Supplementary Information

Remarkable Effects of Solvent and Substitution on Photo-dynamics of Cytosine: A Femtosecond Broadband Time-resolved Fluorescence and Transient Absorption Study

Chensheng Ma,*[1] Chopen Chan-Wut Cheng,[2] Chris Tsz-Leung Chan,[2] Ruth Chau-Ting Chan[2] and Wai-Ming Kwok*[2]

[1] *College of Chemistry and Chemical Engineering, Shenzhen University, Shenzheng, Guangdong, P. R. China.*
[2] *Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong, P. R. China.*

E-mail: macs@szu.edu.cn; wm.kwok@polyu.edu.hk

Experimental details:

Cytosine nucleobase (Cyt), nucleosides of Cyt including cytidine (Cyd) and 2'-deoxycytidine (dCyd), and nucleotides of Cyt including cytidine 5'-monophosphate (CMP) and 2'-deoxycytidine 5'-monophosphate (dCMP) were purchased from Sigma Chemical Company. C5-derivatives of Cyt including 5-methylcytidine (m5Cyd) and 5-methyl-2'-deoxycytidine (m5dCyd) were purchased from TCI. They were used without further purification. 50 mM pH7 potassium aqueous buffer and spectroscopic grade methanol (CH_3OH) were used to prepare sample solution. To prepare pH7 buffered water (H_2O), appropriate amounts of potassium dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were dissolved in 18.2 MΩ double deionized (DDI) water produced by a MILLIPORE (Milli-Q Synthesis) system. In the steady state measurements, UV-vis absorption and steady state fluorescence spectra was respectively recorded by using a Hitachi U-3900H spectrometer and a Jobin Yvon Fluoromax-4 spectrofluorometer.

Time-resolved experimental setups for the broadband fs time-resolved fluorescence (fs-TRF), fs-TRF anisotropy (fs-TRFA), and fs-transient absorption (fs-TA) measurements have been described in detail elsewhere.[1-5] Briefly, the measurement were done based on a commercial ultrafast Ti:Sapphire regenerative amplifier laser system and home-built fs-TRF and fs-TA spectrometers. The sample solutions (with concentration of ~2-4 mM) were excited by a femtosecond pump laser pulse with wavelength at 267 nm, which was generated by an optical parametric amplifier pumped by part of the 800 nm fundamental (1 kHz repetition rate, ~35 fs duration) from the regenerative amplifier of Ti:Sapphire laser system. The subsequent excited-state processes were probed by a second pulse to monitor the temporal evolution of the broadband transient fluorescence and the transient absorption spectrum in the measurement of fs-TRF and fs-TA, respectively.

In the measurements of fs-TRF and fs-TRFA, the Kerr-gate technique was employed.[1-6] With this method, a Kerr-gate device consists of a 1 mm thickness of quartz plate (Kerr medium) equipped between a crossed polarizer pair was driven by an 800 nm gating pulse to function as an ultrafast shutter to sample the transient fluorescence signal at varied selected pump/probe delays. For the measurement of total TRF spectrum, in order to eliminate the effect of rotational diffusion, the polarization direction of the pump laser was set at the magic angle in relative to that of the first polarizer in the Kerr-gate devise. To acquire the spectra of fs-TRFA (r(t)), the TRF measurements at a certain time delay were done by setting the polarization direction of the pump laser to be either parallel or perpendicular to that of the first polarizer in the Kerr-gate device to allow measurement of spectrum $I_{para}(t)$ and $I_{perp}(t)$, respectively. The r(t) was then derived according to Equation 1.[4-6]

$$r(t) = (I_{para}(t) - I_{perp}(t)) / (I_{para}(t) + 2I_{perp}(t)) \quad \text{Equation 1}$$

The fs-TRFA at around time zero (r(0), Figure 1, Figure S2-S3, Table S1) were obtained based on the above equation using $I_{para}(t)$ and $I_{perp}(t)$ spectra recorded at time interval of ~100 fs after the excitation.[4,5] The measurements of $I_{para}(t)$ and $I_{perp}(t)$ and therefore the r(t) derived at all the selected time intervals (Figure 1, Figure S2-S4, Table S1) were not affected by the solvents and the cell windows for the experiments were done using spectrophotometric grade solvents and high quality CaF_2 windows that showed negligible fluorescence signal across the spectral range monitored and the temporal regime of the measurements.

In the fs-TA measurements, the sample was probed by a white-light-continuum (WLC) pulse created by a CaF_2 plate pumped by an 800 or 400 nm laser pulse to allow spectra to be

recorded over wavelength range of 340-700 nm or 240-340 nm, respectively. The presented fs-TA spectra which cover wavelengths from 240-700 nm were obtained by combining the 800 nm and the corresponding 400 nm pump spectra recorded at selected delay times after the photo-excitation.

The instrument response function (IRF) for the measurements is wavelength-dependent, better at longer wavelength. The IRF varies from ~50 to ~200 fs for the measurements of fs-TRF and fs-TRFA which cover wavelengths from ~300 nm to 700 nm. In the measurement of fs-TA, the IRF varies from ~100 to 350 fs as wavelength changes from 700 nm to 240 nm.

