Staff Members of the Institute of Biochemistry, TU Graz

Tel.: +43-(0)316-873-ext.; Mail: office.ibc@tugraz.at

Professors

Peter Macheroux (Full Professor & Head of the Institute)
(peter.macheroux@tugraz.at; Tel.: ext. 6450)

Juliane Bogner-Strauß (Associate Professor, on leave)
(juliane.bogner-strauss@tugraz.at; Tel.: ext. 5337)

Michael Murkovic (Associate Professor)
(michael.murkovic@tugraz.at; Tel.: ext. 6495)

Gustav Oberdorfer (Assistant Professor)
(andreas.winkler@tugraz.at; Tel.: ext. 4526)

Andreas Winkler (Assistant Professor & Deputy Head of the Institute)
(andreas.winkler@tugraz.at; Tel.: ext. 6457)

Senior Research Scientists

Dr. Shalinee Jha
(shalinee.jha@tugraz.at; Tel.: ext. 4529)

Dr. Silvia Wallner
(silvia.wallner@tugraz.at; Tel.: ext. 6955)

Assistants

Grazia Malovan
(grazia.davidovic@tugraz.at; Tel.: ext. 4529)

Julia Messenlehner
(julia.messenlehner@tugraz.at; Tel.: ext. 6466)

Marina Toplak
(marina.toplak@tugraz.at)

Office

Tinkara Kristovic
(tinkara.kristovic@tugraz.at; Tel.: ext. 6451)

Technical Staff

Macheroux Group:	Eva Maria Frießer (Tel.: ext. 4522), Rosemarie Trenker-El-Toukhy (Tel.: ext. 4522)
Murkovic Group:	Claudia Hrastnik (Tel.: ext. 6996)
Oberdorfer Group:	Birgit Grill and Alma Makic (both Tel.: ext. 6454)
Winkler Group:	Elfriede Zenzmaier (Tel.: ext. 6996), Nadine Galler (Tel.: ext. 6460)

Brief History of the Institute of Biochemistry

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

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

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

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

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

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

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

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

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

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

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

2003 P. MACHEROUX was appointed full professor of biochemistry in September 2003 and head of the *Institute of Biochemistry* in January 2004. His research interests revolve around topics in protein biochemistry and enzymology.

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

2009 The *Institute of Food Chemistry and Technology* was disbanded and the research group of Prof. M. MURKOVIC joined the *Institute of Biochemistry*.

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

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

2015 After more than 40 years at the *Institute of Biochemistry* 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 was sworn in as Minister for Women, Families, and Youth of the Austrian government at the end of 2017 and, after the end of the coalition in mid-2019, continued her political career at the provincial government of Styria as Councilor for Research, Society, Health and Care.

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

Highlights of 2019

iGEM NAWI Graz

General

iGEM – short for "international Genetically Engineered Machine" - is an annually held student competition hosted since 2004 by a team originating from MIT, Boston, Massachusetts. Students from universities, colleges, and high schools are encouraged to solve local problems with interdisciplinary methods complementing the field of synthetic biotechnology. A typical iGEM project lasts for about 10 months. It starts with the first general meeting of interested persons, includes in-silico and wet lab project design, finance planning, continuous presenting of the work progress to the general public and culminates in a wiki webpage (http://2019.igem.org/Team:NAWI_Graz) and the participation at the Giant Jamboree – the final scientific congress held in Boston. At this congress over 3000 students from over 300 teams worldwide present their diagnostic, therapeutic, environmental, hardware, software, etc. track projects and are evaluated by iGEM officials and other participating PIs resulting in awards of two categories. On the one hand, medals as general ratings and, on the other hand, special prizes like best track project, best public relations, or best project overall, with teams competing in three age dependent classes called High school, Undergraduate and Overgraduate.

Our Project

Some iGEM NAWI_Graz 2019 participants are passionate beekeepers and they successfully convinced the others to focus on a very challenging problem, the disease American Foulbrood (AFB) caused by the gram-negative bacterium *Peanibacillus larvae*. Today's mechanisms to detect and treat this disease are slow and drastic, as test samples require weeks to be analyzed and evaluated. Outbreaks cause 3 km^2 quarantine zones around the beehives, which prohibits any beehive moving in this area for up to several months. Since industrial beekeepers rely on placing their hives next to blooming plants and agricultural workers rely on the bee-mediated pollination, far-reaching and existence-threatening problems are induced by AFB (especially for the beekeepers). Besides, AFB outbreaks are still stigmatic in rural areas due to the notion that AFB is provoked by bad hygiene and careless beekeeping. Therefore, hives with positive results from quick tests, known as match stick tests, are often kept in secrecy and the hive is burned immediately, since this is also the treatment after AFB has been diagnosed.

Thirteen students from Molecular Biology, Biotechnology, Biomedical Engineering, Technical Chemistry and Information and Computer Engineering decided to develop a novel preventive diagnostic device, the "beeosensor". It consists of gold electrodes on a self-designed measurement chip that is used to immobilize bacteriophages (HB10c2) that specifically bind *P. larvae* cells (of strain ERIC I). Eventually, a voltage is applied and impedance measurements are used to evaluate the presence of bound cells and possibly bound spores as well, which would enable the detection of *P. larvae* cells in their dormant state.

The team found his principal investigator in Professor Peter Macheroux and a place to carry out the biological experiments at the Institute of Biochemistry. From July till October, the students could use the existing infrastructure at the institute extensively and test many different procedures to cultivate *P. larvae* and the corresponding phages and to purify the latter. While the slow cultivation of the bacteria was tedious, one inoculated batch needed four days of shaking, especially the phage purification made the team face quite some problems since the required techniques were new to all members of the team. Also, a once successful phage purification that used ultracentrifugation and CsCl-density-gradient centrifugation could not be reproduced. However, with blood and tears, eventually a working production and purification procedure based on FPLC could be established. These key experiments were essential to prepare the electrochemical measurements at the Institute of Analytical Chemistry of the Karl-Franzens University Graz.

Results

With our limited capacities and a lot of additional tasks required by iGEM parallel to the research itself, ten months were by far not enough to develop a completely new device from scratch. But the team was able to gain insights into previously unknown binding events between phages and spores which was a very important piece of information for our collaborators at the Zoological Institute of the KFU, which provided us with *P. larvae*. The self-designed measurement chip was eventually tested successfully.

In terms of human practices, aka public relations, the team raised a lot of awareness for AFB through several presentations in schools, beekeeper training sessions, and public events. We mainly addressed to eradicate the AFB stigma, but this will take many years of future work for which we could hopefully pave the way with our collaborative efforts.

In the end, the team sent five participants to America and was positively surprised when they came home with three prizes and two nominations. Unexpectedly, the iGEM NAWI_Graz 2019 was awarded a gold medal, won the overgraduate prizes "Best Diagnostic Project" and "Best Integrated Human Practices", was nominated for "Best Presentation" and "Best Poster" and placed under the five best overgraduate teams in total. This is a drastic improvement in comparison to the Austrian teams of the last years. Therefore, the Giant Jamboree will be one of the most intense memories of all time for the participants in addition to participating at their first big scientific conference in general.

Note of thanks

Altogether, the iGEM competition provided the opportunity to design and realize a real scientific project completely independent and free, to learn a tremendous amount of new hard and soft skills and to build worldwide scientific networks through the iGEM competition. Therefore, the team would like to say thanks to all the advisors, supervisors, collaborators and all other people who helped us fulfill this project. It would not have been possible without them.

The iGEM Team (from left to right): Wolfgang Schühly, Peter Macheroux, Eda Mehmeti, Karl Lohner, Oskar May, Henrik Seyfried, Nikola Vinko, Lucija Sovic, Felix Schweigkofler, Vera Wasserbacher, Sarah Hopf, Markus Kreuzer, David Schuster, Farah Farzi, Sebastian Modl, Alexander Fürlinger. not shown, but essential members: Denise Hovorka, Hannes Beims (provided HB10c2 phages)

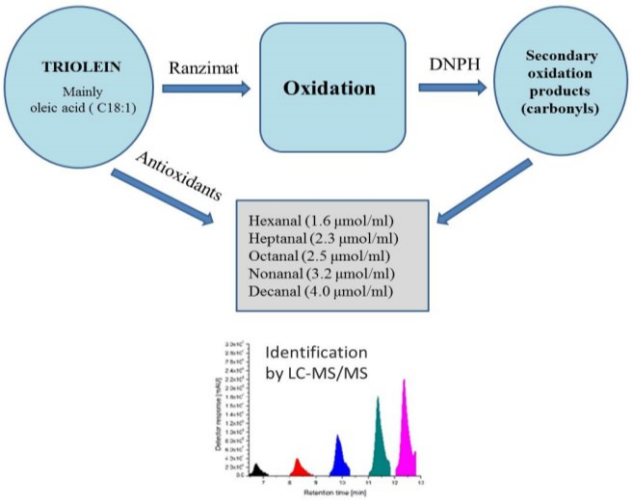
Additional highlights of 2019

In February, Robert Kourist from the *Institute of Molecular Biotechnology* and **Peter Macheroux** represented the newly formed PhD program "CATalytic AppLications of OXidoreductases (CATALOX) at a hearing in Vienna organized by the FWF. The program received a top ranking and will be funded for four years. The consortium comprises a total of ten research groups at the Graz University of Technology and the University of Graz. This will further strengthen our role in promoting applied enzymology and biocatalysis as an important factor to contribute to sustainable development.

