Enhanced Rigidity Changes Ultraviolet Absorption: Effect of a Merocyanine Binder on G-Quadruplex Photophysics

Davide Avagliano, Sara Tkaczyk, Pedro A. Sánchez-Murcia,* and Leticia González*

ABSTRACT: The urge to discover selective fluorescent binders to G-quadruplexes (G4s) for rapid diagnosis must be linked to understand the effect that those have on the DNA photophysics. Herein, we report on the electronic excited states of a bound merocyanine dye to c-Myc G4 using extensive multiscale quantum mechanics/molecular mechanics calculations. We find that the absorption spectra of c-Myc G4, both without and with the intercalated dye, are mainly composed of exciton states and mixed local/charge-transfer states. The presence of merocyanine hardly affects the energy range of the guanine absorption or the number of guanines excited. However, it triggers a substantial amount (16%) of detrimental pure charge-transfer states involving oxidized guanines. We identify the rigidity introduced by the probe in G4, reducing the overlap among guanines, as the one responsible for the changes in the exciton and charge-transfer states, ultimately leading to a redshift of the absorption maximum.

G-quadruplexes (G4s) are noncanonical secondary structures formed in nucleic acids where groups of four guanines interact via Hoogsteen base-pairing to form square structures, tetrads, that stack and are stabilized by a central metal cation,1,2 see Scheme 1. The formation of G4 motifs appears throughout the human genome and evinces essential functions in transcription, replication, stability, epigenetic regulation, as well as in cancer formation.3,4 G4s are present in the promoter regions of oncogenes, like in the protooncogene c-Myc,5 which regulates several elongation factors in cellular transcription. They also appear in viruses6 and have been discussed in the context of the pathogenicity of SARS-CoV-2 currently ravaging the world.7 While in the presence of UV radiation DNA/RNA nucleobases are able to efficiently dissipate the gained energy into heat in the environment,8 G4s can be damaged by the generation of guanine radical cations,9-13 thereby potentially affecting regulation of the transcription by c-Myc.14 Conversely, there is a strong drive to exploit the fluorescence of G4s in order to develop biomarkers for rapid non-destructive diagnosis.15 In this sense, small-molecule fluorescent probes are highly desirable tools to develop real-time diagnostics and also to monitor photo-oxidative lesions,6 as well as to understand the photophysics and photochemistry of G4 motifs themselves.16,17 However, the binding of chemical probes can affect the intrinsic chemical and physical properties of the G4, in both its electronic ground and excited states. Thus, only if the nature of the perturbation (structural, chemical, physical) induced by the probe is clearly identified is it possible to understand the native properties developed in G4 after light excitation. In other words, it might be possible to connect the difference in the absorption after binding, with the perturbation induced by the fluorescent probe.

Received: October 8, 2020
Accepted: November 11, 2020
Published: November 18, 2020

https://dx.doi.org/10.1021/acs.jpclett.0c03070
J. Phys. Chem. Lett. 2020, 11, 10212−10218
reversibly convert into a ring-opened merocyanine form. Intriguingly, the recently developed spiropyran decorated with a quinolizidine moiety (QSP, Scheme 2) does not bind DNA, but once it isomerizes to its open and protonated merocyanine form (QMCH), it binds strongly with c-Myc G4 in vivo.29 The closed QSP form emits at 458 nm and after QSP isomerizes to QMCH in the presence of c-Myc G4, its emission is drastically shifted to 610 nm. This visible change from blue to red allows the in vivo detection of G4 DNAs by the QSP/QMCH system. We recently investigated the QSP → QMCH isomerization reaction mechanism and the most probable binding mode of QMCH to G4, showing that QMCH "rigidiﬁes" G4, reducing its conformational ﬂexibility.30 In this binding mode, the probe is stacked to the upper tetrad of the G4 pocket, at the 3′-end, interacting mainly with the four guanines via π−π stacking, but also with the side nucleobases with non-covalent interactions30 (see Figure 1c). Without the probe, the guanines of G4 present high mobility, as shown in Figure 1a, but the presence of QMCH reduces the mobility of G4 so that the guanines stack more compactly and have less degree of movement—notice the unoccupied space between bases in Figure 1b, c. We hypothesize that the change on the mobility of c-Myc G4 may alter the overlap between the guanine electron densities, inﬂuencing the nature of its excited states, which is in line with recent experiments that suggest that restrained conformational changes are more important than the nature of the central cation or the folding topology in governing the excitation deactivation. In order to unveil this possibility, we characterize the nature of the electronic excited states of c-Myc G4 in the presence and absence of the ﬂuorescent merocyanine probe QMCH.

