CHAPTER 12

ISOLATED DNA BASE PAIRS, INTERPLAY BETWEEN THEORY AND EXPERIMENT

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Abstract: Simultaneous advances in gas phase spectroscopy and computational chemistry have made it possible to study isolated DNA base pairs. This account focuses on three specific topics that have emerged from this research, namely (i) the use of experimental data as benchmarks for theory, (ii) base pair structures, and (iii) the dynamics of the electronically excited state. The lowest energy nucleobase pair structures are not always observed in gas phase spectroscopy. One possible reason may be short excited state lifetimes in certain structures. This explanation is consistent with theoretical models and with the observation that the isolated guanine cytosine (GC) Watson-Crick structure exhibits a different photochemistry than other hydrogen bonded GC structures

Keywords: DNA Bases, Base Pair, Laser Desorption, REMPI, IR-UV Double Resonance

12.1. INTRODUCTION

Life, as we know it, is based on replication, which on a molecular level is realized by DNA base pairing. However, the scheme with four nucleobases of DNA does not necessarily represent the only way to achieve molecular replication [1]. Furthermore, there are alternate pairing schemes and structures and interactions between the bases that can lead to mutations, for example by proton transfers that lead to different tautomers. For all these reasons the study of interactions between individual nucleobases and of the properties of isolated base pairs at the most fundamental level is important and such studies are possible in the gas phase.

Cognizant of the fact that biology takes place in solution, we can summarize the motivation for studying biomolecular building blocks in the gas phase in three points:

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1. Comparison between theory and experiment: Gas phase data are of great value for direct comparison with the highest level quantum chemical computations.
2. Gas phase studies focus on isolated molecules, thus omitting elements of the biological environment, such as macromolecular structure, solvent, and enzymes. This allows for the study of intrinsic properties and their separation from other factors. These studies represent a reductionist approach biomolecular chemistry.
3. The opportunity to study isolated molecules also offers an avenue into questions of prebiotic chemistry. To determine the rules of chemistry that may have been important on an early earth, before the onset of life, one needs to observe interactions between biomolecular building blocks in the absence of biology.

In recent years two parallel developments in experiment and in theory have lead to a body of new research on the properties of isolated bases and their pairing. Experimentally the field has moved forward by new capabilities for placing fragile and low vapor pressure molecules in the gas phase. At the same time theoretical chemistry has seen advances in computational technique that allow investigations of larger systems at higher levels. Together these developments form a great example of interplay between theory and experiment and create a driving force for the study of isolated bases and base pairs.

This account focuses on three specific topics that have emerged from this research, namely (i) the use of experimental data as benchmarks for theory, (ii) base pair structures, and (iii) the dynamics of the excited state.

12.2. TECHNIQUES

Theoretical and computational methodologies are treated in detail elsewhere in this book. Experimental techniques for studying isolated molecules rely on their observation in the gas phase, where molecules can be studied free of interactions. This is different from single molecule studies in which molecules can interact with their environment, but are studied one by one [2]. In the gas phase one may study a large ensemble of molecules or clusters, but each one of those is isolated and does not interact with its environment. Clusters represent a transition area between gas phase and bulk by allowing *intra*-cluster interactions, while being isolated from *inter*-cluster interactions.

Until recently gas phase studies have been limited to molecules that can be heated without degradation. For the nucleobases this includes adenine (A), but not, for example guanine (G) and none of the nucleosides. Since the first report of the resonance enhanced multiphoton ionization (REMPI) spectrum of laser desorbed guanine in 1999, laser desorption has essentially rendered all bases and their clusters amenable to study as neutral species in the gas phase [3].

In this approach material is laser desorbed from a sample probe in front of a pulsed nozzle. The desorption laser is typically (but not exclusively) a Nd:YAG laser operated at its fundamental wavelength of 1064 nm. At this wavelength one does not expect photochemical interaction with any of the nucleobases. Laser desorption involves heating of the substrate, rather than the adsorbate. Therefore it is typically

desirable to match the wavelength of the desorbing light with the absorption characteristics of the substrate, while avoiding overlap with the absorption spectrum of the adsorbate. We routinely use graphite as a substrate, although we have also successfully used metal substrates. Typical laser fluences are of the order of 1 mJ/cm^2 or less, which is significantly less than the fluences normally used for ablation. The laser is focused to a spot of the order of 0.5 mm diameter within 2 mm in front of the nozzle. This is important because in a supersonic expansion most of the cooling takes places close to the nozzle by collisions with the drive gas along a distance of about 10 nozzle diameters. In earlier work we have optimized the geometry for effective entrainment by mapping entrained perylene with laser induced fluorescence [4, 5]. In that work we found that it is possible to entrain a portion of the desorbed material on the axis of the supersonic beam, such that the ionizing laser downstream can interact with a fraction of about 10^{-5} of the desorbed material.

The gas phase approach prescribes techniques for analysis that are germane to the gas phase and this constraint in turn determines what properties are accessible for measurement. Mass spectrometry has traditionally been an important domain of gas phase studies [6, 7], but another major tool is spectroscopy, which provides an indirect but often high resolution measure of structure, can provide insights in dynamics, and provides frequencies for direct comparison with computations.

State of the art for spectroscopic analysis in the gas phase is currently IR-UV double resonant spectroscopy [8–12], schematically depicted in Figure 12-1. This

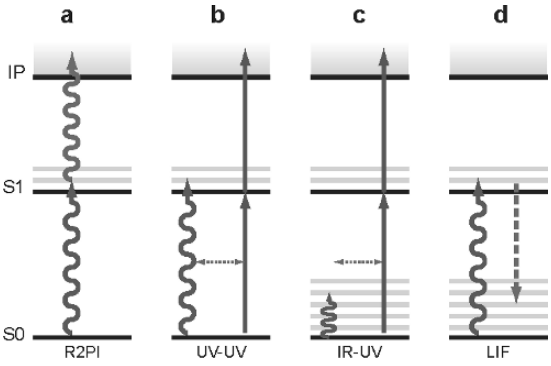

Figure 12-1. Schematic diagram to illustrate double resonance techniques. (a) REMPI 2 photon ionization. The REMPI wavelength is scanned, while a specific ion mass is monitored to obtain a mass dependent S1 ← S0 excitation spectrum. (b) UV-UV double resonance. One UV laser is scanned and serves as a "burn" laser, while a second REMPI pulse is fired with a delay of about 100 ns and serves as a "probe". The probe wavelength is fixed at the resonance of specific isomer. When the burn laser is tuned to a resonance of the same isomer it depletes the ground state which is recorded as a decrease (or ion dip) in the ion signal from the probe laser. (c) IR-UV double resonance spectroscopy, in which the burn laser is an IR laser. The ion-dip spectrum reflects the ground state IR transitions of the specific isomer that is probed by the REMPI laser. (d) Double resonance spectroscopy can also use laser induced fluorescence as the probe, however that arrangement lacks the mass selection afforded by the REMPI probe

approach offers the great advantage of providing isomer selective ground state IR frequencies for comparison with theory and to serve as a structural tool. Mass selection in these experiments enables the spectroscopy of selected clusters, while in some cases fragmentation can offer additional insights in dynamics. Limitations of this technique include the fact that some electronic states may go unobserved because of unfavorable wavelength dependence or Franck-Condon overlap, because of high ionization potentials, necessitating multicolor excitations, and because of short excited state lifetimes. The latter is especially important because it is a direct consequence of excited state dynamics and will be discussed in more detail below.