For both the fs-TRF(A) and fs-TA, the temporal delay of pump to probe pulse was varied using a computer controlled optical delay line. In each of the measurements, sample solution (with concentration of ~2-4 mM) of ~30 ml was flowed in a cell with 0.5 mm path length to avoid photo-degradation. The transient signals produced by the photo-excitation were collected by a monochromator and detected with a liquid nitrogen cooled CCD detector before readout by computer for further analyses. In order to avoid saturation effect and unwanted multi-photon, the energy of the pump laser beam was kept low with a typical laser pulse peak power of ~2 GW/cm^2.

All fs time-resolved spectra were obtained from subtraction of the appropriately scaled probe-before-pump time delay signal from the pump-probe time delay signal, and were corrected for the detection system transmission/sensitivity variation with wavelength and the wavelength-dependent time shift due to the group velocity dispersion (GVD). The wavelength sensitivity variation correction curve was obtained by comparing the 20 ps TRF spectrum of trans-stilbene (this covers the spectral range of interest in the present study and shows very little change on the shape of the TRF spectra at difference time delays) with its sensitivity corrected steady-state spectrum from a commercial fluorescence spectrometer. The GVD of the system was corrected by equation $\delta t = A\lambda^2 - B\lambda + C$, where δt is time shift (fs), λ is wavelength (nm), and A, B, and C are scaling factors determined by the optics arrangement and solvent used in the measurement.[1] The scaling factors were estimated by peak fitting of the TRF spectra of white light continuum created by intense pump pulse and the values of A, B, and C were estimated to be 0.0033, 4.512, and 1254 in the current measurement.

The samples were monitored by UV-vis absorption and revealed no degradation after the fs time-resolved experiments.

Kinetic analysis:

For the analysis of the kinetic decay profiles of the fs-TRF and fs-TA intensities, the experimental time profiles (*F(t)*) were fitted by convolution of the instrument response function (*g(t)*) with a multiple exponential function (*f(t)*) as showed in equations 1-3:[1-4]

$$\boldsymbol{F(t) = \int_{-\infty}^{t} g(t')\, f(t-t')\, dt'} \qquad \text{Equation 2}$$

with $$\boldsymbol{f(t) = \sum_{i}^{n} a_i \exp(-t/\tau_i)} \qquad \text{Equation 3}$$

$$\boldsymbol{\sum_{i}^{n} a_i = 1} \qquad \text{Equation 4}$$

Where τ_i is time constant and a_i the relative amplitude associated to τ_i.

Bi-exponential function (n = 2) was used for $f(t)$ in the fitting of the decay profiles of fs-TRF. For the analysis of the time profiles in fs-TA, tri-exponential (n = 3) and bi-exponential (n = 2) function with a minute long-lived offset represented by an additional component with fixed time constant of 100 ns was used, respectively, for the case of Cyt and its N1 derivatives and the case of the C5 derivatives.

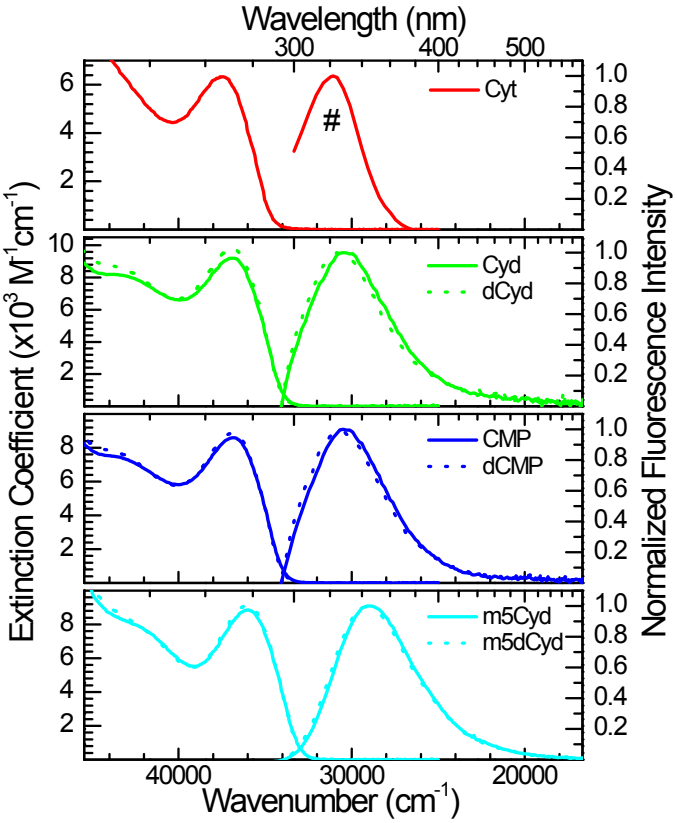

Figure S1. Steady state absorption (left) and normalized fluorescence spectra (right) of Cyt, its nucleosides (Cyd and dCyd) and nucleotides (CMP and dCMP) and its C5-substituted derivatives (m5Cyd and m5dCyd) in pH7 buffered H_2O. The fluorescence spectra were recorded with excitation at 267 nm wavelength. [#]Spectrum from reference 7

