

Determination of G-quadruplex DNA cleavage preference and identification of a perylene diimide G-quadruplex photocleavage agent using a rapid fluorescent assay

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SI.1. PAGE analysis versus solution fluorescence of G-quadruplex cleavage reactions

Analysis of the TMPyP4 photochemical cleavage reactions of F21T in both sodium- and potassium-containing buffers demonstrates that the solution normalized fluorescence is directly proportional to the percent of DNA cleavage prior to piperidine/heat treatment as determined by PAGE. PAGE analysis was carried out on photochemical cleavage reactions of K⁺F21T and Na⁺F21T after 30 min of irradiation and for K⁺F21T after 120 min irradiation in the presence of various concentrations of TMPyP4. Visualization of the gels by FAM fluorescence revealed that samples that had not undergone piperidine/heat treatment afforded diffuse bands corresponding to DNA cleavage localized to the G-residues (e.g., Figure 6 in the paper and Figure S3, left side of gels). No significant cleavage bands were observed for samples incubated with TMPyP4 that had not been irradiated, or for samples that were irradiated in the absence of TMPyP4. The percent cleavage of the DNA was determined as the intensity of the cleavage bands as a proportion of the total band intensity for each sample (see equations 2 and 3, Section 4.8 in the paper). The percent cleavage of the DNA from PAGE analysis shows a strong correlation with the solution normalized fluorescence determined for all these samples ($r^2 = 0.9195$, slope = 1.02, see Figure S2 below).

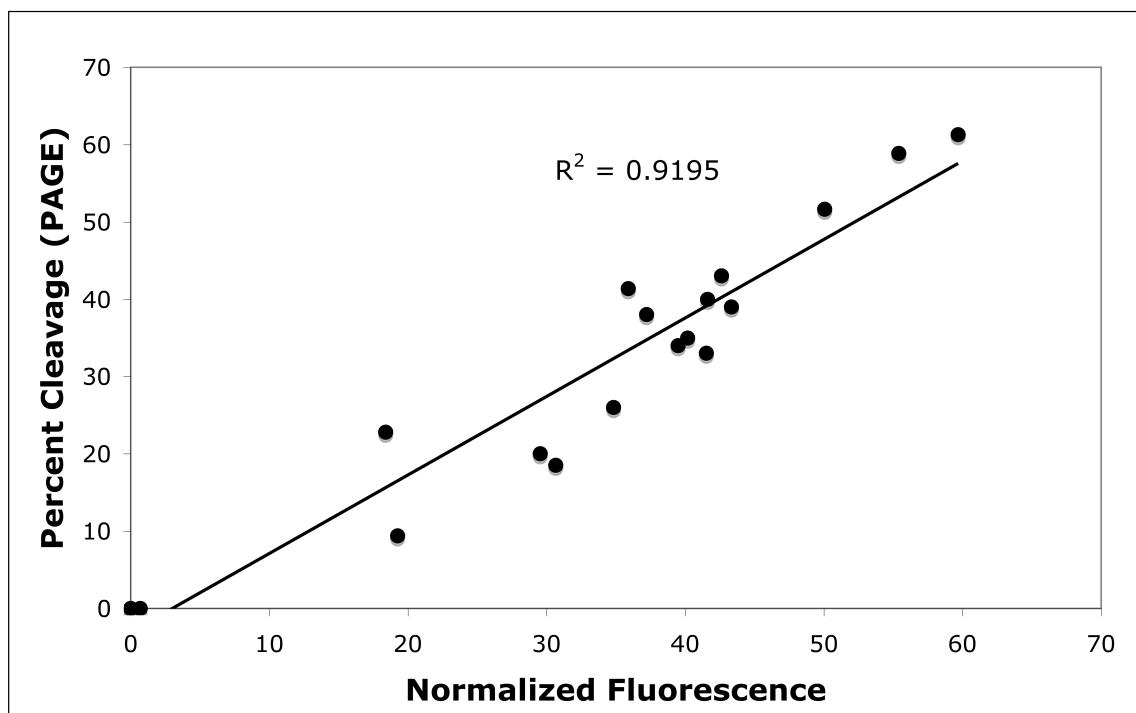


Figure S1. Plot of the normalized solution fluorescence of photocleavage reactions of F21T by TMPyP4 versus the percent DNA cleavage as determined by PAGE analysis before piperidine/heat treatment of the samples.

