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PERSPECTIVE

How nature covers its bases

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Abstract

The response of DNA and RNA bases to ultraviolet (UV) radiation has been receiving increasing attention for a number of important reasons: (i) the selection of the building blocks of life on an early earth may have been mediated by UV photochemistry, (ii) radiative damage of DNA depends critically on its photochemical properties, and (iii) the processes involved are quite general and play a role in more biomolecules as well as in other compounds. A growing number of groups worldwide have been studying the photochemistry of nucleobases and their derivatives. Here we focus on gas phase studies, which (i) reveal intrinsic properties distinct from effects from the molecular environment, (ii) allow for the most detailed comparison with the highest levels of computational theory, and (iii) provide isomeric selectivity. From the work so far a picture is emerging of rapid decay pathways following UV excitation. The main understanding, which is now well established, is that canonical nucleobases, when absorbing UV radiation, tend to eliminate the resulting electronic excitation by internal conversion (IC) to the electronic ground state in picoseconds or less. The availability of this rapid "safe" de-excitation pathway turns out to depend exquisitely on molecular structure. The canonical DNA and RNA bases are generally short-lived in the excited state, and thus UV protected. Many closely related compounds are longer lived, and thus more prone to other, potentially harmful, photochemical processes. It is this structure dependence that suggests a mechanism for the chemical selection of the building blocks of life on an early earth. However, the picture is far from complete and many new questions now arise.

Introduction

Without a fossil record of the prebiotic chemical world we are left to conjecture to understand the road map that led to RNA and DNA.[1-3] The key components of the reproductive machinery are unlikely to have been selected by a biological evolution that requires that very machinery to begin with. Instead it is conceivable that the choice of the molecular building blocks of life was mediated by a chemical selection that preceded biology. This hypothesis implies that molecular properties of nucleobases, preserved unchanged from prebiotic times, may serve as molecular fossils of 4-billion-year old chemistry.

One of those properties is the photochemistry. The nucleobases that are involved in replication generally exhibit short excited state lifetimes which provide high intrinsic stability against otherwise harmful UV photo-damage.[4-14] UV protection comes about when electronic excitation is converted to heat by internal conversion and safely dissipated to the environment at rates too fast for other more harmful reactive pathways to occur. This property would have been highly advantageous for the first self-replicating molecules in prebiotic times before modern enzymatic repair and before the formation of the ozone layer that later attenuated the high levels of UV

radiation penetrating the early atmosphere. The intrinsic damage resistance is exquisitely sensitive to molecular structure. The canonical nucleobases generally decay in a few picoseconds or less, orders of magnitude faster than many of the other heterocyclic compounds, which would likely also have been present on an early earth and could have been candidates for alternative genetic building blocks.[15-17]

Figure 1 shows examples of alternative base pairs. Figure 1(a) shows selected examples based on purine and pyrimidine structures and Figure 1(b) shows two of many possible examples with the pyrimidine analogues pyrazine and triazine. These examples form a subset of the alternative genetic lexicon proposed by Benner,[15, 18] of the set of 82 alternative bases considered by Hud,[16, 19] and discussed by Rios and Tor[17]. These base pairs have hydrogen bonding patterns and geometries that are very similar to those of the canonical base pairs and, aside from other chemical properties, could act as replacements for the canonical base pairs in DNA and RNA. The small green pentagon symbols indicate the position of the sugar moiety in the corresponding nucleoside and for the bare nucleobase these would be hydrogens, indicating the equivalent tautomeric form. For diaminopyrimidine or pyrazine the sugar moiety would have to be bound to a carbon rather than a nitrogen for the aromaticity of the ring to be preserved.

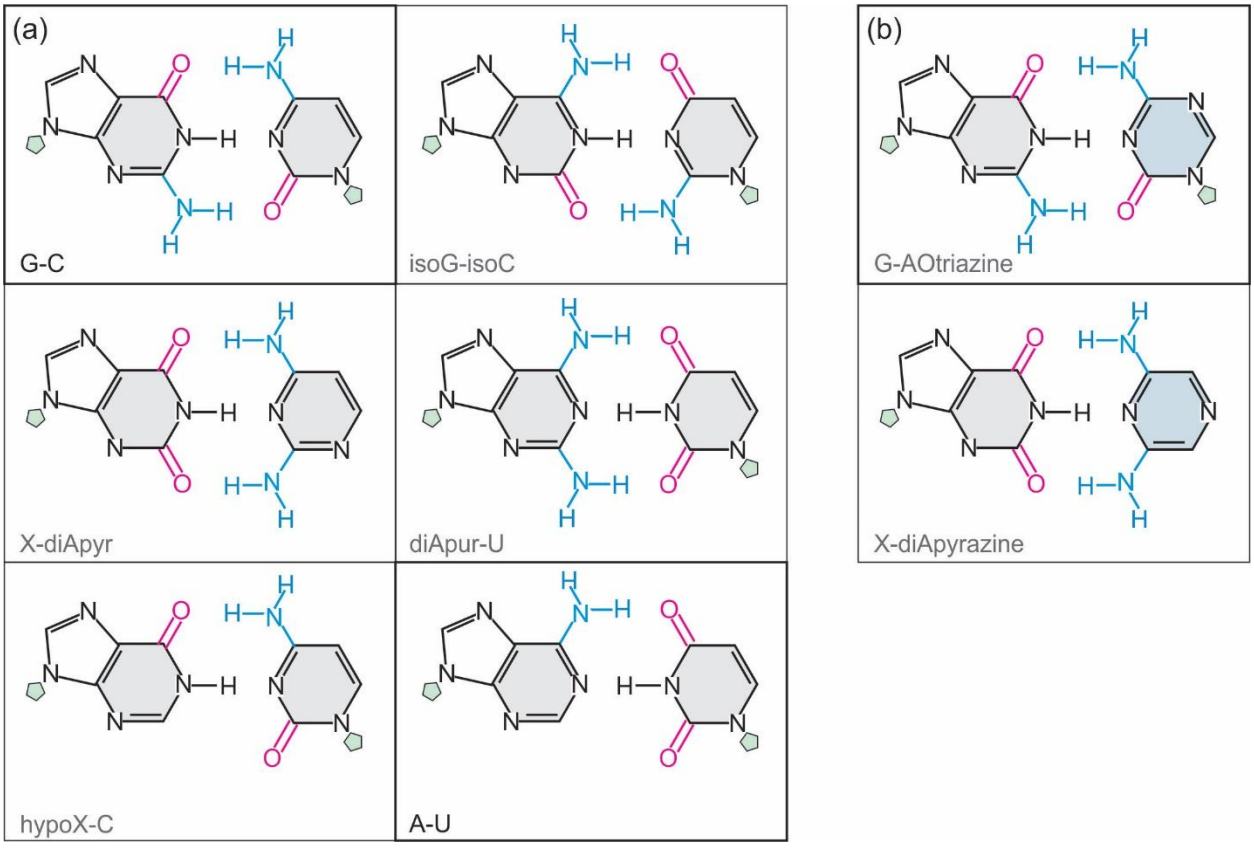

Figure 1: Examples of alternative base pairs. diA=diamino, AO=amino-oxo, X=xanthine, pur=purine, pyr=pyrimidine.

