# ABSTRACTS

## 1) How to prepare samples for electron microscope without killing yourself

The process of sample preparation for electron microscopes requires many steps including possible dangerous procedures. In this presentation, two sample preparation processes will be briefly introduced with a special focus on potentially dangerous steps, namely, the preparation of plant chromosomes for electron microscope scanning and protein sample preparation for transmission cryoelectron tomography. The difference between scanning and transmission electron microscope requirements will be addressed.

## 2) Piece by piece: Creating the picture of organism response to metal nanoparticles with different methods

Lead and cadmium are environmental pollutants causing harmful effects in multiple organ systems. Although the toxic effects of their nanoforms are not yet fully known, it has been determined that they have a unique inflammatory blueprint. In this presentation, the design of inhalation experiments using whole-body inhalation chambers with female mice will be introduced, together with the wide spectrum of different methods used for investigating organism response to metal nanoparticles exposure. Various methods for the proper evaluation of samples include: biochemical analyses, immunohistochemistry and immunofluorescence, Western blot, qPCR, RNA Scope, transmission electron microscopy, atomic absorption spectrometry, LA-ICP-MS and lipid LC-MS. A combination of these advanced methodological approaches will expand knowledge about pathological changes in the organs.

## 3) A new insight into molecular functions of AGR2 in colorectal cancer

In recent years, the role of Anterior gradient protein AGR2 in tumour development and progression has been studied more intensively. The

contribution of AGR2 to malignant transformation, drug resistance and the development of metastases has already been reported by others as well as our group. However, the mechanism of the/its actions and the scope of AGR2 functions remain largely unclear especially in colorectal cancer (CRC) where the role of AGR2 has been minimally studied. This project aims to i) elucidate the molecular reprogramming of AGR2 expression during metastasis, ii) clarify the molecular pathways associated with AGR2 in cell adhesion and iii) describe the role of AGR2 in regulating gene expression at the post-transcriptional level through RNA-protein interactions. Together these could bring new findings contributing to the understanding of these complex mechanisms and may help in future research or even diagnosis and treatment of colorectal cancer on which this project is focused

**4) Know your enemy: Bringing the infection cycle of human enteroviruses to light**

Echovirus 18 (E18) is a common cause of encephalitis and meningitis. However, the structure and life cycle of E18 and many other viruses from Enterovirus genera are still unknown. The in vitro approach of virus purification lacks information about/regarding the replication cycle of the virus in natural conditions. In this project, the focused-ion beam milling (FIBM) is used to create lamellas within the infected cells and image them using cryogenic electron tomography (cryo-ET). A 3D reconstruction of cell sections identifies the structure of the replicating viruses and their intermediate states and addresses the morphological changes of the infected cells in situ. Data regarding human echovirus 18 might serve as a reference for further experiments on other enterovirus species. This research may lead to a better understanding of virus biology and enabling the development of antiviral treatment

**5) Exploring cell wall proteins in plants**

The cell wall is an important structure for plants as it determines the shape of cells, enables a connection of cells, and provides a basic mechanical strength. The cell wall consists of biopolymers such as cellulose, pectins and lignin. Cellulose and lignin are the most abundant polymers on Earth and their modifications have an application in a wide range of industries such as food, paper, textiles and pharmaceutical industries. In the cell wall, many proteins can be found including expansins. Expansins are cell wall-loosening proteins activated during cell wall acidification, first described in cucumber seedlings. Expansins are known to disrupt bonds in the cell wall structure. To investigate the cell wall biomechanical properties of plants, Gateway cloning strategy was used, as well as, a chemically inducible activation system which was devised to regulate transgene expression in plants. In addition to these methods, confocal laser scanning microscopy and GUS staining were applied and will be included in this presentation. Initial results show the expression pattern of expansin 1 in different tissue layer of plants.

**6) Can we do science without animal testing?**

Research involving laboratory animals is considered necessary to ensure human and animal health and to protect the environment. In the absence of human data, it is the most reliable methodology when detecting important toxic properties of chemicals and estimating risks to human and environmental health. Animal research can be debated on many grounds: ethical reasons, utility, reliability, price. These points are crucial to deciding whether animal testing should be conducted, but it does not stop the fact that it is currently happening. In 2014, approximately 7 million animals were used in research and teaching in Australia. In the U.S, approximately 1 million animals were used. However, this value excludes mice, birds, and rats- these other excluded animals could account for up to 90% of the actual testing done. Computer modeling is a promising field. Modeling can be used to do disease and treatment in silico tests and interpret data from human clinical trials. Bioinformatics is also a growing field using similar

technologies. In vitro technologies like organs-on-chips aim to mimic the functions and microstructure of human's living organs. There are several new technologies being used and the question still remains: can we do science without animal testing?

