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Book of Abstracts for Posters

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Magnetically Responsive Enzyme and Hydrogen-bonded Organic Framework Biocomposites for Biocatalytic Applications

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In this study, we explore the impact of magnetic nanoparticles on a biocomposite composed of enzymes encapsulated within a hydrogen-bonded organic frameworks (HOF, namely BioHOF-1). It is well known that enzymes are powerful catalysts, useful for their high selectivity and efficiency[1]. In this regards, hydrogen-bonded organic frameworks have shown interesting properties which can be exploited with enzymes. These self-assembling materials can grow around enzymes, effectively encapsulating them. This can help with an easier recovery of the biocatalyst, where having a free enzyme would lead to complications[2]. The biocatalyst is synthesized through a one-pot method in water at room temperature. The HOF phase is stable during storage and biocatalytic activity. Moreover, the presence of different buffers and solvents showed no effect on it.

Two different enzymes were investigated, namely glucose oxidase (GOx) and catalase (CAT), both of which exhibited an enhanced activity when encapsulated in presence of iron oxide nanoparticles. Better activity retention was observed also after treatment with surfactants, such as SDS.

The innovation on introducing magnetic nanoparticles confers dynamic localization properties to the biocatalyst[3]. We were able to easily collect the powder using a magnet with a minimal loss of product and high precision, multiple times. This feature contributes to improved recoverability, recyclability, and precise localization control for highly specific applications.

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Structure-guided investigation of parameters determining enzyme-catalysed reactions

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Enzymes play an indispensable role in all molecular processes in life. Their substrate and regio-specificity are embedded in their three-dimensional structure. In addition, the structure also contains information on the biochemical reaction to be performed on the substrate. Thus, knowing the three-dimensional structure paves the way to a deeper understanding of enzyme properties. Equally important are kinetic and thermodynamic parameters in the study of enzyme reaction mechanisms. Therefore, in our research, we integrate these different aspects of enzymology to better understand enzyme reaction mechanisms in order to make enzymes amenable to biocatalytic applications and drug discovery.

Design, synthesis and catalytic application of water-soluble dppf-ligands

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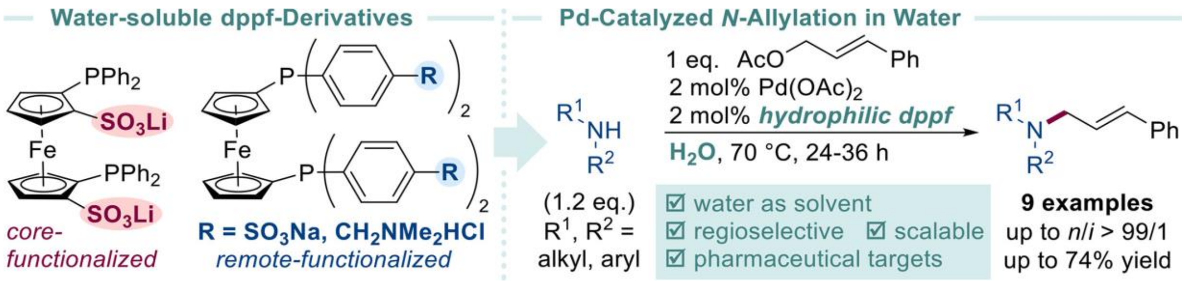
Hydrophilic phosphine ligands have been extensively used for the translation of metal-catalyzed reactions into aqueous solvent systems. Water-soluble variants of several well-known ligands and ligand classes have been reported.[1,2] However, no water-soluble derivative of dppf has been reported to date and only monophosphinylated ferrocenyl ligands bearing hydrophilic groups have been synthesized.[3]

We successfully developed both core- as well as remote-functionalized dppf-derivatives using sulfonates and amines as the hydrophilic groups. Core-functionalized dppf ligands were prepared by a directed metalation strategy starting from diisopropyl ferrocene-1,1´-disulfonate furnishing interesting *meso*-2,2´- and 2,5-disubstitued variants of dppf. Hydrophilic sulfonate or ammonium groups have also been introduced at the phenyl substituents of dppf starting from commercially available 1,1´-bis(dichlorophosphino)ferrocene.

All novel ligands have been spectroscopically characterized. Furthermore, the ligand modified with a tetraammonium salt at the phenyl groups was successfully used in the Pd-catalyzed Tsuji-Trost allylation of amines in water without use of organic co-solvents furnishing the *N*-allylated products, including two pharmaceutically relevant molecules (cinnarizine & naftifine), in good yields and excellent *n*/*i*-ratios.[4]



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Investigation on the development of water electrolysis technologies

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Water electrolysis (WE) is a cornerstone for the development of a sustainable energy industry, as it represents a zero-emission route for storing energy in the form of hydrogen. Over the past decades, significant developments have been carried out in this field, achieving high efficiencies, low operating temperatures and fast response times. Nevertheless, this technology is not exempt of challenges that must be assessed to achieve a process that can compete with the fossil fuel alternatives, which currently dominate the scene. The requirement for Iridium as a catalyst, sensitivity to contaminants, operating lifetime and component degradation are some of the main challenges.

The evaluation of performance, durability and resilience is approached by specifically designed accelerated stress test (AST) protocols based on the main degradation effects in PEMWE components**.** Therefore, the sources for degradation in individual components in a PEMWE single cell is investigated [1,2]. In- and ex-situ analysis is carried out to allow the benchmark of each component based on their performance and durability.

As the cost of iridium is a major drawback, major efforts are devoted to the reduction of catalyst loading in the membrane electrode assembly (MEA). In order to achieve lower loadings, the durability of the MEA at these loadings has to be improved. To facilitate this, an AST profile, targeting catalyst durability, is developed where the cell is cycled between different oxidation states, aiming to induce catalyst layer degradation in order to define the boundaries for catalyst loadings and develop mitigation strategies to prevent catalyst degradation [3].

Another challenge in PEMWE degradation lies in the feed water quality requirements. The ultra-pure water that is needed for PEMWE applications has excellent solvent properties, leading to the leaching of contaminants from the system components. In order to understand the mechanisms governing the degradation routes induced by the cationic contaminants, a PEMWE single cell is operated with different concentrations of various contaminants introduced. Electrochemical and morphological impact of the suggested contaminants on the cell performance and the components of MEA are investigated by various in-situ and ex-situ methods [4].

Alternatively, other routes for water electrolysis are also being investigated. Through the depolarization of the anode by the fed of SO_2 into the cell, the overall theoretical voltage for the generation of H_2 is lowered from 1.23 V vs RHE to 0.156 V vs RHE. This is achieved by replacing the oxygen evolution reaction with the SO_2 oxidation reaction. The boundaries of the reaction and the operating conditions were studied with the purpose of obtaining a functional electrolyser. The cell components were analyzed by SEM/EDX and the liquid products by GC/MS and titration [5].

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P05

Towards Biological Solid-Liquid Interfaces: Chitosan Thin-Film Modification with Small Fluorescent Molecules

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Chitosan, a polysaccharide consisting of β-1,4-D-glucosamine, has found extensive use in different fields, from water & waste treatment over food additives to biopharmaceutical applications such as tissue engineering. This biopolymer as such already has many desirable properties, like good biocompatibility and intrinsic antibacterial properties. However, its chemical modifications can tailor the properties for specific purposes [1]. Various methods have already been developed to attach different residues to the polysaccharide, either by one of the alcohols or the primary amino group at position C-2. [2]

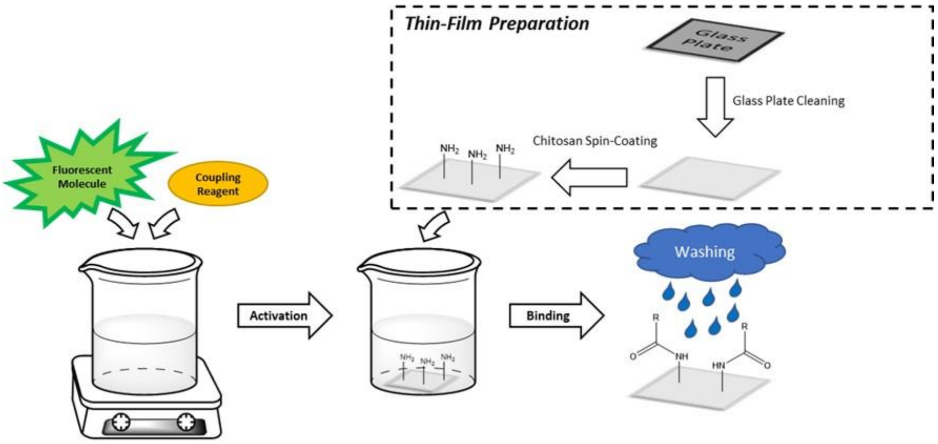

Figure 1: Preparation and chemical modification of chitosan thin-films.

We are interested in the study of solid-liquid interfaces of modified oligo- and polysaccharides, which play an important role in e.g. cell adhesion and biological recognition processes [3]. We already successfully prepared thin-film coatings on various substrates and modified them with simple amino acids [4]. In a proof-of-concept study, we synthesized fluorescently marked small molecules as well as iminosugar derivatives and covalently modified chitosan thin-films with these compounds employing common amide coupling chemistry [5]. The surfaces were analysed by fluorescence spectroscopy, atomic force microscopy, X-ray photoelectron spectroscopy, time-of-flight secondary ion mass spectrometry and QCM-D. Synthetic details and the results of the surface analysis will be presented.

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Single-Molecule Insights into Cellulose Deconstruction

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Ligno(-cellulose), the most abundant source of fixed CO_2 on earth, holds significant promise for producing sustainable chemicals. Its man component, cellulose, is composed of linearly polymerized glucose that forms three-dimensionally organized fiber bundles. This architecture makes cellulose an ideal model for heterogeneous catalysis but also contributes significantly to its high chemical and biological resistance, complicating cost-efficient depolymerization (*1*).

In nature, cellulose degradation is achieved by cellulases, which can function in either free (*2*) or complexed forms (*3*), often in combination with auxiliary enzymes such as xylanases or lytic polysaccharide monooxygenases (*4*, *5*). To fully understand the mechanisms of these synergistically acting enzyme ensembles, we need more than just measurements of soluble products; the state of the cellulose surface is crucial to their performance.

In this spirit, our research focuses on the interplay between the evolving cellulose surface and enzymatic activity on it. We are particularly interested in the three-dimensional degradation patterns of individual enzymes or specific enzyme systems and, how these changes, in turn affect the enzymes (or systems). To this end, we use Atomic Force Microscopy (AFM), a high-resolution technique, to observe topographical changes and individual enzyme activities in real-time (*5*–*8*). By correlating surface changes with enzymatic activity at the single molecule level, we provide new insights into the cellulose deconstruction process. For example, in recent studies, we revealed distinct mechanisms of different cellulase systems and even uncovered a previously unknown degradation mode (*6*, *8*).

This research underscores the importance of surface analysis in enzymatic cellulose degradation studies and highlights the potential of AFM in uncovering the detailed dynamics of cellulose depolymerization at the molecular level. Our findings not only advance the fundamental understanding of cellulose deconstruction but also have implications for developing more efficient industrial processes for sustainable chemical production.

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P07

Generation of quaternary stereocenters via ene-reductase mediated desymmetrization of cyclohexadienones

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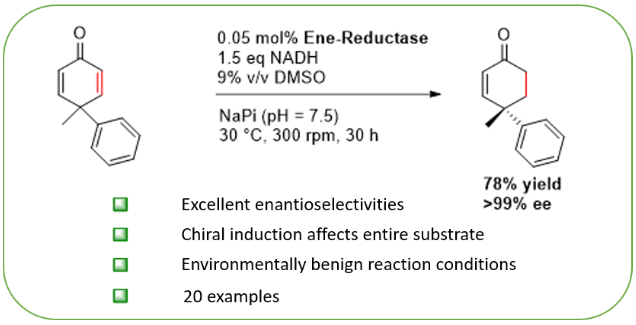
Molecules containing quaternary stereocenters often show interesting biological activities.[1] However, the stereoselective assembly of such molecules still represents a challenging task in organic chemistry, especially when the quaternary stereocenter in question shall be formed in a remote position from a functional handle such as a carbonyl group. One solution to this problem is the desymmetrization of cyclohexadienones. This strategy allows the establishment of a quaternary stereocenter in γ-position to the carbonyl moiety.

Previously, this strategy has been demonstrated for metal hydride catalyzed systems.[2,3] Due to our ongoing interest in the field of non-natural ene-reductase catalyzed biotransformations,[4] we established an ene-reductase mediated desymmetrization of cyclohexadienones.[5] The investigated ene-reductases (OPR3 and YqjM) are able to perform these transformations in >99% ee. Furthermore, overreduction to the fully saturated cyclohexanones is observed only in small amounts, even when the biotransformations are carried out with as much as two equivalents of NADH cofactor. In summary, our biocatalytic system allows the formation of cyclohexenones equipped with a quaternary stereocenter in nearly enantiopure form. The products itself bear the potential of diverse follow up chemistry, without erosion of enantiopurity.



