Article:
Prokhorenko, VI, Picchiotti, A, Pola, M et al. (2016) New Insights into the Photophysics of DNA Nucleobases. Journal of Physical Chemistry Letters, 7 (22). pp. 4445-4450. ISSN: 1948-7185

https://doi.org/10.1021/acs.jpclett.6b02085

New Insights into the Photophysics of DNA Nucleobases

Valentyn I. Prokhorenko[1#], Alessandra Picchiotti[1], Martina Pola[2], Arend G. Dijkstra[1], and R.J. Dwayne Miller[1, 3, 4 #]

[1]Max Planck Institute for the Structure and Dynamics of Matter, Luruper Chaussee 149, 22761 Hamburg, Germany

[2]Institute for Theoretical Physics, University of Hamburg, Jungiusstrasse 9, 20355 Hamburg, Germany

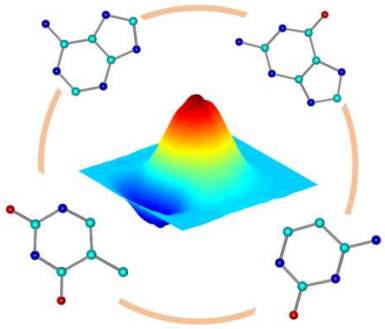
[3]The Departments of Chemistry and Physics, University of Toronto, 80 St. George Street, Toronto, Canada M5S 1H6

[4]The Hamburg Center for Ultrafast Imaging, Luruper Chaussee 149, 22761 Hamburg, Germany

[#]corresponding authors: dwayne.miller@mpsd.mpg.de, valentyn.prokhorenko@mpsd.mpg.de

Abstract

We report the results of an extended time-resolved study of DNA nucleobases in aqueous solutions conducted in the deep UV using broadband femtosecond transient absorption and electronic two-dimensional spectroscopies. We found that the photodeactivation in all DNA nucleobases occurs in two steps – fast relaxation (500-700 fs) from the excited state ππ* to a "dark" state, and its depopulation to the ground state within 1-2 ps. Our experimental observations and performed theoretical modeling allow us to conclude that this dark state can be associated with the nπ* electronic state, which is connected to the excited and ground states via conical intersections.



TOC

Unraveling of the photodeactivation mechanisms in nucleobases and nucleotides is a key factor for understanding DNA photostability in the UV. It is well established that photoexcitation of UV-induced population decays within a few ps or less in DNA nucleobases, whereas in DNA takes much longer time (from tens to hundreds of ps[1-3]). However, the underlying mechanism of this significant difference in deactivation time of the electronic population is still poorly understood and remains debated. Development of ultrafast spectroscopic techniques allowed direct resolution of fast photodeactivation in DNA nucleobases, nucleosides, nucleotides, and DNA oligo-polynucleotides. These have all been intensively studied using femtosecond transient absorption[1-2,4-13] (TA), time-resolved fluorescence[7,13-16] (FS) and photoelectron spectroscopies[3,17-20] (PS). It was established that photodeactivation occurs very rapidly, i.e. within of 0.5-1.5 ps (shorter decay components of <70 fs were also reported in the PS studies). TA spectroscopy plays a major role, since it allows monitoring the dynamics of not only excited state(s), such as in the FS, but also in the ground state (not accessible by PS) and, more importantly, the dynamics of dark states via excited-state absorption (ESA). Due to technical challenges, all reported TA studies to date have been performed in the lowest absorption band (centered around 250-260 nm), either in a single- or two-color fashion (different excitation and monitoring wavelengths) except of a very recent study of thymine[24].

Although a multitude of scenarios, sometimes even controversial[25,26], based on experimental findings and theoretical simulations has been proposed to explain the nature of such a fast photodeactivation in nucleobases (see recent review[23]), a commonly accepted picture is an ultrafast internal conversion in vicinity of conical intersection (CI) connecting potential surfaces of excited and ground states.

To clarify and refine the mechanism of such fast photodeactivation, we performed TA and two-dimensional electronic spectroscopy (2DES) studies of all DNA bases in a large UV-window of 250 – 300 nm (~6000 cm^{-1}), covering their lowest absorption band and excited-state emission band (Fig. 1, see also Fig. 1S in the Supporting Information). The benefits of a broad spectral window, together with extremely short pulse duration (6-7 fs FWHM) are resolution of the entire population dynamics and photodeactivation pathways, allowing establishment of an appropriate spectroscopic model. We found that all bases investigated exhibit similar population kinetics, which can be satisfactorily characterized by two fast decay components with large amplitudes (500-750 fs and 1-2 ps, depending on the compound), as well as one much slower component with a very small amplitude and a lifetime ranges in a 5-50 ps window. The lifetimes of all investigated compounds, retrieved from a global analysis of TA-data, are summarized in Table 1.

