

Exploring the vibrational coherences in the ultrafast electronic relaxation of pyrimidine nucleobases and nucleosides

Shutaro Karashima and Toshinori Suzuki*

Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-Ku, Kyoto 606-8502, JAPAN

KEYWORDS: *Nucleobase, Conical intersection, Internal conversion, Ultrafast photoelectron spectroscopy, High harmonic generation, Extreme ultraviolet*

ABSTRACT: We studied the vibrational coherences during the ultrafast internal conversions (ICs) of pyrimidine nucleobases in aqueous solutions and the gas phase, with an instrumental resolution of 14 fs. The coherence of the same ring-breathing vibrational mode with a frequency of 750 cm^{-1} was observed. In the gas phase, the vibrational coherence was transferred during IC from the ${}^1\pi\pi^*$ state to the ${}^1n\pi^*$ state, and it survives for approximately 1 ps. In an aqueous solution, the vibrational dynamics develop in the ${}^1\pi\pi^*$ state because the ${}^1n\pi^*$ state is energetically upshifted via hydrogen bonding with H_2O , and the vibrational coherence is lost on a similar timescale as that of IC to the ground electronic state.

Nucleobases strongly absorb deep ultraviolet light, which can lead to photochemically induced structural damage to deoxyribonucleic acid (DNA), e.g., the formation of cyclobutane pyrimidine dimers.¹⁻⁵ Nucleobases minimize these risks via ultrafast electronic relaxation to their ground electronic states (S_0), which may be a key factor responsible for the evolutionary selection of these molecules as carriers of genetic data.⁶ However, cyclobutane pyrimidine dimers should also form on an ultrashort timescale.⁷⁻¹² Thus, observing nuclear motion during the ultrafast electronic relaxation of nucleobases is of considerable interest, and pioneering attempts to realize this have been reported.¹³⁻¹⁴ Such experiments are highly challenging because they require extremely and sufficiently high temporal and spectroscopic resolutions, respectively, to observe the vibrational coherence and differentiate between signals with different origins. Borrego-Varillas et al. performed ultraviolet transient absorption spectroscopy (UV-TAS) using aqueous uridine (Urd) and 5-methyluridine with a temporal resolution of <30 fs, identifying the vibrational quantum beat associated with ring-breathing vibrations based on the stimulated emission signal from an excited state to S_0 .¹⁵ To the best of our knowledge, no similar detailed ultrafast spectroscopic studies of gaseous nucleobases have been conducted. Therefore, to comprehensively understand the coherent dynamics of photoexcited pyrimidine-based nucleobases, we applied extreme ultraviolet time-resolved photoelectron spectroscopy (EUV-TRPES) to nucleobases in the gas phase and aqueous solutions, with a temporal resolution of 14 fs, which is the highest reported to date.¹⁶

Figure 1(a) shows the EUV-TRPE spectra of thymine (Thy) molecules in the gas phase. The vertical axis represents the electron binding energy (eBE), which is the difference between the observed electron kinetic (eKE) and probe photon energies (21.7 eV). The horizontal axis represents the pump-probe delay. The bandwidth of the extreme ultraviolet radiation (~ 0.4 eV) exceeds that employed in our previous study (~ 0.1 eV)¹⁷, providing broader spectral features. The short-lived signal at

approximately 5 eV is associated with the ionization from the ${}^1\pi\pi^*$ to the π^{-1} cationic state, and the signal at approximately 6 eV is due to the ionization from the ${}^1n\pi^*$ state generated via internal conversion (IC) to the n^{-1} cationic state. The ${}^1\pi\pi^*$ state is deactivated on an ultrashort timescale, and thus, no quantum beat can be identified for the ${}^1\pi\pi^*$ state. The ${}^1n\pi^*$ state exhibits no latency time prior to exponential decay in 36 ± 3 fs, which is consistent with recent computational results.¹⁸ In contrast, the signal representing the ${}^1n\pi^*$ state clearly reveals a vibrational quantum beat that lasts for >500 fs. The ionization signal at approximately 8 eV also reveals a quantum beat, which is discussed later. Figure 1(b) shows the Fourier transforms of these waveforms. The signals at 5.7–6.0 and 6.8–7.1 eV, corresponding to the lower and higher eBE boundaries of the signal associated with ionization from the ${}^1n\pi^*$ state, display the same vibrational frequency, with a prominent peak at 750 cm^{-1} . These quantum beats exhibit a phase difference of π (Figure S8), indicating that these signals represent ionization from the opposite turning points during vibrational motion. This mode corresponds to the ring-breathing vibration associated with a large change in the $\text{N}_1\text{C}_6\text{C}_5$ angle.¹⁵ We performed a Fourier transform within a short time window to estimate the vibrational dephasing time, which is 0.75 ± 0.12 ps for the $\sim 750\text{ cm}^{-1}$ beat of gaseous Thy (Figure 1(c)).

