

## Modulating the pH Dependent Photophysical Properties of Green Fluorescent Protein

David P. Broughton<sup>†</sup>, Chloe G. Holod<sup>†</sup>, Angelica Camilo-Contreras, Darcy R. Harris, Scott H. Brewer\*, and Christine M. Phillips-Piro\*

Department of Chemistry, Franklin & Marshall College, P.O. Box 3003, Lancaster, PA 17604-3003

<sup>†</sup> Indicates equal contributions.

\* Indicates corresponding author.

Corresponding authors emails: sbrewer@fandm.edu, cpiro@fandm.edu

### Supporting Information

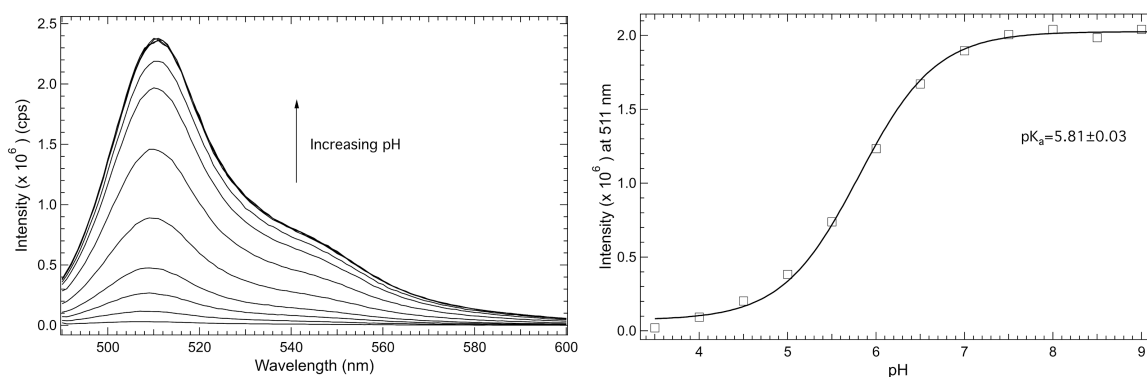


Figure S1. Room temperature fluorescence spectra of wt-sfGFP (A) as a function of pH in the 490 - 630 nm region with an excitation wavelength of 489 nm. wt-sfGFP ( $10 \mu\text{M}$ ) was dissolved in 50 mM potassium phosphate buffers with pH values ranging from 3.50 - 10.00. The pH dependence of the fluorescence intensity of wt-sfGFP measured at 511 nm (open squares, B) was fit (solid curve) to Eqn. (1) to yield a  $pK_a$  of  $5.18 \pm 0.03$  of the phenolic hydrogen of tyrosine at site 66 in the protein.

