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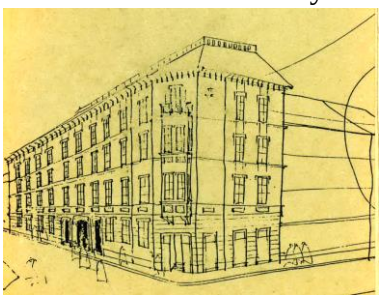
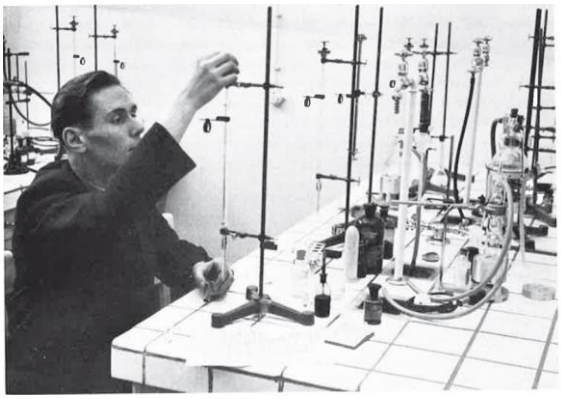
Extended History of 50 years (plus) of the Institute of Biochemistry

1973 marks the year of official establishment of the Institute of Biochemistry when Fritz PALTAUF was appointed professor of the newly founded Institute originating from the division of the former Institute for Biochemical Technology, Food Technology and Microchemistry. Nevertheless, the history of the Institute in the context of the development of biological sciences in Graz dates back much longer. As part of the 50 years' anniversary in **2023**, we present a detailed history of this development below.

1929 Together with all the other Chemistry Institutes, the initial Technical Biochemistry and Microbiology was located in the old Chemistry Building on Baron MANDELL's ground at the corner of Technikerstraße and Mandellstraße. Due to space limitations caused by the increased number of students after World War I, the Institute moved to three refurbished floors in the building of the Fürst-Dietrichstein-Stiftung, Schlögelgasse 9 (right picture), in which all the Biosciences were then concentrated.



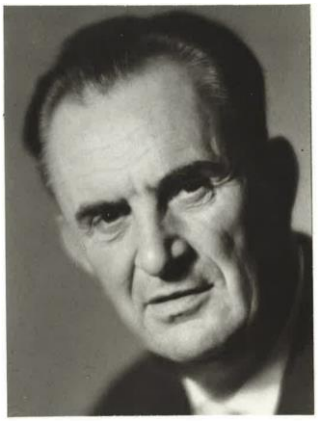
1945 Georg GORBACH - initially in the rank of a docent and soon thereafter as a.o. Professor - took over to lead the Institute. The Institute was renamed Institute of Biochemical Technology and Food Chemistry. In 1948, G. GORBACH was nominated o. Professor and head of the Institute. In succession of the famous Graz School of Microchemistry founded by PREGL and EMICH, G. GORBACH was one of the most prominent and active leaders in the field of Microchemistry. With help of the microchemical working methods and enrichment procedures developed at the Institute and their applications towards organic and inorganic micro-analysis, it was already at that time possible to determine the nature of substances down to microgram scale without relying on the use of sophisticated instruments (see picture on the right). In the field of microbiology, the effects of inhibitors and other bio-active compounds on living beings, most notably microorganisms, were analyzed. In Nutritional Sciences, the effects of vitamins and trace elements were studied and new methods for food manufacturing at an industrial scale were developed.



1950 In the course of increasing industrialization after World War II, questions of water quality und waste water disposal became urgent; hence, the group of the future Prof. K. STUNDL, which at that time was part of the Institute, gained importance. In addition, a division to fight dry-rot, at first supervised by Dr. KUNZE and after his demise 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 laboratories were called EMICH-Laboratories and extended in size to reflect the increased demand for working area. At the same time, the institute was renamed Institute of Biochemical Technology, Food Chemistry and Microchemistry.

1970 After the decease of G. GORBACH (picture on the right), H. GRUBITSCH was appointed head of the Institute. In the following years, the heavy teaching duties were carried by the assistants of the Institute which were appointed as lecturers. Due to the broad working area of the Institute, the teaching duties carried by the Institute were heavy. Lectures were held in Biochemistry, Biochemical Technology, Food Chemistry and Food Technology, Technical Microscopy, Microchemistry and its accompanying laboratory courses. In addition, the Institute covered Technical Microbiology together with Biological and Bacteriological Analysis - with the exception of Pathogenic Microorganisms - and a lecture in Organic Raw Materials Sciences.



1970 Already towards the end of the sixties, the division for water- and waste water disposal headed by K. STUNDL was drawn out of the Institute and established as an Institute of its own in the newly constructed building on the ground of the former Chemistry Building. Already in 1963, H. SPITZY was nominated o. Prof. of General Chemistry, Micro- and Radiochemistry. At first located in two rooms of the building at Schlögelgasse, he covered Quantitative Microchemistry and in 1972 also Qualitative Microchemistry. This Division was thus also drawn out of the mother Institute and at the end of the sixties moved to a new building.

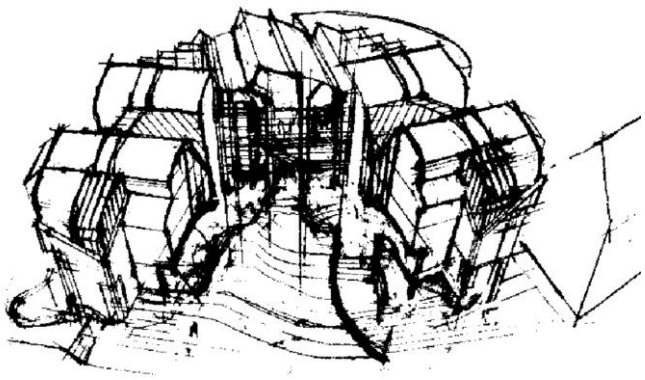
1973 The final division of the Institute for Biochemical Technology, Food Technology and Microchemistry took place. At first, Biochemical Technology together with Food Technology formed a new Institute now called Institute of Biotechnology and Food Chemistry to which the newly nominated Prof. Dr. LAFFERTY was appointed head of the Institute.

1973 In the same year, as stated initially reflecting the foundation of the Institute resembling its current form, F. PALTAUF (right picture), docent of the Karl-Franzens-University Graz, was nominated Professor and head of the newly established Institute of Biochemistry. The interest of F. PALTAUF in studying biological membranes and lipids, with emphasis put on the biochemistry and function of ether lipids and the metabolism of phospholipids in yeast, laid the foundation for the future direction of research at the Institute.

1975 and 1978, G. DAUM and S. D. KOHLWEIN, respectively, joined the Institute to write their diploma and doctoral theses. A. HERMETTER joined the group in 1976. He was given the position of an assistant in recognition of his PhD degree "*sub auspiciis praesidentis*" that he received from the Karl-Franzens University Graz. His prime interest was in studying the biophysical properties of ether lipids in both model and biological membranes. All three young scientists were given the chance to work as Post Docs in renowned laboratories in Switzerland and the USA: G. DAUM with the groups of G. Schatz (Basel) and R. Schekman (Berkeley), A. HERMETTER with J.R. Lakowicz (Baltimore) and S. D. KOHLWEIN with S. A. Henry (New York). Working

in the stimulating atmosphere of such excellent laboratories, the three post docs increased their knowledge of their respective fields of research and became familiar with new techniques which they subsequently brought to Graz. Consequently, independent research groups specialized in cell biology (G. D.), biophysics (A. H.) and molecular biology (S. D. K.) evolved at the Institute in Graz, with the group of F. PALTAUF still focusing on the chemistry and biochemistry of lipids. In spite of this segmentation of the Institute, close collaboration between the individual groups was maintained.

1990



The Institute was still housed in the Fürst-Dietrichstein-Stiftungshaus, Schlögelgasse 9, an old building with historical charm, but otherwise unsuited for laboratory work. This situation changed when the Institute moved to the new building at Petersgasse 12. More space to accommodate a larger number of graduate students and guest scientists became available. Special laboratories were adapted for the use of more delicate equipment such as time resolved fluorescence spectrometry and confocal laser scanning microscopy. This expansion of the size of the individual research groups and the acquisition of new equipment were essential for the Institute to participate in novel collaborative efforts at the national and the international level including joint projects and EU-projects. Thus, the Institute of Biochemistry, together with partner institutes from the Karl-Franzens-University were the driving forces that helped to establish Graz as a center of competence in lipid research.

1993 Moving to the new building was also a prerequisite for the appointment of W. PFANNHAUSER as Professor of Food Chemistry. Through his own enthusiasm and engagement and that of his collaborators, this new section of the Institute rapidly developed and offered students additional opportunities to receive a timely education. The two sections, Food Chemistry and Biochemistry, however, were independent of each other with respect to personnel, teaching, and research.

2001 After Prof. PALTAUF's retirement, in September 2001, G. DAUM was elected interim head of the institute and S. D. KOHLWEIN was appointed full professor of biochemistry at the Karl-Franzens University Graz.

2003 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, which should shape the development of the institute in the years to come. Especially his strong interactions with the biocatalysis community in Graz further influenced the positioning of the institute in the scientific landscape of Graz as well as beyond. Due to his efforts also the long-term perspective of the institute changed more towards protein biochemistry, with an emphasis on basic research, but also connections to industrial partners.

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

2009 With the retirement of W. PFANNHAUSER the *Institute of Food Chemistry and Technology* was disbanded and the research group of Prof. M. MURKOVIC joined the *Institute of Biochemistry*.