- 🟩 Excellent enantioselectivities
- 🟩 Chiral induction affects entire substrate
- 🟩 Environmentally benign reaction conditions
- 🟩 20 examples

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NMR at extreme temperatures – from cryogenic to blazing hot

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Nuclear magnetic resonance offers plenty of advanced techniques, such as 1D and 2D EXSY, to study dynamic features of solid-state lithium-ion and sodium-ion conductors. Provided ultrafast ion dynamics is present at temperatures around ambient, i.e., dealing with jump rates in the order of some GHz, extremely low, if not cryogenic, temperatures are needed to completely freeze any Li^+ ion dynamics on the NMR time scale. On the other hand, slow dynamics need extremely high temperatures to be captured by NMR. Taking advantage of two model compounds, viz. Ge-bearing Li_6PS_5I [1] and $Na_4P_2S_6$ [2] we measured $^{7}Li/^{23}Na$ NMR line shapes and spin-lattice relaxation rates over a wide temperature range down to 9 K and up to 700 K. In the case of Ge-Li_6PS_5I, the NMR experiments resolve the various Li^+ exchange processes that ultimately give rise to overall, fast ionic transport at ambient conditions. As an example, for Ge-Li_6PS_5I, we identified the spin-lock NMR rate peak appearing at 163 K as the one reflecting the rate-limiting intercage Li^+ diffusion process that enables the ions to be transported over long distances. The corresponding Einstein-Smoluchowski diffusion coefficient excellently agrees with that indirectly probed by macroscopic conductivity spectroscopy. For $Na_4P_2S_6$, taking advantage also of variable-temperature ^{31}P NMR, we were able to separately study Na^+ translational ion dynamics and $P_2S_6^{4-}$ rotational jump processes, see Figure 1.

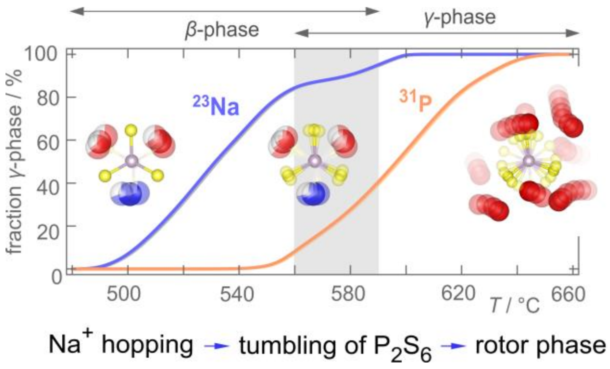

Figure 1 | As revealed by ^{23}Na and ^{31}P NMR, the dynamic processes in the so-called rotor phase $Na_4P_2S_6$ are consecutively switched on with increasing temperature. Rapid Na^+ hopping processes seem to initiate the β-γ phase transformation at approximately 580 K. At higher temperatures, ^{31}P spin-lattice relaxation NMR senses fast rotational motions of the polyanions.

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Synthesis and Characterization of Group 14 Metal *meso*-Tetraalkynyl Porphyrins

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Group 14 metal porphyrins are well known in literature due to their high photocatalytic activity. Their applications range from photodynamic therapy in medicine to environmental technologies such as bulk-hetero-junction solar cells.[1-3] Strong excited state absorption, high triplet yields and long excited-state lifetimes of *meso*-tetraalkynyl porphyrins are favourable for a variety of applications such as optical limiters.[4] This work reports the synthesis of various *meso*-tetra alkynyl tin(IV) porphyrins (Figure 1), their characterization *via* ^{1}H-, ^{119}Sn-, ^{29}Si-NMR and single crystal X-ray diffraction. Furthermore, we compare the UV/Vis absorption behaviour of the presented *meso*-tetraalkynyl tin(IV) porphyrins to the more researched *meso*-tetraaryl tin(IV) porphyrins.

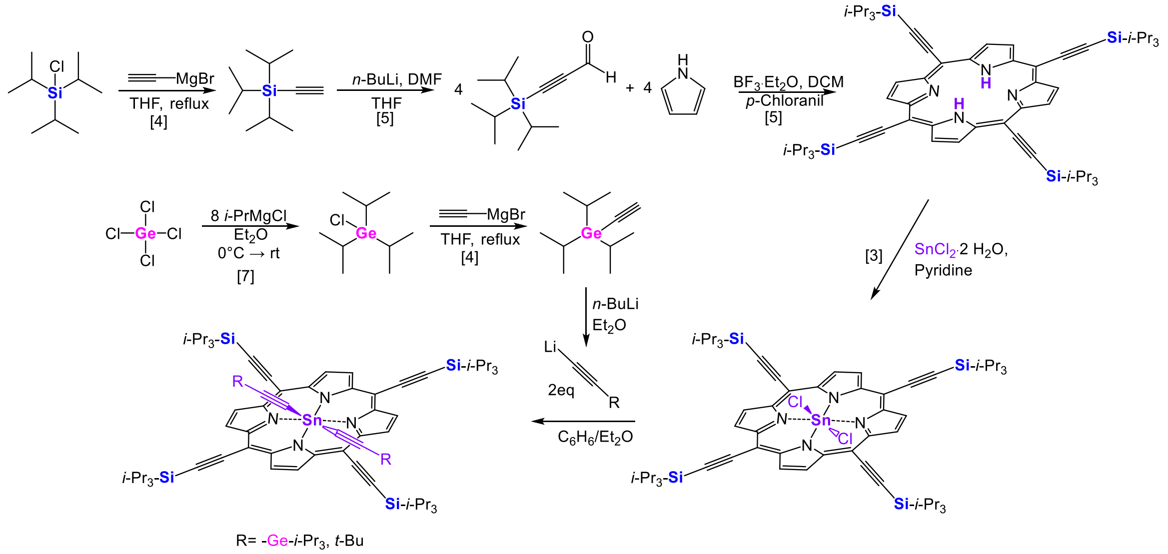

Figure 1. Synthetic pathway towards *meso*-tetraalkynyl tin(IV) porphyrins.[4-7]

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P10

Tracking of liquid movement in bulky viscose fibre networks using infrared thermography

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Infrared thermography is used in pulp and paper research, as a versatile technique to describe liquid interactions in porous cellulosic networks, or for observing failure during mechanical testing of paper. In this work we will show how an infrared camera can be used to track a liquid applied to viscose fibre cylinders of several millimetres in diameter. The pressed cylinders have a density of 0.40 g/cm³ and are made of 40 mm long viscose fibres. During this measurement, a difference between the optical liquid front and the front, visible in thermography can be observed. We assign this difference in the detected fronts, to either the liquid movement in the inner segments of the cylinders below the surface, thus below the optical visible movement, or to mechanical energy induced in the production process of the cylinders. Both ideas are reasonable, as the measured temperature increase, due to liquid application, can be as high as 5°C for these bulky materials and the increase in temperature is low or gone, when testing the same cylinders multiple times.

Monosilane as a Cheap Feedstock for Branched Oligohydridosilanes

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Recently, liquid phase deposition (LPD) attracted attention, due to its reduced production costs as compared to standard vacuum-based approaches. In this context, oligohydridosilanes such as compounds **1** (Figure 1) are ideal precursors and decompose to elemental silicon upon heating to T > 300 °C[1]. However, the high production cost of compound **1**, prevented its implementation as industrial precursors, so far. Therefore, this study was aimed to investigate possible synthetic routes, starting with a cheaper feedstock, to higher silicon hydrides Si_nH_{2n+2}. The method of *Sundermeyer et al*. and *Fehér at al.* was revisited where monosilane is used to generate potassiumsilanide **2** and further potassiumisotetrasilanide **3**[2]. The generation of compound **3** enabled the selective generation of compound **4** and **5** which are oligohydridosilanes (Figure 2).

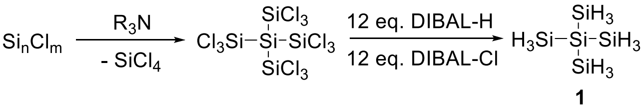

Figure 1: Synthesis of NPS 1 from Chlorosilanes

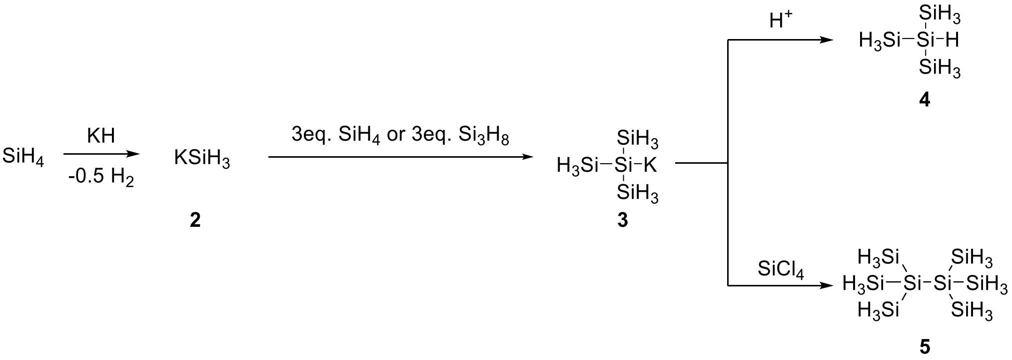

Figure 2: Optimized synthesis of higher silicon hydrides starting from potassiumhydride and monosilane

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Investigations on (semitransparent) organic solar cells with a special focus on stability

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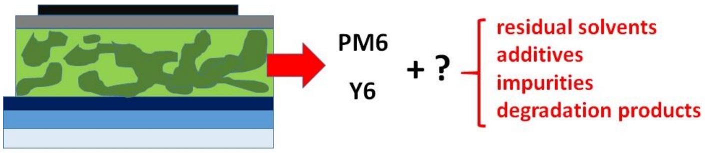
Organic solar cells experienced remarkable improvements of their power conversion efficiencies in recent years and currently values above 19% are reached. In our work we currently focus on two aspects, the preparation of semitransparent organic solar cells, that is of great interest in building integrated photovoltaics (BIPV) as well as for tandem devices, and the investigation of degradation and impurities in OPVs in order to reveal strategies to improve the long-term stability of this technology.

In the search for suitable materials for organic solar cells with high semitransparency in the visible wavelength region of the solar spectrum, the focus fell on efficient conjugated polymers such as D18, PTQ10 or PM6 and non-fullerene acceptors (NFA) such as M3, L8-BO and IEICO-4F, which absorb light in the near infrared range. Different strategies like reducing the layer thickness or donor:acceptor ratios were tested to increase the semi-transparency.

Another important area of current research is the improvement of the stability of OSCs. Since single-junction devices have achieved efficiencies above 19% in recent years, it becomes increasingly important to focus more on longevity. Common active layer materials in organic solar cells are fluorinated or chlorinated to adjust their optical, electronic, and morphological properties. However, fluorinated semiconductor can react with aluminium during the fabrication of solar cells. This leads to degradation of the semiconductor and an enrichment of fluorine as aluminium fluoride or aluminium oxofluoride at the semiconductor/Al interface, which was clearly identified by electron microscopy. In addition, impurities in organic semiconductors and/or solvents used for the fabrication of solar cells are a possible source for faster degradation or lower performance. In order to identify common impurities we carried out a comprehensive pyrolysis/GC-MS study directly on complete solar cells.



Enzymatic cascade to natural ligustrazine

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Ligustrazine (2,3,5,6-tetramethylpyrazine; TMP), a Maillard reaction product that contributes to nutty, roasted, and toasted tonalities of heat-processed food, is widely used in the food industry as a flavor ingredient [1,2]. Additionally, TMP is a natural product found in *Ligusticum wallichii*, a herb used in traditional Chinese medicine. It contributes to cardio-and cerebrovascular health, has anti-cancer properties, and serves as a precursor for other pharmacologically active compounds [3]. The demand for natural TMP exceeds its supply from raw materials, favoring biotechnological methods [2]. Several chemoenzymatic methods rely on enhanced microbial production of acetoin followed by spontaneous condensation with ammonia, often at high temperatures [1,2,4]. We set out to develop an enzymatic cascade towards TMP where all the steps are performed under mild conditions (Figure 1). A carboligase (CL) / transaminase (TA) sequence [5,6] was applied for the first time in synthesis of a short-chain vicinal amino alcohol. For the 3rd step, we tested the ability of a panel of alcohol dehydrogenases (ADHs) to catalyze the challenging oxidation of a vicinal amino alcohol to an aminoketone, which spontaneously forms TMP. 46% of TMP was produced from racemic amino alcohol standard, whereas a sequential one-pot cascade yielded up to 18% TMP. Further optimization will ensure enantiocompatibility of applied enzymes and complete consumption of acetaldehyde, which otherwise forms side-products.

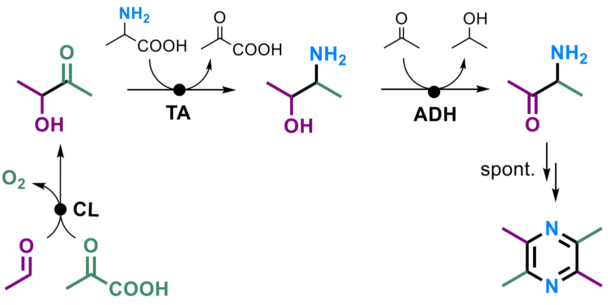

Figure 1. 3-step 1-pot sequential enzymatic cascade for the synthesis of TMP.

Acknowledgements: This work was funded by Axxence Slovakia s. r. o. We thank the COMET center acib: Next Generation Bioproduction is funded by BMK, BMDW, SFG, Standortagentur Tirol, Government of Lower Austria and Vienna Business Agency in the framework of COMET - Competence Centers for Excellent Technologies. The COMET-Funding Program is managed by the Austrian Research Promotion Agency FFG.

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Photoresponsive covalently linked dextran networks – towards functional hydrogels

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Functional materials that can take up and release guest molecules upon stimulation with light can find applications in the area of targeted drug release. [1] Such systems are often based on azobenzene, a molecular photoswitch that can be photoisomerized between its cis and trans isomers. [2] The bacterial polysaccharide dextran and its modifications have been used in a wide variety of drug delivery systems. [3] Here a previously unreported doubly-covalent ester link by azobenzene moieties opens the way for a photo-triggered porous device for the release and capture of small molecules. We have been exploring the potential of such materials by varying the degree and fashion of crosslinking. The preparations of photochromic "functional" hydrogels *via* this procedure has been demonstrated.