Another approach of great importance for studies of excited state dynamics is sub-picosecond time resolved spectroscopy. A number of authors have reported femtosecond pump-probe measurements of excited state lifetimes in A, C, T, and G [13–16] and base pair mimics [17]. Schultz et al. have reported time resolved photoelectron spectroscopy and electron-ion coincidence of base pair mimics [18]. these studies can also be compared with similar measurements in solution [19–24]. While time resolved measurements provide direct lifetime data, they do have the limitation that the inherent bandwidth reduces the spectral resolution, required for selecting specific electronic states and for selecting single isomers, such as cluster structure and tautomeric form.

12.3. INTERPLAY OF THEORY AND EXPERIMENT

An important parameter for comparison with theory as well as for understanding many properties would be relative binding energies or stabilities. Unfortunately those are hard to assess in the gas phase. One of the few experiments to report thermodynamic binding energies between base pairs is the work by Yanson et al. in 1979, based on field ionization [25]. Relative abundances of nucleobase clusters in supersonic beams are an unreliable measure of relative stability for a two reasons: First, supersonic cooling is a non-equilibrium process and thus comparison with thermal populations is tenuous at best. Secondly, ionization probabilities may be a function of cluster composition. The latter is certainly the case for multi photon ionization, as will be discussed in detail below.

The first reported gas phase electronic spectra of DNA base pairs described hydrogen bond frequencies of GC clusters, measured by REMPI [26]. On the one hand these frequencies agreed quite well with theoretical predictions. On the other hand, the six hydrogen bonding modes between two molecules of a given mass are only very weakly dependent on cluster structure and can therefore neither serve as a structural tool nor as a good benchmark for theory. Moreover, REMPI only measures excited state vibrations, while the best calculations apply to the ground state.

To access the ground state the IR-UV double resonant technique has proven to be very powerful. This approach provides ground state vibrations in the 500–4000 cm^{-1} range *with isomer selection*. This has made it possible to obtain tautomer selective and cluster structure selective spectroscopy. This ability to obtain

spectral frequencies of not only isolated base pairs, but even of unique isomers, greatly facilitates meaningful comparison with theory. Some practical considerations constrain possible experiments. Available table top lasers (OPO/OPA systems) typically operate in the near infrared between 2000 and 4000 cm^{-1}. Modes that can be most easily measured in this wavelength range are OH, NH and NH_2 stretching modes. New extensions of these lasers below 2000 cm^{-1} now also include the C = O stretch at about 1800 cm^{-1} in some laboratories [27, 28]. This technique in this wavelength range forms a great structural tool by distinguishing between free *vs.* hydrogen bonded modes. The latter are characterized by typical red-shifts of the order of up to hundreds of wavenumbers, depending on the strength of the hydrogen bonding, and by significant broadening and increase in intensity. However, that also points to a current limitation in computation, namely that generally the extent of these red-shifts is very poorly predicted. Figure 12-2 shows the example of three structures of GC. The free modes are very well reproduced by theory, but the fit of the hydrogen bonded (H-bonded) modes is only qualitatively represented.

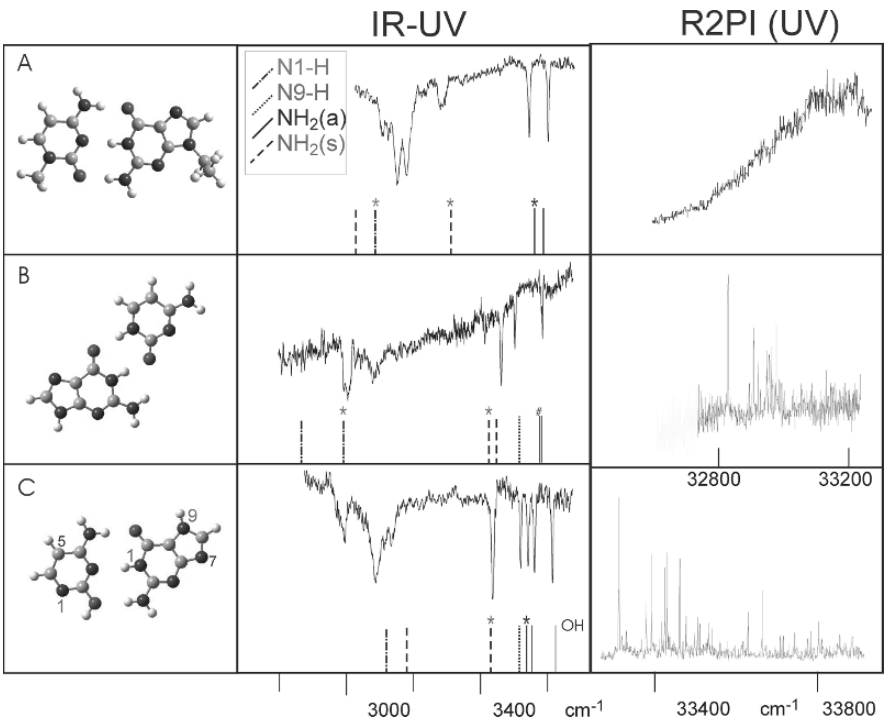

Figure 12-2. IR-UV double resonant and R2PI spectra of three guanine-cytosine cluster structures. Stick spectra show calculated frequencies for modes, indicated in line types according to the key in the top panel. The relevant numbering is indicated in the structure (c). Structure (a) is corresponds to the Watson-Crick structure and is not observed for the unmethylated bases

Validation of theory by experiment is less satisfactory in the mid IR range below 2000 cm^{-1}. Part of the problem is that anharmonicity plays an increasingly important role at shorter wavelengths, requiring often ill defined empiric scaling factors. Gerber and coworkers have reported new algorithms to include anharmonicity [29, 30]. While the near IR above 2000 cm^{-1} provides structural information by virtue of H-bonding shifts, the mid IR, in principle, provides the more subtle and detailed information provided by lower frequency C-H stretches and bending vibrations. Figure 12-3 shows