SI.2. Comparison of dissociation buffers

Samples of F21T (250 nM) in potassium cacodylate buffer (50 mM, pH 7.4) in a 96-well plate were incubated with various concentrations of TMPyP4 for 30 min, followed by irradiation for 60 min in a photoreactor fitted with lamps with a maximal emission at 420 nm. Prior to carrying out the heating/reannealing step, two different dissociation buffers were employed: the samples were either treated with a large excess of calf thymus DNA (10 µg final concentration/well), or with SDS (2 % final concentration). In both cases, the plates were then sealed, heated to 85 °C for 30 min, and allowed to slowly cool to room temperature. The FAM fluorescence for each well was determined, the raw fluorescence was corrected for the background fluorescence due to intact F21T and TMPyP4, and the background-corrected fluorescence was then normalized to a sample of F21T that had been completely cleaved by prolonged incubation with S1 nuclease (see equation 1 in Section 4.7 of the paper).

Regardless of which dissociation buffer was employed, the observed normalized fluorescence after the heating/reannealing step was the same (see Figure S2).

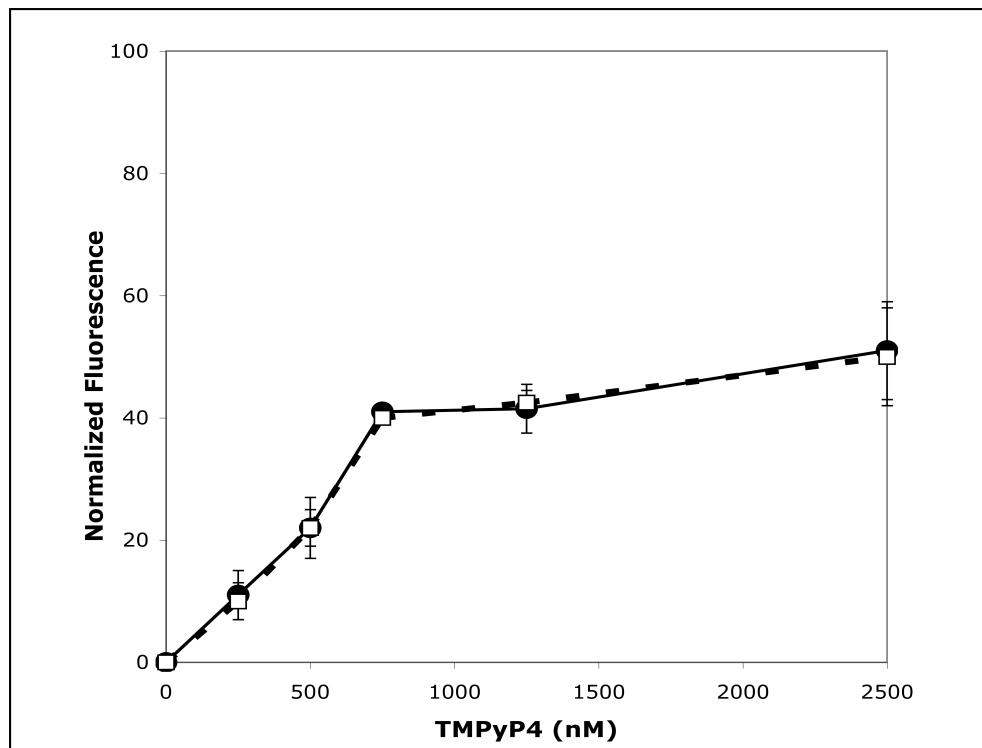


Figure S2. Comparison of normalized solution fluorescence of F21T (250 nM) incubated with various concentrations of TMPyP4, subjected to irradiation for 60 min, and treated with dissociation buffers containing either 10 µg/well final concentration of calf thymus DNA (filled circles) or 2% final concentration of SDS (open squares). All samples were subjected to heating at 85 °C for 30 min and slow cooling to room temperature before the solution fluorescence was measured.

SI.3. PAGE analysis of TMPyP4 photochemical cleavage of Na⁺F21T.

