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POSTER

Role of c-di-GMP as a Regulator for the Production of Natural Compounds in Streptomyces

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The secondary messenger cyclic-dimeric-Guanosinmonophosphate (c-di-GMP) is known to play an important role in the regulation of several different pathways including motility, virulence, growth and development of Gram-negative bacteria. In Gram-positive bacteria on the other hand, the regulation pathways are only beginning to be elucidated (1). The research in Streptomyces focused on the influences on the morphological development, but there were also evidences that the production of natural products is under the control of the c-di-GMP level.
We are investigating the role of c-di-GMP in the regulation of biosynthetic gene clusters in Streptomyces. This work focuses on the strain Streptomyces sp. Tu6071. The strain is known for producing Phenalinolactones, but was not yet analyzed for effects of c-di-GMP. Using the Redirect® Technology we performed knockouts of the genes STTU_RS08525 and STTU_RS21020, encoding c-di-GMP metabolizing enzymes. These proteins have high sequence identities with RmdB and CdgA from the Streptomyces model organism for c-di-GMP studies, Streptomyces coelicolor.
Through a knockout of either a c-di-GMP synthesizing enzyme (diguanylate cyclase) or a c-di-GMP degrading enzyme (phosphodiesterase) we wanted to change the intracellular level of c-di-GMP and consequently investigate the influence on the production of new natural products. In previous investigations, in different Streptomyces strains, this led to an increasing amount of natural products and also activated previously silent gene clusters (2). However, some of the knockouts diminished production of natural products almost completely (3).
Preliminary results of the secondary metabolites profile will be presented.

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mRNA containing non-canonical bases: *In vitro* transcription, translatability, and intracellular processing

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The use of N^1-Methylpseudouridine for *in vitro* transcription of both currently approved COVID-19 vaccines has proven crucial for effectiveness,[1] conferring a multitude of beneficial characteristics to the mRNA.[2,3] Among the few other nucleosides that have been characterised with regard to their influence on translation and cellular metabolism, most occur physiologically.[4] Data on other nucleosides—especially ones bearing non-canonical base moieties—has been lacking.[5]

In collaboration with the Andexer and Jessen groups, we have established a highly adaptable system for the triphosphorylation of modified nucleosides, and investigated their respective effects on translational recoding *in vitro* using dual luciferase assays. While this method is robust and adaptable,[6] it is less suited for studies of mRNA-dynamics *in cellulo*, leading us to develop a new, EGFP-based reporter vector suitable for transcription with recombinant T7 RNA polymerase. After validation of transcribability and translatability *in vitro,* mRNA was produced, containing either 7-Deazaadenosine (7-Deaza-A), 2-Fluoroadenosine (2-Fluoro-A), or N^1-Methylpseudouridine. Incorporation of these nucleosides was verified by LC-ESI-MS/MS analysis. After liposome-mediated transfection of the mRNA into two human cell lines, EGFP expression was confirmed in all cases by fluorescence microscopy as well as western blotting. Interestingly, analysis of gDNA isolated from cells 48 h post transfection revealed incorporation of 7-Deaza-A and 2-Fluoro-A, suggesting their ability to enter salvage pathways upon intracellular metabolism of the administered mRNA.

Our results highlight the potential for further optimisation of mRNA-based therapy by incorporation of non-canonical bases, while concurrently pointing towards the need for stringent monitoring of their intracellular metabolism and potential downstream effects.

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SAM regeneration goes radical

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S-adenoslymethionine (SAM) is a highly versatile cofactor used by methyltransferases, amino(carboxy)propyltransferases and radical SAM enzymes. Hence, it is involved in a wide range of reactions with polar or radical mechanisms.[1] However, the instability and high cost of SAM and byproduct inhibition impedes the usage of SAM-dependent enzymes.[2] Therefore, *in situ* SAM regeneration systems have been developed. These systems are highly effective and are able to support up to 500 turnovers with catalytic amounts of cofactor.[3–5] Nevertheless, in all systems SAM regeneration relies on *S*-adenosylhomocysteine (SAH), the byproduct of methyltransferase reactions. Amino(carboxy)propyltransferases and radical SAM enzymes are not supported as they produce 5'-methylthioadenosine (MTA) and 5'-deoxyadenosine as byproducts, respectively.

The goal of the work presented was to develop a versatile SAM regeneration system that supports the vast catalytic spectrum of methyltransferases, amino(carboxy)propyltransferases and radical SAM enzymes. The system relies on byproduct cleavage by MTA/SAH nucleosidase and a polyphosphate-fuelled biomimetic production of phopshoribose pyrophosphate. We could show that methyltransferases, aminopropyltransferases and radical SAM enzymes are functional in this system, supporting up to 80, 57 and 29 turnovers, respectively.

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Relaxation of Asymmetry Stress through Vesicle Budding

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Lipid membranes are an essential component of all life. The lipid membrane undergoes continuous and dynamic changes in a multitude of cellular processes, such as exo- and endovesiculation, asexual reproduction or the forming of organelles. Although various protein-centered mechanisms have been identified to induce curvature changes, the mechanical properties of the lipid bilayer cannot be neglected when talking about membrane remodeling processes. For example, an overpopulation of one leaflet leads to area asymmetry of the inner and outer leaflet, ultimately creating spontaneous curvature. How does the membrane deal with area asymmetry stress? To answer this question, lysolipids can be used to induce asymmetry stress. Dissolved or dispersed lysolipids in the outer medium insert into the accessible membrane leaflet and expand its area, but show only very slow translocation to the trans leaflet. This resulting asymmetry stress is partially relaxed by vesicle budding even in the absence of proteins and contributes to the multiple biological functions of lysolipids and other membrane-impermeant amphiphiles.
Using Asymmetric Flow Field Flow Fractionation (AF4), the extent of vesicles budded from liposomes as a model membrane can be quantified and assessed. Budding can proceed at sufficient lysolipid concentration until the mother vesicle has become a sphere. In addition, further area can bud off as the mother vesicle is compressed by water outflux. The resulting osmotic pressure in the interior of the liposome limits the budding process. Our studies provide a detailed picture of the lipid-related phenomena and parameters governing membrane remodeling.

ePharmaLib: A Versatile Library of e-Pharmacophores to Address Small-Molecule (Poly-)Pharmacology

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Bioactive compounds oftentimes bind to several target proteins, thereby exhibiting polypharmacology [1]. Experimentally determining these interactions is however laborious, and structure-based virtual screening (SBVS) of bioactive compounds could expedite drug discovery by prioritizing hits for experimental validation. Here, we present ePharmaLib [2], a library of 15,148 e-pharmacophores modeled from solved structures of pharmaceutically relevant protein–ligand complexes of the screening Protein Data Bank (sc-PDB) [3]. ePharmaLib can be used for target fishing of phenotypic hits, side effect predictions, drug repurposing, and scaffold hopping. In retrospective SBVS with compounds of the Streptomyces natural products database (StreptomeDB) [4], a good balance was obtained between computational efficiency and predictive accuracy. As a proof of concept, we carried out prospective SBVS in conjunction with a photometric assay, which inferred that the mechanism of action of neopterin (an endogenous immunomodulator) putatively stems from its inhibition (IC_{50} = 18 µM) of the human purine nucleoside phosphorylase. This ready-to-use library is freely available at http://www.pharmbioinf.uni-freiburg.de/epharmalib.

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Investigations on Archaeal Geranylgeranyl Reductases for Enzymatic Asymmetric Reduction of Isolated C=C Bonds

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Parallel to the rising demand for renewable raw materials and environmentally friendly processes, asymmetric synthesis has become increasingly important for fine chemistry in the last decades.[1] Both aspects are addressed by biocatalysis, as the catalysts are derived from biological sources and reactions are performed stereoselectively under mild conditions.[2] While biocatalysis has proven to be a valuable alternative to "classical" chemistry, the reduction of isolated C=C bonds remains unadressed, which has encouraged us to investigate this field.
Geranylgeranyl reductases (GGRs) are a promising starting point as they are distributed across different biosynthetic pathways in the three domains of life.[3] The targeted C=C bonds lack neighbouring groups which excludes interfering reactions.[4]

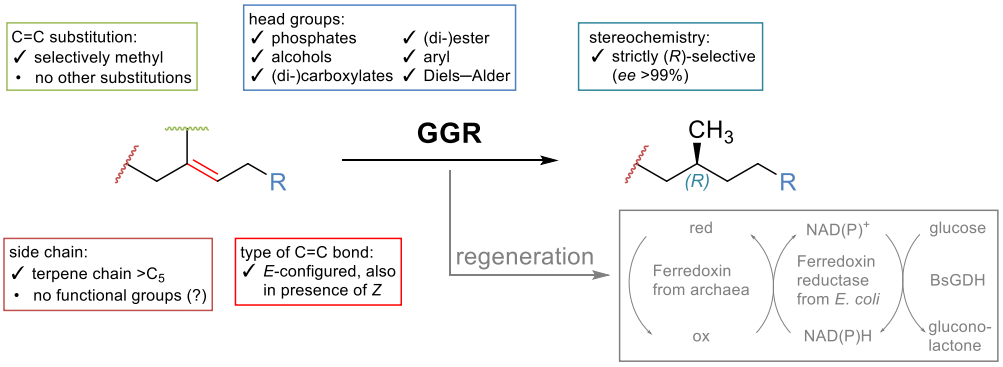

Figure 1. Substrate scope of geranylgeranyl reductases and a new regeneration system which can be coupled with established regeneration systems for NAD(P)H.

We investigated three archaeal GGRs from *S. acidocaldarius*, *A. fulgidus*, and *T. acidophilum* with focus on the substrate scope. GGRs tolerate a variety of different head groups and selectively reduce isolated, methyl-branched, *E*-configured C=C bonds stereoselectively and chemoselectively in presence of other types of C=C bonds.[5] Conversions could be improved by substrate engineering. In addition, we found a regeneration system for one GGR which can be further investigated regarding their use for coupled or *in vivo* reactions. Our results show a possible use of GGRs for asymmetric, chemo- and stereoselective reduction of isolated C=C bonds in methyl-branched olefins.

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Flexible enzymatic alkylation with a four-enzyme-cascade – from smelly thiols to aromatic flavours

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Regio- and stereoselective alkylation of small molecules can impact their physicochemical and pharmaceutical properties.[1,2] Highly selective methyltransferases (MTs) are promising tools for the transfer of alkyl chains onto various substrates.[1,3,4] They require stoichiometric amounts of the instable and expensive cofactor *S*-adenosyl-L-methionine (SAM) for the native methylation reaction and SAM analogues for alkylations. SAM analogues can be synthetised enzymatically from the corresponding L-methionine analogues and ATP by the utilisation of an L-methionine adenosyltransferase (MAT).[5] The analogues are usually chemically generated from L-homocystine and alkyl halogenides followed by purification.[6] Here we explore an alternative approach, following the synthesis of L-ethionine, the simplest L-methionine analogue, with the *O*-acetyl-L-homoserine sulfhydrolase from *S. cerevisiae* (*Sc*MET17). Starting from L-homocysteine and alkyl thiols, different L-methionine analogues could be synthetised. Based on these results we developed a four-enzyme-cascade for selective alkylation. The applicability is demonstrated by the synthesis of alkylated vanillin derivatives from the corresponding alkanthiols and protocatechuic aldehyde.

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Mechanistic insights into the physiological relevant adduct formation of methylglyoxal: Peptides, metabolites and therapeutic drugs

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Methylglyoxal, a highly reactive precursor of advanced glycation endproducts (AGEs), is of significant recent research interest with pathophysiological relevance.[1] These AGEs are not only strongly related to diabetic complications, but they also play a crucial role in age-related diseases.[2–4] Physiologically relevant molecules/drugs containing a guanidine moiety are of specific interest because of their reactivity with the dicarbonyl methylglyoxal. There is a lack of molecular insights into the structure and mechanism of methylglyoxal/guanidine adducts.
We established a peptide model for studying methylglyoxal-derived adducts and their prevention by scavengers. MS/MS peptide analyses provide a tool for identifying and characterising early AGE adducts. In addition, the interplay between short-lifetime cysteine adducts and long-term arginine adducts was investigated.
With the help of the peptide model, differences in the scavenging ability of pharmacologically active molecules with altered guanidine subgroups was observed. Furthermore, we report divergences in the formed methylglyoxal/guanidine adducts comparing different monoguanidines and biguanidines.
These mechanistic and methodological advances enable a better understanding of the formation and prevention of early AGEs with potential relevance for pathophysiology.

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m^6A_m in snRNA is demethylated by FTO in an oxygen-dependent manner

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The Fe^{2+} and 2-OG dependent dioxygenase superfamily is involved in oxygen sensing and DNA/RNA demethylation.[1] As a member of this family, the fat mass and obesity associated protein FTO has been found to demethylate m^6A and m^6A_m in various RNA species.[2] Still, its biological functions and natural substrates are essential areas of epitranscriptomics.[3] Recent cellular research identified m^6A hypermethylation and increased FTO expression as a feature of AML and several solid cancers.[4][5] The hypoxic microenvironment of such tumours may impact the demethylation capabilities of FTO.

For this reason, our research examines how O_2 availability affects m^6A_m demethylation by FTO. We conducted in vitro experiments with recombinant enzyme and isolated total RNA in a hypoxia chamber with controlled O_2 levels. For quantitative RNA modification analysis, we developed an LC-MS/MS method capable of detecting 23 natural occurring nucleosides in the low nM range.

Our results show that at least 1.8% O_2 is required to observe a significant decrease of m^6A_m in total RNA from MCF-7 cells. We confirmed these findings with cell culture experiments in two different cell lines. MCF-7 and HepG2 cells showed a 31% increase in m^6A_m levels in total RNA when exposed to severe hypoxia (0.5% and1% O_2). Together these results imply that RNA demethylation via FTO is hindered by severe hypoxia in solid tumours.

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Biochemical consequences of the clinically relevant mtND1-gene triple mutation in *Escherichia coli* respiratory complex I

NADH:ubiquinone oxidoreductase (respiratory complex I) plays a major role in energy metabolism by coupling electron transfer from NADH to quinone with proton translocation across the membrane. Complex I deficiencies were found to be the most common source of human mitochondrial dysfunction that manifest in a wide variety of neurodegenerative diseases. Seven subunits of human complex I are encoded by mitochondrial DNA (mtDNA) that carry an unexpectedly large number of mutations discovered in mitochondria from patients' tissues. However, whether or how these genetic aberrations affect complex I at a molecular level is mainly unknown. Here, we used *Escherichia coli* as a model system to biochemically characterize a triple mutation that found in mtDNA of patients: D199G/L200K/A201V$^{MT\text{-}ND1}$, of which only D199$^{MT\text{-}ND1}$ is conserved. The triple mutation led to the assembly of a stable but inactive complex in *E. coli*. On the other hand, the single mutation D199G$^{MT\text{-}ND1}$ led to the assembly of a stable complex capable to catalyze redox-driven proton translocation. However, quinone reduction seemed to be perturbed, leading to a diminished activity. Since D199$^{MT\text{-}ND1}$ is part of a cluster of charged amino acid residues that are suggested to be important for the coupling mechanism in complex I, its role for energy conservation in complex I is discussed.

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Functional investigations in *Escherichia coli* complex I

The *Escherichia coli* NADH:ubiquinone oxidoreductase, respiratory complex I, is a key enzyme in cellular energy metabolism. It couples electron transfer from NADH to ubiquinone in its peripheral arm with proton translocation across the membrane and contributes to the protonmotive force. The coupling of these two processes remains, however, elusive [1]. Redox-difference UV/vis spectra of complex I in various redox-states showed the presence of a yet unidentified redox component [2]. This redox-difference spectrum was shown to be similar to that of a quinol anion [3]. Investigation of the quinol anion's re-oxidation kinetics showed a significantly slower reaction in the variant D213G^{H}, associated with a disturbed quinone chemistry [4]. In the presence of the quinone-site inhibitor piericidin A the absorption peaks in the anion's difference spectrum were decreased. A mechanism of proton-coupled electron transfer with the quinol anion as catalytic intermediate is proposed [3, 5].