The nucleobases are characterized by UV absorption with onsets typically in the 260-320 nm range. It is worth noting that for life, as we know it, such near-UV absorption may be unavoidable:

The base-pairing mechanism requires rigid molecular structures, provided by aromatic rings, which are characterized by strong $\pi\pi^*$ absorption. It is this transition that causes the nucleobase absorption of UV radiation. It is also what dominates RNA and DNA absorption, since the backbone absorbs further to the UV, outside the range of the solar spectrum that reaches the surface of the earth. If UV absorption is an unavoidable aspect of a self-replicating molecular system this also necessitates the ability to safely dispose of that energy. On an early Earth, in the absence of an ozone layer, photochemistry would have been possible further to the blue than today.[20-22] This consideration suggests that the natural bases were selected in part due to their high intrinsic photostability or UV hardiness and that their photochemistry is a vital property of the set of prebiotic molecules that could give rise to life on Earth or elsewhere in the universe. The nucleobase photochemistry may thus be a relic of some of the earliest stages of prebiotic chemistry. Even if nucleosides were synthesized directly without nucleobases as intermediate steps, as proposed in recent literature,[23-25] the bases still constitute the chromophore for prebiotic photochemistry whereby addition of the sugar moiety may modify the dynamics.

It is therefore of great interest to study the excited state dynamics of both the canonical nucleobases and the derivatives and analogues of those that could have functioned as alternative bases in prebiotic scenarios. As it turns out, the UV protection of the nucleobases is not perfect: it depends on wavelength and there are in some cases non-zero quantum yields for triplet state formation or population of other dark states. Increasing complexity, such as base pairing, solvent interactions, and macromolecular structure, modifies the dynamics. Furthermore, not all alternative bases are vulnerable to UV photochemistry. From a growing body of work in both gas phase experiments [5, 10-11, 26-48] and computational theory, [46, 49-57] a nuanced and detailed picture is beginning to emerge, though still incomplete, and this perspective, rather than serving as an exhaustive review, aims to briefly summarize the state of our current understanding.

Techniques

Many gas phase techniques rely on formation of supersonic beams of neutral nucleobases coupled with optical spectroscopy. Such molecular beams ensure collision free conditions to isolate the molecules and achieve low internal temperatures to enable high resolution spectroscopy.[58] Brady et al. reported the first molecular beam spectra of uracil and thymine in1988, which were broad without resolved features.[59] Nir et al. reported a first resolved spectrum of guanine in 1999,[26] followed by those of adenine[27] and cytosine.[28] These experiments employed laser desorption, which can volatilize low vapor pressure molecules from a surface into the gas phase without fragmentation or thermal degradation[60-63]. Unlike the MALDI technique, this approach produces neutral molecules. Following desorption, neutral molecules are entrained in a supersonic expansion, which lowers the internal temperature to several tens of degrees Kelvin or below[64]. By controlling the source conditions, it is also possible to generate clusters of desorbed molecules with each other or with water molecules to mimic base pairs and microsolvation.[65-72] Downstream, the jet cooled molecules are excited and photo-ionized and the ions are detected in a time-of-flight mass spectrometer.[64, 73-74] Figure 2 shows a schematic overview of the most common laser spectroscopy approaches to studying neutral molecules in the gas phase. The techniques include laser induce fluorescence, LIF (a) and resonance enhanced multi-photon ionization (REMPI) (b-e). In double resonant experiments (c, d) a REMPI probe signal at a single resonant wavelength is monitored while another laser (often called burn laser), fired some 100 ns earlier, is scanned. Each time the burn laser wavelength is resonant with a transition, this modifies the ground state

population and changes the REMPI signal. The result is a ground state absorption spectrum, obtained with optical selection, by virtue of the choice of REMPI resonant wavelength, and with mass selection, by virtue of the time-of-flight detection. When the burn laser is in the UV range (c), the technique can establish REMPI spectra of individual isomers, such as tautomers or cluster structures. When the burn laser is in the IR range (d), the IR spectra of selected isomers can be obtained and used to identify those isomers. Once the isomeric structures are identified, peaks can be chosen in the REMPI spectrum for isomer specific measurements of excited state lifetimes by pump-probe spectroscopy (e). LIF and REMPI are forms of action spectroscopy. By contrast, microwave absorption and He droplet IR experiments, not depicted in figure 2, are absorption techniques. Most of these approaches can also be implemented for cold ions in cryogenic traps, which is outside the scope of this perspective.[75-79] It is worth stressing that the isomer specificity afforded by double resonant techniques constitutes a great strength of gas phase spectroscopy.

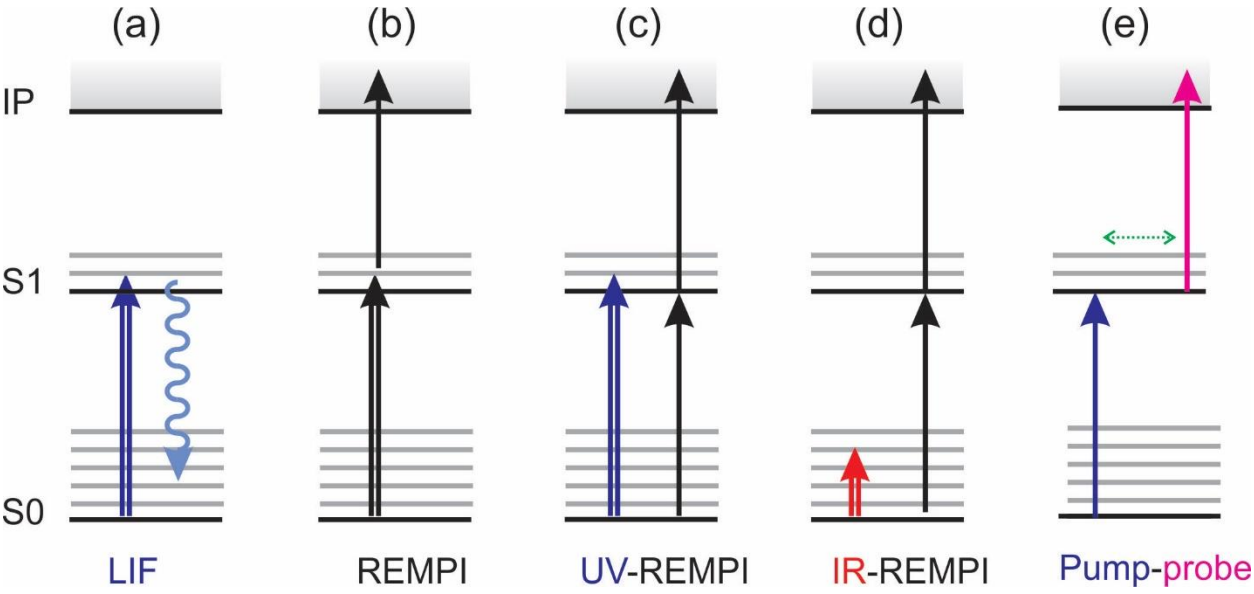

Figure 2: Schematic summary of most common laser spectroscopy techniques for studying neutral nucleobases in the gas phase. Double arrows indicate wavelengths that are being scanned.