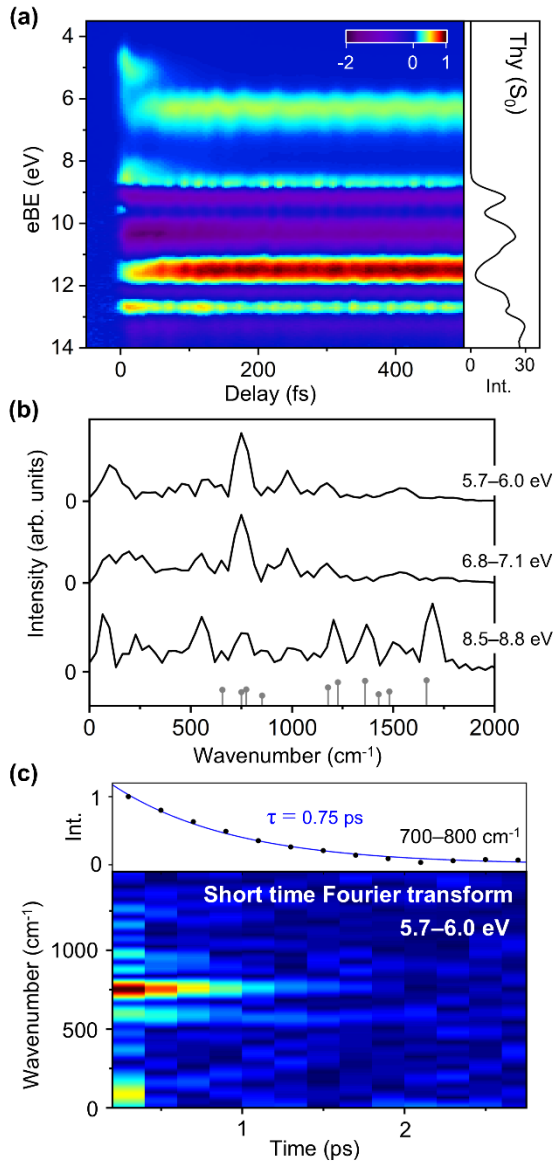


Figure 1. (a) Time-resolved photoelectron spectra of gaseous thymine (Thy), as measured using 267 nm pump and 21.7 eV probe pulses. The photoelectron spectrum in the electronic ground state (S_0) is shown on the right. (b) Frequency spectra of the 5.7–6.0, 6.8–7.1, and 8.5–8.8 eV regions obtained via Fourier transform analysis. The sticks at the bottom show band positions and intensities of the resonance Raman spectra, taken from Ref. 19. (c) Two-dimensional time-frequency map obtained via the short-time Fourier transform of the 5.7–6.0 eV region with a time window of 400 fs. The time profiles integrated over 700–800 cm^{-1} and results of single-exponential fitting are shown on top.

Figure 2 shows the (a) EUV-TRPE spectra, (b) frequency components of the quantum beats, and (c) 2D time-frequency map of gaseous uracil (Ura). The overall spectral features are very similar to those of Thy. The $^1\pi\pi^*$ state exhibits no latency time prior to exponential decay in 17 ± 2 fs. The signals in the 5.7–6.0 and 6.8–7.1 eV regions reveal a phase shift for π (Figure S9). The signal at 8.1–8.4 eV in the time profile exhibits a slow oscillation, which is a clear difference from Thy. The signals at 5.7–6.0 and 6.8–7.1 eV exhibit the same frequency, with a prominent peak at ~ 750 cm^{-1} that lasts for 0.93 ± 0.12 ps.

