MS03-1-4 Structural and Functional Studies of TBEV Non-Structural Protein 5
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Abstract
Tick-borne encephalitis virus (TBEV) is a major human pathogen, transmitted by ticks from family Ixodidae. TBEV is an enveloped virus with a ~ 11 kb positive-sense single-strand RNA genome, encoding a single 375 kDa polyprotein. During the infection, the polyprotein is cleaved into three structural and seven non-structural (NS) proteins. While structural proteins are involved in the assembly of new virions, non-structural proteins are responsible for the virus replication [1].
NS5 is a large conserved protein comprising of two domains connected by a highly flexible linker, which is important for the activity as well as for the overall shape of the protein. N-terminal methyltransferase (MTase) domain is involved in the capping process. C-terminal RNA-dependent RNA polymerase (RdRp) is crucial for virus replication [2].
This project focuses on structural and functional studies of TBEV NS5 protein. Various constructs were designed – NS5 full length, RdRp domain and MTase domain. Expression and purification of individual constructs have been optimized and pure samples were used for initial crystallization screening, cryo-EM analysis and functional assays.
So far, we have obtained cryo-EM data for RdRp domain, using Titan Krios equipped with Falcon 4 camera and Relion processing pipeline yielded a reconstruction of 6 Å resolution. Tiny protein crystals of RdRp grew in several crystallization conditions. Furthermore, fluorescence-based binding assays revealed substrate affinity and specificity.

References
1. Mackenzie, J. (2005). Traffic. 6, 967-977.
2. Bollati, M. et al. (2009). Antiviral Res. 87, 125-148.
This research is supported by ERDF No. CZ.02.1.01/0.0/0.0/15_003/000041, GAJU 106/2021/P and GAJU 04-17/2019/P.