**UV-excitation from an experimental perspective I: frequency resolved**

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**Abstract**
Electronic spectroscopy of DNA bases in the gas phase provides detailed information about the electronic excitation, which places the molecule in the Franck-Condon region in the excited state and thus prepares the starting conditions for excited state dynamics. Double resonant or hole burning spectroscopy in the gas phase can provide such information with isomer specificity, probing the starting potential energy landscape as a function of tautomeric form, isomeric structure, or hydrogen bonded or stacked cluster structure. Action spectroscopy, such REMPI, can be affected by excited state lifetimes.

**keywords**
DNA bases, nucleotides, REMPI, R2PI, hole burning, clusters, conical intersections

## 2.1. Introduction

Understanding the response of DNA bases to UV radiation is critical for both practical and fundamental reasons. [1-5] Nucleobase photochemistry following UV absorption constitutes a fundamental step in radiation-induced DNA damage. Short excited state lifetimes are often described as nature's strategy to protect the building blocks of life against UV photodamage. Furthermore, UV photo-selection may have played a key role in prebiotic chemistry on an early earth. This photochemistry schematically involves two steps: absorption of a photon prepares the nucleobase in an excited state, followed by ensuing chemistry. The first step, the excitation, can be probed by various forms of electronic spectroscopy. The second step, the dynamics of the excited state once it is formed, can often be followed in the time domain, which is the topic of the next chapter. This current chapter focuses on the frequency domain, probing primarily the excitation step. However, some time domain information also follows from the frequency domain because both steps are connected by Heisenberg's uncertainty principle: $\Delta E \Delta \tau \geq h/2\pi$ such that a shorter excited state lifetime, $\tau$, corresponds to a larger linewidth, $\Delta E$.

The fate of a molecule upon irradiation depends on the shape of the excited state potential energy landscape [6]. Electronic excitation places the molecule in the Franck-Condon region of the excited state, as the starting point of a number of competing possible processes, such as fluorescence, internal conversion, intersystem crossing or exciplex formation [7, 8]. The availability of these pathways and their relative rates, or associated excited state lifetimes, determines the outcome of this competition and thus the product of the photochemistry. One molecule can therefore be intrinsically more resistant to damage than another even though they absorb UV light equally. Furthermore, the same molecule can have a different UV response, depending on the molecular environment. Of particular interest in the case of the nucleobases is the rate of internal conversion, compared to other possible processes. Internal conversion returns the molecule to the electronic ground state in a radiationless way, and thus converts electronic energy to heat in the form of ground state vibrational

energy. Heat can subsequently be safely dissipated to the environment. When internal conversion is fast enough to prevent other photochemical reactions from taking place, the molecule will have a very short excited state lifetime, τ*, and be stable against UV photodamage [9-12].

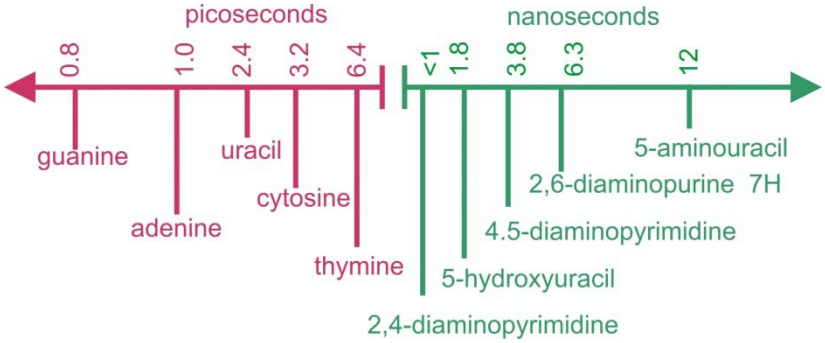


**Figure 1:** Compilation of gas phase excited state lifetimes of selected nucleobases and derivatives.

Rapid internal conversion, when available to a molecule, diffuses electronic energy by converting it to internal energy in the ground state and therefore minimizes access to other photochemical pathways. Generally the biologically most relevant forms of the purines and pyrimidines exhibit the shortest excited state lifetimes, thus selectively minimizing the chances for photochemical damage in the molecular building blocks of life. In stark contrast, many other nucleobase derivatives, even isomers, have orders of magnitude longer lifetimes (See Figure 1). In fact, ultrafast internal conversion — the same property found in many UV sunscreens — is observed for all nucleobases implicated in replication today and, intriguingly, there are no examples of canonical nucleobases that are highly fluorescent [13]. Strengthening the correlation between excited state lifetime and the propensity for photoreaction is the observation that the minor DNA base 5-methylcytosine, with its 10-fold longer lifetime than cytosine, is a hot spot for photodamage [14].

Evolution requires the existence of self-replicating molecules; the selection of the nucleobases as building blocks of those macromolecules would logically have taken place prior to any biological processes. Therefore, if the excited state properties have played a role in a chemical selection of today's nucleobases, they may be a relic of prebiotic chemistry on an early earth.

The intramolecular mechanism governing the ultrafast internal conversion in single purines and pyrimidines is now emerging. The key is the occurrence of conical intersections that connect the excited state potential energy surface, reached by photon absorption, to the ground state energy surface. The dramatic lifetime differences between derivatives of nucleobases appear to be due to variations in the excited state potential surfaces that restrict or slow access to these conical intersections [15].

The excited state potential energy surface, and thus the corresponding electronic absorption and dynamics, is affected by molecular structure and by interactions, such as those with solvent molecules. To disentangle the different effects it is desirable to study isolated molecules, which is possible in the gas phase under collision free conditions. First, this makes it possible to observe intrinsic properties and explore the potential energy

surfaces of the individual molecules. Secondly, by employing double resonant techniques, it is possible to perform isomer specific spectroscopy. Some details of how interactions affect excited states can be studied by cluster spectroscopy in the gas phase. Once intrinsic properties are mapped out, these may be extrapolated to bulk conditions, which are the subject of later chapters.

## 2.2 Vibronic spectra

The nucleobases are heterocyclic aromatic compounds which absorb to their first excited electronic state in the 31,000-37,000 $cm^{-1}$ range. This range also forms the onset of absorption in the liquid phase. Figure 2 shows resonance enhanced multiphoton ionization (REMPI) spectra of all of the canonical bases and some derivatives, as well as some of their clusters, representing base pair combinations. Asterisks indicate origins of different tautomers for the monomers and different hydrogen bonded structures for the clusters. Table 1 lists the respective $S_1 \leftarrow S_0$ origins.

