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2015

Lech, C. J., Phan, A. T., Michel-Beyerle, M. E., & Voityuk, A. A. (2015). Influence of base stacking geometry on the nature of excited states in G-quadruplexes : a time-dependent DFT study. *The Journal of Physical Chemistry B*, 119(9), 3697-3705.

<https://hdl.handle.net/10356/98114>

<https://doi.org/10.1021/jp512767j>

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Influence of Base Stacking Geometry on the Nature of Excited States in G-Quadruplexes: A Time-Dependent DFT Study

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Keywords: G-quadruplex, excited states, exciton delocalization, charge transfer, charge separation, TDDFT, Molecular dynamics

Abstract

G-quadruplexes are four-stranded structures that are formed from the association of guanine nucleobases into cyclical arrangements known as tetrads. G-quadruplexes are involved in a host of biological processes and are of interest in nanomaterial applications. However, not much is known about their electronic properties. In the paper, we analyze electronic excited states of G-quadruplexes using a combination of time-dependent DFT calculations and molecular dynamics calculations. We systematically consider experimentally observed arrangements of stacked guanine tetrads. The effects of structural features on exciton delocalization and photo-induced charge separation are explored using a quantitative analysis of the transition electron density. It is shown that collective coherent excitations shared between two or more guanine nucleobases dominate in the low-energy region of the spectrum. These excitations may also include a significant contribution of charge transfer states. Large variation in exciton localization is also observed between different structures with a general propensity towards localization between two bases. We reveal large differences in how charge separation occurs within different nucleobase arrangements, with some geometries favoring separation within a single tetrad and others favoring separation between tetrads. We also demonstrate that the coordinating K^+ ion located in the central cavity of G-quadruplexes does not have a large influence on the relative excited state properties of such systems. Our results demonstrate how the nature of excited states in G-quadruplexes depends on the nucleobase stacking geometry resulting from the mutual arrangement of guanine tetrads.

Introduction

Electronic interactions between stacked nucleobases within oligonucleotides significantly alter their excited-state properties, in particular excited-state dynamics and photostability.¹⁻⁵ In addition, electronic interactions between bases lead to exciton delocalization and partial charge separation.⁶⁻⁸ Isolated nucleobases have excited state lifetimes in the sub-picosecond regime, which are responsible for DNA photostability.¹⁻⁴ However, in DNA oligomers, long-lived states of up to a nanosecond are observed.¹⁻⁴ Charge transfer states and excited state delocalization over two or more nucleobases appear to be responsible for excited state decay occurring on picosecond to nanosecond timescales. The role of such states has been explored in both experimental¹⁻⁹ and theoretical¹⁰⁻¹³ studies.

Figure 1. (A) G-tetrad formed from the cyclical hydrogen bonding of four guanine bases. (B) Stacked G-tetrads with coordinating cations constitute the core of G-quadruplex nucleic acids.

In comparison to duplex DNA, not much is known on the nature of excited states of G-quadruplexes, which are known to play important roles in biological systems^{14,15} G-quadruplexes are four-stranded nucleic acid structures formed from the association of guanine bases.

Sequences that adopt G-quadruplexes structures are found throughout the human genome, including gene promoters, telomeres, and G-rich repeat sequences.^{15,16} Additionally, the small-molecule targeting of G-quadruplex forming sequences in the telomere on oncogenic promoters is an attractive anti-cancer strategy.¹⁷ Understanding the electronic properties of G-quadruplexes is valuable considering the relatively low oxidation potential of guanines within these structures. In vivo, the formation of G-quadruplexes could act as thermodynamic sinks for electron holes, preventing the formation of radical cation states and resulting DNA damage to information-rich regions of the genome.^{18,19} The electronic properties of nucleobase geometries within these structures is also important from a technological perspective, as G-quadruplexes can form conducting nano-scale wires.²⁰

Unlike the Watson-Crick base pairing found in double-stranded DNA, the building block of the G-quadruplex architecture is the G-tetrad in which four guanine bases are bound in a cyclical and planar fashion (Fig. 1A).²¹ Within a G-quadruplex, multiple G-tetrads stack upon one another through π -stacking interactions and are stabilized by cation coordination (Fig 1B). There are five different discrete modes of G-tetrad stacking experimentally known to occur within G-quadruplexes (Fig. 2).²² Stacking modes *A*, *B* and *C* arise within the core of a single G-quadruplex unit, whereas stacking modes *D* and *E* have only been observed to occur at the interface of two stacked G-quadruplex. Moreover, mode *A* occurs from tetrads stacked with a similar directionality of their hydrogen bond pattern, whereas modes *B-E* arise from tetrads stacking with opposite hydrogen bond directionality.

