



HAL
open science

Deprotonation dynamics of guanine radical cations

Evangelos Balanikas, Akos Banyasz, Gérard Baldacchino, Dimitra Markovitsi

► **To cite this version:**

Evangelos Balanikas, Akos Banyasz, Gérard Baldacchino, Dimitra Markovitsi. Deprotonation dynamics of guanine radical cations. *Photochemistry and Photobiology*, In press, 10.1111/php.13540 . cea-03383354

HAL Id: cea-03383354

<https://hal-cea.archives-ouvertes.fr/cea-03383354>

Submitted on 18 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Deprotonation dynamics of guanine radical cations

Evangelos Balanikas¹, Akos Banyasz^{1,2}, Gérard Baldacchino¹ and Dimitra Markovitsi*¹,

¹. Université Paris-Saclay, CEA, CNRS, LIDYL, F-91191 Gif-sur-Yvette, France,

². Univ Lyon, ENS de Lyon, CNRS UMR 5182, Université Claude Bernard Lyon 1, Laboratoire de Chimie, F-69342 Lyon, France

*Corresponding author e-mail: dimitra.markovitsi@cea.fr (Dimitra Markovitsi)

9 ABSTRACT

10 This review is dedicated to guanine radical cations (G^+) \bullet that are precursors to oxidatively
11 generated damage to DNA. (G^+) \bullet are unstable in neutral aqueous solution and tend to lose a
12 proton. The deprotonation process has been studied by time-resolved absorption experiments
13 in which (G^+) \bullet radicals are produced either by an electron abstraction reaction, using an
14 external oxidant, or by low-energy/low-intensity photoionization of DNA. Both the position of
15 the released proton and the dynamics of the process depend on the secondary DNA structure.
16 While deprotonation in duplex DNA leads to ($G-H1$) \bullet radicals, in guanine quadruplexes the
17 ($G-H2$) \bullet analogues are observed. Deprotonation in monomeric guanosine proceeds with a time
18 constant of ~ 60 ns, in genomic DNA is completed within $2 \mu\text{s}$ and spans from at least 30 ns to
19 over $50 \mu\text{s}$ in guanine quadruplexes. Such a deprotonation dynamics in four-stranded
20 structures, extended over more than three decades of times, is correlated with the anisotropic
21 structure of DNA and the mobility of its hydration shell. In this case, commonly used second
22 order reaction models are inappropriate for its description.

23

24 INTRODUCTION

25 Guanine radical cations (G^+) \bullet are precursors to DNA damage under oxidative stress (1). These
26 species are unstable in neutral aqueous solutions and tend to lose a proton, giving rise to
27 deprotonated guanine radicals ($G-H$) \bullet (2). Alternatively, (G^+) \bullet may follow other reaction path;
28 for example, undergo hydration, which subsequently leads to the well-known oxidation marker
29 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (1). The relative yields of the final lesions
30 resulting from various reactions in competition depend, among others, on the dynamics of the
31 associated reactions. In other terms, the faster the reaction rate, the higher the probability to
32 observe lesions resulting from this reaction (3).

33 Information about the deprotonation dynamics of (G^+) \bullet in room temperature solutions has been
34 obtained by time-resolved techniques, pulsed radiolysis (4, 5) and flash photolysis (6-13),
35 probing the absorption of guanine radicals on the UV-visible spectral domain. Depending on
36 the way in which the (G^+) \bullet are generated, we can distinguish two main experimental
37 approaches. On the one hand, (G^+) \bullet may be produced by electron abstraction from DNA
38 through a reaction with an external oxidant; this approach is named hereafter “indirect”. On the
39 other, (G^+) \bullet may result from photoionization of DNA, without mediation of other molecules,
40 which we coin “direct” approach.

41 In 1992, Candeias and Steenken observed that radicals of monomeric guanosine derivatives
42 generated by photoionization in neutral aqueous solutions are already deprotonated in 200 ns
43 (14). The first determination of (G^+) \bullet deprotonation dynamics was achieved a decade later by
44 Kobayashi and Tagawa (4). This study, as well as subsequent ones performed for different

45 types of DNA multimers, revealed that the $(G^+)^\bullet$ deprotonation rate strongly depends on the
46 secondary structure (4, 6-9, 11-13). Despite the qualitative agreement of the findings published
47 by various groups, important discrepancies regarding the reported rate or time constants
48 appeared, mostly in the case of guanine quadruplexes (G-Quadruplexes). These four-stranded
49 DNA structures are formed by folding and/or association of guanine rich DNA/RNA strands in
50 the presence of metal cations (Figure 1); the latter affect both the folding geometry and the
51 stability of the resulting scaffold (15). G-Quadruplexes are extensively studied because of their
52 important role in numerous biological functions (16, 17) and their potential applications in
53 biosensors (18-20).

54 >Figure 1<

55 The objective of the present review is to (i) highlight the major outcomes on the dynamics of
56 $(G^+)^\bullet$ deprotonation in neutral aqueous solutions reported from different groups, (ii) compare
57 the conclusions reached following different experimental methodologies and (iii) discuss the
58 origins for the observed inconsistencies. As experiments providing information on reaction
59 dynamics require the knowledge of the absorption spectra of the radicals, we start our review
60 by presenting their features. In a next step, the basic principles underlying direct and indirect
61 $(G^+)^\bullet$ generation are outlined. Then, we present the main results acquired by both indirect and
62 direct approaches. The latter were obtained in our group exploiting a recently discovered
63 unexpected phenomenon: absorption directly by DNA of single photons with energies
64 significantly lower compared to the vertical ionization potential of nucleobases (21) may
65 provoke electron ejection (22-24, 13, 25). The photoionization process as well as the general
66 methodology adopted for the quantification of the ejected electrons and guanine radicals have

67 been recently highlighted (13). Here, we insist on quantitative aspects related to the
68 deprotonation dynamics, enriching the existing picture with a novel methodology for the
69 determination of the $(G^+)^\bullet$ survival probability in G-Quadruplexes. Finally, after comparing the
70 various findings, we discuss the occurrence of non-constant reaction rates involving DNA.

71 **ABSORPTION SPECTRA: A PREREQUISITE FOR DYNAMICAL** 72 **STUDIES**

73 The absorption spectra of both $(G^+)^\bullet$ and the resulting deprotonated radicals were first
74 determined by Candeias and Steenken (26). In the case of the nucleoside (dG) and nucleotide
75 (dGMP), they found that the proton in position 1 of the guanine is lost upon deprotonation; but
76 if the release of this proton is not possible, as happens in the case of 1-methyl guanine, the
77 proton in position 2 of the amino group takes over (Figure 2, insets). The ensued deprotonated
78 radicals, noted $(G-H1)^\bullet$ and $(G-H2)^\bullet$, respectively, exhibit quite different absorption spectra.
79 As the relevant feature for the determination of the deprotonation dynamics is the spectral
80 change occurring during this process, we present in Figure 2 the differential spectra
81 corresponding to $(G^+)^\bullet \rightarrow (G-H1)^\bullet$ and $(G^+)^\bullet \rightarrow (G-H2)^\bullet$ deprotonations.

