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Electronic relaxation dynamics in DNA and RNA bases studied by time-resolved photoelectron spectroscopy

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We present femtosecond time-resolved photoelectron spectra (TRPES) of the DNA and RNA bases adenine, cytosine, thymine, and uracil in a molecular beam. We discuss in detail the analysis of our adenine TRPES spectra. A global two-dimensional fit of the time and energy-resolved spectra allows for reliable separation of photoelectron spectra from several channels, even for overlapping bands. *Ab initio* calculations of Koopmans' ionization correlations and He(I) photoelectron spectra aid the assignment of electronically excited states involved in the relaxation dynamics. Based upon our results, we propose the following mechanism for electronic relaxation dynamics in adenine: Pump wavelengths of 250, 267 and 277 nm lead to initial excitation of the bright $S_2(\pi\pi^*)$ state. Close to the band origin (277 nm), the lifetime is several picoseconds. At higher vibronic levels, *i.e.* 250 and 267 nm excitation, rapid internal conversion ($\tau < 50$ fs) populates the lower lying $S_1(n\pi^*)$ state which has a lifetime of 750 fs. At 267 nm, we found evidence for an additional channel which is consistent with the dissociative $S_3(\pi\sigma^*)$ state, previously proposed as an ultrafast relaxation pathway from $S_2(\pi\pi^*)$. We present preliminary results from TRPES measurements of the other DNA bases at 250 nm excitation.

Introduction

The excited state dynamics of biomolecules almost invariably involves the non-adiabatic coupling of vibrational with electronic degrees of freedom, leading to the redistribution of both charge and energy within the molecule. Time-Resolved Photoelectron Spectroscopy (TRPES) is emerging as an important technique for the study of non-adiabatic dynamics in polyatomic molecules and has been applied to a range of problems including internal conversion, photoisomerization, excited state proton and electron transfer, and photodissociation.[1] As photoelectron spectroscopy is sensitive to both molecular orbital configurations and vibrational dynamics, it seems well suited to the general study of electronic relaxation processes in molecules. Here we report the application of femtosecond TRPES to the study of excited state dynamics in the DNA and RNA bases adenine, cytosine, thymine, and uracil, isolated in a molecular beam.

We begin with a review of excited state dynamics in adenine. To date, *ab initio* quantum mechanical studies on 9H adenine suggest two different electronic relaxation pathways. Broo[2] determined the avoided crossing point of the lowest $n\pi^*$ and $\pi\pi^*$ states with a barrier amounting to 0.6 kJ mol^{-1} (calculated as the energy difference between the equilibrium geometry of the $\pi\pi^*$ state and the lowest excited state in the avoided crossing). Coupling of both excited states follows through six membered ring puckering. Further out-of-plane distortion was suggested to promote internal conversion to the S_0 ground state. Sobolewski and Domcke[3] presented another possible pathway for internal conversion along the azine NH stretch coordinate. The repulsive $\pi\sigma^*$ potential energy function intersects with the first excited $\pi\pi^*$ state and the S_0 state. These symmetry allowed crossings are converted into conical intersections through out-of-plane motion of the dissociating H atom. Despite the numerous experimental studies on adenine, at present these deactivation pathways still remain under discussion. In the following, we will present new experimental evidence for the coexistence of both relaxation pathways.

Experimental support for close proximity of the $n\pi^*$ state to the $\pi\pi^*$ state and involvement of the former in the excited state relaxation process has been reported in several spectrally resolved and time-resolved studies. The $\pi\sigma^*$ states, however, are dark in absorption and their potential energy surfaces are dissociative along the NH coordinate, making them difficult to detect experimentally. The resonance enhanced multi-photon ionization (REMPI) spectrum of 9H adenine is dominated by a transition to the first $\pi\pi^*$ state at 36 105 cm^{-1} but evolves into a broad band at excitation energies above 37 000 cm^{-1}.[4,5] A few considerably smaller features further to the red were assigned to vibrationally excited states of the first $n\pi^*$ transition of the 9H tautomer.[6] The lifetimes of DNA bases have been determined to be generally very short. Recent transient absorption measurements of DNA nucleosides in aqueous solution revealed lifetimes of a few hundred fs at an excitation wavelength of 263 nm.[7] In the gas-phase, exponential decay times of a few ps for the DNA bases were measured by photoionization yield spectroscopy at 267 nm.[8] For adenine, additionally the lifetime of the lowest $\pi \rightarrow \pi^*$ transition at 276.9 nm (36 105 cm^{-1}) was determined to be 9 ps.[9] The experimental techniques employed in these studies, however, provide no direct information as to the electronic character of the excited states involved in the deactivation process. To gain further insight, Kang *et al.*[10] used substitution and deuteration experiments on adenine to selectively perturb $n\pi^*$ and $\pi\sigma^*$ states. The failure to observe significant effects on the decay times led them to conclude that the $\pi\sigma^*$ state is not actively involved in the deactivation process.

