Abstract Submitted for the MAR08 Meeting of The American Physical Society

Crystallographic and solution studies of fluorescence photocycle of GFP GEORGI GEORGIEV, Assumption College, JASPER VAN THOR, Oxford University, J. TIMOTHY SAGE, Northeastern University, OXFORD UNI-VERSITY COLLABORATION, NORTHEASTERN UNIVERSITY COLLABO-RATION, ASSUMPTION COLLEGE COLLABORATION — Phototransformation in GFP is a competing process to the excited state proton transfer (ESPT) ionizing the chromophore, and decarboxylating Glu222. The fluorescence photocycle itself is an ESPT from chromophore to Glu222, ionizing chromophore and neutralizing Glu222. Cryotrapped reaction intermediates of the phototransformed GFP 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. 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.

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Date submitted: 27 Nov 2007 Electronic form version 1.4