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Substrate-induced conformational changes in respiratory complex I

Complex I is the largest enzyme in the respiratory chain that catalyzes the transfer of two electrons from NADH to (ubi-)quinone coupled with the translocation of four protons across the membrane [1]. It has an L-shape structure consisting of a peripheral and a membrane arm. Electron transfer takes place in the peripheral arm, whereas the membrane arm catalyses proton translocation [2]. The mechanism of proton translocation by the complex is still under debate. Here, we inserted a small label in close proximity of one of the putative proton channel in the membrane arm. Conformational changes in these positions upon NADH and ubiquinone binding were visulized by surface-enhanced infrared absorption spectroscopy (SEIRAS) [3]. Individual positions were genetically changed to cysteine residues that were labelled with cyanides. Nitrile labels are attractive IR probes as they appear in the clear region of IR spectra and do not overlap with protein signals. These labels are sensitive towards hydrogen bond interactions making it possible to follow reaction-induced conformational changes. The mutations and the labelling resulted in fully assembled variants with a mildly diminished activity. Binding of NADH and ubiquinone led to different spectral shifts. Interestingly, a characteristic pattern was observed labelled residues on the cytoplasmic and periplasmic sides of the proposed channel.

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The role of His224^{CD} for inhibitor binding in *Escherichia coli* complex I

NADH:ubiquinone oxidoreductase, respiratory complex I, couples the electron transfer from NADH to ubiquinone (Q) with the translocation of protons across the membrane. *Escherichia coli* complex I consists of 13 subunits called NuoA to NuoN. Electrons are transferred from NADH *via* FMN through a series of iron-sulfur (FeS) clusters to Q, that is reduced in a unique binding chamber. This chamber is located at the junction between the peripheral and the membrane arm and comprises the hydrophobic subunits NuoA and NuoH and the hydrophilic subunits NuoB and NuoCD. Mutagenesis of complex I from several species showed that His224^{CD} (*E. coli* numbering) is essential for ubiquinone binding [1]. Its role in the binding of piericidin A, a complex I-specific inhibitor, is still under discussion [2]. The Ala/Met/Arg variants of this position in *Yarrowia lipolytica* showed a strongly decreased catalytic activity, while the replacement of the histidine by an arginine residue in *E. coli* led to an enzyme with near wild-type activity [3]. Hence, mutations His224Ala/Met/Arg ^{CD} were introduced into the *E. coli* enzyme and their effect on ubiquinone reduction and piericidin A inhibition were tested. In conclusion, this study shows that His224^{CD} in *E. coli* is indeed involved in binding of ubiquinone as well as of piericidin A.

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Structure of *Escherichia coli* cytochrome *bd*-II type oxidase with bound aurachin D

Cytochrome *bd* quinol:dioxygen oxidases are respiratory terminal reductases so far exclusively found in prokaryotes, including several pathogenic bacteria [1]. They endow pathogens resistance to cellular stressors. The *bd* oxidases transfer two electrons from a quinol to oxygen. This is coupled with the vectorial generation of a proton motive force across the membrane, that is used for the production of ATP. *Escherichia coli* contains two *bd* oxidases of which only the *bd*-I type is structurally characterized [2]. Here, we present the structure of the *E. coli* cytochrome *bd*-II type oxidase with the bound inhibitor aurachin D as obtained by cryo-electron microscopy at 3 Å resolution [3]. The oxidase consists of subunits AppB, C and X that show an architecture similar to the homologous subunits of *bd*-I. The three heme cofactors are found in AppC, while AppB is stabilized by a structural ubiquinone-8 at the homologous positions. Heme b_{595} is exposed to the membrane due to the lack of a fourth subunit but the *d*-heme embedded within the protein is the catalytically active centre. The structure of the Q-loop is fully resolved, revealing the specific binding of aurachin D.

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Cluster N1b of *Escherichia coli* complex I is selectively damaged by hydrogen peroxide

A variety of neuromuscular and neurodegenerative diseases are associated with complex I dysfunctions [1]. It is a popular assumption that reactive oxygen species such as hydrogen peroxide contribute to such dysfunctions. Superoxide for example, is believed to oxidize the iron-sulfur clusters of complex I [2]. Here, we show that incubation of isolated complex I with hydrogen peroxide results in the selective damage of the binuclear cluster N1b at concentrations of 1 mM hydrogen peroxide. However, the activity of complex I is not diminished at this concentration. The NADH:decyl-quinone activity is not inhibited up to a concentration of 1.25 mM hydrogen peroxide. The NADH/ferricyanide oxidoreductase activity and NADH oxidase activity of membranes is inhibited by hydrogen peroxide with an IC_{50} of 14 mM. However, these concentrations are not of physiological importance, as the intracellular hydrogen peroxide concentration in *Escherichia coli* is generally in the nanomolar range [3]. Furthermore, loss of cluster N1b did not influence tunneling rates that are still fast enough to support rapid catalytic turnover [4,5]. This leads to the conclusion that selective damage of cluster N1b by reactive oxygen species does not lead to complex I dysfunction.

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The Three-dimensional Structure of Fe-only nitrogenase

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Nitrogenase are two-component iron-sulfur enzymes that convert atmospheric dinitrogen to bioavailable ammonium, making it accessible for higher organisms including plants. Nitrogenases form a family of three structurally similar isoenzymes that differ primarily in the architecture of their active site cofactor.[1] The most common form of the enzyme is the Mo-dependent nitrogenase. It shows the highest dinitrogen reduction activity of the three classes and is present in all diazotrophic organisms known to date. Its active site is FeMo cofactor is a [Mo:7Fe:9S:C] moiety with a bidentate homocitrate ligand at the apical Mo ion.[2,3] Under Mo-limited conditions, many diazotrophs produce an alternative, V-dependent nitrogenase[4] and/or a third variant that solely relies on iron for its cofactor.

With a high-resolution crystal structure of Fe-dependent nitrogenase from the model diazotroph *Azotobacter vinelandii* we now complete the structural analysis of all known nitrogen-fixing enzymes. The active site, a FeFe cofactor, is a D3-symmetric moiety with a composition of [8Fe:9S:C] that also includes a homocitrate ligand. Comparing functional and structural data on all three isoforms of nitrogenase provides valuable clues for the common mechanism of this important class of metalloenzymes.

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Sara Zipfel, AK Einsle, BC

Structural and mechanistic insights into

quinol dehydrogenases from the nitrogen cycle

Nitrogen is an important element of life with the nitrogen cycle representing the different bioavailable forms of nitrogen. Two enzymes taking part in the nitrogen cycle are the periplasmic nitrate reductase NapA and the N_2O reductase NosZ. For those two enzymes a rather complex protein machinery is required to enable those reductions. Interestingly, both clusters contain two quinol dehydrogenases which are thought to translocate protons through the cytoplasmic membrane into the periplasm. NosB and NosGH for the N_2O reductase and NapC and NapGH for the nitrate reductase, respectively.

Here we report the structure of NapGH solved *via* Cryo-EM at 2.61 Å. It reveals the location of all four [4Fe-4S] clusters in NapG as well as three in NapH. The quinol visible in the Cryo-EM map was isolated and analyzed *via* HPLC. For N_2O reductase, NosGH is the homolog of NapGH. Furthermore, we could isolate the quinol out of NosB showing that two quinol dehydrogenases are present in the assembly machinery of N_2O reductase. As NosB can form a complex with the cytochrome-containing protein NosC2, it might serve as an electron carrier. This protein complex might therefore be a homolog to NapC, a quinol dehydrogenase containing four cytochromes for nitrate reductase.

In conclusion, we have identified the function of both NapGH as well as NosBC2 by showing structural and mechanistic insights. It will be interesting to determine why two quinol dehydrogenases are present in the two systems and why those two systems appear to be so similar.

Investigations on the FeMo Cofactor maturation system

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Structural insights of enzymes involved in biological nitrogen fixation are limited and focus mainly on the mechanism of the catalytically active enzyme - the nitrogenase. On the other hand, information on the process of cofactor maturation for this enzyme is rather limited, due to poor accessibility and stability of the enzymes involved. Looking for stabilizing and destabilizing factors was not considered before, which resulted in an inconvenient isolation of the nifB gen product located in inclusion bodys. Here we take a step back and ask ourselves the more basic question of why the enzyme was less accessible or stable in the first place and what the parameters are that have to be adjusted to obtain a sample for structural analysis. Heterologous expression in Escherichia coli was realized considering degrading and stabilizing accessory factors as well as environmental parameters. Building up on these findings a more efficient way for further research on the next level of cluster maturation is now possible with the additional benefit of an isolated system that now can easily be manipulated.

Julia Seifermann, AK Einsle, BC

Bacterial cytochrome *c* maturation system I

Almost all life on Earth contain covalently bound heme associated proteins called cytochromes *c*. They are electron carriers, key components in most of the metabolic pathways and involved as well in cell processes like apoptosis or detoxification. *C*-type cytochromes are characterized by the covalent linkage of the vinyl groups of the Fe-protoporphyrin to cysteine residues of the polypeptide chain. The formation of the thioether bonds is a post-translational modification which requires a complex membrane-bound protein machinery to assemble the heme group in the periplasm to the reduced heme binding motif CxxCH. In *E. coli* this maturase system is called cytochrome *c* maturation system I, encoded by the operon *ccmABCDEFGH*. While CcmF is the actual heme lyase and CcmGH are required to provide the reduced apocytochrome *c*, the subunits CcmABCDE are in charge for the translocation of heme *b* across the membrane and further to CcmF. The CcmAB complex represents an ABC-transporter with the ATP binding site in CcmA. The remaining subunits CcmCD are required for the transmembrane pathway for loading the cofactor to the heme chaperone CcmE. In this work, we present the isolation of the CcmABCDE complex from *E. coli* as *wild type* and as non-ATP hydrolyzing variant $E145Q^{A}$, as well as their structural characterization by Cryo-EM and functional characterization by *in vivo* maturation assays. With the obtained data we were able to postulate a mechanism by which CcmABCDE transports heme across the membrane and to the heme lyase CcmFH.

Architecture of the NADH:ferredoxin oxidoreductase RNF that drives Biological Nitrogen Fixation

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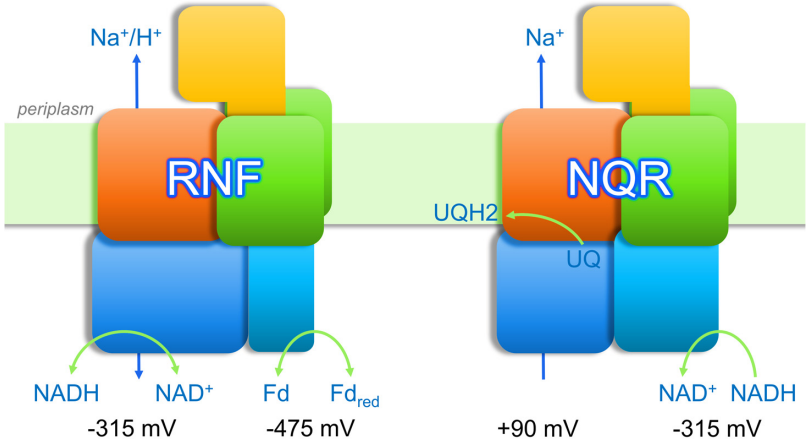
The *rnf* gene cluster was first discovered in ***R**hodobacter capsulatus* for their essential role in **n**itrogen **f**ixation[1], and soon found present in many prokaryotes[2]. The RNF complex is a redox-driven ion (Na^+ or H^+) pumping transmembrane oxidoreductase which mediates the following reaction[3]: NADH + 2 Fd_{ox} + $\Delta\mu H^+/Na^+$ ⇋ NAD^+ + 2 Fd^-_{red} + H^+. In most cases, the Rnf complex has been considered to oxidize reduced ferredoxin, transfer the electron across the cell membrane and back to reduce NAD^+, and contribute to the formation of an ion gradient coupled with ATP synthesis[4, 5]. In diazotrophs, the reverse electron flow from NADH to ferredoxin driven by exploiting the proton motive force facilitates the generation of low-potential electron for nitrogen fixation. However, the architecture of the RNF complex has not been elucidated. Here we report the cryo-EM structure of the nitrogenase-associated RNF complex of *Azotobacter vinelandii*, a seven-subunit membrane protein assembly that contains multiple flavin and iron-sulfur cofactors. Although the RNF complex shares certain similarity to the NQR complex[6], their functionalities work in different redox spans.



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Understanding the determinants of complex formation between the *Archaeoglobus fulgidus* Ammonium transporter Amt2 and its regulatory partner GlnK2

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The genome of the hyperthermophile euryarchaeon *Archaeoglobus fulgidus* (*Af*) contains three *amt* genes encoding for Ammonium Transport (Amt) proteins. These are trimeric integral membrane proteins that selectively mediate the uptake of the most reduced form of nitrogen, ammonium (NH_4^+) for bio-assimilation. As is frequently observed amongst prokaryotes, *amt* genes are present in an operon along with a *glnk* gene **[1]**. GlnK proteins are members of the broad PII protein family and their main function is the regulation of nitrogen metabolism. In particular, upon ADP binding to GlnKs, conformational changes occur in the protein which promotes a specific GlnK:Amt interaction that functions to prevent ammonium uptake into the cytoplasm. Conversely, ATP and 2-oxoglutarate binding to GlnKs are signals for GlnK:Amt complex dissociation and thus transport ammonium into the cell **[2]**. Structural and functional analysis of these proteins in our group revealed that operon-2 contains the most unusual proteins. Ligand binding to Af-GlnK2 confirmed the expected nucleotide recognition but, most intriguingly, revealed a unique incapacity of this PII protein to recognize 2-oxoglutarate **[3]**. The characterization of Amt proteins that have evolved from highly selective transporters to ammonium receptors, such as the ammonium sensor histidine kinase *K*s-Amt5 from "*Candidatus Kuenenia stuttgartiensis*" **[4]** or the *Sd*-Amt1 from *Shewanella denitrificans* **[5]**, allowed us to identify, from amino acid sequence comparisons, the presence of two potential binding sites for ammonium in *Af*-Amt2. Here we describe investigations on the *Af*-Amt2 and *Af*-GlnK2 pair to understand what effectors molecules and events affect their interaction. The structure of the complex, solved by cryo-EM, reveals the presence of ADP and we can infer that NH_4^+ is bound to *Af*-Amt2. Our results so far validate the particularities of these two very interesting proteins in ammonium transport, sensing and homeostasis in *Archaeoglobus fulgidus* cells.

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Ammonium transporters and receptors in anammox bacteria

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Anaerobic ammonium oxidation (anammox) is a process in which bacteria gain energy by oxidizing ammonium with nitrate to produce dinitrogen. It is carried out by extraordinary organisms from the planctomycetes clade, the anammox bacteria. In order for this reaction to take place, ammonium must first cross the outer membrane and several internal membrane systems before entering the anammoxosome, a unique organelle where the anammox process occurs. The selec-tive translocation of ammonium is mediated by ammonium transport (Amt) proteins and our work-ing anammox organism, "*Candidatus* Kuenenia Stuttgartiensis" (*Ks*), encodes seven putative Amts.

These proteins are integral membrane proteins that typically form trimers of 11-12 trans-membrane helices per monomer and selectively transport ammonium across membranes. Inter-estingly, Amt orthologues also exist in combination with additional functional domains within the same open reading frame. Based on sequence analysis, many of these soluble domains are pre-dicted to be involved in signal transduction cascades and belong to different classes of transducer systems. Interestingly, in many of these proteins, the Amt domain and its intrinsic ability to recog-nize ammonium, have been reutilized to function as receptors instead.

Using a combination of structural, biochemical and electrophysiology methods, we will pre-sent here two *Ks* proteins that share similar receptor domains for ammonium cations but convey the message to significantly different downstream partners.

