Prof. dr hab. Elżbieta Kierzek
SARS-CoV-2 RNA, target for inhibition of virus replication

The scientific goal of this project is to find efficient inhibitors of SARS-CoV-2 proliferations via targeting of viral RNA. SARS-CoV-2 is a (+)ssRNA virus containing a 29,903 nt genome. The first two-thirds of the genome encode two overlapping polyproteins associated with virus the replication and transcription processes. The remaining part of genome codes for four structural proteins: the spike (S), envelope (E), membrane (M), and nucleocapsid (N). Our group has long term experiences in the study of Influenza Virus RNA. For ten years we have worked on Influenza Virus RNA structure and replication inhibition via antisense oligonucleotides (ASO), small interfering RNAs (siRNA), peptide nucleic acids, CRISPR/Cas system, ribozymes, and small molecules (SM). We propose to apply those experiences for inhibition of SARS-CoV-2, especially at time of world-wide pandemic caused by this virus. In presented project, we intend to focus on SARS-CoV-2 RNA, specifically on its 5'- and 3'-terminal RNA fragments, membrane (M) and nucleocapsid (N) mRNAs regions. Our advanced structural analyses of the folding of these four viral RNA regions indicate theirs conserved structure. Moreover, all those viral RNA regions are crucial for SARS-CoV-2 virus proliferation.

The goal of this project is the discovery of oligonucleotide and SM inhibitors of SARS-CoV-2 virus replication. Potential oligonucleotide inhibitors (ASO, siRNA and CRISPR/Cas13 system) will be designed based on predicted and experimentally determined secondary structure of viral RNA. SMs will be selected from ligand libraries based on their abilities binding to selected conserved SARS-CoV-2 RNA hairpins via high-throughput screening (HTS) and high-throughput virtual screening (HTVS). We will prepare noninfectious SARS-CoV-2 replicon which will allow in 293T cells for quantitative determination of the inhibitory activity of the selected oligonucleotides and SMs. Within those four RNA regions, we selected 28 structurally conserved targets for oligonucleotide driven virus inhibition. We also selected 8 conserved SARS-CoV-2 RNA hairpins as targets for HTS and HTVS with libraries of physical and virtual SMs.

We intend to test three categories of oligonucleotides: ASO, siRNA and CRISPR/Cas13 system. We will use 2'-O-methyl, LNA, 2'-fluoro and thiophosphate of the oligonucleotide modifications to enhance theirs inhibitory efficiencies. The design of the sequence of these potentially therapeutic oligonucleotides was based on RNAs folding predictions of 5'- and 3'-terminal fragments as well as M and N mRNA regions. Sequence alignments of the entire viral SARS-CoV-2 RNA genome and these four selected RNA regions were conducted using over two thousand SARS-CoV-2 genomes deposited in GISAID database. Moreover, we will perform microarray mapping of N and M mRNAs to experimentally define their secondary structures *in vitro* and design additional oligonucleotides fitting to N and M mRNAs experimental secondary structures.

For choose the SMs capable to inhibit SARS-CoV-2 proliferation we will perform HTS and HTVS based selections. HTS will be based on fluorescent indicator displacement (FID) assay. In this assay indicator become fluorescent only after binding to target RNA and when indicator is displaced from RNA with a stronger than indicator binding ligand it results in the quenching of the fluorescence indicator. HTSV approach is based on bioinformatic docking of ligands to the 3D structure of selected motifs of the four regions of SARS-CoV-2 RNA.

We will construct a noninfectious SARS-CoV-2 replicon suitable for evaluation of all designed oligonucleotides and selected SM inhibitors. The engineering of this replicon is based on the finding that deletion of S and E genes from virus genome does not block replication and transcription in cells but make it noninfectious. Moreover, enhanced green fluorescent protein (eGFP) gene will be inserted at S gene position to constitute a replication reporter. After preparation of the replicon, selection of SMs, and synthesis of the designed ASOs, siRNAs and CRISPR/Cas13 systems, we will test their inhibition activity in 293T cells.

We intend to select 4-6 the most efficient in replicon system oligonucleotide and ligand inhibitors for tests against native SARS-CoV-2 in cells. Experiments with native SARS-CoV-2 need to be performed in BSL3 lab and for this reason these inhibition tests will be conducted in collaboration with Professor Luis Martinez-Sobrido in Texas Biomedical Research Institute (San Antonio, USA). The most efficient oligonucleotides and ligands can be optimal candidates as therapeutics to cure COVID-19 cases related with SARS-CoV-2 virus infection.