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Brief History of the Institute of Biochemistry

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

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

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

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

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

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

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

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

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

1993 W. PFANNHAUSER was appointed as professor of food chemistry. A few years later he was elected head of the newly established Institute of Food Chemistry & Technology

2001 After Prof. PALTAUF's retirement, in September 2001, G. DAUM was elected head of the institute.

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.

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

2009 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 revolve around the development and metabolism of adipocytes.

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

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

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

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

2017 J. BOGNER-STRAUSS was sworn in as Minister for Women, Families, and Youth of the Austrian government at the end of 2017 and, after the end of the coalition in mid-2019, continued her political career at the provincial government of Styria 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

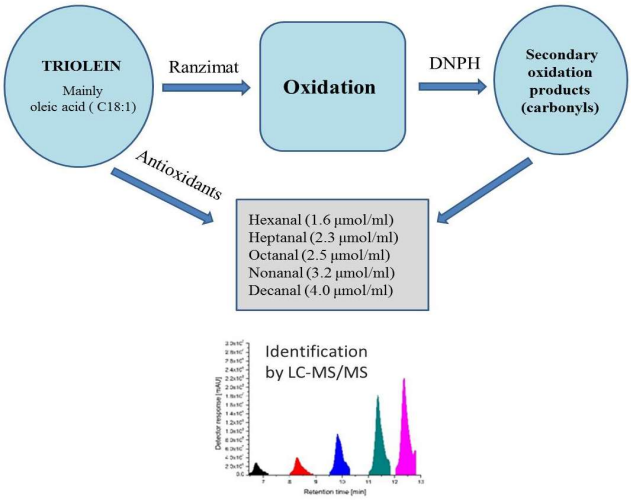
Highlights of 2020

In 2020, the collaboration with the groups of Robert Zimmermann and Karl Gruber at the University of Graz to unravel the physiological role of dipeptidylpeptidase 3 was crowned by a publication in the Journal of Biological Chemistry. The study, mainly conducted by joint first authors Drs. Shalinee Jha and Ulrike Taschler, achieved a major breakthrough in our understanding of the enzyme's role in health and disease.

We have also engaged in a collaboration with the group of Prof. Martinez at the Max Perutz Laboratories in Vienna to characterize the role of the recently discovered mammalian flavoprotein PYRDOX1. During several visits to our laboratory, Igor Asanovic and Dr. Strandback discovered several important properties of PYRDOX1 that turned out to be essential for an understanding of the flavoprotein in the tRNA ligase complex (paper under revision in Molecular Cell).

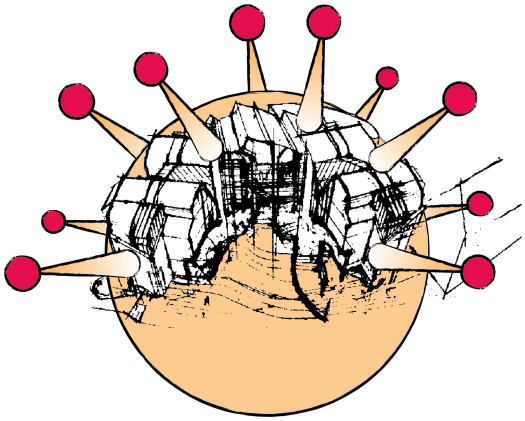
The group of **Gustav Oberdorfer** welcomed a new postdoc in 2020. Horst Lechner started in April. He is the lead scientist on the FET-Open project 'ARTIBLED'. Besides him, the Oberdorfer group grew by two more PhD students. Adrian Tripp, a former masters student stayed with the group to continue working on his doctorate and Massimo Totaro started as a joined PhD student between the Oberdorfer and Winkler groups.

In the group of **Andreas Winkler** several new students started their PhD. **Ursula Vide** followed up on her promising MSc results in the blue-light regulated cyclases field and **Hieu Tran** started to work on the asymmetric red-light activated cyclases. **Massimo Totaro** (joint with AGO) is working on computational aspects of phytochrome engineering for applications in optogenetics. With many new students the Corona restrictions were quite challenging for getting all the projects up and running.



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

The year 2020 was of course not only providing highlights, but also many challenges. Lock-downs followed by partial lock-downs and alternating shifts systems for working at the institute were demanding for all members of the institute. Teaching and research efforts were complicated by the regulations imposed by the government and the university, but in the end helped to keep our institute Corona-free for all 2020. In this respect, at least, this was another highlight and we are all hoping that 2021 will soon bring back some more routine working conditions for all students and permanent staff.



(2019)

Biochemistry Group

Group leader: Peter Macheroux
Secretary: Christine Rother
Senior research scientist: Silvia Wallner
Postdoctoral fellows: Alexandra Jammer, Shalinee Jha
PhD students: Reinmar Eggers, Katharina Fuchs, Bettina Hierzberger, Bianca Kerschbaumer, Sami Ullah Khan, Grazia Malovan
Master students: Stefanie Baldauf, Alexandra Csamay
Technical staff: Eva Maria Frießer, Rosemarie Trenker-El-Toukhy

General description

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

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

Berberine bridge enzyme-like enzymes in plants

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

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

To further unveil the role of BBE-like enzymes, we have teamed up with Professor Dr. Tomas Werner from the Institute of Biology at the University of Graz to investigate the *in planta* functions of the monolignol oxidase sub-family of the *Arabidopsis* BBE-like enzymes (*AtBBE-like 13, 15, 24, 25, 26*). Gene expression in all types of plant organs via qPCR has been carried out for three of the genes (*AtBBE-like 13, 15, 26*) and is currently underway for *AtBBE24* and *AtBBE25*. In addition, GUS reporter lines have been generated to investigate gene expression patterns on the tissue level throughout the plant's life cycle. GUS analyses have largely been completed, and GUS staining patterns have provided valuable information and will serve as basis for the design of further experiments. Furthermore, we are working on the generation of single and multiple knockout lines for our genes of interest. Several T-DNA insertion mutants were ordered from the Nottingham Arabidopsis Stock Centre, and homozygous single knockout lines for *AtBBE-like 13* and *15*, as well as a homozygous double knockout line for the two genes, have been generated. A CRISPR/Cas9 approach is currently underway to generate single, double, and triple knockouts for *AtBBE-like 24, 25,* and *26,* as these genes are located in close proximity on the same chromosome and the individual mutations can, thus, not be combined in a multiple knockout line via crossing approaches. A detailed phenotyping of the single and multiple knockout mutants, combined with the results from the expression analyses (qPCR, GUS plants) and the results of biochemical and physiological studies, will provide the basis for the elucidation of the *in planta* functions of the sub-family members (thesis project of Reinmar Eggers supported by Dr. Alexandra Jammer).