Figure 2. Guanine base stacking geometries for G-tetrad stacking modes experimentally observed within G-quadruplex structures. In this work, stacking modes are referred to by their corresponding *A-E* designation.

The impact of different factors on the electronic properties of G-quadruplexes has been explored in several experimental²³⁻²⁵ and computational studies^{26,27} (see also review²⁸ and references therein). A comprehensive TD-DFT study²⁷ showed that the photoexcited dynamics of G-quadruplexes is strongly affected by their topology. In particular, excited states are found to be delocalized over multiple bases, whereas the emission involves a stacked dimer or a monomer.²⁷ The heavily overlapped aromatic rings of mode *E* gives rise to unique electronic interaction between stacked G-tetrads. This particular stacking mode has been previously computed to have enhanced coupling and electron hole transfer between stacked bases²³ and has been shown experimentally to cause nanosecond excimer fluorescence in the 400 nm regime.²³ These fluorescence effects were only observed when the geometry of mode *E* was adopted between two G-quadruplex units, the interaction of which could be modulated by varying parameters including DNA flanking sequence, salt concentration and temperature.²³

Characterizing the nature of electronic excitation in a system of stacked G-tetrads is made complicated by the large number of chemically identical nucleobases and the ways in which they interact. Both exciton delocalization and inter-base charge transfer arise due base-base interactions occurring within a single tetrad and between stacked tetrads. It remains unclear how large the contributions of charge transfer states are in the low-energy region of the spectrum, and how these contributions depend on the stacking pattern of G-tetrads. In a model G-quadruplex consisting of two stacked G-tetrads, all guanine bases are almost equivalent, although their energies vary due to structural fluctuations. These energetic similarities make it difficult to explore their excited states. To illustrate the problem, let us consider the simple 4-state system shown in Figure 3.

Figure 3. Local excitations (A^*B and AB^*) and charge transfer states (A^-B^+ and A^+B^-) within a system containing two molecules A and B.

Only single excitations between HOMO and LUMO of each site are considered. Adiabatic excited states Ψ_i ($i=1\dots4$) of the system are represented by linear combinations of two locally excited states A^*B and AB^* and two charge transfer states A^+B^- and A^-B^+ .

$$\Psi_i = c_{1i}\psi_{A^*B} + c_{2i}\psi_{AB^*} + c_{3i}\psi_{A^+B^-} + c_{4i}\psi_{A^-B^+} \quad (1)$$

Several distinct situations can be obtained: **(I)** An excitation can be completely localized to the excited fragment. For instance, if $|c_{1i}| \approx 1$ then Ψ_i corresponds to the locally excited state A^*B . **(II)** Excitonic states can be delocalized over A and B and described by a combination of A^*B and AB^* states. For example, when $|c_{1i}| \approx |c_{2i}| \approx 1/\sqrt{2}$, the excitonic state Ψ_i is evenly delocalized over fragments A and B. **(III)** If contributions of both A^*B and AB^* are small, then Eq. (1) represents a pure charge transfer state. When the charge separated (CS) states A^+B^- and A^-B^+ have different energies, they do not mix and the states $\Psi_i = \psi_{A^+B^-}$ and $\Psi_j = \psi_{A^-B^+}$ can be referred to as charge transfer (CT) states, the excitation to which will result in a net charge separation (CS). These states can be easily identified by comparing the dipole moment of the ground and excited states. **(IV)** If the molecules A and B are energetically similar, the situation is more complicated because the states A^+B^- and A^-B^+ contribute equally to the CT states, $\Psi_{1,2} = \frac{1}{\sqrt{2}}(\psi_{A^+B^-} \pm \psi_{A^-B^+})$. In this case, the net charges on A and B are equal to zero and no charge separation occurs despite the CT that occurs during excitation. Such CT states are difficult to distinguish from excitonic states of situations **I** and **II**.