82 >Figure 2<

83 The spectral features of the various guanine radicals were recently rationalized by quantum
84 chemistry calculations (27, 28). In addition, these studies showed, that their main
85 characteristics in the visible spectral domain are maintained upon hydrogen-bonding and base-
86 stacking. Computations also indicated subtle differences in duplex spectra depending on

87 whether the H1 proton is transferred either to the bulk water or to the cytosine (Figure 3a).
88 However, the sensitivity of time-resolved measurements does not allow a clear distinction
89 between these two transfers.

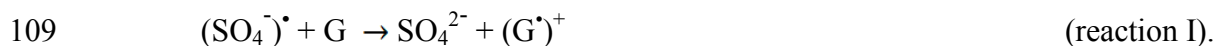
90 Globally, the spectra of deprotonated radicals in DNA multimers match quite well those of
91 monomeric guanosine in the visible spectral domain (9, 13). But moving towards the UV, the
92 agreement may be less good, because of the contribution of the “initial” multimer absorption
93 (see Figure 9 in reference (7)). As a matter of fact, due to the electronic coupling among
94 nucleobases, the Franck-Condon excited states of DNA multimers differ from those of non-
95 interacting monomers (29, 30, 28, 31) and this is reflected in their time-resolved absorption
96 spectra.

97 >Figure 3<

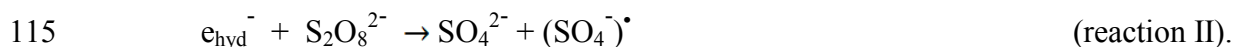
98 The absorption spectra of $(G^+)^\bullet$ in certain G-Quadruplexes were determined experimentally for
99 solutions with pH 3, where deprotonation is known to be hindered for monomeric guanosine
100 derivatives (26). It was observed that the spectral intensity below 450 nm is significantly lower
101 than that of the monomeric analogue (7, 9). This finding is supported by quantum chemistry
102 computations (7, 28), which also showed that the electron hole may be delocalized over
103 parallel stacked guanines (32-36, 28). Such a delocalization could influence the deprotonation
104 rate. However, its occurrence has not been clearly established on the time-scales and
105 temperatures discussed here.

106 **GENERATION OF RADICAL CATIONS**

107 All the studies that reported deprotonation dynamics following the indirect approach, used
108 $(\text{SO}_4^-)^\bullet$ as external oxidant to produce $(\text{G}^\bullet)^+$ radicals.



110 The oxidant itself is formed from a peroxodisulfate salt via a two-step or one-step process. In
111 pulsed radiolysis (4, 5), first, the interaction of an electron beam with water gives rise to
112 hydrated electrons e_{hyd}^- and HO^\bullet radicals. The latter are scavenged by tert-butyl alcohol, which
113 is also added in the solution so that to avoid their reaction with DNA. Then, hydrated electrons
114 react with peroxodisulfate anions:



116 If laser irradiation is used instead of an electron beam, the oxidant is generated by a single
117 photochemical reaction (6, 10):



119 In the low-energy photoionization studies, $(\text{G}^\bullet)^+$ generation takes place at 266 nm (13). The key
120 point of this approach is the use of laser intensities ($\leq 2 \times 10^6 \text{ Wcm}^{-2}$) significantly lower
121 compared to those used to study final lesions (37). Under these conditions, the e_{hyd}^- originate
122 only from DNA, without interference from solvent ionization. Consequently, they can be
123 quantified and compared with the radical concentration through the corresponding molar
124 absorption coefficients (ϵ) as explained in reference (13). For the experiments reported here,
125 DNA was dissolved in the phosphate buffer, composed of an equimolar mixture of
126 $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ or $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ in concentrations of 0.015 - 0.15 $\text{mol} \cdot \text{L}^{-1}$ each. Under
127 these conditions, e_{hyd}^- decay in less than 2 μs , reacting with $\text{H}_2\text{PO}_4^{-2}$ (13).

128 We remark that, going from pulse radiolysis to photosensitized electron abstraction and further
129 to photoionization, both the number of reaction steps necessary to generate $(\text{G}^\bullet)^+$ and the
130 number of chemicals present in the solution, decrease. The counterpart is that, in parallel, the

131 intensity of the transient absorption signals decreases, typically by two orders of magnitude
132 (compare, for example, Figure 5 in reference (4) and Figure 4 in this manuscript). Therefore,
133 low-energy photoionization studies require long measurement times. In addition, both DNA
134 and the buffer ingredients need to be purified so that to avoid spurious signals from impurities
135 and/or their interaction with radicals.

136 We stress that the initial electron holes generated by either the indirect or the direct method are
137 not necessarily located on guanine sites. On the one hand, $(\text{SO}_4^-)^\bullet$ may oxidize other
138 nucleobases (38, 39), and, on the other, low-energy photoionization takes place also in DNA
139 multimers devoid of guanines (23, 24). But, as guanine has the lowest oxidation potential
140 among nucleobases (40), the electron hole is rapidly trapped by guanines following a charge
141 transport process (41-47) and only guanine radicals are observed in the experiments described
142 here.

143 **MAIN FINDINGS: INDIRECT APPROACH**

144 In their seminal work, Kobayashi and Tagawa (4) performed pulse radiolysis and probed the
145 transient absorption at 625 nm (see Figure 2a). They found that $(\text{G}^+)^\bullet \rightarrow (\text{G-H1})^\bullet$ deprotonation
146 in dG proceeds with a rate constant of $1.8 \times 10^7 \text{ s}^{-1}$ (56 ns). Recent flash photolysis
147 measurements yielded a similar rate constant ($1.5 \times 10^7 \text{ s}^{-1}$ / 67 ns) (10).

148 Kobayashi and Tagawa also studied the deprotonation dynamics of $(\text{G}^+)^\bullet$ in a series of model
149 duplexes with 12-13 base-pairs containing G, GG and GGG steps at different positions (4). In
150 this case, the fit of the transient signals required two-exponential functions, provided rate
151 constants of $1.3 \times 10^7 \text{ s}^{-1}$ (78 ns) and $3 \times 10^6 \text{ s}^{-1}$ (330 ns). The fastest one was attributed to

152 guanines located close to the ends of the duplex, while the slowest was assigned to internal
153 guanine sites.

154 The first study on G-Quadruplexes was performed by Su and co-workers, using
155 photosensitized electron abstraction (6). They examined four different sequences forming
156 monomolecular, bimolecular and tetramolecular structures (Figure 1). They came to two
157 important conclusions. First, they showed that, in contrast to what was observed for duplexes,
158 $(G^+)^{\bullet}$ deprotonation in these systems does not involve release of the H1 proton but of the H2
159 proton (Figure 1b). This conclusion was attested by the appearance in the transient absorption
160 spectra of a band peaking in the red, which is typical of the $(G-H2)^{\bullet}$ radicals (26, 48). It was
161 explained by the fact that H1 protons are engaged in the Hoogsteen hydrogen bonds connecting
162 the guanines within the guanine tetrads (Figure 3b). Later, quantum chemistry calculations
163 found that, indeed, $(G-H2)^{\bullet}$ radicals in G-quadruplexes are more stable than the $(G-H1)^{\bullet}$
164 analogues (9). The second conclusion was that $(G^+)^{\bullet} \rightarrow (G-H2)^{\bullet}$ deprotonation in G-
165 Quadruplexes is slower compared to $(G^+)^{\bullet} \rightarrow (G-H1)^{\bullet}$ in duplexes. Rate constants ranging
166 from $1.4 \times 10^6 \text{ s}^{-1}$ (0.7 μs) and $2 \times 10^5 \text{ s}^{-1}$ (5 μs) were reported.