Dipeptidylpeptidase 3

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

Despite the intriguing involvement of DPP3 in metabolic disease pathogenesis, the underlying physiology of this enzyme is poorly understood. In collaboration with Prof. Dr. Robert Zimmerman and Prof. Dr. Karl Gruber from the University of Graz, Assoc. **Prof.** Dr. Tobias Madl and Assoc. Prof. Sasa Frank at the Medical University of Graz and Attoquant Diagnostics in Vienna, we uncovered that DPP3 regulates the RAS pathway and water homeostasis by degrading circulating angiotensin peptides. Characterization of a mouse model

lacking DPP3 at the genetic and proteomic level showed that this enzyme is associated with heightened water consumption, equilibrium angiotensin peptide levels and renal oxidative stress in mice. Furthermore, we established that DPP3 plays a sex-specific role, where only the male mice presented the phenotype on deletion of DPP3. The female mice were protected from the deleterious effects of DPP3 knockout, indicating a link between the endocrine system and the physiological role of DPP3. The characterization of DPP3 in this study established that it has strong metabolic implications through the modulation of the RAS pathway, a property that could be useful in the management of several cardiovascular and related pathologies. Currently, we are investigating the underlying mechanisms involved in the regulation of these physiological functions by DPP3 using the knockout mouse model and mammalian cell culture. In addition, we are also identifying specific inhibitors of this enzyme through virtual screening and evaluating the obtained hits *in vitro* for their binding affinity to the enzyme as well as their inhibitory properties.

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

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

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

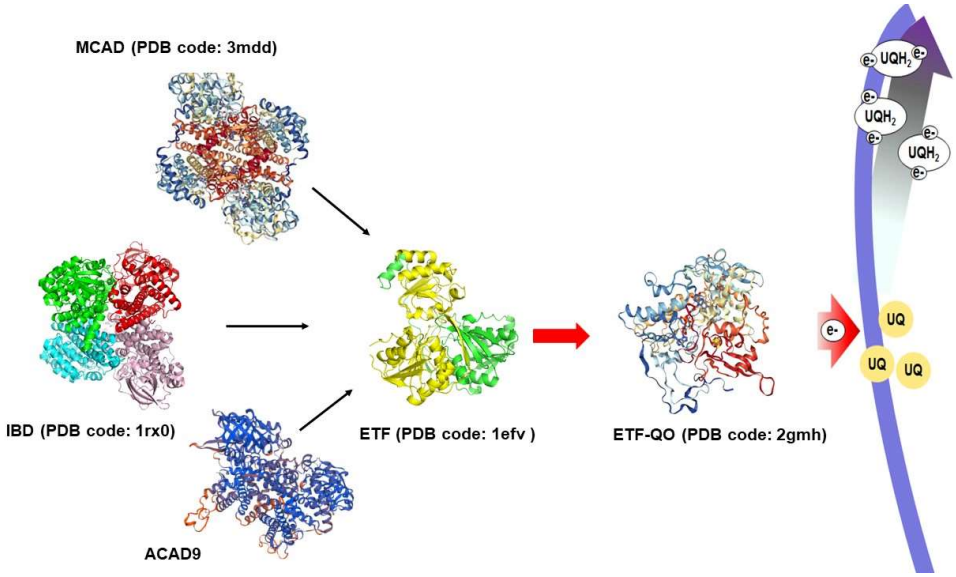
In order to study the oxidative cyclization in chanoclavine biosynthesis and the involved enzymes and cofactors, we will have a closer look at EasE – a flavin adenine dinucleotide (FAD) dependent homologue of the berberine bridge enzyme-like (BBE) enzyme, – and EasC – a heme dependent catalase – originating from *Aspergillus japonicus*. CnsA (BBE-like) and CnsD (a putative catalase) will be also investigated, which represent a similar model in *Penicillium expansum*. The primary goal will be the production of the active enzymes by recombinant biotechnology. Furthermore, the obtained proteins will be characterized with regard to their kinetic parameters and their substrate scope. Eventually, the enzymes will be employed to explore their utility in producing derivatives of the fungal alkaloid chanoclavine (thesis project of Bianca Kerschbaumer).

Flavin-dependent electron transfer in human mitochondria

Electron transferring flavoproteins are found in a variety of eukaryotic and prokaryotic organisms including humans. Human electron transferring flavoprotein (hETF) is involved in the oxidative energy metabolism by accepting electrons from at least fourteen different flavin dehydrogenases and passing them on to the mitochondrial respiratory chain. In previous studies by Drs. Peter Augustin and Marina Toplak it was discovered that the flavin cofactor of hETF can undergo a pH-dependent and irreversible oxidation leading to the formation of an 8-formyl-FAD (8fFAD) moiety.

ETFs are heterodimeric proteins, where extensive protein movement to an open conformation is a prerequisite for effective electron transfer. It is known that the lack of the salt bridge between αN259 and βE165 results in an open form of hETF. Interestingly, the rate of formylation is different between hETF wild type and the αN259A and ßE656A variant proteins.

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



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, IBD or ACAD9) and passes them on to the respiratory chain via membrane bound ETF-ubiquinone oxidoreductase.

Role of bacterial aspartate β-decarboxylase in alamandine biosynthesis

The renin-angiotensin system (RAS) plays a pivotal role in cardiovascular and renal pathophysiology. Angiotensin (Ang) II, the active component of the RAS, is an octapeptide resulting from angiotensin-converting enzyme (ACE)-mediated cleavage of angiotensin I. Ang II binds to its receptors, AT1 and AT2 to mediate its effects. The ACE/Ang II/AT1 receptor axis mediates vasoconstriction, renal sodium reabsorption, thirst, release of vasopressin and aldosterone, inflammation, fibrosis and oxidative stress. Angiotensin-converting enzyme 2 (ACE2), a homolog of ACE with mono-carboxypeptidase activity, converts Ang II to Ang (1–7), contributing to the major cardioprotective arm of RAS, counteracting the pro-hypertensive effects of the classical ACE/Ang II/AT1R axis. Recently, a heptapeptide alamandine and its receptor MrgD were identified as novel RAS components. Alamandine and Ang (1–7) differ by only one amino acid residue, an alanine instead of aspartic acid at the N-terminal position. A similar observation of an alanine[1] substituted Ang II, Ang A, was reported previously. *In-vivo* and *in-vitro* studies demonstrate that alamandine subserves similar functions to Ang (1–7), e.g., vasodilation, blood pressure reduction, anti-inflammation and antifibrosis. Alamandine also reduced blood pressure in spontaneously hypertensive rats equivalent to Ang (1–7), and reduced cardiac fibrosis in Sprague–Dawley rats.

