UOS SUMMER SCHOOL

Biomembranes & Cellular Microcompartments

Osnabrück University, July 7-29, 2017

Scientific Program Organizers

Dr. Heiko Harten – UOS | Prof. Joost Holthuis – UOS | Prof. Sabine Hunke – UOS

General Program Organizers

Selina Vohlken | Malte Paolo Benjamins | International Office UOS

Funding

www.uni-osnabrueck.de/en/prospective_students/summer_schools
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WELCOME FROM THE ORGANIZERS

This Summer School offers a unique opportunity to acquire an integrated perspective on the architecture, biogenesis, dynamics and physiological functions of biomembranes and cellular microcompartments. The School is organized by the Natural Sciences Faculty at Osnabrück University, which combines a strong tradition in biomembrane research with the development of new technologies at the interface of nanoscience and cell biology. You will gain direct insight into a wide array of experimental approaches and model systems to visualize microcompartments and elucidate their function and dynamics. The School combines theory (lectures, tutorials) with experiments, laboratory tours, and a scientific symposium organized by the Integrated Research Training Group of the CRC944, bringing together Summer School participants with local group leaders and PhD students. The program also includes a career development workshop with a special focus on how to make your next move in or outside of academia.

We wish you a rewarding and inspiring time in Osnabrück.

Heiko Harten,
Joost Holthuis &
Sabine Hunke
SUMMER SCHOOL PROGRAM

FRIDAY 7 JULY

Arrival day

SATURDAY 8 JULY

09:00 – 10:30 Welcome Breakfast
Location: “Alex”, Nicolaiort 1-2, Osnabrück City Center

10:30 – 12:30 Icebreaking Games with student tutors

12:30 – 14:00 Lunch break

14:00 – 15:00 Osnabrück City-Tour with tutors
Location: Meet in front of Schloss-Mensa (University Cafeteria, City Center)

SUNDAY 9 JULY

09:00 – 19:45 Free Time or International Facetime

19:45 – 22:00 Nightwatchman Tour
Location: Meet in front of City Hall

MONDAY 10 JULY

10:00 – 11:30 Welcome Session UOS
International Office, University of Osnabrück
Location: 64/E10, Helikonienssaal, Bohnenkamphaus, Botanical Garden

11:30 – 13:00 Campus Tour with student tutors

13:00 – 14:30 Lunch Break

14:30 – 17:30 Intercultural Awareness Workshop
Location: 01/EZ04 (unless indicated otherwise)

19:00 Welcome Dinner
Location: “Kartoffelhaus”, Bierstrasse 38, Osnabrück City Center
meet in front of Schloss main entrance at 18:30

TUESDAY 11 JULY

09:00 – 09:45 Welcome & Introduction to School
Prof. Joost Holthuis & Dr. Heiko Harten, co-directors of School
Location: 64/E10, Helikonienssaal, Bohnenkamphaus, Botanical Garden

BLOCK I – Lectures “Ultrastructural Organization of Biomembranes”
Location: 64/E10, Helikonienssaal, Bohnenkamphaus, Botanical Garden

09:45 – 10:30 Dr. Katherina Psathaki & Apl. Prof. Günter Purschke: A journey into the inner life of a cell: a practical course in transmission electron microscopy – Part I

10:30 – 11:00 Coffee Break
11:00 – 11:45  Apl. Prof. Günter Purschke & Dr. Katherina Psathaki: *A journey into the inner life of a cell: a practical course in transmission electron microscopy – Part II*

**BLOCK I – Lectures “Cellular Microcompartments”**

11:45 – 12:30  Prof. Roland Brandt: *Stress, stress granules and neuronal degeneration*

12:30 – 14:00  Lunch break

14:00 – 15:30  **Taster Course German Language & Culture – Part I**
Eva Bohnet, German teacher, UOS Language Center
**Location:** 69/E18

**WEDNESDAY 12 JULY**

**BLOCK I – Lectures “Cellular Microcompartments”** (continued)
**Location:** 35/E23-24

09:00 – 10:30  Prof. Jacob Piehler & Dr. Changjiang You: *Spatiotemporal organization of multiprotein complexes resolved by fluorescence imaging techniques*

10:30 – 11:00  Coffee Break

11:00 – 12:30  Prof. Achim Paululat & Dr. Heiko Harten: *In vivo labeling of subcellular compartments in Drosophila*

12:30 – 14:00  Lunch break

14:00 – 15:30  **Taster Course German Language & Culture – Part II**
Eva Bohnet, German teacher, UOS Language Center
**Location:** 69/E18

**THURSDAY 13 JULY**

09:00 – 17:00  **BLOCK I – 2Day Practical**
Ultrastructural Organization of Biomembranes / Cellular Microcompartments

Group I (4-5 students)
Purschke/Psathaki lab: *Transmission electron microscopy of the endosomal system of an annelid*
**Location:** 35/244

Group II (4-5 students)
Piehler lab: *Resolving signaling complex assembly & dynamics in living cells by single molecule imaging techniques*
**Location:** 36/136

