

Supplemental Figure S1

The 600 MHz jump-return ^1H -NMR spectrum of S65T/H148D GFP (pH 5.6 28 °C) does not reveal any unusually downfield shifted resonances diagnostic of a solvent exchange-protected hydrogen in a LBHB between the oxygens of Asp148 and the chromophore. The resolved signals between 10 and 13 ppm all arise from nitrogen-bonded protons in imidazole or amide moieties, as evidenced by ~ 95 Hz scalar couplings in a uniformly ^{15}N -labeled sample of the protein (data not shown). No additional downfield signals were detected upon reducing the temperature range of to 5 °C. The excitation maximum for the jump-return pulse sequence was ~ 15 ppm.

