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SCS-FH Awards at the ILMAC 2013

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The ILMAC, the trade fair for Laboratory and Process Technology, specializes strongly on innovation and application in industry. The Swiss Universities of Applied Sciences also focus on these aspects, both in education and in their research and development activities. As a logical consequence, the Swiss Chemical Society has created for the first time in 2013 a prize for 'Innovation & Application' for collaborative projects between the Universities of Applied Sciences and industry partners. The prizewinners will be honored at the ILMAC and their projects displayed.

A total of 13 projects were entered into the competition, which were assessed by a committee of experts from industry and academia. The assessment process looked at the innovative potential and the practical application and realization by the industrial partner. All projects were placed in one of the following three categories:

Molecules for the Life Sciences. Development and production techniques of (bio)molecules for applications in the Life Sciences.

Analytical Methods in the Life Sciences. Development of analysis equipment and methods for applications in the Life Sciences.

Chemistry and Environment. Chemistry and biochemistry in environmental and process technology.

All projects entered in the competition will be displayed at the booth dedicated to the Universities of Applied Sciences and the Swiss Chemical Society at the ILMAC 2013. Practical examples will be used wherever possible. In addition the winners of the three categories will have the opportunity to introduce themselves and their projects in short presentations at the ILMAC Forum.

We would be very happy to receive you as a guest at our booth or as a visitor to one of our project presentations in the Forum.

1. Nanobiocatalytic Depolymerization of Lignin for the Production of Platform Phenolic Chemicals

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Background

Growing concern over greenhouse gas emissions and depleting stocks of fossil fuels calls for increased research for renewable substitutes for petroleum.[1] Biomass constitutes the only known renewable replacement for the production of both fuels and chemicals.[2] One component of biomass that is not effectively used at present is lignin. However, due to its chemical structure it could be a source for valuable chemicals if it can be degraded into smaller molecular units.[3] Up until now studies focusing on lignin degradation have shown that it is very recalcitrant to degradation and most approaches lead to the formation of very high amounts of solid residues.[4] Biocatalytic conversion approaches making use of lignin modifying enzymes (LMEs) are being viewed as promising for lignin conversion.[5] In nature white-rot basidiomycetes efficiently degrade lignin making use of extracellular oxidative enzymes.[4] In order to design efficient processes making use of these enzymes the development of biocatalytic nanomaterials is necessary to increase the enzymatic stability and facilitate reuse.

Aims of the Project

The present project aims to develop nanobiocatalytic materials in order to degrade lignin to oligomers and monolignols, which can be used in the production of green polymers. Specifically, the LMEs will be immobilized on fumed silica nanoparticles (fsNP), magnetic fumed silica (mFS) and ultrafiltration (UF) membranes (Fig. 1). Subsequently, these materials will be applied to membrane filtration-based processes for the depolymerization of lignin with simultaneous fractionation of the reaction products. Finally, the suitability of the obtained products concerning their usability for the formulation of polymers will be evaluated.

Project Partners

A total of four economic partners will be involved in the project.

• ***INOFEA GmbH***, a young company aiming to commercialize innovative nanomaterials for environmental applications.

• ***Representa AG***, the exclusive distributor for Novoflow technology in Switzerland. Novoflow GmbH is market leader in rotating single shaft filter systems.

• ***MMS*** provides membrane systems and solutions to a number of industries and applications.

• ***Huntsman Advanced Materials*** is a leading global supplier

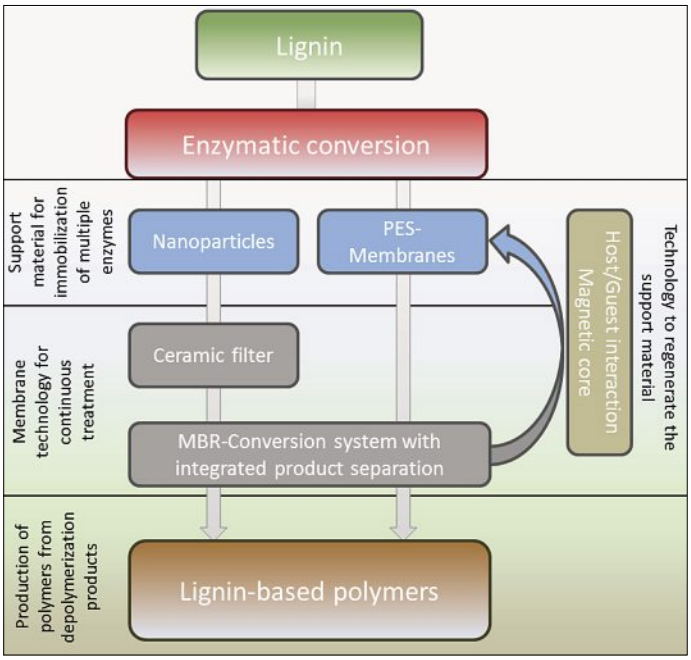

Fig. 1. Schematic overview of the project.

of synthetic and formulated polymer systems for customers requiring high performance materials which outperform the properties, functionality and durability of traditional materials.

Innovation Content of the Project

The proposed project can be regarded as highly innovative on the basis of following aspects:

• Biocatalytic approaches for the degradation of lignin making use of LMEs are still in their infancy and can be expected to be more environmentally reconcilable than chemical processes since they usually work at lower temperatures and are conducted under less harsh reaction conditions.

• The use of nanostructured material for the immobilization of LMEs is recent and was pioneered by FHNW.

• The combination of filtration processes and nanobiocatalysts for the depolymerization of lignin is unprecedented, allows recycling of the biocatalysts and prevents the nanomaterial from being released thereby addressing possible nanosafety issues.

• The reversible immobilization of enzymes onto nano-structured UF membranes is brand new.

• The use of phenolics produced from renewable biomass/biowaste for the formulation of novel green polymers is one important point of innovation strategies in the chemical industry.

The CTI (grant PRNM-NM 12608.1) is gratefully acknowledged for funding the ongoing project.

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2. Skin Bioprinting: An Innovative Approach to Produce Standardized Skin Models on Demand

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The continually rising numbers of compound failures and increasing costs of drug and substance development are fostering the use of biologically more complex tissue models. In addition, European regulations postulate alternative test methods since animal experiments for cosmetic products and their ingredients are forbidden since March 2013. Thus, there is an urgent demand by the cosmetic industry for standardized and customized *in vitro* artificial organomimetic skin models for substance testing. Today, mainly epidermal skin models are commercially available. In order to better represent the complexity of native skin the dermis needs to be included. Commercially available so-called full-thickness skin models are rather simple, composed of fibroblasts in a collagenous scaffold topped with an epidermis. Since the skin model production process is challenging and time-consuming a histological analysis is carried out at the end to verify successful tissue production. In order to reduce costs and time it is necessary to control the entire process *in situ* to select for properly built skin models at any time point of production.

