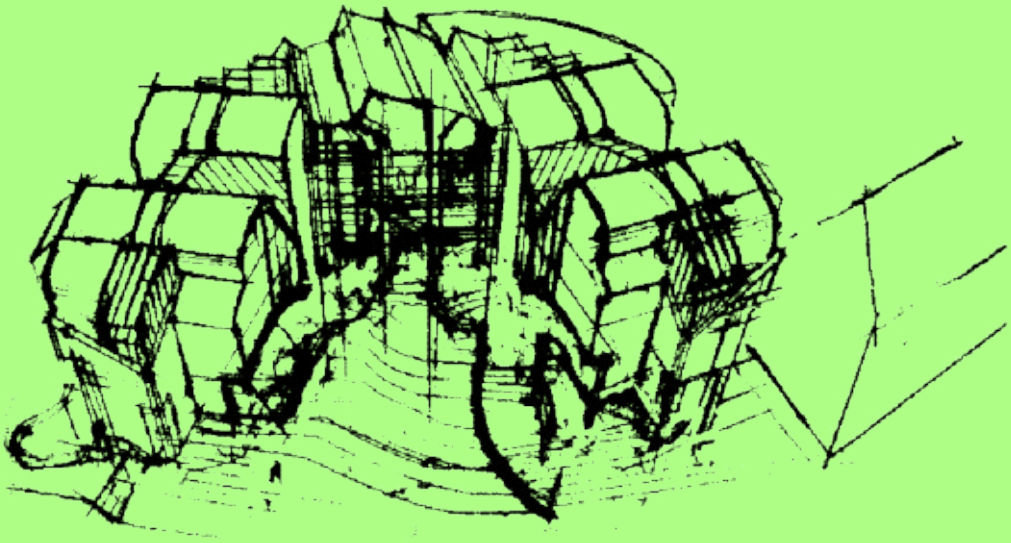
Graz University of Technology
Austria

Institute of Biochemistry



Annual Report 2017

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Brief History of the Institute of Biochemistry

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

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

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

1948 G. GORBACH was nominated full professor and head of the institute. In succession of the famous *Graz School of Microchemistry* founded by PREGL and EMICH, Prof. GORBACH was one of the most prominent and active leaders in the fields of microchemistry, microbiology and nutritional sciences. After World War II, questions of water quality and wastewater disposal became urgent; hence, the group of Prof. K. STUNDL, which at that time was part of the institute, was gaining importance. In addition, a division to fight dry-rot supervised by Dr. KUNZE and after his demise by H. SALOMON, was also affiliated with the institute.

1955 In honor of the founder of microchemistry and former professor at *Graz University of Technology*, the extended laboratory was called EMICH-Laboratories. At the same time, the institute was renamed *Institute of Biochemical Technology, Food Chemistry and Microchemistry*.

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

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

1973 Dr. F. PALTAUF from the University of Graz was appointed professor and head of the newly established *Institute of Biochemistry*. The interest of Prof. PALTAUF to study biological membranes and lipids laid the foundation for the future direction of research. G. DAUM, S. D. KOHLWEIN, and A. HERMETTER joined the institute and had the opportunity to carry out postdoctoral research in renown laboratories: G. DAUM with the groups of G. Schatz (Basel, Switzerland) and R. Schekman (Berkeley, USA), A. HERMETTER with J. R. Lakowicz (Baltimore, USA) and S. D. KOHLWEIN with S. A. Henry (New York, USA). Independent research groups in cell biology (G. D.), biophysics (A. H.) and molecular biology (S. D. K.) were established, with the group of Prof. F. PALTAUF still focusing on the biochemistry of lipids.

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

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

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

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

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

2013 As a result of inter-faculty reorganization, the research group of Prof. J. BOGNER-STRAUSS (formerly at the *Institute of Genomics and Bioinformatics*) joined the *Institute of Biochemistry*. Her main interests revolve around the development and metabolism of adipocytes.

2014 A. WINKLER, who was one of the first students to receive his PhD in the DK "Molecular Enzymology" in 2009, returned to the institute after conducting postdoctoral research with Prof. Schlichting at the Max-Planck-Institute of Medical Research in Heidelberg, Germany for five years.

2015 After more than 40 years of service for the *Institute of Biochemistry* Prof. HERMETTER retired on September 30. Albin started as a graduate student in Prof. PALTAUF's group and moved through the ranks to become an associate professor at the institute. As an active member of the institute he published more than 120 papers in peer-reviewed journals and graduated 25 PhD students.

2016 A. WINKLER was appointed assistant professor at the institute to establish an independent research group on photoreceptor science.

2017 G. DAUM, who had been at the institute for 42 years, retired at the end of the academic year. G. DAUM was one of the leading scientists in the field of yeast lipid research in Graz enjoying worldwide recognition for his seminal achievements. Günther was also a very enthusiastic academic teacher supervising 36 PhD students and acting as director of the Doctoral School for Biosciences.

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

Highlights of 2017

42 successful years in biochemistry and cell biology - Günther Daum retired in 2017

Günther Daum was born in 1951 in Graz, Austria. He is married to his beloved wife Christa, and has 3 children (Michael, Eva and Lukas) and 4 grandchildren (Klaara, Valentina, Joonas and Gwendolyn). Besides his family science was his major interest. On September 30th, Günther Daum retired after 42 years of a successful scientific career, which never would have been possible without the support by his family, especially by his wife.

Already in high school (1961-1969) Günther Daum had made the decision to study chemistry at the Graz University of Technology. He was encouraged to do so by his excellent chemistry teacher Stefan Winkler to whom he owes a lot. Thus, after finishing his military service, Günther Daum enrolled at the Graz University of Technololgy as a chemistry student. In those days, the biosciences were still in their infancy. Nevertheless, Günther Daum realized that the society could benefit from upcoming scientific insights into this field.

Günther Daum took the studies of chemistry seriously. He finished his studies in the minimum of time (1970 – 1975). During these years, he met two persons, who were very important for his future career: Prof. Fritz Paltauf, a classical biochemist, and Prof. Robert Lafferty, a biotechnologist. Günther Daum was excited about both fields, but finally decided that he should specialize in the more academically oriented field of biochemistry. Therefore, he performed his Diploma Thesis (1975) entitled "Influence of myo-inositol on growth of *Saccharomyces carlsbergensis* ATCC 9080 - Metabolism of inositol-containing compounds" at the Institute of Biochemistry at the Graz University of Technology under the guidance of Prof. F. Paltauf. This work laid the foundation for Günther's interest in the field of lipid research, which he followed up on during his Doctoral Thesis (1975 – 1978). For the following two years, he received an assistant position at the Institute of Biochemistry at the Graz University of Technology. From this time on teaching has become another major task, which he enjoyed from the first to the last day as a member of the institute.

After two years as an assistant, Günther Daum had the opportunity to join the group of Prof. Gottfried "Jeff" Schatz at the Biocenter Basel (Switzerland) supported by an EMBO Long-term Fellowship. Jeff Schatz, who died much too early in 2015, taught him two things: first how to perform experiments in cell biology, and secondly, how to present scientific results. Both things were valuable gifts and very important for Günther's future career.In 1982, he returned to Graz and continued his work as Assistant Professor at the Institute of Biochemistry. In 1985, he completed his "Habilitation" in Biochemistry ("Biogenesis of cellular membranes") and was appointed docent, receiving the "*venia legendi*" – the right to teach independently. In 1986, Günther got another excellent chance to broaden his scientific interests: He was accepted as a postdoctoral fellow in the lab of Prof. Randy Schekman, University of California, Berkeley, USA, who received the Nobel Prize for Physiology and Medicine in 2013 together with James Rothman and Thomas C. Südhof for their ground-breaking work on cell membrane vesicle trafficking. For his studies that focused on the secretory pathway in yeast, he received a Max-Kade Fellowship.

In 1987, Günther returned to Graz, with the desire to contribute to the scientific landscape of Austria, the country that had supported him throughout his initial career. With Prof. Paltauf's retirement in 2001, Günther Daum became interim head of the Institute of Biochemistry. In 2003, Prof. Peter Macheroux took over as head of the Institute and introduced a new focus, namely enzyme biochemistry and molecular biology. Nevertheless Günther Daum and his dear colleague Albin Hermetter (who retired in 2015) continued their research on various aspects of lipid biochemistry.

The main topics studied in Günther's group were dedicated to the synthesis and intracellular dynamics of lipids in yeast. He employed methods of biochemistry, cell biology and molecular biology and used the baker's yeast *Saccharomyces cerevisiae* as eukaryotic model. Major research interests were the identification of novel genes and gene products involved in yeast lipid metabolism and lipid assembly into organelles, as well as the characterization of these processes. One successful field was the discovery of triacylglycerol lipases, which contributed significantly to the successful lipid research in Graz. Günther was always a well-respected teacher and 36 PhD students as well as numerous diploma/master students were trained in his lab. He felt always very privileged to work with some of the best students with respect to research performance as well as social dynamics in his group.

As mentioned above, it was very important for Günther to give back for what he had received by the scientific community. Therefore, he organized several conferences, reviewed articles for many renowned journals and served as reviewing board member in several international funding agencies. He also actively participated in the development of university related aspects as well as in the representation of scientific agencies at the interface of science and society. Major highlights of his career are the foundation of the Yeast Lipid Conference series, his membership at the board of the Austrian Science Fund (FWF), his work as Director of the Doctoral School Molecular Biosciences and Biotechnology at the Graz University of Technology and his duty as the president of the German Society of Fat Science (DGF). A complete list of activities can be found in the detailed description of the cell biology group.

In recognition of his efforts for the scientific community, Günther has received several prizes and awards. Among them the Unilever Award, an EMBO-longterm Fellowship, a Max Kade Fellowship and the Normann Medal of the German Society of Fat Science are the most important that also reflect the various stages of his scientific career.

According to Google Scholar Günther Daum has published 178 papers (two more will follow); his publications received more than 11000 citations giving rise to an h-index of 59. More than 390 presentations were contributed to international conferences, among them invited talks and poster presentations. Over the years Günther had many very fruitful collaborations that contributed significantly to the success of his research.

Günther Daum's research was always well funded. Most of the 34 projects were stand-alone projects funded by the Austrian Science Fund (FWF), some were funded by the Austrian National Bank or the Austrian Government. Among these 34 projects there were 9 joint projects. Without the funding by these agencies the science performed by Günther Daum would never have been so successful.

Günther Daum always liked his work at the Institute of Biochemistry of the Graz University of Technology. Especially the work with young people made himself feel young which was the basis for his fruitful career.

In 2017 two doctoral students completed their PhD thesis in the **Macheroux group**. Peter Augustin joined GL Pharma as pharmaco-vigilance officer and Karin Koch started an academic position at the University of Gent in Belgium.

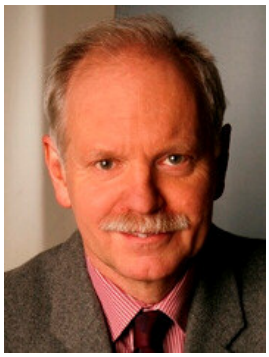
The conference highlight of the year was the 19th International Symposium on Flavins and Flavoproteins held in Groningen (The Netherlands) from the 2nd-7th of July (participants from the institute are shown on the picture to the left).

At this conference it was also decided to hold the 20th meeting in 2020 in Graz! We are very much looking forward to this challenge and to the opportunity to present us as a hot spot in flavoprotein biochemistry and enzymology!

Dina Hofer of the **Bogner-Strauss group** completed her research stay in Lausanne, Switzerland. She returned with a job offer for a PostDoc position in the lab of Johan Auwerx. Three more PhD students – Katharina Huber, Wenmin Xia, Furkan Alkan – started their stay abroad in the USA.
In March we welcomed two new PhD students – Katharina Eva Walter and Gabriel Zirkovits. Katharina started her work on cancer, while Gabriel investigates liver metabolism.
Katharina Küntzel and Irene Svoboda successfully finished their master theses.

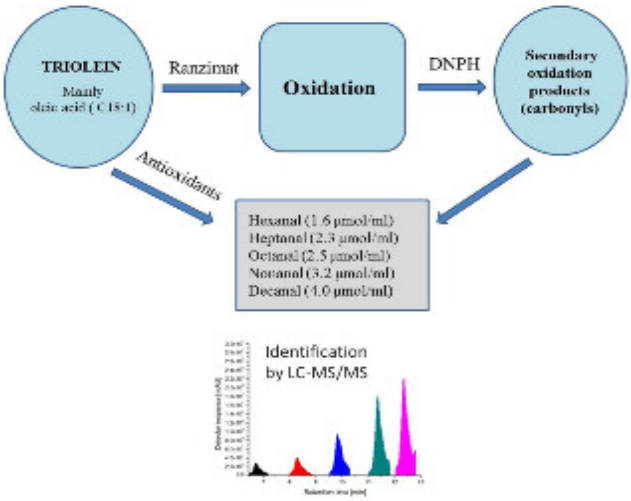
On Dezember 18th 2017, **Juliane Bogner-Strauß** was sworn in as Minister for Women, Families, and Youth in the Austrian government. We congratulate her to this achievement and wish her all the best for her new adventure!

In 2017 the lab of **Günther Daum** was closed. After 42 years of work at the Institute Günther Daum retired, being now able to dedicate his time to his hobby, the painting. All his research projects were successfully finished and even in the last year of Günther Daum's scientific career 5 papers were published. By no means Günther Daum wants to miss the opportunity to thank all his coworker for the successful cooperation over the four decades. More details of Günther Daum's career are summarized on pages 4 and 5 of this Annual report.



The group of **Andreas Winkler** temporarily grew to eight members in 2017. With several Bachelor, Master and PhD students working together this was a very stimulating scientific year. Presenting research output from the group, Andreas Winkler was invited to give a talk at the 13th International Conference on Tetrapyrrole Photoreceptors of Photosynthetic Organisms (ICTPPO) in Chigao (USA) as well as a poster pitch talk at the 19th International Symposium on Flavins and Flavoproteins in Groningen (NL).

In the Functional Food Group of **Michael Murkovic** the main projects were focused on the quality of homemade fruit juices. Several concepts of improving the stability of micronutrients of freshly squeezed fruit juices were developed. In a second project the formation of health threatening components was investigated. These were oxidised lipids and furfuryl alcohol. Both might be related to cancer and a reduction of alimentary exposure should improve food safety.



In 2017, **Grit Straganz** finished her FWF project about the role of the protein environment in O_2 activation at mononuclear nonheme Fe(II) sites. Johannes Niederhauser from her group successfully completed his PhD thesis, with several publications waiting in the pipeline.

Biochemistry Group

Group leader: Peter Macheroux
Secretary: Annemarie Lehsl / Tinkara Kristovic
Senior research scientists / postdoctoral fellows: Bastian Daniel, Alexandra Jammer, Wolf-Dieter Lienhart, Silvia Wallner (on maternal leave)
PhD students: Peter Augustin, Eveline Brodl, Reinmar Eggers, Shalinee Jha, Sami Ullah Khan, Karin Koch, Majd Lahham, Barbara Konrad, Emilia Strandback, Marina Toplak.
Master students: Julia Brunner, Julia Messenlehner, Sabine Pils
Technical staff: Eva Maria Frießer, Rosemarie Trenker-El-Toukhy, Alma Makic

General description

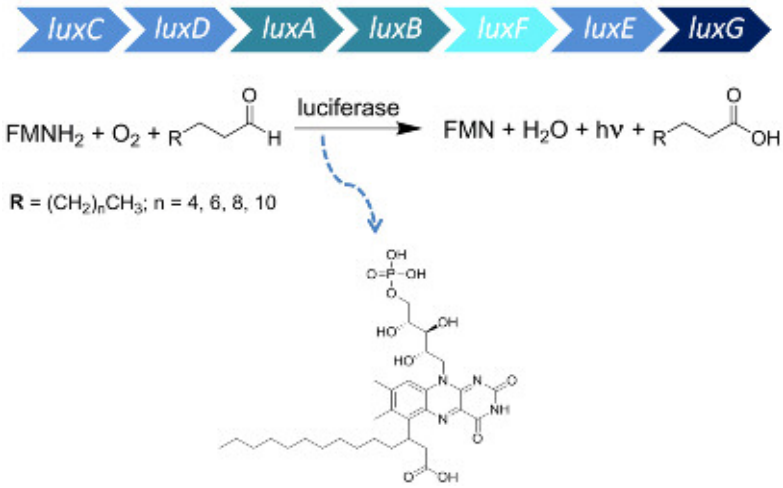
The fundamental questions in the study of enzymes, the bio-catalysts of all living organisms, revolve around their ability to select a substrate (substrate specificity) and subject this substrate to a predetermined chemical reaction (reaction and regio-specificity). In general, only a few amino acid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

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

Bacterial Bioluminescence

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

In our laboratory, we are interested in the bioluminescence of marine bacteria. In these bacteria, the enzyme luciferase (a heterodimeric protein consisting of a 40 kDa α-subunit and a 37 kDa β-subunit) binds to the reduced flavin mononucleotide ($FMNH_2$), which then reacts with dioxygen 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 intermediate is generated (luciferin), which emits the blue-green light peaking at 490 nm. Many strains in the genus *Photobacterium* carry an additional gene, termed *luxF*. The main function of LuxF is supposedly the binding of 6-(3'-(*R*)-myristyl-FMN) (myrFMN), a possible side product of the luciferase reaction.