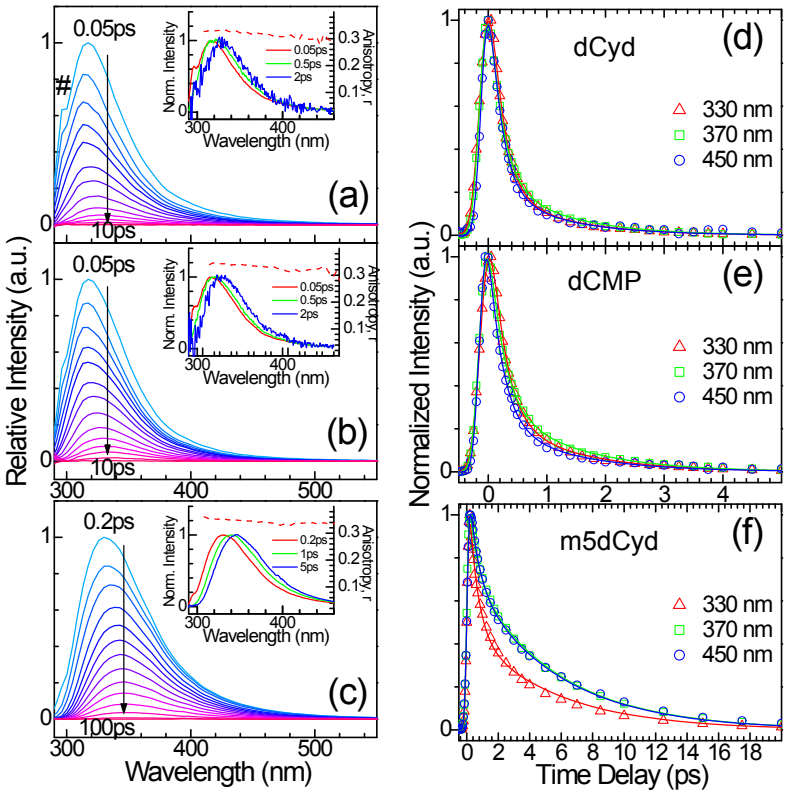

Figure S2. (a-c) Fs-TRF spectra and (d-f) fluorescence decay profiles at selected wavelengths obtained for (a, c) dCyd, (b, d) dCMP and (c, f) m5dCyd in pH7 buffered H_2O. The spectra were recorded at time intervals of (a)-(b) 0.05-10 ps (0.05, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 0.7, 1, 1.4, 2, 3, 5 and 10 ps) and (c) 0.2-100 ps (0.2, 0.35, 0.5, 0.85, 1.25, 1.75, 2.5, 3.5, 5, 7, 10, 15, 25 and 100 ps) after 267 nm photo-excitation. The insets show around teim zero TRF anisotropy spectra and intensity normalized TRF spectra at (a, b) 0.05, 0.5 and 2 ps and (c) 0.2, 1 and 5 ps after the photo-excitation. The arrows indicate directions of spectral evolutions. [#]Raman line of water.

