Intersystem crossing rates of $S_1$ state keto-amino cytosine at low excess energy

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The amino-keto tautomer of supersonic jet-cooled cytosine undergoes intersystem crossing (ISC) from the $v = 0$ and low-lying vibronic levels of its $S_1(1\pi\pi^*)$ state. We investigate these ISC rates experimentally and theoretically as a function of $S_1$ state vibrational excess energy $E_{exc}$. The $S_1$ vibronic levels are pumped with a ~5 ns UV laser, the $S_1$ and triplet state ion signals are separated by prompt or delayed ionization with a second UV laser pulse. After correcting the raw ISC yields for the relative $S_1$ and $T_1$ ionization cross sections, we obtain energy dependent ISC quantum yields $Q_{ISC}^{S_1\rightarrow T_1}$ = 1%–5%. These are combined with previously measured vibronic state-specific decay rates, giving ISC rates $k_{ISC} = 0.4\times1.5\times10^9$ s$^{-1}$, the corresponding $S_1 \rightsquigarrow S_0$ internal conversion (IC) rates are 30–100 times larger. Theoretical ISC rates are computed using SCS-CC2 methods, which predict rapid ISC from the $S_1; v = 0$ state with $k_{ISC} = 3\times10^9$ s$^{-1}$ to the $T_1(1\pi\pi^*)$ triplet state. The surprisingly high rate of this El-Sayed-forbidden transition is caused by a substantial admixture of $1\nu_7\pi^*$ character into the $S_1(1\pi\pi^*)$ wave function at its non-planar minimum geometry. The combination of experiment and theory implies that (1) below $E_{exc} = 550$ cm$^{-1}$ in the $S_1$ state, $S_1 \rightsquigarrow S_0$ internal conversion dominates the nonradiative decay with $k_{IC} \geq 2\times10^{10}$ s$^{-1}$, (2) the calculated $S_1 \rightsquigarrow T_1 (1\pi\pi^*\rightarrow3\pi\pi^*)$ ISC rate is in good agreement with experiment, (3) being El-Sayed forbidden, the $S_1 \rightsquigarrow T_1$ ISC is moderately fast ($k_{ISC} = 3\times10^8$ s$^{-1}$), and not ultrafast, as claimed by other calculations, and (4) at $E_{exc} \sim 550$ cm$^{-1}$ the IC rate increases by ~50 times, probably by accessing the lowest conical intersection (the C5-twist CI) and thereby effectively switching off the ISC decay channels. © 2015 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4937375]

I. INTRODUCTION

The canonical pyrimidine nucleobase cytosine (Cyt) pairs with guanine within double-stranded DNA, and its derivatives play an important role in epigenetics and medicine. In aqueous solution, cytosine is highly stable against UV irradiation. The fact that cytosine has survived photolysis by the intense UV solar radiation on early Earth has been taken to imply the fact that cytosine has survived photolysis by the intense high rate of this El Sayed-forbidden transition is caused by a substantial admixture of $1\nu_7\pi^*$ character into the $S_1(1\pi\pi^*)$ wave function at its non-planar minimum geometry. The combination of experiment and theory implies that (1) below $E_{exc} = 550$ cm$^{-1}$ in the $S_1$ state, $S_1 \rightsquigarrow S_0$ internal conversion dominates the nonradiative decay with $k_{IC} \geq 2\times10^{10}$ s$^{-1}$, (2) the calculated $S_1 \rightsquigarrow T_1 (1\pi\pi^*\rightarrow3\pi\pi^*)$ ISC rate is in good agreement with experiment, (3) being El-Sayed forbidden, the $S_1 \rightsquigarrow T_1$ ISC is moderately fast ($k_{ISC} = 3\times10^8$ s$^{-1}$), and not ultrafast, as claimed by other calculations, and (4) at $E_{exc} \sim 550$ cm$^{-1}$ the IC rate increases by ~50 times, probably by accessing the lowest conical intersection (the C5-twist CI) and thereby effectively switching off the ISC decay channels. © 2015 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4937375]
Numerous theoretical studies of the excited-state dynamics and decay mechanisms of Cyt have been reported, most of which have focused on the keto-amino tautomer 1 (see Figure 1) because of its biological importance. The minimum of the lowest $1\pi\pi^*$ state of 1 has been shown to be connected to three different conical intersections (CIs) with the $S_0$ surface; all three involve some degree of out-of-plane deformation of the pyrimidine ring. Tomic et al. calculated the vertical and adiabatic absorption energies of the keto, enol, and keto-imino tautomers of cytosine. They determined density functional/multi-reference configuration interaction (DFT/MRCI) energies along the time-dependent density functional (TDDFT) reaction path that connects the excited-state $1\pi\pi^*$ minimum and this conical intersection and found that the $1\pi\pi^*$ minimum is separated by a 1600 cm$^{-1}$ (0.2 eV) barrier from the CI. Two recent excited-state dynamics studies including non-adiabatic as well as spin–orbit coupling claimed the decay of the $S_1$ population via ISC to the $T_2$ state to be ultrafast. At least for vibrationally cold $S_1$ cytosine, this result contradicts the recent experimental findings by Lobsiger et al. For the pyrimidine bases uracil and thymine, Etinski et al. carried out combined coupled-cluster and DFT/MRCI calculations to determine ISC rate constants. They predicted ISC to play an important role in the electronic relaxation from the initially excited $1\pi\pi^*$ state in the gas phase. This was later confirmed experimentally. The predictive power of that theoretical study motivated us to reinvestigate the excited-state processes in the biologically relevant keto-amino cytosine by means of approximate coupled-cluster methods, with a focus on ISC.

Femtosecond (fs) pump-probe time-resolved ionization and photoelectron spectroscopic experiments have been performed on thermally vaporized and jet-cooled cytosine. The early measurements found sub-picosecond to picosecond lifetimes with mono- or biexponential decay profiles. Ulrich et al. noted that both keto and enol tautomers of cytosine contribute to the signals observed. However, these measurements employed excitation at UV wavelengths (250-267 nm), at which several gas-phase tautomers can be excited. Kosma et al. attempted to distinguish the decay of the keto-amino tautomer 1 from those of the enol-amino (2a, 2b) and keto-imino (3a, 3b) tautomers (see Figure 1) by exciting at 280-290 nm, where only 1 absorbs UV light. At 280 nm they fitted lifetimes of $\tau = 1.2$ ps and at 290 nm of $\tau = 1.1$ ps. Cheng and co-workers performed fs pump-probe measurements up to 300 nm and observed $\tau = 1.5$ ps. However, even at 300 nm the keto-amino tautomer 1 is excited far above its lowest conical intersection (the C5-C6 “twist” CI) in the $S_1(1\pi\pi^*)$ state.