Overview of bacterial bioluminescence. The upper part shows the order of genes in the lux operon. LuxA and LuxB form the luciferase; LuxC,D,E generate the aldehyde substrate and LuxG the reduced flavin. Together with molecular oxygen these are all the requirements for light generation. The myrFMN side-product scavenged by LuxF is shown at the bottom.

We hypothesized that LuxF is the putative scavenger of myrFMN, due to a higher affinity to LuxF than to the luciferase, thereby preventing inhibition. Replication of the bioluminescent reaction *in vitro* was achieved by applying an enzyme cascade with a cofactor recycling system. In multiple turnover reactions, producing light for more than 48 hours, the formation of myrFMN was confirmed via HPLC/MS analysis. *In vivo* analysis of a range of bioluminescent bacteria ($luxF^+$ and $luxF^-$) revealed that myrFMN formation is independent of *luxF* occurrence. However, there seems to be a correlation between light intensity, myrFMN formation and *luxF* occurrence. Therefore, we are further investigating the role of LuxF, on the one hand *in vitro* via heterologous expression and on the other hand *in vivo* by comparing various bioluminescent bacterial strains ($luxF^+$ and $luxF^-$) to elucidate the influence of LuxF on light emission.

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 (thesis project of Eveline Brodl).

Yeast flavoproteins

Analysis of the genome of the yeast *Saccharomyces cerevisiae* identified 68 genes encoding flavin-dependent enzymes. This organism is one of the best-investigated eukaryotic model systems for molecular and cell biology. Nevertheless many flavoproteins are poorly characterized. In our recent studies, we conducted a detailed biochemical and structural investigation of Pst2p, a member of the flavodoxin-like family. It was demonstrated that Pst2p is a very efficient NAD(P)H:quinone oxidoreductase rapidly reducing quinones *in vitro*

and *in vivo*. However, in contrast to other quinone reductases Pst2p displays an unusually positive redox potential. This affects its substrate spectrum, *e.g.* Pst2p possesses neither azo reductase nor FMN reductase activity. Furthermore, Pst2p binds reduced FMN ca. five orders of magnitude tighter than oxidized FMN. The structure of Pst2p was elucidated by X-ray crystallography in collaboration with Altijana Hromic and Prof. Karl Gruber and revealed that Pst2p adopts the flavodoxin-like fold. It was also found by size exclusion chromatography that Pst2p forms tetramers independent of cofactor binding. In summary our data suggest that Pst2p possesses more similarity to quinone reductases than flavodoxins and that Pst2p enables yeast cells to cope with quinone-induced damage suggesting a role of the enzyme in managing oxidative stress.

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

Berberine bridge enzyme-like enzymes in plants

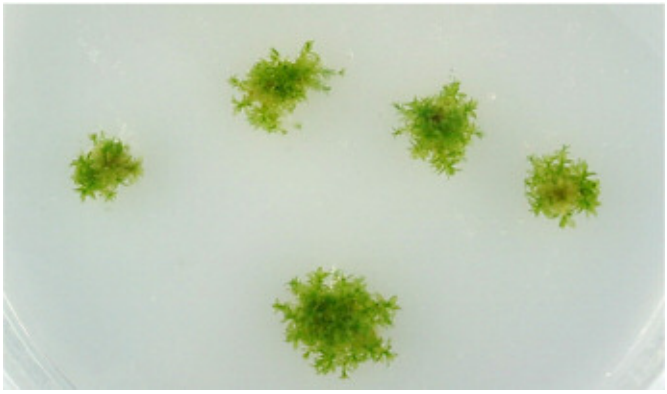
The berberine bridge enzyme- (BBE-) like protein family is a large enzyme family found in bacteria, fungi and plants. It is named after its best characterized member, the berberine bridge enzyme from *Eschscholzia californica* (*Ec*BBE), which is a key enzyme of the biosynthesis of benzylisoquinoline, where it catalyses the important cyclization reaction from (*S*)-reticuline to (*S*)-scoulerine. *Ec*BBE was first characterized by Andreas Winkler, a former PhD student in our group, who showed that the protein exhibits a very unusual protein fold that goes along with a bicovalent attachment of its FAD cofactor. Since then, we have continued our efforts to track down the mysteries around the multi-gene family of BBE-like enzymes. To do so, we have specialized on BBE-like enzymes from plants, which seemed especially interesting to us as the number of BBE-like genes found in various plant species appears to correlate with the development of basal to higher plants, i.e. the moss *Physcomitrella patens* possesses only two BBE-like protein(s), whereas *Arabidopsis thaliana* has 27 genes encoding BBE-like proteins.

In the course of our studies of the BBE-like enzymes from *A. thaliana*, we have identified two BBE-like enzymes (*At*BBE-like 13 and 15) as monolignol oxidoreductases. In 2017, we have focused on a site directed mutagenesis program to elucidate the enzymatic mechanism of these enzymes (master thesis Julia Messenlehner). We have also continued our efforts to develop a better understanding of the physiological function of monolignol oxidoreductases based on a combination of *in vitro* and *in vivo* studies. For that purpose, we are working closely with the research group of Dr. Alexandra Jammer from the *Institute of Plant Biology* at the University of Graz. Together with Reinmar Eggers (PhD thesis project), Alissa Pichler (master thesis), Jasmin Stocker (project student), Janina-Maria Worba (bachelor thesis) and Simon Schernthanner (bachelor thesis), she is carrying out the

characterization of knock out plants, visualizing expression patterns of the enzymes and preparing plant material for analyses of the content of lignin and soluble phenolics that are carried out by our international cooperation partners Wout Boerjan (VIB-UGent Center for Plant Systems Biology, Gent) and Catherine Lapierre (INRA Centre de Versailles-Grignon, Versailles).

With the goal of getting a better understanding of the evolution and development of the gene family of BBE-like proteins, we recently also started working with the BBE-like protein from *Physcomitrella patens* (*Pp*BBE), which is the most basal plant possessing a *BBE-like* gene. Like the BBE-like enzymes from *A. thliana,* we also expressed *Pp*BBE in *Komagataella phaffii*, which enabled further biochemical and structural characterization. We found that the enzyme exhibits very similar structural and spectral features as previously shown for other BBE-like proteins, whereas the catalytic role was found to be rather different. In screening assays, we could show that *Pp*BBE acts on the disaccharide cellobiose, which gets oxidized at the anomeric carbon to yield the corresponding lactone as the sole product. With the help of the structure guided mutagenesis program, we were able to reveal the role of various active site residues in catalysis, which further enabled us to postulate a reaction mechanism.

Our *in vitro* findings are further supported by the *in vivo* results obtained from the characterization of a *Pp*BBE knock-out strain, which was done in cooperation with the research group of Prof. Dr. Ralf Reski from the Department of Plant Biotechnology at the Albert-Ludwigs-University Freiburg. It was found that the enzyme is highly expressed in the chloronema phase, an early stage of the plant's life cycle, where carbohydrate metabolism is strongly upregulated and that the protein is secreted to the extracellular space. Hence, it is possible that *Pp*BBE is involved in the later steps of cellulose degradation, thereby allowing the moss to most efficiently use cellulose for energy production (thesis project of Marina Toplak). Regarding the findings concerning the molecular mechanism of *Pp*BBE and *At*BBE-like 15, we can conclude that the majority of BBE-like enzymes appear to be alcohol oxidases. A common enzymatic mechanism for all of these enzymes can be anticipated involving a catalytic base that is activating the alcohol to form an alcoholate intermediate prior to hydride transfer to the covalently linked FAD.



The moss Physcomitrella patens grown on solid standard agar. The knockout of the BBE-like gene does not result in a clear phenotype under these artificial laboratory conditions.

Human dipeptidylpeptidase III

Dipeptidyl peptidase III (DPPIII) is a zinc-dependent metalloenzyme involved in degrading shorter peptides with 4–12 amino acid residues. It exhibits high affinity to opioid

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

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

Flavin-dependent electron transfer in human mitochondria

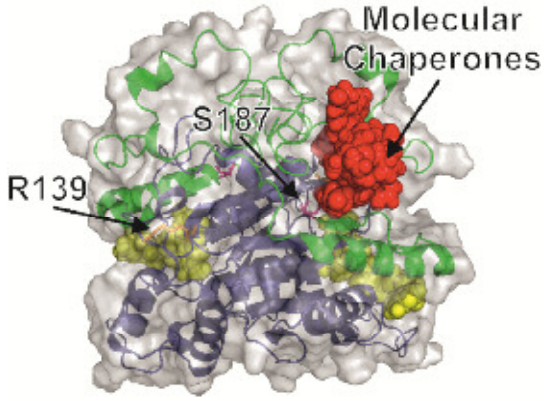
The human electron transferring flavoprotein (hETF) is a versatile and important electron carrier in the mitochondrial matrix and mediates the electron transfer from at least thirteen distinct flavin dehydrogenases to the hETF-ubiquinone oxidoreductase (hETF-QO). The inner mitochondrial membrane bound hETF-QO reduces ubiquinone to ubiquinol and thus directly feeds electrons into the mitochondrial respiratory chain. During our work with hETF, we have discovered that the bound FAD undergoes slow oxidation to an unusual FAD derivative, 8-formyl FAD (8fFAD). Furthermore, we observed that this process strongly depends on the pH and several amino acid residues in the FAD binding pocket. This is the first human flavoenzyme where the active formation of 8fFAD was observed. Previously, we have evaluated the effect of 8fFAD formation on the interaction with dimethylglycine dehydrogenase (hDMGDH) and detected that the affinity to hDMGDH was substantially enhanced for hETF harboring the formylated cofacter. In order to investigate a potential role of 8fFAD in mitochondrial electron handling, we are now investigating the interaction of formylated and unformylated hETF with three other flavin-dehydrogenases, the acyl-CoA dehydrogenase family member 9 (ACAD9), the medium-chain-acyl-CoA dehydrogenase (MCAD) and the isobutyryl-CoA dehydrogenase (IBD) as well as the interaction with hETF-QO (thesis project of Peter Augustin, Sami Ullah Khan and Marina Toplak).

Human quinone reductase NQO1

The human NAD(P)H:quinone oxidoreductase 1 (NQO1) is a FAD dependent enzyme catalyzing the two-electron reduction of quinones to hydroquinones. NQO1 binds to the 20*S* proteasome and recruits important tumor suppressor proteins such as p53 and p73α. The level of NQO1 is also increased in several tumors, making it an important target for anti-cancer prodrugs. Interestingly, the *NQO1* gene is polymorphic and the two most common variants in humans, excluding the wildtype, feature single amino acid replacements (P187S and R139W). Currently we are investigating the detailed molecular reasons for the loss of function and the reduced stability of NQO1 P187S and NQO1 R139W both of which are implicated in tumor development.

Our former studies not only provided the first crystallographic structure of NQO1 P187S but also an explanation for the biochemical dysfunction of this variant. Since the crystal structure of the NQO1 P187S variant was identical to the wild-type NQO1, the observable differences seem to arise from structural instability in particular in the C-terminal domain (shown in green in the figure below), which is in contact to the site of the amino acid replacement.

The current study investigates the possibility to restore the activity and stability of the NQO1 P187S variant by using small molecular-chaperones with the view to use these in cancer chemotherapy for individuals carrying the NQO1*2/*2 allele (thesis project of Emilia Strandback in collaboration with Dr. Wolf-Dieter Lienhart).



Structure of NQO1 showing the cofactor FAD as a yellow sphere model, the C-terminus in green, molecular chaperones in red and the two sites of the amino acid replacements in orange (R139) and pink (S187).

Doctoral Theses completed

Peter Augustin: *Novel biochemical aspects of flavoprotein-mediated electron transport in human mitochondria*

The characterization of the two human flavoproteins dimethylglycine dehydrogenase (hDMGDH) and electron transferring flavoprotein (hETF) symbolizes exemplary the versatility of flavoproteins and their important roles in mitochondrial electron transport. hDMGDH is able to perform one- and two-electron transfer reactions and combines the three metabolic pathways folate one-carbon metabolism, choline catabolism and electron transfer in the mitochondrial matrix. We performed a detailed characterization of hDMGDH, identified the biochemical background that triggers the metabolic disorder dimethylglycine

dehydrogenase deficiency and for the first time solved the crystal structure of the human enzyme. hETF on the other hand stabilizes a semiquinone flavin redox state and lies at a key metabolic branch point of the mitochondrial respiratory chain. Our work with the already well characterized protein brought new insights in the protein mechanism and above all the surprise finding that the enzyme catalyzes the formation of 8-formyl FAD. This unusual FAD analog changes key functions of the enzyme, however, a detailed characterization of the potential physiological relevance must await further studies. Furthermore, we investigated the interaction mechanism of hETF with hDMGDH which is so far only the second interaction study of hETF with one of its thirteen partner proteins. Last, we evaluated the physiological role of hDMGDH in the sarcosine metabolism of human cells, especially in connection with prostate cancer.

Karin Koch: *Flavoproteins from the yeast Saccharomyces cerevisiae*

Analysis of the flavoproteome of the yeast Saccharomyces cerevisiae identified 68 genes encoding flavin-dependent enzymes. This organism is one of the best-investigated eukaryotic model systems for molecular and cell biological studies. Nevertheless, many flavoproteins from Saccharomyces cerevisiae are poorly characterized. This work was initiated to get a deeper insight into the characteristics of two different flavoproteins, the microtubule-associated Irc15p and the flavodoxin-like protein Pst2p. The proteins were heterologously expressed in *Escherichia coli* and purified for a detailed investigation. Cell biological studies indicate a functionality of Irc15p on microtubule dynamics and cell cycle progression. In my studies, I could demonstrate that Irc15p is a flavoprotein and its cofactor can be reduced with NAD(P)H and reoxidized by several artificial electron acceptors. Although Irc15p has a high structural similarity to the disulphide reducing lipoamide dehydrogenase, it lacks the protein disulfide but retained the catalytic diad, which are important for the enzymatic activity of lipoamide dehydrogenase. Even though my data do not directly support the disulphide reducing activity of Irc15p it is conceivable that the interaction with other proteins unmasks this catalytic activity. Alternatively, our findings suggest that Irc15p is efficiently reduced in yeast cells to deliver electrons to an as yet unidentified electron acceptor that is potentially related to Irc15p's function in regulating microtubule dynamics and cell cycle progression. The yeast genome encodes three highly similar flavodoxin-like proteins, namely Pst2p, Rfs1p and Ycp4p, however none of these proteins were functionally characterized. Therefore, I conducted a detailed investigation of Pst2p. It could be demonstrated that Pst2p is a very efficient NAD(P)H:quinone oxidoreductase rapidly reducing quinones in vitro and in vivo. However, in contrast to other quinone reductases Pst2p displays an unusually positive redox potential. This affects its substrate spectrum as well as its cofactor binding properties. The structure of Pst2p was elucidated by X-ray crystallography and revealed that Pst2p adopts the flavodoxin-like fold and forms tetramers independent of cofactor binding. In summary, my data suggest that Pst2p possesses more similarity to quinone reductases than flavodoxins and that Pst2p enables yeast cells to cope with quinone-induced damage suggesting a role of the enzyme in managing oxidative stress.

