Ischemic Heart Disease (IHD) is a leading cause of death worldwide, accounting for almost 1.8 million deaths or 20% of all deaths in Europe annually. The main symptom of IHD is heart attack (myocardial infarction) followed by heart failure. During heart attack, large number of heart muscle cells (cardiomyocytes, CMs) is lost, typically affecting about 25% of the left ventricle. For those, who are fortunate to survive a heart attack, the massive death of CMs nevertheless triggers inflammatory response that activates repair mechanisms. Unfortunately, the human heart has very low regenerative capacity - instead of making new CMs, the repair mechanisms induce the recruitment and activation of fibroblasts, cells that are responsible for making the extracellular matrix and collagen, leading to the replacement of lost myocardium with collagen-rich scar tissue. Even though the scar provides a rapid solution to cardiac injury by stabilizing the wound area, it also weakens cardiac output due to its adverse effect on heart contractility. This incapability of heart to replenish lost CMs leads to heart failure in the long run. Heart transplant has been the only widely successful cure for heart failure, but the lack of available organs, the demanding nature of the surgery and anti-rejection therapies have driven the pursuit of alternative treatment methods. An alternative approach is to identify successful examples of organ regeneration in nature, dissect the mechanism underlying this regenerate capacity and then try to apply gained knowledge to humans via the provision of the appropriate regenerative stimuli. In contrast to mammals, lower vertebrates such as the zebrafish (*Danio rerio*), possess remarkable regenerative capacity of the heart. The zebrafish can fully regenerate its heart following amputation of up to 20% of the ventricle. After injury the wound area is sealed by blood clot which is progressively replaced by CMs over the course of 7-9 days post-injury. After two months, the size and shape of the ventricle, as well as the contractile properties of beating heart, appear normal.

In zebrafish, new CMs are generated from differentiated cells. During renewal of the injured heart, cells residing in the vicinity of the wound revert back to an earlier developmental state, proliferate, and subsequently differentiate to become CMs. It has been demonstrated that proteins regulating embryonic heart development play a crucial role during this process. Among these, Hand2 and Gata4 have been recently reported to be the key players in regenerative response. Both, Hand2 and Gata4, are transcription factors - proteins responsible for turning on the expression of genes involved in certain biological processes. Nevertheless, our understanding of how Hand2 and Gata4 regulate heart regeneration is still incomplete because many of the genes regulated by these proteins have not been identified. A thorough understanding of the pathways regulating heart regeneration would represent the initial step in the exploration of new methods for treatment of heart diseases in humans.

The difficulties in elucidating biological mechanisms are often posed by their highly complex nature – multiple genetic factors interact with each other at different stages and at different subcellular locations to regulate a particular biological process, such that studying individual pathways or genes is often unrepresentative of the whole regulatory process. We propose to characterize the process of heart regeneration in zebrafish using a genomics approach, allowing the observation of multiple biological interactions at the same time. In our proposed study, we will utilize several genomics methods based on the next-generation sequencing (NGS) technology, that allow us to sequence DNA and RNA much more quickly and cheaply than conventional sequencing methods. To identify target genes of Hand2 and Gata4 in CMs from injured as well as regenerating zebrafish heart, we will apply the chromatin immunoprecipitation followed by NGS (ChIP-seq) method. This analysis will be performed at four time points during regeneration: several hours after injury when the regenerative processes are still poorly induced, 7 and 14 days post-injury (dpi) when regeneration is significant and 2 months post-injury when cardiac muscle is fully reconstructed. In order to conduct our study, we will generate a zebrafish line carrying Hand2 and Gata4 proteins tagged with a small peptide which will facilitate their selective "fishing" using a simple method. We will further compare the identified target genes with that involved in embryonic heart development in both zebrafish (currently being generated, funded by NCN OPUS grant Nr. 2014/13/B/N22/03863) and published data on heart regeneration in embryonic mouse. This comparison will be essential to understand the reason behind the decreasing regenerative capacity over developmental progression in mammals and to explore the feasibility of developing methods to increase the regenerative capacity of the adult heart through application of the knowledge gained from zebrafish study. Our study represents a revolutionary approach to dissect into the molecular mechanisms regulating heart regeneration. Identification of the genome-wide binding sites for Hand2 and Gata4 combined with profiling of chromatin landscape changes will allow us to identify their targets and elucidate the gene regulatory network underlying heart regeneration in the adult zebrafish. By understanding how this model organism regenerate a damaged heart and comparing the mechanisms regulating heart regeneration in zebrafish and neonatal mouse, this study is envisaged to contribute valuable insights into how regeneration can be augmented in injured human hearts. Such insights would lead to an enormous social and economic impact as therapies that could facilitate survival or replacement of infracted myocardium are urgently needed.