The main component of every living organism are proteins. They play diverse functions as enzymes digesting food, sensors, switches, to name a few. They are also structural elements of each cell in the body and can produce movements in the muscles. Proteins are long strings or chains of building blocks, chemicals called amino acids (there are twenty of these building blocks in human). These long strings collapse into unique three-dimensional shapes – gears of the cellular mechanism. Each protein with the unique amino acid sequence performs a different function. Proteins are made based on the genetic information stored in the DNA molecules which also have a form of long strings. These strings are made of four types of chemicals termed nucleotides. They can be considered as long texts made of four letters. A group of three such letters codes for a particular amino acid. The information in the DNA is first converted into another very similar molecule termed mRNA. Next, a complex machine termed ribosome sequentially reads the information in the mRNA and based on it synthesizes the protein chain. The key component in this process are specialized RNA molecules termed transfer RNAs or tRNAs. Each type of tRNA has a part which decodes the three letter word of the genetic code and its other end carries the amino acid which corresponds to it. As the ribosome exposes the three letters in mRNA, the tRNA that fits to them comes in and brings the appropriate amino acid which is then added to the growing protein chain. This process termed translation is at the heart of life.

tRNAs are central to this process, but they need to be properly prepared to fulfill their function. Some of them contain additional stretches of nucleotides which need to be removed to form the functional form of tRNA. This so-called tRNA splicing involves two proteins (enzymes). One cuts out the unnecessary nucleotides and the other, called ligase, joins the two ends of tRNA at the resulting gap. This joining is a quite complex chemical reaction and its details are not known. The first aim of this project is to visualize this reaction and all its steps at the level of single atoms. We plan to use a method called crystallography, in which small crystals of the protein are obtained and X-rays are shined on them. Based on how these X-rays are reflected on the crystal, the positions of all atoms in the protein can be calculated. Knowing these positions at various stages of the reaction, we will be able to visualize it with great detail.

In higher organisms such as humans, tRNA ligase is a complicated assembly of several proteins. Only one performs the chemical reaction and the function of the others is not clear. We plan to use another method– cryogenic electron microscopy, to visualize tRNA ligase assembly. We will use a powerful electron microscope which provides magnifications large enough to visualize individual protein molecules. These are large magnifications indeed - if a protein molecule was to be enlarged to the size of 10 cm the human body would have to be proportionally enlarged to the length of 20 000 km, half of the length of the equator. Over the last few years technical developments in electron microscopy allowed it to provide pictures of molecules which are as detailed as the ones from protein crystallography. This breakthrough was awarded a Nobel prize in 2017.

Human tRNA ligase has also a function outside of tRNA processing. It plays a role in cell’s response to problems and defects in protein synthesis. This response mechanism is involved in cancer and other serious diseases. It is thought that the entire response mechanism and human tRNA ligase in particular can be considered as a targets for development of new therapies.

In summary this project will provide detailed information how interesting and important tRNA ligase proteins work, which is currently not fully understood.