Encapsulin as nanoreactor scaffold for transition metal-catalyzed reaction cascades

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Precise control of their metabolism and the associated chemical reactions is a central feature of living organisms.[1] In addition to the sophisticated selectivity of natural enzymes, this control depends largely on the formation of defined reaction spaces. Researchers from a wide variety of fields are trying to harness these properties of natural systems.[2] By combining proteins with the versatile reactivity of transition metal complexes, artificial metalloenzymes are opening up new possibilities in bioorthogonal chemistry and biocatalysis.[3] Protein-based nanocompartments are one platform for compartmentalization that is receiving increasing attention from synthetic biologists and biological chemists.[4]

In this work, a nanoreactor was developed that catalyzes two-step, sequential, and fully bioorthogonal reaction cascades.[5] In the first step of the reaction sequence, a ruthenium-catalyzed allyloxycarbonyl cleavage takes place.[6] The amine released in this process serves as a nucleophile in the subsequent gold-catalyzed hydroamination reaction.[7] For this purpose, various catalyst and substrate combinations were investigated in aqueous systems. A fusion protein of HaloTag and monomeric avidin was identified as a suitable scaffold for the artificial, metalloenzyme.[8,9] Coproduction with encapsulin nanocompartments that automatically host the artificial metalloenzyme as a guest protein allowed the catalytic system to be spatially separated from its environment and a third coordination sphere to be added. Products of the nanoreactor described here are functionalized indoles, phenanthridines and phenantridinium cations. These form the core structure of several biologically active substances that are already used as molecular probes and antitumor reagents.[7]

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Visible Light Initiated Photorelease of Mitochondria Targeting Inorganic Polyphosphate

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Inorganic polyphosphate (polyP), an ubiquitous molecule composed of multiple orthophosphates linked by high-energy phosphoanhydride bonds, is no longer regarded as a “forgotten polymer”.[1] In recent years, many different regulatory functions of polyP have been discovered and very recently, the mammalian mitochondrial F_0F_1-ATP synthase was shown to be capable of synthesizing and degrading polyP.[2,3] However, most enzymes involved in mammalian polyP metabolism have not yet been identified[4], although the delivery of modified polyPs into mammalian cells promises significant advances in polyP research. Here we show the synthesis and photophysical properties of photocaged mitochondria targeting polyP10. The clickable DEACM-450 photocaged polyP10 precursor was accessed from polyP8 using a bisphosphorylation procedure with the corresponding P-amidite. This provides the first chemical synthesis of defined polyP10 and exceeds the previously possible maximum chain length by two phosphate units. A photolysis study with 490 nm light demonstrated that the photorelease of polyP10 competes with a [2+2] cycloaddition, thus slowing down the polyP10 release. After the addition, however, of the mitochondria targeting triphenyl-phosphonium residues by click chemistry, the photolysis finished in less than 25 min. The delivery of this new probe into mammalian cells with molecular transporters, and the organelle specific release of active polyP10 in the mitochondria, will help to rescue polyP depletion effects in the potential site of the polyP synthase in mammals.

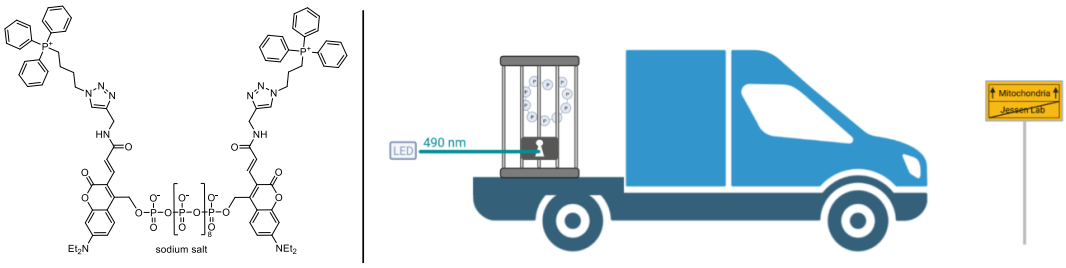

Figure 1. Chemical structure of photocaged mitochondria targeting polyP10 and schematic representation of the concept created with BioRender.com.

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Light-controllable DNA-hydrogels – An approach towards adaptive material systems

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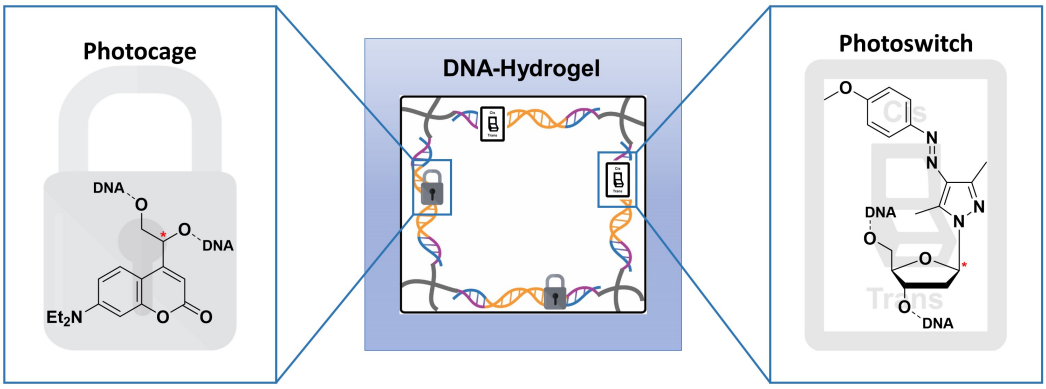
Hydrogels, described as water-swollen networks of cross-linked polymers, are soft matter materials that found diverse applications, such as drug delivery systems, tissue engineering, soft robotics and cell growth supports.[1] In particular, DNA-hydrogels possess a high flexibility regarding sol/gel temperatures, tunable bond lifetimes and interactions with biological systems due to the precise structural control and relatively easy synthesis of DNA strands.[2] Implementing light sensitive molecules, such as photocages or photoswitches, into the original DNA building blocks allows the construction of light-responsive networks.[3]
Here, we present the synthesis of photosensitive molecules ready to use for oligonucleotide application. More precisely, we developed an enantioselective route for a coumarin based photocage and a diastereoselective approach for an arylazopyrazole based photoswitch. Such light-controllable networks could enable a pathway towards adaptive and self-learning material systems.



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Arylazobenzoxazole-derived NHC ligands as a versatile platform for photoswitchable Au(I) catalysis

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A photoswitchable catalyst allows the non-invasive spatiotemporal control over the rate or selectivity of a chemical transformation by light.[1–3] Based on *in-silico* ligand design, we developed a straightforward synthetic route to prepare a series of arylazobenzoxazole-derived (NHC)Au(I) complexes (Figure 1, middle). Photochemical properties of these complexes were thoroughly studied by UV-Vis and NMR spectroscopy revealing high *Z*/*E* isomer ratios (up to 90%) with long thermal half-lives of the *Z*-isomers (up to days). Preliminary results in Au(I) catalysis showed significant photomodulation of activity in cycloisomerization of internal alkynes (Figure 1, left). Furthermore, photocontrolled divergent selectivity could be achieved in cycloisomerization of 1,6-enynes in presence of a nucleophile (Figure 1, right).

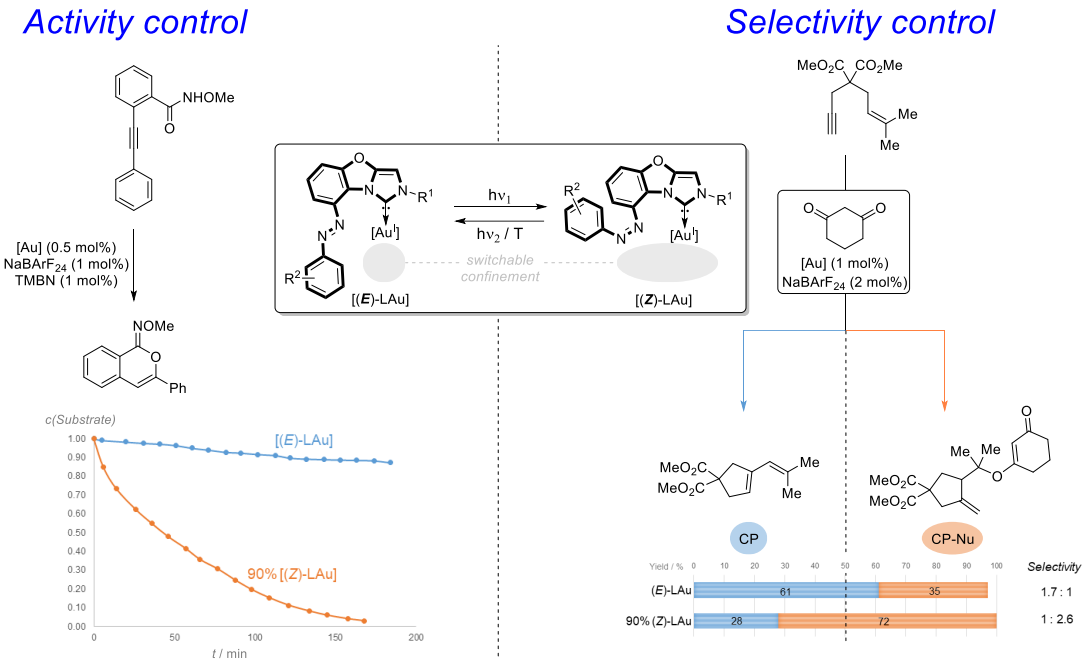

Figure 1. Arylazobenzoxazole-derived (NHC)Au(I) complexes for light-controllable catalytic transformations.

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New Inositol Pyrophosphate Prometabolites for *in vivo* Release

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Inositol pyrophosphates (PP-InsPs) are highly phosphorylated messenger molecules, which appear as different isomers. Many important biological functions like phosphate homeostasis and insulin sensitivity are associated to this molecules. The metabolic connection between the different isomers with high turnover and low concentrations complicates the investigation of their biological function. We introduced a prometabolite approach to modify the messenger's concentrations *in vivo* (**Figure 1** shows an 1,5-PP_2-$InsP_4$ derivative as an example). The prometabolites has protecting groups (AB) to mask the negative charges, which inhibit the cellular uptake. These modifications are biolabile, as they are cleaved by enzymes after they entered the cell. The activity of the messenger is still blocked by a photo removable protection group (photocage). A short irradiation with UV-light removes the photocage and PP-InsP is set free. New photocages were introduced, as they can be cleaved by higher wavelength (450 nm and higher) to overcome phototoxicity. The alkyne-group of the photocage enables to couple different modifications via click-chemistry, which can improve the cellular uptake or target specific organelles.

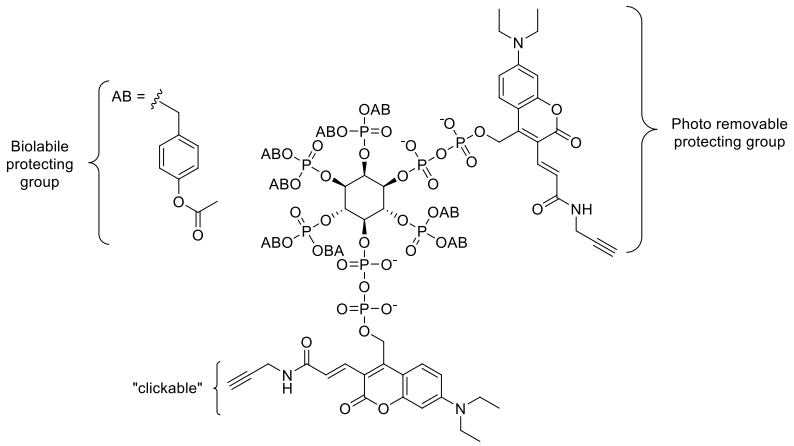

Figure 1. A prometabolite of the isomer 1,5-PP_2-$InsP_4$, with the photocage DEAC450

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Formylation as key step for new tandem reactions – Towards BODIPY dyes

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4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes experience an increasing demand due to their excellent photo- and thermo-, as well as their chemical stability. Therefore, they are utilized in a number of future-oriented applications such as organic lasers, OLEDs, fluorescent sensors, photosensitizers, solar cells etc.[1]
Conventional BODIPY syntheses show low overall yields. Therefore, an one-pot reaction to BODIPY dyes was developed involving literature known hydroformylation[2] or formylation[3] reaction conditions and subsequent substitution reaction with pyrrole nucleophiles under organo-catalytic conditions to obtain dipyrromethanes which are then straight-forward transformed to BODIPYs.

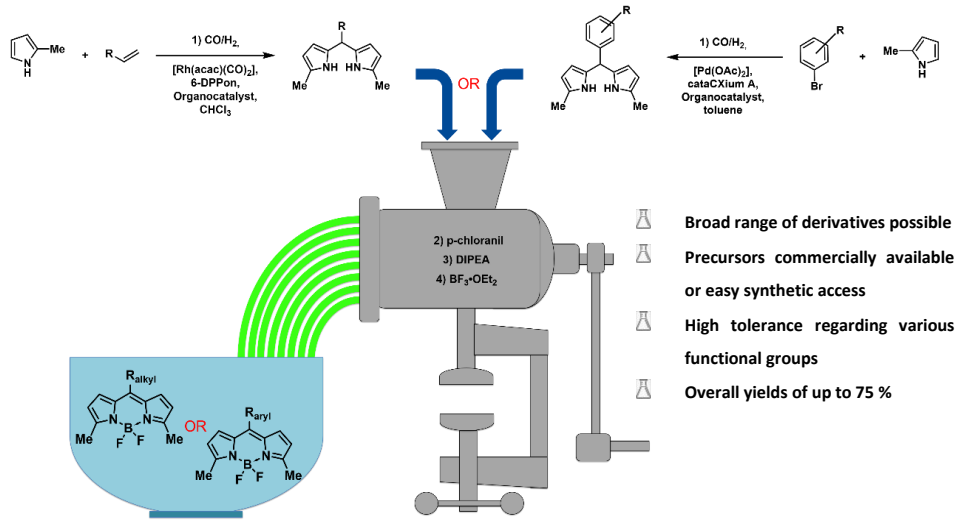

Figure 1: One pot synthesis scheme of BODIPY dyes from available precursors.

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Delivery of Caged Magic Spot Nucleotides into Escherichia coli

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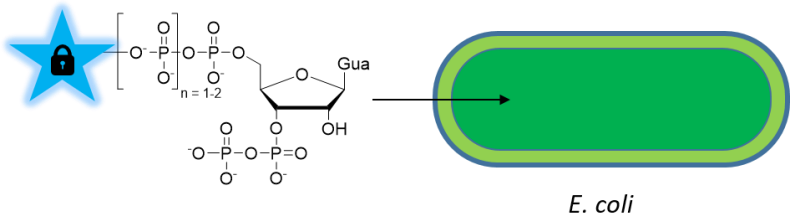
Magic spot nucleotides (MSN) are bacterial alarmones involved in the highly conserved stringent response, a bacterial stress response mechanism enabling survival in challenging environments. New chemical tools such as photocaged MSN-analogues are important to better understand the cellular implications of these signalling molecules. Here we describe the synthesis of caged and clickable MSN analogues and their delivery into *E. coli* cells. These highly phosphorylated nucleotides contain multiple negative charges and cannot permeate bacterial cell membranes spontaneously. Hence, the synthetic nucleoside triphosphate transporter described by ZAWADA *et al.* in 2020 was efficiently applied for cellular MSN delivery. The novel probes will enable studies of MSN involvement in the stringent response with spatial and temporal control.



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Detection and Quantification of Magic Spot Nucleotides

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Bacteria are able to adapt their metabolism to various stress conditions like starvation, antibiotics, changes in pH or in temperature.[1] The initiation of this stress response, known as stringent response, is mediated by the magic spot nucleotides guanosine 3'5'-bispyrophosphate (ppGpp) and guanosine 3'-diphosphate 5'-triphosphate (pppGpp). Detection and quantification of (p)ppGpp have, however, been a challenging task due to low cellular concentrations and fast turnovers.[2]

One potential detection method is capillary electrophoresis coupled to mass spectrometry (CE-MS). This highly sensitive analytical method is known for the excellently separation of highly charged components at low concentrations.[3] Quantification of the aforementioned compounds can be performed by spiking with heavy standards prior to extraction as shown in figure 1.

