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New approach for modelling two-photon absorption spectra of photoactive proteins

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Abstract

Two-photon excitation microscopy is one of the most powerful tools for bioimaging. To fully realize the potential of using two-photon absorption (TPA) of fluorescent proteins, it is important to understand the factors that affect the TPA spectra as compared to those obtained following one-photon absorption (OPA). Here, we develop a sum over states approach employing a two-level model for calculating TPA spectral shapes of fluorescent proteins at a high level of theory.

We simulate the S_0-S_1 TPA and OPA spectral profiles of the GFP chromophore anion inside the protein. The parameters used for modeling vibronic band shapes are obtained using the XMCQDPT2 theory coupled to the EFP method. The TPA and OPA spectra are calculated using the double harmonic parallel-mode approximation and include both Franck-Condon and Herzberg-Teller couplings.

By using the two-level model, we show that the TPA cross-section and the contribution from each vibrational mode can be estimated using simple parameters, such as a difference between permanent dipole moments in the ground and excited states, a transition dipole moment, and their derivatives with respect to normal modes. We show that the non-Condon effects are responsible for the observed shift between the TPA and OPA absorption maxima in GFP. The major contribution to the shift comes from those vibrational modes that modulate permanent dipole moments, correlating with IR-active modes. We conclude that the intensity of these modes can be significantly enhanced when switching from conventional one-photon excitation to a non-linear two-photon absorption regime.

The work is supported by RSF №17-13-01276.