Bioprinting is an upcoming technology in the field of tissue engineering where cell-containing scaffolds are produced in a layer by layer deposition process in order to create 3D tissue-specific models based on a CAD drawing. This technique allows the creation of a biological composite system by controlling the exact deposition of cells, growth factors and extracellular matrix (ECM) molecules in a spatially controlled manner.

In close collaboration with engineers, chemist and biologists we aimed to establish a bioprinting process for human organ-like skin models including hard- and software, suitable bioink and an *in situ* quality control. In the framework of the competence center TEDD (*www.icbc.zhaw.ch/tedd*) and financed by CTI these innovative goals could be reached. The industrial partner regenHU is now able to market several bioprinting products.

We developed a printable hydrogel called BioInk that fulfills important criteria: It is viscous to maintain the structure, before it is photo-polymerized within seconds; it provides cell adhesion sites, matrix-cleavage sites and is cell-compatible. In order to produce a dermis equivalent alternating layers of BioInk (semi-synthetic PEG-based hydrogel) and primary human dermal fibroblasts in medium were printed. After each printed BioInk layer the structure was instantaneously photo-polymerized with an integrated UV LED (365 nm ± 10 nm, 5 mW/cm^2,10 s). These cell-containing scaffolds were cultivated for 14 days in medium. The cell-containing scaffolds were stable throughout the whole time period and the fibroblasts remained viable. The cells were able to interconnect inside the printed hydrogel. In a next step, primary human dermal keratinocytes were printed in media on top of the printed dermis where they remained viable (Fig. 1).

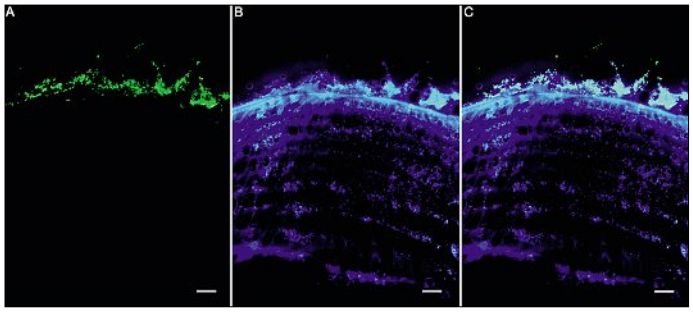

Fig. 1. Fluorescent representation of a printed skin model. Dermis equivalent containing human dermal fibroblasts was printed with BioInk in a layer by layer fashion. On top of the dermis green fluorescent human dermal keratinocytes were printed as a thin layer. Cell nuclei are stained with DAPI (blue). A) keratinocytes, B) DAPI-stained cells and C) overlay of A) and B). Prior analysis the skin model was incubated for 48 h. Scale bar = 200 μm.

In order to provide an *in situ* quality control an optical coherence tomography (OCT) system was implemented into the bioprinting device. It is possible to discriminate between epidermal and dermal skin layers with images obtained with the OCT-system. This finding is crucial to analyze the differentiation progress of keratinocytes in the epidermis and to ensure the production of a physiological relevant and reliable skin model.

To conclude, we developed a BioInk to print an artificial dermis containing primary human dermal fibroblast in a 3D structure. Also, viable primary human dermal keratinocytes were printed on top of the dermis. The BioInk comes with a suitable packaging in a ready to use syringe format. An optimized Bioprinter called '3D Discovery' is manufactured by regenHU. It is tailored to print photo-polymerizable hydrogels and ensures the production of tissue models under sterile conditions using standardized cell culture devices. The OCT system is suitable for *in situ* quality control.

Next steps deal with the improvement of keratinocyte adhesion on top of the printed dermal equivalent in order to initiate the differentiation into a fully stratified epidermis generating the first printed full-thickness skin model.

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3. Remineralization of Sub-surface Carious Lesions Initiated by a Self-assembling Peptide – Development of a Bioceramic Tooth Model

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Non-invasive interventions in dental medicine such as remineralization of carious lesions are rather limited. Currently dental caries is mainly treated by mechanical techniques, like dental fillings or tooth extraction, or by applying protective barriers to the tooth surface, like varnishes, containing *e.g.* fluoride. Therefore biomimetic mineralization is a very promising field to combine biocompatible materials with a non-invasive treatment.

In this study our work focuses on the application of a short peptide, which self-assembles *in situ* under acidic conditions into a supramolecular 3D network after treatment of early carious lesions (white spots). The peptide is assumed to trigger the nucleation of hydroxyapatite nanocrystals and consequently results in remineralization of the lesion.[1] This results in a novel biomimetic mineralization approach.

Studies of diffusion, assembly and remineralization processes were performed *in vitro* on artificial sub-surface caries-like cavities on human teeth.[2] However, due to the limited availability and great structural variability of human teeth, a bioceramic tooth model was developed. Based on this artificial tooth model the study of the demineralization and remineralization processes of sub-surface lesions in the enamel will be simplified and standardized to gain comparability of the results.[3]

Primary results with computer tomography (μ-CT) showed a significant increase of density, and therefore a remineralization, in the area of artificially induced sub-surface lesions when a non-peptide treated reference and a peptide treated sample are compared (Fig. 1A,B,C). Furthermore matrix-assisted desorption/ionization – time-of-flight (MALDI-TOF) analysis proved that the peptide remained inside the lesion, even after two weeks incubation time in remineralization buffer.

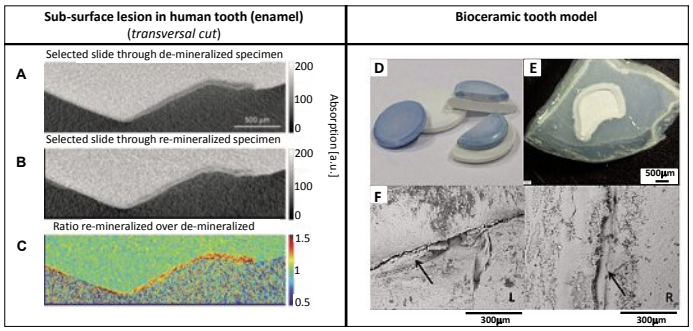

Fig. 1. Left: μCT images of a transversal profile through a sub-surface lesion in enamel. (A) Specimen imaged after demineralization. (B) Specimen imaged after peptide treatment and remineralization for 14 days. (C) Ratio of remineralized over demineralized specimen. Right: (D) Final tooth models: blue - the enamel-like and white - the dentin-like material. (E) Enamel-like tooth model with an artificial lesion (white area). (F) The two lower SEM images showed the enamel-dentin junction of the assembled tooth model. (L) SEM image of enamel (upper) / dentin junction (arrow) of an extracted human tooth. (R) SEM image of assembled enamel (left) / dentin tooth model, showing similar morphology (arrow).