Here, we describe a method, in which spiking with heavy internal standards before extraction eliminates the losses of specific analytes during extraction. The combination with the sensitive CE-MS analysis provides a powerful tool for the determination of magic spot nucleotides with concentrations below 1 µM. This workflow is expected to reveal the bacterial metabolism not only during stress response and could aid in the development of more effective antibiotics.

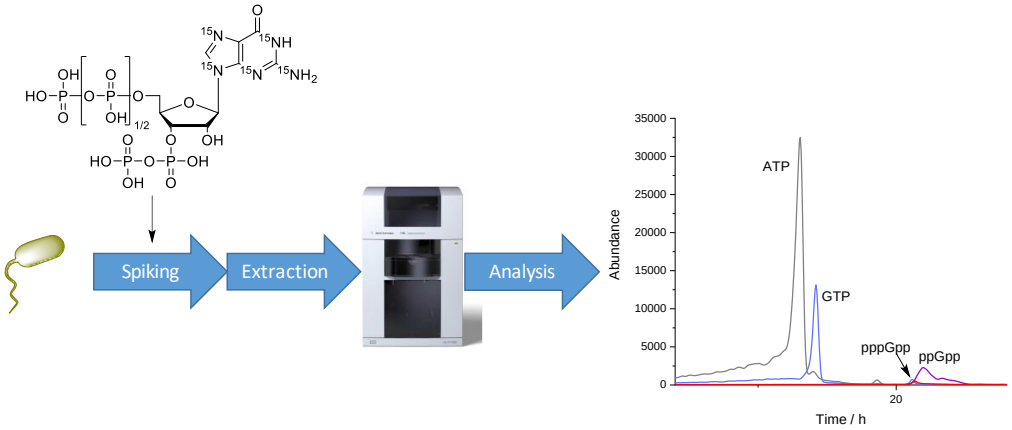

Figure 1. Structural formula of (p)ppGpp and workflow of quantification. Adenosine (ATP) and guanosine (GTP) triphosphates are shown for comparison.

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Mariia Zmyslia, AK Jessen-Trefzer, OC

Encapsulin nanocompartment – a modular nanoreactor

Compartmentalization of chemical reactions inside cells are a fundamental requirement for life. Encapsulins are self-assembling protein-based nanocompartments from the prokaryotic repertoire that present a highly attractive platform for intracellular compartmentalization of chemical reactions by design.

We characterize Encapsulin from *Mycobacterim smegmatis* and validate its cellular uptake. By equipping these capsules with a synthetic ruthenium catalyst *via* covalent attachment to a non-native host protein, we are able to perform *in vitro* catalysis and go on to show that engineered encapsulins can be used as hosts for transition metal catalysis inside living cells in confined space.

Furthermore, we encapsulate nitroreductase NfsB from *E. coli* (NTR). As NTR is an obligate dimer, only when NTR is encapsulated in tandem-dimer configuration does it function correctly and has comparable activity to free, non-encapsulated enzyme. Combination of encapsulated NTR with nitroaromatic prodrugs is a promising approach in Enzyme Prodrug Therapy by minimizing toxicity at healthy cells and increasing concentration of drugs at cancer cells. Surface modification/optimization of Enc_{SM} shell are necessary to influence cellular fate of nanoparticles and direct encapsulated enzyme to cytosol, where it can unravel its action.

4/6-InsP$_7$ – Rediscovery of an Old Friend in New Environment

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Highly charged diphosphoinositol polyphosphates (PP-InsPs or InsP$_x$) are an important class of signaling molecules associated to a wide range of cellular processes, such as apoptosis, DNA repair, energy- and phosphate homeostasis. Even so, the exact mechanisms of action and signaling pathways of PP-InsPs are still poorly understood. Low concentrations and a very fast metabolism make the elucidation of functions and mechanisms extremely challenging. The most important representatives are 5-InsP$_7$ and 1,5-InsP$_8$. The unsymmetric isomer 6-InsP$_7$ was discovered in 1997 in the slime mold *Dictyostelium discoideum*. More than 20 years later the occurrence of 4/6-InsP$_7$ in plants was observed by CE-MS measurements.[1] There are also indications for the presence of those isomers in mammals. So far, little is known about these isomers. Using the tools of chemical biology, the naturally occurring enantiomer will be identified. Pull-down samples will be used to determine protein interactions. By using stable isotope labelled InsPs, concentrations in a wide variety of organisms and tissues can be easily monitored and determined.

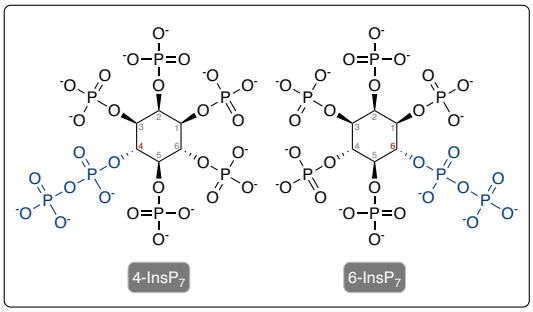

Figure 1. Structures of 4- and 6-diphosphoinositol pentakisphosphate (4/6-InsP$_7$).

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Direct DME Synthesis: Screening & Insights on Deactivation

Dustin Kubas[1,2]; Felix Hilfinger[1,2]; Erdogan Kasisari[2]; Prof. Dr. Ingo Krossing[1,2].

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Introduction:

As part of the NAMOSYN project we investigate the effective CO_2 hydrogenation to directly gain highly selective dimethyl ether (DME; Figure 1, left)[1] In comparison to standard methanol (MeOH) synthesis, direct synthesis of DME significantly increases the theoretically possible CO_2 conversion.[2,3,4] A bifunctional catalyst is needed and finding best suited catalyst materials is key. Here we present an extract of our wide catalyst screening and insights on deactivation.

Research:

The hybrid catalyst system is based on an amorphous Cu/ZnO/ZrO_2 (CZZ) hydrogenation catalyst prepared by co-precipitation.[2,5] In a 1:1 mixture with a solid acid catalyst for dehydration the hybrid catalyst is formed (Figure 1, right). The solid acid catalyst consists of a porous (Lewis acidic) carrier material e.g. Al_2O_3, ZrO_2 or Zeolites (Ferrierite, β-Zeolite), which can be coated with a Brønsted acid.[2] Here we used $H_4SiW_{12}O_{40}$ (HPA).

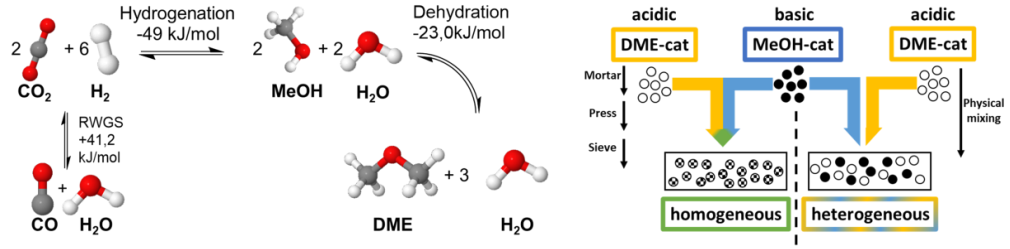

Figure 1: Left: Reaction scheme for the direct DME synthesis by CO_2 hydrogenation. CO_2/H_2 feed gas enables the unwanted endothermic reverse water gas shift (RWGS) side reaction. Right: Schematic illustration of two hybrid catalyst synthesis approaches. Left: homogeneous, leading to high intra-particle interfaces. Right: heterogeneous, leading to separated catalyst particles

In a wide catalyst screening Zeolites (FER, Zeo-β), Al_2O_3 and ZrO_2 were tested pure and coated with HPA in different mass ratios (wt% HPA: 33, 50, 66, or SA_{BET} based monolayer) as well as homogeneously and heterogeneously prepared. Heterogeneous preparation is overall equal or far superior (Figure 2). Coating with HPA results in higher CO_2 conversion and DME yield. Low SA_{BET} catalyst ZrO_2/HPA 31 het (87m²/g) produces 3x more DME per m² than most CO_2 active catalyst FER/HPA 33 het (275m²/g) tested. Surface quality overcomes quantity.

Homogeneously prepared hybrid catalysts containing Zeolites suffer from severe deactivation. Coating with HPA increases resilience for all tested materials. Side-by-side comparison shows benefits of heterogeneous preparation and coating for zeolite catalysts. Uncoated FER hom shows ¾ of FER het's CO_2 conversion with almost no DME yield. After ca. 40h FER hom lost another 25% activity, while FER het remains at ca. 90%. Effects are less pronounced on coated catalysts.

Development of photorechargeable photosupercapacitors

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[2]*Institute of Inorganic and Analytical Chemistry, University of Freiburg, Germany.*
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Off-grid electronic devices require energy autonomous power supplies which can be realized via coupling a solar cell (SC) with an electrochemical energy storage. Such hybrid device should be able to harvest energy from the environment, store and release it on-demand while having the lowest foot-print and being environmentally and economically friendly. One of the most promising way to realize such a hybrid system with high level of integration is by using a three-electrode interconnection scheme, with a shared/common electrode between the SC and the electrochemical storage unit. In this case, charge carriers photogenerated by the solar cell migrate through the shared electrode, which acts simultaneously as a charge acceptor for the storage device. The two remaining electrodes close the circuit in the photovoltaic cell and the storage unit.
The sporadic nature of the energy source and consumption require the storage device to operate efficiently under fast charging/discharging, which is a characteristic of a capacitive system but not a battery. Specifically, electric double layer capacitors (EDLCs) offer much faster power outputs but also large energy densities. This making them suitable for the integration with SCs into photosupercapacitors.
As an electroactive material for EDLCs we used mesoporous N-doped carbon nanospheres (MPNCs) produced via a hard-template approach based on aniline polymerization in the presence of SiO_2.[1,2] This resulted in 140 nm highly monodisperse MPNCs with large specific surface area (825 $m^2\ g^{-1}$) and defined mesopores. Benefiting from the well-defined mesoporous network, MPNC-based EDLC delivered a high capacitance (400 F g^{-1} at 1 A g^{-1}), resulting in large energy and power densities and high (95 %) coulombic efficiency.
As the solar cell, we used a 1 cm^2 large photosensitive area *p-i-n* halide ($FA_{0.75}Cs_{0.25}Pb(I_{0.8}Br_{0.2})_3$) perovskite SC with an optimized layer sequence for improved cell stability. The SC could deliver a high V_{oc} up to 1 V and current density up to 17.9 mA/cm^2. Its further integration with the MPNC-EDLC through a shared electrode in a three-electrode configuration resulted in the monolithic free-standing photosupercapcaitor. The resulting photosupercapacitor showed fast (< 5 s) photocharging up to 1 V under 1-sun illumination and an outstanding overall energy conversion efficiency of 11.8 %.[3] The photosupercapacitor could deliver up to 2.2 mW/cm^2 and 4.27 mWh/cm^2.
The proposed strategy of integration via three-electrode scheme with shared electrode was extended to produce MPNC based monilithic photosupercapacitors with other types of solar cells, including Si-SCs[4] and organic SCs.

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Crystallized Pb(II)- and Sn(II)-ammine complexes as intermediates from the interaction of CH_3NH_2 with BX_2 and $CH_3NH_3BX_3$ (B = Pb, Sn; X = I, Br, Cl)

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The reaction of CH_3NH_2 with $CH_3NH_3PbI_3$ leads to the formation of a yellowish viscous liquid. Upon removal of the CH_3NH_2 gas via slow heating, pristine $CH_3NH_3PbI_3$ can be obtained again.[1] Through higher concentrations of CH_3NH_2, crystals begin to emerge out of the viscous liquid. Our group was able to identify these crystals as $[Pb(CH_3NH_2)_6]I_2$, the first homoleptic lead-ammine complex. By reducing the CH_3NH_2 concentration, $[Pb(CH_3NH_2)_4I]I$ could be isolated as an additional compound.[2] In continuation of this investigation, we herein report on the findings of the interactions of CH_3NH_2 with BX_2 and $CH_3NH_3BX_3$ (B = Pb, Sn; X = I, Br, Cl).[3] We were able to characterize cubic ($Fm\bar{3}m$) $[Pb(CH_3NH_2)_6]Br_2$ (see figure 1) and $[Sn(CH_3NH_2)_6]I_2$, which crystallize isotypic to the analogous lead iodide compound and sport close similarities to the K_2PtCl_6-type. Characteristic for all three compounds are partially disordered CH_3NH_2 ligands (see figure 2). Formal release of CH_3NH_2 in these cases yields $[Sn(CH_3NH_2)_4I]I$ and $Pb(CH_3NH_2)_3Br_2$. Here, Pb^{2+} is octahedrally coordinated by three CH_3NH_2 ligands and three Br^- as a *fac*-isomer. Charge neutrality is achieved by one terminal and two bridging Br^-, forming edge-sharing dimers. $[Sn(CH_3NH_2)_4I]I$ is isotypic to its heavier lead analog, but exhibits significantly larger metal-halide distances. In the case of the lead chlorides, there is only a partial exchange of the halide ligands with CH_3NH_2. One compound obtained is $Pb(CH_3NH_2)_2Cl_2$, with a novel cubic ($Pa\bar{3}$) structure, showing two different octahedral coordination patterns for Pb^{2+}: $[PbCl_6]$ and $[Pb(CH_3NH_2)_3Cl_3]$, which are corner shared connected via a chloride. The other is $Pb(CH_3NH_2)_3Cl_2$, where Pb^{2+} has a *fac*-coordination with one terminal and two bridging chlorides, forming chains in the direction of the *c*-axis. Since all chains possess the same orientation of the methylamine ligands, an acentric (Cc) structure results. Only $[Sn(CH_3NH_2)_5]X_2$ (X = Br, Cl) could be isolated in the case of the tin bromides and chlorides. The structures are isotypic and contain Sn^{2+} square-pyramidally coordinated by CH_3NH_2, boasting a strong stereo active lone-pair effect distorting the polyhedra. Furthermore, there is no direct interaction whatsoever between the tin(II) and the halide atoms, which are bound via hydrogen-bridges. All compounds are sensitive to heat and humidity readily reacting with moisture forming CH_3NH_3X and a metal hydroxide species like $Pb(OH)X$ (X = I, Br, Cl) in the case of lead.

