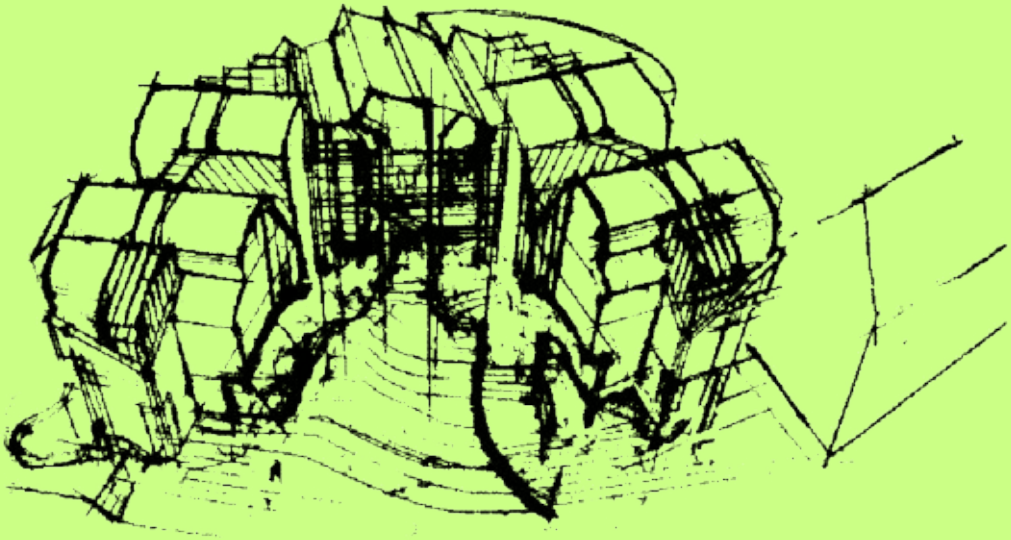
Graz University of Technology
Austria

Institute of Biochemistry



Annual Report 2016

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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 building of the Fürstlich-Dietrichstein-Stiftung, Schlögelgasse 9, in which all the biosciences were then concentrated.

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

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. This division was also drawn out of the mother institute and at the end of the sixties moved to a new building.

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

1973 Dr. F. PALTAUF from the Karl-Franzens-University Graz was appointed professor and head of the newly established *Institute of Biochemistry*. The interest of Prof. PALTAUF to study biological membranes and lipids laid the foundation for the future direction of research. G. DAUM, S. D. KOHLWEIN, and A. HERMETTER joined the institute and were given the opportunity to carry out postdoctoral research in renown laboratories in Switzerland and the USA: 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 chemistry and biochemistry of lipids.

Teaching was always a major task of the institute. Lectures, seminars and laboratory courses in basic biochemistry were complemented by special lectures, seminars, and courses held by the assistants who became docents in 1985 (G. D.), 1987 (A. H.), and 1992 (S. D. K.).

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.

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

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

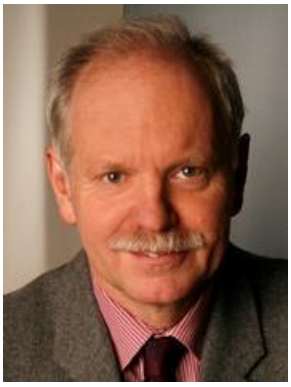
2013 As a result of inter-faculty reorganization, the research group of Prof. J. BOGNER-STRAUSS (formerly at the *Institute of Genomics and Bioinformatics*) joined the *Institute of Biochemistry*. Her main interests revolve around the development and metabolism of adipocytes.

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

2015 After more than 40 years of service for the *Institute of Biochemistry* Prof. A. HERMETTER retired on September 30, 2015. After receiving his PhD sub auspiciis Praesidentis at the Karl Franzens University Graz he started as a University Assistant in Prof. PALTAUF's group and moved through the ranks to become an Associate Professor at the institute. As an active member of the institute he published more than 120 papers in peer-reviewed journals and graduated 25 PhD students.

Highlights of 2016

In 2016 two PhD students from the group of **Peter Macheroux** completed their PhD thesis, Chanakan Tongsook and Chaitanya Tabib. The conference highlight of the year was the Oxizyme meeting in Wageningen, The Netherlands in July. Finally, before the academic year came to a close, Dr. Andreas Winkler, who graduated at the institute as one of the first PhD students of the DK "Molecular Enzymology" was appointed assistant professor. This was the first appointment under the recently established qualification schemes for tenure track professorships at our university.



In 2016 research in the lab of **Günther Daum** was continued as in the years before. Two research projects were supported by the Austrian Science Fund FWF, and one project (ACIB) was funded by the industry. Günther Daum was elected President of the Deutsche Gesellschaft für Fettwissenschaft (German Society for Fat Science) and will serve this position for 3 years.

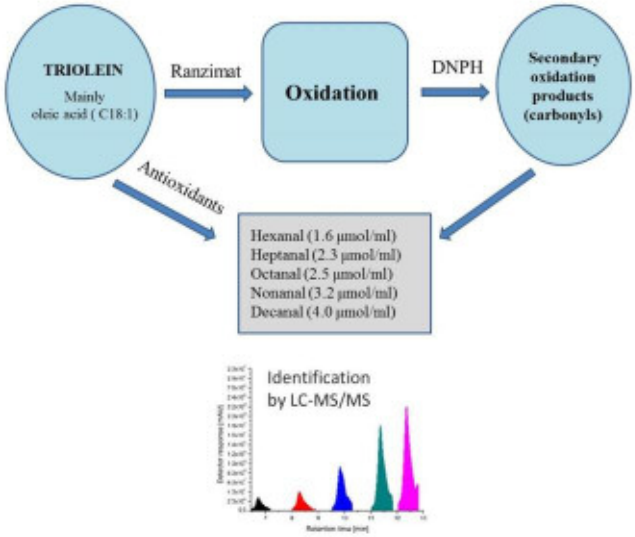
In 2016 **Karin Athenstaedt** was invited to join the "Young Investigators in Lipid Science" meeting in Düsseldorf, Germany. At this meeting Karin met many experts in the field, and had the opportunity to set the stage for collaborative activities.

In 2016, Helmut Pelzmann completed his PhD with a doctoral thesis about „Characterization of the N-acetylaspartate pathway in adipocyte metabolism in vitro and in vivo“. Parts of his work were published in Scientific Reports. Bernhard Gadermaier completed his Master Thesis with the functional characterization of a transmembrane protein. Furkan Alkan received a Marshall Plan scholarship for his current one year abroad stay in the Vander Heiden Lab at the Massachusetts Institute of Technology. Dina Hofer presented her work about “Pex16 in adipocyte development” that was recently published in BBA Lipids at the conference “Peroxisystem” in Israel. Katharina Huber presented her work about “Nat8l in autophagy” at the Keystone meeting in Canada, and Wenmin Xia at the Gordon conference in USA. Further, two more PhD students were recruited who will start in March 2017. Last but not least, Ariane Pessentheiner received a PostDoc position at UCSD, USA.

In 2016 Andreas Winkler was appointed assistant professor at the Institute of Biochemistry to give him the opportunity to build up a new research group in the field of integrative structural biology. The scientific projects of the two PhD students (Stefan Etzl and Geoffrey Gourinchas) developed extraordinarily well and, at the end of the year, the first publication originating from the young group was accepted for publication in Science Advances; the new open access journal of the renowned publisher of Science. Hopefully more to follow …

In 2016, **Grit Straganz** gave an invited talk at , 2nd Small Molecule Activation Conference, Cancun, Mexico, 18th-25th May, 2016, about the role of the protein environment in O_2 activation at mononuclear nonheme Fe(II) sites. Furthermore **Grit Straganz** and **Johannes Niederhauser** finalized work on the ‘automated simulation package for enzyme redesign’, which is designed as an extension of the Yasara suite. Concomitant with the respective publication, the plugin will be made available publicly for download.

In the Functional Food Group directed by **Michael Murkovic** in 2016 the main projects were 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 of them might be related to cancer and a reduction of alimentary exposure should improve the safety of these foods.



Biochemistry Group

Group leader: Peter Macheroux
Secretary: Annemarie Lehsl
Senior research scientists / postdoctoral fellows: Bastian Daniel, Wolf-Dieter Lienhart, Silvia Wallner (on maternal leave)
PhD students: Peter Augustin, Eveline Brodl, Shalinee Jha, Sami Ullah Khan, Karin Koch, Majd Lahham, Barbara Steiner, Emilia Strandback, Chaitanya Tabib, Chanakan Tongsook.
Master students: Julia Messenlehner, Sabine Pils
Technicians: Eva Maria Frießer, Rosemarie Trenker-El-Toukhy

General description

The fundamental questions in the study of enzymes, the bio-catalysts of all living organisms, revolve around their ability to select a substrate (substrate specificity) and subject this substrate to a predetermined chemical reaction (reaction and regio-specificity). In general, only a few amino acid residues in the "active site" of an enzyme are involved in this process and hence provide the key to the processes taking place during enzyme catalysis. Therefore, the focus of our research is to achieve a deeper understanding of the functional role of amino acids in the active site of enzymes with regard to substrate-recognition and stereo- and regiospecificity of the chemical transformation. In addition, we are also interested in substrate-triggered conformational changes and how enzymes utilize cofactors (flavin, nicotinamide) 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 flavinmononucleotide ($FMNH_2$), 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* also carry an extra gene, termed *luxF*. The main function of LuxF supposedly is the binding of 6-(3'-(R)-myristyl-FMN (myrFMN), a possible side product of the luciferase reaction. MyrFMN is also thought to bind sufficiently tight in the active site of luciferase, thus leading to inhibition of the bioluminescent reaction.

