

## ELECTRONIC SUPPLEMENTARY INFORMATION

### Is 1-methylcytosine a faithful model for ultrafast deactivation dynamics of cytosine nucleosides in solution?

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<b>Figure S1</b> Absorption spectra of 1mCyt in H <sub>2</sub> O and CH <sub>3</sub> CN before and after time-resolved experiments (TR expt).....	5
<b>Kinetic analysis and decay associated analysis</b> .....	5
<b>Details of simulation of experimentally measured fluorescence spectra and decay associated spectra by log-normal functions</b> .....	6
<b>Table S1</b> Calculated thermodynamic parameters of 1mCyt at the ground state (S <sub>0</sub> ) by TPSS/6-31G+(d,p) calculations and excited state (S <sub>1</sub> ) by B3LYP/6-31G+(d,p) calculations, under the bulk solvent condition of H <sub>2</sub> O, CH <sub>3</sub> CN and CH <sub>3</sub> OH, and inclusion of one water molecule (1W) and one methanol molecule (1M) .....	7
<b>Table S2</b> The atom coordinates of 1mCyt in S <sub>0</sub> in the bulk solvent condition of H <sub>2</sub> O by TPSS/6-31G+(d,p) calculations .....	7
<b>Table S3</b> The atom coordinates of 1mCyt in S <sub>1</sub> in the bulk solvent condition of H <sub>2</sub> O by B3LYP/6-31G+(d,p) calculations .....	7

<b>Table S4</b> The atom coordinates of 1mCyt in $S_1$ with inclusion of one water molecule (1W) by B3LYP/6-31G+(d,p) calculations .....	8
<b>Table S5</b> The atom coordinates of 1mCyt in $S_0$ in the bulk solvent condition of $\text{CH}_3\text{CN}$ by TPSS/6-31G+(d,p) calculations .....	8
<b>Table S6</b> The atom coordinates of 1mCyt in $S_1$ in the bulk solvent condition of $\text{CH}_3\text{CN}$ by B3LYP/6-31G+(d,p) calculations .....	8
<b>Table S7</b> The atom coordinates and frequencies of 1mCyt in $S_1$ with inclusion of one methanol molecule (1M) by B3LYP/6-31G+(d,p) calculations .....	9
<b>Figure S2</b> Absorption transition Kohn-Sham orbitals for 1mCyt by TPSS/6-31G+(d,p) calculations in the bulk solvent condition of $\text{H}_2\text{O}$ and $\text{CH}_3\text{CN}$ .....	9
<b>Figure S3</b> Fluorescence transition Kohn-Sham orbitals for 1mCyt by B3LYP/6-31G+(d,p) calculations, with the inclusion of one water molecule (1W) and one methanol molecule (1M), and in the bulk solvent condition of $\text{CH}_3\text{CN}$ . .....	10
<b>Figure S4</b> Optimized top-view and side-view structures of 1mCyt at the ground state ( $S_0$ ) by TPSS/6-31G+(d,p) calculations and the lowest energy excited state ( $S_1$ ) by B3LYP/6-31G+(d,p) calculations, with the keto oxygen hydrogen-bonded to one methanol molecule (1M). .....	10
<b>Table S8</b> The fitting parameters of log-normal simulation of steady-state fluorescence spectra and decay associated spectra (DAS) of 1mCyt in $\text{H}_2\text{O}$ and $\text{CH}_3\text{CN}$ after 267 nm excitation: $\nu_p$ is the peak frequency, $\gamma$ the asymmetry, and $\Delta$ the bandwidth parameter of the simulated spectra.....	11
<b>Table S9a</b> Calculated absorption electronic transition nature, energies and the corresponding oscillation strengths for 1mCyt at the ground state by TPSS/6-	

31G+(d,p) calculations under the bulk solvent condition of H <sub>2</sub> O and CH <sub>3</sub> CN, and inclusion of one water molecule (1W) .....	11
<b>Table S9b</b> Calculated absorption electronic transition nature, energies and the corresponding oscillation strengths for 1mCyt at the ground state by B3LYP/6-31G+(d,p) calculations under the bulk solvent condition of H <sub>2</sub> O and CH <sub>3</sub> CN, and inclusion of one water molecule (1W) .....	12
<b>Table S10</b> Calculated fluorescence electronic transition nature, energies and the corresponding oscillation strengths for 1mCyt at the excited state under the bulk solvent condition of H <sub>2</sub> O, CH <sub>3</sub> CN and CH <sub>3</sub> OH, and inclusion of one water molecule (1W) and one methanol molecule (1M) by B3LYP/6-31G+(d,p) calculations .....	13
<b>Figure S5</b> 2D fs-TA spectra at the late time delays (~0-6000 ps) after 267 nm excitation of 1mCyt in H <sub>2</sub> O. ....	13
<b>Figure S6</b> (a) 2D fs-TRF spectra, (b) experimental (○, □, ◇) and fitted (solid lines) TRF intensity decay profiles, (c) 2D fs-TRFA spectra, and (d) decay associated spectra (DAS) from global analysis of fs-TRF spectra after 267 nm excitation of 1mCyt in CH <sub>3</sub> CN. The time delays for 2D spectra shown in (a) are 0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.6, 0.85, 1.25, 1.75, 2.5 and 10 ps. Insert in (a) shows intensity normalized TRF spectra at the denoted time delays. # due to solvent Raman scattering.....	14
<b>Table S11</b> Photophysical parameters obtained from fs-TRF for 1mCyt in H <sub>2</sub> O and CH <sub>3</sub> CN with 267 nm excitation .....	15
<b>Table S12</b> Photophysical parameters obtained from fs-TA for 1mCyt in H <sub>2</sub> O with 267 nm excitation.....	15

**Figure S7** Normalized fluorescence spectra ( $\lambda_{\text{ex}} = 267$  nm) of 1mCyt in H<sub>2</sub>O and CH<sub>3</sub>OH.

