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Valence Photoionization of Thymine: Ionization Energies, Vibrational Structure, and Fragmentation Pathways from the Slow to the Ultrafast

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Abstract

The photoionization of thymine has been studied using vacuum ultraviolet radiation and imaging photoelectron photoion coincidence spectroscopy after aerosol flash vaporization and bulk evaporation. The vaporization techniques are compared based on the photoelectron spectrum and the breakdown diagram. The adiabatic ionization energies for the first four electronic states are 8.922 ± 0.008 , 9.851 ± 0.008 , 10.30 ± 0.02 and 10.82 ± 0.01 eV. Vibrational features have been assigned for the first three electronic states with the help of Franck–Condon factor calculations, based on density functional theory and wave function theory vibrational analysis within the harmonic approximation. The breakdown diagram of thymine, as supported by composite method ab initio calculations, suggests that the main fragment ions are formed in sequential HNC⁺O⁺, CO⁺, and H-loss dissociation steps from the thymine parent ion, with the first step corresponding to a retro-Diels–Alder reaction. Dissociation rate constants were extracted from the photoion time-of-flight distributions and used together with the breakdown curves to construct a statistical model to determine 0 K appearance energies as 11.15 ± 0.16 and 11.95 ± 0.09 for the m/z 83 and 55 fragment ions, respectively. The results allow us to revise previously proposed fragmentation mechanisms and to propose a model for the final, non-statistical H-loss step in the breakdown diagram, yielding the m/z 54 fragment ion at the appearance energy of 13.24 eV.

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Graphical abstract:

When the thymine is right: exploring vaporization techniques, photoelectron spectra, potential energy surfaces, breakdown curves and reaction mechanisms helps us better understand nucleobases and their ion chemistry.

Introduction

The electronic structure, energetics and bond energies of molecules are of general interest to understand the driving forces of chemical reactivity. Charge transfer and fragmentation processes are basic steps in reaction mechanisms. The reactive behavior of deoxyribonucleic acid (DNA) bases has attracted special attention, in particular because they are fundamental building blocks of life. It was, *e.g.*, found that the reactivity of the nucleic acid bases (NABs) towards methylating agents is higher for bases with lower ionization energy.^[1] Electronic structure changes of DNA bases following photoabsorption have mutagenic and carcinogenic effects,^[2] and, as such, are a driving force in evolution. They can lead to the dimerization of thymine with a neighboring thymine or cytosine, consequently disturbing the conformation of the DNA strand as well as its replication. Ionizing radiation not only leads to cancer, but it is also targetedly used to treat it. Thus, a better understanding of its interaction with the hereditary substance may help us improve its therapeutic outcome.^[3] X-ray crystallography was originally developed in the context of DNA structure elucidation, and is still a crucial tool to reveal the structure of macromolecules. However, the radiation that sheds light on complex structures also causes sample damage. Interestingly, DNA has been found to be comparably resistant to such damage,^[4] but understanding the mechanism of radiation damage may help in lifting sample damage limitations of crystallographic methods.

Another area where photophysical and -chemical properties of nucleic acid bases are of interest is the molecular origin of life. The presence of purines and pyrimidines in Comet Halley suggests that these molecules were transported to Earth by cometary dust.^[5] While vacuum ultraviolet (VUV) radiation is absorbed by oxygen and water, and does not propagate in air, it is present in the vacuum of outer space. In fact, the emission spectrum of B-type stars with a surface temperature of ca. 20000 K peaks in the VUV, and this radiation field is responsible for ionizing up to 50% of interstellar H.^[6] Therefore, VUV photochemistry may play an important role in outer space and the study of VUV radiation effects on the building blocks of life may shed light on their genesis and survival under extreme conditions. Mechanisms have been proposed for the synthesis and growth of homocyclic aromatics in the isolated phase,^[7,8] and even for the growth of N-substituted analogues.^[9] However, the introduction mechanism of nitrogen into the ring scaffold remains elusive,^[10,11] because larger barriers and unfavorable energetics make the N-analogue growth reactions unlikely. Furthermore, even condensed phase processes are deemed less likely to form thymine from pyrimidine than, *e.g.*, adenine from purine.^[12]

The most stable thymine tautomer is the diketo form in the gas phase, and the abundance of the most stable enolic form, lying ca. 45 kJ mol⁻¹ above the diketo isomer,^[13] is negligible at temperatures at which thymine is stable. The electronic structure of thymine (Fig. 1) was examined, among other

methods, by measuring its photoelectron or photoionization spectrum.^[1,14,15] The resulting ionization energies (IEs) are strewn in the 8.82–9.18 eV range and the early spectra did not allow much insight into the vibronic structure of the thymine cation. Choi et al. were the first to present a well-resolved VUV threshold ionization spectrum of thymine in the energy range of the ground state, which allowed them to determine the definitive IE and also to assign peaks in the photoelectron spectrum to vibrational transitions.^[16] Bravaya et al. calculated harmonic Franck–Condon factors (FCFs) for vibrational transitions in the $\tilde{X}^+ \leftarrow \tilde{X}$ band.^[17] Their computational approach was justified by comparison with the high-resolution spectrum by Choi et al. The first vibrationally resolved thymine slow photoelectron spectrum for a wider energy range was measured at synchrotron SOLEIL by Majdi et al. and Hochlaf et al. up to a photon energy of 10.3 eV.^[18,19] They explored numerous ab initio approaches to find the most appropriate way to calculate ionization energies. Additionally, they assigned vibrational transitions in the ground state, relying also on the FC calculations of Bravaya et al.^[17] However, anharmonic frequency calculations at the CASSCF/aug-cc-pVDZ level of theory led to different assignments than the ones proposed by Bravaya et al. in some cases. While the assignments of fundamental bands were on quite safe grounds, they emphasized that insecurities remained for the assignment of overtones and combination bands because of overlapping peaks. The assignment of vibrational transitions to the first electronically excited cation state relied solely on anharmonic frequency calculations.^[19] As far as we know, FCFs have not yet been calculated to simulate the vibronic structure of the excited state bands of the photoelectron spectrum of thymine. Here, we will model the first four electronic states and assign the most important vibronic transitions for the excited electronic states for the first time.

The fragmentation of thymine following energy uptake and ionization was observed using different “energy sources.” Fragmentation spectra were recorded upon electron ionization,^[20–23] ion impact^[24–28] as well as after VUV photoionization,^[15] core ionization,^[29] and multiphoton ionization.^[30] Wolf et al. recently reported a novel experiment, in which ultrafast thymine fragmentation was induced by resonance-enhanced three-photon ionization using 4.65 eV photons, *i.e.*, at a total energy of 13.95 eV, followed by a 570 eV free electron laser pulse to produce an oxygen 1s hole, after which the Auger spectrum was recorded. They could show how the isocyanic acid (HNCO) Auger spectrum develops as a function of delay time between 200 and 1500 fs.^[31] They later prepared the $\pi\pi^*$ state by UV excitation and used transient near edge x-ray absorption fine structure spectroscopy (TR-NEXAFS) at the oxygen 1s edge to study its decay.^[32]

Jochims et al.^[15] analyzed the dissociative photoionization of thymine and other nucleic acid bases comprehensively and reported the IEs as well as the appearance energies (AEs) of their fragments. They also suggested fragmentation pathways, which they used to calculate heats of formation for the

nucleic acid bases and some of their fragments. Isocyanic acid (HNCO) loss followed by sequential carbon monoxide (CO) loss was identified as the main dissociative photoionization channel of thymine. The lack of information on how thermal and kinetic shift are accounted for limits the interpretation of previously reported AEs. On the one hand, Jochims et al. estimated the kinetic shift and the mean thermal energy to add up to a shift of 0.5 ± 0.2 eV in a study on C_7H_8 ,^[33] incompatible with the error bars given for thymine. On the other, they assumed that thermal and kinetic shifts cancel out approximately in a study on N-methyl formamide.^[34] Most of the thymine AEs reported by Jochims et al.^[15] are lower than our results (see below), possibly because this assumption does not hold. Herein, we denote thresholds obtained taking into account dissociation rates and the product energy distribution as E_0 in contrast to AEs, where kinetic and thermal shifts have only been considered qualitatively if at all. We will also elucidate the fragmentation mechanism further by exploring the potential energy surface (PES) of the thymine cation to find the constitutional isomers that are energetically allowed at the experimentally determined thresholds. The results presented here are based on threshold photoionization, *i.e.*, photoelectron kinetic energy analysis in delayed coincidence with photoion time-of-flight mass analysis. Coincidence studies based on threshold photoionization have the benefit that the most important factor limiting their resolution is the energy bandwidth of the light source, in our case a few meV.^[35] Thus, we can expand on previous results by applying internal energy selection to record the breakdown diagram (BD) in threshold photoionization.