To test these hypotheses, we first developed a method to isolate myrFMN from *Photobacterium leiognathi* S1. Isolated and purified myrFMN was then used to determine its binding affinity to recombinantly produced apo-LuxF and luciferase. We exploited the tight binding of myrFMN to recombinant apo-LuxF to explore the occurrence of myrFMN in various bioluminescent bacterial strains in the genera *Photobacterium* (*luxF*$^+$ and *luxF*$^-$, *Aliivibrio* and *Vibrio*. These findings suggested that myrFMN generation is not only restricted to the genus *Photobacterium* and, moreover, it is independent of the occurrence of *luxF*. Furthermore, we were successful in replicating the bioluminescence reaction *in-vitro* by using the enzyme luciferase from various genera with abundant substrate and a cofactor recycling system under aerobic conditions. In various multiple turnover reactions the formation of myrFMN was confirmed via HPLC analysis with UV/Vis and MS detection. We successfully demonstrated the generation of myrFMN by comparing the myrFMN reference of *Photobacterium leiognathi* S1 with the isolated and purified myrFMN sample of the *in vitro* reaction. Currently, we are attempting to better understand the correlation between total light emission and the amount of myrFMN formed in bioluminescent bacteria. Toward this end, the *luxF* gene from photobacteria will be introduced into the operon of various *Aliivibrio* and *Vibrio* strains to investigate the impact of the myrFMN scavenging LuxF on light emission. Furthermore, the crystal structure of various luciferases in complex with FMN/$FMNH_2$ is of high interest to better understand the mechanism of bacterial bioluminescence (thesis project of Chaitanya Tabib and 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 by NADPH. Several artificial electron acceptors are capable of oxidizing the reduced FAD, however disulfide, such as cysteine, glutathione or lipoamide were inactive. Taken together our findings suggest that Irc15p is efficiently reduced in yeast cells to deliver electrons to an as yet unidentified electron acceptor that is related to Irc15p's function in regulating microtubule dynamics and cell cycle progression (thesis project of Karin Koch).

Berberine bridge enzyme-like enzymes in plants

Berberine bridge enzyme (BBE) is a central enzyme in the oxidation of the *N*-methyl group of (*S*)-reticuline with concomitant formation of a carbon-carbon bond (the "berberine bridge") to yield (*S*)-scoulerine. Using bioinformatics we found that homologs of BBE are widespread among plants, fungi and bacteria. The model plant *Arabidopsis thaliana*, for example, possesses 28 genes that apparently encode BBE-like enzymes although the plant does not entertain alkaloid biosynthesis. We have recombinantly produced BBE-like homologs, *At*BBE-like proteins 13 and 15, from *A. thaliana* in *Komagataella phaffii* and identified monolignols and their glycosylated derivatives as potential substrates. We have solved the X-ray structure of *At*BBE-like 15 and the topology was found to be very similar to that of the BBE from *Eschscholzia californica* previously resolved by Dr. Andreas Winkler and Prof. Karl Gruber. However, the residues that form the active site are distinct from those found in BBE from *E. californica*. The amino acid residues apparently involved in the oxidation of monolignols to the corresponding aldehydes are currently under investigation by a site-directed mutagenesis program (project of Dr. Bastian Daniel). The active site architecture of the monolignol oxidoreductase is conserved in approximately half of the BBE-like enzymes suggesting that this reaction plays an important role in plant metabolism. To further unveil the role of BBE-like enzymes we have teamed up with Dr. Alexandra Jammer from the Institute of Plant Sciences led by Professor Dr. Maria Müller at the University of Graz in order to investigate the phenotypes of *Atbbe* gene knock-out plants (thesis project of Barbara Steiner).

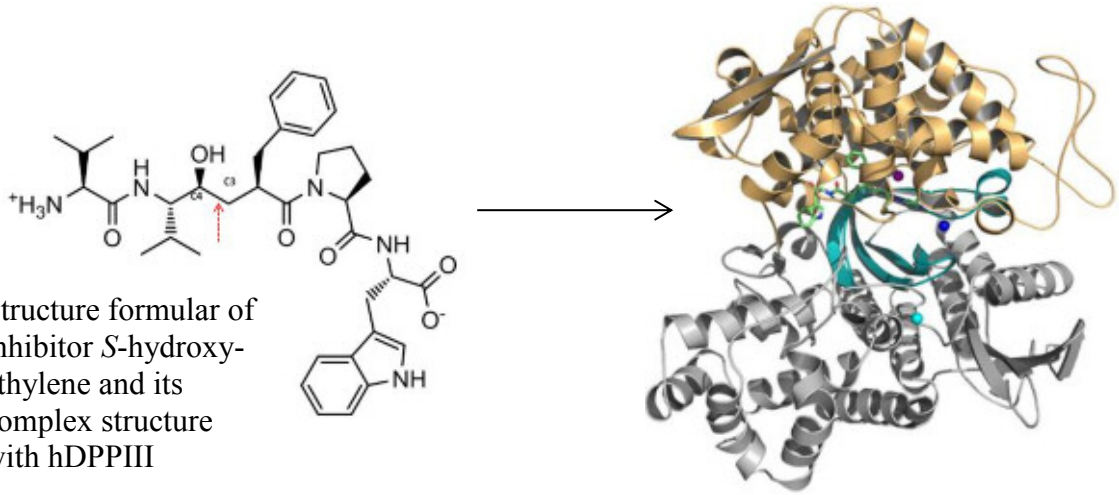
In contrast to *Arabidopsis thaliana*, the genome of the model moss *Physcomitrella patens* encodes a single BBE-like enzyme. As of now, the role of this particular protein is unknown; we have initiated a research program to elucidate the biochemical properties of this enzyme, such as substrate specificity and the type of chemical reaction catalyzed, as this will provide insight into the early functional role of the BBE-like enzyme superfamily in plants. In cooperation with the research group of Prof. Dr. Ralf Reski from the Department of Plant Biotechnology at the Albert-Ludwigs-University Freiburg we have generated a knock-out plant, which is currently characterized in his laboratory. In our team, we currently focus on the biochemical characterization of recombinant *Pp*BBE and have initiated a site-directed mutagenesis program to investigate the role of amino acid residues in the active site of the enzyme (thesis project of Marina Toplak).

Human dipeptidyl peptidase III

Dipeptidyl peptidases III (DPPIII) are zinc-dependent metalloenzymes involved in degrading tetra- to dodecapeptides. The human DPPIII (hDPPIII) 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. However, the precise

functions of hDPPIII are still ill defined. To better understand the physiological roles of the enzyme, we teamed up with the group of Prof. Robert Zimmermann from the University of Graz to generate and characterize a DPPIII gene knock-out mouse model (DPP3KO). In addition, pseudopeptide inhibitors of hDPPIII were developed in collaboration with Profs. Gruber (University of Graz) and Breinbauer (TUG) to exploit this enzyme as a potential drug target for pain intervention strategies. Furthermore, comparison of angiotensin levels in tissue extracts and plasma of wild type and knock-out mice was conducted by our industry partner, Attoquant Diagnostics in Vienna.

Our studies revealed that there are major metabolic differences in the DPP3KO in terms of body weight, feeding behavior and activity. Measurement of angiotensin levels in kidney tissue homogenates and serum showed significantly lower levels of angiotensin I in the DPP3KO mice. Angiotensin II, which is the main metabolite of the renin-angiotensin-system, is also altered in the knockouts, albeit not to a significant level, thus demonstrating that DPPIII has an influence on cardiovascular functions. Kinetic characterization of the inhibitors synthesized in Prof. Breinbauer's group yielded two compounds, which stably inhibit DPPIII activity (below the structure of the inhibitor *S*-hydroxyethylene in complex with hDPPIII is shown). This is a starting point for the design of molecular tools specific for inhibiting hDPPIII as an alternative approach to conventional treatments in the nociceptive filed. Currently, we are investigating the underlying mechanisms involved in the regulation of these physiological functions by hDPPIII.



