Dr Ben Lehner: We were interested in the basic question of whether mutations are equally likely to occur in different regions of the genome. What we (and others) realised is that we could use the data coming from cancer genome projects to address this problem. Most of the mutations that happen in cancer cells have nothing to do with causing cancer – they are simply mutations that have accumulated during the history of the tumour cells. Therefore you can use these ‘passenger’ mutations to look at where mutations are most likely to occur in the genome. The human genome is 3 billion bases long and yet is compacted into a tiny space inside the nucleus of each cell. What we (and others) found was that not all regions of the genome (and so not all genes) are equally likely to mutate. Somehow – and we still don’t understand what molecularly causes this – these differences relate to how the genome is packaged in the nucleus.

Can you give us an overview of the mechanisms of genetic interaction that have been known to date?

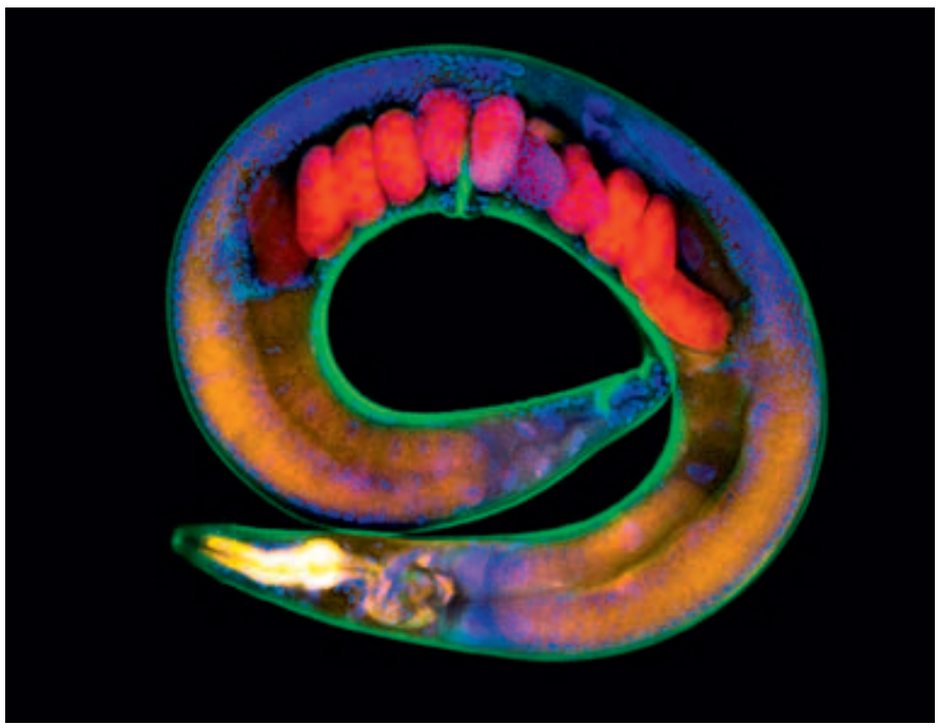
Normally when people are talking about genetic interactions they mean an interac-

tion that occurs between two or more different mutations in a genome. When you combine two mutations together in the same individual there is often a much stronger (or much weaker) outcome than expected – each mutation alone may not cause disease, but the combination does. This is a fascinating problem because it's something that we don't understand very well at a molecular level – why does this happen for particular combinations of mutations and genes? It's also a very important problem because we each inherit mutations in thousands of different genes and so there are a huge number of potential interactions that could be going on in each of us.

More recently we have been interested in the problem of why there is still variation in the outcome of mutations even without any additional mutations. What we thought was that the same thing could be going on with one mutation as when you combine two different mutations. For example, if when you mutate gene A and gene B at the same time something bad happens, the same bad thing would happen if you only mutate gene A but, for some chance or environmental reason, gene B is not switched on enough at a critical time in an individual. Using the simple worm *C. elegans* we showed that this is what happens – that variation in the extent to which particular genes are switched on and off during the very early development of embryos determines to some extent whether an inherited mutation has an effect or not.

You studied in Cambridge, where Crick and Watson worked on the decoding of DNA and revolutionised biology 60 years ago with their description of the DNA structure. Watson was one of the initiators of the Human Genome Project, which made history at the start of the millennium with the complete sequencing of the human genome. To what extent have you been influenced by these milestones?

