

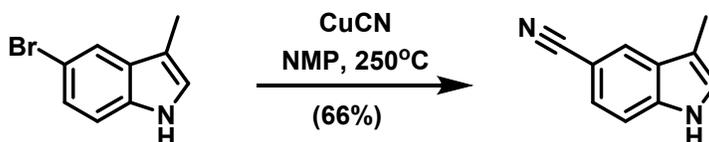
# C≡N Stretching Vibration of 5-Cyanotryptophan as an Infrared Probe of Protein Local Environment: What Determines Its Frequency?

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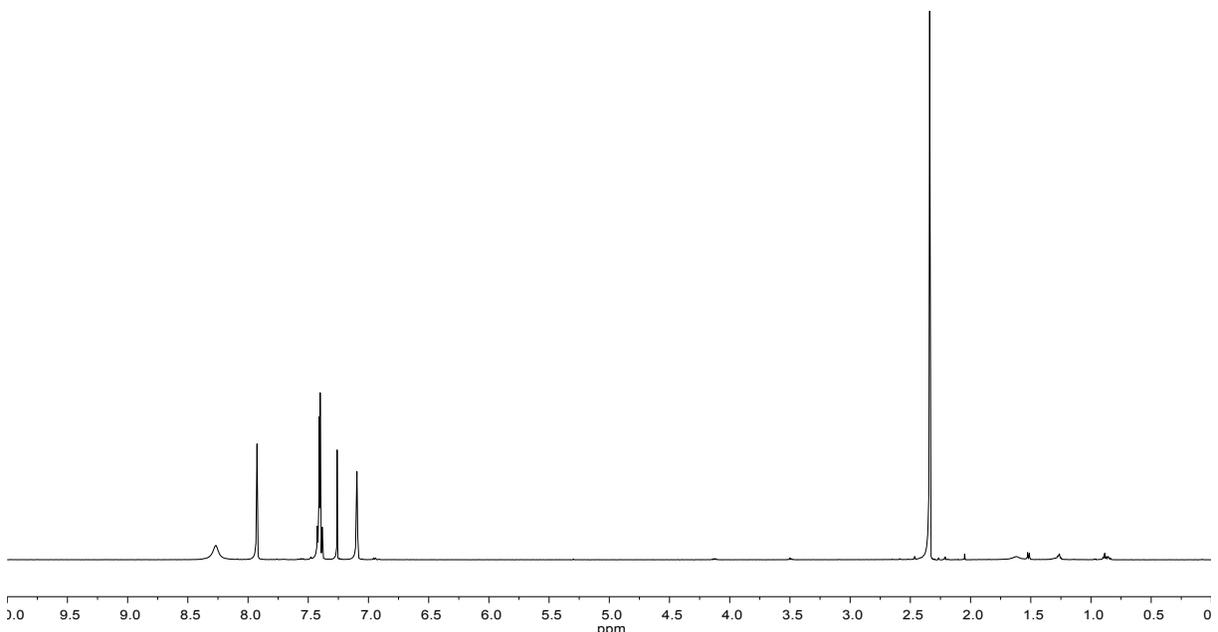
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## Supporting Information

### Synthesis of 3-Methyl-5-Cyanoindole (3M5CI)



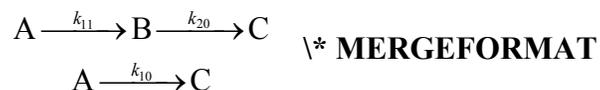
3M5CI was prepared from 3-methyl-5-bromoindole using the above reach scheme. Specifically, a conical heavy-walled microwave tube was charged with 3-methyl-5-bromoindole (500 mg, 2.39 mmol, 1 equiv), CuCN (320 mg, 3.59 mmol, 1.5 equiv), and anhydrous N-methyl-2-pyrrolidone (NMP) (5 mL). Vigorous shaking of the microwave tube was employed to completely dissolve the reactants. The solution turned green during dissolution. The microwave tube was crimped and sealed and then inserted into a microwave (Biotage Initiator). The contents were heated to 250 °C for 1.5 hours at high absorption level. The reaction solution was allowed to cool, then diluted with Et<sub>2</sub>O (30 mL) and then partitioned with water (15 mL) and brine (3 × 15 mL); the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. The oil was loaded onto a silica-gel column and purified by flash chromatography using hexanes/ethyl acetate gradient to yield 246 mg (66%) of a white, amorphous solid. HRMS (ES) found *m/z* 157.0767 [(M+H)<sup>+</sup>]; calcd. for C<sub>10</sub>H<sub>9</sub>N<sub>2</sub>: 157.0766]; <sup>1</sup>H NMR (500 MHz, Chloroform-*d*) δ 8.27 (s, 1H), 7.93 (dt, *J* = 1.6, 0.8 Hz, 1H), 7.41 (d, *J* = 1.4 Hz, 1H), 7.40 (d, *J* = 0.9 Hz, 1H), 7.09 (dd, *J* = 2.3, 1.1 Hz, 1H), 2.34 (d, *J* = 1.2 Hz, 3H). IR (KBr, cm<sup>-1</sup>) 2218.7 (s).



