Laboratory praxis at Ruder Boskovic Institute, Zagreb, Croatia in 2022

CRISPR/Cas gene editing *in vitro* and *in vivo* for studies of DNA repair mechanisms

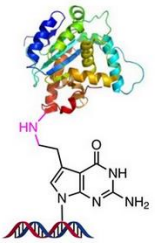
Description

Lab praxis is available during April-October 2022 to work on the DPC project (see below). Students will learn RT-CR, RNA isolation, Western blot, CRISPR/Cas gene editing in cells and zebrafish embryos and other molecular biology methods. This is a full-time laboratory internship praxis for the duration of at least 3 months. Interested applicants should send their application including the CV and motivation letter by email to mpopovic@irb.hr.

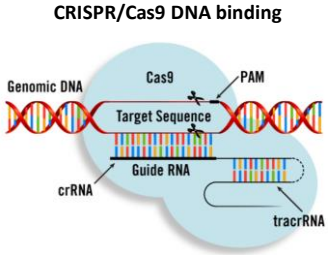
Project abstract

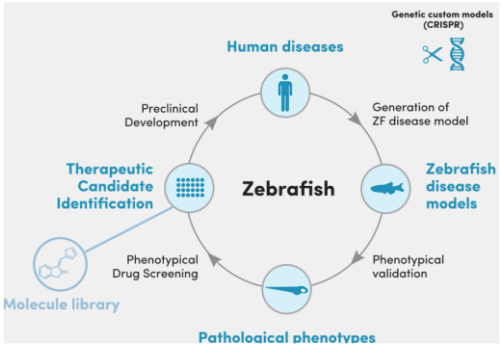
DNA-protein crosslink (DPC) is a type of DNA lesion where a protein becomes irreversibly covalently bound to DNA upon exposure to endogenous or exogenous crosslink inducers. Endogenous DPC inducers are products of normal cellular metabolism such as reactive oxygen species, aldehydes and DNA helical alterations, while exogenous inducers include UV light, ionizing radiation and various chemicals. DNA-protein crosslinks are common DNA lesions which present a physical blockage to all DNA transactions: replication, transcription, recombination and repair. If not repaired, DPCs cause genomic instability and adverse phenotypes in humans including premature aging, neurodegeneration and cancer. Despite the frequency and severe outcomes of DPCs, DNA-protein crosslink repair (DPCR) has been sparsely studied, mostly because it has not been considered a separate DNA damage repair pathway until recently. In 2014 and 2016, we and others have identified novel proteases which initiate the removal of DPCs through the proteolytic digestion of crosslinked proteins. The discovery of proteolysis-coupled DPC repair lead to recognition of the DNA-protein crosslink repair as a separate DNA damage repair pathway. We currently do not know how is the pathway orchestrated and which factors (apart from proteases) are involved, while almost nothing is known of DPCR *in vivo*.

Mechanism of DPC repair will be addressed *in vitro* through the repair quantification of artificially created crosslink of protein OGG1 in the background of targeted mutations in CRISPR/Cas9 edited cell lines. The orchestration and contribution of DPCR factors will be quantified *in vivo* using zebrafish (*Danio rerio*) vertebrate model. With CRISPR/Cas9 gene manipulation tools we knock-out or mutate target genes in zebrafish which we suspect are involved in the removal of DNA-protein crosslinks. Contribution of each protein (and their combinations) to the DNA-protein crosslink repair will be quantified after DPC isolation from transgenic zebrafish embryos and adults.



DNA-protein crosslink





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