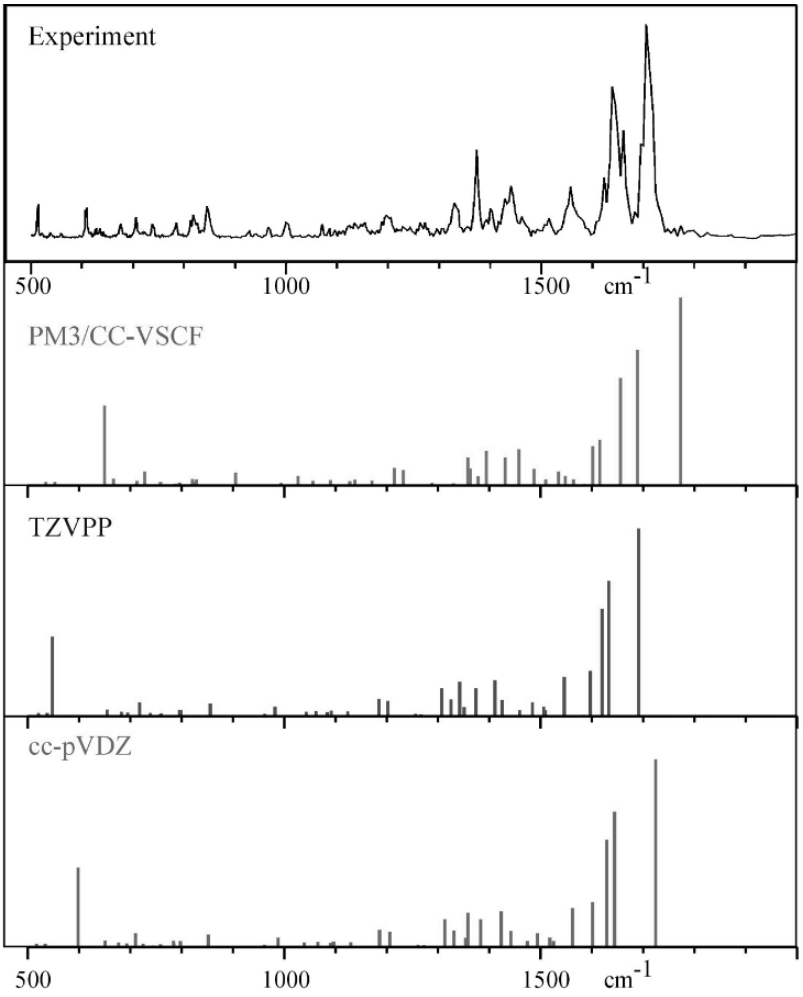

Figure 12-3. IR-UV double resonance spectrum of GC (structure C) in the mid-IR frequency range (recorded at the FELIX free electron laser facility), compared with three types of ab intio calculations. Harmonic frequencies were obtained at the RI-MP2/cc-pVDZ, RI-MP2/TZVPP, and semiempirical PM3 levels of electronic structure theory. Anharmonic frequencies were obtained by the CC-VSCF method with improved PM3 potential surfaces [30]

the IR-UV double resonance spectrum of GC obtained at the FELIX free electron laser and its comparison with calculated frequencies [30, 31]. Harmonic frequencies were obtained at the RI-MP2/cc-pVDZ, RI-MP2/TZVPP, and semiempirical PM3 levels of electronic structure theory. Anharmonic frequencies were obtained by the CC-VSCF method with improved PM3 potential surfaces. Comparison of the data with experimental results indicates that the average absolute percentage deviation for the methods is 2.6% for harmonic RI-MP2/cc-pVDZ (3.0% with the inclusion of a 0.956 scaling factor that compensates for anharmonicity), 2.5% for harmonic RI-MP2/TZVPP (2.9% with a 0.956 anharmonicity factor included), and 2.3% for adapted PM3 CC-VSCF; the empirical scaling factor for the ab initio harmonic calculations improves the stretching frequencies but decreases the accuracy of the other mode frequencies [30].

Reha et al. have reported new computational approaches to account for dispersive forces [32]. Figure 12-4 shows an IR-UV spectrum of Guanosine-cyclic-Phosphate. This is the largest form of a DNA base for which IR-UV data have been reported so far and this still constitutes a considerable challenge for computational comparison, not only because of its size but also because of the large number of closely spaced modes with only small differences between different conformations. Another example of the current limitations on computational resolution is the inability in many cases to distinguish between N7H and N9H tautomers in purines. In spite of all these limitations progress in computational level and in agreement with experiment has been rapid and further advances may be expected.

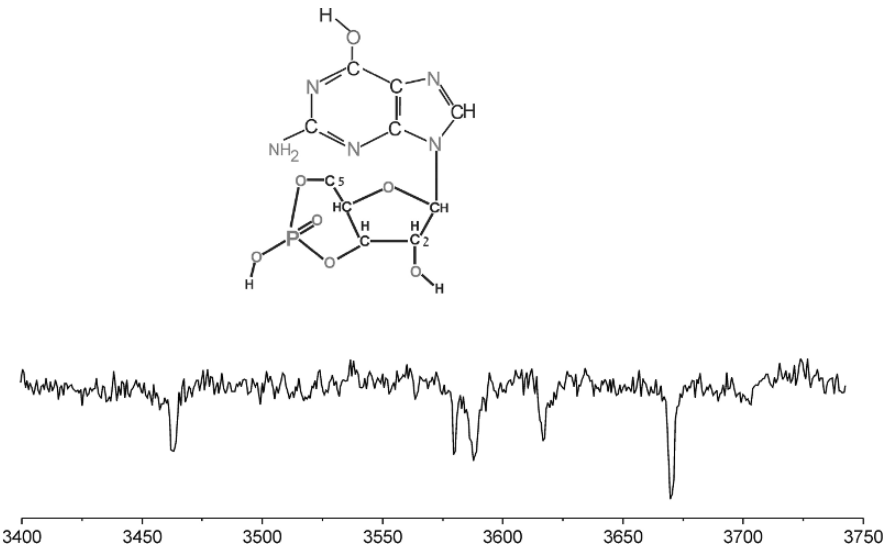

Figure 12-4. IR-UV double resonance spectrum of guanosine-cyclic-phosphate

12.4. STRUCTURES

In the macromolecular context of DNA two types of non-covalent interactions are especially important. Roughly speaking, dispersive forces are responsible for π stacking, which stabilizes the helical structure, while hydrogen bonding is instrumental in molecular recognition between complimentary bases [33–37]. Both types of interactions are implicated in excited state dynamics as well, as we will elaborate in the next section below. In solution individual bases are mostly stacked. In the gas phase individual nucleobases tend to exclusively hydrogen bond, which permits the study of this parameter in isolation. According to computations we expect to observe stacked structures with 2–8 water molecules [38, 39], as summarized in Table 12-1. Methylation of the bases can affect the structure and in some cases even dramatically change the interactions, causing stacked structures to be favored over H-bonded ones in most cases even without any water molecules. One such gas phase stacked base pair structure has been reported by Kabeláč et al. who reported evidence for a stacked structure in 9-methyladenine dimers, while 7-methyladenine dimers form hydrogen bonded structures [38–40]. It has been pointed out that the most stable stacking geometries of individual bases in the gas phase coincide with those that occur in DNA.

One of the most intriguing questions is whether the Watson-Crick (WC) structures that dominate in DNA are intrinsically the most stable structures, even in the absence of the backbone and the solvent. In other words is the biological context required for these structures to be preferred? It is noteworthy that theory predicts the WC structure for AT in the gas phase not to be the lowest in energy [41].

