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Spectroscopic Monitoring of Proton Transfer in Green Fluorescent Protein J. TIMOTHY SAGE, MANNIS O'BRIEN, BRIDGET SALNA, BENABBAS ABDELKRIM, PAUL M. CHAMPION, Northeastern Univ, JASPER VAN THOR, Imperial College — Vibrational spectroscopy is an ideal probe for proton transfer in biological molecules because of its sensitivity to the motion of protons, which are difficult to track with more direct structural methods such as X-ray crystallography. Previous time-resolved infrared measurements provided direct experimental evidence for Glu 222 as the excited state proton acceptor following excitation of green fluorescent protein (GFP). Here, we use infrared cryospectroscopy to characterize a low quantum yield photochemical channel that leads to decarboxylation of Glu 222 coupled with proton transfer to complete the methyl group on the resulting α-aminobutyric acid residue. The *irreversible* nature of this process allows us to obtain infrared data at much higher sensitivity and over an extended frequency range. Difference spectra recorded over the full 1000-4000 cm^{-1} range at 100 K probe perturbations of internal water molecules and nearby amino acids as well as the chromophore. We identify vibrational frequencies that probe hydrogen bonding along the "proton wire" that connects the chromophore to Glu 222.

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