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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 institute. The institute was renamed *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. After World War II, questions of water quality and wastewater disposal became urgent; hence, the group of Prof. K. STUNDL, which at that time was part of the institute, was gaining importance. In addition, a division to fight dry-rot 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 extended laboratory was called EMICH-Laboratories. At the same time, the institute was renamed *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 Dr. F. PALTAUF from the Karl-Franzens-University Graz 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 carried out postdoctoral research in renown laboratories. Independent research groups in cell biology (G. D.), biophysics (A. H.) and molecular biology (S. D. K.) were established.

1990 The institute moved to a new building in Petersgasse 12. The move enabled the expansion of individual research groups and the acquisition of new equipment, 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.

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

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 Prof. 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* Prof. HERMETTER retired in September. Starting as a graduate student in Prof. PALTAUF's group he 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.

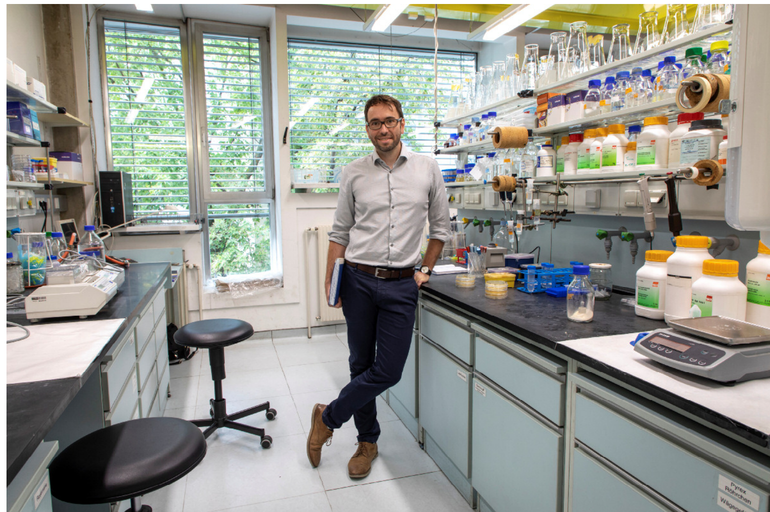
2017 J. BOGNER-STRAUSS from the institute was sworn in as Minister for Women, Families, and Youth of the Austrian government at the end of 2017.

2018 Gustav OBERDORFER received an ERC Starting grant and decided to build up his research group at the Institute of Biochemistry. With his research interests in the fields of protein design and protein engineering, this further strengthened the new protein biochemistry focus of the institute.

Highlights of 2018

Tackling challenges in protein design – ERC Starting Grant to Gustav Oberdorfer

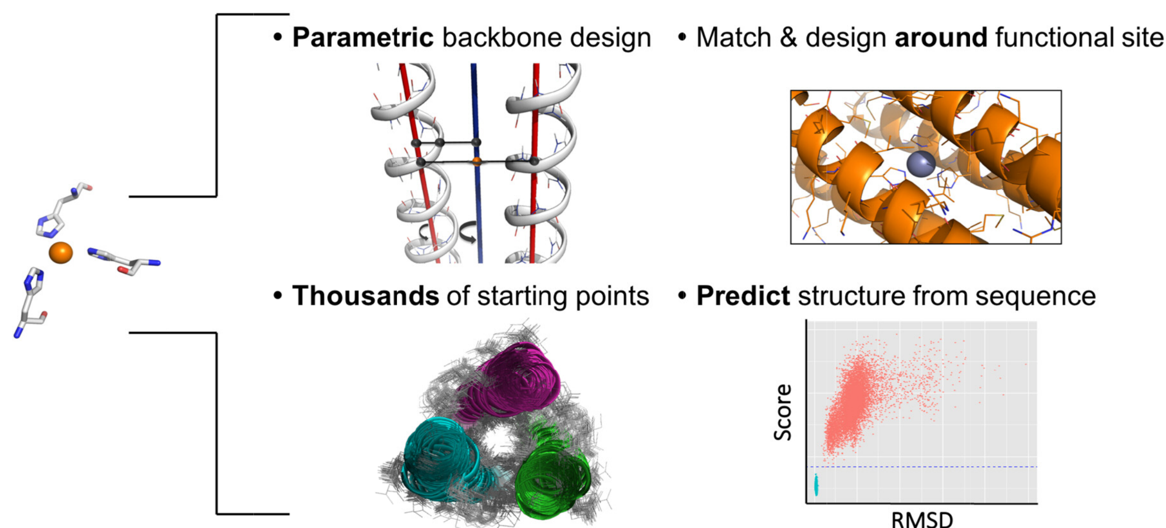
Proteins, nature's building blocks of live, have distinct three-dimensional structures that are built up from a string of amino acids. In a highly regulated process, the amino acids are strung together and arranged into a distinct structure by a cell to make proteins. The amino acid sequence is called the proteins primary structure. It tends to build stable local structures, called secondary structure elements. Those can be spiral structures (helices), elongated stretches (strands) or swirly random structures (loops). With only these three building blocks, nature can build all the proteins we know, through a process called protein folding, in which the helices, strands and loops fold into a stable configuration that represents a low energy state for a particular amino acid sequence. Remarkable, even for small protein structures, this process would take longer than the present age of the universe, if all the conformations it could adopt were explored. This also means, if we can solve the protein folding problem efficiently, we could make proteins from scratch according to our needs – the ultimate goal of all protein design efforts.



A number of parallel developments have rapidly accelerated the pace of protein design research in the last couple of years. Vast computational power has become available at most academic research institutions and the algorithms for performing protein design calculations have become more efficient. Most importantly, synthetic DNA fragments, which are used to produce artificial proteins in the lab, have become cheaper. Thus, it is feasible now to carry out large-scale testing of *de novo* designed proteins, which means that computer-aided protein design will fully unfold its potential and opened up new approaches to solving biotechnological and biomedical problems of the 21st century. For example, present day applications in nanomaterials and general protein engineering require solid building blocks, but naturally occurring proteins are only marginally stable – a shortcoming which could be solved with designed proteins.

During his postdoc Oberdorfer co-developed a general procedure for designing new protein structures, by taking a set of equations originally derived by Francis Crick in 1953, which describe helical protein structures, and combining them with computational modeling. This enabled him to generate new protein structures of more or less arbitrary size and with unprecedented stabilities. The designed proteins are stable above 95°C - a temperature at which most natural proteins have long started to degrade - and in highly degrading conditions.

In his ERC funded project, Oberdorfer together with his team aims to use this technology to produce proteins with cavities that can bind a specific small molecule and subsequently catalyze a reaction. The focus is not put on answering a particular question, but rather developing a general method that can be used to produce such proteins. His team is looking at specific applications, one of which involves trying to bind and break down the pesticide glyphosate.



Overview of the protein design process. From parametric design to prediction of structures.

Biography

Gustav Oberdorfer is the newest addition to the Institute of Biochemistry in February 2018. His group is still establishing itself and focuses on computational protein design. Gustav received a master's degree in Biochemistry and Molecular Biology in 2007 and a PhD in Molecular and Structural Biology in 2011 from the University of Graz, Austria. In 2011-2012 he was a Senior Researcher with the Austrian Centre of Industrial Biotechnology (ACIB GmbH) gaining experience in an industry related working environment. In 2012, he moved to Seattle to work at the Institute of Protein design, an endeavor for which he was awarded a Marie Curie International Outgoing Fellowship from the European Commission. His time in in Seattle resulted in several high-profile publications and two patent applications. In 2016 he received the ASciNA Award (Austrian Scientist & Scholars in North America) from the federal ministry of Education and Science, Austria. Since returning back to Graz, Oberdorfer's main scientific interest lies in designing and engineering biomolecular structures and their functions - a strongly collaborative effort that combines structural biology, computational biology, bioorganic chemistry and biophysics approaches.

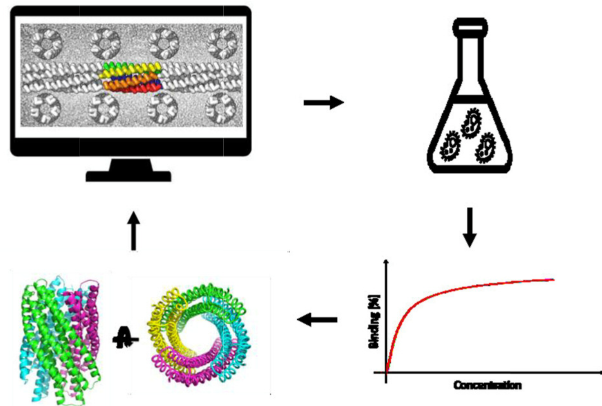
Additional highlights of 2018

In 2018 the Institute of Biochemistry was very active again in training bachelor, master and PhD students with a record number of completed theses! In addition, the institute also hosted guest students from Italy, Iran and Syria. This called for a celebration, which took place at the Buschenschank Sattler.

In June, the annual institute outing took place. As always we roamed through the hills and vineyards of southern Styria refreshing ourselves in a traditional Buschenschank before we returned to Graz!

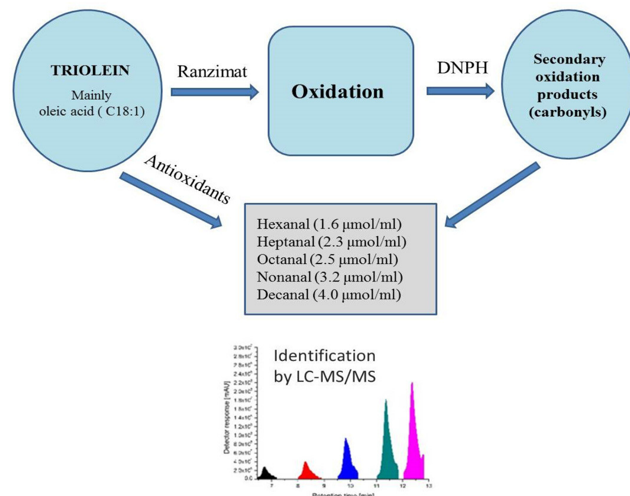
In the group of **Andreas Winkler** three thesis projects were finished in 2018. Both Geoffrey Gourinchas and Stefan Etzl obtained their PhD and Julia Färber finished her Master thesis.
Andreas Winkler obtained follow-up funding for his research via an FWF stand-alone project and acquired an infrastructure grant by the government of Styria for purchasing a mass spectrometry system for protein analytics.
In addition, a new lecture focusing on aspects of integrative structural biology was launched by Andreas Winkler and colleagues.

The group of **Gustav Oberdorfer** was newly founded in 2018 and grew from one to six members within a year. Working together in a highly interdisciplinary environment, several Bachelor, Master and one PhD student try to solve protein folding problems. Overall, this was a very exciting year for the Oberdorfer group, since it moved into new lab space and established itself. In addition, Gustav Oberdorfer got the chance to present his scientific findings from previous years on several occasions, including talks at the Technical University of Munich and at the University of Bristol.



Katharina Huber, Wenmin Xia, Furkan Alkan of the **Bogner-Strauss group** completed their research stays in the USA (University of Pennsylvannia, University of California, Massachusetts Institute of Technology).
Dina Hofer, Wenmin Xia and Katharina Huber successfully finished their PhD theses as well as Jürgen Novak successfully finished his master thesis. Congratulations!

In the Functional Food Group of **Michael Murkovic** the main projects were focused on the quality of homemade fruit juices. Several concepts of improving the stability of micronutrients of freshly squeezed fruit juices were developed. 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.



Biochemistry Group

Group leader: Peter Macheroux
Secretary: Tinkara Kristovic
Senior research scientists / postdoctoral fellows: Bastian Daniel, Alexandra Jammer, Silvia Wallner (on maternal leave)
PhD students: Eveline Brodl, Grazia Davidovic, Reinmar Eggers, Shalinee Jha, Sami Ullah Khan, Barbara Konrad, Majd Lahham, Emilia Strandback, Marina Toplak.
Master students: Alexandra Csamay, Tamara Berger, Julia Brunner, Julia Messenlehner, Alissa Pichler, Florentina Rieger
Technical staff: Eva Maria Frießer, Rosemarie Trenker-El-Toukhy, Alma Makic

General description

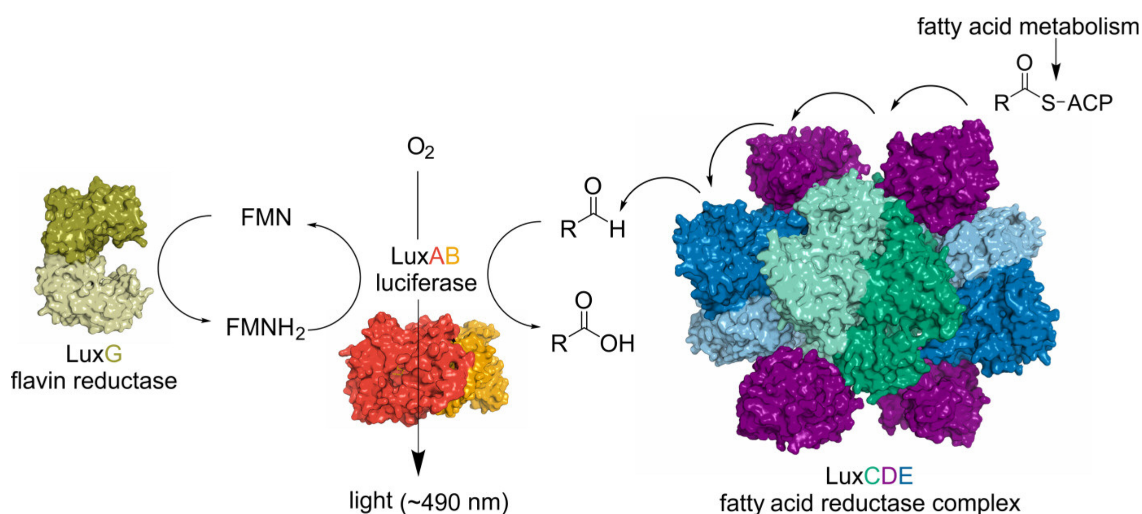
The fundamental questions in the study of enzymes, the bio-catalysts of all living organisms, revolve around their ability to select a substrate (substrate specificity) and subject this substrate to a predetermined chemical reaction (reaction and regio-specificity). In general, only a few amino acid 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.