Recently, Lobsiger et al. have determined the lifetimes of the lowest $S_1(1\pi\pi^*)$ state vibronic levels with excess energies $E_{\text{exc}} = 0-550$ cm$^{-1}$ by measuring the Lorentzian broadening of the rovibronic band contours. The lifetime at the $0^+$ band is $\tau \geq 44 \pm 5$ ps and remains in the 30–45 ps range up to the $2\nu^*_S$ level at $+205$ cm$^{-1}$. This decay rate is $\sim 30-40$ times slower than the nonradiative rates measured by fs time-resolved pump-ionization measurements, signaling much slower nonradiative dynamics for the vibrational levels that are localized near the $S_1(1\pi\pi^*)$ minimum. The levels in the range 308–447 cm$^{-1}$ have lifetimes $\tau \sim 25$ ps, the $6a^e$ level lifetime at $E_{\text{exc}} = 530$ cm$^{-1}$ was estimated to be 3–6 ps. No vibronic bands are observed above 530 cm$^{-1}$, implying an upper lifetime limit of $\tau < 2$ ps for these levels. Trachsel et al. have performed analogous lifetime measurements for 5-methylcytosine.

II. COMPUTATIONAL METHODS AND RESULTS

A. Computational methods

We employed the spin-component scaled coupled-cluster method with approximate treatment of doubles (SCS-CC2) for electronic structure calculations. Spin-component scaling assumes different scaling of energy contributions of the same- and opposite-spin components. This scaling enhances the accuracy of 0-0 transition energies both for $\pi\pi^*$ and $\pi\pi^*$ states. In a recent benchmark study, it was shown that the standard deviation of 0-0 transition energies for a set of organic molecules is 0.06 eV. For the calculations of SCS-CC2 energies we used the standard scaling factors 1/3 for the same-spin and 6/5 for the opposite-spin components.

The calculations were performed with the TURBOMOLE program. We used the resolution-of-identity (RI) CC2 implementation in TURBOMOLE for the ground state and the corresponding linear response theory version for excited-state optimizations and vertical excitation energies and the calculation of properties. In all calculations, valence electrons were correlated. Throughout, Dunning\textsuperscript{50,51} augmented correlation-consistent basis set (aug-cc-pVTZ)
was employed. Auxiliary basis sets for the RI approximation of the two-electron integrals were taken from the TURBOMOLE library. The SNF program was used for numerical calculations of vibrational frequencies in harmonic approximation.

In previous work on thymine and uracil, we had combined potential energy surfaces from CC2 calculations and spin–orbit matrix elements (SOMEs) obtained from DFT/MRCI wave functions to determine ISC rate constants. This procedure seemed inappropriate here because the SCS-CC2 and the DFT/MRCI calculations gave substantially different electronic structures of the excited states. In particular, the relative weights of $\pi\pi^*$ and $n\pi^*$ configurations, which are crucial for the size of the SOMEs, were found to differ largely. For this reason, we decided to use the amplitudes of the SCS-CC2 wave functions to determine the SOMEs.

The optimized geometries of all states are presented in Figure 2. Adiabatic excitation energies and 0-0 transition energies of the $S_1(\pi\pi^*)$, $T_1(\pi\pi^*)$, and $T_2(n_\sigma\pi^*)$ states are

\[
\langle \hat{b} | \hat{H}_{SO} | \hat{a} \rangle = \langle b | \hat{a} \rangle - \langle a | \hat{b} \rangle
\]

and a prefactor of $-1/\sqrt{2}$ from the spin part have to be taken into account in the evaluation of the SOMEs. Spin–orbit integrals of the underlying Hartree-Fock molecular orbitals were evaluated using the SPOCK program employing a one-center mean-field approximation to the Breit-Pauli Hamiltonian.

With the electronic coupling matrix elements, vibrational frequencies and wave functions at hand, intersystem crossing (ISC) rates were calculated using the VIBES program. The calculation of rates makes use of the Fermi golden-rule and harmonic multimode approximation including Dushinsky rotations. Theoretical details of the method are provided elsewhere. In all ISC rate calculations a Gaussian damping function of width 1.0 cm$^{-1}$ was employed.

**B. Theoretical results**

As stated above, we used SCS-CC2 for computing the potential energy surfaces of the ground and excited states. The reason for this choice of method was the optimized geometry of the first excited singlet state of keto-amino cytosine. SCS-CC2 was the only method that resulted in a non-planar equilibrium structure of the $S_1$ state, in agreement with the UV spectroscopic results. Regular CC2 as well as TDDFT employing the B3LYP functional yielded an $n_\sigma\pi^*$ electronic structure for the $S_1$ state with planar minimum geometry. For a comparison of the geometric parameters obtained at these levels of theory, see Figures S1 and S2 of the supplementary material. In the following, only the SCS-CC2 results will be discussed.

The optimized geometries of all states are presented in Figure 2. Adiabatic excitation energies and 0-0 transition energies of the $S_1(\pi\pi^*)$, $T_1(\pi\pi^*)$, and $T_2(n_\sigma\pi^*)$ states are...
collected in Table I together with the experimental results. A comparison of vertical excitations using different quantum chemical methods is given in Table S1 of the supplementary material. In the \( S_0 \) state the minimum nuclear arrangement is nearly planar, only the two hydrogen atoms connected to the \( N_2 \) atom are displaced out of ring plane. Comparing the geometry parameters to those obtained at MP2/DZP level we find that our bond lengths are shorter by 0.01-0.02 Å. The adiabatic excitation energies \( S_1 \), \( T_1 \), and \( T_2 \) and the experimental adiabatic ionization energy are subsequently shown in Figure 3. A comparison of the calculated adiabatic excitation energies with results of previous theoretical work is given in table S2 of the supplementary material.