To produce bioceramic tooth models, a suitable technology was developed to process raw ceramic material accordingly (Fig. 1D,E,F). The requirements to achieve the characteristics of natural tooth enamel and dentin (comparable mechanical and chemical properties) were confirmed *via* analysis by mercury intrusion porosimetry (MIP), Brunauer, Emmett, Teller (BET) gas adsorption, X-ray diffraction (XRD), and Vickers hardness (VH). Artificial lesions were generated by acid treatment similar to the process causing caries in human teeth. The samples were analyzed by scanning electron microscopy (SEM) and μ-CT.

Further studies on the remineralization therapy by 3D-self-assembled peptide supramolecular networks, either in natural teeth or in artificial tooth model are in process.

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[3] G. H. Dibdin, D. F. Poole, *Arch. Oral Biol.* **1982**, *27*, 235.

4. Click Chemistry for DNA Functionalization

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[b]Microsynth AG, CH-9436 Balgach.

Click chemistry is a new versatile approach that can be used for the synthesis of a great variety of (complex) biomolecules. In short, click chemistry is a chemical reaction between an alkyne and an azide moiety to yield a triazol conjugate that connects two different biochemical species.

Due to the nature of the bond chemistry (strong thermodynamic reaction in water, no side reactions, lack of functional group interference *etc.*) this new type of chemistry is perfectly suited to label DNA and RNA oligonucleotides. In combination with state-of-the-art nucleic acid labelling technologies (NHS-ester and phosphoramitide chemistry), it is now possible to label or tag DNA and RNA at any position. If multiple modifications with different labels within the DNA/RNA strand (internal modifications) and/or the termini (5' and 3' modifications) are desired, click-chemistry will make it feasible. Hence click chemistry has opened a door to an entire new world of so far unimaginable biomolecules.

To date, click chemistry is usually applied for the synthesis of more complex DNA/RNA oligonucleotides. The complexity is even more increased since click chemistry distinguishes between a copper-catalyzed ligation of azides and alkynes and a copper-free ligation. The latter has been developed to allow the use of DNA/RNA oligonucleotides under physiological conditions (copper is difficult to remove or purify; residual copper is highly toxic for bacterial as well as mammalian cells).

In this collaboration project, an optimized coupling process has been established for copper-catalyzed and copper-free ligation at the 3' and at the 5' ends of the oligonucleotide. In addition, different labels have been introduced, using three different alkyne nucleosides, one with no alkyne protection, the second with TMS protection and the third with TIPS protection (Fig. 1). The first click reaction was performed directly on the resin. The singly modified oligonucleotide is subsequently cleaved from the support with concomitant cleavage of the TMS group. The TIPS protecting group is retained during this procedure. The second click reaction is performed in solution. Cleavage of the TIPS group with TBAF and a subsequent click reaction in solution results in the desired modified oligonucleotides in excellent overall yields.

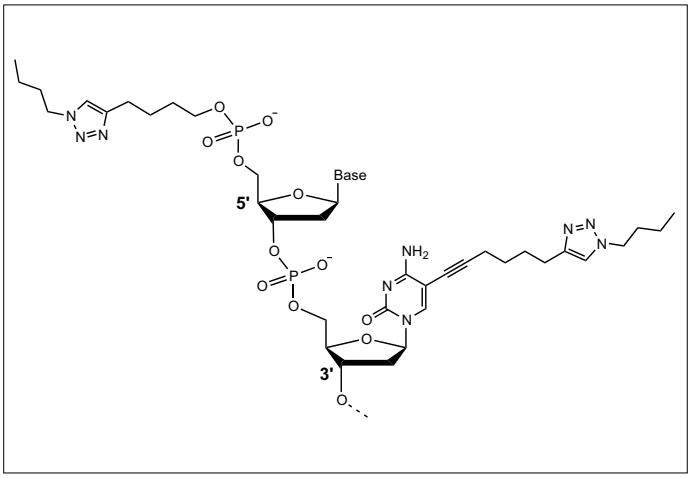

Fig. 1. 5' and internal modification of oligonucleotides.

The excess of dye-azide needed for high coupling efficiencies could be reduced down to 1.1× equivalents. The coupling time could be shortened substantially from the published 'overnight' to a short incubation time.

The very high coupling efficiency of this process has been proven using carefully performed analytics (HPLC, MALDI-TOF MS).

Several different combinations of clicking reactions have been achieved during this project. Different fluorescence resonance energy transfer (FRET) systems have been successfully synthesized. These couplings have been performed at the 3', 5' and/or as internal modifications of oligonucleotides with different dyes in combination with state-of-the-art technologies.

The variety of fluorophores available and introduced in oligonucleotides as phosporamidites or coupled on the CpG (controlled pore glass, *i.e.* starting material of the oligonucleotide synthesis) is quite limited. Therefore the vast majority of modifiers are coupled to an amino-modified oligonucleotide using NHS-ester linking chemistry. Nearly all commercially available fluorophores with absorption/emission from UV to IR on the market have been synthesized as NHS-ester. Unfortunately, only one of these numerous modifiers could be coupled at a distinct position.

Click chemistry multiplies the options to produce oligonucleotides with different modifiers at distinct positions with the choice of the vast variety of chemicals available with azide moiety. This can lead to so far unimaginable FRET systems, FISH/EMSA/FACS applications, qPCR assays, DNA microarrays or probes for fluorescence microscopy.

Theoretically, it is now possible to 'click' three additional different modifiers in combination with modifiers attached to oligonucleotides using common techniques like NHS-ester or phosphoramidite chemistry at Microsynth (for requests: info@microsynth.ch).

5. Recycling of Phosphorus from Sewage Sludge Ash

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Phosphorus is one essential element of life, which can neither be produced synthetically nor substituted by any other substance. Its importance as a plant nutrient is emphasized by the huge amount of 975 000 tonnes of mineral phosphorus annually imported into Europe to sustain good harvests. While phosphorus is a limited fossil element, its extensive recovery from 'secondary deposits' is of paramount importance and follows the principles of the European Roadmap for Resource Efficiency. Municipal wastewater represents a relevant phosphorus reserve and has the potential to cover about 20% of the demand. Technology concepts have been developed in recent European projects to tap into this local resource

The EU-project P-Rex – Sustainable sewage sludge management fostering phosphorus recovery and energy efficiency – aims to demonstrate full-scale applications of technologies for phosphorus recycling from waste water. A systematic assessment of these technologies will be an important result of the P-Rex project. Additionally market aspects for recycled phosphorus fertilizers will be analyzed. Strategies and recommendations will be developed for efficient and wide-spread phosphorus recovery and market penetration with regards to specific regional

conditions, aiming to increase the European phosphorus recycling rate from municipal wastewater up to 80%.