167 A subsequent study on two G-quadruplexes, also performed by photosensitized electron
168 abstraction reached a different conclusion (49). The transient absorption spectra recorded at 50
169 μs were attributed to $(G-H1)^{\bullet}$ deprotonated radicals but the discrepancy with previously
170 reported studies (6, 7) was not discussed.

171 **MAIN FINDINGS: DIRECT APPROACH**

172 In photoionization studies, a full spectral characterization of the radicals is possible only after
173 2-3 μs . This is due, on the one hand, to the low intensity of the transient absorption signals,
174 which are noisier on shorter times, and, on the other, to the presence of hydrated ejected
175 electrons, whose broad and intense absorption is extended over the whole visible domain (13).
176 Yet, some delicate measurements were performed on the sub-microsecond time-scale by
177 adding in the solution efficient electron scavengers, N_2O or NO_3^- (50).

178 For all the DNA systems studied by this method, the guanine radical concentration at 2-3 μs
179 was found to be equal to that of the hydrated ejected electrons $[\text{e}_{\text{hyd}}^-]_0$, determined by
180 independent experiments on the sub-microsecond time-scale (13). Thus, it was possible to
181 quantitatively follow the entire radical population during the time. We note that these “initial”
182 concentrations correspond to electron and holes that escaped recombination (51) and may
183 provoke DNA damage.

184 The differential absorption spectra recorded on the microsecond time-scale for model duplexes
185 and purified calf thymus DNA (CT-DNA) (52, 8, 13) correspond to that of $(\text{G-H1})^\bullet$ radicals,
186 showing that $(\text{G}^+)^\bullet \rightarrow (\text{G-H1})^\bullet$ deprotonation takes place earlier. The deprotonation dynamics
187 was determined in the case of CT-DNA by recording transient absorption signals at 700 nm.
188 The latter exhibit a rise, which can be described by a mono-exponential function with a time
189 constant of 320 ± 30 ns; after $2\mu\text{s}$, the signal starts decreasing slowly (Figure 4) and
190 subsequently it decays on the millisecond time-scale (13).

191 >Figure 4<

192 A more complex picture was found in the case of G-Quadruplexes. The study of 7 different
193 systems showed that a significant population of the initially generated $(G^+)^\bullet$ is still present at 3
194 μs (Table 1). Depending on the G-quadruplex type, the concentration of this “long-lived” $(G^+)^\bullet$
195 population varies from 25% to 60% of $[e_{\text{hyd}}^-]_0$ while the remaining part corresponds to $(G\text{-H}2)^\bullet$
196 radicals.

197 >Table 1<

198 The percentages presented in Table 1 were determined by reconstructing the transient
199 absorption spectra obtained for the studied G-Quadruplexes by linear combinations of the
200 spectra reported for monomeric guanosine radicals the $(G^+)^\bullet$ and $(G\text{-H}2)^\bullet$. This reconstruction
201 takes into account not only the spectral shape but also the spectral intensities. To this end, the
202 differential absorption ΔA , observed over an optical path-length of 1 cm, was divided by the
203 concentration of hydrated ejected electrons, while the monomer spectra were considered with
204 their ϵ (26). An example is given in Figure 5a where the transient absorption spectrum of
205 OXY/Na⁺ at 3 μs is presented together with that corresponding of a linear combination of $(G^+)^\bullet$
206 and $(G\text{-H}2)^\bullet$ radicals at a ratio of 45/55 while the sum of their concentrations is equal to the
207 detected $[e_{\text{hyd}}^-]_0$.

208 >Figure 5<

209 For some G-Quadruplexes, deprotonation toward $(G\text{-H}2)^\bullet$ becomes much more obvious on
210 later times. For example, the spectrum of $(\text{TG}_4\text{T})_4/\text{Na}^+$ at 50 μs (Figure 5 in reference (9))
211 strongly resembles that of monomeric $(G\text{-H}2)^\bullet$. At even longer times, $(G\text{-H}2)^\bullet \rightarrow (G\text{-H}1)^\bullet$

212 tautomerization, which is in competition with other reaction paths, may be observed (13). But
213 this aspect is out of the scope of the present review.

214 >Figure 6<

215 For a few cases, the early deprotonation dynamics could be resolved. This is presented in
216 Figure 6 for TEL21/K⁺, OXY/Na⁺ and OXY/K⁺: the transient signals recorded at 620 nm
217 exhibit a rise, corresponding to the appearance of (G-H2)[•] radicals (see Figure 2b). Although
218 these signals are very noisy, it is clear that a fast deprotonation process takes place in less than
219 1 μs; the time constants, derived from fits with mono-exponential functions (150 ± 15 ns in
220 Figure 6a and 200 ± 30 ns in Figure 6b), are smaller than that found for CT-DNA (320 ± 30 ns,
221 Figure 4).

222 Coming to the slow deprotonation process, it could be visualized directly by transient
223 absorption signals only in the case of tetramolecular G-quadruplexes (9, 11). For these
224 systems, the (G-H2)[•] population at 50 μs is still equal to that of the total radical population at 3
225 μs. Thus, the decay of the differential absorbance at 500 nm and the rise at 600 nm reflect
226 directly the deprotonation dynamics. This is shown in Figure 7a for (TG₄T)₄/Na⁺, where both
227 the decay and the rise are approximated by single exponential functions, with the same time
228 constant of 6 ± 1 μs (9). The deprotonation is practically completed at 20 μs. For other
229 systems, the total radical population, determined by the transient absorption decays at 510-515
230 nm as explained in reference (13), starts decreasing before completion of the deprotonation
231 processes. For this reason, a different method was adopted in order to determine the reaction
232 dynamics of (G⁺)[•]. Several time-resolved spectra were recorded on the microsecond time-scale
233 and, subsequently, reconstructed in the same way as that followed for the 3 μs spectra. As

234 example is presented in Figure 5b the reconstruction of the spectra recorded for TEL21/Na⁺ at
235 25 μs. At this time, the total radical concentration corresponds to 85% of the hydrated ejected
236 electrons; the missing part was associated with formation of 8-oxodG which was detected by
237 analytical methods (7). Finally, the (G⁺)[•] survival probability, determined as a percentage of
238 the initially generated electron holes that have escaped recombination, equal to [e_{hyd}⁻]₀, was
239 plotted. Figures 7b and 7c show the results derived from such an analysis performed for the
240 OXY/Na⁺ and TEL21/Na⁺. At times longer than 50 μs for the former system and 40 μs for the
241 latter, the errors related with the spectral analysis increase to provide reliable data. At those
242 times, about ~10% of the initial population is still present in the G-Quadruplex.

243 >Figure 7<

244 **COMMON CONCLUSIONS AND DISCREPANCIES**

245 The above described studies carried out by different groups using different approaches lead to
246 some common conclusions regarding the (G⁺)[•] deprotonation dynamics.

247 First, similar rate constants were found for deprotonation in dG via the indirect method using
248 either pulse radiolysis (4) and flash photolysis (6). Second, the characteristic time determined
249 by photoionization of CT-DNA (320 ± 30 ns; Figure 4) (13) matches well the longest time
250 constants (330 ns) derived from the study of model duplexes by pulsed radiolysis (4). The
251 shortest time constants reported in the latter study had been correlated with guanines located
252 close to the ends of the duplexes and it is normal that they are not detected for genomic DNA
253 which is very long macromolecule.