Group III (4-5 students)
Paululat lab: *In vivo labeling of subcellular compartments in Drosophila*
**Location:** 35/242

19:30  **Night out with Tutors**
**Location:** t.b.a.
**FRIDAY 14 JULY**

09:00 – 17:00 **BLOCK I – 2Day Practical** (continued)

Ultrastructural Organization of Biomembranes / Cellular Microcompartments

Group I (4-5 students)
Purschke/Psathaki lab: *Transmission electron microscopy of the endosomal system of an annelid*
Location: 35/244

Group II (4-5 students)
Piehler lab: *Resolving signaling complex assembly & dynamics in living cells by single molecule imaging techniques*
Location: 36/136

Group III (4-5 students)
Paululat lab: *In vivo labeling of subcellular compartments in Drosophila*
Location: 35/242

**SATURDAY 15 JULY**

04:45 **EXCURSION - I**
Mudflat hiking tour to the island “Baltrum”
Meet at bus stop "Universität/OsnabrückHalle" (direction outside of the city, vis-à-vis the Schloss)

**SUNDAY 16 JULY**

Free time or International Facetime

**MONDAY 17 JULY**

**BLOCK II – Lectures “Biomembranes, biomimetic membranes & membrane protein dynamics”**
Location: 35/E016

09:00 – 10:30 Prof. Joost Holthuis: *Mechanisms & biological impact of membrane lipid homeostasis*

10:30 – 11:00 Coffee Break

11:00 – 12:30 PD. Dr. Armen Mulkidjianian: *Emergence of new functions upon co-evolution of membranes and membrane proteins*

12:30 – 13:30 Lunch break

13:30 – 14:15 Prof. Helmut Rosemeyer: *Nucleolipids: natural occurrence, synthesis & applications in the molecular life sciences*

14:15 – 15:00 Prof. Martin Steinhart: *Converging materials science & nanobiology: biomimetic membranes & microcompartments*

15:00 – 15:30 Tea break

15:30 – 17:00 Prof. Heinz-Jürgen Steinhoff: *Using EPR spectroscopy to analyse structural dynamics of lipids and membrane proteins*
**TUESDAY 18 JULY**

09:00 – 17:00 **BLOCK II – 2Day Practical**  
Biomembranes & Biomimetic Membranes

Group I (4-5 students)  
Holthuis lab: *Bifunctional lipid technology*  
Location: 67/147

Group II (4-5 students)  
Mulkidjanian/Steinhoff lab: *Membrane protein evolution and dynamics*  
Location: 32/348

Group III (4-5 students)  
Rosemeyer lab: *Nucleolipid synthesis & applications*  
Location: 34/E04

18:30 **International Picnic**  
Location: 15/130

**WEDNESDAY 19 JULY**

09:00 – 17:00 **BLOCK II – 2Day Practical** (continued)  
Biomembranes & Biomimetic Membranes

Group I (4-5 students)  
Holthuis lab: *Bifunctional lipid technology*  
Location: 67/147

Group II (4-5 students)  
Mulkidjanian/Steinhoff lab: *Membrane protein evolution and dynamics*  
Location: 32/348

Group III (4-5 students)  
Rosemeyer lab: *Nucleolipid synthesis & applications*  
Location: 34/E04

**THURSDAY 20 JULY**

09:00 – 12:30 **BLOCK III – Lectures “Top-models in biomolecular and medical research”**  
Location: 64/E10, Helikoniensaal, Bohnenkamphaus, Botanical Garden

09:00 – 10:30 Prof. Sabine Zachgo: *Shaping the beauty: Nuclear mechanisms generating plant diversity* – incl. Botanical Garden Tour

10:30 – 11:00 Coffee Break

11:00 – 12:30 Prof. Chadi Touma: *Behavioural phenotyping of mice in biomedical research*

12:30 – 14:00 Lunch break

14:00 – 17:00 **Poster presentations** by Summer School participants and PhD students/postdocs of the Collaborative Research Center 944 “*Physiology & Dynamics of Cellular Microcompartments*”  
Location: Foyer of Building 67

17:00 **BBQ** with PhD students/postdocs of the Collaborative Research Center 944  
Location: t.b.a.
**FRIDAY 21 JULY**

**BLOCK III – Lectures “Top-models in biomolecular and medical research”**  
*Location: 35/E23-24*

09:00 – 09:45  
Prof. Jürgen Heinisch: *Yeast genetics as valuable tool to study membrane microdomains & facilitate gene expression across all biological kingdoms*

09:45 – 12:30  
**INTERACTIVE SEMINAR “Giving scientific presentations”**

09:45 – 10:30  
Prof. Joost Holthuis: *How to give an effective scientific talk? – part I*

10:30 – 11:00  
Coffee Break

11:00 – 12:30  
Prof. Joost Holthuis: *How to give an effective scientific talk? – part II*

12:30 – 14:00  
Lunch break

14:00 – 17:00  
**Visit to Felix-Nussbaum-Haus**  
*Location: Lotter Str. 2, Osnabrück*

