

Abstract Submitted
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Fluorescence photocycle of GFP studied by trapping phototransformation intermediates GEORGI GEORGIEV, Assumption College, TIMOTHY SAGE, Northeastern University, JASPER VAN THOR, Oxford University, OXFORD/NORTHEASTERN COLLABORATION, NORTHEASTERN/ASSUMPTION TEAM — The fluorescence photocycle consists involves excited state proton transfer (ESPT) from chromophore to Glu222, ionizing chromophore and neutralizing Glu222. A competing low quantum yield phototransformation process ionizes chromophore, and decarboxylates Glu222. We report spectroscopic measurements on cryotrapped reaction intermediates of the phototransformed GFP that electrostatically mimic intermediates in the fluorescence photocycle. Illumination at 100K leads to appearance of both a visible absorption band at 497nm, indicating an ionized chromophore in a non-equilibrium environment, and of multiple IR bands due to photogenerated carbon dioxide, confirming the decarboxylation of Glu222. Additional changes in the IR spectrum reflect numerous rearrangements of surrounding side chains and internal water molecules even at 100K. Polarized IR measurements on single crystals, measurements on mutant GFP, and solvent exchange measurements allow us to identify IR difference signals due to Gln69, Cys70, and weakly hydrogen-bonded internal water molecules. These spectroscopic changes highlight the role of the chromophore environment in preventing nonradiative decay of the excited state, and thereby enhancing the quantum efficiency.

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