Levy and coworkers reported the first REMPI spectra for uracil and thymine in 1988 [16]. The spectra were very broad, merely showing an onset of unstructured absorption around 36,000 $cm^{-1}$ for T and somewhat higher energy for U. These results suggested the absence of useful resolved spectroscopy for nucleobases. However that picture changed with the first report of the resolved guanine REMPI spectrum [17], followed by those of adenine [18] and cytosine [19]. Observation of these spectra became possible with the employment of laser desorption jet cooling techniques, optimized for the study of these types of molecules [20-23].

The vapor pressure of the nucleobases at room temperature is too low for gas phase spectroscopy and heating to obtain sufficient vapor pressure causes thermal degradation. With some care, adenine can still be sufficiently heated in an oven to form a seeded supersonic beam, but this does not work satisfactorily for the other bases. Pulsed heating at a rate of the order of 1000 degrees in 10 ns allows fragment-free vaporization of all bases and this heating rate can be achieved with pulsed laser desorption. Typical experiments use a Nd:YAG laser at 1064 nm with 10 ns pulses of a fraction of a $mJ/cm^2$ to desorb from a graphite substrate, which is moved to expose fresh material in subsequent shots [21, 24, 25]. To permit spectroscopy, the desorbed molecules are entrained in a pulsed supersonic expansion, cooling them to internal temperatures corresponding to the order of 20 K [21]. Such temperatures are low enough for the vibronic spectroscopy described in this chapter, but it may be noted that ions in a cold trap can be cooled to lower temperatures by about an order of magnitude and entrainment in helium droplets leads to even lower temperatures [26, 27].

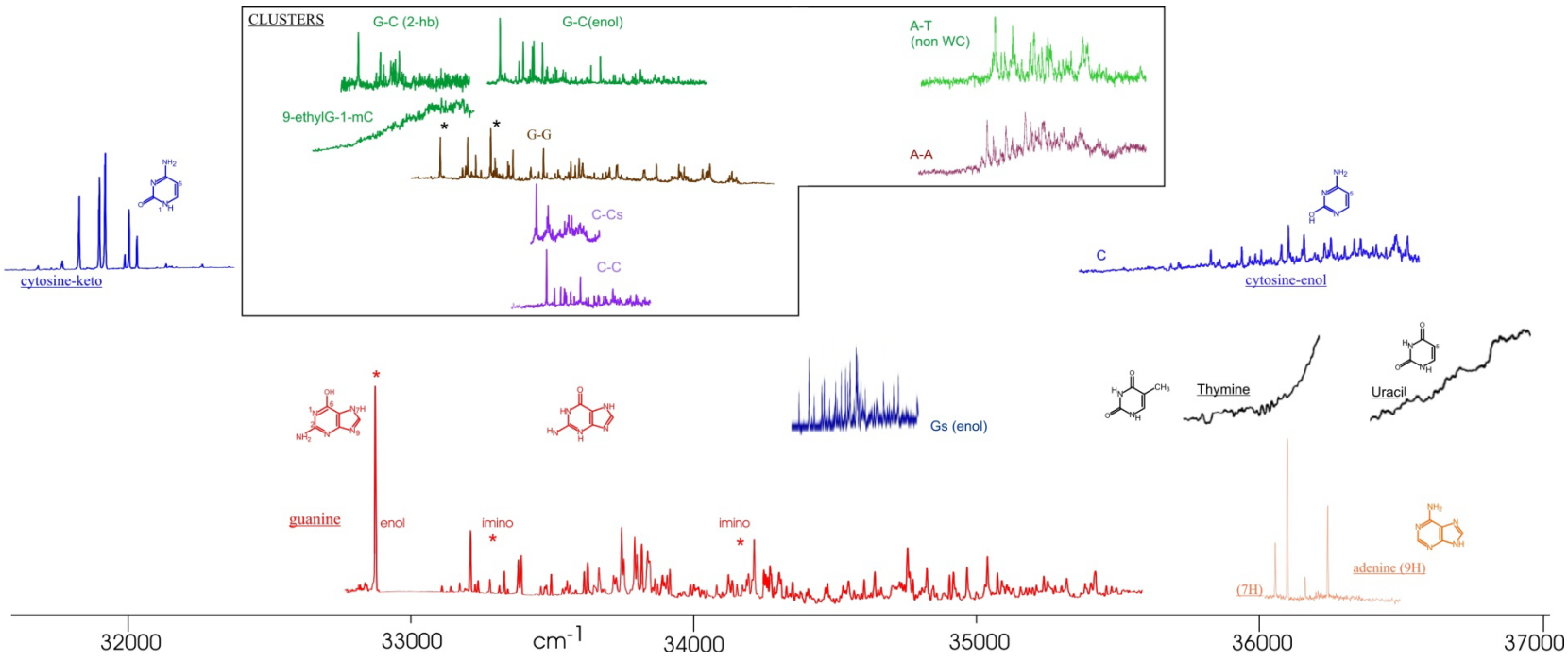


**Figure 2:** REMPI spectra of the canonical bases and their base pairs. Asterisks indicate origins of different tautomeric or structural isomers.

| | Compound | origin $cm^{-1}$ | remarks | references |
|---|---|---|---|---|
| **nucleobases** | guanine | 32 864 | 7H enol *syn* | 28 |
| | | 33 269 | oxo-imino | 28 |
| | | 33 910 | oxo-imino | 28 |
| | | 34 755 | N9H hydroxyl-amino | 28 |
| | adenine | 36 105 | π-π* | 29, 30 |
| | | 36 062 | n-π* | 29, 30 |
| | cytosine | 31 826 | keto | 31 |
| | | ~36 000 | enol | 31 |
| | thymine | 36 000 | broad onset | 16 |
| | uracil | 36 700 | broad onset | 16 |
| **nucleosides** | guanosine | 34 443 | enol | 32 |
| | 2' deoxyguanosine | 34 436 | enol | 32 |
| | 3' deoxyguanosine | 34 443 | enol | 32 |
| **base-pairs** | G-C | 33314 | C in enol form | 33 |
| | | ~32 800 | doubly H-bonded | 34 |
| | A-T | 35 064 | | 35 |
| | G-G | 33 103, 33 282 | | 36 |
| | A-A | 35 040 | | 29, 37 |
| | C-C | 33 483 | | 31 |
| **derivatives** | 1-methylcytosine | 31 908 | | 31 |
| | 5-methylcytosine | 31 269 | | 31 |
| | 5-OH-uracil | 34 440 | | 38 |
| | 5-$NH_2$-uracil | 31 939 | | 38 |
| | 9-methylguanine | 34 612 | | 36 |
| | 2,4-diaminopyrimidine | 34 459 | diamino | 39 |
| | 2,6-diaminopurine | 34 881<br>32 215 | 9H diamino<br>7H diamino | 39 |
| **derivative pairs** | 9-mG-1mC | 33 000 | broad | 34 |
| | C-1mC | 33 419 | | 31 |
| | C-5mC | 32 500, 32 691, 32 916 | | 31 |
| | 5mC-5mC | 32 493, 32691, 32 872 | | 31 |

