# Tag der Forschung

# Fakultät für Chemie und Pharmazie

## 30. Juni 2023

**POSTER**

# Epigenetics of Eukaryotic Parasites: A Potential Avenue

M. Amann, R. Warstat, A. Walter, O. Einsle, B. Breit, M. Hügle, S. Günther

Marius Amann, Pharmaceutical Bioinformatics, Albertstraße 21, 79104 Freiburg im Breisgau

Various potentially lethal diseases for humans, such as malaria and leishmaniasis, are caused by eukaryotic protozoa [1] Recent studies suggest that disrupting epigenetic pathways may provide new treatment approaches [1]. Here we show our recent advances for LmxBDF5, an essential bromodomain protein (BRD) with two bromodomains (BDs) from *Leishmania Mexicana* [2]. Based on the recently published pan-selective BD inhibitor (BDi) MPM6 [3], we confirmed first hits for both BDs by ITC and solved first cocrystal structures.

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[2] N. Jones, et al. *Nat. Commun.* **2022, 13, 4071.**

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# Advanced Insights into Electrocatalysis Studies through X-ray Absorption Near Edge Structure (XANES) and Extended X-ray Absorption Fine Structure (EXAFS)

S. Esmael Balaghi[1,2,3], Zhiqiang Zeng[1,3], Sven Küspert[1,3], Anna Fischer[1,2,3]

[1]Institute for Inorganic and Analytical Chemistry (IAAC), University of Freiburg; [2]Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), University of Freiburg; [3]Freiburg Materials Research Center (FMF), University of Freiburg

The quest for sustainable and efficient electrochemical systems required a fundamental understanding of the processes at the electrochemical interface, where the electrocatalytic reactions occur. This investigation aims to elucidate these mechanisms, particularly the role of local atomic structure and electronic state, using X-ray Absorption Spectroscopy (XAS) which includes X-ray Absorption Near Edge Structure (XANES) studies and Extended X-ray Absorption Fine Structure (EXAFS).[1] These advanced synchrotron-based spectroscopy techniques provide key insights into the material properties under realistic operation conditions, thus bridging the gap between laboratory-scale research and industrial applications.[2]

Operando XAS approaches permit real-time, in-situ observation of the local electronic structure and chemical state of the electrocatalysts, thereby allowing us to understand the dynamic and structural changes on the working electrode interface during electrocatalysis.[1] Moreover, deep analysis of the EXAFS region, as a vital part of XAS, enables the investigation of local structural transformations, providing information on bond lengths, coordination numbers, and structural disorders. By complementing each other, XANES and EXAFS offer a comprehensive toolkit for unveiling the detailed structure-function relationships in electrocatalysis.

This work particularly highlights our recent research on the application of XAS in studying different electrocatalytic processes, such as hydrogen evolution reaction (HER).[2] The understanding derived from these investigations will guide the rational design and optimization of future electrocatalysts, thereby contributing to the advancement of sustainable energy technologies.

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**Chemical probes to study the role of the lysine demethylase LSD1 in Leishmania infection**

Adina A. Baniahmad, Freiburg/Ger, Dr. Johannes Seitz, Freiburg/Ger, Dr. Johannes Schulz-Fincke, Freiburg/Ger, Prof. Dr. Manfred Jung, Freiburg/Ger, Prof. Dr. Gerald Spaeth, Paris/FR

Adina Baniahmad University of Freiburg, Albertstraße 23, 79104 Freiburg, Germany

Epigenetic mechanisms are involved in numerous diseases, including cancer development and immunological microbial infections. In order to promote their own survival, many viral, bacterial, and eukaryotic pathogens that infect mammalian cells have developed co-evolved ways to alter the expression profile of their host cells. The epigenetic mechanisms causing such host cell subversion are still poorly understood, despite the grave repercussions for human health. Parasites of the genus *Leishmania* subverts the function of the macrophage lysine specific demethylase 1 (LSD1) to establish permissive conditions for intracellular parasite survival [1]. LSD1 (aka KDM1A) is a FAD-dependent amine oxidase that removes methyl groups from mono- and demethylated lysine 4 and 9 residues of histone H3 (H3K9/4me1me2) [2]. It was shown that a leishmania infection causes histone H3 demethylation at the activating H3K4 and repressive H3K9 marks which is linked with the suppression of inflammatory gene expression [3]. By developing chemical inhibitors of LSD1, the pathogenic mechanism of *Leishmania* could be reversed and these could then be recognized and destroyed by macrophages without, however, destroying the macrophage itself.
Therefore, chemical degraders (proteolysis targeting chimeras – PROTACs) were synthesized.
PROTACs are heterobifunctional molecules that simultaneously bind a target protein and an E3 ubiquitin ligase. Through such a ternary complex, the target protein and ligase are brought into close distance by the PROTAC. This allows multiple ubiquitinylation of the target protein by the E3 ligase. Modification with polyubiquitin is recognized by the proteasome and leads to proteasomal degradation.
Additionally for cellular target-engagement analysis, biotinylated LSD1 inhibitors scaffold, based on tranylcypromine, was designed, and synthesized.

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# Rhodium-Catalyzed Stereoselective Cyclization of 3-Allenylindoles and *N*-Allenyltryptamines

A. Becker, C. P. Grugel, B. Breit
Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79104 Freiburg

Herein we report a highly enantio- and diastereoselective rhodium-catalyzed cyclization of *N*-allenyltryptamines and 3-allenylindoles to 6-membered spirocyclic indolenines.[1] This hydrofunctionalization methodology offers the advantage of using a comparably cheap commercially available ligand with low loadings of an affordable rhodium precursor. It was previously reported that cyclization of 3-allenylindoles with a shorter linker, resulted in the formation of functionalized tetrahydrocarbazoles via a transient 5-membered spirocyclic intermediate.[2] Due to their lower ring strain and thus higher intrinsic stability these 6-membered spirocyclic indolenines don't undergo an acid-catalyzed rearrangement to the corresponding annulated heterocycles. Interestingly, an inverted diastereoselectivity can be observed, compared to the before mentioned 5-membered derivatives.[3] The versatility of the reported methodology was shown by applying malonate-tethered substrates as well as tryptamine derivatives with a broad functional group tolerance. Additionally noteworthy is the potential to transform the cyclization products to valuable building blocks for natural product synthesis, such as functionalized spirooxindoles and spiroindolines.

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# EPR study of singlet fission in pentacene doped polymer films

M. Bonengel, M. Mayländer, S. Richert

Max Bonengel, Institut für Physikalische Chemie, Universität Freiburg, Albertstraße 21, 79104 Freiburg

The theoretical maximum efficiency that can be achieved in the generation of electrical power from sunlight using photovoltaic cells is approximately 33%, which is known as the Shockley-Queisser limit. A promising strategy to raise this limit is the formation of two photo-excited correlated triplet excitons by singlet fission (SF). By using the process of SF, the Shockley-Queisser limit can be increased up to 50%. However, SF is a quite complex process and the dynamics between the initial and final states are still not completely understood [1,2].
Transient electron paramagnetic resonance (EPR) is a suitable method to gain valuable information about the dynamics and intermediate states involved in the SF process. In contrast to optical methods, the different involved spin states can clearly be distinguished by EPR spectroscopy. Here, we apply different transient EPR methods to investigate the SF process in a variety of pentacene doped polymer films. We show that we are able to identify and characterise the different occurring spin-species as well as their kinetics at room temperature.

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# Rhodium-Catalyzed Enantioselective Intramolecular Hydroalkoxylation of Allenes towards Tetrahydropyranes, Tetrahydrofuranes and Morpholines

M. Daiger, D. Berthold, B. Breit

Martin Daiger, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79104 Freiburg

Oxygen and nitrogen containing cyclic compounds are widely found in natural and pharmaceutical products[1]. Therefore, procedures for the enantioselective synthesis of these compounds are of utmost importance[2-3].

Herein, we report the development of a Rhodium-catalyzed cyclization of alcohols towards allenes. This methodology provides selective access towards tetrahydropyranes, tetrahydrofuranes and morpholines. High yields, high enantiomeric excess and a good functional group tolerance were achieved.

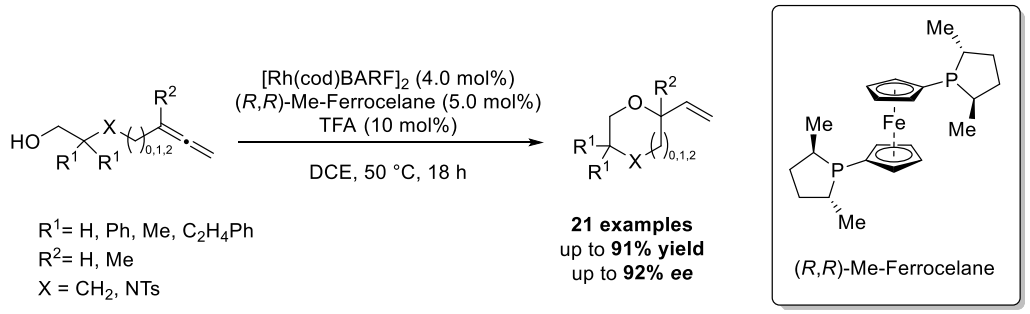


**Figure 1**. Reaction conditions of the Rhodium-catalyzed cyclization of alcohols towards allenes.

Furthermore, the utility of this method was demonstrated by the asymmetric total synthesis of $\alpha$-Tocopherol.

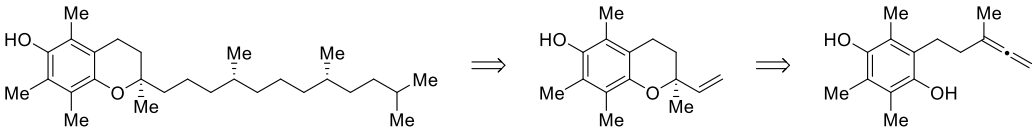

**Figure 2**. Retrosynthetic analysis of $\alpha$-Tocopherol.

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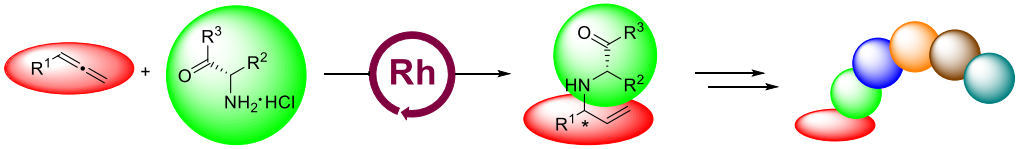
# Rh-catalyzed Hydroamination of Allenes: Asymmetric Functionalization of Amino Acids and Peptides

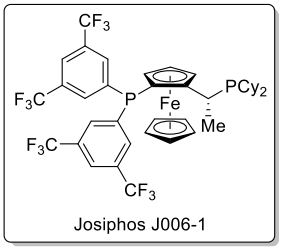
*Edward Damer and Bernhard Breit*
*Institut für Organische Chemie, Albert-Ludwigs-Universität Freiburg*
*Albertstr. 21, 79104 Freiburg i. Br., Germany*
*bernhard.breit@chemie.uni-freiburg.de*

Since the synthesis of insulin in 1921 and its use as first commercial therapeutic peptide in 1923, development of peptide drugs has become an important field in pharmaceutical research.[1] To further improve pharmaceutical properties like metabolic stability, bioavailability, receptor activity and selectivity, the use of peptidomimetics has become a growing field in drug discovery likewise. The strategy of the peptidomimetic approach is to perform focused modifications to the peptidic backbone or side chains.[2] In our group, numerous methods to hydrofunctionalize allenes with C-, N-, O-, and S-nucleophiles to obtain branched allylic compounds stereoselectively have been developed.[3] However the use of aliphatic amines as nucleophiles remained an unsolved problem due to basicity of the amine moiety and possible overalkylation.

In this work a catalytic method is described, which enables stereoselective and atom-economic allylation of aliphatic amines focusing on amino acids and small peptides. As possible application the syntheses of two peptidomimetic compounds utilizing solid phase peptide synthesis (SPPS) were performed.




- 32 examples
- yields up to 98%
- *d.r.* up to >95:5
- catalyst-controlled stereoselectivity
- gram-scale reactions
- workup without column chromatography
- pentapeptides as suitable substrates



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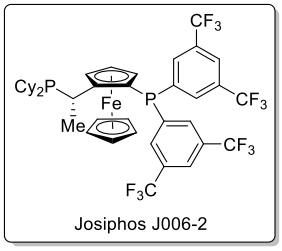

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# Modulation and integration of host cell signalling pathways *via* mycobacterial substrate-binding lipoproteins

T. Dao, M. Li, O. Gorka, O. Groß, C. Jessen-Trefzer

Trinh Dao, Institute for Organic Chemistry, University Freiburg, Alberstr. 21, 79104 Freiburg

Mycobacterium tuberculosis (Mtb) is a highly significant infectious agent responsible for millions of deaths each year, despite antibiotic treatment. Our project aims to analyze the interactions between substrate binding lipoproteins (SBPs) and macrophages, as well as their downstream signaling pathways. By understanding how SBPs interact with host cells, we can potentially target them for the development of new antibacterial agents and anti-tuberculosis vaccines. SBPs are proteins that anchor in membranes using a lipid tail and play a crucial role in nutrient and substrate transport [1]. They also act as important virulence factors, interacting with host cells and activating the immune system through Toll-like receptors (TLRs) [2], [3].

We have chosen to initially investigate PstS1, an SBP of a phosphate ABC-transporter [4], out of the 16 known Mtb SBPs. The gene encoding the lipoprotein of interest was expressed in Mycobacterium smegmatis (Msm), a fast-growing member of the Mycobacteriaceae family. The expressed lipoprotein was then isolated and purified for downstream assays with macrophages.

In our second approach, we plan to express Mtb lipoproteins without the signal peptide for lipid posttranslational modification in E.coli. The purified non-lipidated lipoprotein will be subsequently ligated with the lipid residue in vitro, using lysate from Msm or Mtb H37Ra. This modified lipoprotein will be used to study the signaling pathways in macrophages triggered by SBPs. We will use knock-out mutants of the respective SBP genes as controls.

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# The Three-dimensional Structure of Fe-only nitrogenase

Franka Detemple, Christian Trncik, Oliver Einsle*

*Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Albertstraße 21, 79104 Freiburg, Germany, E-Mail: einsle@biochemie.uni-freiburg.de*

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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# Non-hydrolysable analogues of cyclic and branched condensed phosphates for chemical proteomics

Tobias Dürr-Mayer, Andrea Schmidt Luther, Stefan Wiesler, Tamara Huck, Andreas Mayer, Henning J. Jessen

Tobias Dürr-Mayer, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79100 Freiburg im Breisgau

Studies on the biology of condensed phosphates almost exclusively cover linear polyphosphates.[1] However, evidence was given for the presence of cyclic polyphosphates (metaphosphates) in bacterial phosphate granules[2] and interaction of alkaline phosphatase with branched phosphates (ultraphosphates) has been demonstrated.[3] Efforts to delineate the elusive interactome of non-linear polyphosphates are yet challenged by the sensitivity of modified meta- and ultraphosphates.[3-5] Here we present syntheses of modified, non-hydrolysable analogues of cyclic and branched condensed phosphates, called meta- and ultraphosphonates, and their application in a chemical proteomics approach with yeast cell extracts. We identify putative receptors with overlapping hits for structurally related capture compounds underlining the quality of our results. The datasets serve as starting point to study the biological relevance and functions of meta- and ultraphosphates in further investigations. In addition, we examine the reactivity of meta- and ultraphosphonates: Efforts to increase the ring-sizes of meta- or cyclic ultraphosphonates revealed a strong preference to form trimetaphosphate-analogue structures by cyclization or ring-contraction. Using carbodiimides for condensation, the hitherto inaccessible dianhydro product of ultraphosphonate, corresponding to $P_4O_{11}^{2-}$, was selectively obtained and then ring-opened by different nucleophiles yielding modified, cyclic ultraphosphonates.

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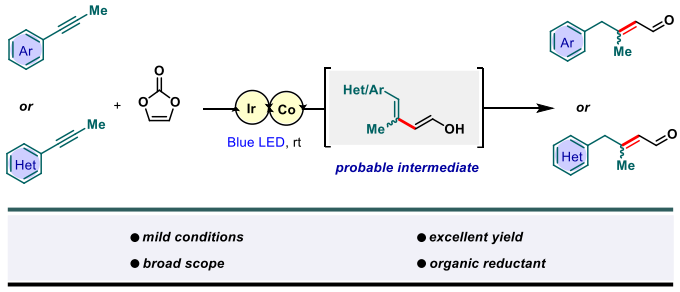
# Light Mediated Photoredox/Cobalt Dual Catalysis for Trisubstituted Enal Synthesis from Alkyne Feedstock

Uttam Dutta, Bernhard Breit*

Institut für Organische Chemie,
Albert-Ludwigs-Universität Freiburg, Albertstr. 21, 79104 Freiburg, Germany
*bernhard.breit@chemie.uni-freiburg.de

**Abstract:**

Over the last few decades, organocatalysis has emerged as a powerful synthetic tool to synthesize valuable structural motifs. Vast majority of these organocatalytic reactions utilize $\alpha,\beta$-unsaturated aldehydes, i.e. enals as substrates. Considering it's huge applicability in synthetic chemistry, development of a novel, straightforward and efficient method to prepare enal under mild and operationally simple reaction conditions is highly desirable. Herein, we report a light-mediated reductive ene-yne coupling reaction between alkyne and vinylene carbonate to furnish enals under dual catalytic activity of cobalt and Ir-based photocatalyst in association with sacrificial organic reductant, Hantzsch ester. The developed method is compatible with a broad range of substituted arenes as well as different classes of heteroarenes.



