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Brief History of the Institute of Biochemistry

The *Institute of Biochemistry and Food Chemistry* was born out of the division of the *Institute of Biochemical Technology, Food Chemistry and Microchemistry* of the former *School of Technology Graz*. Together with all the other chemistry institutes, it was located in the old Chemistry Building on Baron Mandell's ground at the corner Technikerstraße-Mandellstraße.

1929 The *Institute of Technical Biochemistry and Microbiology* moved to the Fürstlich-Dietrichstein-Stiftung building, Schlögelgasse 9, the focus of biosciences at that time.

1945 Georg GORBACH - initially in the rank of a docent and soon thereafter as a.o. Professor - took over to lead the new *Institute of Biochemical Technology and Food Chemistry*.

1948 G. GORBACH was nominated full professor and head of the institute. In succession of the famous *Graz School of Microchemistry* founded by PREGL and EMICH, Prof. GORBACH was one of the most prominent and active leaders in the fields of microchemistry, microbiology and nutritional sciences. Questions of water quality and wastewater disposal were the focus of the group of Prof. K. STUNDL. In addition, a division to fight dry-rot supervised by Dr. KUNZE and later by H. SALOMON, was also affiliated with the institute.

1955 In honor of the founder of microchemistry and former professor at *Graz University of Technology*, the labs were called EMICH-Laboratories and the institute was renamed to *Institute of Biochemical Technology, Food Chemistry and Microchemistry*.

1970 After the decease of Prof. GORBACH, Prof. GRUBITSCH was appointed head of the institute. At the end of the 1960s, the division for water and wastewater disposal headed by Prof. STUNDL was established as an independent institute. Prof. SPITZY was nominated professor of general chemistry, micro- and radiochemistry.

1973 Division of the *Institute for Biochemical Technology, Food Technology and Microchemistry* took place. At first, biochemical technology together with food technology formed a new institute now called *Institute of Biotechnology and Food Chemistry* headed by Prof. LAFFERTY.

1973 F. PALTAUF was appointed professor and head of the newly established *Institute of Biochemistry*. The interest of Prof. PALTAUF to study biological membranes and lipids laid the foundation for the future direction of research. G. DAUM, S. D. KOHLWEIN, and A. HERMETTER joined the institute and, after carrying out postdoctoral research in renowned laboratories, established independent research groups in cell biology (G. D.), biophysics (A. H.) and molecular biology (S. D. K.).

1990 The institute moved to a new building in Petersgasse 12. This enabled the expansion of individual research groups, essential for the participation in novel collaborative efforts at the national and international level. Thus, the *Institute of Biochemistry*, together with partner institutes from the Karl-Franzens-University, was the driving force to establish Graz as a center of competence in lipid research.

2003 After Prof. PALTAUF's retirement in 2001, P. MACHEROUX was appointed full professor of biochemistry in September 2003 and head of the *Institute of Biochemistry* in January 2004. His research interests revolve around topics in protein biochemistry and enzymology.

2007 K. ATHENSTAEDT, a long-time associate of Prof. DAUM, received the *venia legendi* for biochemistry. Karin is the first woman to complete the traditional habilitation at the *Institute of Biochemistry*!

2013 Due to faculty reorganizations, the group of J. BOGNER-STRAUSS (formerly at the *Institute of Genomics and Bioinformatics*) joined the *Institute of Biochemistry*. Her main interests revolve around the development and metabolism of adipocytes.

2014 A. WINKLER, who was one of the first students to receive his PhD in the DK "Molecular Enzymology", returned to the institute after 5 years postdoctoral research with Prof. Schlichting at the Max-Planck-Institute for Medical Research in Heidelberg.

2015 After more than 40 years at the *Institute of Biochemistry* A. HERMETTER retired in September. After a postdoctoral fellowship at the Max Planck Institute in Mühlheim, he joined the institute and moved through the ranks to become associate professor. As an active scientist he published more than 120 papers in peer-reviewed journals and graduated 25 PhD students.

2016 A. WINKLER was appointed assistant professor at the institute to establish an independent research group in the field of photoreceptor research.

2017 G. DAUM, who had been at the institute for 42 years, retired at the end of the academic year. He was one of the leading scientists in the field of yeast lipid research, enjoying worldwide recognition. He also was an enthusiastic academic teacher supervising 36 PhD students and being director of the Doctoral School of Biosciences.

2017 J. BOGNER-STRAUSS left the institute to start a political career first at the federal government and then at the Styrian state government as Councilor for Research, Society, Health and Care.

2019 G. OBERDORFER, who received an ERC Starting grant in 2018, was appointed assistant professor at the Institute of Biochemistry to establish an independent research group in the field of protein design and engineering further strengthening the focus of the institute on protein biochemistry

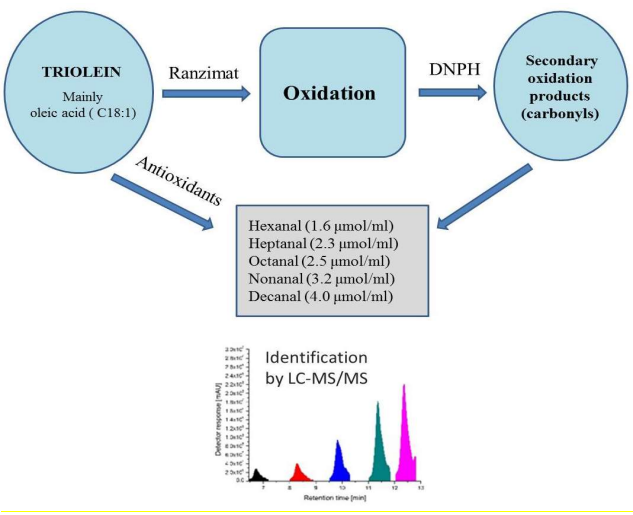
2021 A. WINKLER received his *venia legendi* in *Structural Biochemistry* and was appointed associate professor at the institute.

Highlights of 2021

Following two postponements owing to the Corona virus pandemic, the 20th International Symposium on Flavins and Flavoproteins was finally held in Graz in September 2021! The conference was organized by Peter Macheroux, Gustav Oberdorfer and Andreas Winkler from our institute as well as Karl Gruber and Wolfgang Kroutil from the University of Graz. We were happy to welcome 75 colleagues from around the world for this traditional five-day event. In addition, 65 flavin enthusiasts followed the 32 oral presentations on-line. Although organizing a hybrid conference caused several sleepless nights, we were rewarded by many happy faces and kind comments. The traditional conference banquet in the aula of the University of Graz was the perfect setting for the Vincent-Massey Young Investigator and Poste awarding ceremonies.

The group of **Gustav Oberdorfer** seems to have found its equilibrium size in 2021 and is now comprised of around 14 people. Additions to the group were Henrik Seyfried working on his master theses as well as Sigrid Kaltenbrunner and Katharina Leitner, both performing their bachelor thesis in the group. Besides these three, the Oberdorfer group grew by one more PhD students. Markus Braun, a former masters student and lab technician re-joined the group to work on his doctorate.

In the group of **Andreas Winkler,** the retirement of Elfriede Zenzmaier left a big scar. In a joint team effort all members worked hard to compensate for the now missing help and input in all aspects of lab life – from science to food supplies in group meetings. Especially with the start of two additional PhD students (Oliver Eder and Maximilian Fuchs) everyone was eventually longing for some support in general lab routines since also Nadine Galler was on lab rotation for half a year. Eventually, Philipp Pelzmann started roughly one year after Elfriede had left and only shortly before Nadine also left the institute at the end of her apprenticeship.



In the Functional Food Group of **Michael Murkovic** the main projects were focused on the use of food wastes for cultivation of edible fungi and the development of food ingredients on basis of microalgae. In addition, the cultivation of insects for food is also of interest. In a second project the formation of health threatening components was investigated. These were oxidised lipids and furfuryl alcohol. Both might be related to cancer and a reduction of alimentary exposure should improve food safety. A project on lipophilic alimentary cyclic peptides started.

Some impressions from the International Symposium on Flavins and Flavoproteins

Biochemistry Group

Group leader: Peter Macheroux
Secretary: Christine Rother
Senior research scientist: Silvia Wallner
Postdoctoral fellows: Aleksandar Bijelic, Alexandra Jammer, Shalinee Jha
PhD students: Reinmar Eggers, Katharina Fuchs, Bettina Hierzberger, Bianca Kerschbaumer, Sami Ullah Khan, Grazia Malovan
Guest fellow: Samuele Suraci
Master students: Stefanie Baldauf, Agnes Geher, Christina Horn, Anna Lueger
Technical staff: Eva Maria Frießer, Rosemarie Trenker-El-Toukhy

General description

The fundamental questions in the study of enzymes, the bio-catalysts of all living organisms, revolve around their ability to select a substrate (substrate specificity) and subject this substrate to a predetermined chemical reaction (reaction and regio-specificity). In general, only a few amino acid residues in the "active site" of an enzyme are involved in this process and hence provide the key to the processes taking place during enzyme catalysis. Therefore, the focus of our research is to achieve a deeper understanding of the functional role of amino acids in the active site of enzymes with regard to substrate-recognition and stereo- and regiospecificity of the chemical transformation. In addition, we are also interested in substrate-triggered conformational changes and how enzymes utilize cofactors (flavin, nicotineamide) to achieve catalysis. Towards these aims we employ a multidisciplinary approach encompassing kinetic, thermodynamic, spectroscopic and structural techniques. In addition, we use site-directed mutagenesis to generate enzyme variants to probe their functional role in the mentioned processes. Furthermore, we collaborate with our partners in academia and industry to develop inhibitors for enzymes, which can yield important new insights into enzyme mechanisms and can be useful as potential lead compounds in the design of new drugs.

The methods established in our laboratory comprise kinetic (stopped-flow and rapid quench analysis of enzymatic reactions), thermodynamic (isothermal titration microcalorimetry) and spectroscopic (fluorescence, circular dichroism and UV-vis absorption) methods. We also frequently use MALDI-TOF and ESI mass spectrometry, protein purification techniques (chromatography and electrophoresis) and modern molecular biology methods to clone and express genes of interest. A brief description of our current research projects is given below.

Berberine bridge enzyme-like enzymes in plants

Berberine bridge enzyme (BBE) is a central enzyme in alkaloid metabolism and catalyzes the oxidation of the *N*-methyl group of (*S*)-reticuline with concomitant formation of a carbon-carbon bond (the “berberine bridge”) to yield (*S*)-scoulerine. Using bioinformatics, we found that homologs of BBE are widespread among plants, fungi and bacteria. The model plant *Arabidopsis thaliana*, for example, possesses 27 genes that apparently encode BBE-like enzymes although the plant does not synthesize complex alkaloids. We have recombinantly produced BBE-like homologs, *At*BBE-like proteins 13 and 15, from *A. thaliana* in *Komagataella phaffii* and identified monolignols and their glycosylated derivatives as potential

substrates. We have solved the X-ray structure of *At*BBE-like 15 and the topology was found to be very similar to that of the BBE from *Eschscholzia californica* previously solved by Dr. Andreas Winkler and Prof. Karl Gruber. However, the residues that form the active site are distinct from those found in BBE from *E. californica*. The active site architecture of the monolignol oxidoreductase is conserved in approximately half of the BBE-like enzymes suggesting that this reaction plays an important role in plant metabolism.

To further unveil the role of BBE-like enzymes, we have teamed up with Professor Dr. Tomas Werner from the Institute of Biology at the University of Graz to investigate the *in planta* functions of the monolignol oxidase sub-family six of the *Arabidopsis* BBE-like enzymes (*AtBBE-like 13, 15, 24, 25 and 26).* Gene expression analysis employing qRT-PCR has been carried out in all types of plant organs. All genes from subgroup six show expression in roots and flowers. In addition, GUS reporter lines have been generated to investigate gene expression patterns on the tissue level throughout the plant's life cycle. GUS staining patterns have provided valuable information and will serve as basis for the design of further experiments. Furthermore, we are working on the generation of single and multiple knockout lines for our genes of interest. Several T-DNA insertion mutants were ordered from the Nottingham Arabidopsis Stock Centre, and homozygous single knockout lines for *AtBBE-like 13* and *15,* as well as a homozygous double knockout line for the two genes, have been generated. A CRISPR/Cas9 approach is currently underway to generate single, double, and triple knockouts for *AtBBE-like 24, 25,* and *26*, as these genes are located in close proximity on the same chromosome and the individual mutations can, thus, not be combined in a multiple knockout line via crossing approaches. A detailed phenotyping of the single and multiple knockout mutants, combined with the results from the expression analyses (qPCR, GUS plants) and the results of biochemical and physiological studies will provide information for the elucidation of *in planta* functions of the members of *At*BBE-like subgroup six (thesis project of Reinmar Eggers supported by Dr. Alexandra Jammer).

