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ABSTRACT OF DISSERTATION

The use of biophysical methods in the search and study of selective inhibitors of proteins responsible for the degradation of the 5' end of mRNA

In eukaryotes mRNA is modified by the addition of 7-methylguanosine, also known as the cap. This unique structure serves several critical functions in the organism, with the most important being the protection of messenger RNA from premature degradation by nucleases and participation in the translation initiation. However, mRNA is inherently unstable and, after fulfilling its role, undergoes degradation. This degradation may occur through two alternative pathways – either from the 5' to the 3' end or in the reverse direction. The first of these is initiated by a process called decapping, which involves the removal of the cap structure and further degradation of the transcript by specific exonucleases. The rate of cap hydrolysis directly affects the control of mRNA stability and thus plays a key role in gene expression. Enzymes involved in this mechanism are often therapeutic targets in the treatment of various diseases, and the search for their inhibitors can be beneficial from the perspective of drug design aimed at targeting these enzymes.

The main objective of the doctoral project was to develop a new method that could be used to monitor the activity of enzymes responsible for the degradation of the 5' end of mRNA and adapt it to a high-throughput format. This enabled rapid and efficient screening of compound libraries to identify new, selective inhibitors of two decapping enzymes – the viral D9 enzyme from the Vaccinia virus and the human DCP2 enzyme, which is part of the larger decapping complex PNRC2-DCP1-DCP2. The dissertation presents the development of the method based on fluorescence intensity measurement (FLINT) to track enzyme activity in real-time, as well as its adaptation to a high-throughput format, including method optimization and its validation. In the next step, the developed method was used for screening small-molecule compound libraries – a small nucleotide-derived compound library and the commercially available LOPAC$^{®1280}$ library of pharmacologically active compounds. The hits identified in the screening experiments were further evaluated, including the determination of their IC_{50} values. These results were then confirmed by an independent method through monitoring the decapping reaction on a short RNA substrate *in vitro*. The selectivity of the identified compounds against other decapping enzymes was also tested. Finally, structural studies were described, allowing the characterization of the interaction between one of the enzymes and the FLINT-identified inhibitor at the atomic level.

The obtained results provided valuable insights into the structural preferences of both enzymes towards potential inhibitors and allowed the selection of several compounds that exhibited strong inhibitory properties against the studied enzymes. These findings will contribute to the better design of new compounds with desirable inhibitory properties and potential therapeutic significance.