**7) Exons in, introns out: The importance of correct splicing**

Splicing is one of the post-transcriptional modifications that transforms the precursor mRNA to mature mRNA that can be translated into functional protein. During splicing, introns (non-coding regions) are removed and exons (coding regions) are joined together due to the splicing machinery known as spliceosome. The correct recognition of exons is driven by many factors, especially by splice sites quality, exon length, secondary mRNA structure, or presence of splicing regulatory elements. Even a single point mutation can influence the spliceosomal function. In this presentation, the basic conditions of the splicing process will be introduced with a more detailed focus on pseudoexons –activation events, methodology, and effects of pseudoexon inclusion on human genetic disorders. Deeper insight into the splicing process and pseudoexon recognition may bring valuable information, which could be used in clinical practice.

**8) Gap Junction Intercellular Communication : A Biomarker of Testicular Toxicity**

Effects of the individual chemical responses are well reported in the literature; yet, overlooking the consequences of mixture exposure. There is worldwide decline in male fertility and exposure to organochlorines and their mixture pose a risk to the male reproductive health. The male reproductive system is an understudied system and previously conducted studies have shown the interreference of the organochlorine cocktail with reproductive health. Testicular GJIC plays a vital role in spermatogenesis and steroidogenesis; however, they are often overlooked due to the lack of HCS (High Content Screening) method. In this study, we assessed the effects of organochlorine cocktails on gap junction intercellular communication (GJIC) in murine male prepubertal testicular (Leydig Tm3 and Sertoli Tm4) cells using

the multiparametric scrape load dye transfer (SLDT) assay. The organochlorine mixture inhibited testicular GJIC at the non-cytotoxic concentrations in both Leydig (Tm3) and Sertoli cells (Tm4), respectively. In conclusion, GJIC is an important marker of the testicular toxicity as the inhibition in GJIC could lead to the cancer formation in further stages.

**9) Antimicrobial peptides: A solution to bacterial antibiotic resistance?**

The discovery of antibiotics has enabled the development of our society as we know today. Previously deathly diseases have become a mere discomfort. However, in the 21st century, we may experience the end of this antibiotic era due to the rapid emergence of antibiotic-resistant bacterial strains. According to the WHO, resistant bacteria might cause more than 10 million deaths a year worldwide by 2050. To prevent such a scenario from happening, new types of antibiotics are desperately needed. Antimicrobial peptides constitute a class of potential novel therapeutics. Despite being highly active against various pathogens, their clinical use is often prevented for multiple reasons, cytotoxicity being one of them. The presented project aims to reveal amino acid patterns responsible for the peptide selectivity towards bacterial cells, exploiting the differences in cellular membrane lipid composition. Such knowledge will enable modifications of existing antimicrobial peptides to enhance their selectivity as well as the design of novel antimicrobial peptides with low cytotoxicity. Such an improvement of the peptides would increase their chances of progressing into the clinical trials.

**10) Telomeres in plants and humans – not so similar, not so different**

Although the principles of telomere biology are conserved and point to common evolutionary roots of eukaryotes, their implications for cell and organism survival, senescence, and aging are not shared among kingdoms. In particular, plants show specific features of their growth and development. The requirement to finish the incomplete

replication of chromosome ends is common for all organisms with linear chromosomes. In eukaryotes, this requirement is commonly solved by a specific nucleoprotein enzyme complex called telomerase. It consists of two major subunits in most of the organisms: telomerase RNA (TR) and telomerase reverse transcriptase (TERT). However, the holoenzyme of functional telomerase is composed of many other proteins that can regulate telomerase biogenesis or its access to the telomeres. We recently demonstrated that some protein homologues participate in TERT biogenesis also in plants. Since the genuine plant RNA subunit of telomerase has been identified only recently, it has yet to be found out if the assembly of the telomerase holoenzyme into catalytically active complex is aided by snoRNA proteins, as it is in mammals. In this presentation, the differences between human and plant telomere biology will be shown, with an emphasis on the comparison of telomere associated proteins acting in humans and in A. thaliana model plant. The knowledge of plant telomere biology could help us to understand how efficient are plant molecular systems in ensuring maintenance of genome stability.

