Microfluidics assisted studies of molecular pathway leading to stress-induced synaptic plasticity

Major Depressive Disorder (MDD) is classified as affective disorder and manifests, among other symptoms, with continuous low mood, lack of motivation and suicidal thoughts. It is predicted that MDD affects an estimated 3.8% of the global population, significantly burdening healthcare system. Despite increasing economic and social costs, the success rate of medications is about 60% due to the very complex nature of the disease, that include genetic predisposition and environmental factors. One of such factors is chronic stress, that significantly contributes to the development of depression through affecting stress hormones-sensitive limbic structures in the brain. In detail, it was found that the shape of neuron cells, specifically parts called dendritic spines that form functional contacts with neighboring neurons, is abnormally affected in stress-induced depression. Dendritic spines are very plastic and their size and shape are constantly changing in response to neuronal activity. Therefore, in search for improved diagnostic and therapeutic strategies, the molecular basis of abnormal plasticity of dendritic spines is intensively studied.

Recently, it was found that stress-induced depression causes aberrant synaptic plasticity through signaling facilitated by serotonin receptor 5-HT7R. Serotonin is a neurotransmitter in the centre of development of pharmacological antidepressant therapies, and drugs termed s*elective serotonin reuptake inhibitors* (SSRIs) inhibit the reuptake of serotonin, maintain its high concentration, thereby modulating the plasticity of synaptic connections. The 5-HT7R signaling cascade comprises multiple steps that eventually result in aberrant neuronal growth.

This project sets out to study the biophysical characteristics of 5-HT7R signaling pathway with a variety of experimental approaches, with the emphasis on microfluidic techniques. Miniaturization of liquid handling protocols in microfluidic devices has led to advances in high-throughput and parallel sample handling and detection. By bringing together novel engineering approaches and molecular techniques from the fields of chemistry, physics and biology, new microfluidic methods can be applied to quantitative measurements in protein research. Microfluidic assays perform under native conditions without the need for surface immobilization, requires only a small volume of sample for a single experiment (a few microlitres) and allows for the detection of individual species in a heterogeneous mixture. The goal of this project is to use novel microfluidic techniques to characterise in details the interactions between molecules involved in pathway regulating the synaptic plasticity. A particularly valuable knowledge will be delivered from studies including lipids – a main structural and functional component of cellular membranes – that can regulate the function and interactions between proteins. In addition, the elevated levels of MMP-9 enzyme (element of the studied pathway) in the hippocampus have been recently found in postmortem brain isolates from patients suffering from MDD. We further aim to extend on current microfluidic techniques by a development of method for sensitive quantification of MMP-9 enzymatic activity.

The currently available pharmacological treatment for depression often relies on modification of serotonin uptake by interactions with serotonin receptors. Better understanding of the participation of one of the serotonin receptors in synaptic plasticity will be helpful in explaining not yet fully understood action of drugs, their side effects, and propose improvements in therapeutic strategies. What is more, exploring the diagnostic potential of recently identified pathway, might have major consequences in the care of patients affected by MDD. The proposed research will rely on multidisciplinary experimental approaches. Microfluidics will be employed to answer important questions in neurobiology. Development of new assays will result in improvement of existing microfluidic technologies towards tackling specific research problems. Work will be focused on implementing of devices for in vitro reconstitution of proteins in lipid bilayers, along with platforms for quantification of enzymatic activity of metalloprotease. This would not only offer new research tools but also pave the way for a potential future commercialization of the methods developed during the project.