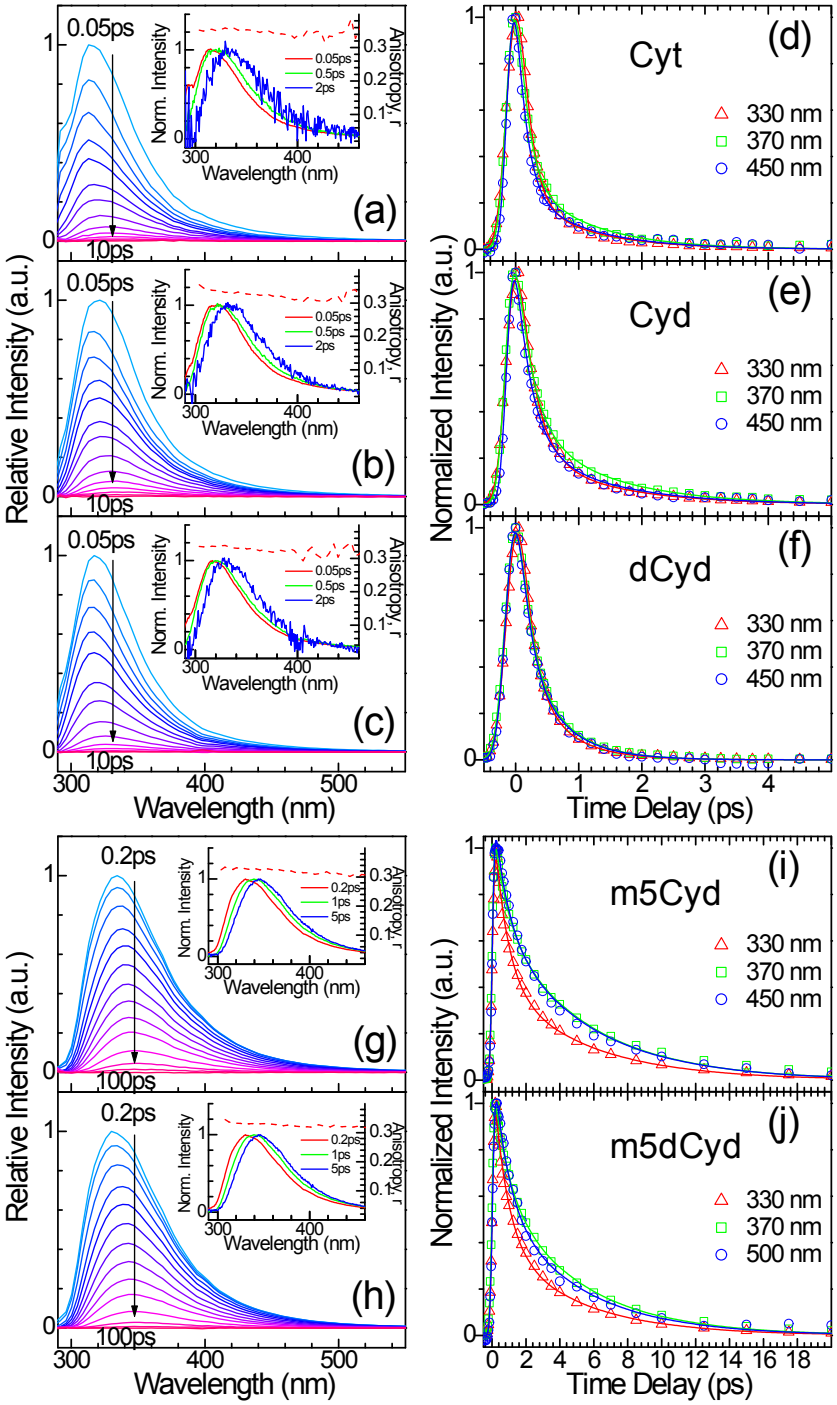

Figure S3. (a-c) and (g-h) Fs-TRF spectra and (d-f) and (i-j) fluorescence decay profiles at selected wavelengths obtained for (a, d) Cyt, (b, e) Cyd, (c, f) dCyd, (g, i) m5Cyd and (h, j) m5dCyd in methanol. The spectra were recorded at time intervals of (a-c) 0.05-10 ps (0.05, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 0.7, 1, 1.4, 2, 3, 5 and 10 ps) and (g-h) 0.2-100 ps (0.2, 0.3, 0.4, 0.6, 0.85, 1.25, 1.75, 2.5, 3.5, 5, 8.5, 15, 25 and 100 ps) after 267 nm photo-excitation. The insets show around time zero TRF anisotropy spectra and intensity normalized TRF spectra at (a-c) 0.05, 0.5 and 2 ps and (g-h) 0.2, 1 and 5 ps after the photo-excitation. The arrows indicate directions of spectral evolutions.

