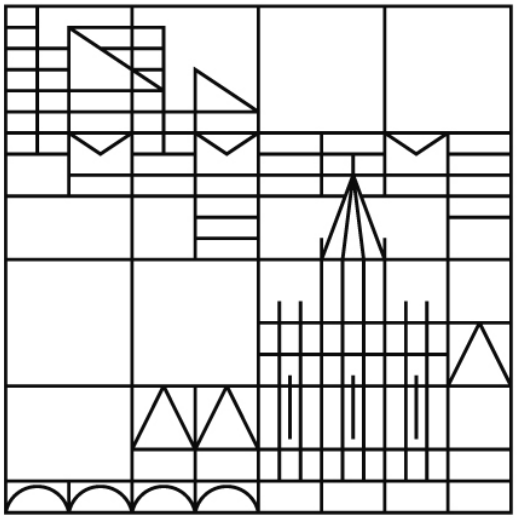
Universität Konstanz



Department of Biology

Advanced / Intensive Courses within the Master Program of the Department of Biology

Department of Biology
Departmental Officer:
Dr. Roland Kissmehl
Tel.: 0049-7531- 88-2414
Fax: 0049-7531- 88-2966
E-mail: pruefungsverwaltung.bio@uni-konstanz.de
FB-Referent.Bio@uni-konstanz.de
Information: www.biologie.uni-konstanz.de

September 2023

Contents

Advanced / Intensive Courses 2023/24

Winter term 2023/2024:
1st Half

06.11.23 – 15.12.23	Immunology (Basler, Schmidtke)
06.11.23 – 15.12.23	Advanced Technologies for the Life Sciences (Core Facility Leaders)
06.11.23 – 15.12.23	Collective Animal Behavior (Couzin)
13.11.23 – 22.12.23	Human and Environmental Toxicology (Dietrich)
06.11.23 – 15.12.23	Environmental Genomics (Epp)
06.11.23 – 15.12.23	Applied Bioinformatics for Studying Health and Disease (Gruber)
13.11.23 – 22.12.23	Molecular Genetics: Cell cycle regulation – from mechanisms to disease (T. Mayer)

2nd Half (advanced course will exceed the semester end for one/two weeks**)**

15.01.24 – 23.02.24	Cell Biology – Cell Adhesion and Signal Transduction (Hauck)
15.01.24 – 23.02.24	Novel *in vitro* Methods in Pharmacology & Toxicology (Leist)
08.01.24 – 16.02.24	Bioinformatics and X-Ray Structural Analysis (Mayans)
08.01.24 – 16.02.24	Microbial Ecology (Schleheck)
08.01.24 – 16.02.24	Chemical Ecology (Spiteller)
08.01.24 – 16.02.24	The role of microbes in stress response and resilience of aquatic metaorganisms (Voolstra)

Summer term 2024:
1st Half

02.04.24 – 24.05.24	Going Wild: Behavior and Ecology of Animals (Crofoot, Wikelski, Dechmann, Kalbitzer) includes *field course: Going wild* (Jordan, Safi, Strandburg Peshkin)
15.04.24 – 24.05.24	Theoretical and Experimental Ecology and Evolution (Becks)
15.04.24 – 24.05.24	Biochemical Pharmacology (Brunner)
15.04.24 – 24.05.24	Physiology and Biochemistry of Plants (Isono, Nagel)
15.04.24 – 24.05.24	Physiology, Ecology and Molecular Biology of Algae (Kroth)
15.04.24 – 24.05.24	Molecular Evolutionary Biology (Meyer)
15.04.24 – 24.05.24	Developmental Biology (P. Müller)

2nd Half

03.06.24 – 12.07.24	Systems Toxicology (Amelio, Bürkle)
03.06.24 – 12.07.24	Behavioral Neurobiology (Bahl, Kleineidam and others)
03.06.24 – 12.07.24	Molecular Microbiology and Cell Biology: Chaperone functions in health and disease (Deuerling)
03.06.24 – 12.07.24	Global change ecology and plants (van Kleunen and others)
03.06.24 – 12.07.24	Dynamics of aquatic ecosystems (Peeters, Martinez Cruz)
03.06.24 – 12.07.24	Biochemistry (Scheffner, Stengel)

Advanced / Intensive Courses 2024/25

Winter term 2024/2025:
1st Half

11.11.24 – 20.12.24	Systems Toxicology (Amelio, Bürkle)
04.11.24 – 13.12.24	Immunology (Basler, Schmidtke)
04.11.24 – 13.12.24	Advanced Technologies for the Life Sciences (Core Facility Leaders)
04.11.24 – 13.12.24	Collective Animal Behavior (Couzin)
04.11.24 – 13.12.24	Environmental Genomics (Epp)
04.11.24 – 13.12.24	Applied Bioinformatics for Studying Health and Disease (Gruber)
11.11.24 – 20.12.24	Molecular Genetics: Cell cycle regulation – from mechanisms to disease (T. Mayer)

2nd Half (advanced course will exceed the semester end for one/two weeks**)**

13.01.25 – 21.02.25	Cell Biology – Cell Adhesion and Signal Transduction (Hauck)
13.01.25 – 21.02.25	Novel *in vitro* Methods in Pharmacology & Toxicology (Leist)
07.01.25 – 14.02.25	Bioinformatics and X-Ray Structural Analysis (Mayans)
07.01.25 – 14.02.25	Microbial Ecology (Schleheck)
07.01.25 – 14.02.25	Chemical Ecology (Spiteller)
07.01.25 – 14.02.25	The role of microbes in stress response and resilience of aquatic metaorganisms (Voolstra)

Summer term 2025:
1st Half

07.04.25 – 30.05.25	Going Wild: Behavior and Ecology of Animals (Crofoot, Wikelski, Dechmann, Kalbitzer) includes *field course: Going wild* (Jordan, Safi, Strandburg Peshkin)
22.04.25 – 30.05.25	Theoretical and Experimental Ecology and Evolution (Becks)
22.04.25 – 30.05.25	Biochemical Pharmacology (Brunner)
22.04.25 – 30.05.25	Physiology and Biochemistry of Plants (Isono, Nagel)
22.04.25 – 30.05.25	Physiology, Ecology and Molecular Biology of Algae (Kroth)
22.04.25 – 30.05.25	Molecular Evolutionary Biology (Meyer)
22.04.25 – 30.05.25	Developmental Biology (P. Müller)

2nd Half (with Pentecost break 16.06. – 22.06.25) advanced course without Pentecost break

02.06.25 – 18.07.25	Behavioral Neurobiology (Bahl, Kleineidam and others)
10.06.25 – 18.07.25	Molecular Microbiology and Cell Biology: Chaperone functions in health and disease (Deuerling)
02.06.25 – 18.07.25	Global change ecology and plants (van Kleunen and others)
02.06.25 – 18.07.25	Dynamics of aquatic ecosystems (Peeters, Martinez Cruz)
02.06.25 – 18.07.25	Integrative animal behaviour (Richter)
10.06.25 – 18.07.25	Biochemistry (Scheffner, Stengel)

Advanced Course "**Systems and Molecular Toxicology": System level analyses of Gene – Environment interactions in the toxic response**

Coordinator (in 2024): Prof. Dr. Ivano Amelio together with Prof. Dr. Alexander Bürkle

1. Introduction

Insult arising from environmental, occupational or therapeutic exposure to toxicants is a primary cause of disease and the treatment of these disorders has significant medical, social and economic implications. Human well-being is determined both by genetic factors (G) and environmental influences (E). Both factors have reciprocal interactions (GxE), and these together determine the outcome on human health. For instance, environmental toxicants (lifestyle, chemicals, radiation) affect genes and their expression. Vice versa, the specific genetic settings impact the response to virtually any extrinsic factor. In toxicology and disease biology, this principle implies that eventual harm/damage is not only determined exclusively by exposure to a toxicant, but the toxicity or disease is rather the outcome of a multifactorial equation which considers many levels of GxE interactions. GxE interactions account for example for inter-individual differences in drug side effects, large differences in tumour incidences between groups of smokers and a wide band width of responses to psychological stressors or viral infection.

Increasing evidence indicates that the outcome of exposure to toxicants is the result of large networks of molecular and functional changes, occurring across multiple levels of biological organization as a result of GxE interactions. Systems Toxicology is an approach towards the assessment of potential health risks associated with exposure to toxicants by integrating concepts from the classic toxicology with quantitative analysis at multiple level of biological systems. This Advanced Course will provide an overview of the research conducted in this area by the group and the field.

2. Contents

a. Theoretical part

The theoretical part will provide a comprehensive overview of the research priorities and methodologies of Systems Toxicology, with in-depth focuses in specific areas as examples of GxE in the toxic response. This part will include background lectures, followed by specific scientific seminars on relevant research topics and completed by a set of journal clubs on recent literature in the field. The topic discussed will include:

- Overview of the research priorities of the Systems Toxicology
- Principles of the Gene – Environment interaction in Toxicology and Biomedicine
- Methods in Systems Biology and Toxicology: Omics approaches
- Genomic integrity and mechanisms of DNA Repair
- GxE interaction: p53 germline and sporadic mutations
- GxE interaction: BAP1 germline and sporadic mutations
- GxE interactions: genetic alterations of the hypoxia response pathways (i.e. VHL)
- Epigenetic basis of the response to stressors
- Scientific publishing: How to prepare and evaluate a scientific article
- Laboratory safety, including fire protection, chemical safety and biohazards

The Interactive Journal Club sessions will have the goal of training the students in the critical evaluation of recent scientific publications in the area of study.

b. Practical part

The students involved in the practical components will be integrated in the research activity of the group under the guidance of a tutor. The students will be assigned a specific experimental task (i.e. small project) that will represent part of a larger research project conducted in the laboratory. The student will be coached and trained for the experimental procedures, and the analysis and interpretation of the data with the goal of preparing her/him to future research activities in the area.

Current research projects in the Chairs for Systems Toxicology and Molecular Toxicology:

- Molecular mechanisms for maintenance of genome integrity
- Identification of novel genomic loci for hypersusceptibility to genotoxicants (Fbox proteins).
- Context-dependent cell death decisions in response to cytotoxic drugs
- Role of p53 variants in tumorigenesis
- Epigenetic regulations of stress response by BAP-1
- Novel in-vitro methods in genetic toxicology
- *C. elegans* as an emerging model system in toxicology

Techniques used in the laboratory:

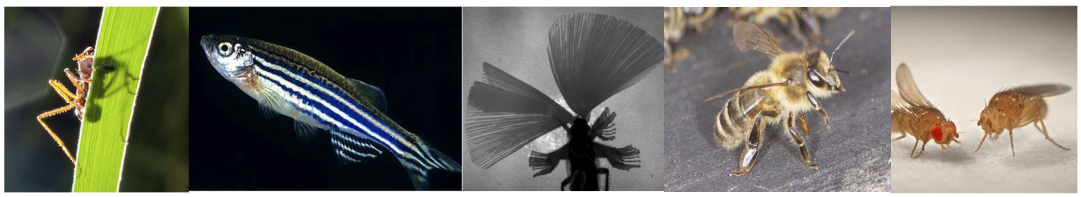
- Cell Biology: cell culture manipulation and analysis of primary and stable cell lines
- Imaging: Flow cytometry, Confocal microscopy and fluorescence microscopy
- Molecular biology: Nucleic acids isolation, Standard and Quantitative PCR, chromatin conformation and binding analyses, innovative methods for detection and quantification of various kinds of DNA damage
- Protein Biochemistry: Western blotting, ELISA, immunocytochemistry, FACS
- Omics: Analysis of large genomic, transcriptomic and metabolomic datasets
- *C. elegans*: Biological readouts with relevance for toxicology

4. Requirements
Basic knowledge in toxicology, biochemistry and cellular and molecular biology.

5. Exam
To pass the course, the students will have to regularly attend the theoretical part, will have to actively participate to the Journal Clubs and provide a final presentation of their research work. Moreover, the students will have to regularly conduct the practical activity and correctly maintain a record in the lab journal.

Advanced Course „Behavioral Neurobiology"

Coordinator:	Prof. Dr. Armin Bahl
Principal investigators:	Prof. (apl.) Dr. Christoph Kleineidam. Prof. Dr. Giovanni Galizia, Dr. Katrin Vogt, Dr. Einat Couzin-Fuchs, Dr. James Foster, Dr. Morgane Nouvian, and others.
Website:	www.neurobiology-konstanz.com



Introduction

The aim of our research is to unravel how neural circuits enable animals to sense their environment, how such information is processed in the brain and used to guide behavior, and how an animal's internal state modulates the underlying network dynamics. To this end, we study how animals make decisions in different contexts and states, how they learn and remember, and how they interact when in a group.

We are generally driven by the idea that biological systems, in their beautiful diversity and complexity, follow relatively simple principles that are commonly shared across species. To explore these common principles, we use a variety of model organisms, including larval zebrafish, locusts, bees, ants, cockroaches, adult flies, and fly larvae. Each one of these model organisms has its specific experimental advantage or shows a particularly interesting behavior:

- Larval zebrafish are tiny and almost completely transparent vertebrates, making it easy to use functional imaging techniques to characterize the activity of the entire brain at cellular resolution, while animals can still make behavioral decisions. Larval zebrafish are genetically tractable, allowing us to manipulate circuit function and test its effect on behavior.
- Locusts show complex group dynamics, they often march or fly in huge groups, which can be mimicked in a laboratory setting. Using electrophysiological recordings and imaging techniques we can ask how such different behavioral states arise and how this affects sensory processing.
- Bees are masters in olfactory processing, spatial memory, and communication, and can make complex decisions as a collective. It is possible to use imaging and electrophysiological techniques to dissect the combinatorial code in the olfactory system and explore how memories are stored. In behavioral experiments, the division of labor and the organization of the colony can be explored.
- Ants are highly social insects and can collectively shape their environment by cutting grass and leaves, and by keeping their environment tidy. Through behavioral experiments, immunohistochemical staining methods, as well as mass spectrometry, we explore how nervous system activity changes as a function of the animal's state.
- Cockroaches have a very fine sense of odor and actively sample their environment using their long antennae. Through behavioral quantification and electrophysical recordings, we aim to understand their behavioral strategies in odor plume detection and how this information is represented on the level of the brain.

- Fruit flies perform sophisticated navigation behaviors towards olfactory or visual cues, which are also modulated by their internal state. Furthermore, they are able to associate rewarding and punishing events with different contextual stimuli, allowing them to adapt their behavior for future behavioral decisions. Using the most powerful genetic toolkit available across all our model organisms, we can dissect the neural circuits underlying all these behaviors on the level of single-cell types. Additionally, we can investigate how genetic modulations affect the activity of other cells in the brain by functional imaging.

Contents of the Course

Students will join one of our currently active research projects, using one of our animal models. Working closely together within our scientific community, we will provide students with hands-on exposure in state-of-the-art experimental techniques, including high-throughput behavioral tracking, two-photon functional calcium-Imaging, electrophysiology, mass spectrometry, immunohistochemistry, molecular biology, and computational modeling.

The project work includes the development of a specific research question, literature research, planning of experimental design, experimental execution, data acquisition, and data analysis. The project ends with a detailed protocol in the form of a manuscript and a final presentation of results in the form of a talk.

The course is accompanied by lectures covering basics in Behavioral Neuroscience and general experimental techniques. Moreover, invited speakers give students the opportunity to learn about ongoing research in the field outside our department. In addition, we will hold a paper seminar where supervisors introduce their own research topic and where we discuss recent publications related to our projects. This will happen during a retreat in the Alps on one of the first weekends during the course.

Lectures, project completion, as well as seminar attendance, are mandatory parts of the course. This course is held in English only.

Interested students should contact one of our principal investigators 2–4 weeks prior to the course to discuss possible research projects.

Recommended reading material

Kandel E, Schwartz J, Jessell T (2000) Principles of Neural Science. McGraw-Hill.

Greenspan RJ (2007) An Introduction to Nervous Systems. Cold Spring Harbor.

Carew T (2000) Behavioral Neurobiology. Sinauer Associates.

Galizia CG, Lledo PM (2013) Neurosciences. Springer.

Zupanc GKH (2010) Behavioral Neurobiology - An Integrative Approach. Oxford University.

Advanced Course (Vertiefungskurs, VTK) "Immunology"

Coordinator: PD Dr. Michael Basler
PD Dr. Gunter Schmidtke

1. Introduction

We are interested in the intracellular processing of antigens and the presentation of the antigens to cytotoxic T-lymphocytes. Protein antigens are degraded in cells by a large protease complex, the proteasome. We determine how the subunit composition of proteasomes affects the immune response of the T cells against viruses and tumors. Recently, we have discovered a new function of a subtype of the proteasome in immune cells, the immunoproteasome, in autoimmune diseases. The mechanistic basis and therapeutic implications of this finding will be investigated.
Poly(D,L-lactide-co-glycolide) (PLGA) microspheres are biodegradable antigen delivery devices for loading of dendritic cells with antigen and toll-like receptor ligand in vitro and in vivo. They possess useful characteristics such as an adjustable drug release profile and a very high encapsulation efficiency. Vaccination of mice with these two compound microspheres led to robust CTL responses strong enough to eliminate pre-established model tumors and to protect mice from vaccinia virus infection. Currently, we are testing whether PLGA microspheres are suitable vaccines against SARS-CoV2 and influenza virus.
The T cell receptor (TCR) is where the T cell-mediated immune response begins and its density at the cell surface, which directly relies on endocytic trafficking, fine tunes the probability and extent of T cell activation. The questions we want to tackle is how membrane organisation and cell forces regulate T cell activation.
Red blood cells, so-called erythrocytes, are essential for life because their main task is to supply the entire organism with oxygen. Erythrocytes make up the main component of our blood. In an adult, up to 200 billion red blood cells are produced in the bone marrow every day. By identifying the erythroid-specific signaling molecules and intracellular transportation of chemokine receptors that determine the signaling pathways of the chemokine receptors in erythroid cells, we want to contribute to more efficient and drug-controlled blood production in the future.
Dendritic cells can be viewed as the sentinels of the immune system, as they constantly sample tissues for invading pathogens. Upon pathogen encounter, dendritic cells start expressing a cell surface receptor (CCR7) that recognises chemokines, which are specific attractant molecules. We are especially interested in the differences between the migration phenotype of cancer and immune cells. The aim of this research is to contribute to the development of new therapeutic strategies, focused on preventing cancer cells to follow the path of immune cells to the lymphatic system.

Please note that some of the participants will do their experimental work at the Biotechnology Institute Thurgau (BITg) in Kreuzlingen, Switzerland (www.bitg.ch), which is 6 km away from the Chair for Immunology on floor P11 of the Konstanz University Campus.

Our current work focus:

a) The role of proteasome regulator PA28α/β and the interferon gamma induced subunits of the 20S immunoproteasome in the immune response against murine lymphocytic choriomeningitis virus and other pathogens.
b) The function of the immunoproteasome in the pathogenesis of autoimmunity, cancer and transplant rejection.

c) The function and conjugation of a ubiquitin-like modifier called FAT10, which is induced by interferon gamma and is encoded in the MHC.
d) T cell targeted immunization with biodegradable PLGA microspheres for tumor immunotherapy and antiviral (influenza, SARS-CoV-2) vaccination.
e) The regulation of migration of dendritic cells and T cells through chemokines
f) The signal transduction via the T cell receptor and the role of membrane tension for T cell activation.
g) The role of Nub1 in proteostasis and in neurodegenerative diseases

2. Course schedule

a) Theoretical part

Week 1	Theoretical part: Daily from 8.30 – 16.00 o'clock: Lectures for recapitulation of basic immunological knowledge and on new developments in Immunology with main focus on the current research topics and techniques.
Week 3 to 5	Each student has to present a recent top publications in immunology. The presentations will be held in English.
Week 4 to 5	Alumni talks. Two talks of former members of the chair of immunology. One working in academia and one working in industry.
Week 6	Oral presentation of the students' experimental data.

b) Experimental part and methods

Week 2 to 5/6 Work in the lab

Every student is working with a scientist or PhD-student on his/her project. The experimental scheduling is adapted to our current scientific research projects and the ongoing experiments. We use methods of molecular biology (plasmid preparation, restriction analysis, real time RT-PCR, molecular cloning), of protein chemistry (immuno-precipitation, Western blot, isolation of proteins with various chromatographic methods), and immunological methods (ELISA, ELISPOT, flow cytometry, proliferation assays, adoptive transfer, cytolytic assays, immunofluorescence microscopy) and for some experiments work with mice.

c) Requirements

To pass the course the following requirements need to be met by students doing theory and practical work:
Participation in the lectures, lab work and presentation of the project results, power point presentation of a recent immunological publication for the course members, submission of a comprehensive work protocol in English language formatted like a research article in *The Journal of Immunology* (https://www.jimmunol.org/info/authors). After the first week of lectures we will have a written exam to inform students (and us) where we stand.
Students who only take part in the theoretical part will need to write a summary of a recent publication in English and also participate in the VTK exam; it is mandatory for all participants to attend all lectures and talks.