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

Master Thesis completed

Alexandra Csamay: *Studies on bioluminescence in different marine bacteria with focus on luciferase and the impact of LuxF on light intensity*

Bioluminescence is defined as the enzymatic production of light in living organisms. Different organisms ranging from microbes to animals have this ability, though abundance is especially high in the marine environment. This master thesis focused on the bioluminescence in marine bacteria which can be found free-living or in symbiosis with fish and other sea life. The reac-tion producing light is catalysed by a flavin-dependent monooxygenase, bacterial luciferase. However, other structural and regulatory genes are involved in the process. These structural genes are organised in a single operon luxCDAB(F)EG. In this study, special attention is drawn to the heterodimeric bacterial luciferase (encoded by luxAB) and the influence of luxF, which is only present in some Photobacterium species, on total light emission.

Luciferase from four marine bacteria was produced recombinantly in Escherichia coli and purified using affinity chromatography. Different approaches were tested in order to find an ideal experimental procedure leading to native heterodimer. All luciferases could be purified in their native form; however, yields were very low. Three of the four enzymes were proven to be active in an in-vitro assay. Analytical size exclusion chromatography showed that pure protein was obtained, although a majority was present in aggregates. For this reason, a realistic comparison of the bacterial luciferases could not be achieved.

The lux operons of P. leiognathi ATCC 25521 and P. mandapamensis ATCC 27561 were compared in an in-vivo assay. The genes luxCDAB(F)EG were expressed recombinantly in E. coli and exclusion of luxF led to a decrease in light emission. This supported prior studies, which found that LuxF acts as a scavenger of an inhibiting side product (an FMN derivative) produced during the light-emitting reaction.

International cooperations

Karine Bourgeois, 4Teen4 Pharmaceuticals GmbH, Hennigsdorf, Germany

Erika Kioshima and Flavio Seixas, Universidade Estadual de Maringa, Brasil

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

Robin Teufel, University of Freiburg, Germany

Research projects

FWF P26341: “The family of berberine bridge enzymes in plants”

FWF-doc.funds “CATALOX”

Talks at national and international conferences/meetings

1. Reinmar S. Eggers, *Characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in* Arabidopsis thaliana*.* 23rd DocDay (NAWI Graz), February 2020
2. Shaline Jha, *Dipeptidyl peptidase 3 modulates the renin-angiotensin-aldosterone system*. Gordon Research Seminar (Angiotensin), Lucca (Barga), Italy, February 2020
3. Reinmar S. Eggers, *Characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in* Arabidopsis thaliana*.* 12th ÖGMBT Annual Meeting (”Science flash” - short presentation), November 2020

Publications

1. Strandback, E., Lienhart, W.-D., Hromic-Jahjefendic, A., Bourgeois, B., Högler, A., Waltenstorfer, D., Winkler, A., Zangger, K., Madl, T., Gruber, K., Macheroux, P.: Rescuing the stability and activity of a cancer-associated variant of human NAD(P)H:quinone oxidoreductase 1 (NQO1) by small-molecular chaperones (2020) ***FEBS Lett.*** 594, 424-438. DOI:10.1002/1873-3468.13636

2. Brodl, E., Csamay, A., Horn, C., Niederhauser, J., Weber, H.-J., Macheroux, P.: The impact of LuxF on light intensity in bacterial bioluminescence (2020) ***J. Photobiol. Photochem. B: Biol.*** 207 111881, DOI: 10.1016/j.jphotobiol.2020.111881

3. Jha, S., Speth, R. C., Macheroux, P.: The possible role of a bacterial aspartate β-decarboxylase in the biosynthesis of alamandine (2020) ***Med. Hypoth.*** 144 110038, DOI: 10.1016/j.mehy.2020.110038

4. Jha, S., Taschler, U., Domenig, O., Poglitsch, M., Bourgeois, B., Pollheimer, M., Madl, T., Gruber, K., Zimmermann, R., Macheroux, P.: Dipeptidyl peptidase 3 modulates the renin-angiotensin system in mice (2020) ***J. Biol. Chem.*** 295 13711-13723, DOI: 10.1074/jbc.RA120.014183

5. Assil-Companioni, L., Büchsenschütz, H., Solymosi, D., Dyczmons-Nowaczyk, N. G., Bauer, K. K. F., Wallner, S., Macheroux, P., Allahverdiyeva, Y., Nowaczyk, M. M., Kourist, R.: Engineering of NADPH supply boosts photosynthesis-driven biotransformations (2020) ***ACS Catal.*** 10, 11864-11877. DOI: 10.1021/acscatal.0c02601

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

7. Messenlehner, J., Hetmann, M., Tripp, A., Wallner, S., Macheroux, P., Gruber, K., Daniel, B.: Reaction mechanism of monolignol oxidoreductase *At*BBE-like protein 15 and related enzymes from the berberine bridge enzyme-like protein family (2021) ***Arch. Biochem. Biophys.*** *In press*.

8. Asanovic, I., Strandback, E., Pasajlic, D., Meinhart, A., Tsung-Pin, P., Djokovic, N., Kroupova, A., Anrather, D., Schutz, T., Suskiewicz, M., Kocher, T., Beveridge, R., Nikolic, K., Schleiffer, A., Jinek, M., Hartl, M., Clausen, T., Penninger, J., Macheroux, P., Weitzer, S., Javier Martinez, J.: Oxidoreductase PYROXD1 enables the unfolded protein response and pre-tRNA splicing by preserving the activity of the tRNA ligase complex (2021) ***Mol. Cell***, 2021, *accepted*.