254 Third, the specificity of $(G^+)^\bullet$ deprotonation towards $(G-H2)^\bullet$ in G-Quadruplexes, initially
255 deduced from photosensitized electron abstraction studies, carried out by Su and co-workers
256 (6), was largely confirmed by our photoionization studies (11, 13, 12, 9, 8, 7). And it is
257 important to note that oxidatively generated lesions to DNA resulting from $(G-H2)^\bullet$ radicals
258 have not been documented so far. Yet, a study Merta et al. using, as the Su group,
259 photosensitized electron abstraction (reaction I), failed to detect the presence of $(G-H2)^\bullet$ in
260 telomeric G-Quadruplexes (49).

261 Another common conclusion drawn by the Su group and our work is that $(G^+)^\bullet$ radicals in G-
262 Quadruplexes are still observed on the microsecond time-scale. But quantification of $(G^+)^\bullet$
263 population, possible only through photoionization studies, revealed that these “long-lived”
264 species, whose decays were characterized in the 3 - 50 μ s interval (Figure 7), represent only
265 part of the initially generated electron holes (see Table 1). As shown in Figures 6a and 6b, an
266 important part of $(G^+)^\bullet$ deprotonates on much shorter times, even faster compared to CT-DNA
267 (Figure 4).

268 **REACTION RATES IN ANISOTROPIC STRUCTURES**

269 In order to understand the discrepancies regarding the rate constants determined for $(G^+)^\bullet$
270 deprotonation in G-Quadruplexes, a closer look at the reaction mechanism underlying their
271 determination may be informative. Proton transfer from $(G^+)^\bullet$ to water is considered to be a
272 second order reaction. Given that the bulk concentration of water molecules is much higher
273 than that of $(G^+)^\bullet$, this second order reaction is approximated by a pseudo-first order one, as
274 indicated by the fact that the rate constants are expressed in s^{-1} (4, 6, 10). However, the validity
275 of such an approximation requires certain conditions to be fulfilled. The reaction rate needs to

276 be controlled by diffusion of the reactants and the probability of their encounter should be
277 uniform over the three-dimensional space over the time-window of the experimental
278 measurements (53). This is the model of a well-stirred reactor.

279 The conditions corresponding to the simplified pseudo-first order reaction kinetics are far from
280 valid in G-Quadruplexes. As a matter of fact, analysis of several crystal structures revealed the
281 existence of well-defined water networks, whose properties depend on the topology of G-
282 Quadruplex; the primary water molecules may be hydrogen bonded to phosphate and/or sugar
283 groups and even to guanines at the edges of the G-quadruplexes (54).

284 Heterogeneities are encountered not only in the static arrangement of the DNA hydration shell
285 but also on the lability of its water molecules which may be modulated by slow conformational
286 motions of the nucleic acids (55). Interestingly, the hydration cell has been found to be thicker
287 around G-quadruplexes than duplexes and more difficult to be disrupted by solute molecules
288 (56). According to two-dimensional NMR experiments, the residence times of the water
289 molecules belonging to the hydration shell of model duplexes do not exceed 1 ns at room
290 temperature (57, 58). Consequently, these effects should be averaged on the time-scale on
291 which $(G^+)^{\bullet}$ deprotonation dynamics is detected for CT-DNA by transient absorption (Figure
292 4). In contrast, longer water rotation and residence times, extended on the microsecond time-
293 scale, have been reported for OXY/Na⁺ G-Quadruplexes (59), explaining, at least partly, the
294 observed slower $(G^+)^{\bullet}$ deprotonation.

295 Second order kinetics in systems with spatial and dynamical heterogeneity may give rise to
296 multiscale survival probabilities of the reactants (60). Such reaction patterns artificially appear
297 to be mono-exponential if the observation and/or analysis of the time-resolved signals is
298 restricted over a small time-window. In addition, different time-windows result to different

299 time constants. It is worth-noticing that multiscale dynamics characterizes also the excited state
300 relaxation in DNA, where electronic coupling, affected by conformational motions among
301 nucleobases (61), is operative (62).

302 Multiscale reaction rates may also concern $(G^+)^\bullet$ generation in DNA multimers using $(SO_4^-)^\bullet$.

303 Reaction I takes place between negatively charged electron acceptors and spatially correlated
304 electron donors located on a polyelectrolyte. It is possible that the rate constants determined for
305 the oxidation process reflect only part of the reaction. In this case, a simple change in the
306 concentration ratios between DNA and the oxidant or the duration of the laser pulse may
307 modify the oxidation rates and, hence, those of deprotonation. This could provide an
308 explanation why $(G-H2)^\bullet$ radicals have gone undetected in one study (49) while they were
309 readily detected in another (6).

310 Focusing on the deprotonation process determined by the photoionization method, the time
311 constants derived from the fits with exponential functions (Figures 4, 6 and 7) do not
312 necessarily correspond to reaction rate constants; they are simply useful for a
313 phenomenological comparison of the transient absorption traces on a given time-window. In
314 this respect, the first photoionization study of G-Quadruplexes reported that $(G^+)^\bullet$ survive in
315 TEL21/ Na^+ on the millisecond time-scale signals (7). This conclusion was based on time
316 constants derived from exponential fits of the transient absorption signals which, following the
317 above reasoning, may lead to erroneous results. The methodology presented here for the
318 determination of the $(G^+)^\bullet$ survival probability (Figure 5b), based on the transient absorption
319 spectra, provided shorter lifetimes. Therefore, we conclude that deprotonation in G-
320 Quadruplexes spans over three orders of magnitude of time, from at least 30 ns to over 50 μ s,
321 the limits being determined by the time resolution and the sensitivity of the experimental set-

322 up. This anisotropic deprotonation dynamics can be also viewed as the occurrence of
323 dynamically anisotropic pKa values.

324 As analytical formulas derived from simple reaction patterns cannot describe correctly the
325 $(G^+)^\bullet$ deprotonation dynamics, specific theoretical developments are needed blatantly. A first
326 step toward this direction was made in a recent quantum chemical study: it was shown that a
327 cluster of four water molecules must be taken into account to correctly reproduce the
328 temperature dependence of the reaction rates $(G^+)^\bullet \rightarrow (G-H1)^\bullet$ in dG determined
329 experimentally as a function of temperature (10). Regarding DNA multimers, theoretical
330 studies should encompass, in addition, molecular dynamics simulations on tens of
331 microseconds, already used to describe interaction of DNA with biomolecules (63, 64). In this
332 way, it should be possible to establish a correlation between DNA topology and $(G^+)^\bullet$
333 dynamics.

334

335 **ACKNOWLEDGMENTS:** This work has received funding from the European Union's
336 Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant
337 agreement No. 765266 (LightDyNAMics).