3. Qualification of course members

Students need to have attended the lectures of Immunology in the 4th semester at Konstanz University and must have passed the associated exam. Equivalent qualifications of students from outside Universities are accepted. The content of the lectures must be refreshed using the script and the textbook "Immunobiology" by Charles Janeway et al. before the start of the course. Basic knowledge in biochemistry, cell biology, molecular biology and good command of English is required. We expect a higher-than-average interest in Immunology and immunological research projects.

4. Literature

Janeway, C., Travers, P., Walport, M. and Shlomchik, M. (2001). "Immunobiology" Garland, New York, (ISBN 0 8153 3642 X).

Muchamuel, et al. (2009) A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. *Nature Med.* 15: 781-787.

Basler, M., Mundt, S., Bitzer, A., Schmidt, C., and Groettrup, M. (2015) The immunoproteasome: a novel drug target for autoimmune diseases. *Clin. Exp. Rheumatol.* **33**, S74-79

Aichem, A., Anders, S., Catone, N., Rossler, P., Stotz, S., Berg, A., Schwab, R., Scheuermann, S., Bialas, J., Schutz-Stoffregen, M. C., Schmidtke, G., Peter, C., Groettrup, M., and Wiesner, S. (2018) The structure of the ubiquitin-like modifier FAT10 reveals an alternative targeting mechanism for proteasomal degradation. *Nature Commun.* **9**, 3321

Aichem, A., and Groettrup, M. (2020) The ubiquitin-like modifier FAT10 - much more than a proteasome-targeting signal. *J. Cell Sci.* **133**, jcs246041

Koerner, J., Horvath, D., Herrmann, V.L., MacKerracher, A., Gander, B., Yagita, H., Rohayem, J., and Groettrup, M. (2021) PLGA-particle vaccine carrying TLR3/RIG-I ligand Riboxxim synergizes with immune checkpoint blockade for effective anti-cancer immunotherapy. *Nature Commun.* **12**, 2935

PLGA Particles in Immunotherapy. Horvath D, Basler M. *Pharmaceutics.* (2023) 11;15(2):615. doi: 10.3390/pharmaceutics15020615.

Membrane Compartmentalization and Scaffold Proteins in Leukocyte Migration. Samson GPB, Legler DF. *Front Cell Dev Biol*. (2020);8:285. doi: 10.3389/fcell.2020.00285.

Role of Mechanotransduction and Tension in T Cell Function. Rossy J, Laufer JM, Legler DF. Front Immunol. (2018);9:2638. doi: 10.3389/fimmu.2018.02638.

Advanced course "**Theoretical and Experimental Ecology and Evolution**"

Coordinator: Prof. Dr. Lutz Becks

1. Introduction

We are interested in the basic processes and mechanisms of species interactions that drive ecological and evolutionary changes over time and space. Species interactions such as between predator and prey, host and pathogen, competitors and symbionts are an integral part of any community and are the major drivers of evolutionary and ecological changes. They often exert strong selection on the interacting species, leading to rapid and significant evolutionary changes in important traits like anti-predatory defenses, resistance or a shift in resource usage. At the same time, species interactions typically result in significant and rapid changes in population sizes, with for example regular or irregular fluctuations in population sizes or the extinction of a population. Different processes and mechanism can drive and regulate the evolutionary and ecological changes (e.g, density-dependent and frequency-dependent processes) and several occur typically simultaneously and influence each other.

Over the last years, it became more and more apparent that rapid evolutionary change (i.e., within a few generations) is an important driver for ecological changes and vice versa, which has led to the development of the research field of eco-evolutionary dynamics. In this important and rapidly growing research field, we aim to understand the importance of these eco-evolutionary dynamics. Understanding the mechanisms and processes that control ecological and evolutionary changes is important to predict how populations or communities react to environmental changes.

In our research we combine experimental approaches (e.g., experimental evolution where we can observe evolutionary change in real time) with mathematical modelling and sequencing approaches to test hypotheses and mechanisms that determine changes in population sizes and traits over time and space. We work with plankton communities and study predator-prey, host-virus, mutualistic and competitive interactions, as well as the combination of those.

2. Objectives

You will learn

- about the novel research field of eco-evolutionary dynamics
- ecological and evolutionary theory, community ecology
- to identify research question and design your own experiments and analyses of mathematical model to answer research questions
- a large range of methods used in ecology and evolution

3. Contents of the course

The course includes lectures, practicals, seminars and a fieldtrip. The lectures will introduce you to the important theories of ecology and evolution with respect to species interactions in communities as well as eco-evolutionary dynamics. In the practicals, you will learn experimental design, mathematical modelling, statistical analyses, scientific writing and presentation. We will teach you how to work with planktonic and microbial communities (culture methods; experimental evolution; enumeration by quantitative PCR methods, flow cytometry, automated High Content Microscopy, optical density measurements) as well as running analyses of mathematical models in R. During the seminars you will read and discuss recent scientific publications. Based on seminars and lectures, you will identify a research question, design and run your own experiment and model analyses in groups of two to put the skills and knowledge into practice.

4. Requirements

Interest and basic knowledge in ecology and evolution.

5. Exam

To pass the course, you have to be actively participating, present and discuss during seminars as well as your project results (poster), and write a paper on your research project.

For more information on our group, visit our webpage (https://www.limnologie.uni-konstanz.de) or contact lutz.becks@uni-konstanz.de

Advanced Course "Biochemical Pharmacology"

Coordinators: Prof. Dr. Thomas Brunner
Prof. Dr. Mohamed Elmesery

1. Introduction

The course intends to introduce various theoretical and research-associated aspects in the field of biomedicine, especially biochemistry, pharmacology, cell biology and inflammation. The compact course contains lectures, seminars, journal presentations and practical work in the laboratory under the guidance of members of the research group.

2. Content of the compact course

a) Theoretical part:

The theoretical part consists of lectures and seminars. In the first week the scientific background of the research conducted at the chair of biochemical pharmacology will be introduced in a possibly interactive manner. The lectures aim at introducing the scientific background, the different seminars specific research topics and projects, while in the journal clubs recent literature in the field will be presented and discussed. The lectures continue in the following weeks with 1.5 hours lectures daily in the morning.

The following subjects will be discussed:

- General lab safety
- Apoptosis-Necrosis-programed necrosis
- Detection methods of cell death
- The extrinsic apoptosis pathway-TNF Receptor family
- The intrinsic apoptosis pathway-Bcl-2 family
- Flow cytometry
- Immunopathologies of the intestine, skin and liver
- Immune homeostasis in the intestine
- Inflammatory bowel disease
- Steroid synthesis
- Extra-adrenal steroid synthesis in the intestine, lung and skin
- Nuclear receptors
- Tumor biology
- Pharmacology

The journal clubs consists of the presentation and discussion of recent publications of subjects covered by this advanced course.The publications will be presented by the participating students, with the aim to analyze and interprete scientific publications. Finally, each student will discuss the data obtained during the practical work of the course in a short presentation at the end of the course.

b) Practical part

In the practical part, the participating students will work on small projects, which are integrated into general projects and research topics of our research group. Students will be coached and supervised by postdocs, PhD students and master students, working on these specific subject, aiming at introducing the students to various aspects of biochemical, cell biological and pharmacological research in an intense and realistic manner. The goal is to give the participating students a deeper insight into the planing, conduction, analysis and interpretation of experiments and research projects. With the participation in this Advanced Course the students should obtain basic skills for future scientific work during the Master thesis.

Actual research subjects at the chair of biochemical pharmacology:

- Regulation of apoptotic and necrotic cell death in tumor cells and primary cells
- Interaktions between the extrinsic and intrinsic apoptosis pathway
- Activation of Bcl-2 homologs
- Thiazolides, Glutathion-S-transferase P1 and apoptosis in colon carcinoma cells
- Regulation of extra-adrenal glucocorticoid synthesis in intestine, lung and skin
- Role of extra-adrenal glucocorticoid synthesis in the regulation of immune homeostasis
- Regulation of steroid synthesis by nuclear receptors
- The role of nuclear receptors in the regulation of apoptotic cell death
- Primary intestinal and tumor organoids

Used techniques:

- Biochemical and molecular standard methods
- Isolation, culture, activation and analaysis of primary cells
- Organ cultures of lung and intestinal tissue
- Culture and transfection of cell lines
- Apoptosis assays
- Flow cytometry for the phenotyping and sorting of immune cells
- Detection of proteins and their activation by Western blotting
- Luciferase reporter assays to study gene regulation
- Radioimmunoassay
- Immunofluorescence and immunohistochemistry
- Microscopy and histology
- Laser Capture Microdissection
- Quantitative PCR

3. Expected background knowledge

Basic knowledge in biochemistry, immunology and molecular biology is expected.

4. Literature

General literature:

- Ahmed A, Schmidt C, **Brunner T**. Extra-adrenal glucocorticoid synthesis in the intestinal mucosa: between immune homeostasis and immune escape. Front. Immunol. Epub ahead 2019, doi: 10.3389/fimmu.2019.01438. eCollection 2019
- Delbridge AR, Strasser A. The BCL-2 protein family, BH3 mimetics and cancer therapy. Cell Death Differ 22:1071-80, 2015
- Wajant H, Siegmund D. TNFR1 and TNFR2 in the control of the life and death balance of macrophages. Front. Cell Dev. Biol. Epub ahead. doi: 10.3389/fcell.2019.00091. eCollection 2019
- Fayard. “LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis”. Trends Cell Biol. 14: 250-60. 2004

Specific literature:

- Phan T S, Schink L, Mann J, Merk V M, Zwicky P, Mundt S, Simon D, Kulms D, Abraham S, Legler D F, Noti M, Brunner T. Keratinocytes control skin immune homeostasis through de novo-synthesized glucocorticoids. Science Advances. Cell Biology. 7:eabe0337. January 2021.
- Phan TS, Merk VM, Brunner T. Extra-adrenal glucocorticoid synthesis at epithelial barriers. Genes Immunity. Doi.org/10.1038/s41435-019-0058-z. 2019.
- Ripani P, Delp J, Bode K, Delgado ME, Dietrich L, Betzler VM, Yan N, von Scheven G, Mayer TU, Leist M, Brunner T. Thiazolides promote G1 cell cycle arrest in colorectal cancer cells by targeting the mitochondrial respiratory chain. Oncogene. 39:2345-2357. 2019
- Seitz C, Huang J, Geiselhöringer AL, Galbani-Bianchi P, Michalek S, Phan TS, Reinhold C, Dietrich L, Schmidt C, Corazza N, Delgado E, Schnalzger T, Schoonjans K, Brunner T. The orphan nuclear receptor LRH-1/NR5a2 critically regulates T cell fucntions. Sci Adv. 5:eaav9732. 2019
- Grabinger T, Bode K, Demgenski J, Seitz C, M. Delgado E, Kostadinova F, Reinhold C, Etemadi N, Wilhelm S, Schweinlin M, Hänggi K, Knop J, Hauck C, Walles H, Silke J, Wajant H, Nachbur U, Wong W-L, Brunner T. Inhibitor of Apoptosis Protein-1 Regulates Tumor Necrosis Factor-mediated Destruction of Intestinal Epithelial Cells. Gastroenterology, 152:867-879. 2017
- Brockmann A, Bluwstein A, Kögel A, May S, Marx A, Tschan MP, Brunner T. Thiazolides promote apoptosis in colorectal tumor cells via MAP kinase-induced Bim and Puma activation. Doi:10.1038 Cell Death and Disease. 2015
- Sidler D, Brockmann A, Mueller J, Nachbur U, Corazza N, Renzulli P, Hemphill A, Brunner T. Thiazolide-induced apoptosis in colorectal cancer cells is mediated via the Jun kinase-Bim axis and reveals glutathione-S-transferase P1 as Achilles’ heel. Oncogene 31,4095-4106. 2012
- Grabinger T, Luks L, Kostadinova F, Zimerberlin C, Medema JP, Leist M, Brunner T. Ex vivo culture of intestinal crypt organoids as a model system for assessing cell death induction in intestinal epithelial cells and enteropathy. Doi:10.1038/cddis.2014.183. Cell Death and Disease. 2014
- Brockmann A, Strittmatter T, May S, Stemmer K, Marx A, Brunner T. Structure-Function Relationship of Thiazolide-Induced Apoptosis in Colorectal Tumor Cells. ACS chemical biology. Dx.doi.org/10.1021/cb500209a. 05/2014.

- Noti M, Corazza N, Mueller C, Berger B, Brunner T. TNF suppresses acute intestinal inflammation by inducing local glucocorticoid synthesis. J Exp Med. 207(5):1057-66. 2010
- Noti, M, Sidler, D., and Brunner, T. Extra-adrenal glucocorticoid synthesis in the intestinal epithelium: more than a drop in the ocean? Sem. Immunopathol. 31:237-48. 2009

Advanced Course „Advanced Technologies for the Life Sciences“

Offered by the Core Facilities (Gerätezentren) of the Department of Biology:
Bioimaging Center (BIC)
Electron Microscopy Center (EMC)
Flow Cytometry Facility (FlowKon)
Next-Generation Sequencing Facility (SequAna)
and the Chair for Cellular Bioimaging

This Advanced Course offers the student insights into four technologies broadly used in life science research: electron and light microscopy, flow cytometry, and next-generation sequencing. After the first week of lectures and active learning assignments, students will start a four-week rotation during which they will stay in each facility for one week in groups of three. During the final week, they will give presentations, prepare their reports, and give feedback for the evaluation. The course aims to give students basic theoretical background and first hands-on experiences in all four technologies, make them aware of their limitations and potential, and introduce them to the working environment of research infrastructures.

BIC: Light Microscopy
The BIC will give students an overview of the current light microscopy approaches, e.g. polarization microscopy, fluorescence microscopy (widefield illumination and laser scanning), holographic microscopy. Lectures will introduce the different methods, explain the fundamental principles and their applications and limitations. They will also introduce approaches to assess the microscope performance and cover ethical and data management issues when working with scientific image data. In hands-on assignments, students will learn how to prepare biological samples for different imaging approaches, carry out microscopy performance checks, or perform image analysis tasks.

EMC: Electron Microscopy
The EMC will introduce techniques and methods used in electron microscopy through lectures, demonstrations, and hands-on exercises in short students projects. Using different biological samples the following techniques in electron microscopy will be covered: Scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive x-ray spectroscopy (EDX) and Focused Ion Beam (FIB). The short students' hands-on projects will cover one or two of the above-named techniques, including methods for sample preparation from the living organism to the imaging and data analyses.

Flowkon: Flow Cytometry
The practical-oriented training course at the FlowKon will introduce techniques and methods used in flow cytometry. The theoretical part of the course outlines essential principles of flow cytometers including optics, fluidics, and electronics, the properties

of fluorophores and explains basic rules of panel design and compensation. The hands-on projects will cover common applications of flow cytometry and demonstrate instrument construction, operation, and data acquisition on different flow cytometric instruments.

SequAna: Sequence Analysis

The advent of next-generation sequencing (NGS) technologies over recent years have transformed the life sciences: an entire human genome can now be sequenced in a matter of days for US$1,000, changing the way we conduct research and analyze data. The new Sequencing Analysis (SequAna) Core Facility will introduce techniques and methods associated with NGS technologies, covering the wet lab (sequencing library generation and quality control) and in silico aspects (sequencing platforms, sequencing files, filtering/trimming/assembly/annotation of sequencing data). About 30% of the course will cover molecular biology wet lab techniques and the remaining 70% will cover bioinformatics analysis, mainly in the form of UNIX command line work. Students will become acquainted with common NGS workflows and data frameworks.

The Chair for Cellular Bioimaging is a joint Professorship of the University of Konstanz and the German Cancer Research Center in Heidelberg. In the frame of this Advanced Course interested students will have the possibility to perform hospitations at the Core Facilities of the DKFZ in Heidelberg. The duration of the hospitation has to be agreed upon with the receiving core facility at the DKFZ. Students need to participate in the theoretical parts of the course in Konstanz to complete the course.

Advanced Course "Collective Animal Behavior"

Coordinator: Prof. Iain Couzin
Lecturers: Iain Couzin, Einat Couzin, Michael Griesser, Liang Li

Supervisor:
Prof. Dr. Iain Couzin
(Chair of Biodiversity and Collective Behavior, University of Konstanz and Max Planck Institute of Animal Behavior)

Overview:

„This course will focus on the quantitative analysis of collective animal behavior in the laboratory and the field. Introductory lectures will cover key concepts and techniques, including automated tracking of animal movements and postures in 2D and 3D space, how social interactions scale to collective dynamics, how information flows through social networks and the resulting transmission of socially learned knowledge and the relation to cumulative culture in animal populations and societies. Theoretical concepts will be expounded through the development of simulations of collective dynamics using video game engines and immersive virtual reality. Subsequently students, and the faculty, will work together to design analyses of models or existing data, resulting in novel research projects.

Please check our website www.collectivebehavior.com for latest information about our current research."

Advanced Course (VTK): Going Wild: Behavior & Ecology of Animals

Field Course: Going Wild: Behavior and Ecology of Animals

-

Main responsibles for the VTK: Meg Crofoot, Dina Dechmann, Urs Kalbitzer, Martin Wikelski
Main responsibles for "Going Wild: Behavior and Ecology of Animals": Alex Jordan, Kamran Safi, Ari Strandburg-Peshkin

Additional instructors: Sections of this course will be taught by Hannah Williams, Andrea Flack, Wolfgang Fiedler and members from the AG Crofoot, the AG Wikelski and the Jordan Lab.

Course content and teaching goals:
Observational and experimental studies on animal behavior in the field (i.e, in situ) are essential to understand animal behavior in the context of life history, ecology and evolution. Yet, such studies present unique challenges in study design, data collection, data analysis, and data interpretation. Our goal for this course is to equip you with the skills required to conduct behavioral ecology research by providing you with hands-on experience in designing and executing your own field study. During theoretical and practical sessions you will learn how to formulate research questions, how to collect data in the field, how to analyze these data, and how to report your results to a scientific audience. As part of this process, you will spend 2-3 weeks in the field during which time you will conduct your own, independent field research projects. Therefore, this VTK and the course "Going Wild: Behavior and Ecology of Animals" must be taken together.

To learn how to design a study, we will review and discuss some basic concepts of behavioral ecological research, the kind of questions that are asked in behavioral ecology, and how data are collected and analyzed to address these questions. As such, this part will be closely linked to a series of hands-on modules during which you will learn the analysis of quantitative behavioral and ecological data, including a general introduction to scientific programming (in R or Python), data wrangling, and visualization. We will build up from the basics, therefore no prior knowledge of programming is expected. Students should, however, bring enthusiasm for picking up new skills and a positive attitude towards diving into computational challenges.

Throughout the course, you will also have a lot of time to go outside to address your independently-formulated research questions and to conduct a field project by collecting data from free-ranging animals. Then, you will analyze the collected data by implementing effective analytical techniques, and interpret the results of your analyses. In addition to technical skills, you will learn problem-solving skills, lateral thinking and resilience in data collection and analysis, which is essential for fieldwork where things inevitably don't go completely as planned, and when working with exciting, but sometimes 'messy' data.

Finally, you will present your findings in a talk to an audience of researchers from the University of Konstanz and the Max Planck Institute of Animal Behavior and write them up in a scientific report, with the potential for subsequent publication. We will support you during this process by offering sessions on scientific writing throughout the course, to help you develop your communication skills.

.

Advanced Course "Molecular Microbiology and Cell Biology: Chaperone functions in health and disease"

Coordinator: Prof. Dr. E. Deuerling

Function and Mechanisms of Molecular Chaperones in Health and Disease

1. Introduction

The generation of biologically active proteins is a basic process which has to proceed in all cells – from bacteria to humans – with a maximum of precision to ensure the survival of the cell. The biosynthesis of proteins at the ribosomes is an exceedingly complex and fast process. For example, already in a bacterial cell approx. 30.000 new proteins per minute are produced at 20.000 ribosomes.

Proteins are functional – i.e. as enzymes or structural components – only if they adopt a defined three-dimensional structure (folding). Proteins are synthesized at the ribosomes as linear polymers composed of amino acids and fold into their defined three-dimensional structure after or during their synthesis. This folding crucially depends on supporting helper proteins, called the Molecular Chaperones. All cells contain a network of different chaperones which cooperate with each other and thus control and assist the folding processes in space and time. Hence, protein folding and the function of Molecular Chaperones are of fundamental importance for all fields of biology and the subject of intense research. In addition, they are also highly relevant for their medical aspects since defects in protein folding and chaperone function lead to protein aggregation. These protein aggregates are characteristic pathological markers for neurodegenerative diseases like prion diseases, Alzheimer's dementia or Chorea Huntington disease, and they are presumably responsible for the neuronal cell death.