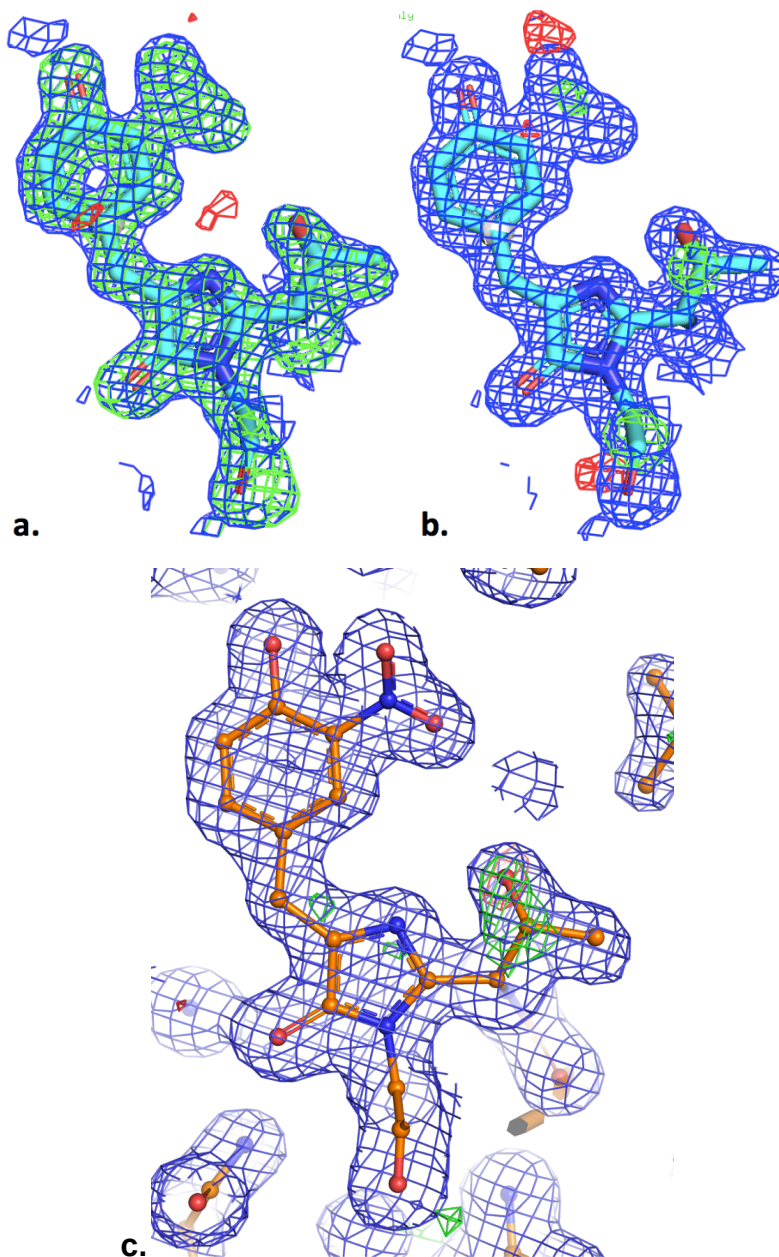


Figure S2. Refinement of mNO<sub>2</sub>Y incorporated chromophore. **a.)** Electron density for the chromophore directly following initial refinement after molecular replacement, but before the atoms in the chromophore were added to the model (atoms are included here for context).  $2F_o-F_c$  map ( $1.0\sigma$ ) in blue,  $+3.0\sigma F_o-F_c$  density in green, and  $-3.0\sigma F_o-F_c$  density in red. **b.)** Electron density (same maps as in **a**) of the Y66mNO<sub>2</sub>Y-sfGFP structure after the wt-sfGFP chromophore atoms were added to the model. **c)** Final refined structure of mNO<sub>2</sub>Y chromophore in Y66mNO<sub>2</sub>Y-sfGFP structure (PDB ID 9c74).

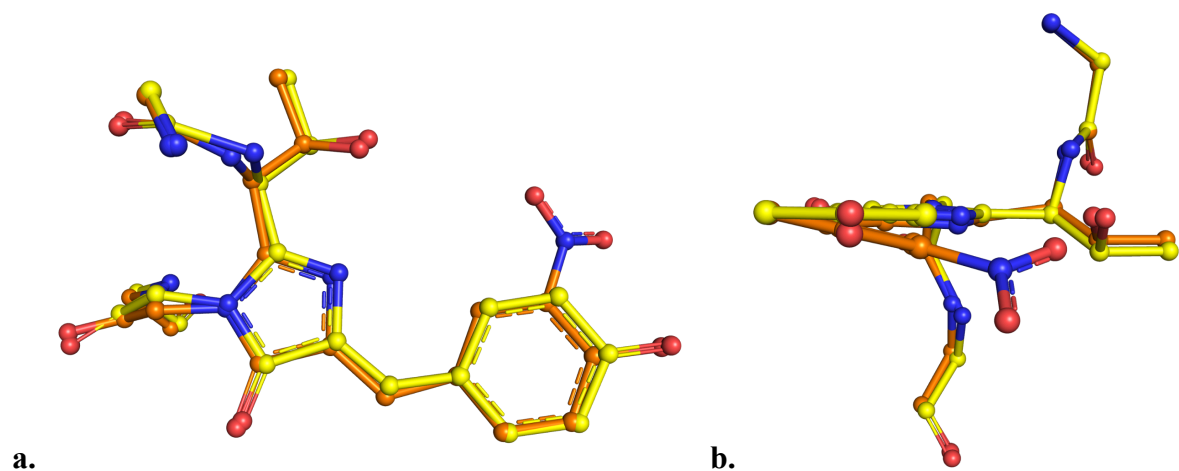


Figure S3. Alignment of the cyclized chromophores of Y66mNO<sub>2</sub>Y-sfGFP (carbons in orange) and wt-sfGFP (2B3P, carbons in yellow). a. Top view of chromophore, b. Side view of chromophore with a view down the phenol O-C bond, illustrating the 14° rotation of the mNO<sub>2</sub>Y ring out of plane with the 5-membered ring of the chromophore.