Excited state dynamics

As sketched in Figure 3, the fate of a molecule following absorption of a UV-VIS photon depends on the potential energy landscape in the excited state. After a photon causes an electronic transition, the excitation energy can be disposed of in many ways. Fluorescence typically occurs at the nanosecond timescale and thus is only important for the cases in which faster decay channels are not available. The canonical bases are essentially non-fluorescent. Of all photochemical pathways by far the least hazardous process is internal conversion to the electronic ground state, which converts the electronic energy, imparted by the photon, to heat, in the form of ground state vibrational and rotational energy. Assuming that this heat can be dissipated by exchange with the environment, the molecule can avoid other potentially more harmful photo-induced reactions. Pecourt et al. have observed very fast dissipation of internal energy in water, suggesting that water

is the preferred solvent of life also for this process.[80] It is not enough that this IC pathway is available; in order to provide UV protection, IC to the ground state must be the dominant process, competing favorably with any other processes. Generally, high quantum yields and high rates for IC – implying short excited state lifetimes – are a good molecular strategy for UV hardiness.

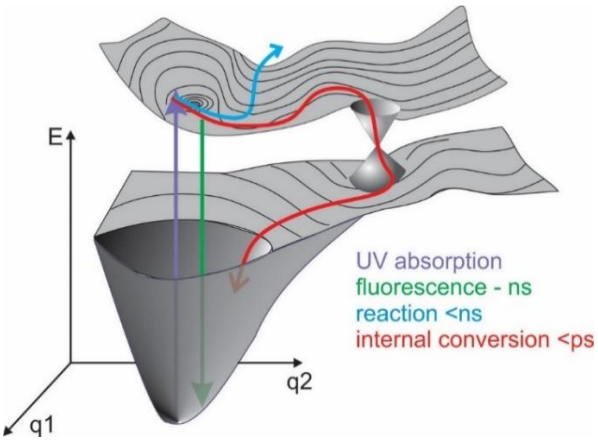

Figure 3: Schematic diagram of three major pathways in excited state dynamics, proceeding on potential energy surfaces as a function of molecular coordinates q1 and q2

It also matters where the photodynamics starts on the excited state potential surface. Barriers for selected pathways may exist and even without those the available excess energy affects the outcome at conical intersections. Therefore, the response to UV excitation is wavelength dependent. Especially, excitation with little or no energy above the vibrationless excited state may lead to significantly longer lifetimes than at higher energies. Consequently, single data points at short absorption wavelengths may not tell a complete story of UV response.

The schematic representation in Figure 3 sketches a conical intersection between two potential energy surfaces (PES) as a function of intramolecular coordinates q1 and q2. As can be recognized from this diagram, such a conical intersection (CI) can exist only at molecular geometries that differ from the ground state equilibrium geometry; in other words, a CI always involves a deformation of the molecular frame. The fact that CIs necessitate a non-equilibrium molecular geometry explains the exquisite dependence of this process on molecular structure.

Derivatives

What distinguishes short lived, and thus UV-robust, compounds from their long lived counterparts are variations in excited state potential landscapes, resulting from structural variations. Figure 4 shows examples with three of the canonical nucleobases and hypoxanthine in the left column and some of their derivatives in the right column [5, 31-32, 81-82]. When probed at energies close to the onset of absorption, all compounds in the left column have excited state lifetimes of the order of picoseconds or less, while those in the right column have lifetimes of the order of nanoseconds. This trend exists both in the gas phase and in solution.[7, 12, 83-84] The red and blue shaded areas on the molecular frame indicate where the geometry change takes place that leads to a major conical intersection. It should be stressed that the excited state potential energy landscape is a

multidimensional function of many internuclear coordinates leading to many possible pathways involving a number of conical intersections and seams[85-86]. Nevertheless, it is a useful simplification to highlight two structural motifs that appear to dominate the dynamics of these compounds. For the pyrimidines the area shaded in blue indicates the C5=C6 bond that twists and stretches. For the purines the most critical geometry change is puckering or pyrimidization at C2, indicated in red. Therefore, substitutions at C5 and C2 can often cause variations in excited state lifetimes by orders of magnitude. The effect is due to modifications of the potential energy surfaces, which, in addition to modifying the CIs themselves, can create barriers or otherwise change the trajectories towards them.

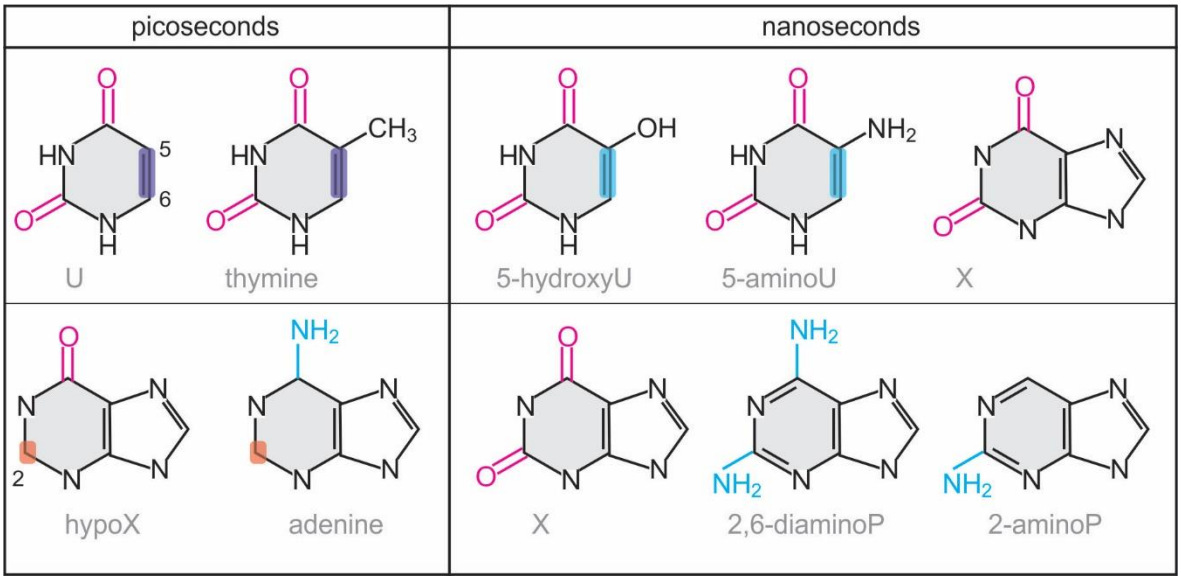

Figure 4: Examples of how excited state lifetime depends on structure. Blue and red shaded areas in left column indicate parts of the molecular structure that deform to lead to conical intersections. U=uracil, X=xanthine, P=purine.