Table 1. Lifetimes (in ps) retrieved from the global fitting of TA-data of adenine (Ade), deoxyadenosine triphosphate (dATP), thymine (Thy), cytosine (Cyt), and guanosine (Guo). Uncertainties of fast and medium lifetimes are ~5%, and of slow ~10%.

compound	fast	medium	slow
Ade	0.75	1.51	38.2
dATP	0.7	1.31	8.6
Thy	0.63	1.74	54
Cyt	0.75	1.8	48.3
Guo	0.5	0.9	5.2

However, the most important observation in the TA-kinetics is the appearance of two distinct spectral bands of opposite sign. As an example, Figure 1 demonstrates the TA for Ade, and two cuts at 263 and 280 nm illustrate the remarkable difference in kinetics at different sides of the spectrum (the TA data for other compounds can be inspected in the Supporting Information Fig. 2S). While at shorter wavelengths close to the maximum of absorption spectrum, the population of ππ* excited state decays, on the red side of the TA-spectra, we initially observe fast population growth (differential transmission ΔT becomes negative), which then decays to zero.

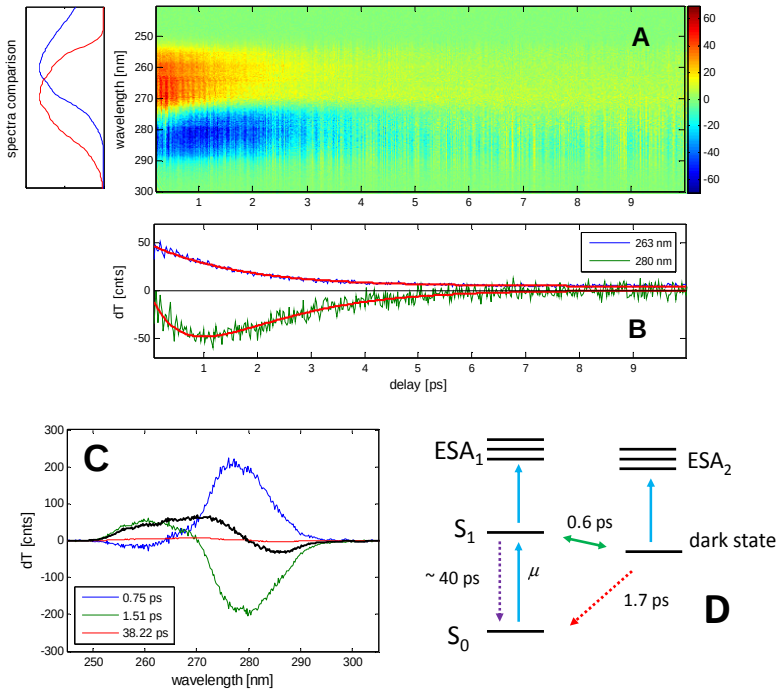


Figure 1. Transient absorption spectra of Ade (A) together with two decay cuts (B) starting from a 50 fs probe delay at 263 and 280 nm. Solid red lines represent fitted transients. The left panel compares normalized absorption (blue) and laser (red) spectra. Panel C shows decay-associated spectra, retrieved from the TA. The black line corresponds to the sum of all DAS, i.e. reflects an initial transient change directly after excitation. Negative shoulder at >280 nm indicates the ESA from the excited state. A phenomenological spectral model describing the TA kinetics is shown in panel D.