Overall, both local excitations and CT states may give comparable contributions into the adiabatic states. Therefore, a special computational approach is required for a quantitative analysis of the nature of excited states in systems containing several guanines. It was shown that the transition electronic density can be used to compute the difference of electron density distribution in molecular systems by electronic excitation.²⁹⁻³¹ In this work, we apply a recently developed approach³¹ to describe excited state properties of stacked G-tetrads adopting different stacking modes.

The computed electronic properties of a complex system may significantly depend on the choice of model employed in the calculation. In particular, using the standard geometry for nucleobases in quantum mechanical calculations of G-quadruplexes will provide excited states for idealized systems. Data of idealized systems can deviate from the results obtained for models that consider the dynamic nature of structure by including the effects of structural fluctuations of the system.^{13,26} With this in mind, we use Molecular Dynamics (MD) simulations as a starting point for our quantum chemical calculations. MD simulations have been used in the past to provide valuable information about the structure of G-quadruplexes.^{32,33} In the current study, we consider 10 sets of atomic coordinates for each of the five modes of G-tetrad stacking (Fig 2) extracted from the MD trajectories for a total of 50 tetrad stacks. On the basis of TD DFT calculations, we analyze in detail the nature of electronically excited states in different G-tetrad stacks.

We show that collective excitations shared between several bases may include significant contributions of CT states, the nature of which depends on the stacking geometry. The corresponding oscillator strengths, indicating the propensity for excitation, are found to be strong enough to populate these states. Our calculations suggest that the stacking mode *E* favors the formation of CT excited states in the low-energy region. The propensity for CS to occur during excitation along with how charge separates within the system varies highly amongst stacking modes.

Methods

Molecular Dynamics Simulation

The starting point for obtaining stacked G-tetrads for our quantum chemical study were molecular dynamic (MD) simulations of three different systems containing G-quadruplex oligomer. The NMR-based structure of the sequence $d[T_2(G_3T)_4]$ ³⁴ was modified by removing the flanking nucleotides to generate a parallel G-quadruplex formed by the sequence $d[(G_3T)_3G_3]$ with exposed external tetrads. Two single G-quadruplex units were then stacked on their 5'-5' tetrad interface (System 1) or their 3'-3' tetrad interface (System 2) to form dimeric G-quadruplex structures that underwent MD simulation. The anti-parallel G-quadruplex formed by the sequence $d[(G_4T_4G_4)_2]$ ³⁵ also underwent MD simulation (System 3). The parallel G-quadruplexes of Systems 1 and 2 allow the extraction of stacking mode *A* while the anti-parallel G-quadruplex of System 3 allows for the investigation modes *B* and *C*. Stacking mode *D* is obtained from the 3'-3' stacking interface of two G-quadruplex units in System 2 and mode *E* is obtained from the 5'-5' interface of System 1.

Potassium cations (K^+) were added to the central channel of starting structures in between stacked G-tetrads. Systems were then neutralized by adding additional K^+ to the exterior of the G-quadruplexes. Systems were then solvated using a truncated octahedral box of TIP3P³⁶ water molecules added to a 10Å radius from the solute. Systems were simulated using periodic boundary conditions on constant pressure (1 bar) and temperature (300 K) using the algorithm of Berendsen et al.³⁷ Covalent hydrogen bonds were constrained using SHAKE³⁸ at 2 femtosecond time step. Long-range electrostatic interactions were calculated using the particle mesh Ewald method,³⁹ with non-bonded interactions considered within a 9Å cutoff and a non-bonded pair list updated at 10 picosecond intervals in which the center-of-mass motion was removed.

Prior to unrestrained minimization, Systems underwent a series of restrained minimizations. They were first minimized using 25 kcal.mol⁻¹.Å⁻² restraints on DNA and central K^+ ions through 500 steps of steepest decent minimization followed by 500 steps of conjugated gradient minimization. Systems were then heated from 100 K to 300 K over 10 ps then maintained at constant volume over 90 ps at 300 K while maintaining aforementioned solute restraints.