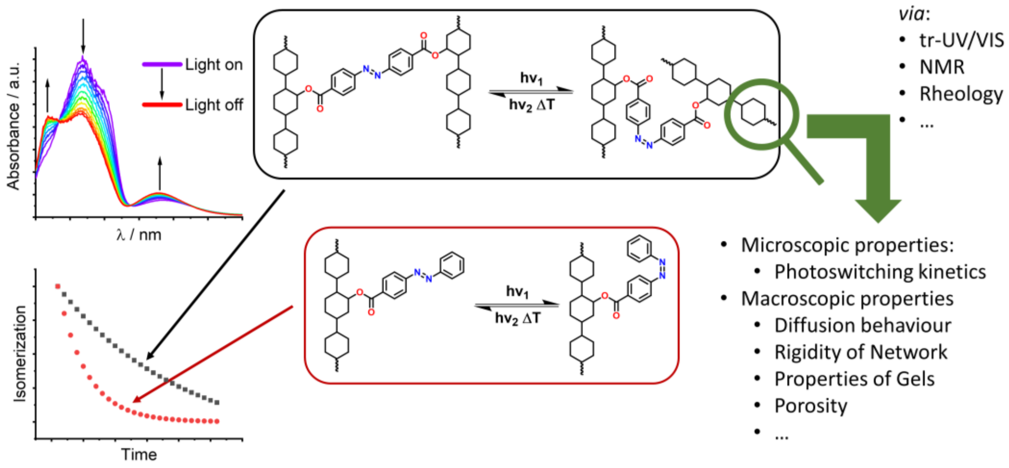

Figure 3. Azobenzene-crosslinked dextran molecules show photoswitching activity

Control experiments indicate remarkably different material properties when the azobenzene linker is attached by one or two covalent bonds. We have been following the kinetics of trans↔cis isomerizations of the azo-moiety and the micro- and macroscopic properties of the crosslinked dextran network using a wide variety of analytical techniques, including time-resolved UV/VIS spectroscopy, NMR spectroscopy and rheological measurements.

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Computational design of new proteases

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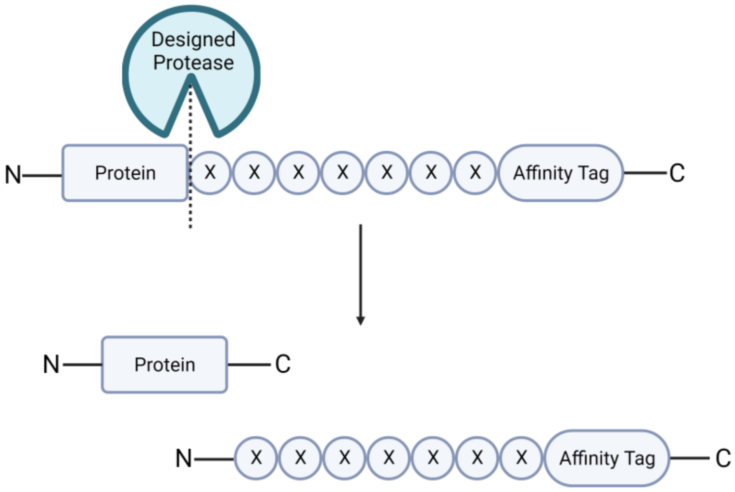
Efficient recombinant protein purification is pivotal in biological research, predominantly relying on affinity tags. However, complete removal of C-terminal tags is problematic since most endoproteases cut towards the C-terminal end of their recognition sequence [1]. Nonetheless, the additional residues on the recombinant protein from the protease recognition sequence after protein tag removal can be adverse to the proper folding or crystallization of the target protein [2]. Here, we harness the power of *de novo* computational design to develop sequence-specific proteases tailored for C-terminal tag removal without leaving any residues from the recognition sequence, a challenge unmet by currently used proteases. A FACS-based screening method will be used to select highly efficient *de novo* proteases. Our preliminary computational results show a gradual improvement of the designed models, which signifies the efficiency of our pipeline. Computational enzyme design is advantageous over conventional directed evolution techniques in developing proteases with customized applications, and our approach will open a new scope for developing proteases for a wide range of applications.



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Exploring the Role of Lytic Polysaccharide Monooxygenases in Human Pathogenicity

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Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent redox enzymes originally identified in saprobiontic fungi and bacteria [1]. These enzymes degrade biopolymers such as cellulose, starch or chitin through an oxidative process that consumes external reductants and H_2O_2 [2,3]. Recent proteomic and biochemical studies have shown that LPMOs fulfil a multiplicity of biological roles beyond biomass degradation. Notably, recent landmark studies have linked LPMO activity to the survival of pathogens in human blood [4].

In our ongoing research, a panel of LPMOs from pathogenic bacteria is recombinantly produced and biochemically characterised. We employ a novel sensor that accurately detects H_2O_2 in continuous measurements [5] as a new method to assess LPMO activity based on its consumption of H_2O_2. This method enabled us to measure LPMO kinetics and, in conjunction with microscale thermophoresis – which measures protein-ligand interactions – allows us to investigate the substrate spectrum and kinetics of the newly produced LPMOs. Additionally, we are generating LPMO knockout and complementation strains targeting the two LPMOs found in the human pathogen *Vibrio cholerae*. These strains will be used in serum resistance assays and *in vivo* assays to investigate changes in virulence and clearance of wildtype and the *lpmo*-deletion strain. This research aims to elucidate how bacteria activate and regulate LPMO activity. Ultimately, this project seeks to clarify whether LPMOs in pathogenic bacteria facilitate infectious processes or if their primary role is to support bacterial survival outside the host.

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Diarylhalonium-based Probes for Activity-based Protein Profiling of Oxidoreductases

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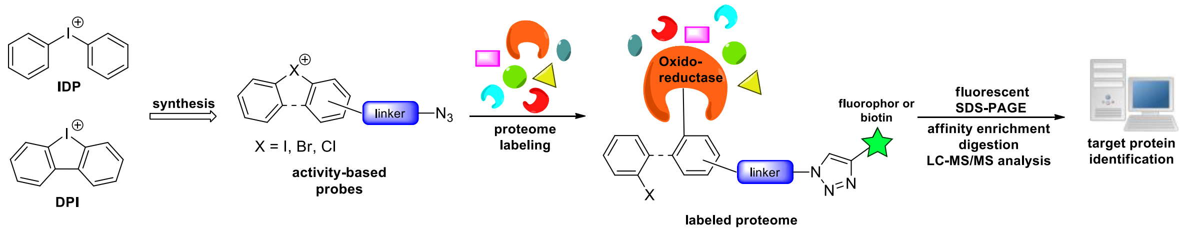
Activity-based protein profiling (ABPP) represents an intriguing and powerful proteomic method for the *in vitro* and *in vivo* identification and evaluation of proteins in its active state.[1] Throughout all enzyme classes, oxidoreductases (EC 1) have received much less attention, although they play a role in essential metabolic processes and a more detailed insight into their function and activity could be beneficial to the understanding of cellular biochemistry and the role of these enzymes in diseases.[2]

In order to expand the ABPP toolbox for oxidoreductases,[3] we designed and synthesized several novel ABPs for the simultaneous labeling of various subclasses of oxidoreductases based on the potent ALDH2 inhibitor and hypervalent iodine compound diphenyleneiodonium (DPI) and its analogue diphenyliodonium (IDP),[4] making use of their unique reactivity via reductive activation by the target proteins. The probes were then used for *in vitro* ABPP labeling experiments with selected enzymes and fresh mouse liver. After proteome labeling and initial SDS-PAGE in-gel fluorescence assays, promising candidates were further used in biotin/streptavidin affinity enrichment, whereupon labeled proteins could be identified by LC-MS/MS after tryptic digestion.



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3D Printing of Polysaccharides Imitating Porcine Aortae

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Nature exhibits an inherent capability to grow complex, anisotropic multilateral structures bottom up from simple molecules like glucose. This allows the construction of highly complex shapes with outstanding properties and functions. For instance, the microstructural anisotropy of plant cells enables tree leaves to responsively react to external stimuli, such as humidity, touch, or light.[1] In human aortae, circumferentially oriented collagen and elastin fiber networks enable unique strain stiffening anisotropic response in billions of repetitions.[2]

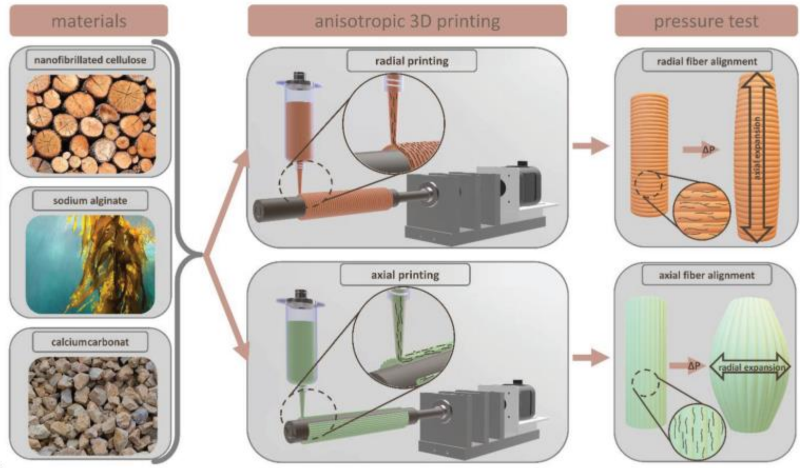

Figure 1 Overview of anisotropic 4-axis 3D printing process from [3]

Herein we present the research results of our 3D printing experiments to create anisotropic structures from a nanofibrillated cellulose/alginate nanocomposite. The bioink received from mixing of the biopolymers and $CaCO_3$ nanoparticles could be processed with an extrusion-based 3D printer, allowing in-situ fiber alignment to produce scaffolds with anisotropic properties. The subsequent crosslinking with weak acids additionally enables the precise control of mechanical properties within one single printing process, to create sophisticated structures with tunable properties.[4] Various tubes with different fiber alignment patterns could be 3D printed to demonstrate the anisotropic and water-resistant behavior of our material. Further the material was characterized to assess important biomedical parameters like cell compatibility, nutrient diffusion and controlled degradability.

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Advanced gas fermentation strategies for CO_2 utilization by the microbial bioplastics producer *Cupriavidus necator*

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Gas fermentation is an upcoming technology utilizing microorganisms to convert CO_2 or CO-rich gases into a wide range of products. The hydrogen-oxidizing bacteria *Cupriavidus necator* efficiently uses CO_2 as its sole carbon source, H_2 as an electron donor and O_2 as electron acceptor. The cultivation of C. necator on CO_2 for the production of bioplastics (poly-(*R*)-3-hydroxybutyrate (PHB)) has become a central focus of both research and industry. Current bottlenecks in cultivating this organism relate to the design of bioreactors that can be used for explosive gas mixtures of the needed substrate gases and the precise control of dissolved oxygen concentrations due to biological limits. Here, we report on the bioreactor design and parameter optimizations for high cell density production of *C. necator* H16. We show how safe lab-scale fermentations can be established in order to cultivate hydrogen-oxidizing bacteria without gas limitations. By implementing continuous gas fermentation set ups, bacteria were grown under reproducible conditions with biomass yields of up to 15 g/L [1]. Precise automation and control models were applied to finely adjust gas concentrations for optimal cell growth and PHB production. We have shown that low concentrations of 0.75 mg/L dissolved oxygen (dO_2) significantly enhance biomass and PHB production under chemolitotrophic conditions [2]. Further optimisation of the media composition led to a final biomass concentration of 53 g/L dry cell weight with a PHB content of 75 %. We want to emphasize that all fermentations were carried out without pH control or additional feeding. With this study we contribute to the development of efficient and sustainable gas fermentation processes and gain new insights into critical growth parameters under chemolithotroph conditions.

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Iron-based structures as sustainable, non-toxic and widely available energy carriers

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Chemical looping as a novel approach to energy conversion holds immense promise for sustainable hydrogen storage. Among the various materials used in chemical looping processes, iron stands out as a particularly effective and versatile candidate. Harnessing the unique properties of iron, chemical looping opens doors to efficient hydrogen storage, carbon capture, and beyond, offering a pathway to a cleaner and more sustainable energy landscape [1].

At the heart of chemical looping with iron lies the principle of redox reactions, where iron-based materials undergo cyclic oxidation and reduction reactions to facilitate the conversion of energy carriers such as hydrogen or carbonaceous fuels [2]. In the context of energy storage, iron serves as an oxygen carrier for the chemical looping hydrogen process HyLoop. The reduction reaction of these iron-based structures is carried out by feeding reducing gases like CO, contaminated H2 or syngas mixtures. During the reduction, the chemical energy of these compounds is stored while being oxidised themselves, being safe species to be released as condensed water or captured as a pure gas stream of CO2. By consecutive oxidation of the iron-based material with steam, an ultra-high purity hydrogen stream is produced on demand, ready to be utilised by fuel cells or other technology [3], [4]. This allows the HyLoop technology to overcome challenges associated with traditional hydrogen storage methods.

One of the key advantages of iron-based chemical looping for hydrogen storage is its inherent safety and stability. Unlike pressurized hydrogen tanks or cryogenic storage, chemical looping with iron eliminates the need for high-pressure or low-temperature conditions, reducing the risk of accidents or leaks. Moreover, recent developments in iron-based materials exhibit excellent chemical stability, ensuring long-term performance and reliability in hydrogen storage applications [5].