**SATURDAY 22 JULY**

Free time or International Facetime

**SUNDAY 23 JULY**

09:15 - 21:00  
**EXCURSION - II**  
Visit to Bremen  
*Meet in front of Osnabrück train station at 09:15*

**MONDAY 24 JULY**

**BLOCK IV – Lectures “Organelle identity & protein-lipid crosstalk”**  
*Location: 35/E016*

09:00 – 10:30  
Prof. Christian Ungermann: *Organelle identity & membrane fusion*

10:30 – 11:00  
Coffee Break

11:00 – 11:45  
Dr. Gabi Deckers-Hebestreit: *Molecular interplay of lipids and membrane proteins in Escherichia coli*

11:45 – 12:30  
Dr. Florian Fröhlich: *Mechanisms and physiology of sphingolipid homeostasis*

12:30 – 13:30  
Lunch break

13:30 – 15:00  
Prof. Christian Kost: *The role of membranes for the origin of life*

15:00 – 15:30  
Tea break

15:30 – 17:00  
Prof. Michael Hensel: *Imaging infection: current approaches to understand the dynamic interplay between bacterial pathogens and their host cells*
**TUESDAY 25 JULY**

09:00 – 17:00 **BLOCK IV – 2Day Practical**
Organelle identity & protein-lipid crosstalk

Group I (4-5 students)
Ungermann lab: *Reconstitution of membrane fusion*
Location: 67/047

Group II (4-5 students)
Fröhlich lab: *Analysing lipid profiles in cells using mass spectrometry*
Location: 67/147

Group III (4-5 students)
Hensel lab: *Live cell imaging of bacterial invasion*
Location: 36/114

**WEDNESDAY 26 JULY**

09:00 – 17:00 **BLOCK III – 2Day Practical** (continued)
Organelle identity & protein-lipid crosstalk

Group I (4-5 students)
Ungermann lab: *Reconstitution of membrane fusion*
Location: 67/047

Group II (4-5 students)
Fröhlich lab: *Analysing lipid profiles in cells using mass spectrometry*
Location: 67/147

Group III (4-5 students)
Hensel lab: *Live cell imaging of bacterial invasion*
Location: 36/114

18:30 **Miniature Golf**
Location: meeting point at 18:20 at Neumarkt, Osnabrück City Center

**THURSDAY 27 JULY**

09:00 – 17:00 Introduction to and preparation for **Career Development Workshop**
Prof. Joost Holthuis & Prof. Sabine Hunke
Location: 35/E23-24

**FRIDAY 28 JULY**

09:00 – 15:00 **Career Development Workshop**
Prof. Joost Holthuis & Prof. Sabine Hunke
Location: 35/E23-24

15:00 – 17:00 Goodbye drinks with lecturers and supervisors
Location: 67/140

**SATURDAY 29 JULY**

14:00 – 15:30 **Summer School Evaluation**
Location: 15/323-324
19:00  Farewell Dinner  
**Location:** “Rampendahl”, Hasestrasse 35, Osnabrück City Center

**SUNDAY 30 JULY – TUESDAY 31 JULY**

Optional  **EXCURSION - III**  
3Day visit to Berlin

**WEDNESDAY 1 AUGUST**

Departure day
OUTLINE OF LECTURES & PRACTICALS

A journey into the inner life of a cell: a practical course in transmission electron microscopy

Lecturers: Dr. Katherina Psathaki & Apl. Prof. Dr. Günter Purschke
Divisions: Microbiology & EM-Facility; Zoology
Website: www.mikrobiologie.uni-osnabrueck.de/

Lecture topics
For learning how cells function, the understanding of the structural organization is a fundamental prerequisite. Light microscopy is limited in resolution by the wavelength of the visible light but has the important advantage that we can observe the dynamics of cell structures in a living cell due to the fact that light is relatively non-destructive. In contrast electron microscopy (EM) permits cell imaging far beyond the resolution of light microscopy and allows an approximately 1000-fold higher resolution than light-dependent microscopy. Electron microscopy can image the subcellular architecture such as organelles, cytoskeleton, biomembranes down to macromolecular complexes within the cell at almost atomic resolution. However, since specimens are exposed to a high vacuum in the electron microscope and have to be rather thin, specimens have to be fixed, preserved, embedded and sectioned by complex specimen preparation protocols. The lecture will give an overview of current EM techniques starting from prerequisites of sample preparation, standard TEM, Cryo-EM, 3D-EM (tomography, serial block-face EM, FIB-SEM) and analytical EM (ESI/EELS, EDX, WDX).

Practical (2 days)
The practical course will be a 2-day introduction and training into basic EM-applications (trimming, ultrathin sectioning ultramicrotomy, basic use of the TEM) with samples prepared by the participants and ends with the first EM images taken by the applicants. As an example we will study the endocytotic system in the epidermis of an annelid, Astomus taenioides. In order to save time we will start with fixed and embedded specimens.

