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*2017 Release of Journal Citation Reports®: Thomson Reuters 2016 Citation Data*
It isn’t the first time, that Qatar has faced opposition from its neighbors, including embargos. Why is this so? Qatar is a peninsula in the Persian Gulf, surrounded only by the sea and Saudi Arabia. The weather is extremely hot and despite the high humidity of ~85% it almost never rains there (<100mm/a). This was the reason why only 50,000 people lived in the area in 1950. In 1939 oil was found and in 1971 the biggest oil field in the world was discovered in Qatar. Since then the number of inhabitants has increased to more than 2.5 million. Due to the political interest of other states like Great Britain, Qatar was able to keep its independence from both, the United Arab Emirates and Saudi Arabia and this is indeed, what bothers Saudi Arabia’s tycoons.

The original inhabitants are mostly orthodox Sunnites, following the teaching of Muhammad ibn Abd al-Wahhab. This orthodox orientation is the basis for the accusation that Qatar supports radical Sunnite groups like the Taliban, IS and others. A basic ideology is the denial of all other Islamic groups, which makes it extremely unlikely that there are closer contacts to Iran, which is dominated by Shites.

In fact Saudi Arabia, which claimed that Qatar supports terrorists, is dominated by the same religious orientation. 15 of the 19 known terrorists who committed the 9/11 attacks were Saudis, like Osama Bin Laden and his family, too. It is also well-known that financial support for the mentioned terrorist groups mainly comes from Saudi Arabia and not from Qatar. Dealing with Saudi Arabia, like Donald Trump announced recently, is much more supportive for future terrorism than free trading with Qatar.

If the alleged support of terrorists is not the reason for Saudi Arabia’s sanctions against Qatar, what might be the real reasons? For sure it is the wish of the Saudi Arabian Dictators to spread their political influence as far as possible and to risk the consequences, a further destabilization of the area.

All efforts to interfere in the numerous conflicts in this part of the world by the so-called western world cause or support other conflicts. The elimination of Saddam Hussein’s strong Iraq for example enabled the IS to rise. It is dangerous to support a party in a foreign region just because one trades with it. It may happen that one supports its own enemies. To give them weapons is mad.

Choose your friends as well as you can, live in peace with the others and trade with all. Do not interfere in conflicts in other areas, which you don’t understand. This always ends up badly.

Dr. Arne Kusserow
Editor-in-Chief
Light can conduct the play of atoms and molecules in the microcosm. Humans manage to interfere with this play. Researchers have now used light to reconfigure hydrocarbons. Using ultrashort laser pulses they removed an outer hydrogen atom from one side of a hydrocarbon molecule and directed it to the opposite side, where it rebounded. The method could be used in the future to synthesize new substance by controlling chemical reactions.

More on page 37
Mira M-3 handheld Raman Spectrometer
True single-handed operation

Barely larger than a smartphone, the Mira M-3 is one of the most convenient to use handheld Raman spectrometers in the market. The Mira M-3 is ...

- **Super compact** – 13 (h) x 8.5 (w) x 4 (d) cm
- **Super fast** – verify identity of materials in seconds
- **Fully compliant** with FDA 21 CFR Part 11

www.metrohm.com/mira
Completing the course of antibiotics may be counterproductive

The deeply embedded message that patients should „complete the course“ of antibiotics to avoid antibiotic resistance is not backed by evidence and should be dropped, argue experts in The BMJ. In fact, patients are put at unnecessary risk from antibiotic resistance when treatment is given for longer than necessary, not when it is stopped early, say Professor Martin Llewelyn at Brighton and Sussex Medical School and colleagues.

Antibiotics are vital to modern medicine and antibiotic resistance is a global, urgent threat to human health. Public communication about antibiotics often emphasises that patients who fail to complete prescribed antibiotic courses put themselves and others at risk of antibiotic resistance. However, the idea that stopping antibiotic treatment early encourages antibiotic resistance is not supported by evidence, while taking antibiotics for longer than necessary increases the risk of resistance, explain the authors.

There is evidence that, in many situations, stopping antibiotics sooner is a safe and effective way to reduce antibiotic overuse. There are notable exceptions for some types of antibiotic, such as those used to treat tuberculosis. Completing the course also goes against one of the most fundamental and widespread medication beliefs people have, which is that we should take as little medication as necessary, they add. Finally, they say, clinical trials are required to determine the most effective strategies for optimising duration of antibiotic treatment.

Original publication: DOI: 10.1136/bmj.j3418

Using Computer-Like Logic to Program Cells

Synthetic biologists are converting microbial cells into living devices that are able to perform useful tasks ranging from the production of drugs, fine chemicals and biofuels to detecting disease-causing agents and releasing therapeutic molecules inside the body. To accomplish this, they fit cells with artificial molecular machinery that can sense stimuli such as toxins in the environment, metabolite levels or inflammatory signals. Much like electronic circuits, these synthetic biological circuits can process information and make logic-guided decisions. Unlike their electronic counterparts, however, biological circuits must be fabricated from the molecular components that cells can produce, and they must operate in the crowded and ever-changing environment within each cell. So far, synthetic biological circuits can only sense a handful of signals, giving them an incomplete picture of conditions in the host cell. They are also built out of several moving parts in the form of different types of molecules, such as DNAs, RNAs, and proteins, that must find, bind and work together to sense and process signals. Identifying molecules that cooperate well with one another is difficult and makes development of new biological circuits a time-consuming and often unpredictable process. As reported in Nature, a team at Harvard’s Wyss Institute for Bio-logically Inspired Engineering is now presenting an all-in-one solution that imbues a molecule of ribonucleic acid or RNA with the capacity to sense multiple signals and make logical decisions to control protein production with high precision. The study’s approach resulted in a genetically encodable RNA nano-device that can perform an unprecedented 12-input logic operation to accurately regulate the expression of a fluorescent reporter protein in E. coli bacteria only when encountering a complex, user-prescribed profile of intra-cellular stimuli. Such programmable nano-devices may allow researchers to construct more sophisticated synthetic biological circuits, enabling them to analyze complex cellular environments efficiently and to respond accurately.

The team’s approach evolved from its previous development of so-called „Toehold Switches“ -- first published in 2014 -- which are programmable hairpin-like nano-structures made of RNA. In principle, RNA toehold switches can control the production of a specific protein: when a desired complementary „trigger“ RNA, which can be part of the cell’s natural RNA repertoire, is present and binds to the toehold switch, the hairpin structure breaks open. Only then will the cell’s ribosomes get access to the RNA and produce the desired protein. Beyond their use in different living organisms, Ribocomputing Devices could also be included in cell-free applications. „These logic-based RNAs could be freeze-dried on paper and thus boost the possibilities of paper-based biological circuits, including diagnostics that can sense and integrate several disease-relevant signals in a clinical sample,“ said Collins.

Original publication: DOI:10.1038/nature23271

Removing Heavy Metals from Water with Quartz Fibres

Plain quartz fiber, top, gains the ability to remove toxic metals from water, according to researchers at Rice University.

Similar to how computer scientists use logical language to have their programs make accurate AND, OR and NOT decisions towards a final goal, „Ribocomputing Devices“ (stylized here in yellow) developed by a team at the Wyss Institute can now be used by synthetic biologists to sense and interpret multiple signals in cells and logically instruct their ribosomes (stylized in blue and green) to produce different proteins.

Carbon nanotubes immobilized in a tuft of quartz fiber have the power to remove toxic heavy metals from water, according to researchers at Rice University.

Prize-winning filters produced in the lab of Rice chemist Andrew Barron by then-high school student and lead author Perry Alagappan absorb more than 99 percent of metals from test samples laden with cadmium, cobalt, copper, mercury, nickel and lead. Once saturated, the filters can be washed and reused.

The robust filters consist of carbon nanotubes grown in place on quartz fibers that are then chemically epoxidized. Lab tests showed that scaled-up versions of the „supported-epoxidized carbon nanotube“ (SENT) filters proved able to treat 5 liters of water in less than one minute and be renewed in 90 seconds. The material retained nearly 100 percent of its capacity to filter water for up to 70 liters per 100 grams of SENT.
Making Chemistry ‘Click’

A team of researchers has developed a faster and easier way to make sulfur-containing polymers that will lower the cost of large-scale production. The achievement, published in Nature Chemistry and Angewandte Chemie, opens the door to creating new products from this class of polymers while producing far less hazardous waste. The researchers’ reaction technique, dubbed SuFEx for sulfur(VI) fluoride exchange, combined with a newly identified class of catalysts that speed up the reactions, could be used to make everything from water bottles and mobile phone cases to medical devices and bulletproof glass. When a useful molecule is discovered, there are few reactions that chemists can use that are simple and efficient enough to meet the industrial production requirements for cost-effectively scaling up. In 2001, Nobel laureate K. Barry Sharpless introduced a new concept to organic chemistry known as ‘click chemistry,’ describing a suite of controllable, highly reactive reactions that are high-yielding and require little to no purification.

Scientists at Lawrence Berkeley National Laboratory’s (Berkeley Lab) Molecular Foundry, a facility that specializes in nanoscale science, worked with a team led by Sharpless and Peng Wu, professors at the Scripps Research Institute (TSRI). The team created long chains of linked sulfur-containing molecules, termed polysulfates and polysulfonates, using a SuFEx click reaction. The SuFEx reaction, introduced as a new family of click reactions in 2014, reliably and quickly creates new chemical bonds, connecting compounds together with sulfates or sulfonates. While polysulfates have shown great potential as competitors to polycarbonates (strong plastics used for eyewear lenses and water bottles, for example), they have been rarely used for industrial applications due to a lack of reliable and easily scalable synthetic processes.

To overcome the challenges of mass-manufacturing polysulfates and polysulfonates, the TSRI team explored various catalysts and starting reagents to optimize the SuFEx reaction. They relied on their collaborators at the Molecular Foundry to assess physical properties and determine if the newly created polymers were thermally stable products. In creating a polysulfonate with SuFEx, the researchers identified ethenesulfonfluoride-amine/aniline and bisphenol ether as good monomers to use and found that using bifluoride salt as a catalyst made the previously slow reaction ‘click’ into action. Researchers found that the high efficiency of the reaction results in a remarkable 99 percent conversion, from starting reactants to products, in less than an hour. Researchers found that the new reaction requires 100 to 1,000 times less catalyst than other known methods, resulting in significantly less hazardous waste. Bifluoride salts are also much less corrosive than previously used catalysts, allowing for a wider range of starting substrate, which researchers said they hope could lead to its adoption for a range of industrial processes.

Original publications: DOI: 10.1002/anie.201701160/full (Angewandte Chemie) and DOI: 10.1038/nchem.2796 (Nature Chemistry)

Global Travel Helped Spreading Dengue in Asia

While the incidences of many other infectious diseases have declined over the past decade, the number of cases and outbreaks of dengue virus have continued to increase. The spread of dengue to new areas is likely due in large part to trends in air travel, researchers now report in PLOS Neglected Tropical Diseases. Dengue virus affects an estimated 390 million people around the globe each year, and can cause symptoms ranging from a mild fever and headache to severe low blood pressure. The virus has mostly caused disease in tropical and subtropical areas of the world, but a 2014 outbreak in Japan broke that pattern. Overall, the geographic area affected by dengue has been growing in recent years.