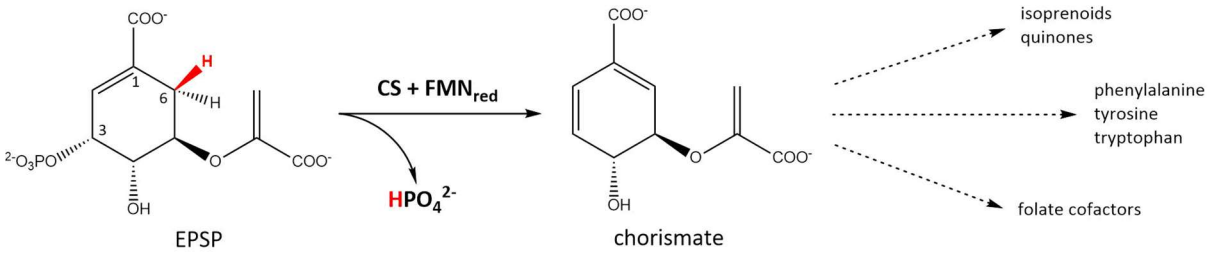
Chorismate synthase as a potential target for pharmaceutically useful inhibitors

Chorismate synthase (CS) is the last enzyme of the shikimate pathway, a biosynthetic route that produces aromatic compounds such as the aromatic amino acids phenylalanine, tryptophan, and tyrosine. As this metabolic pathway is only present in prokaryotes, fungi, and plants, mammals depend on a dietary supply. Inhibition of this pathway has severe consequences for the above organisms because each step of the shikimate pathway comprises an essential reaction in chorismate synthesis, which cannot be bypassed by any alternative enzyme. The absence of the shikimate pathway in mammals make this metabolic pathway a promising target for the development of antibacterial agents and herbicides.

Chorismate synthase was chosen as a drug target because of its unique catalytic mechanism. Potential inhibitors for CS were selected using a combination of virtual screening and molecular dynamics (Rodrigues-Vendramini *et al.*, 2019; Bueno *et al.*, 2019). For the design of new inhibitors, the extremely positively charged binding pocket of CS was considered. In the course of testing several compounds, azo-dyes turned out to be the most promising candidates, which was confirmed by various methods. Within the class of azo-dyes 5-amino-3-[(*E*)-(3-chloro-2-hydroxy-5-nitrophenyl)diazenyl]-4-hydroxy-2,7-naphthalenedisulfonic acid) showed the best results, which can be explained by the structure of this compound containing several negatively charged substituents.

Furthermore, the interaction of the obtained inhibitory compounds with different CSs was evaluated by dissociation constants (1.1-4.4 µM) and IC50 values (10-16 µM), which were determined by binding and inhibition assays, respectively. To get a better insight into the binding mode, crystallization of CS-inhibitor complexes is under way.

By investigating the active site of CS, it was observed that the binding pocket contains several invariant arginine residues. However, structural studies of the active site from different CSs showed one arginine residue that differs, suggesting differences in how the substrate is bound to the active site between CSs. To elucidate the binding mode, it will be necessary to obtain structural information of more CS-inhibitor complexes. Furthermore, new inhibitory compounds should be synthesized and analyzed to improve their binding to CS (thesis project of Katharina Fuchs supported by Dr. Aleksandar Bijelic).



Schematic representation of the seventh and last step of the shikimate pathway. The reaction is catalyzed by chorismate synthase and includes the conversion of EPSP (5-enolpyruvylshikimate-3-phosphate) to the final product of the pathway, chorismate. Chorismate is the starting material for the synthesis of a plethora of aromatic secondary metabolites.

References

Rodrigues-Vendramini, F. A. V., Marschalk, C., Toplak, M., **Macheroux, P.**, de Souza Bonfim-Mendonca, P., Svidzinski, T. I. E., Seixas, F. A. V., Kioshima, E. S.: Promising new antifungal treatment targeting chorismate synthase from *Paracoccidioides brasiliensis*, Antimicrob. Agents Chemother., 2019, **63**:e01097-18. DOI:10.1128/AAC.01097-18

Bueno, P. S. A., Rodrigues-Vendramini, F. A. V., Toplak, M., **Macheroux, P.**, Kioshima Cotica, E. S., Seixas, F. A. V.: New inhibitors of chorismate synthase present antifungal activity against *Paracoccidioides brasiliensis*, Future Microbiology, 2019, **14**:969-980. DOI:10.2217/fmb-2019-0052

Dipeptidylpeptidase 3

Dipeptidyl peptidase 3 (DPP3) is a zinc-dependent metalloenzyme involved in the catabolism of several bioactive peptides, including the vasoconstrictor peptide Angiotensin II. In view of this, it has been associated with blood pressure regulation and enhancement of cancer cell defense against oxidative stress. The documented efficacy of DPP3 in lowering the blood pressure in angiotensin II-infused hypertensive mice is indicative of its role in cardiovascular diseases, particularly in the protection of hypertension-sensitive organs such as heart and kidney. Recent findings strongly indicate that hemodynamically compromised patients, especially those suffering from myocardial infarction, sepsis, cardiogenic shock and heart failure, display elevated levels of DPP3 in circulation. This increase in circulating DPP3 level

was associated to a higher mortality rate in the patients. Higher circulating DPP3 concentration at admission was also associated with an increased risk of death, circulatory failure, and acute kidney injury in severely burnt patients.

Despite the intriguing involvement of DPP3 in metabolic disease pathogenesis, the underlying physiology of this enzyme is poorly understood. In collaboration with Prof. Dr. Robert Zimmerman and Prof. Dr. Karl Gruber from the University of Graz, Assoc. **Prof.** Dr. Tobias Madl and Assoc. Prof. Sasa Frank at the Medical University of Graz and Attoquant Diagnostics in Vienna, we uncovered that DPP3 regulates the RAS pathway and water homeostasis by degrading circulating angiotensin peptides. Characterization of a mouse model lacking DPP3 at the genetic and proteomic level showed that this enzyme is associated with heightened water consumption, equilibrium angiotensin peptide levels and renal oxidative stress in mice. Furthermore, we established that DPP3 plays a sex-specific role, where only the male mice presented the phenotype on deletion of DPP3. The female mice were protected from the deleterious effects of DPP3 knockout, indicating a link between the endocrine system and the physiological role of DPP3. The characterization of DPP3 in this study established that it has strong metabolic implications through the modulation of the RAS pathway, a property that could be useful in the management of several cardiovascular and related pathologies. Currently, we are investigating the underlying mechanisms involved in the regulation of these physiological functions by DPP3 using the knockout mouse model and mammalian cell culture. In addition, we are also identifying specific inhibitors of this enzyme through virtual screening and evaluating the obtained hits *in vitro* for their binding affinity to the enzyme as well as their inhibitory properties.

Additional DPP3 *in vitro* expermiments were conducted to complement our *in vivo* experiments in collaboration with Dr. Julia Kargl (Medical University of Graz). This includes the use of three different cell lines are used (MCF-10A, MCF-7 and MDA-MB-231) to explore the cellular processes impacted by DPP3. Currently, we are employing CRISPR/Cas to generate *dpp3* knock-out cell lines to determine the role of DPP3 in viability, migration and apoptosis. In addition, we are interested to check the expression levels of selected proteins and their respective genes that are believed to interact with DPP3, such as Keap1, Nrf2, NQO1 and SOD2. The cell lines are also used to determine the localization of DPP3 (master project of Anna Lueger; PhD project of Grazia Malovan and Bettina Hierzberger; postdoctoral project of Shalinee Jha).

Oxidative C-H activation for C-C bond formation using the enzymes EasC and EasE

Ergot alkaloids (EAs) are natural products produced by different fungi and are widely used in the pharmaceutical industry for drug formulation, and therefore attract attention in various research fields. However, total synthesis of alkaloids is difficult, which is why production of intermediates of the alkaloid synthesis pathway is desired. A crucial step in EA synthesis is a carbon-carbon (C-C) bond formation. In synthetic organic chemistry this step is still challenging as well as time consuming and expensive. Hence, special emphasis has been placed on the field of enzyme catalyzed C-C bond formation in the last two decades.

In order to study the oxidative cyclization in chanoclavine biosynthesis and the involved enzymes and cofactors, we will have a closer look at EasE – a flavin adenine dinucleotide (FAD) dependent homologue of the berberine bridge enzyme-like (BBE) enzyme, – and EasC – a heme dependent catalase – originating from *Aspergillus japonicus*. CnsA (BBE-like) and

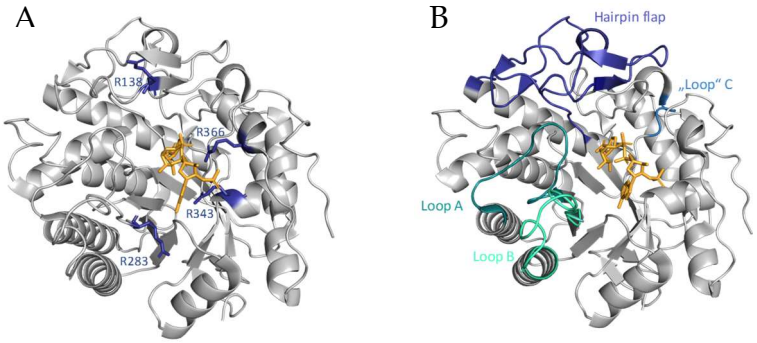
CnsD (a putative catalase) will be also investigated, which represent a similar model in *Penicillium expansum*. The primary goal will be the production of the active enzymes by recombinant biotechnology. Furthermore, the obtained proteins will be characterized with regard to their kinetic parameters and their substrate scope. Eventually, the enzymes will be employed to explore their utility in producing derivatives of the fungal alkaloid chanoclavine (thesis project of Bianca Kerschbaumer).

Ene-reductases: shifting the cofactor preference of *Le*OPR3 from NADPH to NADH

Ene-reductases (ERs) are remarkable enzymes since they show high regio-, stereo-, and enantioselectivity and accept a broad variety of alkenes as substrates. The most investigated class of ERs are the oxidoreductases of the old yellow enzyme family (OYEs), which are flavoproteins using a nicotinamide cofactor as hydride donor and a conserved tyrosine residue for proton delivery.

Intriguingly, the majority of ERs prefer NADPH over NADH, constituting a major drawback for industrial applications. In recent years, a broad spectrum of enzymes of the OYE group was investigated in terms of their catalytic cycle and toward their cofactor preference, revealing that all currently known OYEs exhibit the same tertiary structure. However, the origin of the coenzyme specificity remains a mystery.

In order to study the coenzyme specificity, FMN-dependent 12-oxophytodienoate reductase 3 originating from tomato (*Sl*OPR3) has been chosen as a model enzyme. Two different strategies have been chosen to alter nicotinamide cofactor acceptance of the enzyme: (I) rational design in which only single or multiple amino acids have been exchanged and (II) a semi-rational approach, where whole parts of the protein have been swapped with parts from ERs that prefer NADH as cofactor (thesis project of Bianca Kerschbaumer).



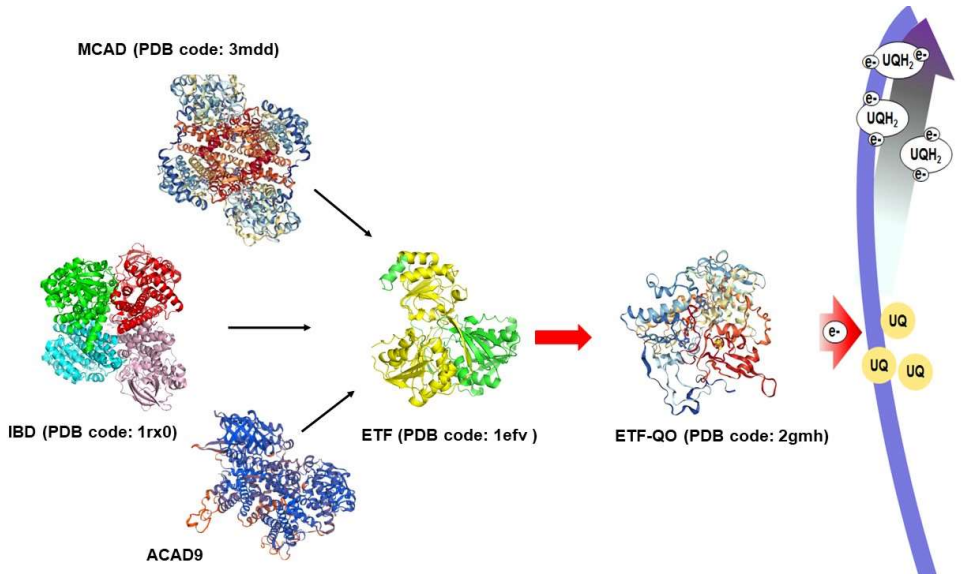
Crystal structure of *Le*OPR3 with NADPH (yellow) and labelled mutation target sites (blue, teal): the cofactor is shown as integrated in Dbr2 (PDB: 5dxy), the crystal structure of the closest homolog available with bound NADPH: (A) representation of the mutation sites of the rational design approach, (B) representation of the loop swap approach.