Systems then underwent cycles of minimization and equilibration during which the restraints were gradually reduced to 5, 4, 3, 2, 1, and 0.5 kcal.mol⁻¹.Å⁻². Unrestrained simulations were then run for Systems 1 and 2 for 200 ns in order to properly sample base stacking modes *D* and *E*. System 3 was simulated unrestrained for 5 ns.

MD simulations were carried out using AMBER 10 software⁴⁰ with the Parmbsc0 correction to the Cornell et al. force.^{41,42} More recent parameterizations including corrections to χ ,⁴³ ϵ/ζ backbone angles,⁴⁴ and recent refinement to base stacking energetics⁴⁵ were not considered. Choice of MD force field made in order to maintain consistency with our recently published study on electron hole transport through G-quadruplex.²⁶ Exclusions of these corrections should not have substantial effects on the geometric sampling of stacked G-tetrads or their relative electronic properties.

Model Building

Atomic coordinates of G-tetrads were extracted from MD simulations to generate the systems investigated in our quantum chemical study. Segments of MD trajectory were identified from in which neighboring guanine tetrads adopted one of the five G-tetrad stacking geometries depicted in Figure 2. The coordinates of stacked G-tetrads and their central coordinating K⁺ ion were extracted at 100ps intervals over 1ns of MD simulation time for a total of 10 coordinate sets for each stacking mode. In the conversion from oligomer to isolated nucleobases, the C1' of the ribose sugar was replaced with a hydrogen atom with an adjusted bond length. Therefore, the entire atomic system considered in our quantum chemical study includes 8 guanine bases and 1 coordinating K⁺ ion as shown in Supporting Figure S1.

The choice of taking 10 coordinate sets was made to balance time consuming QM calculations while investigating the effects of thermal fluctuations on the electronic properties of our systems. Sampling over a 1 ns segment of MD trajectory allowed us to examine the effects of base fluctuations occurring in ps time scales on electronic properties, while maintaining a single stacking mode, which may vary between two tetrads over the entirety of an MD simulation.

Quantum Chemical Computations

Excited states of the systems were calculated using the TD DFT method⁴⁶ with the CAM-B3LYP functional.^{47,48} The B3LYP functional performs well when computing excited states of individual molecules.⁴⁹ However, this scheme underestimates the energy of CT transitions. The long-range corrected functional CAM-B3LYP provides an appropriate description of the CT states. The standard 6-31G* basis set was employed in our calculations. Computations were performed using Gaussian 9 software.⁵⁰

To compare the performance of the TD DFT method with the benchmark EOM-CCSD methodology,⁵¹ we computed the spectrum of guanine using the MBPT(2)//cc-pVDZ optimized geometry (Atomic coordinates were taken from the Supporting information of Ref. 51). The spectrum obtained for isolated guanine using CAM-B3LYP data is similar to that based on the EOM CCSD results,⁵¹ but is shifted by 0.15 eV towards higher energies (Fig. 4). Such shifts will affect the absolute energies of excited states but should not largely impact the relative energies computed for different stacking orientations.

The CAM-B3LYP calculations were performed in the absence of environment as the inclusion of the sugar-phosphate back bone, surrounding counter-ions, or water molecules does not significantly affect the electronic properties of the G4 stacks.²⁶ Additionally, environment should have no effect on the relative behavior of excited states by passing from one stacking mode to another.

Figure 4. Absorption spectra of idealized guanine geometry calculated using the CAM-B3LYP approach (solid line) and benchmark EOM-CCSD approach (dashed line).

Analysis of Excited States

The quantitative analysis of exciton delocalization and charge separation in G-quadruplexes was carried out in terms of transition density.³¹ A key quantity is the parameter Ω :

$$\Omega(A,B) = \frac{1}{2} \sum_{\alpha \in A, \beta \in B} \left[(SP^{0i})_{\alpha\beta} (P^{0i}S)_{\alpha\beta} + P_{\alpha\beta}^{0i} (SP^{0i}S)_{\alpha\beta} \right], \quad (2)$$

where $\mathbf{S}$ is the overlap matrix and $\mathbf{P}^{0i}$ the transition density matrix for $\psi_0 \rightarrow \psi_i$ excitation. The extent of exciton localization on the site A is termed $X_i(A)$ and is defined by $\Omega(A,A)$. Alternatively, the charge transfer from site A to B is represented by $\Omega(A,B)$.³¹ The weight of CT states in Ψ_i is equal to $\Omega(A,B) + \Omega(B,A)$ whereas the CS between sites A and B may be determined as the difference $\Omega(A,B) - \Omega(B,A)$. The quantities $\Omega(A,A)$ for single fragments and $\Omega(A,B)$ between fragments were obtained for 32 excited states of each of 10 snapshots per stacking mode.