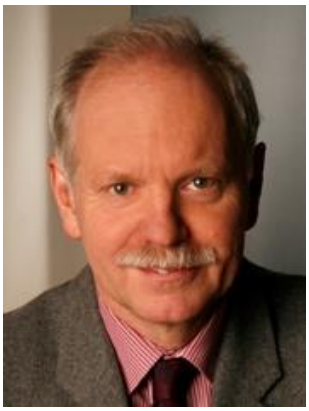
2013 Due to faculty reorganizations, the group of J. BOGNER-STRAUSS (formerly at the *Institute of Genomics and Bioinformatics*) joined the *Institute of Biochemistry*. Her main interests revolved around the development and metabolism of adipocytes.

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 I. Schlichting at the Max-Planck-Institute for Medical Research in Heidelberg. His research interests revolve around the interaction of light with biomolecules and how these signal integration and long range signal transduction are controlled on a molecular level.

2015 After more than 40 years of service for the *Institute of Biochemistry* Prof. A. HERMETTER retired on September 30, 2015. After receiving his PhD *sub auspiciis Praesidentis* at the Karl Franzens University Graz he started as a University Assistant in Prof. PALTAUF's group and moved through the ranks to become an Associate Professor at the institute. As an active member of the institute 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. Supported by two FWF grants, an institute-funded technician and highly motivated students, the group quickly developed its independent character and extended the research portfolio of the institute.

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. The picture below reflects the large number of scientists at the institute focusing on protein biochemistry during that time.

2021 A. WINKLER received his *venia legendi* in *Structural Biochemistry* and was appointed associate professor at the institute. With the help of an infrastructure grant from the county of Styria a new mass spectrometry infrastructure was established at the Institute enabling state-of-the-art protein analytics (intact mass, native mass spectrometry) as well as contributing to the integrative structural biology community in Graz with the in solution structural characterization method hydrogen deuterium exchange-coupled to mass spectrometry (HDX-MS).

2022 H. LECHNER was awarded a BioTechMed Graz Young Researcher Group installation grant. This allowed him to establish his own research group focusing on "*Active site transfer to generate new enzymes*" in 2023

Short group summaries for 2023

 **acheroux group**: 2023 was a very special anniversary for **Peter Macheroux** since he started his research group 20 years ago at the institute of biochemistry. This called for a celebration! Silvia Wallner, a long-time associate of Peter, organized a retreat in southern Styria where the whole group enjoyed food specialties along with the exquisite white wine that is produced in the wineries of the region. In addition, an alumni meeting took place in July where many former PhD students of Peter gathered in Graz from all over Europe.

 **inkler group:** One highlight in the group of **Andreas Winkler** was Ursula Vide's publication reporting findings on the blue light regulated diguanylate cyclase systems in *Science Advances*. In addition, efforts to establish working routines for automated mass spectrometry measurements for protein characterization were an important aspect during 2023. To this end, Philipp Pelzmann created protocols for standardized intact mass determination, native mass spectrometry with online desalting and streamlined the process for the analysis of conformational dynamics using hydrogen-deuterium exchange experiments. These analyses are now easily accessible to members of the institute, the faculty and other collaboration partners.

 **berdorfer group:** the group entered a transition time in 2023. A lot of projects were slowly nearing their end, so a lot of the PhD students in the group started to write up their theses and manuscripts. In fact, Veronica Delsoglio, one of first PhD students in the group, finished here PhD at the end of July 2023. Additions to the group were several bachelor students, including Morakot Chakatok, who stayed as cloning and protein expression expert ever since. In addition, the group hosted several visitors – from February to April, Joseph Phillips from the University of Birmingham joined the group to work on the design of silver coordinating peptides. In fall, the group hosted Liher Gonzales from the CIC BioMagune in San Sebastian for three months in the course of the ARTIBLED project. And close to the end of the year, Shlomo-Yakir Hoch, a PhD student from the Weizmann Institute of Science in Rehovot joined the group to work on a collaborative project (with the group of Prof. Sarel Fleishmann).

 **echner group: Horst Lechner** started a BioTechMed YoungResearcherGroup (BTM YRG) in April 2023 to design new proteases and enzymes. His PhD student Sajith Kolathuparambil from Kerala, India started in September. Additional support joined the group with Ahmed Alagic, doing previously his bachelor thesis at the institute.

 **urkovic group:** In the Functional Food Group the main projects 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 are oxidized 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 and specific fungal non-canonical amino acids as food supplements started.

Biochemistry Group

Group leader: Peter Macheroux
Secretary: Christine Rother
Senior Scientist: Silvia Wallner
Postdoctoral fellow: Aleksandar Bijelic
PhD students: Stefanie Baldauf, Reinmar Eggers, Katharina Fuchs, Bianca Kerschbaumer, Grazia Malovan
Master students: Hannah Gasser, Anja Lex, Silvia Kheir
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. In addition, we use MALDI-TOF and ESI mass spectrometry, X-ray crystallography, 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 catalyzing 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. For example, the model plant *Arabidopsis thaliana* possesses 27 genes that apparently encode BBE-like enzymes, although the plant does not synthesize complex alkaloids. We have recombinantly produced BBE-like homologs from *A. thaliana* (*At*BBE-like proteins 13 and 15) 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 is 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 (*At*BBE-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 a basis for the design of further experiments. Furthermore, we are working on generating 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 *At*BBE-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 *At*BBE-like *24, 25*, and *26*. 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).

Additionally, we aim to investigate BBE-like enzymes from the model fungus *Neurospora crassa*. During flavoproteome analysis, it became obvious that *N. crassa* does not only possess a high number of core flavoproteins participating in housekeeping reactions but also a substantial number of accessory flavoenzymes required for secondary metabolism [1]. Among these auxiliary flavoproteins, 17 belong to the group of BBE-like enzymes, whereas no information on their exact enzymatic function has been available until now. Therefore, we aim to heterologously express and characterize members of this interesting protein family to deepen our understanding of the function of BBE-like enzymes in fungi (thesis project of Stefanie Baldauf and master thesis project of Silvia Kheir supported by Dr. Silvia Wallner).

[1] Kerschbaumer *et al*.: *FlavoFun: Exploration of fungal flavoproteomes* (2022) ***Front Catal***. 2, 1021691.

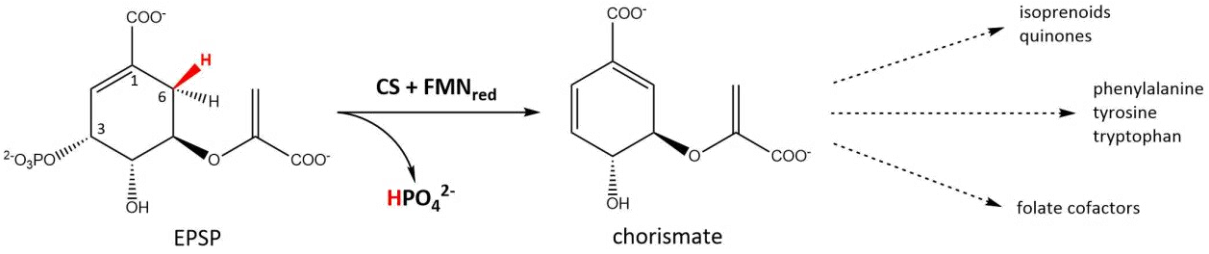
Chorismate synthase as a potential target for pharmaceutically useful inhibitors

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 makes this metabolic pathway a promising target for developing antibacterial agents and herbicides.

CS 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 [2, 3]. For the design of new inhibitors, the highly 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 that are similarly distributed to those of the natural substrate 5-enolpyruvylshikimate 3-phosphate.

Furthermore, the interaction of the obtained inhibitory compounds with different CSs was evaluated by determining their dissociation constants (1.1-4.4 µM) and IC50 values (10-16 µM) with binding and inhibition assays, respectively. To get a better insight into the binding mode, the 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, we need to obtain structural information on 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.

[2] Rodrigues-Vendramini *et al*. *Promising new antifungal treatment targeting chorismate synthase from Paracoccidioides brasiliensis* (2019) ***Antimicrob. Agents Chemother***. 63:e01097-18.

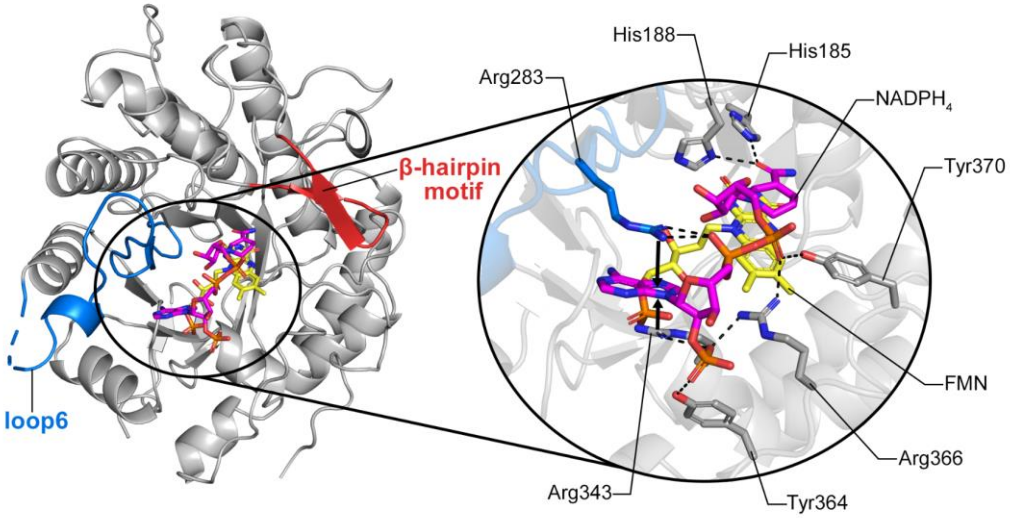
[3] Bueno *et al*. *New inhibitors of chorismate synthase present antifungal activity against Paracoccidioides brasiliensis* (2019) ***Future Microbiol.*** 14:969-980.

