Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
This journal is © the Owner Societies 2019

Electronic Supplementary Information

PET and FRET Utility of an Amino Acid Pair: Tryptophan and 4-Cyanotryptophan

Ismail A. Ahmed[1], Jeffrey M. Rodgers[2], Christina Eng[3], Thomas Troxler[1,2], and Feng Gai[2, *]

[1]Department of Biochemistry and Biophysics, [2]Department of Chemistry, and [3]Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

*Correspondence: gai@sas.upenn.edu; +1-215-573-6256 (F.G.)

Experimental Methods

Förster Distance Calculation

The Förster distance (R_0) of the Trp (donor) and 4CN-Trp[1] (acceptor) FRET pair was calculated using the following equation:[2]

$$R_0^6 = \left(\frac{9000(ln10)\kappa^2 Q_D}{128\pi^5 N\eta^4}\right)J(\lambda), \tag{S1}$$

where κ^2 is the orientation factor, assumed to be 2/3, Q_D is the fluorescence quantum yield of the donor in the absence of the acceptor which is 0.14 for Trp in water,[3,4] N is the Avogadro's number, η is the refractive index of the medium (1.33 for water), and $J(\lambda)$ is the overlap integral, determined by the following equation:[2]

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda, \tag{S2}$$

where $F_D(\lambda)$ is the area-normalized emission spectrum of the donor and $\varepsilon_A(\lambda)$ is the wavelength-dependent molar absorption coefficient of the acceptor.

Stern-Volmer Titration and Numerical Fitting

Static fluorescence quenching experiments (λ_{ex} = 330 nm) was carried out using free 4CNI-3AA and NATA. To determine the underlying quenching rate[5], we employed a diffusion model as described in detail elsewhere[6,7] to numerically fit the experimental Stern-Volmer curve (Figure 4 in the main text). Briefly, the decaying fluorescence signal $I(t)$ is described by:

$$I(t) = I_0 exp\left(-\frac{t}{\tau_0} - C_Q^0\int_0^t k(t')dt'\right), \tag{S3}$$

where τ_0 is the fluorescence lifetime of the fluorophore, C_Q^0 is the quencher bulk concentration, and $k(t)$ is the time-dependent quenching rate which is calculated using:

$$k(t) = \frac{4\pi}{C_Q^0}\int_{a_0}^\infty r^2 k_Q(r)C_Q(r,t)dr, \tag{S4}$$

In the above equation, $C_Q(r,t)$ is the concentration of quencher at distance r from the fluorophore at time t, and $k_Q(r)$ is the distance-dependent quenching rate, defined as:[2]

$$k_Q(r) = k_0 e^{-\beta(r-a_0)}, \tag{S5}$$

where k_0 is the quenching rate when the pair are in van der Waals contact (when $r = a_0$), and β is a constant. For this study, the value of a_0 was set to 7.0 Å, which corresponds to the sum of the

van der Waals radii of two tryptophan sidechains. By defining a normalized concentration of the quencher as:

$$y(r,t) = C_Q(r,t)/C_Q^0, \tag{S6}$$

we can model the time- and distance-dependent quenching using the following equation:

$$\frac{\partial}{\partial t}y(r,t) = -D\nabla^2 y(r,t) - k_Q(r)y(r,t), \tag{S7}$$

We numerically solved for $C_Q(r,t)$ using the following initial conditions and boundary conditions:

$$y(r,t=0) = 1, \tag{S8}$$

$$\left(\frac{\partial}{\partial t}y(r,t)\right)_{r=a_0} = 0, \tag{S9}$$

$$\lim_{r\to\infty} y(r,t) = 1, \tag{S10}$$

and subsequently obtained $k(t)$ and $I(t)$ for each bulk quencher concentration C_Q^0. For the Stern-Volmer data presented in Figure 4, the best fit yielded the following parameters: $k_0 = 6.8\ ns^{-1}$, $\beta = 1.3\ \text{Å}^{-1}$, and $a_0 = 7.0\ \text{Å}$.

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations on the 4CN-Trp-Trp peptide were carried out to characterize its conformational distribution in aqueous solution. First, the force field parameters for the 4-cyanoindole moiety were determined using the Force Field Toolkit Plugin[8] v1.1 for VMD[9] v1.9.4 with the initial charge, bond, and angle parameters from CHARMM36[10] and CGenFF v4.1 for Small Molecule Drug Design.[11] The peptide model was built using Vega ZZ[12] v3.1.1 with an initial structure of a fully extended conformation ($\phi = \varphi = 180°$) and then solvated using the Automatic PSF Generation Plugin v.1.3 in VMD. Simulations were carried out using the NAMD[13] v.2.12 software package. Following a 1 ns equilibration run at 298 K and 1 atm in the NPT ensemble, subsequent production runs totaling 150 ns were performed on the equilibrated system at 298 K in the NVT ensemble. A trajectory was built by saving a snapshot every 500 fs, resulting in 300,000 total frames. Analysis of the MD trajectory was accomplished using VMD; for each frame, the minimum distance between any atoms in the 4-cyanoindole ring and the indole ring in the peptide was determined and used for constructing the distance distribution plot.

