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Title: Excited state structural evolution in fluorescent proteins and their model chromophores

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Abstract:

The discovery of green fluorescent protein (GFP) has revolutionized molecular and cellular biology. Photocycle in wt-GFP involves generation of a bright fluorescent deprotonated chromophore from feebly fluorescent protonated form via excited-state proton transfer (ESPT). The chromophore loses its fluorescence outside the protein matrix due to ultrafast structural changes (ring twisting motion) during ESPT. To identify the key vibrational modes leading to such structural changes, we recently demonstrated how a systematic analysis of spectral data from non-resonant/resonant impulsive excitation followed by spectral dispersion disentangles vibrations arising in ground/excited electronic states of solute and solvent. We apply this method to fluorinated GFP chromophore exploring real-time interplay between its structure and function. In addition to the usual photocycle, FPs also undergo photoconversion upon illumination. Using spectral kinetics of absorbance and fluorescence, global fluorescence lifetime analysis and time-resolved fluorescence anisotropy, we study the photophysics of enhanced GFP (S65T/F64L mutant of wt-GFP) and Venus (a yellow fluorescent protein variant) which exist predominantly in deprotonated form.

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