Background Papers
Stress, stress granules and neurodegeneration

Lecturer: Prof. Roland Brandt
Division: Neurobiology
Website: www.neurobiologie.uni-osnabrueck.de/

Lecture topics
Stress is the body's method of reacting to a challenge, e.g., a change in environmental conditions. Stress has also been implicated in the development and progression of neurodegenerative diseases such as Alzheimer's disease. An understanding of the molecular mechanisms of how stress and disease are influencing each other is just beginning to emerge. An important mechanism how neurons cope with stressful conditions is by the formation of specialized intracellular microcompartments, so-called 'stress granules' (SGs). SGs are cytosolic, non-membranous RNA-protein complexes, which form and behave like liquid droplets and which can be considered as dynamic sorting stations for mRNAs and associated proteins. The lecture will focus on the role of SGs as adaptive but also potentially pathological mechanism of stress response during neurodegenerative disease. The liquid-liquid phase separation model, which has been developed to explain the formation and features of SGs, will be introduced. The application of novel imaging methods will be presented and discussed, which permit to analyze the dynamics and distribution of individual stress granule components in living neuronal cells. It will be highlighted how these approaches may help to analyze the role of stress and SGs as double-edged sword during neurodegeneration.

Background Papers
Spatiotemporal organization of multiprotein complexes resolved by fluorescence imaging techniques

Lecturers: Dr. Changjiang You & Prof. Jacob Piehler
Division: Biophysics
Website: www.biophysik.uni-osnabrueck.de/

Lecture topics
The dynamic organization of biomolecules into functional cellular microcompartments cannot be resolved by traditional, diffraction-limited light microscopy. Recent developments in fluorescence microscopy, however, have overcome these limitations. Fundamental concepts of these techniques will be introduced with a particular focus on single molecule localization microscopy (SMLM), which provides versatile means to visualize the assembly of dynamic cellular microcompartments with highest spatial and temporal resolution. Basic concepts of SMLM techniques including photoactivation localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM) as well as single molecule tracking (SMT) and tracking and localization microscopy (TALM) will be explained and discussed with respect to their application to unravelling nanoscale organization of proteins in cells. These techniques critically depend on the incorporation of suitable probes such as synthetic fluorescence dyes or nanoparticles. Strategies for site-specific labelling as well as novel tools for single cell handling and manipulation techniques opening exciting possibilities for qualitative and quantitative protein interaction analysis will be covered. Emerging strategies for spatiotemporal controlled re-organization of signalling protein complexes in live cells will be highlighted and their applications for quantitative biology will be discussed.

Practical (2 days)
During the practical, single molecule imaging techniques will be applied for quantifying the assembly of signalling complexes in living cells. Different posttranslational fluorescence labelling techniques will be applied for extracellular and intracellular labelling. Single molecule total internal reflection fluorescence (TIRF) microscopy for probing receptor dimerization in living cells will be performed followed by quantitative image analysis, with a particular focus on protein mobility, molecular interactions and complex stoichiometries. Functional surface micropatterning for probing protein-protein interactions at single cell level will be introduced. Spatially resolved capturing of proteins in the plasma membrane of living cells will be applied on these surfaces. The stability of protein complexes will be quantified by fluorescence recovery after photobleaching (FRAP).

Background Papers
In vivo labeling of subcellular compartments in Drosophila

Lecturers: Prof. Achim Paululat & Dr. Heiko Harten
Division: Developmental Biology
Website: www.home.uni-osnabrueck.de/apaulula

Lecture topics
The ability to label and thus identify individual subcellular compartments represents an essential aspect of modern cell biology. In this lecture, we will focus on non-invasive ways to label such compartments in living animals and introduce the “UAS-Gal4-system”, which represents a powerful and versatile tool for in vivo labeling in Drosophila. Due to its genetic amenability, the fruit fly represents an ideal model organism for such an approach. In addition to discussing the genetic basis and the multifaceted applicability of the system, we will report on the benefits of using genetically encoded fluorophores and corresponding fusion proteins as key components of the methodology. In this context, particular attention will be paid to optical readouts of cell activity in real time. Such measurements are based on the specific properties of certain fluorophores. Analyzed parameters include pH (pHluorins), redox state (roGFP), calcium transients (GcaMP), or protein lifetime (tandem fluorescent protein timers, tFTs).

Practical (2 days)
In the course of this practical session we will specifically label the membranes of subcellular compartments within salivary gland cells of live transgenic Drosophila melanogaster. Targeted compartments include plasma membrane, endoplasmic reticulum, Golgi, Golgi - ER intermediates, early endosomes, late endosomes, recycling endosomes, lysosomes, peroxisomes, mitochondria, and nuclear membranes. The corresponding compartment specific fluorescence signals will be visualized by laser scanning microscopy. In addition to a detailed evaluation of respective images, background information on the genetic and molecular bases of fluorescence-labeling in live cells will be provided.