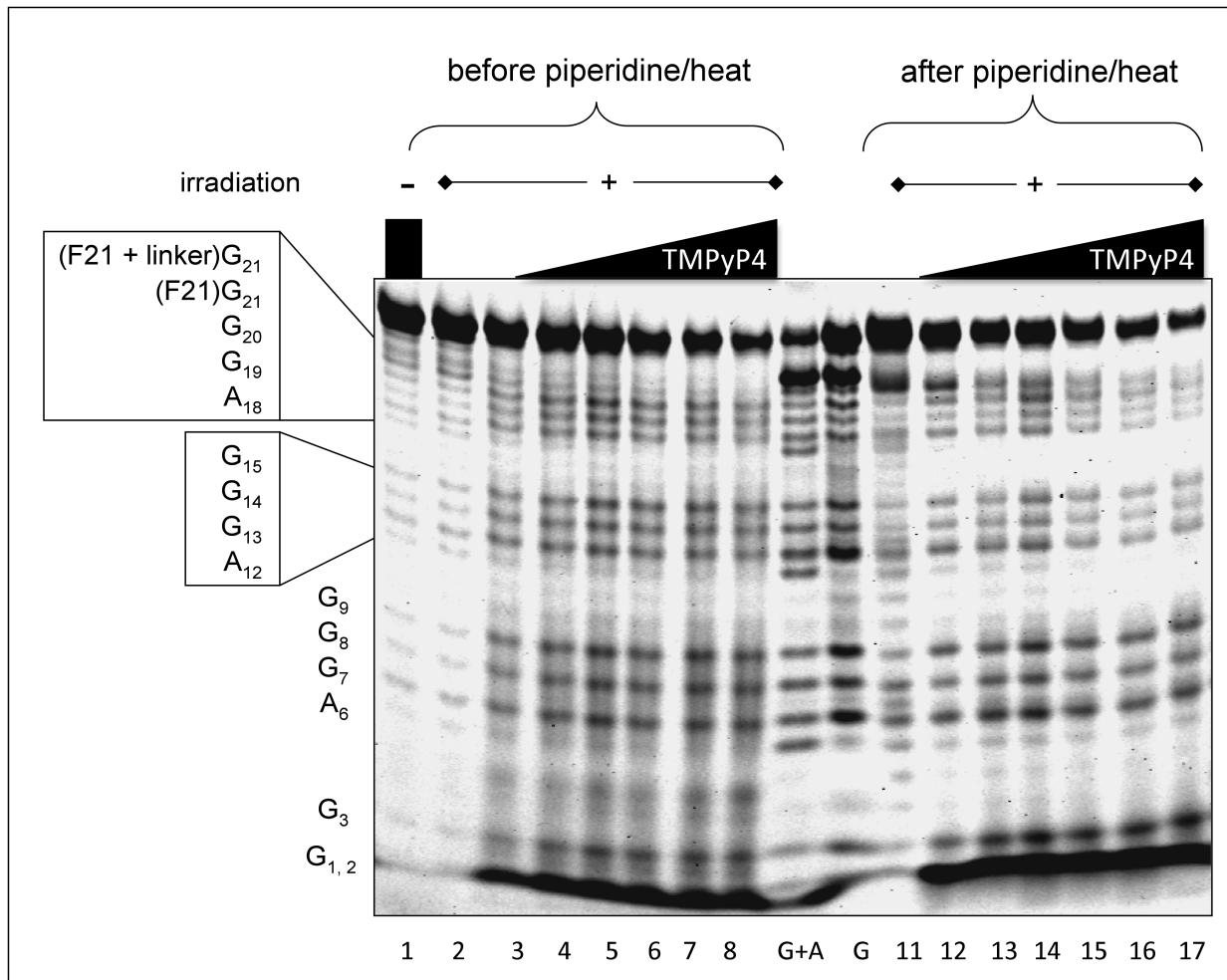


Figure S3. PAGE analysis of Na⁺F21T (250 nM) photocleavage in sodium cacodylate buffer with 0–2.5 μ M TMPyP4 irradiated for 30 minutes (420 nm) and then subjected to heating/reannealing. Samples in lanes 1–8 were directly subjected to PAGE. Samples in lanes 11–17 were treated with piperidine at 90 °C for 30 min before PAGE analysis. Reactions contained 0 nM (lanes 2, 11), 250 nM (lanes 3, 12), 500 nM (lanes 4, 13), 750 nM (lanes 5, 14), 1000 nM (lanes 6, 15), 1.25 μ M (lanes 7, 16), or 2.5 μ M (lanes 1, 8, 17) of TMPyP4. Control sample in lane 1 was not subjected to irradiation.