Furthermore, iron-based chemical looping offers a versatile platform for integrated energy systems, enabling synergies between hydrogen production, carbon capture, and renewable energy sources. By coupling chemical looping with renewable energy sources such as solar or wind, excess energy can be stored in the form of hydrogen during periods of low demand and subsequently released when needed, enhancing grid stability and reliability. Chemical looping hydrogen technologies with iron-based structures offer a sustainable pathway towards a cleaner and more resilient energy future. Continued research and investment in iron-based chemical looping technologies are essential to unlock their full potential and accelerate the transition towards a low-carbon economy.

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In-Situ Alignment of Dielectric Fibers with Electric Fields in Extrusion Processes

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Biological systems found in nature exhibit highly complex hierarchically ordered 3D structures with many degrees of organization and functionalization to achieve their unique mechanical and biological properties. While living organisms grow theses anisotropic reinforcement architectures, the fiber alignment in polymer processing and especially 3D printing requires external forces such as shear force, electric – or magnetic fields and is often limited to specific scenarios.[1]

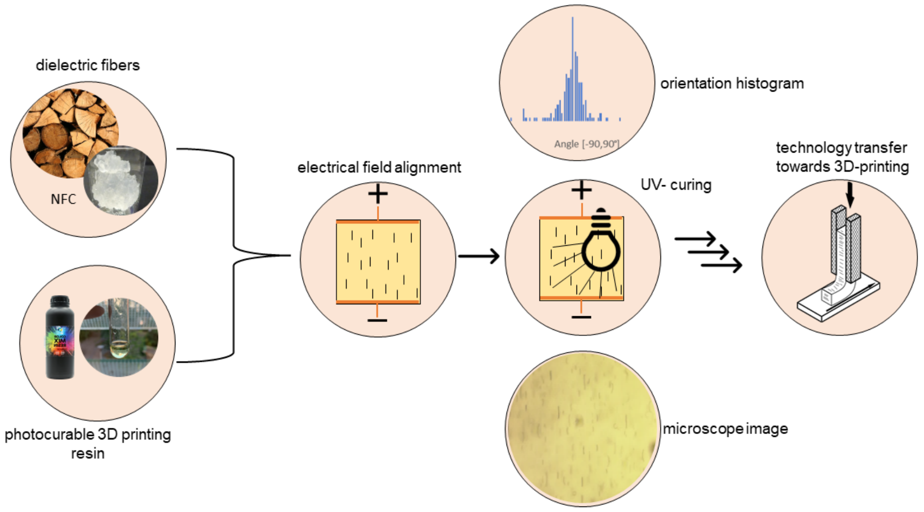

Figure 1. Schematic workflow overview

Herein, the research results for a nanofiber alignment technology transferable to extrusion-based 3D printing employing electrical fields is reported. Modified fibres with dielectric properties like nano fibrillated cellulose (NFC) suspended in commercial photo resins are aligned in electrical fields with tuneable strength. Subsequent UV curing enables the fixation of the aligned fibres and image software assisted orientation analysis. The influence of flow velocity and electrical field strength on alignment quality could be evaluated. Effective solutions against common dendrite formations are presented by using combined AC-DC fields. Furthers we present the concept to transfer this alignment technology towards extrusion-based 3D printing.

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Thioxanthone Dioxide Triplet States have Low Oxygen Quenching Rate Constants

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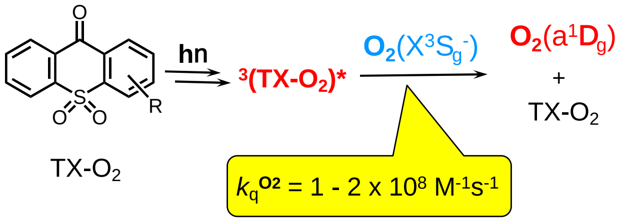
With few exceptions, triplet excited states of organic molecules, $^3M_1$, are quenched by ground state molecular oxygen, $O_2(X^3\Sigma_g^-)$, with rate constants k_q greater than $\sim 10^9$ $M^{-1}s^{-1}$ in fluid solutions. If the energy of the triplet state is above 94 kJ/mol, such quenching can result in the sensitized production of singlet oxygen, $O_2(a^1\Delta_g)$. In the interaction between $^3M_1$ and $O_2(X^3\Sigma_g^-)$, the magnitudes of both k_q and the $O_2(a^1\Delta_g)$ yield depend appreciably on mixing with the M-O_2 charge-transfer state. Here, we report that triplet states of several thioxanthen-9-one-10,10-dioxide derivatives have unusually low k_q values (as low as $\sim 1 \times 10^8$ $M^{-1}s^{-1}$), but have quantum yields for the photosensitized production of $O_2(a^1\Delta_g)$ that approach unity. Because these molecules possess high oxidation potentials (~3.5 V *vs.* SCE), we suggest that charge transfer character in the $^3M_1$-$O_2(X^3\Sigma_g^-)$ encounter complex is reduced, thereby lowering k_q while maintaining high $O_2(a^1\Delta_g)$ yields. These results provide important experimental support for existing models for the quenching of organic molecule excited states by $O_2(X^3\Sigma_g^-)$.

Although the present study focused on an oxygen-specific issue, the results obtained provide insight into the general photophysical phenomenon of CT-mediated transitions between different electronic states, particularly transitions that are formally forbidden. The information obtained also further elucidated how CT-mediated processes can influence, and thereby be used to control, the kinetic competition between different pathways of excited state deactivation [1].



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A Computational Model Study of the Interaction of Methylorange Dye with Doped Polypyrrole

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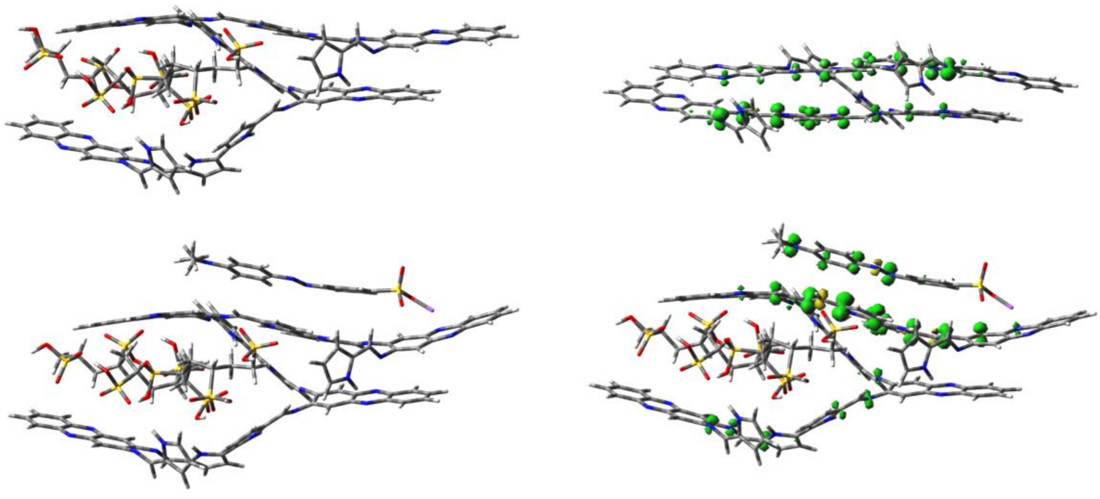
Polymer-based actuators have interesting applications in conducting films, drug delivery and sensors [1,2]. Actuation in conducting polymers is based on redox switching between the doped conducting and undoped insulating states and connected with large volume changes responsible for actuators bending. Zig zag conformations of polypyrrole polymers linked by small organic molecules show a significant deformation and high current density [3]. We investigated an accordion type polymer, poly(phenazine 2,3-diimino(pyrole-2-yl)) (DPP) doped with polyvinyl sulfonic acid (PVSA), upon interaction with the redox dye methyl orange, which serves as an actuator for dye sensing.

In this poster, we support the experimental data [3] with new computational model calculations on the conformational flexibility and redox properties of the doped polymer before and after adsorption of the methyl orange dye to learn more about the volume and electronic changes necessary for actuation.



Geometries of neat PVSA-doped 4DPP and with adsorbed Methylorange (left), and spin density upon reduction of the polymer model (right)

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Fabrication of oriented MOF films and patterns by conversion from oriented ceramic nanostructures

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The fabrication of porous materials with oriented pore channels is a current challenge that could enable applications in anisotropic functional devices, for example for preferential orientation of guests [1]. In particular, for metal-organic frameworks (MOFs) superstructures such as polycrystalline films and patterns, the simultaneous control of crystal orientation and positioning on a substrate is highly desired for their implementation in microdevices. However, the fabrication of precisely oriented MOF films and patterns on commercially relevant scales (cm^2) is currently a challenge. In recent years, the fabrication of MOF films by conversion from a sacrificial ceramic template has emerged as a powerful tool to obtain MOFs as films with precise pore-channel orientation, thickness and positioning [2]. In particular, highly oriented copper-based MOFs can be synthesized by immersing a film of aligned $Cu(OH)_2$ nanobelts in an ethanolic solution containing organic linkers. When linkers are carefully selected to ensure lattice-matching between the $Cu(OH)_2$ and the target MOF, the MOF grows heteroepitaxially retaining the original crystalline orientation. Recently, we reported a semi-automatic method that affords a 100% yield of high-quality ceramic films, at the cm scale, for the fabrication of precisely oriented and homogenous Cu-MOF coatings [3].

A further step required for the implementation of MOF films into microdevices is achieving precise control of the local positioning of the MOF crystals, i.e. patterning [1]. We recently demonstrated the microfabrication of 3D oriented Cu-MOF patterns by deep X-ray lithography (DXRL, Elettra Synchrotron) [4]. By integrating a brominated dicarboxylic ligand (Br_2BDC) into a copper-based MOF Cu_2L_2DABCO ($L=BDC/Br_2BDC$), we achieved sensitivity to radiation, while a mixed-linker strategy was used to ensure the film's orientation and homogeneity. The lithographed samples act as diffraction gratings upon irradiation with a laser, confirming the quality of the extended MOF micropatterns. Additionally, the oriented crystalline micropatterns display anisotropic optical properties. Such progress in the fabrication of films and patterns could facilitate the application of MOFs to molecular separators and micro-opto-electronic devices.

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P25

Enhanced Enzymatic Performance with Multivariate Metal Azolate Framework-Biocomposites

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Metal-organic frameworks (MOFs) are extended materials characterized by porous structures [1] synthesized through a modular approach, combining inorganic centers (metal ions) with organic linkers (ligands). [2] They possess notable properties such as high surface areas, adjustable crystalline symmetry, and versatile pore chemistry. [1, 2] Multivariate MOFs (MTV-MOFs) integrate different constituents as building blocks while maintaining material periodicity. [1] This approach enables the optimization of the pore environment, enhancing specifically tailored properties [3], such as the hydrophobicity of the MOF-matrix. [3, 4] A particularly interesting subclass of MOFs are Zeolitic Azolate Frameworks (MAFs), where the MTV-approach can be implemented. [3] Two widespread examples of MAFs are ZIF-8, composed of Zn^{2+} cations and 2-methylimidazole (2-HmIm) ligands, which develops a hydrophobic surface, and MAF-7, assembled from Zn^{2+} cations and 3-Methyl-1H-1,2,4-triazole (3-Mtz) ligands, which is highly hydrophilic. [4] Both MAFs can be used for enzyme encapsulation, protecting the enzyme against high temperatures, organic solvents, and chaotropic agents. [4] In particular, the use of MOFs to immobilize lipases such as the enzyme *Candida rugosa* lipase (CRL) has been suggested to improve the sensitivity and robustness of these enzymes [5], which are of interest for their ability to detect organic pesticides and herbicides. [6] However, current research efforts are needed to develop synthesis protocols to enhance the MAF-biocomposite's activity and eliminate additives like ammonia, which is commonly used in MAF-7 synthesis, that can reduce enzyme efficiency. In this study, we adopt the MTV-approach to synthesize CRL@MTV-MAF-biocomposites using two distinct ligands, 2-HmIm and 3-Mtz, resulting in an isoreticular structure. For a fundamental understanding of the interaction between CRL and the MTV-MAFs, we evaluated different ligand ratios to determine the optimal activity for the encapsulated enzyme. We show the impact of the different ligand ratios and additive use (ammonia) on the resulting biocomposite's crystallinity, enzymatic activity, encapsulation efficiency and crystal structure aiming to improve the biocomposite's performance. Our findings reveal a non-linear relationship between enzymatic activity and the MOF-matrix's hydrophilicity, with the highest achievable activity surpassing that of the free enzyme by 41%, and without the use of ammonia. This progress in the development of additive-free highly active CRL@MTV-MAF systems will facilitate the implementation of MAF-biocomposites in high-performing sensing applications.

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P26

Alternative Methods for Covalent Cross-linking of Alginates

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Plant based alginic acids ([D-ManA(β1→4)]$_m$-[L-GulA(α1→4)]$_n$) and their salts are very often used to create hydrogels for tissue culture and regeneration [1] [2]. These materials are gelled by multivalent ions, or covalently e.g. by (radical) polymerization of previously attached reactive functional groups. Both approaches have their limitations with respect to either stability or biocompatibility. This work therefore investigates new gelation methods for alginates, based on small molecular crosslinkers comprising *N*-terminal glycine, forming three dimensional networks of amide bonds within the gel. With these crosslinkers, sodium alginate has been chemically gelled via amide bonds using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling reagent in water (**Figure 1**). With this method, stable hydrogels with a storage modulus (G') of approximately 655 kPa and a loss modulus (G'') of 299 kPa are produced. Time dependent measurements showed that the gel transition occurred after 440 s. The materials used are potentially biocompatible, but the bonds formed a most likely susceptible to targeted enzymatic cleavage by esterases or proteases. This could lead to interesting properties for potential applications in tissue culture and regeneration.

