1 Objective of the project

TS (thymidylat synthase) is an ubiquitous enzyme (a protein that catalyses particular reaction), that has a very important role. It is responsible for the synthesis of dTMP, a molecule that is used for DNA building. In the mammalian cells, TS is the sole *de novo* source of dTMP. Resulting form its function, it is a molecular target in the anti-cancer, anti-viral and anti-parasite therapies. As a cause of that very fact, it is also in the center of interest of this project. TS has a specific amino acids (protein's building molecules), that after being exposed on the UV radiations can emit light (a so called fluorescence). An observation of this phenomenon is possible with a special equipment. Moreover, under the binding of small molecules (substrates, inhibitors) or other interactions, fluorescent properties can be modified. This allows for an interpretation of e.g., structural changes of the protein, thus better mechanism understanding. The TS catalytic mechanism is quite complicated and still not fully understood. Moreover, recent studies show that TS can conduct a one step of the reaction with utilisation of different than substrate molecule. This project is aimed at investigation of this unusual and surprising features. An another objective of this project is an inhibition mechanism analysis of the very unusual TS inhibitor - N^4-OH-dCMP that causes an abortive reaction. By comparison of the data obtained from enzyme complexed with N^4-OH-dCMP, with the results from complexes with other inhibitor - FdUMP - which mechanism is known, solution of the questions posed in this project will probably be possible.

In addition, due to the solved 3D structures of the TS solely and in complexes, that are available in the PDB database, coupling of the fluorescence changes with the structural differences visible in aforementioned structures is planned. Moreover, the knowledge of particular structural changes effects on the enzyme's fluorescence will allow to verify if these modifications are indeed equivalent to crystallographic (structures) data and *vice versa*. The concatenation of obtained results with the crystallographic structure analysis will facilitate a creation of a broad analytical project, that in further perspective will serve as a model for interpreting TS interactions from different sources with ligands.

Thymidylate synthases are a group of proteins derived from various organisms that are extremely conservative (ie, proteins are very similar to each other). Hence, mouse thymidylate synthase (mTS) was chosen as the model for studying fluorescence parameters under molecular bonding.

2 Description of research

The objectives of this project will be achieved with an absorption, emission and time-resolved spectroscopy. All of these methods are connected with aforementioned fluorescence. In order for a molecule to emit a photon (radiate), first it has to absorb light. The absorption spectroscopy is used for the measurement of the amount of absorbed photons. As a result of two (e.g., enzyme and substrate or enzyme and inhibitor) or more interacting molecules, alterations in the absorption spectra can be observed. Similarly, the technique that utilizes aforementioned fluorescence (emission spectroscopy) and its changes during complex formation will help with clarification of the striking issues. Third technique (time-resolved spectroscopy) is dedicated for the measurement of the time within which molecule radiates (fluorescence lifetime). This measurements are very sensitive and provide with number of valuable informations about the strength and type of interactions, which will be utilized in the analysis of the proposed problems.

Aforementioned protein structure analysis will be performed with a program called VMD MultiSeq. It is designed for the analysis and alignments of the sequences and structures of the proteins.

3 Reasons for choosing the research topic

Describing the catalytic and inhibition mechanism of mTS in the complexes may have a positive impact on the development of therapy, disease treatment and drug design, which, from the society point of view, is a great advantage. In the wider perspective, this provides the opportunity for more effective cancer treatment and rational anti-cancer therapy. At the same time, and it should be emphasized, due to the conservatism of the enzyme, the results obtained will be widely used as a model for interpreting TS interactions from different sources with ligands.