# CryB from *Rhodobacter sphaeroides* – an iron-sulfur flavoprotein with photochemical activity

Christopher Einholz, Johannes Berger, Andreas Günter, Stefan Weber and Erik Schleicher

Christopher Einholz, Albert-Ludwigs-Universität Freiburg, Albertstraße. 21, 79104 Freiburg

Flavoproteins are ubiquitous and hence can be found in every domain of life. While flavoproteins display a wide variety of functions and structural features, they all contain a flavin cofactor, usually flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). A special family of flavoproteins, the photolyase/cryptochrome superfamily (PCSf), derives its function from light-induced processes such as formation of triplet states at the flavin or the formation of radical pairs between the flavin cofactor and an electron donor such as DNA lesions or aromatic amino acids such as tryptophan or tyrosine [1][2].

CryB from *Rhodobacter sphaeorides* (*Rs*CryB) belongs to a recently discovered PCSf subclass that harbors two additional cofactors: a 6,7-dimethyl-8-ribityllumazine (Lum) and an iron-sulfur cluster (FeS) [1]. Albeit several studies employed a multitude of techniques ranging from molecular biology to *in-vivo* photorepair assays and thereby provided valuable information about this new subclass, an in-depth spectroscopic characterization is still lacking [1][2]. In the presented work, steady-state techniques such as fluorescence and UV-Vis spectroscopy are combined with time-resolved methods such as transient absorption (TA) and transient EPR (trEPR) spectroscopy to elucidate the reactions and photochemical processes following light-excitation of *Rs*CryB. State-of-the-art data analysis allowed the drawing of a photocycle, while open questions will be critically discussed. Finally, the role of Lum-FeS-flavoproteins – both in the biological and evolutionary sense – is discussed in light of these new and previously published results from other members of the PCSf.

Funding: RTG 1976 (Functional Diversity of Cofactors in Enzymes)

**Literatur :**

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# Precious-metal free Catalysts for Anion Exchange Membrane Fuel Cells

Patrick Elsässer[1,2,3], Esmael Balaghi[1,2], Jan Ochsler[1,2,3], Eliot Petitdemange[1,2], Caroline Schneider[2], and Anna Fischer[1,2,4]*

[1] Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), Freiburg, Germany, [2] Institute of Inorganic and Analytical Chemistry, Freiburg, Germany, [3] Cluster of Excellence livMatS@FIT, Freiburg, Germany, [4] Freiburg Materials Research Center, Freiburg, Germany

Hydrogen technologies such as low-temperature fuel cells are, besides batteries, the most promising technology to decarbonize the energy production and storage. Even though the Proton Exchange Membrane Fuel Cell (PEMFC) is the State-of-the-Art low-temperature fuel cell, the highly corrosive environment (pH 1) makes it necessary to use noble metals as catalysts for both the hydrogen oxidation reaction (HOR) and oxygen reduction reaction (ORR). However, catalyst degradation is still a major issue and limits the broad application of PEMFCs. In contrast, the Anion Exchange Membrane Fuel Cell (AEMFC) has gained increasing interest as a potent alternative in recent years since it combines the advantages of the PEMFC, like low-temperature operation and high-power density, with potentially significant cost reductions.[1,2] Especially the possibility to use non-noble metal catalysts could significantly decrease the fuel cell costs and increase the longevity due to the less corrosive environment.[1,3,4]

Among the variety of non-noble metal catalysts, Fe- and N-doped carbons (Fe-N-C) with molecular iron sites (Fe-$N_x$) were intensively investigated for the ORR. These materials show comparable catalytic activities to the expensive Pt-electrocatalysts in both acidic and alkaline media.[5–7]. For the HOR, nanoparticular Ni and its alloys supported on conductive carbons show the highest activities and stabilities for non-noble metal catalysts in alkaline media.[8-10] Especially alloying Ni with molybdenum (NiMo) or copper (NiCu) results in catalysts with promising activities. However, the HOR activities of these materials are still not comparable to commercial Pt-based catalysts and further research is required to improve their activity.

As ORR cathode catalysts, we synthesized Fe/$ZnN_x$-N-C materials using Fe- and Zn-doped MOFs as multi-component Fe-, Zn-, N-, and C-precursors and carbonized them at high temperatures in an inert atmosphere. The obtained Fe/$ZnN_x$-N-C catalysts exhibit a high dispersion of molecular $FeN_x$ and $ZnN_x$ sites as revealed by XRD, XAS, EDX, and XPS. Benefiting from this, the Fe/$ZnN_x$-N-C catalysts show high ORR activity in aqueous alkaline electrolyte (0.1 M KOH) as demonstrated by rotating disk electrode (RDE) experiments. These materials were also investigated in an AEMFC, where the best performing catalyst showed a high peak power density of 850 mW $cm^{-2}$, which is the highest reported peak power densities for non-noble metal catalysts in combination with a commercially available ionomer.

To increase the HOR activity of NiMo anode catalysts as well as the stability against agglomeration of the nanoparticles (NPs), we developed a synthesis strategy for NiMo alloy NPs supported on mesoporous N-doped carbon spheres (MPNCs). We investigated the influence of different MPNC pore sizes (7, 12, and 23 nm) as well as different metal loadings on the HOR activity in alkaline media (0.1 mol/L KOH) using RDE measurements. The synthesized catalysts show promising HOR activities compared to other NiMo catalysts reported in literature.

## Conformational protection of Mo nitrogenase by the FeSII (Shethna) protein

*Philipp Franke*,[1] *Julia Netzer*,[1] *Michael Rohde*,[1] *Oliver Einsle*,[1]

[1] *Institute for Biochemistry, Albert-Ludwigs-Universitat Freiburg, Albertstrasse 21, 79104 Freiburg, Germany*

E-mail: franke@bio.chemie.uni-freiburg.de

The two-component metalloprotein nitrogenase catalyzes the reductive fixation of atmospheric dinitrogen into bioavailable ammonium in diazotrophic prokaryotes. [1] The process requires an efficient energy metabolism, so that although the metal clusters of nitrogenase rapidly decompose in the presence of dioxygen, many free-living diazotrophs are obligate aerobes. In order to retain the functionality of the nitrogen-fixing enzyme, some of these are able to rapidly “switch-off” nitrogenase, by shifting the enzyme into an inactive but oxygen-tolerant state. [2] Under these conditions the two components of nitrogenase form a stable, ternary complex with a small [2Fe:2S] ferredoxin termed FeSII or the “Shethna protein II”. [3] The crystal structure of FeSII from *Azotobacter vinelandii* revealed two distinct states that differ in the conformation of an extended loop in close proximity to the iron−sulfur cluster. This rearrangement is redox-dependent and forms the molecular basis for oxygen-dependent conformational protection of nitrogenase. [4]

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# X-Ray crystallography of inhibitor complexes of the human NAD$^+$-dependent lysine deacylase Sirtuin 2

F. Friedrich, Freiburg DE, A. Vogelmann, Freiburg DE, M. Schiedel, Erlangen DE, S. Hammelmann, Freiburg DE, L. Zhang, Freiburg DE, O. Einsle, Freiburg DE, M. Jung Freiburg DE

Florian Friedrich, University of Freiburg, Albertstraße 25, 79104 Freiburg i. Br.

Sirtuins are NAD$^+$-dependent protein deacylases. They cleave off acetyl and other acyl groups from the ε-amino group of lysines in histones and other substrate proteins. The human isotype Sirt2 has been implicated in the pathogenesis of cancer, inflammation, and neurodegeneration, making Sirt2 a promising target for pharmaceutical intervention. In our group several Sirt2 inhibitors have been developed that are characterized by a selective ligand-induced structural rearrangement of the active site and are referred to as Sirtuin-Rearranging ligands (SirReals). [1] Here, we present a crystal structure of a SirReal-like inhibitor with enhanced potency and dual inhibition of Sirt2-mediated deacetylation and defatty-acylation in cells. [2] The crystal structure reveals pH-dependent inhibitor-specific interactions with side chain residues of Sirt2 that may be responsible for the more potent cellular effect compared to previous SirReals. These data provide the opportunity for further optimization of the inhibitor scaffold.

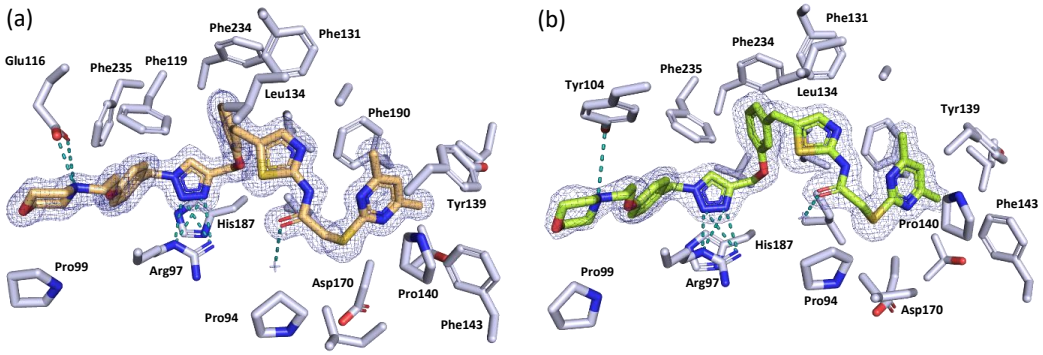


Figure 1: Two crystal structures of the same Sirt2-**Inhibitor** complex that were crystallized at pH 5.5 (a) and at pH 6.5 (b). The pH-dependent protonation status of the morpholine residue protruding from the acyl-lysine binding channel induces a shift of several amino acids of the co-factor binding loop. As a result, polar interactions are formed between the inhibitor and Glu116 at pH 5.5 and Tyr104 at pH 6.5, respectively.

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# Investigation of the antimicrobial potential of Streptomyces from the bark beetle microbiome

A. Goll, A. Bechthold

Anne K.C. Goll, Institute for Pharmaceutical Biology and Biotechnology, Albert-Ludwigs-University Freiburg/Germany, anne.goll@pharmazie.uni-freiburg.de

Actinobacteria, particularly the genus Streptomyces, are an excellent source of potent natural products with antibiotic and antifungal properties. In the face of increasing antibiotic resistance, sources other than soil are being sought. It is hoped that challenging environments (such as marine sediments, plant roots or insects) will force Streptomyces to adapt well and thus offer great potential for the formation of bioactive secondary metabolites. It has already been shown that Streptomyces living with insects such as fungus-growing ants, bees and bark beetles, e.g. the North American Southern pine beetle, are able to produce interesting new antimicrobial compounds [1].
In this context, the microbiome of central European bark beetles is isolated and incubated to search for novel species. Our aim is to screen these Streptomyces by a genome mining approach for the presence of putative biosynthetic gene clusters (BGCs) and to associate them with compounds found in the extracts. 71 Streptomyces from the microbiome of European bark beetles, such as the European spruce bark beetle, were already analyzed by 16S rRNA sequencing and taxonomic analysis were performed through phylogenetic tree construction. Three potentially novel Streptomyces were identified and whole genome sequencing was performed. Crude extracts were purified and screened for bioactive compounds. So far three novel Streptomyces species were identified. Bioinformatic analyses suggest that they have a high potential for the production of antimicrobial agents.

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# Photoactivated inactivation of mycobacteria

M. Grimmeisen, C. Jessen-Trefzer

Michael Grimmeisen, University of Freiburg, Albertstraße 21, Freiburg im Breisgau

Mycobacteria, belonging to the phylum Actinomycetota, possess a distinct and fascinating cell structure comprising a cytoplasmic membrane and an overlaid peptidoglycan layer. Additionally, they exhibit other structural elements, including an arabinogalactan layer directly attached to the mycomembrane. The inner leaflet of the membrane is composed of long mycolic acids linked to arabinogalactan, while the outer leaflet primarily consists of TMM (trehalose monomycolate) and TDM (trehalose dimycolate). These constituents are synthesized through the extracellular Ag85 enzyme complex. In this project, the potential of this enzyme complex to incorporate not only trehalose but also trehalose analogs is being explored. As previously demonstrated by various research groups, mycobacteria can be specifically modified using trehalose derivatives[1,2].

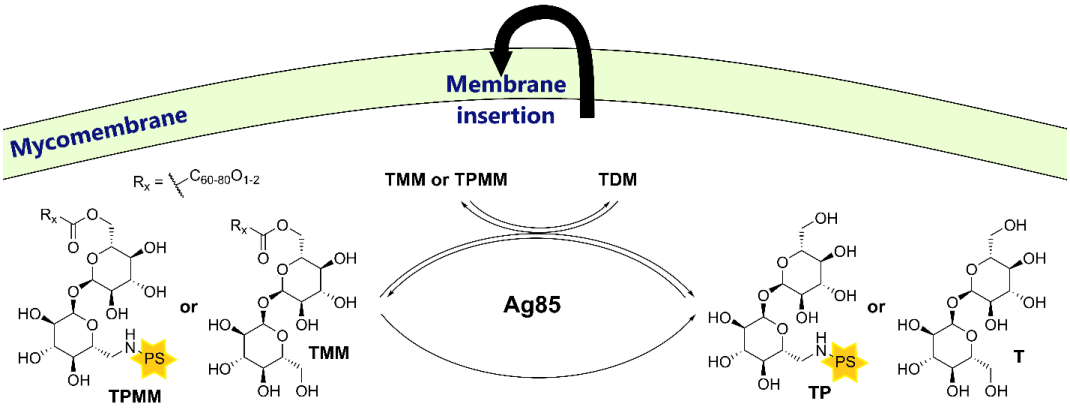


Figure 1: Schematic illustration of the incorporation of trehalose derivatives into the mycomembrane. **T** is for trehalose, **TP** for trehalose-photosensitizer derivative, **TMM** for trehalose monomycolate, **TDM** for trehalose dimycolate and **TPMM** for trehalose-photosensitizer monomycolate. (Adapted from Dutta *et al.* (2019)[1])

The work published by our group in 2019, already demonstrated the potential of bacterial functionalization, allowing for photoinduced inactivation of mycobacteria through the generation of reactive oxygen species (ROS) upon exposure to light irradiation[1,3]. Building upon this foundation, our objective is to develop enhanced photosensitizer-trehalose conjugates specifically tailored for the treatment of skin infections caused by pathogens like *Mycobacterium marinum*. Additionally, we aim to explore the potential of utilizing photosensitizer-modified *Mycobacterium bovis* BCG for the treatment of bladder cancer therapy[4]. Our current synthesis endeavors are primarily centered around BODIPY-photosensitizers and Nile-Blue derivatives. Through our efforts, we have successfully established an enhanced synthesis and purification protocol for known BODIPY-photosensitizers. Presently, our focus is on creating a diverse range of compounds to construct a small library. This library will enable us to evaluate the light-induced killing efficiency against the aforementioned mycobacterial strains, both *in vitro* and within macrophages.

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# Selective Phosphorylation of 1-OH-InsP$_5$ *via* transient phosphitylation

Markus Häner, Henning J. Jessen
Institute of Organic Chemistry, Albert-Ludwigs University, Freiburg
Albertstr. 21, 79104, Freiburg i. Breisgau, Germany
markus.haener@oc.uni-freiburg.de

Inositol-Phosphates (InsP$_x$) play a vital role in all living systems, synthesis of these molecules is crucial to study the various processes they partake[1]. They possess an enormous diversity due to the number of stereocenters and possible phosphorylation-patterns found *in vivo,* leading to either elaborate protecting group strategies or to the strict reliance on enzymatic procedures. However, an amalgamation of the aforementioned strategies is unusual, since the functionalization of free alcohols in the presence of unprotected phosphate-moieties in the same molecule is still a challenging endeavor. Here we show the functionalization of the free alcohol of 1-OH-InsP$_5$ in the presence of five unprotected phosphates using a novel “transient phosphitylation” approach. 1-OH-InsP$_5$ is available on a gram scale by enzymatic dephosphorylation using InsP$_6$ and *XopH*[2]. Reaction of the free alcohol of the unsymmetric 1-OH-InsP$_5$ was achieved by global phosphitylation, exploiting the inherent instability of P(III)-P(V)-bonds, thus allowing the introduction of a protected phosphate-ester in the presence of five unprotected phosphates. Initial de-phosphorylation experiments indicated stability of the protected phosphate towards enzymatic de-phosphorylation, effectively inverting the phosphorylation pattern of the starting material. The novel transient phosphitylation protocol enabled the phosphorylation of free alcohols in the presence of unprotected phosphates, paving the road for new synthetic routes in the field of phosphate chemistry.

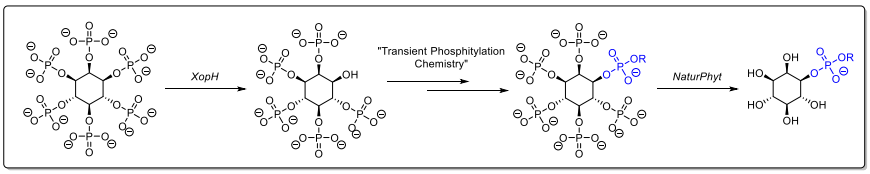


**Figure 1**. Transient Phosphitylation Chemistry of 1-OH-InsP$_5$.

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# A trick of the tail:
# computing the entropic contribution
# to the energetics of
# quinone-protein unbindung

J.Haxhija, F. Guishard, T. Koslowski

Albert-Ludwigs-Universität Freiburg, Albertstr. 21, Freiburg

We estimate the entropic contributions to the free energy of quinone binding in bacterial and mitochondrial respiratory chains using molecular dynamics (MD) and Monte Carlo (MC) computer simulations. For a varying length of the isoprenoid side chain, MD simulations in lipid bilayers and in unpolar solvents are used to assess the dihedral angle distributions along the chain. These form the basis of a MC estimate of the number of molecular structures that do not exhibit steric self-overlap and that are confined to the bilayer. We obtain an entropy drive of $T\Delta S$ = 1.4 kcal/mol for each isoprene unit, which in sum is comparable to the redox potential differences involved in respiratory chain electron transfer. We postulate an entropy-driven zipper for quinone unbinding and discuss it in the context of the bioenergetics and the structure of complex I, and we indicate possible consequences of our findings for MD-based free energy computations.

