Resume of doctoral thesis titled

"Modelling and characterisation of one- and two-photon absorption for chosen green and yellow fluorescent proteins with theoretical chemistry methods"

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Fluorescent proteins (FPs) form a versatile set of tools utilized for visualisation of various processes *in vivo*. Owing to their intrinsic fluorescence, nontoxicity and ability to create stable constructs with host's native proteins, the FPs have found a plethora of applications in modern biochemistry, medicine and general science.

The aim of this PhD thesis is to describe impact of a FPs chromophore environment on its one- (OPA) and two-photon absorption (TPA) spectra. The vital part of each and every FP is its chromophore buried inside the protein cavity. This implies that one could rationally develop novel FPs if one understands the mechanism by which chromophore's environment influences its spectral characteristic. Herein, the author uses the theoretical chemistry methods to gain insight into nature of chromophore – environment interactions and describe their impact on vertical OPA and TPA spectra.

To achieve this goal, the author divides the research into three stages. In stage one, the author calculates OPA and TPA spectra for a set of FPs chromophores differing in structure *in vacuo* (thus neglecting the protein environment). The purpose is two-fold. Firstly, the author determines an efficient exchange-correlation functional (XCF) for OPA and TPA spectra calculations within time-dependent density functional theory (TDDFT) ansatz. Secondly, the author investigates how the chromophore structure itself influences the absorption spectra.

In the second stage, the author looks for an optimal size of FP described by quantum mechanics (QM) for electrostatic and polarizable embedding calculations of OPA and TPA spectra. By systematically adding amino acid (a.a.) residues and water molecules creating the chromophore's environment to the QM subsystem, he identifies the composition of a quantum subsystem that provides a good balance between required computational resources and spectra accuracy.

In stage three, the author investigates chromophore's protein environment impact on OPA and TPA spectra. It is achieved by comparing the absorption spectra for (i) a series of FPs differing in a.a. sequence and (ii) different models of the same FP. In particular, the author reveals the role of a.a. residues hydrogen-bonded to chromophore in absorption spectra tuning. Moreover, electrostatic tuning of absorption spectra is addressed.

The author proposes that TPA can be enhanced by creating more positive electric field acting on the chromophore's phenyl oxygen. This can be achieved by introducing more hydrogen bonds with the environment. Furthermore, it seems that screening the chromophore from distant electric field sources, e.g. through increasing the dielectric constant of the chromophore's immediate environment, may lead to brighter TPA process. The author believes that this thesis is a step forward in a rational and directed design of novel FPs with a special emphasis on enhancing the TPA process.