Table S1. Fluorescence lifetime (τ) and relative amplitude (A) determined from fitting the fluorescence decay of each peptide in Figure S1 to a single or bi-exponential function. The data for 4CN-Trp-Met were taken from Ref. 1.

Peptide Name	Peptide Sequence	τ (ns)	A (%)
4CN-Trp-Gly	4CN-Trp-G	12.8	100
4CN-Trp-His	4CN-Trp-H	12.5	100
4CN-Trp-Arg	4CN-Trp-R	12.5	100
4CN-Trp-Ser	4CN-Trp-S	12.6	100
4CN-Trp-Glu	4CN-Trp-E	12.6	100
4CN-Trp-Met	4CN-Trp-M	12.2	100
4CN-Trp-Phe	4CN-Trp-FAAKKK	11.5	100
4CN-Trp-Tyr	4CN-Trp-YAAKKK	10.5	100
4CN-Trp-Trp	4CN-Trp-WAAKKK	4.1	64
		0.3	36

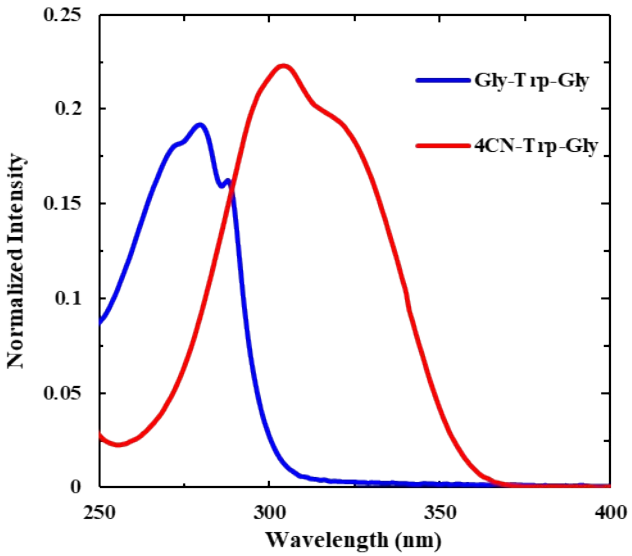

Figure S1. Absorption spectra of Gly-Trp-Gly (33 μM) and 4CN-Trp-Gly (33 μM) in water, as indicated.