This observation indicates rapid excited-state deactivation due to intramolecular population transfer between at least two states. Moreover, since the differential transmission finally decays to zero at all wavelengths, the ground state population is fully recovered and all involved excited states become unpopulated. A negative ΔT on the red side of the spectrum further indicates that the origin of this signal is the ESA from a state to which the excited state population is transferred. These processes are well resolved in the decay-associated spectra (DAS) retrieved from a global analysis of TA data shown in Fig. 1c for Ade. The DAS of major components display the opposite signs at the red- and blue spectral edges and a "butterfly"-like shape typical of transfer processes. In our TA-experiments a broadband excitation pulse (same as a probe pulse) significantly overlaps the absorption band located at 260 nm; therefore, it simultaneously excites all states having allowed transitions. Taking this into account, we can conclude that the TA-signal, growing at the red part of the spectrum (trace @280 nm in Fig. 1b), can only be associated with growing of the dark state population, which is not directly accessible from the ground state but monitored via the ESA. The observed population kinetics can be summarized in a framework of phenomenological spectroscopic model schematically represented in Fig. 1d in terms of a Jablonski diagram, where a key feature is the ESA channel from the dark state. Moreover, without including ESA from the bright $\pi\pi^*$ state, it is not possible to explain the "butterfly-like" shapes of DAS. This simple spectroscopic model satisfactory reproduces measured TA-kinetics and DAS shapes (see Fig. 3S in the Supporting Information). The slow-decaying channel is not included in this model due to its insignificant contribution to the population kinetics as indicated by corresponding DAS in Fig. 1c. It is not clear to which process this long-lived transient can be attributed. One can speculate that it can be originated from the tautomers present in the solution, due to decay of a weakly-populated underlying electronic state not connected to the dark state (e.g., L_b or L_a states, depending on their spectral location), or due to a weak population of a triplet state as proposed for pyrimidines[9,27]. On the other hand, this slow transient, common to all the compounds investigated, can be associated with the cooling of the ground state, which would have undergone heating due to the release of the excess energy after relaxation of the population from the dark state[28]. It should be pointed out that this cooling of the hot ground electronic state was previously attributed by Kohler et al.[4] to the medium component in TA-kinetics (0.9-1.8 ps, Table 1). However, cooling of a solute has non-trivial temporal behavior which cannot be

reduced to a simple exponential function (see eq. 10 in Ref. 29). In addition, since the electronic oscillator strength is temperature-independent and thus the area of the absorption spectrum is conserved, a cooling of a solute should lead to a much larger magnitude in the corresponding DAS at the absorption peak position as compared to the red side. This effect has been demonstrated[28] for the TIN chromophore using femtosecond TA spectroscopy. In our experimental DAS, associated with the medium relaxation times, the situation is either the opposite (Fig. 1 c) or the DAS magnitudes at these wavelengths are similar except for Guo, which has two well-resolved electronic transitions, covered by the laser spectrum. However, we cannot exclude some cooling contribution to these dynamics but rather refer to the dominant processes involved in the excited state relaxation.

Another benefit of extremely short pulses is direct resolution of the fine oscillatory structure visible "by eye" in the TA in Fig. 1. However, it is much better resolved in the heterodyne transient grating measurements due to higher SNR. We detect two long-lasting oscillations with close frequencies for all nucleobases in a whole spectral range of the probe pulse used (Table 1S and Fig. 4S in the Supporting Information). For Ade these frequencies of ~1340 and ~1494 cm^{-1} can be associated with Raman modes corresponding to the stretching modes of a six-member ring (*b2u*) and (*e1u*), respectively[30].

The experimental results of 2DES, performed in the same spectral window, fully support our findings derived from the TA experiments. Since a projection of a 2D spectrum onto an "observation" axis ν_t results in a pump-probe spectrum, one can expect the appearance of two lobes in the 2D spectra with opposite signs along ν_t. Indeed, all experimental 2D spectra of all investigated nucleobases display this remarkable feature (Fig. 2) which can be considered a unique "marker" of a dark electronic state.