338

339 **REFERENCES**

- 340 1. Cadet, J., T. Douki and J.-L. Ravanat (2015) Oxidatively Generated Damage to Cellular
341 DNA by UVB and UVA Radiation. *Photochem. Photobiol.* **91**, 140-155.
- 342 2. Steenken, S. (1989) Purine-Bases, Nucleosides and Nucleotides - Aqueous-Solution Redox
343 Chemistry and transformation Reactions of their Radical Cations and e- and OH Adducts.
344 *Chem. Rev.* **89**, 503-520.
- 345 3. Balanikas, E. and D. Markovitsi (2021) DNA photoionization: from high to low energies. In
346 *DNA Photodamage*. (Edited by R. Improta and T. Douki), pp. 37-54. RSC, Cambridge.
- 347 4. Kobayashi, K. and S. Tagawa (2003) Direct observation of guanine radical cation
348 deprotonation in duplex DNA using pulse radiolysis. *J. Am. Chem. Soc.* **125**, 10213-10218.
- 349 5. Choi, J., C. Yang, M. Fujitsuka, S. Tojo, H. Ihee and T. Majima (2015) Proton Transfer of
350 Guanine Radical Cations Studied by Time-Resolved Resonance Raman Spectroscopy
351 Combined with Pulse Radiolysis. *J. Phys. Chem. Lett.* **6**, 5045-5050.
- 352 6. Wu, L. D., K. H. Liu, J. L. Jie, D. Song and H. M. Su (2015) Direct Observation of Guanine
353 Radical Cation Deprotonation in G-Quadruplex DNA. *J. Am. Chem. Soc.* **137**, 259-266.
- 354 7. Banyasz, A., L. Martinez-Fernandez, C. Balty, M. Perron, T. Douki, R. Improta and D.
355 Markovitsi (2017) Absorption of Low-Energy UV Radiation by Human Telomere G-
356 Quadruplexes Generates Long-Lived Guanine Radical Cations. *J. Am. Chem. Soc.* **139**, 10561-
357 10568.
- 358 8. Balanikas, E., A. Banyasz, G. Baldacchino and D. Markovitsi (2019) Populations and
359 Dynamics of Guanine Radicals in DNA strands: Direct versus Indirect Generation. *Molecules*
360 **24**, 2347.

- 361 9. Banyasz, A., E. Balanikas, L. Martinez-Fernandez, G. Baldacchino, T. Douki, R. Improta
362 and D. Markovitsi (2019) Radicals generated in tetramolecular guanine quadruplexes by
363 photo-ionization: spectral and dynamical features. *J. Phys. Chem. B* **123**, 4950-4957.
- 364 10. Zhang, X., J. Jie, D. Song and H. Su (2020) Deprotonation of Guanine Radical Cation
365 G(center dot+) Mediated by the Protonated Water Cluster. *J. Phys. Chem. A* **124**, 6076-6083.
- 366 11. Behmand, B., E. Balanikas, L. Martinez-Fernandez, R. Improta, A. Banyasz, G.
367 Baldacchino and D. Markovitsi (2020) Potassium Ions Enhance Guanine Radical Generation
368 upon Absorption of Low-Energy Photons by G-quadruplexes and Modify Their Reactivity. *J.*
369 *Phys. Chem. Lett.* **11**, 1305–1309.
- 370 12. Balanikas, E., A. Banyasz, G. Baldacchino and D. Markovitsi (2020) Guanine Radicals
371 Generated in Telomeric G-quadruplexes by Direct Absorption of Low-Energy UV Photons:
372 Effect of Potassium Ions. *Molecules* **25**, 2094.
- 373 13. Balanikas, E., A. Banyasz, T. Douki, G. Baldacchino and D. Markovitsi (2020) Guanine
374 Radicals Induced in DNA by Low-Energy Photoionization. *Acc. Chem. Res.* **53**, 1511-1519.
- 375 14. Candeias, L. P. and S. Steenken (1992) Ionization of purine nucleosides and nucleotides
376 and their components by 193-nm laser photolysis in aqueous solution: model studies for
377 oxidative damage of DNA. *J. Am. Chem. Soc.* **114**, 699-704.
- 378 15. Hud, V. and J. Plavec (2006) The role of cations in determining quadruplex structure and
379 stability. In *Quadruplex nucleic acids*. (Edited by S. Balasubramanian and S. Neidle), pp. 100-
380 130. RSC Publishing, Cambridge.
- 381 16. Rhodes, D. and H. J. Lipps (2015) G-quadruplexes and their regulatory roles in biology.
382 *Nucleic Acids Res.* **43**, 8627-8637.

- 383 17. Kharel, P., G. Becker, V. Tsvetkov and P. Ivanov (2020) Properties and biological impact
384 of RNA G-quadruplexes: from order to turmoil and back. *Nucleic Acids Res.* **48**, 12534-12555.
- 385 18. Xiang, X., Y. Li, L. Ling, Y. Bao, Y. Su and X. Guo (2019) Label-free and dye-free
386 detection of target DNA based on intrinsic fluorescence of the (3+1) interlocked bimolecular
387 G-quadruplexes. *Sens. Actuators B Chem.* **290**, 68-72.
- 388 19. Liu, T. L., D. X. Sun, M. M. Gu, X. M. Wu and G. L. Wang (2020) Intercalative methylene
389 blue as an efficient signal molecule of tremella-like Bi₂WO₆: Toward high performance
390 photoelectrochemical biosensing. *Sens. Actuators B Chem.* **317**.
- 391 20. Gu, M., D. Sun, T. Liu, X. Wu and G.-L. Wang (2020) Homogenous cathodic
392 photoelectrochemistry for DNA binding protein analysis. *Sens. Actuators B Chem.* **324**.
- 393 21. Pluharova, E., P. Slavicek and P. Jungwirth (2015) Modeling Photoionization of Aqueous
394 DNA and Its Components. *Acc. Chem. Res.* **48**, 1209-1217.
- 395 22. Marguet, S., D. Markovitsi and F. Talbot (2006) One and two photon ionization of DNA
396 single and double helices studied by laser flash photolysis at 266 nm. *J. Phys. Chem. B* **110**,
397 11037-11039.
- 398 23. Banyasz, A., T. Ketola, A. Muñoz-Losa, S. Rishi, A. Adhikary, M. D. Sevilla, L. Martinez-
399 Fernandez, R. Improrta and D. Markovitsi (2016) UV-induced Adenine Radicals Induced in
400 DNA A-tracts: Spectral and Dynamical Characterization *J. Phys. Chem. Lett.* **7**, 3949-3953.
- 401 24. Banyasz, A., T. Ketola, L. Martinez-Fernandez, R. Improrta and D. Markovitsi (2018)
402 Adenine radicals generated in alternating AT duplexes by direct absorption of low-energy UV
403 radiation. *Faraday Disc.* **207**, 181-197.