9. Lahham, M., Jha, S., Goj, D. Macheroux, P., Wallner, S.: The family of sarcosine oxidases – same reactions, different products (2021) ***Arch. Biochem. Biophys.*** *accepted.*

Awards

1. **Reinmar S. Eggers**, Best talk award: *Characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in* Arabidopsis thaliana*.* 23rd DocDay (NAWI Graz), 06.02.2020

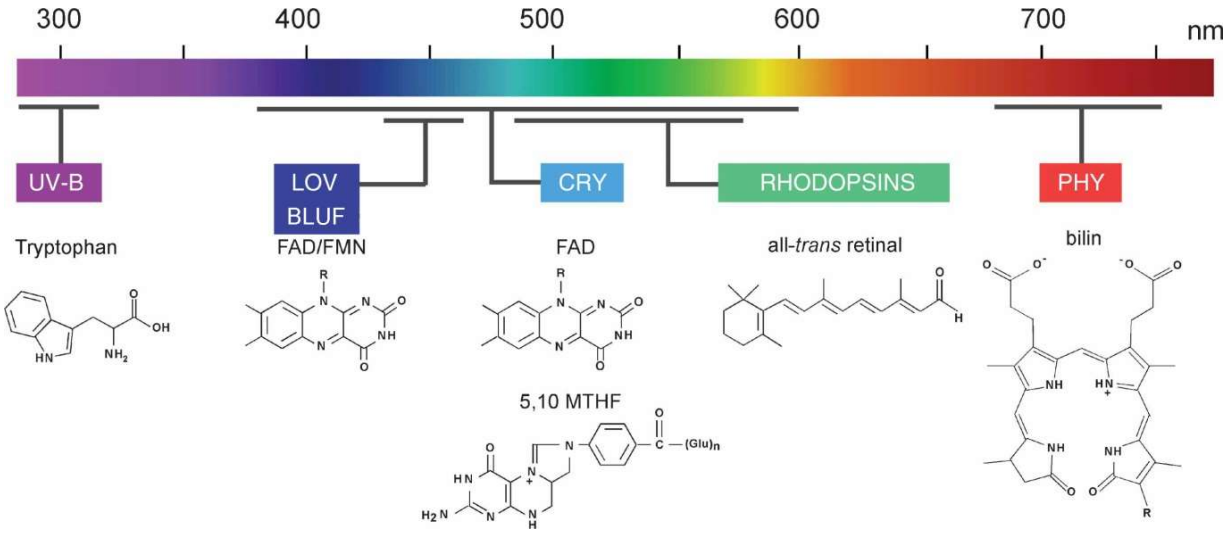
2. **Sami Ullah Kha**n was awarded a performance scholarship (Leistungsstipendium) by the Faculty of Technical Chemistry, Process Engineering and Biotechnology, Graz University of Technology, 03.12.2020.

Photoreceptor Group

Group leader: Andreas Winkler
PhD students: Cornelia Böhm, Massimo Totaro (joint with AGO), Hieu Tran, Ursula Vide
Lab rotation student: Oliver Eder
Master students: Maximilian Fuchs
Bachelor student: Elvira Hujdur, Darja Stjepanovic
Technical staff: Elfriede Zenzmaier, Nadine Galler

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.

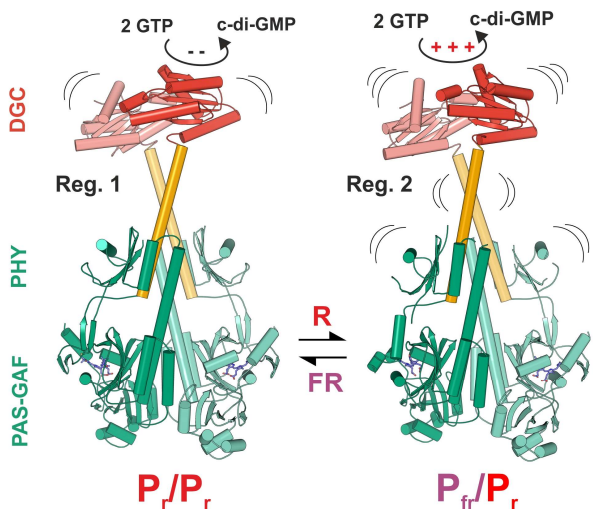


Overview of photoreceptor families and their corresponding cofactors covering the UV/Vis range of the electromagnetic spectrum (adapted from Heintzen WIREs Membrane Transport and Signaling 2012, 1:411–432).

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. Secondary structure predictions suggested a similar architecture to adenylate/guanylate cyclases, which are the focus of another project in the group. Elucidation of corresponding crystal structures later supported this hypothesis but also highlighted some differences. Especially the N-terminal extension is of interest due to the highly conserved residues present in this region that project towards the active site. In view of the direct connection of this element to the linker helix of upstream sensory modules (e.g. blue- and red-light photoreceptors), this might indicate a relatively direct signaling mechanism. The understanding of how evolution has accomplished this astonishing modularity of sensor-effector couples is important for the identification of functionally relevant structural elements with the goal of guiding the efficient design of rationally engineered light-regulatable systems. Since diguanylate cyclases are distantly related to adenylate/guanylate cyclases, the comparison of signal transduction mechanisms between the two protein families is one central aspect of our research interests (thesis projects of Cornelia Böhm and Hieu Tran).

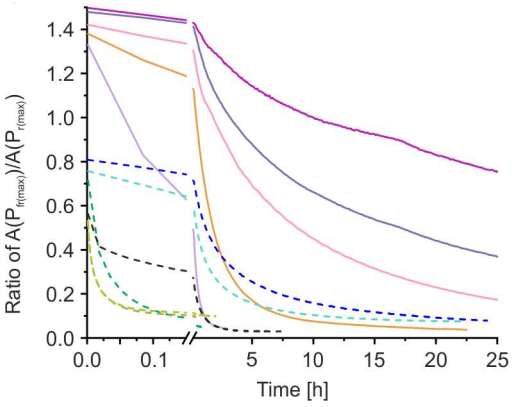
With the elucidation of the first full-length crystal structure of a naturally occurring phytochrome linked to its functional effector domain, important new insights into signal transduction in phytochromes were obtained. The structure revealed a parallel dimeric arrangement of the sensor and effector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



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

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

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



Asymmetry indications of the photoactivated spectra (figure on the left). Biochemical characterization of various chimeras exchanging functionally important elements of PadCs, revealed that two groups can be observed for all constructs. One featuring an asymmetric photoactivated spectrum (starting the recoveries at ratios around 0.75) and the second with a classical symmetric activation (recoveries starting close to a ratio of 1.5). Molecular determinants for which group each construct belongs to can, interestingly, not be assigned to a single structural element.

