

# Photoswitching of the kindling fluorescent protein: a theoretical study

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Fluorescent protein asCP (also known as kindling fluorescent protein) is a member of photoswitchable fluorescent proteins class. Its ability to be reversibly switched between a nonfluorescent “off”-state ( $\lambda_{\text{abs}} = 565 \text{ nm}$ ) and a fluorescent “on”-state ( $\lambda_{\text{abs}} = 576 \text{ nm}$ ,  $\lambda_{\text{em}} = 610 \text{ nm}$ ) upon intense green light irradiation has been attributed to photochemical E $\rightarrow$ Z isomerization of its chromophore. A reverse isomerization proceeds either thermally or photochemically by irradiation at 445 nm. The details of this interconversion remain poorly characterized. Here, by using high-level quantum chemistry methods we explore the asCP photocycle at atomic level.

Structures, spectra and properties of asCP have been modelled by using *ab initio* based QM/MM and molecular cluster approaches. The results of these simulations favour a mechanism describing a majority of photoinduced asCP transformations solely relying on protein structures with the anionic form of the chromophore. The computed energies of S<sub>0</sub> $\rightarrow$ S<sub>1</sub> (561 nm) and S<sub>1</sub> $\rightarrow$ S<sub>0</sub> (605 nm) vertical electronic transitions for the model system with the anionic chromophore, as well as calculated vibronic structure of the absorption band correlate well with the available experimental data. Internal conversion is shown to proceed through two competing radiationless channels in the off-state of the protein corresponding to twisted S<sub>0</sub>/S<sub>1</sub> conical intersections. Both conical intersections are related to internal rotation of the chromophore in the central bridge moiety. One of them corresponds to the photoinduced E $\rightarrow$ Z isomerization, and the other leads to relaxation back to the “off”-state. Small quantum yield of the asCP photoactivation originates from different topographies of S<sub>1</sub> along the two branches in internal conversion. Photochemical quenching of the “on”-state of asCP is traced to the excited-state Z $\rightarrow$ E isomerization of the neutral form of the chromophore. The estimated value of the absorption maximum (437 nm) is in close agreement with the experimental maximum of quenching efficiency (445 nm). Furthermore, the neutral chromophore concentration increases upon “off”-“on” photoswitching as it has been shown by the QM/MM-based molecular dynamics study, whereas the ground state Z $\rightarrow$ E isomerization proceeds exclusively through the anionic form of the chromophore.

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