Supporting Information

Expression and Purification of GFP66ONBY:

To produce ONBY photocaged GFP, we started with a pET GFP vector containing the stable GFP variant '10+1'[17] and a C-terminal 6-His Tag. Codon 66 was converted to TAG through site directed mutagenesis to produce pET GFP66TAG. *E. coli* BL21(DE3) cells were cotransformed with pET GFP66TAG and pSUPAR ONBY A10.[18] Cells were grown in Terrific Broth and incubated at 37° with shaking at 280 RPM until OD_{600}= 0.7. At this point, ONBY dissolved in 2 eqs NaOH was added to a final concentration of 0.2 mM, and the culture was moved to a 30° shaker. After 1 hour, protein production was induced by the addition of 1 mM IPTG. After 20 hours, the culture was harvested by centrifugation at 5000 x G for 10 minutes. After lysis with lysozyme followed by freeze thawing, the cell lysate was clarified with centrifugation at 20000 x G for 20 minutes and GFP was purified using Ni-NTA affinity chromatography. For WT GFP, protein yields were 62 mg/L and GFP66TAG was produced at 38 mg/L. To verify the identity of each protein, both were subjected to liquid chromatography coupled electrospray ionization mass spectrometry. For WT GFP, the expected mass was 26675 and observed mass 26675, while GFP66ONBY had a calculated mass of 26809, with observed mass 26810 (figure S1).

Crystallization of GFP66ONBY:

Ni-NTA purified GFP66ONBY was further purified using a Q-sepharose column, dialyzed into 40 mM Tris pH 8.0, and then loaded onto the column in this buffer. Protein was eluted as the concentration of NaCl was increased from 0 to 1 M over 10 column volumes.The FPLC trace can be seen in figure S2. GFP66ONBY elutes off the column as four separate peaks. We hypothesized that each peak may represent a different oligomeric state of GFP66ONBY, and the protein in each peak was kept separate. All 4 samples were concentrated down to 10 mg/ml and then used to set crystal trays. After two weeks, crystals formed in crystallization buffer containing 0.1 M HEPES pH 7.0, 20 % PEG 6k. Only protein from the second peak (figure S2) was competent to form crystals.

Photochemical Methods:

UV-visible spectra were taken on an Agilent 8453 UV-visible spectrophotometer. Steady-state fluorescence measurements were performed on a FluoroLog 3 fluorometer (HORIBA Jobin Yvon). The fluorescence quantum yield was determined by using 9,10-diphenylanthracene in ethanol as standard and standard correction methods for the different refractive indexes of ethanol and aqueous buffer. Fluorescence lifetimes were measured by time correlated single photon counting using an OB920 spectrometer (Edinburgh Analytical Instruments) in conjunction with a pulsed H_2 lamp as excitation source.

Laser flash photolysis experiments employed the pulses from a dye laser (Fl 3002, Lambda Physik; 390 nm; ca. 10 mJ/pulse; 15 ns pulse duration Excimer laser (Lextra 50, Lambda Physik, ca. 50 mJ/ pulse; 20 ns pulse duration) and a computer-controlled system which has been described elsewhere. Ref.: Yagci, Y.; Jockusch, S. Turro, N. J. *Macromolecules* **2007**, *40*, 4481-4485.

The photoactivation quantum yield was determined by continuous irradiation of a 1.5 mL solution of caged GFP66ONBY in Tris buffer, pH = 8, in a 1 x 1 cm quartz cell at 350 nm (15 nm bandwidth, 30 mW/cm^2) employing a Xe lamp (LX300 UV) in conjunction with a monochromator (Kratos, Schoeffel Instruments). The growth of the absorption of the uncaged GFP at 483 nm was use to determine the quantum yield. The absorbed light dose was determined by actinometry using Aberchrome 540 (Aberchromics Ltd., U.K.). Ref.: Wintgens, V.; Johnston, L. J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 511.

