# 1st Student Conference “Zebrafish as an animal model”

12th of April 2022

# ABSTRACT BOOK

**Organizers: The Polish Zebrafish Society**

Organizing committee:

Katerina Makarova (Medical University of Warsaw)

Krzysztof Rakus (Jagiellonian University in Krakow)

## Program of the 1st Student Conference "Zebrafish as an animal model"

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|---|---|---|
| 16:00-16:40 | Dr. Marta Migocka-Patrzałek (University of Wroclaw) | Zebrafish: a model for biomedical research |
| 16:45-17:00 | Emilia Wysocka (Adam Mickiewicz University in Poznań) | CRISPR-Cas9 F0 knockout approach using quadruple *in vitro*-transcribed guideRNAs recapitulates Rx3 function in eye morphogenesis |
| 17:00-17:15 | Paula Bartecka (Jagiellonian University in Krakow) | Intracellular localization of DDX1 in ZF4 cell line during Poly (I:C) stimulation and SVCV infection |
| 17:15-17:30 | Bartosz Michno (Jagiellonian University in Krakow) | The role of phagocytes in *Streptococcus pneumoniae* infection in zebrafish model |
| 17:30-17:45 | Filip Glista (Poznan University of Medical Sciences) | Functional studies of selected GLI3 pathogenic variants harboured by patients affected with Greig cephalopolysyndactyly or Pallister-Hall syndrome |
| 17:45-18:00 | BREAK | |
| 18:00-18:15 | Adrianna Gabryś (Medical University of Warsaw) | Application of chemometric methods to predict the toxicity of xenoestrogens towards embryos of the Danio rerio species |
| 18:15-18:30 | Martyna Mika (Medical University of Warsaw) | In silico, in vitro, and in vivo studies of curcumin |
| 18:30-18:45 | Weronika Andrzejczyk (Medical University of Warsaw) | Toxicity Analysis of the selected products of degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin using molecular docking and *Danio rerio* embryo model |
| 18:45-19:00 | Martyna Pogórska (Warsaw University of Life Sciences) | Influence of antidepressants on liver morphology of zebrafish (Danio rerio) |
| 19:00-19:15 | Patryk Bujarski (Warsaw University of Life Sciences) | The effect of nanoparticles and silver ions on zebrafish testes |
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# CRISPR-Cas9 F0 knockout approach using quadruple *in vitro*-transcribed guideRNAs recapitulates Rx3 function in eye morphogenesis

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## Abstract

The CRISPR-Cas9 technique allows targeted genome perturbation and/or editing. Application of CRISPR-Cas9-based F0 knockout (KO) approach in zebrafish, permits relatively simple and rapid generation of homozygous knockouts and allows quick investigation of gene functions. Using zebrafish as vertebrate model organism, we tested 1) how effective is the CRISPR-Cas9-based F0 KO approach using quadruple *in vitro* transcribed guide RNA's; 2) how penetrant are the resulting phenotype at later developmental stages, and 3) how robust are such phenotypes in a hybrid AB-Tupfel long-fin (TL) background. We targeted the *rx3* gene that is required for the formation of the eye, a structure that exhibits robustness and can quickly recover from early phenotypes. Our results indicate that, in majority of the samples, injection of Cas9 protein complex with 4 different *in vitro* transcribed guide RNA's targeting *rx3* results in decrease in eye size. Thus, the CRISPR-Cas9-based F0 KO approach using quadruple *in vitro* transcribed guide RNA's can recapitulate the function of a gene even in hybrid genetic backgrounds until 5-dpf stage of larval zebrafish.

