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## DNA photoionization: from high to low energies

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1 3. DNA photoionization: from high to low energies

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13 ABSTRACT

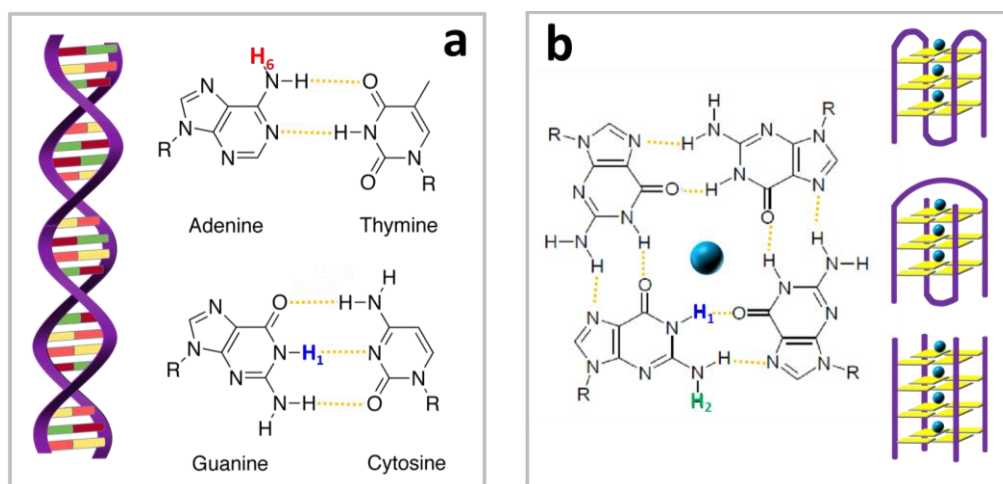
14 This Chapter discusses DNA photoionization in aqueous solution resulting from direct  
15 absorption of ultraviolet radiation. While DNA photoionization at wavelengths shorter  
16 than 200 nm was reported in the 1990s, recent studies showed that it also takes place  
17 at much longer wavelengths, with efficiencies depending strongly on the secondary  
18 structure. The quantum yield of one-photon ionization determined for duplex genomic  
19 DNA at 266 nm is  $2 \times 10^{-3}$  and significantly higher, reaching  $10^{-2}$ , for guanine  
20 quadruplexes. The transient species issued from photoionization are studied by  
21 nanosecond flash photolysis from  $\sim 30$  nanoseconds to 300 milliseconds. At this time-  
22 window, the ejected electrons are hydrated and the radicals are located on guanines  
23 or adenines. The quasi entire population of radical cations in genomic DNA undergoes  
24 deprotonation within 2  $\mu$ s. Deprotonation in guanine quadruplexes is a highly  
25 anisotropic process, taking place from 30 ns to over 50  $\mu$ s.

26

27 **3.1 Introduction**

28 Absorption of one or more UV photons directly by DNA may lead to its ionization: an  
 29 electron is ejected generating an electron hole (radical cation) on the nucleic acid<sup>1-3</sup>.  
 30 Subsequently, these primary species undergo a cascade of chemical transformations,  
 31 which may ultimately damage DNA<sup>4-6</sup>. The transient species issued from the  
 32 photoionization of various DNA systems, spanning from isolated bases in the gas  
 33 phase to genomic DNA in aqueous solution, are studied by spectroscopic techniques  
 34 using a large range of excitation wavelengths and detection methods.

35 Water molecules, which are key structural elements of DNA, are known to affect the  
 36 photoionization process<sup>7, 8</sup> and interact with both electrons and radicals<sup>9</sup>. Therefore,  
 37 this Chapter is dedicated to the photoionization of aqueous DNA solutions.



38  
 39 **Figure 1.** DNA structures whose low-energy photoionization has been studied<sup>3</sup>. (a) Duplexes composed  
 40 of adenine-thymine and/or guanine cytosine base-pairs. (b) G-quadruplexes, characterized by vertical  
 41 stacking of guanine tetrads (in yellow); they are formed by folding of a single DNA strand  
 42 (monomolecular), association of two single strands (bimolecular) or association of four single strands  
 43 (tetramolecular) in aqueous solution containing either Na<sup>+</sup> or K<sup>+</sup> cations (blue spheres). The phosphate  
 44 deoxyribose backbone is indicated in violet. For simplicity, nucleobases at the loops, joining the guanine  
 45 tetrads, and the ending groups have been omitted in (b). The red, blue and green protons are discussed  
 46 in Section 3.5 in respect to the deprotonation of radical cations.

47

48 In a first part (Section 3.2), we examine the mechanisms underlying one-photon  
49 ionization, because they are potentially involved in the damage provoked by the solar  
50 light, in contrast to multiphoton ionization, attained by intense lasers. We discuss the  
51 energies associated in this process, determined by photoelectron spectroscopy, and  
52 introduce the quantum yield  $\phi_i$ , representing the probability of an ionization event per  
53 absorbed photon, determined by nanosecond flash photolysis. Those at high energy,  
54 6.42 - 6.20 eV (193 - 200 nm), were mainly reported about 30 years ago<sup>10-12</sup>. More  
55 recently, a series of studies in our laboratory evidenced that, in the case of duplexes  
56 and guanine quadruplexes (G-quadruplexes), represented schematically in Figure 1,  
57 one-photon ionization is also operative at lower energies<sup>3, 13</sup> and determined  $\phi_i$  values  
58 at 266 nm (4.66 eV). We also discuss the factors indicating that low-energy  
59 photoionization occurs via a different mechanism (indirect) from that involved in the  
60 high-energy process (direct).

61 In a subsequent section (3.3), we focus on the nanosecond flash photolysis. This time-  
62 resolved spectroscopic technique, in addition to the determination of  $\phi_i$  mentioned  
63 above, allows the study of the transient species stemming from ionization. The latter  
64 are identified by their absorption spectra and quantified with respect to the number of  
65 absorbed photons. Their evolution is followed from the nanosecond to the millisecond  
66 time-scales, providing precious indications about possible reaction paths.

67 After examining the fate of ejected electrons (Section 3.4), we focus on purine radicals.  
68 Guanine is the nucleobase with the lowest oxidation potential<sup>14</sup>. Consequently,  
69 following a charge migration process<sup>15-19</sup>, the electron hole finds itself on a guanine  
70 site. Nevertheless, in absence of guanines, the electron hole is trapped by adenines<sup>13,</sup>  
71 <sup>20</sup>. In Section 3.5, we present the transformations that the adenine and guanine

72 radicals, stemming from photoionization, undergo during the time. We present the  
73 absorption spectra of the transient species and discuss changes observed when going  
74 from monomeric radicals to those in duplexes and G-quadruplexes. We show how the  
75 populations of various radical species at a given time are determined in respect to the  
76 ejected electrons. We also stress the anisotropic nature of the reactions taking place  
77 in DNA; for this reason, photoionization studies give a more reliable picture of the  
78 “intrinsic” radical reaction dynamics compared to methods using external oxidants,  
79 such as photosensitized electron abstraction<sup>21</sup>.

