Sensitive, Site-Specific, and Stable Vibrational Probe of Local Protein Environments: 4-Azidomethyl-L-Phenylalanine

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Supporting Information

Sequence of wt-sfGFP.

The sequence for wt-sfGFP is provided in Figure S1.

MVSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKL
PVPWPTLVTTLTYGVQCFSR*Y*PDHMKRHDFFKSAMPEGYVQERTISFKDDGTYK
TRAEVKFEGDTLVNRIELKGIDFKE*D*GNILGHKLEYNFNSHNVYITADKQKNGIK
ANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRD
HMVLLEFVTAAGITHGMDELYKGSHHHHHH

Figure S1. Amino acid sequence of wt-sfGFP with residues 75 and 134 in italics.

ESI-Q-TOF Mass Analysis.

Incorporation of pN_3CH_2Phe or pN_3Phe into sfGFP in response to the amber codon was verified by electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) mass analysis (see Table S1). This analysis was performed on the same purified protein samples used for the FTIR measurements. ESI-Q-TOF mass analysis was performed at the Mass Spectrometry Facility at the University of Illinois Urbana-Champaign under the direction of Dr. Furong Sun. Prior to the analysis, the protein samples were desalted into a 20 mM ammonium acetate buffer (pH 7) using PD10 gel filtration columns, lyophilized, and resuspended in 1:1 H_2O:CH_3CN with 0.2% formic acid.

The mass difference between wt-sfGFP and sfGFP-75-pN_3CH_2Phe is indicative of the replacement of Y75 with pN_3CH_2Phe, and the mass difference between wt-sfGFP and sfGFP-134-pN_3CH_2Phe is indicative of the replacement of D134 with pN_3CH_2Phe, within the error of the measurement. Similarly, the mass difference between wt-sfGFP and sfGFP-75-pN_3Phe is indicative of the replacement of Y75 with pN_3Phe, and the mass difference between wt-sfGFP and sfGFP-134-pN_3Phe is indicative of the replacement of D134 with pN_3Phe, within the error of the measurement.

Table S1. ESI-Q-TOF observed molecular weights of protein constructs.

Protein Construct	Observed Molecular Weight (g/mol)
wt-sfGFP	27829±1
sfGFP-75-pN_3CH_2Phe	27868±1
sfGFP-75-pN_3Phe	27854±1
sfGFP-134-pN_3CH_2Phe	27917±1
sfGFP-134-pN_3Phe	27902±1

Equilibrium UV/Vis Absorbance Measurements.

The equilibrium UV/Vis absorbance spectra were recorded on a PerkinElmer Lambda 25 UV/Vis spectrometer using a 1 cm quartz sample holder. The spectra were recorded at room temperature with 1 nm increments and a scan speed of 240 nm/min. The UV/Vis absorbance spectra for pN_3Phe and pN_3CH_2Phe dissolved in water are shown in Figure S2 illustrating the significant decrease in absorbance at 254 nm for pN_3CH_2Phe compared to pN_3Phe.

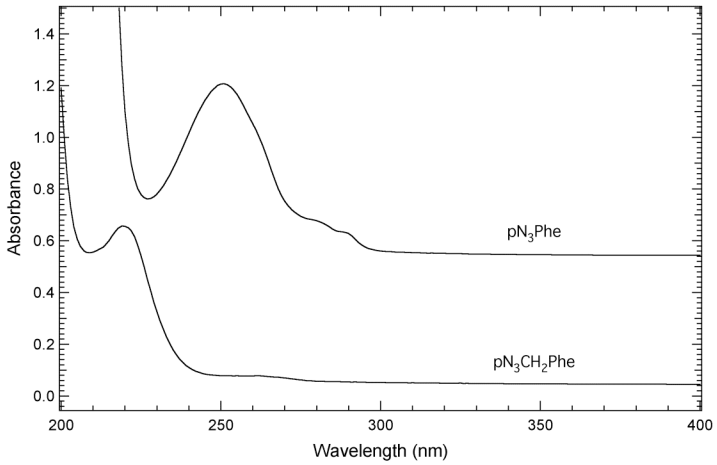

Figure S2. The UV/Vis absorbance spectra of pN_3Phe and pN_3CH_2Phe dissolved in water at a concentration of 50 μM.