In the new work, Huaiyu Tian and Bing Xu, both of Beijing Normal University, China, together with colleagues from the University of Oxford and elsewhere analyzed the spread of dengue viruses in Asia from 1956 to 2015. They used 2,202 genetic sequences of dengue viruses, collected in 20 countries or regions of Asia over the 59 years, to determine how different strains were related. They also investigated trends in air travel, maritime mobility, migration, and socio-economics to determine what factors impact the spread of dengue. The spread of three different dengue virus serotypes, DENV-1, -2, and -3, is associated with air traffic more so than any other factors, the data revealed. Air traffic hubs such as Thailand and India, the researchers found, help seed dengue epidemics, while China, Cambodia, Indonesia, and Singapore help diffuse the virus to other Asian countries.

Original publication: DOI: 10.1371/journal.pntd.0005694

Gene Transfer in Microbial Communities Living on Cheese

Researchers at the University of California San Diego have found that microbial species living on cheese have transferred thousands of genes between each other. They also identified regional hotspots where such exchanges take place, including several genomic ‘islands’ that host exchanges across several species of bacteria. The researchers used the rinds of artisanal cheese varieties as simple model systems to study microorganisms, or communities of microorganisms. Microbiomes are known to play a key role in cheese aging and affecting cheese quality.

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function in many areas, including human health, protecting us from some diseases and amplifying others.

Cheese rinds offer a novel way to study how genes in microbial communities are passed from one organism to another in a process known as „horizontal gene transfer.” Details of the study were published in the journal eLife.

A large percentage of transferred genes involved functions dealing with acquiring nutrients, especially iron, which is known to be in short supply on the surface of cheese. Competition for iron is an important theme for microbes in many environments, including during infections of humans by pathogenic microbes. Based on the new results, Dutton and her colleagues are now probing the intricate dynamics of horizontal gene transfer and how the process unfolds on cheese.

Original publication: DOI: 10.7554/eLife.22144

Porphyridium Boosts the Performance of Electrodes for Rechargeable Batteries

Chlorophyll, blood, and vitamin B12 are all based on the porphyrin molecule. But porphyrin can also be used as an electrode material where it speeds up the charging process of rechargeable batteries. In the „Angewandte Chemie International Edition” journal, researchers from KIT now present the new material system that could mark the beginning of an era of high-performance energy storage and supercapacitors. Currently, the most widely used battery technology is based on lithium ions. No other rechargeable storage device for electric energy has comparable application properties. Thus, lithium ion batteries are currently indispensable for devices such as laptops, smartphones, or cameras, even though improved properties such as quick-charging would be desirable. Many materials that improve the properties of lithium ion batteries in the lab, however, are no sustainable options because they are rare, expensive, toxic or harmful to the environment. Ideally, high-performance energy storage materials would be based on renewable resources.

An interdisciplinary research group now presents a new energy storage material that allows a very fast and reversible inclusion of lithium ions. For this purpose, functional groups were added to the organic copper porphyrin molecule that produce structural and electro-conductive crosslinking of the material when the battery cell is charged for the first time. This significantly stabilizes the structure of the electrode in lab tests and allows several thousands of charge-discharge cycles. With this material, storage capacities of 130-170 miliamp-hours per gram (mAh/g) were measured in the lab - at a medium voltage of 3 Volt - and charging-discharging times of only one minute. Current experiments suggest that the storage capacity can be increased by another 100 mAh/g and that the storage system can be operated not only with lithium, but also with the much more abundant sodium.

Original publication: DOI: 10.1002/ange.201702805

Emerson expands clean-room manufacturing facility for miniature valves

Emerson has greatly expanded the clean room facility at its Asco valve manufacturing plant in Ölbronn-Dürrn, Germany. Clean room manufacturing is essential for miniature valves, which are often used in analytical and medical machinery. Emerson manufactures per ISO Class 6 equivalent cleanliness in every work region. An increasing demand for greater miniaturisation has driven growth in the volume of valves that are required to be manufactured under clean room conditions. The Ölbronn-Dürrn, Germany site also saw an expansion to the R&D and engineering capabilities. The new 720 m² clean room was part of a major expansion at the Analytical & Medical Centre of Excellence, located close to Stuttgart. The site has been producing miniature valves for almost 20 years. The valves are used in super critical medical applications, such as patient respirators, incubators, and dialysis machines, as well as in laboratory applications such as DNA research. The valves being produced at the site are often only a few centimetres or less in size, which means that the orifice, or area through which the media flows, is in some cases only a fraction of a millimetre in diameter. At this scale, particles that may normally be present in the atmosphere can lead to functional impairment or loss of performance. Increasing miniaturisation of the machines in which the valves are used has led to a greater demand for smaller and smaller valves. The expansion has led to a significant increase in the number of employees working in the clean room area. As you would expect from a company involved at the forefront of Industry 4.0, the clean room utilizes paperless manufacturing, with production control managed through an infra-red communication module on the production hall ceiling; the orders are continuously updated as they are processed. The scanning of the electronic tags enables personnel to obtain precise information about the specific assembly steps required and loads the software for the semi-automated final testing of the valves. This prevents any errors.
Chemometrics

Chemometrics is the bridge between chemistry and mathematics, meaning that it uses mathematical methods to analyse the results of analytical measurements. It is applied for interpreting, evaluating, and analysing results of analytical measurements to obtain a maximum of information from the data. It is widely used from experiment design over signal processing up to exchange of data. The author, Matthias Otto, introduces the statistical-mathematical evaluation of chemical measurements, especially analytical ones, going on to provide a modern approach to signal processing, designing and optimizing experiments, pattern recognition and classification, as well as modeling simple and nonlinear relationships. Analytical databases are equally covered as are applications of multiway analysis, artificial intelligence, fuzzy theory, neural networks, and genetic algorithms. With this new edition, coverage is expanded to topics such as orthogonal signal correction and new data exchange formats. Latest developments such as dynamic neural networks and new directives for quality assurance in regulated environments (GLP) round off this book.

Erratum

As you may have noticed, we have managed to negotiate a rebate for the books we are presenting in the Read and Win feature in every issue of the G.I.T. Laboratory Journal. However, due to technical problems, you may not have been able to order the book with the 30% off. Therefore, we are extending the validity of the rebate code for all books we have presented so far this year until the end of September 2017. So, this is your chance to get the following books at a bargain price:


The code for the books is GIT17 and it is redeemable only when ordering from wiley.com or wiley-vch.com
The Basics of PCR

Polymerase Chain Reaction (PCR for short) is used to increase very small and highly specific amounts of individual sections of DNA. The following figure shows a flow chart of the PCR method. The PCR-standard method delivers good results but when problems occur it needs to be optimized bei changing the parameters.

**Components**
- Thermostable DNA-Polymerase, Original DNA (Matrices or templates), buffer (MgCl₂), nucleotides, primer, thermocycler

**Primer**
- Two short single strands of DNA (10 - 35 nucleotides) with complementary sequences for increasing the strand.

**Temperature Cycle**
1. **Denaturing**: at 95°C the two DNA strands are completely separated
2. **Annealing**: at +55°C the primer connects to the highly specific target sequence
3. **Elongation**: at 72°C the polymerase elongates the primer to the end of the target sequence.

This cycle is repeated until the required amount of the target sequence has been created. In theory the amount of targeted DNA should double in each cycle. In practice this amount is never obtained.

**Standard-PCR-Method**

<table>
<thead>
<tr>
<th>Components</th>
<th>Reaction buffer</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x PCR-Reaction buffer</td>
<td>5 µL</td>
<td>1×</td>
</tr>
<tr>
<td>Taq-DNA-Polymerase (1-2 units/µL)</td>
<td>1 µL</td>
<td>1-2 units pro reaction</td>
</tr>
<tr>
<td>10 mM dNTPs (dATP, dCTP, dGTP und dTTP)</td>
<td>1 µL</td>
<td>200 µM per dNTP</td>
</tr>
<tr>
<td>10 µM Forward Primer</td>
<td>1 µL</td>
<td>200 nM</td>
</tr>
<tr>
<td>10 µM Reverse Primer</td>
<td>1 µL</td>
<td>200 nM</td>
</tr>
<tr>
<td>Template-DNA</td>
<td>variable</td>
<td>10-200 ng (for genomic DNA)</td>
</tr>
<tr>
<td>Nuclease-free, sterile water</td>
<td>Fill up to 50 µL</td>
<td></td>
</tr>
</tbody>
</table>

**Standard PCR Program**

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>2-5 min</td>
</tr>
<tr>
<td>Duplication</td>
<td>Denaturing 94°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Annealing 55°C</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td>Elongation 72°C</td>
<td>90 sec</td>
</tr>
<tr>
<td>Final Elongation</td>
<td>72°C</td>
<td>5-7 min</td>
</tr>
<tr>
<td>Cooling Process</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

Take Note!
- Keep pipettes and appliances clean.
- Mix reagents well before use.
- Always prepare a negative control without a template-DNA as well (proof of possible contamination).
- In the case of thermocycler without a heatable lid, cover the reaction mixture with 1-2 drops of mineral oil.
- The fragment of DNA that is to be amplified should not be longer than 2kbp.
- The primer should have a length of 18 - 30 bases, include 40-60% of guanidine and cytosine (G+C) and have almost the same melting point.
- Prepare the Master-Mix with all components except for the template-DNA. Add the DNA as the final component. Make reserves for 1 - 2 more reactions (possible inexact pipetting).
Nowadays, PCR can be robust and easy – but you can still run into difficulties. Make sure to get the most out of your PCR with some simple tricks.

1. Measure your DNA concentration
Determine the concentration and purity of your DNA. Ideal DNA purity range ($A_{260}/A_{280}$) = ~1.7 – 2.0

Take note of the absorbance reading (not just concentration values). It should be between 0.1 – 1.0 $A$ for reliable reading in accordance with the Beer-Lambert Law.

2. Preparation of Mastermix
Prepare the mastermix in only one tube to prevent pipetting variations that can occur from preparing multiple mastermixes. Use a tube large enough (e.g. 5 mL) to sufficiently hold the entire volume of the mastermix. Aliquot immediately afterwards to avoid multiple-thawing that can have a negative impact on the reproducibility of your PCR.

3. In case of non-specific amplifications
Use Hot-start protocols. Make sure your cycler is properly calibrated and reaches the designated temperatures quickly during the run.

For new primers, run optimization with a single primer (i.e. forward or reverse primer only) controls to determine non-specificity

Titrate Mg$^{2+}$ to optimize the concentrations for your PCR reaction.

4. In case of no or low amplification
Optimize denaturation and/or annealing temperature with a gradient

Use PCR enhancers (e.g. DMSO, BSA, etc.) each require empirical testing for the specific combination of template and primer
Researchers hope that they will soon be able to fight cancer cells without damaging healthy tissue, thanks to so-called biologicals. These new active components spot specific molecular characteristics of abnormal cells and help to destroy them systematically. A large number of pharmaceutical candidates must be examined to find customized biologicals for various types of cancers. Gyros, a Swedish company, has developed an analytical device which facilitates the search: Gyrolab xplore automatically and quickly runs tests of multiple samples in parallel, saving time, work force and material. Faulhaber motors provide the speed and precision needed for handling the tests.

If you saw Gyrolab xplore in an office, you would probably take it for a large laser printer. However, lifting the cover reveals a miniature laboratory. A plastic disk, the size of a compact disk, is located at the heart of the device – this is where the samples are analysed. This CD contains a system made up of channels, each with a diameter of less than one millimetre. Capillary and centrifugal forces transport the samples through the channel system, analysing them in the process.