80 Finally, we evoke the final lesions potentially resulting from photoionization (Section  
81 3.6). In fact, the studies presented in this Chapter do not allow their characterization,  
82 which requires analytical chemistry methods. However, they provide information  
83 regarding their extent as well as the type of radicals responsible for them. These  
84 studies also explain the oxidative damage observed upon absorption of UVB/UVA  
85 radiation directly by DNA<sup>22, 23</sup>.

## 86 **3.2 Energies and quantum yields**

### 87 **3.2.1 Direct high-energy photoionization**

88 Direct photoionization takes place when the photon energy is sufficiently high to  
89 detach an electron from the molecule; this energy corresponds to the ionization  
90 potential. Experimental ionization potentials ( $IP_{\text{exp}}$ ) of DNA/RNA components in  
91 aqueous environment were reported only recently<sup>24, 25</sup>. They were determined for  
92 liquid jets combining synchrotron radiation and photoelectron spectroscopy.  
93 Photoelectron spectra were recorded between 6 and 10 eV and  $IP_{\text{exp}}$  values were  
94 derived from the band peaks. In parallel, IP were determined by quantum chemistry  
95 methods<sup>8</sup>. These theoretical studies showed that the  $IP_{\text{exp}}$  match the so-called Vertical

96 Ionization Potential (VIP) (Table 1), which correspond to electron detachment prior to  
 97 any geometrical modification of the molecule accompanying the excited state  
 98 relaxation. Computations also found that base-pairing and base-stacking have only a  
 99 weak effect on VIPs, in line with the experimental finding that the photoelectron  
 100 spectrum obtained for herring sperm DNA resembles that of an equimolar of the four  
 101 constitutive mononucleotides<sup>26</sup>.

102 **Table 1.** *Experimental Ionization Potentials ( $IP_{exp}$ ) and computed lower Vertical*  
 103 *Ionization Potentials (VIP) in eV, determined for DNA/RNA components in water by*  
 104 *photoelectron spectroscopy and quantum chemistry calculations, respectively<sup>25</sup>.*

	ribose	deoxyribose	dT	TMP	Cyt	CMP	Ado	dAMP <sup>-</sup>	Guo	dGMP <sup>-</sup>
$IP_{exp}$	9.4	9.4	-	8.1	8.1	-	7.6	7.7	7.3	-
VIP	9.2	9.1	7.9	7.8	7.8	7.8	7.7	7.7	7.4	7.1

105  
 106 DNA photoionization in solution is still observable at energies somewhat lower than  
 107 the values in Table 1, where flash photolysis measurements are possible (Figure 2).  
 108 Selected one-photon ionization quantum yields determined by this method with  
 109 excitation at 193 nm (6.42 eV) are presented in Table 2.

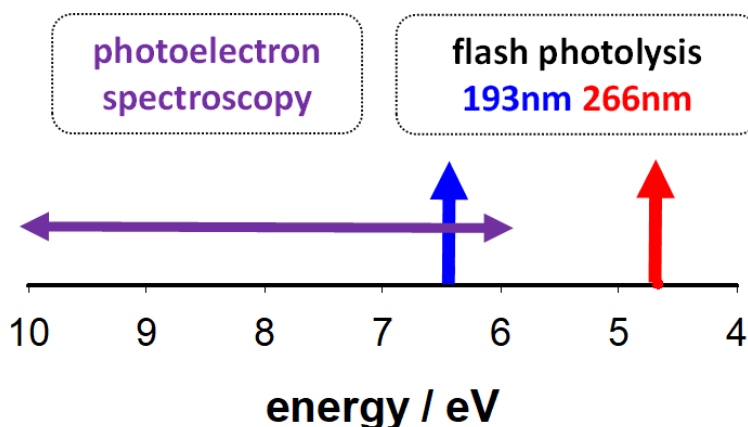
110 **Table 2.** *One-photon ionization quantum yields ( $\phi_i \times 10^3$ ) determined at 193 nm (6.42*  
 111 *eV) for aqueous solutions.*

dT	dC	dA	dG/dGMP	CT-DNA <sup>a</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> /HPO <sub>4</sub> <sup>2-</sup>	H <sub>2</sub> O
55 <sup>11</sup>	17 <sup>11</sup>	24 <sup>11</sup>	73/70 <sup>11</sup>	36 <sup>1</sup> /58 <sup>2</sup>	330/520 <sup>11</sup>	13 <sup>27</sup>

112 a) *calf thymus DNA in water<sup>1</sup> and NaClO<sub>4</sub> aqueous solution<sup>2</sup>*

113  
 114 As found by photoelectron spectroscopy, the  $\phi_i$  determined for duplex genomic DNA  
 115 corresponds roughly to the average of the  $\phi_i$  values found for its monomeric  
 116 constituents suggesting that the same direct photoionization mechanism is operative.

117



118

119

120 **Figure 2.** Energies corresponding to experimental studies of one-photon ionization of DNA in water:  
 121 photoelectron spectra recorded between 10 and 6 eV (violet region); nanosecond flash photolysis  
 122 measurements with excitation at 193 nm (blue arrow) and 266 nm (red arrow).

123 **3.2.2 Indirect low-energy photoionization**

124 The  $\phi_i$  values determined with excitation at 266 nm, are more than one order of  
 125 magnitude smaller (Tables 3-5) than those obtained at 193 nm (Table 2). In contrast  
 126 to what is observed for high-energy photoionization, the low-energy process strongly  
 127 depends on the DNA secondary structure. While it is not detectable for  
 128 mononucleotides and poorly stacked single strands (Table 3), the  $\phi_i$  may be up to 30  
 129 times higher for well-structured DNA multimers.

130 **Table 3.** One-photon ionization quantum yields ( $\phi_i \times 10^3$ ) determined at 266 nm / 4.66  
 131 eV for DNA single strands in phosphate buffer.