In September, the annual institute outing took place. Awesome scenery and beautiful weather made this a memorable excursion to southern Styria with the traditional dinner at a typical Buschenschank. Healthy food and good drinks for everyone before we headed back to Graz.

In the group of **Andreas Winkler** Cornelia Böhm started as a new PhD student at the beginning of 2019. She continues the PhD and Postdoc project of Geoffrey Gourinchas, who later during the year left the group to start a new Postdoc position in Munich.
Another highlight was the participation at the 17th International Congress on Photobiology in Barcelona, where latest insights into light regulated processes were discussed.

The group of **Gustav Oberdorfer** fully established itself in 2019 and grew to a substantial size. In addition, Gustav Oberdorfer was asked to be part of a FET-Open application, which got funded and thus provides a further project for the lab. In this new project the Oberdorfer group is aiming to design artificial fluorescent proteins, which will be used as down-converting filters in LEDs.



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

Biochemistry Group

Group leader: Peter Macheroux
Secretary: Tinkara Kristovic
Senior research scientists / postdoctoral fellows: Alexandra Jammer, Shalinee Jha, Emilia Strandback, Silvia Wallner
PhD students: Eveline Brodl, Reinmar Eggers, Bianca Hengel, Sami Ullah Khan, Grazia Malovan, Marina Toplak.
Master students: Julia Brunner, Alexandra Csamay, Katharina Fuchs, Julia Messenlehner
Technical staff: Eva Maria Frießer, Rosemarie Trenker-El-Toukhy

General description

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

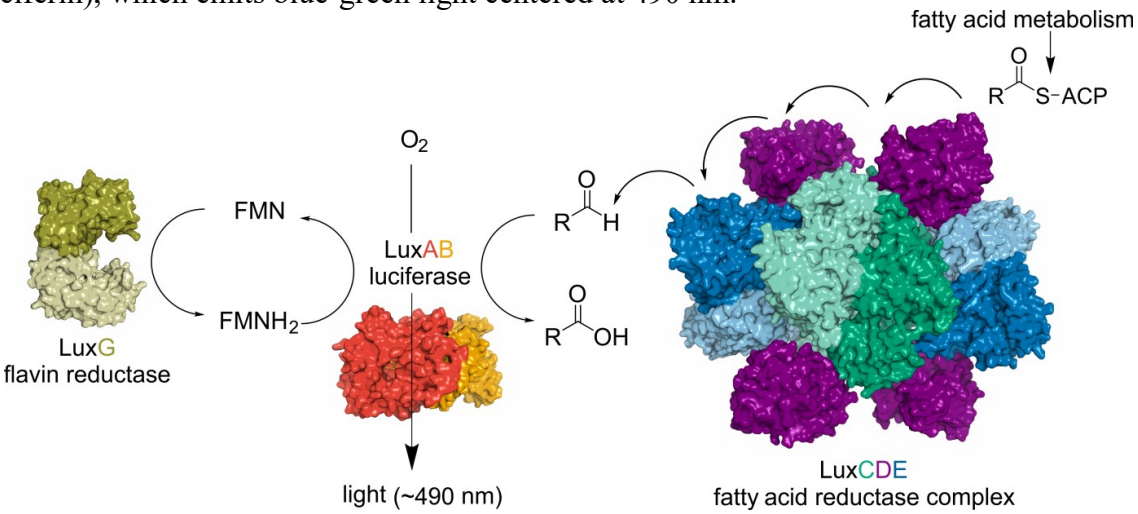
The methods established in our laboratory comprise kinetic (stopped-flow and rapid quench analysis of enzymatic reactions), thermodynamic (isothermal titration microcalorimetry) and spectroscopic (fluorescence, circular dichroism and UV-vis absorption) methods. We also frequently use MALDI-TOF and ESI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

Bacterial Bioluminescence

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 of Alexandra Csamay).

Berberine bridge enzyme-like enzymes in plants

Berberine bridge enzyme (BBE) is a central enzyme in alkaloid metabolism and catalyzes the oxidation of the *N*-methyl group of (*S*)-reticuline with concomitant formation of a carbon-carbon bond (the "berberine bridge") to yield (*S*)-scoulerine. Using bioinformatics, we found that homologs of BBE are widespread among plants, fungi and bacteria. The model plant *Arabidopsis thaliana*, for example, possesses 28 genes that apparently encode BBE-like enzymes although the plant does not synthesize alkaloids. We have recombinantly produced BBE-like homologs, *At*BBE-like proteins 13 and 15, from *A. thaliana* in *Komagataella phaffii* and identified monolignols and their glycosylated derivatives as potential substrates. We have solved the X-ray structure of *At*BBE-like 15 and the topology was found to be very similar to that of the BBE from *Eschscholzia californica* previously solved by Dr. Andreas Winkler and Prof. Karl Gruber. However, the residues that form the active site are distinct from those found in BBE from *E. californica*. The active site architecture of the monolignol oxidoreductase is conserved in approximately half of the BBE-like enzymes suggesting that this reaction plays an important role in plant metabolism.

To further unveil the role of BBE-like enzymes, we have teamed up with Dr. Alexandra Jammer and Professor Dr. Maria Müller from the Institute of Biology at the University of Graz to investigate the *in planta* functions of the supposed monolignol oxidase sub-family of the *Arabidopsis* BBE-like family. Gene expression in all types of plant organs via qPCR has been carried out for three of the genes (*At*BBE-like 13, 15, 26) and is currently underway for *At*BBE24 and *At*BBE25. In addition, GUS reporter lines have been generated to investigate gene expression patterns on the tissue level throughout the plant's life cycle; these plants are currently under investigation. Furthermore, several T-DNA insertion mutants for all five genes were ordered from the Nottingham Arabidopsis Stock Centre, from which we are currently generating homozygous offspring that will then be tested for the gene knockouts via qPCR. A detailed phenotyping of these mutants (and, eventually, multiple knockouts obtained by crossing), combined with the results from the expression analyses (qPCR, GUS plants) in combination with physiological studies, will provide the basis for the elucidation of the *in planta* functions of the sub-family members (thesis project of Reinmar Eggers supported by Dr. Alexandra Jammer).

Dipeptidylpeptidase 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 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 compared to 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 Malovan; postdoctoral project of Shalinee Jha).

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

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

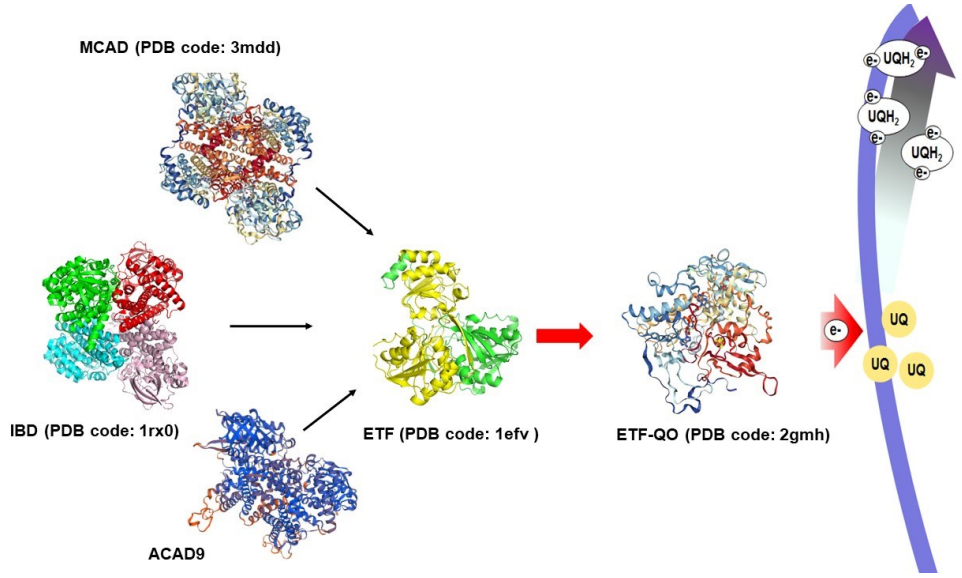
In order to study the oxidative cyclisation in chanoclavine biosynthesis and the involved enzymes and cofactors, we will have a closer look at EasE – a flavin adenine dinucleotide (FAD) dependent homologue of the berberine bridge enzyme-like (BBE) enzyme, – and EasC – a heme dependent catalase – originating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

Flavin-dependent electron transfer in human mitochondria

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

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

Currently, we study the effect of cofactor formylation on the interaction of hETF with selected client dehydrogenases, such as the medium-chain acyl-CoA dehydrogenase (MCAD), acyl-CoA dehydrogenase family member 9 (ACAD9) and isobutyryl-CoA dehydrogenase (IBD). We investigate the electron transfer from these dehydrogenases to the non formylated and formylated forms of hETF and the αN259A and ßE656A protein variants and we also focus on possible conformational differences between wild type and variant proteins with and without 8fFAD formation (thesis project of Sami Ullah Khan supported by Dr. Silvia Wallner).



