Expanding the Utility of 4-Cyano-L-Phenylalanine as a Vibrational Reporter of Protein Environments

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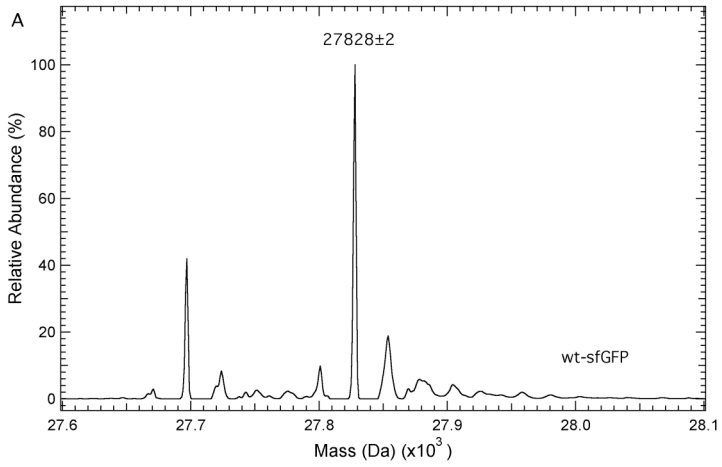
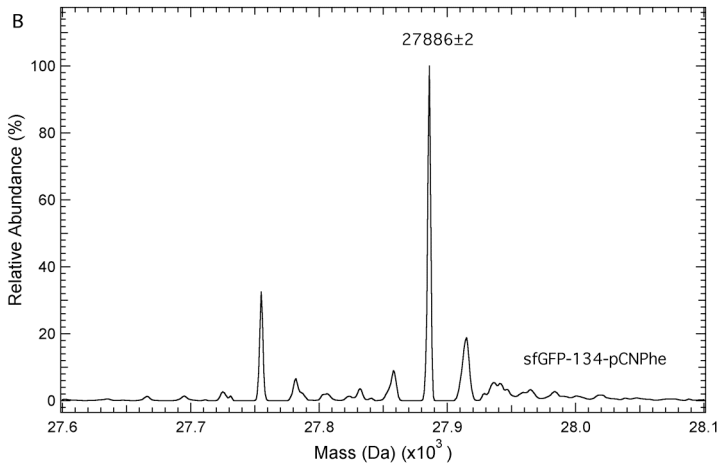
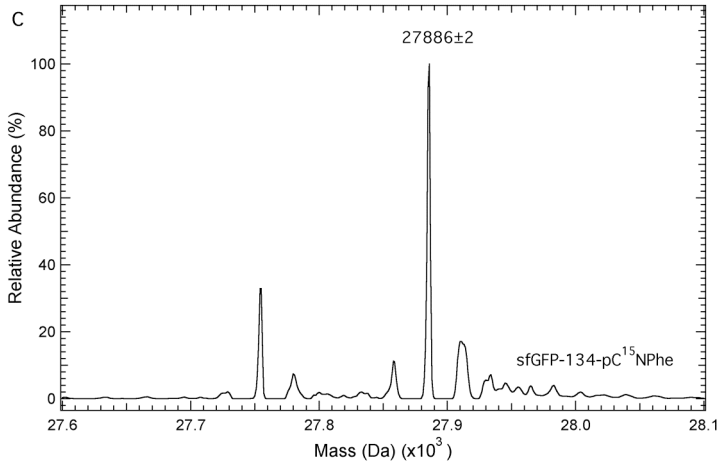
Supporting Information

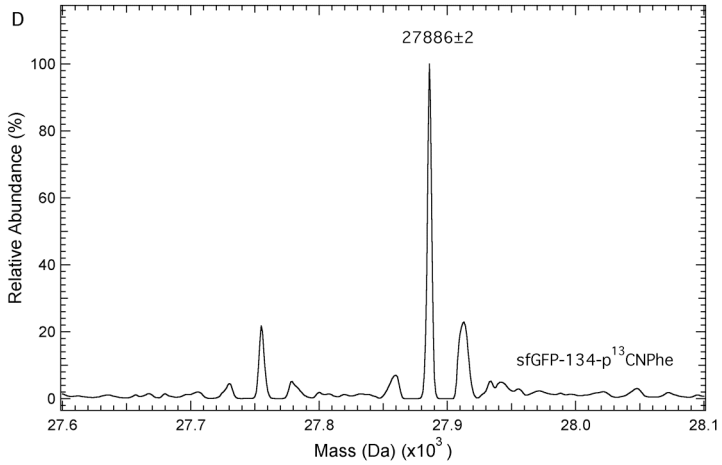
ESI-Q-TOF Mass Analysis.