We evaluated the branching between $^1\pi\pi^* \rightarrow ^1n\pi^*$ and $^1\pi\pi^* \rightarrow S_0$ IC via global fitting analysis (See Supporting Information for details). The estimated quantum yields of the $^1n\pi^*$ states

($QY(^1n\pi^*)$) of Thy and Ura are 0.9 ± 0.1 and 0.5 ± 0.1 , respectively. These values are reasonably consistent with the corresponding values of approximately 1 and 0.45 by Miura et al., respectively.¹⁷ The high temporal resolution in this study enables the clearer separation of the $^1\pi\pi^*$ and $^1n\pi^*$ signals, leading to a more accurate estimation of the $QY(^1n\pi^*)$. The ground-state bleach signals in the eBE region of 10.2–10.7 eV reveal respective recoveries of 20–40% and 30–50% for Thy and Ura, which are broadly consistent with the estimated $QY(^1n\pi^*)$ values.

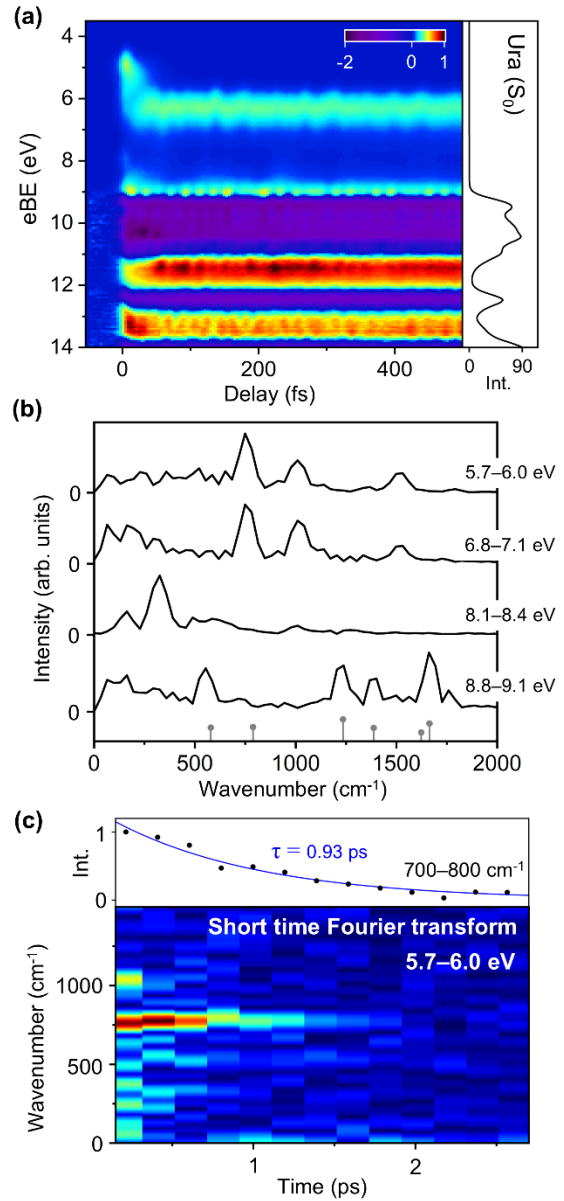


Figure 2. (a) Time-resolved photoelectron spectra of gaseous uracil (Ura), as measured using 267 nm pump and 21.7 eV probe pulses. The photoelectron spectrum in the electronic ground state (S_0) is shown on the right. (b) Frequency spectra in the 5.7–6.0, 6.8–7.1, 8.1–8.4, and 8.8–9.1 eV regions, as obtained via Fourier transform analysis. The sticks at the bottom show band positions and intensities of the resonance Raman spectra, taken from Ref. 20. (c) Two-dimensional time-frequency map obtained via the short-time Fourier transform of the 5.7–6.0 eV region with a time window of 400 fs. The time profiles integrated over 700–800 cm^{-1} and results of single-exponential fitting are shown on the top.