An example is the series of C5 derivatives of uracil (U), shown in the top row of Figure 4. Both U and 5-methylU (thymine) have excited state lifetimes of the order of ps while 5-hydroxyU and 5-aminoU have lifetimes of the order of 1 and 12 ns, respectively.[55, 81] Immobilizing the C5=C6 bond by a five membered ring, as in xanthine, leads to an excited state lifetime of nanoseconds. Leutwyler and coworkers have demonstrated similar trends for cytosine (C), where immobilizing the C5=C6 bond by a five membered ring leads to orders of magnitude longer excited state lifetime.[43]

The bottom row compares a series of purines in which those with an unsubstituted C2 – adenine and hypoxanthine – have ultrashort lifetimes. Those with a substituent at C2 have much longer excited state lifetimes. This pattern is strikingly exemplified in the difference between the isomers adenine (6-aminopurine) and 2–aminopurine. The former does not fluoresce and the latter does, while its excited state lifetime is further affected by hydrogen bonding in solution.[36] Therefore 2-aminopurine is used as an alternative base that serves as a fluorescent marker.[87]

Cytosine, with an amino substituent at C2, has two dominant pathways; in addition to "twisting" of the C5=C6 bond there is a CI associated with a C4-NH_2 out of plane "sofa" configuration.[88] The

5-methyl-2-pyrimidone analogue has similar conical intersections but is fluorescent because the absorption maximum is at lower energies, preparing the molecule in the excite state below a barrier and with less vibrational energy.[37-38, 43-47, 88-89]

In spite of the trends described so far, the precise effect of structure modification is impossible to predict without high level computations. For example, guanine (G) has an amino group at C2, like 2-aminopurine, but some of its tautomers are short lived, while others are long-lived, as discussed below.

In the analogues from Figure 1(b) the C5-C6 motif is replaced by a C-N bond, affecting the excited state dynamics. For 5-azacytosine, Zhou et al. found a excited state lifetimes in solution of 1.5 ps and 15 ps, representing a 40 fold increase over cytosine.[90] The authors argue that the N5 substitution leads to an N5-C6 bond that is shorter than the C5-C6 bond in cytosine, thus modifying the CI associated with that internuclear coordinate. Zhang et al. found in solution that for melamine, the excited-state population decays by internal conversion with a lifetime of 13 ps. The excited-state lifetime of the lysine derivative is slightly longer (18 ps), but the dominant deactivation pathway is otherwise the same as for melamine. In both cases, the vast majority of excited molecules return to the electronic ground state on the aforementioned time scales, but a minor population is trapped in a long-lived triplet state [91].

Typically sulfur substitution increases intersystem crossing rates, as can be seen for thioguanine.[92] Siouri et al. reported data on two thiol tautomers, which decay to a dark state, possibly a triplet state, with rates depending on tautomer form and on excitation wavelength, with the fastest rate on the order of 10^{10} s^{-1}. They also compared 6-TG with 9-enolguanine, for which they observed decay to a dark state with a 2 orders of magnitude smaller rate. At increased excitation energy ($\sim$500 cm^{-1}) an additional pathway appears for the predominant thiol tautomer.

Wavelength dependence

The response to absorbed radiation depends on wavelength, especially when barriers exist on the trajectory towards CIs. Excitation at higher energies increases internal conversion rates while the dynamics at lower excitation energies is very sensitive to the shape of the excited state potential landscape. Figure 5 shows two REMPI spectra for 2,6-diaminopurine, both in the N9H tautomeric form. The black trace was obtained with 2 color REMPI with ns laser pulses (6 ns pulse width, 193 nm as second color);[82] the blue trace was obtained with 2 color REMPI with ps laser pulses (30 ps pulse width, 213 nm as second color). Lifetimes obtained for individual peaks with ps pump-probe measurements appear in blue at each respective REMPI peak. An additional long lifetime component obtained with ns pump-probe at the origin is indicated in black. There appears to exist a barrier at about 150 cm^{-1}. The excited states with lifetimes of less than 40 ps are ionized by the ps pulses but cannot be ionized by the ns pulses. This observation re-emphasizes the fact that REMPI is action spectroscopy and is blind for excited states with lifetimes significantly shorter than the laser pulse-width.

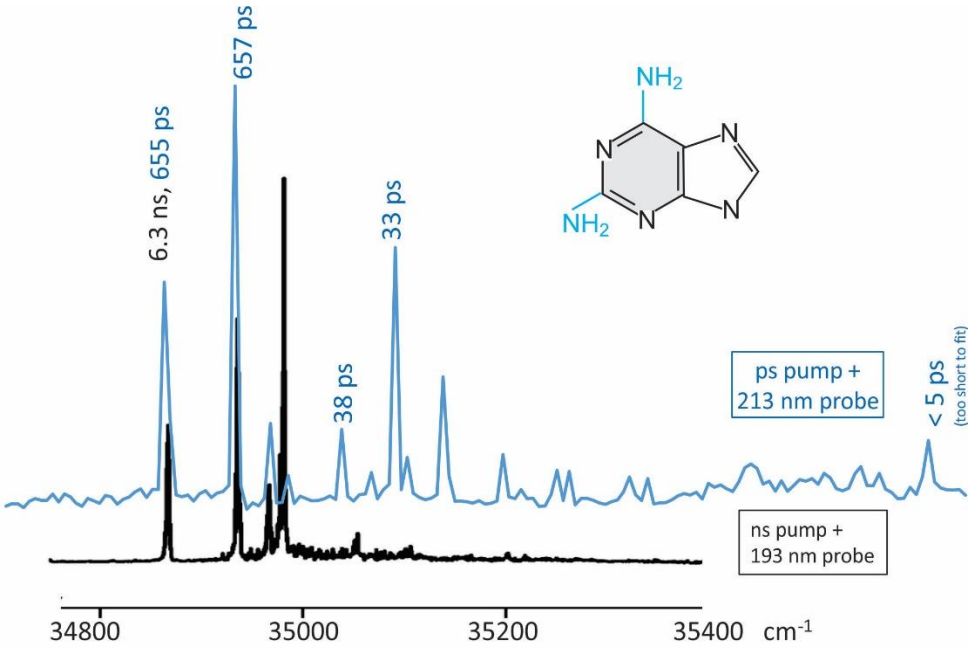

Figure 5: REMPI spectra of 2,6-diaminopurine with ns (black) and ps (blue) laser pulses. Excited state lifetimes from pump-probe measurements appear above selected peaks.

Leutwyler and co-workers reported the wavelength dependence of the excited state dynamics of cytosine and a series of derivatives, showing how the barrier for internal conversion is affected by structure.[45] The limited range of ns REMPI spectra of other nucleobases, such as adenine,[27, 93-95] is also indicative of the existence of barriers to internal conversion pathways.

Generally speaking, spectroscopy at a single wavelength or limited range can only probe a small part of the exited state potential energy surface. Such a strategy is akin to watching a play by observing only one corner of the stage: part of the story may be missed.

Tautomers

One of the great advantages of gas phase spectroscopy is the potential for isomer selective spectroscopy. Tautomers, in particular, can be distinguished by their IR signatures, observed in IR-UV double resonance spectroscopy.