Bacterial Bioluminescence

The emission of light by living organisms (bioluminescence) is a fascinating process found in bacteria, fungi, insects, fish, limpets and nematodes. In all cases, the bioluminescent process is based on a chemiluminescent reaction in which the chemical energy is (partially) transformed into light energy (“cold light”). All bioluminescent processes require a luciferase, *i.e.* an enzyme catalysing the chemiluminescent reaction, and a luciferin, the light-emitting molecule.

In our laboratory, we are interested in the bioluminescence of marine bacteria. In these bacteria, the enzyme luciferase (a heterodimeric protein consisting of a 40 kDa α-subunit and a 37 kDa β-subunit) binds to reduced flavin mononucleotide ($FMNH_2$), which then reacts with molecular oxygen to form a flavin-4a-hydroperoxide intermediate with subsequent oxidation of a long-chain fatty aldehyde (*e.g.* tetradecanal) to the corresponding fatty acid (*e.g.* myristic acid). During this oxidation process, an excited flavin-4a-hydroxide intermediate is generated (luciferin), which emits blue-green light centered at 490 nm.



Bacterial bioluminescence in a nutshell. The central player in bacterial bioluminescence is the heterodimeric luciferase (red/orange), which carries out the oxidation of long-chain fatty aldehydes to the corresponding acid accompanied by light emission. The required reduced FMN is provided by an NAD(P)H-dependent FMN reductase (LuxG, on the left side the structure of the closely related enzyme Fre of *E. coli* is shown in olive; PDB 1QFJ) and the fatty aldehyde is synthesized through the multifunctional complex consisting of LuxCDE (green, violet and blue model on the right).

Many *photobacterial* strains carry an additional gene termed *luxF*. The exact role of *luxF* and its encoded protein LuxF is still uncertain. However, crystallographic analysis of LuxF revealed the presence of four molecules of a flavin derivative, *i.e.* 6-(3'-(*R*)-myristyl) FMN (myrFMN), non-covalently bound to the homodimer. This molecule combines two components of the bioluminescent reaction, FMN and a long-chain aliphatic acid. The elucidation of the role of *luxF* and LuxF and its bound flavin derivative in light emission was the main focus of this project.

In vitro analysis revealed that myrFMN is produced as a side product of the luciferase-catalyzed reaction. *In vivo* analysis of a range of bioluminescent bacteria (with and without *luxF*) demonstrated that myrFMN formation is independent of *luxF* occurrence. MyrFMN binds to the luciferase and inhibits the bioluminescent reaction. It was elucidated that apo-LuxF captures myrFMN and thereby relieves the inhibitory effect on luciferase activity, due to a higher affinity of myrFMN to LuxF than to the luciferase. Development of an *E. coli* based *lux-rib* expression system where the *lux-rib* operon was cloned into a single expression vector allowed the heterologous expression of the complete *lux-rib* operon. Comparing *E. coli* based *lux-rib* expression system with or without *luxF* revealed that the presence of *luxF* enhances light intensity by a factor of 1.5. Furthermore, isolation and analysis of the flavin derivative revealed the presence of more than just the previously investigated myrFMN and led to the discovery of three different flavin derivatives with different alkyl chains.

In conclusion, these findings suggest that LuxF not only plays a role in preventing inhibition but also influences the catalytic activity of the bacterial luciferase and thereby light production (PhD thesis project of Eveline Brodl; master thesis project Alexandra Csamay).

Yeast flavoproteins

Analysis of the genome of the yeast *Saccharomyces cerevisiae* identified 68 genes encoding flavin-dependent enzymes. This organism is one of the best-investigated eukaryotic model systems for molecular and cell biology. Nevertheless many flavoproteins are poorly characterized. In our recent studies, we conducted a detailed biochemical and structural investigation of Pst2p, a member of the flavodoxin-like family. It was demonstrated that Pst2p is a very efficient NAD(P)H:quinone oxidoreductase rapidly reducing quinones *in vitro* and *in vivo*. However, in contrast to other quinone reductases Pst2p displays an unusually positive redox potential. This affects its substrate spectrum, *e.g.* Pst2p possesses neither azo reductase nor FMN reductase activity. Furthermore, Pst2p binds reduced FMN ca. five orders of magnitude tighter than oxidized FMN. The structure of Pst2p was elucidated by X-ray crystallography in collaboration with Altijana Hromic and Prof. Karl Gruber and revealed that Pst2p adopts the flavodoxin-like fold. It was also found by size exclusion chromatography that Pst2p forms tetramers independent of cofactor binding. In summary our data suggest that Pst2p possesses more similarity to quinone reductases than flavodoxins and that Pst2p enables yeast cells to cope with quinone-induced damage suggesting a role of the enzyme in managing oxidative stress.

Another potential flavoprotein from *S. cerevisiae* called Irc15p raised our interest because it is a homolog of lipoamide dehydrogenase (LPD). However, it lacks the two cysteines in the active site that undergo reversible oxidation to a disulfide. Cell biological studies indicated an effect of Irc15p on microtubule dynamics and cell cycle progression. In our studies we have demonstrated that Irc15p is indeed a flavoenzyme that is rapidly reduced by NADH and to a lesser degree by NADPH. Several artificial electron acceptors are capable of oxidizing the reduced FAD, however disulfide, such as cysteine, glutathione or lipoamide were inactive. Taken together our findings suggest that Irc15p is efficiently reduced in yeast cells to deliver electrons to an as yet unidentified electron acceptor that is related to Irc15p's function in regulating microtubule dynamics and cell cycle progression (thesis project of Karin Koch in collaboration with Emilia Strandback and Shalinee Jha).

Berberine bridge enzyme-like enzymes in plants

Berberine bridge enzyme (BBE) is a central enzyme in 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 28 genes that apparently encode BBE-like enzymes although the plant does not entertain alkaloid biosynthesis. 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 resolved 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 amino acid residues apparently involved in the oxidation of monolignols to the corresponding aldehydes are currently under investigation by a site-directed mutagenesis program (master thesis of Julia Messenlehner in collaboration with Dr. Bastian Daniel). 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 Dr. Alexandra Jammer and Professor Dr. Maria Müller from the Institute of Biology at the University of Graz in order to investigate the phenotypes of *Atbbe* gene knock-out plants (thesis project of Reinmar Eggers and Barbara Konrad; master theses of Alissa Pichler and Florentina Rieger).

In contrast to *Arabidopsis thaliana* the genome of the model moss *Physcomitrella patens* encodes a single BBE-like enzyme (*Pp*BBE), which is particularly interesting as the moss is the most basal plant possessing a *BBE-like* gene. Like the BBE-like enzymes from *Arabidopsis* we also recombinantly expressed *Pp*BBE in *Komagataella phaffii*, which enabled further biochemical and structural characterization. We found that the enzyme exhibits very similar structural and spectral features as previously shown for other BBE-like proteins, whereas the catalytic role was found to be rather different. In screening assays we showed that *Pp*BBE acts on the disaccharide cellobiose, which gets oxidized at the anomeric carbon to yield the corresponding lactone. With the help of a structure guided mutagenesis program we were able to reveal the role of various active site residues in catalysis, which further enabled us to postulate a reaction mechanism.

Our *in vitro* findings are supported by the *in vivo* results obtained from the characterization of a *Pp*BBE knock-out strain, which was done in cooperation with the research group of Prof. Dr. Ralf Reski from the Department of Plant Biotechnology at the Albert-Ludwigs-University Freiburg. It was found that the enzyme is highly expressed in the chloronema phase, an early stage of the plant's life cycle, where carbohydrate metabolism is strongly upregulated and that the protein is secreted to the extracellular space. Hence, it is possible that *Pp*BBE is involved in the later steps of cellulose degradation, thereby allowing the moss to most efficiently use cellulose for energy production (thesis project of Marina Toplak supported by Dr. Bastian Daniel)

Human dipeptidylpeptidase III

Dipeptidyl peptidase III (DPPIII) is a zinc-dependent metalloenzyme involved in degrading shorter peptides with 4–12 amino acid residues. It exhibits high affinity to opioid peptides and to some of the vasoconstrictor peptides from the renin-angiotensin-aldosterone system. In view of this it has been associated with pain signaling, blood pressure regulation and enhancement of cancer cell defense against oxidative stress, but the precise function of DPPIII is still unknown. To better understand the physiological function of this peptidase, we teamed up with Professor Dr. Robert Zimmerman from the University of Graz to generate and characterize DPPIII knockout mice (DPPIIIKO). Nuclear magnetic resonance (NMR) spectroscopy for metabolic analyses of urine and tissue homogenates in DPPIIIKO and control mice was performed in collaboration with Assoz. Prof. Tobias Madl at the Medical University Graz. Furthermore, LC-MS-based comparison of angiotensin levels in the tissue homogenates and plasma of mice was performed by our industry partner, Attoquant Diagnostics in Vienna.

Wild-type (WT) and KO animals (male and female) at the age of 16-weeks were fed on standard chow diet and their metabolic parameters were investigated. The male KO mice showed significantly reduced body weights than the WT mice. This change was less pronounced in female KO mice. Detailed analysis of the mice in metabolic cages revealed that the male KO mice have higher food and water intake. Additionally, their oxygen consumption and carbon dioxide production was also increased, indicating a higher rate of metabolism. No significant metabolic differences between the genotypes were observed in female mice. NMR-based metabolite profiling in the kidney tissue homogenates and urine indicate metabolites of the TCA cycle to be downregulated in the male KO mice. These effects were less prominent in the female mice. Quantification of the hypertensive peptide hormone angiotensin II in plasma showed elevated level in male KO mice. In contrast, the female KO mice displayed reduced angiotensin II levels. These results indicate that loss of DPP3 implicates significant sex-specific alterations in metabolism, weight loss, cardiovascular functions and oxidative stress. Currently, we are investigating the underlying mechanisms involved in the regulation of these physiological functions by DPPIII (thesis project of Grazia Davidovic and Shalinee Jha).

Flavin-dependent electron transfer in human mitochondria

Electron transferring flavoproteins have not only been found in humans, but also in a variety of other eukaryotic and prokaryotic organisms. While the human protein has been studied in detail in terms of its biochemical properties as well as its interaction with client dehydrogenases and the ETF-QO, hardly anything is known about the mitochondrial electron transfer process in yeast. It was proposed that the yeast ETF (yETF) receives electrons form D-lactate dehydrogenase 2 (Dld2) and delivers them to an ETF-QO homolog named Cir2p, however, this interaction has not been confirmed experimentally thus far.

In order to study the possible electron transfer between Dld2 and yETF, we produced both proteins in *Komagataella phaffii* and *Escherichia coli*, respectively. In a detailed biochemical characterization of yETF, we could show that the protein exhibits spectral and biochemical properties strongly diverging from hETF, though sharing rather high sequence identity and structural similarity. Kinetic studies revealed that Dld2 has a much higher catalytical efficiency for the oxidation of D-2-hydroxyglutarate as compared to D-lactate and that tight binding of the reaction product, *i.e.* α-ketoglutarate or pyruvate, is used to suppress reoxidation of the reduced enzyme by molecular oxygen.

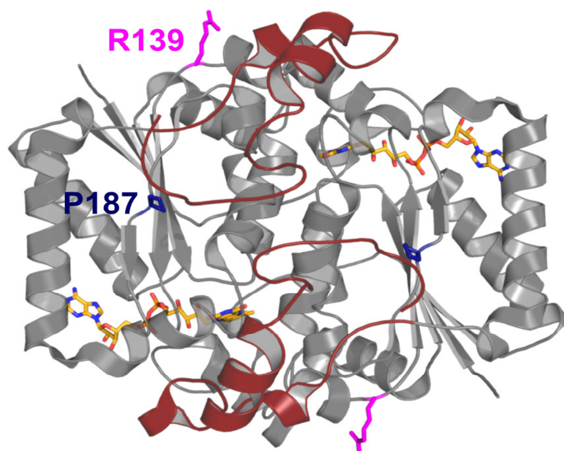
In line with the biochemical properties of both yETF and Dld2, we could finally confirm the successful electron transfer from the dehydrogenase to yETF, indicating that Dld2 is the natural electron donor of yETF (thesis project of Marina Toplak and master thesis of Julia Brunner).

Human quinone reductase NQO1

The human NAD(P)H:quinone oxidoreductase 1 (NQO1) is a FAD dependent enzyme catalyzing the two-electron reduction of quinones to hydroquinones. NQO1 binds to the 20*S* proteasome and recruits important tumor suppressor proteins such as p53 and p73α. The level of NQO1 is also increased in several tumors, making it an important target for anti-cancer prodrugs. Interestingly, the *NQO1* gene is polymorphic and the two most common variants in

humans, excluding the wildtype, feature single amino acid replacements (P187S and R139W). Currently we are investigating the detailed molecular reasons for the loss of function and the reduced stability of NQO1 P187S and NQO1 R139W both of which are implicated in tumor development.

Our former studies not only provided the first crystallographic structure of NQO1 P187S but also an explanation for the biochemical dysfunction of this variant. Since the crystal structure of the NQO1 P187S variant was identical to the wild-type NQO1, the observable differences seem to arise from structural instability in particular in the C-terminal domain (shown in green in the figure below), which is in contact to the site of the amino acid replacement. The current study investigates the possibility to restore the activity and stability of the NQO1 P187S variant by using small molecular-chaperones with the view to use these in cancer chemotherapy for individuals carrying the NQO1*2/*2 allele (thesis project of Emilia Strandback).



Overview of the NQO1 structure. The structure of NQO1 with the cofactor FAD displayed in yellow, the C-terminus in maroon, and the sites of the amino acid replacements in magenta (R139) and blue (P187).

Doctoral Theses completed

Eveline Brodl: *A short story of living lights: Functional studies on the role of LuxF and flavin derivatives in bacterial bioluminescence*

Bioluminescence is the production of light by living organisms. The enzymes encoded by the *lux* operon, with the gene order *luxCDABE(G)*, catalyze a cascade of reactions enabling cold light emission. The *luxA* and *luxB* genes encode the α- and β-subunits, respectively, of the enzyme luciferase. LuxC, LuxD and LuxE constitute the fatty acid reductase complex, responsible for the synthesis of the long-chain aliphatic aldehyde substrate and *luxG* encodes for a flavin reductase. In bioluminescent bacteria, the heterodimeric luciferase catalyzes the monooxygenation of long-chain aliphatic aldehydes to the corresponding acids utilizing reduced flavin mononucleotide ($FMNH_2$) and molecular oxygen. The energy released as a photon results from an excited state flavin-4a-hydroxide, emitting light centered around 490 nm. Some photobacterial strains carry the additional gene *luxF* with the altered gene order *luxCDABFEG*. The exact role of *luxF* and its encoded protein LuxF is still uncertain. However, crystallographic analysis of LuxF revealed the presence of four molecules of a flavin derivative, i.e. 6-(3’-(*R*)-myristyl) FMN (myrFMN), non-covalently bound to the homodimer. This molecule combines two components of the bioluminescent reaction, FMN and a long-chain aliphatic acid.