(A) <sub>20</sub>	(T) <sub>20</sub>	5'-TTAGGG-3'	S1 <sup>a)</sup>
1.1 ± 0.1 <sup>28</sup>	<0.5 <sup>13</sup>	<0.3 <sup>29</sup>	1.1 ± 0.3 <sup>21</sup>

132 a) S1: 5'-CGTACTCTTTGGTGGGTCGGTCTTTCTAT-3'

133

134 Model duplexes containing twenty base-pairs of the same type in a repetitive  
 135 sequence (homopolymeric adenine-thymine, alternating adenine-thymine or



136 alternating guanine-cytosine), exhibit quite similar  $\phi_i$  values,  $1.0 \times 10^{-3}$  -  $1.5 \times 10^{-3}$  (Table  
 137 4). If both types of base-pairs are present in a random sequence the  $\phi_i$  increases by  
 138 ca. 40% and remains practically the same, when going from a duplex with 30 base-  
 139 pairs to a very long genomic DNA ( $2.0 \times 10^{-3}$ ). This suggests that rather the base  
 140 sequence than the duplex size is a decisive parameter, as it will be discussed later.

141 **Table 4.** One-photon ionization quantum yields ( $\phi_i \times 10^3$ ) determined at 266 nm / 4.66  
 142 eV for DNA duplexes in phosphate buffer.

(A) <sub>20</sub> •(T) <sub>20</sub>	(AT) <sub>10</sub> •(AT) <sub>10</sub>	(GC) <sub>5</sub> •(GC) <sub>5</sub>	S1•S2 <sup>a)</sup>	CT-DNA <sup>b)</sup>
1.4 ± 0.1 <sup>28</sup>	1.1 ± 0.1 <sup>20</sup>	1.2 ± 0.2 <sup>30</sup>	2.1 ± 0.4 <sup>21</sup>	2.0 ± 0.2 <sup>3</sup>

143 <sup>a)</sup> S1: 5'-CGTACTCTTTGGTGGGTCGGTCTTTCTAT-3'; S2: 3'-  
 144 GCATGAGAAACCACCCAGCCAAGAAAGATA-5'; <sup>b)</sup> CT-DNA: calf thymus DNA.

145

146 The propensity of G-quadruplexes to undergo low-energy photoionization is  
 147 significantly larger compared to duplexes: not only their  $\phi_i$  values are higher but also  
 148 exhibit a more important dispersion, varying from  $3.5 \times 10^{-3}$  to  $9.8 \times 10^{-3}$  (Table 5). Such  
 149 a dispersion arises from their structural diversity. Despite their common feature of  
 150 vertically stacked guanine tetrads (Figure 1b), they contain additional loops joining the  
 151 tetrads and/or dangling ends, whose length and/or base sequence vary from one  
 152 system to the other. In addition, the metal cations (Na<sup>+</sup> or K<sup>+</sup>) located in their central  
 153 cavity are constitutive elements of these structures, contributing to their stability.  
 154 Therefore, the type of cation is noted in their abbreviations in Table 5.

155 From the studies performed so far, it appears that the number of tetrads composing  
 156 the G-quadruplex core does not play a role on their capacity to photo-eject an electron.  
 157 For example, the  $\phi_i$  of (TG<sub>4</sub>T)<sub>4</sub>/Na<sup>+</sup> with four tetrads ( $3.5 \times 10^{-3}$ ) is lower than that of  
 158 TEL25/Na<sup>+</sup>, characterized by three tetrads ( $5.2 \times 10^{-3}$ ). The same lack of correlation is  
 159 observed in Table 5 between the  $\phi_i$  values and the molecularity of the four-stranded

160 structure, *i.e.* the number of DNA strands that are associated together (Figure 1b). A  
 161 small decrease of  $\phi_i$  is detected when the dangling groups TA at the 5' end and TT at  
 162 the 3' end are removed from the telomeric sequence ( $4.5 \times 10^{-3}$  for TEL21/Na<sup>+</sup> vs  $5.2$   
 163  $\times 10^{-3}$  for TEL25/Na<sup>+</sup>).

164 In contrast to the structural parameters mentioned above, the metal cations located in  
 165 the central cavity of G-quadruplexes have a strong influence on their photoionization:  
 166 for all the examined sequences the presence of K<sup>+</sup> leads to higher  $\phi_i$  values compared  
 167 to Na<sup>+31</sup>. This behaviour is not encountered for duplex genomic DNA, whose  
 168  $\phi_i$  remains the same when Na<sup>+</sup> ions are replaced by K<sup>+</sup> ions in the buffer in which it is  
 169 dissolved<sup>3</sup>.

170 **Table 5.** One-photon ionization quantum yields ( $\phi_i$ ) determined at 266 nm / 4.66 eV  
 171 for G-quadruplexes in phosphate buffer containing either Na<sup>+</sup> or K<sup>+</sup> cations.

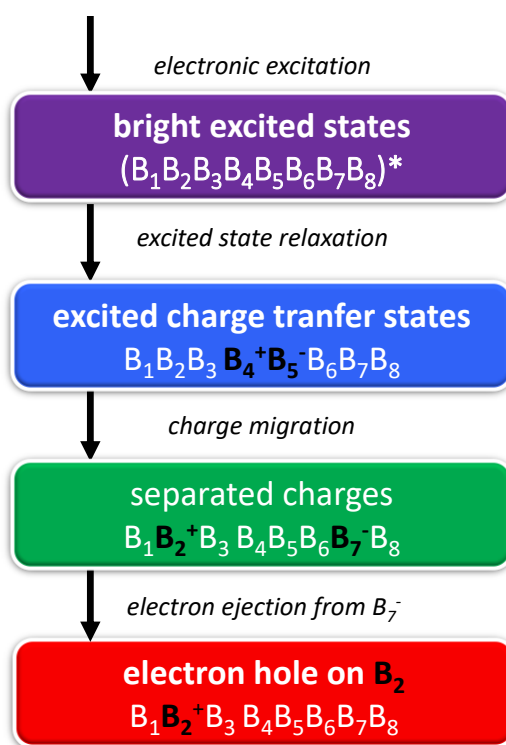
Type (Figure 1)	sequence	system	$\phi_i \times 10^3$	reference
Monomolecular	TAGGG(TTAGGG) <sub>3</sub> TT	TEL25/Na <sup>+a)</sup>	$5.2 \pm 0.3$	21
		TEL21/Na <sup>+ a)</sup>	$4.5 \pm 0.6$	29
	GGG(TTAGGG) <sub>3</sub>	TEL21/K <sup>+ a)</sup>	$9.4 \pm 0.1$	32
Bimolecular	GGGGTTTTGGGG	OXY/Na <sup>+ b)</sup>	$6.0 \pm 0.2$	3
		OXY/K <sup>+ b)</sup>	$7.9 \pm 0.1$	3
Tetramolecular	TGGGGT	(TG <sub>4</sub> T) <sub>4</sub> /Na <sup>+</sup>	$3.5 \pm 0.5$	33
		(TG <sub>4</sub> T) <sub>4</sub> /K <sup>+</sup>	$8.1 \pm 0.5$	31

