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# Cation-Mediated Energy Transfer in G-Quadruplexes Revealed by an Internal Fluorescent Probe

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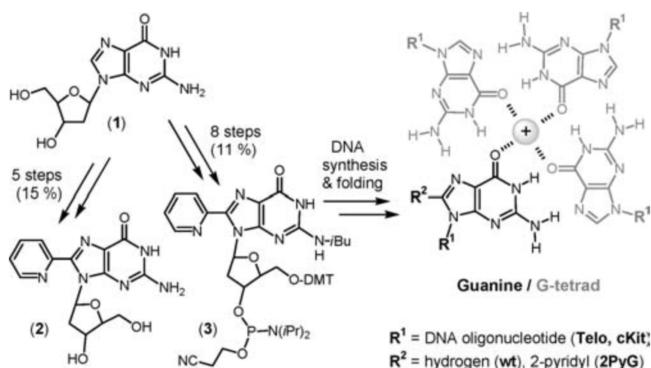
***Abstract:*** A single pyridine unit incorporated into G-quadruplex DNA has revealed efficient energy transfer reactions in cation-containing G-quadruplexes. 8-(2-Pyridyl)-2′-deoxyguanosine, "2PyG", is a highly sensitive internal fluorescent probe of G-quadruplex folding and energy transfer. 2PyG was minimally disruptive to G-quadruplex folding and exhibited intense fluorescence, even when it was base-stacked with other guanine residues. Using 2PyG we have quantified energy transfer efficiencies within G-quadruplex structures prepared under conditions of excess $Na^+/K^+$ (110 mM) or in 40% polyethylene glycol (PEG) under salt deficient conditions. G-quadruplex structures containing coordinated cations exhibited efficient DNA-to-probe energy transfer reactions ($\eta_t = 0.11-0.41$), while PEG-folded G-quadruplexes exhibited very little energy transfer ($\eta_t = 0.02-0.07$). Experiments conducted using unmodified G-quadruplexes suggest that cation coordination at the $O^6$ position of guanine residues results in enhanced quantum yields of G-quadruplex nucleobases that, in turn, serve as efficient energy donors to 2PyG. Given the growing interest in G-quadruplex-based devices and materials, these results will provide important design principles toward harnessing the potentially useful photophysical properties of G-quadruplex wires and other G-rich structures.

G-quadruplexes are intriguing nanostructures possessing potential biological relevance[1,2] and interesting photophysical and material properties.[3−5] Energy and electron transfer reactions involving G-quadruplexes can mediate DNA damage and repair[4] and might facilitate the development of new molecular devices.[5] Previous studies have suggested that G-G base-stacking, hydrogen bonding, and/or restricted motions within G-quadruplexes can enhance the photoexcited lifetimes and energy transfer properties of guanine residues.[6] However, the poor quantum yield ($\Phi \approx 10^{-4}$), short-lived excited state ($\tau \approx 1$ ps), and spectral overlap of 2′-deoxyguanosine (**1**) with other nucleobases prevent the quantification of energy transfer efficiencies ($\eta_t$) within unmodified G-quadruplexes.[6]

Internal fluorescent probes serving as energy acceptors can provide powerful means to characterize energy and electron transfer processes within DNA.[7] Fluorescent base analogs, like 2-aminopurine (2AP), can exhibit useful fluorescence properties when inserted into the loops or ends of G-quadruplexes,[8] but attempts by Mergny and co-workers to introduce fluorescent guanine mimics at positions directly involved in G-tetrad formation resulted in nonemissive products.[9] This result was consistent with numerous studies involving duplex DNA where 2AP, 6-methylisoxanthopterin, 3-methylisoxanthopterin, and other fluorescent guanine analogs were quenched 100-fold or more by close proximity and base-stacking interactions with purines, the same factors needed for efficient energy transfer.[6,7,10] To address this apparent conundrum, here we report 8-(2-pyridyl)-2′-deoxyguanosine, "2PyG" (**2**), as a "turn-on" fluorescent probe of G-quadruplex folding and energy transfer. Using 2PyG we have quantified energy transfer efficiencies within G-quadruplexes prepared under conditions of excess NaCl or KCl (110 mM) or in 40% polyethylene glycol 200 (PEG) under salt-deficient conditions (10 mM lithium cacodylate). Interestingly, the G-quadruplex structure is necessary but not sufficient for promoting efficient intramolecular energy transfer. The binding of ions to the $O^6$ positions of guanine residues is also needed for the unusually efficient energy transfer reactions within G-quadruplex structures.

