Zika virus (ZIKV) is an emerging mosquito-borne pathogen, belonging to the *Flavivirus* genus that causes febrile illness in humans but is also linked to Guillain- Barré syndrome as well as to **microcephaly in new-borns. Giving the risk of development of microcephaly in fetuses, ZIKV poses a serious problem for pregnant women.** Until now, 84 countries had reported Zika virus cases since first big outbreak in 2007. The rapid transmission of ZIKV was mostly caused by the spread of a competent mosquitoes, which geographical range as well as Zika virus and other viruses is still expanding e.g. active transmission of ZIKV has been reported in Europe. Up to date no vaccine or antiviral therapy has been approved, despite the fact that after pandemic in 2015-2016, numerous studies have been conducted all over the world.

ZIKV is an enveloped virus and two proteins: prM/M and E are anchored in the envelope. These proteins during replication cycle, undergo one of the most common post-translational modification, which is glycosylation. The most important type of glycosylation is N-glycosylation and N-linked glycans on the surface of virus proteins are involved in virus entry to the host cell, assembly of progeny viruses and also in immune evasion, by shielding the virus from neutralizing antibodies. The second most important type of glycosylation is O-glycosylation. **However, up to this date there is no information about the role of O-glycans in those processes of ZIKV replication cycle. Therefore, this project aims in evaluating the role of O-glycosylation of Zika virus envelope proteins (prM/M and E) in virus entry, assembly and immune evasion.** Both glycoproteins may be decorated with multiple putative O-glycosylation sites, according to bioinformatic analysis of those proteins, thus different approaches will be used to determine the role of O-glycans in replication cycle of ZIKV.

First stage of the project will focus on the evaluation of O-glycans role in the entry to the host cell. To achieve this goal, we will used special molecules called lectins, which bind various and specific sugar moieties of O- as well as N-glycans on glycoproteins to neutralize ZIKV. With the use of lectins we will examine the level of virus neutralization using different *in vitro* cell culture models. **In the next stage, O-glycans role in the virion assembly will be determined**. Mutations in the sequence of prM/M and E proteins will be introduced in order to abolish O-glycosylation. For this aim we plan to use virus-like particles (VLPs), which are built of prM/M and E proteins, as a model for virus assembly in order to minimise to risk of virus genetic modification. Additionally, chemical inhibitors of glycosylation process or cell lines without O-glycosylation will be used. Using this approach, experiments with viruses will also be performed to directly assess influence of O-glycans on the replication cycle. Next, the assembly of modified VLPs will be analysed with various immunological techniques. **Last step of the project will be focused on the evaluation of O-glycans impact on the antigenicity of Zika virus proteins/VLPs**. PrM/M, E proteins and VLPs with mutations of O-glycans will be used to assess whether introduced changes influence recognition of virus proteins by various neutralizing antibodies. Moreover, mutated VLPs will be used to analyse the binding to specific receptors, which are present on the host cells and are involved in the attachment and the entry of the Zika virus.

In light of this, it would be of great importance to study the role of O-glycans in order to design new antiviral compounds targeting entry and assembly through interfering with glycans or glycosylation of proteins against Zika virus. Furthermore, if O-glycans take part in the immune evasion, analysis of their role, would help to design rational vaccine antigen candidates. This project would be the first in the field of flaviviruses and as flaviviruses have high structural and functional similarity, it may help to unravel the role of O-glycans in other viruses from this genus.