The elucidation of the role of *luxF* and LuxF and its bound flavin derivative in light emission was the main focus of my work. As a first step, I have evaluated the substrate scope of a bacterial luciferase and tried to relate substrate preferences as a potential determinant for myrFMN formation. Even numbered saturated and α,β-unsaturated aldehydes with eight, ten, twelve and fourteen carbon atoms were examined as potential substrates *in vitro*. Both saturated and unsaturated aldehydes were accepted as substrates, while those with longer chain lengths had higher activity in terms of total bioluminescent light emission than shorter chain lengths. Nevertheless, the mechanism of the flavin derivative formation remained obscure. Further in-depth analysis revealed that myrFMN is produced as a side product of the luciferase-catalyzed reaction. Based on these findings a reaction mechanism for generation of this flavin derivative was postulated. Additionally, it was elucidated that apo-LuxF captures myrFMN and thereby relieves the inhibitory effect on luciferase activity. This suggested that LuxF acts as a scavenger of myrFMN in bioluminescent bacteria. Novel insights into the role of LuxF in bioluminescent light emission were gained via application of an *E. coli* based *lux-rib* expression system where the *lux-rib* operon of *Photobacterium leiognathi* subsp. *mandapamensis* 27561 was cloned into a single expression vector, which allowed the heterologous expression of the complete *lux* operon. To evaluate this novel bioluminescence expression system, a plate reader assay that combines recording of cell growth and bioluminescent light emission intensity over time was developed. Comparing *E. coli* based *lux-rib* expression system with or without *luxF* revealed that the presence of *luxF* enhances light intensity by a factor of 1.5. Furthermore, isolation and analysis by UV/Vis absorption and NMR spectroscopy as well as mass spectrometry of the flavin derivative revealed the presence of more than just the previously investigated myrFMN and led to the discovery of three different flavin derivatives with different alkyl chains.

Shalinee Jha: *Biochemical and metabolic studies towards the characterization of dipeptidyl peptidase-3 knockout mice*

Dipeptidyl peptidase 3 (DPP3) is a metallopeptidase containing a catalytic center with bivalent metal, such as Zn^{2+} or Co^{2+} as cofactor. It belongs to the M49 family, which is characterized by the presence of two signature motifs: HEXXGH and EEXRAE/D, involved in the coordination of the metal ion. DPP3 preferentially hydrolyzes peptides from 4 to 12 amino acids in length. Several bioactive peptides have been identified as DPP3 substrates. These include endogenous opioid peptides and peptides from the renin-angiotensin system. DPP3 has been implicated in an array of pathophysiological processes. It is upregulated in cancer cells and involved in apoptosis modulation and blood pressure regulation. There are strong evidences suggesting its role in endogenous pain regulation and defense against oxidative damage. However, the precise molecular mechanisms underlying the mediation of these effects by DPP3 are still unknown.

This thesis embarks on the metabolic and behavioral characterization of DPP3 knockout mice. Using an amalgamation of biochemical, biophysical and physiological studies, the knockout mice were compared to control wildtype mice. It was found that DPP3 deletion results in altered levels of several angiotensin peptides, thereby consolidating its involvement in the renin-angiotensin pathway, which affects the reno-cardiovascular circuit in mammals. There is also an indication of oxidative stress in the DPP3 knockout mice due to angiotensin II-mediated reactive oxygen species (ROS) production.

The second focus of this thesis revolved around the design and characterization of specific inhibitors against DPP3. These inhibitors can be exploited to gain structural and mechanistic insights into ligand binding to DPP3, which can ultimately reveal its substrate specificity and selectivity. Kinetic screening yielded two compounds – (*R*)-hydroxyethelene (HER) and (*S*)-hydroxyethyelene (SHE), which effectively inhibit DPP3 in vitro. Additionally, a comparison of HER with tynorphin, the strongest inhibitor reported against DPP3, showed that HER is more effective in stably inhibiting DPP3 over a period of time. Towards the end, the thesis presents an outlook into utilizing peptidomic approaches to identify natural substrates of DPP3. Since a peptidase may cleave many substrates in vitro, traditional biochemical approaches for determining endogenous substrates are time consuming and may be unreliable. Therefore, assessing the global changes in the peptidome following the knockout of DPP3 may facilitate identification of peptides likely to be substrates or products of DPP3.

Barbara Konrad: *Biochemical and physiological characterization of the berberine Bridge enzyme-like family in Arabidopsis thaliana*

Biological oxidations play an essential role in life and are involved in a variety of different metabolic pathways. The plant kingdom harbors a large number of proteins catalyzing oxidative reactions and among them is a very interesting family, the berberine-bridge enzyme like (BBE-like) superfamily (Pfam 08031). The BBE-like superfamily belongs to the FAD-linked oxidases (SCOPe d.58.32), which exhibit a characteristic feature, the typical fold of vanillyl-alcohol oxidase (VAO-fold) possessing a FAD and a substrate binding domain. A well-known representative among the BBE-like enzymes in plants is the berberine bridge enzyme from California poppy (*Eschscholzia californica*), which is a branch-point enzyme in the benzylisoquinoline alkaloid biosynthesis.

In the first section of my work, *Ec*BBE variants G164A, V169I and G164AV169I were successfully generated and produced in *Komagataella phaffii* to analyze the oxygen reactivity. A specific oxygen binding pocket was identified on the re-side of the FAD cofactor opposite to the substrate binding pocket and the gatekeeper amino acid residue isoleucine controls oxygen access and reactivity. An inverse exchange of a valine in the oxidase *Ec*BBE to the gatekeeper residue isoleucine resulted in a decrease of O2 reactivity by a factor of 500 and led to the conversion into a dehydrogenase. The substitution of a glycine at the gatekeeper position of *Ec*BBE with an alanine had a slightly stronger effect on oxygen reactivity. The analysis of the double variant revealed no significantly stronger influence. The biochemical function of many BBE-like enzymes in the plant kingdom is still unexplored. Therefore, we chose the BBE-like protein family of Arabidopsis thaliana to broaden our understanding of BBE-like enzymes in plants. The genome of *Arabidopsis thaliana* contains 27 genes encoding for BBE-like proteins, and the main objective of the presented thesis was the biochemical characterization of *At*BBE-like protein 13 and the investigation of the in planta function mainly of *At*BBE-like protein 13 and 15. *At*BBE-like protein 15 was previously identified as a monolignol oxidoreductase, and the same function was shown for *At*BBE-like protein 13. Both enzymes catalyze the oxidation of monolignols to the corresponding aldehydes, suggesting a role in monolignol metabolism and lignin formation. Analysis of the active site composition by multiple sequence alignments revealed four different active site types, which appeared frequently in *A. thaliana* but also in the whole *Brassicaceae* family.

Due to the fact that the physiological function of *At*BBE-like enzymes is largely unknown, we decided to study the phenotype of T-DNA insertional mutant lines. Seedlings from putative loss-of function mutants for *At*BBE-like protein 13 and 15 showed developmental defects in vitro under salt stress compared to wild type. The increased salt sensitivity of *At*bbe-like protein 13 mutants could be confirmed in experiments with soil-grown plants. Another important part of this project was a detailed lignin analysis of the putative *At*bbe-like protein 13 and 15 mutant plants. Thioacidolysis showed a lower aldehyde content and a reduction of the total lignin amount, suggesting a role of monolignol oxidoreductases in providing extracellular aldehydes for the subsequent polymerization of lignin.

A further objective was the physiological investigation of *At*BBE-like protein 28. The oxidase *At*BBE-like protein 28 features a novel active site composition with a mono-covalent instead of a bi-covalent FAD tethering and multiple sequence alignments showed that *At*BBE-like protein 28 exhibits an active site type II. First phenotyping experiments of a putative *At*BBE-like protein 28 loss-of-function mutant showed a 10% reduction of biomass under standard growth conditions on soil, and in vitro salt stress experiments showed a reproducible phenotype with a significantly lower number of healthy green seedlings compared to wild type.

Majd Lahham: *Characterization of a fungal and a yeast flavoprotein*

Flavin-dependent enzymes catalyze many oxidations, including formation of ring structures in natural products. The gene cluster for biosynthesis of fumisoquins, secondary metabolites structurally related to isoquinolines, in the filamentous fungus *Aspergillus fumigates* harbors a gene that encodes a flavoprotein of the amine oxidase family, termed fsqB ("fumisoquin biosynthesis gene B"). This enzyme catalyzes an oxidative ring closure reaction that leads to the formation of isoquinoline products. This reaction is reminiscent of the oxidative cyclization reported for berberine bridge enzyme and tetrahydrocannabinol synthase. Despite these similarities, amine oxidases and berberine bridge enzyme-like enzymes possess distinct structural properties, prompting us to investigate the structure-function relationships of FsqB.

In this thesis, I report the recombinant production and purification of FsqB, elucidation of its crystal structure, and kinetic analysis employing five putative substrates. The crystal structure at 2.6 Å resolution revealed that FsqB is a member of the amine oxidase family with a covalently bound FAD cofactor. N-methyl-dopa was the best substrate for FsqB and was completely converted to the cyclic isoquinoline product. The absence of the *meta*-hydroxyl group, as e.g. in L-*N*-methyltyrosine, resulted in a 25-fold lower rate of reduction and the formation of the demethylated product L-tyrosine, instead of a cyclic product. Surprisingly, FsqB did not accept the D-stereoisomer of N-methyltyrosine, in contrast to N-methyl-dopa, for which both stereoisomers were oxidized with similar rates. On the basis of the crystal structure and docking calculations, I postulate a substrate-dependent population of distinct binding modes that rationalizes the stereospecific oxidation in the FsqB active site.

Emilia Strandback: *Biochemical and biophysical characterization of cancer-associated variants of human NAD(P)H:quinone oxidoreductase 1*

NAD(P)H:quinone oxidoreductase 1 (NQO1; EC 1.6.99.2) is a human FAD-dependent enzyme that catalyzes the two-electron reduction of quinones to hydroquinones. NQO1 plays a crucial role in the antioxidant defense system, where it lowers quinone levels and thereby prevents the formation of reactive oxygen species (ROS). The level of NQO1 is also increased in several tumors making it an important target for quinone based anti-cancer prodrugs.

There are two commonly occurring single nucleotide polymorphisms in the nqo1 gene, NQO1*2 and NQO1*3, that result in protein variants with single amino acid replacements, P187S and R139W, respectively. The distribution of the homozygous NQO1*2 among the population varies between 2 and 20%, whereas NQO1*3 is less frequent with a distribution of 0 to 5%. The amino acid exchange P187S has been shown to severely compromise the activity and stability of the enzyme in vitro and the NQO1*2 genotype has been linked to a higher risk for several types of cancer. Additionally, this mutation is associated with poor prognosis after anthracycline-based chemo-therapy. On the other hand, involvement of NQO1*3 in disease development is not well characterized. In the first part of this thesis the behavior of the R139W variant was investigated. It could be established that the variant protein displays similar biochemical and structural properties as the wild-type enzyme. Consequently, the diminished enzyme activity reported in cancer cells homozygous to NQO1*3 could be attributed to an erroneous splicing event. The second part of this thesis explores the possibility of restoring the activity and stability of the NQO1 P187S variant by using small-molecular chaperones. A combination of methods was employed to first identify potential ligands by virtual screening and to characterize the properties of the most promising hit, N-(2-bromophenyl)pyrrolidine-1-sulfonamide (BPPSA), on the stability and activity of the protein in solution. Several biophysical and biochemical measurements indicated that binding of BPPSA to the P187S variant repopulated the native wild-type conformation. As a consequence of the stabilizing effect, the activity of the variant protein is also strongly improved. These results demonstrate that development of molecular chaperones is a promising concept for the stabilization of unstable protein variants, where the designed molecules can be exploited as a treatment alternative in human diseases caused by protein instability.

Master Thesis completed

Tamara Berger: *Cloning, expression and characterization of two enzymes involved in nicotine biosynthesis*

Among the secondary metabolites, produced by plants, nicotine is one of the most prominent ones. It is mainly found in tobacco (*Nicotiana tabacum*), where it is synthesized as a defense against herbivores. Its synthesis can be upregulated in case the plant gets hurt. As tobacco has been cultivated for at least 3 millennia, it was subject of numerous studies, in particular to characterize the synthesis of nicotine. Despite its intense investigation it was not yet possible to elucidate the nicotine biosynthesis. Some important enzymes involved had already been identified. Two of them were subject of my master´s thesis. The BBE-like enzyme BBLa and A622, an isoflavone reductase-like enzyme. The proteins were expressed in different microorganisms and purified for further investigation. Even though their exact role could not be determined, some pioneering results could be achieved. Copper could be identified as the metal cofactor of A622.

Alissa Pichler: *Mutant characterisation and gene expression analysis in Arabidopsis mutants of the berberine-bridge-enzyme-like family*

BBE-like enzymes are flavoenzymes that received their name from the Berberine Bridge Enzyme (BBE). BBE is found in California poppy (*Eschscholzia californica*) and catalyses the ring closure from (*S*)-reticuline to (*S*)-scoulerine. This oxidoreductase plays an important role in benzylisoquinoline alkaloid biosynthesis. BBE-like genes were also discovered in several different plant families that do not synthesise alkaloids. Besides their involvement in alkaloid biosynthesis, BBE-like enzymes are able to oxidise a variety of substrates. Many BBE-like enzymes were found to be carbohydrate oxidases. For *Arabidopsis thaliana*, 28 BBE-like enzymes were identified. For the presented thesis, three AtBBE-like genes of the same BBE-like subfamily in Arabidopsis, BBE13, BBE15 and BBE26, were chosen for further research. BBE13 and BBE15 are known to be monolignol oxidoreductases and BBE26 could catalyse similar reactions. These three enzymes are suggested to play a role in lignin formation or modification. The aim of the presented thesis was to analyse wild type gene expressions of BBE13, BBE15 and BBE26 in tissues of *A. thaliana* at different developmental stages and gene expression in seedlings under salt stress over 24 hours. Additionally, phenotypes of wild type and triple T-DNA insertional mutant plants were compared over their whole life cycle. BBE13, BBE15 and BBE26 show similar expression patterns throughout the plant's development. Overall these genes seem to mostly play a role in roots and stems of seedlings and adult plants. This supports the hypothesis of an involvement in lignin formation or modification. Over 24 hours of salt stress, the genes show different expression patterns, suggesting different roles in stress response. Results of the presented thesis form a starting point for more experiments, to determine subcellular locations of enzymes, involvement in the plant's development and finally identify the functions of BBE-like genes in Arabidopsis.

