All biological processes starting from bacteria fighting viruses to heart development in humans are controlled by changes in the expression of genetic material and dynamic interactions between protein and RNA molecules. We already know a lot about how these proteins are shaped structurally, which RNA features they recognize and what is the effect of these interactions. In recent years, more and more attention is being paid to understanding molecular mechanisms governing biological processes. Thanks to this type of research, we will better understand how these mechanisms evolve, but also, we can easier manipulate them e. g. to develop vaccines.

The goal of this project is to study the molecular mechanisms governing the communication between particular processes such as RNA targeting, RNA degradation, and translation in bacteria. Post-transcription gene regulation enables responses to stress, e. g. switching the metabolism to utilize available nutrients or adjusting to the host in case of pathogenic bacterial strains. This regulation is enabled by small RNAs (sRNAs) interacting with messenger RNAs (mRNAs) based on complementarity. Base pairing between sRNAs and mRNAs is facilitated by Hfq chaperone protein. sRNA-mRNA pairing often leads to degradation of both molecules, thus it downregulates the expression of target mRNA. In turn, the degradation of most bacterial RNAs, including those paired by Hfq, is carried out by a protein complex called the degradosome, containing the enzyme catalyzing RNA cleavage: ribonuclease E (RNase E). Interestingly, sRNAs have been identified in cells in complexes with Hfq, but also with Hfq and the degradosome. It is not known what functional differences divide these complexes. The degradosome often associates with RhlB helicase which unwinds structured RNAs, therefore, we hypothesize that the presence of the degradosome allows sRNA to access previously inaccessible mRNA target sites. Moreover, it is not known whether the formation of a stable mRNA-degrading complex depends on the order in which the individual components are assembled.

Previous studies have shown that most, but not all sRNAs are degraded together with target mRNA, which would lead to the silencing of the regulator. However, it is not known what factors determine sRNA degradation and whether Hfq is present in this process. If so, short fragments after RNA degradation may remain bound to Hfq, affecting the downstream fate of target mRNAs. We plan to reconstitute a catalytically active degradosome that also can interact with proteins. Using fluorescently labeled components, we will be able to track the assembly of the complexes simultaneously with RNA catalysis.

The coordination of these processes is even more complicated since mRNA is undergoing translation. It is known that sRNAs frequently target the ribosome binding region on mRNA, but it is unclear whether there is direct competition between the ribosome and the sRNA-Hfq complex. In addition, degradosomes that cleave mRNA in the coding regions must directly coordinate their work with the ribosomes. We want to visualize how dynamic these processes are.

The results of this project will provide insight into the communication between key cellular processes involved in the expression of genetic information. We will monitor the dynamics of these mechanisms on the scale of milliseconds - on the timescales relevant in cells. By monitoring single molecules, we will also gain insight into the heterogeneity of these processes. All this information will be valuable in designing artificial regulators of gene expression and simulating their effects.