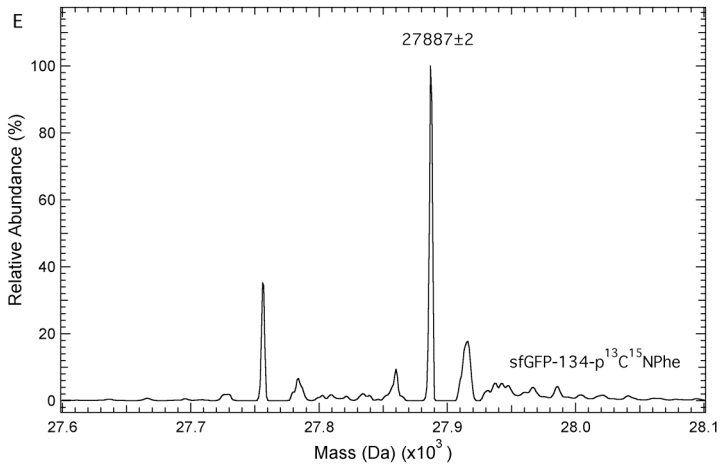
Incorporation of **1**, **1a**, **1b**, or **1c** into sfGFP in response to the amber codon was verified by electrospray ionization quadrupole time-of-flight (ESI-Q-TOF) mass analysis (see Figure S1, Panels A – I). This analysis was performed on the same purified protein samples used for the FTIR measurements. 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.

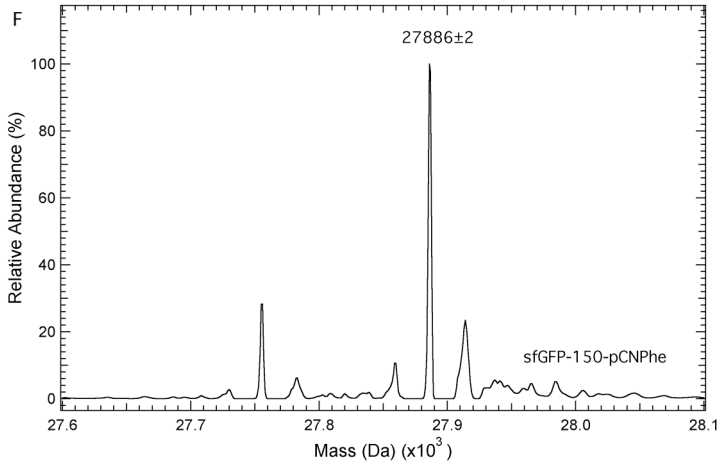


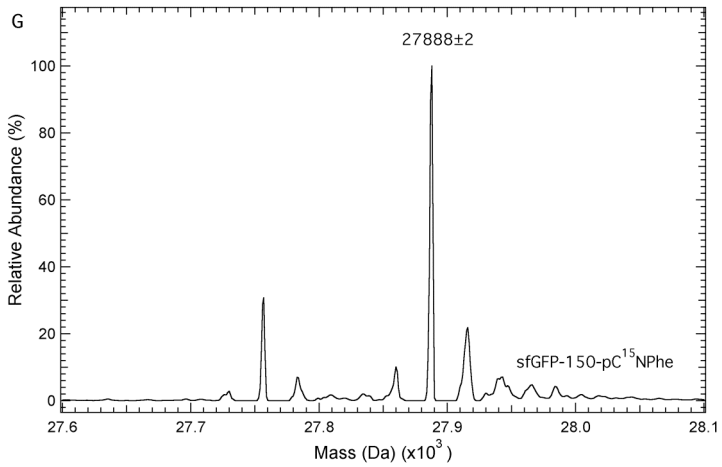

B
27886±2
sfGFP-134-pCNPhe
Relative Abundance (%)
Mass (Da) (x10^3)


C
27886±2
sfGFP-134-pC15NPhe
Relative Abundance (%)
Mass (Da) (x10^3)


D
27886±2
sfGFP-134-p13CNPhe
Relative Abundance (%)
Mass (Da) (x10^3)


E
27887±2
Relative Abundance (%)
sfGFP-134-p^{13}C^{15}NPhe
Mass (Da) (x10^3)


F
27886±2
Relative Abundance (%)
sfGFP-150-pCNPhe
Mass (Da) (x10^3)


G
27888±2
Relative Abundance (%)
sfGFP-150-pC^{15}NPhe
Mass (Da) (x10^3)