# The last steps of heme $d_1$ biosynthesis

Pia Hebecker, Heike Meyer, Gunhild Layer

Pia Hebecker, University of Freiburg, Institute of Pharmaceutical Sciences, Pharmaceutical Biology, Stefan-Meier-Str. 19, 79104 Freiburg

The opportunistic pathogen *Pseudomonas aeruginosa* is able to use nitrate or nitrite as electron acceptors instead of oxygen to generate energy under anaerobic conditions. The reduction of nitrite to nitric oxide is catalyzed by the cytochrome c$d_1$ nitrite reductase NirS, which contains the unique cofactor heme $d_1$. The biosynthesis of heme $d_1$ starts from the common tetrapyrrole precursor uroporphyrinogen III, which is transformed into 12,18-didecarboxy-siroheme (DDSH) by several enzymatic steps. Then, DDSH is converted into the heme $d_1$ precursor dihydroheme $d_1$. First, the radical SAM enzyme NirJ catalyzes the removal of two propionate side chains of DDSH.[1] It is postulated that the NirJ reaction product carries two hydroxyl groups instead of these propionate groups. Second, the hydroxyl groups must be oxidized to yield the keto functions of dihydroheme $d_1$. Our hypothesis is that NirF and NirC are responsible for this oxidation reaction. NirF could act as dehydrogenase with the *c*-type cytochrome NirC as the electron acceptor.[2] In order to test this hypothesis, we produced and purified recombinant NirF and NirC, and performed enzyme activity assays together with the NirJ reaction product. By HPLC analysis of the assay mixtures we observed the formation of dihydroheme $d_1$. Moreover, the reduction of NirC in the course of the reaction was detected by UV/Vis absorption spectroscopy.

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# Investigation of variants in the proton channel of the Escherichia coli bd-I and bd-II oxidase

L. Heger, J. Kägi, T. Friedrich

Lucia Heger, Jan Kägi und Prof. Dr. Thorsten Friedrich, Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, D-79104 Freiburg, Germany

The cytochrome *bd* oxidases are members of the terminal oxidases in the oxidative phosphorylation of *E. coli* and couple the reduction of oxygen to water, with the oxidation of quinol. Thus, they generate a proton motive force, which is then further used for ATP-production. As the *bd* oxidases are insensitive for respiratory chain inhibitors (e.g. NO, $CN^-$) and also expressed under oxygen limited conditions, they promote virulence in bacteria for example in *Mycobacterium tuberculosis*. Therefore, they are potential drug targets and special interest lies on their structure-function relationship.

Due to that, also the proton channel of the *bd* oxidases, that leads to heme *d*, their active center, is investigated. However, the question of which amino acid residues are crucial for the proton transport, is not fully clarified yet. Here, the proton channel of the *bd* oxidases was investigated by point-mutations of single amino acids. Cloning, purification, redox-difference spectra and activity measurements revealed, that the interaction of the superimposed amino acid residue pair $W57^{cydB}$/$D58^{cydB}$ in the *bd*-I oxidase proton channel, seems to be crucial for a sufficient proton transport. Moreover, does the residue $D105^{appB}$ in the *bd*-II oxidase seem to have a catalytic effect on the proton transfer activity.

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# Atomic layer deposition of titanium oxide over mesoporous nitrogen-doped carbon

S. Heidari[1,2,3,4], N. Ortlieb[1,3], A. Fischer[1,2,3,4]

[1] Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany
[2] Cluster of LivMats@FIT, University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany
[3] Institute for Inorganic and Analytical Chemistry, University of Freiburg, Albertstraße 21, 79104 Freiburg, Germany
[4] Freiburg Materials Research Center (FMF), University of Freiburg, Stefan-Meier-Straße 19, 79104 Freiburg, Germany

Developing functional coatings on high aspect ratio powders has garnered significant attention in various fields, from energy storage to catalysis.[1,2] In this study, we present an atomic layered deposition (ALD) approach to deposit a $TiO_2$ coating on mesoporous nitrogen-doped carbon (MPNC), using titanium(IV) isopropoxide (TTIP) and water ($H_2O$) as the reactants.

The ALD process allows for precise control over the thickness and uniformity of the $TiO_2$ coating over the highly porous MPNC. The interconnected pore structure of MPNC offers enhanced accessibility for reactant diffusion, implementing uniform $TiO_2$ coating. The experimental setup involved a thermal ALD reactor with a rotating canister for powder materials, where TTIP and $H_2O$ were sequentially introduced as alternating pulses. The precursor exposure time and purge times were carefully optimized to achieve a conformal and uniform $TiO_2$ coating. The growth rate of the $TiO_2$ was monitored in situ using a quartz microbalance (QCM) across the reaction chamber. Furthermore, characterization techniques such as transmission electron microscopy (TEM), and scanning transmission electron microscopy/energy dispersive X-ray analysis (STEM/EDX) were employed to investigate the chemical composition and structural properties of the $TiO_2$-coated MPNC. The results revealed $TiO_2$ deposition with no apparent change in the structure of the carbon material.

In conclusion, the $TiO_2$ coating on highly porous mesoporous carbon, by applying the ALD technique and using TTIP and $H_2O$ as reactants, offers a promising approach for developing advanced functional materials.[1,2] The conformal and uniform coating, combined with the unique properties of the carbon substrate, presents opportunities for various applications in energy storage, as well as electrocatalysis.

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**Characterization of the cobalamin-dependent *Radical* SAM methyltransferase QCMT involved in maturation of methyl-coenzyme M reductase**

Hannah Antonia Herrmann[1], Gunhild Layer[1]

[1]*Institute of Pharmaceutical Science, University of Freiburg, 79104 Freiburg, Germany*

The enzyme methyl-coenzyme M reductase (MCR) plays an important role in the global carbon cycle due to its ability to catalyze both, the formation and consumption of methane in methanogenic archaea and anaerobic methane-oxidizing archaea, respectively [1]. This enzyme possesses several unusual post-translational amino acid modifications, including 2-C-(*S*)-methylglutamine [2]. The *Radical* SAM methyltransferase (QCMT) is responsible for this chemically challenging methylation of the unreactive $sp^3$ carbon atom at C2 of glutamine [3]. QCMT belongs to the subfamily of cobalamin-dependent Radical SAM methyltransferases and was initially characterized in a recent study.

In this project, we aim to solve the structure QCMT through crystallization and x-ray. Using the sitting drop method, first protein crystals were generated under anaerobic conditions, however, they have not been analyzed yet.

The second aim is the elucidation of the reaction mechanism. It has been demonstrated that QCMT catalyzes the transfer of a methyl group to the glutamine residue of a peptide substrate via a radical-based mechanism. During the methylation reaction, two molecules of SAM are consumed, and 5'-deoxyadenosine (DOA) and *S*-adenosyl-L-homocysteine (SAH) are formed as coproducts in a nearly 1:1 ratio. The methyl group itself originates from enzyme-bound methylcobalamin, which is re-methylated by SAM. It was also shown that peptides of different lengths can be used by QCMT. In further studies, the role of amino acid resides within the active site as well as of the peptide substrate will be investigated.

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# Auf dem Weg zu „anodenfreien“ Ca- und Mg-organischen wiederaufladbaren Batterien

E. Jacob, H. Koger, N. Dorrani, M. A. Epting, A. Schmidt, I. Krossing

Albert-Ludwigs-Universität Freiburg, IAAC, Albertstr. 21, Freiburg
und Freiburger Materialforschungszentrum, Stefan-Meier-Str. 21, Freiburg

Um die Anwendung von gut verfügbarem und bivalentem Mg oder Ca (sehr niedriges Reduktionspotential als negative Elektrode in wiederaufladbaren Batterien zu ermöglichen, müssen verschiedene Voraussetzungen an der Elektrolyt-Elektroden-Grenze erfüllt sein: (1) der ungehinderte $M^{2+}$ Transport zwischen Elektrolyt und Elektrode, (2) keine Nebenreaktionen des Metalls mit dem Elektrolyten, (3) die chemische und mechanische Stabilität während der elektrochemischen Prozesse.
Da die nativen Oberflächenverunreinigungen nicht $M^{2+}$-permeabel sind, muss zunächst die Oberfläche der eingesetzten Metalle von Kontaminationen aus der Reaktion mit Atmosphärilien oder Prozessölen befreit werden. Hierfür werden sie mit mechanischen Mitteln und ggf. organischen Lösungsmitteln unter inerten Bedingungen behandelt. Die dadurch erhaltenen Metalloberflächen sind hochreaktiv. Die eingesetzten Elektrolyte müssen ihnen gegenüber daher thermodynamisch oder kinetisch stabil sein, oder bei Reaktion mit ihnen stabile $M^{2+}$-permeable Grenzphasen (*SEI*s) bilden, um (1+2) zu erfüllen.[1] Die Inertheit gegenüber $M^0$ soll dabei aber nicht auf Kosten der Inertheit gegenüber anderen Zellkomponenten oder der Oxidationsstabilität der Elektrolyte erreicht werden.[1] Daher werden nichtkorrosive Salze mit stabilen schwach koordinierenden Anionen (*WCA*s) eingesetzt.[2] Einfache *WCA*s (bspw. $[PF_6]^-$ oder $[TFSI]^-$), die häufig in Lithiumbatterien eingesetzt werden reagieren jedoch oft mit Ca oder Mg unter Bildung isolierender Grenzphasen.[3] Wir konnten zeigen, dass polyfluorierte Alkoxyborate und -aluminate – WCAs der *Krossing Group* – in ihrer Reaktivität abgestufte Eigenschaften gegenüber Magnesium zeigen.[4] Während das perfluorierte $[pf]^-$-Anion ($[Al(OC(CF_3)_3)_4]^-$) isolierendes $MgF_2$ bildet, ist die Zersetzung von $[B(OC(H)(CF_3)_2)_4]^-$ kinetisch gehemmt.[4] $[Al(OC(H)(CF_3)_2)_4]^-$ ist thermodynamisch stabil und für Mg die optimale Wahl.[4] Hieraus ergeben sich auch Rückschlüsse für die Wahl geeigneter Anionen in Ca-Elektrolyten.
Eine ergänzende Idee ist es, gezielt schützende $M^{2+}$-leitfähige Grenzphasen (artifizielle *SEI*s) auf den Metallen zu erzeugen.[5] Diese können durch Beschichtungsreaktionen aus Lösung direkt auf das Metall aufgebracht oder durch geeignete Additive im Elektrolyten *in operando* generiert werden.[6] Die Wahl geeigneter Elektrolytadditive soll dabei durch die Analytik geeigneter Beschichtungen abgeleitet werden.
Das übergeordnete Ziel ist es, die reaktiven Metalle an geeigneten Stromabnehmern erst beim Laden aus der metallierten Form einer organischen positiven Elektrode zu gewinnen und beim Entladen wieder vollständig aufzulösen (hohe *Coulomb*-Effizienz), sodass man ohne Metallüberschuss und Beschichtungen auszukommt („anodenfrei“).[7]

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# New Inositol Pyrophosphate Prometabolites for *in vivo* Release

N. Jork, J. Ma, H. J. Jessen

Nikolaus Jork, *Albert-Ludwigs-University Freiburg*, *Albertstr. 21, 79104 Freiburg*

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.

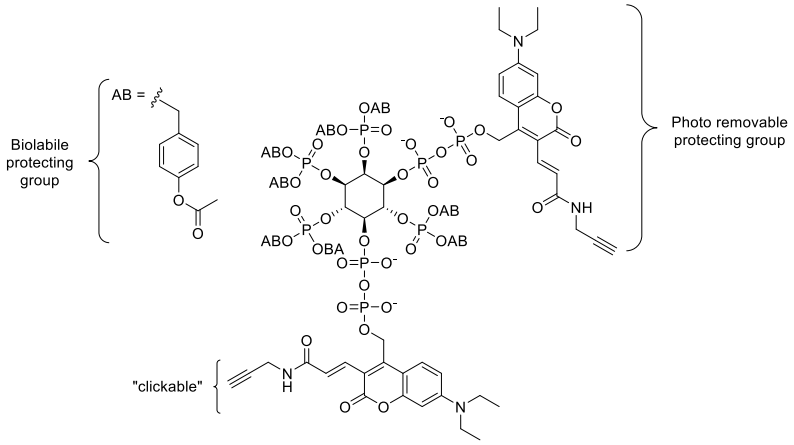


**Figure 1.** A prometabolite of the isomer 1,5-$PP_2$-$InsP_4$, with the photocage DEAC450

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# Characterisation of *S*-Adenosyl-L-homocysteine Hyrolases: Structure, Function and Substrate Preferences

Lars-Hendrik Köppl[1], Désirée Popadić [1], Raspudin Saleem-Batcha[1], Jennifer N. Andexer[1]

[1] University of Freiburg, Institute of Pharmaceutical Sciences
Albertstr. 25, 79104 Freiburg, Germany
lars-hendrik.koeppl@pharmazie.uni-freiburg.de

*S*-Adenosyl-L-homocysteine hydrolase (SAHH) catalyses the reversible cleavage of *S*-adenosyl-L-homocysteine (SAH), a by-product and potent feedback-inhibitor of *S*-adenosyl-L-methionine (SAM) dependent methylation reactions (**Figure 1**) [1,2]. Their regulatory role in the methylation potential of living cells from eukaryotes, bacteria and archaea makes SAHHs an interesting target for drug development [3]. In *Methanocaldococcus jannaschii*, an alternative pathway for recycling of SAM metabolites was elucidated, including a 5'-deoxyadenosine deaminase (DadD) followed by a SAHH homologue with a preference for *S*-inosyl-L-homocysteine (SIH), the product of SAH deamination [4].

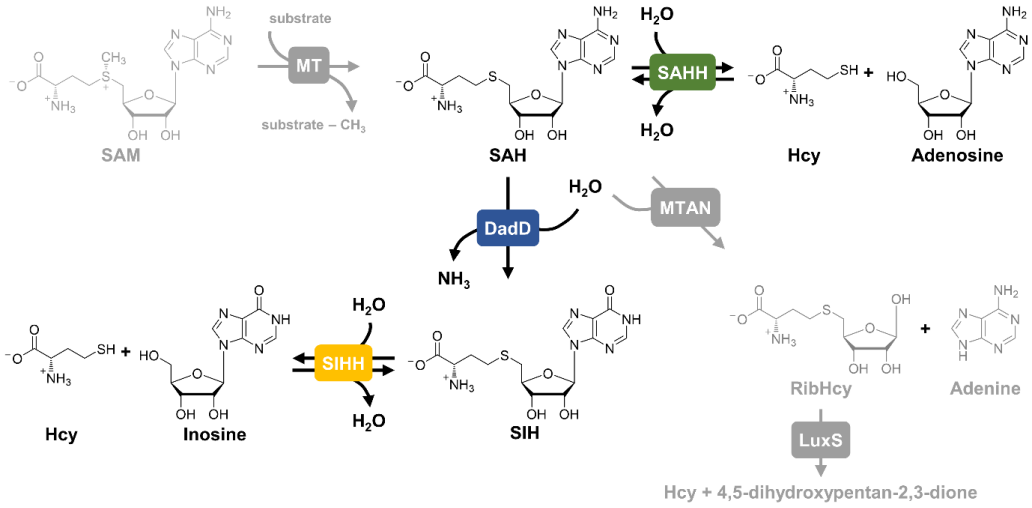


**Figure 1.** SAH is either reversibly cleaved to adenosine and L-homocysteine (Hcy), or degraded in two steps catalysed by 5'-methylthioadenosine nucleosidase (MTAN) and *S*-ribosyl-L-homocysteine lyase (LuxS). The pathway discovered in *Methanocaldococcus janaschii* contains an additional deamination step of SAH to SIH, which is cleaved to inosine and Hcy by SIHH.

This discovery steered us to biochemically characterise various SAHH homologues from organisms of different kingdoms or phyla within the three domains of life. Our studies revealed that some homologues from eukaryotes, bacteria and archaea are catalytically active with SIH. Enzymes from Euryarchaea and evolutionary closely related thermophilic bacteria exhibit a notable substrate preference for SIH. To identify the reason for different substrate preferences within SAHHs/SIHHs, protein crystallisation and structure determination were applied together with bioinformatical methods for sequence comparison, which led to the identification of a signature sequence differing between enzymes with different substrate preferences. In addition, the first crystal structures of archaeal SAHHs in complex with inosine or SIH offered insights into the binding mode of those untypical substrates [5].

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# Skipping the diketone: AOS enzymatic activity generates novel C–C bond in biocatalytic cathinone synthesis

M. Krause, A. Behrens, K. Witte, M. Müller

Markus Krause, Albert-Ludwigs-Universität Freiburg, Albertstraße 25, Freiburg

Phenylalkylamines such as ephedrine, cathinone and their derivatives are pharmaceutically active alkaloids produced by plants, for instance *Ephedra sp*., *Pinellia ternata* or *Catha edulis*.[1] Although commonly utilized in traditional medicine or modern medication, the biosynthetic pathway of ephedrine and cathinone remains to be elucidated. Currently, most studies on the putative biosynthetic route are based on a transaminase reaction of the aromatic diketone 1-phenylpropane-1,2-dione originated from a benzoyl derivative substrate (Figure 1). However, a respective gene regarding the proposed biosynthesis has not yet been identified.[2] We hypothesize a contrary biosynthetic route circumventing the transamination.

