Contribution ID: **25** Type: **Invited**

Time-resolved serial femtosecond crystallography on the fluorescent protein rsEGFP2-V151A in different photon excitation regimes

Thursday 5 June 2025 09:30 (30 minutes)

Time-resolved serial femtosecond crystallography (TR-SFX) enables the visualization of ultrafast structural changes in crystalline macromolecules [1]. For light-sensitive proteins, optical excitation lasers represent a convenient means to trigger the reaction. Generally, high excitation-laser fluences are used to maximize light-induced features in the Fourier difference electron density maps [2,3]. However, these fluences generally correspond to nominally more than one absorbed photon per chromophore on average, significantly increasing the risk of unwanted multiphoton effects that convolute with the functionally-meaningful single-photon process of interest [4]. Hence, the choice of excitation laser fluence is a topic of intense discussion in the TR-SFX field [5-7] and a first systematic study on myoglobin indeed evidenced different CO dissociation mechanisms in the single- and the multi-photon regimes [4].
Here, TR-SFX experiments were conducted on the V151A variant of the reversibly photoswitchable fluorescent protein rsEGFP2 [8]. The off- (trans chromophore) to on-state (cis chromophore) photoswitching process was probed at two different time delays (1 and 500 ps) following 150-femtosecond excitation at low, medium and high fluences (0.05, 0.15 and 0.5 mJ/mm² at the Gaussian peak, respectively) corresponding nominally to 0.8, 2, and 8 absorbed photons per chromophore on average. At the high fluence, only a marginal further increase is observed in light-induced Fourier difference electron density peaks compared to the medium fluence, and the expected cis conformer is either absent (1 ps) or occupied below expectation (500 ps). Fluence-dependent time-resolved absorption spectroscopy suggested a chromophore radical species only formed at the high fluence. Our findings suggest that multi-photon induced radical formation at high fluence alters the functional photoisomerization process in rsEGFP2.

References

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Session Classification: Pump-Laser Excitation Conditions in Time-Resolved Serial Femtosecond Crystallography