Importantly, most PadC homologs investigated, show indications of being structurally heterodimeric upon illumination. In most of these systems, asymmetric activation leads to a significant increase in DGC activity. In contrast, symmetric activation and no increase in c-di-GMP production upon illumination is observed in one subgroup of phytochrome linked diguanylate cyclases that also features an unexpected linker length with respect to the functional role of the linker requiring heptad repeat patterns for inhibiting and stimulating coiled-coil registers. In an attempt to address the functional relevance of individual structural elements, a range of chimeras between a symmetrically and one asymmetrically activated phytochrome were created. Spectral data and activity measurements showed that asymmetric phytochrome activation, while potentially advantageous for substrate binding and product formation, does not appear to be an absolute requirement for the red light induced increase in GTP turnover. More detailed analyses are in progress to unravel the molecular determinants of the asymmetry/symmetry transition in bacteriophytochromes and to understand the role of asymmetry in the biological context (manuscript in preparation - project of Cornelia Böhm).

Optogenetic tools based on red-light sensing phytochromes

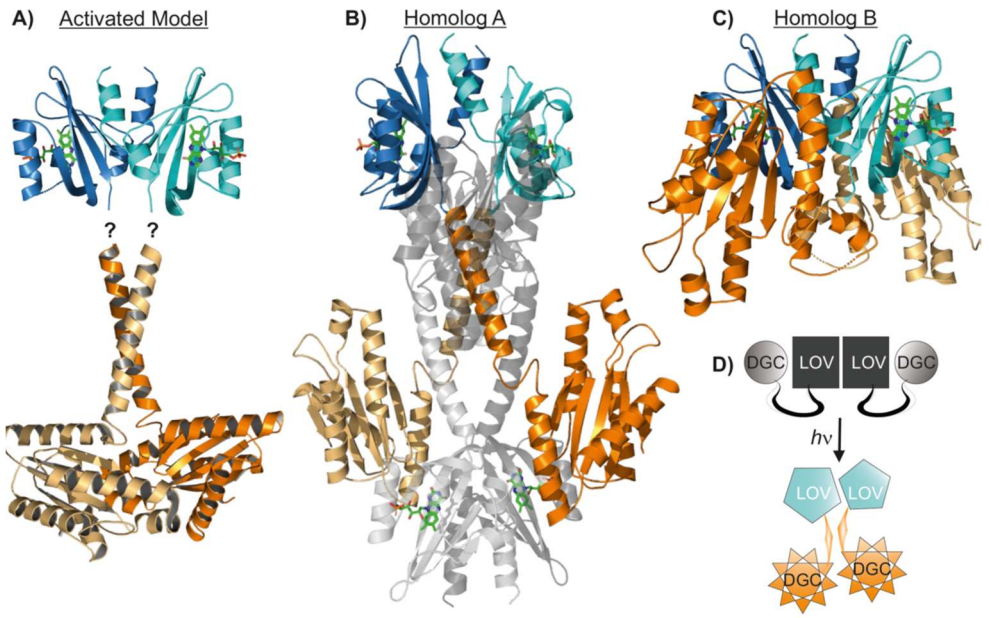
Due to deep tissue penetration of red light and the availability of the biliverdin cofactor as heme-degradation product in mammalian cells, bacteriophytochromes are interesting building blocks for optogenetic tools. To assess the suitability of PadC derived systems, we are testing different constructs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

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

Blue-light-regulated diguanylate cyclases

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

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

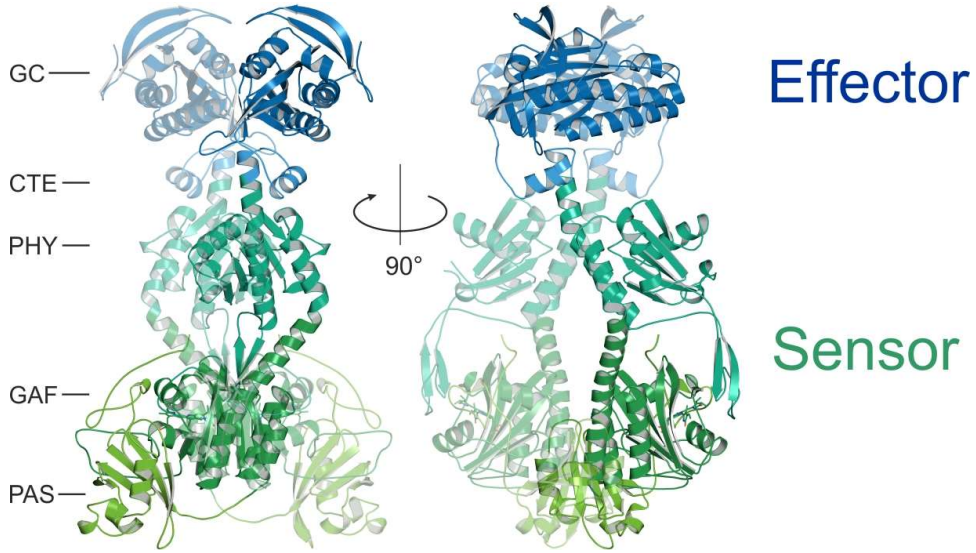


Crystal structures of LOV-regulated diguanylate cyclases. (**A**) Model of an activated conformation based on the observations in the phytochrome regulated GGDEF systems. (**B** and **C**). Crystal structures of two homologs with identical linker lengths, but different quaternary assemblies. (**D**)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

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

Light-activated adenylate/guanylate cyclases

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



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

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

Master thesis completed

Ursula Vide: *Molecular mechanisms of light regulation in LOV-diguanylate cyclases*

The importance of light as an external stimulus is reflected in the abundance of various photoreceptors found across all three kingdoms of life. Photoreceptors sensing blue light were early on identified as key players of plant phototropism and in recent years progress was made in understanding molecular mechanisms of light regulation in this family of flavin-dependent light-oxygen-voltage (LOV) domains. Since different effector domains can be coupled to them, they also contributed significantly to the field of optogenetics using natural and artificial LOV-regulated systems. The effector domain of interest in this work is the diguanylate cyclase (DGC) GGDEF domain, which catalyzes the formation of the bacterial second messenger 3',5'-cyclic dimeric guanosine monophosphate (c-di-GMP). C-di-GMP regulates fundamental physiological processes in bacteria, however, its synthesis requires an appropriate assembly of the active site at the dimer interface of two GGDEF protomers. According to the predictions, LOV-GGDEF proteins are constitutive dimers. The formation of the catalytically active dimers thus depends on the architecture of the LOV domain and the linker region between LOV and GGDEF domains.