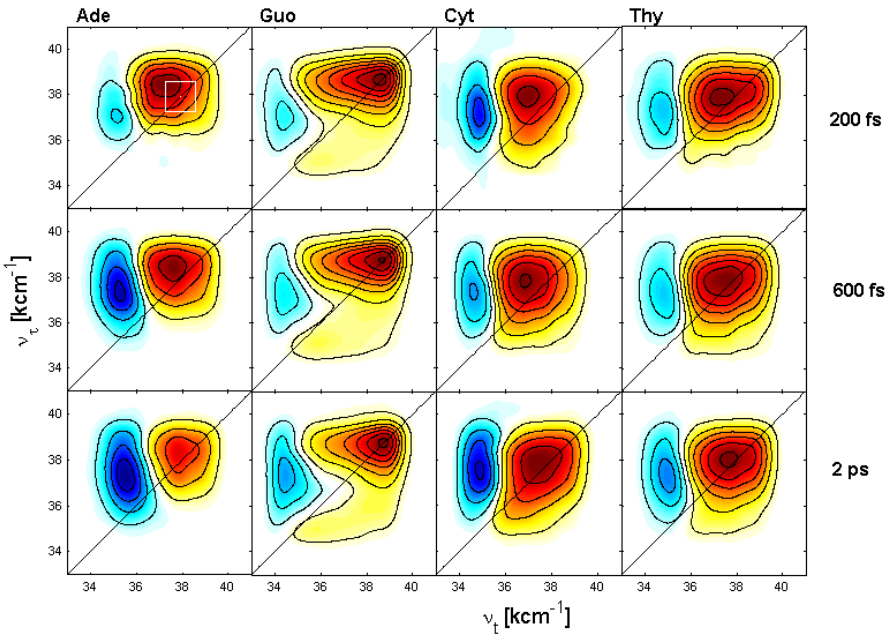


Figure 2. 2DES spectra (real part) of investigated DNA nucleobases for selected waiting times as indicated. The contours steps are in 10% magnitudes; each spectrum is normalized to its maximum. White square indicates a spectral bandwidth realized in[21].

It should be noted that in recently reported 2DES studies of Ade[21-22] the realized spectral bandwidth was approximately 6-12 times smaller (a few nm) which significantly restricts observation capabilities of photodeactivation dynamics. Therefore, these two peaks were not resolved. The mentioned above oscillations are also well resolved in a series of 2D spectra taken with small waiting-time step. Figure 3 shows as an example the oscillations with remarkable magnitudes for Ade monitored at ν_t = 37410 cm^{-1} and ν_τ = 36010 cm^{-1}. Although a detailed analysis is beyond the scope of present paper, we have to note that the phase relations between the oscillations are very different across the 2D spectrum.

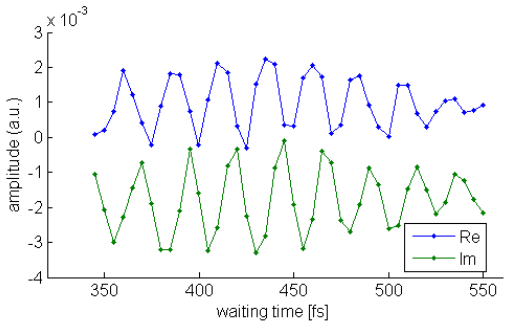


Figure 3. The oscillations observed in 2D spectra of Ade at ν_t = 37410 cm^{-1} and ν_τ = 36010 cm^{-1}.

The information retrieved from 2DES is richer compared to that from TA spectroscopy. In particular, these two lobes in the 2D-spectra of Ade are noticeably tilted with respect to the diagonal, whereas for Cyt and Thy they are arranged in parallel. Moreover, although Ade and Guo are both purines, their 2D-spectra exhibit notable shape differences. The 2D-spectra of Guo manifest two clearly resolved diagonal peaks along with a cross-peak located at around ν_τ = 36000 cm^{-1}, ν_t = 38000 cm^{-1}. This reflects the presence of two electronic transitions (distinguishable also in the absorption spectrum of Guo) sharing the same ground state which leads to a cross-peak[31]. This low-lying diagonal positive peak "shields" a contribution of the negative peak to the pump-probe spectrum and thus decreases the negative amplitudes in TA of Guo at the red side (cf. Fig. 2S in the Supporting Information). Moreover, we can see from these 2D spectra that population transfer to the dark state in Guo occurs from the above-located bright electronic state. We are not detecting any pronounced inhomogeneous broadening in the 2D spectra; they do not exhibit any perceptible elongation along the diagonals. The off-diagonal width of 2D spectra directly relates to the magnitude of homogeneous broadening, which is large (~3000 cm^{-1}) in all nucleobases, so that the associated electronic dephasing time is very short (6-8 fs depending of nucleobase); thus, the system-bath interaction in these compounds is also very strong.