**3-methyl-5-cyanoindole ( $^1\text{H-NMR}$ ,  $\text{CDCl}_3$ , 500 MHz)**

### Decay Kinetics of the Excited State of the $\text{C}\equiv\text{N}$ Stretching Vibration

According to the kinetic scheme in the main text, the  $(\text{C}\equiv\text{N})_{\nu=1}$  state (A), initially prepared by the IR excitation pulse, has two decay channels: one to  $(\text{C}\equiv\text{N})_{\nu=0}$  (C), and the other to  $(\text{Dark State})_{\nu=1}$  (B) which then converts to  $(\text{C}\equiv\text{N})_{\nu=0}$ . This kinetic model, as indicated below,



gives rise to the following rate equations:

$$\begin{aligned} \frac{d[\text{A}]}{dt} &= -k_{11}[\text{A}] - k_{10}[\text{A}] \\ \frac{d[\text{B}]}{dt} &= k_{11}[\text{A}] - k_{20}[\text{B}] \\ \frac{d[\text{C}]}{dt} &= k_{20}[\text{B}] + k_{10}[\text{A}] \end{aligned}$$

Solving these differential equations leads to the following population kinetics:

$$\begin{aligned}
[A] &= [A]_0 e^{-(k_{11}+k_{10})t} \\
[B] &= [A]_0 \frac{k_{11}}{(k_{11}+k_{10})-k_{20}} (e^{-k_{20}t} - e^{-(k_{11}+k_{10})t}) \\
[C] &= [A]_0 - [A] - [B] = [A]_0 \left\{ 1 - \frac{k_{11}e^{-k_{20}t} + (k_{10}-k_{20})e^{-(k_{11}+k_{10})t}}{(k_{11}+k_{10})-k_{20}} \right\}
\end{aligned} \tag{1}$$

The ground state bleach recovery signal is given by  $[C] - [A]_0$ , i.e.,

$$\text{GSB}(t) = -[A]_0 \frac{k_{11}e^{-k_{20}t} + (k_{10}-k_{20})e^{-(k_{11}+k_{10})t}}{(k_{11}+k_{10})-k_{20}}, \tag{2}$$

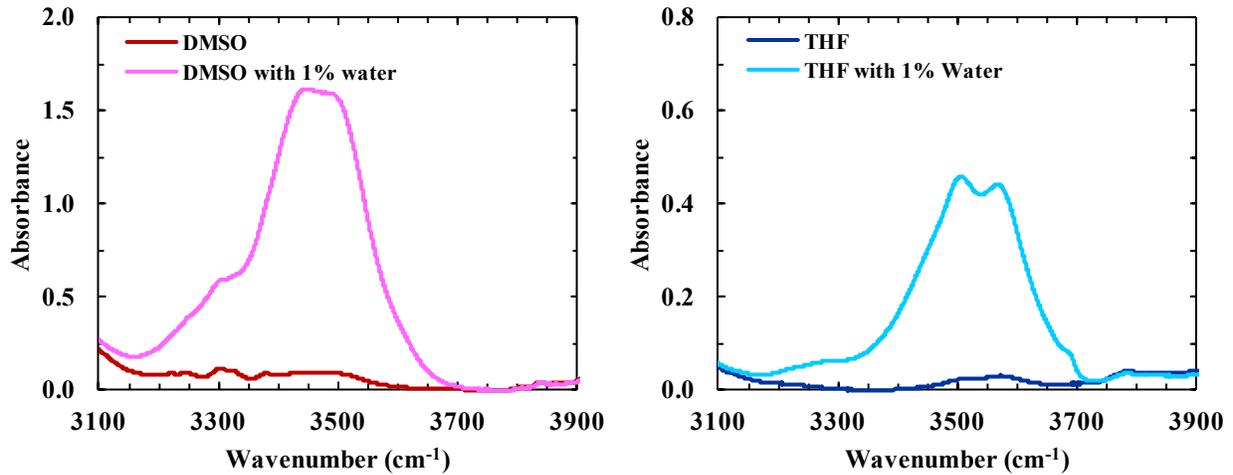
which indicates that the percentage of the slow component is