SI.4. TMPyP4 incubation time-dependent photochemical cleavage of F-c-Myc22m-T.

Further insight into the photochemical cleavage of F-c-Myc22m-T by TMPyP4 was obtained by monitoring the normalized solution fluorescence and the extent of DNA as determined by PAGE analysis for samples of F-c-Myc22m-T pre-incubated with difference concentrations of TMPyP4 for increasing times prior to irradiation for 30 min. The normalized solution fluorescence was found to decrease with increasing pre-incubation times (Figure S4).

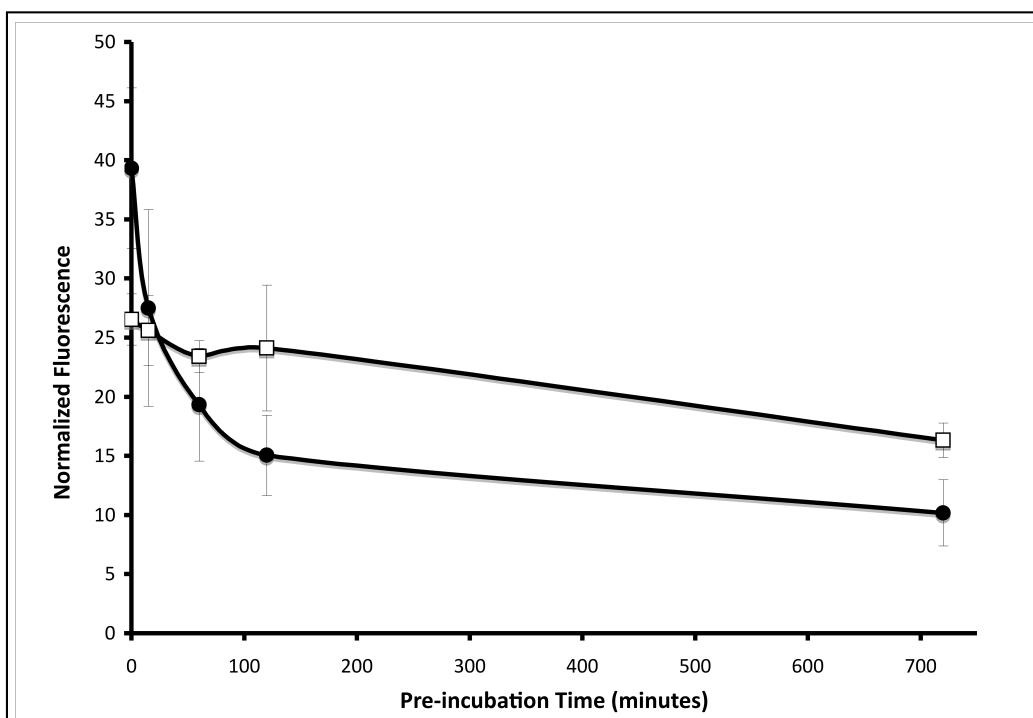


Figure S4. Normalized fluorescence intensity of 250 nM F-c-Myc22m-T preincubated with 250 nM (open squares) or 1250 nM (filled circles) TMPyP4 for 0-720 min prior to irradiation for 30 min (420 nm lamps), addition of calf thymus DNA dissociation buffer (10 μ g/well final concentration) followed by heating at 85 °C for min and cooling to room temperature.

In contrast to the solution fluorescence results (Figure S4) for these samples, PAGE analysis prior to piperidine/heat treatment reveals no change in the extent of photocleavage with preincubation (Figure S5B). Rather, there is a pronounced decrease in the overall intensity of the bands with increasing preincubation (Figure S5C). This is particularly evident after piperidine/heat treatment; the bands from PAGE analysis the samples treated with the highest concentration of TMPyP4 for the longest preincubation time are barely visible (Figure S5A). This loss of FAM fluorescence is not accompanied by a change in the cleavage pattern.