2. Contents of the course

a) Theoretical part
Protein folding, function and mechanisms of molecular chaperones, protein folding defects, molecular basis of neurodegenerative diseases and aging, yeast and *C. elegans* as genetic model systems; biochemical methods for the analysis of protein-protein interactions: crosslinking techniques and fluorescence spectroscopy; detailed structural and functional insights into ribosomes and translation regulation.

b) Practical part
The practical part of this advanced course orients itself at our current research projects. Our major goal is to enhance our understanding of protein synthesis and folding in health and disease. Thus, the major topics of our research are

1. to illuminate the structural, functional and mechanistic principles of molecular chaperones with a particular emphasis on investigating the role of ribosome-associated chaperones that control protein folding and regulate translation;
2. to study cotranslational folding pathways of nascent polypeptides;
3. to investigate protein processing and quality control mechanisms that ensure that the newly made protein is either correctly modified and folded or rapidly degraded by proteolytic systems;
4. to understand the functions of ribosome-associated chaperones in aging and diseases related to protein misfolding.

c) Model organisms and range of methods
The questions raised above are worked on using three different model organisms: the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae* as well as the nematode *C. elegans*. For our work, we combine demanding genetic analyses of chaperone and ribosome mutants *in vivo* with protein analysis *in vitro*. This includes RNAi experiments in *C. elegans*, knockout mutations in *E. coli* and yeast and fluorescence microscopy analysis with all three model systems. Furthermore, state-of-the-art kinetic and mechanistic investigations of translation and chaperone-assisted protein folding *in vitro* are performed using translation systems, ribosome profiling, qPCR, fluorescence spectroscopy and crosslinking techniques.

3. State of knowledge of the participants

a) Compact course Molecular Microbiology;

b) Elementary knowledge in microbiology, biochemistry and molecular biology including all the techniques like protein purification methods, PCR, cloning, etc. is a prerequisite.

4. Literature

a) General

- Lubert Stryer "Biochemie"
- Michael T. Madigan, John M. Martinko und Thomas D. Brock "Mikrobiologie"
- Bruce Alberts, Alexander Johnson und Julian Lewis " Molekularbiologie der Zelle"
- Friedrich Lottspeich und Haralabos Zorbas "Bioanalytik"

b) Special

Gamerdinger, M., et al. (2019) Early scanning of nascent polypeptides inside the ribosomal tunnel by NAC. Mol. Cell 75, 996-1006

Shen, K., et al. (2019) Dual role of ribosome-binding domain of NAC as a potent suppressor of protein aggregation and aging-related proteinopathies. Mol. Cell 74, 729-741

Martin, E.M., et al. (2018) Conformational flexibility within the nascent polypeptide-associated complex enables its interactions with structurally diverse client proteins. J. Biol. Chem. 293, 8554-8568

Hanebuth, M.A., et al. (2016) Multivalent contacts of the Hsp70 Ssb contribute to its architecture on ribosomes and nascent chain interaction. Nat. Commun. 7:doi:10.1038/ncomms13695

Preissler, S., et al. (2015) Not4-dependent translation repression is important for cellular protein homeostasis in yeast. EMBO J. 34, 1905-1924

Gamerdinger, M., et al. (2015) The principle of antagonism ensures protein targeting specificity at the endoplasmic reticulum. Science 348, 201-207

Kirstein-Miles, et al. (2013) The nascent polypeptide-associated complex is a key regulator of proteostasis, EMBO J. 32, 1451-1468

Preissler, S., and Deuerling, E. (2012) Ribosome-associated chaperones as key players in proteostasis. Trends Biochem. Sci. 37, 274-283

Eichmann, C., et al. (2010) Co-translational structure acquisition of nascent polypeptides monitored by NMR spectroscopy. Proc. Natl. Acad. Sci. USA, 107(20), 9111-9116.

Koplin, A., et al. (2010) A dual function for chaperones SSB/RAC and the NAC nascent polypeptide-associated complex on ribosomes. J. Cell Biol. 189(1), 57-68.

Ferbitz, L., et al. (2004). Trigger Factor in complex with the ribosome forms a molecular cradle for nascent proteins. Nature 431, 590-596.

Gamerdinger, M. et al. (2023) NAC controls cotranslational N-terminal methionine excision in eukaryotes. Science 380, 1238–1243

Jaskolowski, M. et al. (2023) Molecular basis of the TRAP complex function in ER protein biogenesis. Nat. Struct. Mol. Biol., published online 11/05/2023

Jomaa, A. et al. (2022) Mechanism of signal sequence handover from NAC to SRP on ribosomes during ER-protein targeting. Science 375, 839-844

Fries, S. et al. (2021) Deciphering molecular details of the RAC–ribosome interaction by EPR spectroscopy. Sci. Rep. 11, 8681

Nikolay, R. et al. (2021) Snapshots of native pre-50S ribosomes reveal a biogenesis factor network and evolutionary specialization. Mol. Cell 81, 1200-1215

Fürsch, J. et al. (2020) Proteome-wide structural probing of low-abundant protein interactions by cross-linking mass spectrometry. Anal. Chem. 92, 4016-4022

Schneider, T. et al. (2019) Conformational and functional characterization of artificially conjugated non-canonical ubiquitin dimers. Sci. Rep. 9, 19991

Advanced Course „Human and Environmental Toxicology”

Date: 13.11.23 – 22.12.23

Location: Lab-1014, Level 10 M-Building, Universitätsstrasse 10, D-78457 Konstanz, Germany

Coordinators: Prof. Dr. Daniel Dietrich (Daniel.Dietrich@uni-konstanz.de)

Dr. Sascha Beneke (Sascha.Beneke@uni-konstanz.de)

1. Introduction

With a continuously growing knowledge about the limitation of our natural resources the needs were arising to assess comprehensively the risks of single individuals and communities from damages caused by an increasing population density, industrialisation, and modernisation of the society. Particularly, of growing concern is the increasing number of ubiquitously distributed xenobiotics at low concentrations, mostly of anthropogenic origin, which potentially could be toxic for humans and animals. The xenobiotics are not only equally distributed in the environment, but can also be distributed and accumulated in various organisms including humans, e.g. via direct (water, drinking water) or indirect exposure (food chain). Primarily human and environmental toxicology focus on the acquisition, assessment (and at best in the future on prediction and prevention) of interactions between anthropogenic agents and the ecosystem including species/individual organisms and humans. In view of the large number of chemical agents, the complexity of the ecosystem and the rapidly changing living and eating habits of our society this goal seems to be difficult to achieve. Therefore, environmental toxicology is a multidisciplinary field of science that is not only concerned with the study of the harmful effects of various chemical, biological and physical agents on individual living organisms, but also considers aspects of environmental chemistry and ecology and put them in a common context. The major goal is the determination of the risk potential of specific substances, whether of natural (e.g. cyanobacterial toxins) or of anthropogenic (e.g. pharmaceuticals) origin, while considering also factors like physicochemical characteristics and amount released, property and size of the affected environmental compartment, period and route of exposure of individual organisms and closely associated different substance effects. Furthermore, the research also encompasses developments of effects caused by global warming like the potential selection of temperature-tolerant toxin-producing cyanobacterial strains or resp. the invasion of toxic neobiota into the temperate climate zone along with the problems of toxin contamination of surface waters and drinking water.

Since humans, as part of the living environment, are primary organism to be protected from adverse effects incurred by exposure to toxic substances, the field of environmental toxicology cannot be separated from the medically oriented human toxicology. In order to address the many issues of environmental and human toxicology, numerous research methods for application in the laboratory, but also model ecosystems and field studies have been developed.

2. Research topics

The research of the Human and environmental toxicology group focuses on

- environmental behaviour and toxicokinetics and -dynamics of cyanobacterial toxins, e.g. microcystins or anabaenopeptolins, or marine biotoxins, e.g. ciguatoxins, on various organs, like liver, lungs, kidneys and the CNS
- nephrotoxic and cancerogenic effects of plant toxins, e.g. aristolochic acid, or mycotoxins, e.g. ochratoxin
- development of a suitable test-system for nephrotoxins in humans, with a special focus on physiological oxygen concentrations (PhysOx)

3. Course

The participants will work under supervision on subprojects of current research work. The course begins every morning at 8:15 a.m. sharp. On the first day of the Advanced Course participants will be informed about individual projects, the respective supervisors and the experimental methods. Additionally, all organisational details will be discussed (notification also on our home page https://www.biologie.uni-konstanz.de/dietrich/ and the information board of Fachbereich Biologie). The first week will cover basic topics such as “Introduction into Cell Culture” and “General Aspects of Lab Work”. During this first week a project plan will be worked out by the participants and presented at the end of the week to the group members. During week 2 to 5 from 8.15 – 10.00 a.m. daily lectures and workshops with different speakers of the team will take place. Optionally, seminars with external speakers will amend the seminar schedule. Literature seminars will take place on a regular basis with the aim to discuss methodical aspects, learn to judge publication in regard of their respective scientific impact, and intensify the knowledge in the chosen research topic. The practical part of the project will be performed between week 2 and 5, finalised and the project results will be presented in week 6 in form of a poster. At the end of the course each participant will submit a scientific protocol of his/her work in the style of a publication. This course can be used as preparation for a future master thesis.

Theoretical part (lectures, seminars and workshops)

- Part A: laboratory safety, scientific writing and test documentation, math & statistics
- Part B: general toxicology, e.g. behaviour of xenobiotics in the organism (ADME), organ toxicology and pathology (liver, kidney, lung, nervous and hormone system), carcinogenesis, molecular toxicology
- Part C: environmental toxicology, transport and environmental behaviour of xenobiotics, aquatic toxicology, terrestrial toxicology, risk assessment of toxic substances
- Part D: „case studies“ in the field of environmental toxicology, applied toxicology, seminars on related scientific topics with speakers from different research groups and academic institutes

Experimental part (applied methodology)

- Analytics (HPLC, UV-VIS, Mass Spectrometry)
- Cell biology: stable cell lines, primary cells from various species and organs, cytotoxicity assays, cell proliferation analysis, transfection
- Protein biochemistry: Western blotting, ELISA, immunocytochemistry, FACS analyses, enzyme kinetics, cellular transport studies
- Molecular biology: DNA/RNA isolation from cells or tissue, PCR, real-timePCR, screening of a cDNA library
- Data evaluation and interpretation, statistics

For lab course participants of the VTK:

In order to pass this course, lab course participants need to fulfil the following requirements:

- regular participation in all lectures, including introductory project presentation (week 1), final presentation of the project results (week 6),
- power-point presentation of specific publications at the literature seminars
- practical work under supervision (week 1-6)
- continuous maintenance of a lab journal and timely delivery to the supervisor
- final presentation of course work as a power-point based talk or as a poster (decided dependent on pandemic situation, for example) at the end of the course (graded for Life Science students in accordance with study requirements)
- timely submission of a complete work report in English in manuscript form, compiled in accordance with the general rules for scientific writing and the guidance for authors provided at: https://www.journals.elsevier.com/chemico-biological-interactions

For students taking part only in lectures and not part of the VTK:

Participation in **ALL** lectures and seminars and a final written exam of 1 hour is mandatory in order to achieve the 5 ECTs.

Desirable state of knowledge of the participants

Prerequisites for a successful participation of this course are basic knowledge in “Molecular Toxicology” or “Disease Biology” (Prof. A. Bürkle), “In vitro Toxicology” (Prof. M. Leist), and “Environmental Toxicology” (Prof. D. Dietrich). Basic knowledge of statistics, profound expertise in using of computers, especially of current standard programs, like MS-Office, digital imaging and graphics. In particular, to allow experimentation with primary cell cultures, sufficient protection from hepatitis and tetanus via prior vaccination is mandatory.

4. Recommended literature

- “Molekulare Pharmakologie und Toxikologie“ Thomas Efferth, Springer Verlag (2006)
- “Toxikologie“, Hans Marquardt, Siegfrid G. Schäfer und Holger Barth, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 4. Auflage (2019)
- “Allgemeine und Spezielle Pharmakologie und Toxikologie“ W. Forth, D. Henschler, W. Rummel, Elsevier, München (2013)

Project related literature:

- Piossek F, Beneke S, Schlichenmaier N, Mucic G, Drewitz S, Dietrich DR. (2022): Physiological oxygen and co-culture with human fibroblasts facilitate in vivo-like properties in human renal proximal tubular epithelial cells. ***Chem Biol Interact***. 361: 109959. doi: 10.1016/j.cbi.2022.109959.
- Weisbrod B, Riehle E, Helmer M, Martin-Creuzburg D, Dietrich DR. (2020): Can toxin warfare against fungal parasitism influence short-term Dolichospermum bloom dynamics? - A field observation. ***Harmful Algae*** 99:101915. doi: 10.1016/j.hal.2020.101915.
- Bastek, H, Zubel, T., Stemmer, K., Mangerich, A., Beneke, S., Dietrich, D.R. (2019): Comparison of Aristolochic acid I derived DNA adduct levels in human renal toxicity models. ***Toxicology***, 420:29-38. doi: 10.1016/j.tox.2019.03.013.

- Altaner, S., Puddick, J., Fessard, V., Feurstein, D., Zemskov, I., Wittmann, V., and Dietrich, D.R. (2019): Simultaneous detection of 14 microcystin congeners from tissue samples using UPLC- ESI-MS/MS and two different deuterated synthetic microcystins as internal standards. ***Toxins***, 10.3390/toxins11070388.
- Altaner, S., Jaeger, S., Fotler, R., Zemskov, I., Wittmann, V., Schreiber, F., and Dietrich, D.R. (2019): Machine learning prediction of cyanobacterial toxin (microcystin) toxicodynamics in humans. ***ALTEX***, doi: 10.14573/altex.1904031.
- Secker, P.F., Schlichenmaier, N., Beilmann, M., Deschl, U. and Dietrich, D.R. (2019): Functional transepithelial transport measurements to detect nephrotoxicity *in vitro* using the RPTEC/TERT1 cell line. ***Arch. Toxicol.***, doi: 10.1007/s00204-019-02469-8.
- Basteck, H., Zubel, T., Stemmer, K., Mangerich, A., Beneke, S., and Dietrich, D.R. (2019): Comparison of Aristolochic acid I derived DNA adduct levels in human renal toxicity models. Toxicology, 420: 29-38, doi: 10.1016/j.tox.2019.03.013.
- Kleinteich, J., Puddick, J., Wood, S.A., Hildebrand, F., Laughinghouse IV, H.D., Pearce, D.A., Dietrich, D.R. and Wilmotte, A. (2018): Toxic cyanobacteria in Svalbard: Chemical diversity of microcystins detected using a liquid chromatography tandem mass spectrometry precursor screening method. ***Toxins***, *10*(4):147-161, doi: 10.3390/toxins10040147.
- Secker, P.F., Beneke, S., Schlichenmaier, N., Delp, J., Gutbier, S., Leist, M., and Dietrich, D.R. (2018): Canagliflozin mediated dual inhibition of mitochondrial glutamate dehydrogenase and complex I: an off-target adverse effect. ***Cell Death and Disease***, 9(2):226-238, doi: 10.1038/s41419-018-0273-y
- Secker, P.F., Luks, L., Schlichenmaier, N., and Dietrich, D.R. (2018): RPTEC/TERT1 cells form highly differentiated tubules when cultured in a 3D matrix. ***ALTEX***, 35(2):223-234. doi: 10.14573/altex.1710181.
- Maier, M.Y., Luks, L., Baudendistel, O., Wittmann, V., and Dietrich, D.R. (2017): Identification of d-amino acid oxidase and propiverine interaction partners and their potential role in the propiverine-mediated nephropathy. ***Chemico-Biological Interactions***, 281:69-80, doi: 10.1016/j.cbi.2017.12.023.
- Kleinteich, J., Hildebrand, F., Bahram, M., Voigt, A.Y., Wood, S.A., Jungblut, A.D., Küpper, F.C., Quesada, A., Camacho, A., Pearce, D.A., Convey, P., Vincent, W.F., Zarfl, C., Bork, P., Dietrich, D.R. (2017): Pole-to-pole connections: Similarities between Arctic and Antarctic microbiomes and their vulnerability to environmental change. ***Frontiers in Ecology and Evolution***, doi: 10.3389/fevo.2017.00137.
- Steiner, K., Wood, S.A., Puddick, J., Hawes, I., Dietrich, D.R. and Hamilton, D.P. (2017): Shifts in bacterial community structure, activity and microcystins associated with formation and breakdown of a cyanobacterial scum. ***Aquatic Microbial Ecology***, Vol. 80: 243–256, 2017, doi: 10.3354/ame01852.
- Bond, G.G. and Dietrich D.R. (2017): Further thoughts on limitations, uncertainties and competing interpretations regarding chemical exposures and diabetes. ***J Epidemiol Community Health***, doi:10.1136/jech-2017-209451.
- Bond, G.G. and Dietrich D.R. (2017): Human Cost Burden of Exposure to Endocrine Disrupting Chemicals — A Critical Review. ***Arch. Toxicol.,*** 91(8), 2745-2762. doi: 10.1007/s00204-017-1985-y.
- Luks, L., Maier, M. Y., Sacchi, S., Pollegioni, L., and Dietrich, D. R. (2017): Understanding renal nuclear protein accumulation: an in vitro approach to explain an in vivo phenomenon. ***Arch. Toxicol.,*** doi: 10.1007/s00204-017-1970-5.
- Altaner, S., Puddick, J., Wood, S.A., and Dietrich, D.R. (2017): Adsorption of ten microcystin congeners to common laboratory-ware is solvent and surface dependent. ***Toxins,*** 9(4), 129; doi:10.3390/toxins9040129.
- Klaus, V., Bastek, H., Damme, K., Collins, L., Frötschl, R., Benda, N., Lutter, D., Ellinger-Ziegelbauer, H., Swenberg, J.A., Dietrich, D.R., and Stemmer, K. (2017): Time-matched analysis of DNA adduct formation and early gene expression as predictive tool for renal carcinogenesis in methylazoxymethanol acetate treated Eker rats. ***Arch. Toxicol.***, 91(10):3427-3438. doi:10.1007/s00204-017-1953-6.

Advanced Course "Environmental Genomics"

Coordinator: Prof. Dr. Laura Epp
Supervisors: Prof. Dr. Laura Epp, Dr. Gisela Kopp

General research topic

Current advances in molecular genetic techniques offer the possibility to investigate present and past biodiversity using DNA extracted directly from environmental samples, such as water or sediments. This environmental DNA (eDNA) is shed into the environment by all organisms, both microbial and macrobial, and it can be used to identify species and gain genomic information. We can thus analyse (genomic) diversity in space and through time across centuries and millennia, both at the level of species composition of biotic communities and within single species.
We apply these techniques to study questions of ecosystem history and current environmental change.

Structure and objectives of the course

The course is structured around small research projects related to ongoing work, in which you will learn the **practical steps** of environmental DNA analyses, from sampling through wet-lab methods to bioinformatics.
In accompanying **lectures and workshops**, you will be introduced to the underlying theoretical principles of environmental DNA analyses and will learn analytical skills as well as scientific writing and project planning.
In a **literature seminar** you will present and discuss papers highlighting current applications of environmental DNA in ecology and paleoecology.

Objectives

In this course you will

- gain a thorough current understanding and practical experience of the application and uses of environmental DNA in ecological research
- learn how to acquire and analyze different types of eDNA data by designing your own reactions for eDNA (bioinformatically and in the lab)
- acquire skills in designing and conducting a research project
- gain an understanding of the scales and causes of ecosystem changes at different timescales (centennial, millenial).
- expand your presentation skills

Techniques and theoretical concepts covered

- sampling and extraction of environmental DNA.
- specificities of working with ancient and degraded DNA.
- bioinformatic design of reactions to trace target organisms in environmental samples.
- wet-lab evaluation and application of these reactions: (quantitative) PCR and DNA-metabarcoding.
- bioinformatic analyses of NGS sequencing data from environmental samples
- taxonomic assignments of DNA sequences using reference databases

Literature

Book

Taberlet P, Bonin A, Zinger L, Coissac E (2018) *Environmental DNA. For Biodiversity Research and Monitoring* Oxford University Press, Oxford, UK.

Articles

Domaizon I, Winegardner A, Capo E, Gauthier J, Gregory-Eaves I (2017) DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. *Journal of Paleolimnology* **58**, 1-21.

Ficetola GF, Coissac E, Zundel S*, et al.* (2010) An *In silico* approach for the evaluation of DNA barcodes. *Bmc Genomics* **11**, 434.

Parducci L, Bennett KD, Ficetola GF*, et al.* (2017) Ancient plant DNA in lake sediments. *New Phytologist* **214**, 924-942.