Electron transfer from dehydrogenases to the mitochondrial transport chain via the human electron transferring flavoprotein (hETF). hETF accepts electrons from up to fourteen different dehydrogenases (such as MCAD, IBD or ACAD9) and passes them on to the respiratory chain via membrane bound ETF-ubiquinone oxidoreductase.

Doctoral Thesis completed

Marina Toplak: *Flavin-dependent reactions in carbohydrate metabolism and mitochondrial electron transport*

Flavin-dependent proteins constitute one of the largest and most diverse protein families. Due to the redox activity of their vitamin B2-derived cofactors, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), flavoproteins participate in one- and two-electron transfer reactions, and play important roles in a great variety of metabolic pathways.

An especially interesting subfamily of flavoenzymes is the group of so-called berberine bridge enzyme- (BBE-) like proteins. They bind their FAD cofactor in a mono- or bicovalent manner and thereby strongly enhance their reduction potential, which enables them to catalyze demanding oxidation reactions in various metabolic pathways ranging from carbohydrate catabolism to alkaloid biosynthesis. While BBE-like enzymes are only found in a few bacterial and fungal species, they are present in all land plants, with the number of homologs increasing from basal to higher plants. To identify the primordial role of BBE-like proteins in plants, I

heterologously produced the BBE-like protein from the most basal plant *Physcomitrella patens* (PpBBE1) and studied its biochemical and structural properties. Consistent with the results obtained from the in planta characterization of the protein, it was shown that PpBBE1 is a cellobiose oxidase involved in carbohydrate metabolism.

In contrast to the function of flavoenzymes in carbohydrate metabolism, their role in mitochondrial electron transport of eukaryotic organisms has been studied rather extensively. Upon oxidizing their natural substrates, flavin-dependent dehydrogenases involved in fatty acid and amino acid degradation, take up electrons, which they deliver to electron transferring flavoproteins (ETFs). These important electron carriers, in turn, interact with the membrane protein ETF-ubiquinone oxidoreductase and thereby pass the electrons on to the respiratory chain. In order to guarantee efficient electron transfer along the cascade, the electrochemical properties of the protein-bound cofactors need to be very well-tuned, which is the reason why already small changes in the nature or environment of the cofactors can have adverse effects on the overall process.

This phenomenon could nicely be observed in the course of my studies of the hETF and its electron donor human dimethylglycine dehydrogenase, which showed that the spontaneous modification of the FAD cofactor in hETF strongly affects the interaction as well as electron transfer efficiency between the two proteins. While mitochondrial electron transport in humans is very well studied, hardly anything is known about the electron transfer reactions in yeast. It was speculated that a single enzyme, named D- lactate dehydrogenase 2 (Dld2), serves as electron donor of the yeast ETF (yETF), however, this interaction has never been confirmed experimentally. The detailed biochemical characterization of the two proteins, as well as the analysis of their possible interaction finally revealed Dld2 to be a much better D-2-hydroxyglutarate dehydrogenase than D-lactate dehydrogenase and confirmed Dld2 to be the natural electron donor of yETF. The finding that humans suffering from defects in hETF or hETF-QO exhibit increased levels of D-2-hydroxyglutarate (D-2HG), prompted us to search the human genome for the presence of a Dld2 homolog. As a matter of fact, a human protein exhibiting more than 50% sequence identity with Dld2 (hD2HGDH) was identified and it could be shown that the recombinantly produced protein indeed transfers electrons to hETF. In the course of further biochemical characterizations of hD2HGDH, I also analyzed the effect of two pathogenic single amino acid exchanges on the properties of the enzyme, which helped to get a better understanding of the molecular basis of D2HGDH deficiency.

Master Theses completed

Julia Brunner: *Electron transferring flavoproteins link degradation of D-2-hydroxy-glutarate and D-lactate with energy production in humans and yeast*

Electron transferring flavoproteins (ETFs) are a group of proteins found in all organisms of life. They function as electron carriers, deliver electrons to the electron transport chain and therefore play an important role in energy production. The aim of this thesis was to gain more knowledge on the characteristics of the ETF from *Saccharomyces cerevisiae* (yETF) and of its electron donor D-lactate dehydrogenase (Dld2), as well as their putative interaction. Furthermore, human D-2-hydroxyglutarate dehydrogenase in humans (hD2HGDH) was found to be a human protein homologous to Dld2 and was therefore tested for its ability to function as an electron donor for hETF. Analyses of the electron transfer system in Saccharomyces cerevisiae using Dld2 and yETF have shown different biochemical and kinetic properties compared to the electron transfer system found in humans. Using D-2-hydroxyglutarate (D-2HG) as the preferred substrate, the conversion of the substrate using Dld2 leads to two electrons being transferred to yETF in a single step. Therefore, the electron transfer in

Saccharomyces cerevisiae does not show the expected transfer of single electrons to ETF, like it is known for the human system and all previously tested eukaryotic ETFs. Considering the biochemical properties of yETF, it may also transfer two electrons at once to the following receptor enzyme, Cir2p, which would again be different compared to the homologous human system, as only single electrons get transferred from hETF to hETF-QO. Additionally, experiments on hD2HGDH have shown that while this enzyme is a protein homologue of Dld2, the electron transfer from hD2GDH to hETF is slower than the electron transfer from Dld2 to yETF. Under steady-state conditions using DCPIP as final electron acceptor, this may be explained by the number of electrons transferred at once; while Dld2 transfers two electrons in a single step, hD2HGDH transfers one electron after the other. Nevertheless, the rates we determined in our assay are in the same range as shown for the electron transfer from other client dehydrogenases to hETF. In the course of this thesis, new information about the electron transfer processes occurring in *Saccharomyces cerevisiae* could be gathered and information about its energy metabolism could be obtained. Additionally, hD2HGDH was identified as electron donor for hETF, making it the fourteenth enzyme found in humans to carry out this function.

Katharina Fuchs: *Investigating the inhibitory effect of azo-dyes on the activity of chorismate synthases from various organisms*

The disease paracoccidioidomycosis (PCM) caused by the dimorphic fungus *Paracoccidioides*, is endemic in Latin America and recent studies have shown that it is responsible for approximately 50 % of deaths caused by systemic mycoses. Because of the frequent occurrence in Brazil local laboratories try to find and develop efficient drugs for the treatment of PCM. As a target for new therapeutics, an enzyme of the shikimate pathway was chosen, chorismate synthase.

Virtual screening and molecular modelling were used to identify new potential antifungal compounds, which specifically bind to chorismate synthase. Two promising candidates - CP1 ((1*S*,2*S*,3a*S*,4*S*,9b*R*)-1-chloro-6-nitro-2-(2-nitrophenyl)sulfanyl-2,3,3a,4,5,9b-hexahydro-1H-cyclopenta[c]quinoline-4-carboxylic acid) and CaCS02 (2-(2,4-dinitrophenylazo)-1-hydroxynaphthalene-3,6-disulfonic acid disodium salt) - were identified and investigated. The compound CaCS02 seemed to be a more promising antifungal drug because of the lower K_D- and IC50-values compared to CP1. Therefore, CaCS02 was used as a lead structure for the development of further antifungal compounds. To this end, several azo-dyes with similar structure to CaCS02 were tested, of which the compound PH011 (5-amino-3-[(E)-(3-chloro-2-hydroxy-5-nitrophenyl)diazenyl]-4-hydroxy-2,7-naphthalenedisulfonic acid) turned out to be the most promising candidate. The binding assay as well as the inhibition assay, performed with chorismate synthases from 12 different species, showed strong binding of the inhibitor to the proteins and an inhibitory effect on the enzymatic activity could be observed. In the binding assay, dissociation constants in the range of 1.1-4.4 µM were obtained, confirming the effective binding of the inhibitor to chorismate synthases. These values are quite near to the nanomolar range, thus further modifications of the compound may lead to potent antibiotics or fungicides. The efficiency of this compound was corroborated also by the inhibition assay, in which 50% inhibitory constants (IC50) of 10-16 µM were obtained, proving that the inhibitor is able to significantly reduce CS activity.

My experiments have confirmed the assumption that chorismate synthases may efficiently be inhibited by compounds similar to PH011. Thus, further in vitro studies/cytoxicity studies should be performed in order to get one step closer to the ultimate goal of finding and developing a drug that can be used to efficiently treat PCM and other fungal or bacterial diseases.

