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Temperature dependence of proton transfer kinetics in the green fluorescent protein[1] BRIDGET SALNA, ABDELKRIM BENABBAS, PAUL CHAMPION, J. TIMOTHY SAGE, Northeastern University, JASPER VAN THOR, ANN FITZPATRICK, Imperial College London — In green fluorescent protein (GFP), near UV photoexcitation leads to proton transfer from the chromophore phenolic oxygen along a proton "wire" consisting of an internal water molecule, Ser205 and Glu222. Using transient absorption kinetics, the complete cycle, including the picosecond excited-state proton transfer, the nanosecond radiative emission, and the slower ground state proton back-transfer reactions have been studied holistically as a function of temperature. This experiment was performed for both the hydrogenated and deuterated forms of GFP. We have extracted the Arrhenius prefactors and activation energy barriers for both the forward and back proton transfer kinetics. A large kinetic isotope effect for the ground state proton back-transfer has been observed at high temperatures suggesting that tunneling plays an important role. At lower temperatures the data suggest a cross-over to a different pathway for the back-transfer reaction. To investigate this hypothesis we studied the E222D mutant of GFP, which substitutes aspartate for glutamate on the proton wire. The H/D kinetics of this mutant explicitly test for the source of proton donors and indicate that proton transfer proceeds along the same pathway in the native protein at room temperature.

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