Keywords: CRISPR Cas9, F0 knockout, *rx3*, eye, Zebrafish

**Intracellular localization of DDX1 in ZF4 cell line during Poly (I:C) stimulation and SVCV infection.**

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Upon infection, the innate immune mechanisms are activated immediately after pathogen recognition. Pathogens are recognized by pattern recognition receptors (PRR), which sense pathogen-associated molecular patterns (PAMP). The main viral PAMPs are viral nucleic acids. In the cytoplasm, viral RNA is recognized by RIG-I-like receptors (RLR), which belong to the DExD/H-box RNA helicases family. In mammals, some non-RLR DExD/H-box RNA helicases (e.g. DDX1) were also shown to sense viral RNA and activate antiviral response, but their role in the immune response of fish is poorly understood. The aim of this study was to investigate the intracellular localization and expression of DDX1 in zebrafish ZF4 cell line. Localization of DDX1 was studied in non-stimulated ZF4 cells as well as during Poly (I:C) stimulation and spring viraemia of carp virus (SVCV) infection using immunocytochemistry technique. Furthermore, expression of *ddx1* was studied in Poly (I:C) stimulated ZF4 cells and in zebrafish larvae stimulated with recombinant zebrafish type I interferon (zfIFNϕ1) using RT-qPCR. This study revealed the presence of the DDX1 in the cell nucleus of non-stimulated cells, Poly (I:C) stimulated cells and SVCV infected cells. A small presence of DDX1 was also found in the cytoplasm of SVCV infected cells. Significant up-regulation of the expression of *ddx1* was observed in ZF4 cell line, 24 hours after Poly (I:C) stimulation. No significant changes in the expression of *ddx1* after zfIFNϕ1 injection was demonstrated in zebrafish larvae. Our data suggest that DDX1 might be involved in sensing viral RNA and activated antiviral response in fish.

This work was supported by the National Science Centre of Poland under Sonata Bis 5 project (Grant number UMO-2015/18/E/NZ6/00516).

**Authors:** Bartosz Michno[1], Tomasz Prajsnar[1]

**Title:** “The role of phagocytes in *Streptococcus pneumoniae* infection in zebrafish model”.

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## Abstract

Zebrafish larvae have been widely used as a model organism in biomedical research due to particular set of features, such as amenability to genetic manipulation and relatively high homology of many genes with humans. Their transparency during early life stages allows visualization of the different structures including immune cells – macrophages and neutrophils to examine their biology *in vivo.*

A Gram-positive, facultative anaerobic bacterium *Streptococcus pneumoniae* is one of the major human pathogens causing invasive diseases such as pneumonia, sepsis or meningitis. It has been described that bacterial capsule is an important virulence factor promoting bacterial evasion of phagocytosis.

The aim of this study was to determine the role of professional phagocytes in response to *S. pneumoniae* infection and to confirm the involvement of the bacterial capsule using larval zebrafish model.

Confocal observation showed that macrophages are primarily responsible for internalization of pneumococci. Interestingly, the encapsulated bacteria were able to avoid phagocytosis, whereas the non-capsulated were easily engulfed and neutralized. To confirm the role of macrophages their genetic ablation was performed, and it led to proliferation of bacteria *in vivo* causing death of all infected larvae.

Subsequently, to unravel killing mechanisms of phagocytosed pathogens, zebrafish larvae with inhibited v-ATPase were infected with non-capsulated pneumococci. It led to impaired acidification of bacteria-containing phagosomes, increasing bacterial burden and finally causing host death.

Summarizing, the presented study illustrates role of macrophages as a key effectors of antibacterial host defence in zebrafish infection model and highlights acidification of internalized pneumococci as a crucial killing mechanism.

**Functional studies of selected *GLI3* pathogenic variants harboured by patients affected with Greig cephalopolysyndactyly or Pallister-Hall syndrome**

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**Introduction:** GLI3, encoded by *GLI3* gene, is a transcription factor that co-occurs either as GLI3-full length or GLI3-repressor, acting on highly conserved Hedgehog (Hh) pathway. Pathogenic variants located within the first and last third of the *GLI3* result in Greig cephalopolysyndactyly (GCPS), whereas in the middle third of *GLI3* - in Pallister-Hall syndrome (PHS).

The clinical features of GCPS encompass craniofacial abnormalities and preaxial polydactyly type IV. The variable PHS phenotypes include hypothalamic hamartoma, impairment of pituitary gland, visceral malformations, and postaxial polydactyly (type A/B).

**Aim of the study:** We aim to reconstruct the human GCPS/PHS phenotypes resulting from unpublished *GLI3* pathogenic variants in the *Danio rerio* model. In the following step we plan to analyze the expression levels of genes associated with the Hh pathway.

**Materials and methods:** After successful mutagenesis and cloning (using plasmids pCS2Dest/pENTR/D-TOPO and E.coli), mRNA was injected into *Danio rerio* eggs.

**Results:** The wild-type *gli3*-Danio rerio and c.2255C>G p.Ser752* mutants were reconstructed. Besides, different mRNA concentrations were tested - 2-20 ug/ul.

**Conclusions:** We have shown the successful mutagenesis, cloning and mRNA injections to *Danio rerio* eggs. In the nearest future we will aim to obtain the following *gli3* mutants: p.Gly674Valfs*19, p.Asp896Asn,p.Ser907Arg, p.Ser1006Arg. All mutants & wild types will be stained to visualize bone and cartilaginous elements. In the following step we plan to isolate RNA from all derived mutants & wild types, and analyze the expression levels of selected Hh pathway genes.