Ene-reductases: shifting the cofactor preference of *Sl*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). We recently solved the crystal structures of *Sl*OPR3 in complex with $NADPH_4$ and $NADH_4$ (redox-inactive homologs of NAD(P)H) and revealed that while the nicotinamide group of the cofactors binds in the same way (i.e., above the flavin), their (2'-phosphate)-adenosine tails bind to different sites within the active site cavity [4]. This structural information is currently being leveraged to design *Sl*OPR3 variants with switched cofactor preferences.



Crystal structure of *Sl*OPR3 in complex with $NADPH_4$: $NADPH_4$ (purple sticks) binds with its nicotinamide group above the flavin (yellow sticks) and with its adenosine tail to a positively charged site within the loop 6 region (blue loop). The key to NADPH preference in *Sl*OPR3 is the electrostatic stabilization of the coenzyme's adenosine tail. The 2'-phosphate group is bound by Arg343, Tyr364, and Arg366 (dashed lines) while the adenine ring is stabilized by cation-π interactions with Arg343 and Arg283 (black arrows).

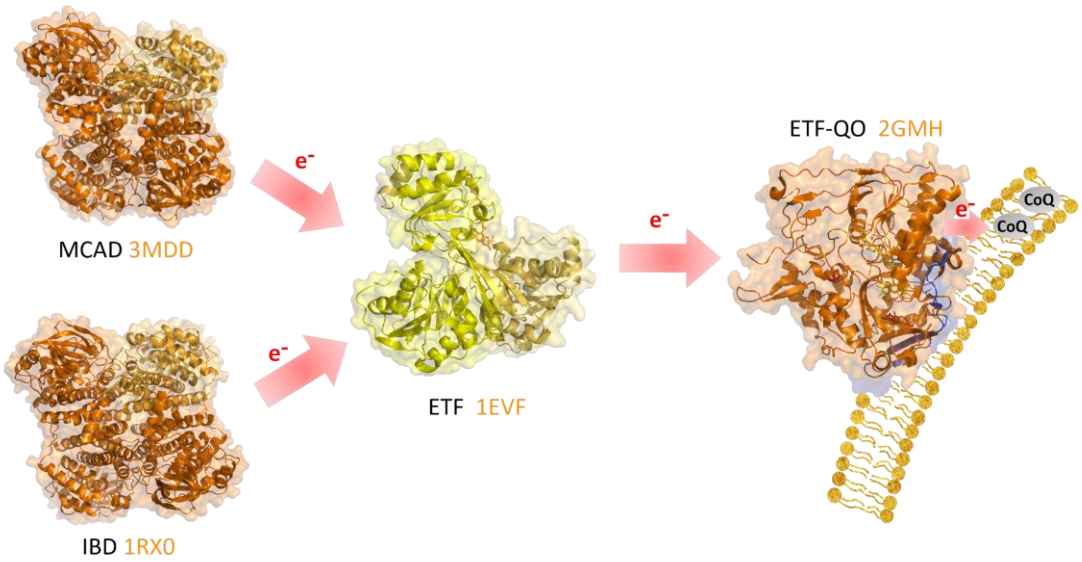
[4] Kerschbaumer et al. *Loop 6 and the β-hairpin flap are structural hotspots that determine cofactor specificity in the FMN-dependent family of ene-reductases* (2024) ***FEBS J.*** in press. DOI:10.1111/febs.17055

Flavin-dependent electron transfer in human mitochondria

In humans, the oxidative energy metabolism and thus mitochondrial electron transport is of utmost importance for the survival of every individual. Electron-transferring flavoproteins (ETFs) accept electrons from different flavin dehydrogenases and pass them on to the mitochondrial respiratory chain, more precisely to ETF-quinone oxidoreductases (ETF-QOs). ETF-QOs, in turn, accept electrons coming from ETF and feed them to the quinone pool in the electron transport chain.

ETFs are heterodimeric proteins in which 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 amino acids αN259 and βE165 results in a strong preference for the open form of hETF. In previous studies by Drs. Peter Augustin, Marina Toplak, and Sami Ullah Khan, 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 and that this formylation occurs at different rates when ETF wild type and the αN259A and βE165A variant proteins are compared.

Currently, we want to shed light on the mechanism of electron transfer to and from hETF. We aim to understand whether interaction with client dehydrogenases, such as medium-chain acyl-CoA dehydrogenase (MCAD) and isobutyryl-CoA dehydrogenase (IBD), affects the rate of cofactor modification in hETF. Additionally, we test protein design strategies for heterologous production of ETF-QO in E. coli to obtain this protein for mechanistic studies (Master thesis project of Anja Lex supported by Dr. Silvia Wallner).



Electron transfer from dehydrogenases to the mitochondrial transport chain via the human electron transferring flavoprotein (hETF). hETF accepts electrons from up to fourteen different dehydrogenases (such as MCAD or IBD) and passes them on to the respiratory chain via membrane-bound ETF-ubiquinone oxidoreductase.

Doctoral Thesis completed

Grazia Malovan: *The role of dipeptidyl peptidase in mammalian physiology*

Dipeptidyl peptidase 3 (DPP3), present across several kingdoms of life and highly conserved among higher animals, is a multifaceted protein that cleaves dipeptides sequentially from the N-terminus of peptide chains. DPP3 is the sole member of the M49 family of metalloproteases with a mass of approximately 80-85 kDa, and it is mostly described as a cytosolic protein, although some studies advocate its membranous localization. It is a zinc-dependent aminopeptidase with several important roles in physiological and pathophysiological processes in mammals, such as involvement in oxidative stress, pain, cell cycle regulation, carcinogenesis, and inflammation. However, the exact role of DPP3 in physiology and disease-related processes remains elusive. In addition, recent data have indicated that DPP3 also affects the Keap1-Nrf2 signal pathway by competing with Nrf2 for binding to Keap1 through a highly conserved ETGE motif found both in DPP3 and Nrf2. Binding of DPP3 to Keap1 may enhance the function of Nrf2 by blocking its ubiquitination, which usually leads to uncontrolled transcriptional activation of Nrf2. The project mainly focused on investigating the role of DPP3 in oxidative stress and its role in carcinogenesis. Tissues isolated from WT and KO mice were used for this project, and the KO and WT mice were also used to generate mouse embryonic fibroblast cells (MEFs). The generated MEF cell line, a non-differentiated cell line (stem cells), can be used as a system to investigate the basic role of DPP3 in mammals. Furthermore, two breast cancer cell lines, MCF-7 and MDA-MB-231, were used in order to determine the role of DPP3 in carcinogenesis as well as its role in oxidative stress. For this purpose, breast cancer cells were subjected to hyperoxia treatment, after which the parameters of oxidative stress, in correlation to DPP3, were checked. In order to gain a deeper insight into the role of DPP3 in mammals, the CRISPR/Cas9 system was used to generate a dpp3-knockout breast cancer cell line. In conclusion, the results obtained indicate that DPP3 might be localized not only in the cytosol but also in the nucleus. This could raise new questions related to translocation and the role of DPP3 in the nucleus. Furthermore, a potentially essential role of DPP3 in the survival and cell cycle of breast cancer cells could be shown during the generation of the KO breast cancer cell lines.

Master Thesis completed

Silvia Kheir: *Investigation of berberine bridge enzyme-like enzymes from Neurospora crassa*

Generally, oxidations are vital reactions for all living organisms. Oxidases are a versatile group of enzymes involved in numerous metabolic pathways. Flavin adenine dinucleotide (FAD), a vitamin B2 derivative, operating as an electron acceptor during oxidation reactions, confers a key role to enzymes in energy metabolism and biosynthesis of metabolites. There, the cofactor can be either covalently or non-covalently attached to the enzyme. An FAD-dependent oxidase with bi-covalent flavin attachment is the berberine bridge enzyme (BBE). The enzyme from *E. californica* catalyzes the synthesis of (S)-scoulerine in benzylisoquinoline alkaloid biosynthesis by the formation of the so-called berberine bridge, thus introducing an additional fourth ring into the molecule. In plants, the number of genes encoding BBE-like enzymes varies from one (*Physcomitrella patens*) to dozens (*Populus trichocarpus*), and several BBE-like plant enzymes have already been characterized. Yet, the fungal “universe” remained largely unexplored. During this thesis, genes from the red bread mold *N. crassa*

(Ascomycota) encoding BBE-like proteins were structurally analyzed. Among 17 genes, four genes were selected for heterologous protein production in the *Komagataella phaffii* expression system. The KM71H [pPICK-PDI] strain of *K. phaffii* with a deletion of the aox1 gene and an expression plasmid for protein disulfide isomerase was chosen. Expression in this strain is achieved by induction of the strong AOX1 promoter with methanol. Two flavoenzymes could be successfully produced by fermentation, namely *Nc*BBE4 and *Nc*BBE9. These proteins can now be subjected to further biochemical and structural studies.

International cooperation

Asaph Aharoni, Weizmann Institute of Science, Rehovot, Israel.