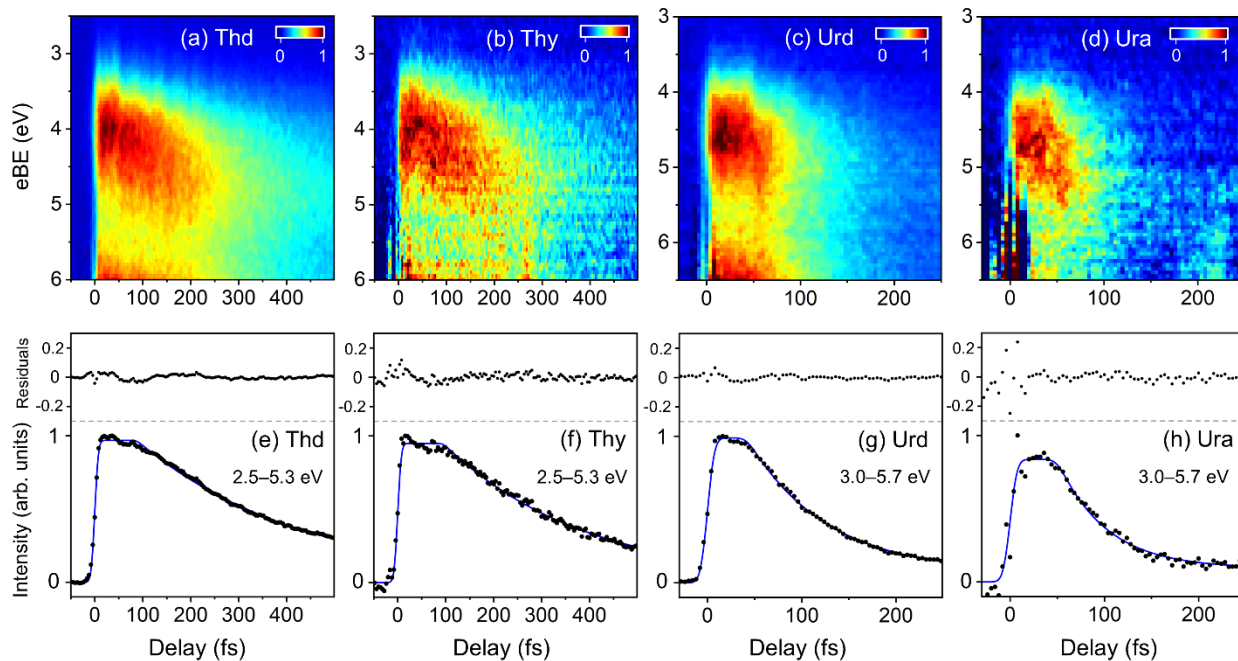


Figure 3. (a)–(d) Time-resolved photoelectron spectra of 150 mM thymidine (Thd), 10 mM thymine (Thy), 150 mM uridine (Urd), and 10 mM uracil (Ura) in H_2O , as measured using 267 nm pump and 21.7 eV probe pulses. The coherent artifact at the time origin has already been subtracted. (e)–(h) Intensity-time profiles integrated over 2.5–5.3 eV for Thd and Thy and 3.0–5.7 eV for Urd and Ura. The experimental results and best-fit curves are shown as black dots and solid blue lines, respectively, and the residuals are shown at the top.

Figures 3(a)–(d) show the TRPE spectra of aqueous 150 mM thymidine (Thd), 10 mM Thy, 150 mM Urd, and 10 mM Ura, respectively. The coherent artifact observed at the time origin has already been subtracted from these spectra (Supporting Information). Very similar spectra are observed for Thd and Thy, and Urd and Ura, indicating that ultrafast dynamics are dictated by nuclear motion within the pyrimidine ring. The signal-to-noise ratios of nucleosides exceed those of nucleobases, primarily because the sample concentrations of the former are higher. Previous experimental and theoretical studies indicate that methyl substitution at the C5 position hinders nuclear (puckering) motion toward the conical intersection between the $^1\pi\pi^*$ and S_0 states and increases the lifetimes of the $^1\pi\pi^*$ states of Thy and Thd.¹⁵ Figures 3(e)–(h) show the time profiles of the integrated photoelectron intensities over the selected eBE ranges, along with the results of least-squares fitting (Supporting Information). Aqueous Thd exhibits a latency time of 88 ± 15 fs prior to decay, with a time constant of 350 ± 10 fs. Aqueous Thd, Thy, Urd, and Ura exhibit similar latencies, with larger values for Thy derivatives. The exponential decay time constants of Thy derivatives are also larger (Table I). The decay profiles shown in Figures 4(a) and 4(b) were measured at the lower eBE boundaries of the $^1\pi\pi^*$ photoelectron bands. The Fourier transforms of the oscillatory components display peaks at ~ 750 cm^{-1} , which are consistent with the corresponding gas-phase results shown in Figures 1 and 2, in addition to the results of UV-TAS of 5-methyluridine reported by Borrego-Varillas et al.¹⁵ (Thd exhibits a deoxyribose component, whereas 5-methyluridine displays a ribose component). Vibrational coherence is generated upon photoexcitation, and it is maintained in the $^1\pi\pi^*$ state in an aqueous solution, whereas it is transferred from the $^1\pi\pi^*$ state to the $^1n\pi^*$ state in the gas phase.