Classical and mixed quantum mechanics/molecular mechanics (QM/MM) molecular dynamics were used to sample the conformational space of a c-Myc G4-folded 22-mer single-stranded DNA chain and provided 100 initial conditions on which the lowest 60 excited states are calculated with time-dependent density functional theory (TD-DFT).32 Here, an electrostatic embedding QM/MM scheme was employed, where the MM part was represented as point charges and the QM region was calculated with the CAM-B3LYP33 functional with a def2-SVP34 basis set. The QM region includes the 12 guanines involved in the three tetrads, the QMCH probe (see Figure 1c), and two additional frontier nucleobases to avoid spurious effects (see the Supporting Information for further details). In summary, our ad-hoc protocol relies on extensive sampling to reproduce an experimental-like ensemble of geometries and on a quantitative analysis of the electronic excitations. The high number of excitations calculated (6000 for the whole ensemble of geometries) allows for statistical analysis of the electronic effect induced by absorption of light in the G4. Ultimately, the applicability of the same protocol to both the unprobed and probed system gives the possibility of a direct comparison of the electronic excitations of the G4 in the absence and presence of the external probe.

A simple way of visualizing electronic excitations is to consider that when light absorption promotes an excited electron (E) to an upper electronic state, an electron hole (H)
is created at the initial location of the electron. Depending on where H and E are located, the electronic excitations can be classified (see Figure 2) as (a) monomer-like or local excitations, where both H (empty circles) and E (full circles) are located on the same nucleobase; (b) exciton states, when more than one local excitation is present in more than one nucleobase; and (c) charge-transfer states, where the H and E are on different nucleobases. As implemented in the wavefunction analysis software TheoDORE, we additionally employ two parameters to discriminate between these states (also in Figure 2): the charge-transfer number (CT) and the number of donor (D) and acceptor (A) units. In a monomer, or local excitation, the H and E are fully localized on the same guanine unit, and thus, there is only one donor and one acceptor (D = A = 1) with no charge-transfer component (CT = 0, Figure 2a). In an exciton, the local excitations take place on different guanines, so that the number of D and A are still the same but larger than one (D = A > 1, Figure 2b).

The analysis of these descriptors in the c-Myc G4 shows that in the exciton states, the involved guanines also interact with each other. This implies that a fraction of the total density transfers from one monomer to another. Thus, CT can be anything in the range 0.1 to 0.9 (Figure 2b). In contrast, pure charge-transfer states have CT > 0.9 and the H and E are separated on different D and A units, but the number of D and A is the same; they are labelled with D = A = 1 (Figure 2c). These three scenarios usually coexist after light absorption in flexible multi-chromophoric systems, as was found by studying the fluorescent behavior of human telomeric G4 DNA. Here, the H and E can be localized on a different number of D and A units (D ≠ A) and different CT (0.1 < CT < 0.9) are possible. Accordingly, we label these states as mixed local/charge-transfer states (Figure 2d). In this case, some guanines are responsible of a local excitation while others induce electron density transfer between nucleobases, leading to different number of D and A participating units. Thus, these states are a combination of local, exciton, and charge-transfer states. As we will show below, this rich mosaic of excited states with diverse charge-transfer values and diverse spatial localizations will contribute differently to the UV spectrum of c-Myc G4 with or without the fluorescent probe.

