Messenger RNA (mRNA) is a natural molecule, which serves as a template for protein biosynthesis and thus is of key importance for gene expression. In vitro transcribed mRNA has recently emerged as a potential therapeutic with the promise to revolutionize gene therapy-based approaches. The cellular fate of mRNA depends, among others, on the presence and stability of natural regulatory elements at 5’ and 3’ ends: mRNA cap and polyA tail, respectively. The 5′ cap, composed of 7-methylguanosine (m^7G) linked by a 5′,5′-triphosphate bridge to the first transcribed nucleotide, is the hallmark of mRNA that is recognized by several specialized proteins involved in mRNA processing, transport, translation, and degradation. One of the major functions of the cap is to prevent premature mRNA degradation within the cells. Thus, the cleavage of phosphoanhydride bonds within the mRNA 5′ cap structure, known as decapping, is a key step in the regulation of mRNA stability and turnover. Several canonical and non-canonical decapping enzymes have been discovered over the years, which differ in substrate preferences and regioselectivity of cleavage. Dysregulation of decapping activity has been linked to various genetic disorders. Other enzymes that do not target cap or capped mRNA, but participate in turnover of initial decapping products have also been discovered and may participate in the regulation of mRNA homeostasis and are also within interest of this proposal.

Despite intense research into regulation of decapping enzymes and their contributions into mRNA degradation pathways the processes are not fully understood. In this project we aim to develop a set of nucleotide- and oligonucleotide-derived molecular probes as chemical tools enabling specific and sensitive visualization and real-time monitoring of selected decapping enzymes in the cell.

The key step towards this goal are: (i) chemical synthesis of the probes, (ii) biochemical characterization of the probes, (iii) application of selected probes for experiments in living cells.

The molecular probes developed in this project may aid us in understanding of decapping processes at a molecular and cellular level. Application of these probes in experiments in living cells or even living organisms may contribute to gaining new insights into spatial and temporal control of various decapping enzymes. This in turn may result in new ideas on how to control particular decapping enzymes for advanced research applications and for therapeutic purposes. Moreover, the probes developed here, may be also applied to develop high throughput screening assays for the discovery of small molecule ligands that modulate decapping enzyme activity.