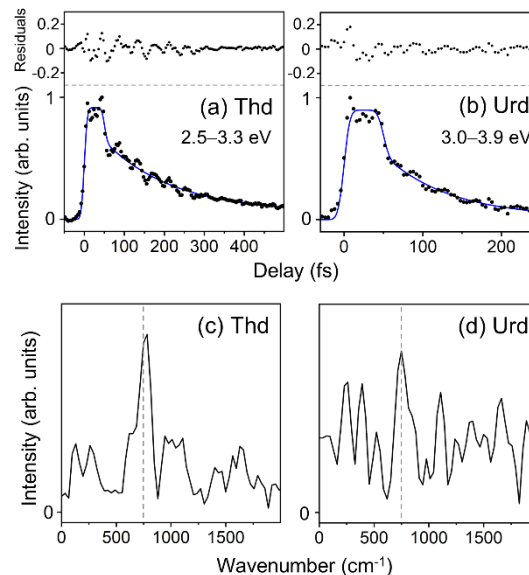


Figure 4. (a), (b) Intensity-time profiles integrated over 2.5–3.3 and 3.0–3.9 eV for thymidine (Thd) and uridine (Urd), respectively, in H_2O . The experimental results and best-fit curves are shown as black dots and solid blue lines, respectively, and the residuals are shown at the top. (c), (d) Frequency spectra of Thd and Urd, as obtained via Fourier transform analysis of the residuals after 14 fs.

The high-frequency beats of gaseous Thy in the 8.5–8.8 eV region and Ura in the 8.8–9.1 eV region clearly differ from those in lower eBE regions.²¹ These spectral features, with characteristic intensities in the 1200–1700 cm⁻¹ region, are consistent with the resonance Raman spectra previously measured at 266 nm.^{19–20} Thus, these beats are attributed to the wavepacket in S₀ generated via impulsive Raman scattering induced by ultrashort pump pulses.

As previously noted by Miura et al., gaseous Ura exhibits a characteristic beat with a low frequency of ~300 cm⁻¹ in the region eBE = 8.1–8.4 eV.¹⁷ Similar results are observed for 6-methyluracil (6MU, Supporting Information).²² This vibrational coherence is likely associated with the motion of the C5–H moiety, which is the major structural difference between Ura, Thy, and 6MU. The different manifestations of the vibrational quantum beats in the eBE regions 5.7–7.1 and 8.1–8.4 eV are

tentatively attributed to the differences in the topographies of the cationic potential energy surfaces responsible for these ionization transitions.

In summary, EUV-TRPES with a temporal resolution of 14 fs enabled the observation of vibrational coherence during the ultrafast electronic relaxation of gaseous and aqueous nucleobases and -sides. The similarity in the spectral features of Ura/Urd and Thy/Thd indicated that ultrafast dynamics occurred primarily in the pyrimidine ring. The vibrational coherence in the ring-breathing mode (750 cm⁻¹) generated in the ¹ππ* state was maintained in an aqueous environment, whereas it was transferred to the ¹nπ* state in an isolated molecule. This vibrational coherence was more clearly observed for Thd compared to that of Urd. These findings deepen our understanding of nuclear motion in the ultrafast IC of pyrimidine nucleobases, -sides, and -tides and possibly in DNA photolesions.

Table I. Experimental cross-correlation times (XCT) between the pump and probe pulses, lifetimes (τ), latency times (t_{lat}), and quantum yields (QYs) of the ¹nπ* states of the gaseous and aqueous nucleobases.

Molecule		XCT (fs)	Dynamics in the ¹ ππ* state		QYs
			τ (fs)	t _{lat} (fs)	
Thy (gas)	Ref. [17]	47	39(1)		~1.0
	This work	14	36(3) ^a		0.9(1) ^a
Ura (gas)	Ref. [17]	47	17(1)		0.45
	This work	14	17(2) ^a		0.5(1) ^a
Thy (aq)	Ref. [17]	100	150(90), 160(90)	-	
	This work	14	310(20) ^a	94(9) ^a	
Ura (aq)	Ref. [17]	100	67(2)	-	
	This work	14	50(10) ^a	49(5) ^a	
Thd (aq)	Ref. [17]	100	87(3), 250(10)	-	
	This work	14	350(10) ^a	88(15) ^a	
Urd (aq)	Ref. [17]	60	120(20)	-	
	This work	14	80(10) ^a	41(6) ^a	

^a Values in parentheses represent the standard deviation evaluated from multiple experimental data sets.