The absorption spectrum of c-Myc G4 alone (without probe), obtained from 6000 excited states calculated from an ensemble of 100 geometries, is shown in Figure 3a (black line). It displays two peaks centered at 4.8 and 5.4 eV, respectively. We additionally deconvoluted the spectrum according to the contributions given by the monomer local excitations (blue line), exciton states (violet line), and mixed states (green line). Noteworthy, pure charge-transfer states (D = A = 1, CT > 0.9) do not exist in our ensemble of vertical excitations because a small contribution of local excitations is always present throughout all the excitations. The contribution of monomer or local excitations alone is small; it peaks at circa 5.0 eV and corresponds to local guanine π → π* excitations. Thus, the relevant electronic excitations underlying both peaks are excitonic and/or mixed states excitations but with an important difference: whilst the excitonic excited states are significant at lower energies, the mixed states dominate the spectrum at high energies.

We can compare the computed absorption spectrum with a convoluted spectrum of the density of excited states (Figure 3b), which includes all excited states regardless of their brightness. We decompose it also into local, exciton, and mixed excitations. Additionally, we discriminate exciton and mixed states according to their amount of charge-transfer contributions (CT < 0.5 and CT > 0.5). As observed in the absorption

**Figure 2.** Schematic representation of the excited states formed in a G-quadruplex. Each rectangle represents a single guanine in a tetrad. Empty and full circles denote an electron hole and an excited electron, respectively. The number of electron donor (D) and acceptor (A) fragments involved in each type of excited state and their charge-transfer number (CT) is indicated for each type of electronic state.

**Figure 3.** (a) UV absorption spectra of c-Myc G4 (black) and decomposition according to different excitations contributions. (b) Corresponding density of states decomposed according to the nature of excitations, also attending to the amount of charge transfer. (c) UV absorption spectrum of c-Myc G4 in the presence of QMCH (black line) and decomposition according to different excitations contributions. (d) Corresponding density of states decomposed according to the nature of excitations, also attending to the amount of charge transfer.
spectrum, exciton states prevail at low energies, but they have mostly low charge transfer (CT < 0.5, dashed line) while only a little amount of excition states with high charge transfer (CT > 0.5, dotted line) is found at high energies. The mixed states, which dominate the density of states spectrum, have significant charge-transfer character at high energy (dotted line), while those with small charge transfer (dashed line) are equally distributed behind the two peaks.

We can therefore conclude that the two peaks observed in the absorption spectrum of c-Myc G4 correspond to excited states that differ in their excitation length and in their amount of charge transfer. The first one at 4.8 eV, less intense, is dominated by local, exciton and mixed states with low charge-transfer character. The second one at 5.4 eV is mostly composed of mixed states with strong charge-transfer character between different guanines. In the molecular orbital picture it means that a high fraction of density transfer (CT > 0.5) leads to a blue-shift in the absorption peak with respect of the local π → π* guanine absorption band. That signifies that the G4 acts as a H-like molecular aggregate, shifting the maximum of the absorption to the blue, with respect to the single local guanine excitation, once the guanines are compacted in the tetrads. States with small charge-transfer character can be found in any region of the spectrum, although they are most relevant at low energies. In spite of the large amount of charge-transfer character contributing to the high energy peak at 5.4 eV, something that is known to lead to dark excited states in systems, highly prompted to absorb UV/vis light. Indeed, in extended conjugated system, QMCH is, as other cyanine radicals, as a precursor of oxidative damage of the genetic code, can be generated by absorption of low energy UV light. Since G4 is a structure with propensity to form such experimentally observed guanine photo-oxidation, this would be enhanced by the interaction with the probe. On the other hand, the possible induced damage of the genetic code could have important consequences for applications in photoinduced therapy. Therefore, we believe that despite challenges, an investigation of the dynamics of such process will be of high interest in the future.

