In recent years rapid expansion of tick-borne diseases has been observed in Europe. The most important causes of this phenomenon will certainly include the warming climate and the lack of effective ways of controlling ticks and their ability to carry pathogenic agents. In Europe, *Ixodes ricinus* is the most widespread tick species. It is responsible for both human and animal diseases. It transmits pathogenic agents responsible for zoonotic diseases such as borreliosis (Lyme disease), tick-borne encephalitis, babesioses, anaplasmoses or rickettsioses. The most prevalent tick-borne infection of humans in the northern hemisphere is borreliosis. The incidence of this disease has strongly increased in European countries over the last decade and more than 50 000 cases are reported each year. Other tick-borne diseases also take a heavy toll in Europe.

Ticks have developed complex mechanisms enabling them to overcome both the host's defense systems during a few days' engorgements and surviving the intervals between feeding periods. At each every stage of the life cycle, a specific set of factors is produced in ticks' bodies. Interestingly, the mechanisms evolved by ticks to improve the parasitism are also used by the tick-transmitted pathogens to infect its hosts during tick feeding and to facilitate the colonization of the vector. These mechanisms and the proteins involved in tick-pathogen-host interactions are poorly known as reflected by very few known protein structures from *Ixodes* ticks or lack of effective tick-borne disease prevention methods. A better understanding of tick-pathogen-host interactions is the only chance to overcome these adverse trends in the field of tick-borne diseases prevention. The overall objective of this project is a structural study of proteins that are both involved in colonization of the *Ixodes ricinus* tick by *Borrelia burgdorferi* and used by the pathogenic bacteria to transfer from ticks to animals.

The *Borrelia* spirochete colonizes the tick during the engorgement of the latter on infected vertebrate. In this process a pair of proteins plays a crucial role. These are: TROSPA (Tick Receptor for Outer Surface Protein A) from *I. ricinus* and OspA (Outer Surface Protein A) from *B. burgdorferi*. The basic function of TROSPA is unknown, as well as its structure and the nature of its interaction with OspA from *B. burgdorferi*. Interestingly, it was also reported, that TROSPA may be involved in tick colonization by the other pathogenic microorganism *Babesia bigemina*. In the course of our previous studies we showed that the recombinant TROSPA from I*xodes ricinus* produced in E. coli retains the ability to interact with OspA proteins from different species of *Borrelia*. Our recent, unpublished results suggest that TROSPA exhibits the features of intrinsically disordered protein (IDP). IDPs were discovered in the 1990s and since then learning about the rules of their ordering is a major challenge for modern structural biology. According to the recent bioinformatics data, 25–30% of all eukaryotic proteins are mostly disordered, and they play their roles mainly as signaling and receptor molecules. IDPs, as opposed to globular proteins, in their native environment are devoid of a stable tertiary structure. These proteins, however, can easily change their conformational state in response to changing environmental conditions, or form stable spatial structure upon the interaction with another molecule. The capability of binding a variety of partners is a unique feature of IDPs. Therefore, the TROSPA--OspA pair of protein appears to be a very interesting and promising object of studies, because it is a key factor in the colonization of the tick by at least two microorganisms. During the implementation of the present project we will verify, if the possibility of colonization of tick by different pathogens is related to disordered character of TROSPA receptor. By studying TROSPA--OspA complex we will also provide insight for learning about the mechanisms governing IDP ordering and protein folding.

Ticks normally take several days to feed, and this is long enough to allow the host to mount an immune response against the tick antigens. Therefore, tick saliva, introduced into the host's skin during the feeding process, contains a wide range of proteins suppressing the host's immunological response. This allows the parasite for successful engorgement. Factors evolved by ticks to suppress the local host immune response and to improve the parasitism are also used by tick-transmitted pathogens to effectively infect their hosts during tick engorgement. The second pair of proteins selected as the object of structural studies planned herein is the Iric-1 salivary protein from *I. ricinus* interacting with the OspC protein (Outer Surface Protein C) from *B. burgdorferi*. Iric-1 is a member of Salp15 tick protein family. In tick salivary glands, the bacteria with OspC proteins exposed on their surface bind Iric-1 and in this coated form penetrate the host's bloodstream together with the tick saliva. This interaction with Iric-1 protects *B. burgdorferi* from antibody-mediated killing. Recently it was shown that salivary glands of *I. ricinus* ticks secrete a wide spectrum of Salp15 homologues. In spite of this, the rules of the interactions of Salp15 family members with the OspC protein or the factors present in the blood of vertebrates still remain unknown. During the implementation of the present project, we are going to select the other Salp15 members from *I. ricinus*, verify if they are capable of interaction with OspC and study and compare their structures. The goal of this approach is to discover the structural motifs within poorly known Salp15 protein family, involved in *Borrelia burgdorferi* transmission.

During our earlier studies we have developed protocols for the efficient production of a TROSPA, OspA from different *Borrelia* species, Iric-1 and OspC from different *Borrelia* species including both full length proteins and their derivatives in bacterial system. We have also elaborated protocols for the production of recombined TROSPA and Iric-1 in insect cells so as to obtain the tick proteins with a very similar structure as those produced by ticks. We have also carried out the preliminary studies of TROSPA--OspA and Iric-1--OspC interactions and we have confirmed that these proteins are able to form the complexes. Thus, we already have got all the necessary proteins to complete the presented project at our disposal.

To sum up, the structural studies planned in the scope of the present project will provide insight for learning about the mechanisms of tick-pathogen-host interactions, which is important in view of tick-borne diseases prevention. TROSPA--OspA pair of proteins is a new and valuable model to study the rules of IDPs ordering, which is one of the major challenges for modern biochemistry. The structural studies of Salp15--OspC proteins interaction will reveal the structure-conserved sites of Salp15 which help to understand the diversity and function of this interesting group of proteins as well as might be useful during the development of improved vaccines based on Salp15. Current knowledge of these issues is poor, and the results obtained during the implementation of this project will become a source of valuable publications.