Figure 12-5 summarizes the observed structures of various DNA base pairs in the gas phase, as determined by IR-UV double resonance spectroscopy. Open circles indicate the sites at which the ribose group is attached in the nucleosides. The structures in columns (b) and (c) are the ones observed experimentally. The structures in column (a) were not observed. For G-C pairs the structure in column (a) is the Watson-Crick structure. Abo-Riziq et al. observed this structure when the bases were derivatized in the ribose position (N9 for guanine and N1 for cytosine), however, in that case the UV spectrum was very broad [42]. One of the most remarkable features of these data is thus that some of the biologically most important structures so far remain unobserved in the gas phase. The structures of column (a)

Table 12-1. Minimum number of water molecules predicted to cause at least 50% of the cluster population to be stacked rather than hydrogen bonded for various base pair combinations [38]

2 H_2O	4 H_2O	6 H_2O	8 H_2O
AA	AC	GC	CC
AG	CT		TT
AT	GG		
	GT		

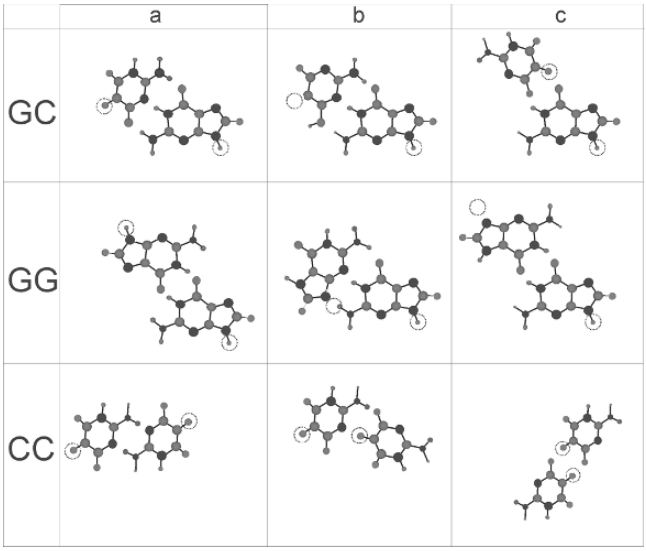

Figure 12-5. Low energy structures of guanine and cytosine nucleobase pairs. Open circles indicate location of R group in nucleosides

are the forms in which these bases pair in the biological context and these are also the structures that theory predicts to be the lowest in energy in the gas phase. (We note that the GG pair is important in telomer structures and that its symmetrical structure, according to theory, is lower in energy even than the WC form of AT). In the case of adenine dimers the lowest energy structure is also symmetric and was not observed experimentally in the gas phase while the next two higher energy structures were identified [43]. However in this case the lowest energy structure is not biologically relevant and the WC structure, which was not observed, is higher in energy by 5–10 kcal/mol. The phenomenon of unobserved isomers is also apparent for monomers. The case of the tautomers of guanine is the topic of the next chapter in this book. REMPI experiments fail to detect the keto tautomers [44]. The same holds for all 9-substituded guanines, including all guanosines (Gs). Double resonant experiments reveal only the enol form of Gs, 2 deoxyGs, and 3 deoxyGs [45]. This is remarkable because the keto form is dominant in biological context: it is the preferred form in solution and it is the form in which base pairing occurs in DNA. In the case of clusters of GG pairs with one and with two water molecules, we only observed the structure in column (b), as shown in Figure 12-6. For the bare dimer we also show the calculated frequencies and once again the agreement for the free modes is excellent, while the hydrogen bond shifts are reproduced quantitatively [46]. All modes are color coded in correspondence to be structures in the insets.

Figure 12-7 shows clusters involving 9-methylguanine, demonstrating how tautomeric blocking can be used to confirm structural assignments [47]. Figure 12-8 shows REMPI spectra of cytosine dimers and their methyl derivatives [48]. We used

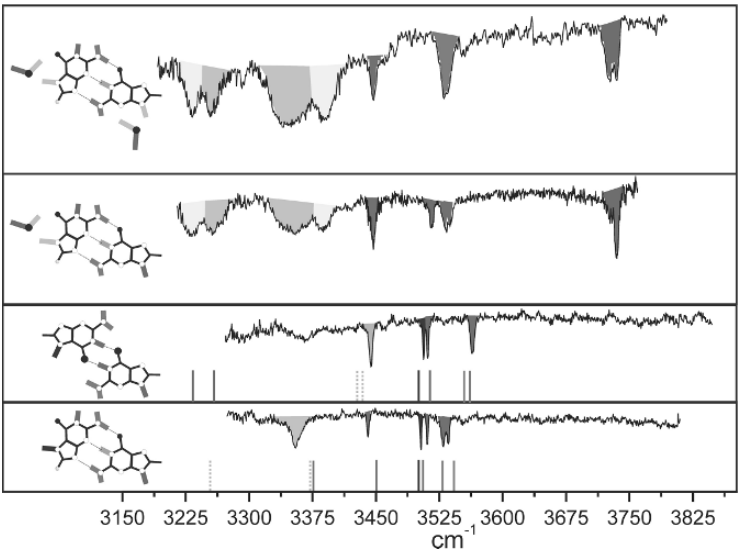

Figure 12-6. IR-UV double resonance spectra of guanine dimers with 0, 1, and 2 water molecules