..... 15

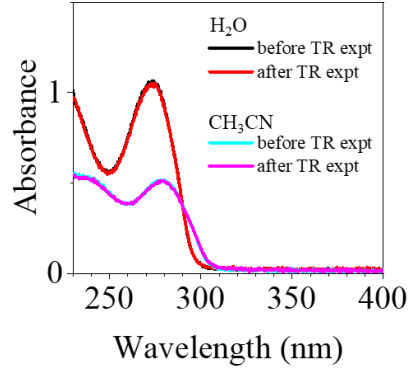
**Figure S8** (a) 2D fs-TRF spectra, (b) experimental ( $\circ$ ,  $\square$ ,  $\diamond$ ) and fitted (solid lines) TRF

intensity decay profiles from global analysis of fs-TRF spectra after 267 nm excitation of Cyt in H<sub>2</sub>O. And (c) Experimental ( $\square$ ) and fitted (solid lines) TRF intensity decay profiles at 330 nm from global analysis of fs-TRF spectra after 267 nm excitation of 1mCyt and Cyt in H<sub>2</sub>O. The time delays for 2D spectra shown in (a) are 0, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.7, 1, 1.8 and 10 ps. # due to solvent Raman scattering..... 16

**Figure S9** (a-b) 2D fs-TA spectra, (c-d) experimental ( $\circ$ ,  $\square$ ,  $\diamond$ ) and fitted (solid lines)

TA intensity decay profiles at the denoted wavelengths in (c) (~0-3 ps), (d) (~0-10 ps) the early time delays after 267 nm excitation of Cyt in H<sub>2</sub>O. And (e) Experimental ( $\square$ ) and fitted (solid lines) TA intensity decay profiles at 451 nm from global analysis of fs-TA spectra after 267 nm excitation of 1mCyt and Cyt in H<sub>2</sub>O. The time delays for 2D spectra shown in (a) are 0, 0.1, 0.15, 0.2, 0.3 and 0.6 ps, while those in (b) are 0.6, 1.25, 2, 3, 6 and 25 ps..... 17

**References**..... 18



**Figure S1** Absorption spectra of 1mCyt in H<sub>2</sub>O and CH<sub>3</sub>CN before and after time-resolved experiments (TR expt).

### Kinetic analysis and decay associated analysis

In order to obtain the kinetic decay profiles of fs-TRF and fs-TA intensities, the instrument response function ( $g(\tau)$ ) represented by the Gaussian function was convoluted with a multiple exponential function ( $f(t)$ ) to do global fitting for the experimental time profiles ( $F(t)$ ), as showed in equations 1-4:<sup>1-5</sup>

$$F(t) = \int_{-\infty}^t g(\tau)f(t - \tau)d\tau \quad \text{Equation 1}$$

$$g(\tau) = \frac{1}{\pi^{1/2}\sigma} \exp[-\{(t - \tau_0)/\sigma\}^2] \quad \text{Equation 2}$$

$$f(t - \tau) = \sum_i a_i \exp(t - \tau)/\tau_i \quad \text{Equation 3}$$

$$\sum_i a_i = 1 \quad \text{Equation 4}$$

Where  $\tau_0$  and  $t$  are the time-shift which can be used to determine the absolute time-zero, and delay time after photo-excitation, respectively. While  $\tau_i$  and  $a_i$  represent time constants and relative amplitudes associated with the time constants, respectively.

The spectra of time constants fitted from fs-TRF, called decay associated spectra (DAS),<sup>6</sup> were obtained by using decay associated analysis. This method is achieved by using the multi-exponential function to get the relative amplitudes corresponding to the time constants at every wavelength for spectral plotting.

The average fluorescence lifetime ( $\langle \tau \rangle$ ) is defined by the following equation:

$$\langle \tau \rangle = \sum_i a_i \tau_i \quad \text{Equation 5}$$

### **Details of simulation of experimentally measured fluorescence spectra and decay associated spectra by log-normal functions**

Log-normal line shape function is expressed as following equations:<sup>1</sup>

$$F(\nu) = h \begin{cases} \exp[-\ln(2)\{\ln(1 + \alpha)/\gamma\}^2] & \alpha > -1 \\ 0 & \alpha \leq -1 \end{cases} \quad \text{Equation 6}$$

$$\alpha = 2\gamma(\nu - \nu_p)/\Delta \quad \text{Equation 7}$$

A nonlinear least-squares fitting was used to simulate the fluorescence spectra by adjusting the four parameters, the peak height  $h$ , the peak frequency  $\nu_p$ , the asymmetry  $\gamma$  and the bandwidth parameter  $\Delta$ . The first step of the spectral simulation was to represent the spectra in wavenumber ( $\nu$  in  $\text{cm}^{-1}$ ) units by using a relation equation  $F(\nu) = \lambda^2 F(\lambda)$ . For readers' convenience, the fitting result of  $\nu_p$  was shown in units of wavelength (nm) in Table S8 which summarized the fitting parameters ( $\gamma$ ,  $\nu_p$ , and  $\Delta$ ).