It's true that Cambridge has a unique history when it comes to science and molecular biology in particular – it's not just the structure of DNA and the sequencing of the genome, but also the isolation of embryonic stem cells, protein crystallography, DNA sequencing, monoclonal antibodies, in vitro fertilisation, and more than 80 Nobel prizes in total. There are two things I appreciated about this: first, that it helps you put your own work and achievements (and those of others) in perspective – compared to what has already been achieved we are only making small steps; second, that even the 'famous' people at Cambridge were normally very down to earth and only had small labs. So it creates this culture that as long as you work on important problems there is a chance that you might progress towards something important.



***C. elegans* – with its deceptively simple structure, the nematode shows surprisingly wide phenotypic variability.**

Photo: A. Klosin / B. Lehner Lab, CRG, Spain

Angelina Jolie's drastic prophylactic decision has stimulated a lot of discussion worldwide, after a test for BRCA mutations apparently showed that she had an 87% risk of contracting breast cancer. Will your research make it easier in future to predict accurately whether and when a disease will be contracted and whether it can be avoided?

We hope so. Actually examples like the hereditary BRCA mutations where most individuals carrying the mutations will eventually be affected are quite rare. For most common diseases, we already know from studying identical twins that we'll never be able to predict accurately what happens to individuals from their genome sequences alone. So I think that a really important challenge that should receive much more attention is how to combine the genetic information with other measurements that are made on the individuals. Just as in *C. elegans* we could make much more accurate predictions if we combine the genetic information with measurements made in each individual, so this should also be possible in humans. It will be the combination of genetics and clinical measurements that will be most effective in predicting what will happen to individuals.

What tips, Dr Lehner, would you give to young scientists who are just at the start of their career?

Inspired by your question above, one tip would be to read something about the history of your field. I think this helps you to distinguish questions that are important from techniques or approaches that are just popular or fashionable at the moment. Also, one of the frustrating things about science is that you have to be able to cope with almost continuous disappointment and rejection – both in the lab and when trying to publish papers. So it's important not to be too affected by that!

Dr Lehner, many thanks for the interview – we wish you every continuing success.

(Interview: Claudia Schiller)

ANALITICA LATIN AMERICA

The mission of NürnbergMesse Brasil is to create experiences, connect people and provide knowledge. Within this concept, the 13th Analitica edition, has once more become a business platform of the chemical industry in Latin America, which brings together the main suppliers, manufacturers, distributors and purchasers of this sector.

The chemical industry plays an important role in the economy. In Brazil, the chemical sector is the second greatest contributor for Industrial GDP. The biennial fair growth is a consequence from the strong market fostering in combination with the economic growth achieved in Brazil over the past years. Considering all the segments, the chemical industry had in 2012, according to ABIQUIM – Brazilian Chemical Industry Association, in 2012, the chemical industry achieved a net turn-over estimated in R$ 293 billion – growth of 12,4% in comparison to 2011.

Analitica Latin America is one of the main worldwide meeting points of the chemical and analytical industry. Along three days of exhibition, multi-sectorial brands will remain within reach in a single location, and will present the latest news and trends of the sector.

And in order to ensure a high quality content and integrate different projects during the Analitica Latin America, the 1º Circuito de Conhecimento e Inovação (1st Circuit of Knowledge and Innovation) will present the content, trends and the most recent Analytical Chemistry existing, and its different application to the congressmen and visitors audience of the trade fair.

The project will integrate Trade Fairs, Knowledge Arena which will provide visitors and guests the opportunity to participate of short presentations with innovative subjects through lectures and interaction in the trade fair area, the Analitica Latin America Congress, where the main goal is the interaction between the academic community and the industrial sector and the LiveLab, a platform which will have a full equipped laboratory with daily live demonstrations. From the preparation of the sample to the measurement and assessment of the results, visitors will have access to the most different procedures and techniques. The Circuit of Knowledge and Innovation will offer several areas and activities which will provide experiences, information exchange, new ideas presentation, services and products providing a relationship among many players who compose the Brazilian Analytical Chemistry scenario.

Visitors and opinion formers like you can appreciate this event and have access to the trends focused on solutions, products and services for laboratories and industries of the sectors: pharmaceutical, cosmetic, food, petrochemical, chemical, energy, mining, environmental, biotechnology, etc.