The index L can be used to describe the distribution of an exciton over a system. Defined as the inverse participation ratio, the index L gives the number of sites over which the exciton is delocalized. To characterize exciton delocalization between two tetrads, we define the $L2$ parameter:

$$L2 = \frac{[X(T_1) + X(T_2)]^2}{X(T_1)^2 + X(T_2)^2} \quad (3)$$

Here, $X(T_1)$ and $X(T_2)$ represent the weight of local excitations on the first (T_1) and second (T_2) G-tetrads. The $L2$ parameter has the bounds $1 \leq L2 \leq 2$. Similarly, the parameter $L8$ characterizes exciton distribution over the 8 guanines G_k in the stack

$$L8 = \frac{\left[\sum_k X(G_k) \right]^2}{\sum_k X^2(G_k)}, k = 1, \dots, 8 \quad (4)$$

The $L8$ parameter has the bounds $1 \leq L8 \leq 8$.

RESULTS AND DISCUSSION

In the sections below we detail the examination of excited state character for five unique G-tetrad stacking modes which occur in experimental G-quadruplex structures. These geometries of these stacking modes have been previously characterized and described in terms of their aromatic-ring overlap.²² For simplicity, we refer to them throughout this work using stacking modes *A-E* shown in Fig. 2.

Absorption Spectra

Absorption spectra were computed for various arrangements of guanine nucleobases including isolated guanines, G-tetrads, and stacked G-tetrads (Fig. 5). The guanine spectrum was obtained by isolating nucleobases from snapshots of stacked G-tetrads. The broadening observed in this spectrum compared to that calculated for the optimized geometry (Fig. 4) reflects the effect of structural fluctuations on such calculations. More details on how structural fluctuations influence the excited state properties are provided in Supporting Figures S2-S3. The absorption spectrum of guanine (Fig. 5 top panel) is found to have two maxima at 5.2 and 5.7 eV. The assembly of guanine bases into a G-tetrad via Hoogsteen hydrogen bonding induces large changes in the absorption spectrum, with the two maxima converging to a single maximum at 5.4 eV. These changes demonstrate the effect of such hydrogen bonding on the excited state properties of guanine bases.

Figure 5. Computed absorption spectra in arbitrary units for isolated guanine bases (white), non-stacked G-tetrads (grey) and stacked G-tetrads (colored according to their description in Figure 2). Oscillatory Strengths summed over 0.05 eV are represented as bars. Spectra profiles are obtained by summing Gaussian functions representing individual excitation oscillatory strengths with half-width at half maximum to be 0.1 eV.

In contrast, the stacking of G-tetrads does not induce such significant changes in the absorption spectrum. This situation is similar to that observed for duplex DNA in which base stacking does not heavily influence absorption spectra.¹ The formation of complexes A-E leads to a slight shift of the absorption maximum towards higher energies. The spectra of the five G-quadruplexes exhibit a dominant maximum in the range of 5.4-5.6 eV. A local maximum at 5.1 eV is observed for mode C, whereas a less defined shoulder in the same energy region is found for the stacking modes B and D. However, no significant absorption in this region is found for stacks A and E.