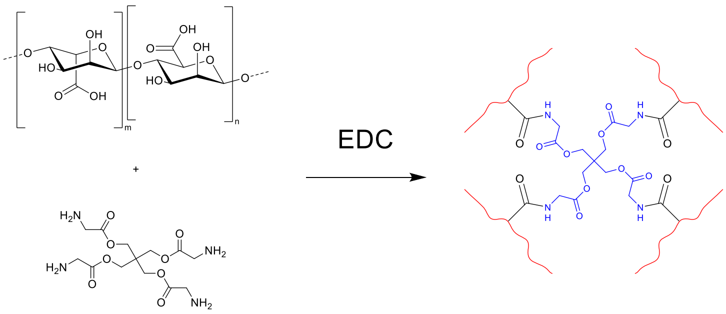

Figure 4: Covalent cross-linking of alginic acid with pentaerythritol tetrakis (glycinate) as a multifunctional spacer molecule

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Hydrogen-based processing of mineral iron carbonate; iron production combined with catalytic CO/CO2 hydrogenation

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The direct reduction of Styrian carbonate iron ore (siderite) with hydrogen offers a high potential for reducing greenhouse gas emissions and thus represents a green alternative to carbon-based technologies for the future sustainable production of iron from carbonaceous ores. [1,2] Mineral iron carbonate is usually refined by sintering before it can be fed into the blast furnace. During sintering, iron carbonate (e.g. three tonnes of ore with an iron content of 33% by weight) is converted into hematite (e.g. 0.45 t of Fe2O3) in an oxidising atmosphere, releasing 1.18 t of CO2 per tonne of pig iron. Together with the CO2 emissions from the blast furnace process, this results to around 2.2 tonnes of CO2 per tonne of pig iron (or 1.9 tonnes of CO2 per tonne of pig iron, based on FeCO3). When, alternatively, mineral iron carbonate is processed in reducing atmosphere to form elemental iron instead of hematite (FeCO3 + H2 ⇌ Fe + CO2 + H2O) carbon dioxide emissions could be cut by 50% (except for the energy demand for the smelter).

Sufficient availability of renewable hydrogen is crucial for industrial implementation. With pure hydrogen from electrolysis complete conversion of FeCO3 is obtained, which raises the question of whether lean reducing gas (e.g. coker gas) would also be suitable. Therefore, the effects of hydrogen quality and the impact of matrix constituents in the hydrogen source, such as methane and carbon dioxide, on the direct reduction of mineral iron carbonate were investigated. It was shown that with hydrogen concentrations as low as 55 vol% complete iron carbonate conversion is possible. Carbon dioxide in the feed gas (27–63 vol%) favors magnetite formation. Methane in the feed gas has no negative impact on the composition of the reductively calcined product. These results suggest that even low-grade hydrogen sources are viable options for direct iron carbonate reduction. [3] However, carbon dioxide emission reduction is limited to 50% compared to the state-of-the-art process. Advanced carbon dioxide emission reduction needs process gas treatment by using it for energy storage (actually hydrogen storage). Hydrogenation of carbon dioxide may contribute to the reduction of carbon-based emissions. The high carbon dioxide concentrations of the process gas from the direct mineral iron carbonate reduction with hydrogen recommend the conversion of carbon dioxide to methane or methanol. [4]

Acknowledgement: This research was funded in part by the The Austrian Research Promotion Agency (FFG) [Energieforschungsprogramm, PN 86543]. Moreover, the financial support from the ‘Zukunftsfonds Steiermark’ (grant number: PN 1418) is acknowledged.

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P28

Multifunctional 2D Polysaccharide-based Biomaterials

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The development of multifunctional 2D polysaccharide-based biomaterials marks a significant advancement in biomedical engineering, with a wide range of applications from tissue engineering to drug delivery[1]. Polysaccharides and biodegradable polyesters are ideal candidates for creating innovative vascular graft biomaterials due to their biocompatibility, biodegradability, and structural versatility. Although polyester biomaterials like polycaprolactone (PCL) are crucial in vascular surgery, their success is often hindered by issues such as nonspecific protein adsorption, thrombogenicity, and poor endothelial cell response[2]. To overcome these challenges, this study investigates the functionalization of polyester biomaterials with various polysaccharide coatings using a quartz crystal microbalance with dissipation (QCM-D)[3]. A two-step, water-based method was used to modify cationized PCL surfaces with different sulfated polysaccharides (e.g., cellulose sulfate, heparin, fucoidan), varying in sulfate content and origin (see **Figure 1)**. These polysaccharide coatings demonstrated long-term stability, tunable morphology, controlled roughness, film thickness, chemical composition, and hydration levels. The coated surfaces exhibited enhanced protein adsorption (e.g., with fibrinogen), increased anticoagulation activity, and prolonged coagulation time compared to uncoated PCL. The adhesion and viability of human primary endothelial cells cultured on these modified PCL surfaces were also investigated, correlating with the type and sulfate content of the adsorbed polysaccharides[4]. These findings pave the way for developing novel (bio)coatings with tailored functionality, charge, and biochemical properties, thereby extending the applicability of PCL-based implants in complex vascular grafts.

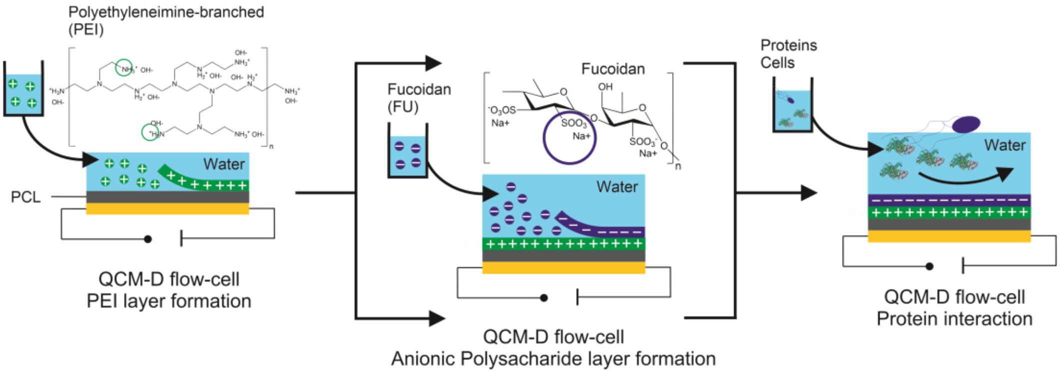

Figure 1. Functionalization of polyester biomaterials (PCL) with sulfated polysaccharide (Fucoidan) coatings using a quartz crystal microbalance with dissipation (QCM.D).

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Light-induced chemical transformations in condensed matter.

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Photo-induced chemical reactions, where the energy of photons is converted into molecular reactivity, play an important role in nature, technology, and medicine. Light activation enables a wide variety of chemical processes which would have been impossible otherwise using thermal activation. Spatial, time, and energetic selectivity of light excitation make it a unique tool for controlling chemical reactivity in different conditions.

In the research group of Prof. Georg Gescheidt, we use custom-built light sources of different frequencies (LEDs, UV-vis lamps, lasers) in combination with modern spectroscopic techniques like NMR, EPR, FT-IR, cyclic voltammetry, ENDOR, and UV-vis to explore various topics which include photo-triggered processes:

- Light-induced nanocomposites preparation
- Photo-induced free radical polymerization
- Photocatalysis
- Light-driven molecular motors
- Bio-molecules oxidation
- Radical processes in biomimetic systems
- Antioxidants
- Novel organic materials

In parallel, we develop new technologies and methods to follow look at those processes. Some of our developments are:

- high-intensity LED-based multi-wavelength photo-reactors

- multi-wavelength NMR probe for the observation of photochemical reactions in situ.[1]

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P30

Piezoelectric hardening: Mechanisms, Processing, Characterization and Applications

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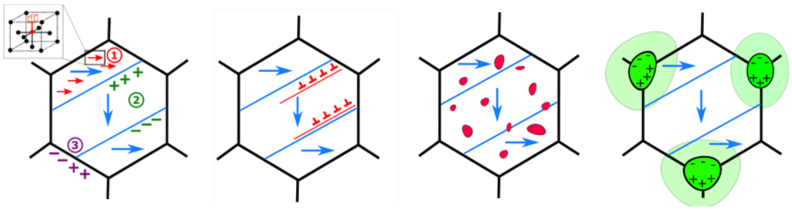
Piezoelectric materials enable the coupling between electrical and mechanical signals and are widely used in various electronic applications. A large part of piezoceramics is used in high-power devices, which among others include voltage transformers, ultrasonic motors, ultrasonic welding, cutting, cleaning devices, and therapeutic medical applications [1]. In these devices the piezoelectric is driven in the vicinity of its resonance frequency by a comparatively-high AC electric field, which induces a high vibration velocity. These harsh conditions require piezoelectrically hard materials with low losses and high stability.

We start by describing the underlying piezoelectric hardening mechanisms, which are based on hindering the movement of ferroelectric domain walls by various defects. The first mechanism includes point defects, which are introduced by acceptor doping and resulting charge-compensating vacancies. We are developing a new concept of Fermi level engineering in piezoceramics, whereby targeted co-doping enables to increase the concentration of dopants [2]. The second mechanism is based on the introduction of dislocations, i.e., 1-dimensional (line) defects with charged cores and space-charge layers [3]. These enable elastic and electrostatic pinning of ferroelectric/ferroelastic domain walls. The third mechanism utilizes 2-dimensional defects, mostly grain boundaries, which are restricting domains by intragranular stresses and direct pinning [4]. The fourth mechanism includes 3-dimensional defects, such as second phase precipitates [5]. Selected material compositions exhibiting the above-mentioned mechanisms will be presented and their synthesis routes will be detailed.

In the final part, we present the structural and electro-mechanical characterization methods used for the evaluation of hard-type piezoelectric materials. In particular, we focus on measurements of the piezoelectric resonance, which enables amplification of the displacement. In order investigate the mechanisms and directly follow the interactions between the various defects and domain walls, we developed a method combining resonance electro-mechanical excitation with high-energy X-ray diffraction [6]. Finally, an outlook on further material development and potential applications of the newly-developed materials will be provided.



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Synthesis and Characterization of New Counterion Substituted Triacylgermenolates and Investigation of Selected Metal-Metal Exchange Reactions

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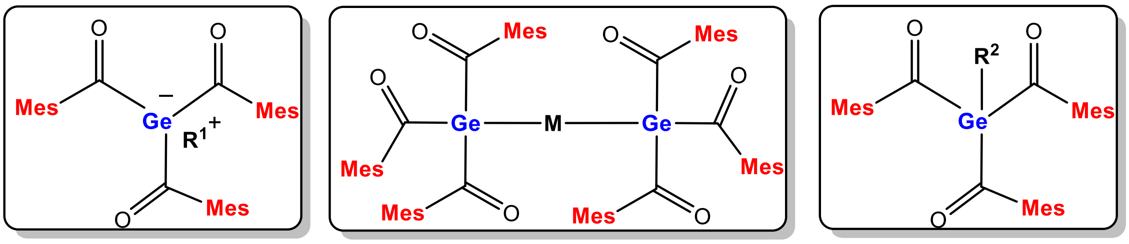
Recently, we introduced triacylgermenolates as innovative building blocks for the formation of radical photoinitiators. These enolates are generated either through single electron transfer (SET) with metallic potassium or the KOtBu mediated desilylation of tetrakis(trimethylsilyl)germanium and the reaction with the corresponding acyl fluoride. [1] Building on this, other counterions with the 2,4,6-trimethylbenzyl (Mes) moiety at the carbonyl group were investigated. Different alkali metals, namely sodium, rubidium and cesium, were examined through SET and the corresponding tert-butoxides. Moreover, we reacted the potassium substituted triacylgermenolate **1** with nBu_4NBr leading to the ammonia substituted derivative. Additionally, selected metal-metal exchange reactions were carried out with **1**. Furthermore, the hydrido-substituted triacylgermane was obtained by reacting **1** with HCl/Et2O. In Figure 1 an overview of the obtained products is depicted. [2]



$\mathbf{R^1}$ = Na, Rb, Cs,
K/$ZnCl_2$, $n\mathrm{Bu}_4\mathrm{N}$,
Zn*t*Bu

M = Mg, Hg

$\mathbf{R^2}$ = H, Cl

Figure 5: Isolated compounds.

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Efficient and Sustainable Enzymatic Synthesis of the Crucial mRNA Vaccine Building Block Pseudouridine

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β-Pseudouridine (Ψ) is the *C*-nucleosidic isomer of uridine. Recently, Ψ has gained attention as a crucial component of RNA vaccines.[1] Substituting uridine with Ψ or its N1-methylated derivative (N1m-Ψ) reduces the immunogenicity of the synthetic mRNA, resulting in increased biological stability and efficient translation. As mRNA vaccines have proven highly effective in combating COVID-19 and show promising results against other infectious diseases and cancer, demand of the mRNA building blocks Ψ -5'-triphosphate (ΨTP) or N1m-ΨTP is expected to rise. To enable its supply, expedient access to Ψ is necessary. Consequently, there is a high demand for efficient and environmentally friendly synthesis routes. Here, we present the development of sustainable and highly efficient biocatalytic synthesis routes.