The presence of the QMCH also leads to the appearance of few mixed guanine/probe states, where the electron density can be both transferred from the guanine to the probe (G → P) and vice versa (P → G). These states show a very small oscillator strength and represent a small percentage of the total amount of excitation (3% each). Altogether, from an electronic point of view, merocyanine does not affect particularly the energy range of the guanine absorption because the largest group of excitations (60%) is represented by excited states involving only guanines (local/exciton/mixed states within G4, Table 1), whose absorption energy range is unshifted upon binding. In the following, we shall analyze in detail this region of the QMCH:G4 electronic absorption spectrum in order to discern the effect of the probe on the character of the G → G excitations. Example excitations of the G4:QMCH complex can be found in Figure S3.

We now analyze whether the number of guanines that participate in the electronic excitations changes, in the absence or presence of the probe, and whether it leads to differences in the two spectra. To this purpose, we employ the electron delocalization length (DEL) descriptor, which indicates over how many guanines an excitation is delocalized (see Supporting Information). We focus on the exciton and mixed states, as they are the relevant states in the absorption spectrum of c-Myc G4. Figure 4 displays the number of guanines involved in each of the states of G4 (Figure 4a) and of G4:QMCH (Figure 4b). Unexitingly, the differences are negligible, meaning that QMCH does not affect the DEL distributions, neither for exciton nor for mixed states. However, much more interesting is to see that the number of excited guanines is very different in the exciton and in the mixed states. While in the former, half of the population is found in two guanines (D = A = 2), the mixed states are mostly delocalized in three guanine units (ca. 30 %) but can reach up to nine different guanines, i.e., at least three tetrads can be simultaneously involved in one excitation. Excluding the role of

| Table 1. Excitations Found in the Electronic Absorption Spectrum of the Complex G4:QMCH |
|---|
| excitation | E (eV) | $f_{osc}$ | percentage of state (%) |
| local excitations within probe (P, first absorption band) | 2.4–3.4 | ~1 | 11 |
| local excitations within probe (P, second absorption band) | 4.5–5.9 | <0.100 | 7 |
| pure charge-transfer states (G → P) | 3.1–3.9 | <0.006 | 16 |
| mixed states (P → G) | 3.9–4.5 | <0.004 | 3 |
| mixed states (G → P) | 4.7–6.0 | <0.013 | 3 |
| local/exciton/mixed excitations within G4 (G) | 4.3–6.0 | <0.400 | 60 |

*Energy range of absorption (E, eV), oscillator strength ($f_{osc}$), and their percentage with respect of the total number of excitation (%). P is the probe (QMCH) and G is guanine.*
the DEL, we can conclude that it is the rigidity imposed by the probe (recall Figure 1a, b) that is responsible for the different absorptions at 4.5 and 5.5 eV of the two systems.