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We have used femtosecond TRPES to investigate the excited state dynamics of isolated adenine, cytosine, thymine, and uracil in a molecular beam. As opposed to time-resolved ion yield measurements, TRPES allows for direct identification of the states involved in the electronic relaxation process through projection of the excited state dynamics onto cationic states.[11] In a simple picture, emission of an independent outer electron occurs without simultaneous electronic reorganization of the ion core (Koopmans' theorem or frozen core approximation). Ionization into specific ion electronic states varies with respect to the molecular orbital nature of the neutral electronic states. Hence photoelectron spectroscopy can provide information on the character of the excited state and, since ionization is always allowed, states appearing 'dark' in absorption can be probed. TRPES requires that the cationic states have been well characterized *e.g.* by He(I) photoelectron spectra and high level *ab initio* calculations. We recently reported fs TRPES spectra of adenine.[12] Comparison of spectra recorded at 250 and 267 nm excitation showed, that although the main decay pathways can be attributed to $S_2(\pi\pi^*) -> S_1(n\pi^*)$, a minor second decay pathway might be present at 267 nm excitation. Speculatively, we assigned this decay channel to the $\pi\sigma^*$ pathway predicted by Sobolewski and Domcke.[3] In this paper we will present the detailed analysis of our adenine data and add further support from simulations of Franck–Condon structures of photoelectron bands corresponding to ionization of the $\pi\pi^*$ and $\pi\sigma^*$ states. In addition, we present new experimental time-resolved photoelectron spectra of the DNA/RNA bases cytosine, thymine, and uracil, and a preliminary discussion of their analysis.

Experimental and computational methods

Experimental details

The TRPES apparatus used in this study has been described in detail elsewhere.[13] A simple high temperature glass nozzle was used to introduce the sample into a magnetic bottle photoelectron spectrometer. The nozzle assembly consists of a glass tube, a Vespel plug with an orifice of 500 μm diameter and an Ultra-Torr Cajon fitting to the carrier gas inlet. The DNA bases (Sigma-Aldrich, used without further purification) are applied (as a saturated solution) onto pre-cleaned fibreglass wool. The glass wool was then packed tightly into the glass tube nozzle. This simple method serves to significantly increase the surface area, thus enhancing the thermal desorption rate over the thermal decomposition rate. The whole assembly is heated to 220–250 °C using cartridge heaters and a heating lamp, which additionally kept the molecular beam skimmer at high enough temperatures to avoid clogging. Helium at a stagnation pressure of 120 Torr was used as a carrier gas for the expansion. The simplicity of this design brings several benefits: (1) O-rings, that potentially disintegrate at high temperatures in a chemical environment, are avoided. (2) The highly stable continuous flow allows for pump–probe experiments at kHz rates and, consequently, extensive signal averaging. (3) The source is easy to set up and to clean. This source has been successfully employed for studies on all DNA bases except for guanine. [We have recently developed a general kHz laser ablation source for biomolecule spectroscopy of more demanding samples and demonstrated its successful use for stable (>12 hours) kHz rate guanine desorption.][14] Before photoelectron data acquisition, the spectrometer was switched to ion time-of-flight detection in order to confirm that the monomer of the sample was the dominant species in the molecular beam. We observed neither significant decomposition/fragmentation of the sample nor the formation of higher clusters. Previous ns-double resonance and high resolution spectroscopic studies led us believe that we observe the 9H tautomer of adenine,[6,15] but it is currently unclear which tautomers were observed for cytosine, thymine and uracil. A recent REMPI study showed the presence of keto and enol forms of cytosine under molecular beam conditions.[16]

Two time-delayed fs laser pulses interact with the doubly skimmed molecular beam in the ionization region of the spectrometer. A laser cross-correlation (instrument response function) of 160 fs FWHM was determined *via* probing 1,3-butadiene and NO. The cross-correlation function was used in the subsequent deconvolution of the time-resolved data and generally permits unambiguous determination of time constants down to about 20% of the cross-correlation FWHM (in other words, ~40 fs). In addition, the well known photoelectron spectra of NO and butadiene were used for electron energy calibration. The energy resolution of our apparatus is generally limited to ≥30 meV by the experimental set up and the bandwidth of the fs laser pulses. Excitation wavelengths of 250 nm and, for adenine, 267 and 277 nm were additionally employed. Ionization was promoted in all cases reported here by a fs 200 nm pulse. A photoelectron spectrum was recorded for different pump–probe delays. Pump-only and probe-only (*i.e.* one color, multi-photon) photoelectron spectra are simultaneously recorded by chopping the individual lasers. These dynamically subtracted TRPES spectra contain time-dependent pump–probe (and sometimes probe–pump) signals only, without any static background.