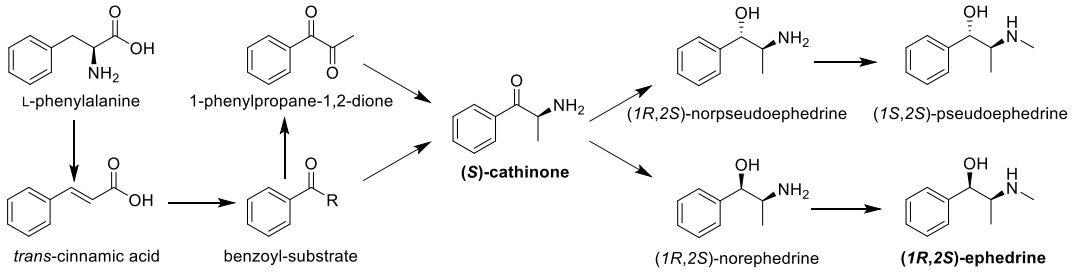


**Figure 1.** Putative biosynthesis of (*S*)-cathinone and ephedrine (arrows indicate enzymatic synthesis).

The pyridoxal phosphate-dependent enzymes of the α-oxoamine synthase (AOS) enzyme family are capable of performing a decarboxylative claisen-like condensation that generates an α-oxoamine. As a member of this enzyme family the promiscuous TTHA1582 from *Thermus thermophilus* was used in our studies.[3] Our experiments with TTHA1582 show the conversion of a variety of aromatic thioesters with different amino acids (Figure 2).

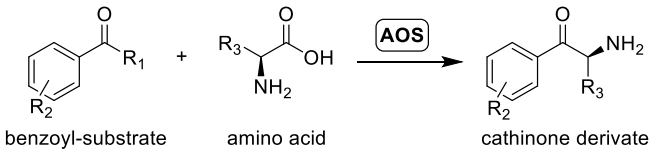


**Figure 2.** Enzymatic conversion of various substrates by AOS activity.

Focusing on cathinone, an α-oxoamine in the biocatalytic route, the formation succeeds in the conversion of benzoyl-CoA or benzoyl-SNAC with L-alanine. Hence, a putative alternative route to ephedra alkaloids in one enzymatic step is indicated.

# An Enzymatic Toolbox Towards SAM and SAM Analogues

M. Kuge, L. Gericke, D. Mhaindarkar, L. Karst, S. Jahn, J. Gagsteiger, N. V. Cornelissen, M. Mohr, S. Mordhorst, A. Rentmeister, F. P. Seebeck, G. Layer, C. Loenarz, J. N. Andexer

Marco Kuge, University of Freiburg, Albertstr. 25, 79104 Freiburg

*S*-Adenosyl-L-methionine (SAM) is a fundamental cofactor employed by a vast number of SAM-dependent enzymes. The scope of catalysed reactions with defined regio- and stereochemical outcome under mild reaction conditions makes these enzymes desirable tools for chemical synthesis [1]. For technical application of these enzymes, as well as studies aimed at characterisation with cofactor analogues, numerous cofactor supply- and regeneration systems have been developed. The latter mainly focus on SAM-dependent methyltransferases (MTs), as they represent the most prominent class of SAM-dependent enzymes. [2]
Here, we present a flexible in vitro SAM supply and regeneration system suitable for a wide range of SAM-dependent enzymes. The cascade was successfully applied to MTs, radical SAM enzymes and aminopropyltransferases (**Figure 1**). By combining established polyphosphate-driven ribose- and nucleotide phosphorylation cascades [3,4] with SAM derived byproduct recycling, we realised a modular cofactor regeneration system that is applicable to the synthesis of SAM and its analogues [5]. The exchange of single cofactor building blocks enables the synthesis of SAM analogues with different nucleobases and alkyl chains at the sulfonium centre of the cofactor. Due to the modular set-up, the system offers access to commercially not available cofactor derivatives, which can be used to investigate the substrate scope of different SAM dependent enzymes and might be of interest for the development of biorthogonal systems.

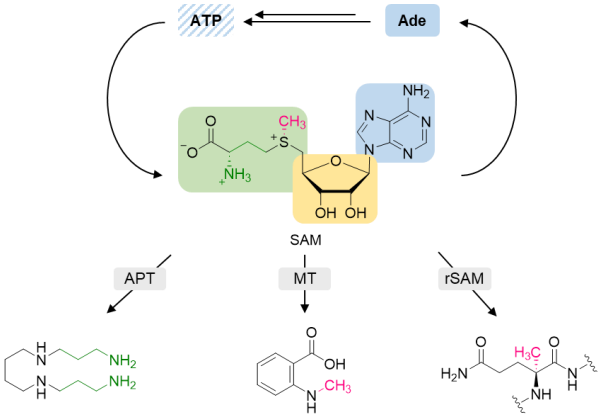


**Figure 1**: Modular S-adenosyl-L-methionine (SAM) supply system. The in situ generated cofactor was successfully employed by aminopropyltransferases (APT), methyltransferases (MT), and radical SAM (rSAM) methyltransferases. The system can be used to exchange different cofactor building blocks, as SAM is composed of: the amino acid-derived side chain (green box), the pentose sugar moiety (yellow box) and the nucleobase (blue box).

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# $F_2$- and HF-Chemistry in the Krossing Group

J. Kulenkampff, H. Schuster, J. Drolshagen, L. Ernst, I. Krossing*

University of Freiburg, Department of Inorganic and Analytical Chemistry,
Albertstraße 21, 79104 Freiburg

The handling of elemental fluorine and anhydrous hydrogen fluoride as well as hydrofluoric acid is a unique field in synthetic chemistry, that requires special training, equipment and safety precautions in order to be performed properly.


Figure 1: Monel Vacuum Line and Reaction Setup for Fluorinations in anhydrous HF.

In in our contribution, we would like to give insight in our fluorine lab and our daily work with those compounds. This includes our equipment, our capabilities and safety measures. Furthermore, we would like to present an overview of our current fields of research with fluorine and hydrogen fluoride, as the fluorine lab plays a key role in our group for multiprofessional applications, from fundamental research on novel cationic species stabilized by weakly coordinating anions[1] to applied science as improving solid state catalysts for synthetic fuel synthesis via carbon dioxide hydrogenation.[2]

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# Ultrahigh Mass Activity Pt Entities composed of Pt Single atoms, Clusters and Nanoparticles for improved Hydrogen Evolution Reaction

*S. Küspert[1,2], Z. Zeng[1,2], S. E. Balaghi[2,3], H. E. M. Hussein[4], N. Ortlieb[1], M. Knäbbeler-Buß[5], P. Hügenell[5], S. Pollitt[6], N. Hug[1], J. Melke[1,2,3], A. Fischer[1,2,3*]*

[1]Institute of Inorganic and Analytical Chemistry (IAAC), University of Freiburg, Albertstraße 21, 79104 Freiburg, Germany.

[2]Freiburg Materials Research Center (FMF), University of Freiburg, Stefan-Meier-Straße 21, 79104 Freiburg, Germany.

[3]Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany.

[4]Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom.

[5]The Fraunhofer Institute for Solar Energy Systems ISE, Heidenhofstraße 2, 79110 Freiburg, Germany.

[6]Paul Scherrer Institute, Forschungsstrasse 111, 5232 Villigen PSI, Switzerland.

Platinum is one of the best-performing catalysts for the hydrogen evolution reaction (HER). However, its high cost and scarcity severely limit the large-scale application of Pt electrocatalysts. Constructing highly dispersed ultrasmall Platinum entities is a very effective strategy to increase Pt utilization and its mass activities and thereby reduce costs. Here, we synthesized highly dispersed Pt entities consisting of a mixture of Pt single atoms, clusters and nanoparticles supported on mesoporous N-doped carbon nanospheres. The presence of Pt single atoms, clusters and nanoparticles is demonstrated by a combined approach involving XRD, XPS, ac-ADF-STEM, XAS and electrochemical CO stripping. The best catalyst exhibits an excellent HER geometric and Pt-based mass activity which is respectively ~4 and 26 times higher than that of a commercial Pt/C reference and a Pt/NC-nanofiber catalyst with similar Pt loadings. Noteworthily, after optimization of the geometrical Pt electrode loading, the best catalyst exhibits ultrahigh Pt- and catalyst-mass activities (56±3 A $mg^{-1}_{Pt}$ and 11.7±0.6 A $mg^{-1}_{Cat}$ at -50 mV vs. RHE), which are respectively 1.5 and 58 times higher than the highest Pt- and catalyst-mass activities reported so far for Pt single atom and cluster-based catalysts.[1,2,3]

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# Structure and function of the nitrogenase-like reductase CfbC/D involved in coenzyme $F_{430}$ biosynthesis

**Alena Kussler** [a], Lin Zhang [b], Oliver Einsle[b], Gunhild Layer[a]
[a] *Albert-Ludwig-University, Institute of Pharmaceutical Sciences*
*alena.kussler@pharmazie.uni-freiburg.de*
[b] *Albert-Ludwig-University, Institute of Biochemistry*

The metallo-enzyme nitrogenase and its functional and structural homologs are involved in essential biological processes, such as nitrogen fixation, photosynthesis and biological methane formation (methanogenesis). Nitrogenase catalyzes the biological nitrogen fixation, and thus, the reduction of dinitrogen to ammonia [1]. A structurally simpler, iron-sulfur cluster containing nitrogenase-like enzyme system called CfbC/D (Coenzyme $F_{430}$ Biosynthesis) is involved in the biosynthesis of coenzyme $F_{430}$, the essential cofactor of methyl-coenzyme M reductase in methanogenesis. During the biosynthesis of coenzyme $F_{430}$ the tetrapyrrole macrocycle of $Ni^{2+}$-sirohydrochlorin a,c-diamide (Ni-SHC-D) is reduced by CfbC/D to $Ni^{2+}$-hexahydrosirohydrochlorin a,c-diamide (Ni-$H_6$-SHC-D) by the addition of 6 electrons and 7 protons. In homology to nitrogenase, CfbC/D consists of a reductase component and a catalytic component. The reductase component is a homodimer of CfbC carrying an intersubunit [4Fe-4S] cluster. The catalytic component is composed of a homodimer of CfbD bridged by an intersubunit [4Fe-4S] cluster, in contrast to the heterotetrameric catalytic component of nitrogenase [2].
CfbC and CfbD from the hyperthermophilic methanogen *Methanocaldococcus jannaschii* were produced in *Escherichia coli*. In contrast to nitrogenase, the reductase $CfbC_2$ showed ATP hydrolysis activity in the absence of the catalytic component $CfbD_2$. However, ATP hydrolysis was stimulated by adding $CfbD_2$. Also, chimeric complexes of $CfbC_2D_2$ from *Methanosarcina barkeri* and *M. jannaschii* showed ATPase activity and the highest activity was observed with $CfbC_2$ from *M. barkeri*. Using microscale thermophoresis, the $K_d$ values for the interaction between $CfbC_2$ and $CfbD_2$ from *M. jannaschii* with and without nucleotides were determined. In the absence of nucleotides, a $K_d$ value of 123 ± 3 nM was determined. The addition of nucleotides (non-hydrolysable ATP analog or ADP) led to a substantial increase of the $K_d$ value. The exchange of leucine 125 to proline in the switch II motif of CfbC led to a slight increase in the affinity of the proteins. Further, this exchange led to a lower tetrapyrrole conversion but nearly the same ATPase activity. Structural analysis using Cryo-EM indicated two different conformations of the $CfbC_2D_2$ complex with or without substrate.

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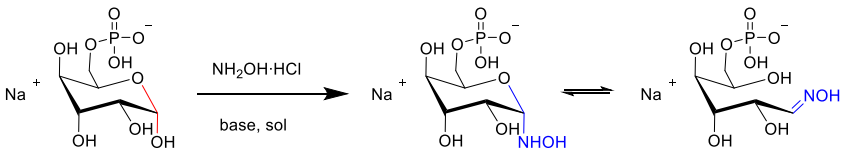
# Identification of inositol phosphates and sugar phosphates isomers

Mengsi Lu, H J.Jessen

Mengsi Lu, Universität Freiburg, Albertstr. 21, Freiburg

Inositol phosphates play an essential role in biology world and they are a type of molecule with numerous isomers, chromophore-free and a high charge density structures. Capillary electrophoresis mass spectrometry (CE-MS) has been shown to be a powerful analysis approach for those salts.[1,2] However, in biosamples, it is common to find mixtures of inositol phosphates and sugar phosphates, which share the same mass and become problematic for assignment by CE-MS. Here we introduce one possibility for solving the problem: the hemiacetal (carbonyl group) in glucose is specifically transformed into other mass-variable functional groups in a synthetic way. Hydroxylamine was introduced to covert carbonyl to oxime, which left the inositol phosphates unchanged and identified in CE-MS.



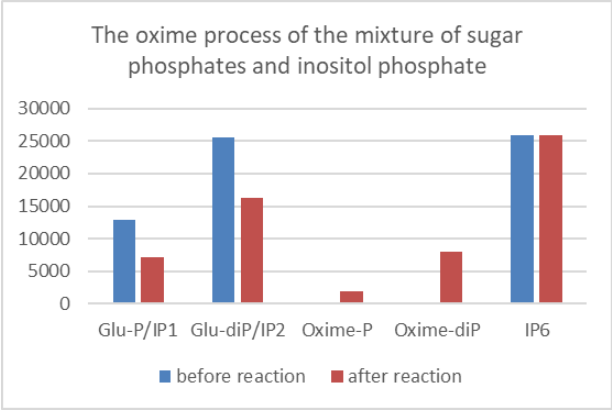

G-6-P

$C_6H_{12}O_9P^-$:
259.0224

Oxime

$C_6H_{13}NO_9P^{--}$:
274.0333

Data analysis: normalized to the [$^{18}$O]-$IP_6$ internal standards and compared with the peak area.

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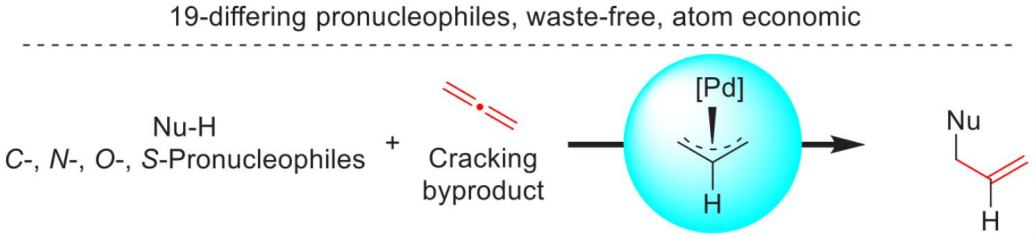
Ilja Lubins, AK Breit, Organische Chemie

# Hydrofunctionalization of Propadiene – New Life for a Previously Unwanted Product




A highly versatile palladium-catalyzed allylation reaction of several pronucleophiles is reported. The use of propadiene in toluene provides an atom economic and waste-free access to allylated nucleophiles, a structural motif with almost unlimited possibilities for further functionalization. In addition to *N*-, *O*-, and *S*-pronucleophiles, the Pd/BINAP system is capable of adding a *C*-pronuceophile to allene. A plausible mechanism is supported by deuterium labeling experiments.

# *Approach to improve $SiO_2$-based Electrets with $AlO_x$ deposited by Atomic layer deposition (ALD)*

Niklas Maier [1,2,3,4], Manuel Larsson[4], Ali Yassine[3,5], Peter Woias[1,3], Anna Fischer[1,2,4]
Niklas.maier@livmats.uni-freiburg.de

[1]Cluster of Excellence: Living, Adaptive and Energy-autonomous Materials Systems, liv*Mat*S*; [2]Institute of Inorganic and Analytical Chemistry*; [3]Department of Microsystems Engineering – IMTEK*; [4]Freiburg Materials Research Center – FMF*; [5]Department of Sustainable Systems Engineering – INATECH*; *University of Freiburg, Germany

**Abstract:** To improve $SiO_2$-based electrets for mechanical energy harvesters (MEH), very thin $AlO_x$ layers are deposited on thermal $SiO_2$. For the deposition, ALD was chosen because of extremely uniform coatings even at thicknesses under 10 nm leading to favorable electrical properties for electrets. The charging is done by corona discharge and surface potential is measured contactless under a controlled environment. The layers are further characterized with SEM, X-Ray Reflectivity (XRR), XPS and electrically with a Mercury Probe (CV and IV).

**Keywords:** atomic layer deposition, layered electret, corona discharge

## Introduction

Mechanical energy harvesters (MEHs) offer the possibility to have wireless sensor nodes at places like machine housings, tires etc. where e.g. solar cells would be useless. For MEHs, an alternative to piezoelectric or magnetic harvesters is electrostatic systems with electrets. Their unique selling point is the easy integration into micro-electronic mechanical systems (MEMS). In electret research, mostly fluorinated polymers are used as they can be charged up to thousands of volts [1]. However, most polymers are unsuited for MEMS due to high process temperatures and harsh chemicals during production. Hence, we are focusing on $SiO_2$ as an electret material, which already has a good performance [2] and try to improve its charge density and retention. Therefore, thin $AlO_x$ layers were deposited on $SiO_2$ by ALD.