International cooperations

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

Matthias Mack, Hochschule Mannheim, Germany

Ralf Reski, Albert-Ludwigs University, Freiburg, Germany

Research projects

FWF P26341: "The family of berberine bridge enzymes in plants"

FWF PhD program "Molecular Enzymology" DK-Molecular Enzymology (W901)

Talks at international conferences

1. Brodl, E.: *Teaching E. coli to shine.* 19th Doc Day, Graz, February 2018.
2. Brodl, E.: *LuxF: a rescue factor in bacterial bio-luminescence?* 20th International Symposium on Bio- and Chemiluminescence, Nantes, May 2018.
3. Eggers, R.: *Characterization of monolignol oxidoreductases from the berberine bridge enzyme-like protein family in* Arabidopsis thaliana. Graduate Seminar, Graz
4. Toplak, M.: *D-lactate dehydrogenase 2 – the natural electron donor of the electron transferring flavoprotein of* Saccharomyces cerevisiae? Graduate Seminar, Graz

Publications

1. Augustin, P., Toplak, M., Fuchs, K., Gerstmann, E. C., Prassl, R., Winkler, A., Macheroux, P.: Oxidation of the FAD cofactor to the 8-formyl-derivative in human electron transferring flavoprotein, (2018) ***J. Biol. Chem.***, 293:2829-2840. DOI:10.1074/jbc.RA117.0008
2. Toplak, M., Wiedemann, G., Ulicevic, J., Daniel, B., Hoernstein, S., Kothe, J., Niederhauser, J., Reski, R., Winkler, A., Macheroux, P.: The single berberine bridge enzyme homolog of *Physcomitrella patens* is a cellobiose oxidase. (2018) ***FEBS J.***, 285:1923-1943; DOI: 10.1111/febs.14458
3. Heckenbichler, K., Schweiger, A., Brandner, L., Binter, A., Toplak, M., Macheroux, P., Gruber, K., Breinbauer, R.: Asymmetric reductive carbocyclization using engineered ene reductases, (2018) ***Angew. Chem. Int. Ed.***, 57:7240-7244. DOI: org/10.1002/anie.201802962.
4. Brodl, E., Niederhauser, J., Macheroux, P.: *In situ* measurement and correlation of cell density and light emission of bioluminescent bacteria. (2018) ***J. Vis. Exp.***, 2018, 136. DOI:10.3791/57881
5. Lahham, M., Pavkov-Keller, T., Fuchs, M., Niederhauser, J., Chalhoub, G., Daniel, B., Kroutil, W., Gruber, K., Macheroux, P.: Oxidative cyclization of *N*-methyl-dopa by a fungal flavoenzyme of the amine oxidase family. (2018) ***J. Biol. Chem.***, 2018, 293:17021-17032. DOI:10.1074/jbc.RA118.004227
6. Brodl, E., Winkler, A., Macheroux, P.: Molecular mechanisms of bacterial bioluminescence. (2018) ***Comput. Struct. Biotechnol. J.***, 16:551-564. DOI:10.1016/j.csbj.2018.11.003
7. Iqbal, N., Stewart, J. D., Macheroux, P., Rudroff, F., Mihovilovic, M.: Novel concurrent redox cascades of (*R*)- and (*S*)-carvones enables access to carvo-lactones with distinct regio- and enantioselectivity. (2018) ***Tetrahedron***, 74:7389-7394. DOI:10.1016/j.tet.2018.11.005
8. Rodrigues, F. A. V., Marschalk, C., Toplak, M., Macheroux, P., de Souza Bonfim-Mendonca, P., Svidzinski, T. I. E., Seixas, F. A. V., Kioshima, E. S.: Promising new antifungal treatment targeting chorismate synthase from *Paracoccidioides brasiliensis*, (2019) ***Antimicrob. Agents Chemother.***, 63:e01097-18. DOI:10.1128/AAC.01097-18
9. Koch, K., Strandback, E., Jha, S., Richter, G., Bourgeois, B., Madl, T., Macheroux, P.: Oxidative stress induced structural changes in the microtubule-associated flavoenzyme Irc15p from *Saccharomyces cerevisiae*. (2019) ***Protein Science***, 28:176-190. DOI:10.1002/pro.3517
10. Rehfeld, L., Funk, E., Jha, S., Macheroux, P., Melander, O., Bergmann, A.: Novel methods for the quantification of dipeptidyl peptidase 3 (DPP3) concentration and activity in human blood samples. (2019) ***J. Appl. Lab. Med.***, *in press*. DOI:10.1373/jalm.2018.027995

Award

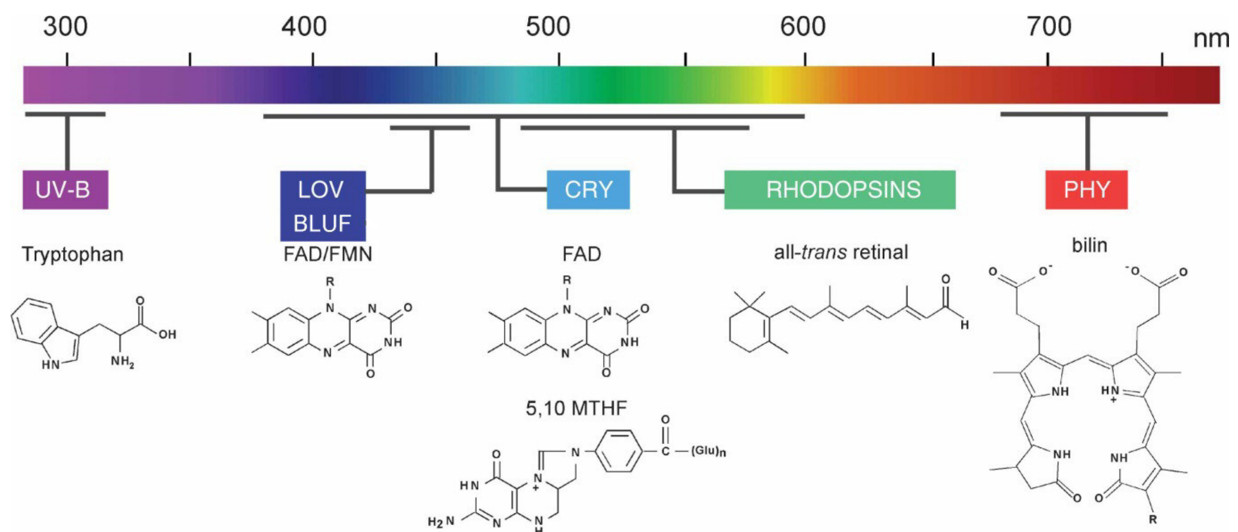
1. **Marina Toplak** was awarded the best presentation prize at the DK Molecular Enzymology graduate seminar, Graz November 2018.

Photoreceptor Group

Group leader: Andreas Winkler
PhD students: Stefan Etzl, Geoffrey Gourinchas
Master student: Julia Färber
Bachelor students: Maximilian Fuchs, Martina Reiter, Sara Trestenjak
Technical staff: Elfriede Zenzmaier

General description

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



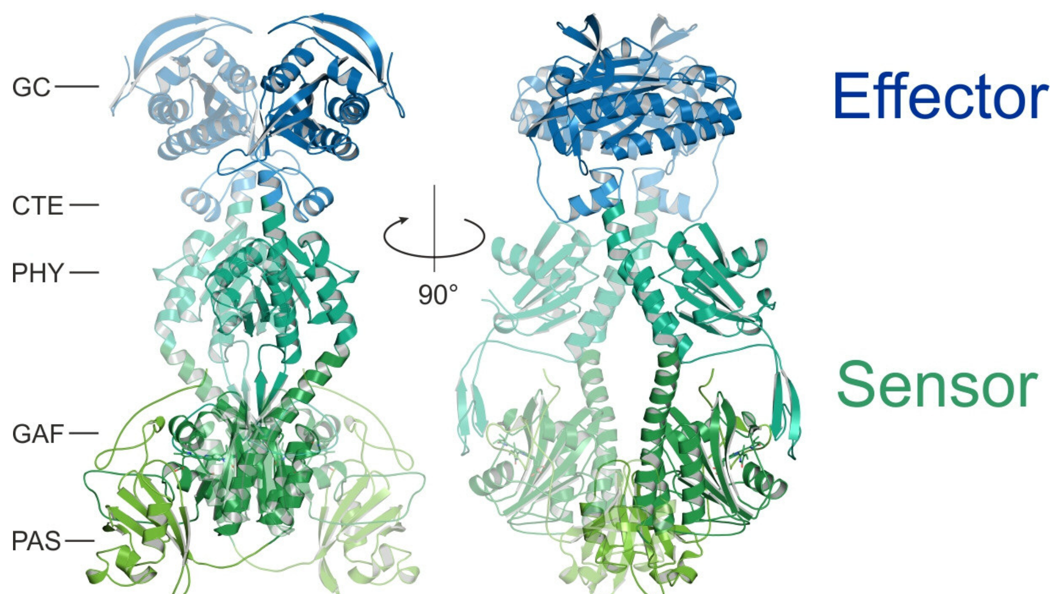
Overview of photoreceptor families and their corresponding cofactors covering the UV/Vis range of the electromagnetic spectrum (adapted from Heintzen WIREs Membrane Transport and Signaling 2012, 1:411–432).

Light-activated cyclases

One 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.*, JMB, 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 will provide new insight into the coupling mechanism of sensor-effector modules. A successful characterization of these systems requires 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 are 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 (thesis project of Stefan Etzl).

Highlights in this project were the elucidation of a full-length structure of a red-light activated adenylate cyclase (see figure below) and the demonstration that it can be used as an effective optogenetic tool in the animal model system *Caenorhabditis elegans*. Starting from a series of constructs featuring a red light sensing bacteriophytochrome linked to an adenylate cyclase domain, we identified several red light-regulated fusions with promising properties. One light-activated construct with high dynamic range and low dark state activity was analyzed in detail. The full-length crystal structure of this phytochrome-linked cyclase revealed molecular details about the photoreceptor-effector coupling, and highlights the importance of a specific regulatory element of adenylate cyclases that precedes the core cyclase domain and was previously overlooked. In combination with the analysis of conformational dynamics by in different functional states, we improve our understanding of phytochrome signaling and integration of the light signal by effector domains. Light-induced conformational changes in the phytochrome destabilize the coiled coil sensor-effector linker element, which in turn releases the cyclase regulatory element from an inhibited conformation, resulting in increased cyclase activity of our artificial system. Future designs of new optogenetic functionalities can benefit from our work on red light-activated adenylate cyclases, in that rational considerations for the effector improve the success rate of initial designs to provide optogenetic tools with superior properties.

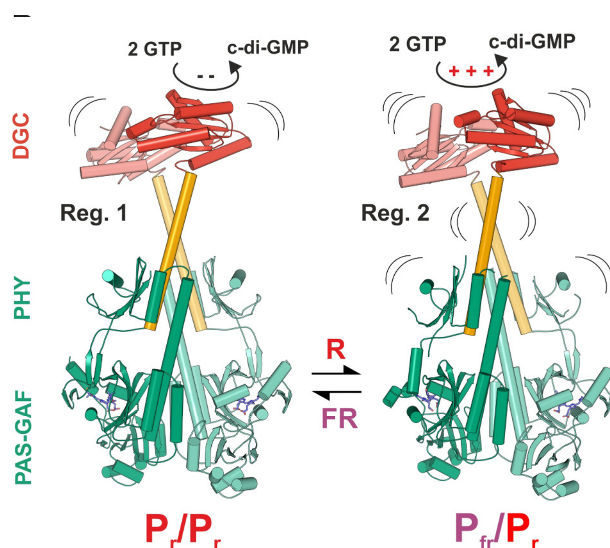


Crystal structure of the bacteriophytochrome-linked adenylate/guanylate cyclase. PAS, GAF and PHY domains are part of the red light regulated phytochrome sensor with its biliverdin chromophore shown as green stick model. The CTE corresponds to the Cyclase Transducer Element, required for efficient signal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

Light-regulated diguanylate cyclases

Another 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. Based on secondary structure predictions a similar architecture to adenylate/guanylate cyclases, which are the focus of the project described above, was proposed for GGDEF domains. 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 project of Geoffrey Gourinchas).

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).



Recently, 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). More research in the direction of asymmetric activation of bacteriophytochromes is part of a recently accepted FWF project.

Doctoral Theses completed

Geoffrey Gourinchas: *Functional Cross-talk of Sensor and Effector Modules in Phytochrome Activated Diguanylyl Cyclases*

The development of organisms is constantly modulated by intricate regulatory networks. Such networks include an array of interacting components that monitor changes in environmental condition to trigger physiological adaptations. Light is a ubiquitous actuator which allosterically affects many lifestyle decisions in all kingdoms of life. The perception of light is allowed by a collection of modular proteins, called photoreceptors, adapted to the broad visible spectrum of light. Phytochromes, which respond to red and far-red light, have attracted special attention due to the deep tissue penetration of red light. Recently, they have been extensively used as building blocks for the generation of near-infrared-based optogenetics tools to control biological process *in vivo* with high spatiotemporal precision. However, the complexity behind the light signal transduction mechanism in phytochromes remains largely unexplained and precludes the rational design of innovative phytochrome-modulated biological tools.

In order to characterize the mechanism of long-range signal transduction in phytochrome, the focus of this thesis was set on naturally occurring Phytochrome activated diguanylyl Cyclases (PadCs) to better understand the structural requirements of enzymatic effector modulation by phytochrome sensors based on naturally evolved systems. We found that fine-tuned conformational dynamics between the various functional domains constituting full-length PadC cause a broad diversification of photoresponses. Supported by new mechanistic insights, the sensor-effector linker region which bridges the two functional moieties is presented as a critical actor involved in light signal transduction and modulation of the diguanylyl cyclase (DGC) effector. The 3D structure of a PadC homolog in the dark-adapted condition as well as in a signalling active conformation revealed the complexity of the molecular cross-talk between the phytochrome and the DGC that in the case of PadC appears to require structural asymmetry in the dimer. Single protomer activation allows the signal transduction to the DGC effector, thereby highlighting asymmetric phytochrome homodimers as a potential evolutionary bisection of bacteriophytochromes to adapt to the modulation of specific effector domains. Beyond the apparent similarity of global photoactivation mechanisms between bacteriophytochromes and other phytochromes subfamilies, we show that phytochromes have evolved a fine-tuning of specific interactions between functional domains allowing an astonishing diversification of photoresponses and regulation of diverse effectors.

