SUMMARY OF DOCTORAL DISSERTATION

Molecular structures and spectral properties of chosen fluorescent proteins and rhodopsin chromophores Quantum chemical calculations

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The phenomena of absorption, conversion and sometimes emission of light are often breakthrough stage of many fundamental biological processes in which photoactive proteins often play a major role. One of the most important examples of such biomolecules is visual purple - rhodopsin, which belongs to the family of G-protein-coupled receptors (GPCR). Visual purple is responsible for scotopic vision and can be found in the rod cells of molluscs, arthropods and vertebrates eye retina. Rhodopsin chromophore, which is protonated Schiff Base of 11-cis-retinal (rPSB), after photon absorption isomerises to all-trans conformation (rPSBT). As a result of that photoisomerisation, conformational changes occur in the protein structure and the nerve impulse is passed to the brain.

The other important group of photoactive biomolecules ale fluorescent proteins (FP). One of the best studied members of FP family is Green Fluorescent Protein (GFP) from the jellyfish *Aqueorea victoria*. GFP, as well as other FPs, consists of a β-barrel with a fluorophore placed inside. They are commonly used in genetic engineering and molecular biology as marker genes to organelles, cells and tissues visualisation. Moreover, they found application in fluorescent microscopy and as biodetectors and nanosensors.

Because of biological significance and practical applications, it is very desirable to determine the spectral properties of FP and factors affecting them, e.g. impact of the closest amino acid residues, solvent effect and also spectral properties of isolated chromophore. Currently, there is only one experimental group that is able to determine absorption spectra of such fluorophores *in vacuo*. Unfortunately, the experimental method they use is able to investigate ionized molecules only, what is an inconvenient limitation. Fortunately, quantum chemistry methods can study all protonation states of the chromophores.

Doctoral dissertation presents the results of calculations, that proved, that appropriately selected and properly applied theoretical methods can be very useful tool for protein chromophores spectral properties examination *in vacuo*. Calculations described in doctoral dissertation were carried out for a few models of rhodopsin chromophore and for fluorophore models of three FPs: GFP, GFP-W7 and DsRed. The whole work was divided into three research projects.

The main goal of the first task was to determine the usefulness and performance of second-order approximate coupled-cluster method (CC2) to explore the reaction paths of photochemical reactions, especially in conical intersection space and its closest neighbourhood. For many minimal rhodopsin chromophore model (PSB3) structures of which are composed reactions paths on S_0 and S_1 potential energy surfaces, energy calculations were made. Obtained values were compared with reference data (MRCISD+Q). Interestingly, CC2 method gave very good results not only for stationary points of reaction, but also for the whole paths, including the conical intersection region. In

conclusion, the CC2 method works very well for all examined aspects of studying photoreaction energy paths, predicting correctly topography as well as topology of potential energy surface.

The final effect of the second research task was to obtain a complete set of vertical electronic excitation energies for full-size models of retinal molecule. They were calculated using multireference perturbation theory (CASPT2) methodology. According to our results, the choice of the theoretical method used to find an equilibrium geometry does affects electronic excitation energies. Especially, there exists the linear dependence between bond-lenght alternation parameter values (BLA) and absolute energy deviations. However, even larger influence on electronic excitation energies is due to the choice of the IPEA-shift empirical correction parameter, that modifies the zeroth-order Hamiltonian in CASPT2 scheme. Using the default value of the IPEA-shift (0.25 a.u.) in calculations of vertical excitation energies leads to 0.2-0.3 eV deviations from the experimental data. It was shown, that optimal value of this parameter in case of investigated retinal models ranges between is 0.00-0.10 a.u.

The third project focused on studying the absorption and emission properties of GFP, GFP-W7 and DsRed fluorescent proteins chromophore models. CC2-based transition energies were obtained for all aforementioned molecules. Additionally, for GFP and GFP-W7 chromophore models calculations were also carried out at CASPT2 level of theory. In effect, the set of best-estimates of vertical electronic excitation energies was achieved. For some molecular models vertical electronic emission and adiabatic energies were calculated. In case of CASPT2 method, the influence of the IPEA-shift parameter value on electronic transition energies was discussed. For studied group of molecules its optimal value is 0.15 a.u.

The results of the second and the third research tasks led to a general conclusion. Contrary to the expectations of the author, the zeroth-order Hamiltonian with the default value of the IPEA-shift parameter, cannot play the role of universal correction to the CASPT2 systematic errors. Optimal value of this parameter has to be individually determined for each family of structures. IPEA-shift value also clearly depends on the size of studied molecule. However, it should be emphasized, that CASPT2 is nowadays one of the best methods available, that can be applied to determine electronic transition energies of chromophores of biological origin.