Flavin-dependent electron transfer in human mitochondria

Electron transferring flavoproteins are found in a variety of eukaryotic and prokaryotic organisms including humans. Human electron transferring flavoprotein (hETF) is involved in the oxidative energy metabolism by accepting electrons from at least fourteen different flavin

dehydrogenases and passing them on to the mitochondrial respiratory chain. In previous studies by Drs. Peter Augustin and Marina Toplak it was discovered that the flavin cofactor of hETF can undergo a pH-dependent and irreversible oxidation leading to the formation of an 8-formyl-FAD (8f-FAD) moiety.

ETFs are heterodimeric proteins, where extensive protein movement to an open conformation is a prerequisite for effective electron transfer. It is known that the lack of the salt bridge between αN259 and βE165 results in an open form of hETF. Interestingly, the rate of formylation is different between hETF wild type and the αN259A and ßE656A variant proteins. Currently, we study the effect of cofactor formylation on the interaction of hETF with selected client dehydrogenases, such as the medium-chain acyl-CoA dehydrogenase (MCAD), acyl-CoA dehydrogenase family member 9 (ACAD9) and isobutyryl-CoA dehydrogenase (IBD). We investigate the electron transfer from these dehydrogenases to the non formylated and formylated forms of hETF and the αN259A, ßE156A as well as the αN259A ßE156A variants with a focus on possible conformational differences between wild type and variants as a function of cofactor formylation. In the course of our study, we discovered that the flavin cofactor can be further oxidized to its 8-carboxy form, which was confirmed using HR-MS. By means of spectrophotometry and mass spectrometry, we now aim to shed light on the interplay of cofactor modifications, protein conformation and the rate of electron transfer in the crucial human electron carrier ETF (thesis project of Sami Ullah Khan supported by Dr. Silvia Wallner).



Schematic representation of the interaction of hETF with various client dehydrogenases and ETF-QO. ETF accepts electrons from different flavin dehydrogenases and passes them on to the mitochondrial respiratory chain via ETF-QO.

Role of bacterial aspartate β-decarboxylase in alamandine biosynthesis

The renin-angiotensin system (RAS) plays a pivotal role in cardiovascular and renal pathophysiology. Angiotensin (Ang) II, the active component of the RAS, is an octapeptide resulting from angiotensin-converting enzyme (ACE)-mediated cleavage of angiotensin I. Ang II binds to its receptors, AT1 and AT2 to mediate its effects. The ACE/Ang II/AT1 receptor axis mediates vasoconstriction, renal sodium reabsorption, thirst, release of vasopressin and aldosterone, inflammation, fibrosis and oxidative stress. Angiotensin-converting enzyme 2 (ACE2), a homolog of ACE with mono-carboxypeptidase activity, converts Ang II to Ang (1–

7), contributing to the major cardioprotective arm of RAS, counteracting the pro-hypertensive effects of the classical ACE/Ang II/AT1R axis. Recently, a heptapeptide alamandine and its receptor MrgD were identified as novel RAS components. Alamandine and Ang (1–7) differ by only one amino acid residue, an alanine instead of aspartic acid at the N-terminal position. A similar observation of an alanine[1] substituted Ang II, Ang A, was reported previously. *In-vivo* and *in-vitro* studies demonstrate that alamandine subserves similar functions to Ang (1–7), e.g., vasodilation, blood pressure reduction, anti-inflammation and antifibrosis. Alamandine also reduced blood pressure in spontaneously hypertensive rats equivalent to Ang (1–7), and reduced cardiac fibrosis in Sprague–Dawley rats.

Alamandine as well as Ang A are found in human plasma, with increased levels reported in patients with end-stage renal disease (ESRD) or renal failure, however, their physiological significance in humans remains largely unexplored. It was shown that alamandine can be synthesized in rat hearts perfused with Ang (1–7), but the enzyme responsible for endogenous alamandine biosynthesis under these circumstances is still unknown. It is believed that both Ang A and alamandine are produced by the N-terminal aspartate decarboxylation of Ang II and Ang (1-7) respectively. Aspartate β-decarboxylase (EC 4.1.1.12), first discovered in the 1950s, catalyzes the conversion of L-aspartic acid to L-alanine. The majority of the aspartate β-decarboxylases reported till now have a bacterial origin, which points towards the possible involvement of a bacterial symbiont in alamandine formation. Using recombinant production and purification of aspartate β-decarboxylases from four different gut bacterial strains, we confirmed their ability to decarboxylate the amino terminal aspartic acid of Ang (1-7) to alanine, thus forming alamandine. Currently we are investigating the kinetics of alamandine production and the role played by the cofactor pyridoxal 5'-phosphate in this enzymatic process (Master thesis of Stefanie Baldauf, supported by Dr. Shalinee Jha).

Master Theses completed

Agnes Geher: *Identification of potential pathogenic variants in cardiac disease and epilepsy-related genes in sudden infant death syndrome (SIDS) and sudden unexplained death (SUD) cases*

Sudden unexpected death of a loved one is a tragic and painful event, especially because it mainly affects infants, children or young adults. The sudden and unexplained death of an apparently healthy infant up to 12 months of age is defined as sudden infant death syndrome (SIDS). If sudden death occurs in people older than one year of age and a diagnosis cannot be made despite a thorough investigation, including an autopsy, the cause of death is called sudden unexplained death (SUD). It is known from previous genetic studies that 15-30% of sudden deaths that remained elusive in conventional forensic autopsy examinations can be explained by genetic variants. In particular, cardiomyopathies, ion channelopathies or metabolic disorders are often associated with SIDS or SUD, but recent studies suggest that epilepsy may also explain some of the sudden deaths.

The aim of this work was to identify possible pathogenic single nucleotide variants (SNVs) and structural variants (SVs) in the SIDS and SUD cohorts of the Zurich Institute of Forensic Medicine. The cohorts included 151 SIDS and 45 SUD cases. Exome data from all cases were available from previous studies. The focus was set on 244 genes associated with cardiogenic diseases and 365 genes associated with epilepsy. Analyses of the exome data according to current ACMG guidelines identified 22 heterozygous pathogenic or likely

pathogenic SNVs associated with cardiogenic diseases in 14.6% of the SIDS cases. In addition, 52 heterozygous pathogenic or likely pathogenic SNVs associated with epilepsy were found in 27.8% of these cases. In the SUD cohort, 23 heterozygous pathogenic or likely pathogenic SNVs in epilepsy-associated genes were found in 40.0% of the SUD cases. All pathogenic or likely pathogenic SNVs with a coverage of ≥ 20 and ≤ 50 as well as potential deletions were confirmed by Sanger sequencing. The SUD cohort included nine SUDEP cases that had a known medical history of epilepsy. Within these nine cases, five cases showed pathogenic or likely pathogenic variants in epilepsy-related genes (COL4A1, KCNT1, PIGN, RMND1, SCN5A). The pathogenic variants and the cases with known epilepsy were discussed in detail. In the exome data of the SIDS cohort two potentially pathogenic or likely pathogenic SVs associated with epilepsy were identified (FKRP, KCNA1). However, the two SVs could not be confirmed by MLPA and/or Sanger sequencing. No other pathogenic or likely pathogenic SVs were identified in the cardiac disease related genes in the SIDS cohort and in the epilepsy-related genes in the SUD cohort.

This study supports the hypothesis that cardiac disease and epilepsy-related genetic variants may contribute to SIDS and SUD pathology and, therefore, genetic testing should be routinely performed in such cases.

This master's thesis was carried out in collaboration with the Zurich Institute of Forensic Medicine at the University of Zurich, Zurich, Switzerland.

Christina Horn: *Preparation and characterization of archaeosomes encapsulating cannabidiol and ferrous iron*

Liposomes represent an interesting and advantageous option for drug delivery in the human body due to their bipolar character. Not only a transport of encapsulated hydrophilic or lipophilic substances is possible, but also improvements with regard to the stability under storage conditions as well as the stability in the gastrointestinal tract can be significantly enhanced. Archaeosomes are liposomes that are partly composed of tetraether lipids (TEL) in addition to conventional lipids such as 1-palmitoyl-2-oleoylphosphatidylcholine (POPC). TELs, which are derived from archaea, are more stable against low pH, high temperatures and enzymatic degradation due to their ether linkage. Due to these properties, they can increase the bioavailability of orally administered substances.

In this work, the advantages of TEL in liposomal formulation in terms of encapsulation efficiency (EE) and stability were demonstrated. The formulations were performed with microfluidic mixing technique, which proved to be a fast and simple technique for preparing liposomes and archaeosomes. Cannabidiol (CBD) and ferrous iron were used as model substances for the encapsulation of a lipophilic and a hydrophilic cargo. Particle size, EE, cargo:lipid ratio and the stability in the gastrointestinal tract were selected as characterization parameters. The encapsulation of CBD in archaeosomes (100 mol-% TEL) achieved an EE of 94 ± 2% compared to liposomes (87 ± 11%). An *in vitro* digestion assay showed that 40% of encapsulated CBD could be recovered in the intestinal phase and could potentially be absorbed (liposomes: 20%). Concerning the encapsulation of ferrous iron, higher encapsulation efficiencies using tetraether lipids were obtained, though the encapsulation of hydrophilic substances turned out to be more challenging. Only a small amount of ferrous iron were encapsulated in archaeosomes (EE: 2.38 ± 0.21%) as well in liposomes (EE: 2.04 ± 0.02%).

The results of this thesis showed that the application of TEL in the formulation of liposomes lead to an increase in stability during storage and digestion in the gastrointestinal tract. Further, the microfluidic mixing technique provided reproducible results and enables a production on a large scale.

This master's thesis was carried out in collaboration with Assoc.-Prof. Dr. Oliver Spadiut at the Vienna University of Technology, Vienna, Austria.

International cooperations

Karine Bourgeois, 4Teen4 Pharmaceuticals GmbH, Hennigsdorf, Germany

Robert Speth, Nova Southeastern University, Florida, U.S.A.

Research projects

FWF-doc.funds "CATALOX"

Talk at national and international conferences/meetings

1. Eggers, R.: *Berberine bridge enzyme-like proteins in* Arabidopsis thaliana*: Fine-tuning of the extracellular phenolics pool?* 23rd meeting of the Austrian Society of Plant Biology, Seitenstetten, Austria, November 2021.

Publications

1. Matic, S., Kekez, I., Tomin, M., Bogar, F., Supljika, F., Kazazic, S., Hanic, M., Jha, S., Gruber, K., Brkic, H., Bourgeois, B., Madl, T., Macheroux, P., Matkovic-Calogovic, D., Matovina, M., Tomic, S.: *Binding of dipeptidyl peptidase III to the oxidative stress cell sensor Kelch-like ECH-associated protein 1 is a two-step process* (2021) ***J. Biomol. Struct. Dyn.*** 39, 6870-6881*,* DOI: 10.1080/07391102.2020.1804455

2. Messenlehner, J., Hetmann, M., Tripp, A., Wallner, S., Macheroux, P., Gruber, K., Daniel, B.: *Reaction mechanism of monolignol oxidoreductase* At*BBE-like protein 15 and related enzymes from the berberine bridge enzyme-like protein family* (2021) ***Arch. Biochem. Biophys.*** 700 108766. DOI:10.1016/j.abb.2021.108766

3. Lahham, M., Jha, S., Goj, D. Macheroux, P., Wallner, S.: *The family of sarcosine oxidases – same reactions, different products* (2021) ***Arch. Biochem. Biophys.*** 704 108868. DOI: 10.1016/j.abb.2021.108868

4. Macheroux, P.: *Current Topics in Flavins and Flavoproteins (Proceedings of the 20th International Symposium on Flavins and Flavoproteins)* (2021) ***Arch. Biochem. Biophys.*** 707 108908. DOI:10.1016/j.abb.2021.108908

5. Asanovic, I., Strandback, E., Kroupova, A., Pasajlic, D., Meinhart, A., Tsung-Pin, P., Djokovic, N., Anrather, D., Schuetz, T., Suskiewicz, M. J., Sillamaa, S., Köcher, T., Beveridge, R., Nikolic, K., Schleiffer, A., Jinek, M., Hartl, M., Clausen, T., Penninger, J., Macheroux, P., Weitzer, S., Javier Martinez, J.: *Oxidoreductase PYROXD1 uses NAD(P)$^+$ as an antioxidant to sustain tRNA ligase activity in pre-tRNA splicing and unfolded*

protein response (2021) ***Mol. Cell*** 81 (12) 2520-2532.e16, DOI:10.1016/j.molcel.2021.04.007

6. Eggers, R., Jammer, A., Jha, S., Kerschbaumer, B., Lahham, M., Strandback, E., Toplak, M., Wallner, S., Winkler, A., Macheroux, P.: *The scope of flavin-dependent reactions and processes in the model plant Arabidopsis thaliana* (2021) ***Phytochemistry*** 189 112822. DOI:10.1016/j.phytochem.2021.112822

7. Ivkovic, J., Jha, S., Lembacher-Fadum, C., Puschnig, P., Kumar, P., Gruber, K., Macheroux, P., Breinbauer, R.: *Efficient entropy-driven inhibition of dipeptidyl peptidase III by hydroxyethylene transition state peptidomimetics* (2021) ***Chem. Eur. J.*** 27 (56) 14108-14120, DOI:10.1002/chem.202102204

Awards

1. **Reinmar S. Eggers**: *Characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in* Arabidopsis thaliana*.* Best Flash Presentation, 24th DocDay (NAWI Graz), 04.-05.02.2021.