It is a pleasure to held a new edition of Analitica Latin America. The event is organized and promoted for your business success. Participate of these three days of event actively and discover the best of analytical chemistry industry.

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Analitica Latin America
September, 24th – 26th, 2013
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www.analiticanet.com.br

Asia's largest bio event

In Tokyo, from May 8th to 10th, Asia's largest bio event "BIOtech 2013 in Japan" took place showing a significant growth both in the scale and the quality. Check the key figures below to see how successful the show was: 603 exhibitors from 25 countries (UP from 490 exhibitors from 15 countries in 2012),10.362 visitors from around the world, 237 presentations, 4.845 attendees to the Keynote & Special Sessions.

One of the many factors which lead BIOtech 2013 Japan to the huge success was that the Japanese bio industry is showing signs of recovery and further growth. Dr. Yamanaka's creation of iPS cells not only generated the new market for iPS cell research and regenerative medicine, also became a cause for the government to enhance its support toward the bio/pharma-related research. Also fueled by newcomers from other industries, business and innovation in the Japanese bio industry is rejuvenated. Growing interests toward the Japanese market also resulted in an increased number of attendees from overseas. Due to the economic stagnation in Europe, more overseas biotech companies have chosen to exhibit at the show, setting their target to Japan. Partly because of that, the number of overseas exhibitors almost doubled from the previous show. Also, increased numbers of overseas visitors were seen having active business talks, attracted by the latest research achievements/products presented at the show.

This year, BIOtech Japan has further established its position as an international event where the Japanese/Asian bio industry and those who are targeting it all get together, as THE STATE OF ILLINOIS (exhibitor) said: "This is THE must-attend event for those targeting the Japanese market. " BIOtech 2014 Japan will take place from May 14 to 16, 2014 at Tokyo Big Sight, becoming larger and more international.

www.bio-t.jp/en

Ukrainian laboratory market

Ukrainian market of laboratory equipment shows recently the constant development. The market growth is 30–40% per year. The main trends are characterized with the shift of demand to the high quality equipment. The opportunity to learn more about Ukrainian laboratory market, novelties of laboratory equipment and innovational technology will be on the VI International Forum "Complex support of laboratories", that will be held on 15–17 October 2013 at the exhibition center "KyivExpoPlaza", Kyiv, Ukraine. The International Forum is the only one large-scale international event in Ukraine, which covers all the aspects of the comprehensive support of laboratories and laboratory diagnosis in all branches of industry, scientific researches and medicine. It has the official government support, international visiting and participation and organized in associated with industries associations and business unions. The organizers of the International Forum "Complex support of laboratories" are the National Academy of science and the company "LMT". The unique structure of the Forum allows joining together the leading experts and players of the laboratory industry in the same place and at the same time and also provides the ideal platform for the dialog between the business-visitors and exhibitors.

The General partner is company "CHIMLABORREACTIV". The General sponsor is Tokyo Boeki Technology LTD. The partners of the Forum became: Expert TM, ULAB TM, ALT- Ukraine LTD, Intertech Corporation, Aurum LAB, Fisher Scientific, Bruker Corporation, HBO «Nikomed», Riana alliance, ShimUkraine, Labor-Technik, LECO, Instron, Alsi LTD, DiaVeritas, Donau Lab Kiev, Alsi-Chrom, Makrolab, CEM, Retsch, Ukrdiagnostika, Sartorius Group, Termo Techno, Melitec-Ukraine, Novations LLS, ShelTec-Ukraine, Perkin Elmer, IKA WERKE GmbH, Octanorm. International specialized partner – "labor&more" magazine.

www.labcomplex.com

analytica Anacon India 2013

While the Government is looking for a system guaranteeing food security, the quality of food remains rudimentary. This has been seen in recent incidents across the country where the quality of food being served in certain institutions was compromised and led to unfavourable results. As a result, food safety has become a growing concern for consumers and industry leaders.

Taking place from 12th to 14th November, 2013, at the Bombay Convention and Exhibition Centre, Mumbai, analytica Anacon India will provide methodologies to detect, screen, identify and quantify chemicals in ingredients and products at various stages of the food chain. It will also offer analytical technologies and resources that will help the manufactures and distributors of food. The event presents a number of new systems for chromatography (GC, HPLC), Spectrometry, like mass spectrometry or atomic absorption spectrometers, lab centrifuges and other analysis techniques that play an important role in food testing.