Rice–Ramsperger–Kassel–Marcus (RRKM) theory is routinely used to support the interpretation of mass spectra, also in the model applied in this study. However, such statistical approaches need, among other data, *a priori* knowledge of the fragmentation channels as well as ion and transition state (TS) vibrational frequencies to compute state functions. Grimme et al. pioneered the ab initio prediction of the fragmentation mechanism by predicting electron ionization mass spectra from first principles trajectory calculations.^[36,37] They also applied this approach to predict the electron ionization mass spectra of NABs, including thymine.^[38]

Aerosol flash vaporization has previously been used as a soft vaporization technique to bring involatile organics,^[39,40] including vitamins^[41] and oligopeptides^[42] into the gas phase. Koh et al. reported that certain hypergolic ionic liquids can only be vaporized intact from the aerosol phase.^[43] Two effects may contribute to the mildness of aerosol flash vaporization. First, the gaseous sample may be inherently colder with respect to bulk evaporation and, second, the sudden vaporization process may suppress pyrolysis of the sample. We also set out to compare aerosol flash and bulk vaporization based on the peak widths in the photoelectron spectrum and by directly measuring the internal energy distribution in the breakdown diagram.

Figure 1. Thymine molecular structure (left) shown with the lowest-energy dissociative photoionization step, the loss of HNCO, corresponding to a retro-Diels–Alder reaction, yielding a keteneimine (3-imino-2-methylprop-1-en-1-one) cation and isocyanic acid.

Results and Discussion

Threshold Photoelectron Spectrum and Ionization Energies

Choi et al.^[16] presented the high-resolution mass analyzed threshold ionization spectrum (MATI) of thymine up to 0.2 eV above the adiabatic IE and assigned the vibrational transitions. They also determined the definitive IE of thymine to be 8.9178 ± 0.0010 eV, which is significantly higher than the value of 8.82 ± 0.03 eV obtained by Jochims et al. from photoionization mass spectrometry (PI-MS).^[15] Bravaya et al.^[17] published the differentiated photoionization spectrum (PIE) of thymine up to a photon energy of 11.3 eV and compared the ground state band in the 8.5–9.7 eV range with FCF calculations based on ω B97X-D/6-31+G(d,p) frequency analysis within the double harmonic approximation for the neutral and the ground state of the ion. While the low resolving power of the differentiated PIE precluded a detailed analysis of the observable transitions, the results confirmed the previous assignment by Choi et al. in the 8.9–9.0 eV energy range.^[16]

The threshold photoelectron spectrum of thymine is shown in Fig. 2 up to a photon energy of 11.5 eV, *i.e.*, past the third excited electronic state band recorded by both bulk evaporation and aerosol flash vaporization. Aside from the better signal-to-noise ratio for the bulk evaporation TPES, the vaporization technique does not have a major impact on the TPES fine structure, which implies a similar rotational temperature in both cases. The rotational envelope is broader than in the TPES of Majdi and Hochlaf et al.,^[18,19] who could resolve somewhat more features in the photoelectron spectrum taken in a supersonic expansion thanks to rotational cooling. However, comparing the spectral resolution of the spectrum of Hochlaf et al.^[19] to our spectrum recorded at ca. 390 K, it can be concluded that thymine probably experienced only moderate rotational cooling in the supersonic expansion, as well.

As confirmed by the calculations, the geometry relaxation is small in every cation electronic state, and the TPES bands are characterized by a sharp resonance at the origin transition. The experimental

adiabatic IEs (Table 1) were obtained based on the Franck–Condon simulations of the bulk evaporation spectra and taking the Stark broadening into account, which is $6 \text{ cm}^{-1} \cdot (F/(\text{V} \cdot \text{cm}^{-1}))^{1/2}$ in the diabatic limit, with F being the continuous extraction field of $250 \text{ V} \cdot \text{cm}^{-1}$.^[44]

Figure 2. Thymine TPES with the electronic and some of the strongest vibrational transitions assigned, as measured with bulk evaporation (light blue) and aerosol flash vaporization (dark blue). The red bar spectrum are the FCFs for the vibrational transitions calculated with the harmonic model at the EOM-IP-CCSD/cc-pVDZ level of theory and convoluted with a 36 meV FWHM Gaussian to account for thermal broadening (thick red line). The thin red line for the $\tilde{\mathbf{X}}^+$ state is an FC-simulation based on a DFT calculation with a lower cut-off to include more transitions. The thin black trace for the $\tilde{\mathbf{A}}^+$ state corresponds to a convolution with a 20 meV FWHM Gaussian to show fine structure in the 10.15–10.25 eV range. The notation for the vibrational modes follows Majdi et al.^[18]

The uncertainty in the IE for the $\tilde{\mathbf{B}}^+$ and $\tilde{\mathbf{C}}^+$ states is larger than for the $\tilde{\mathbf{X}}^+$ and $\tilde{\mathbf{A}}^+$ states because their origin transition is not as well resolved, possibly because of the short lifetime of these states. The vicinity of the strong ν_{25} transition further increases the uncertainty for the $\tilde{\mathbf{B}}^+$ state.

Table 1. Adiabatic ionization energies to the ground and excited electronic states of thymine.

IE [eV]	experimental		theoretical	
	this work ^a	literature	this work ^b	literature
$\tilde{X}^+ \ ^2A''$	8.922 ± 0.008	8.9178 ± 0.0010 , ^c 8.913 ± 0.005 ^d	8.842	$8.917^{d,e}$
$\tilde{A}^+ \ ^2A'$	9.851 ± 0.008	9.842 ± 0.005 ^f	9.892	$9.868^{e,f}$
$\tilde{B}^+ \ ^2A''$	10.30 ± 0.02		10.285	
$\tilde{C}^+ \ ^2A'$	10.82 ± 0.01		10.869	

^aOn the basis of Franck–Condon modeling of the bulk evaporation TPES, ^b(EOM-IP-)-CCSD/cc-pVTZ with (EOM-IP-)-CCSD/cc-pVDZ ZPVE, ^cChoi et al.,^[16] ^dMajdi et al.,^[18] ^e(R)CCSD(T)-F12(b)/cc-pVTZ-F12 (+CV+SR+ZPVE), ^fHochlaf et al.^[19]

The FC stick spectrum at the (EOM-IP-)CCSD/cc-pVDZ level of theory and convoluted with 36 meV FWHM Gaussian (thick red line) represents the shape of the photoelectron bands at their onsets very well. This is despite the large number of large-amplitude low-frequency vibrations (ten below 70 meV in the $\tilde{X}^+ \ ^2A''$ state, of which five are totally symmetric and therefore active), for which the harmonic approximation is least accurate. However, FC simulations predict the origin transition to be the most intense peak in the band, which is at odds with the experimental results for the first two electronic states. We also carried out a DFT Franck–Condon simulation with a lower cut-off value to include thousands more transitions, mostly due to combination bands of the large-amplitude motions. As the thin red line in Fig. 2 for the $\tilde{X}^+$ state shows, including these transitions may account for the higher overall intensity at higher photon energies. Having said this, harmonic FCF-based intensities frequently diverge from the experimental results in organic molecules, which may also exhibit an autoionization baseline in threshold photoionization.^[45,46] Then there are further limitations to the FC approach, such as filling up of the TPES in medium sized FC gaps due to autoionization of Rydberg states^[47,48] and vibrational coupling.^[47,49] For adamantane, major discrepancies between the relative intensity of the peaks in the TPES and the FC simulation were also explained by rovibrational autoionization.^[45] Thus, the higher observed intensity at the high-energy ends of the $\tilde{X}^+$ and $\tilde{A}^+$ state bands may be due to the numerous possible transitions to large-amplitude vibrations and internal rotations as well as to rovibrational autoionization. Hochlaf et al. observed a well-resolved series of vibrational transitions for the $\tilde{A}^+$ state in the 10.15–10.25 eV energy range,^[19] which is washed out in our TPES. Our confidence in the overall validity of the FC model was reinforced when we convoluted the stick spectrum with a 20 meV Gaussian (thin black line) and these transitions were clearly predicted as combination bands.

