Influenza viruses (FLUV) are one of the main causes of global morbidity and mortality. The ability of influenza viruses to constantly adapt to immune pressure results in annually recurring influenza epidemics, which pose a significant health threat, in particular to infants and the elderly: 250 000 to 500 000 annual deaths are attributed to influenza epidemics and are associated with costs of 87.1 billion USD per year in the US alone. One of the critical steps of influenza virus infection is the virus entry, mediated by the FLUV hemagglutinin (HA), which hijacks cellular proteases for its own activation. It was shown that TMPRSS2, a member of type II transmembrane serine proteases (TTSPs) can proteolytically cleave HA and activate the influenza virus, and the TMPRSS2 activity is essential for spread and pathogenesis of several FLUV in rodent models. Moreover, polymorphisms in the human TMPRSS2 gene which increase TMPRSS2 expression are associated with increased risk for severe influenza. Therefore, TMPRSS2 is a host factor critical for FLUV infection, and becomes an interesting candidate for antiviral treatment. However, it is still unknown why only some TTSPs members (i.e. TMPRSS2) can cleave HA and activate FLUV, while others (i.e. TMPRSS3) cannot. Additionally, it is unclear how FLUV changes the cellular machinery for its own purpose.

Therefore, the proposed project will:

- i) Determine the role of TMPRSS2-related proteases in the FLUV spread and pathogenesis
- ii) Investigate how the analyzed TMPRSS2 structural domains determines HA activation
- Analyze the secondary structure of the TMPRSS2 mRNA, its changes after FLUV infection and conserved motifs among all TTSPs members
- iv) Check whether antisense oligos developed on the basis of the mRNA secondary structure inhibit FLUV spread and pathogenesis in the ex vivo experiments, using non-human primates precise cut lung slices, and determine the mechanisms underlying the inhibition.

In the proposed research, we will employ the classical virology, molecular biology, biochemistry, cell biology and structural biology techniques, both in vitro as well as ex vivo. Using the cell transfection and infection, protein analysis (i.e. Western blotting), flow cytometry and confocal microscopy we will screen other TTSPs members for their HA cleavage ability and FLUV activation, as well as determine the structural domains responsible for TTSPs-HA interaction. Additionally, by the isolation of RNA from the cells endogenously expressing TTSPs and co-infected with FLUV, and chemical mapping of RNA, we will check the TMPRSS2 mRNA secondary structure, and how its changes after influenza mediated cell shut down.

The obtained results allow for deeper understanding the influenza virus entry to the host cells.