The first excited singlet state in the adiabatic spectrum is \( \pi\pi^* \), however, due to the nonplanarity of the \( S_1 \) minimum geometry the wave function contains \( \approx 30\% \) of \( n_O\pi^* \) character. The dihedral angles \( C_2\pi N_2 C_3 \), \( C_2\pi N_1 C_2 \), and \( C_2\pi N_1 C_3 \) are \(-17.3^\circ\), 165.0°, and \(-19.8^\circ\), respectively. The largest bond length changes with respect to the \( S_0 \) ground state occur for the \( C_2\pi O \) (+0.14 Å), \( C_2\pi N_3 \) (−0.10 Å), and \( N_3\pi C_4 \) (+0.12 Å) bonds.

Table II gives the calculated rotational constants of the \( S_0 \) and \( S_1 \) states and the \( x/y/z \) ratios of the \( S_0 \rightarrow S_1 \) transition dipole moment components in the molecule-fixed inertial-axis frame \((a/b/c)\), computed at the SCS-CC2 level. The values are in excellent agreement with experiment, thus supporting our choice of method. In that experiment, the rotational contours of the \( 0^0_0 \) band and nine vibronic bands as well as the orientation of the transition dipole moment vector were found to be \( >95\% \) in-plane \((a/b\)-oriented\) thereby proving that the \( S_1 \) is dominantly a \( \pi\pi^* \) state.

In contrast, complete active space self-consistent field (CASSCF) yields a \( n_O\pi^* \) electronic structure for the \( S_1 \) state with planar minimum nuclear arrangement. The previously reported MS-CASPT2 optimized \( 1\pi\pi^* \) geometry is the \( S_1 \) global minimum as in the SCS-CC2 case. However, the MS-CASPT2 geometry is planar and its \( C_2\pi O \) bond (1.28 Å) is shorter than in the SCS-CC2 case.

The SCS-CC2 adiabatic \( 1\pi\pi^* \) state energy (4.04 eV) can be compared to other theoretical results: It is close to the MS-CASPT2 value of 3.98 eV and lower than the MRCI and DFT/MRCI values of 4.31 and 4.18 eV, respectively. CC2 yields an adiabatic energy of 3.78 eV for the \( 1\pi\pi^* \) state while TDDFT/B3-LYP gives 3.94 eV. Note, however, that the \( 1\pi\pi^* \) state corresponds to the \( S_2 \) state in both the latter cases. The SCS-CC2 zero-point energy corrected adiabatic energy is 3.87 eV, which is close to the experimental value of 3.947 eV. Our attempts to optimize the singlet \( \pi\pi^* \) state failed at the SCS-CC2 level of theory due to its close proximity with the \( \pi\pi^* \) state.

The \( T_1 \) state in the adiabatic spectrum exhibits mainly \( \pi\pi^* \) character. The most elongated bonds are \( C_4\pi C_5 \) and \( N_1\pi C_6 \). The ring is not planar, the \( C_2\pi N_1 C_2 \) dihedral angle being 21.9°. Furthermore, the hydrogen atoms bound to \( C_2 \) and \( C_3 \) are twisted strongly out of plane with a \( H_2 C_2\pi H \) dihedral angle of about 67°. The \( 3\pi\pi^* \) adiabatic and zero-energy corrected energies are 3.42 and 3.30 eV, respectively. The experimentally determined \( T_1 \) energy lies in the range 3.26-3.37 eV and nicely agrees with the SCS-CC2 \( T_1 \) energy.

The second triplet state in the adiabatic spectrum is an \( n_O\pi^* \) state. Its geometry is also not planar, but the out-of-plane distortions are significantly smaller than in the \( S_1 \) and \( T_1 \) states. The largest displacement with respect to the \( S_0 \) geometry is for the \( C\pi O \) bond length which is elongated to 1.40 Å in the \( T_2 \) state. Its adiabatic energy amounts to 4.06 eV. The zero-point energy corrected adiabatic energy is 3.95 eV. To our knowledge, experimental results are not available for this state.

With regard to ISC, we are mainly interested in the decay rate from the vibrationless \( v^0 = 0 \) level of the \( S_1 \) state (initial state). At the SCS-CC2 level, only the \( S_0 \) and \( T_1 \) states are located energetically below this state (Table I). According to our calculations, the lowest vibronic level of the \( T_2 \) state is only 0.08 eV (645 cm\(^{-1}\)) higher than the lowest vibronic level of the initial state. For this reason, we considered the ISC via the \( T_2 \) state as an additional possible decay channel of the \( S_1 \) population.

A detailed overview over SCS-CC2 wave function amplitudes and spin-orbit integrals over molecular orbitals is presented in Fig. S3 and Tables S3–S6 of the supplementary material.
Due to the dominant $n_O\pi^*$ character of the $T_2$ state, the $S_1-T_2$ spin–orbit interaction is stronger than the $S_1-T_1$ coupling. We obtain 34.61i ($x$), 18.15i ($y$), and 2.95i cm$^{-1}$ ($z$), respectively, for the Cartesian components of the spin–orbit Hamiltonian. Since the $S_1\sim T_2$ transition is an activated process according to our SCS-CC2 calculations, we computed the ISC rate at elevated temperatures, thus populating higher vibrational levels of the $S_1$ state. At 300 K, the $S_1\sim T_2$ ISC rate is found to be $2.3 \times 10^{10}$ s$^{-1}$. The small energy difference between the computed $S_1$ and $T_2$ adiabatic energies lies within the confidence range of the SCS-CC2 method. Therefore we cannot exclude the possibility that the $3n_O\pi^*$ state is in fact degenerate with or lower than the $1\pi\pi^*$ state. For this reason we tested the influence of this energy separation on the ISC rate constant. When the $T_2$ potential energy surface is lowered by values between 800 and 1000 cm$^{-1}$, the rate constants for the vibrationally cold $S_1$, $v = 0 \sim T_2$ transition are obtained in the range between $10^{10}$ and $10^{11}$ s$^{-1}$. It should be mentioned, however, that the computed rate constants vary substantially with the energy shift due to the low density of vibrational states in the $T_2$ state in this energy regime.