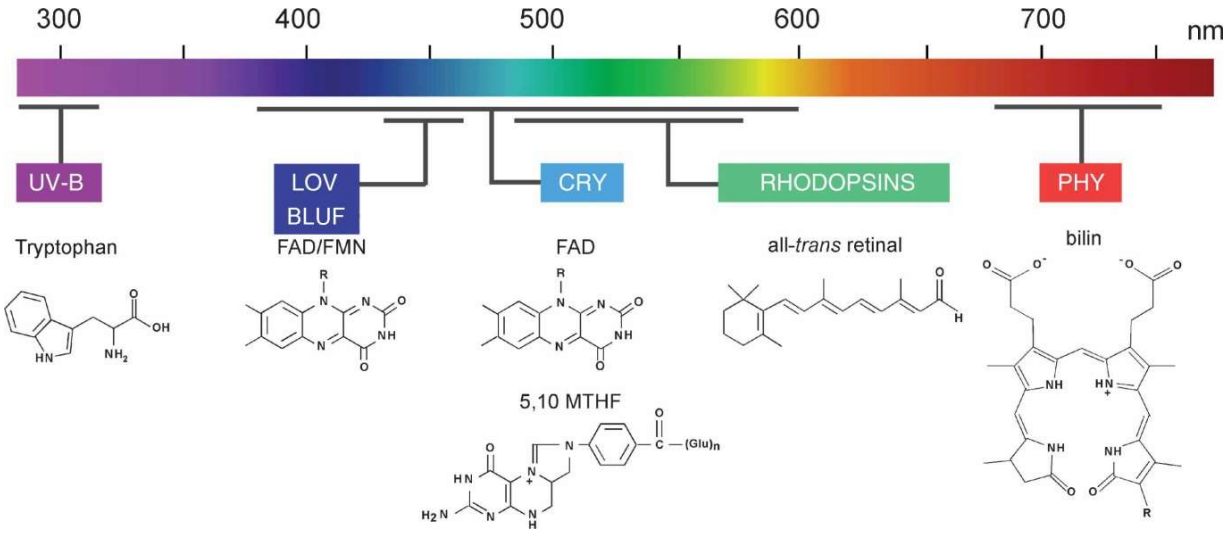
2. **Katharina Fuchs**: *Investigating the inhibitory effect of azo-dyes on the activity of chorismate synthases from various organisms.* Best poster award*,* 20th International Symposium on Flavins and Flavoproteins, Graz, Austria, 05.- 09.09.2021.

Photoreceptor Group

Group leader: Andreas Winkler
PhD students: Cornelia Böhm, Massimo Totaro (joint with AGO), Hieu Tran, Ursula Vide, Oliver Eder, Maximilian Fuchs
Master students: Martina Reiter (external student at Qualizyme Diagnostics)
Bachelor students: Martin Heimböck, Dzenita Kasapovic, Sophie Zweytick
Technical staff: Nadine Galler, Philipp Pelzmann, Elfriede Zenzmaier

General description

The ability to perceive and integrate environmental stimuli is essential for all living organisms. One important ambient factor is light that is sensed by a variety of photoreceptors (Figure below). Many light-responsive proteins are directly connected to enzymatic functionalities and enable photo-activation or -inhibition of various cellular processes. Recently, the interest in light-triggered systems has increased significantly due to the establishment of 'optogenetics', which refers to the concept of genetically targeting biological systems to enable optical control of diverse processes. However, the growing demand for light controlled systems goes beyond the possibilities of naturally occurring photoreceptors. Even though substantial progress has been made in understanding the concepts of light activation in several photoreceptor families, the rational design of synthetic tools is not straight forward. Since mechanistic descriptions of signal transduction to effector domains differ even within photoreceptor families, it is obvious that a more detailed understanding of the underlying principles of sensor-effector coupling is required.

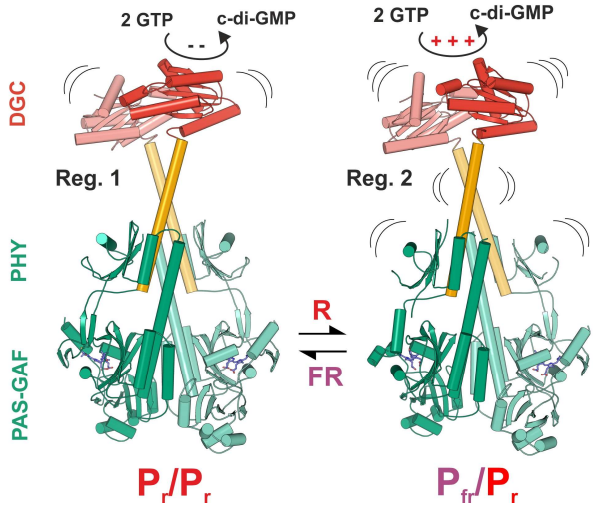


Overview of photoreceptor families and their corresponding cofactors covering the UV/Vis range of the electromagnetic spectrum (adapted from Heintzen WIREs Membrane Transport and Signaling 2012, 1:411–432).

Red-light-regulated diguanylate cyclases

One key approach towards a better understanding of sensor-effector coupling is to appreciate how nature has accomplished its remarkable modularity of sensor-effector combinations. To this end, we focus on the light-regulation of cyclic-dimeric-GMP formation by GGDEF diguanylate cyclases that are ubiquitous in bacteria and that naturally occur covalently linked to various photoreceptors. The understanding of how evolution has accomplished this astonishing modularity of sensor-effector couples is important for the identification of functionally relevant structural elements with the goal of guiding the efficient design of rationally engineered light-regulatable systems. Since diguanylate cyclases are distantly related to adenylate/guanylate cyclases, the comparison of signal transduction mechanisms between the two protein families is one central aspect of our research interests (thesis projects of Cornelia Böhm and Hieu Tran).

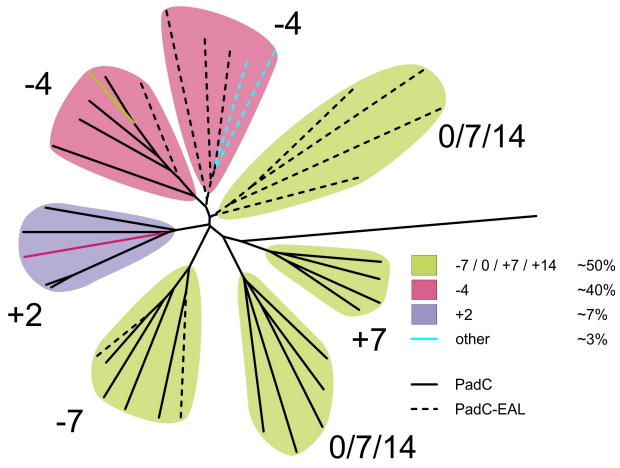
With the elucidation of the first full-length crystal structure of a naturally occurring phytochrome linked to its functional effector domain, new insights into signal transduction in phytochromes were obtained. The structure revealed a parallel dimeric arrangement of the sensor and effector domains. Based on an in-depth characterization of the involvement of functionally important elements, our studies provide a foundation for increasing our understanding of the fine-tuned coupling mechanism of phytochrome sensors with various effector domains. The characterization of in-solution conformational dynamics substantiates the involvement of several structural elements of phytochromes and highlights their dynamic interplay with the coiled-coil sensor-effector linker region. Our results demonstrate how allosteric light regulation of enzymatic effectors is fine-tuned by the architecture and composition of the coiled-coil linker and by the central helical spine of phytochromes without direct interaction of the sensory module (Gourinchas *et al.*, Sci Adv, 2017).



Additional insights into the mode of phytochrome activation have been obtained by the elucidation of the crystal structure of a constitutively active variant. In particular, the observation of an asymmetric dimer that induces conformational changes in the central sensor-effector linker region allowed us to postulate mechanistic concepts of phytochrome activation that were previously not considered (Gourinchas *et al.*, eLife, 2018). Understanding the role of asymmetry in these systems is the major focus of the PhD theses of Cornelia Böhm and Hieu Tran.

Understanding molecular details of how structural asymmetry is realized was the focus of a collaboration with the group of Peter Hildebrandt. By combining crystallography, HDX-MS and vibrational spectroscopy, we could show that the photoactivated state is an equilibrium of a classical Pfr protomer with an intermediate "Pfr-like" state that combines features of Pfr and Meta-R states. We identified the N-terminal segment (NTS) of bacteriophytochromes to be a key determinant for the structural rearrangements and we showed that the PHY-tongue conformation is partially uncoupled from the initial changes in the NTS in the group of asymmetrically activated phytochromes (Buhrke, Gourinchas *et al.*, J Biol Chem, 2020).

In an attempt to further characterize molecular determinants of asymmetry in *Is*PadC, Hieu Tran is currently trying to employ NMR for looking into the conformational dynamics of these complex dimeric systems. Identifying appropriate constructs of *Is*PadC that express well enough for isolating the amounts required for NMR, together with addressing the role of specific amino acids in controlling asymmetric properties as well as Pr/Pfr stabilities are the major efforts in his thesis at the moment. Based on complementary experiments performed on chimeric proteins of *Is*PadC with a related PadC homolog that features a symmetric activation by red light (Böhm *et al*. J Mol Biol, 2021), it turns out that the symmetry/asymmetry determinants are not located on a specific structural element, but are rather influenced by the interplay of conformational dynamics of multiple functionally important regions.



Linker-length clustering in the evolutionary relationship of PadCs (left figure). The phylogenetic characterization carried out by Cornelia Böhm (PhD student) together with Christoph Sensen, revealed a clustering of linker lengths and a strong conservation of multiples of 7 residues – in line with the coiled-coil character of the sensor-effector linker. The similarly abundant -4 linkers also function as light activated cyclases, albeit with lower dynamic ranges of light activation due to the less optimal coiled-coil architecture. The +2 family features pronouncedly different properties in many aspects, like symmetric Pfr state formation, high light state stabilities and light-inhibition upon illumination.

The central role of asymmetry on PadC function was observed in the biochemical characterization of several homologs from the phylogenetic tree shown in the figure above. While not a strict requirement for PadC functionality, as recently observed in synthetic chimeras (Böhm *et al*. J Mol Biol, 2021), the naturally occurring PadCs with light activation all feature asymmetric red light illuminated spectra. A full characterization of interesting representatives from all major branches of PadCs is currently in progress. While the biochemical and phylogenetic aspects have been submitted recently (Böhm *et al*. under revision, 2022) we are also following up on the structural diversity of these homologs with an emphasis on the conformational dynamics that appear to influence the spectroscopic signatures of the sensory modules and eventually also the regulatory properties of the bacteriophytochrome-linked effectors.

Optogenetic tools based on red-light sensing phytochromes

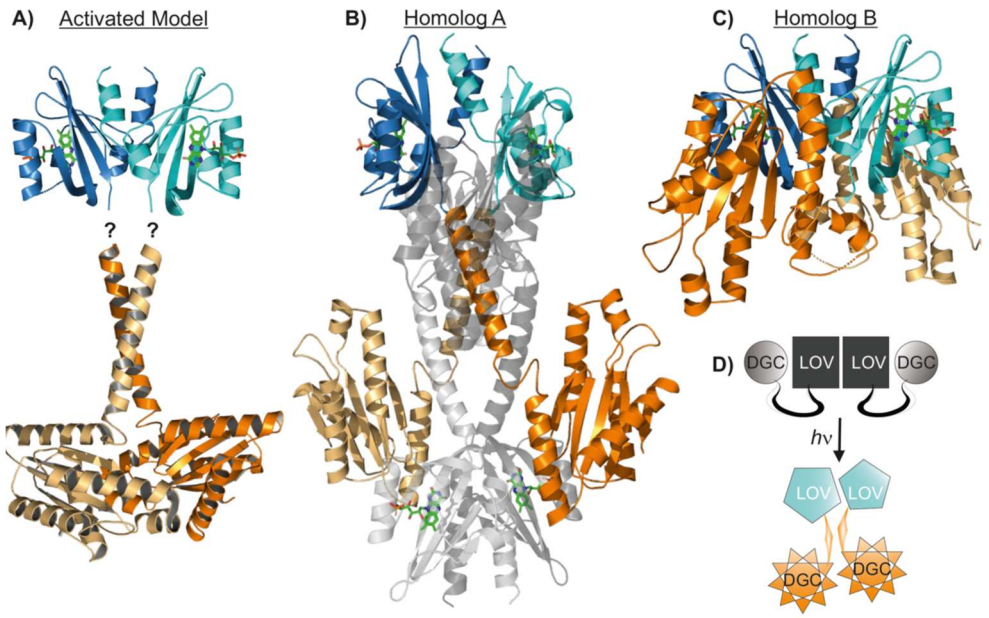
Due to deep tissue penetration of red light and the availability of the biliverdin cofactor as heme-degradation product in mammalian cells, bacteriophytochromes are interesting building blocks for optogenetic tools. To assess the suitability of PadC derived systems, we are testing different constructs for their oligomerization characteristics to obtain useful red light regulatable dimerization tools. In addition to the native dimer interface of bacteriophytochromes, artificial interfaces can be employed for improving specificity and bringing together different target functionalities.