Biological Agents as Weapon Against Cancer

The companies use the system to test their biologicals. Biologicals are molecules which are too large and complex to be created synthetically. This is why they are produced by living – usually genetically engineered – cells, which are cultivated in nutrient fluid in the laboratory. Most biologicals are proteins. Cancer researchers have been pinning their hopes on one type of protein in particular: antibodies. These molecules are produced by specialized cells of the immune system. They recognize and bind foreign proteins – for example of bacterial or viral origin – that enter the body during an infection. Thus, the pathogens can be eliminated or marked for degradation by phagocytes. The same principle can also be applied to fight cancer cells.

Detection on CD

The system can be used to analyze the new drug during any development phase – for example, in the cells’ nutrient solution or in the blood of test animals and patients. Up to 112 data points can be generated in parallel using a single CD. Thanks to the CD microstructures, a run requires very low sample volumes and minimizes reagent consumption. The sample fluid is pipetted into the wells of a microtiter plate, which is then placed into the instrument. Inside, the samples are transferred onto the CD by a robotic arm. They are then introduced into the appropriate channels by a capillary force. Only a minute amount of sample fluid is needed for the test – between 20 and 200 nanolitres, depending on the CD type.

The exact volume of the sample liquid is measured on the CD itself. For this purpose, the channel expands to form a chamber, which is the right size for the required volume. There is a hydrophobic barrier at the lower end, which stops the liquid from flowing further into the channel. Next, the CD starts to rotate. The centrifugal force diverts the sample fluid, which is located above the chamber, through an overflow channel. Then, the rotational speed is increased so that the sample overcomes the hydrophobic barrier and moves into the next section.

The same principle is used to run wash cycles and add further reagents to the experiment. The entire test process is fully automatic – each individual step is controlled by the software included with the device. Automation does not only reduce the amount of work, but it also minimizes the risk of errors.

Binding According to the Lock And Key Model

The special binding characteristics between an antibody and its target protein – the so-called antigen – are utilized to detect the active component. Similar to the lock and key model, antigen and antibody bind very specifically: they invariably recognize each other even among millions of other molecules. To determine the concentration of an antibody, for example, in sample fluids, its antigen is

Fig. 1: Analyzer Gyrolab xplore tests biologics.
closely bound to a short section of the CD’s channel wall. When the antibody passes the antigen in the channel, it is extracted by the antigen and retained in the channel. Following the same principle, a second antibody, which is marked with a fluorescence dye, subsequently binds to the first one. The dye is then excited by a laser. Detection of the emitted light is used to determine the concentration of the protein within the sample – in this case, the antibody.

**Speed For High Throughput**

Gyros launched Gyrolab xplore in 2015. At that time, many companies were already working with the new device’s big brother, Gyrolab xp workstation, which is able to analyze five CDs in one run. However, companies with relatively low throughput rates and small departments of large pharmaceutical companies often found the device a little too large. With Gyrolab xplore, Gyros now brings these customers a tailored alternative.

When designing the new device, developers made sure that it matches its predecessor in analytical speed. The robot arm therefore has to be able to transport samples just as quickly and safely. Unfortunately, the extremely fast stepper motors which move the bigger model’s robotic arm were no longer being produced. The search for an alternative led Gyros to Compotech Provider. The motors must be very fast without compromising the torque. This is why it was decided to replace the stepper motors with powerful servomotors. The model finally selected was Faulhaber’s BX4 series brushless DC-servomotor with 4-pole technology and high torque. The motors are equipped with incremental encoders and, thanks to their compact design, are just a little larger than the stepper motor used in the previous model. Another advantage is their price-performance ratio.

The system contains three servomotors from the BX4 series, two of which have been mounted on a linear table. They move the robotic arm horizontally to transfer the samples and control the laser’s movements during analysis. The third motor has a planetary gear box, which lifts and lowers the pipetting head. The high-precision control electronics with more than 3,000 position set points and low torque ripple ensure that the samples are positioned accurately on the CD, directly at the inlet of the respective channel. Equipped with BX4 motors, the system also meets the speed requirements, as generating 112 data points takes less than an hour.

**Fig. 2:** Gyrolab xplore TM tests automatically tiny quantities of up to 112 samples in parallel, saving time, labor and material.

**Fig. 4:** Due to precise control electronics and constant torque, the samples can be positioned precisely on the plastic dish at the entrance of the respective channel.
In times of rising personnel costs and the general trend of increasing efficiency in the analytical laboratory, methods for automated sample preparation like e.g. online solid phase extraction (online-SPE) are an attractive alternative to increase throughput and reduce the costs per analysis. Again, miniaturization shows a clear advantage over classic procedures, which will be discussed in the following.

**Sample Throughput**

The increase of sample throughput in all areas of analytical chemistry and life sciences is a criterion of high priority. In many cases “only” the step of measurement, for example HPLC-UV or HPLC-MS, will be considered and the sample throughput is just correlated with the time of the chromatographic run. Besides that, the remaining work steps such as sample preparation, evaluation and plausibility control of results as well as report generation take significantly more time. Therefore, it is useful to consider the overall process. The automation yields a valuable contribution to increase the sample throughput, especially if the system can shift personal intensive working steps in night or weekend hours where the lab business in many cases rests or is at least strongly restricted.

**Online-SPE**

A fully automated sample preparation system (online-SPE-HPLC-MS/MS) including clean-up and enrichment steps is shown in figure 1. This system consists of a “prep and load robotic tool change” (PAL, RTC) autosampler with syringes of a volume of 100 µL, 1 mL and 10 mL, two injection valves equipped with different loops and an enrichment unit with a cartridge exchanger for the automated online-SPE. The first sample loop has a volume of 50 µL. Therefore, the system can be used as a conventional HPLC system with the possibility of injecting smaller volumes. The second sample loop has a volume of 10 mL. Herewith the enrichment of the sample takes place on a disposable SPE cartridge. Optionally, washing steps to remove salts or polar matrix constituents can be performed. As it is apparent from the instrumental setup depicted in figure 1, a large space must be provided to properly arrange all system components. Furthermore, it can be clearly seen that relatively complex valve circuits and long transfer capillaries are necessary to connect all tools and modules.

Theoretically, the system configuration shown in figure 1 can also be understood as a two-dimensional HPLC system by using the SPE cartridge not only for enrichment or clean-up but also for a pre-separation, if a cartridge with orthogonal selectivity compared to the HPLC column is used. Furthermore, two SPE cartridges can be coupled serially. By means of frontal elution with an organic solvent the analytes trapped on an SPE cartridge can be eluted in a small plug which is then transferred directly to the HPLC column. Via an auxiliary pump or, as shown in figure 1, via the high pressure dispenser (HPD), the organic plug can be diluted with water to ensure a sample focusing on the analytical column. Figure 2 exemplarily shows two flow paths for sample enrichment as well as elution.

**2D-Micro-LC**

In the field of microscale two-dimensional HPLC, there are first approaches to develop compact systems including gradient pumps and switching valves. Such a system is shown in figure 3. A critical point is the connection between the column and the emitter tip of the mass spectrometer ion source. As shown in figure 3, an approximately 25 cm long capillary is needed for this connection. Especially those volumes behind the column have a great influence on the extra-column band broadening because the chromatographic bands cannot be refocused. A big advantage of this system is that the required laboratory space can be reduced significantly compared to the setup shown in figure 1. The optimal solution in terms of space requirements would be a separation unit on a chip, which can be placed directly in front of the ion source inlet of the mass spectrometer. Such a design circumvents the problem of extra-column band broadening and thus represents the best possible hyphenation between chromatography and detection.
Even Smaller

Current approaches of research are aimed at not only miniaturizing the two dimensional unit on chip size, but also to integrate sample preparation steps. Such a design is shown in figure 4. Establishing this approach would lead to an enormous reduction of needed laboratory space.

In principle, the microfluidic chip is built of two microstructured layers with cavities and channels, which can be used as reservoirs and flow channels. By sliding these layers, micro cavities of upper- and lower level can be united and closed again. Moreover, flow channels can be generated by adding complements of structures. This approach is extremely innovative because it does not need chip integrated valves and thus promises an increased degree of robustness. Beyond that, extra-column volumes are extremely low, because this chip architecture includes the injection unit and a low dead volume connection between the chip and the MS is possible if the emitter is also integrated on the chip. It is to be noted that the miniaturization of the separation unit is only one important step for designing portable systems. To achieve the overall goal of miniaturization, all peripheral components such as pumps and detectors have to be miniaturized.

Conclusion

Despite the enormous advantages which are provided by chip technology, it is still a long way from research to routine. It can be clearly seen that the strength of integrated miniaturized systems is that all components such as sample clean-up and enrichment, one- or two-dimensional chromatography and possibly further functionalities like for example the integration of mixing or auxiliary channels for feeding ionization additives for sensitive MS detection can be combined on a single chip.

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Figure 2: Flow paths for the online-SPE system: a) enrichment step and b) elution step.

Figure 3: Overview of a compact two-dimensional HPLC system including the injection unit, switching valves and pumps.

Figure 4: Schematic presentation of the modular microfluidic chromatography chip (SlipChip).
Multidisciplinary Approach to SNP Mechanisms
Personalized Medicine from Computational Modeling Discovery

D. N. Meijles¹ and B. J. Howlin²

Reactive oxygen species (ROS) produced by the NADPH oxidase (Nox) enzymes play a central role in age-related diseases, including Alzheimer’s disease, cancer, arthritis, type-2 diabetes, and cardiovascular diseases including atherosclerosis and hypertension. Despite regulating normal cellular function, ROS, either as a result of cellular stress or from a chronic overload of antioxidant defense mechanisms, negatively impacts cellular health by damaging macromolecules, such as DNA. By extension, ROS cause oxidative damage to tissues and, therefore, remain a dominant proponent for age-related organismal decline. Using a multi-disciplinary approach employing computational protein modeling, molecular and cellular biology, clinical sample phenotyping and characterization, and rational drug design with structure activity relationships, we identify a route of enquiry that uncovers novel mechanisms or drug candidates, which can identify new routes for preventing oxidative stress-linked diseases that acutely correlate with ageing.

Age-Related Diseases and ROS

Advanced human age is an independent risk factor underlying many degenerative diseases, such as Alzheimer’s disease, cancer, arthritis, type-2 diabetes, and cardiovascular diseases including atherosclerosis and hypertension. Moreover, diseases associated with ageing and co-morbidity presentation are acutely correlated. By year 2035, it is predicted that 23% of the UK population (24.3% of EU member states) will be > 65yrs and present clinically with one or more age-associated disease, costing health care providers ~£4.4 billion more per year for social care and medical costs [1, 2]. Understanding the mechanisms that lead to tissue degeneration and disease, therefore, are of paramount importance.