Structure formular of inhibitor *S*-hydroxy-ethylene and its complex structure with hDPPIII

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

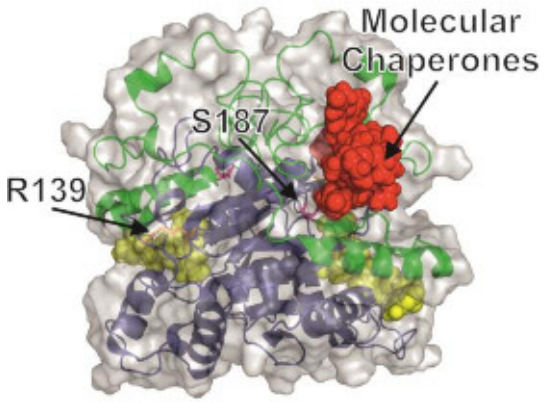
dehydrogenase (hDMGDH) and detected that the affinity to hDMGDH was substantially enhanced for hETF harboring the formylated cofacter. In order to investigate a potential role of 8fFAD in mitochondrial electron handling, we are now investigating the interaction of formylated and unformylated hETF with 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 and Sami Ullah Khan).

Human quinone reductase NQO1

The human NAD(P)H:quinone oxidoreductase 1 (NQO1) is a FAD dependent enzyme catalysing the two-electron reduction of quinones to hydroquinones. NQO1 binds to the 20*S* proteasome and recruits important tumour suppressor proteins such as p53 and p73α. Interestingly, the *NQO1* gene is polymorphic and the two most common variants, next to the wild-type, 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.

Currently, we are investigating the potential of molecular chaperones to stabilise the structure in the NQO1 P187S variant 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).

***S*-Adenosyl-methionine-dependent methyltransferases**

Methylation is a biologically important process that affects the properties of various molecules such as nucleic acids and proteins as well as a plethora of small molecules. In many of these methylation reactions *S*-adenosylmethionone (SAM) serves as a methyl group donor. In our group we are interested in the dimethylation of 8-amino-riboflaivn to the *N*,*N*-

dimethylamino derivative, known as roseoflavin, catalyzed by SAM-dependent methyltransferase RosA. This antibacterial compound is produced by *Streptomyces davavensis* in an as yet unknown biosynthetic pathway (collaboration with prof. Mack from the Mannheim University in Germany). In collaboration with Dr. Michael Uhl and Prof. Karl Gruber the X-ray structure of RosA was solved and the reaction was characterized biochemically by Chanakan Tonsook (see publication #2).

Currently, we are also interested in two human methyltransferases, methyltransferase-like enzyme 23 (METTL23) and histamine *N*-methyltransferase (HNMT) associated with inborn intellectual disability (ID) in collaboration with Prof. Christian Windpassinger from the Medical University Graz and Prof. Vincent John from the Centre for Addiction and Mental Health in Toronto, Canada). At present the physiological function and enzymatic properties of METTL23 are unknown and attempts are under way to identify potential substrates in order to better understand its involvement in human disease. On the other hand, the function and structure HNMT is well known, however, the finding that certain mutations in the human gene are associated with ID was surprising. Chanakan Tongsook (PhD thesis) in our laboratory has studied two point mutations discovered in human patients suffering from ID. Both point mutations lead to single amino acid exchanges, one in the active site and the other in a helical domain of the protein. Chanakan has generated both protein variants and characterized these in terms of their thermal stability, substrate binding propensities and catalytic properties. Her results clearly demonstrate that both variants are severely compromised rationalizing the observed phenotype in human subjects (see publication #5).

Doctoral Theses completed

Chaitanya Tabib: Biochemical studies on the mechanism of bacterial bioluminescence *in vivo* and *in vitro*

Bioluminescence is the production of light by living organisms using enzyme-catalyzed reactions as a key factor to release the energy. Generally, the bioluminescent reaction in bacteria is catalysed by a luciferase, an enzyme employing FMN as a redox cofactor to drive the mono-oxygenation of an aldehyde substrate to its corresponding acid product. The free energy released during the oxidation of the aldehyde gives rise to an excited state FMN-4a-hydroxide, which in turn serves as the light emitting molecule luciferin. The genes responsible for the light production are present as an operon, luxCDABEG: luxA and luxB encode the α and β subunits of luciferase; luxC, luxD, and luxE specify the enzymatic components of a fatty acid reductase complex; and luxG encodes a flavin reductase. Many strains of Photobacteria also carry an extra gene, termed luxF, having a lux operon gene order of luxCDABFEG. Sequence similarity to luxB suggests that luxF has evolved by gene duplication, however, its role in bacterial bioluminescence is obscure. The hypothesized function of LuxF is to bind 6-(3'-(R)-myristyl)-FMN (myrFMN, a possible side product of the luciferase reaction), which otherwise is thought to bind the active site of luciferase sufficiently tight thus leading to inhibition of the bioluminescence reaction. The generation of myrFMN in the Photobacterium is a largely unexplored phenomenon. In the present study, we have developed a method to isolate myrFMN from *Photobacterium leiognathi* S1. Using isolated and purified myrFMN we could show that binding to apo-LuxF (Kd = 80 nM) was fifty times tighter than to luciferase (Kd = 4 μM) by using isothermal titration calorimetry. In addition, we exploited this tight binding of myrFMN to recombinant apo-LuxF, to explore the occurrence of myrFMN in various bioluminescent bacterial strains (luxF+ and luxF-) in

Photobacterium. This analysis showed that myrFMN is present in all photobacterial strains tested, suggesting that myrFMN production is independent of the occurrence of luxF. Similarly, finding of trace amounts of myrFMN in Vibrio and *Aliivibrio* indicates that myrFMN generation is not restricted only to Photobacterium. To study the effect of myrFMN on the bioluminescence yield, an inhibition assay was performed using single turnover reactions. With increasing myrFMN concentration the total light yield went down dramatically. Addition of LuxF helped in scavenging the myrFMN to substantial levels bringing back the lost activity of luciferase, thereby confirming our hypothesis that LuxF serves as a scavenger of myrFMN in bioluminescent bacteria. The creation of luxF, presumably by gene duplication of luxB, was an important evolutionary invention that provided an enormous advantage over other bioluminescent bacteria. Finally, in order to investigate the formation of myrFMN and to analyze the role of luciferase and LuxF in this process, we established a cofactor regeneration enzyme-catalyzed cascade reaction that supports the luciferase reaction for up to 72 hours. This approach enabled to unambiguously demonstrate by UV-Vis absorption spectroscopy and mass spectrometry that myrFMN is generated in the bacterial bioluminescent reaction. Based on this finding we have postulated a reaction mechanism for myrFMN generation that is compatible with the proposed radical mechanism for the luciferase reaction (CIEEL mechanism).

Chanakan Tongsook: Biochemical characterization and kinetic studies on S-adenosylmethionine-dependent methyltransferases

Methyltransferases catalyze methylation processes involved in metabolism, signal transduction, protein/DNA repair and biosynthesis. *S*-Adenosylmethionine (SAM) is the major biological methyl donor in reactions catalyzed by methyltransferases. Several human SAM-dependent methyltransferases in genes encoding polymorphisms are connected to inherited diseases. Moreover, many small-molecule SAM-dependent methyltransferases are applied for industrial bioprocessing, i.e. biosynthesis of antibiotics. This work focuses on the functional and structural characteristics of two SAM-dependent methyltransferases and their genetic polymorphisms linked to intellectual disability and on a small-molecular SAM-dependent methyltransferase essential for antibiotic biosynthesis. Histamine N-methyltransferase (HNMT) catalyzes the degradation of histamine by transferring a methyl group from SAM to histamine. HNMT is critically important for the maintenance of neurological processes. Recently, two mutations in the encoding human gene were reported to give rise to dysfunctional protein variants (G60D and L208P) leading to intellectual disability. Determination of biochemical and structural properties of the wild-type and variants were conducted and we confirmed that G60D disrupts the SAM binding site rendering the variant catalytically inactive. On the other hand, the L208P variant showed reduced protein stability. This finding rationalizes the loss of enzymatic activity observed in the L208 variants. METTL23 is a putative SAM-dependent methyltransferase. Nonsense mutation and a 5 bp frame shift deletion lead to truncation of the putative METTL23 protein disrupting the predicted catalytic domain and altering the cellular localization. Expression analysis of METTL23 indicated a strong association with heat shock proteins, which suggests that these may act as putative substrates for methylation by METTL23. Disruption of METTL23 presented here supports the importance of methylation processes for intact neuronal function and brain development. N,N-8-demethly-8-amino-d-ribolfavin demethyltransferase (RosA) catalyzes the final dimethlyation of 8-demethyl-8-amino-d-rioflavin (AF) the antibiotic roseoflavin (Roof) in *Streptomyces. davawensis*. In the present study, we have solved the X-ray structure of RosA, and determined its biochemical properties. The structure of RosA is similar to that of previously described SAM-dependent methyltransferase featuring two domains: a mainly a-helical “orthogonal bundle” and a Rossmann-like domain. Both

substrates, AF and SAM, bind independently to their respective binding pocket. This finding was confirmed by kinetic experiments that demonstrated a random-order 'bi-bi' reaction mechanism. Both products, RoF and S-adenosylhomocyseine (SAH, bind more tightly to RosA compared to the substrates, AF and SAM. This suggests that RosA may contribute to reseoflavin resistance in *S. davawensis*. The tighter binding of products is also reflected by the results of inhibition experiments, in which RoF and SAH behave as competitive inhibitors of AF and SAM, respectively. We also showed that formation of a ternary complex of RosA, RoF and SAH (or Sam) leads to drastic spectral changes that are indicative of a hydrophobic environment.