Master Thesis completed

Sabine Pils: *Characterization of a monolignol oxidoreductase from Arabidopsis thaliana for biocatalytic applications*

Flavoproteins are a large and diverse group of proteins, which use either flavin mononucleotide or flavin adenine dinucleotide as cofactor for their catalysis. They can be classified due to their binding of the cofactor. The flavin cofactor can either be covalent or non-covalently attached to the enzyme. In rare cases the cofactor is even bi-covalently attached to the enzyme. A common example for a bi-covalently attached enzyme is the berberine bridge enzyme (BBE) from the California poppy. In various plants BBE-like enzymes were identified in recent years. In many cases, their specific functions are not known yet. In this study a flavin-dependent BBE-like monolignol oxidoreductase from *Arabidopsis thaliana* (*At*BBE-like 15) was characterized to identify its potential as biocatalyst for oxidation reactions. As a dehydrogenase the enzyme shows sluggish activity towards oxygen. The resulting slow reaction velocities hamper the use of this enzyme as a biocatalyst. The exchange of a single amino acid resulted in a higher activity of *At*BBE-lke 15 towards oxygen, which makes this enzyme interesting for industrial applications. The enzyme was tested under various conditions, the pH and the temperature optimum of the enzyme were determined. Furthermore, the stability in organic co-solvents and the substrate scope was measured. The enzyme shows the highest activity at pH 7 and 50 °C. Between pH 5 and 10 it retains more than 50% of its activity. In the presence of acetonitrile, 2-propanol, 1,4-dioxane, THF, 1-butanol, DMSO and ethanol the enzyme shows an increased activity in comparison to the activity in buffer. Long-term stability in the presence of various organic solvents was tested. The highest long term stability is given in DMSO. As substrates, primary and secondary allylic alcohols and primary benzylic alcohols are accepted by the enzyme. The investigation of the enantioselectivity of the enzyme towards secondary allylic alcohols yielded good results with E-values of 34 to >200. These results make the enzyme a possible biocatalyst for oxidation reactions and enantioselective conversion, which cannot be performed by organic chemical synthesis.

International cooperations

Marjia Abramic & Marjia Luic, Ruder-Boskovic Institute, Zagreb, Croatia

Wout Boerjan, VIB-UGent Center for Plant Systems Biology, Gent, Belgium

Catherine Lapierre, INRA Centre de Versailles-Grignon, Versailles, France

Ralf Reski, Albert-Ludwigs University, Freiburg, Germany

Research projects

FWF P24189: “Bacterial bioluminescence”

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

FWF-PhD program “Molecular Enzymology” DK-Molecular Enzymology (W901)

Talks at international conferences

1. Strandback, E.: *Rescuing the stability of a cancer associated variant of human NQO1 by small-molecular chaperones*. 17th DocDay, Graz, February 2017.
2. Toplak, M.: *The origin of the berberine bridge enzyme-like protein family in plants*. 19th International Symposium on Flavins and Flavoproteins, Groningen, June 2017.
3. Khan, S. U.: *The interaction of human dimethylglycine dehydrogenase with electron transferring flavoprotein*. 19th International Symposium on Flavins and Flavoproteins, Groningen, June 2017.
4. Daniel, B.: *Berberine bridge enzyme-like proteins: From characterization to application*. 19th International Symposium on Flavins and Flavoproteins, Groningen, June 2017.
5. Lienhart, W.-D.: *Drug development for cancer treatment and prevention targeting the human NAD(P)H:quinone oxidoreductase 1*. 19th International Symposium on Flavins and Flavoproteins, Groningen, June 2017.
6. Toplak, M.: *Structural and functional characterization of the berberine bridge enzyme-like protein from Physcomitrella patens*. 18th DocDay, Graz, July 2017.
7. Jha, S.: *Dipeptidyl peptidase 3- an emerging key player in Renin-Angiotensin System and Oxidative stress*. 18th DocDay, Graz, July 2017.

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1. Tongsook, C., Niederhauser, J., Kronegger, E. M., Straganz, G., Macheroux, P.: Leucine 208 in human histamine *N*-methyltransferase emerges as a hotspot for protein stability rationalizing the role of the L208P variant in intellectual disability, Biochim. Biophys. Acta: Mol. Basis Dis., 2017, 1863:188-199; DOI:10.1016/j.bbadis.2016.10.005.
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5. Tabib, C., Brodl, E., Macheroux, P.: Evidence for the generation of myristylated FMN by bacterial luciferase, Mol. Microbiol., 2017, 104:1027-1036; DOI:10.1111/mmi.13676.
6. Koch, K., Hromic, A., Sorokina, M., Strandback, E., Reisinger, M., Gruber, K., Macheroux, P.: Structure, biochemical and kinetic properties of recombinant Pst2p from *Saccharomyces cerevisiae*, a FMN-dependent NAD(P)H:quinone reductase, Biochim. Biophys. Acta: Proteins & Proteomics, 2017, 1865:1046-1056. DOI:10.1016/j.bbapap.2017.05.005.

7. Daniel, B., Konrad, B., Toplak, M., Lahham, M., Messenlehner, J., Winkler, A., Macheroux, P.: The family of berberine bridge enzyme-like enzymes: treasure-trove of oxidative reactions, invited review, Arch. Biochem. Biophys., 2017, 632:88-103; DOI:10.1016/j.abb.2017.06.023.

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9. Payer, S. E., Marshall, S. A., Bärland, N., Sheng, X., Reiter, T., Dordic, A., Steinkellner, G., Wuensch, C., Kaltwasser, S., Fisher, K., Rigby, S. E. J., Macheroux, P., Vonck, J., Gruber, K., Faber, K., Himo, F., Leys, D., Pavkov-Keller, T., Glueck, S. M.: Regioselective *para*-carboxylation of catechol compounds by a prenylated flavin dependent decarboxylase, Angew. Chem. Int. Ed., 2017, 56:13893-13897; DOI:10.1002/anie.201708091.

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12. Augustin, P., Toplak, M., Fuchs, K., Gerstmann, E. C., Prassl, R., Winkler, A., Macheroux, P.: Oxidation of the FAD cofactor to the 8-formyl-derivative in human electron transferring flavoprotein, J. Biol. Chem., 2018, *in press*. DOI:10.1074/jbc.RA117.0008.

Award

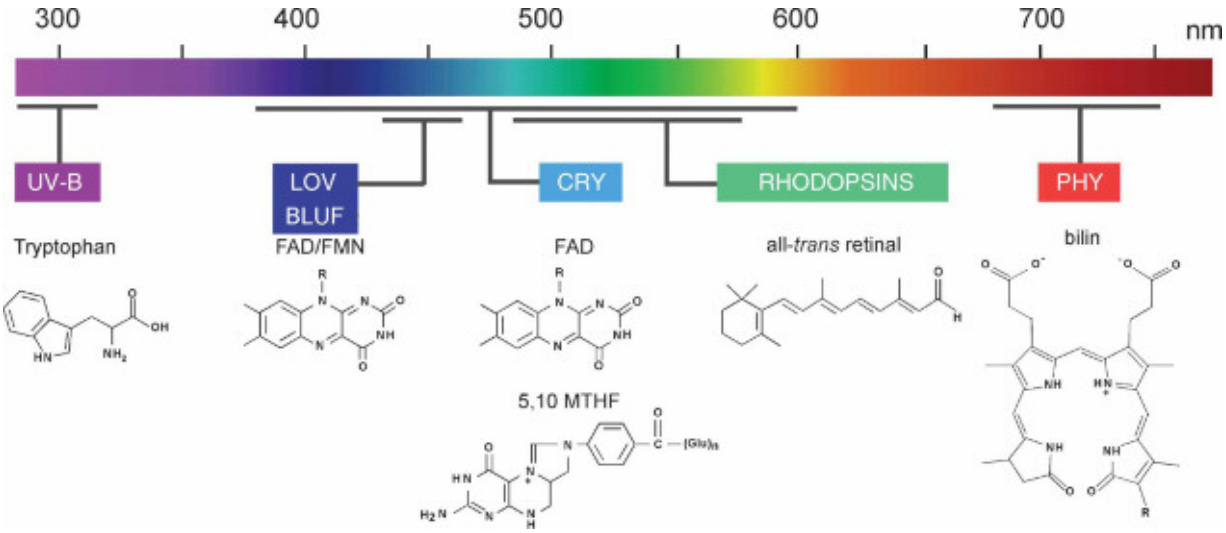
1. **Wolf-Dieter Lienhart** received the *Vincent Massey Award* at the 19th International Symposium on Flavins and Flavoproteins, Groningen, June 2017.

Photoreceptor Group

Group leader: Andreas Winkler
PhD students: Stefan Etzl, Geoffrey Gourinchas
Master student: Julia Färber
Bachelor students: Sara Trestenjak, Corina Maller
Technical staff: Elfriede Zenzmaier

General description

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



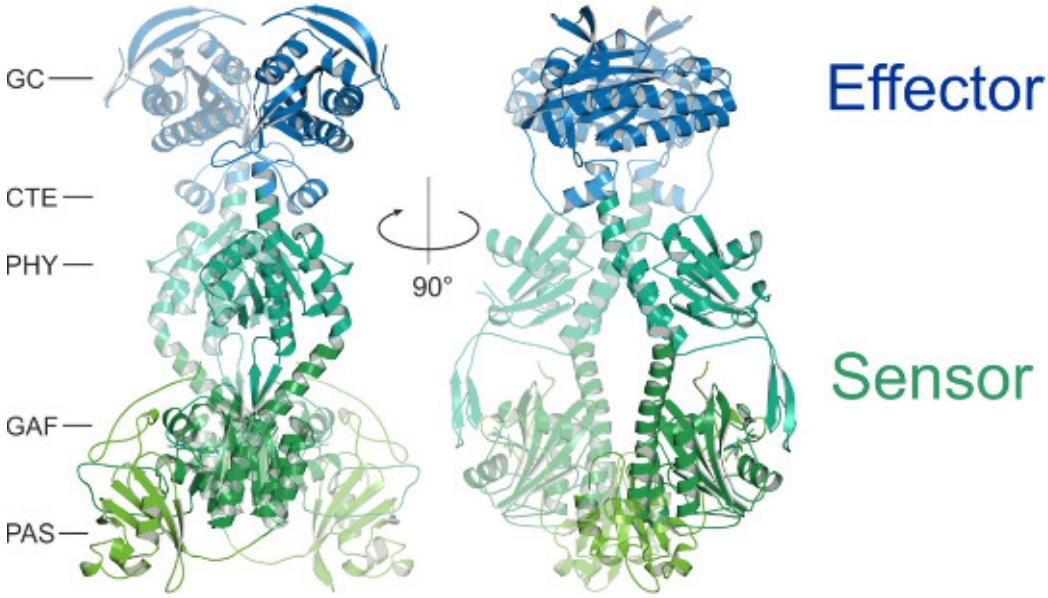
Overview of photoreceptor families and their corresponding cofactors covering the UV/Vis range of the electromagnetic spectrum (adapted from Heintzen WIREs Membrane Transport and Signaling 2012, 1:411–432).

Light-activated cyclases

One area of research are blue- and red-light activatable guanylate and adenylate cyclases, respectively. These represent artificial light-regulatable tools that are designed based on previous functional data of a blue-light regulatable adenylate cyclase and the evolutionary

conservation of elements required for signal transduction. The identification of specific signaling elements for the closely related effector systems and the comparison of functionalities of different artificial chimeras will provide new insight into the coupling mechanism of sensor-effector modules. A successful characterization of these systems requires an interdisciplinary approach combining biochemistry with tools of structural biology (in collaboration with Prof. Karl Gruber, KFU Graz). Atomic models obtained from x-ray crystallography are functionally extended by the in-solution method hydrogen-deuterium exchange (HDX, experiments performed at the Max Planck Institute for Medical Research in Heidelberg) to obtain structural information of elements that are involved in photo-activation and signal transmission (thesis project of Stefan Etzl).

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

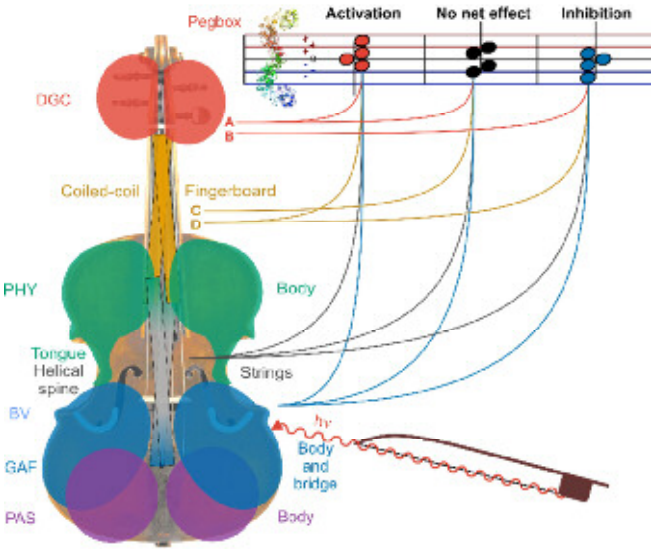


Crystal structure of the bacteriophytochrome-linked guanylate cyclase. PAS, GAF and PHY domains are part of the red light regulated phytochrome sensor with its biliverdin chromophore shown as green stick model. The CTE corresponds to the Cyclase Transducer Element, required for efficient signal integration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

Light-regulated diguanylate cyclases

Another key approach towards a better understanding of sensor-effector coupling is to appraciate how nature has accomplished its remarkable modularity of sensor-effector combinations. To this end, we focus on the light-regulation of cyclic-dimeric-GMP formation by GGDEF diguanylate cyclases that are ubiquitous in bacteria and that naturally occur covalently linked to various photoreceptors. Based on secondary structure predictions a similar architecture to adenylate/guanylate cyclases, which are the focus of the project described above, was proposed for GGDEF domains. Elucidation of corresponding crystal structures later supported this hypothesis but also highlighted some differences. Especially the N-terminal extension is of interest due to the highly conserved residues present in this region that project towards the active site. In view of the direct connection of this element to the linker helix of upstream sensory modules (e.g. blue- and red-light photoreceptors) this might indicate a relatively direct signaling mechanism. The understanding of how evolution has accomplished this astonishing modularity of sensor-effector couples is important for the identification of functionally relevant structural elements with the goal of guiding the efficient design of rationally engineered light-regulatable systems. Since diguanylate cyclases are distantly related to adenylate/guanylate cyclases, the comparison of signal transduction mechanisms between the two protein families is one central aspect of our research interests (thesis project of Geoffrey Gourinchas).

With the elucidation of the first full-length crystal structure of a naturally occurring phytochrome linked to its functional effector domain, important new insights into signal transduction in phytochromes were obtained. The structure revealed a parallel dimeric arrangement of the sensor and effector domains, similar to the observations in the project described above. Based on an in-depth characterization of the involvement of functionally important elements, our studies provide a foundation for increasing our understanding of the fine-tuned coupling mechanism of phytochrome sensors with various effector domains. The characterization of in-solution conformational dynamics substantiates the involvement of several structural elements of phytochromes and highlights their dynamic interplay with the coiled-coil sensor-effector linker region (Figure below). 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.*, 2017).



The characteristic dimeric structure suggests a model of signal transduction corresponding to the violin model. Instead of a linear cascade of structural changes resulting in enzyme activation, the conformational dynamics of the whole system define the population of functionally relevant states leading to activation. In the case of the phytochrome-violin the pegbox corresponds to the effector domain whose activity is tuned by the sensory module. PAS, GAF and PHY are subdomains of the phytochrome and DGC is the Diguanylate Cyclase effector.

International cooperations

Mark Gomelsky, University of Wyoming, U.S.A.

Ilme Schlichting, Max Planck Institute for Medical Research, Germany

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

Research projects

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

Talks at international conferences

1. Winkler, A.: *Molecular mechanisms of light regulation in LOV-diguanylate cyclases - implications for sensor-effector modularity*. 19th International Symposium on Flavins and Flavoproteins, Groningen, June 2017.
2. Winkler, A.: *Mechanistic insights into phytochrome regulation of c-di-GMP production in red light-regulated diguanylyl cyclases*. 13th International Conference on Tetrapyrrole Photoreceptors of Photosynthetic Organisms (ICTPPO), Chicago, July 2017

Publications

1. Gourinchas G., Etzl S., Göbl C., Vide U., Madl T., Winkler A.: Long-range Allosteric Signaling in Red Light-Regulated Diguanylyl Cyclases. Sci. Adv., 2017, 3: e1602498; DOI:10.1126/sciadv.1602498
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3. Daniel B., Konrad B., Toplak M., Lahham M., Messenlehner J., Winkler A., Macheroux P.: The family of berberine bridge enzyme-like enzymes: treasure-trove of oxidative reactions. Arch. Biochem. Biophys., 2017, 632: 88-103; DOI:10.1016/j.abb.2017.06.023.
4. Augustin P., Toplak M., Fuchs K., Gerstmann E. C., Prassl R., Winkler A., Macheroux P.: Oxidation of the FAD cofactor to the 8-formyl-derivative in human electron transferring flavoprotein. J. Biol. Chem., 2018, *in press*; DOI:10.1074/jbc.RA117.0008.

