Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

p-Cyanophenylalanine and Selenomethionine Constitute a Useful Fluorophore-Quencher Pair for Short Distance Measurements: Application to Polyproline Peptides

Supporting Information

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Mass Results:

Gly-Phe_{CN}-Gly: expected 304.3, observed 305.1. SeMet-Phe_{CN}: expected 368.3, observed 369.2. Met-Phe_{CN}: expected 320.4, observed 321.3. SeMet-Pro-Phe_{CN}: expected 465.4, observed 466.3. SeMet-$(Pro)_2$-Phe_{CN}: expected 562.5, observed 561.4. SeMet-$(Pro)_3$-Phe_{CN}: expected 659.6, observed 660.7. SeMet-$(Pro)_4$-Phe_{CN}: expected 756.7, observed 755.4.

UV-Vis Spectroscopy

UV-Vis spectra were collected on a Perkin Elmer Lambda 25 UV/Vis spectrometer. To prepare the free SeMet amino acid sample, an appropriate amount of SeMet was dissolved in Millipore water for a concentration of 100 μM.

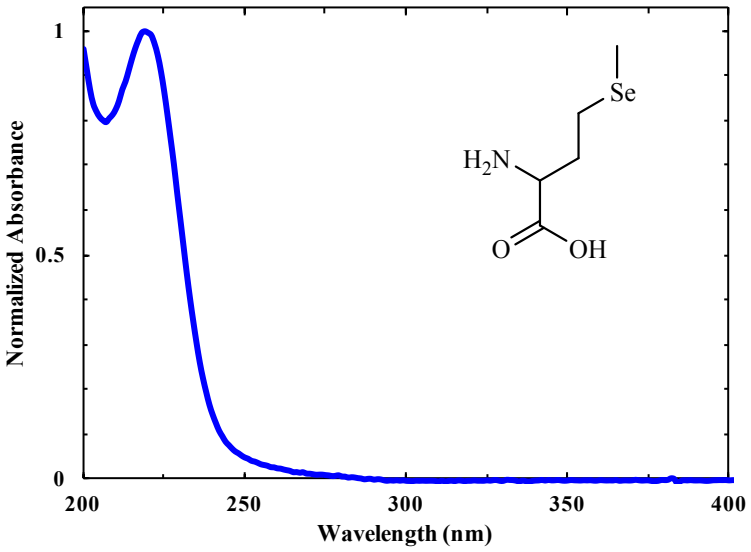

Figure S1: UV-Vis spectrum of free SeMet, structure as shown, in Millipore water. The absorbance was normalized to the peak maximum of 219 nm. There is no absorbance overlap with the fluorescence emission of Phe_{CN}, whose peak emission is centered at 300 nm.

Nanosecond Transient Absorption Spectroscopy

Excitation pulses of 266 nm (~1 mJ/pulse) were generated from the 4th harmonic of a 10 Hz NdYAG laser (~10 ns pulse width) and slightly focused on the sample with a cylindrical UV lens (f = 100 mm). Details of the experimental setup have been reported elsewhere.[1] The samples were prepared by dissolving the desired amino acid in Millipore water. The samples were then degassed with argon for at least 30 minutes. The optical densities at 266 nm of the Phe_{CN} sample (165 µM), the Tyr sample (180 µM), and the Phe_{CN} (165 µM) and SeMet (8 mM) mixture were ~0.2, ~0.2 and ~0.3, respectively.

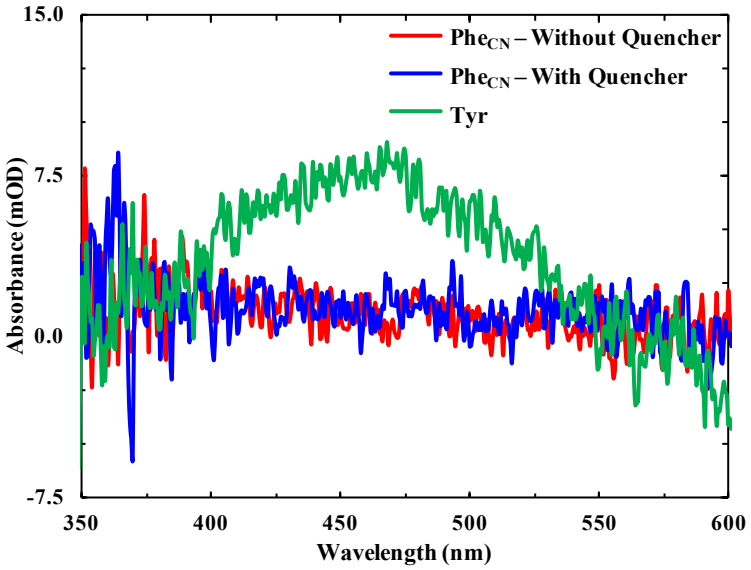

Figure S2: Transient absorption spectra measured at a delay time of 1 µs of the free amino acids Tyr (green), Phe_{CN} (red), and a Phe_{CN}/SeMet mixture (blue). For the Tyr sample, a broad band centered at ~475 nm exists, indicating the formation of the triplet state. For both Phe_{CN} samples, no detectable triplet state formation was observed.

Cyclic Voltammetry

Cyclic voltammetry measurements were collected on a CHI630E workstation (CH Instruments, Inc., Austin, TX). The glassy carbon working electrode, the Pt wire counter electrode, and the calomel reference electrode were contained in a Faraday cage. The free Phe_{CN} amino acid (500 µM) was dissolved in degassed phosphate buffer (pH 7.0) containing 0.2 M KCl. Measurements were conducted under argon to minimize O_2 reactions.

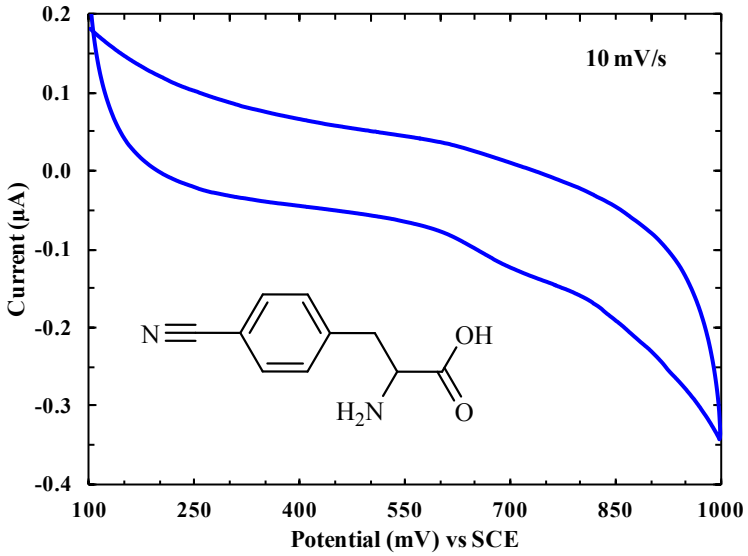

Figure S3: Cyclic Voltammogram of free Phe_{CN}, structure as shown, in 0.2 M KCl-containing phosphate buffer at pH 7.0 and room temperature, with a scan rate of 10 mV/s. The oxidation potential was approximately 680 mV vs. SCE (921 mV vs. NHE). This value is comparable to other aromatic amino acids.[2]

References

1 S. Papp, J. M. Vanderkooi, C. S. Owen, G. R. Holtom, and C. M. Phillips, *Biophys. J.*, 1990, **58**, 177-186.

2 A. Harriman, *J. Phys. Chem.*, 1987, **91**, 6102-6104.