Eukaryotic mRNA molecules and other RNAs transcribed by RNA Polymerase II possess at their 5′ ends a unique structure termed ‘cap’. In a so-called cap0 structure, a methylated guanosine is linked via an unusual 5′–5′ triphosphate bridge to the 5′-end of an RNA transcript. This cap0 is critical for mRNA interactions with many nuclear and cytoplasmic proteins and plays multiple roles in gene expression, including the enhancement of RNA stability, splicing, nucleocytoplasmic transport, and translation initiation. In higher eukaryotes, mRNAs and small nuclear RNAs are further modified at their 5' ends by methylation of the ribose on the first and second transcribed nucleosides (i.e., cap1 and cap2, respectively). Although capping is one of the fundamental processes in preparation of mRNA molecules for translation, our knowledge about it still remains incomplete. Especially, the function of cap1 and cap2 methylations still remains obscure. This may be due to the fact, that the enzymes introducing cap1 and cap2 structures have been unknown until recently. In human, cap0 and cap1 methylations are present on all mRNA molecules, whereas only approximately half of the capped and polyadenylated RNA molecules contain cap2 methylation. In order to understand, why not all mRNA transcripts are modified at cap2 it is crucial to study and compare the specificities and structures of the cap1- and cap2-introducing enzymes CMTr1 and CMTr2.

Therefore, the proposed research project focuses on the identification of CMTr2 RNA substrates and the analysis of sequence and secondary structure influences on substrate recognition. Furthermore, protein X-ray crystallography will have an essential part in verifying these results and in providing new insights into the mode of CMTr2-RNA interactions. I expect to reveal key differences between CMTr1 and CMTr2 RNA substrates and structures that explain their different selectivities. Thus, the proposed project will essentially contribute to the understanding of the roles of cap1 and cap2 RNA modifications. It will help us broaden the knowledge about fundamental metabolic processes like translation and splicing and further exploit cellular RNA metabolism.