The aim of the project is the investigation of the **routes of fast direct transmission of coronavirus between cells** in infected organisms. Viruses can infect cells from outside, binding to the receptors on the surface of a permissive cell and gradually penetrating into host cell. Inside the cell , after uncoating of the viral particle , the released genetic material (DNA or RNA) is replicated, viral proteins are synthesized and new progeny viral particles are assembled. These new particles can exit from the cells and infect next surrounding cells. However, some viruses can be transmitted to neighbor cells without being released to the extracellular environment, via cell-cell contacts of adjacent cells or / and by exploitation of various types of intercellular connections . This mode of direct cell-to-cell transmission facilitates faster spread of the virus and protects viral particles from contact with immune response of the host. It has been shown for some enveloped viruses (e.g. measles virus, HIV or human leukemia virus) that this hidden route of transmission may constitute over 60% of infection. Therefore, the inhibition of cell-to-cell spread is considered to be a promising therapeutic target, especially in neurological diseases. Moreover, viral mutants devoid of genes essential for direct transmission are used as vaccine strains in case of some animal herpesviruses.

Until now, **it has not been reported that coronaviruses can be transmitted between cells by direct fast route. However, taking into account the exceptional efficiency of SARS-CoV-2 infection and the variety of organs which may be infected – not only lungs, but also heart, intestines, kidneys- it can be assumed that this virus, apart from regular mode of infection , may also use the other mode of spread**. We have studied for many years the mechanism of cell-to-cell spread occurring during herpesviruses and hepatitis C (HCV) virus infection. We demonstrated that such transmission occurs more frequently in primary cells than in laboratory cell lines and we discovered that herpesvirus can be transmitted between distant cells via long thin actin protrusions, tunneling nanotubes (TNT), specialized intercellular bridges which have been recently identified. Moreover, we were able to show that virus infection stimulates the TNT formation and one of the virus kinases is involved in this process. **In the presented PhD project we plan to perform similar investigation for SARS-CoV-2 virus, searching for hidden pathways of virus transport between adjacent and distant cells**. We will start the project with studies of the entry of coronavirus to various types of cells and analysis of the speed of plaque formation (which reflects the efficacy of virus spread). Using confocal microscopy and immunofluorescence, we will analyze the connections between infected and non-infected cells, searching for virus presence in structures like TNT as well as in junctions and close contact areas (similar to “virological synapses” discovered for HIV). Another question which we want to ask is, whether SARS-CoV-2 is able to move between cells of different types and origin, eg. between epithelial and blood (PBMC) cells. Such mode of transport could possibly enable virus to access even those cells which do not contain the receptor (like ACE2). For these experiments we will use co-cultures of various cells: epithelial or endothelial cells , lymphocytes, cells of neuronal origin. We also plan to use 3D type of cultures (organoids) , especially for the analysis of (less known) intestinal SARS-CoV-2 infections. Expression of SARS-CoV-2 individual genes will help to find viral factors involved in modification of cellular cytoskeleton, what usually leads to formation of intercellular connections. The important part of the project will be the search for specific inhibitors of coronavirus cell-to-cell transmission, which could have possible therapeutic application.