***Scheme 1.*** Structure and Synthesis of 8-(2-pyridyl)-2′-Deoxyguanosine (**2**), the 2PyG Phosphoramidite (**3**), and a Cation-Stabilized G-Tetrad[11]




To furnish a guanosine mimic for G-quadruplex folding and energy transfer studies, we synthesized 8-(2-pyridyl)-2′-deoxyguanosine (**2**) (Scheme 1).[11] Compound **2** exhibits a much higher quantum yield in acetonitrile ($\Phi \approx 0.71$) than in water ($\Phi \approx 0.02$).[12] The environmental sensitivity of **2** and its spectral overlap with 2′-deoxyguanosine (**1**) suggested that 2PyG might serve as a useful internal fluorescent probe of G-quadruplex folding and energy transfer.[12] The corresponding 5′-DMT-3′-phosphoramidite of 2PyG (**3**) was therefore synthesized in eight steps with 11% overall yield and introduced into single sites of G-quadruplexes derived from the human telomeric repeat "Telo" and the cKit promoter (Scheme 1).[11] Importantly, 2PyG is minimally disruptive to G-quadruplex folding and can exhibit good quantum yields ($\Phi = 0.04-0.15$) even when base-stacked with other guanine residues. G-quadruplexes containing 2PyG can adopt wild-type folds that exhibit efficient DNA-to-probe energy transfer reactions ($\eta_t = 0.11-0.41$).[13] Interestingly, these efficiencies are highly sensitive to DNA folding as well as the presence of cations within the folded G-quadruplexes.

Modification of the 8-position of guanine offers an attractive avenue to fluorescent probes because it is not involved in the extensive base-pairing interactions of G-tetrads ($R^2$, Scheme 1). Arylation of the 8-position of guanine can yield fluorescent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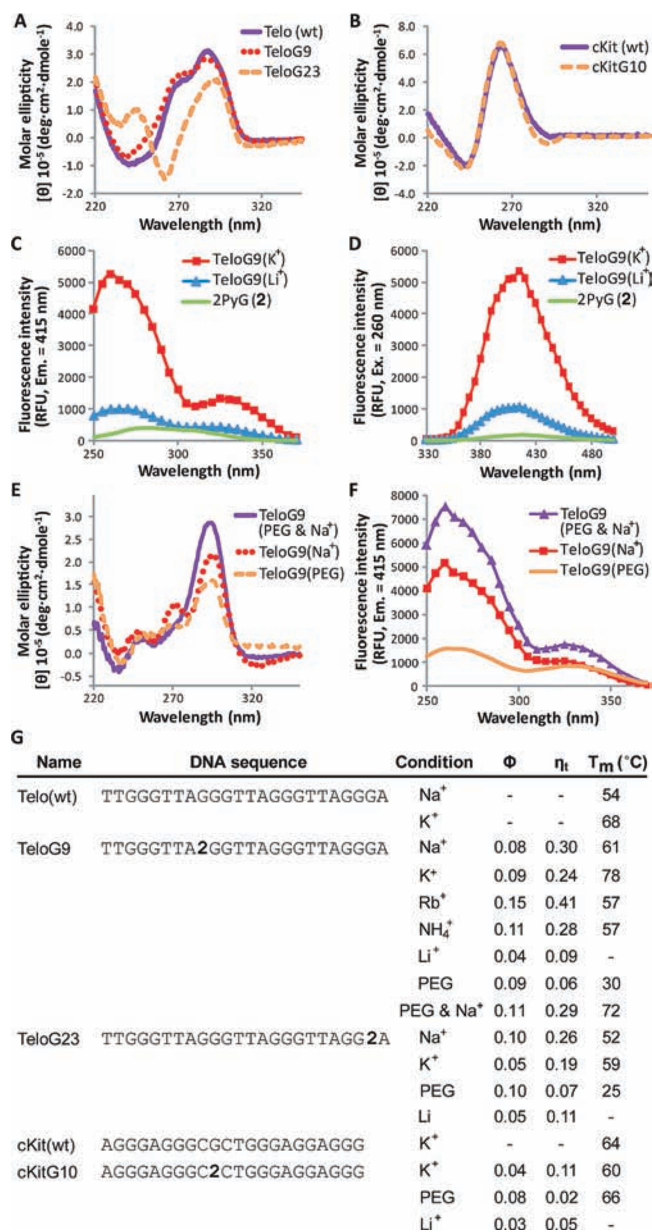
products,[14] and 8-aryl and pyridyl derivatives have been used for the self-assembly of intermolecular G-tetrads.[3a,f,g] While the addition of bulky groups to the 8-position of guanosine can shift the conformational equilibrium of the glycosidic bond from *anti* to *syn*,[15−17] G-quadruplex folding can force 8-modified guanosines to adopt *anti* conformations with relatively small energetic penalties to quadruplex folding ($\leq$1 kcal/mol).[15b,c,e] We used circular dichroism and thermal denaturation experiments to determine the impact of 2PyG incorporation on the global structure and stability of G-quadruplexes carrying 2PyG. We selected sites known to be directly involved in G-tetrad formation for modification.[18,19] As compared to the wild-type sequences, the incorporation of 2PyG into the G9 position of Telo caused very little, if any, change to the global secondary structure of Telo in 110 mM KCl (Figure 1A). A very similar result was observed for cKitG10 (Figure 1B). Consistent with the direct involvement of 2PyG in folded G-tetrads, the substitution of G9 in Telo with 2PyG caused a significant thermal stabilization ($\Delta T_m = +10$ °C) of the "(3 + 1) hybrid" structure due to the *syn* conformational preference of this position (Figure 1A,G).[15,17,18a] In contrast, the G10 position of cKit is known to favor an *anti* conformation,[18b] and its substitution with 2PyG caused a small thermal destabilization ($\Delta T_m = -4$ °C) as compared to the unmodified sequence (Figure 1G). Interestingly, 2PyG caused TeloG23 to fold into an "antiparallel" structure even in $K^+$ solutions (Figure 1A). This structure, where G23 adopts a *syn* conformation,[19] is normally only observed in $Na^+$-containing solutions. TeloG23 was therefore characterized in $Na^+$ buffer, and it exhibited nearly the same global structure and thermal stability as the wild-type sequence in $Na^+$ ($\Delta T_m = -2$ °C, Figure S4B Supporting Information). Taken together, these data indicate that 2PyG can be incorporated into the G-tetrads of these natively folded G-quadruplexes with little, if any, negative impact on their structure or stability.[20]

