

*Electronic Supplementary Information for:*

## **Comparison of biological chromophores: photophysical properties of cyanophenylalanine derivatives**

Joshua P. Martin,<sup>a</sup> Natalie R. Fetto,<sup>b</sup> and Matthew J. Tucker\*<sup>b</sup>

<sup>a</sup> Present Address: Department of Chemistry, Metropolitan State University of Denver, 80217 USA

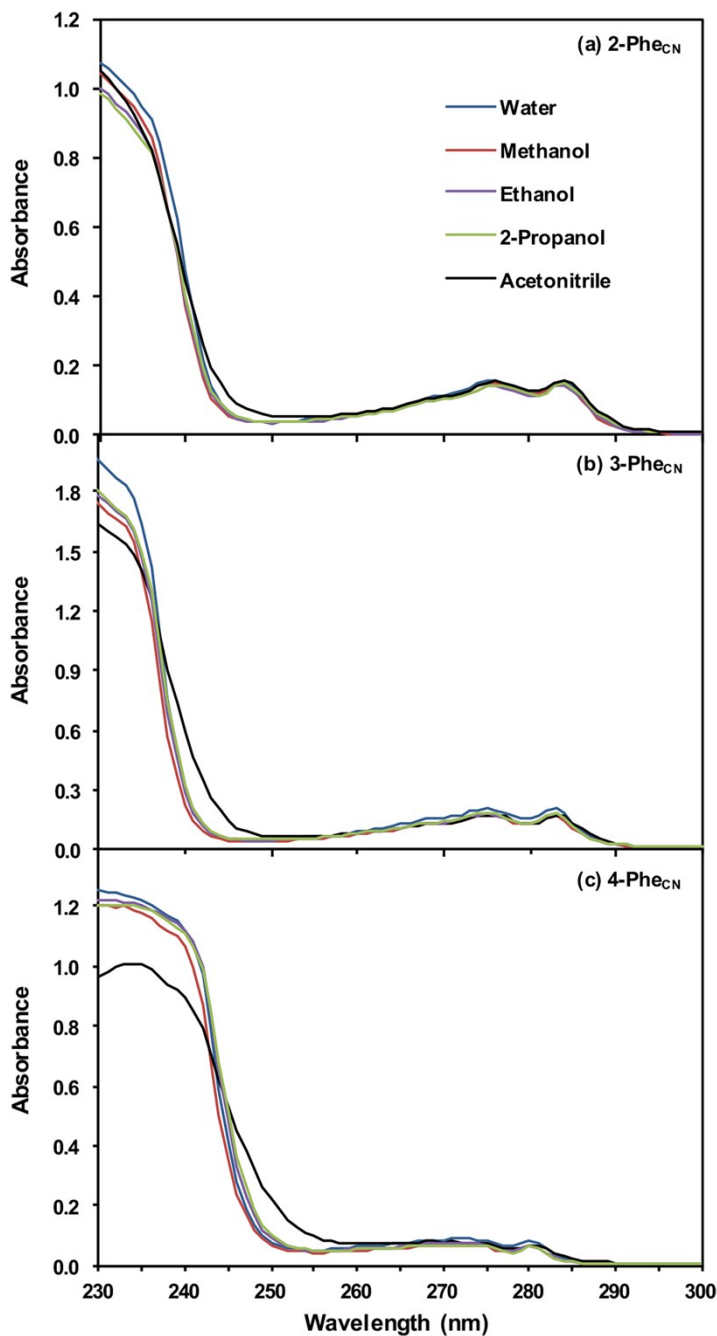
<sup>b</sup> Department of Chemistry, University of Nevada, Reno, 89557 United States of America

\*Corresponding Author: [mtucker@unr.edu](mailto:mtucker@unr.edu)

### **Contents:**

- 1 Absorption spectra of 2-Phe<sub>CN</sub>, 3-Phe<sub>CN</sub>, and 4-Phe<sub>CN</sub> in various solvents
- 2 Efficiency of energy transfer between 4-Phe<sub>CN</sub>, 2-Phe<sub>CN</sub> FRET pair
- 3 Fluorescence spectra of 2-Phe<sub>CN</sub>, 4-Phe<sub>CN</sub>, and F<sub>2CN</sub>-(Pro)<sub>12</sub>-F<sub>4CN</sub>

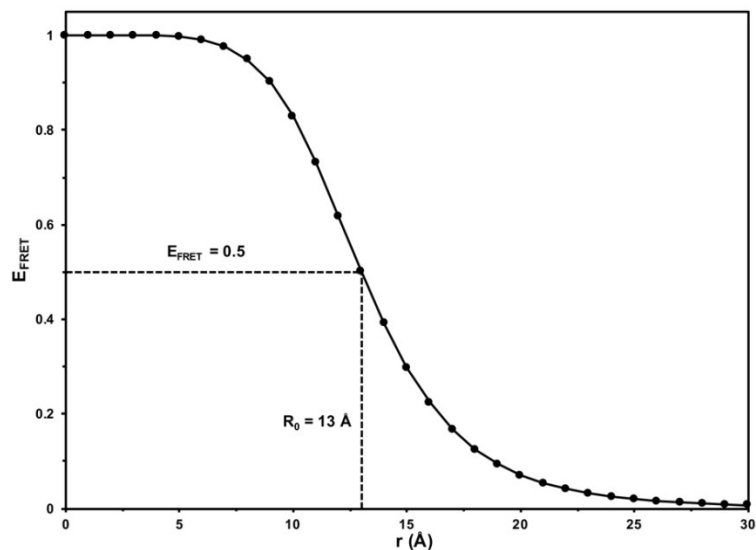
# 1 Absorption spectra of 2-Phe<sub>CN</sub>, 3-Phe<sub>CN</sub>, and 4-Phe<sub>CN</sub> in various solvents



**Figure S1.** Absorption spectra of (a) 2-Phe<sub>CN</sub>, (b) 3-Phe<sub>CN</sub>, and (c) 4-Phe<sub>CN</sub> in various solvents, as indicated.