Stefan Etzl: *Design and Functional Characterization of Artificial Phytochrome-linked Cyclases*

During the last decades, the advances in the field of optogenetics have led to impressive applications and ground-breaking findings. The concept of optogenetics is to employ naturally occurring or engineered photoreceptor proteins as tools to manipulate cellular signalling in a highly defined temporal and spatial manner. The generation of new optogenetic tools still proves a delicate task, as the underlying molecular processes of signal transduction in photoreceptors are frequently not understood in enough detail.

In order to investigate the rationales for successful signal transduction between photosensors and their enzymatic effectors, this work focused on the generation of artificial fusion proteins consisting of red light-sensitive phytochromes and cyclases. For the structure-guided design process of these fusion constructs, current findings about specific structural elements important for cyclase regulation were considered. In a first series of fusions, the photosensory part of a bacteriophytochrome from *Deinococcus radiodurans* (*Dr*BphP) was linked to a cyanobacterial guanylyl cyclase domain, including the cyclase transducer element (CTE). The presumed alpha-helical linker part was sequentially altered by single amino acid insertions or deletions. Several light-regulated constructs with promising properties for applications as optogenetic tools could be identified and characterized with biochemical and biophysical methods. Interestingly, two light-activated constructs were separated by seven amino acids in linker length, indicating that the helical register is crucial for functional light-regulation. The crystal structure of a light-activated construct with a truncated C-terminus of the cyclase part showed the first structure of *Dr*BphP with an enzymatic effector attached and revealed a coiled coil linker between the photosensory module and the cyclase effector. HDX-MS analyses of the light-regulated constructs indicated a pronounced structural change in the coiled coil linker upon light-illumination. The crystal structure of an additional light-activated construct confirmed a coiled coil linker and revealed the structure of the cyclase's C-terminus as an unexpected domain. Derivatives of this fusion construct with the same overall linker length, but altered linker sequence lost light-responsiveness. The analysis of further variants, featuring uncoupling from the protein's signalling state from the photocycle of the chromophore, highlighted the relevance of the linker composition for signalling. To compare, how the observations made in the initial fusion series translate to other sensor-effector combinations, additional series were designed in a corresponding manner with another bacteriophytochrome and a second guanylyl cyclase. In total, series from four combinations of phytochromes and cyclases were available for comparative analysis. Despite the different components, light-regulation and the typical heptad repeat pattern was observed in all series.

In summary, the data suggest that the helical register, i.e., the relative positioning of sensor and effector domains is important for functional light-regulation. The signal transduction process is based on a complex interplay of conformational dynamics of several structural elements, in which the composition of the linker plays a key role for the determination of activity and dynamic range of the regulated effector.

Master Thesis completed

Julia Färber: *Biochemical characterization of blue light-regulated LOV-diguanylate cyclases*

LOV-diguanylate cyclases are light-sensitive proteins, responding to blue light. Bacteria use this environmental stimulus for the regulation of their cellular functions, like enzymatic activities that correlate with the production of the bacterial second messenger c-di-GMP. At the C-terminus of these photoreceptors, GGDEF domains work as effectors, featuring the catalytic activity of diguanylate cyclases. As output domains, they are responsible for downstream signaling. In addition, such proteins contain an N-terminal LOV sensor as input domain, which uses flavin as cofactor. Upon photon absorption at 445 nm, this results in a light-activated adduct state of the photosensory domain. Sensor and effector domains are covalently linked to each other via specific supercoiled α-helices that are essential for signal transduction within LOV photoreceptors.

In this project, two naturally occurring as well as two engineered LOV-GGDEF versions were structurally and functionally characterized to get knowledge about the modulation of blue light-regulated LOV-diguanylate cyclases. In addition, the chimeras LPadC_A and LPadC_B are combinations of one of the two constructs with *Is*PadC (phytochrome-activated diguanylate cyclase) that originates from *Idiomarina species* A28L.

Full-length LadCs show aggregation tendencies and oligomerization after size-exclusion chromatography, influenced by different light conditions. In contrast, all the other tested systems exhibit light-independent dimeric states, leading to high protein stability. In addition, some LadCs show a high 100-fold activation upon illumination, whereas LPadC_B is about 10-fold activated by blue light exposure in comparison to darkness. In contrast, LPadC_A offers low enzymatic activity under light conditions and but no detectable activity in the dark. Therefore, all tested proteins appear to be light-activated systems.

International cooperations

Ilme Schlichting, Max Planck Institute for Medical Research, Germany

Matthew Nelson, Saint Joseph's University, Philadelphia, U.S.A.

Peter Hildebrandt, TU Berlin, Berlin, Germany

Research projects

FWF P27124: “Structure-function studies on signal transduction in photoactivatable cyclases”

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

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

Talks at international conferences

1. Winkler, A.: *Molecular mechanisms involved in asymmetric activation of bacteriophytochromes*. SFB 1078 - International Phytochrome Workshop 2018, Berlin, October 2018.
2. Etzl, S.: *Signal transduction in artificial red light-regulated cyclases*. 20th DocDay, Graz, July 2018.
3. Winkler, A.: *Asymmetric Activation Mechanism of a Homodimeric Red Light Regulated Photoreceptor*. Gordon Research Conference: Photosensory Receptors & Signal Transduction, Barga, March 2017.
4. Etzl, S.: *Molecular mechanisms of red-light regulation in phytochrome linked adenylate/guanylate cyclases*. Gordon Research Seminar: Photosensory Receptors and Signal Transduction, Barga, March 2017.

Publications

1. Gourinchas, G., Heintz, U., Winkler, A.: Asymmetric activation mechanism of a homodimeric red light-regulated photoreceptor. (2018) ***eLife***, 7, e34815; DOI:10.7554/eLife.34815

2. Etzl S., Linder R., Nelson M. D., Winkler A.: Structure-guided design and functional characterization of an artificial red light-regulated guanylate/adenylate cyclase for optogenetic applications. (2018) ***J. Biol. Chem.***, 293(23), 9078-89; DOI:10.1074/jbc.RA118.003069

3. Augustin P., Toplak M., Fuchs K., Gerstmann E. C., Prassl R., Winkler A., Macheroux P.: Oxidation of the FAD cofactor to the 8-formyl-derivative in human electron transferring flavoprotein. (2018) ***J. Biol. Chem.***, 293(8), 2829-40; DOI:10.1074/jbc.RA117.000846

4. Toplak, M., Wiedemann, G., Ulicevic, J., Daniel, B., Hoernstein, S., Kothe, J., Niederhauser, J., Reski, R., Winkler, A., Macheroux, P.: The single berberine bridge enzyme homolog of *Physcomitrella patens* is a cellobiose oxidase. (2018) ***FEBS J.***, 285:1923-1943; DOI: 10.1111/febs.14458

5. Brodl, E., Winkler, A., Macheroux, P.: Molecular mechanisms of bacterial bioluminescence. (2018) ***Comput. Struct. Biotechnol. J.***, 16:551-564. DOI:10.1016/j.csbj.2018.11.003

6. Gourinchas, G., Vide, U., Winkler, A.: Influence of the N-terminal segment and the PHY-tongue element on light-regulation in bacteriophytochromes (2019) ***J. Biol. Chem***. In press. DOI:10.1074/jbc.RA118.007260

7. Gourinchas, G., Etzl, S., Winkler, A.: Bacteriophytochromes - from informative model systems of phytochrome function to powerful tools in cell biology (2019) ***Curr. Opin. Struct. Biol.*** in press

Award

1. **Geoffrey Gourinchas** was awarded the Best Poster Prize at the Gordon Research Conference: Photosensory Receptors and Signal Transduction 2018 in Barga, Italy.

Protein Design Group

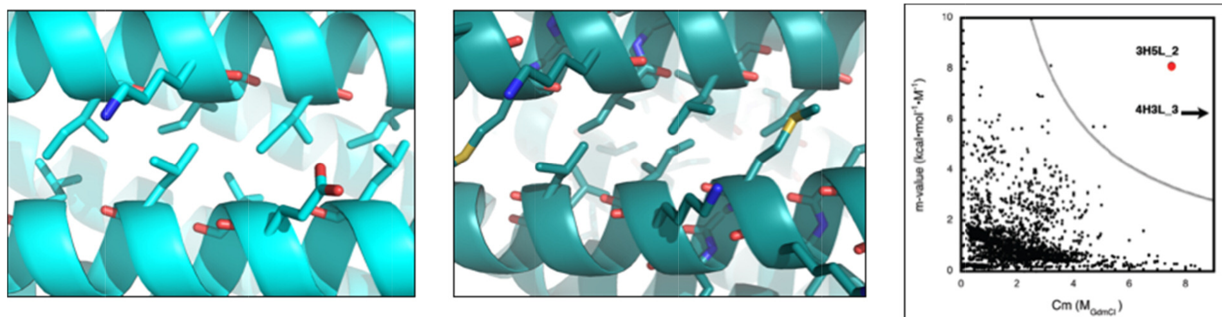
Group leader: Gustav Oberdorfer
PhD students: Wael Elaily
Master students: Dominik Fridrich, Adrian Tripp
Bachelor students: Stella Ebner, Markus Braun
Technical Staff: Alma Makic

General description

Computational design of novel protein structures is a promising tool to make superior biological materials with tailor-made properties, new pharmaceuticals or complex fine chemicals. Tremendous progress has been made over the last five years in the field of *de novo* protein design, ranging from a set of rules for designing small, ideal α,β-proteins to design protocols for α-helical proteins with ultra-fine control of backbone geometry and unprecedented thermodynamic stabilities. In addition, proteins with repeating units exhibiting more diverse repeats than observed in nature as well as an ideal version of nature's most prolific fold - the TIM-barrel – have been designed and characterized. Moreover, while computational tools to make these types of novel proteins are becoming more robust, yielding thousands of hypothetical proteins, high-throughput biochemical testing has become feasible as well. Together, all these advancements paved the way to investigate functionalization of *de novo* proteins. Over the last year we focused on two different protein design problems – the design of helical protein pores and the catalytic functionalization of single chain *de novo* proteins.

Computational design of de novo protein pores with custom geometries

In recent years an exceptionally well working software suite – Rosetta (Leaver-Fay, A., et.al. (2011) Methods in Enzymology, pp 545-574.) – has been developed, which has had significant success in designing protein structures, protein catalysts and protein-protein interactions from scratch. During my postdoctoral training in the group of Prof. David Baker at the University of Washington in Seattle, we further developed Rosetta and added methods that use equations originally derived by Francis Crick in 1953 to accurately describe the geometries of α-helical protein structures and to sample the folding space of helical bundle proteins computationally. We established a computational method to iteratively sample this parameter space with different levels of detail. First, a wide range of helical parameters is screened in a coarse-grained fashion to find reasonable design starting points. This is followed by finer sampling around those identified parameters. Combinatorial design calculations then identify low energy sequences for various helix supercoil arrangements, and the designed helices in the lowest energy arrangements are connected by loop modeling. By applying this method, we designed an antiparallel monomeric untwisted three-helix bundle with 80 residues per helix, an antiparallel monomeric right handed four-helix bundle, and a pentameric parallel left handed five-helix bundle. The designed proteins were extremely stable (extrapolated $\Delta G_{fold} > 60$ kcal mol^{-1}), and their crystal structures are close to identical with the design models with nearly perfect core packing between the helices (knobs-into-holes, Figure below, Oberdorfer, G., Huang, P. S., Xu, C., et. al., (2014) Science 346, 481-485).

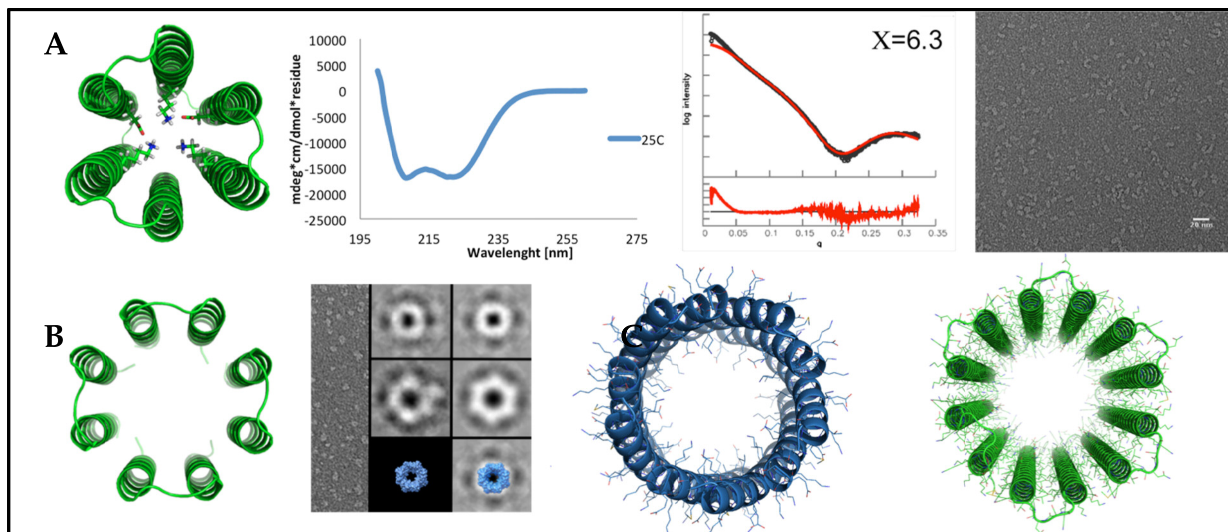


Computationally designed helix-helix interface. (left and center panel) Through iterative fine sampling of backbone geometries, the computations converged on 'ideal' knobs-into-holes packing arrangements, without enforcing of sequence motifs to achieve this type of packing. (right panel) Figure taken from (Oberdorfer, G., Huang, P. S., Xu, C., et. al., (2014) Science 346, 481-485). A plot showing data (black dots) harvested from ProTherm (Kumar, M. D., et. al. (2006) Nucleic acids research. 34, D204-206.). X axis - GdmCl denaturation midpoint (Cm); Y axis - dependence of folding free energy on GdmCl concentration (m value); red circle and black arrow indicate stability of two previously reported designed helical proteins. The free energy of folding in the absence of denaturant is the product of the m-value and the Cm; the curve m-value × Cm = 25 kcal/mol (gray) separates almost all native proteins from the two designs. 4H3L_3 (black arrow) does not denature in GdmCl.