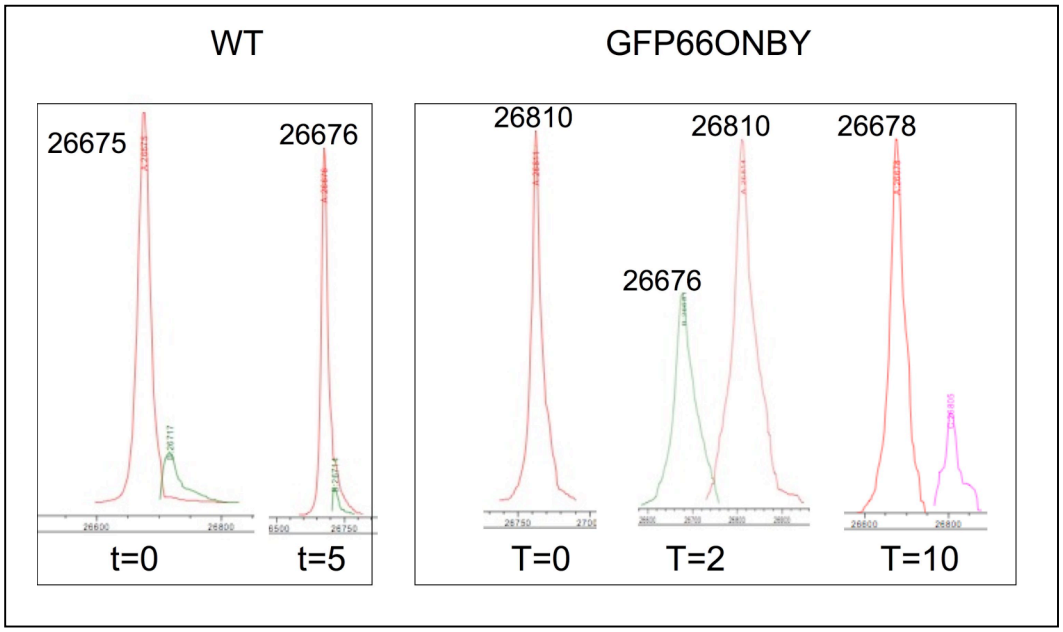

Figure S1. Masses of WT GFP and GFP66ONBY undergoing photolysis.
Samples were purified using Ni-NTA affinity chromatography and then desalted using a Zeba column. Masses were analyzed using LC-MS with electrospray ionization with a mass analyzer operating in positive ionization mode. Raw spectra were deconvoluted to produce the masses shown.

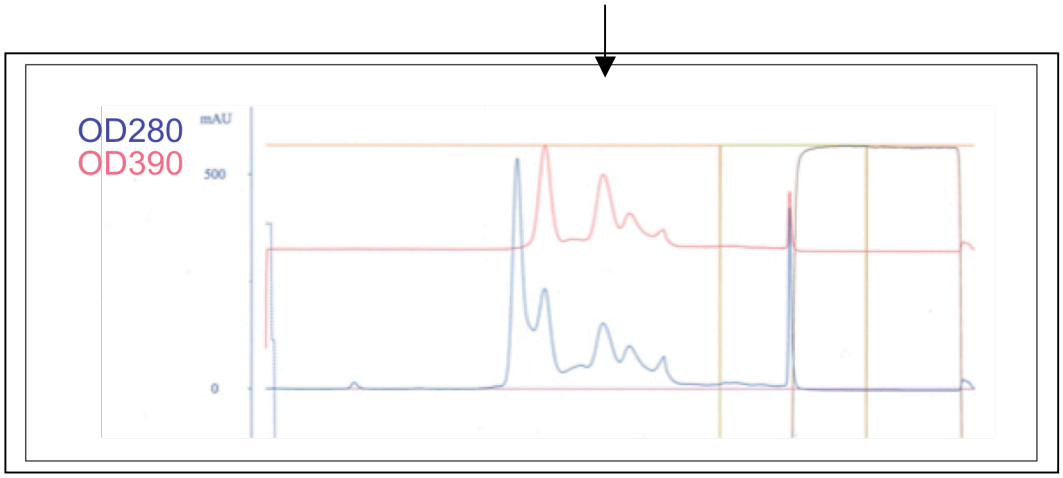

Figure S2. Q-sepharose purification of GFP66ONBY.
10 mgs of GFP66ONBY was loaded onto the column in 2 ml of into 40 mM Tris, pH 8.0, and then eluted with a gradient from 0-1 M NaCl over 10 column volumes. The red trace shows absorbance at 390 nm and the blue trace at 280 nm. Only protein from peak 2 formed protein crystals.

Table S1. Data Collection and Refinement Statistics for GFP66ONBY

Protein	GFP66ONBY
Space Group	$P2_1$
a Å	48.04
b Å	70.86
c Å	60.79
Wavelength Å	1.2815
Resolution Å	2.1
R_{merge} (Highest Shell) %	0.07 (0.20)
Unique Refs (observed)	39505 (119501)
Completeness (Highest Shell) %	100 (99.6)
Redundancy (Highest Shell)	3.2 (3.1)
R_{factor} (R_{free} *) %	22.7 (29.3)
No. protein atoms	3548
No. water atoms	68
rmsd bonds Å	0.019
rmsd angles º	1.9
Mean B factor $Å^2$	34