172 <sup>a)</sup> TEL: containing the human telomeric repeat TTAGGG <sup>b)</sup>OXY: containing the oxytricha nova  
 173 telomeric repeat TTTTGGGG

174

175 The ensemble of the results obtained for low-energy photoionization point toward a  
 176 mechanism different than that underlying the high-energy process discussed in

177 previous section. This is further supported by quantum chemistry calculations  
 178 performed for the tetramolecular G-quadruplexes  $(TG_4T)_4/Na^+$  and  $(TG_4T)_4/K^+$ : the  
 179 VIPs, computed for these two systems using the same computational method, differ  
 180 less than 3%<sup>31</sup> while the their  $\phi_i$  values vary by a factor of two (Table 5). On the basis  
 181 of these studies, combined with the knowledge accumulated since the beginning of  
 182 the 21<sup>st</sup> century on the relaxation of electronic excited states and charge transport in  
 183 DNA (see Chapter 5), a complex indirect mechanism has been proposed to explain  
 184 low-energy photoionization of DNA.



185

186 **Figure 3.** Successive steps potentially leading to DNA photoionization at low-energies.  $B_i$  designate  
 187 stacked nucleobases.

188

189 The main steps of the proposed mechanism are schematically depicted in Figure 3.

190 Initially, photon absorption populates excited states which may be delocalized over a

191 few nucleobases. Very rapidly, an important part of the excited state population

192 evolves toward excited charge transfer states, in which negative and positive charges

193 are located on adjacent stacked nucleobases. These “charged” nucleobases normally  
194 undergo a geometrical rearrangement, including modification in their solvation, so that  
195 to minimize their energy, and, subsequently, they recombine to the ground state.  
196 However, it is possible that, prior to such a modification, a small part of the excited  
197 charge transfer states undergoes charge separation<sup>34</sup>. As the VIP of “anionic”  
198 nucleobases is lower than that of “neutral” ones<sup>35</sup>, electron ejection may take place  
199 from the negatively charged moiety under the effect of conformational motions, leaving  
200 an electron hole at some other part of the system.

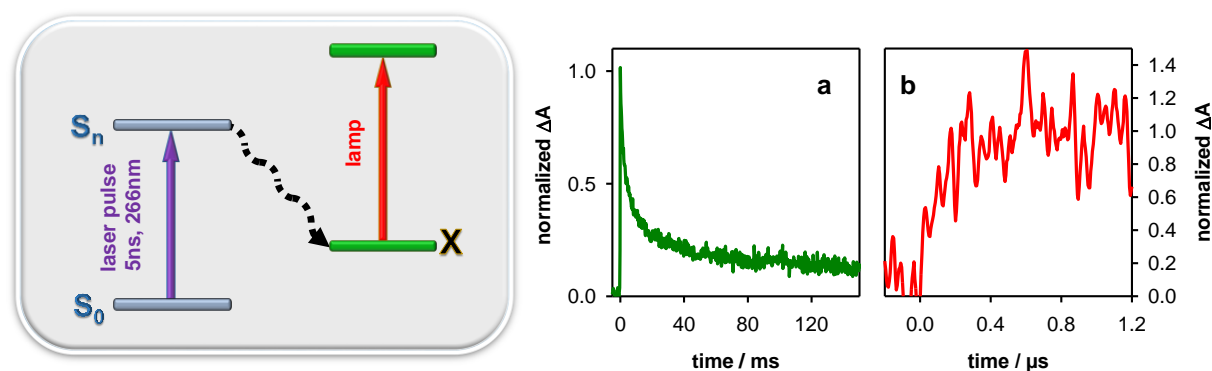
201 A key step of this mechanism is charge migration leading to charge separation<sup>34</sup>. The  
202 higher propensity of G-quadruplexes to undergo low-energy photoionization compared  
203 to duplexes is attributed to trapping of the positive charge by the guanine core,  
204 potentially accompanied by charge delocalization<sup>36, 37</sup>, while the negative charge may  
205 be located on a nucleobase of a loop or an ending group. The presence of Na<sup>+</sup> ions, which are smaller and more mobile than K<sup>+</sup> ions, favour geometrical  
206 cavity of Na<sup>+</sup> ions, which are smaller and more mobile than K<sup>+</sup> ions, favour geometrical  
207 stabilization of the excited charge transfer states and, consequently, charge migration  
208 to neighbouring nucleobases becomes less effective, leading to lower  $\phi_i$  values.

209 Although duplexes are devoid of such distinct structural elements, GG or GGG steps  
210 are known to behave as traps for electron holes<sup>38, 39</sup>, the charge separation being  
211 ensured by conformational motions. Thus, it is understandable that S1:S2 and calf  
212 thymus DNA, which contain such traps, have higher  $\phi_i$  values than duplexes with  
213 simpler repetitive base sequence (Table 4).

### 214 **3.3 Nanosecond flash photolysis: advantages and limitations**

215 Before discussing the evolution of various transient species issued from  
216 photoionization, we present the basic concept of such measurements. Although the

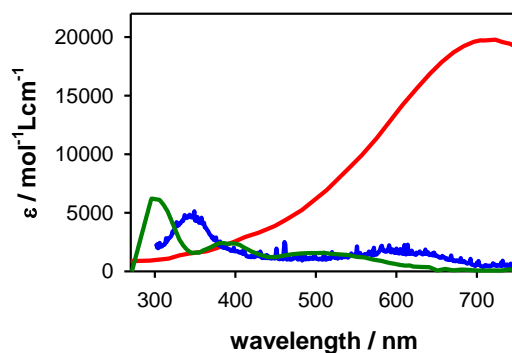
217 general lines employed for experiments with low-energy excitation were inspired from  
 218 older studies with high-energy excitation, we put emphasis on the more recent ones,  
 219 which profited from technical and methodological improvements and allowed the study  
 220 of several duplexes and G-quadruplexes.



221  
 222 **Figure 4.** Left panel: schematic representation of flash photolysis measurements. Right panel:  
 223 examples of transient absorption signals obtained for TEL25/Na<sup>+</sup> at 500 nm<sup>21</sup> (a; decay) and TEL21/K<sup>+</sup>  
 224 at 620 nm<sup>32</sup> (b; rise).