Helix bundles usually are comprised of repeating protein backbones. These repeating geometries are good targets for design since there are fewer distinct side-chain packing problems to be solved. We identified three distinct repeating geometries that require deviation of less than three degrees from an ideal unstrained helix. First, a Cα-step size of 102.85° (which is 2.85° from the ideal value of 100.0°) results in the classic heptad repeat (after seven residues the helix has completed two full turns (720°)). Second, with a step size of 98.2° the helix has completed three full turns (1080°) after 11 residues and third, if the step-size is exactly 100°, the helix has completed five full turns (1800°) after 18 residues. We refer to these three cases as 2-layer, 3-layer, and 5-layer designs, respectively, corresponding to the number of distinct helix-helix interacting layers that must be designed.

We explored the design of 2-layer, 3-layer and 5-layer helix bundles with six, eight, ten and twelve helices surrounding the supercoil axis. Parametric generation of the protein backbones is very fast and each individual computational design calculation takes only about 50 seconds for modelling problems with repeating units and symmetric helical arrangements. The approach enabled the custom design of hyperstable proteins with fine-tuned backbone geometries and the results validated the computational approach and represented a significant advance in the field of *de novo* protein design (Figure below). Biochemical and biophysical characterization of one 6- and one 8-helix bundle design show that they are readily expressed in *E. coli* and show α-helical circular dichroism signals. Small-angle X-ray scattering measurements display very close fits of theoretical versus measured scattering curves, indicating that the overall structures of the designs are similar to the ones occurring in solution. In addition, negative-stain EM images show monodisperse particles with averages that could resemble the designed molecules.

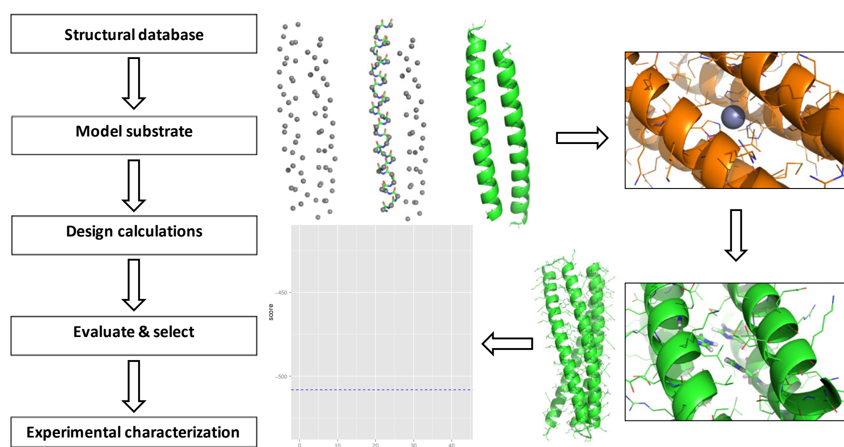
Since the parametric generation of helical backbones is very fast, it is now possible to generate thousands of helical protein structures with feasible backbone geometries in hours. Combined with the emerging technological advances in oligonucleotide synthesis, this approach opens the possibility to generate and test large computational design libraries experimentally.



Experimental verification of designs. **(A)** Computationally designed 6-helix bundle without supercoil twist. A synthetic gene, encoding for the designed sequence was ordered and expressed in *E. coli*. The resulting protein was purified and analyzed using circular dichroism (CD), small-angle X-ray scattering (SAXS) and negative stain electron microscopy (EM). All biophysical data indicate that this protein is similar to its design model. **(B)** A designed antiparallel 8-helix bundle. Biophysical analysis gave α-helical CD-signals that showed no transition even at 95°C and monodisperse particles on a negative stain EM grid. 2D class-averages show design-like properties. **(C)** First computational design of a 2-layer 10-helix (blue, left) and a 5-layer 12-helix bundle (green, right). Both designs followed the same design strategies as described in our original paper (Huang et al., 2014), using my iterative generation and design protocol. Due to the size of both designs, ab-initio structure prediction didn't converge on the designed structures, which can mainly be attributed to sampling issues.

Design of single chain helical proteins with local deformations

We are developing a new computational protocol to design *de novo* proteins harboring a binding/functional site. To achieve this goal, we use the parametric design pipeline developed previously and devise a strategy that uses it to generate ensembles of backbones with a particular functional site in mind.



Schematic overview of the design workflow. (left) According to a rough geometric constraint, pertinent to the diameter of the envisioned binding site, a structural database is generated, followed by placement of the binding site and downstream design with constraints on keeping the binding site in place. This will deform the designed structures around the binding site, but will enable ideal packing around it. (right) Graphic representation of the design workflow.

To test different levels of deviation from ideal coiled-coil geometry in the helical backbones, we chose functional sites of different sizes, in particular we designed a type-2 copper center and a bis-his coordinated heme-moiety. Both of which have been designed into single stranded three and four helix bundles. We started to characterize the copper center designs, as a minimal model system and after characterization thereof attempt to characterize the bis-his coordinated heme group designs. The type-2 copper center is an ideal candidate site to start with, as it can act as a nitrate reductase in addition to binding Cu^{2+} and other divalent cations. With these designed proteins our goal is to design the first genetically encodable *de novo* catalytic protein. To accomplish this, we will follow a general design workflow that we refer to as 'Active Site Repurposing', which is based on the notion that during evolution, naturally occurring enzymes inherit their function from family members and precursors and are able to develop new functionalities by repurposing their own active site to catalyze a new reaction or bind to a novel ligand. This is usually facilitated by sequence optimization. We are following a similar approach *in silico* by utilizing characterized functional sites derived from naturally occurring proteins for our design calculations. From a design perspective, this means that it is possible to only search around an energy minimum, in which the best geometries for binding/catalysis have already been identified and the remaining interactions in the designed protein are optimized for packing only.

International cooperations

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

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

William DeGrado, Institute for Neurodegenerative Diseases, UCSF, United States

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

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

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

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"

Talks at international conferences

1. Oberdorfer, G.: *Functionalization of de novo helix bundles by deviating from ideal geometries*. TU Munich Physics winter school, Tux, Austria, January 2018
2. Oberdorfer, G.: *Proteins made to order: Computational design of de novo proteins with custom geometries*. CuPiD – Computational Protein Design Workshop, Bristol, UK, May 2018

Publications

1. Marcos, E., Chidyausiku, T. M., McShan, A. C., Evangelidis, T., Nerli, S., Carter, L., Nivón, L. G., Davis, A., Oberdorfer, G., Tripsianes, K., Sgourakis, N. G. and Baker, D. *De novo* design of a non-local β-sheet protein with high stability and accuracy. (2018) ***Nat. Struct. Mol. Biol***., 25, 1028–1034; DOI: 10.1038/s41594-018-0141-6

2. Shen, H., Fallas, Jorge A., Lynch, E., Sheffler, W., Parry, P., Jannetty, N., Decarreau, J., Wagenbach, M., Vicente, J. J., Chen, J., Wang, L., Dowling, Q., Oberdorfer, G,. Stewart, L., Wordeman, L., De Yoreo, J., Jacobs-Wagner, C., Kollman, J. and Baker, D. (2018) *De Novo* Design of Self-assembling Helical Protein Filaments. (2018) ***Science***, 362 (6415), 705-709; DOI: 10.1126/science.aau3775

Cellular Metabolism Group

Group leader: Juliane Bogner-Strauss (on leave)
Postdoctoral fellow: Melina Amor
PhD students: Furkan Alkan, Dina Hofer, Katharina Huber, Katharina Walter, Wenmin Xia, Gabriel Zirkovits
Master students: Jürgen Novak
Administration / Technical Staff: Claudia Gaug (part time), Thomas Schreiner

General description

Over the last 5 years we focused on the investigation of new candidate genes/proteins with respect to adipogenic development, energy metabolism and associated disorders. We use various murine/human white/brown adipogenic and cancer cell lines and several mutant mouse models to uncover the molecular circuits that control fat cell development and cancer cell proliferation by sustaining their energetic and biosynthetic needs.

N-acetyltransferase 8-like (Nat8l)

In the brain, Nat8l is known to catalyze the formation of *N*-acetylaspartate (NAA) from acetyl-CoA and L-aspartate. NAA acts as a carrier of acetyl groups that can subsequently be incorporated into neutral lipids. The metabolic importance of NAA has been shown in two inborn human neurodegenerative disorders, where defects in NAA biosynthesis as well as catabolism lead to reduced lipid synthesis. Our group was the first to show that Nat8l is highly expressed and active in brown and white adipocytes and thereby impacts energy and lipid metabolism. Nat8l seems to be a promising target to treat obesity and it is associated with disorders by activating brown adipocyte metabolism and thereby dissipating energy as heat instead of storing it. Furthermore, levels of NAA have also been found to be elevated in lung and ovarian tumors compared to corresponding healthy tissues. This increase of NAA correlates with worse patient outcome. Katharina Eva Walter is investigating the impact of NAA/Nat8l/Aspa on cancer energy metabolism. The presence of NAA reduces ER-stress and increases protein synthesis upon nutrient starvation. An understanding of how the NAA pathway supports tumor growth will path the way for novel potential therapeutic strategies to combat cancer. Further studies will unravel the mechanism of NAA in cancer.

Aralar (AGC1)

AGC1 (mitochondrial **A**spartate-**G**lutamate **C**arrier 1) exports aspartate from mitochondria to cytosol. Aspartate is a non-essential amino acid that is produced in mitochondria as a result of tricarboxylic acid (TCA) cycle and potentially an important precursor of macromolecule biosynthesis in cancer cells. Furkan Alkan has been working on understanding the metabolism of cancer cell proliferation. Cancer cells mostly require extracellular nutrients -such as glucose and glutamine- for growth and survival. Yet, some cancer cells have adapted their metabolism to survive and proliferate even in nutrient limiting conditions. In this project, we have found that expressing AGC1 helps sustaining survival and proliferation of cancer cells in glutamine-limiting environments. Mechanistically, we clarified that cancer cells suffer from aspartate shortage following glutamine limitations and the aspartate exported from mitochondria to cytoplasm via AGC1 is crucial for maintaining protein and DNA synthesis.

Aspartoacylase (Aspa)

While Nat8l catalyzes the formation of N-acetylaspartate (NAA) from acetyl-CoA and L-aspartate, Aspa deacetylates NAA and produces acetate and L-aspartate. Thereafter, the acetate moiety is reutilized for acetyl-CoA synthesis and subsequently incorporated into lipids. Additionally, aspartate is released after cleavage of NAA by Aspa and available in the cytosol for nucleotide/amino acid synthesis. Both, acetyl-CoA and aspartate play an important role in cancer cell proliferation and survival. We showed that silencing or overexpression of Aspa in cancer cell lines impact their proliferation capacity and tumor growth in xenograft models. Moreover, Katharina Eva Walter could show (part of her PhD thesis) that apoptosis and ER-stress marker are increased upon Aspa overexpression compared to controls when nutrients are scarce. These effects could be reduced with additional NAA treatment upon low nutrient conditions. Further studies are planned to investigate the mechanism behind.

Matrix metalloproteinase 12 (MMP-12)

Previous studies and our preliminary data point to matrix metalloproteinase 12 (MMP12) as an interesting molecular target for the treatment of obesity-associated Type 2 Diabetes and cardiovascular diseases. MMP12 abundance in several metabolic tissues is highly increased in the obese state when compared to the lean state and has been related to the development of a wide spectrum of pathological conditions, including insulin resistance and atherosclerosis, main features behind Type 2 Diabetes and cardiovascular diseases, respectively. We hypothesize that blocking MMP12 may lead to a general improvement in metabolic and cardiovascular parameters associated with obesity. In this project, Melina Amor is exploring mechanistic aspects associated with the pathological role of MMP12. *In vitro* neutralization of MMP12 showed improved insulin sensitivity and decreased pro-inflammatory gene expression in murine and human adipogenic cell lines. In line with these results, MMP12 levels have been measured in serum from severely obese patients and significantly correlated with insulin resistance parameters and inflammatory markers. Within the framework of this project, Manca Zupan completed her international research stay (February-September 2018). Her work was mainly focused on the overexpression of MMP12 in brown and white adipocytes. In the upcoming future we aim to investigate the role of MMP12 in a mouse model that simultaneously develops both pathological conditions giving the advantage to better mirror the human disease. This will be performed via whole-body genetic deletion of MMP12 and additionally by using a novel, vaccination-based therapeutic strategy. In conclusion, this project will provide a comprehensive investigation of a promising target, which could directly support further studies in humans.

PhD Theses completed

Dina Hofer: *Messing up with fat – Investigation of novel genes involved in adipogenesis and adipocyte metabolism*

Adipose tissues (AT) are critical regulators of whole-body energy homeostasis. Imbalances in AT metabolism are therefore associated with the development of a variety of devastating diseases, including type 2 diabetes, cardiovascular diseases, and even cancer. To develop targeted treatment strategies for obesity and its comorbidities, it is important to get a broader understanding of AT physiology. Microarray experiments proposed peroxisomal biogenesis factor 16 (PEX16, gene: Pex16) and aspartate N-acetyltransferase (Asp-NAT, gene: Nat8l) as promising candidates to investigate in adipocytes.

PEX16 is important for peroxisome de novo biogenesis at the endoplasmic reticulum. Although suggested by previous studies, the relevance of peroxisomes for adipocyte differentiation and function remained poorly understood. We showed that Pex16 is robustly expressed in AT and upregulated during adipogenic differentiation of human and murine cells. Further, we demonstrated that Pex16 is a direct and functional target gene of the adipogenesis "master-regulator" PPARγ. Pex16-silencing in white adipogenic 3T3-L1 cells diminished peroxisome number and peroxisome-associated lipid metabolism. Furthermore, Pex16-silencing reduced cellular respiration and increased fatty acid release. Importantly, silencing of Pex16 impaired adipogenesis. However, treatment with PPARγ agonist rosiglitazone and peroxisome produced ether lipids rescued adipogenesis upon Pex16-silencing. Contrarily, overexpression of Pex16 in 3T3-L1 cells increased peroxisome number and activity. Altogether, our data demonstrate that PEX16 is crucial for peroxisome formation in adipocytes and highlight the importance of peroxisomes for adipocyte differentiation and lipid homeostasis.