Thomsen PF, Willerslev E (2015) Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* **183**, 4-18.

Valentini A, Taberlet P, Miaud C*, et al.* (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology* **25**, 929-942.

Advanced Course **"Applied Bioinformatics for Studying Health and Disease"**

Coordinator: Prof. Dr. Andreas Gruber

Topic of the course

Understanding the molecular basis of healthy cells enables the identification of alterations that are key to human disease. Molecular cell biology can be studied on a systems scale by sequencing whole genomes and/or measuring the activity of thousands of genes in parallel, both of which can be achieved using next-generation sequencing (NGS). Over the past decade, NGS technologies significantly advanced and became broadly available for basic research and clinical diagnostics. However, even though the availability of NGS datasets increases on a fast pace, the information content that NGS datasets harbour is far from being exploited. In recent years, a large toolbox of data science approaches has been developed. These tools make it now possible to study many aspects of molecular cell biology, and have great potential to drastically improve our understanding of healthy cells, but also of molecular dysregulations underlying human disease.

In our research we analyse high-throughput sequencing data by applying available as well as in-house developed, machine learning-based data science approaches to study healthy and diseased cellular states in human (*Homo sapiens*) and mouse (*Mus musculus*). In particular, we are interested in gene expression regulation in the context of:

(i) basic cellular processes and their molecular regulation (e.g. cell adhesion, differentiation and identity as well as transcription, splicing and 3' end processing)
(ii) the immune system (e.g. immune cell activation and function as well as the cellular response to viral infections and virus-host cell interactions)
(iii) cancer development and progression (e.g. mutational patterns, dysregulated transcript isoform expression programs)

Aims and contents of the course

This course aims to provide you with the theoretical background and practical fundamentals of NGS data analysis. In lectures and seminars, you will gather theoretical knowledge on high-throughput sequencing technologies and data science tools for the analysis of large-scale NGS datasets. For the practical hands-on sessions, you will get assigned a small research project from one of the above mentioned topics. In this course you will:

1. get skilled in **searching databases** for genomes, gene annotations, and datasets
2. understand **NGS data** and get experienced in data **quality control**
3. learn **Bash** and **R programming** basics in a Linux environment
4. **map NGS data** to a reference genome (human or mouse) using command line tools
5. learn how to **infer global gene expression** from NGS data
6. understand the concepts behind identifying **differential expressed genes**
7. gain data science skills in the R programming language, enabling you to investigate **gene expression at systems scale** (e.g. by applying **clustering** algorithms)
8. identify **transcriptional regulators** that explain global changes in gene expression
9. read, understand and present **bioinformatics literature**
10. relate your data analysis results to the **current scientific knowledge / publications**

11. design a **research project**, **follow-up analyses** and **validation experiments**
12. **document**, **write-up** and **present** your **analysis and research results**, using FAIR (Findable, Accessible, Interoperable, Reusable) data principles, which will be introduced, discussed and applied throughout the course.

Requirements to pass the course

- The course is structured such that the practical tasks of every course day build upon the theory taught in the morning lecture and the results achieved within the previous day(s). That's why the attendance and active participation in all the theoretical lectures, seminars and practical sessions is inevitable for following the course and completing the practical exercises, which are required for writing and handing in the scientific report (see below). If you have any questions or needs, please get in contact (gruber[at]uni-konstanz.de) to discuss it prior registering for the course. Thank you!
- Presentation and discussion of an assigned scientific publication (literature seminar).
- Submission of a scientific report and presentation of the research project results.

In principle, all of the requirements mentioned above can be completed during the course. However, the final reports can be handed in up to four weeks after the course has ended.

Qualification / Required pre-knowledge

It is expected, that students have basic cell biology knowledge and an interest in the molecular foundations of health and disease. Before every practical hands-on course the required background knowledge will be presented and extensively discussed within theoretical sessions (lectures and seminars). Importantly, the practical hands-on sessions will build up from the very basics and thus no prior programming or data science skills are required. However, to be qualified for the course students should be interested in gaining data science skills and be willing to touch base with data analysis challenges. In the course we will be using bash commands (Linux) and the programming languages R and Python. However, the overarching goal of the performed analyses is cell biology research, ultimately aiming to extend our knowledge about the molecular foundations of health and disease.

Further reading

Reviews on Relevant Topics and Example Data Science Tools for Analysing NGS Data

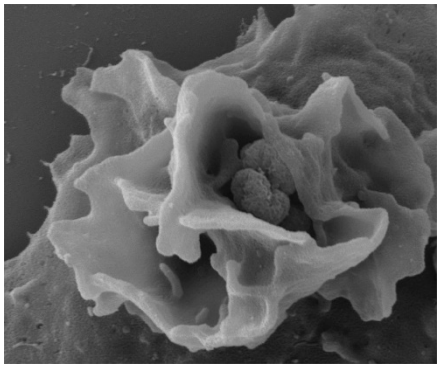
- Gruber, A. J. et al. (2019) "Alternative cleavage and polyadenylation in health and disease", *Nature Reviews Genetics.* doi: 10.1038/s41576-019-0145-z.
- Gruber, A. J. et al. (2013) "Modulation of epigenetic regulators and cell fate decisions by miRNAs", *Epigenomics*. doi: 10.2217/epi.13.65.
- Stark, R. et al. (2019) "RNA sequencing: the teenage years", *Nature Reviews Genetics*. doi: 10.1038/s41576-019-0150-2.
- Gruber, A. J. et al. (2018) "Terminal exon characterization with TECtool reveals an abundance of cell-specific isoforms", *Nature Methods*. doi: 10.1038/s41592-018-0114-z.
- Dobin, A. et al. (2013) "STAR: ultrafast universal RNA-seq aligner", *Bioinformatics.* doi: 10.1093/bioinformatics/bts635.
- Gruber, A. J. et al. (2018) "Discovery of physiological and cancer-related regulators of 3' UTR processing with KAPAC", *Genome Biology*. doi: 10.1186/s13059-018-1415-3.

Advanced Course "Cell Biology – Cell Adhesion and Signal Transduction"

Coordinator: Prof. Dr. Christof R. Hauck

1. Background

Cellular adhesion receptors are critical for many cellular processes by providing stable connections between cells and the extracellular matrix. Cellular adhesion receptors also mediate cell-cell interactions, together supporting the formation of tissues and organs in multicellular animals. Besides their mechanical function, adhesion receptors of the integrin, cadherin or immunoglobulin protein families help to organize the intracellular cytoskeleton and contribute to signal transduction processes that guide cell proliferation, differentiation and survival, as well as cell motility. Moreover, the surface exposed nature of cell adhesion receptors as well as their functional connection to the cytoskeleton makes these proteins preferred targets for viral and bacterial pathogens.



In the frame of this advanced laboratory course, we try to answer some pertinent questions in the field of cell adhesion receptors. In particular, we will focus on integrins and the regulation of their function as well as on immunoglobulin-related cell adhesion molecules of the CEACAM family and their role as receptors for pathogenic bacteria.

In this context, we will employ molecular biological methods to generate recombinant DNA and proteins, which will be used for plasmid transfection, viral or protein transduction, and RNAi- or CRISPR/Cas-mediated knock-down of protein expression. Genetically manipulated cells will be employed in functional assays of cell adhesion, cell migration, cytoskeletal organization, or endocytosis/phagocytosis and will be further investigated by advanced microscopy techniques or flow cytometry (FACS analysis). As protein-protein interactions are a prominent characteristic of signalling cascades in eukaryotic cells, we apply a diverse set of methods (pull-down analyses, co-immunoprecipitation, FRET measurements, protein domain microarrays) to identify and monitor the regulated association of proteins. Finally, signal transduction pathways often regulate characteristic gene expression events, which will be studied by quantitative real-time PCR and luciferase-based promoter assays.

2. Content of the Advanced Laboratory Course

A) Theoretical Part: Lectures and Seminar

The lecture part is spanning the first four weeks of the course.

In the first part, the lectures **cover** the following **areas of cell biology** : adhesion molecules: integrins, IgCAMs; focal adhesions, protein phosphorylation: kinases/ phosphatases, adapter proteins/ protein-protein-interaction domains/ SH3-domains/ SH2-domains / ITAMs/ITIMs, lipid phosphorylation: kinases/ phosphatases, endocytosis, vesicle trafficking, autophagy, dynamics of the actin cytoskeleton, regulation of cell migration, phagocytosis, innate immunity, cellular microbiology. Selected pathogenic bacteria will be presented: *Neisseria*:

medical aspects, microbiology, host adaptation, virulence factors: LOS, pili, opa proteins, IgA protease, porin, *Staphylococci* and *Streptococci*: microbiology, medical aspects, FnBP: structure and function.
In the second part of the lecture series, common **experimental strategies**, and the **principles, application and pitfalls of** the used **methodology** will be discussed, in particular we talk about cell culture, hybridoma cells, monoclonal antibodies, manipulation of cells – transfection, transduction, RNA-interference (RNAi) with shRNA, generation of transgenic and conditional knock-out mice, genome editing strategies via CRISPR/Cas9, fluorescence labeling and –detection, flow cytometry, electron microscopy, advanced fluorescence microscopy, protein detection, epitope-tagging, GFP-fusion, detection of protein-protein-interactions, affinity purification of proteins: GST, His-tag, antibodies.
In the last two weeks of the course, a literature seminar is held, where current publications and breakthrough findings in the above mentioned areas will be presented and discussed in detail. Each student presents one original paper.

B) Experimental Part

Individual projects will be conducted alongside existing lines of investigation in the field of cell adhesion receptors and address the topics discussed in the lectures. Examples of recent projects include: CEACAM-initiated signalling in granulocytes; Phosphatase PPM1F in leukocyte adhesion; CRISPR/Cas-mediated knock-out of LRIG-2; From human CEA to chimpanzee CEA via site-directed mutagenesis; Light-induced protein-protein interactions in living cells; Expression of functional protein kinases in bacteria; Role of PPM family phosphatases in cell adhesion

3. Requirements

Participation in the practical part of this course requires the physical ability to conduct experimental research in a laboratory operating on Security level 2 (S2), which entails increased exposure to potential biological hazards. A specific introduction into laboratory safety is mandatory and will be given on the first day of the course. To participate in the practical and/or theoretical part, the lectures Cell Biology I and II, Biochemistry II, and Immunology or equivalents to these lectures must have been followed and passed. There will be a qualifying written exam prior to the course, which needs to be passed.
To pass the course and obtain the credits (for life science students in addition being marked) the following requirements need to be met: Active participation in the lectures, power point presentation in the literature seminar, research project and presentation of the project results in the form of a scientific poster or a written report.

4. Literature

Pollard/Earnshaw „Cell Biology“ 2nd edition (Sauners/Elsevier)
Easy Reading edition by Spektrum Akademischer Verlag, Berlin – Heidelberg
Signatur der UB Konstanz: lbs 670/p65b

Advanced Course "Physiology and Biochemistry of Plants"

Coordinator: Prof. Dr. Erika Isono

For the **lab work**, we offer small projects from our ongoing research projects that uses diverse methods in **molecular biology**, **protein biochemistry** and **cell biology**. Projects will be presented on the first day of the course and can be chosen by the participants. **Lectures** will focus on state-of-the-art techniques as well as current topics in the research field of **ubiquitin biology, endocytosis and autophag**y and are complemented with a journal club and final presentations held by participants.

Keywords: *ubiquitin, membrane trafficking, endocytosis, autophagy, posttranslational modifications*

Methods and Techniques:

Molecular biology: Isolation of RNA and DNA, PCR, qRT-PCR, molecular cloning (overexpression constructs, epitope- or GFP-tagged proteins).

Protein biochemistry: Recombinant protein expression and purification, *in vitro* binding assays, MicroScale Thermophoresis, analysis of posttranslational modifications, *in vitro* DUB assays, SDS-PAGE and Western blotting, yeast two-hybrid, clathrin coated vesicle (CCV) isolation.

Imaging: Epifluorescence and confocal microscopy using Arabidopsis seedlings of Arabidopsis cell cultures. Depending on the project, electron microscopic analysis will be applied.

Genetics: Isolation and characterisation of knockout and knockdown mutants.

***Projects*:**

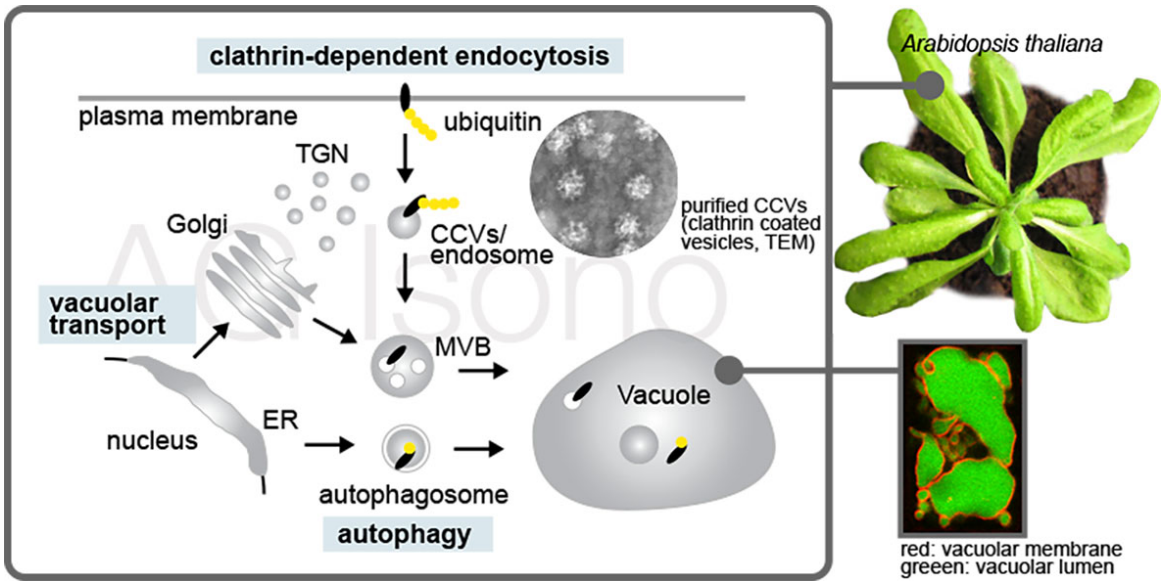


Figure 1: Ubiquitin-dependent membrane trafficking. Membrane proteins are endocytosed via clathrin coated vesicles CCVs) upon ubiquitination and transported via the endosomal trafficking pathway to the vacuole for degradation. Proteins for selective autophagic degradation are similarly transported to the vacuole by autophagosomes upon ubiquitination. The regulation of protein stability is crucial for almost all physiological pathways.

***Background*:** Plasma membrane receptors and transporters play crucial roles in the coordination of extracellular stimuli and intracellular responses that enable plants to react readily to environmental changes and stress. Regulation of the abundance of plasma membrane receptors by endocytosis-dependent protein degradation in the vacuole is an important regulatory mechanism in a variety of signaling pathways such as phytohormone signaling and biotic- and abiotic stress responses.

Regulation of ubiquitination in intracellular membrane trafficking of plants

We aim to understand the molecular mechanisms by which ubiquitin is regulating protein degradation in the model plant *Arabidopsis thaliana* with a special focus on endocytosis and the subsequent protein transport to the vacuole (**Figure 1**). Ubiquitination is a post-translational process in which ubiquitin, a highly conserved small protein of 76 aa, is covalently attached to substrate proteins and is, among others, a signal for selective protein degradation. Depending on the modification type (mono-ubiquitination, K48, K63 and other linkages), ubiquitination can serve as signals in various cellular processes. Since many of these target proteins are key regulatory factors of different signaling pathways, it is essential that the processes of ubiquitin conjugation and deconjugation are strictly regulated.

Function of deubiquitinating enzymes in plants

The conjugation of ubiquitin molecules to its target proteins is a process that is mediated by the activity of E1, E2 and E3, in which the combination of the E2 and E3 enzymes usually defines the ubiquitin chain-type to be conjugated as well as substrate specificity. Deubiquitination, on the other hand, is mostly carried out by a single DUB (**Figure 2**). Though the ubiquitination machinery plays a key role in determining target protein stability, DUBs can also influence target protein fate by removing the ubiquitin signals from the protein. In most of the cases the interaction of the DUB with the conjugated ubiquitin chain, rather than with the target protein itself, is sufficient for target deubiquitination. The spatio-temporal regulation of DUBs is therefore crucial for deubiquitination of the correct target proteins. Our aim is to characterize Arabidopsis DUBs that are involved in the regulation of protein stability and to understand their physiological functions.

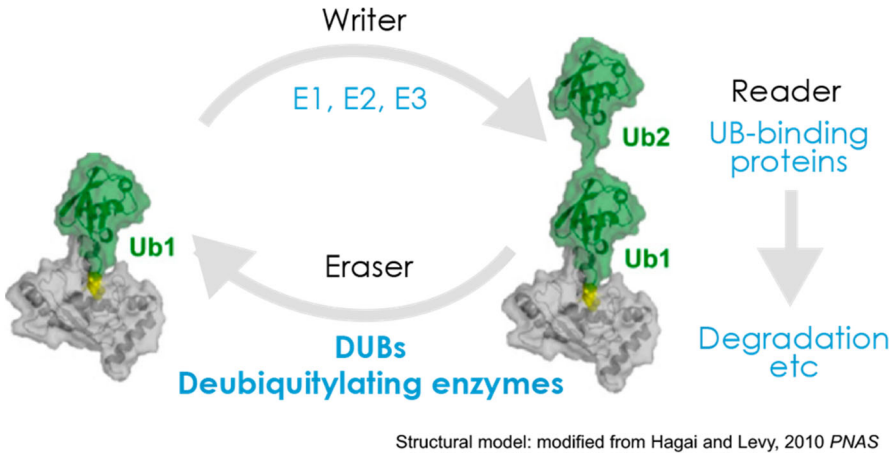


Figure 2: Ubiquitination is a reversible process. A protein, targeted for degradation, is labeled by ubiquitin through the enzyme cascade of E1, E2 and E3. Deubiquitinating enzymes (DUBs) counteract this enzyme cascade and remove the ubiquitin from the target protein.

Advanced Course "Global change ecology and plants"

Coordinator: Prof. Dr. Mark van Kleunen

1. Introduction

We are living in the ***Anthropocene***, a period of unprecedented rapid environmental change. Human actions result in climate change, habitat destruction and fragmentation, pollution, eutrophication and biological invasions. These changes impose a challenge to many organisms, and opportunities to others. However, the exact ecological and evolutionary consequences of global change, and the impacts on ecosystem functions and services (e.g. supply of food and clean water) are still poorly understood.

A few impressions of our research. (a) our global-warming simulation facility, (b) a typical greenhouse experiment, (c) experimental manipulation of nutrient availability.

We are interested in the basic questions of how phenotypic variation in functional traits is shaped by environmental and genetic variation, and how these traits interact with extrinsic factors to determine the success of organisms - plants in particular. As ecology and evolution are intrinsically related, we also address evolutionary questions. The approaches that we use include experiments in greenhouse and garden as well as field studies, but we also perform global syntheses of available knowledge through database studies and meta-analyses of published research.

2. Objectives

You will learn the “what” and “how” of Global Change Biology and Plant Ecology:

- What are the major…
    - **drivers** and **impacts** of global environmental change.
    - **questions** in global-change and plant ecology.
    - **methods** and approaches in plant (evolutionary) ecology.
- How to:
    - **test hypotheses** in ecology.
    - design, set-up, and run **experiments**.
    - **analyse the data** that you collect.
    - **present** your results (poster presentation, oral presentation, paper).

3. Contents of the course

The course consists of a combination of lectures, workshops, seminars, excursions and doing experiments. In the lectures, you will learn the major theories in global-change and plant ecology. In the workshops, you will learn important skills, such as **experimental design**, **statistical analysis**, **scientific writing** and how to make a poster. In the seminars, you will present and discuss recent publications. During a one-day excursion to the mountains, you will learn about alpine ecology and take inspiration for a research proposal, and during another excursion you will gain insights in managing a nature reserve. In a small group, **you will design and run your own research project**, during which the acquired methods, skills and knowledge will be put into practice. Overall, you will obtain experience in all aspects of scientific research – from observing the natural world to presenting your completed research.

4. Requirements and passing the course

Requirements are basic knowledge of and a keen interest in ecology and evolution. To pass the course, you have to actively participate, present and discuss papers and your project results, and write a paper on your research project.