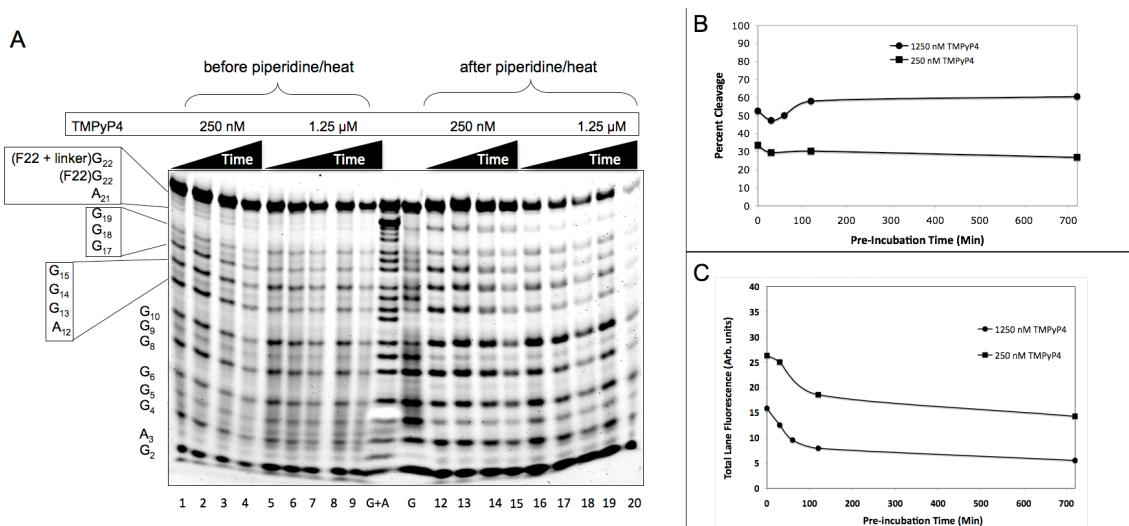


Figure S5. (A) PAGE analysis for G-quadruplex photocleavage reactions of F-c-Myc22m-T subjected to pre-incubation with 250 nM or 1.25 μ M TMPyP4 prior to irradiation for 30 min. Samples were pre-incubated for 0 (lanes 1, 5, 12, 16), 30 (lanes 2, 6, 13, 17), 60 (lanes 7, 14), 120 (lanes 3, 8, 14, 19), or 720 min (lanes 4, 9, 15, 20) prior to irradiation for 30 minutes and analyzed directly (lanes 1–9) or after piperidine/heat treatment (lanes 12–20). Lanes G and G+A are Maxam Gilbert sequencing reaction samples. The gel was visualized using FAM fluorescence. (B) Plot of percent DNA photocleavage of F21T as determined by PAGE analysis versus pre-incubation time with 250 or 1.25 μ M prior to irradiation for 10 min. (C) Plot of total band intensity for each lane in the PAGE analysis of photocleavage of F21T versus pre-incubation time with 250 or 1.25 μ M prior to irradiation for 10 min.