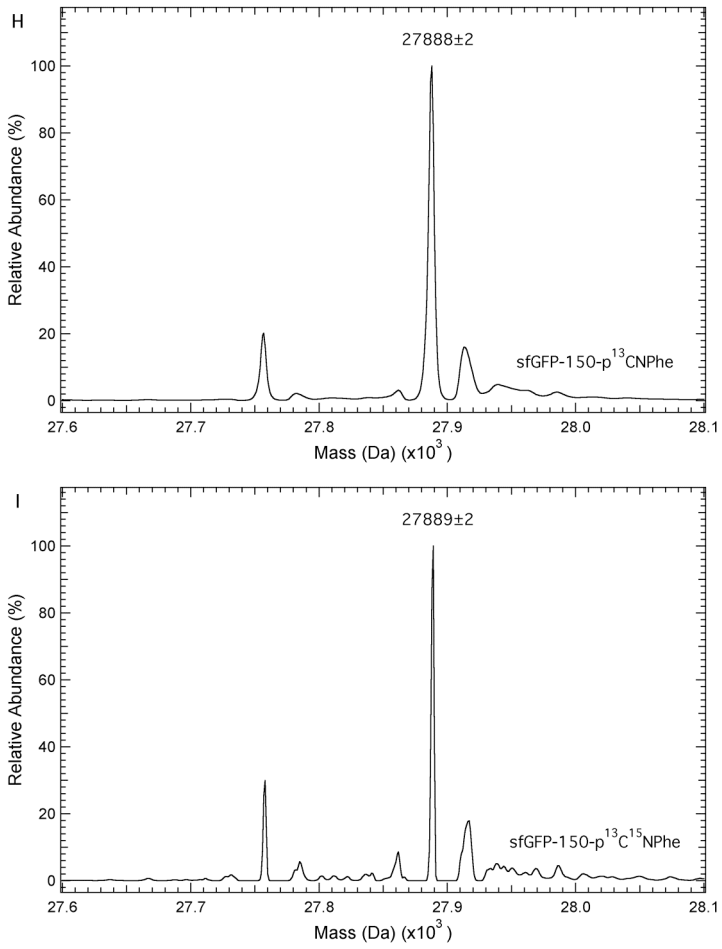


Figure S1. ESI-Q-TOF MS of wt-sfGFP (A), sfGFP-134-pCNPhe (B), sfGFP-134-pC^{15}NPhe (C), sfGFP-134-p^{13}CNPhe (D), sfGFP-134-p$^{13}C^{15}$NPhe (E), sfGFP-150-pCNPhe (F), sfGFP-150-pC^{15}NPhe (G), sfGFP-150-p^{13}CNPhe (H), and sfGFP-150-p$^{13}C^{15}$NPhe (I) showing UAA incorporation (with the exception of wt-sfGFP) into sfGFP at either site 134 or site 150 with high fidelity in response to the amber stop codon (TAG). The mass difference between wt-sfGFP and sfGFP-134-pCNPhe is indicative of the replacement of D134 with **pCNPhe**, and the mass difference between wt-sfGFP and sfGFP-150-pCNPhe is indicative of the replacement of N150 with **pCNPhe**, within the error of the measurement.

SDS-PAGE.

Incorporation of **1** and **1b** into site 150 of sfGFP in response to the amber codon and the incorporation of **1**, **1a**, **1b**, and **1c** into sites 134 and 150 in sfGFP in response to both amber codons was verified by SDS-PAGE (see Figure S2). This analysis was performed on the same purified protein samples used for the FTIR measurements.

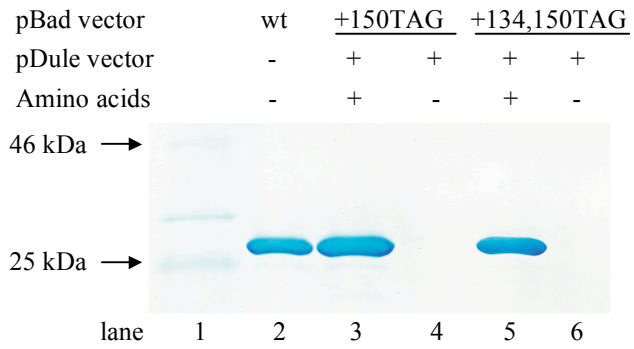


Figure S2. Coomassie blue stained tris-glycine SDS-PAGE illustrating efficient, site-specific incorporation of **1**, **1a**, **1b**, and/or **1c** with high fidelity into sfGFP. The protein constructs were expressed from *pBad-sfGFP* (wt-sfGFP, lane 2); *pBad-sfGFP-150TAG* and *pDule-pCNPhe* (lanes 3 and 4) in the presence (lane 3) or absence (lane 4) of **1** and **1b**; or *pBad-sfGFP-134,150TAG* and *pDule-pCNPhe* (lanes 5 and 6) in the presence (lane 5) or absence (lane 6) of **1**, **1a**, **1b**, and **1c**.

DFT Optimized Structures.

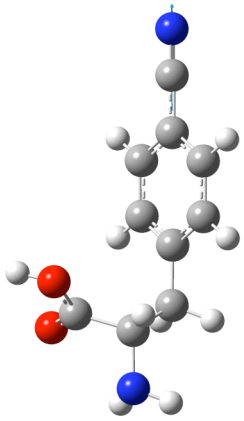


Figure S3. DFT calculated eigenvector projection of the nitrile symmetric stretching mode of 4-cyano-L-phenylalanine.

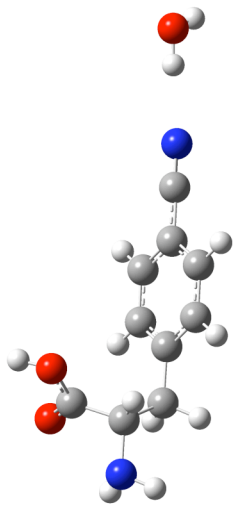


Figure S4. DFT optimized structure of 4-cyano-L-phenylalanine in the presence of a single water molecule in a H-bonding configuration with the nitrile group of **pCNPhe**.