# Application of chemometric methods to predict the toxicity of xenoestrogens towards embryos of the Danio rerio species.

Adrianna Gabryś, Katerina Makarova

Department of Physical Chemistry, Medical University of Warsaw

Zebrafish is cheap, robust in vivo model widely used to study the toxicity of various compounds. The results, obtained with zebrafish are often highly correlated with higher vertebrates.
The aim of this study is to find a model that will predict the mortality of zebrafish embryos after exposure to xenoestrogen compounds. Xenoestrogens are considered toxic for living organisms and can disrupt the functioning of the reproductive system. Danio rerio are usually used for in vivo studies on the toxicity and properties of xenoestrogens, therefor we will test the predictions of mortality of zebrafish embryos after 24, 48, 72, 96 and 120 hours of exposure using several types of Artificial Neural Network to find the most effective one. The research involved the collecting and checking the data describing the mortality of zebrafish embryos after exposure to xenoestrogens and distribution coefficients of the compounds as well as using PCA methods for analysis of the relationship between the collected data. In the next stage we will create the model using Artificial Neural Network. The use of artificial intelligence is becoming more and more popular also in the field of pharmacy, so the model may prove useful for researchers planning in vitro toxicity testing of xenoestrogen compounds on zebrafish embryos. The results can help with selecting the concentrations of compounds.

# In silico, in vitro, and in vivo studies of curcumin

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Curcuminoids are a class of organic compounds that are primarily responsible for the yellow colour of turmeric, the spice commonly used in Indian cuisine. Curcumin is the most studied and most potent compound among curcuminoids. It is a polyphenol that is derived from the rhizomes and plant stems of the herb, turmeric. Curcumin has antioxidant, anti-inflammatory, and anticancer activities. The biological action of curcumin can be due to its binding to various molecular targets( Aurora A kinase or estrogen receptors).

As a first step, we studied curcumin toxicological safety in comparison to constituents of peppers spices. In this work capsaicin, dihydrocapsaicin, nonivamide (chili pepper), curcumin (turmeric), and piperine (black pepper) were selected as a set of ligands for molecular docking. Molecular docking (MD) was performed using α and γ-estrogen receptor and Aurora A kinase (human and zebrafish isoform). The MD studies showed a high affinity of dihydrocapsaicin and capsaicin to Zebrafish γ -estrogen-related receptor. The high value of the R2 factor (0.823) for the correlation of total score functions between human and zebrafish receptors was observed. Zebrafish studies revealed that the least toxic compounds at the concentration of 5 mg/l were piperine and curcumin. Only at the concentration of 10mg/l, they lead to high mortality (around 50-75%) in the first 48h of zebrafish embryo development.

As a second step, we tested 11 samples of beverages/drinks with curcumin available on Polish market. The antioxidant activity determined by FRAP test ranges from 255 to 6314 μM $Fe^{2+}$/ml. The highest one was for S! Metabolizm shot (6314 $Fe^{2+}$/ml). The polyphenol content ranges from 127 to 1315 mg GAE/l, and the highest one was determined for Curcumin tumeric juice 100% sample.

As a natural product with low side effects, curcumin is a safe and effective alternative to many synthetic chemical products targeting similar receptors. The curcumin residues are also safe for the aquatic environment.

**Toxicity Analysis of the selected products of degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin using molecular docking and *Danio rerio* embryo model.**

**Weronika Andrzejczyk, Katerina Makarova, Paweł Siudem**

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Dioxins pose a serious threat nowadays being toxic for both humans and the environment. Their effect is long-term and transmitted to next generations. That is why we need the knowledge about toxicity and characteristics of both main dioxins and the products of their degradation.

In this study, we screened the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and products of its degradation. We used molecular docking (*in silico* method) and examination on Danio rerio (*in vivo* method).

The main purpose of molecular docking was to estimate the interaction of TCDD and its metabolites with estrogen receptor (ER) and aromatic hydrocarbon receptor (AhR). We screened TCDD, tri-CDD (tri-chlorinated dibenzodioxin), 1,3-dihydro-2H-inden-2-one, 4,5-DCC (4,5-dichloro-1,2-dihydroxybenzene), 3,4-DCP (3,4-dichlorophenol), benzaldehyde, 4,5-dichloro-1,2-benzochinone and DCB (1,2-dichlorobenzene). Generally, the binding affinity to human receptors was higher than to fish receptors. The highest biding affinity to fish estrogen receptor was observed for 3,4-DCP and 4,5-DCC, whereas to human – for tri-CDD and 1,3-dihydro-2H-inden-2-one. The highest binding affinity to AhR receptors was obtained for tri-CDD and *4,5-DCC. Thus*, 3,4-DCP and 4,5-DCC were selected for the further in vivo studies.