The Institute of Ecopreneurship (IEC), School of Life Sciences, University of Applied Sciences, Northwestern Switzerland is involved intensively in the different work areas of this European project. One important task of the institute is an experimental study of a process for the recycling of phosphorus from sewage sludge ash. This pilot study of phosphorus recycling from sewage sludge ash is done in cooperation with the company BSH who operated the process in full scale for several months in 2012 using the infrastructure of the municipal solid waste incineration plant in Bern before its demolition. Presently, the IEC is installing the technology in pilot scale at the wastewater treatment plant ARA Rhein in Pratteln. The basic principle of the technology under investigation is a leaching step to dissolve the phosphate bound in the sewage sludge ash. In a subsequent step the liquid phase is separated from the remaining solids using filtration in form of a vacuum belt filter.

The full scale trials have shown the feasibility of all steps, made a first estimation of costs and production of phosphorus product for bioavailability and processing tests possible. The pilot plant will be used in order to perform an extended parameter test and address problems encountered in the full scale trials. Some components of the process mass balance will be confirmed in the pilot experimental study. Different phosphorus products will be produced under varying process conditions. These product types will again be object to further investigations regarding the quality and suitability as fertilizer or for other markets.

6. Online Process Control of the De-alcoholization of Beer with Inline Refractometry

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Alcohol-free beer is increasingly becoming a life-style soft drink, especially if the typical beer taste and flavor can be maintained after the alcohol removal. The applicability of membrane technology has been proven for the de-alcoholization of beer.[1] However, the membrane process control requires use of off-line analytic resources like gas-chromatographic analysis. Using an innovative online-refractometer, the degree of de-alcoholization can be monitored and in the event of membrane damage, shut-down procedures can simply be applied.

Nanofiltration (NF) is a pressure-driven membrane process using the specific membrane structure for the rejection of molecules with a molecular weight >100 g/mol, *e.g.* beer aroma, flavors and fragrances. Smaller molecules such as water and ethanol, however, can pass through the membrane as a clear permeate. Thus, the membrane separation leads to an alcohol-free beer retaining almost all beer aroma components. Currently, the process is controlled by offline gas chromatography of the alcohol concentration. Alcohol concentration measurement by means of refractive index fails, since beer is a multi-component mixture of several hundred substances and the obtained signal cannot be associated with any particular component. However, in binary mixtures like the membrane permeate the refractive index can be straightforwardly used for the control of the de-alcoholization process (Fig. 1). Hence, process control takes place by the online-measurement of the refractive index on the permeate side. In the event of a membrane failure, beer enters

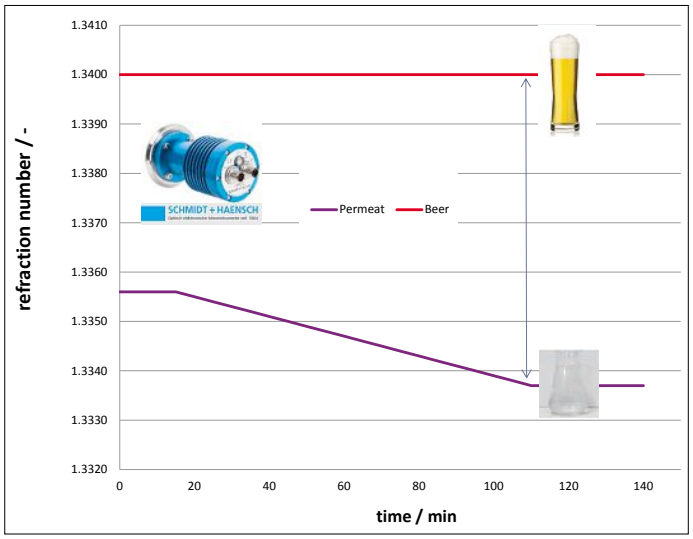

Fig. 1. Refractive index for the control of a nanofiltration beer de-alcoholization process.

the permeate side unhindered and will be detected immediately. In addition, the refractometer features a programmable turn-off function that is triggered when the inspected solution reaches certain threshold values (*e.g.* refractive index of pure beer). Since it is directly linked to a regulation system, the membrane unit turns itself off automatically and reverts to a safe sleep mode.

With a state-of-the-art online refractometer, ethanol content can be measured with a precision of up to 0.01 wt.%. A comparable accuracy can also be obtained by conventional analytical methods like gas chromatography but neither in an online-mode nor in combination with a regulation function (start/stop of the process).

With the combination of NF membranes for the separation of alcohol and a refractometer for online measurement and controlling on the permeate side, the industrial partner was able to plan and construct a compact skid-mounted unit which enables particularly mid-scale breweries to use an innovative technology for the generation of 50 hl/day alcohol-free beer without any advanced knowledge of membrane technology.[2] This unit was presented to the public at the international fair 'brau beviale' 2011 in Nuremberg, Germany.

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[2] W. Riedl, *Brewing and Beverage Industry International* **2012**, *4*, 38.

7. Tackling Antibiotic Resistance by Transcription Repressor Inhibitory Compounds

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The past decade has seen a dramatic worldwide increase in human-pathogenic bacteria that are resistant to one or multiple

antibiotics. More and more infections caused by resistant micro-organisms fail to respond to conventional treatment. In the hospital environment, increasing numbers of patients are infected by highly-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*.

Researchers at the Center for Organic and Medicinal Chemistry at the ZHAW are collaborating with the biopharmaceutical company BioVersys AG in order to tackle antibiotic resistance. They have identified the need for certain types of small organic molecules, called Transcription Repressor Inhibitory Compounds (TRICs), which are designed and synthesized by the organic and medicinal chemists at the ZHAW before they are tested at BioVersys for their biological activity and at the FHNW for their binding characteristics. In contrast to a wide range of traditional antibiotics for which bacteria have developed resistance, TRICs do not interfere with the bacterial metabolism but work on the bacteria's genetic level. These TRICs switch off the bacterial defense program and the original antibiotic can kill the bacteria again. This has two beneficial effects: they disable the possibility for further development of antibiotic resistances and they reopen the door for conventional antibiotics. The formulation of those small organic TRICs could be a combination with conventional antibiotics in one pill. Bacterial resistance, although being a complex and flexible mechanism, follows a general principle, which is genetically encoded. Resistance genes are clustered and regulated by global transcriptional regulators, which either recognize the antibiotic or its derivatives or act as stress sensors. Based on structural information of the transcription factors, the project partners develop compounds, which specifically inhibit global transcriptional regulators of bacterial resistance genes (Fig. 1).