Julia Messenlehner: *Characterization of flavin-dependent oxidoreductases within the family of berberine bridge enzyme like proteins from Arabidopsis thaliana*

Flavoproteins are a diverse class of enzymes that use flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as a cofactor and facilitate a large variety of catalytic tasks. Among the flavoproteins, the multigene family of berberine bridge enzyme-like (BBE-like) proteins is defined by the BBE(-like) domain (pfam 08031), which implicates a mono- or a unique bi-covalent attachment of the cofactor. Despite their frequent occurrence, little is known about the biochemical properties of these proteins, in particular with regard to their substrate range. Recently, a member of this protein family from *Arabidopsis thaliana* named *At*BBE-like 15 has been identified as a monolignol oxidoreductase. To generate a deeper understanding of the role of this enzyme in planta, three groups from the fields of biochemistry, structural biology and plant physiology have teamed up. In this collaborative effort it was the aim of my master's thesis to identify the roles of amino acids in the active site in the course of monolignol oxidation. To probe the catalytic mechanism of the enzyme, putative catalytic active residues were identified within the crystal structure (4UD8). Loss of function-variants were created by a site directed mutagenesis approach. A spectrophotometric assay was performed to evaluate the impact of the respective variations on the kinetic parameters of the enzyme as a function of pH. For each variant, the pH optimum and the relative turnover number was determined. The strongest impact on the pH optimum was observed in the variants Q438V, Y139F and R292M with a ΔpH of +1.5, +1.2 and +1.2, respectively. The former two variants also showed the lowest residual activity with 4% and 1%, respectively while the R292M variant retains 50% of the activity. Based on the structural information and the kinetic data the following mechanism is proposed: The catalytic machinery consists of the bicovalent attached FAD-cofactor, the catalytic base that activates the substrate by deprotonation (Y479) and the preorganized alkoxide binding site, formed by the amino acids Q438 and R292, which stabilize the negative charge prior to oxidation. Since the active site composition is largely conserved within the family consisting of approximately 6000 members, the proposed mechanism for *At*BBE-like 15 can be considered a paradigm for the whole protein family.

International cooperations

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

Sphingotec Therapeutics GmbH, Hennigsdorf, Germany

Research projects

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

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

FWF-doc.funds "CATALOX"

Talks at national and international conferences/meetings

1. Jha, S.: *Emerging role of dipeptidyl peptidase 3 as a modulator of the reninangiotensin-aldosterone system*, DK Molecular Enzymology Final Retreat, Seggauberg, May 2019.

2. Strandback, E.: *Biochemical and biophysical characterization of cancer-associated variants of human NAD(P)H:quinone oxidoreductase 1*, DK Molecular Enzymology Final Retreat, Seggauberg, May 2019.

3. Toplak, M.: *Studying the molecular causes of D-2-hydroxyglutaric aciduria*, DK Molecular Enzymology Final Retreat, Seggauberg, May 2019

Publications

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

2. 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

3. 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.***, **3**:943-953. DOI:10.1373/jalm.2018.027995

4. Toplak, M., Brunner, J., Tabib, C., Macheroux, P.: Closing the gap: Product binding controls electron transfer in D-lactate dehydrogenase 2 from *Saccharomyces cerevisiae.* (2019) ***FEBS J.***, **286**:3611-3628. DOI:10.1111/febs.14924

5. Toplak, M., Brunner, J., Schmidt, J., Macheroux, P.: Biochemical characterization of human D-2-hydroxyglutarate dehydrogenase and two disease related variants reveals the molecular cause of D-2-hydroxyglutaric aciduria. (2019) **Biochim. Biophys. Acta: Proteins & Proteomics**, 140255. DOI:10.1016/j.bbapap.2019.07.008

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

7. Gandomkar, S., Jost, E., Loidolt, D., Swoboda, A., Pickl, M., Elaily, W., Daniel, B. Fraaije, M. W., Macheroux, P., Kroutil, W.: Biocatalytic enantioselective oxidation of *sec*-allylic alcohols with flavin-dependent oxidases. (2019) ***Adv. Synth. Catal.***, **361**:5264-5271. DOI:10.1002/adsc.201900921

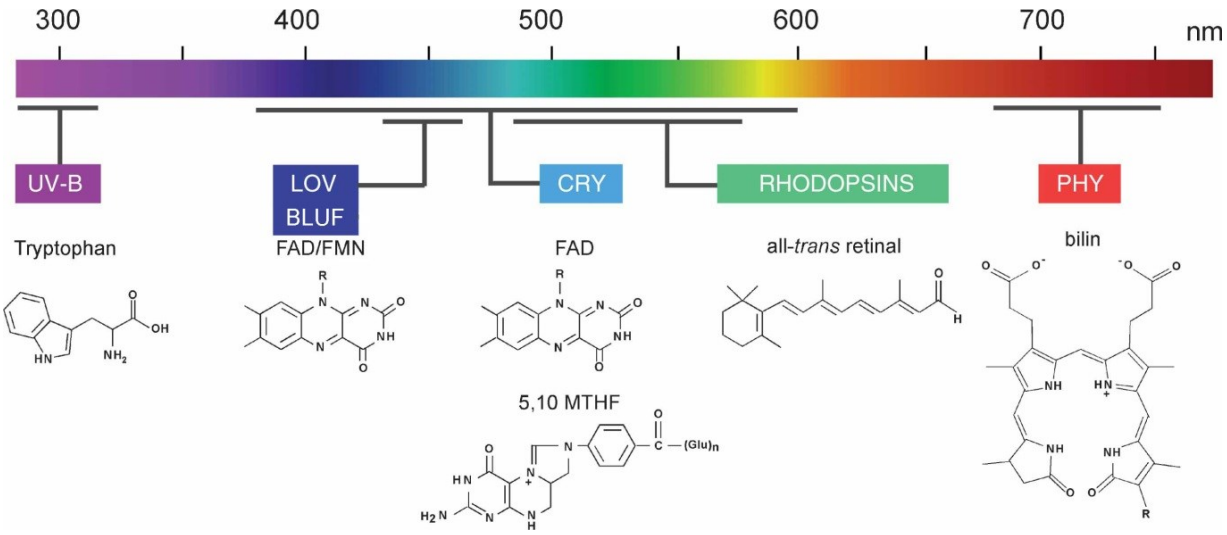
8. Strandback, E., Lienhart, W-D., Hromic-Jahjefendic, A., Bourgeois, B., Högler, A., Waltenstorfer, D., Winkler, A., Zangger, K., Madl, T., Gruber, K., Macheroux, P.: A small molecule chaperone rescues the stability and activity of a cancer-associated variant of NAD(P)H:quinone oxidoreductase 1 *in vitro* (2019) ***FEBS Lett***. 594 (3) 424-438, DOI: 10.1002/1873-3468.13636.

Photoreceptor Group

Group leader: Andreas Winkler
Post-Doc: Geoffrey Gourinchas
PhD student: Cornelia Böhm
Master students: Uršula Vide, Nikolina Todorović
Bachelor student: Marco Balasso
Summer/Project students: Nina Fasching, Maximilian Fuchs
Technical staff: Elfriede Zenzmaier, Nadine Galler

General description

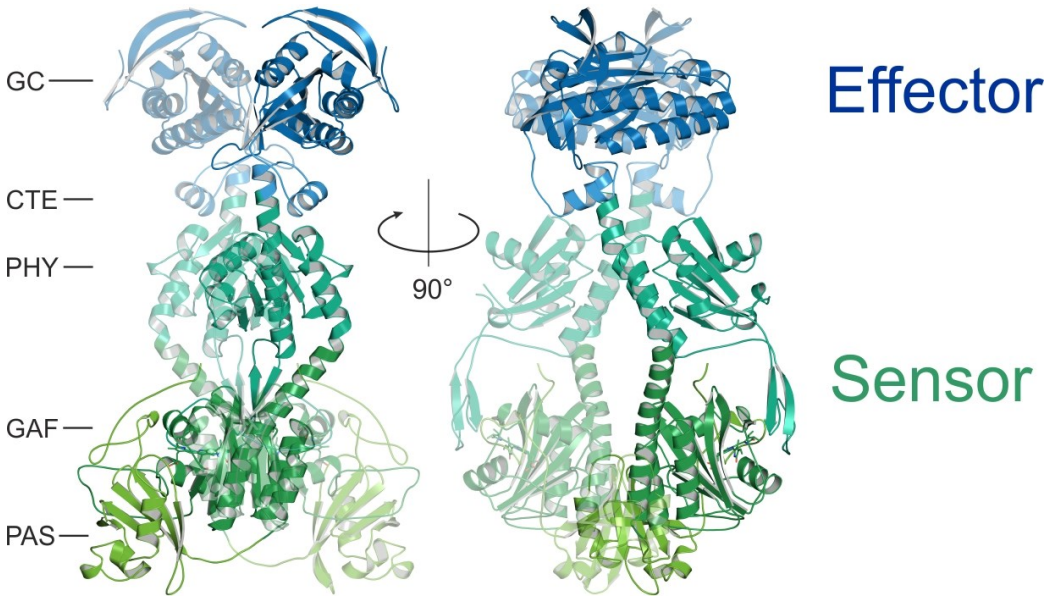
The ability to perceive and integrate environmental stimuli is essential for all living organisms. One important ambient factor is light that is sensed by a variety of photoreceptors (Figure below). Many light-responsive proteins are directly connected to enzymatic functionalities and enable photo-activation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



