Our organs are made of multiple types of cells which cooperate and interact to perform a particular function. The heart is an example of an organ which consists of many cell types which cooperate to perform its function to circulate blood to the whole body. In order for the heart to pump blood, heart pacemaker cells generate electrical impulses to stimulate the heart muscle cells or cardiomyocytes to contract in a rhythmic fashion. In addition, other cell types such as the endocardial cells make up other important structures such as the heart valves to ensure unidirectional flow of blood.

In order to form an organ, complex processes at the molecular, cell, and tissue levels, have to be precisely coordinated. Cell which gives rise to the heart (heart progenitors) are initially found at the left and right side of the early embryo. As development progresses, heart progenitors migrate to the midline and form a tube structure, known as the primitive heart tube. This structure subsequently expands and undergoes looping which gives rise to distinct chambers of the heart. One key question in the field of organ development relates to how the many types of cells required to make an organ are generated from a pool of progenitor cells with initially similar characteristics. More specialized characteristics evolve over time through molecular changes within the cells which results in the expression of certain genes and specific changes to the DNA molecule known as epigenetics. These factors can be utilized to determine the identity of the cells and their state of differentiation. Heart progenitor cells are located close to those that will also generate blood and blood vessels. At very early stages of embryonic development, they express a common gene known as Nkx2.5. Later on, each type of cells expresses different sets of genes and adopts particular epigenetic state which signifies their characteristic identity.

The goal of our study is to understand how the Nkx2.5-expressing progenitor cells are diversified throughout development, giving rise to cells with different characteristics in the heart, blood, and blood vessels. In order to achieve this, it is essential to follow the journey of each individual cell to determine how their molecular make-up changes over time – a feat which has only recently been possible thanks to the development of single cell sequencing technology. In this proposal, we plan to employ a range of state-of-the-art methodology which combines an experimental biology approach with the power of computational analyses to trace the process of cell diversification based on the expression of different genes and epigenetic state in each individual cell. Using the expression of Nkx2.5 as an indicator, we will specifically isolate this population of progenitors and then trace the process of diversification of each single cell into the heart and blood/vessel cell types using the single cell sequencing technology. Sophisticated computational algorithms will be developed to predict and model the cell “family tree” which describes the origins and evolution of identity for each individual progenitor cell over time. Ultimately, this interdisciplinary approach will allow us to determine the mechanism which governs the process of cell identity diversification, the knowledge of which would be essential for the development of targeted cell-based therapies for various heart diseases.

A unique strength of this study design lies in the use of zebrafish (*Danio rerio*) as a model, which allows for easy genetic manipulations necessary to apply the methods for cell tracing. The zebrafish heart exhibits remarkable similarities with the human heart in terms of form and function. The study of heart development also poses a unique challenge due to the importance of the organ for survival. In mammals, disruption to factors regulating the early steps of heart formation can result in early embryonic lethality. The use of zebrafish alleviates this problem by allowing access to developing embryos immediately after fertilization and its ability to survive without a functioning heart up to a comparatively late stage of development. Thus it is an ideal model organism to study heart development and to model human heart diseases.