International cooperations

Catherine Lapierre, Institut National de la Recherche Agronomique (INRA), Paris, France
Matthias Mack, Hochschule Mannheim, Germany
Ralf Reski, Albert-Ludwigs University, Freiburg, Germany
Thomas Roitsch and Eric van der Graaff, Copenhagen University, Denmark
John Vincent, Centre for Addiction and Mental Health, Toronto, Canada

Research projects

FWF P22361: "Mechanism of redox controlled protein degradation"
FWF P24189: "Bacterial bioluminescence"
FWF P26341: "The family of berberine bridge enzymes in plants"
FWF-PhD program "Molecular Enzymology" DK-Molecular Enzymology (W901)

Invited Lectures

1) Macheroux, P.: Bacterial bioluminescence: the mystery of myristylated flavins. Hochschule Mannheim, Germany, 15.04.2016

2) Daniel, B.: The family of berberine bridge enzyme-like proteins from *Arabidopsis thaliana*. Oxizymes, Wageningen, The Netherlands, 3.7.-6.7.2016

3) Jha, S.: Towards low-risk painkillers: human dipeptidyl peptidase III (hDPPIII) as a novel target for therapeutic pain intervention. XXIV EFMC International Symposium on Medicinal Chemistry**,** Manchester, UK, 28.8.-1.9.2016

4) Strandback, E.: On the way to rescue the stability and activity of a cancer associated variant of human NQO1. Joint Meeting of DK "Molecular Enzymology" and "Biomolecular Technology of Proteins" at Grand Hotel Panhans, Semmering, 29.9.-30.9.2016

Publications

1. Kumar, P., Reithofer, V., Reisinger, M., Wallner, S., Pavkov-Keller, T., **Macheroux, P.**, Gruber, K.: Substrate complexes of human dipeptidyl peptidase III reveal the mechanism of enzyme inhibition, Sci. Rep., 2016, 6:23787; DOI: 10.1038/srep23787.

2. Tongsook, C., Uhl, M. K., Jankowitsch, F., Mack, M., Gruber, K., **Macheroux, P.**: Structure and mechanism of RosA, the enzyme catalyzing the methylation of 8-demethyl-8-amino-D-riboflavin to the antibiotic roseoflavin, FEBS J., 2016, 283:1531-1549; DOI:10.1111/febs.13690.

3. Daniel, B., Wallner, S., Steiner, B., Oberdorfer, G., Kumar, P., Sensen, C., van Graaff, E., Roitsch, T., Gruber, K., **Macheroux, P.**: Structure of a berberine bridge enzyme-like enzyme with an active site specific to the plant family of *Brassicaceae*, PLoS One, 2016, 11:e0156892; DOI:10.1371/journal.pone.0156892.

4. Augustin, P., Hromic, A., Pavkov-Keller, T., Gruber, K., **Macheroux, P.**: Structure and biochemical properties of recombinant human dimethylglycine dehydrogenase and comparison to the disease-related H109R variant, FEBS J, 2016, 283:3587-3603; DOI:10.1111/febs.13828.

5. 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. Bas. Dis., 2017, 1863:188-199; DOI:10.1016/j.bbadis.2016.10.005.

6. Aigner, M., Kalcher, K., **Macheroux, P.**, Lienhart, W.-D., Wallner, S., Edmondson, D., Ortner, A.: Determination of total monoamines in rat brain via nanotubes based human monoamine oxidase B biosensor, Electroanalysis, 2017, *in press*; DOI:10.1002/elan.201600326.

7. Pils, S., Schnabl, K., Fuchs, M., Rocha, R., Wallner, S., Kljajic, M., Kupresanin, N., Breinbauer, R., Schrittwieser, J., Kroutil, W., Daniel, B., **Macheroux, P.**: Characterization of a monolignol oxidoreductase *At*BBE-like protein 15 L182V for biocatalytic applications, J. Mol. Cat. B: Enzymatic, 2017, *in press*.; DOI:10.1016/j.molcatb.2016.10.018.

8. Brodl, E., Ivkovic, J., Tabib, C., Breinbauer, R., **Macheroux, P.**: Synthesis of α, β-unsaturated aldehydes as potential substrates for bacterial luciferases, Bioorg. Med. Chem., 2017, *in press;* DOI:10.1016/j.bmc.2017.01.013.

Awards

Peter Augustin won the best oral presentation prize at the 15th DocDay on February 5th held by the NAWI Graz Doctoral School for Molecular Biosciences.

Barbara Steiner won the best poster prize at the 16th DocDay on July 7th held by the NAWI Graz Doctoral School for Molecular Biosciences.

Photoreceptor Group

Group leader: Andreas Winkler
PhD students: Stefan Etzl (since June 2015), Geoffrey Gourinchas (since August 2015)
Bachelor student: Uršula Vide (since June 2016)
Technician: Elfriede Zenzmaier (since November 2015)

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 1). 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.

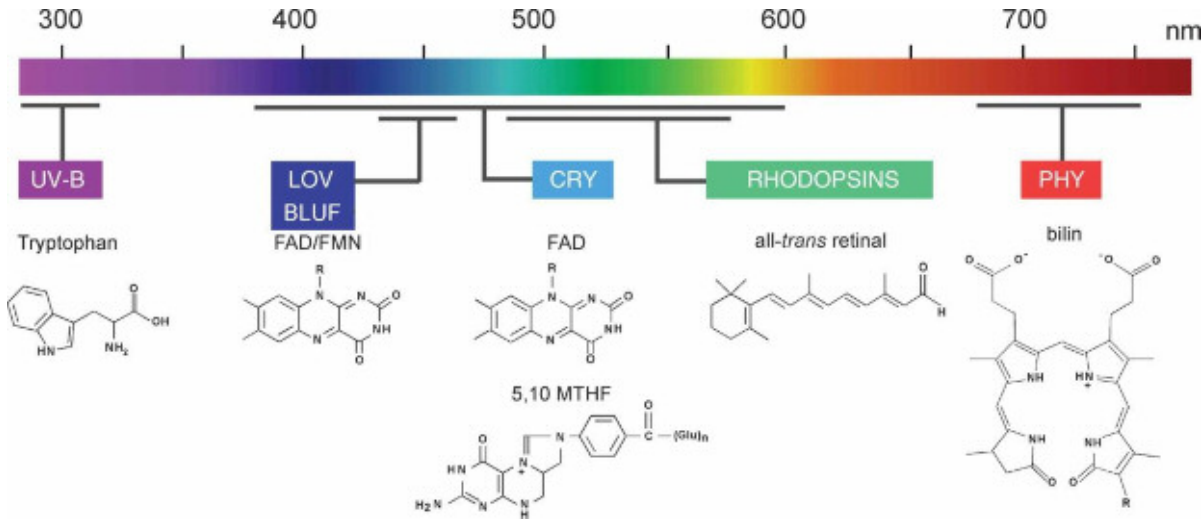


Fig. 1.: Overview of photoreceptor families and their corresponding cofactors covering the UV/Vis range of the electromagnetic spectrum (adapted from Heintzen WIREs Membr Transp Signal 2012, 1:411–432).

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 will be functionally extended by the in-solution method hydrogen-deuterium exchange (HDX, experiments will be 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).

Light-regulated diguanylate cyclases

One key approach for the better understanding of sensor-effector coupling is the appreciation of how nature has accomplished its remarkable modularity of sensor-effector combinations. To this end, we will 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 will be important for the identification of functionally relevant structural elements and also for guiding the efficient design of artificial light-regulatable systems. Since diguanylate cyclases are distantly related to adenylate/guanylate cyclases, it will be interesting to compare signal transduction mechanisms between the two protein families (thesis project of Geoffrey Gourinchas).

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”

Invited Lectures

1) Gourinchas, G.: Characterization of long-range signal transduction in red-light modulated diguanylyl cyclases. 8th ÖGMBT meeting, Graz, September 2016.

2) Winkler, A.: Allosteric signaling in light-regulated enzymatic functionalities. 1st Field of Expertise Day Human & Biotechnology, Graz, November 2016.