Comparison of the absorption spectrum computed for stacking mode E with and without a coordinating K⁺ ion (Supporting Figure S4) shows that the coordinating cation does not heavily

influence the spectrum profile. We note that the maxima of computed spectra of assembled guanine bases are blue-shifted by 0.6-0.8 eV compared to the experimental absorption maxima at roughly 4.77 eV. This difference is caused by several limitations of our computational model (1) The effects of polar surroundings were excluded; (2) The CAM-B3LYP functional used in TD DFT calculations is known to overestimate excitation energies by 0.25 eV;⁵² (3) The vibronic effects were not accounted for, leading to a shift of computed spectra by 0.1-0.3 eV to higher energies.⁵³

Exciton Delocalization

We use the parameters $L2$ and $L8$ (Eq. 3 and 4) in order to characterize the delocalization of excitons across the G-tetrad stack. The index $L2$ describes the delocalization of excited states between two G-tetrads whereas $L8$ describes the exciton distribution over all eight guanine bases. The dependence of the $L2$ and $L8$ parameters on the excitation energy for stacking Geometries A-E is shown in Figure 6.

Figure 6. Exciton delocalization over two tetrads ($L2$) and over eight guanine bases ($L8$). The means ($\bullet$) and standard deviations ($|$) of $L2$ (left) and $L8$ (right) are calculated as a function of energy at 0.1 eV intervals. Parameter bounds are $1 \leq L2 \leq 2$ and $1 \leq L8 \leq 8$.

The $L2$ index is observed to be generally low for stacking mode *A* and high for modes *D* and *E* (Fig 6 left). The behavior of $L2$ converges for mode *A* to low values close to 1 in the high-energy regime > 5.5 eV. Stacking modes *B-D* have more variable behavior in this range with a sharp increase in $L2$ for excitons in the range > 6.0 eV suggesting these excitons to be delocalized between stacked G-tetrads. Stacking mode *E* is observed to display notably high $L2$ values in the low-energy regime of < 5.0 eV. When considering the $L8$ description of exciton localization over the eight guanine bases in the tetrad stack, it is observed that most excited states are delocalized in the range 2 ± 1 . Variation in $L8$ converges in the high-energy region > 5.7 eV with mean values mostly in the range of 1.5-2. The stacking mode *E*, unlike other geometries, shows exciton delocalization close to two for low-energy excited states between 4.6-4.8 eV.

The lowest extent of exciton delocalization is found for stacking mode *A*. As the extent of exciton delocalization depends on the excitonic coupling,⁵⁴ the increasing value of $L2$ when passing from the stacking mode *A* to *E* implies an increase of the electronic interaction between G-tetrads. A similar situation was also found for electron hole transfer through G-quadruplexes.²⁶ The $L8$ index depends considerably on the energy of exciton and the stacking mode. The extent of delocalization changes in the range of 1.0-2.5 and thus the excited states delocalized over 4 or more guanine bases are rarely observed.

Delocalization of the excited states over guanine bases in G-quadruplexes depends on electronic coupling and the energy mismatch of electronic states localized on individual bases. There are several methods to calculate the matrix elements from adiabatic excited state properties (see Ref. ⁵⁵ and references therein). Our model systems consist of 8 guanines and each guanine has two closely lying $\pi\pi^*$ excited states, 1L_a and 1L_b . An accurate estimation of the matrix elements from adiabatic properties of the corresponding 16 states is quite difficult. **Error! Bookmark not defined.** Thus we applied a less accurate approach based on transition charges.^{56,57} The performance of the transition charge model was recently discussed.⁵⁸ Within this scheme, the coupling V_{AB} of two fragments *A* and *B* is computed as the electrostatic interaction between transition atomic charges (see the Supporting Information). Using transition charges derived

from CAM-B3LYP calculations of the 1L_a and 1L_b excited states of guanine, we computed the exciton couplings between different bases within the G-tetrad stack for different stacking modes (Figure S5 and Table S1-S3). Overall, excitons are more strongly coupled between G-tetrads. Guanine bases that are hydrogen bonded within the same G-tetrad demonstrate relatively low coupling (Figure S5). Thermal fluctuations of the G-tetrad stack structure significantly affect the coupling value (Supporting Table S3).

