The nervous system consists of a network of neuronal cells that can make connections with each other due to their special shape and the position in the brain (or other structure of the nervous system). Therefore, it is important for a proper function of the nervous system that the neuronal cells obtain their correct shape and position during the development to set up a functional neuronal networks. Neuronal cells, also called neurons derive their shape during differentiation process in which they extend neuronal protrusions in various directions. The shape of a neuronal cell is usually characteristic for each type.

mTOR protein is essential in the differentiation and positioning of neuronal cells. Yet, comprehensive understanding of its role in a complicated process of development of complex neural networks composed of several types of neurons is missing, although scattered pieces of evidence suggest that mTOR controls in fact every aspect of this process. **The general aim of the proposed project is, therefore, to unravel the role of mTOR in the development of complex and highly structured neuronal networks in the living organism.** The project will focus on development of retina of the model organism D. rerio, also called Zebrafish. Zebrafish belongs to the vertebrates, and is thus close to humans, and what is more the Zebrafish retina is composed of the same types of neurons as the human retina. It was shown previously that mTOR is necessary for neuronal shape and for proper neuronal positioning. The obtained preliminary data suggests that **mTOR controls development of retinal neurons, including shape and position, by yet not fully understood mechanisms to orchestrate most optimal connectivity and function of neuronal networks.** This hypothesis will be tested using a method developed by us (in collaboration with Prof. W. Harris, Cambridge University), which is aimed to image single retinal neurons in the living organism. The extended shapes of neuronal cells, which usually form intermingled nets, make monitoring of the shape of single neuronal cell impossible, therefore, there is a need for methods to tackle this problem. We developed a method that employs transplantations of a few cells from Zebrafish line called SoFa to fish of pigment-free line. The pigment reflects the light making the imaging of the retina impossible. The transplantations need to be performed in the very early stage of development, called blastula, when all the cells are still undifferentiated and exhibit stem-like features. The SoFa line has every type of retinal neuron expressing different combination of fluorescent proteins and therefore exhibiting different colour. The transplantations of SoFa cells to the pigment-free blastula enables imaging of a few fluorescently labeled retinal neurons, therefore making possible the simultaneous observations of changes in shape in different neuronal types and in their positioning across time. These processes will be imaged by Single-plane Illumination microscopy (SPIM), which enables high resolution 3D imaging of whole living organisms for long time. The principle of SPIM is that the illumination is done by laser sheet and not a laser line, and therefore it is more effective and much faster, reducing photo-toxicity (caused by long-lasting exposure to light) and providing possibility to image live specimens for very long time. After establishing the role of mTOR in the development of retinal neurons, the analysis of impact of the mTOR protein on neuronal networks activity in the retina, and in the connections between retina and brain will be conducted. Subsequently, the behavioral tests will be performed to examine neuro-motor skills of the fish lacking mTOR or with hyperactive mTOR protein. Altogether, these experiments will lead to our further understanding of the mTOR function on the levels of single cell, cell networks and inter-organ connections, and finally on the level of the whole organism. The research will be conducted in Zebrafish lacking mTOR and with hyperactive mTOR, which will bring complementary results followed by our better understanding of the mTOR-dependent molecular pathways and cellular processes that control neuronal development and neuronal network formation. Moreover, this project will extend our knowledge also in the field of neuro-developmental diseases like tuberous sclerosis complex.