

# Time-Resolved Spectroscopic Study of the ON → OFF Photoswitching Reaction Pathway of the Fluorescent Protein Dreiklang

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Reversibly switchable fluorescent proteins (RSFPs) are able to switch reversibly between a fluorescent ON state and a non-fluorescent OFF state. Of particular interest for advanced imaging, their use is in most cases hindered because excitation of the fluorescent ON form simultaneously triggers the decay of the ON population. Dreiklang is one of the rare RSFPs, whose photophysics is fully decoupled: three different excitation wavelengths respectively lead to ON→OFF photoswitching, OFF→ON photoswitching and fluorescence emission. This property is explained by a unique photoswitching mechanism based on hydration/dehydration of the chromophore (Figure 1)<sup>[1]</sup>. However, the nature and kinetics of the involved elementary steps are still poorly known. We present here the first transient absorption spectroscopy experiments on Dreiklang ON-state. Different setups were used to access relevant timescales from 100 fs to milliseconds and disclosed OFF formation within 30 ns (Figure 2)<sup>[2]</sup>. Comparison of the photoswitching properties and dynamics of four single-point variants of Dreiklang plus recent theoretical studies brought insights into the role of neighboring amino acids in the mechanism and enabled us to entangle ON → OFF photoswitching from side reaction pathways<sup>[3],[4]</sup>.

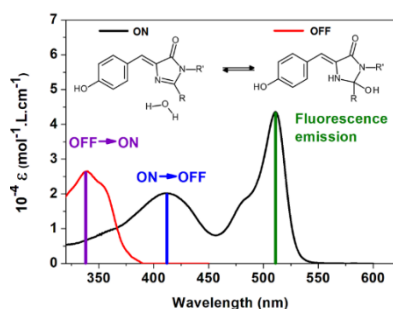


Figure 1. Absorption spectra of Dreiklang

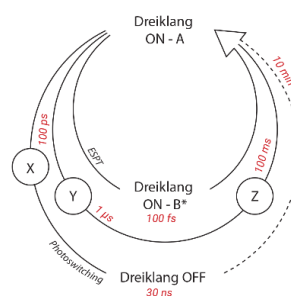


Figure 2. Photoinduced reactions on Dreiklang

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- [4] Sen, T. et al., *J. Phys. Chem. B*, **2021**, 125 (3), 757–770.