![Graph](image)

**FIG. 3.** Experimental (red) and SCS-CC2/aug-cc-pVTZ calculated adiabatic energies (in black) of the low-lying singlet and triplet states of the keto-amino forms of (a) cytosine and (b) 5-methylcytosine. The EOM-IP-CCSD calculated adiabatic ionization energy in (a) is taken from Ref. 75.

III. EXPERIMENTAL METHODS AND RESULTS

The experimental setup has been described previously. Briefly, Ne (Linde, >99.995%) at $p = 1.8$ bar backing pressure is passed through a pulsed nozzle (0.4 mm diameter) containing cytosine (Sigma, >99% purity) heated to 235°C. The jet-cooled keto-amino cytosine is excited with 200 µl/pulse UV pulses from a frequency-doubled Radiant Dyes NarrowScan dye laser and ionized by tunable UV pulses in the 215-226 nm range (~150-200 µl, ~10 cm$^{-1}$ bandwidth) from an Ekspla NT342B ultraviolet optical parametric oscillator (UV-OPO). Delayed ionization measurements involved first exciting at the 0$_{01}$ band or one of the seven vibronic bands up to +437 cm$^{-1}$ and then ionizing with a nanosecond time delay that was varied from 0-1000 ns, controlled by a DG535 digital delay unit. The $S_1$ population of 1 undergoes ISC to a triplet state that gives rise to an ion signal that decays with a lifetime of several 100 ns. The resolution of the delayed ionization measurements is determined by the pulse widths of the two lasers (5-7 ns) and the relative trigger jitter, giving an instrumental response function of ~9 ns. Although internal conversion and intersystem crossing occurs about 10-15 times faster, the ratio of these rate constants $k_{IC}/k_{ISC}$ can be accurately fitted from the relative amplitudes of the $1\pi\pi^*$ and triplet state contributions to the total ion signal, see below.

Figure 4 shows the R2PI spectrum of jet-cooled Cyt in the frequency range 31 200–32 400 cm$^{-1}$ when ionizing at 226 nm. The spectrum shows a sharp 0$_{01}$ band at 31 835 cm$^{-1}$ (3.947 eV) and five medium to strong vibronic bands up to +205 cm$^{-1}$, with ten weaker bands between +277 and +530 cm$^{-1}$. The spectrum is similar to that reported by de Vries and co-workers, who also reported that a part of the keto-amino cytosine population excited at the 0$_{01}$ band relaxes to a long-lived state that decays with a lifetime of...
TABLE II. SCS-CC2/aug-cc-pVTZ calculated rotational constants (MHz) of the $S_0$ state, rotational constant changes relative to the $S_1\left(\pi\pi^*\right)$ state, the respective $S_0\rightarrow S_1$ transition dipole moment orientations in the molecule-fixed inertial axis frame, and comparison to the experimental data, for cytosine, 5-methylcytosine, and 5-fluorocytosine. The estimated experimental errors are given in parentheses.

| Molecule          | $A''$ | $B''$ | $C''$ | $A'-A''$ | $B'-B''$ | $C'-C''$ | $|\mu_a|^2:|\mu_b|^2:|\mu_c|^2$ |
|-------------------|-------|-------|-------|----------|----------|----------|-------------------------------|
| Cytosine          | 3857  | 2012  | 1323  | −80      | −26      | −9       | 7:91:2                        |
| Expt.\(^a\)       |       |       |       |          |          |          |                               |
| 5-Methylcytosine   | 3137  | 1412  | 980   | −103     | 1        | −3       | 13:75:12                      |
| Expt.\(^b\)       |       |       |       |          |          |          |                               |
| 5-Fluorocytosine   | 3188  | 1407  | 976   | −127     | 6        | 0        | 15:81:4                       |
| Expt.\(^c\)       |       |       |       |          |          |          |                               |

\(^{a}\)Reference 16.  
\(^{b}\)Reference 41.  
\(^{c}\)Reference 72.

several 100 ns, long enough to be measured by temporally delaying the ionization laser pulse. When photoionizing at 193 nm, Nir et al. measured a lifetime of $\tau_T = 290$ ns, based on which they tentatively assigned it to a triplet state.\(^{13}\) In our experiments we tune the 5 ns pulses from the UV OPO from the ionization threshold of the $S_1$ state, which lies at 38 600 cm\(^{-1}\) (4.79 eV) beyond the ionization threshold of the long-lived state at 44 200 cm\(^{-1}\) (5.48 eV). From this energy difference, the energy of the long-lived state has been determined as 3.26-3.37 eV above the $S_0$ state, in good agreement with our theoretical SCS-CC2 values of the $T_1\left(\pi\pi^*\right)$ energy and previous TDDFT results for keto-amino cytosine.\(^{15}\) Figure 5 shows the analogous (R2PI) spectrum of jet-cooled 5-methylcytosine.

The photoionization efficiency (PIE) curves following excitation at the 0\(^0\) band for prompt ionization (0 ns delay) and with delayed ionization (100 ns delay) have been given and discussed,\(^{15}\) and we only briefly review the salient points: With prompt ionization, an onset in the PIE curve is observed at 38 600 cm\(^{-1}\). The sum of the $S_0\rightarrow S_1$ 0\(^0\) frequency (31 386 cm\(^{-1}\)) and the PIE threshold frequency (39 510 ± 78 cm\(^{-1}\)) corresponds to an adiabatic ionization potential of 8.73 ± 0.02 eV. In contrast, the onset of the PIE curve for delayed ionization is offset by about 4600 cm\(^{-1}\) to higher energy with a slow initial rise above the background. The rise of the delayed ionization PIE curve corresponds to ionization out of vibrationally hot levels that are produced by relaxation to the long-lived state.\(^{15}\)

Figure 6(a) shows a time-delay scan of the ionization pulse relative to the excitation pulse on the 0\(^0\) band. The rise and fall around 0 ns (prompt ionization) reflect the ion signal contribution from the optically excited $S_1$ state $v=0$ level, the later near-constant part of the signal reflects the contribution from triplet state(s) that are populated by ISC out of the $v=0$ level.\(^{13,15}\) For a quantitative evaluation of the ISC rate constant, we assume that the only decay channels accessible

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FIG. 4. Two-color resonant two-photon ionization spectrum of jet-cooled cytosine with vibronic band assignments, based on CC2 calculations (adapted from Figure 3 of Ref. 16). The wavenumber scale is relative to the $0^0_0$ band at 31 835 cm\(^{-1}\). Reproduced by permission of IOP Publishing Ltd.
to $1^\pi^*$ Cyt at low excess energy are fluorescence, $1^\pi^* \rightarrow S_0$ internal conversion and ISC with rate constants $k_{rad}$, $k_{IC}$, and $k_{ISC}$, respectively. The observed $S_1$ state decay rate constants, which have been determined in Ref. 16 via the vibronic-specific lifetimes $\tau_{exc} = (k_{n,S_1})^{-1}$, are then given by the sum of the decay rates $k_{exc} = k_{rad} + k_{IC} + k_{ISC}$. Since the radiative rate constant $k_{rad}$ for keto-amino cytosine is $k_{rad} \sim 3 \times 10^7$ s$^{-1}$ we will neglect it relative to $k_{IC} + k_{ISC}$.