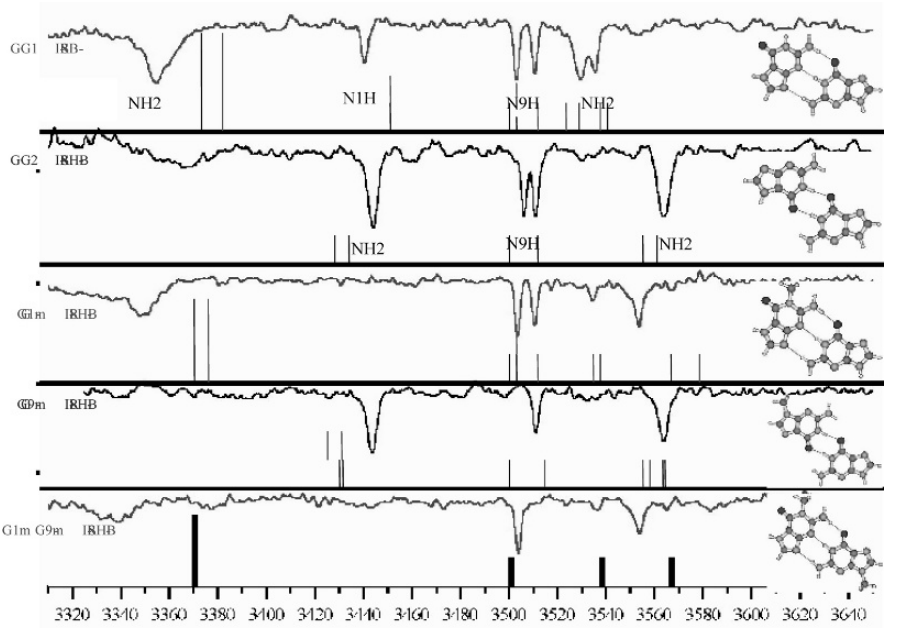

Figure 12-7. Tautomeric blocking in GG clusters

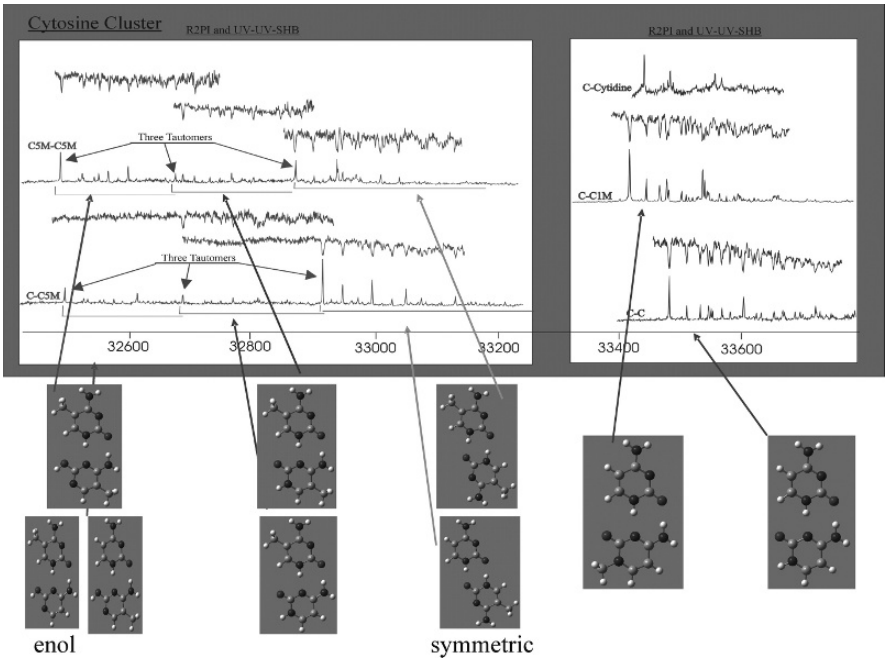

Figure 12-8. REMPI spectra of various cytosine dimers. Asterisks indicate origins of different structures as determined by UV-UV double resonance spectroscopy. The corresponding structures, as determined by IR-UV double resonance spectroscopy are shown below

UV-UV double resonance spectroscopy to determine origins of separate isomers, followed by IR-UV double resonance to determine their structures. The resulting structures appear in the figure.

When predicted molecular forms go unobserved in the gas phase there are two possibilities: Either these forms are less stable in the gas phase or they actually do exist but the detection method discriminates against them. In REMPI experiments at least four parameters can adversely affect detection efficiency. (1) The molecule may not absorb or absorb poorly to the S1 state at the employed wavelengths because of low oscillator strength or poor Frank-Condon overlap. (2) The ionization potential may be more than twice the employed photon energy. In that case single color REMPI cannot ionize the molecule, but two color REMPI still could. (3) The ion may be unstable. In that case spectroscopy may be possible with detection at a fragment mass. (4) The S1 excited state lifetime may be several orders of magnitude shorter than the laser pulse length, significantly reducing two-photon ionization cross sections. This could be the case for sub-picosecond excited state lifetimes of states excited with nanosecond laser pulses. The possible implications of this option are the topic of the next section.

12.5. EXCITED STATE DYNAMICS

There are examples of gas phase structures that REMPI does not detect, but that do show up with other techniques. Guanine keto tautomers do not show up in double resonant experiments with a REMPI probe, but they do appear in helium droplets [49–51]. There are a number of differences between the two experiments, including details of both vaporization and cooling. However, probably the most significant difference is the fact that the IR absorption in the He droplet is detected by its heating effect on the droplet and thus it does not involve the S1 excited state. There are also some examples that suggest more directly that excited state dynamics is implicated in the failure to observe key structures by REMPI in the gas phase. Kang et al. have reported adenine$(H_2O)_n$ with multiple water adducts when ionizing with 100 *fs* laser pulses at 266 nm, while not observing any adenine water clusters when ionizing with ns laser pulses at the same wavelength [14]. We have observed the same effect for hypoxanthine, ionized with short (100 *fs*) pulses but defying detection with long (10 *ns*) pulses. He et al. have reported a decrease of excited state lifetime of uracil and thymine up on successive addition of water molecules [52].

All DNA bases implicated in replication have short excited state lifetimes. The expanding field of condensed phase time resolved experiments has recently been extensively reviewed by Kohler [23]. It has long been speculated that this property is nature's defense against photochemical damage [53]. The idea is that a doorway state couples S_1 with S_0 through conical intersections. This provides a pathway for ultrafast conversion of electronic excitation to heat (in the form of ground state vibrational excitation) which can subsequently safely be transferred to the environment, thus preventing chemistry to be initiated by the electronically excited molecule. Heterocyclic molecules provide several candidates for the doorway state and considerable theoretical effort has recently been invested in detailed computations of the potential energy landscape that can lead to this phenomenon [18, 54–63].

12.5.1. Monomers

The most intensely studied nucleobase excited state to date is that of adenine. Recently Marian and Perun et al. [61] showed that puckering of the six-membered ring of adenine at the C2-H group provides an essentially barrierless pathway for efficient internal conversion of the mixed $n\pi^*$-/$\pi\pi^*$- states to the electronic ground state [56]. The NH_2 group in position 2 in 2-aminopurine (2AP) creates a barrier for this mechanism and as a result 2AP has a large fluorescence lifetime [54]. Our observation that hypoxanthine can only be photoionized with short laser pulses, while we detect xanthine with nanosecond REMPI, parallels this observation, as the difference between the two molecules is the C2 substituent of the purine in the case of xanthine. A weak absorption can be observed for adenine close to that of the dominant spectrum, which can be ascribed to the $n\pi^*$ transition, consistent with this model. Sobolewski and Domcke have proposed a doorway state of $\pi\sigma^*$ character, associated with motion along an N-H coordinate; For adenine this would be the N9-H coordinate [64]. Consistently with this mechanism, Hünig et al. directly

observed N-H photodissociation in adenine at 243 nm photolysis energy via resonant ionization of product H atoms arising from N9-H but also to some extent from the NH_2 group [65]. Zierhut et al. obtained similar results at 239 and 266 nm photolysis energy [66]. On the other hand Kang et al. measured the excited state lifetimes in femtosecond 267 nm pump-probe experiments and found them to be similar for all adenine derivatives, including 9-methyl adenine [67]. Based on this result these authors argued against a $\pi\sigma^*$ character for the doorway state. The onset of the $\pi\sigma^*$ state therefore is probably at around 266 nm.

The internal conversion pathways of the other nucleobases are less well investigated. They seem to involve C-C twisting in cytosine with a low barrier for keto-cytosine (producing a short vibronic spectrum) and a considerable energy gap to the S0 state even at extended twisting for enol-cytosine [55, 68] (producing an extensive vibronic spectrum [69]). For 9-methyl guanine the internal conversion pathway seems to involve a strongly bent amino group in position 2. It should be noted that treatments in which the potential surfaces are traced along a single coordinate are necessarily simplifications.