The two dimensional (time and energy) data were fit with the following global function

$$S(E,t) = \sum_i I_i(E)[P_i(t) \otimes g(t)]$$

in order to extract the excited state population dynamics $P_i(t)$ and the corresponding photoelectron spectra $I_i(E)$ for each ionization channel i. We assumed exponential rise and decay for all excited states and a Gaussian time-profile $g(t)$ for the laser cross-correlation. We used a Levenberg–Marquardt algorithm to simultaneously fit spectra and dynamics to the two-dimensional data.

Computational details

We employed *ab initio* quantum chemical calculations to support the analysis of our time-resolved photoelectron spectra. For adenine, Koopmans' ionization correlations (*i.e.* the one-photon, one-electron propensity rules) were determined at the B3LYP/6-31++G** level of theory. We analyzed valence molecular orbitals of the geometry-optimized ground S_0 and D_0 states. Vertical excitation energies were computed using TDDFT methods.

For the Franck–Condon (FC) simulations of adenine photoelectron bands, geometries and force-fields for each electronic state (S_0, $S_{\pi\sigma^*}$, $S_{\pi\pi^*}$, D_0 and D_1) were obtained in HF and CIS calculations with the 6-31++G** basis set. The FC structure of electronic transitions (absorption and photoionization) is governed by the displacement parameters calculated for each *final* electronic state vibration. The last specification ensured that the mode mixing is taken care of in a simple way, for each transition, and to a good approximation.[17]

The dimensionless displacement parameter for the ith a_1 mode in the S_k state is defined as:[18]

$$B_i(S_k) = 0.1725\omega_i\{(S_k)^{1/2}[\boldsymbol{X}_{S_0} - \boldsymbol{X}_{S_k}]\boldsymbol{\mu}^{1/2}L\}$$

where X_{S_k} is the $3N$-dimensional vector of the Cartesian coordinates of the N atoms in the S_k electronic state, $\boldsymbol{L}$ is the $3N \times (3N-6)$ matrix that transforms mass-weighted coordinates into normal coordinates, ω_i is the frequency of the vibration in cm^{-1}, and $\boldsymbol{\mu}$ is the $3N \times 3N$ diagonal matrix of atomic masses. To calculate the continuous FC structure of the spectrum, we assign the same Lorentzian linewidth $\Gamma = 300\ cm^{-1}$

to each individual vibronic transition. If the frequency of a given mode is the same in the neutral and ionized species, then the relative intensity of the $0 \rightarrow n$ vibronic transition for this mode with respect to the intensity of the $0 \rightarrow 0$ line is:

$$\frac{I_{0-n}^{(i)}}{I_{0-0}} = \frac{B_i^{2n}}{2^n n!}$$

Results and discussions

Time-resolved photoelectron spectra of adenine

In a recent communication[12] we presented time-resolved photoelectron spectra of adenine recorded at 250, 267 and 277 nm. Here, we provide more detail on the analysis of these data and draw additional support from *ab initio* calculations. In Fig. 1(a) we show the "raw" (background subtracted) data as recorded with our magnetic bottle photoelectron spectrometer at 267 nm excitation.

We simultaneously fit both spectra and dynamics, allowing us to separate the two dimensional time-resolved photoelectron spectrum into time-resolved photoelectron spectra for each contributing channel (see Fig. 1(c)–(f)). The residuals remaining (Fig. 1(b)) when the fitted time-resolved photoelectron spectra are subtracted from the two dimensional data are an indication of the quality of the fit. For adenine, four channels with different dynamics were required to obtain a good fit of the experimental data. The residuals (see Fig. 1 (b)) show only statistical noise and no systematic errors. The quality of the fit is critically dependent on the number of channels included. To ensure the necessity for inclusion of a ns channel we have performed survey scans to hundreds of ps pump–probe delays. A pump–probe signal was still observed at these long delays, however, as the scan range was not long enough to provide an accurate lifetime measurement for this ns channel, we set the lifetime fixed to 1 ns during the fit and extracted lifetimes of <50 fs and 750 fs for the other channels that result from a pump–probe process. The necessity for a probe–pump channel is apparent from the probe–pump (negative delay) signal in the data and is additionally supported by the strong 200 nm absorbance of adenine reported in the literature.[19] The 277 nm TRPES spectrum looks qualitatively similar to that at 267 nm but, due to the low absorption cross-section at the S_2 origin, suffers from poor signal-to-noise ratios. Nevertheless, in agreement with Lührs *et al.*,[9] the integrated photoelectron signal must be fit with a double exponential decay. An excited state lifetime of >2 ps was extracted and an additional very long-lived (ns) channel, tentatively attributed to a triplet state is observed.