Based on El-Sayed’s rules,67 ISC from the $1^\pi^*$ state is expected to occur most efficiently to $3^\pi^*$ triplet states, that is to the $T_2$ state and not directly to the $T_1$ state, which is $3^\pi^*$.

For the closely related 5-methyl-2-hydroxypyrimidine, Pohler et al. have recently shown that the rapid ISC from the $S_1$ state66 indeed proceeds by a related $S_1 \rightarrow T_2$ El-Sayed-allowed ISC mechanism.68 In the latter case, the $T_2$ state rapidly relaxes to $T_1$, so that our determination of $k_{ISC}$ via ionization of the $T_1$ state includes the $T_2 \rightarrow T_1$ internal conversion rate. Finally, the $T_1 \rightarrow S_0$ reverse ISC rate is denoted $k_{T_1} = (\tau_1)^{-1}$. The time dependence of the sum of the singlet and triplet-state populations can then be modeled as:

$$I(t) = \frac{[S_0]}{k_{obs} - k_{T_1}}[(k_{n,S_1} - k_{ISC} - k_{T_1})e^{-t/\tau_S} + k_{ISC}e^{-t/\tau_T}]$$

$$= Ae^{-t/\tau_S} + Be^{-t/\tau_T}. (1)$$

In order to account for the pulse width of the two lasers as well as the excited state lifetime (which is shorter than the laser pulses) the singlet and triplet parts of the equation were multiplied with the convolution of the instrument response function $E(t')$.69

$$I(t) = Ae^{-t/\tau_S} \int_0^t E(t')e^{(t'/\tau_S)}dt'$$

$$+ Be^{-t/\tau_T} \int_0^t E(t')e^{(t'/\tau_T)}dt'. (2)$$

The instrument response function (IRF) is given by the convolution of the laser excitation and ionization pulse widths, which are approximate Gaussians of $\sim 6$ ns full width at half-maximum (FWHM), resulting in a Gaussian with $\sim 8.5$ ns FWHM. This has in turn to be convoluted with a third Gaussian that represents the time distribution of the trigger jitter of the two pulses. The width of the IRF was determined at an ionization wavelength $> 226$ nm, at which only singlet (and no triplet) ionization occurs, and was found to be $\sim 9$ ns FWHM.

The quantum yield for ISC can then be expressed as:

$$Q_{ISC} = \frac{k_{ISC}}{k_{obs}} = B \frac{A + B}{A + B} \left(1 - \frac{k_{T_1}}{k_{obs}}\right) \approx \frac{B}{A + B}. (3)$$

The intersystem crossing quantum yields $Q_{ISC}$ were determined for the $v = 0$ level and seven following $S_1$ state vibronic levels up to $E_{exc} = +437$ cm$^{-1}$. The time-delay scans for these eight vibrations are shown in Figure 6. On this time scale up to 50 ns after the pump pulse the $T_1 \rightarrow S_0$ ISC rate is almost unmeasurable, the experimental lifetime being $\tau = 290$ ns.13 The level-specific ISC quantum yields were fitted to Equations (1) and (2) with a home-written IDL program using a Levenberg-Marquardt non-linear least-squares fit. The $S_1$ (green) and $T_1$ (blue) population contributions to the fit are indicated in Figure 6, where the total fit is plotted in red. The analogous time-delay scans for five lowest vibronic levels of 5-methylcytosine,41 with ionization at 215 nm are shown in Figure 7.

In Equations (1) and (2) the ionization cross sections of the $S_1$ and $T_1$ states are implicitly assumed to be identical, $\sigma_{ion}(S_1) = \sigma_{ion}(T_1)$. If the energy of the ionization photon is increased far beyond the adiabatic IP, these cross sections must become similar, $\sigma_{ion}(S_1)/\sigma_{ion}(T_1) \equiv \eta_{rel} \approx 1$, since the electron is ionized out of the same $\pi^*$-orbital. At the low ionization energies employed in our experiments this assumption is not justified, since (1) the geometries of the $S_1$ and $T_1$ states differ, hence the Franck-Condon factors in ionization to the ion ground states $D_0$ are different. (2) Ionization from the $S_1$ state occurs from the optically excited vibronic levels, while ISC produces the triplet cytosine with $\sim 4600$ cm$^{-1}$ internal energy.15 (3) The 215 nm ionization pulses ionize this “hot” $T_1$ state to about 3000 cm$^{-1}$ above the triplet photoionization.

Figure 5. Two-color resonant two-photon ionization spectrum of jet-cooled 5-methylcytosine with vibronic band assignments, based on CC2 calculations (adapted from Figure 4 of Ref. 41). The wavenumber scale is relative to the $0^0$ band at 31 277 cm$^{-1}$. Reproduced by permission of IOP Publishing Ltd.
threshold,\textsuperscript{15} while the $S_1$ state is ionized to \~7900 cm$^{-1}$ above the adiabatic ionization threshold. Therefore, the intersystem crossing quantum yields given in Figure 6 for cytosine and Figure 7 for 5-methylcytosine are uncorrected values $Q_{\text{raw}}^{\text{ISC}}$.