Research projects

FWF P35858: "The family of monolignol oxidoreductases in *Arabidopsis thaliana*"
FWF-doc.funds "CATALOX, Catalytic applications of oxidoreductases"

Publications

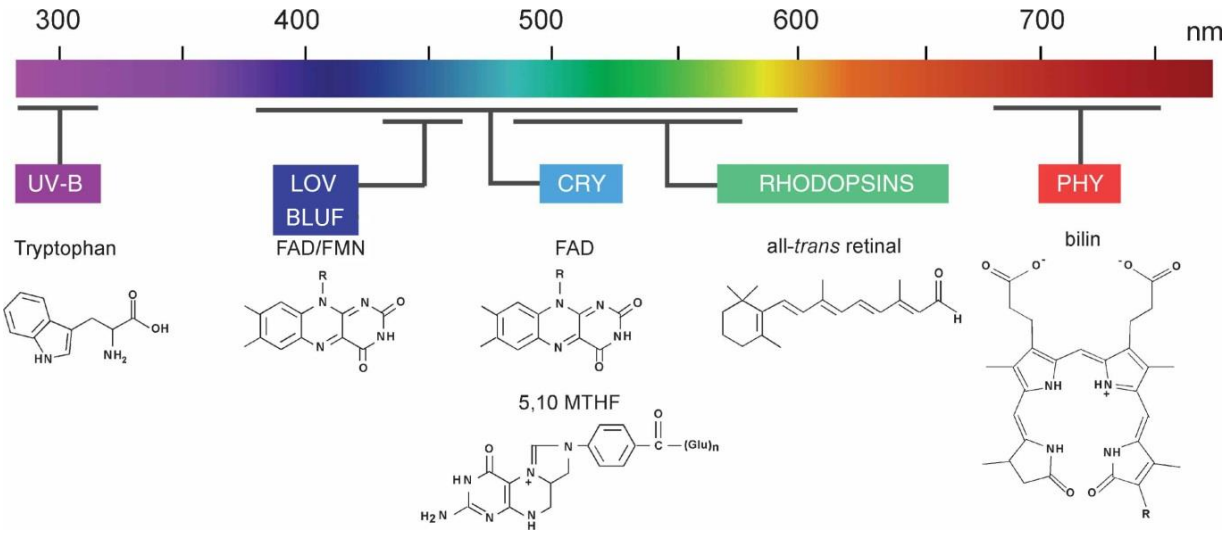
1. Skourti, E., Macheroux, P.: In conversation with Peter Macheroux (2023) ***FEBS J.***, 290, 2208-2213, DOI:10.1111/febs.16646
2. Malovan, G., Hierzberger, B., Suraci, S., Schaefer, M., Santos, K., Jha, S., Macheroux, P.: *The emerging role of dipeptidyl peptidase 3 in pathophysiology* (2023) ***FEBS J.***, 290, 2246-62, DOI:10.1111/febs.16429
3. Boehm, T., Martin-Higueras, C., Friesser, E., Zitta, C., Wallner, S., Walli, A., Kovacevic, K., Hubmann, H., Klavins, K., Macheroux, P., Hoppe, B., Jilma, B.: Simple, fast and inexpensive quantification of glycolate in the urine of patients with primary hyperoxaluria type 1 (2023) ***Urolithiasis***, 51, 49, DOI:10.1007/s00240-023-01426-6
4. Carmona, G. O., Lahham, M., Poliak, P., Goj, D., Friesser, E., Wallner, S., Macheroux, P., Oostenbrink, C.: Understanding the riddle of amine oxidase flavoenzyme reactivity on the stereoisomers of *N*-methyl-dopa and *N*-methyl-tyrosine (2023) ***Mol. Recognit.***, e3068, DOI:10.1002/jmr.3068
5. Kerschbaumer, B., Totaro, M. G., Friess, M., Breinbauer, R., Bijelic, A., Macheroux, P.: Loop 6 and the β-hairpin flap are structural hotspots that determine cofactor specificity in the FMN-dependent family of ene-reductases (2023) ***FEBS J.***, *in press.* DOI:10.1111/febsj.17055

Photoreceptor Group

Group leader: Andreas Winkler
PhD students: Oliver Eder, Maximilian Fuchs, Massimo Totaro (joint with AGO), Hieu Tran, Uršula Vide
Master student: Regina Zausinger
Bachelor student: Stefan Minnich
Technical staff: Philipp Pelzmann
Apprentice: Christoph Schaffer

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.

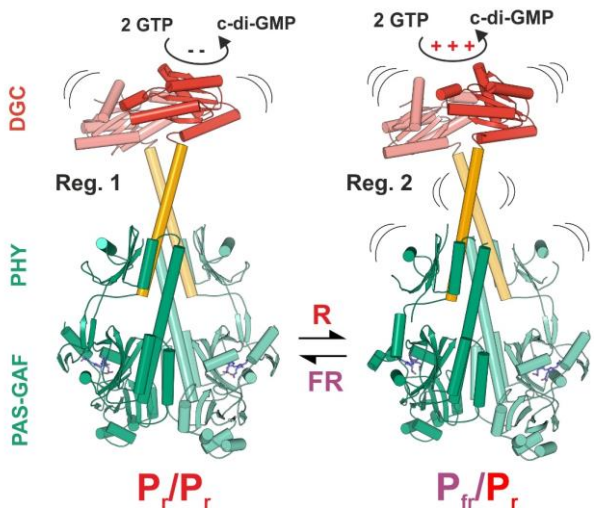


Overview of photoreceptor families and their corresponding cofactors covering the UV/Vis range of the electromagnetic spectrum (adapted from Heintzen WIREs Membrane Transport and Signaling 2012, 1:411–432).

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.

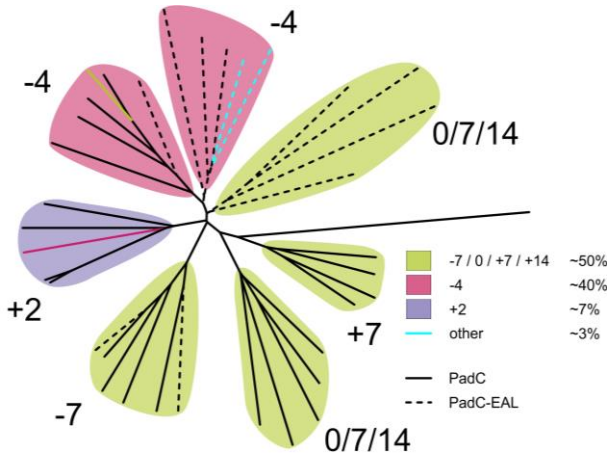
With the elucidation of the first full-length crystal structure of a naturally occurring phytochrome linked to its functional effector domain, new insights into signal transduction in phytochromes were obtained. The structure revealed a parallel dimeric arrangement of the both domains. Based on an in-depth characterization of the involvement of functionally important elements, our studies provide a foundation for appreciating 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 [1].



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 [2]. Understanding the role of structural asymmetry and how they correlate with non-canonical spectroscopic signatures (only partial Pfr formation) in these systems is the focus of the PhD thesis of Hieu Tran.

In an attempt to further characterize molecular determinants of asymmetry in *Is*PadC, Hieu Tran has attempted to use NMR for looking into the conformational dynamics of these complex dimeric systems. Initial analyses using Methyl-TROSY by labelling with ^{13}C isoleucine and methionine did not show significant shifts in NMR spectra due to the limited activation from a fiber-coupled LED via top-down illumination. However, roughened optic fibers and immersion of the fiber into the NMR tube resolved the issue of low activation. However, the propensity of *Is*PadC to transiently form tetrameric assemblies via an "artificial interface" caused severe losses in signal intensity and, thereby, stopped current efforts to follow up on asymmetric properties of light activation via NMR. Other homologs with similar activation properties, but lacking the artificial interface, might be interesting candidates for follow up experiments with NMR.

In an effort to better understand the interplay of specific amino acids with the biliverdin cofactor and to characterize their role in stabilizing Pr or Pfr, Hieu is has performed an in-depth mutagenesis programme of *Is*PadC. Particularly interesting variants are ones stabilizing Pfr for days, but still featuring the non-canonical spectra of *Is*PadC wild-type. Hence, even if Pfr is stabilized via amino acid substitutions that destabilize the Pr conformation, a homodimeric Pfr/Pfr system appears to be unfavorable in the context of *Is*PadC. Another very interesting variant destabilized Pfr formation substantially, allowing only 5 % of Pfr to be present during constant illumination with red light. Still, the enzymatic activity of the effector domain can be stimulated in a comparable manner to the wild-type protein. This allows the conclusion that no Pfr/Pfr dimer is needed for stimulation of output activity and that an asymmetric intermediate/Pfr state is sufficient for signal integration (manuscript in revision).



Linker-length clustering in the evolutionary relationship of PadCs (left figure). The phylogenetic characterization carried out by Cornelia Böhm (former 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.

While non-canonical spectra appear to be no strict requirement for PadC functionality, as recently observed in synthetic chimeras [3], naturally occurring PadCs with light activation all feature the characteristic partial-Pfr spectra upon red light illumination. A full characterization of interesting representatives from all major branches of PadCs has been performed by former PhD student Cornelia Böhm. The biochemical and phylogenetic aspects have recently been published [4] and support the notion that linker length strongly correlates with functional properties of PadCs. According to the characterization of multiple homologs and the chimeras, additional structural and/or functional elements in the PAS-GAF bidomain of phytochromes should play a role in defining the characteristic spectral properties of *Is*PadC and many other phytochromes.

Since first shell residues surrounding the cofactor are mostly conserved or were already targeted by the mutational programme from Hieu Tran, Massimo Totaro (joint PhD student with AGO) and Oliver Eder teamed up to ask the question: “Are there also residues further away from the cofactor that still influence the optical properties?”. To this end, Massimo Totaro used deep learning computational tools (Protein MPNN) to get hints on the functional/structural involvement of residues that appear non-obvious in sequence alignments, but might still have an important role in phytochromes. Oliver Eder together with Stefan Minnich then produced variants probing identified residues and trying to infer their influence on the spectroscopic properties of the protein variants. Interesting candidates were identified and are currently under detailed investigation. This line of research will ultimately deepen our understanding of these fascinating light sensors, and might help us to appreciate their intricate molecular mechanisms to allow future development of fluorescent probes or optogenetic tools.