The uridine isomerization cascade involves four enzymatic steps.[2] The *N*-glyosidic bond of uridine is cleaved by uridine phosphorylase via phosphorolysis to give uracil and D-ribose (Rib)-α-1-phosphate. Phosphopenotse mutase catalyzes the isomerization to Rib-5-phosphate, which serves as the substrate for Ψ-5'-phosphate (ΨMP) glycosidase (YeiN). YeiN reconnects the Rib-5-phosphate with uracil via a C-glycosidic linkage, producing the *C*-nucleotide ΨMP. Lastly, a specific nucleotide phosphatase removes the phosphate, yielding the desired Ψ. The phosphate released, can be utilized by the phosphorylase, enabling phosphate recycling within the cascade. The optimized cascade yields a supersaturated product solution (~250 g/L) from which the pure Ψ crystallizes enabling product recovery by filtration (90% recovery). Scalability of the cascade was demonstrated by performing a 100 mL batch reaction. The synthesis of Ψ achieved a productivity of 36 g/L/h, and 24.3 g of product was isolated with a purity of 99%.[2]

To enable easy access to modified Ψ derivatives, we developed an alternative kinase-dependent cascade starting from a pentose, which is phosphorylated at the 5-hydroxyl position by ribokinase using ATP as the phosphate donor. Acetate kinase is employed for ATP recycling, utilizing acetyl phosphate as an inexpensive phosphate donor. YeiN couples the added nucleobase to produce the corresponding ΨMP derivative and phosphate ester hydrolysis by a phosphatase yields the desired *C*-nucleoside product. This approach allows the synthesis of stable isotope (^{15}N, ^{13}C) labeled Ψ[3], as well as novel base and pentose modified Ψ derivatives[4], with yields ranging from 20% to 95%.[3–4]

Synthesis of ΨTP, the substrate needed for mRNA production, was also considered. Starting from ΨMP, ΨTP was produced using a phosphorylation cascade with acetate kinase and uridine 5'-phosphate kinase as biocatalysts. Acetyl phosphate served as the phosphate donor, and ATP, provided in substoichiometric amounts (5 mol%), was regenerated by acetate kinase. Isolation of 240 mg of ΨTP was achieved with a yield of 85% from a 6 mL reaction. The isolated ΨTP was then used in in vitro transcription to produce uridine-to-Ψ substituted mRNA.[4]

The presented cascades for Ψ synthesis establish biocatalytic methods for *C*-nucleoside synthesis. The developed routs meet both the criteria for "green chemistry" and the requirements for industrial implementation.

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P33

Glycomimetics as Selective Ligands for Ligand Directed Protein Profiling of Glycoside Hydrolases

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Glycoside hydrolases together with glycosyl transferases represent the two largest families of so-called carbohydrate-active enzymes (CAZymes) [1,2]. Due to the essential role of glycoside hydrolases in cells, including metabolism, antibacterial defense, and pathogenesis, profiling of the location and amount of active enzyme rather than abundance is essentially important. In this context, ligand directed chemistry (LDC) [3,4] as a variant of well-known activity-based protein profiling (ABPP) [5,6] enables such quantification by covalent chemical modification of a protein of interest (POI). Essential for the LDC probe design is the availability of a selective ligand (A, Fig. 1) for the target protein. The linker region, connecting the ligand (A) and the terminal reporter tag (C), is equipped with a cleavable electrophilic reactive group (B), which enables covalent bond formation between the target enzyme and the reporter tag (C) [3]. As a consequence of the mechanism of LDC and the use of reversible inhibitors as ligands, the labeled protein remains its activity after covalent tagging [3,4].

Here we present the design, synthesis and biological evaluation of glycomimetics based probes for selective profiling of glycoside hydrolases, applying the ligand directed chemistry approach. Experimental details and results of biological activity will be presented.

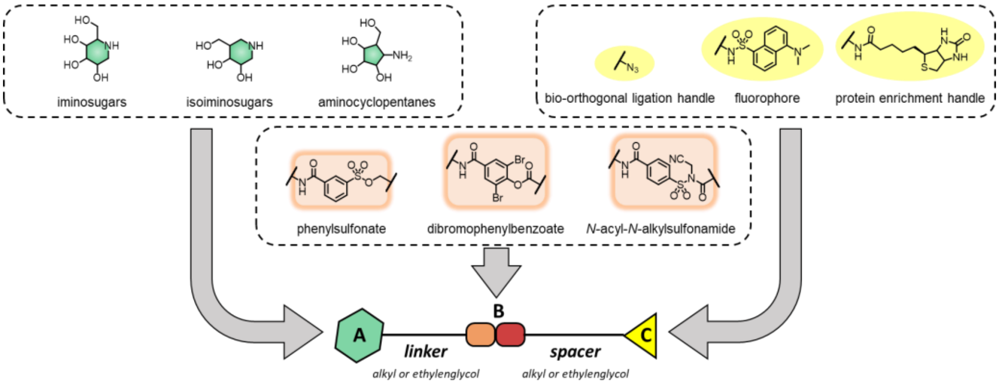

Figure 6: Building block concept for ligand directed chemistry (LDC) probes targeting glycoside hydrolases. (A) reversible inhibitor as ligand; (B) linker with electrophilic reactive group; (C) reporter tag.

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P34

Predictive Power to the Particles

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Production processes involving particles and powders are extremely important in industry – simply because particles are often the product. Examples include pharmaceutics (tablets), or recycled batteries. Smart calibration of computer models (e.g., for the powder's ability to flow) with experimental data is a game changer to increase their predictive power. We present digital workflows that streamline calibration tasks and use machine learning to give true (predictive) power to particle models. We focus on battery recycling processes, in which the cells are grinded to a hard-to-flow granular material that is reactive, cancerogenic and potentially self-igniting. Specifically, we present a novel simulation strategy that uses a specific shape of the parcels (i.e., a computational particle that represents the granular material): tetrapods, which are four rigidly connected spheres placed on a tetrahedral skeleton. Our strategy is computationally efficient (in terms of number of parcels, as well as the requirements on the time step for integration), is able to model interlocking grains in granular materials, and can be applied to a plurality of situations. We show examples of how tetrapods can be used to predict (i) granular flow at different stress levels, as well as (ii) heat conduction within a granular material.

Influence of Perovskite Composition and Crystallization on the Properties of Tin Halide Perovskite Solar Cells

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Perovskite solar cells have made major progress in the last years, reaching efficiencies over 26%. [1] However, the toxicity of lead used in absorber layers poses environmental and health risks. Therefore, research is focused on developing lead-free perovskite materials with comparable performance to their lead-based analogs. Tin is a very good candidate for this replacement, as it is significantly less toxic, and Sn^{2+} shows similar electronic properties to Pb^{2+}. Tin halide perovskite based solar cells already achieve efficiencies around 15%. [2-4]

However, tin halide perovskites not only show good potential, they also come with some new challenges, such as the oxidation of Sn^{2+} to Sn^{4+} and limited environmental stability. Here, we place our focus on the material composition and the crystallization step of the tin perovskite films. The crystallization step is influenced by the spin coating parameters and an antisolvent treatment, and is important for photovoltaic performance of tin perovskite absorbers. Thus, we explore alternative antisolvents as our main focus. Initial results using the green antisolvent diethyl carbonate show promise in terms of perovskite film morphology and photovoltaic properties. Using diethyl carbonate is therefore a potential alternative to conventional antisolvents like chlorobenzene. This contributes to a greener manufacturing process for tin-based perovskite solar cells. In addition, well-suited antisolvents might improve the crystallinity of the perovskite grains, which would positively impact their environmental stability. In addition, we studied and optimized the interface of the perovskite film to the hole transport layer. Through tailoring the chemical composition of the 2D/3D tin perovskite absorber we were able to achieve power conversion efficiencies above 12%. Moreover, our solar cells show promising stability under operational conditions at the maximum power point and one sun illumination under inert conditions. Our results contribute to overcome the challenges of lead-free perovskite solar cells, marking a step forward in the development of a more sustainable solar energy conversion technology.

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P36

Exploiting retro oxa-Michael chemistry in polymers

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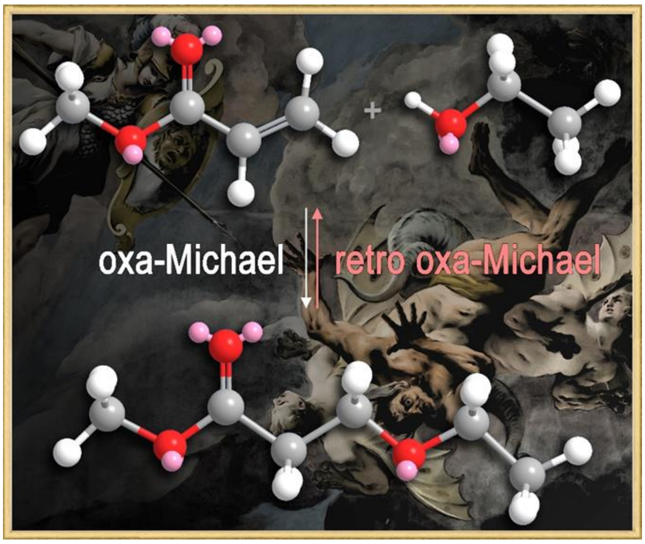
One way to obtain recyclable polymeric materials is to include reversible bonds in polymers. Herein, we study the reversibility of the oxa-Michael reaction and explore its scope and limitations in simple model systems and further in linear polymers as well as in polymer networks. The results show that the retro oxa-Michael reaction of sulfone, acrylate or acrylonitrile based adducts is considerably fast at elevated temperatures (>100 °C) if Brønsted bases (e.g. KOH) are used as catalysts. Under these conditions, alcohols can easily be exchanged in oxa-Michael adducts within minutes. Furthermore, poly(ether)s derived from oxa-Michael reactions can be depolymerized into small fragments in the presence of alcohols and show self-healing characteristics in networks.[1]



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LPMOs as tools for signal peptide evaluation in *Pichia pastoris*

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An advantage of using *Pichia pastoris* as the host for protein production is the organism's ability to secrete high yields of recombinant protein while hardly secreting endogenous proteins. To export proteins to the extracellular environment, leading sequences, known as signal peptides (SP), are fused to the N-terminus of the to-be-secreted target protein. For protein production using *P. pastoris*, the secretion signal of the α-factor mating pheromone (MFα-SP) from *S. cerevisiae* is the gold standard. Unfortunately, the processing, *i.e.* cleavage, of the MFα-SP by the STE13 protease is often incomplete, resulting in a Glu-Ala overhang at the N-terminus of the secreted protein. This circumstance is problematic for various proteins, *e.g.,* antibodies, that require a perfectly processed N-terminus for their activity. Hence, SPs combining good secretion capacities and perfect processing are highly desirable.

A perfect new model for evaluating alternative SPs concerning N-terminal processing and secretion efficiency in *P. pastoris* is *Nc*AA9C, a cellulose-degrading lytic polysaccharide monooxygenase [2]. This is because of the active site architecture of LPMOs in which the N-terminal histidine is needed to coordinate the catalytic copper ion [3]. Thus, incomplete processing of the SP will result in secreted enzymes that are catalytically inactive, which suggests in return, that if enzyme activity is observed, the SP has been cleaved correctly [4], [5].

Here, we present our approach for assessing SP processing in *P. pastoris* by using the cellulose oxidation activity of *Nc*AA9C in an agar plate-based high throughput assay.

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P38

A robust fuel cell operating on lunar water derived fuels

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The space-proven fuel cell technology will play a crucial role in the forthcoming expansion of human presence into the solar system. Hydrogen fuel and oxygen can be derived from lunar water, which is believed to be trapped in permanently shadowed craters at the lunar poles. However, compounds such as H_2S are detected alongside the water ice [1], and these impurities are known to cause irreversible damage to the platinum catalyst and other components of the membrane electrode assembly (MEA) [2]. This research aimed to explore if fuel cell robustness could be enhanced by improving the platinum utilization using optimized electrode manufacturing processes. A comprehensive comparison of different electrode manufacturing methods [3] identified electrospinning as a promising technique that produces electrodes with superior platinum utilization. Electrospinning creates fibres at the nanometre scale, resulting in a nanofiber electrode with an extremely high porosity.

MEAs featuring these innovative electrospun electrodes with a platinum loading of 0.3 $mg_{Pt}\cdot cm^2$ were compared to MEAs made by ultrasonic spray coating, a common manufacturing method considered the laboratory standard. Performance evaluations showed that ultrasonic spray-coated MEAs achieved a maximum performance of 260 $mW\cdot cm^2$. In contrast, the nanofiber MEAs exhibited a maximum performance of 455 $mW\cdot cm^2$ under similar conditions, indicating a 75% increase in platinum utilization.

Subsequently, sulphur contamination tests were conducted for both the anode and cathode, as the water-splitting process transfers contamination to both hydrogen and oxygen. These tests were performed under galvanostatic conditions with an H_2S concentration of 2 ppm. Cathodic contamination trials revealed that electrospun MEAs operated for longer durations under all tested conditions. At a current density of 0.4 $A\cdot cm^2$, the nanofiber MEA took 62 minutes to fall below the cut-off voltage of 200 mV, whereas the ultrasonic MEA reached this voltage in just 29 minutes. However, anodic contamination trials painted a different picture. The nanofiber MEA required 40 minutes to reach the cut-off voltage, while the ultrasonic MEA took 320 minutes. These findings suggest that platinum utilization is not the sole factor in optimizing fuel cell resilience against contamination. At the anode, where H_2 competes with H_2S, the diffusion properties of both molecules become crucial. Less porous electrodes are more resilient because the smaller H_2 molecules are less hindered by the electrode's bulk compared to the larger H_2S molecules.

These experiments underscore the complexity of enhancing fuel cell robustness in the presence of contaminants, indicating that a multifaceted approach considering both platinum utilization and electrode porosity is essential for advancing fuel cell technology for lunar applications.