**Table 1:** Observed $S_1$ band origins of nucleobases, nucleosides, base-pairs, and assorted nucleobase derivatives. M (as in 1mC) designates methyl.

*Spectroscopic techniques*

In molecular beams, direct absorption is generally not measurable because the optical density is too low. Intracavity techniques can sometimes be used, such as cavity ringdown [40] and microwave spectroscopy [41], but the common approach is a form of action spectroscopy, especially laser induced fluorescence (LIF) and resonant two photon ionization (REMPI). A consequence of this technical limitation is that the excited sate becomes part of the detection method and thus the dynamics of the excited state can influence the outcome of the measurement. In particular, both LIF and REMPI are blind for transitions to an excited state with a lifetime significantly shorter than the laser pulse duration. The following sections will discuss some implications of this limitation for understanding excited state behavior.

A major advantage of gas phase spectroscopy is the ability to perform isomer specific measurements by double resonant, or hole-burning techniques. Figure 3 schematically outlines this approach for the case of REMPI. Purple arrows indicate wavelengths that are scanned to obtain a wavelength dependent spectrum. In (a) this produces a vibronic REMPI spectrum. In (b) the blue arrow represents the probe laser, set to a single resonant wavelength in the REMPI spectrum. Its ion signal comes from one specific isomer. The purple arrow represents the burn laser, which precedes the probe laser by typically 100 ns. Whenever the burn laser is resonant with a transition it depletes the ground state and alters the Franck-Condon area, resulting in a dip in the ion signal from the probe laser, but only if both lasers interact with the same isomer population. Therefore a scan with the probe laser produces an ion dip spectrum that amounts to an isomer selected rovibronic spectrum. In (c) the same procedure is followed with an IR laser as probe laser to generate an isomer selected ground state IR spectrum. In Figure 2 asterisks denote origins of different isomers, identified by double resonant spectroscopy.

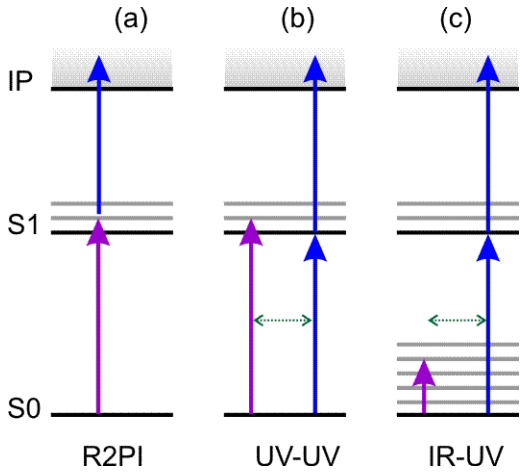


**Figure 3:** Schematic diagram of spectroscopic techniques. Purple arrow indicates wavelength that is being scanned to obtain a spectrum. Blue arrows indicate fixed wavelength. Green arrows indicate time delay between two laser pulses. (a) REMPI provides vibronic spectra. (b) UV-UV hole burning separates isomeric contributions to the UV spectrum. (c) IR-UV hole burning provides isomer selected ground state IR spectra.

Electronic spectra essentially contain information about the energy difference between the ground state and the excited state. By reducing the ground state vibrational population to v=0 through cooling, vibronic spectral structure can provide additional information on the excited state. IR-UV hole burning on the other hand provides ground state vibrational frequencies which can be used to identify the starting structures of the excitation, especially tautomeric form and cluster structure. Further information can be derived from line contours for identifying excited state symmetries and lifetimes.

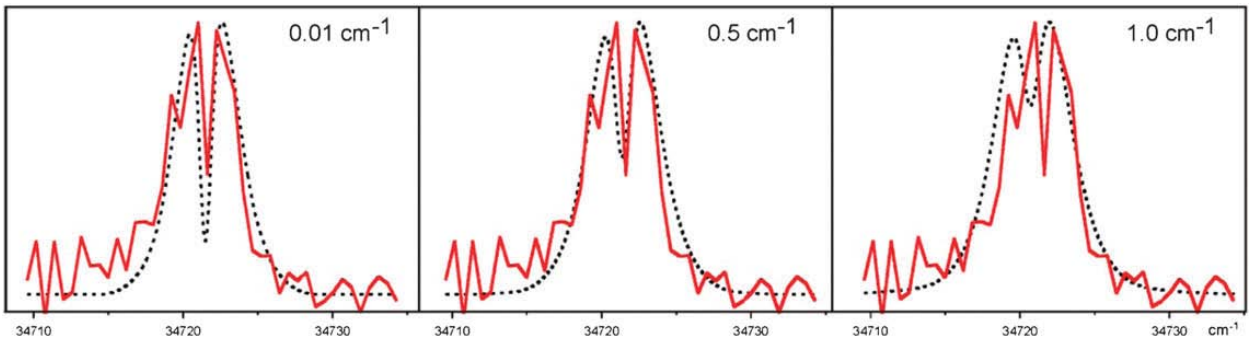


**Figure 4:** Detail of the 34,720 $cm^{-1}$ peak in the 2,4-DAPy REMPI spectrum, compared with simulations of the rotational envelope at three different Lorentzian linewidths [39].

The latter is demonstrated in Figure 4, showing the comparison of a peak in the REMPI spectrum of 2,4-diaminopyrimidine with simulated lineshapes based on different Lorentzian linewidths [39]. The upper limit to the linewidth of 0.5 $cm^{-1}$ thus observed in this case corresponds to a lower limit in the excited state lifetime of 10 ps.