Time-resolved photoelectron spectroscopy, as opposed to energy-integrated ion yield measurements, provides spectral information on the electronic character of the excited states of each channel. We extracted the character of the electronically excited states through (1) assignment of the photoelectron band by comparison with previously reported He(I) photoelectron spectra or *ab initio* ionization potentials and (2) projection back from the cationic to the neutral excited states through Koopmans' ionization correlations.

In Fig. 2 we compare the photoelectron spectra of the channels with <50 fs and 750 fs lifetimes at 250 and 267 nm excitation. Assuming a $S_2(\pi\pi^*)$ band origin at 4.477 eV (36 105 cm^{-1}) we calculate a vibrational energy of 0.483 and 0.167 eV for 250 and 267 nm excitation, respectively. He(I) photoelectron spectra report vertical ionization energies of $IP_0 = 8.48$ eV (π-hole) and $IP_1 = 9.58$ eV (n-hole) shown as two dotted lines.[20] For rigid molecules, we expect ionization to the electronic states of the cation with similar vibrational energy as in the excited state. The red and blue stars indicate our estimated positions for the vibrationally shifted ionization energies of the first π-hole and for the first n-hole. We can therefore assign the photoelectron bands with a <50 fs, and 750 fs decay time to the π-hole and n-hole channel, respectively. An unambiguous assignment of the ns channel

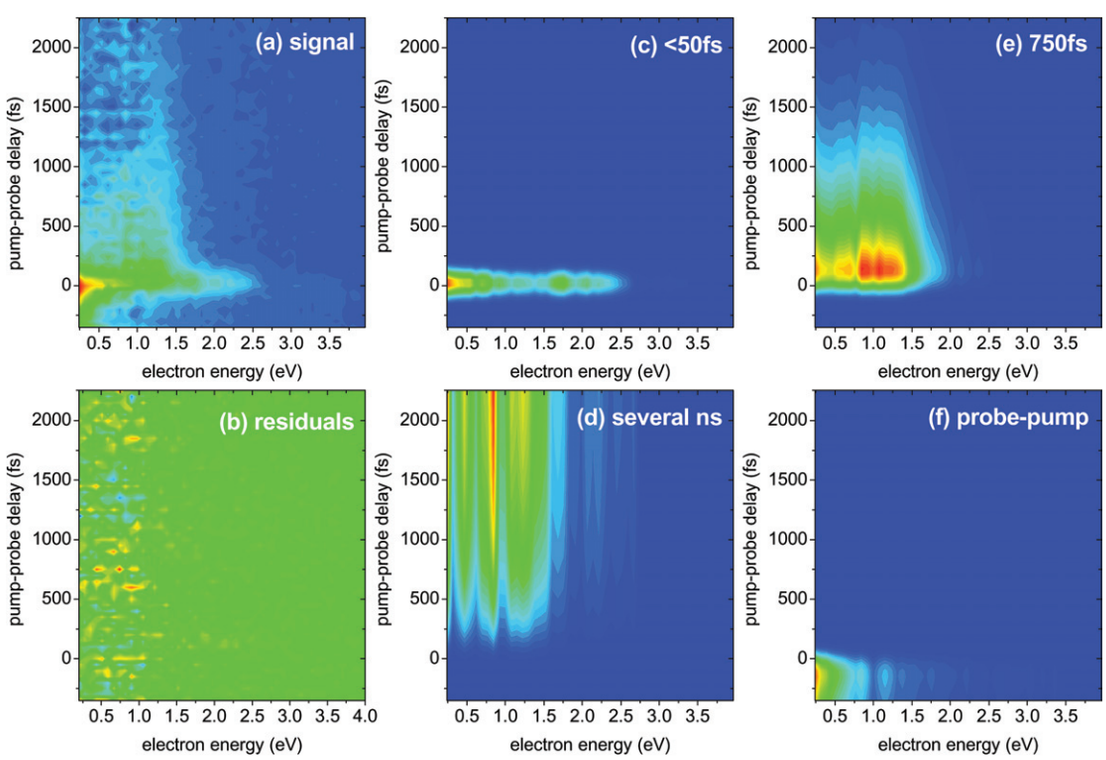

Fig. 1 The recorded two dimensional TRPES spectra of adenine with 267 nm excitation and 200 nm probe are displayed in (a). Our global fit provides time-resolved photoelectron spectra of different channels that show excited state lifetimes of (c) <50 fs, (d) 750 fs, (e) several ns. In (f) we show the extracted probe–pump dynamics which evolves towards negative time delays. The residuals (b) are an indication of the quality of the fit.