**11) Structural insight into lectins: finding a new substitution for antibiotics**

Lectins are ubiquitous proteins and glycoproteins with the ability to specifically, non-covalently and reversibly bind to mono-, oligo- and polysaccharides. These sugar-binding proteins play an important role in many processes occurring in nature such as cell-cell interaction or recognition of the host by the pathogen. Since lectins are involved in the host-pathogen interaction, we can use our results and knowledge for drug development in a so-called antiadhesive therapy to overcome this antibiotic resistance related issue. To understand the lectin function and mechanism of action, we need to obtain the high-resolution structure of the studied protein. Structural data describes key residues involved in interaction and allows us to find substances for antiadhesive therapy.

**12) Making Good's Buffers Good for Freezing: The Acidity Changes and their Elimination via Mixing with Sodium Phosphate**

Three Good's buffers (HEPES, MOPS, and MES), both pure and mixed with sodium phosphate buffers (Na-P), are investigated in terms of the freezing-induced acidity changes in their operational pH ranges. The Good's buffers have the tendency to basify upon freezing and, more intensively, at lower pHs. The acidity levels vary most prominently in MES, where the change may reach the value of two. It is significant that the Good's buffers are shown to mitigate the strong acidification in the Na-P buffer. Diverse concentrations of the Good's buffers are added to cancel out the strong, freezing-induced drop in 50mM Na-P that markedly contributes to the solution's acidity; These buffer blends are, therefore, proposed to be applied in maintaining approximately the acidity of solutions even after the freezing process and, as such, should limit the stresses for frozen chemicals and biochemicals.

**13) Characterization of CA IX enzyme structural and interaction implications for targeting tumors and metastases – Killing two birds with one stone**

Human carbonic anhydrases (CAs) are a family of zinc metalloenzymes that play an essential role in the acid-base balance. Up till now, 15 human isoforms of CA have been identified, some of them are involved in cancer growth. Unfortunately, CAs share high sequence homology in enzymatic active sites, where most inhibitors are designed. This homology causes inhibitor binding to off-target isoforms present in healthy tissue. Unlike other human carbonic anhydrases, the carbonic anhydrase IX (CAIX) has a unique intrinsically disordered part (IDP) that participates in catalytic activity. The cancer cells upregulate CAIX to preserve physiological conditions inside the cancer cells while maintaining extracellular acidic pH. CAIX expression is essential to cancer cell growth, metastasis, and contributes to increased resistance to conventional radiation therapy and chemotherapy. While the catalytic domain of CAIX has been successfully crystallized, attempts to obtain crystal structure of extracellular CAIX have ended in failure and the structure of the IDP region CAIX remains unresolved despite

rising evidence of its biological relevance. Nuclear magnetic resonance (NMR) will be employed to characterize the structure of IDP region of CAIX and its interaction with isoform-specific inhibitors.

**14) Molecular Dynamics (MD) Simulation on DNA 4-way Junction, Reliable or Not?**

Molecular Dynamics (MD) simulation is a widely used technique analyzing the physical movement of molecules at the atomistic level. In nucleic acids studies. MD simulation promotes our understanding in the key interactions stabilizing standard-form DNA/RNA, the structural mechanism of conformation transitions, the interactions between nucleic acids and proteins, etc. However, compared with other molecules like proteins, this modelling technique is not mature enough to return fully reliable results of nucleic acids simulations. The abundant nucleic acids structures contribute to the major difficulties of MD simulation on nucleic acids. From our simulation research on DNA 4-way junction, a special DNA structure appears in gene recombination and DNA reparation, we have noticed/noticed incorrect descriptions of MD simulation on the junction dynamics. In order to reproduce the practical junction behaviour, several strategies that bias the simulation balance can be applied. Nevertheless, even if these strategies correct the junction simulation, they are still limited to the scenario of DNA 4-way junction application. The simulation of DNA 4-way junction works as an example to show the obstacle of acquiring a general MD simulation parameter set or protocol to have a satisfying performance on nucleic acids MD simulation.