Further information about the excited state dynamics may be derived by comparing these electronic spectra, for example by comparing frequency shifts between various isomers and derivatives and by contrasting broad versus sharp spectra. Furthermore, since all of these REMPI spectra were obtained with nanosecond pulses, absences of signal from selected species may point to ultrafast internal conversion.

## 2.3 Isomers

The origins on the UV spectra, obtained by REMPI, reflect the energies of the $S_1$ excited state relative to $S_0$ ground state, which is difficult to obtain computationally with any reasonable accuracy. Figure 2 shows that both the purine and pyrimidine bases absorb in the same frequency range, but significant shifts exist between different related compounds. For example, adenine absorbs 3000 $cm^{-1}$ to the blue of the lowest energy observed tautomer of guanine [39, 42]. Adenine, which is 6-aminopurine (6AP), also absorbs about 3500 $cm^{-1}$ to the blue of its isomer 2-aminopurine (2AP). Comparing those two isomers, one notices not only the large difference in origin, but also the fact that the adenine spectrum features only a few lines while the 2-aminopurine spectrum is very extensive and covers a large frequency range [43]. These observations are consistent with a model that is

summarized schematically in Figure 5 [42]. This model assumes excitation to an $S_1$ state, which interacts with a second excited state through a curve crossing. This picture represents potential curves as a function of a single internuclear coordinate. In the more general case of multidimensional potential surfaces, to be discussed below, the curve crossing will be replaced by a conical intersection. The second excited state may couple with the electronic ground state to quench fluorescence and phosphorescence. In the case of the purines, the $S_1$ state is likely to be of π-π* character and the second excited state is likely to be of n-π* character, to be discussed below. The absorption characteristics of the purines and their excited state lifetimes then depend strongly on two parameters: (i) the relative energies of these two states and (ii) the amount of electronic coupling between them.

Possible arrangements are shown schematically in Figure 5. A curve crossing, as sketched schematically for 6AP, will typically lead to spectra that are diffuse or show a cutoff at absorption energies larger than that of the crossing. If, on the other hand, as sketched for 2AP, the interaction between the two excited states is weak, or if the $S_1$ state is significantly lower in energy, then we may expect a sharp and extended vibronic spectrum. A lower energy $S_1$ state is also consistent with the large observed red-shift. Finally, the other extreme is sketched in the inset: If the interaction is strong and the $S_1$ state is higher in energy, then it is possible to have a barrierless curve crossing, resulting in a completely structureless vibronic spectrum and an extremely short excited state lifetime.

Within this picture the striking photochemical difference of purine and adenine as compared to 2-aminopurines is the result of the relative energies of the π-π* and the n-π* states. The fact that the origin for the 2AP spectrum is significantly to the red of the adenine spectrum, implies that the $S_1$ state is lower in this case. Furthermore, the extent of the spectrum suggest that 2AP does *not* exhibit a curve crossing as sketched in Figure 5 2b, as opposed to adenine. Therefore the gas-phase spectra suggest that the amino substitution in the 2 position of purine lowers the excited state potential of the π-π* state, while substitution in the 6 position has little effect. This is fully consistent with solution results, as well as with circular dichroism measurements by Holmen et al. which identify a π-π* state about 4000 $cm^{-1}$ below the n-π* state for 2AP [44].

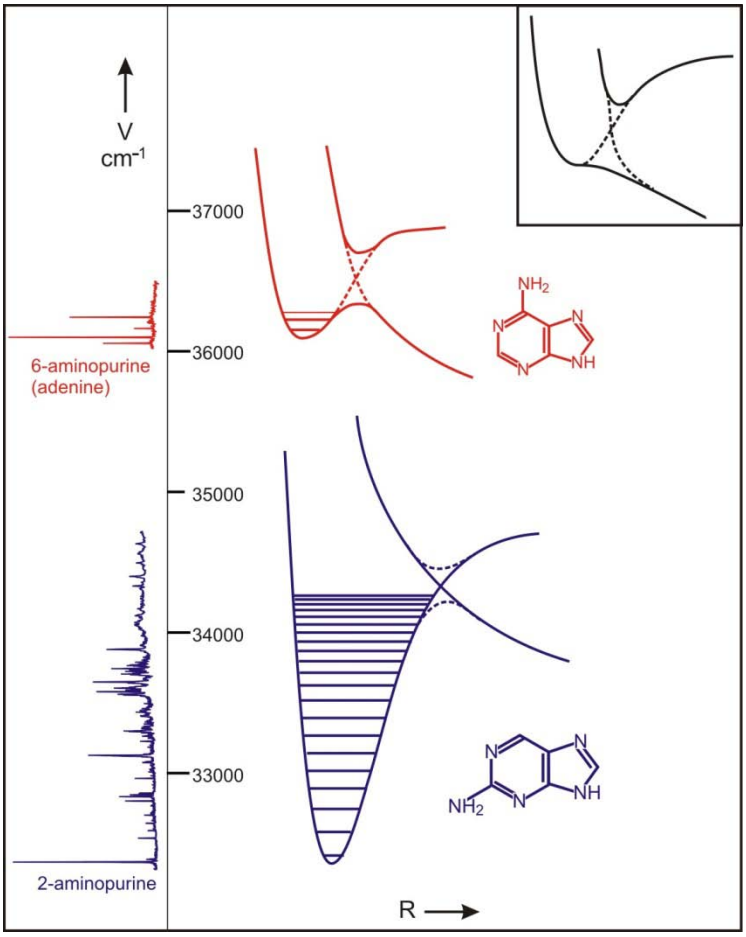


**Figure 5:** Left along vertical axis, REMPI spectra of 6-aminopurine (adenine) in red and 2-aminopurine in blue. Right, schematic potential energy diagrams of the excited state as a function one internuclear coordinate, representing a model with a curve crossing between $S_1$ and another excited state. The inset shows the case without a barrier, which would not produce a discrete spectrum; see text for details.

