Sunday 30 June
8:30–10:30, Yellow Room

Genome Editing and Gene Therapy – Part A

S-04.1–2
Identification and evolution of novel CRISPR-Cas systems from the human microbiome

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CRISPR technologies are transforming the bio-medicine field by providing new therapeutic concepts for the treatment of diseases through genetic repairs and deployment of disease-protecting factors. Nonetheless, the currently available CRISPR nucleases and derived technologies do not address the hurdles related to genome modification in gene therapy applications. Challenges are imposed by specific properties of CRISPR tools which includes high molecular weight limiting their compatibility with most commonly delivery vectors including lipid nanoparticles, target sequence constraints, immunogenicity and heterogeneous efficiency and precision throughout the genome. We recently focused on the development of new technologies by retrieving CRISPR systems from a large databank of the human microbiome and through a directed evolution approach to enhance the activity of the prokaryotic enzymes to eukaryotic environment. This work led us to the discovery of new CRISPR systems and the enhancement of Cas nucleases with compelling features for genome editing applications.

S-04.1-1
Deciphering DNA–protein crosslink repair *in vivo* using CRISPR/Cas genome editing in a zebrafish model

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DNA–protein crosslinks (DPCs) are very common DNA lesions that interfere with all DNA transactions including replication and transcription. The consequences of impaired DNA–Protein Crosslink Repair (DPCR) are severe. At the cellular level, impaired DPCR leads to the formation of double strand breaks, genomic instability and/or cell death, while at the organismal level, deficiency in DPCR is associated with cancer, aging and neurodegeneration. Induction of DPCs is used in medicine to treat many cancers and understanding the repair at the organismal levels could provide an impetus for the development of new drugs and combination therapies with currently used chemotherapeutics. We use zebrafish (*Danio rerio*), an established vertebrate model to study cancer, neurodegenerative and cardiovascular diseases, and CRISPR/Cas gene editing to knock-out or mutate genes of interest in order to study the interplay of DPCR factors and sub-pathways including proteolysis-, and tyrosyl-DNA phosphodiesterase-dependent repair at the biochemical and cellular level. I will present our recent discoveries from three new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR, as well as a transgenic strain with the inactive DPCR factor, tyrosyl-DNA phosphodiesterase 1 (TDP1). We have found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality. By injecting ACRC (WT) mRNA constructs into mutant embryos, we were able to grow the transgenic line and perform DPCR analysis. We found that ACRC is a DPCR protease with many cellular substrates and that the SprtT domain is essential for repair, while the intrinsically disordered region is dispensable. We also show that TDP1 is required for the resolution of topoisomerase 1- and histone-DPCs at the organismal level and we further characterise a novel TDP1-mediated repair pathway for histone-DPC repair.

ShT-04.1-1
CRISPR CGBE1-based editing on CD34+ stem cells to grow Bombay blood group compatible red blood cells

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Bombay blood group is the result of a rare genetic disorder, where individuals with the Bombay phenotype do not express the H-antigen on their red blood cells (RBC); therefore, they cannot receive blood from any member of the ABO blood group system. The objective of this project is to create an artificial blood type which can be transfused to individuals with Bombay blood group in emergency situations. For this reason, the use of CRISPR based-editing technology to mutate the FUT1 gene is proposed, which is responsible for the formation of the H-antigen in hematopoietic CD34+ stem cells, and induce the cells to produce RBCs lacking the H-antigen. We propose an *in vitro* experiment, which is comprised of designing guide-RNA (gRNA) and base editor constructs, delivering base editor and gRNA to the CD34+ stem cells, verifying on- and off-target mutations by whole genome sequencing, growing RBCs in a G-Rex medium, validation of CRISPR editing using leukoreduction filters and preparing for transfusion by cryopreservation. The project was implemented within the frameworks of IBO Challenge 2020 International Group Project. *The authors marked with an asterisk equally contributed to the work.

ShT-04.1-2
Viral and non-viral vectors for the delivery of genome editing tools to mammalian cells

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While the discovery of CRISPR-Cas systems and their application to mammalian cells has rapidly expanded genome editing tools, their medical implementation remains constrained. A key