## Theory and Methods

For our electrets, different silicon substrates with $SiO_2$ are coated with amorphous $AlO_x$ in a thermal or plasma-enhanced (PE)ALD process. Because of the extremely uniform coatings of ALD, every possible defect on the $SiO_2$ should be effectively covered, increasing the resistivity of the electret. $AlO_x$ also has a higher permittivity than $SiO_2$ lowering the E-field in the latter after charging. Besides, in [3] it was mentioned that the increasing bandgap from $AlO_x$ to $SiO_2$ could have a positive impact on the charge stability by limiting the migration of charges. The electrets are made by charging to different surface potentials via corona discharge. Then, the resulting charge stability is measured with a surface voltmeter in a chamber with controlled humidity and temperature. To be able to interpret the results, the thickness (SEM, XRR), density (XRR), bandgap (XPS), permittivity (CV) and electrical breakdown (IV) of the oxide layers (thermal and PE-ALD) are determined.

# Next Generation Batteries

S. Burger, J. Meierl, N. Schmidt-Meinzer, F. Wortelkamp

Julia Meierl, AK Krossing, Universität Freiburg, Materialforschungszentrum Freiburg
julia.meierl@ac.uni-freiburg.de

Lithium-Ionen-Akkumulatoren haben sich seit den 80er Jahren als führende Variante der Batterien etabliert, als positive Elektroden auf Basis von Übergangsmetalloxiden, allen voran $LiCoO_2$ mit seinen damals hohen Kapazitäten von 140mAhg$^{-1}$, eingeführt wurden. Diese Technik ist so entscheidend für die Energiefrage der Weltbevölkerung, dass sie im Jahr 2019 sogar mit dem Chemie-Nobelpreis gewürdigt wurde.[1][2]
Trotz allem steigen stetig die Anforderungen an Batterien, vor allem durch das Wachstum der Elektromobilität, und die bisher verwendete Technik der Lithium-Ionen-Akkus stößt an ihre Grenzen. Deshalb wird schon seit längerem nach der nächsten Generation an Batterien, den „next generation batteries“ oder „beyond Li-ion batteries“, gesucht, die die bisherigen Batterien ablösen sollen.[3]
Die Anforderungen an die nächste Generation von Batterien sind vielfältig und variieren stark in Abhängigkeit von dem jeweiligen Anwendungsgebiet. Im Allgemeinen werden unter anderem hohe Kapazitäten, hohe Spannungen, große Zyklenstabilitäten und eine günstige Herstellung angestrebt.[3]
Auch der Arbeitskreis Krossing widmet sich der Suche nach neuen Batteriematerialien. In verschiedenen Forschungsprojekten wird an verschiedenen Materialien und den damit verbundenen Vor- und Nachteilen geforscht. Neben der Suche nach preiswerten und dabei leistungsstarken Elektrolyten werden auch verschiedene Elektrodenmaterialien untersucht. Als vielversprechende Elektrodenmaterialien gelten elementares Lithium und Silizium, als negative Elektrode sowie Schwefel als positive Elektrode, die allerdings alle ihre eigenen Herausforderungen mit sich bringen.
Auf diesem Poster soll ein Überblick über diese Materialien und die im Freiburger Materialforschungszentrum innerhalb der Battery-Subgroup des Arbeitskreises Krossing verfolgten Lösungsansätze und die damit einhergehenden Probleme gegeben werden.

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**Functional investigations in *Escherichia coli* complex I**

Luca Mérono, Franziska Hoeser, Sabrina Oppermann, Johannes Schimpf, Daniel Wohlwend and Thorsten Friedrich

Institute of Biochemistry, Albert-Ludwigs-University, Albertstr. 21, Freiburg

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

*L. Miller, F. Bauer, A. Impelmann, Bernhard Breit[1],*
*[1]Universität Freiburg, Institute for organic chemistry,*
*Albertstrasse 21, 79104 Freiburg, Germany*
*lukas.miller@chemie.uni-freiburg.de*

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, a 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.

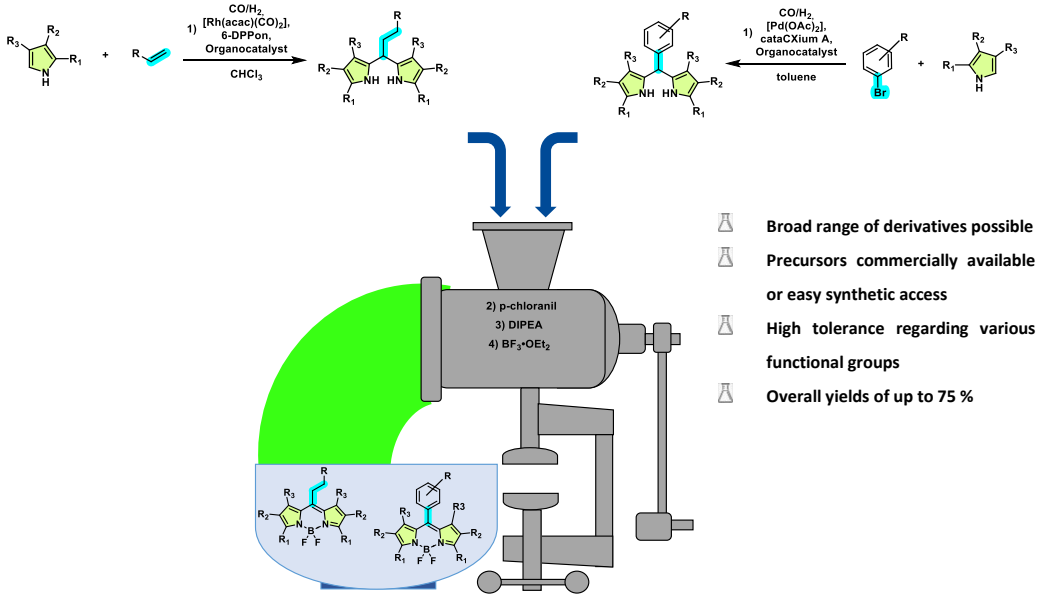


*Figure 1: One pot synthesis scheme of BODIPY dyes from available precursors.*

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# Synthesis of Fluorescent Inorganic Polyphosphate to Study Lysine Polyphosphorylation

S. Moser, G. Liu, H. J. Jessen

Sandra Moser, Institute of Organic Chemistry, Albert-Ludwigs-University Freiburg, Albertstrasse 21, 79104 Freiburg, Germany

Inorganic polyphosphate (polyP), an ubiquitous molecule composed of multiple orthophosphates linked by phosphoanhydride bonds, is no longer regarded as a "forgotten polymer".[1] In recent years, the synthesis of defined polyPs was established[2] and many different biological functions of polyP have been discovered.[3] One of them is the covalent attachment to lysine residues, a new nonenzymatic post-translational modification. It is known that lysine polyphosphorylation occurs in polyacidic serine and lysine (PASK) rich clusters, however, the mechanism is not yet understood. The state-of-the-art method for analyzing lysine polyphosphorylation is a band shift-up on NuPAGE which requires long polyP-chains to be detectable.[4] Here, we show the synthesis of fluorescent and FRET labelled short-chain inorganic polyphosphate to study lysine polyphosphorlyation in model proteins. A bidirectional approach, which uses the triphosphorylation reagent cyclic pyrophosphoryl-phosphoramidite (*c*-PyPA) **1**, enables fast access to symmetrical-modified polyPs, while the monodirectional approach allows to install different modifications at the termini (Figure 1). Synthetic fluorescent labelled polyP with a defined chain length facilitates analysis by mass spectrometry and the fluorescence makes the detection on NuPAGE independent on the length of the polyP. Furthermore, FRET labelled polyP will help to monitor lysine polyphosphorylation in living cells. The synthesized modified polyPs will hopefully enable to understand how polyP modifies target proteins non-enzymatically.

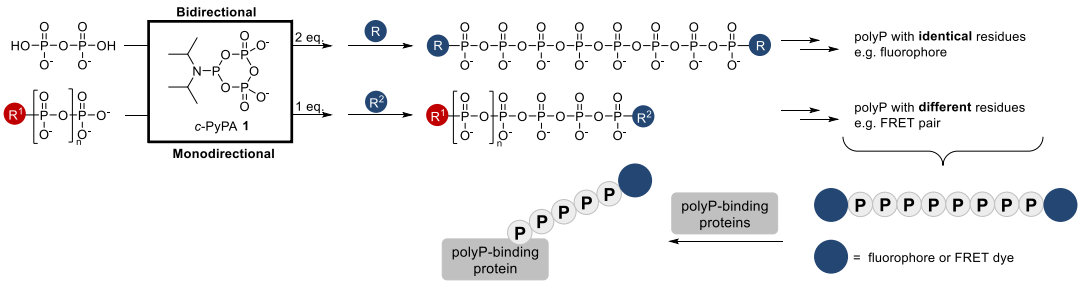


***Figure 1:*** *Overview of the mono- and bidirectional approach using c-PyPA* ***1*** *to obtain polyP chains of defined length with modified ends and schematic presentation of lysine polyphosphorylation.*

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# The Atypical Amt2:GlnK2 Pair from *Archaeoglobus fulgidus*

A. Mukherjee, F. Ormeño and S. L. A. Andrade

Institute of Biochemistry, University of Freiburg, Alberstrasse 21, 79104 Freiburg, Germany

PII regulatory proteins sense the energy and nitrogen/carbon levels of prokaryotic, archaea, and plant cells, by selectively binding ADP, Mg-ATP, and Mg-ATP:2-oxoglutarate (2-OG) **[1].** The integration of these signals results in conformational changes in the protein that affect its interaction with downstream partner proteins. Amongst these an increasing number of enzymes, transcription factors, cofactors, signaling molecules and transporters have been reported **[2**,**3]**. Our project focuses on PII proteins (GlnK) that are involved in ammonium uptake regulation by direct interaction with Ammonium transport (Amt) proteins, thereby preventing the accumulation of toxic concentrations of ammonium while meeting the carbon and nitrogen demands of the cell **[4-6]**. In our model organism, *Archaeoglobus fulgidus* (*Af*), three *glnk* gene copies are organized in distinct operons with *amt* genes. While *Af*-GlnK1 and *Af*-GlnK3 are typical proteins involved in Amt regulation **[8**,**9]** and *Af*-Amt1 and *Af*-Amt3 are canonical $NH_4^+$ transporters **[7]**, *Af*-GlnK2 does not recognize 2-OG, the signal for GlnK:Amt complex dissociation **[8]** and *Af*-Amt2 is also not a typical transporter [**10**] .

We have recently identified the signals that drive complex formation and dissociation **[10]** and with this information, we are now investigating the function of the two proteins in *A. fulgidus*. Our immediate goal in to identify downstream partner(s) to understand the physiological consequences of their interaction in as a function of the Mg-ATP/ADP ratio. In parallel, we are also doing *in vitro* assays to understand how these extremely similar proteins sustain such distinct sensing characteristics.

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# 3D Printed Electrodes for Supercapacitors

Jan Oechsler[1,2,3], Taisiia Berestok[1,2,3], Anna Fischer[1,2,3]

[1]Cluster of Excellence *liv*MatS@ FIT – Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, [2]Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, [3]Institute of Inorganic and Analytical Chemistry, University of Freiburg

Electrochemical double layer (EDLC) supercapacitors are electrochemical energy storing devices, where charges are stored via ionic capacitive processes in the electrochemical double layer formed at high surface area electrodes. In contrast to batteries, supercapacitors have a much lower energy density, but a far higher power density and can thus be charged and discharged very quickly. Recently, energy autonomous photo-rechargeable supercapacitors, monolithically integrating solar cells with supercapacitors, are gaining more and more attention, as these devices are able to harvest and convert sunlight into stored electrical energy for decentralized small off-grid devices, such as sensors and IoT devices.[1]
Classical electrode preparation techniques such as doctor blading result in unstructured flat film electrodes. When increasing the mass loading on these film electrodes the areal capacitance is increasing sub linearly with the loading due to mass transport limitations of the electrolyte through the electrode material, thereby severely limiting the achievable areal energy density.
With 3D Printing it is possible to create 3D architectured electrodes with high surface areas and open porous architectures which are accessible to the electrolyte even at very high mass loadings, resulting in a linear correlation between the mass loading and the resulting areal capacitance, vastly increasing the areal capacitance compared to thin film electrodes.[2]
In here, we will present our recent results on 3D printing of 3D architectured carbon based electrodes with high surface area and eventually improved power and energy density.

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# Molecular Determinants in *Archeoglobus fulgidus* Amt2:GlnK2 complex formation

F. Ormeno, L. Zhang and S. Andrade

Institute of Biochemistry, University of Freiburg, Albertstrasse 21, 79104 Freiburg, Germany

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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# Reevaluating an old paradigm: Evidence for a 5-deazaflavin radical provided by photo-CIDNP

S. Panter, J. Wörner, B. Illarionov, M. Fischer, A. Bacher, S. Weber

Sabrina Panter, Institute of Physical Chemistry, University of Freiburg, Albertstr. 21, 79104 Freiburg

Flavins are found in a range of blue light receptor proteins as versatile coenzymes [1]. To unravel and manipulate reaction mechanisms of these proteins, the replacement of the flavin cofactor by a 5-deazaflavin analogue has proven to be valuable [2]. It was frequently reported that one-electron-transfer reactions in flavoproteins are impeded with 5-deazaflavin as cofactor. Consequently, the 5-deazaflavin radical was assumed to be significantly less stable than the flavin semiquinone [2-5]. The long-standing paradigm of 5-deazaflavin being solely a two-electron / hydride acceptor / donor needs now to be reevaluated with the indirect observation of a one-electron reduced (paramagnetic) species using photo-chemically induced dynamic nuclear polarization (photo-CIDNP) $^{1}$H NMR.

Photo-CIDNP is a versatile tool to investigate mechanisms in the context of light-induced radical pair reactions. This NMR technique allows the investigation of transient radical pairs that are difficult to probe by EPR. Crucial for this process is a spin-correlated radical pair (SCRP) undergoing nuclear spin dependent intersystem crossing which leads to a process of spin-sorting. The resulting hyperpolarization pattern of the diamagnetic product is proportional to isotropic hyperfine coupling constants of the respective paramagnetic intermediate if a time-resolved experiment is used. Additionally, the sign of the hyperpolarization is dependent on the multiplicity of the precursor and successor species of the SCRP. Analysis of this range of information gives access to important mechanistic details of the investigated radical pair reactions [6].

By means of a photo-CIDNP study, a radical pair formed from L-tryptophan and 5-deazaFMN was characterized over a wide pH range. Proton isotropic hyperfine coupling constants of the 5-deazaFMN radical were extracted and correlated with predictions from density functional theory calculations. Analysis of the data suggests radical pair formation from a singlet precursor contrasting the radical pair formation of FMN which involves a triplet precursor.

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# Do unspecific peroxygenases dream of selectivity?

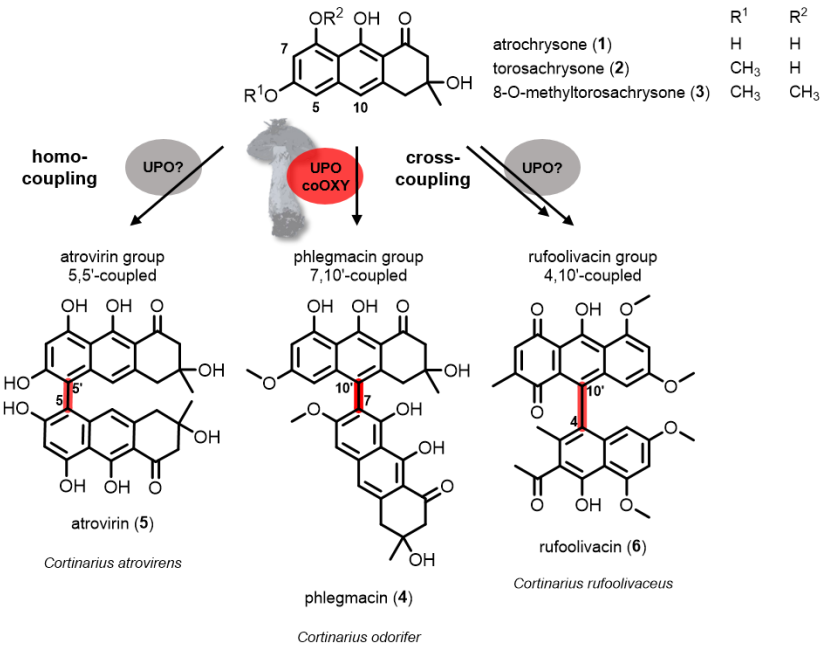
*Lukas Platz[a], Nikolai Andreas Löhr[b], Wolfgang Hüttel[c], Prof. Dirk Hoffmeister[b], Prof. Michael Müller[a],*

[a]*Institute for Pharmaceutical Sciences, Albert-Ludwigs-Universität Freiburg, Germany*

[b]*Institute of Pharmacy, Friedrich-Schiller-Universität Jena, Germany*

[c]*Institute of Organic Chemistry, Albert-Ludwigs-Universität Freiburg, Germany*

Dimeric pre-anthraquinones are widespread in nature with remarkable diversity among different kingdoms such as fungi or plants[1] and possess bioactivities such as antibacterial, antifeedant, and antiplasmodial properties.[2] They include pigments of the phlegmacin, rufoolivacin, and atrovirin groups of different coupling types. However, the regio- and stereoselective control of their biosynthesis remains elusive (Scheme 1).[3]




**Scheme 1** Oxidative phenol coupling of pre-anthraquinones catalyzed by 'unspecific' peroxygenases. Monomeric atrochrysone (**1**) or its derivatives (**2**, **3**) are dimerized to phlegmacin (**4**) or atrovirin (**5**) or can even be cross-coupled with a heptaketide to rufoolivacin (**6**).