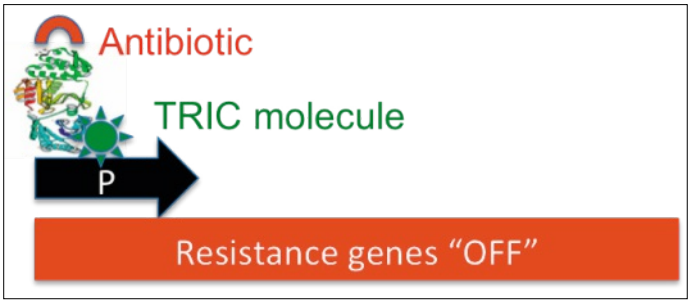

Fig. 1. Transcription Repressor Inhibitory Compounds (TRIC) (green star) inhibit the activation of resistance genes by blocking the release of the transcriptional regulator in response to the antibiotic. (P: Promoter; 3D structure comic: Repressor protein).

As a result of this collaboration, a first patent application for TRIC compounds was filed in 2013.

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8. Printing Enzymes for Colorimetric Assays on Paper

Sina S. Saxer*[a], Fabienne Wildi[a], Marcus Walser[a], Olivier Boss[b], Reiner Zimmer[b], Mariana Spulber[c], Wolfgang Meier[c], Peter Paul[b], Nico Bruns[c], and Uwe Pieles[a]

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Diagnostic enzymes are important for the diagnosis and monitoring of diseases and are used for the detection of disease-specific, low-concentrated biomolecules in urine, blood plasma, *etc*.[1] In general, colorimetric enzymatic assays are applied for the detection of such molecules. Thereby enzymes convert substrates, which lead then to a quantifiable color change.[2] However the highly sensitive and selective method is usually performed in solution and requires trained personnel. Our aim is to implement such enzymatic assays on paper, where enzymes would be applied by general printing processes *e.g*. screen, flexo, pad printing. The printed enzyme patch changes color as soon as substrate is present and the resulting color intensity allows an approximate quantification. Enzymatic reactions are usually performed under physiological conditions since most enzymes are sensitive to changes in ionic strength, pH, temperature, solvents, *etc*.[3] Thus printing enzymes requires appropriate carrier ink and/or additional stabilization, for example by encapsulation of the enzyme.[4] Although such capsules should ideally maintain the physiological environment, they should also be permeable for substrate diffusion, to enable the color assay.[5] Polyethylene imine (PEI) capsules synthesized *via* a water-in-oil emulsion method[6] were used to encapsulate horseradish peroxidase (HRP), a relatively stable enzyme that oxidizes substrate in presence of hydrogen peroxide. 3,5,3,5'-Tetramethylbenzidine (TMB) was used as substrate and turned the solution blue if exposed to HRP and hydrogen peroxide.[7] Capsules consist of HRP in PEI surrounded by a thin, cross-linked PEI membrane (Fig. 1A). They are spherical in solution but collapse when dried (Fig. 1A+B). Capsule diameters ranged from 2–25 µm and were adjusted by variation of homogenizer velocity. Encapsulation of approx. 10 µg HRP (active) per mg capsules was measured for capsules with a diameter of 25 µm. HRP in capsules was stable for a minimum of 24 days under dry, room temperature conditions, while free HRP was stable for only 12 hours in comparison. First experiments with capsules implemented in binder, proved that HRP remained active and that printed capsule patches turned blue upon addition of TMB (Fig. 1C). Finally capsules were printable and showed promising results. Future steps are detailed studies of the printing process and various parameters such as capsule size, stability of printed HRP and adsorption of TMB in binder.

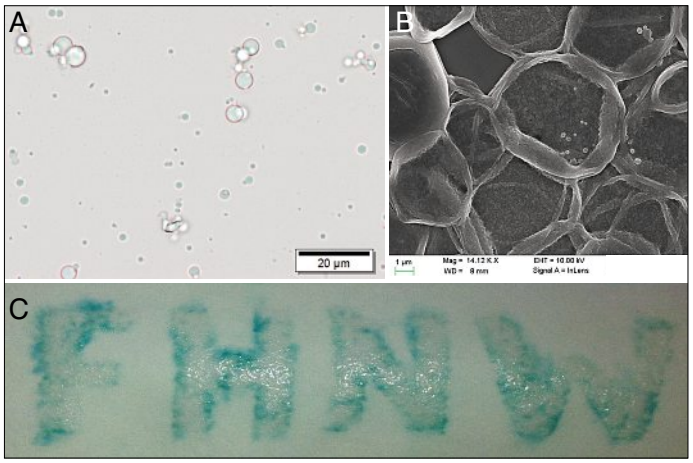

Fig. 1. A) PEI capsules in phosphate buffer pH = 7.4 measured with transmission microscope Olympus BX51. B) PEI capsules dried and gold coated for scanning electron microscopy with Zeiss Supra SEM. C) Capsules in binder screen-printed on Whatman filterpaper no.1 and incubated with TMB ready to use solution (Invitrogen) for 30 min at 25 °C in the dark.

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9. Synergy of Metabolic Profiling, Pharmacological Testing and Process Engineering Enables Inauguration of a Bamboo Pilot Plant Extraction Unit in Fujian, China

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In Europe, bamboo is mainly known as an ornamental plant, as a source for application in the wood and fiber industry and as an energy crop. However, a growing interest in alternative uses of bamboo can be observed because this fast growing plant provides a rich natural source of promising phytochemicals which exhibit many beneficial physiological effects such as anti-inflammatory, anti-oxidant, anti-viral, anti-aging properties or prevention against cardio vascular diseases. Despite this, there is still a lack of information on the active secondary metabolites present in the many different bamboo species around the world. Thus, a great need for fully characterized, controlled and qualified bamboo extracts for therapeutic, cosmetic and beverage applications exists.

In this research project, we focused on three main key aspects. Firstly, we started with the investigation of the qualitative and quantitative chemical compositions of leaves of morphologically heterogeneous bamboo species. For this we developed a tailored high-performance liquid chromatography–high-resolution mass spectrometry (HPLC-MS/MS) method for metabolic profiling of bamboo. In combination with statistical data analysis tools like principal component analysis (PCA) we were able to differentiate bamboo according to genera (*Phyllostachys*, *Fargesia* and *Sasa*) as well as bamboo leaves according to leaf-age (Fig. 1). Secondly, we developed physiological test systems to investigate potential health beneficial properties of bamboo. Starting from simple assays to test for anti-oxidative properties of bamboo we ended with cell-based assay systems to investigate anti-inflammatory effects and wound healing properties of bamboo on human keratinocytes. Interestingly bamboo leaves of different physiological age exhibit pronounced differences in their anti-inflammatory capabilities. Young leaves showed considerable positive effects, whereas old leaves did not. Thus, the combination of pharmacological data with analytical profiling information results in a consistent differentiation of bamboo leaves due to growth state. This allows information on molecular features present in young leaves to be linked to physiological properties. Thirdly, the extraction of leaf material was optimized on a pilot plant scale. In the basic engineering phase, the focus was to find environmentally friendly process parameters which enable a maximum extraction yield with a minimum amount of solvent and energy. The next step was to find equipment which fits these requirements and to design the process chain from the delivery of leaf material, *via* spray-dried extracts to waste disposal. With these results a concept for a 100 kg/hour extraction plant in Fujian, China was engineered.