For more information on our group, visit our https://www.biologie.uni-konstanz.de/kleunen/

Advanced Course "Physiology, Ecology and Molecular Biology of Algae"

Coordinator: Prof. Dr. Peter G. Kroth

Algae in their variety of forms, colors and genetic constitutions are fascinating organisms that contribute considerably to life on this planet. We therefore are working on physiological, cellular, molecular and ecological aspects of diatoms to increase our understanding of their molecular and physiological functions. The current projects include photoreceptors, regulation of photosynthesis, the metabolism of storage carbohydrates, genomic aspects of diatoms, biotechnological issues, and the role of diatoms in biofilms in Lake Constance.

Genetic methods: Cloning in *E. coli*, Transformation of diatoms (Particle Gun, conjugation), Real-Time-qPCR, RNA-Seq, Genome editing of algae via TALEN and CRISPR/Cas9, expression of GFP fusion proteins, Northern blots, Southern blots, yeast-1-hybrid analyses, and Transcriptomics.

Biochemical methods: Isolation of organelles, isolation of proteins, Western Blots, heterologous expression and purification of proteins in *E.coli*, SDS gel electrophoresis, 2D-electrophoresis, centrifugation, gel filtration, HPLC analyses of pigments, fluorescence quantification of lipids, enzymatic quantification of carbohydrates, extraction and characterization of signal molecules.

Further methods: fluorescence microscopy and confocal laser-scanning microscopy, particle- and fluorescence-based cell counting, biofilm growth chambers, cell cultivation in bioreactors.

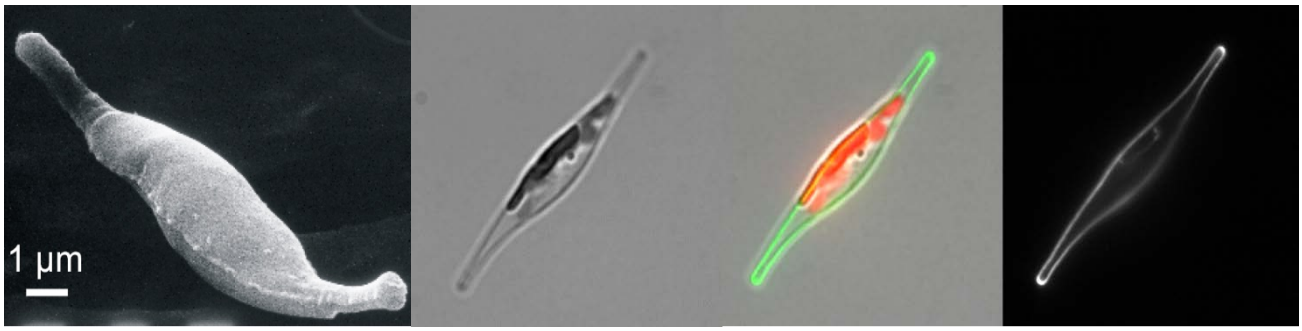


Fig 1: The diatom *Phaeodactylum tricornutum* is one of the model systems in our lab. Left: SEM image. Next images: expression of GFP fused to a plasma membrane protein; from left to right: bright field, GFP and chlorophyll fluorescence, GFP fluorescence only.

Carbohydrate Storage in Diatoms Occurs in Vacuoles

Chrysolaminarin is the main storage compound in diatoms, a glucan consisting of linear 1,3-β-chains with 1,6-ß-branches. In diatoms, chrysolaminarin is stored in intracellular vacuoles in a non-crystalline form. The biosynthetic pathway of chrysolaminarin in diatoms as well as the involved enzymes so far are poorly investigated. Therefore, we aim at studying this pathway in the diatom model system *Phaeodactylum tricornutum*. We screened the respective genome and identified genes encoding enzymes that are potentially involved in chrysolaminarin synthesis or modification, including UDP glucose pyrophosphorylases, a ß-glucan synthase and ß-1,6-transglycosylases. By expression of GFP fusion proteins in *P. tricornutum*, we determined the respective intracellular localizations of the proteins. We also investigate the functionality of the glucan synthase and the putative transglycosylases from *P. tricornutum*, by applying gene silencing techniques or by complementation of transglycosylase-deficient yeast strains. Silencing of the glucan synthase yielded a number of phenotypic cellular changes including reduced growth, a higher NPQ and a changed thylakoid morphology. Phylogenetic analyses finally revealed that these proteins are conserved between the Stramenopiles, a taxonomic group including diatom, brown algae and non-photosynthetic Oomycetes.

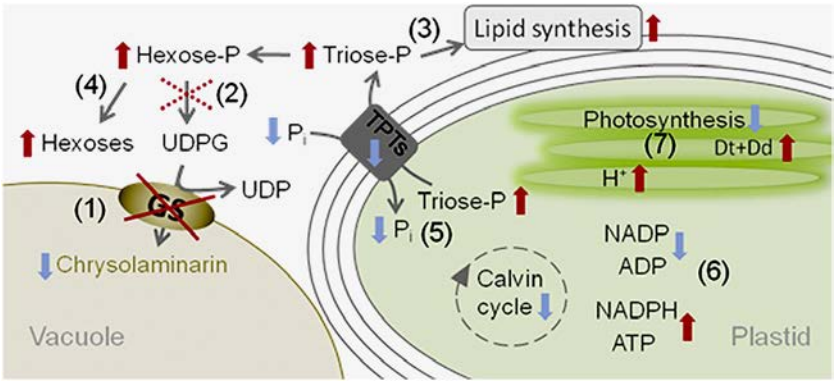


Fig. 2: Chrysolaminarin storage in diatoms. Proposed scenario explaining the observed effects of reduced glucan synthase (*Pt*BGS) activity in *P. tricornutum*. From: Huang et al. (2018), PNAS.

Aureochromes, new and very fast blue light photoreceptors in algae

Recently, a new type of photoreceptor has been discovered, which is only found in stramenopile algae. Aureochromes are unique blue light receptors as they simultaneously represent transcription factors, thus light driven gene switches. We have generated TALEN-based (Transcription activator-like effector nucleases) aureochrome deletion mutants. Characterization of Aureochrome 1a knockout cell lines indicates non-overlapping functions, which cannot be compensated for by the other isoforms. Moreover, knocking out the Aureochrome 1a gene revealed that the protein is one of the most effective master switches in the eukaryotic kingdom. We are using transcriptomic data as well as cell biology tools to identify interaction partners and transcription factors to understand how light is converted into a cellular response.

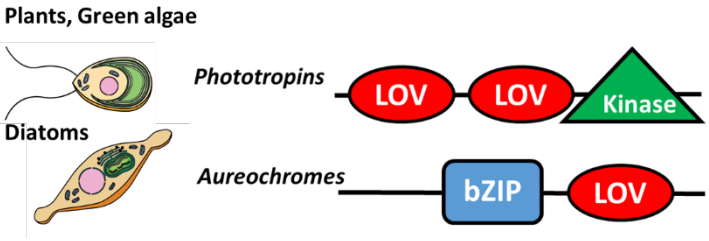


Fig. 5: While plants use phototropins as photoreceptors, diatoms have aureochromes. Both photoreceptors contain LOV domains (Light, oxygen, voltage), however, while phototropins initiate signaling cascades via a kinase, aureochromes are transcription factors that can start transcription of a target gene immediately.

The role of Aureochromes for the diatom clock

Diatoms like other algae have internal clocks that help them to regulate diel programs that control the expression of gens during the day. We have evidence that the bZIP factor Aureochrome, together with the bHLH transcription factor RITMO1 are required to keep the daily rhythmic expression of genes. We therefore recentl started to use knockout mutants to identify further components of the molecular clock of the diatoms, and compare transcriptomic responses of wild type and mutant lines.

Annotation of Diatom Genomes and Development of Molecular Tools for Algae

Meanwhile a number of diatom genomes have been published, and our group is involved in the annotation process of several genome and metagenome projects on eukaryotic algae. The analysis of the genome uncovered a series of surprises. For example, many of the Calvin Cycle enzymes can be found in the genome in multiple copy numbers. We are also involved in developing a number of molecular tools for diatoms including gene silencing, complementation, and genome editing via TALENs and CRISPR/Cas9.

Characterization of diatoms in biofilms of Lake Constance

Phototrophic, epilithic biofilms are a typical feature of aquatic ecosystems. In order to study interactions between diatoms and bacteria in biofilms, we have isolated and identified different diatoms and associated satellite bacteria from Lake Constance. Interestingly, purified diatom cultures showed significant differences with respect to growth and biofilm formation when compared to the corresponding xenic cultures. The diatom *Achnanthidium minutissimum* forms capsule-like structures in the presence of an isolated bacterial strain, but not in axenic state. Interestingly, formation of capsules can be induced by adding substances secreted by the satellite bacteria. We recently sequenced the genome of this bacterium (Dow et al., 2020). Transcriptome studies show that *P. tricornutum* strongly responds to a presence of bacteria. We furthermore try to identify diatom receptors that identify bacterial signalling substances based on leucine-rich repeat (LRR) structures.

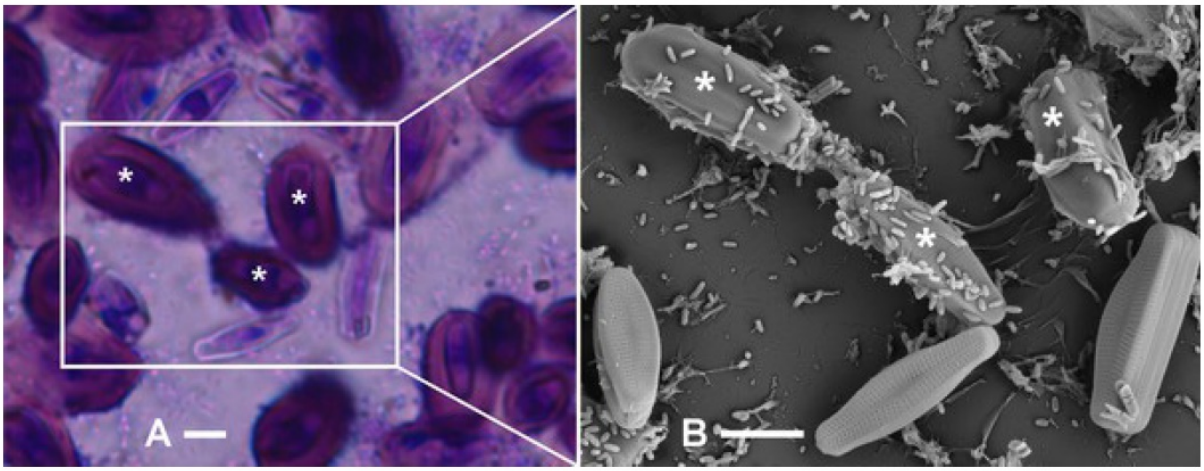


Fig. 6: Identification of *A. minutissimum* capsules (asterisks) by successive observation of cell clusters by first bright-field and then scanning electron microscopy of a xenic biofilm (scale bars: 5 µm).
(A) Bright-field micrograph of crystal violet (CV) stained, 31 days old culture. Encapsulated cells (asterisks) are strongly stained, while weak staining indicates few extracellular polymeric substances (EPS) on the frustule surfaces. (B) Scanning electron micrograph of the same cell cluster. Encapsulated cells (asterisks) are surrounded by an opaque material. Frustule pores are visible on cells that did not possess a capsule in the hydrated biofilm. Note also the unequal distribution of bacteria cells on capsules versus non-encapsulated frustules (Leinweber and Kroth, 2015).

Dinotoms are peculiar dinoflagellates using diatoms as endosymbionts

Dinotoms are dinoflagellates, unicellular eukaryotes, that have tertiary plastids derived from diatoms. They are excellent model organisms for studying the early evolutionary stages of plastids. Dinotom plastids are in evolutionary intermediate stages, which all other phototrophs had already passed through long time ago: all diatom organelles are still preserved within dinotoms, not just the plastids. Each dinotom species contains different species of diatoms, and these diatoms show different evolutionary stages from temporarily-maintained kleptoplastids to permanently-maintained endosymbionts (Yamada et al., 2019). Studying these host/endosymbionts with molecular tools allows insights into the very first processes of organelle establishment.

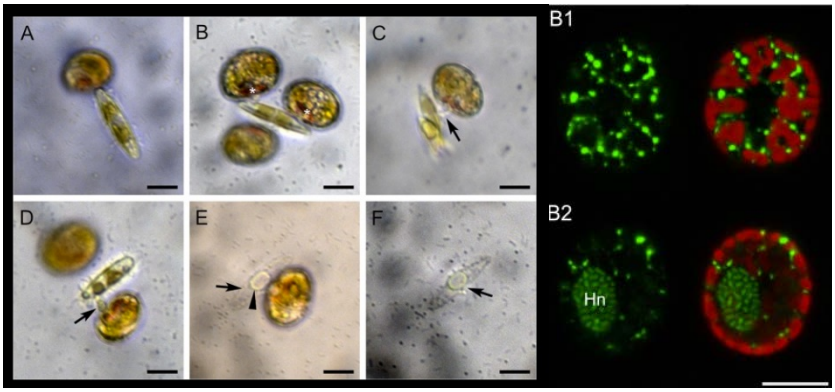


Fig. 7: (A-F) Diatom *Nitzschia cf. agnita* (strain IRTA-CC-152) being taken up by the dinoflagellate *Durinskia capensis*. (from Yamada et al., 2019). (B1-B2) Diatom nuclear dynamics in *Durinskia kwazulunatalensis* observed with CLSM. SYBR-Green stained nucleus = Green, MitoTracker Green stained mitochondria = green, Chlorophyll *a* autofluorescence = Red. located in the middle of the cell (C2); host dinoflagellate nucleus (Hn). Scale bar = 10 µm.

If you should be interested in algae and diatoms, you can find some of our recent publications here:

Yamada N, Lepetit B, Mann D, Sprecher B, Buck J, Bergmann P, Kroth PG, Bolton J, Dąbek P, Witkowski A, Kim S-Y, Trobajo R (2023) Prey preference in a kleptoplastic dinoflagellate is linked to photosynthetic performance. ISME J, https://doi.org/10.1038/s41396-023-01464-3

Yu G, Nakajima K, Gruber A, Rio Bartulos C, Schober A, Lepetit B, Yohannes E, Matsuda Y, Kroth PG (2022) Mitochondrial PEP Carboxylase contributes to carbon fixation in the diatom *Phaeodactylum tricornutum* at low inorganic carbon concentrations. New Phytologist 235: 1379-1393. https://doi.org/10.1111/nph.18268

Dow L, Morrissey KL, Willems A, Kroth PG (2020) Complete genome sequence of Dyadobacter sp. 32, isolated from a culture of the freshwater diatom *Cymbella microcephala*. Marine Genomics 100720. doi: 10.1016/j.margen.2019.100720.

Schober AF, Río Bártulos C, Bischoff A, Lepetit B, Gruber A, Kroth PG (2019) Organelle studies and proteome analyses on mitochondria and plastids fractions from the diatom *Thalassiosira pseudonana*. Plant and Cell Physiology 60: 1811-1828.

Yamada N, Bolton JJ, Trobajo R, Mann DG, Dąbek P, Witkowski A, Onuma T, Horiguchi T, Kroth, PG (2019) Discovery of a kleptoplastic 'dinotom' dinoflagellate and the unique nuclear dynamics of converting kleptoplastids to permanent plastids. Scientific Reports 9: 10474.

Huang W, Haferkamp I, Lepetit B, Molchanova M, Hou S, Jeblick W, Río Bártulos, Kroth PG (2018) Reduced vacuolar β-1,3-glucan synthesis affects carbohydrate metabolism as well as plastid homeostasis and structure in *Phaeodactylum tricornutum*. Proc Natl Acad Sci USA 115: 4791-4796.

Hess S, Lepetit B, Kroth PG, Mecking S (2017) Production of Chemicals from Microalgae Lipids - Status and Perspectives. Eur. J. Lipid Sci. Technol. 120: 1700152. DOI: 10.1002/ejlt.201700152.

Kroth PG, Wilhelm C, Kottke T (2017) An Update on Aureochromes: Phylogeny - Mechanism - Function. Journal of Plant Physiology 217: 20-26.

Serif M, Lepetit B, Weißert K, Kroth PG, Bartulos CR (2017) A fast and reliable strategy to generate TALEN-mediated gene knockouts in the diatom *Phaeodactylum tricornutum*. Algal Research 23: 186-195.

Leinweber K, Kroth PG (2015) Capsules of the diatom *Achnanthidium minutissimum* arise from fibrillar precursors and foster attachment of bacteria. PeerJ 3:e858 dx.doi.org/10.7717/peerj.858.

Windler M, Leinweber K, Rio Bartulos C, Philipp B, Kroth PG (2015) Biofilm and Capsule Formation of the Diatom *Achnanthidium minutissimum* are affected by a Bacterium. Journal of Phycology 51: 343-355.

Kroth PG (2015) The Biodiversity of Carbon Assimilation. Journal of Plant Physiology 172: 76-81.

Kroth, PG (2007) Genetic Transformation - a Tool to Study Protein Targeting in Diatoms. In: "Methods in Molecular Biology - Protein Targeting Protocols" (Van der Giezen, M., Ed.) Humana Press, 257-268.

Advanced Course "Novel *in vitro* methods in pharmacology and toxicology"

Coordinator: Prof. Dr. M. Leist

Introduction:

We develop human cell culture models to examine disease processes and toxicity in the nervous system. For models of developmental neurotoxicity, we use stem cells that differentiate to neural cells (neural stem cells, neurons, glial cells). We use different approaches to characterize the developmental changes that the cells undertake over time, and new methods for better control and characterization of the differentiation are being developed. We use such models to study toxicity of drugs and environmental compounds (in particular pesticides). We are working with EFSA, the OECD, the US EPA and we are partners in the biggest European research projects in this area. The course will give insight into those methods and into the projects with international partners. Methods will involve stem cell biology, cellular metabolism and signal transduction, neurobiology, epigenetic changes of developing cells and of cells exposed to stress, as well as characterizations of toxicity and cell death. Also, the current research in toxicology and activities of large industry and governmental organisations will be represented. Some of our analyses are performed on systems wide (e.g. whole transcriptome, metabolome) levels to get a comprehensive overview of cellular responses on a systems biology level. This also involves the use of some bioinformatics approaches. We are particularly interested in 1. characterizing stress response pathways in cells, related to oxidative, mitochondrial and proteotoxic stress (as observed in Parkinson's disease), and changes in neuronal functionality (impaired electrical activity).

Our current research interest, and therefore also the projects offered during the course are:

1. Development of a human neuronal model reflecting degeneration of dopaminergic neurons (relevant for Parkinson's disease). Introduction of reporter genes into human neurons by switchable vectors and examination of downstream pathomechanisms.
2. Cocultures of glial cells and neurons (also in 3D - organoids) to better study toxic processes e.g. of pesticides or proteasome inhibitors.
3. Examination of the pathways that determine the generation and activity of glial cells in the brain. Modulation of gliogenesis and neuroinflammation.
4. Differentiation of pluripotent stem cells to neuronal precursor cells and mature neurons: examination of transcriptional changes associated with differentiation; examination of differentiation processes sensitive to certain toxicants.
5. Examination of functional properties of "young neurons", such as the migration of neural crest cells or the neurite growth of neural precursors as targets of toxicity.
6. Study of calcium regulation and toxicity mechanisms on human peripheral neurons triggered by natural toxins from bacteria and algae.
7. Distribution and metabolism of chemicals in cell culture systems to better predict the fate in patients/exposed populations
8. Study of neuronal electrical networks with novel methods and new software tools.

The projects use cell culture technology, fluorescent staining and imaging technologies (high content automated imaging, pattern recognition and quantitative microscopy), FACS analysis, RT-PCR and SeaHorse mitochondrial analysis methods. Some involve lentiviral vector construction, Western blotting, electrical activity recording and several projects are linked to our interest in transcriptome and metabolome changes. This implies also data mining, statistics and visualization approaches.

Course theory:

The lectures will be in English. In the lecture part, a lot of cross-sectional topics from biochemistry, pharmacology, cell biology, immunology and molecular biology will be covered as they form the basis for cell culture models. We will cover many internationally-used toxicological test systems and explain the 3R (reduce, replace, refine) principle of moderate and rational animal protection, and its scientific basis. A particular focus will be our own research interest, i.e. human cell-based models for the pharmacology of neurodegenerative diseases (e.g. Parkinson's disease), and models that would predict chronic and developmental neurotoxicity in humans.
The course introduces the drug discovery processes and selected aspects of neuropharmacology and neuropathology. Some pre-knowledge in pharmacology and toxicology is STRONGLY advisable, as alternative methods cut across these disciplines in many examples. The lectures will cover the whole range of 3R methods, but they focus particularly on specific scientific interests of our group. Some lectures will be given by international guest researchers in forms of seminars or a minisymposium. In week 4 and 5 of the course, selected publications from the scientific literature will be presented by students (English presentations). The discussion will clarify methodic aspects, and the relation of the chosen topic to actual research problems. Students are guided to critically judge third party data. Ethical aspects and the presentation style will also be addressed.
Literature: all material will be provided during the lectures. Some introductory literature/movies on alternative methods, modern toxicology and on the use of stem cells will be provided on Ilias. Some literature overview can be gained from our website: http://cms.uni-konstanz.de/leist/research/ (if we manage to get it updated), from PubMed or Web of Science (https://publons.com/wos-op/researcher/2838554/marcel-leist/)

Requirements to enter the course:

The knowledge covered by the lecture series Human Biology, Pharmacology and Toxicology I, and Biochemistry II (or any equivalent) is required. Basic knowledge of immunology would be desirable. The status of general knowledge and the capability to perform simple lab routine calculations may be tested at the beginning of the course.