12.5.2. Base Pairs

It is possible that the absence of some of the most stable structures of base pairs in the gas phase is due to short excited state dynamics. Direct experimental proof of this explanation requires fast time resolved measurements. For an indirect measure one can consider the linewidths in UV spectra. Of the 27 base pairs analyzed in the gas phase so far, 24 structures do not form WC type pairs and all of those exhibit sharp structured REMPI spectra, as shown in Figure 12.9(a). Only three

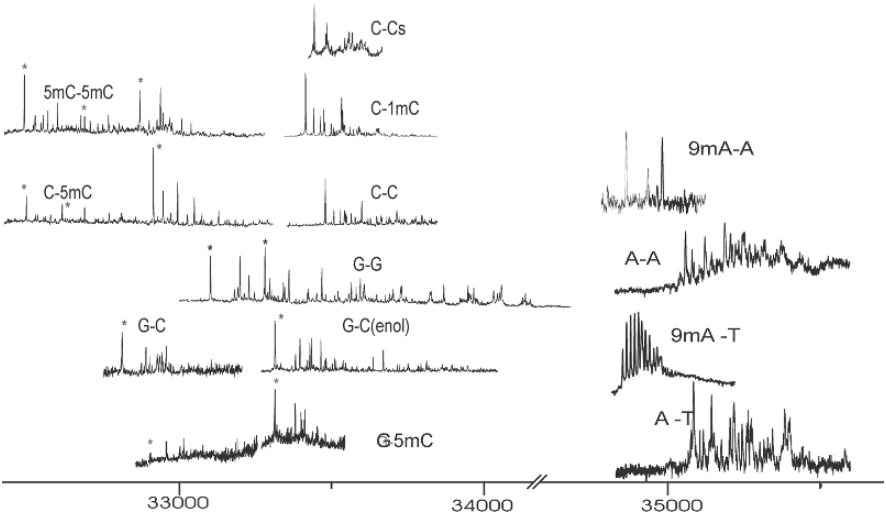

Figure 12.9a. REMPI spectra of various nucleobase pairs. Asterisks indicate origins of different structures as determined by double resonance spectroscopy

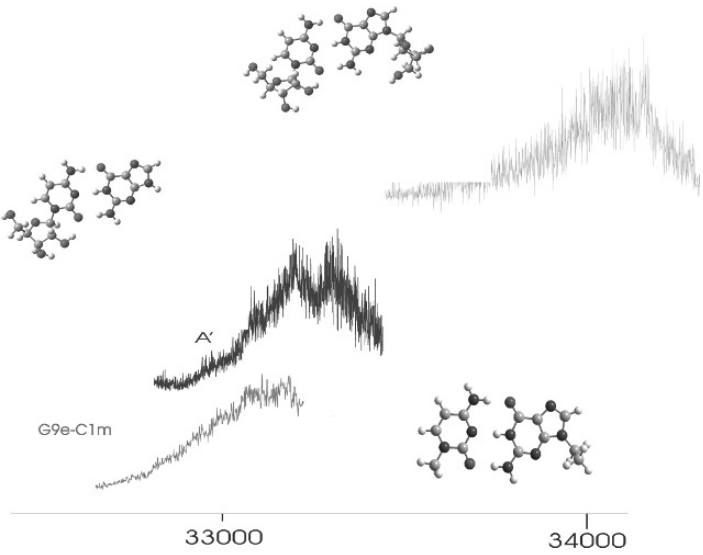

Figure 12.9b. REMPI spectra of GC base pairs

structures have been observed that correspond to Watson-Crick pairing and all of these three exhibit broad, unstructured REMPI spectra, as shown in Figure 12.9(b). This forms direct evidence for a different photochemistry for this special structure, and as such it is an intriguing finding. One possible explanation could be in the excited state dynamics and thus this could be interpreted as indirect evidence of structure selective shorter excited state lifetimes. This interpretation is consistent with theoretical modeling.

Figure 12-2 shows data we obtained for three isolated GC base pair structures. Row A shows results for the Watson-Crick (WC) structure, while rows B and C represent the second and third lowest energy structures, respectively, which are not WC. The second column shows the IR-UV double resonance data, compared with the *ab initio* calculations of the vibrational frequencies. These data allow us to assign the structures. The third column shows the UV excitation spectra, measured by resonant two-photon ionization (R2PI). The UV spectrum is broad for the WC structure (A) and exhibits sharp vibronic lines for the other structures.

Figure 12.10 shows schematic potential energy diagrams, as calculated by Sobolevski and Domcke [70, 71]. In the WC structure the excited state (S1) is coupled to the ground state (S0) via an intermediate state (of charge transfer character – CT) with barrierless conical intersections. For the other structures small differences in relative energies cause the existence of barriers that lead to discrete spectra and lifetimes that can be two orders of magnitude longer. In the B and C structures the curve crossings between the lowest locally excited $^1\pi\pi^*$ state and the CT state occur about 10 kcal/mol above the $^1\pi\pi^*$ energy minimum, while in the WC structure the crossing is close to the $^1\pi\pi^*$ state minimum. Another reaction

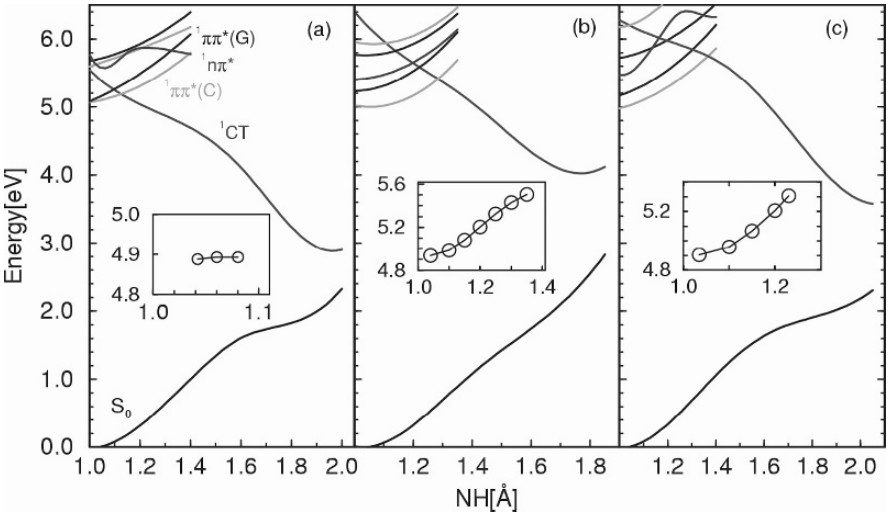

Figure 12.10. Potential-energy functions of the S_0 state, the locally excited $^1\pi\pi^*$ states of guanine and cytosine, the lowest $^1n\pi^*$ state, and the $^1\pi\pi^*$ charge-transfer state of the WC conformer (a), the conformer B (b), and the conformer C (c) of the CG dimer. The PE functions have been calculated along the linear-synchronous-transit proton-transfer reaction path from the S_0 minimum to the biradical minimum. Insets show the potential-energy function of the locally excited $^1\pi\pi^*$ state of guanine calculated along the minimum-energy path for stretching of the NH bond

coordinate may involve out-of plane deformation of guanine or cytosine, analogous to the excited state dynamics of cytosine monomer.

We have observed an indication of hydrogen or proton transfer from guanine to cytosine in the B cluster (analogous to the Watson-Crick structure up with the cytosine in the enol form). We recorded a REMPI spectrum on the mass of cytosine plus one (CytH) that was identical to the REMPI spectrum recorded on the mass of the GC cluster. Therefore the CytH fragment must have been formed after excitation of the GC cluster, either by hydrogen transfer in the excited state or by proton transfer in the ionic state. Attempts to determine which hydrogen is involved by isotope labeling failed because of isotopic scrambling.