Figure 3c shows the computed UV absorption spectrum of G4 in the presence of QMCH (black line). It appears in the same energy range as G4 (Figure 3a), but there is an inversion on the relative intensities of the two absorption peaks. The absolute absorption maximum (~5.0 eV) is now the lowest-energy peak, and it is more intense than the one still centered at 5.4 eV. The low-energy region though has a higher density of states (black line, Figure 3d). In detail, we see that the number of exciton and mixed states with CT < 0.5 increases at low energies (dashed lines), while at high energy, mixed and exciton states with CT > 0.5 show comparable density of states (dotted lines) as without the probe. In the same region, the net number of exciton and mixed states with CT < 0.5 decreases (dashed lines), leading to an absolute higher number of exciton and mixed states in the low energy peak. The presence of QMCH affects mainly the oscillator strength of the mixed states at high energy, reducing their brightness, and promotes the formation of exciton and mixed states of small charge-transfer character in the low energy region. The intrinsic flexibility of the G4 promotes bright excited states at high energies with a strong charge-transfer character (mainly mixed states) and, thereby, with a high transfer of electron density among the involved fragments. Nevertheless, this situation changes upon binding of the probe. There is a reduction in the absorbance of the high energy peak due to the rigidity imposed by the dye, which stabilizes excitonic and mixed states with small charge transfer, thereby red-shifting the main absorption peak to 5.0 eV. The presence of the probe induces an external perturbation, which in turn induces a different absorption of the DNA. Knowing the nature of the perturbation, in this case the reduced mobility of the guanines, we are able to trace back the properties responsible of the UV/vis absorption of the system. The overlapping of numerous chromophores leads to the presence of mixed states, combining local, exciton, and charge-transfer states, and with a strong coupling between exciton and charge-transfer states. We showed how this overlapping is necessary for the population of mixed states with high charge transfer, responsible for the absorption at 5.4 eV of the unprobed G4 and the aggregate-like behaviour. Once the probe is bound, the absorption is shifted to the red, missing this requirement for the main absorption at those energies.

In conclusion, we have investigated for the first time the UV/vis absorption spectrum of c-Myc G4. We have characterized the absorption spectrum in terms of local, exciton, and mixed local/charge-transfer states and evaluated the impact of binding of a merocyanine binder on the photophysics of c-Myc G4. The probe does not affect either the energy range of G4 guanines absorption or the extent of the delocalization of the excited states, but its oxidative nature induces the formation of guanine oxidative states. Accordingly, the binding changes remarkably the photophysics of c-Myc G4 in the UV region. The probe induces an enhanced conformational rigidity on G4, altering the yield of exciton and mixed states absorption, ultimately leading to a global redshift in the G4 absorption maximum. These finding points out the importance of the structural flexibility in the photophysics of G4 DNA structures.

The characterization of the excitations involving the G4 binder will be helpful to functionalize fluorescent probes with optimally tuned photophysical properties. For instance, if the probe is modified so that the population of mixed/charge-transfer states is promoted, this species could evolve via non-emissive pathways and potentially react with G4. On the contrary, if functionalization promotes the population of electronic states that can relax to lower energy states, then fluorescence will be reinforced. Identifying these mixed states is thus interesting to rationalize the effect on the fluorescence of the probe. This could be an attractive avenue to explore in the future, both theoretically and experimentally. Further, our results can contribute to understand the photochemistry of related G4-binders as well as motivate the study of the temporal evolution of these excited states from both computational and experimental points of view.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.0c03070.
Extended computational details, additional UV spectra, and natural transition orbitals (PDF)

- **AUTHOR INFORMATION**

**Corresponding Authors**
Pedro A. Sánchez-Murcia – Institute of Theoretical Chemistry, Faculty of Chemistry, University of Vienna, A-1180 Vienna, Austria; orcid.org/0000-0001-8415-870X; Email: pedro.murcia@medunigraz.at

Leticia González – Institute of Theoretical Chemistry, Faculty of Chemistry and Vienna Research Platform on Accelerating Photoaction Discovery, University of Vienna, A-1180 Vienna, Austria; orcid.org/0000-0001-5112-794X; Email: leticia.gonzalez@univie.ac.at

**Authors**
Davide Avagliano – Institute of Theoretical Chemistry, Faculty of Chemistry, University of Vienna, A-1180 Vienna, Austria

Sara Tkaczyk – Institute of Theoretical Chemistry, Faculty of Chemistry, University of Vienna, A-1180 Vienna, Austria

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jpcllett.0c03070

**Notes**
The authors declare no competing financial interest.

- **ACKNOWLEDGMENTS**

The authors are thankful for funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 765266 (LightDynAMics). The Vienna Scientific Cluster (VSC) is gratefully acknowledged for its generous allocation of computational resources. Basile F.E. Curchod and Lea M. Ibele are thanked for stimulating discussions.