To correct the $Q_{\text{raw}}^{\text{ISC}}$ values we calculated the dependence of the $\sigma_{\text{ion}}(S_1)$ and $\sigma_{\text{ion}}(T_1)$ cross sections of cytosine on the ionization energy by evaluating the Franck-Condon factors (FCFs) from the $S_1$ and $T_1$ states to the ion ground state $D_0$. Figure 8(a) shows the $S_1(v = 0) \rightarrow D_0$ FCFs in black and the $T_1(v = 0) \rightarrow D_0$ FCFs in red. The $S_1$, $T_1$, and $D_0$ geometries were optimized and their respective harmonic vibrational frequencies and eigenvectors calculated at the SCS-CC2/aug-cc-pVDZ level. The ionization FCFs were calculated using PGOPHER 7.0.\textsuperscript{70} Vibronic transitions from the $S_1$ and $T_1$ state $v = 0$ levels were calculated to all vibrational levels of the ion within the space of the four vibrations with the largest relative displacements (Huang-Rhys factors) between $S_1$ and $D_0$, or $T_1$ and $D_0$. The FCFs are convoluted with an ionization threshold function that is assumed to be a 0 $\rightarrow$ 1 step function at the adiabatic ionization threshold\textsuperscript{15,71} in this near-threshold region. Figure 8(b) shows the respective calculated $S_1(v = 0) \rightarrow D_0$ and $T_1(v = 0) \rightarrow D_0$ PIE curves. The calculated $S_1$ PIE curve is very similar to the experimental PIE curve, which is shown in Figure 8(c). On the other hand, the calculated $T_1$ PIE curve in Figure 8(b) is offset by about +1000 cm$^{-1}$ relative to the experimental curve in Figure 8(c). According to point (2), the $S_1 \leadsto T_1$ ISC produces vibrationally hot $T_1$ molecules with \~4600 cm$^{-1}$ internal energy. The reason for the shift of the experimental $T_1$ PIE curve is the quasi-thermal PIE tail to low energy that is not included in the simulation.

Based on Figure 8(b) we estimate the relative singlet and triplet ionization cross sections as $\sigma_{\text{ion}}(S_1)/\sigma_{\text{ion}}(T_1) \sim$ 3 at the ionization wavelength of 215 nm. This estimate may be on the low side, because the geometry difference between $T_1$ and $D_0$ is larger than that between $S_1$ and $D_0$, as the width of the FCF distribution in Figure 8(a) shows. Since our PIE curve simulation can only account for the geometry change along the four normal modes with the largest Huang-Rhys factors, we expect the relative importance of the neglected vibrations.
(with smaller Huang-Rhys factors) to be larger for \( T_1 \rightarrow D_0 \) ionization.

We note that in Ref. 13, ionization of the cytosine excited states was performed with an ArF excimer laser at a wavelength of 193 nm. At this higher ionization energy (51800 cm\(^{-1}\)), Figure 8(b) predicts that the triplet photoionization cross section increases further, relative to the singlet photoionization cross section. Since \( \sigma_{\text{ion}}(S_1)/\sigma_{\text{ion}}(T_1) \) now approaches \( \sim 1 \), the \( S_1 \) and \( T_1 \) states are ionized with almost the same probability. This should remove the “shoulders” in the time-delay profiles, as compared to Figs. 6 and 7. This prediction of our Franck-Condon model is in perfect agreement with the experimental 193 nm time-delay profile in Fig. 3 of Ref. 13, which does not exhibit a shoulder.

Based on Figure 8, we corrected the \( Q_{\text{ion}}^{\text{corr}} \) values in Figures 6 and 7; the corrected ISC yields \( Q_{\text{ISC}}^{\text{corr}} \) are given in Tables III and IV. Combining these \( Q_{\text{ISC}}^{\text{corr}} \) values with the experimental \( S_1 \) state lifetimes \( \tau_{\text{obs}} \) determined for the same vibronic levels of cytosine,\(^{16} \) which are given in column 2 of Table III then allows to determine the internal conversion and intersystem crossing rate constants \( k_{\text{IC}} \) and \( k_{\text{ISC}} \) as a function of vibrational excess energy \( E_{\text{exc}} \). As Table III shows, the experimental ISC rate constants are in the range

\[
\begin{array}{cccccc}
\text{Vibronic transition} & \tau_{\text{obs}}/5 \text{ ps} & Q_{\text{ion}}^{\text{corr}} & Q_{\text{ISC}}^{\text{corr}} & k_{\text{ISC}}/10^9 \text{ s}^{-1} & k_{\text{IC}}/10^{10} \text{ s}^{-1} \\
0_0^0 & 44 (5) & 0.09 & 0.03 & 0.68 & 2.2 \\
1_1^1 (71 \text{ cm}^{-1}) & 33 (5) & 0.13 & 0.05 & 1.5 & 2.9 \\
2_2^0 (92 \text{ cm}^{-1}) & 33 (5) & 0.09 & 0.03 & 0.91 & 2.9 \\
1_2^1 + 2_0^0 (162 \text{ cm}^{-1}) & 38 (4) & 0.13 & 0.05 & 1.3 & 2.5 \\
1_3^0 (177 \text{ cm}^{-1}) & 33 (3) & 0.12 & 0.04 & 1.2 & 2.9 \\
3_2^0 (205 \text{ cm}^{-1}) & 44 (4) & 0.13 & 0.05 & 1.1 & 2.2 \\
308 \text{ cm}^{-1} & > 25 & 0.07 & 0.03 & 1.2 & < 3.9 \\
437 \text{ cm}^{-1} & > 25 & 0.03 & 0.01 & 0.40 & < 3.9 \\
\end{array}
\]

\( ^a \)Assuming relative ionization cross section \( \sigma_{\text{ion}}(S_1)/\sigma_{\text{ion}}(T_1) = 3 \).

\[
\begin{array}{cccccc}
\text{Vibronic transition} & \tau_{\text{obs}}/\text{ps} & Q_{\text{ion}}^{\text{corr}} & Q_{\text{ISC}}^{\text{corr}} & k_{\text{ISC}}/10^9 \text{ s}^{-1} & k_{\text{IC}}/10^{10} \text{ s}^{-1} \\
0_0^0 & 60 (5) & 0.04 & 0.02 & 0.33 & 1.6 \\
1_0^1 (49 \text{ cm}^{-1}) & 60 (5) & 0.03 & 0.01 & 0.17 & 1.6 \\
1_0^1 (69 \text{ cm}^{-1}) & 60 (5) & 0.06 & 0.02 & 0.33 & 1.6 \\
6_0^0 + 2_0^0 (385 \text{ cm}^{-1}) & \ldots & 0.05 & 0.02 & \ldots & \ldots \\
6_0^0 + 2_0^0 (458 \text{ cm}^{-1}) & \ldots & 0.03 & 0.01 & \ldots & \ldots \\
\end{array}
\]

\( ^a \)Assuming relative ionization cross section \( \sigma_{\text{ion}}(S_1)/\sigma_{\text{ion}}(T_1) = 3 \).