In this respect, Massimo Totaro (joint PhD student with AGO) is using Rosetta-based tools to optimize such artificial interfaces and to destabilize the native dimer interface. Thereby, the complexity of bacteriophytochromes can be reduced and the specificity of the interactions can be increased. Hence the goal of the project is to shift the equilibrium towards a monomeric form in dark conditions and towards an exclusive dimer upon light activation. Additional efforts in this direction also address the possibility to render artificial interfaces specific for heterodimeric interactions. Thereby, one could think of additional applications that are based on the recruitment of different functionalities to induce biological responses. In order to move from the in silico-based approach of computational protein design to *in vitro* testing and ultimately *in vivo* applications, Oliver Eder (PhD student), is establishing a screening system in *E. coli* that is based on a bacterial two hybrid system. In parallel, he is testing promising constructs from the calculations that will ultimately serve as controls for the larger scale screening efforts.

Blue-light-regulated diguanylate cyclases

Photoreceptors sensing blue light were early on identified as key players of plant phototropism and in recent years progress was made in understanding molecular mechanisms of light regulation in this family of flavin-dependent light-oxygen-voltage (LOV) domains (Losi & Gärtner, Photochem Photobiol, 2017). Members of this family also contributed significantly to the field of optogenetics using natural and artificial LOV-coupled effector domains (Pudasaini *et al*., Front Mol Biosci 2015).

In order to complement our efforts in understanding the modularity of sensors regulating diguanylate cyclases described above, we also work on LOV-regulated GGDEF domains. To elucidate molecular mechanisms of light regulation we are studying naturally occurring LOV-GGDEF systems and integrate our results with data obtained for the phytochrome-GGDEF systems. Since c-di-GMP formation requires appropriate assembly of the active site at the dimer interface of two GGDEF protomers, our focus lies on how such catalytically active dimers are modulated by the sensory domain and the linker region between the two domains. Notably, the linker between LOV and GGDEF domains shows a high conservation of heptad repeats involved in coiled-coil formation. Taken together with the observations for related phytochrome-linked GGDEF systems, the coiled-coil linker is proposed to play an important role in integrating the light signal and activating the GGDEF domains. So far, we successfully solved the full-length crystal structures of two homologous LOV-GGDEF systems, featuring identical linker lengths yet distinct oligomeric arrangements of the individual protomers and distinct spectral and biochemical properties. We are currently in the process of complementing the structural data with in solution structural analyses and site-directed mutagenesis approaches to address functional details of how the sensor-effector linker integrates the incoming light signal and enables the modulation of enzymatic activity with dynamic ranges >10,000-fold.

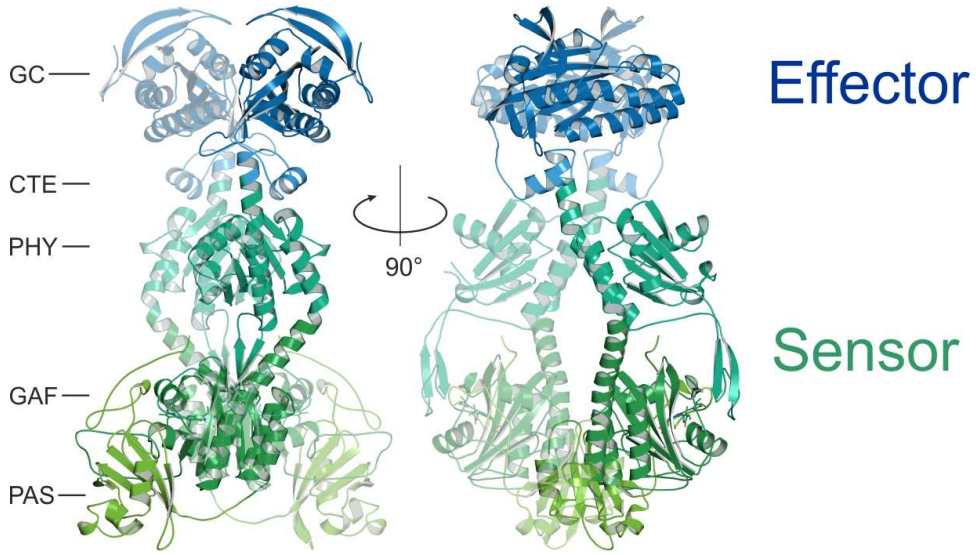


Crystal structures of LOV-regulated diguanylate cyclases. (**A**) Model of an activated conformation based on the observations in the phytochrome regulated GGDEF systems. (**B** and **C**). Crystal structures of two homologs with identical linker lengths, but different quaternary assemblies. (**D**) Working hypothesis for mechanistic aspects of DGC regulation by LOV domains.

The functional implications of the structures (shown above) combined with their biochemical and spectral characterization are the focus of the PhD thesis of Ursula Vide. Focusing on the role of specific residues at the sensor-effector linker and the functionally important A'-alpha helix of the LOV domain, she is in the finalizing a manuscript with the detailed functional characterization of LOV-GGDEF systems. In the long run, also more complex LOV-GGDEF-EAL or REC-LOV-GGDEF system will be interesting to address molecular logics of sensor-effector systems with multiple inputs or output functionalities (PhD project of Maximilian Fuchs).

Light-activated adenylate/guanylate cyclases

Another area of research are red-light activatable guanylate and adenylate cyclases, respectively. These represent artificial light-regulatable tools that are designed based on functional data of a blue-light regulatable adenylate cyclase (Lindner *et al*., J Mol Biol, 2017) and the evolutionary conservation of elements required for signal transduction. The identification of specific signaling elements for the closely related effector systems and the comparison of functionalities of different artificial chimeras provided new insight into the coupling mechanism of sensor-effector modules. The successful characterization of these systems required an interdisciplinary approach combining biochemistry with tools of structural biology. Atomic models obtained from x-ray crystallography were functionally extended by the in-solution method hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) to obtain structural information of elements that are involved in photo-activation and signal transmission. Highlights in this project were the elucidation of a full-length structure of a functional red-light activated adenylate/guanylate cyclase (see figure below) and the demonstration that it can be used as an effective optogenetic tool in the animal model system *Caenorhabditis elegans* (Etzl *et al*., J Biol Chem, 2018).



Crystal structure of the bacteriophytochrome-linked adenylate/guanylate cyclase. PAS, GAF and PHY domains are part of the red light regulated phytochrome sensor with its biliverdin chromophore shown as green stick model. The CTE corresponds to the Cyclase Transducer Element, required for efficient signal integration by the adenylate/guanylate cyclase (GC) effector.

In addition to these efforts, specific sensor variants were created that are supposed to result in functionally uncoupled enzymatic activities (Takala *et al*., J Biol Chem 2018). In order to further address the role of the sensor-effector linker, different linker length constructs were analyzed in detail. While showing a high dynamic range of activation in the wild-type form, the uncoupled variants displayed either complete loss of enzymatic activity or a relatively high basal activity. While this further supports the functional importance of the linker element in regulation of enzymatic activity, further experiments are needed to fully understand the molecular basis for these observations. To this end, infrared spectroscopy experiments are currently performed in collaboration with Janne Ihalainen at the University of Jyväskylä (Finland) and will eventually be integrated with a more detailed biochemical characterization.

Master thesis completed

Maximilian Fuchs: *Expression and characterization of dual sensor diguanylate cyclases*

Using light as a signal to adapt to environmental changes is widespread across all kingdoms of life. Through evolution a multitude of light sensitive protein domains developed, which react to different parts of the visible spectrum. The systems used to perceive light are oftentimes modular and signal transduction can be achieved by non-covalent interactions leading to conformational or structural changes. Investigating these systems, modifying them and finding new ways to integrate them into organisms led to the rise of the field of optogenetics. One way to stock up the tools that are available in this field is to characterize native systems and try to understand the processes involved in signal transduction and how to couple domains from different organisms producing chimeras.

This study focuses on two uncharacterized homologs of photoreceptors, which can be classified as dual sensor diguanylate cyclases (DGCs) and will be referred to as RecLadC. The enzymes feature two input domains combined with one output domain. Both input domains react to independent signals. The N-terminal receiver domain features a conserved aspartate residue, which can be phosphorylated. *In vivo* receiver domains are part of two component

signaling systems, these domains rely on a signal transducer/receptor, which activates the protein via phosphorylation. The second input domain is a light-oxygen-voltage (LOV) domain, which contains a non-covalently bound blue light sensitive flavin mononucleotide (FMN) cofactor. Upon light exposure a covalent bond between a conserved cysteine residue and the cofactor is formed, this leads to a change in the absorption spectrum and initiates signal transduction, through a coiled-coil linker connecting it to the output domain. The output domain, a so called GGDEF domain, catalyzes the formation of cyclic dimeric guanosine monophosphate (c-di-GMP) an important bacterial second messenger controlling bacterial lifestyle decisions. The aim of this thesis was to elucidate how the input domains sense two different signals and integrate this information to affect the output domain.

Seeing the two input domains as binary logic elements, which can either be switched "on" or "off", the way these two signals are combined and affect the output domain remains elusive. Three homologs of these photoreceptors were chosen initially, but two were characterized in more detail. We found that the two homologs show different characteristics regarding their LOV domain. *La*RecLadC does not seem to be able to form the photoadduct characteristic for these domains, although the conserved cysteine residue is present. *Ap*RecLadC on the other hand shows the characteristic change from dark- to light-adapted spectrum. What both homologs have in common is a high basal activity and low dynamic range of activation. The phosphomimic $BeF3^-$ was used to mimic the phosphorylation of the N-terminal receiver domain. However, only a weak influence on catalytic activity was observed. Additional experiments are needed to confirm, if an *in vitro* phosphorylation of the receiver domain is possible without the presence of the cognate kinase. We were nonetheless able to gain insight into fundamental biochemical characteristics of both homologs. This knowledge will accelerate future projects studying similar proteins.

International cooperations

Janne Ihalainen, University of Jyväskylä, Jyväskylä, Finland

Jochen Reinstein, Max Planck Institute for Medical Research, Germany

Vladislav Verkhusha, Albert Einstein College of Medicine, New York, USA

Research projects

FWF P32022: "Signal Integration in Phytochrome-linked Diguanylate Cyclases"

FWF P34387: "Molecular Mechanisms of LOV-regulated Diguanylate Cyclases"

Zukunftsfonds Styria PN 1105: "Structural analyses by HDX-MS"

FWF DOC130: "BioMolStruct – Biomolecular Structures and Interactions; doc.funds"

Talks at national and international conferences/meetings

1. Böhm, C.: *Phytochrome-Activated Diguanylyl Cyclases: The Functional Relevance of Asymmetry*. 24th Doc-Day of the Doctoral School of Molecular Biomedical Sciences and Biotechnology, Graz, Austria, February 2021.

2. Vide, U.: *Activation mechanism in blue light-sensing LOV-diguanylate cyclases*. 2nd Frontiers in Integrative Structural Biology and Biophysics Symposium, Graz, Austria, April 2021.
3. Böhm, C.: *Characterising Phytochrome-Activated Diguanylyl Cyclases*. 19th Congress of the European Society for Photobiology, Salzburg, Austria, September 2021.
4. Winkler, A.: *Bridging the language barrier between sensor and effector domains in bacteriophytochromes*. 19th Congress of the European Society for Photobiology, Salzburg, Austria, September 2021.