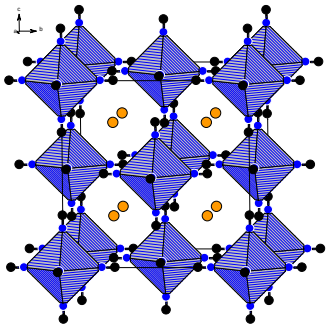

Fig 2: Unit cell of $[Pb(CH_3NH_2)6]Br_2$; disorder of N-atoms not considered; H-positions not determined

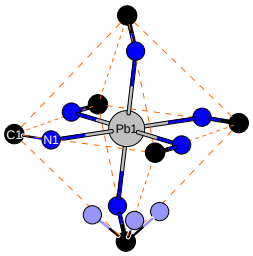

Fig 1: Coordination of Pb in $[Pb(CH_3NH_2)6]Br_2$; disorder of N-atoms partially considered

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Investigations into the Highly Selective Synthesis of Heterodinuclear $Au^{I}Pt^{II}$ Complexes

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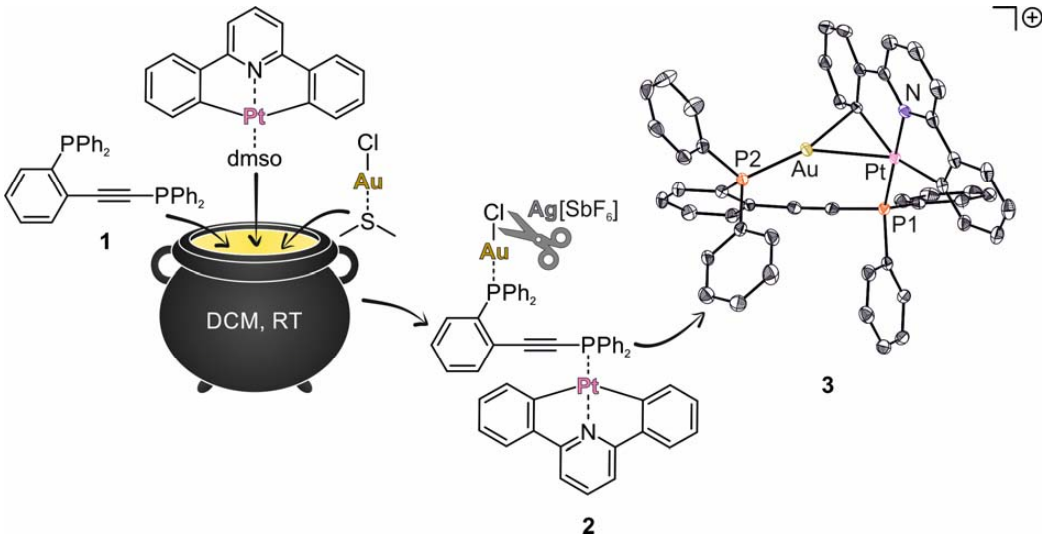

Besides the formation of mononuclear complexes, chelating ligands also allow for the stabilization of small transition-metal clusters. This property can be advantageous for the application in catalytic processes since chelating ligands might prevent the decomposition of such multinuclear complexes even if the metal–metal bond is temporarily cleaved during a catalytic process.[1]
Based on the idea of maintaining the metal atoms in close proximity by the aid of a bridging ligand, the coordination chemistry of the L-shaped long-range "*cis*-spanning" ligand **1** (L_{PP}) has been investigated. AuCl and Pt(CNC) (CNC = 2,6-diphenylpyridine-*o,o'*-diate) can be selectively coordinated to the *phenylic* and the *alkinylic* site of **1**, respectively, in a one-pot reaction, thus yielding complex **2**. The observed selectivity is remarkable since L_{PP} can also support the complexes $[L_{PP}(AuCl)_2]$ and $[L_{PP}\{Pt(CNC)\}_2]$, thus confirming the ability of both donor atoms of **1** to coordinate both gold and platinum. The formal $Au^{I}Pt^{II}$ complex $[L_{PP}Au^{I}Pt^{II}(CNC)]^+$ (**3**), which is neither sensitive to air nor moisture, can be obtained by chloride abstraction from **2**. In contrast to the inspiring literature-known complex $[(Ph_3P)Au^{I}Pt^{II}(CNC)(PPh_3)]^+$ as synthesized by MARTÍN and co-workers,[2,3] the metal atoms in **3** are additionally bridged by the chelating ligand L_{PP} (**1**).
Complementary DFT computations – including population and bond-critical point analyses – provided deeper insights into the bonding situation within cationic $Au^{I}Pt^{II}$ complexes such as **3**. In contrast to $Au^{I}Pt^{0}$ complexes, in which the metal–metal contact can be described in terms of a so-called "metal-only Lewis-pair",[4] the intermetallic interaction in **3** is rather weak. The bonding situation is better described as an interaction, in which the Au–C bond acts as a donor for the formal Pt^{II} center.

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P-XTRACT: Phosphate recycling from a local wastewater treatment plant

Lorenz Bier-Schorr, Christian Wildschut, Philipp Kurz, Institut für Anorganische und Analytische Chemie, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, Germany

Since 2017, the European Union has defined phosphate as a critical raw material.[1] The EU itself has no convenient deposits of phosphate rock as primary material for an economic production of P-fertilisers. In the course of a phosphate recycling offensive of the German federal government, communal sewage plant operators are obliged to recover high amounts of phosphate from the sewage water. However, numerous procedures for achieving this goal are currently being explored, but as a standard route has not been established yet, phosphate recycling from wastewater is a currently very active research field.

Our group supports a regional newly formed project called P-XTRACT, which aims to develop a possible procedure to recycle phosphate from sewage water.[2] The project coordinator is the association for sewage treatment Staufener Bucht, which is building a combustion plant for sewage sludge in Grezhausen close to Freiburg. The operation of this plant is planned to start in autumn 2022. We support the project by analysing the composition of the raw materials and the combustion products in laboratory experiments.

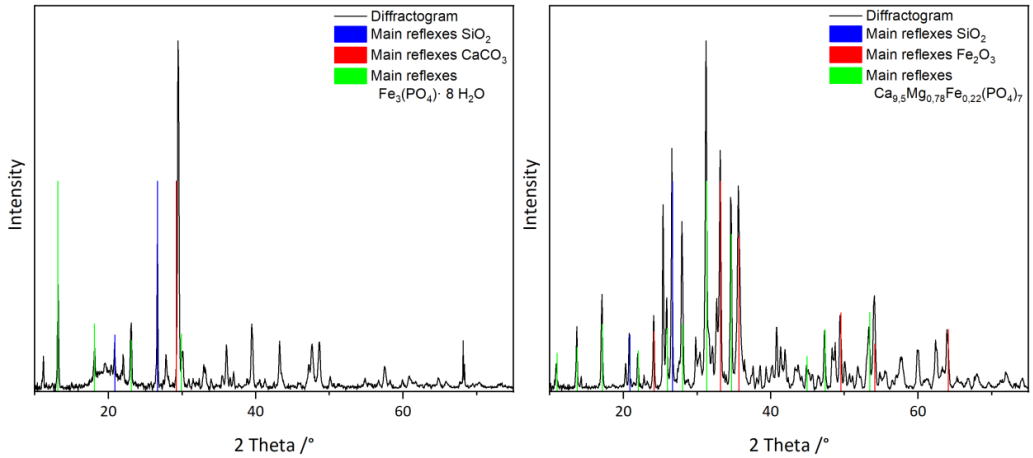

Figure 1: *Left* - X-ray analysis of a sewage sludge starting material. *Right* - X-ray analysis of the ash obtained after combustion in a pyrolysis furnace at 815°C.

Design of carbon materials for electrochemical applications

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The rational design of active electrode materials plays an important role in improving the electrochemical properties of both, energy storage and conversion devices. For most applications, not only well-defined porous structures but also control over morphology and particle size is desired. While processing onto substrates, the nanoparticles (NPs) monodispersity determines their packing and as a result influences percolation and interaction with the electrolyte.
Here, we report a strategy to produce mesoporous N-doped carbon nanospheres as a versatile platform adaptable for applications need that allows to control morphology and porous network, wall-thickness, but also chemical and surface composition.
The process implies an oxidative polymerization of aniline in the presence of a silica template yielding spherical composites. Its further carbonization and template removal results in mesoporous N-doped carbon nanospheres with homogeneous sizes and a porous structure directed by the template, as well as N content imprinted by the polyaniline.
Using different sizes of silica allowed us to tailor pore sizes in the range of 7 to 100 nm but keeping the particle size constant. The obtained MPNC have large specific surface areas (1061-305 $m^2\ g^{-1}$) and large pore volumes (1.5-0.25 $cm^3\ g^{-1}$). The approach possess a good degree of reproducibility and scalability, representing a technologically competitive solution.

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Synthesis of cationic low-valent main-group complexes starting from [(AlCp*)$_4$]

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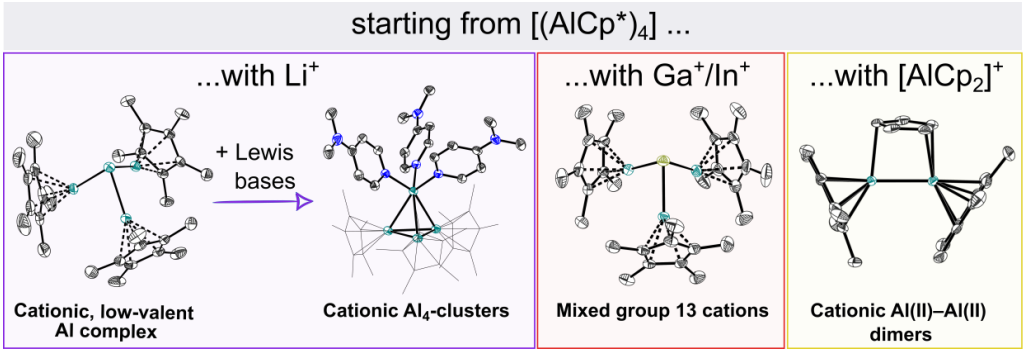
Low-valent aluminium complexes are among the most widely researched main-group complexes. In recent years, isolation of highly nucleophilic aluminyl anions as well as mono-coordinate aluminylenes significantly expanded the field of Al(I) complexes.[1,2] Yet, an accessible cationic, low-valent aluminium compound combining the nucleophilicity of low-valent compounds with the electrophilicity of aluminium is hitherto unknown. Here, we present the preparation of the cationic, low-valent aluminium complex salt $[Al(AlCp^*)_3]^+[Al(OR^F)4]^-$ (OR^F = $C(CF_3)_3$) via reaction of easily accessible $Li^+[Al(OR^F)_4]^-$ with $[(AlCp^*)_4]$ (Cp^* = C_5Me_5). As suggested by the structural and computational analysis, $[Al(AlCp^*)_3]^+$ is best described as cationic Al atom coordinated by three strongly electron-donating AlCp* ligands. Interestingly, dimerisation of the cation to dicationic, purple $[Al_2(AlCp^*)_6]^{2+}$ can be observed in solution and solid state. Moreover, the unique Al atom in $[Al(AlCp^*)_3]^+$ can be coordinated by Lewis bases, which induces reformation of the tetrameric Al4 clusters. Here, Al–Al bond lengths and cluster stability can be fine-tuned by the donor strength of the added Lewis base.

With AlCp* being able to stabilize the elusive Al^+ cation, we were interested to expand AlCp* chemistry to various other main-group metal-cations. Addition of $[(AlCp^*)_4]$ to complex salts $[M(PhF)_{2\text{-}3}]^+[Al(OR^F)_4]^-$ (M = Ga, In, Tl) allows for isolation of the Ga-, In- and Tl derivatives $[M(AlCp^*)_3]^+[Al(OR^F)_4]^-$. Surprisingly, reaction of these mixed group 13-complex salts with an amine-ligand results in isomerization of the initially formed clusters to the respective amine-coordinated Al_4^+ cluster salt. Moreover, we reacted $[(AlCp^*)_4]$ with the highly electrophilic $[AlCp_2]^+$ cation (Cp = C_5H_5). Surprisingly, two species of rare cationic dialanes could be isolated. The molecular structures of the complex salts revealed a novel coordination of a Cp-ligand, being coordinated “side-on” to the Al–Al single bond.



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Next Generation Batteries

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Seit in den 80er Jahren Kathoden auf Basis von Übergangsmetalloxiden, allen voran $LiCoO_2$ mit seinen damals hohen Kapazitäten von 140mAhg^{-1}, etabliert wurden haben die Lithium-Ionen-Akkumulatoren sich als führende Variante der Batterien etabliert. Diese Technik ist so wichtig, dass sie im Jahr 2019 sogar mit einem Nobelpreis gewürdigt wurde[1][2].
Dennoch steigen die Anforderungen an Batterien, vor allem aufgrund von Ausbau in der Elektromobilität, stetig weiter und die bisher verwendete Technik der Lithium-Ionen-Akkus stößt an ihre Grenzen. Deshalb hat die Suche nach der nächsten Generation der Batterien, den „next generation batteries“ oder „beyond Li-ion batteries“, die die bisherigen Batterien ablösen sollen, schon lange begonnen[3].
Die Anforderungen an die nächste Generation von Batterien sind vielfältig und hängen stark von ihrem Anwendungsgebiet ab. Generell gewünscht sind unter anderem hohe Kapazitäten, hohe Spannungen, große Zyklenstabilität und eine günstige Herstellung[3].

Auch im Arbeitskreis Krossing wird sich der Suche nach neuen Batteriematerialien gewidmet. In verschiedenen Forschungsprojekten wird an verschiedenen Materialien und damit verbundenen Vor- und Nachteilen geforscht. Elementares Lithium, Silizium, Magnesium oder Calcium als Anode sowie Schwefel als Kathode sind vielversprechende Kandidaten, die alle jedoch ihre eigenen Herausforderungen mit sich bringen.
Auf diesem Poster soll ein Überblick über diese Materialien und die innerhalb der Battery-Subgroup des Arbeitskreises Krossing verfolgten Lösungsansätze für die damit einhergehenden Probleme gegeben werden.

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Transition metal-mediated P_4 conversion using cations stabilized by perfluorinated weakly coordinating anions

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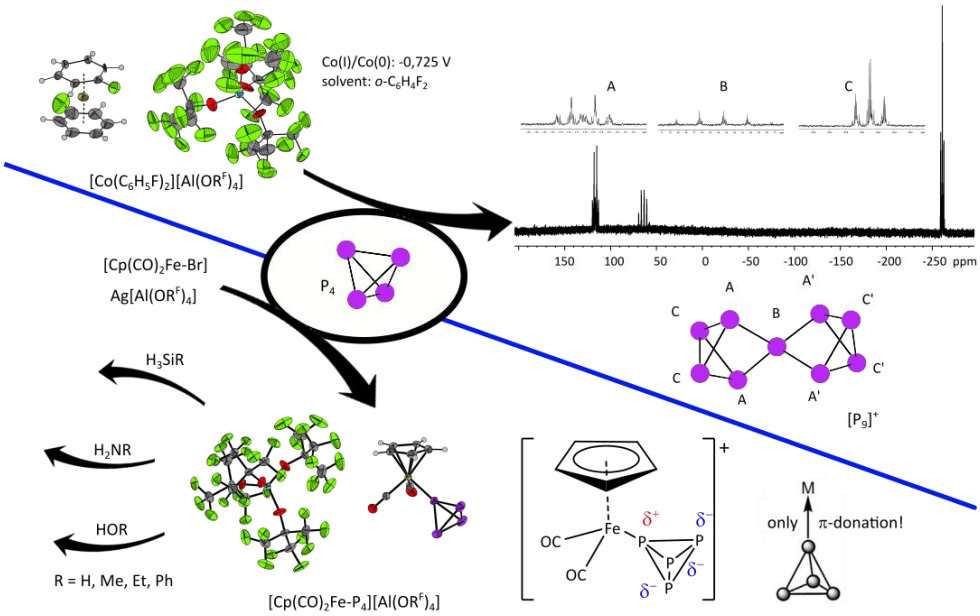
Stabilizing non-coordinated metal cations in solution has been targeted for years, using elevated weakly coordinating anions that are sterically demanding and often perfluorinated generating improved properties. Recent work in our group included the „naked“ $[Co(o\text{-}C_6H_5F)_2][Al(OR^F)_4]$ ($R^F = C(CF_3)_3$) [1] which is capable of converting white phosphorous towards the homopolyatomic $[P_9]^+$-cation.



In $[Cp(CO)_2Fe\text{-}\eta^1\text{-}P_4][Al(OR^F)_4]$, which has been a targeted complex in our group, the lack of π-back donation results in an umpolung of the typically phosphidic P_4 ligand to a phosphonium character,[2] that allows to functionalize P_4 using nucleophiles, rather than electrophiles that are typically associated with P_4-cotaining transition metal complexes. Here we present a flock of nucleophiles that are promising candidates to build useful phosphorus containing molecules PNu_3 and provide further elucidation about the reactivity and the binding situation in this P_4 complex.