SI.4. Oxidative cleavage of K⁺-F21T and Na⁺-F21T by Mn²⁺TMPyP4

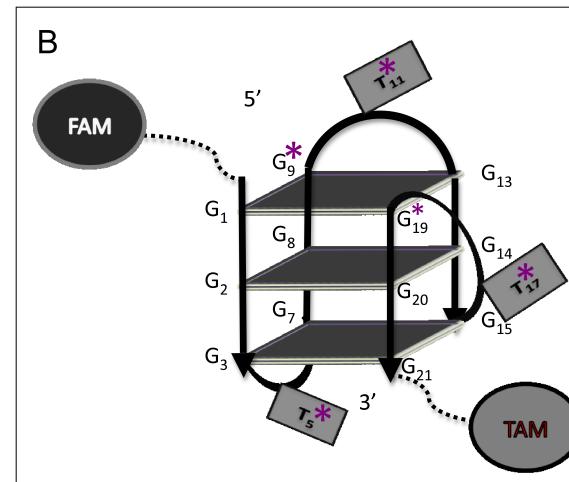
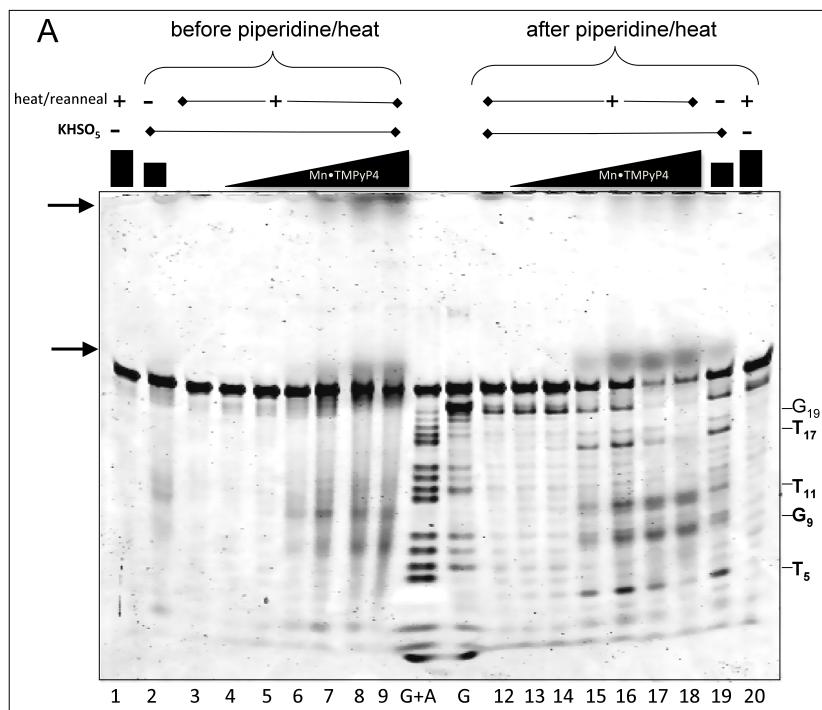


Figure S6. (A) PAGE analysis of G-quadruplex oxidative cleavage reactions of K⁺F21T (250 nM) in potassium cacodylate buffer with 0-1.25 μM Mn²⁺TMPyP4 and KHSO₅ (100 μM) for 15 min. Samples in lanes 1-9 were directly subjected to PAGE. Samples in lanes 12-20 were treated with piperidine at 90 °C for 30 min before PAGE analysis. Reactions contained 0 nM (lanes 3, 12), 67.5 nM (lanes 4, 13), 125 nM (lanes 5, 14), 250 nM (lanes 6, 15), 500 nM (lanes 2, 7, 16, 19), 750 nM (lanes 8, 17), or 1.25 μM (lanes 1, 9, 18, 20) of Mn²⁺TMPyP4. Samples in lanes 2 and 19 did not undergo the heat/reanneal cycles. Control samples in lanes 1 and 20 contained no KHSO₅. Lanes G and G+A are Maxam-Gilbert sequencing reaction samples. The gel was visualized using FAM fluorescence. (B) Cartoon representation of F21T in a mixed antiparallel/parallel G-quadruplex structure with the major sites of Mn(=O)TMPyP4 cleavage as determined by PAGE analysis marked with a “*”.