**Table S1** Calculated thermodynamic parameters of 1mCyt at the ground state ( $S_0$ ) by TPSS/6-31G+(d,p) calculations and excited state ( $S_1$ ) by B3LYP/6-31G+(d,p) calculations, under the bulk solvent condition of  $H_2O$ ,  $CH_3CN$  and  $CH_3OH$ , and inclusion of one water molecule (1W) and one methanol molecule (1M)

		$H_2O$	1W	$CH_3CN$	$CH_3OH$	1M
$S_0$	$E_{tot}/\text{Hartree}$	-434.458	-510.769	-434.457	-434.195	-550.098
$S_1$	$E_{tot}/\text{Hartree}$	-434.140	-510.588	-434.139	-434.139	-549.886

**Table S2** The atom coordinates of 1mCyt in  $S_0$  in the bulk solvent condition of  $H_2O$  by TPSS/6-31G+(d,p) calculations

Number	Atom	X	Y	Z	Number	Atom	X	Y	Z
1	C	-0.2681	1.502017	0.000405	9	H	-3.01188	0.161498	0.890831
2	C	-0.62016	-0.896494	-0.00033	10	H	-2.78774	1.699588	0.003607
3	C	1.556398	-0.02536	-0.00073	11	H	-3.01344	0.164862	-0.88895
4	C	1.086135	1.325912	-0.00067	12	O	-1.44279	-1.82988	-0.00016
5	H	-0.72941	2.484474	0.0021	13	N	0.732114	-1.0813	0.001753
6	H	1.760656	2.173846	-0.00257	14	N	2.889453	-0.28201	-0.02646
7	N	-1.1203	0.438844	-0.00013	15	H	3.206033	-1.23808	0.075115
8	C	-2.5803	0.627961	0.001449	16	H	3.565315	0.459909	0.094211

**Table S3** The atom coordinates of 1mCyt in  $S_1$  in the bulk solvent condition of  $H_2O$  by B3LYP/6-31G+(d,p) calculations

Number	Atom	X	Y	Z	Number	Atom	X	Y	Z
1	C	-0.26775	1.495963	-0.000114	9	H	-3.01234	0.165933	0.887935
2	C	-0.62045	-0.88958	-0.000056	10	H	-2.78577	1.695272	-0.000807
3	C	1.555139	-0.02845	-0.000079	11	H	-3.01277	0.164325	-0.886654
4	C	1.082721	1.323213	-0.000197	12	O	-1.43594	-1.82782	0.000094
5	H	-0.72531	2.478473	-0.000034	13	N	0.729046	-1.0755	0.000163
6	H	1.756257	2.170021	-0.00054	14	N	2.882886	-0.28016	-0.002699
7	N	-1.12028	0.434962	-0.000153	15	H	3.209133	-1.2351	0.008967
8	C	-2.57385	0.626167	0.000103	16	H	3.561873	0.464614	0.011259

**Table S4** The atom coordinates of 1mCyt in  $S_1$  with inclusion of one water molecule (1W) by B3LYP/6-31G+(d,p) calculations

Number	Atom	X	Y	Z	Number	Atom	X	Y	Z
1	C	1.908251	0.784801	0.388348	11	H	-0.43623	2.97197	-0.605926
2	C	-0.41514	0.441219	-0.069527	12	O	-1.56163	0.98606	-0.177783
3	C	0.991393	-1.41593	-0.050788	13	N	-0.29416	-0.87894	-0.003601
4	C	2.085497	-0.61449	0.279767	14	N	1.070151	-2.75757	-0.371002
5	H	2.685232	1.479423	0.67179	15	H	0.240848	-3.29714	-0.149625
6	H	3.063037	-1.04412	0.477001	16	H	1.931466	-3.22998	-0.12604
7	N	0.684609	1.311594	-0.013256	17	O	-3.91491	-0.60282	0.236211
8	C	0.562637	2.745993	-0.241531	18	H	-3.67684	-1.53557	0.316198
9	H	0.737286	3.289483	0.696472	19	H	-3.06869	-0.13443	0.1018
10	H	1.316201	3.05932	-0.971696					

**Table S5** The atom coordinates of 1mCyt in  $S_0$  in the bulk solvent condition of  $\text{CH}_3\text{CN}$  by TPSS/6-31G+(d,p) calculations

Number	Atom	X	Y	Z	Number	Atom	X	Y	Z
1	C	-0.268104	1.502017	0.000405	9	H	-3.01188	0.161498	0.890831
2	C	-0.620155	-0.896494	-0.000332	10	H	-2.78774	1.699588	0.003607
3	C	1.556398	-0.02536	-0.000726	11	H	-3.013438	0.164892	-0.888947
4	C	1.086135	1.325912	-0.00067	12	O	-1.442786	-1.829878	-0.000159
5	H	-0.729414	2.484474	0.0021	13	N	0.732114	-1.081304	0.001753
6	H	1.760656	2.173846	-0.002565	14	N	2.889453	-0.282009	-0.026458
7	N	-1.120296	0.438844	-0.000127	15	H	3.206033	-1.23808	0.075115
8	C	-2.580298	0.627961	0.001449	16	H	3.565315	0.459909	0.094211