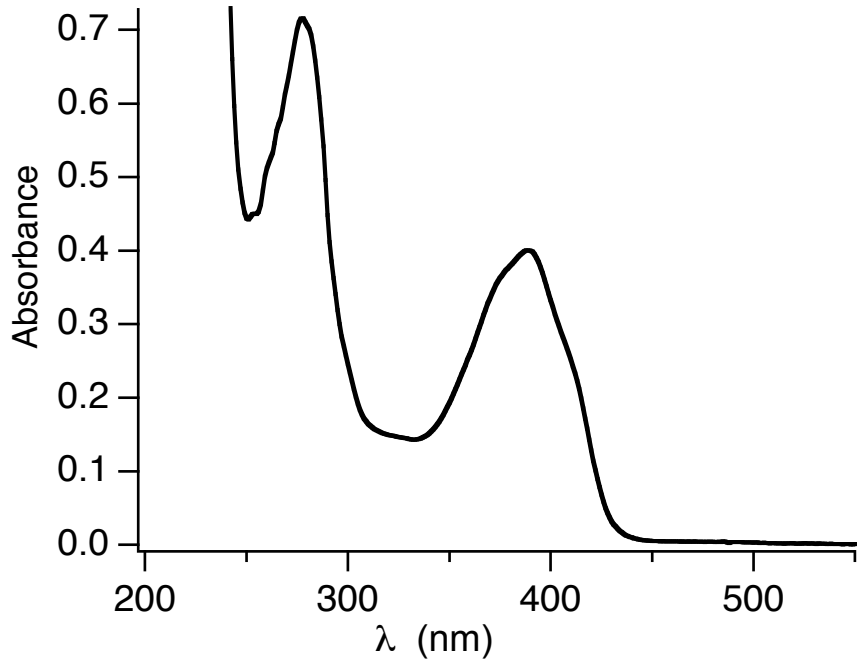

Figure S3: Absorption spectrum of caged GFP66ONBY in Tris buffer, pH = 8.

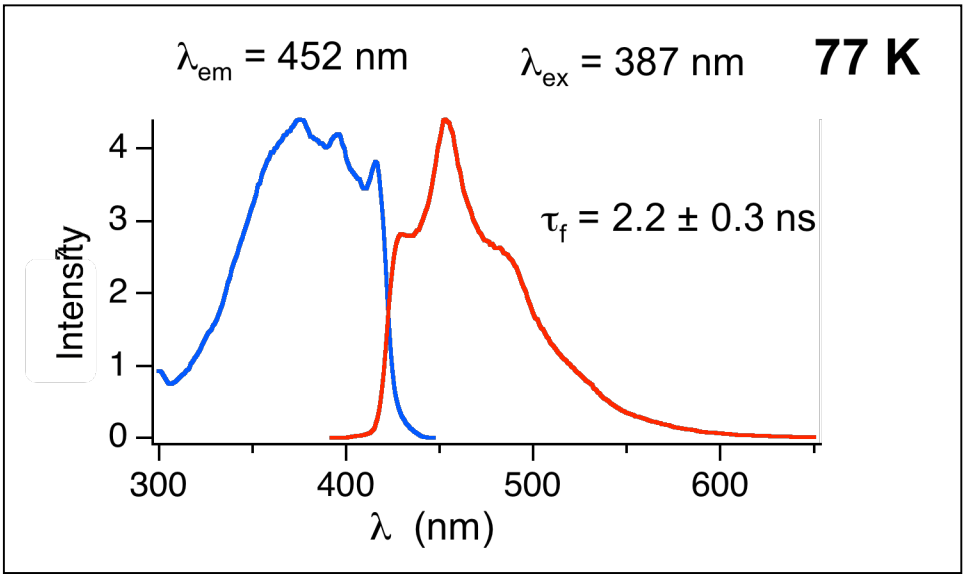

Figure S4: Fluorescence excitation (blue) and emission (red) spectra of caged GFP66ONBY in frozen matrix at 77 K (top). For caged GFP66ONBY in frozen matrix at 77 K, strong fluorescence was observed with a long lifetime (2.2 ns). This increased

fluorescence when frozen indicates that the quenching mechanism requires some activation energy and/or some steric flexibility.

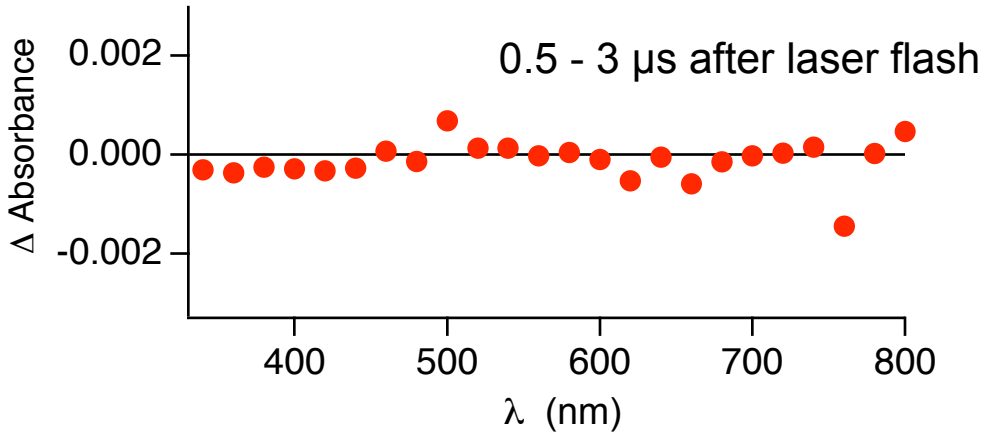

Figure S5: Transient absorption spectrum recorded 0.5 - 3 µs after the laser flash (390 nm, 15 ns pulse length, ~ 10 mJ/pulse) of caged GFP66ONBY in Tris buffer, pH = 8, at room temperature. Because no transient absorption was observable on the µs time scale, the intramolecular electron transfer quenching probably reverses fast to the ground state. If radical ions were formed, which are stable in the µs timescale, transient absorption would be expected.