Course projects:

Projects are within the areas of nervous system degeneration and re-generation, including pathobiochemical mechanisms implicated in chronic neurodegenerative disease and neurotoxicity. They will follow the research projects run at that time in the research group (see above, also for techniques used). Knowledge of mechanisms will be translated into the development of improved in vitro test methods and potential treatment strategies of disease. The results of the student projects will be presented and discussed in the final week.
Students following only the theoretical part of the course will investigate a new or important technology, and present it to others in form of a poster

Performance evaluation:

To obtain a "Schein" (for life science students: obtain a mark); the following requirements need to be met: Attendance and active participation in the lectures; research project and presentation of its results; power point presentation at the literature seminar and subsequent discussion of the paper and its background (!). In case of good progress in the lab project, a poster on the results can be presented at the end of the course instead of the protocol preparation. Technology posters will be presented by 'theory students' as main assignment. A test of theoretical knowledge may be performed during the course, if performance cannot be judged otherwise. Missing time may need to be compensated, e.g. with a theory assignment.

Advanced Course "Bioinformatics and X-Ray Structural Analysis"

Coordinator: Prof. Dr. Olga Mayans

In order to understand living systems, it is essential to understand the function of their macromolecular constituents -such as proteins and nucleic acids. Macromolecules adopt defined three-dimensional architectures that dictate their functions and their mechanistic mode of action. Studying these 3D-structures at the atomic level allows gaining a deep insight into biological processes - for example, how molecules interact with their ligands, how substrates are processed by enzymes, how molecules recognize and bind to other protein partners, how DNA is processed by the cell, and how macromolecular activity is regulated. Such molecular, mechanistic knowledge underpins most areas of modern biology. It also has applications in biotechnology and biomedicine, being critical for the rational design of therapeutic drugs, the (re)engineering of enzymes of industrial interest and, in some cases, to determine and understand the molecular cause of human disease.

Revealing the 3D-structure of a macromolecule implies that the coordinates (i.e. location in space) of each of its thousands of individual atoms must be determined. Visualizing a molecule at such atomic resolution requires a microscopic enlargement of approximately 10^7. This is not achievable by conventional microscopy and special methodologies are required. The most popular and successfully employed technique for this task is X-ray crystallography, which has yielded *ca* 90% of all protein structures elucidated to date. The technique undisputedly reaches the highest spatial resolution currently attainable, is economical and user-friendly. However, its application requires that the sample under study is first prepared in the form of a crystal, where multiple copies of the molecule are arranged in a highly regular lattice. The growth of such crystals is deterministic of the success or failure of the technique and, thus, the technique is not always applicable. Crystallographic structural analysis is closely accompanied by bioinformatic analysis of protein sequences and other sample data - jointly, these methods can produce an enriched view of the molecular world.

A fascinating discipline, X-ray crystallography brings together aspects of physics, chemistry, engineering, and computer programming in order to allow the study of the structure of biological matter. In this course you will gain a broad understanding of the theory and practice

of X-ray crystallography, as well as sample production, crystal growth and the bioinformatics analysis of the macromolecular structures produced.

2. Contents of the course:

The lectures and practical work held during the course cover the following topics:
Atomic structure of proteins; interpretation of a structural model; purification of proteins; crystallisation methods; diffraction of X-rays by crystals; the phase problem and its solution by heavy atom derivatives and Molecular Replacement; the calculation of electron density maps revealing the position of atoms in space; modelling of a protein structure using computer graphics; refinement and validation of a 3D-structure; sequence analysis and alignment; computational exploration of protein models, functional annotation of structural models.

3. Previous knowledge

No previous knowledge is required. However, students wishing to engage in projects with a laboratory component would benefit from previous experience in general microbiological and biochemical preparation techniques: *E. coli* cultivation, chromatographic purification of proteins, electrophoresis.

Advanced Course "Molecular Genetics: Cell cycle regulation - from mechanisms to disease "

Coordinator: Prof. Dr. T.U. Mayer

Introduction:

The genetic integrity of each organism depends on the faithful segregation of its genome during mitosis and meiosis. Errors in this pathway can result in cancer formation or developmental defects. The aim of our research is to dissect the function and regulation of mitotic and meiotic proteins. Insights into mitotic and meiotic processes are not only important for a better understanding of the basic concepts of cell division, but also for the development of novel strategies for the treatment of cell cycle relevant diseases such as cancer.

Our research covers the following major lines of investigation:

1. Which are the key components required for the assembly of the mitotic spindle and how are they regulated?
2. What is the role of motor-proteins in chromosome congression and segregation?
3. How is the ubiquitin ligase APC regulated to ensure switch-like cell cycle transitions?

Course theory:

Eukaryotic cells – like any organism – face the challenge to make the right decisions, i.e. they have to adapt their cell cycle program to internal as well as external cues. Furthermore, once decisions are made they have to be irreversible to ensure that the different cell cycle events occur in the right order. Typical examples are the entry into M-phase or the metaphase-to-anaphase transition where cells have to wait until the last chromosome is correctly attached. Our interest is to understand the mechanisms underlying the decision process of mitotic as well as meiotic divisions, e.g. how do cells sense unattached chromosomes? How are the antagonistic activities of kinases and phosphatases regulated to ensure switch-like entry into M-phase? How do kinesins find the spindle equator? For these studies we combine classical cell-biology and molecular-biology approaches with state-of-the-art time-resolved 3D-microscopy techniques. In addition to classical approaches, the lab uses small molecules to modulate protein function in living cells. This approach, termed Chemical Biology, facilitates the modulation of protein function on a fast time scale. Compounds identified in our lab are not only of interest for basic research but also for the development of novel therapies in tumor treatment. During the course of the VTK, we invite 4 - 5 internationally renowned scientists from e.g. Stanford University, Yale University, Kings College London to present their work. This talks provide not only access to the latest scientific achievements and breakthroughs but also the opportunity to ask experts about their career and scientific history.

Literature:

“The Cell Cycle” by D. Morgan, Oxford University Press or our website: https://www.biologie.uni-konstanz.de/mayer/

Requirements:

Before the start of the VTK we will have a short meeting (please, check the black-board / your university email account for the announcement) where we will discuss the general outline of the course. Basic knowledge of cell biology, biochemistry, molecular genetics, and physiology (comparable to the content of respective courses in the Bachelor study courses “Biological Sciences” and “Life Science” at the University of Konstanz).

You will learn:

Scientific skills:

- Cell biological methods, e.g. tissue culture, transfections, stable cell line generation, cell engineering (CRISPR/Cas9), immunofluorescence
- Molecular biological methods, e.g. PCR, cloning, mutagenesis
- Biochemical methods, e.g. protein expression and purification in bacterial as well as in eukaryotic systems, in vitro enzyme assays
- Microscopy techniques, e.g. fluorescence time-resolved 3D imaging, high-resolution microscopy, quantitative image analysis,
- Chemical biology methods, e.g. compound handling, in vitro and in vivo small molecule inhibition studies, data handling, design and analyses of compound screens

Transferable Skills:

- You will learn how to prepare a poster and present your scientific data in our lab seminar
- You will have the chance to meet and talk to experts in the field of cancer drug development as well as young researchers working in the area of cell cycle research at internationally renowned research institutes
- You will learn how to present a manuscript in our journal club
- You will learn how to critically analyze and discuss scientific data
- You will be introduced to Excel, PowerPoint, and Adobe Illustrator to present your data in a professional manner

Advanced Course "Molecular Evolutionary Biology"

Coordinator: Prof. Dr. Axel Meyer

1. Topic of the course:

This course focuses on fundamental issues in evolutionary and developmental biology. The central aim of the course is to contribute to our understanding of the evolution of biodiversity. Specifically, the projects in the course will focus on the developmental, molecular, as well as genomic mechanisms of morphological and behavioral traits that differentiate species and other major groups of organisms. Students will have the unique opportunity to contribute to on-going studies of the molecular evolutionary biology underlying morphological adaptation and speciation. As part of the course, participants will take part in multidisciplinary research that integrates modern approaches in population genetics, molecular evolution, and molecular phylogenetics, comparative genomics and bioinformatics as well as includes work on the connections between developmental and evolutionary biology. The organisms that will provide the primary taxonomic focus of many projects include the zebrafish, live-bearing fish from the Neotropics, and the evolutionary highly successful cichlid fishes. Using these models, we will study how species form and how genomes change over evolutionary timescales. The course will be conducted in English, and it will have daily lectures on topics spanning the breadth of developmental and evolutionary biology. We will also conduct a weekly journal club in which we will discuss recent literature and students will also receive structured guidance on all steps from planning of experiments, to executing their study, to writing the final papers that detail their empirical findings. We hope to provide a stimulating and educational experience that highlights the excitement of doing organismal biology in the genomic age.

2. Techniques:

A large variety of questions and approaches will be utilized in the course. Student projects will exploit a diversity of techniques that are not only at the cutting edge of evolutionary and developmental biology but also increasingly essential in disciplines such as human genetics, toxicology, and personalized medicine. A large component of all projects will involve molecular techniques to determine and analyze population genetic data, characterize phenotypic and genomic variation among close relatives, and DNA sequence analysis. Instruction will also be provided for how to handle the large datasets (up to several million nucleotides) that often make up the core of biological research today. Several of the molecular biological techniques taught in this course will include the extraction of DNA, PCR-amplification of genes, and construction of c-DNA libraries. For gene expression analyses and developmental investigations, techniques such as whole mount *in situ* hybridization, quantitative PCR, microinjection into oocytes, and transcriptome assembly and analyses will all be taught. Computer-based investigations will include statistical evaluations of population genomic and phylogenomic data, and will also emphasize the analysis of genetic information retrieved from genomic databases for questions in comparative genomics and bioinformatics. Depending on the interests of the students, almost entirely “bench“ or “computer“ projects will be offered.

Relevant Literature:

Meng Qu, Yali Liu, Y., Zhang, Y., Wan, S., Ravi, V., Qin, G., Wang, X., Jiang, H., Zhang, H., Zhang, B., Gao, Z., Huysseune, A., Zhang, Z., Zhang, H., Chen, Z., Yu, H., Wu, Y., Tang, L., Li, C., Zhong, J., Yin, J., Witten, P.E., Meyer, A., Venkatesh, B. and Q. Lin. 2021. Immune system complexity and male pregnancy coevolved in the seadragons. ***Science Advances.***

Hoch, R., Schneider, R.F., A. Meyer and J. Woltering. 2021. From fin to fin; spiny and soft-rayed fin domains in acanthomorph fish are established through a BMP-gremlin-shh signaling network. ***Proceedings National Academy of Sciences.***

Meyer, A., S. Schloisnig, P. Franchini, K. Du, J. Woltering, I. Irissari, N. Wong, S. Nowoshilow, S. Kneitz A. Kawaguchi, A. Fabrizius, P. Xiong, C. Dechaud, H. Spaink, J.-N. Volff, O. Simakov, T. Burmester, E.M. Tanaka, and M. Schartl. 2021. Giant lungfish genome elucidates the conquest of land by vertebrates. ***Nature*** 590: 284-289.

Rhie, A., et al. Jarvis. 2021. Towards complete and error-free genome assembly of all vertebrate species. ***Nature*** 592: 737-746.

Schartl, M., S. Kneitz, J. Ormanns, C. Schmidt, J.L. Anderson, A. Amores, A. Meyer, J. Postleithwait. 2021. The developmental and genetic architecture of the sexually selected male ornament of swordtails. ***Current Biology*** 31: 911-922.

Li, C., Olave, M., Hou, Y., Quin, G., Schneider, R., Gao, Z. Tu, X., Wang, X., Qi, F., Nater, A., Kautt, A., Wan, S., Zhang, Y., Liu, Y., Zhang, H., Zhang, B., Zhang, H. Qu, M., Liu, S., Chen, Z., Zhong, J., Zhang, H., Meng, L., Wang, K., Yin, J., Huang, L., Venkatesh, B., Meyer, A., and X. Lu. 2021. Genome sequences of 21 seahorse species shed light on global dispersal routes and suggest convergent developmental mechanisms of unusual bony spines. ***Nature Communications*** 12: 1094.

Kautt, A.F., C.F. Kratochwil, A. Nater, G. Machado-Schiaffino, M. Olave, F. Henning, J. Torres-Dowdall, A. Härer, C.D. Hulsey, P. Franchini, M. Pippel, G. Myers, and A. Meyer. 2020. Contrasting signatures of genomic divergence in rapidly speciating crater lake cichlid fishes. ***Nature*** 588: 106-111.

Woltering, J.M., Irisarri, I., Ericsson, R., Joss, J.M.P., Sordino, P., and A. Meyer 2020. Sarcopterygian fin ontogeny elucidates the origin of hands with digits. ***Science Advances*** 6: eabc3510.

Real, F., et al. 2020. Reorganization of regulatory landscapes as an evolutionary mechanism for true mammalian XX hermaphroism in moles. ***Science*** 370: 208-214.

Franchini, P., Xiong, P., Fruciano, C., Schneider, R.F., Woltering, J.M., Hulsey, C.D. and A. Meyer. 2019. MicroRNA gene regulation in the extremely young and parallel adaptive radiations of Nicaraguan crater lake cichlid fish. ***Molecular Biology and Evolution*** 36: 2498-2511.

Franchini, P., Jones, J., Xiong, P., Kneitz, S., Gompert, Z., Warren, W.C., Walter, R.B., Meyer, A., and M. Schartl. 2018. Long-term experimental hybridisation results in the evolution of a new sex chromosome in swordtail fish. ***Nature Communications*** 9: 5136.

Kratochwil, C.F., Liang, Y., Gerwin, J., Woltering, J.M., Urban, S., Henning, F., Machado-Schiaffino, G., Hulsey, C.D., and A. Meyer. 2018. Agouti-related peptide 2 facilitates convergent evolution of stripe patterns across cichlid fish radiations. ***Science*** 362: 457-460.

General literature:

Futuyma, D. and Kirkpatrick, M. 2017. Evolutionary Biology (4th Ed.). Sinauer Assoc.

Grauer, D. 2016. Molecular and Genome Evolution. Sinauer Assoc.

Gilbert, S.F. and Barresi, M.J.F. Developmental Biology. (11th Ed.). Sinauer Assoc.

Hillis, D.M, et al. (ed.) 1996. Molecular Systematics (2nd Ed.). Sinauer Assoc.

Li., W.-H. 1997. Molecular Evolution (2nd Ed.). Sinauer Assoc.

Advanced Course "Developmental Biology"

Coordinator:

Prof. Dr. Patrick Müller

Keywords:

Signaling molecules, patterning, vertebrate development

Course overview:

We offer training in modern approaches to the central question in developmental biology: How is a ball of nearly equal cells transformed into a structured embryo? And how does cellular communication via signaling molecules mediate this process?

In small research projects on the role of signaling molecules in patterning during vertebrate development, you will use a variety of experimental and theoretical techniques. The available projects, centered around ongoing research in our lab, will be presented on the first day of the course.

You will be trained and accompanied in your project by experienced scientists and technicians. You will be mentored to present your research findings to an international audience and practice your skills by giving a presentation to the participants at the end of the course. To get trained in critically assessing scientific publications, each student will present one paper describing a major finding in developmental biology.

In accompanying lectures, you will learn about exciting new areas in developmental biology and ongoing research in our lab. In tutorials, we will practice and discuss classical techniques as well as quantitative tools for image-based studies in developmental biology, from image processing and data analysis to computational modeling.

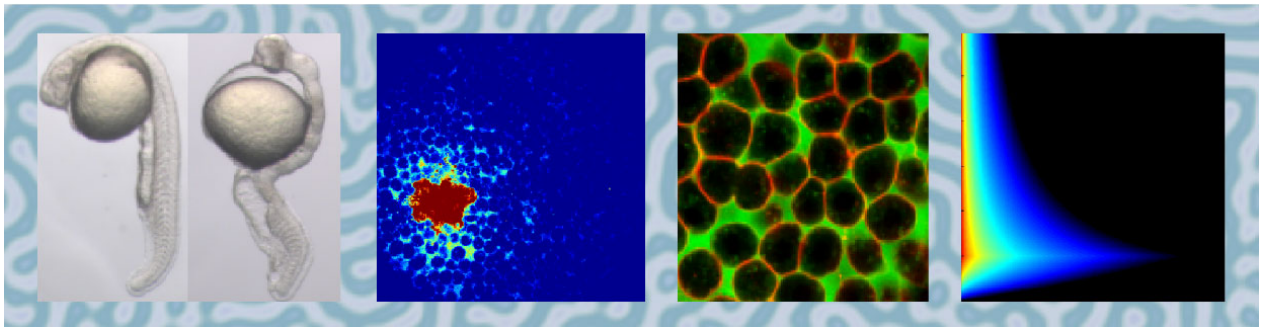
Methods and techniques:

Molecular biology: mRNA extraction, cDNA synthesis, PCR, cloning, *in vitro* transcription for mRNA synthesis

Embryology: Handling fish embryos, embryo injections, phenotypic characterization

Imaging: Bright-field microscopy, fluorescence microscopy, light-sheet microscopy

Image analysis: Basic image processing, macro programming in Fiji, programming in Python



Advanced Course "Dynamics of aquatic ecosystems"

Coordinators: Prof. Dr. Frank Peeters, Dr. Karla Martinez-Cruz

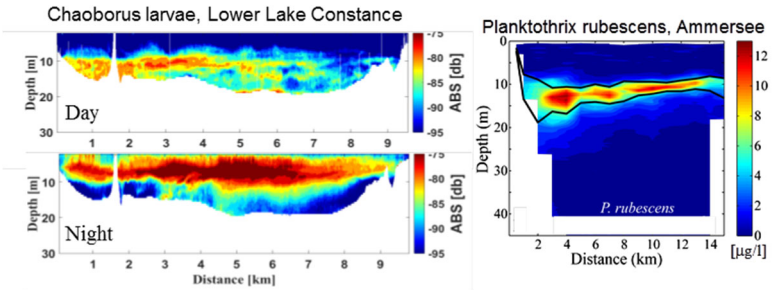
Research goals of the environmental physics group

Our research aims at a process based understanding of the dynamic interactions within aquatic ecosystems. Climate change, reduction in nutrient supply, and immigration of invasive species influence not only the abiotic conditions in the aquatic habitat but also the conditions for growth and the food web structure and thus alter the organismic interactions in lakes. To assess and predict the consequences of environmental change for lake ecosystems, we work on a mechanistic understanding of the role of nutrient and temperature distributions and of hydrodynamic conditions and mixing processes for the development and interactions of organisms within the ecosystem. In recent years a major research focus has also been on greenhouse gases in lakes and especially on the factors that influence the release of methane from lakes and reservoirs. Methane emissions from lakes and reservoirs are one the most important natural sources of methane at global scale.

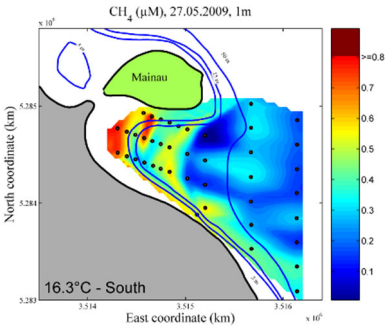
Our research is strongly based on empirical data, which are collected in field experiments. Thereby, we combine high-resolution measurements using probes equipped with in-situ measuring sensors that are deployed in moorings or operated from ships in a profiling mode, with laboratory analyses of water, sediments and plankton samples collected in the field. Lake research investigating ecosystem interactions under natural conditions differs from the research on laboratory systems, because it is usually impossible to control the conditions affecting entire ecosystems. Therefore, we typically base our research on intensive empirical field investigations and combine data sets with numerical modelling to allow generalization of the results. In addition to field measurements, we also conduct experiments in mesocosms to test hypothesis under more controlled conditions.