A decline in tissue function and a central hallmark of ageing is senescence, a process whereby cells withdraw from mitotic division and lose proliferative responses to growth factors or mitogens. As an irreversible form of cell-cycle arrest, cellular senescence is initiated by a variety of stresses including genotoxic and oxidative stress [3]. However, as first observed by Harman and colleagues, oxidative stress has long been considered a key driver of the ageing process [4]. Since their discovery as professional reactive oxygen species (ROS; i.e. superoxide and hydrogen peroxide) producing enzymes, the role of the highly conserved NADPH oxidase (Nox) family is well defined in myriad pathologies where oxidative stress is central in disease aetiology [5]. Only recently, however, has the notion of Nox-derived ROS been proposed as a potential source for age-related oxidative stress [6]. Of the 7 Nox family members, Nox2 remains a prominent source of damaging superoxide in vascular cells and cardiovascular disease. Nox2 generates ROS when the organizing p47phox protein interacts with the Nox2 catalytic core via anchoring with the membrane bound p22phox subunit [7]. Nox2 complex activation, therefore, is highly dependent on post-translational protein modification (e.g. glycosylation and phosphorylation), and occurs in response to multiple stimuli, such as angiotensin-II, tumor necrosis factor-alpha, or elevated glucose levels [8]. Genetic studies using clinical samples identified that the Nox2 complex proteins are highly polymorphic, with many single nucleotide polymor-
phisms (SNPs) translating to the protein [9]. Recently, natural variation in human ROS levels was hypothesized to result from the effects of SNPs. However, how Nox2-linked SNPs affect oxidative stress-linked diseases with age remains unknown.

From Protein Modeling to Clinical Characterization

The p22phox is an essential component of the Nox1-4 isoforms and contains 7 SNPs, of which only 2 translate to the protein. Structurally, using a consensus computational homology modeling approach as no crystal structures exist, it was identified that the p22phox topology contains three N-terminal transmembrane spanning helices and a C-terminal cytoplasmic domain [10], with an extensive extracellular domain located between helix 2 and 3. As membrane proteins are notoriously challenging to crystallize, a computational modeling approach provided the rational means for understanding structure-function relationship for SNPs of the p22phox, and to generate further hypothesis-driven discovery.

Of the domains in p22phox, the extracellular region positions the site for the clinically relevant C242T SNP, which results in a histidine to tyrosine substitution. Genetically, the C242T SNP is associated with reduced atherosclerotic or hypertensive prevalence in some studies. However, other reports document no effect or that the C242T is linked to vascular disease progression. Given that little consensus existed for the effects of the C242T SNP, a multi-disciplinary approach was used to interrogate the functional consequence of the tyrosine substitution on Nox2 function [8]. Computational protein modeling identified that the tyrosine substitution resulted in a significant structural change in the extracellular loop of p22phox. Using molecular biology approaches and subsequent over-expression of p22phox C242T in human endothelial or p22phox-deficient tumor cells, our work identified an inhibited effect for stimulus-induced Nox2 ROS production. An important regulatory role for the p22phox subunit alongside its ability to anchor p47phox for Nox2 complex assembly is to assist in Nox2 maturation and cytochrome b558 complex formation. Mechanistically, we identified that the altered structure and inhibited ROS production due to the C242T SNP resulted from reduced maturation and expression of the Nox2 subunit by cell biology and antibody phenotyping techniques. Further, this discovery was functionally interrogated using clinical samples to confirm our in vitro studies and provide clinical characterization. This multi-disciplinary research direction elucidated a novel mechanism for a common SNP mutation and how it is protective against cardiovascular disease via inhibited activation of Nox2.

As the p22phox is essential to agonist-induced ROS production for Nox1 and Nox2 enzymes, both of which are culprits in cardiovascular diseases, we returned to a computational platform to exploit the p22phox-p47phox interface for novel drug discovery [7]. Interestingly, the region for p47phox anchoring by p22phox is located within a region separate to the C242T SNP and has been crystalized. Further work using this multi-disciplinary approach will be described in a forthcoming study.
platform is currently being undertaken to fully characterize potent and entirely novel candidate compounds by structure-activity relationship studies. Our work characterizes for the first time mechanisms that can be exploited by novel drug discovery approaches to inhibit the Nox2 enzyme activity based on inhibited maturation or complex assembly inhibition, thereby providing novel medicaments for attenuating age-related vascular diseases.

Personalized Medicine and Clinical Relevance

Successful treatment of age-related vascular diseases is expected to improve quality of life and reduce the financial burden confronting health services. By elucidating the molecular mechanism of a common Nox2-linked SNP, our research approach enables the design of drugs that will prevent the Nox2 activation process in conditions of stress, including ageing. Importantly, our work has direct implications in support of personalized medicine, which aims to utilize an individual’s genetic makeup for predicting treatment strategies and improve diagnosis. Despite this new branch of medicine being in the discovery phase, multiple examples of targeted strategies exist that are already clinically viable, e.g. trastuzumab (Herceptin) treatment for breast cancer [11]. Given that personalized medicine can be divided into two areas (i.e. influence of genetic variation on drug response or influence of phenotype switches in diseased tissue for variation on drug response) we are able to extend our current knowledge to the effects for inhibiting Nox2 in age-related disease. Firstly, however, viable isoform selective assembly inhibitors (i.e. those currently being studied by our research direction) are essential to global treatment regimens for Nox enzymes in disease aetiology [12]. Next, using the garnered knowledge of the C242T SNP we could tailor efficacy of specific inhibitors to genetic makeup or the need for activity versus expression inhibitors. But, as a consequence of the current research direction in age-related diseases, we must be aware of the interplay between genetics and environment.

Fig.1: Depicted interaction between the p47phox (in yellow) super-SH3 domain (3D representation in green) and proline-rich region of the p22phox (in red) of the inducible Nox family members. Results generated using molecular operating environment (MOE; ChemComp Inc.) and depicted by www.SciCommStudios.co.uk.

Fig.2: Representation of inhibited p47phox-p22phox interaction using our in silico designed Nox assembly inhibitors.
Tracking Silver Nanoparticles

Ultra-Trace Analysis of Silver Nanoparticles in the Environment in the Parts Per Trillion-Range

A. Wimmer and M. Schuster

Analytical Challenges

So far, detecting AgNPs in environmental samples, like WWTP influents and effluents, river and lake water, or sewage sludge, was very challenging for scientists. The concentration of nanoparticulate silver in the samples is often too low to be measurable using conventional analytical methods. Furthermore, AgNPs occur in presence of ionic silver species and are embedded into very complex matrices. The research group Analytical Chemistry around Prof. Dr. Michael Schuster of the Technical University of Munich was aware of this problem and able to solve it: Using a special enrichment technique, the so called Cloud-Point-Extraction (CPE), AgNPs can be species selectively separated from environmental samples and enriched by a factor of 100 [1].

This method is based on a micellar mediated separation of AgNPs from aqueous samples. The aqueous sample is mixed with a special surfactant and heated over a certain temperature specific to this surfactant. The surfactant molecules form micelles and thereby enclose AgNPs in the micelle’s hydrophobic inside. From a macroscopic perspective, the solutions become clouded because the so-called cloud-point temperature is reached. Ionic silver species are retained in the aqueous phase, which works almost quantitatively using special ligands. After centrifugation, the aqueous and surfactant rich phase are separated and the AgNPs can finally be found in an unmodified but strongly enriched form.

Application on Real Samples [3]

Wastewater Treatment Plants (WWTPs)

So far, existing data addressing AgNPs in environmental samples is based on model calculations or laboratory and techni-
cal experiments, respectively, using high AgNP concentrations. The presented technique enabled us to measure real environmental samples and collect reliable data on AgNPs in the environment for the first time. Within a research project financed by the Bavarian State Ministry of the Environment the actual way of AgNPs, passing WWTPs with different wastewater treatment processes, to natural water bodies was tracked. As an example for running waters, the river Isar – a 292 km long river in Southern Germany originating in the alps, passing Munich (1.5 million inhabitants), and finally reaching the river Danube near Deggendorf – was examined on the content of AgNPs. Additionally, water samples from several surrounding pre-alpine lakes were collected and their AgNP concentration was also determined. The aim of the project was to examine the influence of WWTP effluents on the occurrence of AgNPs in surface waters.

The wastewater treatment of all examined WWTPs works very well because the sewage sludge retains up to 96% of the AgNPs. This observation is consistent with the data presented in Fig. 2, which shows the AgNP concentrations in effluent of two WWTPs over the seasons, with LOD = limit of detection. (b) and (c): Size distribution of AgNPs in wastewater influent (b) and effluent (c) measured by spICPMS [3].

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with experiments using a laboratory WWTP at the Bavarian Environmental Agency [4]. Depending on the season, the influent carries 350 ng/L (winter) to 10 ng/L (summer, after heavy rain) AgNPs into the WWTPs whereby the effluent contains AgNPs up to a maximum of 11 ng/L (fig. 2a). The average particle size varies between 20 nm in the influent (fig. 2b) and 15 nm in the effluent (fig. 2c).

Based on the data of different WWTPs the estimated flux of AgNPs referred to WWTP effluent discharge is 33 kg per year in Germany. In the following, it is therefore analyzed how WWTP effluents influence the occurrence of AgNPs in running waters and lakes.

**AgNPs Along the River Isar and in Pre-Alpine Lakes**

For this purpose, surface water samples of the river Isar were taken at randomly chosen sites along the river. Furthermore, water samples were collected exactly at those points where WWTPs dispose their effluent into the Isar. After each of the disposal points an additional sample was taken 1.5 km downstream to observe dissolution effects. All water samples were subjected to CPE and silver was measured using ETAAS. In the upstream areas before Munich (sites 1-9) no AgNPs are detectable but as soon as the first WWTP after Munich discharges effluent into the Isar (site 10) 1.9 ng/L AgNPs are found (fig. 3). Such AgNP load peaks are detectable at every disposal point into the Isar. Due to dissolution effects at the sampling sites downstream the discharge points, the concentration decreases again reaching a constant level until the next WWTP effluent is disposed. Nevertheless, there is a slow progressive increase in the AgNP concentration in the river from the downstream areas to its confluence with the river Danube leading to a final concentration of 2 ng/L AgNPs near the river Danube (site 33). Consequently, AgNPs in the river Isar are clearly anthropogenic but, however, AgNP concentrations remain very low.

AgNPs could also be monitored in several pre-alpine lakes. Here, the concentration of nanoparticulate silver is in a range from 0.5 to 1.3 ng/L. All examined lakes are protected against WWTP effluents in the immediate surroundings by using ring sewer systems but some lake tributaries are WWTP influenced. In summary, all examined lakes in Southern Germany contained very low concentrations of AgNPs.

It is surprising that lakes with and without WWTP influence show similar AgNP concentrations. It can thus be concluded that AgNPs in natural water bodies are not only of anthropogenic origin. Measurements indicate that there is also a natural background. These NPs are most likely attributable to a natural formation of AgNPs from dissolved silver species.

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Introduction

To determine organic contamination levels, wastewater and drinking water treatment facilities are moving toward using the sum parameter total organic carbon (TOC) as opposed to traditional methods of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). In contrast to these traditional methods, TOC analysis is fast and has the advantages of better accuracy, lower sample volume requirements and the ability for complete automation through the use of online instrumentation. Additional advantages of TOC include low waste production and reduced interferences compared to COD and BOD. Hazardous waste is a significant disadvantage of COD, where highly toxic metals (Hg, Ag, Cr) are used and released in sulphuric acid results.

Additional qualitative and quantitative analysis of wastewater is often performed using chromatography coupled with mass spectrometry methods such as LC-MS, LC/MS/MS, GC-MS and GC-MS/MS to specifically detect certain regulated and non-regulated contaminants. The aim of this article is to discuss advantages and limitations of the classical TOC method in comparison with new enhanced techniques like Total Organic Carbon Size Exclusion Chromatography (TOC-SEC).

Fig. 1: Size Exclusion Chromatography (SEC) Column. Path of big molecules (red) without any diffusion into column particles, medium size molecules (green) diffusing into column particles, and small molecules (blue) diffusing into pores of the particles taking the longest to elute from the column.