[1] Gourinchas *et al*.: *Long-Range Allosteric Signaling in Red Light-Regulated Diguanylyl Cyclases* (2017) ***Sci. Adv.*** 3, e1602498

[2] Gourinchas *et al*.: *Asymmetric activation mechanism of a homodimeric red light-regulated photoreceptor* (2018) ***eLife***, 7, e34815

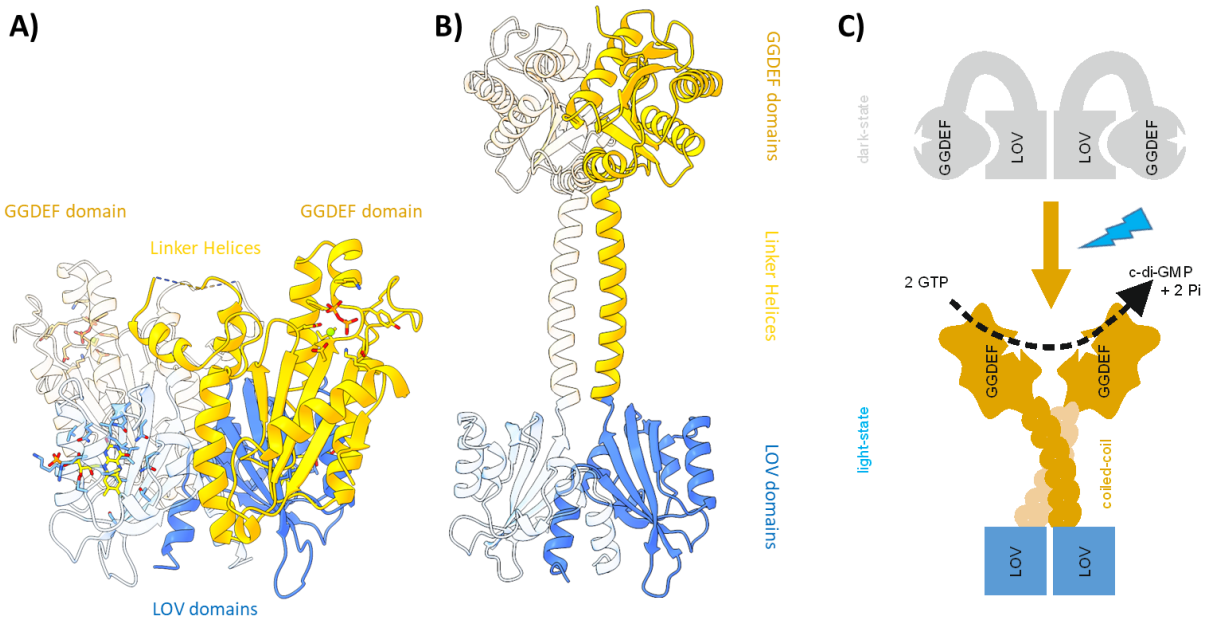
[3] Böhm *et al*. *The PHY domain dimer interface of bacteriophytochromes mediates cross-talk between photosensory modules and output domains* (2021) ***J. Mol. Biol.***, 433, 15, 167092

[4] Böhm *et al*. *Characterisation of sequence–structure–function space in sensor–effector integrators of phytochrome-regulated diguanylate cyclases* (2022) ***Photochem. Photobiol. Sci.***, 21 (10), 1761-1779

Blue-light-regulated diguanylate cyclases

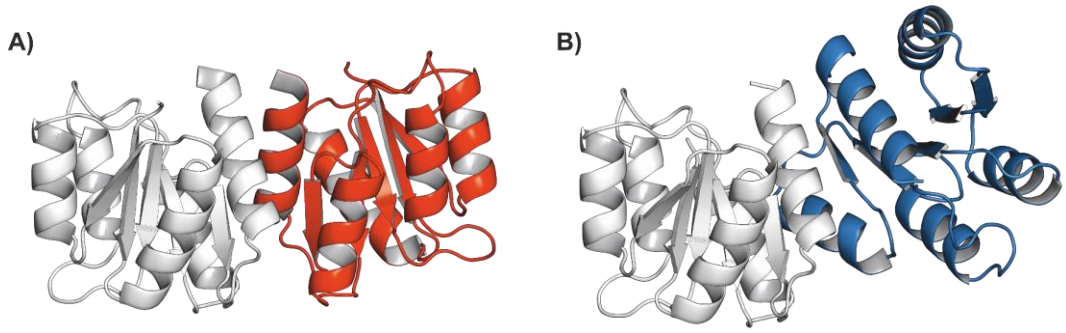
Photoreceptors sensing blue light were early on identified as key players in plant phototropism, and belong to the family of flavin-dependent light-oxygen-voltage (LOV) domains [5]. LOV domains have a high potential in applied biosciences, as they contributed significantly to the field of optogenetics using a variety of LOV-coupled effector domains [6].

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 (PhD projects of Uršula Vide and Maximilian Fuchs). To elucidate molecular mechanisms of light regulation we are studying naturally occurring LOV-GGDEF systems and integrate our results with data obtained for other LOV- or GGDEF-containing 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. We employed an integrative structural biology approach combining X-ray crystallography, solution scattering, computational methods and hydrogen-deuterium exchange coupled to mass spectrometry to reveal how a subfamily of LOV-GGDEF proteins, with extraordinary high dynamic range of activation by light, operates on a molecular level [7]. Additional aims of the PhD thesis of Uršula Vide go in the direction of addressing the light activated state on a molecular level and using this system for optogenetics and other applications that require precise temporal and spatial control of enzymatic activity or other biological outputs. Besides focusing on the role of specific residues in the sensor-effector linker and at the extensive inhibitory interface between the two domains, she is currently also exploring various possibilities for computer-aided optimization of the LOV-GGDEF switch. To further characterize the system and enhance the understanding of GGDEF domains, we attempted to eliminate negative feedback product inhibition (Master thesis project of Regina Zausinger), incorporating hints towards for successful amino acid substitutions from a computational tool developed by Massimo G. Totaro [8].



Basis for the LOV-activated diguanylate cyclase switch mechanism. Crystal structure (**A**) of a LOV-GGDEF homolog and its predicted AlphaFold model (**B**) showing a coiled-coil sensor-effector linker. The dark, inhibited conformation (**A**) is characterized by a tight association of the sensory LOV and catalytic GGDEF domains in the dimeric assembly, which prevents the productive encounter of GGDEF domains as required for catalysis. The efficient caging of the effector domains is only released upon structural rearrangements induced by blue light illumination (**C**).

Furthermore, diguanylate cyclases can be regulated by a variety of other stimuli. Several complex domain architectures like LOV-GGDEF-EAL or Rec-LOV-GGDEF can be found in nature. Studying these systems might offer insights into the molecular logic of sensor-effector systems with multiple input or output functionalities (PhD project by Maximilian Fuchs). Our current focus lies on studying Rec-LOV-GGDEF systems, referred to as dual-sensor diguanylate cyclases. Unlike the light-regulated LOV domain, phosphorylation of the Rec domain provides an additional layer of complexity that could result in complex molecular logics resulting in on how active or inactive conformations of the effector are regulated. Various methods, including in-house mass spectrometry and Phos-Tag gels, are employed to confirm the phosphorylation status of the receiver domain. In vitro, phosphorylation of our model system appears feasible only for the isolated Rec domain. This led us to investigate the influence of the coiled-coil linker between Rec and LOV domains on phosphorylation inhibition in the full-length protein. With few dual-sensor systems characterized, understanding the tightness of their regulation remains challenging. Hence, our focus is on deciphering the molecular mechanisms of signal processing in these proteins, aided by structural data obtained through X-ray crystallography. Promising preliminary data has been obtained for Rec-only and Rec-LOV truncation constructs. The dimer interface observed in the Rec-LOV construct is not present in the stand-alone Rec domain. Therefore, the conformational changes in the receiver domain upon phosphorylation could be inhibited by the rigid dimer interface seen in the Rec-LOV structure. Insights into cooperative domain interplay could facilitate the development of molecular tools for implementing additional regulatory networks in optogenetics.



Distinct dimer interfaces within the receiver domain of a dual-sensor diguanylate cyclase. The crystal structure revealed that the dimer interface of the receiver domain varies depending on the adjacent domain. In the structure of the Rec-LOV truncation (A) a two-fold symmetry between the protomers is observable. For the Rec truncation (B) the second protomer is turned upside down.

[5] Losi & Gärtner: Solving Blue Light Riddles: New Lessons from Flavin-binding LOV Photoreceptors (2016) ***Photochem. Photobiol.***, 93, 1, 141-158

[6] Pudasaini *et al.*: *LOV-based optogenetic devices: light-driven modules to impart photoregulated control of cellular signaling* (2015) ***Front. Mol. Biosci.***, 2, 18

[7] Vide *et al.*: *Illuminating the inner workings of a natural protein switch: Blue-light sensing in LOV-activated diguanylate cyclases* (2023) ***Sci. Adv.***, 9, 31, eadh4721

[8] Totaro et al.: *ESM-Scan-a tool to guide amino acid substitutions* (2023) ***bioRxiv***, DOI: 10.1101/2023.12.12.571273

Methodological developments and mass spectrometry-based services offered

HDX-MS: With the acquisition of a high-resolution time-of-flight mass spectrometer in 2021, the foundations were set to establish the technique of hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) in Graz. As one complementary structural analysis technique in the field of integrative structural biology, the idea was to complement existing expertise in Graz and to also offer this methodology to other groups interested in addressing conformational dynamics in proteins. Insights into dynamics and secondary structure stability in different functional states of a protein can provide valuable information as to which regions are involved in a variety of biological processes. The first systems to be characterized included processes like allosteric regulation (light regulation – see above), biomolecular interactions (together with Monika Oberer, UG), or immobilization on carriers (collaboration with Bernd Nidetzky).