Research questions in the course

Currently, we work on several topics that involve field experiments on Lake Constance and on small lakes in the Upper-Swabian Lake District, but we also work with mesocosms and with sediment cores in the lab. One research area is on plankton. We want to understand the influence of local sources of nutrients and water column properties on the spatial distribution and structure of the plankton community and the role of transport processes for the generation of heterogeneous plankton distributions, i.e. plankton patchiness. Another research topic concentrates on the question, why the toxic cyanobacteria *P. rubsecens*, which is a dominant species in several pre-alpine lakes, does not establish large abundances in Lake Constance.



A second research area is on methane, particularly on the heterogeneous release of methane from sediments. The investigations are based on installed instruments measuring methane and CO_2, and on ship based profiling with in-situ sensors, together with water and sediment incubations in laboratory to estimate methane production and oxidation rates. Recently, we started investigating the methane contribution from littoral sediments to the total methane budget of lakes. This study includes the effect of (i) lake reeds, (ii) bioturbation, (iii) heterogeneity of sediments, (iv) methane production



and oxidation in water and sediments, and (v) mass transfer across the lake. We study the impact of these processes on the total methane budget in lakes with different morphology and trophic status. The research has especial relevance with respect to the consequences of climate warming and trophic change on the methane release from lakes.

The third area of current research is the impact of surface waves on the littoral zone. Particular focus is on surface waves generated by ship traffic and the connection of these waves to erosion and organisms in the shallow water zone. Wave properties and information on erosion can be derived from moored instruments in the shallow water zone. We also intend to test a new instrument for measuring wave motions off-shore and combine this with measurements on turbidity and erosion near shore.

Besides the observational research, another major focus of our research is numerical modelling addressing e.g. competition and predator-prey interactions in vertical water columns, the simulation of transport and ecosystem dynamics in three dimensions and surface waves and their impact. It is also possible to conduct projects in this area of research.

Work program

Practical part:

The students work in groups of two. They develop a work plan for their project, conduct the field and laboratory work and analyse the data with the support of a project supervisor. All projects are integrated part of our current research projects. After three weeks, intermediate results are presented by the research groups. The discussion of the results with the other participants and supervisors support the adjustment of the remaining research program. At the end of the course the project results will be presented by the research groups in a poster session. Each group compiles and documents their data to make them available for further research. After the course, the students provide a summary of their project work in the format of a scientific manuscript.

Theoretical part:

Lectures:
Basic principles in physical limnology (exchange and transport processes, tracer techniques), relevance and release of methane, utilization of acoustic techniques in aquatic systems, plankton patchiness, waves and their ecological relevance, basic ocean dynamics, climate change, introduction to ecological modelling, case studies from specific lakes. The lectures not only present basic principles but will also show recent results from the current projects of the research group. We will have additional presentations from invited guests addressing specific research topics.

Seminar:
In the seminar the participants present selected articles relevant for their projects.

Additionally, the students will be introduced in a hands-on tutorial to the software MATLAB. This software is excellently suited for the analyses of large data sets and for numerical modelling and is not only used by our working group but also in many other research groups and in engineering.

Aims of the course

Development of an expertise to independently design and conduct limnological projects, that consider biotic and abiotic factors; experience with a wide variety of field techniques and the analysis of large data sets; familiarity with the interpretation of field data and the assessment of the relevance of abiotic conditions and physical processes for organismic interactions in lakes; understanding of the causes and consequences of dynamic interactions and of the implications of environmental change (climate warming, oligotrophication) on aquatic ecosystems.

Advanced Course "Integrative animal behaviour"

Coordinator: Prof. Dr. Xiang-Yi Li Richter

1. Introduction

Did you know that starlings, with their vibrant and joyful songs, once charmed the legendary Mozart into keeping them as pets? But what prompts a starling to belt out its melodies? To truly unravel this mystery, researchers focused on several different aspects:

The **function** perspective: From an evolutionary standpoint, researchers have delved into how these songs increase a starling's ability to defend its territory and attract mates.
The **mechanism** perspective: Anatomically curious minds study the structure of the starling's syrinx, seeking to understand how air passage results in such melodious sounds.
The **ontogeny** perspective: When tracing the development of these songs, some researchers investigate the learning process - how starling babies learn to sing, and from whom?
The **phylogeny** perspective: On a broader taxonomical spectrum, by comparing starlings to their feathery relatives, some researchers aim to discover if their ancient ancestors also had a penchant for singing when they first took their place in the Tree of Life.

For any animal behaviour, like a starling's song, there are layers of reasons, both immediate and deeply rooted in evolutionary history. To truly unravel the mystery behind an animal's actions, we must take an integrative approach to journey through intertwining pathways of research. So, the next time you hear a starling sing, remember: there's a world of wonder behind every note!

2. Objectives

- Understand the concepts of proximate and ultimate causes of animal behaviour
- Understand why animal behaviours need to be studied using different approaches
- Collaborate in groups to discuss and propose an integrative research project

3. Contents of the course

The course consists of lectures and seminars in the first 5 weeks. After that, students will work on a writing project in the form of a research proposal. In the lectures, I will introduce Tinbergen's four proximate and ultimate questions about how to explain animal behaviour with different foci (i.e., function, phylogeny, mechanism, and ontogeny), and use case studies to illustrate each of the fundamental approaches. In the seminars, we focus on five important topics in animal behaviour research (i.e., communication & signalling, cooperation, foraging & predator avoidance, sexual selection & reproductive behaviours, and mating system & parental care). Students will form groups to choose and present research papers that use at least two of the research approaches to study each topic. We then discuss the benefits (if any) of integrating the different approaches, and the possible reasons why other approaches have not been used in the study. We finally summarize the different papers studied under each topic and discuss what insights the integrative approach can bring us that cannot be achieved by each of the four approaches in isolation. In the writing project, each group of students will propose a small research project that uses at least two approaches to study a type of animal behaviour that we covered in the lectures and seminars.

4. Requirements

Interest and basic knowledge of animal behaviour.

5. Exam

To pass the course, students must actively participate, present and discuss during the seminars (50%) and collaborate with each other to write a research proposal in groups and present the project on the examination day (50%).

Advanced Course "Biochemistry"

Coordinators: Profs. M. Scheffner & F. Stengel

1. Introduction

Cells need to control a virtually infinite number of processes and biochemical reactions in time and space. A key concept is the organization of the cellular space into functional units and compartments. Cellular compartmentalization scales from single molecules over protein complexes of varying complexity to membrane-surrounded organelles. On the level of single proteins and protein complexes, posttranslational modifications affect the functionality of proteins and lead to the generation of functional "proteoforms" which confer complexity and dynamics to the system. In addition, liquid-liquid phase separation has been identified as a mechanism to assemble biomolecular condensates that in turn facilitate distinct biochemical reactions and contribute to the organization of a functional proteome. In our groups, we are studying the impact of distinct posttranslational modifications (e.g. acetylation, ubiquitination) on proteome organization in general and on the fate/properties of individual proteins in particular. We are using various methods of biochemistry combined with bioorthogonal chemistry and are applying and developing integrated mass spectrometry-based approaches in order to address the modular proteome at the molecular level and at a timescale that facilitates probing of dynamics.

2. Content

a) Theoretical part:

1. Enzymology and physiology of the modification of proteins by ubiquitin and ubiquitin-like proteins.
2. Methods used in the analysis of protein modification (e.g., mass spectrometry, unnatural amino acids).
3. Methods used in proteomics and structural mass spectrometry (principles of mass spectrometry, basic proteomic workflows, methods in structural mass spectrometry).
4. Introduction into hybrid methods in structural biology.
5. Aspects of cancer biology including the role of human papillomaviruses and the ubiquitin-conjugation system.

b) Experimental part:

The actual topics of the student projects depend on ongoing research activities of the supervising doctoral researchers and postdoctoral fellows. In general, we are studying the impact of distinct posttranslational modifications (ubiquitination, acetylation) on the function of selected proteins as well as on proteome organization in health and disease. To this end, we

are using various techniques of biochemistry and molecular biology in combination with bioorthogonal chemistry and are developing and applying novel integrated mass spectrometry-based approaches.

Current research activities include:

1. Elucidating how single molecules organize themselves in non-membrane bound structures via phase separation and how this relates to the organization and formation of the function-centric modular proteome
2. Unraveling the influence of posttranslational modifications on protein-protein interactions, protein complex composition and dynamics by applying bioorthogonal chemistry in combination with proteomic profiling.
3. Role of the ubiquitin system in pathophysiological processes (cervical cancer, Angelman syndrome).
4. Expansion of the structural mass spectrometry toolbox.

c) Methods:

- Methods in proteomics and structural mass spectrometry:
  Cross-linking, data-dependent and data-independent acquisition, quantitation, computational analysis and data processing.
- Bioinformatics: cross-linking guided integrative modeling.
- Biochemical and cell biological methods include:
  PCR, PCR-directed mutagenesis, cloning; expression of recombinant proteins in *E. coli* or mammalian cells (transient und stable transfections); co-precipitation analyses, gel electrophoresis, Western-Blot, immunofluorescence; determination of the activity of enzymes of the ubiquitin system.
- Preparative and analytical methods include:
  Isolation and purification of proteins expressed in *E. coli* or mammalian cells by various chromatographic methods (e.g., ion exchange, size exclusion, affinity); mass spectrometry.

3. Requirements

Basic knowledge of biochemistry (with an emphasis on protein structure and function), cell biology, molecular genetics, and physiology (comparable to the content of respective courses in the Bachelor study courses "Biological Sciences" and "Life Science" at the University of Konstanz).

4. Literature (preparatory)

de Hoffman & Stroobant, Mass Spectrometry; Stryer, Biochemistry; Voet, Voet, Pratt, Biochemistry.

Advanced Course "Microbial Ecology"

Coordinators: Prof. Dr. David Schleheck and Dr. Nicolai Müller

1. Introduction

The majority of life on planet Earth is microbial, and the survival and growth of these organisms in complex communities and in diverse habitats require biochemical transformations. Hence, one central goal of the research done in our group is to discover and understand bacterial metabolism. For example, we investigate degradation pathways in anaerobic bacteria of the human gut for a transformation of dietary organosulfur compounds into harmful hydrogen sulfide (H_2S) (see example below), and pathways in aerobic and anaerobic environmental bacteria for a transformation of natural compounds, xenobiotic industrial chemicals and “bioplastic”. Other research done in the Schleheck group involves the phyto-, bacterio- and virioplankton community of Lake Constance and microbial biofilm formation and biofilm control. Therefore, our laboratory methods comprise bacterial cultivation and physiology, biochemistry, analytical chemistry, genomics and proteomics, but we analyse microbial communities also directly in their natural habitat. Ultimately, the accumulated knowledge should inform new approaches to improve human and environmental health as well as biotechnology.

Our present research covers the following areas:

- The **microbial biodegradation** projects cover organosulfonate ($R\text{-}SO_3^-$) substrates, such as taurine and sulfoquinovose, and organophosphonate ($R\text{-}PO_3^{2-}$) substrates, such as ciliatine. Further projects involve syntrophic fermentation in co-cultures and anaerobic utilization of acetone and of phosphite (projects led by Dr. Nicolai Müller). Here, as for one example shown in the Figure below, emphasis is put on a recently discovered process, that is ‘organosulfonate respiration’. This process is relevant in anoxic environmental habitats as well as in the human intestinal community and leads to the production of hydrogen sulfide: as opposed to using sulfate as electron acceptor for anaerobic respiration, these microbes make use of the organosulfonates as electron acceptor for their energy conservation. One pathway discovered in the human gut bacterium and opportunistic pathogen *Bilophila wadsworthia* employs a new, desulfonating glycyl-radical enzyme.

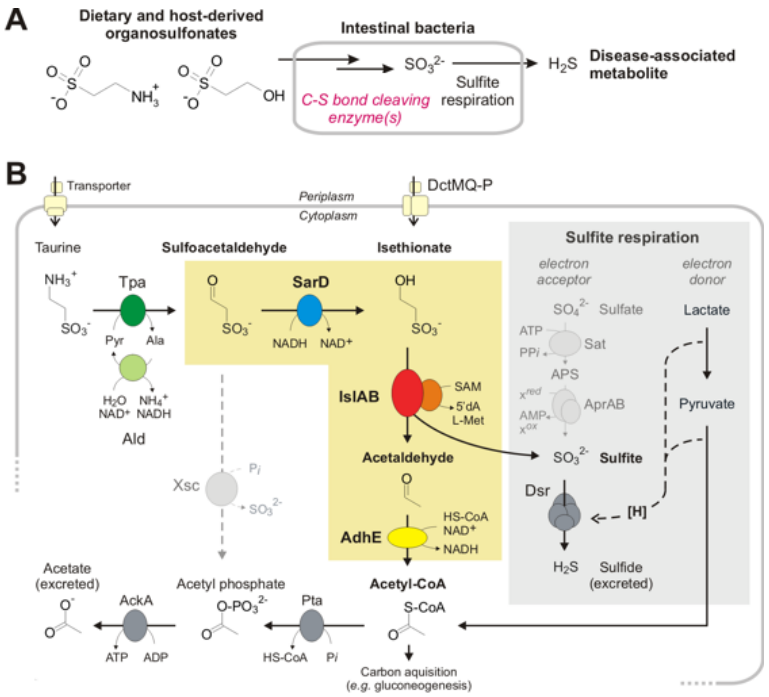


Figure 1. Metabolism of taurine and isethionate by the human gut bacterium *Bilophila wadsworthia*. (A) *B. wadsworthia* and other intestinal bacteria degrade dietary and host-derived organosulfonates in order to access sulfite as electron acceptor for their anaerobic respiration and produce harmful hydrogen sulfide (H_2S). (**B**) *B. wadsworthia* utilizes taurine and isethionate as electron acceptors through the glycyl radical enzyme (GRE) isethionate sulfite-lyase (IslA) with its GRE-activase component (IslB). The sulfite released by the GRE is reduced to sulfide by dissimilatory sulfite reductase (Dsr) and coupled to proton translocation for ATP synthesis (symbolized as [H]), when using electrons from oxidation of an alternative electron donor such as lactate (grey box). For comparison, the ATP-consuming activation of sulfate to sulfite is also shown. Other enzyme abbreviations: Tpa, taurine-pyruvate aminotransferase; Ald, alanine dehydrogenase; AdhE, CoA-acylating acetaldehyde dehydrogenase; Pta, phosphotransacetylase; AckA, acetate kinase; Sat, sulfate transferase; Apr, APS reductase.

- The diversity and seasonal succession of the zoo- and phytoplankton in Lake Constance is being well-studied since many years as part of a routine sampling program. However, the phylogenetic diversity and seasonal succession of the **bacterioplankton community** (size class 5 µm – 0.2 µm) in this lake has not yet been explored in such detail. We are establishing a much more detailed view on the recurrence and resilience of taxa within the annual plankton succession in Upper and Lower Lake Constance, by application of contemporary next-generation sequencing methods: the two lake basins differ in their morphology and physicochemical (nutritional) conditions, and we expect that the plankton succession in both basins differ due to differences in abiotic and biotic conditions. Furthermore, a much more fine-scaled correlation analyses is feasible regarding positive and negative interactions between phytoplankter, other protists and bacteria, reflecting competition, grazing, and other interactions. Most recently, we started collecting also the virioplankton of Lake Constance (size class 200 nm – 10 nm).

- Biotic and abiotic surfaces in the environment are rapidly colonized by complex microbial communities. The process of **surface colonization and biofilm formation** provides numerous advantages to these organisms and supports critical ecological and biogeochemical functions. It also contributes to deleterious effects such as biofouling, biocorrosion, and the persistence and transmission of harmful or pathogenic microorganisms and of their genetic determinants. In the euphotic zone of aquatic habitats - such as in Lake Constance - these biofilm communities comprise photoautotrophic microorganisms, such as diatoms, green algae and cyanobacteria, which produce the organic carbon that fuels the life of a heterotrophic contingent of microorganisms, mostly bacteria. We study the initial colonization and formation of complex photoautotrophic-heterotrophic biofilm communities in our lake, as opposed to its planktonic microbial contingent (see above), and biofilms in the lake that are formed on bioplastic surfaces, supposedly degrading these materials.

2. Contents of the advanced course

a. Theoretical part

Cultivation of aerobic and anaerobic bacteria in the lab. Analytical-chemical, biochemical and molecular methods for Microbial Ecology research. Dissimilatory and assimilatory metabolism. Aerobic and anaerobic degradation of organic matter. Fermentations, sulfate reduction, methanogenesis, syntrophic associations, dehalorespiration. Roles of microbes in biogeochemical carbon, nitrogen, sulfur and phosphorus cycling. Limits of microbial transformation (e.g. pesticides, plastic). Starvation and survival. Microbial communities and microbial biofilms. Cell-cell interactions, chemical communication and signalling (quorum sensing). Cultivation-independent techniques of microbial community analysis. Microbial ecology of specific environments, e.g., lake water column and sediment, soil, intestinal systems of humans and animals, extreme environments.

b. Experimental part

We study environmental bacteria, e.g. as isolated from water, soil, or the human gut, in our lab for their biodegradation capabilities and the underlying biochemical transformations, enzymes and genes. This includes growth of the strains in batch or continuous culture and quantifying biomass formation, substrate disapperance and product formation. In the past, bacterial pathways for degradation of industrial chemicals and natural organo-sulfur compounds have been studied in aerobic as well as anaerobic strains, including the enzymes catalyzing these transformations. Our research involves also genome sequencing, proteomic and transcriptomic

analysis, and production of enzymes by heterologous expression. Another research avenue aims at characterizing microbial communities directly in their environment (cultivation-independent), by total DNA extraction and meta-genomic sequencing (Bodensee plankton and biofilms).

Projects offered in the Advanced Course will be chosen based on the research currently being done in the lab. However, you are also strongly encouraged and highly welcome to propose your own research project; please discuss this with us in advance.

c. Methods

Microbiological work, cultivation of aerobic and anaerobic bacteria. Chemical and biochemical analyses using GC-MS, HPLC-MS, chromatography and/or spectrophotometry. Molecular techniques for analysis of degradation pathways, bacterial genome-sequencing, total proteome and transcriptomic analysis. Analysis and purification of enzymes, heterologous production of candidate enzymes, enzyme assays. Molecular characterization of natural microbial communities and of bacterial isolates based on PCR, sequencing, phylogenetic and bioinformatic analyses. Plankton filtration and DNA extraction. Biofilm cultivation.

3. Required experience of participants

Participants should have taken at least one course in basic microbiology and should have experience in basic microbiological lab work (e.g., Microbiology-I lectures and the Kompaktkurs). Basic knowledge in chemistry and biochemistry is required for projects covering these areas. Experience in molecular biology and analytical chemistry is helpful.

4. Literature

Textbooks for general study:

Fuchs, G.: Allgemeine Mikrobiologie, begr. v. H. G. Schlegel. Thieme, Stuttgart, 10. Aufl. (2017)

Madigan et al.: Brock Biology of Microorganisms., Pearson, New York, USA, 15. Aufl. (2018)

For publications on our current projects, please see our homepage, https://www.biologie.uni-konstanz.de/schleheck/publications/

For specific literature on the VK research projects, this will be provided at the beginning of our course.

Advanced Course in Chemical Ecology/Biological Chemistry

Coordinator: Prof. Dr. Dieter Spiteller

Introduction:

Chemical Ecology is an interdisciplinary research at the interface of biology and chemistry. It aims to reveal the **chemical background of interactions between organisms**. We identify compounds, such as antibiotics, toxins or signalling molecules. Next, we want to understand the **biological function** of identified metabolites.

Microorganisms, in particular *Actinomyces*, are well known as producers of both structurally highly diverse and pharmaceutically invaluable secondary metabolites. However, the role of such compounds for the producing organisms in their natural environment is largely unknown. Indeed, most natural products from microorganisms have been isolated from pure cultures. From whole genome sequencing projects, it was realised that most microorganisms have much more genes for secondary metabolite production than the number of compounds known from them. Microorganisms respond to abiotic factors or to other organisms - in symbiotic to pathogenic relationships - with induction of secondary metabolism and morphological changes. Thus, it is obvious that environmental factors trigger the production of secondary metabolites (Figure 1).
Our research about microbial chemical ecology contributes to the understanding of secondary metabolite formation, regulation and their function for the producing organisms.