Publications

1. Böhm, C., Todorovic, N., Balasso, M., Gourinchas, G., Winkler, A..: *The PHY domain dimer interface of bacteriophytochromes mediates cross-talk between photosensory modules and output domains* (2021) ***J. Mol. Biol.*** 433 (15) 167092 DOI: 10.101/j.jmb.2021.167092.
2. Eggers, R., Jammer, A., Jha, S., Kerschbaumer, B., Lahham, M., Strandback, E., Toplak, M., Wallner, S., Winkler, A., Macheroux, P.: *The scope of flavin-dependent reactions and processes in the model plant Arabidopsis thaliana* (2021) ***Phytochemistry*** 189 112822. DOI: 10.1016/j.phytochem.2021.112822
3. Christiansen, A., Weiel, M., Winkler, A., Schug, A., Reinstein, J.: *The trimeric major capsid protein of Mavirus is stabilized by its interlocked N-termini enabling core flexibility for capsid assembly* (2021) ***J. Mol. Biol.*** 433 (7) 166859 DOI: 10.1016/j.jmb.2021.166859.

Protein Design Group

Group leader: Gustav Oberdorfer
Postdocs: Horst Lechner
PhD students: Veronica Delsoglio, Wael Elaily, Julia Messenlehner, Florian Wieser, Massimo Gregorio Totaro, Adrian Tripp, Markus Braun
Rotation students: Tobias Fleiß
Master students: Markus Braun (finished November 2021), Nina Grujicic, Lena Parigger (finished September 2021), Henrik Seyfried
Bachelor students: Sigrid Kaltenbrunner, Katharina Leitner
Technical Staff: Alma Makic, Alexandra Grebe, Birgit Grill

General description

De novo protein design is the attempt to use our best understanding of protein biochemistry and biophysics – how proteins fold into their shapes by burial of hydrophobic amino acid residues, or what the typical inter- and intramolecular interactions of amino acids are and how they interact with their environment or targets/substrates – to identify a minimum energy amino acid sequence composition that allows the protein to fold exactly into a desired shape. This is essentially the protein structure prediction problem turned upside down, where a minimum energy structure for a given amino acid sequence is computed. Computationally, protein design represents two interconnected problems: a) How do we score conformations of an amino acid chain and b) How can we sample all its degrees of freedom efficiently? These problems are difficult to solve, because sequence space for a typically sized protein (~200 amino acids) is vast (20^{200}) and comprehensive sampling of it remains a challenge even with current computational power. Besides that, the free energy of such a large system is very difficult to compute with absolute accuracy.

So why try to design a protein, if the odds of success are against the experimenter? With the advent of protein sequence databases and their ever-increasing growth, it has become evident that nature only sampled an infinitesimal small subset of all possible sequences available. Protein design on the contrary allows for the exploration of this 'dark matter' of amino acid sequence space (Figure below). However, the question remaining is: Is it possible to find something new in this pool of unexplored sequences? Given the sheer number of available and yet unexplored sequences, it is reasonable to argue that there are thousands of possibilities for designing novel proteins of high stability and arbitrary shape. All of these bare the potential to go beyond classical biochemical approaches and could ultimately provide solutions to biomedical and biotechnological challenges much faster than nature can. Over the last couple of years, tremendous progress has been made in this direction with many novel protein structures designed from scratch. This can be attributed to advances in understanding the fundamental processes underlying protein folding and concomitant improvements in computational methods. In addition, breakthroughs in the field of synthetic DNA manufacturing and the increase in computational power were key aspects for these successes.

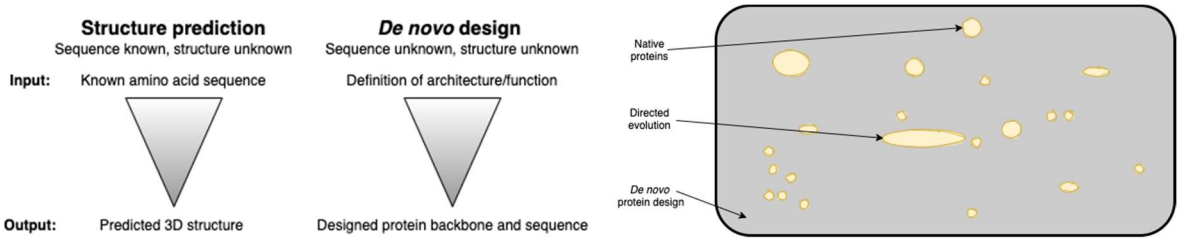


Figure (left) *Differences in structure prediction and de novo protein design* ***(right)*** *Illustrative representation of protein sequence space (grey). Sequence analysis of native proteins shows that tight clusters of protein families can be identified (beige). (c) Gustav Oberdorfer*

Highlight: Artificial fluorescent proteins for the next generation of sustainable and safer LEDs (ARTIBLED)

The European Union (EU) priority action lines state the need of efficient electricity consumption, expecting >20% reduced consumption using inorganic white light-emitting diodes (WLEDs) present in the LED lamps of our homes [1]. They consist of a blue-chip covered by color down-converting filters based on toxic and/or rare-earth elements (inorganic phosphors or IPs) that are, indeed, listed as one of the 27 critical raw materials by the EU. It is expected that reserves of IPs will run out in 10-15 years if global demand for LEDs rises as expected, while the recycling process is still quite inefficient. The rising costs and ecological impact (mining/refining/toxicity) along with the control of the limited rare-earth resources put a high burden on the long-term sustainability of WLEDs [2]. Finally, the IP filters do not efficiently convert blue light, causing serious implications on visual acuity in children and elderly people and non-visual effects, such as sleep disorder in adults. We must be aware that we are using artificial illumination for long periods of time throughout the day (8-14 h per day).

In this context, EU/US Governments command to replace IPs by non-toxic and sustainable organic phosphors (OPs) towards a new generation of hybrid WLEDs. Since their first introduction in 1995, polymers, carbon nanodots, and fluorescent dyes embedded into polymer matrices have been applied as OPs without meeting customer requirements, showing, in general, low efficiencies and stabilities. This is related to i) the reduction of the photoluminescence quantum yield (PLQY) due to aggregation-assisted quenching and ii) the degradation upon both UV/thermal curing of the matrix and photo-induced oxidation under ambient operation conditions.

A joint research FET-OPEN initiative (ARTIBLED) between Spain (CIC-biomaGUNE-Dr. Cortajarena; CFM-CSIC-Dr. Coto), Italy (UNITO-Dr. Barolo; ABIEL-Dr. Ghersi), Austria (TU Graz-Dr. Oberdorfer), and Germany (TUM-Dr. Costa) aims at replacing IPs by protein filters based on engineered fluorescent proteins (FPs) for lighting applications. The first milestone was realized by the research team of Dr. Costa (TUM) that introduced a new concept to stabilize FPs into different polymer matrices for long periods of time under ambient storage and operating conditions in WLEDs [3]. This new technology is called Bio-LED and has already achieved up to 6 months of stability with a high efficiency [4]. In order to further improve this technology, ARTIBLED promises to achieve a second milestone focused on developing a new family of artificial fluorescent proteins customized for our lighting needs.

The key element for this vision is a true cross-disciplinary work. Within ARTIBLED we are combining expertise in organic synthesis of fluorescent dyes, quantum chemistry calculations, protein design and synthetic biology, material sciences as well as process

development. To achieve our goal, we first simulate thousands of different hypothetical protein scaffolds that are designed to bind specifically to new synthetic dyes without affecting their photophysical properties. A subset of all designed and *in silico* evaluated structures are then ordered as synthetic DNA constructs and experimentally tested for dye binding and photophysical properties. As soon as lead candidates are identified, these new, artificial fluorescent proteins are integrated into a polymer matrix and tested for their suitability as color down-converting filters with regards to Bio-LEDs (Figure below) This all is made possible through recent research and technology advances in quantum chemistry calculations, protein design and synthetic biology, the synthesis of fluorescent dyes, and the invention of novel polymer coating for Bio-LEDs.

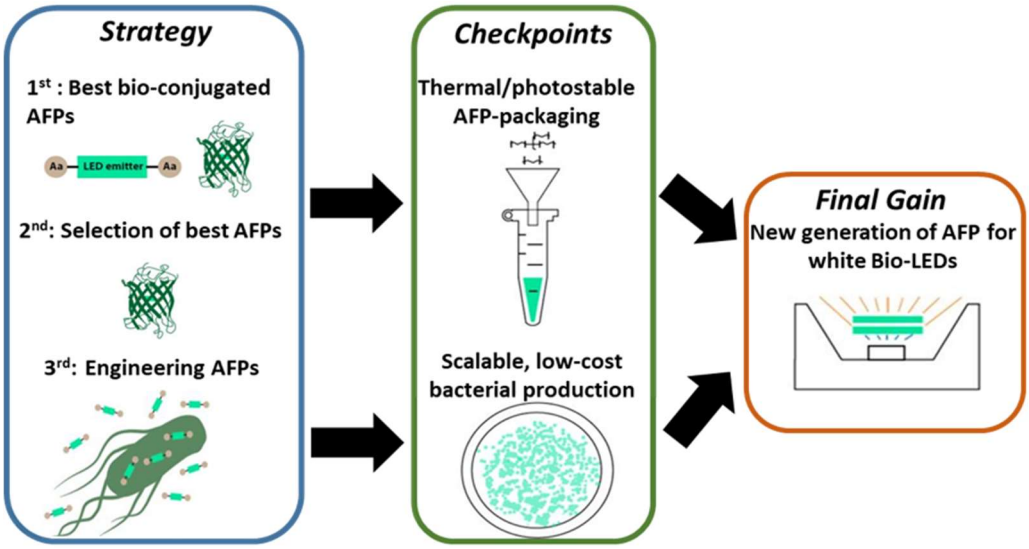


Figure. *The ARTIBLED concept – We start by engineered artificial amino acids into selected protein scaffolds for covalent labelling with the fluorescent dyes. This is followed by experimental testing of this host-guest system for its fluorescent properties. Next, the labelled protein is packaged into a polymer matrix, which is subsequently put in front of the LED chip as down-converting filter.*

Fluorescent dyes and Quantum Chemistry

A key aspect in the development of new biomaterials for lighting applications is the design and synthesis of optimized dyes featuring excellent photoluminescence and photostability. Well-designed emitters, when incorporated in the protein environment, should keep or even enhance their optical features, attaining higher stability. Therefore, the synthetic chemist group (UNITO-Dr. Barolo) plays a key role in the development of compact biocompatible emitters of proven photostability. The second important step is to develop an easy scale-up and low cost synthetic pathway within a greener process. In this respect, theory and simulation (CFM-CSIC-Dr. Coto; TU Graz-Dr. Oberdorfer) play an important role in the development of new dyes for their use in protein-based lighting systems. On the one hand, they can provide a link between molecular and electronic structure of the dye, its stability and optical properties, and how these are modified by the surrounding environment, thus facilitating the design and synthesis of dyes exhibiting specific characteristics. On the other hand, they can provide detailed information on the processes that occur during device operation, assisting the interpretation of experimental results and providing the underlying mechanistic information that cannot be straightforwardly obtained from experiments. (Figure below).

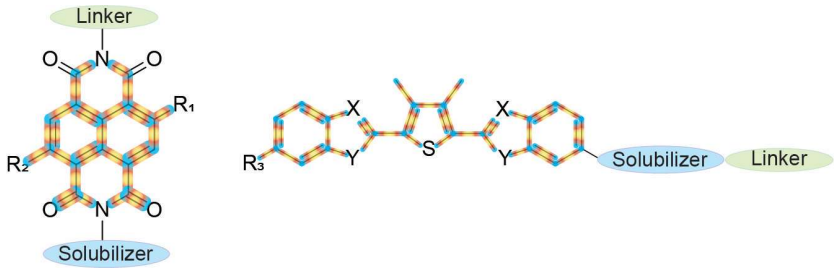


Figure. *Backbones of the synthesized dyes with fine-tuned photophysical features, able to specifically tag protein scaffolds*

Protein Design and Synthetic Biology

In the last decade, the fields of protein design and synthetic biology are driving significant advances towards robust engineering of novel synthetic biomolecular systems [5]. These approaches have been applied mostly to the fabrication of synthetic systems for biomedical applications and less effort has been put on using this technology for the biotech sector. However, the state-of-the-art in those fields suggests their readiness to face relevant technological challenges. In this sense, computational protein design presently enables the generation of stable *ad hoc* protein scaffolds both *de novo* as well as based on existing protein templates. Especially *de novo* proteins have proven to be of very high thermal stability (melting temperatures above 100°C) [6]; a property often highly desired for biotechnological applications. Similarly, synthetic biology tools, including the unnatural amino acids (UAA) technology, in which an orthogonal tRNA-Synthetase system is used to introduce a novel/unnatural amino acid into a protein synthesized by the ribosome, permit the incorporation of novel chemical functionalities into proteins, thereby expanding the molecular toolbox for potential synthetic systems enormously. Altogether, the biomolecular engineering groups (CIC-biomaGUNE-Dr. Cortajarena; TU Graz-Dr. Oberdorfer) are well equipped to tackle the current challenges faced in the technological application of protein-based components for lighting applications (Figure directly below).