Background Papers
Mechanisms and biological impact of membrane lipid homeostasis

Lecturer: Prof. Joost Holthuis
Division: Molecular Cell Biology
Website: www.holthuis-lab-uos.de

Lecture topics
Biological membranes form the boundaries of cells and cellular organelles, creating barriers that separate in from out. Their basic structure is a thin film of many different lipid and protein molecules, held together by mainly non-covalent interactions. Membrane lipids and proteins co-evolved since the origin of life. Understanding how they influence each other’s behavior to sustain vital cellular processes (e.g. respiration, signaling, transport) poses a major enigma in current cell biology. In this lecture, I will focus on the evolutionary origin of biological membranes; the physicochemical properties of membrane lipids in relation to their cellular functions; how cells maintain lipid diversity and non-random lipid distributions; the underlying protein machinery of lipid converters, sensors and flippases; how dysfunction of this machinery disrupts key cellular processes, resulting in system failure and disease. In a second lecture, I will discuss the development and application of photo-actuated lipids as unique experimental tools to crack the lipid code and for imaging lipid-protein interactions in live cells and organisms.

Practical (2 days)
Sphingolipids are unusually versatile membrane components that contribute to mechanical stability, sorting and signaling. This hands-on practical starts with metabolic labelling of cells using a photoactivatable and ‘clickable’ sphingoid base (pacSph) as precursor for de novo synthesis of a variety of bifunctional sphingolipids. UV-irradiation of cells allows in vivo cross-linking of proteins in direct contact with sphingolipids. Next, click chemistry is used to label sphingolipid-protein complexes with a reporter molecule, allowing their visualization and identification. These approaches provide a starting point for visual and chemical screens to search for novel sphingolipid effectors and homeostatic machinery.

Seminar "How to give an effective scientific talk"
The scientific talk, like the scientific paper, is integral part of the scientific communication process. In many ways, your research reputation will be enhanced (or diminished!) by your scientific talk. While few of us are experts at public speaking, all of us are experts at listening to scientific talks. We can easily distinguish a good talk from a bad one. To give an effective scientific presentation, we just need to learn to translate what we want as an audience member into how we give a talk as individuals. This is not an easy task! In this seminar, I will draw from your personal experience to assemble a toolkit that should enable you to give a scientific talk that sticks and enhances your scientific reputation. Questions to be addressed include: how do I make my topic accessible to a broad audience; how do I structure my talk; how do I attract and retain attention; how do I make effective slides; how do I prepare for my talk; how do I control my nerves, handle questions, etc.

Background Papers
Emergence of new functions upon co-evolution of membranes and membrane proteins

**Lecturer:** PD. Dr. Armen Mulkidjanian  
**Division:** Physics/Biophysics  
**Website:** www.macromol.uni-osnabrueck.de

**Lecture topics**  
Modern biological membranes are almost perfect barriers that help keep the chemical composition within cells constant; this composition may dramatically differ from the chemistry of the habitats. Phospholipids - which make biological membranes tight - are chemically complex molecules; their synthesis proceeds in many steps and involves diverse enzymes. The very first cell membranes, most likely, were built of simpler amphiphilic molecules and would have been impermeable only to large biopolymers, at best. Accordingly, an increase in the tightness of membranes would provide conditions for the emergence of new biological functions that demanded membranes to be impermeable for small molecules and ions. It will be shown how a combination of phylogenomic and structural analyses allows one to reconstruct the evolutionary origin of some key membrane systems.

**Practical (1 day)**  
In this practical, students will learn to apply bioinformatics tools to gain insight into the evolutionary origin of membrane proteins.

**Background Papers**  
   www.pnas.org/cgi/doi/10.1073/pnas.1117774109  
Lecture topics
Definition of Nucleolipids; Nucleoside Antimetabolites; synthetic and naturally-occurring lipids (e.g. farnesol and its role in nature); nucleolipids combat cancer: biomedical application of nucleolipids in the treatment of human cancer types from the NCI-60 panel; effect of nucleolipids on PMA-differentiated THP-1 macrophages as a measure of nucleolipid side effects; synthesis of auto-fluorescent nucleolipids and their interaction with natural (human stratum corneum of the skin) and artificial lipid membranes. Nucleolipids as hydrophobization tools of oligonucleotides (e.g. siRNA) for a better cell membrane transfer; biophysical studies on the interaction of artificial lipid bilayers with lipo-oligonucleotides carrying different nucleolipid headgroups. Developed working techniques, demonstrated by an mp4 video.

Practical (2 days)
Day 1: Two-step syntheses of two auto-fluorescent nucleolipids, carrying different lipophilic groups (each first-step compound will be provided by the group); each second-step compound including their chromatographic purification should be performed by the summer school members, guided by a group member ((a) M.Ed. C. Knies; (b) M.Ed. T. Abakumov).
Day 2: Transfer of the summer school students, together with their compounds to the Ionovation GmbH, Westerbreite 7, Osnabrück and on the spot study of membrane incorporation of the auto-fluorescent nucleolipids into an artificial lipid bilayer by fluorescence spectroscopy using a confocal fluorescent microscope.
Requested deliverable: a short report at the end of the summer school period.