$$\frac{A_2}{A_1 + A_2} = \frac{k_{11}}{k_{11} + (k_{10} - k_{20})} \tag{3}$$

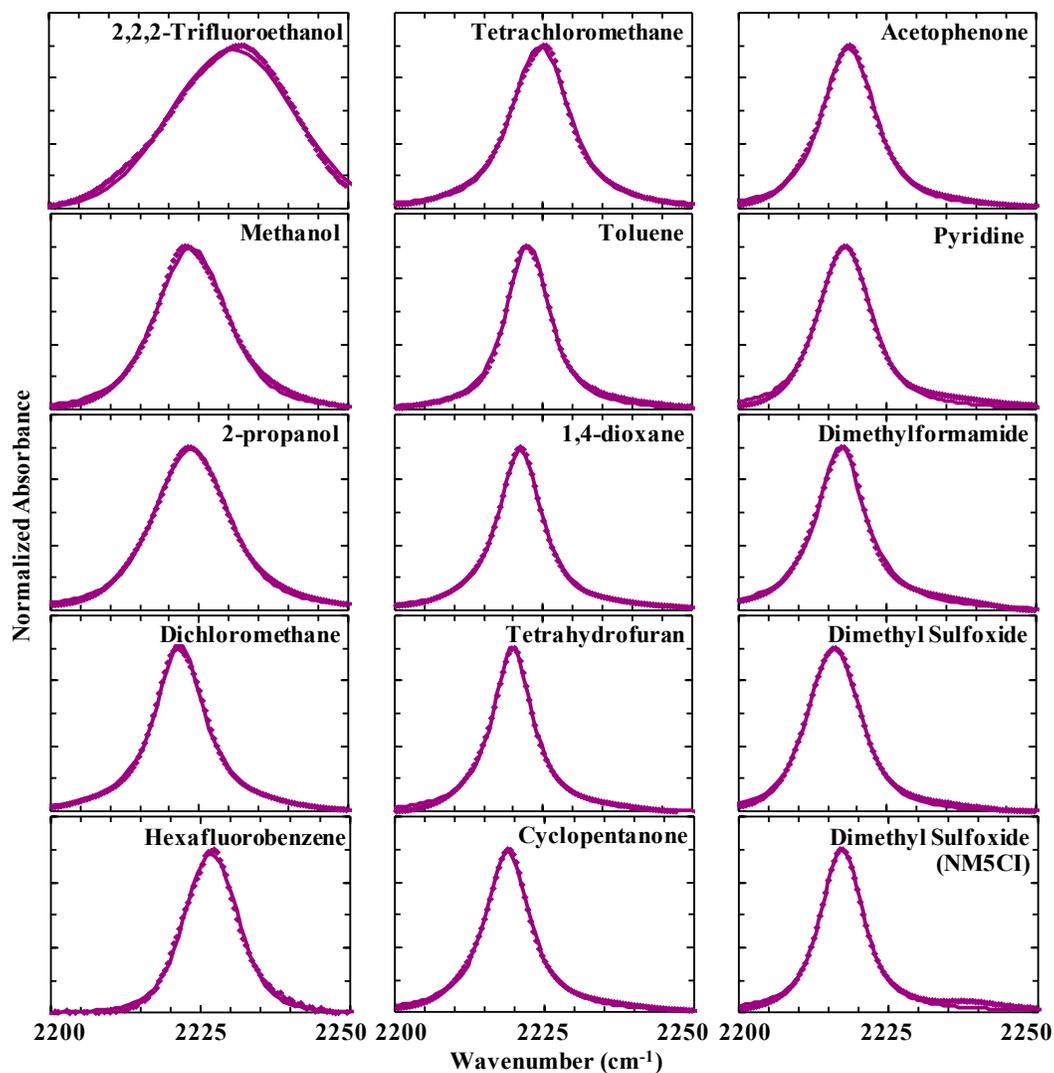
Adding the stimulated emission contribution to Eq. (2) gives rise to the decay kinetics of the negative-going pump-probe signal,  $S(t)$ :

$$S(t) = -[A]_0 \frac{k_{11}e^{-k_{20}t} + (2k_{10} - 2k_{20} + k_{11})e^{-(k_{11}+k_{10})t}}{(k_{11}+k_{10})-k_{20}} \tag{4}$$

