In recent decades, aquaculture has become the fastest growing food production sector in the world, with fish providing an extremely important source of nutrients for humans, including unsaturated fatty acids. Unfortunately, a major problem in global aquaculture is the massive mortality of farmed fish caused by infections with various pathogens. **Disease prevention and control are therefore crucial for the development of sustainable aquaculture.** Vaccination is the most effective way to prevent infectious disease of fish. Importantly, vaccination of fish also allow to reduce the use of chemotherapeutics and antibiotics, which are still used to treat and even prevent diseases in aquaculture. The application of chemotherapeutics in the aquatic environment is highly undesirable due to their residues in fish but also due to increased bacterial resistance to antibiotics.

This project aims to develop a unique and innovative strategy for fish vaccination by using safe and highly-effective mRNA-based vaccines. The project will focus on the development of an effective vaccine against TiLV (tilapia lake virus), which is responsible for massive mortality of Nile tilapia (*Oreochromis niloticus*). In freshwater aquaculture, Nile tilapia is the third most produced freshwater fish in the world and they losses due to TiLV infection has wreaked havoc on the global tilapia industry.

Our research will be based on two animal models: (i) laboratory model organism, zebrafish (*Danio rerio*), for which a TiLV infection model has been developed in our laboratory, and (ii) the Nile tilapia, which is the natural host of TiLV. As a first step, we will investigate the effect of different modifications and purification protocols on mRNA stability and translational efficiency in fish cells using the zebrafish model. In the next step, we will examine the protective efficacy of 14 mRNA vaccine candidates (encoding 14 different TiLV virus proteins). This will allow us to select the 2 most promising mRNA vaccines encoding viral proteins with the greatest potential to stimulate the antiviral response in fish. Based on this knowledge, we will conduct studies on Nile tilapia. We will be testing the protective effect of different types of vaccines containing: (i) previously selected mRNAs, (ii) viral proteins encoded by these mRNAs, and (iii) nanoparticles coated with selected viral proteins. We will test the most effective route of vaccine delivery. In addition, we will determine the age at which Nile tilapia should be vaccinated and the duration of protection after vaccination.

This project will be conducted in collaboration between two research units which have the expertise and infrastructure to perform planned studies: (1) Department of Evolutionary Immunology of the Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, performing research on the mechanisms of fish immune response, (2) Biological Chemistry Laboratory of the Center for New Technologies at the University of Warsaw, an expert in RNA biology, synthesis, properties and applications of modified nucleotides and mRNA vaccines. **This collaboration will ensure that the planned research is carried out properly and will contribute to the development of the first mRNA vaccines for use in aquaculture.**