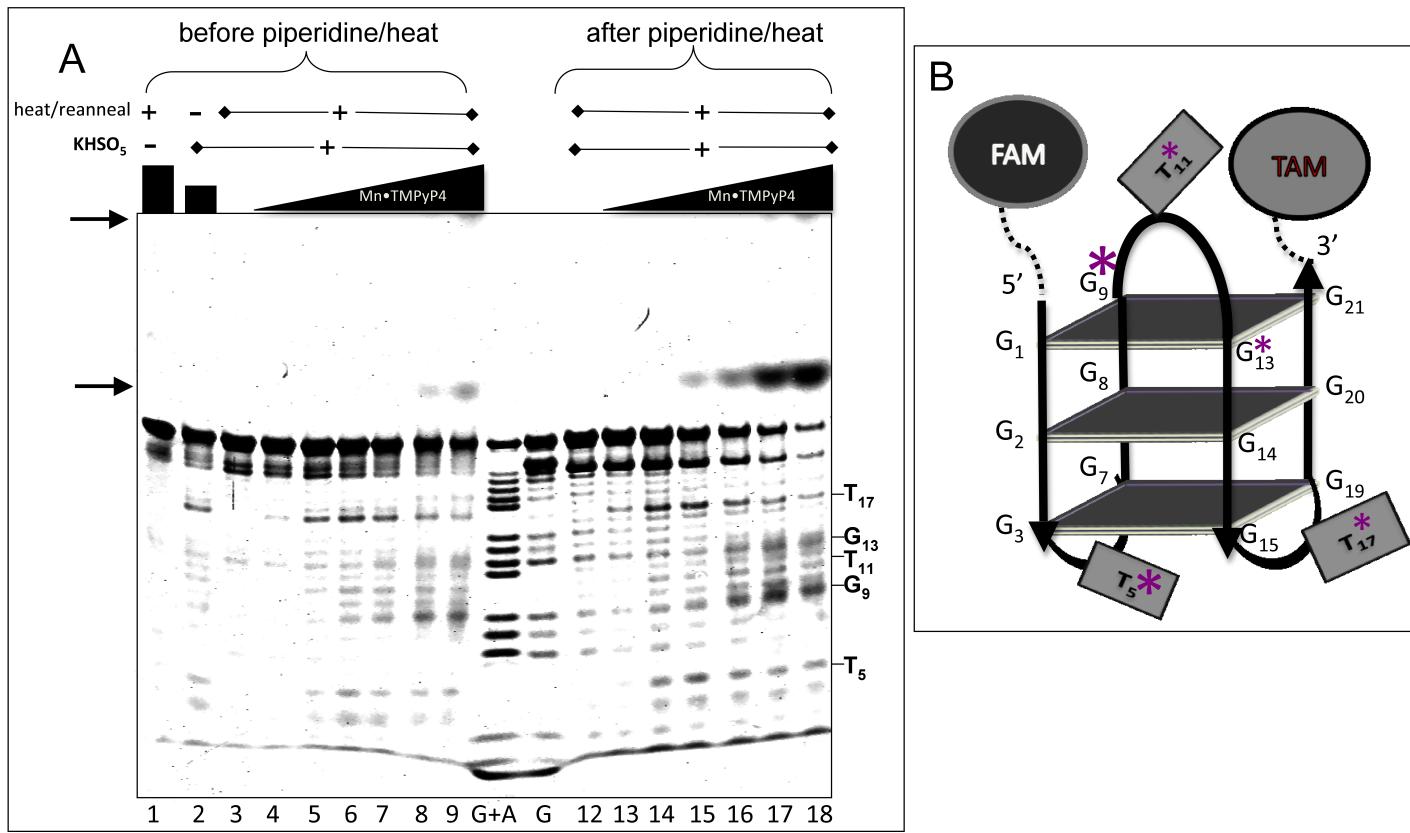


Figure S7. (A) PAGE analysis of G-quadruplex oxidative cleavage reactions of Na⁺F21T (250 nM) in sodium cacodylate buffer with 0–1.25 μM Mn•TTPyP4 and KHSO₅ (100 μM) for 15 min. Samples in lanes 1–9 were directly subjected to PAGE. Samples in lanes 12–18 were treated with piperidine at 90 °C for 30 min before PAGE analysis. Reactions contained 0 nM (lanes 3, 12), 67.5 nM (lanes 4, 13), 125 nM (lanes 5, 14), 250 nM (lanes 6, 15), 500 nM (lanes 2, 7, 16), 750 nM (lanes 8, 17), or 1.25 μM (lanes 1, 9, 18) of Mn•TTPyP4. Control sample in lane 1 was not subjected to KHSO₅. Sample in lane 2 did not undergo the heat/reanneal cycle. Lanes G and G+A are Maxam-Gilbert sequencing reaction samples. The gel was visualized using FAM fluorescence. (B) Cartoon of F21T in the antiparallel quadruplex structure formed by GGG(TTAGGG)₃ in the presence of sodium ions with sites of Mn(=O)TTPyP4 cleavage as determined by PAGE analysis marked with a “*”.