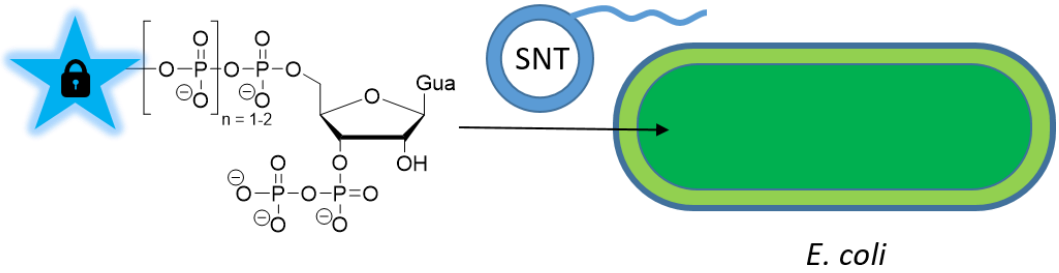
The kingdom of fungi, particularly the phylum of *Basidiomycota*, is a producer of a large variety of these compounds that employ various types of modifications such as dehydration, methylation, or oxidation of the pre-anthraquinone scaffold atrochrysone (**1**).[3] However, the knowledge of their biosynthesis is still elusive and is only recently advancing with more genomic information being accessible.[4] Whole genome

**Delivery of Caged Magic Spot Nucleotides into *Escherichia coli***

Christoph Popp, Henning J. Jessen

Christoph Popp, Institute of Organic Chemistry, University of Freiburg, Albertstraße 21, 79104 Freiburg, Germany

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. Cellular transport was facilitated by conjugation to a cell-penetrating peptide and through a cyclodextrin based synthetic nucleotide transporter. 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

I. Prucker, M. Milanov, H. J. Jessen

University of Freiburg, Albertstraße 21, 79104 Freiburg

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.

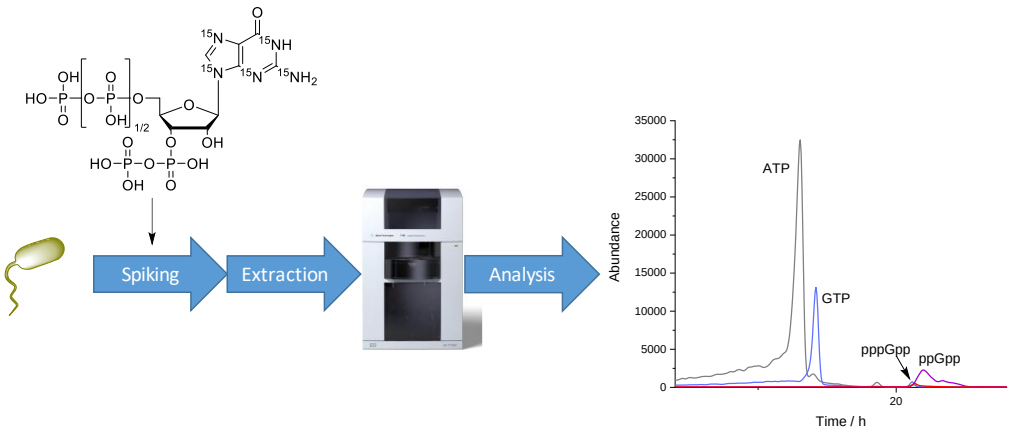


**Figure 1**. Structural formula of (p)ppGpp and workflow of quantification. Adenosine (ATP) and guanosine (GTP) triphosphates are shown for comparison.

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# CPRiL: compound-protein relationships in literature

A. Qaseem, S. Günther

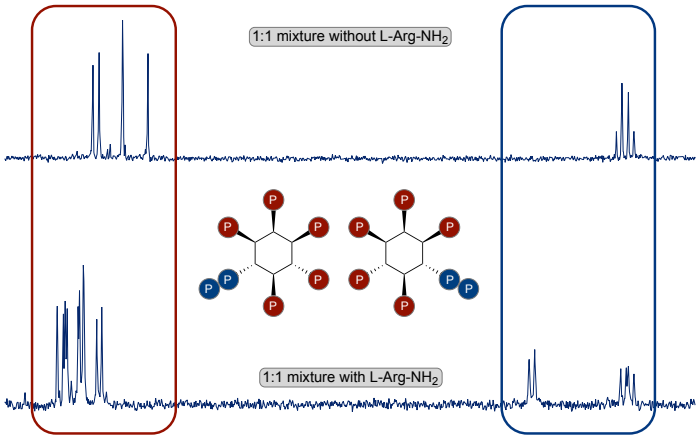
Ammar Qaseem, Albert-Ludwigs-Universität Freiburg, Hermann-Herder-Strasse 9, D-79104 Freiburg i. Br.

Newly discovered functional relationships of (bio-)molecules are a key component in molecular biology and life science research. Especially in the drug discovery field, knowledge of how small molecules associated with proteins plays a fundamental role in understanding how drugs or metabolites can affect cells, tissues and human metabolism. Finding relevant information about these relationships among the huge number of published articles is becoming increasingly challenging and time-consuming. On average, more than 25,000 new (bio-)medical articles are added to the literature database PubMed weekly. To support literature research, we developed a new web server [compound-protein relationships in literature (CPRiL)] that provides information on functional relationships between small molecules and proteins in literature [1]. Currently, CPRiL contains ~465,000 unique names and synonyms of small molecules, ~100,000 unique proteins and more than 9 million described functional relationships between these entities. The applied BioBERT machine learning model [2] for the determination of functional relationships between small molecules and proteins in texts was extensively trained and tested. On a related benchmark [3], CPRiL yielded a high performance with an F1 score of 86.0%, precision of 85.2%, and recall of 86.8%.

# Assigning the Absolute Configuration of Inositol Poly- and Pyrophosphates by NMR Using a Single Chiral Solvating Agent

K. Ritter, N. Jork, A.-S. Unmüßig and H. J. Jessen*

Kevin Ritter, Institute of Organic Chemistry, University of Freiburg, Albertstr. 21, 79104 Freiburg, Germany




Inositol phosphates constitute a family of highly charged messenger molecules that play diverse roles in cellular processes. The various phosphorylation patterns they exhibit give rise to a vast array of different compounds. To fully comprehend the biological interconnections a precise molecular identification of each compound is crucial. Since the *myo*-inositol scaffold possesses an internal mirror plane, enantiomeric pairs can be formed. Most commonly employed methods for analyzing InsPs have been geared towards resolving regioisomers, but they have not been capable of resolving enantiomers. In this study, we present a general approach for enantiomer assignment using NMR measurements. To achieve this goal, we used $^{31}$P-NMR in the presence of L-arginine amide as a chiral solvating agent, which enables differentiation of enantiomers. Using chemically synthesized standard compounds allows for an unambiguous assignment of the enantiomers. This method was applied to highly phosphorylated inositol pyrophosphates, as well as to lower phosphorylated inositol phosphates and bisphosphonate analogs. By isolating naturally occurring compounds from cells, our method will facilitate the assignment of biologically relevant isomers.[1,2]

**Literature :**

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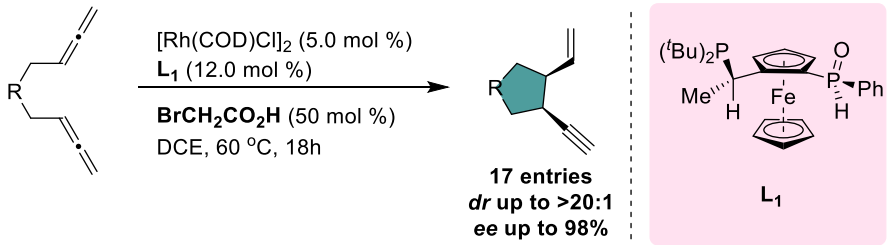
# Rhodium-Catalyzed Diastereo- and Enantioselective Cycloisomerization of 1,5-*Bis*-(allenes) to 1,2-Enyne Cyclic Skeletons

Farhad Panahi,[#] Marzieh Samadani,[#] Felix Bauer, and Bernhard Breit*

Institut für Organische Chemie, Albert-Ludwigs-Universität Freiburg, Albertstraße 21, 79104 Freiburg im Breisgau, Germany

*bernhard.breit@chemie.uni-freiburg.de

We present here a novel enantioselective Rh-catalyzed cycloisomerization of 1,5-*bis*-(allenes), which provides a versatile route for the synthesis of chiral five-membered ring (hetero)cycles. This method is suitable for the synthesis of spiro carbocyclic compounds, which are valuable chiral building blocks in organic synthesis. The reaction provides high yields of the desired products with excellent diastereoselectivity and high enantioselectivity. The newly formed products possesses both a branched alkene and a terminal alkyne moiety, providing opportunities for further functionalization [1]. This developed scaffold is similar to the structures of drugs such as prostaglandins and natural products such as kainic acid [2]. Furthermore, we demonstrate the scalability of the reaction through a gram-scale synthesis. We also illustrate the synthetic utility of the resulting 1,2-enyne cyclic products through several transformations. Density functional theory (DFT) calculations and deuterium labelling experiments support the proposed reaction mechanism.

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# ZIF-8 based FeNCs for electrochemical ammonia production: What they can do and what not

C.Schneider, N. Ortlieb, P. Elsässer, E. Balaghi, Prof. A. Fischer

Caroline Schneider, Albert-Ludwigs-Universität Freiburg, Albertstr. 21, Freiburg i. Br.

The electrochemical reduction of $N_2$ in aqueous media and ambient conditions would present a great advancement in sustainability and defossilization of the fertilizer and energy sector if the obstacles to this technology were not as great as they are at present. It is even doubted if the electrochemical nitrogen reduction reaction (NRR) is possible in aqueous media [1]. Herein, we present an exemplary catalyst, a metal-organic framework-based Fe and Zn single atomic site catalyst and revisit its NRR activity in aqueous neutral electrolyte. ZIF-8 based Fe- and N-co-doped carbon materials (FeNCs) are well known catalyst materials that have been claimed active for the NRR [1]. However, our results, when following a strict gas scrubbing and electrochemical measurement routine avoiding any type of easily reduced N-containing contaminations, point towards the opposite. On the other hand, FeNCs show good activity for the reduction of NOx species, which could possibly explain previous (false) positive NRR results. Furthermore, the electrochemical nitrate reduction activity of the presented FeNC materials is investigated and a faradaic efficiency of 100% at -0.7 V vs. RHE is obtained. The interplay of Fe and Zn atomic sites possibly enhancing the selectivity of the catalyst is also discussed.

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# Investigation on the FeMo Cofactor maturation system

*Florian Schneider, Marc Broghammer, Oliver Einsle*

*Institute of Biochemistry, Albert-Ludwigs-University Freiburg, Albertstr. 21, D-79104 Freiburg, Germany*
E-mail: schneiderf@bio.chemie.uni-freiburg.de

Structural insights of enzymes involved in biological nitrogen fixation are limited and focus mainly on the mechanism of the catalytically active enzyme - the nitrogenase [1][2]. Structural studies on the maturation system are rather underrepresented. Three key techniques are employed to study the structural features of enzymes: X-ray crystallography, NMR and cryo electron microscopy, each of which with its unique limitations. The recent advent of AI prediction systems such as Alphafold[3][4] offer a way of simulating and predicting structures. To test Alphafolds reliability published structures were simulated and compared with the experimental data. In direct comparison prediction and experimental structure look strikingly accurate;
yet we want to highlight the differences at the metal cofactor binding sites, interdomain distances and the domain-domain interface. Also structurally unexplored enzymes were simulated. Keeping in mind identified blind spots, the results still suggest additional features such as flexible linkers and terminal regions. As not
all questions in structural biology can be answered by conventional methods, Alphafold with the limitation of being purely predictive still is a powerful tool to sort missing puzzle pieces.

*References*

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# The Schnitzer Lab – Rethinking Catalyst Development

T. Schnitzer

Tobias Schnitzer, Universität Freiburg, Abertstraße 21. 79104, Freiburg.

Tailor-made catalysts that exhibit reactivity exclusively towards specific targets – substrates, sites, or sequences – are currently a hallmark of nature. Harnessing this level of precision on-demand and applying it to small molecule catalysts would boost efficacy and sustainability of chemical synthesis. Recent advancements in data-driven workflows have sparked a revolution in catalyst development, offering a departure from traditional "trial-and-error" and rational design approaches.

In our group, we rethink the conventional methods of small molecule catalyst development. We envision that by analyzing vast amounts of data, we can now embark on a more directed path towards identifying catalysts with high specificity for a certain substrate, site or sequence. Towards this goal, our group will harness peptides as powerful organocatalysts. Their modular structure enables rapid, automated and scalable synthesis, making peptides ideal for generating extensive libraries with unparalleled functional and structural diversity. By subjecting such a library to a diverse range of reactions and substrates, we will map essential substrate/reaction features and thereby paving the way for the development of a computer-aided workflow that predicts and identifies catalysts with target selectivity.

Check out our poster to learn more about our research and join us at the Schnitzer Lab as we embark on this exciting journey to small molecule catalyst development.

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# Mit fluorierten Cu/ZnO-Katalysatoren zu nachhaltigem Methanol

H. Schuster, L. D. Ernst, I. Krossing

Universität Freiburg, Freiburger Materialforschungszentrum,
Stefan-Meier-Straße 21, 79104 Freiburg

Als Beitrag zum *Power-to-Methanol*-Konzept wurden binäre Cu/ZnO und ternäre Cu/ZnO/$FeO_x$-Katalysatoren[1] mit $F_2$ fluoriert und deren Aktivität zur $CO_2$-Hydrogenierung untersucht.[2] Der Fluoridgehalt wurde zwischen 1.2 und 8.7 Gew.-% variiert. In dieser Varianz zeigt der Katalysator einen deutlichen Anstieg in der MeOH-Produktivität, in der MeOH-Selektivität und im $CO_2$-Umsatz. Durch Variation der Feedgas-Zusammensetzung von 100 % $CO_2$ / 3 $H_2$ zu 100 % CO / 2 $H_2$ (fossiles CO / 2 $H_2$ wird industriell eingesetzt)[3], ist ein Trend in Richtung bevorzugter $CO_2$-Hydrogenierung erkennbar.[4] Eisenhaltige Systeme sind besonders durch eine geringe CO-Hydrogenierung gekennzeichnet, während fluorierte binäre Cu/ZnO-Katalysatoren die höchste Methanol-Produktivität liefern.

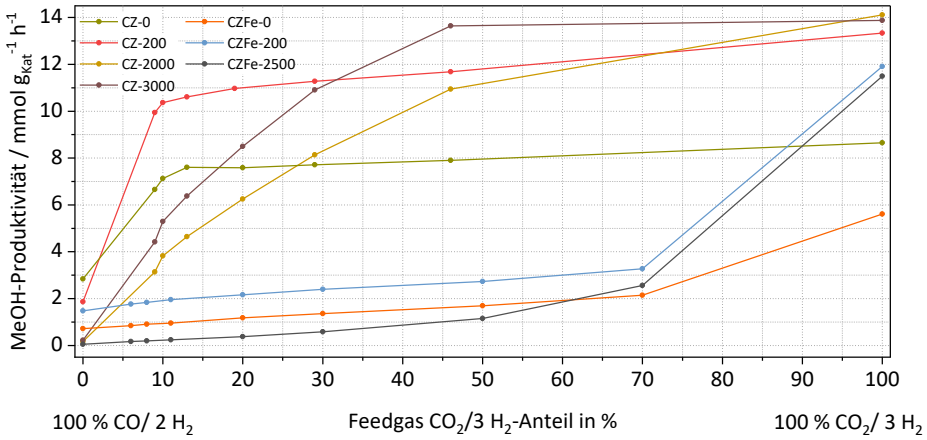


Abbildung 1: Methanol-Produktivität in Abhängigkeit der Feedgas-Zusammensetzung, gemessen von 100 % $CO_2$ / 3 $H_2$ bis 100 % CO / 2 $H_2$. Untersucht wurden fluorierte und unfluorierte eisenhaltig ternäre bzw. binäre Cu/ZnO-Katalysatoren CZ(Fe)-*x*. *x* entspricht dem eingesetzten Fluordruck in mbar pro 1.5 g Präkatalysator.

In Anbetracht der dringend notwendigen $CO_2$-Neutralität in der Industrie bieten die Ergebnisse einen Beitrag zur katalysatoroptimierten Umsetzung von $CO_2$ aus Industrieemissionen und Wasserstoff.

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## Ag(I)/Bi(III)-based double perovskites as alternatives detector materials

Catherine Schweinle, Sahsuvar, Nazire Simay , Harald Hillebrecht,Michael Fiederle

Catherine Schweinle, Albert-Ludwigs-Universität, Albertstraße 21, 79104 Freiburg im Breisgau

X-ray is a very common diagnostic tool, not only in human medicine, but also in science and industry.[1] Common x-ray detector materials include silicon, cadmium zinc telluride and other compounds that have a high atomic number. [2] The new generation of detectors being developed by researchers have a very low detection limit and high sensitivity, allowing the necessary dose of X-rays to be decreased.
Having a closer look at this new generation, halide perovskites are emerging. First research has been done on lead containing materials, such as $(MA)PbX_3$ (X=Br,I).[3,4] Lead, however, is a toxic metal, not only for humans, but also for the environment. In 2018, $Cs_2AgBiBr_6$ was introduced as a promising candidate for x-ray detection.[5]
One figure of merit for an x-ray detector is the density of traps. Corn boundaries can behave as such traps.[6] so researchers are developing large single crystals.
Using a controlled cooling method which was adopted by Yin et al.[7], testing different additives and seed crystals, we are now able to grow single crystals of $Cs_2AgBiBr_6$ with a minimum size of 10 mm. During our research, we've found that using toluene or sodium acetate are good additives for the crystal growth. It is also recommended to use a temperature program which begins to cool down from higher temperatures.
Halide perovskites are not the only materials used for x-ray detection, however. Low-dimensional perovskite-like phases, such as Ruddlesden-Popper or Dion-Jacobson phases, can also be used.[8]
To the best of our knowledge, the two known single crystal structure of a Dion-Jacobsen phase found in literature uses 1,4-butanediammonium (BDA) [9] and 1,4-bis(ammoniomethyl)cyclohexane [10]
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, constructed from corner-sharing $AgBr_6$ and $BiBr_6$ octahedra, separated by organic spacer cations. All of the amine groups are protonated, which allows hydrogen bonds to form with the bromide ions.
All the products have been crystallized from a hot hydrobromic acid solution, with a stoichiometric amount of AgBr, Bi2O3, and the corresponding amine.