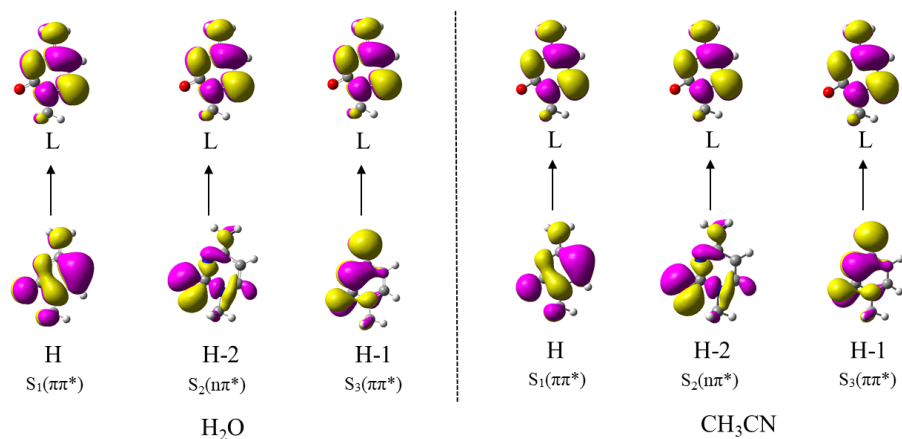
**Table S6** The atom coordinates of 1mCyt in  $S_1$  in the bulk solvent condition of  $\text{CH}_3\text{CN}$  by B3LYP/6-31G+(d,p) calculations

Number	Atom	X	Y	Z	Number	Atom	X	Y	Z
1	C	-0.333392	1.500473	0.238029	9	H	-3.022741	-0.343369	-0.575579
2	C	-0.576767	-0.869099	0.089672	10	H	-3.063241	0.983963	0.609733
3	C	1.567713	0.033399	-0.022866	11	H	-2.687353	1.337799	-1.095103
4	C	1.064271	1.319894	0.148161	12	O	-1.381436	-1.860253	0.109724
5	H	-0.819243	2.445902	0.430654	13	N	0.728928	-1.064545	0.194721
6	H	1.722101	2.179974	0.234691	14	N	2.879812	-0.272741	-0.334837
7	N	-1.146052	0.400724	-0.039629	15	H	3.20203	-1.164649	0.0239
8	C	-2.567371	0.603067	-0.292693	16	H	3.554396	0.471947	-0.209627

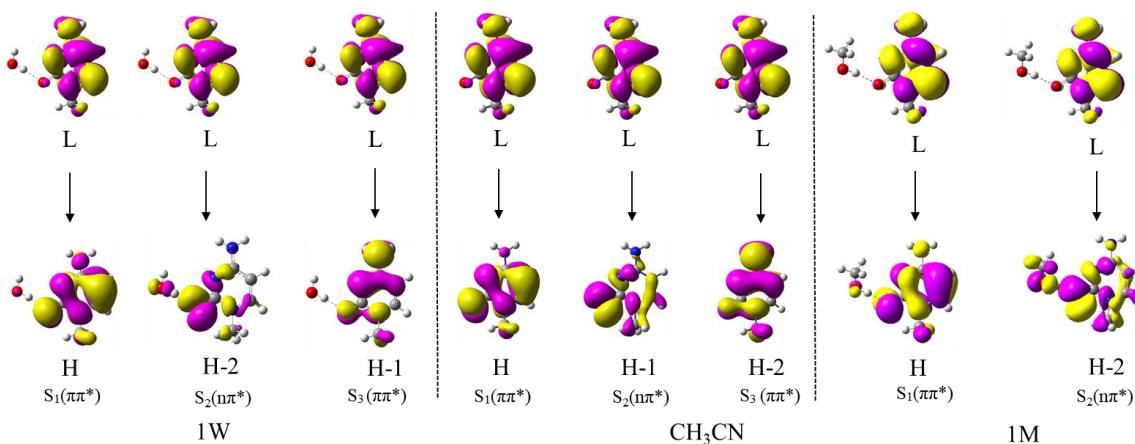


**Table S7** The atom coordinates and frequencies of 1mCyt in  $S_1$  with inclusion of one methanol molecule (1M) by B3LYP/6-31G+(d,p) calculations

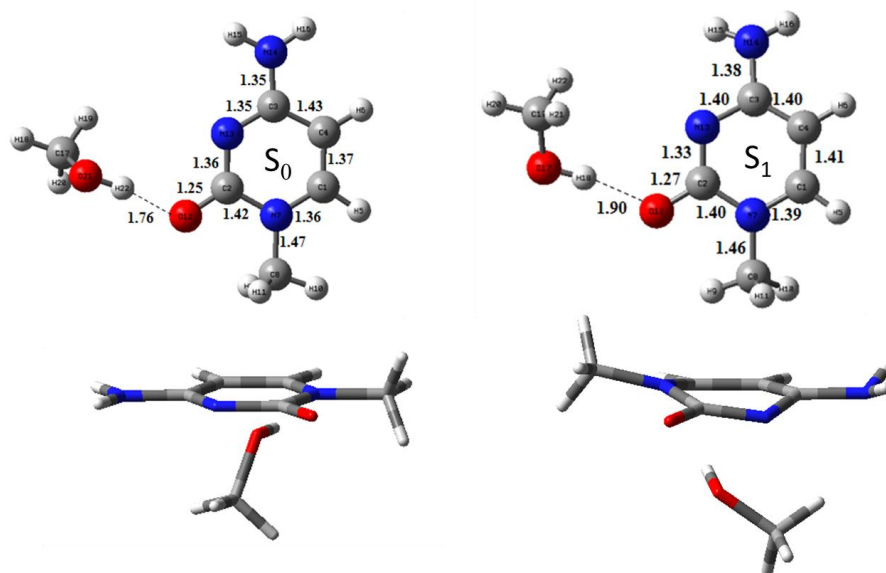
Number	Atom	X	Y	Z	Number	Atom	X	Y	Z
1	C	2.432905	-0.386099	0.496926	12	O	-0.860268	-1.328468	-0.313334
2	C	0.135522	-0.550616	-0.14013	13	N	-0.02833	0.764434	-0.108827
3	C	1.119236	1.559602	-0.09729	14	N	0.910039	2.909645	-0.314828
4	C	2.324108	1.014392	0.350845	15	H	0.088967	3.125157	-0.867515
5	H	3.315222	-0.89663	0.853626	16	H	1.72122	3.451279	-0.583581
6	H	3.168199	1.644948	0.613990	17	O	-3.6413	-0.634872	-0.185805
7	N	1.384581	-1.167886	0.019277	18	H	-2.674113	-0.754541	-0.189904
8	C	1.579899	-2.602362	-0.15082	19	C	-3.957432	0.588893	0.476095
9	H	0.689984	-3.039302	-0.59698	20	H	-5.044863	0.697029	0.458193
10	H	2.450987	-2.772389	-0.7918	21	H	-3.624407	0.585052	1.522423
11	H	1.763951	-3.074355	0.823422	22	H	-3.512024	1.454266	-0.031817