- 404 25. Balanikas, E., L. Martinez-Fernandez, R. Improta, P. Podbevsek, G. Baldacchino and D.
405 Markovitsi (2021) The Structural Duality of Nucleobases in Guanine Quadruplexes Controls
406 Their Low-Energy Photoionization. *J. Phys. Chem. Lett.* **12**, 8309–8313.
- 407 26. Candeias, L. P. and S. Steenken (1989) Structure and acid-base properties of one-electron-
408 oxidized deoxyguanosine, guanosine, and 1-methylguanosine. *J. Am. Chem. Soc.* **111**, 1094-
409 1099.
- 410 27. Kumar, A. and M. D. Sevilla (2019) Excited States of One-Electron Oxidized Guanine-
411 Cytosine Base Pair Radicals: A Time Dependent Density Functional Theory Study. *J. Phys.*
412 *Chem. A* **123**, 3098-3108.
- 413 28. Martinez-Fernandez, L., L. Esposito and R. Improta (2020) Studying the excited electronic
414 states of guanine rich DNA quadruplexes by quantum mechanical methods: main achievements
415 and perspectives. *Photochem. Photobiol. Sci.* **19**, 436-444.
- 416 29. Emanuele, E., K. Zakrzewska, D. Markovitsi, R. Lavery and P. Millie (2005) Exciton
417 states of dynamic DNA double helices: alternating dCdG sequences. *J. Phys. Chem. B* **109**,
418 16109-16118.
- 419 30. Nogueira, J. J., F. Plasser and L. Gonzalez (2017) Electronic delocalization, charge transfer
420 and hypochromism in the UV absorption spectrum of polyadenine unravelled by multiscale
421 computations and quantitative wavefunction analysis. *Chem. Sci.* **8**, 5682-5691.
- 422 31. Ibele, L., P. Sánchez-Murcia, S. Mai, J. Nogueira and L. González (2020) Excimer
423 Intermediates en Route to Long-Lived Charge-Transfer States in Single-Stranded Adenine
424 DNA as Revealed by Nonadiabatic Dynamics. *J. Phys. Chem. Lett.* **11**, 7483–7488.

- 425 32. Adhikary, A., D. Khanduri and M. D. Sevilla (2009) Direct Observation of the Hole
426 Protonation State and Hole Localization Site in DNA-Oligomers. *J. Am. Chem. Soc.* **131**,
427 8614-8619.
- 428 33. Adhikary, A., A. Kumar, S. A. Munafo, D. Khanduri and M. D. Sevilla (2010) Prototropic
429 equilibria in DNA containing one- electron oxidized GC: intra-duplex vs. duplex to solvent
430 deprotonation. *Phys. Chem. Chem. Phys.* **12**, 5353-5368.
- 431 34. Kumar, A. and M. D. Sevilla (2011) Density Functional Theory Studies of the Extent of
432 Hole Delocalization in One-Electron Oxidized Adenine and Guanine Base Stacks. *J. Phys.*
433 *Chem. B* **115**, 4990-5000.
- 434 35. Livshits, G. I., A. Stern, D. Rotem, N. Borovok, G. Eidelstein, A. Migliore, E. Penzo, S.
435 J. Wind, R. Di Felice, S. S. Skourtis, J. Carlos Cuevas, L. Gurevich, A. B. Kotlyar and D.
436 Porath (2014) Long-range charge transport in single G-quadruplex DNA molecules. *Nat.*
437 *Nanotechnol.* **9**, 1040-1046.
- 438 36. Capobianco, A., T. Caruso, A. M. D'Ursi, S. Fusco, A. Masi, M. Scrima, C. Chatgililoglu
439 and A. Peluso (2015) Delocalized Hole Domains in Guanine-Rich DNA Oligonucleotides. *J.*
440 *Phys. Chem. B* **119**, 5462-5466.
- 441 37. Cadet, J., J. R. Wagner and D. Angelov (2019) Biphotonic Ionization of DNA: From
442 Model Studies to Cell. *Photochem. Photobiol.* **95**, 59-72.
- 443 38. Candeias, L. P., P. O'Neill, G. D. D. Jones and S. Steenken (1992) Ionization of
444 polynucleotides and DNA in aqueous solution by 193 nm pulsed laser light: identification of
445 base derived radicals. *Int. J. Radiat. Biol.* **61**, 15-20.

446 39. Candeias, L. P. and S. Steenken (1993) Electron transfer in di(deoxy)nucleoside
447 phosphates in aqueous solution: rapid migration of oxidative damage (via adenine) to guanine.
448 *J. Am. Chem. Soc.* **115**, 2437-2440.

449 40. Palecek, E. and M. Bartosik (2012) Electrochemistry of Nucleic Acids. *Chem. Rev.* **112**,
450 3427-3481.

451 41. Kanvah, S., J. Joseph, G. B. Schuster, R. N. Barnett, C. L. Cleveland and U. Landman
452 (2010) Oxidation of DNA: Damage to Nucleobases. *Acc. Chem. Res.* **43**, 280-287.

453 42. Douki, T., D. Angelov and J. Cadet (2001) UV laser photolysis of DNA: effect of duplex
454 stability on charge-transfer efficiency. *J. Am. Chem. Soc.* **123**, 11360-11366.

455 43. Meggers, E., M. E. Michel-Beyerle and B. Giese (1998) Sequence dependent long range
456 hole transport in DNA. *J. Am. Chem. Soc.* **120**, 12950-12955.

457 44. Saito, I., T. Nakamura, K. Nakatani, Y. Yoshioka, K. Yamaguchi and H. Sugiyama (1998)
458 Mapping of the hot spots for DNA damage by one-electron oxidation: Efficacy of GG doublets
459 and GGG triplets as a trap in long-range hole migration. *J. Am. Chem. Soc.* **120**, 12686-12687.

460 45. Genereux, J. C. and J. K. Barton (2010) Mechanisms for DNA Charge Transport. *Chem.*
461 *Rev.* **110**, 1642-1662.

462 46. Kawai, K. and T. Majima (2013) Hole Transfer Kinetics of DNA. *Acc. Chem. Res.* **46**,
463 2616-2625.

464 47. Lewis, F. D., R. L. Letsinger and M. R. Wasielewski (2001) Dynamics of photoinduced
465 charge transfer and hole transport in synthetic DNA hairpins. *Acc. Chem. Res.* **34**, 159-170.

466 48. Chatgililoglu, C. (2021) The Two Faces of the Guanyl Radical: Molecular Context and
467 Behavior. *Molecules* **26**.

468 49. Merta, T. J., N. E. Geacintov and V. Shafirovich (2019) Generation of 8-oxo-7,8-
469 dihydroguanine in G-Quadruplexes Models of Human Telomere Sequences by One-electron
470 Oxidation. *Photochem. Photobiol.* **95**, 244-251.

471 50. von Sonntag, C. (2006) *Free-Radical-Induced DNA damage and its Repair* Springer-
472 Verlag, Berlin-Heidelberg.

473 51. Peon, J., G. C. Hess, J. M. L. Pecourt, T. Yuzawa and B. Kohler (1999) Ultrafast
474 photoionization dynamics of indole in water. *J. Phys. Chem. A* **103**, 2460-2466.

475 52. Banyasz, A., L. Martinez-Fernandez, R. Improta, T. M. Ketola, C. Balty and D. Markovitsi
476 (2018) Radicals generated in alternating guanine-cytosine duplexes by direct absorption of
477 low-energy UV radiation. *Phys. Chem. Chem. Phys.* **20**, 21381-21389.

478 53. Blumen, A., J. Klafter and G. Zumofen (1986) Models for reaction dynamics in glasses. In
479 *Optical spectroscopy of glasses*. (Edited by I. Zschokke), pp. 199-265. Springer: Dordrecht, the
480 Netherlands.

481 54. Li, K., L. Yatsunyk and S. Neidle (2021) Water spines and networks in G-quadruplex
482 structures. *Nucleic Acids Res.* **49**, 519-528.