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# Developing an Orthogonal Assay Platform to Screen for Inhibitors of the Protein Methyltransferase METTL21A

*Maximilian Staudt[1], Ling Peng[2], Sheng Wang[2], Eric Metzger[2], Roland Schüle[2], and Manfred Jung[1]*

*[1]Institute of Pharmaceutical Sciences, University of Freiburg, Albertstraße 25, 79104 Freiburg*

*[2]Freiburg/DE*

Heat shock proteins (HPSs) serve as molecular chaperones and hence contribute to key cellular processes, such as correct protein folding and protein homeostasis. (1) Within the last years, increasing interest of post-translational modifications of Hsp70 family members and their clinical significance has emerged. Methylation of a conserved lysine in HSPA1 and HSPA8 has been shown to be involved in diseases such as Parkinson's disease and cancer. (2, 3) In 2013, the protein methyltransferase METTL21A was identified to methylate this conserved lysine. (2) Hence, METTL21A might play a role in several diseases associated with enhanced Hsp70 methylation.
In order to study biological and biochemical functions of METTL21A, an orthogonal assay platform to screen and characterize potential new inhibitors should be developed. We developed the first fluorescent thermal shift assay (FTSA) to study thermal stabilization of METTL21A by potential ligands. We adapted Promega's MTaseGlo kit to METTL21A and determined the Z' factor to be 0.69. A $K_m$ of 40 µM for SAM and of 0.5 µM for the substrate HSPA8 was determined. The functionality of the assays was validated using S-adenosyl homocysteine and Sinefungin.

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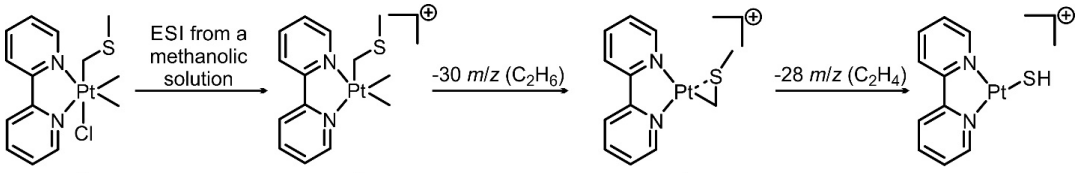
**Synthesis and Reactivity of Key Intermediates of Ion-Molecule Reactions –A Gas-Phase Mimetic Mechanistic Study**

G. Stebens, L. Jakob, and B. Butschke
*Albert-Ludwigs-Universität Freiburg, Institut für Anorganische und Analytische Chemie*

In the gas-phase reaction of "rollover"-cyclometalated $[(bipy - H)Pt]^+$ (bipy = 2,2'-bipyridine) with dimethylsulfide, the loss of ethylene from the encounter complex is observed as the main reaction channel.[1,2] The three-membered heterometallacycle $[(bipy)Pt(CH_2SCH_3)]^+$ (**1**) has been identified as a key intermediate in this ion-molecule reaction by the aid of computational studies.[2] In a combined gas-phase and solution-phase approach, the synthesis and fragmentation of $[(bipy)Pt(CH_2SCH_3)]^+$ (**1**) was thus envisaged in order to probe the hypothesis of **1** as an intermediate in the above process experimentally. The analysis of the decomposition products of **1** in solution was supposed to provide insights into the question in how far chemistry in the gas-phase and in solution are related.

While the cationic Pt(II) complex $[(bipy)Pt(CH_2SCH_3)]^+$ (**1**) itself could not be selectively synthesized, the corresponding Pt(IV) dimethyl complex $[(bipy)PtCl(CH_3)_2(CH_2SCH_3)]$ (**2**) is formed by the reaction of $[(bipy)Pt(CH_3)_2]$ with $CH_3SCH_2Cl$. The reaction of **2** with $NH_4PF_6$ gives rise to the formation of $[(bipy)Pt(CH_3)_2(CH_2SCH_3)]_2(PF_6)_2$ ($\mathbf{3}_2(PF_6)_2$). Electrospray ionization (ESI) of both Pt(IV) complexes generates the cation $[(bipy)Pt(CH_3)_2(CH_2SCH_3)]^+$ (**3**). Collision-induced dissociation (CID) of **3** selectively results in the C–C bond reductive elimination of ethane thus generating the postulated intermediate **1** in the gas-phase. CID of **1**, finally, causes the loss of ethylene (see Figure).




**2** **3** **1**

Postulated intermediate of the ion-molecule reaction of "rollover"-cyclometalated $[(bipy-H)Pt]^+$ with dimethylsulfide

These observations are in agreement with the previous gas-phase and computational findings of the ion-molecule reaction of $[(bipy - H)Pt]^+$ with dimethylsulfide.[1,2] Obviously, **1** indeed serves as a key intermediate in this reaction. To investigate whether the observed gas-phase processes also take place in the condensed phase, the complexes $[(bipy)PtCl(CH_3)_2(CH_2SCH_3)]$ (**2**) and $[(bipy)Pt(CH_3)_2(CH_2SCH_3)]_2(PF_6)_2$ ($\mathbf{3}_2(PF_6)_2$) were heated in deuterated dimethylsulfoxide to 170°C. The formations of both ethane and ethylene were observed by NMR spectroscopy. This result provides evidence that intermediate **1** has also been formed in solution by C–C reductive elimination (thus generating ethane), followed by the fragmentation of **1** to generate ethylene. A defined product complex, however, could not be isolated. Still, these results bridge the gap between the mass-spectrometric experiments and corresponding chemistry in solution. The intramolecular processes causing **1** to eject ethylene are obviously not significantly altered by the solvent molecules or by the counter ions.

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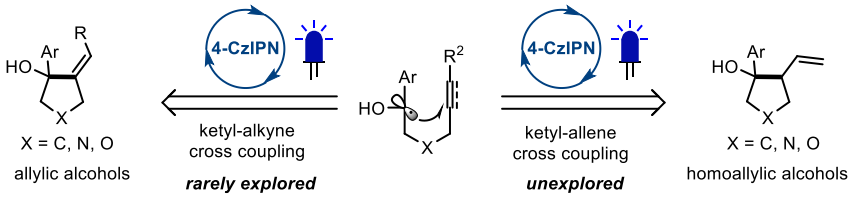
# Visible-Light Induced Metal-Free Intramolecular Reductive Cyclisations of Ketones with Alkynes and Allenes

Nana Tang,[§] Raphael Jonas Zachmann,[§] Hui Xie, Jun Zheng,* Bernhard Breit*

Institut für Organische Chemie, Albert-Ludwigs-Universität Freiburg
Albertstraße 21, 79104 Freiburg im Breisgau, Germany
bernhard.breit@chemie.uni-freiburg.de




Saturated five-membered rings (e.g. pyrrolidine, tetrahydro-furan and cyclopentane) bearing tertiary alcohols are common structural motifs in various biologically active compounds.[2] Consequentially, numerous methods have been established for the preparation thereof.[3] Among them, formation of the tertiary alcohol via direct cross coupling between the corresponding carbonyl and a π-system is highly desirable.[4] Herein, we report a visible-light-induced, intramolecular, reductive cyclisation of ketones with an unsaturated hydrocarbon moiety. In contrast to conventional protocols requiring resource precious or hazardous metal sources, this method enables facile access to ketyl radicals under metal-free and mild reaction conditions. By polarity-reversed, ketyl radical hydroalkoxylation of alkynes and allenes, a variety of five-membered (hetero-)cyclic products were generated in good yields with good to excellent stereoselectivities. The embedded homoallylic tertiary alcohol could be transformed into other useful functionalities, highlighting the synthetic utility of this reaction. This efficient and sustainable ketyl-alkynes/allenes cross coupling also features broad functional group tolerance and scalability.

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# Influence of the substitution position on spin communication in light-induced multi-spin systems

P. Thielert, M. Mayländer, M. El Bitar Nehme, T. Quintes, M. Franz, S. Zimmermann, M. Rickhaus, P. Gilch, S. Richert

Philipp Thielert, Institute of Physical Chemistry, University of Freiburg, Albertstraße 21, 79104 Freiburg, Germany

Molecular assemblies consisting of a chromophore and stable radical may form versatile multi-spin systems upon photoexcitation [1,2]. Because of their modular nature, such molecules are excellent candidates for the investigation of structural variations on spin communication properties [1].

Within this study, we investigate multiple perylene–nitroxides, whereby the radical is connected to different positions of the perylene core, namely the *bay* (1), *ortho* (2) and *peri* (3) positions. The nitroxide radical differs from a conventional TEMPO radical by one unsaturated bond in the six-membered ring.

The multi-spin systems are investigated using theoretical methods, with the exchange interaction parameter between chromophore triplet state and radical ($J_{TR}$) being the crucial parameter regarding spin communication [3]. The experimental investigation is carried out using transient electron paramagnetic resonance (trEPR) spectroscopy, to characterize the spin species, as well as optical techniques, including femtosecond transient absorption (fsTA) spectroscopy, to investigate the excited state dynamics and evaluate the triplet yield.

Based on the theoretical investigations, the sign and magnitude of $J_{TR}$ varies depending on the substitution position. FsTA studies demonstrate triplet state formation on the picosecond time scale for all investigated structures, whereby differences in the excited singlet state lifetimes and triplet yields are observed. TrEPR spectra suggest differences in the exchange interaction although quartet state formation is observed for all the investigated perylene–nitroxides. Structure optimizations reveal the presence of multiple energetically accessible conformations for the *bay* and *ortho* isomers, resulting in a spread in the magnetic parameters as evidenced by numerical simulations of the trEPR data. All results taken together, we can conclude that substitution in *peri* position seems most promising for a systematic study of spin information transfer involving perylene chromophores.

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**Novel Functionalities of Ammonium Transport (Amt)-like Proteins**

A. Volkov and S. Andrade

Institute of Biochemistry, University of Freiburg, Albertstraße 21, 79104 Freiburg, Germany

Ammonium is a highly relevant cation for living systems. Microorganisms utilise it as direct source for the element nitrogen and the ammonium produced from human catabolism requires thorough detoxification. In both examples, highly selective and dedicated membrane proteins called ammonium transport (Amt) or Rhesus (Rh) proteins are required to mediate the transport of the $NH_4^+$cation across lipid bilayers. These trimeric proteins share a core of 400 – 500 amino acids residues that fold into 11 – 12 transmembrane helices per monomer [1].

In our group we have characterised various Amt transporters but also Amt-like proteins that work as $NH_4^+$ receptors instead. The latter, utilise conserved features of transporters to selectively recruit and conduct the cation halfway into the pore, but then deviate it into a binding pocket for two $NH_4^+$ (the N1/N2 site) that is absent in the transporters [2].

Based on the Pfam Amt/Rh family database [3], we selected for family members that contain a conserved Amt-like transmembrane core fused to soluble domain(s) but lack the N1/N2 site. Our immediate goal is to understand Nature´s portfolio of Amt-like proteins and extend the current knowledge of how cell deal and make use of ammonium.

Here, we present two of these novel target proteins and describe our current efforts to produce, isolate and study them *in vitro*.

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# Assembly and Dynamic Interactions of Protein Machineries by Single-molecule Fluorescence Methods

Leonie Vollmar, Veronika Frank, Julia Schimpf, Sophie Weyrauch, Abhinaya Anandamurugan, Bianca Hermann, Thorsten Hugel

Proteins are important building blocks that play a crucial role in all areas of life. However, they rarely work alone; instead, they form complexes and assemble into molecular machines to fulfill their respective functions.

Hsp90 is a so-called chaperone, which means it is a helper protein that assists in the folding or activation of other proteins, such as kinases. Hsp90 forms various molecular machineries with different cochaperones (i.e., "helper helpers") for a variety of clients. Its involvement in neurodegenerative diseases like Alzheimer's, as well as various types of cancer, requires a deep understanding of these machineries. The first step on this path is understanding the assembly of these machineries and the dynamics of their interactions.

To address these questions, we use single-molecule fluorescence techniques like Förster Resonance Energy Transfer (FRET), nanosecond Fluorescence correlation spectroscopy (FCS), single-molecule tracking and bleaching step analysis. Through these methods, we can investigate protein dynamics both *in vitro* and *in vivo*, studying conformational changes across different timescales (ranging from nanoseconds to minutes), protein complex formation, and cluster formation within living cells.

Collectively, these approaches allow us to directly observe this fascinating molecular machinery and quantify the interplay between its components both in and out of equilibrium.

**Mutations F408D$^{M}$ and F396D$^{N}$ effect the activity of *E. coli* complex I**

**Maximilian Weiß, Caroline Harter, Prof. Dr. Thorsten Friedrich**

Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Albertstraße 21, 79104 Freiburg im Breisgau.
Kontakt: weiss@bio.chemie.uni-feiburg.de

NADH:ubiquinone oxidoreductase, respiratory complex I, is the first and largest enzyme of most respiratory chains. It couples 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. The unique L-shape leads to its bipartite structure, consisting of peripheral and membrane arm. Electrons are transferred from NADH via FMN through a series of iron-sulfur (FeS) clusters to ubiquinone (Q) [1]. It is of significant importance to understand how this enzyme functions, as mitochondrial dysfunctions lead to neurodegenerative diseases such as Parkinson's or Alzheimer's disease [2].

The electron transport mechanism from NADH to Q is understood in broad terms [3], while several proton pumping mechanisms are under discussion. There are two main theories, one based on molecular dynamics (MD) simulations [4] and one based on structural analysis [5]. The first theory proposes an electric wave that travels along the membrane arm taking up protons in the forward mode and releasing protons in the backward mode. Protons are expected to be transported through each antiporter like subunit (NuoM, N and L) and the E-channel (NuoA, H, J and K) [4]. The latter theory ("ND5 only" theory) includes the presence of two electric waves and proton uptake from the membrane arm to Q to produce the product ubiquinol. Here, protons are taken up by NuoL and M but they are exclusively released to the outside via NuoL (ND5 in human complex I) [5].

Residues F408$^{M}$ and F396$^{N}$ are located at the exit of the putative proton pathways of NuoM and NuoN, which are thought to be inactive in the framework of the "ND5 only" theory. Replacement of these phenylalanine residues by an aspartic acid influenced the activity of E. coli complex I. Proton translocation is affected and the electron transfer activity of the F408D$^{M}$ variant is diminished. Thus, these residues seem to play a role in proton translocation.

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## Scaling Down the Universe: Navigating the Nanoscale World with Atomic Force Microscopy

R. Schlatterer, C. Wenzel, N. Ranjan, T. Hugel and B. N. Balzer

University of Freiburg, Albertstr. 21, Freiburg

In our everyday life, the nanoscale world is hidden from us, concealed by the resolution of our eyes. Nowadays, the understanding of the nanoscale world expands, revealing secrets that go unnoticed in the macroscopic world. One versatile tool to discover the nanoscale world is Atomic Force Microscopy (AFM) where not only the nanoscale-, but also the microscale and even the sub-nanoscale world is accessible. [1,2]

Here, we show how AFM can be used to investigate the structures formed by self-assembling building blocks through AFM-based imaging. The autonomous assembly of such building blocks is a common process in nature and often controlled by chemical fuels such as ATP. [3] Mimicking this process in an artificial peptide-based system, we use aminoacyl phosphates as high energy building blocks. [4] Sub-nanometer spatial resolution allows us to monitor and understand these assembly processes, which is important for the synthesis of novel materials in adaptive and sustainable materials systems.

Unlike other microscopic methods, AFM does not rely on light or electrons, but on interaction forces. Therefore, it is not limited to imaging and can additionally be used for Single Molecule Force Spectroscopy (SMFS). Here, this is used to investigate the adhesion properties of mucin, a high-molecular-weight glycoprotein, mostly known for being a key-component of mucus. As a biolubricant, it reduces mechanical damage in body parts, e.g., the eye lid, by reducing friction. [5-6] But where does the ability to act as a biolubricant stem from? SMFS is used to investigate the impact of different mucin moieties on adhesion.

Applying voltage opens the door for Kelvin Probe Force Microscopy (KPFM) [7]. It combines the principle of AFM with Kelvin probe measurements to investigate the surface potential and electrical properties of materials at the nanoscale. This method is useful in a broad range of applications such as corrosion studies of metals and alloys, surface potential analysis of photovoltaic cells and electronic devices. Here, we use KPFM and SMFS modes jointly to understand the charge generation and separation mechanism of organic thin film surfaces on contact electrification.