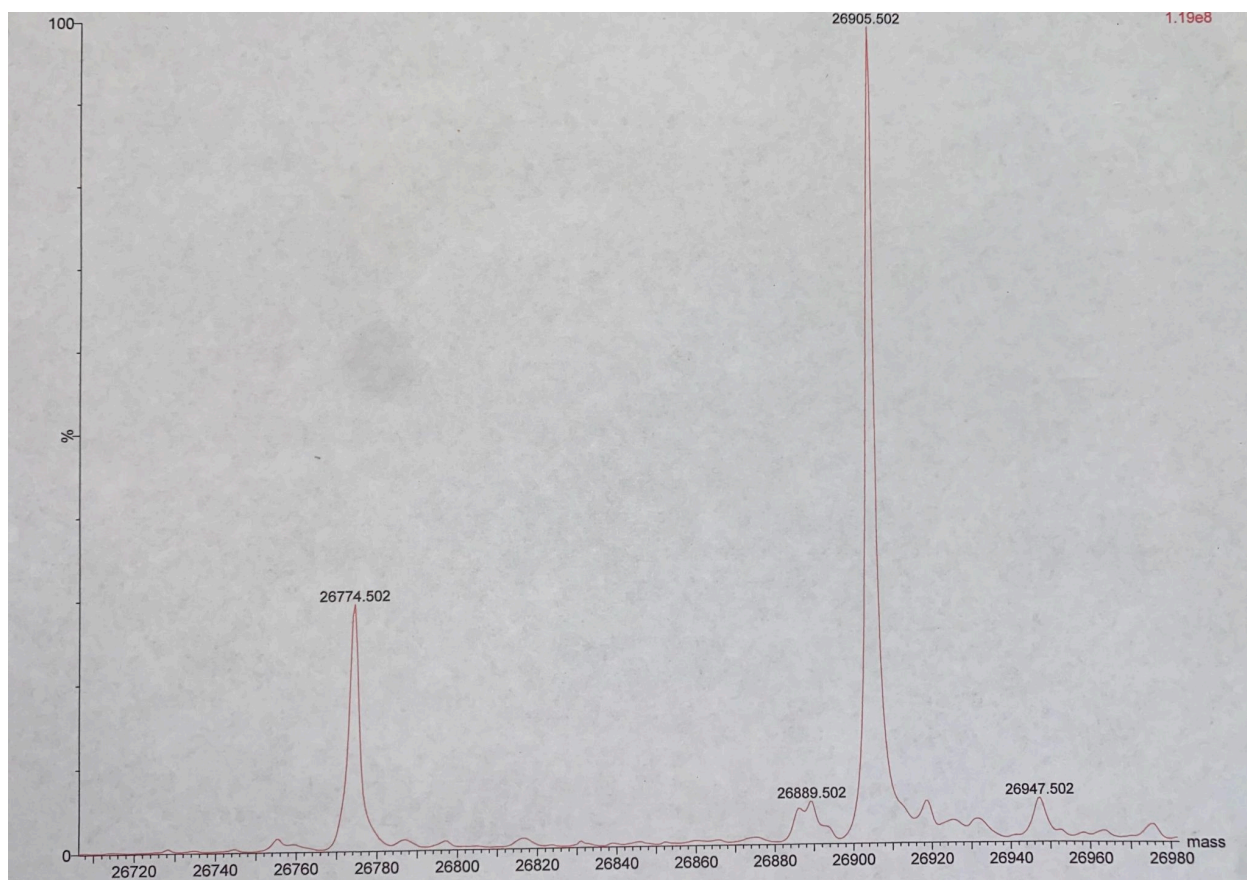


Figure S4. ESI-Q-TOF MS of Y66mNO<sub>2</sub>Y-sfGFP illustrates the successful incorporation of mNO<sub>2</sub>Y at site 66 in the protein.