Native MS: Another powerful bioanalytical technique that can be performed in our laboratory is native mass spectrometry. Addressing the stoichiometry of protein-protein interactions is frequently an important aspect of a functional characterization of biomolecular complexes. Standard methodologies to address the molecular weight of intact species, such as size-exclusion chromatography, are frequently flawed by the variability of protein structures that often deviate from the spherical approximations used for the mass calibrations. Due to the accuracy of modern mass spectrometry-based techniques, native MS can serve as a powerful alternative to other light scattering-based absolute mass determination techniques, for example, multi-angle light scattering.

Intact mass measurements: An additional standard technique for an initial characterization of newly expressed proteins is the confirmation of its exact mass. Based on this, heterogeneity during sample preparation, amino acid substitutions and/or modifications can be readily identified. Considering the famous 4th commandment of enzymology „Thou shall not waste clean thinking on dirty proteins“ (Kornberg, TiBS, 2003), precise knowledge of what is present in your sample preparation can save valuable time in unnecessary follow-up experiments.

Master thesis completed

Regina Zausinger: *Functional characterization of LOV-activated diguanylate cyclase variants addressing the GGDEF inhibitory site*

LOV-activated diguanylate cyclases (LadCs) are proteins, which are composed of two domains. An effector domain for the formation of 3',5'-cyclic dimeric guanosine monophosphate (c-di-GMP) and a sensor domain to perceive blue-light. c-di-GMP functions as a second messenger modulating certain intracellular pathways in bacteria. These pathways include for example the cell cycle, the regulation of biofilm formation and it is responsible for the dispersion, motility and virulence of bacteria. Therefore, functioning as a key component through regulating cellular responses regarding the lifestyle of several bacterial species.

The enzymatic activity of the effector GGDEF domain is controlled by the sensory LOV domain, which has a blue light sensitive flavin chromophore incorporated in its structure. This chromophore is non-covalently bound within the domain in the enzymes inactive state (dark-state). As soon as it is exposed to blue light, a highly reactive triplet state flavin is formed. The co-factor then binds covalently to a conserved cysteine within the LOV domain. This leads to a structural rearrangement of both domains and enables the formation of c-di-GMP by the effector domain. For the successful production of c-di-GMP, the domain, and therefore the whole enzyme, needs to adapt a dimeric conformation to bring two substrate molecules of GTP into close proximity. To maintain a controlled catalysis of the second messenger, DGCs contain a conserved inhibitory site (I-site). This I-site can be recognised via an RxxD sequence motif, which is present in roughly 50 % of all sequenced DGCs and binds c-di-GMP allosterically. This allosteric inhibition prevents futile conversion of GTP to c-di-GMP.

The aim of this master thesis was to get a better insight in the enzyme´s light-induced, activated confirmation and to characterize it in more detail. This was implemented by trying to eliminate the allosteric inhibition and disrupting the inhibitory interface, by substituting the conserved arginine and aspartic acid with other amino acids. The residues for the substitution were either chosen from previous publications on related GGDEF proteins or via an in-house prediction tool for amino acid substitutions.

The results have shown, that two tested LadC variants addressing the I-site exhibit a two-fold faster recovery mean lifetime, whereas other variants almost took twice as long when compared to the wildtype. Enzyme kinetic analysis revealed a significant decrease in cyclase activity upon illumination, a reduced product formation and an accelerated allosteric inhibition. Moreover, the fold-change of all active variants was reduced considerably. Therefore, a successful elimination of the allosteric inhibition could not be achieved for any of the tested variants. The results suggest that other important parts, besides the I-site, of the enzymes conformation might have gotten altered, which resulted in the observed changed characteristics.

International cooperations

Jon Hughes, Justus-Liebig-University Giessen, Giessen, Germany

Research projects

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

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

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

Talks at national and international conferences/meetings

1. Vide, U.: *LOV-activated diguanylate cyclase: a high dynamic-range switch for optogenetics*. Hot Topics in Contemporary Crystallography 5, Dubrovnik, Croatia, April 2023.
2. Eder, O.: *Developing novel red light sensitive protein dimerization tools. 2*nd Scientific Retreat - BioMolStruct, Seggau, Austria, June 2023.
3. Fuchs, M.: *Dual sensor diguanylate cyclases. 2*nd Scientific Retreat - BioMolStruct, Seggau, Austria, June 2023.
4. Tran, Q.H.: *Analysis of conformational dynamics of red/far-red light activated bacteriophytochromes*. 29th Doc-Day of the Doctoral School of Molecular Biomedical Sciences and Biotechnology, Graz, Austria, July 2023.
5. Vide, U.: *Characterization of Sequence-Structure-Function Relationships in Phytochrome Regulated Diguanylate Cyclases*. 20th Congress of the European Society for Photobiology, Lyon, France, August 2023.
6. Fuchs, M.: *Dual Senor Diguanylate Cyclases.* Advanced Methods in Ambient Crystallography at ESRF-EBS, Grenoble, France, November 2023.
7. Winkler, A.: *Signal integration in dark- and light-state "locked" bacteriophytochrome variants.* SFB 1078 International Phytochrome Workshop, Berlin, Germany, October 2023.

Publications

1. Vide, U., Kasapović, D., Fuchs, M., Heimböck, M.P., Totaro, M.G., Zenzmaier, E., Winkler, A.: *Illuminating the inner workings of a natural protein switch: Blue-light sensing in LOV-activated diguanylate cyclases* (2023) ***Sci. Adv.*** 9, 31, eadh4721
2. Hughes, J., & Winkler, A.: *New insight into phytochromes: Connecting structure to function* (2024) ***Annu. Rev. Plant. Biol.***, *in press*, DOI: 10.1146/annurev-arplant-070623-110636
3. Totaro M., Vide, U., Zausinger, R., Winkler, A., Oberdorferg, G.: *ESM-Scan-a tool to guide amino acid substitutions* (2023) ***bioRxiv***, DOI: 10.1101/2023.12.12.571273
4. Tran, H., Eder, O., Winkler A.: *Dynamics-driven allosteric stimulation of diguanylate cyclase activity in a red light regulated phytochrome* (2024) ***J. Biol. Chem.***, *in press*, DOI: 10.1016/j.jbc.2024.107217

Protein Design Group

Group leader: Gustav Oberdorfer
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PhD students: Markus Braun, Veronica Delsoglio, Wael Elaily, Julia Messenlehner, Anna Schrüfer, Massimo Totaro, Adrian Tripp, Florian Wieser
Master students: Melanie Moser, Henrik Seyfried
Bachelor students: Amelia Rieder, Morakot Chakatok, Nikolina Karpic
Technical Staff: Alma Makic, David Stoll, Morakot Chakatok, 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.

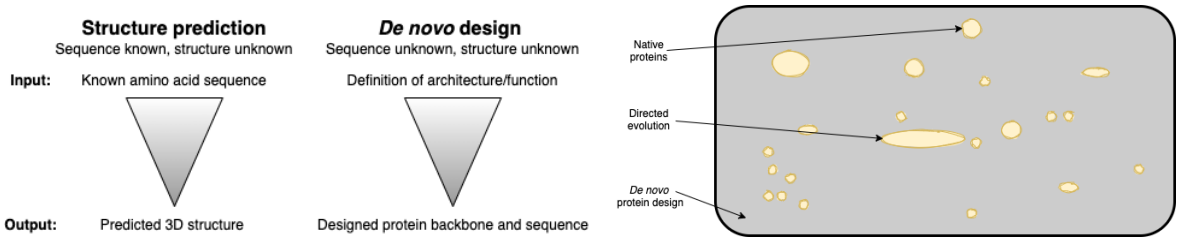


Figure (left) *Differences in structure prediction and de novo protein design* ***(right)*** *Illustrative representation of protein sequence space (grey). Sequence analysis of native proteins shows that tight clusters of protein families can be identified (beige).*

Functionalizing helical *de novo* proteins by deviating from ideal geometries

Coiled-coils, a particular group of protein structures, has seen big advances in terms of design over the last several years. These usually parallel and oligomeric protein assemblies present ideal targets for protein design studies, as they are very regular and follow a repeating sequence, which, in the canonical case, is seven residues long. We could show that it is possible to design genetically encoded, single chain helix bundle structures with atomic level accuracy. To do so, a novel method that uses equations, originally derived by Francis Crick in 1953, which accurately describe the geometries of α-helical protein structures, was established, and used to sample the folding space of helical proteins computationally. The resulting designed proteins were highly idealistic in terms of geometry and showed very high thermodynamic stability (extrapolated $\Delta G_{fold} > 60$ kcal mol^{-1}), with their experimentally determined structures close to identical to the design models and nearly perfect packing of amino acid side chains between the helices. However, it is obvious that in nature, most protein functional sites sit at the end of structural elements or in unstructured regions and therefore are not placed at positions of ideal protein geometry. It has been shown that this can be a result of selective pressure, where the ancestral proteins had more regular structural elements, exhibited higher thermodynamic stabilities and less dynamics, in comparison to their contemporary versions. This is why it is still unclear if idealized protein structures can be functionalized.

In order to address this question, research in the Oberdorfer group is focusing on designing large proteins with topologies not observed in nature. Key elements we hope to find with these studies are whether they exhibit similar rigidity and stability as observed for the small ideal proteins we designed previously. We are also investigating to which extend we can harness, or trade some of the very high thermodynamic stability of these parametrically designed helical bundles to introduce deviations from ideal geometry for the gain of catalytic function. To test different levels of deviation from ideal coiled-coil geometry in helical backbones and to check if this is concomitant with reduction of thermodynamic stability, functional sites of various sizes have been chosen. In particular, the lab is working on metal complexation and cofactor binding (Figure 2). The ability to sample hundreds of thousands of potential protein backbones, which can be used as starting points to introduce catalytic or ligand binding sites into *de novo* designed helical proteins is a big advantage over previous attempts in designing functional proteins. Initial results from this research shows that there might be a tradeoff between high stability and degree of idealism as far as the protein backbone is concerned, however, many more designs have to be made and characterized before we can draw definite conclusions. In answering these questions though, we hope to pave the way for downstream applications of *de novo* protein design to biocatalysis, biomedical and biotechnological problems.

