Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world with the highly growing prevalence rate, and insufficiently effective therapy, which in the ageing populations represent enormous clinical and economic burdens. The current definition of PD assumes that it belongs to a group of diseases commonly called synucleinopathy, in which the intracellular alpha-synuclein (ASN) aggregates induce neurotoxicity. According to the current hypothesis of the cell to cell 'pathogenic spread', the ASN aggregates can be transmitted to the other brain regions, similarly to prion diseases. Another neuropathological feature of PD is uncontrolled activation of the glial cells inflammatory response. Due to the multifaceted nature of PD, potential neuroprotective therapy for slowing the progression of PD should act on multiple therapeutic targets. Disturbed signalling dependent of sphingosine kinase 1 and its pro-survival product sphingosine-1-phosphate (SPHK1/S1P), similar to many other neurodegenerative pathologies, appears dysregulated in PD. This enzyme controls the homeostasis of bioactive sphingolipids with an opposite function, i.e. pro-survival S1P and pro-apoptotic ceramide, which makes SPHK1 a valuable therapeutic target in the cytoprotective strategy of many diseases. It seems likely that SPHK1 could be an essential negative regulator of both the formation of ASN aggregates and the cytotoxic inflammation in the CNS. Our previous studies have shown a decreased expression/activity of SPHK1 and a neuroprotective role of S1P receptors activation in the cellular and animal model of PD. However, the significance of SPHK1-ASN crosstalk in neuronal cell biology in PD model is not fully elucidated. Similarly, the role of SPHK1, its isoform (SPHK2) and other enzymes crucial for S1P metabolism and action, i.e. S1P lyase (enzyme degrading S1P) and S1P receptors, in neuronal and glial cells, in the course of PD remain not fully elucidated. **Current project aims to understand the importance of SPHK1/S1P signalling in PD models, induced by ASN intracellular toxicity.** Interestingly, our previous studies in the *in vitro* PD model have indicated a positive relationship between SPHK1 inhibition and extracellularly added ASN toxicity, which may suggest that this interaction is involved in the formation of toxic aggregates of the other protein, i.e. beta-amyloid oligomers. We indicated that inhibition of SPHK1 leads to the release of ASN outside of the cell. On the other hand, exogenously added ASN reduces SPHK1 expression/activity, causing oxidative stress and neuronal death. Those mentioned above, exciting observations point to the critical regulatory role of the SPHK1/S1P signalling pathway in synucleinopathies. Hence, our working hypothesis assumes that SPHK1 can inhibit ASN aggregation and its toxicity. Subsequently, we suppose that the signal dependent on S1P in astrocytes and microglia can switch their response from the classical to alternative (neuroprotective) phenotype, thus lowering the cytotoxicity of ASN and slowing the cell death.

To verify these hypotheses, we intend to conduct comprehensive research in animal and cellular model of PD, combining interdisciplinary methods in the field of molecular biology and biomedical engineering. Using fluorescence microscopy and flow cytometry combined with a fluorescence-activated cell sorter (FACS), we will examine the distribution of SPHK1 and other proteins critical for S1P metabolism and action in neurons and individual types of glial cells in the brain and spinal cord of transgenic mice with ASN mutation. Moreover, the neuronal colocalization of SPHK1 and ASN will be analysed. This task will be partially completed as part of the internship at the Institute of Neurobiology of the Slovak Academy of Sciences. Furthermore, we will investigate the level of proteins important in the metabolism and action of S1P in cells with genetically induced ASN overexpression and mice brain regions with high density of ASN inclusions. Next, we will try to answer the question: What is the impact of pharmacological activation/inhibition of SPHK1 on ASN intracellular expression, the process of ASN secretion outside of the cell and its cytotoxicity? Subsequently, we will attempt to explain the role of S1P in the classical and alternative activation of glial cells and the role of signal transmitted via S1P receptors on selected signalling pathways, crucial for the process of protein folding, regulation of mitochondrial function, and the other cell survival/death pathways in the course of PD. For this purpose, neuronal cells culture will be treated with siponimod, exerted efficacy in phase III clinical trials of Multiple Sclerosis (MS). Siponimod is a selective agonist of S1P1 and S1P5 receptors, a new generation based on Fingolimod (FTY720) structure. FTY720 is the first oral immunosuppressive drug approved in MS, with proven efficacy in preclinical studies of the other neuronal diseases, including PD, which is confirmed by our previous research.

Current project may contribute to a better understanding of the molecular mechanism of both synucleinopathy and diseases with co-existing neuroinflammation. We suppose that the activation of the S1P receptor-dependent signal in neuronal cells under ASN-induced toxicity and uncontrolled inflammatory response will exert the protective effect, which can be used in designing effective pharmacotherapy of PD and other synucleinopathies.