Decoding the code: Unraveling the role of human methyltransferase Trm5 in tRNA modification

Transfer RNA (tRNA) plays a crucial role in protein biosynthesis, transporting amino acids to the ribosome during translation. For messenger RNA (mRNA) to be decoded correctly, tRNA must undergo a series of modifications. One of these is the addition of a methyl group to the first nitrogen of guanine at position 37 of tRNA (m^1G37). This process is essential for maintaining the correct reading frame; its disruption can contribute to cell death. In eukaryotes and archaea, the methyltransferase Trm5, possessing a Rossmann fold active site, performs this process. Conversely, bacterial m^1G37 is catalyzed by the Trm5 ortholog, TrmD, characterized by a deep trefoil knot. Both enzyme groups utilize S-adenosylmethionine (SAM) as a methyl donor. Despite performing the same modification, Trm5 and TrmD exhibit significant evolutionary, structural, and mechanistic differences.

Furthermore, in one archaea (*Pyrococcus abyssi*), Trm5 also modifies tRNA with a phenylalanine anticodon, transforming m^1G37 into wyosine derivatives (specifically, 4-demethylwyosine to isowyosine). This suggests potential bifunctional activity in other organisms. The lack of a deposited structure for human Trm5 and the insufficient understanding of its activity mean that research on it will significantly broaden knowledge in the field of enzyme evolution and the mechanisms of methyltransferases. **Therefore, the main goal of this project is an in-depth structural and biochemical analysis of the methyltransferase Trm5 from *Homo sapiens* (HsTrm5)**, which will make it possible to answer the following questions: How does HsTrm5's structure compare to its orthologs? What active site changes occur during SAM binding? Is Trm5's bifunctional character unique to select prokaryotes? Why did such significant structural divergence occur between enzymes performing the same reaction?

Advanced techniques from the interface of structural biology, biochemistry, biophysics, and molecular biology will be used during the project's implementation (Fig. 1). This will allow me to determine not only the single structure of human Trm5 but its performance over time and the properties of the tRNA modification it performs. Beyond classical crystallography, I will use time-resolved crystallography, which is a technique that allows real-time tracking of structural changes in molecules, making it possible to record molecular "movies" of proteins in action. It allows observation of the dynamics of chemical reactions, conformational changes of proteins, or other molecular processes that occur over short time intervals. Additionally, I will use DFT calculations, which will allow for the understanding of crucial catalytic aspects that are difficult or even impossible to access by experiment. The implementation of this grant will help solve this important puzzle for our quality of life.

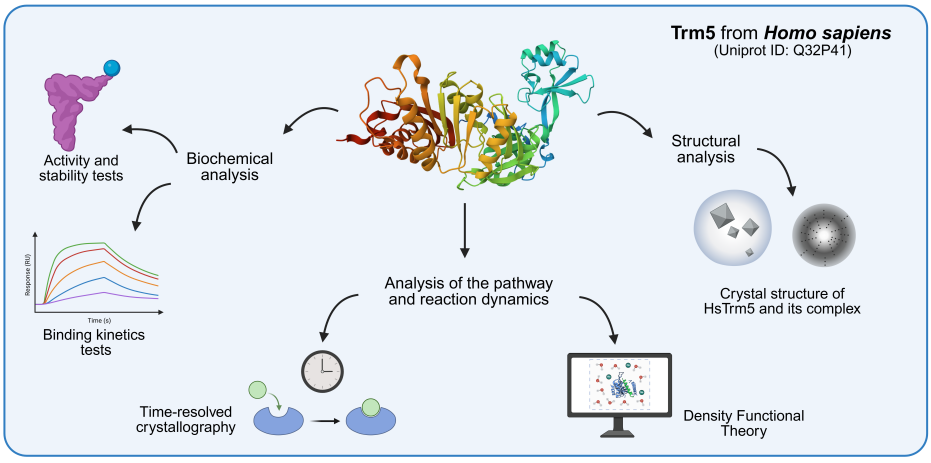

Figure 1. Schematic representation of research tasks assigned within this project.

m^1G37 is one of two tRNA modifications found throughout the entire domain of bacteria, including human pathogens such as *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*. Antimicrobial resistance has been identified by the World Health Organization as one of the greatest threats to human health and development. TrmD is recognized as one of the targets for a new generation of antibiotic therapies – it has been shown that SAM-like molecules prevent tRNA methylation, thereby inhibiting bacterial growth. We believe that the results we obtain regarding the activity of human methyltransferase Trm5 will have a crucial impact on the further development of research into drugs targeting TrmD and will positively contribute to the fight against antibiotic resistance.