Vibrational progressions are often trivially identified in small molecules. In thymine, the high number of possible transitions in the spectrum makes the unambiguous assignment of peaks to a single strong

vibrational transition or to the sum of several weaker transitions challenging. Majdi et al.^[18] assigned 17 peaks within the ionic ground state region based on calculated vibrational frequencies and symmetries. However, they could also rely on the high-resolution spectrum by Choi et al.^[16] and on the FC calculations by Bravaya et al.^[17] As our work also uses harmonic FCF calculations, it is not unexpected that the dominant transitions identified in our Franck–Condon simulation concur with those of Majdi et al. Higher in energy and in the absence of previous high-resolution spectra or FCF calculations, the assignment of a congested spectrum based on even state-of-the-art anharmonic frequency calculations appears to be somewhat lacking. From the 25 peaks assigned by Hochlaf et al.^[19] for the $\tilde{A}^+$ state in the photon energy range of 9.84–10.22 eV, the FC calculations in this work could only confirm the origin transition. Based on our (EOM-IP-)CCSD FC calculations, the rest of the dominant band features, as listed in Fig. 2, are due to different vibrational transitions than previously proposed. This result emphasizes the inherent difficulty in interpreting photoelectron spectra without FCF calculations: not only may vibrational transitions be misidentified, but the nature of the electronic states, too,^[50] and even the adiabatic ionization energies may lie significantly below or above the ionization onset, depending on the geometry change upon ionization and the source temperature.^[51–53]

Dissociative Photoionization

Threshold photoionization mass spectra confirm the previously established main fragment ion peaks at m/z 83, 55, and 54 below a photon energy of 15 eV (Fig. 3). Additionally, at ca. 13.8 eV, two minor channels open up parallel to the dominant m/z 55 peak, yielding fragment ions m/z 82 and m/z 56. Up to a photon energy of 15.4 eV, only trace amounts of the fragment with mass m/z 28 were observable. Jochims et al.^[15] also report the m/z 39 ion. No such fragment could be detected in the energy range of this work. On the whole, the main mass spectral peaks detected in this study coincide with the most intense peaks from previous studies, irrespective of the ionization technique.^[15,20,21,38] The low intensity of further, possibly minor thymine peaks, such as m/z 71, 77, and 80, visible also at some energies in Fig. 3 does not allow us to assign these as transient fragment ions of dissociative photoionization or sample impurities. The fractional abundance of the parent and major fragment peaks in threshold photoionization is shown in the breakdown diagram in Fig. 4. The parent ion disappears from the BD at $h\nu \approx 11.6$ eV. Yet, the m/z 126 peak is still the most dominant one in the 70 eV EI-MS of Rice et al.^[20] Therefore, even 70 eV electron ionization predominantly yields parent ions at energies below 11.6 eV relative to the neutral, as also pointed out by Jochims et al.^[15] The first, 43 amu loss suggests isocyanic acid (HNCO) as neutral fragment. The relatively narrow crossover region yielding the next fragment ion is indicative of a sequential loss of 28 amu, *i.e.*,

carbon monoxide. This is followed by another sequential unit mass loss, namely a hydrogen atom leaves to form the m/z 54 fragment ion. Thus, the neutral fragments as inferred from the mass spectra and the BD are in agreement with the suggestions of Rice et al.^[20] and Jochims et al.^[15] The time-of-flight distributions and the breakdown diagram were modeled in the light of the potential energy surface calculations (see below) to obtain the dissociation mechanism and appearance energies listed and compared with previous results in Table 2.

Figure 3. Threshold photoionization mass spectra at representative photon energies. The m/z 83 and 55 peaks are asymmetric at their onset, indicating that their precursor ion is metastable at low excess energies. The m/z 54 fragment ion peak is always symmetric, indicating fast dissociation at threshold.

Figure 4. (a) Breakdown diagram of thymine. Symbols correspond to measured values and continuous lines represent the statistical model, which also yielded the E_0 values as indicated. The dotted line corresponds to the model assuming statistical kinetic energy release for the CO-loss step. The continuous lines above 13 eV assume that a constant fraction (0.24) of the excess energy is released when the system passes the CO-loss transition state (see PES in Fig. 5 and Eq. 1). Panel (b) shows the breakdown diagram recorded using the aerosol flash vaporization source. Lowering the model temperature by ca. 30 K improves the agreement with the experiment in the < 11.4 eV photon energy range where the most energetic parent ions (highlighted) dissociate. This suggests that aerosol flash vaporization probably yields ca. 30 K colder sample than bulk evaporation at the same thermodesorber temperature (see Temperature Comparison in text).

Table 2. Comparison of the fragments and their appearance energies [eV] with results from previous works. The E_0 s were determined from the statistical model experimentally and as the average computational result using CBS-QB3, G4 and W1 as well as CBS-APNO energies for minima.

m/z	Formula	Neutral fragment(s)	E_0		Literature (AE)
			expt.	calc'd	
126	$C_5H_6N_2O_2^+$	(ionization energy)	8.922 ± 0.008	8.90 ± 0.07	8.82 ± 0.03^a , 8.9178 ± 0.0010^b
83	$C_4H_5NO^+$	HNCO	11.15 ± 0.16	10.96 ± 0.05	10.70 ± 0.05^a
82	$C_4H_4NO^+$	HNCO + H	*	13.04 ± 0.17	13.20 ± 0.05^a
55	$C_3H_5N^+$	HNCO + CO	11.95 ± 0.09	11.99 ± 0.04^c	11.7 ± 0.1^a
54	$C_3H_4N^+$	HNCO + CO + H	13.24^d	13.35 ± 0.16	12.09^a
43	HNCO $^+$	C_4H_5NO	*	14.07 ± 0.08	11.9 ± 0.1^a
39	$C_3H_3^+$	2 HNCO + H	*	14.34 ± 0.08^e	14.4 ± 0.1^a
		HNCO + HCN + OH		15.06 ± 0.13	
28	HCNH $^+$	HNCO + CH_3CCO	*	13.38 ± 0.13	13.6 ± 0.1^a
		HNCO + CO + H + C_2H_2		15.99 ± 0.15	
	$C_2H_4^+$	HNCO + CO + HCN		13.92 ± 0.15	

^aJochims et al.,^[15] ^bChoi et al.,^[16] ^ccorresponds to the barrier height, the reaction energy is 11.15 ± 0.10 eV, ^dbased on the optimized kinetic energy release factor, no error bar given, ^eno feasible m/z 82 intermediate found, *not observed as a major peak herein.

Potential Energy Surface

Figure 5. Reaction energy profiles for thymine cation fragmentation. Likely active dissociation pathways are plotted in black. Previously proposed but energetically or kinetically unlikely pathways are plotted in grey.^[15,20,38] Dotted connections between stationary points indicate submerged intermediate transition states and minima, left out for simplicity. The pathways to the $C_3H_3^+$ ion at m/z 39 and the cyclic isomerization pathway of m/z 83 have not been located, only the reaction energy is shown (dashed line).