To check the toxicity of compounds in fish embryos, the embryos were exposed to 0.5 to 30 mg/l concentrations of 3,4-DCP and 4,5-DCC for 72 hours post fertilization. We examined mortality rate, delay of development, yolk edema, cardiac edema, heartbeat rate, spontaneous motions and fish length. As a result, 3,4-DCP was more toxic than 4,5-DCC. Still, exposure to both compounds resulted in cardiac edema, decrease of heartbeat rate and shorter fish length.

**Acknowledgement:** The research was supported by the National Science Centre (NSC grant SONATA 2013/11/D/NZ7/02346)

# Influence of antidepressants on liver morphology of zebrafish (Danio rerio)

Authors: Martyna Pogórska, Wydział Hodowli, Bioinzynierii I Ochrony Zwierząt , Szkoła Główna Gospodarstwa Wiejskiego w Warszawie, engineer's thesis wrote under the tutelage of dr hab. Maciej Kamaszewski, profesor of SGGW, Samodzielny Zakład Ichtiologii i Biotechnologii w Akwakulturze, Instytut Nauk o Zwierzętach, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie.

A good part of xenobiotics released into the aquatic environment through sewage treatment plants are drugs, including antidepressants. Despite low concentrations of antidepressants in water enviroment (ng), they may affect water species living in such enviroment. Adverse effects of these substances on both invertebrate and vertebrate organisms have been demonstrated in a shape of development dysfunctions, changes in metabolism, behaviour, biochemistry and genes expression. The following study focuses on the effects of various antidepressants on liver histology of zebrafish (*Danio rerio*). Fish were administered antidepressants such as fluoxetine (FLUO), sertraline (SER), paroxetine (PAR), mianserin (MIAN) and a mixture (R) of the mentioned antidepressants at concentrations: 10, 20 or 40 ng/ml, where individual antidepressants were administered at 10 and 40 ng/ml concentrations and mixture of antidepressants were administered at 10 and 20 ng/ml concentrations. Microscopic analysis of the liver of zebrafish showed the increased numer of melanomacrophage centers in FLUO 20 and PAR 10 groups. Inflammation shown by increased inflow of lymphocytes was visible in the MIAN 40 group. Increased liver vacuolization was also observed in fish from FLUO 40, PAR 10 and SER 40 groups. Histomorphometric measurements showed a reduced ratio of cell nucleus area to hepatocyte area in PAR 40, MIAN 40 and R 10 groups. Likewise, enlarged ratio of cel nucleus area to hepatocyte area have been observed in FLUO 20. These results indicate stimulation of the immune system visible in liver of analised zebrafish and possible bioaccumulation of antidepressants in body tissues, which are signs of disturbed homeostasis in zebrafish body caused by xenobiotics.

**Title:** The effect of nanoparticles and silver ions on zebrafish testes.

**Authors:** Patryk Bujarski, Kacper Kawalski, Jakub Martynowm, Maciej Kamaszewski

**Speaker:** Patryk Bujarski

**Abstract:**
Nanoproducts are a new xenobiotic which concentration in aquatic ecosystems is gradually increasing. Nowadays, the impact of silver nanoparticles on water animal's community is not fully understood. Therefore, the purpose of our analyses is to investigate the effects of nanoparticles and silver ions on germinal cells and fertility in zebrafish (*Danio rerio*). Mature zebrafish individuals at 6 months of age were exposed to aqueous solutions of silver nanoparticles at concentrations of: 0.01; 0.05; 0.1; 0.5; 1.0 and silver ions at a concentration of 0.01 for 7 days. Fish in the control group were maintained in water without the tested xenobiotics. On the last day of the experiment, the fish were sedated and subjected to standard histological processing. Then testicular morphology was analysed. The study showed that high mortality was observed in groups with higher xenobiotic concentrations. The analysis of male gonads showed that in all xenobiotic-exposed groups, the percentage of spermatogonia was lower compared to the gonads of control fish, and the proportion of spermatids, was significantly higher to the control group. No histopathological changes were found in the morphology of the testis. Silver nanoparticles at concentrations higher than according to expected mathematical models in the environment negatively affect the meiosis process in zebrafish gonads. The results indicate that chronic exposure to nanoxenobiotics may lead to reproductive disruption in naturally occurring populations and disruption of demographic structure in populations.

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