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

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



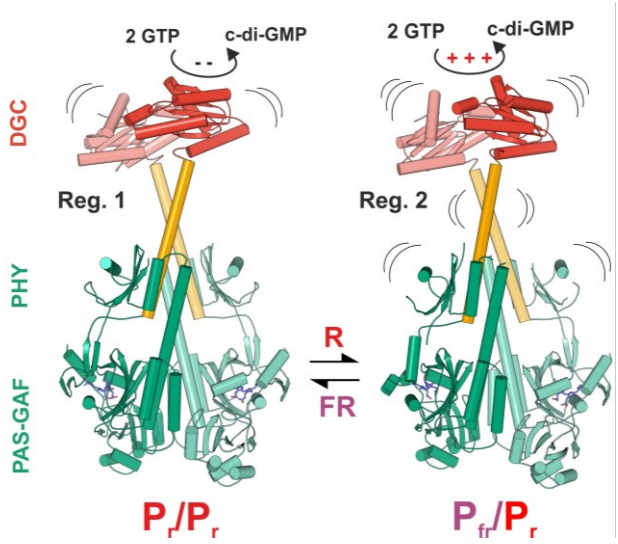
Crystal structure of the bacteriophytochrome-linked adenylate/guanylate cyclase. PAS, GAF and PHY domains are part of the red light regulated phytochrome sensor with its biliverdin chromophore shown as green stick model. The CTE corresponds to the Cyclase Transducer Element, required for efficient signal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

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

Red-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 Cornelia Böhm).

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). Understanding the role of asymmetry in these systems is the major focus of the PhD thesis of Cornelia Böhm.

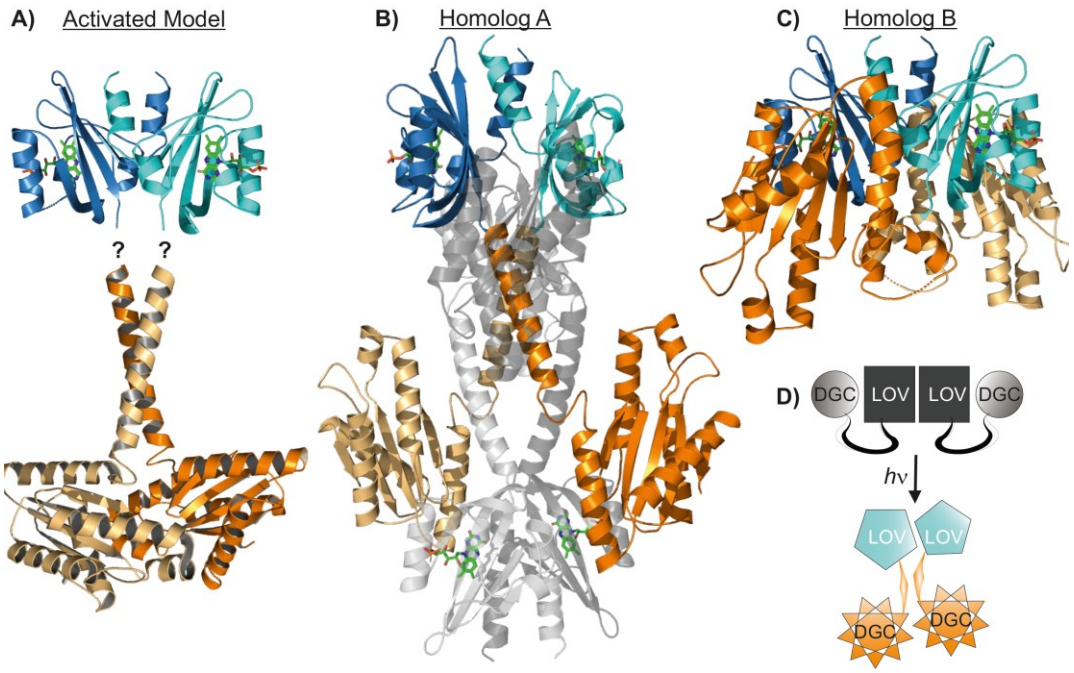
Understanding molecular details of how structural asymmetry is realized was the focus of a collaboration with the group around Peter Hildebrandt in Berlin. By combining crystallography, hydrogen-deuterium exchange coupled to MS and vibrational spectroscopy, we could show that the photoactivated state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

Interestingly, all homologs investigated so far, but one, show indications of being structurally heterodimeric upon red light illumination. In most of these systems, asymmetric activation leads to a significant increase in DGC activity. In contrast, symmetric activation and no increase in c-di-GMP production upon illumination is observed in one subgroup of phytochrome linked diguanylate cyclases. In an attempt to address the functional relevance of individual structural elements, a range of chimeras between symmetrically and asymmetrically activated phytochromes were created. Spectral data and activity measurements showed that asymmetric phytochrome activation, while potentially advantageous for substrate binding and product formation, does not appear to be an absolute requirement for the red light induced increase in GTP turnover. More detailed analyses are in progress to unravel the molecular determinants of the asymmetry/symmetry transition in bacteriophytochromes and to understand the role of asymmetry in the biological context (thesis project of Cornelia Böhm).

Blue-light-regulated diguanylate cyclases

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

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



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

The functional implications of the structures (shown above) combined with their biochemical and spectral characterization are the focus of the Master thesis of Ursula Vide. Additional efforts are currently on the way to obtain funding for a PhD student to follow up on these promising preliminary data and to further characterize molecular details of the mechanistic model described in panel D of the figure above.

Master Thesis completed

Nikolina Todorovic: *Biochemical characterization of molecular determinants involved in photoconversion, signal transduction and stability of homodimeric phytochrome-activated diguanylyl cyclases*

Light is one of the basic foundations on which today known life is formed. Many organisms, whether phototrophic or not, use light not only to initiate a variety of metabolic processes but also as a signal for food intake, avoidance of enemies or adverse environmental conditions. During evolution, protein sensors have adapted to light changes in the environment to facilitate effective control of light-conditioned processes, therefore light perception is enabled by a large number of photoreceptors that have developed the ability to use a wide range of ambient light. These photoreceptors represent complexes of proteins and cofactors which are acting together in order to absorb a photon that will trigger a physiological response. Therefore, the idea of using light in precise stimulation of biological processes and formation of certain biological information in living organisms enabled the development of optogenetics. Among other photoreceptors, phytochromes that respond to light in the red and far-red light range were found in plants, algae, fungi, and bacteria. With their chromophores providing the ability of reversible light activation and red-light tissue penetration, these phytochromes present a good basis for potential application in the control of biological processes, using the near-infrared

light. In order to use red-light sensing phytochromes in optogenetic tool design, it is essential to provide a detailed understanding of fundamental events occurring in phytochrome photoactivation and signal transduction. Considering the availability of the crystal structure of the dimeric bacterial phytochrome from *Idiomarina sp*. A28L in the ground and activated states (Gourinchas et al., 2017;2018), this phytochrome-activated diguanylate cyclase represents a good starting point for understanding bacteriophytochrome activation. In order to provide better insights into the role of conserved domains in photoconversion, signal transduction and bacteriophytochrome stability, this work focused on bacteriophytochromes from two organisms *Idiomarina sp*. A28L and *Marinobacter persicus*, which feature characteristic differences in their primary structure and photoresponses. Swapping individual domains between the two homologs, expression, purification and biochemical characterization, which involves spectrophotometric and kinetic characterization of the designed artificial proteins, of these constructs provided further information about the impact of different structural elements on photoactivation and regulation of signal transduction and stability. Consideration of my new insights contributes to new approaches for studying phytochromes with the aim of optimizing optogenetic tools in the future.

International cooperations

Ilme Schlichting, Max Planck Institute for Medical Research, Germany

Peter Hildebrandt, TU Berlin, Berlin, Germany

Janne Ihalainen, University of Jyväskylä, Jyväskylä, Finland

Research projects

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

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

Talks at national and international conferences/meetings

1. Winkler, A.: *Molecular determinants of asymmetric phytochrome activation*. World Congress on Light and Life: 18th Congress of the European Society for Photobiology, Barcelona, Spain, August 2019.
2. Vide, U.: *Molecular mechanisms of light regulation in LOV-diguanylate cyclases*. 11th ÖGMBT Annual meeting: Inside the World of Biomolecules, Salzburg, Austria, September 2019. (poster pitch talk)
3. Gourinchas, G.: *Asymmetric structural rearrangements involved in light activation of a dimeric red light photoreceptor*. Frontiers in Integrative Structural Biology, Graz, Austria, April 2019. (local symposium)

Publications

1. 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***. 294 (12), 4498-4501, DOI: 10.1016/j.sbi.2019.02.005.

2. 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.*** 57, 72-83, DOI: 10.1074/jbc.RA118.007260.

3. Strandback, E., Lienhart, W-D., Hromic-Jahjefendic, A., Bourgeois, B., Högler, A., Waltenstorfer, D., Winkler, A., Zangger, K., Madl, T., Gruber, K., Macheroux, P.: A small molecule chaperone rescues the stability and activity of a cancer-associated variant of NAD(P)H:quinone oxidoreductase 1 *in vitro* (2019) ***FEBS Lett***. 594 (3) 424-438, DOI: 10.1002/1873-3468.13636.