The exploration of the potential energy surface is guided by mechanistic insights from the breakdown diagram, the assumption that internal conversion is fast compared with fragmentation, and dissociation takes place on the ground state surface. The increasing peak width and disappearing fine structure in the excited state TPES bands below 11 eV, at energies where the parent ion is stable with respect to dissociation, hint at a short electronic excited state lifetime and fast internal conversion and lend weight to this assumption. Only ground electronic state reactivity was assumed in the trajectory calculations to predict EI-MS spectra by Bauer and Grimme, as well.^[38]

The first dissociation step, the loss of HNCO to yield the m/z 83 keteneimine fragment ion is straightforward, although constrained geometry optimizations show that it takes place via a submerged barrier, located 100 meV below the threshold. As shown in Fig. 1, this step corresponds to a retro-Diels–Alder reaction. Jochims et al.^[15] propose that the prompt m/z 83 fragment ion may

isomerize to a cyclic structure, the 4-methyl-isoxazole cation. However, we calculate this to lie more than 1 eV higher in energy than the open-chain product, making isomerization improbable. There are numerous more stable $C_4H_5NO^+$ isomers, for instance, oxazines. However, in view of the small barrier to sequential CO loss, the m/z 83 ion is unlikely to undergo significant constitutional isomerization, apart from H transfer, prior to further fragmentation.

Further sequential decay channels have also been proposed from the HNCO-loss fragment ion besides CO loss. H-loss from the methyl group, as proposed by Jochims et al., may only take place at 13.11 eV, which is more than 1 eV above the CO-loss transition state. We also considered an alternative H-loss product at 12.74 eV, but as the system is required to pass over a transition state slightly above 13 eV, it is equally unlikely to contribute. While sequential H-loss does not compete with CO-loss at low energies, it does appear as a minor fragment ion peak at close to 14 eV. This may be due to (i) a looser H-loss transition state compared with the isomerization transition state of CO loss, (ii) H-loss from a more stable m/z 83 isomer ion, or (iii) a non-statistical process on an excited state surface. Because of the low abundance of m/z 82, (i) and (ii) appear to be more likely explanations, but the experimental data are insufficient to validate either.

The m/z 56 ion can be formed by HCN or HNC loss. Bauer and Grimme^[38] propose HNC/HCN loss and H transfer to yield the CH_3CHCO^+ ion. While the reaction energy is low at 11.78 eV, a tight transition state has to be passed at 13.08 eV. Jochims et al. propose the m/z 56 ion to arise by loss of HCN.^[15] The product CH_3CCOH^+ may be formed at energies above 13.4 eV. Similar to H loss, the low observed fragment ion abundance at high excess energies suggests a weakly competing parallel channel, but the experimental data do not allow for quantitative analysis.

The trivial carbon monoxide loss path from the m/z 83 ion involves simple C=C bond breaking. In fact, the resulting CH_3CCHNH^+ product ion features prominently in all previous dissociation schemes. However, our potential energy surface suggests that this bond breaking step is rather unlikely. In the nascent conformation of the m/z 83 ion, C=C bond breaking involves a high-lying transition state at 13.99 eV and yields the m/z 55 fragment ion over a 0.57 eV reverse barrier at 13.42 eV, far above the CO-loss onset. Furthermore, the high-energy CH_3CCHNH^+ fragment ion is unstable and, considering that it is formed over a reverse barrier, would almost always be energetic enough to dissociate further, and could never be a dominant mass spectral peak. Alternatively, an H-transfer may yield the most stable m/z 55 minimum found in this study, CH_3CHCNH^+ , at 11.03 eV. However, the computed activation energy, at 13.28 eV, is still much in excess of the appearance energy of the (HNCO + CO)-loss fragment ion.

Our proposed 28 amu loss mechanism relies on conformational isomerism, *i.e.*, internal rotation around the C-CH bond, followed by a five-membered ring transition state at 11.90 eV (see starred

structure in Fig. 5), shifting a methyl-hydrogen to the nitrogen atom yielding an amino-allene fragment ion. This may readily yield the CO-loss product, $\text{CH}_2\text{CCHNH}_2^+$ at 11.15 eV. The blue rectangle in Fig. 5 shows three additional low-energy isomers, which may interconvert depending on the available internal energy. $\text{CH}_2\text{CCHNH}_2^+$ may also decay by H loss to yield m/z 54 at 13.35 eV, while some of the other isomers could also lose HCN to yield C_2H_4^+ , *i.e.*, m/z 28, albeit not as the lowest-energy decay channel. As we have excluded $\text{CH}_3\text{CCHNH}^+$ as CO-loss product ion, we also rule out $\text{CH}_2\text{CCHNH}^+$ as sequential H-loss product ion as proposed by Jochims et al. as well as Bauer and Grimme. This is also supported by its calculated energy at 15.42 eV, 2 eV above its experimental onset.

Jochims et al.^[15] suggested the m/z 83 and 54 ions as immediate precursors for the formation of 28 m/z , which they assigned as HCNH^+ . The path over the m/z 54 fragment involves the loss of HNCO, CO, H, and C_2H_2 , and requires ca. 16 eV energy. Since there is a lower lying channel already at 14 eV from m/z 54 leading to C_2H_4^+ , also at m/z 28, we rule out that m/z 54 yields HCNH^+ . However, HNCH^+ can be formed along a viable pathway from m/z 83 already at $13.4 \text{ eV} \pm 0.1 \text{ eV}$.

Compared with the rather straightforward fragmentation pathways suggested previously,^[15,20,38] Fig. 6 gives a more complex impression of the thymine cation dissociation mechanism. In fact, calculations could only confirm the parent ion and the immediate HNCO-loss fragment ion structure among the seven non-trivial structures in the proposed mechanism by Jochims et al.^[15]

Statistical Model

The statistical model^[54] was also needed to guide the exploration of the potential energy surface, especially for the second, CO-loss step, which can take place from several isomeric m/z 83 ions and the reaction path is not obvious *per se* (Fig. 5). It was refined until the experimental appearance energies were consistent with the calculations. The optimized statistical model reproduced the breakdown diagram (Fig. 4(a)) and the TOF distributions (see Figs. 3 and 6 for representative TOF distributions) simultaneously and yielded 11.15 ± 0.16 as well as 11.95 ± 0.09 eV threshold energies to HNCO and sequential CO losses, respectively. The calculated E_0 for the m/z 82, 55, 43, 39, and 28 channels is also listed in Table 2, for comparison with the results of Jochims et al.,^[15] although their low intensity did not allow for statistical analysis of the experimental data. Among these, the calculated m/z 82 and 28 E_0 agree well with the measured appearance energy, assuming sequential H and CH_3CCO loss from m/z 83, respectively. The calculated reaction energies to yield m/z 43 and – due to a lack of feasible reaction path – m/z 39 could not be reconciled with the reported appearance energy of these fragments.

The formation of m/z 54 by H loss from the m/z 55 fragment ion is a major process, for which the statistical model predicts a faster rising breakdown curve than experimentally observed. A tentative explanation of a slower rising fractional abundance could be that the channel in question is parallel to HCO loss. In parallel processes, the often slowly changing dissociation rate constant ratio is measured in the breakdown diagram as opposed to the faster changing abundance of the precursor internal energy distribution above a sequential barrier. However, HCO is about 0.6 eV more energetic than H + CO, which renders its competitive parallel formation unlikely. Also, computations confirm that the H-loss channel from the CO-loss product opens up at ca. 13.35 eV, *i.e.*, the existence of another m/z 54 production channel could only increase its abundance and make the breakdown curve even steeper.^[55]

Thus, the reason for this discrepancy must be that the internal energy of the intermediate m/z 55 ion is lower and its distribution broader than predicted by statistical theory, and some of the excess energy in the intermediate steps is lost non-statistically. This could be an isolated dissociative state which imparts suprathermal kinetic energy to the leaving isocyanic acid neutral, thereby reducing the total energy available for CO and sequential H loss, similar to the recently reported non-statistical CO-loss behavior of $\text{Cr}(\text{CO})_6^+$.^[56] However, the photoelectron spectrum of thymine lacks Franck–Condon gaps, often associated with isolated state behavior and time-dependent density functional theory calculations also suggest that all excited electronic states within 4 eV of the ionization energy are bound with respect to HNCO loss. The larger than expected energy loss is rather rooted in the shape of the fragmentation energy curve (Fig. 5). A high-energy transition state has to be overcome at ca. 0.8 eV higher than the final product to form the 55 m/z fragment ion. This repulsive reaction energy curve inherently leads to an impulsive dissociation and a higher fraction of the excess energy is released as kinetic energy (KER).^[57]