Related results were obtained by Schultz et al. for 2-aminopyridine dimers, which serve as a base-pair mimetic [18]. Femtosecond time-resolved mass spectrometry of 2-aminopyridine clusters revealed an excited-state lifetime of 65 ± 10 picoseconds for the near-planar hydrogen-bonded dimer, which is significantly shorter than the lifetime of either the monomer or the 3- and 4-membered nonplanar clusters. Ab initio calculations of reaction pathways and potential-energy profiles identify the same mechanism of the enhanced excited-state decay of the dimer: Conical intersections connect the locally excited singlet $\pi\pi^*$ state and the electronic ground state with a singlet $\pi\sigma^*$ charge-transfer state that is strongly stabilized by the transfer of a proton [18]. This is the same type of $\pi\sigma^*$ state that is proposed for the N9-H reaction coordinate in adenine. In fact,

Sobolewski and Domcke have proposed that this mechanism may be more general in bio molecular excited state dynamics [72].

Crespo-Hernandez et al. have recently reported femtosecond pump-probe measurements in the liquid phase, pointing to the role of stacked structures in the rapid deactivation of excited states [24]. This points to the complexity of the excited state dynamics, requiring further detailed experiments to determine the contributions of possibly competing pathways.

12.6. PREBIOTIC CHEMISTRY

One of the enticing consequences of the excited state dynamics of base pairs is the possible role this property may have played in chemistry on the early earth. Prior to the existence of living organisms photosynthesis would have been absent. Consequently there would have been no free oxygen in the atmosphere and no ozone layer would have existed. The earth's surface would have been exposed to deeper (more energetic) UV irradiation than is the case today. Therefore UV photochemistry is part of the set of rules that may have governed the chemistry that could take place at that time.

The DNA bases involved in reproduction have short S_1 excited state lifetimes of the order of one picosecond or less [13, 15, 19, 23, 73–75]. It has been argued that this phenomenon serves to protect these bases against photochemical damage, because following excitation they do not cross to the reactive triplet state, but instead they rapidly internally convert to the electronic ground state [76]. This may have been particularly significant under the conditions of the early earth when purines and pyrimidines presumably were assembled into the first macromolecular structures, producing RNA.

Curiously protection against photochemical damage is no longer as critical under today's conditions as it may have been in the past. DNA today is not only protected by the ozone layer, but also by enzymatic repair mechanisms. Most cellular photo damage today is caused indirectly as light is absorbed by other molecules in the cell which produce free radicals, which in turn react with DNA. Furthermore, deactivation by charge transfer along stacked bases, today can compete with dynamics along the hydrogen bond coordinates. Therefore it seems reasonable to assume that the protective mechanism of internal conversion for all crucial bases was important only earlier in the history of life, particularly prior to the formation of the ozone layer.

The implication is that under the conditions of deep UV irradiation, which likely existed on the early earth, selective chemistry may have taken place in favor of species with the shortest excited state lifetimes. Benner and coworkers, for example, have proposed a molecular lexicon of 12 alternate bases that can produce 6 base pairs with virtually identical geometries as the guanine-cytosine (GC) triply hydrogen bonded base pair [1]. Several of these "alternate" bases have been observed as products in simulation experiments that test the feasibility of synthesis of such compounds under primitive conditions [77, 78]. This raises the

question what properties would possibly have made the bases that from today's DNA most suitable for that development. Moreover, beyond a selection of certain base pair *combinations* it seems possible that certain base pair *structures* have unique photochemical properties. Of all possible GC structures the WC structure is the most energetically stable, but not by much (See Figure 12-3). Energetics alone, even when including entropy, do not suffice to explain the fact that this structure is special [35, 79]. Therefore it is conceivable that relative populations of different base pair structures in equilibrium were affected by their photochemical stabilities.

12.7. OUTLOOK

At least four directions suggest themselves in which these investigations can progress. (a) It is desirable to further probe dynamics at sub-picosecond time scales. Much of our understanding of excited state dynamics, so far, comes from theoretical models and from inferences from indirect experimental evidence. A number of femtosecond pump-probe and time resolved photoelectron experiments have already been reported, but most are hampered by the fact that at those time scales spectral bandwidth is large. Structure selective time resolved experiments by double resonance, currently under way in our laboratory, will be one way to address that issue. (b) The field of study of DNA base pair dynamics has been driven by simultaneous progress in both experimental and computational technique. As this trend continues we may expect studies of larger systems, such as not only nucleosides, which have already been reported, but also nucleotides and even oligonucleotides. A practical problem posed by the latter to experimentalists is the charge on the phosphate groups, suggesting either studies of ions, rather than neutrals or studies of alternate structures, such as peptide nucleic acid (PNA). (c) Microhydration in the form of clusters with water constitutes a bridge between the gas phase and the solution phase. Mass resolved water cluster spectroscopy offers the opportunity to probe the role of the solvent essentially one successively added water molecule at a time. To date work has been reported with small numbers of water molecules and we may expect extensions to numbers that will make the system approach bulk properties. (d) In spite of impressive achievements in recent years, there still is ample room for increases in spectral resolution. At this time it is probably fair to state that computational resolution in many cases still lacks experiment. Considerable further progress may be expected from continued coordinated computational and experimental efforts.