**Figure S2** Absorption transition Kohn-Sham orbitals for 1mCyt by TPSS/6-31G+(d,p) calculations in the bulk solvent condition of  $H_2O$  and  $CH_3CN$ .



**Figure S3** Fluorescence transition Kohn-Sham orbitals for 1mCyt by B3LYP/6-31G+(d,p) calculations, with the inclusion of one water molecule (1W) and one methanol molecule (1M), and in the bulk solvent condition of CH<sub>3</sub>CN.



**Figure S4** Optimized top-view and side-view structures of 1mCyt at the ground state ( $S_0$ ) by TPSS/6-31G+(d,p) calculations and the lowest energy excited state ( $S_1$ ) by B3LYP/6-31G+(d,p) calculations, with the keto oxygen hydrogen-bonded to one methanol molecule (1M).

**Table S8** The fitting parameters of log-normal simulation of steady-state fluorescence spectra and decay associated spectra (DAS) of 1mCyt in H<sub>2</sub>O and CH<sub>3</sub>CN after 267 nm excitation:  $\nu_p$  is the peak frequency,  $\gamma$  the asymmetry, and  $\Delta$  the bandwidth parameter of the simulated spectra

Solvent		$\gamma$	$\nu_p$ (nm)	$\Delta$ (cm <sup>-1</sup> )
H <sub>2</sub> O	Steady-state fluorescence	-0.39±0.01	324±1	5129±10
	DAS- $\tau_1$	-0.33±0.01	315±2	5154±50
	DAS- $\tau_2$	-0.31±0.01	324±2	4748±20
CH <sub>3</sub> CN	Steady-state fluorescence	-0.31±0.01	336±1	5731±10
	DAS- $\tau_1$	-0.31±0.01	315±2	5051±60
	DAS- $\tau_2$	-0.46±0.01	333±1	5516±20

**Table S9a** Calculated absorption electronic transition nature, energies and the corresponding oscillation strengths for 1mCyt at the ground state by TPSS/6-31G+(d,p) calculations under the bulk solvent condition of H<sub>2</sub>O and CH<sub>3</sub>CN, and inclusion of one water molecule (1W)

Solvent	State <sup>a</sup>	$\lambda$ <sup>b</sup> (eV <sup>c</sup> ) f <sup>d</sup>	Transition orbitals <sup>e</sup>
H <sub>2</sub> O	S <sub>1</sub> ( $\pi\pi^*$ )	279 (4.4495) 0.0769	H→L(85%)
	S <sub>2</sub> ( $n\pi^*$ )	277 (4.4799) 0.0004	H-2→L(97%)
	S <sub>3</sub> ( $\pi\pi^*$ )	250 (4.9523) 0.0985	H-1→L(66%)
1W	S <sub>1</sub> ( $\pi\pi^*$ )	275 (4.5109) 0.0743	H→L(79%)
	S <sub>2</sub> ( $n\pi^*$ )	264 (4.7043) 0.0001	H-1→L(99%)
CH <sub>3</sub> CN	S <sub>1</sub> ( $\pi\pi^*$ )	279 (4.4402) 0.0752	H→L(86%)
	S <sub>2</sub> ( $n\pi^*$ )	278 (4.4603) 0.0005	H-2→L(96%)
	S <sub>3</sub> ( $\pi\pi^*$ )	251 (4.9457) 0.0929	H-1→L(61%)

<sup>a</sup> Electronic transition nature; <sup>b</sup> Calculated absorption energy in wavelength and <sup>c</sup> electron volt; <sup>d</sup> Calculated oscillator strength at the maximum absorption; <sup>e</sup> Molecular orbital of the corresponding electronic transition with H donating for HOMO, L for LUMO and the bracketed value for percentage contribution of the correlated transition.

