DESCRIPTION FOR THE GENERAL PUBLIC (IN ENGLISH)

Methods based on fluorescence phenomenon are often used in biochemical investigations. These techniques are characterized by high sensitivity, therefore, they do not consume a large amounts of reagents. As a research objects may be used either particles which are inherently fluorescent as well as chemical compounds labeled with suitable fluorophore.

In this project we plan to develop a method based on fluorescence polarization for searching inhibitors of m^7G nucleotide binding proteins. The technique uses fluorescently labeled ligand and its specific protein. In the experiment fluorescence polarization changes are observed due to the formation of a protein-ligand complex. The monitored signal change is observed because fluorescent ligand in the complex is characterized by reduced rotation, resulting in an increase in fluorescence polarization.

Molecular targets interesting for us are m^7G nucleotides binding proteins. These proteins have a specific function, m^7G base occurs only at the 5' end of the mRNA as a structural element of cap (m^7GpppN_n), or in a cell as a cap product hydrolysis. Accordingly, the nucleotide comprising m^7G -binding proteins are involved in all steps involved in mRNA metabolism, such as pre-mRNA splicing, translation, transport, and degradation.

For the project we selected model protein - the translation initiation factor eIF4E. High levels of factor eIF4E have been observed in a tumor cells. Its overexpression contributes to increased protein production and cancer growth. High affinity ligands for eIF4E are potential therapeutics, their activity leads to reduction of eIF4E availability for transcripts. Many ligands, in particular based on the cap structure, have been characterized in the literature, therefore we choose eIF4E as a model protein, to check the accuracy of the obtained results.

In addition, we selected two enzymes, DcpS - involved in mRNA degradation and CN-IIIB - responsible for the metabolism of nucleotides. DcpS enzyme has been identified as a therapeutic target in the treatment of spinal muscular atrophy. In the case of the enzyme CN-IIIB biological significance is not known, its inhibitors can be used in research on cN-IIIB biological function.

The method based on fluorescence polarization represent an excellent tool to search ligands with high affinity for the tested protein, which can also be used in high-throughput approaches. The use of 96-well plates in combination with a microplate reader allow a quick examination of multiple samples simultaneously at short time. An additional advantage of high throughput approach is to examine the impact of multiple structural ligand fragments on their inhibitory efficiency. This analysis makes possible to design a compound with the desired properties and targeted search for ligands in the future. In particular, we hope that the proposed project will allow us to find selective inhibitors for tested proteins. These inhibitors may be used as drugs in anticancer therapies and neurodegenerative disorders.