Further, we investigated the role of Asp-NAT and its product N-acetylaspartate (NAA) in AT and whole-body energy homeostasis. NAA is highly abundant in brain and regarded as important storage form for acetyl-CoA used for lipid synthesis. It is synthesized by Asp-NAT from L-aspartate and acetyl-CoA and catabolized by aspartoacylase (ASPA) to aspartate and acetate. In white adipocytes, we found that Asp-NAT localizes to mitochondria and ASPA to the cytosol in white adipocytes. Further, whole-body or adipocyte-specific loss of Nat8l/Asp-NAT in mice reduced body weight, increased energy dissipation, and improved glucose tolerance on chow diet, high-fat diet, and fat-free diet. Moreover, Nat8l-deficient adipocytes and AT showed increased cellular respiration, elevated ATP synthesis, and an induction of browning. However, lipid turnover was impaired upon Nat8l-deficiency. Accordingly, 75% of Nat8l-knockout mice died on fat-free diet after weaning, but could be rescued by NAA supplementation, suggesting NAA as a powerful energy metabolite. In line, NAA-treatment of mice reduced energy dissipation, glucose tolerance, and insulin sensitivity. Furthermore, NAA-treated adipocytes showed reduced cellular respiration and lipolysis, but increased de novo lipogenesis. Collectively, these data revealed the NAA pathway and NAA per se as important players in the regulation of adipocyte energy metabolism and overall determination of metabolic health.

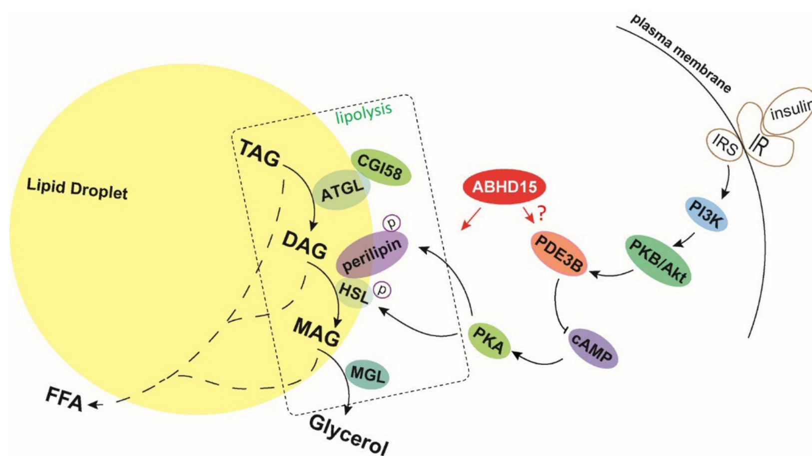
Wenmin Xia: *The role of alpha/beta hydrolase domain containing 15 (ABHD 15) in lipid metabolism*

Adipose tissue (AT) is an important multifunctional organ which serves as a commander of energy homeostasis. Dysregulated lipid and glucose metabolism in AT result in insulin resistance and the development of obesity and type 2 diabetes. There are still remaining questions on insulin-regulated lipolysis, which is highly relevant as elevated circulating fatty acids (FA) contribute to obesity-associated metabolic complications.

My thesis shows that α/β-hydrolase domain-containing 15 (ABHD15) is indispensable for the stability of phosphodiesterase 3B (PDE3B) and insulin-inhibited lipolysis in white adipose tissue (WAT). ABHD15 is highly expressed in energy metabolism tissues, such as brown adipose tissue (BAT) and WAT. WAT ABHD15 expression is strongly downregulated by fasting, while upregulated by refeeding. The nutritional transition from fasting to refeeding is tightly controlled by insulin in WAT. Thus we applied insulin injection and glucose gavage to investigate which insulin-related pathways are changed by Abhd15 deletion in mice. We found that neither insulin nor glucose treatments can suppress free fatty acids (FFAs) release

in total and AT-specific Abhd15 knockout (Abhd15-ko and Abhd15-ako) mice. In addition, insulin-regulated glucose uptake via protein kinase B (PKB/Akt) signaling was impaired in Abhd15-ko adipocytes, thus de novo lipogenesis from glucose was also reduced. Our in vitro data revealed that ABHD15 associates with PDE3B to stabilize its protein level. Consistently, PDE3B expression is decreased in the absence of ABHD15 in mouse. This mechanistically explains the increased protein kinase A (PKA) activity, HSL phosphorylation and undiminished FFAs release by insulin in Abhd15-ko mice. Due to the unsuppressed FFAs release and disrupted insulin signaling, Abhd15-ko mice eventually develop insulin resistance upon aging and high fat or glucose diet. To further support our animal results, we found that ABHD15 expression is decreased in obese, diabetic patients compared to obese, non-diabetic patients. Our results identified ABHD15 as a potential therapeutic target to mitigate insulin resistance.

Despite the fact that ABHD15 plays a key role in the node of insulin-regulated lipolysis pathway in WAT, ABHD15 might play a different role in BAT. We found that Abhd15-ako mice are catecholamine sensitive. With an acute β3 adrenergic receptor agonist administration, Abhd15-ako showed a higher O2 consumption and energy expenditure than control mice. Our preliminary data suggest that this might be due to the increased FFAs release in WAT which promotes the FA oxidation in BAT of Abhd15-ako mice.
In summary, my studies provide an important contribution to understand the physiological function of ABHD15, also including comprehensive information on the newly generated Abhd15 total and AT-specific deleted animal models which will benefit future studies.



Proposed working model of ABHD15, Copyright Bogner-Strauss Lab

Katharina Huber: *Analyzing cellular metabolism in adipose tissue and sub-cellular compartments of biological systems*

Cellular metabolism refers to all life-sustaining chemical reactions occurring within living organisms. Abnormalities in cellular metabolism perturb normal physiology and cause e.g. adipose tissue (AT) dysfunctions resulting in obesity, type 2 diabetes and cancer. Brown adipose tissue (BAT) is well recognized for its ability to dissipate energy in form of heat and its subsequent contribution to increased energy expenditure and regulation of insulin sensitivity renders it a promising target for anti-obesity strategies. In order to develop targeted therapies, it is of critical importance to uncover metabolic perturbations and to understand novel metabolic pathways that control energy metabolism.

Aspartate N-acetyltransferase (Asp-NAT, encoded by Nat8l) and its product N-acetylaspartate (NAA) are highly abundant in BAT and increased Nat8l expression was reported to accelerate energy expenditure and lipid turnover. However, the accurate mechanism and physiological stimuli of the NAA pathway are not well defined. In this thesis, I demonstrate that increased nutrient provision and particular high glucose concentrations activate the entire NAA pathway. NAA is preferentially synthesized from glucose-derived acetyl-CoA and aspartate. Increased NAA pathway activity drains glucose-derived acetyl-CoA into NAA thus influencing lipid turnover, levels of TCA cycle intermediates and amino acid degradation. Rewiring of cellular metabolites might be responsible for the observed upregulation of compensatory mechanisms. Mechanistically, increased Asp-NAT activity caused a combined activation of neutral lipolysis and lipophagy to facilitate increased lipid degradation and subsequently elevated mitochondrial respiration. Collectively, the NAA pathway is an important regulator of energy homeostasis in brown adipocytes. Therefore, the NAA pathway is suggested as a promising tool to fight metabolic complications.

Advances in metabolite profiling contribute to characterization of various diseases. However, whole-cell extracts paint an incomplete picture of metabolism by providing only average metabolite concentrations. The established fractionation method allows the analysis of compartmentalized metabolic changes that cannot be resolved upon whole-cell analysis. Acyl-CoA thioesters are central metabolic intermediates in numerous anabolic and catabolic pathways, including fatty acid biosynthesis, β-oxidation, TCA cycle, and cholesterol and isoprenoid biosynthesis. The sub-cellular fractionation approach was validated in different cell systems and demonstrated the applicability of this technique to a variety of cells and even human tissue. The method allowed the detection of different distribution pattern of acyl-CoA-species in distinct cellular compartments. Tracing studies revealed acyl-CoA thioester detection in mitochondrial, cytosolic and nuclear compartments with distinct kinetics of label incorporation. This method might substantially contribute to the understanding of cellular metabolism, elucidating the role of acyl-CoA thioesters in various disease settings, and targeting of physiologically important enzymes that utilize acetyl-CoA substrates.

Master Theses completed

Jürgen Novak: *The role of α/β-hydrolase domain containing protein 15 (ABHD15) in brown adipose tissue*

Overweight and obesity became an increasing worldwide problem. A sedentary lifestyle and excess of food led to an increase morbidity of non-communicable diseases. Hence, research focused with growing interest on the physiology of adipose tissue (AT). Mammals possess at least two types of AT, which can be distinguished into white adipose tissue (WAT) as storage organ and energy provider and brown adipose tissue (BAT) that dissipates energy to generate heat. This thesis intended to investigate the role of α/ß hydrolase domain containing protein 15 (ABHD15) in BAT and brown adipocytes. Studies have so far concentrated on the role of ABHD15 in WAT or white adipocytes. The aims of this thesis were to: 1) elucidate potential effects of constitutively Abhd15-knockout at the single gene and pathway levels in BAT, 2) investigate the role of ABHD15 in immortalized brown adipocytes during proliferation and differentiation, and 3) analyze the regulation of ABHD15 in murine BAT upon different nutritional status or cold exposure.

The first part of this thesis comprised results from microarray experiments and gene set enrichment analysis. We were able to identify an interesting pool of dysregulated genes and biological processes in BAT from Abhd15-knockout mice. These results indicate a potential influence of Abhd15 in a variety of biological processes such as energy homeostasis, reactive oxygen species production, and hormone metabolism. Additionally, we investigated the metabolic phenotype of an immortalized brown adipocyte cell line stably overexpressing Abhd15 by proliferation and differentiation experiments. Finally, we studied the effects of cold exposure, dietary composition, and fasting and refeeding on ABHD15 in BAT depots. Recent studies in WAT revealed that ABHD15 stabilizes phosphodiesterase 3B (PDE3B) and influences its expression. Mice with deletion of ABHD15 in WAT showed a reduced expression of PDE3B and induced an unrestrained fatty acid mobilization, accompanied by a reduced glucose uptake, which promotes insulin resistance. We could show that PDE3B levels were also decreased in BAT from Abhd15-KO mice compared to their WT littermates. These results suggest a similar effect of ABHD15 on PDE3B expression in BAT. Hence, it could be possible that ABHD15-KO evocates insulin resistance in BAT as well as in WAT. However, further studies remain necessary in order to draw a complete picture of the role of Abhd15 in BAT and in whole-body energy metabolism.

International cooperations

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Research projects

FWF-PhD program: “Metabolic and Cardiovascular Disease” (W1226)

FWF P27108: “Nat8l: a new player in brown adipose tissue development and energy metabolism”

FWF SFB-Lipotox: Ab-hydrolase Domain Containing 15 (ABHD15) – a key factor in lipid metabolism and apoptosis

Talk at international conference

1. Walter K.: N-Acetylaspartate – a brain metabolite found in tumors. 20th DocDay of NAWI Graz, July 2018.

Posters at international conference

1. Walter K.: N-Acetylaspartate – a brain metabolite found in tumors. 7th International Graz Symposium on Lipid and Membrane Biology, Graz, April 2018.
2. Zirkovits G.: α/ß hydrolase domain containing protein 15 (ABHD15) influences liver metabolism. 7th International Graz Symposium on Lipid and Membrane Biology, Graz, April 2018.

Publications

1. Duta-Mare M, Sachdev V, Leopold C, Kolb D, Vujic N, Korbelius M, Hofer DC, Xia W, Huber K, Auer M, Gottschalk B, Magnes C, Graier WF, Prokesch A, Radovic B, Bogner-Strauss JG, Kratky D.: Lysosomal acid lipase regulates fatty acid channeling in brown adipose tissue to maintain thermogenesis. (2018) ***Biochim Biophys Acta Mol Cell Biol Lipids***. 1863(4):467-478. DOI: 10.1016/j.bbalip.2018.01.011.
2. Xia W, Pessentheiner AR, Hofer DC, Amor M, Schreiber R, Schoiswohl G, Eichmann TO, Walenta E, Itariu B, Prager G, Hackl H, Stulnig T, Kratky D, Rülicke T, Bogner-Strauss JG.: Loss of ABHD15 Impairs the Anti-lipolytic Action of Insulin by Altering PDE3B Stability and Contributes to Insulin Resistance. (2018) ***Cell Rep.*** 23(7):1948-1961. DOI: 10.1016/j.celrep.2018.04.055.
3. Skorobogatko Y, Dragan M, Cordon C, Reilly SM, Hung CW, Xia W, Zhao P, Wallace M, Lackey DE, Chen XW, Osborn O, Bogner-Strauss JG, Theodorescu D, Metallo CM, Olefsky JM, Saltiel AR.: RalA controls glucose homeostasis by regulating glucose uptake in brown fat, (2018) ***Proc Natl Acad Sci U S A***. 115(30):7819-7824. DOI: 10.1073/pnas.1801050115.
4. Amor M, Itariu BK, Moreno-Viedma V, Keindl M, Jürets A, Prager G, Langer F, Grablowitz V, Zeyda M, Stulnig TM.: Serum Myostatin is Upregulated in Obesity and Correlates with Insulin Resistance in Humans., (2018) ***Exp Clin Endocrinol Diabetes***. DOI: 10.1055/a-0641-5546
5. Alkan HF, Walter KE, Luengo A, Madreiter-Sokolowski CT, Stryeck S, Lau AN, Al-Zoughbi W, Lewis CA, Thomas CJ, Hoefler G, Graier WF, Madl T, Vander Heiden MG, Bogner-Strauss JG.: Cytosolic Aspartate Availability Determines Cell Survival When Glutamine Is Limiting., (2018) ***Cell Metab***. 28(5):706-720.e6. DOI: 10.1016/j.cmet.2018.07.021.
6. Huber K, Hofer DC, Trefely S, Pelzmann HJ, Madreiter-Sokolowski C, Duta-Mare M, Schlager S, Trausinger G, Stryeck S, Graier WF, Kolb D, Magnes C, Snyder NW, Prokesch A, Kratky D, Madl T, Wellen KE, Bogner-Strauss JG., N-acetylaspartate pathway is nutrient responsive and coordinates lipid and energy metabolism in brown adipocytes., (2018) ***Biochim Biophys Acta Mol Cell Res***. 1866(3):337-348. DOI: 10.1016/j.bbamcr.2018.08.017.