Figure 6. (a) Threshold ionization TOF distributions (crosses) and the statistical model results (continuous lines) showing the thymine parent ion (m/z 126, at 12.8 μs), m/z 83 (HNCO loss at 10.4 μs), and m/z 55 (HNCO + CO loss at 8.5 μs) daughter ions at representative photon energies. (b) Modeled rate curve for HNCO loss and sequential CO loss as a

function of the internal energy of the respective parent ion. The internal energy is given relative to the neutral ground state. The red circle denotes the approximate HNCN-loss rate determined by Wolf et al., at the maximum attainable energy after three-photon absorption in their experiment.^[31]

As observed, *e.g.*, for CF₃SF₅, a constant fraction of excess energy may be released as translational energy over a broad energy range in impulsive dissociations.^[58] While this assumption may not hold as strongly for thymine, because the parent ion is not prepared at a repulsive section of the potential energy surface, we adapted the statistical model assuming that the product energy distribution is statistical in all degrees of freedom except the reaction coordinate, in which a constant fraction of the excess energy is deterministically released. While the threshold to *m/z* 55 fragment ion formation is modelled to be 11.95 eV, the product energy is calculated to be 11.15 ± 0.10 eV. If the kinetic energy release factor (KERF) is constant, it is possible to account for this by removing one translational degree of freedom from the statistical calculation of the product energy distribution and rescaling the breakdown diagram in the photon energy range of H loss, *i.e.*, at $h\nu > 12.99$ eV, as follows:

$$E(h\nu > 12.99 \text{ eV}) = 11.15 \text{ eV} + \frac{h\nu - 11.15 \text{ eV}}{1 - \text{KERF}} \quad (1)$$

The adapted model was fitted to reproduce the experimental data by optimizing both the E_0 for H loss and the KERF in Eq. 1. This yielded an optimized KERF of 0.24 at a 0 K appearance energy of 13.24 eV. As seen in Fig. 4(a) and Table 2, the computed and modelled E_0 agree and the model reproduces the low-energy part of the H-loss breakdown curve quite well, although it starts to overestimate fragmentation at higher photon energies.

Temperature Comparison

We applied two different approaches, aerosol flash vaporization and effusive bulk evaporation, to bring a non-volatile biomolecule, thymine, intact into the gas phase. Koh et al. demonstrated aerosol flash vaporization to be milder than bulk evaporation in a study of hypergolic ionic liquids.^[43] In the light of this result and further uses of aerosol flash vaporization,^[39–42,59] we wanted to determine whether a lower final internal energy or the sudden vaporization play a more important role in the mildness of the aerosol flash vaporization approach. Peak widths in the threshold photoelectron spectrum are in part determined by the rotational envelope and can be compared directly. Furthermore, the shape of the first crossover region in the breakdown diagram, HNCN loss, is in part determined by the internal energy distribution of the sample, broadened and blue-shifted because of

the metastable parent ion and the resulting kinetic shift. Thus, the rotational temperatures and the internal energy distribution of gaseous thymine can be directly compared across the vaporization techniques.

Aside from the better signal to noise ratio in the bulk evaporation spectrum, the two TPES traces in Fig. 2 are indistinguishable, indicating at most a small difference in the rotational temperature. Bear in mind that Majdi and Hochlaf and co-workers were able to resolve the vibrational fine structure in a supersonic expansion better.^[18,19] Nevertheless, the HNCO-loss crossover region in the BD is ever so slightly narrower in aerosol flash vaporization compared to bulk evaporation, suggesting a slightly lower effective temperature in the former at the same, ca. 390 K thermodesorber temperature (Fig. 4b).^[60]

The best fit model temperature for bulk evaporation was a little higher than the thermodesorber temperature at 410 K. The aerosol flash vaporization breakdown diagram was fitted using the same statistical model and by varying only the temperature to reproduce the rising HNCO-loss breakdown curve below 11.4 eV (as highlighted in Fig. 4b). This yielded 380 K as the sample temperature in aerosol flash vaporization, although the signal-to-noise ratio in the aerosol flash vaporization breakdown diagram is insufficient to determine the temperature difference quantitatively. Thus, the aerosol flash vaporization source yielded neutrals with an internal temperature probably only ca. 30 K colder than bulk evaporation, which, while not insignificant, underlines the importance of sudden vaporization in suppressing possibly also condensed phase pyrolysis processes in aerosol flash vaporization.

Conclusions

We report the threshold photoelectron spectrum of thymine in the 8.5–11.5 eV photon energy range together with double harmonic Franck–Condon simulations for the ground, and, for the first time, also for the first three excited states. The derived IEs for the $\tilde{\mathbf{X}}^+$ and $\tilde{\mathbf{A}}^+$ states, 8.922 ± 0.008 and 9.851 ± 0.008 eV, respectively, agree with literature results. Those for the $\tilde{\mathbf{B}}^+$ and $\tilde{\mathbf{C}}^+$ states, 10.30 ± 0.02 and 10.82 ± 0.01 eV, respectively, are newly reported herein. The vibrational structure of the ground, $\tilde{\mathbf{X}}^+$ state band has already been analyzed by Choi et al.,^[16] relying on accurately measured vibrational transitions, by Bravaya et al.,^[17] using Franck–Condon simulations, and by Majdi et al.,^[18] based on the first complete high-resolution photoelectron spectrum of the band and anharmonic frequency calculations. Our work mostly confirms the literature results. However, most previous vibrational assignments for the $\tilde{\mathbf{A}}^+$ state,^[19] based solely on state-of-the-art frequency calculations, are revised. This emphasizes that Franck–Condon simulations are indispensable to understand congested spectra. Our results show that the ν_9 vibrational mode, its overtones and combination

transitions determine the shape of the $\tilde{\text{A}}^+$ band. Furthermore, the most intense transition besides the origin transition was assigned to the ν_{25} fundamental for the $\tilde{\text{B}}^+$ state.

Threshold photoionization mass spectra of thymine exhibited three dominant peaks in the 11–15 eV photon energy range besides the parent ion at m/z 126, namely at m/z 83, 55, and 54. Based on the breakdown diagram, these correspond to sequential HNCO, CO, and H loss processes at experimentally determined thresholds of 11.15 ± 0.16 , 11.95 ± 0.09 , and 13.24 eV, respectively. The experimental data could only be analyzed simultaneously with ab initio potential energy surface exploration, in particular the second, CO-loss step, which involves supraprecursor kinetic energy release after an isomerization transition state. The fact that these mechanistic details have not been previously discussed accentuates the need for synergic computational and experimental approaches to understand fragmentation processes of even medium sized species. Thanks to internal energy selection in the PEPICO experiment and to composite method calculations, we could revisit previously proposed fragmentation mechanisms.^[15,20]

Finally, we quantified the temperature difference of the gaseous thymine sample generated by effusive bulk evaporation and aerosol flash vaporization. The latter yielded noisier data but was found to generate a colder sample by only ca. 30 K in our experiment. This points to the importance of sudden vaporization in the aerosol source, which may play an important role in preventing thermal decomposition of labile samples. Compared with aerosol flash vaporization, effusive bulk evaporation requires much less sample and is simpler to use, which may make it the method of choice whenever feasible.

As for the astrochemical aspects, we can confirm that thymine does not yet dissociatively ionize at the Lyman- α line at 10.2 eV, although it may form neutral fragments after photoabsorption. Its survival thus depends on environments that shield radiation, *e.g.*, in meteoritic pores, so that its production rate is higher than its rate of destruction. The inverse process of the cation fragmentation is the recombination of the fragments. In the case of thymine, the Diels–Alder cycloaddition of isocyanic acid and the m/z 83 keteneimine 3-imino-2-methylprop-1-en-1-one cation may readily yield the thymine ion. However, the formation of said keteneimine in the cationic state by CO addition to $\text{C}_3\text{H}_5\text{N}^+$ is unlikely because of the small impact parameter and the saddle point along the reaction energy curve.