ASSOCIATED CONTENT

Supporting Information.

Supporting Information is available free of charge on the ACS Publications website at DOI:.

Further information regarding the experimental methods and analysis of the results of TRPES (PDF).

AUTHOR INFORMATION

Corresponding Author

***Toshinori Suzuki** – Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan; Email: suzuki@kuchem.kyoto-u.ac.jp

Funding Sources

This study was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant No. 21H04970.

Notes

The authors declare no competing financial interests.

REFERENCES

1. Crespo-Hernández, C. E.; Cohen, B.; Hare, P. M.; Kohler, B., Ultrafast Excited-State Dynamics in Nucleic Acids. *Chem. Rev.* **2004**, *104* (4), 1977-2019, DOI: 10.1021/cr0206770.
2. Schreier, W. J.; Schrader, T. E.; Koller, F. O.; Gilch, P.; Crespo-Hernández, C. E.; Swaminathan, V. N.; Carell, T.; Zinth, W.; Kohler, B., Thymine Dimerization in DNA Is an Ultrafast Photoreaction. *Science* **2007**, *315* (5812), 625-629, DOI: 10.1126/science.1135428.
3. Middleton, C. T.; de La Harpe, K.; Su, C.; Law, Y. K.; Crespo-Hernández, C. E.; Kohler, B., DNA Excited-State Dynamics: From Single Bases to the Double Helix. *Annu. Rev. Phys. Chem.* **2009**, *60*, 217-239, DOI: 10.1146/annurev-physchem.59.032607.093719.
4. Improta, R.; Santoro, F.; Blancafort, L., Quantum Mechanical Studies on the Photophysics and the Photochemistry of Nucleic Acids and Nucleobases. *Chem. Rev.* **2016**, *116* (6), 3540-3593, DOI: 10.1021/acs.chemrev.5b00444.
5. Schreier, W. J.; Gilch, P.; Zinth, W., Early Events of DNA Photodamage. *Annu. Rev. Phys. Chem.* **2015**, *66*, 497-519, DOI: 10.1146/annurev-physchem-040214-121821.
6. Serrano-Andrés, L.; Merchán, M., Are the Five Natural DNA/Rna Base Monomers a Good Choice from Natural Selection? *J.*

Photochem. Photobiol. C: Photochem. Rev. **2009**, *10* (1), 21-32, DOI: 10.1016/j.jphotochemrev.2008.12.001.

7. Huix-Rotllant, M.; Schwinn, K.; Pomogaev, V.; Farmani, M.; Ferré, N.; Lee, S.; Choi, C. H., Photochemistry of Thymine in Solution and DNA Revealed by an Electrostatic Embedding Qm/Mm Combined with Mixed-Reference Spin-Flip Tddft. *J. Chem. Theory. Comput.* **2023**, *19* (1), 147-156, DOI: 10.1021/acs.jctc.2c01010.

8. Conti, I.; Martínez-Fernández, L.; Esposito, L.; Hofinger, S.; Nenov, A.; Garavelli, M.; Improta, R., Multiple Electronic and Structural Factors Control Cyclobutane Pyrimidine Dimer and 6-4 Thymine-Thymine Photodimerization in a DNA Duplex. *Chem. Eur. J.* **2017**, *23* (60), 15177-15188, DOI: 10.1002/chem.201703237.

9. Lee, W.; Matsika, S., Mechanistic Aspects of the Effect of Flanking Nucleotide Sequence on Cpd Formation and Cpd Self-Repair in DNA. *J. Phys. Chem. B* **2023**, *127* (1), 18-25, DOI: 10.1021/acs.jpcc.2c06680.

10. Roca-Sanjuán, D.; Olaso-González, G.; González-Ramírez, I.; Serrano-Andrés, L.; Merchán, M., Molecular Basis of DNA Photodimerization: Intrinsic Production of Cyclobutane Cytosine Dimers. *J. Am. Chem. Soc.* **2008**, *130* (32), 10768-10779, DOI: 10.1021/ja803068n.