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Novel routes towards Oxymethylene Dimethyl Ethers (OME_n) – A 2nd Generation E-Fuel

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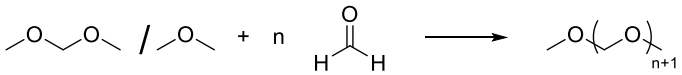
Introduction

Oxymethylene Dimethyl Ethers (OME_n; $CH_3(\text{-}OCH_2\text{-})_nO\text{-}CH_3$, n = 3-5) are promising candidates as synthetic fuels/eFuels since they hold soot-free combustion properties and possess comparable physical properties like the proscribed Diesel.[1] This novel class of synthetic fuels can sustainably be synthesized based on CO_2 and H_2, consequently avoiding an increase in the global share of CO_2. In general, a distinction is made between aqueous and non-aqueous routes and in the choice of starting molecules for OME_n syntheses.[2,3] Methanol, Dimethyl Ether (DME) or OME_1 serve as methyl-group provider for chain termination whereas para-formaldehyde (p-FA), trioxane (TRI) or monomeric gaseous formaldehyde (FA) are used as chain propagating units. Monomeric gaseous FA is rarely used as substrate although thermodynamically desirable since the production of anhydrous TRI is complex and energy-intensive.[4] Using gaseous FA (synthesized by dehydrogenation of methanol) instead of trioxane the overall H_2-consumption can be reduced by 22 % resulting in an energy conversation of 87 % (instead of 67 % for conventional anhydrous syntheses using TRI).[2,5] Consequently, our research focuses on the anhydrous reaction of molecular FA with different chain terminating groups, Scheme 1.



Scheme 1: Reaction of OME_1 / DME with molecular FA to OME_n.

A biphasic reaction concept was established for the reaction of molecular FA with OME_1 in a batch process which allows easy catalyst separation and reuse of the catalyst.[6] Based on this, recent studies as part of the NAMOSYN project focus on the transfer of the batch reactor concept to a continuous flow reactor concept as well as the use of DME as chain terminating reagent.

The Protoelectric Potential Map (PPM): An Absolute Chemical Potential Scale

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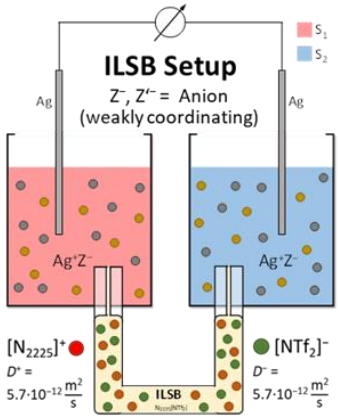
The comparison of acidities and redox potentials of different species is always limited to a specific medium. The Protoelectric Potential Map (PPM) was created to overcome this limitation. It allows not only to compare acidities and redox potentials across different solvents but can also be applied to gaseous and solid states. A change in the reference state to either the ideal proton gas or the ideal electron gas eliminates the solvent dependence. The chemical potential of these ideal gaseous states is arbitrarily set to zero. Thus, the chemical potential of the proton/electron corresponds to their Gibbs free energy of solvation $\Delta_{solv}G°(H^+)$. The experimental measurements rely on single-ion thermodynamics, specifically the Gibbs energy of transfer from one solvent to another.

$$\Delta_{trans}G°(H^+, S_1 \rightarrow S_2) = \Delta_{solv}G°(H^+, S_2) - \Delta_{solv}G°(H^+, S_1)$$

The drawback of the conventional determination of single-ion transfer energies lies in the need for *extra-thermodynamic* assumptions.[1] To free the transfer energies of these assumptions, we employ an 'Ideal-Ionic Liquid Salt Bridge (ILSB).[2] The word 'ideal' refers to the cations and anions almost identical diffusion coefficients, both in the two solvents and the ionic liquid itself.



The ILSB salt $[N_{2225}][NTf_2]$ was used to obtain transfer energies of the silver ion $\Delta_{tr}G°(Ag^+)$ in a variety of solvents.[3] This data set will be expanded with commonly used solvents. Additionally, the experimental research is supported by quantum chemical calculations with COSMO-RS[4] and continuum solvation models.

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From Low-Valent Carbonyl Cations to Novel Piano-Stool and Sandwich Complexes

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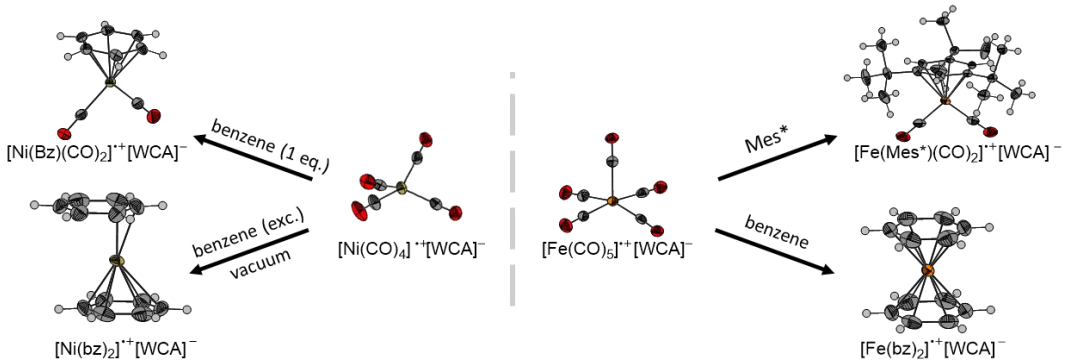
Transition metal carbonyl cations have been studied extensively in the gas phase by mass spectrometry (e.g. ref.[1]). However, for the stabilization of these reactive cations in the condensed phase, the interaction with the environment has to be minimized. These “pseudo gas-phase conditions” can be achieved with the choice of an appropriate weakly coordinating anion and solvent. The nickel tetracarbonyl radical cation $[Ni(CO)_4]^{\bullet+}$ was generated by deelectronation (single electron oxidation) of nickel powder under CO atmosphere with the synergistic $Ag^+[F\text{-}\{Al(OR^F)_3\}_2]^-$ / 0.5 I_2 system (R^F = $C(CF_3)_3$).[2] Attempted deelectronation of neutral iron pentacarbonyl using NO^+ and Ag^+ resulted in ligand substitution and coordination. Only when employing the innocent deelectronator $[phenazine^F]^{\bullet+}$ in combination with the weakly coordinating aluminate anions $[Al(OR^F)_4]^-$ and $[F\text{-}\{Al(OR^F)_3\}_2]^-$ is it possible to generate the $[Fe(CO)_5]^{\bullet+}$ cation in condensed phase.[3] By substituting the carbonyl ligands with arenes one has access to the piano stool and sandwich complexes $[M(arene)(CO)_2]^{\bullet+}$ and $[M(arene)_2]^{\bullet+}$ (M =Fe,Ni).



Scheme 1: Ligand exchange reactions of the $[Ni(CO)_4]^{\bullet+}$ and $[Fe(CO)_5]^{\bullet+}$ radical cations.

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Dual atom Fe-Zn cathode catalysts for Alkaline Membrane Fuel Cells

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With the recent development and increasing availability of stable and performant hydroxide conductive ionomers, the Anion Exchange Membrane Fuel Cells (AEMFCs) are promising candidates to replace the current state-of-the-art Proton Exchange Membrane Fuel Cells (PEMFCs) since they combine the advantages of PEMFCs, like low-temperature operation and high-power density with the low component costs of alkaline fuel cells.[1,2] One major advantage of switching from the acidic environment of a PEMFC (~pH 1) to an alkaline environment of an AEMFC (~pH 13) is the possibility of replacing the expensive platinum-based electrocatalysts with cheaper electrocatalysts like nickel-based materials for the anode and iron-based materials for the cathode.[1,3,4]
Over the last decades, various materials were investigated to replace the expensive platinum on carbon electrocatalysts at the cathode. Among these materials, iron- and nitrogen-doped carbons (Fe-N-C) with molecular iron sites (Fe-N_x) show comparable catalytic activities to Pt and decent stabilities for the oxygen reduction reaction (ORR) in alkaline media.[5,6,7]
These Fe-N-C catalysts are usually synthesized by co-pyrolysis of Fe-, N-, and C-sources at high temperatures (700-1000°C) and subsequential acid leaching. Lately, new synthesis strategies for Fe-N-C catalysts with superior activity focus on using Fe-doped metal-organic frameworks (Fe-MOF's) as precursor-templates resulting in materials with a high dispersion of Fe-N_x sites as well as high porosity and high specific surface areas.[8]
To produce Fe-N-C catalysts with a large number of Fe-N_x single sites, we synthesized Fe-, and Zn-doped MOFs (Fe-Zn-MOF) as multicomponent Fe-, Zn-, N-, and C-precursors and pyrolyzed them in the presence of additional nitrogen sources at high temperatures in an inert atmosphere. The resulting Fe-Zn-N-C catalysts revealed high dispersion of Fe and Zn, high specific surface areas (400-600 m^2/g), and high porosity as revealed by XRD, XPS, EDX, and N_2 physisorption. Depending on the pre-treatment of the Fe-Zn-MOF, the Fe content and Fe species of the resulting Fe-N-C catalyst could be varied. Benefiting from the increased Fe-dispersion, high specific surface area, and additional presence of Fe species (Fe_3C), the best performing Fe-Zn-N-C catalyst shows high activity towards ORR in alkaline media (0.1 mol/L KOH) as demonstrated by RDE measurements featuring a more positive half-wave potential (0.87 V vs. RHE) than a commercial 50 wt.% Pt/C catalyst (0.83 V vs. RHE) and high mass activity (47 mA/mg_{cat}).
Further investigation in an AEMFC, using the Fe-Zn-N-C catalysts at the cathode, a commercial PtRu/C catalyst (40 wt.% Pt, 20 wt.% Ru on carbon black, AlfaAesar) at the anode, and a commercial ionomer in the catalyst layer and in the membrane (Ionomr Innovations) revealed a high peak power density of 850 mW/cm^2 for the best performing Fe-Zn-N-C catalyst. This performance is among the highest reported peak power densities for non-precious metal cathode catalysts in combination with a commercially available ionomer so far and represents the huge potential of this class of materials.

The project AlkaCell was funded by the Vector Stiftung. The authors are grateful for generous funding.

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Theoretical studies investigating the mechanism of methanol formation over Cu/ZnO based catalysts

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MeOH production from syngas ($CO/CO_2/H_2$) is a major industrial process, reaching 311 Mt/a in 2030 and growing.[1] The mechanism of MeOH formation is still under discussion. Key areas of debate are the role of O defects/ vacancies on the ZnO surface, Cu-Zn synergism, the formation of Cu/Zn alloys, the role of ZnO (i.e. SMSI), etc.[2,3,4] Here, we present a computational study of the possible intermediates relevant to MeOH synthesis on a Cu cluster on a reconstructed polar ZnO model.[5] We analysed stabilities on multiple sites, activation of the intermediates on the surface and activation barriers for relevant elementary processes.

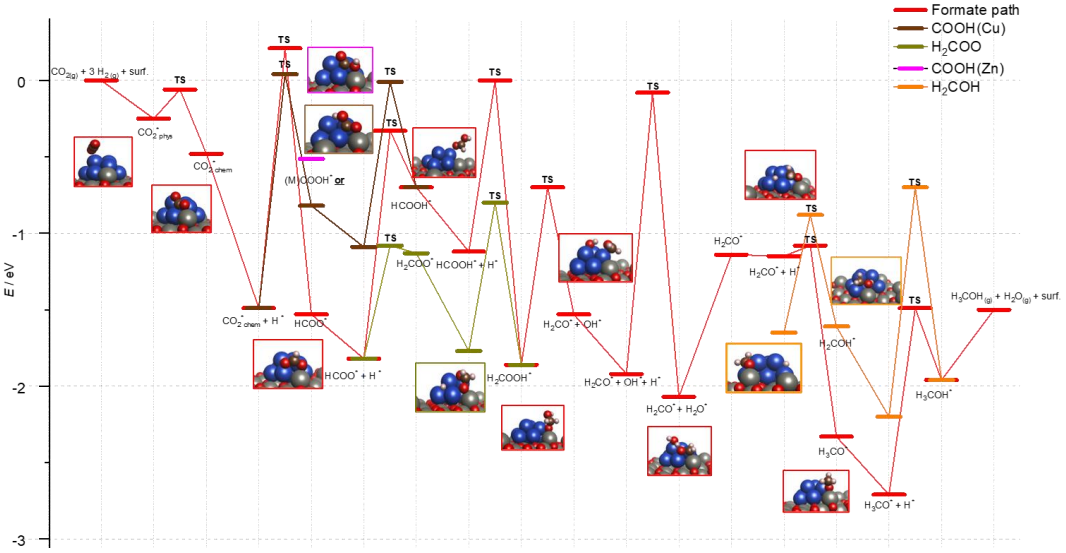

Figure 1: Energetic pathway of the CO_2 hydrogenation via HCOO pathway to form MeOH on Cu/ZnO system. Red are O atoms, brown for C, blue for Cu, grey for Zn and white for H. Transition states are described as TS. On top legend describing the different pathways associated to the formate formation.

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Fluorination of Methanol Catalysts to improve Productivity and Selectivity

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Introduction

The largest part of todays produced methanol is synthesized based on fossil resources *via* a catalytical process. Regarding the current climate problem, a more sustainable synthesis route such as the hydrogenation of CO_2 would be desirable. In almost all industrial MeOH catalyst systems, the actual catalysis is mainly based on the catalytically active Cu/ZnO pair, with one or more supporting oxides (such as Al_2O_3, called support or promotor[1]) being added. For a MeOH synthesis from CO_2 and H_2 instead of CO and H_2, Al_2O_3 is unsuitable as a promotor, here ZrO_2 was discovered as a promising alternative (CZZ system).[2] Investigations on binary Cu / Zn systems have shown that treatment with fluorine increases MeOH productivity and selectivity.[3,4] When trying to transfer the positive effect of fluorination to the ternary CZZ system, it was found that Zr acts as a "fluorine magnet" and prevents fluorination at the copper and zinc centers and therefore no positive effect on productivity or selectivity is observed.[5]

Research

Iron has been identified as a promising candidate to create an active system that could benefit from fluorination. Several different CZFe catalysts with different compositions (Cu: Zn: Fe = 3.75 : 1.73 : X, X = 0.25, 0.5, 1.0, 1.5) and under different synthesis conditions (variation of synthesis temperature and aging time) were produced.

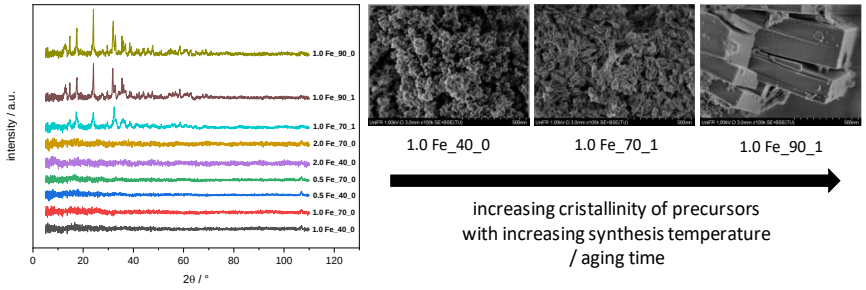

Figure 1: Variability of the properties (here: crystallinity) of the catalyst systems by varying the synthesis conditions. Nomenclature: X Fe_Y_Z, X = share of iron (Cu : Zn : Fe = 3.75 : 1.73 : X), Y = synthesis temperature, Z = aging time.

The typical synthesis process includes co-precipitation from an aqueous solution of the corresponding nitrate salts, subsequent spray drying and calcination at 300 ° C. The calcination is followed by a reaction with elemental fluorine. A total of 13 different systems were produced, all of which were treated with about 1.4 wt% F_2, thus 26 different systems were obtained. The catalyst systems show good tunability in their physical and chemical properties (see fig. 1) as well as in their catalytic properties. It was found that a high iron content is detrimental to

Spectroelectrochemical studies of O_2-tolerant [NiFe]-hydrogenase immobilized on tin-rich indium tin oxide with alteration in catalytic bias

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The ability of hydrogenases to reversibly catalyze the production and oxidation of hydrogen with minimal overpotential makes them attractive electrocatalysts for hydrogen energy conversion devices. The oxygen tolerance demonstrated by the membrane-bound [NiFe] hydrogenase from *Ralstonia eutropha* (MBH) provides a further advantage; however, this enzyme is well-known as being strongly biased towards hydrogen oxidation and shows little promise towards hydrogen production. Here, we have immobilized the MBH after genetically attaching two different affinity tags to the C terminus of the enzyme - a His-tag (MBH_{His}) and a Strep-tag (MBH_{Strep}). The differences in adsorption and electrocatalytic behavior were investigated when wired to an amorphous, transparent, and planar tin-rich indium tin oxide (ITO_{TR}) surface with a Sn:In ratio of 1:1. As demonstrated by ATR-IR spectroelectrochemical studies, the affinity of the His-tag for the tin-rich ITO allows the quantitative immobilization of MBH_{His} in a direct electron transfer configuration. Remarkably, once immobilized on the tin-rich ITO surface, hydrogen oxidation as well as an unusually high proton reduction current is observed - an effect that is enhanced under illumination. Herein we demonstrate conditions that promote catalytic bidirectionality in [NiFe]-hydrogenases, and how the latter is related to favorable, direct enzyme-semiconductor interactions.