In Figure S1, the absorbance of 2-Phe<sub>CN</sub> (a), 3-Phe<sub>CN</sub> (b), and 4-Phe<sub>CN</sub> (c) is shown in five different solvents of equal concentration of 0.11 mM, 0.17 mM, and 0.09 mM, respectively. The molar absorptivity does not vary for each solvent and it is found to be equivalent at the excitation wavelength of 280 nm. Low solubility of all three Phe<sub>CN</sub> species in acetonitrile results in a slight deviation from the expected profile from approximately 230 to 260 nm.

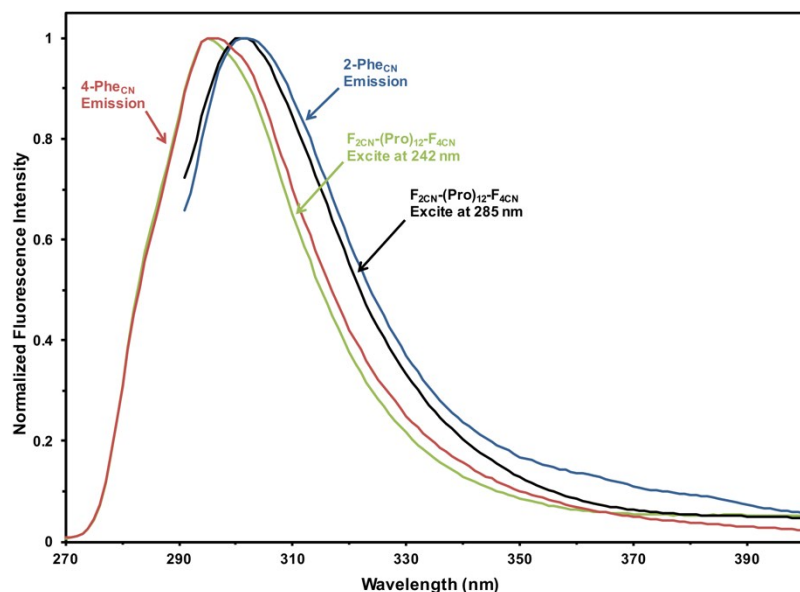
## 2 Efficiency of energy transfer between 4-Phe<sub>CN</sub>, 2-Phe<sub>CN</sub> FRET pair



**Figure S2.** Efficiency of Förster Resonance Energy Transfer,  $E_{\text{FRET}}$ , as a function of distance between 4-Phe<sub>CN</sub> (donor) and 2-Phe<sub>CN</sub> (acceptor),  $r$ .

The Förster distance,  $R_0$ , is determined when  $E_{\text{FRET}}$  reaches 0.5, or 50%, energy transfer. The efficiency of energy transfer decreases as the distance between the FRET pair increases. At distances shorter than  $R_0$ , the efficiency of energy transfer continues to increase and eventually approaches one hundred percent. The distance between the 2-Phe<sub>CN</sub> and 4-Phe<sub>CN</sub> fluorophores in the F<sub>2CN</sub>GGF<sub>4CN</sub> peptide, discussed in this work, was determined from Gaussian09 calculations to be  $\sim 7 \text{ Å}$ , giving an efficiency of roughly 0.97. Thus, it is expected that upon excitation of the 4-Phe<sub>CN</sub> donor chromophore the energy will be almost fully transferred to the 2-Phe<sub>CN</sub> acceptor. At distances larger than around  $20 \text{ Å}$ , the efficiency of energy transfer approaches zero, allowing for selective excitation.

### 3 Fluorescence spectra of 2-Phe<sub>CN</sub>, 4-Phe<sub>CN</sub>, and F<sub>2CN</sub>-(Pro)<sub>12</sub>-F<sub>4CN</sub>



**Figure S3.** Normalized emission spectra of 2-Phe<sub>CN</sub> excited at 285 nm, 4-Phe<sub>CN</sub> excited at 242 nm, and F<sub>2CN</sub>-(Pro)<sub>12</sub>-F<sub>4CN</sub> excited at both 242 nm and 285 nm, as indicated.

As seen in Figure S3, the emission spectra of a short double labelled peptide, F<sub>2CN</sub>-(Pro)<sub>12</sub>-F<sub>4CN</sub>, demonstrates selective excitation. Following excitation at 242 nm, the fluorescence profile is similar to that of 4-Phe<sub>CN</sub> while the spectra following 285 nm excitation is similar to that of the 2-Phe<sub>CN</sub> fluorescence profile. Molecular Dynamics simulations were computed using NAMD (Nanoscale Molecular Dynamics) with a CHARMM force field solvated in a TIP3P water model. From these simulations, the average value of the distance between the first carbon of each phenyl ring (*i.e.* the phenyl carbon closest to the amino group) was determined to be  $\sim 30$  Å from trajectories over a 150 ns simulation. This distance is greater than the  $R_0$  (13 Å) calculated for the pair. It is expected that the efficiency of FRET at this distance is less than one percent, suggesting that energy transfer for this system is negligible. Thus, each chromophore can be selectively excited as a function of wavelength.