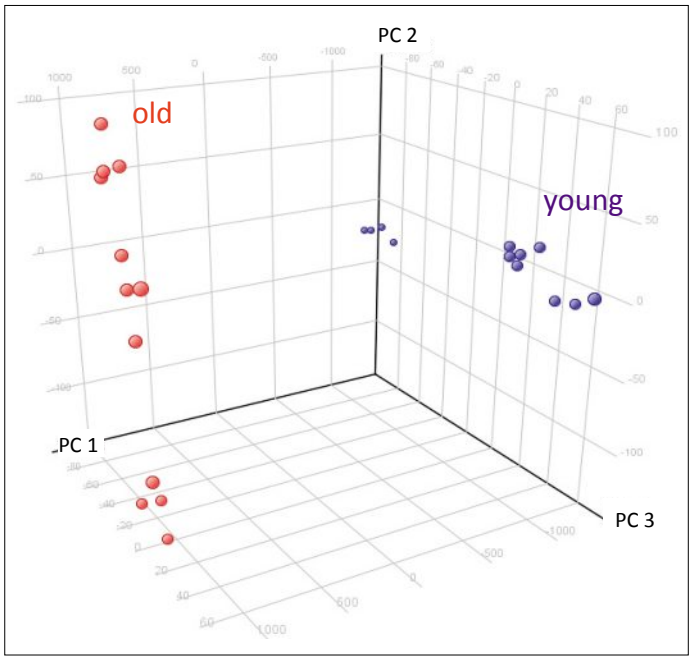

Fig. 1. Score plot of principal component (PC) analysis of young vs. old bamboo leaves.

The application of high-end analytical technologies for exact characterization of the plant material achieves great synergy in combination with pharmacology and process technology. On basis of the combination of the three disciplines, analytical chemistry, process technology and pharmacology, not only criteria for an appropriate selection of bamboo species with beneficial health properties could be determined. In fact differences in pharmacological properties of bamboo extracts could be directly linked on a molecular level with results of metabolic profiling.

This enabled our project partner Organic Bamboo Industries AG (St. Gallen) to successfully inaugurate a pilot plant extraction unit in Fujian, China.

10. Conversion of a Radioenzymatic Vitamin B6 Assay into a Rapid Colorimetric Enzymatic Assay

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Pyridoxal 5' phosphate (PLP) is the biologically active form of vitamin B6 and is required as a co-factor by a large number of enzymes. Low PLP levels in blood generally indicate a nutritional deficiency. In this work, we successfully converted a commercial PLP radioenzymatic assay into a rapid non-radioactive enzymatic assay based on the activity of tyrosine decarboxylase. The assay developed has a detection limit below 7 nmol/L PLP and respectively correlates with a R^2 of 0.95 and

0.99 with the radioenzymatic and HPLC methods in comparative validation studies involving EDTA blood from 41 and 44 subjects, respectively.

Vitamin B6 is a water-soluble compound found in many different foods. The biologically active form, pyridoxal 5' phosphate (PLP) is essential as a co-factor for the function of more than hundred enzymes. Low PLP status is typically associated with nutritional deficiency, but also with several very different diseases including cardiovascular disease or cancer.[1]

The radioenzymatic assay, formerly provided by BÜHLMANN Laboratories AG, relied on PLP-dependent conversion of tritium-labeled tyrosine into labeled tyramine and carbon dioxide catalyzed by tyrosine apo-decarboxylase (EC 4.1.1.25) from *Streptococcus faecalis* (Reaction (1)). The radioactive tyramine was selectively extracted and quantified by scintillation counting. The detected amount of labeled tyramine correlates linearly with PLP concentration, which remains invariable, since PLP is catalytically constant during the process.

For the development of a non-radioactive rapid alternative, we considered coupling PLP-dependent enzymatic reactions with colorimetric or UV-active detection reactions. We evaluated several PLP-dependent enzymes for this purpose. We eventually decided upon tyrosine decarboxylase, not least because it is involved in the previously established commercial radioactive assay and therefore was expected to demonstrate higher consistency in method comparison for validation. Nevertheless, instead of the *Streptococcus faecalis* crude cell extract containing tyrosine decarboxylase involved in the radioenzymatic assay, we used the recombinant product of a synthetic gene encoding this enzyme that we expressed in *E. coli* and purified.

In the colorimetric assay, tyramine produced by PLP-driven decarboxylation of tyrosine (Reaction (1)) is deaminated in a coupled reaction by a primary-amine oxidase (1.4.3.21) from *Arthrobacter sp.* while producing hydrogen peroxide (Reaction (2)). A third enzyme, horseradish peroxidase, catalyzes the peroxide-dependent conversion of a pro-dye into a colored compound, whose formation rate directly correlates with PLP concentration and can be easily monitored by absorbance at 546 nm (Reaction (3)).

Reaction (1) tyrosine $\rightarrow$ tyramine + CO_2 (tyrosine decarboxylase, PLP-dependent)

Reaction (2) tyramine+H_2O+$O_2$$\rightarrow$4-hydroxyphenylethanal + NH_3 + H_2O_2 (primary-amine oxidase)

Reaction (3) 2 H_2O_2 + 4-aminoantipyrine + TOOS $\rightarrow$ quinoneimine dye + 4 H_2O (horseradish peroxidase)

The assay was successfully validated in comparison to a reference HPLC method and the original radioenzymatic assay using EDTA blood from 44 and 41 subjects, respectively. The correlations resulted in a R^2 of 0.99 and 0.95, respectively. The detection limit of this new rapid enzymatic assay in plasma lies below 7 nmol/L PLP.[2]

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11. A Novel Synthetic Virus Recognition Nanomaterial for Diagnostic and Environmental Applications

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The development of synthetic materials capable of selective molecular recognition is an ongoing challenge in molecular sciences. Despite the recent advances in supramolecular chemistry, the recognition of large biomolecular assemblies remains unsolved. We have recently developed a novel method to produce synthetic nanoparticles possessing remarkable virus recognition properties. This method provides the possibility to produce virus-imprinted nanoparticles (VIPs) with control over both the size and the chemical composition of the imprints. The so-produced VIPs have been shown to possess a relevant affinity and specificity for the virus used as template for their synthesis. Work is underway to implement this new class of nanomaterial to develop a commercial kit for the detection of waterborne viruses.