Awards

1. **Stefan Etzl** received the *Best Talk Award* at the DK Molecular Enzymology Graduate Seminar 2017.
2. **Geoffrey Gourinchas** was awarded the Best Poster Prize at the Designer Biology Symposium 2017 in Vienna by the microbiology society.
3. **Geoffrey Gourinchas** received the *Best Poster Prize* at the 17th DocDay of the NAWI Graz Doctoral School of Molecular Biosciences and Biotechnology (2017).

Metalloprotein Group

Group leader: Grit D. Straganz
PhD student: Johannes Niederhauser

General description

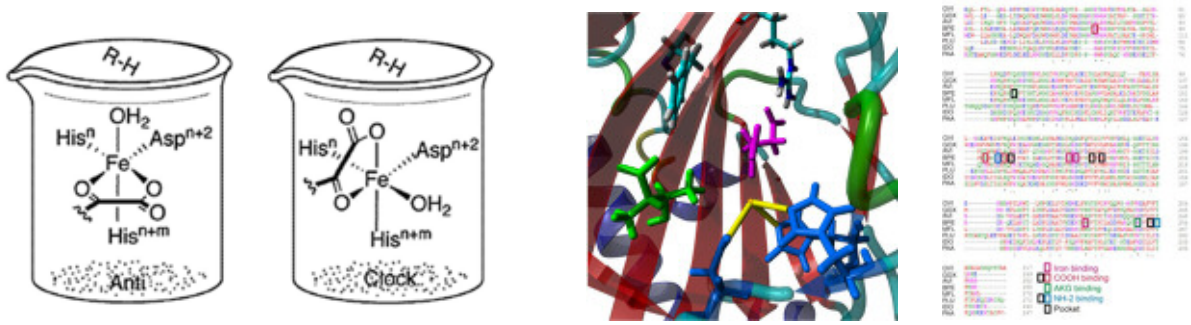
Metal ions play central structural and functional roles in biological systems. They are involved in the regulation and processing of DNA and RNA, in environmentally important biogeochemical cycles, and in signaling events that trigger or terminate biochemical processes. Metal ions are present in about half of all proteins as they occur in nature. Metal cofactor dependent enzymes are involved in a number of important pathways including the biosynthesis of antibiotics, DNA, and an array of important metabolites. In fact, in nature the chemically most challenging transformations, such as the hydrogenation of N_2 to NH_3, the oxidation of H_2O to O_2 during photosynthesis and the conversion of electrochemical to chemical energy rely on enzymatic metal centers. Also the metabolism of O_2 largely depends on metalloenzymes. The underlying mechanisms of the catalyzed reactions are still not well understood. In depth studies of the catalytic metallocenters and their interaction with substrates and protein environments can help elucidate the molecular basis of catalysis, in order to understand and ultimately expand the catalytic potential of metalloenzymes.

Our current focus is on the catalytic mechanisms that bring about O_2 dependent reactions at enzymatic nonheme Fe(II) centers. These metal centers can oxidize, halogenate, desaturate and decarboxylate organic structures, including the non-activated carbon-hydrogen bonds in methane and alkyl chains. The often complex and highly selective reactions cannot be mimicked by conventional synthetic chemistry. Their ability to bring about diverse and selective catalysis under mild conditions makes nonheme Fe(II) enzymes promising platforms for 'green' synthetic reactions.

The methods established in our group comprise kinetic (stopped-flow analysis of ligand binding events and enzymatic reactions), spectroscopic (fluorescence, circular dichroism and UV/VIS absorbance) methods and ESI mass spectrometry, as well as standard biochemical and molecular biology methods, including protein purification, cloning, expression and mutation of genes of interest. In order to gain further structural insights, we employ computational methods (DFT calculations, MD simulations, docking, *in silico* mutational analysis) that we correlate with experimental data with the aim of gaining a detailed understanding of the structure-activity relationships in chemical catalysis. A brief description of our current research projects is given below.

Structure-function relationships in α-ketoglutarate dependent hydroxylases

α-Ketoglutarate dependent nonheme Fe(II) hydroxylases (α-KG-MNH) are enzymes that transform cell metabolites via oxidative hydroxylation. They all share a cupin fold and a common metal center organization. Yet, particular members of this family show high but diverse stereo-, regio-, chemo- and substrate-selectivities. In this project the structural basis of this diversity is explored. Therefore, exponents of α-KG-MNHs are subjected to a combination of experimental and computational investigations. Mutational analyses combined with kinetic and spectral characterizations are correlated with molecular dynamic studies in order to gain insights into the impact of the protein structure on particular steps of catalysis.



(a) (b)

Fig. 1: (a) There are two naturally occurring principal structures (anti-clockwise and clockwise) found in α-KG-MNHs. (b) Via primary, secondary and tertiary structure analysis of Bpe1 in relation to the MNHE consensus model, the Bpe1 metal center could be classified as clockwise. On this basis the catalytically competent Bpe1- α-KG -substrate model complex from the apo-enzyme crystal structure was constructed. Note that substrate binding displaces the coordinated water and opens a coordination site for O_2. According to recent research, constitutes the coordination site at which hydroxylation ultimately takes place. (Figure adapted from Hangasky J.A.et al. Metallomics. 2013).

Leucine dioxygenase (Bpe1): Based on the crystal structure of Bpe1, solved in collaboration with the group of Monika Oberer, a mutational analysis of the active site was performed in order to identify structural features that determine regio- and substrate selectivity and indeed enzymes with distinct and so far unknown regio- and substrate-selectivities were obtained. The crystal structure was then used to build models of Bpe1 and its variants that were subjected to MD simulations and results were correlated with experimentally found catalytic properties of the enzyme and its variants. Intriguingly, our hypothesis that regio (γ)-selectivity in Bpe1 is determined by the positioning of an acidic residue that coordinates the substrate's amino group was not confirmed. Instead, according to our simulations, like its β-selective counterparts, the substrate's amino-group interacts with the metal coordinating aspartate residue. By contrast, regio-selectivity appears to be driven by the positioning of an arginine residue that coordinates the substrate's carboxy group. The positioning of this arginine is in turn tuned by steric interactions with outer sphere residues lining the substrate binding pocket, which can in turn be engineered to obtain distinct region-selectivities.

Metalloenzymes – towards tools for computational redesign

Selective O_2 dependent hydroxylations are key reactions in the biosynthesis of bioactive secondary metabolites and the ability to harness enzymes with selectivities that do not occur in nature could open new, biosynthetic routes for the production of high value compounds such as non-natural amino acids and, ultimately, synthetic antibiotic libraries. However, *in vitro*-evolution methods are not suitable for some enzyme groupings. In the case of α-KG-MNHEs, Fe(II) instability highly complicates the establishment of a screening method for the free enzyme. Regarding whole cell essays, hydrophilic products, such as amino acids, often cannot be detected, either because they do not efficiently pass through the cell wall, or because they are further metabolized. A computational platform could circumvent this problem.

Based on previous work on *Hydroxymandelate Synthase* (HMS) an automated platform for α-KG-MNHs, was developed. It was applied to the model enzyme *Proline-4-hydroxylase (P4H)*: P4H, an enzyme with known protein structure. The system now automatically generates single, double and triple variants by permutations of defined positions and their respective variation lists. It subsequently runs MD simulations, extracts user-defined potentially relevant descriptors (e.g. atom distances, angles, energies) produces an output file that lists all calculated variants with the respective statistic analysis of said descriptors (e.g. average values and standard deviations). In parallel a screening system for P4H was established and variants of interest were constructed and their region-selectivity towards proline was characterized by HPLC-MS. Based on our results our program appears to be a suitable tool for the redesign of KG-MNHs, however, ^{13}C-NMR studies to ultimately confirm the identity of some metabolites are still in progress.

Beyond metals: MD simulations elucidate structure-function relationships in enzymes

In the Macheroux group's quest for an in depth understanding of why Leucine 208 in human histamine N-methyltransferase emerges as a hotspot for protein stability, we performed MD simulations and could thus help rationalize, how a rather distant mutation propagates structural destabilization of the active site.

PhD Thesis completed

Johannes Niederhauser: *Smart Enzyme Engineering: From Structure-Function Relationships towards Redesign of Mononuclear Nonheme Fe(II) Enzymes*

Currently, 'clean and green' chemical and biocatalytical processes are a hot topic in pharmaceutical and chemical industry. Establishment and utilization of novel routes to generate valuable products from renewable sources is a primary target of biosynthetic chemistry: Selective oxygenations of non-activated carbon atoms by O2 are a major target in green chemistry, as they are often inaccessible by abiotic synthetic means. Mononuclear O2 and nonheme iron dependent enzymes (MNHE) constitute a very versatile group of enzymes which are capable of highly selective oxidative transformations. MNHEs generally share a common metal binding motif consisting of two histidines and one carboxylate residue ligating an Fe(II) cofactor. These enzymes display an outstanding diversity, which is brought about by the respective protein environment. Unravelling the structure-function relationships and thereby the mechanism which allows the metal-center and the respective protein's structure to catalyze highly challenging stereo- and regio-selective transformations is an important step on the way to rational (re)design of novel O2 and Fe(II) dependent biocatalysts, thus expanding their biocatalytic potential. Cutting-edge computational methods including sequence analysis, molecular docking and molecular dynamics (MD) simulations represent promising tools for in-depth structural characterization of enzymes. In this study, the structurefunction contributions of particular exponents of the grouping of substrate hydroxylating MNHEs via aforementioned computational methods are investigate.Combined cycles of in-silico benchmarking and in-vitro mutagenic probing of these enzymes will ultimately enable us to rationally redesign the investigated proteins. The overarching aim is to use the gained and combined knowledge to establish a platform for in-silico MNHE redesign that can be used to predict how structural changes in the protein yield altered functions, which will then enable us to generate novel tailor-made biocatalysts. Their integration into whole-cell catalysis systems

will open new biosynthetic routes for the production of fine-chemicals and pharmaceutical building blocks from 'renewable and green' sources. In this study, several exponents of the group of MNHEs, namely hydroxy mandelate synthase (HMS), isoleucine dioxygenase (IDO) and diketone dioxygenase (Dke1) are investigated in-vivo and in-silico in order to gain insights into their structure-function relationships.

International cooperations

Edward I. Solomon, Stanford University, Stanford, U. S. A.

Samuel de Visser, University of Exeter, Exeter, U. K.

Gideon Grogan, University of York, York, U. K.

Research project

FWF P26539: "Nonheme Fe(II) Hydoxylases"

Publication

1. Tongsook, C., Niederhauser, J., Kronegger, E., Straganz, G. D., Macheroux P. Leucine 208 in human histamine N-methyltransferase emerges as a hotspot for protein stability rationalizing the role of the L208P variant in intellectual disability. Biochim Biophys Acta. 2017; 1863(1):188-199. DOI:10.1016/j.bbadis.2016.10.005.

Cell Biology Group

Group leader: Günther Daum
Postdoctoral fellows: Karlheinz Grillitsch (ACIB), Isabella Klein
PhD students: Ariane Wagner (Fankl), Francesca Di Bartolomeo, Martina Korber
Research associate: Andreas Grutsch (ACIB)
Technical staff: Claudia Hrastnik (part time), Alma Makic (part time)

General description

Lipids are important biomolecules. They can serve as storage molecules which are mobilized under conditions of energy requirements, but also as important building blocks of cellular membranes. Biogenesis of organelles is closely linked to lipid synthesis and lipid dynamic processes.

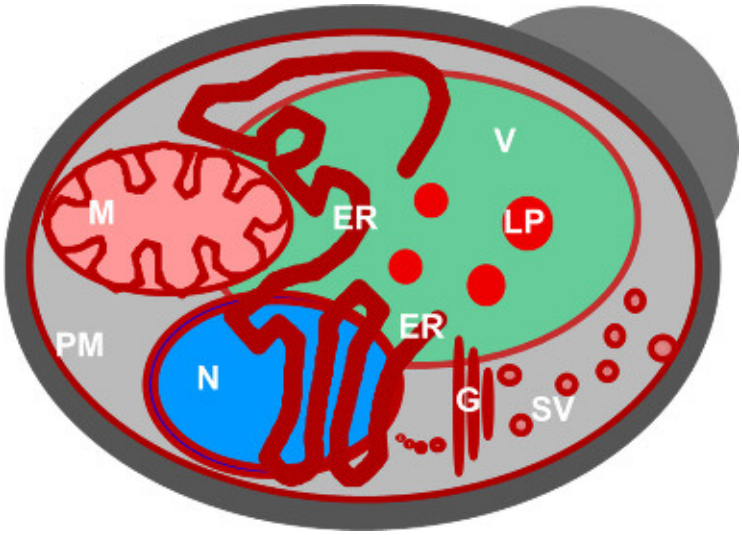


Fig. 1: Yeast organelles: LP, lipid particles/droplets; M, mitochondria; ER, endoplasmic reticulum; N, nucleus; G, Golgi; V, vacuole; PM, plasma membrane; SV, secretory vesicles.

Our laboratory has a long-standing tradition to study lipid biosynthesis, storage and degradation in relation to maintenance of biological membranes using the yeast as an experimental model system. The yeast has become a most valuable tool to investigate principles of cell biology, molecular biology and biochemistry, but also to address applied biotechnological aspects.

Specific investigations performed recently in our laboratory were (i) molecular enzymology of phosphatidylserine decarboxylase 1, a central enzyme of phospholipid metabolism in the yeast; (ii) non-polar lipid metabolism in the yeast and regulatory aspects affecting formation and mobilization of lipid depots; and (iii) secretory pathway in the yeast *Pichia pastoris*.

Phosphatidylserine decarboxylase, an important enzyme of yeast lipid metabolism

Phosphatidylethanolamine (PE) is one of the major phospholipids of yeast membranes. It is highly important for membrane stability and integrity and thus also for cell function and proliferation. PE synthesis in the yeast is accomplished by four different pathways, namely (i) synthesis of phosphatidylserine (PS) in the endoplasmic reticulum and decarboxylation by mitochondrial phosphatidylserine decarboxylase 1 (Psd1p); (ii) synthesis of PS and conversion to PE by the Golgi localized phosphatidylserine decarboxylase 2 (Psd2p); (iii) the CDP-ethanolamine pathway (Kennedy pathway) in the endoplasmic reticulum, and (iv) the lysophospholipid acylation route catalyzed by Ale1p and Tgl3p.

The major player in the PE biosynthetic network is phosphatidylserine decarboxylase 1 (Psd1p) of the inner mitochondrial membrane (IMM), an enzyme forming PE by decarboxylation of phosphatidylserine (PS). A number of cellular functions, especially in mitochondria, require the activity of Psd1p.