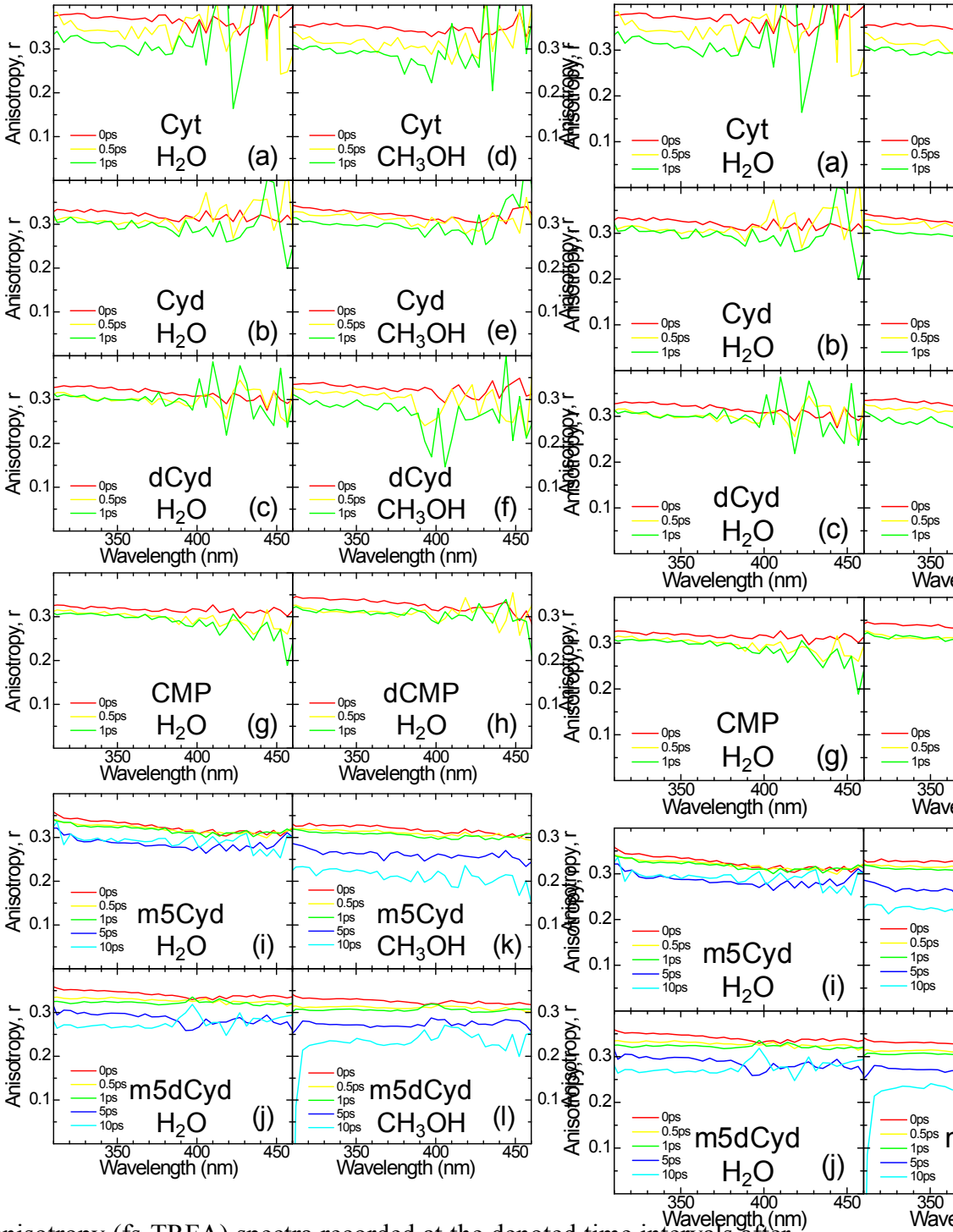

Figure S4. Fs-TRF anisotropy (fs-TRFA) spectra recorded at the denoted time intervals after 267 nm excitation of Cyt and its N1 and C5 derivatives in water and methanol.

Table S1. Spectral and dynamic parameters obtained from fs-TRF and fs-TRFA measurements for cytosine and its N1- and C5-substituted derivatives with 267 nm excitation

	Solvent	[a]Probe/nm	[b]τ_1/ps (a_1)		[b]τ_2/ps (a_2)		[c]r(0)
		330		(0.89±0.02)		(0.11±0.02)	
Cyt	H_2O (pH 7)	370	0.17±0.03	(0.85±0.02)	1.46±0.1	(0.15±0.03)	0.37±0.01
		450		(0.90±0.02)		(0.10±0.02)	
		330		(0.90±0.02)		(0.10±0.03)	
Cyt	CH_3OH	370	0.16±0.03	(0.83±0.02)	1.05±0.1	(0.17±0.03)	0.35±0.01
		450		(0.86±0.02)		(0.14±0.02)	
		330		(0.88±0.02)		(0.12±0.03)	
Cyd	H_2O (pH 7)	370	0.24±0.03	(0.79±0.03)	1.79±0.1	(0.21±0.03)	0.33±0.01
		450		(0.84±0.02)		(0.16±0.02)	
		330		(0.86±0.02)		(0.14±0.02)	
Cyd	CH_3OH	370	0.25±0.03	(0.77±0.03)	1.29±0.1	(0.23±0.03)	0.33±0.01
		450		(0.81±0.02)		(0.19±0.03)	
		330		(0.88±0.02)		(0.12±0.02)	
dCyd	H_2O (pH 7)	370	0.21±0.03	(0.83±0.02)	1.13±0.1	(0.17±0.03)	0.33±0.01
		450		(0.87±0.02)		(0.13±0.02)	
		330		(0.88±0.02)		(0.12±0.02)	
dCyd	CH_3OH	370	0.22±0.03	(0.81±0.02)	0.84±0.1	(0.19±0.03)	0.33±0.01
		450		(0.82±0.02)		(0.18±0.03)	
		330		(0.82±0.02)		(0.18±0.02)	
CMP	H_2O (pH 7)	370	0.27±0.03	(0.75±0.02)	1.84±0.1	(0.25±0.02)	0.32±0.01
		450		(0.83±0.02)		(0.17±0.02)	
		330		(0.85±0.02)		(0.15±0.03)	
dCMP	H_2O (pH 7)	370	0.24±0.03	(0.78±0.02)	1.29±0.1	(0.22±0.02)	0.34±0.01
		450		(0.85±0.02)		(0.15±0.03)	
		330		(0.69±0.03)		(0.31±0.02)	
m5Cyd	H_2O (pH 7)	370	0.73±0.05	(0.33±0.03)	7.62±0.3	(0.67±0.02)	0.34±0.01
		450		(0.39±0.03)		(0.61±0.02)	
		330		(0.62±0.02)		(0.38±0.03)	
m5Cyd	CH_3OH	370	0.78±0.05	(0.38±0.03)	5.10±0.3	(0.62±0.02)	0.33±0.01
		450		(0.43±0.03)		(0.57±0.02)	
		330		(0.62±0.02)		(0.38±0.03)	
m5dCyd	H_2O (pH 7)	370	0.56±0.05	(0.37±0.03)	5.43±0.3	(0.63±0.02)	0.35±0.01
		450		(0.41±0.02)		(0.59±0.02)	
		330		(0.58±0.02)		(0.42±0.02)	
m5dCyd	CH_3OH	370	0.60±0.05	(0.35±0.03)	4.14±0.3	(0.65±0.02)	0.33±0.01
		450		(0.36±0.02)		(0.63±0.02)	

[a]Wavelength for fluorescence examined in the kinetic analysis. [b]Time constant (τ_i, i = 1, 2) and corresponding pre-exponential factor (a_i, i = 1, 2) obtained from kinetic analysis of the TRF decay. [c]Values of fluorescence anisotropy at around time zero after photo-excitation.