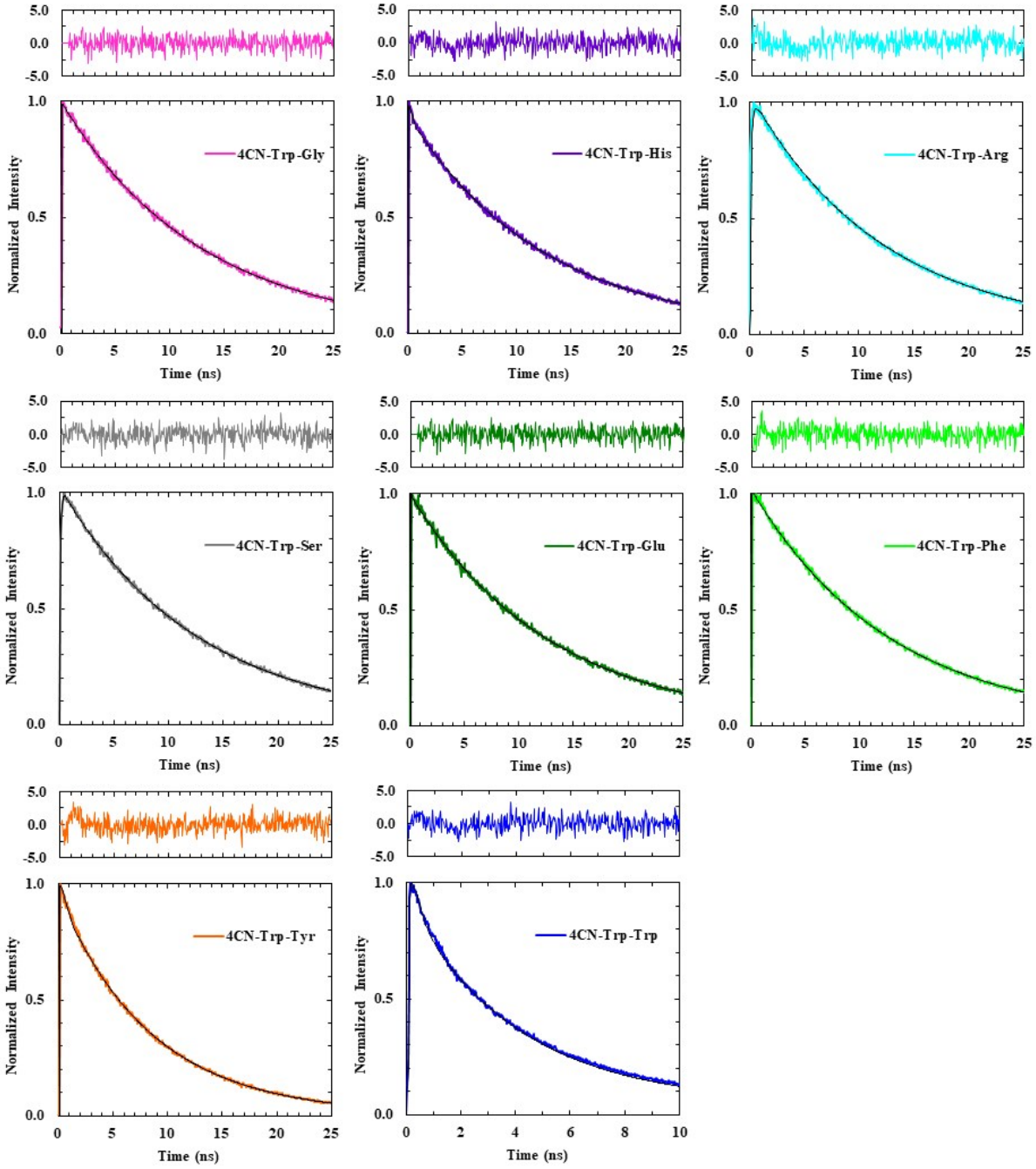

Figure S2. Fluorescence decay kinetics of 4CN-Trp-X peptides, as indicated. In each case, the smooth black line corresponds to the best fit of the respective data to a single-exponential or a bi-exponential function and the resultant lifetime(s) is given in Table S1. Residuals of the fits are on top of each respective panel.

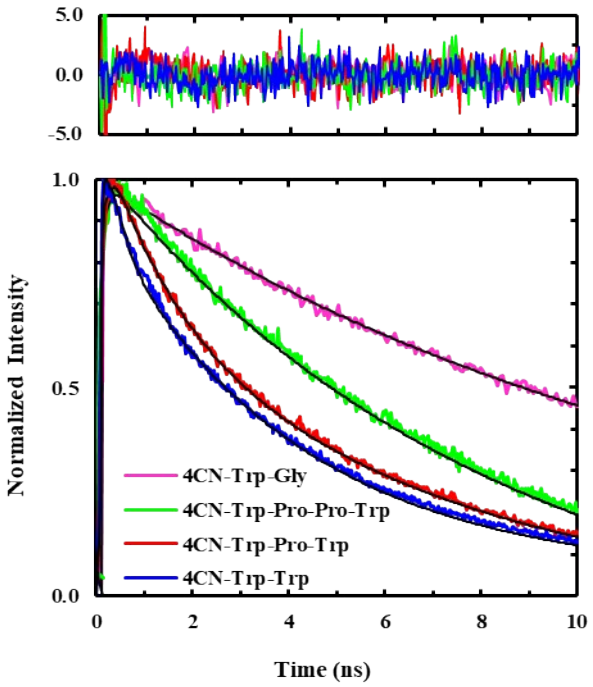

Figure S3. Fluorescence decay kinetics of 4CN-Trp-Gly, 4CN-Trp-Pro-Pro-Trp, 4CN-Trp-Pro-Trp, and 4CN-Trp-Trp, as indicated. The smooth line in each case correspond to the best fit of the data to either a single-exponential function (4CN-Trp-Gly and 4CN-Trp-Pro-Pro-Trp) or a bi-exponential function (4CN-Trp-Pro-Trp and 4CN-Trp-Trp) and the resultant lifetime(s) are listed in Table 1 of the main text.