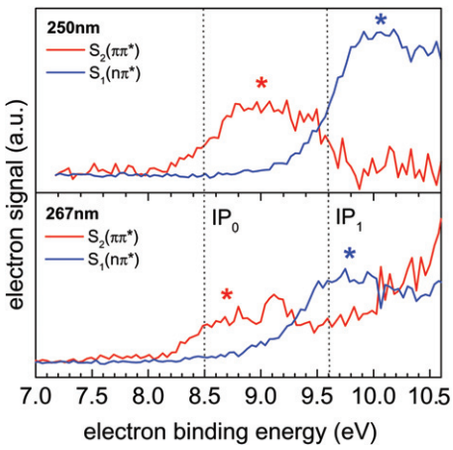

Fig. 2 Time-integrated photoelectron spectra of $S_2(\pi\pi^*)$ (red) and $S_1(n\pi^*)$ (blue) states at 250 nm (top) and 267 nm (bottom) excitation using a 200 nm probe. The dotted lines denote the He(I) vertical ionization potential[20] for IP_0 (π-hole) and D_1 (n-hole) and the vertical IPs expected for the vibrationally excited states of the $S_2(\pi\pi^*)$ (red) and $S_1(n\pi^*)$ (blue) states. At 267 nm, an additional ionization channel contributes to the nominal $S_2(\pi\pi^*)$ band which leads to the strong increase in electron signal at high electron binding energies.

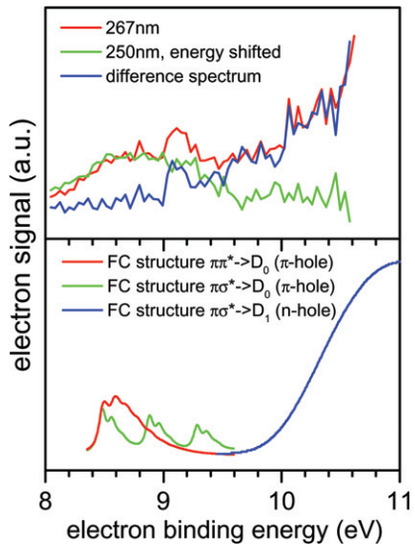

Fig. 3 (Top) Experimental photoelectron spectrum of the nominal πσ* state extracted from the 267 nm cross-correlation spectrum through substraction of the energy-shifted 250 nm spectrum. The experimental data is compared to *ab initio* Franck–Condon structure simulations of $\pi\pi^* \rightarrow D_0$, $\pi\sigma^* \rightarrow D_0$, and $\pi\sigma^* \rightarrow D_1$ simulations.

is currently not possible. Although the photoelectron spectrum of the ns channel appears, at first sight, similar to the 750 fs channel (*i.e.* ionization into D_1 (n-hole), ionization of highly vibrationally excited lower-lying ππ* triplet states into a highly vibrationally excited D_0 (π-hole) would be expected in the same photoelectron energy range. Assuming the long-lived signal originates from a triplet state and is populated *via* the S_1 (nπ*) state, we would expect ππ* triplet electronic character (''El-Sayed's rule'').

The following Koopmans' ionization correlations are provided by *ab initio* calculations (TD-B3LYP/6-31++G**): S_1, the lowest nπ* state, preferentially ionizes into the D_1 (n-hole) cation excited state, whereas S_2, the lowest ππ* state, and S_3, a πσ* state, both preferentially ionize into the D_0 (π-hole) cation ground state. This allows us to correlate the dynamics of the <50 fs channel (π-hole) with the initially excited, bright S_2 (ππ*) and the 750 fs dynamics (n-hole) to the dark (S_1 nπ*) state. This assignment supports Broo's picture[2] of the excited state relaxation process.