Publications

1) Wang H., Vilela M., Winkler A., Tarnawski M., Schlichting I., Yumerefendi H., Kuhlman B., Liu R., Danuser G., Hahn KM.: LOVTRAP: An Optogenetic System for Photoinduced Protein Dissociation. Nat. Methods 13 (2016) 755-758.
2) Gourinchas G., Etzl S., Göbl C., Vide U., Madl T., Winkler A.: Long-range Allosteric Signaling in Red Light-Regulated Diguanylyl Cyclases. Sci. Adv. (2016) *accepted*.

Awards

1) Geoffrey Gourinchas received the *Best Poster Award* at the FEBS Advanced Methods in Macromolecular Crystallization VII workshop in Nové Hrady, Czech Republic.

Metalloprotein Group

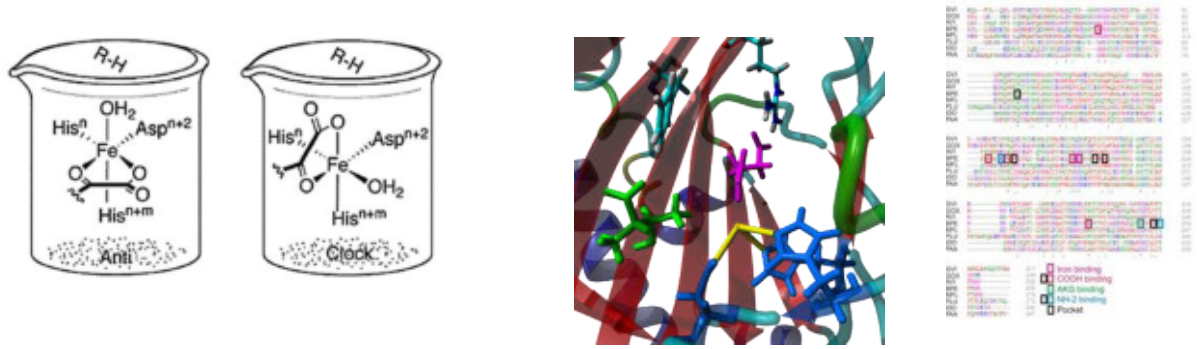
Group leader: Grit D. Straganz
PhD student: Johannes Niederhauser

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 often still not well understood. In depth studies of the catalytic metallocenters and their interaction with their respective substrates and protein environments can help us elucidate the molecular basis of catalysis, in order to fully 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 an enzyme grouping that transforms cell metabolites via oxidative hydroxylation. They all share a cupin fold and a common metal center organization. Yet, particular exponents of this enzyme grouping 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.

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 projects

FWF P26539: "Nonheme Fe(II) Hydoxylases"

Invited Lecture

1) Straganz, G. D.: Enzymatic Mononuclear Nonheme Fe(II) Centers: The Role of the Protein Environment in Tuning O_2 Dependent Catalysis, 2nd Small Molecule Activation Conference, Cancun, Mexico, 18th-25th May, 2016.

Publications:

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 Jan;1863(1):188-199.
2) Pratter, S., Straganz, G. D., (2016) Halogenation of Non-Activated Fatty Acyl Groups by a Trifunctional Non-Heme Fe(II)-Dependent Halogenase. In J. W. Whittall, P. W. Sutton, W. Kroutil, Practical Methods for Biocatalysis and Biotransformations: Volume 3, 204-210, John Wiley & Sons, Inc., Hoboken, NJ, USA.

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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)
Technicians: Claudia Hrastnik (part time), Alma Ljubijankic (part time)
Guest Student: Jennifer Vazquez Gonzalez, Universitat Rovira i Virgili, Food Science, Microbiology, Tarragona, Spain,

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.

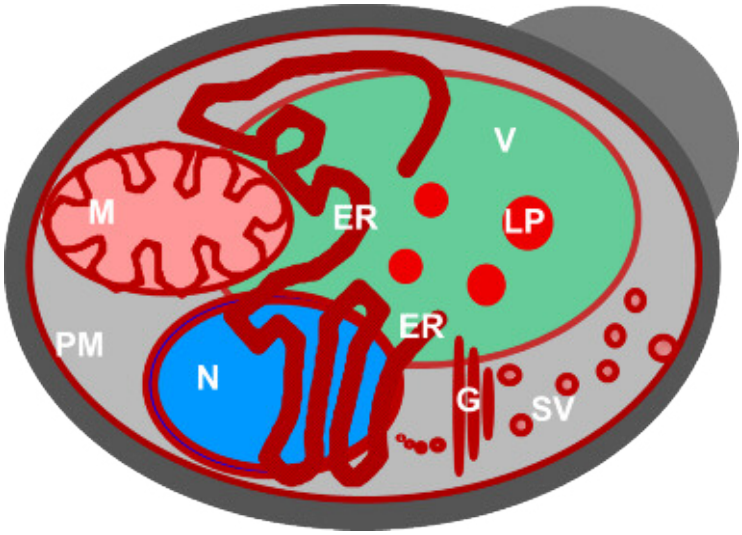


Fig. 1: Yeast organelles: LP, lipid particles/droplets; M, mitochondria; ER, endoplasmic reticulum; N, nucleus; G, Golgi; V, vacuole; PM, plasma membrane; SV, secretory vesicles.

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.

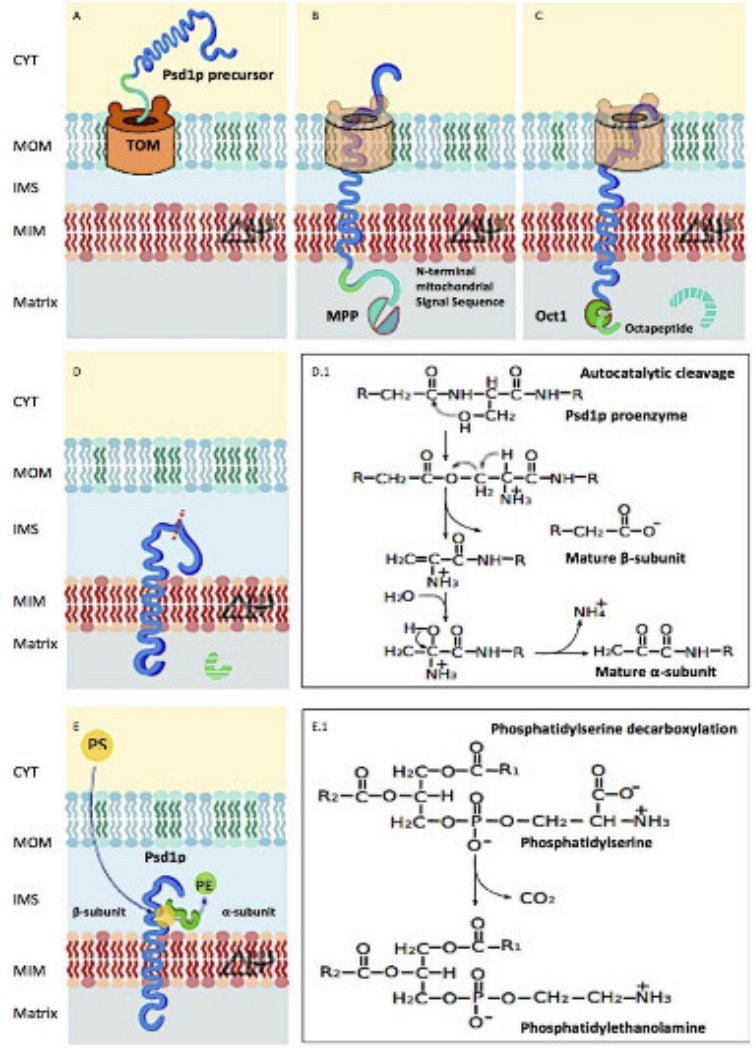


Fig. 2: Import and assembly of phosphatidylserine decarboxylase 1 in mitochondria from the yeast.

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 out 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

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.

Tgl3p, Tgl4p and Tgl5p are the major triacylglycerol lipases of the yeast *S. cerevisiae*. Recently, we demonstrated that properties of the major yeast triacylglycerol lipase Tgl3p were regulated by the formation of non-polar lipids. In a more recent study we extended these investigations to the two other yeast triacylglycerol lipases, Tgl4p and Tgl5p. We showed that Tgl4p and Tgl5p, which are localized to lipid droplets in wild type, were partially retained to the endoplasmic reticulum in cells lacking TG, and localized exclusively to the endoplasmic reticulum in a mutant devoid of lipid droplets. To investigate the regulatory network of yeast triacylglycerol lipases in some more detail, we also examined properties of Tgl3p, Tgl4p and Tgl5p, respectively, in the absence of the other lipases. Surprisingly, lack of two lipases did not affect expression, localization and stability of the remaining Tgl-protein. These results indicate that Tgl3p, Tgl4p and Tgl5p although exhibiting similar functions act as independent entities.