- **REFERENCES**

(1) Williamson, J. R. G-Quartet Structures in Telomeric DNA. *Annu. Rev. Biophys. Biomol. Struct.* 1994, 23, 703–730.

(2) Burge, S.; Parkinson, G. N.; Hazel, P.; Todd, A. K.; Neidle, S. Quadruplex DNA: Sequence, Topology and Structure. *Nucleic Acids Res.* 2006, 34 (19), 5402–5415.

(3) Varshney, D.; Spiegel, J.; Zyen, K.; Tannahill, D.; Balasubramanian, S. The Regulation and Functions of DNA and RNA G-Quadruplexes. *Nat. Rev. Mol. Cell Biol.* 2020, 21 (8), 459–474.

(4) Guilbaud, G.; Murat, P.; Recolin, B.; Campbell, B. C.; Maiter, A.; Sale, J. E.; Balasubramanian, S. Local Epigenetic Reprogramming Induced by G-Quadruplex Ligands. *Nat. Chem.* 2017, 9 (11), 1110–1117.

(5) Siddiqui-Jain, A.; Grand, C. L.; Bearss, D. J.; Hurley, L. H. Direct Evidence for a G-Quadruplex in a Promoter Region and Its Targeting with a Small Molecule to Repress c-MYC Transcription. *Proc. Natl. Acad. Sci. U. S. A.* 2002, 99 (18), 11593–11598.

(6) Ruggiero, E.; Richter, S. N. G-Quadruplexes and G-Quadruplex Ligands: Targets and Tools in Antiviral Therapy. *Nucleic Acids Res.* 2018, 46 (7), 3270–3283.

(7) Hognon, C.; Miclot, T.; García-Iriepa, C.; Francés-Monerris, A.; Grandemanche, S.; Terenzi, A.; Marazzi, M.; Barone, G.; Monari, A. Role of RNA Guanine Quadruplexes in Favoring the Dimerization of SARS Unique Domain in Coronavirus. *J. Phys. Chem. Lett.* 2020, 11 (14), 5661–5667.

(8) Barbatti, M.; Barone, V.; Borin, A. C.; Crespo-Hernández, C. E.; Vries, M. S. de; Giusansen, A.; González, L.; Hochlafl, M.; Improta, R.; Mai, S.; et al. Photoinduced Phenomena in Nucleic Acids I; Barbatti, M., Ullrich, S., Borin, A. C., Eds.; Springer: Heidelberg, 2015.

(9) Balanikas, E.; Banyasz, A.; Baldacchino, G.; Markovitsi, D. Populations and Dynamics of Guanine Radicals in DNA Strands-Direct versus Indirect Generation. *Molecules* 2019, 24 (13), 2347.

(10) Banyasz, A.; Balanikas, E.; Martinez-Fernandez, L.; Baldacchino, G.; Douki, T.; Improta, R.; Markovitsi, D. Radicals Generated in Tetramolecular Guanine Quadruplexes by Photoionization: Spectral and Dynamical Features. *J. Phys. Chem. B* 2019, 123 (23), 4950–4957.

(11) Hall, J. P.; Poynton, F. E.; Keane, P. M.; Gurung, S. P.; Brazier, J. A.; Cardin, D. J.; Winter, G.; Gunnlaugsson, T.; Sazanovich, I. V.; Towrie, M.; Cardin, C. J.; Kelly, J. M.; Quinn, S. J. Monitoring One-Electron Photo-Oxidation of Guanine in DNA Crystals Using Ultrafast Infrared Spectroscopy. *Nat. Chem.* 2015, 7 (12), 961–967.

(12) Wu, L.; Liu, K.; Jie, J.; Song, D.; Su, H. Direct Observation of Guanine Radical Cation Deprotonation in G-Quadruplex DNA. *J. Am. Chem. Soc.* 2015, 137 (1), 259–266.