For the $S_1(n\pi^*)$ state, the form of the Franck–Condon envelope is very similar at 267 and 250 nm (Fig. 2, blue spectra). For $S_2(\pi\pi^*)$, however, this is not the case (Fig. 2, red spectra). To analyze this further, we shift the 250 nm spectrum by the difference in vibrational energy (*i.e.* by 0.316 eV, the energy difference between 267 and 250 nm) and obtain the difference spectrum (Fig. 3, blue line). This photoelectron difference band appears to be due to an additional channel at 267 nm. Furthermore, the amplitude of $S_1(n\pi^*)$ relative to $S_2(\pi\pi^*)$ is ~2× smaller at 267 nm than at 250 nm. We speculate as to the origin of this additional channel. Sobolewski and Domcke[3] proposed an additional decay pathway for $S_2(\pi\pi^*)$ *via* the dissociative $S_3(\pi\sigma^*)$ state along the NH-stretch coordinate. According to the Koopmans' correlations, the $S_3(\pi\sigma^*)$ state should ionize into D_0 (π-hole). This places this new band in the energy range observed and, additionally, the dissociative nature of $S_3(\pi\sigma^*)$ should result in a broad and diffuse photoelectron band, as observed. The state lifetime would be within the time resolution of our experiments ($\tau < 50$ fs). However, our calculations suggest that 200 nm excitation of higher states would show Koopmans' ionization to D_0, D_1 and D_2. Therefore we caution that *probe*-*pump* processes, evolving towards *negative* time delays, also conceivably contribute to this photoelectron band within the laser cross-correlation time.

Simulated FC structures of adenine photoelectron spectra

In Fig. 3 we compare our experimental photoelectron bands (top) to FC structure simulations (bottom). The experimental spectrum measured at 250 nm (Fig. 3 top, green line) is energy-shifted by 0.316 eV (the energy difference between 250 and 267 nm) and subtracted from the 267 nm spectrum (red line) providing the photoelectron difference band (blue line). To line up the *ab initio* FC structures (Fig. 3 bottom) with the experimental photoelectron bands, we use experimental He(I) photoelectron ionization potentials: $IP_0 = 8.48$ eV for the S_2 ($\pi\pi^*$) and S_3 ($\pi\sigma^*$) → D_0(π-hole) (red and green lines) and $IP_1 = 9.58$ eV for S_3 ($\pi\sigma^*$) → D_1(n-hole) (blue line). The vertical scaling is arbitrary and (for all structures) independently adjusted to appear similar to the experimental photoelectron spectrum. The FC structures are composed of C–C stretches for the $\pi\pi^* \rightarrow D_0$ transition, N–H stretches for $\pi\sigma^* \rightarrow D_0$, and C–C and N–H stretches and C–C–C bends for $\pi\sigma^* \rightarrow D_1$. These results are for a NH distance of *ca.* 1.2 Å and a NH stretch frequency of *ca.* 1500 cm^{-1} in the $S_{\pi\sigma^*}$ state. If the state is really dissociative, as suggested by TDDFT calculations,[3] then the FC structure due to photoionization from this state could be more diffuse and spread over a larger energy range than is shown here.

Our simulations seem to show intensity at the correct electron energies and appear to mimic the observed photoelectron band (Fig. 3 top, red line). However, within our simple picture of single excitations of the excited πσ* state, no Koopmans' ionization correlation to D_1 (blue line) is expected. However, the σ* orbital is a diffuse, Rydberg-like orbital with rather peculiar localization and bonding properties.[21] It is therefore conceivable that multiple configurations are required to adequately describe the πσ* state, possibly including configurations that will preferentially ionize to D_1. The large amplitude NH-stretch motion associated with the πσ* state can also lead to distortion of the single configuration molecular orbital picture.

Lifetimes of pyrimidine bases

In Fig. 4 we present time-resolved photoelectron spectra (colourmap) of the DNA bases, adenine, cytosine, thymine, and uracil recorded for 250 nm excitation with 200 nm probe