Fig. 2: Example TOC-SEC System (own representation).
ulated contaminants of concern [1]. Due to low concentrations of organic contaminants in water (ng/L), an enrichment (i.e., pre-concentration) technique before these actual analyses is needed. Additionally, significant methods development is necessary for these mass spectrometry techniques for optimal quantification of each individual organic compound. Since the number of chemicals in the environment is continuously increasing and real-time monitoring is intended, composite parameters are becoming increasingly important. Nevertheless, analyzing wastewater is challenging due to its variable composition and complexity: ranging from biodegradable natural organic matter (NOM) to anthropogenic organic pollutants. Even though NOM itself is not toxic, it affects mobility and toxicity of organic contaminants [2, 3, 4]. Performing TOC analysis allows for a quantification of the sum total of the organic species in the water; however, a more complete picture of the organic composition of the sample can be useful and can be determined by performing separation of the organics by molecular size. Size exclusion chromatography (SEC) was applied to gather information about quality and treatability of organic matter. Therefore, a modified TOC detector for characterization of NOM was introduced [4].

Analytical Equipment

TOC Analyser

All TOC analysers oxidize organic material to CO₂ using either wet chemical oxidation or high temperature combustion. The resulting CO₂ is then measured to determine a TOC concentration. Before oxidizing organic carbon, the inorganic carbon (IC), atmospheric CO₂, carbonate, and bicarbonate in the sample, is removed. This removal is accomplished through lowering the pH by adding acid and then either purging with a carrier gas or diffusion through a CO₂ permeable membrane. If the former method is used, then the
resulting TOC value is referred to as non-purgeable organic carbon (NPOC). If the latter method is used, typically the resulting total carbon (TC) is measured as along with any remaining IC, and TOC is obtained through subtraction (TOC = TC – IC). The TOC measurement and DOC detection referred to in this article is called UV-Persulfate oxidation whereby organic carbon is converted to CO₂ via UV light and the addition of a persulfate oxidizing reagent. The resulting TC and IC are determined using the Sievers Membrane Conductometric Detection (MCD). MCD involves the separation of the CO₂ produced through the oxidation of organic matter through a gas-permeable CO₂-selective membrane, which prevents interference from other ionic species in the sample.

Modified TOC with HPLC SEC (TOC-SEC)
The applied SEC coupled TOC system combines a size selective separation system with two detectors, a UV detector and a modified TOC analyser operated as a dissolved organic carbon (DOC) detector. The system provides not only TOC, but also size information and UV activity. The separation is driven by differential permeation of molecular sizes when a solution flows through a column with porous packing [5]. Larger molecules elute off the column earlier than smaller molecules, which are held back due to diffusion into the packing (fig. 1). With this system, the molecular weight distribution of NOM can be estimated [6].

Other mechanisms like hydrophobic interactions, ion exchange, ion-exclusion, and intramolecular electrostatic repulsive interactions, might be relevant in the separation process [7, 8]. As humic substances comprise a large majority of NOM entering a treatment plant, the application of TOC-SEC to NOM and specifically humic substances has been investigated in past [7].

The TOC-SEC system provides a method for size fractionation of organics based on molecular weight. This application is cost-effective, easy to use, and simple to integrate. Coupling HPLC SEC UVA and SEC DOC detector leads to a total representation of all the dissolved organic compounds, not only those that are aromatic or have a UV or fluorescent signal. Figure 2 shows an example system in the laboratory.

TOC-SEC provides a multi-detector combination system and insights in NOM molecular fractions [4, 6, 7, 9]. The set-up is described in detail in table 1.

Results and Discussion
As an example, a drinking water treatment facility in the United States sources its water from a newly built reservoir. The reservoir is filled from creek water that is downstream of a wastewater treatment plant. The creek provides ample residence time for contaminants originating in the wastewater to degrade therefore making the drinking water facility a pseudo indirect reuse plant. The water is treated with chemical coagulation and flocculation followed by membranes and disinfected with chloramines.

While the overall TOC was similar from the wastewater treatment plant (WWTP) effluent to water treatment plant (WTP) influent as shown in table 2, concentrations of each size fraction changed dramatically due to environmental degradation as shown in figure 3. The WTP effluent had less overall TOC and consequently lower concentrations in each size fraction. The presence and concentration of organics in different size fractions varies from WWTP effluent to WTP influent to WTP effluent.

TOC monitoring and DOC speculation can be used to optimize treatment processes, prove reliability, and meet target contaminant removal. Monitoring bulk TOC in addition to characterizing DOC allows facilities to ensure efficient operations that protect public health and the environment.

Conclusion
Since efficiency of organic matter removal from wastewater is one of the major challenges in water treatment processes, characterization of the remaining organic matter in wastewater effluent by size can indicate treatment quality without having to know the concentration of every single compound. The TOC-SEC system is a useful tool to describe the nature and changes of source water as well as effluent characteristics for discharge. TOC-SEC can also help operators establish optimal treatment settings when bringing up a new treatment system or troubleshooting when a treatment system is down.

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Silicon-Based Tandem Solar Cells
Boosting the Performance Using Non-Toxic Metal Oxides

Today, wafer-based crystalline silicon solar cells dominate the photovoltaic (PV) market, with a market share of more than 90%. Further cost reductions for this technology can be achieved by developing silicon-based tandem solar cells employing low-cost, abundant, and non-toxic metal oxide materials. Such tandem cells can increase the conversion efficiency of silicon solar cells beyond their conventional limitations with obvious economic and environmental benefits.
Introduction

Many candidate materials have been proposed for the next generation of solar cells, and metal oxides are considered among the most promising ones. For instance, cuprous oxide (Cu$_2$O) is an attractive material for photovoltaic applications since it’s an earth-abundant and non-toxic p-type semiconductor with high optical absorption and a direct bandgap of approximately 2 eV, yielding a theoretical conversion efficiency limit of 20% [1]. To construct a p-n heterojunction, various n-type oxide materials, such as ZnO and SnO$_2$, can be combined with Cu$_2$O, and accordingly, one can foresee heterojunction solar cells completely based on low-cost metal oxides [2]. In addition, and perhaps even more exciting, a metal oxide heterojunction solar cell can be combined with a conventional c-Si bottom subcell in a tandem architecture in order to boost the conversion efficiency of crystalline silicon (c-Si) solar cells beyond the conventional limitations of this technology [3]. Figure 1 shows an illustration of a tandem solar cell, combining a conventional c-Si bottom subcell with a ZnO/Cu$_2$O top subcell in a four-terminal configuration, i.e. a mechanical stack of independently connected cells. The ZnO/Cu$_2$O top subcell, which is deposited on a transparent quartz substrate by reactive magnetron sputtering, enables low-energy photons to be transmitted through the top subcell for subsequent absorption in the c-Si bottom subcell. In this way, the solar spectrum can be more efficiently utilized in the wavelength range from ultraviolet to near-infrared.

Experimental

Cu$_2$O and Al-doped ZnO (AZO) thin films were deposited on 10 x 10 x 0.5 mm$^3$ quartz substrates using a direct current/radio frequency (DC/RF) magnetron sputtering system (Semicore Triaxis). 500 nm thick Cu$_2$O film were deposited by reactive sputtering of a 99.999% Cu target in O$_2$/Ar (6/49 sccm) at a substrate temperature of 400°C. The power was fixed at 100 W. As-grown Cu$_2$O films were annealed at 900°C for 3 minutes in vacuum (pressure ~10$^{-1}$ Torr). 200 nm thick AZO films were deposited by co-sputtering of a 99.99% pure ZnO ceramic target at 50 W and a 99.999% Al target at 3 W in Ar at a substrate temperature of 400°C, yielding an aluminum content of approximately 4 wt% in the deposited layers. During the magnetron sputtering deposition, the base pressure was below 3.0 x 10$^{-7}$ Torr. The optical properties and surface morphology of the AZO and Cu$_2$O thin films were analyzed using a Horiba Jobin Yvon Uvisel spectroscopic ellipsometer and a Quanta Inspect F 50 scanning electron microscope (SEM), respectively. The optical transmittance spectrum was measured using a setup with spectrophotometers, a deuterium–halogen light source, and an integrating sphere. Also, room temperature Hall effect measurements (LakeShore 7604) were carried out using the van-der Pauw configuration.

Results

Table 1 shows the majority carrier mobility, film resistivity, and carrier concentration for Cu$_2$O (as-grown and annealed) and AZO thin films deposited on quartz, derived from Hall effect measurements.

<table>
<thead>
<tr>
<th>Material</th>
<th>Mobility (cm$^2$/V·s)</th>
<th>Resistivity (Ω·cm)</th>
<th>Carrier concentration (cm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu$_2$O (as-grown)</td>
<td>10</td>
<td>560</td>
<td>3x10$^{15}$</td>
</tr>
<tr>
<td>Cu$_2$O (annealed)</td>
<td>50</td>
<td>200</td>
<td>1x10$^{15}$</td>
</tr>
<tr>
<td>AZO</td>
<td>20</td>
<td>5x10$^{-4}$</td>
<td>3x10$^{19}$</td>
</tr>
</tbody>
</table>

Tab. 1: Carrier mobility, film resistivity, and carrier concentration for Cu$_2$O (as-grown and annealed) and AZO thin films deposited on quartz, derived from Hall effect measurements.

<table>
<thead>
<tr>
<th>Subcell</th>
<th>$J_{sc}$ (mA/cm$^2$)</th>
<th>$V_{oc}$ (V)</th>
<th>Fill factor (%)</th>
<th>Power density (mW/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO/Cu$_2$O top</td>
<td>11.1</td>
<td>1.42</td>
<td>75.9</td>
<td>12.0</td>
</tr>
<tr>
<td>c-Si bottom</td>
<td>21.6</td>
<td>0.60</td>
<td>80.4</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Tab. 2: I-V parameters for the ZnO/Cu$_2$O top and c-Si bottom subcell in a four-terminal tandem configuration.
tation for Cu$_2$O (as-grown and annealed) and AZO thin films deposited on quartz. The data suggests that the electrical properties for the Cu$_2$O thin film are enhanced after thermal annealing. The increase in carrier mobility after annealing can, at least partly, be attributed to the increase in grain size and reduced grain-boundary scattering. SEM images of the Cu$_2$O thin film confirm that the average grain size increases from about 70 nm for the as-grown film to about 600 nm for the annealed film [3]. The carrier mobility and concentration are comparable to values previously reported for sputter-deposited Cu$_2$O thin films on quartz [4] and suggest that the annealed Cu$_2$O thin film is well suited for photovoltaic applications.

The complex refractive index of Cu$_2$O and AZO the thin films were derived from spectroscopic ellipsometry measurements and implemented in a ray tracing model for calculation of the optical characteristics of the ZnO/Cu$_2$O subcell [3]. The absorbed and transmitted spectral intensities for the ZnO/Cu$_2$O subcell are shown in figure 2. The short-wavelength photons below ~550 nm are absorbed in the top subcell, whereas the long-wavelength photons above ~600 nm are transmitted through the top subcell and onto the c-Si bottom subcell.

The electrical performance of the four-terminal tandem solar cell was evaluated based on device modeling in Silvaco Atlas, and both experimental and tabulated materials parameters were adopted in the model [5]. Typical current-voltage (I-V) parameters for the top and bottom subcells are summarized in Table 2. The data suggests an overall power conversion efficiency of 22.5% for the tandem solar cell under 1 sun (100 mW/cm$^2$) illumination, whereas the corresponding efficiency for the c-Si solar cell is 18.2% at 1 sun. This implies that a metal oxide heterojunction solar cell can be used in a tandem configuration in order to boost the conversion efficiency of c-Si solar cells.