To study the impact of G-quadruplex folding on the fluorescence properties of 2PyG, DNA samples were folded in the presence of variable cation salts. In the presence of $K^+$, $Na^+$, $Rb^+$, or $NH_4^+$, three diverse and well-defined G-quadruplex structures were observed (Figures 1A−B and S4). Surprisingly, the quantum yields for selective excitation of 2PyG ($\lambda_{ex} = 330$ nm) in these structures were 2- to 7-fold higher than that of the 2PyG nucleoside monomer (**2**) under the same conditions ($\Phi = 0.02$, Figures 1G and S1). The modest quantum yields for 2PyG in ion-folded G-quadruplexes ($\Phi = 0.04-0.15$) were compensated by the relatively large molar extinction coefficient of 2PyG ($\epsilon_{310\ nm} \approx 25\,000$ cm$^{-1}$ M$^{-1}$) and its ability to act as an emissive energy acceptor when base-stacked with other guanine residues.[20] In contrast, the most commonly used internal probe, 2-aminopurine, is a poor guanine mimic and not very bright due to its low molar extinction coefficient ($\epsilon_{305\ nm} = 5600$ cm$^{-1}$ M$^{-1}$) and low quantum yield ($\Phi \approx 0.005$) when base-stacked with guanine residues.[7a]