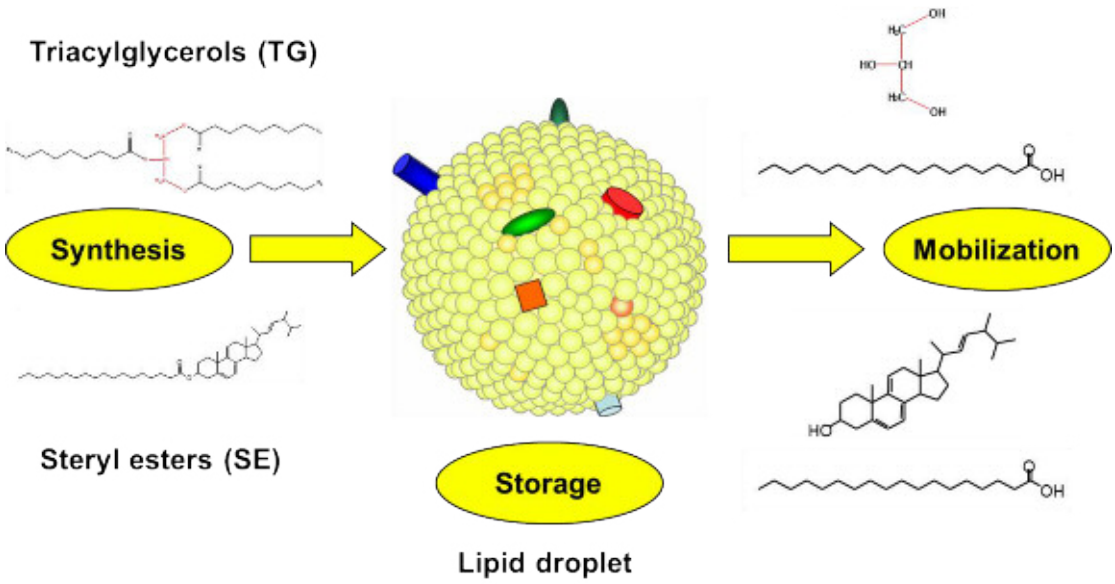


Fig. 3: Life cycle of yeast non-polar lipids

Besides TG, SE are major storage lipids in the yeast. Recently, we extended our studies of 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 are 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.

International cooperations

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

I. Feussner, Department of Plant Biochemistry, Georg-August-University Göttingen, Albrecht-von-Haller-Institute for Plant Sciences, Göttingen, 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. Roier S., Zingl F.G., Cakar F., Durakovic F., Kohl P., Eichmann T.O., Klug L., Gadermaier B., Weinzerl K., Prassl R., Lass A., Daum G., Reidl J., Feldman M. and Schild S.
   A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria
   Nat. Commun. 2016 Jan 25;7:10515. doi: 10.1038/ncomms10515.
2. Klein I., Klug L., Schmidt C., Zandl M., Korber M., Daum G. and Athenstaedt K.
   Regulation of the yeast triacylglycerol lipases Tgl4p and Tgl5p by the presence/absence of nonpolar lipids.
   Mol Biol Cell. 27 (2016) 2014-2024
3. Schuler M.-H., Di Bartolomeo, F, Mårtensson, C. U., Daum, G. and Becker, T.
   Phosphatidylcholine affects inner membrane protein translocases of mitochondria
   J. Biol. Chem. 291 (2016) 18718-18729

Award

Francesca Di Bartolomeo received the Best Poster Award at the 57th International Conference on the Bioscience of Lipids (ICBL), Chamonix, Mont-Blanc, France, September 4-8, 2016

Molecular Biology Group

Group leader: Karin Athenstaedt
Research Associate: Bernadette Kiegerl

Biosynthesis of phosphatidic acid in yeast

Our research is focused on studying the regulation of phosphatidic acid biosynthesis. Phosphatidic acid serves as a precursor for the formation of all glycerophospholipids and triacylglycerols. Glycerophospholipids are major constituents of biomembranes, whereas triacylglycerols are important storage molecules. Beside its central role in glycerolipid metabolism, phosphatidic acid functions in cell signaling. Because of these important functions, the formation of phosphatidic acid has to be tightly adjusted to the cellular requirements.

The first and committed reaction of phosphatidic acid biosynthesis is catalyzed by a glycerol-3-phosphate acyltransferase. This enzyme links an activated fatty acid to the *sn-1* position of the precursor glycerol-3-phosphate yielding 1-acyl glycerol-3-phosphate (lyso-phosphatidic acid). In the following, lyso-phosphatidic acid is converted to phosphatidic acid by another acylation reaction which is mediated by a 1-acyl glycerol-3-phosphate acyltransferase (Fig. 1).

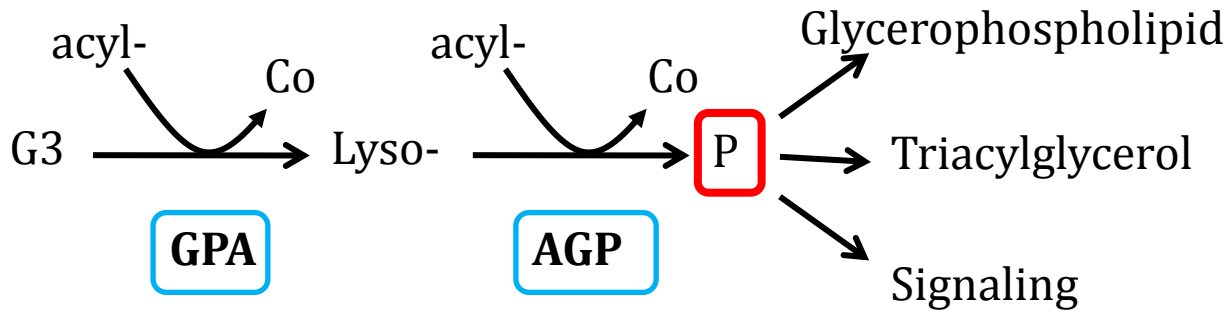


Figure 1: *De novo* synthesis of phosphatidic acid and its central role in cell metabolism. AGPAT: 1-acyl glycerol-3-phosphate acyltransferase; G3P: glycerol-3-phosphate; GPAT: glycerol-3-phosphate acyltransferase; PA: phosphatidic acid.

In all eukaryotic organisms at least two glycerol-3-phosphate acyltransferases and 1-acyl glycerol-3-phosphate acyltransferases exist. It is speculated that these enzymes with overlapping functions synthesize different pools of phosphatidic acid which serve in different pathways. In our studies we examine this hypothesis by using the budding yeast *Saccharomyces cerevisiae* as our model system.

In *Saccharomyces cerevisiae* two glycerol-3-phosphate acyltransferases, Sct1p (Gat2p) and Gpt2p (Gat1p), catalyze the first and rate determining reaction of phosphatidic acid biosynthesis. Both enzymes are modified by phosphorylation, and localize to the endoplasmic reticulum. In addition, Gpt2p is associated with lipid droplets, cell compartments destined for

the storage of nonpolar lipids. Most interestingly, in the absence of the respective counterpart the distribution pattern as well as the phosphorylation status of either glycerol-3-phosphate acyltransferase is altered. Similarly, the degree of phosphorylation of both acyltransferases changes during cell growth. These observations suggest that phosphorylation regulates and coordinates the contribution of both glycerol-3-phosphate acyltransferases to phosphatidic acid biosynthesis. Currently, we focus on elucidating the meaning of phosphorylation at individual phosphorylation sites of the glycerol-3-phosphate acyltransferases.

International Cooperation

V. Zaremberg, Department of Biological Sciences, University of Calgary, Canada

Research project

FWF P26308: Regulatory aspects of phosphatidic acid biosynthesis in yeast

Invited Lectures

1) Athenstaedt K., Nagler B., Shabits B., Tavassoli M., and Zaremberg V.
Regulation of phosphatidic acid biosynthesis via phosphorylation of the glycerol-3-phosphate acyltransferase Gpt2/Gat1 in the yeast.
Young Investigators in Lipid Science
Düsseldorf, Germany, May 10-11, 2016

Publications

1) Klein I., Klug L., Schmidt C., Zandl M., Korber M., Daum G. and Athenstaedt K.
Regulation of the yeast triacylglycerol lipases Tgl4p and Tgl5p by the presence/absence of non polar lipids.
Mol. Biol. Cell 27 (2016) 2014-2024.

2) Athenstaedt K.
Isolation and characterization of lipid droplets from yeast.
In Hydrocarbon and Lipid Microbiology Protocols, Springer Protocols Handbooks (eds T.J. McGenity et al.), Springer-Verlag Berlin Heidelberg (2016) 81-91.

3) Athenstaedt K.
Nonpolar lipids in yeast: synthesis, storage, and degradation.
In Handbook of Hydrocarbon and Lipid Microbiology (ed. O. Geiger), Springer International Publishing AG, accepted.

4) Athenstaedt K.
Players in the nonpolar lipid game: proteins involved in nonpolar lipid metabolism in yeast.
In Handbook of Hydrocarbon and Lipid Microbiology (ed. O. Geiger), Springer International Publishing AG, accepted.