## 2.4 Derivatives

When we consider more than one internuclear coordinate we can describe the more general situation as illustrated schematically in Figure 6. The crossings of multidimensional potential surfaces are conical intersections. These features can only occur in regions of the potential energy landscape that represent a deformation of the molecular frame from the ground state equilibrium geometry. The following case study of adenine derivatives serves as an example [18, 45-57]. For 4-aminopyrimidine (Figure 7 top left), surface hopping calculations identified two conical intersections [58]: deformation at the C2 position (red circle) leads to deactivation of the excited state with a lifetime, τ*, of 1 ps and deformation at the C5=C6 bond (blue rectangle) leads to deactivation with τ* of 400 fs. Immobilizing the latter with a 5 membered ring forms adenine with a single conical intersection due to the C2 deformation and τ* of 1 ps. Figure 7 4 (bottom left) shows the geometry at this conical

intersection as calculated by Marian et al. [59]. Substitution at the C2 position further modifies the excited state potential and eliminates this conical intersection. Consequently, both 2,6-diaminopurine and 2-aminopurine have fluorescent excited states with lifetimes of the order of nanoseconds. In fact, the latter is used as a fluorescent tag in DNA research. In the same way, C5 substituents in pyrimidines alter excited state lifetimes over a range of picoseconds to nanoseconds, by modification of the topography of the potential energy surfaces around C5=C6 torsion and stretching coordinates [60-62]. Interestingly, the same coordinates are found to play a role in thymine photo-dimerization in DNA [63]. Other excited states also need to be considered: N9-H motion forms an additional coordinate in adenine that can lead to a conical intersection with a πσ* state [29, 55]. A weakly absorbing nπ* state plays a role as well, in addition to the ππ* state, and can sometimes serve as a "dark state" with an extended lifetime [30, 64].

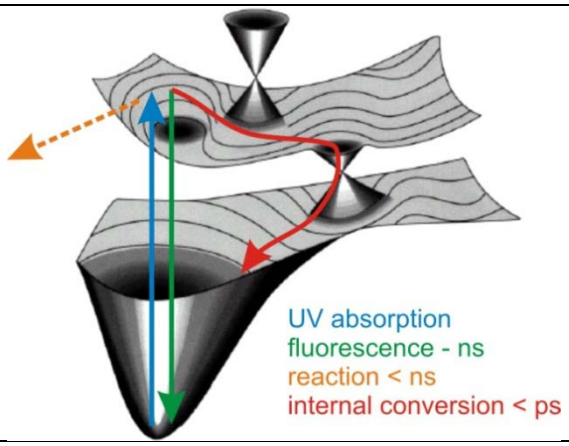


**Figure 6:** Schematic potential energy diagram (as function of two inter-nuclear coordinates) with two conical intersections connecting the $S_1$ excited state, via an intermediate state, with the $S_0$ ground state. Arrows indicate competing de-excitation pathways.

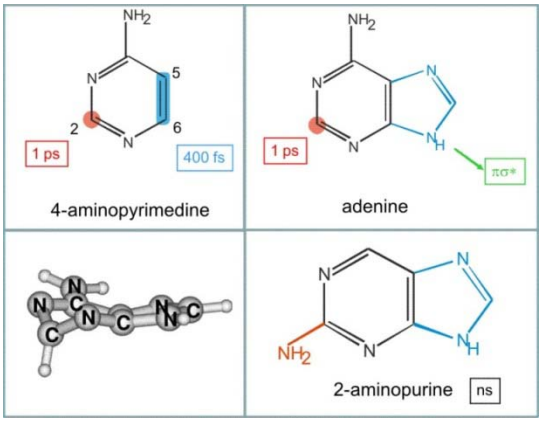


**Figure7:** Ring deformations at C2 or C5=C6 lead to conical intersections that mediate internal conversion at different time scales depending on molecular structure: on the order of 1 ps for C2 pyramidalization (bottom left) and 400 fs for C5=C6 stretch and torsion. Details in text .

Furthermore, purine, the parent compound of adenine as well as guanine, forms triplet states in high yield [65]. Lifetimes also depend strongly on tautomeric forms, as discussed below in section 2.6 [28, 66-70]. Understanding these mechanisms helps explain how subtle structural differences between substituted nucleobases can produce excited state lifetime differences of orders of magnitude.

A similar strong effect can be observed in uracil (U) derivatives upon substitution in the C5 position [38]. Two-photon ionization and IR/UV double-resonant spectra of the uracil analogues, 5-OH-U and 5-$NH_2$-U show that there is only one tautomer present for each with an excited state lifetime of 1.8 ns for 5-OH-Ura and 12.0 ns for 5-$NH_2$-Ura as determined from pump–probe experiments. These lifetimes are 3 and 4 orders of magnitude longer, respectively, than that for unsubstituted uracil [71]. Nachtigallova et al. determined vertical excitation energies, excited state minima, minima on the crossing seam and reaction paths

towards them by means of multi-reference ab initio methods [38]. They found sizeable barriers on these paths that provide an explanation for the lifetimes of several nanoseconds observed in the experiment.

Kistler and Matsika reported a similar effect for cytosine derivatives as well [61, 72]. For example, 5-methyl-2-pyrimidin-(1*H*)-one (5M2P, with a methyl substituent in the C5 position instead of the amino group in the C4 position) has a fluorescence lifetime that is orders of magnitude longer than the picosecond lifetime of cytosine [5, 64, 71, 73]. This excited state lifetime difference correlates directly with a lower $S_1$ excitation energy for 5M2P, analogous to the energetic considerations for adenine *vs.* 2-aminopurine.

### 2.5 Character of the excited state

The character of the first excited states is not always entirely clear but generally the UV spectra contain contributions to two excited states, of ππ* and nπ* character respectively [30, 74]. The π-π* transition carries most oscillator strength and the nπ* state can function as a dark state for internal conversion, depending on whether the relative energies lead to the occurrence of conical intersections. In the case of adenine, Lee et al. characterized the excited states based on rotational band contour analyses [30]. They showed that the lowest nπ* and ππ* states can be labeled with their excited-state vibronic symmetry, and exhibit a strong ππ*-nπ* vibronic coupling via an out-of-plane vibrational mode. They assigned the band at 36,062 cm-1 as the n-π* transition, and the 36,105 cm-1 band as the π-π* transition by symmetry analysis. The band at 36,248 cm-1 provides evidence of the strong ππ*-nπ* vibronic coupling via an out-of-plane vibrational mode.

Sobolewski and Domcke showed that in the excited state dynamics a πσ* can also play a role along a NH nuclear coordinate [75]. Motion along this coordinate can lead to hydrogen loss and Hunig et al. demonstrated this channel by detecting the dissociating hydrogen using Lyman-α excitation and analyzing the kinetic energy from the Doppler shift [55].