For guanine, Figure 6(b) shows the lowest energy tautomers with energies given in kJ/mol,[96] and excited state lifetimes where available. The interpretation of the gas phase spectra was initially confused since the imino and amino structures have very similar IR spectra and the amino forms seemed a more likely interpretation because they are lower in energy. However, subsequent experiments and careful analysis unraveled the puzzle.[26, 49, 67, 96-100] Only the tautomers indicated in yellow have been observed in ns REMPI experiments. The keto form has been observed in helium droplets by IR absorption and in microwave spectroscopy in molecular beams so it is clear that the keto form exists in the gas phase.[101-102] Both those techniques measure absorption directly. On the other hand, REMPI is a form of action spectroscopy, detecting excitation by ionizing the excited state. If the ionization laser pulse is significantly longer than the excited state lifetime the

detection is suppressed. With typical ns laser pulses, ps or fs excited states can therefore be obscured. Thus, it is likely that failure to observe this lowest energy tautomer in REMPI experiments is due to short excited state lifetime. However, the absence of data remains an unsatisfactory form of evidence. Notably, it is the elusive keto form which is biologically relevant for DNA. The enol and imino forms have much longer lifetimes, producing well resolved UV spectra[66]. For the enol tautomer, EA2 Siouri et al. have observed excited state lifetimes of 13 ns and 40 ns.[92] The former is consistent with fluorescence decay reported by Mons et al.[98]

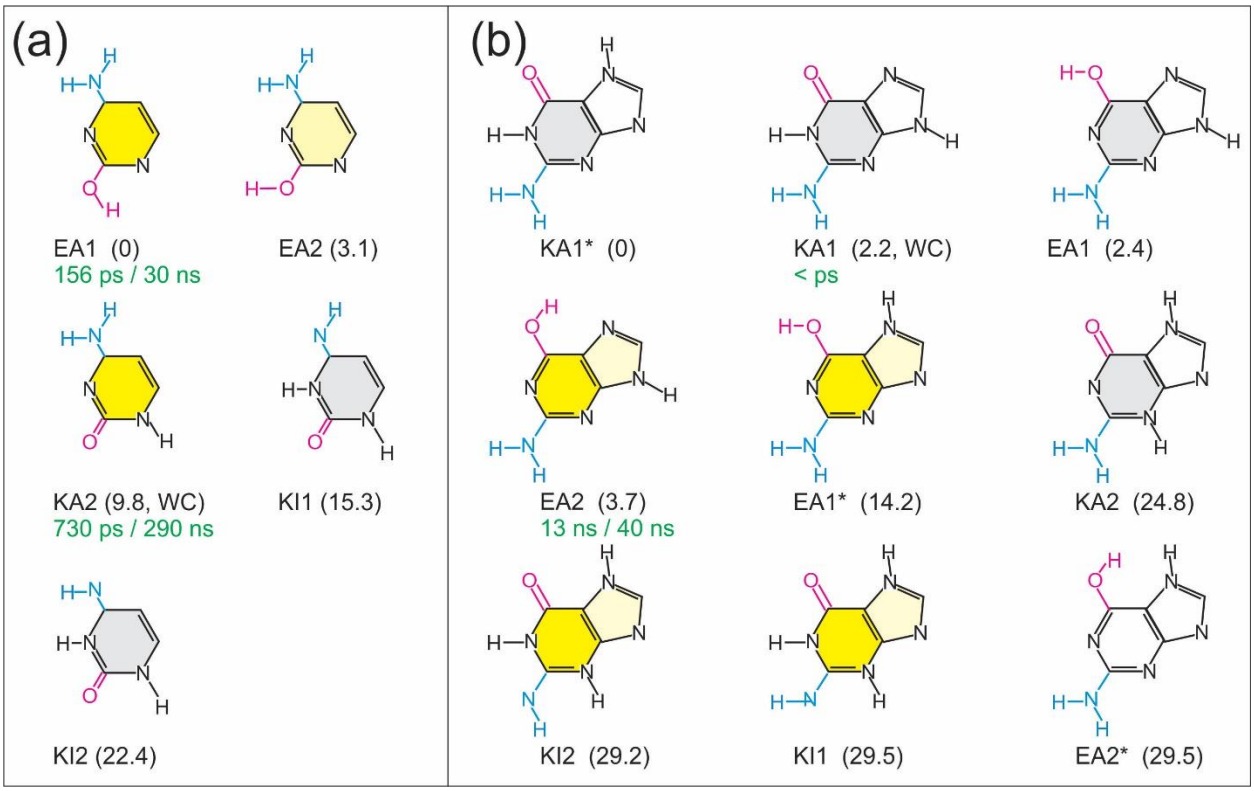

Figure 6: Lowest energy tautomers of (a) cytosine and (b) guanine with energies in kJ/mol.[96] Yellow tautomers are observed in ns REMPI experiments with the excited state lifetimes indicated below. (WC) indicates Watson-Crick structure. (*) indicates N7H as opposed to N9H tautomer.

Figure 6(a) shows tautomers of cytosine (C) while Figure 7 shows those of (a) iscoC and (b) isoG. The observed lifetimes are given in the figures and summarized in table 1. Figure 8 shows an example of the pump-probe data on which the lifetime numbers are based, in this case for isocytosine.[103] The resolution of pump-probe measurements is limited to the instrument response function, IRF shown in red, which limits the observable lifetime to the order of the laser pulse width. On the other hand, often lifetimes orders of magnitude longer than the IRF can also not be resolved, as they fall outside the experimental scanning range. Therefore, these experiments used two different laser set-ups with 30 ps and 5ns pulses, respectively, in order to cover both the ps and ns timescales. The lifetime of ~ps for isoG enol is merely inferred from the fact that it is shorter than the IRF but likely not sub-picosecond, which would have made it unlikely to have been observed by REMPI at all. The isoG enol tautomers cannot be distinguished by their IR signatures and they are very close in energy, so it is possible that a mixture of both is present in the beam. Similarly, presence of KA2 cannot be entirely excluded.

The excited state characteristics of isoC appears to defy the generalization of trends associated with structural motifs. In fact, isoC is an isomer of C, in which the oxo and amino groups are merely switched leaving the C5=C6 bond available for forming a conical intersection. However, this motif appears to be unimportant in isoC, based both on experiment and computation. Instead, the excited state dynamics more closely corresponds to that of G, which contains the isoC moiety as part of its structure. It appears that the amino group at C2 tends to provide an efficient access to a conical intersection while oxygen substitution at C2 tends to have the opposite effect. This observation is consistent with computations by Szabla et al.[104] By contrast, the isoG dynamics does resemble that of C with the C5-C6 bond immobilized, similar to the "planarized" cytosine structure of trimethylenecytosine, reported by Traschel et al.[43]