225  
 226 Flash photolysis is a time-resolved absorption technique (Figure 4). DNA is excited by  
 227 a nanosecond laser pulse. Absorption of the laser photons leads, among others, to the  
 228 formation of a transient species X. Its presence in the solution is probed with the help  
 229 of a lamp allowing the determination of differential absorbance, corresponding the  
 230 difference in the absorbance before and after the excitation:  $\Delta A = A_{\text{after}} - A_{\text{before}}$ . As X  
 231 is unstable, its  $\Delta A$  decreases with the time (Figure 4a). If, instead, X is formed during  
 232 the time-window of the observation, a rise is detected (Figure 4b).

233



234

235 **Figure 5.** Comparison of the absorption spectra of ejected electrons (red;  $e_{\text{hyd}}^-$ <sup>40</sup>) and the corresponding  
 236 holes on adenine (blue;  $dA^{28}$ ) and guanine (green:  $dGMP^{41}$ ) moieties.

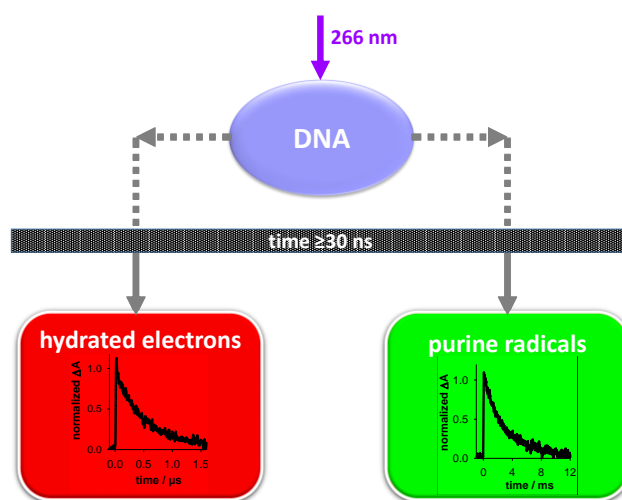
237

238 Although several transient species may co-exist in photoexcited DNA, the knowledge  
 239 of their absorption spectra helps disentangling them. It is well-established that  
 240 electrons ejected in aqueous solution become hydrated within a few picoseconds<sup>42</sup>;  
 241 the spectrum of hydrated electrons  $e_{\text{hyd}}^-$  is characterized by a large absorption band  
 242 peaking at 720 nm (Figure 5)<sup>40</sup> and extending all over the whole visible spectral  
 243 domain. The absorption of radical cations located on adenines or guanines, whose  
 244 spectra are also shown in Figure 5, is very weak compared to that of  $e_{\text{hyd}}^-$ . Their  
 245 relatively intense UV bands cannot be exploited because they may interfere with the  
 246 absorption of both DNA<sup>28, 29</sup> and dimeric photoproducts<sup>20, 43, 44</sup> (see Chapter 2).  
 247 Consequently, the radical cations and the subsequent deprotonated radicals (see  
 248 Section 3.5.1) can be properly detected only when  $e_{\text{hyd}}^-$  have decayed.

249 Figure 6 illustrates the time-domains on which nanosecond flash photolysis provides  
 250 information about the transient species issued from photoionization. Due to a time-  
 251 resolution of 30 ns, all the steps related with complex mechanism depicted in Figure  
 252 3 are not accessible. Their direct observation, which could unambiguously validate the  
 253 proposed mechanism, is a real challenge. As a matter of fact, femtosecond setups,

254 which have appropriate time-resolution, experience difficulties in detecting transient  
 255 species corresponding to only  $10^{-3}$  of the excited state population.

256 The  $\Delta A$  signals determined around 700 nm provide the lifetime of  $e_{\text{hyd}}^-$ , which, under  
 257 the conditions used in these experiments, disappear within a few microseconds  
 258 (Figure 6 in red). However, their decays become faster in presence of efficient  
 259 scavengers ( $\text{O}_2$ ,  $\text{NO}_3$ ,  $\text{N}_2\text{O}\dots$ ), rendering possible the study of nucleobase radicals  
 260 over a larger time window (see for example Figure 4b). We note that, because of  
 261 technical reasons, the sensitivity of the method is smaller at shorter times. The  
 262 nucleobase radicals decay completely on the millisecond time-scale (Figure 6 in  
 263 green).



264

265 **Figure 6.** Schematic illustration of the time-domains on which nanosecond flash photolysis provides  
 266 information about the transient species issued from DNA photoionization. As examples are given the  
 267 decays of  $e_{\text{hyd}}^-$  and adenine radicals determined for alternating adenine-thymine duplexes<sup>20</sup>.

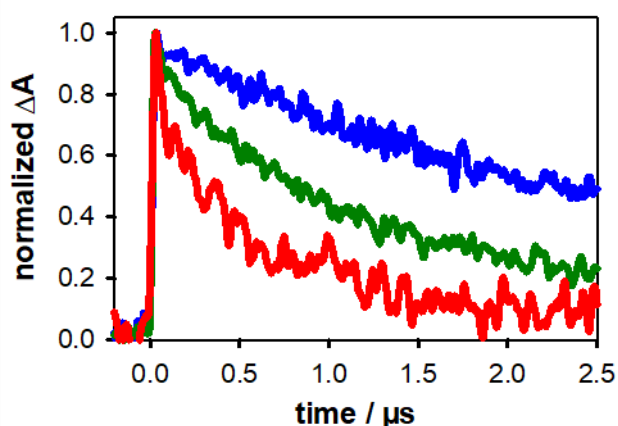
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269 These experiments are not only performed with low-energy excitation but also using  
 270 low-intensity laser pulses to circumvent saturation effects, as explained in detail in  
 271 reference<sup>3</sup>. As a result, the transient absorption signals are very weak, requiring long  
 272 measurements in order to reduce the signal-to-noise ratio. In addition, frequent

273 replacement of the DNA solutions is indispensable so that to avoid exciting the DNA  
 274 that has been already damaged. This condition, associated to the importance of using  
 275 purified nucleic acids, makes such studies particularly long and expensive.

### 276 3.4 Evolution of ejected electrons

277 We already pointed out that the only form of electrons observable by nanosecond flash  
 278 photolysis is the hydrated one, which disappears within a few microseconds. In fact,  
 279 their lifetime depends on the molecules present in the solution. In most of the  
 280 experiments on low-energy photoionization, DNA is dissolved in phosphate buffer  
 281 composed of an equimolar mixture of  $\text{MH}_2\text{PO}_4$  and  $\text{M}_2\text{HPO}_4$  ( $\text{M} = \text{Na}^+$  or  $\text{K}^+$ ) in  
 282 concentrations of  $0.15 \text{ mol}\cdot\text{L}^{-1}$  each, which is four orders of magnitude higher than the  
 283 concentration of model DNA systems. It is well-known that  $\text{H}_2\text{PO}_4^-$  ions react with  $e_{\text{hyd}}^-$   
 284 ( $e_{\text{hyd}}^- + \text{H}_2\text{PO}_4^{2-} \rightarrow \text{H}^\bullet + \text{HPO}_4^{2-}$ <sup>45</sup>). This reaction takes indeed place in the case of the  
 285 reported experiments as attested by the variation of the  $e_{\text{hyd}}^-$  decay with the buffer  
 286 concentration (Figure 7).