Conclusion

In conclusion, the conversion efficiency of crystalline silicon solar cells can be boosted by adopting a tandem solar cell architecture, incorporating low-cost, abundant, and non-toxic metal oxide materials. The sputter-deposited Cu$_2$O and Al-doped ZnO thin films presented in this work show good potential for application in a silicon-based tandem solar cell.

Acknowledgments

This work was conducted under the research project “High-performance tandem heterojunction solar cells for specific applications (SOLHET)”, financially supported by the Research Council of Norway (RCN) and the Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI) through the M-Era.net program. RCN is also acknowledged for the support to the Norwegian Micro- and Nano-Fabrication Facility, NorFab, project number 245963/F50.

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Spider Silk’s Molecular Architecture

The mechanical properties of spider silk arise from a refined architecture on different length scales. A single thread has a skin-core structure with numerous fibrils (fig. 1a-c), which are composed of a phase-separated matrix of two spider silk proteins (major ampullate spidroin I and II, MaSpI and MaSpII, fig. 1d). Both exhibit a block-like primary structure with alternating polyalanine ($A_n$) and glycine-rich segments, $(GGX)_n$ and $(GPGXX)_n$. During spinning the proteins experience shear force which orients them and induce a structural conversion. The $A_n$ parts adopt a β-sheet secondary structure forming nanometer-sized crystallites (5×5×7 nm$^3$) and the glycine-rich segments embed the crystals in a matrix, while the orientation and order of all molecular segments is well preserved on macroscopic scale (fig. e) [1]. Furthermore, IR spectroscopy in combination with external mechanical fields performed by Prof. Dr. Friedrich Kremer and co-workers [3, 4] proved that the nanocrystals are interconnected through pre-stressed protein chains [3, 4]. Thereby, the tendency to contract is counterbalance by the crystal surrounding protein matrix. As a consequence of this refined construction macroscopically applied load is transferred...
through the soft matrix down to the molecular scale where it acts on the much stronger crystallites, which finally dissipate the impinging energy. This exceptional mechanism manifests itself in the load dependence of a molecular vibration that is exclusively located within those crystallites. In case tensile stress is applied to the silk fiber, the molecular stress acting on the nanocrystals is increased and a red shift of the crystal-specific IR absorption band occurs (fig. 1f). In the opposite case, when hydrostatic pressure is applied, the molecular stress is reduced and the crystal band shows a blue shift.

Manufacturing of Artificial Silk

In order to elevate spider silk production to industrial scale, the manufacturing of recombinant proteins has to be developed. In one approach Prof. Dr. David L. Kaplan and coworkers inserted DNA encoding spider silk proteins into prokaryotes as *Escherichia coli* bacteria [5] or by other researchers into eukaryotes as *Pichia pastoris* yeast [6], which then expressed these proteins. The transfection and expression works also with mammalian cells like bovine mammary epithelial alveolar and baby hamster kidney cells as Dr. Anthoula Lazaris and coworkers has demonstrated [7]. Alternatively, transgenetic goats, which produced milk including spider silk proteins, and transgenetic plants has been tested by Prof. Dr. Randolph V. Lewis and coworkers [8], but both projects were put on hold in the meantime. However, performing sericulture with transgenetic silkworms that produce chimeric silkworm/spider silk fibers as published by Prof. Dr. Malcolm J. Fraser Jr., Prof. Dr. Randolph V. Lewis, Prof. Dr. Donald L. Jarvis, and coworkers may be another promising solution. The first recombinant spider silk with comparable toughness as natural major ampullate silk was published in 2015 by Prof. Dr. Thomas Scheibel and coworkers [9].

Applications in Technique

Silk has a long history as protective material. Except for body armors, silkworm silk has also been employed in parachutes. Although spider silk provides superior mechanical resistance compared to silkworm silk, problems as spiders’ cannibalism did prevent industrial farming comparable to sericulture in the past. By means of the new production methods, spider silk will become more prominent; actually the U.S. Army is testing spider silk for body armors (The Washington Times, July 12, 2016).

Besides mechanical demanding applications, spider silk may play an important role in future photonics and electronics. Nowadays, silkworm silk can be prepared as optical device like contact lenses, optical waveguides, and 3D dif-
fraction patterns [10]. When casting silk solution over a template of close-packed colloidal particles a 3D photonic crystal of silk invers opals is obtained. Its color may be modified by the casting template or filling. In addition it is suited as optical humidity sensor [11, 17].

Application in Medicine

Spider silk holds great potential in the medical sector. Native silk is excellently biocompatible while suppressing inflammations and promoting cell adhesion. It is suited as suture (fig. 2; fibers, meshes) or replacement for tissues as Prof. Dr. Kerstin Reimers-Fadhlaoui and coworkers figured out [12-14]. As published by Prof. Dr. Thomas Scheibel and coworkers, a coating of recombinant spider silk proteins onto a silicone implant prevents post-operative inflammatory and fibrotic complications [15].

Nowadays, silkworm silk-based materials provide an ideal matrix for stabilizing enzymes or antigens, which may work as disease detecting biosensors (films) or drug delivery systems (particles) [10]. Prof. Dr. David L. Kaplan, Prof. Dr. Fiorenzo G. Omenetto, and coworkers demonstrated the formation of conformal electronics as biointegrated devices for diagnosing or brain/machine interfaces [11, 16, 18]. Moreover, silkworm silk implants are tested by Prof. Dr. Fritz Vollrath and coworkers as bone or cartilage growing templates (foams).

Summary

Spider silk provides versatile opportunities for high tensile robustness applications but also for excellent biocompatibility requirements. Repairing traumatically interrupted nerve cells appears possible as well as integrate electronic into living organisms. As soon as spider silk will be available in industrial quantities, it will capture a share in the market, which is at the moment dominated by silkworm silk.

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Iron(III) (oxyhydr)oxides exist in a variety of phases, differing in their crystallinity, shape, composition, physical, and chemical properties. As a result, the possible applications of iron(III) (oxyhydr)oxides in various fields of industry are very diverse. They are used as pigments, sorbents, catalysts, and as coating for protection from corrosion, just to name a few [1]. In order to control the phase identity, the potential introduction of guest ions, crystallinity, shape and therefore the properties of the resulting particles, the onset of phase separation needs to be understood. Control of the precipitation mechanism is the key to control the precipitation product. Another field where knowledge of the precipitation pathway would be beneficial is the recycling of acid mine drainage. Here, the precipitation or iron(III) oxides is used for the removal of heavy metal ions via co-precipitation. As much as the process of iron(III) oxide precipitation constitutes a central point in many industrial applications, the mechanisms underlying the pathway are not understood.

Despite the widespread occurrence of iron(III) (oxyhydr)oxides and their numerous applications in industry, no general pathway for their precipitation mechanism has been established. An understanding of the key steps and the occurring intermediate phases would be highly interesting, as it would enable controlled syntheses of materials with desired properties. In this article we present how the precipitation mechanism and the underlying chemical and physical chemical processes can be elucidated, and used for the development of synthetic strategies.

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Classical Nucleation Theory (CNT)

The initial phase separation event, e.g. nucleation, can follow different mechanisms. Most systems are described using Classical Nucleation Theory, CNT. This framework was established in the 1920s and divides the formation of nuclei and their subsequent growth to a bulk material into three stages; the pre-critical, the critical and the post-critical regime. In the pre-critical regime, prior to phase separation, small nuclei form upon statistical addition of single ions. These pre-critical nuclei are already structured like the final crystal lattice and are therefore assumed to possess the same properties. Owing to their small size of only a few atoms, they exhibit an extremely high surface to bulk ratio. The high surface area is energetically unfavorable and cannot be balanced by bulk energy, due to the small size of the nuclei. Due to this, the pre-critical nuclei are thermodynamically unstable and dissolve again, and thus exist in a very small population. This scenario changes with size, however. With increasing radius of the nucleus, the surface energy grows with $r^2$, while the bulk energy increases at a faster rate with $r^3$. The radius at which the favorable bulk energy starts to balance the unfavorable surface energy is called the critical radius and defines the so-called critical nucleus. It is a metastable state, i.e., both dissolution and growth result in more stable states. Consequently, post-critical nuclei possessing a radius larger than the critical radius grow without limit.

Non-classical PNC Pathway

CNT has been challenged repeatedly in the recent years, since experimental observations made in many systems display a significant discrepancy with the predictions of this theory. One major issue of CNT is the assumption of bulk properties for pre-critical nuclei, as material properties change significantly when particle sizes reach the nanometre-regime. In contrast to the notions of CNT, it was shown that prior to phase separation, a significant population of thermodynamically stable clusters can be present, so-called pre-nucleation clusters (PNCs) [2]. They are highly dynamic and exhibit a high water content. Due to these characteristics, PNCs are conceived of as solutes, where an interface with the surrounding solution does not exist, because there is no difference in dynamics between the clusters and the solution. The phase separation event in the PNC pathway is then not governed by a critical size, but rather by the development of an interface due to a distinct decrease in the cluster dynamics. Thereby, the clusters become nanodroplets that aggregate in order to decrease their thermodynamically unfavorable interfacial surface area.

When it comes to the elucidation of nucleation mechanisms, especially the very early stages prior to the precipitation event are of high interest. Prenucleation species represent the primary stage to the forming crystal. Their investigation, however, is challenging due to their small size and high reactivity. Another difficulty arises from the fact that an isolation of the early compounds is likely to induce changes in their structure. To overcome these problems a special titration experiment (fig. 1) was designed for the preparation of reaction solutions that contain the early stages. By mixing very dilute solutions, controllable, reproducible and homogeneous reaction conditions can be provided. In situ analytical techniques, i.e., techniques that allow an investigation of the components without requiring isolation from the solution, were applied for detailed characterizations of the distinct nucleation stages; the special titration experiment enabled access to the different stages of iron(III) (oxyhydr)oxide precipitation, especially the pre-nucleation regime. Moreover, it can be modified easily, allowing studies on different systems, leading to different iron(III) (oxyhydr)oxide phases, such as akaganéite or ferrihydrite [3,4].

Results

It was shown that the precipitation of iron(III) (oxyhydr)oxides follows the non-classical PNC pathway [4]. This means that very dynamic, solute, stable, low-density iron(III) clusters, PNCs, are present in the early stage of the precipitation (fig. 2). The chemical reactions that govern and direct the phase separation event were identified. The formation of the PNCs proceeds via olation reactions, i.e., the bridging of iron(III) centres with hydroxo bridges. This reaction is reversible and the reaction is in (metastable) chemical equilibrium at this stage. The properties of the reaction solution change significantly with the onset of another chemical reaction, oxolation. During oxolation, more stable and more rigid oxo-bridges are formed. As a result, the clusters become denser and much less dynamic, and develop an interface with the surrounding solution. Thus, the onset of oxolation within olation PNCs marks the onset of phase separation. Particles precipitate and aggregate and further oxolation processes take place resulting in the final structures.