### 2.6 Tautomers

A complication in the study of nucleobase structure and dynamics is the fact that these compounds exhibit multiple tautomeric forms. The electronic spectra, such as those in Figure 2, may contain contributions of all the tautomers present in the beam.

As shown in Figure 2, the $S_1 \leftarrow S_0$ transitions of the two most stable cytosine tautomers, keto and enol, are a remarkable 4000 $cm^{-1}$ apart, while energetically they differ by only 0.03 eV [19, 31]. The third major tautomer, keto-imine, is significantly higher in energy [76-78] and not observed in these spectra. The keto form is the biologically important one, with Watson-Crick base pairing in DNA, and predominant in solution. In matrix isolation Szczesniak et al. observed both keto and enol forms with higher abundances for the latter and small contributions from the imino form [79]. Brown et al. have obtained rotational

constants for all three tautomeric forms by microwave spectroscopy [80]. Schiedt et al. also identified the existence of keto and enol tautomers of neutral cytosine in the gas phase by anion spectroscopy [81].

Kosma et al. reported excited state dynamics by probing with different excitation wavelengths in a range from 260 nm to 290 nm, which allowed them to distinguish between keto only versus mixed populations [82]. They conclude that the deactivation pathways are quite different for the different tautomers, with three time constants for keto-cytosine and two for the others. Theoretical treatments in the literature had focused on modeling the latter, while the new results point to the importance of tautomeric distinction in these analyses. For the keto form the two faster channels, with femtosecond and picosecond lifetimes are consistent with fast, but not barrierless excited-state relaxation via conical intersections between the $^1\pi\pi^*$ and ground state [76]. The third channel with a lifetime of hundreds of picoseconds at 290 nm is proposed to involve excited-state tautomerization to a low-lying $^1n\pi^*$ state of the keto-imino tautomer [83]. The authors propose that in the condensed phase this channel would be quenched by rapid back hydrogen-transfer, catalyzed by hydrogen-bound water molecules [84, 85]. Consequently this pathway would not alter the inherent photostability.

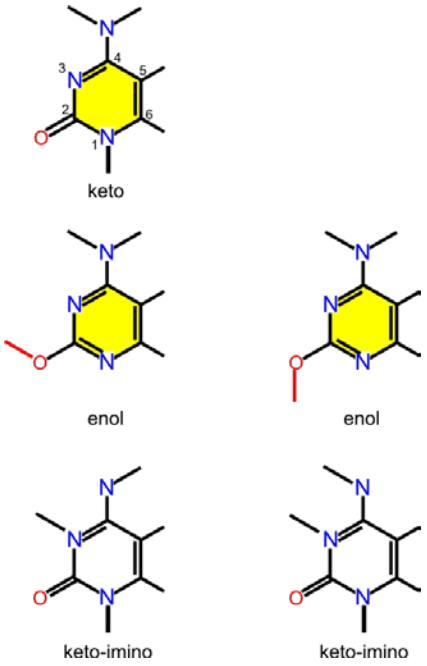


**Figure 8:** The major tautomers of cytosine. The IR-UV double resonant experiments observe the keto and enol forms (yellow) but do not distinguish between the two enol tautomers.

In most cases electronic spectra of different tautomers do not exhibit significant shifts and thus vibronic spectra potentially consist of overlapping contributions from a number of tautomers. Such overlapping electronic spectra can be disentangled by IR-UV double

resonant spectroscopy. However, an additional complication with this technique is that it is based on action spectroscopy in which the probe signal is multiphoton ionization. As a result this approach is blind for tautomers for which the excited state life time is significantly shorter than the laser pulse. Such tautomers do not show up in the electronic spectrum to begin with.

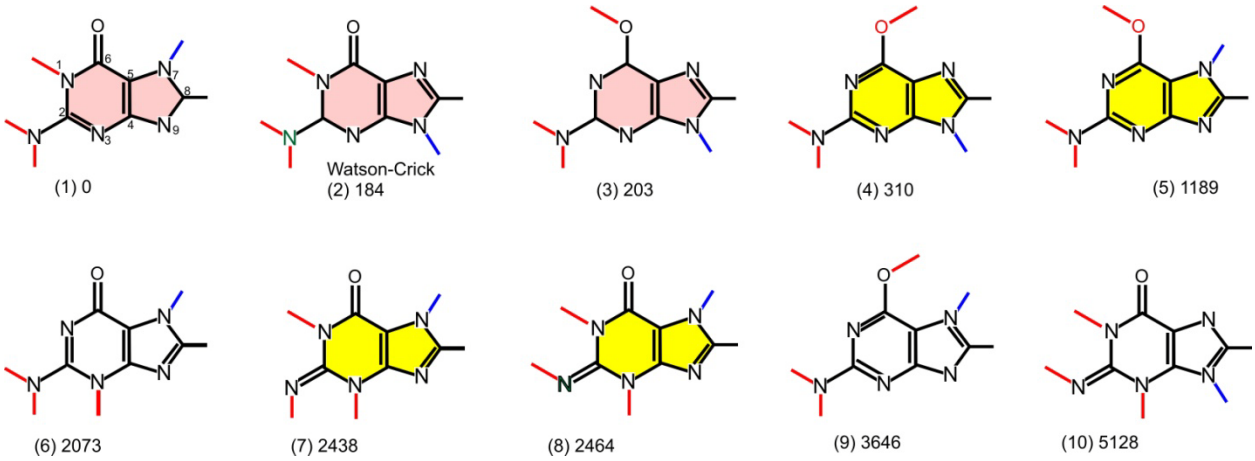


**Figure 9:** Ten of the lowest energy tautomers of guanine. Relative energies in wavenumbers from reference [67]. Yellow structures are observed in gas phase nanosecond REMPI experiments. Red structures have sub picosecond excited state lifetimes. Structures 1-4 are observed in helium droplets. Keto-N9H is the Watson-Crick structure.

