PROJECT SUMMARY

Trimethylcap (TMG-cap) present at the 5' end of the small nuclear RNA is a nuclear localization signal (NLS). It is recognized by the adaptor protein snurportin 1, which in a complex with an importin-β transports TMG-capped snRNA to the nucleus. Nuclear transport can be successfully used for the delivery of therapeutic oligonucleotides to the nucleus. The approach assumes the use of a natural transport system to deliver chemically modified structures into the cell.

However, to be an effective mediator of nuclear transport, synthetic TMG-cap analogs should be stable in vivo and should be recognized by cap binding proteins. Because the cap structure contains 5'-5'-triphosphate bridge, which is prone to hydrolysis by decapping enzymes that cleave triphosphate bridge between α/β or β/γ position, it is necessary to introduce a modification in order to improve the TMG-cap stability in vivo. In the project, we plan to synthesize 5'-phosphorothiolate TMG cap analogs, modification -which has never been studied in the context of cap function. We expect that, besides improved stability, introduced modification will also positively affect the interaction with nuclear proteins.

In addition, the project involves also labeling of the most stable compounds with molecular rotors. These compounds will provide tools to study interactions between synthetic TMG-cap analogs and proteins using fluorescence based methods. Molecular rotors mechanism of action is based on the "turn on" fluorescence as a result of interaction with a protein/enzyme. Such system (zero-one, On-Off), in which the signal appears only during an important process is very useful in biological research. First of all, this allows estimating desired parameters (K_{AS}, IC_{50}), which are usually time-consuming, complicated or difficult to estimate using standard fluorescence techniques. For example, the fluorescence probe signal can be masked by the protein fluorescence. The fluorescence of molecular rotors is based on the twisted intramolecular charge transfer phenomenon (TICT). TICT is characterized by rotation one of the segments around the σ bond, which, depending on the molecular rotor structure, results in non-radiative relaxation process (no fluorescence, OFF) or radiative relaxation process (fluorescence, ON). The intramolecular rotation strongly depends on the type of solvent, its polarity, hydrogen bonds, isomerization and steric hindrance, thus, is dependent on the major forms of interaction between a molecule and a protein.

In the project, we plan to use TMG cap labeled with such molecular rotors to study its interactions with snurportin 1.We want to develop a high throughput fluorescence based method, which can be used to identify and estimate affinity parameters of synthesized compounds with snurportin 1. These studies will help to identify the key structural elements necessary for a recognition by the protein and will form the basis for the future design of new TMG cap analogs as NLS probes.

Molecular rotors modified TMG cap analogs can be also used to visualize the functional TMG-cap-protein complexes using life-time fluorescence imaging microscopy - FCS-FLIM (*Fluorescence Correlation Spectroscopy-Life-time Fluorescence Imaging Microscopy*). These studies will help to determine whether synthesized TMG capped oligonucleotides are functional and whether they penetrate the cell nucleus mediated by natural proteins.