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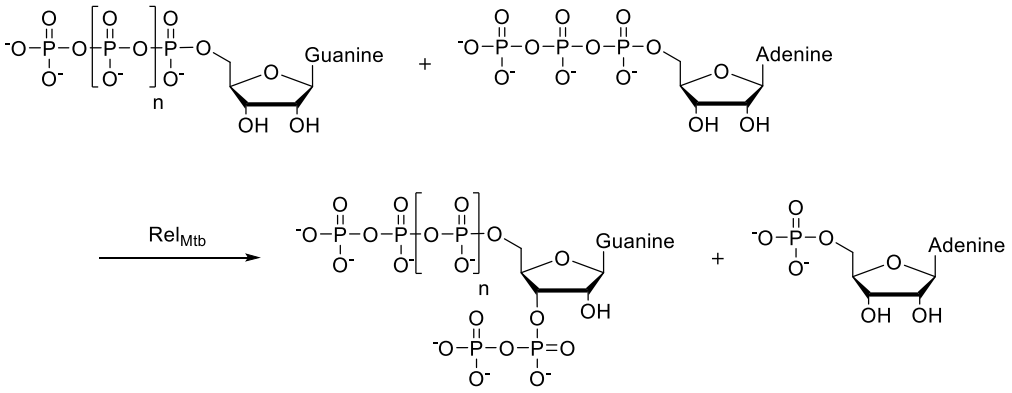
# Quantification of Magic Spot Nucleotides Produced by $Rel_{Mtb}$ from *Mycobacterium tuberculosis*

*Felix Wollensack, Henning J. Jessen*
*Institute of Organic Chemistry, Albert-Ludwigs-University of Freiburg,*
*Albertstraße 21, 79104 Freiburg*
*felix.wollensack@ocbc.uni-freiburg.de*

Bacteria can resist diverse stress like nutritional deficiency, heat or antibiotics by using the stringent response (SR). During SR, a protein of the RelA/SpoT Homolog (RSH) Superfamily synthesizes magic spot nucleotides (MSN) like pppGpp or ppGpp. MSN are able to regulate gene expression, whereby the metabolism is shut down, until the stress factor is not affecting the bacteria anymore[1]. Since antibiotics do not work as intended during SR, the synthesis of MSN is a good target to prevent bacteria from bypassing the antibiotic effects[2]. In this project, we show a possibility to quantify both educts and products from $Rel_{Mtb}$ taking part in the stringent response.




***In vitro* synthesis of (p)ppGpp**. $Rel_{Mtb}$ from *M. tuberculosis* transfers a pyrophosphate from ATP to either GDP (n=0) or GTP (n=1) to form AMP and either ppGpp (n=0) or pppGpp (n=1).

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# Modular Access to Homoallylic Alcohols via Organophotoredox/Ni-Cocatalyzed Allylation of Allenes

Hui Xie[a], Bernhard Breit[a*]

Institute of Organic Chemistry, University of Freiburg,
Albertstrasse 21, 79104, Freiburg im Breisgau, Germany
xiehui1270@gmail.com

We herein describe a general, modular reductive allylation of aldehydes upon coupling with allenes using a new organophotoredox/nickel dual catalysis process. Preliminary mechanistic studies indicate a key π-allylnickel intermediate generated from Ni−H insertion of allene: the coupling with an aldehyde occurs *via* a preferred Zimmerman-Traxler transition state, resulting in branch selective anti-homoallylic alcohols (up to 91% yield, >95:5 dr). This synthetic methodology has also provided a diverse way to access intermediates of medicinally relevant drugs. We hope that this novel methodology will complement and expand the field of metallaphotoredox-catalyzed C−C bond-forming reactions.

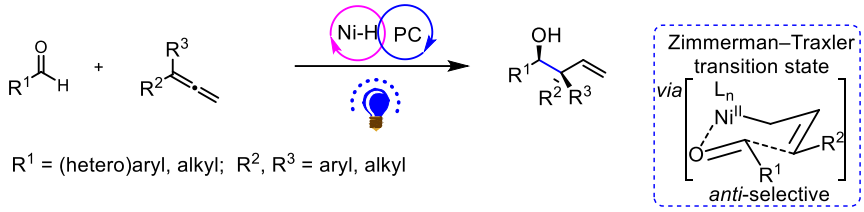


**Figure 1**. Strategies for homoallylic alcohols synthesis

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

*Zhiqiang Zeng[1,2#], Sven Küspert[1,2#], S. Esmael Balaghi[2,3], Haytham E. M. Hussein[4], Niklas Ortlieb[1], Markus Knäbbeler-Buß[5], Philipp Hügenell[5], Stephan Pollitt[6], Niclas Hug[1], Julia Melke[1,2,3], Anna Fischer[1,2,3*]*

[1]Institute of Inorganic and Analytical Chemistry (IAAC), University of Freiburg, Albertstraße 21, 79104 Freiburg, Germany.

[2]Freiburg Materials Research Center (FMF), University of Freiburg, Stefan-Meier-Straße 21, 79104 Freiburg, Germany.

[3]Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany.

[4]Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom.

[5]The Fraunhofer Institute for Solar Energy Systems ISE, Heidenhofstraße 2, 79110 Freiburg, Germany.

[6]Paul Scherrer Institute, Forschungsstrasse 111, 5232 Villigen PSI, Switzerland.

**Abstract**

Platinum is one of the best-performing catalysts for the hydrogen evolution reaction (HER). However, its high cost and scarcity severely hinder the large-scale application of Pt electrocatalysts. Constructing highly dispersed ultrasmall Platinum entities is a very effective strategy to increase Pt utilization and its mass activities and thereby reduce costs. Here, we synthesized highly dispersed Pt entities composed of a mixture of Pt single atoms, clusters and nanoparticles supported on mesoporous N-doped carbon (MPNC) nanospheres. The presence of Pt single-atoms, clusters and nanoparticles is demonstrated by a combined approach involving XRD, XPS, ac-ADF-STEM, XAS and electrochemical CO stripping. The best catalyst exhibits an excellent HER geometric and Pt-based mass activity which is respectively 4.2 and 26 times higher than that of a commercial Pt/C reference and a Pt/NC-nanofiber catalyst with similar Pt loadings. Importantly, after optimization of the geometrical Pt electrode loading, the best catalyst exhibits ultrahigh Pt- and catalyst-mass activities (56±3 A $mg^{-1}_{Pt}$ and 11.7±0.6 A $mg^{-1}_{Cat}$ at -50 mV vs. RHE), which are respectively 1.5 and 58 times higher than the highest Pt- and catalyst-mass activities reported so far for Pt single-atom and cluster-based catalysts.[1,2]

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# PrrA/B and MSMEG_0243 potentially regulate phosphatidylinositol mannoside acetylation in mycobacteria

Y. Zhang, M. Weldle, M. Li, C. Jessen-Trefzer

Yu Zhang, Albert-Ludwigs University of Freiburg, Albertstraße 21, Freiburg

Two-component systems (TCSs) are a common signal transduction mechanism in many bacteria. They consist of a membrane-bound histidine kinase that is autophosphorylated upon signal molecule binding with ATP consumption at a histidine residue. The kinase can then phosphorylate a response receiver at an aspartic acid residue, which triggers the cellular response.[1] Bacterial TCSs play important roles in virulence and drug resistance, and can be potential drug targets.[2] The histidine kinase MSMEG_0246 (PrrB) and the response receiver MSMEG_0244 (PrrA) constitute a two-component system (TCS) within *Mycobacterium smegmatis*. Adjacent to *msmeg_0244* and *msmeg_0246* in the operon lies a periplasmic protein gene called *msmeg_0243*, accompanied by its paralog *msmeg_0242* upstream. Both of these proteins are annotated as heme-binding proteins. Knockout mutants showed that biofilm formation was abrogated when *msmeg_0243*, *msmeg_0244*, and *msmeg_0246* were knocked out, compared to the wildtype *M. smegmatis*. The lipid analysis of this knockout mutant showed a defect in processing acetylated phosphatidylinositol mannosides.[3]
The aim of this project is to test the heme binding properties of MSMEG_0242 and MSMEG_0243 and investigate potential interaction partners of MSMEG_0246. This was tested with proteins purified in *Escherichia coli* but also over-expressions of MSMEG_0243, MSMEG_0244 and MSMEG_0246 in *M.smegmatis* were achieved and pure proteins were obtained.
Heme binding was tested using UV/Vis spectrometer and biolayer interferometry (BLI), interaction to MSMEG_0246 was tested by BLI and co-purification experiments.
While MSMEG_0242 showed no binding to heme, MSMEG_0243 bound heme with a KD value of ~300 nM. Neither MSMEG_0243 nor MSMEG_0242 showed direct binding to MSMEG_0246. The extracellular protein Ag85B, which is involved in cell wall construction in mycobacteria, showed binding to MSMEG_0246, but it has to be examined if it is relevant in cells.
Several open questions remain, especially regarding the signal transduction mechanisms and downstream gene regulation, which will be subject of further studies.
Since MSMEG_0243 and MSMEG_0244/0246 are involved in biofilm formation and lipid acetylation, abrogation of the physical interaction between these proteins on the extracellular side of the cell provides a handle to reduce biofilm formation and potentially colonialization of bacteria, even with small molecule drugs that are not able to penetrate the mycobacterial plasma membrane.

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**Molecular interplay of an assembly machinery for nitrous oxide reductase**

Christoph Müller[1,4], Lin Zhang[1,4],Sara Zipfel[1], Annika Topitsch[1], Marleen Lutz[1], Johannes Eckert[1], Benedikt Prasser[1], Mohamed Chami[2], Wei Lü[3], Juan Dü[3] & Oliver Einsle[1]

[1] Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany.

[2] BioEM Lab, Biozentrum, Universität Basel, Basel, Switzerland.

[3] Department of Structural Biology, Van Andel Institute, Grand Rapids, MI, USA.

[4] These authors contributed equally: Christoph Müller, Lin Zhang.

Emissions of the critical ozone-depleting and greenhouse gas nitrous oxide ($N_2O$) from soils and industrial processes have increased considerably over the last decades (refs. [1-3]). As the final step of bacterial denitrification, $N_2O$ is reduced to chemically inert $N_2$ (refs. [1,4]) in a reaction that is catalysed by the copper-dependent nitrous oxide reductase ($N_2OR$) (ref. [5]). The assembly of its unique [4Cu:2S] active site cluster $Cu_Z$ requires both the ATP-binding-cassette (ABC) complex NosDFY and the membrane-anchored copper chaperone NosL (refs.[4,6]). Here we report cryo-electron microscopy structures of *Pseudomonas stutzeri* NosDFY and its complexes with NosL and $N_2OR$, respectively. We find that the periplasmic NosD protein contains a binding site for a $Cu^+$ ion and interacts specifically with NosL in its nucleotide-free state, whereas its binding to $N_2OR$ requires a conformational change that is triggered by ATP binding. Mutually exclusive structures of NosDFY in complex with NosL and with $N_2OR$ reveal a sequential metal-trafficking and assembly pathway for a highly complex copper site. Within this pathway, NosDFY acts as a mechanical energy transducer rather than as a transporter. It links ATP hydrolysis in the cytoplasm to a conformational transition of the NosD subunit in the periplasm, which is required for NosDFY to switch its interaction partner so that copper ions are handed over from the chaperone NosL to the enzyme $N_2OR$ (ref. [7]).

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# Harnessing Encapsulins for Intracellular Organometallic and Enzymatic Catalysis

M. Zmyslia., P. Lohner, M. Grimmeisen, Dr. Claudia Jessen-Trefzer
Mariia Zmyslia, University of Freiburg, Albertstraße 21, Freiburg im Breisgau

Subcellular compartmentalization is a fundamental strategy used by all organisms to enable regulation and fine-tuning of metabolic processes. Cells utilize compartmentalization to create specific microenvironments, segregate incompatible biochemical reactions and pathways, or protect themselves from toxic by-products [1]. Encapsulins (Enc), self-assembling protein-based bacterial nanocompartments, are a highly attractive platform for enzyme compartmentalization. Encapsulins are temperature and pH-stable [2] and promote highly selective packaging of co-expressed proteins through a conserved amino acid sequence (encapsulin localization sequence, ELS) at their C-terminus [3–5]. Non-native cargo proteins can be directed inside Enc via simple tagging with ELS, enabling self-assembly of the new functional compartment [3–5].
In this study, we describe the first targeted incorporation of an organometallic catalyst to the interior of Enc from *Mycobacterium smegmatis* ($Enc_{SM}$) enabled by a HaloTag-ELS construct ($Enc_{SM}${HaloTag}). The approach is versatile, as the HaloTag-ELS strategy enables the introduction of many different metal/ligand systems new to biology, provided they operate in water. We explore, as a proof of concept reaction, the ruthenium-catalyzed deallylation of a profluorophore inside the Enc nanoreactor.
Upon installing a transition metal catalyst inside the capsule, we show that catalysis inside this capsule is possible and that encapsulation affects reaction yields. The deallylation of a profluorophore takes place with lower yields with the HaloTag-$PEG_3$-Ru construct outside of the capsule compared to within encapsulin. We go on to demonstrate that the same reaction is also efficiently catalyzed inside a living cell line that takes up the engineered Enc and produces localized fluorescent vesicles [6].
To investigate enzyme catalysis in $Enc_{SM}$ nanocompartment, we explore encapsulation of nitroreductase NfsB from *E. coli* (NTR). The combination of encapsulated NTR with nitroaromatic prodrugs is a promising approach in enzyme prodrug therapy by minimizing toxicity to healthy cells and increasing the concentration of drugs against cancer cells.
Since NTR is an obligate dimer, we show that a tandem-dimer configuration (tdNTR), where NTR monomers are connected by a floppy 22-residue linker, enhances enzyme stability [7] and ensures correct packaging of the enzyme inside the nanocompartment, retaining its activity. In vitro prodrug activation is confirmed by LC/MS analysis after incubation with free and encapsulated tdNTR ($Enc_{SM}${td-NTR}).
In line with this, extracellular prodrug activation proves that $Enc_{SM}${td-NTR} and 1-(4-Nitrobenzoyl)piperidine prodrug are a promising combination and prodrug activation leads to a decrease in cell viability. On the other hand, in cellulo prodrug activation is not sufficient for a significant decrease in cell viability.
A performed co-localization study shows that the taken-up Enc particles are found together with Lysotracker dye. Therefore, we hypothesize that the encapsulated enzyme is trapped in lysosomes, where it cannot activate the prodrug, as the required co-factor NADH is not present in the lysosomal environment. Surface modification/optimization of $Enc_{SM}$ shell is necessary to influence the cellular fate of nanoparticles and direct the encapsulated enzyme to the cytosol, where it can unravel its action.

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# Crystal Structure and Expanded Substrate Spectrum of the α-keto acid *C*-Methyltransferases SgvM and MrsA

Juliane Breiltgens, Ziruo Zou, Christina Sommer-Kamann, Florian Kemper, Jennifer N. Andexer, Oliver Einsle, Michael Müller

*S*-adenosylmethionine (SAM)-dependent methyltransferases (MTs) are involved in the *C*-methylation of a variety of natural products such as DNA, proteins, or small molecules.[1] Enzymatic *C*-methylation requires activation of the substrate's carbon atom by an adjacent functional group to form a nucleophilic intermediate carbanion and to enable nucleophilic attack on the methyl residue of SAM. A few examples of *C*-MTs have been described that methylate enolizable β-branched α-keto acids, leading to precursors of non-proteinogenic amino acids.[2] The MTs SgvM from *Streptomyces griseoviridis* and MrsA from *Pseudomonas syringae* pv. syringae catalyze the methylation of the β-carbon atom of α-keto acids in the biosynthesis of the antibiotic natural products viridogrisein and 3-methylargenine, respectively.[3] MrsA shows high substrate selectivity for its native substrate 5-guanidino-2-oxovalerate, while other α-keto acids, such as the SgvM substrates 4-methyl-2-oxovalerate, 2-oxovalerate, and phenylpyruvate, were not accepted.

Here, we report on the crystal structures of SgvM and MrsA in the apo form, in complex with their substrates, SAM, and methyladenosine, a degradation product of SAM, respectively. By investigating key substrate recognition residues in the active site of both enzymes and through site-directed mutagenesis, the substrate spectrum of MrsA was extended to accept the α-keto acid substrates of SgvM with uncharged and lipophilic β-residues. Our results showcase the possibility to transfer the substrate promiscuity of α-keto acid MTs from distinct biosynthetic pathways by rational design.

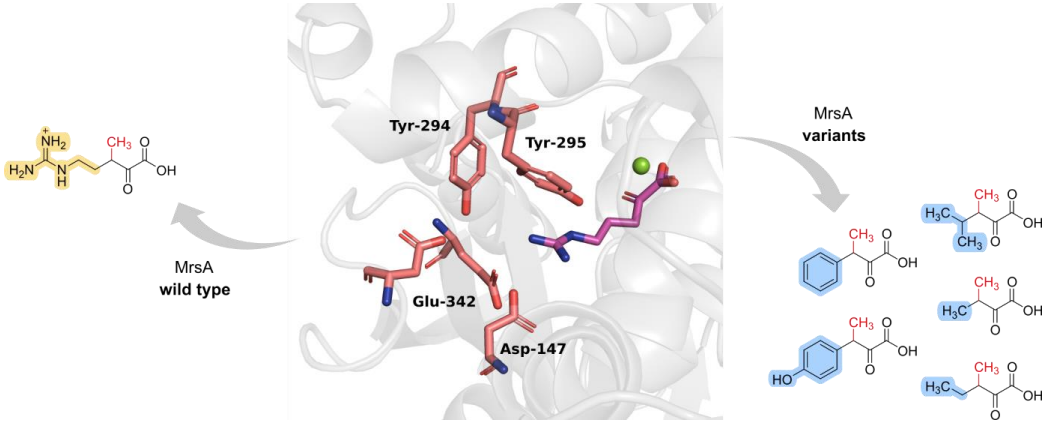


**Figure 1.** Active site of MrsA wild type in complex with its native substrate 5-guanidino-2-oxovalerate (dark grey) and $Mg^{2+}$ ion (green). The residues of the mutation sites for MrsA variants are highlighted in red.

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