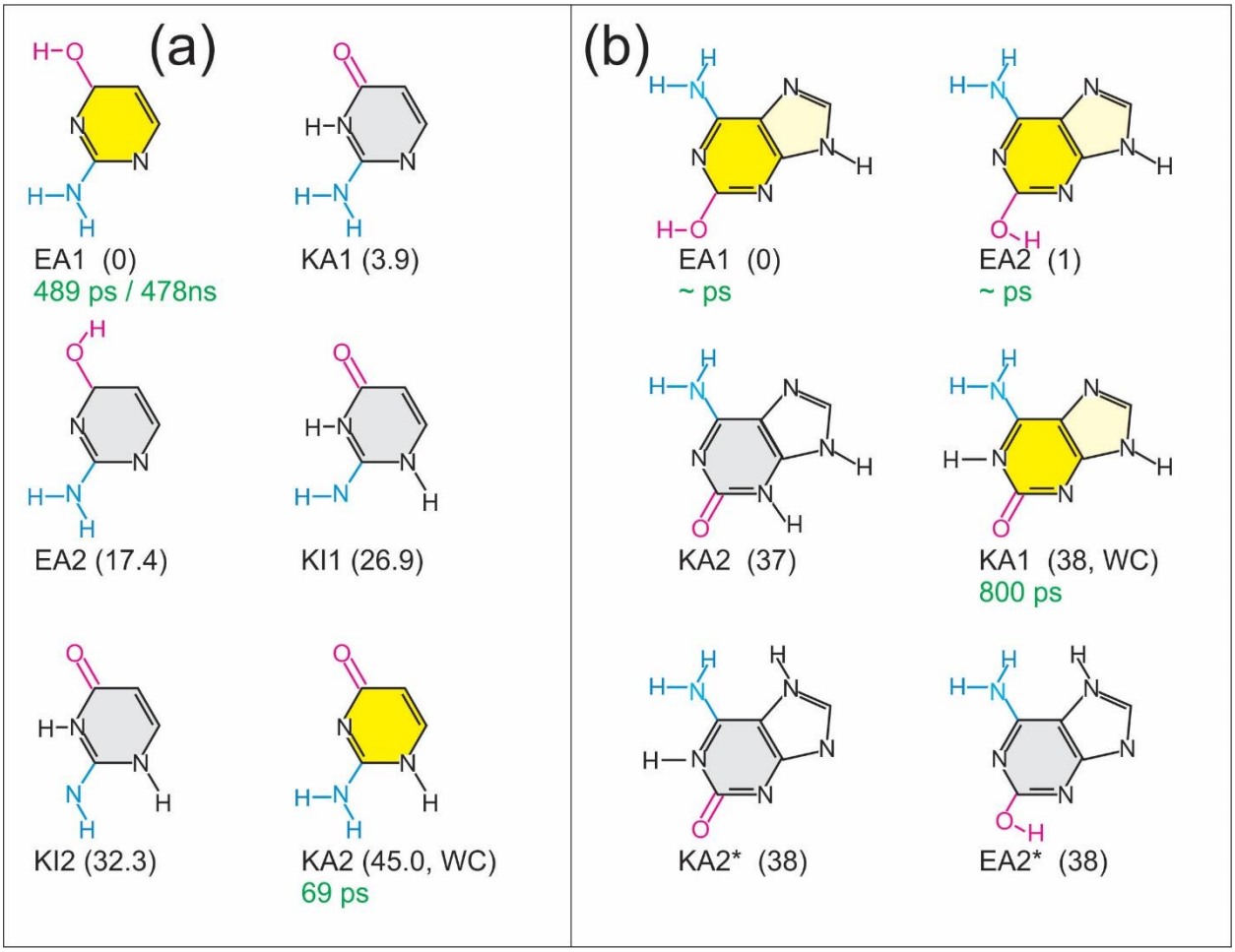

Figure 7: Lowest energy tautomers of (a) isocytosine and (b) isoguanine with energies in kJ/mol. Yellow tautomers are observed in ns REMPI experiments with the excited state lifetimes indicated below. For Isoguanine we cannot be sure whether we observe EA1, EA2, or a mixture of both. (WC) indicates Watson-Crick structure. (*) indicates N7H as opposed to N9H tautomer.

	keto KA1	keto KA2	enol
guanine	N/O	N/O	13 ns – 40 ns[92]
cytosine	N/O	730 ps[45] – 290 ns[28]	56 ps* – 30.5 ns* [103]
isocytosine	N/O	69 ps[103]	489 ps – 478 ns[103]
isoguanine	800 ps	N/O	~ps

Table 1. Vibrationless Excited-State Lifetimes. N/O = not observed. (*) The reported pump−probe results for enol C were not obtained at the 0−0 transition but rather on the rising edge of its broad initial absorption region. (**) For Isoguanine we cannot be sure whether we observe KA1, KA2, or a mixture of both.

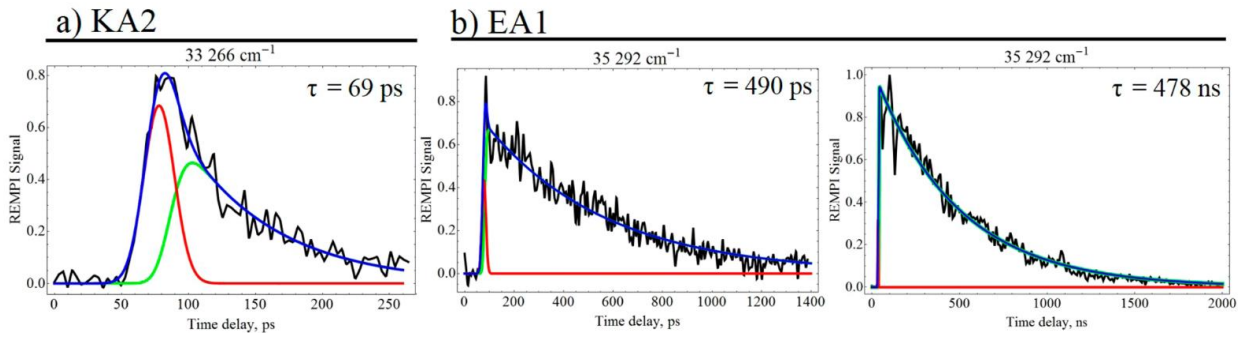

Figure 8. Pump−probe results from the origin bands of isocytosine in (a) the KA2 and (b) the EA1 tautomer forms. The data are fit to a curve (blue) which is the sum of a single exponential decay convolved with a Gaussian component (green) representative of our instrument response function (IRF) and the IRF itself (red).[103]

Dark states.

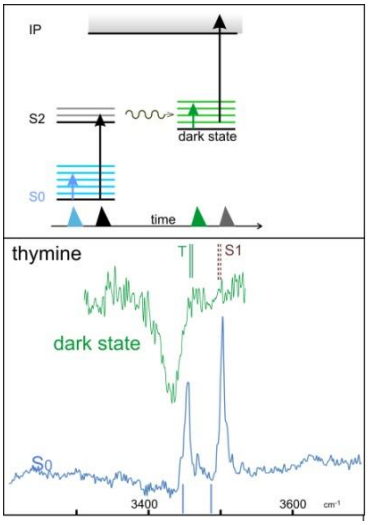

Figure 9: IR-UV double resonant spectra of ground state (blue) and dark excited state (green) of thymine. Pulse sequences in top panel. Computed frequencies as stick spectra.

Unlike with purine nucleobases, for pyrimidine bases the internal conversion does not occur with maximum quantum yield and the UV damage resistance is thus not complete. A small portion of excited state molecules appears to populate a "dark state". Ligare et al. report IR spectra of both

thymine and uracil, both for the ground state and for the dark state, as shown in Figure 9.[105] The top panel shows the pulse sequences employed to obtain these spectra. For the ground state, an initial IR pulse (blue) is scanned in the NH and OH stretch frequency region and followed by two step ionization probing (black pulses). When the IR pulse is resonant with a vibrational frequency this modifies the ground state vibrational population producing a modified Franck-Condon landscape. Usually this reduces the ion probe signal, but in this case it increases the ion probe signal. This suggests a strong geometry change between S_0 and S_2.[59]

For the dark state the pulse sequence starts with excitation to S_2 (black pulse) followed by rapid relaxation to the dark state. After 20 ns the IR laser is fired (green pulse) followed after another 30 ns by the ionization pulse from the excimer laser (black pulse), serving as the probe. In this sequence the IR laser modifies the dark state vibrational population, producing the green ion-dip spectra. Harmonic and partly anharmonic quantum calculations of the frequencies match well with the experiments and show the ground state to be of diketo character while the dark state matches with a triplet state.