(13) Banyasz, A.; Martinez-Fernandez, L.; Balty, C.; Perron, M.; Douki, T.; Improta, R.; Markovitsi, D. Absorption of Low-Energy UV Radiation by Human Telomere G-Quadruplexes Generates Long-Lived Guanine Radical Cations. *J. Am. Chem. Soc.* 2017, 139 (30), 10561–10568.

(14) Fleming, A. M.; Burrows, C. J. Interplay of Guanine Oxidation and G-Quadruplex Folding in Gene Promoters. *J. Am. Chem. Soc.* 2020, 142 (3), 1115–1136.

(15) Kozitsina, A. N.; Svalova, T. S.; Malysheva, N. N.; Ohkohkonin, A. V.; Vidrevich, M. B.; Brainina, K. Z. Sensors Based on Bio and Biomimetic Receptors in Medical Diagnostic, Environment, and Food Analysis. *Biosensors* 2018, 8 (2), 35.

(16) Li, Q.; Xiang, J. F.; Yang, Q. F.; Sun, H. X.; Guan, A. J.; Tang, Y. L. G4LDB: A Database for Discovering and Studying G-Quadruplex Ligands. *Nucleic Acids Res.* 2013, 41 (D1), D1115–D1123.

(17) Mannia, S.; Srivatsan, S. G. Fluorescence-Based Tools to Probe G-Quadruplexes in Cell-Free and Cellular Environments. *RSC Adv.* 2018, 8 (45), 25673–25694.

(18) Dai, J.; Carver, M.; Hurley, L. H.; Yang, D. Solution Structure of a 2:1 Quindoline-c-MYC G-Quadruplex: Insights into G-Quadruplex-Interactive Small Molecule Drug Design. *J. Am. Chem. Soc.* 2011, 133 (44), 17673–17680.

(19) Deiana, M.; Mettra, B.; Martinez-Fernandez, L.; Mazur, L. M.; Pawlik, K.; Andraud, C.; Samoc, M.; Improta, R.; Monnereau, C.; Matczynski, K. Specific Recognition of G-Quadruplexes over Duplex-DNA by a Macromolecular NIR Two-Photon Fluorescent Probe. *J. Phys. Chem. Lett.* 2017, 8 (23), 5915–5920.

(20) Di Antonio, M.; Ponjavic, A.; Radzevičius, A.; Ranasimghe, R. T.; Catalano, M.; Zhang, X.; Shen, J.; Needham, L. M.; Lee, S. P.; Klenerman, D.; Balasubramanian, S. Single-Molecule Visualization of DNA G-Quadruplex Formation in Live Cells. *Nat. Chem.* 2020, 12 (9), 832–837.

(21) Improta, R. Quantum Mechanical Calculations Unveil the Structure and Properties of the Absorbing and Emitting Excited Electronic States of Guanine Quadruplexes. *Chem. - Eur. J.* 2014, 20 (26), 8106–8115.

(22) Saravanan, V.; Rajamani, A.; Subramaniam, V.; Ramasamy, S. Interaction of (G4)2 and (X4)2 DNA Quadruplexes with Cu+, Ag+ and Au+ Metal Cations: A Quantum Chemical Calculation on Structural, Energetic and Electronic Properties. *Struct. Chem.* 2020, 31 (1), 465–484.

(23) Martinez-Fernandez, L.; Changenet, P.; Banyasz, A.; Gustavsson, T.; Markovitsi, D.; Improta, R. Comprehensive Study of Guanine Excited State Relaxation and Photoactivity in G-Quadruplexes. *J. Phys. Chem. Lett.* 2019, 10 (21), 6873–6877.

(24) Martinez-Fernandez, L.; Esposito, L.; Improta, R. Studying the Excited Electronic States of Guanine Rich DNA Quadruplexes by Quantum Mechanical Methods: Main Achievements and Perspectives. *Photochem. Photobiol. Sci.* 2020, 19 (4), 436–444.