The behavior of $L8$ suggests that excitons are generally localized on one or two guanine bases. Exciton delocalization between stacked G-tetrads is described by $L2$. The low $L2$ value observed for mode A points to relatively weak interaction between the stacked tetrads. In contrast, high values of $L2$ found for modes D and E correspond to significant exciton delocalization over stacked guanines. In idealized G-quadruplex structures, where all guanines have the same electronic properties, excited states are delocalized over the entire stack. However, thermal fluctuations of internal geometries of the nucleobases and their surroundings lead to structural and dynamic disorder in the systems. The main factor, which reduces the exciton length in homogeneous DNA stacks, is the vibronic interactions within nucleobases whereas environment fluctuations play a relatively minor role.⁵⁹ Because of the vibronic interactions, the delocalization index $L8$ computed for the structures A-E (Fig. 6) is considerably smaller than 8 ($L8=8$ in G8 complexes of the idealized geometry). It was demonstrated for duplex DNA that the extent of exciton delocalization is not sensitive to the length of the stack.⁵⁹ Therefore, in larger G-quadruplexes consisting of 3 and more tetrads, the exciton localization is expected to be similar to that shown in Fig. 6

Charge transfer character of excited states

The transition from the ground state to an excited state of any molecular system results in a shift of electron density between its fragments. In G-quadruplexes, the shift of electron density from one guanine molecule to another is referred to as charge transfer (CT). In stacked G-tetrads, guanines have similar electronic properties which leads to delocalization of electron density over multiple sites in both ground and excited states. As explained in Fig. 3 and related discussion, one should distinguish the quantities that describe CT and charge separation (CS). The last term

characterizes net charges on the guanine bases. By definition (see the section “Analysis of excited states”), the CT term is always larger than the CS term. The CT character of excited states in G-quadruplexes may have important implications for excited state lifetimes, relaxation pathways, and chemical reactions.

The CT contributions as a function of excitation energy for modes *A-E* are shown in Figure 7, together with the computed absorption spectrum. It is observed that excitations of high CT character are found predominately in the region of 5.6-6.1 eV for all stacking modes. Importantly, the CT content of excitations with energies <5.0 eV varies for different geometries (Fig. 7 *right*), with mode *E* having the largest CT content among the stacked G-tetrad geometries. Alternatively, mode *C* is found to have the lowest contribution of CT in this region. The direct generation of CT states by irradiation appears to be possible considering the partial overlap or absorption spectra with states containing high CT contributions (Fig. 7 *left*).

As the rearrangement of electron density by excitation does not always result in charge separation, it is valuable to look at the differences between the CT and CS quantities. Photoinduced CS within the stacks was characterized for different energy regions with special attention paid to whether CS was occurring within one G-tetrad, “Intra-tetrad”, or between stacked G-tetrads, “inter-tetrad” (Fig. 8). For all 5 stacking modes, intra-tetrad CS occurs primarily for excitation energies > 5.5 eV (Fig. 8 *left*), mimicking the energy range observed for CT. However, some drastic differences are observed in the character of CS occurring in different systems.

The magnitude of intra-tetrad CT varies greatly among stacking modes. Substantial intra-tetrad CS occurs for mode *A*, consistent with CT behavior observed at energies > 5.5 eV. Sizeable, but more dynamic behavior is observed for modes *B* and *C* as well. However, modes *D* and *E* have a very low content of intra-tetrad CS. Inter-tetrad CS is found to also occur in excited states with energies > 5.5 eV. The dependence of the inter-tetrad CS on the stacking geometry is opposite to that observed for intra-tetrad CS (Fig. 8). The stacking modes *A-C* have relatively low contributions of CS occurring between the stacked tetrads; while much higher inter-tetrad CS is obtained for modes *D* and *E*. A comparison of the propensity for Inter-tetrad CS to the entire CS

content for the various stacking modes illustrates the relative likelihood of CS to occur between stacked tetrads (Fig. 8 *right*), with the propensity in mode *A* being 20%, modes *B* and *C* about 40%, and modes *D* and *E* about 80%.

Figure 7. (*Left*) Histogram of total exciton CT contributions as a function of excited state energy for G-tetrad stacks *A-E*. Computed absorption spectra are shown for reference. (*Right*) CT contributions for excited states with energies less than 5.0 eV.

Figure 8. Photoinduced CS occurring within a single tetrad (*left panel*) and between two stacked tetrads (*middle panel*) for Geometries (A-E). Means ($\bullet$) and standard deviations ($-$) of CS are calculated as a function of energy. The fraction of CS contribution that is Inter-tetrad in nature is quantified for each geometry (*right panel*).