The key findings for directed syntheses are that the materials originate from PNCs and that cluster transformation
from solutes to a solid phase determines their appearance. This can be illustrated by means of the akaganéite system. Akaganéite is an iron(III) oxyhydroxide exhibiting a very specific tunneled structure. The tunnels are filled with and stabilized by chloride ions. The mechanism by which chloride ions are introduced into the iron(III) phase were debated. Using the above described experiment, it was shown that they bind already in the pre-nucleation stage to PNCs [3]. They compete with hydroxo-ligands and are thus incorporated into the olation clusters. Upon the onset of phase separation, i.e., oxolation, the chloride ions are released from the iron(III) centers. However, due to their presence not only on the surface but also within the clusters, complete expulsion is not possible. This leads to a microphase separation within the material and to the formation of the characteristic tunneled crystal structure.

The knowledge of the precipitation pathway can also be used to design novel materials, employing the experimental setup designed to investigate the iron(III) (oxyhydr)oxide system, monitoring the formation of the material. By adding the polymer poly(aspartic acid) to the iron(III) system, a novel material, consisting of nanoscopic, polydisperse spheres can be obtained (Fig. 3). Again, the PNCs play a key role in the formation of this material. While the polymer shows no interaction with unhydrolyzed iron(III) ions, it does significantly affect the fate of the PNCs. Due to their interaction with the polymer, and the resulting close proximity of the relevant atoms in the PNC-polymer hybrid structure, the oxolation reaction is facilitated. The resulting composite material contains both organic and inorganic compounds and is formed by the strong interaction of the PNCs with the polymer. It exhibits different properties than both the polymeric and the inorganic compounds and might serve in the future for interesting applications.

**Summary**

These studies serve as excellent examples for how the understanding of precipitation pathways can be utilized to not only understand and improve the formation of known materials but also for the directed synthesis of interesting, new materials. As the experimental setup can be used for the investigations of different systems, future studies will be used to understand the formation of specific materials and improve the synthesis protocols in order to optimize the reaction products with respect to their application.

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Fig. 3: Scanning electron microscopy (SEM) image of the composite material formed in the presence of poly(aspartic acid).
Hybrid Materials for Artificial Skins
Developing a Single Multi-Stimuli Responsive Material

A. M. Coclite

The goal of the just-funded ERC starting project “Smart Core” is to develop a single multi-stimuli responsive material, which would allow a simplification of the artificial skin matrix and enable unprecedented spatial resolution. The material will be comprised of a smart core, responsive to temperature and humidity, and a piezoelectric shell for pressure sensing. The swelling of the smart core upon stimuli will be sensed by the piezoelectric shell and produce a measurable potential. This architecture will be achieved thanks to the use of novel vapor-based technologies for material processing that allow fabrication at the nanoscale.

Introduction

The skin of humans and animals regulates the cycle of information from the external world to the body. The stimuli detected by the skin are then transformed into useful real world information content.

Artificial skins are made of three main components -the sensor, the signal processing & control unit and the actuator. The sensor responds to the stimuli coming from the environment, producing a measurable signal. The signal processing & control unit detects the signal. The actuator transforms the signal into an action. Each tactile mechanoreceptor in the human skin is a highly sophisticated biological circuit capable of detecting and transducing stimuli into a series of discrete electrical pulses that can be sent to the brain to generate the action [1].

Simplified solutions for artificial skins can be offered by multi-responsive materials, i.e. one material that will respond to different stimuli at the same time. A material like this can be obtained by combining the functionalities of polymers and inorganics into a hybrid structure. Our material will be comprised of a polymer core, responsive to temperature and humidity, and an inorganic zinc oxide (ZnO) piezoelectric shell for pressure sensing. The choice of ZnO as shell material resides on its interesting optoelectronic and piezoelectric properties that can easily be

Fig. 1: Schematics of the core-shell structure of the hybrid material and of the mechanism of stimuli detection.
used for functional devices. The stimuli coming from the environment, such as humidity or temperature changes, will be sensed by the smart material, inducing changes in size and shape. The changes in shape and size of the core will be detected by the piezoelectric ZnO shell and transformed in measurable electric current. An array of such sensors can be used as a platform for the creation of an artificial skin.

The core-shell architecture of the hybrid materials will be achieved thanks to the use of innovative vapor-based technologies, which allow controlling the growth of the materials at the nanoscale.

**Methods to Deposit Conformal Coatings**

Arrays of core-shell structures can be obtained by lithographic techniques or by subsequent deposition in porous templates. The template-deposition of polymers or of inorganic materials requires coating high-aspect-ratio nanopores with high conformality.

Liquid-phase based or line-of-sight deposition methods (sputtering, plasma assisted CVD, evaporation) may not fill uniformly the pores, resulting in low quality nanorods. Therefore, new techniques are required to fabricate such structures. We will use initiated Chemical Vapor Deposition (iCVD) for the core and plasma assisted atomic layer deposition (plasma-ALD) for the ZnO shell. Both these techniques are driven by surface-limited reactions, which ensure highly conformal coating and/or filling of the template pores.

**Initiated Chemical Vapor Deposition**

The iCVD technique is well suited for the production of organic polymers: it is a solvent-free method therefore it can be easily implemented in the manufacturing steps of integrated circuits or electronic device production. Its conceptual development was carried out mainly in Prof. Karen K. Gleason’s group at MIT, Boston. In terms of polymerization steps, iCVD is very similar to the conventional free radical polymerization, but with the advantage of being free of solvents. This allows to easily obtaining copolymers from monomers that have different solubilities.

iCVD involves the reaction between a radical-generating species (i.e. an initiator) and a monomer with unsaturated bonds to create monomer radicals adsorbed on the substrate surface where they polymerize [2, 3]. The initiator and

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**Fig 2:** The hybrid material will be obtained by template deposition. First, the shell material will be conformally deposited in the pores of the template. Secondly, the polymer will fill the pores. The electrical contact will be then glued on top and bottom.
monomer species enter the iCVD chamber as vapors. The initiator is decomposed by interaction with a relatively hot filament (150-300°C). These temperatures are enough to selectively break only labile bonds present in the initiator structure, (e.g. the O-O bond in the tert-butyl peroxide, TBPO). The monomer decomposition temperatures are > 500°C, therefore the monomer fully retains its chemical structure. In analogy to solution-phase synthesis, in iCVD, the initiator radicals activate the chain growth polymerization of the monomer.

The result is a surface of well-defined chemical composition having a high density of functionalities.

**Plasma Assisted Atomic Layer Deposition**

The ALD methods are based on sequential self-limiting surface reactions, which allow sub-monolayer thickness control [4]. The surface is first activated either by thermal energy or by plasma with concurrent flow of water vapors or oxygen. The subsequent step consists in purging the chamber from the oxidizer vapors and exposing the surface to the precursor vapors. Another purging step follows to remove the unreacted precursor molecules and the cycle restarts from the oxidant exposure. Purging time is a very important factor. It is the time that separates the introduction of the first precursor and the second (e.g. the oxidant) to the reactor. If precursors are introduced simultaneously, we have a CVD-like process. In such cases, growth rates are significantly higher from those in the ALD process but films are rougher. The ALD mechanism of growth limits growth rate up to maximum 1 monolayer/ cycle. The self-limiting surface reactions of the ALD processes result in very high conformality.

**Conclusions and Outlook**

The goal of the Smart Core project is to integrate temperature, humidity and pressure sensing in a single novel hybrid material that will detect the stimuli in terms of location and type. The outcome will be used to fabricate an efficient device for artificial skin applications. The successful development of the hybrid materials that are the hearth of this project will have consequences in different disciplines, such as sensing, biotechnology, tissue engineering.

The insights gained will lead to new approaches to the manufacture of nanocomposites materials for various applications (e.g. structural materials, responsive materials, drug delivery, membranes, sensors). It will also offer new, alternative ways to control the chemistry and molecular organization of polymers by deposition from the vapor phase.

The approach suggested in this project has the potential to be a competitive alternative to the commonly used techniques. The development of the iCVD technique has high potentials to be implemented in many new emerging technologies, thus advancing the field of material research. Due to its easy scalability, iCVD can also foster the collaboration and knowledge transfer between academia and industry. iCVD is well established in the US but, apart from few centers, it is not very present in Europe. This makes it highly important to establish a large research group in Europe, on this topic. TU Graz is the first Austrian research institution that has adopted this emerging technology and certainly one of the few in Europe.

The funding of the ERC project will be highly beneficial to study new applications for iCVD polymers and open new research area in the field of nanostructured materials.

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Fig 3: Picture of the iCVD reactor built at the TU Graz.
Restructuring of Molecules with Lasers
Hydrogen Migration Controlled with Laser Pulses

M. Kübel1,2, C. Burger1,2, R. Siemering3, T. Naeser1,2, R. de Vivie-Riedle3, M.F. Kling1,2

Light can conduct the play of atoms and molecules in the microcosm. Humans manage to interfere with this play. Researchers from the Laboratory of Attosecond Physics (LAP) of the Max Planck Institute of Quantum Optics (MPQ) and the Ludwig-Maximilians-Universität (LMU) and from the Department of Chemistry of the LMU have now used light to reconfigure hydrocarbons. Using ultra-short laser pulses they removed an outer hydrogen atom from one side of a hydrocarbon molecule and directed it to the opposite side, where it rebounded. The method could be used in the future to synthesize new substances by controlling chemical reactions.

Fig. 1: Artistic impression of hydrogen migration in acetylene. The exact waveform of the laser pulses determines if a hydrogen atom migrates from the left to the right side or from the right to the left side.
Photo-Isomerization

Everything in these experiments happens unbelievably fast – within just a few millionths of a billionth of a second. An ultrashort laser pulse hits an acetylene molecule. The symmetric, linear hydrocarbon molecule with one hydrogen atom on each outer side starts to wobble, and is ionized. On an extremely short timescale, a hydrogen atom on one side becomes loose and migrates to the other side, where it rebounds. Vinylidene is formed from acetylene.

Such a restructuring of atoms in a molecule, induced by light, is generally called photo-isomerization. Photo-isomerization plays a large role in nature, for example in the visual process in human eyes and other vertebras, as well as in vitamin-D synthesis in human skin. The restructuring of atoms often results in clearly different chemical and physical properties of a molecule. The possibility to control photo-isomerization can therefore open up new perspectives in the catalysis and synthesis of new compounds.

Hydrogen Migration with Tailored Laser Pulses

In experiments it was now achieved to control the direction of hydrogen migration with tailored laser pulses [1], where a bound hydrogen atom is steered from one side of the molecule to the other. Quantum chemical simulations show that this process is enabled by controlled laser-coupling of various vibrational states of the molecule.

Besides acetylene (ethyne), also the larger molecule allene (propyne) was studied. In both cases the hydrogen migration in the ionized state of the molecule was initiated and controlled via phase-stable laser pulses, which consist of about one oscillation of the electrical field (fig. 2). The phase stability results in an exactly reproducible electric field waveform for each of the pulses. A certain waveform corresponds to a laser phase \( \phi \), with a value that can be freely chosen. A change in the laser phase then results in a modification of the electric field waveform. The influence of the laser phase on the hydrogen migration process was studied with a so-called reaction microscope. This instrument measures the movement of charged particles and allows determining if a hydrogen atom migrated from the right to the left or from the left to the right side.

The vinylidene molecular ion, which is formed upon hydrogen migration from acetylene, is unstable and decays asymmetrically: one of the two carbon atoms has two hydrogen atoms bound to it, whereas the other carbon atom has no hydrogen attached anymore. This asymmetric decay is visualized with the reaction microscope and the direction of hydrogen migration is determined for each molecule. The number of registered hydrogen migrations for each side is recorded and analyzed as a function of the laser phase, and then brought into relation. The result is shown in fig. 3(a), which demonstrates the control of hydrogen migration with the laser phase.

