Proper functioning of the brain is fundamental to our health and life. During brain diseases, the organism attempts to neutralize their cause and to assure recovery. Glial cells, in particular astrocytes and microglia, play a key role in reacting to any brain disease or damage. In response to pathological changes in the brain, glial cells undergo activation leading to the secretion of biologically active proteins that may control the immune response, stimulate production of new blood vessels or rebuild the extracellular matrix, i.e. the substance surrounding the cells. Intriguingly, **glial cells may behave differently in the course of distinct diseases**, which is associated with the secretion of various proteins, and in consequence leads to a variety of effects, promoting recovery or, on the contrary, stimulating inflammation and destruction of nerve cells. Acquisition of these distinct properties by glial cells depending on the type of stimulus is called **phenotypic polarization**. This process, which triggers various states of microglia and astrocyte activation, is extremely important for the course of brain diseases, but the mechanisms controlling it are still not fully understood.

Our preliminary results indicate that the activity of so-called **VPS10P domain (VPS10P-D) sorting receptors** may contribute to different glial cell functions associated with phenotype polarization. VPS10P-D receptors, which include SorLA, sortilin, SorCS1, SorCS2 and SorCS3, are present in the brain and are known primarily for their function in neuronal cells. They contribute to the proper sorting of proteins inside neurons, i.e. they direct their protein ligands to the appropriate cellular compartments. As a result, the proteins produced by neurons reach the appropriate location within the cell or are secreted to the extracellular space. We expect that VPS10P-D receptors may also perform similar functions in astrocytes and microglia, thereby contributing to the secretion of proteins crucial for the disease progression.

The aim of the project is therefore to determine **the importance of VPS10P-D receptors for glia function in various activation states** and, in consequence, for the course of brain diseases. We plan to explore mechanisms controlling the level of VPS10P-D receptors in astrocytes and microglia and to uncover new proteins sorted by these receptors and secreted by activated glial cells. Finally, we will examine the importance of these processes for the pathogenesis of brain diseases such as gliomas, Alzheimer's disease or ischemic stroke.

In particular, we will first identify mechanisms that control the transcription of genes encoding VPS10P-D receptors in course of phenotypic polarization of microglia and astrocytes. Towards this end, we will use three complementary approaches: (1) **bioinformatic analysis** of publicly available databases derived from single-cell RNA-seq studies; (2) ***in silico*** **analysis** of potential regulatory elements important for the expression of genes encoding VPS10P-D receptors, and (3) ***in vitro*** **cell culture experiments**. Next, we will apply a **high-throughput mass spectrometry based method** to identify new ligands for VPS10P-D receptors in astrocyte and microglia. We will then examine the molecular mechanisms of sorting these new ligands by VPS10P-D receptors. We will finally verify the significance of these newly discovered mechanisms for the course of brain diseases in ***in vivo*** **models**. Depending on the results of the previous stages of our research, we will use mouse models of selected brain diseases, such as glioblastoma, ischemic stroke or Alzheimer's disease, in combination with mouse lines lacking expression of selected VPS10P-D receptors specifically in astrocytes or microglia. In addition, we will check whether key results of our study are reflected in tissue samples from patients suffering from selected brain diseases.

In summary, we will use a combination of *in silico*, *in vitro* and *in vivo* methods to build a **comprehensive model of VPS10P-D receptors regulation and function in glia during phenotypic polarization** in the course of brain diseases. This new knowledge will contribute to a better understanding of the mechanisms protecting the brain against diseases or contributing to their progression. In addition, implementation of this project will lead to generating new cell and animal models that will be used in future studies of VPS10P-D receptors conducted by us and by other research groups worldwide.