Viruses represent major human and animal health issues. These small infectious agents are the cause of a number of severe pathologies including HIV, cholera, influenza, typhus, smallpox, tuberculosis, to name but a few. In a world facing global change pressure and because of the increasing demand in potable water, many countries are experiencing more and more frequent droughts and water stress conditions. The reclamation of treated effluents is a very promising solution to mitigate water shortages and to lower freshwater demand. However reclamation faces the problem of water-related vector-borne diseases. The World Health Organization (WHO) reports that 1.8 million people die every year from diarrheal diseases caused by viral infections, of which more than 88% have a water-related origin. State-of-the-art methods to detect viruses in waters rely on sophisticated molecular biology techniques, they are costly and time-consuming. Consequently, they cannot be applied on a systematic basis and new solutions are needed.

The challenge in the design of artificial virus-recognition systems lies in the large size of the target and its fragile self-assembled structure,[1] and only few examples of polymers possessing virus-recognition properties have been reported.[2,3] We have recently developed a novel approach to produce silica nanoparticles (SNPs) possessing selective recognition properties of icosahedral non-enveloped viruses. Based on an approach that can be generally categorized as *molecular imprinting*, the synthetic strategy consists in growing at the surface of inorganic nanoparticles, a hybrid organic–inorganic recognition layer possessing open partial viral replicas (or imprints).

The synthesis was carried out by first anchoring covalently the target virus at the surface of SNPs. This step was achieved by using a widely used homo-difunctional cross-linker, glutaraldehyde. The next step consisted in growing from the surface of the SNPs, and intimately surrounding the immobilized virus, a layer of organosilica (*i.e.* polysilsesquioxane). This step was successfully achieved by incubating the SNPs in a mixture of silanes. At this stage, it was important to have the possibility to control the kinetics of layer growth in order to produce a 'recognition layer' (RL) that does not bury the virus completely. This was achieved by controlling the reaction conditions (*i.e.* temperature, concentration of silanes). The final step of the synthesis consisted

in releasing the virus to free the produced imprints. This was achieved by submitting the produced particles to an ultrasonic treatment in acidic conditions. The produced virus-imprinted particles (VIPs) were characterized by field-emission scanning electron microscopy. Two representative micrographs are shown in Fig. 1.

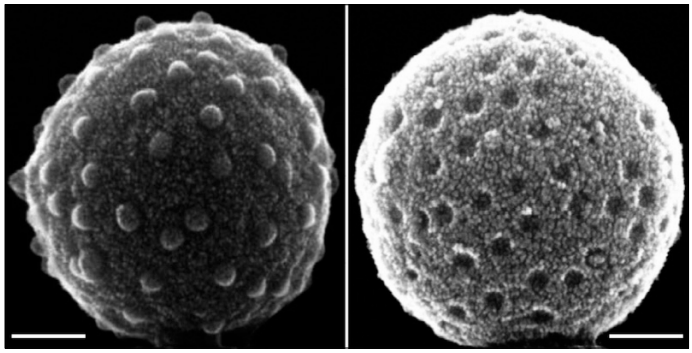

Fig. 1. Scanning electron micrographs of VIPs before (left) and after (right) virus removal. [scale bar: 100 nm]

The binding properties of the VIPs produced using as template a plant virus, namely turnip yellow mosaic virus (TYMV) were studied using an ELISA (enzyme-linked immunosorbent assay)-based batch-rebinding assay. The results clearly showed the selectivity of the produced VIPs for their targets, even in a real matrix such as blood serum. The control over the affinity of the produced VIPs for its target, achieved by controlling the recognition layer thickness, was also demonstrated. The work is underway to adapt the VIP nanomaterial to a virus diagnostic kit.

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12. Green Synthesis of Mono- and Disubstituted Pyridine *via* Aromatic Nucleophilic Substitution

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Introduction

The proven importance of pyridine derivatives in many biological and chemical systems led to their large-scale industrial production. Most of the current industrial processes are not environmentally friendly. The synthesis of substituted pyridines needs new alternative reaction conditions that respect the principles of green chemistry, without compromising quality and yield. The classical ways to produce pyridine derivatives are nucleophilic aromatic substitution (SNAr) or palladium-catalyzed cross-coupling reaction. From a process chemistry point of view, a metal-free procedure is highly preferred (cost for metal, usually palladium, problems related to Pd-removal, *etc*.). Indeed the SNAr route is undoubtedly designed as the most appropriate way to go in order to design synthesis processes that meet the new requirements of image, economy and sustainability.

Experimental Strategy

The SNAr reaction is a simple one-pot reaction, where the leaving group of a substituted pyridine is replaced by an alkyl-oxy function. This nucleophilic substitution occurs in the presence of an alcohol (the nucleophile), with a base as the catalyst, in an appropriate solvent. Usually mono-substituted pyridines are prepared with chloropyridine as the chosen nucleophile, potassium hydroxide as the base and toluene or DMSO as the solvent. Our main objective was to find greener solvents from renewable resources and easy to recycle, while maintaining reaction yield as high as possible. These new conditions could require changing the base to maintain high reactivity. Different combinations of substrates/solvents/bases were tested with several screening experiments. An assortment of twenty different solvents was tested, including classical, hazardous, exotic or sustainable ones. After selecting the most promising solvent, their interactions with nine different bases were studied. The best combinations were applied on seventeen different pyridines with leaving groups in position 2 or in position 4. The leaving groups were halides, sulfonates, sulfinates, nitro compounds, phosphates, or carbonates. The resulting optimal conditions were applied to five 2,4-disubstitued pyridines to check their leaving group reactivity and to find a way to control the substitution position selectivity. With 2-chloropyridine and 2,4-dichloropyridine as starting materials, thermal safety was studied with DSC while calorimetric parameters were obtained with reactions performed in RC1e with a volume of 250 ml, which constituted at the same time a first scale-up step. The main by-products formed with the substitution reaction on 2,4-dichloropyridines were isolated and characterized. A final scale-up on a volume of 10 liters was done with 2-chloropyridine.

Results and Outlook

Compared to the classical use of DMSO, the conversion degree was very low in 2-MeTHF (9% versus 93%, with KOH as base and 2-chloropyridine as substrate). Nevertheless, we continued with a base screening in 2-MeTHF, as well as in other solvents (classical or green). Surprisingly we observed that in 2-MeTHF the conversion degree reached 82% when KOtBu was used as base instead of KOH. A comparable effect on reactivity was observed with 2,4-disubstituted pyridines, with a high selectivity on position 2. Several additional adjustments enabled a degree of conversion of more than 99% (Fig. 1). During the scale-up phase we also noted the work-up improvements with 2-MeTHF, the product being quickly isolated and the solvent being easily recovered.