The total CS and CT contributions to the excited states of different G-tetrad stacking modes are compared in Table 1. All stacking modes exhibit similar average CT contributions, with values in the range of 25.5-29.6 % CT contribution per exciton. As expected, the average CS character is slightly lower than the average CT character per exciton, as sometimes CT can occur without resulting in CS. Interestingly, the difference between CT and CS character is more pronounced for stacking mode *E* compared to modes *A-D*.

Table 1. Average CT and CS contribution per exciton for different G-tetrad stacking modes.

Geometry	Avg. per Exciton (%)		
	CT	CS	$\Delta(\text{CT-CS})$
A	26.3	23.6	2.7
B	29.6	26.0	3.6
C	25.5	22.4	3.1
D	26.4	22.1	4.4
E	27.7	19.0	8.7

Our analysis suggests also that excitations with high CT content occur in a similar spectral region for all geometries, far from lower-energy transitions. Some overlap between the CT region and the absorption spectra points to the possibility of generating excited states with CT by

direct irradiation of G-quadruplexes. The degree of this overlap depends on the stacking mode. While total CT content for different geometries shows a similar dependence on excitation energy, the character of CS within the G-tetrad stacks shows strikingly different behavior. The dependence on inter-tetrad CS on stacking geometry may have implications for charge transport in supramolecular G-quadruplex assemblies.

Earlier we found that stacking mode *E*, with a high degree of 5/6-ring overlap, shows exceptional electron hole transfer rates compared to other geometries owing to strong electronic coupling of stacked guanine bases.²⁶ Also, mode *E* differs significantly from other types of complexes in terms of absorption spectra. In the energy region < 5.2 eV, the absorption probability of *E* model is relatively weak. In particular, a shoulder at 5.0 eV is absent (unlike B, C and D models). Furthermore, in mode *E*, the low-energy excitons are strongly delocalized between two tetrads compared to other stacking modes as quantified by the *L2* parameter. Also, the contribution of CT states in this region is found to be larger.

Despite these differences, the profiles of absorption spectra and overall CT character of all types of stacking modes are similar. Previous experimental studies²³ demonstrated stacking mode *E* to play a unique role in the generation of red-shifted, long-lived (ns) excimer fluorescence. According to our data, the stacking mode influences the CT character of the low energy excitations. However, given the similarities in absorption spectra and general CT behavior across stacking modes, the structural relaxation that accompanies electronic excitations of stacked G-tetrads may be a key factor generating experimentally observed emissive states, rather than the inherent differences in Franck-Condon excited states,

Conclusions

Time-dependent DFT calculations have been carried out for a number of stacked guanine assemblies extracted from molecular dynamics trajectories of G-quadruplexes. Using a recently developed tool³¹ we have analyzed in detail the electronic excited states of stacked guanine tetrads adopting different stacking geometries. In particular, exciton delocalization and photo-induced charge separation within tetrad stacks have been examined. In most excited states of G-quadruplexes, the extent of exciton delocalization over nucleobases is found primarily in the range 2 ± 1 similar to that observed for duplex DNA. Large variations in the nature of excited states are found within different stacking geometries. It has been shown that the excitations in the low-energy region of the absorption spectrum may include a significant contribution of charge transfer states. Several electronic transitions to excited states with relatively large contributions of CT states are found to be strong enough to directly induce charge separation by UV absorption. The character of the CT states within the stacks is heavily dependent on the mutual position of G-tetrads; in particular, the inter-tetrad charge separation is significantly favored by stacking modes *D* and *E*. The results reported here provide valuable insight into how the structure of G-quadruplexes influences their excited state properties.

Acknowledgments

This research was funded by Nanyang Technological grants (A.T.P and M.-E.M.-B.) and by the grant CTQ2011-26573 from Ministry of Science and Innovation, Spain (A.A.V.).

Supporting Information Available

Supporting information includes: figures illustrating the structure of stacked G-tetrads; effects of thermal fluctuations on absorption spectra and exciton localization; the effect of K⁺ ion coordination on calculated spectra; exciton couplings. This information is available free of charge via the Internet at <http://pubs.acs.org>

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