Background Papers
Converging materials science and nanobiology: biomimetic membranes and compartments

**Lecturer:** Prof. Martin Steinhart  
**Division:** Institute of Chemistry of New Materials  
**Website:** www.ifc.uni-osnabrueck.de/physikalische-chemie/

**Lecture topics**
To elucidate functionalities of biomolecules, it might be helpful to create controlled artificial environments that allow tuning specific environmental parameters. By doing so, the response of complex molecules to intentionally adjusted triggers under well-controlled model conditions may be monitored and compared to the molecules' behaviour under physiological conditions. Moreover, artificial surfaces for biological-synthetic interfacing that possess either tailor-made chemical or tailor-made topographic (Figure 1) patterns may allow gaining enhanced understanding of cellular interactions. The lecture tries to present the thrusts material science can offer to create such artificial model environments. These include artificial cavities, membranes and surfaces with tailored properties.

**Fig. 1.** Fibroblast cell grown on an artificial array of biodegradable polymer nanorods.

**Background Papers**
Using EPR spectroscopy to analyse structural dynamics of lipids and membrane proteins

Lecturer: Prof. Heinz-Jürgen Steinhoff
Division: Physics/Biophysics
Website: www.macromol.uni-osnabrueck.de

Lecture topics

**Topic 1:** Electron paramagnetic resonance (EPR) spectroscopy in combination with site-directed spin labeling (SDSL) has emerged as a powerful method to study the structure and conformational dynamics of membrane proteins. EPR spectroscopy of spin label side chains introduced into a protein provides structural and dynamic information for restraint modeling of protein domains, protein-protein or protein-lipid interaction and for following conformational changes with high temporal and spatial resolution. Double electron-electron resonance (DEER) spectroscopy not only reveals average interspin distances but also provides distance distributions making this approach very valuable for determining heterogeneity of complex systems, e.g., of membrane protein complexes in their native environment or for characterizing thermodynamic equilibria of protein conformations. The first lecture will cover the basics of SDSL EPR, the second lecture will present applications with examples on the signal transfer mechanism in the halobacterial phototaxis sensory rhodopsin-transducer complex, the conformational dynamics of rhodopsins and of ABC-transporters.

**Topic 2:** Signal transduction mechanisms in chemotaxis and phototaxis. Chemotaxis and phototaxis of bacteria and archaea regulate cell motility through two-component signal transduction pathways. These sensory pathways have been studied in detail during the past 40 years. Structural and functional studies applying site-directed spin labelling and electron paramagnetic resonance spectroscopy have yielded insights into the structure, the mechanisms of signal perception, the signal transduction across the membrane and provided information about the subsequent information transfer within the transducer protein towards the components of the intracellular signalling pathway. The lecture will provide an overview about the findings of the last decade, which served to understand the basic principles microorganisms use to adapt to their environment. We document the time course of a signal being perceived at the membrane, transferred across the membrane and, for the first time, how this signal modulates the dynamic properties of a HAMP domain, a ubiquitous signal transduction module found in various protein classes.

**Practical (1 day)**
A first set of experiments will show how EPR spectroscopy can be used as a tool to quantify the dynamics of spin labeled proteins and lipids. This tool will be applied to determine the reorientational dynamics of lipids in different lipid mixtures with the nitroxide spin label located at different distances from the head groups.

**Background Papers**
Shaping the beauty: Nuclear mechanisms generating plant diversity

Lecturer: Prof. Sabine Zachgo, Director of the Botanical Garden
Division: Botany
Website: www.home.uni-osnabrueck.de/szachgo/home & www.bogos.uni-osnabrueck.de/

Lecture topics
Among extant plants, angiosperms are by far the dominant group, since they comprise more species than all the other living land plant groups combined. This phenomenon had already been noted by Charles Darwin and puzzled him so much that he referred to it as an ‘abominable mystery’. Our lab investigates the mechanisms that contributed to establish the diversification of plants with a focus on crucial nuclear events. Gene duplications enable the generation of functional redundancies, the raw material for further sub- and neofunctionalization processes, which can give rise to novel innovations. We investigate morphological and biochemical key innovations in land plants by employing a comparative molecular approach. Covering over 450 MYs of evolution, we analyze a broad spectrum of evolutionary informative species such as mosses, basal land plants, which left an aquatic environment and adapted to novel biotic and abiotic stresses. Thereby, plants successively increased their complexity yielding finally the enormously diverse angiosperms. The molecular mechanism to generate biodiversity are presented and subsequently illustrated by a tour through our Botanical Garden. The Garden hosts more than 8,000 plant species, including an agrobiodiversity area demonstrating how nuclear mechanisms impacted during the domestication of wild plants to generate our cultivated, edible crops.

Background Papers
Lecturer: Prof. Dr. Chadi Touma  
Division: Behavioural Biology  
Website: www.verhaltensbiologie.uni-osnabrueck.de

Lecture topics

Rodents represent the vast majority of all vertebrates used for research worldwide. Particularly in the biomedical sciences, laboratory mice are extensively used, as they share many physiological and genetic characteristics with humans. Furthermore, genetic manipulations such as knocking out or overexpressing certain gene products are possible in this species, making mice ideal animal models for investigating the molecular mechanisms and novel therapeutic strategies for human disorders. Particularly for mouse models of psychiatric diseases, an in-depth characterization of the emotional behaviour and cognitive functions of these animals is of major importance.

