STRUCTURAL
BIOLOGY

Volume 77 (2021)

Supporting information for article:

Structural and spectrophotometric investigation of two unnatural amino-acid altered chromophores in the superfolder green fluorescent protein

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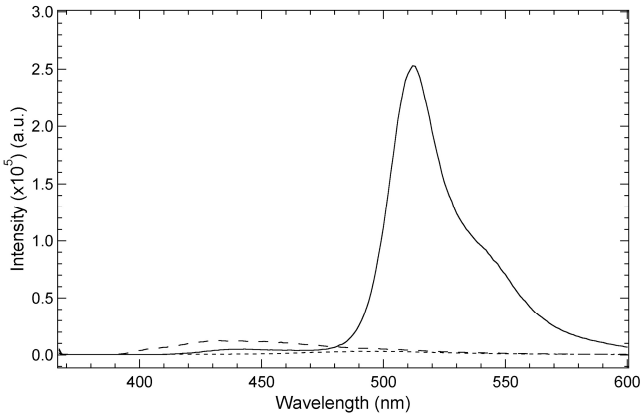

Figure S1 Emission spectra for wt sfGFP (solid curve), Tyr66pNO_2Phe-sfGFP (short-dashed curve), and Tyr66pCNPhe-sfGFP (long-dashed curve) recorded with an excitation wavelength of 365 nm. The proteins were dissolved in a 20 mM HEPES buffer (pH 7.5) at a concentration of 4 μM.

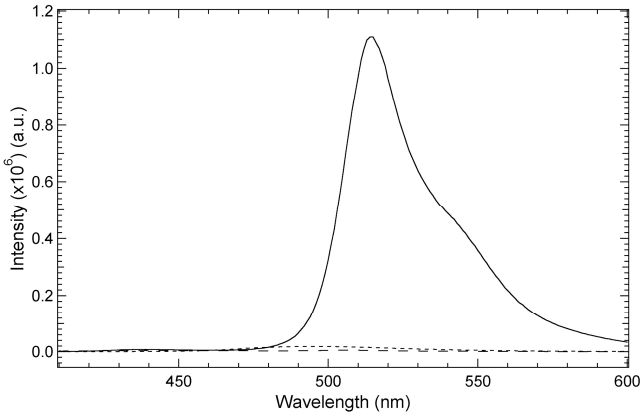

Figure S2 Emission spectra for wt sfGFP (solid curve), Tyr66pNO_2Phe-sfGFP (short-dashed curve), and Tyr66pCNPhe-sfGFP (long-dashed curve) recorded with an excitation wavelength of 406 nm. The proteins were dissolved in a 20 mM HEPES buffer (pH 7.5) at a concentration of 4 μM.

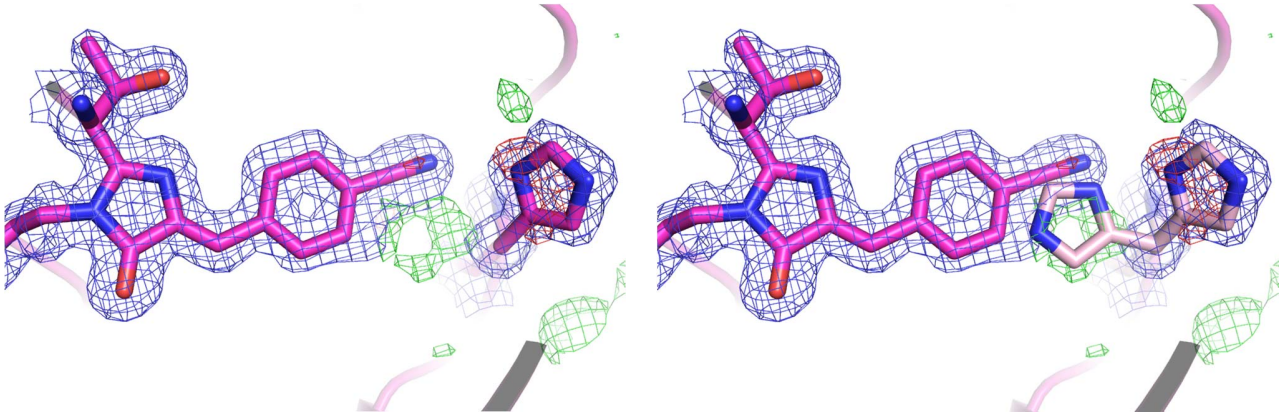

Figure S3 Structure of the chromophore and nearby His148 of the Tyr66pCNPhe-sfGFP structure with F_o-F_c difference density shown at +3.0σ (green) and –3.0σ (red) and 2F_o-F_c at 1.0σ in blue. The structure on the left is deposited in the PDB. Illustration on the right with two conformations of His148 illustrates possible alternate conformation of His148 in difference density, but which is not possible given proximity to the Tyr66pCNPhe chromophore.

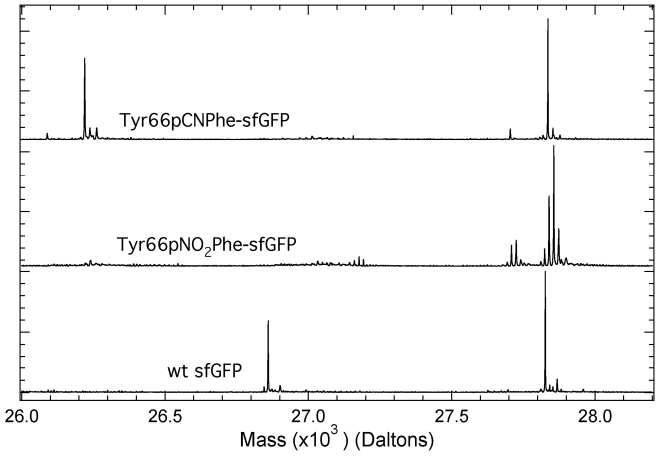

Figure S4 Deconvoluted mass spectra of wt sfGFP, Tyr66pNO_2Phe-sfGFP, and Tyr66pCNPhe-sfGFP pre-trypsin digest. The primary peak at 27827 Da for wt sfGFP corresponds to the full length protein while the minor peak at 26860 Da corresponds to the protein with the His_6 tag cleaved after Lys238. The primary peak at 27857 Da for Tyr66pNO_2Phe-sfGFP corresponds to full length protein. The primary peak at 27836 Da for Tyr66pCNPhe-sfGFP corresponds to full length protein while the minor peak at 26220 Da corresponds to the protein cleaved after residue Met234.