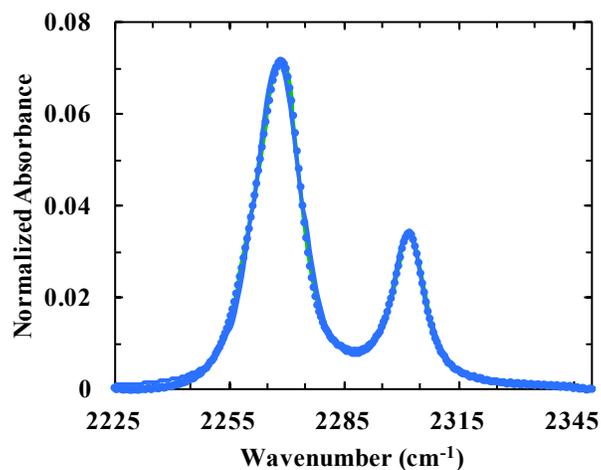
It is easy to show, based on Eq. (4), that the ratio of the two exponential components in the negative-going pump-probe decay kinetics is  $k_{11}/(2k_{10} - 2k_{20} + k_{11})$ .



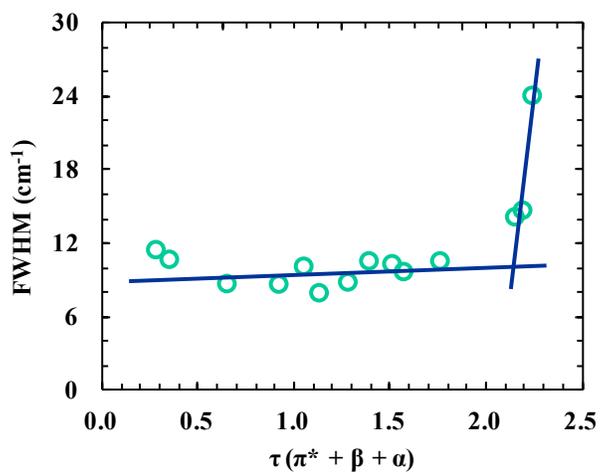
**Figure S1.** FTIR spectra of DMSO and THF with and without addition of 1% water, as indicated, in the OH stretching band region of water. These spectra indicate that the water content in the original solvents is negligible.



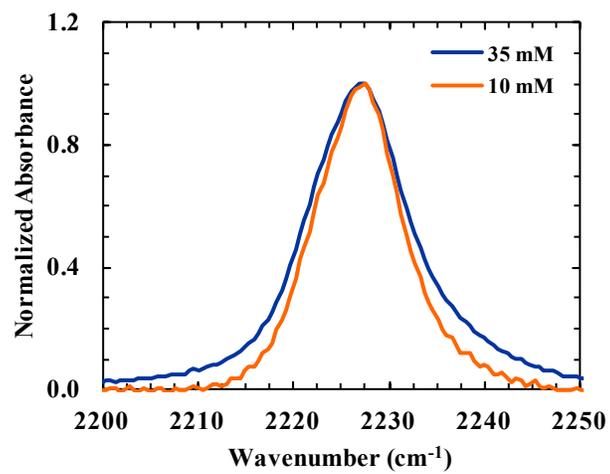
**Figure S2.**  $\text{C}\equiv\text{N}$  stretching bands of 3M5CI and NM5CI in different solvents, as indicated. The solid line in each case is the fit of the data to a Voigt profile and the resulting peak frequency and FWHM are given in Table 1.



**Figure S3.** FTIR spectrum of acetonitrile in 2,2,2-trifluoroethanol, where the solid line is a fit of the spectrum to a function composed of two Voigt profiles. The peak frequency and FWHM of the C≡N stretching band are 2267.7 cm<sup>-1</sup> and 14.3 cm<sup>-1</sup>, respectively. The second, lower intensity band located at 2301.9 cm<sup>-1</sup> arises from a Fermi resonance.



**Figure S4.** Plot of FWHM versus  $\tau = \pi^* + \beta + \alpha$ .



**Figure S5.** C≡N stretching bands of 3M5CI in hexafluorobenzene at 35 mM and 10 mM, as indicated.