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P39

Phosphanyl-Supported Low-Valent Compounds of the Heavier Tetrels: An Experimental and Computational Study on Formation and Reactivity

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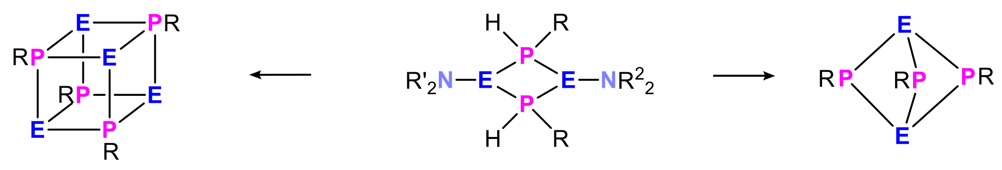
In conjunction with Lappert’s landmark syntheses of amide based ditetrylenes $[((Me_3Si)_2N)_2E]_2$ (E = Ge, Sn, Pb),[1a,b] the isolation of the first stable N-heterocyclic carbene (NHC) by Arduengo during the 1990s is commonly acknowledged as a pivotal achievement in the investigation of main group compounds in nontraditional bonding environments.[2] Currently, a wide range of nitrogen-substituted derivatives is documented in literature,[3] while the number of phosphatetrylenes is limited to just a handful of compounds[4a-d] and examples of heteroleptic N/P-substituted representatives are even more scarce.[5a,b] Herein, we present the isolation of the dimeric amino-phosphino tetrylenes $[(\mu 2\text{-}R(H)P)E(NR')]_2$ (E = Sn, Pb; R = $C(SiMe_3)_3$; R’ = Et, $SiMe_3$), including the first plumbylene of this kind. The remaining amide, as well as the hydrogen function within the molecule allowed follow-up reactions, which led to the formation of heteronuclear cubanes $[(RP)E]_4$ and propellanes $[(RP)_2E_2]$ with heavier atoms at the bridgehead positions. Experimental results, including crystallographic and spectroscopic data will be presented. Additionally, computational data will aid in providing a more comprehensive understanding of the reaction pathway leading to the isolated products.



E = Sn, Pb; R = $C(SiMe_3)_3$; R' = Et, $SiMe_3$

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Innovative approaches to the manufacture of membrane electrode assemblies

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Fuel cells offer a promising solution for reducing greenhouse gas emissions, as they convert chemical energy into electrical energy high efficiently through electrochemical reactions. The heart of the fuel cell is the membrane electrode assembly (MEA), comprised of two electrodes (anode and cathode) and a membrane responsible for ion transport. Various methods, including slot die coating, ultrasonic spray coating, or electrospinning, are utilized in the manufacturing of electrodes or MEAs. However, optimizing electrodes and achieving straightforward large-scale production of MEAs with high performance and long-term stability are crucial to making fuel cells appealing for the global market. [1]

Three innovative approaches were pursued to pave the way for the production of high-performance MEAs. Firstly, we focused on examining the influence of electrode layer thickness produced with ultrasonic spray coating on performance and resistance [2, 3]. The findings indicate that mass transport resistance increases, while electrolyte resistance decreases with thicker catalyst layers. This latter phenomenon is attributed to increased water retention. [2, 3] Secondly, we directed our attention towards the electrode deposition method, recognizing it as another critical challenge in electrode fabrication that significantly affects fuel cell performance. [4] We successfully investigated the influence of various electrode deposition methods of N-rGO-based catalyst inks, which were ultrasonic spray coating, drop coating, brush coating, and roll coating, on the morphology and hydrophobicity, thereby affecting performance. Notably, the restacking of graphene sheets emerged as a major influencing factor. Furthermore, integrating graphene derivatives into the MEA led to a doubling of long-term durability compared to commercial MEAs. [4] Finally, the focus was on the storage of catalyst inks for the optimization of large-scale MEA manufacturing. [5] It was observed that the ink should be re-dispersed prior to every use after longer periods of storage, as the catalyst particles sediment in the ink. Additionally, it became evident that acetone is produced as an oxidation product, which affects both the electrochemically active surface area and the pore structure of the electrode. Fuel cell measurements have demonstrated that catalyst ink for MEA production can be stored for four weeks in cool dark conditions without significant chemical decomposition of the components and subsequent significant performance losses. [5]

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Effect on Paper Properties by Adding and Removing Xylan from Chemical Pulp

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Xylan, one of the most abundant renewable polymers, is found primarily in the secondary cell wall, which gives plants their support and strength. To take advantage of the reinforcing effect of xylan in papermaking, it is essential to understand the role of xylan in pulp fibers, as it undergoes substantial changes during pulping. Removal and addition of xylan from pulps have been extensively investigated, but few studies have explored the combined effect of xylan adsorption and degradation at the same time, leaving the role of xylan on pulp and paper properties unclear. To address this knowledge gap, we investigated the role of xylan in pulp and paper sheets by (i) partially degrading xylan from refined elemental chlorine free bleached softwood kraft pulp (BSKP) using an endo-β-xylanase and by (ii) adsorbing beechwood xylan onto refined BSKP. Adsorption of 3% xylan resulted in improved fiber-fiber bonding concomitant with an increase in sheet density and a slight increase in mechanical performance. Enzymatic degradation of about 1% xylan from BSKP resulted in lower bonded areas and decreased sheet densities. The enzymatic degradation led to a significant weakening of the fiber network in the hand sheets, which cannot be regained by adding xylan to the pulp fibers. These results show that the xylan location in pulps is critical for the resulting properties of paper hand sheets.

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Charge carrier dynamics of the mixed conducting interphase in all-solid-state batteries: lithiated $Li_{1.3}Al_{0.3}Ti_{1.7}(PO_4)_3$ as a case study

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All-solid-state batteries relying on Li metal as negative electrode material and a ceramic electrolyte may severely suffer from unwanted interfacial processes. Here, $Li_{1.3}Al_{0.3}Ti_{1.7}(PO_4)_3$ (LATP) serve as a model electrolyte [1] which is known to form an ionic-electronic, that is, mixed conducting interphase (MCI) when in contact with metallic Li or any other Li source. $Li_{1.3+x}Al_{0.3}Ti_{1.7}(PO_4)_3$ with $x = 0.2$, 0.6 and 1.3 is prepared via ex situ chemical lithiation to mimic the formation of MCIs taking place otherwise *operando* (see Figure 1). The preparation of large amounts of lithiated LATP with controlled Li contents allowed us to use nuclear and electric techniques to study local structures and ionic/electronic dynamics in detail [2]. The results point to the formation of a core-shell two-phase morphology with the Li-rich $Li_3Al_{0.3}Ti_{1.7}(PO_4)_3$ phase covering the non-lithiated Li-poor regions. The originally poor electronic conductivity σ_{eon} of 6.5×10^{-12} S cm^{-1} (293 K) increases by ≈3 orders of magnitude, hence reaching the order of 6.6×10^{-9} S cm^{-1} for $x = 0.6$. At even higher loadings ($x = 1.3$), a decrease in conductivity is seen, i.e., not exceeding alarming values for σ_{eon}. Quantifying electronic and ionic transport processes will help assessing the extent of damage through MCI formation and discussing whether any strategies to mitigate such formation is necessary at all.

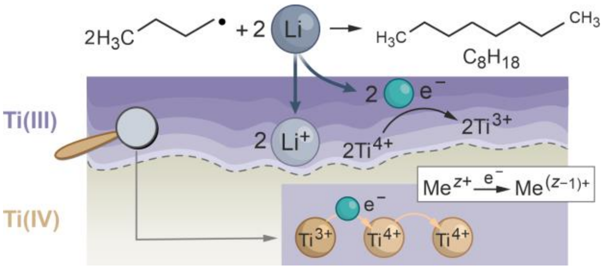

Figure 1 | Illustrating the formation of an MCI at the LATP surface that propagates into the interior of the electrolyte, drawing according to that introduced by Janek and Zeier [3]. The MCI forms when LATP is in contact with metallic Li as shown. Bottom: Illustration of the same formation process but caused by the reaction with n-butyllithium, as it is used in this work

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Aryl Terminated Germanium Nanoparticles: Preparation and Properties

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Due to low toxicity, semi conductivity and optical absorption, numerous applications for germanium nanoparticles have been established.[1,2] While dehydrogenative coupling is an established process for the polymerization of tin hydrides, it has seen no utilization for germanium derivatives so far. Here we present a process for the dehydrogenative coupling of arylgermanium trihydrides to produce aryl decorated germanium nanoparticles (Scheme 1).

$$\mathrm{RGeH_3} \xrightarrow[\mathrm{-H_2,\ -RH}]{\text{Diglyme, }\Delta T} \mathrm{Ge@R}$$

Scheme 1: Dehydrocoupling of the starting material to nanoparticles

During this reaction, both hydrogen and aryl residues are cleaved off. The resulting formation of new germanium-germanium bonds leads to increasing catenation, observable as deepening coloration of the reaction mixture (Figure 1).

0 h 4 h 12 h 24 h 72 h

Figure 7: Development of the color over the course of the reaction

Insights into the reaction mechanism will be presented as well as the possibility to shape the properties of the nanoparticles. Analytical strategies for the characterization of the material include UV/Vis, TOF-MS, GPC, TEM and ^{1}H-NMR, among many others.

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Synthesis of porous metal sulfides and titania/metal sulfide photocatalysts

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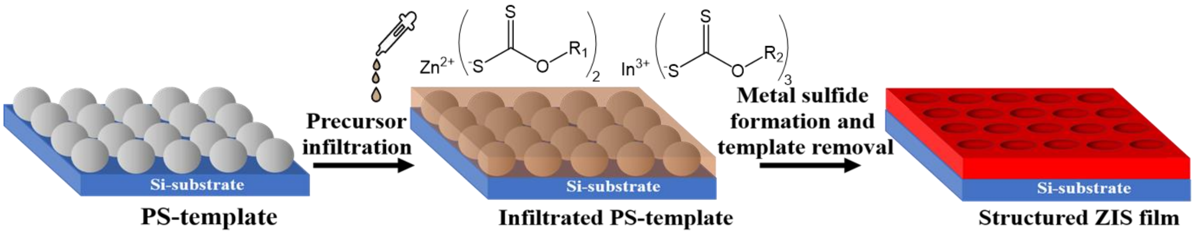
Porous metal sulfides and titania/metal sulfides are an interesting class of materials in photocatalysis. Within the lead project Porous Materials@Work for Sustainability we investigate metal xanthates as precursors for metal sulfides. In a microsphere colloidal lithography approach using monodisperse polystyrene spheres, we successfully prepared hierarchically porous zinc indium sulfide thin films with macropores in the 300 nm regime with microspores around 1.6 nm. Furthermore, we performed photocatalytic dye degradation tests with Rhodamine B to confirm a 3.3-fold increase in specific catalytic activity compared to a bulk film. These results suggest promise to optimize photocatalytic activity via multiscale porosity.

In a second approach, we modified mesoporous titania films with a thin layer of nickel sulfide using nickel xanthates as single-source precursors. Xanthates provide a simple method to prepare homogeneous metal sulfide films with tunable stoichiometry, phase, and morphology, depending on the xanthate's ligand. We prepared and characterized a range of different nickel xanthates, and evaluated the photocatalytic performance of our modified catalysts using hydrogen evolution experiments with methanol as sacrificial electron donor. The first hydrogen evolution experiments demonstrated a significant increase of 90 times the efficiency for the NiS modified films, compared to the pristine titania.

This work was financially supported by the TU Graz lead project Porous Materials@Work for Sustainability.



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Automating chemical process optimization

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Chemical processes have conventionally been optimized by using manual and iterative laboratory work. Research chemists will usually vary one variable at a time until ideal conditions have been found. More recently however, the use of complex strategies for process optimization, such as the design of experiments (DoE) approach, as well as algorithms from the field of mathematical optimization, have been described in literature. The combination of such optimization strategies with automated laboratory equipment has allowed for self-optimizing chemical systems. The algorithmic control of these continuous setups allows for autonomous closed-loop execution of experiments without the need of human input. Its goal is to find the ideal set of parameters to maximize a desired process step outcome, such as the yield of chemical reactions.

In this project, we aim the utilization of these new technologies in the field of chemical engineering via the use of automation and advanced optimization strategies. Its scope includes automated continuous flow reactions with discrete variables employing the DoE system[1] and the benchmarking of currently unused optimization algorithms against current literature via in-silico and real-life optimization problems[2]. As such, a Suzuki-Miyaura coupling reaction was optimized with the setup seen in Figure 8. Present focus lies on expanding the self-optimization field from its main area of reaction optimization onto further chemical processes, such as continuous crystallization.

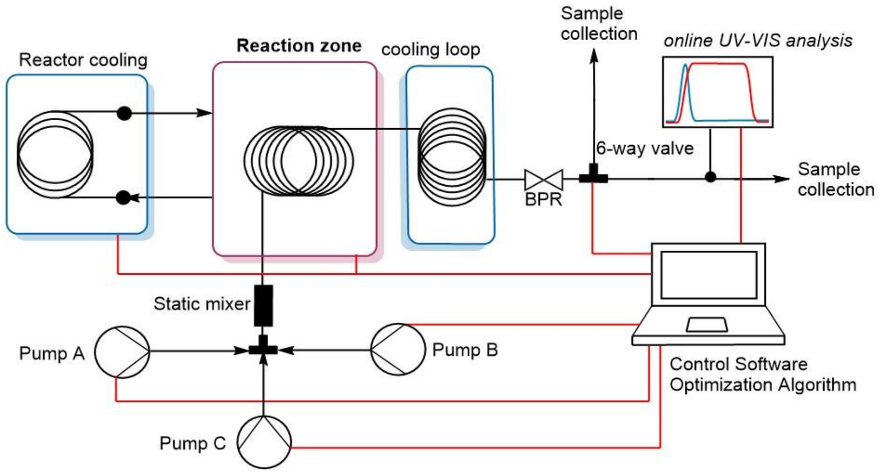

Figure 8: Automated reaction setup for performing self-optimized experiments in continuous flow

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P46

A remarkable influence on biological behaviours of isofagomine induced by suitable N-modifications

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Isoiminosugars (**A**) are glycomimetics in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group. [1] This class of compounds is highly biologically active. For example, these structures provide outstanding selectivity and inhibition potency towards respective carbohydrate processing enzymes connected to various diseases such as Alzheimer´s [2], Parkinsonism [3], HIV [4] or lysosomal storage diseases [5]. Consequently, broad interest in investigations towards such compounds has been gained since their discovery in 1994 [6]. Despite great synthetic efforts introduced from dedicated leaders in this field, the organic and/or chemoenzymatic synthesis of isoiminosugars still remains challenging.