Notably, the linker shows a high conservation of heptad repeats involved in coiled-coil formation. The coiled-coil linker is proposed to play an important role in integrating the light signal and activating the GGDEF domain by reorienting the two protomers properly. To understand the molecular mechanisms of light regulation and to improve the rational design of other LOV-coupled effectors, naturally occurring LOV-GGDEF homologs were characterized in this work. With regard to the linker region, two clusters were identified with common properties in each cluster. The biochemical characterization included measuring the spectral, enzymatic and in-solution assembly properties. LOV-GGDEF homologs belonging to the cluster with a longer linker showed attractive catalytic properties with very high dynamic ranges of activation. In addition, a new dark-state crystal structure of a natural LOV-GGDEF homolog with long linker was solved. The new structure revealed a dimeric assembly in the crystal lattice, whereas a previously solved structure of a homologous protein assembled as a tetramer. Combined with additional data from this work and insights from related phytochrome-linked GGDEF systems, functional implications of both structures suggest an interesting regulatory mechanism, where light induced rearrangements of the inhibited dimer allow extension of the linker helices into a coiled-coil. Thereby the GGDEF domains adopt a conformation suitable for more efficient synthesis of c-di-GMP

International cooperations

David Buhrke, University of Zurich, Switzerland

Geoffrey Gourinchas, Max Planck Institute of Neurobiology, Munich, Germany

Peter Hildebrandt, TU Berlin, Berlin, Germany

Janne Ihalainen, University of Jyväskylä, Jyväskylä, Finland

Jochen Reinstein, Max Planck Institute for Medical Research, Germany

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

Research projects

FWF P32022: “Signal Integration in Phytochrome-linked Diguanylyl Cyclases”

Zukunftsfonds Styria PN 1105: “Structural analyses by HDX-MS”

BioTechMed Lab Rotation: “Optogenetic tools based on bacteriophytochromes” (Oliver Eder)

Talks at national and international conferences/meetings

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

Publications

1. Strandback, E., Lienhart, W-D., Hromic-Jahjefendic, A., Bourgeois, B., Högler, A., Waltenstorfer, D., Winkler, A., Zangger, K., Madl, T., Gruber, K., Macheroux, P.: A small molecule chaperone rescues the stability and activity of a cancer-associated variant of NAD(P)H:quinone oxidoreductase 1 *in vitro* (2020) ***FEBS Lett***. 594 (3) 424-438, DOI: 10.1002/1873-3468.13636.

2. Buhrke, D., Gourinchas, G., Müller, M., Michael, N., Hildebrandt, P., Winkler, A.: Distinct chromophore-protein environments enable asymmetric activation of a bacteriophytochrome activated diguanylate cyclase (2020) ***J. Biol. Chem.*** 295 (2) 539-551, DOI: 10.1074/jbc.RA119.011915.

3. Christiansen, A., Weiel, M., Winkler, A., Schug, A., Reinstein, J.: The trimeric major capsid protein of Mavirus is stabilized by its interlocked N-termini enabling core flexibility for capsid assembly (2021) ***J. Mol. Biol.*** in press, DOI: 10.1016/j.jmb.2021.166859.

Habilitation

1. Winkler, A.: Structure-Function Studies in the Field of Light-Regulated Sensor-Effector Couples (2020) - *scientific field*: **Structural Biochemistry** *submitted at Graz University of Technology (Austria); Faculty of Technical Chemistry, Chemical and Process Engineering, Biotechnology.*

Award

1. **Nikolina Todorović** was awarded the Best Diploma Thesis Award 2020 by the Nenad M. Kosćic foundation in Serbia for her Master thesis performed in the group in 2019.

2. **Andreas Winkler** received an honorable citation together with the second mentor of Nikolina by the Nenad M. Kostić foundation and the offer to participate in the ceremony, which, disappointingly, due to Corona restrictions could not be followed up on.

Protein Design Group

Group leader: Gustav Oberdorfer
Postdocs: Horst Lechner
PhD students: Veronica Delsoglio, Wael Elaily, Julia Messenlehner, Florian Wieser, Massimo Gregorio Totaro, Adrian Tripp
Rotation students: Tobias Fleiß
Master students: Markus Braun (co-supervision with Birgit Wiltschi), Dominik Fridrich (finished July 2020), Nina Grujicic, Lena Parigger (co-supervision with Toni Glieder), Dominik Schwarz,
Bachelor students: Stefanie Ferstl, Laura Rammer
Technical Staff: Alma Makic, Stella Ebner, Birgit Grill

General description

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

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

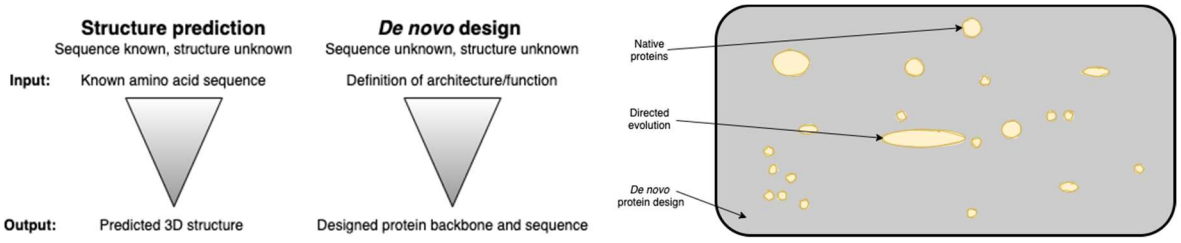


Figure (left) *Differences in structure prediction and de novo protein design* ***(right)*** *Illustrative representation of protein sequence space (grey). Sequence analysis of native proteins shows that tight clusters of protein families can be identified (beige). (c) Gustav Oberdorfer*