The excitation spectra of 2PyG-containing quadruplexes exhibited maxima at ~260 and ~330 nm (Figures 1C and S1). The excitation peak at 260 nm revealed the presence of DNA-to-2PyG energy transfer.[7b,e] Interestingly, the efficiencies of energy transfer ($\eta_t$) were always much higher in G-quadruplex-folded structures as compared to the same, mostly unfolded DNAs prepared in LiCl-containing buffers (Figure 1C−D,G).[11] Due to their small size and large desolvation energies, lithium ions are not normally compatible with G-quadruplex folding.[1] DNA samples prepared in aqueous 110 mM LiCl therefore gave CD data typical of G-stacked single-stranded DNA (Figure S4). Energy transfer efficiencies were 70−350% higher for cKitG10, TeloG23, and TeloG9 G-quadruplexes when folded in 110 mM solutions of $Na^+$, $K^+$, $NH_4^+$, and $Rb^+$ ($\eta_t$ = 0.11−0.41) as compared to the same DNAs prepared in 110 mM $Li^+$ ($\eta_t = 0.05-0.11$, Figure 1G).[13,21] The combination of strong DNA molar absorptivity ($\epsilon_{260\ nm} \approx 250\,000$ cm$^{-1}$ M$^{-1}$) with efficient energy transfer resulted in fluorescence enhancements up to 15-fold for ion-containing G-quadruplexes as compared to the corresponding unfolded DNAs in LiCl (Figures 1D,G and S1−S2).




| Name | DNA sequence | Condition | Φ | $\eta_t$ | $T_m$ (°C) |
|---|---|---|---|---|---|
| Telo(wt) | TTGGGTTAGGGTTAGGGTTAGGGA | Na⁺ | - | - | 54 |
| | | K⁺ | - | - | 68 |
| TeloG9 | TTGGGTTA**2**GGTTAGGGTTAGGGA | Na⁺ | 0.08 | 0.30 | 61 |
| | | K⁺ | 0.09 | 0.24 | 78 |
| | | Rb⁺ | 0.15 | 0.41 | 57 |
| | | NH₄⁺ | 0.11 | 0.28 | 57 |
| | | Li⁺ | 0.04 | 0.09 | - |
| | | PEG | 0.09 | 0.06 | 30 |
| | | PEG & Na⁺ | 0.11 | 0.29 | 72 |
| TeloG23 | TTGGGTTAGGGTTAGGGTTAGG**2**A | Na⁺ | 0.10 | 0.26 | 52 |
| | | K⁺ | 0.05 | 0.19 | 59 |
| | | PEG | 0.10 | 0.07 | 25 |
| | | Li | 0.05 | 0.11 | - |
| cKit(wt) | AGGGAGGGCGCTGGGAGGAGGG | K⁺ | - | - | 64 |
| cKitG10 | AGGGAGGGC**2**CTGGGAGGAGGG | K⁺ | 0.04 | 0.11 | 60 |
| | | PEG | 0.08 | 0.02 | 66 |
| | | Li⁺ | 0.03 | 0.05 | - |

***Figure 1.*** Molar ellipticity ($\theta$) of Telo(wt), TeloG9, and TeloG23 (A) or cKit(wt) and cKitG10 (B) in 110 mM KCl. Excitation (C) and emission (D) spectra of TeloG9 and the 2PyG nucleoside in 110 mM KCl or 110 mM LiCl. Molar ellipticity (E) and excitation spectra (F) of TeloG9 folded in the presence of 40% PEG, 110 mM NaCl, or 40% PEG and 110 mM NaCl. DNA sequences, names, quantum yields ($\Phi$), energy transfer efficiencies ($\eta_t$), and thermal denaturation melting temperatures ($T_m$) of the oligonucleotides studied (G). All samples contained 2 $\mu$M of DNA or 2PyG (**2**) in an aqueous 10 mM cacodylate buffer (pH 7.4). Excitation spectra were collected using emission at 415 nm, and emission spectra were collected using excitation at 260 nm. Thermal melting temperatures ($T_m$) were determined by monitoring $\Delta\theta$ vs temperature. $\Phi$ values and energy transfer efficiencies ($\eta_t$) were measured using $\lambda_{ex} = 330$ or 260 nm, respectively.[11,13]