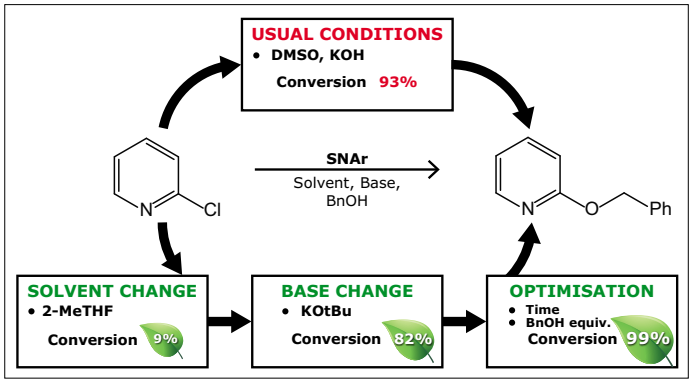

Fig. 1. SNAr, classical versus improved reaction.

The sustainability analysis took place throughout the entire project, as it was a major criterion during the solvent screening and selection. At a very early stage, our work led us to the choice of 2-MeTHF as solvent, mainly because of its green properties.

If DMSO is safer than 2-MeTHF in terms of toxicity and thermal hazard, 2-MeTHF presents large advantages in the fields of environment and process sustainability. The main problems of DMSO are product separation and recycling issues. Its miscibility with water complicates the work-up, and its distillation requires a large amount of energy while presenting non-negligible hazards of exothermic decomposition. 2-MeTHF could be easily recycled, and it is mainly produced from agricultural waste. Its main disadvantage is the formation of peroxides when exposed to oxygen. However this could be easily prevented by the addition of low levels of BHT.

This project is a strong demonstration that it is worthwhile to integrate green solvents in classical chemical processes. Even if sometimes the use of a green solvent shows poor results during synthesis, perseverance in adapting other reaction conditions could lead to unexpected findings. In our case, it happened that the combination between solvent and base played a major role in reactivity, selectivity and yields.

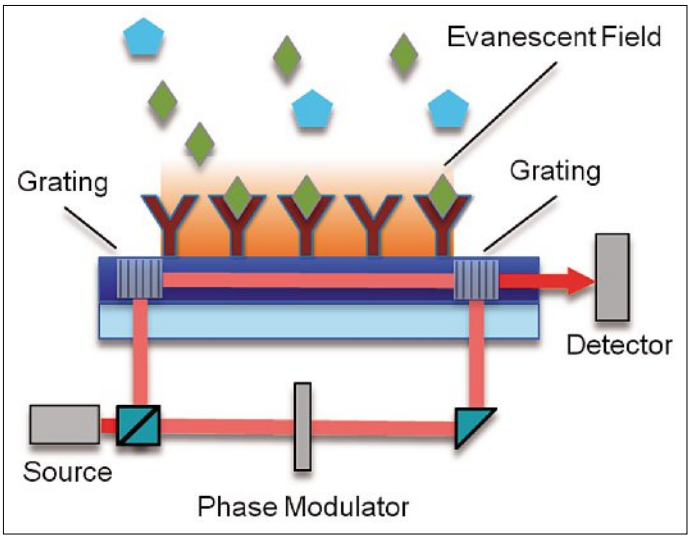

Fig. 1. Principle set-up of the grating-coupled interferometer

13. Development of a High-sensitivity Label-free Waveguide Interferometry Instrument: A Project between the Center for Biochemistry at the Institute of Chemistry and Biological Chemistry ZHAW as Main Research Partner and Creoptix GmbH

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Creoptix GmbH has developed a novel and innovative instrument for label-free detection of molecules based on grating-coupled interferometry (GCI). GCI is a proprietary technology characterized by a very high sensitivity at low technical complexity. One main application will be the measurement of binding affinities in research and development projects such as drug discovery. The market for label-free instruments has an estimated volume of 250 Mio US$ and is growing steadily. The main advantage of label-free methods over conventional methods based on absorption, fluorescence or radioactivity is that the interaction between the molecules is not disturbed by any label. Furthermore, labeling and washing steps are not needed and thus reducing reagent use, costs and time. Compared to other label-free sensors such as those based on surface plasmon resonance (SPR), grating-coupled interferometers show higher sensitivity due to a longer interaction with the sample (some millimeters compared to micrometers with SPR). In addition, their evanescent fields have a shorter penetration depth of 80–100 nm compared to 200–300 nm with SPR, and thus interferometric waveguide sensors are less sensitive to matrix effects caused by changes in bulk refractive index.

Fig. 1 shows the principle set-up of the grating-coupled interferometer developed by Creoptix. The central element is a chip with a tantalum pentoxide optical waveguide which is produced with conventional etch and surface coating technologies. The surface of the chip is functionalized to allow immobilization of ligands or receptors to the chip. Binding of analyte occurs when the analyte is passed over the chip in a microfluidic system (not shown). The readout is achieved by detecting a time-dependent interference signal which is generated by the superposition of a phase-modulated reference wave and an unmodulated signal wave within the waveguide. The binding of the analyte to the immobilized receptor in the evanescent field results in a phase change of the signal wave, thereby shifting the interference pattern. The innovative integration of the microfluidics into a disposable cartridge will lower device down-time and together with the modern software will improve the ease of use with respect to existing instrumentation.

Goal of an interdisciplinary CTI project with the partners from ZHAW, FHNW and CSEM together with Creoptix is the development of a first GCI instrument including disposables and the optimization of its functionality, followed by field tests to pave the way to market introduction. CSEM implemented the fluidics concept of Creoptix and FHNW will coordinate the field tests.

The Center for Biochemistry at the ZHAW developed a concept for the Operation Qualification which was used to test and optimize the fluidic system. In a second work package sensor chips with different surface coatings were evaluated and finally feasibility studies with a diverse set of model systems were performed. For comparison measurements were performed on a SPR-based Biacore T200 (GE Healthcare), currently the most sensitive label-free instrument on the market. The determination of the binding of low molecular weight drug candidates for target proteins requires highest sensitivity. The GCI prototype QUBE CX-3 showed equivalent kinetic data as Biacore T200 for the binding of sulfanilamide (172 Da) to carbonic anhydrase, however with an about 4-fold higher signal to noise ratio. The GCI prototype was also capable to measure the very high affinity (K_D = 90 pM) of the interaction of human interleukin-1β and a monoclonal antibody which is in clinical development. Importantly, for this interaction, the very low dissociation rate constant ($k_d = 5.7\times10^{-5}$ s^{-1}) could reliably be measured on the GCI prototype demonstrating a high stability of the instrument.

In this project a grating-coupled interferometer with highest sensitivity was developed in collaboration between a young innovative company with universities of applied sciences and CSEM. The feasibility studies for the use in life sciences helped to pave the way to the market launch planned for end of 2013.

The project partners acknowledge the CTI for funding the ongoing project Nr. 13390.1 PFFLE-LS.