In the condensed phase, be it in ices or in biological systems, nucleobases may dissipate excitation energy by vibrational energy transfer, which plays a crucial role in suppressing fragmentation. We can now compare the time-dependence of the Auger spectra reported by Wolf et al.^[31] and our statistical model. At a total energy of 14 eV, our model predicts HNCO-loss rate from the parent ion to be $3 \cdot 10^{11} \text{ s}^{-1}$. This is at the maximum feasible energy of the system after three-photon absorption

in the Wolf experiment, and, as seen in Fig. 6(b), our extrapolated rate agrees quite well with an estimated effective rate based on the evolution of the Auger spectrum. However, it is possible that more than three photons are absorbed in multiphoton UV absorption, and also that kinetic electrons are released, which raise and lower the available internal energy, respectively. At any rate, the time scales in question are commensurate with the expected energy dissipation times, which implies that such fragmentation processes may be fast enough to take place in the condensed phase, as well.

Experimental Section

Photoelectron Photoion Coincidence

Thymine (Sigma-Aldrich, $\geq 99\%$) was used as received. The experiments were conducted in the 8.7–15.5 eV energy range and repeated using two vaporization techniques. They were first performed by vaporizing a thymine aerosol using a thermodesorber in the imaging Photoelectron Photoion Coincidence (iPEPICO) endstation^[59,61] at the VUV beamline of the Swiss Light Source (SLS). A thymine aerosol of 20–400 nm particle size was generated by nebulization of an aqueous solution of thymine by nitrogen. Afterwards, the particles were dried and introduced into the vacuum of the ionization chamber with the help of an aerodynamic lens system,^[62,63] collimating and guiding the aerosol beam onto a thermodesorber where the particles were vaporized.

In the CRF-PEPICO endstation,^[64] thymine was brought into the gas phase by bulk evaporation: The thermodesorber used for aerosol flash vaporization was heated to the same, ca. 390 K temperature after modification to include a cavity at the end, which was capped by a 0.5 mm nozzle. This cavity held the bulk sample and the evaporated molecules exited the nozzle in direct vicinity of the VUV beam.

Detailed information on the beamline^[65] and the two endstations^[61,64] can be found elsewhere, and only a concise description is given here. Bending magnet synchrotron radiation is collimated, dispersed by a monochromator in grazing incidence using a 150 or 600 lines/mm blazed or laminar grating, respectively, and focused into a differentially pumped gas filter to suppress higher harmonic radiation. Using a 200 μm exit slit, the 600 lines/mm grating yields a photon energy resolution of 3–5 meV, whereas the 150 lines/mm grating produces almost an order of magnitude more flux at a resolution of 10–30 meV. The monochromatic light reaches the ca. $4 \times 2 \text{ mm}^2$ interaction region of the endstation, where the gaseous sample is ionized and, depending on the photon energy, the parent ion may also fragment. A constant electric field extracts photoelectrons and photoions in opposite directions. Photoelectrons are velocity map imaged (VMI) onto a delay-line detector and also start the time-of-flight measurement of the photoion.

Both endstations contain a long first acceleration region at the extraction field of 120–250 V cm⁻¹, which affords the parent ion a residence time of several μ s in this field and results in exponentially decaying peak shapes if the rate constant for a dissociation process is on this time scale. The CRF-PEPICO endstation is a double imaging setup also offering velocity map imaging for the photoions. However, ion images are not analyzed herein.

After photoabsorption, the total energy of the system, relative to the zero-point energy of the neutral, consists of the initial internal energy of the sample molecule and the photon energy. Electron removal consumes the ionization energy, and the remainder is distributed among the kinetic energy of the photoelectron and the internal energy of the parent ion. Photoion internal energy selection is thus made possible by kinetic energy analysis of the photoelectron. In threshold photoelectron photoion coincidence spectroscopy (TPEPICO), only threshold photoelectrons, *i.e.*, photoelectrons emitted with negligible kinetic energy, are accepted as start signal. In VMI, these electrons are focused onto the center of the detector. Kinetic electrons with no off-axis momentum are also detected there, which leads to hot electron contamination of the center signal. This can be suppressed based on a ring area around the central threshold focus. Assuming the ring represents the hot electron contamination of the center signal faithfully, the hot electron contamination can be subtracted from the center signal after multiplication with the area ratio of the center and the ring regions of interest.^[66] The exact subtraction factor is dependent on local collection efficiencies and is fine-tuned empirically to achieve on average zero mean parent ion fractional abundance in the breakdown diagram above the disappearance energy of the parent ion. This approach has already been tested in a large number of experiments.^[54,67]

Thanks to ion TOF analysis, TPEPICO allows for obtaining photoion mass selected threshold photoelectron spectra (ms-TPES) by plotting the photoion signal of selected mass channels as function of the photon energy. The sum of the ms-TPES belonging to a parent ion gives the TPES. The breakdown diagram can be plotted based on the fractional abundances of the parent and daughter ions in threshold photoionization as a function of the photon energy. Modelling the breakdown diagram and the experimental rate curves allows us to account for thermal, kinetic, and competitive shifts quantitatively to extract threshold energies, E_0 , for dissociative ionization, which are independent of instrument parameters.

Ab Initio Calculations

Calculations supported this investigation in three distinct ways. First, frequency calculations on the neutral as well as the ground and excited electronic states of the cation yield the nuclear wave function overlap between the neutral and ionic states, *i.e.*, the Franck–Condon factors (FCF). These can be

used to simulate and assign the photoelectron spectrum. Second, the potential energy surface was explored to find the stationary points, namely transition states and (intermediate) minima, driving the fragmentation of the cation. Afterwards, ionization and dissociative ionization thresholds could be calculated reliably using composite methods and compared with experiment. Third, the statistical model for dissociative photoionization (see below) relies on densities and numbers of states of the fragmenting species and the transition states, respectively. Density of states functions are also used to partition the excess energy among the ionic and neutral fragments as well as translational degrees of freedom, which plays a role in sequential dissociations. Ab initio state functions are obtained based on rotational constants and harmonic vibrational frequencies and are used to construct the statistical model, which is then adjusted to reproduce the experiment and obtain the experimental 0 K appearance energies.

The FCFs were calculated at the optimized geometries of neutral thymine and its ground and excited cationic states and in the harmonic approximation at a temperature of 380 K. We used a coupled cluster singles and doubles (CCSD) approach with the cc-pVDZ basis set for the neutral and the equation-of-motion EOM-IP-CCSD theory with the same basis set for the cation calculations. The FCFs for transitions into the ionic ground state were also calculated using density functional theory (DFT) geometry optimization and frequency analyses with a low FCF cut-off value to include a large number of transitions. The EOM-IP-CCSD calculations were performed with Q-Chem 4.3,^[68] and the DFT calculations with Gaussian16.^[69] We used eZspectrum^[70] to calculate the FCFs and the resulting stick spectrum was compared with the experimental results after convolution with a 36 meV full width at half maximum (FWHM) Gaussian function to account for rotational broadening and experimental resolution.^[45]

Several electronically excited states are accessible in the dissociative photoionization energy range. However, as long as the ergodic hypothesis holds, the system may explore the whole phase space prior to fragmentation, which is always dominated by the ground electronic state functions. Therefore, the ground electronic state drives the dissociation processes of the cation and only its energetics is of relevance. Ab initio calculations were therefore used to investigate the thymine cation ground state PES to locate the fragmentation reaction coordinates. The stationary points, *i.e.* minima and saddle points, on the PES of thymine were located using constrained optimizations and density functional theory (DFT) with the B3LYP functional and the 6-311++G(d,p) basis set. The search for saddle points corresponding to transition states was supported by synchronous transit-guided quasi-Newton calculations.^[71] In frequency calculations and in difficult geometry optimizations, the force constants were also calculated analytically.

The energies of the stationary points were further refined using composite methods. The CBS-

QB3,^[72] G4,^[73] and W1U^[74] methods were applied to transition states, and CBS-APNO was also used for minima. The spin contamination corrected W1Usc values were taken for open shell species. The recipes for the composite methods are sufficiently different, and twice the standard deviation is reported as error bar around the average of the composite method results. However, a note of caution is required regarding the transition states, because all applicable methods rely on B3LYP geometries albeit with different basis sets. The PES exploration yielded insights into dissociation pathways and thresholds, in the light of which we could re-examine the fragmentation mechanism.