Although the majority of test paradigms available for the behavioural phenotyping of mice are relatively simple, their proper performance and the valid interpretation of the findings are not straightforward. In order to achieve good reproducibility and biological relevance of the results, ample experience and expertise in behavioural phenotyping of mice is as important as keeping high quality standards of the test settings.

In this lecture, I will introduce behavioural test paradigms frequently used for assessing emotionality and cognitive functions in mice, including important aspects of the test design, readout parameters, data analysis and interpretation as well as limitations and confounding factors.

Background Papers

Yeast genetics as valuable tool to study membrane microdomains & gene expression across all biological kingdoms

Lecturer: Prof. Jürgen J. Heinisch
Division: Genetics
Website: www.genetik.uni-osnabrueck.de/

Lecture topics
The yeast *Saccharomyces cerevisiae* is the eukaryotic model organism, due to its well established classical genetics (i.e. tetrad analysis), the ease of its genetic manipulation, and its enormous capacity for homologous recombination. The latter has been employed in the construction of various “triple-shuttle vectors”, which allows cloning and expression of genes in *E. coli* and *S. cerevisiae* in combination with different hosts, such as *Drosophila melanogaster*, *Mus musculus*, and *Arabidopsis thaliana*. The CRISPR/Cas9 system is currently being adapted to these vectors. In a first part, I will give an overview on these basic applications of modern yeast genetics. The yeast plasma membrane contains several proteins, which reside in distinct patterns ranging from patches to network-like structures. Different sensors of the cell wall integrity signaling pathway reside in different patterns. In the second part, we will show how yeast genetics and cell biology can be employed to study the *in vivo* distribution of membrane proteins and relate the patterns to their physiological functions.

Background Papers
Organelle Identity and membrane fusion

Lecturer: Prof. Christian Ungermann
Division: Biochemistry
Website: www.biochemie.uni-osnabrueck.de

Lecture topics
Cellular membranes separate cells from the outside, but also provide selected environments to facilitate biochemical pathways such as protein degradation. In eukaryotic cells, organelles of the endomembrane system exchange proteins and lipids via vesicles, but can also change identity by a process named organelle maturation. This is particularly important in the endocytic branch of the endomembrane system, along which membrane proteins are finally degraded in the lysosome. In my lecture, I will focus on the mechanism of membrane fusion at endomembranes, in particular at the lysosome like vacuole. I will describe how peripheral membrane proteins are recruited to endomembranes, how they shape the organelle, organize recycling of receptors and downregulation of selected membrane proteins. I will then highlight recent insights, how proteins involved in membrane fusion cooperate in tethering and bilayer mixing, how maturation of organelles is likely regulated and sensed, and how this eventually leads to a change in identity. I will further provide insights how lysosomes are linked to other organelles, and how lysosomal signalling affects its biogenesis.

Practical (2 days)
Endosomes and lysosomes recruit peripheral membrane proteins to their surface in a coordinated manner. In this hands-on practical, we begin by isolation of a dimeric protein complex involved in the recruitment of a switch-like GTPase, the Rab7 like Ypt7. They will determine its concentration and purity. Students will then learn how to determine activity of the complex by using a fluorimeter-based activity assay. Furthermore, activity will be determined in a reconstituted membrane fusion system, which relies on content mixing of fused proteoliposomes. The approaches taught in the practical exemplify how the order and control of organelle maturation can be dissected in the test tube.

Background Papers
Molecular Interplay of Lipids and Membrane Proteins in *Escherichia coli*

**Lecturers:** Dr. Gabriele Deckers-Hebestreit  
**Division:** Microbiology  
**Website:** www.mikrobiologie.uni-osnabrueck.de/

**Lecture topics**
To enable life in a wide range of physical environments, *Escherichia coli* needs a precise control of the biophysical properties of its glycerophospholipid composition. The inner membrane of *E. coli* consists of only three different phospholipids phosphatidylethanolamine (70-75%), phosphatidylglycerol (20-25%) and cardiolipin (3-10%) with mainly three different fatty acid chains, C16:0, C16:1 and C18:1. Nevertheless, the lipid composition is exactly adjusted by changing the types of fatty acids produced in the biosynthetic pathways as well as by modifying pre-existing phospholipids in the membrane. The lecture will focus on the *E. coli* phospholipid biosynthesis and its regulation. Especially, changes in the phospholipid composition in dependence on the growth phase or temperature and due to acid or oxidative stress, respectively, will be discussed in detail and compared to mechanisms used by other Bacteria. Due to its comparably low complexity, *E. coli* phospholipids form an ideal model membrane to study the influence of phospholipid class variations on membrane protein function and the effects of membrane fluidity and thickness on protein dynamics *in vitro* as well as *in vivo*. Experimental tools to analyse the lipid micro-environment of membrane proteins, its influence on dynamics, localization and activity will be described.