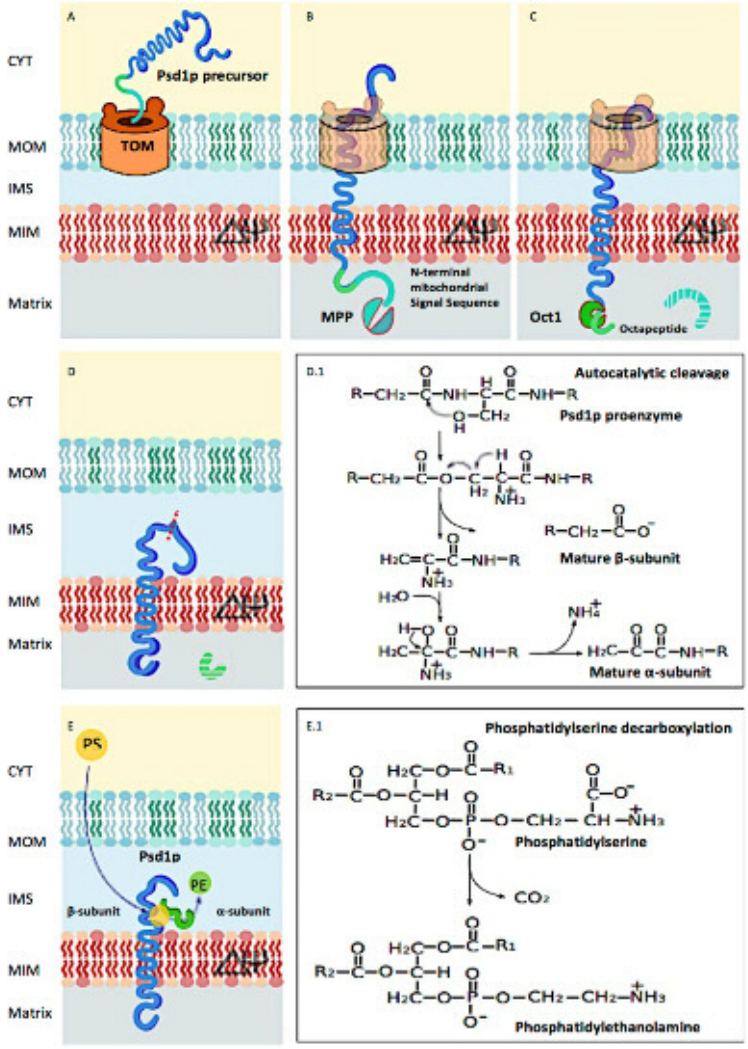


Fig. 2: Import and assembly of phosphatidylserine decarboxylase 1 in yeast mitochondria.

A specific feature of Psd1p is its structural arrangement in the form of an α- and a β-subunit. The latter domain anchors the protein to the IMM. The α-subunit which is separated from the β-subunit during membrane assembly is largely responsible for the enzymatic activity and is per se soluble. Our previous investigations indicated that well balanced interaction of targeting sequences, membrane anchor domains and the active domain of the protein were prerequisite for correct assembly of Psd1p and formation of an enzymatically active protein. However, several details about these interactions and detailed information about position and orientation of some critical domains of the protein are still unknown.

To obtain more insight into structure and function of Psd1p we analyzed recently two specific domains of the protein in more detail. One of these relevant functional domains is a consensus motif FXFXLKXXXKXR in the α-subunit in close proximity to the active site which is assumed to be involved in substrate binding. To address the specific role of this consensus motif in the biogenesis of Psd1p we constructed a series of *S. cerevisiae* mutant strains with specific point mutations along the consensus motif. These mutants were characterized by lipid profiling, Western blot analysis of mitochondrial extracts and enzymatic activity. We found that this consensus motif contributes to processing, maturation, stability and functionality of Psd1p.

Other domains of interest within Psd1p are two putative membrane spanning domains IM1 and IM2 which are involved in the anchoring of the Psd1p β-subunit into the inner mitochondrial membrane. IM1 was previously characterized. More recently, we focused on the specific role of IM2. Mutations within this membrane binding domain led to mislocalization of the enzyme, but also to defects in processing of the protein and to compromised import into mitochondria.

Storage of non-polar lipids in lipid droplets and mobilization

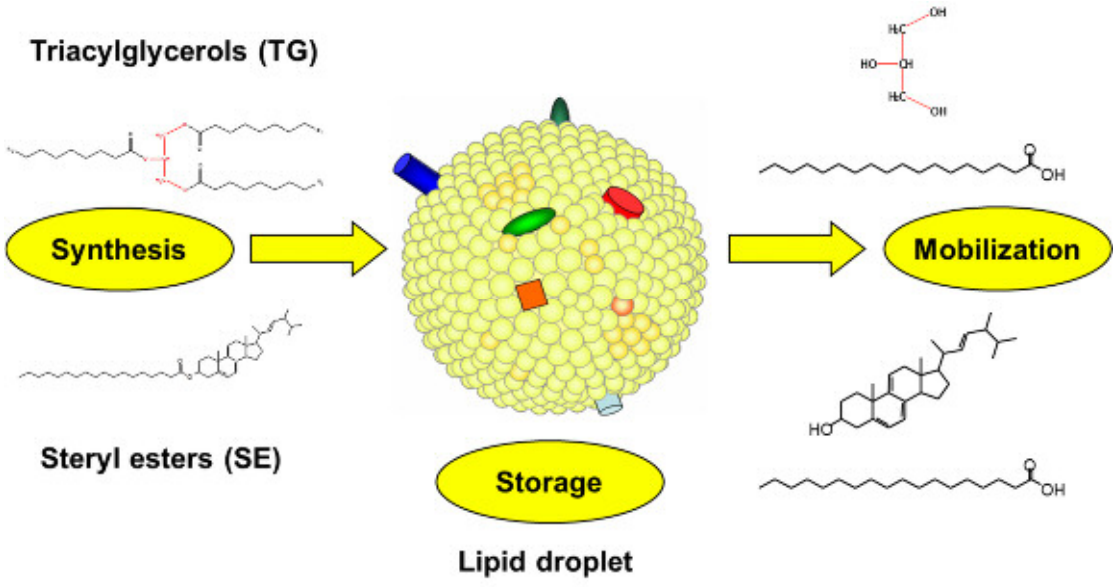


Fig. 3: Life cycle of yeast non-polar lipids

Yeast cells like most other cell types have the capacity to store non-polar lipids. In *Saccharomyces cerevisiae,* triacylglycerols (TG) and steryl esters (SE) are the predominant lipid storage molecules which accumulate in subcellular structures named lipid droplets/particles. Upon requirement, TG and SE can be mobilized and serve as building blocks for membrane biosynthesis. Recent studies in our laboratory focused on regulatory aspects of non-polar lipid formation, storage and mobilization.

Besides TG, SE are major storage lipids in the yeast. Recently, we studied regulation within the non-polar lipid storage network to these SE. SE are formed in the endoplasmic reticulum by the two acyl-CoA:sterol acyltransferases Are1p and Are2p, whereas SE hydrolysis is catalyzed by the three SE hydrolases Yeh1p, Yeh2p and Tgl1p. To shed light on the regulatory link between SE formation and hydrolysis in the maintenance of cellular sterol and free fatty acid levels we studied the fate of the three SE hydrolases under specific conditions. We demonstrated that in the absence of their substrate and in the absence of lipid droplets Yeh1p and Tgl1p, which are normally localized to lipid droplets are retained in the endoplasmic reticulum. Under these conditions the stability of the enzymes is also decrease. We conclude that these changes contribute to a balanced SE metabolism in the yeast.

Pichia pastoris secretion pathway

In an industry related research project we are studying the secretory pathway of the yeast *Pichia pastoris*. This yeast is an important experimental system for heterologous expression of proteins. The aim of this study is to generate strains with improved protein expression and secretion. Current investigations were focused on the isolation of secretory vesicles. This study may help to get a better view of cargo specific processes and to construct mutant strains to overcome bottlenecks in the secretory pathway.

Doctoral theses completed

Francesca Di Bartolomeo: *Phospholipids in mitochondrial membranes from Saccharomyces cerevisiae*

Phosphatidylethanolamine (PE) is one of the most abundant phospholipids found in yeast, plant and mammalian cells. PE can be synthesized by (i) decarboxylation from the precursor phosphatidylserine (PS) through the action of the PS decarboxylases type I and II, (ii) reacylation of lyso-PE catalyzed by Ale1p and Tgl3p or (iii) the de novo biosynthesis starting from ethanolamine via CDP-ethanolamine (CDP-Etn) or Kennedy pathway. In Saccharomyces cerevisiae, the mitochondrial phosphatidylserine decarboxylase 1 (Psd1p) is the key enzyme as it produces the largest amount of cellular PE. During its biogenesis Psd1p is synthesized as a larger precursor on cytosolic ribosomes and follows a unique processing pathway upon import into mitochondria. The transition to an active form of the enzyme goes through an autocatalytic cleavage which separates Psd1p into an intermembrane space localized α- and an inner membrane-bound β-subunit. The α-subunit harbors a highly conserved motif, which was proposed to be implicated in the catalysis through a specific interaction with phosphatidylserine (PS). In the thesis I present a molecular analysis regarding the significance of this consensus motif in Psd1p, making use of mutant forms bearing either deletions or point mutations in this region of the α-subunit. The biochemical analysis performed showed that any modification in this motif differently affects the processing and stability of Psd1p. From these observations we conclude that the putative substrate

recognition site of Psd1p α-subunit has a crucial role in assuring the structural integrity and the correct biogenesis of Psd1p influencing, as a consequence, also the catalytic activity of the enzyme. Beside the work on Psd1p, my scientific interested included also some important aspects related to the interaction between phospholipids and protein complexes in mitochondrial membranes. It is well known that mitochondria contain a complex machinery that cathalyses the import of precursors proteins into the mitochondrial outer membrane. Regarding the β-barrel proteins, the outer membrane translocase (TOM complex) and the assembly machinery (SAM complex) are responsible for their proper positioning in the mitochondria. The phospholipids PE and cardiolipin (CL) are both required for the proper function of these complexes. In this context our findings demostrate that also the bilayer-forming phosphatidylcholine (PC) is required for stability and function of the SAM complex in the biogenesis of β-barrel precursors. Additional studies have also focused on the two protein translocases involved in the transport of precursor proteins into or across the inner mitochondrial membrane. In this case we have again shown that, unlike PE and CL, PC is able to interact directly with the complex TIM23. In fact the experiments performed revealed that the lack of PC in S. cerevisiae pem1Δpem2Δ mutant strain, causes destabilization of the TIM23 complex without influencing the stability and activity of the respiratory supercomplexes and consequentially impacting the membrane potential.

Ariane Wagner (Fankl): *Formation and subcellular distribution of phospholipids in the yeast Saccharomyces cerevisiae*

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant phospholipids found in yeast, plant and mammalian cells. In eukaryotes, PE can be synthesized via two different major pathways: (i) phosphatidylserine (PS) can be decarboxylated by phosphatidylserine decarboxylases type I (Psd1) and type II (Psd2), and (ii) ethanolamine can be activated by CTP and incorporated into PE via the so-called Kennedy pathway. The majority of PE is formed in the mitochondria by Psd1. PSDs are found in many cell types, including yeasts, plants, mammalians and parasites. In the yeast Saccharomyces cerevisiae Psd1 is anchored to the inner mitochondrial membrane (IMM) through membrane spanning domains and oriented towards the mitochondrial intermembrane space. The incorporation of phosphatidylserine decarboxylase 1 (Psd1) in yeast into the mitochondrial membrane and its need for functionality is only poorly understood. Therefore we analyzed in this study the membrane sorting signals of Psd1 and found that the enzyme harbors at least two membrane spanning domains, named IM1 and IM2. Deletions in the second membrane sorting signal IM2 lead either to mislocalization of Psd1 to the matrix site of the mitochondria or to the outer mitochondrial membrane and to defects in import, processing and activity of Psd1. Deletion of Psd1 results in depletion of phosphatidylethanolamine and in up-regulation of the glycogen phosphorylase GPH1, which is catalyzing the degradation of glycogen to glucose in yeast. Deletion of this gene causes decreased levels of phosphatidylcholine (PC), triacylglycerols and steryl esters. Depletion of the two non-polar lipids in a Δgph1 strain lead to a lack of lipid droplets, and a decrease of the PC level results in instability of the plasma membrane. In the yeast Saccharomyces cerevisiae, PC can be synthesized via two different pathways: (i) methylation of phosphatidylethanolamine (PE) catalyzed by the methyl transferases Cho2p/Pem1p and Opi3p/Pem2p and (ii) incorporation of choline through the CDP-branch of the Kennedy pathway. Formation of PC via both pathways is negatively affected in a Δgph1 strain, although expression of the involved genes is not down regulated. Altogether, Gph1p besides its function as a glycogen mobilizing enzyme appears to play a regulatory role in yeast lipid metabolism. To determine the contribution of the two PC forming pathways to the supply of PC to peroxisomes, yeast strains bearing defects in the two pathways, namely cho2Δopi3Δ (mutations in the methylation pathway) and cki1Δdpl1Δeki1Δ

(mutations in the CDP-choline pathway), were analyzed regarding their lipid composition and membrane properties. We found that both pathways produce PC for the supply to peroxisomes, although the CDP-choline pathway seemed to contribute with higher efficiency than the methylation pathway. We defined the origin of peroxisomal PC and demonstrated the importance of PC for peroxisome membrane formation and integrity.

Thomas Gatternig: *Recombinant Expression, Purification and Characterization of a Host Cell Protein*

During the downstream purification of recombinantly expressed proteins in mammalian cell culture, host cell proteins (HCP) usually account for a significant part of total impurities present. Despite the use of different orthogonal purification steps, including but not limited to chromatography, filtration or precipitation, a certain amount of host cell proteins is still detectable in bulk drug substance (BDS) preparations. Detailed analysis of the impurity profile of a recombinantly expressed anti-coagulation factor identified a single host cell protein ("target protein") accounting for a large portion of total HCP in the BDS material. In the course of this doctoral thesis this target protein was studied in detail by recombinant expression in suitable hosts, purification of the expression product and characterization of the recombinant protein including in-silico predictions of protein structure and post-translational modifications, analytical evaluation of the protein´s structure and general properties as well as functional assays. Recombinant expression entailed sequencing of the corresponding cDNA from a genomic library of the host cell line, amplification and cloning of full-length, fragments and fusions of the target protein into suitable plasmids. Out of the host systems Escherichia coli, Pichia pastoris, CHO and HEK293, which were tested for efficient recombinant expression of target protein variants, only HEK293 expression yielded sufficient titers of stable and adequately processed full-length target protein. Purification of a his-tag fusion of the target protein in a two- or three-step process included immobilized metal affinity, ion-exchange and/or size exclusion chromatography. Purified preparations of the target protein were analyzed by N-terminal sequencing, differential scanning calorimetry, analytical size exclusion chromatography and peptide mapping using HPLC/MS. The glycosylation structure was evaluated using HPLC methods for the qualitative and quantitative determination of the N-glycans as well as the sialisation structure. Functional assays included hydrophobic interaction chromatography and binding studies using surface plasmon resonance technologies. In silico evaluation of the DNA and protein sequences of the target protein showed high homology to variants of the target protein in different mammalian systems including humans. Secondary and tertiary structure predicitons for the target protein were mostly comparable to its human analogue. Analytical characterization and functional studies on the target protein generally confirmed properties published in scientific literature for its human counterpart.

Martina Korber: *Regulation of steryl ester metabolism in the yeast Saccharomyces cerevisiae*

The two major storage lipids in the yeast Saccharomyces cerevisiae are the nonpolar lipids triacylglycerol and steryl esters. Both lipid species are synthesized in the endoplasmic reticulum. Dga1p and Lro1p are responsible for the formation of triacylglycerols and Are1p and Are2p catalyze the synthesis of steryl esters. The latter enzymes also contribute to the formation of triacylglycerols, although to a minor amount. Once steryl esters and triacylglycerols are built up, they can be stored in an organelle-like structure termed lipid droplet. In times of starvation or growth, these nonpolar lipids serve as important building blocks for membrane lipid formation and as energy pool. Therefore steryl esters and

triacylglycerols need to be mobilized by hydrolytic enzymes. Whereas triacylglycerols are hydrolyzed by triacylglycerol lipases, our focus was set on the three steryl ester hydrolases Tgl1p, Yeh1p and Yeh2p. Tgl1p and Yeh1p are known to be lipid droplet resident enzymes, in contrast of Yeh2p which is located at the cell periphery. The overall aim of this work was to shed light on regulatory aspects of steryl ester metabolism. On the one hand, we investigated the steryl ester synthases Are1p and Are2p under conditions of deprived steryl ester mobilization. We clearly demonstrated a feedback regulation on the two steryl ester forming enzymes. Although gene expression and protein levels of the two acyltransferases were not affected in a strain lacking all three steryl ester hydrolases, the in vitro activity of Are1p and Are2p and the in vivo incorporation of radio-labelled fatty acids into steryl esters was significantly reduced under the tested conditions. On the other hand, we were curious about the fate of the three steryl ester hydrolases in the absence or presence of nonpolar lipids. For these studies we used strains either lacking steryl esters, triacylglycerols or lipid droplets at all. Based on the performed experiments we showed that Tgl1p and Yeh1p are retained to the endoplasmic reticulum solely in the strain lacking lipid droplets. As a consequence of this relocalization, the enzymes lost their hydrolytic activity and were highly unstable. Yeh2p was confirmed to be a protein localized at the plasma membrane, even in a strain lacking lipid droplets. Furthermore, Yeh2p is subject to a posttranslational modification, namely phosphorylation. In summary, results shown in this Thesis are one step forward to understand how steryl ester metabolism is regulated in the yeast S. cerevisiae.