**Table S9b** Calculated absorption electronic transition nature, energies and the corresponding oscillation strengths for 1mCyt at the ground state by B3LYP/6-31G+(d,p) calculations under the bulk solvent condition of H<sub>2</sub>O and CH<sub>3</sub>CN, and inclusion of one water molecule (1W)

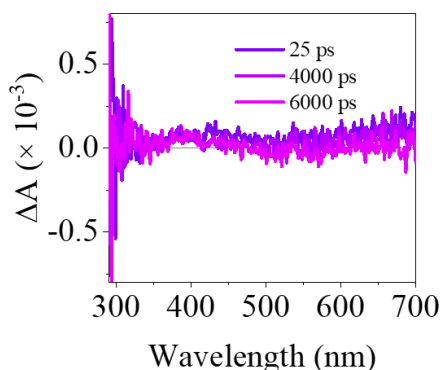
Solvent	State <sup>a</sup>	$\lambda$ <sup>b</sup> (eV <sup>c</sup> ) f <sup>d</sup>	Transition orbitals <sup>e</sup>
H <sub>2</sub> O	S <sub>1</sub> ( $\pi\pi^*$ )	262 (4.7322) 0.1285	H→L(92%)
	S <sub>2</sub> ( $n\pi^*$ )	235 (5.2777) 0.0064	H-2→L(57%)
1W	S <sub>1</sub> ( $\pi\pi^*$ )	256 (4.8422) 0.1303	H→L(79%)
	S <sub>2</sub> ( $n\pi^*$ )	230 (5.3936) 0.0024	H-1→L(99%)
CH <sub>3</sub> CN	S <sub>1</sub> ( $\pi\pi^*$ )	262 (4.7254) 0.1272	H→L(92%)
	S <sub>2</sub> ( $n\pi^*$ )	236 (5.2623) 0.0058	H-2→L(52%)

<sup>a</sup> Electronic transition nature; <sup>b</sup> Calculated absorption energy in wavelength and <sup>c</sup> electron volt; <sup>d</sup> Calculated oscillator strength at the maximum absorption; <sup>e</sup> Molecular orbital of the corresponding electronic transition with H donating for HOMO, L for LUMO and the bracketed value for percentage contribution of the correlated transition.

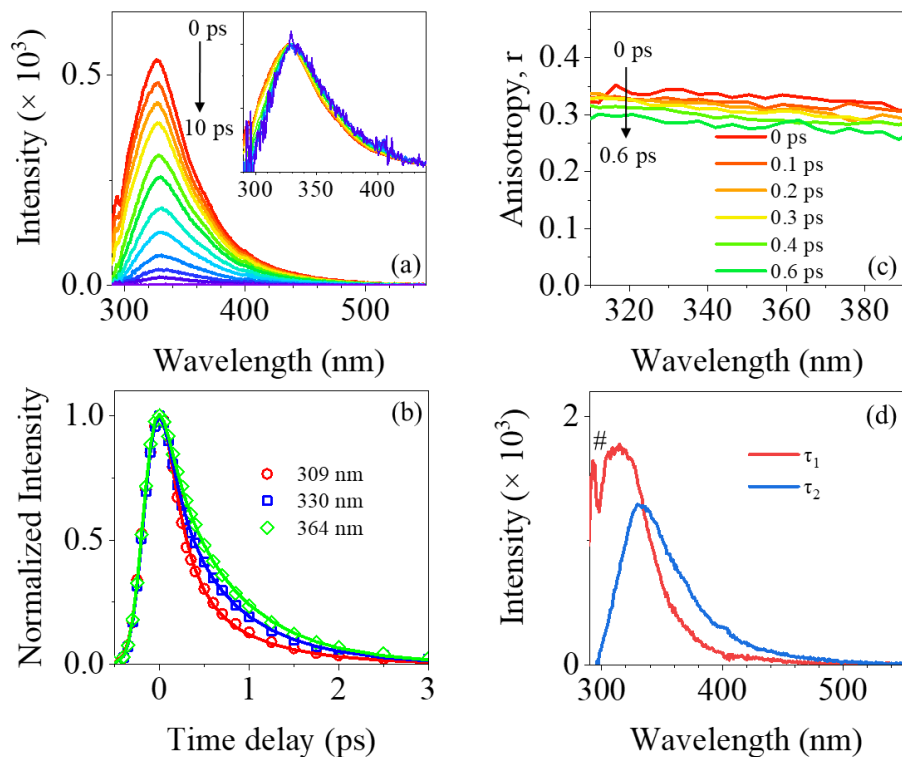
**Table S10** Calculated fluorescence electronic transition nature, energies and the corresponding oscillation strengths for 1mCyt at the excited state under the bulk solvent condition of H<sub>2</sub>O, CH<sub>3</sub>CN and CH<sub>3</sub>OH, and inclusion of one water molecule (1W) and one methanol molecule (1M) by B3LYP/6-31G+(d,p) calculations

Solvent	State <sup>a</sup>	$\lambda$ <sup>b</sup> (eV <sup>c</sup> ) f <sup>d</sup>	Transition orbitals <sup>e</sup>
H <sub>2</sub> O	S <sub>1</sub> ( $\pi\pi^*$ )	338 (3.6692) 0.0886	H←L(97%)
	S <sub>2</sub> ( $n\pi^*$ )	292 (4.2522) 0.0165	H-1←L(99%)
	S <sub>3</sub> ( $\pi\pi^*$ )	268 (4.6270) 0.2240	H+1←L(71%)
1W	S <sub>1</sub> ( $\pi\pi^*$ )	323 (3.8337) 0.1181	H←L(97%)
	S <sub>2</sub> ( $n\pi^*$ )	271 (4.5704) 0.0212	H-2←L(96%)
	S <sub>3</sub> ( $\pi\pi^*$ )	264 (4.7004) 0.2294	H-1←L(74%)
CH <sub>3</sub> CN	S <sub>1</sub> ( $\pi\pi^*$ )	340 (3.6433) 0.0814	H←L(97%)
	S <sub>2</sub> ( $n\pi^*$ )	295 (4.2057) 0.0167	H-1←L(99%)
	S <sub>3</sub> ( $\pi\pi^*$ )	269 (4.6146) 0.2128	H-2←L(69%)
CH <sub>3</sub> OH	S <sub>1</sub> ( $\pi\pi^*$ )	339 (3.6560) 0.0824	H←L(96%)
	S <sub>2</sub> ( $\pi\pi^*$ )	294 (4.2205) 0.0132	H-2←L(97%)
1M	S <sub>1</sub> ( $\pi\pi^*$ )	326 (3.8020) 0.1051	H←L(97%)
	S <sub>2</sub> ( $\pi\pi^*$ )	276 (4.4937) 0.0138	H-2←L(94%)