Summarizing our experimental observations and findings, we can conclude that there has to be a universal mechanism of photodeactivation that is equally valid for all DNA bases, independent of their structural peculiarities (purines vs. pyrimidines). Plenty of scenarios have been already proposed and considered[23]; however, all of them assume different pathways involving different electronic states for different nucleobases. A universal mechanism of such a two-step photodeactivation for *all* nucleobases and investigated nucleotides may be offered: the population passes from an initially excited bright state $\pi\pi^*$ through an $n\pi^*$ (dark) state to the ground state via two conical intersections as schematically depicted in Fig. 4a. It should be noted that a similar scenario has been proposed and discussed for Cyt[32] and Ade[33]. However, we believe that this scenario should be valid for all DNA nucleobases since the only dark state common to all nucleobases is the $n\pi^*$ state. To support this mechanism, we performed theoretical modeling of 2D spectra in vicinity of two CI's and ESA's (see details in the Supporting Information). Despite some model restrictions, the calculated 2D spectra match the observed spectra very well. Figure 4b shows the calculated 2D spectrum at T = 330 fs (more can be found in the Supporting Information in Fig. 5S). The main feature of the 2DES experimental spectra – two peaks with the opposite signs – is clearly reproduced, along with the overall spectral shape. We should point out that both heating and cooling of the ground state are automatically included in these simulations as time-dependent displacement of the tuning coordinate. However, if we restrict the spectroscopic model to only one CI (i.e. direct crossing of the $\pi\pi^*$

and ground states), we don't find reasonable agreement between the simulated and measured 2D spectra.

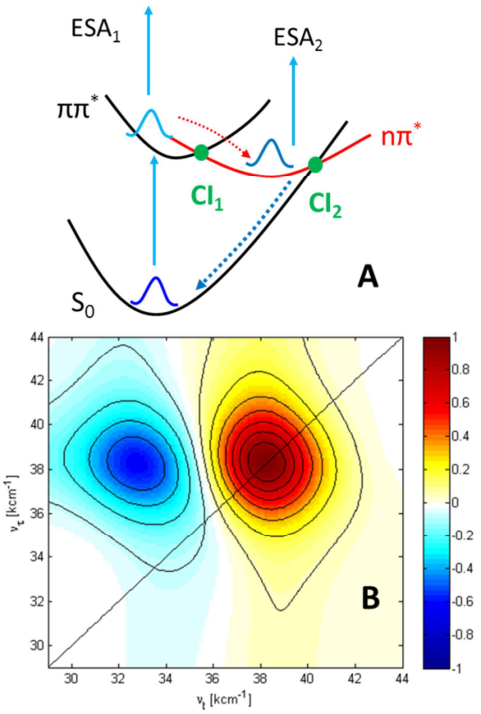


Figure 4. (A) Quantum-mechanical spectroscopic model for DNA nucleobases: population from initially excited bright state $\pi\pi^*$ flows within 500-750 fs through a conical intersection CI_1 to dark state $n\pi^*$ and then, within 1.5-2 ps, is transferred back to ground state S_0 via second conical intersection CI_2. (B) The 2DES spectrum, calculated in framework of this model, clearly displays two peaks with the opposite signs observed in the experimental 2D spectra.

The possible origins of CI's in nucleobases were extensively investigated in numerous quantum-chemistry theoretical studies. Yet we would like to stress that these calculations were performed starting from perfectly optimized molecular structures. In a "living" molecule, immersed in a fluctuating environment (water at RT), a large number of CI's can be realized so that the population transfer between the excited $\pi\pi^*$ state and the dark $n\pi^*$ state is actually unavoidable.

In summary, we experimentally demonstrated that all DNA nucleobases have identical photodeactivation mechanism characterized by two-step relaxation of induced electronic population through the dark state which, on the basis of our modeling and experimental findings, we can associate with the $n\pi^*$ state. This state connects the excited state $\pi\pi^*$ and ground state via two conical intersections. The established spectroscopic model for single DNA bases can help to develop the spectroscopic models for DNAs and thus in unraveling of their photodeactivation mechanism and understanding the DNA photostability.

Experimental Methods

Supporting Information is available that provides detailed information regarding the samples, the experimental setup and the data treatment.

Notes

The authors declare no competing financial interest.

Acknowledgments

This work was funded by the Max Planck Society (Germany). A.P. acknowledges a scholarship from IMPRS-UFAST, A.G.D. acknowledges support by a Marie Curie International Incoming Fellowship grant #627864. V.I.P. thanks for the helpful discussions with M. A. Kochman regarding quantum-chemistry modeling of nucleobases.

Supporting Information Description

Detailed information regarding the sample's preparation and measuring conditions, the absorption- and TA-spectra of all investigated compounds (Figures 1S, 2S), description of the experimental setup used and the data processing, numerical simulation of the TA kinetics (Figure 3S), the long-lasting oscillations observed in the transient grating signals from the nucleobases (Figure 4S and Table 1S), modeling of the two-dimensional electronic spectra along with the calculated 2D- and TA spectra (Figures 5S and 6S).

