Application ID: 645657; principal investigator MSc. Krzysztof Polaczek

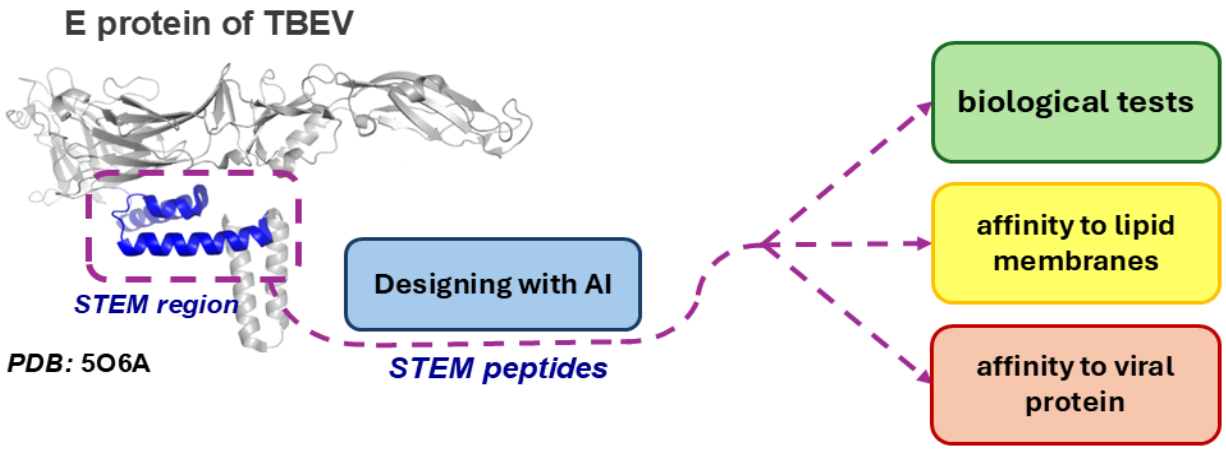
Development and study of peptide inhibitors of membrane fusion in flaviviruses, based on the STEM region of the tick-borne encephalitis virus E protein

Flaviviruses are a family of viruses, which includes pathogens such as ZIKA virus and Dengue virus. However, in Europe and northern Asia, the predominant representative of this family is the tick-borne encephalitis virus (TBEV) the presence of which is a serious health problem due, among other things, to the lack of specific treatments. Moreover, progressive climate change is leading to an expansion of tick habitats - the main vector of transmission of this virus, causing a steady increase in the number of new infections. Our proposal to fight the TBEV is to use peptides, which are fragments of the structural E protein. This protein forms the outer coat of the virus, which undergoes conformational changes induced by low pH just after entering the cell via endocytosis. As a result of these changes, protein trimmers necessary for the formation of pores, through which the viral RNA is transferred into the host cell, are formed on the surface of the virus. Previous studies have shown that analogous protein fragments of Dengue or ZIKA virus, effectively block virus-host membrane fusion. Considering the structural similarity and common entry mechanism of flaviviruses, they can also be used as a new type of therapeutic or prophylactic strategies to treat TBEV and other viruses within this family.

Therefore, the main goal of our project is to develop new peptide compounds capable of disrupting the membrane fusion process of the TBEV and the host cell, as well as to study their mechanism of action in detail. We believe that the molecules designed in this way will exhibit a similar mechanism to that described so far for other flaviviruses. These compounds are designed to simultaneously bind to the viral membrane and surface proteins and, in effect - inhibit pore formation and transfer of viral RNA into the cell. Due to their relatively low toxicity, high structural diversity and ease of synthesis, peptides are valuable tools for in vitro studies of the mechanisms of action of in example antiviral compounds. They also provide a great tool for searching lead structures, and their further modification, which allows to obtain proteolytically and metabolically stable potential therapeutics.

The project includes three main research areas: **1) the study of interactions with lipid membranes**, which aims to track the conformational changes of peptides in the presence of liposomes using circular dichroism spectroscopy, identify key amino acid residues that anchor peptides in the membrane using NMR spectroscopy, and study the mechanism of interaction with lipid membranes using impedance spectroscopy (EIS); **2) biological studies** that will determine the cytotoxicity of the obtained compounds and the inhibitory of cytopathic effect against cell lines infected with TBEV and other flaviviruses; **3) Analysis of the affinity of peptides for surface E protein**, using microscale capillary thermophoresis and, for the most active compounds, Biotin-Streptavidin pull-down assay coupled to SDS-PAGE-type electrophoresis, enabling assessment of affinity for E protein in different conformational states. In addition, we plan to use artificial intelligence (AI)-assisted computational design methods to rationalize the design and optimization of new peptide sequences.

The research enable to develop compounds capable of blocking viral RNA transfer at the viral membrane fusion stage and to better understand their mechanism of action. This type of approach may lead to the development of new types of therapeutics against flaviviruses in the future.



Conceptual diagram of the project