<sup>a</sup> Electronic transition nature; <sup>b</sup> Calculated fluorescence energy in wavelength and <sup>c</sup> electron volt; <sup>d</sup> Calculated oscillator strength at the maximum fluorescence; <sup>e</sup> Molecular orbital of the corresponding electronic transition with H donating for HOMO, L for LUMO and the bracketed value for percentage contribution of the correlated transition.



**Figure S5** 2D fs-TA spectra at the late time delays (~0-6000 ps) after 267 nm excitation of 1mCyt in H<sub>2</sub>O.



**Figure S6** (a) 2D fs-TRF spectra, (b) experimental ( $\circ$ ,  $\square$ ,  $\diamond$ ) and fitted (solid lines) TRF intensity decay profiles, (c) 2D fs-TRFA spectra, and (d) decay associated spectra (DAS) from global analysis of fs-TRF spectra after 267 nm excitation of 1mCyt in  $\text{CH}_3\text{CN}$ . The time delays for 2D spectra shown in (a) are 0, 0.1, 0.15, 0.2, 0.3, 0.4, 0.6, 0.85, 1.25, 1.75, 2.5 and 10 ps. Insert in (a) shows intensity normalized TRF spectra at the denoted time delays. # due to solvent Raman scattering.

**Table S11** Photophysical parameters obtained from fs-TRF for 1mCyt in H<sub>2</sub>O and CH<sub>3</sub>CN with 267 nm excitation

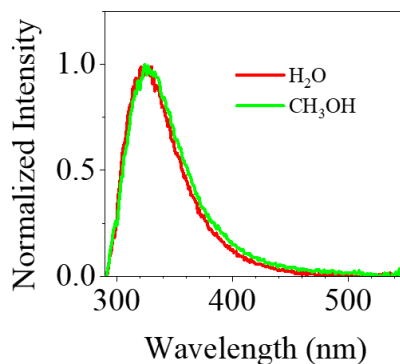
Solvent	Probe <sup>a</sup> /nm	$\tau_1^b$ /ps ( $a_1$ )		$\tau_2^b$ /ps ( $a_2$ )	
H <sub>2</sub> O	309		(0.90)		(0.10)
	330	0.18±0.01	(0.81)	0.76±0.03	(0.19)
	364		(0.77)		(0.23)
CH <sub>3</sub> CN	309		(0.77)		(0.23)
	330	0.20±0.01	(0.54)	0.77±0.01	(0.46)
	364		(0.34)		(0.66)

<sup>a</sup>Wavelength for TRF time profile examined in the kinetic analysis; <sup>b</sup>Time constant ( $\tau_i$ ,  $i=1-2$ ) and the corresponding pre-exponential factor ( $a_i$ ,  $i=1-2$ ) obtained from kinetic analysis of the TRF decay.

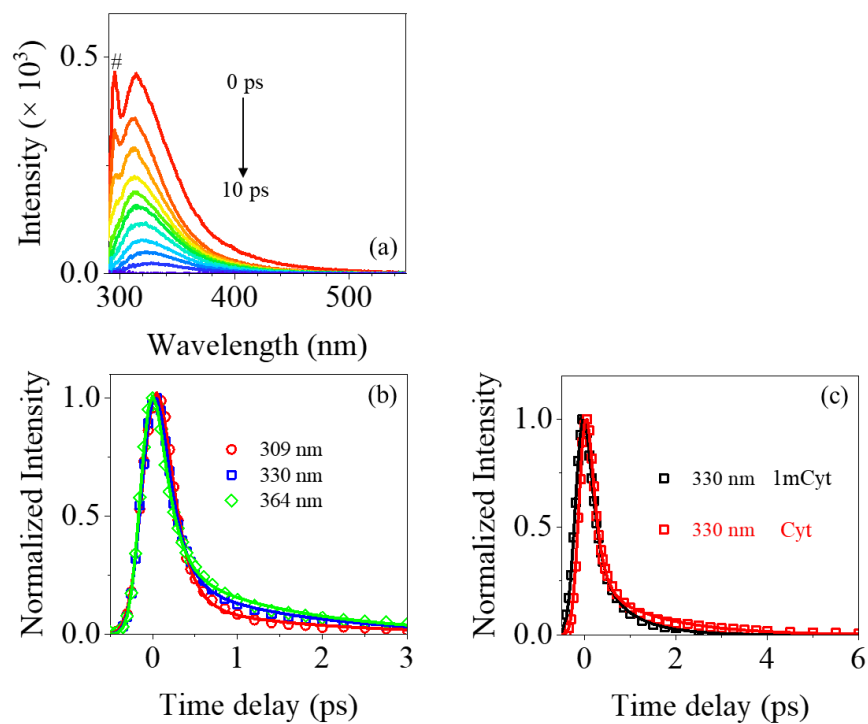
**Table S12** Photophysical parameters obtained from fs-TA for 1mCyt in H<sub>2</sub>O with 267 nm excitation