Master Thesis completed

Stefan Kühberger: *Hyperthermia as a supporting strategy for cancer immunotherapy*

Fever is a naturally occurring phenomenon of our body which stimulates the immune system to fight infections. High fever periods have been linked to spontaneous regression of tumors. This finding provided a rationale to induce artificial fever, or external heat application, in cancer therapy. Today, hyperthermia is clinically applied as supportive strategy to sensitize tumor cells to chemo- and radiotherapy. The biological effects of hyperthermia include macroscopic effects such as increased perfusion, cytotoxic effects on tumor cells and immunostimulation. However, the cellular and molecular mechanisms mediating the effects remain largely unclear. The aim of this project was to study the effects of hyperthermia on the killing potential of cytotoxic T lymphocytes (CTL), a cell population of the immune system which is capable of specifically detecting and eliminating cancer cells. Particularly, the definition of the range of temperatures and treatment durations which are required to enhance CTL killing efficiency and the cellular mechanisms which mediate the effect were in the focus of this study. Using 3D organotypic collagen-based cytotoxicity assays and live cell microscopy, we showed that fever-range temperatures of 38.5 and 39.5 C are sufficient to significantly enhance CTL-mediated killing of murine melanoma cells. Further, clinically relevant treatment schemes of 1 hour per day and repeated on 2 consecutive days were capable of increasing CTL killing efficiency. We further identified a direct cytotoxic and proliferation-inhibiting effect on the tumor cells which contributed to the increased CTL killing efficiency. To validate the results in live tumors, an in vivo system was set up to treat mice with whole-body hyperthermia followed by intravital imaging to evaluate the response of tumor and immune cells at cellular level. Application of 1 hour of whole-body hyperthermia at 39.5 °C induced an immediate inhibition of mitosis which confirmed the direct cytotoxic effect on tumor cells observed in vitro. Thus, the results obtained during this project support a combination of fever-range hyperthermia with adoptive CTL transfer.

Academic career - Günther Daum

As described at the beginning of this booklet Günther Daum retired in September 2017. Therefore, a short CV shall be presented here describing the highlights of his career.

Born on 27th Nov. 1951 in Graz, Austria
Austrian citizen
Married, 3 children

Education and Career History

1957 - 1961	Elementary School (Volksschule) in Koeflach, Austria
1961 - 1969	High School (Gymnasium) in Graz and Koeflach, Austria
1969 - 1970	Military Service in the Austrian Army
1970 - 1975	Studies of Technical Chemistry at the University of Technology, Graz, Austria
1975	Diploma Thesis at the Institute of Biochemistry at the University of Technology, Graz, Austria, under the guidance of Prof. F. Paltauf Assistant at the Institute of Biochemistry at the University of Technology, Graz, Austria Subject of the Diploma Thesis: "Influence of myo - inositol on growth of Saccharomyces carlsbergensis ATCC 9080. Metabolism of inositol - containing compounds"
1975 - 1978	Doctoral Thesis at the Institute of Biochemistry at the University of Technology, Graz, Austria, under the guidance of Prof. F. Paltauf Subject of the Doctoral Thesis: "Lipid metabolism in inositol deficient yeast"
1978 - 1980	Assistant at the Institute of Biochemistry at the University of Technology, Graz, Austria
1980 - 1981	Post - doctoral studies at the Biocenter Basel, Switzerland, in the group of Prof. G. Schatz; studies on the import of proteins into mitochondria of the yeast; EMBO Long-term Fellowship
1982 - 1986	Assistant at the Institute of Biochemistry at the University of Technology, Graz, Austria
1985	"Habilitation" in Biochemistry ("Biogenesis of cellular membranes") and appointment as Docent
1986 - 1987	Sabbatical visitor at the University of California, Berkeley, USA, in the group of Prof. R. Schekman; studies on the secretory pathway in yeast; Max-Kade Fellowship
1987	Assistant Professor and tenure at the Institute of Biochemistry at the University of Technology, Graz, Austria
1995 - dato	Associate Professor at the Institute of Biochemistry at the University of Technology, Graz, Austria
2001 - 2003	Head of the Institute of Biochemistry at the University of Technology, Graz, Austria
2003 - dato	Associate Professor at the Institute of Biochemistry at the University of Technology, Graz, Austria, and group leader of the Cell Biology Group

Career-related Activities

1988 - 1990	Secretary of the Austrian Biochemical Society
1990 - dato	Reviewer for various international scientific journals, e.g., Biochim. Biophys. Acta, Biochem. J., EMBO J., Science, Nature Cell Biol., Mol. Microbiol. FEBS Lett., J. Cell Biol., Eur. J. Biochem., J. Bacteriol., J. Biol. Chem., Mol. Biol. Cell, Mol. Cell. Biol., Biochem. J., Yeast, PLOS ONE
1991 –1995	Local representative of the Austrian Biochemical Society in Graz
1995 - 1997	Sub-coordinator in the EU project EUROFAN (Function analysis of yeast genes)
1995	Organizer and Founding Father of the Yeast Lipid Conference
1995 - 2015	Chairman of the Yeast Lipid Conference
2000 - 2003	Coordinator AUSTROFAN-Cell Structures (Function analysis of yeast genes)
2000 - dato	Project Reviewer for the Academy of Sciences, Finland; Biotechnology and Biological Sciences Research Council, UK; National Science Foundation, USA; Earth & Life Sciences Council, The Netherlands; Swiss National Science Foundation, Switzerland, Barth Foundation, USA; DFG Deutsche Forschungsgemeinschaft, Germany
2002	Organizer of the 43th International Conference on the Bioscience of Lipids
2003 - 2009	Coordinator of the Key Research Area Life Science Technology at the TU Graz, Austria
2005 - 2014	Member of the Board of the Austrian Science Fund (FWF)
2005 - 2010	Member of the Editoral Board of The Journal of Biological Chemistry
2005 - 2016	Associate Editor of FEMS Yeast Research
2005 - 2009	Vice President of the International Conference on the Bioscience of Lipids
2007 - 2009	Vice President of the Austrian Society of Biochemistry and Molecular Biology
2007 - 2016	Director of the Doctoral School Molecular Biosciences and Biotechnology at the TU Graz
2009	Organizer of the 7th Euro Fed Lipid Congress, "Lipids, Fats and Oils: From Knowledge to Application",
2010 - 2011	Advisory Board Member of Prog. Lipid Res.
2010 - dato	Advisory Board Member of Eur. J. Lipid Sci. Technol.
2010 - 2012	President of the International Conference on the Bioscience of Lipids
2011 - dato	Executive Editor of Prog. Lipid Res.
2011 – dato	Editor of Prog. Lipid Res.
2012 - dato	Chairman of the Section Microbial Lipids of Euro Fed Lipid (European Federation of the Science and Technology of Lipids)
2012 - 2016	Vice Dean of Studies for Molecular Biomedical Sciences and Biotechnology at the TU Graz
2014 – 2016	Vice President of the Deutsche Gesellschaft für Fettwissenschaft (German Society of Lipid Research) (DGF)
2016 – dato	President of the Deutsche Gesellschaft für Fettwissenschaft (German Society of Lipid Research) (DGF)

Awards

1975	Unilever Award
1980	EMBO Fellowship
1986	Max Kade Fellowship
2011	Normann Medal of the Deutsche Gesellschaft für Fettwissenschaft (German Society of Lipid Research)

Memberships

Austrian Society for Molecular Biology, Biochemistry and Biotechnology
Deutsche Gesellschaft für Fettwissenschaft (German Society of Lipid Research)

Publications

Number of publications: 172
Number of presentations at meetings: more than 394
Hirsch Index: 59

International cooperations

N. Pfanner and T. Becker, Institute of Biochemistry and Molecular Biology, ZBMZ, University of Freiburg, Germany

R. Schneiter, Department of Biology, University of Fribourg, Fribourg, Switzerland

P. Ferrer, Department of Chemical, Biological and Environmental Engineering, Escola d'Enginyeria, Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain.

Research projects

FWF W901-305 PhD Program Molecular Enzymology

FWF P 26133: Phosphatidylserine decarboxylase 1 of the yeast (continued)

FWF P 27346: Steryl ester hydrolases of the yeast

Austrian Centre of Industrial Biotechnology (ACIB): Cell engineering of *Pichia pastoris*

Publications

1. Wagner A, Di Bartolomeo F, Klein I, Hrastnik C, Doan KN, Becker T, Daum G.: Identification and characterization of the mitochondrial membrane sorting signals in phosphatidylserine decarboxylase 1 from *Saccharomyces cerevisiae*. Biochim. Biophys. Acta. 1863 (2018) 117-125. DOI:10.1016/j.bbalip.2017.11.003.
2. Korber M, Klein I, Daum G.: Steryl ester synthesis, storage and hydrolysis: A contribution to sterol homeostasis. Biochim. Biophys. Acta. 1862 (2017) 1534-1545. DOI:10.1016/j.bbalip.2017.09.002.
3. Klein I, Korber M, Athenstaedt K, Daum G.: The impact of nonpolar lipids on the regulation of the steryl ester hydrolases Tgl1p and Yeh1p in the *yeast Saccharomyces cerevisiae*. Biochim. Biophys. Acta. 1862 (2017) 1491-1501. DOI:10.1016/j.bbalip.2017.08.009.

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Award

1. **Martina Korber** received the *Best Poster Prize* at the 17th DocDay of the NAWI Graz Doctoral School of Molecular Biosciences and Biotechnology (2017).

Cellular Metabolism Group

Group leader: Juliane Bogner-Strauss
Postdoctoral fellow: Melina Amor
PhD students: Katharina Huber, Dina Hofer, Wenmin Xia, Furkan Alkan, Katharina Walter, Gabriel Zirkovits
Master students: Katharina Küntzel, Irene Svoboda
Adminsitration / Technical Staff: Florian Stöger (part time), Claudia Gaug (part time), Thomas Schreiner
Apprentice: Wolfgang Krispel, Nadine Galler

General description

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

N-acetyltransferase 8-like (Nat8l)

In the brain, Nat8l is known to catalyze the formation of *N*-acetylaspartate (NAA) from acetyl-CoA and L-aspartate. NAA acts as a carrier of acetyl groups that can subsequently be incorporated into neutral lipids. The metabolic importance of NAA has been shown in two inborn human neurodegenerative disorders, where defects in NAA biosynthesis as well as catabolism lead to reduced lipid synthesis. Recently, our group was the first who showed that Nat8l is highly expressed and active in brown and white adipocytes and thereby impacts energy and lipid metabolism.

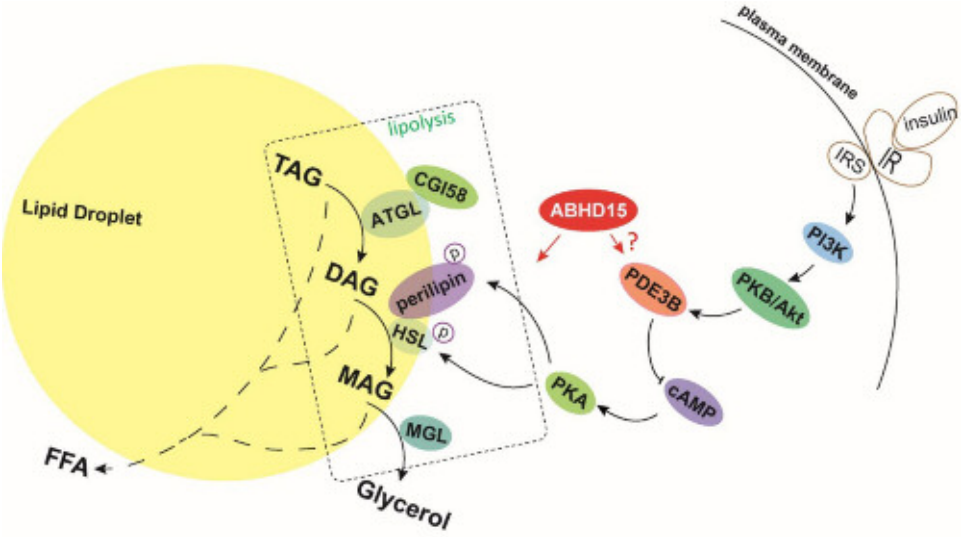
With these data we hypothesize that the Nat8l/NAA pathway also exists outside the brain and is a functional and parallel pathway to ATP-citrate lyase in providing cytosolic acetyl-CoA. Cytosolic acetyl-CoA can be used for lipid/cholesterol synthesis and protein acetylation and its availability controls a lot of processes such as de novo lipogenesis, DNA transcription, and autophagy. However, the trigger(s) that influence Nat8l expression *in vivo* are recently under investigation in the Nat8l-ko mouse model in a close collaboration with the group of Atsumi Nitta (University of Toyama, Japan). Further, this mouse model is used to investigate energy and lipid metabolism in white and brown adipose tissue after deletion of Nat8l (PhD thesis of Helmut Pelzmann and Dina Hofer). The PhD thesis of Katharina Huber deals with the availability of acetyl-CoA in the cytosol of brown fat cells after overexpression and silencing of Nat8l and the subsequent impact of changed acetyl-CoA levels on autophagy, longevity, and histone acetylation. Nat8l seems to be a promising target to treat obesity and its associated disorders by activating brown adipocyte metabolism and thereby dissipating energy as heat instead of storing it. Furthermore, levels of NAA have also been found to be elevated in lung and ovarian tumors compared to corresponding healthy tissues. This increase of NAA correlates with worse patient outcome. Katharina Eva Walter is investigating the impact of NAA/Nat8l on cancer energy metabolism. An understanding of how the NAA pathway supports tumor growth will path the way for novel potential therapeutic strategies to combat cancer.

Aralar (AGC1)

AGC1 (mitochondrial **A**spartate-**G**lutamate **C**arrier 1) exports aspartate from mitochondria to cytosol. Aspartate is a non-essential amino acid that is produced in mitochondria as a result of tricarboxylic acid (TCA) cycle and potentially an important precursor of macromolecule biosynthesis in cancer cells. During his ongoing PhD project, Furkan Alkan discovered that blocking AGC1 transporter activity using RNAi technology impairs cells' ability to utilize aspartate for macromolecule production and leading to reduced cell growth. Furthermore, in collaboration with Matt Vander Heiden lab at Massachusetts Institute of Technology, Furkan Alkan found that aspartate transport from mitochondria becomes more important for cancer cells when exogenous carbon sources are limiting. In fact, cancer cells lacking AGC1 start dying rapidly following glutamine withdrawal from cell culture media, which is the predominant carbon donor for TCA cycle in cancer cells. Moreover, inhibiting glutamine metabolism together with aspartate transporter significantly impairs tumor growth in vivo suggesting a therapeutic potential for targeting chemo-resistant tumors.

Aspartoacylase (Aspa)

While Nat8l catalyzes the formation of *N*-acetylaspartate (NAA) from acetyl-CoA and L-aspartate, Aspa deacetylates NAA and produces acetate and L-aspartate. Thereafter, the acetate moiety is reutilized for acetyl-CoA synthesis and can subsequently be incorporated into lipids. Additionally, asparate is released after cleavage of NAA by Aspa and available in the cytosol for nucleotide/amino acid synthesis. Both, acetyl-CoA and aspartate have been shown to play an important role in cancer cell proliferation and survival. Preliminary results show that silencing or overexpression of Apsa in cancer cell lines impact their proliferation capacity and tumor growth in xenograft models. Further studies are planned to investigate the mechanism behind.



Proposed working model of ABHD15, Copyright Bogner-Strauss Lab

Alpha-beta hydrolase domain containing protein 15 (ABHD15)

During the last years, the mammalian a/ß hydrolase domain containing (ABHD) proteins have emerged as potential regulators of lipid metabolism. There are at least 19 ABHD family members that all possess a similar tertiary protein fold of α-helices and β-sheets. However, they do not share obvious sequence similarities, leading to a widespread variety of enzyme subclasses, such as lipases, esterases, dehydrogenases, dehalogenases, peroxidases, and epoxide hydrolases. It is therefore expected that ABHD15 possesses a hydrolytic active site but its distinct function has not been defined so far.

