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Excited State Electronic Structure of Fluorescent Proteins Revealed by Two-Dimensional Double Quantum Coherence Spectroscopy PATRICK KONOLD, University of Colorado-Boulder — Red fluorescent proteins (RFPs) are nearly ideal probes for monitoring subcellular processes with extremely high spatial and temporal precision. Modern derivatives with increased brightness are sought to further enhance imaging applicability, however, photostability issues represent a universal obstacle towards RFP development. In this work, we employed Two-Dimensional Double Quantum Coherence (2D2Q) spectroscopy to probe the excited state electronic structure of mKate, a widely used RFP. Our results help explain the excited state absorption contributions observed in spectrally resolved transient grating measurements that ultimately relate to excited state photophysics thought to modulate the dark state conversion and irreversible photobleaching processes leading to poor brightness. Moreover, we contrast results across a panel of point mutants of the S158 residue and find a connection between chromophore-sidechain interactions and the position of energy levels in the doubly excited manifold. Such observations highlight the role of the protein environment in tuning excited state photophysics and may provide a clue for engineering more photostable RFPs.

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