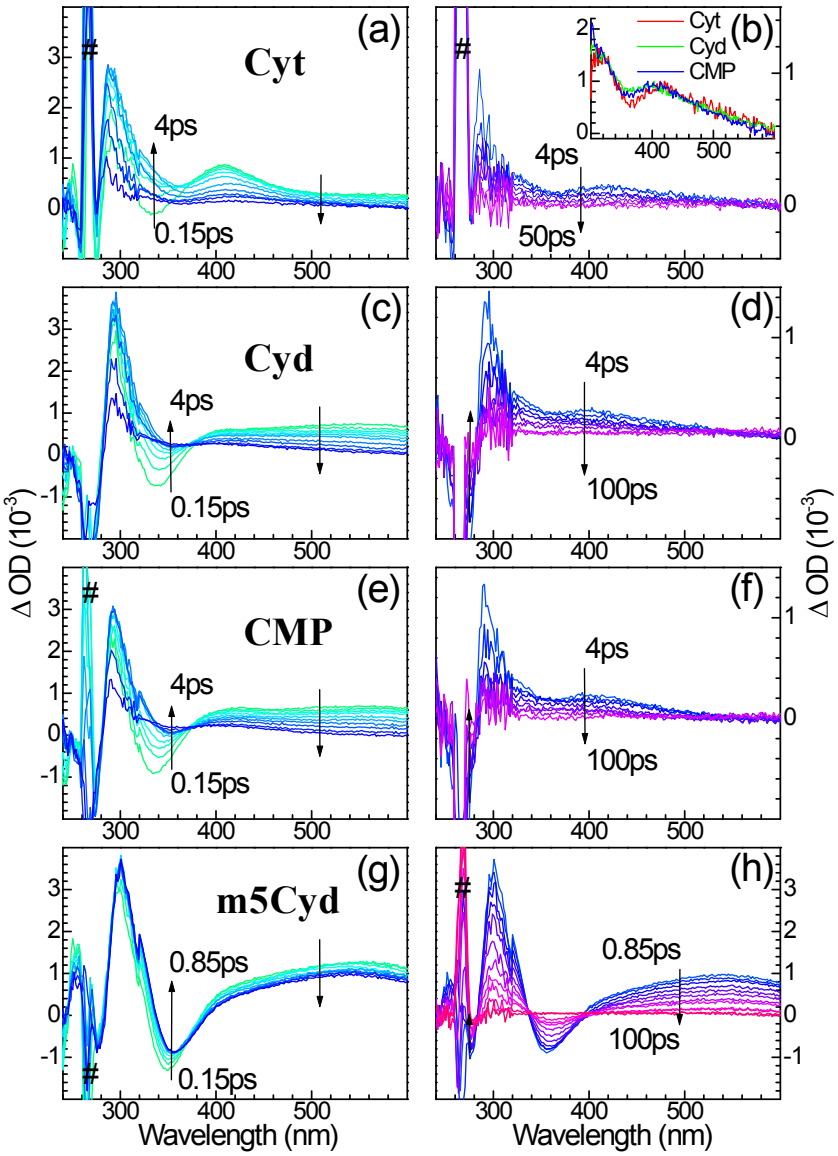

Figure S5(a). Temporal evolutions of fs-TA spectra recoded at early (left) and late (right) time intervals after 267 nm excitation of (a, b) Cyt, (c, d) Cyd, (c, f) CMP and (g, h) m5Cyd in pH7 buffered water. The arrows indicate directions of spectral evolutions. The inset in (b) compares the spectra at ~4 ps after the excitation for Cyt, Cyd and CMP in water. #Due to the pulse of excitation laser.

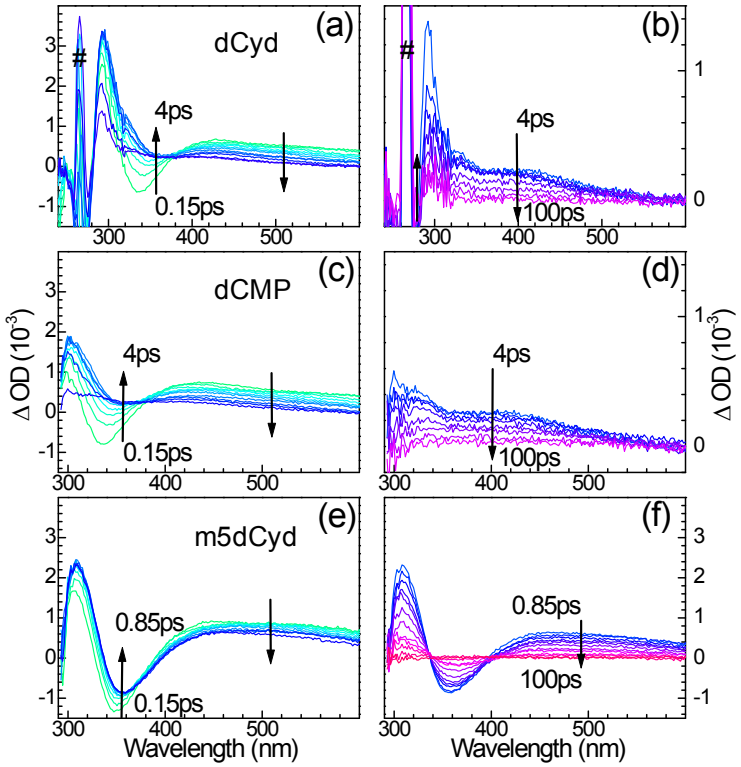

Figure S5(b). Fs-TA spectra recorded with 267 nm excitation of (a, b) dCyd, (c, d) dCMP and (e, f) m5dCyd in pH7 buffered H_2O. The spectra were recorded at time intervals of (a) 0.15-4 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.7, 1, 2.5 and 4 ps) and (b) 4-100 ps (4, 7, 8.5, 10, 20, 40, 60 and 100 ps); (c) 0.15-4 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.7, 1, 1.5 and 4 ps) and (d) 4-100 ps (4, 5, 7, 10, 20, 30, 60 and 100 ps); (e) 0.15-0.85 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.6, 0.7 and 0.85 ps) and (f) 0.85-100 ps (0.85, 1.25, 1.75, 2.5, 3, 4, 6, 8.5, 10, 15, 30 and 100 ps) after the photo-excitation. #Due to the laser for photo-excitation. The arrows indicate directions of the spectral evolutions.