References

(1) Crespo-Hernandez, C. E.; Cohen, B.; Hare, P. M.; Kohler, B. Ultrafast Excited-State Dynamics in Nucleic Acids. *Chem. Rev.* **2004**, *104*, 1977-2019.

(2) Middleton, C. T.; de La Harpe, K.; Su, C.; Law, Y. K. Crespo-Hernandez, C. E.; Kohler, B. DNA Excited-State Dynamics: From Single Bases to the Double Helix. *Ann. Rev. Phys. Chem.* **2009**, *60*, 217-239.

(3) Bisgaard, C. Z.; Satzger, H.; Ulrich, S.; Stolow, A. Excited-State Dynamics of Isolated DNA Bases: A Case Study of Adenine. *ChemPhysChem* **2009**, *10*, 101-110.

(4) Pecourt, J-M. L.; Peon, J.; Kohler, B. DNA Excited-State Dynamics: Ultrafast Internal Conversion and Vibrational Cooling in a Series of Nucleosides. *J. Am. Chem. Soc.* **2001**, *123*, 10370-10378.

(5) Kang, H.; Lee, K. T.; Jung, B.; Ko, Y. J.; Kim, S. K. Intrinsic Lifetimes of the Excited States of DNA and RNA Bases. *J. Am. Chem. Soc.* **2002**, *124*, 12958-12959.

(6) Crespo-Hernandez, C. E.; Cohen, B.; Kohler, B. Base Stacking Controls Excited-State Dynamics in A·T DNA. *Nature* **2005**, *436*, 1141-1144.
(7) Kwok, W-M.; Ma, C.; Phillips, D. L. Femtosecond Time- and Wavelength-Resolved Fluorescence and Absorption Spectroscopic Study of the Excited States of Adenosine and an Adenine Oligomer. *J. Am. Chem. Soc.* **2006**, *128*, 11894-11905.
(8) Buchvarov, I.; Wang, Q.; Raytchev, M.; Trifonov, A.; Fiebig, T. Electronic Energy Delocalization and Dissipation in Single- and Double-Stranded DNA. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4794-4797.
(9) Hare, P. M.; Crespo-Hernandez, C. E.; Kohler, B. Internal Conversion to the Electronic Ground State Occurs via Two Distinct Pathways for Pyrimidine Bases in Aqueous Solution. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 435-440.
(10) Kwok, W-M.; Ma, C.; Phillips, D. L. A Doorway State Leads to Photostability or Triplet Photodamage in Thymine DNA. *J. Am. Chem. Soc.* **2008**, *130*, 5131-5139.
(11) Su, C.; Middleton, C. T.; Kohler, B. Base-Stacking Disorder and Excited-State Dynamics in Single-Stranded Adenine Homo-Oligonucleotides. *J. Phys. Chem. B* **2012**, *116*, 10266-10274.
(12) Chen, J.; Kohler, B. Base Stacking in Adenosine Dimers Revealed by Femtosecond Transient Absorption Spectroscopy. . *J. Am. Chem. Soc.* **2014**, *136*, 6362-6372.
(13) Ma, C.; Cheng, C. C-W.; Chan, C. T-L.; Chan, R. C-T.; Kwok, W-M. Remarkable Effects of Solvent and Substitution on the Photo-Dynamics of Cytosine: A Femtosecond Broadband Time-Resolved Fluorescence and Transient Absorption Study. *Phys. Chem. Chem. Phys.* **2015**, *17*, 19045-19057.
(14) Peon, J.; Zewail, A. H. DNA/RNA Nucleotides and Nucleosides: Direct Measurement of Excited-State lifetimes by Femtosecond Fluorescence Up-Conversion. *Chem. Phys. Lett.* **2001**, *348*, 255-262.
(15) Onidas, D.; Markovitsi, D.; Marguet, S.; Sharonov, A.; Gustavsson, T. Fluorescence Properties of DNA Nucleosides and Nucleotides: A Refined Steady-State and Femtosecond Investigation. *J. Phys. Chem. B* **2002**, *106*, 11367-11374.
(16) Pancur, T.; Schwab, N. K.; Renth, F.; Temps, F. Femtosecond Fluorescence Up-Conversion Spectroscopy of Adenine and Adenosine: Experimental Evidence for the $\pi\sigma^*$ State? *Chem. Phys.* **2005**, *313*, 199-212.
(17) Ulrich, S.; Schulz, T.; Zgierski, M. Z.; Stolow, A. Direct Observation of Electronic Relaxation Dynamics in Adenine via Time-Resolved Photoelectron Spectroscopy. *J. Am. Chem. Soc.* **2004**, *126*, 2262-2263.
(18) Ulrich, S.; Schulz, T.; Zgierski, M. Z.; Stolow, A. Electronic Relaxation Dynamics in DNA and RNA Bases Studied by Time-Resolved Photoelectron Spectroscopy. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2796-2801.