In the case of allene, hydrogen migration leads to the formation of a propyne ion, which has three hydrogen atoms on one side, and only one on the other. The formation of the propine ion results with high probability in the split off of a trihydrogen ion, which informs about the direction of the preceding hydrogen migration. As depicted in fig. 3(b), the hydrogen migration in the larger allene can be controlled analogously to acetylene. This shows that the method also works for longer-chain hydrocarbon molecules.

Underlying Mechanism

The experimental observations and the underlying mechanism were explained with quantum mechanical simulations. These compute the motions of atoms in the respective molecule during and after the interaction with the intense laser field. To cope with the computational effort in view of the many possible motions, the theoretical chemists used a trick: first, the influence of the laser field and its phase were computed. After the interaction of the molecule with an ultrashort laser pulse, the description of the molecule is switched to a carefully chosen coordinate system, in which the hydrogen migration can be described. The motion of migrating hydrogen atoms can then be depicted as a path on a potential surface, as shown in fig. 4. This way, the relation between the laser phase and the directional motion of the hydrogen atoms can be evaluated. The simulations reveal that the steering of the hydrogen migration becomes possible since the phase of the laser pulses is transferred to certain molecular vibrations. In combination with the ionization process, vibrational wave packets can be created, where an initial kick (to the left or right) is given to the migrating hydrogen. The kick depends on the laser phase and leads to directional hydrogen migration.

Outlook

The work shows that it is possible to utilize intense laser pulses to not only control
electrons in the microcosm, but also the about 2000-times heavier hydrogen atoms. Since the method is based on the excitation and manipulation of molecular vibrations, it can be used with all kinds of molecules consisting of many atoms. In the future it may thus be feasible to restructure complex molecules and synthesize new compounds. In particular in medicine and for the design of new pharmaceutics this perspective is very appealing.

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Biopharmaceutical Industry Cooperation

Sartorius Stedim Biotech, an international supplier to the biopharmaceutical industry, has announced an agreement with Nova Biomedical, a well-known US manufacturer of cell culture analyzers, to integrate their Bioprofile FLEX2 into the ambr multi-parallel bioreactor systems for automated, at-line cell culture analytics. Together they create a tool able to simultaneously run, sample and analyze a massive number of cell culture conditions during high-throughput cell line, media and process development. This will allow biopharmaceutical companies to develop cell culture processes in less time while preventing the process development bottleneck being shifted to the analytical laboratory.

Sartorius
www.sartorius.com

High Force, High Resolution Linear Actuator

Designed for highly accurate and fast repetitive industrial applications, the L-239 compact linear actuator is the newest addition to the suite of motion control solutions available from precision positioning systems industry leader PI (Physik Instrumente). The actuator has a positioning range of 52mm (2”), pushing force of 300N (66lbs) and 50mm/sec (2”/sec) maximum velocity. The non-rotating tip enables uniform motion as it prevents wobble, torque and wear at the point of contact. For vacuum-applications, special versions to 10-9 hPa are available. Resolution of 100 nanometers (0.1 microns) and repeatability down to 0.5 microns is achieved by a closed-loop servo motor for higher velocities or a micro-stepped 2-phase direct-drive motor.

Physik Instruments
www.pi-usa.us

Antibodies Product Portfolio Expanded

BBI Solutions have launched over 20 new antibodies in a range of monoclonal and polyclonal varieties, extending their portfolio to include new cardiac, cancer, inflammation and fertility markers. The unprecedented numbers of couples who desire pregnancy later in life as well as the high morbidity rates of both cardiac and cancer conditions have inspired the industry to research to find ways of improving diagnosis. The new products include CYFRA 21-1, a tumor marker of non-small cell lung cancer, prostate specific antigen, H-FABP used as a biomarker for myocardial ischemia and estradiol and others. Many of the antibodies also have a recommended matched pair, which will save customers time and money on antibody screening.

BBI Solutions
www.bbisolutions.comn
Vibration Free Chilling/Heating Incubators
Torrey Pines Scientific announces two larger Echotherm vibration-free benchtop chilling incubators that are ideal for protein crystallography. The incubators have 100 liter capacity and can hold 66 assay plates without stacking. They are Peltier-based for heating and chilling and are vibration-free, making them ideal for protein crystallizations. Other applications include incubating marine samples below room temperature, enzyme reactions and deactivations, hybridizations, ligations and general lab incubations. They are settable from 4.0°C to 70.0°C and have temperature control to ± 0.1°C. They are UL, CSA, and CE compliant.

Torrey Pines Scientific

Fast Scanning Add-On
The FLIMbee galvo scanner add-on has been released for the Microtime 200 time-resolved microscopy platform by Picoquant. This new scanner provides high flexibility in scanning speed, ranging from very slow to fast, while maintaining the excellent spatial precision of the instrument. It is designed to minimize vignetting of the image field and to ensure a constant focal volume over a wide scan range. The fast scanning option enables imaging of quickly occurring changes in samples and of fast chemical or physical processes - it is even possible to acquire more than 15 FLIM images per second. Slower scanning speeds are optimal for applications such as Phosphorescence Lifetime Imaging (PLIM), which can provide deeper insights into the charge carrier dynamics of semi-conductors, for example.

Pico Quant
www.picoquant.com

Protein A Kit for Biotherapeutics
Gyros Protein Technologies, a provider of automated nanoliter-scale immunoasays and peptide synthesizers and reagents, has launched a new version of its Gyrolab Protein A Kit. This gives increased efficiency and throughput for quantification of residual Protein A ligands, with 96 microstructures. Two ready to use versions of the kit – Native Protein A and Mabselect Sure – are designed for use with Gyrolab systems, and include an enhanced protocol for automated acid pretreatment of harvested samples from downstream purification of biotherapeutics.

Gyros
www.gyrosproteintechnologies.com

Small High Stability, High Voltage Power Supply
Hamamatsu Photonics has announced the development of a very small high-voltage power supply (HVPS) module with high stability and low noise. The module is less than half the size and weight of the company’s conventional product, made possible by a newly developed proprietary manufacturing process for layering high isolation circuits on high voltage circuits. The new HVPS module can be used to power photomultiplier tubes inside compact, portable instruments for applications such as hygiene monitoring, rapid medical tests and security.

Hamamatsu
www.hamamatsu.com

Metals Quality Analyzer Detects Production Problems
The Thermo Scientific Explorer 4 Analyzer with MQA software now offers improved throughput for analysis of non-metallic inclusions in steel. The platform is a fourth-generation scanning electron microscope (SEM)/x-ray spectroscopy (EDX) for industrial manufacturing. The device provides metals quality analysis such as the size, shape, number and elemental composition of non-metallic inclusions in metals. The system can automatically characterize thousands of inclusions per hour to provide a firm statistical foundation for critical production decisions. The system provides increased beam stability and can distinguish smaller features and finer variations in composition faster than previous generations.

Thermo
www.thermofisher.com

To have a chance of winning the book find the original figure in this issue from which the image below is taken. Send the title of the article to team@laboratory-journal.com with the subject line Read & Win! All correct answers will be entered in a prize draw and the lucky winner will receive a copy of “Chemometrics”, which is featured on page 9.

Closing date: October 9th, 2017
**New 3 µm Particle Size Adds HPLC Scalability**

Phenomenex is introducing the 3 µm Polar C18 and the 3 µm PS C18 to its fully porous Luna Omega LC column line. The Polar C18 stationary phase is a robust selectivity bonded to an innovative silica particle that delivers high loadability and retention for both polar and nonpolar analytes. This new phase is 100 percent aqueous-stable due to a polar-modified surface, providing the flexibility in solvent and gradient system selection needed to achieve desired polar/nonpolar analyte separation. The new 3 µm particle joins the existing 1.6 µm and 5 µm sizes to provide full scalability from UHPLC to HPLC to preparative chromatography. The PS C18 delivers two distinct and useful separation mechanisms and offers 100 percent aqueous stability. The particle surface of the PS C18 contains a positive charge that facilitates greater acidic compound retention through ionic interaction, while the C18 ligand delivers general reversed-phase retention.

Phenomenex
www.phenomenex.com

**Rapidly Desalting and Purifying Tool**

Microsaic Systems, a developer of chip-based mass spectrometry (MS) instruments, has developed a technology to enable real-time analysis of proteins in bioprocessing applications, such as in the manufacture of biologic molecules (proteins, antibodies and peptides) for therapeutic and diagnostic uses. The technology is a tool for rapidly desalting and purifying protein samples prior to their analysis using Microsaic’s 4000 MiD MS instrument. This has the potential to significantly accelerate the industrial purification of biologics. Analysis times can be reduced from days or even weeks to minutes.

Microsaic Systems
www.microsaic.com

**Tensile Stage Selected For Correlative AFM and SEM Product**

Deben, a provider of in-situ testing stages together with innovative accessories and components for electron microscopy, reports on the use of the AFSEM correlative AFM and SEM system with their tensile stage for in situ mechanical test and measurement. Manufactured by GETec, it will be sold by NanoSurf and other selected sales channels worldwide. Austrian instrument company, GETec Microscopy, has developed AFSEM — a high-end atomic force microscope (AFM) for seamless integration into most of the commercially available scanning electron microscopes (SEM and SEM/FIB). Benefits include the ability to make tensile, indentation and electrical measurements, all on the nanoscale, allowing detection in the early stages of crack or fracture formation, sample deformation, breakage analysis and other surface defects.

Deben
www.deben.co.uk

**Gas Chromatograph**

Shimadzu has released their Nexis GC-2030 gas chromatograph (GC). It can be equipped with various detectors providing high sensitivity and reproducibility with analysis productivity. The Labsolutions software ensures compliance with FDA 21 CFR Part 11 and supports the laboratory management workflow. The device can be operated intuitively via its full-color LCD touch panel.

Shimadzu
www.shimadzu.eu

**Alternative CRISPR Genome Editing Method**

Merck has developed a new genome editing tool that makes CRISPR more efficient, flexible and specific, giving researchers more experimental options and faster results that can accelerate drug development and access to new therapies. The new technique, called ‘proxy-CRISPR’ and subject to patent applications, provides access to previously unreachable areas of the genome. Merck’s research on proxy-CRISPR, “Targeted Activation of Diverse CRISPR-Cas Systems for Mammalian Genome Editing via Proximal CRISPR Targeting,” was published in the April 7, 2017 edition of Nature Communications. The company’s next suite of genome editing tools for the research community, to be launched later in 2017, will include novel and modified versions of Cas and Cas-like proteins.

Merck
www.merckgroup.com

**Affordable Digital Imaging and Multi-Mode Detection**

Biotek Instruments has introduced their Cytation 1 Cell Imaging Multi-Mode Reader as an affordable entry into automated digital quantitative microscopy along with multi-mode microplate detection. This patented combined solution provides quantitative phenotypic cellular information and well-based quantitative data. The modular architecture allows users to satisfy both their current research needs and future upgrades as required. The digital microscopy mode includes fluorescence and high contrast brightfield channels for visualization from 1.25x to 60x. Automated XY stage, focus and LED intensity facilitate imaging throughput while a multi-mode detection module includes sensitive filter-based fluorescence and luminescence along with monochromator-based UV-Vis absorbance for flexibility in a wide range of endpoint, kinetic, inject/read and other assay types.

Biotek
www.biotek.com
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