TABLE III. Experimental lifetimes, intersystem crossing quantum yields \( Q_{\text{ISC}} \) and \( k_{\text{ ISC}} \) parameters of keto-amino cytosine.

TABLE IV. Experimental lifetimes, intersystem crossing quantum yields \( Q_{\text{ISC}} \) and \( k_{\text{ ISC}} \) parameters of keto-amino 5-methylcytosine.

FIG. 8. (a) Calculated ionization Franck-Condon factors (FCFs) from the \( S_1 \) and \( T_1 \) excited-state vibrationless \( \nu = 0 \) levels to the ion ground state (\( D_0 \)): \( S_1(\nu = 0) \rightarrow D_0 \) FCFs in black, \( T_1(\nu = 0) \rightarrow D_0 \) FCFs in red, plotted vs. the energy of the ionization photon in cm\(^{-1}\). (b) Calculated photoinization efficiency (PIE) curves of the \( S_1 \) and \( T_1 \) states using the FCFs from panel (a). Vertical black red and blue dashed lines indicate the adiabatic ionization threshold (vibrationless \( S_1 \rightarrow D_0 \) and \( T_1 \rightarrow D_0 \) transitions, respectively. The dashed blue line indicates the experimental ionization energy (215 nm). (c) Experimental PIE curves of amino-keto cytosine \( S_1 \) state (undelayed or “prompt” ion signal) and \( T_1 \) state, data taken from Ref. 15. For further details see the text.
0.4–1.6 \cdot 10^9 \text{s}^{-1}, while the experimental IC rate constants are much higher, being in the range 2.2–3.9 \cdot 10^{10} \text{s}^{-1} or 30–100 times the ISC rate constants. Upon correcting the raw ISC yields shown in Figure 7 for 5-methylcytosine with the same relative ionization efficiency factor \( \sigma_{\text{崇尚}}(S)/\sigma_{\text{崇尚}}(T) \sim 3 \), we obtain the corrected \( Q_{\text{ISC}} \) values and the ISC and IC rate constants given in Table IV.

### IV. DISCUSSION

If the energetic ordering of the \( S_1 \) and \( T_2 \) states of keto-amino Cyt obtained from the SCS-CC2 calculations is correct, then only the \( S_1 \rightarrow T_1 \) ISC channel is open from the vibrationally cold \( v = 0 \) \( S_1 \) state. Our calculated ISC rate constant for this process (3 \( \cdot \) 10^9 \text{s}^{-1}) is four times the experimental value \( k_{\text{ISC}} = 0.7 \cdot 10^9 \text{s}^{-1} \). Given the uncertainties involved in both theory and experiment, this agreement is remarkably good. Results obtained for cytosine derivatives support this mechanism, which was also advocated by Merchán and co-workers. The calculated and experimental adiabatic excitation energies and vibrationless (0\( \rightarrow \)) transition energies of the \( S_1, T_1, \) and \( T_2 \) states of cytosine, 5-methylcytosine, and 5-fluorocytosine are collected in Table I. It is seen that the adiabatic energy differences between the \( S_1 \) and \( T_1 \) states of the three compounds are similar; in contrast, the energy gap between the \( S_1 \) and \( T_2 \) states of cytosine and 5-methylcytosine differs substantially. While it is debatable whether the \( v = 0 \) level of the \( T_2 \) state is located energetically above \( S_1 \) (\( v' = 0 \)) in cytosine, this is clearly not the case in the 5-methyl derivative where these levels are 0.36 eV apart. Hence, it can safely be assumed that the \( S_1 \rightarrow T_2 \) ISC can only occur as a thermally activated process in 5MCyt. Nevertheless, the experimental 0\( \rightarrow \) (1\( \pi \pi \)) lifetimes of jet-cooled 5-methylcytosine (60 ps) and 5-fluorocytosine (75 ps) are of the same order of magnitude as that of cytosine (45 ps). We therefore conclude that the dominant ISC mechanism of the vibrationally cold \( S_1 \) state of cytosine in the gas phase is the direct \( S_1, v = 0 \rightarrow T_1 \) intersystem crossing.

As soon as the \( T_2 \) ISC channel becomes energetically accessible, the calculated ISC rates increase by one to two orders of magnitude, depending on the exact energy separation between the initial and final states. Despite the considerable spread in our theoretical results, we are confident that the decay rates are substantially smaller than the values predicted by Richter et al. and Mai et al. These authors claimed the \( S_1 \rightarrow T_2 \) transition to occur on an ultrafast (subpicosecond) time scale. However, their excited-state surface hopping dynamics treatment is based on state-averaged CASSCF energies and wave functions. At that level of theory, the global minimum of the \( S_1 \) potential energy surface corresponds to a \( ^1n_\delta \pi^* \) electronic structure, which is proven to be not correct by recent experimental investigations.

According to our present calculations, the \( S_1 \rightarrow T_2 \) ISC cannot be responsible for the experimentally observed ultrafast decay of the \( S_1 \) state, which begins about 530 cm\(^{-1} \) above the origin. For the vibrational levels located above this threshold, ultrafast internal conversion to the \( S_0 \) state via a conical intersection is believed to be the dominating process.

Overall, we note that the intersystem crossing quantum yields and rates are similar to that measured for thymine isomer 5-methyl-2-hydroxyuracil, for which ISC is the dominant nonradiative process out of the \( S_1 \) state, with \( k_{\text{ISC}} \sim 1/\tau_{\text{ISC}} \sim 2 \cdot 10^9 \text{s}^{-1} \).