11. Schreier, W. J.; Kubon, J.; Regner, N.; Haiser, K.; Schrader, T. E.; Zinth, W.; Clivio, P.; Gilch, P., Thymine Dimerization in DNA Model Systems: Cyclobutane Photolesion Is Predominantly Formed Via the Singlet Channel. *J. Am. Chem. Soc.* **2009**, *131* (14), 5038-5039, DOI: 10.1021/ja900436t.

12. Rauer, C.; Nogueira, J. J.; Marquetand, P.; Gonzalez, L., Cyclobutane Thymine Photodimerization Mechanism Revealed by Nonadiabatic Molecular Dynamics. *J. Am. Chem. Soc.* **2016**, *138* (49), 15911-15916, DOI: 10.1021/jacs.6b06701.

13. Xue, B.; Yabushita, A.; Kobayashi, T., Ultrafast Dynamics of Uracil and Thymine Studied Using a Sub-10 fs Deep Ultraviolet Laser. *Phys. Chem. Chem. Phys.* **2016**, *18* (25), 17044-17053, DOI: 10.1039/c5cp07861j.

14. Prokhorenko, V. I.; Picchiotti, A.; Pola, M.; Dijkstra, A. G.; Miller, R. J., New Insights into the Photophysics of DNA Nucleobases. *J. Phys. Chem. Lett.* **2016**, *7* (22), 4445-4450, DOI: 10.1021/acs.jpclett.6b02085.

15. Borrego-Varillas, R.; Nenov, A.; Kabaciński, P.; Conti, I.; Ganzer, L.; Oriana, A.; Jaiswal, V. K.; Delfino, I.; Weingart, O.; Manzoni, C.; Rivalta, I.; Garavelli, M.; Cerullo, G., Tracking Excited State Decay Mechanisms of Pyrimidine Nucleosides in Real Time. *Nat. Commun.* **2021**, *12* (7285), DOI: 10.1038/s41467-021-27535-7.

16. Karashima, S.; Chen, C. J.; Suzuki, T., Generation of Sub-10-Fs Deep and Extreme Ultraviolet Pulses for Time-Resolved Photoemission Spectroscopy. *Opt. Lett.* **2024**, *49* (13), 3777-3780, DOI: 10.1364/OL.528323.

17. Miura, Y.; Yamamoto, Y. I.; Karashima, S.; Orimo, N.; Hara, A.; Fukuoka, K.; Ishiyama, T.; Suzuki, T., Formation of Long-Lived Dark States During Electronic Relaxation of Pyrimidine Nucleobases Studied Using Extreme Ultraviolet Time-Resolved Photoelectron Spectroscopy. *J. Am. Chem. Soc.* **2023**, *145* (6), 3369-3381, DOI: 10.1021/jacs.2c09803.

18. Park, W.; Lee, S.; Huix-Rotllant, M.; Filatov, M.; Choi, C. H., Impact of the Dynamic Electron Correlation on the Unusually Long Excited-State Lifetime of Thymine. *J Phys Chem Lett* **2021**, *12* (18), 4339-4346, DOI: 10.1021/acs.jpclett.1c00712.

19. Zhu, X.-M.; Wang, H. G.; Zheng, X.; Phillips, D. L., Role of Ribose in the Initial Excited State Structural Dynamics of Thymidine in Water Solution: A Resonance Raman and Density Functional Theory Investigation. *J. Phys. Chem. B* **2008**, *112* (49), 15828-15836, DOI: 10.1021/jp806248b.

20. Yarasi, S.; Ng, S.; Loppnow, G. R., Initial Excited-State Structural Dynamics of Uracil from Resonance Raman Spectroscopy Are Different from Those of Thymine (5-Methyluracil). *J. Phys. Chem. B* **2009**, *113* (43), 14336-14342, DOI: 10.1021/jp9053378.

21. Karashima, S.; Humeniuk, A.; Suzuki, T., Vibrational Motions in Ultrafast Electronic Relaxation of Pyrazine. *J. Am. Chem. Soc.* **2024**, 11067-11071, DOI: 10.1021/jacs.4c02886.

22. Orimo, N.; Yamamoto, Y. I.; Karashima, S.; Boyer, A.; Suzuki, T., Ultrafast Electronic Relaxation in 6-Methyluracil and 5-Fluorouracil in Isolated and Aqueous Conditions: Substituent and Solvent Effects. *J. Phys. Chem. Lett.* **2023**, *14* (11), 2758-2763, DOI: 10.1021/acs.jpclett.3c00195.

TOC Graphic

Vibrational Coherence in Pyrimidine Nucleobases