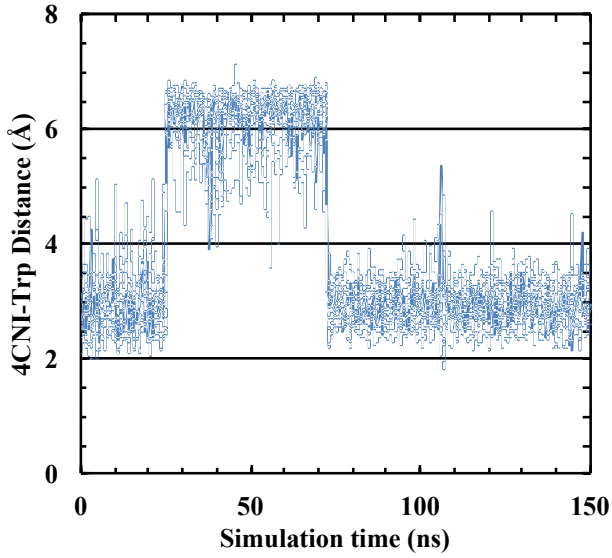

Figure S4. The trajectory of the separation distance between the two fluorophores in the 4CN-Trp-Trp peptide obtained from the MD simulations.

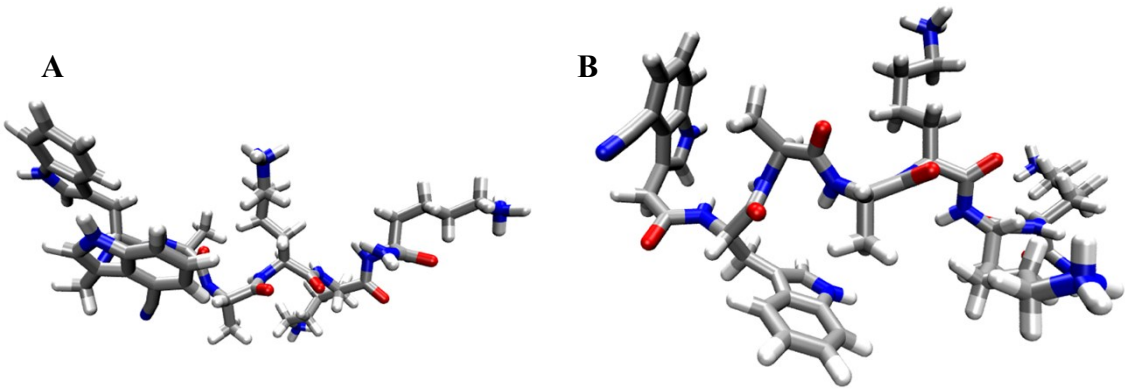

Figure S5. Representative structures of the two conformational populations of the 4CN-Trp-Trp peptide. The distances between the 4-cyanoindole and indole moieties are 2.96 Å (A) and 6.62 Å (B), respectively.

References

1. M. R. Hilaire, I. A. Ahmed, C. W. Lin, H. Jo, W. F. DeGrado and F. Gai, *Proc. Natl. Acad. Sci. U.S.A.*, 2017, **114**, 6005-6009.
2. J. R. Lakowicz, Principles of Fluorescence Spectroscopy. Springer: New York, USA, 2nd edn. 1999.
3. R. N. Chen, *Anal. Lett.*, 1967, **1**, 35–42.
4. K. Suzuki, A. Kobayashi, S. Kaneko, K. Takehira, T. Yoshihara, H. Ishida, Y. Shiina, S. Oishi and S. Tobita, *Phys. Chem. Chem. Phys.*, 2009, **11**, 9850–60.
5. B. N. Markiewicz, D. Mukherjee, T. Troxler and F. Gai, *J. Phys. Chem. B*, 2016, **120**, 936–944.
6. J. R. Lakowicz, B. Zelent, I. Gryczynski, J. Kusba and M. L. Johnson, *Photochem. Photobiol*., 1994, **60**, 205–214.
7. M. R. Mintzer, T. Troxler and F. Gai, *Phys. Chem. Chem. Phys*., 2015, **17**, 7881–7887.
8. C. G. Mayne, J. Saam, K. Schulten, E. Tajkhorshid and J. C. Gumbart, *J. Comput. Chem*. 2013, **34**, 2757–2770.
9. W. Humphrey, A. Dalke and K. Schulten, *J. Mol. Graph.*, 1996, **14**, 33–38.
10. R. B. Best, X. Zhu, J. Shim, P. E. M. Lopes, J. Mittal, M. Feig and A. D. MacKerell, *J. Chem. Theory Comput.*, 2012, **8**, 3257–3273.
11. K. Vanommeslaeghe, E. Hatcher, S. K. Acharya, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov and A. D. J. Mackarell, *J. Comput. Chem*., 2010, **31**, 671–690.
12. A. Pedretti, L. Villa and G. Vistoli, *J. Mol. Graph. Model.*, **2002**, *21*, 47–49.
13. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé and K. Schulten, *J. Comput. Chem*., 2005, **26**, 1781–1802.