We found Abhd15 expressed in brown and white adipose tissue and strongly upregulated during adipogenesis in various murine and human cell lines. In vitro ABHD15 is required for proper adipogenesis and seems to protect from apoptosis. Wenmin Xia and Ariane Pessentheiner investigated the ABHD15-ko mice in detail and found out that ABHD15 regulates the expression and activity of PDE3B, an inhibitor of intracellular lipolysis. ABHD15-ko mice show a strongly decreased PDE3B expression in white adipose tissue (WAT). Additionally, ABHD15 does not react to insulin-elicited repression of lipolysis in the fed state but still show high lipolytic activity in WAT. Confirming this data, insulin injection also cannot inhibit lipolysis in WAT. When investigating the molecular mechanism behind, we found that HSL phosphorylation is strongly increased in ABHD15-ko mice in the refed/insulin injected state suggesting that the PDE3B-cAMP-PKA signaling pathway is deregulated in mice upon ABHD15 depletion (see depicted scheme).

The liver is the central metabolic organ in vertebrates, regulating not only glucose homeostasis in the fed/fasted state, but also lipid synthesis and corresponding lipoprotein metabolism. We could show that the hepatic response upon refeeding a diet rich in carbohydrates is dysregulated in ABHD15-ko mice. As a consequence, in these experiments, plasma Very Low Density Lipoprotein – Triglyceride (VLDL-TG) levels are reduced in mice lacking ABHD15. Gabriel Zirkovits focuses on the underlying molecular dysregulations.

Matrix metalloproteinase 12 (MMP-12)

Previous studies and our own preliminary data point to matrix metalloproteinase 12 (MMP12) as an interesting molecular target for the treatment of obesity-associated Type 2 Diabetes and cardiovascular diseases. MMP12 abundance in several metabolic tissues is highly increased in the obese state when compared to the lean state and has been related to the development of a wide spectrum of pathological conditions, including insulin resistance and atherosclerosis, main features behind Type 2 Diabetes and cardiovascular diseases, respectively. In this project, Melina Amor is currently exploring mechanistic aspects associated with the pathological role of MMP12 *in vitro*. In the upcoming future, we will investigate the role of MMP12 in a mouse model that simultaneously develops both pathological conditions giving the advantage to better mirror the human disease. This will be performed via whole-body genetic deletion of MMP12 and additionally by using a novel, vaccination-based therapeutic strategy. We hypothesize that blocking MMP12 in mice may lead to a general improvement in metabolic and cardiovascular parameters that are associated with obesity. In conclusion, this project will provide a comprehensive investigation of a promising target, which could directly support further studies in humans.

Adipocyte plasma membrane associated protein (APMAP)

The identification of genes that predispose individuals to obesity, insulin resistance, and type 2 diabetes could provide tools for developing strategies and/or therapeutics to combat obesity and its consequences. We identified APMAP as an important player in fat cell development. APMAP expression is up-regulated in murine and human adipogenic cell models and in a genetic mouse model of obesity. Silencing of APMAP in a white adipogenic cell line strongly impaired the maturation of these cells into fat cells. Moreover, we could show that APMAP is a direct and functional target of PPARgamma, the master regulator of adipogenesis. Ariane Pessentheiner investigated this mouse model in detail. The results revealed that the APMAP-ko mice show a so called "healthy adipositas" upon high fat diet feeding, reflected in increased glucose tolerance, smaller and more adipocytes, reduced extra cellular matrix formation, reduced inflammation and fibrosis. Currently, this work is under review in FASEB.

Master Theses completed

Katharina Küntzel: *N-acetylaspartate metabolism in white adipocytes*

Obesity and overweight have become a growing problem worldwide. Thus, research increasingly focuses on understanding adipose tissue metabolism. Adipose tissues can be distinguished in two different types fulfilling different functions: 1) white adipose tissue which is responsible for the storage of excess calories and for the release of fatty acids in case of nutrient depletion (fasting condition) and 2) brown adipose tissue which dissipates energy in form of heat. This work focuses on aspartate N-acetyltransferase 8-like (NAT8L), an enzyme that has been investigated in brain since decades, but has only been found in adipose tissues in 2013. Nat8l catalyzes the formation of N-acetylaspartate from acetyl-coenzyme-A and aspartate. Thereafter, N-acetylaspartate is cleaved into acetate and aspartate by aspartoacylase (ASPA). Until today, research on NAT8L in adipose tissues was performed in brown adipocytes, the main cell type in brown adipose tissue; this thesis deals with the role of Nat8l in white adipocytes. To investigate the function of NAT8L in white adipocytes, we stably overexpressed Nat8l in 3T3-L1 cells, an established in vitro model for white adipocytes. Nat8l overexpression led to a very poor adipocyte phenotype manifested in reduced intracellular lipid content and decreased de novo lipogenesis gene and protein expression. We hypothesized that these cells might accumulate NAA, which, in high concentrations could be toxic for these cells, speculating that this is one reason for the lack of adipogenic differentiation. However, addition of Rosiglitazone (ROSI), a PPAR agonist that is known to increase adipocyte differentiation, could rescue the differentiation defect of Nat8l overexpressing 3T3-L1 cells, contradicting the toxicity hypothesis. In particular, addition of ROSI to Nat8l o/e 3T3-L1 cells made the lipid content and the expression of lipid synthesizing genes/proteins comparable to control cells. However, ATP-citrate-lyase the canonical reaction for lipid synthesis, was still significantly decreased in these cells. This data indicates that Nat8l overexpression in 3T3-L1 cells, like in brown adipocytes, decreases cytosolic acetyl-coenzyme-A production suggesting the Nat8l/NAA pathway as an alternative route for lipid synthesis in all adipogenic cell types. Further experiments showed reduced autophagy, responsiveness to insulin and increased browning (conversion of white into brown adipocytes). To compare Nat8l overexpression to a model with Nat8l-knockdown, primary adipocytes of Nat8l-knockout mice were differentiated and showed exactly the opposite effects of Nat8l overexpression arguing for a crucial role of Nat8l in energy homeostasis.

Irene Svoboda: *The role of Pex16 in brown adipocytes*

Peroxisomes (PO) are organelles that maintain abundance by division or de novo biogenesis. Peroxin 16 (PEX16) is a membrane protein involved in both processes and essential in mammals. In the mature state, PO perform α- and β-oxidation of very long-chain and poly-unsaturated fatty acids, synthesis of ether lipids and bile acids, as well as detoxification of H2O2. In adipocytes, PO number is upregulated during adipogenesis owing to their central role in lipid metabolism. Specific functions of brown adipocytes are lipid storage and dissipation of energy as heat. The aim of this thesis was to investigate the function of PEX16 in immortalized brown adipocytes (iBACs). Overexpression of PEX16 in iBACs led to increased mRNA expression of PPARα and PPARγ, transcription factors activated by PO-derived lipids. Expression of Cidea was increased, encoding a protein involved in lipid storage and mobilization. Even though no changes in PO abundance could be observed, activity of the peroxisomal marker protein catalase, catalyzing the detoxification of H2O2, was significantly increased. As a consequence, oxidative stress remained unchanged despite increased mitochondrial respiration that was indicated by significantly increased oxygen consumption. This argues for an elevated lipid metabolism as the basal rate of glycolysis remained unchanged. Transient silencing with anti-Pex16 siRNA conducted before or during differentiation proved to yield low silencing efficiency, which did not allow further analysis. Establishment of stable Pex16 silencing by transfection with shRNA-carrying lentiviral particles also resulted in poor silencing on protein and mRNA level. Therefore, no further experiments were conducted. This data implicate a crucial role for Pex16 in enhancing lipid metabolism in mature brown adipocytes.

International cooperations

Atsumi Nitta, Department of Pharmaceutical Therapy & Neuropharmacology, Faculty of Pharmaceutical Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Japan

Dayoung Oh, School of Medicine, Division of Endocrinology & Metabolism, UCSD, California, USA

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

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

Research projects

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

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

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

Talk at international conference

1. Huber K.: *The role of N-acetyltransferase in energy metabolism and autophagy.* 17th DocDay, Graz, February 2017

Publications

1. Bogner-Strauß, J. G.: N-Acetylaspartate Metabolism Outside the Brain: Lipogenesis, Histone Acetylation, and Cancer. Front. Endocrinol., 2017, 8:240, DOI:10.3389/fendo.2017.00240
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Awards

1. **Wenmin Xia** received the Marshall Plan scholarship for her one year stay abroad in the lab of Alan Saltiel (UC San Diego, USA).
2. **H. Furkan Alkan** received the Marshall Plan scholarship for his one year stay abroad in the lab of Matthew Vander Heiden (MIT, USA).

Chemistry of Functional Foods

Group leader: Michael Murkovic
PhD students: Marini Damanik, Sandra Holzer, Nicole Pabi, Abdullatif Albouchi
Master students: Verena Buchgraber, Laura Sonnleitner, Christopher Hartl, Maria Hulla, Tamara De Zuani, Daniela Krieg, Christoph Trummer
Technical staff: Alma Makic, Claudia Hrastnik

General description

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

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

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

PhD Thesis completed

Damanik Marini: *Secondary lipid oxidation: Analysis of lipid oxidation and antioxidants interaction in coffee oil and some edible oils*

Vegetable oils consist of triglycerides between 95-99%. They also contain soluble vitamins (A, D, E, and K), phytosterols, natural pigments and phospholipids. The increasing content of unsaturated fatty acids the possibility of oxidation increases as well. The heat influences the oxidation of the oils which leads to the formation primary oxidation products and secondary oxidation products. Hydroperoxides are the primary oxidation products of lipid oxidation. These products are generally unstable and decompose into a variety of secondary oxidation products, including the carbonyls compounds which were compounds focusing in this

dissertation. Carbonyl compounds are more stable than hydroperoxides and the measurement is a good index of oxidative changes in lipids. The derivatization of carbonyls with 2,4-dinitrophenylhydrazine and HPLC measurement of the aldehydes is correlated with rancidity. The oxidation experiments were done in a Ranzimat at 120 °C with an air flow of 20 l/h to have reproducible and comparable conditions. The formation of carbonyls was measured in coffee oil, triolein, palm oil, rapeseed oil, and sunflower oil. The aldehydes formed during roasting of coffee were hydroxypentanal and heptatrienal. In triolein – a model substance – hexanal, heptanal, octanal, nonanal, and decanal were identified. In rapeseed oil and sunflower oil hexanal, octanal, nonanal, decenal, and 2-undecanal were identified. In rapeseed oil the maximum was observed after 7.5 h. Sunflower oil oxidised 1 hour earlier. In addition, two antioxidants commonly present in edible oils were investigated. These showed a slight reduction of the aldehyde formation in the experiments.

Master Theses completed

Wollner Oliver: *Method development and validation to determine free and protein-bound fractions of drugs in biological fluids with LC/MS analysis*

The analysis of drug binding by endogenous proteins plays a major role in pharmaceutical drug development. Current binding studies are mainly made with plasma as it is presumed that unbound drugs can move freely between tissues. As this hypothesis has its limits it would be necessary to take the interstitial fluid into account as well. The interstitium is the area where most of the drugs mediate their effect. This fluid, however, is difficult to access due to the narrow space between tissue cells, but modern sampling approaches have shown promising results. It is unclear whether binding essays can also function in fluids with low protein content such as the interstitium. In this thesis drug protein binding essays were evaluated with several biological fluids in order to highlight possible differences in drug binding capacities between tissues. Therefore, several techniques were investigated and compared. The rapid equilibrium dialysis was found to be the most suitable technique for drug-protein binding analysis and was additionally validated via various experiments. Moreover, it could be shown that ultrafiltration, a standard technique for plasma protein binding experiments, is not in line with the demands for low-protein fluid analysis.

Trummer Christopher: *Comparison of liquid chromatographic methods and spectrophotometric assays for polyphenol analysis – validation and quantification in selected food*

Polyphenols are secondary plant substances that significantly contribute to the many plant characteristics, for instance stability, colour or flavour. They are known to exert benefits on human health due to their interaction with free radicals (reducing properties). Therefore, they are thought to be beneficial in cancer protection and for prevention of cardiovascular diseases. Due to this, consumers and food industry likewise have become increasingly interested into this class of chemical compounds and analytical methods for determining polyphenols have improved during the last decades which are commonly employed in food science and research. Researchers are interested in both, the amounts of polyphenols in selected groceries as well as their identity and therefore their influence on human health. In this thesis, different analytical strategies were investigated regarding their suitability for polyphenol analysis in food samples (e.g. grain samples, coffee, extracts of *Pinus cembra*, etc.). Amongst the most commonly employed methods are such that generate a sum parameter e.g. total antioxidant capacity (TAC) or total polyphenol content (TPC). Analytically, methods are separated into

spectrophotometric (e.g. Folin Ciocalteu assay) assays which can be performed in 96-well plates to increase sample throughput and chromatographic techniques e.g. thin layer chromatography for method development where e.g. a DPPH staining can be used. Here, the DPPH radical reacts with antioxidants and therefore not only a qualitative statement regarding the number of separated compounds and their retention factor (R_f value) can be made, but also signal intensity can be read either directly or fluorometrically. For identification of individual antioxidative substances, mass spectrometry is the most commonly used strategy. Here, ions are generated following separation by a chromatographic (for polyphenols high performance liquid chromatography is most commonly used) system, whose mass over charge ratio is determined and additionally the molecule is fragmented and based on the fragmentation pattern, the molecular structure can be derived.

Sonnleitner Laura: *Method development and validation to determine the antidiabetic drug metformin in human lithium heparin plasma with RP-HPLC analysis*

This thesis focusses on the establishment of a suitable and reliable method for the determination of the antidiabetic drug metformin in human lithium heparin plasma. Samples (n=44) from patients affected by T2DM and PCOS were obtained from the Medical University of Graz. Samples were deproteinized with acetonitrile and MF concentrations were determined with RP-HPLC with pre-column fluorescence derivatization. Analysis was run with a linear gradient elution with increasing concentrations of acetonitrile from 5% up to 65%. MF fluorescence maxima were detected at 279 nm for excitation and at 440 nm for emission. The formed metformin fluorophore was stable over 24 h with a retention time of 8.8 minutes, no endogenous plasma substances interfered with the detection. The calibration curve of metformin in human plasma was linear in the range of 0.95 - 4.76 µg/ml with a correlation coefficient of 0.997. Recovery was calculated to be 109%. LOD and LOQ were determined to be 190 ng/ml and 650 ng/ml, respectively. Mean MF concentration in patient samples was determined to be 3.18 ± 1.41 µg/ml. The majority of the obtained results were within the expected range of 1-4 µg/ml. The applied method has proven to be simple and suitable for metformin quantification.

Sattler Reingild: *Bile binding capacity of beta-glucan from oat porridge*

Recently there has been significant interest in dietary fibers, especially β-glucan (BG), and their bile-binding ability. Bile is produced in the liver and gets secreted by the gallbladder into the gut during fat digestion. By binding bile, dietary fibers reduce the amount of bile getting reabsorbed and this in turn leads to positive health effects such as reduced cholesterol levels in the blood. In this project the bile binding capacity of the BG from oat porridge was determined, using bovine bile. Oat porridge in cooked and uncooked form was digested by INFOGEST in vitro static as well as semi-dynamic digestion method and subsequently 24-hour dialysis on the digesta was performed. The bovine bile concentration was determined via HPLC-UV quantitative analysis. For comparison, the matrices BG90 and OatWell28® were used. The viscosity of all digesta was also determined. The results showed that without any other components involved, the BG concentration needs to be ≥0.5% for the matrix to sequester bile. For whole oat porridge, a BG concentration of ≥0.14% is sufficient. Cooked oat samples showed higher bile-binding capacity when semidynamically digested, whereas uncooked oat samples showed a higher capacity when statically digested. The BG concentration and the bile-binding capacity were only proportional for the static digested samples. Therefore, the influence of the in vitro digestion method on the amount of

sequestered bile is significant. The correlation of high viscosity and high bile-binding capacity could only be seen at the static digested samples, while the semi-dynamically digested samples showed no correlation. The equilibrium of the bile concentration in the dialysis was reached medially between 5 and 24 hours. The simulated digestion method and the preparation of the food sample seem to have a huge impact on the amount of bile that gets sequestered by oat porridge.