(19) Evans, N. L.; Ulrich, S. Wavelength Dependence of Electronic Relaxation in Isolated Adenine Using UV Femtosecond Time-Resolved Photoelectron Spectroscopy. *J. Phys. Chem. A* **2010**, *114*, 11225-11230.
(20) Buchner, F.; Nakayama, A.; Yamazaki, S.; Ritze, H-H.; Lübcke, A. Excited-State Relaxation of Hydrated Thymine and Thymidine Measured by Liquid-Jet Photoelectron Spectroscopy: Experiment and Simulation. *J. Am. Chem. Soc.* **2015**, *137*, 2931-2938.
(21) West, B. A.; Womick, J. M.; Moran, A. M. Probing Ultrafast Dynamics in Adenine with Mid-UV Four-Wave Mixing Spectroscopies. *J. Phys. Chem. A* **2011**, *115*, 8630-8637.
(22) Tseng, C-h.; Sándor, P.; Kotur, M.; Weinacht, T. C.; Matsika, S. Two-Dimensional Fourier Transform Spectroscopy of Adenine and Uracil Using Shaped Ultrafast Laser Pulses in the Deep UV. *J. Phys. Chem. A* **2012**, *116*, 2654-2661.
(23) Improta, R.; Santoro, F.; Blancafort, L. Quantum Mechanical Studies on the Photophysics and the Photochemistry of Nucleic Acids and Nucleobases. *Chem. Rev.* **2016**, *116*, 3540-3593.
(24) 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*, 17044-17053.
(25) Broo, A. A Theoretical Investigation of the Physical Reason for the Very Different Luminescence Properties of the Two Isomers Adenine and 2-Aminopurine. *J. Phys. Chem. A* **1998**, *102*, 526-531.
(26) Sobolewski, A. L.; Domcke, W. On the Mechanism of Nonradiative Decay of DNA Bases: Ab Initio and TDDFT Results for the Excited States of 9H-Adenine. *Eur. Phys. J.* **2002**, *D 20*, 369-374.
(27) Merchán, M.; Serrano-Andrés, L.; Robb, M. A.; Blancafort L. Triplet-State Formation along the Ultrafast Decay of Excited Singlet Cytosine. *J. Am. Chem. Soc.* **2005**, *127*, 1820-1825.
(28) Frey, W.; Elsaesser, T. Femtosecond Intramolecular Proton Transfer of Vibrationally Hot Molecules in the Electronic Ground State. *Chem. Phys. Lett.* **1992**, *189*, 565-570.
(29) Iwata, K.; Hamaguchi, H-o. Microscopic Mechanism of Solute-Solvent Energy Dissipation Probed by Picosecond Time-Resolved Raman Spectroscopy. *J. Phys. Chem. A* **1997**, *101*, 632-637.
(30) Hirakawa, A. Y.; Okada, H.; Sasagawa S.; Tsuboi, M. Infrared and Raman Spectra of Adenine and Its ^{15}N and ^{13}C Substitution Products. *Spectrochim. Acta A* **1985**, *41*, 209-216.
(31) Jonas, D. M. Two-Dimensional Femtosecond Spectroscopy. *Annu. Rev. Phys. Chem.* **2003**, *54*, 425-465.

(32) Ismail, N.; Blancafort, L.; Olivucci, M.; Kohler, B.; Robb, M. A. Ultrafast Decay of Electronically Excited Singlet Cytosine via a π,π^* to n_0,π^* State Switch. *J. Am. Chem. Soc.* **2002**, *124*, 6818-6819.
(33) Chen, H.; Li, S. Theoretical Study toward Understanding Ultrafast Internal Conversion of Excited 9H-Adenine. *J. Phys. Chem. A* **2005**, *109*, 8443-8446.