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"The role of glycosylation of structural proteins in the life cycle of SARS-CoV-2 coronavirus".

For centuries viral diseases have been posing an imminent threat to humanity. The extremely grave epidemiological situation due to the COVID-19 unprecedented spread calls for immediate measures such as construction of new genetic and immunologic tests for SARS-CoV-2 detection. However, in the long run, it is crucial to develop therapeutic agents which in the near future may extinguish the epidemic and will provide long-lasting immunity. In this respect vaccines are the most important elements of combatting many infectious diseases. Rapid progress in scientific fields such as molecular biology, genetic engineering and virology contributed to the development of many vaccines against dangerous viral pathogens. In some, very rare cases, already approved vaccines have failed for large-scale administration and had to be withdrawn; this was the case with the anti-dengue vaccine. Due to immense efforts of scientists all over the world, great commitment of pharmaceutical companies and a huge financial input of governments of rich countries, four vaccines against SARS-CoV-2 have been approved by FDA until today. However, despite extensive clinical trials only one drug - Veklury (remdesivir) has been approved for the treatment for SARS-CoV-2 infection. Therefore, the ongoing COVID-19 pandemic requires the urgent development of new, effective antiviral therapies.

Glycoprotein S (spike) of coronaviruses is the main neutralizing component of SARS-CoV-2. This viral envelope protein is highly modified by the addition of complex glycan structures that represent half of its molecular mass. Thanks to the extremely intense efforts of scientists from around the world, it was possible to obtain a wealth of data on the structure of N-glycans present in the S protein. However, our knowledge about the role of these glycans in the SARS-CoV-2 infection process is still very limited. Most of these data are inferred from computational models or resemblance to other viruses. As spike glycoprotein is the target of most vaccines and treatments under development due to its outermost location and essential role in SARS-CoV-2 life cycle, in the proposed project, we plan to thoroughly characterize the role of N-glycosylation of this protein in viral infection using various experimental approaches. Additionally, the role of N-glycosylation of other structural proteins M and E on viral infection will be examined.

As a molecular virology group, specializing for years in antiviral activity studies using a wide array of N-glycosylation inhibitors against many important animal and human pathogens such as influenza virus, tick-borne encephalitis virus, hepatitis C virus and Zika virus, in this project we plan to use our knowledge and methodology to examine the role of N-glycosylation of all SARS-CoV-2 proteins on virus entry, assembly and antigenicity. In the proposed project, we plan to characterize the role of various N-glycosylation sites of SARS-CoV-2 S, M and E proteins in the formation and infectivity of progeny viral particles using various approaches such as: coronavirus propagation in *in vitro* cell culture in the presence of different N-glycosylation inhibitors (mouse MHV-1 and human CoV-NL63 and SARS-CoV-2), SARS-CoV-2 virus-like particles (VLPs) and SARS-CoV-2 pseudo-particles production (both native and carrying the mutations in N-glycosylation sites of proteins). Some preliminary work has already started in our laboratory.

We hope that the knowledge obtained during this project will contribute to the development of new strategies of drug design. We anticipate that the studies using our novel, synthesized compounds will help to develop novel routes to exploit the N-glycosylation inhibition for COVID-19 treatment. In our opinion the approaches targeting N-glycans may be more advantageous than polypeptide-based ones as N-glycosylation is more conserved, independent protein modification less prone to changes which may adversely affect therapeutic effects of inhibitors.