### V. CONCLUSIONS

We show experimentally and by \textit{ab initio} calculations that the keto-amino tautomers of supersonically cooled cytosine and 5-methylcytosine undergo moderately efficient intersystem crossing from their photoexcited \( S_1(\pi\pi^*) \) states\(^{3,13}\) to the triplet \( T_1(\pi\pi^*) \) state. We experimentally quantify the ISC quantum yields \( Q_{\text{ISC}} \) and rates \( k_{\text{ISC}} \) as a function of the \( S_1 \) state vibrational excess energy \( E_{\text{ex}} \) by separately detecting the \( S_1 \) and \( T_1 \) states using prompt and delayed laser photoionization. The relative \( S_1 \) and \( T_1 \) ionization cross sections are calculated from the SCS-CC2 calculated \( S_1, T_1 \) and ion \( D_0 \) state geometries and frequencies, followed by calculation of the photoionization Franck-Condon factors. The ionization-corrected ISC quantum yields are in the range \( Q_{\text{ISC}} \) from 0.01–0.05 for the lowest eight vibronic levels of cytosine and \( Q_{\text{ISC}} \) from 0.01–0.02 for the lowest three levels of 5-methylcytosine.

Combining these \( Q_{\text{ISC}} \) values with the experimental \( S_1 \) state lifetimes that were previously determined for the same vibronic levels of cytosine\(^{16}\) and of 5-methylcytosine\(^{41}\) allows to determine both the ISC and IC rates \( k_{\text{ISC}} \) and \( k_{\text{IC}} \). The ISC rates for \( S_1 \) state cytosine up to \( E_{\text{ex}} \sim 550 \text{cm}^{-1} \) are \( k_{\text{ISC}} \sim 0.4–1.5 \cdot 10^9 \text{s}^{-1} \). For 5-methylcytosine the ISC rates of the lowest three levels are \( k_{\text{ISC}} \sim 0.3 \cdot 10^9 \text{s}^{-1} \).

Theoretical ISC rates are calculated using the spin-component-scaled SCS-CC2 method with the aug-cc-pVTZ basis set. These predict rapid ISC from the \( S_1, v = 0 \) state with \( k_{\text{ISC}} = 3 \cdot 10^9 \text{s}^{-1} \) to the \( T_1(\pi\pi^*) \) triplet state. The surprisingly high rate of this El Sawy-forbidden transition is caused by a substantial admixture of \( ^1n_\delta \pi^* \) character into the \( S_1(\pi\pi^*) \) wave function at its non-planar minimum geometry. Results on 5-methylcytosine and 5-fluorocytosine support this mechanism. The vibrational ground state of the \( T_2(\pi\pi^*) \) triplet state of cytosine is located \( \sim 650 \text{cm}^{-1} \) above the \( S_1, v = 0 \) level. As soon as the \( T_2 \) channel becomes energetically accessible, ISC rates increase by one to two orders of magnitude, depending on the excess energy.

For the low-lying vibronic levels of cytosine and 5-methylcytosine investigated, the level-specific \( S_1 \rightarrow S_0 \) IC rate constants are 20–50 times faster than the corresponding ISC rate constants, so internal conversion is clearly the dominant nonradiative process near the minimum of the \( S_1 \) state. Above \( E_{\text{ex}} \sim 600 \text{cm}^{-1} \) no further vibronic bands are observed, implying that the IC rate increases by a factor of \( \geq 50 \) within about 150 cm\(^{-1}\). This agrees\(^{16}\) with the experimental observation of short lifetimes \( \tau \leq 2 \) ps measured by femtosecond two-step laser photoionization techniques at higher \( E_{\text{ex}} \). Above this energy, we detect no triplet state ion signals (ISC quantum yield \( Q_{\text{ISC}} \leq 0.01 \), which implies that \( S_1 \rightarrow S_0 \) internal conversion effectively shuts off both the \( S_1 \rightarrow T_1 \) and \( S_1 \rightarrow T_2 \) ISC decay channels. The sudden
increases in $k_{IC}$ imply that the barrier towards the lowest conical intersection (C5-twist CI) is surmounted around $E_{exc} = 550 \text{ cm}^{-1}$.

Our calculated and experimental ISC rate constants are $1000–10\,000$ times smaller than those of previous calculations that predicted ultrafast ISC of cytosine with $k_{ISC} = 10^{13}$–$10^{14}$ s$^{-1}$.32,33 Our calculations address the low-lying vibronic levels of $S_1$ cytosine, while the previous predictions were computed using broad-band excitation in the range from $4–7$ eV ($177–310$ nm), corresponding to excitation at very high energy.32,33 While broadband excitation extending far into the deep UV may be interesting from a theoretical point of view, the solar UV radiation that leads to modern-day DNA photodamage is cut off by the earth's ozone layer around 295–300 nm. In fact, the region of low $E_{exc}$ probed in our experiments and calculations corresponds to the long-wavelength "red edge" absorption of cytosine in solution.5,73 This is the primary region of photobiological and -medical interest with respect to UV lesions and skin cancer,73 and not the vertical excitation to the absorption band maximum at $\sim 260$ nm.32,33

In summary, we show that upon exciting gas-phase $S_1$ state keto-amino cytosine from the $0_0^0$ band up to $E_{exc} \sim 440 \text{ cm}^{-1}$, neither $S_1 \rightarrow S_0$ internal conversion or $S_1 \rightarrow T_1$ intersystem crossing are ultrafast. Internal conversion is the most efficient nonradiative channel, being $30–40$ times faster than ISC. The ISC quantum yield of cytosine and cytidine in aqueous solution is $Q_{ISC} = 0.02$,10,11 very close to that determined here for cold gas-phase cytosine. However, the IC rate of cytosine and cytidine in aqueous solution measured with excitation at 263–270 nm (that is, at photon energies $\sim 5600 \text{ cm}^{-1}$ higher than in this work) is $k_{IC} \sim 6–13 \cdot 10^{10}$ s$^{-1}$ (Refs. 6–8) or about $30$ times faster than in the gas-phase at low excess energy $E_{exc}$.10 This implies that at this much higher excess energy, the $k_{ISC}$ in room temperature aqueous solution also increases by $\sim 30x$. It will be interesting to investigate the nonradiative properties of cytosine close to its electronic origin also in aqueous solution, and also to probe the effects of H-bonding interactions with proximal water molecules74 on the gas-phase photophysics of cytosine.

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