Description for the general public

Fluorescent proteins (FPs) distinguish themselves among others with ability to absorb light of proper wavelength and subseqeunt light emition. For this reason, naturally occuring FPs are involved in e.g. communication between the organisms or repelling the predators. This extraordinary ability to absorb and emit light is also utilized in modern chemistry and biology for visualization of various processes taking place in living cells, tissues and even whole organisms.

Chromophore is "a heart" of every fluorescent protein. It is responsible for light absorption and emition. Chromophore is a set of modified amino acids and it is created in reactions catalyzed by its protein environment. What is more, chromophore's environment is the key element responsible for its spectral properties (light absorption and emission intensity as well as wavelengths at which this intensity reaches its maximum), structural and physico-chemical.

In conjuction with strong chromophore's environment impact on listed spectral properties, it is possible to engineer and obtain fluorescent proteins with desired absorption and emission spectra by introducing proper mutations in protein's amino acid sequence. So far, experimental efforts allowed to obtain whole range of fluorescent proteins which absorption and emission spectra cover whole visual spectrum as well as near ultraviolet and near infrared.

One of the modern directions of fluorescent proteins' development is their utilization in two-photon fluorescent microscopy. This technique relies on absorption of not one, but two photons. The energy of photons absorbed in two-photon microscopy is twice smaller than in case of one-photon visualization techniques. This phenomenon allows for visualization of chosen molecules and processes in living tissues and organisms with considerably smaller impact on investigated system than in case of one-photon absorption-based methods. What is more, two-photon microscopy provides images of much enhanced resolution and contrast.

Currently, fluorescent proteins seem to be well characterized in term of one-photon properties. However, in case of two-photon absorption intensity, experimental results obtained with various techniqes may differ even by two orders of magnitude. What is more, chromophore's protein environment impact on its formation rate is usually unknown. Thus determining the concentration of proficient fluorescent protein is a difficult task. Due to difficulties with experimental measurements, it is necessary to use theoretical chemistry methods to obtain consistent results that are independent from other factors. What is more, descprition of chromophore's protein environment impact on its absorption properties is the most convincing thanks to simultaneous analysis of experimental measurements and theoretical calculations results.

Objective of the present project is to systematically characterize chromophore's protein environment impact on its spectral properties, and in particular on two-photon absorption intensity. According to literature data, chromophore's environment influences the two-photon absorption intensity to a much greater extent than one-photon absorption intensity. It is pivotal to understand factors responsible for high value of two-photon absorption cross-section so that one may rationally design and obtain fluorescent proteins that are applicable for two-photon microscopy.

Our investigations will utilize so called hybrid technique. The part of protein responsible for light absorption is described with quantum mechanics (QM) and the rest of the system is described with molecular mechanics (MM). Firstly, we will perform calculations to choose the most reliable QM methodology in a sense of quality of obtained results as well as required computational time and resources. Next, chromophore along with amino acid residues and water molecules from chromophore's immediate environment will be described with QM method in order to describe chromophore's environment impact on its spectral properties as precisely as possible. Then, we will perform decomposition of interaction energy between the chromophore and its environment into contributions from individual residues. In this way, we will obtain qualitative or semi-quantitative image of protein environment influence on chromophore's spectral properties. In the next stage, selected amino acid residues will be substituted by other ones and effects of those mutations on chromophore's spectral properties will be investigated. Such a systematic research will allow us to understand reliationship between chromophore's environment and spectral properties. This should allow for rational engineering of novel fluorescent proteins in the future. What is more, based on calculations results, we will propose novel fluorescent proteins, in particular those having large two-photon absorption cross-section value.

The final effect of the present project will be proposition of procedure for obtaining novel fluorescent proteins. It will be based on prediction of their properties based on molecular modelling investigations. Such an approach will considerably accelerate and increase efficiency of obtaining novel markers.