Statistical Model of Dissociative Photoionization

The Boltzmann distribution determines the internal energy of the neutral at the experimental temperature. This distribution is assumed to be transposed into the ion manifold by the photon energy in threshold photoionization, which relies on the approximation that the threshold photoionization cross section is constant for (mostly rotational) sequence transitions.^[54] The breakdown diagram of a single prompt fragmentation channel is, thus, directly related to the neutral internal energy distribution: the integral part of the distribution above the barrier will yield a fragment ion, while the integral part below will be detected as parent ion. If the temperature is known, the only adjustable parameter in the fit to the breakdown diagram is the dissociative photoionization threshold, E_0 . In the case of slow dissociations, a certain amount of excess energy is required for the fragmentation to take place on the time scale of the experiment, which is the kinetic shift. Such processes are recognized experimentally by the resulting asymmetric daughter ion TOF peaks, which are a result of fragmentation inside the acceleration region and are a direct measure of the unimolecular rate constant. The RRKM rate curve is given as

$$k(E) = \frac{\alpha N^\ddagger(E - E_0)}{h\rho(E)} \quad (2)$$

with α being the reaction degeneracy and h Planck's constant.^[75] $N^\ddagger(E - E_0)$ is the number of quantum states of the transition state in the internal energy interval $[0; E - E_0]$, and $\rho(E)$ is the density of states of the precursor ion. In such cases, the ab initio rate curve is fitted to the experiment by scaling the lowest five transitional frequencies in the transition state number of states calculation. The BD and the TOF distributions are modelled simultaneously by varying the 0 K appearance energy and the frequency scaling factor until the experimental data are best reproduced.

The first two major fragmentation steps in the dissociative photoionization of thymine, isocyanic acid and carbon monoxide loss are slow at threshold, and the kinetic shift has to be quantified to derive accurate dissociation thresholds. In sequential dissociations, the excess energy above the barrier is partitioned statistically between the ionic and neutral fragments as well as the newly created

translational degrees of freedom, *i.e.*, the kinetic energy release. This partitioning relies solely on the density of states, which are based on harmonic vibrational frequencies for the fragments.

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References

- [1] S. Urano, X. Yang, P. R. LeBreton, *J. Mol. Struct.* **1989**, *214*, 315–328.
- [2] E. C. Friedberg, G. C. Walker, W. Siede, *DNA Repair and Mutagenesis*, ASM Press, **1995**.
- [3] M. Schwell, M. Hochlaf, in *Photoinduced Phenom. Nucleic Acids*, Springer, **2014**, pp. 155–208.
- [4] C. Bury, E. F. Garman, H. M. Ginn, R. B. G. Ravelli, I. Carmichael, G. Kneale, J. E. McGeehan, *J. Synchrotron Radiat.* **2015**, *22*, 213–224.
- [5] A. H. Delsemme, in *Mol. Orig. Life Assem. Pieces Puzzle* (Ed.: A. Brack), Cambridge University Press, **1998**, pp. 101–118.
- [6] J. Vallergera, *Astrophys. J.* **1998**, *497*, 921–927.
- [7] D. S. N. Parker, F. Zhang, Y. S. Kim, R. I. Kaiser, A. Landera, V. V. Kislov, A. M. Mebel, A. G. G. M. Tielens, *Proc. Natl. Acad. Sci.* **2012**, *109*, 53–58.
- [8] T. Yang, R. I. Kaiser, T. P. Troy, B. Xu, O. Kostko, M. Ahmed, A. M. Mebel, M. V. Zagidullin, V. N. Azyazov, *Angew. Chemie* **2017**, *129*, 4586–4590.
- [9] D. S. N. Parker, R. I. Kaiser, O. Kostko, T. P. Troy, M. Ahmed, A. M. Mebel, A. G. G. M. Tielens, *Astrophys. J.* **2015**, *803*, 53.
- [10] J. Bouwman, B. Sztáray, J. Oomens, P. Hemberger, A. Bodi, *J. Phys. Chem. A* **2015**, *119*, 1127–1136.
- [11] J. Bouwman, A. Bodi, P. Hemberger, *Phys. Chem. Chem. Phys.* **2018**, *20*, 29910–29917.
- [12] S. A. Sandford, P. P. Bera, T. J. Lee, C. K. Materese, M. Nuevo, *Top. Curr. Chem.* **2014**, *356*, 123–164.
- [13] J. Rejnek, M. Hanus, M. Kabeláč, F. Ryjáček, P. Hobza, *Phys. Chem. Chem. Phys.* **2005**, *7*, 2006–2017.
- [14] C. Lifschitz, E. D. Bergmann, B. Pullman, *Tetrahedron Lett.* **1967**, *8*, 4583–4586.
- [15] H.-W. W. Jochims, M. Schwell, H. Baumgärtel, S. Leach, *Chem. Phys.* **2005**, *314*, 263–282.
- [16] K.-W. Choi, J.-H. Lee, S. K. Kim, *J. Am. Chem. Soc.* **2005**, *127*, 15674–15675.
- [17] K. B. Bravaya, O. Kostko, S. Dolgikh, A. Landau, M. Ahmed, A. I. Krylov, *J. Phys. Chem. A* **2010**, *114*, 12305–12317.
- [18] Y. Majdi, M. Hochlaf, Y. Pan, K.-C. Lau, L. Poisson, G. A. Garcia, L. Nahon, M. M. Al-Mogren, M. Schwell, *J. Phys. Chem. A* **2015**, *119*, 5951–5958.
- [19] M. Hochlaf, Y. Pan, K.-C. Lau, Y. Majdi, L. Poisson, G. A. Garcia, L. Nahon, M. M. Al Mogren, M. Schwell, *J.*

Phys. Chem. A **2015**, *119*, 1146–1153.