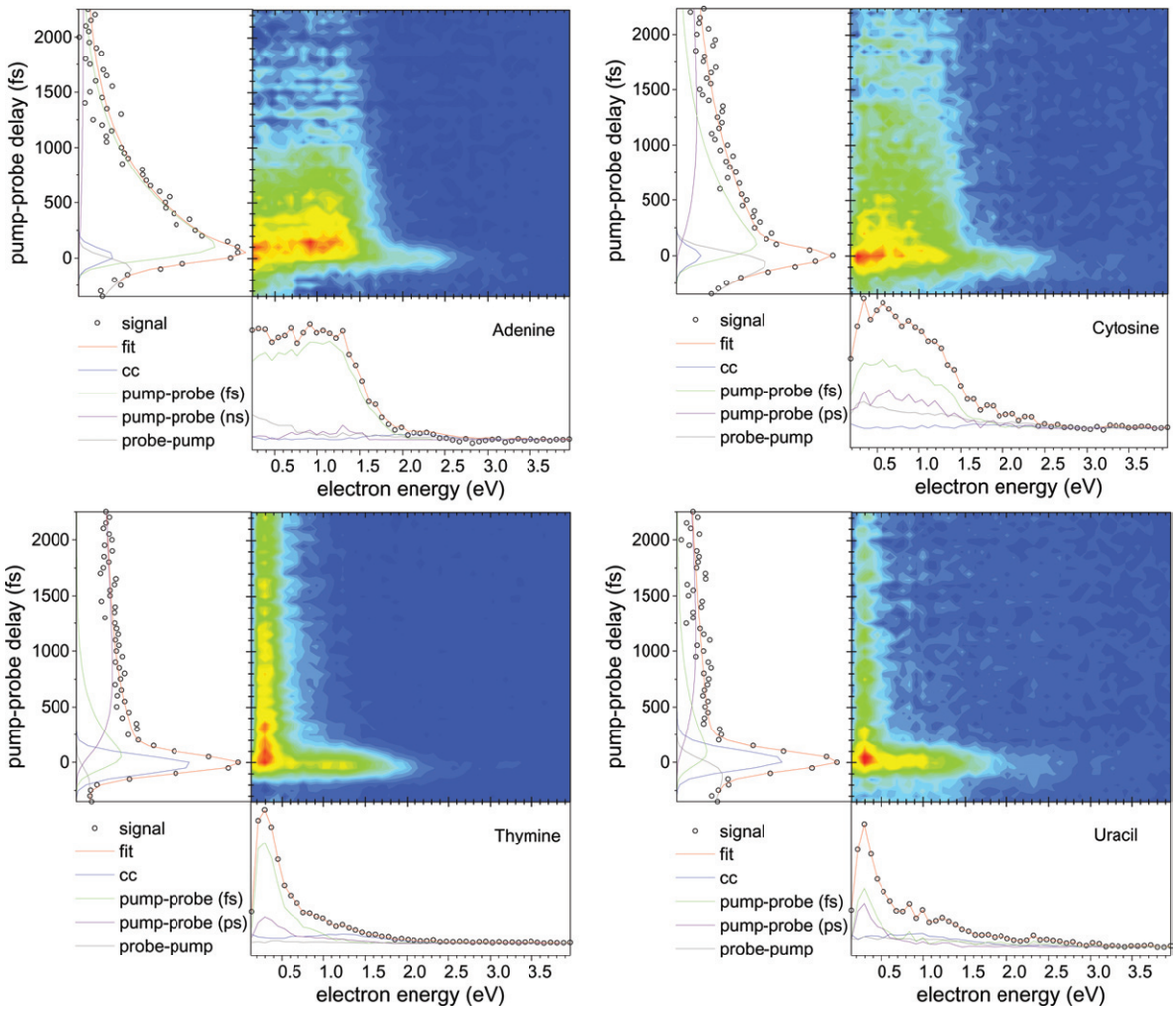

Fig. 4 TRPES spectra of adenine, cytosine, thymine, and uracil, displayed as 2D color maps. Projection of the different dynamics onto the pump–probe delay axis (*i.e.* energy integration) and projection onto the energy axis (*i.e.* time delay integration) are attached to the color maps. The results of the 2D global fitting procedure lead to the assignment of several channels. For details, see the text.

ionization. We used the same two dimensional global fit procedures as described above for adenine and using supporting information.[16,22] We again need four channels to achieve a reasonably good fit for the pyrimidine bases: (1) a Gaussian cross-correlation component centered at zero delay time (blue trace, labeled cc), (2) an exponential decay on the hundreds of fs timescale (green trace), (3) a long-lived signal of several ps (purple trace) that we fixed to values previously reported in the literature,[8] and (4) a probe–pump signal evolving towards negative time delays due to strong absorbance of the DNA bases around 200 nm (gray trace).[19] Using the global fitting procedure, we extracted exponential decay traces and photoelectron spectra for each channel. Projection of the fits onto the energy axis, *i.e.* integration over delay times, yields the energy-resolved photoelectron spectrum of each channel (bottom of colourmap). Projection onto the time axis, *i.e* energy integration over each photoelectron channel, yields the lifetime associated with each channel (left of colourmap).