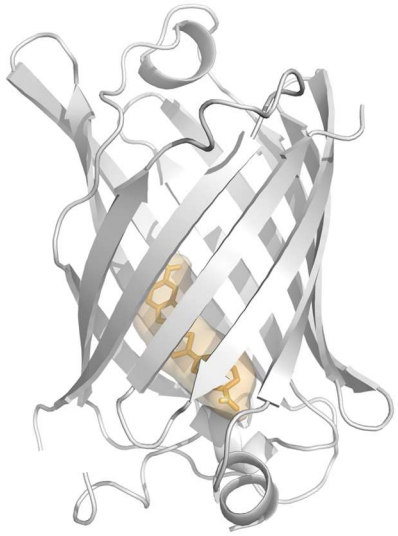


Figure. *Rendering of a fluorescent dye bound to a designed beta-barrel protein. The fluorescent dyes are covalently attached to the protein via the introduction of an artificial amino acid residue. During the design process all surrounding residues will be optimized computationally to generate an ideal environment for the dye.*

Environmentally friendly polymer coatings for lighting purposes

The interest in using protein scaffolds stems from i) their ability to provide an excellent shield of the chromophore to the ambient surrounding, and ii) an essentially limitless bacterial production in-place and highly optimized. However, the main bottleneck is the low stability of protein scaffolds out of the cellular environment and/or physiological conditions in aqueous solutions. This has been solved by a material scientist group (TUM; Dr. Costa) using an innovative stabilization method combining branched/linear polyethylene derivatives to replace the water molecules surrounding the protein skeleton using physical cross-linking upon drying. In this way, the protein scaffold holds its bio-functionality in an easy-to-manipulate polymer material to further fabricate filters for lighting applications. First Bio-WLEDs consisted of a blue-LED chip covered by several natural FPs like enhanced green fluorescent protein and mCherry, which partially converts blue into green and red, achieving efficiencies >50 lm/W, a color rendering index (CRI) >80, and stabilities >100 h (Figure below) [3]. Further optimization (architecture/polymers/proteins) led to >3000 h at >130 lm/W [4]. Thus, solutions to pH, temperature, and oxidative driven degradations are at hand. However, the chromophores present in natural FPs show a prominent photodeactivation (H-transfer/*cis-trans* isomerization) in the polymer matrix. Thus, the redesign of the protein with respect to its chromophore could be key towards highly stable and efficient bio-based lighting systems.

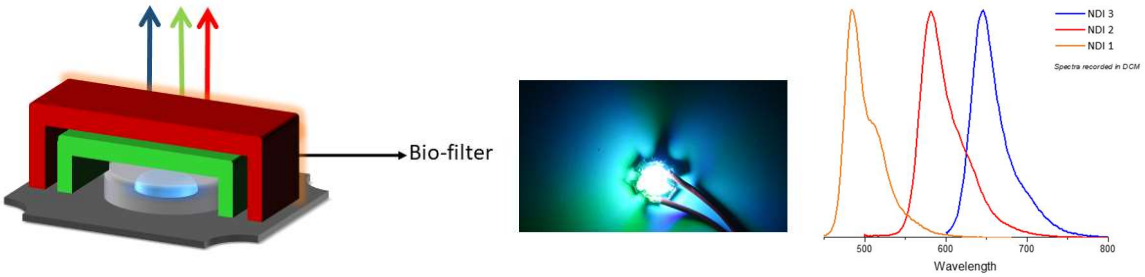


Figure. (left) *Schematic drawing of Bio-WLED and picture of a running prototype.* ***(right)*** *Emission spectra of the dyes currently used.*

Large scale production

To test our artificial fluorescent proteins for larger scale production, the industrial partner (ABIEL-Dr. Ghersi) will test the productive transfer of the new fluorescent proteins from lab scale batch production in shaker flasks to a fermenter based scale-up in order to determine their pre-industrial production in terms of quantity and quality. It is the goal of the project to come up with a production and purification processes with highly competitive costs compared to current standards. ABIEL has acquired experience in the field of macromolecule synthesis that has led to the patenting of this procedure for some enzymes of which it is a producer [7], representing a high yield production and purification procedure with low costs.

Conclusion

The invention of WLEDs is considered the future of artificial lighting, as we are now replacing all the old-fashioned lamps/bulbs by the highly efficient and stable LED technology. However, this comes at high ecological cost by utilizing scarce materials that are not sustainable in the long-term. Within ARTIBLED we have compiled a cross-disciplinary consortium to

develop the next generation of color filters with the aim to boost the emerging Bio-LED technology. Based on our innovative approach, our goal is to keep the actual device performance combining artificial fluorescent proteins and polymers that are environmentally friendly, low cost, and sustainable. Thus, the ARTIBLED consortium expects to realize a significant thrust to all the above subfields, while achieving a well-oriented technological goal: cheap/in-place production of bio-materials for lighting. This is a unique approach that, if successful, will shape the future of our home illumination.

References

[1] a) Eurostat, June **2017**; b) EU Commission, Dec. **2017**.

[2] a) CADMUS, *http://ma-eeac.org/wordpress/wp-content/uploads/MA-Task-5b-LED-Incremental-Cost-Study_FINAL_01FEB2016.pdf*, Feb. **2016**; b) US Department of Energy https://energy.gov/sites/prod/files/2017/09/f37/ssl_suggested-research-topics_sep2017.pdf; Sep. **2017**.

[3] a) M. D. Weber, *et al. Adv. Mater.*, **2015**, *27*, 5493; b) L. Niklaus, *et al. Adv. Funct. Mater.* **2017**, *27*, 1601792; c) C. Fernández *et al. ACS Omega*, **2018**, *3*, 15829; d) V. Fernández-Luna, *et al. Angew. Chem., Int. Ed.* **2018**, 57, 8826.

[4] *A. Espasa et al. Nat. Commun.* **2020***, 11, 879*

[5] a) P. Huang, *et al. Nature* **2016**, 537, 320–327 b) Chin, J. *et al. Nature* **2017**, 550, 53–60

[6] P-S. Huang*, G. Oberdorfer*, *et al. Science* **2014***,* 346, 481-485. *equal contribution

[7] *Bertuzzi, F., Cuttitta, A., Ghersi, G., Mazzola S., Salamone M., Seidita G., PCT WO 2011/073925 USA, Europe patent no. EP10818077.9, Canada patent n. 2784050 and Israel patent no. 220359)*

Master thesis completed

Lena Parigger: *Modelling artificial 2A sequences for polycistronic expression in yeast*

Viral 2A sequences comprise a promising method for separate expression of several genes within one open reading frame, enabling condense expression constructs for facilitated gene transfer and stability. Their self-processing mechanism, which is often referred to as “ribosomal skipping” or “stop-carry on”, is not yet fully understood. It is, however, assumed that the structure of 2A sequences in the ribosomal exit tunnel influences translation so that a certain peptide bond formation is skipped. This leads to a stop codon independent release of the peptide chain, while the ribosome continues with translation. As 2A sequences originate from viruses, their application in pharmaceutical and food industry might cause criticism and longer discussions before approval. To facilitate their use and thus new applications in these industries, an approach to create artificial 2A sequences via peptide structure-based design using the Rosetta software is presented in this work. Self-processing activities of selected designs were evaluated upon implementation in specific reporter systems expressed by the methylotrophic yeast *Pichia pastoris*. Remarkably, functional artificial sequences were generated, with and without the presence of natively conserved residues, suggesting that peptide structure is the key for skipping the formation of a peptide bond. (Finished: October 2021)

Markus Braun: *Design of a Novel PylRS Mutant to Genetically Encode Methylated Arginine*

In recent years, engineering of the archaeal pyrrolysyl-tRNA synthetase (PylRS) – $tRNACUAPyl$ orthogonal pair using randomization techniques enabled the genetic encoding of structurally diverse non-canonical amino acids (ncAAs). This expansion of the genetic code to novel ncAAs allowed to add unique functionalities to proteins, such as selectively reactive groups, spectroscopic probes, or post-translational modifications. However, engineering of PylRS by randomization often produced enzymes with weak activities towards their targeted ncAAs. In this thesis we applied computational protein design using the Rosetta macromolecular software suite to shift the substrate scope of PylRS towards the ncAA asymmetric dimethylarginine (ADMA). Guided by established enzyme-design practices, we compiled a RosettaScripts design protocol that allowed us to probe multiple design parameters for the remodeling of the PylRS amino acid binding pocket. We ranked the designed PylRS mutants based on Rosetta scoring parameters and screened binding of arginine in silico to retain orthogonality. Although eGFP incorporation assays showed that our mutants were catalytically inactive, our work represents the first steps to develop a computer-based method to alter the substrate scope of the PylRS-$tRNACUAPyl$ orthogonal pair. (Finished: November 2021)

International cooperations

David Baker, Institute of Protein Design, University of Washington, Seattle, United States

Sinisa Bjelic, Department of Chemistry and Biomedical Sciences, Linnæus University, Småland, Sweden

Hendrik Dietz, Department of Physics, Technical University of Munich, Germany

Anna Peacock, School of Chemistry, University of Birmingham, United Kingdom

Research projects

FWF P30826: “Design of de novo protein pores with custom geometries”

ERC StG (GA: 802217): “HelixMold: Computational design of novel functions in helical proteins by deviating from ideal geometries”

ERC FET-OPEN (863170), Participant: “Artificial fluorescent proteins for the next generation of sustainable and safer LEDs”

FWF DOC130: “BioMolStruct – Biomolecular Structures and Interactions” doc.funds

Talk at national and international conferences/meetings

1. Oberdorfer, G.: *Proteins made to order: Computational design of de novo proteins with custom geometries.* NextGenBiocat – An International Young Investigator Symposium, March 2021

Publications

1. Wieser F, Stryeck S, Lang K, Hahn C, Thallinger GG, Feichtinger J, Hack P, Stepponat M, Merchant N, Lindstaedt S, Oberdorfer G.: *A local platform for user-friendly FAIR data management and reproducible analytics*. (2021) ***J Biotechnol*** 341 43-50. DOI: 10.1016/j.jbiotec.2021.08.004
2. Wagner, G.E., Totaro, M.G., Volland, A., Lipp, M., Saiger, S., Lichtenegger, S., Forstner, P., von Laer, D., Oberdorfer, G., Steinmetz, I.: *A novel high-throughput nanopore-sequencing-based strategy for rapid and automated s-protein typing of sars-cov-2 variants* (2021) ***Viruses***, 13 (12) 2548. DOI: 10.3390/v13122548
3. Lechner, H., Oberdorfer, G. *Derivatives of natural organocatalytic cofactors and artificial organocatalytic cofactors as catalysts in enzymes*. (2022) ***ChemBioChem***, *accepted*.

Chemistry of Functional Foods

Group leader: Michael Murkovic
PhD students: Monika Grasser
Master students: Iris Stalzer, Zehra Gromilic, Elke Aichhorn, Maria-Luise Deflorian, Marcel Wilhelm, Monika Kozlowska, Adnan Fojnica, Vildana Fazlic, Zerina Duhovic
Technical staff: Claudia Hrastnik

General description

Antioxidants have different functions depending on the location of action. Is it the protection of biological systems maintaining the integrity of the system or the protection of foods against oxidation leading to health threatening substances? The exposure to oxidation products is either described as oxidative stress or the oxidized substances have an acute or chronic toxicity or are carcinogenic. The production of healthier and safer foods is of primary interest of this research group.

The antioxidants of interest are polyphenols including anthocyanins and carotenoids. The evaluation of their occurrence in food and their behavior during processing and cooking is important especially when these substances are used as food additives. The safety evaluation of these compounds includes the evaluation of possible degradation products.

Heating of food is a process that is normally done to improve the safety and digestibility and improve the sensory attributes like texture, color, and aroma. During the heating reactions occur that lead to the degradation of nutritive constituents like carbohydrates, proteins, amino acids and lipids. Some of the reaction products are contributing to the nice aroma, color, and texture of the prepared food and many of them are highly toxic and/or carcinogenic. However, these hazardous compounds occur in rather low concentrations being normally not acute toxic. The substances have a very diverse chemical background like heterocyclic amines, polycondensated aromatic compounds, acrylamide, or furan derivatives. The aim of the research is to investigate the reaction mechanisms that lead to the formation of these hazardous compounds and establish strategies to mitigate the formation and thereby reducing the alimentary exposure. Recent results show that oxidized lipids are related to either colon cancer or hepatic inflammation. With this background a project was started to investigate the formation of non-volatile aldehydes during lipid oxidation in edible oils and during roasting of coffee.

A new research area was started in which the presence of cyclic lipophilic alimentary peptides is investigated. These cyclic peptides can have specific physiological properties with a positive effect on e.g. malaria.