Probe <sup>a</sup> /nm	$\tau_1^b$ /ps ( $a_1$ )	$\tau_2^b$ /ps ( $a_2$ )	$\tau_3^{b,c}$ /ps ( $a_3$ )	$\tau_4^{b,c}$ /ps ( $a_4$ )
337	0.18	(-0.68)	0.76 (0.29)	5.7 (0.02) (0.01)
451	±0.01	(0.38)	±0.03 (0.46)	±0.3 (0.11) 100000 (0.05)
551		(0.59)	(0.41)	

<sup>a</sup>Wavelength for TA time profile examined in the kinetic analysis; <sup>b</sup>Time constant ( $\tau_i$ ,  $i=1-4$ ) and the corresponding pre-exponential factor ( $a_i$ ,  $i=1-4$ ) obtained from kinetic analysis of the TA decay; <sup>c</sup>Time-independent offset.

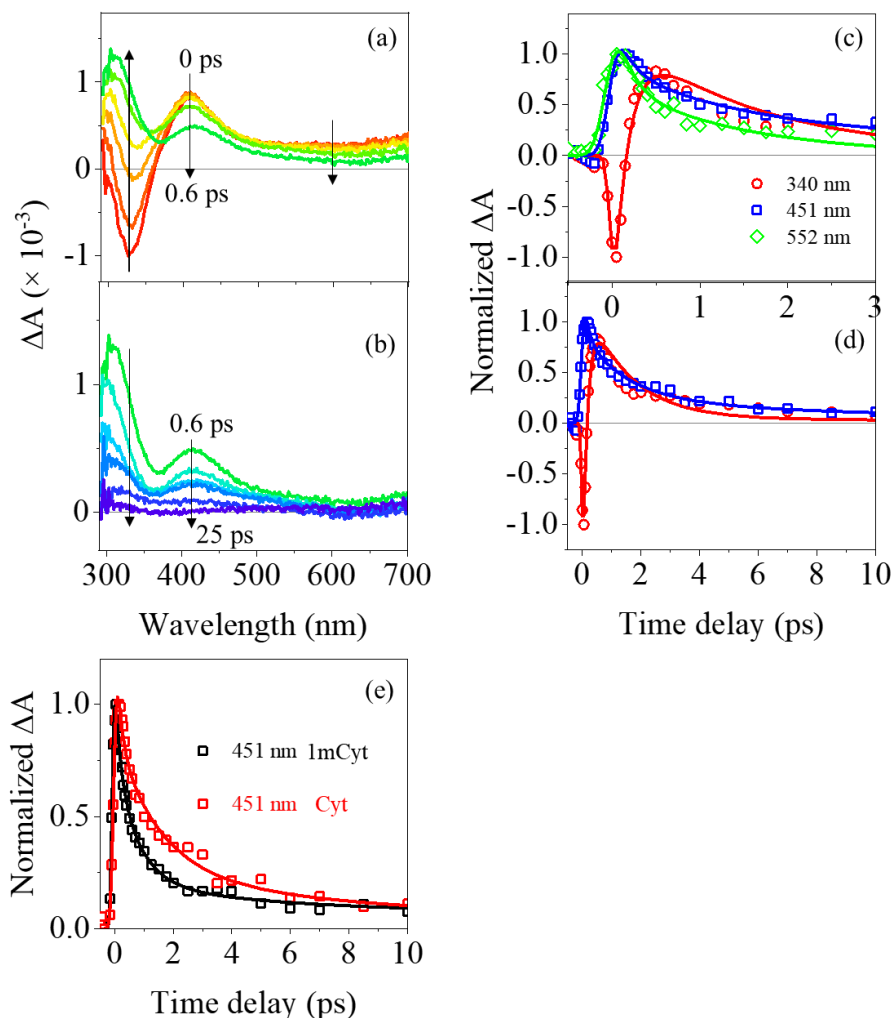


**Figure S7** Normalized fluorescence spectra ( $\lambda_{ex} = 267$  nm) of 1mCyt in H<sub>2</sub>O and CH<sub>3</sub>OH.



**Figure S8** (a) 2D fs-TRF spectra, (b) experimental ( $\circ$ ,  $\square$ ,  $\diamond$ ) and fitted (solid lines) TRF intensity decay profiles from global analysis of fs-TRF spectra after 267 nm excitation of Cyt in  $\text{H}_2\text{O}$ . And (c) Experimental ( $\square$ ) and fitted (solid lines) TRF intensity decay profiles at 330 nm from global analysis of fs-TRF spectra after 267 nm excitation of 1mCyt and Cyt in  $\text{H}_2\text{O}$ . The time delays for 2D spectra shown in (a) are 0, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.7, 1, 1.8 and 10 ps. # due to solvent Raman scattering.





**Figure S9** (a-b) 2D fs-TA spectra, (c-d) experimental ( $\circ$ ,  $\square$ ,  $\diamond$ ) and fitted (solid lines) TA intensity decay profiles at the denoted wavelengths in (c) ( $\sim$ 0-3 ps), (d) ( $\sim$ 0-10 ps) the early time delays after 267 nm excitation of Cyt in H<sub>2</sub>O. And (e) Experimental ( $\square$ ) and fitted (solid lines) TA intensity decay profiles at 451 nm from global analysis of fs-TA spectra after 267 nm excitation of 1mCyt and Cyt in H<sub>2</sub>O. The time delays for 2D spectra shown in (a) are 0, 0.1, 0.15, 0.2, 0.3 and 0.6 ps, while those in (b) are 0.6, 1.25, 2, 3, 6 and 25 ps.

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