287  
 288 **Figure 7.** Photoionization of CT-DNA: variation of the  $e_{\text{hyd}}^-$  decay with the concentration of  $\text{H}_2\text{PO}_4^-$  ions;  
 289  $0.15 \text{ mol}\cdot\text{L}^{-1}$  (red),  $0.015 \text{ mol}\cdot\text{L}^{-1}$  (green) and 0 (DNA dissolved in ultrapure water: blue).

290



291 The decays of  $e_{\text{hyd}}^-$  stemming from oligomeric DNA systems can be described by  
292 mono-exponential functions. The lifetimes derived from the fits are independent of the  
293 type of the studied system, depending only on the buffer concentration (0.5  $\mu\text{s}$  in usual  
294 conditions). Such a behaviour means that the reaction with  $\text{H}_2\text{PO}_4^-$  ions constitutes by  
295 far the dominant reaction path. Incidentally, the high concentration of the phosphate  
296 buffer used in these studies protects DNA from being attacked by  $e_{\text{hyd}}^-$  <sup>6</sup>.

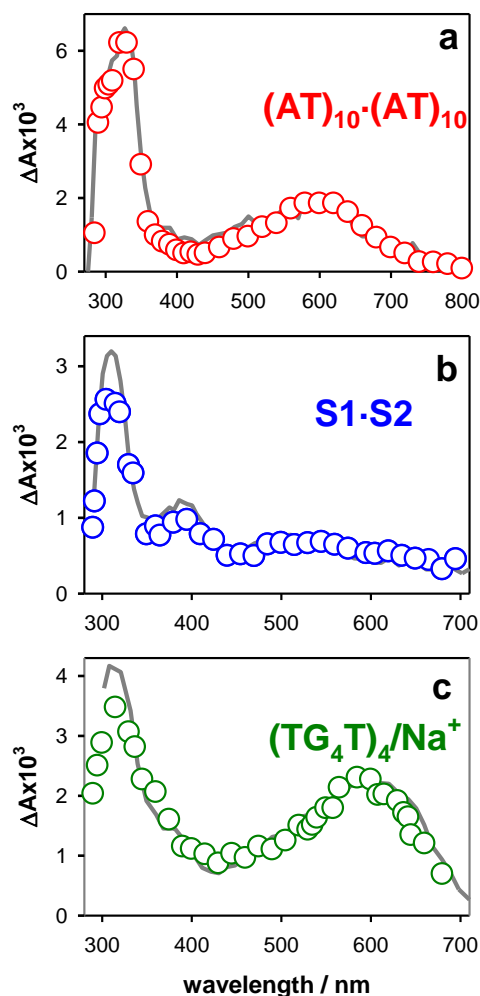
297 The situation changes when going from model systems to genomic DNA. In this case,  
298 the lifetime of  $e_{\text{hyd}}^-$  becomes significantly shorter than those observed for oligomeric  
299 structures<sup>3</sup>. In addition, it can no more be described by a mono-exponential functions.  
300 Hence, it was deduced that additional reaction paths, involving the DNA itself, are also  
301 operative. Their occurrence was explained by the flexibility of the very long natural  
302 macromolecule<sup>46</sup> which could facilitate the encounter between components of the  
303 nucleic acid and  $e_{\text{hyd}}^-$  that are generated in their vicinity.

### 304 **3.5. Evolution of purine radicals**

#### 305 **3.5.1 Deprotonation and tautomerization**

306 Adenine and guanine radical cations,  $(\text{A}^+)^\bullet$  and  $(\text{G}^+)^\bullet$ , are stronger acids than the  
307 parent nucleobases. Consequently, when they are generated in neutral aqueous  
308 solution they tend to lose a proton<sup>11</sup> (see Figure 1). For dAMP, the proton at position  
309 6 (H6) is transferred to the bulk water, giving rise to the deprotonated radical  $(\text{A-H6})^\bullet$ .  
310 In the case of dGMP, the loss of the proton at position 1 (H1) leads to the formation of  
311 the  $(\text{G-H1})^\bullet$  deprotonated radical. The spectra of these deprotonated radicals have  
312 been determined by high-energy photo-ionization and/or using oxidizing agents  
313 (Figures 8a and 8b)<sup>11, 41</sup>. They exhibit only subtle differences from those of the

314 corresponding radical cations (Figure 5), obtained in low temperature glasses<sup>17</sup> or in  
 315 pH 3<sup>14</sup>, where deprotonation is avoided.



316

317 **Figure 8.** Comparison of the transient absorption spectra obtained for DNA multimers (circles) with  
 318 those of monomeric deprotonated radicals (grey lines):  $(AT)_{10} \bullet (AT)_{10}$  duplex at  $200 \mu s$ <sup>20</sup> and  $(A-H6) \bullet$ <sup>11</sup>  
 319 (a);  $S1 \bullet S2$  duplex (see Table 4) at  $5 \mu s$ <sup>21</sup> and  $(G-H1) \bullet$ <sup>11</sup> (b);  $(TG_4T)_4 / Na^+$  G-quadruplex at  $50 \mu s$ <sup>33</sup> and  
 320  $(G-H2) \bullet$ <sup>47</sup>. The intensity of monomeric radical spectra has been arbitrarily normalized to that of the  
 321 corresponding multimer.

322

323 As the H6 proton of adenine is not perturbed by base-pairing, deprotonation in  
 324 duplexes is expected to lead also to  $(A-H6) \bullet$ . This is attested by the perfect overlap of

325 the spectra obtained for dAMP and (AT)<sub>10</sub>•(AT)<sub>10</sub> following high-energy<sup>11</sup> and low-  
326 energy photoionization<sup>20</sup>, respectively (Figure 8a).