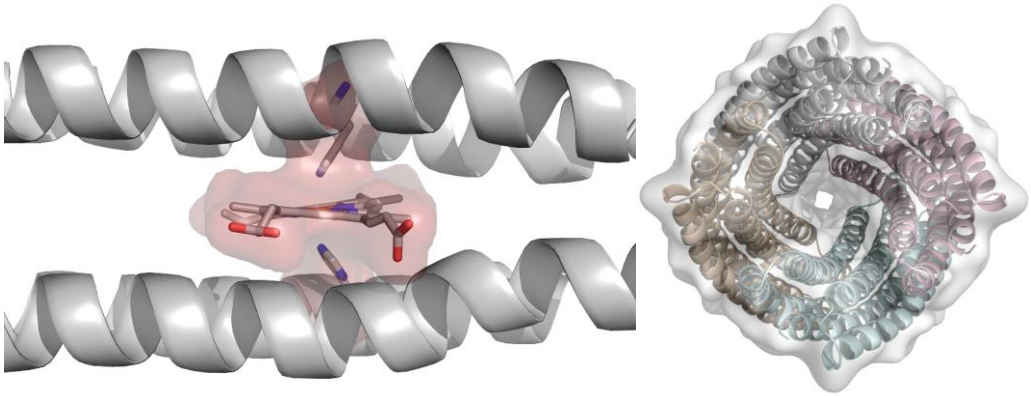


Figure 2. (left) *Computationally designed 4-helix bundle with a designed binding site for b-type heme. It can clearly be seen how much the otherwise straight helices had to be bent, to accommodate the heme cofactor.* ***(right)*** *top view of a computationally designed 20-helix bundle. This is a completely unknown topology to nature.*

Doctoral Thesis completed

Veronica Delsoglio: *Computational design of dimeric de novo heme-binding helical bundles*

Proteins mediate the fundamental processes of life and have been the focus of much biomedical research for decades. Indeed, protein-based materials can solve a vast array of technical challenges in biotechnology and biomedicine. Recently, advances in the field of computational protein design have shown that the small-molecular simulation can help to predict sequences with new and improved functions (*de novo* protein design). Moreover, the computational methodology has advanced to the point that a wide range of structures can be designed from scratch with atomic-level accuracy. One major remaining challenge in de novo protein design is small-molecule induced homo-oligomer formation. To investigate and potentially solve this design challenge, I am working on homodimeric bundles of three □ helices – homodimeric TH_3H5L_4_3-3 and homodimeric TH_3H5L_4_3-4. These two designs are unique in their amino acid composition and are constituted by two antiparallel, straight (18-residue repeat units) three-helix bundles (3H5L_2) arranged around a two-fold symmetry axis. Moreover, they are coordinated to heme via a histidine residue that points towards the homo dimer interface. The monomeric structure, 3H5L_2, was designed using parametric equations first derived by Francis Crick in 1953. This approach has gained a lot of attraction for peptide design efforts due to its simplicity and the regularity of the generated designs. The aim of the project is to optimize and fully characterize the generated designs biochemically, biophysically and structurally. RosettaScripts, as implemented in the Rosetta Macromolecular Modelling suite, is used for all design approaches. Several rounds of designs are being tested. This includes cloning of the respective genes into expression plasmids and protein expression and purification in *E. coli* Biophysical and structural characterization is performed by means of FPLC-SEC, SEC-MALS, UV-visible spectroscopy, CD-spectroscopy, DSF spectroscopy, small angle X-ray scattering (SAXS) and X-ray crystallography. The biophysical results confirm the system´s thermo- and chemical stability. The structural studies prove the correct fold of the designs and their capability to specifically bind b-type heme. The HIS side chains are found to play an essential role in coordinating the ligand, even if other residues interact with the porphyrin ring. The truncated structures, generated after several cycles of protein design-optimisation/production, are obtained in high yields, and display the biochemical features of interest. They represent the starting point for further studies with the goal to extend the bundle´s length and allow for multiple bis-HIS heme coordination events. In that way, they can be used as scaffolds for electron transfer mechanism.

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

Anabella Ivancich, Directeur de Recherche CNRS, CNRS et Aix-Marseille Université, France

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

Sarel Fleishman, Department of Biomolecular Sciences, Weizmann Institute of Science, Israel

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

Talks at national and international conferences/meetings

1. Oberdorfer, G.: *Ideal is pretty, but irregular more interesting* - functionalization of parametrically designed helix bundles by deviating from ideal geometries. Designed Metalloproteins, July 2023, Paris, France
2. Oberdorfer, G.: *Ideal is pretty, but irregular more interesting* - functionalization of parametrically designed helix bundles by deviating from ideal geometries. Alpbach Meeting on Coiled Coils, Fibrous and Repeat Proteins, September 2023, Portorož, Slovenia
3. Oberdorfer, G.: *Ideal is pretty, but irregular more interesting* - functionalization of parametrically designed helix bundles by deviating from ideal geometries. European Rosettacon, September 2023, Leipzig, Germany
4. Oberdorfer, G.: *Ideal is pretty, but irregular more interesting* - functionalization of parametrically designed helix bundles by deviating from ideal geometries. COZYME-Network Meeting, September 2023, online
5. Oberdorfer, G.: Practically useful: application driven protein design to tackle challenges of the 21st century. Biophysics Seminar, October 2023, Graz, Austria

Publications

1. Totaro, M. G., Vide, U., Zausinger, R., Winkler, A., Oberdorfer, G. ESM-Scan - a tool to guide amino acid substitutions (2023) ***bioRxiv*** 2023.12.12.571273, DOI:10.1101/2023.12.12.571273
2. Wirnsberger, G. Pritišanac, I., Oberdorfer, G., Gruber, K. Flattening the curve - How to get better results with small deep-mutational-scanning datasets (2023) ***Proteins*** accepted

3. Braun, M., Gruber, C. C., Krassnigg, A., Kummer, A., Lutz, S., Oberdorfer, G., Siirola, E., Snajdrova, R. Accelerating Biocatalysis Discovery with Machine Learning: A Paradigm Shift in Enzyme Engineering, Discovery, and Design. (2023) ***ACS Catal.***, Oct 26, 13(21), 14454-14469. DOI:10.1021/acscatal.3c03417

4. Bernauer, L., Berzak, P., Lehmayer, L., Messenlehner, L., Oberdorfer, G. Zellnig, G., Wolinski, H., Augustin, C., Baeck, M., Emmerstorfer-Augustin, A. Sterol interactions influence the function of Wsc sensors. (2023) ***JLR,*** 64(12), 100466. DOI:10.1016/j.jlr.2023.100466

5. Sjöström, D. J., Grill, B., Ambrosetti, E., Alikkam Veetil, A., Mohlin, C., Teixeira, A. I., Oberdorfer, G., Bjelic, S. Affinity Maturated Transferrin Receptor Apical Domain Blocks Machupo Virus Glycoprotein Binding. (2023) ***J. Mol. Biol.***, 435(20), 168262, DOI:10.1016/j.jmb.2023.168262.

BioTechMed YoungResearcherGroup “Enzyme Design”

Group leader: Horst Lechner
PhD students: Sajith Kolathuparambil
Technical Staff: Ahmed Alagic

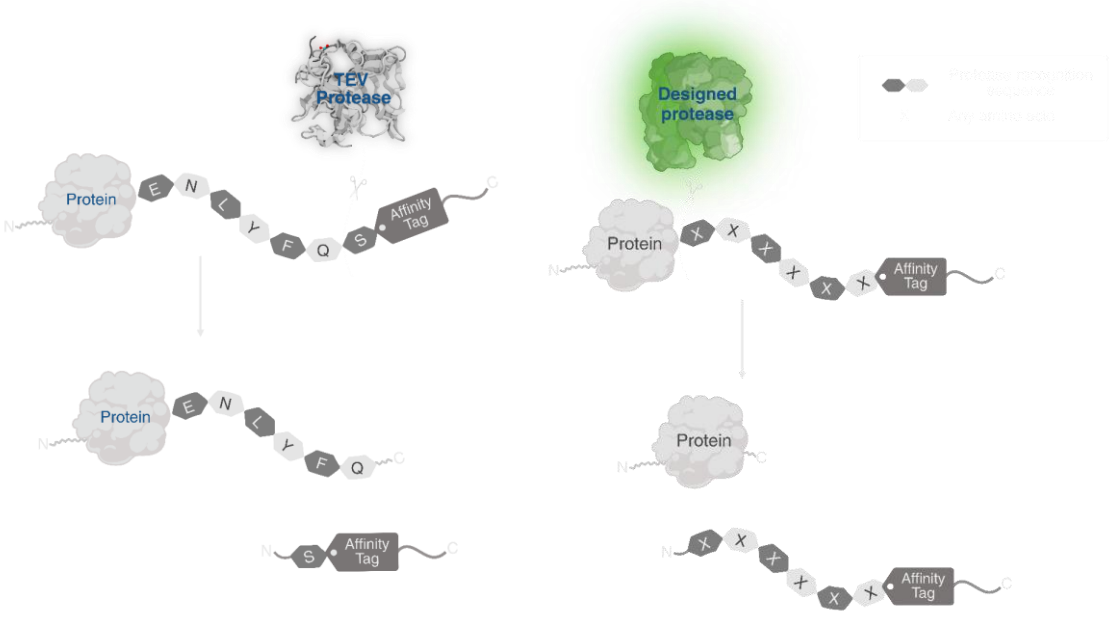
General description

Enzyme engineering is often characterized by its tedious, time-consuming and labor-intensive nature. Computational enzyme engineering and *de-novo* design can be supportive to increase the success rates and speed up the process. One of the reasons for recently grown interest to apply more computational methods in this area is a consequence of the recognition that AI methods can be applied to many challenges in this field and new, powerful methods were developed over the last few years. We use those programs to design new enzymes or redesign existing ones to change their properties.