483 55. Duboue-Dijon, E., A. C. Fogarty, J. T. Hynes and D. Laage (2016) Dynamical Disorder in
484 the DNA Hydration Shell. *J. Am. Chem. Soc.* **138**, 7610-7620.

485 56. Nakano, M., H. Tateishi-Karimata, S. Tanaka, F. Tama, O. Miyashita, S.-i. Nakano and N.
486 Sugimoto (2015) Thermodynamic properties of water molecules in the presence of cosolute
487 depend on DNA structure: a study using grid inhomogeneous solvation theory. *Nucleic Acids*
488 *Res.* **43**, 10114-10125.

489 57. Phan, A. T., J. L. Leroy and M. Gueron (1999) Determination of the residence time of
490 water molecules hydrating B'-DNA and B-DNA, by one-dimensional zero-enhancement
491 nuclear Overhauser effect spectroscopy. *J. Mol. Biol.* **286**, 505-519.

492 58. Denisov, V. P., G. Carlstrom, K. Venu and B. Halle (1997) Kinetics of DNA hydration. *J.*
493 *Mol. Biol.* **268**, 118-136.

494 59. Snoussi, K. and B. Halle (2008) Internal Sodium Ions and Water Molecules in Guanine
495 Quadruplexes: Magnetic Relaxation Dispersion Studies of d(G(3)T(4)G(3)) (2) and
496 d(G(4)T(4)G(4)) (2). *Biochemistry* **47**, 12219-12229.

497 60. Benichou, O., C. Chevalier, J. Klafter, B. Meyer and R. Voituriez (2010) Geometry-
498 controlled kinetics. *Nat. Chem.* **2**, 472-477.

499 61. Bouvier, B., J. P. Dognon, R. Lavery, D. Markovitsi, P. Millié, D. Onidas and K.
500 Zakrzewska (2003) Influence of conformational dynamics on the exciton states of DNA
501 oligomers. *J. Phys. Chem. B* **107**, 13512-13522.

502 62. Gustavsson, T. and D. Markovitsi (2021) Fundamentals of the Intrinsic DNA Fluorescence.
503 *Acc. Chem. Res.* **54**, 1226-1235.

504 63. Dai, L., Y. Xu, Z. Du, X.-D. Su and J. Yu (2021) Revealing atomic-scale molecular
505 diffusion of a plant-transcription factor WRKY domain protein along DNA. *Proc. Natl. Acad.*
506 *Sci. USA* **118**.

507 64. Armeev, G. A., A. S. Kniazeva, G. A. Komarova, M. P. Kirpichnikov and A. K. Shaytan
508 (2021) Histone dynamics mediate DNA unwrapping and sliding in nucleosomes. *Nature*
509 *Comm.* **12**.

510

511

512 **Table 1.** Concentrations of $(G^+)^\bullet$ and $(G-H2)^\bullet$ radicals present in G-Quadruplexes at 3 μ s after
 513 photoionization, expressed as percentage of $[e_{\text{hyd}}^-]_0$; error: $\leq \pm 5\%$.

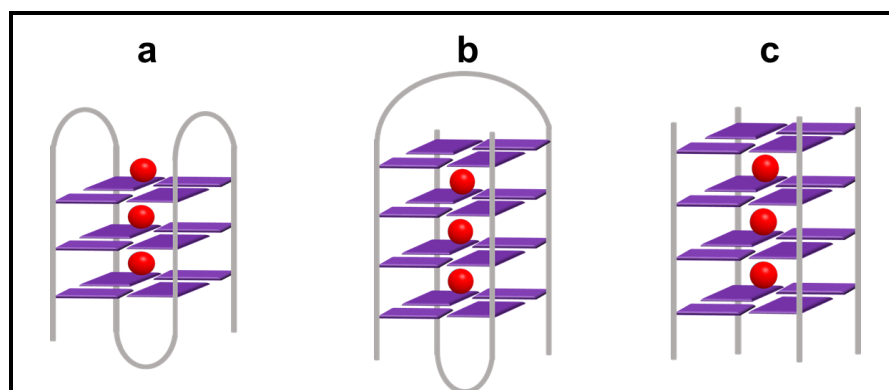
type	Sequence	G-Quadruplex	$(G^+)^\bullet$	$(G-H2)^\bullet$
monomolecular	TAGGG(TTAGGG) ₃ TT GGG(TTAGGG) ₃	TEL25/Na ⁺ † (8)	45	55
		TEL21/Na ⁺ † (7)	50	50
		TEL21/K ⁺ † (12)	60	40
bimolecular	GGGGTTTTGGGG	OXY/Na ⁺ ††	45	55
		OXY/K ⁺ (13) ††	30	70
tetramolecular	TGGGGT	(TG ₄ T) ₄ /Na ⁺ (9)	25	75
		(TG ₄ T) ₄ /K ⁺ (11)	40	60

514 †TEL: containing the human telomeric repeat TTAGGG; ††OXY: containing the
 515 oxytricha nova telomeric repeat TTTTGGGG.

516

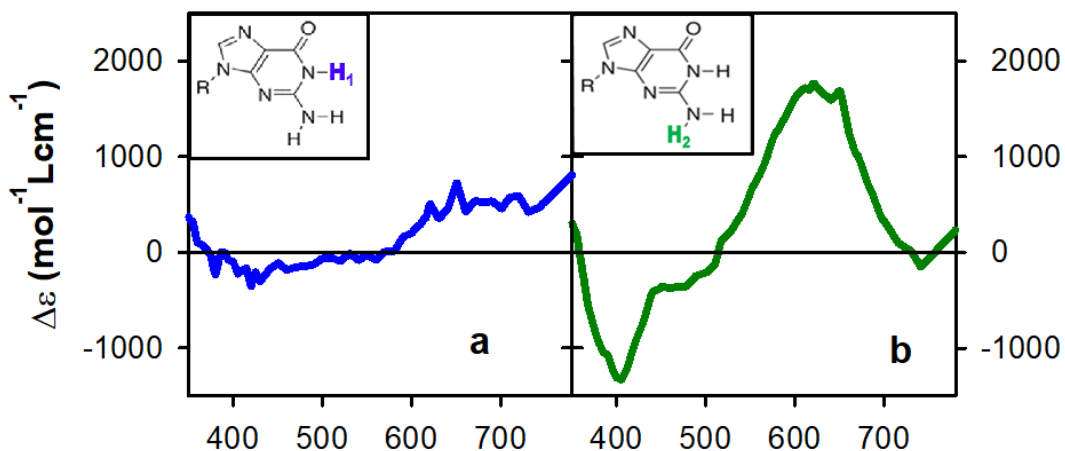
517 **FIGURE CAPTIONS**

518 **Figure 1.** Schematic illustration of the G-Quadruplexes discussed in this review: (a)
 519 monomolecular, formed by folding of a single strand, (b) bimolecular, formed by folding and
 520 association of two single strands and (c) tetramolecular, formed by association of four single
 521 strands; red spheres represent metal cations (Na^+ , K^+) located in the central cavity. Guanines
 522 are shown in violet and the backbone in grey.



