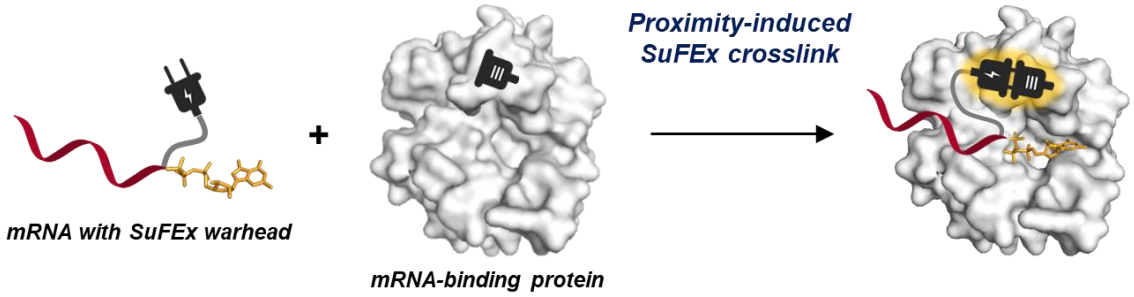
Feel the chemistry if you're close enough – proximity-induced SuFEx ligation for mRNA biology and medicine

Nucleotides perform a number of critical functions in cells, the most obvious being building blocks of nucleic acids. In addition, their presence is necessary for the proper functioning of many enzymes (as cofactors) and they participate in intra- and intercellular signalling. Due to the key role of nucleotides, their synthetically modified analogues are an extremely valuable tools for biochemistry and biology. Their use contributes to the uncovering of the greatest mysteries of our body (e.g. inheritance) and drives the development of modern medicine. Modified nucleotides and nucleic acids are increasingly becoming the basis for pharmacotherapy of diseases that until recently were considered difficult to treat or even considered incurable. In particular, RNA (including mRNA, which is our cellular protein recipe) has nowadays become extremely interesting for developing new therapies against cancer, infectious diseases and rare genetic diseases. Great successes in this field include therapeutic siRNA (patisiran, givosiran) and mRNA vaccines against SARS-Cov-2. In this project, we propose the use of modern biochemical tools that allow the more in-depth understanding of the processes related to mRNA metabolism.



Our idea for research in this field is based on the use of the SuFEx (Sulfur-Fluorine Exchange) reaction described by the two-time Nobel Prize winner Barry K. Sharpless. This is a new and very attractive "click chemistry" reaction, the application of which in the field of nucleotide and mRNA chemistry has not been explored so far. SuFEx type functional groups, i.e. containing bonds between sulfur (VI) and fluorine, have many unique chemical properties that are successfully applied in general organic chemistry and polymer chemistry. The most remarkable feature of these compounds is the possibility of precise and controlled activation of the generally stable S-F bond. One of the factors triggering such activation is the binding of a molecule possessing a SuFEx group in the binding pocket of the protein. In a situation of confined space, forced proximity of various functional groups present in proteins and various chemical factors (e.g. hydrogen bond networks), the S-F bond can be broken to form a new bond between the sulfur and the side chain of the amino acid. The SuFEx molecule then connects to the protein with a covalent bond, permanently changing its properties (e.g. inhibiting enzymatic activity). This phenomenon is often used for the design of small molecule inhibitors, however, in the case of nucleotides and nucleic acids, it still unexplored. In order to initially recognize the potential of such an approach in the context of nucleotide chemistry and biochemistry, we introduced a SuFEx group at the appropriate position of guanosine monophosphate, resulting in a very potent inhibitor of the human endonuclease CNIIIB. Using mass spectrometry, we proved that the inhibition of enzyme activity resulted, as expected, from the covalent (i.e. permanent) ligation between enzyme and the inhibitor. This result is the starting point for the presented project, which covers an extensive research on the use of SuFEx-type chemistry to modify nucleotides and nucleic acids (primarily mRNA) and studies on proteins involved in their recognition and metabolism.

The successful implementation of this project will, first of all, make it possible to indicate which proteins interact with individual nucleotides or mRNA fragments in cellular conditions, and secondly, it will allow to affect the processes in which mRNA participates. The positive results of this project will provide new knowledge and tools for a better understanding of cellular processes related to mRNA and the function of enzymes involved in its metabolism (degradation, translation). Understanding the factors affecting the selectivity of mRNA binding to individual proteins will potentially facilitate the rational design of safe therapeutics with improved effectiveness.