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

Award

1. **Ursula Vide** was awarded the Best Poster Prize at the OeGMBT annual meeting "Inside the World of Biomolecules" 2019 in Salzburg, Austria.

Protein Design Group

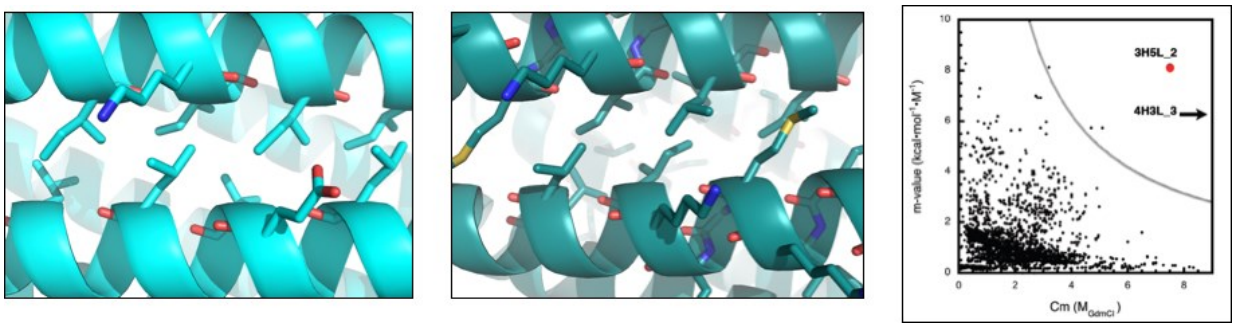
Group leader: Gustav Oberdorfer
PhD students: Veronica Delsoglio, Wael Elaily, Julia Messenlehner, Florian Wieser
Master students: Dominik Fridrich, Dominik Schwarz, Adrian Tripp
Bachelor students: Julius Hochrinner, Henrik Seyfried
Technical Staff: Alma Makic (maternity leave), Markus Braun, Stella Ebner, Henrik Seyfried (all maternity leave replacements), Birgit Grill

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).

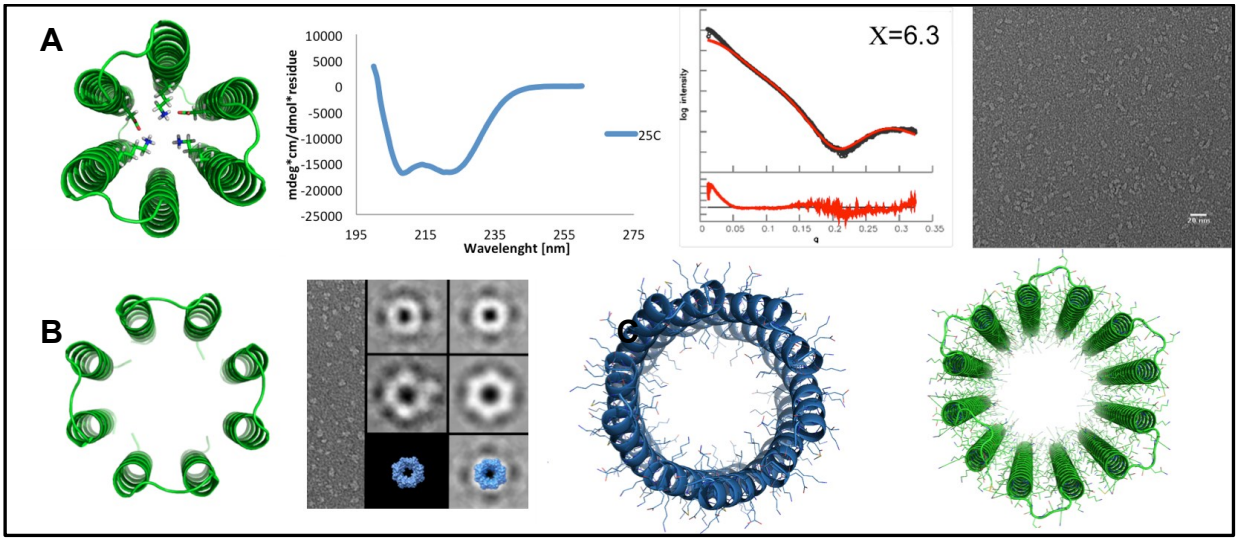


Computationally designed helix-helix interface. (left and center panel) Through iterative sampling of backbone geometries, the computations converged on 'ideal' knobs-into-holes packing arrangements, without enforcing of sequence motifs to achieve this type of packing. (right panel) Figure taken from (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.

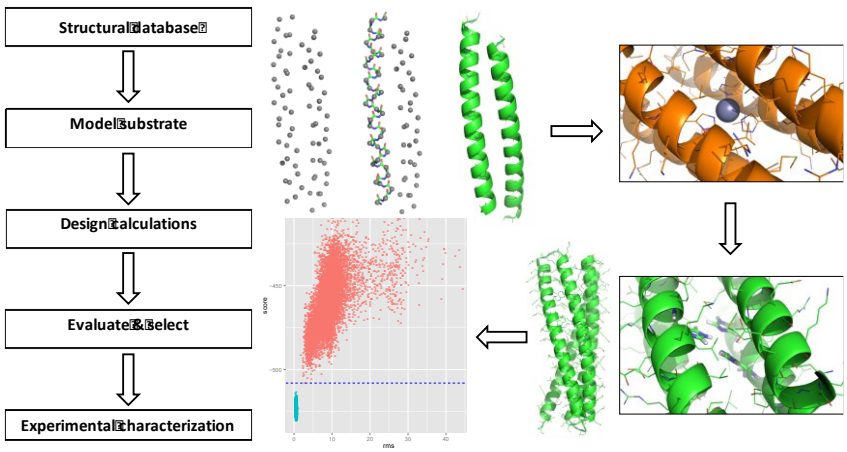
Since the parametric generation of helical backbones is very fast, it is now possible to generate thousands of helical protein structures with feasible backbone geometries in hours. Combined with the emerging technological advances in oligonucleotide synthesis, this approach opens the possibility to 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. If successful, these designed proteins will add to the very few genetically encodable *de novo* catalytic proteins. 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

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

Research projects

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

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

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

Talks at national and international conferences/meetings

1. Oberdorfer, G.: *Proteins made to order: Computational design of de novo proteins with custom geometries.* Biophysics Colloquium Johannes Kepler University Linz, Linz Austria, January 2019

2. Oberdorfer, G.: *Proteins made to order: Computational design of de novo proteins with custom geometries.* BZMB seminar, University of Bayreuth, Bayreuth, Germany, February 2019

3. Oberdorfer, G.: *Computational design of a novel ene-reductase by repurposing the active site of an aldo-, keto-reductase.* Symposium: Towards Functional Biomaterials, Lund University, Lund, Sweden, June 2019

4. Oberdorfer, G.: *Computational design of a novel ene-reductase by repurposing the active site of an aldo-, keto-reductase.* RosettaCon 2019, Leavenworth, Sleeping Lady Resort, WA, USA, August 2019

5. Oberdorfer, G.: *Proteins made to order: Computational design of de novo proteins with custom geometries.* Biomolecular Interactions and Structures Seminar Series, Biocentre Basel, Basel, Switzerland, December 2019

Publication

1. Park, J., Selvaraj, B., McShan, A.C., Boyken, S.E., Wei, K.Y., Oberdorfer, G., DeGrado, W., Sgourakis, N.G., Cuneo, M.J., Myles, D.A.A., Baker, D.: *De novo* design of a homo-trimeric amantadine-binding protein. (2019) ***eLife***, 8:e47839, DOI: 10.7554/eLife.47839

Cellular Metabolism Group

Group leader: Juliane Bogner-Strauss (on leave)
Postdoctoral fellow: Melina Amor
PhD students: Furkan Alkan, Katharina Walter
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 and aspartoacylase in cancer

N-acetylaspartate (NAA) is the second most abundant metabolite in the brain after glutamate and is synthesized from acetyl-CoA and L-aspartate by the enzyme aspartate-N-acetyltransferase (ASP-NAT, gene name: Nat8l). While, N-acetyltransferase 8-like (Nat8l) catalyzes the formation of NAA, aspartoacylase (ASPA, gene name Aspa) deacetylates NAA producing acetate and L-aspartate. In brain, 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. Thus, Nat8l impacts energy and lipid metabolism and presents a promising target to combat obesity. Moreover, previous studies could detect increased levels of NAA in lung and ovarian tumors compared to their corresponding tissue of origin, which was associated with worse patient outcome. Thus, since little is known about the role of NAA in cancer, we are investigating the impact of NAA/Nat8l/Aspa on cancer energy metabolism. In this project, we have found that the presence of the metabolite NAA helps sustaining survival and proliferation of cancer cells in glucose limiting environments. Mechanistically, we clarify that cancer cells overexpressing Nat8l/exhibiting high levels of NAA reduce ER-Stress and improve protein synthesis maintaining cell growth when nutrients are scarce.