Hulla Maria: *Storage stability and antioxidative properties of astaxanthin from Haematococcus pluvialis*

Over the past few years, astaxanthin has gained considerable interest due to its biological functions. This xanthophyll is able to protect against degenerative diseases and provides high antioxidant activity. The production of astaxanthin for food use is only permitted by cultivation of the microalgae *Haematococcus pluvialis*, which is a unicellular biflagellate microorganism. Due to astaxanthin´s conjugated polyene chain it is susceptible to degradation which results in the loss of product quality as well as bioactivity. The factors influencing the stability of astaxanthin are temperature, light, oxygen, and pH. The main aim of this study was to determine the effect of different environmental parameters on the (storage) stability of astaxanthin, and to find strategies to enhance its stability. In addition to that, the antioxidative properties were also looked into. The stability of astaxanthin was evaluated qualitatively as well as quantitatively via high performance liquid chromatography, thin layer chromatography, and UV-vis spectrophotometry. Furthermore, the algal biomass was analyzed via mass spectrometry and scanning electron microscopy. The antioxidative properties of the extracts were evaluated via the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the ferric reducing power assay. As a result, astaxanthin was relatively stable when the *H. pluvialis* biomass was stored at 4 °C or minus 30 °C over a period of 18 weeks. Adding antioxidants such as α-tocopherol, butylated hydroxytoluene (BHT), trolox and ascorbyl palmitate enhanced the stability. Better stability was also obtained when astaxanthin was stored without oxygen (vacuum, nitrogen) compared to storage in the presence of air. The addition of oxygen absorbers to the packaged biomass (whole cells) led to an accelerated degradation of astaxanthin. Astaxanthin portrayed enhanced stability when stored in oil, in an alginate matrix, or as an inclusion complex with hydroxypropyl-β-cyclodextrin. Astaxanthin was stable for 2 hours when heated up to 100 °C. When increasing the temperature beyond 100 °C the degradation started. In the biomass sample mainly astaxanthinmonoesters could be found. The obvious difference of the appearance of spray-dried and belt dried algal biomass was seen in the electron microscope pictures. The spray-dried sample showed more singular particles compared to the belt dried product which had multi-layered plates.

De Zuani Tamara: *Stability of selected nutrients in home-made fruit juices under oxygen-free storage conditions*

Aim of this master thesis was to analyze the influence of oxygen on the stability of selected nutrients in home-made fruit juices. Therefore, the juices were either stored for 24 hours at 4 °C under oxygen atmosphere or under nitrogen atmosphere in desiccators. Juices from different fruits and vegetables (orange, strawberry, carrot and red grape) and three different juice extractors (centrifuge, blender, and masticating juicer) were compared. The following nutrients and parameters were analyzed: vitamin C, 5-methyltetrahydrofolate, vitamin B_6, total phenolic content, anthocyanins, and hesperidin. In addition, the colony forming units of yeasts, molds, and bacteria were determined. There was no optimal juicer for all fruits and

vegetables, as every device has its advantages and disadvantages. All in all it can be said that vitamin C and hesperidin were not stable over 24 hours when stored under oxygen atmosphere, whereas the stability was enhanced by oxygen exclusion. The other parameters measured were stable for 24 hours. The highest vitamin C concentrations in orange- and strawberry juice were 50.2 and 50.0 mg/100 ml and were found in juices from the masticating juicer and the centrifuge. The vitamin C content in juices from the blender was reduced by 70 % and 80 % during the storage under oxygen. When stored under oxygen, a degradation of the vitamin C content was detected in juices from all three extractors, while storing under nitrogen led to an enhanced stability. The content of 5-methyltetrahydrofolate in orange and strawberry juice was stable over 24 hours when exposed to oxygen. The highest content in orange juice was achieved by using the blender (25.4 mg/100 ml) and the highest content in strawberry juice was achieved by using the centrifuge (771 mg/100 ml). The vitamin B6 content in carrot juice was stable under oxygen and nitrogen atmosphere. The highest content was detected in juices from the centrifuge (177 mg/100 ml). The total phenolic content in strawberry, carrot and grape juice was stable under both storage conditions. The highest concentration in strawberry juice was 366 mg/100 ml, in carrot juice 32.3 mg/100 ml and in grape juice 45.0 mg/100 ml. They were all detected in juices processed with the help of the blender. The content of anthocyanins in strawberry and grape juice was stable under oxygen and nitrogen storage conditions. The obtained values of the three juicers were similar for strawberry juice, while the anthocyanin content in grape juices from the blender was twice as high as in juices from the centrifuge and the masticating juicer. The hesperidin concentration decreased in orange juices from all three extractors when stored under oxygen. The highest detected concentration was 108 mg/100 ml, and was found in juices from the blender, whereas 60 % of the initial content was degraded over 24 hours. Storing under nitrogen atmosphere enhanced the stability during the storage period. The average storage temperature in domestic fridges was assumed to be 3.9 ± 4.7 °C. Carrot juices from all three juice extractors showed high contaminations of bacteria with values greater than 300,000 colony forming units (CFU)/ml. Juices from the centrifuge showed the highest bacterial growth in orange, strawberry and grape juice, with values up to 24,000 CFU/ml. The highest number of yeasts was found in carrot juices from the masticating juicer (151,000 CFU/ml), whereas for all other fruits the highest contamination was, again, found in juices from the centrifuge. Molds were only detected in significant amounts in grape juices from the masticating juicer (14,000 CFU/ml). Storing under nitrogen led to a reduced growth of both, bacteria and yeasts, in all juices.

Buchgraber Verena: *Development and validation of a chromatographic method for analysis of amines with low reactivity in biological matrices*

As type 2 diabetes cases still rise the development of individual therapies becomes more important. The identification of certain single nucleotide polymorphisms (SNPs) on genes of organic cation transporters (OCTs) that influence drug uptake is of great scientific interest. For this reason, the current study in cooperation with the Medical University Graz is crucial for improvement of metformin medication. Possible SNPs are identified clinically, genetically and chemically. This thesis is focused on the chemical metformin determination in urine via HPLC. A C18 reversed phase column (150 × 3 mm) was used for this method. For the gradient elution, solvent 1 consists of acetonitrile, 0.5 M Tris(hydroxymethyl)aminomethane (TRIS) pH 8 and water (5:21:74 v/v), solvent 2 contains acetonitrile, tetrahydrofuran, 0.5 M TRIS pH 8 and water (65:10:10:15 v/v). The flow rate was 0.5 ml/min and the run time 10 min. The retention time of metformin was found to be 8.8 min. The detection of the drug was carried out via fluorescence. The derivatization of metformin with benzoin is a reliable pre-

column derivatization procedure. In comparison to desyl bromide, benzoin was more sensitive. Derivatized samples should not be stored, because of degradation. The current HPLC method was validated for selectivity, linearity, precision, accuracy and robustness. The recovery was determined to be 96 %. The limit of detection is 30 µg/ml, the limit of decision is 60 µg/ml and the limit of quantification is 110 µg/ml. The found concentrations in urine samples range from 0.1 mg/ml to 2.1 mg/ml metformin, which is comparable to other studies.

International cooperations

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. Damanik, M.: *The antioxidants effect to the formation of carbonyls during lipid oxidation in some edible oils.* 18th DocDay, Graz, July 2017.

Publications

1. Passos, C.P., Kukurová, K., Basil, E., Fernandes, P.A.R., Neto A., Nunes, F.M., Murkovic, M., Ciesarová, Z., Coimbra, M.A. (2017) Instant coffee as a source of antioxidant-rich and sugar-free coloured compounds for use in bakery: Application in biscuits. Food Chemistry 231 (2017) 114–121.
2. Damanik M, Murkovic M (2017) Formation of potentially toxic carbonyls during oxidation of triolein in the presence of alimentary antioxidants. Monatshefte für Chem (2017) 148:2031-2035.
3. Pedreschi F., Murkovic M. (2017) Chapter 7: Potentially toxic food components formed by excessive heat processing. In: Dietary AGEs and Their Role in Health and Disease, Uribarri J (Ed) CRC Press, pp 88-101.

Lectures and Laboratory Courses

Winter Semester

Course no.	Title	Hours	Type	Lecturers
CHE.154_1	Biochemistry Laboratory Course I	5.33	LU	Team
CHE.155	Biochemistry II	1.5	VO	Macheroux P
CHE.191	Bioanalytics	2.25	VO	Klimant I, Winkler A
CHE.192	Biochemistry Laboratory Course II	4	LU	Team
CHE.890	Food Biotechnology	1.33	VO	Murkovic M
CHE.892	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MAS.420UF	Biomaterials	2	VO	Bogner-Strauß J
MOL.833_1	Project laboratory	9	LU	Team
MOL.845_UF	Master Thesis Seminar	2	SE	Macheroux P, Winkler A
MOL.855UF	Molecular physiology	2	VO	Macheroux P
MOL.881UB	Biophysical Methods	3	VO	Winkler A, […]
MOL.933	Food Biotechnology	1.3	VO	Murkovic M
MOL.959	Enzymatic and Microbial Food Processing	2	VO	Murkovic M
MOL.961	Food Chemistry and Technology II	2	VO	Murkovic M
648.001	Fundamentals of molecular biology	2	VO	Bogner-Strauß J
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	Bogner-Strauß J,
648.020	Teaching Experience	2	SE	Macheroux P
648.059	Fundamentals of Pharmacology	2	VO	Dittrich P
648.092	Cell biology of lipids	2	PV	Daum G
648.100	Cell Biology 1	1	SE	Daum G
648.601	Cellular Metabolism	2	PV	Bogner-Strauß J
649.027	Chemical Reactions in Foods I	2	PV	Murkovic M

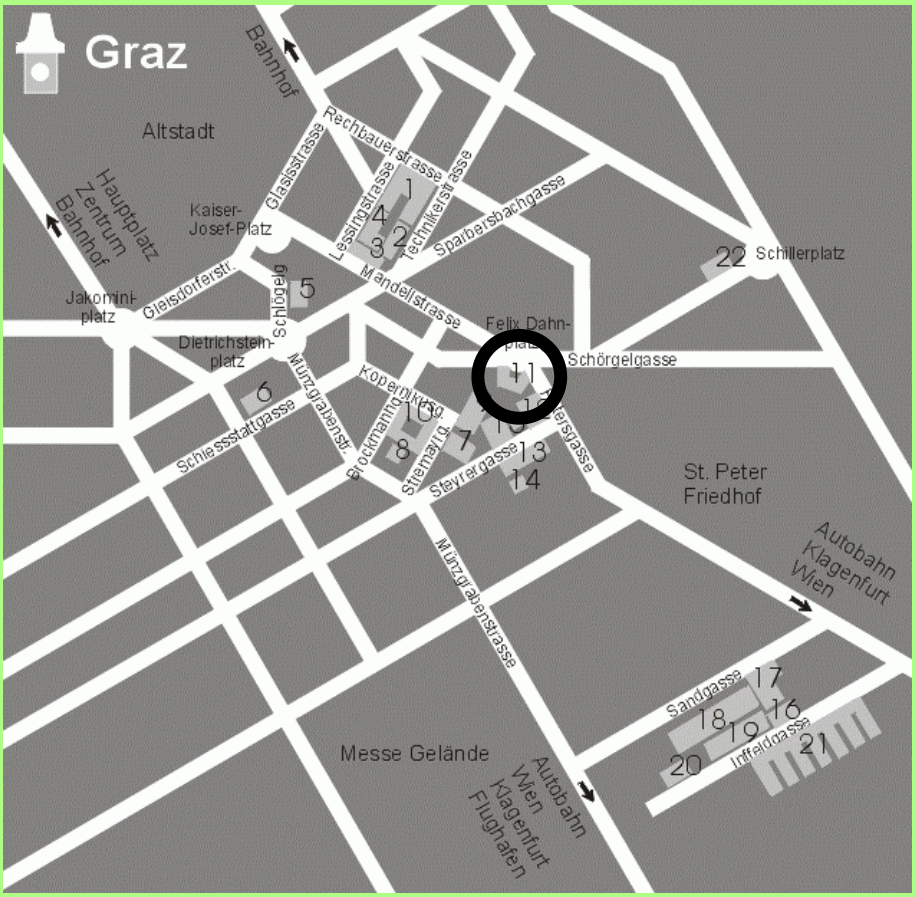
VO: Lecture Course; LU Lab Course; SE Seminar; PV: Privatissimum

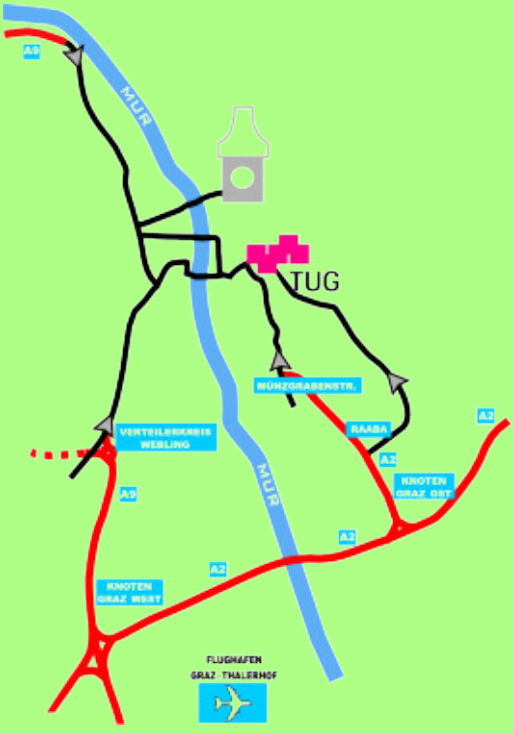
Lectures and Laboratory Courses

Summer Semester

Course no.	Title	Hours	Type	Lecturers
CHE.147	Biochemistry I	3.75	VO	Macheroux P
CHE.193	Molecular biology laboratory course	3	LU	Lienhart W, Daniel B
CHE.194	Seminar for Molecular biology laboratory course	1	SE	Lienhart W, Daniel B
CHE.195	Cell Biology	1.5	VO	Daum G
MOL.101	Introduction to Bachelor Study	1	OL	
MOL.406	Methods in Immunology	2	VO	Daum G
MOL.407	Methods in Immunology	2	LU	Team
MOL.606_1	Bachelor Thesis	1	SE	
MOL.833_1	Project laboratory	9	LU	Team
MOL.845_1	Seminar for undergraduate students	2	SE	Team
MOL.851	Special Topics in Biochemistry	1	VO	Daum G, Macheroux P
MOL.880	Mechanistic Enzymology	2	VO	Gruber K, Macheroux P, Nidetzky B
MOL.886	Biophysical Methods - Lab Course	3	LU	Winkler A, Lienhart W
648.000	Laboratory Practice	4	PT	Macheroux P
648.002	Molecular diagnostics	2	VO	Bogner-Strauß J
648.004	Molecular Enzymology II	2	PV	Macheroux P
648.006	Introduction to Biochemistry	2	VO	Macheroux P, Lienhart W
648.008	Graduate Seminar 2	1	SE	Team
648.010	Scientific Colloquium for Graduate Students 2	1	SE	Team
648.013	Advanced cell culture training course II	1	SE	Bogner-Strauß J
648.016	Chemical Reactions in Foods II	2	PV	Murkovic M
648.020	Teaching Experience	2	SE	Macheroux P
648.093	Cell biology of lipids	2	PV	Daum G
648.200	Cell Biology 2	1	SE	Daum G
648.302	Methods in Immunology	1	VO	Daum G
648.602	Cellular Metabolism	2	PV	Bogner-Strauß J

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


Graz
Bahnhof
Altstadt
Hauptplatz
Zentrum
Bahnhof
Kaiser-
Josef-Platz
Glacisstrasse
Rechbauerstrasse
Lessingstrasse
Technikerstrasse
Sparbersbachgasse
Gleisdorferstr.
Jakomini-
platz
Schlögelg.
Mandellstrasse
Felix Dahn-
Platz
Schörgelgasse
Dietrichstein-
platz
Münzgrabenstr.
Kopernikusg.
Grochmanng.
Schiesstattgasse
Stiftingtalstr.
Styrergasse
Leonhardstrasse
St. Peter
Friedhof
Autobahn
Klagenfurt
Wien
Münzgrabenstrasse
Sandgasse
Inffeldgasse
Messe Gelände
Autobahn
Wien
Klagenfurt
Flughafen
22 Schillerplatz


MUR
TUG
MÜNZGRABENSTR.
RAABA
VERTEILERKREIS
WEBLING
KNOTEN
GRAZ OST
KNOTEN
GRAZ WEST
FLUGHAFEN
GRAZ - THALERHOF