In Fig. 5 we compare exponential decay traces of the different pyrimidine bases. The photoelectron spectra are not discussed at this time since they require a detailed analysis including consideration of different tautomers. REMPI spectra of cytosine show the presence of both the keto and the enol form under molecular beam conditions.[16] Since both tautomers have almost identical ionization energies, their photoelectron spectra are expected to overlap. We currently cannot distinguish between tautomers. Only little is known about thymine and uracil.[23] Their REMPI spectra show a broad band without any sharp peaks. We extract lifetimes of <50 fs for channels (1) and lifetimes of 820 fs, 490 fs, and 530 fs for channels (2) of cytosine, thymine, and uracil, respectively, when keeping channels (3, purple) fixed to 3.2 ps for cytosine,

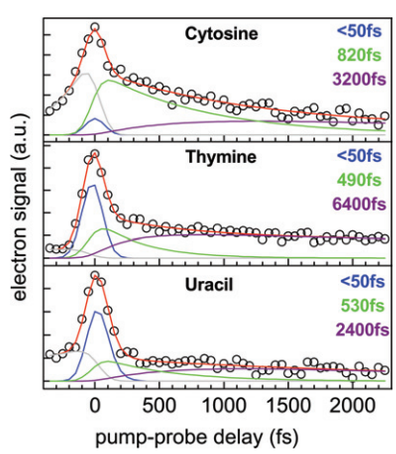

Fig. 5 Comparison of decay traces extracted from two dimensional global fits to TRPES measurements of (1) cytosine, (2) thymine, and (3) uracil. Black circles represent the energy-integrated photoelectron signal for each channel and the red line is the best fit. Four channels are assumed: a Gaussian component (blue), a short (green) and a long-lived (purple) exponential decay, and a probe–pump signal (gray). See text for details.

6.4 ps for thymine and 2.4 ps for uracil. These values for the ps channel were taken from the literature.[8] We would like to point out that these studies were conducted at a different excitation wavelength of 267 nm and with 800 nm multiphoton ionization yield measurements. Kim *et al.*[8] used a single exponential function to fit the pump–probe decay, with the exception being thymine which showed double exponential behavior. The unfavorable signal-to-noise ratios in our long time range TRPES spectra do not permit us to obtain reliable global fits. We note, however, that we reproduce the literature values to within ±0.5 ps by fitting our energy-integrated spectra with a single exponential decay. Inclusion of an additional ns channel, as reported in the literature[24,16] showed no significant contribution to our global fits in the short time range TRPES spectra. (For discussion of other fitting procedures, yielding different lifetimes, see endnote 25.)

A comparison of our gas-phase studies to measurements of nucleosides in aqueous solution by Pecourt *et al.*[7] shows that the lifetimes we extracted for the additional fs channel are comparable to lifetimes measured in the condensed phase (720 fs for cytodine, and 540 fs for thymidine).

Conclusions

We have presented a detailed picture of the gas-phase photophysics of adenine. In our current model, the initially prepared bright S_2 ($\pi\pi^*$) state decays rapidly (<50 fs) to the S_1 ($n\pi^*$) state which has a lifetime of 750 fs. We observe an energy dependent branching ratio of the relaxation pathways. At 267 nm excitation, indications arise for an additional decay pathway consistent with the theoretically predicted $S_3(\pi\sigma^*)$. In addition, we presented preliminary results for the pyrimidine bases. The dynamics are multi-exponential decays as determined using two-dimensional global fitting. All pyrimidine bases have an ultrashort decay <50 fs followed by a fs (820 fs for cytosine, 490 fs for thymine, 530 fs for uracil) and a ps channel (3.2 ps for cytosine, 6.4 ps for thymine, 2.4 ps for uracil). Additionally, ns decays were reported previously in the literature. We wish to point out some difficulties associated with extracting lifetimes from these data. Two numerical fitting procedures, global fitting (used here) and singular value decomposition, are commonly used to fit 2D data of the sort discussed here but do not always lead to the unambiguous interpretation of real experimental data. Real data contains noise and, especially if there are components with comparable time constants but greatly different amplitudes, the results of the fitting procedure may be quite sensitive to the initial input values used (see endnote 25). We anticipate that analysis of the corresponding photoelectron spectra will provide insights into the character of the excited states involved in the complex nonradiative decay dynamics. Further theoretical and experimental studies at different excitation and probe wavelengths are currently underway to aid in the interpretation and hopefully resolve remaining issues regarding contributions due to the presence of several tautomers and possible undesired probe–pump processes.

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25 (*a*) If we fit the TRPES spectra analogously to adenine, we extract a <50 fs channel and a channel with a 1140 fs, 1020 fs, and 750 fs lifetime for cytosine, thymine, and uracil respectively, while keeping the long-lived channel fixed to 1ns. The error of the global fit is slightly worse than with the approach presented in the text; (*b*) If we fit the 250 nm adenine data analogously to the other bases, as described in the text, we fix the long-lived channel fixed to 1 ps as suggested in ref. 8, and extract a <50 fs channel and a 360 fs channel. At 267 nm excitation, however, this fitting procedure fails as it appears to be missing a long-lived (ns) channel..