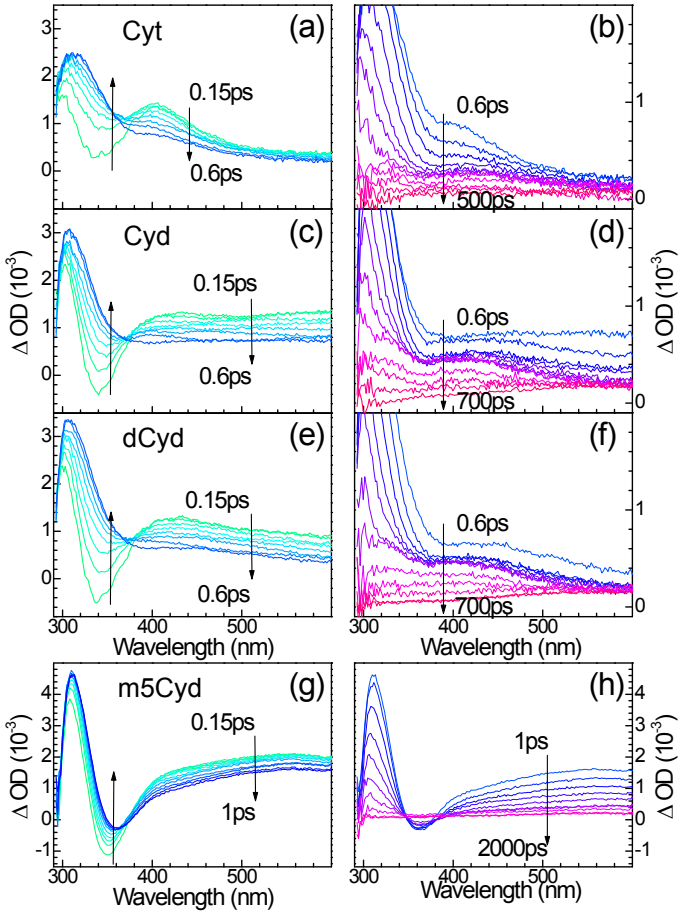

Figure S6. Fs-TA spectra recorded with 267 nm excitation of (a, b) Cyt, (c, d) Cyd, (e, f) dCyd and (g, h) m5Cyd in CH_3OH. The spectra were recorded at time intervals of (a) 0.15-0.6 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5 and 0.6 ps) and (b) 0.6-500 ps (0.6, 0.85, 1.25, 2, 3, 4, 6, 15, 25, 30, 60 and 500 ps); (c) 0.15-0.6 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5 and 0.6 ps) and (d) 0.6-700 ps (0.6, 0.85, 1.25, 1.75, 2.5, 3.5, 5, 8.5, 50, 125, 250 and 700 ps); (e) 0.15-0.6 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5 and 0.6 ps) and (f) 0.6-700 ps (0.6, 1.25, 1.75, 2.5, 3.5, 6, 10, 60, 125, 350, 500 and 700 ps); (g) 0.15-1 ps (0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5, 0.6, 0.85 and 1 ps) and (f) 1-2000 ps (1, 1.75, 3, 5, 7, 10, 15, 30, 500 and 2000 ps) after the photo-excitation. The arrows indicate directions of the spectral evolutions.

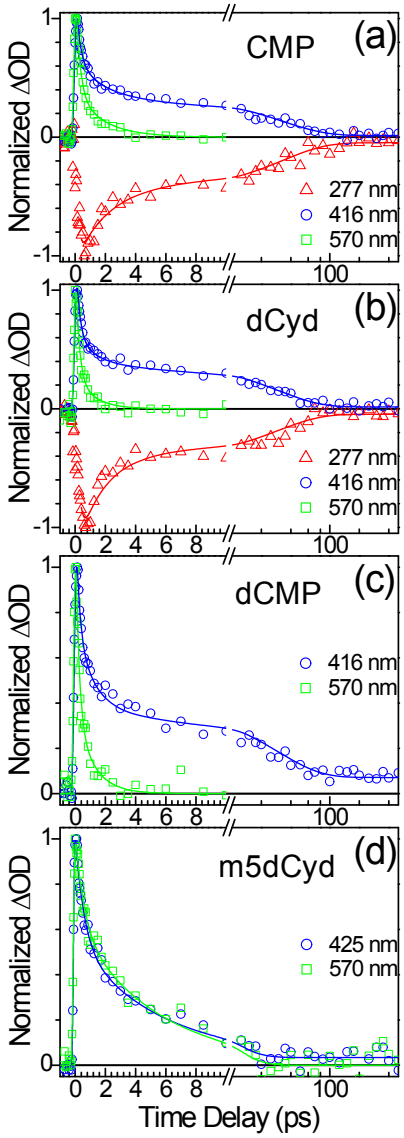

Figure S7. Fs-TA time profiles obtained for (a) CMP, (b) dCyd, (c) dCMP, and (d) m5dCyd with 267 nm excitation in H_2O.

