Viruses are infectious agents that replicate only inside the living cells of an organism. Hundreds of viruses, designated as arthropod-borne viruses (arboviruses), are transmitted by arthropod vectors in complex transmission cycles between the virus, vertebrate host, and the vector. Flaviviruses such as dengue (DENV), West Nile (WNV), and Zika (ZIKV) cause major outbreaks of potentially fatal diseases and affect more than 50 million people every year. In the past years a global (re)emergence of arboviruses, such as ZIKV, was widely reported in the media as though it was a new phenomenon. However, arboviruses have been (re)emerging for centuries. The major difference today is that its emergence and dispersion are more rapid and geographically extensive, largely due to climate change intensive growth of global transportation systems, arthropod adaptation to increasing urbanization, our failure to reduce mosquito population density, and land perturbation. With the effects of climate change, dengue is predicted to be a potential risk in Europe in the near future. Despite the global health problem, there are no specific treatments or vaccines for neither of the three viruses, according to the Centers for Disease Control and Prevention. In the proposed project we turn the focus on the non-coding RNA regions as potential drug targets. Flaviviruses have a (+) sense RNA genome and replicate in the cytoplasm of the host cells. The viral RNA plays an active role in the viral genome amplification, it provides signals that act as promoters, enhancers, and/or silencers of the virus propagation process. The RNA genome contains 5' and 3' untranslated regions (UTRs) and contain several structures that are conserved and restricted to the flavivirus family. Besides, the 5' and 3' regions contain complementary sequences that are involved in long-range RNA–RNA interactions resulting in the cyclization of the viral RNA, critical for viral RNA replication. These different RNA elements have been shown essential for virus-induced cytopathicity and pathogenicity and can be used as targets for potential drug therapies and vaccine development. However, as of yet, no relevant 3D structures are available for the Flaviviruses regulatory RNA elements.

In this project, we propose to answer two main questions: How do the 5' and 3' RNA regions fold in isolation, and what are the structural rearrangements required for their cyclic formation? And what are the key structures of the Flavivirus genus? To answer these questions we will determine the 3D structures of Flaviviruses RNA regions that are necessary for the virus functions, by Cryo Electron Microscopy (CryoEM). This project aims to determine the structures of the 5’ and 3’ RNA regions separately and in the cyclic 5’/3’ complex. The selected targets are: Dengue four subtypes (DENV1 to 4) as well as Zika (ZIKV), and West Nile (WNV) viruses. The different targets will allow us to understand the structural conservation and variability, and to identify common elements that can be used as targets for antiviral drug design in follow-up analyses. The structure determination of the 5’ and 3’ RNA regions separately and in the cyclic 5’/3’ complex will also give us insights to understand the structural rearrangements necessary for the genome circularization, implicated in the viral replication and give a structural platform for the development of more effective vaccines. During this project, we will use a combination of established tools and experimental techniques, as well as prototypes of innovative tools developed very recently in the host laboratory at IIMCB. Our RNA and structural biology expertise can bring new useful insights for the global effort to understand the mechanisms of the Flaviviruses and the proposed results will significantly contribute to the basic understanding of sequence-structure-function relationships in Flaviviruses RNAs, with a strong potential for practical applications in the future.