We have also obtained lifetimes for the dark state by nanosecond pump probe measurements (black). The lifetime varies, depending on wavelength, from 59 to 69 ns for uracil and from 177 ns to 275 ns for thymine. We also observe a very long timescale component, due to ionization out of the hot ground state, resulting from rapid internal conversion out of the ππ* state. Quantum yields (QYs) are inversely proportional to lifetimes so if both the fast and slow decay would originate from the same excited state the slow process would have 4 to 5 orders smaller QY and could not be observed. Therefore the dark state must be populated at a rate similar to that for the internal conversion. As sketched in Figure 10, calculations predict excitation to an $^1S_2(\pi\pi^*)$ state which couples with both the ground state and a $^1S_1(n\pi^*)$ state at sub-picosecond rates.[105] The $^1S_1(n\pi^*)$ in turn intersystem crosses efficiently with a $^3T(\pi\pi^*)$ state according to El-Sayed rules as. Formation of photolesions is too fast for the triplet to be a precursor but the doorway state may be involved.[106-107]

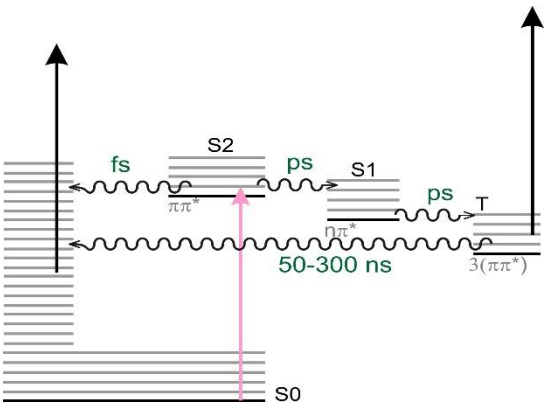

Figure 10: Schematic Jablonski diagram of the processes following UV excitation in thymine and uracil.[105]

Hydrogen bonding / charge transfer

The process of internal conversion via conical intersections is also affected by hydrogen bonding in base pairing, solvation, and internal hydrogen bonds in nucleosides. In many of these cases the internal conversion pathway is mediated by charge transfer states[51, 108-116]. In all these cases isomer

selective data are very valuable because the effect depends on H-bonding structures which we can distinguish in clusters. Figure 11 shows low energy structures of the homodimers and mixed clusters of guanine and cytosine. In nanosecond REMPI experiments we did not observe the structures in column (a), which are predicted to be the lowest energy clusters. We did, however, observe the structures shown in columns (b) and (c).[68, 70] This finding suggests for the GC base pair, that the triply H-bonded Watson-Crick base pair has much faster internal conversion than other GC cluster structures and that for GG and CC dimers the lowest energy, symmetrically H-bound, structures exhibit short excited state lifetimes as well.[37, 117-121] For the GC base pair, Domcke and Sobolewski predict a rapid internal conversion following coupling with a charge transfer state, which causes a proton transfer of the central hydrogen from G to C.[122] The charge-transfer state couples with the ground state with a CI leading to a return of the proton to the guanine, completing the deactivation. A similar process may occur in intramolecularly hydrogen bonded nucleosides. For example, calculations by Tuna, Domcke, and Sobolewski describe a charge transfer state in two separate conformers of adenosine which intersects with the ground state and involves a proton transfer between the sugar and the base[114]. The resulting conical intersections are about 0.6 eV lower than those corresponding to ring puckering in the base, possibly leading to faster de-excitation.

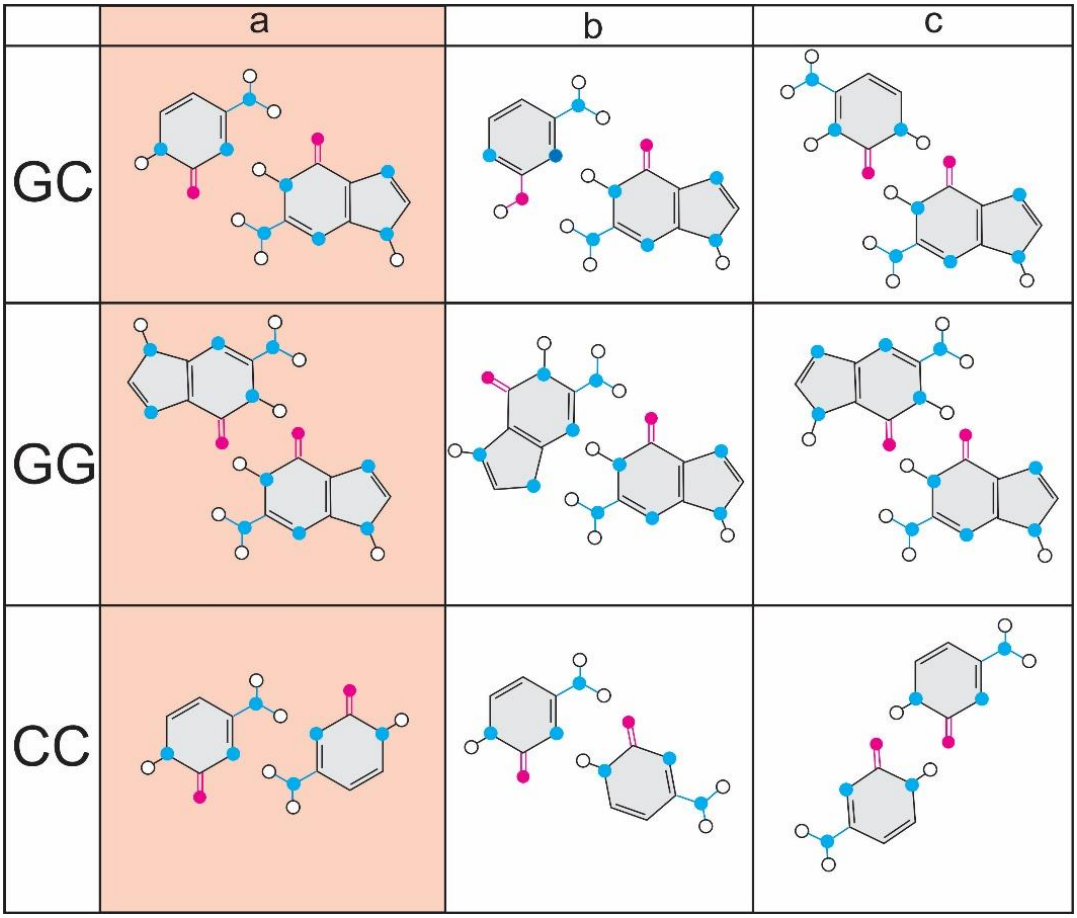

Figure 11: Low energy structures of the homodimers and mixed clusters of guanine and cytosine. In nanosecond REMPI experiments we did not observe the structures in column (a), which are predicted to be the lowest energy clusters. We did observe the structures shown in columns (b) and (c).

Exciplex decay

An extra set of decay channels can exist when the nucleobases are stacked, as in the helix structure. In that case the excited state can exhibit excitons that can form exciplexes or excimers on a ps time scale, which in turn can revert to the ground state by charge recombination on a 100 ps timescale. There is ample evidence for this process in solution phase fs experiments.[7, 83-84, 123-136] It appears that while the timescale slows down to the picosecond regime, compared to the rapid sub-picosecond internal conversion that dominates in monomers, charge separation still keeps the excitation localized and eventually returns the system safely to the ground state. Beckstead et al. have proposed that these different photoproperties that emerge from assemblies of photostable building blocks, as opposed to their monomers, may explain the transition from a world of molecular survival to a world in which energy-rich excited electronic states were eventually tamed for biological purposes such as energy transduction, signaling, and repair of the genetic machinery[126].