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

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

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

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

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

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

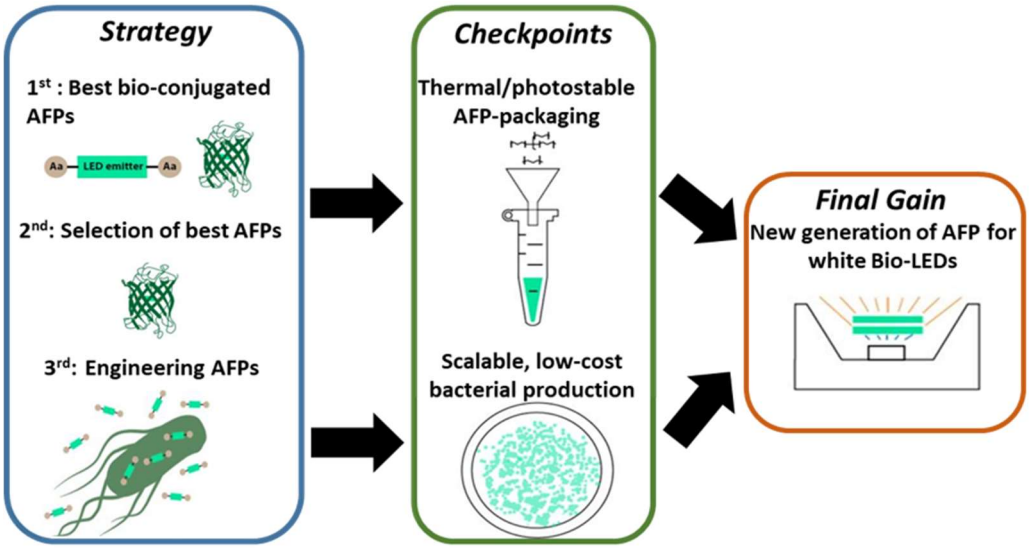


Figure. *The ARTIBLED concept – We start by engineered artificial amino acids into selected protein scaffolds for covalent labelling with the fluorescent dyes. This is followed by experimental testing of this host-guest system for its fluorescent properties. Next, the labelled protein is packaged into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

Fluorescent dyes and Quantum Chemistry

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

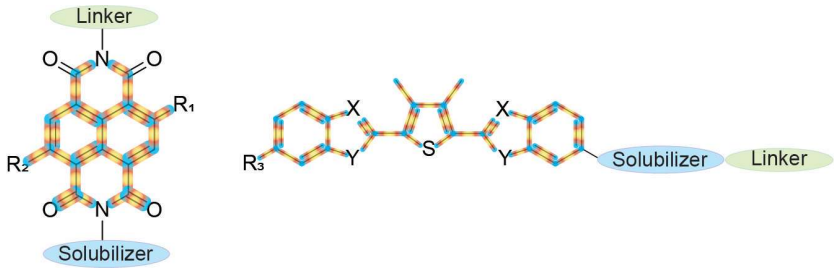


Figure. *Backbones of the synthesized dyes with fine-tuned photophysical features, able to specifically tag protein scaffolds*

Protein Design and Synthetic Biology

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

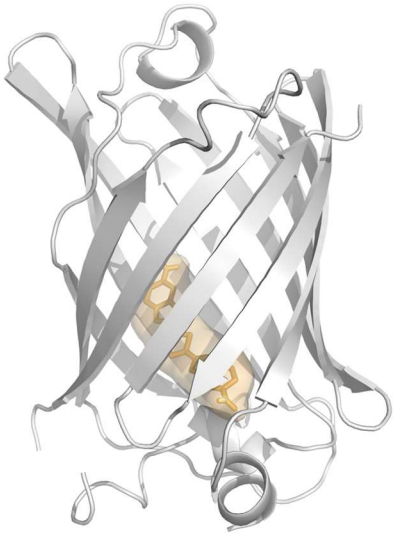


Figure. *Rendering of a fluorescent dye bound to a designed beta-barrel protein. The fluorescent dyes are covalently attached to the protein via the introduction of an artificial amino acid residue. During the design process all surrounding residues will be optimized computationally to generate an ideal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

Environmentally friendly polymer coatings for lighting purposes

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

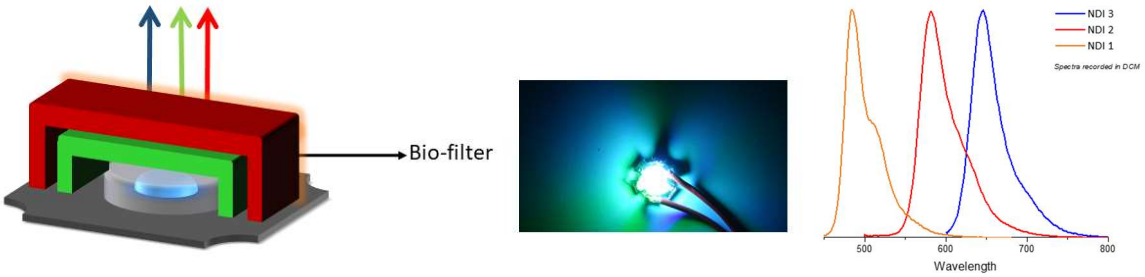


Figure. (left) *Schematic drawing of Bio-WLED and picture of a running prototype.* ***(right)*** *Emission spectra of the dyes currently used.*

Large scale production

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

Conclusion

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

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

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International cooperations

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

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

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

Frank DiMaio, Department of Biochemistry, UW, Seattle, United States

Jens Gundlach, Department of Physics, UW, Seattle, United States

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

Research projects

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

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

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

BioTechMed Lab Rotation: “Computational design of fluorescent alpha-helical barrel proteins” (Tobias Fleiß)

Talk at national and international conferences/meetings

1. Oberdorfer, G.: *Proteins made to order: Computational design of de novo proteins with custom geometries.* R(a)isingChemBioTalents – ITN brainstorming workshop, May 2020

Publication

1. Sjöström, D.J., Berger, S.A., Oberdorfer, G. and Bjelic, S.: Computational backbone design enables soluble engineering of transferrin receptor apical domain (2020) ***Proteins*.** Accepted Author Manuscript, DOI: 10.1002/prot.25974

Patents

1. Park, J., Boyken, S., Wei, K., Oberdorfer, G., Baker, D. (2020) Amantadine Binding Protein. PCT/US2020/028280.
2. Chidyausiku, T., Marcos, E., Nivon, K., Oberdorfer, G., Baker, D., Carter, L. (2019) De Novo Designed JellyRolls aka Cupins Non-Local Beta Sheet Proteins. U.S. Provisional Application No.: 62/926,203

Chemistry of Functional Foods

Group leader: Michael Murkovic
PhD students: Sandra Freudenthaler-Holzer, Monika Grasser
Master students: Iris Stalzer, Zehra Gromilic, Elke Aichhorn, Maria-Luise Deflorian, Marcel Wilhelm
Technical staff: Claudia Hrastnik

General description

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

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

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

PhD Thesis completed

Sandra Freudenthaler-Holzer: *Microbial investigation of milk product quality in different operating levels*

In this comprehensive study, milk products such as whey protein concentrate (WPC), unskimmed milk and low fat milk were investigated during its whole production process. For this purpose samples were taken at specific production steps and microbial contamination was recorded. Also raw-material quality and the final products were proved, considering the different seasons at delivery.