Matrix metalloproteinase 12 (MMP-12)

Matrix metalloproteinase 12 (MMP12), a macrophage-secreted proinflammatory molecule, has been identified as an interesting target for the treatment of obesity-associated Type 2 Diabetes and cardiovascular diseases. Its 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 MMP12 depletion may lead to a general improvement in different metabolic conditions. In this project, Melina Amor is exploring mechanistic aspects associated with the pathological role of MMP12. Murine and human adipocytes treatment with

recombinant MMP12 showed a worsened cell function, denoted by an impaired insulin sensitivity and increased pro-inflammatory gene expression. Additionally, we investigated the transcriptional regulation of MMP12 and we were able to demonstrate that it is tightly regulated by the peroxisome proliferator-activated receptor γ (PPARγ), a major regulator of the adipose tissue homeostasis. In line with these results, we also investigated the effect of different nutritional states on MMP12 levels in several metabolic tissues and we observed a significant increased gene expression and protein levels upon fasting and aging. Within the framework of this project, Johannes Breithofer completed his Bachelor thesis (March-August 2019). His work was mainly focused on the role of MMP12 in white adipocytes.

In the upcoming future we aim to investigate the role of MMP12 in macrophage (dys)function and in a mouse model that simultaneously develops insulin resistance and atherosclerosis giving the advantage to better mirror the human disease. This will be performed by using primary cell cultures from mice and humans and via whole-body genetic deletion of MMP12 respectively. In conclusion, this project will provide a comprehensive investigation of a promising target, which could directly support further studies in humans.

International cooperations

Atsumi Nitta, Dept. of Pharmaceutical Therapy & Neuropharmacology, Faculty of Pharmaceutical Sciences, University of Toyama, Japan

Da Young OH, School of Medicine, Division of Endocrinology & Metabolism, UCSD, California, USA

Andrew Pospisilik, Max Planck Institute of Immunobiology and Epigenetics, Freiburg, GER

Alexander Pfeifer, Institut für Pharmakologie und Toxikologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

Alan R. Saltiel, Department of Medicine, Division of Endocrinology and Metabolism, Institute for Diabetes and Metabolic Health, University of California, San Diego, USA

Johan Auwerx, Institute of Bioengineering, EPFL, Lausanne, Switzerland

Kathryn E. Wellen, Department of Cancer Biology, University of Pennsylvannia, Philadelphia, USA

Matthew Vander Heiden, David Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, USA

Research project

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

Publications

1. Trefely S, Liu J, Huber K, Doan MT, Jiang H, Singh J, von Krusenstiern E, Bostwick A, Xu P, Bogner-Strauss JG, Wellen KE, Snyder NW.: Subcellular metabolic pathway kinetics are revealed by correcting for artifactual *post harvest* metabolism. (2019) ***Mol Metab.*** 30:61-71, DOI: 10.1016/j.molmet.2019.09.004.

2. Hofer DC, Zirkovits G, Pelzmann HJ, Huber K, Pessentheiner AR, Xia W, Uno K, Miyazaki T, Kon K, Tsuneki H, Pendl T, Al Zoughbi W, Madreiter-Sokolowski CT, Trausinger G, Abdellatif M, Schoiswohl G, Schreiber R, Eisenberg T, Magnes C, Sedej S, Eckhardt M, Sasahara M, Sasaoka T, Nitta A, Hoefler G, Graier WF, Kratky D, Auwerx J, Bogner-Strauss JG. N-acetylaspartate availability is essential for juvenile survival on fat-free diet and determines metabolic health. (2019). ***FASEB J.*** 33(12):13808-13824. DOI: 10.1096/fj.201801323R.

3. Alkan HF, Bogner-Strauss JG. Maintaining cytosolic aspartate levels is a major function of the TCA cycle in proliferating cells. (2019). ***Mol Cell Oncol.*** DOI: 10.1080/23723556.2018.1536843.

4. 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. (2019) ***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, Oliver Wollner, Monika Grasse
Master students: Iris Stalzer, Zehra Gromilic, Julia Ruß
Technical staff: Claudia Hrastnik

General description

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

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

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

PhD Thesis completed

Nicole Pabi: *Investigation of the stability of micronutrients in beverages*

Micronutrients represent an important class of substances in the field of nutrition and in the enrichment of food. Their stability, however, is limited and varies within the foodstuff. The producer is obliged to ensure that the labeled micronutrients last by the end of the shelf-life and the flavour of the product should remain unchained. There are several parameters, such as the temperature, the oxygen content, the pH-value and interactions within the product composition, which affect the product stability. In beverages, but especially in carbonated soft-drinks, the pH-value is an invariable parameter, and the parameters temperature and oxygen content should be kept at a minimum to avoid degradation. A decomposition of micronutrients can also emerge likewise by interactions between and within the constituents of the beverage.

Within this work, the stability of the B-vitamins B_2, B_3, B_5, B_6 and B_{12} were examined in three different carbonated and caffeine-containing beverages. For the investigations, analytical methods such as HPLC and LC-MS were employed as the main techniques. The vitamins B_5 and B_{12} and the flavour were mainly affected by increased temperatures during storage. To enhance the stability of these vitamins and the flavour of the product, various natural antioxidants such as rosemary, pomegranate and acerola extracts were added and compared with the most common antioxidant ascorbic acid. Accelerated shelf-life tests (ASLT) were applied for the prediction on the stability of the beverages. For that purpose, products were stored at four different temperatures, namely 5, 20, 30, 40 °C for ten weeks. Every second week, the products were evaluated by analytical and sensory testing. To examine the antioxidant activity of the several antioxidants, different analyzing techniques, e.g., ORAC-, TAC- and Polyphenol-Assay were tested. For that, the total antioxidant capacity and sensory evaluations were executed, whereas the importance of the combination of both methods were emphasized. Besides the addition of antioxidants to improve the vitamin stability, an additional investigation attempted the replacement of the common vitamin B5, calcium-D-pantothenate, with the more stable D-Panthenol.

The results of the investigations are discussed in detail within this thesis. Additionally, comprehensive insights of the theory of vitamins, colors and antioxidants, and a brief overview of the methods are given in the first three chapters of this work.

Master Theses completed

Julia Russ: *Quantitative determination of metformin and melatonin using high performance liquid chromatography*

The first part of this thesis deals with the quantitative analysis of metformin, which is part of a bigger project in cooperation with the Medical University of Graz. Metformin is administered to patients affected by type 2 diabetes mellitus either alone or in combination with other medications. Patient's urine (n = 102) and plasma (n = 78) samples taken 0 and 2 hours after administration of metformin medication are analyzed for their metformin content. The data is correlated with genetic data from the patients by the Medical University. Samples are subjected to precolumn fluorescence derivatization using benzoin and analyzed using a linear gradient elution with a C18-column on the HPLC. A flow rate of 0.5 ml/min for urine and 0.6 ml/min for plasma samples was used. Solvent 1 (5% ACN, 21% 0.5 M HCl/tris buffer pH 8, 74% ultrapure H2O) was decreased from 100% to 0% over the timeframe of 5 minutes, while solvent 2 (65% ACN, 10% 0.5 M HCl/tris buffer pH 8, 10% THF, 15% ultrapure H2O) was increased accordingly for a total run time of 10 and 12 minutes, respectively. The retention time for the metformin derivative was 8.6 minutes for urine samples and 8.1 minutes for plasma. The fluorescence maxima were detected at 280 nm for excitation and 440 nm for emission.

The second part of the thesis is concerned with the quantitative analysis of melatonin. Cranberry and white mustard seeds are analyzed both involving precolumn derivatization and direct measurement with the HPLC using a C18 column. In this thesis, the procedure involving precolumn derivatization did not give any results. Therefore, melatonin was analyzed directly using its own fluorescence at 280 nm (excitation) and 350 nm (emission). With a run time of 15 minutes, the melatonin peak is found at 6.7 minutes. Starting at 20% ACN and 80% ultrapure H2O, the gradient increases to 35% ACN and 65% ultrapure H2O over 6.5 minutes and then to 100% ACN over 1 minute. 100% ACN is maintained for the rest of the running time. The HPLC methods were validated for linearity, precision, accuracy, and robustness.

Anita Reisenbauer: *Development of an Escherichia coli strain to express bacterial antigenic peptide and coexpress Braun's lipoprotein antisense oligonucleotide*

Aquaculture is a really important sector of the food industry. Through the fact that fish in such cultures live together very close, it is super easy for diseases like Vibriosis to transmit between them. Therefore, it is important to develop vaccines for the fish against such diseases. Thus, the aim of this study was to overexpress the OmpK protein, which is widely distributed on the cell envelope of Vibrio species, in a Escherichia coli industrial strain, so that, once purified, an antibody can be raised for fundamental studies and OmpK production optimized to be used as vaccine against Vibriosis. In the beginning, OmpK produced from other recombinant *E. coli* cells that were obtained from a previous study was overexpressed. Although, the overexpression was successful, a purified soluble protein extract revealed high endotoxins content, and so that protein solution could not be used to produce the antibody. A HIC was carried out to see, if that way the endotoxins could be separated from the protein efficiently. The chromatogram showed two isolated peaks which may indicate enough separation. As the concentration of the OmpK protein was too low for a confirmation of this result, this part must be carried out again with a higher concentrated protein solution.