A case in point is the so-called guanine puzzle [28, 69]. The REMPI vibronic spectrum consists of the overlapping spectra from four different tautomers, as determined by UV-UV holeburning [24, 86, 87]. However the identification of the specific tautomers by IR-UV double resonance spectroscopy proved challenging. Two enol tautomers could easily be assigned based on the strong OH stretch around 3600 $cm^{-1}$. However the other two species were initially incorrectly assigned as keto tautomers. Figure 9 shows the ten lowest energy tautomers of guanine with relative energies in wavenumbers from reference [67]. Clearly the two keto tautomers (N7H and N9H) are the lowest energy forms and their IR spectra formed a quite reasonable match with calculated frequencies for these forms, making this a plausible assignment. However, subsequent data from a helium droplet experiment exhibited slightly different IR spectra that formed a better match with the keto forms [27]. This led to a re-analysis of the IR-UV data and a reassignment of the two tautomers from the keto to the imino form. This assignment was subsequently confirmed by IR-UV measurements in the imino stretch region below 2000 $cm^{-1}$ [67]. Gas phase microwave experiments also found the keto tautomers [41].

These observations are summarized in Figure 9 in which the red tautomers were observed by the two techniques that involve direct absorption in the ground state (helium droplets and microwave), while the yellow tautomers were observed by the technique that involves action spectroscopy via the electronic excited state (REMPI). This difference suggests that the lowest energy forms are most abundant in the gas phase in all cases, but are not

observed with REMPI because they have short lived excited states. This explanation is confirmed by computations, showing a rapid internal conversion pathway through a conical intersection, which is not accessible for the higher energy enol and imino tautomers [69].

The tautomeric landscape of adenine is somewhat less varied than in the case of guanine because of the absence of the oxygen. Plützer and Kleinermanns reported IR-UV double resonance spectroscopy and observed two tautomers [52]. Both tautomers are of the amino form, with the 9H form most abundant and a small presence of the 7H form. This finding is consistent with microwave measurements by Brown et al.[88]. At the conditions of jet cooling the imino form appears to be absent, although in the gas phase at elevated temperature the IR spectra seem to comprise multiple tautomers, including imino.

Microwave measurements of uracil in a heated cell suggest the di-keto form as the most abundant. Brown et al. reported to the first microwave measurements in a seeded molecular beam and also concluded that the di-keto form was predominant [89, 90]. Viant et al. reported to the first rotationally resolved gas phase IR spectra of uracil [91]. This work employed a slit nozzle, an IR diode laser, and a multi-pass arrangement to obtain high-resolution IR absorption spectra of the out of phase $\nu_6$ stretching vibration. The rotational analysis unambiguously assigned this species to the diketo tautomer. Brown et al. also observed the diketo form of thymine in a seeded molecular beam, based on hyperfine structure [92].

## 2.7. Intermolecular effects – clusters

It is often assumed that the same internal conversion processes govern the subpicosecond de-excitation timescales of nucleobases in solution, albeit modified by solvent interactions. It is reasonable to postulate that the chromophore is affected primarily by the first solvent shell. Therefore many of the details of the dynamics in solution can be elucidated by studying clusters with small numbers of water molecules [24, 25, 93-98]. It appears that hydrogen bonding can play a role in two ways. First the excited state potential energy surfaces will be modified, thus affecting possible trajectories through conical intersections. Secondly the hydrogen bond itself can provide another coordinate for coupling excited states and the ground state. This effect is not limited to hydrogen bonding with water, but may also play a role in base pairing and in intramolecular hydrogen bonds. This coordinate can be thought of as an analogue of the N-H stretch coordinate correlated with a πσ* state, and its associated conical intersections [75, 99-102]. In the latter case, if the πσ* potential surface fails to reconnect with $S_0$, the hydrogen can actually dissociate. In the hydrogen bonded case the hydrogen is confined to the system and this coordinate can only mediate a single or double proton transfer.

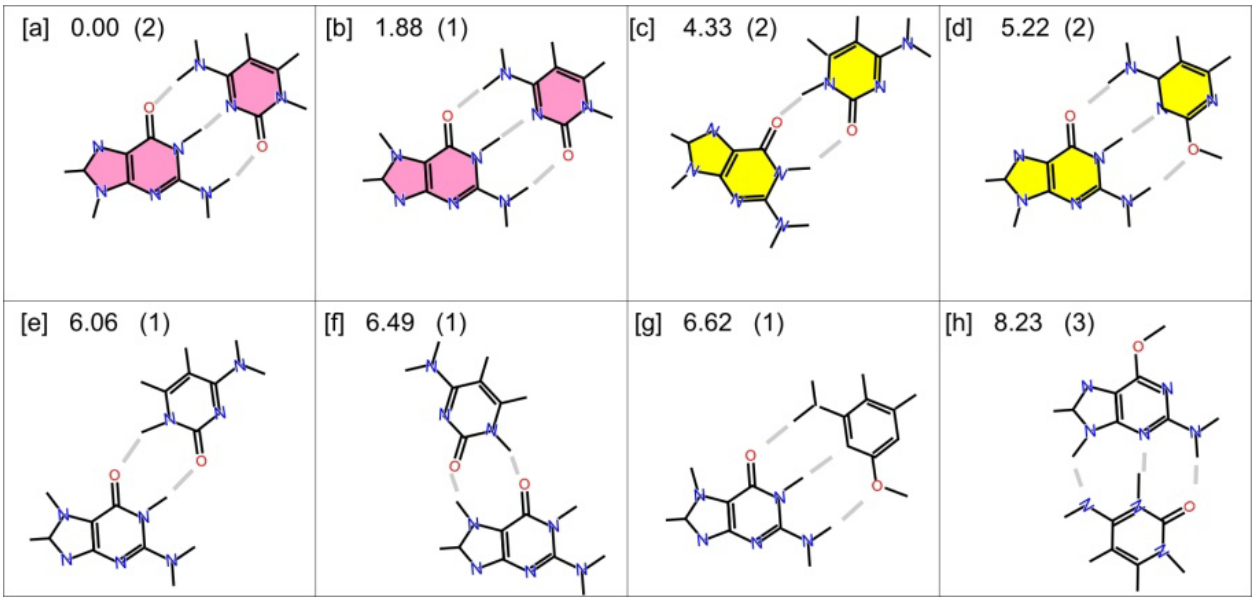


**Figure 10:** The lowest energy structures of hydrogen bonded GC clusters with energies in kcal/mol. Yellow structures were observed in REMPI experiments, while red structures were not.