**Background Papers**
Mechanisms and physiology of sphingolipid homeostasis

Lecturer: Dr. Florian Fröhlich
Division: Molecular Membrane Biology
Website: www.biologie.uni-osnabrueck.de/arbetsgruppen/molekulare_membranbiologie.html

Lecture topics
Every cell is enveloped by a membrane that forms a barrier between the cell and its environment. This membrane contains fat molecules called ‘sphingolipids’, which help to maintain the structure of the membrane and enable it to work correctly. These molecules are also used as signals to send information around the interior of the cell and are required for the cell to grow and divide normally. The levels of sphingolipids in the membrane have to be tightly controlled because any imbalance can cause stress to the cell and can lead to serious diseases. How cells maintain sphingolipid homeostasis remains largely unknown. In this lecture I will focus on recent developments linking intra-cellular trafficking to sphingolipid homeostasis and its implication in neurodegenerative disorders. I will also discuss cutting edge technologies to study lipid homeostasis systematically.

Practical (1 day)
Cells have to regulate their lipid composition according to need. Therefore, different metabolic enzymes have to be regulated accordingly. To explore the function of these enzymes the lipid profile of such mutants have to be quantified. First, lipid standards for the quantification of lipids will be measured. Next, membrane lipids will be extracted from mutants, spiked with lipids standards and identified and quantified by mass spectrometry (MS). Because of the similar or even identical molecular weight of distinct lipid species, lipid extracts will be subjected to Post-Source-Decay analysis (MS/MS). The reassembly procedure of the target molecule structure will be practiced as well as how to handle the MS equipment.

Background Papers
The role of membranes for the origin of life

**Lecturer:** Prof. Christian Kost  
**Division:** Ecology  
**Website:** www.kostlab.com

**Lecture topics**

Life can be defined as a self-sustaining chemical system that is capable of Darwinian evolution. To achieve this, life had to invent chemical molecules that can function as replicators with both informational and catalytic properties. Moreover, to participate in the process of evolution by natural selection, these molecules needed to produce offspring that is heritably different from the parental generation and these differences needed to translate into competitive advantages over other molecules. In the long-run, this process should have favoured replicators that are more stable or can replicate faster than other molecules that compete for the same resources. Likely, RNA was the first genetic material able to evolve by natural selection. However, only the emergence of first protocells, in which self-replicating molecules were encapsulated by a membrane, provided a physical link between the genome and its gene products. This membrane-mediated compartmentalisation was thus likely a necessary condition that allowed different replicators to compete against each other. I will discuss the features that make RNA a likely candidate as the first genetic molecule, describe the difficulties to explain the stable coding of biological information, and illustrate how the emergence of first membranes has set the stage for biological complexity to evolve.

**Background Papers**

3) Deamer D (2017) The role of lipid membranes in life’s origin. *Life* 7(1)
Imaging infection: current approaches to understand the dynamic interplay between bacterial pathogens and their host cells

Lecturer: Prof. Michael Hensel
Division: Microbiology
Website: www.mikrobiologie.uni-osnabrueck.de/

Lecture topics
Bacterial pathogens have evolved sophisticated mechanisms to manipulate cellular functions of mammalian host cells. These manipulations comprise manipulation of the cytoskeleton resulting in invasion of non-phagocytic cells or paralysis of phagocytes, induction or inhibition of apoptosis, redirection of vesicular traffic, reprogramming of cell cycle, and many more. To understand the cellular and molecular mechanisms of these manipulations, imaging of host-pathogen-interactions is of paramount importance. As these interactions highly dynamic and can vary substantially between cells, various approaches for live cell imaging are available. Lecture part a) introduces model systems for live cell imaging of infection. The key virulence traits of *Salmonella enterica* and *Listeria monocytogenes* will be introduced, and the bacteria are used in the practical part. Lecture part b) introduces microscopy setup for live cell imaging of infection in cell culture models. The technical and biological requirements for analysis host-pathogen-interactions in live cells will be discussed (infection formats, cell lines, fluorescent proteins, fluid tracers, inhibitors, etc.). Approaches to experimentally manipulate the interaction (photobleaching, -activation, -switching) are introduced. I will further describe experimental setup for more complex infection models, such as intravital imaging of infection by parasites such as *Plasmodium falciparum* and correlative imaging approaches to link live cell imaging to ultrastructural analyses.

Practical (1 day)

a) Live cell imaging of bacterial invasion. *Salmonella enterica* and *Listeria monocytogenes* expressing fluorescent proteins are used to infect mammalian epithelial cells. Host cells express LifeAct-GFP for labelling the F-actin cytoskeleton. The alterations of the host cell cytoskeleton will be followed during the course of infection. Mutant strains with defects in invasion will be compared to wild-type strains. The effect of pharmacological inhibitors of the actin cytoskeleton on invasion will be analyzed.

b) Live cell imaging of intracellular lifestyle of bacterial pathogens. *S. enterica* and *L. monocytogenes* expressing fluorescent proteins are used to infect mammalian epithelial cells. Host cells express LifeAct-GFP for labelling the F-actin cytoskeleton or LAMP1-GFP for labeling of late endosome/lysosomes. *L. monocytogenes* escapes into the host cell cytosol and initiates intracellular motility by recruiting F-actin. *S. enterica* resides in a membrane-bound compartment, redirects host vesicular transport and induces the formation of a complex tubular network of endosomes.

Background Papers


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