Designing a new method for direct identification and absolute quantification of premature transcription termination events, following the example of bacterial riboswitches.

Bacteria reveal an extraordinary flexibility in adaptation to environmental changes. Many of them are able to survive utilizing various carbon and nitrogen sources in order to synthetize all necessary cellular components. However, such plasticity requires precise and accurate control of gene expression. Bacteria utilize broad spectrum of different mechanism of gene expression regulation. Significant fraction of them is based on transcription termination. Specific environmental condition, like changes in temperature or starvation, might trigger co-transcriptional formation of alternative fold of transcripts with a dominant structure of a termination hairpin. In such cases, RNA polymerase action is halted and the transcription is prematurely terminated. This phenomenon is commonly used by riboswitches - sequences located in 5' untranslated region (5'UTR) of cellular mRNAs, able to directly bind small cellular compounds (like vitamins, nucleotides, amino acids, metal ions) and evoke regulatory effect in a controlled gene. In the most common scenario, when a given cellular compound (ligand) is absent, aniterminator hairpin is a dominant structure in the riboswitch, and transcription may occur undisturbed. However, if the ligand is abundant in the cell, it interacts with the riboswitch, which stimulates the formation of a terminator hairpin and downregulates transcription.

The scale of this phenomenon proves that the transcription termination is an extremely important and crucial process, which requires certain attention. When aiming at detection of this kind of events, one has to take into consideration several problematic issues, though. First of all, prematurely terminated transcripts are assumed to be found at very low basic levels in the cell, since they their presence is temporary, they are less stable and prone to quick degradation. Therefore, the sensitivity of a detection method has to be high enough in order to provide the possibility of identification of even the smallest amount of prematurely terminated transcripts. Another obstacle is the estimation of terminated transcript levels, as most of routinely exploited methods do not allow for accurate, direct and/or absolute quantification, presenting rather relative amounts or levels. I believe that both of these issues are pointing into an urgent need for elaboration of a new method aiming at reliable estimation of premature transcription termination events.

The execution of this project will allow for better understanding of transcription regulation process. Newly developed method of transcription termination analysis with convenient and precise measurement of termination products will provide a novel insight into regulatory mechanisms of gene expression. What is of special significance, this approach is not only limited to riboswitches analysis. It might become an excellent molecular tool for the studies of all transcription termination events in many fields of life sciences. This method will offer the possibility of direct and absolute quantification of terminated and read-through transcripts, which cannot be easily achieved by current methods.

Furthermore, the knowledge about gene regulation in bacteria is of crucial importance not only from scientific but also medical point of view. Riboswitches as antibacterial drug targets are now intensively studied, as they might serve in the future as a new group of antibiotics. It proves that such studies are urgent and necessary, as they might be particularly beneficial for public healthcare, especially taking into consideration constantly increasing antibiotic resistance among pathogenic strains of bacteria. Moreover, there is only a limited number of scientific reports concerning the riboswitches action *in vivo* as a function of time. I believe that the results of this project will definitely contribute to the knowledge about regulatory transcription termination events occurring during bacteria lifespan.