To dissect the multiple roles that metal ions might play in mediating both G-quadruplex folding and energy transfer, we prepared G-quadruplexes under salt-deficient conditions (10 mM

lithium cacodylate) by molecular crowding/partial dehydration in 40% polyethylene glycol 200 (PEG).[22] Under these conditions, cations are absent or only weakly bound in the central cavities of each G-quadruplex.[22] For all DNAs evaluated, PEG-mediated folding resulted in G-quadruplex structures exhibiting cooperative and reversible folding, as well as highly variable thermal stabilities (Figure 1E,G). Interestingly, very little energy transfer was observed in the PEG-folded G-quadruplexes under salt-deficient conditions ($\eta_t \approx 0.02-0.07$, Figure 1F,G). Quadruplexes prepared in the presence of both PEG and 110 mM $Na^+$, in contrast, exhibited energy transfer efficiencies similar to that for the samples prepared in 110 mM $Na^+$ ($\eta_t \approx 0.30$, Figure 1F,G). Nonspecific electrostatic stabilization of backbone phosphate repulsion is not responsible for the enhanced energy transfer, because PEG-folded G-quadruplexes prepared in 110 mM of tetrabutylammonium chloride exhibited approximately the same energy transfer efficiencies as the PEG-only samples. Taken together, these results suggest that the G-quadruplex structure by itself is not sufficient for promoting efficient energy transfer and that ions coordinated directly to the $O^6$ position of guanine residues play a critical role in mediating energy transfer reactions in G-quadruplex structures.

Previous studies proposed that G−G base-stacking, hydrogen bonding, and/or restricted motions within G-quadruplex structures can enhance the photoexcited lifetimes and quantum yields of guanine residues.[6] To evaluate the impact of cation binding on the quantum yields of unmodified guanine residues, we prepared unmodified G-quadruplexes from Telo(wt) in the presence of 40% PEG under salt-deficient conditions or in the presence of 40% PEG and 110 mM NaCl. While both samples were more fluorescent than a comparable mixture of nucleotide monophosphates,[11] samples containing PEG and $Na^+$ exhibited a higher quantum yield and red-shifted emission as compared to the G-quadruplexes prepared in 40% PEG only (Figure S3, Supporting Information). These results suggest that guanine-cation coordination is largely responsible for the enhanced photoexcited lifetimes and quantum yields reported for guanine residues in G-quadruplex structures.[6] The enhanced quantum yields of the unmodified guanine residues, in turn, facilitate the unusually efficient DNA-to-2PyG energy transfer reactions reported here.[21]

In summary, the incorporation of a single pyridine unit into G-quadruplexes has provided a useful fluorescent probe for monitoring G-quadruplex folding and revealed efficient intramolecular energy transfer reactions in ion-containing G-quadruplex structures. We used 2PyG in conjunction with PEG-promoted G-quadruplex folding to dissect the multiple roles metal ions play in mediating both G-quadruplex folding and energy transfer. Given the growing interest in G-quadruplex-based devices and materials,[3,5] these results may provide important design principles toward harnessing the potentially useful photophysical properties of G-quadruplex wires and related structures.[5,6] Elucidating the exact mechanism of cation-mediated energy transfer will require additional studies, but our results suggest that the enhanced quantum yields of unmodified guanine residues upon $O^6$ ion coordination are responsible for the unusually efficient energy transfer reactions mediated by G-quadruplex structures.[21] Interestingly, the $O^6$ carbonyl stretching frequencies of guanine residues are known to decrease upon $NH_4^+$-mediated quadruplex folding,[23] and the "trapped enol" mimic $O^6$,9-dimethylguanine is known to be highly fluorescent when protonated.[24] Taken together, these results suggest that the ion-coordinated guanine residues in G-quadruplexes may electronically resemble emissive enol forms of guanine more than the corresponding ion-free guanine residues.

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**Supporting Information Available:** The synthesis and characterization of **2**−**3**, additional fluorescence and CD data, and the procedures for all experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Interestingly, 2PyG exhibits a 2-fold higher quantum yield in $D_2O$ than in $H_2O$, and 8-phenyl-2′-deoxyguanosine exhibits approximately the same quantum yield in $H_2O$, $D_2O$, and acetonitrile ($\Phi \approx 0.6$). These results suggest that water-mediated quenching of 2PyG involves proton transfer between the pyridyl nitrogen and bulk solvent. We speculated that, upon G-quadruplex folding, the pyridyl nitrogen of 2PyG would become protected from bulk solvent to provide a fluorescence enhancement.

(13) $\eta_t$ is defined as the number of photons transferred from active nucleobase donors to 2PyG divided by the total number of photons absorbed by all nucleobases at 260 nm.[7b,e] The reported efficiencies (Figure 1G) therefore provide a lower limit for the transfer efficiencies of the active donors.

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