Herein, we present an alternative and elegant synthetic approach towards powerful β–glucosidase inhibitor isofagomine, clearly targeting access to sufficient bench-amounts of these highly desired compound. Suitable derivatizations of the ring nitrogen provided a set of N-modified isofagomine analogues. Biological evaluations of these compounds showed a remarkable change in potency as well as α/β-preference for various glycosidases from different sources when compared to the parent compound isofagomine. [7] Herein, synthetic details and biological data will be presented.

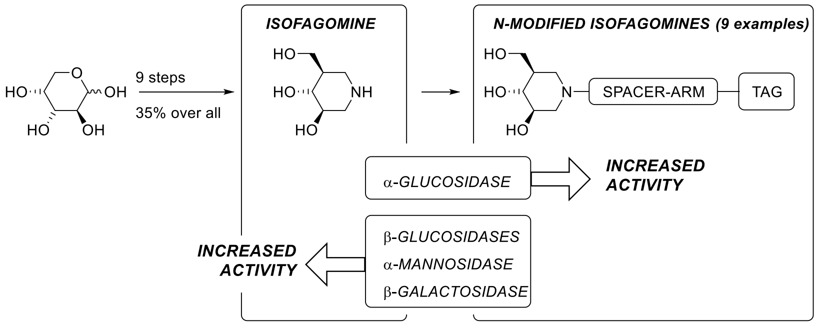

Figure: Schematic depiction of the change in potency as well as α/β-preference for various glycosidases of inhibitor isofagomine provided by suitable N-modifications of this prominent parent compound.

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Trapping of cyclohexynes via cycloaddition reactions

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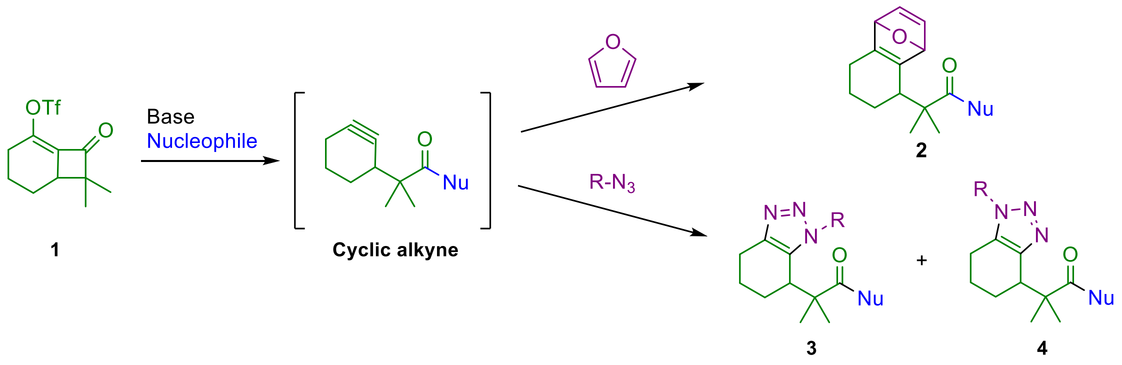
Small rings containing triple bonds have been the subject of investigation for over a century by various research groups.[1] The existence of benzyne was first reported in 1902 and was conclusively validated in 1953 by Roberts.[2] Currently, benzyne is utilized in a variety of synthetic applications. Apart from benzyne, cyclooctyne is arguably the most extensively studied strained alkyne, particularly noted for its applications in bioorthogonal chemistry.[3] In contrast, reactions involving cyclohexyne as a synthetically versatile intermediate have been significantly underrepresented in the literature until today.

To utilize the unique properties of these small strained ring systems, efforts were made to develop a synthesis for a precursor compound (**1**) that can generate the cyclic alkyne *in situ* and subsequently undergo a variety of selective chemical transformations. In this project, we report the nucleophile-induced ring-opening of bicyclic molecules, based on the bicyclo-[4.2.0]-octane structure, as a novel synthetic approach for the synthesis of cyclohexyne-structured compounds. Different nucleophiles, for example benzyl alcohol as well as a biologically interesting derivative of 5-hydroxymethylcytosine are employed to induce the fragmentation of the precursor compound, which generates the respective cyclic alkyne. In initial studies to investigate the formation of the cyclic alkyne as well as its synthetic application in complex ring-formation reactions, the reactive species is subsequently trapped with furan in a [4+2]-cycloaddition and with different azides *via* a strain-promoted azide-alkyne click reaction.



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Concise and efficient access to isoiminosugars and beyond

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Isoiminosugars (**A**) are glycomimetics in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group [1]. In general, these compounds are selective and highly potent inhibitors of glycoside hydrolases [2-4]. Moreover, advanced derivatives of this compound class, e.g. *C*-5a-chain extended entities (**2**) of 4-*epi*-isofagomine (4-*epi*-IFG, **1**) have been proven as highly potent pharmacological chaperones for the treatment of G_{M1} gangliosidosis [2,3]. Synthetic strategies towards these compounds reported so far [2-4] still remain complex and most importantly lack in the possibility for up-scaling.

In context with our interest in the design and synthesis of such structures, we have found an efficient and concise synthetic approach towards isoiminosugars (**A**). [5] This strategy takes advantage of a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated glycopyranoses (**I**) as key step leading to corresponding C-2 carbon chain branched glycofuranosides (**II**) [6]. We applied this ring contraction for the synthesis of isoiminosugars (**A**). Employing different configurations of **I** and variations in the reaction sequence open the avenue to various modifications in the substitution pattern. Herein, synthetic and mechanistic details as well as the scope and limitations of this approach will be presented.

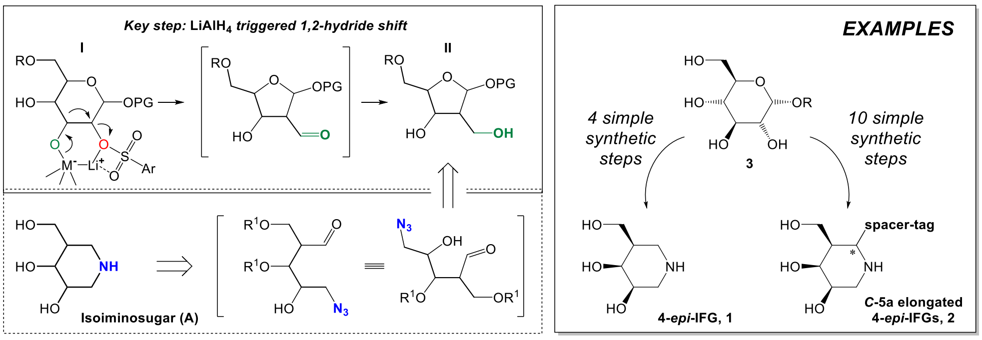

Figure: Schematic overview of a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated pyranosides (**I**) as key-step in our general (retro-) synthetic concept for isoiminosugars (**A**) (left), as well as its demonstration in a 4 step synthesis of 4-epi-isofagomine (**1**) and according conversions to *C*-5a elongated derivatives (**2**) starting from common α-D-glucopyranosides (**3**) respectively (right).

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Advancing solutions for the implementation of biocatalysts in continuous flow

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Biocatalysis has been widely recognized as a green alternative to conventional catalysts in synthetic chemistry. Enzymes have in fact many advantages, such as low toxicity and environmental impact, high activity in mild conditions, and high selectivity. However, still a lot of work needs to be done to increase the stability of biocatalysts and to design continuous flow biocatalytic processes that can be industrially competitive.

The aim of this project [1] is to design continuous, stable and affordable biocatalytic processes for a more sustainable synthesis of active pharmaceutical ingredients (APIs). In order to achieve this goal, supports for enzyme immobilization were designed in order to maximize the surface area available for the reaction and also to be easily fitted and interchanged into HPLC column used as packed bed reactors. The designs were then printed out of ceramics via vat photopolymerization (VPP). The flow behavior inside these inserts was determined with residence time distribution (RTD) experiments enabled by in-house designed and 3D printed inline photometric flow cells. As a proof of concept, these structured inserts were fitted in an HPLC column to serve as solid inorganic supports for the immobilization of the enzyme Phenolic acid Decarboxylase (*bs*PAD), which catalyzes the decarboxylation of cinnamic acids. The conversion of coumaric acid to vinylphenol was chosen as a model system to prove the implementation of these engineered inserts in a continuous biocatalytic application with high product yield and process stability. To increase the level of process understanding, an automated reaction setup was designed and controlled by a Python based algorithm with an integrated Graphic User Interface (GUI). The algorithm was devised to perform a Design of Experiments (DoE) evaluation in order to quickly identify the optimal operating conditions and maximize the space-time-yield. With the designed process it was possible to achieve a space-time-yield (STY) of 39.21 g product/L·h and high process stability. Thus, we could demonstrate that the designed 3D printed supports can be successfully employed as enzyme carriers in continuous flow, making them particularly interesting for industrial applications.

To conclude, with this project we expect to contribute to the implementation of enzymes as powerful and affordable catalysts for the production of fine chemicals on industrial scale.

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Mass Spectrometry-based techniques in Integrative Structural Biology

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While conventional structural techniques such as X-ray crystallography and NMR have allowed the structural elucidation of important protein complexes over the past decades, they frequently cannot deal with dynamic, heterogeneous and very large assemblies. While cryo-EM promises to alleviate some of the challenges, it also has limitations as far as sample preparation requirements and tolerated conformational dynamics are concerned. Additional promising and complementary methodologies for the field of integrative structural biology are mass spectrometry (MS)-based techniques. Recent advances have shown that a range of MS approaches are able to provide information with respect to conformational dynamics (Hydrogen-Deuterium eXchange coupled to MS - HDX-MS) on native complexes or to study the stoichiometry and connectivity of entire assemblies (native MS) rapidly, reliably, and from small amounts of substrate. Here we showcase some of these techniques that are available for testing and application to your projects at the Institute of Biochemistry, Graz University of Technology.

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Production of Functional Cello-oligosaccharide Materials Enabled by Applied Biocatalysis

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Cello-oligosaccharides (cellodextrins) are linear β-1,4-gluco-oligosaccharides with substantial potential for applications in the structural polymers and materials. Conventional top-down approaches for producing cellodextrins encounter significant limitations in efficiency, selectivity, and flexibility. Conversely, bottom-up enzymatic methods offer enhanced precision in structural control and enable multiple functionalization possibilities that are largely inaccessible via top-down processing.[1,2]

Cellodextrin phosphorylase (CdP, EC 2.4.1.49), a carbohydrate-active enzyme belonging to glycoside hydrolase family 94, catalyses the iterative β-1,4-glycosylation of α-D-glucose 1-phosphate to elongate various acceptors, including cellobiose and a spectrum of synthetic glycosides with non-sugar aglycons.[1,2] The iterative glycosylation process results in products with varying degrees of polymerization (DP), yielding either soluble cello-oligosaccharides or insoluble cellulosic materials.[3] Here, we present a summary utilizing CdP as biocatalyst for the bottom-up synthesis of functional cello-oligosaccharide materials. The soluble materials as-synthesised (DP $\leq$ 6) demonstrated a selective prebiotic effect.[4] Oligomer chains with DP $\geq$ 7 formed as insoluble materials exhibiting sheet-like crystalline structures of cellulose allomorph II. In the presence of biopolymers or ions, polymerization-induced gelation was attained,[5] facilitating the in-situ encapsulation of cells or biomolecules into hydrogels, which are of significant interest for applications in tissue engineering and sensor development. Meanwhile, the broad acceptor scope for polymerization also enables reducing-end modification of cellulose chains, which offers possibility to further functionalize cellulose materials.[6] Overall, the cello-oligosaccharides obtained via bottom-up synthesis catalysed by CdP expand the cellulose applications towards product classes and types that are difficult to obtain via top-down processing of natural materials.

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Deciphering Epistatic Effects & Context Dependency in Enzyme Evolution of Borneol-type Dehydrogenases

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The exceptional stereoselectivity of enzymes is a key feature for industrial applications, enabling the design of promising biocatalyst-based synthesis routes for enantiopure compounds. While directed evolution and rational design are powerful tools for the artificial improvement of stereoselectivity [1], it remains unclear how Nature evolved stereoselectivity within enzyme classes. As such, enzymes within the family of plant borneol-type dehydrogenases (BDHs) display striking differences in enantioselectivity towards borneol enantiomers despite close relations [2,3]. Mimicking Nature, we investigated active site and peripheral mutations' impact on BDH selectivity, to rationalize natural enzyme evolution. In this context, ancestral sequence reconstruction (ASR) acts as a guide to construct a plausible evolutionary pathway of BDH towards the enantioselective BDH1 from *Salvia rosmarinus* [2]. Common ancestors of selective and non-selective dehydrogenases were inferred and key mutations contributing to their stepwise diversification were identified. Ancestral enzymes were recombinantly produced, biochemically characterized, and their structures predicted. Consecutively, guided by *de-novo* protein structure prediction, selected residues were substituted to elucidate the structural basis of substrate targeting. This work highlights the importance of context-dependent evolutionary changes in enzymes. It exemplifies how applying computationally guided enzyme engineering tools lead to a deeper understanding of natural evolutionary principles in less investigated enzyme classes.

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