Cellular Metabolism Group

Group leader: Juliane Bogner-Strauss
Postdoctoral fellow: Ariane Pessentheiner
PhD students: Helmut Pelzmann, Katharina Huber, Dina Hofer, Wenmin Xia, Furkan Alkan
Master students: Bernhard Gadermaier
Technician/Adminsitration: Florian Stöger (part time), Su Poldrack(part time), Claudia Gaug (part time), Thomas Schreiner
Apprentice: Wolfgang Krispel

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 and their energetic requirements.

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.

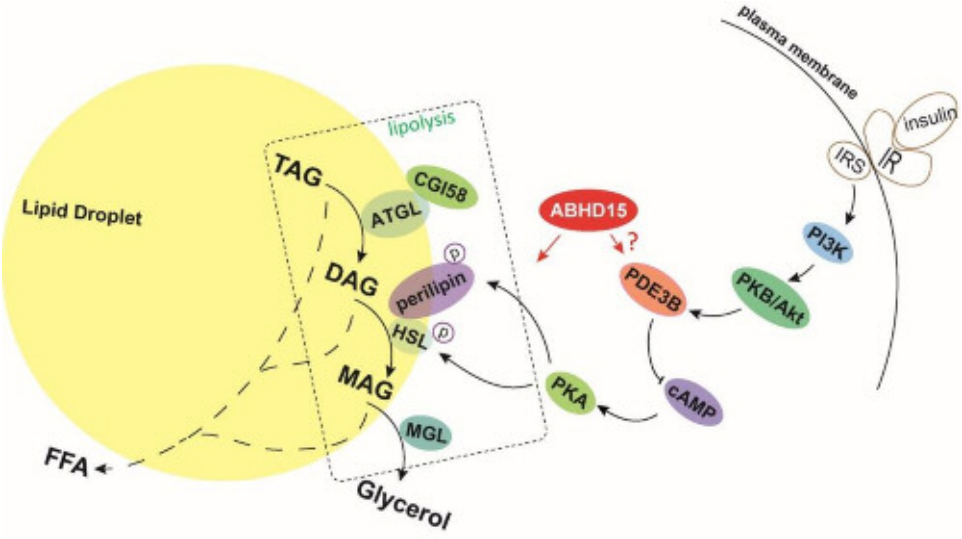
Aralar (AGC1)

Aralar is a glutamate/aspartate carrier that transports glutamate into the mitochondria in exchange of aspartate. As aspartate is required for nucleotide and amino acid synthesis and could thus play a role in proliferation, we hypothesized that AGC1 might play a role in cancer. Furkan Alkan is working on this project. Modulating the amount of Aralar expression

by either silencing or overexpression influences the proliferation in various (cancer) cell lines and xenograft models reveal that the expression of Aralar impacts tumor growth. Currently, metabolite flux analysis is used to delineate the mechanism(s) behind.

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 mainly 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 do 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).

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 Thesis completed

Bernhard Gadermaier: Characterization of Adipocyte Plasma Membrane-Associated Protein (APMAP) regarding regulation and enzymatic function in fat cells. Bernhard Gadermaier investigated the possible physiological regulation of APMAP *in vitro* and *in vivo* by diverse stimuli and triggers.

Doctoral Thesis completed

Helmut Pelzmann: Characterization of the N-acetylaspartate pathway in adipocyte metabolism in vitro and in vivo.
Helmut Pelzmann worked on the characterization of the Nat8l-ko mice. In addition, he investigated the function of aspartoacylase in brown adipocytes *in vitro*.

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-Doktoratskolleg: “Metabolic and Cardiovascular Disease”
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

Talks at international conferences

1) H. Furkan Alkan
The role of an asp/glu carrier in cancer cell proliferation and cancer growth. ÖGMBT meeting, September 2016, Graz, Austria

Publications

1) Hofer DC, Pessentheiner AR, Pelzmann HJ, Schlager S, Madreiter-Sokolowski CT, Kolb D, Eichmann TO, Rechberger G, Bilban M, Graier WF, Kratky D, Bogner-Strauss JG. Critical role of the peroxisomal protein PEX16 in white adipocyte development and lipid homeostasis. Biochim Biophys Acta. 2016 Dec 23;1862(3):358-368. doi: 10.1016/j.bbalip.2016.12.009. [Epub ahead of print]

2) Prokesch A, Graef FA, Madl T, Kahlhofer J, Heidenreich S, Schumann A, Moyschewitz E, Pristoynik P, Blaschitz A, Knauer M, Muenzner M, Bogner-Strauss JG, Dohr G, Schulz TJ, Schupp M. Liver p53 is stabilized upon starvation and required for amino acid catabolism and gluconeogenesis. FASEB J. 2016 Nov 3. pii: fj.201600845R. [Epub ahead of print]

3) Prokesch A, Pelzmann HJ, Pessentheiner AR, Huber K, Madreiter-Sokolowski CT, Drougard A, Schittmayer M, Kolb D, Magnes C, Trausinger G, Graier WF, Birner-Gruenberger R, Pospisilik JA, Bogner-Strauss JG. N-acetylaspartate catabolism determines cytosolic acetyl-CoA levels and histone acetylation in brown adipocytes. Sci Rep. 2016 Apr 5;6:23723. doi: 10.1038/srep23723.

Awards

Wenmin Xia received the “Best Poster Award” at the Doctoral Day NAWI Graz, Austria

H. Furkan Alkan received the Marshall Plan scholarship for his one year stay abroad in the lab of Matthew Vander Heiden. MIT, USA.

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Group Chemistry of Functional Foods

Group leader: Michael Murkovic
PhD students: Marini Damanik, Sandra Holzer, Nicole Pabi
Diploma students: Verena Buchgraber, Laura Sonnleitner, Christopher Hartl, Maria Hulla, Christoph Provasnek, Tamara De Zuani, Carmen Mößlacher, Koller Katrin, Alma Cehic, Florina Vorauer, Sandra Tschurnig, Manuela Schratzer, Daniela Krieg, Christoph Trummer
Technician: Alma Ljubijankic

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.

Master Theses completed

Manuela Schratzer: Characterization of the tanning process in plant tissues after mechanical injury

The aim of the project was to develop a new conservation method which improves flavour, colour, and pulp of the apples. To prevent the browning process, the apples were treated with different chemical methods. First of all the four different kinds of apples were monitored under oxygen and nitrogen conditions while the apples were treated with different chemicals. Especially by using the nitrogen environment, the results of preventing the tanning process

were better than the treatment under oxygen conditions. Probably, the reason for the rate of tanning could be that different types of apples have a different content of polyphenols. Furthermore the contents of polyphenols in treated and untreated apples were analysed by HPLC (High-Performance-Liquid-Chromatography). As it is shown in figures 27, 28 and 29, different percentages of polyphenols were detected in apples. The results of this master thesis show that the conservation of apples is possible by using chemicals. Due to the fact of chemical treatment apples showed a higher content of polyphenols than untreated apples.

Alma Cehic: Stability of Steviosides in Beverages and Emulsions

Consuming large amount of sugars, especially sucrose, has resulted in the increased prevalence of diseases, such as diabetes, obesity, cardiovascular diseases, and dental problems. Therefore, in the last couple of decades, artificial sweeteners are used in large quantities in food industry as sugar substitutes. The artificial sweeteners belong to the class of high-intensity sweetener (HIS). They are many times sweeter then sucrose and have reduced calorie content. The sweeteners such as aspartame, neotame, acesulfame-K, saccharin and sucralose contain very low amount of calories, and are produced synthetically. Therefore, scientists are developing natural sweeteners as alternative for sucrose, but with similar properties. In the last couple of years, stevia, ″the sweet herb from Paraguay″, attracts attention of many researchers. Stevia represents a good alternative to sucrose, because it is natural, and 300 times sweeter than sucrose, and a non-caloric sweetener. Steviol glycosides which are found in stevia leaves are responsible for the sweetness of stevia. Today, extracts of stevia leaves, steviol glycosides, are used in some Asian countries and in South America as a natural, low-calorie sweetener, in some products such as soft drinks, yoghurt, soya and others. Some researchers have found that stevia has beneficial effects on human health, including prevention of diabetes, antihypertensive, antihyperglycemic and, non-cariogenic properties. Emulsions are systems which contain at least two liquids that do not mix perfectly. Emulsions are widely used in food, pharmaceutical and cosmetics industry. They are unstable systems, which can be stabilized by adding emulsifier. An emulsifier is a substance which is responsible for emulsion stability and formation of droplet aggregates. To find a good emulsifier which will enable emulsion stability for a long time is a field of active research. In this study, the stability of rebaudioside A, rebaudioside B, rebaudioside D and rubusoside during incubation at different temperatures, pH values and storage times were analyzed. The second task was to observe steviosides behavior in emulsions (milk, oil and espresso). Incubation of the steviosides in citric acid buffer at room temperature and refrigerator temperature showed good stability in pH range 3-6 up to one week of incubation. After one week of incubation decreasing steviosides stability was noticed. Stevioside incubation in phosphoric acid buffer at room temperature and refrigerator temperature showed good stability in pH range 3-5 up to one month incubation. Steviosides are remarkably stable in a pH range 3-5 under thermal treatment up to 95 °C and 50 minutes of incubation. In the experiment about steviosides behavior in emulsions, results showed that steviosides tends to stay in the aqueous phase. In milk and espresso only rebaudioside D could be detected. It is of importance to study more about steviosides behavior in emulsions.