Master Theses completed

Elke Aichhorn: *Thermal and oxidative stability of edible oils*

Aldehydes and ketones are volatile compounds which can be formed in the oxidation process of edible oils. In food chemistry these compounds play a big role in flavoring. Oxidation processes attack unsaturated fatty acids and change their taste and odor in a negative way considering optical and unhealthy consequences. In this thesis the stability of edible oils was investigated by aldehyde and triglyceride measurement of heated oil samples. The Ranzimat

was used for standardized thermal oxidation. Edible oils were heated at 200 °C with an oxygen supply rate of 3.5 l/h over a period of 10 hours. In the following step the obtained aldehydes were derivatized with 2,4-dinitrophenylhydrazin and analyzed by a reversed phase gradient-HPLC system with UV detector. For comparison also fresh, untreated oils were analyzed. Additionally, the polymerization of triglycerides was measured by an isocratic HPLC system with refractive index detector. Results showed that the stability of edible oils are strongly dependent on heat and oxygen supply. The difference between fresh and several hours heated oils was clearly visible. In general, rapeseed oil showed a lower thermic stability than olive oil. The developed HPLC methods are highly sensitive and precise methods for the identification of volatile oxidation products (carbonyls) and triglycerides in edible oils.

Stefanie Bammer: *Improvement and evaluation of AMF productivity in cultivation systems*

The demand of agricultural products is steadily on the rise and leads through the usage of monocultures with low natural vegetation to a decline of native soil microbiome. A possibility to counteract this process would be the application of arbuscular mycorrhizal fungi (AMF), which acts as a bio stimulant for plants. Therefore, a symbiotic interaction between fungus and roots is necessary, that can result in an enhanced nutrient supply of the plant and thus an increased crop yield. The classical cultivation of AMF is mostly performed in greenhouses based on substrate-dependence, which only allows a controlling of humidity and temperature and unsterile conditions are obtained. Hence, so-called in vitro cultures, which are cultivated in combination with “Hairy roots” (HR) as symbiotic partner, are focused in current research and production processes. Though, the processes are not scalable yet, which makes the price of the end product high.

This master thesis covers substantially four main points for optimization of the current fermentation processes, as well as spore quantification and qualification procedure of AMF in the company “Evologic technologies”. These include the testing of new spore inoculation methods, the application of alternative “Bead” materials, the adaptation of process parameters to enhance the AMF productivity in lab-scale bioreactors, as well as the examining of a new evaluation method for more rapid analysis of spore viability. Conducted distribution experiments for investigation of purified spore suspension as alternative inoculum, lead to a settling of spores to the bottom of the reactor and moreover to an increased risk of spore loss during the process. Due to identification of possible error sources, a first-time closed spore balancing was achieved. In case of the even distributed encapsulated spores in alginate beads, a germination of spores from inside the beads to the outside could be proven and thereby a possible suitability for further experiments. The productivity of AMF could be enhanced by performing a so-called “Airlift-Dry phase fermentation” with the implementation of a 28 days’ airlift-phase and resulted in the highest increase of spores in the company so far. Besides, a novel spore network with a size of around 5x5 cm, which was composed of 25000 spores, hyphae and roots, was found in this setup. In parallel, the testing of different bead materials, including alginate, kappa-carrageenan and gelrite was performed in a down-scale model. In comparison to the typical used alginate beads, a 3-fold increase of spores in gelrite beads could be achieved. The usage of a simple gelrite gel layer as reference, showed a further enhancement of spores up to 3.5-times. The proven growth advantage of gelrite as bead material, thereby provides a novelty within this work. For verification of a correlation between the currently used Germination assay and the Iodonitrotetrazolium (INT) assay, a factor of 0.5 between both methods was determined, as well as a distribution coefficient of 0.99. This indicates a nearly perfect regression and thus can be used as a reference for further experiments.

Marie-Luise Deflorian: *Stability of valuable compounds in edible oils and micro algae*

Edible oils are the main source of lipids in human nutrition. Most of the oils consumed are obtained from fruit or seeds. The oils mainly consist of triacylglycerols, which consist of a glycerol backbone esterified with three fatty acids. They have several purposes in the human body such as serving as an energy source or as a protective layer surrounding organs. Besides the fatty acids which are part of triacylglycerols, free fatty acids are also present in vegetable oils. The fatty acids can be categorized into saturated (no double bonds) and unsaturated (one or more double bonds) fatty acids. The latter are known to have beneficial effects on human health and some of them are essential. The most common fatty acids in plants are palmitic, oleic, linoleic and often also stearic acid. One other important component group in vegetable oils are phenolic compounds, of which tocols (tocopherols and tocotrienols) are of great interest. The plant oils represent the best source for vitamin E. Vitamin E can act as antioxidant. Considering olive oil hydroxytyrosol and tyrosol, which also are phenolic compounds, have to be mentioned. Both compounds – which are antioxidants – have an impact on the oil quality as well as the taste of the oil. During the last years several so called "health foods" were introduced. One of them are microalgae which turned out to be rich in polyunsaturated fatty acids as well as high amounts of tocopherols.

In this thesis, HPLC was used for the analytical characterization of edible oils. Six oil samples produced by the company "Fandler" had to be characterized considering their triacylglycerol and fatty acid composition as well as their α-tocopherol content. In addition, the content of tyrosol and hydroxytyrosol of two different olive oil samples and the fatty acid composition of Euglena gracilis at different growth conditions were measured using the developed methods. The measurement of hydroxytyrosol and tyrosol in olive oil showed concentrations of 9 – 15 mg/kg for tyrosol and 11 – 17 mg/kg for hydroxytyrosol which correspond to the values found in literature. The analysis of α-tocopherol in the oil samples showed, other than expected, that more γ-tocopherol was present in the samples, especially in linseed oil. The method for the measurement of the fatty acid profile of the oils could also be applied to a certain degree to the microalgae Euglena gracilis but should be adjusted if further measurements were needed.

Wan Yi Tina Lin: *Characterization of honey - setting into relation the polyphenols and o-dicarbonyls with the floral origin*

In the recent years, analysis of honey became a topic of interest due to its beneficial properties for the human health. Eleven Austrian and two Poland samples were characterized. The samples vary in geographical location, season, and age. Polyphenols, sugar, 1,2-dicarbonyls and 5-hydroxymethylfurfural (HMF) content were analyzed by HPLC. Therefore, respective extraction or derivatization method are represented for HPLC-DAD or HPLC-RID analysis. Several polyphenols could be identified: catechin, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid and o-coumaric acid. The quantity of total polyphenol content varies from 0.56 µg/g to 25.4 µg/g. Moreover, five different sugars could be identified: fructose, glucose, sucrose, lactose and maltose. On average, the sugar composition of honey consists of 55.0 % fructose, 43.4 % glucose and 1.5 % higher sugars. Furthermore, three different 1,2-dicarbonyl compounds were measured: 3-deoxyglucosone (3-DG), glyoxal (GO) and methylglyoxal (MGO). The values of 3-DG vary between 123 mg/kg and 765 mg/kg, while GO values are between 0.1 mg/kg and 5.3 mg/kg and MGO values range between 0.2 mg/kg and 3.8 mg/kg. Also, 1,2-dicarbonyls formation were analyzed by different storage conditions over a defined period of time for the honey samples, as well as for different sugar syrups. Comparing the honey

samples after storage at 37 °C for 50 days, the 3-DG values increased up to 284 %. HMF concentrations varies between 2.7 mg/kg and 77.5 mg/kg. Also, kinetic measurements of HMF were done at different temperatures in order to see the formation of HMF. A significant acceleration of HMF formation were observed from 80 °C on. Finally, the pollens of the honey samples were evaluated by microscope and the colors of the honey samples were described with CIELAB color data.

International cooperations

I. Cantalejo, Universidad de Navarra, Pamplona, Spain

K. Cejpek, VSCHT Prague, Czech Republic

Z. Cieserova, Food Research Institute, Bratislava, Slowakia

M. Mateus, H. Pinheiro, Instituto Superior Tecnico, Lisboa, Portugal

F. Pedreschi, Pontificia Universidad Catolica de Chile, Santiago, Chile

R. Swasti, Atma Jaya University, Yogyakarta, Indonesia

Talk at international conference

1. Murkovic M.: *Food quality/safety and security: A challenge for the next generation* ICETECH 2021, Madiun, Indonesia.

Publications

1. Kratzer, R., Murkovic, M.: *Food ingredients and nutraceuticals from microalgae: Main product classes and biotechnological production* (2021) **Foods** 10 1626. DOI: 10.3390/foods10071626
2. Murkovic, M.: *Food as a basis for good health and well-being*. (2021) IOP Conf. Series: ***Earth and Environmental Science*** 810 012047. DOI: 10.1088/1755-1315/810/1/012047

Lectures and Laboratory Courses

Winter Semester 2020/21

Course no.	Title	Hours	Type	Lecturers
CHE.154_1UF	Biochemistry Lab Course I	5.33	LU	Team
CHE.155UF	Biochemistry II	1.5	VO	Macheroux P
CHE.191UF	Bioanalytics	2.25	VO	Klimant I, Winkler A
CHE.192UF	Biochemistry Lab Course II	4	LU	Bijelic A, Wallner S
CHE.200_FUF	Project Lab (Bachelor)	5	LU	PIs
CHE.210_FUF	Project Work to the Bachelor Thesis	0.5	SE	PIs
CHE.890UF	Food Biotechnology	1.33	VO	Murkovic M
CHE.892UF	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MOL.101UB	Introduction to Bachelor Study	1	SE	Macheroux P
MOL.606_1UF	Bachelor Thesis	1	SE	PIs
MOL.833_1	Project Laboratory	9	LU	Team
MOL.844_1UF	Journal Club Biochemistry and Molecular Biomedicine	1.5	SE	Team
MOL.845_UF	Master Thesis Seminar	2	SE	PIs
MOL.855UF	Molecular Physiology	2	VO	Lorber B, Macheroux P
MOL.881UB	Biophysical Methods	3	VO	Winkler A, …
MOL.882_1UF	Profession-oriented Research Practices	2	PV	Macheroux P
MOL.933UF	Food Biotechnology	1.3	VO	Murkovic M
MOL.936UF	Food Biotechnology	4	LU	Murkovic M, Leitner E
MOL.959UF	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MOL.961UF	Food Chemistry and Technology II	2	VO	Murkovic M
648.003	Molecular Enzymology I	2	PV	Macheroux P
648.007	Graduate Seminar 1	1	SE	Team
648.009	Scientific Colloquium for Graduate Students 1	1	SE	Team
648.021	Structural Biochemistry I	2	SE	Winkler A
648.030	Soft Skills Doctoral School Molecular Biomedical Sciences and Biotechnology	1	SE	Macheroux P
649.027	Chemical Reactions in Foods I	2	PV	Murkovic M

VO: Lecture Course; LU Lab Course; SE Seminar; PV: Privatissimum

Lectures and Laboratory Courses

Summer Semester 2021

Course no.	Title	Hours	Type	Lecturers
CHE.147UF	Biochemistry I	3.75	VO	Macheroux P
CHE.193UF	Molecular Biology Laboratory Course	3	LU	Kerschbaumer B, Wallner S
CHE.194UF	Seminar for Molecular Biology Laboratory Course	1	SE	Kerschbaumer B, Wallner S
CHE.200_FUF	Project Laboratory Chemistry (Bachelor)	0.5	SE	Macheroux P, Murkovic M, Oberdorfer G, Winkler A
CHE.210_FUF	Project Work to the Bachelor Thesis	0.5	SE	Macheroux P, Murkovic M, Oberdorfer G, Winkler A
MAS.476UB	Biophysical Methods	3	LU	Winkler A, …
MOL.406UF	Methods in Immunology	2	VO	Oberdorfer G
MOL.407UF	Methods in Immunology	2	LU	Team
MOL.606_1UF	Bachelor Thesis	1	SE	Team
MOL.832_1UF	Project Laboratory	9	LU	Team
MOL.844_1UF	Journal Club Biochemistry and Molecular Biomedicine	1.5	SE	Team
MOL.845_1UF	Seminar for Undergraduate Students	2	SE	Team
MOL.880UF	Molecular Enzymology	2	VO	Gruber K, Macheroux P, Nidetzky B
MOL.886UB	Biophysical Methods	3	LU	Winkler A, Oberdorfer G, […]
MOL.969UF	Integrative Structural Biochemistry	2	VO	Winkler A, Madl T, Gruber C
MOL.970UF	Protein Design	2	VO	Oberdorfer G
648.004	Molecular Enzymology II	2	PV	Macheroux P
648.006	Introduction to Biochemistry	2	VO	Wallner S
648.008	Graduate Seminar 2	1	SE	Team
648.010	Scientific Colloquium for Graduate Students 2	1	SE	Team
648.012	Frontiers in Integrative Structural Biology	1	SE	Macheroux P, Winkler A, Oberdorfer G, …
648.013	Advanced cell culture training course II	1	SE	Amor M
648.016	Chemical Reactions in Foods II	2	PV	Murkovic M
648.022	Structural Biochemistry II	2	SE	Winkler A

VO: Lecture Course; LU Lab Course; SE Seminar; PV: Privatissimum