Chemistry of Functional Foods

Group leader: Michael Murkovic
PhD students: Sandra Holzer, Nicole Pabi, Abdullatif Albouchi, Oliver Wollner
Master students: Christopher Hartl, Manuel Deutsch, Nora Rezaeian, Admir Nezerovic
Technical staff: Alma Makic, 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

Abdullatif Albouchi: *Hydroxymethyl-Substituted Furan Derivatives in Coffee: Its Analysis, Formation Kinetics, Exposure, and Mitigation*

Hydroxymethyl-substituted furan derivatives contribute significantly to the total furan derivatives of roasted coffee; furthermore, they receive a lot of attention due to their mutagenic properties after conversion by intracellular sulfotransferases. Their analysis, formation kinetics, exposure and mitigation in coffee are of special importance and thus were the focus of this dissertation.

A validated simple and selective HPLC-DAD method was developed for the simultaneous separation and quantitation of furfuryl alcohol, 2-furoic acid, 5-hydroxymethyl furaldehyde and 5-hydroxymethyl furoic acid in roasted coffee. Different extraction solvents brought about various extraction efficacies, with water being the most suitable while methanol resulted in lacking extraction. Conversely, no significant differences were observed between tested extraction techniques.

Coffee produces more furfuryl alcohol compared to other beans or seeds roasted at the same conditions with observed formation kinetics resembling those of other process contaminants (e.g. HMF, acrylamide). The temperature and duration of applied roasting as well as the species of coffee used affected the level of furfuryl alcohol to be produced, whereas the moisture content of the green coffee had limited effect. Great amounts of furfuryl alcohol (up to 57 %) are evaporating and get released to the atmosphere during roasting of coffee.

An eight weeks storage at varying temperatures did not affect the level of furfuryl alcohol in the ground coffee matrix. On the other hand, the use of different brewing techniques affected the concentration of furfuryl alcohol in the brew and its extraction efficiency from the ground coffee. Furfuryl alcohol was at least 2-fold the amounts of 5-hydroxymethyl furaldehyde and 5-fold the amounts of 2-furoic acid or 5-hydroxymethyl-2-furoic acid in the same prepared brew with an observed variation of up to 22-fold in their concentrations. Stressing furfuryl alcohol with simulated gastrointestinal fluids points out a significant decrease under simulated gastric fluid conditions only, although the same effect could not be reproduced when mimicking a regular coffee ingestion situation.

Fourteen different mitigation agents were incorporated into two dry model systems mimicking important coffee natural constituents and an actual coffee system afore a controlled roasting process to evaluate their mitigation capacity of hydroxymethyl substituted furan derivatives. The agents can be chemically categorized to hydroxy cinnamates and their esters, hydroxy benzoates, flavonoids, as well as other non-phenolic agents. An especially high mitigation capacity could be shown with polyphenolics (hydroxy cinnamates or benzoates derivatives), quinic acid and EDTA. The number and availability of phenolic groups affected the mitigation capacity of polyphenolics. Certain agents exhibited a furan derivative-specific reducing capacity while others exerted a generalized effect. In the coffee system, a comparable mitigation trend to that in chemically-set model systems was observed with taurine and sodium sulfite exerting the highest capacity. The mitigation efficacy decreased gradually by the increasing chemical complexity of the tested model.

Master Theses completed

Manuel Deutsch: *Stability of Selected Nutrients in Home-Made Fruit Juices under Different Ways of Processing*

The aim of this master thesis was to analyse the stability of selected nutrients in self-made fruit juices after processing with a masticator with an antifoaming device and with a blender under different atmosphere conditions: normal air (reference), vacuum, CO_2 and N_2O. Therefore, strawberry, orange, blueberry, kiwi, red pepper, apple, red grape and matcha powder were used for the processing and the juices were stored in capped glass bottles for 24 hours at 3 °C in the refrigerator.

The following nutrients were investigated: vitamin C (total ascorbic acid content), anthocyanins, total phenolic content and in addition different catechins of the matcha powder. The vitamin C of the different fruit juices showed good stability over a storage time of 24 hours. The application of vacuum did not influence the stability of vitamin C, except in the kiwi juice, which showed the only positive trend for vacuum application in regards to vitamin C stability. The application of CO_2 and N_2O led to a very good stabilisation of the vitamin C content of all juices, only negligible losses or no losses were detected. Similar results were obtained for the juices from the masticator with the antifoaming device, which showed very good stability for the vitamin C content.

A huge loss of the anthocyanin content of the grape juice from the blender with normal air was detected. The application of vacuum led to a much better stability of the anthocyanins, as only minor losses of the anthocyanin content were detected. Nevertheless, the application of CO_2 and N_2O increased the stability of the anthocyanins a little bit better than the application of vacuum. Also, the grape juice from the masticator with the antifoaming device showed a very good stability of the anthocyanin content. However, the anthocyanin content was very low in comparison to the blending procedures, due to the separation of the grape skins from the juice, which contain the most anthocyanins.

The total phenolic content of the fruit juices showed less stability over a storage time of 24 hours compared to the ascorbic acid content. Vacuum application did not show any positive effect on the stability of the total phenolic content. In contrast, the application of CO_2 and N_2O increased the stability of the total phenolic content. Again, similar results with regards to increased stability of the total phenolic content were obtained for the juices from the masticator with the antifoaming device. A special mention is the total phenolic content of the matcha juice, which was totally stable, maybe due to matcha being an already processed food product. Catechin and epicatechin had very good stability in the matcha juice over a storage time of 24 hours, whereas rather large decreases of the epigallocatechin-gallate were detected. The application of vacuum, CO_2 and N_2O did not affect the stability of the different catechins. Blending of the matcha powder led to higher catechin contents of the juices than manual mixing.

To conclude, the application of vacuum did not show many positive trends in terms of nutrient stability for the various fruit juices. The only discovered positive effects were the increased stability of the vitamin C content of kiwi juice and especially of the anthocyanin content of grape juice. The application of CO_2 and N_2O always led to better results than the application of vacuum. Reason for this might be the power of the vacuum pump, which is only capable to produce a vacuum of -0.7 bar, hence 0.3 bar of normal air are still present in the jar of the blender. Also very good results in regards to nutrient stability were obtained for the masticator with the antifoaming device. The only disadvantage of the masticator was the overall low anthocyanin content of the grape juice, because the anthocyanin rich grape skins are removed by the masticator during the processing of the juice.

Christoph Hartl: *Characterization of the stability of polysaccharides during baking*

Recently beta glucan is one of the most common and well-studied dietary fibers, especially in terms of health benefits. The alteration of beta glucans molecular weight in baking processes was analyzed by size-exclusion chromatography (SEC). A simulation with thermal and rheological conditions, typical in baking industries, was performed. For analytical separation a TSKgel PWxl column from Tosoh Bioscience GmbH was used and the reduction

of the molecular weight in branched (1.3/1.6)-beta glucan was determined by using a refractive index detector (RID). Furthermore measurements of the water-solubility of branched beta glucan induced by using high mechanical energy for stirring were conducted.

The results show that SEC is a reliable method for the analysis and molecular weight determination of beta glucan in food matrices. Physical and thermal treatments had an effect on the molecular weight. A correlation between the molecular weight and intensity of treatment could be observed. Compared to the calibration curve (PEG Standards) moderately treated beta glucan may have a molecular weight of approximately 32,000 Da while double treated beta glucan has a molecular weight of 28,000 down to 1,000 Da.
Physical forces (thermal and rheological) improve the water solubility of beta glucan. The maximum value of solubility was reached after 3 h of incubation.

Admir Nezirevic: *Sea Buckthorn as a Nutrient – Rich Resource for Food Ingredients Comparison of different samples with sea buckthorn components*

The aim of this Master's thesis was to analyze the influence of sea buckthorn as a component in various food products and to determine the concentrations of selected nutrients. Various samples of sea buckthorn constituents were analyzed for concentration of L – ascorbic acid, total vitamin C content, β-carotene and total phenolic content. In summary, it can be said that the sea buckthorn samples had a much higher concentration of L-ascorbic acid and therefore also vitamin C than β-carotene and total phenol content. Nevertheless, some samples surprised with a higher β-carotene and total phenolic content. Thus, the plant approves its advantages as a nutrient – rich source, which can have a positive effect on the human diet.

The highest vitamin C concentrations were found in the three tinctures in the range of 2,410 to 3,880 mg/ 100 g. As well, the highest ascorbic acid content of 1,293 – 3,308 mg/ 100 g was determined in the tinctures. Also, a very high concentration was found in sea buckthorn juice and sea buckthorn syrup, whose concentrations resembled those of sea buckthorn berry. Surprising were various bakery goods, tea and honey samples and by – products which also contained in total vitamin C and ascorbic acid. The content of β-carotene was highest in the sea buckthorn tea leaves sample with 8.08 mg/ 100 g. Closely followed by milled dry sea buckthorn samples containing 7.08 mg/ 100 g. Interestingly, the content in the sea buckthorn berries was very low at 1.99 mg/ 100 g for crop year 2015 or 1.66 mg/ 100 g crop year 2016. In comparison, the cake with sea buckthorn had a much higher β-carotene content in a concentration of 4.48 mg/ 100 g.

The total phenolic content in the tincture and tea samples, as well as by – products or baked goods were very high. The tea tincture sample was highest at 211 mg/ 100 g, followed by by-product samples from all three batches at concentrations of 137 – 164 mg/ 100 g. Surprisingly small content contained the sea buckthorn berries and samples such as sea buckthorn juice and syrup, honey or jam.

Nora Rezeaian: *Development of HPLC Methods for the Analysis of β-Carotene and β-apo-8'-Carotenal Degradation Kinetics During Thermal Oxidation in Triacylglycerol Model Systems*

β-Carotene and β-apo-8'-carotenal are significant fat soluble carotenoids with antioxidant activity, which are also used in the industry as food colorant. Natural antioxidants in edible oil help to maintain the product quality by inhibiting oxidation processes. The oxidation reactions effect unsaturated lipid components and cause undesirable flavor and aroma. Furthermore, degradation products can influence the safety of lipid-containing food. In this study the stability of β-carotene and β-apo-8'-carotenal as antioxidant in triacylglycerol model systems are investigated by analysis of kinetic models which describe the heat-induced degradation of the two carotenoids. Triolein containing β-carotene and / or β-apo-8'-carotenal was oxidized in the Rancimat at 110 °C with different air flow rates (20 l/h, < 4 l/h, and no air). Degradation of the carotenoids and oxidation products were measured with a developed reversed phase HPLC-DAD method. It was found that β-apo-8'-carotenal had a lower degradation rate and higher stability during thermal oxidation in oil. Different air flow rates effect the formation and degradation of β-carotene oxidation products during thermal oxidation in the Rancimat. A further HPLC-DAD method was also developed for detection of volatile oxidation products (carbonyl compounds), originating from β-carotene thermal oxidation. The developed gradient HPLC-DAD method presents a highly sensitive and accurate analytical method for the detection of DNPhydrazones in the volatile oxidation product of β-carotene degradation in a saturated fatty acid model system.

International cooperations

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R. Swasti, Atma Jaya University, Yogyakarta, Indonesia

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

Talks at international conference

1. M. Murkovic (2018) Third International Conference on Food Science and Technology, Formation of furfurfyl alcohol during roasting of coffee
2. M. Murkoivc (2018) PATPI – SEAFAST International Conference: Science-based Ingredients: The Future for Food in Asia, Antioxidants – Roles and Safety Concerns

Publications

1. Damanik, M., Murkovic, M. The stability of palm oils during heating in a rancimat. (2018) ***European Food Research and Technology***. 244, 1293-1299.
2. Albouchi, A., Russ, J.,Murkovic, M. Parameters affecting the exposure to furfuryl alcohol from coffee. (2018) ***Food and Chemical Toxicology***. 118, 473-479, 2018
3. Murkovic, M., Pedreschi, F. & Ciesarova, Z. (2018) Process contaminants: A review. (2018) ***Reference Module in Food Science***. Elsevier B.V., pp 1-6

Lectures and Laboratory Courses

Winter Semester 2017/18

Course no.	Title	Hours	Type	Lecturers
CHE.154_1	Biochemistry Laboratory Course I	5.33	LU	Team
CHE.155	Biochemistry II	1.5	VO	Macheroux P
CHE.191	Bioanalytics	2.25	VO	Klimant I, Winkler A
CHE.192	Biochemistry Laboratory Course II	4	LU	Team
CHE.890	Food Biotechnology	1.33	VO	Murkovic M
CHE.892	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MAS.420UF	Biocompatible Materials	2	VO	Amor M
MOL.101	Introduction to Bachelor Study	1	SE	Macheroux P
MOL.833_1	Project laboratory	9	LU	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.933	Food Biotechnology	1.3	VO	Murkovic M
MOL.959	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MOL.961	Food Chemistry and Technology II	2	VO	Murkovic M
648.001	Fundamentals of molecular- and cell biology	2	VO	Amor 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
648.013	Advanced cell culture training course II	1	SE	Amor M
648.014	Biomaterials	2	VO	Amor M
648.020	Teaching Experience	2	SE	Macheroux P
648.059	Fundamentals of Pharmacology	2	VO	Dittrich P
648.601	Cellular Metabolism	2	PV	Bogner-Strauß J
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 2018

Course no.	Title	Hours	Type	Lecturers
CHE.147UF	Biochemistry I	3.75	VO	Macheroux P
CHE.193UF	Molecular biology laboratory course	3	LU	Daniel B
CHE.194UF	Seminar for Molecular biology laboratory course	1	SE	Daniel B
MOL.406UF	Methods in Immunology	2	VO	Oberdorfer G
MOL.407UF	Methods in Immunology	2	LU	Team
MOL.606_1	Bachelor Thesis	1	SE	Team
MOL.833_1	Project laboratory	9	LU	Team
MOL.845_1	Seminar for undergraduate students	2	SE	Team
MOL.880UF	Molecular Enzymology	2	VO	Gruber K, Macheroux P, Nidetzky B
MOL.886	Biophysical Methods	3	LU	Winkler A, Oberdorfer G, […]
648.000	Laboratory Practice	4	PV	Macheroux P
648.002	Molecular diagnostics	2	VO	Amor M
648.004	Molecular Enzymology II	2	PV	Macheroux P
648.005	Molecular Diagnostics	2	LU	Amor M
648.006	Introduction to Biochemistry	2	VO	Lienhart W
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
648.013	Advanced cell culture training course II	1	SE	Bogner-Strauß J
648.016	Chemical Reactions in Foods II	2	PV	Murkovic M
648.020	Teaching Experience	2	SE	Macheroux P
648.302	Methods in Immunology	1	VO	Oberdorfer G, Macheroux P
648.602	Cellular Metabolism	2	PV	Amor M

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