327 Guanine deprotonation in duplexes and G-quadruplexes is more complex because the  
328 H1 proton is engaged in hydrogen bonding (Figure 1). In duplexes, the H1 proton may  
329 be transferred either to cytosine or to the bulk water<sup>1</sup>; quantum chemistry calculations  
330 on GC pairs showed that the absorption spectra of the resulting deprotonated radicals  
331 exhibit only weak differences<sup>31</sup>. A bigger spectral modification is observed if the H2  
332 proton is lost<sup>31</sup>. Absorption spectra of (G-H2)• deprotonated radicals were determined  
333 experimentally by flash photolysis and pulse radiolysis experiments on monomeric  
334 guanine derivatives<sup>41, 47</sup> (Figure 8c).

335 The transient absorption spectra recorded for both model duplexes<sup>21, 30</sup> and genomic  
336 DNA<sup>3</sup> by low-energy photoionization strongly resemble those of the monomeric (G-  
337 H1)• in the visible spectral domain (Figure 8b). It is possible that the radicals  
338 corresponding to H1 proton transferred to the cytosine were missed due the  
339 insufficient time-resolution and/or the poor sensitivity of the measurements.

340 A completely different picture emerged for deprotonation of guanine radical cations in  
341 G-quadruplexes. In agreement with the results obtained by photosensitized electron  
342 abstraction from these systems<sup>48</sup>, photoionization studies revealed the formation of  
343 (G-H2)• radicals<sup>3, 21, 29, 32, 33</sup> (Figure 8c). In most cases, (G<sup>+</sup>)• → (G-H2)• deprotonation  
344 is followed by (G-H2)• → (G-H1)• tautomerization<sup>3, 29, 33</sup>. These findings were  
345 rationalized by quantum chemistry calculations showing that (G-H2)• radicals in G-  
346 quadruplexes are more stable than (G-H1)•<sup>33</sup>. In addition, theoretical studies found  
347 that the general features of the guanine radical spectra are maintained within four-  
348 stranded structures<sup>49</sup>.

### 349 **3.5.2 Radical populations**

350 The spectral similarity of the various deprotonated radicals of purines in duplexes and  
351 G-quadruplexes with those determined for their monomeric analogues, allows their  
352 quantification. This is achieved via the Beer Lambert law, using the molar absorption  
353 coefficients  $\epsilon$  determined for the monomeric species in the visible spectral domain<sup>11</sup>.  
354 Moreover, the spectra recorded following photoionization of G-quadruplexes in pH 3  
355 match, in the 450-700 nm domain, the spectrum of monomeric (G<sup>+</sup>)• both in shape  
356 and in intensity<sup>3</sup>.

357 The quantification of the radical population in duplexes is straightforward, as only one  
358 type of deprotonated radical is detected in these systems at times  $\geq 3\mu\text{s}$  (Figures 8a  
359 and 8b). In contrast, two or three types of radicals may coexist in G-quadruplexes. In  
360 this case, the total radical population is determined considering that at 510-515 nm the  
361  $\epsilon$  of (G<sup>+</sup>)•, (G-H2)• and (G-H1)• is the same<sup>3</sup>. Subsequently, the transient absorption  
362 spectra of G-quadruplexes are reconstructed as a linear combinations of the  
363 corresponding monomeric spectra and the population of each type of radical is  
364 determined<sup>3, 32</sup>.

365 Following the above methodology, it was found that the radical population in all the  
366 examined duplexes and G-quadruplexes at 3  $\mu\text{s}$  equals that of the hydrated ejected  
367 electrons; the associated error, depending on the system, was estimated to be lower  
368 than 5%. A straightforward conclusion is that no major reaction besides deprotonation  
369 takes place before 3  $\mu\text{s}$ .

370

371

372 **3.5.3 Reaction dynamics**

373 The decays of the radical population in DNA multimers span over at least four orders  
374 of magnitude of time. Although they can be described by multi-exponential functions,  
375 it is not appropriate to assign the time-constants derived from such fits to specific  
376 species. The reason is that radical formation and/or decay underlie bimolecular  
377 reactions taking place in a highly anisotropic space. For example, deprotonation of  
378 radical cations involves water molecules whose approach to the reactive site depends  
379 on its location. Under these conditions, the kinetic models developed for reactions in  
380 homogenous solutions are not valid<sup>43,44</sup>. It should be noted that other processes, such  
381 as DNA fluorescence<sup>50</sup> or solvation<sup>51, 52</sup> in DNA multimers also undergo multiscale  
382 dynamics.

383 Given the above described difficulty, the radical reaction dynamics in various DNA  
384 multimers was simply compared by considering their half-life ( $\tau_{1/2}$ ), *i.e.* the time at  
385 which the entire radical population has decreased by a factor 2. For all the examined  
386 systems  $\tau_{1/2}$  amounts to a few milliseconds.<sup>3</sup> Base-pairing slows down the radical  
387 decays, with  $\tau_{1/2}$  increasing, for example, from 1 to 4 ms for adenine tracts<sup>28</sup>. Such a  
388 change in the reaction rate is explained by the higher degrees of freedom  
389 characterizing single strands compared to duplexes, allowing deprotonated radicals to  
390 reach faster reactive conformations, and their larger exposure to water. In G-  
391 quadruplexes, the  $\tau_{1/2}$  values depend strongly on the type of the metal cations located  
392 in their central cavity: they are at least twice as high for Na<sup>+</sup> compared to K<sup>+</sup>, but the  
393 mechanism responsible for such different dynamics is not yet clear.

394 Focusing on the deprotonation dynamics occurring before 2  $\mu\text{s}$ , a few delicate  
395 photoionization experiments, using electron scavengers ( $\text{N}_2\text{O}$  or  $\text{NO}_3^-$ ), provided  
396 information for guanine radicals. Thus, a rise of transient absorption was observed  
397 (Figure 4) at wavelengths at which the absorbance of deprotonated radicals is more  
398 intense than that of  $(\text{G}^+)^\bullet$ <sup>3, 32</sup>. Hence, it was found that deprotonation in calf thymus-  
399 DNA is completed within 2  $\mu\text{s}$ <sup>3</sup>. A faster deprotonation process, occurring in less than  
400 1  $\mu\text{s}$ , was detected for TEL21/ $\text{K}^+$  and OXY/ $\text{K}^+$ . But this rapid step concerns only part  
401 of the  $(\text{G}^+)^\bullet$  population in G-quadruplexes which ranges from 40% to 75%, depending  
402 on the system. The remaining part of  $(\text{G}^+)^\bullet$  survives in G-quadruplexes for much longer  
403 times, at least several tens of microseconds.

404 The decays of deprotonated radicals are not affected neither by the presence of  
405 oxygen nor by the buffer ingredients. Consequently, the associated reactions should  
406 involve only the nucleic acid itself and/or water molecules. Under these conditions, the  
407 decays represent, in a certain way, the “intrinsic” radical reaction dynamics, which can  
408 be obtained only via low-energy/low-intensity excitation, triggering solely DNA  
409 photoionization. In contrast, high-energy photoionization generates also electrons  
410 from the aqueous solvent (Table 2) which are known to react nucleic acids<sup>9</sup> altering  
411 the transient absorption signals.