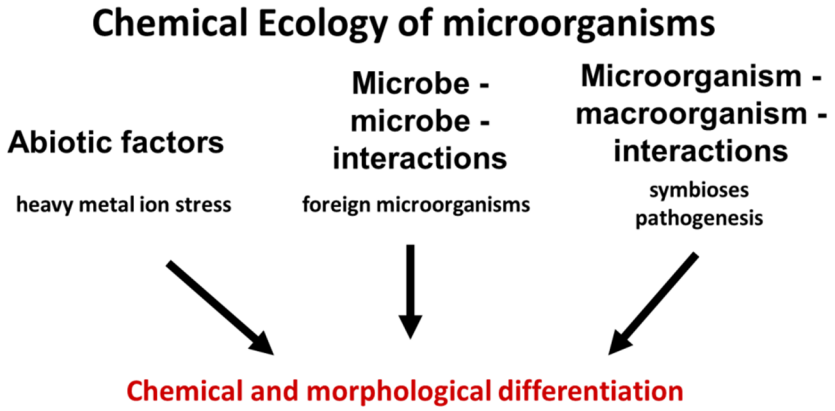


Figure 1: Microbial Chemical Ecology – Organismic interactions

Current major research topics:

Chemical defence by microbial symbionts

In order to study the chemistry of microorganisms in symbiotic interactions we mainly investigate the symbiosis of leaf cutting ants with microorganisms. Leaf cutting ants grow a symbiotic fungus with leaf material. In turn the ants use their fungus garden as major food source. This mutualistic relationship is threatened by pathogens. However, leaf cutting ant associated microorganisms help to protect the fungus garden of the ants. We identified the first antifungal compounds from microbial symbionts in this interaction. Using bioassay-guided fractionation we found that many of the symbionts produce the well-known antifungal candicidin macrolides (Figure 2).
However, the system is much more complex. There are many microorganisms involved and many pathogenic diseases. Thus, a multitude of antibiotic and antifungal compounds can be

expected from this ecosystem. Indeed, we already identified antimycins, valinomycins and actinomycins as additional antibiotics from leaf cutting ant symbionts (Figure 2).
The specific fungal pathogen *Escovopsis weberi* produces secondary metabolites that support its attack of the garden fungus such as emodin, cycloarthropsone and shearinines.
Besides the leaf cutting ants as the most studied model system, many more interactions in which microorganisms play a crucial role that have just not been studied so far can be expected. A systematic screening for symbionts among insects may reveal their microbial symbionts and their chemistry as well as novel antibiotics.

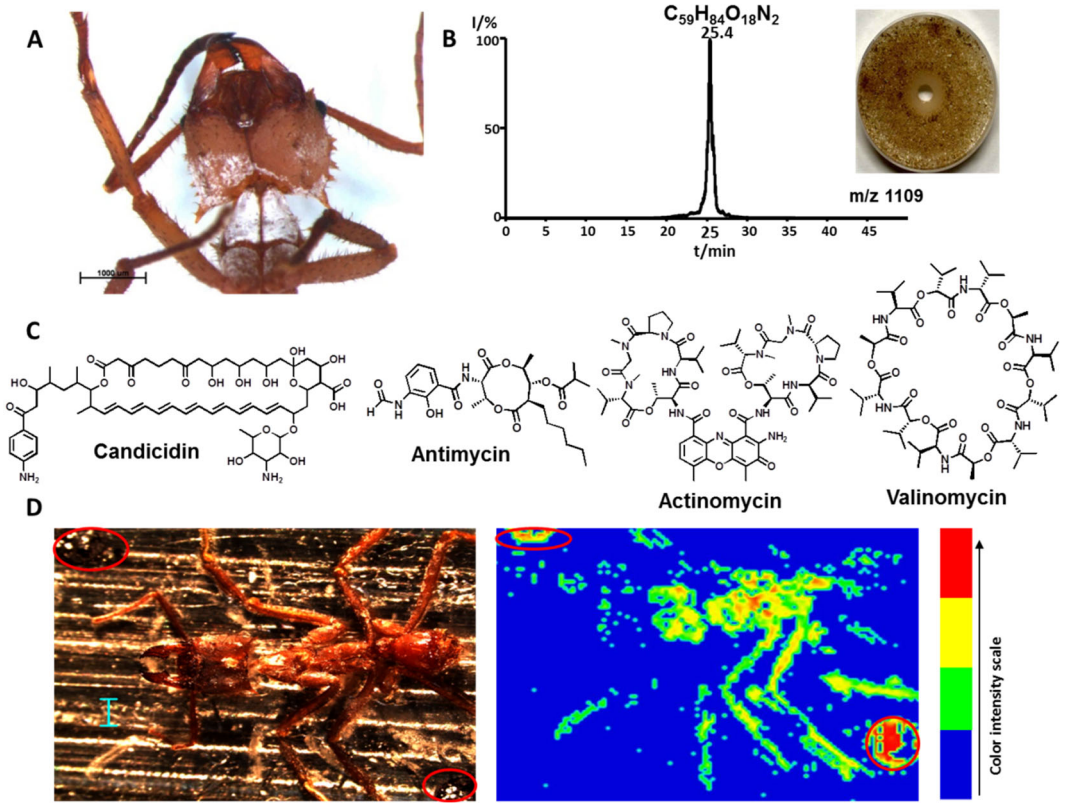


Figure 2: Antibiotics from microbial symbionts of leaf cutting ants: A) Biofilms on leaf cutting ants; B) LC-MS analysis and agar diffusion assay of an antifungal made by microbial symbionts; C) Antibiotics identified so far from *Streptomyces* symbionts of leaf cutting ants; D) Direct detection by MALDI (matrix assisted laser desorption ionisation) imaging

Molecular basis of epiphytic microorganisms to protect their host plants against pathogens
In this project we intend to reveal the molecular basis of the biocontrol function of selected epiphytic microorganisms that protect plants e.g. soy bean against pathogenic infections.
For example, we recently identified the highly polar toxin 3-methylarginine using bioassay-guided fractionation in combination with HILIC HPLC (hydrophobic interaction liquid chromatography high performance liquid chromatography) (Figure 3). Since the chemistry of microbial epiphytes and endophytes is quite little studied, we expect that there is great potential to identify novel active substances from promising biocontrol organisms. Therefore, microorganisms associated with plants need to be isolated, characterised and tested for their biocontrol potential. Then extracts will be made and active compounds will be purified and identified. In order to understand the *in vivo* mode of action of biocontrol strains, knock-out mutants could be prepared and *in planta* experiments performed.

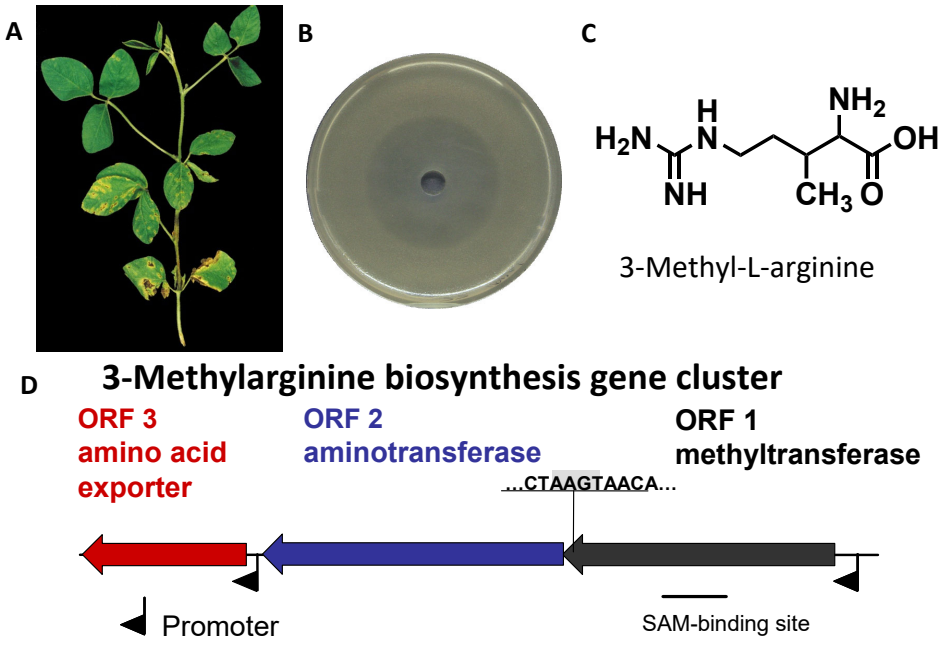


Figure 3: Epiphytes as biocontrol organisms: A) Bacterial blight of soy bean caused by the pathogen *Pseudomonas syringae* pv. *glycinea*, B) inhibition of *P. syringae* pv. *glycinea* by the epiphyte *Pseudomonas syringae* pv. *syringae 22d/93*, C) structure of the toxin 3-Methyl-L-arginine that inhibits the growth of *P. syringae* pv. *glycinea*, and D) 3-Methyl-L-arginine biosynthesis gene cluster

Microbial reactions and adaptations to stress

Abiotic factors such as heat, drought or heavy metal ions influence the growth of microorganisms and force them to adapt. We focused on *Streptomyces coelicolor* exposed to heavy metal ion stress. Stress by heavy metal ions can be studied under controlled conditions because heavy metal ions are just one defined factor that induces reactions in contrast to, for example, cocultivation where many possible factors may induce metabolic responses.

Testing a variety of heavy metal ions, Co^{2+}-ions turned out to cause the most pronounced effects leading to drastic metabolic changes that even led to new phenotypes. So far, we started metabolic profiling of the red phenotype. The red phenotype overproduces prodigiosins and in addition forms novel prodigiosin derivatives, coeligiosins.

Further experiments aim to fully characterise the induced secondary metabolites in response to heavy metal ion stress and to address their biological role.

Induction of secondary metabolite production in coculture

Microorganisms use intra- and interspecific chemical signals to coordinate their interactions with their environment. Using cocultivation assays we screen for metabolic or morphological changes that are induced when strains grow together (mimicking natural conditions).

Both bioassays and differential metabolic profiling by LC-MS are applied to identify the effects of cocultivation. Again we make use of bioassay-guided fractionation in order to isolate and characterise novel, induced secondary metabolites or the inducing signals.

Degradation/Detoxification of natural products or xenobiotics by microorganisms

We are also interested in how microorganisms degrade secondary metabolites and xenobiotics. For example, how anaerobic bacteria degrade sulfonates or if microbial symbionts contribute to detoxify xenobiotics.

Methods:
As interdisciplinary science at the interface between chemistry and biology Chemical Ecology requires **an open minded, flexible approach** to address research questions. In a problem-orientated way we combine techniques of organic chemistry, analytical chemistry, biochemistry, microbiology, molecular biology and ecology.

- Analytical chemistry (chromatography, HPLC, mass spectrometry, LC-MS, GC-MS, NMR)
- Organic synthesis (labelled compounds for biosynthetic studies, structure elucidation or material for biological testing)
- Microbiology (cultivation, isolation of microorganisms, PCR, cloning, mutagenesis, heterologous expression and functional characterisation of enzymes, genome mining)
- Ecology (biological function of natural products, bioassays)

Research Practical:
The course consists of a **practical** and **theoretical** part. You will work in close collaboration with a PhD student or Postdoc on a small research project of our current work that complements your supervisor's work. Besides you will attend lectures/discussions, the group seminar and our literature seminar. At the end of your practical you will give an oral presentation and write a report of your experiments in the style of a paper. After the final submission of your report we will meet to discuss it.

The course aims to

- provoke further **interest in the chemistry of biological systems**
- provide experience in chemical ecology/chemical biology and interdisciplinary work
- provide **hands-on experience in research work** (experimental design, experimental practise, data analysis, critical interpretation of results, writing up results)
- make you familiar with **new methods** such as state of the art mass spectrometry
- prepare you for future **independent research work** (master, PhD thesis)

Your research project will be chosen as much as possible based on your interests, e.g. you may want to focus on analytical chemistry or you may decide to concentrate on molecular biology methods or a mixture of both.

Requirements:
In order to have a solid background for the course prior attendance of my lectures in Chemical Ecology and Chemistry and Biochemistry of Natural Products is required.

Solid knowledge and keen interest in **organic chemistry, analytical chemistry, biochemistry**, **microbiology**, and **molecular biology** is essential.

Literature:
At the beginning of the practical you will be provided with some key literature about your specific project and you will perform a literature search yourself.

P. M. Dewick, Medicinal natural products: a biosynthetic approach, 2009, 3rd edition, Wiley

B. Dhodary, M. Schilg, R. Wirth, D. Spiteller, Secondary Metabolites from Escovopsis weberi and Their Role in Attacking the Garden Fungus of Leaf-Cutting Ants Chemistry - A European Journal, **2018**, *24*, 4445-4452.

K. Schmidt, D. Spiteller, Ammonia Released by *Streptomyces aburaviensis* Induces Droplet Formation in *Streptomyces violaceoruber* Journal of Chemical Ecology, **2017**, *43*, 806-816.

A. Morgenstern, C. Paetz, A. Behrend, D. Spiteller, Divalent Transition-Metal-Ion Stress Induces Prodigiosin Biosynthesis in *Streptomyces coelicolor* M145: Formation of Coeligiosins. *Chemistry - A European Journal* **2015**, 21, 6027-6032.

I. Schoenian, C. Paetz, J. S. Dickschat, B. Aigle, P. Leblond, D. Spiteller, An unprecedented 1,2-shift in the biosynthesis of the 3-aminosalicylate moiety of antimycins, *ChemBioChem* **2012**, *13, 769-773*

I. Schoenian, M. Spiteller, M. Ghaste, R. Wirth, H. Herz, D. Spiteller, Chemical basis of the synergism and antagonism in microbial communities in the nests of leaf cutting ants, *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 1955-1960.

S. D. Braun, J. Hofmann, A. Wensing, M. S. Ullrich, H. Weingart, B. Völksch, D. Spiteller, Identification of the biosynthetic gene cluster of 3-methylarginine, a toxin produced by *Pseudomonas syringae* pv. *syringae* 22d/93, *Appl. Environ. Microbiol.* **2010**, *76*, 2500-2508.

S. Haeder, R. Wirth, H. Herz, D. Spiteller, Candicidin-producing *Streptomyces* support leaf-cutting ants to protect their fungus garden against the pathogenic fungus *Escovopsis*. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106,* 4742-4746.

Advanced Course "The role of microbes in stress response and resilience of aquatic metaorganisms"

Coordinators: Prof. Dr. Christian R Voolstra
Dr. Anny Cardenas
Dr. Claudia Pogoreutz
Dr. Nils Rädecker

1. Introduction

Recent years have brought a changing imperative in life sciences sparked by the revolution of genomic tools to study the molecular setup of organisms. The development of next-generation sequencing changed our understanding of microbial diversity associated with organisms and environments. There is now a multitude of studies that support the notion that host-specific microbial consortia ('microbiomes') are associated with all multicellular organisms and provide functions related to metabolism, immunity, and environmental adaptation. Consequently, the biology of animals and plants must be understood in light of the host and its associated microbial consortia, the so-called 'metaorganism'. The research interest of our group builds around this new concept. Our focus is to understand the structure and function of microbial associates and how they support host physiology and adaptation to changing environments. In particular, we study coral metaorganisms (composed of the coral animal host, intracellular photosynthetic dinoflagellate algae, and associated bacteria), which form the keystone species of reef ecosystems. Broadly, our lab follows three research areas: **Research area 1** focuses on the development of novel methods to delineate the function and identity of aquatic metaorganism member species. **Research area 2** is centered around the employment of model system-assisted approaches (manipulate gene function, microbial association, nutrient exchange) using seawater and freshwater anemones (Aiptasia and *Hydra*) to understand building principles and regulatory mechanisms of phototroph-heterotroph symbioses. **Research area 3** is focused on the development of frameworks and diagnostics that support climate change resilience using field-based physiological and genomic coral reef surveys.

2. Contents

Students will have the unique opportunity to participate and contribute to on-going research projects. The course will be in English and it will consist of daily lectures on topics related to metaorganism structure/function/adaptation, coral reef ecosystems, climate change, symbiosis, genomics, mining 'big data', bioinformatics, and statistics. We will conduct a 'journal club' where participants present research papers to discuss current literature, and students will present their results in a 'lab seminar' designed to learn to critically examine the research conducted and the results obtained. Based on their research projects, participants are expected to write a concise research paper (Introduction, Material & Methods, Results, Discussion) that detail their empirical findings. For all aspects, students will receive structured guidance and supervision.

The course will introduce participants to the Coral Bleaching Automated Stress System (CBASS), a rapid climate test to assess coral stress tolerance as a predictor for climate change survival. The project was recently supported with >4 Mio. USD by the Paul G. Allen Family Foundation (https://pgafamilyfoundation.org/Press-Room/2021/Coral-grants.aspx). In the course, we will

assess the effect of removing and adding bacteria to Aiptasia sea anemones, a model system for corals, to assess the prospect of probiotics to increase coral thermal tolerance. The course will feature a physiology, molecular, and bioinformatics component.

3. Expected background knowledge / Requirements

- Basic knowledge in molecular biology/genetics is expected.
- To pass the course, the following requirements need to be met:
    - Active participation in the lectures
    - Power point presentation in the journal club and lab seminar
    - Actively conducting research and presentation of the project results in the form of a (concise) scientific paper

4. Literature

Metaorganism framework

Jaspers C, Fraune S, Arnold AE, Miller DJ, Bosch T, and Voolstra CR. **2019**. Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. ***Zoology***.

Voolstra CR, Ziegler M (**2020**) Adapting with Microbial Help: Microbiome Flexibility Facilitates Rapid Responses to Environmental Change. ***Bioessays*** 42:e2000004

Coral Bleaching & Climate Change

Hoegh-Guldberg O (**1999**) Climate change, coral bleaching and the future of the world's coral reefs. ***Mar Freshwater Res*** 50:839–866

Kleypas J, Allemand D, Anthony K, Baker AC, Beck MW, Hale LZ, Hilmi N, Hoegh-Guldberg O, Hughes T, Kaufman L, Kayanne H, Magnan AK, Mcleod E, Mumby P, Palumbi S, Richmond RH, Rinkevich B, Steneck RS, Voolstra CR, Wachenfeld D, Gattuso J-P (**2021**) Designing a blueprint for coral reef survival. ***Biol Conserv 257:109107***

Voolstra CR, Suggett DJ, Peixoto R, Parkinson JE, Quigley K, Silveira C, Sweet M, Muller EM, Barshis DJ, Bourne DG, M, Aranda. **2021**. Extending the adaptive capacity of corals to survive climate change. ***Nature Reviews Earth & Environment***

Rädecker N, Pogoreutz C, Gegner HM, Cárdenas A, Roth F, Bougoure J, Guagliardo P, Wild C, Pernice M, Raina J-B, Meibom A, Voolstra CR (**2021**) Heat stress destabilizes symbiotic nutrient cycling in corals. ***Proc Natl Acad Sci*** U S A 118:

Aiptasia model system

Baumgarten S, Simakov O, Esherick LY, Liew YJ, Lehnert EM, Michell CT, Li Y, Hambleton EA, Guse A, Oates ME, Gough J, Weis VM, Aranda M, Pringle JR, and Voolstra CR. **2015**. The

genome of Aiptasia, a sea anemone model for coral symbiosis. ***Proceedings of the National Academy of Sciences*** 112:11893-11898

Voolstra CR. **2013**. A journey into the wild of the cnidarian model system Aiptasia and its symbionts. ***Molecular Ecology*** 22:4366–4368

Prokaryotes & stress resilience

Costa RM, Cárdenas A, Loussert-Fonta C, Toullec G, Meibom A, Voolstra CR. **2021**. Surface Topography, Bacterial Carrying Capacity, and the Prospect of Microbiome Manipulation in the Sea Anemone Coral Model Aiptasia. ***Front Microbiol*** 12:637834

Peixoto RS, Sweet M, Villela HDM, Cardoso P, Thomas T, Voolstra CR, Høj L, Bourne DG. **2021**. Coral Probiotics: Premise, Promise, Prospects. ***Annu Rev Anim Biosci*** 9:265–288

Ziegler M, Seneca FO, Yum LK, Palumbi SR, and Voolstra CR. **2017**. Bacterial community dynamics are linked to patterns of coral heat tolerance. ***Nature Communications*** 8:14213. 10.1038/ncomms14213

Santoro EP, Borges RM, Espinoza JL, Freire M, Messias CSMA, Villela HMD, Mattos LP, Vilela CLS, Rosado JG, Cardoso PM, Rosado PM, Assis JM, Duarte GAS, Perna G, Rosado AS, Macrae A, Dupont CL, Nelson KE, Sweet MJ, Voolstra CR, Peixoto RS. **2021**. Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. ***Science Advances***

Symbiodiniaceae & stress resilience

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, and Santos SR. **2018**. Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. ***Current Biology*** 28:2570-2580.e2576.

Hume BCC, Smith EG, Ziegler M, Warrington HJM, Burt JA, LaJeunesse TC, Wiedenmann J, and Voolstra CR. **2019**. SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. ***Molecular Ecology Resources*** 19:1063-1080.

CBASS / Climate Stress Test

Voolstra CR, Buitrago-López C, Perna G, Cárdenas A, Hume BCC, Rädecker N, Barshis DJ (**2020**) Standardized short-term acute heat stress assays resolve historical differences in coral thermotolerance across microhabitat reef sites. ***Glob Chang Biol*** 26:4328–4343