523

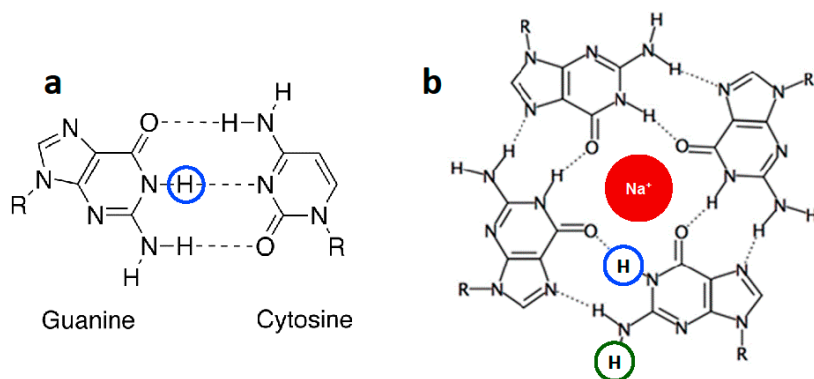
524 **Figure 2.** Spectral differences corresponding to $(\text{G}^+)^{\bullet} \rightarrow (\text{G-H1})^{\bullet}$ (a) and $(\text{G}^+)^{\bullet} \rightarrow (\text{G-H2})^{\bullet}$ (b)
 525 deprotonation, obtained by subtracting the spectrum of the radical cation from those of
 526 deprotonated radicals determined for monomeric guanosine derivatives (2, 48). The position of
 527 H1 and H2 hydrogen atoms is indicated in the insets.



528

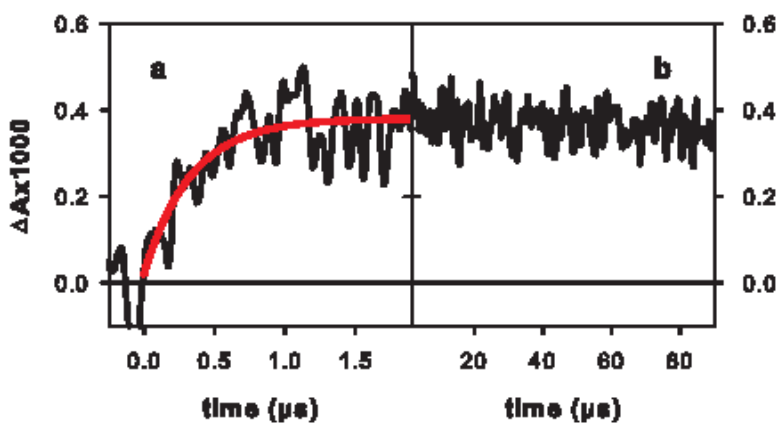
529

530 **Figure 3.** Location of the hydrogen atoms H1 and H2 in (a) a Watson-Crick guanine-cytosine
531 pair; (b) a tetrad composed of four guanines interconnected via Hoogsteen hydrogen bonds.



532

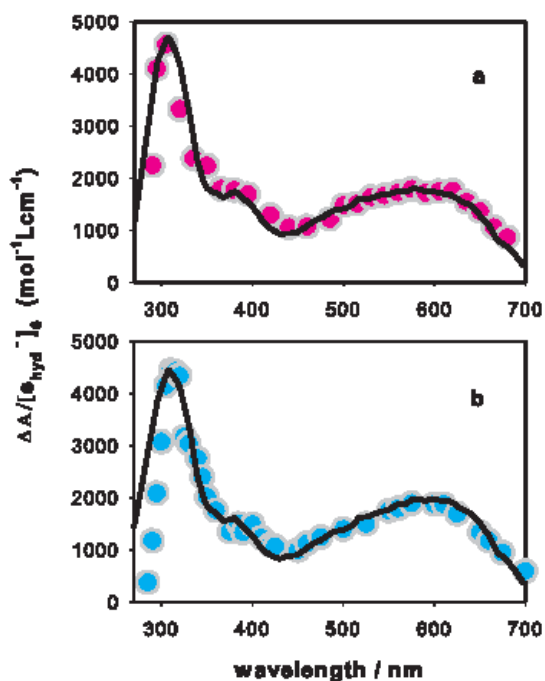
533 **Figure 4.** Transient absorption signals (black) recorded for N₂O-saturated solutions of CT-
534 DNA at 700 nm. The red line in (a) is derived from a fit with the model function $A_1(1-\exp(-$
535 $t/\tau_1))+A_0$ with $\tau_1 = 320 \pm 30$ ns. Adapted from reference 9 with ACS permission.



536

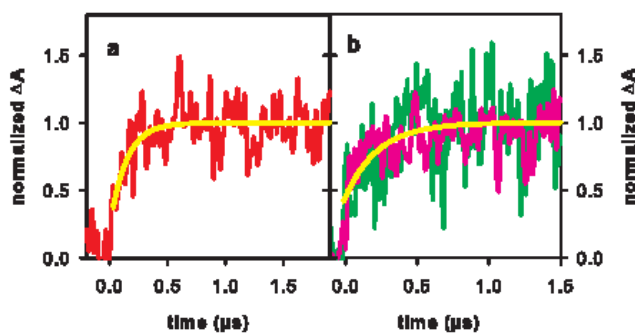
537

538 **Figure 5.** Reconstruction of the transient absorption spectra determined for G-Quadruplexes
 539 (circles) with linear combinations of the $(G^+)^{\bullet}$ and $(G-H2)^{\bullet}$ spectra reported for monomeric
 540 guanosine derivatives (solid lines), represented with their molar absorption coefficient (26, 48).
 541 (a) OXY/Na⁺ at 3 μs; $[(G^+)^{\bullet}]/[(G-H2)^{\bullet}] = 45/55$, $[(G^+)^{\bullet}] + [(G-H2)^{\bullet}] = [e_{\text{hyd}}^-]_0$. (b) TEL21/Na⁺
 542 at 25 μs; $[(G^+)^{\bullet}]/[(G-H2)^{\bullet}] = 20/80$, $[(G^+)^{\bullet}] + [(G-H2)^{\bullet}] = 0.85[e_{\text{hyd}}^-]_0$.



543

544 **Figure 6.** Deprotonation of in G-Quadruplexes on the sub-microsecond time scale. Transient
 545 absorption signals recorded for N₂O-saturated solutions of TEL21/K⁺ (red) (12), OXY/Na⁺
 546 (green) and OXY/K⁺ (pink) (13). Yellow lines correspond to fits with mono-exponential
 547 functions, $A_1(1-\exp(-t/\tau))+A_0$; $\tau = 150 \pm 15$ ns (a) and 200 ± 30 ns (b; adapted from reference
 548 (13) with ACS permission).



549

551 **Figure 7.** Reaction dynamics of $(G^+)^*$ in G-Quadruplexes on the microsecond time-scale. (a)
552 transient absorption signals recorded at 500 nm (green) and 600 nm (red) for $(TG_4T)_4/Na^+$;
553 their intensity has been arbitrarily scaled; reproduced from reference 24 with ACS permission.
554 Survival probability of the $(G^+)^*$ population (circles) in OXY/ Na^+ (b) and TEL21/ Na^+ (c),
555 determined from reconstruction of the time-resolved spectra, as shown in Figure 5. Black lines
556 correspond to fits with exponential functions $A_1(1-\exp(-t/\tau_1))+A_0$ (rise) and $A_1 \exp(-t/\tau_2)+A_0$
557 (decays) with time constants: $6 \pm 1 \mu s$ (a); $22 \pm 2 \mu s$ (b) and $7 \pm 0.5 \mu s$ (c).