Nucleosides

In this context of prebiotic chemistry, focusing on the photochemistry of nucleobases might be insufficient as it seems difficult to effectively glycosilate nucleobases, that is form the N-glycosidic bond between ribose and a nucleobase, under prebiotically plausible conditions. In fact, several proposed efficient pathways towards pyrimidine nucleosides bypass the glycosidation step in favor of indirect synthesis.[23-25] Therefore, the photochemical prebiotic selection might have occurred at the level of nucleosides, or their small-molecule precursors. The work on the nucleobases has provided valuable understanding in the photochemical properties of the chromophores of the nucleosides but so far we have limited insights in how the excited state dynamics may vary with the added complexity of the of the sugar moiety. Nucleosides in solution exhibit sub-picosecond relaxation after excitation around 260 nm.[80, 137-138] Recently, de Camillis et al. found similar lifetimes in the gas phase for A, C, and T nucleosides and a longer time for guanosine.[139] Lifetimes were a factor 2 shorter than those obtained for the bases,[140-142], again with the exception of G. However, these data are for a single wavelength of 267 nm, likely well above any possible barrier for IC. Furthermore, the spectra were obtained for fragment ions rather than at the parent mass and there was no jet-cooling in the experiment and no option to observe longer time scale processes. There was no isomer selection, which may be crucially important since in our earlier ns experiments on a series of guanosines, we have observed only enol tautomers.[143-145] Furthermore, nucleosides can exist in at least two conformations, anti and *syn.*[146] The addition of the sugar moiety to the nucleobase chromophore can affect the excited state dynamics in a number of interesting ways, such as charge transfer between the sugar and the base, described above.[114] It has also been speculated that following intramolecular vibrational redistribution (IVR), the system can be trapped in the excited state due to the increased number of vibrational modes in the larger compound. At the same time, IVR can slow down with low excess energy.[147]

Summary and outlook

Figure 12 summarizes the major motifs that have emerged for nucleobase excited state dynamics with orders of magnitude of typical timescales. The safest deactivation process is rapid internal

conversion directly to the ground state but other, potentially more harmful, processes may compete. The outcome of this competition depends on many factors, especially the shape of the potential energy surfaces. There may be numerous possible trajectories, the starting point of which differs with excitation wavelength, with barriers to overcome and multiple conical intersections to navigate. Since the potential energy landscape depends on the molecular structure, the dynamics may differ strongly for analogues and for derivatives of the same basic structure and even for different tautomers. Some of the effects can be understood by observing some general structural motifs for formation of conical intersections, but that intuition needs to be aided by high level quantum dynamics computation.

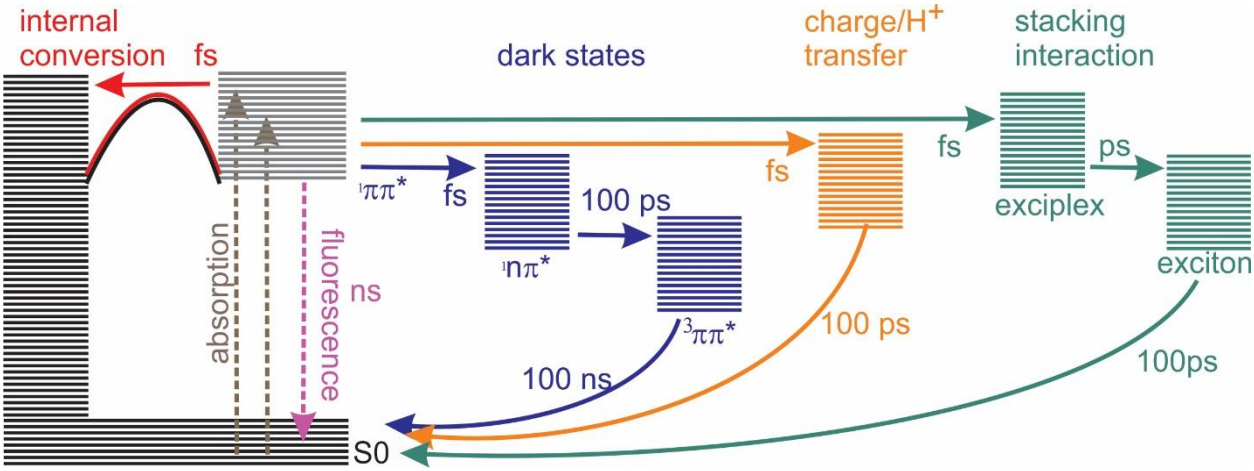

Figure 12: schematic summary of major motifs that are now emerging of nucleobase excited state dynamics with typical time scales.

The UV protection of the canonical bases is not perfect. Not all non-canonical bases have long excited state lifetimes so it could be possible that rapid internal conversion to the ground state was a necessary but not sufficient condition for survival under UV conditions. Therefore, it is necessary to extend these studies to more derivatives and analogues, especially those that have been proposed as having played a role in early prebiotic chemistry. The work on isoC and isoG is one such example. Similarly, there is a need for more gas-phase studies of nucleosides. Since the nucleobase is still the chromophore, the work so far can serve as a basis for understanding nucleoside excited state dynamics, but a full understanding is lacking of how the addition of the sugar moiety may alter the properties.

In spite of the remarkable progress in forming a comprehensive picture of nucleobase excited state dynamics, there remain significant gaps in our understanding. First, most sub-ps excited sate species have never been directly observed in the gas phase. Many conclusions have been drawn based on their absence in comparison with other techniques, which is a very inconclusive and unsatisfactory approach. Direct studies at faster time-scales are needed. Second, more needs to be learned about the wavelength dependence. Several nucleobases, such as adenine, exhibit a narrow section of the spectrum with a longer lived excited state. The fact that below a barrier for IC to the ground state deactivation slows down does not always have to imply that there are competing harmful pathways, but this regime needs to be investigated. Most work so far has concerned itself with energies well above those for CIs and the excited state dynamics is expected to slow down at

lower energies. Studies in the picosecond time regime at those energies can most sensitively probe the critical areas of the potential energy landscape, involving barriers, different pathways and conical intersections. Third, exciplex formation and decay has been observed in solution but not yet in the gas phase, as isolated nucleobases tend to hydrogen bond rather than stack. Finally, there is a need to determine accurate quantum yields for the competing decay processes and these will depend on wavelength, structure, tautomeric form, and the character of the various states. Such data can help in modeling prebiotic chemistry as well as in modeling the detailed shapes of the potential energy surfaces involved and the resulting photodynamics. Gas phase experiments have proven to be important for comparison with the highest levels of theory. Progress in this interplay between theory and experiment has been driven by simultaneous advances in the ability to study molecules of increasing size both in the gas phase and in silico. Fourth, further insights are called for in order to elucidate by what processes and with what products longer lived excited states could lead to harmful outcomes. Finally, to extrapolate these findings to better models of bulk properties will be helpful to extend gas phase experiments to larger clusters with water and further to nucleosides, nucleotides and small model oligonucleotides.

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