Sandra Tschurnig: Oxidation of polyphenols in hops

Beer is the most consumed alcoholic beverage and contains hops as an essential compound. The prenylated chalcone Xanthohumol (XH) occurs in lupulin glands of the hop plant and obtained much attention in the last decades due to its beneficial and biological effects in human health. During brewing the XH content decreases due to isomerisation reaction which

converts the chalcone into its isomeric form called isoxanthohumol (IXH). However the prenylated flavanone IXH has less positive and healthy effects compared to XH. Not only isomerization reaction decreases the XH content but also fermentation, separation and filtration steps are responsible for this process. Since the advantages of XH are known, the brewing industry researches for suitable production technologies to produce XH-enriched beer. Investigators also look for an appropriate enrichment of XH with the addition of hops in other edibles like for example in herbal tea. Furthermore the hop plant itself is applied in extract capsules as dietary supplement and it is also utilized in the cosmetic industry for several beauty products. The aim of this master thesis was to determine the ratio of hop polyphenols (XH and IXH) in different hop pellet species and beer samples. XH and IXH were also analyzed during wort boiling to show the isomerization reaction which occurs in the brewing process. Finally the maltose and glucose concentrations were determined in boiled wort samples.

Daniela Krieg: Characterization of the stability of home-made fruit juices
The aim of this project was, to analyze the nutrients of different fruit and vegetable juices (oranges, strawberries, apples, grapes, spinach and tomatoes), which were produced by different types of juice extractors. The juices were analyzed immediately after squeezing and after 24 h, stored in the fridge at 4 °C. The stability of the fruit juices, produced using three different technologies, was analyzed during a 24 h period. The three juice extractors have different kinds of setup. The first one works like a centrifuge with sharp knives, the second one is a blender and the third one is an extruder. The concentration of vitamin C, β-carotene and anthocyanins plus the pH-value, the electric conductivity and the concentration of oxygen were analyzed. The total anti-oxidative capacity of the juices and the number of bacterial, yeast and fungal colonies (cfu = colony forming unit) were also tested. The institute of analytical chemistry and food-chemistry analyzed the sensory quality of the produced juices. In summary, it can be said, that all nutrients and the other parameters, expect a few juices, were stable during the storage conditions of 24 hours and 4 °C. There was no ideal juice extractor for all fruits and vegetables, because every machine has its own advantages and disadvantages.

International cooperations

T. Husoy, National Institute of Public Health, Oslo, NO
C. Svendsen, National Institute of Public Health, Oslo, NO
H. Pinheiro, Instituto Superior Tecnico, Lisboa, Portugal
V. Piironen, Department of Applied Chemistry and Microbiology, Helsinki, FI
Z. Cieserova, Food Research Institute, Bratislava, SL
K. Cejpek, VSCHT Prague, CZ
C. Thongkraung, Prince of Songkla University, Hatyai, Thailand
R. Swasti, Atma Jaya University, Yogyakarta, Indonesia
F. Pedreschi, Pontificia Universidad Catolica de Chile, Santiago, Chile

Lectures

1) Murkovic, M.:
   Formation of Furfuryl Alcohol During Roasting of Coffee, EFFOST Conference, Vienna, 2016

2) Murkovic, M.:
Wie sicher sind neue Lebensmittel – in: Jahrestagung der ÖGE Süd, 2016

Publications

1) C. Svendsen, A.H. Høie, J. Alexander, M. Murkovic, T. Husøy
The food processing contaminant glyoxal promotes tumour growth in the multiple intestinal neoplasia (Min) mouse model.
Food and Chemical Toxicology 94 (2016) 197-202.
2) Huffman M.P., Høie A.H., Svendsen C., Brunborg G., Murkovic M., Glatt H., Husøy T; An in vitro study on the genotoxic effect of substituted furans in cells transfected with human metabolizing enzymes: 2,5-dimethylfuran and furfuryl alcohol.
Mutagenesis, 31, 597-602, 2016.

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, Macheroux P, Wallner S
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
MOL.833_1	Project laboratory	9	LU	Team
MOL.855	Molecular physiology	2	VO	Macheroux P
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
648.000	Biomaterials	2	VO	Bogner-Strauß J
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
648.012	Microarray Workshop	1	SE	Bogner-Strauß J, Pessentheiner A
648.013	Advanced cell culture training course II	1	SE	Bogner-Strauß J, Pessentheiner A
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

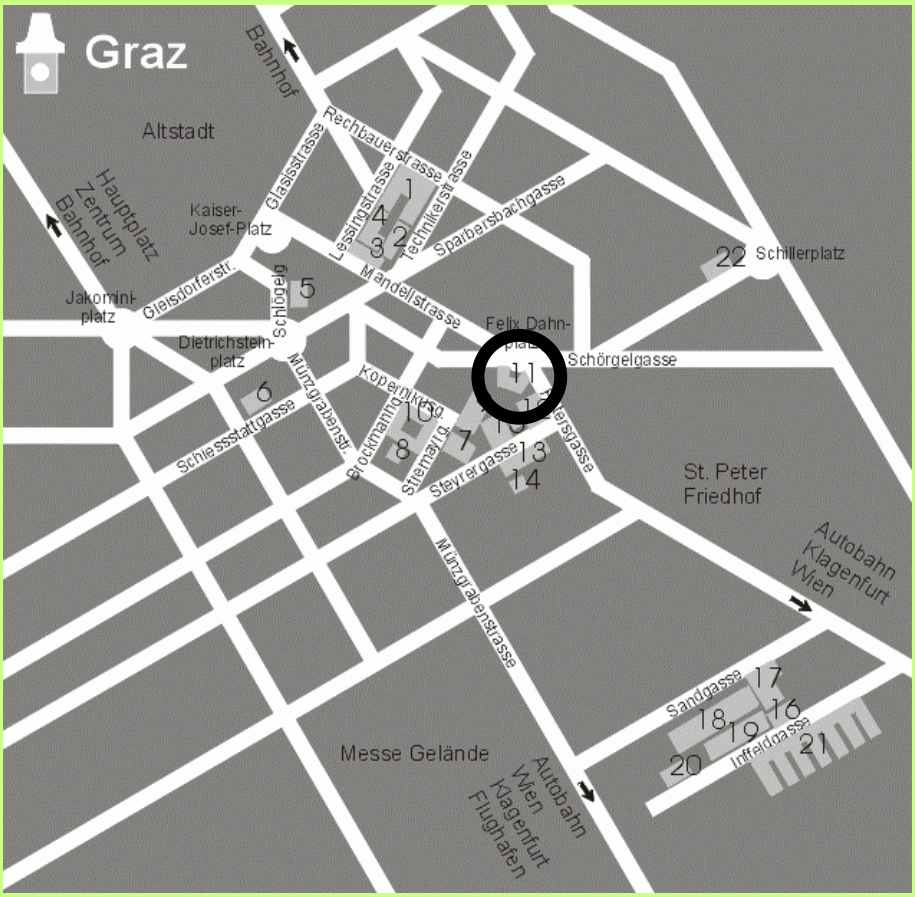
VO: Lecture Course; LU Lab Course; SE Seminar; PV: Privatissimum

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	Steiner B, Wallner S
CHE.194	Seminar for Molecular biology laboratory course	1	SE	Athenstaedt K, Wallner S
CHE.195	Cell Biology	1.5	VO	Becker W, Daum G
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	Becker W, Daum G, Hermetter A, Macheroux P
MOL.880	Mechanistic Enzymology	2	VO	Gruber K, Macheroux P, Nidetzky B
MOL.885	Biophysical Methods	2	VO	Macheroux P
MOL.886	Biophysical Methods - Lab Course	3	LU	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, Wallner S
648.008	Graduate Seminar 2	1	SE	Team
648.010	Scientific Colloquium for Graduate Students 2	1	SE	Team
648.011	Biology, Society and Human Values	2	SE	Becker W
648.012	Microarray Workshop	1	SE	Bogner-Strauß J
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	Becker W, 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.
Stiftingtal-
straße
Steyrergasse
Schiesstattgasse
Münzgrabenstrasse
St. Peter
Friedhof
Autobahn
Klagenfurt
Wien
Sandgasse
Inffeldgasse
Messe Gelände
Autobahn
Wien
Klagenfurt
Flughafen
22 Schillerplatz


A9
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VERTEILERKREIS
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GRAZ WEST
FLUGHAFEN
GRAZ - THALERHOF