Design of new proteases

Efficient recombinant protein purification is pivotal in biological research as well as in biotechnology, often relying on affinity tags. However, complete removal of C-terminal tags is problematic since most endoproteases cut towards the C-terminal end of their recognition sequence. Nonetheless, the additional residues on the recombinant protein from the protease recognition sequence after protein tag removal can be detrimental to the proper folding, activity or crystallization of the target protein.
We want to develop a sequence-specific proteases tailored for C-terminal tag removal without leaving any residues from the recognition sequence, a challenge unmet by currently used proteases. Therefore, we harness the power of *de novo* computational design to create new protein backbones using RFDiffusion (Watson et al., 2023), harboring a catalytically active fragment of a known protease and a binding cleft for the target sequence. ProteinMPNN (Dauparas et al., 2022) is used to derive sequences for these backbones.
A FACS-based screening method will be used to select and further improve the *de novo* proteases. (Denard et al., 2021) Our preliminary computational results show a gradual improvement of the designed models, which signifies the efficiency of our pipeline.

Computational enzyme design is advantageous over conventional directed evolution techniques in developing proteases with customized applications, and our approach will open a new scope for developing proteases for a wide range of applications.



Enzyme design approach. Left: Currently available proteases (as example the Tobacco Etch Virus (TEV)-protease) cleave after their recognition site leaving their recognition sequence on the target protein. Right: Proposed protease cleaving before its recognition sequence.

Research projects

BioTechMed YRG: “Active site transfer to generate new enzymes”

Talks at national and international conferences/meetings

1. Lechner, H.: Proteins binding Dyes meet Light-Emitting Diodes, European Rosettacon: Protein design in the age of artificial intelligence, Leipzig, Germany, Sept 2023

Chemistry of Functional Foods

Group leader: Michael Murkovic
PhD students: Monika Grasser
Master students: Iris Stalzer, Zehra Gromilic, Monika Kozlowska, Vildana Fazlic, Zerina Duhovic, Franz Lindbichler
Technical staff: Claudia Hrastnik, Lena Pfeiffer

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 and specific non-canonical amino acids – as metabolites in edible mushrooms – are investigated. These cyclic peptides can have specific physiological properties with a positive effect on e.g. malaria. Especially ergothioneine which is an antioxidant amino acid being actively transported from the gut to the circulation can reach high concentrations in the blood.

Master Theses completed

Zehra Gromilic: *Development and validation of a chromatographic method for analysis of calystegines isolated from potatoes*

The structure of calystegines might relate to substances that are potential glucosidase inhibitors. These inhibitors can be used to reduce the post prandial glucose blood levels and thereby reduce the consequences of diabetes. These could also be used for prevention and therapy of diabetes Type II.

The incidence of type-2-diabetes mellitus is constantly increasing with a concomitant development of respective therapies. The aim of this study was to isolate calystegines from potatoes and use these substances as analytical references for determination of the concentration in the foods. The calystegines had to be isolated and purified and analyzed by HPLC and TLC. For detection after TLC separation a derivatisation using alkalinized $AgNO_3$.

It could be shown that calystegines are present in the potato plant and especially in the roots and the tubers. Several orthogonal analytical methods were developed for identification and quantification. The derivatisation was developed using amino sugars as model compounds.

Franz Lindbichler: *Apple quality in relation to climate change*

Global warming and climate change affects us more every year. Recent reports disclose an increase of the global average temperature by 1 °C since the 1950s. Moreover, weather related catastrophes got more frequent over the last 50 years. Naturally, research pivoted more towards future climate predictions, its consequences and countermeasures against a further increase in global temperature. Another object of concern is the reduced availability and access to food. While the quantity of food is of supreme importance, its quality should also not be neglected. Therefore, this master thesis aimed at finding a possible correlation between different climate factors and their effects on the quality of apples, which was determined by their polyphenol concentration. To simulate climate change, Crimson Crisp and Topaz apples were harvested once a week, from early August to late October in 2021 and 2022, from 3 locations in Austria, which were in 3 different climate regions (G.1, A.2, A.5). The apple samples were freeze-dried, and the total polyphenol content measured with a Folin-Ciocalteu assay, while individual polyphenol concentrations were measured with HPLC-DAD. Total polyphenol concentration was highest in apples from the most northern location (3.7 µg/mg dw) and became lower in more southern regions of Styria (3.4 and 3.2 µg/mg dw). Individual polyphenols behaved the same in this regard. It was also possible to detect a decrease of the polyphenol concentration over time. The results from HPLC-DAD and Folin-Ciocalteu measurements were then correlated with weather data through principal component analysis. Cluster plots showed that weather factors are unlikely to be correlated to the polyphenol concentration found in apples. Additionally, a negative correlation between (-)-epicatechin and the other measured polyphenols, phlorizin, (+/-)-catechin, chlorogenic acid and p-coumaric acid was found.

Elena Maria Maggauer: *Changes in Human Milk Oligosaccharides in Maternal Serum during Oral Glucose Tolerance Test (OGTT) and Associations with Maternal Glucose Metabolism*

Human milk oligosaccharides (HMOs) are structurally diverse glycans in breastmilk with known health benefits for the breastfed infant and the mother. HMOs are also present in maternal serum during pregnancy. Previous studies suggest an impact of HMOs on the regulation of maternal glucose metabolism. Whether transient hyperglycemia as can be observed in an oral glucose tolerance test (OGTT) also might have an influence on HMO serum concentrations is unknown. Hypotheses: We hypothesized that glucose load directly affects HMO concentrations in maternal serum in an OGTT. Further, we hypothesized that serum HMO concentrations vary with maternal glucose and insulin metabolism. Methods: Serum samples were available at three time points (0 h, 1 h and 2 h) during a 75 g OGTT conducted at 24-28 weeks of gestation from a cohort of 120 healthy women. HMOs were isolated by solid phase extraction, fluorescently labeled with 2-aminobenzamide and separated by HPLC using a linear gradient. Associations of HMOs with maternal metabolic parameters were analyzed by Spearman correlation. Results: We quantified the five most abundant HMOs (2'FL, LDFT, 3'SL, 3'SLN and 6'SLN) in serum. 3'SL showed a significant increase upon glucose load during an OGTT. Correlation analyses revealed significant associations of sialylated HMOs, in particular 3'SL, with glucose and C-peptide levels at all three time points during OGTT. We found many associations with C-peptide, but hardly any with insulin. 3'SL concentration was associated with HOMA-IR C-peptide index, Matsuda C-peptide index and C-peptide index 1, estimating insulin sensitivity and β-cell function. 3'SLN concentration was positively associated with insulin clearance at fasting state and with the respective area under the curves (AUCs). 3'SL and 3'SLN concentrations, in particular one-hour post glucose load, were positively correlated with BMI (prepregnancy and delivery) and SAT thickness. Conclusion: This is the first study to examine potential changes in maternal serum HMO concentrations during the OGTT. Our result that HMO concentrations change upon glucose load suggest that future studies might consider potential postprandial effect on HMOs and might be performed at fasting state. Further research is needed to determine whether the change in HMO concentration is due to a rise in plasma glucose, or whether hormones or other mediators contribute.

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.: *Recent results on heat induced carcinogens – formation of furfuryl alcohol during roasting of coffee.* Euro Food Chem, Belgrade.

Publications

1. Fojnica, A., Gromilic, Z., Vranic, S. & Murkovic, M.: *Anticancer Potential of the Cyclolinopeptides* (2023) ***Cancers***. 15, 13, 3874
2. Kratzer, R. & Murkovic, M.: *Microalgae in nutrition.* (2023) ***Universum Innere Medizin*** 9, 4.
3. Oz, E., Aoudeh, E., Murkovic, M., Toldra, F., Gomez-Zavaglia, A., Brennan, C., Proteos, C., Zeng, M. & Oz, F.: (2023) Heterocyclic aromatic amines in meat: Formation mechanisms, toxicological implications, occurrence, risk evaluation, and analytical methods (2023) ***Meat Science***. 205, 19, 109312
4. Kukurova, K., Ciesarova, Z., Jelemenska, V., Orolinova, M., Duhovic, Z., Kobincova, J., Dubova, Z., Horvathova, J., Tobolkova, B., Murkovic, M. & Siegmund, B.: *Qualitative parameters of monoflora honeys from Slovakia and Austria and the role of water activity* (2023***) Journal of Microbiology, Biotechnology and Food Sciences***. 13, 1, e9938.
5. Wang, Y., Kratzer, R., Murkovic, M., Eibinger, M., Machado Charry, F. E., Li, S., Zhang, T., Zhang, X., Zhang, M. & Chen, H.: Fabrication and characterization of a novel zein/pectin/pumpkin seed oil Pickering emulsion and the effects of myricetin on oxidation stability (2023) ***International Journal of Biological Macromolecules***. 253, 7, 127386.

Conference / workshop organized

1. *Food chemistry updated*, on-line lecture series

Lectures and Laboratory Courses

Winter Semester 2022/23

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.832_1UF	Project Laboratory	9	LU	Team
MOL.844_1UF	Journal Club Biochemistry and Molecular Biomedicine	1.5	SE	Team
MOL.845_1UF	Master Thesis Seminar	2	SE	PIs
MOL.855UF	Molecular Physiology	2	VO	Lorber B, Macheroux P
MOL.881UB	Biophysical Methods	2	SE	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	PV	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 2023

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)	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
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, …
648.016	Chemical Reactions in Foods II	2	PV	Murkovic M
648.022	Structural Biochemistry II	2	PV	Winkler A

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