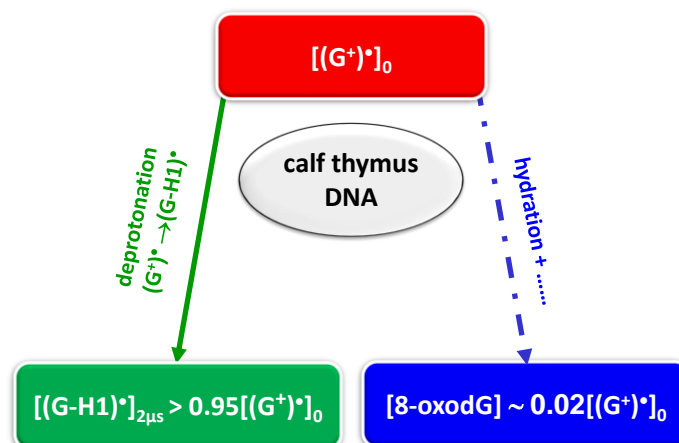
412 The time evolution of nucleobase radicals has been largely studied by flash photolysis  
413 and pulse radiolysis, using electron abstraction mediated by other molecules.  
414 According to these methodologies, a laser pulse or a pulsed electron beam triggers a  
415 redox reaction between the DNA and an external oxidant. Although such studies have  
416 brought important insights, in particular regarding the deprotonation of electron  
417 holes<sup>48, 53, 54</sup>, they fail to correctly describe radical dynamics over long time-scales<sup>21</sup>.

418 This happens because they involve bimolecular reactions requiring the approach  
419 between an oxidant, such as  $\text{SO}_4^{\bullet-}$ , and the DNA, which is an anionic polyelectrolyte  
420 with a highly inhomogeneous structure. As a result, the generation of radicals may  
421 interfere with their decay, artificially changing their reaction dynamics. A detailed  
422 comparison of guanine radical reaction dynamics in duplexes and G-quadruplexes  
423 obtained by an external oxidant<sup>55, 56</sup> and low-energy photoionization is presented in  
424 reference 21.

### 425 **3.6 Final lesions**

426 Although the studies presented in this Chapter concern the primary species issued  
427 from photoionization, they provide some information regarding the associated final  
428 DNA damage. The total quantum yield of the final lesions  $\phi_t$  resulting from purine  
429 radicals, irrespectively of their nature, should be equal to the  $\phi_i$  at the irradiation  
430 wavelength. Additional damage may also be provoked via reactions involving  $e_{\text{hyd}}^-$ .  
431 The existence of two different photoionization mechanisms (direct and indirect) shows  
432 that such DNA damage may be provoked by irradiation over a large spectral domain.  
433 Starting from 180 nm,  $\phi_t$  should decrease with increasing wavelength, in line with the  
434 behaviour of direct ionization<sup>24, 26</sup>. Then, upon reaching the absorption band peaking  
435 at 260 nm, where the indirect process takes over, a non-monotonous variation is  
436 expected. In this case, radical generation depends only on the formation of excited  
437 charge transfer states, which is the main relaxation path in DNA multimers (see  
438 Chapter 5). It should even be extended over the UVA spectral domain, where DNA  
439 exhibits a weak absorption tail<sup>57, 58</sup>, correlated with excited states having partial charge  
440 transfer character<sup>59, 60</sup>. The wide spectral efficiency of low-energy photoionizaion is  
441 corroborated by the detection of DNA lesions stemming from guanine radicals after

442 UVB<sup>22</sup> and even UVA<sup>23</sup> irradiation. Despite a few efforts to characterize specific  
 443 lesions resulting from one-photon ionization of DNA at either high- or at low-energies<sup>22</sup>,  
 444 <sup>29, 61</sup>, the determined quantum yields were significantly lower than  $\phi_t$ , meaning that an  
 445 important part of final lesions remained unidentified.



446

447 **Figure 9.** Competition between two reactions involving guanine radical cations: deprotonation and  
 448 hydration, ultimately leading the formation of 8-oxodG.

449

450 A second important outcome of the time-resolved studies on DNA photoionization  
 451 concerns the interplay between dynamics and populations. In other terms, if there is  
 452 competition between two reactions, the most important part of the reactant population  
 453 evolves along the faster path. This is illustrated in Figure 9 for two reactions involving  
 454  $(G^+)^\bullet$ : on the one hand deprotonation and, on the other, hydration which ultimately  
 455 leads to formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG)<sup>62</sup>. In the case of  
 456 calf thymus DNA, deprotonation is a fast reaction, being completed within 2  $\mu$ s; at this  
 457 time the quasi-entire  $(G^+)^\bullet$  population (>95%) has been transformed to deprotonated  
 458  $(G-H1)^\bullet$  radicals. Thus, it is not surprising that the quantum yield determined for the  
 459 formation of 8-oxodG by analytical methods corresponds only 2% of the  $\phi_t$ <sup>22</sup>. In



460 contrast, as part of  $(G^+)^{\bullet}$  survives in TEL21/ $Na^+$  for longer times, a higher level of 8-  
461 oxodG (corresponding to 7% of the  $\phi_r$ ) has been detected for this G-quadruplex<sup>29</sup>. The  
462 corollary is that 8-oxodG, widely used as marker of oxidative damage, is not  
463 representative of the extent of this damage when it takes place by one electron  
464 oxidation leading to the formation of radical cations.

465 Under the above conditions, the great majority of oxidative lesions are expected to  
466 stem from deprotonated radicals. The fact that the radical decays are not sensitive  
467 versus oxygen shows that neither imidazolone nor oxazolone constitute major lesions  
468 since their formation requires aerated conditions<sup>63</sup>. In contrast, oxygen does not affect  
469 strand breakage. As a matter of fact, strand breakage was detected following both 193  
470 nm irradiation<sup>2, 61</sup> and UVA irradiation<sup>23</sup>.

471 The reactions involving  $(G-H2)^{\bullet}$ , which represents the only deprotonated radical in  
472 some G-quadruplexes, have never been explored. Yet, the fingerprint of a reaction  
473 intermediate has been observed in the transient absorption spectra of TEL21/ $K^+$   
474 around 350-450 nm<sup>32</sup>. Finally, the role of metal cations in the central cavity of G-  
475 quadruplexes, which affect the radical reaction rate, needs also to be assessed.

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