- [20] J. M. Rice, G. O. Dudek, M. Barber, *J. Am. Chem. Soc.* **1965**, *87*, 4569–4576.
- [21] J. Ulrich, R. Teoule, R. Massot, A. Cornu, *Org. Mass Spectrom.* **1969**, *2*, 1183–1199.
- [22] C. J. Tiessen, J. A. Trocchi, J. D. Hein, J. Dech, W. Kedzierski, J. W. McConkey, *J. Phys. B At. Mol. Opt. Phys.* **2016**, *49*, 125204.
- [23] P. J. M. van der Burgt, F. Mahon, G. Barrett, M. L. Gradziel, *Eur. Phys. J. D* **2014**, *68*, 151.
- [24] Z. Deng, M. Imhoff, M. A. Huels, *J. Chem. Phys.* **2005**, *123*, 144509.
- [25] A. Le Padellec, P. Moretto-Capelle, M. Richard-Viard, J. P. Champeaux, P. Cafarelli, *J. Phys. Conf. Ser.* **2008**, *101*, 012007.
- [26] J. Tabet, S. Eden, S. Feil, H. Abdoul-Carime, B. Farizon, M. Farizon, S. Ouaskit, T. D. Märk, *Phys. Rev. A* **2010**, *82*, 022703.
- [27] J. Tabet, S. Eden, S. Feil, H. Abdoul-Carime, B. Farizon, M. Farizon, S. Ouaskit, T. D. Märk, *Int. J. Mass Spectrom.* **2010**, *292*, 53–63.
- [28] T. Schlathölter, R. Hoekstra, R. Morgenstern, *Int. J. Mass Spectrom.* **2004**, *233*, 173–179.
- [29] E. Itälä, D. T. Ha, K. Kooser, E. Rachlew, M. A. Huels, E. Kukk, *J. Chem. Phys.* **2010**, *133*, 154316.
- [30] R. Pandey, M. Ryszka, T. da Fonseca Cunha, M. Lalande, M. Dampc, P. Limão-Vieira, N. J. Mason, J. C. Pouilly, S. Eden, *Chem. Phys. Lett.* **2017**, *684*, 233–238.
- [31] T. Wolf, F. Holzmeier, I. Wagner, N. Berrah, C. Bostedt, J. Bozek, P. Bucksbaum, R. Coffee, J. Cryan, J. Farrell, et al., *Appl. Sci.* **2017**, *7*, 681.
- [32] T. J. A. Wolf, R. H. Myhre, J. P. Cryan, S. Coriani, R. J. Squibb, A. Battistoni, N. Berrah, C. Bostedt, P. Bucksbaum, G. Coslovich, et al., *Nat. Commun.* **2017**, *8*, 29.
- [33] M. Schwell, F. Dulieu, C. Gée, H.-W. Jochims, J.-L. Chotin, H. Baumgärtel, S. Leach, *Chem. Phys.* **2000**, *260*, 261–279.
- [34] S. Leach, N. Champion, H.-W. Jochims, H. Baumgärtel, *Chem. Phys.* **2010**, *376*, 10–22.
- [35] T. Baer, R. P. Tuckett, *Phys. Chem. Chem. Phys.* **2017**, *19*, 9698–9723.
- [36] S. Grimme, *Angew. Chemie Int. Ed.* **2013**, *52*, 6306–6312.
- [37] V. Ásgeirsson, C. A. Bauer, S. Grimme, *Chem. Sci.* **2017**, *8*, 4879–4895.
- [38] C. A. Bauer, S. Grimme, *Eur. J. Mass Spectrom.* **2015**, *21*, 125–140.
- [39] E. R. Mysak, K. R. Wilson, M. Jimenez-Cruz, M. Ahmed, T. Baer, *Anal. Chem.* **2005**, *77*, 5953–5960.
- [40] F. Gaie-Levrel, G. A. Garcia, M. Schwell, L. Nahon, *Phys. Chem. Chem. Phys.* **2011**, *13*, 7024–7036.
- [41] H. Dossmann, A. Schwarzenberg, D. Lesage, M. Pérot-Taillandier, C. Afonso, B. Cunha de Miranda, G. A. Garcia, *J. Phys. Chem. A* **2014**, *118*, 11185–11192.
- [42] K. R. Wilson, M. Jimenez-Cruz, C. Nicolas, L. Belau, S. R. Leonet, M. Ahmed, *J. Phys. Chem. A* **2006**, *110*, 2106–2113.
- [43] C. J. Koh, C.-L. Liu, C. W. Harmon, D. Strasser, A. Golan, O. Kostko, S. D. Chambreau, G. L. Vaghjiani, S. R. Leone, *J. Phys. Chem. A* **2011**, *115*, 4630–4635.
- [44] W. A. Chupka, *J. Chem. Phys.* **1993**, *98*, 4520–4530.

- [45] A. Candian, J. Bouwman, P. Hemberger, A. Bodi, A. G. G. M. Tielens, *Phys. Chem. Chem. Phys.* **2018**, *20*, 5399–5406.
- [46] X. Wu, X. Zhou, P. Hemberger, A. Bodi, *J. Phys. Chem. A* **2017**, *121*, 2748–2759.
- [47] S. Y. Truong, A. J. Yench, A. M. Juarez, S. J. Cavanagh, P. Bolognesi, G. C. King, *Chem. Phys.* **2009**, *355*, 183–193.
- [48] A. J. Yench, A. J. Cormack, R. J. Donovan, A. Hopkirk, G. C. King, *Chem. Phys.* **1998**, *238*, 109–131.
- [49] G. A. Garcia, L. Nahon, S. Daly, I. Powis, *Nat. Commun.* **2013**, *4*, 2132.
- [50] R. P. Tuckett, J. Harvey, P. Hemberger, A. Bodi, *J. Mol. Spectrosc.* **2015**, *315*, 172–183.
- [51] A. Bodi, M. Daniel Brannock, B. Sztáray, T. Baer, *Phys. Chem. Chem. Phys.* **2012**, *14*, 16047.
- [52] M. F. Heringa, J. G. Slowik, A. S. H. Prévôt, U. Baltensperger, P. Hemberger, A. Bodi, *J. Phys. Chem. A* **2016**, *120*, 3397–3405.
- [53] F. Holzmeier, M.-P. Herbert, I. Fischer, M. Steglich, A. Bodi, P. Hemberger, *J. Anal. Appl. Pyrolysis* **2017**, *124*, 454–460.
- [54] B. Sztáray, A. Bodi, T. Baer, *J. Mass Spectrom.* **2010**, *45*, 1233–1245.
- [55] J. Harvey, P. Hemberger, A. Bodi, R. P. Tuckett, *J. Chem. Phys.* **2013**, *138*, 124301.
- [56] K. Voronova, K. G. Torma, J. P. Kercher, A. Bodi, B. Sztáray, *Int. J. Mass Spectrom.* **2019**, *438*, 63–71.
- [57] J. Harvey, A. Bodi, R. P. Tuckett, B. Sztáray, *Phys. Chem. Chem. Phys.* **2012**, *14*, 3935.
- [58] A. Bodi, P. Hemberger, R. P. Tuckett, *Phys. Chem. Chem. Phys.* **2017**, *19*, 30173–30180.
- [59] M. F. Heringa, J. G. Slowik, M. Goldmann, R. Signorell, P. Hemberger, A. Bodi, *ChemPhysChem* **2017**, *18*, 3595–3604.
- [60] K. Voronova, K. M. Ervin, K. G. Torma, P. Hemberger, A. Bodi, T. Gerber, D. L. Osborn, B. Sztáray, *J. Phys. Chem. Lett.* **2018**, 534–539.
- [61] A. Bodi, M. Johnson, T. Gerber, Z. Gengeliczki, B. Sztáray, T. Baer, *Rev. Sci. Instrum.* **2009**, *80*, 34101.
- [62] S. Amanatidis, B. L. Yoder, R. Signorell, *J. Chem. Phys.* **2017**, *146*, 224204.
- [63] B. L. Yoder, A. H. C. West, B. Schläppi, E. Chasovskikh, R. Signorell, *J. Chem. Phys.* **2013**, *138*, 044202.
- [64] B. Sztáray, K. Voronova, K. G. Torma, K. J. Covert, A. Bodi, P. Hemberger, T. Gerber, D. L. Osborn, *J. Chem. Phys.* **2017**, *147*, 013944.
- [65] M. Johnson, A. Bodi, L. Schulz, T. Gerber, *Nucl. Instruments Methods Phys. Res. Sect. A Accel. Spectrometers, Detect. Assoc. Equip.* **2009**, *610*, 597–603.
- [66] B. Sztáray, T. Baer, *Rev. Sci. Instrum.* **2003**, *74*, 3763–3768.
- [67] A. Bodi, P. Hemberger, *Phys. Chem. Chem. Phys.* **2014**, *16*, 505–515.
- [68] Y. Shao, Z. Gan, E. Epifanovsky, A. T. B. Gilbert, M. Wormit, J. Kussmann, A. W. Lange, A. Behn, J. Deng, X. Feng, et al., *Mol. Phys.* **2015**, *113*, 184–215.
- [69] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, et al., *Gaussian 16 Revision A.03*, **2016**.
- [70] V. A. Mozhayskiy, A. I. Krylov, EzSpectrum, [Http://Iopenshell.Usc.Edu/Downloads](http://Iopenshell.Usc.Edu/Downloads), **2016**.
- [71] C. Peng, P. Y. Ayala, H. B. Schlegel, M. J. Frisch, *J. Comput. Chem.* **1996**, *17*, 49–56.

- [72] J. W. Ochterski, G. A. Petersson, J. A. Montgomery, *J. Chem. Phys.* **1996**, *104*, 2598–2619.
- [73] L. A. Curtiss, P. C. Redfern, K. Raghavachari, *J. Chem. Phys.* **2007**, *126*, 84108.
- [74] E. C. Barnes, G. A. Petersson, J. A. Montgomery, M. J. Frisch, J. M. L. Martin, *J. Chem. Theory Comput.* **2009**, *5*, 2687–2693.
- [75] T. Baer, W. L. Hase, *Unimolecular Reaction Dynamics: Theory and Experiments*, Oxford University Press, New York, **1996**.