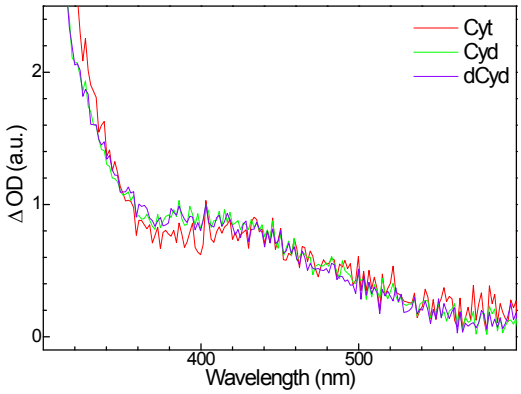

Figure S8. Comparison of fs-TA spectra recorded at ~4 ps after 267 nm excitation of Cyt, Cyd and dCyd in CH_3OH. The spectra were intensity normalized at ~410 nm.

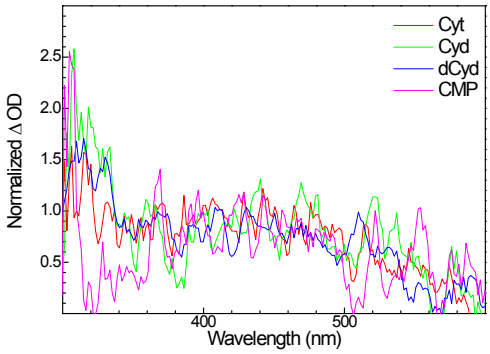

Figure S9. Fs-TA spectra of Cyt, Cyd, dCyd, and CMP in pH7 buffered H_2O obtained at ~85 ps after excitation at 267 nm.

Table S2. Dynamic parameters obtained from fs-TA measurements for Cyt and its N1- and C5-derivaitves with excitation at 267 nm wavelength

Molecule	Solvent	[a]Probe/nm	[b]τ_1/ps (a_1)		[b]τ_2/ps (a_2)		[b]τ_3/ps (a_3)		[b,c]τ_4/ps (a_4)	
Cyt	H_2O	258			2.90±0.1	(-0.75±0.02)		(-0.25±0.04)		
		410	0.17±0.03	(0.58±0.03)	1.46±0.1	(0.31±0.03)	7.74±0.6	(0.10±0.06)	100000	(0.01±0.08)
		570		(0.71±0.02)		(0.29±0.03)				
	CH_3OH	410	0.16±0.03	(0.67±0.02)	1.05±0.1	(0.23±0.03)	51.85±3	(0.08±0.05)		(0.02±0.05)
Cyd	H_2O	277			2.10±0.1	(-0.49±0.04)		(-0.49±0.04)		(-0.02±0.07)
		416	0.24±0.03	(0.52±0.04)	1.79±0.1	(0.20±0.04)	35.00±2	(0.25±0.04)	100000	(0.03±0.06)
		570		(0.76±0.02)		(0.24±0.03)				
	CH_3OH	416	0.25±0.03	(0.61±0.05)	1.29±0.1	(0.13±0.03)	144.03±6	(0.21±0.03)		(0.05±0.06)
dCyd	H_2O	277			1.72±0.1	(-0.66±0.03)	29.67±2	(-0.33±0.05)		(-0.01±0.05)
		416	0.21±0.03	(0.58±0.04)	1.13±0.1	(0.17±0.04)		(0.24±0.03)	100000	(0.01±0.07)
		570		(0.81±0.02)		(0.19±0.05)				
	CH_3OH	416	0.22±0.03	(0.67±0.03)	0.84±0.1	(0.11±0.05)	186.22±6	(0.19±0.04)		(0.03±0.07)
CMP	H_2O	277			2.14±0.1	(-0.60±0.03)		(-0.39±0.05)		(-0.01±0.06)
		416	0.27±0.03	(0.52±0.05)	1.84±0.1	(0.24±0.03)	33.52±2	(0.23±0.04)	100000	(0.01±0.04)
		570		(0.76±0.03)		(0.24±0.02)				
dCMP	H_2O	410	0.24±0.03	(0.50±0.04)	1.29±0.1	(0.24±0.02)	30.00±2	(0.21±0.05)	100000	(0.05±0.07)
		570		(0.80±0.02)		(0.20±0.02)				
m5Cyd	H_2O	279				(-0.99±0.01)				(-0.01±0.04)
		425	0.73±0.05	(0.55±0.03)	7.62±0.3	(0.40±0.04)			100000	(0.05±0.06)
		570		(0.35±0.03)		(0.65±0.03)				
	CH_3OH	570	0.78±0.05	(0.26±0.03)	5.10±0.3	(0.69±0.03)				(0.05±0.05)
m5dCyd	H_2O	425	0.56±0.05	(0.51±0.04)	5.43±0.3	(0.46±0.03)			100000	(0.03±0.06)
		570		(0.44±0.02)		(0.56±0.02)				

[a]Wavelength for the fs-TA time profile examined in the kinetic analysis; [b]Time constants (τ_i, i = 1 – 4) and corresponding pre-exponential factors (a_i, i = 1 – 4) derived from the kinetic analysis of the TA time profiles; [c]Time-independent offsets.

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