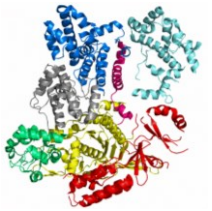
Chiroptical properties of proteins and their complexes (CRISPR/Cas-RNA), and their relationship to biological activity



CRISPR associated Cas proteins are enzymes, called also 'molecular scissors' that are a key element of an innovative system used in genetic engineering for a precise genome modification. CRISPR stands for *Clustered Regularly Interspaced Short Palindromic Repeats* that are naturally occurring genetic features of bacteria used together with Cas nucleases to fight against invading viruses. Thus, CRISPR/Cas system is a natural protective mechanism adopted by scientist as a molecular biology tool allowing for precise gene editing in a broad range of organisms. Developing the CRISPR/Cas-based method gives researchers the ability to easily add, remove, or change regions of an organism's DNA, reducing the time and cost of the process and increasing its potential application in the field of agriculture, biology, and medicine. In **2020**, *the Royal Swedish Academy of Sciences* has awarded **the Nobel Prize in Chemistry** for the **discovery**, development and research on **the CRISPR/Cas system**, which automatically highlights the importance and opportunities of this method. As mentioned above, t**he CRISPR/Cas works like a pair of molecular scissors that cuts DNA at specific locations and either deletes sections or replaces them with alternate sequences**. It involves two main biological macromolecules: first, **a single guide RNA** molecule (gRNA) that contains a short fragment complementary to target DNA sequence and acting as a guide to the system, and second, **a Cas protein** that does the cutting. In the functional CRISPR/Cas system the gRNA molecule, designed in the lab, binding to the Cas nuclease results in a significant change of the protein geometry and leads to form a ribonucleoprotein complex (RNP) revelling directed nucleolytic activity.

Hence, the principal motivation of the project is to find how, and to what extent, conformational changes of Cas proteins affect their biological activities. For this purpose, **chiroptical spectroscopic techniques** such as *Electronic Circular Dichroism* (**ECD**), *Vibrational Circular Dichroism* (**VCD**) and *Raman Optical Activity* (**ROA**) will be used as leading research methods, additionally accompanied by conventional ones, e.g., *absorption spectroscopy in the UV-Vis* (UV-Vis), *absorption spectroscopy in the infrared range* (IR), and *Raman spectroscopy* (RS). Realization of the project would **reveal structural variation among Cas proteins**, as well as **transfer developed research methodology to study biological activity and structure of other important enzymes** and **their cofactors**, as well as **globular proteins** upon binding pigments, or vitamins.

Scientific literature shows that chiroptical techniques are very suitable to study proteins and nucleic acids interactions because these methods are very sensitive to three-dimensional structure of natural biomolecules. Both proteins, and nucleic acids, like RNA and DNA, are chiral and optically active, as well as interact with circularly polarized light in a specific way. ECD and VCD are based on the differential absorption of left- and right-handed component of circularly polarised light by chiral compounds. In turn, ROA technique is related with the observation of a small difference in intensity of Raman scattering of right and left circularly polarized light due to molecular chirality. It is also surprising that so far **chiroptical methods have been hardly used for structural analysis of the Cas proteins** and RNP complexes. Hence, all together, i.e., UV-Vis, IR and Raman spectroscopy, ECD, VCD and ROA could **create a set of ultra-sensitive spectroscopic tools to study secondary and tertiary structure of proteins and to monitor conformational changes in the structure of their complexes in solution**, and in the case of ECD and ROA even in the natural water environment.