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obstacle is the scarcity of suitable delivery tools for Cas nucleases and their sgRNA, particularly for advanced applications. Current adeno-associated viral vectors, being limited in size, struggle to accommodate both Cas and sgRNA. Addressing this limitation, liposomes and herpes viral (HSV) vectors emerge as promising alternatives, capable of overcoming size constraints and adaptable for *in vitro*, *ex vivo*, and *in vivo* applications. This study focuses on leveraging proprietary liposomes for Cas9 and sgRNA delivery to mammalian cells. Assessment involves the delivery of Cas9 RNPs encapsulated in liposomes, with Cas9-GFP fusion facilitating the visualization of protein localization in various cells. Variable Cas9 concentrations in liposomes and diverse incubation times are tested to optimize delivery efficiency. Additionally, we explore the potential of proprietary liposomes to traverse the blood-brain barrier using an *in vitro* BBB model. The second delivery approach involves HSV, targeting more intricate genome editing tools, such as prime-editors. A singular HSV vector, encompassing all prime-editor elements, was constructed using an extensible mammalian modular kit. Validation of this designed HSV vector was performed on an established reporter cell line within the laboratory, with the objective of editing genome-encoded GFP to BFP. These endeavours underscore the potential of liposomes and HSV vectors as robust delivery platforms for advancing CRISPR/Cas-based therapies, bringing us closer to overcoming existing barriers in genome editing applications.

ShT-04.1–3
Rad51-based editing of mitochondrial DNA via CRISPR approach

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Previously, we developed a CRISPR/Cas9-based method to excise the mitochondrial mutation 15059G > A. Subsequent mitochondrial DNA analysis revealed instances of double-strand break repair via homologous recombination. Exploiting this, we aimed to edit mitochondrial DNA by inserting a "barcode" sequence of either 20 or 100 nucleotides at the mutation site 1555A > G. This was achieved using two specific nickases, D10A and H840A, facilitating double-strand breaks by targeting the leading and lagging strands, respectively. Each nickase was paired with a guide RNA, one matched to the mutation and the other to a mitochondrial DNA conserved region. To enable efficient mitochondrial delivery of the nickases, the Cox8a signal peptide, a Mitochondrial Targeting Signal (MTS), was added to their domains, promoting cytoplasm-synthesized protein translocation into mitochondria. The delivery system comprised RNA encoding the nickases, the MTS, and a sequence for Streptavidin-SpyCatcher, forming a complex with biotinylated guide RNA for transport across mitochondrial membranes. The barcode insertion utilized an mRNA sequence of Rad51, tagged with an MTS, and a single-stranded oligonucleotide (ssODN) bearing unique sequence AAATTTAAA. Rad51's binding to a specific ssODN sequence facilitated its mitochondrial delivery. Our integrated system, involving mitochondrially targeted nickases and Rad51, successfully delivered guide RNA and ssODN into the mitochondrion, enabling precise genome editing. We verified the integration of both 20 and 100 nucleotide sequences into the mitochondrial genome, noting higher efficiency with the 20-nucleotide sequence. Integration was confirmed through PCR and sequencing, highlighting the system's potential for precise mitochondrial DNA mutation correction. This work was supported by the Russian Science Foundation (Grant #22–15-00064). *The authors marked with an asterisk equally contributed to the work.

Sunday 30 June
17:00–19:00, Silver Room

Translational Proteomics

S-01.3-2
Translational proteomics of rare diseases and the journey towards precision medicine

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Rare diseases have their own diagnostic and therapeutic challenges that genetic information alone cannot solve entirely. Proteomics, the global study of proteins, offers dynamic insights into protein expression, modifications, and interactions hence contributing to the understanding of disease mechanism. Translational proteomics in particular, play a pivotal role in patients' stratification to enhance diagnostic accuracy and prognostic assessment. It also contributes to understanding the cellular pathways altered in the disease and to elucidate the relationship between phenotype and genotype. In this talk, through the results of translational proteomics studies in different rare diseases, cystinuria, cystic fibrosis, cystinosis and idiopathic nephrotic syndrome, I will highlight the significant contribution of translational proteomics to the long journey towards precision medicine. Despite challenges like data complexity and limited sample availability, translational proteomics holds promise in advancing precision medicine, contributing to personalized and targeted therapies for rare diseases.

S-01.3-1
Antivenomics: a translational venomics platform for the preclinical efficacy evaluation of antivenoms

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Snakebite envenoming is a WHO class A neglected tropical disease that claims over 100 000 human lives annually worldwide. Snakebite envenoming represents a major issue for impoverished populations living in rural areas of tropical and subtropical regions across sub-Saharan Africa, South to Southeast Asia, Latin America and Oceania. Antivenoms constitute the only scientifically validated therapy for snakebite envenomings, provided they are safe, effective, affordable, accessible and administered appropriately. The assessment of the capacity of antivenoms to neutralize the lethal activity of snake venoms is the gold standard in the preclinical analysis of antivenom efficacy. To aid in the preclinical testing of antivenoms, our group has developed "antivenomics", a venomics-guided affinity chromatography-based platform for the quantitative toxin-resolved assessment of the immunorecognition landscape of antivenoms towards homologous and heterologous venoms. Antivenomics is translational venomics. In this talk we will discuss the

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