The first main part of this thesis was to build a plasmid with the sequence of a Lpp antisense, with the objective of increasing the outer membrane permeability of the expressing cells. After the cloning steps in E. coli αDH5, which obtained the pMLBAD vector with the sequence for the Lpp antisense, the plasmid was transformed into E. coli BL21 (DE3) and some physiological tests were carried out to check the effect of the plasmid on the cells. None of the tests showed the expected results. Just afterwards the result of the sequencing arrived, which revealed that the insert in the plasmid was not the desired one.

The second main part was to express the outer membrane protein itself. As for the production of antibodies it is not necessary to use the whole protein but an antigenic fragment of it, just the N-terminal part of OmpK with the natural signal peptide was cloned. When the pET23a+ vector containing the correct sequence was transformed into E. coli BL21 (DE3), several different attempts of overexpression were carried out. Unfortunately, none of them was successful and different attempts of the overproduction of the protein still have to be tried.

Cathrina Neuhauser: *Identification and characterization of phytogenic substances with potential health beneficial properties*

As the number of patients suffering from diabetes mellitus and insulin resistance is increasing worldwide and current pharmaceutical therapies often come along with severe side effects, alternative treatment and prevention strategies are of critical importance. Reasons for diabetes mellitus include improper insulin production/secretion or insulin resistance. In healthy human beings, insulin binds to its corresponding receptor on the cell surface. As a result, the PI3K pathway is activated, which induces the translocation of glucose transporter 4 (GLUT4) to the plasma membrane and finally leads to glucose uptake in muscle and adipose tissue.

Several plant extracts, used as nutraceuticals or in traditional medicine are known to contain active ingredients, which are capable of translocating GLUT4 to the plasma membrane in the absence of insulin. Hence, these extracts appear to be candidates for alternative treatment and prevention strategies of diabetes mellitus as they could potentially lower the blood glucose level. In the course of this study, a primary screen for identifying phytogenic extracts, which contain bioactive compounds that could be integrated into functional food and food supplements for the prevention and therapy of diabetes, was performed. To efficiently quantitate the translocation of GLUT4 from intracellular storage compartments to the plasma

membrane, GLUT4 was co-labelled with a myc and a GFP tag and stably expressed in HeLa cells. Translocation of GLUT4 was imaged by total internal reflection microscopy (TIRFM) and signal intensity change before and after substance addition was determined.

Overall, 254 phytogenic extracts were tested for their capability of translocating GLUT4 to the plasma membrane in the absence of insulin. More than 30 of these non- to medium auto-fluorescent phytogenic compounds showed a signal increase of plasma membrane localized GLUT4 higher than 3% and are possible candidates for future in vivo tests including the hens egg test (HET).

Claudia Kieler: *Biological Activity of a Pt(II) Metallacycle Aimed at Targeting G-Quadruplex Nucleic Acids*

G-quadruplexes (G4) are structurally very polymorphic non-canonical DNA/RNA secondary structures, which can readily form in guanine-rich sequences both in vitro and in vivo. As their location was found to be non-random and enriched in biologically relevant sites of the genome, such as telomeres, oncogene promoters as well as 5′ and 3′ untranslated regions (UTRs), G4s attracted increasing attention during the last years as novel promising targets for anticancer therapy. Thus, the rational design of small molecule ligands selective for G4s has become a promising strategy in the development of potential antitumor agents. Amongst them, metal-based compounds have emerged as particularly strong and selective G4 binders. Based on a recent report of a self-assembly strategy of Pt2L2 boxes aimed at targeting G-quadruplex structures, a new, improved Pt2L2 metallacycle displaying intrinsic fluorescence was synthesized.

The aim of this study, thus, was to explore the biological applicability of this Pt2L2 compound for potential antitumor therapy and investigate its intracellular behavior. Moderate cytotoxic effects and anti-proliferative activity were observed in different human cancer cell models. Live cell imaging and confocal laser scanning microscopy (CLSM) further revealed that this compound is stable in the intracellular milieu, taken up by cancer cells and delivered to the nucleus displaying a strong blue fluorescence. This fluorescence signal was more resistant to UV photobleaching in nucleoli, nuclear substructures known to be particularly rich in G4-forming sequences, which indicates that the Pt2L2 metallacycle can detect both double-stranded and G-quadruplex DNA structures, but has a higher affinity for G4s. Immunostaining with a G4-selective antibody further proved that the Pt2 compound shares the identical final molecular target. Competition assays showed that the metallacycle replaces other well-established G4 ligands. Moreover, photophysical changes including fluorescence quenching and increased absorption were observed upon G4 binding, indicating, once again, that the compound displays increased selectivity for G4s compared to dsDNA. Finally, via whole genome gene expression microarray and gene set enrichment analysis (GSEA) it was revealed that the Pt2 compound indeed targets G4 regulated genes, especially genes located in chromosomal regions enriched for G4 structures.

Summing up, we were able to show that the novel Pt2L2 metallacycle is a promising candidate for targeting and detecting G-quadruplex DNA/RNA structures in cancer cells and aids in the development of new G4-selective Pt-based anticancer compounds.

International cooperations

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

V. Piironen, Department of Applied Chemistry and Microbiology, Helsinki, Finland

Z. Cieserova, Food Research Institute, Bratislava, Slowakia

K. Cejpek, VSCHT Prague, Czech

R. Swasti, Atma Jaya University, Yogyakarta, Indonesia

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

Talk at international conference

1. M. Murkovic (2019) 1st International GHI Conference, Leyden NL, Furfuryl alcohol a potentially toxic compound formed during roasting of coffee

Publications

1. Albouchi, A., Murkovic, M. LC method for the direct and simultaneous determination of four major furan derivatives in coffee grounds and brews. (2019) ***Journal of Separation Science***, 1-7.
2. Zeb, A., Haq, A., Murkovic, M. Effects of microwave cooking on carotenoids, phenolic compounds and antioxidant activity on Cichorium intybus L. (chicory) leaves. (2019) ***European Food Research and Technology***, 245, 365-374.

Lectures and Laboratory Courses

Winter Semester 2018/19

Course no.	Title	Hours	Type	Lecturers
CHE.154_1UF	Biochemistry Lab Course I	5.33	LU	Team
CHE.155UF	Biochemistry II	1.5	VO	Macheroux P
CHE.191UF	Bioanalytics	2.25	VO	Klimant I, Winkler A
CHE.192UF	Biochemistry Lab Course II	4	LU	Team
CHE.210_FUF	Project Work to the Bachelor Thesis	0.5	SE	Macheroux P, Murkovic M
CHE.890UF	Food Biotechnology	1.33	VO	Murkovic M
CHE.892UF	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MAS.420UF	Biocompatible Materials	2	VO	Amor M
MOL.101UB	Introduction to Bachelor Study	1	SE	Macheroux P
MOL.606_1UF	Bachelor Thesis	1	SE	Macheroux P, Murkovic M
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.933UF	Food Biotechnology	1.3	VO	Murkovic M
MOL.959UF	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MOL.961UF	Food Chemistry and Technology II	2	VO	Murkovic M
648.000	Laboratory I	4	PV	Macheroux P
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 2019

Course no.	Title	Hours	Type	Lecturers
CHE.147UF	Biochemistry I	3.75	VO	Macheroux P
CHE.193UF	Molecular biology laboratory course	3	LU	Jha S
CHE.194UF	Seminar for Molecular biology laboratory course	1	SE	Wallner S
CHE.210_FUF	Project Work to the Bachelor Thesis	0.5	SE	Macheroux P, Murkovic M
MOL.406UF	Methods in Immunology	2	VO	Oberdorfer G
MOL.407UF	Methods in Immunology	2	LU	Team
MOL.606_1UF	Bachelor Thesis	1	SE	Team
MOL.833_1UF	Project laboratory	9	LU	Team
MOL.845_1UF	Seminar for undergraduate students	2	SE	Team
MOL.880UF	Molecular Enzymology	2	VO	Gruber K, Macheroux P, Nidetzky B
MOL.886UB	Biophysical Methods	3	LU	Winkler A, Oberdorfer G, […]
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	Wallner S
648.008	Graduate Seminar 2	1	SE	Team
648.010	Scientific Colloquium for Graduate Students 2	1	SE	Team
648.011	Integrative Structural Biochemistry	2	VO	Winkler A, Madl T, Gruber C
648.012	Frontiers in Integrative Structural Biology	1	SE	Macheroux P, Winkler A, …
648.013	Advanced cell culture training course II	1	SE	Amor M
648.016	Chemical Reactions in Foods II	2	PV	Murkovic M
648.020	Teaching Experience	2	SE	Macheroux P
648.302	Methods in Immunology	1	VO	Oberdorfer G
648.602	Cellular Metabolism 2	2	PV	Bogner-Strauss J

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