Application of In-situ Electrochemical Liquid (Scanning) Transmission Electron Microscopy in understanding Materials Dynamics under Electrochemical Conditions

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The project “In-situ EChem-(S)TEM” funded by the Volkswagen Foundation in the Momentum funding scheme is dedicated to studying the dynamics of energy materials for electrochemical energy conversion and storage applications under operational conditions at the nanoscale using in-situ electrochemical transmission electron microscopy techniques.

Recent advances in the micro-fabrication of electrochemical MEMS devices and their combination with electron microscopy methods have opened new horizons allowing us to study the complex and dynamic behavior of electrochemical energy materials at the nanoscale under electrochemical conditions. Materials of interest are, for example, electrocatalysts for electrochemical energy conversion[1,2] and fuel cells[3], as well as battery materials for electrochemical energy storage[4]. The knowledge gained in such in-situ studies will improve the understanding of the materials “under operational condition” and therefore help to pave the way to design and manufacture materials with improved performance in terms of activity, storage capacity, or long-term stability.

Here the working principles of “In-situ EChem-(S)TEM” will be presented as well as some preliminary results of a case study regarding the morphological evolution of CO_2 reduction electrocatalysts. These in-situ studies will be complemented by ex-situ characterizations (such as X-ray absorption spectroscopy (XAS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (PXRD), Raman spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), cyclic voltammetry (CV), linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS)) to gain an overall mechanistic understanding of the materials dynamics and catalytic mechanism.

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$Rb_4CuSb_2Br_{12}$: A new vacancy-ordered quadruple perovskite

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The introduction of $MAPbI_3$ as a new absorber material for solar cells [1] resulted in an enormous boost in research on metal halide perovskites and related compounds. Such compounds include divalent halides ABX_3, double perovskites $A_2BB'X_6$ with mono and trivalent cations, or similar representatives with only trivalent ($A_3M_2X_9$) or even tetravalent (A_2MX_6) cations. A relatively new contender, in this context, is the class of so-called vacancy-ordered quadruple perovskites, with the general formula $A_4B\square B'_2X_{12}$ [2], containing one divalent cation, two trivalent cations and one vacancy. The crystal structure can be seen as an alternating order of the B/B' cations and the vacancies, forming layers that are perpendicular to the direction (111), with respect to the cubic (double) perovskite structure. Besides $Cs_4BB'_2Cl_{12}$ (B=Cd, Mn; B'=Sb, Bi) [3] and $K_4Fe_3F_{12}$ [4], which can be described in the rhombohedral space group *R*-3*m*, also $Cs_4CuSb_2Cl_{12}$, [5] which takes on a lower symmetry (*C*2/*m*), was found some time ago.

During our systematic investigations, we were able to isolate $Rb_4CuSb_2Br_{12}$ ($P2_1/c$, a=13.656(3), b=7.4280(16), c=13.633(3) Å and β=114.604(3)°) as black polyhedral crystals from Sb_2O_3, CuO and RbBr in concentrated aqueous hydrobromic acid. The lower symmetry, compared to the other representatives of this class, results from the fact that Cu^{2+}, as a d^9 system, typically prefers a 4+2 (Jahn–Teller effect) coordination. The smaller Rb^+ cations of $Rb_4CuSb_2Br_{12}$, in contrast to $Cs_4CuSb_2Cl_{12}$, favor, in combination with the larger $CuSb_2X_{12}$-Framework, an additional rotation of the octahedra along one direction, which further decreases symmetry. The relationship between the different representatives of these kinds of “cation-deficient Perovskites”, as well as to the cubic (double) perovskites, will be shown via group-subgroup relations using the Bärnighausen formalism.

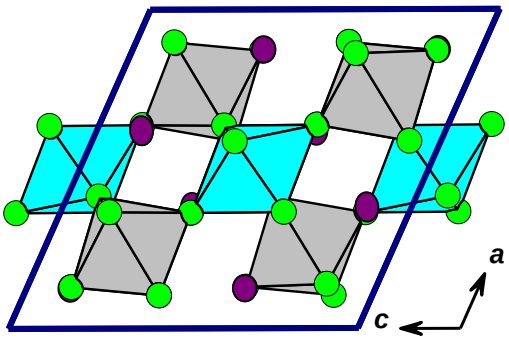

Figure 1: Crystal structure of $Rb_4CuSb_2Br_{12}$. Color code: Rb = violet, Cu = turquoise, Sb = grey, Br = green.

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Symmetric Cells as an efficient Tool in Lithium-Ion-Batteries Research

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Lithium-Ion batteries (LIBs) have been widely employed in portable electronics over the last decades and are currently introduced in automotive and grid energy. The demand to extend their energy density and longevity for thousands of cycles can be achieved by active material modification, adaption of electrolytes and well-developed fabrication.

Classic LIB investigation on these factors take months or years and the influences from the material level, fabrication and measurement techniques are commonly convoluted. New efficient approaches to evaluate the cell degradation mechanisms therefore are highly required in LIBs research. Because positive/positive or negative/negative Li-Ion symmetric cells (+/+ or -/-) (SCs) separately operate in the same potential ranges as in a Li-Ion full cell (+/-), ageing and degradation mechanism are easier and faster to disentangle. This special cell arrangement allows the isolation of parasitic reactions belonging exclusively to the positive/negative electrode and can exaggerate these.

Two factors, lithium salt (typical commercial $LiPF_6$ and $LiBF_4$) and electrolyte additive (pyridine boron trifluoride, PBF) were studied, and the electrochemical performance of Lithium and NMC622 SCs were investigated parallel with Lithium::NMC622 half-cells. The Li::NMC622 half-cells and Li::Li SCs with $LiPF_6$ exhibited better capacity retention than with $LiBF_4$ in EC/EMC while NMC622 SCs with $LiPF_6$ resulted in poor capacity retention compared to their $LiBF_4$ analogue. The PBF generally improved the capacity retention in Li::NMC622 half-cells and reduced polarization in Li SCs with $LiPF_6$ and $LiBF_4$ EC/EMC electrolyte. NMC622 SCs maintained their initial capacity only for a few cycles in $LiPF_6$ EC/EMC with 1%PBF additive while the capacity retention in $LiBF_4$ EC/EMC was similar with or without 1%PBF additive.

These results of SCs are highly related to the chemical and electrochemical stability of electrolytes against reduction and oxidation where Li or NMC622 are operating at its characteristic potential ranges in Li::NMC622 half-cells. This case study provides us with a new very fast methodology to evaluate LIBs EC-performance factors such as surface modification, electrolyte additives, temperature, deep of charge, cycling number.

Highly Dispersed Pt Entities Consisting of Pt Single-atoms, Clusters and Nanoparticles on Mesoporous N-doped Carbon Nanospheres for Improved Hydrogen Evolution Reaction

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Abstract
Platinum is one of the best-performing catalysts for the hydrogen evolution reaction (HER), but its high cost and scarcity severely hinder the large-scale application of Pt catalysts. Constructing highly dispersed ultrasmall platinum species to increase mass activities is a very effective strategy to increase its utilization and hence decrease costs. Here, we synthesized highly dispersed Pt species composed of a mixture of Pt single atoms, clusters and nanoparticles on mesoporous N-doped carbon (MPNC) supports. The presence of Pt single atoms, clusters and nanoparticles was demonstrated by a combined approach of x-ray diffraction, aberration-corrected high-angle annular dark-field scanning transmission electron microscopy and electrochemical CO stripping. The mesoporous structure and N-doping of the MPNC supports played a key role for the formation of highly dispersed Pt species which endowed the catalyst with a large electrochemical active surface area and excellent HER geometric and mass activities. Such excellent HER activity turned out to be respectively 3 and 30 times higher than that of a commercial Pt/C and a Pt/NC-nanofiber catalyst with similar Pt loadings, i.e. 20 wt%. Noteworthily, after optimization of the geometrical Pt loading on the electrode, higher mass activity than for state-of-the-art Pt single-atom and cluster catalysts are achieved.[1,2]

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New Dion-Jacobsen phases in the system $A_2AgBiBr_8$

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Organic-inorganic hybrid perovskite, such as $(CH_3NH_3)PbI_3$, are well known in literature. these types of compounds have been the subject of intensive research for some time due to their excellent optoelectronic properties.[1] Perovskites have the general formula ABX_3. Their structure can be described as a three-dimensional network of vertex-sharing BX_6 octahedra, with *A* in the cuboctahedral void.[2] For *X* = halide, typically, *B* is a divalent cation which builds up the network in perovskites, though this can be replaced by a mono- and a trivalent cation. When these two species replace the divalent one, the structure can be called a double-pervoskite. Formally removing layers along either (100), (111), or (110) results in two-dimensional structures. These can be separated using organic spacer cations. When the “cut-out” was along (100) and a monovalent cation is used Ruddlesden -Popper Phases (RP) are resulting while in Dion-Jacobsen (DJ) phases[3], it is divalent. Herein, we present some examples of new Dion-Jacobsen phases in the system $A_2AgBiBr_8$, using linear and pyridinium cations.
To the best of our knowledge, the only known single crystal structure in the literature uses 1,4-butanediammonium (BDA).[4]
Different diamines have been used in the search for new DJ phases. Using 1,3-Diaminopropane (DPA) results in the DP phase $(DPA)_2AgBiBr_8$ ($P2_1/n$ a = 8.357(3) Å, b = 7.803(4) Å, c = 18.421(7) Å, ß = 99.076(7)°) as yellow plates. Using the pyridinium cations 2-Picolylamine (2-PCA), 3-Picolylamine (3-PCA) and 4-Picolylamine (4-PCA), $(2\text{-PCA})_2AgBiBr_8$ ($P2_1$ a = 8.283(6 Å, b = 17.389(14) Å, c = 9.172(7) Å, ß = 102.704(10)°), $(3\text{-PCA})_2AgBiBr_8$ ($P2_1/c$ a = 8.195(3) Å, b = 17.370(7)(4) Å, c = 18,469(8) Å, ß = 102,090(19)°), $(4\text{-PCA})_2AgBiBr_8$ ($P2_1/c$ a = 8.149(5) Å, b = 17.649(12) Å, c = 18.292(11) Å, ß = 101.775(11)°) results in yellow plates. All $AgBr_6$ octahedra can be described with a 2+4 coordination (compressed octahedra). The degree of compression differs in the different compounds. The structure of all these new compounds can be described as alternating layers built up of corner-sharing $AgBr_6$ and $BiBr_6$ octahedra, separated by organic spacer cations. All amine groups are protonated, which builds up hydrogen bonds with the bromide ion. No pi-stacking can be observed in any of the structures with the pyridinium cations, since the distance between the rings is higher than five Ångstroms.[5]
All the products have been crystallized from a hot hydrobromic acid solution, with a stoichiometric amount of AgBr, Bi_2O_3, and the corresponding amine.

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Protein vibrations and their localization behaviour. A numerical scaling analysis

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Using a classical force field, we investigate the localization properties of protein normal modes. For a set of eighteen proteins that cover five classes of increasing size, we compute the participation ratio as a measure of the spatial extent of protein vibrations. In this scaling analysis, we find extended low-frequency far-infrared and Terahertz modes, in contrast to localized high-frequency near-infrared vibrations. These regimes are separated by a broad crossover around a wave number of 260 cm^{-1} . Biophysical and biochemical implications are discussed, and the vibrational localization properties are compared to those of amorphous solids.

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Diffusion of Cocaine through a Model Membrane: A Computational Approach

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We study the diffusion of cocaine through a DMPC lipid bilayer as an example of a protonable, amphiphilic molecule passing a biological membrane. Using classical molecular dynamics simulations, the free energy surfaces are computed applying the umbrella sampling technique for the protonated and the neutral molecule. For the combined surface, we numerically solve the diffusion equation at constant flow and for time-dependent concentrations. We find a potential of mean force dominated by a barrier of 3.5 kcal/mol within the membrane, and a pH-dependent entry and exit barrier of 2.0 kcal/mol and 4.1 kcal/mol, respectively. This behaviour can be rationalized chemically by the amphiphilic nature of the molecule and the change of its protonation state while passing the membrane. Diffusion through the barriers is 3.5 times slower than along the membrane, and the typical time scale of passage amounts to 0.1 ms. We discuss biochemical and medical implications of our findings, such as the mechanism of the drug passing the blood-brain barrier.

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Investigation of spin communication in PDI–TEMPO systems for applications in quantum information science

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One of the current major challenges in quantum information science (QIS) is the identification of new materials to be used in quantum devices. To be suitable, these materials have to comply with the DiVincenzo criteria [1]. Since photogenerated molecular spin systems fulfil most of these criteria, they are promising candidates. A further advantage of molecular systems is the possibility to modify, and thereby optimise, their structure [2,3].
In this contribution, we investigate the interactions in photogenerated multi-spin systems and how they can be manipulated. The molecules investigated here consist of a chromophore covalently linked to a stable radical. The stable radical, TEMPO, represents the first spin centre, while the second spin centre is generated by light excitation of a perylene diimide (PDI) chromophore.
We use optical spectroscopic techniques, such as femtosecond UV-vis transient absorption spectroscopy, to observe the ultrafast photochemical processes. This provides information on the yield and kinetics of the formation of the photogenerated spin centre. To study the magnetic interactions between the spins, we use transient electron paramagnetic resonance techniques.
The comparison of different systematically modified PDI–TEMPO molecules finally leads us to propose design guidelines for molecular systems in which the photogenerated spin centre can be formed efficiently. In addition, we found that the spin state formed in PDI–TEMPO after light excitation has magnetic properties suitable for applications in QIS.

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Investigation of the Triboelectric Effect with AFM-based Force Spectroscopy and Kelvin Probe Force Microscopy

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For thousands of years, the triboelectric effect has been present in our daily life (e.g., lightning or ignition of dust explosions). It describes a charge separation process, when two (different or same) materials are contacted or are in motion against each other. However, the physical origins of the triboelectric effect are still not well understood. In our work, two different AFM-based techniques are combined to study charge separation after contacting various materials at a microscopic scale. This enables us to probe several physical parameters for the triboelectric effect and determine their impacts on triboelectrification. Atomic Force Microscopy (AFM)-based force spectroscopy [1] is used to contact the respective materials and Kelvin Probe Force Microscopy (KPFM) [2] is performed to obtain the surface potentials before and after such contacting experiments. This work can provide a new approach for the characterization and understanding of the triboelectric effect, and will help us to test new materials for efficient energy-harvesting systems based on the triboelectric effect, such as triboelectric nanogenerators (TENGs) [3].