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REFERENCES

1. Geyer CR, Battersby TR, Benner SA (2003) Structure 11: 1485–1498.
2. Weiss S (1999) Science (USA) 283:1676–1683.
3. Nir E, Grace LI, Brauer B, de Vries MS (1999) J Am Chem Soc 121:4896–4897.
4. Arrowsmith P, de Vries MS, Hunziker HE, Wendt HR (1988) Appl Phys B 46:165–173.
5. Meijer G, de Vries MS, Hunziker HE, Wendt HR (1990) Applied Physics B 51:395–403.
6. Gidden J, Bushnell JE, Bowers MT (2001) J Am Chem Soc 123:5610–5611.
7. Wyttenbach T, Bowers MT (2003) Gas Phase Conformations: The Ion Mobility/Ion Chromatography Method. In: Topics in Current Chemistry, Schalley CA (ed) Springer, Berlin, Top Curr Chem 225:207–232.
8. Pribble RN, Zwier TS (1994) Faraday Discuss 97:229–241.
9. Pribble RN, Garrett AW, Haber K, Zwier TS (1995) J Chem Phys 103:531–544.
10. Dian BC, Florio GM, Clarkson JR, Longarte A, Zwier TS (2004) J Chem Phys 120:9033–9046.
11. Kim W, Schaeffer MW, Lee S, Chung JS, Felker PM (1999) J Chem Phys 110:11264–11276.
12. Schmitt M, Muller H, Kleinermanns K (1994) Chem Phys Lett 218:246–248.
13. Luhrs DC, Viallon J, Fischer I (2001) Phys Chem Chem Phys 3:1827–1831.
14. Kang H, Lee KT, Kim SK (2002) Chem Phys Lett 359:213–219.
15. Kang H, Jung B, Kim SK (2003) J Chem Phys 118:6717–6719.
16. Canuel C, Mons M, Piuzzi F, Tardivel B, Dimicoli I, Elhanine M (2005) J Chem Phys 122:7.
17. Douhal A, Kim SK, Zewail AH (1995) Nature (UK) 378:260–263.
18. Schultz T, Samoylova E, Radloff W, Hertel IV, Sobolewski AL, Domcke W (2004) Science (USA) 306:1765–1768.
19. Peon J, Zewail AH (2001) Chem Phys Lett 348:255–262.
20. Pal SK, Peon J, Zewail AH (2002) Chem Phys Lett 363:57–63.
21. Pecourt JML, Peon J, Kohler B (2000) J Am Chem Soc 122:9348–9349.
22. Pecourt JML, Peon J, Kohler B (2001) J Am Chem Soc 123:10370–10378.
23. Crespo-Hernandez CE, Cohen B, Hare PM, Kohler B (2004) Chem Rev 104:1977–2019.
24. Crespo-Hernandez CE, Cohen B, Kohler B (2005) Nature (UK) 436:1141–1144.
25. Yanson IK, Teplitsky AB, Sukhodub LF (1979) Biopolymers 18:1149–1170.
26. Nir E, Kleinermanns K, de Vries MS (2000) Nature (UK) 408:949–951.
27. Fricke H, Gerlach A, Gerhards M (2006) Phys Chem Chem Phys 8:1660–1662.
28. Gerhards M (2004) Opt Commun 241:493–497.
29. Chaban GM, Jung JO, Gerber RB (1999) J Chem Phys 111:1823–1829.
30. Brauer B, Gerber RB, Kabelac M, Hobza P, Bakker JM, Riziq AGA, de Vries MS (2005) J Phys Chem A 109:6974–6984.
31. Bakker JM, Compagnon I, Meijer G, von Helden G, Kabelac M, Hobza P, de Vries MS (2004) Phys Chem Chem Phys 6:2810–2815.
32. Reha D, Valdes H, Vondrasek J, Hobza P, Abu-Riziq A, Crews B, de Vries MS (2005) Chem-Eur J 11:6803–6817.
33. Hobza P, Sponer J (2002) J Am Chem Soc 124:11802–11808.
34. Jurecka P, Hobza P (2003) J Am Chem Soc 125:15608–15613.
35. Sponer J, Jurecka P, Hobza P (2004) J Am Chem Soc 126:10142–10151.
36. Dabkowska I, Gonzalez HV, Jurecka P, Hobza P (2005) J Phys Chem A 109:1131–1136.
37. Sponer J, Leszczynski J, Hobza P (2001) Biopolymers 61:3–31.
38. Kabelac M, Ryjacek F, Hobza P (2000) Phys Chem Chem Phys 2:4906–4909.
39. Hanus M, Kabelac M, Rejnek J, Ryjacek F, Hobza P (2004) J Phys Chem B 108:2087–2097.
40. Kabelac M, Hobza P (2001) J Physl Chem B 105:5804–5817.
41. Plutzer C, Hunig I, Kleinermanns K, Nir E, de Vries MS (2003) ChemPhysChem 4:838–842.

42. Abo-Riziq A, Grace L, Nir E, Kabelac M, Hobza P, de Vries MS (2005) Proc Natl Acad Sci USA 102:20–23.
43. Plutzer C, Nir E, de Vries MS, Kleinermanns K (2001) Phys Chem Chem Phys 3:5466–5469.
44. Mons M, Piuzzi F, Dimicoli I, Gorb L, Leszczynski J (2006) J Phys Chem A 110:10921–10924.
45. Nir E, Hunig I, Kleinermanns K, de Vries MS (2004) ChemPhysChem 5:131–137.
46. Abo-Riziq A, Crews B, Grace L, de Vries MS (2005) J Am Chem Soc 127:2374–2375.
47. Nir E, Janzen C, Imhof P, Kleinermanns K, de Vries MS (2002) Phys Chem Chem Phys 4:740–750.
48. Nir E, Hunig I, Kleinermanns K, de Vries MS (2003) Phys Chem Chem Phys 5:4780–4785.
49. Choi MY, Miller RE (2006) J Am Chem Soc 128:7320–7328.
50. Choi MY, Douberly GE, Falconer TM, Lewis WK, Lindsay CM, Merritt JM, Stiles PL, Miller RE (2006) Int Rev Phys Chem 25:15–75.
51. Choi MY, Dong F, Miller RE (2005) Philos T Roy Soc A 363:393–412.
52. He YG, Wu CY, Kong W (2004) J Phys Chem A (USA) 108:943–949.
53. Broo A, Holmen A (1996) Chem Phys 211:147–161.
54. Seefeld KA, Plutzer C, Lowenich D, Haber T, Linder R, Kleinermanns K, Tatchen J, Marian CM (2005) Phys Chem Chem Phys 7:3021–3026.
55. Tomic K, Tatchen J, Marian CM (2005) J Phys Chem A 109:8410–8418.
56. Marian CM (2005) J Chem Phys 122:10.
57. Sobolewski AL, Domcke W (1999) Chem Phys Lett 315:293.
58. Sobolewski AL, Domcke W, Dedonder-Lardeux C, Jouvet C (2002) Phys Chem Chem Phys 4:1093–1100.
59. Sobolewski AL, Domcke W (2000) Chem Phys 259:181–191.
60. Sobolewski AL, Domcke W (1999) Chem Phys Lett 300:533–539.
61. Perun S, Sobolewski AL, Domcke W (2005) J Am Chem Soc 127:6257–6265.
62. Sobolewski AL, Domcke W (2006) Phys Chem Chem Phys 8:3410–3417.
63. Perun S, Sobolewski AL, Domcke W (2006) J Phys Chem A 110:9031–9038.
64. Sobolewski AL, Domcke W (2002) Eur Phys J D 20:369–374.
65. Hünig I, Plutzer C, Seefeld KA, Lowenich D, Nispel M, Kleinermanns K (2004) ChemPhysChem 5:1427–1431.
66. Zierhut M, Roth W, Fischer I (2004) Phys Chem Chem Phys 6:5178–5183.
67. Kim NJ, Kang H, Jeong G, Kim YS, Lee KT, Kim SK (2000) J Phys Chem A 104:6552–6557.
68. Kistler KA, Matsika S (2007) Journal of Physical Chemistry A 111: 8708–8716.
69. Nir E, Muller M, Grace LI, de Vries MS (2002) Chem Phys Lett 355:59–64.
70. Sobolewski AL, Domcke W (2004) Phys Chem Chem Phys 6:2763–2771.
71. Sobolewski AL, Domcke W, Hättig C (2005) Proc Natl Acad Sci USA 102:17903–17906.
72. Sobolewski AL, Domcke W (2006) ChemPhysChem 7:561–564.
73. Daniels M, Hauswirt.W (1971) Science (USA) 171:675-&.
74. Callis PR (1983) Annu Rev Phys Chem 34:329–357.
75. Kang HY, Jung BY, Kim SK (2003) J Chem Phys 118:11336–11336.
76. Broo A (1998) J Phys Chem A 102:526–531.
77. Ehrenfreund P (2006) Abstr Pap Am Chem Soc 231: 38–38.
78. Pizzarello S (2004) Abstr Pap Am Chem Soc 228:U695–U695.
79. Abo-Riziq A, Grace L, Nir E, Kabelác M, Hobza P, de Vries MS (2005) Proc Natl Acad Sci USA 102:20–23.