An example is the finding that the specific Watson-Crick (WC) structure in isolated GC nucleobase pairs has a short excited state lifetime that is explained by proton motion in the middle hydrogen bond [34, 103-105]. By contrast, in other GC base pair structures this coordinate does not lead to accessible conical intersections [34, 106]. Figure 10 shows the eight lowest energy structures of hydrogen bonded GC clusters, with the very lowest energy structure representing the WC structure. The two lowest energy structures, marked in red, were not directly observed in gas phase experiments with nanosecond timescale REMPI detection, while the next two higher energy structures, marked in yellow, were observed and identified by IR-UV hole burning [33, 34, 106].

For AT base pairs the Watson-Crick structure was also not identified, however in that case it is not the lowest energy structure so it is possible that it was insufficiently populated in the molecular beam [35]. In REMPI spectra of homo base pair combinations the predicted lowest energy structure was not observed. This absence occurred for GG dimers [107], CC dimers [31], and AA dimers [37]. In these homodimers the missing structure is symmetric so it is possible that an excimer state is formed with a considerable splitting causing a shift in the UV band outside of the experimental range. However, it is also possible that the absence of those cluster structures in the REMPI spectra is due to short lived excited states, that are dark in nanosecond timescale REMPI detection. For 9-methyladenine-adenine clusters Plützer et al. identified a stacked structure, which they detected on the mass of protonated adenine. They interpreted their finding as involving hydrogen transfer from the 9H position of the adenine. Schultz and coworkers reported indications of this process in time resolved photoelectron spectroscopy studies of adenine and adenine containing clusters [95, 108-110] and in nucleobase pair analogues [103, 111, 112].

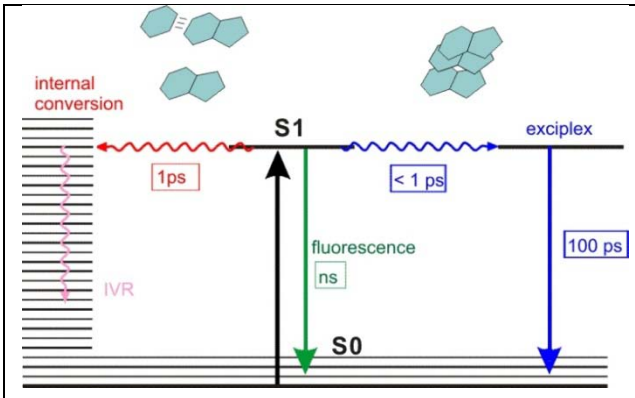


**Figure 11:** Simplified Jablonski diagram, showing major possible processes for deactivation of the excited state in nucleobases: Fluoresence is typically slow, in the nanosecond range. Internal conversion back to the ground state in the (sub)picosecond time scale occurs for selected single bases and some hydrogen bonded base pairs. Exciton formation in stacked bases can lead to exciplex states in less than a picosend for specific stacked structures. Those states subsequently decay to the ground state by charge recombination on the 100 ps time scale.

Another structure in which hydrogen bonding occurs and possibly affects dynamics is in nucleosides in the *syn* configuration. Nir et al. found by double resonant spectroscopy that isolated guanosine indeed forms an internal hydrogen bond between 5'OH and 3N [113]. Nevertheless they found a sharp REMPI spectrum implying a relatively long lived excited state. By contrast, an REMPI spectrum for adenosine has not been observed, suggesting a possibly short excited state lifetime [114, 115]. It should be noted that for guanosine in the gas phase only the enol tautomer has been observed, so far, which suggests that the keto tautomer is selectively short lived, analogously to the nucleobase. So whether and to what extent the shortened lifetime observed in solution is an intrinsic property of nucleosides remains an open question. Other aspects of the role of the sugar moiety in excited state dynamics may also play a role. It has been proposed that the additional degrees of freedom provide a bath for quenching excited state excitation. Furthermore, in solution the dynamics may be further complicated because the sugar also provides additional hydration sites.

Another intermolecular interaction is base stacking, which provides much of the stability in helices. In the π stacked structural motif, exciplex formation appears to play a major role in the photodynamics[115, 116]. The overlap of the π clouds depends critically on the relative position of the molecules. For example, Schreier et al. have shown that thymine dimer formation in DNA is determined largely by conformational properties at the instant of excitation [117]. In solution phase experiments Crespo-Hernández and Kohler and coworkers have shown that base stacking in single- and double-stranded DNA favors non-radiative decay via the formation of charge-transfer or exciplex states [118-120]. Such states decay much more slowly than the ππ* states in single bases (see Figure 11). These considerations are the topic of the next chapter.

## Summary

Electronic and vibrational spectroscopy of isolated nucleobases sheds light on excited state dynamics indirectly in a number of ways. UV-VIS excitation spectroscopy probes the potential energy landscape in the Franck-Condon region, which establishes the starting conditions of any ensuing photochemistry. The combination with IR hole burning to obtain vibrational spectroscopy as well, offers the important opportunity to obtain isomer selected data. This approach makes it possible to probe individual tautomers, structural conformers, isomers, and cluster structures. This isomer selectivity as well as the fact that nucleobases can be studied free of interactions provides unique insights in their intrinsic properties, unperturbed by any secondary effects of the biological environment. Therefore such gas phase experiments offer the best data for comparison with computational chemistry at the highest levels. The frequency domain is coupled with the time domain by the inverse relationship between energy and line width. Particularly in the 10-100 ps range excited state lifetimes may be derived from spectra line widths. Shorter lifetimes not only lead to extensive broadening but often render excited states unobservable by action spectroscopy, such as REMPI or LIF, with nanosecond timescale laser pulses. Possibly future experiments in the frequency domain will provide additional insight in excited state dynamics. Examples may include spectroscopy with shorter laser pulses, including a trade-off between time and frequency resolution, spectroscopic characterization of the hot ground state products of internal conversion, and more elaborate studies of clusters with water to detail the role of micro hydration.

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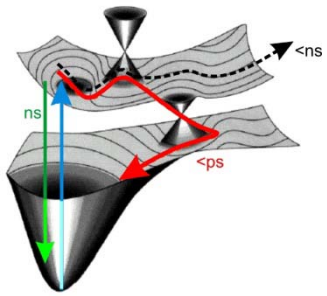
## TOC GRAPHIC:




Following electronic excitation, probed in the frequency domain (blue), internal conversion (red) can compete with fluorescence (green) and other photochemical pathways (black).

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