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Regulation of fluctuations in a multi-domain protein

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Um Proteindynamiken auf einer Zeitskala von Millisekunden bis Minuten besser zu verstehen, wurden bisher hauptsächlich fluoreszenzbasierte Methoden genutzt – insbesondere bei großen Proteinen. Diese Zeitskala möchten wir erweitern, indem wir fortgeschrittene Fluoreszenzmethoden mit Molekulardynamiksimulationen (MD-Simulationen) kombinieren, um für das Hitzeschockprotein Hsp90 Zeitskalen von Nanosekunden bis Millisekunden zu untersuchen [1].
Das als Homodimer vorliegende Hsp90, dessen Monomere ein Molekulargewicht von etwa 90 kDa pro Monomer haben, verfügt über eine ATPasefunktion. Seine Assoziation mit Onkoproteinen macht das Verständnis dieser Proteinmaschinerie für die gezielte Anwendung von Medikamenten (Drug Targeting) in der therapeutischen Medizin von großer Bedeutung [2].
Weiterhin soll die Fluoreszenzlöschung durch Tryptophan mittels photoinduziertem Elektronentransfer (PET) genutzt werden, um diese Nanosekundenfluktuationen weiter zu quantifizieren und zu lokalisieren.
Insgesamt möchten wir zeigen, wie Kinetiken im Nanosekundenbereich ein Multidomänenprotein - beispielhaft am bekannten Hitzeschockprotein Hsp90 - regulieren.

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AFM-based detection of polystyrene microparticle interaction with lipid bilayers

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We have based our modern life on the benefits of polymers, enjoying the prosperity while simultaneously harming the environment and possibly our health. Few threats to today's nature are as visible as the burden of plastic waste while the impact on our health is harder to quantify. Since plastic has long been proven to be part of the food chain and is used for biomedical applications, the question of how and how far plastic particles can penetrate the body inevitably arises.[1] So far, there is no clear answer and experimental data is needed to shed light into the interaction of polymeric materials at different length scales with living organisms, in particular with cell membranes.[2-4]

Here, we study the interaction of polystyrene colloids of different sizes with solid supported lipid bilayers (SLBs) as model systems for polymeric microparticles interacting with lipid membranes. The quality of the SLBs can be assessed using a combination of AFM-based imaging, AFM-based force spectroscopy and fluorescence microscopy. This makes precise positioning of cantilevers with colloidal probes on homogenous SLBs possible. Then, AFM-based force spectroscopy is used to investigate the interaction mechanism of the colloidal probe with a SLB. Our techniques allow us to investigate the interaction of a single polystyrene microparticle and helps to better understand possible mechanisms for the interaction and entry of polysytrene microparticles into lipid membranes. This will serve us to assess the impact of plastics in the environment to biological systems and might lead to the design of more environmentally friendly types of polymeric materials in the future.

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Assembly and Dynamic Interactions of the Hsp90 Chaperone Machinery by Multi-Colour Single Molecule FRET

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Proteine sind wichtige Bausteine, die in allen Bereichen des Lebens eine große Rolle spielen. Sie arbeiten jedoch selten allein, sondern bilden Komplexe und setzen sich zu molekularen Maschinen zusammen, um ihre jeweiligen Funktionen zu erfüllen.

Hsp90 ist ein sogenanntes Chaperon, also ein Helferprotein, das bei der Faltung von Proteinen, wie zum Beispiel Kinasen, hilft.

Hsp90 bildet verschiedene Chaperon-Maschinen mit unterschiedlichen Co-Chaperonen (also ‚Helfershelfern') für eine Vielzahl von Klienten. Insbesondere seine Beteiligung an neurodegenerativen Krankheiten wie Alzheimer sowie an verschiedenen Krebsarten erfordert ein tiefgreifendes Verständnis dieser Maschinen. Der erste Schritt auf diesem Weg ist die Kenntnis über den Zusammenbau dieser Maschinen und der Dynamik ihrer Wechselwirkungen. Daher muss geklärt werden, ob die beteiligten Proteine zufällig aufeinandertreffen und zufällige Komplexe bilden, oder ob es einen zugrundeliegenden, sequenziellen Mechanismus gibt, bei dem ein Protein ein anderes rekrutiert und dann das nächste und so weiter.

Mittels mehrfarbigem Einzelmolekül-FRET untersuchen wir die Wechselwirkung zwischen Hsp90, einem Co-Chaperon und einem Substrat in Echtzeit. Um geringe gegenseitige Affinitäten zu überwinden, werden verknüpfte Proteine mit orthogonalen Markierungsstellen verwendet. Dreifarbige Einzelmoleküldaten zeigen Wechselwirkungen zwischen allen drei Proteinen in vitro. Erste Ergebnisse deuten auf einen hochdynamischen Rekrutierungsprozess durch Hsp90 hin. Zusammengenommen ermöglicht uns dies nun die direkte Beobachtung und Quantifizierung von Komplexbildungswegen.

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Simulating EPR Spectra of Photogenerated Triplet-Radical Systems

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The rapidly expanding field of quantum information science deals with the usage of the quantum nature of matter or photons for applications in computing, communication and sensing. The design of suitable materials, which should be characterized by pure, well-defined and long-lived spin states, is highly sought-after. Well suited molecular systems are *triplet-radical systems*. These are molecular systems consisting of a chromophore, that is attached via a covalent linker to a stable radical. The exited singlet state of the chromophore, that is generated by absorption of light, is able to interact with the radical. The chromophore triplet state is formed rapidly after photoexcitation by *radical enhanced intersystem crossing*. This way, a well-defined three-spin system is created.

Transient electron paramagnetic resonance spectroscopy (TREPR) is an ideal method for the analysis of the behavior of spins in high magnetic field. It can help to gather a fundamental understanding of the spin system. For the interpretation of rather complex TREPR spectra of multi-spin systems, simulations are of great value [1].

It is our aim to develop python-based software, that can be used to *predict* the time-dependent TREPR spectra of multi-spin systems (in particular triplet-radical-systems) depending on the initial population of the spin states (determined by the photoactivation mechanism), the strength of the interaction between triplet and radical, and relaxation processes. Two theoretical models are included in the simulation, and are presented.

Firstly, we describe the theory of the spin system as needed for the calculation of the energies of the spin states. The multi-spin Hamiltonian is set up and a system consisting either of a doublet and a triplet state or, in the strong-coupling case, of a doublet and a quartet state is calculated [2]. Secondly, the calculation of a TREPR spectrum is described. Here, we make use of the density matrix formalism and the *Von-Neumann* equation [3].

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Distance determinations in a LOV-fusion-protein using dipolar EPR-spectroscopy

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Dipolar EPR-spectroscopy is an important tool for determination of distances and dynamic studies, e.g. conformational changes or the formation of multimers in biomolecules. Diamagnetic proteins are usually labeled by attaching nitroxide spinmarkers to selective cysteine residues. For proteins harboring a paramagnetic cofactor the mutational and labeling effort can be reduced by using the cofactor as an intrinsic spinmarker. LOV (light oxygen voltage) domains are small blue-light photoreceptor domains, containing a Flavin mononucleotide (FMN) as chromophore. The photochemical properties of FMN can be modulated by introducing point mutations in the binding pocket: Either stable semiquinone radicals or photogenerated triplet states can be formed [1], [2]. Both paramagnetic species can be used as an intrinsic spinmarkers for dipolar EPR-experiments.
In this study, a heterodimeric LOV-fusion-protein was examined by two dipolar EPR-techniques: the widely used 4-pulse-ELDOR (electron-electron double resonance) and the recently introduced ReLaserIMD (refocused laser-induced magnetic dipole) experiments [3]. The first mentioned experiment was also used to determine the radical-radical distances *in cell*. Interestingly, the obtained distances differ depending on the experiment and the according spin states of the flavins. For further understanding of these results, the protein was analyzed by X-ray crystallography and DFT calculations.

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Phenothiazine derivatives as model compounds to study radical-dependent phenomena

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Electron paramagnetic resonance (EPR) spectroscopy has been used since the 1940s to study – as the name implies – paramagnetic centers of different nature, most notably organic radicals and transition metals. While the last decades have increased the availability and usage of pulsed EPR techniques like DEER and ENDOR, continuous wave EPR (cw-EPR) is still a powerful and versatile technique to study the surrounding and interactions of paramagnetic centers. In recent years, pulsed and cw-EPR were used to study a series of phenothiazine derivatives (PTs) in different oxidation and polymerization states [1][2]. While PTs have been used since 1891 as pharmaceutical agents, only recently their potential as organic cathode materials has attracted some attention. Already demonstrating discharge potentials of over 3 V *vs.* Li/Li^+ thus being “competitive” with commercial Li-ion battery cathodes, while also displaying ultra-high cycling stability, phenothiazine-based cathode materials suffer from their comparatively low to moderate capacity (<100 mAh/g). This combination of properties was analyzed by a variety of techniques like EPR and X-ray photoelectron spectroscopy, DFT calculations and surface electron microscopy (SEM). The presented poster will focus on the EPR spectroscopy and will draw a bigger picture in exploring its capabilities to investigate processes like radical stabilization, spin-spin interaction, spin delocalization and other related phenomena while being rather “hands-on” in regards to spectroscopic data instead of long theoretical and/or mathematical derivations.

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Calculation of exchange couplings in the excited state of molecular three-spin systems

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Photogenerated organic triplet-doublet systems hold great promise as building blocks for applications in the emerging field of molecular spintronics, which is a research area that focuses on the use of the electron spin for the generation, transport, and storage of quantum information.

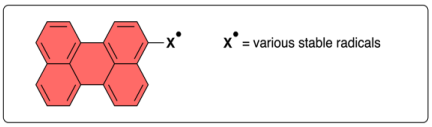

Figure 1: The investigated series are composed of a PDI chromophore covalently bound to a nitroxide radical, and an unsubstituted perylene covalently bound to various stable radicals.

To find suitable molecular materials for any particular application, we need to know how the magnetic properties of the molecules can be controlled. Consequently, it is of great importance to investigate how and to which extent the exchange interaction between the chromophore triplet and the stable radical, J_{TR}, can be manipulated in such systems.

The present study is based on theoretical calculations, allowing us to determine the exchange interactions for several series of molecules and to learn from the observed trends. Since the description of the exchange interactions in three-centre-three-electron systems is intrinsically a multi-determinantal problem,[1] we use the CASSCF method (complete active space self-consistent field) to calculate the electronic states in combination with QD-NEVPT2 (quasi-degenerate *n*-electron valence state perturbation theory) to account for dynamical electron correlation. With the obtained multi-determinantal wavefunctions, the ab initio Hamiltonian is represented in the basis of determinants. The individual exchange coupling constants can then be extracted from the off-diagonal elements in the subspace of neutral determinants, which corresponds to the Heisenberg-Dirac-Van-Vleck Hamiltonian.

Here we focus on a series of of unsubstituted perylenes that are covalently bound to different radicals. From the calculated electronic states, we can draw conclusions on the influence of the linker length, and the radical compound on the excited state exchange interactions and consequently the magnetic properties of the system.

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Drosophila Cryptochrome Photo-reaction Characterization with incorporated Unnatural Amino Acids and Modified Flavins

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Cryptochromes are a class of flavoproteins found in plants and animals that are sensitive to blue light. They are involved in the circadian rhythm and sensing of magnetic field in a number of species. Cryptochrome plays the major role in the Transcription-Translation Negative Feedback loop regulating the expression of the circadian clock genes. *Drosophila melanogaster* Cryptochrome (DmCry) photo-activation requires the Flavin Adenine Dinucleotide (FAD) cofactor. Subsequently, stepwise sequential electron transfer (ET) takes place along the Trp-tetrad until the terminal surface–exposed tryptophan cation radical is formed, which corresponds to the [$FAD^{\bullet -}$–$TrpH^{\bullet +}$] **(1)**. RP1 is further stabilized by deprotonation, thus forming the secondary [$FAD^{\bullet -}$–$Trp^{\bullet}$] radical pair = RP2. In this project, we show the ability to genetically encode additional redox-active unnatural amino acid (UNNA) which would significantly enhance our ability to engineer the electron-transfer process in the protein. We report that *p*-Amino phenylalanine (*p*AF) can be genetically, selectively and efficiently incorporated into different positions in the Trp-tetrad (Trp-394, 342, 397 *p*AF) using orthogonal tRNA-aminoacyl tRNA synthetase pairs. The incorporated *p*AF has shown a contribution in the redox-reaction with radical pair formation and modified the photo-reduction of the protein in response to the blue-light illumination and its distance/orientation from the electron acceptor FAD cofactor. We report also the possibility of incorporating a number of modified FAD cofactors *in vivo* using genetically modified riboflavin auxotrophic strain **(2)**. The incorporated modified FADs can dramatically change the dark/light protein sensitivity and increase our understanding of the protein photoreaction.

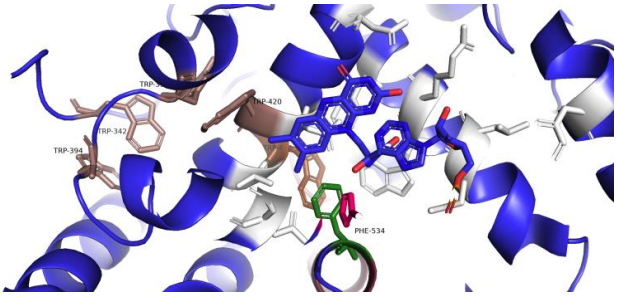

Fig1: Overview of *Drosophila* Cryptochrome binding pocket and the tryptophan-tetrad (TRP-420, 397, 342 and 394) involved in the protein photo-reduction.

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Synthesis and Characterization of a Novel Photocurable Bioink for Printing of Complex Structures

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3D bioprinting (3DBP) offers many unique advantages in the processing of soft materials [1]. Hydrogels possess many necessary attributes to mimic cellular microenvironment and have been extensively explored in cell encapsulation, tissue engineering, and also as bioinks in 3DBP. Printing hydrogels into physically stable complex structures however presents several challenges due to the stringent viscosity requirements during the various stages of extrusion-based bioprinting (EBB). Furthermore, physical or chemical crosslinking has to be employed in order to preserve the geometry of the printed hydrogel. Carboxylated agarose (CA) represents a novel class of bioinks that possess tunable mechanical properties and can yield complex and anatomically relevant structures without the need for support phases [2]. In this study a novel CA-based photocurable bioink (CAPA) with controlled chemical modification of photocurable groups is presented. Through rigorous rheological testing and numerical simulation, the rheological behavior of CAPA during printing was established. Employing a in-house developed print-head equipped with elements to process the bioink in real-time using light, free-standing structures of various degrees of complexity were printing with bioinks of low mass-fraction of CAPA. The real-time light-processing of the bioink supports the printing of human cells within such structures with high viability. Printed cells present protrusion that are important for cell migration and cell-contact and undergo proliferation. Thus, CAPA-based bioinks are very attractive for various applications such as regenerative medicine and organ-on-a chip.

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3D-Printing as a Paradigm for Sustainability in Biomedical Research - Hanging Drop Devices for Multicellular Spheroid Engineering

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While the benefits of modern biomedical research is evident in the enhanced quality of healthcare, biomedical research is also a source of significant plastic pollution as many of the labware used on a daily basis is made of petroleum-based polymers. Much of the labware is single-use plastic that has to be discarded properly and cannot be recycled and hence accumulates in landfills. Sustainability in biomedical research therefore needs to incorporate elements such as point of use and on-demand production of labware, and the use of compostable materials in manufacturing. This can be potentially implemented by combining degradable polymers with 3D-printing (3DP).
In cancer research, systems to recapitulate 3D cell growth are essential for understanding development and cancer biology, as cells organized in 3D environments can evolve certain phenotypic traits that cannot be accessed in 2D culture. Cellular spheroids therefore constitute an important aspect of in vitro tumor biology. Spheroids are prepared the hanging-drop cell culture plate which is highly customized, expensive and hard to recycle and therefore a good model for demonstrating a sustainable solution based on 3DP. Here, the development of bespoke hanging drop devices using 3DP is presented and their utility in the engineering of multicellular spheroids is demonstrated. The design attributes of the hanging drop device take into account the need for high-throughput, high efficacy in spheroid formation, and automation. The spheroids were characterized using light microscopy and histology and showed good morphological and structural integrity and high viability throughout the entire workflow. The systems and workflow presented here represent a user-focused 3DP driven spheroid culture platform which can be reliably reproduced in any research environment and scaled to- and on-demand. The standardization of spheroid preparation, handling and culture should eliminate user-dependent variables and have a positive impact on translational research and enable direct comparison of scientific findings [1].

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