ABSTRACT FOR THE GENERAL PUBLIC

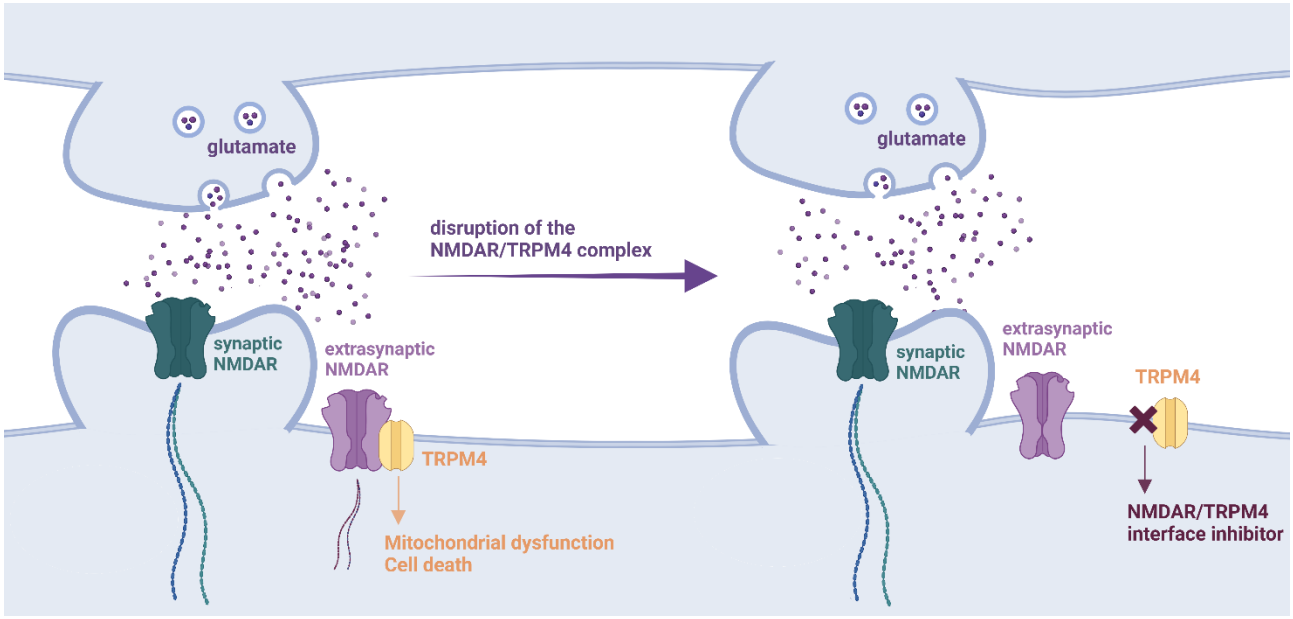
Disrupting the Toxic NMDAR/TRPM4 Protein Dialogue as a Novel Neuroprotective Strategy: Development of Small-Molecule Inhibitors of Protein–Protein Interactions for Ischemic Stroke Therapy

Ischemic brain stroke is one of the leading **causes of death and disability** in adults. Currently, therapeutic strategies in the acute phase of brain stroke are primarily limited to the administration of clot-dissolving agents. However, this approach has several limitations, such as the requirement for rapid administration (max. 4.5 hours after stroke onset).

It is crucial to highlight that the stroke is not limited only to the cell death within the so-called *ischemic core*. Dysfunctions also progress in the region surrounding the stroke core which is known as the ***penumbra***. The ongoing cell death in this area is linked to the **excess of glutamate**. In an effort to protect neurons from glutamate-induced excitotoxicity – primarily mediated through **overactivation of NMDA receptors** (NMDARs) – neuroprotective strategies focused on blocking NMDARs have been extensively explored over recent decades, but without promising results.

The lack of satisfying outcomes emerges from the fact that the NMDAR blockade suppresses not only its toxicity, but also the receptor's physiological activity, vital for the proper body functions. Here a new approach to this topic arises: a selective **inhibition of the NMDAR/TRPM4 complex formation** rather than general NMDAR blockade.

Research shows that the neuronal cell death after stroke is associated with the escape of the glutamate outside the synapse. There, it binds to the **extrasynaptic NMDARs** that form **complexes with TRPM4 proteins**. Signallisation induced by those complexes leads to **excitotoxicity** and **cell death** progression. Therefore, blockade of the NMDAR/TRPM4 complex formation could mitigate its excitotoxicity. Simultaneously, it would not affect the (physiologically essential) synaptic NMDA transmission (*see scheme*).



Scheme. Excitotoxicity of NMDAR/TRPM4 complex and mechanism of action of NMDAR/TRPM4 interface inhibitors.

The project focuses on this innovative approach and proposes **design, synthesis and biological evaluation of new chemical molecules targeting the inhibition of NMDAR/TRPM4 complex formation**.

During the project, original chemical entities will be designed and obtained. Then, after preeliminary physicochemical analyses, they will be further evaluated *in vitro* and *in vivo*. The neuroprotective potential of the molecules will be assessed in a cellular model of brain stroke, in which stroke conditions are induced by the deprivation of oxygen and glucose. Next, the most promising compounds will be further pharmacologically tested to confirm their activity: mitochondrial membrane potential (which is an indicator of cell viability) and levels of apoptotic markers (such as caspase 3/7) will be checked. Based on the results, one the most promising compound will be selected. The study will be finalised by evaluation of its pharmacokinetic properties and neuroprotective activity in an animal model of ischemic brain stroke.

This project broaden the knowledge in the area of the potential neuroprotective strategies for the ischemic brain stroke therapy. It will enrich the underexplored niche of NMDAR/TRPM4 complex inhibitors. It will also contribute to the field of small modulators of protein-protein interactions, which is one of the most trending therapeutic approaches in the last years.