The methods used for this study are EN ISO/IEC 17025 standard methods and all sample investigations were conducted in an accredited laboratory. One of the several advantages of an accredited laboratory is the comparability of the results to other accredited labs. Quality of the results, hygiene instructions, methods and the correct mode of operation are frequently audited.

In general, raw-material quality is much lower than final product-quality, in particular WPC raw-materials. In this case, also a relatively high percentage was tested Cronobacter sakazakii positive. Furthermore, the total bacteria count, Enterobacteria and Coliform bacteria were present in a high number in all raw-material samples. After two or even three heating steps, the number of not-heat-resistant bacteria decreased. Cronobacter sakazakii was detected just in one of the final products. In contrast, the number of thermophilic bacteria and thermophilic spores increased in the final product samples in a huge way.

The high standard deviation at all results show a wide range of raw material quality, which also influences the resulting final product. If the raw material quality could be improved, the food producer would have less trouble to produce contamination free final product. The drying process eliminates about 99 % of bacterial contamination, but the point is how high the counts are at the beginning. All in all, a proper hygienic monitoring, cleaning and sterile handling prevent unnecessary contaminations at the factory and support the production of high quality dried milk products.

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

Talks at international conference

1. Murkovic M.: *Food as a basis for good health and well-being*. Synergy in Science: Environmental for Global Movement to Achieve SDGs, Palembang, Indonesia, Oct 2020.
2. Murkovic M.: *Furfuryl alcohol (FFA) formation and mitigation in coffee based products*. 6th FiAConference 2020 on Food Science, Nutrition and Health, Jakarta, Indonesia, Oct 2020.

Publications

1. Albouchi, A., Murkovic, M.: Investigation on the mitigation effects of furfuryl alcohol and 5-hydroxymethylfurfural and their carboxylic acid derivatives in coffee and coffee-related model systems (2020) ***Food Res. Int.*** 137, 1-9, DOI: 10.1016/j.foodres.2020.109444.

2. Ahmad, S., Zeb, A., Ayaz, M., Murkovic, M.: Characterization of phenolic compounds using UPLC–HRMS and HPLC–DAD and anti-cholinesterase and anti-oxidant activities of *Trifolium repens* L. leaves. **Europ. Food Res. Techn.** 246 (2020) 485-496, DOI: 10.1007/s00217-019-03416-8.

3. Tatzber, F., Wonisch, W., Lackner, S., Lindschinger, M., Pursch, W., Resch, U., Trummer, C., Murkovic, M., Zelzer, S., Holasek, S., Cvirn, G.: A Micromethod for Polyphenol High-Throughput Screening Saves 90 Percent Reagents and Sample Volume (2020) **Antioxidants.** 9, 1-12, DOI: 10.3390/antiox9010011.

4. Ciesarová Z, Murkovic M, Cejpek K, Kreps F, Tobolková B, Koplík R, Belajová E, Kukurová K, Daško L, Panovská Z, Revenco D, Burčová Z.: (2020) Why is sea buckthorn (*Hippophae rhamnoides* L.) so exceptional? A review. **Food Res. Int.** 133, 109170, DOI: 10.1016/j.foodres.2020.109170.

Lectures and Laboratory Courses

Winter Semester 2019/20

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	Jha S, Wallner S
CHE.210_FUF	Project Work to the Bachelor Thesis	0.5	SE	Macheroux P, Murkovic M, Winkler A, Wallner S
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	Macheroux P, Murkovic M, Winkler A, Wallner S
MOL.833_1	Project laboratory	9	LU	Team
MOL.844_1UF	Journal Club Biochemistry and Molecular Biomedicine	1.5	SE	Team
MOL.845_UF	Master Thesis Seminar	2	SE	Macheroux P, Winkler A, ...
MOL.855UF	Molecular Physiology	2	VO	Macheroux P
MOL.881UB	Biophysical Methods	3	VO	Winkler A, …
MOL.933UF	Food Biotechnology	1.3	VO	Murkovic M
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
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 2020

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
CHE.200_FUF	Project Laboratory Chemistry (Bachelor)	0.5	SE	Macheroux P, Murkovic M, Oberdorfer G, Winkler A
CHE.210_FUF	Project Work to the Bachelor Thesis	0.5	SE	Macheroux P, Murkovic M, Oberdorfer G, Winkler A
MAS.476UB	Biophysical Methods	3	LU	Winkler A, …
MOL.406UF	Methods in Immunology	2	VO	Oberdorfer G
MOL.407UF	Methods in Immunology	2	LU	Team
MOL.606_1UF	Bachelor Thesis	1	SE	Team
MOL.832_1UF	Project laboratory	9	LU	Team
MOL.844_1UF	Journal Club Biochemistry and Molecular Biomedicine	1.5	SE	Team
MOL.845_1UF	Seminar for undergraduate students	2	SE	Team
MOL.880UF	Molecular Enzymology	2	VO	Gruber K, Macheroux P, Nidetzky B
MOL.882_1UF	Profession-oriented research practice	2	PV	Macheroux P
MOL.886UB	Biophysical Methods	3	LU	Winkler A, 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
648.011	Integrative Structural Biochemistry	2	VO	Winkler A, Madl T, Gruber C
648.012	Frontiers in Integrative Structural Biology	1	SE	Macheroux P, Winkler A, …
648.013	Advanced cell culture training course II	1	SE	Amor M
648.016	Chemical Reactions in Foods II	2	PV	Murkovic M
648.020	Teaching Experience	2	SE	Macheroux P
648.040	Soft skills	2	SE	Macheroux P

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