Along with all the big national and multinational exhibitors, the fair has also developed a competent and focused gathering for conferences. The theme this year is "Research and Regulations: Challenges for the Food, Life Science and Pharmaceutical Industries" and focuses on the regulatory and patenting issues, as well as on standards and safety, drug discovery and biosimilars. The special session on "Food Standards and Safety – Priorities for India" will be held by speakers from Vitma Labs, Coca Cola India and research institutions.

www.messe-muenchen.de

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www.analytik-jena.com

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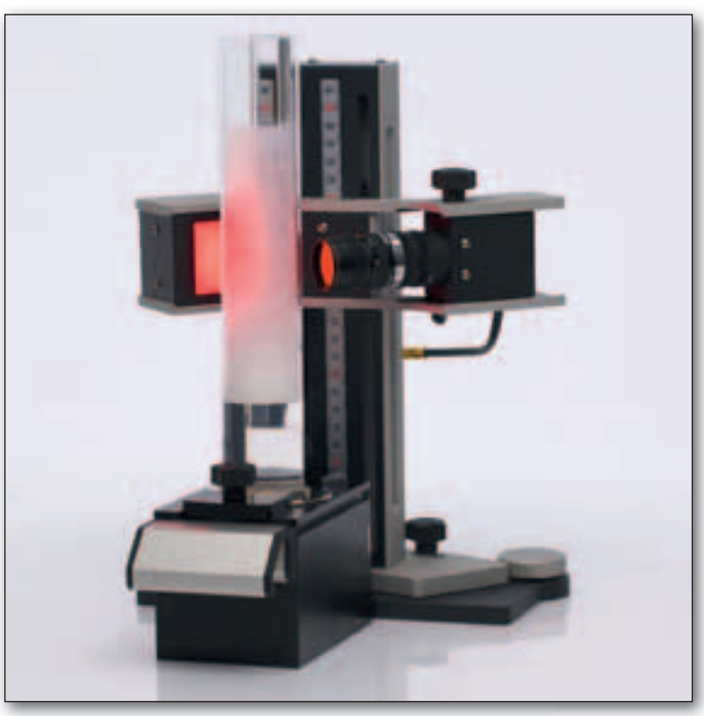
www.cybio-ag.com



New solution for optical foam structure analysis

KRÜSS GmbH presented a new module for the DFA100 Dynamic Foam Analyzer intended for the optical testing of fluid foam structures at the European Coatings Show 2013 in Nuremberg. The instrument uses image analysis to determine the number, size and size distribution of the bubbles of a foam generated under control in the instrument. The foam structure module features a height-adjustable camera with rapid image sequence for measuring the structural changes over time, thus making the inner destabilisation of the foam visible long before it actually decays.

Initially this modular instrument focussed on measuring the decay of instable foams. A component for measuring liquid content presented in 2012 plus the new optical module are both geared towards stable and metastable foams.

www.kruss.de

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With this innovation, Reichelt Chemietechnik presents a flowmeter meeting highest requirements, with integrated data display indicating the instantaneous value and the volume passed through. The measuring range lies between 0.1 to 25 ml/min, the permissible temperature range is -20 °C to +100 °C with a maximum working pressure of 25 bar. Particular mention deserves its high measuring accuracy which is ±3 % of the instantaneous value at a viscosity up to approx. 15 cSt. The mode of operation is simple as well as ingenious! The rotor contained in the flowmeter is set in a rotary motion by the medium to be measured. A Hall sensor scans the rotor and generates a frequency signal according to the flow rate. The signal is then analyzed and digitally indicated.

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New features implemented in Metrohm's latest high-performance IC system greatly increase ease of use. Based on the new 941 Eluent Production Module, the 940 Professional IC Vario enables inline preparation of eluents of any composition and in any concentration. Even more convenience is possible, as an ELGA PURELAB flex5/6 ultrapure water system can be directly connected to the system. In this setup, conventional tap water can be used for ion chromatography.

When it comes to product quality, the 940 Professional IC Vario follows in the tradition of the Metrohm brand. Metrohm's new flagship in ion chromatography comes with a 3-year instrument warranty, a 10-year warranty on the anion suppressor, and 10 years guaranteed availability of spare parts.

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