SI.6. PAGE analysis of photochemical cleavage of K⁺F21T by Tel11

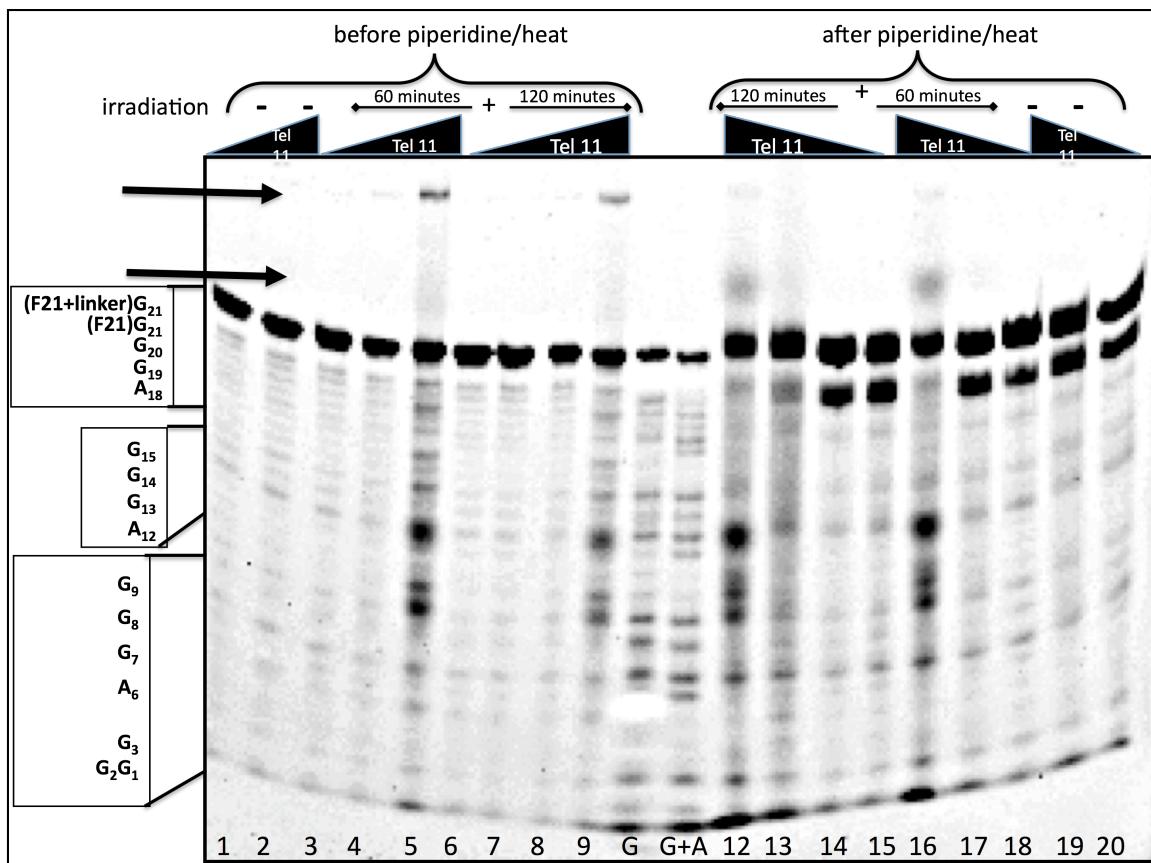


Figure S8. PAGE analysis of G-quadruplex photocleavage reactions of K⁺F21T (250 nM) in potassium cacodylate buffer with 0-100 μM Tel 11 irradiated for 0-120 minutes (420 nm). Samples in lanes 1–9 were directly subjected to PAGE. Samples in lanes 12–20 were treated with piperidine at 90 °C for 30 min prior to PAGE. Reactions contained 0 nM (lanes 1, 3, 6, 15, 18, 20) 1 μM (lanes 7, 14), 10 μM (lanes 4, 8, 13, 17) and 100 μM (lanes 2, 5, 9, 12, 16, 19) of Tel 11. Control samples in lanes 1, 2, 19, 20 were not irradiated. Samples in lanes 3–5 and 16–18 were irradiated for 60 min, while those sample in lanes 6–9 and 12–15 were irradiated for 120 min. Maxam Gilbert sequencing lanes are indicated by the G and G+A. The gel was visualized by FAM fluorescence.